JEM

Supplemental material

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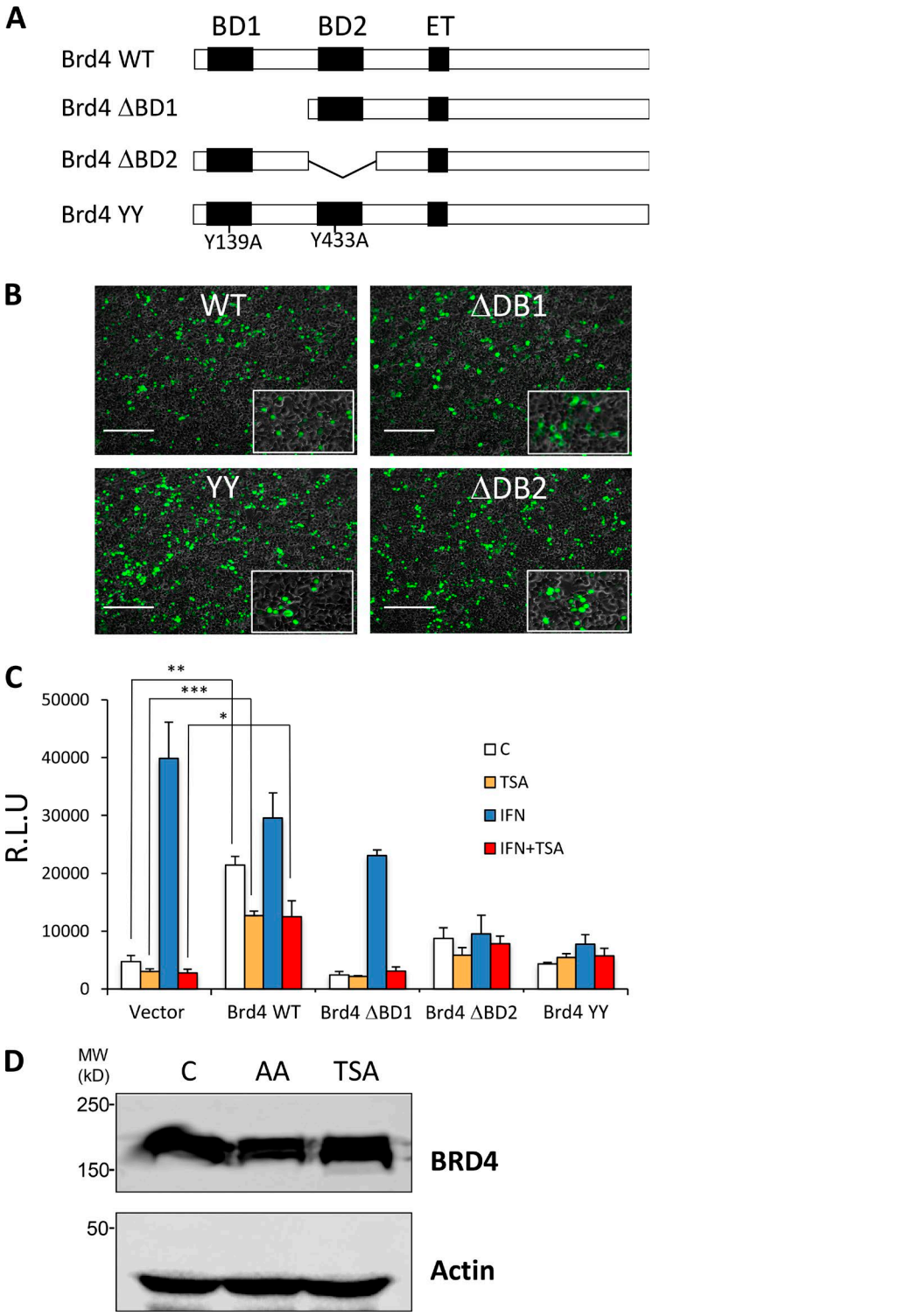

Figure S1. **Acetyl-lysine–binding bromodomains of BRD4 are required for ISG regulation. (A)** Diagram of Brd4 WT and mutant constructs. BD, bromodomain; ET, extraterminal domain. **(B and C)** HEK293T cells were transfected with empty vector (Vector), Brd4 wild type (Brd4 WT), or mutants (as shown in A) as GFP fusion expression constructs, along with a luciferase reporter driven by the ISG54 promoter. 24 h after transfection, cells were imaged to monitor expression (B) and were either left untreated or treated with IFN-α for 12 h in the absence or presence of TSA before being assayed for luciferase activity (C). Bars, 500 µm; boxed insert, additional 2× magnification. Luciferase data are shown as the mean and standard deviation of triplicate biological samples and represent an example of two experiments. *, $P < 0.01$; **, $P < 0.001$; ***, $P < 0.0001$. R.L.U., relative light units. **(D)** Abundance of total nuclear BRD4 was assessed by immunoblot in nuclear extracts of cells untreated or treated for 2 h with anacardic acid (AA) or TSA.

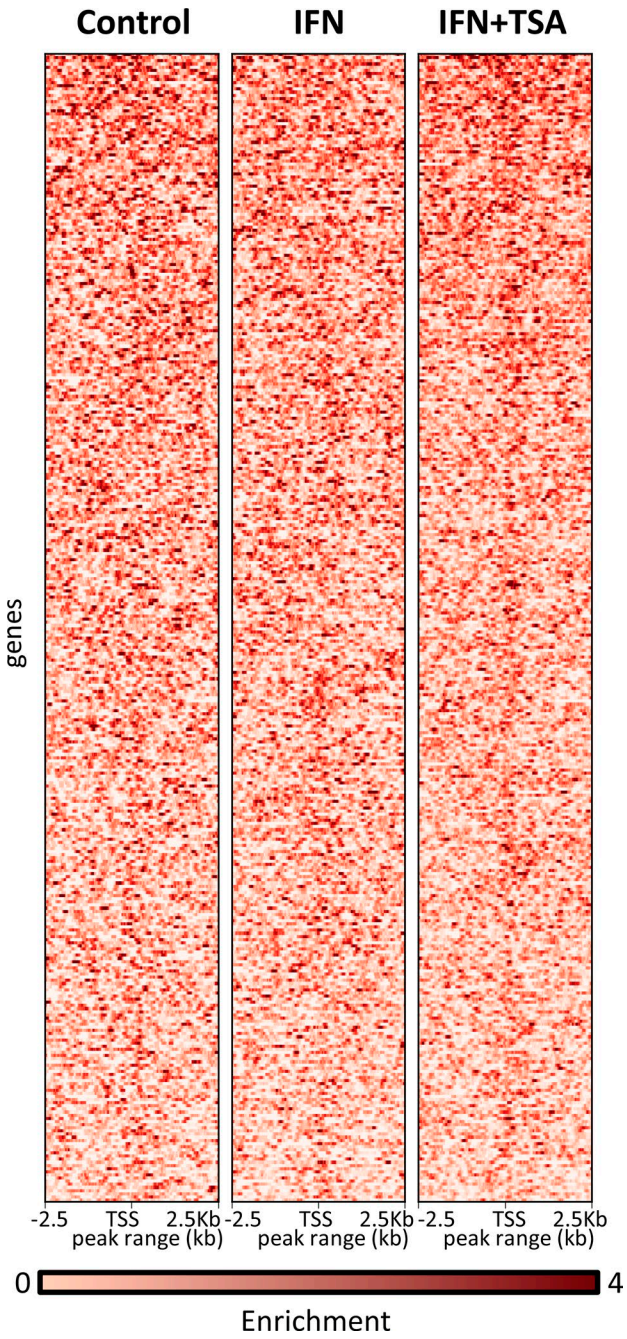

Figure S2. **BRD4 recruitment to control genes is not affected by IFN treatment.** Heat map of BRD4 binding at gene segments ±2.5 kb relative to TSS for genes showing no consistent modulation of BRD4 binding following IFN treatment. Each row represents a unique gene segment.

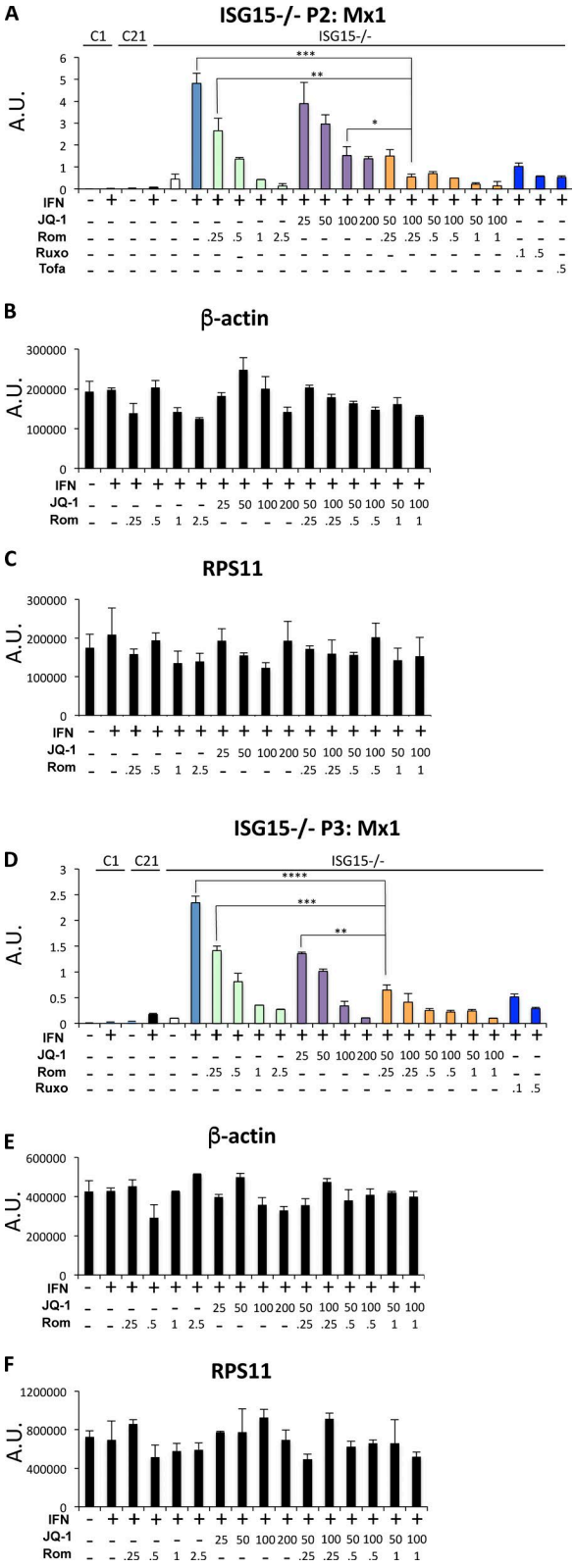

Figure S3. **Analysis of cells from additional normal and ISG15 mutant patients. (A and D)** hTert-immortalized human fibroblasts from two independent ISG15-deficient patients (P1 and P3) and two independent healthy donors (C1 and C21) were treated with IFN-α for 8 h, washed with PBS, and incubated in the absence of IFN for 3 d. Where indicated, cells were treated with JQ-1, romidepsin, or a combination of the two drugs (nM) for the final 24 h before RNA extraction. Ruxolitinib and tofacitinib treatments (μM) were included for comparison. Mx1 mRNA was quantified using real-time RT-PCR and normalized to GAPDH mRNA abundance. **(B and E)** β-Actin mRNA was quantified by real-time RT-PCR. **(C and F)** RPS11 pre-mRNA abundance was quantified by real-time RT-PCR using primers spanning an exon-intron junction. *, P < 0.08; **, P < 0.04; ***, P < 0.02; ****, P < 0.005 for representative experiments (of two) performed in duplicate, and error bars represent +SD. A.U., arbitrary units.

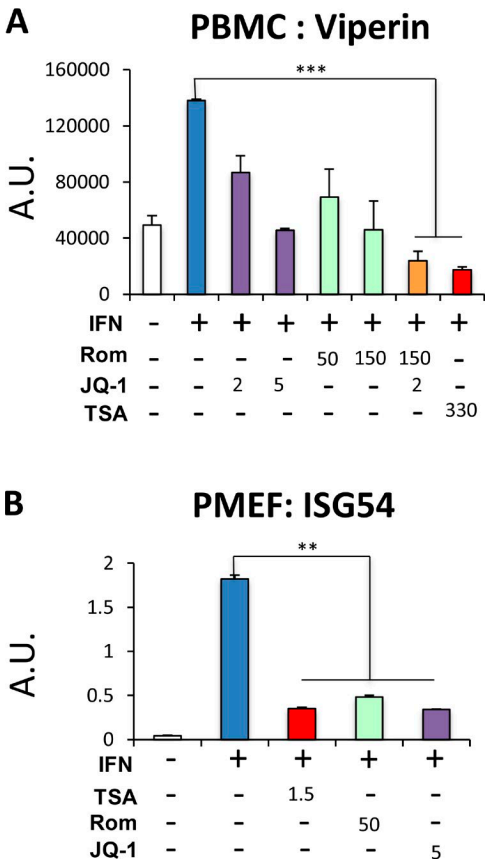

Figure S4. **ISG expression in primary cells analyzed ex vivo is sensitive to HDAC and BRD4 inhibitors. (A)** PBMCs from a healthy donor were treated with 250 U/ml IFN-α for 5 h as indicated along with romidepsin (Rom, nM), JQ-1 (μM), TSA (nM), or a combination of Romidepsin and JQ-1 as indicated, before RNA extraction. Viperin mRNA was quantified using real-time RT-PCR and normalized to GAPDH mRNA abundance. **(B)** Primary mouse embryonic fibroblasts (PMEFs) were treated with 500 U mouse IFN-α for 6 h in combination with TSA (1.5 μM), romidepsin (50 nM), or JQ-1 (5 μM) as indicated, before RNA extraction. ISG54 mRNA was quantified using real-time RT-PCR and normalized to GAPDH mRNA abundance. **, $P < 0.004$; ***, $P < 0.005$ for representative experiments (of two) performed in duplicate, and error bars represent +SD. A.U., arbitrary units.

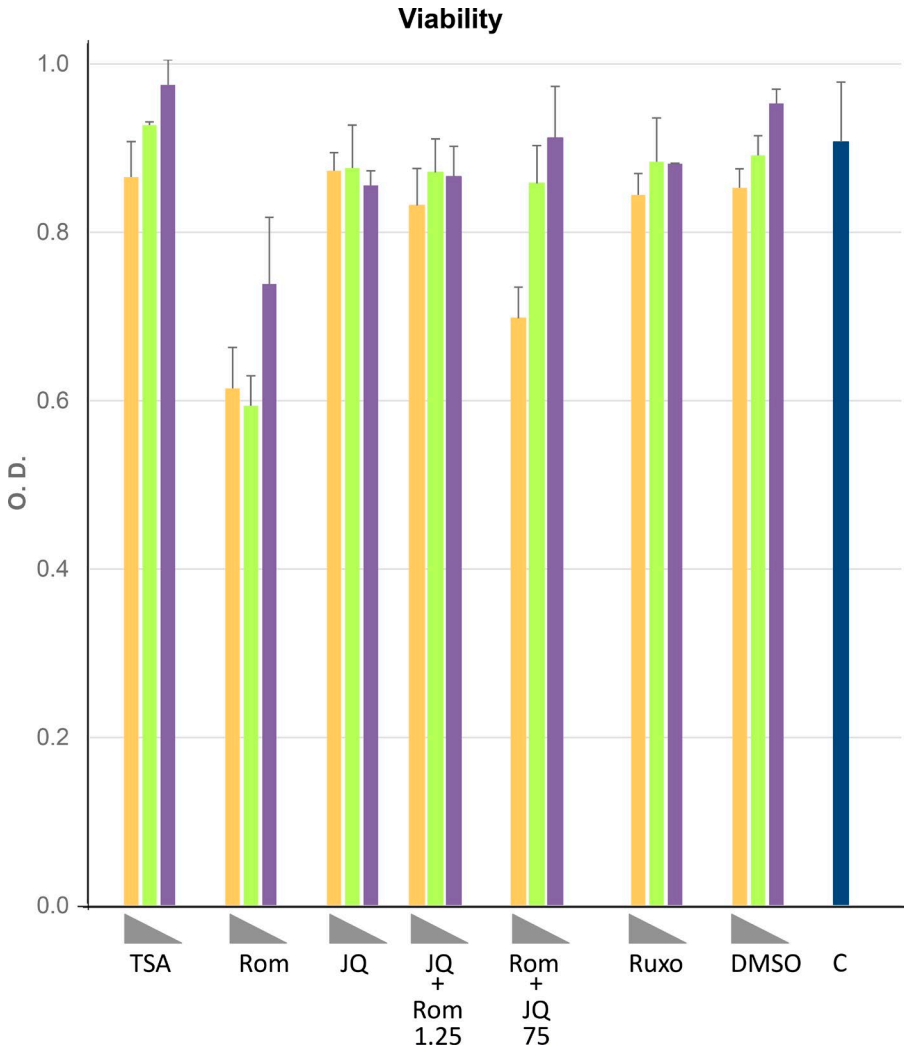

Figure S5. **HDAC and BRD4 inhibitors display minimal toxicity.** hTERT-immortalized normal human fibroblasts were treated with various concentrations of inhibitors for 2 h before measuring total cell viability. Concentrations tested were as follows: TSA, 750, 375, 187.5 nM; romidepsin, 5, 2.5, 1.25 nM; JQ-1, 300, 150, 75 nM; ruxolitinib, 250, 125, 62.5 nM; and DMSO, 5%, 2.5%, 1.25%. Cell viability of triplicate samples was measured by the absorbance at 450 nm after incubation for 1.5 h with the tetrazolium salt WST-8. Data are from a representative experiment (of two) performed in triplicate, and error bars represent +SD.

Tables S1–S4 are provided online as Excel files. Table S1 lists genes associated with IFN-induced ChIP-Seq peaks that were abrogated by HDAC inhibition. Table S2 lists genes associated with "Virus perturbations from GEO up" pathways enriched for IFN-induced ChIP-Seq peaks. Table S3 lists genes associated with "Virus perturbations from GEO down" pathways enriched for IFN-induced ChIP-Seq peaks. Table S4 lists genes regulated by IFN enriched for IFN-induced ChIP-Seq peaks.