Inventory of supplemental materials

Supplemental figure legends

Figure S1 (related to Figure 1). Gating strategies and quality control metrics for single cell RNA-seq analyses in Figure 1. (A) Gating strategies for HSCs and HPCs are shown. Representative flow cytometry plots are provided for each age. The plots show c-kit selected cells. (B, C) Transcript counts as unique molecular identifiers (UMI) and numbers of genes identified per cell for HSC analyses in Figure 1. (D, E) Transcript counts as UMI and numbers of genes identified per cell for HPC analyses in Figure 1. (F) Reactome pathway analysis for the adult identity genes. (G, H) GFP+ HSC and HPC chimerism in recipients of EGFP control, IGFBP1-2A-EGFP or IGFBP2-2A-EGFP HSCs at 16 weeks after transplantation. n=10-18, **p<0.01, ***p<0.001 by two tailed Student's t-test.

Figure S2 (related to Figure 1). Quadratic programming reveals a linear, gradual, global transition from fetal to adult identity. (A, B) Adult identity scores for HSCs and HPCs when quadratic programming was performed with all expressed genes. (C, D) Distribution of total absolute deviation of all genes (green) or non-randomly selected genes (purple). Dashed vertical lines indicated the thresholds for selecting low and high variability genes for random quadratic programming. (E) Average adult identity scores in HPCs using randomly selected low or high variability genes.

Figure S3 (related to Figure 2). Fetal identity genes are inactivated, and adult genes are activated, in an uncoordinated manner. Additional dot plots are shown for

representative fetal and adult identity genes. (A, B) Fetal identity genes *Hmga2* and *Arid3a* are expressed in subsets of HSCs and HPCs that have high adult identity scores. (C, D) Adult identity genes *H2-Q7* and *Sdsl* are expressed in subsets of HSCs and HPCs that have low adult identity scores. (E, F) The fractions of cells that express *Hmga2* and *Arid3a* decline with age. (G, H) The fractions of cells that express *H2-Q7* and *Sdsl* increase with age. (I) Heat map showing the 20 most differentially expressed adult and fetal identity genes in individual HSCs (columns). For each adult identity score quintile, shown at the bottom of the plot, the 100 HSCs with the highest UMI scores are shown in order of increasing adult identity. Ages for each HSC are shown at the top of the heatmap.

Figure S4 (related to Figure 5). Quality control metrics for single cell RNA-seq analyses in Figure 5. (A-D) Transcript counts as UMI and numbers of genes identified per cell for single cell RNA-seq analyses in Figure 5. HSC results are shown in A and B, HPC results are shown in C and D. (E, F) Canonical correlation analysis was used to merge E16.5 and P7 HPC expression data from the first and second single cell RNA-seq experiments (Figures 1 and 5, respectively). There were strong correlations in average gene expression levels between the two experiments. (G, H) t-SNE plots are shown for the merged HPC data at E16.5 and P7. Identically-aged HPCs do not segregate based on experiment.

Figure S5 (related to Figure 6). A late perinatal type I interferon spike promotes adult identity gene expression. (A) Heatmap showing expression of type I interferon target genes (from Figure 6A) in HPCs at E16.5, P0, P7, P14 and 8 weeks after birth. (B) Fold changes for four representative genes, *Irf7*, *Ifi27l2a, Ifit1* and *Ifit3*, are shown to the right. Data reflect averages from 4 independent biological replicates per age. (C) IFNα expression in sera from pregnant mothers at E14.5 and E18.5. As controls, sera were collected from non-pregnant adult mice treated with vehicle or poly-inosine:poly-cytosine (pIpC). n=3-5 per group. ***p<0.001; two-tailed Student's t-test; error bars reflect standard deviations. (D, E) HSC and HPC numbers in livers from standard "SPF" and germ free "GF" fetal mice at the indicated ages. (F) Venn diagram indicating overlap of genes downregulated in *Ifnar*$^{-/-}$ HSCs and HPCs at P0. (G, H) Transcript counts as UMI and numbers of genes identified per cell for scRNA-seq analyses in Figure 6. (I) Adult HPC ATAC-seq peaks from Figure 3A were reanalyzed in P7 wild type and *Ifnar*$^{-/-}$ HPCs. Aggregate peak heights were lower in *Ifnar*-deficient HPCs, as indicated in the histogram.

Figure S6 (related to Figure 7). The prenatal interferon pulse promotes HPC expansion while sparing HSCs.

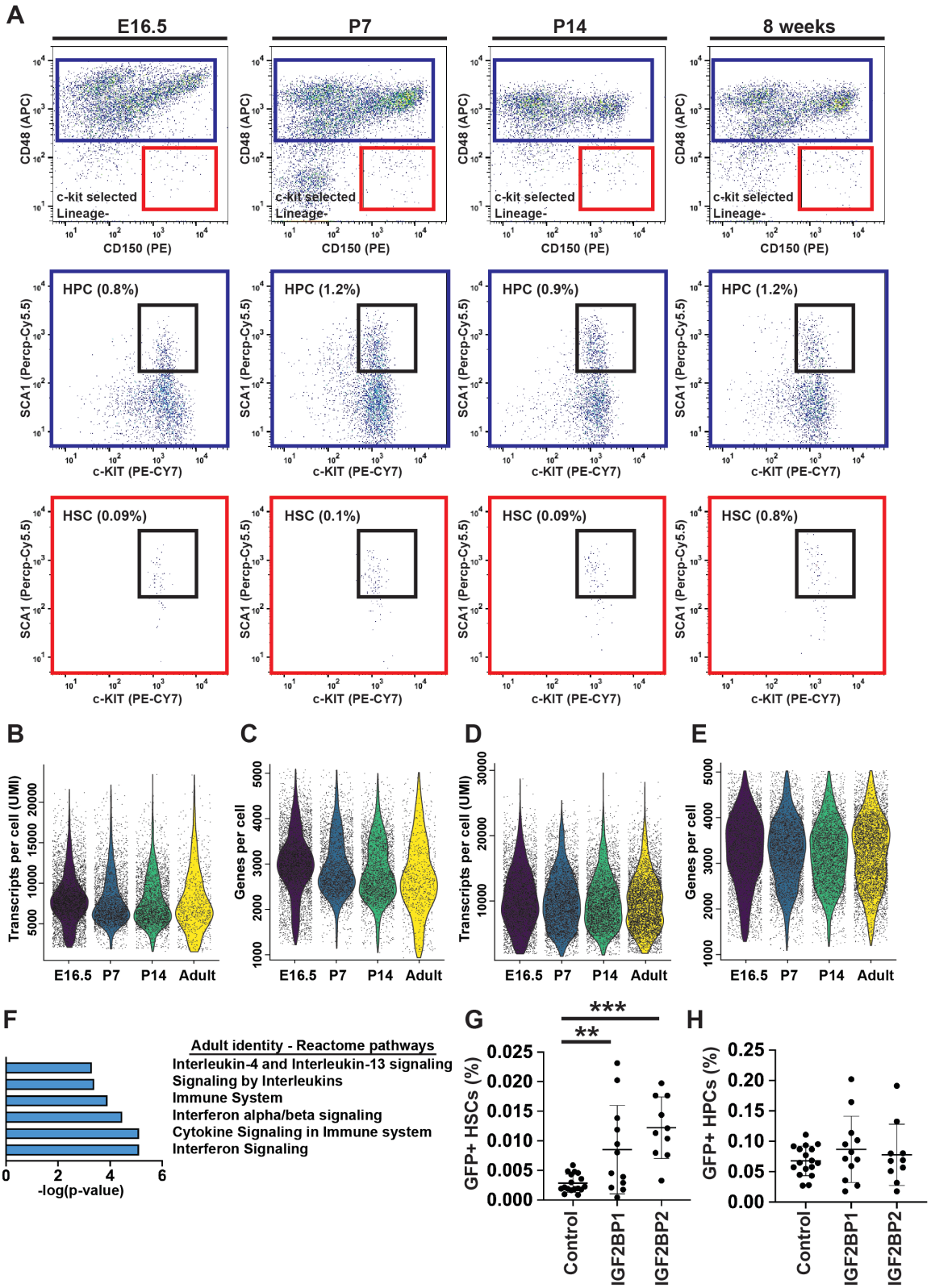
(A-C) Reconstitution of peripheral blood myeloid cells, B-cells and T-cells after transplantation of 20 wild type or *Ifnar*$^{-/-}$ P0 HSCs and 300,000 competitor bone marrow cells. (D) Peripheral blood leukocyte reconstitution after transplantation of 20 wild type or *Ifnar*$^{-/-}$ P14 HSCs and 300,000 competitor bone marrow cells. (E) Twenty-four-hour BrdU incorporation in HSCs from P7 wild type and *Ifnar*$^{-/-}$ mice. N=6-7 per genotype. (F)

Percentages of CD45.2 bone marrow cells, spleen follicular B-cells and peritoneal B1a B-cells in recipients of P14 wild type or *Ifnar*$^{-/-}$ HSCs. Donor chimerism was measured 8 weeks after transplantation. n=12-13 recipients per donor genotype from independent donors per genotype. (G-I) Frequencies of HPC-2, pGM and GMPs in wild type and *Ifnar*$^{-/-}$ mice at the indicated ages. n=5-8 per genotype and age. For all panels, error bars indicate standard deviations. **p<0.01, ***p<0.001 as calculated by Student's t-test with Holm-Sidak post-hoc test to correct for multiple comparisons.

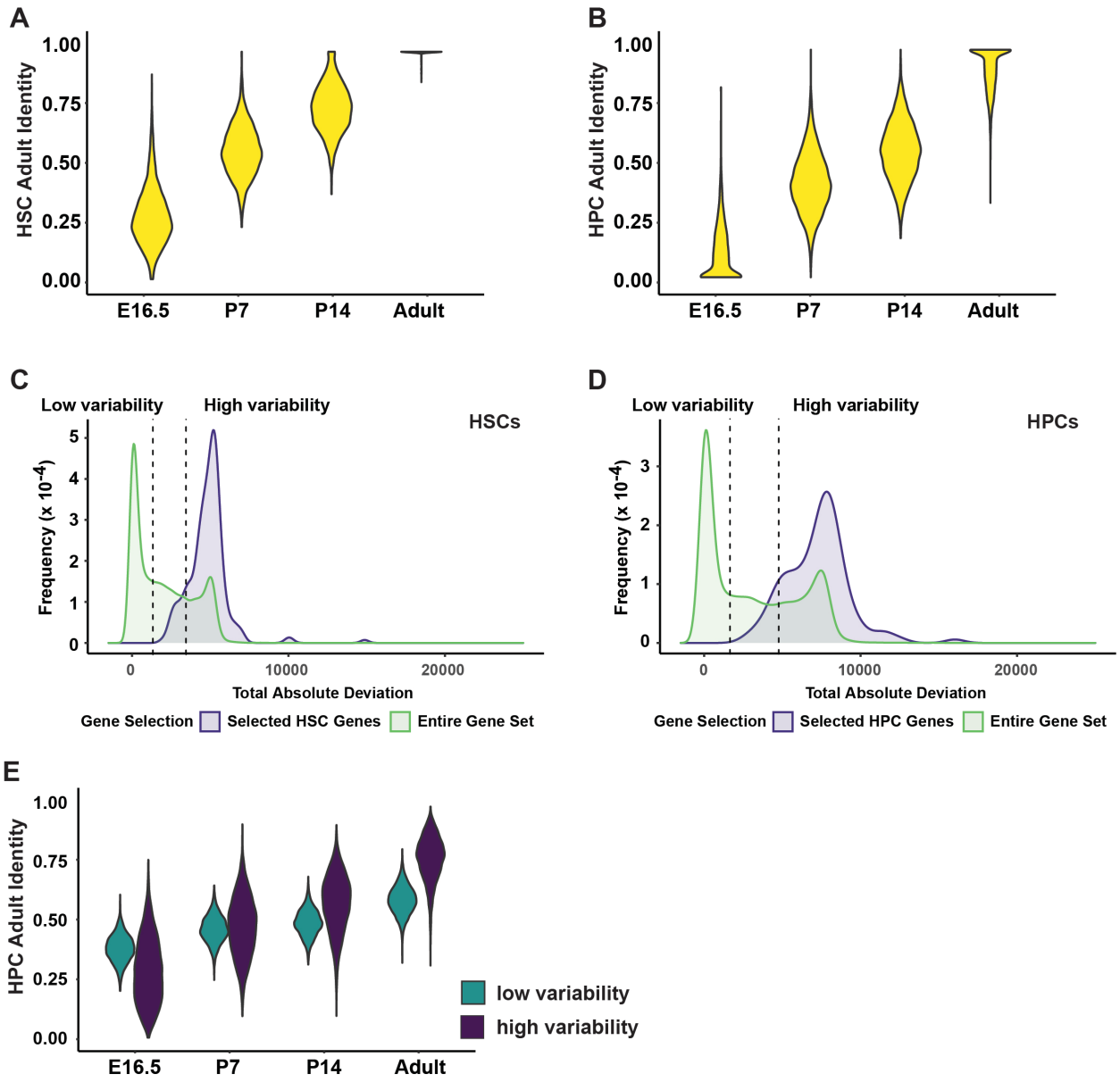
Figure S7 (related to Figure 7). Interferon signaling drives expression of MHC-I complex genes while sparing lineage commitment in neonatal hematopoietic progenitors.

(A, B) Detected gene and transcript levels (UMI) in the scRNA-seq experiments from Figure 7. (C) Cluster specific expression of differentiation markers in adult Lineage^{-}c-kit^{+} cells. These expression patterns were used to annotate clusters identified by Seurat analysis, as shown on the y-axis. Genes are listed on the x-axis. Circle size corresponds to increased expression of the indicated gene within the indicated cluster. (D) Heat demonstrating clusters generated by ICGS analysis of adult wild type and *Ifnar*$^{-/-}$ Lineage^{-}c-kit^{+} cells. The genotypes of each cell/column are indicated, and each cluster is populated by both genotypes. (E, F) UMAP plot indicating clusters generated by ICGS (F) and annotated based on lineage-specific markers (F). (G, H) Distribution of wild type "WT" and *Ifnar*$^{-/-}$ "Ifnar ko" cells within the adult clusters. (I-L) expression of H2-D1 in adult wild type and *Ifnar*$^{-/-}$ Lineage^{-}c-kit^{+} cells. Similar patterns were observed for other MHC-1 genes (e.g. *H2-K1*, *B2m*).

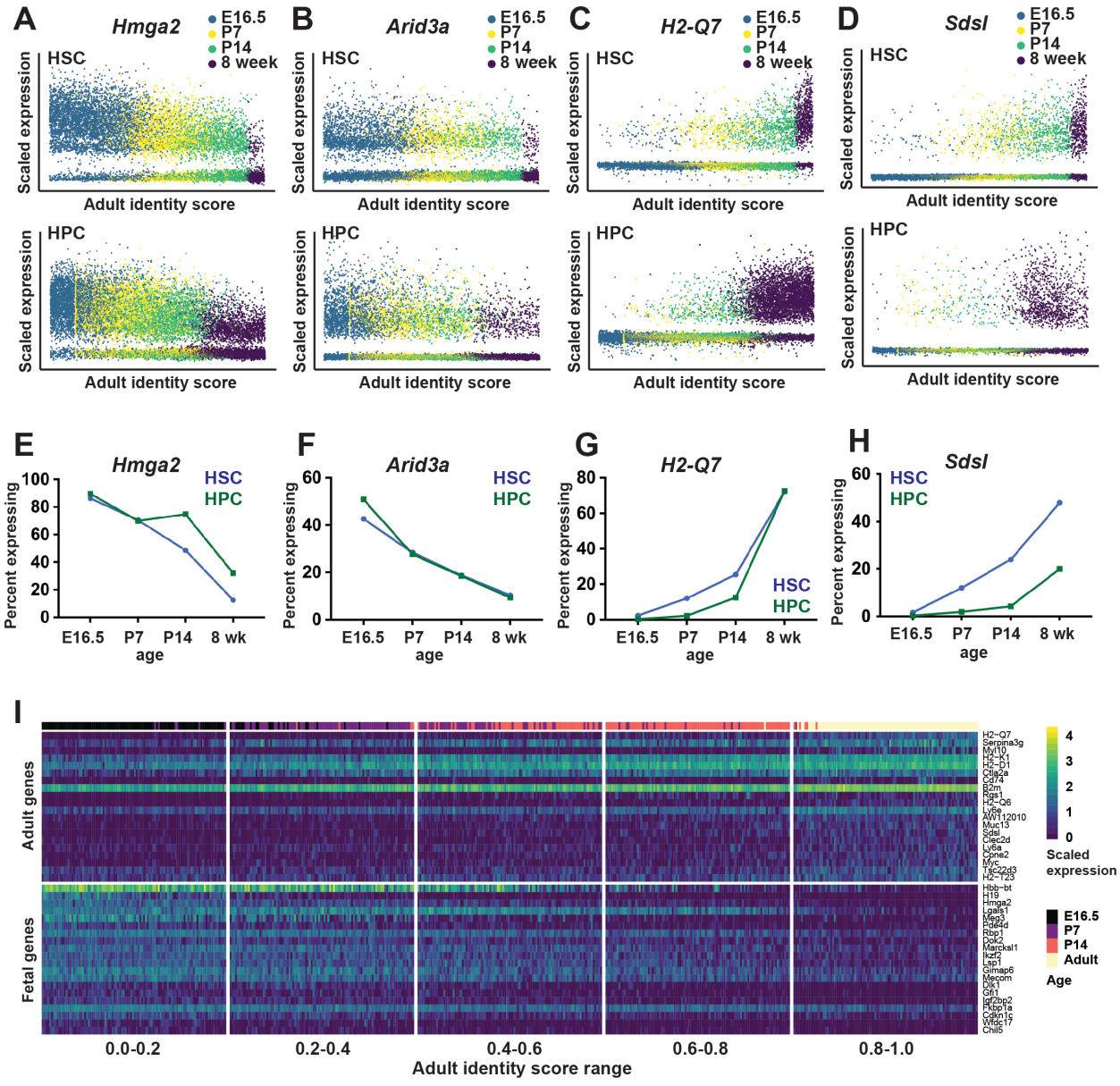
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



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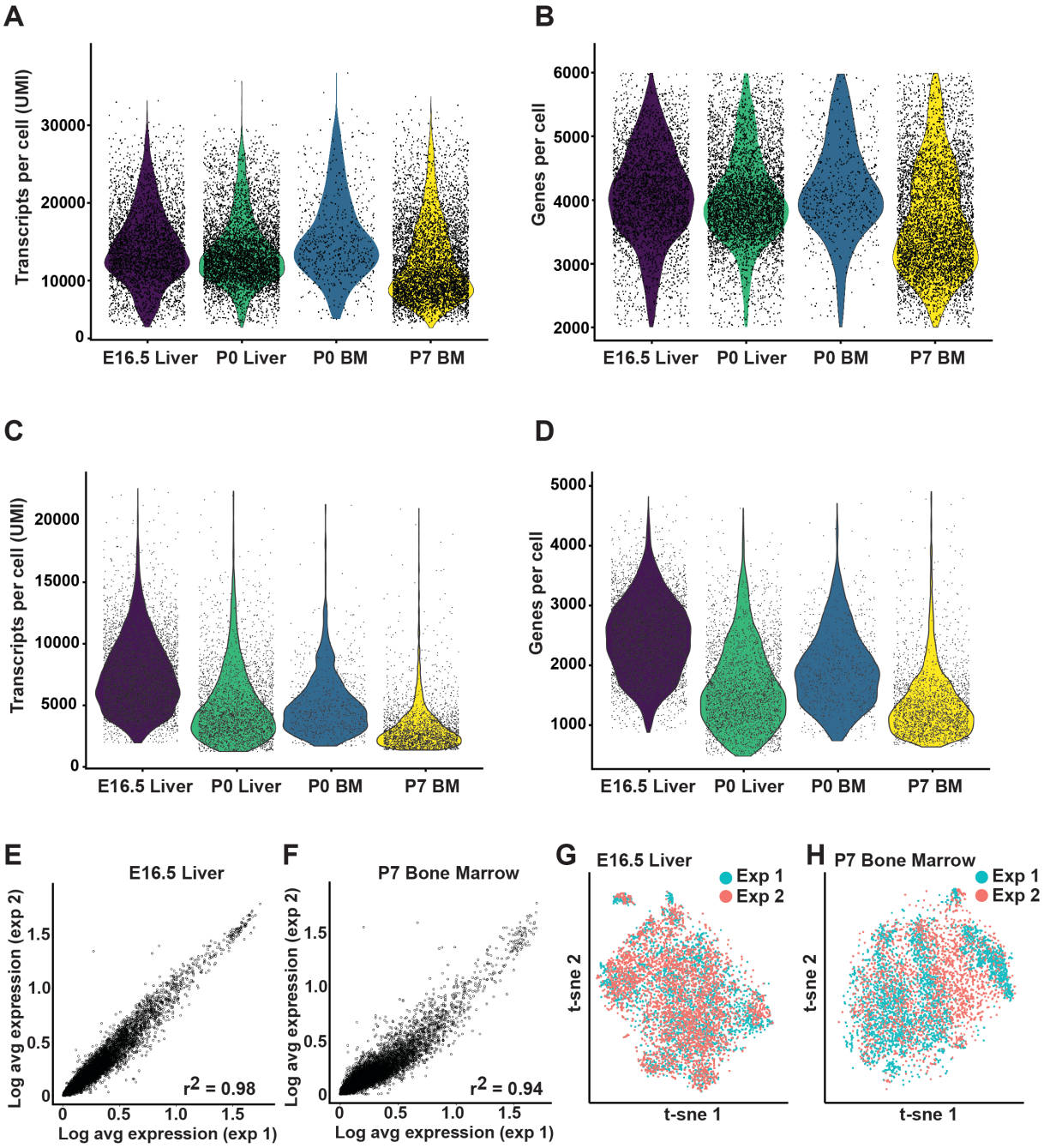

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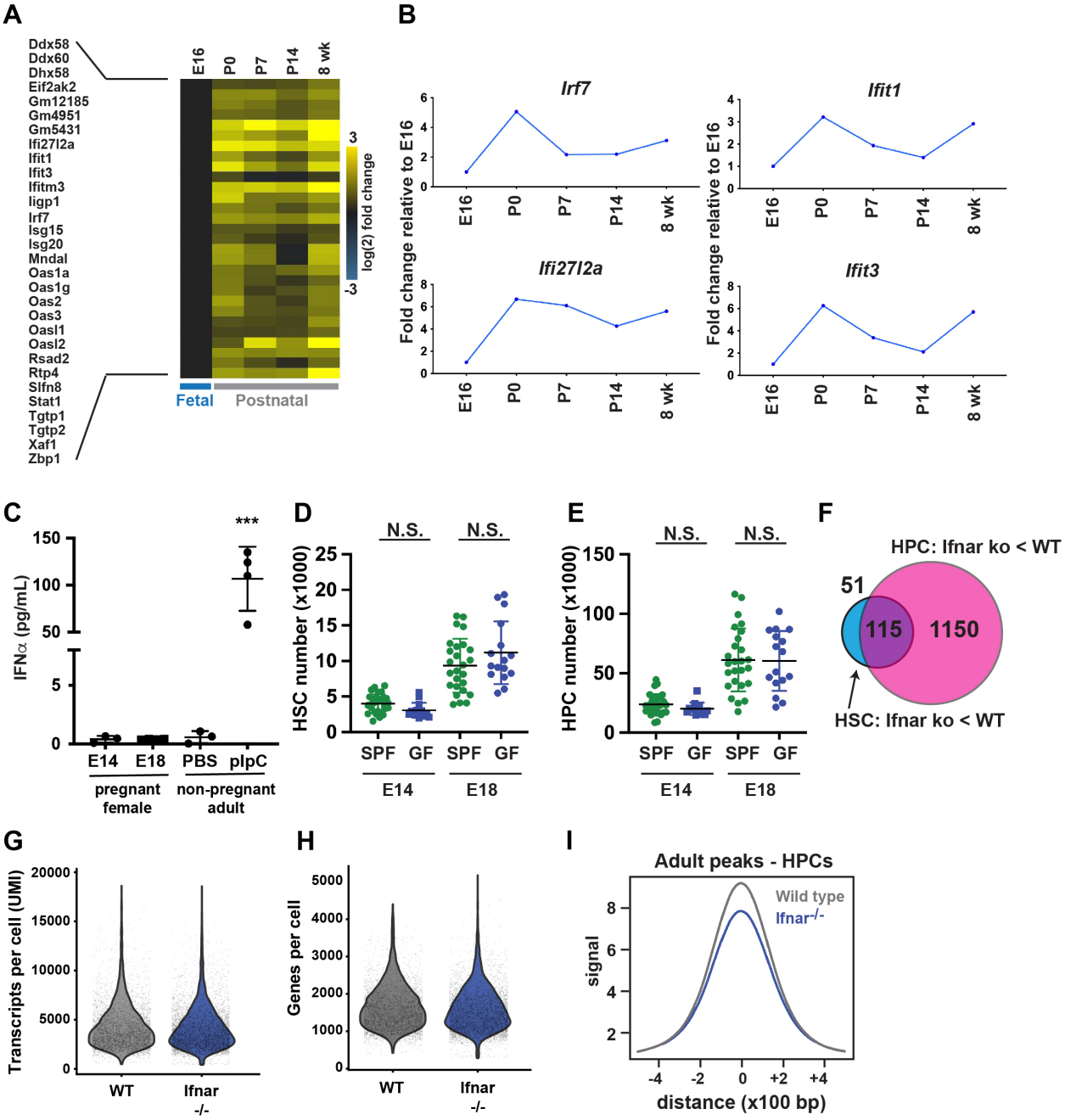
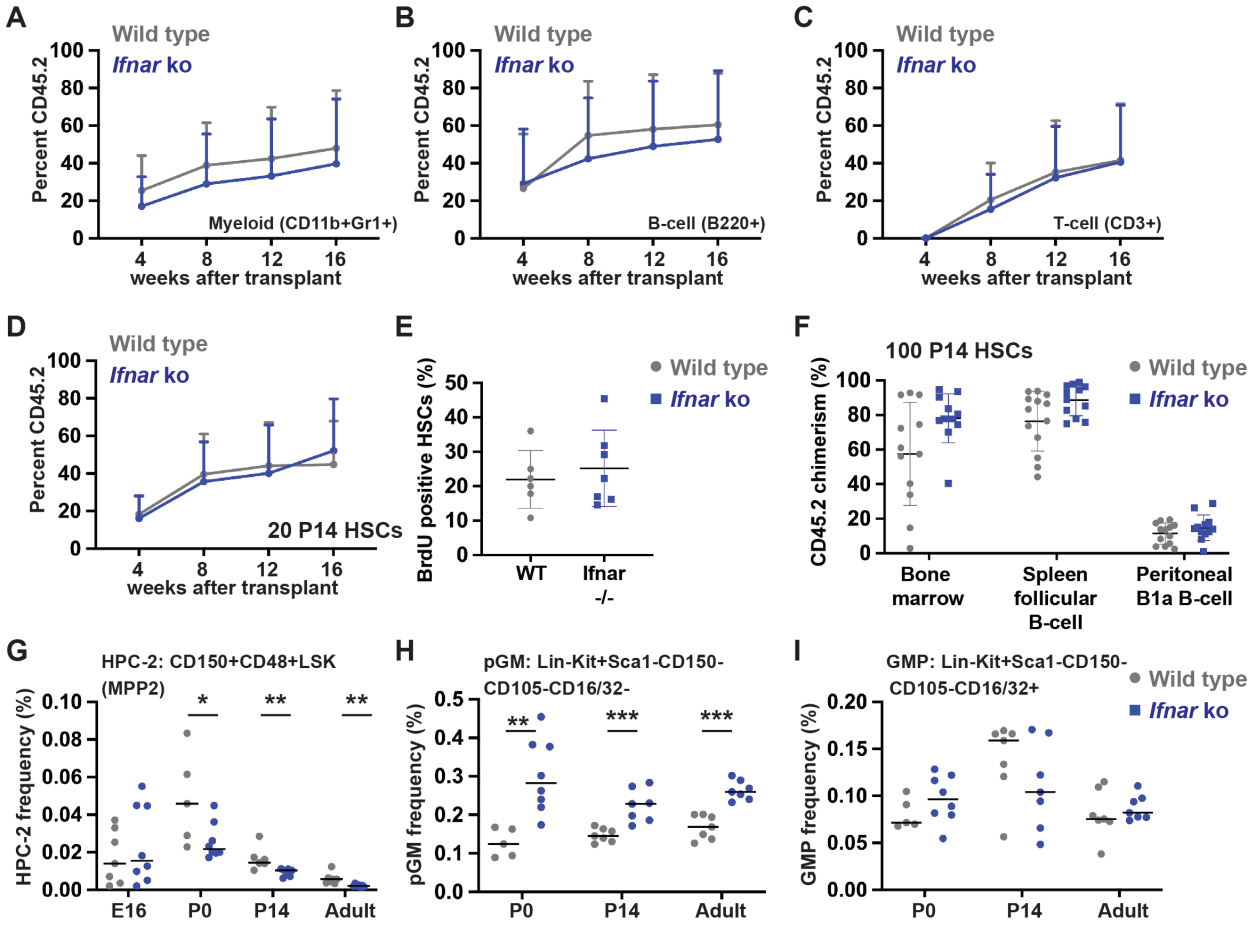


Supplemental Figure S6



Supplemental Figure S7