1 **Title: Dosing interval is a major factor determining the quality of T cells induced**
2 **by SARS-CoV-2 mRNA and adenoviral vector vaccines**

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26 **Abstract:**

27 Functional T cell responses are crucial for protective immunity induced by COVID-19
28 vaccination, but factors influencing the quality of these responses are incompletely
29 understood. We employed an activation induced marker (AIM) assay and single-cell

transcriptomic sequencing to analyze SARS-CoV-2 spike-responsive T cells following mild SARS-CoV-2 infection or following one or two doses of mRNA-LNP or adenoviral vectored COVID-19 vaccines. Our findings revealed broad functional and clonal heterogeneity in T cells generated by vaccination or infection, including multiple distinct effector populations. T cell function was largely conserved between COVID-19 vaccine platforms but was distinct compared to SARS-CoV-2 infection. Notably, the dosing interval greatly influenced the quality of T cells after two vaccine doses, particularly after mRNA-LNP vaccination, where a longer interval led to reduced inflammatory signaling and increased secondary proliferation. These insights enhance our understanding of SARS-CoV-2 specific T cells and inform the optimization of mRNA vaccination regimens.

One Sentence Summary:

Antigen-specific T cell functionality is dependent on dose-interval between COVID-19 vaccines, particularly mRNA-LNP vaccination.

45 **Main Text:**

46 **INTRODUCTION**

47 In addition to binding antibody titers (*1*), T cell responses are correlated with protection
48 from severe COVID-19 (*2*). But fewer studies have investigated T cell responses to
49 COVID-19 mRNA-LNP and adenoviral vector vaccines encoding SARS-CoV-2 spike
50 antigens compared with those looking at antibodies. The complexity and heterogeneity
51 of the T cell response following COVID-19 vaccination remains largely unexplored.
52 While often referred to as a single aggregate response, T cell responses represent the
53 activation of multiple heterogeneous cell subsets (i.e., $CD8^+$, $CD4^+$, T_H1, T_H2, T_H17, T_{FH}
54 and T_{reg}) with diverse effector functions (e.g., cytokine production, cytotoxicity, cell-cell
55 contact-dependent functions)(*3*). Thus, detailed studies are required to capture the
56 multiple facets of an effective T cell response.

57 Enumeration of IFN-γ^+ T cells, by ELISpot or intracellular cytokine staining (ICS), is the
58 most widely used measure of vaccine-induced T cell responses and has provided key
59 insights into differences in the immunogenicity of vaccine platforms and in the intervals
60 between vaccine doses. In randomized clinical trials (RCTs), ChAdOx1 nCoV-19
61 induced stronger T cell responses after the priming dose than BNT162b2, but two of
62 three trials reported higher responses with BNT162b2 following a booster (i.e., second)
63 vaccination (*4-6*). Observational studies have also shown higher magnitude T cell
64 responses after one or two doses of adenovirus vector vaccines (ChAdOx1 or Ad26)
65 compared with mRNA-LNP (BNT162b2 or mRNA-1273) vaccination (*7-9*). These
66 studies indicate differences in the quantity of SARS-CoV-2 spike-specific T cells but
67 crucially lack insights into the quality or multi-faceted functionality of these T cells.

68 Along with vaccine platform, the interval between vaccine doses has a demonstrable
69 impact on vaccine-induced T cell responses. To ensure maximum delivery of the first
70 COVID-19 vaccine across the population and based on evidence of improved vaccine
71 immunogenicity in early clinical trial data, the UK and Canada implemented an
72 “extended” interval between the first and second dose of mRNA-LNP vaccination. This
73 increased the dosing interval from the RCT-defined 3–4-week interval (short interval) to
74 an 8–12-week interval (long interval). Both dosing intervals were found to provide high

efficacy in population-level studies (*10*) and RCTs (*11*), with equivalent or increased efficacy in long compared with short dosing intervals in both vaccine types (*12, 13*). Regardless of vaccine type, RCTs and observational cohort studies showed that increased dosing interval marginally decreased the frequency of IFN-γ^+ T cells (*5, 14, 15*). In contrast, using mRNA-LNP vaccines in an extended prime/boost dosing regimen markedly enhanced humoral immunity (*5, 6, 14, 15*), highlighting a disconnect between T cell and antibody responses. Nevertheless, the frequency of IFN-γ^+ T cells is only a single measure of cellular immunity, and further work is required to broaden these results to the full diversity of T cell functionality.

Some studies have begun to address this knowledge gap. Assessment of T cell function using multi-parameter ICS has demonstrated that ChAdOx1 and mRNA-LNP vaccines induce predominantly T_H1-biased CD4$^+$ T cell responses (*4, 16*). However, the ICS assay likely underestimates T cell responses and is limited by its ability to capture only specific cytokine-producing cells with relatively limited functional breadth. Activation-induced marker (AIM) assays have emerged as an approach to quantify the frequency of antigen-specific CD4$^+$ and CD8$^+$ T cells and overcomes limitations in ICS by detecting T cells independent of their cytokine-producing functionality. In addition, the AIM assay can be used to sort live, antigen-responsive T cells for further analysis using flow cytometry. This can be done in a manner agnostic to HLA-type or precise *a priori* knowledge of specific target epitopes, challenges that limit the use of HLA-tetramers to sort antigen-specific T cells. In combination with transcriptomic analysis, the AIM assay may therefore be used to deeply phenotype polyclonal antigen-responsive T cells in various biological settings. This approach has been used previously to reveal lasting transcriptional differences in SARS-CoV-2 responsive T cells over six months after mRNA vaccination (*17*) and demonstrated skewing toward a hyper-effector phenotype after mRNA vaccination in X-linked agammaglobulinemia patients (*18*), thus highlighting its utility in profiling the functional diversity of antigen-responsive T cells.

In this work, we sought to broaden our understanding of how vaccine type and the interval between vaccine doses impacts on the functionality, transcriptional state, and diversity of CD4$^+$ and CD8$^+$ T cell responses induced by vaccination. To accomplish

105 this, we performed multi-modal single-cell RNA, surface protein, and TCR sequencing
106 on sorted AIM$^+$ ("AIM-seq") SARS-CoV-2 spike-reactive T cells using peripheral blood
107 samples taken after one or two doses of BNT162b2 or ChAdOx1 nCoV-19 vaccination.
108 To compare the observed vaccine-induced responses with immune responses following
109 SARS-CoV-2 infection (ancestral strain), we sampled individuals after mild COVID-19 at
110 two timepoints that were analogous to the post-dose one vaccine timepoint for the short
111 or long interval. We show that AIM$^+$ T cell populations induced after exposure to a
112 SARS-CoV-2 spike antigen through vaccination or infection are functionally diverse,
113 including effector populations not usually identified, thus validating the power of an
114 unbiased transcriptomic approach. Whereas vaccine type had an unexpectedly
115 nuanced impact on the phenotype and function of responding T cells, interval between
116 doses had a more marked effect that was dependent on the vaccine platform.
117 Collectively, these data provide insight into the heterogeneity of the effector T cell
118 responses generated by SARS-CoV-2 infection and vaccination and highlight the impact
119 of dosing interval on T cell functionality.

120 RESULTS

121 Study population

122 To assess the impact of vaccine modality, number of vaccine doses given, and interval
123 between doses on T cell phenotype and function, we sampled individuals with no history
124 of symptomatic COVID-19 vaccinated with ChAdOx1 nCoV-19 ("ChAd") or BNT162b2
125 ("BNT") with a short or long dosing interval immediately before ("T1-S" or "T1-L",
126 respectively) and 28-days after ("T2") their second vaccine dose (**Fig. 1A**). Also
127 included were a group of unvaccinated individuals with mild COVID-19, sampled
128 approximately 28 days and 56 days after SARS-CoV-2 PCR positivity ("COVID"),
129 corresponding to the T1 timepoint for the short ("T1-S") and long ("T1-L") vaccine
130 groups respectively. Participants in the BNT groups and ChAd long-interval group were
131 also included in (*19*). Groups were broadly balanced for age and sex (**Table S1**).

132 To capture SARS-CoV-2 spike peptide-reactive T cell responses we used an activation-
133 induced marker (AIM) assay (**Fig. 1B**). AIM markers were CD69 combined with OX-40
134 and/or 4-1BB. On average, 2% of CD3$^+$ T cells were AIM$^+$ (**Fig. 1C**), with little difference

135 between study groups or timepoints (**Fig. S1A**). Median percentages of $CD4^+$, $CD8^+$ and
136 CD4/CD8 double negative (DN) AIM^+ T cells identified by flow cytometry were 86%, 7%
137 and 6%, respectively (**Fig. S1B**). All AIM^+ $CD3^+$ T cells were isolated by flow cytometric
138 cell sorting for multi-modal (RNA, TCR and eight selected cell-surface phenotyping and
139 AIM protein markers) single-cell sequencing, with the largest numbers of AIM^+ $CD3^+$ T
140 cells obtained from BNT vaccinees (**Fig. S1C**).

141 **AIM^+ T cells elicited by COVID-19 vaccines display diverse phenotypes**

142 After sequencing and removal of low-quality cells, the final dataset of T1 and T2 AIM^+ T
143 cells contained 128,017 cells (**Fig. 1D**). Clustering and manual annotation (**Methods**)
144 identified 18 clusters: 11 were identified as $CD4^+$ T cells, two as $CD8^+$ T cells, two as
145 mucosal-associated invariant T (MAIT) cells, and three as γδT cells (**Fig. 1E**). All
146 vaccine types, both timepoints and the majority (80%) of donors contributed to all
147 clusters (**Fig. S1D**). $CD4^+$ T cell clusters comprised multiple populations with clear
148 parallels to known polarized subsets, including a hybrid T_H1/T_{FH} cluster (CD4_Th1_Tfh),
149 a hybrid T_H1/T_H17 cluster (CD4_Th1_Th17), a T_H2 cluster (CD4_Th2), and a T_{reg} cluster
150 (CD4_Treg) (**Fig. 1D-F**). Several clusters exhibited phenotypes not associated with T_H
151 polarization, including a cytotoxic population (CD4_cytotoxic) and memory states
152 (CD4_Tcm and CD4_Tcm2). Finally, four clusters of $CD4^+$ T cells were defined by
153 distinctive expression of specific markers (*TGFB1,* interferon stimulated genes [ISGs],
154 HLA molecules, and *ZBTB16* [encodes PLZF], respectively) and could not be easily
155 assigned to known $CD4^+$ populations (CD4_TGFB1, CD4_ISG, CD4_HLA, and
156 CD4_PLZF, respectively). The two clusters of $CD8^+$ T cells corresponded to activated
157 IFN-γ-producing effector $CD8^+$ T cells (CD8_IFNG) and more quiescent effector
158 memory T_{EM} cells (CD8_Tem) (**Fig. 1D-F**). There was strong positive correlation
159 between flow cytometry and single-cell derived CD4, CD8 and unconventional T cell
160 subsets (**Fig. S1E**). Flow cytometry-based phenotyping of AIM^+ T cells aligned with the
161 diverse T cell populations identified by AIM-seq (**Fig. S2 and S3**).

162 **Baseline AIM^+ T cells have defined phenotypic and transcriptional characteristics**

163 In the conventional AIM assay, background subtraction of the small fraction of T cells
164 that are AIM^+ at rest gives a very good signal-to-noise ratio (*20-22*). However, these

165 cells cannot be excluded from sorting gates when isolating cells by flow cytometric cell
166 sorting. Thus, we sought to identify sources of background in the AIM assay.
167 Unstimulated PBMCs identified biased enrichment of specific T cell populations within
168 the AIM^+ population (**Fig. S3**). Notably, the background was impacted by duration of
169 stimulation and many populations declined in frequency with 24-hours compared to 12-
170 hours of incubation. Unconventional cells, and particularly $V\delta2^+$ γδT cells, were
171 particularly notable in this regard (**Fig. S3C and S3D**). Only AIM^+ T_{reg}s and naïve $CD4^+$
172 T cells significantly increased in absolute frequency with extended incubation in the
173 absence of stimulation. Interestingly, 24-hour spike peptide stimulation had minimal
174 impact on the proportions of AIM^+ T cells as compared to 12-hour stimulation, with the
175 notable exception of increased T_{reg}s and a trend towards decreased $V\delta2^+$ γδT cells.
176 Thus, 24-hour stimulation appears to effectively increase the signal-to-noise ratio
177 primarily by reducing background activation.

178 To directly assign the transcriptional signatures identified in the single-cell RNA
179 sequencing data with the baseline phenotype, we identified baseline AIM^+ cells by
180 surface expression of CD69, CD134 (OX-40), and CD137 (4-1BB) in a published CITE-
181 seq dataset of unstimulated T cells prepared in a similar manner to our dataset (*23*)
182 (**Methods**, **Fig. S4A**). Based on the original cluster annotations, MAIT cells were the
183 population most over-represented in the baseline AIM^+ population (**Fig. S4B**). When our
184 AIM^+ cluster labels were mapped onto the baseline AIM^+ population, nearly all cells
185 mapped to CD4_Tcm, CD8_Tem, VD2, and MAIT_1 cells (**Fig. S4C**). Critically, many of
186 the polarized $CD4^+$ T cell clusters we identified (e.g., T_H1) did not appear to have a
187 corresponding baseline AIM^+ population, and were expanded compared to baseline,
188 and therefore are distinct from AIM^+ "background" T cell populations.

189 **AIM^+ MAIT cells have signatures of cytokine-driven activation**

190 MAIT cells cannot directly recognize peptide antigen (*24, 25*), but the frequency of AIM^+
191 MAIT cells increased after 12- or 24-hour SARS-CoV-2 spike peptide stimulation (**Fig.
192 S3C).** As evidenced by our analysis of baseline AIM^+ T cells, their detection is at least
193 partially attributable to their expression of AIM markers at rest. Baseline AIM^+ MAIT cells
194 only mapped to our MAIT_1 cluster, suggesting the MAIT_2 phenotype was activation

195 induced (**Fig. S4C**). As we report in (*19*), MAIT cells and γδT cells can be activated by
196 IFN-γ released by peptide-specific T cells in a feed-forward cascade. To determine if
197 this was detectable in the AIM assay, we first confirmed that the TCR characteristics of
198 the MAIT_1 and MAIT_2 clusters mirrored those seen previously in sorted MAIT cells
199 (*26*)(**Fig. S5A-D**). Clonal overlap was observed between the two clusters and across
200 timepoints, suggesting the two clusters reflected plastic phenotypes (**Fig. S5E**).
201 Strikingly, the MAIT_2 cluster had a signature of cytokine-mediated or dual TCR and
202 cytokine (IL-12 and IL-18) stimulation, while the MAIT_1 cluster had a signature of TCR-
203 driven activation (**Fig. S5F and S5G**). Thus, the MAIT_2 cluster appears to represent
204 an effector population responding in a secondary manner to cytokines produced by
205 antigen-specific T cells (*19*), while the MAIT_1 cluster represents cells exhibiting
206 residual in vivo activation.

207 **AIM$^+$ T cells have diverse functional characteristics**

208 To investigate how AIM$^+$ T cells may relate to those captured by ICS assays, we next
209 examined the production of an array of cytokines, chemokines, and cytotoxic effector
210 molecules at T1/T2 (**Fig. 1F and 1G**). Canonical effector molecules of given T cell
211 polarization states were produced as expected (**Fig. 1F and 1G**). *TNF* was broadly
212 expressed and in some cases was the only detectable effector cytokine.

213 Different studies use different combinations of AIMs to identify peptide-reactive cells
214 (*20-22, 27*). In addition to CD69, OX-40 and 4-1BB which were included in our staining
215 panel, we performed CITE-seq staining for other commonly used AIM markers:
216 CD107a, CD40L, ICOS and PD-1 (**Fig. 1H**). RNA transcript expression for CD69, OX-
217 40 and 4-1BB was consistent with the expected protein-level expression for each AIM
218 (**Fig. 1E**). Comparison of the surface protein expression of the other AIMs revealed
219 interesting patterns between cell types and clusters, indicating that markedly distinct T
220 cell populations can be recovered depending on the combination of AIMs used (**Fig.
221 1H**).

222 To better understand the function of AIM$^+$ T cells, we examined putative cell-cell
223 interactions between clusters, with the caveat that we could only examine T-T cell
224 interactions in our dataset. Overall, CD4$^+$ T cells sent and received proportionally fewer

225 signals than $CD8^+$ T cells and unconventional T cells (**Fig. 1I and S6A**). Classification of
226 these interactions highlights diverse biology (**Fig. S6B**). The T_H1/T_{FH} cluster sent the
227 greatest number of signals to other clusters, suggesting a "hub" role for these cells (**Fig.**
228 **1I and S6A**). Examination of specific interactions revealed both immune stimulatory
229 (e.g., IFN-γ, TNF and FASL interaction with their receptors) and immunomodulatory
230 (e.g., BTLA and prostaglandin E2 interaction with their receptors) interactions between
231 the T_H1/T_{FH} cluster and all other clusters (**Fig. 1J**).

232 **Antigen-specific and expanded clones are shared across T cell subsets**

233 We next sought to determine the clonal relationship of the AIM^+ $CD4^+$ T cell populations.
234 Clusters did not show specific enrichment of TCRα or TCRβ chains, with the exception
235 of the CD4_PLZF cluster (**Fig. S7A and S7B**). Expanded clones were found across all
236 clusters and at both timepoints, but were proportionally more abundant in T_H2 cells,
237 cytotoxic $CD4^+$ T cells, ISG^{hi} $CD4^+$ T cells and IFN-γ^+ $CD8^+$ T cells (**Fig. 2A**). There was
238 considerable clonal sharing between $CD4^+$ T cell clusters, with notable exception of the
239 T_{reg} population (**Fig. 2B and 2C**). A major contribution to this clonal sharing was
240 population interconversion between timepoints (**Fig. S7C**). Separate analyses of T1-
241 long and T1-short timepoints demonstrated few differences in clonal sharing and
242 expansion with time post SARS-CoV-2 exposure (by infection or vaccination) (**Fig. S7D**
243 **and S7E)**.

244 Excluding MAIT cells, CoNGA clonotype analysis, a graph-based approach to assess
245 the relationship between the transcriptional phenotype and TCR sequence of T cell
246 clones (**Methods**), revealed that there was low overall correlation between TCR metric
247 and gene expression when clonotypes of different cellular origins were aggregated
248 based on their TCR sequence (**Fig. S8A and S8B**), reflecting the functional diversity of
249 the captured clones. Despite this overall trend, one set of clonotypes within the IFN-γ^+
250 $CD8^+$ T cell population did have strong correlation between function and specificity (**Fig.**
251 **S8C**). These clonotypes included one clone that matched a previously published SARS-
252 CoV-2 spike specific clone (CASQETNTGELFF, (*28*)). In contrast to the CoNGA
253 analysis, expanded clones within IFN-γ^+ $CD8^+$ and T_H1/T_{FH} clusters were associated with
254 increased cytotoxicity (published geneset from (*29*)) and *IFNG* production, respectively

255 (**Fig. 2D**). This analysis suggests that for a given effector state, functionality is
256 associated with clonal expansion.

257 Identification and comparison of TCR meta-clonotypes with simulated non-antigen
258 enriched TCR repertoires (**Methods**) identified multiple antigen-enriched meta-
259 clonotypes in both $CD4^+$ and $CD8^+$ T cell clusters and accounted for 0-2.5% of
260 clonotypes within each conventional T cell population (**Fig. 2E and 2F**). Notably, none
261 of the 6,001 unique clonotypes identified in the $CD4^+$ T_{reg} cluster were predicted to
262 belong to an antigen-enriched meta-clonotype (**Fig. 2F**). Furthermore, $CD4^+$ T cells
263 associated with the AIM^+ background (CD4_Tcm, **Fig. S5C**) or with a more resting gene
264 expression phenotype (CD4_TGFB1 and CD4_HLA) had lower frequencies of antigen-
265 enriched clonotypes compared with other $CD4^+$ T cell populations (**Fig. 2F**).

266 173 distinct meta-clonotypes were predicted to be antigen-enriched, including 11 with
267 more than 10 unique participating clonotypes (**Fig. S8D-G**). The antigen-enriched meta-
268 clonotypes included cells from several cell types (**Fig. S8D**). Based on TCR gene
269 usage, the largest antigen-enriched meta-clonotype matched the description of a CD1d-
270 restricted invariant NKT TCR (*30*). This aligned with the CoNGA clonotype analysis,
271 which independently identified clones within the $PLZF^+$ $CD4^+$ T cell cluster that were
272 restricted to *TRBV4-1* gene usage (**Fig S8H)**. These TCR characteristics, combined
273 with *PLZF* expression in this cluster, suggest that CD1c and CD1b auto-reactive $CD4^+$ T
274 cells may additionally be captured in this assay (*31, 32*). The presence of these cells in
275 the AIM^+ T cell population is likely caused by the same processes that result in
276 identification of MAIT cells, as discussed above.

277 Other predicted antigen-enriched meta-clonotypes represent undescribed clusters of
278 likely SARS-CoV-2 spike-specific T cell clones with similar TCR properties and are
279 characterized in **Fig. S8D-G.**

280 **AIM^+ T_{reg} cells are not antigen-specific but have an immune promoting phenotype**

281 Our analysis suggested that the T_{reg} population was not a clonally restricted antigen-
282 specific population and detection of these cells was partially due to spontaneous
283 activation after 24 hours of incubation without stimulation (**Fig. 2 and S3A**). The overall
284 frequency of T_{reg} cells as a fraction of all $CD3^+$ T cells did not change at 12 or 24 hours

285 of incubation **(Fig. S9A).** However, AIM$^+$ T cells were significantly enriched for T_{regs} in all
286 stimulation conditions after 24 hours **(Fig. S9A)**. A previously published scRNA- and
287 TCR-seq dataset (*33*) of AIM$^+$ T cells captured after stimulation of PBMCs from recent
288 COVID-19 vaccinees with SARS-CoV-2 spike and CEF peptides also identified a large
289 proportion of AIM$^+$ T_{regs} to both peptide pools **(Fig. S9B)**. Concordant with our data **(Fig.
290 2B)**, re-analysis of this data identified minimal overlap of TCR clones between T_{reg} and
291 CD4$^+$ effector memory T cells for each stimulus (**Fig. S9B).** Strikingly, there was
292 comparatively more clonal overlap between T_{reg} populations induced by the different
293 peptide pools (**Fig. S9B)**. Together, this data suggests that AIM$^+$ Tregs are
294 spontaneously induced over the stimulation period, but their frequency is further
295 increased by peptide in a non-antigen specific manner.

296 Despite their apparent lack of antigen-specificity, it is possible that T_{regs} play a role in
297 regulating/maintaining the vaccine-induced response. Sub-clustering of spike-
298 responsive T_{regs} identified multiple clusters with graded expression of CCR7, CD278
299 (ICOS), *LEF1* and *CTLA4*, suggesting the presence of both effector T_{regs} (CTLA-4$^+$
300 ICOS$^+$ FoxP3^{int} CD25^{lo}) and memory T_{regs} (LEF1$^+$ CCR7$^+$ FoxP3^{hi} CD25^{hi}) (**Fig. S9C-E**)
301 (*34*). *FOXP3*, *IL2RA* and moderate levels of *IKZF2* (HELIOS) were expressed by all
302 clusters, but there was minimum expression of T_{reg} effector cytokines *IL10*, *TGFB1*,
303 *IL12A* and *EBI3* (**Fig. S9F)**. In contrast to a suppressive response, the T_{reg} populations
304 appeared to have an immune promoting phenotype. Multiple effector T_{reg} clusters had
305 elevated expression of gene sets associated with antigen presentation (clusters 0-3)
306 and positive regulation of inflammatory responses to antigen stimulation (cluster 3),
307 compared with other T_{reg} populations (**Fig. S9G**). A putative reciprocal interaction
308 between the T_{reg} cluster and nearly all populations involved cell-cell interaction via
309 CD62L (*SELL*) binding to the receptor PSGL-1 (*SELPLG*). Additionally, T_{reg} cells
310 uniquely produced IL-7 (*IL7* expression) (**Fig. S9H**).

311 **Pre-existing SARS-CoV-2-reactive T cells are expanded by COVID-19 vaccination**

312 Pre-existing SARS-CoV-2 cross-reactive T cells are well described (*35*), but the extent
313 to which these cells contribute to vaccine-induced immunity remains unclear. To
314 address this, we used a seven-day cell proliferation assay at the pre-vaccine timepoint

315 ("T0") to identify spike-specific T cells in unexposed individuals (*36*) (**Fig. 3A and 3B**).
316 After QC, we recovered 1,050 cells comprising $CD4^+$ and $CD8^+$ T cell populations with
317 naïve and effector phenotypes (**Fig. S10A-D**). Surprisingly, unconventional T cells were
318 also recovered (**Fig. S10A-E**). Reference mapping the T0 dataset to the T1/T2 AIM
319 dataset found only a portion of clusters described in the T0 data, with logical
320 concordance between annotations (**Fig. S10F**).

321 From the T0 timepoint we identified 756 unique paired TCR clonotypes. Only 21 (2.8%)
322 of these overlapped with either of the post-vaccine timepoints and only eight (1.1%)
323 were found at both timepoints (**Fig. 3C**). Strikingly, pre-existing spike-reactive T cells
324 were found post-vaccination only in the BNT group (0 out of 22,368 cells in pre-existing
325 clones ChAd vs 94 out of 40,569 cells in pre-existing clones BNT, $p = 1.46 \times 10^{-18}$,
326 Fisher's exact test) (**Fig. S10G**). Baseline clones identified at post-vaccine timepoints
327 were mostly found in $CD8^+$ T cell clusters (**Fig. 3D**). Post-vaccination, pre-existing
328 clonotypes were not more cytotoxic than ones only identified after vaccination (**Fig. 3E**).
329 Collectively, these data suggest that pre-existing cross-reactive T cells make only a
330 minor contribution to the overall vaccine-induced response.

331 We next examined if clones induced by the primary vaccine and recalled by the second
332 dose (detected at T1 and T2; "recalled clones") were different from clones only detected
333 at T2 (and thus more likely to be a *de novo* response of vaccine dose 2). Differential
334 expression analysis revealed a limited set of genes in each cluster between recalled
335 and *de novo* clones, with the largest number of differences in the T_H1/T_{FH}, T_H1/T_H17,
336 cytotoxic and ISG^{hi} $CD4^+$ T cell clusters (**Fig. 3F**). *SLAMF1* was the only differentially
337 expressed gene between recalled and *de novo* clones in $IFN\gamma^+$ $CD8^+$ T cells. Cytolytic
338 components such as *GZMA*, *GZMB* (Granzyme A & B), *GNLY* and *LGALS1* were
339 amongst the most upregulated genes in recalled versus *de novo* clones in $CD4^+$
340 populations (**Fig. 3G**). In T_H1/T_{FH} cells but not T_H1/T_H17 cells, this corresponded with a
341 more effector-like phenotype, with decreased protein expression of CCR7 and CD45RA
342 (**Fig. 3H**). Thus, effector function and memory phenotype differ across recalled and *de*
343 *novo* clones in $CD4^+$ populations, but not in $CD8^+$ T cells.

344 **Distinct T cell responses are induced by ChAdOx1 nCoV-19 and BNT162b2**
345 **vaccines**

346 Having described the AIM^+ T cells as a complete dataset, we next examined the impact
347 of vaccine type on activated T cell functionality. ChAd vaccination induced a greater
348 proportion of T_H1/T_{FH} $CD4^+$ T cells at both the T1 and T2 timepoints compared with BNT,
349 in addition to select unconventional T cell populations (MAIT_1 and naive $V\delta1^+$ γδT cell
350 clusters) (**Fig. 4A**). Additionally, the ratio of MAIT_2 (cytokine- or TCR+cytokine-
351 stimulated) to MAIT_1 (TCR-stimulated) and effector $V\delta1^+$ γδT to naive $V\delta1^+$ γδT cells
352 was higher in BNT compared with ChAd (**Fig. S11A and S11B**).

353 Differential gene expression analysis per cluster revealed only minor differences
354 between ChAd and BNT vaccination (**Fig. 4B**) that were largely consistent across
355 timepoints **(Fig. S11C)**. Differential expression of AIMs at the protein level was also
356 observed between groups. CD107a (*LAMP*), ICOS and PD-1 (*PDCD1*) expression was
357 increased in the IFN-γ^+ $CD8^+$ T cell population in ChAd compared with BNT (**Fig. 4C**).

358 There were specific gene expression differences of interest between vaccine groups,
359 particularly in the IFN-γ^+ $CD8^+$ T cell cluster. Examination of a signature of cytotoxic
360 function found discordant differential expression based on vaccine type (**Fig. 4D**). *IFNG*
361 expression was higher in response to ChAd vaccination, while BNT induced greater
362 levels of cytotoxic granule molecules (*GZMA* and *GNLY*) (**Fig. 4D**). This was largely
363 consistent between timepoints, however several ISGs were identified which were only
364 upregulated in BNT vs ChAd vaccination at the T2 timepoint **(Fig. S11D)**. These
365 differences corresponded to a more T_{EMRA} phenotype ($CD45RA^+$ $CCR7^-$) in $CD8^+$ T cells
366 induced by BNT compared with a mixed T_{EM} ($CD45RA^-$ $CCR7^-$)/T_{EMRA} phenotype induced
367 by ChAd (**Fig. 4E & S11E**). Comparison of putative cell-cell interactions between
368 vaccine types indicated that the majority of signaling pathways were shared by vaccine
369 type (**Fig. S11F**), with a small number of differences. CD160, a co-inhibitory receptor
370 (*37*), was only expressed by IFN-γ^+ $CD8^+$ T cells and effector $V\delta1^+$ γδT cells from ChAd
371 vaccinated individuals (**Fig. S11G**). Interaction with its receptor HVEM (*TNFRSF14*),
372 expressed on all clusters, represented a broad and distinct feedback mechanism (**Table**
373 **S2**). PD-L1/PD-1 signaling differed based on vaccine type, with a greater number of

374 involved cell types and cell-cell interactions in ChAd vaccination relative to BNT (**Fig.**
375 **4F**).

376 Finally, we examined the impact of vaccine type on TCR usage. A substantially larger
377 proportion of the top expanded $CD4^+$ clonotypes at T2 were also identified at T1
378 following BNT compared with ChAd vaccination (**Fig. 4G**). Amongst the top expanded
379 clonotypes at T2, clonotypes found at both timepoints (i.e. "recalled" clones) increased
380 as a proportion of total cells at T2 compared with T1 within BNT and ChAd vaccinees
381 (**Fig. 4H)**. Across all clones in vaccinees, clones that were recalled at T2 were
382 proportionally larger and more expanded than non-recalled clones at T1 **(Fig. S11H and**
383 **S11I)**. Compared with top clones only found at T2 ("*de novo"* clones), there was an
384 increased proportion of clones with a T_H2 phenotype in the recalled clones in individuals
385 in both vaccine groups (Bonferroni adjusted $p<0.001$, Fisher's exact test) (**Fig. 4I**).
386 Notably, there was a higher proportion of $CD4^+$ T cells with an ISG^{hi} phenotype in top
387 recalled clones in BNT compared with ChAd vaccinees (**Fig. 4J**), highlighting the
388 contribution of this interferon-driven cell type to the recall response, singularly in BNT
389 vaccination.

390 **Different dynamics of AIM^+ T cell response induced by COVID-19 vaccines and**
391 **SARS-CoV-2 infection**

392 We next compared the phenotype of AIM^+ T cells induced at early and late timepoints
393 post SARS-CoV-2 infection and at equivalent timepoints after a priming vaccine dose.
394 There were differences in cell composition between vaccinees and COVID-19
395 convalescent individuals that were largely consistent at both T1-long and T1-short
396 timepoints (**Fig. S12A),** but T_H1/T_{FH}, T_H1/T_H17 and the IFN-γ^+ $CD8^+$ T did not differ in
397 proportion**.** Compared with difference between vaccine vectors (**Fig. 4B)** there were
398 considerably more differentially expressed genes between vaccinees and COVID-19
399 convalescent individuals, particularly at the T1-Short timepoint (**Fig. S12B)**. There was
400 positive enrichment of genesets related to IFN-γ, TNF, and IL2 signaling in COVID-19
401 convalescent individuals at the T1-Short timepoint, which decreased over time and was
402 reduced compared to vaccine induced responses at the T1-Long timepoint (**Fig. S12C)**.

403 These data suggest differences in early effector responses driven by infection versus
404 vaccination which transitions towards a more convergent phenotype over time.

405 **Time interval between vaccine dose 1 and dose 2 impacts on the T cell response**

406 We next examined the impact of dose number on the vaccine-induced T cell response.
407 Despite the boosting dose, a comparison of T1 versus T2 revealed only minor
408 differences in the relative abundance of different cell populations (**Fig. S13A**). $TGFB1^+$
409 $CD4^+$ and $V\delta2^+$ γδT cells decreased at T2 compared with T1 in BNT vaccinees, but no
410 significant differences were observed between timepoints in ChAd vaccinees.
411 Correspondingly, few genes were differentially expressed by timepoint, and increased
412 gene expression at T2 compared with T1 was primarily associated with BNT vaccination
413 (**Fig. S13B**).

414 However, when T2 was separated based on the interval between first and second
415 vaccine dose, substantial differences were observed – many of these were in opposite
416 directions based on interval and thus masked the observations when T2 was analyzed
417 in aggregate (**Fig. 5A)**. The long interval was associated with increased abundance of
418 T_{EM} $CD8^+$ T cells and HLA^{hi} $CD4^+$ T cells in ChAd vaccinees, a phenomenon not
419 observed in BNT vaccinees (**Fig. 5A**).

420 Across clusters, surface effector molecule expression was differentially impacted by
421 vaccine type and interval. CD154 (CD40L) was upregulated in short-interval compared
422 with long-interval BNT, but the opposite was seen for ChAd. This pattern was reversed
423 with CD278 (ICOS), which was upregulated specifically in short-interval ChAd (**Fig. 5B**).
424 Differential gene expression analysis revealed major differences based on interval for
425 both the $CD4^+$ T cell and unconventional T cell clusters (**Fig. 5C**). Strikingly most of
426 these genes were only differentially expressed to either ChAd or BNT, with relatively
427 little overlap. Overrepresentation analysis highlighted broad differences in the strength
428 of geneset enrichment across the clusters in short versus long interval for both BNT and
429 ChAd (**Fig. 5D**). Gene sets related to $IFN\alpha$ and $IFN\gamma$ were more significantly
430 overrepresented in multiple cell types in the short-interval BNT group but less so in
431 short-interval ChAd; whereas processes related to mTOR signaling, hypoxia and
432 glycolysis were more strongly increased in short-interval compared with long-interval

433 ChAd (**Fig. 5D**). Conversely, when assessing long-interval boosting for either vaccine
434 platform, relatively few annotated biologic processes were enriched for the upregulated
435 genes (**Fig. S13C**).

436 Based on these findings, short-interval and long-interval were compared head-to-head
437 for the two vaccines. GSEA analysis identified few differences in the cell processes or
438 signaling pathways induced by long-interval BNT versus long-interval ChAd (**Fig. 5E**). In
439 contrast, short-interval BNT more strongly induced type I and II interferon signatures
440 across the majority of cell types compared with short-interval ChAd (**Fig. 5E**).
441 Examining this in detail in the IFN-γ-producing conventional T cell clusters revealed
442 different patterns based on cell type (**Fig. 5F**). For the T_H1/T_{FH} and T_H1/T_H17 $CD4^+$ T cell
443 clusters, there was stronger induction of this pathway in both vaccine types by the short-
444 interval and downregulation in the long-interval, but it was more strongly induced in
445 short-interval BNT compared with short-interval ChAd (**Fig. 5F**). For IFN-γ^+ $CD8^+$ T
446 cells, it was uniquely induced by short-interval BNT at T2, with decreased signaling for
447 all other regimens at T2 relative to T1 (**Fig. 5F**). This could be seen as coordinated
448 induction of genes in this biologic pathway specifically in this vaccine condition (**Fig.
449 5G**). In contrast, a hypoxia pathway, known to regulate effector T cell function (*38*), was
450 more strongly induced in nearly all $CD4^+$ T cell clusters in short-interval ChAd relative to
451 short-interval BNT (**Fig. 5E**). Together, these data highlight the critical role that interval
452 between first and second dose of vaccine has on resultant T cell functionality, and that
453 the biology is not concordant between vaccine types.

454 **Short dosing interval of BNT162b2 induces a more inflammatory recall T cell
455 phenotype**

456 A particular characteristic of the short interval is that the time since last dose at T1 and
457 T2 is the same (28 days). Thus, we sought to determine how the T cell phenotype
458 changed in the BNT group between first and second dose depending on the interval. In
459 the short-interval but not long-interval BNT group, type I and II IFN signaling were
460 elevated across all cell types at T2 relative to T1 (**Fig. 6A and 6B**). The short interval
461 between doses also led to sustained TNF signaling between T1 and T2, while the long-
462 interval dose resulted in reduced induction of this pathway post-boost (**Fig. 6A and 6B**).

463 At an individual gene level, this reflected coordinated changes in expression of nearly all
464 genes in the signature, with little overlap of the two signatures, suggesting multiple
465 parallel inflammatory pathways (**Fig. 6C**). There were no differences between the
466 phenotypes of AIM$^+$ T cells in unstimulated samples in long- compared with short-
467 interval vaccinees at T1, implying no differences in baseline vaccine associated
468 bystander activation **(Fig. S14A and S14B)**. Unlike BNT vaccination, the short dosing
469 interval in ChAd vaccinees induced a less dramatic type I or type II IFN and TNF
470 response at T2 relative to T1 (**Fig. S14C**).

471 We next investigated the impact of vaccine dosing interval on the expansion and recall
472 of TCR clones. There was less expansion of recalled clones in individuals vaccinated
473 with a short dosing interval of either vaccine (**Fig. 6D**). Overall, across multiple clusters,
474 there was evidence of reduced activation and inflammatory signaling at T2 in long-
475 interval BNT compared to T1, which may be associated with preferential recall of
476 secondary ("recalled") effector cells, while most of these signatures were equally robust
477 (or higher) at T2 in the short-interval BNT group. Thus, increasing the time between
478 doses of mRNA vaccines results in a secondary response that is less inflammatory with
479 lower production of effector molecules, such as *TNF, GZMA, CCL20, CXCL10* (**Fig. 6C**)
480 and a larger average expansion of recalled clones. Compared to the short dosing
481 interval, the long dosing interval was associated with increased expression of stem-cell
482 associated T cell genes in multiple AIM$^+$ T cell clusters (**Fig. 6E**) and enrichment for
483 AIM$^+$ central memory phenotype cells (CD4_Tcm2) (**Fig. 6F**) immediately pre-second
484 vaccine (e.g., T1).

485 **DISCUSSION**

486 In this study we sought to determine how ChAdOx1 and mRNA (BNT162b2) vaccine
487 platforms, number of doses, and interval between vaccines impacts the phenotype,
488 functionality, and clonality of spike-specific T cell populations. To accomplish this, we
489 utilized an AIM assay combined with scRNA-seq and scTCR-seq ("AIM-seq") to gain
490 insight into antigen-specific T cells independent of cytokine production functionality.
491 Validating the use of this unbiased approach, peptide responsive T cells included not
492 only the expected IFN-γ$^+$ CD4$^+$ and CD8$^+$ T cells but also CD4$^+$ T_H2 and CD4$^+$ T_H17

493 cells, in addition to other diverse subpopulations. Interval between doses had the
494 largest impact on T cell phenotype and function, with a short 3–4-week interval between
495 doses resulting in a markedly more pro-inflammatory T cell response induced by
496 BNT162b2 vaccination, and to a lesser extent ChAdOx1 vaccination. These data reveal
497 unexpected functional heterogeneity in vaccine-induced T cell responses and a
498 dominant impact of the interaction between vaccine type and dosing interval on T cell
499 function.

500 The AIM assay is an appealing method for the identification of antigen-responsive T
501 cells in a function-independent and HLA-agnostic manner, but capture of T cells that are
502 activated at baseline ("background") or indirectly activated independently of TCR
503 engagement ("bystander activated") may limit detection of true antigen-specific cells.
504 Flow cytometric assessment and reanalysis of published CITE-seq data (*23*) revealed
505 that "background" cells are associated with very specific phenotypic/transcriptional
506 states, including $CD4^+$ T_{CM} cells, $CD4^+$ T_{reg} cells, and unconventional T cell populations
507 (**Fig. S2**). The detection of activated MAIT and $V\delta2^+$ γδT cell populations, which are not
508 directly peptide responsive (*24, 25*) but can become activated by cytokines over 24-
509 hours (*39, 40*) (*19*), demonstrated the infiltration of bystander activated T cells into the
510 AIM^+ population following peptide stimulation. However, activation of these cells by
511 BNT162b2 immunization is associated with vaccine reactogenicity (*19*), therefore
512 including these cells in future analyses may reveal intriguing biology. AIM^+ T_{regs} also
513 represent a substantial potential "bystander" population (*41*). Our TCR analysis
514 demonstrated they have negligible clonal sharing with effector $CD4^+$ populations, so are
515 unlikely to be SARS-CoV-2 spike-specific, and do not appear to be enriched to any
516 specific antigen. Our time course analysis suggests that spontaneous activation of T_{regs},
517 possibly due to IL-2 signaling (*41*), may result in their detection. However, given their
518 role as a source for the T cell survival cytokine IL-7, further work is required to
519 understand the role of T_{reg} cells in modulating the vaccine response, particularly T cell
520 memory formation (*42*).

521 Comparison of COVID-19 vaccine platforms revealed specific differences. Intriguingly,
522 only BNT vaccination caused detectable recall of pre-existing T cell clones, consistent

523 with data from a large study that identified that BNT162b2 priming induced recall of the
524 cross-reactive $S_{816\text{-}830}$ $CD4^+$ T cell epitope but ChAdOx1 priming did not (*9*). In our study,
525 BNT groups also had greater recruitment of T cells induced by dose 1 into the recall
526 response following dose 2. Together this suggests there is a fundamental difference in
527 the way ChAdOx1 and BNT162b2 vaccines promote recall of memory T cell
528 populations. Increased abundance of *IFNG* expressing T_H1/T_{FH} $CD4^+$ T cells and
529 increased *IFNG* transcript expression in $CD8^+$ T cells from ChAdOx1 nCoV-19
530 compared with BNT162b2 vaccinees demonstrates that the AIM assay can indirectly
531 recapitulate the findings, made using ICS and ELISpot assays, of increased frequencies
532 of IFN-γ^+ T cells after ChAdOx1 nCoV-19 compared with BNT162b2 vaccination (*7-9*).
533 However, the AIM assay also captures additional functional T cell complexity not
534 identified by these previous approaches. This was particularly the case when
535 considering the interval between doses.

536 IFN-γ^+ T cell frequencies measured by ELISpot are modestly impacted by vaccine
537 dosing interval (*5, 15*). However, in our dataset the dosing interval had the greatest
538 impact on T cell phenotype, and this impact was exacerbated in BNT162b2 compared
539 to ChAdOx1 nCoV-19 vaccination. Increasing the interval between BNT162b2 doses
540 resulted in reduced inflammatory signaling in post-boost antigen-responsive T cells and
541 greater proliferative potential in comparison to a short dosing interval. In a parallel study
542 (*19*), we identified that increasing the interval between mRNA vaccine doses reduced
543 memory T cell-induced innate inflammatory signaling. We hypothesize that after short
544 BNT162b2 vaccination the elevated inflammatory environment induced by the second
545 dose leads to a recall T cell response with increased IFN-γ and TNF signaling. By
546 contrast, inflammation induced by Ad vectors is driven primarily by engagement of
547 innate pDCs and myeloid cells (*43, 44*) and thus would vary less between priming and
548 boosting.

549 Inflammatory signaling in T cells is associated with increased effector polarization at the
550 expense of long-lived proliferative memory differentiation (*45*). Given the elevated
551 inflammatory phenotype of short-interval mRNA vaccination, we hypothesize T cells
552 induced by this regimen would have reduced proliferative potential. Consistent with this,

553 IFN-γ T cell responses generated by two doses of mRNA vaccination given in a long-
554 interval regimen increased in magnitude following an additional (third) dose, whereas
555 those generated by a short-interval regimen did not (*46*). In our dataset, we observed an
556 expansion of recalled clones after two doses of vaccine given in a long-interval regimen,
557 but no expansion of recalled clones after a short-interval regimen, and this
558 corresponded to increased expression of stem-associated genes in several $CD4^+$ T cell
559 clusters. Thus, the dosing interval for a primary vaccination regimen has a long-term
560 impact on the anamnestic potential of vaccine induced T cells, which may lead to the
561 increased long-term protection against COVID-19 observed with the extended-dosing
562 interval (*12*).

563 The primary limitation of the current study is the sample size per experimental group.
564 While the overall dataset includes 60 samples for the AIM-seq assay (30 individuals and
565 two timepoints) the inclusion of five experimental groups limits the power of
566 comparisons when performed at the level of individuals. Furthermore, the relatively
567 limited number of cells captured per individual may lead to overestimation of the number
568 of singlet clones identified. As we sorted AIM^+ T cells and do not also have scRNA-seq
569 data on whole peripheral blood samples, we are limited to analysis of cell-cell
570 interactions only between T cells and cannot assess interactions with other important
571 antigen-presenting cells. In addition, the use of 15mer peptide pools may bias towards
572 the detection of $CD4^+$ T cells.

573 In sum, we have demonstrated the utility of AIM-seq to characterize the phenotype,
574 function and clonality of all SARS-CoV-2 spike-responsive T cells. While studies have
575 used this approach in more limited ways, this study fully highlights the use of this
576 approach for studying human T cell responses in vaccination and infection. Most
577 strikingly, interval between vaccine doses 1 and 2 had a marked, and platform-specific,
578 impact on the resultant T cell response. When combined with our companion study (*19*),
579 we have built a model where elevated inflammatory T responses in the short 3-4-wk
580 BNT162b2 mRNA regimen is associated with enhanced innate responses at the time of
581 boosting. These have practical considerations for optimizing dosing regimens of mRNA

582 technology dependent on the context of its use – e.g., for use in infectious diseases or
583 cancer, compared with use in autoimmunity or for gene therapy.

584

585 **MATERIALS AND METHODS**

586 **Study design**

587 We investigated the phenotype, function, and clonality of SARS-CoV-2 spike-specific T
588 cells in humans after COVID-19 vaccination or SARS-CoV-2 infection. Specifically, we
589 compared across different vaccine platforms (mRNA-LNP vs. adenoviral vector) and
590 dosing intervals (3–4 weeks vs. 8–12 weeks). Peripheral blood was collected from
591 healthy donors without previous symptomatic COVID-19 before and after two doses of
592 BNT162b2 or ChAdOx1 nCoV-19 vaccination. Also included were a group of
593 unvaccinated individuals with mild COVID-19, sampled approximately 28 days (range
594 17-28 days) and 56 days (range 53-61 days) after SARS-CoV-2 PCR positivity
595 ("COVID"), corresponding to the T1 timepoint for the short and long vaccine groups
596 respectively. Demographic information is in **Table S1**. Six donors were sampled across
597 multiple timepoints per group. Individuals in the BNT162b2 3–4 weeks ("short") and 8–
598 12 weeks ("long") group and the ChAdOx1 nCoV-19 8–12 weeks ("long") group are also
599 included in the companion work (*19*). The study was not randomized, investigators were
600 aware of study groups and no formal sample size calculation was performed. Single-cell
601 CITE- and TCR-sequencing was performed on sorted AIM^{+} T cells after *ex vivo*
602 stimulation of peripheral blood mononuclear cells with SARS-CoV-2 spike peptides. In
603 addition, we assessed the contribution of pre-existing SARS-CoV-2-responsive T cells
604 to the vaccine response by performing single-cell RNA- and TCR-sequencing on T cells
605 that proliferated in response to SARS-CoV-2 spike peptides in blood sampled prior to
606 vaccination.

607 **Ethics statement**

608 Informed written consent was received from all participants. All work was performed in
609 accordance with relevant ethical regulations and in compliance with the principles of the
610 Declaration of Helsinki (2008). Protective Immunity from T Cells in Healthcare workers
611 (PITC) ethical approval: Oxford GI Biobank Study Ethics Committee (REC Ref:

612 16/YH/0247, Yorkshire & The Humber Sheffield REC, approved on 29 July 2016,
613 amended on 8 June 2020)). COV001 trial registration: NCT04324606; ISRCTN
614 15281137.

615 **Cohort**

616 Participants were those in COV001 Phase 1/2 clinical trial (*47*) and the PITCH (*15*)
617 studies who received two doses of either BNT162b2 or ChAdOx1 nCoV-1 vaccines at
618 long (8-12 weeks) or short (3-4 weeks) dosing intervals, or had mild COVID-19 infection
619 (pre-Alpha COVID-19 strain). Samples from the short ChAd group were from
620 participants in COV001, while samples from all other groups were from the PITCH
621 study**.** Participants in the BNT and ChAd long interval groups were also included in (*19*).
622 Participants were sampled before first vaccine ("T0"), and immediately before ("T1") and
623 four weeks after second ("T2") COVID-19 vaccine. Patients with mild COVID-19 were
624 diagnosed by SARS-CoV-2 PCR positivity and were sampled at timepoints selected to
625 match the short interval vaccine dosing, such that T1-Short was 3-4 weeks and T1-Long
626 was 8 weeks after initial PCR positivity. The age and sex of participants was similar
627 across each experimental group (Table S1). PBMCs were processed and stored as
628 described in (*15, 48*).

629 **Stimulation, flow cytometric staining and sorting of activation induced marker T**
630 **cells**

631 One vial of cryopreserved PBMCs per patient per timepoint was thawed and washed in
632 R10 (RPMI-1640 [Sigma Aldrich] + 10% FBS [Sigma Aldrich] + 1%
633 Penicillin/Streptomycin [Sigma Aldrich]) + Benzonase (500 U; Sigma Aldrich) on the day
634 of use. Both timepoints of a single patient from each experimental group were included
635 on a given day to minimize batch effects across sequencing runs. PBMCs from each
636 vial were split in R10 across up to 8 wells of a U-bottom 96-well plate at 1×10^6 PBMCs
637 per well. Pools of overlapping peptides (15mers with 11 amino acid overlap) which
638 covered the entire SARS-CoV-2 spike S1 and S2 protein regions (JPT Peptide
639 Technologies GmbH; catalog number PM-WCPV-S-2) were added to each well at a
640 final concentration of 1 μg/ml and plates were incubated for 24 hours at 37 °C. One well
641 of unstimulated cells per donor was included for use as a gating control for flow

cytometric cell sorting. After incubation, plates were centrifuged at 706 *g* for 2 min, washed in FACS buffer (PBS [Sigma Aldrich] + 0.05% bovine serum albumin [Sigma Aldrich] + 1 mM EDTA [Thermo Fisher Scientific]), and centrifuged again at 706 *g* for 2 min prior to staining with the following antibodies: FITC-CD19 (HIB19; 1:100 dilution), FITC-CD14 (M5E2; 1:100 dilution), PE-4-1BB (CD137; 4B4-1; 1:100 dilution), PE-OX-40 (CD134; Ber-ACT35; 1:100 dilution), AF700-CD8α (SK1; 1:100 dilution), BV421-CD69 (FN50; 1:100 dilution), BV650-CD4 (OKT4; 1:100 dilution), BV785-CD3 (OKT3; 1:100 dilution), TotalSeq-C0032-CD154 (CD40L; 24-31; 1:100 dilution), TotalSeq-C0155-CD107a (H4A3; 1:200 dilution), TotalSeq-C0088-PD-1 (EH12.2H7; 1:400 dilution), TotalSeq-C0171-ICOS (C398.4A; 1:200 dilution), TotalSeq-C0063-CD45RA (HI100; 1:400 dilution), TotalSeq-C0148-CCR7 (G043H7; 1:100 dilution), TotalSeq-C0080-CD8 (RPA-T8; 1:400 dilution), TotalSeq-C0072-CD4 (RPA-T4; 1:400 dilution) and one of TotalSeq-C anti-human Hashtag antibodies (1-10; LNH-94, 2M2; 1:400 dilution) per donor and timepoint. All antibodies were from BioLegend. TotalSeq antibodies were prepared as per manufacturer instructions and staining was performed in a final volume of 50 µl for 30 min at 4 °C. After staining, cells were washed three times with FACS buffer, resuspended in 100 µl of FACS buffer, and transferred to a 1.5 ml RNase-free Microfuge tube. SYTOX Green (Thermo Fisher Scientific) was pre-diluted 1:60 in PBS + 0.04% BSA, then diluted 1:100 in each sample. Samples were stored at 4 °C until sorting.

Sorting was performed on a BD FACSAria III (BD Biosciences) using an 85-micron nozzle. All AIM$^+$ cells (**Fig. 1B, Fig. S15A**) from all individual samples were sorted into a single collection tube (RPMI-1640 [Sigma Aldrich] + 1% NEAA [Thermo Fisher Scientific] + 1% Na Pyruvate [Thermo Fisher Scientific] + 2.5% HEPES [Thermo Fisher Scientific] + 10% FBS [Sigma Aldrich]), washed twice in collection media and resuspended at 13,700 cells per 38.7 µl of resuspension buffer in preparation for the 10x Genomics Chromium Next GEM Single Cell 5’ v2 (Dual Index) workflow.

For flow cytometry-based characterization of AIM$^+$ T cells, stimulations were performed as described above. Stimulation with an MHC-II-optimized immunodominant peptide pool for cytomegalovirus, Epstein Barr virus, influenza and tetanus toxoid (CEFT-II; JPT

672 Peptide Technologies; catalog number PM-CEFT-MHC-II-1) was included as an
673 additional stimulation. Samples were collected after 12 or 24 hours of stimulation. After
674 incubation, plates were centrifuged at 706 *g* for 2 min, washed in FACS buffer, and
675 centrifuged again at 706 *g* for 2 min prior to staining. Chemokine receptor staining was
676 performed for 30 min at 37 °C with: PerCP-Cy5.5-CCR6 (G034E3; 1:50 dilution),
677 BV786-CCR7 (G045H7; 1:50 dilution), BUV395-CXCR3 (1C6; 1:50 dilution), PE-Cy7-
678 CCR4 (L291H4; 1:50 dilution). Cells were washed once, and surface stained with: FITC-
679 Vδ1-TCR (REAL277; 1:100 dilution), BV421-CD69 (FN50; 1:100 dilution), BV510-
680 CD45RA (HI100; 1:100 dilution), BV605-Vα7.2-TCR (3C10; 1:100 dilution), BV711-Vδ2-
681 TCR (B6; 1:100 dilution), BUV563-CD3 (UCHT1; 1:100 dilution), BUV737-CD127 (HIL-
682 7R-M21; 1:100 dilution), APC-CD161 (191B8; 1:100 dilution), AF700-CD8α (SK1; 1:100
683 dilution), Near-IR vital exclusion dye (1:400 dilution), APC-Cy7-CD19 (HIB19; 1:100
684 dilution), APC-Cy7-CD14 (M5E2; 1:100 dilution), PE-OX-40 (ACT35; 1:100 dilution),
685 PE-4-1BB (4B4-1; 1:100 dilution), and PE-Cy5-CD4 (OKT4; 1:100 dilution). Staining
686 was performed in a final volume of 50 μl for 30 min at 4 °C. Cells were washed once
687 and resuspended in 200 μl True-Nuclear 1× Fix Concentrate (BioLegend) and incubated
688 light-protected for 60 min at room temperature. Cells were washed twice with True-
689 Nuclear 1× Perm Buffer (BioLegend). Transcription factor staining for FoxP3 (PE-
690 CF594, clone 206D; 1:100 dilution) was performed in a final volume of 50 μl for 30 min
691 at 4 °C. Samples were washed twice with True-Nuclear 1× Perm Buffer, resuspended in
692 FACS buffer and stored at 4 °C until data acquisition on a BD LSRFortessa X-20 flow
693 cytometer (BD Biosciences). Flow cytometry data was analyzed in FlowJo v10 (BD
694 Biosciences).

695 **CellTrace Violet assay to detect pre-existing SARS-CoV-2 specific clones**

696 Given its high sensitivity to detect pre-existing SARS-CoV-2 spike cross-reactive T cells,
697 we used a CellTrace Violet (CTV) dilution assay (*36*). One vial of cryopreserved PBMCs
698 per vaccinee at the pre-vaccination (baseline; "T0") timepoint was thawed and washed
699 in R10 (RPMI-1640 [Sigma Aldrich] + 10% FBS [Sigma Aldrich] + 1%
700 Penicillin/Streptomycin [Sigma Aldrich]) + Benzonase (500 U; Sigma Aldrich) on the day
701 of use. 5.25×10^6 cells per donor were collected and labeled with CTV, as described
702 (*36*). Briefly, cells were pelleted and washed twice with sterile PBS. After washing, cells

703 were resuspended in 1 ml of PBS and 0.5 µl of CTV (Thermo Fisher Scientific) was
704 added (1:2000 dilution). Cells were stained for 10 minutes at room temperature and the
705 reaction was quenched using 4 ml of ice-cold FBS. Media was removed and cells were
706 resuspended in 5 ml of RhuAB10 media (RPMI-1640 [Sigma Aldrich] + 10% human AB
707 serum [Thermo Fisher Scientific] + 1% Penicillin/Streptomycin [Sigma Aldrich]). Cells
708 were allowed to rest for 5 min to allow excess CTV to leach away, counted, and
709 resuspended in fresh RhuAB10 media at 2.5×10^6 cells per ml.

710 Cells (2.5×10^5) were added to wells of a 96-well U-bottom plate. Unstimulated control
711 (containing DMSO; Sigma Aldrich) and positive control (PHA at 2 µg/ml; Sigma Aldrich)
712 wells were included. The remainder of the cells were added in sequential wells so that
713 all cells were used and stimulated with S1 + S2 peptide pools (JPT Peptide
714 Technologies GmbH; catolog number PM-WCPV-S-2) (each at 1 µg/ml) in a final
715 volume of 200 µl. Cells were placed in a 37 °C, 5% CO_2 incubator for 7 days. On day 4,
716 the plate was centrifuged (706 *g* for 3 min) and 100 µl of media was removed and
717 replaced with fresh, pre-warmed RhuAB10 media.

718 On day 7, the plate was centrifuged (706 *g* for 2 min), and all media was removed. Cells
719 were washed in FACS buffer and wells were merged (3 into 1) to reduce the number of
720 wells to stain. Staining was performed for 30 min at 4 °C using a cocktail of: FITC-CD19
721 (HIB19; 1:100 dilution), FITC-CD14 (M5E2; 1:100 dilution), AF700-CD8α (SK1; 1:100
722 dilution), BV650-CD4 (OKT4; 1:100 dilution), BV785-CD3 (OKT3; 1:100 dilution), and
723 one of TotalSeq-C anti-human Hashtag antibodies (1-8; LNH-94, 2M2; 1:400 dilution)
724 per donor. TotalSeq-C antibodies were prepared as described above. After staining,
725 cells were washed three times with FACS buffer, resuspended in 100 µl of FACS buffer,
726 and transferred to a 1.5 ml RNase-free Microfuge tube (Axygen Scientific). SYTOX
727 Green (Thermo Fisher Scientific) was pre-diluted 1:60 in PBS + 0.04% BSA, then
728 diluted 1:100 in each sample. Samples were stored at 4 °C until sorting.

729 Sorting was performed as per AIM$^+$, using the gating highlighted in **Fig. 3A and Fig.
730 S15B.**

731 Flow cytometry data was analyzed in FlowJo v10 (BD Biosciences).

732 **Single-cell RNA-sequencing library preparation and sequencing**

733 Samples were loaded onto a 10x Chromium Controller at an average of 23,000 cells per
734 channel (28 channels total). Gene expression, cell surface protein (antibody-derived
735 tag, ADT) and TCR sequencing libraries were prepared as per manufacturer guidelines,
736 and sequencing was performed on an Illumina Novaseq 6000 as per the manufacturer's
737 instructions (Wellcome Sanger Institute, Cambridge, UK).

738 **Data analysis methods**

739 *Statistical comparisons*

740 Where indicated in the figure legends, parametric and non-parametric statistical
741 comparisons were performed after assessing the data for normality by direct
742 visualization of the data distribution and a Shapiro-Wilk test when sample sizes
743 permitted. Boxplots are median and interquartile range and whiskers are 1.5x
744 interquartile range. Statistical tests used are listed in figure legends.

745 *Read mapping, quality control, hashtag demultiplexing and clustering*

746 FASTQ files were generated from BCL files using Illumina bcl2fastq. FASTQ files for all
747 modalities were mapped to the GRCh38-2020-A reference genome and a custom ADT
748 marker list using the Cell Ranger 7.0.0 multi pipeline for count, ADT and VDJ data. The
749 filtered_contig_annotations.csv file was filtered to retain only high-confidence, full-
750 length, productive contigs corresponding to TCRα or TCRβ chains.

751 Data analysis was primarily performed using the Seurat package (v4.3.0) (*49*) in R
752 (v4.3.0). Quality control was performed as follows: low quality cells were removed
753 (based on low UMI and gene count and high percent mitochondrial reads), doublets
754 were flagged and removed using scDblFinder (*50*). Immunoglobulin and TCR gene
755 segments, identified from the IMGT database (imgt.org), were removed from the gene
756 expression object, except for the following genes: *IGHM, IGHG1, IGHG2, IGHG3,*
757 *IGHG4, IGHD, IGHE, IGHA1, TRAV1-2, TRAV24, TRDV1, TRDV2, TRDV3*. Hashtags
758 for each unique individual within a single experiment were demultiplexed using
759 MULTIseqDemux (*51*) and then data from each experiment were merged into a single
760 Seurat object. Gene counts were log-normalized (NormalizeData function), variable

761 features were found (n=2000, FindVariableFeatures function) and features were scaled
762 using default Seurat parameters (ScaleData function). The top variable features were
763 used for nearest-neighbor and Louvain clustering (resolution 0.7) and to generate
764 principal components, the top 30 of which were used to generate uniform manifold
765 approximation and projections (UMAP). One individual was identified in the vaccine
766 cohort that had potential previous asymptomatic COVID-19 infection. A sensitivity
767 analysis was performed that indicated that this individual did not differ from other
768 individuals in the vaccine group at either T1 or T2, consistent with a separate report
769 (*52*), therefore the potentially previously infected donor was retained for further analysis.

770 Following Louvain clustering and manual annotation of identified cell clusters, several
771 developmentally distinct cell populations were found to co-cluster based on gene
772 expression and were manually separated as follows: MAIT_2 were separated from the
773 co-clustered VD2 and CD8_Tem populations based on co-expression of *SLC4A10* and
774 *TRAV1-2*; VD1_Eff were separated from the co-clustered CD8_IFNG population based
775 on lack of expression of $\alpha\beta$ TCR chains.

776 *Baseline spike-responsive T cell data pre-processing*

777 One individual was removed from the baseline spike-responsive T cell data analysis
778 due to detection of potential previous SARS-CoV-2 infection. Single-cell RNA-
779 sequencing data on CTV^{lo} baseline reactive T cells underwent QC as per above.
780 Integration of data from different experiments (one donor per experimental group was
781 included per experiment) was performed using Seurat IntegrateData with 3000 variable
782 features selected. The integrated data slot was used for subsequent data visualization
783 as appropriate. As in the AIM^{+} spike-responsive T cells, after Louvain clustering, we
784 identified developmentally distinct populations which co-clustered. We therefore
785 separated the VD2 population from CD8_IFNG and CD8_GZMA populations based on
786 lack of expression of $\alpha\beta$ TCR chains.

787 *Differential abundance analysis*

788 Differential abundance analysis was performed using the edgeR package (v3.42.2) (*53*),
789 with the function glmQLFtest with flags robust=TRUE and abundance.trend=FALSE and
790 dispersions estimated using estimateDisp with the flag trend=“none”.

791 *Differential gene expression and geneset enrichment, overrepresentation and variation*
792 *analysis*

793 For comparisons between individuals in different groups, differentially expressed genes
794 were defined using Wilcoxon rank sum test with p values corrected using Bonferroni
795 correction based on the number of genes in the dataset which passed filtering with an
796 average fold-change of >0.05 and minimum percent expression within each compared
797 cluster of 25% (FindMarkers function). For within-individual comparisons (e.g. those
798 across timepoints), differential expression of genes was assessed using the Seurat
799 FindMarkers function with the test.use=“LR” flag, with individual as a latent variable.

800 For analysis of the interactions between vaccine, interval and timepoint, sex and age,
801 mixed-effects linear models with estimated precision weights were assessed using the
802 dream method of variancePartition (v1.3.0) (*54*), based on analysis performed in (*55*).
803 Briefly, gene counts within a given individual * timepoint * cluster combination were
804 aggregated using the edgeR function Seurat2PB, were filtered by expression with
805 filterByExpr with min.count=5 and min.total.count=15, and normalized library sizes
806 generated. voomWithDreamWeights was used to normalize data and estimate precision
807 weights for dream analysis. Dream analysis was performed using the formula ~ 0 +
808 group.time + Age + Sex + (1|ID) to estimate regression coefficients for each gene and
809 empirical bayes shrinkage was applied using the eBayes function of variancePartition.

810 Genesets used for GSEA and overrepresentation analyses were obtained from Human
811 MSigDB Hallmark or the Gene Ontology Biological Process database and filtered to
812 include genesets related to cytokines, T helper and cytotoxic subsets, and antigen
813 presentation pathways. A $CD8^{+}$ T cell cytotoxicity associated geneset was obtained
814 from (*29*). Stimulated MAIT cell genesets were obtained from (*26*) by taking the top 100
815 differentially expressed genes from sorted MAIT cells that were most differentiated from
816 unstimulated cells after stimulation with a TCR (MR1/5-OP-RU), cytokine (IL-12 and IL-
817 18), or TCR and cytokine stimulus. Gene lists were pre-ranked based on either average

818 log fold change of differentially expressed genes or using the scaled coefficients (z.std)
819 resulting from the contrasts applied following dream analysis. GSEA and
820 overrepresentation analysis were performed using the fgsea function within the fgsea
821 (v1.26.0) (*56*) or clusterProfiler (v4.8.1) packages (*57*). Published genesets related to
822 $CD8^+$ T cell cytotoxicity (*29*) were quantified using the Seurat AddModuleScore function
823 with default parameters, or with geneset variation analysis (*58*) on aggregate, pseudo-
824 bulked counts of the relevant cell type.

825 *CellPhoneDB*

826 To find putative cell-cell interactions, CellPhoneDB (v5, (*59*)) was applied to the entire
827 pre-processed and filtered dataset, or subsets of cells including only BNT162b2 or
828 ChAdOx1 nCoV-19 vaccinated individuals. The Seurat package FindAllMarkers function
829 was used to define differentially expressed genes for each cluster within each dataset
830 with flags only.pos=T, random.seed=1, logfc.threshold=0.25, return.thresh=0.05,
831 min.pct=0.25. The function pdb_degs_analysis_method with threshold=0.1 was used to
832 find interactions between celltypes. Downstream visualization and analysis were
833 performed in R (v4.3.0).

834 *TCR analysis*

835 Paired αβ TCR chains were compiled for each cell using the scRepertoire package
836 (v2.0.4), which loads filtered_contig_annotation files (output from 10x CellRanger multi)
837 and combines them with the Seurat object for downstream analysis. Briefly,
838 createHTOContigList was used with groups based on the called hashtag IDs and
839 combineTCR with default settings. Clones were called based on paired CDR3 amino
840 acid chain calling within each individual at each timepoint. Visualization of overlapping
841 TCR clones was performed using the circlize package (v0.4.15) (*60*). Visualization of
842 selected TCR amino acid sequences motifs was performed using the ggseqlogo
843 package (v0.1). For analysis over time, clones were called as those with identical paired
844 αβ TCR chains, and 'recalled clones' were defined as those present at more than one
845 timepoint.

846 To identify the relationship between TCR physiochemical properties and gene
847 expression, and identify clones enriched in our dataset compared with simulated
848 ‘background’ TCR repertoires, we performed analysis using the CoNGA package
849 (v0.1.2) (*61*) in Python (v3.9). The filtered and normalized Seurat object was exported
850 from R into an anndata object using the DropletUtils package (v1.20.0) (*62*). Variable
851 features were found and data was scaled using conga.preprocess.filter_and_scale, with
852 parameters set to include all previously filtered cells. Cells were reduced to a single
853 clone using reduce_to_single_cell_per_clone with default settings, which returns a
854 single representative clone using a PCA-based approach to identify the single cell within
855 a paired αβ TCR amino-acid clonotype with the least average gene expression
856 difference to other cells within a clonotype. Within the CoNGA package, TCRdist was
857 used to quantify physiochemical scores of the TCRs of each clonotype and find the
858 distance in TCR-space between two independent TCR clonotypes. This metric was then
859 used in kernel PCA to reduce the cell x cell TCRdist matrix. The 50 top TCRdist kernel
860 PCs were retained for downstream clustering and dimensionality reduction, alongside
861 the top 40 PCs of the variable gene expression. Neighborhoods of gene and TCRdist
862 similarity were defined using conga.preprocess.calc_nbrs, with
863 nbr_frac_for_nndists=0.01 and nbr_fracs=[0.01,0.1]. Comparison of the TCRdist and
864 gene expression nearest neighbor graphs was then performed to compare gene
865 expression and TCR distancing metrics with conga.correlations.run_graph_vs_graph.
866 Significant CoNGA scores represent clonotypes that are present in neighborhoods with
867 significant overlap in gene expression and TCR space.

868 To identify TCR meta-clonotypes that were likely to be antigen-enriched, we performed
869 TCR clumping analysis using conga.tcr_clumping.assess_tcr_clumping with default
870 settings. This analysis simulates a background VDJ repertoire representative of a
871 simple model of VDJ recombination and tests whether individual TCR neighborhoods
872 are overrepresented in the observed data compared with this null model of VDJ
873 recombination. Meta-clonotypes with >10 participating clonotypes were selected for
874 further analysis, and clustalOmega within the msa (v1.32.0) package was used to derive
875 consensus sequences for αβ CDR3 regions separately.

876 **Supplementary Materials**

877 Figures S1 to S15

878 Tables S1 and S2

879 Data file S1

880 MDAR Reproducibility checklist

881 **References and Notes**

882 1. S. Feng, D. J. Phillips, T. White, H. Sayal, P. K. Aley, S. Bibi, C. Dold, M.
883 Fuskova, S. C. Gilbert, I. Hirsch, H. E. Humphries, B. Jepson, E. J. Kelly,
884 E. Plested, K. Shoemaker, K. M. Thomas, J. Vekemans, T. L. Villafana, T.
885 Lambe, A. J. Pollard, M. Voysey, S. Adlou, L. Allen, B. Angus, R. Anslow,
886 M.-C. Asselin, N. Baker, P. Baker, T. Barlow, A. Beveridge, K. R. Bewley,
887 P. Brown, E. Brunt, K. R. Buttigieg, S. Camara, S. Charlton, E. Chiplin, P.
888 Cicconi, E. A. Clutterbuck, A. M. Collins, N. S. Coombes, S. A. C.
889 Clemens, M. Davison, T. Demissie, T. Dinesh, A. D. Douglas, C. J. A.
890 Duncan, K. R. W. Emary, K. J. Ewer, S. Felle, D. M. Ferreira, A. Finn, P.
891 M. Folegatti, R. Fothergill, S. Fraser, H. Garlant, L. Gatcombe, K. J.
892 Godwin, A. L. Goodman, C. A. Green, B. Hallis, T. C. Hart, P. T. Heath, H.
893 Hill, A. V. S. Hill, D. Jenkin, M. Kasanyinga, S. Kerridge, C. Knight, S.
894 Leung, V. Libri, P. J. Lillie, S. Marinou, J. McGlashan, A. C. McGregor, L.
895 McInroy, A. M. Minassian, Y. F. Mujadidi, E. J. Penn, C. J. Petropoulos, K.
896 M. Pollock, P. C. Proud, S. Provstgaard-Morys, D. Rajapaska, M. N.
897 Ramasamy, K. Sanders, I. Shaik, N. Singh, A. Smith, M. D. Snape, R.
898 Song, S. Shrestha, R. K. Sutherland, E. C. Thomson, D. P. J. Turner, A.
899 Webb-Bridges, T. Wrin, C. J. Williams, C. V. T. G. the Oxford, Correlates
900 of protection against symptomatic and asymptomatic SARS-CoV-2
901 infection. *Nature Medicine* **27**, 2032-2040 (2021).
902 2. M. J. Scurr, G. Lippiatt, L. Capitani, K. Bentley, S. N. Lauder, K. Smart,
903 M. S. Somerville, T. Rees, R. J. Stanton, A. Gallimore, J. P. Hindley, A.
904 Godkin, Magnitude of venous or capillary blood-derived SARS-CoV-2-
905 specific T cell response determines COVID-19 immunity. *Nature*
906 *Communications* **13**, 5422 (2022).
907 3. C. Dong, Cytokine Regulation and Function in T Cells. *Annual Review of*
908 *Immunology* **39**, 51-76 (2021).
909 4. A. S. V. Stuart, R. H. Shaw, X. Liu, M. Greenland, P. K. Aley, N. J.
910 Andrews, J. C. Cameron, S. Charlton, E. A. Clutterbuck, A. M. Collins, T.
911 Darton, T. Dinesh, C. J. A. Duncan, A. England, S. N. Faust, D. M.
912 Ferreira, A. Finn, A. L. Goodman, C. A. Green, B. Hallis, P. T. Heath, H.
913 Hill, B. M. Horsington, T. Lambe, R. Lazarus, V. Libri, P. J. Lillie, Y. F.
914 Mujadidi, R. Payne, E. L. Plested, S. Provstgaard-Morys, M. N.
915 Ramasamy, M. Ramsay, R. C. Read, H. Robinson, G. R. Screaton, N.
916 Singh, D. P. J. Turner, P. J. Turner, I. Vichos, R. White, J. S. Nguyen-Van-

917 Tam, M. D. Snape, Immunogenicity, safety, and reactogenicity of
918 heterologous COVID-19 primary vaccination incorporating mRNA, viral-
919 vector, and protein-adjuvant vaccines in the UK (Com-COV2): a single-
920 blind, randomised, phase 2, non-inferiority trial. *Lancet* **399**, 36-49
921 (2022).
922 5. R. H. Shaw, X. Liu, A. S. V. Stuart, M. Greenland, P. K. Aley, N. J.
923 Andrews, J. C. Cameron, S. Charlton, E. A. Clutterbuck, A. M. Collins, W.
924 Dejnirattisai, T. Dinesh, S. N. Faust, D. M. Ferreira, A. Finn, C. A. Green,
925 B. Hallis, P. T. Heath, H. Hill, T. Lambe, R. Lazarus, V. Libri, F. Long, Y.
926 F. Mujadidi, E. L. Plested, E. R. Morey, S. Provstgaard-Morys, M. N.
927 Ramasamy, M. Ramsay, R. C. Read, H. Robinson, G. R. Screaton, N.
928 Singh, D. P. J. Turner, P. J. Turner, I. Vichos, L. L. Walker, R. White, J. S.
929 Nguyen-Van-Tam, M. D. Snape, Effect of priming interval on
930 reactogenicity, peak immunological response, and waning after
931 homologous and heterologous COVID-19 vaccine schedules:
932 exploratory analyses of Com-COV, a randomised control trial. *Lancet*
933 *Respir Med* **10**, 1049-1060 (2022).
934 6. X. Liu, R. H. Shaw, A. S. V. Stuart, M. Greenland, P. K. Aley, N. J.
935 Andrews, J. C. Cameron, S. Charlton, E. A. Clutterbuck, A. M. Collins, T.
936 Dinesh, A. England, S. N. Faust, D. M. Ferreira, A. Finn, C. A. Green, B.
937 Hallis, P. T. Heath, H. Hill, T. Lambe, R. Lazarus, V. Libri, F. Long, Y. F.
938 Mujadidi, E. L. Plested, S. Provstgaard-Morys, M. N. Ramasamy, M.
939 Ramsay, R. C. Read, H. Robinson, N. Singh, D. P. J. Turner, P. J. Turner,
940 L. L. Walker, R. White, J. S. Nguyen-Van-Tam, M. D. Snape, Safety and
941 immunogenicity of heterologous versus homologous prime-boost
942 schedules with an adenoviral vectored and mRNA COVID-19 vaccine
943 (Com-COV): a single-blind, randomised, non-inferiority trial. *Lancet*
944 **398**, 856-869 (2021).
945 7. A. Y. Collier, J. Yu, K. McMahan, J. Liu, A. Chandrashekar, J. S. Maron, C.
946 Atyeo, D. R. Martinez, J. L. Ansel, R. Aguayo, M. Rowe, C. Jacob-Dolan,
947 D. Sellers, J. Barrett, K. Ahmad, T. Anioke, H. VanWyk, S. Gardner, O.
948 Powers, E. A. Bondzie, H. Wan, R. S. Baric, G. Alter, M. R. Hacker, D. H.
949 Barouch, Differential Kinetics of Immune Responses Elicited by Covid-
950 19 Vaccines. *N Engl J Med* **385**, 2010-2012 (2021).
951 8. H. Parry, R. Bruton, C. Stephens, K. Brown, G. Amirthalingam, A. Otter,
952 B. Hallis, J. Zuo, P. Moss, Differential immunogenicity of BNT162b2 or
953 ChAdOx1 vaccines after extended-interval homologous dual
954 vaccination in older people. *Immun Ageing* **18**, 34 (2021).
955 9. L. Henze, J. Braun, L. Meyer-Arndt, K. Jürchott, M. Schlotz, J. Michel, M.
956 Grossegesse, M. Mangold, M. Dingeldey, B. Kruse, P. Holenya, N.
957 Mages, U. Reimer, M. Eckey, K. Schnatbaum, H. Wenschuh, B.
958 Timmermann, F. Klein, A. Nitsche, C. Giesecke-Thiel, L. Loyal, A. Thiel,
959 Primary ChAdOx1 vaccination does not reactivate pre-existing, cross-
960 reactive immunity. *Frontiers in Immunology* **14**, (2023).
961 10. F. T. T. Lai, M. Fan, C. Huang, C. S. L. Chui, E. Y. F. Wan, X. Li, C. K. H.
962 Wong, C.-L. Cheung, I. C. K. Wong, E. W. Y. Chan, Effectiveness of

963 BNT162b2 after extending the primary series dosing interval in
964 children and adolescents aged 5–17. *Nature Communications* **14**, 1845
965 (2023).
966 11. M. Voysey, S. A. Costa Clemens, S. A. Madhi, L. Y. Weckx, P. M.
967 Folegatti, P. K. Aley, B. Angus, V. L. Baillie, S. L. Barnabas, Q. E. Bhorat,
968 S. Bibi, C. Briner, P. Cicconi, E. A. Clutterbuck, A. M. Collins, C. L.
969 Cutland, T. C. Darton, K. Dheda, C. Dold, C. J. A. Duncan, K. R. W.
970 Emary, K. J. Ewer, A. Flaxman, L. Fairlie, S. N. Faust, S. Feng, D. M.
971 Ferreira, A. Finn, E. Galiza, A. L. Goodman, C. M. Green, C. A. Green, M.
972 Greenland, C. Hill, H. C. Hill, I. Hirsch, A. Izu, D. Jenkin, C. C. D. Joe, S.
973 Kerridge, A. Koen, G. Kwatra, R. Lazarus, V. Libri, P. J. Lillie, N. G.
974 Marchevsky, R. P. Marshall, A. V. A. Mendes, E. P. Milan, A. M.
975 Minassian, A. McGregor, Y. F. Mujadidi, A. Nana, S. D. Padayachee, D. J.
976 Phillips, A. Pittella, E. Plested, K. M. Pollock, M. N. Ramasamy, A. J.
977 Ritchie, H. Robinson, A. V. Schwarzbold, A. Smith, R. Song, M. D.
978 Snape, E. Sprinz, R. K. Sutherland, E. C. Thomson, M. E. Török, M.
979 Toshner, D. P. J. Turner, J. Vekemans, T. L. Villafana, T. White, C. J.
980 Williams, A. D. Douglas, A. V. S. Hill, T. Lambe, S. C. Gilbert, A. J.
981 Pollard, Single-dose administration and the influence of the timing of
982 the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19
983 (AZD1222) vaccine: a pooled analysis of four randomised trials. *Lancet*
984 **397**, 881-891 (2021).
985 12. V. Hall, S. Foulkes, F. Insalata, P. Kirwan, A. Saei, A. Atti, E. Wellington,
986 J. Khawam, K. Munro, M. Cole, C. Tranquillini, A. Taylor-Kerr, N.
987 Hettiarachchi, D. Calbraith, N. Sajedi, I. Milligan, Y. Themistocleous, D.
988 Corrigan, L. Cromey, L. Price, S. Stewart, E. d. Lacy, C. Norman, E.
989 Linley, A. D. Otter, A. Semper, J. Hewson, S. D'Arcangelo, M. Chand, C.
990 S. Brown, T. Brooks, J. Islam, A. Charlett, S. Hopkins, Protection against
991 SARS-CoV-2 after Covid-19 Vaccination and Previous Infection. *New*
992 *England Journal of Medicine* **386**, 1207-1220 (2022).
993 13. M. Voysey, S. A. Costa Clemens, S. A. Madhi, L. Y. Weckx, P. M.
994 Folegatti, P. K. Aley, B. Angus, V. L. Baillie, S. L. Barnabas, Q. E. Bhorat,
995 S. Bibi, C. Briner, P. Cicconi, E. A. Clutterbuck, A. M. Collins, C. L.
996 Cutland, T. C. Darton, K. Dheda, C. Dold, C. J. A. Duncan, K. R. W.
997 Emary, K. J. Ewer, A. Flaxman, L. Fairlie, S. N. Faust, S. Feng, D. M.
998 Ferreira, A. Finn, E. Galiza, A. L. Goodman, C. M. Green, C. A. Green, M.
999 Greenland, C. Hill, H. C. Hill, I. Hirsch, A. Izu, D. Jenkin, C. C. D. Joe, S.
1000 Kerridge, A. Koen, G. Kwatra, R. Lazarus, V. Libri, P. J. Lillie, N. G.
1001 Marchevsky, R. P. Marshall, A. V. A. Mendes, E. P. Milan, A. M.
1002 Minassian, A. McGregor, Y. F. Mujadidi, A. Nana, S. D. Padayachee, D. J.
1003 Phillips, A. Pittella, E. Plested, K. M. Pollock, M. N. Ramasamy, A. J.
1004 Ritchie, H. Robinson, A. V. Schwarzbold, A. Smith, R. Song, M. D.
1005 Snape, E. Sprinz, R. K. Sutherland, E. C. Thomson, M. E. Török, M.
1006 Toshner, D. P. J. Turner, J. Vekemans, T. L. Villafana, T. White, C. J.
1007 Williams, A. D. Douglas, A. V. S. Hill, T. Lambe, S. C. Gilbert, A. J.
1008 Pollard, M. Aban, K. W. M. Abeyskera, J. Aboagye, M. Adam, K. Adams,

1009 J. P. Adamson, G. Adewatan, S. Adlou, K. Ahmed, Y. Akhalwaya, S.
1010 Akhalwaya, A. Alcock, A. Ali, E. R. Allen, L. Allen, F. B. Alvernaz, F. S.
1011 Amorim, C. S. Andrade, F. Andritsou, R. Anslow, E. H. Arbe-Barnes, M.
1012 P. Ariaans, B. Arns, L. Arruda, L. Assad, P. D. A. Azi, L. D. A. Azi, G.
1013 Babbage, C. Bailey, K. F. Baker, M. Baker, N. Baker, P. Baker, I.
1014 Baleanu, D. Bandeira, A. Bara, M. A. S. Barbosa, D. Barker, G. D.
1015 Barlow, E. Barnes, A. S. Barr, J. R. Barrett, J. Barrett, K. Barrett, L.
1016 Bates, A. Batten, K. Beadon, E. Beales, R. Beckley, S. Belij-
1017 Rammerstorfer, J. Bell, D. Bellamy, S. Belton, A. Berg, L. Bermejo, E.
1018 Berrie, L. Berry, D. Berzenyi, A. Beveridge, K. R. Bewley, I. Bharaj, S.
1019 Bhikha, A. E. Bhorat, Z. E. Bhorat, E. M. Bijker, S. Birch, G. Birch, K.
1020 Birchall, A. Bird, O. Bird, K. Bisnauthsing, M. Bittaye, L. Blackwell, R.
1021 Blacow, H. Bletchly, C. L. Blundell, S. R. Blundell, P. Bodalia, E. Bolam,
1022 E. Boland, D. Bormans, N. Borthwick, F. Bowring, A. Boyd, P. Bradley, T.
1023 Brenner, A. Bridges-Webb, P. Brown, C. Brown, C. Brown-O'Sullivan, S.
1024 Bruce, E. Brunt, W. Budd, Y. A. Bulbulia, M. Bull, J. Burbage, A. Burn, K.
1025 R. Buttigieg, N. Byard, I. Cabrera Puig, A. Calvert, S. Camara, M. Cao, F.
1026 Cappuccini, R. Cardona, J. R. Cardoso, M. Carr, M. W. Carroll, A. Carson-
1027 Stevens, Y. d. M. Carvalho, H. R. Casey, P. Cashen, T. R. Y. Castro, L. C.
1028 Castro, K. Cathie, A. Cavey, J. Cerbino-Neto, L. F. F. Cezar, J. Chadwick,
1029 C. Chanice, D. Chapman, S. Charlton, K. S. Cheliotis, I. Chelysheva, O.
1030 Chester, E. Chiplin, S. Chita, J.-S. Cho, L. Cifuentes, E. Clark, M. Clark, R.
1031 Colin-Jones, S. L. K. Collins, H. Colton, C. P. Conlon, S. Connarty, N.
1032 Coombes, C. Cooper, R. Cooper, L. Cornelissen, T. Corrah, C. A.
1033 Cosgrove, F. B. Costa, T. Cox, W. E. M. Crocker, S. Crosbie, D. Cullen, D.
1034 R. M. F. Cunha, C. J. Cunningham, F. C. Cuthbertson, D. M. da Costa, S.
1035 N. F. Da Guarda, L. P. da Silva, A. C. da Silva Moraes, B. E. Damratoski,
1036 Z. Danos, M. T. D. C. Dantas, M. S. Datoo, C. Datta, M. Davids, S. L.
1037 Davies, K. Davies, H. Davies, S. Davies, J. Davies, E. J. Davis, J. Davis, J.
1038 A. M. de Carvalho, J. De Jager, S. de Jesus Jnr, L. M. De Oliveira Kalid, D.
1039 Dearlove, T. Demissie, A. Desai, S. Di Marco, C. Di Maso, T. Dinesh, C.
1040 Docksey, T. Dong, F. R. Donnellan, T. G. Dos Santos, T. G. Dos Santos,
1041 E. P. Dos Santos, N. Douglas, C. Downing, J. Drake, R. Drake-Brockman,
1042 R. Drury, J. Du Plessis, S. J. Dunachie, A. Duncan, N. J. W. Easom, M.
1043 Edwards, N. J. Edwards, F. Edwards, O. M. El Muhanna, S. C. Elias, B.
1044 Ellison-Handley, M. J. Elmore, M. R. English, A. Esmail, Y. M. Essack, M.
1045 Farooq, S. Fedosyuk, S. Felle, S. Ferguson, C. Ferreira Da Silva, S. Field,
1046 R. Fisher, J. Fletcher, H. Fofie, H. Fok, K. J. Ford, R. Fothergill, J. Fowler,
1047 P. H. A. Fraiman, E. Francis, M. M. Franco, J. Frater, M. S. M. Freire, S. H.
1048 Fry, S. Fudge, R. Furlan Filho, J. Furze, M. Fuskova, P. Galian-Rubio, H.
1049 Garlant, M. Gavrila, K. A. Gibbons, C. Gilbride, H. Gill, K. Godwin, K.
1050 Gokani, M. L. F. Gonçalves, I. G. S. Gonzalez, J. Goodall, J. Goodwin, A.
1051 Goondiwala, K. Gordon-Quayle, G. Gorini, A. Goyanna, J. Grab, L.
1052 Gracie, J. Green, N. Greenwood, J. Greffrath, M. M. Groenewald, A.
1053 Gunawardene, G. Gupta, M. Hackett, B. Hallis, M. Hamaluba, E.
1054 Hamilton, J. Hamlyn, D. Hammersley, A. T. Hanrath, B. Hanumunthadu,

1055 S. A. Harris, C. Harris, T. D. Harrison, D. Harrison, T. A. Harris-Wright, T.
1056 C. Hart, B. Hartnell, J. Haughney, S. Hawkins, L. Y. M. Hayano, I. Head,
1057 P. T. Heath, J. A. Henry, M. Hermosin Herrera, D. B. Hettle, C. Higa, J.
1058 Hill, G. Hodges, S. Hodgson, E. Horne, M. M. Hou, C. F. Houlihan, E.
1059 Howe, N. Howell, J. Humphreys, H. E. Humphries, K. Hurley, C. Huson,
1060 C. Hyams, A. Hyder-Wright, S. Ikram, A. Ishwarbhai, P. Iveson, V. Iyer,
1061 F. Jackson, S. Jackson, S. Jaumdally, H. Jeffers, N. Jesudason, C. Jones,
1062 C. Jones, K. Jones, E. Jones, M. R. Jorge, A. Joshi, E. A. M. S. Júnior, R.
1063 Kailath, F. Kana, A. Kar, K. Karampatsas, M. Kasanyinga, L. Kay, J. Keen,
1064 J. Kellett Wright, E. J. Kelly, D. Kelly, D. M. Kelly, S. Kelly, D. Kerr, L.
1065 Khan, B. Khozoee, A. Khurana, S. Kidd, A. Killen, J. Kinch, P. Kinch, L. D.
1066 W. King, T. B. King, L. Kingham, P. Klenerman, D. M. Kluczna, F.
1067 Knapper, J. C. Knight, D. Knott, S. Koleva, P. M. Lages, M. Lang, G.
1068 Lang, C. W. Larkworthy, J. P. J. Larwood, R. Law, A. M. Lawrie, E. M.
1069 Lazarus, A. Leach, E. A. Lees, A. Lelliott, N.-M. Lemm, A. E. R. Lessa, S.
1070 Leung, Y. Li, A. M. Lias, K. Liatsikos, A. Linder, S. Lipworth, S. Liu, X. Liu,
1071 A. Lloyd, S. Lloyd, L. Loew, R. Lopez Ramon, L. B. Lora, K. G. Luz, J. C.
1072 MacDonald, G. MacGregor, M. Madhavan, D. O. Mainwaring, E.
1073 Makambwa, R. Makinson, M. Malahleha, R. Malamatsho, G. Mallett, N.
1074 Manning, K. Mansatta, T. Maoko, S. Marinou, E. Marlow, G. N. Marques,
1075 P. Marriott, R. P. Marshall, J. L. Marshall, M. Masenya, M. Masilela, S. K.
1076 Masters, M. Mathew, H. Matlebjane, K. Matshidiso, O. Mazur, A.
1077 Mazzella, H. McCaughan, J. McEwan, J. McGlashan, L. McInroy, N.
1078 McRobert, S. McSwiggan, C. Megson, S. Mehdipour, W. Meijs, R. N. Õ.
1079 Mendonça, A. J. Mentzer, A. C. F. Mesquita, P. Miralhes, N. Mirtorabi, C.
1080 Mitton, S. Mnyakeni, F. Moghaddas, K. Molapo, M. Moloi, M. Moore, M.
1081 Moran, E. Morey, R. Morgans, S. J. Morris, S. Morris, H. Morrison, F.
1082 Morselli, G. Morshead, R. Morter, L. Mottay, A. Moultrie, N. Moyo, M.
1083 Mpelembue, S. Msomi, Y. Mugodi, E. Mukhopadhyay, J. Muller, A.
1084 Munro, S. Murphy, P. Mweu, C. Myerscough, G. Naik, K. Naker, E.
1085 Nastouli, B. Ndlovu, E. Nikolaou, C. Njenga, H. C. Noal, A. Noé, G.
1086 Novaes, F. L. Nugent, G. L. A. Nunes, K. O'Brien, D. O'Connor, S.
1087 Oelofse, B. Oguti, V. Olchawski, N. J. Oldfield, M. G. Oliveira, C. Oliveira,
1088 I. S. Q. Oliveira, A. Oommen-Jose, A. Oosthuizen, P. O'Reilly, P. J.
1089 O'Reilly, P. Osborne, D. R. J. Owen, L. Owen, D. Owens, N. Owino, M.
1090 Pacurar, B. V. B. Paiva, E. M. F. Palhares, S. Palmer, H. M. R. T.
1091 Parracho, K. Parsons, D. Patel, B. Patel, F. Patel, M. Patrick-Smith, R. O.
1092 Payne, Y. Peng, E. J. Penn, A. Pennington, M. P. Peralta Alvarez, B. P.
1093 Pereira Stuchi, A. L. Perez, T. Perinpanathan, J. Perring, R. Perumal, S.
1094 Y. Petkar, T. Philip, J. Phillips, M. K. Phohu, L. Pickup, S. Pieterse, J. M.
1095 Pinheiro, J. Piper, D. Pipini, M. Plank, S. Plant, S. Pollard, J. Pooley, A.
1096 Pooran, I. Poulton, C. Powers, F. B. Presa, D. A. Price, V. Price, M. R.
1097 Primeira, P. C. Proud, S. Provstgaard-Morys, S. Pueschel, D. Pulido, S.
1098 Quaid, R. Rabara, K. Radia, D. Rajapaska, T. Rajeswaran, L. Ramos, A.
1099 S. F. Ramos, F. Ramos Lopez, T. Rampling, J. Rand, H. Ratcliffe, T.
1100 Rawlinson, D. Rea, B. Rees, M. Resuello-Dauti, E. Reyes Pabon, S.

1101 Rhead, T. Riaz, M. Ricamara, A. Richards, A. Richter, N. Ritchie, A. J.
1102 Ritchie, A. J. Robbins, H. Roberts, R. E. Robinson, S. Roche, C. Rollier, L.
1103 Rose, A. L. Ross Russell, L. Rossouw, S. Royal, I. Rudiansyah, K. Ryalls,
1104 C. Sabine, S. Saich, J. C. Sale, A. M. Salman, N. Salvador, S. Salvador,
1105 M. D. Sampaio, A. D. Samson, A. Sanchez-Gonzalez, H. Sanders, K.
1106 Sanders, E. Santos, M. F. S. Santos Guerra, I. Satti, J. E. Saunders, C.
1107 Saunders, A. B. A. Sayed, I. Schim van der Loeff, A. B. Schmid, E.
1108 Schofield, G. R. Screaton, S. Seddiqi, R. R. Segireddy, R. Senger, S.
1109 Serrano, I. Shaik, H. R. Sharpe, K. Sharrocks, R. Shaw, A. Shea, E.
1110 Sheehan, A. Shepherd, F. Shiham, S. E. Silk, L. Silva-Reyes, L. B. T. D.
1111 Silveira, M. B. V. Silveira, N. Singh, J. Sinha, D. T. Skelly, D. C. Smith, N.
1112 Smith, H. E. Smith, D. J. Smith, C. C. Smith, A. S. Soares, C. Solórzano,
1113 G. L. Sorio, K. Sorley, T. Sosa-Rodriguez, C. M. C. D. L. Souza, B. S. D. F.
1114 Souza, A. R. Souza, T. Souza Lopez, L. Sowole, A. J. Spencer, L. Spoors,
1115 L. Stafford, I. Stamford, R. Stein, L. Stockdale, L. V. Stockwell, L. H.
1116 Strickland, A. Stuart, A. Sturdy, N. Sutton, A. Szigeti, A. Tahiri-Alaoui, R.
1117 Tanner, C. Taoushanis, A. W. Tarr, R. Tarrant, K. Taylor, U. Taylor, I. J.
1118 Taylor, J. Taylor, R. te Water Naude, K. Templeton, Y. Themistocleous,
1119 A. Themistocleous, M. Thomas, K. Thomas, T. M. Thomas, A.
1120 Thombrayil, J. Thompson, F. Thompson, A. Thompson, A. Thompson, K.
1121 Thompson, V. Thornton-Jones, L. H. S. Thotusi, P. J. Tighe, L. A. Tinoco,
1122 G. F. Tiongson, B. Tladinyane, M. Tomasicchio, A. Tomic, S. Tonks, J.
1123 Towner, N. Tran, J. A. Tree, G. Trillana, C. Trinham, R. Trivett, A. Truby,
1124 B. L. Tsheko, P. Tubb, A. Turabi, R. Turner, C. Turner, N. Turner, B.
1125 Tyagi, M. Ulaszewska, B. R. Underwood, S. van Eck, R. Varughese, D.
1126 Verbart, M. K. Verheul, I. Vichos, T. A. Vieira, G. Walker, L. Walker, M. E.
1127 Wand, T. Wardell, G. M. Warimwe, S. C. Warren, B. Watkins, M. E. E.
1128 Watson, E. Watson, S. Webb, A. Webster, J. Welch, Z. Wellbelove, J. H.
1129 Wells, A. J. West, B. White, C. White, R. White, P. Williams, R. L.
1130 Williams, S. Willingham, R. Winslow, D. Woods, M. Woodyer, A. T.
1131 Worth, D. Wright, M. Wroblewska, A. Yao, Y. T. N. Yim, M. B. Zambrano,
1132 R. L. Zimmer, D. Zizi, P. Zuidewind, Single-dose administration and the
1133 influence of the timing of the booster dose on immunogenicity and
1134 efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of
1135 four randomised trials. *The Lancet* **397**, 881-891 (2021).
1136 14. V. G. Hall, V. H. Ferreira, H. Wood, M. Ierullo, B. Majchrzak-Kita, K.
1137 Manguiat, A. Robinson, V. Kulasingam, A. Humar, D. Kumar, Delayed-
1138 interval BNT162b2 mRNA COVID-19 vaccination enhances humoral
1139 immunity and induces robust T cell responses. *Nat Immunol* **23**, 380-
1140 385 (2022).
1141 15. R. P. Payne, S. Longet, J. A. Austin, D. T. Skelly, W. Dejnirattisai, S.
1142 Adele, N. Meardon, S. Faustini, S. Al-Taei, S. C. Moore, T. Tipton, L. M.
1143 Hering, A. Angyal, R. Brown, A. R. Nicols, N. Gillson, S. L. Dobson, A.
1144 Amini, P. Supasa, A. Cross, A. Bridges-Webb, L. S. Reyes, A. Linder, G.
1145 Sandhar, J. A. Kilby, J. K. Tyerman, T. Altmann, H. Hornsby, R. Whitham,
1146 E. Phillips, T. Malone, A. Hargreaves, A. Shields, A. Saei, S. Foulkes, L.

1147 Stafford, S. Johnson, D. G. Wootton, C. P. Conlon, K. Jeffery, P. C.
1148 Matthews, J. Frater, A. S. Deeks, A. J. Pollard, A. Brown, S. L. Rowland-
1149 Jones, J. Mongkolsapaya, E. Barnes, S. Hopkins, V. Hall, C. Dold, C. J. A.
1150 Duncan, A. Richter, M. Carroll, G. Screaton, T. I. de Silva, L. Turtle, P.
1151 Klenerman, S. Dunachie, H. Abuelgasim, E. Adland, S. Adlou, H. D.
1152 Akther, A. Alhussni, M. Ali, M. A. Ansari, C. V. Arancibia-Cárcamo, M.
1153 Bayley, H. Brown, J. Chalk, M. Chand, A. Chawla, S. Chinnakannan, J.
1154 Cutteridge, C. de Lara, L. Denly, B. Diffey, S. Dimitriadis, T. M. Drake, T.
1155 Donnison, M. Dupont, D. Eyre, A. Fairman, S. Gardiner, J. Gilbert-
1156 Jarmillo, P. Goulder, C.-P. Hackstein, S. Hambleton, M. Haniffa, J.
1157 Haworth, J. Holmes, E. Horner, A. Jämsén, S. Johnson, C. Jones, M.
1158 Kasanyinga, S. Kelly, R. Kirk, M. L. Knight, A. Lawrie, L. Lee, L. Lett, K.
1159 Lillie, N. Lim, H. Mehta, A. J. Mentzer, D. O'Donnell, A. Ogbe, M. Pace, B.
1160 A. I. Payne, G. Platt, S. Poolan, N. Provine, N. Ramamurthy, N.
1161 Robinson, L. Romaniuk, P. Rongkard, O. L. Sampson, B. Simmons, J. S.
1162 Spegarova, E. Stephenson, K. Subramaniam, J. Thaventhiran, S.
1163 Thomas, S. Travis, S. Tucker, H. Turton, A. Watson, L. Watson, E.
1164 Weeks, R. Wilson, S. Wood, R. Wright, H. Xiao, A. A. T. Zawia,
1165 Immunogenicity of standard and extended dosing intervals of
1166 BNT162b2 mRNA vaccine. *Cell* **184**, 5699-5714.e5611 (2021).
1167 16. K. J. Ewer, J. R. Barrett, S. Belij-Rammerstorfer, H. Sharpe, R. Makinson,
1168 R. Morter, A. Flaxman, D. Wright, D. Bellamy, M. Bittaye, C. Dold, N. M.
1169 Provine, J. Aboagye, J. Fowler, S. E. Silk, J. Alderson, P. K. Aley, B.
1170 Angus, E. Berrie, S. Bibi, P. Cicconi, E. A. Clutterbuck, I. Chelysheva, P.
1171 M. Folegatti, M. Fuskova, C. M. Green, D. Jenkin, S. Kerridge, A. Lawrie,
1172 A. M. Minassian, M. Moore, Y. Mujadidi, E. Plested, I. Poulton, M. N.
1173 Ramasamy, H. Robinson, R. Song, M. D. Snape, R. Tarrant, M. Voysey,
1174 M. E. E. Watson, A. D. Douglas, A. V. S. Hill, S. C. Gilbert, A. J. Pollard, T.
1175 Lambe, T cell and antibody responses induced by a single dose of
1176 ChAdOx1 nCoV-19 (AZD1222) vaccine in a phase 1/2 clinical trial. *Nat
1177 Med* **27**, 270-278 (2021).
1178 17. S. L. Gray-Gaillard, S. M. Solis, H. M. Chen, C. Monteiro, G. Ciabattoni,
1179 M. I. Samanovic, A. R. Cornelius, T. Williams, E. Geesey, M. Rodriguez,
1180 M. B. Ortigoza, E. N. Ivanova, S. B. Koralov, M. J. Mulligan, R. S. Herati,
1181 SARS-CoV-2 inflammation durably imprints memory CD4 T cells. *Sci
1182 Immunol* **9**, eadj8526 (2024).
1183 18. Y. Gao, C. Cai, D. Wullimann, J. Niessl, O. Rivera-Ballesteros, P. Chen, J.
1184 Lange, A. Cuapio, O. Blennow, L. Hansson, S. Mielke, P. Nowak, J.
1185 Vesterbacka, M. Akber, A. Perez-Potti, T. Sekine, T. R. Müller, C.
1186 Boulouis, T. Kammann, T. Parrot, J. R. Muvva, M. Sobkowiak, K. Healy,
1187 G. Bogdanovic, S. Muschiol, G. Söderdahl, A. Österborg, F. Hellgren, A.
1188 Grifoni, D. Weiskopf, A. Sette, K. Loré, M. Sällberg Chen, P. Ljungman, J.
1189 K. Sandberg, C. I. E. Smith, P. Bergman, H. G. Ljunggren, S. Aleman, M.
1190 Buggert, Immunodeficiency syndromes differentially impact the
1191 functional profile of SARS-CoV-2-specific T cells elicited by mRNA
1192 vaccination. *Immunity* **55**, 1732-1746.e1735 (2022).

1193 19. A. Amini, L. C. Garner, R. H. Shaw, N. W. Kelly, S. Adele, D. T. Skelly, W.
1194 Dejnirattisai, M. Greenland, X. Liu, A. Heslington, C.-P. Hackstein, S. M.
1195 Murray, C. R. Vano, L. Stafford, S. Johnson, K. Sayaf, M. F.
1196 Pudjohartono, E. A. Clutterbuck, S. Bibi, C. P. Conlon, T. James, K.
1197 Jeffery, B. Kronsteiner, A. J. Mentzer, D. O'Shea, M. N. Ramasamy, G. R.
1198 Screaton, M. D. Snape, A. E. Hogan, E. Barnes, T. Lambe, S. J.
1199 Dunachie, N. M. Provine, P. Klenerman, MAIT and other innate-like T
1200 cells integrate adaptive immune responses to modulate interval-
1201 dependent reactogenicity to mRNA vaccines. *Sci Immunol*, (2025).
1202 20. G. Bowyer, T. Rampling, J. Powlson, R. Morter, D. Wright, A. V. S. Hill, K.
1203 J. Ewer, Activation-induced Markers Detect Vaccine-Specific CD4⁺ T Cell
1204 Responses Not Measured by Assays Conventionally Used in Clinical
1205 Trials. *Vaccines (Basel)* **6**, (2018).
1206 21. S. Reiss, A. E. Baxter, K. M. Cirelli, J. M. Dan, A. Morou, A. Daigneault, N.
1207 Brassard, G. Silvestri, J. P. Routy, C. Havenar-Daughton, S. Crotty, D. E.
1208 Kaufmann, Comparative analysis of activation induced marker (AIM)
1209 assays for sensitive identification of antigen-specific CD4 T cells. *PLoS*
1210 *One* **12**, e0186998 (2017).
1211 22. A. Lemieux, G. Sannier, A. Nicolas, M. Nayrac, G. G. Delgado, R.
1212 Cloutier, N. Brassard, M. Laporte, M. Duchesne, A. M. Sreng Flores, A.
1213 Finzi, O. Tastet, M. Dubé, D. E. Kaufmann, Enhanced detection of
1214 antigen-specific T cells by a multiplexed AIM assay. *Cell Rep Methods*
1215 **4**, 100690 (2024).
1216 23. B. Zhang, R. Upadhyay, Y. Hao, M. I. Samanovic, R. S. Herati, J. D. Blair,
1217 J. Axelrad, M. J. Mulligan, D. R. Littman, R. Satija, Multimodal single-cell
1218 datasets characterize antigen-specific CD8+ T cells across SARS-CoV-2
1219 vaccination and infection. *Nature Immunology* **24**, 1725-1734 (2023).
1220 24. N. M. Provine, P. Klenerman, MAIT Cells in Health and Disease. *Annu*
1221 *Rev Immunol* **38**, 203-228 (2020).
1222 25. B. E. Willcox, C. R. Willcox, γδ TCR ligands: the quest to solve a 500-
1223 million-year-old mystery. *Nature Immunology* **20**, 121-128 (2019).
1224 26. L. C. Garner, A. Amini, M. E. B. FitzPatrick, M. J. Lett, G. F. Hess, M.
1225 Filipowicz Sinnreich, N. M. Provine, P. Klenerman, Single-cell analysis of
1226 human MAIT cell transcriptional, functional and clonal diversity. *Nature*
1227 *Immunology* **24**, 1565-1578 (2023).
1228 27. A. Tarke, C. H. Coelho, Z. Zhang, J. M. Dan, E. D. Yu, N. Methot, N. I.
1229 Bloom, B. Goodwin, E. Phillips, S. Mallal, J. Sidney, G. Filaci, D.
1230 Weiskopf, R. da Silva Antunes, S. Crotty, A. Grifoni, A. Sette, SARS-CoV-
1231 2 vaccination induces immunological T cell memory able to cross-
1232 recognize variants from Alpha to Omicron. *Cell* **185**, 847-859.e811
1233 (2022).
1234 28. M. Goncharov, D. Bagaev, D. Shcherbinin, I. Zvyagin, D. Bolotin, P. G.
1235 Thomas, A. A. Minervina, M. V. Pogorelyy, K. Ladell, J. E. McLaren, D. A.
1236 Price, T. H. O. Nguyen, L. C. Rowntree, E. B. Clemens, K. Kedzierska, G.
1237 Dolton, C. R. Rius, A. Sewell, J. Samir, F. Luciani, K. V. Zornikova, A. A.
1238 Khmelevskaya, S. A. Sheetikov, G. A. Efimov, D. Chudakov, M. Shugay,

1239 VDJdb in the pandemic era: a compendium of T cell receptors specific
1240 for SARS-CoV-2. *Nature Methods* **19**, 1017-1019 (2022).
1241 29. R. A. Watson, O. Tong, R. Cooper, C. A. Taylor, P. K. Sharma, A. V. de
1242 los Aires, E. A. Mahé, H. Ruffieux, I. Nassiri, M. R. Middleton, B. P.
1243 Fairfax, Immune checkpoint blockade sensitivity and progression-free
1244 survival associates with baseline CD8+ T cell clone size and
1245 cytotoxicity. *Science Immunology* **6**, eabj8825 (2021).
1246 30. J. Liu, B. J. Hill, S. Darko, K. Song, M. F. Quigley, T. E. Asher, Y. Morita,
1247 H. Y. Greenaway, V. Venturi, D. C. Douek, M. P. Davenport, D. A. Price,
1248 M. Roederer, The peripheral differentiation of human natural killer T
1249 cells. *Immunol Cell Biol* **97**, 586-596 (2019).
1250 31. T. Guo, M. Y. Koo, Y. Kagoya, M. Anczurowski, C. H. Wang, K. Saso, M.
1251 O. Butler, N. Hirano, A Subset of Human Autoreactive CD1c-Restricted
1252 T Cells Preferentially Expresses TRBV4-1(+) TCRs. *J Immunol* **200**, 500-
1253 511 (2018).
1254 32. I. Van Rhijn, N. A. Gherardin, A. Kasmar, W. de Jager, D. G. Pellicci, L.
1255 Kostenko, L. L. Tan, M. Bhati, S. Gras, D. I. Godfrey, J. Rossjohn, D. B.
1256 Moody, TCR Bias and Affinity Define Two Compartments of the CD1b–
1257 Glycolipid-Specific T Cell Repertoire. *The Journal of Immunology* **192**,
1258 4054-4060 (2014).
1259 33. J. Davis-Porada, A. B. George, N. Lam, D. P. Caron, J. I. Gray, J. Huang, J.
1260 Hwu, S. B. Wells, R. Matsumoto, M. Kubota, Y. Lee, R. Morrison-Colvin,
1261 I. J. Jensen, B. B. Ural, N. Shaabani, D. Weiskopf, A. Grifoni, A. Sette, P.
1262 A. Szabo, J. R. Teijaro, P. A. Sims, D. L. Farber, Maintenance and
1263 functional regulation of immune memory to COVID-19 vaccines in
1264 tissues. *Immunity* **57**, 2895-2913.e2898 (2024).
1265 34. S. I. Koizumi, H. Ishikawa, Transcriptional Regulation of Differentiation
1266 and Functions of Effector T Regulatory Cells. *Cells* **8**, (2019).
1267 35. S. M. Murray, A. M. Ansari, J. Frater, P. Klenerman, S. Dunachie, E.
1268 Barnes, A. Ogbe, The impact of pre-existing cross-reactive immunity on
1269 SARS-CoV-2 infection and vaccine responses. *Nature Reviews*
1270 *Immunology* **23**, 304-316 (2023).
1271 36. A. Ogbe, B. Kronsteiner, D. T. Skelly, M. Pace, A. Brown, E. Adland, K.
1272 Adair, H. D. Akhter, M. Ali, S. E. Ali, A. Angyal, M. A. Ansari, C. V.
1273 Arancibia-Cárcamo, H. Brown, S. Chinnakannan, C. Conlon, C. de Lara,
1274 T. de Silva, C. Dold, T. Dong, T. Donnison, D. Eyre, A. Flaxman, H.
1275 Fletcher, J. Gardner, J. T. Grist, C. P. Hackstein, K. Jaruthamsophon, K.
1276 Jeffery, T. Lambe, L. Lee, W. Li, N. Lim, P. C. Matthews, A. J. Mentzer, S.
1277 C. Moore, D. J. Naisbitt, M. Ogese, G. Ogg, P. Openshaw, M.
1278 Pirmohamed, A. J. Pollard, N. Ramamurthy, P. Rongkard, S. Rowland-
1279 Jones, O. Sampson, G. Screaton, A. Sette, L. Stafford, C. Thompson, P. J.
1280 Thomson, R. Thwaites, V. Vieira, D. Weiskopf, P. Zacharopoulou, L.
1281 Turtle, P. Klenerman, P. Goulder, J. Frater, E. Barnes, S. Dunachie, T cell
1282 assays differentiate clinical and subclinical SARS-CoV-2 infections from
1283 cross-reactive antiviral responses. *Nat Commun* **12**, 2055 (2021).

37. J. I. Rodriguez-Barbosa, P. Schneider, A. Weigert, K.-M. Lee, T.-J. Kim, J.-A. Perez-Simon, M.-L. del Rio, HVEM, a cosignaling molecular switch, and its interactions with BTLA, CD160 and LIGHT. *Cellular & Molecular Immunology* **16**, 679-682 (2019).
38. E. N. McNamee, D. Korns Johnson, D. Homann, E. T. Clambey, Hypoxia and hypoxia-inducible factors as regulators of T cell development, differentiation, and function. *Immunologic Research* **55**, 58-70 (2013).
39. J. E. Ussher, M. Bilton, E. Attwod, J. Shadwell, R. Richardson, C. de Lara, E. Mettke, A. Kurioka, T. H. Hansen, P. Klenerman, C. B. Willberg, CD161++CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. *European Journal of Immunology* **44**, 195-203 (2014).
40. N. M. Provine, B. Binder, M. E. B. FitzPatrick, A. Schuch, L. C. Garner, K. D. Williamson, B. van Wilgenburg, R. Thimme, P. Klenerman, M. Hofmann, Unique and Common Features of Innate-Like Human Vδ2(+) γδT Cells and Mucosal-Associated Invariant T Cells. *Front Immunol* **9**, 756 (2018).
41. M. Z. M. Zheng, L. Burmas, H.-X. Tan, M.-C. Trieu, H. J. Lee, D. Rawlinson, A. Haque, S. J. Kent, A. K. Wheatley, J. A. Juno, Deconvoluting TCR-dependent and -independent activation is vital for reliable Ag-specific CD4+ T cell characterization by AIM assay. *Science Advances* **11**, eadv3491.
42. L. Pace, A. Tempez, C. Arnold-Schrauf, F. Lemaitre, P. Bousso, L. Fetler, T. Sparwasser, S. Amigorena, Regulatory T cells increase the avidity of primary CD8+ T cell responses and promote memory. *Science* **338**, 532-536 (2012).
43. N. M. Provine, A. Amini, L. C. Garner, A. J. Spencer, C. Dold, C. Hutchings, L. Silva Reyes, M. E. B. FitzPatrick, S. Chinnakannan, B. Oguti, M. Raymond, M. Ulaszewska, F. Troise, H. Sharpe, S. B. Morgan, T. S. C. Hinks, T. Lambe, S. Capone, A. Folgori, E. Barnes, C. S. Rollier, A. J. Pollard, P. Klenerman, MAIT cell activation augments adenovirus vector vaccine immunogenicity. *Science* **371**, 521-526 (2021).
44. J. E. Teigler, M. J. Iampietro, D. H. Barouch, Vaccination with adenovirus serotypes 35, 26, and 48 elicits higher levels of innate cytokine responses than adenovirus serotype 5 in rhesus monkeys. *J Virol* **86**, 9590-9598 (2012).
45. N. S. Joshi, W. Cui, A. Chandele, H. K. Lee, D. R. Urso, J. Hagman, L. Gapin, S. M. Kaech, Inflammation Directs Memory Precursor and Short-Lived Effector CD8+ T Cell Fates via the Graded Expression of T-bet Transcription Factor. *Immunity* **27**, 281-295 (2007).
46. S. C. Moore, B. Kronsteiner, S. Longet, S. Adele, A. S. Deeks, C. Liu, W. Dejnirattisai, L. S. Reyes, N. Meardon, S. Faustini, S. Al-Taei, T. Tipton, L. M. Hering, A. Angyal, R. Brown, A. R. Nicols, S. L. Dobson, P. Supasa, A. Tuekprakhon, A. Cross, J. K. Tyerman, H. Hornsby, I. Grouneva, M. Plowright, P. Zhang, T. A. H. Newman, J. M. Nell, P. Abraham, M. Ali, T. Malone, I. Neale, E. Phillips, J. D. Wilson, S. M. Murray, M. Zewdie, A.

1330 Shields, E. C. Horner, L. H. Booth, L. Stafford, S. Bibi, D. G. Wootton, A.
1331 J. Mentzer, C. P. Conlon, K. Jeffery, P. C. Matthews, A. J. Pollard, A.
1332 Brown, S. L. Rowland-Jones, J. Mongkolsapaya, R. P. Payne, C. Dold, T.
1333 Lambe, J. E. D. Thaventhiran, G. Screaton, E. Barnes, S. Hopkins, V.
1334 Hall, C. J. A. Duncan, A. Richter, M. Carroll, T. I. de Silva, P. Klenerman,
1335 S. Dunachie, L. Turtle, Evolution of long-term vaccine-induced and
1336 hybrid immunity in healthcare workers after different COVID-19
1337 vaccine regimens. *Med* **4**, 191-215.e199 (2023).
1338 47. P. M. Folegatti, K. J. Ewer, P. K. Aley, B. Angus, S. Becker, S. Belij-
1339 Rammerstorfer, D. Bellamy, S. Bibi, M. Bittaye, E. A. Clutterbuck, C.
1340 Dold, S. N. Faust, A. Finn, A. L. Flaxman, B. Hallis, P. Heath, D. Jenkin,
1341 R. Lazarus, R. Makinson, A. M. Minassian, K. M. Pollock, M. Ramasamy,
1342 H. Robinson, M. Snape, R. Tarrant, M. Voysey, C. Green, A. D. Douglas,
1343 A. V. S. Hill, T. Lambe, S. C. Gilbert, A. J. Pollard, J. Aboagye, K. Adams,
1344 A. Ali, E. Allen, J. L. Allison, R. Anslow, E. H. Arbe-Barnes, G. Babbage,
1345 K. Baillie, M. Baker, N. Baker, P. Baker, I. Baleanu, J. Ballaminut, E.
1346 Barnes, J. Barrett, L. Bates, A. Batten, K. Beadon, R. Beckley, E. Berrie,
1347 L. Berry, A. Beveridge, K. R. Bewley, E. M. Bijker, T. Bingham, L.
1348 Blackwell, C. L. Blundell, E. Bolam, E. Boland, N. Borthwick, T. Bower, A.
1349 Boyd, T. Brenner, P. D. Bright, C. Brown-O'Sullivan, E. Brunt, J.
1350 Burbage, S. Burge, K. R. Buttigieg, N. Byard, I. Cabera Puig, A. Calvert,
1351 S. Camara, M. Cao, F. Cappuccini, M. Carr, M. W. Carroll, V. Carter, K.
1352 Cathie, R. J. Challis, S. Charlton, I. Chelysheva, J.-S. Cho, P. Cicconi, L.
1353 Cifuentes, H. Clark, E. Clark, T. Cole, R. Colin-Jones, C. P. Conlon, A.
1354 Cook, N. S. Coombes, R. Cooper, C. A. Cosgrove, K. Coy, W. E. M.
1355 Crocker, C. J. Cunningham, B. E. Damratoski, L. Dando, M. S. Datoo, H.
1356 Davies, H. De Graaf, T. Demissie, C. Di Maso, I. Dietrich, T. Dong, F. R.
1357 Donnellan, N. Douglas, C. Downing, J. Drake, R. Drake-Brockman, R. E.
1358 Drury, S. J. Dunachie, N. J. Edwards, F. D. L. Edwards, C. J. Edwards, S.
1359 C. Elias, M. J. Elmore, K. R. W. Emary, M. R. English, S. Fagerbrink, S.
1360 Felle, S. Feng, S. Field, C. Fixmer, C. Fletcher, K. J. Ford, J. Fowler, P.
1361 Fox, E. Francis, J. Frater, J. Furze, M. Fuskova, E. Galiza, D. Gbesemete,
1362 C. Gilbride, K. Godwin, G. Gorini, L. Goulston, C. Grabau, L. Gracie, Z.
1363 Gray, L. B. Guthrie, M. Hackett, S. Halwe, E. Hamilton, J. Hamlyn, B.
1364 Hanumunthadu, I. Harding, S. A. Harris, A. Harris, D. Harrison, C.
1365 Harrison, T. C. Hart, L. Haskell, S. Hawkins, I. Head, J. A. Henry, J. Hill, S.
1366 H. C. Hodgson, M. M. Hou, E. Howe, N. Howell, C. Hutlin, S. Ikram, C.
1367 Isitt, P. Iveson, S. Jackson, F. Jackson, S. W. James, M. Jenkins, E. Jones,
1368 K. Jones, C. E. Jones, B. Jones, R. Kailath, K. Karampatsas, J. Keen, S.
1369 Kelly, D. Kelly, D. Kerr, S. Kerridge, L. Khan, U. Khan, A. Killen, J. Kinch,
1370 T. B. King, L. King, J. King, L. Kingham-Page, P. Klenerman, F. Knapper,
1371 J. C. Knight, D. Knott, S. Koleva, A. Kupke, C. W. Larkworthy, J. P. J.
1372 Larwood, A. Laskey, A. M. Lawrie, A. Lee, K. Y. Ngan Lee, E. A. Lees, H.
1373 Legge, A. Lelliott, N.-M. Lemm, A. M. Lias, A. Linder, S. Lipworth, X. Liu,
1374 S. Liu, R. Lopez Ramon, M. Lwin, F. Mabesa, M. Madhavan, G. Mallett,
1375 K. Mansatta, I. Marcal, S. Marinou, E. Marlow, J. L. Marshall, J. Martin, J.

1376 McEwan, L. McInroy, G. Meddaugh, A. J. Mentzer, N. Mirtorabi, M.
1377 Moore, E. Moran, E. Morey, V. Morgan, S. J. Morris, H. Morrison, G.
1378 Morshead, R. Morter, Y. F. Mujadidi, J. Muller, T. Munera-Huertas, C.
1379 Munro, A. Munro, S. Murphy, V. J. Munster, P. Mweu, A. Noé, F. L.
1380 Nugent, E. Nuthall, K. O'Brien, D. O'Connor, B. Oguti, J. L. Oliver, C.
1381 Oliveira, P. J. O'Reilly, M. Osborn, P. Osborne, C. Owen, D. Owens, N.
1382 Owino, M. Pacurar, K. Parker, H. Parracho, M. Patrick-Smith, V. Payne, J.
1383 Pearce, Y. Peng, M. P. Peralta Alvarez, J. Perring, K. Pfafferott, D. Pipini,
1384 E. Plested, H. Pluess-Hall, K. Pollock, I. Poulton, L. Presland, S.
1385 Provstgaard-Morys, D. Pulido, K. Radia, F. Ramos Lopez, J. Rand, H.
1386 Ratcliffe, T. Rawlinson, S. Rhead, A. Riddell, A. J. Ritchie, H. Roberts, J.
1387 Robson, S. Roche, C. Rohde, C. S. Rollier, R. Romani, I. Rudiansyah, S.
1388 Saich, S. Sajjad, S. Salvador, L. Sanchez Riera, H. Sanders, K. Sanders,
1389 S. Sapaun, C. Sayce, E. Schofield, G. Screaton, B. Selby, C. Semple, H.
1390 R. Sharpe, I. Shaik, A. Shea, H. Shelton, S. Silk, L. Silva-Reyes, D. T.
1391 Skelly, H. Smee, C. C. Smith, D. J. Smith, R. Song, A. J. Spencer, E.
1392 Stafford, A. Steele, E. Stefanova, L. Stockdale, A. Szigeti, A. Tahiri-
1393 Alaoui, M. Tait, H. Talbot, R. Tanner, I. J. Taylor, V. Taylor, R. Te Water
1394 Naude, N. Thakur, Y. Themistocleous, A. Themistocleous, M. Thomas, T.
1395 M. Thomas, A. Thompson, S. Thomson-Hill, J. Tomlins, S. Tonks, J.
1396 Towner, N. Tran, J. A. Tree, A. Truby, K. Turkentine, C. Turner, N.
1397 Turner, S. Turner, T. Tuthill, M. Ulaszewska, R. Varughese, N. Van
1398 Doremalen, K. Veighey, M. K. Verheul, I. Vichos, E. Vitale, L. Walker, M.
1399 E. E. Watson, B. Welham, J. Wheat, C. White, R. White, A. T. Worth, D.
1400 Wright, S. Wright, X. L. Yao, Y. Yau, Safety and immunogenicity of the
1401 ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of
1402 a phase 1/2, single-blind, randomised controlled trial. *The Lancet* **396**,
1403 467-478 (2020).
1404 48. A. Flaxman, N. G. Marchevsky, D. Jenkin, J. Aboagye, P. K. Aley, B.
1405 Angus, S. Belij-Rammerstorfer, S. Bibi, M. Bittaye, F. Cappuccini, P.
1406 Cicconi, E. A. Clutterbuck, S. Davies, W. Dejnirattisai, C. Dold, K. J.
1407 Ewer, P. M. Folegatti, J. Fowler, A. V. S. Hill, S. Kerridge, A. M.
1408 Minassian, J. Mongkolsapaya, Y. F. Mujadidi, E. Plested, M. N.
1409 Ramasamy, H. Robinson, H. Sanders, E. Sheehan, H. Smith, M. D.
1410 Snape, R. Song, D. Woods, G. Screaton, S. C. Gilbert, M. Voysey, A. J.
1411 Pollard, T. Lambe, Reactogenicity and immunogenicity after a late
1412 second dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy
1413 of two randomised controlled trials (COV001 and COV002). *Lancet* **398**,
1414 981-990 (2021).
1415 49. Y. Hao, S. Hao, E. Andersen-Nissen, W. M. Mauck, III, S. Zheng, A.
1416 Butler, M. J. Lee, A. J. Wilk, C. Darby, M. Zager, P. Hoffman, M.
1417 Stoeckius, E. Papalexi, E. P. Mimitou, J. Jain, A. Srivastava, T. Stuart, L.
1418 M. Fleming, B. Yeung, A. J. Rogers, J. M. McElrath, C. A. Blish, R.
1419 Gottardo, P. Smibert, R. Satija, Integrated analysis of multimodal
1420 single-cell data. *Cell* **184**, 3573-3587.e3529 (2021).

1421 50. P. Germain, A. Lun, C. Garcia Meixide, W. Macnair, M. Robinson,
1422 Doublet identification in single-cell sequencing data using scDblFinder
1423 [version 2; peer review: 2 approved]. *F1000Research* **10**, (2022).
1424 51. C. S. McGinnis, D. M. Patterson, J. Winkler, D. N. Conrad, M. Y. Hein, V.
1425 Srivastava, J. L. Hu, L. M. Murrow, J. S. Weissman, Z. Werb, E. D. Chow,
1426 Z. J. Gartner, MULTI-seq: sample multiplexing for single-cell RNA
1427 sequencing using lipid-tagged indices. *Nature Methods* **16**, 619-626
1428 (2019).
1429 52. R. da Silva Antunes, V. Fajardo-Rosas, E. D. Yu, R. I. Gálvez, A. Abawi,
1430 E. A. Escarrega, A. Martínez-Pérez, E. Johansson, B. Goodwin, A.
1431 Frazier, J. M. Dan, S. Crotty, G. Seumois, D. Weiskopf, P. Vijayanand, A.
1432 Sette, Evolution of SARS-CoV-2 T cell responses as a function of
1433 multiple COVID-19 boosters. *Cell Reports* **44**, 115907 (2025).
1434 53. M. D. Robinson, D. J. McCarthy, G. K. Smyth, edgeR: a Bioconductor
1435 package for differential expression analysis of digital gene expression
1436 data. *Bioinformatics* **26**, 139-140 (2010).
1437 54. G. E. Hoffman, E. E. Schadt, variancePartition: interpreting drivers of
1438 variation in complex gene expression studies. *BMC Bioinformatics* **17**,
1439 483 (2016).
1440 55. M. P. Mulè, A. J. Martins, F. Cheung, R. Farmer, B. A. Sellers, J. A. Quiel,
1441 A. Jain, Y. Kotliarov, N. Bansal, J. Chen, P. L. Schwartzberg, J. S. Tsang,
1442 Integrating population and single-cell variations in vaccine responses
1443 identifies a naturally adjuvanted human immune setpoint. *Immunity*
1444 **57**, 1160-1176.e1167 (2024).
1445 56. G. Korotkevich, V. Sukhov, N. Budin, B. Shpak, M. N. Artyomov, A.
1446 Sergushichev, Fast gene set enrichment analysis. *bioRxiv*, 060012
1447 (2021).
1448 57. T. Wu, E. Hu, S. Xu, M. Chen, P. Guo, Z. Dai, T. Feng, L. Zhou, W. Tang,
1449 L. Zhan, X. Fu, S. Liu, X. Bo, G. Yu, clusterProfiler 4.0: A universal
1450 enrichment tool for interpreting omics data. *The Innovation* **2**, (2021).
1451 58. S. Hänzelmann, R. Castelo, J. Guinney, GSVA: gene set variation
1452 analysis for microarray and RNA-Seq data. *BMC Bioinformatics* **14**, 7
1453 (2013).
1454 59. L. Garcia-Alonso, V. Lorenzi, C. I. Mazzeo, J. P. Alves-Lopes, K. Roberts,
1455 C. Sancho-Serra, J. Engelbert, M. Marečková, W. H. Gruhn, R. A.
1456 Botting, T. Li, B. Crespo, S. van Dongen, V. Y. Kiselev, E. Prigmore, M.
1457 Herbert, A. Moffett, A. Chédotal, O. A. Bayraktar, A. Surani, M. Haniffa,
1458 R. Vento-Tormo, Single-cell roadmap of human gonadal development.
1459 *Nature* **607**, 540-547 (2022).
1460 60. Z. Gu, L. Gu, R. Eils, M. Schlesner, B. Brors, circlize implements and
1461 enhances circular visualization in R. *Bioinformatics* **30**, 2811-2812
1462 (2014).
1463 61. S. A. Schattgen, K. Guion, J. C. Crawford, A. Souquette, A. M. Barrio, M.
1464 J. T. Stubbington, P. G. Thomas, P. Bradley, Integrating T cell receptor
1465 sequences and transcriptional profiles by clonotype neighbor graph
1466 analysis (CoNGA). *Nat Biotechnol* **40**, 54-63 (2022).

1467 62. A. T. L. Lun, S. Riesenfeld, T. Andrews, T. P. Dao, T. Gomes, J. C.
1468 Marioni, J. participants in the 1st Human Cell Atlas, EmptyDrops:
1469 distinguishing cells from empty droplets in droplet-based single-cell
1470 RNA sequencing data. *Genome Biology* **20**, 63 (2019).

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Data and materials availability:

No custom software was generated for this manuscript. The software used for all analyses is listed in the relevant sections of the Methods and sufficient detail is given to reproduce analyses. Tabulated data underlying Figs. 1 to 6 and Figs. S5, S8 & S9 are provided in data file S1. Primary scripts are available on reasonable request.

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Figures:

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1526 **Fig. 1) Identification of transcriptionally and functionally diverse conventional and**
1527 **unconventional T cells by AIM assay after SARS-CoV-2 spike stimulation. A)**
1528 Study overview. **B)** Representative flow cytometric staining for sorting of SARS-CoV-2
1529 spike peptide stimulated peripheral blood by activation induced markers (AIM). **C)**
1530 Proportion of AIM^+ $CD3^+$ T cells following 24h stimulation. **D)** UMAP representation of
1531 gene expression data from sorted AIM^+ $CD3^+$ T cells, with manual annotation. **E)**
1532 Selected surface protein (ADT, antibody-derived tag) and RNA features used for
1533 manual annotation. **F)** Mean normalized RNA expression of selected functional genes.
1534 **G)** Projection of the RNA expression density of selected functional cytokines onto gene
1535 expression UMAP. **H)** Mean ADT expression of surface activation induced markers. **I)**
1536 Pearson correlation of total number of interactions to and from a given cell type. Point
1537 size is scaled by number of cells per cluster. Blue and red text is R^2 with and without
1538 CD4_Th1_Tfh, respectively. **J)** Selected effector and inhibitory interactions that
1539 originate from the CD4_Th1_Tfh cluster. Arrow points in direction of interaction and is
1540 scaled by interaction strength.

1541 **Fig. 2) Expanded and spike-specific clones are found across AIM^{+} T cell subsets**
1542 **and are related to functionality. A)** Proportion of cells within expanded or singlet
1543 clones per cluster and timepoint projected onto gene expression UMAP (left) or as
1544 proportion of each cluster (right). **B)** Sharing of expanded CD4^{+} T cell clones (clone size
1545 >1) across transcriptional clusters. Chord sizes reflect the relative proportion of each
1546 cluster that has shared clones. **C)** Number and proportion of clones that are singlets or
1547 expanded clones that are unique to a given cluster or shared across clusters. **D)** Per
1548 cell effector CD8^{+} cytotoxicity module score ((*29*), left) and effector CD4^{+} normalized
1549 *IFNG* transcript expression (right) as a function of clonal size (Mann-Whitney U test,
1550 Bonferroni adjusted). **E)** Gene expression UMAP colored by TCR meta-clonotype

antigen-enrichment score p value (**Methods**). **F)** Frequency of clonotypes within each T cell cluster which are within an antigen-enriched TCR meta-clonotype (adjusted p value <0.05).

1555 **Fig. 3) Recall of pre-existing or SARS-CoV-2 vaccine/infection induced T cell**
1556 **clones influences the function of AIM$^+$ CD4$^+$ T cells. A)** Representative flow
1557 cytometric staining for sorting of SARS-CoV-2 spike peptide stimulated peripheral blood
1558 by CellTrace Violet (CTV). Positive control (phytohemagglutinin; PHA) plot also shown.
1559 **B)** Frequency of CTV^{lo} T cells after 7 days with (spike) or without (unstim) SARS-CoV-2
1560 spike peptide stimulation. **C)** Number of unique paired αβ TCR clones that are shared
1561 across study timepoints in conventional spike-responsive T cells. Color is scaled to
1562 proportion of clones. **D)** Proportion of post-vaccine spike-responsive conventional T
1563 cell clones that are shared across timepoints within an individual donor. **E)** Cytotoxicity
1564 module score (*29*) of post-vaccination effector CD8$^+$ cells that shared or did not share
1565 clonality with a pre-vaccination spike-responsive T cell (students T-test). **F)** Number of
1566 within-cluster differentially expressed genes (DEGs, average $\log_2$ fold change >0.25 and
1567 adjusted $p < 0.05$) between post-second dose (T2) T cells with (recalled) or without (de
1568 novo) a shared identical paired TCR at an earlier study timepoint. **G)** Significantly
1569 differentially expressed genes (as in F) from selected CD4$^+$ T cell clusters with highest
1570 frequencies of DEGs. The top 15 most upregulated genes are annotated (red dots). **H)**
1571 Surface protein (antibody-derived tag, ADT) expression of T cell memory markers in
1572 selected CD4$^+$ T cell clusters at T2 (Mann-Whitney U test, Bonferroni adjusted).

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1574 **Fig. 4) BNT162b2 and ChAdOx1 nCoV-19 vaccine vectors induce functionally**
1575 **distinct SARS-CoV-2 spike-responsive T cells. A)** Log_2 fold change (FC) in relative
1576 abundance of spike-responsive T cell clusters at pre- (T1) and post- (T2) second
1577 vaccine timepoints. Benjamini-Hochberg derived false discovery rate (FDR) values for
1578 comparisons with an $FDR \leq 0.1$ are denoted. **B)** Number of significantly differentially
1579 expressed genes with an average log_2 FC >0.25 and adjusted $p <0.05$ between ChAd

1580 and BNT vaccinees at T1 and T2. **C)** Average log_2 FC of normalized activation induced
1581 marker surface protein (antibody-derived tag, ADT) expression. **D)** Average log_2 FC in
1582 normalized gene expression between spike-responsive IFNγ$^+$ CD8$^+$ effector T cells of
1583 ChAd and BNT vaccinees. Labelled genes are those included within a cytotoxicity-
1584 associated geneset *(29)*. **E)** Distribution of normalized expression of T cell memory
1585 associated surface proteins (ADT) within spike-responsive IFNγ$^+$ CD8$^+$ effector T cells.
1586 **F)** Number of significant *CD274-PDCD1* interactions between each cell type. Size of
1587 plot is scaled to total number of significant interactions, the arrow and distance of the
1588 chord end to the circle edge denotes the directionality of the interaction. **G)** Proportion
1589 of the top 70 largest paired CD4$^+$ T cell clones at T2 that share an identical clone at the
1590 T1 timepoint (recalled T cell). Mann-Whitney U test, Bonferroni adjusted. **H)** Proportion
1591 of total cells per individual/timepoint of top 70 largest clones (Paired t-test, Bonferroni
1592 adjusted). **I)** Transcriptional cluster phenotype of recalled clones, or clones which are
1593 only found at T2 (de novo) within the top 70 CD4$^+$ T cell clones. **J)** Proportion of recalled
1594 top 70 clones that have a CD4_ISG phenotype at T2. EdgeR comparison (**Methods**).
1595 BNT, BNT162b2 vaccinees; ChAd, ChAdOx1 nCoV-19 vaccinees.

1596

1597 **Fig. 5) Dosing interval differentially impacts BNT162b2 and ChAdOx1 nCoV-19**

1598 **induced spike-responsive T cells. A)** Log_2 fold change (FC) of post- second vaccine
1599 (T2) spike-responsive T cell clusters. Benjamini-Hochberg false discovery rate (FDR)
1600 values for comparisons with an unadjusted p value <0.25 are denoted. L, long interval;
1601 S, short interval. **B)** Mean normalized surface protein expression (antibody-derived tag,
1602 ADT) of activation induced markers in T2 spike-responsive T cell clusters (signif =
1603 Bonferroni adjusted $p < 0.05$). **C)** Number of significantly differentially expressed genes
1604 (DEG) with an average log_2 fold change >0.25 and adjusted $p < 0.05$ within T2 spike-
1605 responsive T cell clusters. **D)** Overrepresentation of genesets in upregulated genes
1606 identified in panel C. **E)** Enrichment of genesets in genes ranked based on the strength
1607 of association of their expression with the given comparison (**Methods**) at T2. Only
1608 enrichments with adjusted p value <0.01 are shown. **F)** Difference between T2 and T1
1609 in the aggregate expression of genes within the Hallmark Interferon Gamma Response
1610 (M5913) geneset in selected spike-responsive T cell subsets. **G)** Mean normalized
1611 expression of genes within the Hallmark Interferon Gamma Response geneset in spike-
1612 responsive $CD8^+$ effector T cells. BNT, BNT162b2 vaccinees; ChAd, ChAdOx1 nCoV-
1613 19 vaccinees.

1614 **Fig. 6) An extended BNT162b2 vaccine dosing interval induces spike-responsive**
1615 **T cells with reduced inflammatory functionality. A)** Enrichment of genesets in genes
1616 ranked based on the strength association of their expression with the given comparison
1617 (**Methods**). Comparisons are made between paired timepoints, controlling for variation
1618 across individuals. Only enrichments with adjusted p value <0.01 are shown. **B)**
1619 Geneset variation analysis (GSVA) scoring of aggregate expression of genes within the

1620 Hallmark Interferon Gamma Response (M5913) and Hallmark TNFA signaling via NFKB
1621 (M5890) genesets. **C)** Mean normalized expression of genes within the Hallmark
1622 Interferon Gamma Response geneset in spike-responsive $CD4^+$ T_H1/T_H17 effector T
1623 cells. **D)** Proportion of total cells per individual/timepoint of each clone that is shared
1624 within an individual at T2 and T1 (recalled) and within the top 70 largest clones at T2
1625 (Paired t-test, Bonferroni adjusted). **E)** Average log_2 fold change of gene expression of
1626 selected stem-associated genes. Genes with log_2 fold change <0.25 or adjusted p >0.05
1627 denoted by ns. **F)** Proportion of T1 AIM^+ T cells represented by CD4_Tcm2, a stem-
1628 associated T cell cluster. Unadjusted p value and false discovery rate (FDR) presented.
1629 EdgeR comparison (**Methods**). BNT, BNT162b2 vaccinees; ChAd, ChAdOx1 nCoV-19
1630 vaccinees.