Supporting Information
Eggenberger et al. 2019

SI Materials and Methods

Cell culture and reagents

HEK293T, 2fTGH, primary human foreskin fibroblast (FF) and primary murine embryonic fibroblast (MEF) cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Human induced pluripotent stem cells (iPSCs) were maintained on Matrigel-coated plates in E8 (Stem Cell Core, Mount Sinai) or StemFlex Medium (Gibco). Mouse embryonic stem cells (mESCs, WT 129-R1 and WT clone 39) were a kind gift from Dr. Nicole C. Dubois and Dr. Ivan Marazzi, respectively (Icahn School of Medicine at Mount Sinai) and were maintained on Matrigel-coated plates in serum-free ES maintenance media (50% DMEM-F12 1:1, 50% Neurobasal media, 0.5x N2 supplement, 0.5x B27 supplement, 0.05% BSA fraction V (7.5%), 1x L-Glutamine medium, 1x penicillin/streptomycin, 150 μM B-Mercaptoethanol, 3 nM CHIR, 1 nM PD325901, 20 ng/mL mouse Lif). All cells were maintained at 37°C in a humidified incubator with 5% CO_2 and were routinely screened for mycoplasma. Transfection of DNA was performed using Lipofectamine 2000 (Thermo Fisher). Transfection of pppRNA was performed using RNAiMax (Thermo Fisher). Where indicated, cells were treated with 300 U/mL of human IFNB or 200 U/mL of mouse IFNB (BEI resources). For 293T and iPSC Dox-induction experiments, Doxycycline (Sigma, D9891) was diluted to a working concentration of 1ug/ml and replaced at 48 hours with fresh media where indicated.

Western blot analysis

Whole-cell lysates were collected using radioimmunoprecipitation (RIPA) buffer (10mM Tris-Cl (pH 8.0), 1mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 140 mM NaCl) supplemented with protease inhibitors (PI and PMSF). Briefly, cells were incubated in buffer for 30 min on ice, and soluble fractions were measured by standard Bradford assay after centrifugation for 10 min at 13,000 rpm. Cell extracts were separated on Mini Protean TGX gels (Bio-Rad). Following electrophoresis, protein was transferred onto a nitrocellulose membrane for 1.5 hours at room temperature. The membrane was blocked in 5% dried milk in PBS for 1 hour at room temperature and then probed with indicated antibodies. Monoclonal antibody specific to A/PR/8/34 NP was a kind gift from Dr. Thomas Moran at the Monoclonal Antibody Facility (Icahn School of Medicine at Mount Sinai). Antibodies specific to ACTIN (Thermo Fisher, MS-

1295-P), hRIG-I (A. Garcia-Sastre), hIFIT1 (Cell Signaling, D2X9Z), hSTAT1 (BD Transduction, 610119), mIFIT1 (Thermo Scientific, PA3-846), mIFIT2 (Thermo Scientific, PA3-845), VSV G (GenScript, A00199), and hISG15 (Santa Cruz, sc-69701) were used at a dilution of 1:1,000 in 5% dried milk in PBS. Antibody specific to Flag-M2 epitope (Sigma, A8592) was used at a dilution of 1:10,000 in 3% BSA in PBS. Incubations were performed overnight at 4°C, and incubation mixtures were washed in PBS five times for a total of 25 minutes. Following washes, the membrane was incubated with peroxidase-conjugated goat anti-rabbit or -mouse antibody (GE Healthcare, 1:5,000) 1h at room temperature. Following incubation with the secondary antibody, the membrane was washed again for 25 minutes and subsequently developed using the Immobilon Western Chemoluminescent HRP (Millipore) and SuperSignal West Femto Maximum Sensitivity (Thermo Fisher) substrates.

Cloning and expression analyses

Mammalian expression plasmids encoding Flag-GFP and Flag-IRF7del247-467 (Flag-IRF7Δ) were generated using the multiple cloning site (MCS) of the pCAGGS plasmid. Lentiviral vectors encoding Flag-GFP and Flag-IRF7Δ were generated using the multiple cloning site (MCS) of the pTRE3G-BFP2-PuroR backbone vector, a kind gift from Dr. Kristen J. Brennand. Third-generation VSV-G pseudotyped HIV-1 lentiviruses were produced by Lipofectamine 2000-based transfection of HEK293T cells. In short, 10 μg of pTRE3G or FUW-M2rtTA plasmid was co-transfected along with 10 μg HIV Gag/Pol and 4 μg VSV-G. At 72 hours post transfection, supernatants were harvested and centrifuged at 1500 rpm and 4°C for 15 minutes. Supernatants were concentrated and purified using Amicon Ultra-15 10K Centrifugal Filter Devices (Millipore), and subsequently stored at -80C. To generate cells stably expressing Flag-GFP or Flag-IRF7Δ, media was pre-warmed in a 37°C water bath. Lentiviral aliquots of TRE3G-Flag-EGFP-BFP2-PuroR, TRE3G-Flag-IRF7D-BFP2-PuroR and FUW-M2rtTA were thawed at room temperature and added to the indicated media. To transduce iPSCs, the media was aspirated and replaced with StemFlex containing the lentiviruses. A 12-well plate of iPSCs plated a day prior as single cells was centrifuged for 1 hour at 1,000 g and 25°C. Cells were placed in the 37°C incubator for 3-4 hours, after which time viral media was replaced with fresh StemFlex. Cells were allowed to recover overnight and were passaged at a 1:2 dilution the following day. Puromycin (Sigma, P7255) was diluted to a working concentration of 1μg/ml in StemFlex and 1 ml was added to each well followed by a 16-24-hour incubation, when wells were washed with PBS and replaced with fresh media.

Virus preparation and infections

Influenza A virus delta NS1 (mouse-adapted A/Puerto Rico/8/34 deficient in the nonstructural protein 1 (NS1)) was propagated in Madine Darby Canine Kidney cells overexpressing NS1 (MDCK-NS1) in virus infection media (EMEM, 35% BSA, 1% penicillin/streptomycin, 1% L-glutamine, 0.15% Sodium Bicarbonate, and 2% HEPES) supplemented with TPCK-treated Trypsin at a concentration of 1μg/mL. Virus stocks were filtered, concentrated and tittered by plaque assay on MDCK-NS1 cells. Infections for iPSCs and FFs were performed at a multiplicity of infection (MOI) of 2 for indicated timepoints. Virus was inoculated onto cells containing infection media (PBS supplemented with 0.3% bovine serum albumin (BSA, MP Biomedicals), 10 mM CaMg, and 1% penicillin/streptomycin) for 1 hour. Inoculum was then aspirated off, washed with PBS and replaced with fresh media. Vesicular Stomatitis Virus (VSV) was propagated in Vero cells in normal culture media. Virus was inoculated onto indicated cells for indicated timepoints.

Immunofluorescence

iPSCs were stained with antibodies against endogenous Nanog, Tra-1-60, Sox2, and Oct4, as previously described (1). Endoderm was stained with antibodies against endogenous AFP, mesoderm was stained with antibodies against endogenous Vimentin, and Ectoderm was stained with antibodies against endogenous beta III tubulin, as previously described (1).

TaqMan hPSC Scorecard Analysis

Total RNA was isolated from IRF7Δ- or GFP-treated iPSCs and was DNase-treated using RNase-free, DNase I recombinant (Roche, 4716728001). cDNA was then generated using a High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, 4374966) and TaqMan qRT-PCR was performed according to the manufacturer's protocol. In short, cDNA was mixed with 2X TaqMan Gene Expression Master Mix and loaded onto each well of a TaqMan hPSC Scorecard Panel 384-well format (Applied Biosystems, A15872). qRT-PCR was performed using the ViiA 7 System under standard conditions. Gene expression data was analyzed using the web-based hPSC Scorecard Analysis Software (www.lifetechnologies.com/scorecarddata).

qRT-PCR

Total RNA was isolated from respective samples by TRIzol extractions (Thermo Fisher) and DNase-treated using RNase-free, DNase I recombinant (Roche, 4716728001). 250 ng to 1 μg

RNA was reverse transcribed into cDNA using oligo-dT primers with Superscript II Reverse Transcriptase (Thermo Fisher). qPCR was performed on indicated cDNA samples using KAPA SYBR FAST qPCR Master Mix (KAPA Biosystems) and the LightCycler 480 (Roche). We calculated delta delta cycle threshold (ΔΔCT) values over replicates using tubulin as the endogenous housekeeping gene and mock-infected or mock-transfected samples as the calibrator in respective experiments. Graphed values represent the fold change for each condition as compared with mock-infected or mock-transfected samples. Error bars indicated ±SD of fold induction. Primers used for qRT-PCR are summarized (**See SI Appendix, Table S10**).

SI References

1. Brennand KJ*, et al.* (2011) Modelling schizophrenia using human induced pluripotent stem cells. *Nature* 473(7346):221-225.
2. Wu X*, et al.* (2018) Intrinsic Immunity Shapes Viral Resistance of Stem Cells. *Cell* 172(3):423-438 e425.

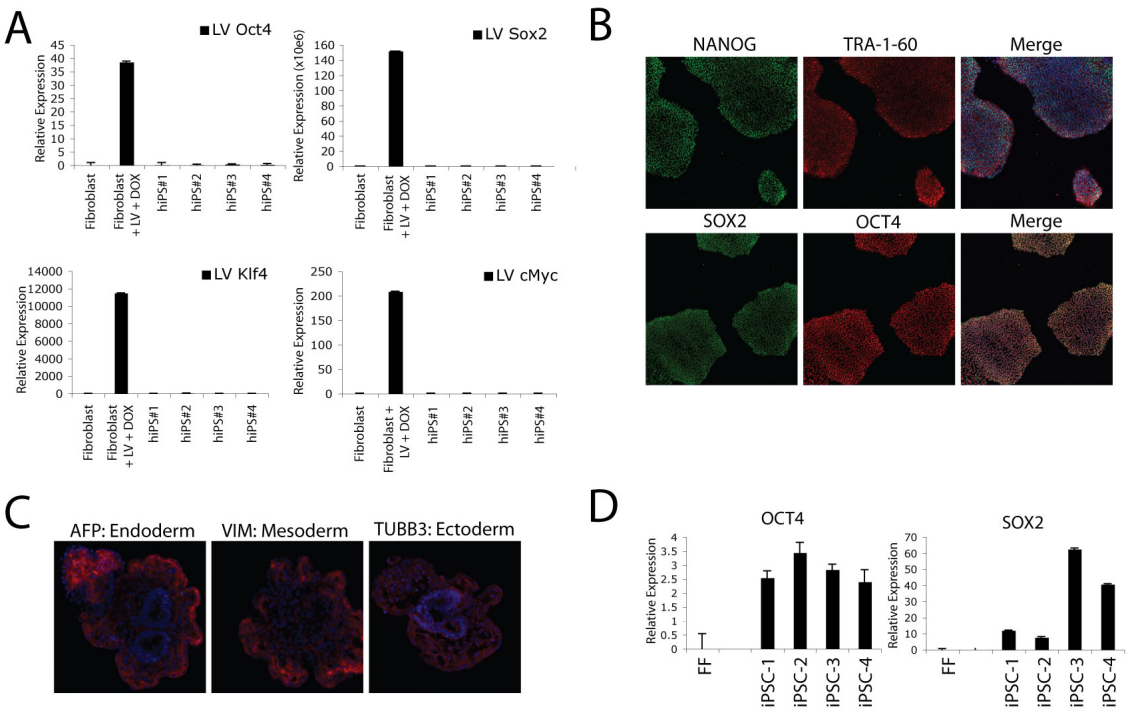

Figure S1. Generation and characterization of induced pluripotent stem cells (iPSCs). (A) qRT-PCR analyses of RNA isolated from untreated foreskin fibroblasts, foreskin fibroblasts transduced with a dox-inducible lentivirus expressing the reprogramming factors Oct4, Sox2, Klf4, and cMyc (OSKM) and treated with doxycycline (1ug/ml), and four hiPSC clones derived from FF. Total RNA was analyzed for relative expression levels of lentiviral-derived Oct4, Sox2, Klf4, and cMyc. (B) iPSC clones were stained with antibodies against endogenous NANOG, TRA-1-60, SOX2, and OCT4. (C) iPSC clones were differentiated into the three germ layers and stained with antibodies against endogenous AFP (endoderm), endogenous Vimentin (mesoderm), and endogenous beta III tubulin (Ectoderm). (D) qRT-PCR analyses of isolated RNA from foreskin fibroblasts and four clones of induced pluripotent cells (iPSC-1-4) were analyzed for relative expression of endogenous OCT4 and SOX2. Error bars depict standard deviation from the mean.

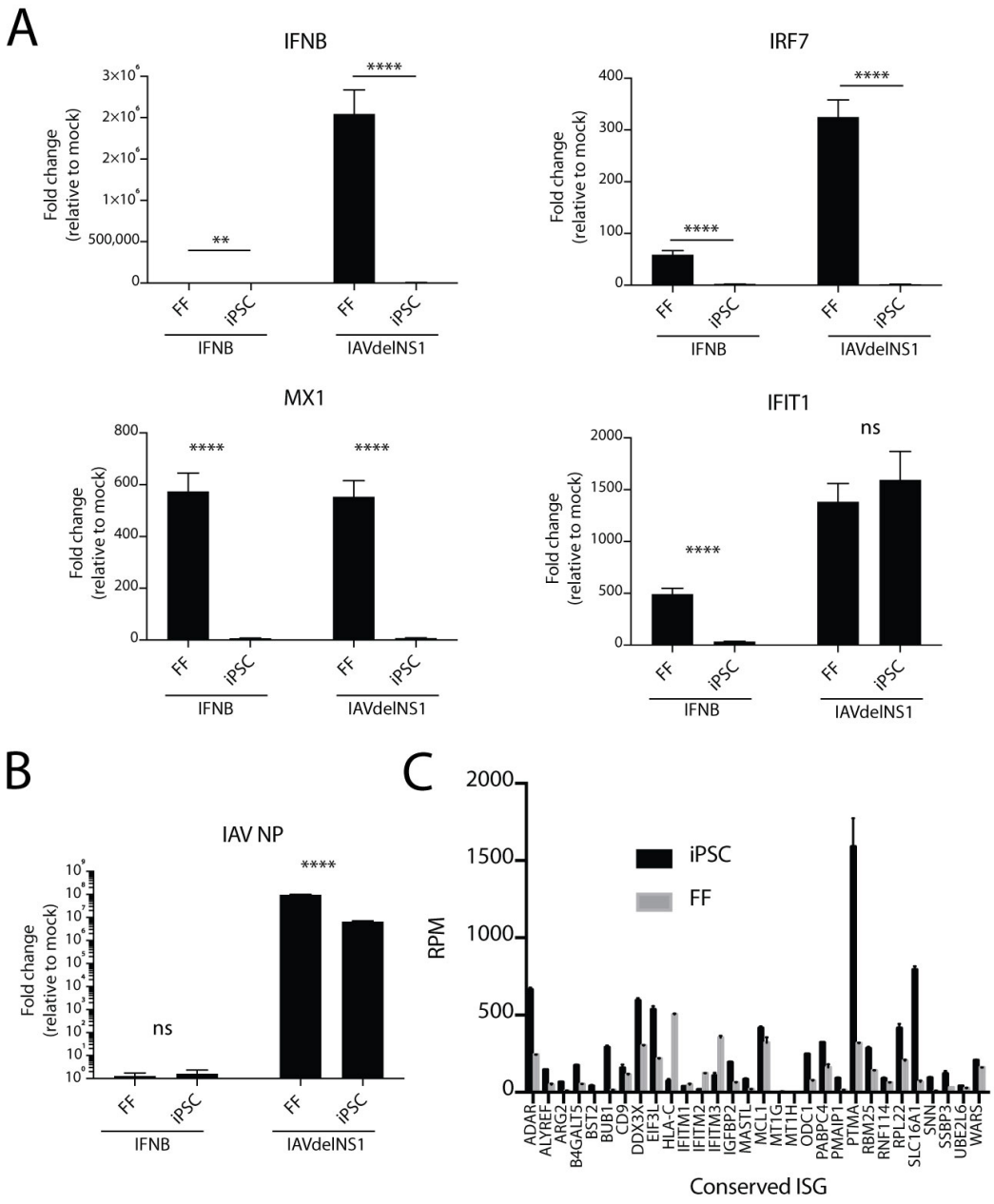

Figure S2. Characterization of FFs and iPSCs treated with IFNB or infected with virus. (A) FFs and iPSCs were either treated with IFNB or infected with IAVdelNS1 for 8 hours and analyzed by qRT-PCR. Graphs depict relative expression levels of IFNB, IRF7, MX1, or IFIT1 transcripts. Error bars represent the standard deviation from the mean. Significance was determined by unpaired Student t-test where * to **** denote p-values of 0.05 to less than .0001, respectively. (B) qRT-PCR analysis of IAV nucleoprotein (NP) transcript levels from FFs and iPSCs at 8 hours post infection. (C) Baseline mRNA levels in FFs and iPSCs of conserved ISGs (2). Raw RNA-Seq read counts were normalized to total read counts for each sample and divided by one million (reads per million, or RPM).

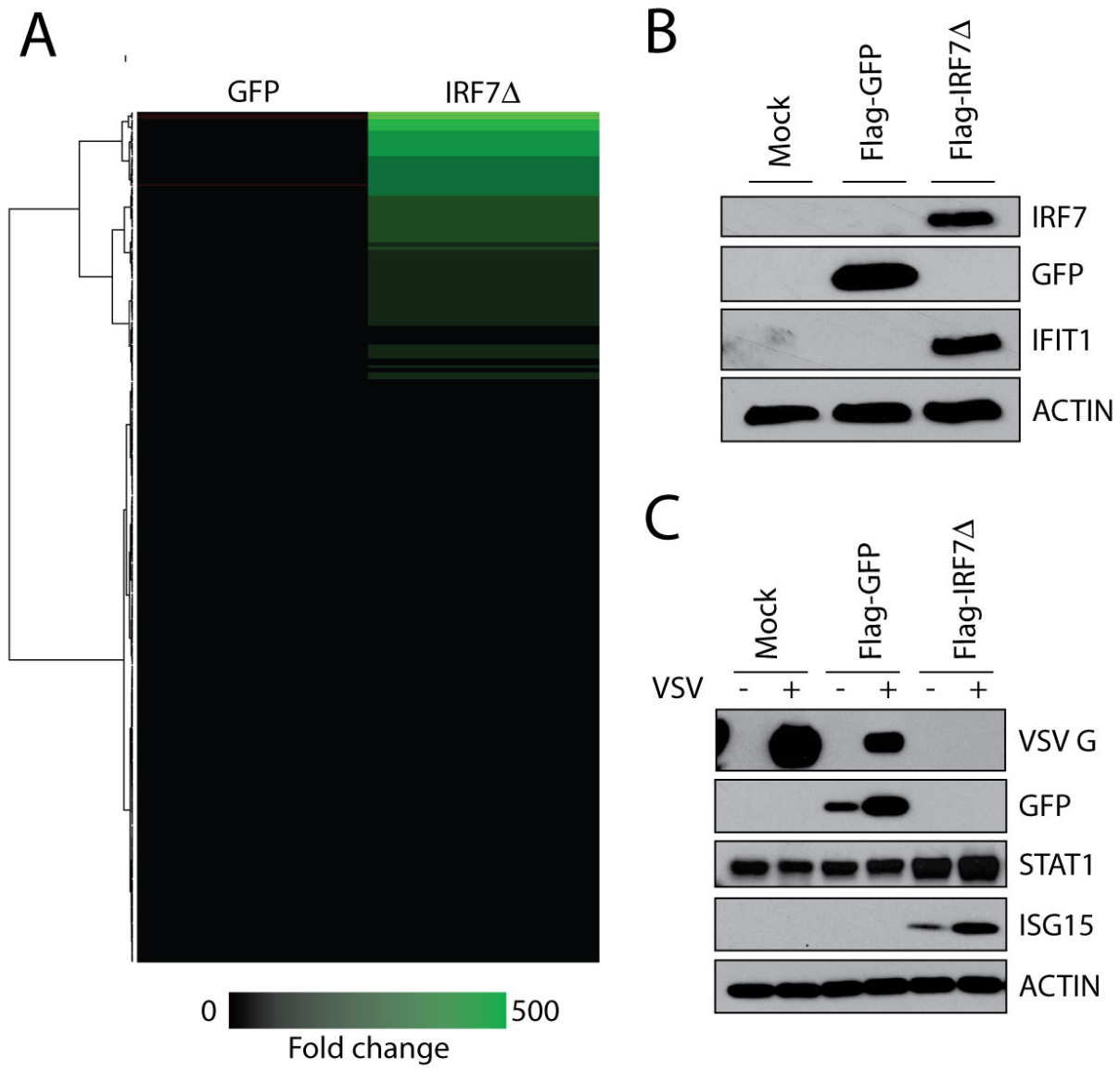

Figure S3. IRF7Δ expression in fibroblasts is sufficient to induce an antiviral response. (A) Heatmap derived from RNA-Seq gene expression profiles in human fibroblasts transfected with plasmids expressing either green fluorescent protein (GFP) or a constitutively active construct of Interferon Regulatory Factor 7 lacking amino acids 247-467 (IRF7Δ). Gene clustering of each condition is represented as fold change over mock. (B) Western blot of whole cell extract from cells described in (A). Immunoblots depict protein levels of IRF7, GFP, IFIT1, and ACTIN. (C) Western blot of whole cell extract derived from cells described in (A) either mock treated (-) or infected with Vesicular Stomatitis Virus (VSV) at a multiplicity of infection of one, twelve hours post infection (+). Immunoblots depict protein levels of VSV glycoprotein (G), GFP, STAT1, ISG15, and ACTIN.

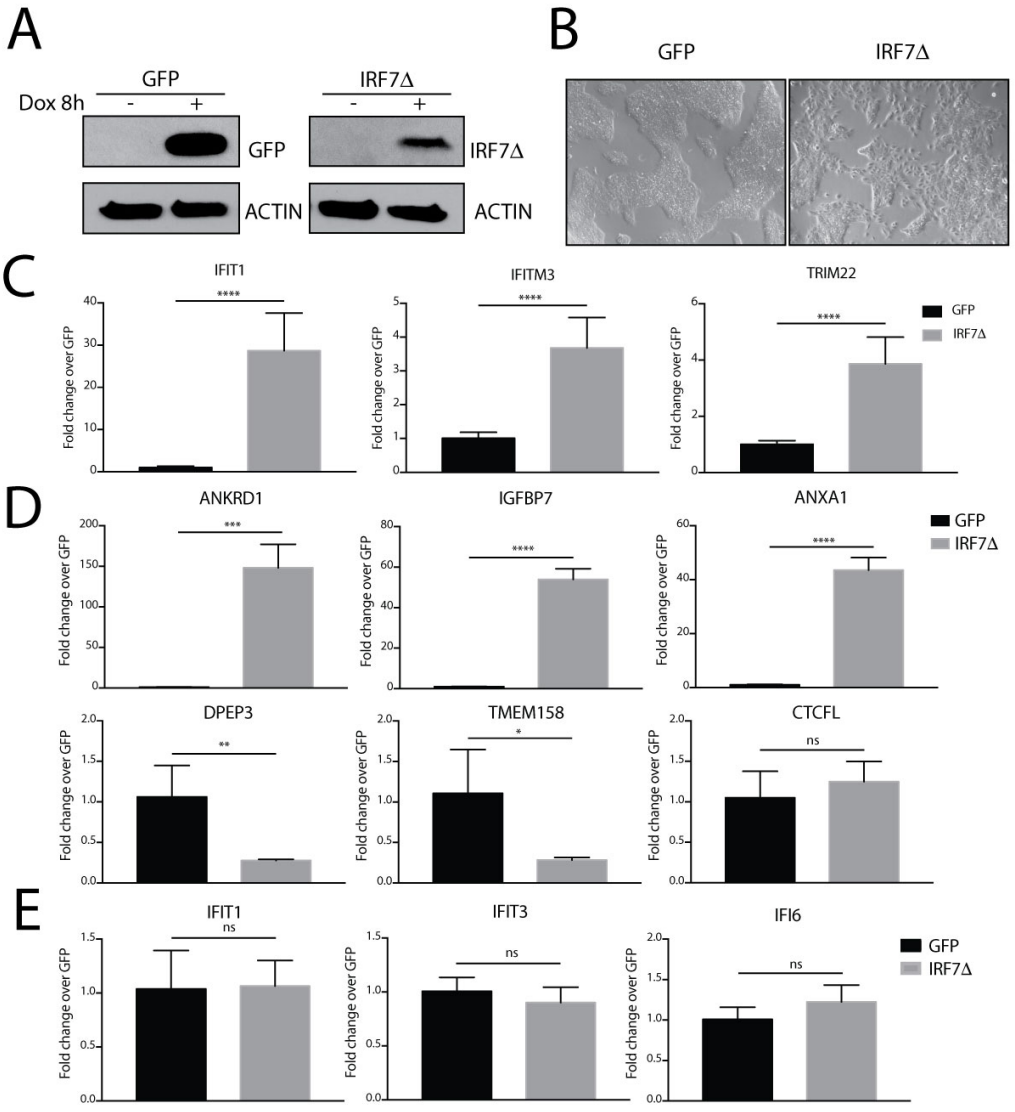

Figure S4. Dox-inducible IRF7Δ expression profiles in differentiated and pluripotent cells. (A) HEK293T cells were transduced with TRE3G-GFP-BFP2-PuroR or TRE3G-IRF7Δ-BFP2-PuroR lentiviruses. Individual clones were mock treated or treated with 1 μg/mL Doxycycline (Dox) for 8 hours and analyzed by Western blot. (B) Visible light microscopy of iPSC clones that were Dox-treated for 48hrs. GFP and IRF7Δ represent pTRE3G-GFP-BFP2-PuroR and pTRE3G-IRF7Δ-BFP2-PuroR transduced lines, respectively. (C) qRT-PCR analysis of RNA derived from iPSC clones treated with Dox for 48hrs to induce GFP or IRF7Δ expression. Graphs depict RNA transcript levels for induced ISGs identified from RNA-Seq data including: IFIT1, IFITM3, and TRIM22. Error bars denote the standard deviation of the mean. Significance was determined by unpaired Student t-test where * to **** denote p-values of 0.05 to less than .0001, respectively. (D) qRT-PCR as in (C) for ANKRD1, IGFBP7, ANXA1, DPEP3, TMEM158, and CTCFL. (E) qRT-PCR as in (C) for IFIT1, IFIT3, and IFI6.

A

Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
GFP (48h plus rest)	-0.54	-0.47	-0.87	-1.31
IRF7Δ (48h plus rest)	-0.37	-0.64	-0.11	-1.39

Gene expression relative to the reference standard

Upregulated						Downregulated
x > 1.5	1.0 < x ≤ 1.5	0.5 < x ≤ 1.0	-0.5 ≤ x ≤ 0.5	-1.0 ≤ x < -0.5	-1.5 ≤ x < -1.0	x < -1.5

B

Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
GFP (48h plus rest, 5d differentiated)	-2.08	-0.63	1.60	-1.29
IRF7Δ (48h plus rest, 5d differentiated)	-2.67	-0.33	1.77	-1.65

Gene expression relative to the reference standard

Upregulated						Downregulated
x > 1.5	1.0 < x ≤ 1.5	0.5 < x ≤ 1.0	-0.5 ≤ x ≤ 0.5	-1.0 ≤ x < -0.5	-1.5 ≤ x < -1.0	x < -1.5

Figure S5. Scorecard analysis of iPSCs treated with GFP or IRF7Δ. Gene expression relative to the reference standard for Dox-treated plus rest (A) and differentiated (B) iPSCs. Each sample represents biological duplicates. Colors correlate to the fold change in expression of the indicated gene relative to the undifferentiated reference set, where blue is downregulated, white is unchanged, and red is upregulated.

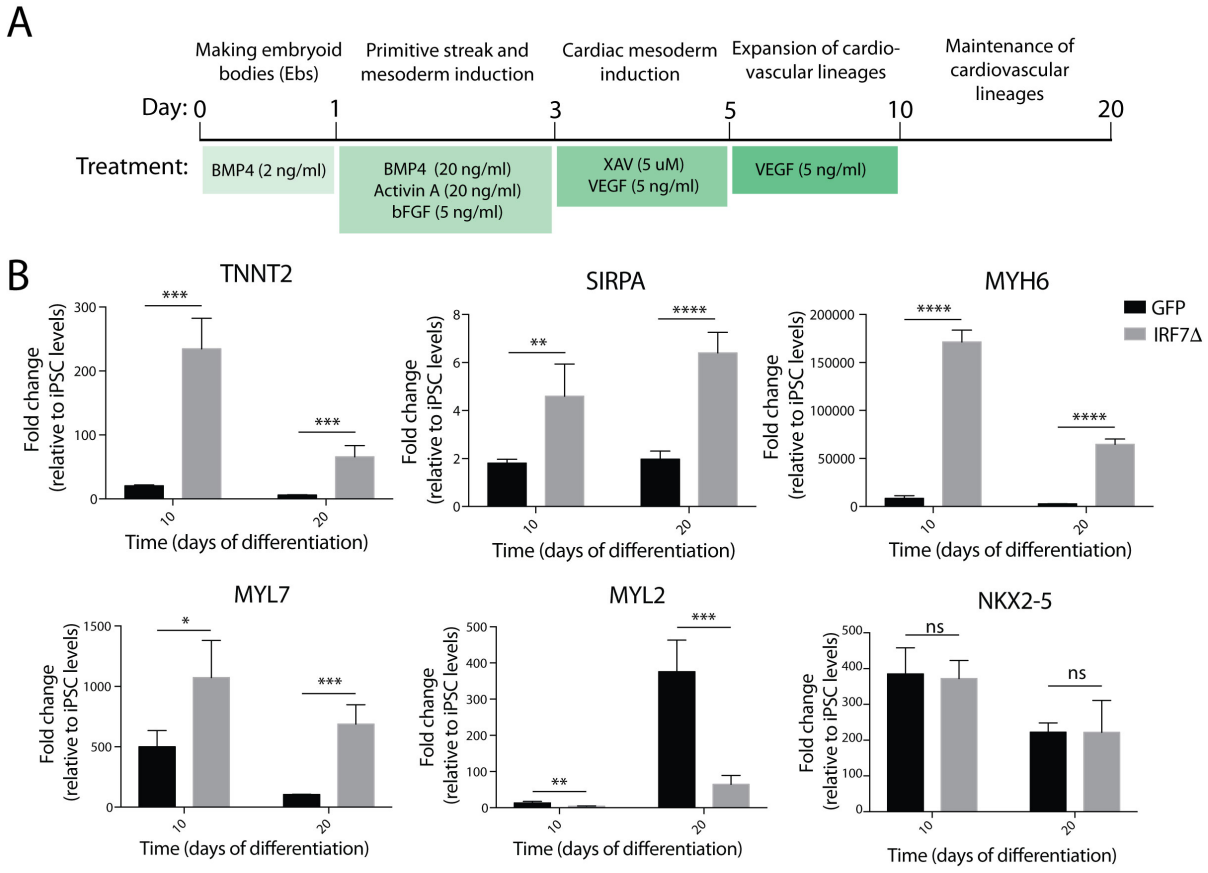

Figure S6. Effects of IRF7Δ expression on cardiomyocyte (CM) differentiations. (A) Schematic of the protocol used to differentiate iPSCs into cardiomyocytes. GFP- or IRF7Δ-transduced iPSCs were treated with Dox for 48 hours, allowed to rest for 5 days, and subsequently differentiated into cardiomyocytes over a 20-day time course. (B) qRT-PCR analysis of CM-specific markers from GFP- or IRF7Δ-pulsed cardiomyocytes relative to GFP- or IRF7Δ-pulsed iPSCs. TNNT2, SIRPA, MYH6, MYL7, MYL2, and NKX2-5 RNA transcript levels are depicted as fold change over levels in respective iPSC lines. Error bars depict the standard deviation of the mean. Significance was determined by unpaired Student t-test where * to **** denote p-values of 0.05- less than .0001, respectively.