

# Heightened resistance to type 1 interferons characterizes HIV-1 at transmission and following treatment interruption

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## **OVERLINE: HIV**

### **One Sentence Summary**

Interferon-mediated pressures vary throughout HIV-1 infection but are the highest during viral rebound after treatment interruption.

## **Abstract**

Type 1 interferons (IFN-I) are potent innate antiviral effectors that constrain HIV-1 transmission. However, harnessing these cytokines for HIV-1 cure strategies has been hampered by an incomplete understanding of their anti-viral activities at later stages of infection. Here, we characterized the IFN-I sensitivity of 500 clonally-derived HIV-1 isolates from the plasma and CD4<sup>+</sup> T cells of 26 individuals sampled longitudinally following transmission or after antiretroviral therapy (ART) and analytical treatment interruption. We determined the concentration of IFN $\alpha$ 2 and IFN $\beta$  that reduced viral replication in vitro by 50% (IC<sub>50</sub>), and found consistent changes in the sensitivity of HIV-1 to IFN-I inhibition both across individuals and over time. IFN-I resistance was uniformly high during acute infection, decreased in all individuals in the first year post-infection, was reacquired concomitant with CD4<sup>+</sup> T cell loss, and remained elevated in individuals with accelerated disease. Isolates obtained by viral outgrowth during suppressive ART were relatively IFN-I sensitive, resembling viruses circulating just prior to ART initiation. However, viruses that rebounded following treatment interruption displayed the highest degree of IFN $\alpha$ 2 and IFN $\beta$  resistance observed at any time during the infection course. These findings indicate a dynamic interplay between host innate immune responses and the evolving HIV-1 quasispecies, with the relative contribution of IFN-I to HIV-1 control impacted by both ART and analytical treatment interruption. Although elevated at transmission, host innate pressures are the highest during viral rebound, limiting the viruses that successfully reactivate from latency to those that are IFN-I resistant.

## Introduction

Type 1 interferons (IFN-I) comprise a family of pro-inflammatory and immunomodulatory cytokines with potent antiviral activity (1, 2). In humans, this family includes 13 IFN $\alpha$  subtypes as well as IFN $\beta$ , IFN $\epsilon$ , IFN $\kappa$ , and IFN $\omega$ , of which IFN $\alpha$  and IFN $\beta$  are the best characterized (3). IFN-I is rapidly up-regulated in response to pathogen exposure and infection (4, 5), and mediates its activity by binding to the heterodimeric interferon- $\alpha/\beta$  receptor (IFNAR) expressed on all nucleated cells (6). This binding results in the activation of signal transduction cascades that trigger the expression of hundreds of interferon-stimulated genes (ISGs), which have direct and indirect antiviral activity, and regulate the activation state, function, proliferation and survival of host immune cells (7, 8). Although all IFN-I subtypes bind to the same receptor, they differ in their affinity for the two IFNAR subunits and elicit different patterns of ISG expression, suggesting that they vary in their in vivo activity and potency (9-12).

Up-regulation of IFN-I expression is one of the earliest innate responses to infection with both human (HIV-1) and simian (SIV) immunodeficiency viruses (13-17). In acute HIV-1 infection, maximal IFN-I concentrations are detected in the plasma prior to peak viremia (18). In acute SIV infection of macaques (SIVmac), plasmacytoid dendritic cells (pDCs) are actively recruited to mucosal sites, where they produce high concentrations of both IFN $\alpha$  and IFN $\beta$  (19, 20). IFN-I is known to control HIV/SIV replication in CD4 $^+$  T cells and macrophages by targeting multiple steps in the viral lifecycle (8). Consistent with this, pre-treatment of rhesus macaques with exogenous IFN $\alpha$ 2 increased the number of intrarectal challenges required to achieve systemic SIVmac infection (21) and vaginal administration of IFN $\beta$  protected rhesus macaques from recombinant simian/human immunodeficiency virus (SHIV) infection (22). Likewise, blocking IFN-I during acute SIVmac infection led to higher viral titers, a more rapid decline of CD4 $^+$  T cells, and faster disease progression (23). Thus, numerous studies in both humans and

primates have shown that IFN-I play a key role in controlling HIV and SIV replication during the earliest stages of infection.

In contrast to the beneficial effects of IFN-I upregulation during acute HIV-1 infection, sustained IFN-I signaling during chronic infection appears to have an overall negative effect (17, 24-28). In untreated individuals, plasma IFN $\alpha$  concentrations are positively correlated with HIV-1 viral load and inversely correlated with CD4 $^{+}$  T cell counts, and individuals with higher viral loads and faster disease progression frequently exhibit higher ISG expression (24, 27) and heightened IFN $\beta$  production in the gut (29). Continuous IFN-I signaling increases the number of susceptible CD4 $^{+}$  target cells, induces CD4 $^{+}$  T cell apoptosis, limits antigen specific CD4 $^{+}$  and CD8 $^{+}$  T cell responses, and contributes to immune exhaustion (15, 30, 31). In SIVmac-infected macaques, prolonged IFN $\alpha$ 2 treatment resulted in decreased antiviral ISG expression, an increased SIVmac reservoir size, and the loss of CD4 $^{+}$  T cells (21). Indeed, sustained IFN-I signaling and ISG up-regulation differentiates pathogenic from non-pathogenic SIV infections (32-34) and drives systemic immune activation in HIV-1 infection, which is associated with poor CD4 $^{+}$  T cell recovery and a predictor of disease progression (35).

Given the pleiotropic and context-dependent effects of IFN-I, it is not surprising that clinical studies evaluating its therapeutic benefits have yielded mixed results (14, 16, 36). IFN $\alpha$ 2 administration can suppress HIV-1 replication, blunt rebound viremia, and extend viral control following the interruption of antiretroviral therapy (37-40). However, various combinations of anti-retroviral and IFN-I treatments have not led to long-term improvements of CD4 $^{+}$  T cell reconstitution or clinical outcome (14). Moreover, blocking rather than stimulating IFN-I signaling, or selectively counteracting only certain ISGs, had beneficial effects in HIV-1-infected humanized mice (41, 42). Thus, it seems clear that effective manipulation of the IFN-I system to prevent, treat, or cure HIV-1 infection will require a much more detailed understanding of the quality of the endogenous IFN-I response.

One approach to gain insight into the in vivo IFN-I response is to test the IFN-I sensitivity of plasma viruses, which are reliable indicators of selection pressures that act on both virions and virus-infected cells (43-48). Such studies have shown that transmitted founder (TF) viruses are highly IFN-I resistant (49) and that this resistance constitutes a major determinant of HIV-1 transmission fitness (50). In contrast, viruses isolated during chronic infection are generally IFN-I sensitive, although this sensitivity appears to revert as individuals progress toward acquired immunodeficiency syndrome (AIDS) (51, 52). Thus, existing data indicate that IFN-mediated pressures vary during the infection course. However, the kinetics and magnitude of these changes, and their uniformity in individuals with different rates of disease progression, have not been determined. Here, we performed a systematic analysis of the IFN-I sensitivity of plasma- and CD4+ T cell-derived viral isolates from prospectively sampled study participants before and after antiretroviral therapy (ART) and following short-term treatment interruption (also called analytical treatment interruption or ATI). We found that the endogenous IFN-I response is much more dynamic than previously assumed and that the relative contribution of IFN-I to HIV-1 control varies at different stages of the infection.

## **Results**

### *Generation of plasma isolates from longitudinally sampled individuals*

To characterize the kinetics of IFN-I resistance over the course of HIV-1 infection, we generated plasma isolates from 10 prospectively sampled individuals, who were followed from shortly after transmission until 4.1 - 12.4 years post-infection (table S1). All participants were men-who-have-sex-with-men (MSM) who presented with symptomatic primary subtype B infection. Peripheral blood was collected at regular intervals immediately following onset of symptoms and continued for 74 to 318 weeks (1.6 to 6.2 years) in the absence of antiretroviral treatment. At each time

point, participants underwent clinical evaluation, including viral load and CD4<sup>+</sup> T cell count determinations (table S1). Six individuals experienced a gradual loss of their CD4<sup>+</sup> T cells over the study period (typical progressors; Fig. 1A), two individuals maintained high CD4<sup>+</sup> T cell counts and low viral loads throughout their 3.6- and 5.6-year clinical follow-up (non-progressors; Fig. 1B), and two individuals developed AIDS (CD4<sup>+</sup> T cells < 300 cell/ $\mu$ l) within 0.7 and 1.5 years of infection (rapid progressors; Fig. 1C). ART was initiated in eight participants between weeks 85 and 323 post onset of symptoms based on the standard-of-care at the time (shaded in Fig. 1).

We also characterized the evolving HIV-1 quasispecies in these individuals. This was done by amplifying viral RNA directly from the plasma using single genome amplification (SGA), which retains genetic linkage across viral genes and generates sequences devoid of PCR-induced artifacts (53, 54). For each individual, 3' half genomes or *env* gene sequences were amplified from sequential plasma samples, with sequences from the earliest time point used for TF enumeration (fig. S1). This analysis showed that seven participants acquired a single TF virus, while the remaining three became infected with two or more TF viruses (table S1). Sequences from subsequent time points exhibited the expected patterns of viral diversification (55-57), except for participants MM14, MM23 and MM33, who became superinfected with additional subtype B strains 5 to 29 weeks following the initial infection (Fig. 1 and fig. S2).

To determine the IFN sensitivity of individual quasispecies members at different stages of HIV-1 infection, we generated 277 limiting dilution plasma isolates from five of the 10 individuals (table S1). Plasma samples were end-point diluted, co-cultured with healthy donor CD4<sup>+</sup> T cells, and the resulting isolates were sequenced to ensure that they were indeed single virion-derived (50). In phylogenetic trees of *env* gene sequences, the limiting dilution isolates were completely interspersed with sequences amplified directly from the plasma, thus confirming their authenticity (fig. S2). To characterize their sensitivity to IFN-I inhibition, we determined the IFN $\alpha$ 2 and IFN $\beta$  concentrations that reduced virus replication in vitro by 50%

(50). Briefly, donor CD4+ T cells were treated with increasing quantities of IFN $\alpha$ 2 and IFN $\beta$ , and then infected with equal amounts of virus (50). Cells were cultured for 7 days, with the IFN-containing media replenished every second day. Virus replication was measured by quantifying p24 antigen in supernatants and used to calculate the half-maximal inhibitory concentration (IC<sub>50</sub>) (fig. S3).

Since the IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values for limiting dilution isolates from the same plasma sample were similar, we reasoned that conventional (bulk) virus isolation was likely sufficient to capture the IFN-I sensitivity of the circulating virus pool. To test this, we generated 18 bulk isolates from 11 plasma samples (table S1) and determined their IFN-I resistance (table S2). All bulk isolates yielded IC<sub>50</sub> values that fell within 5-fold of the mean of the corresponding limiting dilution isolates (fig. S4, A and B). Moreover, the interferon resistance of the individual bulk isolates was highly correlated with the geometric mean of the corresponding limiting dilution isolates (IFN $\alpha$ 2  $r=0.94$  and IFN $\beta$   $r=0.97$ , fig. S4, C and D). We thus used the less labor-intensive conventional approach to generate 79 plasma isolates for the remaining five individuals.

#### *Kinetics of IFN-I resistance in untreated HIV-1 infection*

Analysis of 374 plasma isolates from 10 prospectively sampled individuals revealed differences in their sensitivity to IFN $\alpha$ 2 and IFN $\beta$  inhibition over the course of the infection (Fig. 2). To quantify these dynamic changes, we developed a Bayesian hierarchical change point model (50, 58), which allowed us to infer the temporal patterns of IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values and their associations with CD4+ T cell counts across different individuals while accounting for differences in sampling times and frequencies.

Consistent with previous results (49, 50, 59), isolates obtained during acute HIV-1 infection were uniformly IFN-I resistant, yielding mean IC<sub>50</sub> values for IFN $\alpha$ 2 of 0.90 pg/ml and for IFN $\beta$  of 19 pg/ml (Fig. 2). This resistance decreased in all individuals, with IFN $\alpha$ 2 and IFN $\beta$

IC<sub>50</sub> values falling in typical progressors on average 180-fold (95% Credible Interval [CrI]: 100 - 340-fold) and 3,500-fold (95% CrI: 1500 - 8900-fold) within 340 days (95% CrI: 250 - 490 days) and 460 days (95% CrI: 310 - 700 days), respectively (Fig. 3, A and B). Isolates from non-progressing individuals fell to similar IC<sub>50</sub> values but reached a nadir earlier at an estimated 100 days (95% CrI: 50 - 200 days) for IFN $\alpha$ 2 and 110 days (95% CrI: 50 - 240 days) for IFN $\beta$  (Fig. 3, A and B). The time to nadir for fast progressing individuals was difficult to determine because IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values decreased only 2.8-fold (95% CrI: 0.78 - 10.5-fold) and 2.7-fold (95% CrI: 0.52 - 16-fold), respectively, resulting in 39-fold and 1,000-fold higher values than in individuals with more typical disease progression (Fig. 2 and Fig. 3, A and B).

Plasma virus from typical progressors exhibited a consistent rise in IFN $\alpha$ 2 and IFN $\beta$  resistance during the later stages of infection (Fig. 2). This rise and other fluctuations in resistance during chronic infection, for example as seen in individual MM34 in Fig. 2, appeared to associate with changes in CD4<sup>+</sup> T cell counts. Indeed, the changepoint model estimated that after the initial decline in IFN-I resistance, each decrease of 100 CD4<sup>+</sup> T cells/ $\mu$ l was associated with a 2.3-fold (95% CrI: 1.3 - 4.4-fold) increase in mean IFN $\alpha$ 2 IC<sub>50</sub> values and a 4.0-fold (95% CrI: 1.9 - 8.7-fold) increase in mean IFN $\beta$  IC<sub>50</sub> values (Fig. 2A and Fig. 3, C and D). The two non-progressing individuals maintained relatively high CD4<sup>+</sup> T cell counts and the two fast progressing individuals were largely depleted of CD4<sup>+</sup> T cells early after infection. Thus, there was no apparent association between IFN-I resistance and CD4<sup>+</sup> T cell counts in these participants (Fig. 2, B and C and Fig. 3, C and D).

Overall, the temporal changes in IFN-I resistance were remarkably similar among the different individuals. Viral IFN-I resistance was high during acute infection, decreased during early chronic infection, and increased again during later stages concomitant with CD4<sup>+</sup> T cell loss and disease progression. Notably, neither the number of transmitted founder viruses at transmission nor subsequent superinfection events appeared to influence these kinetics (Fig. 2). Across all individuals and time points with IFN-I resistances spanning several orders of

magnitude, IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values were highly correlated ( $r=0.9$ , fig. S5). These findings thus indicate a highly dynamic interplay between host innate immune responses and evolving HIV-1 quasispecies, with the relative contribution of IFN-I to HIV-1 control varying markedly, but predictably, over the course of HIV-1 infection.

#### *IFN-I sensitivity of ex vivo reactivated latent viruses*

Having characterized the IFN-I response of plasma virus during untreated infection, we next used a quantitative viral outgrowth assay (QVOA) to generate reactivated latent viruses from some of the same individuals after they were placed on therapy. For five of the 10 longitudinally followed individuals, cryopreserved peripheral blood mononuclear cells (PBMCs) collected 1 to 4 years after ART initiation were available (Fig. 1). These were stimulated, co-cultured with primary CD4+ T cells from healthy donors, and tested for viral replication by monitoring p24 concentration in culture supernatants (60). Due to limited numbers of cryopreserved cells, only eight QVOA isolates were recovered (Fig. 2 and table S1). All isolates were sequenced (fig. S2) and their IFN-I resistance determined (table S2). Although participant WEAU received zidovudine (AZT) beginning 1.6 years post infection, this monotherapy was not sufficient to maintain suppression of plasma viral loads (Fig. 1, table S1). Thus, post-treatment samples from this individual were not suitable for QVOA analyses.

As shown in Fig. 2, seven of the eight QVOA isolates were moderately IFN-I resistant, exhibiting IC<sub>50</sub> values very similar to plasma isolates obtained from the same individuals immediately prior to ART initiation. This was true for individuals with single as well as multiple QVOA isolates, including viruses collected several months apart as in samples from individual MM34. A single QVOA isolate from a rapidly progressing individual (MM15) was more IFN-I resistant, but plasma virus obtained just prior to treatment initiation displayed comparable IFN-I resistance. Phylogenetic analyses confirmed these findings, showing that the eight outgrowth

isolates did not cluster with viruses recovered during acute or early infection, but were most closely related to plasma viruses replicating during late infection (Fig. 4 and fig. S2). Thus, both IFN-I and phylogenetic analyses indicated that the QVOA-derived viruses had entered the latent reservoir near the time of therapy initiation.

To increase the number of outgrowth viruses for IFN-I phenotypic analyses, we obtained cryopreserved PBMCs or previously generated QVOA isolates from 9 additional ART suppressed individuals who participated in different HIV-1 treatment interruption or latency studies (table S3). Two individuals underwent leukapheresis after prolonged ART suppression for a qualitative and quantitative analysis of their replication competent HIV-1 reservoir (61), four individuals underwent leukapheresis before (-2 weeks) and during (12 weeks) ATI, but prior to viral rebound while receiving the two human broadly neutralizing antibodies 3BNC117 and 10-1074 (62), and three individuals underwent leukapheresis before and six months after ATI and infusion of the broadly neutralizing antibody VRC01 (63, 64). From the available material, we were able to generate or expand 52 QVOA isolates and test their IFN-I sensitivity (table S4). The results showed a range of IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values that was very similar to that of the eight QVOA isolates from the longitudinally sampled cohort, with most isolates exhibiting low or moderate IFN-I resistance (Fig. 5, A and C). There were only two outgrowth viruses that displayed an unusually high IFN-I resistance, especially for IFN $\alpha$ 2, both of which were isolated from the post-ATI leukapheresis sample of a single individual (A08).

#### *IFN-I sensitivity of rebound viruses following treatment interruption*

Some of the individuals for whom QVOA isolates were available also underwent treatment interruptions, which allowed the comparison of the IFN-I sensitivity of in vitro and in vivo reactivated viruses. Using plasma samples collected shortly after detection of recrudescence viremia, we generated 37 isolates from six such individuals (table S3), most of which were limiting dilution-derived. In addition, we obtained plasma samples from seven individuals who

participated in other treatment interruption trials (table S3). Three of these underwent successive ATI cycles of increasing duration, but without further intervention (65). One individual was infused with the broadly neutralizing human monoclonal antibody 3BNC117 prior to and during ATI (66). Three individuals received a 20 week course of pegylated IFN $\alpha$ 2 (1 $\mu$ g/kg) prior to and during ART interruption (67). Using samples collected shortly after detectable viremia, we were able to generate 29 additional isolates (table S3). All rebound isolates formed individual-specific clusters in a phylogenetic tree (fig. S6) and grew efficiently in CD4<sup>+</sup> T cells isolated from healthy donors (table S4). However, analysis of their IFN $\alpha$ 2 (Fig. 5, A and B) and IFN $\beta$  (Fig. 5 C, D) IC<sub>50</sub> values yielded unexpected results: All 66 rebound isolates were highly IFN-I resistant, exceeding IC<sub>50</sub> values even from highly resistant acute infection isolates.

To quantify differences in IFN-I sensitivity between viral groups, we developed a hierarchical Bayesian model (Supplementary Methods), which combined the data from the QVOA and rebound isolates with results from the longitudinal cohort (Fig. 2) and previously published transmission pairs (50). Specifically, we compared IFN-I IC<sub>50</sub> values of acute infection isolates (<30 days after onset of symptoms) with those from rebound, chronic (>300 days after onset of symptoms) and outgrowth viruses, dividing the latter into pre- and post-ATI isolates (Fig. 5, B and D). Two apparent outliers isolated from the CD4<sup>+</sup> T cells of participant A08 six months after re-initiation of therapy suggested that viruses phenotypically resembling rebound viruses might be present in post-ATI samples (Fig. 5, A and C). To account for this possibility, we included a mixture term into the model that allowed for some proportion of post-ATI viruses to derive from the rebound population. This mixture term was also included for pre-ATI samples to allow comparison between pre- and post-ATI viruses.

Comparing all available IFN-I IC<sub>50</sub> data, we determined the fold-change in IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values of acute, rebound, chronic as well as pre-ATI and post-ATI isolates (Fig. 5, B and D). Chronic infection isolates from untreated individuals were on average 18-fold (95% CrI:

9.3 – 31-fold) less IFN $\alpha$ 2 and 99-fold (33 - 230-fold) less IFN $\beta$  resistant than acute isolates. This was also the case for pre-ATI QVOA isolates, which exhibited 13-fold (7.5 - 21-fold) lower IFN $\alpha$ 2 and 50-fold (19 - 107-fold) lower IFN $\beta$  IC<sub>50</sub> values than acute infection isolates. Remarkably, rebound viruses were the most IFN-I resistant, exhibiting on average 3.0-fold (2.3 - 3.8-fold) and 6.4-fold (3.8 - 10-fold) higher IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values than acute infection isolates, respectively. These differences were observed regardless of whether or not ART interruption was combined with additional interventions, such as the administration of broad neutralizing antibodies. Interestingly, the IFN $\alpha$ 2 IC<sub>50</sub> values of rebound isolates from individuals who were treated with pegylated IFN $\alpha$ 2 (1 $\mu$ g/kg) before and during ART interruption (individuals 004, 030, 044) were on average 1.8-fold (1.2 - 2.6-fold) higher than those of rebound viruses from IFN $\alpha$ 2 untreated individuals, while such differences were not observed for the corresponding IFN $\beta$  IC<sub>50</sub> values. The fact that IFN $\alpha$ 2 administration had a measurable effect suggests that the endogenously produced concentrations of IFN $\alpha$ 2 were not saturating. However, the increase in viral IFN $\alpha$ 2 resistance resulting from exogenous administration was modest compared to that driven by endogenous IFN $\alpha$ 2. Taken together, these data demonstrate that treatment interruption and viral recrudescence trigger an IFN-I response that places considerable selection pressures on the rebounding virus.

#### *IFN-I sensitivity of post-ATI outgrowth viruses*

Given the heightened IFN-I resistance of rebound viruses, we examined post-ATI isolates for an enrichment of such viruses. Post-ATI outgrowth viruses had mean IFN-I resistance values that were very similar to those of pre-ATI viruses, but appeared to comprise two populations (Fig. 5, B and D), primarily because of two post-ATI isolates from one individual (A08) that displayed an unusually high IFN-I resistance. The model estimated the proportion of "rebound-like" viruses in post-ATI isolates to be 13% (2.9 - 29%) for IFN $\alpha$ 2 and 4% (0.1% - 15%) for IFN $\beta$ . While no

“rebound-like” IFN-I resistant viruses were detected in pre-ATI samples, this group had credible intervals that overlapped those estimated for post-ATI samples. Thus, future studies are required to determine whether “rebound-like” isolates are only observed in post-ATI samples.

To further characterize the two post-ATI QVOA isolates with elevated  $IC_{50}$  values, we compared their sequences to those of other QVOA and rebound viruses from the same individual (Fig. 6). As previously reported (63, 64), participant A08 initiated ART during chronic infection, underwent treatment interruption and VRC01 monoclonal antibody treatment, and was restarted on ART after detection of rebound viremia. Phylogenetic analysis of viral sequences obtained before, during, and after treatment interruption identified multiple rebound lineages, some of which were closely related to both pre-ATI and post-ATI QVOA isolates (Fig. 6). When 20 of these QVOA isolates were phenotypically tested, 18 exhibited very low IFN $\alpha$ 2 and IFN $\beta$   $IC_{50}$  values, indicating that they were highly IFN-I sensitive (Fig. 5). The exceptions were the two post-ATI QVOA isolates 3D8 and 6F6, which were not only more IFN-I resistant, but also shared identical *env* sequences with three rebound viruses (highlighted by arrows in Fig. 6). Further sequence analysis confirmed these close genetic relationships, showing that the two QVOA and three rebound isolates differed at 10 or fewer sites across their genomes (fig. S7A). Thus, ATI in participant A08 appeared to have re-seeded the reservoir with IFN-I resistant viruses. However, other post-ATI QVOA isolates from participant A08 were highly IFN-I sensitive, even though they were closely related to rebound isolates. For example, the genomes of post-ATI QVOA.M20 and REB.5D2 exhibited only five nucleotide differences (fig. S7B), but the rebound isolate was over 100-fold more IFN $\alpha$ 2 resistant and over 500-fold more IFN $\beta$  resistant (Fig. 6, red and teal triangles). Thus, in some viruses, very few sequence changes are sufficient to dramatically alter the IFN-I phenotype.

## Discussion

In untreated HIV-1-infected individuals, plasma virus has a half-life of less than one hour, and the cells that are producing most of this virus have a half-life of less than one day (43, 47, 48, 68). Thus, virus circulating in the plasma of infected individuals is a sensitive real time indicator of the in vivo selection pressures that act on viruses and virally infected cells, such as neutralizing antibodies (NAb), cytotoxic T lymphocytes (CTL), and antiviral drugs (43-46). Here, we characterized the resistance of plasma viruses to an additional selection pressure present in vivo, IFN $\alpha$ 2 and IFN $\beta$ , and show that the relative contribution of these cytokines to HIV-1 control varies throughout the infection course (Fig. 2). IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values were uniformly high during acute infection, consistent with the rapid induction of a potent innate antiviral state that selects for founder viruses that are highly IFN-I resistant (18, 50). IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values declined in all individuals in the first year post infection, at least in part because of viral escape from adaptive immune responses (45, 46, 69, 70). Recent studies showed that HIV-1 resistance to interferon-induced transmembrane proteins and to IFN-I itself decreased over the first 6 months of infection as a direct result of acquiring NAb escape mutations (69). Although a similar causal relationship has not yet been shown for escape from cellular immunity, CTL responses are known to place strong pressure on viral quasispecies during early HIV-1 infection (45, 46). Thus, some of the same mutations that allow HIV-1 to successfully evade potent NAb, CTL, and other adaptive immune pressures likely result in a loss of IFN-I resistance, even if IFN-I signaling is increased (26, 32, 71, 72).

Although IFN-I IC<sub>50</sub> values varied by orders of magnitude, the observed temporal changes were surprisingly similar across all individuals. Plasma viruses from all typical progressors displayed the same initial decline of IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values irrespective of the initial multiplicity of infection (number of TF viruses) or subsequent superinfection events, followed by the gradual reacquisition of IFN-I resistance during later stages of infection. In most individuals, a nadir was reached within two years of symptom onset, although in the two rapid progressors the fall and rise of IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values was much less pronounced. Plasma

viruses from the two non-progressors displayed the same initial IC<sub>50</sub> decline, but then maintained low resistance values. Thus, the overall dynamics of IFN-I resistance were very similar, suggesting common patterns of virus-host interactions.

The balance between innate and adaptive immune pressures is also reflected in the IFN-I phenotype of outgrowth and rebound viruses. The latent reservoir is largely comprised of proviruses that are transcriptionally and translationally silent and are therefore not subject to innate or adaptive immune pressures. Moreover, adaptive immune responses are reduced under suppressive ART due to reduced viremia (73, 74). This may explain the importance of rapidly induced innate responses at the time of viral recrudescence (75) and the resulting IFN-I resistance of rebound viruses. Testing rebound plasma for IFN $\alpha$ 2 and IFN $\beta$  protein failed to show consistent increases following treatment interruption, suggesting elevated IFN-I concentrations locally at the sites of viral recrudescence. Whether the superior IFN-I resistance of rebound viruses confers enhanced transmissibility is not known, but the occurrence of transmissions during treatment interruption suggests that this possibility needs to be investigated (76, 77).

Recent studies by Abrahams and colleagues showed that 71% of outgrowth viruses are genetically most similar to viruses circulating in the plasma just prior to ART initiation (78). Since this proportion is far greater than would be expected if the reservoir formed continuously, treatment appears to alter the host environment to facilitate the formation of latently infected cells (78). We also found that outgrowth viruses isolated years after ART suppression were genetically most similar to viruses replicating late in untreated infection. Moreover, these outgrowth viruses exhibited IFN-I IC<sub>50</sub> values that were near identical to those of viruses isolated just prior to ART. Our data thus extend these earlier findings, showing that outgrowth viruses are both genetically and phenotypically most similar to viruses replicating late in infection, which provides additional evidence for the preferential entry of HIV-1 into the latent reservoir near the time of ART initiation.

The viral determinants that confer IFN-I resistance are not known. Studying transmitted founder and six-month consensus viruses from the same individual, a previous study failed to identify resistance or sensitivity signatures that were common among the different virus pairs (59). Sequence changes occurred at many sites throughout the viral genome, suggesting differential responses to numerous ISGs. Given the myriad IFN-I effector pathways and the plasticity of HIV-1 quasispecies members, these results are not surprising, especially since we demonstrate here that IFN-I resistance can be altered by a small number of sequence changes. Thus, the path to resistance for any one HIV-1 strain is likely context-dependent, although certain resistance determinants may be more common than others.

A critical question in cure research is whether treatment interruption and the associated transient viremia have an impact on the composition and size of the latent reservoir. Comparing pre-ATI and post-ATI QVOA isolates, a previous study found only minimal changes in the abundance of replication-competent viruses, with little genetic evidence of rebound viruses enriching the post-ATI reservoir (64). The exception was one individual (A08), who harbored post-ATI viruses that were genetically closely related to rebound viruses (64). Examining additional QVOA isolates from this same individual, we found that pre-ATI and post-ATI viruses had similar IFN-I resistance values. However, the post-ATI QVOAs included two isolates with elevated IFN-I resistance, which were genetically very similar to a subset of rebound viruses. Thus, the post-ATI outgrowth viruses in this individual comprised two viral populations, one of which represented “rebound-like” viruses based on genetic and phenotypic analyses.

Treatment interruptions are the gold standard to evaluate promising cure strategies (79) and thus must be safe and well designed. Consistent with previous results (64, 66), we found that pre-ATI QVOA isolates were neither predictive nor representative of the viruses that subsequently rebounded in vivo. This disconnect reinforces the clinical importance of ATIs since merely testing cure strategies in vitro may generate misleading results. A comprehensive analysis of post-ATI isolates in participant A08 suggested that treatment interruption may have

re-seeded the reservoir with IFN-I resistant viruses, although the existence of these variants prior to ART could not be formally excluded. Although rebound-like, IFN-resistant viruses comprised only a minor subset of the sampled post-ATI reservoir, this possibility requires further exploration to assure the safety of ATIs.

The finding that rebound viruses are uniformly IFN-I resistant is also relevant to the design of cure strategies that directly or indirectly engage IFN-I pathways. These approaches attempt to harness the antiviral activity of IFN-I either by administering these cytokines directly (38) or by eliciting IFN-I responses via immunomodulators, such as Toll-like receptor 7 and 9 agonists (80, 81), and other latency reversal agents (82). The fact that ART cessation likely induces near-maximal IFN-I responses at the sites of virus recrudescence will need to be factored into the design of these strategies. Consistent with this, a recent study found that activation of natural killer cell cytotoxicity, and not the induction of interferon stimulated genes, correlated with exogenous IFN $\alpha$ 2-mediated HIV-1 control (83).

A limitation of the present study is the lack of diversity among study participants, since all but one were men-who-have-sex-with-men from the US and Europe who initiated ART during chronic infection. It will thus be important to examine additional trial participants, including women and minorities, to test the generality of our results. It will also be important to examine participants who initiated ART during acute infection, since rebound viruses from such individuals have been reported to be nearly identical to the corresponding transmitted viruses (84) and may thus not differ in their IFN-I resistance. However, even in acute ART initiators, rebound viruses may have to escape heightened IFN-I pressures. Given the limited genetic diversity, this would provide a unique opportunity to dissect the underlying mechanisms. Future studies should also perform viral outgrowth assays in the presence of IFN-I to see whether pre-treatment of latently infected CD4+ T cells activates rebound-like viruses and conduct integration site analyses to determine whether IFN-I resistant viruses are preferentially found integrated in chromatin regions that restrict viral gene expression in the absence of IFN-I. The

latter finding would suggest that IFN-I stimulation is required to reactivate a subset of latently infected CD4+ T cells, which would have obvious clinical implications. Finally, the high resistance of rebound viruses reinforces the need for nonhuman primate models that faithfully recapitulate this key feature of HIV-1 persistence. If SIVmac- or SHIV-infected rhesus macaques exhibited the same IFN-I kinetics as humans, these models could be used to trace the provenance and activation requirements of rebound viruses.

In summary, we show here that IFN-I play an important role in the control of HIV-1 during acute and later stages of infection. The IFN-I resistance of plasma viruses reflects the contribution of IFN-I mediated activity relative to other antiviral pressures at the site of virus production. The kinetics of the IFN-I response vary over the course of infection, with rebound viruses exhibiting by far the most IFN-I resistance. Although the tissue and cell origins of rebound viruses remain to be determined, our data indicate that they arise either from a cryptic reservoir of highly IFN-I resistant viruses or rapidly evolve at the sites of viral recrudescence to acquire IFN-I resistance.

## **Materials and Methods**

### *Study design*

The role of IFN-I in the control of HIV-1 during infection was determined by generating 500 plasma- and PBMC-derived viral isolates from 26 individuals sampled prospectively before and during ART or following ATI. Viruses were genetically characterized and their susceptibility to IFN $\alpha$ 2 and IFN $\beta$  inhibition (IC<sub>50</sub>) was determined. Sample sizes were dependent on availability and not predetermined by power calculations. The number of samples available and the tests performed are detailed in table S1.

Participants MM14, MM15, MM23, MM33, MM34, MM39, MM40, MM55, and MM62 were recruited to an acute HIV-1 infection cohort at the Mortimer Market Centre for Sexual Health and HIV Research in London, United Kingdom, while participant WEAU was followed at the 1917 Clinic of the University of Alabama at Birmingham. Peripheral blood was collected in EDTA-containing tubes following onset of symptoms and at regular intervals thereafter (table S1). Plasma was separated by centrifugation, while PBMCs were isolated using a Histopaque 1.077 density gradient and cryopreserved. Individuals provided informed consent and were offered antiretroviral therapy based on the standard-of-care at the time. Ethical approval for the London cohort study was provided by the National Health Service Camden/Islington Community Local Research Ethics Committee. The use of stored (de-identified) samples from participant WEAU was approved by the University of Alabama at Birmingham Institutional Review Board. Plasma and viably frozen PBMC samples were also obtained from 16 individuals who participated in six different treatment interruption and latency trials (MNU-0628, NCT02825797, NCT0246322, NCT00051818, NCT02227277, NCT02588586). These materials were obtained from existing repositories and their use was approved by the respective Institutional Review Boards as previously described (61-67).

#### *Virus isolation from plasma*

CD4<sup>+</sup> T cells were isolated from buffy coats of healthy donors (purchased from ZenBio Inc.) by positive selection using human CD4 micro beads (Miltenyi Biotec) as described (50). For limiting dilution isolation, plasma samples were end-point diluted and used to infect  $1 \times 10^6$  activated CD4<sup>+</sup> T cells in 24-well plates (50). To generate bulk isolates, plasma aliquots containing 1,500 - 20,000 viral RNA copies were used to infect  $4 \times 10^6$  healthy donor CD4<sup>+</sup> T cells in 6-well plates. Cultures were maintained for 21 days and tested weekly for the presence of p24 antigen in the supernatant. Virus positive cultures were expanded in  $1 \times 10^7$  pooled healthy donor CD4<sup>+</sup> T cells and the resulting viral stocks were used for all genetic and biological analyses.

### *Quantitative viral outgrowth assay*

Virus isolations were performed as described (60) with minor modifications. CD4<sup>+</sup> T cells were isolated from viably frozen PBMC, incubated in RPMI-1640 media containing 15% fetal bovine serum (FBS) without IL-2 for 24 hours, and stimulated for 24 hours using a combination of anti-CD2, CD3 and CD28 antibody-coated beads (Miltenyi Biotec T Cell Activation/Expansion Kit). Cells were then cocultured with healthy donor CD4<sup>+</sup> T cells (1:10 patient:donor cells) and cultured for 3 weeks with weekly monitoring of p24 production. Positive cultures were expanded and viral stocks generated as described above. In cases where QVOA isolates were already available (61-64), supernatants were obtained and used to generate viral stocks in healthy donor CD4<sup>+</sup> T cells.

### *Single genome amplification*

Single genome amplification of 3' half genomes or viral *env* genes from plasma RNA and proviral DNA was performed as previously described (54, 85). Briefly, ~20,000 copies of viral RNA were extracted from plasma using QIAamp Viral RNA kit (Qiagen) and reverse transcribed using SuperScript III Reverse Transcriptase (Invitrogen). PBMC DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen). Viral cDNA and PBMC DNA were then endpoint diluted and amplified using nested PCR with primers and conditions as previously reported (54, 85).

### *Isolate sequencing*

Viral RNA was extracted from isolate stocks, reverse-transcribed, and the resulting cDNA was used to amplify overlapping 5' and 3' genome halves in separate PCR reactions (50). Amplicons were sequenced using Illumina MiSeq and paired-end reads assembled to generate a sample-specific reference sequence. Viral reads were mapped to this reference, and the extent of genetic diversity was examined for each position along the alignment. Isolates that exhibited

more than 15% diversity at any one position were judged to contain more than one variant. These positions were recorded as ambiguous sites in finished viral sequences.

### *Phylogeny*

Plasma viral-, proviral- and isolate-derived sequences for each individual were aligned with Clustal Omega (86) and inspected for misassemblies, overly truncated sequences and sequences with abundant G to A mutations (considered to be the result of APOBEC hypermutation). Alignments were trimmed to the shortest sequence and positions with gaps in any sequence were masked in all sequences. Trees were generated using RAxML v8.2.12 (87) with model GTRGAMMA and bootstrap values calculated from 1,000 replicates. Potential recombinants from superinfected individuals were identified in alignments using Recco (88) using default parameters; recombinants were reported if they had an alignment p-value <0.05 and contained at least 10 recombination induced mutations.

### *Interferon IC<sub>50</sub> determinations*

To determine the IFN $\alpha$ 2 and IFN $\beta$  concentrations required to inhibit virus replication by 50% (IC<sub>50</sub>), pooled activated CD4+ T cells were left untreated or cultured in the presence of increasing amounts of IFN $\alpha$ 2 or IFN $\beta$  for 24 hours as described (50). Cultures (2.5x10<sup>5</sup> cells) were then infected overnight with equal amounts of virus based on reverse transcriptase (RT) activity (0.25 ng of RT) and maintained for 7 days in IL-2-containing (30 U/ml) RPMI-1640+FBS media, replenishing IFN-I every 48 hours. Virus replication was measured as the amount of p24 produced at day 7 and IC<sub>50</sub> was calculated as the amount of IFN-I necessary to reduce p24 production in the absence of IFN-I by 50% (fig. S3). The same pool of activated CD4+ T cells from four healthy donors (purified by positive selection from buffy coats) was used for all IFN-I IC<sub>50</sub> determinations.

### *Statistical analyses*

We developed hierarchical Bayesian models to analyze the longitudinal changes of interferon resistance while avoiding bias from differential sampling (50, 58). These models assumed that the data were log normally distributed with the resistance observed in each individual deriving from a common population-level distribution (also see Supplementary Methods). For the longitudinal analysis, IFN-I resistance was assumed to start at an acute infection level, fall (or rise) to some nadir change point, and then rise (or fall) based on changes in CD4+ T cell counts (Fig. 3). For comparisons of outgrowth and rebound viruses, each isolate was assumed to be a random sample from the viruses circulating within an individual, and each individual was assumed to be representative of a larger population. Outgrowth isolates were assumed to represent mixed populations that could include rebound-like viruses (Supplementary Methods). Data was processed and analyzed using R v3.4.4 (89). Posterior probability distributions were estimated using Markov chain Monte Carlo sampling as implemented in Stan v2.23 (90).

### **Supplementary Materials**

#### Supplementary Methods

Fig. S1. Transmitted founder virus inference and enumeration.

Fig. S2. HIV-1 quasispecies diversification over time.

Fig. S3. Determination of IFN-I IC<sub>50</sub> values.

Fig. S4. IFN-I resistance of bulk and limiting dilution isolates from the same plasma samples.

Fig. S5. Correlation of IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values.

Fig. S6. Phylogenetic relationships of rebound isolates.

Fig. S7. Closely related rebound and post-ATI QVOA isolates from participant A08.

Table S1. Generation of HIV-1 isolates from plasma and peripheral blood mononuclear cells of 10 individuals sampled from acute infection throughout their clinical course.

Table S2. IFN-I resistance of plasma and QVOA isolates from longitudinally sampled study participants.

Table S3. Generation of viral isolates from ART suppressed individuals with and without treatment interruption.

Table S4. IFN-I resistance of viral outgrowth and rebound isolates.

Data file S1. GenBank accession numbers.

## References and Notes

1. D. B. Stetson, R. Medzhitov, Type I interferons in host defense. *Immunity* **25**, 373-381 (2006).
2. S. Pestka, C. D. Krause, M. R. Walter, Interferons, interferon-like cytokines, and their receptors. *Immunol Rev* **202**, 8-32 (2004).
3. H. M. Lazear, J. W. Schoggins, M. S. Diamond, Shared and Distinct Functions of Type I and Type III Interferons. *Immunity* **50**, 907-923 (2019).
4. E. V. Mesev, R. A. LeDesma, A. Ploss, Decoding type I and III interferon signalling during viral infection. *Nat Microbiol* **4**, 914-924 (2019).
5. G. Schreiber, The molecular basis for differential type I interferon signaling. *J Biol Chem* **292**, 7285-7294 (2017).
6. C. Thomas, I. Moraga, D. Levin, P. O. Krutzik, Y. Podoplelova, A. Trejo, C. Lee, G. Yarden, S. E. Vleck, J. S. Glenn, G. P. Nolan, J. Piehler, G. Schreiber, K. C. Garcia, Structural linkage between ligand discrimination and receptor activation by type I interferons. *Cell* **146**, 621-632 (2011).

7. T. Doyle, C. Goujon, M. H. Malim, HIV-1 and interferons: who's interfering with whom? *Nat Rev Microbiol* **13**, 403-413 (2015).
8. J. W. Schoggins, S. J. Wilson, M. Panis, M. Y. Murphy, C. T. Jones, P. Bieniasz, C. M. Rice, A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* **472**, 481-485 (2011).
9. C. M. Berry, Understanding Interferon Subtype Therapy for Viral Infections: Harnessing the Power of the Innate Immune System. *Cytokine Growth Factor Rev* **31**, 83-90 (2016).
10. T. B. Lavoie, E. Kalie, S. Crisafulli-Cabatu, R. Abramovich, G. DiGioia, K. Moolchan, S. Pestka, G. Schreiber, Binding and activity of all human alpha interferon subtypes. *Cytokine* **56**, 282-289 (2011).
11. H. P. Moll, T. Maier, A. Zommer, T. Lavoie, C. Brostjan, The differential activity of interferon-alpha subtypes is consistent among distinct target genes and cell types. *Cytokine* **53**, 52-59 (2011).
12. R. Szubin, W. L. Chang, T. Greasby, L. Beckett, N. Baumgarth, Rigid interferon-alpha subtype responses of human plasmacytoid dendritic cells. *J Interferon Cytokine Res* **28**, 749-763 (2008).
13. P. Borrow, Innate immunity in acute HIV-1 infection. *Curr Opin HIV AIDS* **6**, 353-363 (2011).
14. S. E. Bosinger, N. S. Utay, Type I interferon: understanding its role in HIV pathogenesis and therapy. *Curr HIV/AIDS Rep* **12**, 41-53 (2015).
15. K. Nganou-Makamdop, D. C. Douek, Manipulating the Interferon Signaling Pathway: Implications for HIV Infection. *Virol Sin* **34**, 192-196 (2019).

16. N. S. Uday, D. C. Douek, Interferons and HIV Infection: The Good, the Bad, and the Ugly. *Pathog Immun* **1**, 107-116 (2016).
17. S. E. Bosinger, D. L. Sodora, G. Silvestri, Generalized immune activation and innate immune responses in simian immunodeficiency virus infection. *Curr Opin HIV AIDS* **6**, 411-418 (2011).
18. A. R. Stacey, P. J. Norris, L. Qin, E. A. Haygreen, E. Taylor, J. Heitman, M. Lebedeva, A. DeCamp, D. Li, D. Grove, S. G. Self, P. Borrow, Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. *J Virol* **83**, 3719-3733 (2009).
19. K. Abel, D. M. Rocke, B. Chohan, L. Fritts, C. J. Miller, Temporal and anatomic relationship between virus replication and cytokine gene expression after vaginal simian immunodeficiency virus infection. *J Virol* **79**, 12164-12172 (2005).
20. Q. Li, J. D. Estes, P. M. Schlievert, L. Duan, A. J. Brosnahan, P. J. Southern, C. S. Reilly, M. L. Peterson, N. Schultz-Darken, K. G. Brunner, K. R. Nephew, S. Pambuccian, J. D. Lifson, J. V. Carlis, A. T. Haase, Glycerol monolaurate prevents mucosal SIV transmission. *Nature* **458**, 1034-1038 (2009).
21. N. G. Sandler, S. E. Bosinger, J. D. Estes, R. T. Zhu, G. K. Tharp, E. Boritz, D. Levin, S. Wijeyesinghe, K. N. Makamdop, G. Q. del Prete, B. J. Hill, J. K. Timmer, E. Reiss, G. Yarden, S. Darko, E. Contijoch, J. P. Todd, G. Silvestri, M. Nason, R. B. Norgren, Jr., B. F. Keele, S. Rao, J. A. Langer, J. D. Lifson, G. Schreiber, D. C. Douek, Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. *Nature* **511**, 601-605 (2014).

22. R. S. Veazey, H. A. Pilch-Cooper, T. J. Hope, G. Alter, A. M. Carias, M. Sips, X. Wang, B. Rodriguez, S. F. Sieg, A. Reich, P. Wilkinson, M. J. Cameron, M. M. Lederman, Prevention of SHIV transmission by topical IFN-beta treatment. *Mucosal Immunol* **9**, 1528-1536 (2016).
23. D. Carnathan, B. Lawson, J. Yu, K. Patel, J. M. Billingsley, G. K. Tharp, O. M. Delmas, R. Dawoud, P. Wilkinson, C. Nicolette, M. J. Cameron, R. P. Sekaly, S. E. Bosinger, G. Silvestri, T. H. Vanderford, Reduced Chronic Lymphocyte Activation following Interferon Alpha Blockade during the Acute Phase of Simian Immunodeficiency Virus Infection in Rhesus Macaques. *J Virol* **92**, (2018).
24. G. A. Hardy, S. Sieg, B. Rodriguez, D. Anthony, R. Asaad, W. Jiang, J. Mudd, T. Schacker, N. T. Funderburg, H. A. Pilch-Cooper, R. Debernardo, R. L. Rabin, M. M. Lederman, C. V. Harding, Interferon-alpha is the primary plasma type-I IFN in HIV-1 infection and correlates with immune activation and disease markers. *PLoS One* **8**, e56527 (2013).
25. A. R. Sedaghat, J. German, T. M. Teslovich, J. Cofrancesco, Jr., C. C. Jie, C. C. Talbot, Jr., R. F. Siliciano, Chronic CD4+ T-cell activation and depletion in human immunodeficiency virus type 1 infection: type I interferon-mediated disruption of T-cell dynamics. *J Virol* **82**, 1870-1883 (2008).
26. H. Rempel, B. Sun, C. Calosing, S. K. Pillai, L. Pulliam, Interferon-alpha drives monocyte gene expression in chronic unsuppressed HIV-1 infection. *AIDS* **24**, 1415-1423 (2010).
27. S. Fernandez, S. Tanaskovic, K. Helbig, R. Rajasuriar, M. Kramski, J. M. Murray, M. Beard, D. Purcell, S. R. Lewin, P. Price, M. A. French, CD4+ T-cell deficiency in HIV

- patients responding to antiretroviral therapy is associated with increased expression of interferon-stimulated genes in CD4+ T cells. *J Infect Dis* **204**, 1927-1935 (2011).
28. G. Silvestri, D. L. Sodora, R. A. Koup, H. M. McClure, S. I. Staprans, M. B. Feinberg, Nonpathogenic SIV Infection of Sooty Mangabeys Is Characterized by Limited Bystander Immunopathology Despite Chronic High-Level Viremia. *Immunity* **18**, 441-452 (2003).
  29. S. M. Dillon, K. Guo, G. L. Austin, S. Gianella, P. A. Engen, E. A. Mutlu, J. Losurdo, G. Swanson, P. Chakradeo, A. Keshavarzian, A. L. Landay, M. L. Santiago, C. C. Wilson, A compartmentalized type I interferon response in the gut during chronic HIV-1 infection is associated with immunopathogenesis. *AIDS* **32**, 1599-1611 (2018).
  30. J. P. Herbeuval, A. Boasso, J. C. Grivel, A. W. Hardy, S. A. Anderson, M. J. Dolan, C. Chougnet, J. D. Lifson, G. M. Shearer, TNF-related apoptosis-inducing ligand (TRAIL) in HIV-1-infected patients and its in vitro production by antigen-presenting cells. *Blood* **105**, 2458-2464 (2005).
  31. D. Sauce, C. Elbim, V. Appay, Monitoring cellular immune markers in HIV infection: from activation to exhaustion. *Curr Opin HIV AIDS* **8**, 125-131 (2013).
  32. S. Lederer, D. Favre, K. A. Walters, S. Proll, B. Kanwar, Z. Kasakow, C. R. Baskin, R. Palermo, J. M. McCune, M. G. Katze, Transcriptional profiling in pathogenic and non-pathogenic SIV infections reveals significant distinctions in kinetics and tissue compartmentalization. *PLoS Pathog* **5**, e1000296 (2009).
  33. S. E. Bosinger, Q. Li, S. N. Gordon, N. R. Klatt, L. Duan, L. Xu, N. Francella, A. Sidahmed, A. J. Smith, E. M. Cramer, M. Zeng, D. Masopust, J. V. Carlis, L. Ran, T. H. Vanderford, M. Paiardini, R. B. Isett, D. A. Baldwin, J. G. Else, S. I. Staprans, G. Silvestri, A. T. Haase, D. J. Kelvin, Global genomic analysis reveals rapid control of a

- robust innate response in SIV-infected sooty mangabeys. *J Clin Invest* **119**, 3556-3572 (2009).
34. B. Jacquelin, V. Mayau, B. Targat, A. S. Liovat, D. Kunkel, G. Petitjean, M. A. Dillies, P. Roques, C. Butor, G. Silvestri, L. D. Giavedoni, P. Lebon, F. Barre-Sinoussi, A. Benecke, M. C. Muller-Trutwin, Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I IFN response. *J Clin Invest* **119**, 3544-3555 (2009).
35. P. W. Hunt, HIV and inflammation: mechanisms and consequences. *Curr HIV/AIDS Rep* **9**, 139-147 (2012).
36. A. Soper, I. Kimura, S. Nagaoka, Y. Konno, K. Yamamoto, Y. Koyanagi, K. Sato, Type I Interferon Responses by HIV-1 Infection: Association with Disease Progression and Control. *Front Immunol* **8**, 1823 (2017).
37. D. M. Asmuth, R. L. Murphy, S. L. Rosenkranz, J. J. Lertora, S. Kottlilil, Y. Cramer, E. S. Chan, R. T. Schooley, C. R. Rinaldo, N. Thielman, X. D. Li, S. M. Wahl, J. Shore, J. Janik, R. A. Lempicki, Y. Simpson, R. B. Pollard, A. C. T. G. A. Team, Safety, tolerability, and mechanisms of antiretroviral activity of pegylated interferon Alfa-2a in HIV-1-monoinfected participants: a phase II clinical trial. *J Infect Dis* **201**, 1686-1696 (2010).
38. L. Azzoni, A. S. Foulkes, E. Papasavvas, A. M. Mexas, K. M. Lynn, K. Mounzer, P. Tebas, J. M. Jacobson, I. Frank, M. P. Busch, S. G. Deeks, M. Carrington, U. O'Doherty, J. Kostman, L. J. Montaner, Pegylated Interferon alfa-2a monotherapy results in suppression of HIV type 1 replication and decreased cell-associated HIV DNA integration. *J Infect Dis* **207**, 213-222 (2013).

39. F. Boue, J. Reynes, C. Rouzioux, D. Emilie, F. Souala, R. Tubiana, C. Goujard, R. Lancar, D. Costagliola, Alpha interferon administration during structured interruptions of combination antiretroviral therapy in patients with chronic HIV-1 infection: INTERVAC ANRS 105 trial. *AIDS* **25**, 115-118 (2011).
40. C. Goujard, D. Emilie, C. Roussillon, V. Godot, C. Rouzioux, A. Venet, C. Colin, G. Pialoux, P. M. Girard, V. Boilet, M. L. Chaix, P. Galanaud, G. Chene, A.-I. S. Group, Continuous versus intermittent treatment strategies during primary HIV-1 infection: the randomized ANRS INTERPRIM Trial. *AIDS* **26**, 1895-1905 (2012).
41. A. Zhen, V. Rezek, C. Youn, B. Lam, N. Chang, J. Rick, M. Carrillo, H. Martin, S. Kasparian, P. Syed, N. Rice, D. G. Brooks, S. G. Kitchen, Targeting type I interferon-mediated activation restores immune function in chronic HIV infection. *J Clin Invest* **127**, 260-268 (2017).
42. L. Cheng, J. Ma, J. Li, D. Li, G. Li, F. Li, Q. Zhang, H. Yu, F. Yasui, C. Ye, L. C. Tsao, Z. Hu, L. Su, L. Zhang, Blocking type I interferon signaling enhances T cell recovery and reduces HIV-1 reservoirs. *J Clin Invest* **127**, 269-279 (2017).
43. X. Wei, J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar-Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong, G. M. Shaw, Antibody neutralization and escape by HIV-1. *Nature* **422**, 307-312 (2003).
44. K. J. Bar, C. Y. Tsao, S. S. Iyer, J. M. Decker, Y. Yang, M. Bonsignori, X. Chen, K. K. Hwang, D. C. Montefiori, H. X. Liao, P. Hraber, W. Fischer, H. Li, S. Wang, S. Sterrett, B. F. Keele, V. V. Ganusov, A. S. Perelson, B. T. Korber, I. Georgiev, J. S. McLellan, J. W. Pavlicek, F. Gao, B. F. Haynes, B. H. Hahn, P. D. Kwong, G. M. Shaw, Early low-titer

- neutralizing antibodies impede HIV-1 replication and select for virus escape. *PLoS Pathog* **8**, e1002721 (2012).
45. P. Borrow, H. Lewicki, X. Wei, M. S. Horwitz, N. Pfeffer, H. Meyers, J. A. Nelson, J. E. Gairin, B. H. Hahn, M. B. Oldstone, G. M. Shaw, Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat Med* **3**, 205-211 (1997).
  46. N. Goonetilleke, M. K. Liu, J. F. Salazar-Gonzalez, G. Ferrari, E. Giorgi, V. V. Ganusov, B. F. Keele, G. H. Learn, E. L. Turnbull, M. G. Salazar, K. J. Weinhold, S. Moore, C. C. C. B, N. Letvin, B. F. Haynes, M. S. Cohen, P. Hraber, T. Bhattacharya, P. Borrow, A. S. Perelson, B. H. Hahn, G. M. Shaw, B. T. Korber, A. J. McMichael, The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. *J Exp Med* **206**, 1253-1272 (2009).
  47. A. S. Perelson, A. U. Neumann, M. Markowitz, J. M. Leonard, D. D. Ho, HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science* **271**, 1582-1586 (1996).
  48. B. Ramratnam, S. Bonhoeffer, J. Binley, A. Hurley, L. Zhang, J. E. Mittler, M. Markowitz, J. P. Moore, A. S. Perelson, D. D. Ho, Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. *Lancet* **354**, 1782-1785 (1999).
  49. N. F. Parrish, F. Gao, H. Li, E. E. Giorgi, H. J. Barbian, E. H. Parrish, L. Zajic, S. S. Iyer, J. M. Decker, A. Kumar, B. Hora, A. Berg, F. Cai, J. Hopper, T. N. Denny, H. Ding, C. Ochsenbauer, J. C. Kappes, R. P. Galimidi, A. P. West, Jr., P. J. Bjorkman, C. B. Wilen, R. W. Doms, M. O'Brien, N. Bhardwaj, P. Borrow, B. F. Haynes, M. Muldoon, J. P.

- Theiler, B. Korber, G. M. Shaw, B. H. Hahn, Phenotypic properties of transmitted founder HIV-1. *Proc Natl Acad Sci U S A* **110**, 6626-6633 (2013).
50. S. S. Iyer, F. Bibollet-Ruche, S. Sherrill-Mix, G. H. Learn, L. Plenderleith, A. G. Smith, H. J. Barbian, R. M. Russell, M. V. Gondim, C. Y. Bahari, C. M. Shaw, Y. Li, T. Decker, B. F. Haynes, G. M. Shaw, P. M. Sharp, P. Borrow, B. H. Hahn, Resistance to type 1 interferons is a major determinant of HIV-1 transmission fitness. *Proc Natl Acad Sci U S A* **114**, E590-E599 (2017).
51. M. S. Kunzi, H. Farzadegan, J. B. Margolick, D. Vlahov, P. M. Pitha, Identification of human immunodeficiency virus primary isolates resistant to interferon-alpha and correlation of prevalence to disease progression. *J Infect Dis* **171**, 822-828 (1995).
52. B. R. Edlin, M. H. St Clair, P. M. Pitha, S. M. Whaling, D. M. King, J. D. Bitran, R. A. Weinstein, In-vitro resistance to zidovudine and alpha-interferon in HIV-1 isolates from patients: correlations with treatment duration and response. *Ann Intern Med* **117**, 457-460 (1992).
53. J. F. Salazar-Gonzalez, E. Bailes, K. T. Pham, M. G. Salazar, M. B. Guffey, B. F. Keele, C. A. Derdeyn, P. Farmer, E. Hunter, S. Allen, O. Manigart, J. Mulenga, J. A. Anderson, R. Swanstrom, B. F. Haynes, G. S. Athreya, B. T. Korber, P. M. Sharp, G. M. Shaw, B. H. Hahn, Deciphering human immunodeficiency virus type 1 transmission and early envelope diversification by single-genome amplification and sequencing. *J Virol* **82**, 3952-3970 (2008).
54. B. F. Keele, E. E. Giorgi, J. F. Salazar-Gonzalez, J. M. Decker, K. T. Pham, M. G. Salazar, C. Sun, T. Grayson, S. Wang, H. Li, X. Wei, C. Jiang, J. L. Kirchherr, F. Gao, J. A. Anderson, L. H. Ping, R. Swanstrom, G. D. Tomaras, W. A. Blattner, P. A. Goepfert,

- J. M. Kilby, M. S. Saag, E. L. Delwart, M. P. Busch, M. S. Cohen, D. C. Montefiori, B. F. Haynes, B. Gaschen, G. S. Athreya, H. Y. Lee, N. Wood, C. Seoighe, A. S. Perelson, T. Bhattacharya, B. T. Korber, B. H. Hahn, G. M. Shaw, Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A* **105**, 7552-7557 (2008).
55. F. Gao, M. Bonsignori, H. X. Liao, A. Kumar, S. M. Xia, X. Lu, F. Cai, K. K. Hwang, H. Song, T. Zhou, R. M. Lynch, S. M. Alam, M. A. Moody, G. Ferrari, M. Berrong, G. Kelsoe, G. M. Shaw, B. H. Hahn, D. C. Montefiori, G. Kamanga, M. S. Cohen, P. Hraber, P. D. Kwong, B. T. Korber, J. R. Mascola, T. B. Kepler, B. F. Haynes, Cooperation of B cell lineages in induction of HIV-1-broadly neutralizing antibodies. *Cell* **158**, 481-491 (2014).
56. M. Bonsignori, E. F. Kreider, D. Fera, R. R. Meyerhoff, T. Bradley, K. Wiehe, S. M. Alam, B. Aussedat, W. E. Walkowicz, K. K. Hwang, K. O. Saunders, R. Zhang, M. A. Gladden, A. Monroe, A. Kumar, S. M. Xia, M. Cooper, M. K. Louder, K. McKee, R. T. Bailer, B. W. Pier, C. A. Jette, G. Kelsoe, W. B. Williams, L. Morris, J. Kappes, K. Wagh, G. Kamanga, M. S. Cohen, P. T. Hraber, D. C. Montefiori, A. Trama, H. X. Liao, T. B. Kepler, M. A. Moody, F. Gao, S. J. Danishefsky, J. R. Mascola, G. M. Shaw, B. H. Hahn, S. C. Harrison, B. T. Korber, B. F. Haynes, Staged induction of HIV-1 glycan-dependent broadly neutralizing antibodies. *Sci Transl Med* **9**, eaai7514 (2017).
57. K. Wagh, E. F. Kreider, Y. Li, H. J. Barbian, G. H. Learn, E. Giorgi, P. T. Hraber, T. G. Decker, A. G. Smith, M. V. Gondim, L. Gillis, J. Wandzilak, G. Y. Chuang, R. Rawi, F. Cai, P. Pellegrino, I. Williams, J. Overbaugh, F. Gao, P. D. Kwong, B. F. Haynes, G. M. Shaw, P. Borrow, M. S. Seaman, B. H. Hahn, B. Korber, Completeness of HIV-1

- Envelope Glycan Shield at Transmission Determines Neutralization Breadth. *Cell Rep* **25**, 893-908 e897 (2018).
58. A. Gelman, J. B. Carlin, H. S. Stern, D. B. Rubin, *Bayesian Data Analysis, Second Edition (Texts in Statistical Science)*. (Chapman & Hall/CRC 2003).
59. A. E. Fenton-May, O. Dibben, T. Emmerich, H. Ding, K. Pfafferott, M. M. Aasa-Chapman, P. Pellegrino, I. Williams, M. S. Cohen, F. Gao, G. M. Shaw, B. H. Hahn, C. Ochsenbauer, J. C. Kappes, P. Borrow, Relative resistance of HIV-1 founder viruses to control by interferon-alpha. *Retrovirology* **10**, 146 (2013).
60. G. M. Laird, E. E. Eisele, S. A. Rabi, J. Lai, S. Chioma, J. N. Blankson, J. D. Siliciano, R. F. Siliciano, Rapid quantification of the latent reservoir for HIV-1 using a viral outgrowth assay. *PLoS Pathog* **9**, e1003398 (2013).
61. J. C. Lorenzi, Y. Z. Cohen, L. B. Cohn, E. F. Kreider, J. P. Barton, G. H. Learn, T. Oliveira, C. L. Lavine, J. A. Horwitz, A. Settler, M. Jankovic, M. S. Seaman, A. K. Chakraborty, B. H. Hahn, M. Caskey, M. C. Nussenzweig, Paired quantitative and qualitative assessment of the replication-competent HIV-1 reservoir and comparison with integrated proviral DNA. *Proc Natl Acad Sci U S A* **113**, E7908-E7916 (2016).
62. P. Mendoza, H. Gruell, L. Nogueira, J. A. Pai, A. L. Butler, K. Millard, C. Lehmann, I. Suarez, T. Y. Oliveira, J. C. C. Lorenzi, Y. Z. Cohen, C. Wyen, T. Kummerle, T. Karagounis, C. L. Lu, L. Handl, C. Unson-O'Brien, R. Patel, C. Ruping, M. Schlotz, M. Witmer-Pack, I. Shimeliovich, G. Kremer, E. Thomas, K. E. Seaton, J. Horowitz, A. P. West, Jr., P. J. Bjorkman, G. D. Tomaras, R. M. Gulick, N. Pfeifer, G. Fatkenheuer, M. S. Seaman, F. Klein, M. Caskey, M. C. Nussenzweig, Combination therapy with anti-HIV-1 antibodies maintains viral suppression. *Nature* **561**, 479-484 (2018).

63. K. J. Bar, M. C. Sneller, L. J. Harrison, J. S. Justement, E. T. Overton, M. E. Petrone, D. B. Salantes, C. A. Seamon, B. Scheinfeld, R. W. Kwan, G. H. Learn, M. A. Proschan, E. F. Kreider, J. Blazkova, M. Bardsley, E. W. Refsland, M. Messer, K. E. Clarridge, N. B. Tustin, P. J. Madden, K. Oden, S. J. O'Dell, B. Jarocki, A. R. Shiakolas, R. L. Tressler, N. A. Doria-Rose, R. T. Bailer, J. E. Ledgerwood, E. V. Capparelli, R. M. Lynch, B. S. Graham, S. Moir, R. A. Koup, J. R. Mascola, J. A. Hoxie, A. S. Fauci, P. Tebas, T. W. Chun, Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption. *N Engl J Med* **375**, 2037-2050 (2016).
64. D. B. Salantes, Y. Zheng, F. Mampe, T. Srivastava, S. Beg, J. Lai, J. Z. Li, R. L. Tressler, R. A. Koup, J. Hoxie, M. Abdel-Mohsen, S. Sherrill-Mix, K. McCormick, E. T. Overton, F. D. Bushman, G. H. Learn, R. F. Siliciano, J. M. Siliciano, P. Tebas, K. J. Bar, HIV-1 latent reservoir size and diversity are stable following brief treatment interruption. *J Clin Invest* **128**, 3102-3115 (2018).
65. E. Papasavvas, J. R. Kostman, K. Mounzer, R. M. Grant, R. Gross, C. Gallo, L. Azzoni, A. Foulkes, B. Thiel, M. Pistilli, A. Mackiewicz, J. Shull, L. J. Montaner, Randomized, controlled trial of therapy interruption in chronic HIV-1 infection. *PLoS Med* **1**, e64 (2004).
66. Y. Z. Cohen, J. C. C. Lorenzi, L. Krassnig, J. P. Barton, L. Burke, J. Pai, C. L. Lu, P. Mendoza, T. Y. Oliveira, C. Sleckman, K. Millard, A. L. Butler, J. P. Dizon, S. A. Belblidia, M. Witmer-Pack, I. Shimeliovich, R. M. Gulick, M. S. Seaman, M. Jankovic, M. Caskey, M. C. Nussenzweig, Relationship between latent and rebound viruses in a clinical trial of anti-HIV-1 antibody 3BNC117. *J Exp Med* **215**, 2311-2324 (2018).
67. L. Azzoni, E. Papasavvas, J. Kostman, P. Tebas, K. Mounzer, I. Frank, K. M. Lynn, L. Lalley-Chareczko, R. Feng, S. Appel, B. Howell, D. Holder, S. L. Goh, G. Wu, L. M.

- Montaner, Interferon  $\alpha$ 2b reduces inducible CD4-associated HIV in ART-suppressed individuals, Abstract 136 presented at the *Conference on Retroviruses and Opportunistic Infections, Seattle Washington, March 4-7, 2019*.
68. M. Markowitz, M. Louie, A. Hurley, E. Sun, M. Di Mascio, A. S. Perelson, D. D. Ho, A novel antiviral intervention results in more accurate assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay in vivo. *J Virol* **77**, 5037-5038 (2003).
  69. T. L. Foster, H. Wilson, S. S. Iyer, K. Coss, K. Doores, S. Smith, P. Kellam, A. Finzi, P. Borrow, B. H. Hahn, S. J. D. Neil, Resistance of Transmitted Founder HIV-1 to IFITM-Mediated Restriction. *Cell Host Microbe* **20**, 429-442 (2016).
  70. D. R. Collins, G. D. Gaiha, B. D. Walker, CD8(+) T cells in HIV control, cure and prevention. *Nat Rev Immunol*, (2020).
  71. L. D. Harris, B. Tabb, D. L. Sodora, M. Paiardini, N. R. Klatt, D. C. Douek, G. Silvestri, M. Muller-Trutwin, I. Vasile-Pandrea, C. Apetrei, V. Hirsch, J. Lifson, J. M. Brenchley, J. D. Estes, Downregulation of robust acute type I interferon responses distinguishes nonpathogenic simian immunodeficiency virus (SIV) infection of natural hosts from pathogenic SIV infection of rhesus macaques. *J Virol* **84**, 7886-7891 (2010).
  72. Q. Li, A. J. Smith, T. W. Schacker, J. V. Carlis, L. Duan, C. S. Reilly, A. T. Haase, Microarray analysis of lymphatic tissue reveals stage-specific, gene expression signatures in HIV-1 infection. *J Immunol* **183**, 1975-1982 (2009).
  73. J. A. Warren, G. Clutton, N. Goonetilleke, Harnessing CD8(+) T Cells Under HIV Antiretroviral Therapy. *Front Immunol* **10**, 291 (2019).

74. M. Bonsignori, M. A. Moody, R. J. Parks, T. M. Holl, G. Kelsoe, C. B. Hicks, N. Vandergrift, G. D. Tomaras, B. F. Haynes, HIV-1 envelope induces memory B cell responses that correlate with plasma antibody levels after envelope gp120 protein vaccination or HIV-1 infection. *J Immunol* **183**, 2708-2717 (2009).
75. J. L. Mitchell, H. Takata, R. Muir, D. J. Colby, E. Kroon, T. A. Crowell, C. Sacdalan, S. Pinyakorn, S. Puttamaswin, K. Benjapornpong, R. Trichavaroj, R. L. Tressler, L. Fox, V. R. Polonis, D. L. Bolton, F. Maldarelli, S. R. Lewin, E. K. Haddad, P. Phanuphak, M. L. Robb, N. L. Michael, M. de Souza, N. Phanuphak, J. Ananworanich, L. Trautmann, R. V. Rv, R. V. S. Groups, Plasmacytoid dendritic cells sense HIV replication before detectable viremia following treatment interruption. *J Clin Invest* **130**, 2845-2858 (2020).
76. A. Ugarte, Y. Romero, A. Tricas, C. Casado, C. Lopez-Galindez, F. Garcia, L. Leal, Unintended HIV-1 Infection During Analytical Therapy Interruption. *J Infect Dis* **221**, 1740-1742 (2020).
77. J. D. Lelievre, L. Hocqueloux, Unintended HIV-1 Transmission to a Sex Partner in a Study of a Therapeutic Vaccine Candidate. *J Infect Dis* **220**, S5-S6 (2019).
78. M. R. Abrahams, S. B. Joseph, N. Garrett, L. Tyers, M. Moeser, N. Archin, O. D. Council, D. Matten, S. Zhou, D. Doolabh, C. Anthony, N. Goonetilleke, S. A. Karim, D. M. Margolis, S. K. Pond, C. Williamson, R. Swanstrom, The replication-competent HIV-1 latent reservoir is primarily established near the time of therapy initiation. *Sci Transl Med* **11**, eaaw5589 (2019).
79. B. Julg, L. Dee, J. Ananworanich, D. H. Barouch, K. Bar, M. Caskey, D. J. Colby, L. Dawson, K. L. Dong, K. Dube, J. Eron, J. Frater, R. T. Gandhi, R. Geleziunas, P. Goulder, G. J. Hanna, R. Jefferys, R. Johnston, D. Kuritzkes, J. Z. Li, U. Likhitwonnawut,

- J. van Lunzen, J. Martinez-Picado, V. Miller, L. J. Montaner, D. F. Nixon, D. Palm, G. Pantaleo, H. Peay, D. Persaud, J. Salzwedel, K. Salzwedel, T. Schacker, V. Sheikh, O. S. Sogaard, S. Spudich, K. Stephenson, J. Sugarman, J. Taylor, P. Tebas, C. T. Tiemessen, R. Tressler, C. D. Weiss, L. Zheng, M. L. Robb, N. L. Michael, J. W. Mellors, S. G. Deeks, B. D. Walker, Recommendations for analytical antiretroviral treatment interruptions in HIV research trials-report of a consensus meeting. *Lancet HIV* **6**, e259-e268 (2019).
80. E. N. Borducchi, J. Liu, J. P. Nkolola, A. M. Cadena, W. H. Yu, S. Fischinger, T. Broge, P. Abbink, N. B. Mercado, A. Chandrashekar, D. Jetton, L. Peter, K. McMahan, E. T. Moseley, E. Bekerman, J. Hesselgesser, W. Li, M. G. Lewis, G. Alter, R. Geleziunas, D. H. Barouch, Publisher Correction: Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. *Nature* **564**, E8 (2018).
81. L. K. Vibholm, C. V. Konrad, M. H. Schleimann, G. Frattari, A. Winckelmann, V. Klastrup, N. M. Jensen, S. S. Jensen, M. Schmidt, B. Wittig, K. Zuwala, K. Mack, R. Olesen, S. Hua, M. Lichterfeld, L. Ostergaard, P. W. Denton, M. Tolstrup, O. S. Sogaard, Effects of 24-week Toll-like receptor 9 agonist treatment in HIV type 1+ individuals. *AIDS* **33**, 1315-1325 (2019).
82. D. M. Margolis, J. V. Garcia, D. J. Hazuda, B. F. Haynes, Latency reversal and viral clearance to cure HIV-1. *Science* **353**, aaf6517 (2016).
83. E. Papasavvas, L. Azzoni, A. V. Kossenkov, N. Dawany, K. H. Morales, M. Fair, B. N. Ross, K. Lynn, A. Mackiewicz, K. Mounzer, P. Tebas, J. M. Jacobson, J. R. Kostman, L. Showe, L. J. Montaner, NK Response Correlates with HIV Decrease in Pegylated IFN-alpha2a-Treated Antiretroviral Therapy-Suppressed Subjects. *J Immunol* **203**, 705-717 (2019).

84. E. M. Cale, H. Bai, M. Bose, M. A. Messina, D. J. Colby, E. Sanders-Buell, B. Dearlove, Y. Li, E. Engeman, D. Silas, A. M. O'Sullivan, B. Mann, S. Pinyakorn, J. Intasan, K. Benjapornpong, C. Sacdalan, E. Kroon, N. Phanuphak, R. Gramzinski, S. Vasan, M. L. Robb, N. L. Michael, R. M. Lynch, R. T. Bailer, A. Pagliuzza, N. Chomont, A. Pegu, N. A. Doria-Rose, L. Trautmann, T. A. Crowell, J. R. Mascola, J. Ananworanich, S. Tovanabutra, M. Rolland, R. V. S. Group, Neutralizing antibody VRC01 failed to select for HIV-1 mutations upon viral rebound. *J Clin Invest* **130**, 3299-3304 (2020).
85. J. F. Salazar-Gonzalez, M. G. Salazar, B. F. Keele, G. H. Learn, E. E. Giorgi, H. Li, J. M. Decker, S. Wang, J. Baalwa, M. H. Kraus, N. F. Parrish, K. S. Shaw, M. B. Guffey, K. J. Bar, K. L. Davis, C. Ochsenbauer-Jambor, J. C. Kappes, M. S. Saag, M. S. Cohen, J. Mulenga, C. A. Derdeyn, S. Allen, E. Hunter, M. Markowitz, P. Hraber, A. S. Perelson, T. Bhattacharya, B. F. Haynes, B. T. Korber, B. H. Hahn, G. M. Shaw, Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. *J Exp Med* **206**, 1273-1289 (2009).
86. F. Sievers, A. Wilm, D. Dineen, T. J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert, J. Soding, J. D. Thompson, D. G. Higgins, Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* **7**, 539 (2011).
87. A. Stamatakis, RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312-1313 (2014).
88. J. Maydt, T. Lengauer, Recco: recombination analysis using cost optimization. *Bioinformatics* **22**, 1064-1071 (2006).
89. The R Project for Statistical Computing, <https://www.r-project.org/>.

90. B. Carpenter, A. Gelman, M. D. Hoffman, D. Lee, B. Goodrich, M. Betancourt, M. Brubaker, J. Guo, P. Li, A. Riddell, Stan: A Probabilistic Programming Language. *Journal of Statistical Software* **76**, (2017).

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### **Author contributions**

M.V.P.G., S.S.M., F.B.R., R.M.R., G.M.S., P.B., K.J.B. and B.H.H. conceived and planned the study; M.V.P.G., F.B.R., S.T., A.G.S., Y.L., W.L., A.N.A., J.C.D., and J.C. performed sequence analyses; M.V.P.G, S.S.M., F.B.R., R.M.R. and S.T. isolated virus and determined IFN-I IC<sub>50</sub> values; L.J.P. and P.M.S. performed evolutionary analyses; S.S.M. performed statistical analyses; A.E.F.M., P.P., I.W., E.P., J.C.C.L., D.B.S., F.M., M.A.M., Y.Z.C., S.H., M.S.S., L.J.M., R.G.C., J.M.S., R.F.S., M.C., M.C.N., P.B., K.J.B. conducted clinical trials, provided patient material, performed serological analyses, and performed outgrowth assays; M.V.P.G., S.S.M.,

G.M.S., P.B., K.J.B. and B.H.H. coordinated the contributions of all authors and wrote the manuscript.

### **Competing interests**

The authors declare no competing financial interests. S.H. is a member of an HIV/Immunology advisory panel for Merck and Co.

### **Data and materials availability**

All data associated with this study are in the paper or supplementary materials. HIV-1 sequences have been deposited in GenBank with accession numbers listed in data file S1. Analysis code is archived on Zenodo at doi:10.5281/zenodo.4273090.

### **Figure Legends**

**Fig. 1. HIV-1 viral loads and CD4+ T cell counts in prospectively studied individuals.** Viral loads (RNA copies/ml; red, left y-axis) and CD4+ T cell counts (cells/ $\mu$ l; blue, right y-axis) are shown for 10 HIV-1-infected individuals, who were followed from the onset of symptoms until 213 - 645 weeks (4.1 - 12.4 years) post-infection (x-axis). Participants are grouped based on disease progression, with (A) including typical progressors, (B) non-progressors, and (C) rapid progressors. Gray shading indicates suppressive antiretroviral therapy (alternating shading in WEAU indicates non-suppressive zidovudine monotherapy). Purple arrows denote the time points of PBMC sample isolation that yielded QVOA isolates, open triangles denote superinfection, and dashed vertical lines indicate termination of the study or loss to follow up.

**Fig. 2. Kinetics of IFN-I resistance over the course of HIV-1 infection.** IFN $\alpha$ 2 (A-C) and IFN $\beta$  (D-F) IC<sub>50</sub> values (pg/ml) are shown for limiting dilution- (circles) and bulk culture-derived

(squares) isolates from the onset of symptoms until 140 - 466 weeks (2.7 – 8.9 years) post-infection. Each data point represents the average of two technical replicates. Colored lines denote the average  $IC_{50}$  values as estimated by a Bayesian model, with darker shading indicating the 95% credible and lighter shading the 95% prediction intervals. Gray shading indicates suppressive antiretroviral therapy (alternating shading in WEAU indicates non-suppressive zidovudine monotherapy). Blue circles indicate isolates obtained by viral outgrowth from CD4+ T cells after 1.2 to 4.1 years of suppressive ART. Open triangles denote superinfection. Panels A and D, B and E, and C and F show typical progressors (orange), non-progressors (green), and rapid progressors (pink), respectively.

**Fig. 3. Dynamic changes in IFN-I resistance.** A hierarchical Bayesian change point model used to estimate the IFN-I resistance of plasma viral isolates from 10 longitudinally sampled HIV-1-infected study participants is shown. The longitudinal patterns of IFN $\alpha$ 2 and IFN $\beta$   $IC_{50}$  values were inferred across individuals by first predicting resistance as a fall from acute IFN-I  $IC_{50}$  values to a nadir point and then as variation from nadir values based on changes in CD4+ T cell counts. (A, B) Predicted mean IFN $\alpha$ 2 (A) or IFN $\beta$  (B)  $IC_{50}$  values (lines) are shown for plasma virus isolates modelled for typical progressor (orange), non-progressor (green) and rapid progressor (pink) participants from the onset of symptoms through early infection (x-axis), along with corresponding 95% credible (darker shading) and prediction (lighter shading) intervals. (C, D) Predicted mean IFN $\alpha$ 2 (C) or IFN $\beta$  (D)  $IC_{50}$  values (lines) are shown for plasma virus isolates modeled for individual typical progressor (orange), non-progressor (green) and rapid progressor (pink) participants based on changes in CD4+ T cell counts following the nadir (x-axis indicates decreases and increases from the nadir which is set to 0), along with corresponding 95% credible (darker shading) and prediction (lighter shading) intervals. Individual data points indicate virus isolates from the respective individuals, with shading

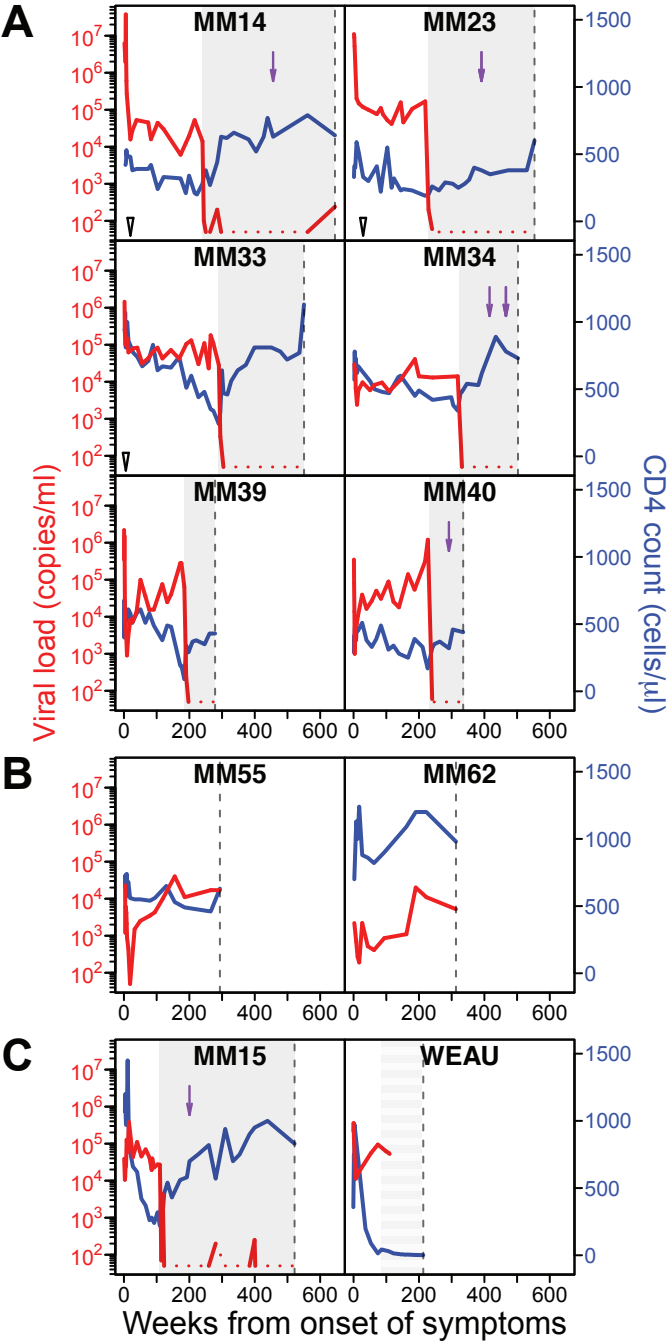
reflecting the estimated posterior probability that the time of nadir preceded the plasma collection time point (from white, probability of 0, to black, probability of 1). Isolates estimated to have less than 5% probability of following the time of nadir are not shown. For display, the nadir CD4+ T cell count was estimated as the posterior mean for that individual.

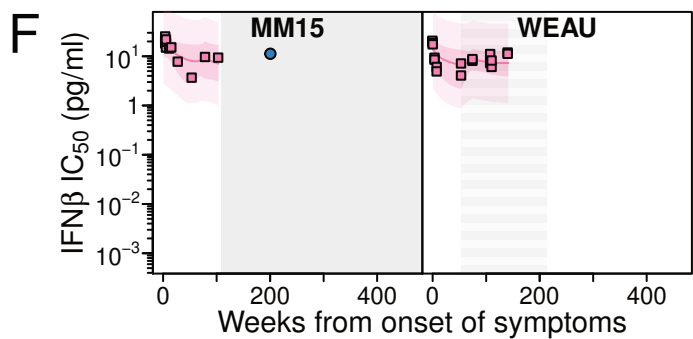
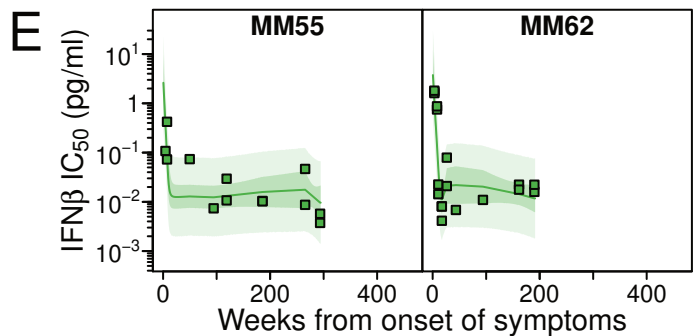
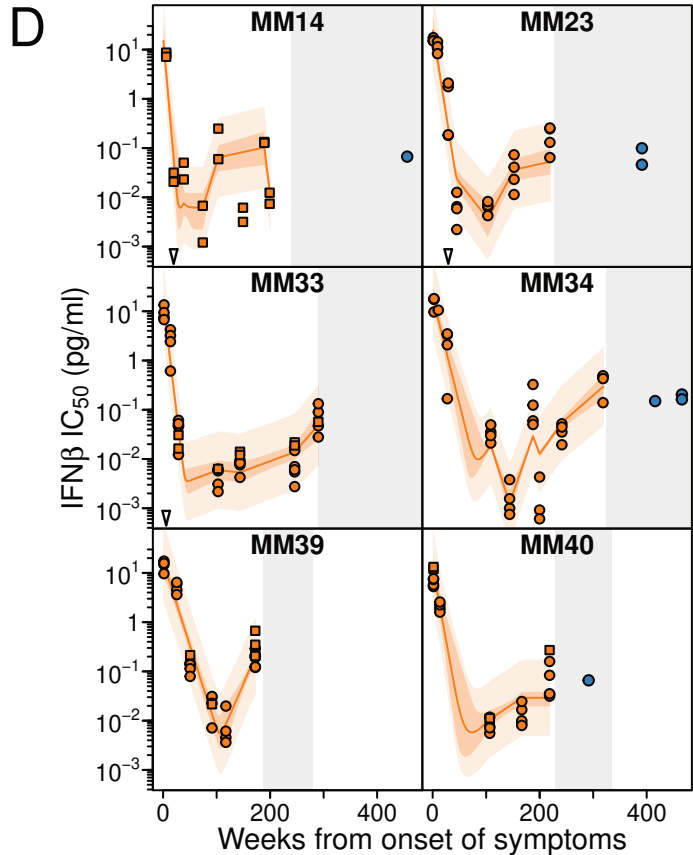
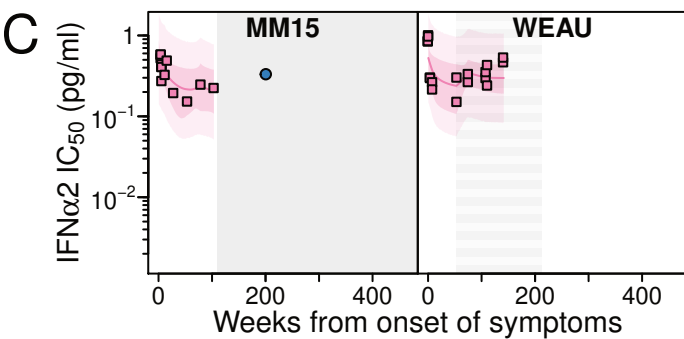
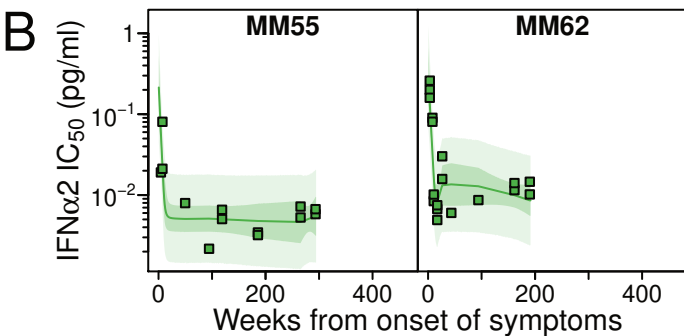
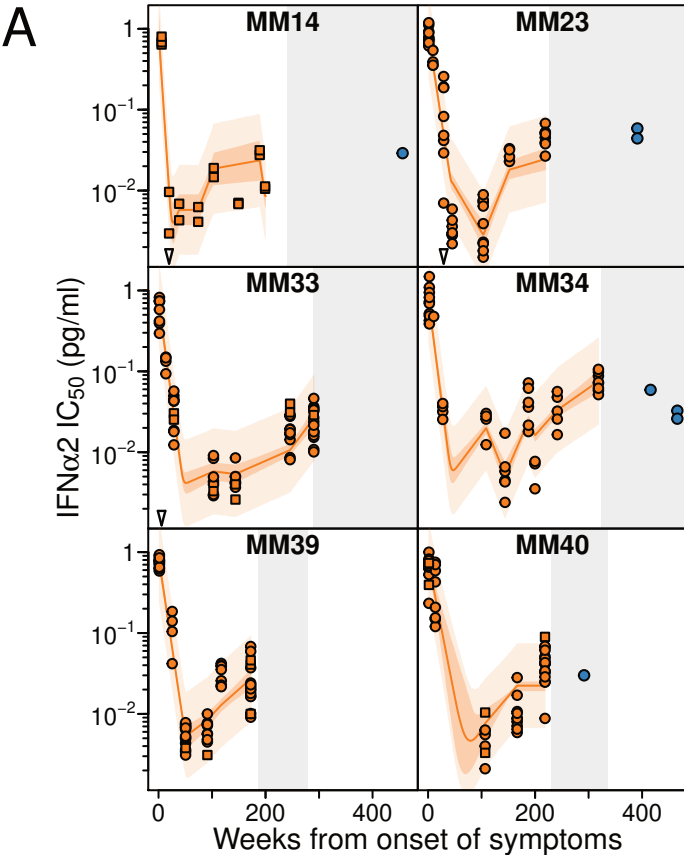
**Fig. 4. Position of QVOA isolates within the evolving HIV-1 quasispecies of participant MM34.** The evolutionary relationships of *env* nucleotide sequences generated by single genome amplification either directly from plasma viral RNA or plasma viral isolates of participant MM34 are shown for a 6-year time period. Samples are colored by time point, with blue sequences derived early and red sequences derived late in infection. Purple leaves indicate the position of QVOA isolates obtained 2 and 3 years following ART initiation, while grey leaves indicate proviral sequences amplified from the corresponding PBMC sample. One hypermutated PBMC-derived sequence is shown with a gap in the branch. The scale bar indicates 0.05 substitutions per site.

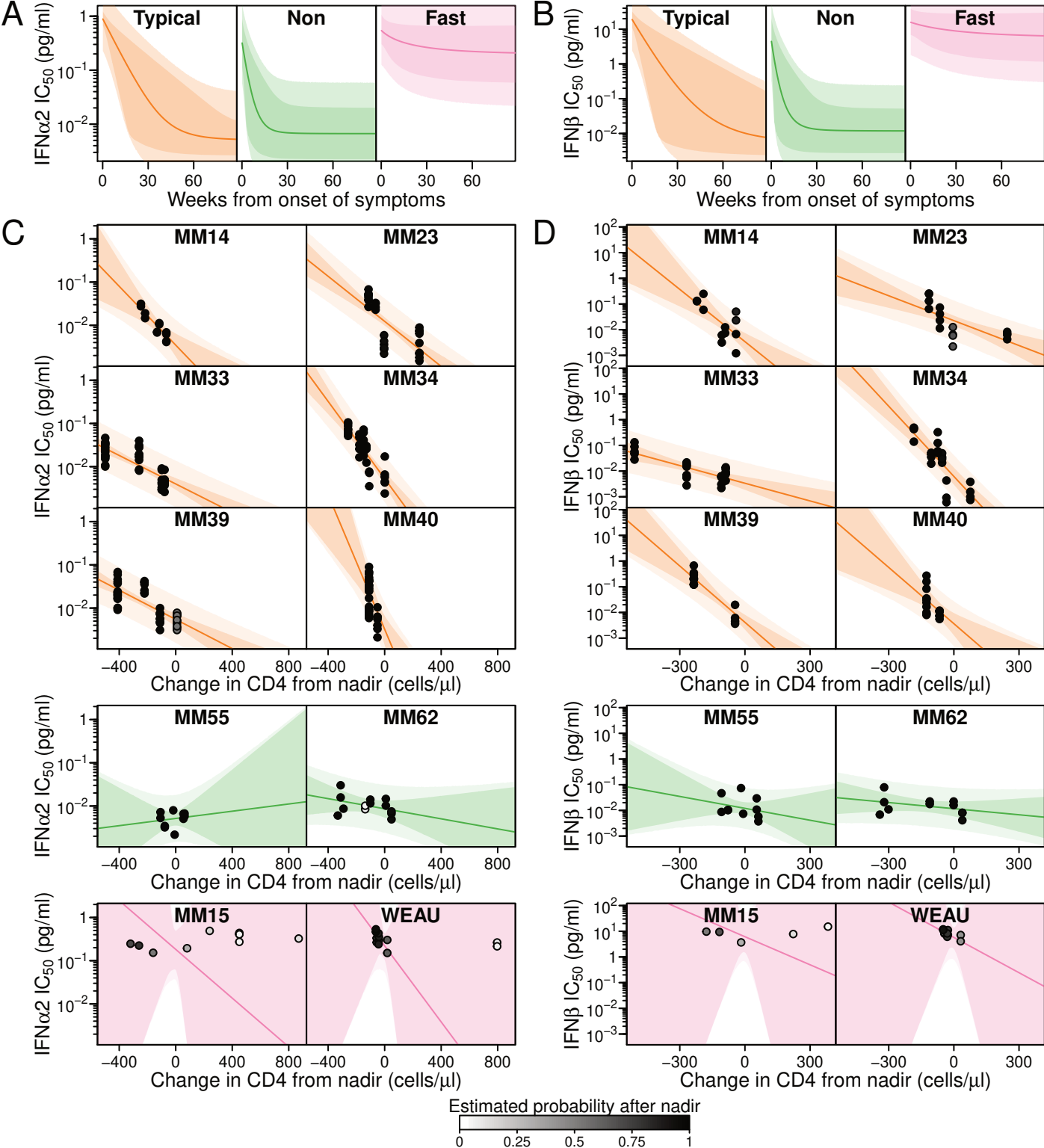
**Fig. 5. IFN-I resistance of QVOA versus rebound viruses.** (A, C) IFN $\alpha$ 2 (A) and IFN $\beta$  (C) IC<sub>50</sub> values are shown for plasma isolates of individuals experiencing rebound viremia after ART cessation (red) and QVOA isolates generated from the PBMCs of ART suppressed individuals before (blue) or after (turquoise) ATI (each data point is the average of two technical replicates). Isolates are grouped by individuals (shaded boxes), with pre-ATI, rebound, and post-ATI isolates depicted in temporal order when available. Two post-ATI QVOA isolates from participant A08 with elevated IFN-I resistance are boxed. Also shown are the IFN $\alpha$ 2 (A) and IFN $\beta$  (C) IC<sub>50</sub> values for outgrowth isolates (blue) and plasma viruses (grey) from the longitudinal cohort (Acute, <30 days since onset of symptoms; Early, >300 days and <Nadir; Nadir, time point with lowest mean IC<sub>50</sub> value; Late, >Nadir and <Last; Last, last time point before ART initiation) as well as previously reported donor-recipient transmission pairs (50). For

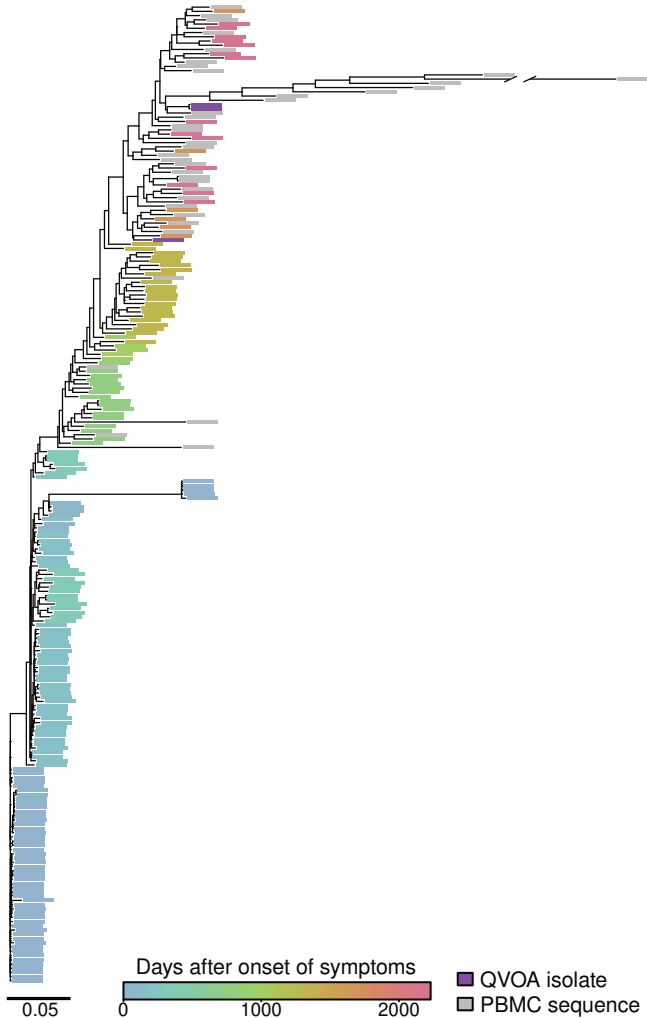
the latter,  $IC_{50}$  values were adjusted to account for potency differences among commercial IFN-I batches. Diamonds indicate isolates from fast progressing individuals. (B, D) The fold-change in  $IFN\alpha_2$  and  $IFN\beta$   $IC_{50}$  values from acute infection isolates is shown for rebound (red), chronic (grey), and pre-ATI (blue) and post-ATI (turquoise) QVOA isolates. Violin plots indicate the estimated posterior probability of the mean fold change between isolate types, with the darker shading indicating the 95% credible interval and the lighter the 95% prediction interval. Dashed horizontal line indicates a fold change of 1, which indicates no change.

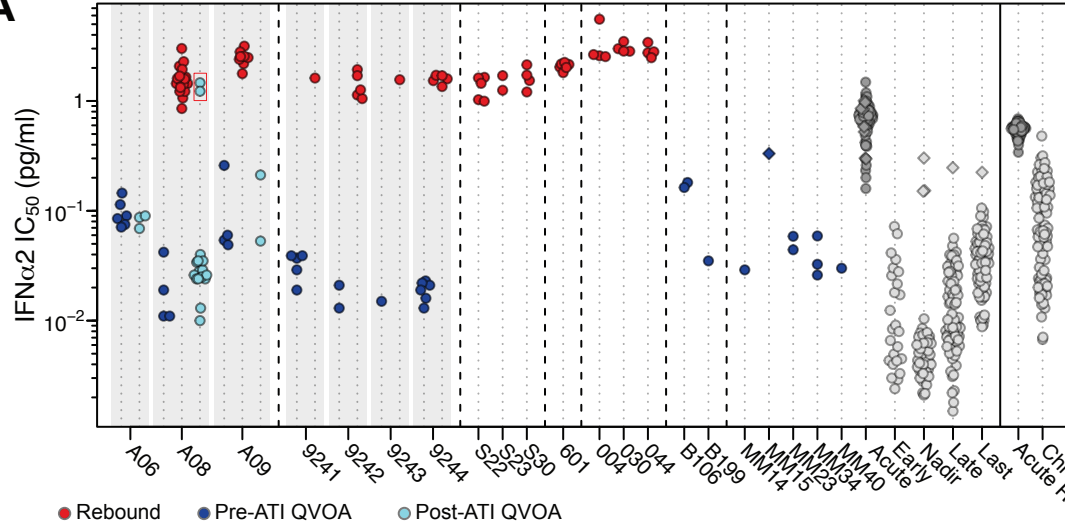
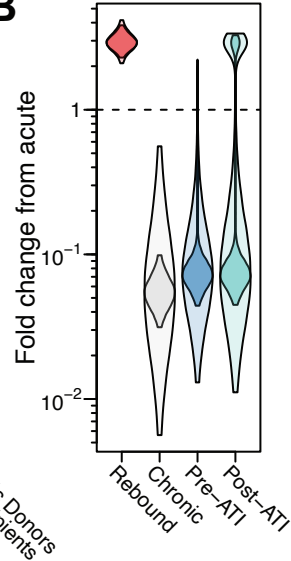
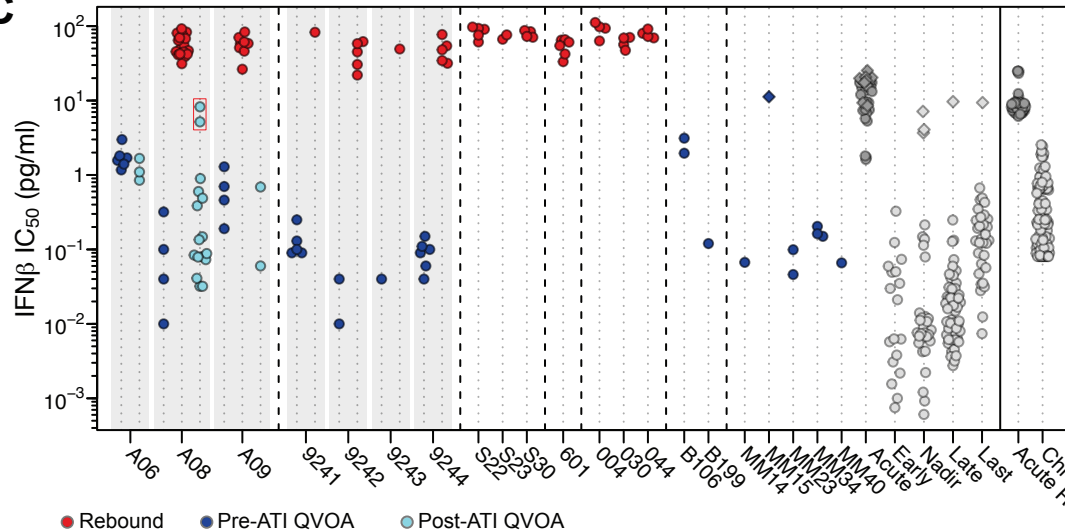
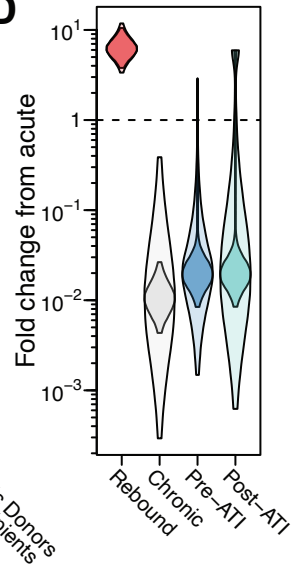
**Fig. 6. Genotype and IFN-I phenotype of rebound and outgrowth viruses before and after treatment interruption of individual A08.** The phylogenetic relationships of *env* gene sequences from pre-ATI QVOA (blue), plasma rebound (red) and post-ATI QVOA (teal) isolates are shown for participant A08, along with available  $IFN\alpha_2$  and  $IFN\beta$   $IC_{50}$  values (pg/ml). Asterisks indicate bootstrap values >90%; the scale bar indicates 0.01 substitution per site. A clade of near identical rebound and post-ATI QVOA isolates is highlighted by bold text and a bracket, with two “rebound-like” post-ATI QVOA isolates with heightened IFN-I resistance denoted by teal arrows. Two other closely related rebound and post-ATI QVOA isolates that differ in their IFN-I resistance are indicated by red and teal triangles.

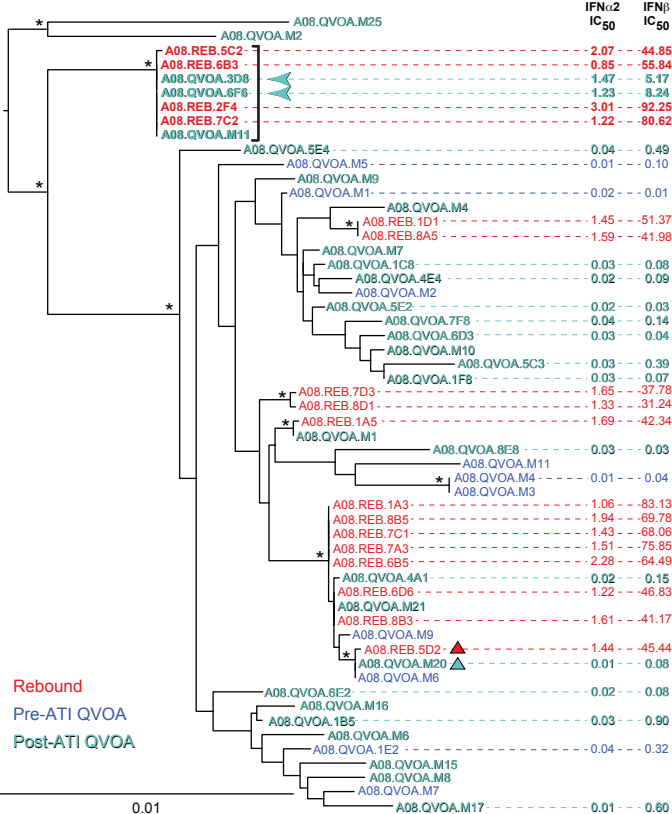








**A****B****C****D**



## Supplementary Materials and Methods

### Supplementary Methods

#### Bayesian models of IFN-I resistance of HIV-1 plasma isolates from longitudinally sampled individuals

To create a simple model of the temporal dynamics of type I interferon (IFN-I) resistance, IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values were each modeled using a Bayesian change point hierarchical model. The model is based on a segmented regression of the log IC<sub>50</sub>, making the following simplifying assumptions:

- Each individual has a degree of resistance at the acute infection stage drawn from separate population-level distributions for typical progressors, non-progressors, or fast progressors.
- Each individual has a decrease in IFN-I resistance from acute values drawn from separate population-level distributions for typical progressors, non-progressors, or fast progressors.
- Each individual has a time to nadir drawn from separate population-level distributions for typical progressors, non-progressors, or fast progressors.
- Resistance changes linearly from onset of symptoms to time of nadir.
- The nadir of IFN-I resistance represents a changepoint in the data. Following this point, changes in IFN-I resistance are modeled as a linear function of CD4+ T cell count changes away from the count found at nadir.
- Where data are not present, CD4+ T cell counts are assumed to be linearly interpolated between adjacent observations.

The log IC<sub>50</sub> observation from each viral isolate  $i$  was modeled as a normal distribution  $IC_{50_i} \sim \text{Normal}(\mu_i, \sigma)$  with mean  $\mu_i$  where:

$$\mu_i = \begin{cases} \alpha_{\text{person}_i} + \delta_{\text{person}_i} \frac{\text{time}_i}{s_{\text{person}_i}} & \text{if } \text{time}_i < s_{\text{person}_i} \\ \alpha_{\text{person}_i} + \delta_{\text{person}_i} + \beta_{\text{person}_i} (\text{CD4}_{\text{person}_i, \text{time}_i} - \text{CD4}_{\text{person}_i, s_{\text{person}_i}}) & \text{if } \text{time}_i \geq s_{\text{person}_i} \end{cases}$$

where the parameters  $\alpha_j$  represent the degree of IFN-I resistance at symptom onset,  $\delta_j$  represents the change from symptom onset to nadir and  $s_j$  represents the time of nadir in individual  $j$ . Study participant data are represented by  $\text{time}_i$  corresponding to the time since onset of symptoms and  $\text{person}_i$  recording the individual from which isolate  $i$  was collected,  $\text{CD4}_{j,k}$  containing the estimated CD4+ T cell count for individual  $j$  at time  $k$ , and  $\text{progression}_j$  is the disease progression type (fast/non/typical) for individual  $j$ . The hierarchical probabilities for these parameters were:

$$\begin{aligned} \sigma &\sim \text{Gamma}(1, 0.1) \\ \alpha_j &\sim \begin{cases} \text{Normal}(\theta_{\alpha, \text{typical}}, \tau_{\alpha}) & \text{if } \text{progression}_j = \text{typical} \\ \text{Normal}(\theta_{\alpha, \text{typical}} + \theta_{\alpha, \text{fast}}, \tau_{\alpha}) & \text{if } \text{progression}_j = \text{fast} \\ \text{Normal}(\theta_{\alpha, \text{typical}} + \theta_{\alpha, \text{non}}, \tau_{\alpha}) & \text{if } \text{progression}_j = \text{non} \end{cases} \\ \delta_j &\sim \begin{cases} \text{Normal}(\theta_{\delta, \text{typical}}, \tau_{\delta}) & \text{if } \text{progression}_j = \text{typical} \\ \text{Normal}(\theta_{\delta, \text{typical}} + \theta_{\delta, \text{fast}}, \tau_{\delta}) & \text{if } \text{progression}_j = \text{fast} \\ \text{Normal}(\theta_{\delta, \text{typical}} + \theta_{\delta, \text{non}}, \tau_{\delta}) & \text{if } \text{progression}_j = \text{non} \end{cases} \\ s_j &\sim \begin{cases} \text{NegativeBinomial}(\theta_{s, \text{typical}}, \tau_s) & \text{if } \text{progression}_j = \text{typical} \\ \text{NegativeBinomial}(\theta_{s, \text{typical}} \exp(\theta_{s, \text{fast}}), \tau_s) & \text{if } \text{progression}_j = \text{fast} \\ \text{NegativeBinomial}(\theta_{s, \text{typical}} \exp(\theta_{s, \text{non}}), \tau_s) & \text{if } \text{progression}_j = \text{non} \end{cases} \\ \beta_j &\sim \begin{cases} \text{Normal}(\theta_{\beta, \text{typical}}, \tau_{\beta}) & \text{if } \text{progression}_j = \text{typical} \\ \text{Normal}(\theta_{\beta, \text{fast}}, \tau_{\beta}) & \text{if } \text{progression}_j = \text{fast} \\ \text{Normal}(\theta_{\beta, \text{non}}, \tau_{\beta}) & \text{if } \text{progression}_j = \text{non} \end{cases} \end{aligned}$$

where  $j$  indicates each individual and **NegativeBinomial(x,y)** represents a negative binomial distribution parameterized such that the expected value is  $x$  and the variance is  $x + \frac{x^2}{y}$ .

All hyperparameters were given prior probabilities of  $\theta_x \sim \text{Normal}(0,10)$  for parameters representing the means of a distribution and  $\tau_x \sim \text{Gamma}(1,0.1)$  for parameters representing standard deviations other than  $\theta_{\alpha,\text{typical}}$  and  $\theta_{s,\text{typical}}$ , which were given a flat prior and  $\tau_s \sim \text{Cauchy}(0,10)$ .

For computational efficiency, the nadir time parameter  $s$  was discretized to weekly intervals, assumed to fall within 1 to 150 weeks after symptom onset and marginalized out of the joint probability:

$$p(\text{IC50}, \dots) = p(\dots) \prod_{i=1}^n \sum_{s=1}^{150} \text{Normal}(\text{IC50}_i | \mu_{i,s}, \sigma) \text{NegativeBinomial}(s | \theta_s, \tau_s)$$

where ... represents all parameters other than  $s$  and  $\mu_{i,s}$  is defined the same as  $\mu_i$ :

$$\mu_{i,s} = \begin{cases} \alpha_{\text{person}_i} + \frac{\text{time}_i}{s} \delta_{\text{person}_i} & \text{if } \text{time}_i < s \\ \alpha_{\text{person}_i} + \delta_{\text{person}_i} + \beta_{\text{person}_i} (\text{CD4}_{\text{person}_i, \text{time}_i} - \text{CD4}_{\text{person}_i, s}) & \text{if } \text{time}_i \geq s \end{cases}$$

Posterior probabilities were estimated with 50 Markov chain Monte Carlo chains of 5,000 iterations each using Stan (90).

## Bayesian models of IFN-I resistance of outgrowth and rebound HIV-1 isolates

To compare the IFN-I resistance of viral isolates derived from plasma samples collected during acute, chronic and rebound infections, as well as from viably frozen PBMCs collected during ART suppression (QVOA), IFN $\alpha$ 2 and IFN $\beta$  values were modeled using a Bayesian hierarchical model with the simplifying assumptions that:

- Isolates found at acute infection form a base level of IFN-I resistance for a given individual. Resistances in virus isolated from chronic, ART suppressed, and rebound infection for this individual are modelled as changes from the initial resistance.
- The mean IC<sub>50</sub> value within each individual for acute isolates and the change from acute values for chronic, QVOA and rebound isolates are drawn from a population-level distribution for that type.
- QVOA isolates are separated into two populations; a “pre” group composed of QVOA viruses isolated from individuals prior to or in the absence of treatment interruption (ATI) and a “post” group of QVOA viruses isolated from individuals following ATI and reinitiation of ART.
- In both QVOA populations, the viruses can include some proportion of rebound-like isolates. This mixture is modeled in both pre- and post-treatment so that differences in mixture proportion between the two populations can be assessed.
- Variation in the potency of IFN-I used to experimentally determine IC<sub>50</sub> values may shift the inferred resistance for isolates tested in other studies. This effect is modeled as a multiplicative shift in IC<sub>50</sub> for all isolates measured outside this study (acute recipient and chronic donor isolates from ref. 50).
- Isolates from individuals who received exogenous IFN $\alpha$ 2 during treatment interruption may display altered interferon resistance. This effect is modeled as a multiplicative shift in IC<sub>50</sub> for all rebound isolates from such individuals (individuals 004, 030, and 044 from ref. 38).

The log IC<sub>50</sub> observation from each viral isolate  $i$  from acute, chronic and rebound isolates was modeled as a normal distribution:

$$\text{IC50}_i \sim \text{Normal}(\mu_{\text{type}_i, \text{person}_i}, \sigma_{\text{type}_j})$$

with the mean resistance for isolate type  $j$  from individual  $k$ :

$$\mu_{j,k} \sim \begin{cases} \text{Normal}(\alpha_k + \beta_{\text{batch}} \text{batch}_k, \psi_j) & \text{if } j = \text{acute} \\ \text{Normal}(\alpha_k + \beta_{j,k} + \beta_{\text{batch}} \text{batch}_k + \beta_{\text{ifn}} \text{IFN}_k, \psi_j) & \text{if } j = \text{rebound} \\ \text{Normal}(\alpha_k + \beta_{j,k} + \beta_{\text{batch}} \text{batch}_k, \psi_j) & \text{otherwise} \end{cases}$$

where  $\text{type}_i$  indicates whether isolate  $i$  was isolated during acute, chronic, QVOA or rebound infection from individual  $\text{person}_i$ ,  $\text{batch}_k$  indicates when isolates from individual  $k$  were tested in another study and  $\text{IFN}_k$  indicates when individual  $k$  was treated with exogenous IFN $\alpha$ 2 prior to and during ATI. Parameters were included for the mean resistance value during acute infection for each individual  $\alpha_k$ , standard deviation of isolates of type  $j$  within an individual  $\sigma_j$ , standard deviation of mean resistance for type  $j$  isolates among people  $\psi_j$ , change from acute values in isolates of type  $j$  in a given individual  $\beta_{j,k}$ , the effects of exogenous IFN-I treatment  $\beta_{\text{ifn}}$  and batch to batch variation in IFN in isolates assayed in previous studies  $\beta_{\text{batch}}$ .

For QVOA isolates, the IC<sub>50</sub> was modeled as a mixture of two populations such that:

$$p(\text{IC50}_i | \mu_{\text{qvoa}, \text{person}_i}, \sigma_{\text{qvoa}}, \mu_{\text{rebound}, \text{person}_i}, \sigma_{\text{rebound}}, \phi_{\text{prePost}_i}) = \phi_{\text{prePost}_i} \text{Normal}(\text{IC50}_i | \mu_{\text{rebound}, \text{person}_i}, \sigma_{\text{rebound}}) + (1 - \phi_{\text{prePost}_i}) \text{Normal}(\text{IC50}_i | \mu_{\text{qvoa}, \text{person}_i}, \sigma_{\text{qvoa}})$$

where  $\text{prePost}_i$  indicates whether isolate  $i$  was isolated pre- or post-ATI, and  $\phi_{\text{pre}}$  and  $\phi_{\text{post}}$  represent the proportion of rebound-like virus present in pre- and post-ATI QVOA isolates.

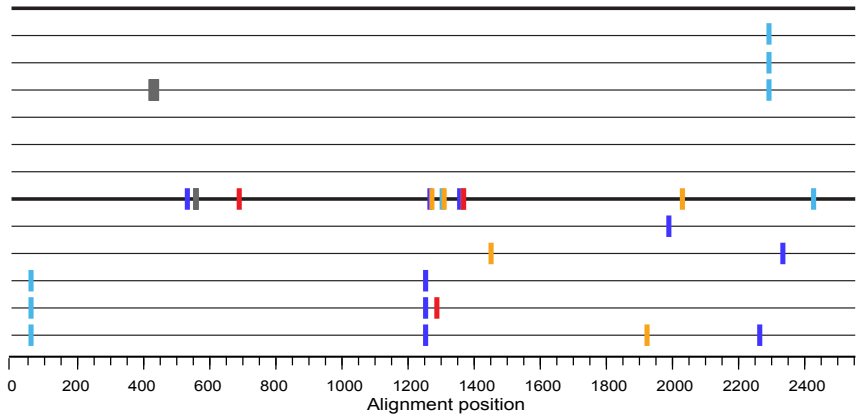
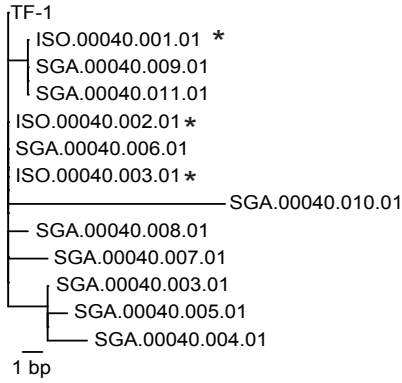
The hierarchical parameter priors were modeled as:

$$\begin{aligned} \sigma_j &\sim \text{Gamma}(1, 0.1) \\ \psi_j &\sim \text{Gamma}(1, 0.1) \\ \phi_{\text{pre}} &\sim \text{Uniform}(0, 1) \\ \phi_{\text{post}} &\sim \text{Uniform}(0, 1) \end{aligned}$$

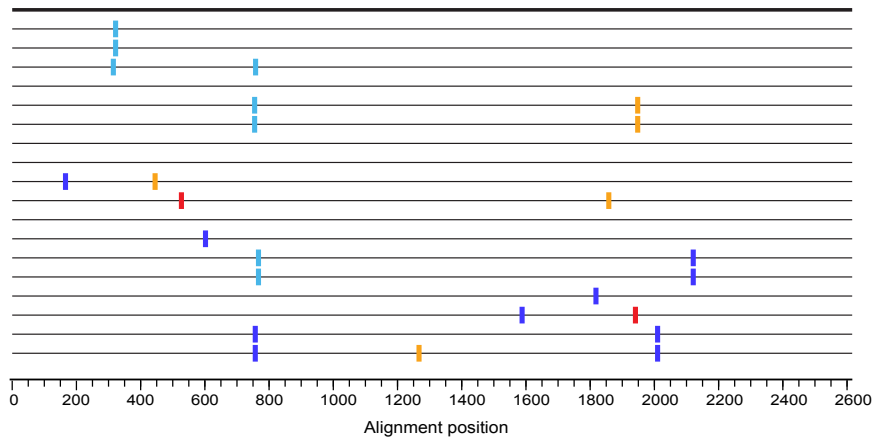
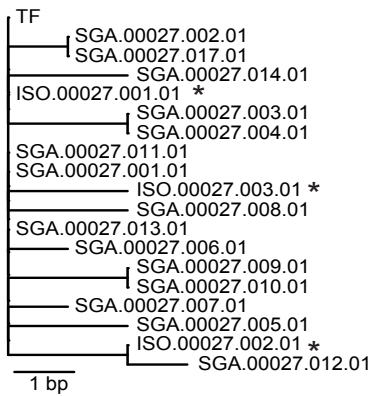
where  $j$  indicates the isolate type (acute, chronic, QVOA, rebound). All  $\alpha_k$ ,  $\beta_{j,k}$  were given flat priors and  $\beta_{\text{ifn}} \sim \text{Normal}(0, 10)$  and  $\beta_{\text{batch}} \sim \text{Normal}(0, 10)$ . Posterior probabilities were estimated with 50 Markov chain Monte Carlo chains of 5,000 iterations each using Stan (90).

# Supplementary Figures

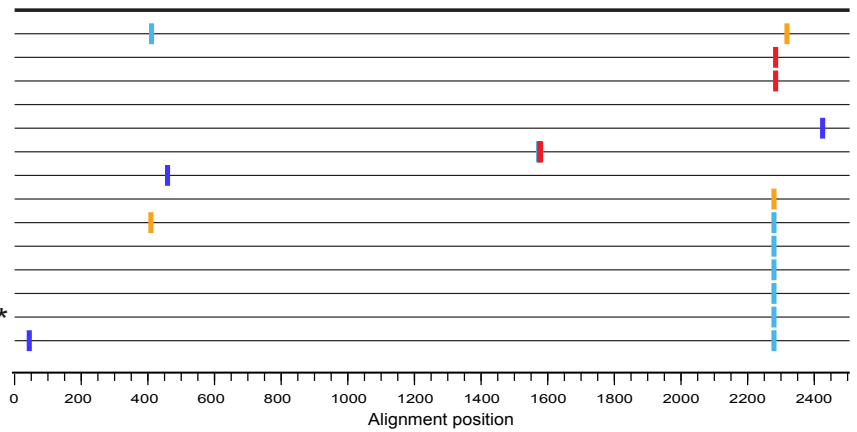
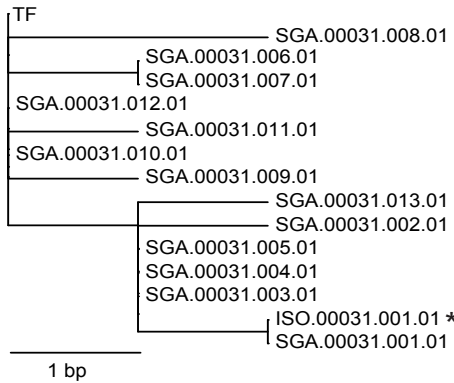
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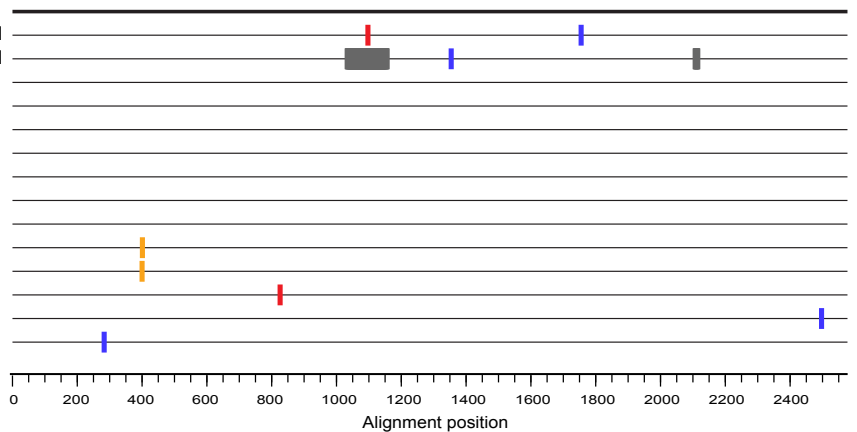
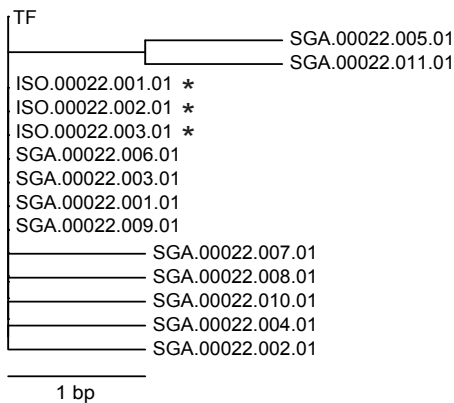
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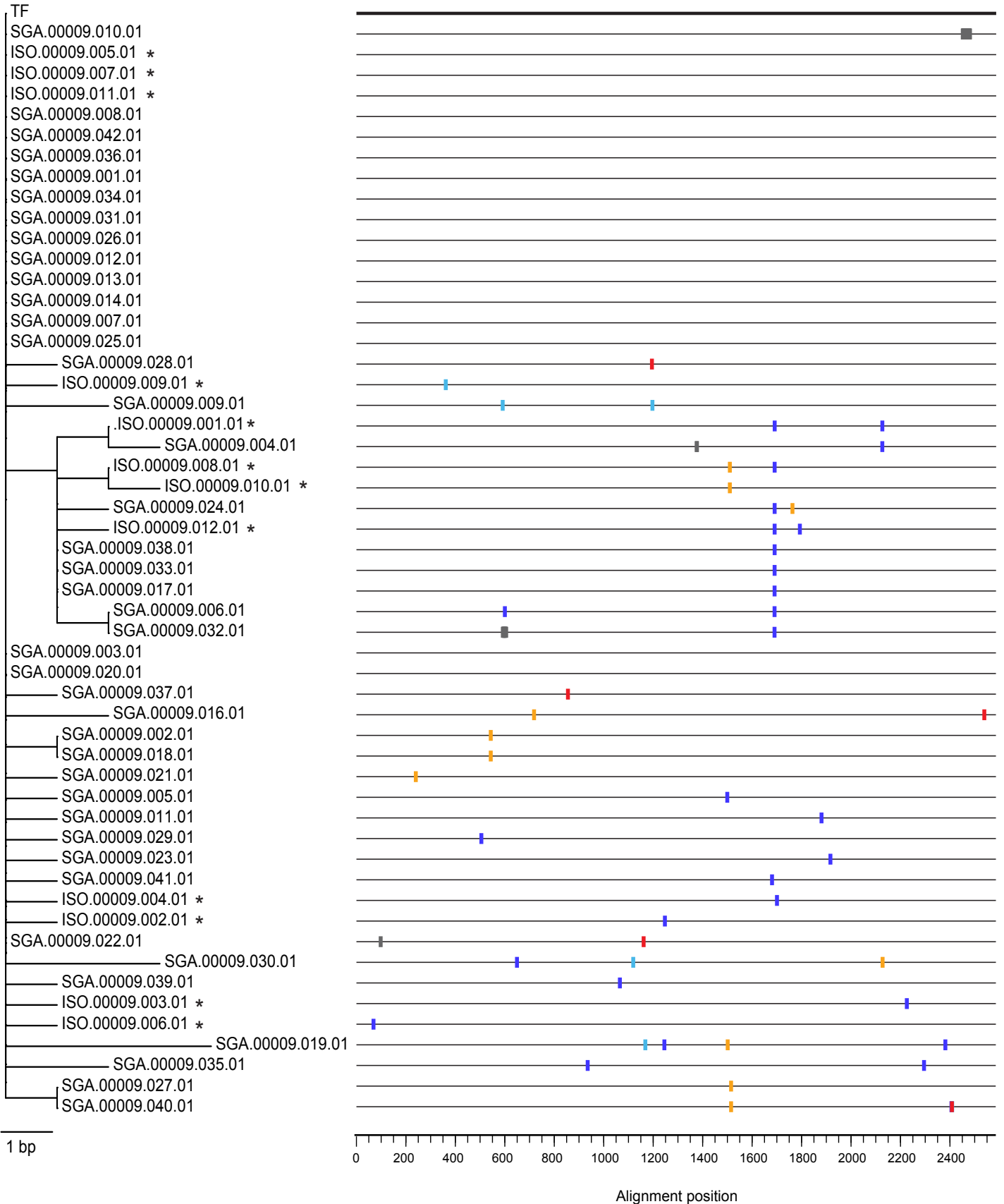
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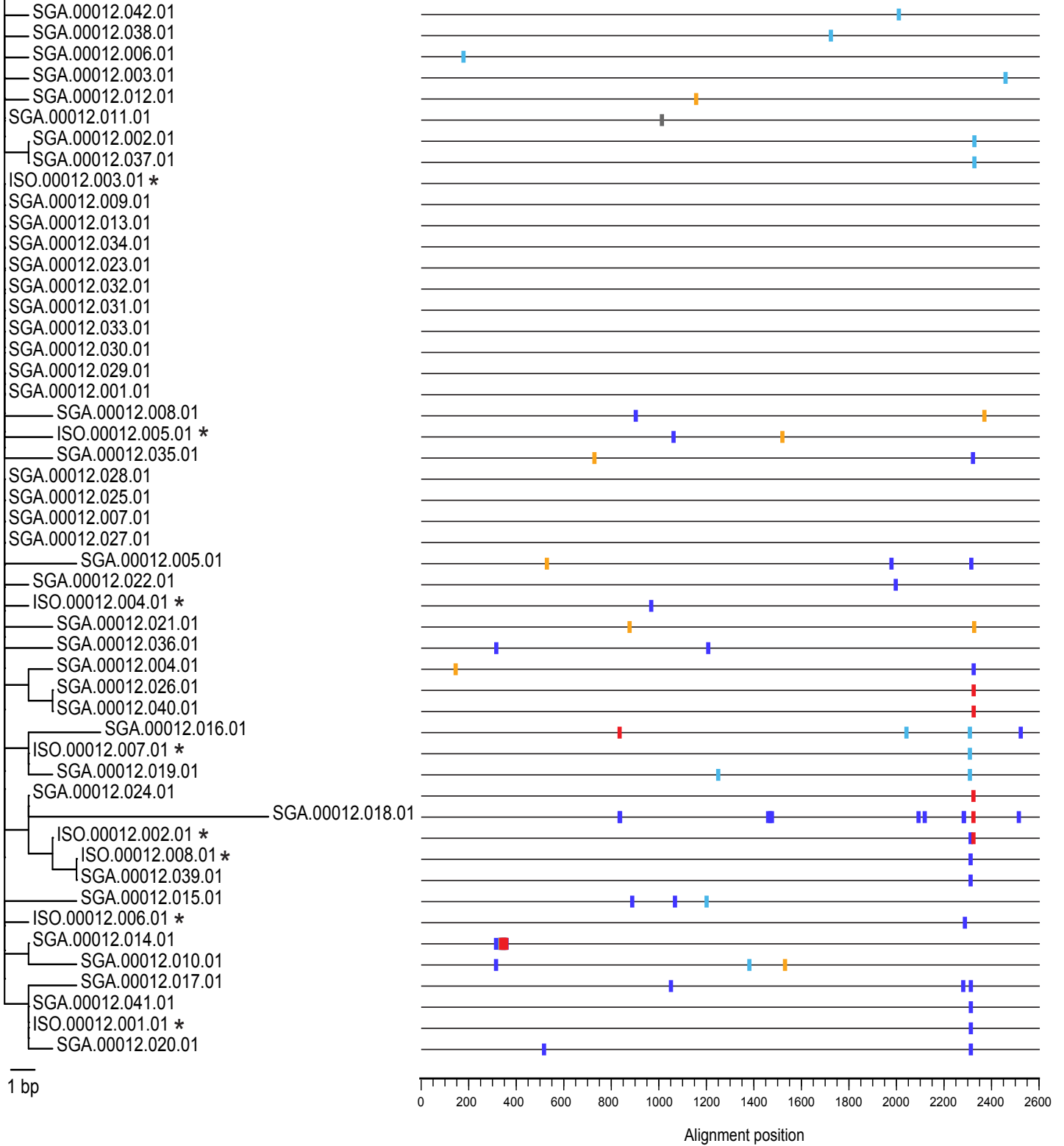


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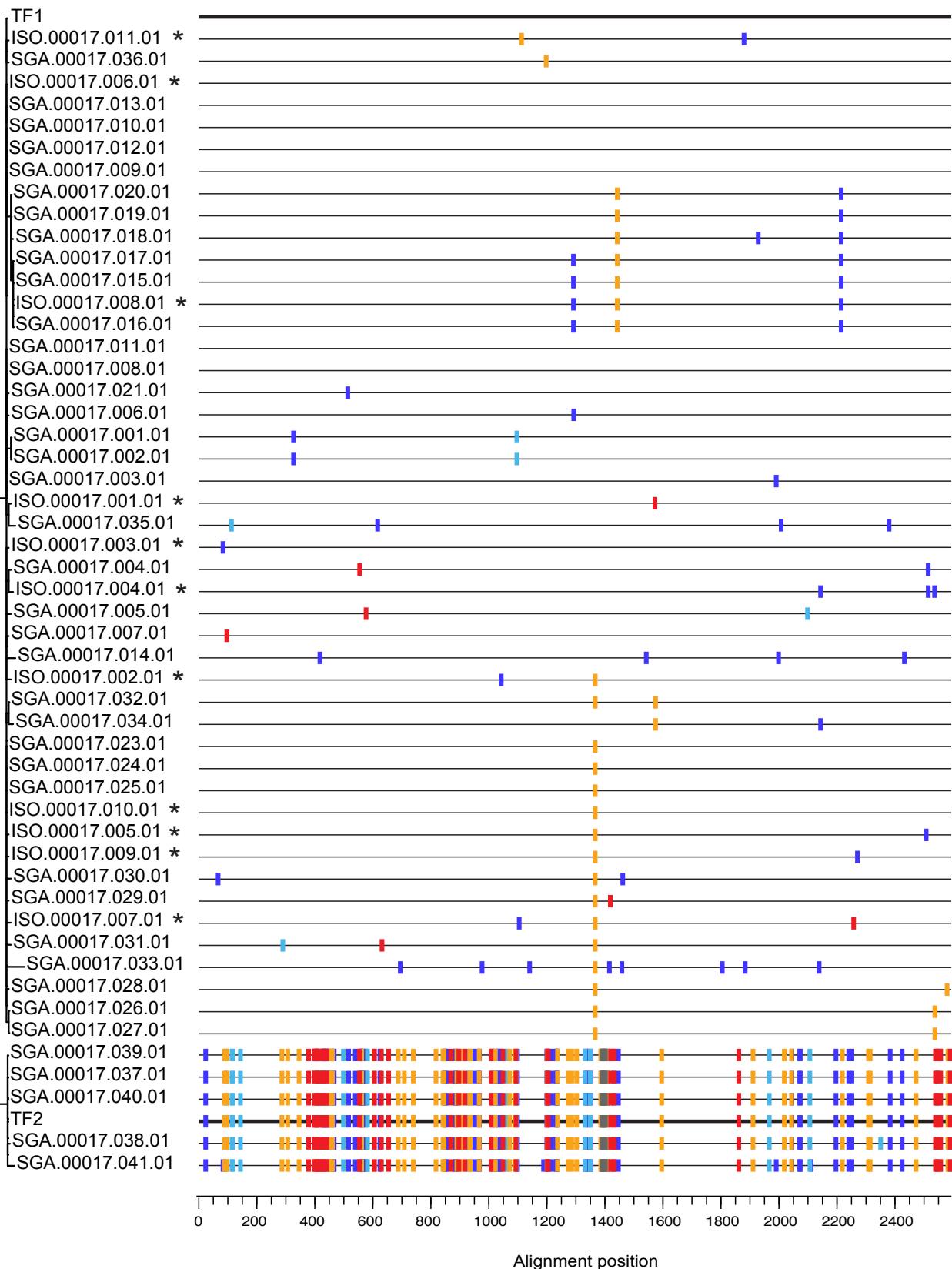


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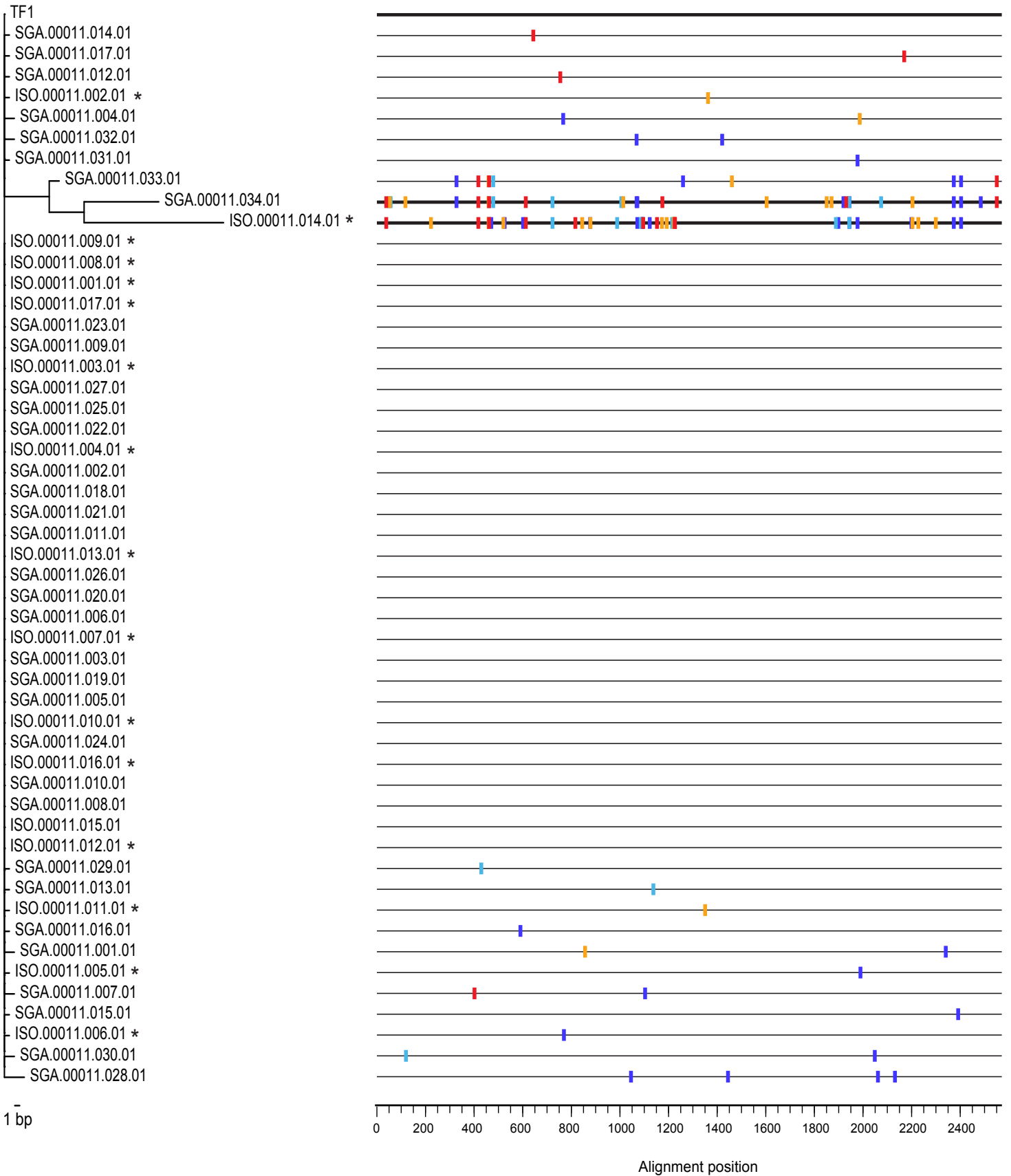
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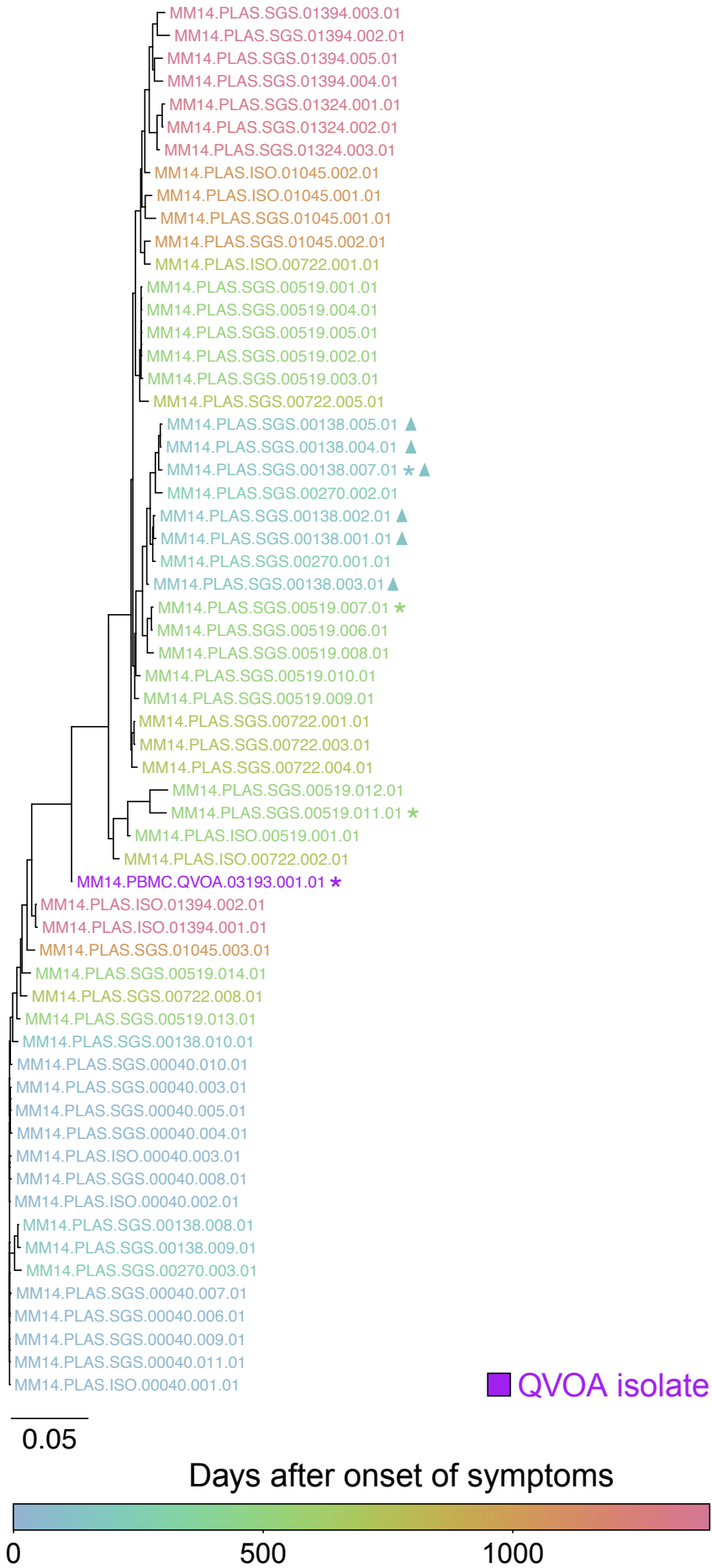


# MM40



**fig. S1. Transmitted founder virus inference and enumeration.** Highlighter (v2.1.1) plots and corresponding phylogenetic trees of *env* gene sequences derived by single genome amplification (SGA) from plasma viral RNA and limiting dilution isolate supernatants are shown for the first available sample of eight study participants. Tick marks indicate differences from the inferred transmitted founder sequence depicted in bold on top (red, T; blue, A; teal, C; orange, G; gray, indel). Participants MM14, MM34 and MM40 were infected with more than one transmitted founder virus (highlighted in bold). Trees were constructed using the neighbor-joining method implemented in the Highlighter v2.1.1 software (hiv.lanl.gov). Sequences obtained from plasma isolates are indicated by an asterisk. The scale bar indicates 1 base pair. Participants MM39 and WEAU were each infected with a single transmitted founder virus as reported previously (54, 57).

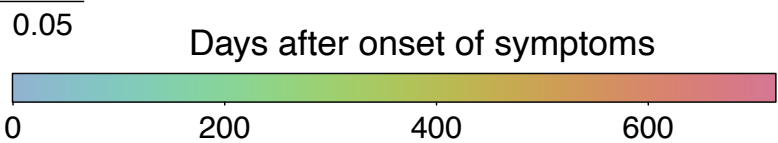
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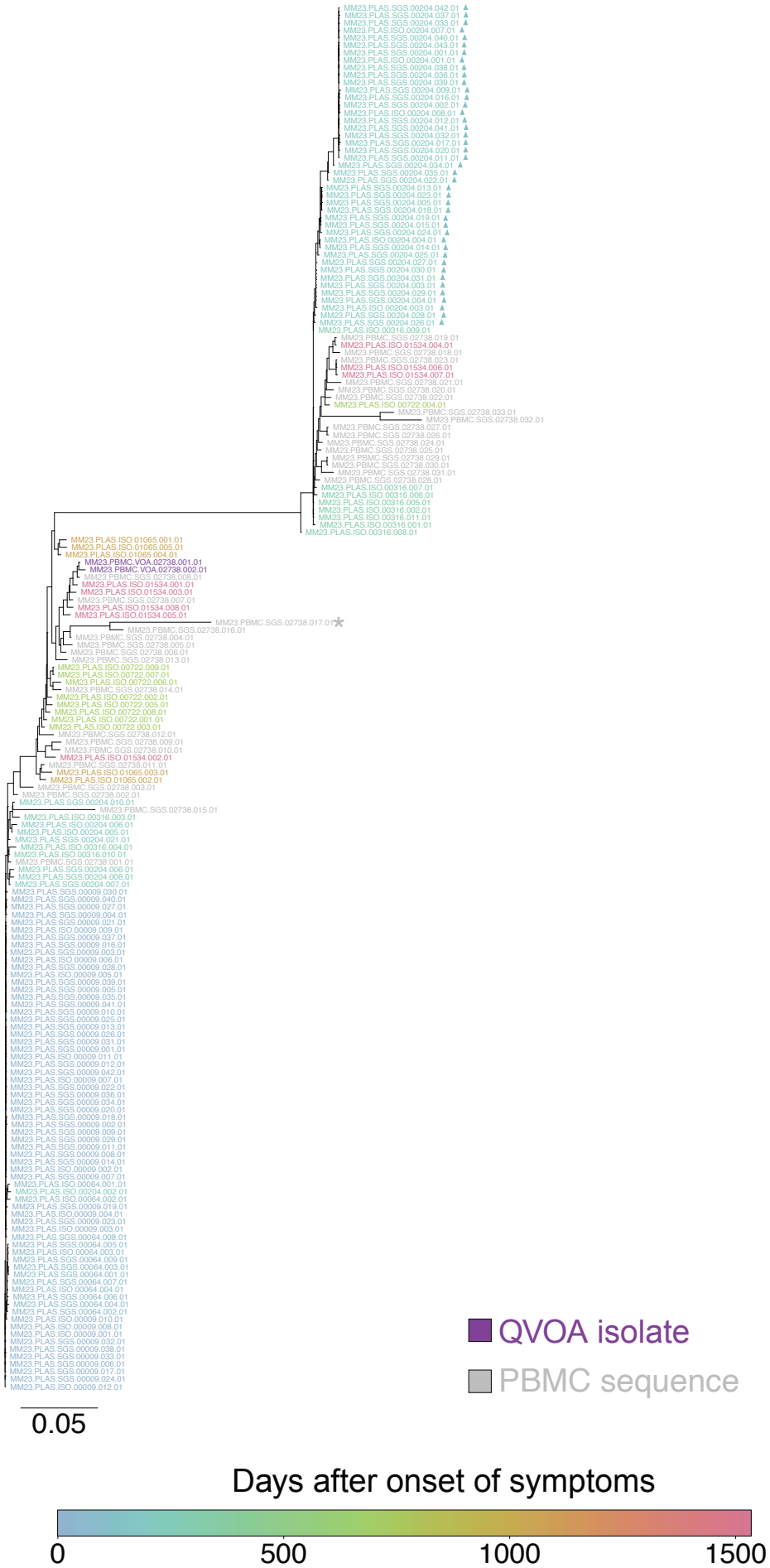
# MM15

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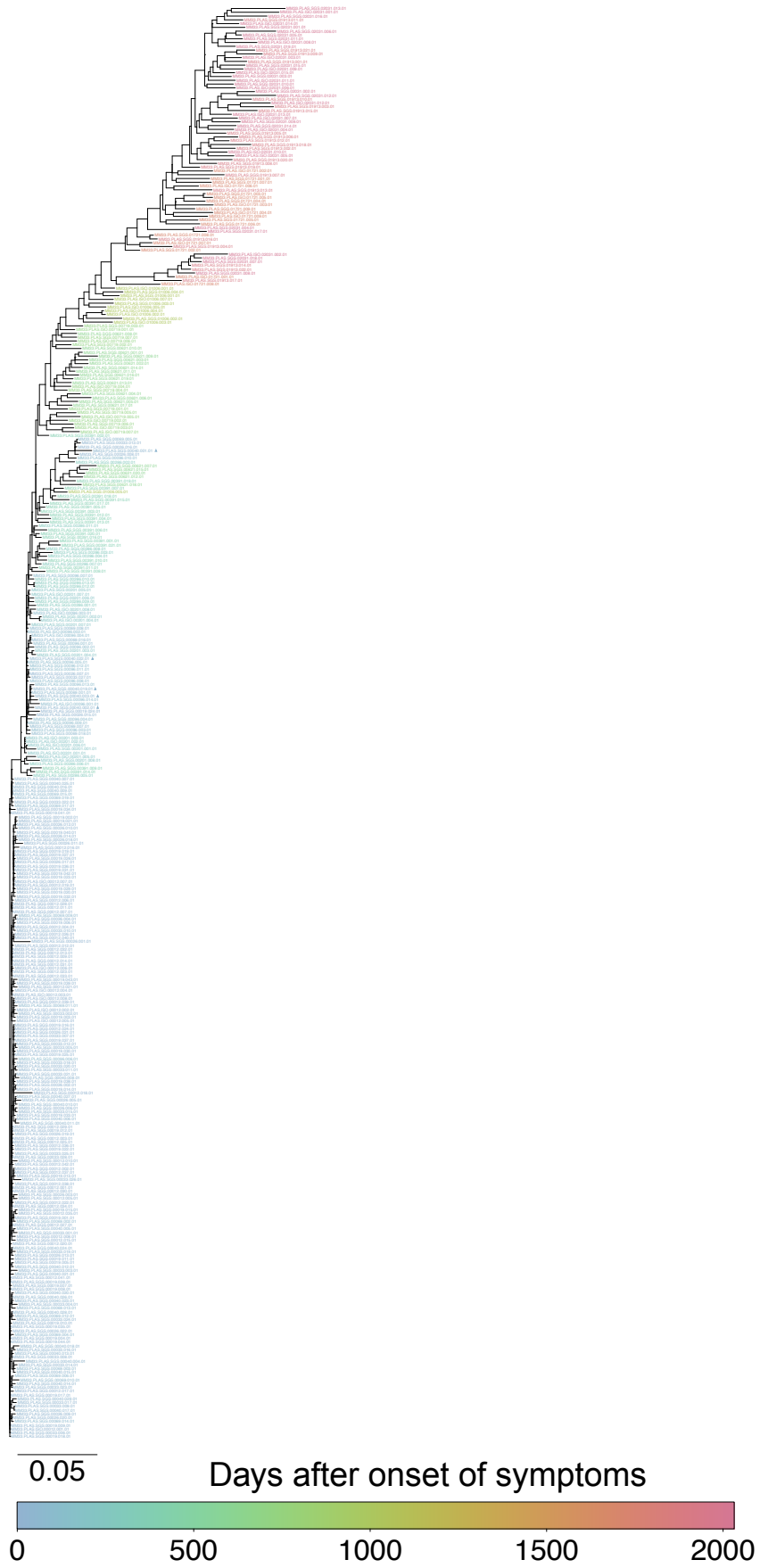
■ QVOA isolate



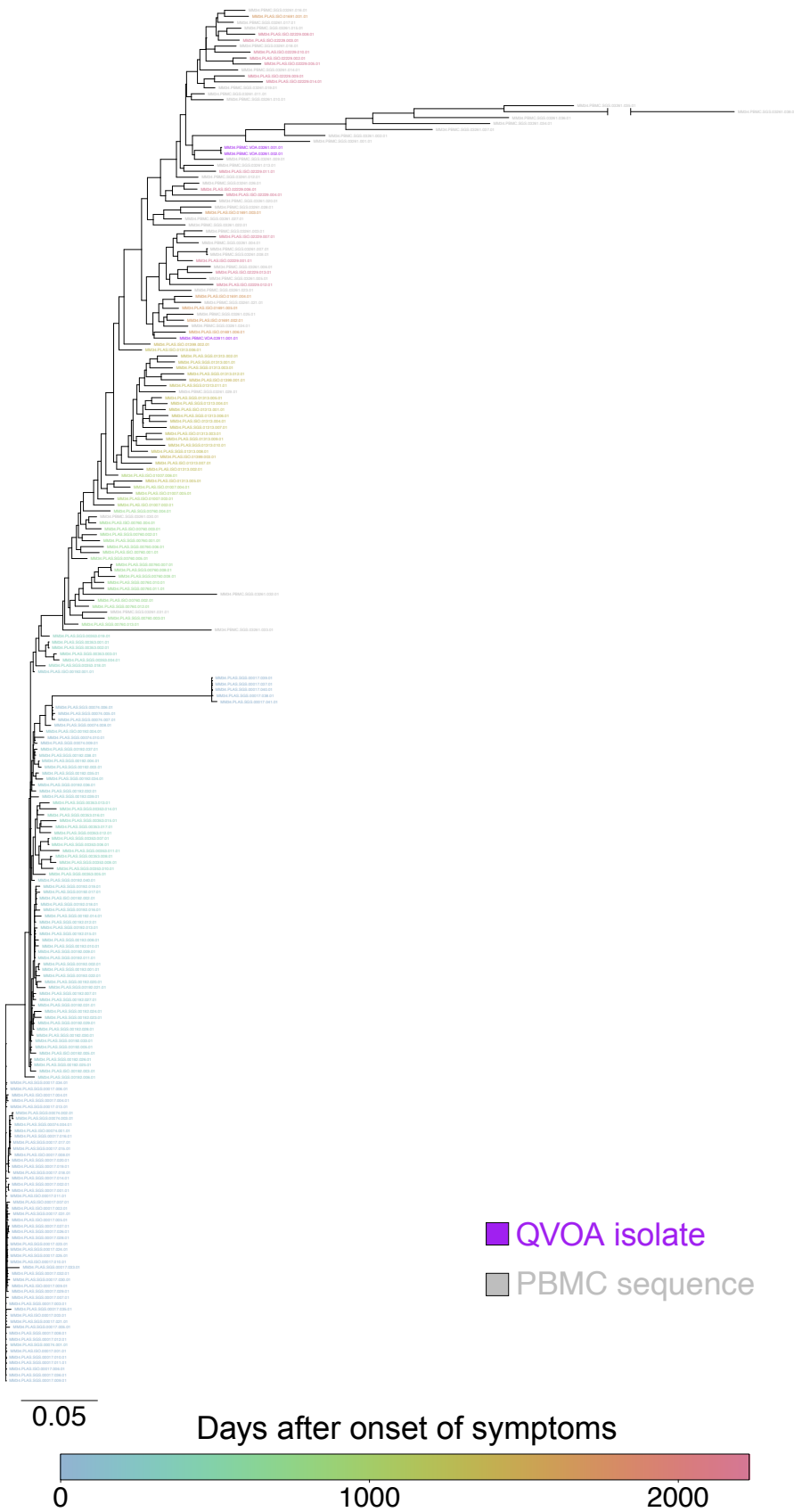
# MM23



# MM33



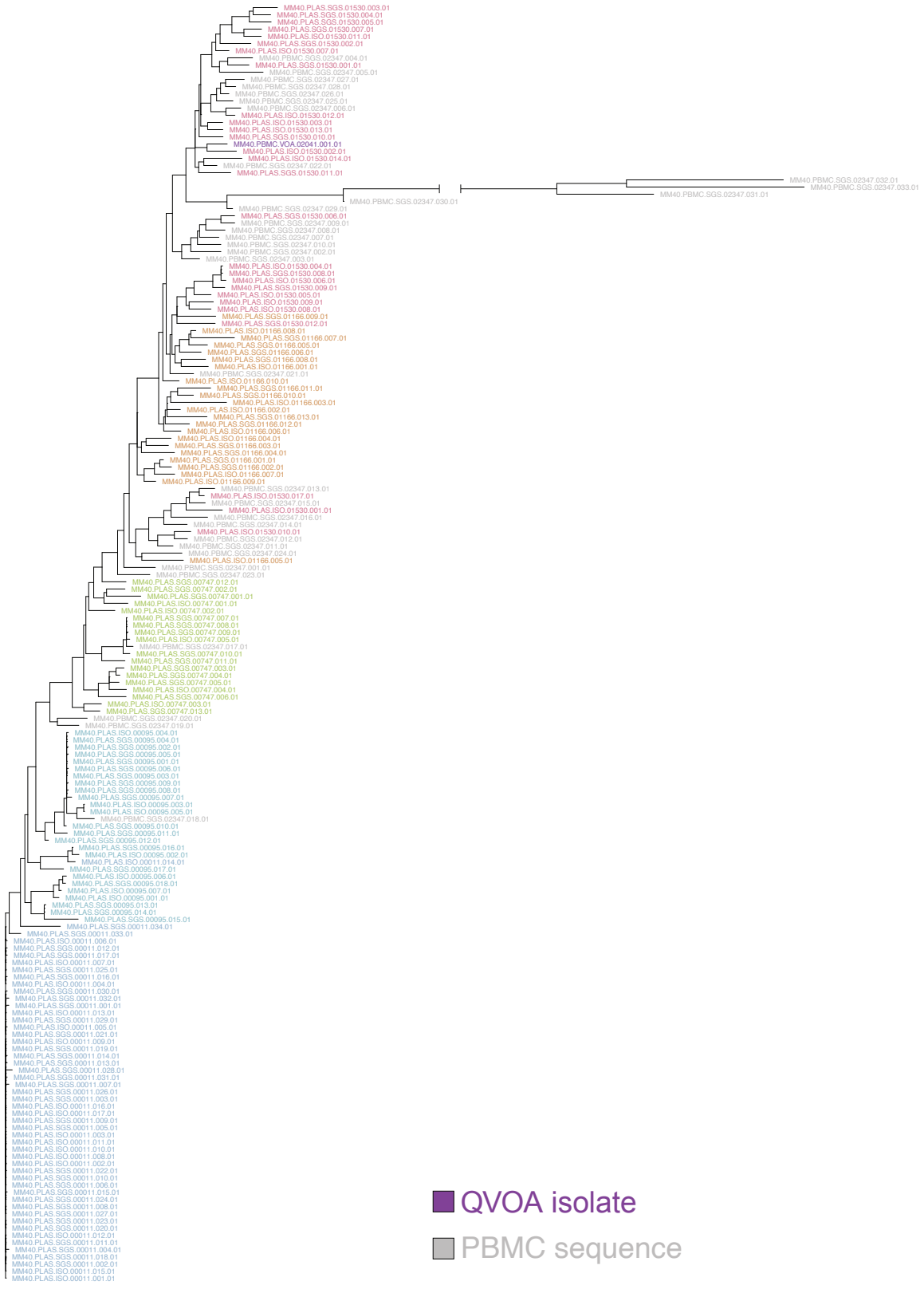
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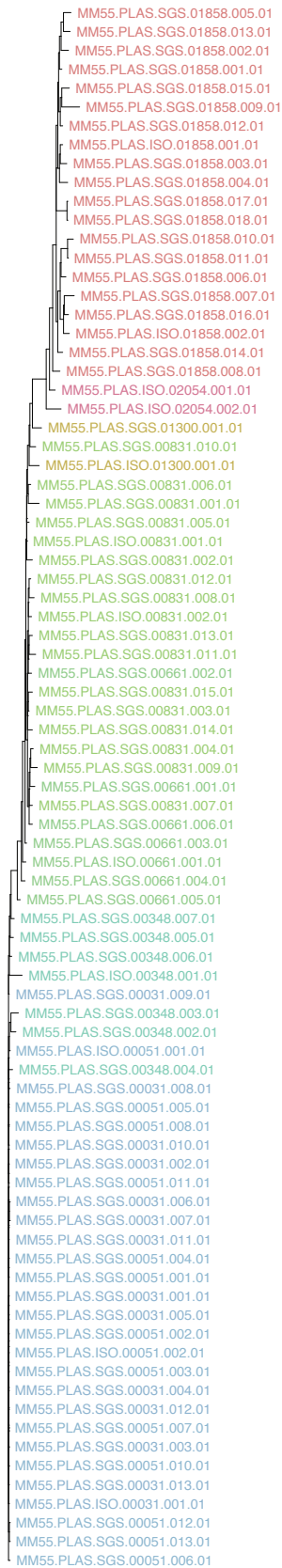
# MM39



# MM40

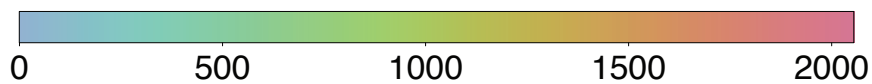


# MM55

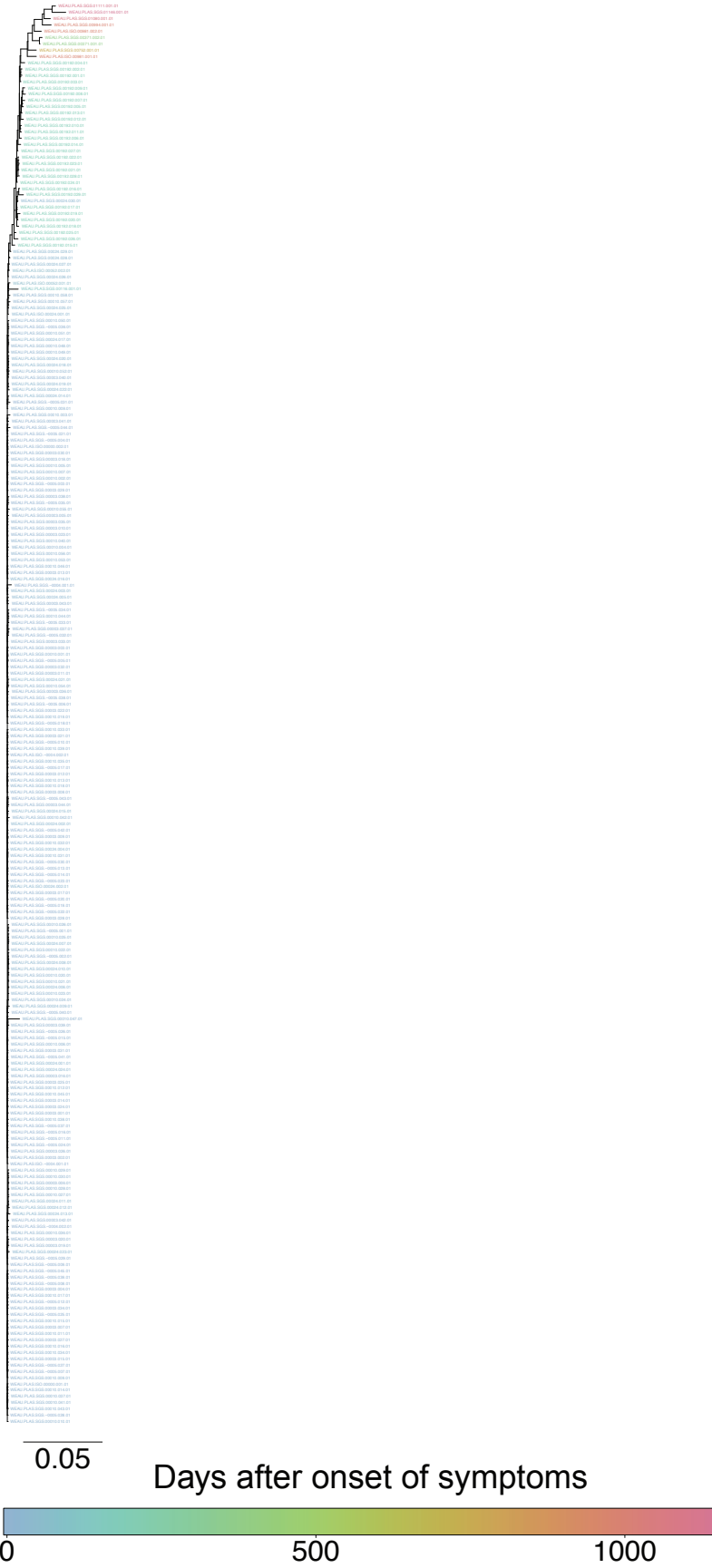


0.05

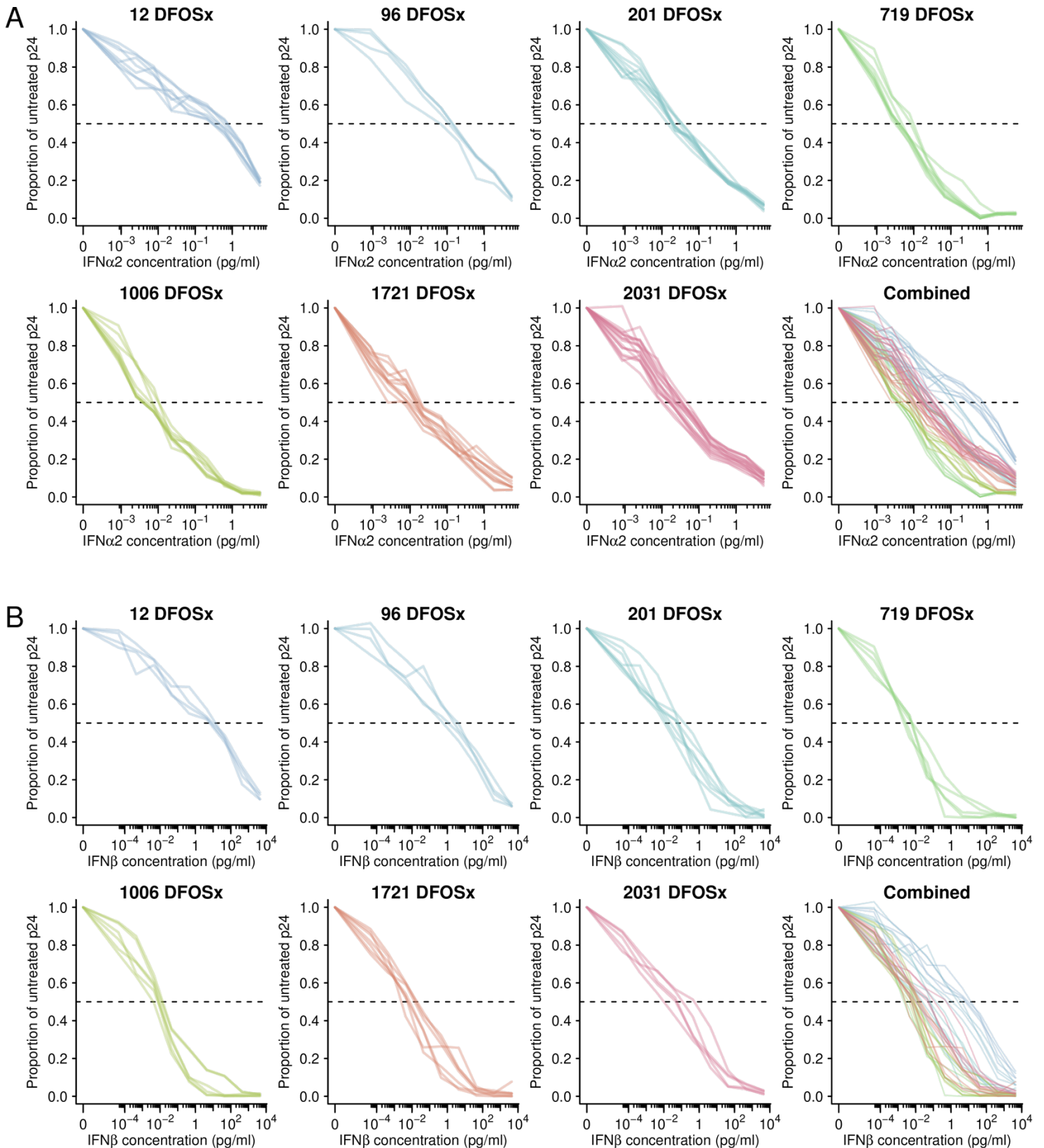
Days after onset of symptoms



# WEAU

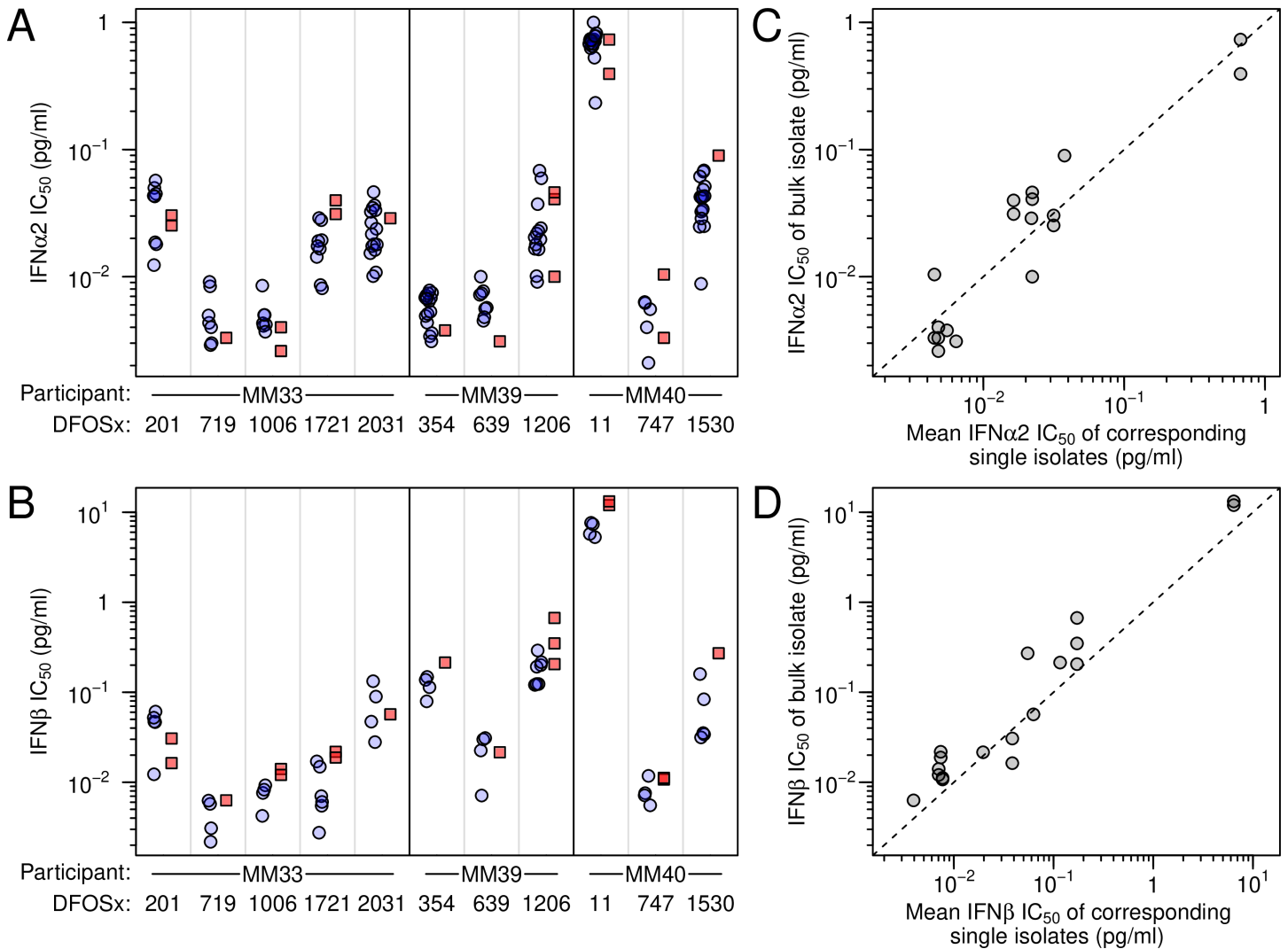


**fig. S2. HIV-1 quasispecies diversification over time.** A maximum likelihood tree depicting the phylogenetic relationships of *env* nucleotide sequences amplified either directly from uncultured plasma (PLAS.SGS) or PBMCs (PBMC.SGS) or generated from plasma (PLAS.ISO) or PBMC (PBMC.QVOA) viral isolates, is shown for each of ten prospectively sampled individuals. Sequences are colored by time point (indicated in the sequence name), with blue (earliest samples) transitioning to red (latest samples). Purple leaves indicate PBMC-derived outgrowth viruses obtained 1.2 to 4.1 years after initiation of suppressive ART, while grey leaves indicate single genome amplification derived sequences generated directly from the corresponding PBMCs. Each leaf is labeled to indicate the individual, source, days post onset of symptoms, and a sequence or isolate identifier. Asterisks indicate sequences inferred as recombinant and triangles indicate superinfecting viruses in participants MM14, MM23 and MM33. A gap in a branch accommodates hypermutated sequences. The scale bar indicates 0.05 substitutions per site.

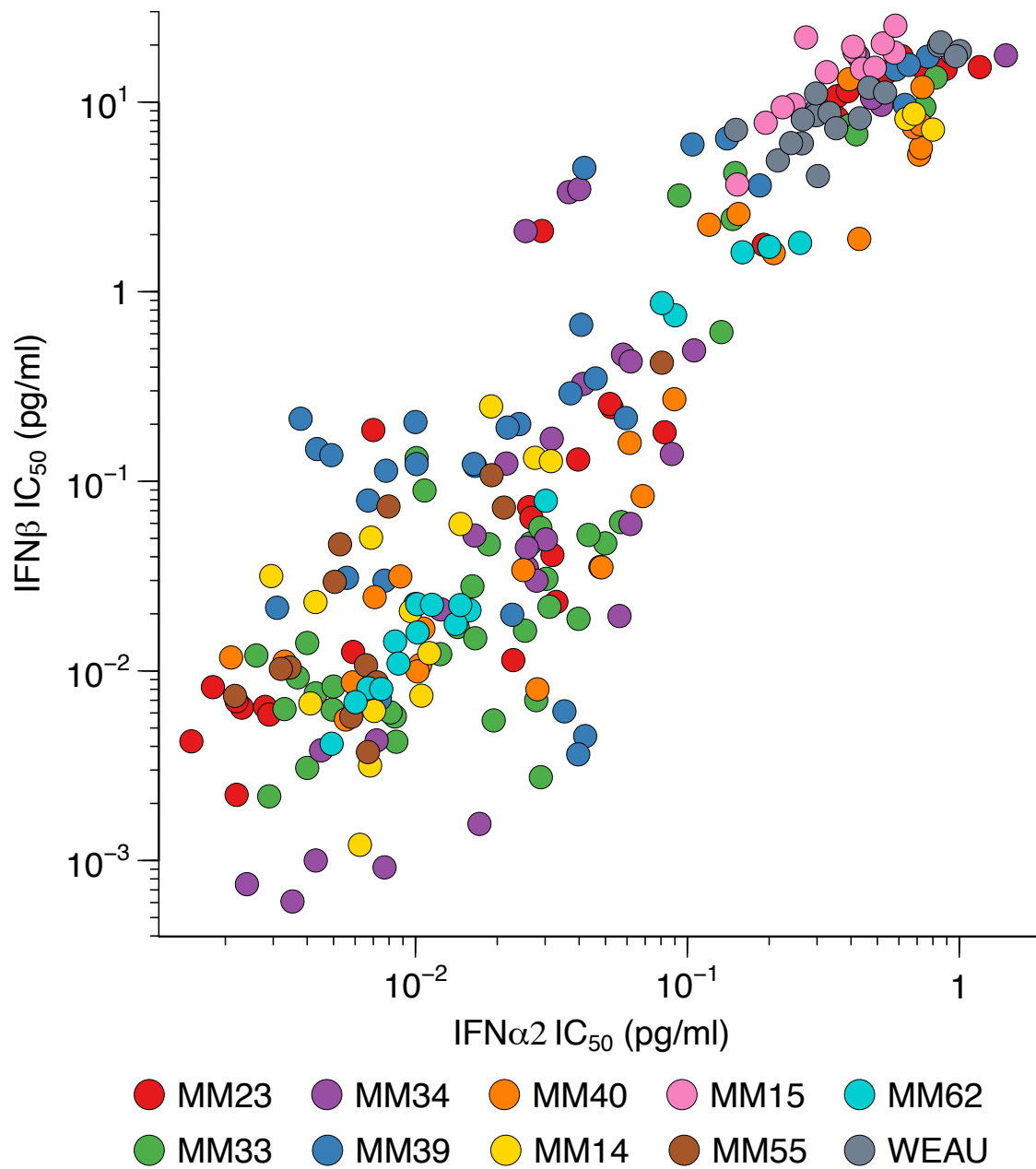


**fig. S3. Determination of IFN-I IC<sub>50</sub> values.** Dose response curves showing the effect of increasing amounts of IFN $\alpha$ 2 (A) and IFN $\beta$  (B) on the replication potential of limiting dilution plasma viral isolates

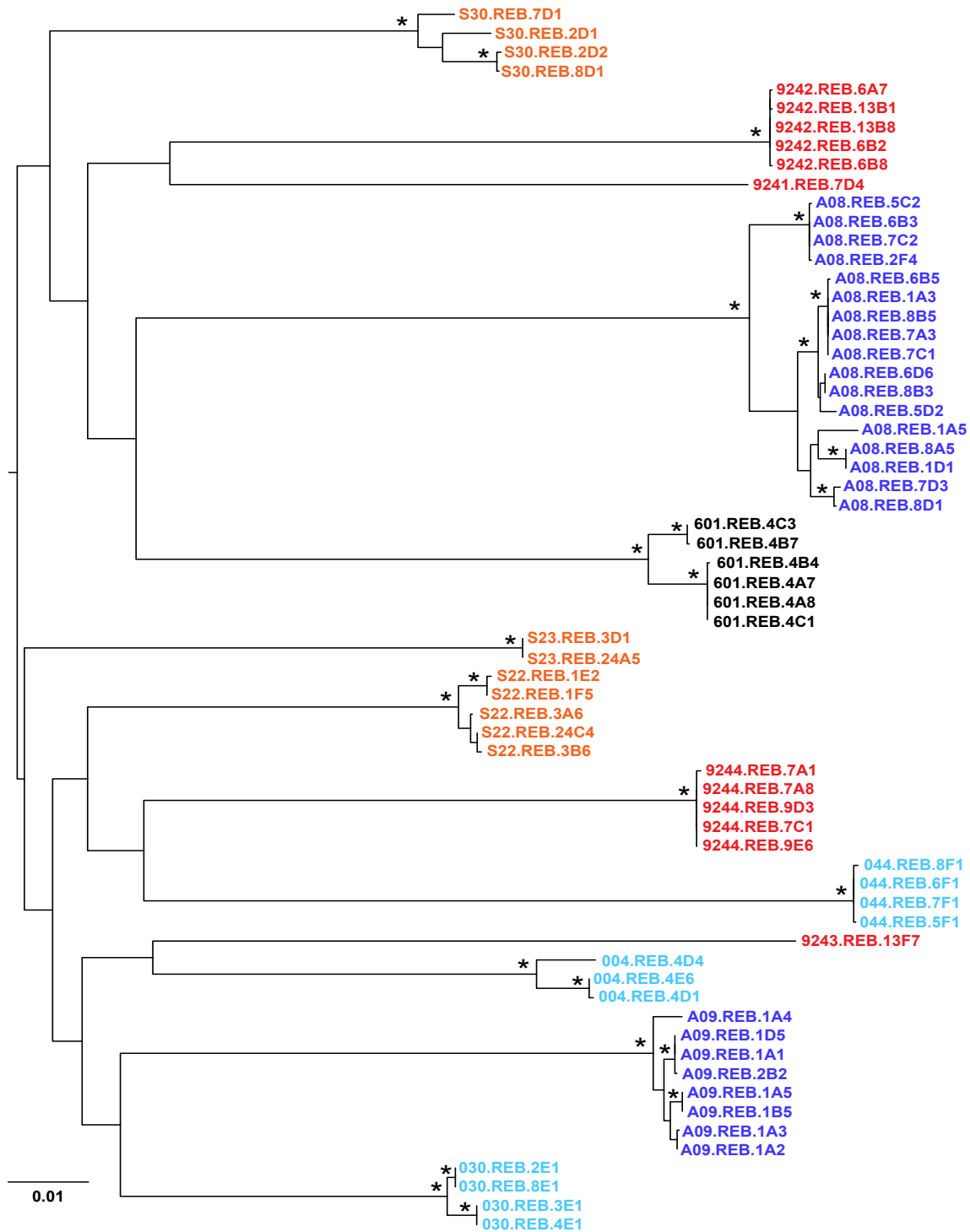
from participant MM33 are shown for individual timepoints with days following onset of symptoms (DFOSx) indicated or combined (last panel). Curves are colored by the timepoint indicated. Dashed horizontal lines indicate the half-maximal inhibitory concentration ( $IC_{50}$ ).



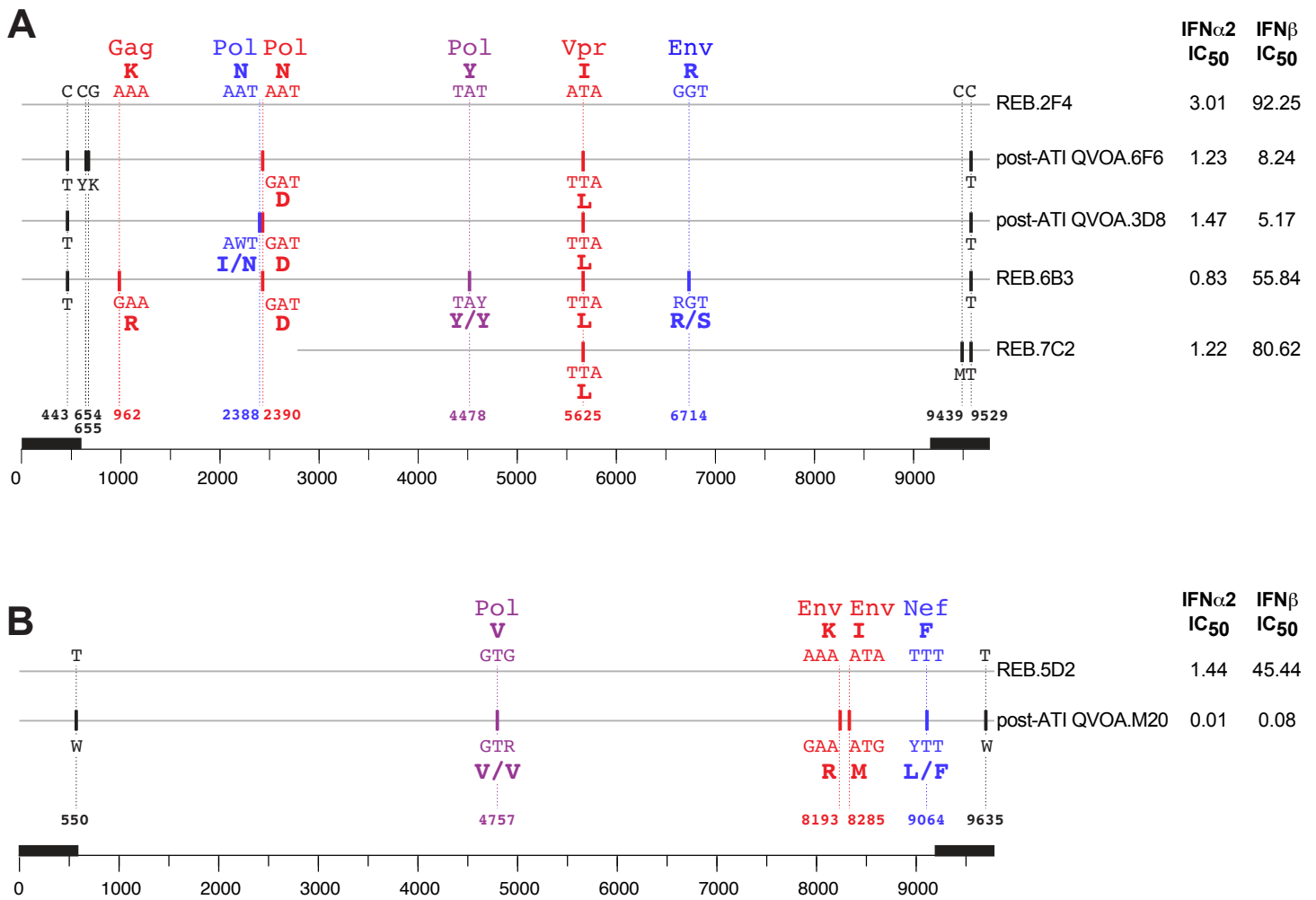
**fig. S4. IFN-I resistance of bulk and limiting dilution isolates from the same plasma samples.** (A, B) IFN $\alpha$ 2 (A) and IFN $\beta$  (B) IC<sub>50</sub> values are shown for limiting dilution derived (blue) and bulk (red) viral isolates from longitudinal plasma samples (days following onset of symptoms; DFOSx) of three participants. (C, D) IFN $\alpha$ 2 (C) and IFN $\beta$  (D) IC<sub>50</sub> values of bulk isolates are compared to the geometric mean IC<sub>50</sub> values of limiting dilution isolates from the same plasma samples (IFN $\alpha$ 2  $r=0.94$  and IFN $\beta$   $r=0.97$ ).



**fig. S5. Correlation of IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values.** The IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values of isolates from ten longitudinally studied participants (represented by colored points) are compared ( $r=0.9$ ).



**fig. S6. Phylogenetic relationships of rebound isolates.** A maximum likelihood tree depicting the phylogenetic relationships of 3' half-genome sequences of rebound isolates from different study participants is shown. Isolates are colored according to the respective study (red, NCT02825797; blue, NCT02463227; orange, NCT00051818; teal, NCT02227277; black, NCT02588586). Asterisks indicate bootstrap values of >90%; the scale bar indicates 0.01 substitution per site.



**fig. S7. Closely related rebound and post-ATI QVOA isolates from participant A08.** (A, B) Highlighter plots of closely related full-length and partial genome sequences of rebound and post-ATI QVOA isolates that differ moderately (up to 20-fold) (A) or markedly (up to 570-fold) (B) in their IFN-I resistance. Tick marks denote differences from the reference sequence shown on top. IFN $\alpha$ 2 and IFN $\beta$  IC<sub>50</sub> values (pg/ml) are listed on the right. Full-length genomes were Illumina sequenced and paired-end reads assembled to generate an isolate-specific reference sequence. Positions with more than 15% diversity (but at least 50-fold coverage) were identified as ambiguous (W, A/T; Y, C/T; R, A/G) and numbered according the HXB2 reference sequence. Red indicates codons with unambiguous nucleotide differences that change the corresponding Gag, Pol, Vpr, Env, and Nef protein sequences; blue indicates codons with ambiguous bases that change the encoded amino acid in a subset of

variants; purple indicates codons with ambiguous bases that do not change the encoded amino acid; black indicates ambiguous and unambiguous bases in non-coding regions; the position of the Long Terminal Repeat (LTR) is indicated by a black bar.

table S1. Generation of HIV-1 isolates from plasma and peripheral blood mononuclear cells of 10 individuals sampled from acute infection throughout their clinical course

| Participant <sup>1</sup> | Number of TF viruses <sup>2</sup> | Date <sup>3</sup> | DFOSx <sup>4</sup> | Viral load (copies/ml) | CD4+ T cell counts (cells/ $\mu$ l) | Number of SGA derived viral sequences (plasma) <sup>5</sup> | Number of SGA derived viral sequences (PBMC) <sup>6</sup> | Number of limiting dilution isolates (plasma) <sup>7</sup> | Number of bulk isolates (plasma) <sup>8</sup> | Number of outgrowth viruses (PBMC) <sup>9</sup> |
|--------------------------|-----------------------------------|-------------------|--------------------|------------------------|-------------------------------------|---|---|--|---|---|
| MM14                     |                                   | 21-Jun-01         | 11                 | 6,235,600              |                                     |   |   |  |   |   |
|                          |                                   | 12-Jul-01         | 32                 | 1,950,600              | 420                                 |   |   |  |   |   |
|                          | 2                                 | 20-Jul-01         | 40                 | 37,611,600             | 430                                 | 9   |   |  | 3 (2)   |   |
|                          |                                   | 27-Jul-01         | 47                 | 2,162,300              | 460                                 |   |   |  |   |   |
|                          |                                   | 2-Aug-01          | 53                 | 613,400                | 530                                 |   |   |  |   |   |
|                          |                                   | 9-Aug-01          | 60                 | 269,000                | 490                                 |   |   |  |   |   |
|                          | SI                                | 26-Oct-01         | 138                | 15,800                 | 480                                 | 9   |   |  | 2   |   |
|                          |                                   | 6-Dec-01          | 179                | 27,200                 | 380                                 |   |   |  |   |   |
|                          |                                   | 7-Mar-02          | 270                | 52,700                 | 390                                 | 3   |   |  | 2   |   |
|                          |                                   | 11-Nov-02         | 519                | 45,300                 | 390                                 | 14  |   |  | 2   |   |
|                          |                                   | 9-Jan-03          | 578                | 16,000                 | 420                                 |   |   |  |   |   |
|                          |                                   | 2-Jun-03          | 722                | 44,400                 | 240                                 | 5   |   |  | 2 (1)   |   |
|                          |                                   | 6-Oct-03          | 848                | 30,100                 | 330                                 |   |   |  |   |   |
|                          |                                   | 20-Apr-04         | 1,045              |                        |                                     | 3   |   |  | 2 (1)   |   |
|                          |                                   | 4-Oct-04          | 1,212              | 6,100                  | 320                                 |   |   |  |   |   |
|                          |                                   | 24-Jan-05         | 1,324              | 12,600                 | 210                                 | 3   |   |  | 2   |   |
|                          |                                   | 4-Apr-05          | 1,394              | 19,700                 | 340                                 | 4   |   |  | 2   |   |
|                          |                                   | 1-Aug-05          | 1,513              | 52,500                 | 210                                 |   |   |  |   |   |
|                          |                                   | 19-Sep-05         | 1,562              |                        | 200                                 |   |   |  |   |   |
|                          |                                   | 16-Jan-06         | 1,681              | 14,000                 | 280                                 |   |   |  |   |   |
|                          |                                   | 13-Feb-06         | 1,709              | 100                    | 340                                 |   |   |  |   |   |
|                          |                                   | 27-Mar-06         | 1,751              | 50                     | 380                                 |   |   |  |   |   |
|                          | 26-Jun-06                         | 1,842             | 50                 | 270                    |                                     |   |   |  |   |   |
|                          | 27-Nov-06                         | 1,996             | 200                | 440                    |                                     |   |   |  |   |   |
|                          | 19-Feb-07                         | 2,080             | 50                 | 630                    |                                     |   |   |  |   |   |
|                          | 18-Jun-07                         | 2,199             | 50                 | 620                    |                                     |   |   |  |   |   |
|                          | 19-Nov-07                         | 2,353             | 50                 | 660                    |                                     |   |   |  |   |   |
|                          | 30-Sep-08                         | 2,669             | 50                 | 610                    |                                     |   |   |  |   |   |
|                          | 9-Mar-09                          | 2,829             | 50                 | 520                    |                                     |   |   |  |   |   |
|                          | 18-Aug-09                         | 2,991             | 50                 | 630                    |                                     |   |   |  |   |   |
|                          | 9-Nov-09                          | 3,074             | 50                 | 770                    |                                     |   |   |  |   |   |
|                          | 8-Mar-10                          | 3,193             | 50                 | 630                    |                                     |   |   |  | 1   |   |
|                          | 15-Mar-12                         | 3,931             | 50                 | 790                    |                                     |   |   |  |   |   |
|                          | 22-Oct-13                         | 4,517             | 240                | 640                    |                                     |   |   |  |   |   |
| MM15                     |                                   | 6-Sep-01          | 5                  | 39,000                 |                                     |   |   |  |   |   |
|                          |                                   | 21-Sep-01         | 20                 | 10,600                 | 1,070                               |   |   |  |   |   |
|                          | 1                                 | 28-Sep-01         | 27                 | 13,200                 | 1,200                               | 15  |   |  | 3 (1)   |   |
|                          |                                   | 8-Oct-01          | 37                 | 28,600                 | 1,030                               | 17  |   |  | 4 (1)   |   |
|                          |                                   | 15-Oct-01         | 44                 | 24,500                 | 970                                 |   |   |  |   |   |
|                          |                                   | 29-Oct-01         | 58                 | 127,200                | 980                                 |   |   |  |   |   |
|                          |                                   | 19-Nov-01         | 79                 | 59,900                 | 1,450                               |   |   |  | 1 (1)   |   |
|                          |                                   | 17-Dec-01         | 107                | 381,500                | 820                                 | 15  |   |  | 1 (1)   |   |
|                          |                                   | 11-Mar-02         | 191                | 43,200                 | 660                                 | 6   |   |  | 1 (1)   |   |
|                          |                                   | 10-Jun-02         | 282                | 110,700                | 620                                 |   |   |  |   |   |
|                          |                                   | 9-Sep-02          | 373                | 45,200                 | 420                                 | 7   |   |  | 1 (1)   |   |
|                          |                                   | 9-Dec-02          | 464                | 69,900                 | 370                                 |   |   |  |   |   |
|                          |                                   | 3-Mar-03          | 548                | 41,700                 | 260                                 | 6   |   |  | 1 (1)   |   |
|                          |                                   | 17-Apr-03         | 593                | 19,400                 | 280                                 |   |   |  |   |   |
|                          |                                   | 30-Apr-03         | 606                | 39,700                 |                                     |   |   |  |   |   |
|                          |                                   | 9-Jun-03          | 646                | 22,800                 | 240                                 |   |   |  |   |   |
|                          |                                   | 22-Aug-03         | 720                | 27,500                 | 320                                 | 9   |   |  | 1   |   |
|                          |                                   | 10-Oct-03         | 769                | 27,000                 | 220                                 |   |   |  |   |   |
|                          |                                   | 10-Nov-03         | 800                | 70                     | 450                                 |   |   |  |   |   |
|                          |                                   | 10-Dec-03         | 830                | 4,300                  | 310                                 |   |   |  |   |   |
|                          |                                   | 9-Jan-04          | 860                | 50                     | 460                                 |   |   |  |   |   |
|                          |                                   | 22-Mar-04         | 933                | 50                     | 540                                 |   |   |  |   |   |
|                          |                                   | 23-Jun-04         | 1,026              | 50                     | 430                                 |   |   |  |   |   |
|                          |                                   | 15-Dec-04         | 1,201              | 50                     | 560                                 |   |   |  |   |   |
|                          |                                   | 11-May-05         | 1,348              | 50                     | 580                                 |   |   |  |   |   |
|                          |                                   | 5-Jul-05          | 1,403              | 50                     | 700                                 |   |   |  |   | 1   |
|                          |                                   | 5-Jan-06          | 1,587              | 50                     | 750                                 |   |   |  |   |   |
|                          | 22-Aug-06                         | 1,816             | 50                 | 820                    |                                     |   |   |  |   |   |
|                          | 16-Jan-07                         | 1,963             | 200                | 570                    |                                     |   |   |  |   |   |
|                          | 9-Aug-07                          | 2,168             | 50                 | 940                    |                                     |   |   |  |   |   |
|                          | 22-Jan-08                         | 2,334             | 50                 | 700                    |                                     |   |   |  |   |   |
|                          | 12-Jun-08                         | 2,476             | 50                 | 750                    |                                     |   |   |  |   |   |
|                          | 9-Jan-09                          | 2,687             | 50                 | 900                    |                                     |   |   |  |   |   |
|                          | 29-Apr-09                         | 2,797             | 250                | 950                    |                                     |   |   |  |   |   |
|                          | 13-May-09                         | 2,811             | 50                 |                        |                                     |   |   |  |   |   |
|                          | 1-Feb-10                          | 3,075             | 50                 | 1,000                  |                                     |   |   |  |   |   |
|                          | 1-Sep-11                          | 3,652             | 50                 | 830                    |                                     |   |   |  |   |   |
| MM23                     | 1                                 | 5-Mar-02          | 9                  | 11,105,300             | 330                                 | 41  |   | 12   |   |   |
|                          |                                   | 11-Mar-02         | 15                 | 8,871,700              | 410                                 |   |   |  |   |   |
|                          |                                   | 18-Mar-02         | 22                 | 5,140,100              | 410                                 |   |   |  |   |   |
|                          |                                   | 2-Apr-02          | 37                 |                        | 400                                 |   |   |  |   |   |
|                          |                                   | 29-Apr-02         | 64                 | 200,500                | 590                                 | 9   |   | 4  |   |   |
|                          |                                   | 17-Jun-02         | 113                | 147,700                | 510                                 |   |   |  |   |   |
|                          | SI                                | 16-Sep-02         | 204                | 117,600                | 330                                 | 43  |   | 8  |   |   |
|                          |                                   | 6-Jan-03          | 316                | 100,100                | 300                                 |   |   | 11   |   |   |
|                          | 7-Jul-03                          | 498               | 76,300             | 410                    |                                     |   |   |  |   |   |
|                          | 30-Sep-03                         | 583               | 111,200            | 220                    |                                     |   |   |  |   |   |

|  |    |           |       |           |       |    |    |   |   |
|--|----|-----------|-------|-----------|-------|----|----|---|---|
|  |    | 17-Nov-03 | 631   | 74,700    | 350   |    |    |   |   |
|  |    | 16-Feb-04 | 722   | 52,400    | 550   |    | 9  |   |   |
|  |    | 18-May-04 | 814   | 41,600    | 250   |    |    |   |   |
|  |    | 7-Jul-04  | 864   |           | 320   |    |    |   |   |
|  |    | 30-Nov-04 | 1,010 | 157,800   | 230   |    |    |   |   |
|  |    | 24-Jan-05 | 1,065 | 45,700    | 240   |    | 5  |   |   |
|  |    | 1-Aug-05  | 1,254 | 105,800   | 230   |    |    |   |   |
|  |    | 8-May-06  | 1,534 | 168,300   | 190   |    | 8  |   |   |
|  |    | 29-Jun-06 | 1,586 |           |       |    |    |   |   |
|  |    | 17-Jul-06 | 1,604 | 200       | 200   |    |    |   |   |
|  |    | 2-Oct-06  | 1,681 | 60        | 260   |    |    |   |   |
|  |    | 26-Feb-07 | 1,828 | 50        | 230   |    |    |   |   |
|  |    | 9-Jul-07  | 1,961 | 50        | 290   |    |    |   |   |
|  |    | 17-Dec-07 | 2,122 | 50        | 280   |    |    |   |   |
|  |    | 15-Apr-08 | 2,242 | 50        | 250   |    |    |   |   |
|  |    | 1-Sep-08  | 2,381 | 50        | 280   |    |    |   |   |
|  |    | 4-Dec-08  | 2,475 | 50        | 310   |    |    |   |   |
|  |    | 19-Mar-09 | 2,580 | 50        | 400   |    |    |   |   |
|  |    | 24-Aug-09 | 2,738 | 50        | 380   | 33 |    |   | 2 |
|  |    | 25-Feb-10 | 2,923 | 50        | 350   |    |    |   |   |
|  |    | 17-Mar-11 | 3,308 | 50        | 380   |    |    |   |   |
|  |    | 19-Apr-12 | 3,707 | 50        | 380   |    |    |   |   |
|  |    | 2-Oct-12  | 3,873 | 50        | 600   |    |    |   |   |
|  | 2  | 7-Jul-03  | 12    | 1,451,400 | 940   | 42 | 8  |   |   |
|  |    | 14-Jul-03 | 19    | 215,700   | 940   | 44 |    |   |   |
|  |    | 21-Jul-03 | 26    | 163,800   | 1,070 | 22 |    |   |   |
|  |    | 28-Jul-03 | 33    | 100,500   | 840   | 28 |    |   |   |
|  | SI | 4-Aug-03  | 40    | 129,800   | 810   | 29 |    |   |   |
|  |    | 2-Sep-03  | 69    | 147,400   | 1,000 | 19 |    |   |   |
|  |    | 29-Sep-03 | 96    | 62,200    | 860   | 14 | 4  |   |   |
|  |    | 12-Jan-04 | 201   | 80,000    | 770   | 8  | 8  | 2 |   |
|  |    | 6-Apr-04  | 286   | 83,200    | 740   | 13 |    |   |   |
|  |    | 20-Jul-04 | 391   | 31,200    | 670   | 21 |    |   |   |
|  |    | 29-Nov-04 | 523   | 43,500    | 710   |    |    |   |   |
|  |    | 7-Mar-05  | 621   | 65,100    | 830   | 20 |    |   |   |
|  |    | 13-Jun-05 | 719   | 83,200    | 640   | 7  | 7  | 1 |   |
|  |    | 26-Sep-05 | 824   | 42,600    | 670   |    |    |   |   |
|  |    | 27-Mar-06 | 1,006 | 72,100    | 660   | 5  | 7  | 2 |   |
|  |    | 19-Sep-06 | 1,182 | 41,800    | 740   |    |    |   |   |
|  |    | 19-Feb-07 | 1,335 | 105,200   | 510   |    |    |   |   |
|  |    | 5-Jun-07  | 1,441 | 131,000   | 600   |    |    |   |   |
|  |    | 5-Nov-07  | 1,594 | 30,000    | 440   |    |    |   |   |
|  |    | 11-Mar-08 | 1,721 | 110,000   | 480   | 9  | 9  | 2 |   |
|  |    | 12-Jun-08 | 1,814 | 23,000    | 390   |    |    |   |   |
|  |    | 24-Jul-08 | 1,856 | 180,000   | 350   |    |    |   |   |
|  |    | 19-Sep-08 | 1,913 | 110,000   | 340   | 22 |    |   |   |
|  |    | 15-Jan-09 | 2,031 | 28,000    | 240   | 19 | 15 | 1 |   |
|  |    | 16-Feb-09 | 2,063 | 330       | 330   |    |    |   |   |
|  |    | 23-Mar-09 | 2,098 |           | 640   |    |    |   |   |
|  |    | 23-Apr-09 | 2,129 | 50        | 470   |    |    |   |   |
|  |    | 17-Jul-09 | 2,214 | 50        | 460   |    |    |   |   |
|  |    | 6-Oct-09  | 2,295 | 50        | 560   |    |    |   |   |
|  |    | 26-Jan-10 | 2,407 | 50        |       |    |    |   |   |
|  |    | 23-Feb-10 | 2,435 | 50        | 640   |    |    |   |   |
|  |    | 16-Aug-10 | 2,609 | 50        | 680   |    |    |   |   |
|  |    | 15-Feb-11 | 2,792 | 50        | 810   |    |    |   |   |
|  |    | 13-Feb-12 | 3,155 | 50        | 810   |    |    |   |   |
|  |    | 23-Aug-12 | 3,347 | 50        | 780   |    |    |   |   |
|  |    | 15-Jan-13 | 3,492 | 50        | 720   |    |    |   |   |
|  |    | 7-Oct-13  | 3,757 | 50        | 770   |    |    |   |   |
|  |    | 13-Jan-14 | 3,855 | 50        | 1,130 |    |    |   |   |
|  | 2  | 21-Jul-03 | 17    | 29,900    | 570   | 40 | 10 | 1 |   |
|  |    | 29-Jul-03 | 25    | 19,900    | 780   |    |    |   |   |
|  |    | 5-Aug-03  | 32    | 21,400    | 660   |    |    |   |   |
|  |    | 14-Aug-03 | 41    | 16,400    | 620   |    |    |   |   |
|  |    | 18-Aug-03 | 45    | 6,800     | 730   |    |    |   |   |
|  |    | 16-Sep-03 | 74    | 2,400     | 620   | 10 | 1  |   |   |
|  |    | 14-Oct-03 | 102   | 5,900     | 670   |    |    |   |   |
|  |    | 12-Jan-04 | 192   | 9,600     | 630   | 40 | 5  |   |   |
|  |    | 21-Jun-04 | 353   | 5,900     | 560   | 19 |    |   |   |
|  |    | 20-Sep-04 | 444   | 8,200     | 500   |    |    |   |   |
|  |    | 3-Mar-05  | 608   | 9,500     | 480   |    |    |   |   |
|  |    | 2-Aug-05  | 760   | 5,500     | 470   | 13 | 4  |   |   |
|  |    | 16-Feb-06 | 958   |           | 590   |    |    |   |   |
|  |    | 6-Apr-06  | 1,007 | 11,400    | 600   |    | 6  |   |   |
|  |    | 6-Feb-07  | 1,313 | 40,700    | 450   | 12 | 7  |   |   |
|  |    | 3-May-07  | 1,399 | 14,000    | 490   |    | 3  |   |   |
|  |    | 19-Feb-08 | 1,691 | 13,000    | 420   |    | 5  | 1 |   |
|  |    | 26-Mar-09 | 2,092 |           | 440   |    |    |   |   |
|  |    | 7-May-09  | 2,134 |           | 380   |    |    |   |   |
|  |    | 10-Aug-09 | 2,229 | 14,000    | 340   |    | 13 | 1 |   |
|  |    | 14-Sep-09 | 2,264 |           |       |    |    |   |   |
|  |    | 12-Oct-09 | 2,292 |           | 480   |    |    |   |   |
|  |    | 9-Nov-09  | 2,320 | 50        | 480   |    |    |   |   |

|      |           |           |        |           |     |     |    |       |       |   |
|------|-----------|-----------|--------|-----------|-----|-----|----|-------|-------|---|
|      |           | 22-Feb-10 | 2,425  | 50        | 540 |     |    |       |       |   |
|      |           | 25-Oct-10 | 2,670  | 50        | 530 |     |    |       |       |   |
|      |           | 10-Jan-11 | 2,747  | 50        | 620 |     |    |       |       |   |
|      |           | 23-Jun-11 | 2,911  |           |     |     |    |       |       | 1 |
|      |           | 3-Nov-11  | 3,044  | 50        | 890 |     |    |       |       |   |
|      |           | 7-Jun-12  | 3,261  | 50        | 780 |     | 38 |       |       | 2 |
|      |           | 21-Feb-13 | 3,520  | 50        | 730 |     |    |       |       |   |
| MM39 | 1         | 21-Apr-04 | 3      | 2,203,800 |     |     |    |       |       |   |
|      |           | 23-Apr-04 | 5      | 350,600   | 610 |     |    |       |       |   |
|      |           | 26-Apr-04 | 8      |           | 400 |     |    |       |       |   |
|      |           | 29-Apr-04 | 11     | 1,508,000 | 680 | 36* |    | 15*   |       |   |
|      |           | 11-May-04 | 23     | 6,700     | 490 |     |    |       |       |   |
|      |           | 25-May-04 | 37     | 7,400     | 520 | 21* |    |       |       |   |
|      |           | 22-Jun-04 | 65     | 900       | 530 |     |    |       |       |   |
|      |           | 19-Jul-04 | 92     | 3,000     | 610 | 14* |    |       |       |   |
|      |           | 2-Sep-04  | 137    | 8,400     | 580 |     |    |       |       |   |
|      |           | 14-Oct-04 | 179    | 7,000     | 510 | 17* |    | 4*    |       |   |
|      |           | 13-Jan-05 | 270    | 13,400    | 580 | 19* |    |       |       |   |
|      |           | 7-Apr-05  | 354    | 99,300    | 610 | 20* |    | 15*   | 1     |   |
|      |           | 21-Jul-05 | 459    | 35,600    | 510 | 21* |    |       |       |   |
|      |           | 13-Oct-05 | 543    | 15,300    | 580 | 21* |    |       |       |   |
|      |           | 17-Jan-06 | 639    | 15,600    | 490 | 19* |    | 8*    | 1     |   |
|      |           | 18-Jul-06 | 821    | 75,400    | 380 | 17* |    | 7*    |       |   |
|      |           | 31-Oct-06 | 926    | 24,700    | 490 |     |    |       |       |   |
|      |           | 23-Jan-07 | 1,010  | 40,300    | 480 |     |    |       |       |   |
|      |           | 7-Aug-07  | 1,206  | 280,000   | 190 | 16* |    | 13*   | 3     |   |
|      |           | 31-Aug-07 | 1,230  | 280,000   | 190 |     |    |       |       |   |
|      |           | 1-Nov-07  | 1,292  | 63,000    | 90  |     |    |       |       |   |
|      |           | 13-Nov-07 | 1,304  |           |     |     |    |       |       |   |
|      |           | 11-Dec-07 | 1,332  | 240       | 340 |     |    |       |       |   |
|      | 29-Jan-08 | 1,381     | 50     | 290       |     |     |    |       |       |   |
|      | 25-Apr-08 | 1,468     | 50     | 370       |     |     |    |       |       |   |
|      | 11-Jul-08 | 1,545     | 50     | 380       |     |     |    |       |       |   |
|      | 9-Jan-09  | 1,727     | 50     | 350       |     |     |    |       |       |   |
|      | 24-Apr-09 | 1,832     | 50     | 430       |     |     |    |       |       |   |
|      | 21-Aug-09 | 1,951     | 50     | 430       |     |     |    |       |       |   |
| MM40 | 3 or more | 21-Jun-04 | 8      | 350,100   | 290 |     |    |       |       |   |
|      |           | 24-Jun-04 | 11     | 79,300    | 410 | 34  |    | 17    | 2     |   |
|      |           | 1-Jul-04  | 18     | 13,700    | 380 |     |    |       |       |   |
|      |           | 8-Jul-04  | 25     | 1,000     | 470 |     |    |       |       |   |
|      |           | 15-Jul-04 | 32     | 2,100     | 490 |     |    |       |       |   |
|      |           | 22-Jul-04 | 39     | 6,500     | 430 |     |    |       |       |   |
|      |           | 5-Aug-04  | 53     | 5,500     | 380 |     |    |       |       |   |
|      |           | 19-Aug-04 | 67     | 6,400     | 470 |     |    |       |       |   |
|      |           | 16-Sep-04 | 95     | 10,800    | 450 | 18  |    | 8     |       |   |
|      |           | 14-Dec-04 | 184    | 39,300    | 510 |     |    |       |       |   |
|      |           | 17-Mar-05 | 277    | 16,400    | 380 |     |    |       |       |   |
|      |           | 7-Jul-05  | 389    | 26,800    | 330 |     |    |       |       |   |
|      |           | 13-Oct-05 | 487    | 50,100    | 390 |     |    |       |       |   |
|      |           | 20-Jan-06 | 586    | 30,600    | 490 |     |    |       |       |   |
|      |           | 30-Jun-06 | 747    | 89,000    | 310 | 13  |    | 5     | 2     |   |
|      |           | 15-Oct-06 | 854    | 25,400    | 340 |     |    |       |       |   |
|      |           | 9-Feb-07  | 971    | 18,000    | 280 |     |    |       |       |   |
|      |           | 23-Aug-07 | 1,166  | 140,000   | 250 | 13  |    | 10    |       |   |
|      |           | 10-Jan-08 | 1,306  | 54,000    | 390 |     |    |       |       |   |
|      |           | 30-Jun-08 | 1,478  |           | 330 |     |    |       |       |   |
|      |           | 21-Aug-08 | 1,530  | 310,000   | 250 | 12  |    | 18    | 1     |   |
|      |           | 16-Oct-08 | 1,586  |           | 170 |     |    |       |       |   |
|      |           | 20-Oct-08 | 1,590  | 1,200,000 |     |     |    |       |       |   |
|      | 10-Nov-08 | 1,611     |        |           |     |     |    |       |       |   |
|      | 20-Jan-09 | 1,682     | 60     | 320       |     |     |    |       |       |   |
|      | 2-Mar-09  | 1,723     | 50     | 350       |     |     |    |       |       |   |
|      | 2-Jul-09  | 1,845     | 50     | 370       |     |     |    |       |       |   |
|      | 14-Jan-10 | 2,041     | 50     | 320       |     |     |    |       | 1     |   |
|      | 7-Apr-10  | 2,124     | 50     | 460       |     |     |    |       |       |   |
|      | 16-Nov-10 | 2,347     | 50     | 440       |     | 33  |    |       |       |   |
| MM55 | 1         | 25-Sep-07 | 24     | 23,000    | 640 |     |    |       |       |   |
|      |           | 2-Oct-07  | 31     | 1,200     | 730 | 13  |    |       | 1     |   |
|      |           | 9-Oct-07  | 38     | 6,100     | 670 |     |    |       |       |   |
|      |           | 22-Oct-07 | 51     | 4,200     | 570 | 12  |    |       | 2 (1) |   |
|      |           | 30-Oct-07 | 59     | 2,100     | 740 |     |    |       |       |   |
|      |           | 6-Nov-07  | 66     | 880       | 630 |     |    |       |       |   |
|      |           | 4-Dec-07  | 94     | 450       | 680 |     |    |       |       |   |
|      |           | 10-Jan-08 | 131    | 50        | 560 |     |    |       |       |   |
|      |           | 16-Apr-08 | 228    | 1,500     | 550 |     |    |       |       |   |
|      |           | 14-Aug-08 | 348    | 2,500     | 550 | 6   |    |       | 1     |   |
|      |           | 12-Mar-09 | 558    | 3,500     | 540 |     |    |       |       |   |
|      |           | 23-Jun-09 | 661    | 4,300     | 560 | 6   |    |       | 1     |   |
|      |           | 10-Dec-09 | 831    | 10,000    |     | 15  |    |       | 2     |   |
|      |           | 24-Feb-10 | 907    |           | 650 |     |    |       |       |   |
|      | 23-Aug-10 | 1,087     | 40,000 | 530       |     |     |    |       |       |   |
|      | 24-Mar-11 | 1,300     | 11,000 | 490       | 1   |     |    | 2     |       |   |
|      | 2-Oct-12  | 1,858     | 17,000 | 460       | 18  |     |    | 2     |       |   |
|      | 16-Apr-13 | 2,054     | 17,000 | 630       |     |     |    | 2 (1) |       |   |

|      |                        |                        |       |         |       |     |   |  |       |
|------|------------------------|------------------------|-------|---------|-------|-----|---|--|-------|
| MM62 |                        | 17-Dec-08              | 16    | 2,200   | 700   |     |   |  |       |
|      | 1                      | 23-Dec-08              | 22    |         |       | 11  |   |  | 3 (1) |
|      |                        | 27-Jan-09              | 57    | 680     | 1,130 |     | 2 |  | 2     |
|      |                        | 13-Feb-09              | 74    |         |       |     |   |  | 2 (1) |
|      |                        | 25-Feb-09              | 86    | 270     | 1,000 |     |   |  |       |
|      |                        | 31-Mar-09              | 120   | 190     | 1,240 |     |   |  | 3 (1) |
|      |                        | 4-Jun-09               | 185   | 2,200   | 880   | 1   |   |  | 2     |
|      |                        | 1-Oct-09               | 304   | 520     | 860   |     |   |  | 1     |
|      |                        | 11-Feb-10              | 437   | 410     | 820   |     |   |  |       |
|      |                        | 20-Sep-10              | 658   | 860     | 900   | 1   |   |  | 1 (1) |
|      |                        | 5-Jan-12               | 1,130 | 1,100   | 1,090 |     |   |  | 2     |
|      |                        | 24-Jul-12              | 1,331 | 20,000  | 1,200 |     |   |  | 2     |
|      |                        | 7-Mar-13               | 1,557 | 11,000  | 1,200 |     |   |  |       |
|      | 2-Dec-14               | 2,192                  | 5,200 | 980     |       |     |   |  |       |
| WEAU | 1                      | 30-May-90              | -5    | 216,415 | 358   | 45* |   |  |       |
|      |                        | 31-May-90              | -4    |         |       | 2*  |   |  | 2 (2) |
|      |                        | 4-Jun-90               | 0     | 355,184 |       |     |   |  | 2 (2) |
|      |                        | 7-Jun-90               | 3     | 355,360 |       | 44* |   |  |       |
|      |                        | 11-Jun-90              | 7     | 146,816 | 748   |     |   |  |       |
|      |                        | 14-Jun-90              | 10    |         |       | 58* |   |  |       |
|      |                        | 18-Jun-90              | 14    | 100,877 | 568   |     |   |  |       |
|      |                        | 24-Jun-90              | 20    |         | 972   |     |   |  |       |
|      |                        | 28-Jun-90              | 24    | 34,737  |       | 30* |   |  | 2 (1) |
|      |                        | 26-Jul-90              | 52    | 11,385  |       |     |   |  | 2 (2) |
|      |                        | 28-Sep-90              | 116   | 17,322  |       | 1*  |   |  |       |
|      |                        | 13-Dec-90              | 192   |         |       | 29* |   |  |       |
|      |                        | 14-Feb-91              | 255   |         | 197   |     |   |  |       |
|      |                        | 10-Jun-91              | 371   | 55,269  | 89    | 2*  |   |  | 2     |
|      |                        | 6-Nov-91               | 520   | 94,474  | 14    |     |   |  | 2     |
|      |                        | 24-Jan-92 <sup>§</sup> | 599   |         | 43    |     |   |  |       |
|      |                        | 3-May-92 <sup>§</sup>  | 699   | 61,667  |       |     |   |  |       |
|      |                        | 25-Jun-92 <sup>§</sup> | 752   | 55,900  | 30    | 1*  |   |  | 2     |
|      |                        | 14-Jul-92 <sup>§</sup> | 771   | 52,745  |       |     |   |  | 2     |
|      |                        | 6-Oct-92 <sup>§</sup>  | 855   |         | 14    |     |   |  |       |
|      |                        | 9-Feb-93 <sup>§</sup>  | 981   |         |       |     |   |  | 2 (2) |
|      |                        | 22-Feb-93 <sup>§</sup> | 994   |         | 8     | 1*  |   |  |       |
|      |                        | 16-Mar-93 <sup>§</sup> | 1,016 |         | 6     |     |   |  |       |
|      |                        | 13-Apr-93 <sup>§</sup> | 1,044 |         | 7     |     |   |  |       |
|      |                        | 19-May-93 <sup>§</sup> | 1,080 |         |       | 1*  |   |  |       |
|      |                        | 15-Jun-93 <sup>§</sup> | 1,107 |         | 4     |     |   |  |       |
|      |                        | 19-Jun-93 <sup>§</sup> | 1,111 |         |       | 1*  |   |  |       |
|      | 24-Jul-93 <sup>§</sup> | 1,146                  |       |         | 1*    |     |   |  |       |
|      | 26-Jul-93 <sup>§</sup> | 1,148                  |       | 5       |       |     |   |  |       |
|      | 1-Jan-94 <sup>§</sup>  | 1,307                  |       | 2       |       |     |   |  |       |
|      | 5-Jul-94 <sup>§</sup>  | 1,492                  |       | 1       |       |     |   |  |       |

<sup>1</sup>All subjects were men-who-have-sex-with-men (MSM) from the United Kingdom (MM) or the United States (WEAU), who were diagnosed with symptomatic primary subtype B infection and longitudinally followed before and after (shading) antiretroviral therapy.

<sup>2</sup>The number of transmitted founder (TF) viruses was inferred as previously described (54) from single genome amplification (SGA) derived viral sequences from the first available plasma sample (fig. S1); subjects MM14, MM23 and MM33 were superinfected (SI) with additional subtype B strains at the timepoints indicated.

<sup>3</sup>Visit date; grey shading indicates time on suppressive antiretroviral therapy; subject WEAU received zidovudine monotherapy (indicated by ¶), which did not reduce viral loads.

<sup>4</sup>Days following onset of symptoms (DFOSx).

<sup>5</sup>Number of SGA derived 3' half genome or *env* gene sequences amplified directly from plasma (GenBank accession number are listed in Data file S1); *env* gene sequences for subjects MM39 and WEAU that have previously been reported (43, 54, 57, 86) are indicated by asterisks.

<sup>6</sup>Number of SGA derived 3' half genome or *env* gene sequences amplified directly from peripheral blood mononuclear cells (PBMCs); GenBank accession number are listed in Data file S1.

<sup>7</sup>Number of limiting dilution derived plasma isolates; all isolates were confirmed to be single virion derived by sequence analysis; GenBank accession number are listed in Data file S1.

<sup>8</sup>Number of conventional (bulk) plasma isolates; most isolates were sequenced and those that represent single virion derived strains are shown in parentheses; GenBank accession number are listed in Data file S1.

<sup>9</sup>Number of outgrowth viruses from peripheral blood mononuclear cells (PBMCs); GenBank accession number are listed in Data file S1.

**table S2. IFN-I resistance of plasma and QVOA isolates from longitudinally sampled study participants**

| Isolate          | Sequence ID <sup>1</sup> | DFOSx <sup>2</sup> | Replicative capacity (ng p24/ml) <sup>3</sup> | IFN $\alpha$ 2 IC <sub>50</sub> (pg/ml) <sup>4</sup> | IFN $\beta$ IC <sub>50</sub> (pg/ml) <sup>4</sup> |
|------------------|--------------------------|--------------------|---|--|---|
| MM14.02.2C3.bulk | MM14.PLAS.ISO.00040.003  | 40                 | 1,148   | 0.639  | 8.160   |
| MM14.02.2B4.bulk | MM14.PLAS.ISO.00040.002  | 40                 | 899   | 0.682  | 8.666   |
| MM14.02.2A1.bulk | MM14.PLAS.ISO.00040.001  | 40                 | 924   | 0.800  | 7.161   |
| MM14.06.2B1.bulk | MM14.PLAS.ISO.00138.001  | 138                | 525   | 0.010  | 0.021   |
| MM14.06.2B2.bulk | MM14.PLAS.ISO.00138.002  | 138                | 394   | 0.003  | 0.032   |
| MM14.08.2B3.bulk | MM14.PLAS.ISO.00270.001  | 270                | 447   | 0.007  | 0.050   |
| MM14.08.2B4.bulk | MM14.PLAS.ISO.00270.002  | 270                | 511   | 0.004  | 0.023   |
| MM14.10.2C1.bulk | MM14.PLAS.ISO.00519.001  | 519                | 449   | 0.004  | 0.007   |
| MM14.10.2C2.bulk | MM14.PLAS.ISO.00519.002  | 519                | 251   | 0.006  | 0.001   |
| MM14.12.1A1.bulk | MM14.PLAS.ISO.00722.001  | 722                | 414   | 0.015  | 0.060   |
| MM14.12.1A2.bulk | MM14.PLAS.ISO.00722.002  | 722                | 476   | 0.019 <sup>5</sup>                                   | 0.248 <sup>5</sup>                                |
| MM14.14.1A1.bulk | MM14.PLAS.ISO.01045.001  | 1,045              | 273   | 0.007  | 0.006   |
| MM14.14.1A2.bulk | MM14.PLAS.ISO.01045.002  | 1,045              | 215   | 0.007  | 0.003   |
| MM14.16.1B1.bulk | MM14.PLAS.ISO.01324.001  | 1,324              | 688   | 0.028  | 0.133   |
| MM14.16.1B2.bulk | MM14.PLAS.ISO.01324.002  | 1,324              | 725   | 0.032 <sup>5</sup>                                   | 0.128 <sup>5</sup>                                |
| MM14.17.1B3.bulk | MM14.PLAS.ISO.01394.001  | 1,394              | 304   | 0.011  | 0.007   |
| MM14.17.1B4.bulk | MM14.PLAS.ISO.01394.002  | 1,394              | 253   | 0.011 <sup>5</sup>                                   | 0.012 <sup>5</sup>                                |
| MM14.23.1A3.QVOA | MM14.PBMC.QVOA.03193.001 | 3,193              | 347   | 0.029  | 0.067   |
| MM15.02.2B5.bulk | MM15.PLAS.ISO.00027.002  | 27                 | 876   | 0.577 <sup>5</sup>                                   | 18.246 <sup>5</sup>                               |
| MM15.02.2A2.bulk | MM15.PLAS.ISO.00027.001  | 27                 | 904   | 0.523  | 20.371  |
| MM15.02.2A4.bulk | MM15.PLAS.ISO.00027.003  | 27                 | 489   | 0.582  | 25.291  |
| MM15.03.2B6.bulk | MM15.PLAS.ISO.00037.002  | 37                 | 729   | 0.412  | 18.431  |
| MM15.03.2A3.bulk | MM15.PLAS.ISO.00037.001  | 37                 | 571   | 0.439  | 14.984  |
| MM15.03.1B2.bulk | MM15.PLAS.ISO.00037.003  | 37                 | 387   | 0.407  | 19.592  |
| MM15.03.2B1.bulk | MM15.PLAS.ISO.00037.004  | 37                 | 389   | 0.273  | 21.927  |
| MM15.06.2A1.bulk | MM15.PLAS.ISO.00079.001  | 79                 | 603   | 0.326  | 14.380  |
| MM15.07.2A3.bulk | MM15.PLAS.ISO.00107.001  | 107                | 1,317   | 0.489  | 15.166  |
| MM15.08.2B1.bulk | MM15.PLAS.ISO.00191.001  | 191                | 668   | 0.194  | 7.782   |
| MM15.11.2B4.bulk | MM15.PLAS.ISO.00373.001  | 373                | 645   | 0.153  | 3.676   |
| MM15.13.2C1.bulk | MM15.PLAS.ISO.00548.001  | 548                | 879   | 0.247 <sup>5</sup>                                   | 9.677 <sup>5</sup>                                |
| MM15.14.2C3.bulk | MM15.PLAS.ISO.00720.001  | 720                | 589   | 0.224  | 9.385   |
| MM15.16.1B2.QVOA | MM15.PBMC.QVOA.01403.001 | 1,403              | 467   | 0.332  | 11.236  |
| MM23.01.2A3      | MM23.PLAS.ISO.00009.011  | 9                  | 142   | 1.114  |   |
| MM23.01.2C2      | MM23.PLAS.ISO.00009.012  | 9                  | 186   | 0.925  |   |
| MM23.01.11A3     | MM23.PLAS.ISO.00009.001  | 9                  | 283   | 0.635  |   |
| MM23.01.11A6     | MM23.PLAS.ISO.00009.002  | 9                  | 249   | 0.784 <sup>5</sup>                                   |   |
| MM23.01.11B5     | MM23.PLAS.ISO.00009.003  | 9                  | 232   | 0.730  |   |
| MM23.01.11C4     | MM23.PLAS.ISO.00009.004  | 9                  | 168   | 0.765 <sup>5</sup>                                   |   |
| MM23.01.13A4     | MM23.PLAS.ISO.00009.005  | 9                  | 321   | 0.708 <sup>5</sup>                                   |   |
| MM23.01.13A5     | MM23.PLAS.ISO.00009.006  | 9                  | 331   | 0.614  | 17.458  |
| MM23.01.13B3     | MM23.PLAS.ISO.00009.007  | 9                  | 524   | 0.735  | 15.166  |
| MM23.01.13C2     | MM23.PLAS.ISO.00009.008  | 9                  | 960   | 1.189  | 15.293  |
| MM23.01.13D2     | MM23.PLAS.ISO.00009.009  | 9                  | 926   | 0.891  | 14.955  |
| MM23.01.14D5     | MM23.PLAS.ISO.00009.010  | 9                  | 1,392   | 0.673  |   |
| MM23.05.2C1      | MM23.PLAS.ISO.00064.004  | 64                 | 245   | 0.542  | 14.380  |
| MM23.05.14B3     | MM23.PLAS.ISO.00064.001  | 64                 | 591   | 0.356  | 10.750  |
| MM23.05.14C3     | MM23.PLAS.ISO.00064.002  | 64                 | 218   | 0.389  | 11.426  |
| MM23.05.14D2     | MM23.PLAS.ISO.00064.003  | 64                 | 317   | 0.355  | 8.262   |
| MM23.07.1C3      | MM23.PLAS.ISO.00204.001  | 204                | 499   | 0.042  |   |
| MM23.07.2A2      | MM23.PLAS.ISO.00204.002  | 204                | 119   | 0.048  |   |
| MM23.07.2A4      | MM23.PLAS.ISO.00204.003  | 204                | 151   | 0.083  | 0.181   |
| MM23.07.2A6      | MM23.PLAS.ISO.00204.004  | 204                | 292   | 0.191  | 1.766   |
| MM23.07.2B2      | MM23.PLAS.ISO.00204.005  | 204                | 177   | 0.029  | 2.090   |
| MM23.07.2B3      | MM23.PLAS.ISO.00204.006  | 204                | 100   | 0.257  |   |
| MM23.07.2B5      | MM23.PLAS.ISO.00204.007  | 204                | 526   | 0.007  | 0.186   |
| MM23.07.2C6      | MM23.PLAS.ISO.00204.008  | 204                | 397   | 0.187  |   |
| MM23.08.13C6     | MM23.PLAS.ISO.00316.001  | 316                | 168   | 0.006  | 0.013   |
| MM23.08.21B6     | MM23.PLAS.ISO.00316.004  | 316                | 362   | 0.004  |   |
| MM23.08.21C2     | MM23.PLAS.ISO.00316.005  | 316                | 144   | 0.003  | 0.006   |
| MM23.08.22A3     | MM23.PLAS.ISO.00316.006  | 316                | 434   | 0.003  | 0.006   |
| MM23.08.22B4     | MM23.PLAS.ISO.00316.007  | 316                | 477   | 0.004  |   |
| MM23.08.22B5     | MM23.PLAS.ISO.00316.008  | 316                | 431   | 0.002  | 0.002   |
| MM23.08.22C6     | MM23.PLAS.ISO.00316.009  | 316                | 427   | 0.003  |   |
| MM23.11.1A1      | MM23.PLAS.ISO.00722.001  | 722                | 291   | 0.007  |   |
| MM23.11.1A5      | MM23.PLAS.ISO.00722.002  | 722                | 358   | 0.002  | 0.006   |
| MM23.11.1C3      | MM23.PLAS.ISO.00722.003  | 722                | 361   | 0.004  |   |
| MM23.11.1D3      | MM23.PLAS.ISO.00722.004  | 722                | 157   | 0.002  | 0.007   |

|                  |                          |       |     |                    |       |
|------------------|--------------------------|-------|-----|--------------------|-------|
| MM23.11.1D4      | MM23.PLAS.ISO.00722.005  | 722   | 165 | 0.002              | 0.004 |
| MM23.11.1D6      | MM23.PLAS.ISO.00722.006  | 722   | 128 | 0.002              | 0.008 |
| MM23.11.2B2      | MM23.PLAS.ISO.00722.007  | 722   | 403 | 0.008 <sup>5</sup> |       |
| MM23.11.2B3      | MM23.PLAS.ISO.00722.008  | 722   | 268 | 0.006 <sup>5</sup> |       |
| MM23.11.2C2      | MM23.PLAS.ISO.00722.009  | 722   | 290 | 0.009              |       |
| MM23.12.21B1     | MM23.PLAS.ISO.01065.001  | 1,065 | 300 | 0.023              | 0.011 |
| MM23.12.22A3     | MM23.PLAS.ISO.01065.002  | 1,065 | 284 | 0.033              | 0.023 |
| MM23.12.22C1     | MM23.PLAS.ISO.01065.003  | 1,065 | 231 | 0.026              | 0.073 |
| MM23.12.22C6     | MM23.PLAS.ISO.01065.004  | 1,065 | 323 | 0.032              | 0.041 |
| MM23.13.1B4      | MM23.PLAS.ISO.01534.001  | 1,534 | 335 | 0.068 <sup>5</sup> |       |
| MM23.13.2D1      | MM23.PLAS.ISO.01534.008  | 1,534 | 157 | 0.053 <sup>5</sup> | 0.246 |
| MM23.13.21A3     | MM23.PLAS.ISO.01534.002  | 1,534 | 290 | 0.045 <sup>5</sup> |       |
| MM23.13.21B5     | MM23.PLAS.ISO.01534.003  | 1,534 | 374 | 0.040 <sup>5</sup> | 0.130 |
| MM23.13.21B6     | MM23.PLAS.ISO.01534.004  | 1,534 | 272 | 0.052              | 0.256 |
| MM23.13.21D4     | MM23.PLAS.ISO.01534.005  | 1,534 | 284 | 0.038              |       |
| MM23.13.22B3     | MM23.PLAS.ISO.01534.006  | 1,534 | 302 | 0.050              |       |
| MM23.13.22C5     | MM23.PLAS.ISO.01534.007  | 1,534 | 427 | 0.027              | 0.064 |
| MM23.18.1A1.QVOA | MM23.PBMC.QVOA.02738.001 | 2,738 | 354 | 0.059              | 0.099 |
| MM23.18.2A1.QVOA | MM23.PBMC.QVOA.02738.002 | 2,738 | 284 | 0.044              | 0.046 |

|                  |                         |       |       |                    |        |
|------------------|-------------------------|-------|-------|--------------------|--------|
| MM33.01.1C1      | MM33.PLAS.ISO.00012.007 | 12    | 418   | 0.734 <sup>5</sup> |        |
| MM33.01.13C1     | MM33.PLAS.ISO.00012.001 | 12    | 1,013 | 0.820              | 13.488 |
| MM33.01.14C4     | MM33.PLAS.ISO.00012.002 | 12    | 558   | 0.394 <sup>5</sup> | 7.507  |
| MM33.01.16A5     | MM33.PLAS.ISO.00012.003 | 12    | 582   | 0.744 <sup>5</sup> | 9.406  |
| MM33.01.16C3     | MM33.PLAS.ISO.00012.004 | 12    | 1,035 | 0.739              |        |
| MM33.01.18A5     | MM33.PLAS.ISO.00012.005 | 12    | 552   | 0.581 <sup>5</sup> |        |
| MM33.01.19C4     | MM33.PLAS.ISO.00012.006 | 12    | 91    | 0.419 <sup>5</sup> | 6.764  |
| MM33.01.20D1     | MM33.PLAS.ISO.00012.008 | 12    | 1,020 | 0.295              |        |
| MM33.07.1A2      | MM33.PLAS.ISO.00096.001 | 96    | 471   | 0.150 <sup>5</sup> | 4.223  |
| MM33.07.1B6      | MM33.PLAS.ISO.00096.002 | 96    | 137   | 0.093 <sup>5</sup> | 3.217  |
| MM33.07.1D4      | MM33.PLAS.ISO.00096.003 | 96    | 367   | 0.133 <sup>5</sup> | 0.612  |
| MM33.07.2D2      | MM33.PLAS.ISO.00096.004 | 96    | 757   | 0.147 <sup>5</sup> | 2.419  |
| MM33.08.1A2      | MM33.PLAS.ISO.00201.001 | 201   | 390   | 0.019 <sup>5</sup> | 0.047  |
| MM33.08.1A3      | MM33.PLAS.ISO.00201.002 | 201   | 372   | 0.050 <sup>5</sup> | 0.047  |
| MM33.08.1A4      | MM33.PLAS.ISO.00201.003 | 201   | 320   | 0.045 <sup>5</sup> |        |
| MM33.08.1D2      | MM33.PLAS.ISO.00201.004 | 201   | 312   | 0.012 <sup>5</sup> | 0.012  |
| MM33.08.1D4      | MM33.PLAS.ISO.00201.005 | 201   | 533   | 0.057 <sup>5</sup> | 0.061  |
| MM33.08.1D5      | MM33.PLAS.ISO.00201.006 | 201   | 791   | 0.043 <sup>5</sup> |        |
| MM33.08.2A3      | MM33.PLAS.ISO.00201.007 | 201   | 436   | 0.018 <sup>5</sup> |        |
| MM33.08.2B6      | MM33.PLAS.ISO.00201.008 | 201   | 587   | 0.043 <sup>5</sup> | 0.052  |
| MM33.08.1A4.bulk | MM33.PLAS.ISO.00201.009 | 201   | 313   | 0.030              | 0.031  |
| MM33.08.1A3.bulk | MM33.PLAS.ISO.00201.010 | 201   | 344   | 0.025              | 0.016  |
| MM33.13.1D3      | MM33.PLAS.ISO.00719.004 | 719   | 681   | 0.008              | 0.006  |
| MM33.13.1D6      | MM33.PLAS.ISO.00719.005 | 719   | 244   | 0.004 <sup>5</sup> |        |
| MM33.13.2A4      | MM33.PLAS.ISO.00719.006 | 719   | 507   | 0.004              | 0.003  |
| MM33.13.2D6      | MM33.PLAS.ISO.00719.007 | 719   | 174   | 0.005 <sup>5</sup> | 0.006  |
| MM33.13.11B1     | MM33.PLAS.ISO.00719.001 | 719   | 608   | 0.003              | 0.002  |
| MM33.13.11B5     | MM33.PLAS.ISO.00719.002 | 719   | 684   | 0.009              |        |
| MM33.13.12A4     | MM33.PLAS.ISO.00719.003 | 719   | 598   | 0.003              |        |
| MM33.13.1.bulk   | MM33.PLAS.ISO.00719.008 | 719   | 121   | 0.003              | 0.006  |
| MM33.14.11A3     | MM33.PLAS.ISO.01006.001 | 1,006 | 682   | 0.009 <sup>5</sup> | 0.004  |
| MM33.14.11B1     | MM33.PLAS.ISO.01006.002 | 1,006 | 682   | 0.005              |        |
| MM33.14.11B4     | MM33.PLAS.ISO.01006.003 | 1,006 | 645   | 0.004              |        |
| MM33.14.11C6     | MM33.PLAS.ISO.01006.004 | 1,006 | 690   | 0.004              |        |
| MM33.14.12A2     | MM33.PLAS.ISO.01006.005 | 1,006 | 580   | 0.004              | 0.008  |
| MM33.14.12A4     | MM33.PLAS.ISO.01006.006 | 1,006 | 559   | 0.004 <sup>5</sup> | 0.009  |
| MM33.14.12C6     | MM33.PLAS.ISO.01006.007 | 1,006 | 639   | 0.005 <sup>5</sup> | 0.008  |
| MM33.14.1A1.bulk | MM33.PLAS.ISO.01006.008 | 1,006 | 106   | 0.004              | 0.014  |
| MM33.14.1A2.bulk | MM33.PLAS.ISO.01006.009 | 1,006 | 536   | 0.003              | 0.012  |
| MM33.17.1D2      | MM33.PLAS.ISO.01721.009 | 1,721 | 493   | 0.019              | 0.005  |
| MM33.17.1D6      | MM33.PLAS.ISO.01721.001 | 1,721 | 260   | 0.014              | 0.017  |
| MM33.17.2A4      | MM33.PLAS.ISO.01721.002 | 1,721 | 639   | 0.017 <sup>5</sup> | 0.015  |
| MM33.17.2B3      | MM33.PLAS.ISO.01721.003 | 1,721 | 140   | 0.019 <sup>5</sup> |        |
| MM33.17.2B4      | MM33.PLAS.ISO.01721.004 | 1,721 | 59    | 0.028 <sup>5</sup> | 0.007  |
| MM33.17.2B5      | MM33.PLAS.ISO.01721.005 | 1,721 | 114   | 0.017 <sup>5</sup> |        |
| MM33.17.2C3      | MM33.PLAS.ISO.01721.006 | 1,721 | 50    | 0.009              |        |
| MM33.17.2D4      | MM33.PLAS.ISO.01721.007 | 1,721 | 356   | 0.029 <sup>5</sup> | 0.003  |
| MM33.17.2D5      | MM33.PLAS.ISO.01721.008 | 1,721 | 484   | 0.008              | 0.006  |
| MM33.17.1A1.bulk | MM33.PLAS.ISO.01721.010 | 1,721 | 381   | 0.031              | 0.022  |
| MM33.17.1A2.bulk | MM33.PLAS.ISO.01721.011 | 1,721 | 615   | 0.040              | 0.019  |
| MM33.19.1A2      | MM33.PLAS.ISO.02031.001 | 2,031 | 565   | 0.015              |        |
| MM33.19.1A4      | MM33.PLAS.ISO.02031.002 | 2,031 | 331   | 0.018              |        |
| MM33.19.1B1      | MM33.PLAS.ISO.02031.003 | 2,031 | 616   | 0.017              |        |
| MM33.19.1C2      | MM33.PLAS.ISO.02031.004 | 2,031 | 274   | 0.011              | 0.089  |

|                  |                          |       |     |                    |                    |
|------------------|--------------------------|-------|-----|--------------------|--------------------|
| MM33.19.2A4      | MM33.PLAS.ISO.02031.005  | 2,031 | 244 | 0.027              | 0.047              |
| MM33.19.2B1      | MM33.PLAS.ISO.02031.006  | 2,031 | 224 | 0.016              | 0.028              |
| MM33.19.2B5      | MM33.PLAS.ISO.02031.007  | 2,031 | 689 | 0.010              | 0.133              |
| MM33.19.2C1      | MM33.PLAS.ISO.02031.008  | 2,031 | 621 | 0.018              |                    |
| MM33.19.2D2      | MM33.PLAS.ISO.02031.009  | 2,031 | 591 | 0.032              |                    |
| MM33.19.3A1      | MM33.PLAS.ISO.02031.010  | 2,031 | 220 | 0.036              |                    |
| MM33.19.3B4      | MM33.PLAS.ISO.02031.011  | 2,031 | 242 | 0.035              |                    |
| MM33.19.3D2      | MM33.PLAS.ISO.02031.012  | 2,031 | 551 | 0.024              |                    |
| MM33.19.4A5      | MM33.PLAS.ISO.02031.013  | 2,031 | 149 | 0.022              |                    |
| MM33.19.4B5      | MM33.PLAS.ISO.02031.014  | 2,031 | 655 | 0.033              |                    |
| MM33.19.4D1      | MM33.PLAS.ISO.02031.015  | 2,031 | 628 | 0.046              |                    |
| MM33.19.1B1.bulk | MM33.PLAS.ISO.02031.016  | 2,031 | 267 | 0.029              | 0.057              |
| MM34.01.1A5      | MM34.PLAS.ISO.00017.002  | 17    | 176 | 0.518              | 9.675              |
| MM34.01.1B3      | MM34.PLAS.ISO.00017.003  | 17    | 154 | 0.425              | 17.497             |
| MM34.01.1B6      | MM34.PLAS.ISO.00017.004  | 17    | 279 | 0.485              |                    |
| MM34.01.1C2      | MM34.PLAS.ISO.00017.001  | 17    | 135 | 1.485              | 17.631             |
| MM34.01.1D1      | MM34.PLAS.ISO.00017.006  | 17    | 128 | 1.094              |                    |
| MM34.01.2A4      | MM34.PLAS.ISO.00017.007  | 17    | 237 | 0.725              |                    |
| MM34.01.2B3      | MM34.PLAS.ISO.00017.008  | 17    | 343 | 0.386              |                    |
| MM34.01.2C2      | MM34.PLAS.ISO.00017.009  | 17    | 200 | 0.944              | 18.025             |
| MM34.01.2D4      | MM34.PLAS.ISO.00017.010  | 17    | 218 | 0.691              |                    |
| MM34.01.2D5      | MM34.PLAS.ISO.00017.011  | 17    | 503 | 0.821              |                    |
| MM34.06.21C6     | MM34.PLAS.ISO.00074.001  | 74    | 266 | 0.478              | 10.531             |
| MM34.08.11B5     | MM34.PLAS.ISO.00192.001  | 192   | 392 | 0.032 <sup>5</sup> | 0.168 <sup>5</sup> |
| MM34.08.11C2     | MM34.PLAS.ISO.00192.002  | 192   | 439 | 0.037 <sup>5</sup> | 3.350 <sup>5</sup> |
| MM34.08.11D3     | MM34.PLAS.ISO.00192.004  | 192   | 409 | 0.04               | 3.475 <sup>5</sup> |
| MM34.08.21A3     | MM34.PLAS.ISO.00192.005  | 192   | 624 | 0.025              | 2.088 <sup>5</sup> |
| MM34.12.21A1     | MM34.PLAS.ISO.00760.001  | 760   | 340 | 0.026              | 0.035              |
| MM34.12.21C5     | MM34.PLAS.ISO.00760.002  | 760   | 236 | 0.012              | 0.021              |
| MM34.12.21D1     | MM34.PLAS.ISO.00760.003  | 760   | 59  | 0.030              | 0.049              |
| MM34.12.21D3     | MM34.PLAS.ISO.00760.004  | 760   | 100 | 0.028              | 0.030              |
| MM34.13.11A1     | MM34.PLAS.ISO.01007.001  | 1,007 | 66  | 0.005              | 0.004              |
| MM34.13.11A2     | MM34.PLAS.ISO.01007.002  | 1,007 | 268 | 0.006              |                    |
| MM34.13.11B1     | MM34.PLAS.ISO.01007.003  | 1,007 | 154 | 0.004              | 0.001              |
| MM34.13.11C5     | MM34.PLAS.ISO.01007.004  | 1,007 | 640 | 0.017              | 0.002              |
| MM34.13.11D2     | MM34.PLAS.ISO.01007.005  | 1,007 | 143 | 0.002              | 0.001              |
| MM34.13.11D5     | MM34.PLAS.ISO.01007.006  | 1,007 | 214 | 0.007              |                    |
| MM34.14.1A4      | MM34.PLAS.ISO.01313.001  | 1,313 | 482 | 0.072              |                    |
| MM34.14.1B2      | MM34.PLAS.ISO.01313.002  | 1,313 | 389 | 0.018              |                    |
| MM34.14.1B3      | MM34.PLAS.ISO.01313.003  | 1,313 | 354 | 0.062              | 0.060              |
| MM34.14.1C6      | MM34.PLAS.ISO.01313.004  | 1,313 | 646 | 0.036              |                    |
| MM34.14.1D4      | MM34.PLAS.ISO.01313.005  | 1,313 | 478 | 0.022              | 0.124              |
| MM34.14.2A1      | MM34.PLAS.ISO.01313.006  | 1,313 | 596 | 0.041 <sup>5</sup> | 0.326 <sup>5</sup> |
| MM34.14.2B1      | MM34.PLAS.ISO.01313.007  | 1,313 | 153 |                    | 0.050              |
| MM34.15.11A1     | MM34.PLAS.ISO.01399.001  | 1,399 | 188 | 0.007              | 0.004              |
| MM34.15.11C1     | MM34.PLAS.ISO.01399.002  | 1,399 | 242 | 0.008              | 0.001              |
| MM34.15.11D3     | MM34.PLAS.ISO.01399.003  | 1,399 | 384 | 0.004 <sup>5</sup> | 0.001 <sup>5</sup> |
| MM34.16.11A5     | MM34.PLAS.ISO.01691.002  | 1,691 | 414 | 0.017              | 0.052              |
| MM34.16.11C1     | MM34.PLAS.ISO.01691.003  | 1,691 | 278 | 0.048              | 0.035              |
| MM34.16.11C3     | MM34.PLAS.ISO.01691.004  | 1,691 | 591 | 0.032              |                    |
| MM34.16.11D1     | MM34.PLAS.ISO.01691.005  | 1,691 | 413 | 0.026              | 0.045              |
| MM34.16.11D2     | MM34.PLAS.ISO.01691.006  | 1,691 | 255 | 0.056              | 0.019              |
| MM34.18.1A4      | MM34.PLAS.ISO.02229.003  | 2,229 | 619 | 0.072              |                    |
| MM34.18.1A6      | MM34.PLAS.ISO.02229.004  | 2,229 | 588 | 0.088              | 0.139              |
| MM34.18.1C5      | MM34.PLAS.ISO.02229.006  | 2,229 | 232 | 0.098              |                    |
| MM34.18.2A4      | MM34.PLAS.ISO.02229.009  | 2,229 | 274 | 0.058              | 0.466              |
| MM34.18.2A6      | MM34.PLAS.ISO.02229.010  | 2,229 | 102 | 0.106              | 0.490              |
| MM34.18.2C2      | MM34.PLAS.ISO.02229.011  | 2,229 | 258 | 0.052              |                    |
| MM34.18.2D1      | MM34.PLAS.ISO.02229.013  | 2,229 | 118 | 0.073              |                    |
| MM34.18.2D2      | MM34.PLAS.ISO.02229.014  | 2,229 | 276 | 0.062              | 0.429              |
| MM34.23.1B2.QVOA | MM34.PBMC.QVOA.02911.001 | 2,911 | 392 | 0.059              | 0.150              |
| MM34.25.3A5.QVOA | MM34.PBMC.QVOA.03261.002 | 3,261 | 303 | 0.033              | 0.204              |
| MM34.25.2B5.QVOA | MM34.PBMC.QVOA.03261.001 | 3,261 | 254 | 0.026              | 0.162              |
| MM39.02.1B4      | MM39.PLAS.ISO.00011.001  | 11    | 424 | 0.584 <sup>5</sup> | 14.857             |
| MM39.02.2A2      | MM39.PLAS.ISO.00011.002  | 11    | 180 | 0.756 <sup>5</sup> |                    |
| MM39.02.2B5      | MM39.PLAS.ISO.00011.003  | 11    | 276 | 0.700 <sup>5</sup> |                    |
| MM39.02.13B5     | MM39.PLAS.ISO.00011.004  | 11    | 437 | 0.799 <sup>5</sup> |                    |
| MM39.02.13C3     | MM39.PLAS.ISO.00011.005  | 11    | 326 | 0.796 <sup>5</sup> |                    |
| MM39.02.13D2     | MM39.PLAS.ISO.00011.006  | 11    | 275 | 0.631 <sup>5</sup> | 9.659              |
| MM39.02.13D3     | MM39.PLAS.ISO.00011.007  | 11    | 249 | 0.620              |                    |
| MM39.02.13D6     | MM39.PLAS.ISO.00011.008  | 11    | 453 | 0.731 <sup>5</sup> |                    |
| MM39.02.14C1     | MM39.PLAS.ISO.00011.010  | 11    | 637 | 0.897 <sup>5</sup> |                    |
| MM39.02.14C2     | MM39.PLAS.ISO.00011.011  | 11    | 566 | 0.787 <sup>5</sup> |                    |

|                  |                         |       |     |                    |                    |
|------------------|-------------------------|-------|-----|--------------------|--------------------|
| MM39.02.14C3     | MM39.PLAS.ISO.00011.012 | 11    | 828 | 0.767 <sup>5</sup> | 17.371             |
| MM39.02.14C5     | MM39.PLAS.ISO.00011.013 | 11    | 758 | 0.652 <sup>5</sup> | 15.713             |
| MM39.02.14D4     | MM39.PLAS.ISO.00011.014 | 11    | 132 | 0.929 <sup>5</sup> |                    |
| MM39.02.14D5     | MM39.PLAS.ISO.00011.015 | 11    | 228 | 0.850 <sup>5</sup> |                    |
| MM39.07.11A3     | MM39.PLAS.ISO.00179.001 | 179   | 288 | 0.104 <sup>5</sup> | 5.980              |
| MM39.07.11A4     | MM39.PLAS.ISO.00179.002 | 179   | 178 | 0.042              | 4.497              |
| MM39.07.11C1     | MM39.PLAS.ISO.00179.003 | 179   | 117 | 0.185 <sup>5</sup> | 3.633              |
| MM39.07.11C2     | MM39.PLAS.ISO.00179.004 | 179   | 195 | 0.140 <sup>5</sup> | 6.435              |
| MM39.10.11B3     | MM39.PLAS.ISO.00354.001 | 354   | 94  | 0.007 <sup>5</sup> |                    |
| MM39.10.11B4     | MM39.PLAS.ISO.00354.002 | 354   | 298 | 0.007 <sup>5</sup> |                    |
| MM39.10.11D1     | MM39.PLAS.ISO.00354.003 | 354   | 415 | 0.006 <sup>5</sup> |                    |
| MM39.10.11D4     | MM39.PLAS.ISO.00354.004 | 354   | 198 | 0.004 <sup>5</sup> | 0.148              |
| MM39.10.11D6     | MM39.PLAS.ISO.00354.005 | 354   | 129 | 0.007              |                    |
| MM39.10.12A2     | MM39.PLAS.ISO.00354.006 | 354   | 127 | 0.007              |                    |
| MM39.10.12A6     | MM39.PLAS.ISO.00354.007 | 354   | 143 | 0.003              |                    |
| MM39.10.12B5     | MM39.PLAS.ISO.00354.008 | 354   | 91  | 0.007              |                    |
| MM39.10.12C3     | MM39.PLAS.ISO.00354.009 | 354   | 121 | 0.004              |                    |
| MM39.10.12C4     | MM39.PLAS.ISO.00354.010 | 354   | 130 | 0.005 <sup>5</sup> | 0.137 <sup>5</sup> |
| MM39.10.12D3     | MM39.PLAS.ISO.00354.011 | 354   | 271 | 0.008              | 0.114              |
| MM39.10.12D4     | MM39.PLAS.ISO.00354.012 | 354   | 80  | 0.005              |                    |
| MM39.10.14A3     | MM39.PLAS.ISO.00354.015 | 354   | 295 | 0.003              |                    |
| MM39.10.14B4     | MM39.PLAS.ISO.00354.013 | 354   | 86  | 0.007              | 0.079              |
| MM39.10.14C5     | MM39.PLAS.ISO.00354.014 | 354   | 206 | 0.005              |                    |
| MM39.10.1C1.bulk | MM39.PLAS.ISO.00354.016 | 354   | 121 | 0.004 <sup>5</sup> | 0.214 <sup>5</sup> |
| MM39.13.1B1      | MM39.PLAS.ISO.00639.001 | 639   | 594 | 0.008              | 0.030              |
| MM39.13.11A2     | MM39.PLAS.ISO.00639.002 | 639   | 180 | 0.006              |                    |
| MM39.13.11A4     | MM39.PLAS.ISO.00639.003 | 639   | 349 | 0.007              |                    |
| MM39.13.11B1     | MM39.PLAS.ISO.00639.004 | 639   | 188 | 0.005              |                    |
| MM39.13.11B2     | MM39.PLAS.ISO.00639.008 | 639   | 124 | 0.007              | 0.007              |
| MM39.13.11C3     | MM39.PLAS.ISO.00639.005 | 639   | 379 | 0.006              | 0.031              |
| MM39.13.11C5     | MM39.PLAS.ISO.00639.006 | 639   | 167 | 0.010              | 0.023              |
| MM39.13.11D1     | MM39.PLAS.ISO.00639.007 | 639   | 247 | 0.005              |                    |
| MM39.13.1C3.bulk | MM39.PLAS.ISO.00639.009 | 639   | 66  | 0.003              | 0.022              |
| MM39.14.1A2      | MM39.PLAS.ISO.00821.001 | 821   | 107 | 0.042              | 0.005              |
| MM39.14.1A5      | MM39.PLAS.ISO.00821.002 | 821   | 339 | 0.040              | 0.004              |
| MM39.14.1B3      | MM39.PLAS.ISO.00821.003 | 821   | 547 | 0.039              |                    |
| MM39.14.2A3      | MM39.PLAS.ISO.00821.004 | 821   | 233 | 0.026              |                    |
| MM39.14.2B1      | MM39.PLAS.ISO.00821.005 | 821   | 455 | 0.035 <sup>5</sup> | 0.006 <sup>5</sup> |
| MM39.14.2B5      | MM39.PLAS.ISO.00821.007 | 821   | 318 | 0.023              | 0.020              |
| MM39.14.2D4      | MM39.PLAS.ISO.00821.006 | 821   | 93  | 0.022              |                    |
| MM39.16.1A4      | MM39.PLAS.ISO.01206.002 | 1,206 | 189 | 0.009              |                    |
| MM39.16.1B2      | MM39.PLAS.ISO.01206.003 | 1,206 | 239 | 0.024 <sup>5</sup> | 0.200              |
| MM39.16.1B4      | MM39.PLAS.ISO.01206.004 | 1,206 | 167 | 0.017 <sup>5</sup> | 0.121              |
| MM39.16.1C4      | MM39.PLAS.ISO.01206.005 | 1,206 | 107 | 0.016 <sup>5</sup> | 0.123              |
| MM39.16.2A5      | MM39.PLAS.ISO.01206.006 | 1,206 | 172 | 0.022 <sup>5</sup> | 0.192              |
| MM39.16.2C3      | MM39.PLAS.ISO.01206.001 | 1,206 | 539 | 0.020 <sup>5</sup> |                    |
| MM39.16.2D2      | MM39.PLAS.ISO.01206.007 | 1,206 | 635 | 0.018 <sup>5</sup> |                    |
| MM39.16.13A2     | MM39.PLAS.ISO.01206.013 | 1,206 | 426 | 0.068              |                    |
| MM39.16.13A5     | MM39.PLAS.ISO.01206.008 | 1,206 | 113 | 0.037              | 0.291              |
| MM39.16.13B1     | MM39.PLAS.ISO.01206.009 | 1,206 | 117 | 0.059              | 0.216              |
| MM39.16.13B3     | MM39.PLAS.ISO.01206.010 | 1,206 | 270 | 0.020              |                    |
| MM39.16.14B3     | MM39.PLAS.ISO.01206.011 | 1,206 | 183 | 0.023              |                    |
| MM39.16.14C1     | MM39.PLAS.ISO.01206.012 | 1,206 | 617 | 0.010              | 0.123              |
| MM39.16.1A1.bulk | MM39.PLAS.ISO.01206.014 | 1,206 | 80  | 0.010              | 0.205              |
| MM39.16.1A2.bulk | MM39.PLAS.ISO.01206.015 | 1,206 | 493 | 0.041              | 0.669              |
| MM39.16.1A3.bulk | MM39.PLAS.ISO.01206.016 | 1,206 | 628 | 0.046              | 0.349              |
| MM40.01.1A1      | MM40.PLAS.ISO.00011.001 | 11    | 518 | 0.712 <sup>5</sup> | 5.287              |
| MM40.01.1A2      | MM40.PLAS.ISO.00011.002 | 11    | 736 | 0.741 <sup>5</sup> |                    |
| MM40.01.1A4      | MM40.PLAS.ISO.00011.003 | 11    | 557 | 0.736 <sup>5</sup> |                    |
| MM40.01.1A5      | MM40.PLAS.ISO.00011.004 | 11    | 709 | 0.722              |                    |
| MM40.01.1A6      | MM40.PLAS.ISO.00011.005 | 11    | 654 | 0.782              |                    |
| MM40.01.1B1      | MM40.PLAS.ISO.00011.006 | 11    | 315 | 0.723 <sup>5</sup> | 5.738              |
| MM40.01.1B2      | MM40.PLAS.ISO.00011.007 | 11    | 727 | 0.999              |                    |
| MM40.01.1B3      | MM40.PLAS.ISO.00011.008 | 11    | 532 | 0.653              |                    |
| MM40.01.1B4      | MM40.PLAS.ISO.00011.009 | 11    | 132 | 0.233              |                    |
| MM40.01.1B5      | MM40.PLAS.ISO.00011.010 | 11    | 685 | 0.627              |                    |
| MM40.01.1C6      | MM40.PLAS.ISO.00011.011 | 11    | 538 | 0.528              |                    |
| MM40.01.1D1      | MM40.PLAS.ISO.00011.012 | 11    | 484 | 0.652              |                    |
| MM40.01.2A4      | MM40.PLAS.ISO.00011.013 | 11    | 629 | 0.821              |                    |
| MM40.01.2B5      | MM40.PLAS.ISO.00011.014 | 11    | 312 | 0.679              |                    |
| MM40.01.2C2      | MM40.PLAS.ISO.00011.015 | 11    | 366 | 0.681              | 7.361              |
| MM40.01.2D4      | MM40.PLAS.ISO.00011.016 | 11    | 657 | 0.722              | 7.624              |
| MM40.01.2D5      | MM40.PLAS.ISO.00011.017 | 11    | 417 | 0.782              |                    |

|                  |                          |       |       |                    |                    |
|------------------|--------------------------|-------|-------|--------------------|--------------------|
| MM40.01.2A1.bulk | MM40.PLAS.ISO.00011.018  | 11    | 1,254 | 0.732              | 11.993             |
| MM40.01.2A2.bulk | MM40.PLAS.ISO.00011.019  | 11    | 583   | 0.394              | 13.197             |
| MM40.07.11A3     | MM40.PLAS.ISO.00095.004  | 95    | 421   | 0.429              | 1.899              |
| MM40.07.11A4     | MM40.PLAS.ISO.00095.001  | 95    | 319   | 0.590              |                    |
| MM40.07.11B1     | MM40.PLAS.ISO.00095.002  | 95    | 314   | 0.750              |                    |
| MM40.07.11B3     | MM40.PLAS.ISO.00095.005  | 95    | 387   | 0.701              |                    |
| MM40.07.11B4     | MM40.PLAS.ISO.00095.003  | 95    | 287   | 0.149              |                    |
| MM40.07.11B5     | MM40.PLAS.ISO.00095.006  | 95    | 266   | 0.208              | 1.598              |
| MM40.07.11C1     | MM40.PLAS.ISO.00095.008  | 95    | 200   | 0.120              | 2.256              |
| MM40.07.12B1     | MM40.PLAS.ISO.00095.007  | 95    | 233   | 0.154              | 2.568              |
| MM40.12.13A6     | MM40.PLAS.ISO.00747.001  | 747   | 166   | 0.006              | 0.008              |
| MM40.12.13C1     | MM40.PLAS.ISO.00747.002  | 747   | 176   | 0.002              | 0.012              |
| MM40.12.13D1     | MM40.PLAS.ISO.00747.003  | 747   | 178   | 0.004              |                    |
| MM40.12.14C5     | MM40.PLAS.ISO.00747.004  | 747   | 192   | 0.006 <sup>5</sup> | 0.006              |
| MM40.12.14D2     | MM40.PLAS.ISO.00747.005  | 747   | 219   | 0.006 <sup>5</sup> | 0.007              |
| MM40.12.1A1.bulk | MM40.PLAS.ISO.00747.006  | 747   | 267   | 0.010              | 0.011              |
| MM40.12.1A2.bulk | MM40.PLAS.ISO.00747.007  | 747   | 529   | 0.003              | 0.011              |
| MM40.13.11A1     | MM40.PLAS.ISO.01166.002  | 1,166 | 289   | 0.009 <sup>5</sup> |                    |
| MM40.13.11A5     | MM40.PLAS.ISO.01166.003  | 1,166 | 237   | 0.017 <sup>5</sup> |                    |
| MM40.13.11B3     | MM40.PLAS.ISO.01166.004  | 1,166 | 208   | 0.008 <sup>5</sup> |                    |
| MM40.13.11D4     | MM40.PLAS.ISO.01166.005  | 1,166 | 238   | 0.011 <sup>5</sup> | 0.017              |
| MM40.13.12A1     | MM40.PLAS.ISO.01166.006  | 1,166 | 182   | 0.007 <sup>5</sup> | 0.025              |
| MM40.13.12B2     | MM40.PLAS.ISO.01166.007  | 1,166 | 189   | 0.006 <sup>5</sup> | 0.009              |
| MM40.13.12B5     | MM40.PLAS.ISO.01166.008  | 1,166 | 195   | 0.009 <sup>5</sup> |                    |
| MM40.13.12C2     | MM40.PLAS.ISO.01166.009  | 1,166 | 263   | 0.010 <sup>5</sup> | 0.010              |
| MM40.13.12C3     | MM40.PLAS.ISO.01166.010  | 1,166 | 251   | 0.006 <sup>5</sup> |                    |
| MM40.13.13D5     | MM40.PLAS.ISO.01166.001  | 1,166 | 162   | 0.028 <sup>5</sup> | 0.008              |
| MM40.15.1A3      | MM40.PLAS.ISO.01530.001  | 1,530 | 192   | 0.048 <sup>5</sup> |                    |
| MM40.15.1A5      | MM40.PLAS.ISO.01530.002  | 1,530 | 241   | 0.032 <sup>5</sup> |                    |
| MM40.15.1B1      | MM40.PLAS.ISO.01530.003  | 1,530 | 230   | 0.025 <sup>5</sup> | 0.034              |
| MM40.15.1B6      | MM40.PLAS.ISO.01530.004  | 1,530 | 251   | 0.042              |                    |
| MM40.15.1C1      | MM40.PLAS.ISO.01530.005  | 1,530 | 423   | 0.067 <sup>5</sup> |                    |
| MM40.15.2A2      | MM40.PLAS.ISO.01530.006  | 1,530 | 126   | 0.043 <sup>5</sup> |                    |
| MM40.15.2B1      | MM40.PLAS.ISO.01530.007  | 1,530 | 257   | 0.051 <sup>5</sup> |                    |
| MM40.15.2B5      | MM40.PLAS.ISO.01530.008  | 1,530 | 247   | 0.025 <sup>5</sup> |                    |
| MM40.15.2B6      | MM40.PLAS.ISO.01530.009  | 1,530 | 606   | 0.033 <sup>5</sup> |                    |
| MM40.15.2C1      | MM40.PLAS.ISO.01530.010  | 1,530 | 235   | 0.009 <sup>5</sup> | 0.031              |
| MM40.15.2C3      | MM40.PLAS.ISO.01530.011  | 1,530 | 465   | 0.069 <sup>5</sup> | 0.084              |
| MM40.15.2D1      | MM40.PLAS.ISO.01530.012  | 1,530 | 290   | 0.042 <sup>5</sup> |                    |
| MM40.15.2D6      | MM40.PLAS.ISO.01530.013  | 1,530 | 267   | 0.048 <sup>5</sup> | 0.035              |
| MM40.15.13B6     | MM40.PLAS.ISO.01530.014  | 1,530 | 217   | 0.041              |                    |
| MM40.15.13C1     | MM40.PLAS.ISO.01530.018  | 1,530 | 210   | 0.043              |                    |
| MM40.15.14B3     | MM40.PLAS.ISO.01530.015  | 1,530 | 212   | 0.061              | 0.159              |
| MM40.15.14C3     | MM40.PLAS.ISO.01530.016  | 1,530 | 273   | 0.034              |                    |
| MM40.15.14D5     | MM40.PLAS.ISO.01530.017  | 1,530 | 175   | 0.029              |                    |
| MM40.15.1.bulk   | MM40.PLAS.ISO.01530.020  | 1,530 | 144   | 0.090              | 0.271              |
| MM40.18.1C7.QVOA | MM40.PBMC.QVOA.02041.001 | 2,041 | 373   | 0.030              | 0.066              |
| MM55.01.1A3.bulk | MM55.PLAS.ISO.00031.001  | 31    | 210   | 0.019 <sup>5</sup> | 0.108 <sup>5</sup> |
| MM55.03.2B4.bulk | MM55.PLAS.ISO.00051.002  | 51    | 265   | 0.021 <sup>5</sup> | 0.073 <sup>5</sup> |
| MM55.03.2A1.bulk | MM55.PLAS.ISO.00051.001  | 51    | 310   | 0.081 <sup>5</sup> | 0.422 <sup>5</sup> |
| MM55.09.2A1.bulk | MM55.PLAS.ISO.00348.001  | 348   | 184   | 0.008              | 0.074              |
| MM55.11.2A3.bulk | MM55.PLAS.ISO.00661.001  | 661   | 133   | 0.002              | 0.007              |
| MM55.12.2B1.bulk | MM55.PLAS.ISO.00831.001  | 831   | 174   | 0.005 <sup>5</sup> | 0.029 <sup>5</sup> |
| MM55.12.2B2.bulk | MM55.PLAS.ISO.00831.002  | 831   | 181   | 0.007              | 0.011              |
| MM55.14.1B4.bulk | MM55.PLAS.ISO.01300.003  | 1,300 | 475   | 0.003              | 0.010              |
| MM55.14.2B5.bulk | MM55.PLAS.ISO.01300.001  | 1,300 | 477   | 0.003              | 0.010              |
| MM55.15.1A3.bulk | MM55.PLAS.ISO.01858.001  | 1,858 | 222   | 0.007              | 0.009              |
| MM55.15.1A4.bulk | MM55.PLAS.ISO.01858.002  | 1,858 | 209   | 0.005              | 0.047              |
| MM55.16.1C2.bulk | MM55.PLAS.ISO.02054.001  | 2,054 | 402   | 0.006              | 0.006              |
| MM55.16.1E1.bulk | MM55.PLAS.ISO.02054.002  | 2,054 | 513   | 0.007              | 0.004              |
| MM62.01.1A3.bulk | MM62.PLAS.ISO.00022.003  | 22    | 404   | 0.200 <sup>5</sup> | 1.723 <sup>5</sup> |
| MM62.01.1C4.bulk | MM62.PLAS.ISO.00022.001  | 22    | 227   | 0.160              | 1.614              |
| MM62.01.2C6.bulk | MM62.PLAS.ISO.00022.002  | 22    | 238   | 0.260              | 1.808              |
| MM62.02.1B1.bulk | MM62.PLAS.ISO.00057.002  | 57    | 295   | 0.090              | 0.751              |
| MM62.02.2D1.bulk | MM62.PLAS.ISO.00057.001  | 57    | 223   | 0.081              | 0.871              |
| MM62.03.1B3.bulk | MM62.PLAS.ISO.00074.001  | 74    | 255   | 0.008              | 0.014              |
| MM62.03.2D2.bulk | MM62.PLAS.ISO.00074.002  | 74    | 245   | 0.010              | 0.022              |
| MM62.04.1C1.bulk | MM62.PLAS.ISO.00120.001  | 120   | 298   | 0.005 <sup>5</sup> | 0.004 <sup>5</sup> |
| MM62.04.1D3.bulk | MM62.PLAS.ISO.00120.002  | 120   | 243   | 0.007              | 0.008              |
| MM62.04.1E5.bulk | MM62.PLAS.ISO.00120.003  | 120   | 279   | 0.007              | 0.008              |
| MM62.05.2C1.bulk | MM62.PLAS.ISO.00185.001  | 185   | 358   | 0.016 <sup>5</sup> | 0.021 <sup>5</sup> |
| MM62.05.2D5.bulk | MM62.PLAS.ISO.00185.002  | 185   | 374   | 0.030              | 0.079              |
| MM62.06.1C3.bulk | MM62.PLAS.ISO.00304.001  | 304   | 267   | 0.006              | 0.007              |

|                  |                         |       |     |                    |                    |
|------------------|-------------------------|-------|-----|--------------------|--------------------|
| MM62.08.2A1.bulk | MM62.PLAS.ISO.00658.001 | 658   | 362 | 0.009              | 0.011              |
| MM62.10.1D6.bulk | MM62.PLAS.ISO.01130.001 | 1,130 | 258 | 0.012              | 0.022              |
| MM62.10.1G6.bulk | MM62.PLAS.ISO.01130.002 | 1,130 | 246 | 0.014              | 0.018              |
| MM62.11.2B5.bulk | MM62.PLAS.ISO.01331.001 | 1,331 | 298 | 0.010              | 0.016              |
| MM62.11.2A2.bulk | MM62.PLAS.ISO.01331.002 | 1,331 | 307 | 0.015              | 0.022              |
| WEAU.02.1A1.bulk | WEAU.PLAS.ISO.0004.001  | -4    | 257 | 0.843              | 19.873             |
| WEAU.02.1A2.bulk | WEAU.PLAS.ISO.0004.002  | -4    | 464 | 0.854              | 20.646             |
| WEAU.03.1A4.bulk | WEAU.PLAS.ISO.00000.001 | 0     | 260 | 1.006              | 18.580             |
| WEAU.03.1A5.bulk | WEAU.PLAS.ISO.00000.002 | 0     | 146 | 0.972              | 17.459             |
| WEAU.08.1B1.bulk | WEAU.PLAS.ISO.00024.001 | 24    | 283 | 0.302              | 9.336              |
| WEAU.08.1B2.bulk | WEAU.PLAS.ISO.00024.002 | 24    | 295 | 0.298              | 8.530              |
| WEAU.11.1B5.bulk | WEAU.PLAS.ISO.00052.001 | 52    | 228 | 0.264              | 6.061              |
| WEAU.11.1B6.bulk | WEAU.PLAS.ISO.00052.002 | 52    | 287 | 0.215              | 4.924              |
| WEAU.14.1C1.bulk | WEAU.PLAS.ISO.00371.001 | 371   | 306 | 0.151 <sup>5</sup> | 7.139 <sup>5</sup> |
| WEAU.14.1C3.bulk | WEAU.PLAS.ISO.00371.002 | 371   | 158 | 0.302              | 4.073              |
| WEAU.15.1C4.bulk | WEAU.PLAS.ISO.00520.001 | 520   | 140 | 0.266              | 8.100              |
| WEAU.15.1C5.bulk | WEAU.PLAS.ISO.00520.002 | 520   | 121 | 0.333              | 8.758              |
| WEAU.17.1D1.bulk | WEAU.PLAS.ISO.00752.001 | 752   | 146 | 0.298              | 11.100             |
| WEAU.17.1D2.bulk | WEAU.PLAS.ISO.00752.002 | 752   | 189 | 0.353              | 7.279              |
| WEAU.18.1D4.bulk | WEAU.PLAS.ISO.00771.001 | 771   | 245 | 0.241              | 6.080              |
| WEAU.18.1D5.bulk | WEAU.PLAS.ISO.00771.002 | 771   | 178 | 0.431              | 8.211              |
| WEAU.19.1A1.bulk | WEAU.PLAS.ISO.00981.001 | 981   | 299 | 0.466              | 11.906             |
| WEAU.19.1A3.bulk | WEAU.PLAS.ISO.00981.002 | 981   | 380 | 0.533              | 11.227             |

<sup>1</sup>Virus isolate sequence identifier

<sup>2</sup>Days following onset of symptoms

<sup>3</sup>Concentration of p24 in culture supernatant at day 7 in the absence of interferon (IFN) treatment.

<sup>4</sup>Concentration of IFN $\alpha$ 2 and IFN $\beta$  that reduced virus replication by 50%.

<sup>5</sup>Average of multiple biological replicates.

**table S3. Generation of viral isolates from ART suppressed individuals with and without treatment interruption**

| Virus   | Subjects <sup>1</sup> | Gender <sup>2</sup> | Years on ART before ATI <sup>3</sup> | Sample time point                    | Viral load (copies/ml) | CD4+ T cell count (cells/ $\mu$ l) | Sample <sup>4</sup>                     | Study Interventions             | Antiretroviral Therapy <sup>5</sup> | Number of virus isolates <sup>6</sup> | Study Number | References                              |
|---------|-----------------------|---------------------|--------------------------------------|--------------------------------------|------------------------|------------------------------------|---|---------------------------------|-------------------------------------|---------------------------------------|--------------|---|
| QVOA    | B199                  | M                   | 4                                    | second leukapheresis                 | <20                    | 200                                | QVOA Supernatant                        | none                            | TDF/FTC, RAL                        | 1                                     | MNU-0628     | Lorenzi et al., 2016                    |
| QVOA    | B106                  | M                   | 7                                    | first and second leukapheresis       | <20                    | 390                                | QVOA Supernatant                        | none                            | TDF/FTC/RPV                         | 2                                     |              |   |
| QVOA    | 9241                  | M                   | 5                                    | during ATI (12 weeks) before rebound | <20                    | 709                                | QVOA Supernatant                        | 10-1074 and 3BNC117             | EVG/cobi/TDF/FTC                    | 1                                     | NCT02825797  | Mendoza et al., 2018                    |
| QVOA    | 9241                  | M                   | 5                                    | 2 weeks pre ATI                      | <20                    |                                    | QVOA Supernatant                        | 10-1074 and 3BNC117             | EVG/cobi/TDF/FTC                    | 4                                     |              |   |
| QVOA    | 9242                  | M                   | 2                                    | during ATI (12 weeks) before rebound | <20                    | 967                                | QVOA Supernatant                        | 10-1074 and 3BNC117             | EVG/cobi/TDF/FTC                    | 2                                     |              |   |
| QVOA    | 9243                  | M                   | 5                                    | during ATI (12 weeks) before rebound | <20                    | 545                                | QVOA Supernatant                        | 10-1074 and 3BNC117             | RPV/TDF/FTC                         | 1                                     |              |   |
| QVOA    | 9244                  | M                   | 5                                    | 2 weeks pre ATI                      | <20                    | 796                                | QVOA Supernatant                        | 10-1074 and 3BNC117             | EFV/TDF/FTC                         | 4                                     |              |   |
| QVOA    | 9244                  | M                   | 5                                    | during ATI (12 weeks) before rebound | <20                    | 796                                | QVOA Supernatant                        | 10-1074 and 3BNC117             | EFV/TDF/FTC                         | 2                                     |              |   |
| Rebound | 9241                  | M                   | 5                                    | 22 weeks post ATI                    | 13,250                 | 500                                | Plasma                                  | 10-1074 and 3BNC117             | EVG/cobi/TDF/FTC                    | 1                                     |              |   |
| Rebound | 9242                  | M                   | 2                                    | 16 weeks post ATI                    | 65,520                 | 450                                | Plasma                                  | 10-1074 and 3BNC117             | EVG/cobi/TDF/FTC                    | 5                                     |              |   |
| Rebound | 9243                  | M                   | 5                                    | 21 weeks post ATI                    | 10,110                 | 350                                | Plasma                                  | 10-1074 and 3BNC117             | RPV/TDF/FTC                         | 1                                     |              |   |
| Rebound | 9244                  | M                   | 5                                    | 22 weeks post ATI                    | 120,330                | 730                                | Plasma                                  | 10-1074 and 3BNC117             | EFV/TDF/FTC                         | 5                                     |              |   |
| QVOA    | A06                   | M                   | >3.6                                 | 2.5 weeks pre ATI                    | <50                    | >470                               | QVOA Supernatant and cryopreserved PBMC | VRC01                           | EVG/cobi/TDF/FTC                    | 6                                     | NCT02463227  | Bar et al., 2016; Salantes et al., 2018 |
| QVOA    | A06                   | M                   | >3.6                                 | 10.5 months post ATI                 | <50                    |                                    | QVOA Supernatant                        | VRC01                           | EVG/cobi/TDF/FTC                    | 3                                     |              |   |
| QVOA    | A08                   | M                   | >3.6                                 | 4 weeks pre ATI                      | <50                    | >470                               | QVOA Supernatant                        | VRC01                           | DTG, TDF/FTC                        | 4                                     |              |   |
| QVOA    | A08                   | M                   | >3.6                                 | 11 months post ATI                   | <50                    |                                    | QVOA Supernatant and cryopreserved PBMC | VRC01                           | DTG, TDF/FTC                        | 16                                    |              |   |
| QVOA    | A09                   | M                   | >3.6                                 | 1 week pre ATI                       | <50                    | >470                               | QVOA Supernatant                        | VRC01                           | rtv/DRV, TDF/FTC                    | 4                                     |              |   |
| QVOA    | A09                   | M                   | >3.6                                 | 12 months post ATI                   | <50                    |                                    | QVOA Supernatant                        | VRC01                           | rtv/DRV, TDF/FTC                    | 2                                     |              |   |
| Rebound | A09                   | M                   | >3.6                                 | 3 weeks post ATI                     | 2,330                  | >350                               | Plasma                                  | VRC01                           | rtv/DRV, TDF/FTC                    | 8                                     |              |   |
| Rebound | A08                   | M                   | >3.6                                 | 5 weeks post ATI                     | 20,000                 | >350                               | Plasma                                  | VRC01                           | DTG, TDF/FTC                        | 17                                    |              |   |
| Rebound | S22                   | M                   | 8                                    | during 2nd ATI                       | 750,000                | 449                                | Plasma                                  | none                            | NFL, ddi, NVP                       | 5                                     | NCT00051818  | Papasavvas et al., 2004                 |
| Rebound | S23                   | M                   | 5                                    | during 2nd ATI                       | 556,372                | 351                                | Plasma                                  | none                            | ddi, d4T, EFZ                       | 2                                     |              |   |
| Rebound | S30                   | M                   | 5                                    | during 3rd ATI                       | 517,473                | 467                                | Plasma                                  | none                            | ABC, d4T, 3TC, EFZ                  | 4                                     |              |   |
| Rebound | 004                   | M                   | >1                                   | 4 weeks post ATI (week 12 of study)  | 33,812                 | 794                                | Plasma                                  | Pegintron (Peg-IFN $\alpha$ 2b) | rtv/ATV, FTC/TDF                    | 4                                     | NCT02227277  | Azzoni et al, 2019                      |
| Rebound | 030                   | M                   | >1                                   | 4 weeks post ATI (week 12 of study)  | 18,056                 | 377                                | Plasma                                  | Pegintron (Peg-IFN $\alpha$ 2b) | EFV/FTC/TDF                         | 4                                     |              |   |
| Rebound | 044                   | F                   | >1                                   | 4 weeks post ATI (week 12 of study)  | 20,171                 | 496                                | Plasma                                  | Pegintron (Peg-IFN $\alpha$ 2b) | ABC/3TC, DTG                        | 4                                     |              |   |
| Rebound | 601                   | M                   | 20                                   | 5 weeks post ATI (week 29 of study)  | 112,160                | 707                                | Plasma                                  | 3BNC117                         | LOP/rtv, ABC/3TC                    | 6                                     | NCT02588586  | Cohen et al., 2018                      |

<sup>1</sup>All participants were recruited in the US and had subtype B infections, except for subject A09, who also harbored a subtype A strain (only subtype B viruses were identified in rebound plasma).

<sup>2</sup>M, male; F, female.

<sup>3</sup>ATI, analytical treatment interruption.

<sup>4</sup>QVOA, quantitative viral outgrowth assay.

<sup>5</sup>EVG - Elvitegravir, cobi - cobicistat, TDF - Tenofovir Disoproxil Fumarate, FTC - Emtricitabine, DRV - Darunavir, RPV - Rilpivirine, EFZ - Efavirenz, RAL - Raltegravir, rtv - ritonavir, ABC - Abacavir, NLF - Nelfinavir, ddi - Didanosine, NVP - Nevirapine, d4T - Stavudine, ATV - Atazanavir, DTG - dolutegravir, 3TC - lamivudine, LOP - Lopinavir. co-formulated pills are indicated with slashes, separate pills by commas. Boosting agents with lower case font.

<sup>6</sup>Most viral isolates were single virion-derived; GenBank accession number are shown in Data file S1.

table S4. IFN-I resistance of viral outgrowth and rebound isolates

| Type <sup>1</sup>           | Isolate ID <sup>2</sup>     | Study number | Replicative capacity (ng p24/ml) <sup>3</sup> | IFN $\alpha$ 2 IC <sub>50</sub> (pg/ml) <sup>4</sup> | IFN $\beta$ IC <sub>50</sub> (pg/ml) <sup>4</sup> |
|-----------------------------|-----------------------------|--------------|---|--|---|
| QVOA                        | B199.2.C.BF.1               | MNU-0628     | 80  | 0.04   | 0.12  |
| QVOA                        | B106.1.C.3.8 <sup>6</sup>   |              | 100   | 0.18   | 3.12  |
| QVOA                        | B106.2.C.D6.S8 <sup>6</sup> |              | 195   | 0.16   | 1.96  |
| QVOA - week 12 <sup>5</sup> | 9241.QVOA.12AU7             | NCT02825797  | 360   | 0.03   | 0.25  |
| QVOA - week -2 <sup>5</sup> | 9241.QVOA.D2                |              | 445   | 0.02   | 0.09  |
| QVOA - week -2 <sup>5</sup> | 9241.QVOA.A10               |              | 315   | 0.04   | 0.13  |
| QVOA - week -2 <sup>5</sup> | 9241.QVOA.C19               |              | 282   | 0.04   | 0.09  |
| QVOA - week -2 <sup>5</sup> | 9241.QVOA.C8                |              | 278   | 0.04   | 0.10  |
| QVOA - week 12 <sup>5</sup> | 9242.QVOA.12F23             |              | 386   | 0.02   | 0.04  |
| QVOA - week 12 <sup>5</sup> | 9242.QVOA.12M6              |              | 641   | 0.01   | 0.01  |
| QVOA - week 12 <sup>5</sup> | 9243.QVOA.12Y10             |              | 679   | 0.02   | 0.04  |
| QVOA - week 12 <sup>5</sup> | 9244.QVOA.12I10             |              | 492   | 0.02   | 0.04  |
| QVOA - week 12 <sup>5</sup> | 9244.QVOA.12J17             |              | 483   | 0.02   | 0.09  |
| QVOA - week -2 <sup>5</sup> | 9244.QVOA.G12               |              | 694   | 0.01   | 0.10  |
| QVOA - week -2 <sup>5</sup> | 9244.QVOA.H1                |              | 496   | 0.02   | 0.06  |
| QVOA - week -2 <sup>5</sup> | 9244.QVOA.K2                |              | 404   | 0.02   | 0.15  |
| QVOA - week -2 <sup>5</sup> | 9244.QVOA.P11               |              | 423   | 0.02   | 0.11  |
| Rebound                     | 9241.REB.7D4                |              | 626   | 1.62   | 82.64   |
| Rebound                     | 9242.REB.6B8                |              | 299   | 1.13   | 45.04   |
| Rebound                     | 9242.REB.6A7                |              | 309   | 1.05   | 21.99   |
| Rebound                     | 9242.REB.6B2                |              | 286   | 1.93   | 30.49   |
| Rebound                     | 9242.REB.13B1               |              | 547   | 1.26   | 61.88   |
| Rebound                     | 9242.REB.13B8               |              | 418   | 1.70   | 58.24   |
| Rebound                     | 9243.REB.13F7               |              | 554   | 1.56   | 49.26   |
| Rebound                     | 9244.REB.7A8                |              | 499   | 1.53   | 54.25   |
| Rebound                     | 9244.REB.7A1                |              | 459   | 1.59   | 31.67   |
| Rebound                     | 9244.REB.7C1                |              | 567   | 1.72   | 34.43   |
| Rebound                     | 9244.REB.9D3                |              | 578   | 1.35   | 48.06   |
| Rebound                     | 9244.REB.9E6                | 535          | 1.70  | 77.03  |   |
| QVOA - Pre ATI              | A06.QVOA.M5                 | NCT02463227  | 139   | 0.15   | 1.70  |
| QVOA - Pre ATI              | A06.QVOA.M10                |              | 144   | 0.11   | 1.57  |
| QVOA - Pre ATI              | A06.QVOA.M30                |              | 158   | 0.09   | 1.81  |
| QVOA - Pre ATI              | A06.QVOA.M28                |              | 97  | 0.09   | 1.17  |
| QVOA - Pre ATI              | A06.QVOA.M19                |              | 150   | 0.08   | 1.40  |
| QVOA - Pre ATI              | A06.QVOA.M29                |              | 124   | 0.07   | 2.99  |
| QVOA - Post ATI             | A06.QVOA.2B                 |              | 143   | 0.07   | 0.85  |
| QVOA - Post ATI             | A06.QVOA.M20                |              | 126   | 0.09   | 1.67  |
| QVOA - Post ATI             | A06.QVOA.M3                 |              | 117   | 0.09   | 1.10  |
| QVOA - Pre ATI              | A08.QVOA.1E2                |              | 99  | 0.04   | 0.32  |
| QVOA - Pre ATI              | A08.QVOA.M1                 |              | 1548  | 0.02   | 0.01  |
| QVOA - Pre ATI              | A08.QVOA.M4                 |              | 965   | 0.01   | 0.04  |
| QVOA - Pre ATI              | A08.QVOA.M5                 |              | 665   | 0.01   | 0.10  |
| QVOA - Post ATI             | A08.QVOA.M17                |              | 977   | 0.01   | 0.60  |
| QVOA - Post ATI             | A08.QVOA.M20                |              | 620   | 0.01   | 0.08  |
| QVOA - Post ATI             | A08.QVOA.1B5                |              | 122   | 0.03   | 0.90  |
| QVOA - Post ATI             | A08.QVOA.1C8                |              | 152   | 0.03   | 0.08  |
| QVOA - Post ATI             | A08.QVOA.1F8                |              | 163   | 0.03   | 0.07  |
| QVOA - Post ATI             | A08.QVOA.3D8                |              | 65  | 1.47   | 5.17  |
| QVOA - Post ATI             | A08.QVOA.4A1                |              | 103   | 0.02   | 0.15  |
| QVOA - Post ATI             | A08.QVOA.4E4                |              | 120   | 0.02   | 0.09  |
| QVOA - Post ATI             | A08.QVOA.5C3                |              | 83  | 0.03   | 0.39  |
| QVOA - Post ATI             | A08.QVOA.5E2                |              | 142   | 0.02   | 0.03  |
| QVOA - Post ATI             | A08.QVOA.5E4                |              | 119   | 0.04   | 0.49  |
| QVOA - Post ATI             | A08.QVOA.6D3                |              | 120   | 0.03   | 0.04  |
| QVOA - Post ATI             | A08.QVOA.6E2                |              | 86  | 0.02   | 0.08  |
| QVOA - Post ATI             | A08.QVOA.6F6                |              | 66  | 1.23   | 8.24  |
| QVOA - Post ATI             | A08.QVOA.7F8                |              | 150   | 0.04   | 0.14  |
| QVOA - Post ATI             | A08.QVOA.8E8                |              | 50  | 0.03   | 0.03  |
| Rebound                     | A08.REB.1A3                 |              | 451   | 1.06   | 83.13   |
| Rebound                     | A08.REB.1D1                 |              | 496   | 1.45   | 51.37   |
| Rebound                     | A08.REB.5C2                 |              | 125   | 1.22   | 80.62   |

|                 |              |             |     |      |        |
|-----------------|--------------|-------------|-----|------|--------|
| Rebound         | A08.REB.5D2  |             | 250 | 1.44 | 45.44  |
| Rebound         | A08.REB.6B3  |             | 125 | 0.85 | 55.84  |
| Rebound         | A08.REB.6D6  |             | 728 | 1.22 | 46.83  |
| Rebound         | A08.REB.7A3  |             | 504 | 1.51 | 75.85  |
| Rebound         | A08.REB.7C1  |             | 707 | 1.43 | 68.06  |
| Rebound         | A08.REB.7C2  |             | 176 | 2.07 | 44.85  |
| Rebound         | A08.REB.7D3  |             | 309 | 1.65 | 38.78  |
| Rebound         | A08.REB.8A5  |             | 768 | 1.59 | 41.98  |
| Rebound         | A08.REB.8B3  |             | 763 | 1.61 | 41.17  |
| Rebound         | A08.REB.8D1  |             | 425 | 1.33 | 31.24  |
| Rebound         | A08.REB.6B5  |             | 510 | 2.28 | 64.49  |
| Rebound         | A08.REB.8B5  |             | 510 | 1.94 | 69.78  |
| Rebound         | A08.REB.2F4  |             | 420 | 3.01 | 92.25  |
| Rebound         | A08.REB.1A5  |             | 506 | 1.69 | 42.34  |
| QVOA - Pre ATI  | A09.QVOA.M25 |             | 194 | 0.26 | 1.29   |
| QVOA - Pre ATI  | A09.QVOA.M22 |             | 206 | 0.05 | 0.19   |
| QVOA - Pre ATI  | A09.QVOA.M2  |             | 254 | 0.05 | 0.46   |
| QVOA - Pre ATI  | A09.QVOA.M6  |             | 171 | 0.06 | 0.70   |
| QVOA - Post ATI | A09.QVOA.M9  |             | 171 | 0.21 | 0.06   |
| QVOA - Post ATI | A09.QVOA.M16 |             | 285 | 0.05 | 0.69   |
| Rebound         | A09.REB.1B5  |             | 275 | 3.16 | 58.67  |
| Rebound         | A09.REB.1D5  |             | 101 | 2.19 | 51.20  |
| Rebound         | A09.REB.2B2  |             | 171 | 2.79 | 26.36  |
| Rebound         | A09.REB.1A1  |             | 376 | 2.40 | 46.03  |
| Rebound         | A09.REB.1A3  |             | 227 | 1.78 | 58.57  |
| Rebound         | A09.REB.1A4  |             | 239 | 2.48 | 70.36  |
| Rebound         | A09.REB.1A5  |             | 249 | 2.55 | 61.27  |
| Rebound         | A09.REB.1A2  |             | 221 | 2.54 | 83.45  |
| Rebound         | S22.REB.24C4 |             | 399 | 1.64 | 90.59  |
| Rebound         | S22.REB.1E2  |             | 389 | 1.03 | 61.14  |
| Rebound         | S22.REB.1F5  |             | 374 | 1.62 | 93.32  |
| Rebound         | S22.REB.3A6  |             | 487 | 1.45 | 97.27  |
| Rebound         | S22.REB.3B6  |             | 378 | 1.00 | 75.65  |
| Rebound         | S23.REB.3D1  | NCT00051818 | 358 | 1.70 | 66.56  |
| Rebound         | S23.REB.24A5 |             | 475 | 1.25 | 76.30  |
| Rebound         | S30.REB.8D1  |             | 407 | 1.21 | 84.20  |
| Rebound         | S30.REB.2D1  |             | 303 | 2.14 | 87.20  |
| Rebound         | S30.REB.2D2  |             | 350 | 1.54 | 70.82  |
| Rebound         | S30.REB.7D1  |             | 336 | 1.72 | 72.69  |
| Rebound         | 004.REB.4D4  |             | 413 | 2.58 | 93.58  |
| Rebound         | 004.REB.4E6  |             | 263 | 2.54 | 63.21  |
| Rebound         | 004.REB.4D1  |             | 259 | 2.65 | 97.87  |
| Rebound         | 004.REB.4E3  |             | 252 | 5.55 | 111.80 |
| Rebound         | 030.REB.3E1  |             | 371 | 2.99 | 56.49  |
| Rebound         | 030.REB.2E1  |             | 396 | 2.84 | 47.24  |
| Rebound         | 030.REB.4E1  | NCT02227277 | 427 | 3.48 | 70.43  |
| Rebound         | 030.REB.8E1  |             | 308 | 2.84 | 69.21  |
| Rebound         | 044.REB.6F1  |             | 283 | 3.42 | 79.99  |
| Rebound         | 044.REB.5F1  |             | 348 | 2.75 | 69.41  |
| Rebound         | 044.REB.7F1  |             | 329 | 2.80 | 72.71  |
| Rebound         | 044.REB.8F1  |             | 355 | 2.48 | 91.45  |
| Rebound         | 601.REB.4B7  |             | 497 | 2.15 | 64.85  |
| Rebound         | 601.REB.4C3  |             | 483 | 2.03 | 33.29  |
| Rebound         | 601.REB.4B4  |             | 468 | 2.17 | 65.81  |
| Rebound         | 601.REB.4A8  | NCT02588586 | 546 | 1.81 | 42.24  |
| Rebound         | 601.REB.4A7  |             | 562 | 2.25 | 60.84  |
| Rebound         | 601.REB.4C1  |             | 536 | 2.02 | 54.66  |

<sup>1</sup>Isolates were obtained by expanding previously generated quantitative viral outgrowth assay (QVOA) supernatants, or generated *de novo* from cryopreserved PBMCs or plasma samples (61-67).

<sup>2</sup>For GenBank accession numbers see Data file S1.

<sup>3</sup>Concentration of p24 in culture supernatant at day 7 in the absence of IFN treatment.

<sup>4</sup>Dose of IFN that reduced viral replication in CD4+ T cells by 50%.

<sup>5</sup>Samples from the NCT02825797 study were collected before (-2 week) and during (12 week) treatment interruption, but prior to any detectable rebound virus.

<sup>6</sup>B106.1.C.3.8 was isolated from the first and 106.2.C.D6.S8 from the second leukapheresis sample.