

Supplementary Materials for

IFITM proteins inhibit placental syncytiotrophoblast formation and promote fetal demise

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Movies S1

Materials and Methods

Plasmids

pLOC_LacZ-Omega and pLOC_LacZ-Alpha were generated as follows. The LacZ- α and LacZ- ω fragments were cloned into a Precision LentiORF™ (Dharmacon) plasmid. pQCXIP-Empty control plasmid and pQCXIP-IFITM1-N-FLAG, pQCXIP-IFITM2-N-FLAG, pQCXIP-IFITM3-N-FLAG, pQCXIP-IFITM3 Δ Palm-N-FLAG, pQCXIP-IFITM3 Δ 1-21-N-FLAG, pQCXIP-IFITM3 Δ Ub-N-FLAG plasmids encoding N-terminal FLAG-tagged IFITM1, IFITM2, IFITM3 and IFITM3 mutants were described previously (15, 16, 22). pQCXIP-BSR-GFP11 and pQCXIP-GFP1-10 were from Yutaka Hata (Addgene plasmid #68716 ; <http://n2t.net/addgene:68716> ; RRID:Addgene_68716 and Addgene plasmid #68715 ; <http://n2t.net/addgene:68715> ; RRID:Addgene_68715). pcDNA3.1_Syn1_Strep-Tag plasmid contains an affinity tag (residues D WSHPQFEK GGSA WSHPQFEK) at the N-ter of syncytin-1. The phCMV-syn1, phCMV-syn2, phCMV-Mus-SyncA, phCMV-Mus-SyncB plasmids have been described previously (23-25).

Lentiviral and Retroviral vectors

For lentiviral production, 293T cells (ATCC) were co-transfected with pLOC-derived plasmids (pLOC_LacZ-Omega and pLOC_LacZ-Alpha), packaging plasmid R8-2 and VSV-G plasmid as described (13). For murine retroviral vector production, the platinum-E (Cell Biolabs) retroviral packaging cell line was transfected with pQCXIP-derived plasmids. Vector containing supernatants were harvested at 36h, 48h and 72h and ultracentrifuged 1h at 4°C at 22000 g.

Cell cultures

VCTs were isolated from term placenta as described (26). Mouse embryonic fibroblasts (MEFs) were isolated from embryos of IfitmDel and C57BL/6JRj mice, at day 13.5-14.5 of gestation. Primary cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (P/S) at 37°C in 5% CO₂. 293T cells, platinum-E retroviral packaging cell line, TZM-bl cells and derivatives were cultured in DMEM supplemented with 10% FCS and 1% P/S. BeWo cells were maintained in DMEM (Gibco) supplemented with 20% FCS and 1% P/S. ExpiCHO cells were grown in ExpiCHO™ Expression Medium (Gibco) at 37°C with 8% CO₂ and constant shaking at 130 rpm. Cell lines transduced with pQCXIP or pLOC-derived vectors were grown with 1 μ g/ml puromycin or 10 μ g/ml blasticidin, respectively (InvivoGen).

Generation of stable cell lines

For lentiviral or retroviral transduction, 2×10^4 293T, TZM-bl or BeWo cells or 5×10^5 MEFs were resuspended in 150 μ l of medium containing 5-25 μ l of ultracentrifuged lentiviral or retroviral vectors. Cells were agitated 30 seconds every 5 minutes for 2h30 at 37°C in a Thermomixer. MEFs were transduced at passage 1 and kept up to 4-6 passages. For cell lines co-

expressing IFITMs and a complementation system, the GFPsplit or β -Gal vectors were transduced before IFITM vectors to avoid differential transduction efficiency due to VSV-G envelope restriction by IFITMs. All cell lines were routinely tested for mycoplasma and found negative.

X-Gal and CPRG assays

β -Gal- α or - ω expressing BeWo cells were resuspended at a 1:1 ratio in medium supplemented with 10 μ M forskolin or DMSO (control) and plated at 2×10^4 per well in 96 well plates for 48h. For X-Gal assay, cells were fixed with 0.5% Glutaraldehyde for 10 min. 100 μ l of X-Gal solution (PBS + 1 mM $MgCl_2$, 5 mM potassium ferrocyanide ($K^4Fe(CN)_6 \cdot 3H_2O$ - Sigma) and 5 mM potassium ferricyanide ($K_3Fe(CN)_6$ - Sigma)) was added per well. Cells were incubated 1h at 37°C and imaged on an EVOS FL (Thermo). For CPRG assay, media was removed and cells were lysed with 100 μ l of lysis buffer (PBS + 1 mM $MgCl_2$ + 0.1% IGEPAL® CA-630 (Sigma)). 10 μ l of cell lysate was used for DC™ protein assay (Bio-Rad) and 50 μ l was used for CPRG assay. 50 μ l of cell lysate was mixed with 50 μ l of CPRG mix (PBS + 1 mM $MgCl_2$, 0.1% IGEPAL® CA-630, 500 μ M CPRG (10884308001 – Roche)). Reaction mix was incubated in the dark at 37°C, 570 nm OD was read every 15 minutes for 1h. 690 nm OD was used as a background signal reference.

GFPsplit fusion assay

GFP1-10 or GFP11-expressing 293T cells were mixed at a 1:1 ratio. 6×10^4 cells in 96 well plates were transfected in suspension using Lipofectamine2000 (Thermo) with 100 ng of DNA. 5 ng of phCMV-syn1 and 30 ng of pQCXIP-Empty or pQCXIP-IFITM-N-FLAG were used and adjusted to 100 ng DNA with pQCXIP-Empty. For the IFITM1+2+3 cotransfection, 30ng of each plasmid was used. After 18h, two images per well were recorded and the area of GFP was quantified using an ImageJ macro. For live imaging, 8×10^4 GFP1-10 or GFP11-expressing BeWo cells were mixed at a 1:1 ratio in the presence of 10 μ M forskolin and seeded into a μ -Dish 35 mm Quad dish (ibidi – #80416). Transmission and fluorescence images were taken at 37°C every 10 min up to 72h using a Nikon Biostation IMQ, with five fields for each condition.

Growth curves

BeWo cells expressing or not IFITMs were seeded at 4×10^4 cells in 24-well plates. Viability and total cell numbers per well (in triplicates) were calculated at 48, 72 and 96h.

Luciferase-based fusion assay

6×10^4 293T cells in 96 well plates were transfected in suspension using Lipofectamine2000 (Thermo) with 100 ng of DNA. 10 ng of pTat, 1ng of phCMV-syn1 and 30 ng of pQCXIP-Empty or pQCXIP-IFITM1-3-N-FLAG were used and adjusted to 100 ng DNA with pQCXIP-Empty. 6h post transfection, cells were detached, washed in PBS and resuspended

in medium. 3×10^3 TZM-bl control or IFITM1-3 cells were added to each well of 293T cells and co-cultured for 20h before lysis. Luciferase signal was measured with the Bright-Glo™ luciferase assay system (Promega - #E2620).

RNA extraction and RT-qPCR

For murine tissue RNA extraction, 40-100 mg of frozen liver or placenta samples were lysed in 1 ml of QIAzol Lysis Reagent (QIAGEN) using Precellys® Tissue Homogenizing. Total RNA extraction was performed by following the QIAzol RNA extraction protocol. For VCT RNA extraction, 1 to 2×10^6 VCTs were lysed in 350 μ l RLT buffer (QIAGEN) supplemented with 1% β -mercaptoethanol. Total RNA extraction was performed with a RNeasy Mini Kit (QIAGEN). Samples (500 ng RNA) were treated with RQ1 RNase-free DNase (Promega) and used for reverse-transcription with SuperScript II Reverse Transcriptase (Thermo Fischer Scientific). qPCR was performed with iTaq Universal SYBR Green Supermix (Biorad) and a QuantStudio 6 Flex Real-Time PCR System. For mouse samples, the following primers were used: *Stat1* forward (5'- CCG AGA ACA TAC CAG AGA ATC -3'), *Stat1* reverse (5'- AGT AGC AGG AAG GAA TCA CAG -3'), *Irf7* forward (5'- CAG CGA GTG CTG TTT GGA GAC -3'), *Irf7* reverse (5'- AAG TTC GTA CAC CTT ATG CGG -3'), *Oas1a* forward (5'- GGA GGC GGT TGG CTG AAG AGG -3'), *Oas1a* reverse (5'- GAA CCA CCG TCG GCA CAT CC -3'), *SynA* forward (5'- TAC TCC TGC CCG ATA GAT GA -3'), *SynA* reverse (5'- CCG TTT TTC TTA ACA GTG GGT -3'), *SynB* forward (5'- CCA CCA CCC ATA CGT TCA AA -3'), *SynB* reverse (5'- GGT TAT AGC AGG TGC CGA AG -3'), *Tbp* forward (5'- AGA ACA ATC CAG ACT AGC AGC -3'), *Tbp* reverse (5'- GGG AAC TTC ACA TCA CAG CTC 3'). For human samples, the following primers were used: HERVW forward (5'- CGG ACA TCC AAA GTG ATA CAT CCT -3'), HERVW reverse (5'- TGA TGT ATC CAA GAC TCC ACT CCA -3'), HERVFRD forward (5'- GCC TGC AAA TAG TCT TCT TT -3'), HERVFRD reverse (5'- ATA GGG GCT ATT CCC ATT AG -3'), SLC1A5 forward (5'- GGC TTG GTA GTG TTT GCC AT -3'), SLC1A5 reverse (5'- GGG CAA AGA GTA AAC CCA CA -3'), MFSD2A forward (5'- CTC CTG GCC ATC ATG CTC TC -3'), MFSD2A reverse (5'- GGC CAC CAA GAT GAG AAA -3'), SDHA forward (5'- TGG GAA CAA GAG GGC ATC TG -3'), SDHA reverse (5'- CCA CCA CTG CAT CAA ATT CAT G -3').

Antibodies

Mouse monoclonal antibodies (mAb): IFITM1 (#60074-1-Ig, Proteintech) 1:200 for FACS, IFITM2/3 (#66081-1-Ig, Proteintech) 1:200 for FACS, Ifitm3 (ab15592, abcam) 1:500 for FACS and 1:200 for IF, StrepMAB-Immo mouse mAb (IBA Biosciences, #2-1517-001) 1:5000 for FACS, GATA3 mouse mAb (HG3-31, sc-268 Santa Cruz) 1:100 for IF. Rat monoclonal antibody: E-Cadherin (ECCD-2, Thermo or ab11512, Abcam) 1:500 for IF. Armenian hamster antibody: CD31 (MAB1398Z, Merck Millipore) 1:500 for IF. Rabbit polyclonal antibodies: FLAG-Tag DYKDDDDK (D6W5B) (#14793, Cell Signaling) 1:800 for FACS, desmoplakin (ab71690, Abcam) 1:300 for IF.

Secondary antibodies coupled with Alexa Fluor 488, 555 or 647 (InvitroGen) or DyLight 549 (anti armenian hamster, Jackson Immunoresearch) were used at 1:500 for FACS and IF.

Flow cytometry

Cells were fixed in 4% PFA for 10 minutes. Intracellular stainings were performed in PBS, 1% BSA, 0.05% sodium azide, 0.05% Saponine. Cells were incubated with primary antibodies, and then with secondary antibodies for 30 minutes at RT. Impact of IFITM on Syncytin-1 levels: 293T cells fuse upon Syncytin-1 transfection. We therefore used the ExpiCHO cell line, which does not express the Syncytin-1 receptor, for flow cytometry experiments. ExpiCHO cells were co-transfected with pcDNA3.1_Syn1_Strep-Tag plasmid and pQCXIP-Empty or pQCXIP-IFITM1-3-N-FLAG plasmids using Expifectamine CHO (Thermo). After 24h, cells were stained intracellularly with StrepMAB-Immo and FLAG-Tag DYKDDDDK (D6W5B) antibodies. Cells were acquired on a BD FACSCanto™ cytometer (BD Biosciences) and data analyzed with FlowJo software.

MEF Fusion assay

Mouse embryonic fibroblasts (MEFs) from C57BL/6JRj or *IfitmDel* mice were seeded at 10^4 cells/well in 96-well plates, and transfected in suspension with Lipofectamine2000. Cells were co-transfected with 30 ng of phCMV-g-Mus-SyncA or pQCXIP-Empty plasmid, 30 ng of *pmaxGFPTM* (Lonza) and adjusted to 100 ng with pQCXIP-Empty. After 24h, GFP-containing syncytia were analyzed by fluorescence microscopy and visually scored.

Mice/Mouse ethics

All procedures with animals were approved by the Animal Ethics Committee on March 9, 2016, under 2016-0018, authorized by the French Ministry of Research under #6466 and performed in compliance with European regulation 2010/63 EU. C57BL/6JRj mice were purchased from Janvier Labs (France). *Ifnar* knock-out mice, carrying the inactivated *Ifnar1* gene (*Ifnar1^{tm1Agt}* allele, MGI:1930950) on C57BL/6J background were bred at Institut Pasteur (hereafter referred to as *Ifnar*^{-/-} mice). *IfitmDel* mice (*I4*) carrying the Del(7Ifitm5-Ifitm6)1Masu allele (MGI:3804041) on C57BL/6J background were obtained from the European Mouse Mutant Archive (EMMA) (EM:04804) and bred at Institut Pasteur. This deletion encompasses *Ifitm1*, *Ifitm2*, *Ifitm3*, *Ifitm5* and *Ifitm6* genes.

Poly-IC challenge

Female mice of 7 to 20 weeks of age were used for timed mating and checked for the presence of a vaginal plug, indicating gestational age E0.5. For fetal resorption experiments, pregnant mice were injected intraperitoneally at E7.5 with 200 µg of HMW VacciGrade Poly-IC (InvivoGen) (100 µl of 2 mg/ml Poly-IC in PBS). Control mice were injected with 100 µl of PBS. Mice were sacrificed at E9.5 and fetuses and placentas were harvested and examined. Fetal resorption was assessed by dissecting individual fetoplacental units for the presence of an

embryo. Some resorbed fetoplacental units were strongly necrosed and thus excluded from downstream qPCR and immunostaining analysis. Images of fetuses were acquired on a ZEISS SteREO Discovery.V20 microscope and embryo size was calculated using ImageJ.

For short term (14 h) Poly-IC challenge, pregnant mice were injected intraperitoneally at E10.5 with 200 µg of HMW Vaccigrade Poly-IC (InvivoGen) (100 µl of 2 mg/ml Poly-IC in PBS) or 100 µl of PBS. Mice were sacrificed 14h post-injection; livers and placentas were harvested and either frozen at -80°C for RNA extraction or fixed overnight at 4°C in 4% PFA for immunostaining.

Tissue section Immunostaining

Fetoplacental units were harvested at day E9.5 and fixed with 4% PFA overnight at 4°C. Thick sections: Tissues were washed in PBS and embedded in agarose for 150-µm-thick vibratome section. Thin sections: After PFA fixation, tissues were washed in PBS, immersed in sucrose and embedded in OCT for 8-µm-thick cryosection. Sections were permeabilized and blocked for 1h in PBS 3% BSA, 0.4% Triton-X100 and stained overnight at 4°C (thick sections) or 2h at RT (thin sections) with primary antibodies. Tissues were washed 4 times in PBS and stained with secondary antibodies and Hoechst for 2h at RT. Sections were mounted in Fluoromount G, (Beckman Coulter) and dried at 37°C for 30 min. Confocal images were acquired on a Zeiss LSM710 inverted confocal microscope with the Zen software.

Human placenta collection and ethical statement

The study was performed according to the principles of the Declaration of Helsinki. Placentas were obtained with the patients' written informed consent. The protocol was approved by the local ethics committee (CPP 2015-mai-13909). Placental tissues were obtained from women that underwent caesarean section at Cochin Port-Royal, Antony, and Montsouris maternity units (Paris, France).

Western Blot

Extracts were prepared from snap-frozen cell pellets using RIPA Buffer (5 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1 % NP-40, 1 % Sodium desoxycholate, 0.1 % SDS, containing freshly added protease inhibitors (#539131, Calbiochem), and phosphatase inhibitors (#524629, Calbiochem)), and sonicated. Lysates were centrifuged 14,000g for 10 min at 4°C, and supernatants transferred in a new tube. Protein concentrations were determined using the micro BCA™ Protein Assay Kit (#23135, Thermo Scientific). Equal amounts of proteins (40 µg) were separated on 4–15 % SDS-PAGE mini-PROTEAN® TGX™ precast protein gel under reducing conditions (DTT) and transferred onto a 0.2 µm nitrocellulose membrane using a Trans-blot® Turbo™ Transfer System (Bio-rad). After blocking with 5% non-fat milk in TBS at 4°C overnight, membranes were incubated with primary antibodies in 1% BSA TBS at RT for 3 h. The membranes were then washed and incubated with secondary antibodies at RT for 1 h. Membranes were washed and scanned with an Odyssey® Imaging System (Li-Cor). The primary

antibodies were: anti-IFITM1 (0.75 µg/ml (1:2000), 60074-1, Proteintech), anti-IFITM2/3 (0.15 µg/ml (1:2000), ab109429, Abcam), anti-vinculin (0.1 µg/ml (1:10000), #V9131, Sigma-Aldrich). The secondary antibodies were: Alexa Fluor® 680-conjugated donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed (1:20000, #A10038, Invitrogen), Alexa Fluor® 790-conjugated donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed (1:20000, #A11374, Invitrogen). Signal intensity was quantified using the Image Studio Lite (version 5.2) software. The densities were normalized to vinculin.

Reverse Magnetofection of primary VCT

The transfection complexes were composed of 30 µl Opti-MEM medium was mixed with 0.6 µl Lipofectamine 2000CD (Invitrogen), 0.3 µl Magnetofection CombiMag (OZ Biosciences), and 0.3 µg of pQCXIP-IFITM3-Nflag or pEGFP-C1 plasmid DNA per well (12-well removable chamber, #81201, Ibidi). The mixture was incubated for 20 min at RT and thereafter distributed into the well. Culture slides were placed on a magnetic plate (OZ Biosciences) at least 10min at 37°C, 5% CO₂ before VCT seeding (8.10⁴/well). Culture slides placed on magnetic plate were transferred back into the incubator. After overnight incubation, transfection was terminated by the substitution of the incubation solution with fresh culture medium. After 48 h, cells were fixed and stained for FLAG, GATA3 and E-Cadherin.

VCT viability assay

VCT viability was assessed by quantification of cleaved cytokeratin 18 (cCK18) as described (27).

VCT GATA3/DAPI fusion assay

Percentage of VCT fusion was calculated by GATA3/DAPI nuclei quantification as described (18).

Statistical analysis

Statistical details and number of replicates are found in the corresponding Figure legends. Analyses were performed using GraphPad Prism 6 Software.

Bioinformatic analysis

Human chorionic villous explants raw fastq reads were retrieved from published data (7) (accession number GSE104349) and analyzed using RNA-seq pipeline from Sequana v. 0.6.2 (28). Quality analysis of FASTQ files was performed using FastQC (29) which provides assessment of GC content, over-abundance of adaptors and overrepresented sequence. Potential residuals of poly (A) and poly (T) tails were removed using Cutadapt (30). Reads were aligned using the two-pass alignment mode of STAR v. 2.5.3a (31) on human hg38 assembly (32) and gene counts were obtained from uniquely mapped reads using the featureCounts module of Subreads v. 1.5.3 (33).

Statistical Analysis of RNA-Seq data

The statistical analysis for RNA-Seq data was performed using R software, version 3.5.0 (34) on raw gene counts. Differential analysis was performed using the negative binomial generalized linear modelling (GLM) implemented in DESeq2 package version 1.20.0 (35), with default parameters for size factors and dispersion estimation as well as outlier detection and low expressed genes. A preliminary filter was applied to discard genes with too low counts, keeping genes with total read count larger than 10, as advised in the vignette of DESeq2. IFITM5 was not included in the analysis as no read mapped on this gene. The statistical model was adjusted on the experimental condition of interest as well as on sex of the foetus and tissue type, in order to account for the pairing across samples taken from the same donor. A Wald test was applied to perform comparisons among conditions. Empirical null modelling was applied to avoid overestimation of the variance in the null distribution using fdrtool package (36). P-values were corrected for multiple testing using Benjamini-Hochberg correction (37) with a risk level set to 0.05.

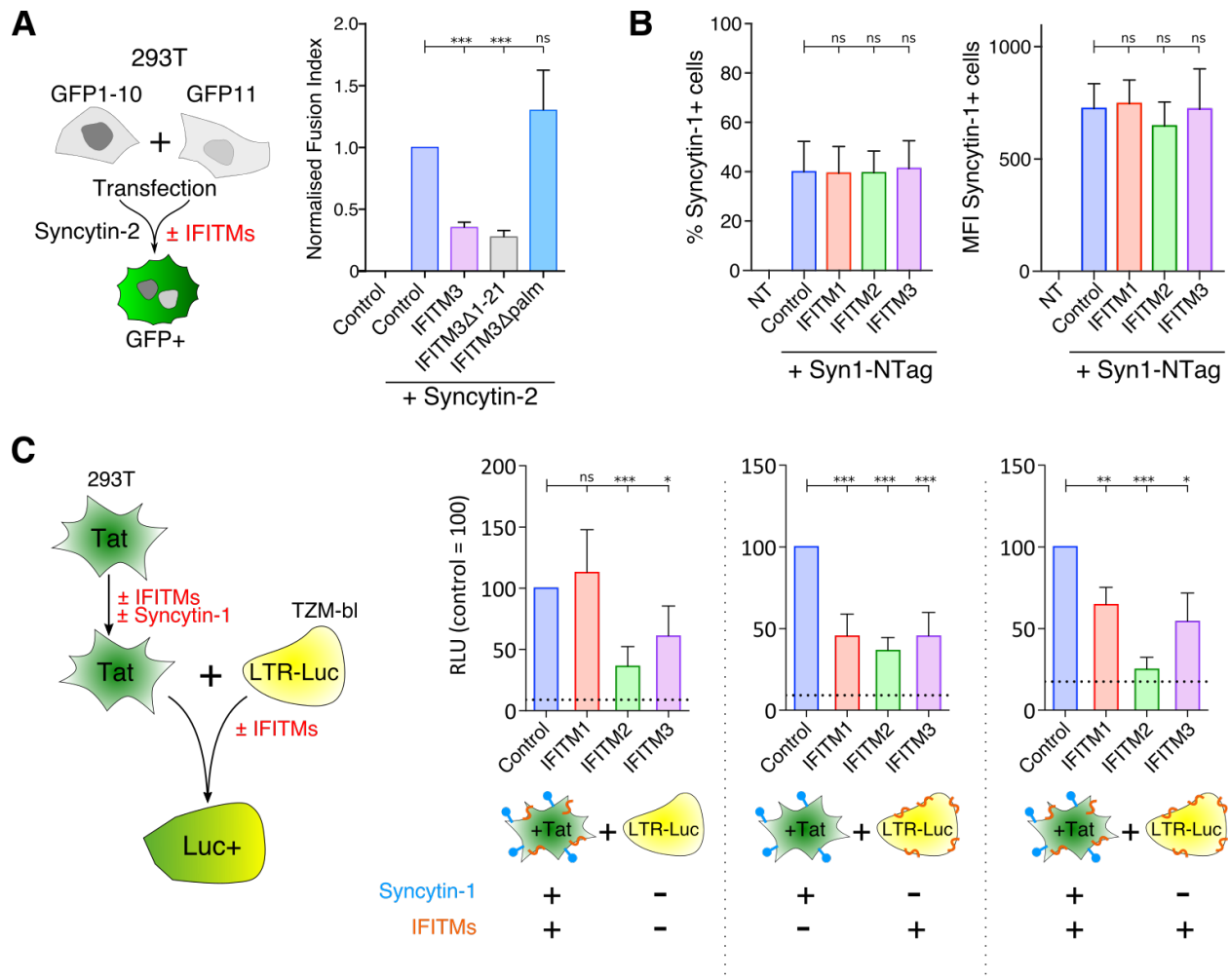


Fig. S1.

(A) IFITMs inhibit Synctin-2 mediated fusion. Left: 293T-GFP1-10 and -GFP11 were co-cultured at a 1:1 ratio and co-transfected with Synctin-2 and IFITM or control plasmids. Cell fusion was quantified by measuring the GFP+ area at 18h. Right: Quantification of GFP areas. Results are mean±sd from 4 independent experiments. **(B) IFITMs do not decrease Synctin-1 levels.** ExpiCHO cells were co-transfected with strep-tagged Synctin-1 and control or IFITM plasmids. After 24h, cells were permeabilized and stained with anti-Tag antibodies, left, percentage of positive cells and, right, mean fluorescence intensity (MFI) measured by flow cytometry. Data are mean±sd of 3 independent experiments. **(C) Effect of IFITMs in donor or target cells.** Right: 293T cells (donor cells) were co-transfected with Tat and Synctin-1 and/or

IFITM plasmids. After 6h, media was changed, TZM-bl cells (target cells), expressing control or IFITM vectors, were added and co-cultured for 20h. Cell fusion will induce Tat-mediated LTR transactivation and luciferase expression. Left: Relative luminometer units (RLU) of luciferase activity measured in the indicated conditions. IFITMs inhibited fusion when present in either donor and/or target cells, with IFITM2 having the strongest effect. Data are mean \pm sd of 6 independent experiments. Statistical analysis: One-Way ANOVA, ns: non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

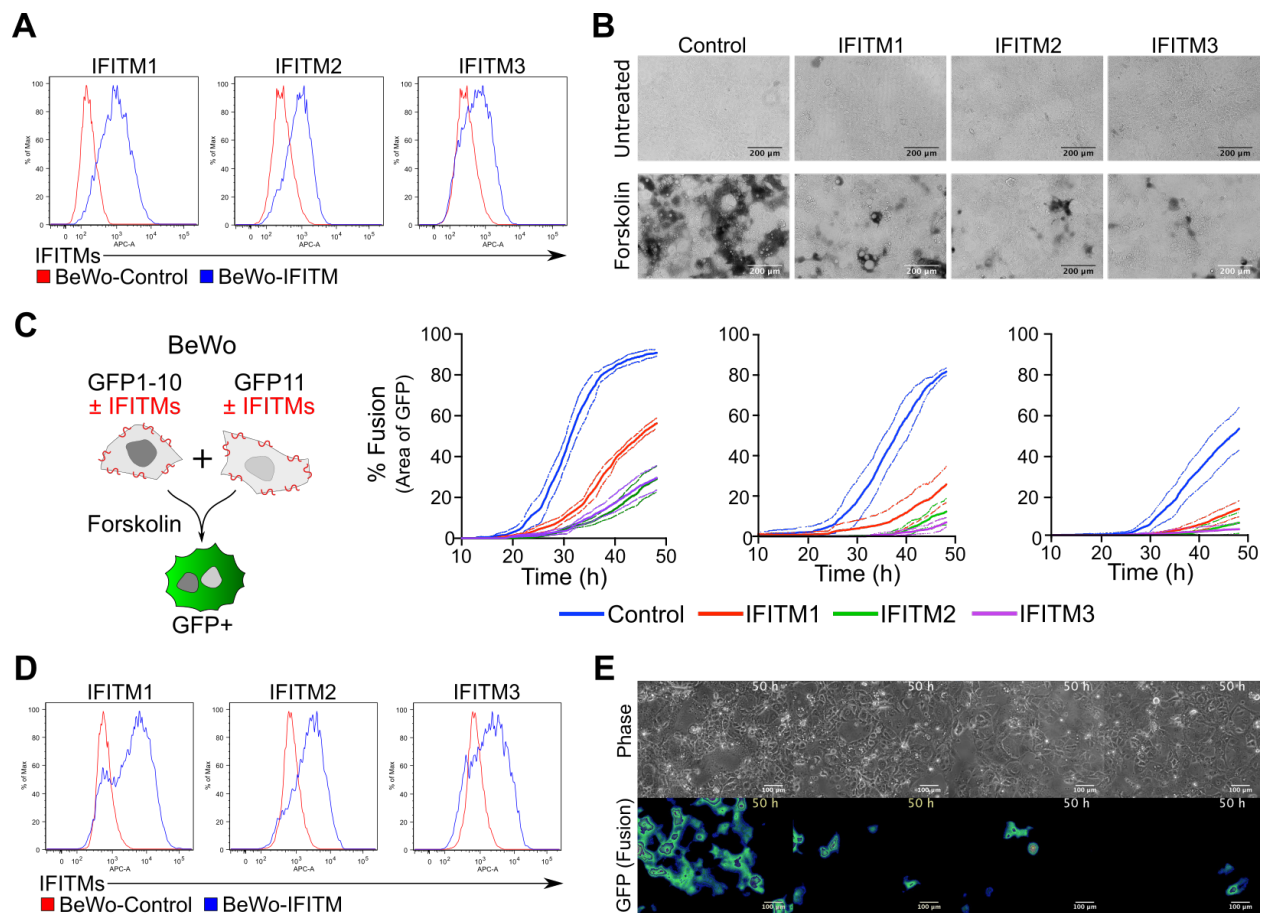


Fig. S2.

(A) Levels of IFITMs in BeWo- β -Gal- α cells. BeWo cells transduced with the indicated IFITM (blue) or control vectors (red) were stained with the corresponding IFITM antibodies and analyzed by flow cytometry. Similar results were obtained with BeWo- β -Gal- ω cells **(B) Fusion in BeWo cells.** BeWo- β -Gal- α and BeWo- β -Gal- ω cells were mixed at a 1:1 ratio and treated with forskolin for 48h. Fusion was visualized by X-gal staining. Syncytia (black) are induced by forskolin and IFITMs inhibit their formation. **(C) BeWo fusion time-lapse microscopy.** Left: BeWo-GFP1-10 and -GFP11 expressing IFITMs or control were co-cultured at a 1:1 ratio and fusion was induced by forskolin for 48h. Right: Cells were monitored by video microscopy and GFP area was quantified over time. Results are mean \pm sd from 5 fields per condition. Three independent replicates are shown. **(D) Levels of IFITMs in Bewo-GFP1-10 cells (GFPSplit**

system). BeWo cells transduced with the indicated IFITM (blue) or control vectors (red) were stained with the corresponding IFITM antibodies and analyzed by flow cytometry. **(E) Still image of GFP levels at the 50h timepoint.** Fused cells are in green (pseudo-colour). IFITM1-3 decrease syncytia formation.

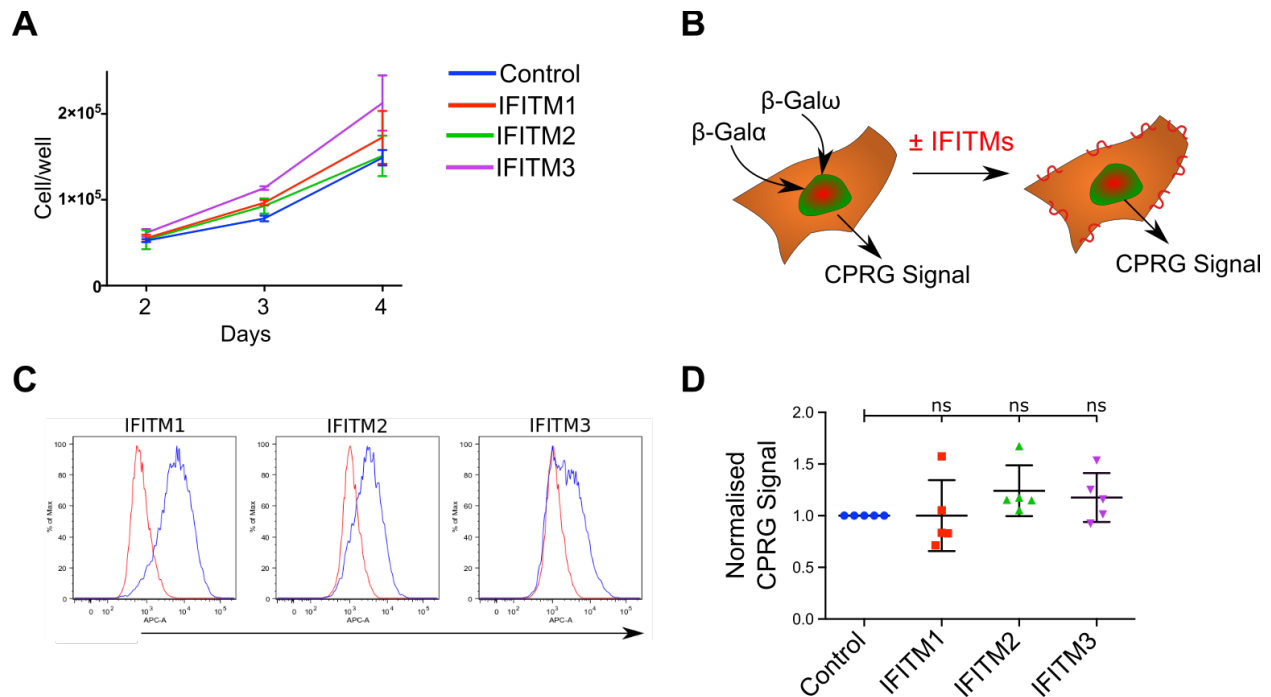


Fig. S3.

(A) Growth of BeWo cells expressing IFITM1-3. IFITM1-3 do not alter cell growth. Data are mean \pm sd of 3 independent experiments. **(B)** BeWo cells were co-transduced with β -Gal- α + ω to generate a constitutively active β -Gal. The cells were then transduced with control or IFITM expressing vectors to assess the impact of IFITM on β -Gal activity in absence of fusion. **(C)** Levels of IFITMs in Bewo cells co-expressing β -Gal- α and ω . BeWo cells, transduced with the indicated IFITM (blue) or control vectors (red) were stained with the corresponding IFITM antibodies and analyzed by flow cytometry. **(D)** β -Gal activity in BeWo cells co-expressing β -Gal- α and ω was measured. IFITMs do not reduce constitutive β -Gal activity. Data are mean \pm sd of 5 independent experiments.

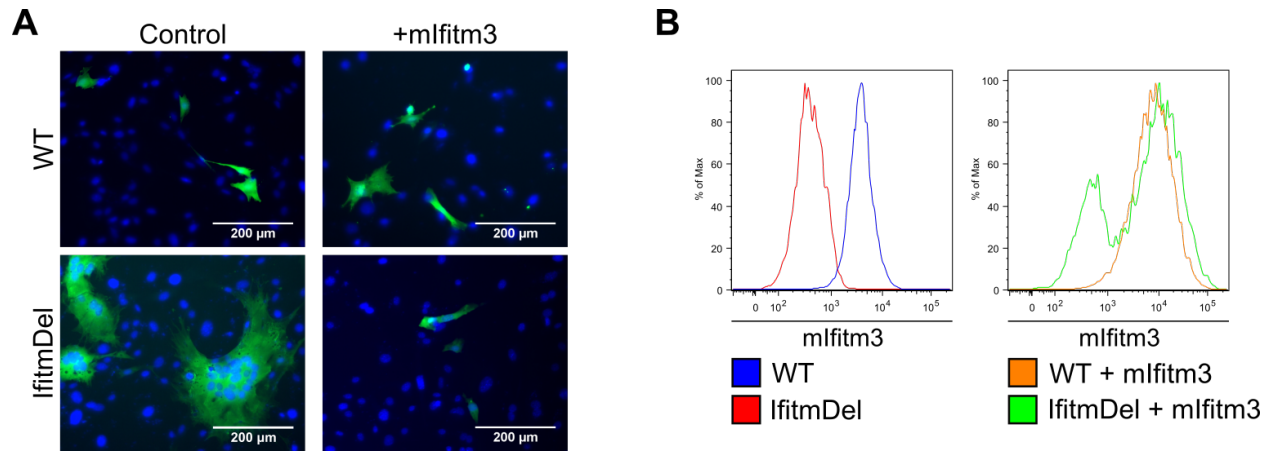


Fig. S4.

(A) Syncytin-A induces large syncytia formation in IfitmDel MEFs, a process counteracted by Ifitm3. Representative images of WT or IfitmDel MEFs co-transfected with GFP and Syncytin-A plasmids. **(B) Levels of murine Ifitm3 in WT (Blue) and IfitmDel (Red) MEFs.** Cells were stained with anti-Ifitm3 antibody and analyzed by flow cytometry.

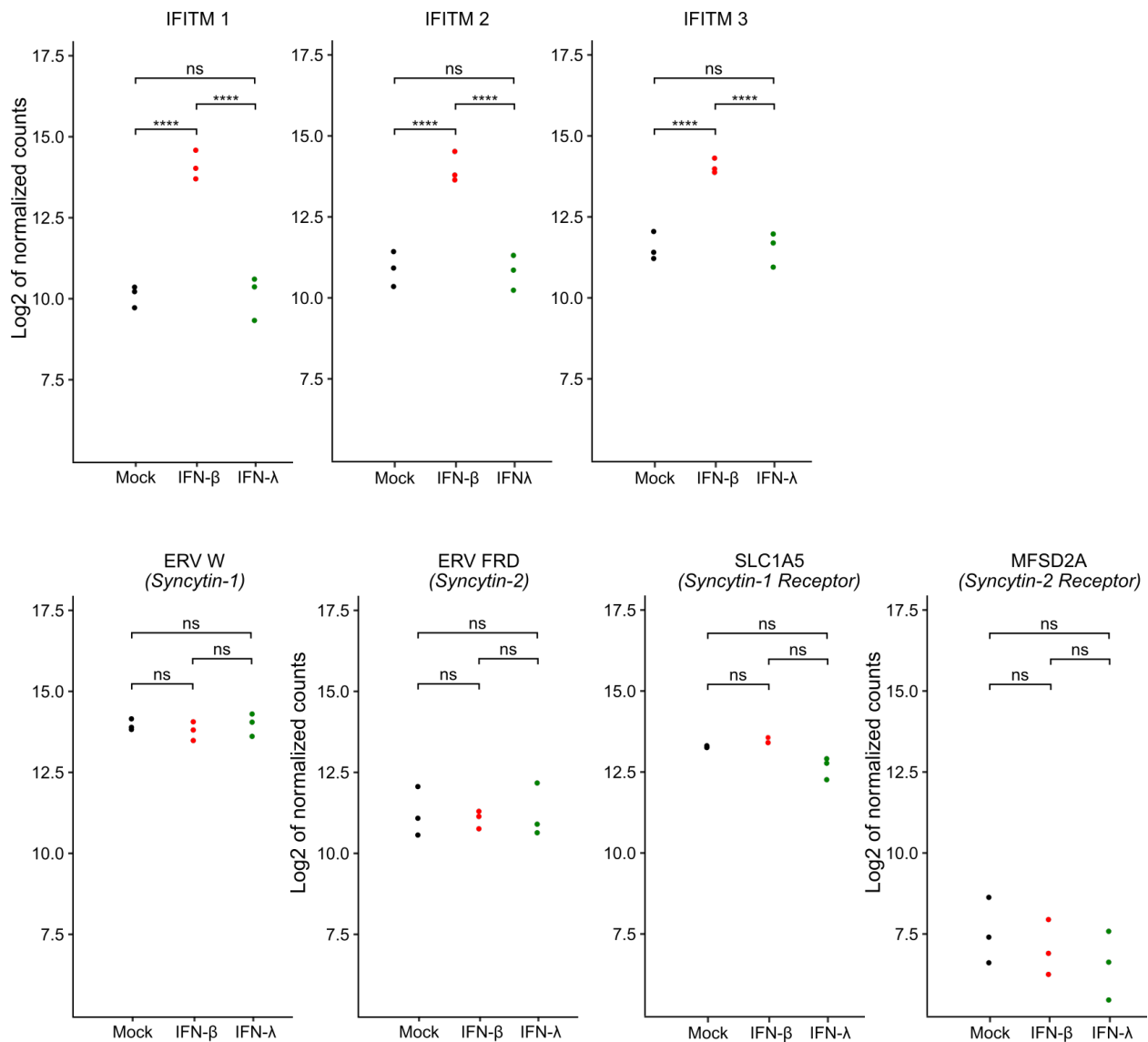


Fig. S5.

Human chorionic villous explants raw fastq reads were retrieved from published data from Yockey et al. 2018 (2) (accession number GSE104349) and a differential analysis was performed. Significance results for IFITM1-3, Syncytin-1/2 (ERV W and FRD) and Syncytin receptors (MFSD2A and SCL1A5) are displayed, with the log₂ of normalized read counts within each condition. As noted by Yockey et al. IFITM1 is upregulated by IFN-β but not IFN-λ. IFITM2 and IFITM3 follow the same pattern. The levels of Syncytin-1 and 2 and their respective receptors are not modified by IFN-β or IFN-λ. ns: non-significant, ****p < 0.0001.

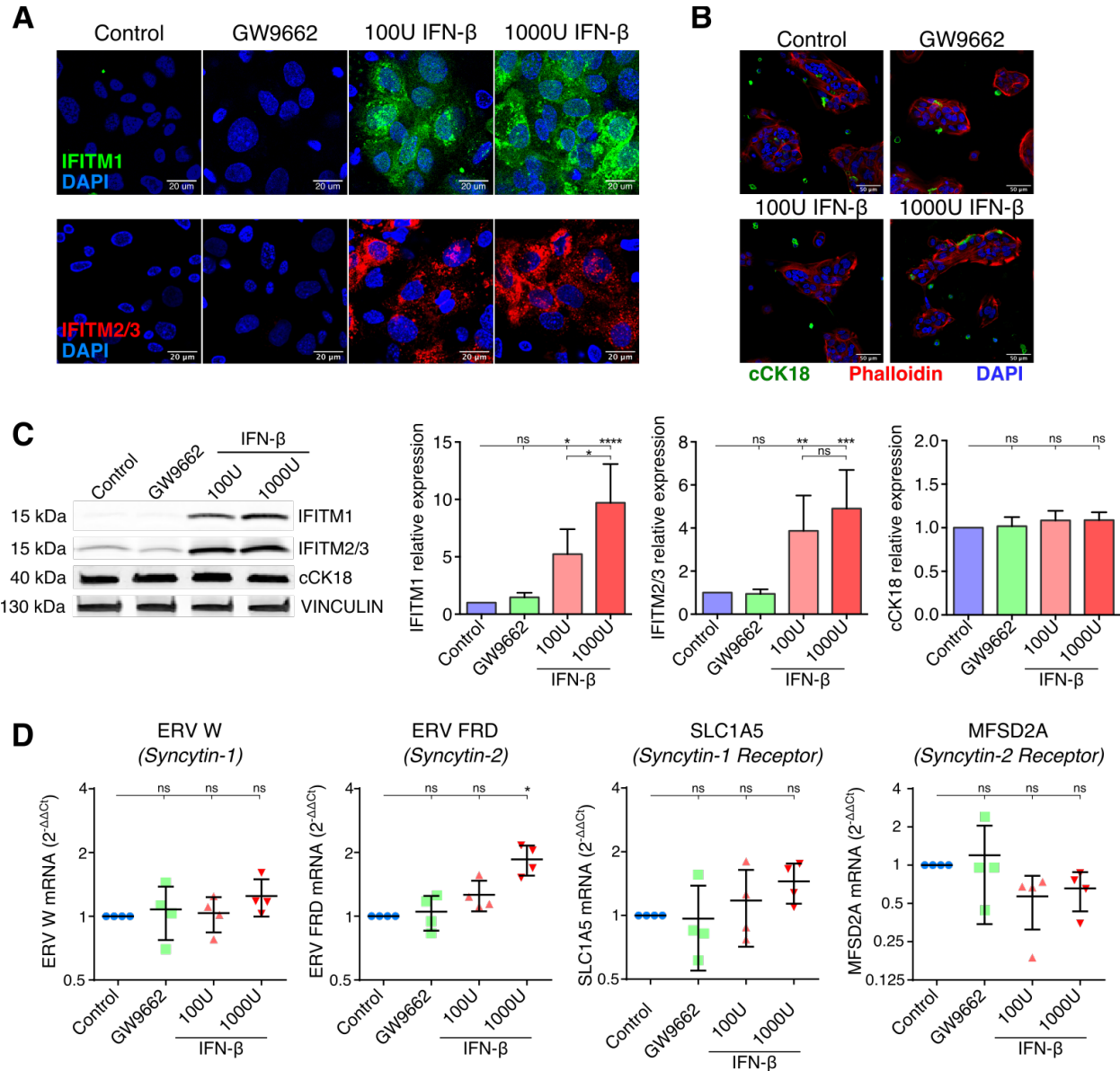


Fig. S6.

(A) VCTs upregulate IFITMs upon IFN- β treatment and (B) IFN- β treatment does not induce cell death. (A-D) 24 h after isolation, cells were incubated for 48 h with vehicle, PPAR γ antagonist (GW9662, 1 μ M) or IFN- β (100 or 1000 U/ml). (A) Representative image of IFITM expression in VCTs. Cells were fixed and immunostained for IFITM1 (green) or IFITM2/3 (red), and DAPI (blue). (B) Representative image of cCK18 expression in VCTs. Cells were fixed and immunostained for cCK18 (Green), phalloidin (red) and DAPI (blue). (C)

Quantification of IFITM and cCK18 expression. Right: Cells lysates were analysed by western blot for IFITM1 and IFITM2/3 and cCK18 expression. One representative experiment (out of 4) is shown. Left: Intensity of IFITM1, IFITM2/3 and cCK18 bands was quantified by densitometry and relative protein expression normalised to VINCULIN control was plotted. Results are mean±sd from 4 independent experiments. Statistical analysis: One-Way ANOVA, *p < 0.05, **p < 0.01, ***p < 0.001. **(D) Levels of ERV-W, ERV-FRD, SLC1A5 and MFSD2A mRNAs in treated VCTs.** Expression data were normalized to SDHA endogenous control, and relative mRNA expression to control VCTs ($2^{-\Delta\Delta CT}$) is plotted. Results are mean±sd from 4 independent experiments. Statistical analysis: One-Way ANOVA, ns: non-significant, *p < 0.05.

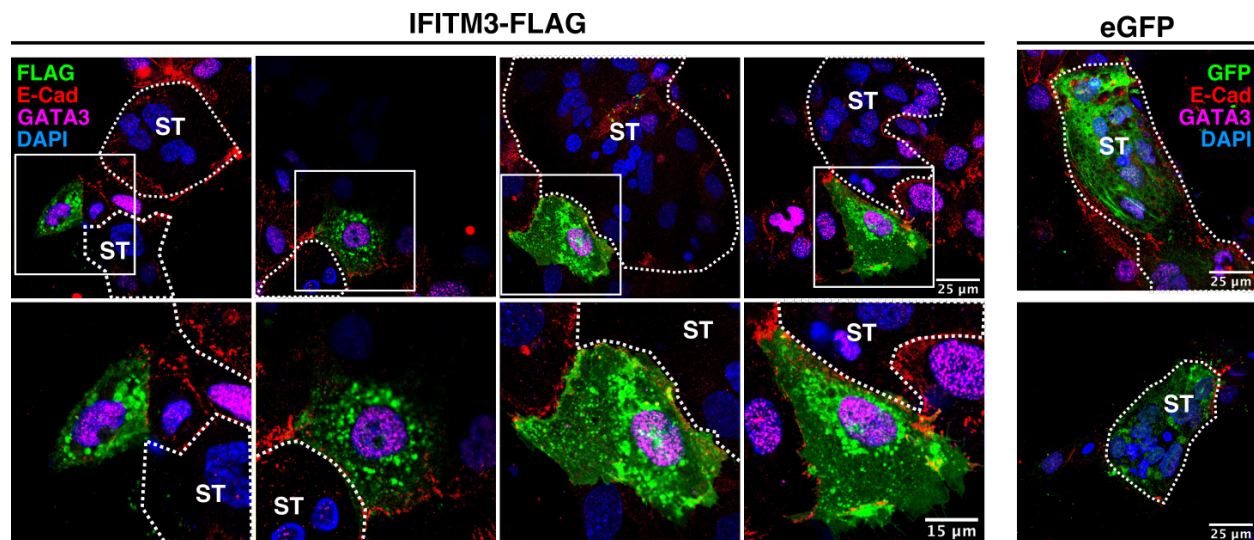


Fig. S7.

IFITM3 inhibits VCT fusion. Representative image of VCTs transfected with either a FLAG-tagged IFITM3 or an eGFP plasmid, fixed and stained for FLAG (Green), E-Cadherin (Red), GATA3 (Magenta) and counterstained with DAPI. IFITM3-transfected cells remain GATA3 positive and do not fuse, whereas GFP-transfected cells form syncytia and lose GATA3 expression. Bottom panels are magnifications of the upper panels and correspond to the indicated white boxes. Representative images from 3 independent experiments are shown.

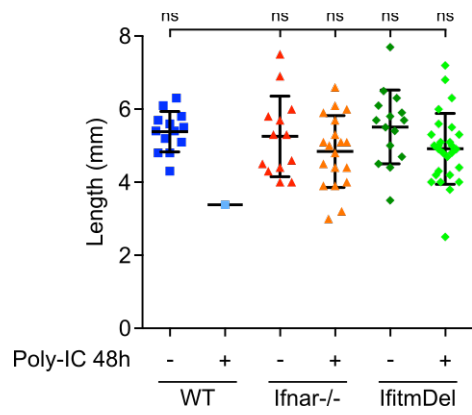


Fig. S8.

Poly-IC challenge does not alter *Ifnar*^{-/-} or *IfitmDel* embryo size. Embryo length from figure 3B-C was measured manually using ImageJ. Length of non-resorbed fetuses was plotted.

Statistical analysis: One-Way ANOVA, ns: non-significant.

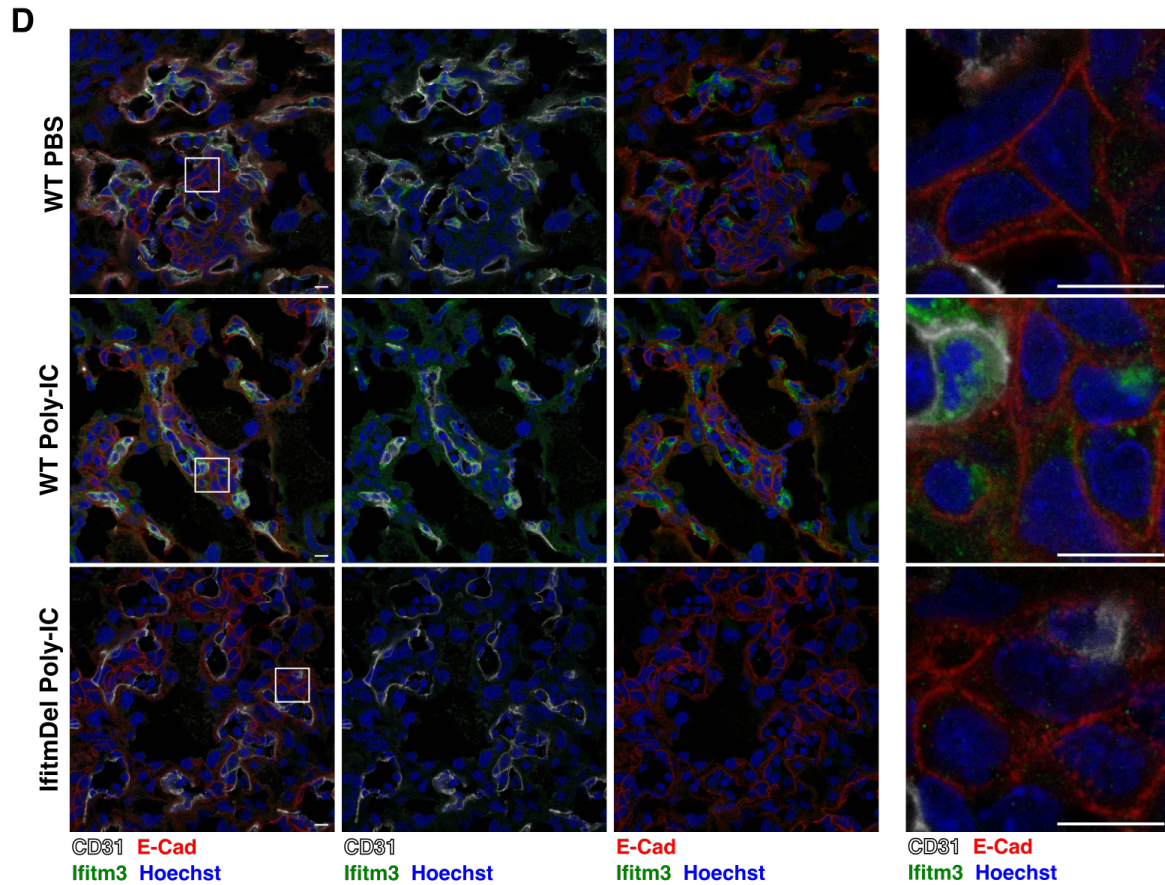
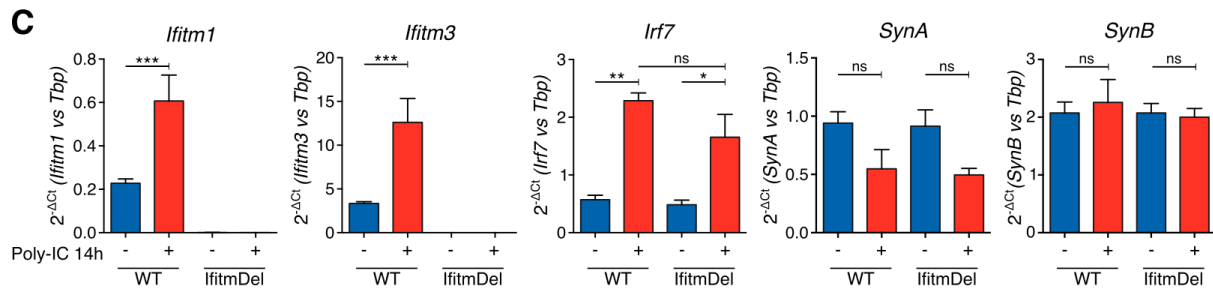
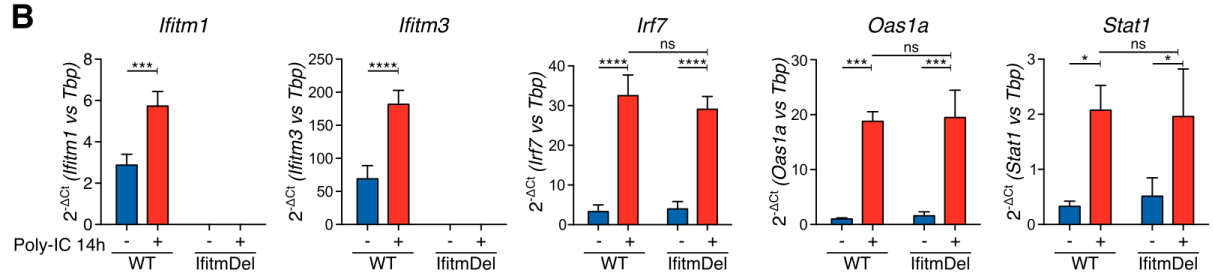
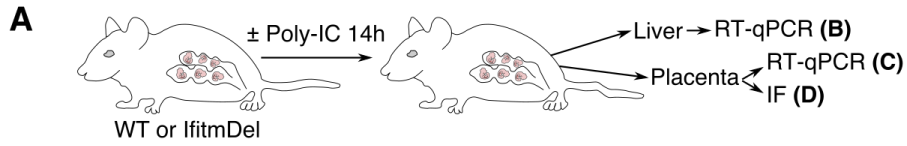


Fig. S9.**Poly-IC injection induces systemic and placental Ifitm and ISG in mice. (A) Experimental**

layout. Gestating mice (E10.5) were injected with 200 ug of Poly-IC or PBS intraperitoneally.

After 14h, liver and placenta were harvested and analyzed by RT-qPCR or by histology. **(B)**

Levels of Ifitm1, Ifitm3, Irf7, Oas1a and Stat1 mRNAs in the liver, analyzed by RT-qPCR .

The relative quantity of mRNA was normalized to TATA Box protein (Tbp) endogenous control and plotted ($2^{-\Delta CT}$). Results are mean \pm sem from 3 animals in each group. **(C) Levels of Ifitm1,**

Ifitm3, Irf7, SynA and SynB mRNAs in the placenta, analyzed by RT-qPCR.

The relative quantity of mRNA was normalized to Tbp endogenous control and plotted ($2^{-\Delta CT}$). Results are mean \pm sem from at least 4 placentas from 2-3 animals in each group. Statistical analysis: One-

Way ANOVA, ns: non-significant, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. **(D)**

Ifitm3 up-regulation in placental cells after Poly-IC injection.

WT and IfitmDel placentas stained for Ifitm3 (green), E-cadherin (E-Cad, red), CD31 (white) and DAPI (blue). Right panels

are magnifications of the left panels and correspond to the indicated white boxes. Representative

images from at least 4 placentas from 2-3 animals in each group. Bars= 10 μ m.

Movie S1.

BeWo-GFP1-10 and -GFP11 expressing IFITMs or control were co-cultured at a 1:1 ratio and fusion was induced by forskolin treatment. Cells were imaged by time-lapse microscopy. White outlined GFP area represents the quantified fusion area.