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Supplemental information

HSC-independent definitive hematopoiesis persists into adult life

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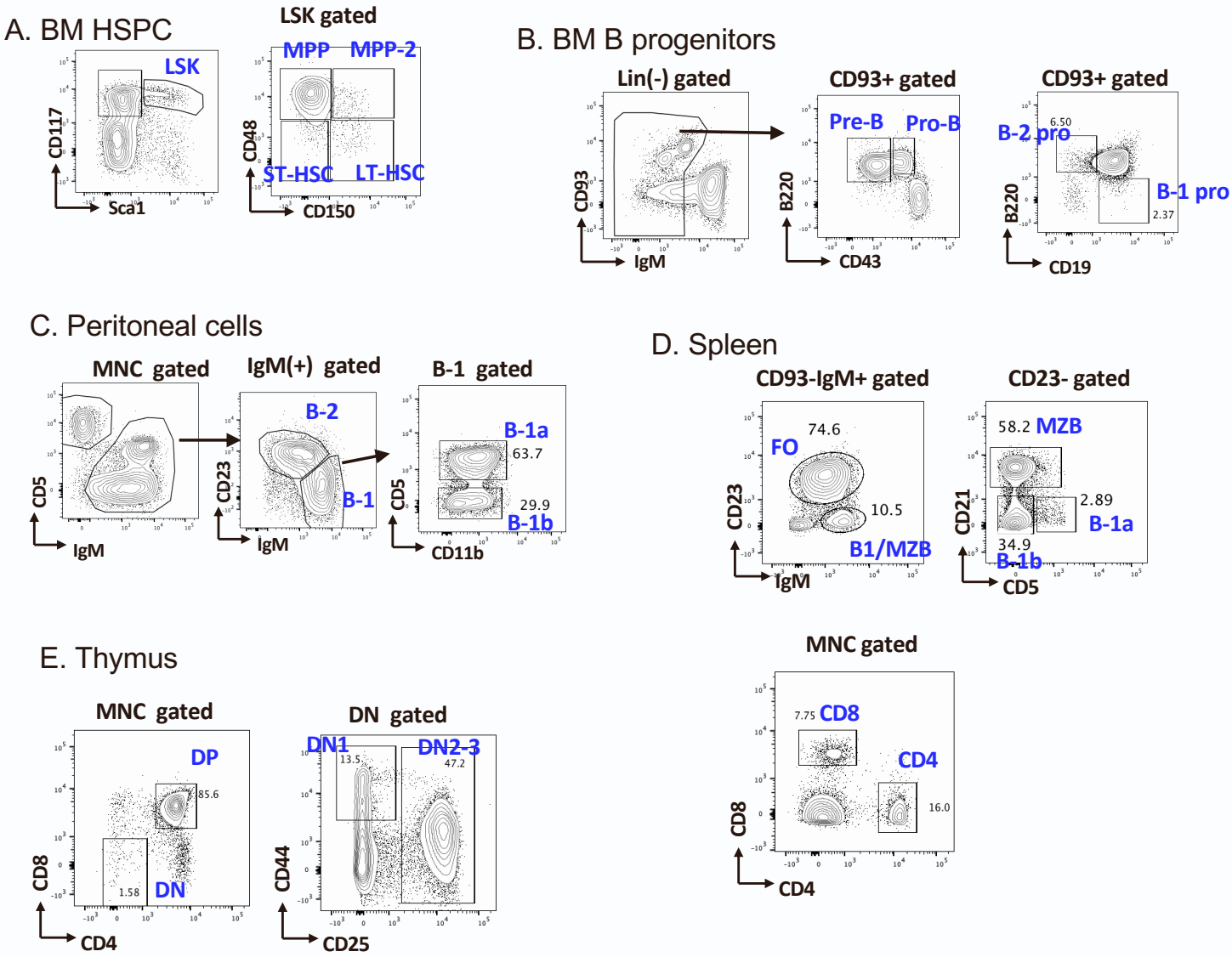


Figure S1 Gating strategies of hematopoietic subsets for lineage tracing. Related to Fig. 1 & 2

Representative FACS plots. (A) BM HSPC (B) BM B-progenitors, (C) peritoneal B cell subsets, (D) spleen B cell subsets and CD4, CD8 T cells, and (E) thymus DP and DN T cells.

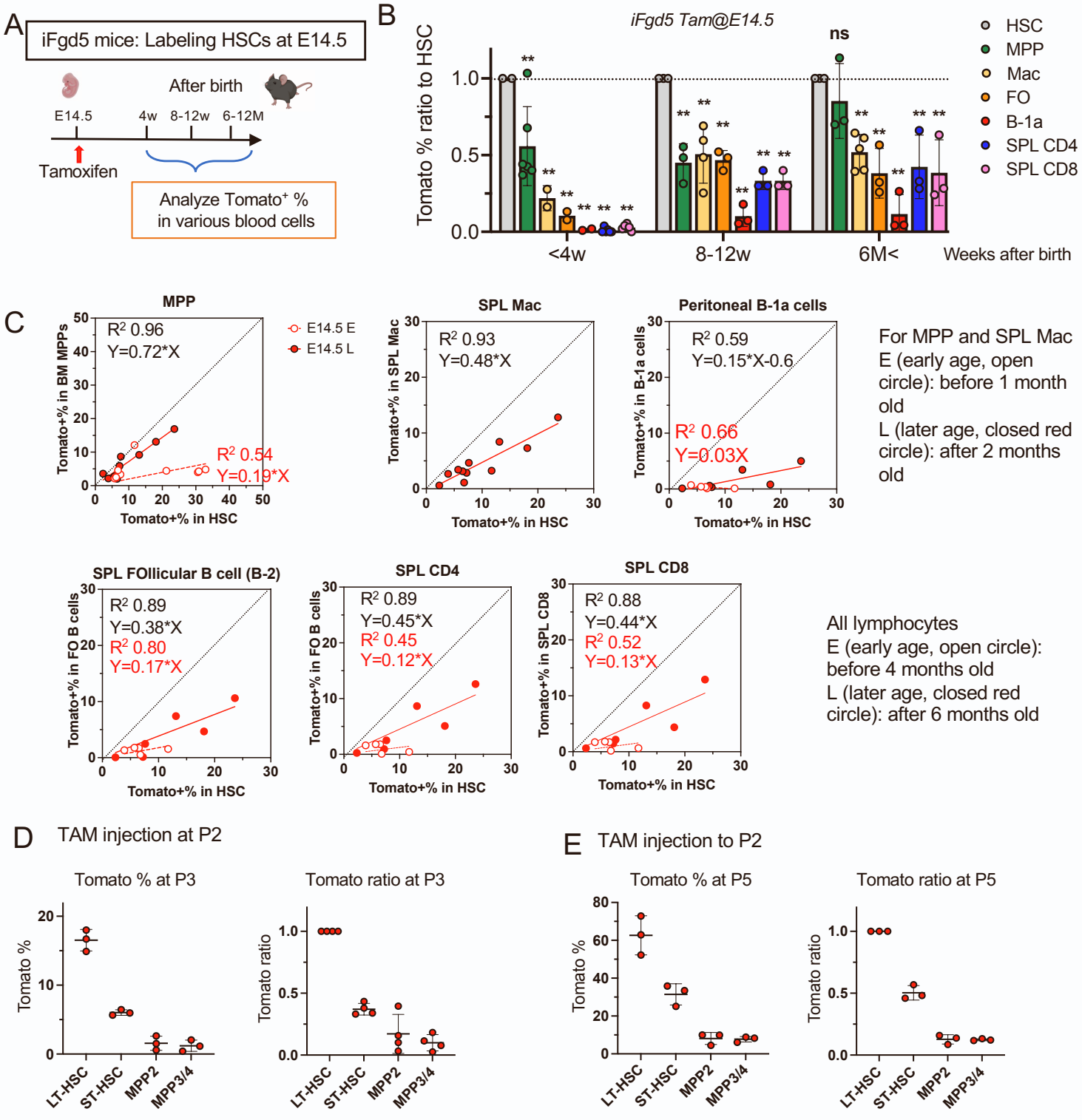


Figure S2 HSC-lineage study revealed HSCs do not fully contribute to lymphopoiesis even in the adult at 6 months of age. Related to Fig. 1 and Discussion

(A) Experimental design. TAM was injected into iFgd5 pregnant mice at E14.5 and pups were analyzed at 4, 8-12 weeks, and 6-12 months after birth. (B) The relative Tomato% ratios of MPP, spleen macrophages, B cell subsets, CD4, and CD8 T cells to LT-HSCs at different time points after birth are depicted when TAM was injected at E14.5 BM: bone marrow, SPL: spleen, PW: peritoneal wash. (C) Fate mapping scatter plots for BM MPPs, spleen macrophages, FO B cells, CD4, CD8 T cells and peritoneal B-1a cells when HSCs are labeled at E14.5. Blue dots indicate that mice were analyzed within 4 months old (N=5-10) and red dots indicate that mice were analyzed after 6 months old (N=6-10). Dots of spleen macrophages include all time points because all the dots showed correlations. Tomato % and Ratio of LT-HSC, ST-HSC, and MPP in the BM at P3 (D) and P5 (E) when TAM was injected into P2 iFgd5 neonates.

A iCdh5 mice analyzed (total 97 mice)

	E7.5	E8.5	E9.5	E10.5	E11.5
<4M	22	25	9	7	6
>6M	11	3	3	5	6

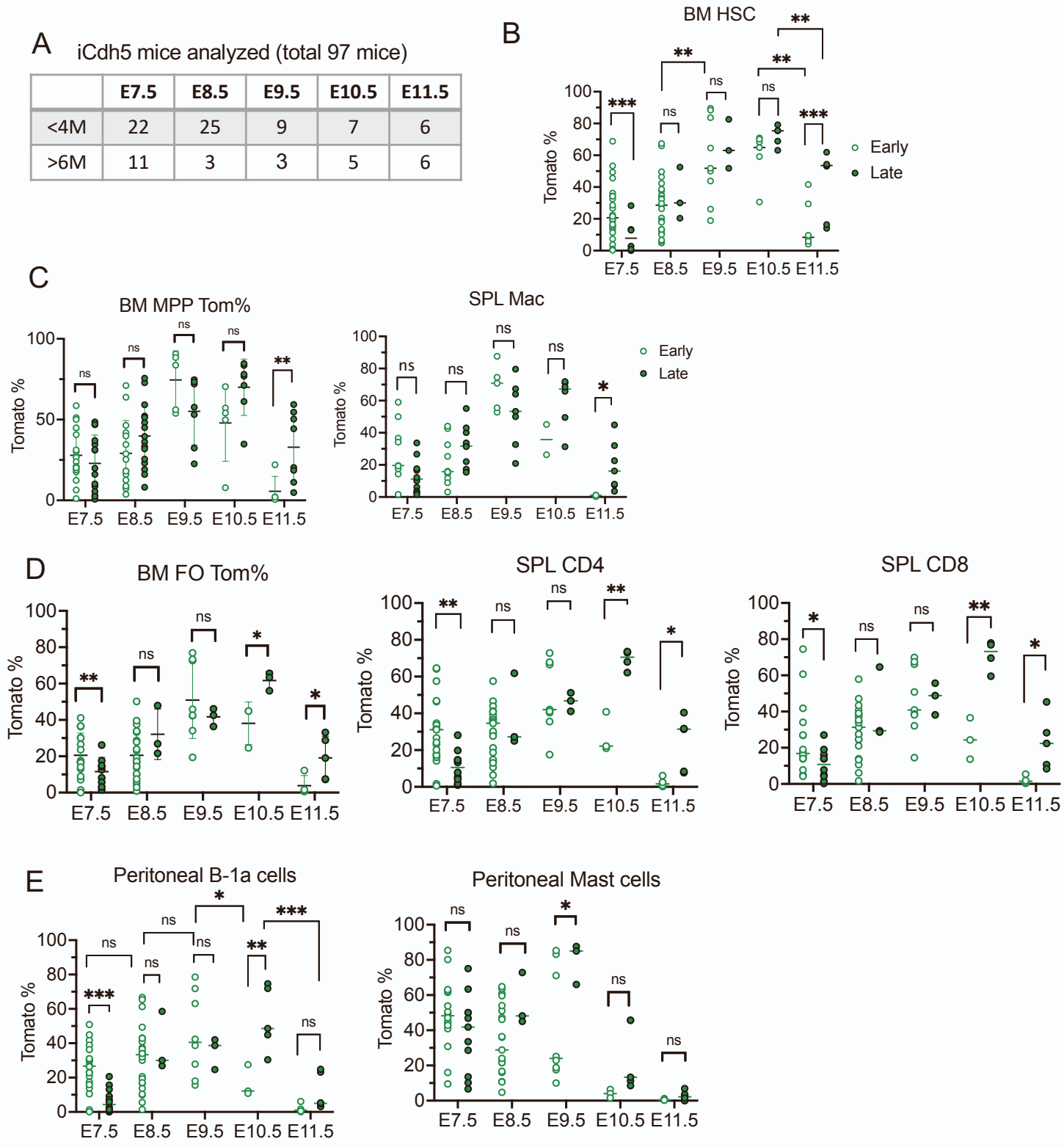


Figure S3. ECs at different embryonic stages mark MPPs and B- and T- lymphocytes in adult mice. Related to Fig. 2.

(A) The numbers of mice injected at TAM at each embryonic day and examined before 4 months (E, early) or after 6 months (L, late). For MPP and Mac, E <4 weeks and L>8 weeks based on the results in Fig. 1. (B-E) Tomato % of each blood subset at different time points after birth following TAM injection at E7.5 (green dots), 8.5 (yellow dots), 9.5 (red dots), 10.5 (blue dots), and 11.5 (orange dots). *p<0.05, **p<0.01, ***p<0.001

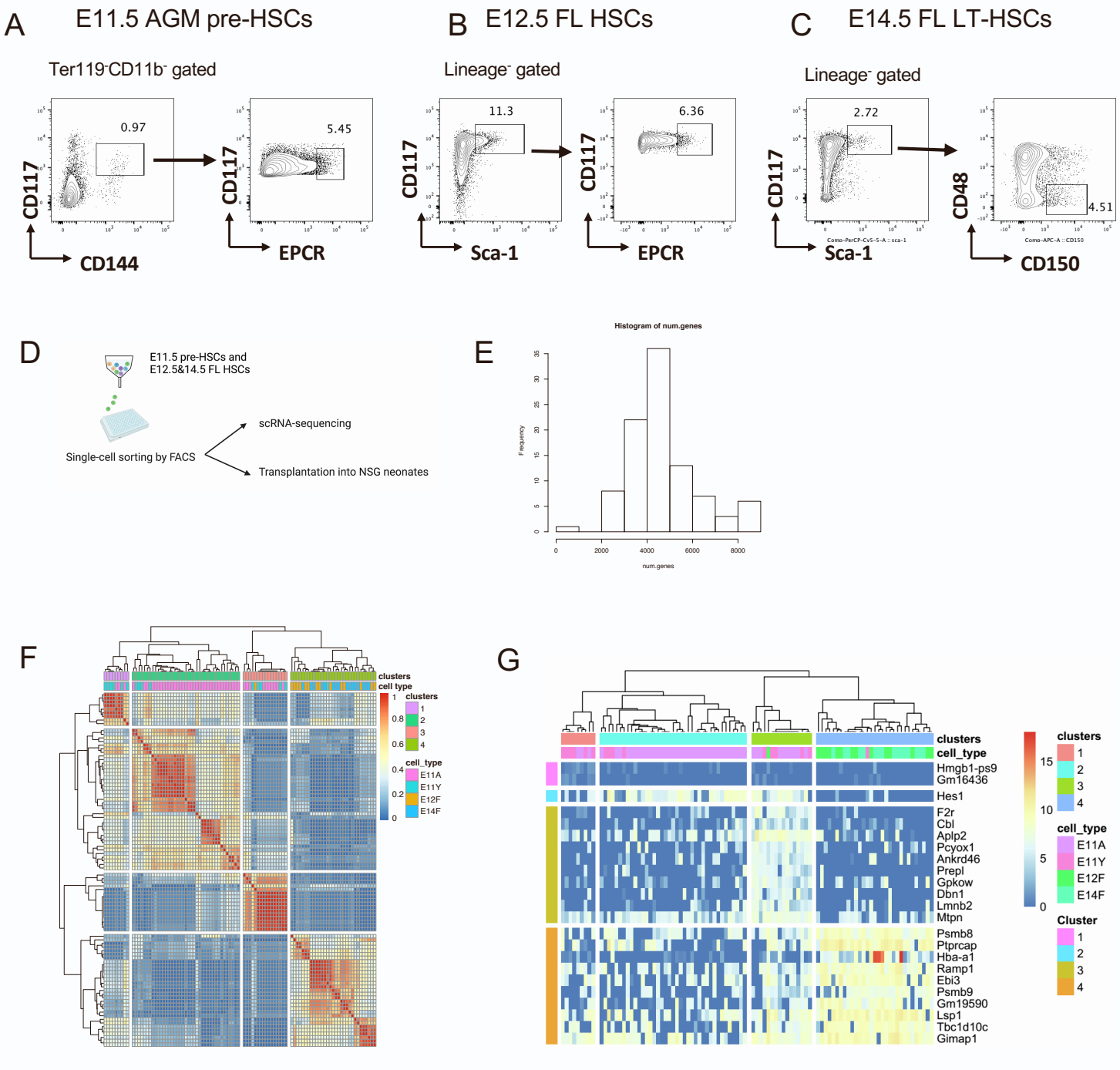
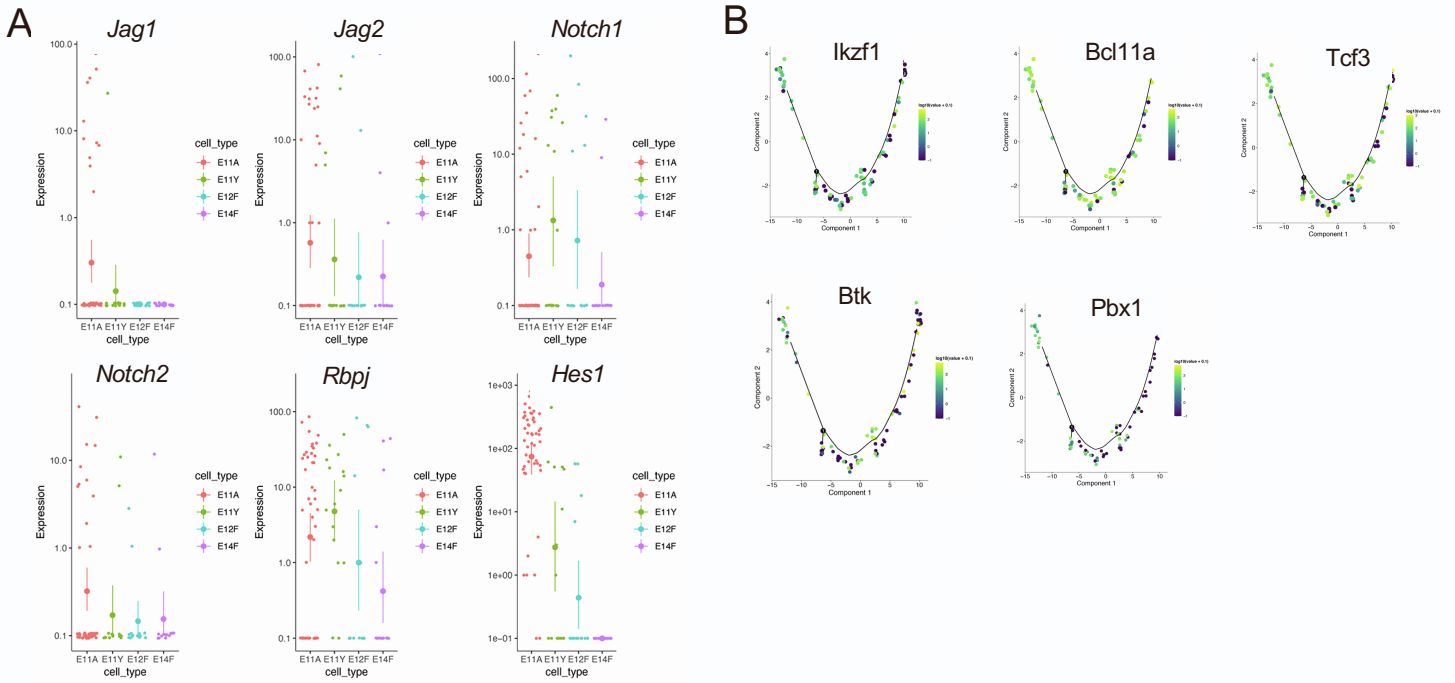
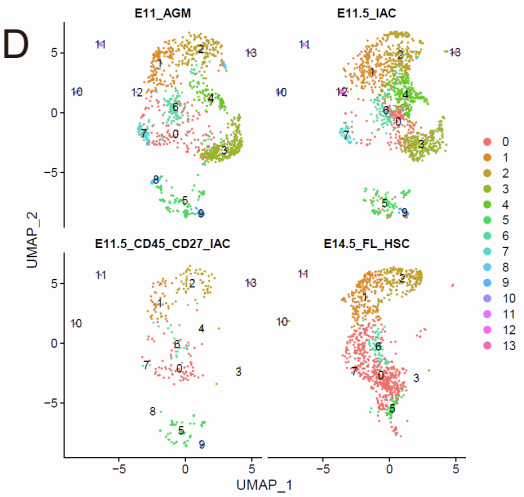


Figure S4. Gating strategies of pre-HSC and FL HSCs at different stages. Related to Fig. 5. Representative FACS plots for E11.5 pre-HSCs (A), E12.5 FL HSCs(B), and E14.5 FL HSCs(C). (D) The experimental design for scRNA-seq. While 96 single cells were sorted, 5-10 pre-HSCs or HSCs were transplanted into NSG mice to validate the biological functions. (E) Histogram of numbers of genes expressed in each cell (read count >10). We filter the cells which have less than 2000 genes (read counts >10) and genes (read counts >10) which are detected in less than 10 cells. At last, 95 cells and 11,814 genes are used for further analysis. (F) SC3 consensus matrix predicted 4 clusters. (G) Heatmap of normalized scRNA-seq expression of markers (the area under the ROC curve (auroc) > 0.85 and the adjusted p-values <0.01). The top 10 differentially expressed genes are depicted



C

Samples	Cell types	Ref.
GSM4338426	E11 AGM V+61+E+primary s1	[S1]
GSM4338427	E11 AGM V+61+E+primary s2	[S1]
GSM4067228	E11.5 IAC replicate 1	[S2]
GSM4067229	E11.5 IAC replicate 2	[S2]
GSM4067230	E11.5 CD45+CD27+IAC	[S2]
GSM4067232	E14.5 FL HSC	[S2]



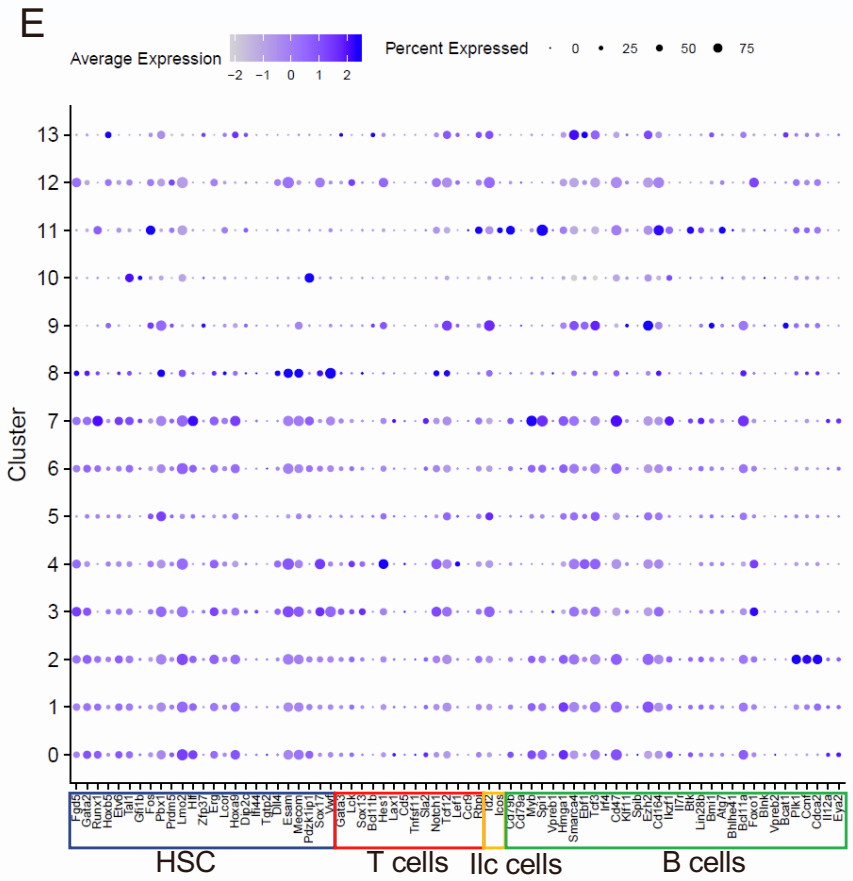


Figure S5. Gene expression profiles in our and published scRNA-seq data using pre-HSPCs. Related to Fig. 5.

(A) Expressions of Notch related genes are shown in each single cells from VE-cadherin^{+}c-kit^{+}CD45^{+} EPCR^{+} cells from E11.5 AGM and YS, Ter119-CD45^{+}c-kit^{+}Sca-1^{+}EPCR^{+}E12.5 FL HSCs, and lin^{-}Sca-1^{+}c-kit $^{+}$ CD48^{-}CD150^{+}E14.5 FL HSCs. (B) HSC-related and early B-cell commitment related genes in Pseudo-time-ordering trajectory of scRNA-seq data using Monocle2. (C) Summary of used public data and cell population [S1][S2]. (D) UMAP of public scRNA-seq of pre-HSCs, showing the clusters and cell populations in the UMAP. (E) Dot plot analysis of scRNA-seq data of phenotypic pre-HSCs from public data sets.

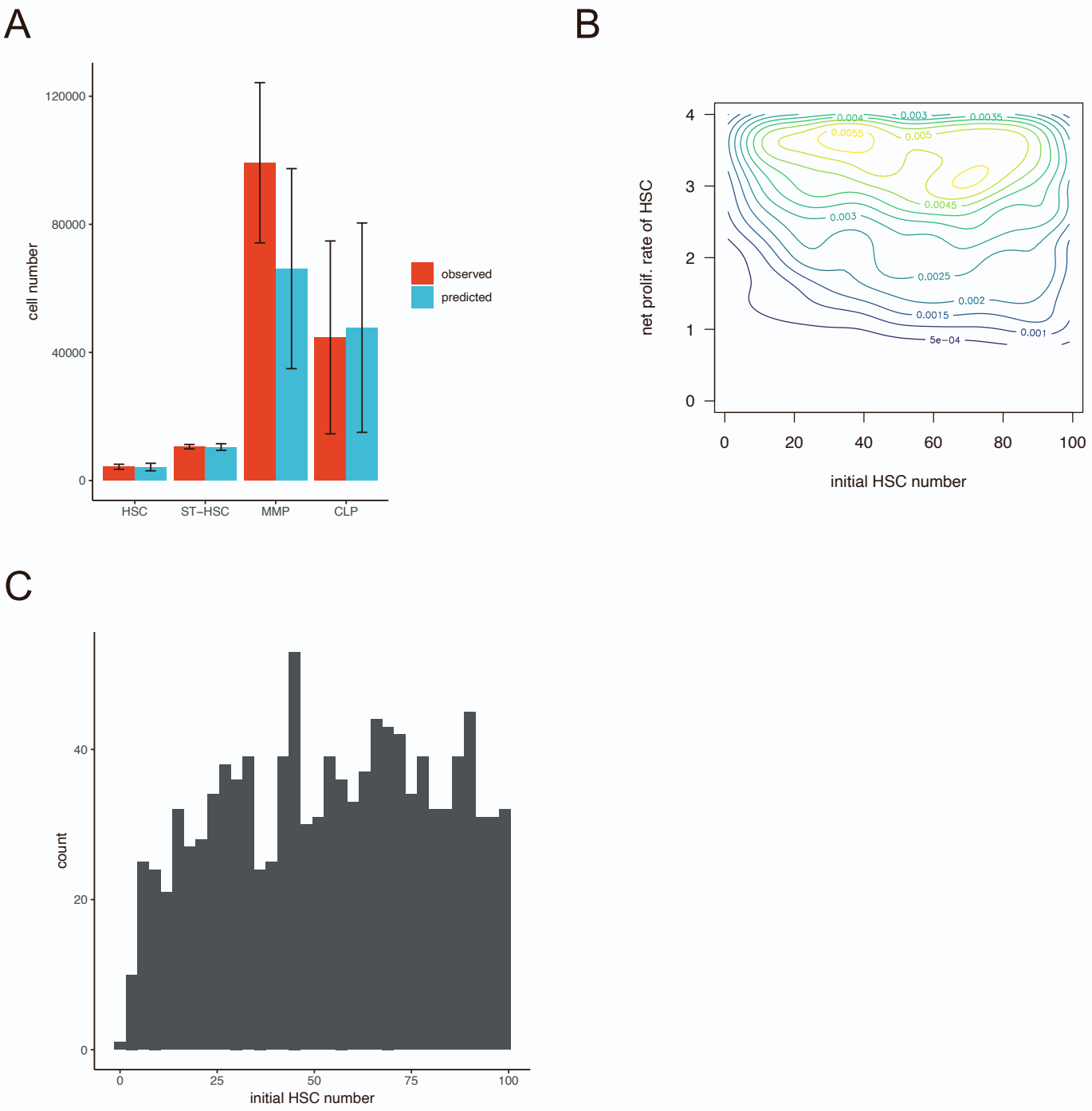


Figure S6. ***The simulation model to produce output stem and progenitor cell numbers.*** Related to Discussion.

(A) abc-fit: Evaluation of model fit for the approximate Bayesian computation (ABC) model of hematopoietic differentiation. Model parameters were estimated by ABC such that the predicted cell counts fall within the 99% confidence interval of the observed cell counts. Bars represent mean cell counts and error bars depict standard deviation. (B) Contour plot of the posterior probability distribution of the fitted ABC model for hematopoietic differentiation. Each contour line depicts a slice of the estimated posterior probability density at particular values for the net proliferation rate parameter, as well as the initial HSC number. (C) Histogram of the estimated initial HSC numbers under the fitted ABC model.

Supplemental references[1,2]

[S1] Hadland B, Varnum-Finney B, Dozono S, et al. Engineering a niche supporting hematopoietic stem cell development using integrated single-cell transcriptomics. *Nat Commun*. 2022;13(1):1584.

[S2] Zhu Q, Gao P, Tober J, et al. Developmental trajectory of prehematopoietic stem cell formation from endothelium. *Blood*. 2020;136(7):845-856.