Title: IFITM3 restricts virus-induced inflammatory cytokine production by limiting Nogo-B mediated TLR responses

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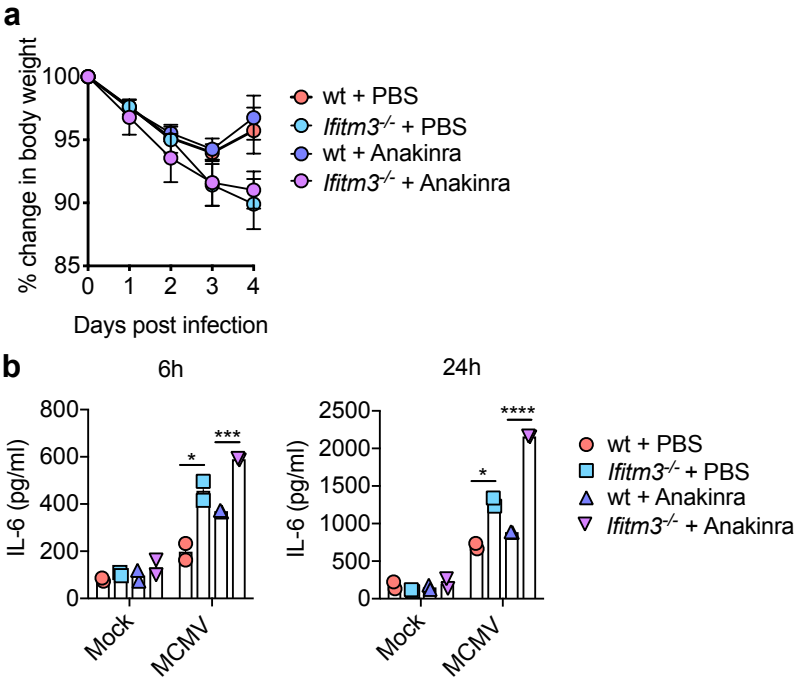
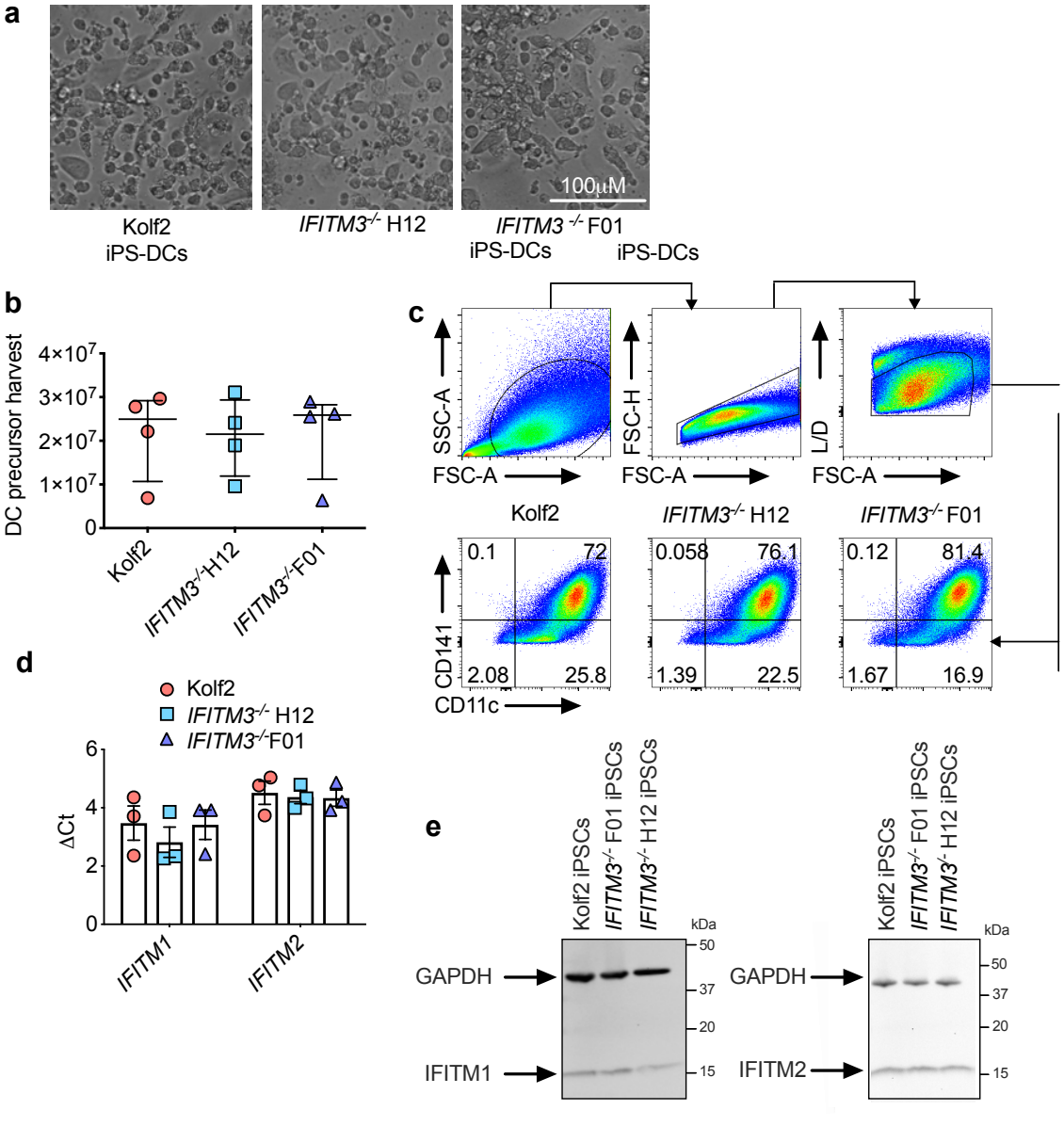
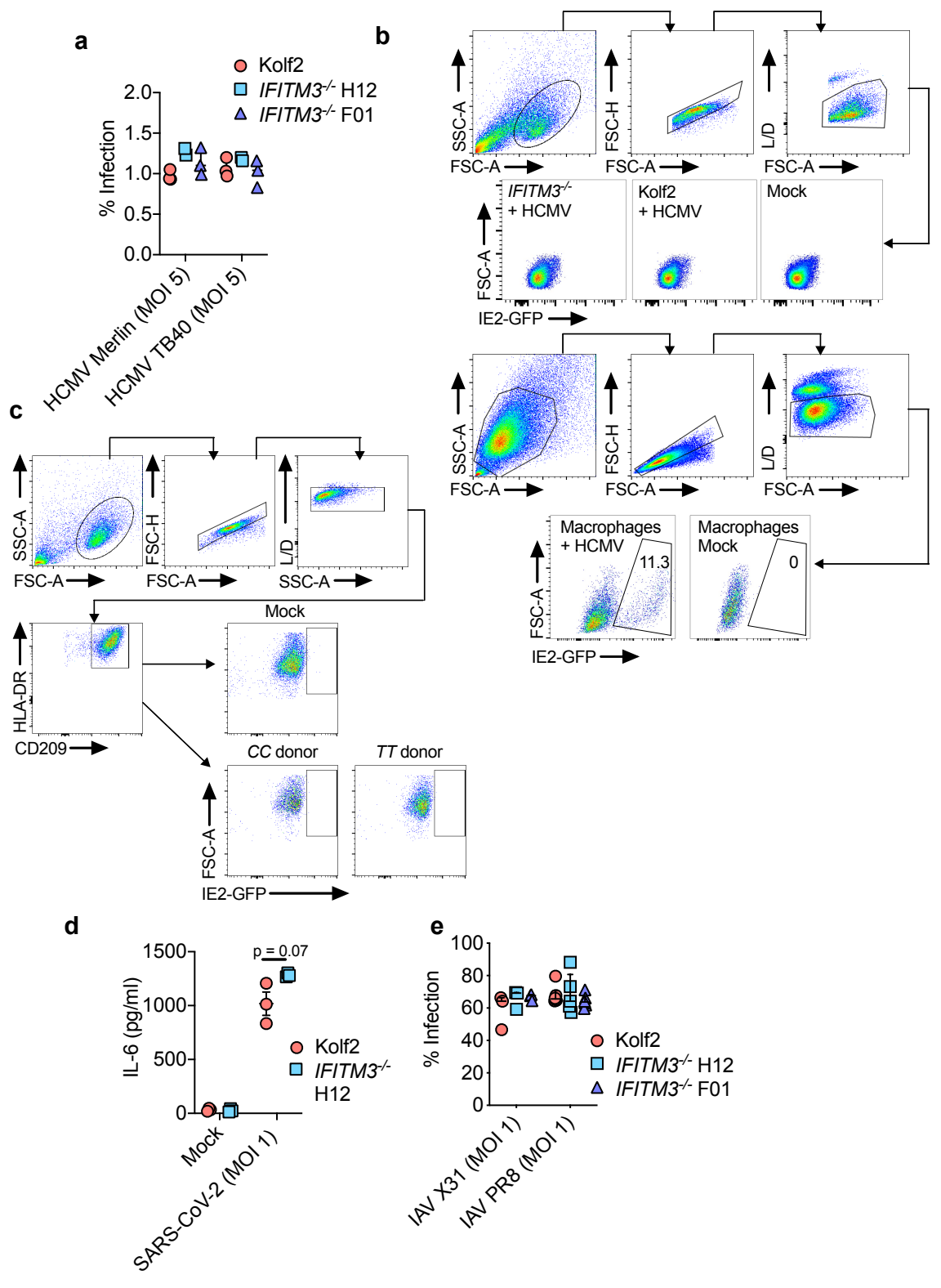


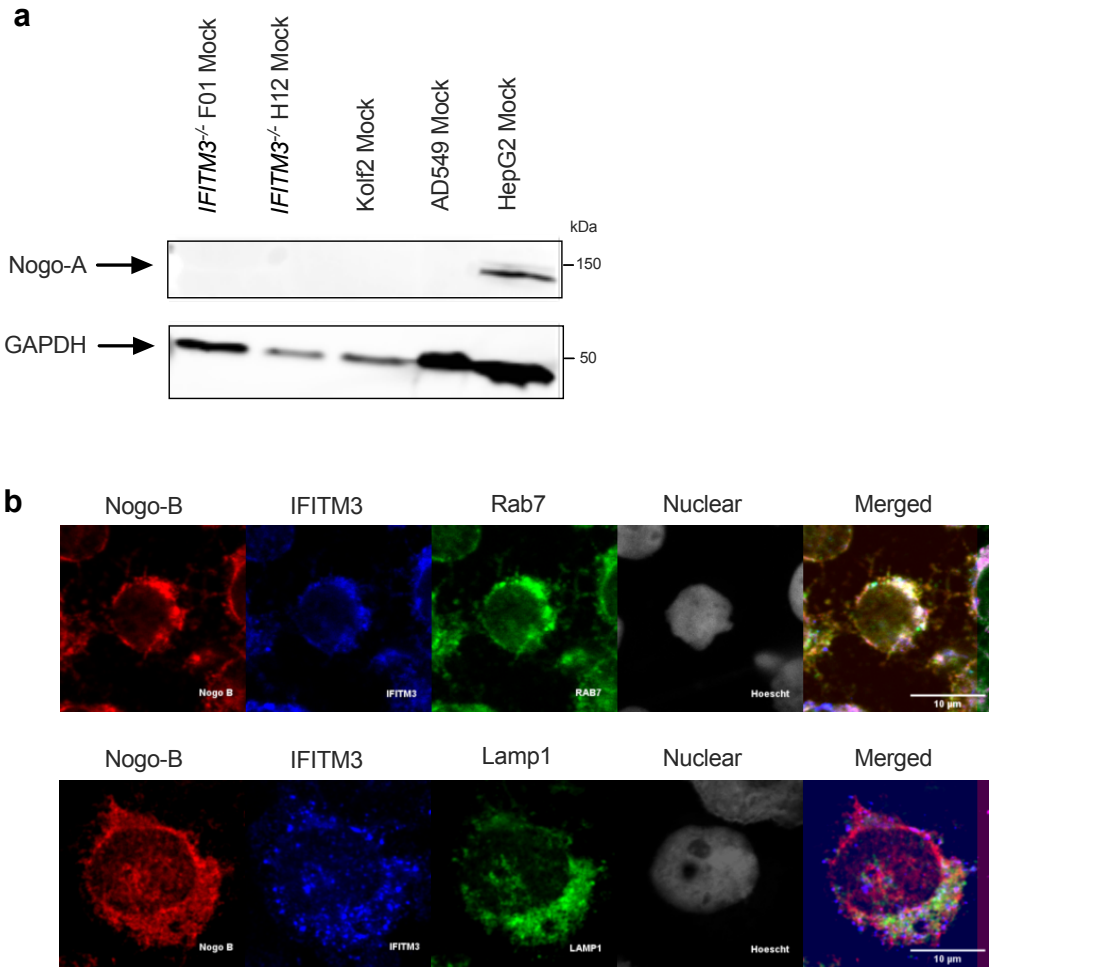
Supplementary Figure 1. **Antagonising IL-1R signalling has no impact on MCMV-induced weight loss or cytokine production.** (**a**) Wt and *Ifitm3*$^{-/-}$ mice were infected with MCMV with either 25mg/kg Anakinra or PBS and weight loss was assessed over time. Data shown represents 4 mice per group. (**b)** BM-DCs from wt and *Ifitm3*$^{-/-}$ mice were pre-incubated with or without Anakinra and then infected with MCMV (MOI 1). Supernatant was harvested at 6h and 24 post infection and IL-6 levels were assayed. Data are presented as mean +/- SEM of 2 biologically separate replicates and statistical significance was assessed using 2-way ANOVA (**a**) or 1-way ANOVA (**b**). *P* values are reported as follows: n.s., >0.05; *, ≤0.05; **, ≤0.01; ***, ≤0.001; and ****, ≤0.0001. Source data are provided as a Source Data file.


a
100μM
Kolf2 iPS-DCs
IFITM3-/- H12 iPS-DCs
IFITM3-/- F01 iPS-DCs
b
DC precursor harvest
4×10^7
3×10^7
2×10^7
1×10^7
0
Kolf2
IFITM3-/-H12
IFITM3-/-F01
c
SSC-A
FSC-A
FSC-H
L/D
Kolf2
IFITM3-/- H12
IFITM3-/- F01
0.1
72
2.08
25.8
0.058
76.1
1.39
22.5
0.12
81.4
1.67
16.9
CD141
CD11c
d
Kolf2
IFITM3-/- H12
IFITM3-/-F01
ΔCt
6
4
2
0
IFITM1
IFITM2
e
Kolf2 iPSCs
IFITM3-/- F01 iPSCs
IFITM3-/- H12 iPSCs
kDa
50
37
20
15
GAPDH
IFITM1
IFITM2

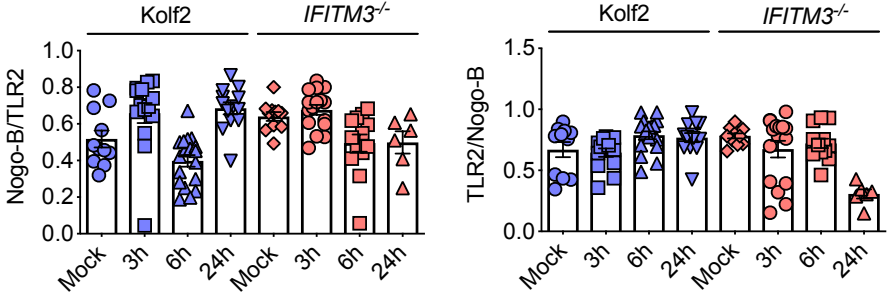
Supplementary Figure 2. IFITM3-deficiency does not affect DC differentiation and phenotype. CRISPR/Cas9 was used to generate biallelic mutations in IFITM3 in Kolf2 iPSCs. iPSCs were differentiated into dendritic cells using defined concentrations of growth factors to generate embryoid bodies (EBs), GM-CSF and IL-4 to generate immature DCs from EBs. (**a**) Morphology of iPS-DCs in culture (100X). (**b**) Total cell numbers of DC precursors harvested from DC differentiation plates. Data shown as individual technical replicates + mean and are from 4 independent differentiations per iPSC line. (**c**) Surface expression of DC markers CD11c and CD141 was examined by flow cytometry. Representative plots presented from one experiment, with experiments performed at least three times. (**d**) Gene expression of *IFITM1* and *IFITM2* by iPS-DCs relative to *GAPDH* was quantified using TaqMan gene expression assays. Data presented shows mean Ct values +/- SEM from 3 biologically independent cell cultures, with assays repeated in triplicate. (**e**) IFITM1 and IFITM2 expression in iPSCs was assayed by Western blot. Data represents 2 separate experiments. Source data are provided as a Source Data file.


a
Kolf2
IFITM3-/- H12
IFITM3-/- F01
% Infection
2.0
1.5
1.0
0.5
0.0
HCMV Merlin (MOI 5)
HCMV TB40 (MOI 5)
b
SSC-A
FSC-A
FSC-H
L/D
IFITM3-/- + HCMV
Kolf2 + HCMV
Mock
IE2-GFP
Macrophages + HCMV
11.3
Macrophages Mock
0
c
HLA-DR
CD209
Mock
CC donor
TT donor
d
IL-6 (pg/ml)
1500
1000
500
0
p = 0.07
Kolf2
IFITM3-/- H12
Mock
SARS-CoV-2 (MOI 1)
e
% Infection
100
80
60
40
20
0
IAV X31 (MOI 1)
IAV PR8 (MOI 1)

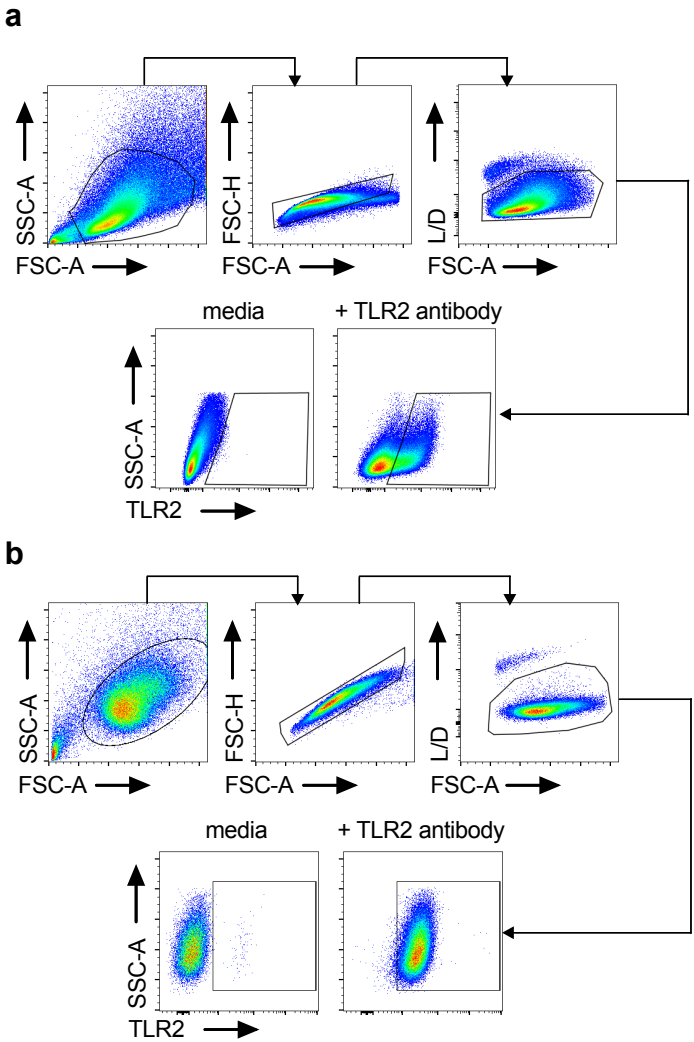
Supplementary Figure 3. IFITM3-deficiency in iPS-DCs does not alter infectivity by IAV or HCMV. (**a&b**) *IFITM3*$^{-/-}$ and Kolf2 iPS-DCs were non-permissive to HCMV using both merlin and TB40, as assayed by expression of IE2-GFP, with representative FACS plots from these assays, and a permissive cell line (macrophages), shown in (**b**). (**c)** Monocyte-derived DCs were non-permissive to HCMV, as assayed by expression of IE2-GFP, with representative FACS plots shown for *CC* or *TT* donors. (**d**) iPS-DCs were infected with SARS-CoV-2 (MOI 1), and IL-6 in supernatants was assayed 6h post infection by ELISA. Individual technical replicates + mean are shown and statistical significance was assessed using Student's *t* test performing paired analysis of SARS-CoV-2 stimulated cells. (**e**) *IFITM3*$^{-/-}$ and Kolf2 iPS-DCs were stimulated with IAV A/X31 (MOI 1) and then stained for IAV NP 24h post-stimulation.



Supplementary Figure 4. iPS-DCs do not express NOGO-A. (**a**) Expression of anti-Nogo-A was assayed in iPS-DCs, A549s and HepG2s from whole cell lysates by Western blot. (**b**) Immunoflourescent images of THP1 cells stained with anti-Nogo B, anti-IFITM3 and anti-RAB7 (top) or anti-Lamp1 (bottom), and Hoescht 33342 nuclear stain taken at x60 magnification using a Zeiss LSM 800 confocal microscope. Data represents 2 (**a**) and 3 (**b**) separate experiments.



Supplementary Figure 5. Nogo-B and TLR2 colocalise after HCMV infection. Immunostaining for TLR2 and NogoB was performed in *IFITM3*$^{-/-}$ and Kolf2 iPS-DCs stimulated for 1h, 3h or 24h with HCMV or mock treated followed by colocalization analysis. Data presented is percentage colocalization for at least 10 cells per condition. Mean +/- SEM are shown from 6-18 different cell cultures. Source data are provided as a Source Data file.



Supplementary Figure 6. Gating strategy of surface TLR2 assessed by FACS in (a) Kolf2 and *IFITM3*$^{-/-}$ iPS-DCs in Figure 7 b&c and (b) THP-1s in Figure 7 d&e .

Supplementary Table 1. Flow cytometry antibodies.

Antibody	**Company/ Clone**	**Catalog number**	**Dilution /amount**	**Validation**
anti-Influenza-A FITC	Abcam (431)	ab20921	1/50	https://www.abcam.com/Influenza-A-Virus-Nucleoprotein-antibody-431-FITC-ab20921.html?intFromAbID=81126
TruStain FcX	Biolegend (CD16 (3G8), CD32 (FUN-2) CD64 (10.1))	422301	1/20	https://www.biolegend.com/en-gb/products/human-trustain-fcx-fc-receptor-blocking-solution-6462
anti-CD11c FITC	Biolegend (Bu15)	337213	1/50	https://www.biolegend.com/en-gb/products/fitc-anti-human-cd11c-antibody-6087
anti-CD141 APC	Biolegend (M80)	344105	1/50	https://www.biolegend.com/en-gb/products/apc-anti-human-cd141-thrombomodulin-antibody-7168
anti-TLR2 PE	Biolegend (TLR2.1)	309707	1/50	https://www.biolegend.com/en-us/products/pe-anti-human-cd282-tlr2-antibody-1505
anti-TLR2 APC	Biolegend (W15145C)	392303	1/100	https://www.biolegend.com/en-gb/products/apc-anti-human-cd282-tlr2-antibody-15332
anti-HLA-DR PB	Biolegend (L243)	307623	1/100	https://www.biolegend.com/en-gb/products/pacific-blue-anti-human-hla-dr-antibody-3335
anti-CD209 DC-SIGN PE-Cy7	Biolegend (9E9A8)	330114	1/50	https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-human-cd209-dc-sign-antibody-7059

Supplementary Table 2. Immunostaining and Imaging antibodies.

Antibody	Company/ Clone	Catalog number	Dilution/ amount	Validation
anti-goat IgG FITC	Abcam	ab6737	1/1000	https://www.abcam.com/rabbit-goat-igg-hl-fitc-ab6737.html
anti-TLR2	Abcam Immunogen CLEIDASD LQSYEPK SLKSIQNV SHLI	ab1655	1/100	https://www.abcam.com/tlr2-antibody-ab1655.html
Goat anti-mouse IgG DyLight™ 488	Biolegend (poly4053)	405310	1/200	https://www.biolegend.com/en-gb/products/dylight-488-goat-anti-mouse-igg-minimal-x-reactivity-5687
Goat anti-mouse IgG DyLight™ 649	Biolegend (poly4053)	405312	1/200	https://www.biolegend.com/en-gb/products/dylight-649-goat-anti-mouse-igg-minimal-x-reactivity-5689
Donkey anti-rabbit IgG DyLight™ 649	Biolegend (poly4064)	406406	1/200	https://www.biolegend.com/en-gb/products/dylight-649-donkey-anti-rabbit-igg-minimal-x-reactivity-5695
Donkey anti-rabbit IgG AF488	Biolegend (poly4064)	406416	1/200	https://www.biolegend.com/en-gb/products/alexa-fluor-488-donkey-anti-rabbit-igg-minimal-x-reactivity-9380
anti-human CD107a FITC	Biolegend (H4A3)	328606	1/50	https://www.biolegend.com/en-gb/products/fitc-anti-human-cd107a-lamp-1-antibody-4966
anti-Rab7A	Biolegend (W16034A)	850401	1/100	https://www.biolegend.com/en-gb/products/purified-anti-rab7a-antibody-14708
Goat anti-rat IgG AF647	Biolegend (poly4054)	405416	1/200	https://www.biolegend.com/en-gb/products/alexa-fluor-647-goat-anti-rat-igg-minimal-x-reactivity-9252
anti-Nogo-B	Biotechne Accession # NP_722550	AF6034	1/100	https://www.rndsystems.com/products/human-mouse-nogo-b-antibody_af6034#product-datasheets
anti-sheep IgG Cy5	Merck Millipore	AP184S	1/1000	https://www.merckmillipore.com/GB/en/product/Donkey-Anti-Sheep-IgG-Antibody-Cy5-conjugate-Species-Adsorbed,MM_NF-AP184S

anti-TLR2	Novus biologicals	NB100-56720	1/200	https://www.novusbio.com/products/tlr2-antibody_nb100-56720#datasheet
anti-Sheep IgG (H+L) AF594	Thermo-Fisher	A-11016	1/200	https://www.thermofisher.com/antibody/product/Donkey-anti-Sheep-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11016
anti-IFITM3	In house	n/a	1/200	Wellington, D. *et al.* IFITM3-specific antibody reveals IFN preferences and slow IFN induction of the antiviral factor IFITM3 in humans. *Eur J Immunol* **51**, 742-745 (2021).

Supplementary Table 3. Protein preparation and western blotting antibodies.

Antibody	Company/ Clone	Catalog number	Dilution/ amount	Validation
anti-fragilis	Abcam	ab15592	1µg/ml	https://www.abcam.com/fragilis-antibody-ab15592.html
Veriblot IP detection reagent	Abcam	ab131366	1/200	https://www.abcam.com/veriblot-for-ip-detection-reagent-hrp-ab131366.html
anti-Nogo-A	Abcam Accession # NP_065393	ab62024	1µg/ml	https://www.abcam.com/nogo-a-antibody-ab62024.html
anti-sheep IgG (H+L)-HRP	Biorad	1721017	1/3000	https://www.bio-rad.com/en-uk/sku/1721017-rabbit-anti-sheep-igg-hl-hrp-conjugate?ID=1721017
anti-rabbit IgG (H+L)-HRP	Biorad	STAR124P	1/3000	https://www.bio-rad-antibodies.com/polyclonal/rabbit-lapine-igg-antibody-star124.html?f=hrp&_ga=2.175517666.1766291682.1655199188-1864392910.1655199187
anti-Nogo-B	Biotechne Accession # NP_722550	AF6034	0.2µg/ml	https://www.rndsystems.com/products/human-mouse-nogo-b-antibody_af6034#product-datasheets
anti-sheep (H+L) AF680	Invitrogen	A-21102	0.2µg/ml	https://www.thermofisher.com/antibody/product/Donkey-anti-Sheep-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21102
IRDye 680LT goat anti-mouse	Li-Cor	926-68020	1/5000	https://www.licor.com/bio/reagents/irdye-680lt-goat-anti-mouse-igg-secondary-antibody
IRDye 800LT goat anti-rabbit	Li-Cor	925-32210	1/5000	https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-rabbit-igg-secondary-antibody
anti-GAPDH	Merck Millipore	MAB374	1µg/ml	https://www.merckmillipore.com/GB/en/product/Anti-Glyceraldehyde-3-Phosphate-Dehydrogenase-Antibody-clone-6C5,MM_NF-MAB374
anti-Actin	Merck Millipore	A2066	1µg/ml	https://www.sigmaaldrich.com/GB/en/product/sigma/a2066?gclid=Cj0KCQjwwJuVBhCAARIsAOPwGASaWVYIB9SqfPapSGQREbytgqIJeJH3003yBBA-k3DpD8xX6kF-S1EaAp3MEALw_wcB

anti-IFITM1	Proteintech (5B5E2)	60074-1-lg	3μg/ml	https://www.ptglab.com/products/IFITM1-Antibody-60074-1-Ig.htm
anti-IFITM2	Proteintech	12769-1-AP	3μg/ml	https://www.ptglab.com/products/IFITM2-Antibody-12769-1-AP.htm

Supplementary Table 4. Proteomic pulldown antibodies.

Antibody	Company/ Clone	Catalog number	Dilution/ amount	Validation
anti-fragilis	Abcam	ab15592	1µg/ml	https://www.abcam.com/fragilis-antibody-ab15592.html
anti-rabbit IgG	Abcam	ab37415	1µg/ml	https://www.abcam.com/rabbit-igg-polyclonal-isotype-control-ab37415.html

Supplementary Table 5. Neutralization assay antibodies.

Antibody	Company/ Clone	Catalog number	Dilution/ amount	Validation
Cytotect	Biotest	626010010	500µg/ml	https://www.biotest.com/gb/en/products/clinical_immunology/cytotect_cp-biotest/product_profile.cfm
Anakinra/ KINERET	Cardiff & Vale NHS Pharmacy	n/a	500ng/ml	https://www.kineretrx.com
anti-TLR2	Invivogen	pab-hstlr2	200µg/ml	https://www.invivogen.com/anti-tlr2

Supplementary Table 6. gRNA sequence for generation of *IFITM3*$^{-/-}$ iPSCs and primer sequences for validation of *IFITM3*$^{-/-}$ iPSCs, and *IFITIM3* genotyping primers.

***IFITM3*$^{-/-}$iPS DC generation**	**Sequence**
gRNA sequence	TGGGGCCATACGCACCTTCA CGG
External Primer (F)	TGTGAGTTCCCTTCTCACTTT
External Primer (R)	CACTGTCCAAACCTTCTTCTC
Internal Primer (F)	AGTCACAGGGACACACAAGTC
Internal Primer (R)	CAACACCCTCTTCATGAACCC
***IFITM3* genotyping primers**	
Forward	GGCAGAGGTGAGGGCTTT
Reverse	GTCCCTTACGAGTCTCCCAC

Supplemental Table 7. Sequence information of *IFITM3*$^{-/-}$ iPSC clones. Underlined sequences are CD225 domain, and alternative methionine start codon in some protein isoforms.

IFITM3	**Sequence**
IFITM3_WT sequence	CCTCTGAGCATTCCCTGGGGCCATACGCACCTTCACGGAGTAGGCGA
IFITM3_F01 MUT sequence	CCTCTGAGCATTCCCTGGGGCCATACGCACCTTTCACGGAGTAGGCGA
IFITM3_H12 First Allele MUT sequence (Insertion of T)	CCTCTGAGCATTCCCTGGGGCCATACGCACCTTTCACGGAGTAGGCGA
IFITM3_H12 Second Allele MUT sequence (Insertion of T)	CCTCTGAGCATTCCCTGGGGCCATACGCACCTCTTCACGGAGTAGGCG
WT Protein	MNHTVQTFFSPVNSGQPPNYEMLKEEHEVAVLGAPHNPAPPTSTVIHIRSETSVPDHVVWSLFNTLFMNPCCLGFIAFAYSVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGILMTILLIVIPVLIFQAYG*
MUT Protein (T Insertion)	MNHTVQTFFSPVNSGQPPNYEMLKEEHEVAVLGAPHNPAPPTSTVIHIRSETSVPDHVVWSLFNTLFMNPCCLGFIAFAYSVKV*
MUT Protein (CT Insertion)	MNHTVQTFFSPVNSGQPPNYEMLKEEHEVAVLGAPHNPAPPTSTVIHIRSETSVPDHVVWSLFNTLFMNPCCLGFIAFAYSVKSLGTGRWLAT*