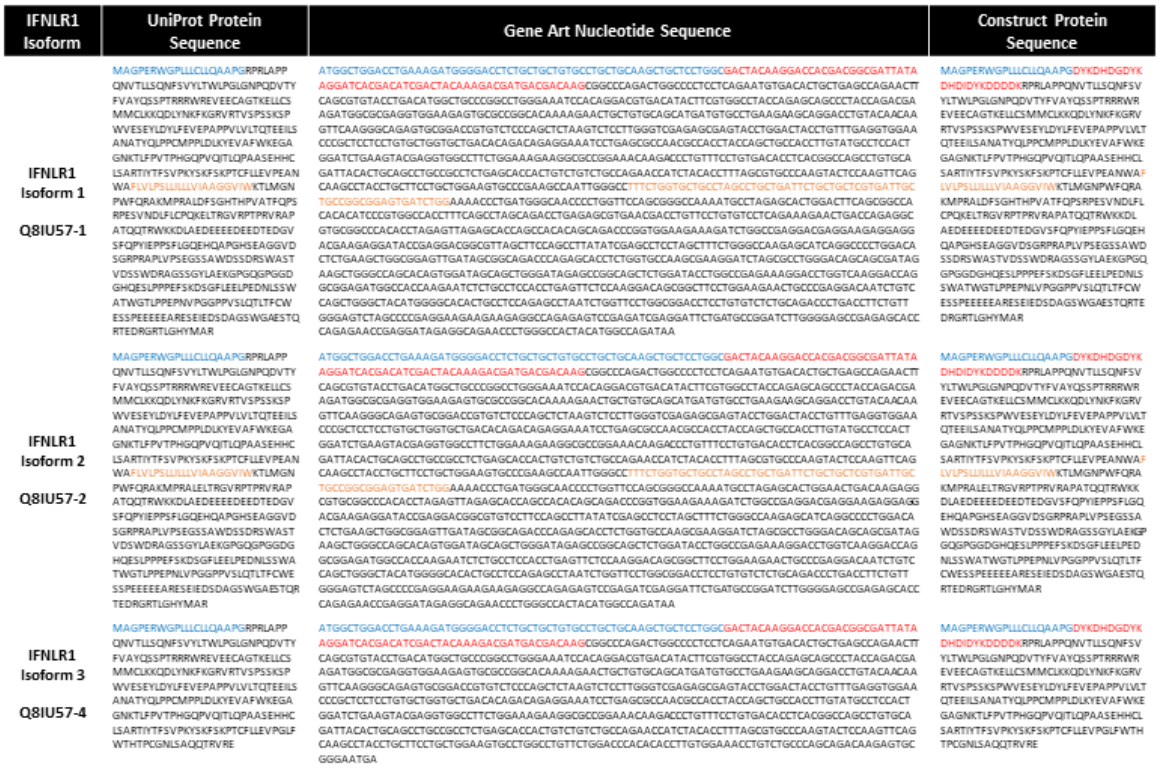
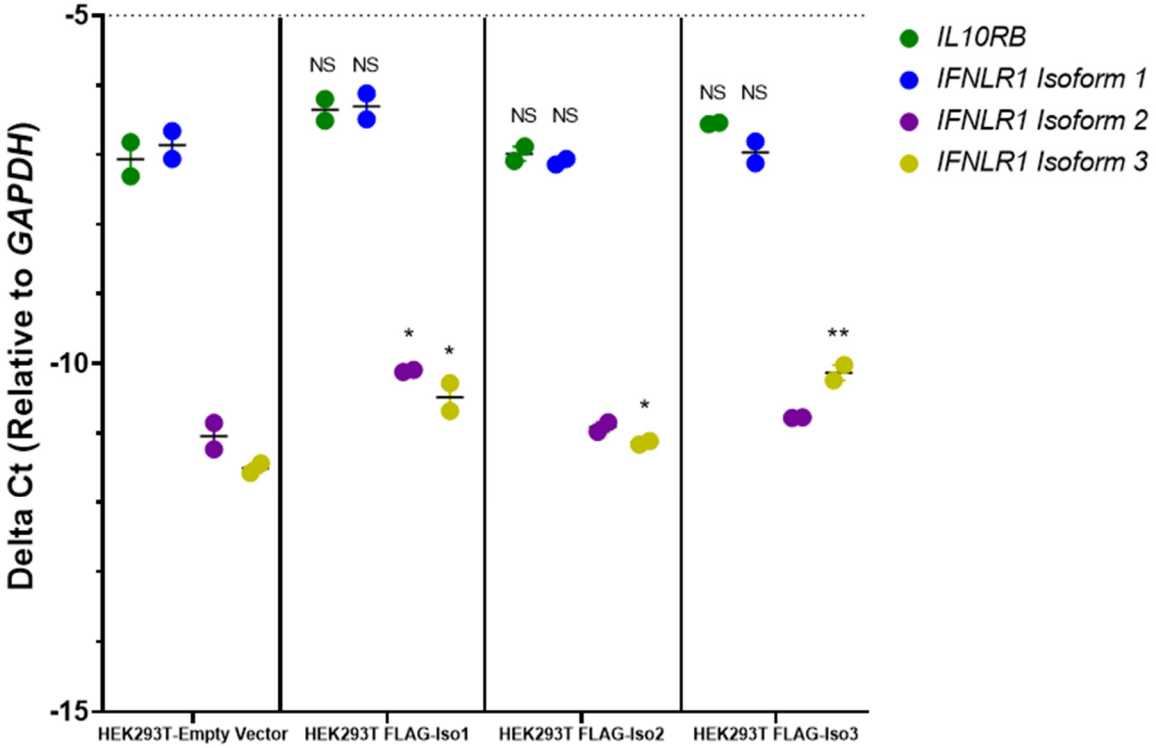
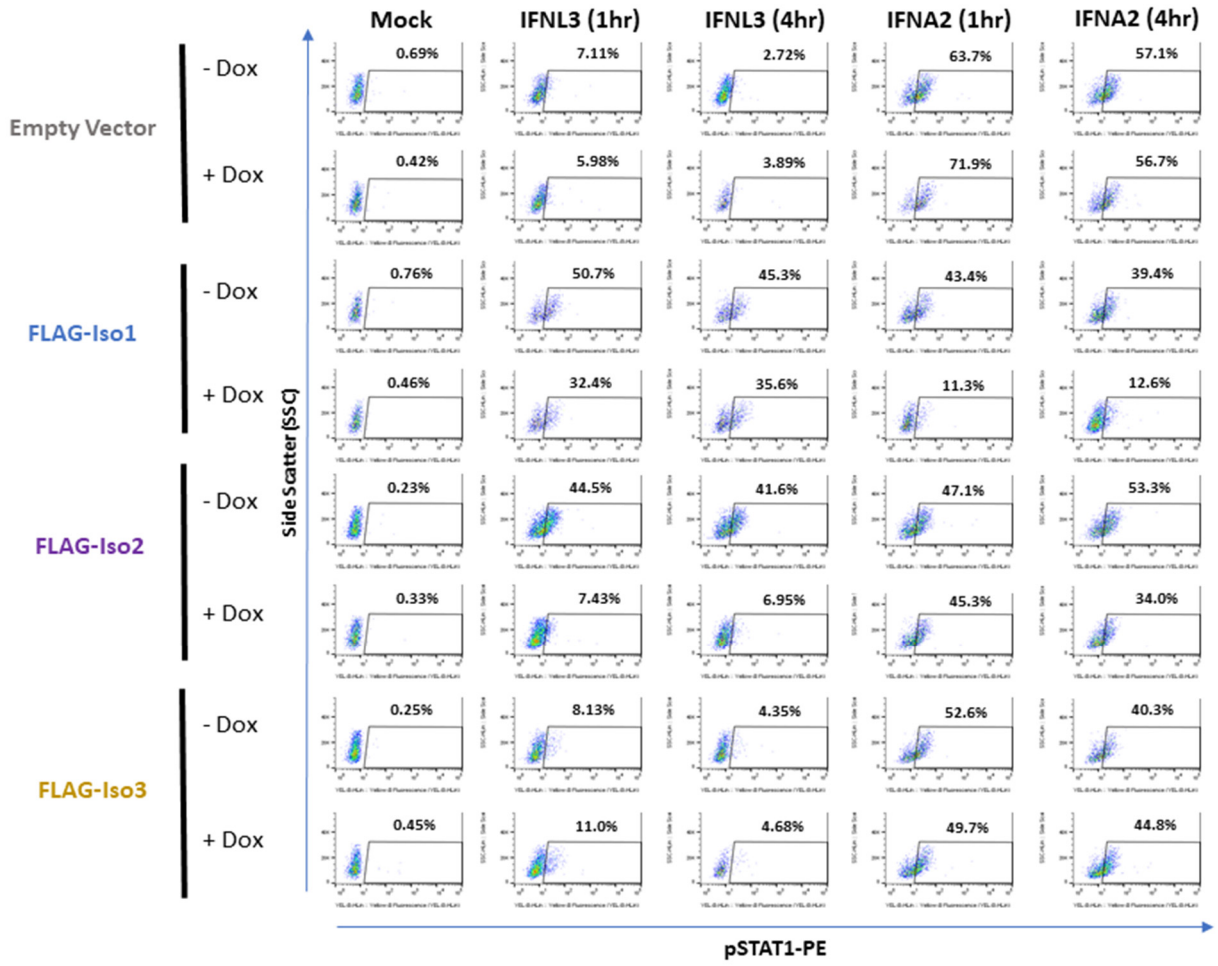
Supplemental Figure



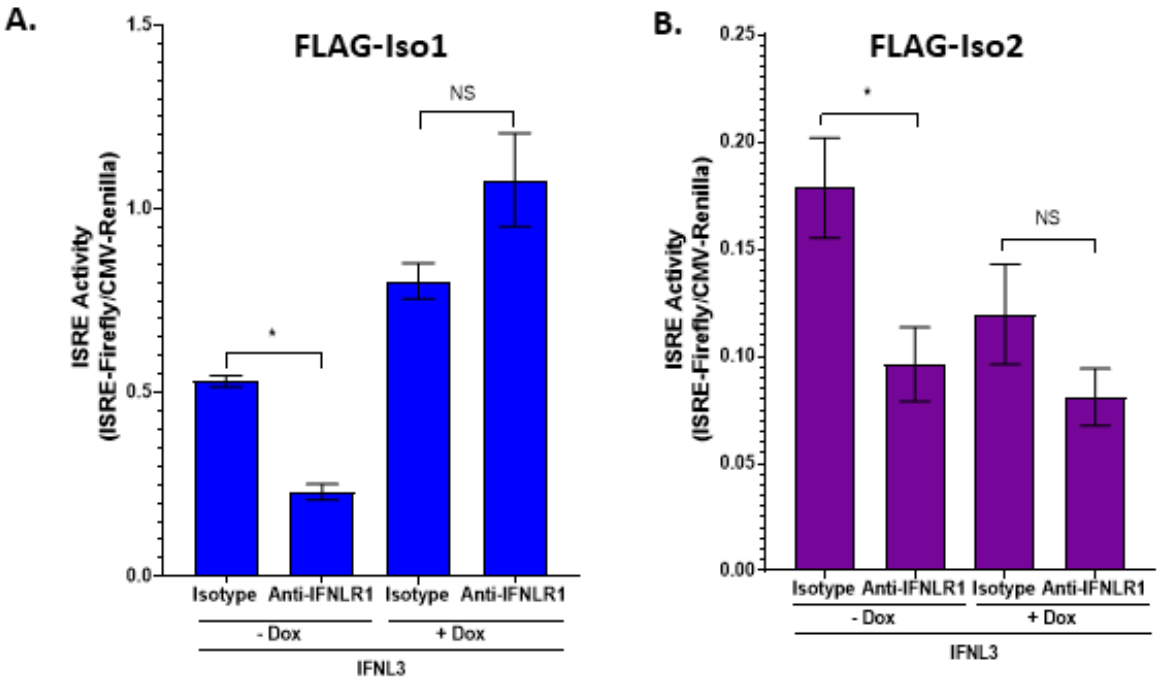
Supplemental Figure S1: Protein and nucleotide sequences of IFNLR1 isoforms. Unaltered protein sequences downloaded from UniProt database (**left** column), codon-optimized nucleotide sequences used for Gene Art synthesis (**middle** column), and FLAG-tagged IFNLR1 isoform protein sequences (**right** column). Signal sequence is denoted in blue; 3x-FLAG tag is denoted in red; the transmembrane domain is denoted in orange.



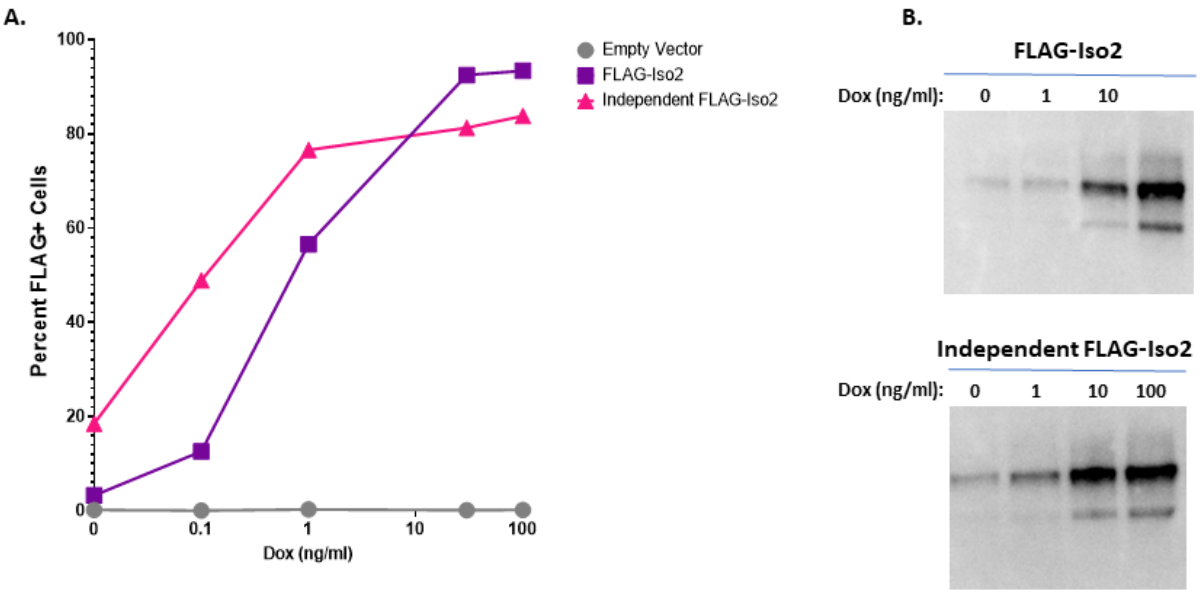
Supplemental Figure S2: Endogenous *IL10RB* and *IFNLR1* isoform expression in HEK293T stable lines. Cellular gene expression was quantitated by qRT-PCR. Statistical significance represented by asterisks reflect comparisons between EV and FLAG-IFNLR1 isoform cells. Error bars represent standard error of the mean. * = $p < 0.05$, ** = $p < 0.01$. NS = not significant.



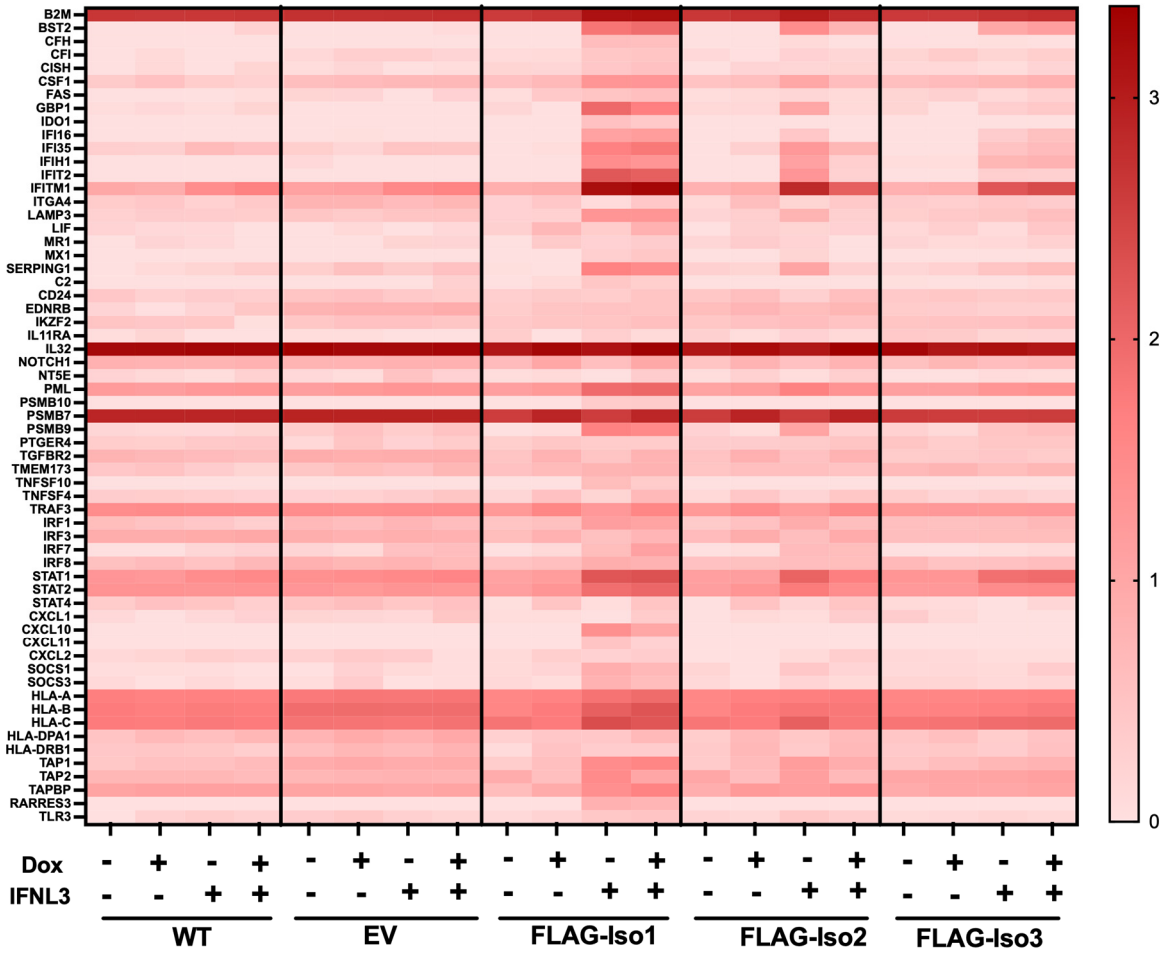
Supplemental Figure S3: Representative flow cytometry plots used for quantification in Figures 3, 4, 6, and 9



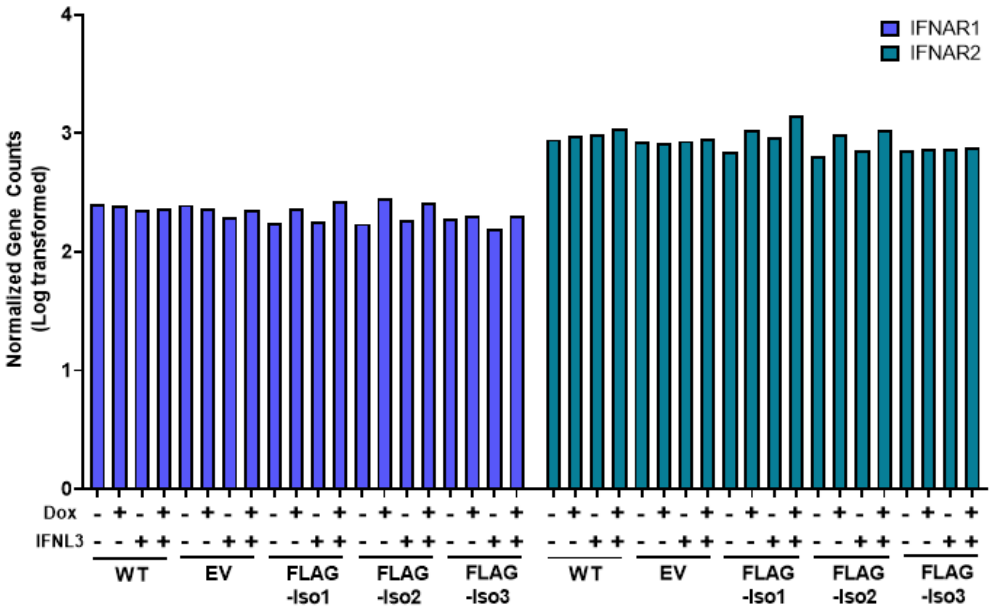
Supplemental Figure S4: Effect of pre-incubation with anti-IFNLR1 antibody on the IFNL3 response in FLAG-Iso1 and FLAG-Iso2 lines. IFNLR1 FLAG-Iso1 **(A)** and FLAG-Iso2 **(B)** cells were treated +/- dox (100 ng/ml) for 24 hrs prior to incubation with anti-IFNLR1 antibody or isotype control (anti-IL-28RA antibody, control IgG, R&D Systems, 4ug/ml) for 1.5 hrs, after which the cells were stimulated with IFNL3 (100 ng/ml) for 24 hrs and harvested for dual luciferase assay (representative data, 2 independent experiments). Error bars represent standard error of the mean. * = $p < 0.05$. NS = not significant.



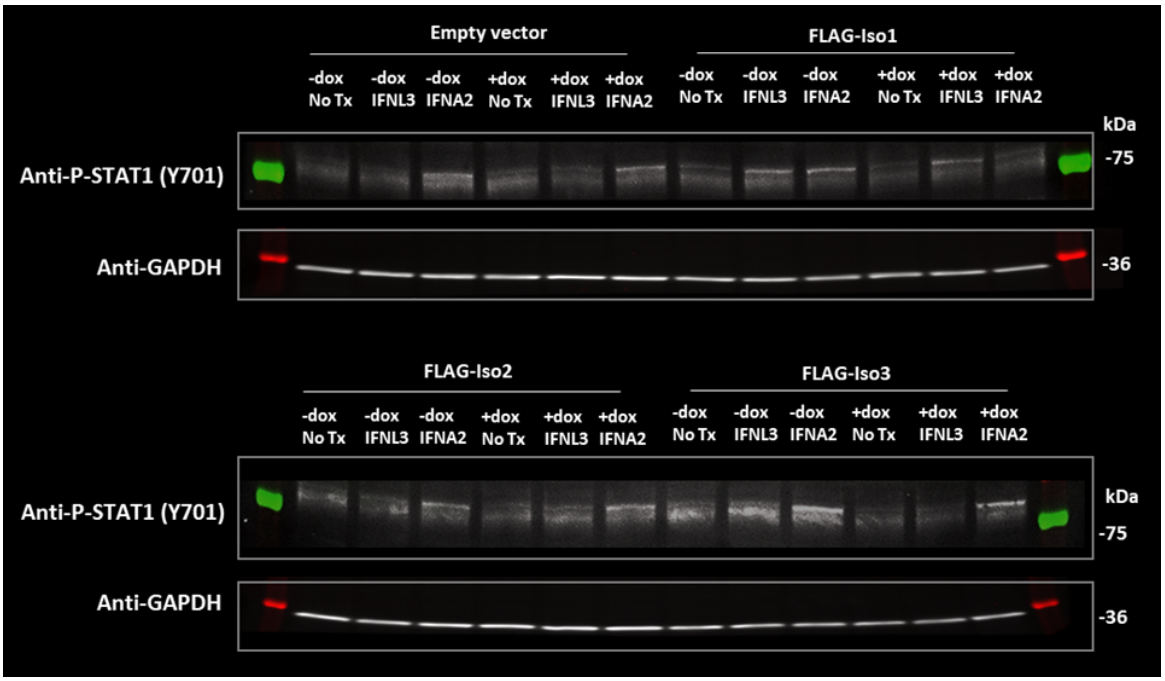
Supplemental Figure S5: Dox-titratable FLAG-Isoform 2 expression in independent HEK293T stable lines. **(A)** HEK293T-EV, the FLAG-Iso2 line characterized in Figure 4, and an independent FLAG-Iso2 line were treated +/- dox (0, 0.1, 1, 10, or 100 ng/ml) for 24 hr prior to analysis of FLAG expression by flow cytometry. **(B)** FLAG-Iso2 expression in each line after treatment +/- dox (0, 1, 10, 100 ng/ml) for 24 hr is shown by western blot of cell lysates using anti-FLAG antibody.



Supplementary Figure S6: Differentially expressed genes among HEK293T cells line. HEK293T WT and stable lines were induced +/- dox (100 ng/ml) for 24 hrs prior to treatment with +/- IFNL3 (100 ng/ml) for an additional 24 hrs. RNA was collected and gene count quantitated by NanoString analysis (nCounter Human Immunology v2 Panel). Genes shown were differentially regulated (>2-fold change) in one or more cell lines after doxycycline and/or IFNL3 treatment. Data are shown as log transformed normalized counts.



Supplemental Figure S7: *IFNAR1* and *IFNAR2* RNA expression levels are unchanged among cell lines and conditions. Data were derived from NanoString analysis (described in Figure 8 and Supplemental Figure S6).



Supplemental Figure S8: Western blot analysis of pSTAT1 in HEK293T stable lines. HEK293T-EV and FLAG-IFNLR1 isoform lines were +/- dox treated (100 ng/ml) for 24 hrs prior to mock, IFNL3 (100ng/ml), or IFNA2 (100 ng/ml) stimulation for 1 hr. Protein was collected from cell lysates and assessed by western blot. The larger of the two observed bands is interpreted to reflect pSTAT1 signal.