**Supplementary Figure Legends**

**Supplemental Figure S1**. **Structures and locations of protein interaction sites on ISG15**. **A.** The ISG15 C-terminal ubiquitin-like domain (PDB 1Z2M) is colored in gray. Residues in the IFN-γ signaling patch (Y96, R99, T101, Q102, T103) are colored and shown as sticks. **B.** ISG15 structures with influenza B virus NS1 (magenta) and USP18 (yellow) are shown (PDB 3SDL and 5CHV, respectively). ISG15 is in gray with the IFN-γ signaling patch shown in red. **C.** ISG15 in gray, with the IFN-γ signaling patch in red and the residues in both the N- and C-lobes that correspond to the position of the ubiquitin hydrophobic patch shown in blue (residues L5, L10, and A46 in the N lobe, and V74, N89, and T125 in the C lobe).

**Supplemental Figure S2. ISG15 signals when bound to a surface, and ISG15 variants retain the ability to be conjugated intracellularly and retain wild-type unfolding characteristics. A.** IL-12 and the indicated concentrations of GST-ISG15 on glutathione beads were incubated with NK-92 cells for 48hrs, and an IFN-g ELISA of the cell culture supernatants was performed in biological duplicate. Error bars indicate standard error of the mean. **B**. FLAG-ISG15 variants were expressed by plasmid transfection in HEK293T cells alone or with ISG15 E1- E-2 and E3-expressing plasmids (UBE1L/UBH8/HERC5). Whole cell lysates were immunoblotted with anti-FLAG antibody. All IFN-g signaling-competent (green labels) and signaling-deficient (red labels) variants were conjugated intracellularly in the presence of the ISG15 conjugation machinery. **C**. ISG15 variants were subjected to thermal denaturation analysis using the Protein Thermal Shift™ assay (Thermo-Fisher). Individual points represent four repeats of the experiment and error bars represent the standard error of the mean. Points in green represent IFN-g signaling-competent variants and points in red indicated signaling-deficient (red labels) variants.

**Supplemental Figure S3. Interaction of ISG15 with the αI domain of CD11a. A**. HEK293T cells transfected with plasmids expressing the indicated proteins (CD11a, FLAG-CD11b, FLAG-CD11c, CD18, IL12R) were examined for the expression of the indicated receptors by immunobloting with antibodies to the IL12 receptor b1 subunit, CD11a, or anti-FLAG antibody. The same transfected cells were used in the ISG15 binding experiment shown in Figure 4B. **B**. $^{32}$P-labeled WT ISG15 and Y96L Q102D ISG15 were incubated with GST-CD11a αI domain on glutathione sepharose. The beads were collected and the supernatant was analyzed for unbound $^{32}$P-labeled ISG15. The $K_d$ was determined according to the methods described by Pollard (Pollard; 2010). Three biological repeats were conducted and error bars indicate standard error of the mean. **C**. Purified GST-CD11a αI domain and MIDAS mutants (S139A S141A, S139A S141A D239A, and D239A E241A, with all indicated residues altered to alanine) on glutathione sepharose were incubated with FLAG-ISG15. Beads were collected, washed, and analyzed for binding of FLAG-ISG15 by immunoblotting.

**Supplemental Figure S4. The effect of ICAM1 on ISG15-LFA-1 binding, signaling, and cell adhesion. A**. Purified GST-CD11a αI domain and MIDAS mutant S139A S141A D239A were incubated with $^{32}$P-labeled ISG15 and increasing concentrations of ICAM1. Beads were collected and the supernatants were analyzed for the presence of $^{32}$P-labeled ISG15. Three biological repeats were conducted and error bars indicate standard error of the mean. **B**. Splenocytes from ICAM1$^{+/+}$ and ICAM1$^{-/-}$ C57B6 mice were treated with mouse IL-12 and the indicated amounts of mISG15 for 48 hours (mISG15 alone was 117nM), and an IFN-γ ELISA of the cell culture supernatants was performed. IFN-γ release is shown as fold increase over that of untreated splenocytes. Three biological repeats of the experiment were conducted with spleens from three animals of each genotype, and the error bars represent the standard error of the mean. **C**. Jurkat cells were activated with PMA and assessed for binding to ICAM1- and

ICAM2-coated plates in the presence of increasing concentrations of ISG15. Three biological replicates were conducted and error bars indicate standard error of the mean.

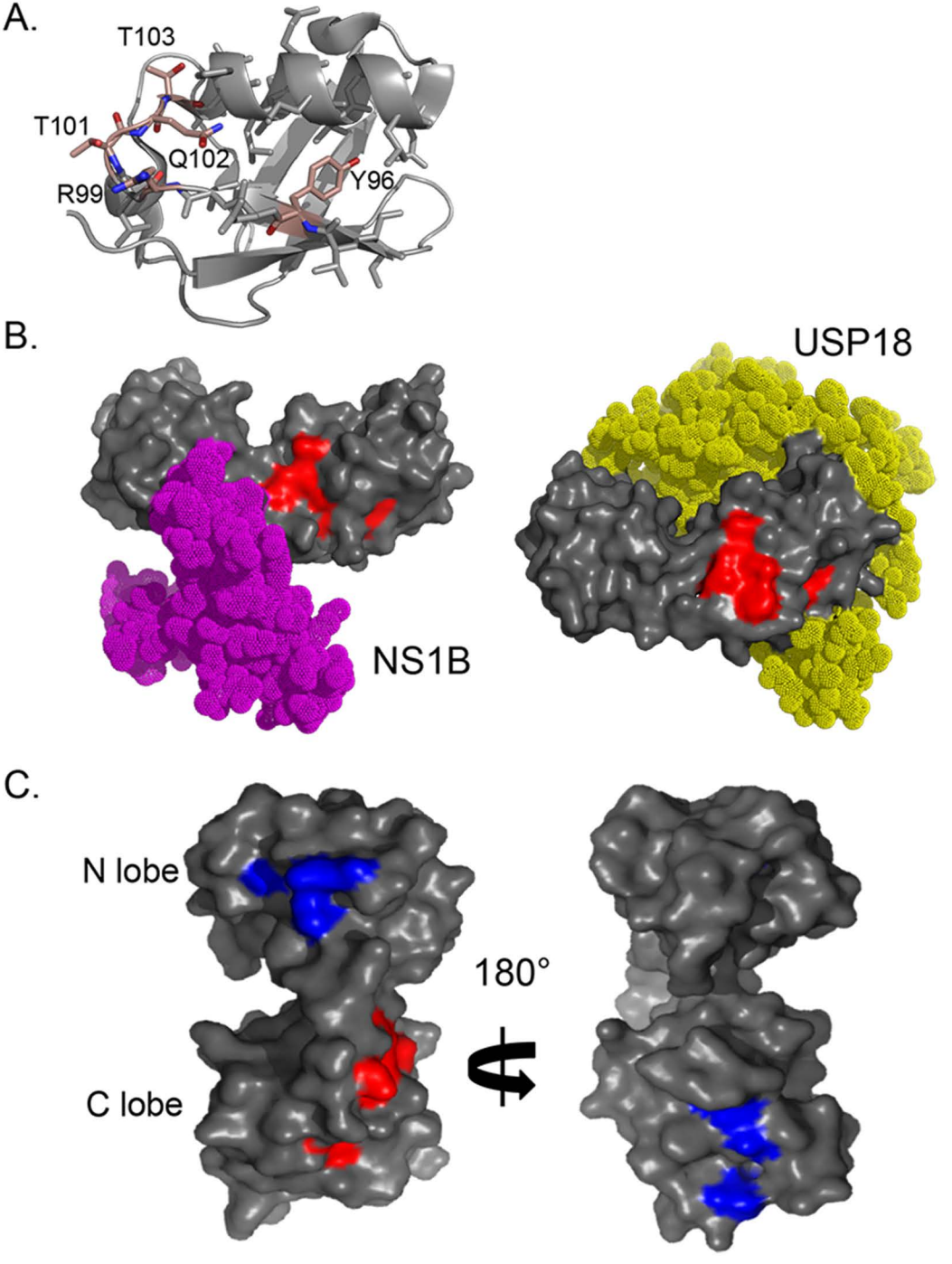
**Supplemental Figure S5**. **ISG15 signaling does not enhance IFN-g expression or STAT4 phosphorylation. A.** Quantitative RT-PCR of IFN-γ-encoding mRNA in NK-92. Cells were treated for either 3 or 16 hours with ISG15, IL-12, or ISG15 and IL-12, and mRNA levels determined relative to untreated cells with TaqMan® IFN-g mRNA probes. Error bars indicate standard error of the mean for three biological replicates. The difference between IL-12 alone and IL-12 plus ISG15, at both treatment times, was not significant as measured by a Student t-Test (p-value 0.19). **B.** NK-92 cells were treated for 2 or 6 hours with ISG15, IL-12, or ISG15 and IL-12. Total cell lysates were immunoblotted with a phosphor-STAT4 specific antibody. for STAT4 phosphorylation Phospho-STAT4 specific antibody. An anti-tubulin antibody was used as to control for equal loading.

**Supplemental Figure S6**. **SRC family kinases in NK-92 cells**. **A.** Immunoblot for LYN, HCK, and FGR in NK-92 cells. **B.** NK-92 cells were treated with ISG15, ISG15 plus IL-12, or IL-12 alone for 10 minutes. Whole cell lysates were made and immunoblotted with an antibody that detects the phospho-activated form of LCK (Y396).

**Supplementary Table. Proteins purified in ISG15-UBAIT experiments.** Spectral counts of proteins (71) that were identified in all three biological repeats of the ISG15-UBAIT screen are shown, in alphabetical order. Proteins highlighted in gray and blue represent those that met the scoring cut-off of having at least two-fold more spectral counts with the WT UBAIT than both the

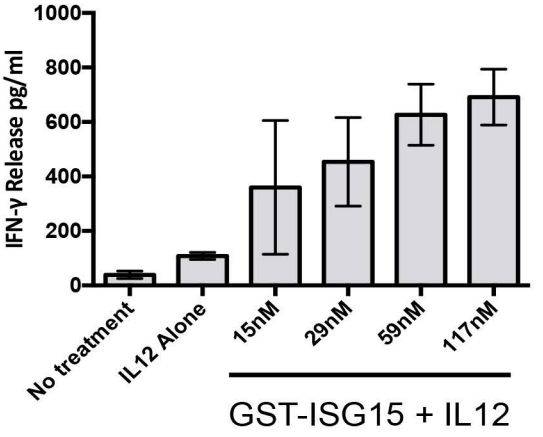
ΔGG UBAIT or the Y96L/Q102D UBAIT. Protein highlighted in blue represent those that met the scoring criteria and are plasma membrane associated cell surface proteins.

Supplemental Figure S1; related to Figure 2



Supplemental Figure S2; related Figures 1 and 2

A.

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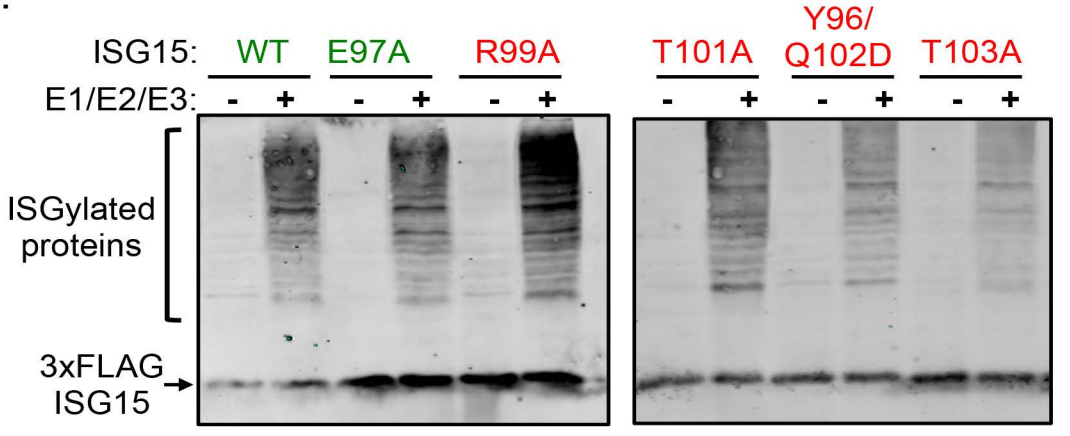


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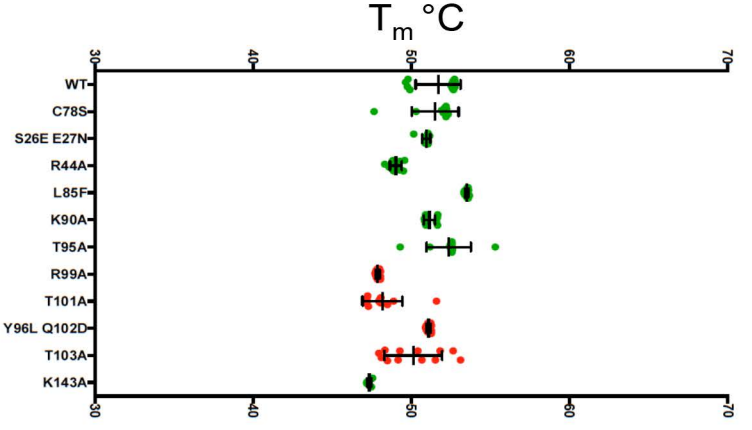
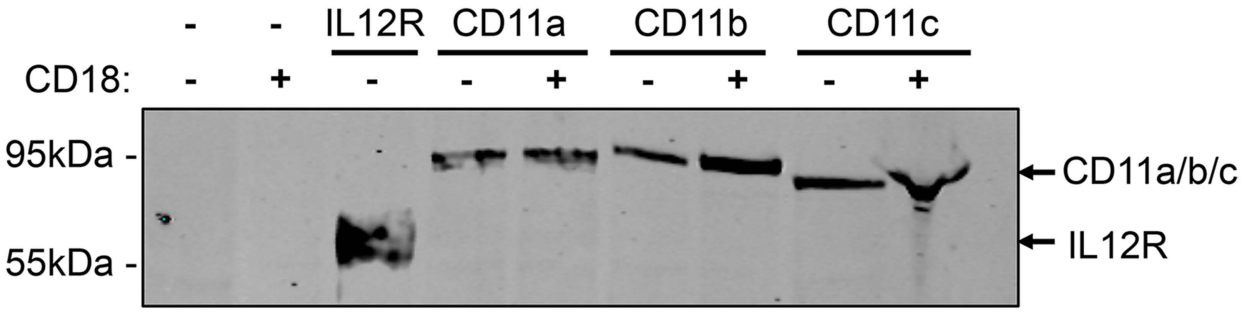


Supplemental Figure S3; related to Figures 3 and 4.

A.

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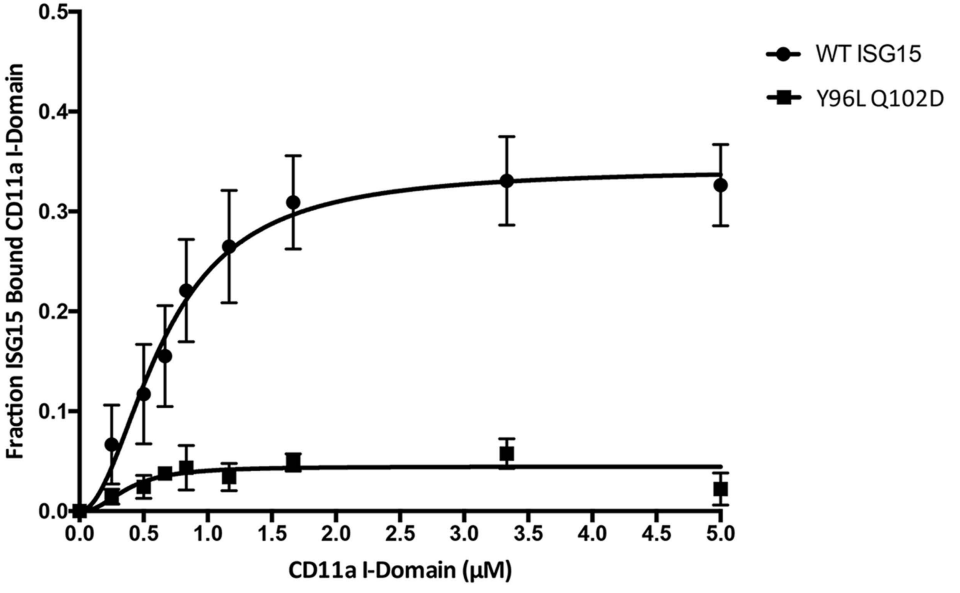


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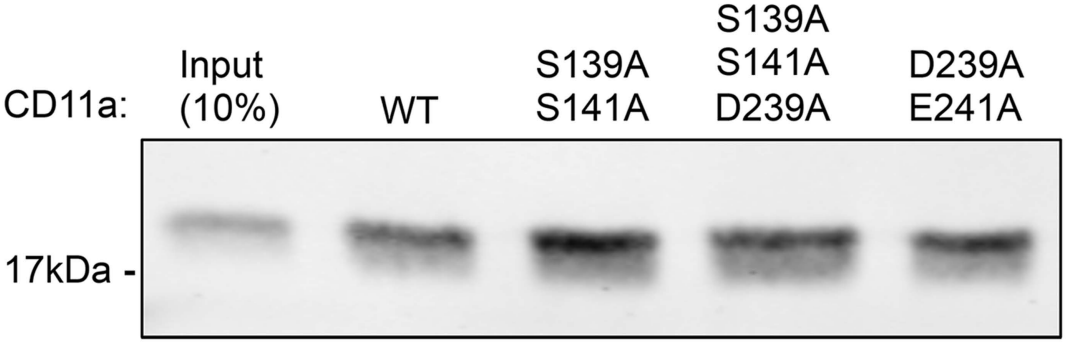
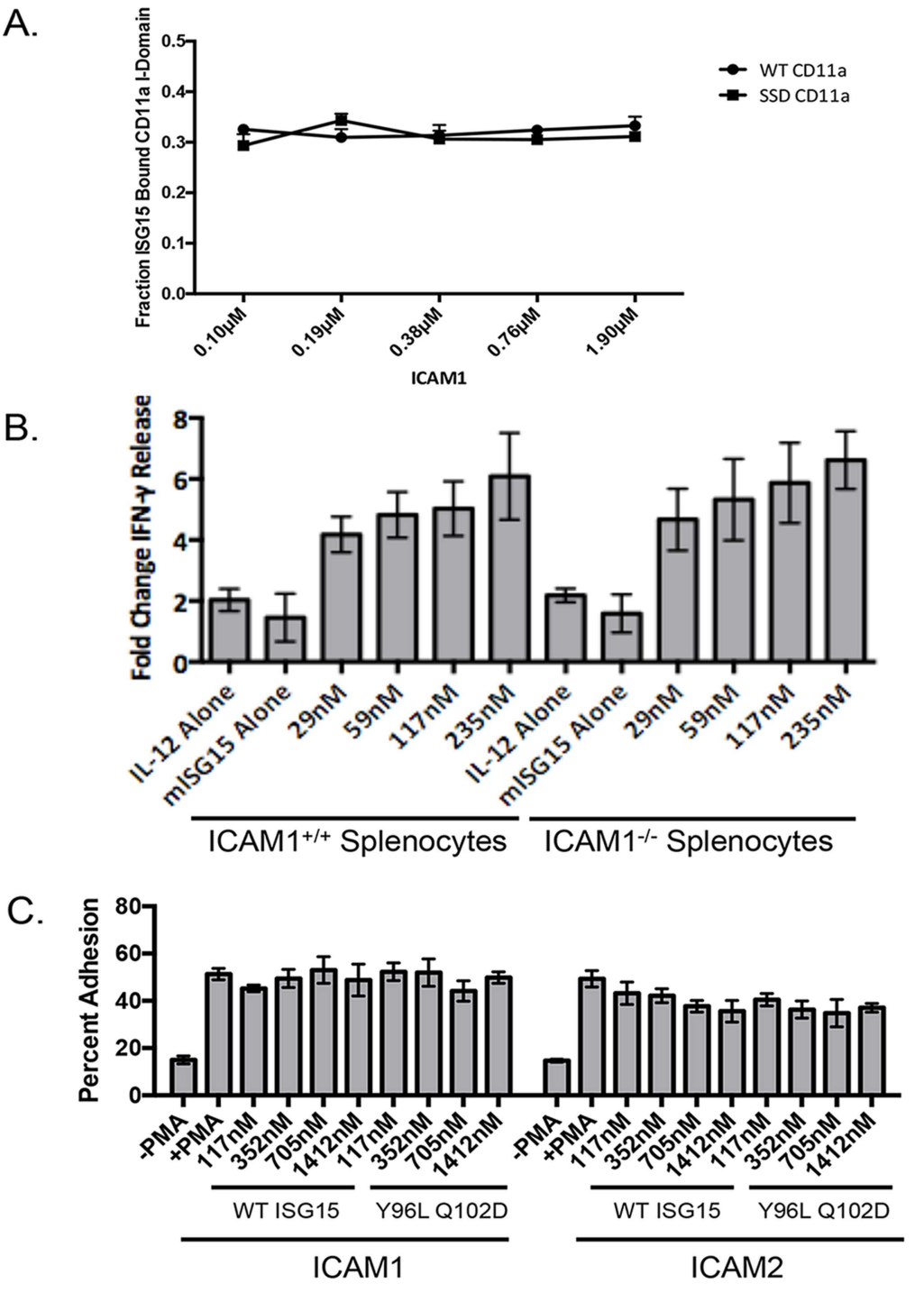
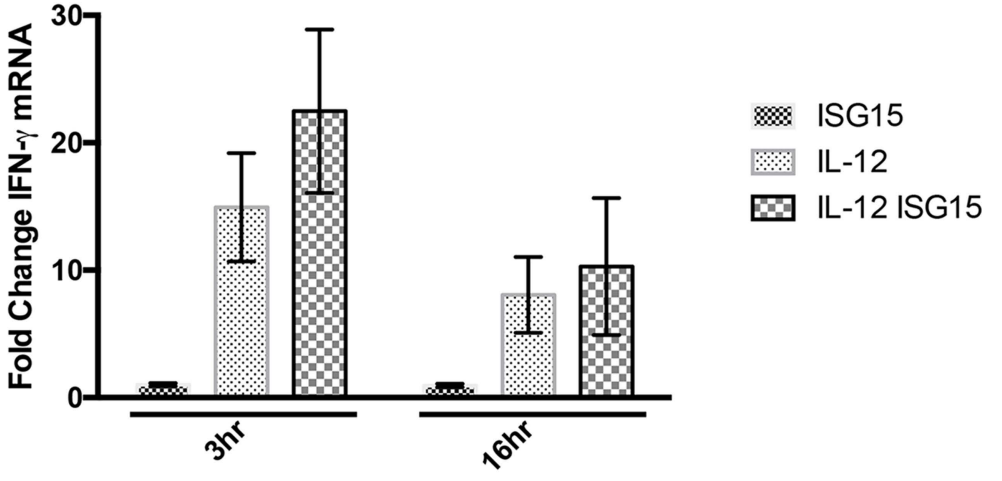


Supplemental Figure S4; related to Figure 5



Supplemental Figure S5; related to Figure 6

A.

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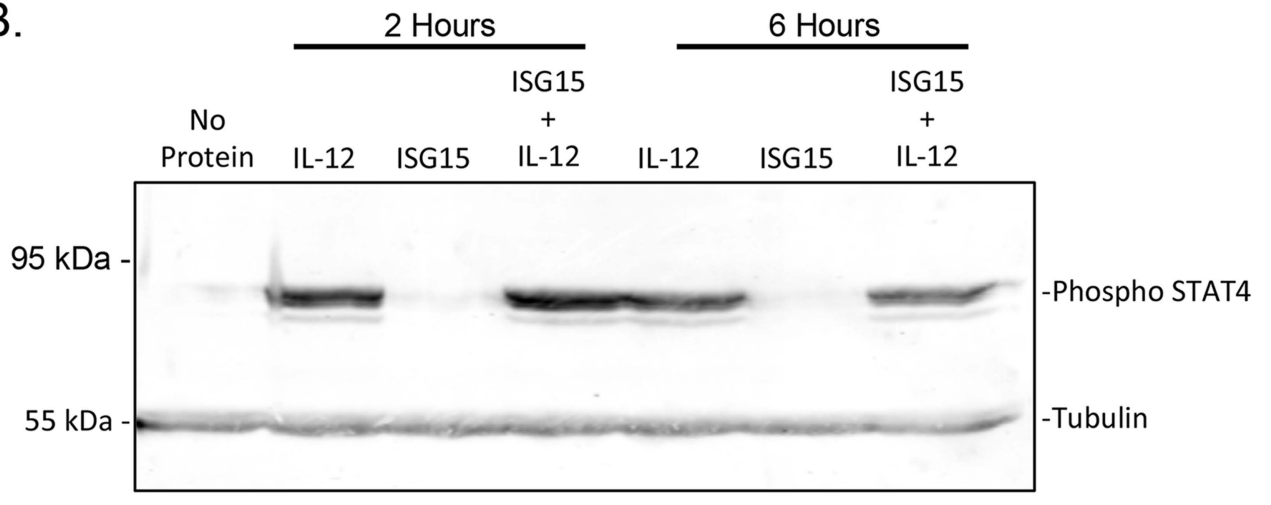

Supplemental Figure S6; related to Figure 7