Supplemental material

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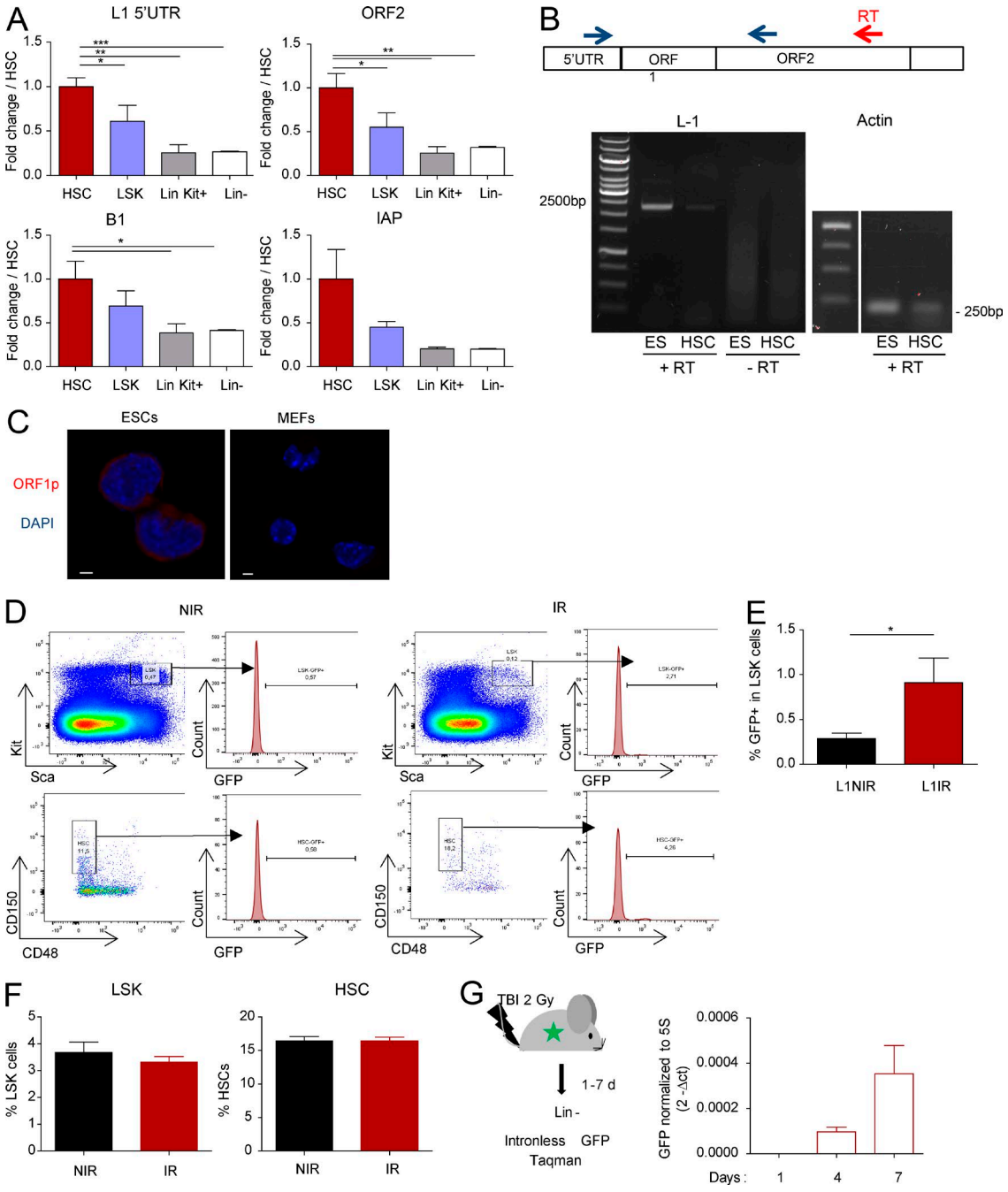

Figure S1. **RE mRNA expression and retrotransposition increase with irradiation. (A)** qRT-PCR analysis of the mRNA expression of LINE, SINE, and IAP elements in HSCs (LSK-CD34$^-$Flk2$^-$) and various hematopoietic populations, as indicated. Values were normalized to the β-actin and Gapdh levels and to the mean value expression in HSCs. *n* = 4 (B1, ORF2), 5 (5′-UTR), and 8 (IAP) mice from three to four independent experiments. One-way ANOVA with multiple comparison test. **(B)** PCR amplification of full-length L1 from HSC- and ESC-purified RNA. The schematic positions of the primers used in the RT and PCR reactions are shown. Amplification without RT treatment and of actin were used as negative and loading controls, respectively. **(C)** Representative images of IF analysis of ORF1p expression in mouse embryonic fibroblasts (MEFs) and ESCs. Bars, 50 µm. **(D)** Gating strategy for the detection of GFP$^+$ LSK and HSCs by FACS analysis in L1-GFP mice, nontreated (NIR), or one 1 mo after TBI (IR). **(E)** GFP$^+$ LSK cells in L1-GFP mice, nontreated and 1 mo after TBI. Means ± SEM, *n* = 8 (NIR) and 9 (IR) mice from two independent experiments. Mann-Whitney test. **(F)** Percentages of LSK and HSCs sorted from L1-GFP mice immediately after TBI (*n* = 3). **(G)** Kinetics of L1 retrotransposition induced by irradiation in L1-GFP mice. Mice were irradiated (2 Gy) and Lin$^-$ progenitor cells were sorted at different times. GFP expression was detected by Taqman assay using exon–exon probe and primers. *n* = 2–3 independent mice/time point. This experiment was performed only one time. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

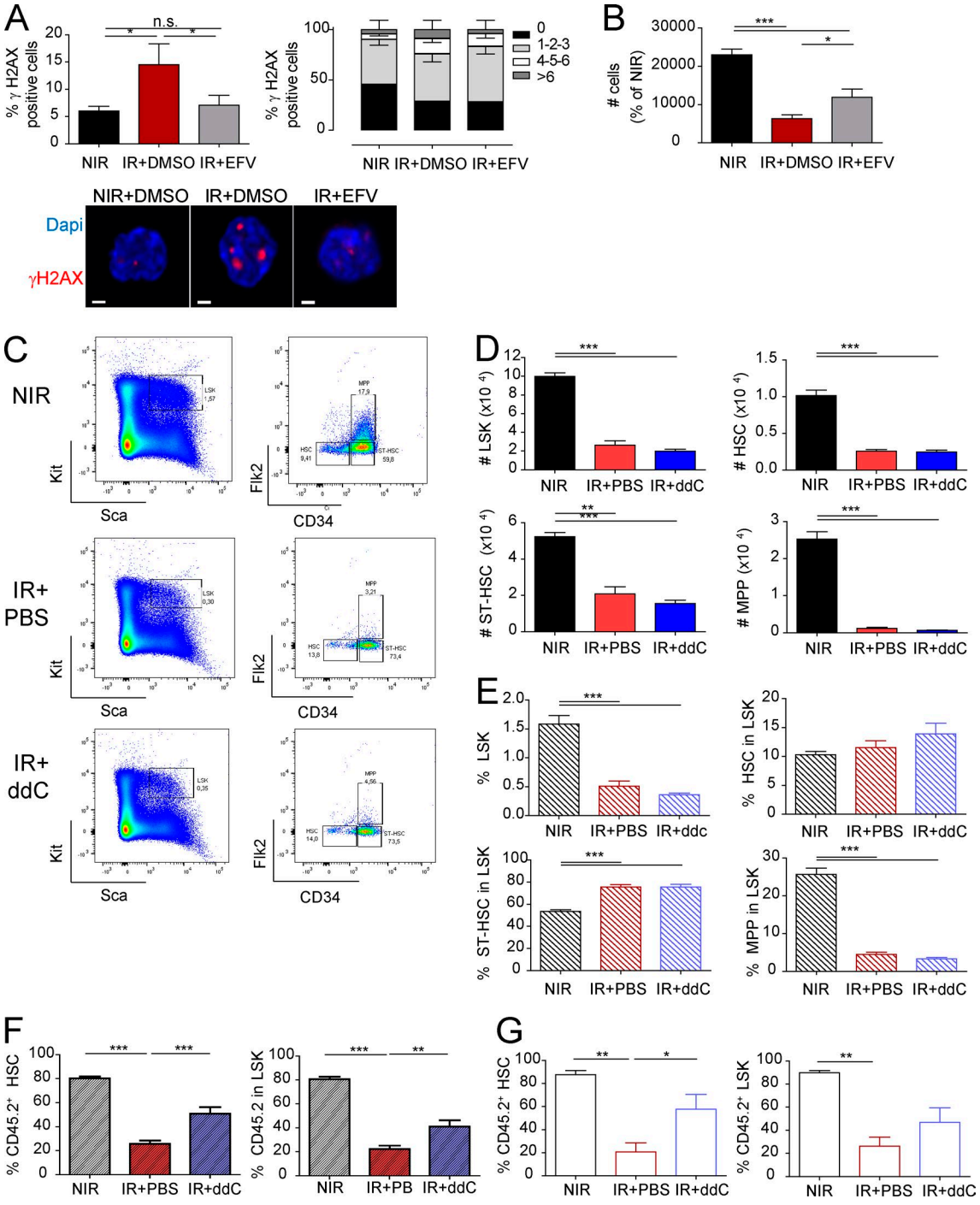

Figure S2. **Reverse transcription inhibitors rescue irradiation-induced HSC loss of function. (A and B)** γH2AX foci and in vitro proliferation of LSK-CD34$^-$Flk2$^-$ HSCs isolated from mice subjected to TBI or not and treated daily for 1 mo with efavirenz (EFV) or DMSO as a control. Means ± SEM, n = 6 (NIR), 4 (IR+DMSO), and 5 (IR+EFV) mice. One-way ANOVA with Dunnett's multiple comparison test. This experiment was performed only once. Bars, 30 µm. **(C–E)** Quantification of HSCs in the bone marrow 1 mo after irradiation and daily injection of ddC or PBS (stage 1, Fig. 3 A). **(C)** Representative FACS analysis for each group. **(D)** Total cell numbers. **(E)** Relative frequencies. Means ± SEM, n = 8 (NIR) and 12 (IR+PBS and IR+ddC) mice from two independent experiments. One-way ANOVA with Dunnett's multiple comparison test. **(F and G)** CD45.2$^+$ donor contribution 4 and 5 mo after primary (F) and secondary reconstitution (G), respectively, with cells from mice irradiated treated with ddC or not as in Fig. 3 A. Means ± SEM. One-way ANOVA with Dunnett's multiple comparison test. **(F)** n = 7 (NIR), 9 (IR+PBS), and 10 (IR+ddC) mice from two independent experiments. **(G)** n = 4 (NIR and IR+PBS) and 5 (IR+ddC). Representative independent experiment out of two performed. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

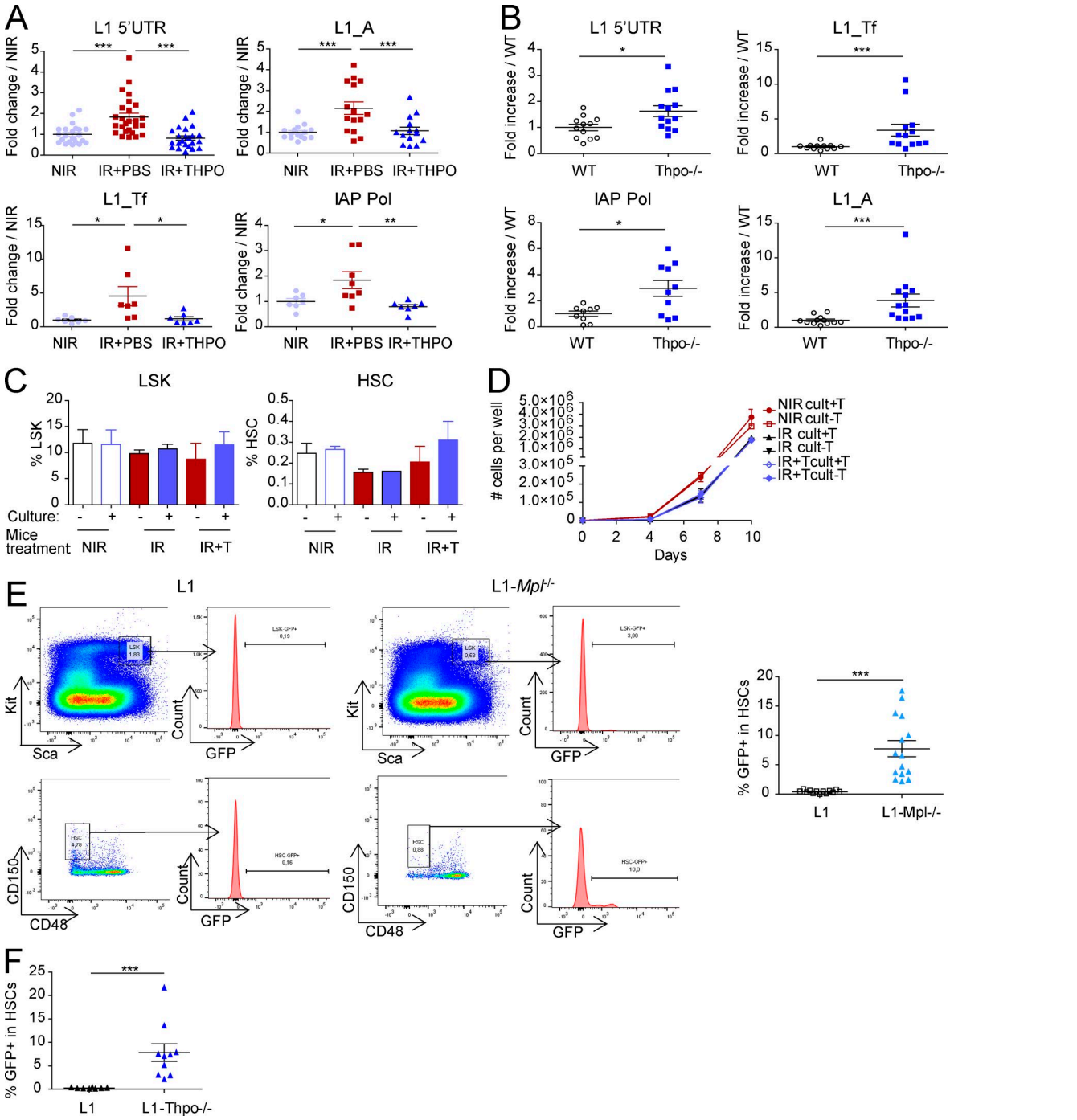

Figure S3. **THPO signaling restrains L1 expression and retrotransposition in HSCs in the absence of irradiation. (A)** RE mRNA expression in LSK cells isolated 1 mo after 2 Gy TBI with (IR+THPO) or without (IR+PBS) THPO injection or nontreated (NIR). Results represent means ± SEM and are expressed as fold change from the NIR mean value after normalization. Each dot represents an individual mouse. Data are pooled from three to four independent experiments. One-way ANOVA with Dunnett's multiple comparison test. **(B)** qRT-PCR analysis of RE expression in LSK cells from WT and *Thpo*$^{-/-}$ mice. Results are normalized to the mean values obtained with WT cells. Each dot represents an individual mouse. Mean ± SEM from two to four independent experiments. Mann-Whitney test. **(C and D)** Percent of HSCs and LSK cells measured by FACS analysis at day 10 (C) and total cell counts (D) in in vitro cultures of L1-GFP HSCs isolated immediately after TBI with or without THPO injection and cultured in the presence (+T) or absence of THPO (−T). Means ± SEM of two to three independent cultures. **(E)** Representative gating strategy and quantification of retrotransposition detected by FACS analysis in HSCs (LSK-CD48^{-}CD150^{+}) from L1-GFP and L1-*Mpl*$^{-/-}$ mice. Means ± SEM from four independent experiments. Mann-Whitney test. **(F)** Retrotransposition detected by FACS analysis in HSCs (LSK-CD48^{-}CD150^{+}) from L1-GFP and L1-*Thpo*$^{-/-}$ mice. Each dot represents an individual mouse. Means ± SEM from two independent experiments. Mann-Whitney test. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

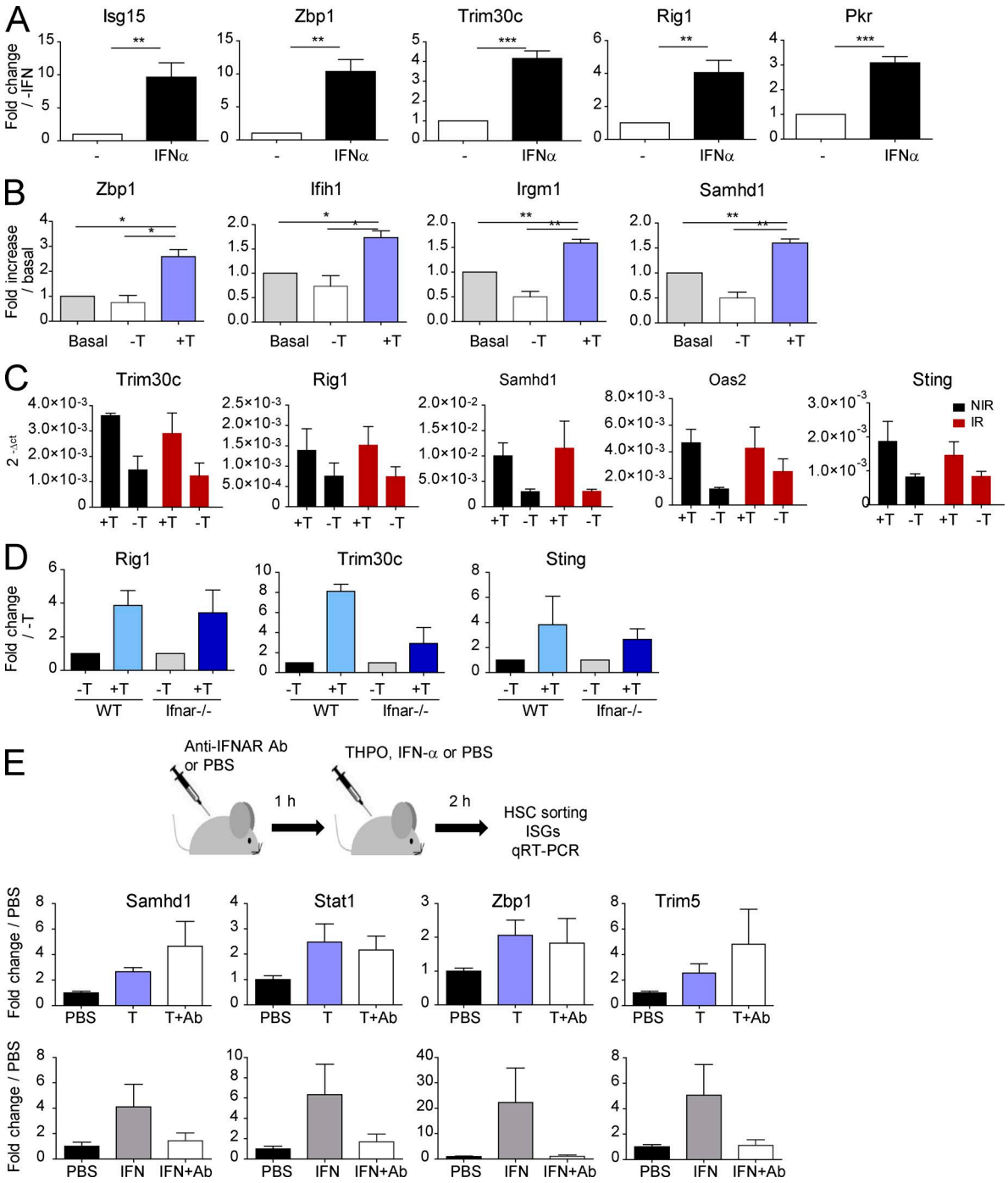

Figure S4. **THPO induces IFN-regulated genes in HSCs. (A)** qRT-PCR analysis of ISG expression in LSK cells cultured for 90 min in a medium containing or not containing 100 ng/ml IFN-α. Ct values were normalized to β-actin and/or Gapdh. Results are expressed as fold change from cells isolated from the same mice and cultured without IFN-α. Means ± SEM. *n* = 5 (Rig1 and Pkr), 7 (Trim30c, Zbp1, and IRF7), 10 (Isg15, Oas2, and Ifi44) mice from two to three independent experiments; paired *t* test. **(B)** qRT-PCR analysis for THPO–up-regulated genes in HSCs just after sorting (Basal) or stimulated for 90 min in vitro in medium containing cytokines with or without THPO as in Fig. 6 A. Data normalized to the mean values of expression in unstimulated cells. Means ± SEM; *n* = 3 pools of 5 mice in two independent experiments. Repeated measure ANOVA with multiple comparison test. **(C)** ISG analysis in HSCs treated as in Fig. 5 A. Means ± SEM of Ct values obtained from three pools of six to eight mice, representative experiment out of two performed. **(D)** THPO-induced ISGs in *Ifnar*$^{-/-}$ HSCs. HSCs from WT and *Ifnar1*-deficient mice were incubated for 90 min in vitro in medium supplemented with (+T) or without (−T) before qRT-PCR analysis. Means ± SEM. *n* = 4–5 pools of five mice in two independent experiments. **(E)** Experimental design for testing the effect of anti-IFNAR blocking antibodies on ISG induction by THPO or IFN-α in vivo. Top: *n* = 7–10 mice from two to three experiments. Bottom: *n* = 3 mice from a representative experiment. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

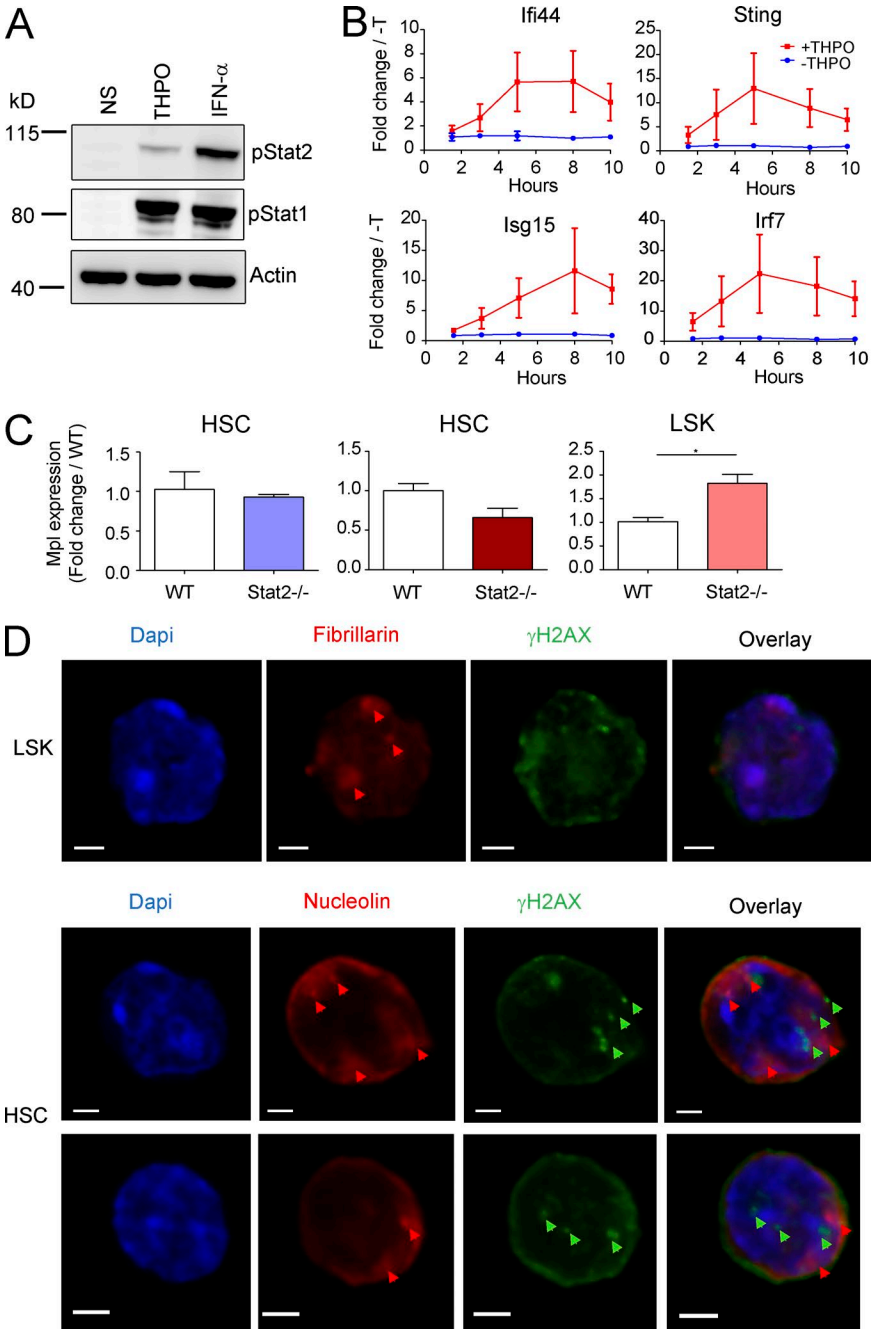

Figure S5. **THPO induces IFN type-I signaling in UT7-Mpl cells. (A)** Human UT7-Mpl cells were stimulated for 10 min with THPO peptide (10 nM) or IFN-α (50 ng/ml) before lysis and Western blot analysis. Representative experiment out of two performed. **(B)** qRT-PCR analysis of ISG induction in UT7-Mpl cells stimulated with 3 nM THPO peptide or not. Means ± SEM from two independent experiments. **(C)** qRT-PCR analysis for Mpl mRNA expression in HSCs (LSK-CD34$^-$Flk2$^-$) or LSK cells isolated from WT or *Stat1*- or *Stat2*-deficient mice. Results are expressed as fold change from the mean $2^{-\Delta CT}$ values of WT mice. n = 2 (*Stat1*$^{-/-}$ HSC) and 4 (*Stat2*$^{-/-}$ HSC and LSK) pools of six mice from two to three independent experiments. Unpaired t test. *, P < 0.05. **(D)** Long-lasting TBI-induced γH2AX foci do not colocalize with the nucleolus. LSK cells and HSCs were sorted 1 mo after TBI and stained with mouse anti-γH2AX and rabbit anti-nucleolin (Ab22758; Abcam) or fibrillarin (2639; Cell Signaling Technolgy), as indicated. The slides were counterstained with Dapi analyzed using a confocal microscope. Arrowheads indicate the position of the nucleolus (red) and γH2AX foci (green). Bars, 30 µm.

Tables S1 and S2 are provided online as Excel files. Table S1 shows up- and down-regulated genes differentially expressed in THPO stimulated HSCs (fold change > 1.5; P > 0.05). Table S2 describes the primers used in this study.