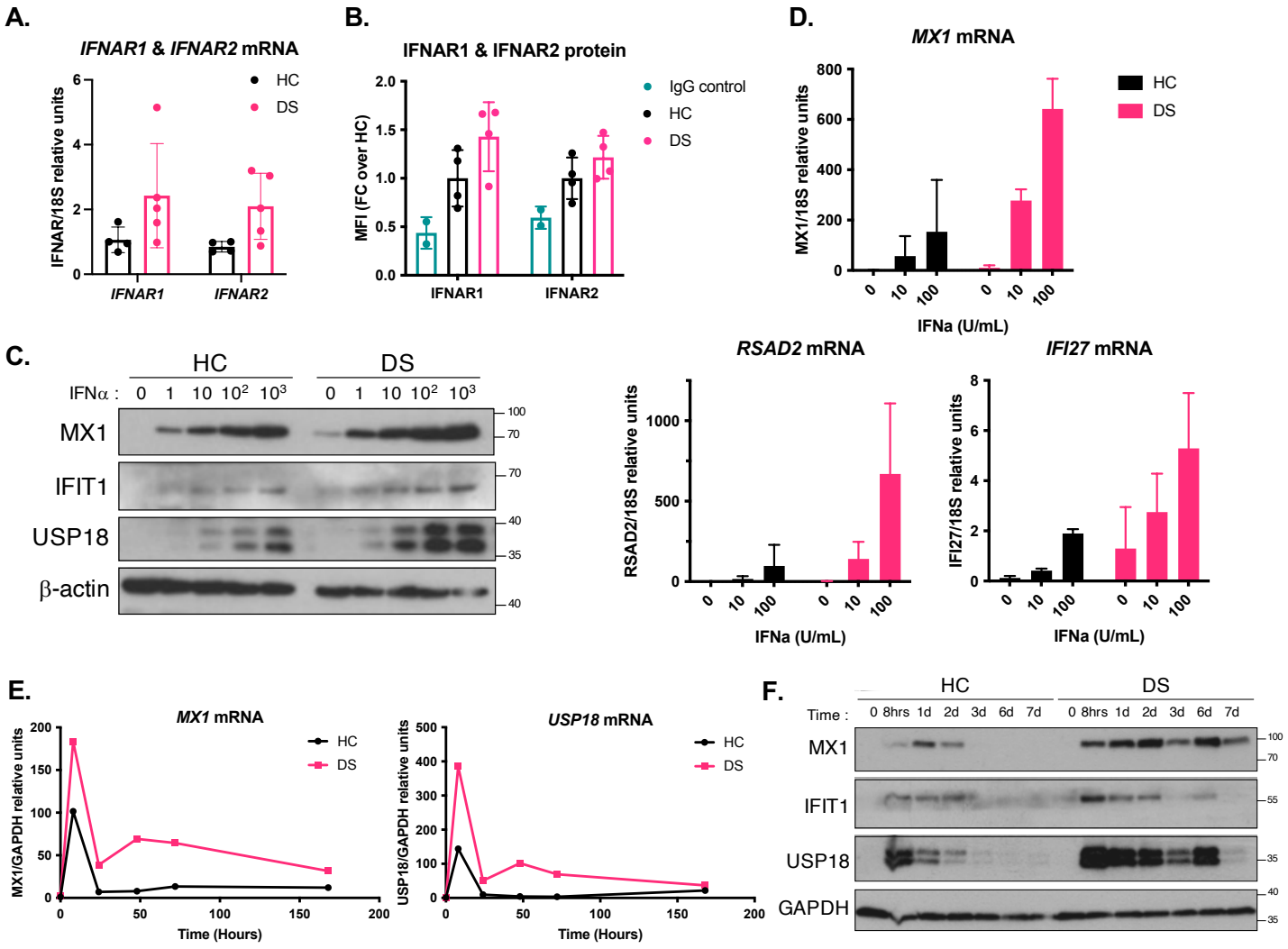
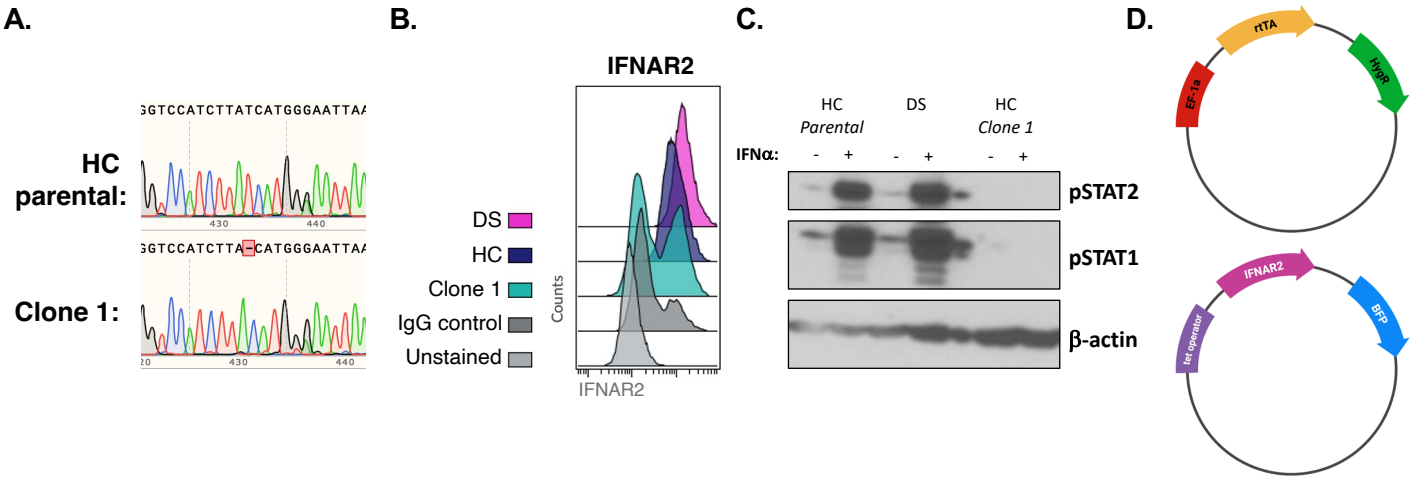
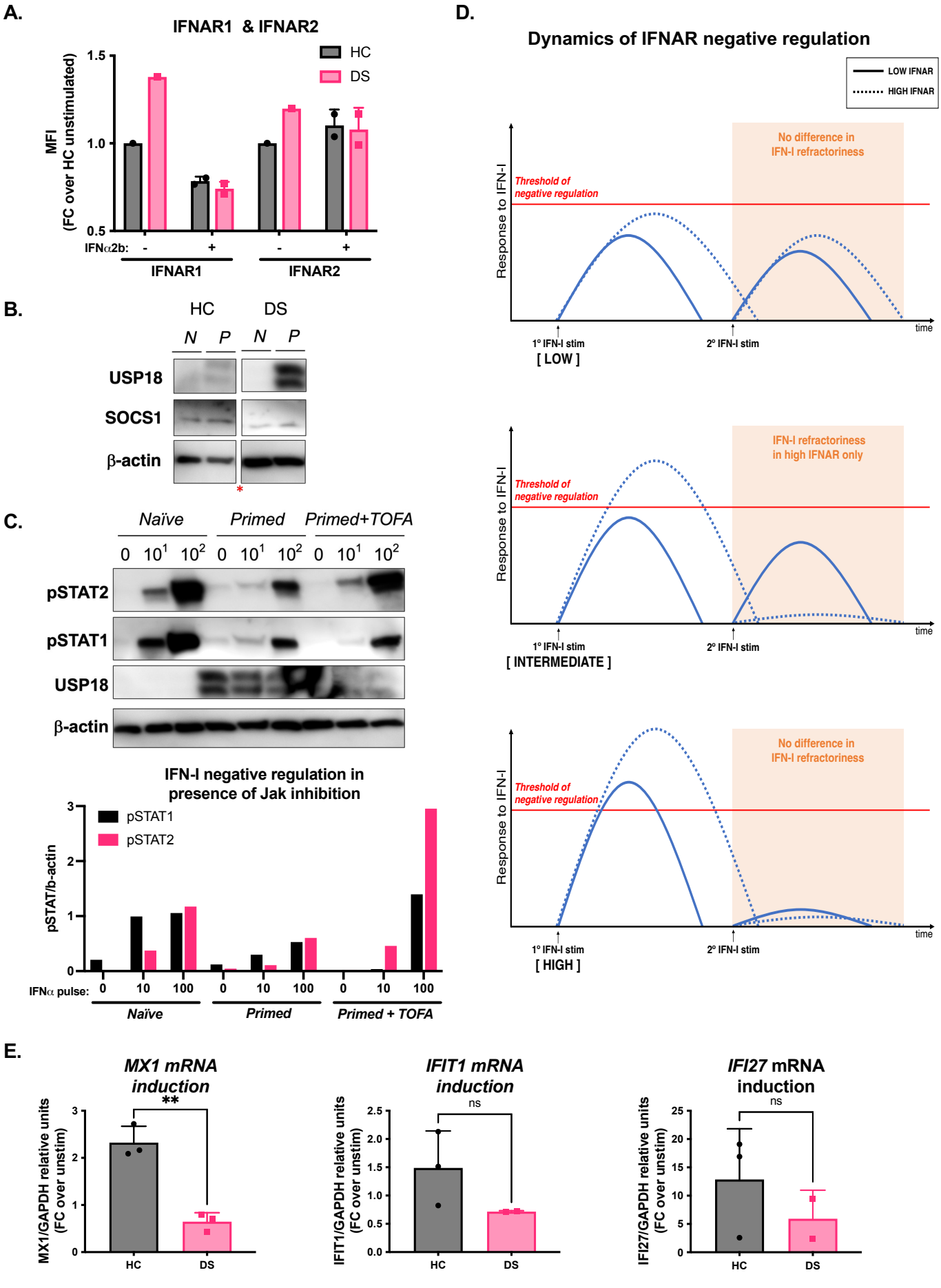


Supplemental Figure 1. Hyper-response to IFN-I in DS hTERT fibroblasts.
(A) qPCR and **(B)** Flow cytometry quantification of *IFNAR1* and *IFNAR2* expression in hTERT-immortalized fibroblasts from healthy controls (HC, *n=4*) or individuals with DS (DS, *n=4*).
(C) Immunoblotting and **(D)** qPCR for ISG induction in HC (*n=4*) and DS (*n=4*) hTERT-immortalized fibroblasts stimulated for 30 min with indicated doses of IFN-α (IU/mL) followed by rest in untreated media for 24 hours (protein, B) or 6 hours (mRNA, C).
(E) qPCR and **(F).** Immunoblotting for ISG induction after stimulation with IFN-α (10 IU/mL) for 8 hours, wash, and rest for indicated times. Result representative of of fibroblasts derived from *n=3* HCs and *n=3* individuals with DS.



Supplemental Figure 2. Doxycycline-inducible *IFNAR2* system in hTERT fibroblasts.
(A) Sanger sequencing of genomic DNA confirming 1 amino acid deletion in *IFNAR2* knockout Clone 1.
(B) Flow cytometry quantification of *IFNAR2* confirming lack of expression in *IFNAR2* knockout Clone 1.
(C) Immunoblotting for STAT phosphorylation after stimulation for 30 min with IFN-α (1000 IU/mL) confirming lack of response in *IFNAR2* knockout Clone 1.
(D) Diagram of 2-plasmid system used for lentiviral doxycycline-inducible *IFNAR2* expression.


A.
IFNAR1 & IFNAR2
HC
DS
MFI
(FC over HC unstimulated)
1.5
1.0
0.5
IFNα2b: - + - +
IFNAR1
IFNAR2
B.
HC DS
N P N P
USP18
SOCS1
β-actin
*
C.
Naïve Primed Primed+TOFA
0 10^1 10^2 0 10^1 10^2 0 10^1 10^2
pSTAT2
pSTAT1
USP18
β-actin
IFN-I negative regulation in presence of Jak inhibition
pSTAT1
pSTAT2
pSTAT/b-actin
IFNα pulse: 0 10 100 0 10 100 0 10 100
Naïve
Primed
Primed + TOFA
D.
Dynamics of IFNAR negative regulation
LOW IFNAR
HIGH IFNAR
Response to IFN-I
Threshold of negative regulation
No difference in IFN-I refractoriness
time
1° IFN-I stim
2° IFN-I stim
[LOW]
IFN-I refractoriness in high IFNAR only
[INTERMEDIATE]
[HIGH]
E.
MX1 mRNA induction
**
MX1/GAPDH relative units (FC over unstim)
HC
DS
IFIT1 mRNA induction
ns
IFIT1/GAPDH relative units (FC over unstim)
IFI27 mRNA induction
IFI27/GAPDH relative units (FC over unstim)

Supplemental Figure 3. Increased IFNAR negative regulation in Down syndrome.
(A) Flow cytometry quantification of *IFNAR1* and *IFNAR2* expression in the HC and DS hTERT-immortalized fibroblasts previously stimulated (+) or not (+) with a primary stimulus of IFN-α (10 IU/mL) for 12 h, washed, and allowed to rest for 36 h.
(B) Immunoblotting for SOCS1 in HC and DS hTERT-immortalized fibroblasts previously stimulated (N) or not (P) with a primary stimulus of IFN-α (10 IU/mL) for 12 h, washed, and allowed to rest for 36 h. *image was cropped to remove irrelevant lanes.
(C) DS hTERT-immortalized fibroblasts were or untreated (Naïve) or stimulated with IFN-α (10 IU/mL) for 8 hours and Tofacitinib (50mM) was added at hour 2 (Primed +TOFA) or not (Primed). Cells were washed, allowed to rest for 36 h, and re-stimulated for 15 min with IFN-α. Immunoblotted for STAT phosphorylation and quantification.
(D) Diagram of engagement of IFN-I refractoriness with increasing levels of IFN-α priming dose and increasing levels of *IFNAR2* expression.
(E) qPCR for ISG induction in PBMCs derived from HCs (*n=2*) and individuals with DS (*n=3*), stimulated with 100 IU/mL IFN-α for 30 min followed by rest in untreated media for 6 hours (mRNA, C). Expressed as fold-induction over individual's baseline.