

Supplementary material to

Interferons lambda 3 and 4 induce identical response in human liver cell lines depending exclusively on canonical signaling

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Supplementary figure legends

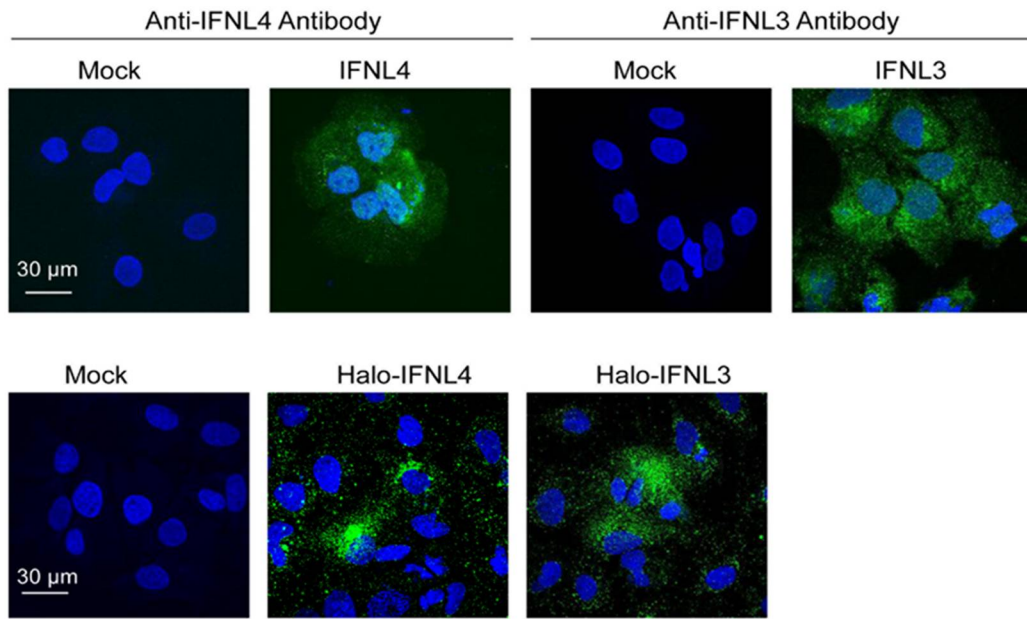
Supplementary Figure 1: Expression of IFNLs and ISGs in HuH7 control cells transfected by IFNL3 and IFNL4 lacking HaloTag. (A) High resolution confocal image of IFNL3 and IFNL4 in wild type HuH7 transfected with tagged and non-tagged *IFNL3* or *IFNL4* for 48 hours. Empty plasmid was used as a Mock control. Blue – nuclei, green - IFNL3 / IFNL4. IFNL3 and IFNL4 were detected either with the corresponding anti-IFNL3/anti-IFNL4 antibody or with anti-Halo tag ligand. Scale bars 30 μ m. (B) Relative expression of *RSAD*, *IFI27*, *IFITM1* and *OAS1* determined in HuH7 cells 48 hours transfected with tagged or non-tagged IFNL3 or IFNL4. GAPDH was used as internal control. Results are presented as mean \pm SEM (n=4). (C) Kinetics of STAT1 and phospho STAT1 activation in HuH7 cells transfected by nontagged *IFNL3* or *IFNL4* expressing plasmids detected by immunoblot analysis. β Actin was used as a loading control.

Supplementary Figure 2: Densitometric quantification of STAT1 and pSTAT1. Wild type HuH7 cells were transfected with non-tagged or Halo-tagged *IFNL3* or *IFNL4* for 48 hours or stimulated with recombinant IFNL3 or IFNL4 protein in the media for 24 hours. Empty plasmid was used as Mock control. Four independent experiments were used for quantification. **p< 0.05 was considered as statistically significant.

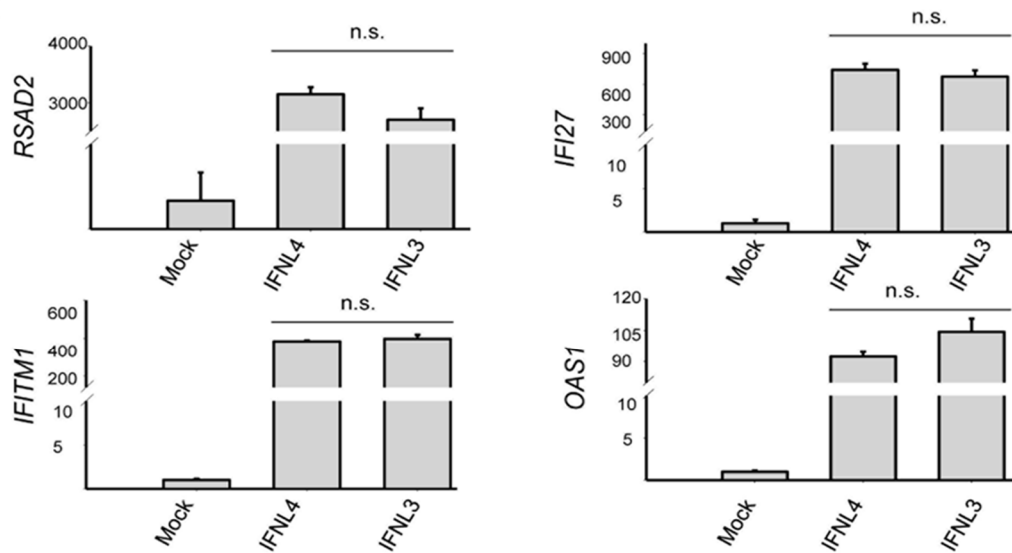
Supplementary Figure 3: Characterization of IFNLR1 and IL10R2 knockout cell clones. Confocal immunofluorescence images of IFNLR1 and IL10R2 were done in control and CRISPR/Cas9 edited HuH7 cells. Nuclei are shown in blue, membrane in green, IFNLR1 and IL10R2 are in red. Scale bar 20 μ m. Representative pictures achieved from several knockout clones are displayed.

Supplementary Figure 4: IFNLR1/IL10R2 receptor complex is indispensable for activation of JAK-STAT signaling pathway by IFNLs in HepG2 cells. Control, IFNLR1 and IL10R2 knockout HepG2 cells were transfected with non-tagged *IFNL3* or *IFNL4* for 48 hours or stimulated with recombinant IFNL3 or IFNL4 for 24 hours. Empty plasmid was used as Mock control. Activation of STAT1 and pSTAT1 was determined by immunoblot analysis. Each lane is representative of 4 biological replicates per transfection/stimulation group. β Actin was used as a loading control.

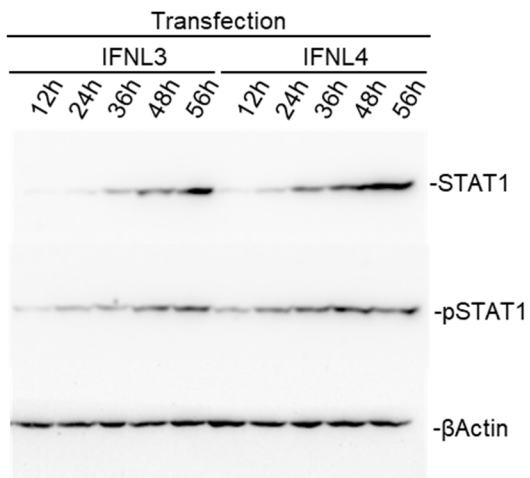
A



B

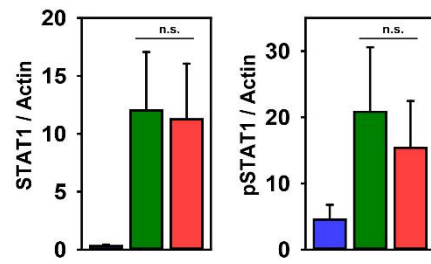


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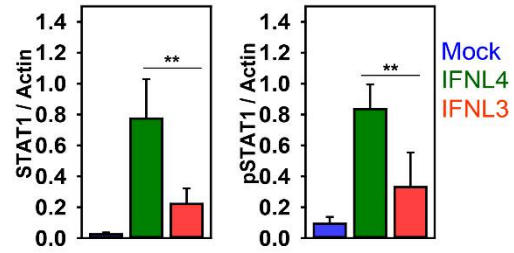


Supplementary Figure 1.

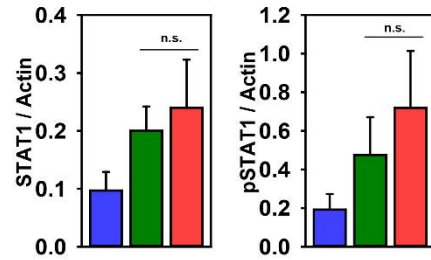
Transfection with non-tagged Interferons



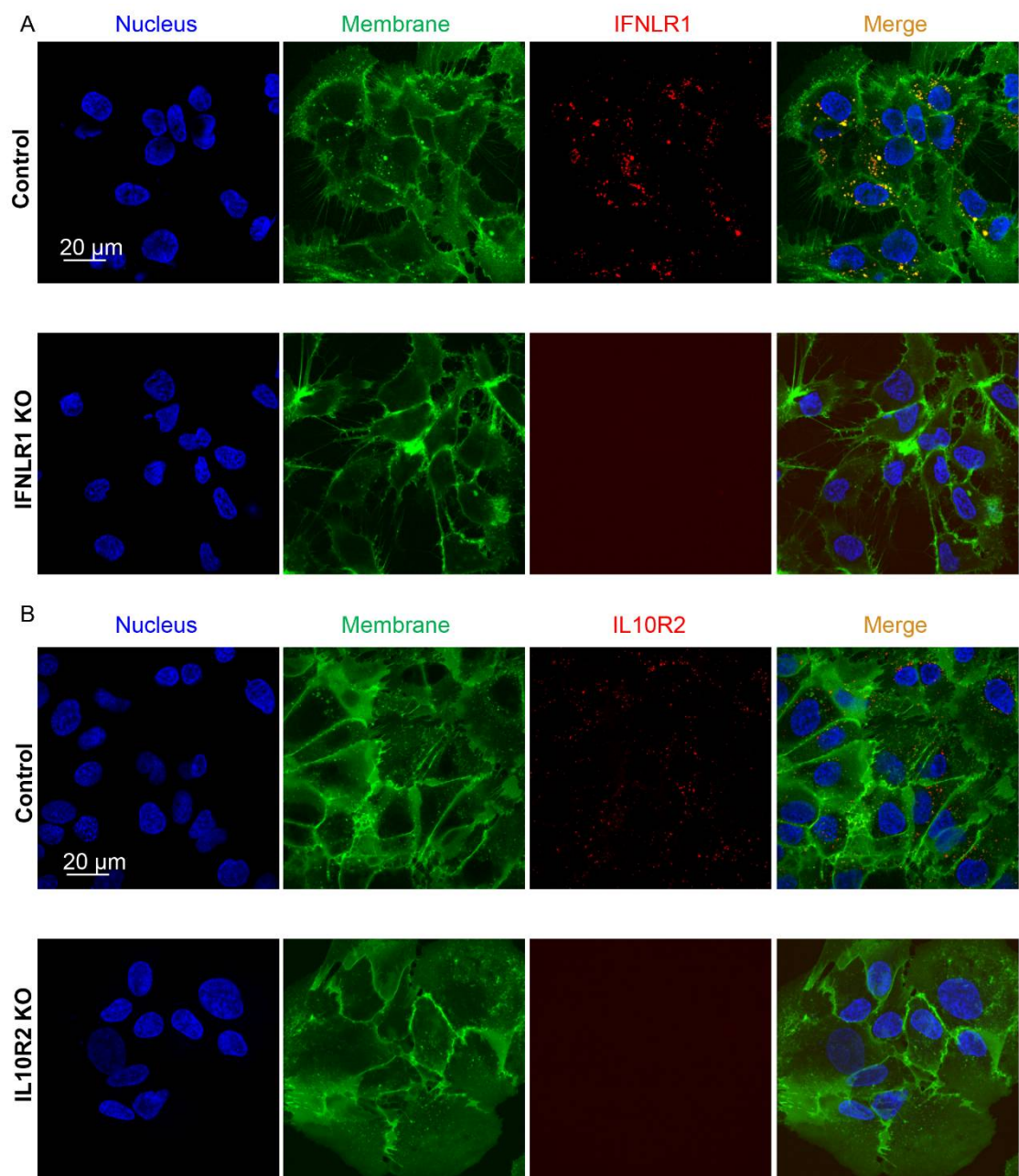
Transfection with Halo-tagged Interferons



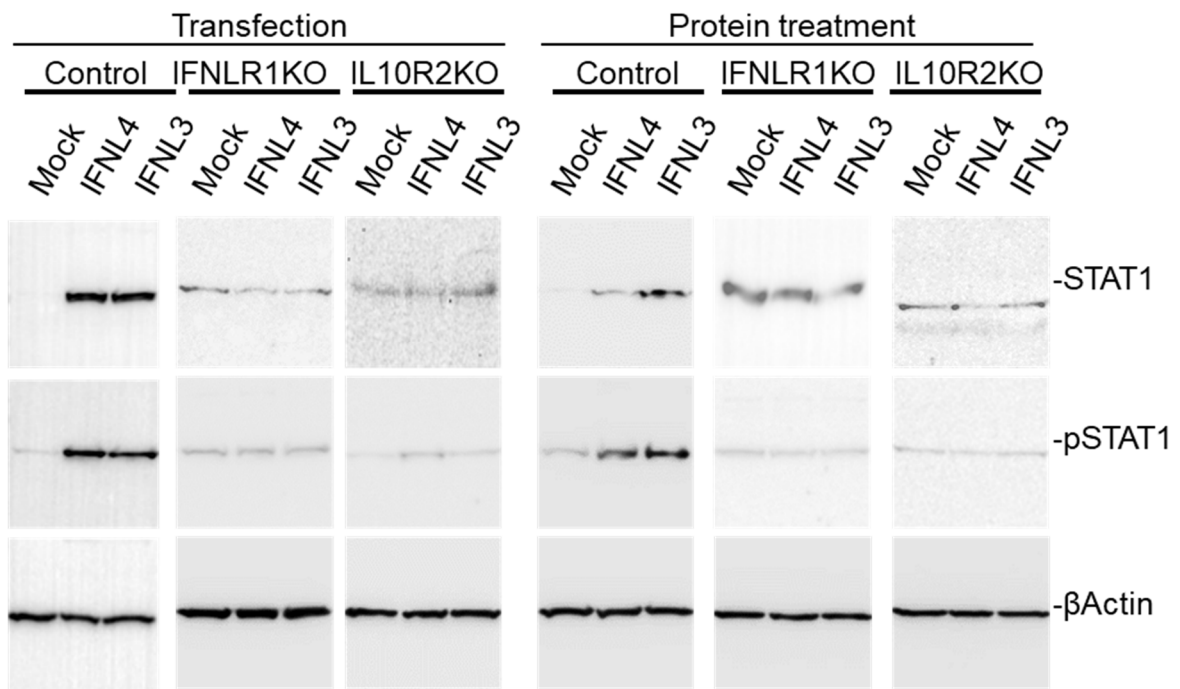
Interferon protein treatment



Supplementary Figure 2.



Supplementary Figure 3.



Supplementary Figure 4.

Supplementary Table 1: Antibodies and chemicals used for immunoblotting and fluorescence labeling.

Name	Company	Cat №	Dilution	
			WB	IF
βActin	ThermoFisher	MA5-15452	1:2000	
STAT1 (C-136)	Santa Cruz	SC-464	1:1000	
pSTAT1	Cell Signalling	8826	1:1000	
IFNL4	Millipore	MABF227		1:200
IFNL3	ThermoFisher	MA5-24254		1:200
IFNLR1	Novus Biologicals	NBP1-69636	1:500	
IL10R2	R&D Systems	MAB874-100	1:1000	
Goat anti mouse IgG	Invitrogen	G21040	1:10000	
Goat anti rabbit IgG	Invitrogen	G21234	1:10000	
Goat anti mouse Alexa 568/488	ThermoFisher	A-11004/A-11029		1:500
Goat anti rabbit Alexa 568/488	ThermoFisher	A-11011/A-11008		1:500
Cell Mask Green	ThermoFisher			1:1000
Hoechst	ThermoFisher	H1398		1:10000

Supplementary Table 2: **Description of IFNLR1 and IL10R2 knockout clones.**

Genotype	Protein	Clones
IFNLR1		
HuH7		
c.123delG	p.Thr42Hisfs*12	9x
c.125_126delCA	p.Thr42Metfs*24	4x
c.123_124insC	p.Thr42Hisfs*25	1x
HepG2		
c.122_126delTGACA	p.Thr42Alafs*23	2x
c.126_130delATGGC	p.Trp43Profs*22	1x
c.127_128delTG	p.Trp43Alafs*23	1x
c.121_125delCTGAC	p.Leu41Metfs*24	1x
IL10R2		
HuH7		
c.69delT	p.Glu24Lysfs*28	1x
c.59_60insA	p.Met20Ilefs*32	1x
HepG2		
c.22_23insT	p.Trp8Leufs*32	3x

Supplementary Table 3: **TaqMan gene expression assays used in real time PCR.**

Gene Name	TaqMan Gene Expression Assay ID
<i>GAPDH</i>	Hs02786624_g1
<i>OAS1</i>	Hs00973635_m1
<i>MX1</i>	Hs00895608_m1
<i>ISG15</i>	Hs00192713_m1
<i>USP18</i>	Hs00276441_m1
<i>IFI6</i>	Hs00242571_m1
<i>IFI27</i>	Hs00271467_m1
<i>IFITM1</i>	Hs01652522_g1
<i>RSAD2</i>	Hs00369813_m1