

1 **Supplemental Materials**

2 **Table S1. FACS staining reagents used in this study.** Marker, format, isotype, clone, source,
3 and catalog number for all monoclonal antibodies (mAbs) used in fluorescent cytometry
4 experiments for this study. Combinations and staining procedures are listed in experimental
5 methods and FMO controls were used to distinguish auto-fluorescent cells from cells expressing
6 low levels of a particular surface marker as described (see methods).

Marker	Format	Isotype	Clone	Source	Catalog No.
B220	BV786	Rat IgG2a, κ	RA3-6B2	BD Biosciences	563894
B220	PE-Cy7	Rat IgG2a, κ	RA3-6B2	BD Biosciences	552772
B220	APC	Rat IgG2a, κ	RA3-6B2	BD Biosciences	553092
B220	AF700	Rat IgG2a, κ	RA3-6B2	BD Biosciences	553092
CD11b	APC-Cy7	Rat IgG2b, κ	M1/70	BD Biosciences	557657
CD11b	BV711	Rat IgG2b, κ	M1/70	BD Biosciences	563168
CD11b	Pacific Blue	Rat IgG2b, κ	M1/70	Invitrogen	RM2828
CD16/32	Purified	Rat IgG2b, κ	2.4G2	BD Biosciences	553142
CD19	BV786	Rat IgG2a, κ	1D3	BD Biosciences	563333
CD19	PE-Cy5.5	Rat IgG2a, κ	1D3	Invitrogen	35-0193-80
CD117	BV421	Rat IgG2b, κ	ACK2	BioLegend	135124
CD127	BV605	Rat IgG2a, κ	A7R34	BioLegend	135041
CD135	Biotin	Rat IgG2a, κ	A2F10	BioLegend	135308
CD150	PE-Cy7	Rat IgG2a, λ	TC15-12F12.2	BioLegend	115914
CD23	PE	Rat IgG2a, κ	B3B4	BD Biosciences	553139
CD23	Biotin	Rat IgG2a, κ	B3B4	BD Biosciences	553137
CD3ε	APC	Armenian Hamster IgG	145-2C11	BioLegend	100312
CD34	AF700	Rat IgG2a, κ	RAM-34	BD Biosciences	560518
CD38	PE-Cy5	Rat IgG2a, κ	90	Invitrogen	15-0381-81
CD41	PE-Cy5.5	Rat IgG1, κ	MWReg30	In-house custom	—
CD41	BV510	Rat IgG1, κ	MWReg30	BioLegend	133923
CD45	BV570	Rat IgG2b, κ	30-F11	BioLegend	103136
CD48	APC-Cy7	Armenian Hamster IgG	HM48-1	BioLegend	103423
CD5	PE-Cy5	Rat IgG2a, κ	53-7.3	BioLegend	100610
CX3CR1	PE	Mouse IgG2a, κ	SA011F11	BioLegend	149006
F4/80	PE-Cy7	Rat IgG2a, κ	BM8	BioLegend	123114
Gr-1	APC	Rat IgG2b, κ	RB6-8C5	BioLegend	108412
Gr-1	AF700	Rat IgG2b, κ	RB6-8C5	BD Biosciences	557979
Gr-1	APC-Cy7	Rat IgG2b, κ	RB6-8C5	BD Biosciences	557661
I-A/I-E	BV650	Rat IgG2b, κ	M5/114.15.2	BD Biosciences	563415
IgK	Biotin	Rat IgG1, κ	187.1	BD Biosciences	559750
IgK	APC-Cy7	Rat IgG1, κ	187.1	BD Biosciences	561353
IgM	AF700		331	In-house custom	—

Ly-6C	APC	Rat IgM, κ	AL-21	BD Biosciences	560595
Sca-1	PE	Rat IgG2a, κ	D7	BioLegend	108108
Sca-1	PE-Cy5.5	Rat IgG2a, κ	D7	Invitrogen	MSCA18
Streptavidin	BV711	—	—	BioLegend	405241
Streptavidin	Qdot605	—	—	Invitrogen	Q10101MP
TER-119	PE	Rat IgG2b, κ	TER119	BD Biosciences	553673
TER-119	APC	Rat IgG2b, κ	TER119	BioLegend	116212
Viability dye	Zombie Aqua	—	—	BioLegend	423102
Vδ6.3/2	PE	Armenian Hamster IgG2, κ	8F4H7B7	BD Biosciences	555321
VH11	Pacific Blue		VH11Id.6e9	In-house custom	—

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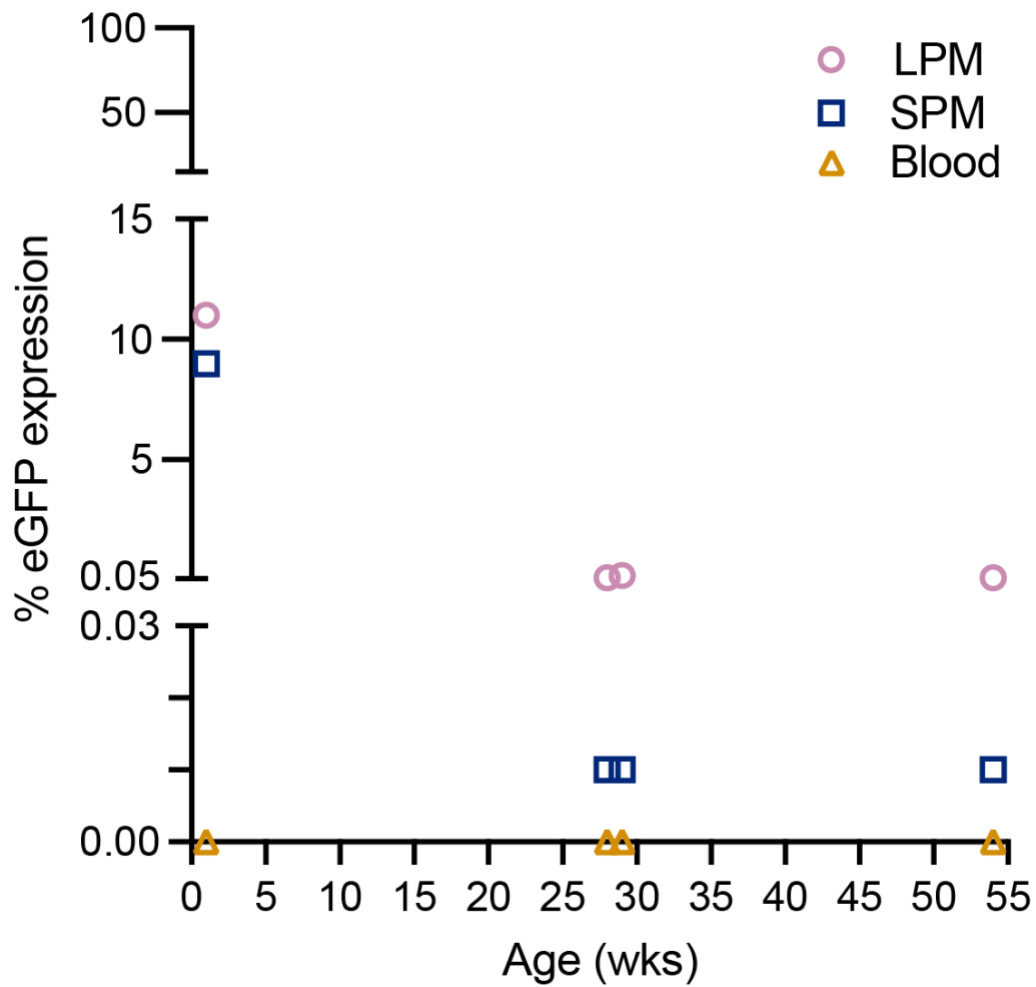
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9 **Table S2. Fluidigm plate design for single cell HT-qPCR assay.** Target, RefSeq accession
10 number, and gene symbol for the 77 targets used in Fluidigm single-cell HT-qPCR assay (in
11 alphabetical order).
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Target	Design RefSeq	Gene Symbol
Actb	NM_007393.3	Actb
Arnt	NM_009709.4	Arnt
Atf1	NM_007497.3	Atf1
Atf2	NM_009715.2	Atf2
Atf3	NM_007498.3	Atf3
Atf4	NM_009716.2	Atf4
Cebpa	NM_007678.3	Cebpa
Cebpb	NM_009883.3	Cebpb
Cebpg	NM_009884.3	Cebpg
Creb1	NM_001037726.1	Creb1
Crebbp	XM_006521751.3	Crebbp
Cttnb1	NM_007614.3	Cttnb1
Dr1	NM_026106.3	DR1
E2f1	NM_007891.4	E2f1
E2f6	NM_033270.1	E2F6
Egr1	NM_007913.5	Egr1
Elk1	NM_007922.4	Elk1
Esr1	NM_007956.4	Esr1
Ets1	NM_001038642.1	Ets1
Ets2	NM_011809.3	Ets2
Fos	NM_010234.2	Fos
Foxa2	NM_010446.2	Foxa2
Foxg1	NM_001160112.1	Foxg1
Foxo1	NM_019739.3	Foxo1
Gapdh	NM_008084.2	Gapdh
Gata1	NM_008089.1	Gata1

Hdac1	NM_008228.1	Hdac1
Hif1a	NM_010431.2	Hif1a
Hprt	NM_013556.1	HPRT1
Hsf1	NM_008296.2	Hsf1
Id1	NM_010495.2	ID1
Irf1	NM_008390.2	Irf1
Jun	NM_010591.2	Jun
Junb	NM_008416.2	Junb
Jund	NM_010592.4	Jund
Klf2	NM_008452.2	Klf2
Klf4	NM_010637.3	Klf4
Max	NM_008558.1	Max
Mef2a	NM_001033713.1	Mef2a
Mef2c	NM_001170537.1	Mef2c
Myc	NM_010849.4	Myc
Myf5	NM_008656.5	Myf5
Neurod2	NM_010895.2	Neurod2
Neurod4	NM_007501.4	Neurod4
Nfat5	NM_018823.2	Nfat5
Nfatc1	NM_016791.4	Nfatc1
Nfatc3	NM_010901.2	Nfatc3
Nfatc4	NM_023699.3	Nfatc4
Nfkb1	NM_008689.2	Nfkb1
Nfyb	NM_010914.1	Nfyb
Nr3c1	NM_008173.3	Nr3c1
Olig1	NM_016968.3	Olig1
Pax6	NM_013627.4	Pax6
Pou5f1	NM_013633.2	Pou5f1
Rel	NM_009044.2	Rel
Rela	NM_009045.4	Rela
Relb	NM_009046.2	Relb

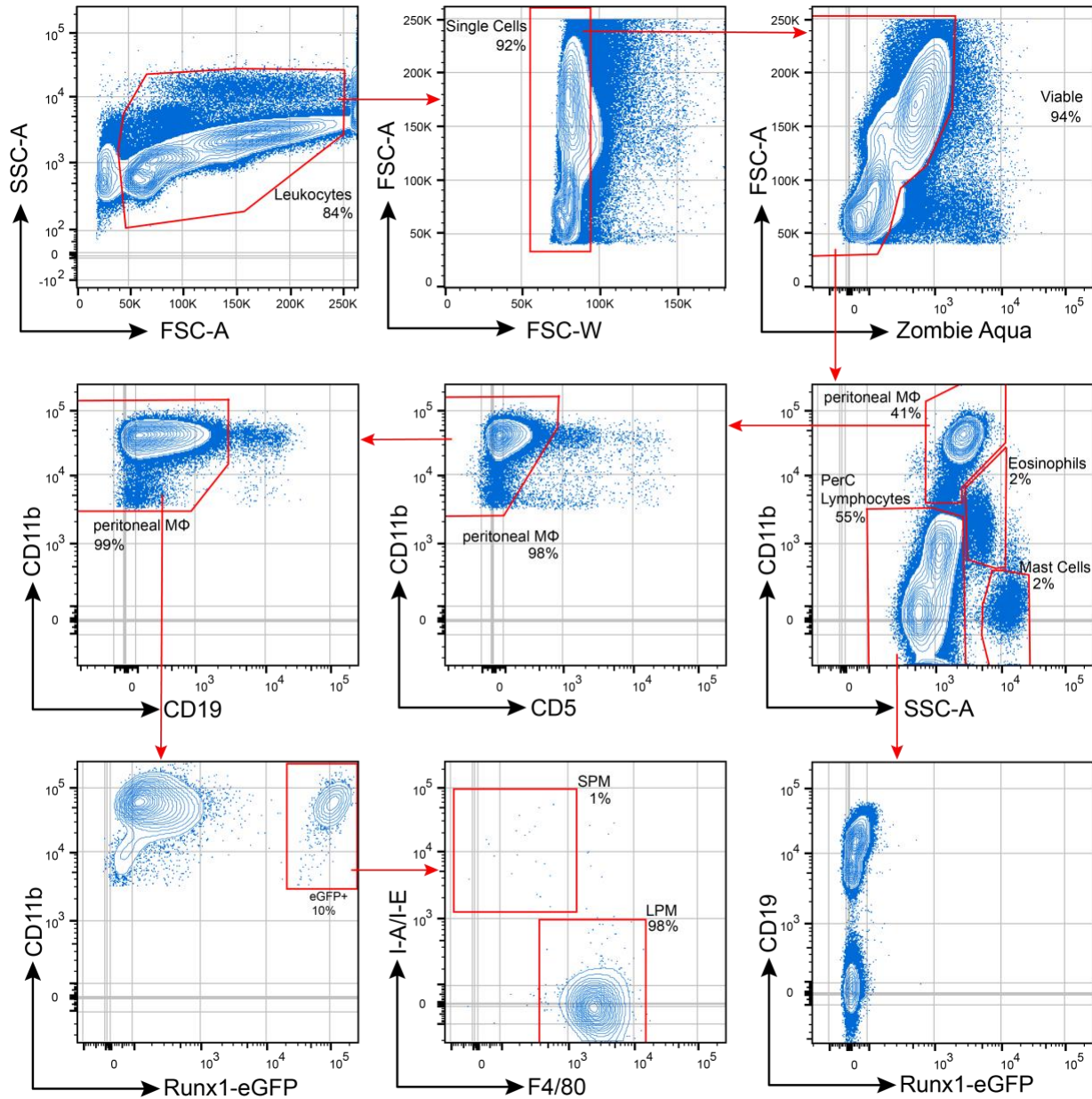
Smad1	NM_008539.3	Smad1
Smad4	XM_001001668.1	Smad4
Smad5	NM_008541.3	Smad5
Smad9	NM_019483.4	Smad9
Sp1	NM_013672.2	Sp1
Sp3	NM_001098425.1	Sp3
Stat1	NM_009283.3	Stat1
Stat2	NM_019963.1	Stat2
Stat3	NM_213659.2	Stat3
Stat4	NM_011487.4	Stat4
Stat5a	NM_011488.3	Stat5a
Stat5b	NM_011489.2	Stat5b
Stat6	NM_009284.2	Stat6
Tbp	NM_013684.3	Tbp
Tcf712	NM_001142918.1	Tcf712
Tcf712	NM_009333.3	Tcf712
Tfap2a	NM_011547.3	Tfap2a
Tgif1	NM_009372.2	Tgif1
Trp53	NM_011640.2	Trp53
Yy1	NM_009537.2	Yy1



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16 **Figure S1. Frequency of eGFP labeled cells in *Runx1* lineage tracing mice at age of**
 17 **sacrifice.** Each individual *Runx1*^{cre/eGFP} mouse shown in Fig. 2 is plotted at age of sacrifice to
 18 show higher proportions of eGFP cells in the peritoneal cavity of young mice (1 wk old) that
 19 declines with age yet persists suggesting some of the yolk-sac-derived population is likely
 20 maintained by self-renewal of eGFP-expressing LPM and SPM.

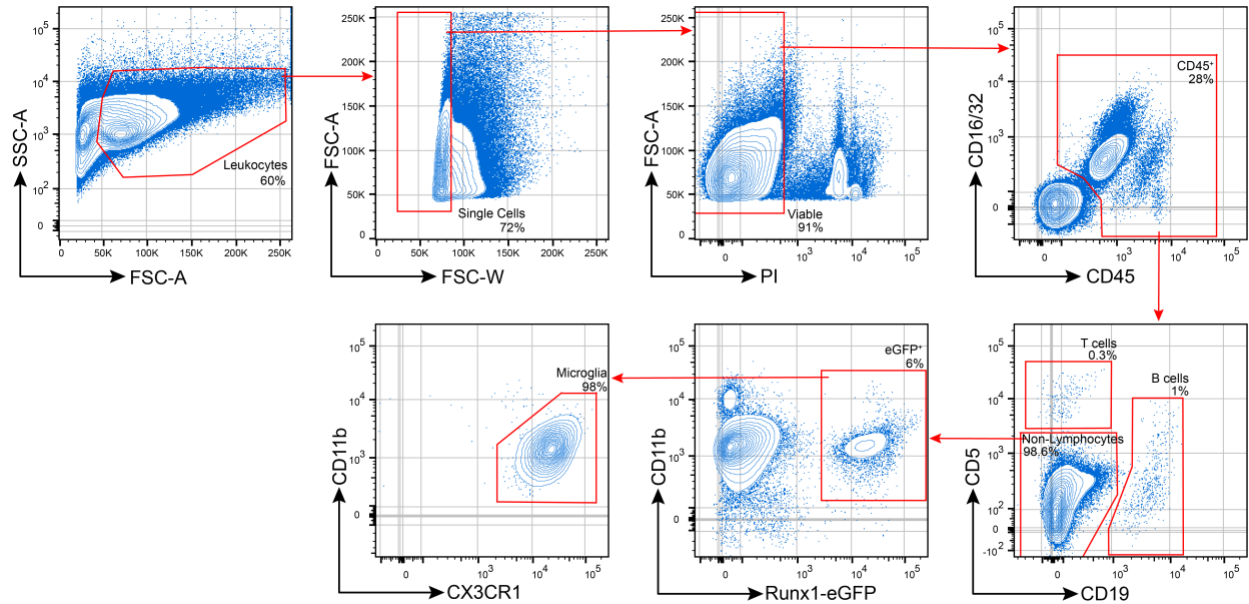
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23 **Figure S2. Representative gating strategy–Peritoneal Cavity (PerC).** Viable, single cells
 24 were gated first using CD11b and SSC-A to identify major immune lineages in the PerC as
 25 previously established (Ghosn et al. 2010). Gated peritoneal macrophages (MΦ) were “cleaned:
 26 for T/B cells doublets (removing CD5 and CD19, respectively) then fluorescent reporters
 27 interrogated in lineage tracing (*Runx1*^{cre/eGFP}) or transplanted mice (RFP & GFP) as shown in
 28 Figs. 2 & 3. SPM and LPM were distinguished using I-A/I-E (MHC II) and F4/80 expression as
 29 previously established (Ghosn et al. 2010). Gated PerC lymphocytes were interrogated for eGFP
 30 expression in *Runx1*^{cre/eGFP} mice to confirm that LT-HSCs were not labeled.

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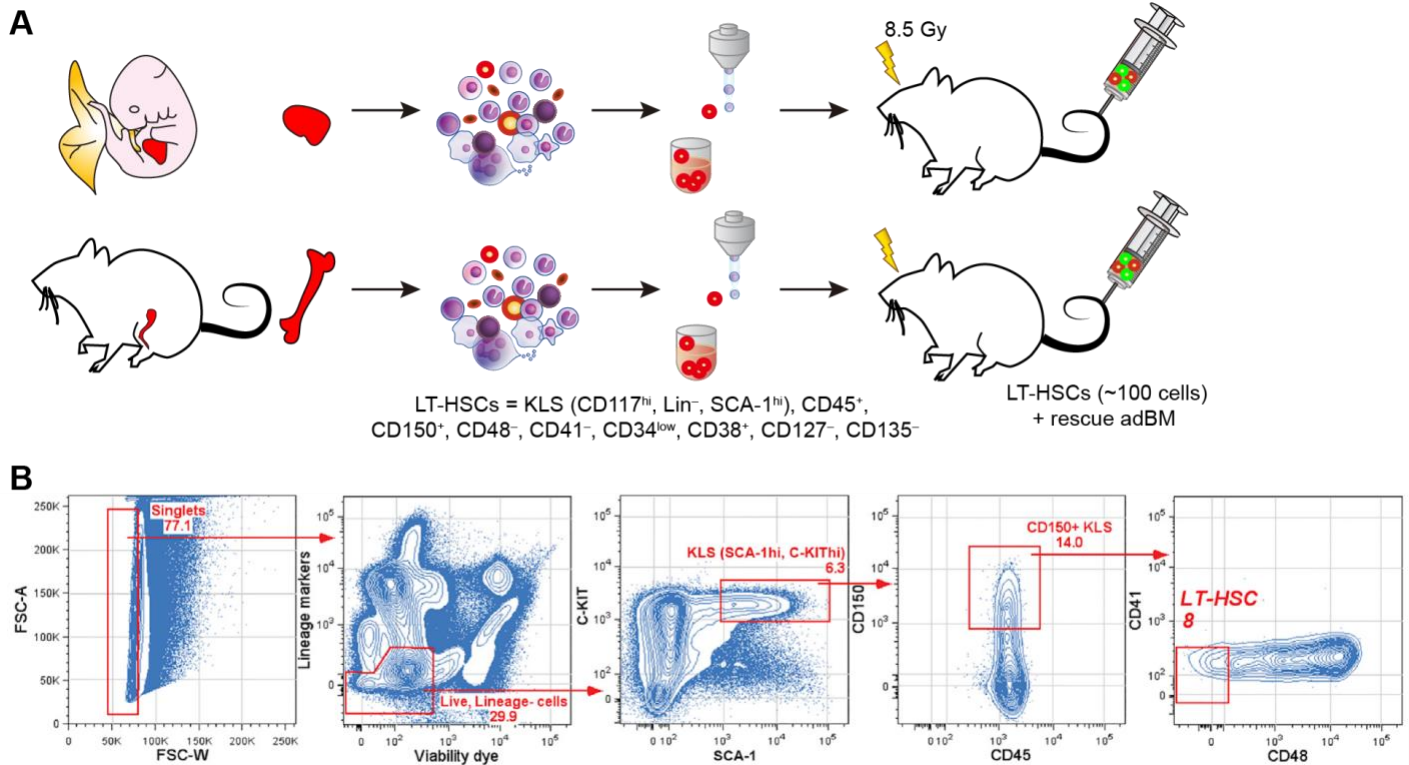
33 **Figure S3. Representative gating strategy–Brain.** Viable, single cells were gated for CD45+

34 cells to analyze immune populations in the brain. CD5+ T cells and CD19+ B cells were gated

35 and remaining CD11b+ cells analyzed for *Runx1*^{cre/eGFP} expression and RFP/GFP expression in

36 transplanted mice in gates displayed in Figs. 2 & 3.

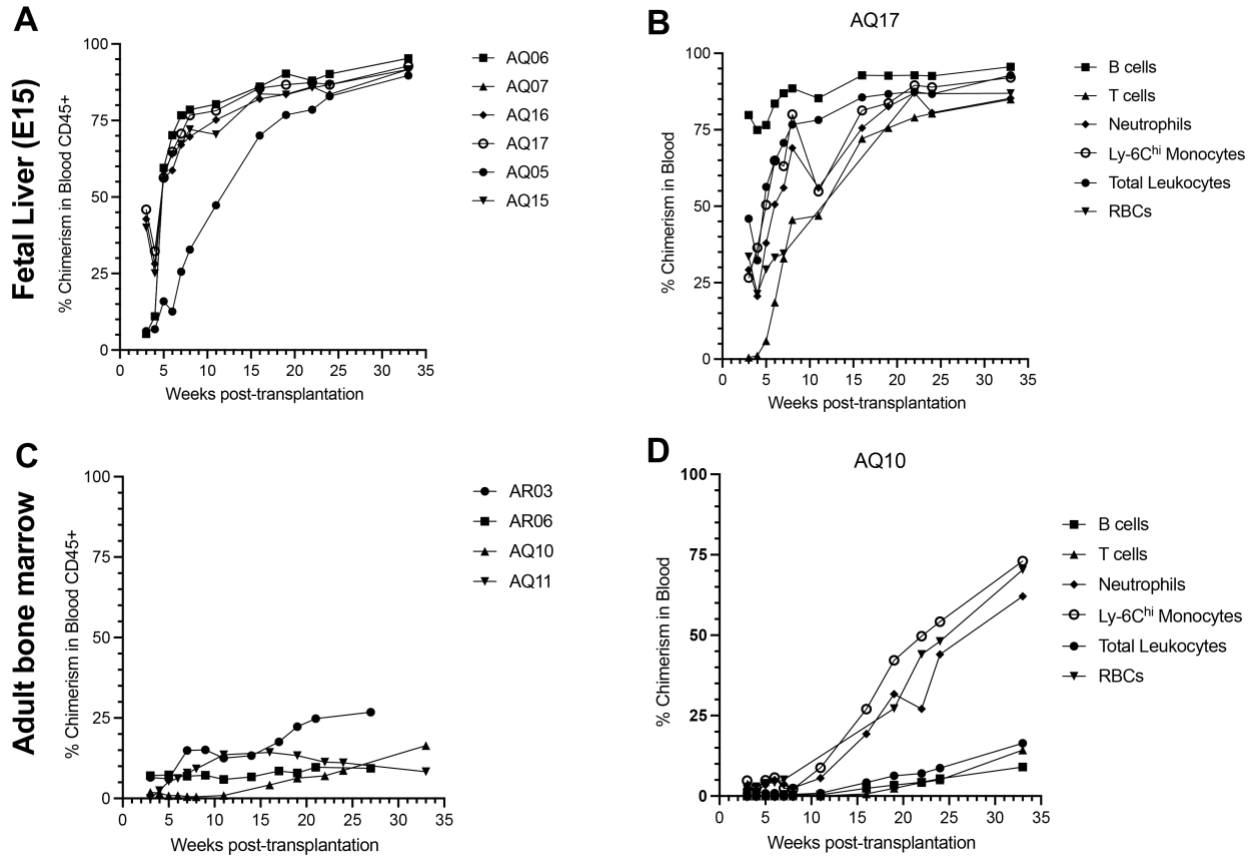
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39 **Figure S4. LT-HSC adoptive transplantation strategy. A.** Fetal liver (E15) and adult BM
 40 (adBM) are isolated from TM7-RFP mice and single LT-HSCs sorted as described (see methods)
 41 for adoptive transplantation into lethally-irradiated recipient mice. Mice also received GFP⁺
 42 adBM rescue cells as described at the time of LT-HSC transplantation (see methods). **B.**
 43 Representative sorting strategy for RFP⁺ LT-HSCs from fetal (shown here) and adBM. Adapted
 44 from Ghosn et al. (2016).

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47 **Figure S5. Representative blood RFP chimerism kinetics of transplant recipient mice. A.**
 48 Chimerism kinetics of total CD45⁺ blood leukocytes for 6 representative mice that received E15
 49 fetal liver (FL) RFP⁺ LT-HSC transplants. **B.** Chimerism kinetics of major immune lineages
 50 from one representative mouse that received E15 FL RFP⁺ LT-HSC transplant. **C.** Chimerism
 51 kinetics of total CD45⁺ blood leukocytes for 4 mice that received adult bone marrow (adBM)
 52 RFP⁺ LT-HSC transplant. **D.** Chimerism kinetics of major immune lineages from one
 53 representative mouse that received adBM RFP⁺ LT-HSC transplant.

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