

**Lack of truncated IFITM3 transcripts in cells homozygous for the rs12252-C variant that is associated with severe influenza infection**

Running head: Lack of truncated IFITM3 transcripts

Summary: The single nucleotide polymorphism rs12252 of *IFITM3* from T to C, which is associated with severe influenza infection, does not result in a truncated form of the protein as previously suggested.

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## **Abstract**

IFITM3 protein is known to restrict the entry of a range of enveloped viruses. The single nucleotide polymorphism rs12252-C within *IFITM3* has been shown to be associated with severe influenza A virus infection. It has been suggested that rs12252-C results in expression of a truncated IFITM3 protein lacking the first 21 amino acids. By performing high throughput RNA sequencing on primary dendritic cells and peripheral blood mononuclear cells isolated from pandemic H1N1 influenza & HIV-1 infected patients we show that full-length IFITM3 mRNA is dominantly expressed (>99%) across all rs12252 genotypes. Full-length IFITM3 protein can be detected in all genotypes.

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## **Key words**

IFITM3, Influenza A virus, rs12252,

## **Abbreviations**

IFITM3, IAV, HIV, SNP, WT, DC, PBMC,

## Introduction

Interferon-induced transmembrane protein 3 (IFITM3) has been shown to restrict the replication of Influenza A Virus (IAV) and other enveloped viruses that enter cells via the endosomal pathway, such as West Nile Virus and Human Immunodeficiency Virus (HIV) <sup>(1)</sup>. The importance of IFITM3 in controlling IAV was highlighted by the work of Everitt *et al* (2012) <sup>(2)</sup> who showed that the severity of infection was significantly increased in *ifitm3* knockout mice, compared to wild type (WT) mice.

In addition to their work on *ifitm3* knockout mice, Everitt *et al* <sup>(2)</sup> also reported that a disproportionately large number of Northern European patients hospitalized with severe seasonal or pandemic influenza H1N1/09 virus were carriers of the minor allele of an *IFITM3* SNP, rs12252 (major allele T, minor allele C). The allele frequencies of rs12252 are varied in human populations, with the allele frequency of the C variant being much higher in the Han Chinese population (minor allele frequency (MAF: 0.53)) compared to the Northern European population (MAF: 0.034) <sup>(3)</sup>.

Previously, we were able to confirm an association between rs12252-C and the severity of IAV infection in a large cohort of Chinese patients. In this cohort, 69% of patients with severe pandemic influenza A H1N1/09 virus infection carried the CC genotype, compared to 25% of those with mild infections <sup>(3)</sup>. In a second study, we observed a significantly higher frequency of the CC and CT genotypes in HIV-1-infected patients who were rapid disease progressors compared to slow disease progressors <sup>(4)</sup>. This SNP was also shown to be associated with severe avian influenza H7N9 and Hantaan virus infections in Chinese populations <sup>(5-7)</sup>, however this association was not observed in two studies on European cohorts <sup>(8,9)</sup> or in a recent American study <sup>(10)</sup>.

It has been suggested that rs12252-C changes the affinity of a splicing acceptor site in the coding sequence of exon 1 of *IFITM3*<sup>(2)</sup>. This is predicted to result in the expression of alternative *IFITM3* mRNA isoforms, (ENST00000602735.2 and ENST00000526811.4) incurred via usage of alternative 5' untranslated regions upstream of the canonical first exon. These transcripts vary from the canonical transcript (ENST00000399808.4) as they have a 21 amino acid deletion at the N-terminus.

Transfection of truncated IFITM3 into WT cells revealed that this version had a different subcellular distribution in comparison to the full-length protein, potentially explaining the differential viral restriction seen with rs12252 mutations<sup>(11)</sup>. Additionally, studies have shown that the first 21 amino acids of the N-terminus are required for attenuation of vesicular stomatitis virus replication, and that truncated IFITM3 protein fails to restrict the replication of various strains of influenza virus<sup>(2, 3)</sup>. The truncated version of IFITM3 has also been shown to have an impact on antiviral activity in HIV-1 infection<sup>(12, 13)</sup>. Conversely, using native and epitope-tagged IFITM3 isoforms, Williams *et al*<sup>(14)</sup> showed that both full-length and truncated IFITM3 restricted entry and replication of H1N1 efficiently. Truncated IFITM3 also had an attenuated yet significant effect on restricting on the restriction of H3N2 IAV. Despite the discrepancies, overall this has led to the hypothesis that the observed effects of rs12252-C in patient cohorts are attributable to the alternative splicing of *IFITM3*<sup>(2)</sup>.

However, expression of truncated IFITM3 protein in primary cells, carrying the rs12252-C polymorphism has not previously been shown. One of the major obstacles in studying IFITM3 expression is the lack of availability of an IFITM3-specific antibody. Due to high homology between IFITM2 and IFITM3, all commercially

available anti-IFITM3 antibodies have high cross reactivity to IFITM2. To overcome this obstacle, we developed an anti-IFITM3 antibody specific to the first 21 amino acids on the N-terminus of IFITM3, and with a very low cross reactivity to IFITM2. We then used this antibody to investigate the presence of full-length IFITM3 protein in primary cells with different rs12252 genotypes. Furthermore, in this study we examined the presence of the truncated IFITM3 isoforms in primary dendritic cells (DCs) carrying the rs12252-C allele, and in peripheral blood mononuclear cells (PBMCs) collected from patients infected with either seasonal IAV or HIV-1.

## **Materials and Methods**

**Ethics Statement:** This study was approved by the local ethics committee of Beijing You'An Hospital and the University of Oxford Tropical Ethics Committee (OXTREC 16-10 and 1001-13). All methods were performed in accordance with the relevant guidelines and regulations, and informed consent was obtained from all human subjects.

**Study Subjects:** Monocyte-derived DCs were isolated from a total of nine healthy UK volunteers, carrying the TT, CT or CC genotype. Cells were infected for 10 hours with non-replicating GFP-expressing influenza virus (S-FLU<sup>(15)</sup>) pseudotyped with H7 protein from A/Anhui/1/2013. S-FLU is only capable of one round of infection; an eGFP gene replaces segment four of the influenza genome (containing the haemagglutinin gene). GFP positive cells were sorted prior to RNA extraction (RNeasy Plus mini kit, Qiagen). RNA was also isolated from stored PBMC samples collected from acute HIV-1 infected (1-2 months after the diagnosis) and pdmH1N1

infected patients from the Beijing You'an Hospital bio-bank as described in our previous publications<sup>(3, 4)</sup>.

#### **RNA Sequencing and Analysis.**

Transcriptome libraries were prepared at the Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, UK (DCs from healthy volunteers), and the Beijing Genomics Institute (BGI), Beijing, China (patient PBMCs) using standard protocols (supplementary material).

#### **IFITM3 Specific Antibody Production and Screening.**

To produce IFITM3-specific antibodies, mice were immunized with the first 21 amino acids on the N-terminus of IFITM3. Hybridoma cell lines were generated from the spleen of the immunized mice and screened for anti-IFITM3 antibody production. The binding activity of each antibody to IFITM3 was verified by ELISA. Binding affinity and cross reactivity with IFITM2 protein were assessed using inhibition ELISA (further details in supplementary material).

#### **Generation of IFITM3 knock out HEK293 cell line:**

Guide RNAs (gtcaaatcgggatttgccg; ggcctggaagatcagcact) were designed with the MIT CRISPR web tool and cloned into px458 plasmids with a mRuby2 fluorescent protein. HEK293 cells were transfected in bulk with Lipofectamine 2000 (Thermo Fisher Scientific). Single cells were sorted after 48 hours based on presence of mRuby2 signal. Clones were probed with anti-IFITM3 antibody and sequenced at guide loci to confirm knock out of the *IFITM3* gene.

#### **Western Blot analysis of IFITM3 protein expression:**

HEK293T, *IFITM3*<sup>-/-</sup> HEK293T and fibroblast cell lines were homogenised using RIPA lysis buffer (Thermo Fisher Scientific). Anti-GAPDH Antibody clone 6C5 (Merck Millipore, MAB374) was used as control antibody; our in house anti-IFITM3

antibody (XA254.3) was compared with commercially available anti-IFITM3 antibody (Clone AF311a, VWR). Primary antibodies were probed by IRDye 680LT Goat Anti-Mouse (Li-Cor, 926-68020) and IRDye 800LT Goat Anti-rabbit (Li-Cor, 925-32210), and visualised using the Li-Cor Odyssey Imaging System.

## Results

### All rs12252 Genotypes Express Full-Length IFITM3 Transcript in H7N1 S-FLU infected DCs

To verify the expression of *IFITM3* isoforms in all rs12252 genotypes, we performed RNA-Seq analysis on DCs infected with non-replicating GFP-expressing H7N1 influenza virus (S-FLU). Splicing analysis showed a predominant expression of full-length *IFITM3* (ENST00000399808.4) in all three genotypes, before and after H7N1 S-FLU infection (Fig. 1A). In all three genotypes, IFITM3 transcript was induced more than 100-fold following S-FLU infection. Furthermore, low-level expression of a transcript predicted to be degraded by nonsense-mediated decay (NMD; ENST00000531688.1) was observed (less than 0.5% of total *IFITM3* transcripts). After S-FLU infection, less than 0.005% of total IFITM3 transcript was truncated *IFITM3* (missing 21 amino acids on N-terminal; ENST00000602735.2, ENST00000526811.4). More importantly, no significant difference was observed when comparing expression ratios of *IFITM3* isoforms across CC and TT rs12252 genotypes. These results indicate full length *IFITM3* transcript is dominantly expressed at a similar level in all three genotypes.

**All rs12252 Genotypes Express Full-Length IFITM3 Transcript in Virus-Infected PBMCs and Fibroblast Cell Lines**

We further investigated the transcript levels of IFITM3 isoforms in PBMCs isolated from HIV-1 positive subjects and individuals infected with pdmH1N1 influenza virus. Similar to our observation in DCs, individuals infected with either virus showed negligible expression of the truncated *IFITM3* isoforms. The full-length *IFITM3* isoform was found to be dominantly expressed across all three genotypes (Fig. 1B, C), which suggests full-length IFITM3 expression is dominant in all major types of peripheral immune cells, regardless of genotype. Similar results were also found in primary fibroblast cell lines before and following IFN- $\alpha$  treatment (Fig. 1D).

**Expression of full-length IFITM3 protein was detected in rs12252 variants using a novel antibody specific to IFITM3 N-terminus**

Since all commercially available IFITM3 reactive monoclonal antibodies cross-react with IFITM2, we developed a highly specific anti-IFITM3 monoclonal antibody targeting the first 21 amino acids on the IFITM3 N-terminus. The calculated IC<sub>50</sub> for this antibody was 1.04 nM, and this antibody showed very low cross reactivity to IFITM2 in ELISA experiments (CR%=0.00066%; Fig. 2A and B).

The specificity of this antibody was further verified by Western blot in WT and *IFITM3*<sup>-/-</sup> HEK293T cell lines probed with our in-house IFITM3-specific antibody and an anti-IFITM3 commercial antibody, highly cross-reactive to IFITM2. Our in house IFITM3-specific antibody detected a single 15kDa band in WT HEK293T cells and not in the *IFITM3*<sup>-/-</sup> cells. However, using the commercial antibody we could detect a 15kDa band in both cell lines, although, at a lower level in the *IFITM3*<sup>-/-</sup> cell line, as expected (Fig. 2C).



195 This antibody was next used to measure the presence of full-length IFITM3  
196 protein in fibroblast cell lines from TT and CC patients. Full-length protein was  
197 detected in both cell lines (Fig. 2D)

## 199 Discussion

200 Various studies have demonstrated that the C allele of rs12252 is associated with  
201 severe IAV infection <sup>(2, 3)</sup>. To explain this observation, it has been hypothesized that  
202 the minor allele rs12252-C results in expression of truncated IFITM3 protein (i.e.,  
203 missing the first 21 amino acids), which is not as effective as full-length IFITM3, in  
204 inhibiting the influenza virus <sup>(11)</sup>. A recent study suggested that the putative rs12252  
205 splice isoform ENST00000526811.4 was not observed in three IFITM3 homozygous  
206 individuals with pdm2009H1N1 infection <sup>(10)</sup>. In the current study, we examined this  
207 hypothesis by verifying the expression of IFITM3 truncated isoforms  
208 (ENST00000602735.2, ENST00000526811.4) in rs12252 genotypes.

209 We measured the expression of *IFITM3* isoforms in DCs before and after infection  
210 with non-replicative H7N1 S-FLU, IFN- $\alpha$ 2 stimulated fibroblasts (data not shown),  
211 and individuals infected with either HIV-1 or H1N1 virus. High throughput RNA  
212 sequencing showed that a negligible level of truncated IFITM3 mRNA was expressed  
213 across all genotypes compared with >99% of the full-length isoform  
214 (ENST00000399808.4). We further showed full-length protein expression in primary  
215 human fibroblasts carrying homozygote rs12252 genotypes. Due to a lack of suitable  
216 antibody to the C-terminus of IFITM3 without cross-reactivity to other IFITM  
217 proteins, the truncated version of the protein could not be detected by Western blot.  
218 Due to the limited number of samples, we could not draw any firm conclusions on the

differences in full-length protein expression level on the cells carrying different genotypes in our western blot analysis; this merits further investigation.

The results of this study indicate that expression of truncated *IFITM3* transcript is negligible, and full-length transcript/protein dominant, in all rs12252 genotypes, making truncated isoforms unlikely to influence viral infections. Our findings do not support the hypothesis that CC genotype carriers express truncated IFITM3 <sup>(2)</sup>.

Although rs12252-C has been consistently associated with IAV, HIV and Hantaan virus infection in Chinese cohorts, contradictory results have been observed in two European cohorts <sup>(8, 9)</sup> and an American paediatric cohort <sup>(10)</sup>. This may be due to population bias (i.e. low frequency of rs12252-C in European populations); however, it is possible that rs12252 is in linkage disequilibrium with a causative SNP near the *IFITM3* locus in Chinese populations. We are currently investigating the functional properties of other closely linked variants that are in linkage disequilibrium with rs12252-C.

Overall, the results of this study showed all rs12252 genotypes dominantly express full-length IFITM3 protein, and a negligible level of truncated transcript, suggesting the truncated isoforms cannot be the underlying explanation for the association between rs12252-C and severe influenza virus infection. Further investigation is required to elucidate the association of rs12252-C with severe viral infection.

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**Author Contributions:**

TD designed and conceptualized the project; SMN, HLS, LW, DW designed the experiments; SMN, HLS, LW, DW, KK performed the experiments; AT provided GFP-expressing influenza virus; HKM, SMN, HLS, DW analysed the data; BQJ and CS generated IFITM3 antibody; YHZ, YZ, QL, HW provided clinical data and samples from acute HIV-1 and H1N1 seasonal cohorts; SMN, TD wrote the manuscript, and HLS, DW and AM contributed in revising the manuscript. All authors have read and approved the final manuscript.

**Additional Information:**

The authors would like to confirm that there is no conflict of interest.

## Figure legends

**Figure 1.** Expression of full-length IFITM3 isoform in various cell types, carrying either rs12252 genotype. (A) Expression of full-length IFITM3 in DCs before and after single cycle H7N1 S-FLU infection (3, 2, and 4 TT, CT and CC individuals, respectively); expression of full-length IFITM3 transcripts in PBMCs collected from acute HIV-1 (8 patients for each genotype, *i.e.*, TT and CC) (B) and pdm2009H1N1 (6, 6, and 5 TT, CT and CC patients, respectively) (C) infected individuals. (D) Full-length IFITM3 transcript was dominantly expressed in primary fibroblast cell lines pre- and post- IFN- $\alpha$ 2 treatment, DC isolated from healthy individuals before and after H7N1 S-FLU infection, PBMCs isolated from pdm2009H1N1 and HIV-1 infected patients carrying CC, CT and TT genotypes.

## Figure 2:

The specificity of our in-house IFITM3 antibody as determined by ELISA and Western blot. (A) An inhibition ELISA to determine the affinity and specificity of anti-IFITM3 monoclonal antibody. Different concentrations of free IFITM3, ranging from 0 to 16.25 nM were used to inhibit the binding of the anti-IFITM3 antibody to coating IFITM3 protein. The calculated IC<sub>50</sub> for this antibody is 1.04 nM. (B) An inhibition ELISA assay showed a very low cross reactivity to IFITM2 (CR%=0.00066%). (C) Wild-type (WT) and *IFITM3* knock out (*IFITM3*<sup>-/-</sup>) HEK293T cell lines were probed for IFITM3 with our in-house antibody (clone XA254.3) or a commercial IFITM3 antibody (clone AF311a) and GAPDH. Using our antibody, IFITM3 was detected in WT HEK293T cells but not in the *IFITM3*<sup>-/-</sup> cells. However, with the commercial antibody a 15kDa band was present in both cell lines, showing that this antibody recognizes both IFITM3 and IFITM2. These results

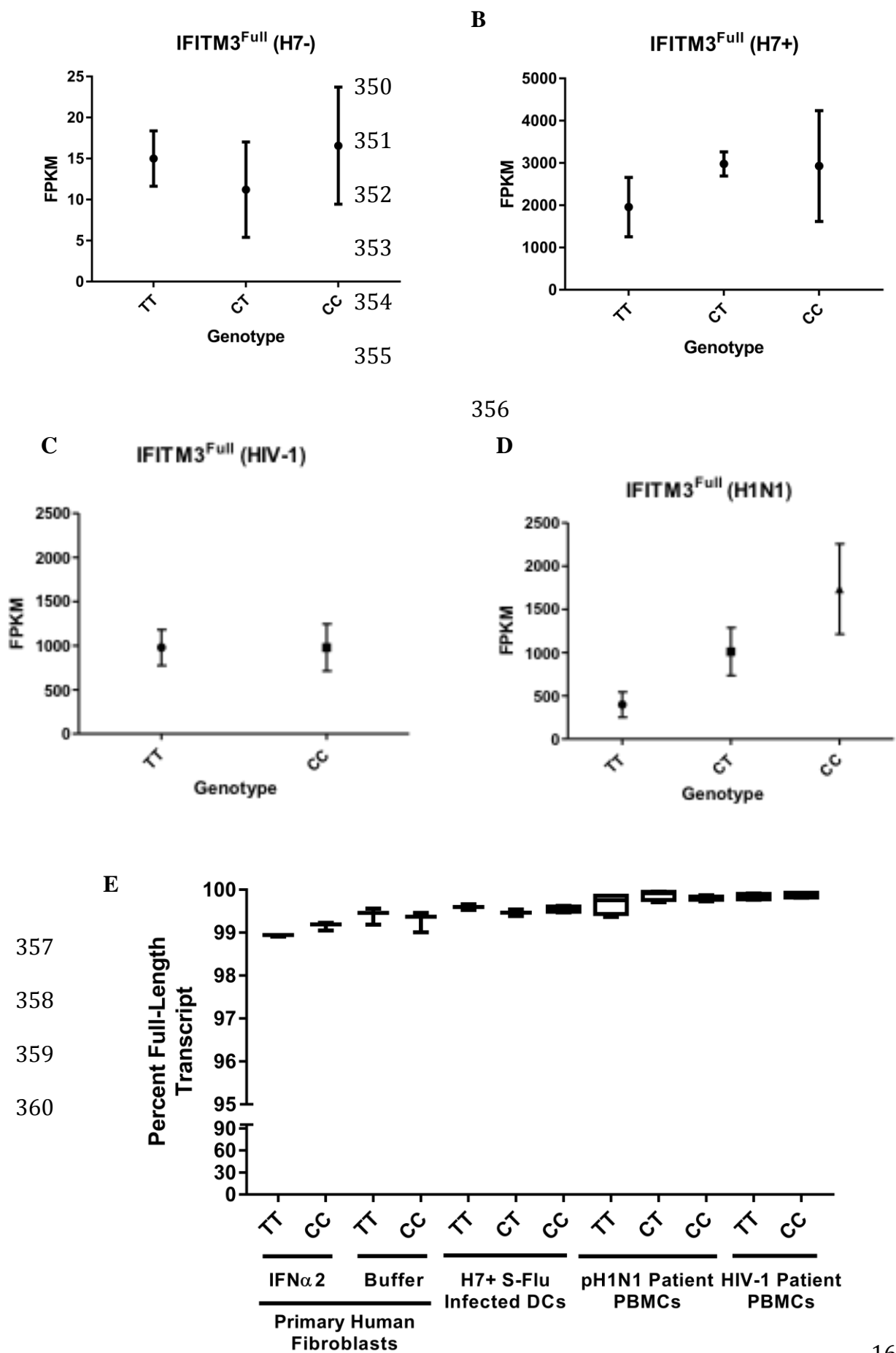
289 were reproducible in the presence and absence of IFN- $\alpha$ 2. (D) Primary human  
290 fibroblasts, carrying CC and TT genotypes were probed in the presence and absence  
291 of IFN- $\alpha$ 2. The expressed IFITM3 in these cells were probed with our in-house  
292 antibody (clone XA254.3). Under both conditions, expression of full length IFITM3  
293 protein was detected.

- 297 1. Brass AL, Huang IC, Benita Y, John SP, Krishnan MN, Feeley EM, et al.  
298 IFITM Proteins Mediate the Innate Immune Response to Influenza A H1N1 Virus,  
299 West Nile Virus and Dengue Virus. *Cell*. 2009;139(7):1243-54.
- 300 2. Everitt AR, Clare S, Pertel T, John SP, Wash RS, Smith SE, et al. IFITM3  
301 restricts the morbidity and mortality associated with influenza. *Nature*.  
302 2012;484(7395):519-23.
- 303 3. Zhang YH, Zhao Y, Li N, Peng YC, Giannoulatou E, Jin RH, et al. Interferon-  
304 induced transmembrane protein-3 genetic variant rs12252-C is associated with  
305 severe influenza in Chinese individuals. *Nat Commun*. 2013;4:1418.
- 306 4. Zhang Y, Makvandi-Nejad S, Qin L, Zhao Y, Zhang T, Wang L, et al.  
307 Interferon-induced transmembrane protein-3 rs12252-C is associated with rapid  
308 progression of acute HIV-1 infection in Chinese MSM cohort. *AIDS*.  
309 2015;29(8):889-94.
- 310 5. Wang Z, Zhang A, Wan Y, Liu X, Qiu C, Xi X, et al. Early hypercytokinemia is  
311 associated with interferon-induced transmembrane protein-3 dysfunction and  
312 predictive of fatal H7N9 infection. *Proc Natl Acad Sci U S A*. 2014;111(2):769-74.
- 313 6. Xu-Yang Z, Pei-Yu B, Chuan-Tao Y, Wei Y, Hong-Wei M, Kang T, et al.  
314 Interferon-Induced Transmembrane Protein 3 Inhibits Hantaan Virus Infection,  
315 and Its Single Nucleotide Polymorphism rs12252 Influences the Severity of  
316 Hemorrhagic Fever with Renal Syndrome. *Front Immunol*. 2016;7:535.
- 317 7. Lee N, Cao B, Ke C, Lu H, Hu Y, Tam HC, et al. IFITM3, TLR3, and CD55  
318 Genes SNPs and Cumulative Ge-netic Risks for Severe Outcomes in Chinese  
319 Patients with H7N9 / H1N1pdm09 Influenza. *The Journal of Infectious Diseases*.  
320 2017.
- 321 8. Mills TC, Rautanen A, Elliott KS, Parks T, Naranbhai V, Ieven MM, et al.  
322 IFITM3 and Susceptibility to Respiratory Viral Infections in the Community. *The*  
323 *Journal of Infectious Diseases*. 2014;209(7):1028-31.
- 324 9. Lopez-Rodriguez M, Herrera-Ramos E, Sole-Violan J, Ruiz-Hernandez JJ,  
325 Borderias L, Horcajada JP, et al. IFITM3 and severe influenza virus infection. No  
326 evidence of genetic association. *European journal of clinical microbiology &*  
327 *infectious diseases* : official publication of the European Society of Clinical  
328 Microbiology. 2016;35(11):1811-7.
- 329 10. Randolph AG, Yip WK, Allen EK, Rosenberger CM, Agan AA, Ash SA, et al.  
330 Evaluation of IFITM3 rs12252 Association with Severe Pediatric Influenza  
331 Infection. *The Journal of Infectious Diseases*. 2017.
- 332 11. Jia R, Pan Q, Ding S, Rong L, Liu SL, Geng Y, et al. The N-terminal region of  
333 IFITM3 modulates its antiviral activity by regulating IFITM3 cellular localization.  
334 *J Virol*. 2012;86(24):13697-707.
- 335 12. Compton AA, Roy N, Porrot F, Billet A, Casartelli N, Yount JS, et al. Natural  
336 mutations in IFITM3 modulate post-translational regulation and toggle antiviral  
337 specificity. *EMBO Rep*. 2016;17(11):1657-71.
- 338 13. Foster TL, Wilson H, Iyer SS, Coss K, Doores K, Smith S, et al. Resistance of  
339 Transmitted Founder HIV-1 to IFITM-Mediated Restriction. *Cell Host Microbe*.  
340 2016;20(4):429-42.
- 341 14. Williams DE, Wu WL, Grotefend CR, Radic V, Chung C, Chung YH, et al.  
342 IFITM3 polymorphism rs12252-C restricts influenza A viruses. *PLoS One*.  
343 2014;9(10):e110096.

344 15. Powell TJ, Silk JD, Sharps J, Fodor E, Townsend AR. Pseudotyped influenza  
345 A virus as a vaccine for the induction of heterotypic immunity. J Virol.  
346 2012;86(24):13397-406.  
347

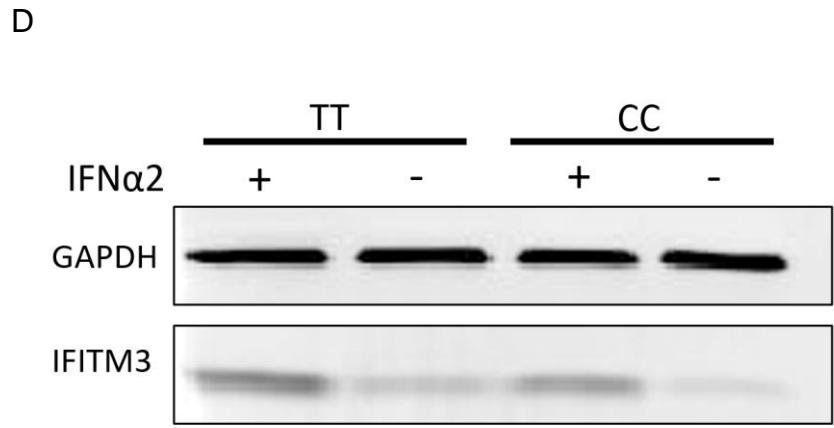
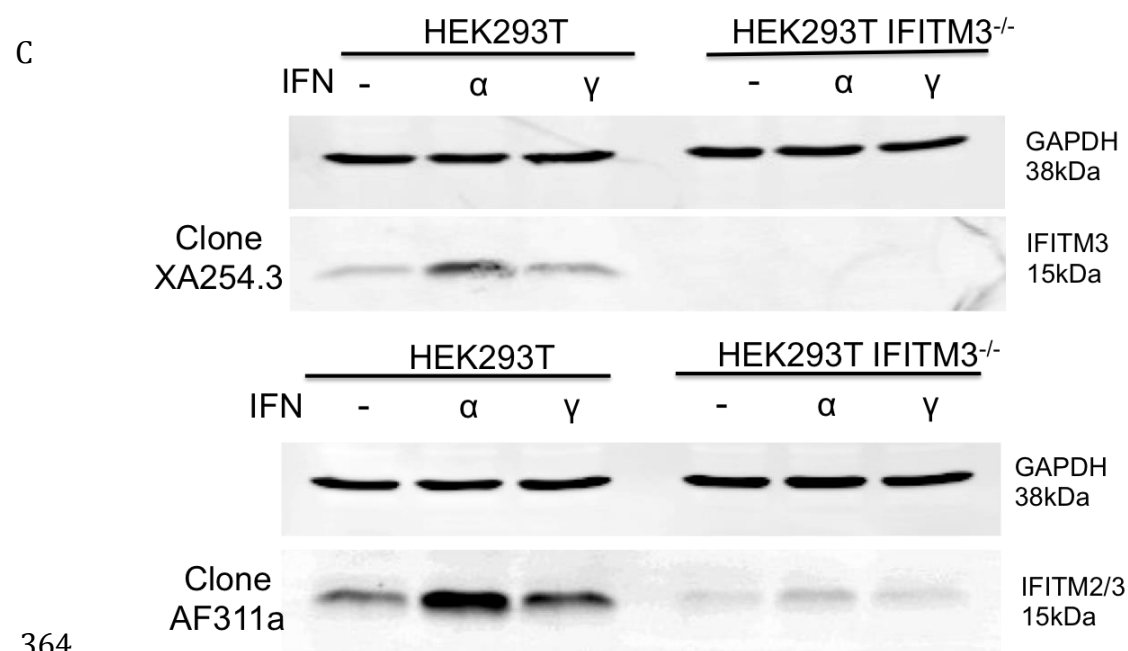
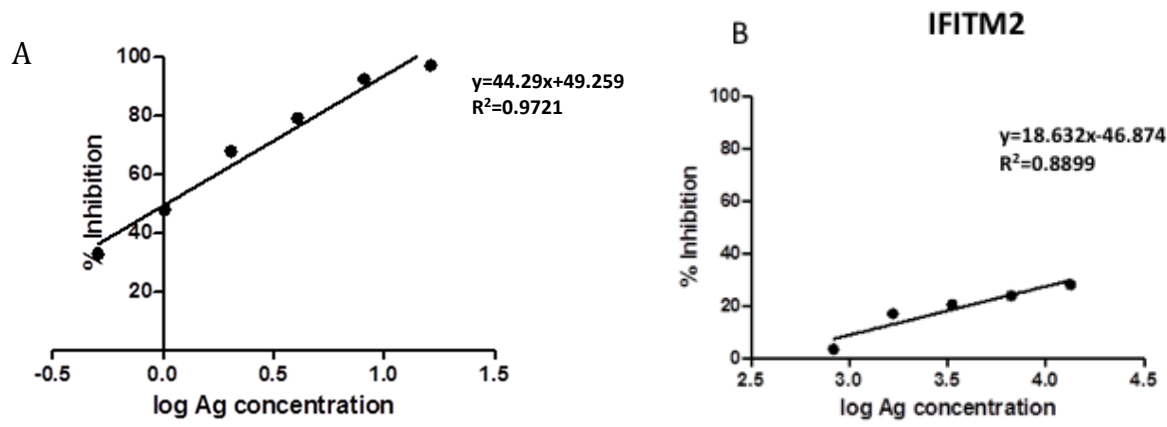
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Figure 1.





**Figure 2**



**Supplementary material:**

**Methods:**

**RNA sequencing and analysis.** Transcriptome libraries were prepared by either the Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, UK (DCs from healthy volunteers) or the Beijing Genomics Institute (BGI), Beijing, China (patient PBMCs) using standard protocols. Each sample was indexed with a separate barcode and pooled into a single library for sequencing. The libraries were sequenced on an Illumina HiSeq platform using 100 base pair, paired-end reads (PE). FastQC along with trimmomatic<sup>12</sup> were used for assessments and trimming low quality bases in order to retain high quality nucleotides. Reads were mapped to the GRCh37 assembly using Bowtie2 (version 2. 2.5)<sup>13</sup> and TopHat (version 2.0.13)<sup>14</sup>. Cufflinks (version 2.2.1)<sup>15</sup> was used to guide transcript assembly and to obtain gene and transcript level expressions.

**IFITM3 Specific Antibody Production and Screening.** To produce IFITM3-specific antibodies, five mice were immunized with the first 21 amino acids on the N-terminus of IFITM3. Hybridoma cell lines were generated from the spleen of the immunized mice and screened for anti-IFITM3 antibody production. The binding activity of each antibody to IFITM3 was verified by ELISA. In brief, the wells of a microtitre plate's first row were coated with IFITM3 ( $1 \mu\text{g mL}^{-1}$ ) in 1X PBS, followed by a serial dilution (1:2 v/v across the plate) in 1X PBS. As a negative control row was coated with either BSA or KLH, the carrier proteins. The coated plates were incubated at 4 °C, overnight. After washing the wells three times with 1X PBS, wells were blocked with 5% MPBS (skim milk in 100 mL of 1X PBS) for 2 h at room temperature. Wells were washed as described above and  $1 \mu\text{g mL}^{-1}$  of monoclonal antibody was diluted in 1X PBS. After adding the diluted antibody to the

first column, a serial dilution (1:2 v/v across the plate) in 1X PBS was performed. The antibodies were allowed to bind to IFITM3 by incubation for 1.5 h at room temperature. Wells were washed five times with 1X PBST and incubated mouse anti-human antibody conjugated to horseradish peroxidase. The signals were detected by adding TMB peroxidase substrate. After 30 min, reactions were stopped by adding 1 M H<sub>2</sub>SO<sub>4</sub> (100 uL) and absorbance (A) was measured at 450 nm.

#### **Assessment of binding affinity**

To verify the specific immune response to free IFITM3, an inhibition ELISA was performed on the selected antibodies against IFITM3. Wells were coated with 0.3 µg mL<sup>-1</sup> IFITM3 and incubated at 4 °C for 16 h. Antibodies at concentration of 0.78 µg mL<sup>-1</sup> were prepared. Their bindings to IFITM3 were inhibited with various concentrations of free IFITM3 (0 to 16.25 nM). The remaining steps were performed as described above. All samples were run in triplicate and each assay was repeated three times for testing its reproducibility.

The cross-reactivity of these antibodies to IFITM2 was tested by ELISA. A similar ELISA was performed as it was described above; except the wells were coated with IFITM3 and the antibodies were inhibited by IFITM2. Percentage of cross-reactivity was calculated according to the following equation: % CR = [IC<sub>50</sub> (IFITM3)/IC<sub>50</sub> (IFITM2)] X 100; where IC<sub>50</sub> is the concentration at which 50% of the antibodies are bound to either IFITM3 or IFITM2. All samples were run in triplicate and each assay was repeated three times for testing its reproducibility.