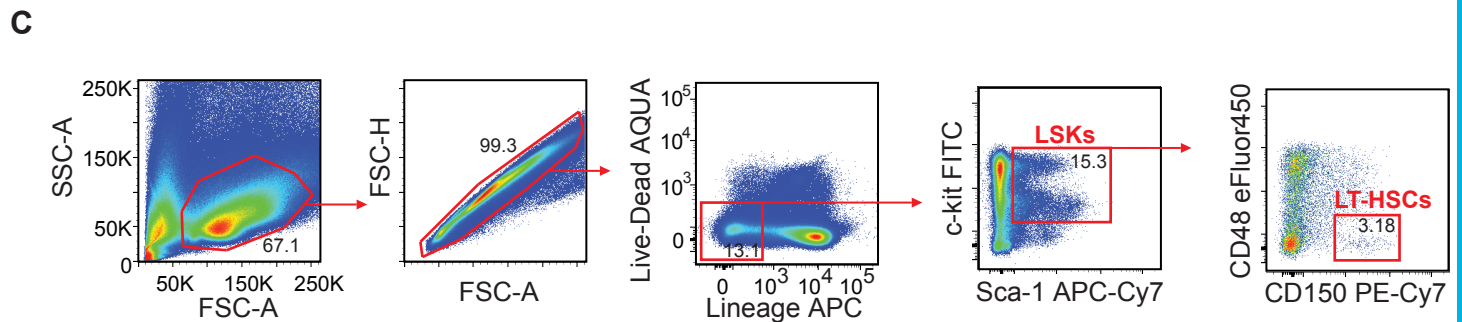
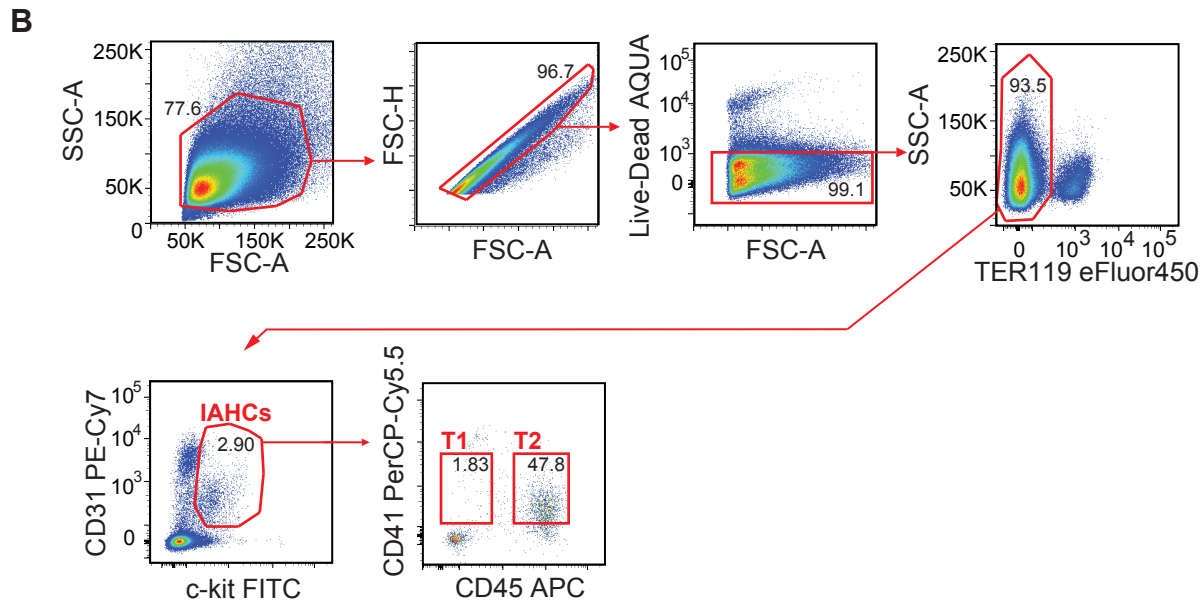
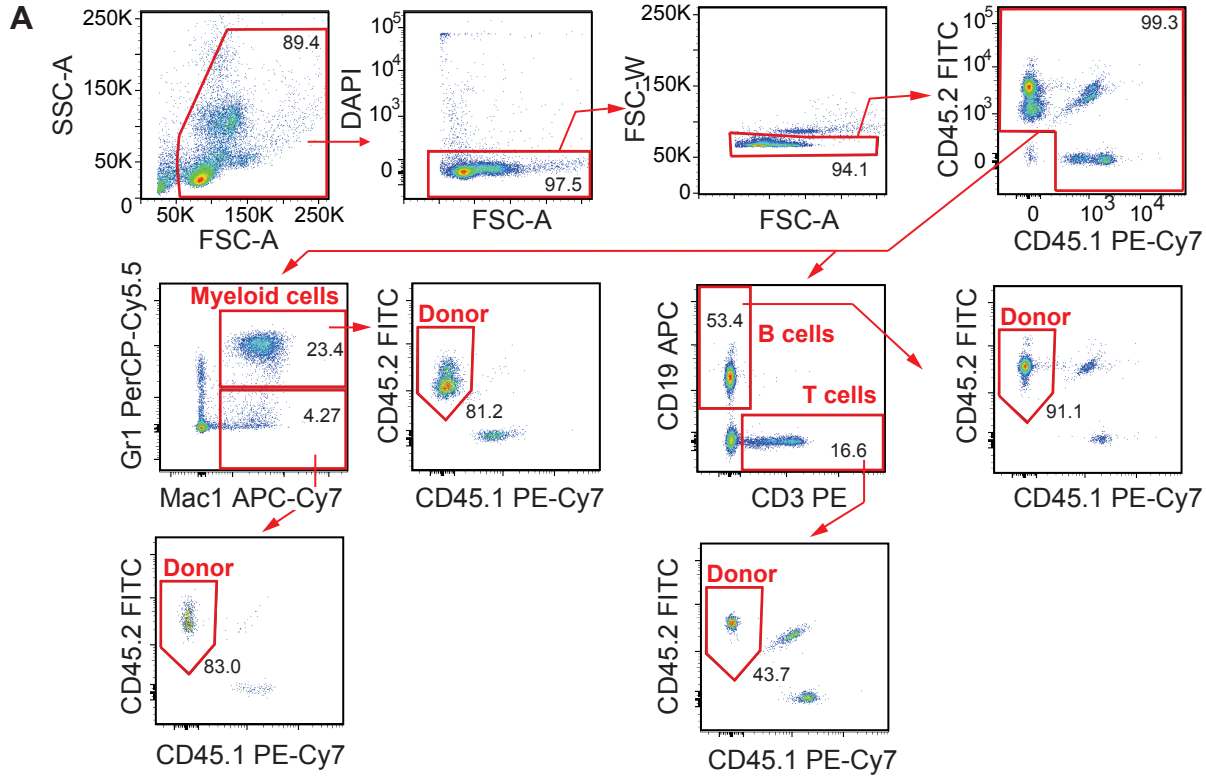


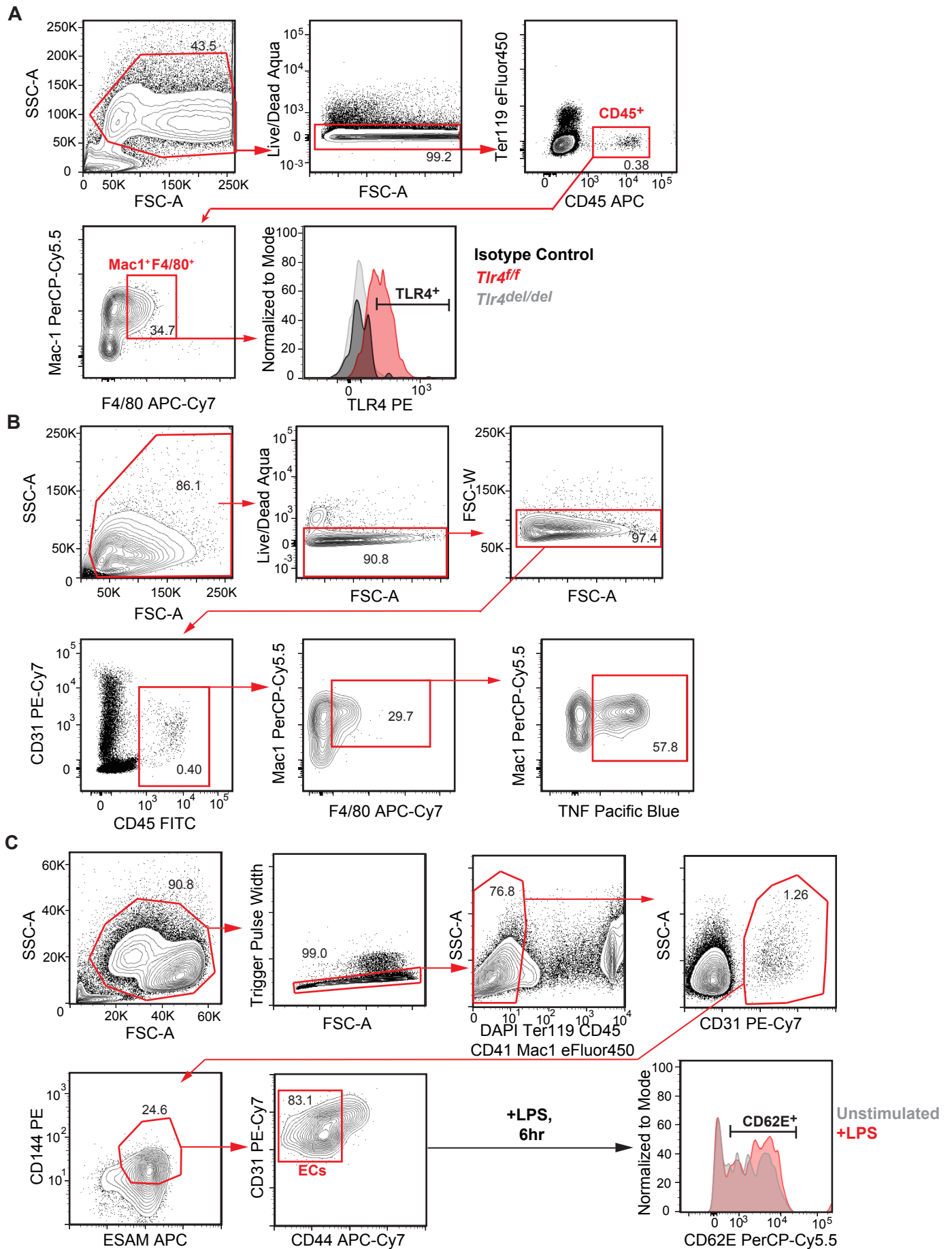
**Fig. S1. HE cell numbers are normal at E9.5, whereas the proliferation of CD45<sup>+</sup> IAHC cells at E10.5 is decreased.**

- A) Representative flow plots depicting the sorting strategy for arterial endothelial cells (ECs) for limiting dilution assays (CD31<sup>+</sup>CD144<sup>+</sup>ESAM<sup>+</sup>CD44<sup>+</sup>CD41<sup>lo/-</sup>Ter119<sup>-</sup>Mac1<sup>-</sup>CD45<sup>-</sup>). Numbers on the x and y-axes are indicated on the first plot and are not shown on subsequent plots if the scale is unchanged. Related to Fig. 1F.
- B) Representative flow plots depicting gating strategy of ECs, CD45<sup>-</sup> and CD45<sup>+</sup> IAHC cells and the percent of EdU<sup>+</sup> cells in each population in *Tlr4*<sup>+/-</sup> and *Tlr4*<sup>-/-</sup> embryos. Related to Fig. 1M,N.



**Fig. S2. Loss of MyD88 increases the number of adult-repopulating HSCs.**

- A) Representative flow plots depicting gating strategy of myeloid, B and T cells in transplant recipient mice and the percentage of donor cells in each lineage. Related to data in Fig. 2C,D.
- B) Representative flow plots depicting gating strategy for phenotypic type I (T1) or type II (T2) pre-HSCs in the AGM regions of E11.0 *Tlr4<sup>+/+</sup>* and *Tlr4<sup>-/-</sup>* embryos. Related to data in Fig. 2E.
- C) Representative flow plots depicting gating strategy for phenotypic long-term repopulating HSCs (LT-HSCs) in E12.5 and E13.5 fetal livers of *Tlr4<sup>+/+</sup>* and *Tlr4<sup>-/-</sup>* embryos. Related to data in Fig. 2F.



**Fig. S3. Csf1r-Cre reduces cell surface TLR4 and TLR4 signaling in embryonic macrophages.**

- A) Representative flow plots depicting gating strategy to examine surface TLR4 on embryonic macrophages. Related to data in Fig. 3A,B.
- B) Representative flow plots depicting gating strategy to examine the percentage of TNF<sup>+</sup> macrophages following LPS stimulation of isolated AGM regions *ex vivo*. Related to data in Fig. 3C-D,G-H.
- C) Representative flow plots depict sorting strategy for ECs (CD31<sup>+</sup>ESAM<sup>+</sup>CD144<sup>+</sup>CD44<sup>-</sup>CD41<sup>lo/-</sup>Ter119<sup>-</sup>Mac1<sup>-</sup>CD45<sup>-</sup>). Related to data in Fig. 3K. Sorted ECs were stimulated with 100ng/mL LPS for 6 hours and CD62E surface expression analyzed by flow cytometry. A representative histogram is shown depicting gating of CD62E<sup>+</sup> cells where the gray histogram is from unstimulated and the red histogram from stimulated EC cells (+LPS).

**Table S1.** List of all antibodies used for flow cytometry and whole mount immunostaining. For each antibody, the clone, fluorophore, dilution, manufacturer, and antibody registry number (or manufacturer's catalog number when no RRID number is available) are provided.

Antibody	Clone	Fluorophore	Supplier	RRID #	Dilution
c-Kit	2B8	Alexa Fluor 700	Thermo Fisher	AB_657583	1:200
c-Kit	2B8	APC- eFluor 780	Thermo Fisher	AB_1272213	1:200
c-Kit	2B8	Unconjugated	Thermo Fisher	AB_467434	1:250
CD3e	145-2C11	APC	Biolegend	AB_312677	1:200
CD3e	145-2C11	PE	Thermo Fisher	AB_465496	1:200
CD19	eBio1D3 (1D3)	APC	Thermo Fisher	AB_1659676	1:200
CD31 (PECAM-1)	390	PE-Cy7	Thermo Fisher	AB_2716949	1:200
CD31	MEC13.3	Unconjugated	BD Biosciences	AB_396660	1:500
CD41a	eBioMWRReg30 (MWRReg30)	eFluor450	Thermo Fisher	AB_1582238	1:200
CD44	IM7	APC-Cy7	BD Biosciences	AB_1727481	1:200
CD45	RA3-6B2	eFluor450	Thermo Fisher	AB_1548761	1:200
CD45	30-F11	PE	Biolegend	AB_312971	1:200
CD45.1	A20	APC-Cy7	Biolegend	AB_313505	1:200
CD45.1	A20	PE-Cy7	Thermo Fisher	AB_469629	1:200
CD45.2	104	FITC	Thermo Fisher	AB_465062	1:200
CD45R/B220	RA3-6B2	APC	Biolegend	AB_312997	1:200
CD48	HM48-1	eFluor450	Thermo Fisher	AB_11151336	1:200
CD61	2C9.G2 (HM $\beta$ 3-1)	FITC	BD Biosciences	AB_10895806	1:200
CD62E	10E9.6	Biotin	BD Biosciences	AB_395030	1:200
CD135 (Flt-3)	A2F10	PE	Biolegend	AB_1877218	1:200
CD144	eBioBV13 (BV13)	APC	Thermo Fisher	AB_10597442	1:200
CD144	eBioBV13 (BV13)	PE	Thermo Fisher	AB_1907346	1:200
CD150	TC15-12F12.2	PE-Cy7	Biolegend	AB_439797	1:200
CD284 (TLR4)	SA15-21	PE	Biolegend	AB_2561873	1:200
ESAM	1G8/ESAM	APC	Biolegend	AB_2101658	1:200
ESAM	1G8/ESAM	PE	Biolegend	AB_1953300	1:200
F4/80	BM8	APC-Cy7	Biolegend	AB_893477	1:200
Gr-1	RB6-8C5	APC	Biolegend	AB_313377	1:200
Gr-1	RB6-8C5	PerCP-Cy5.5	Thermo Fisher	AB_906247	1:200
Ki67		Unconjugated	Abcam	AB_443209	1:500
Mac-1	M1/70	APC	Biolegend	AB_312795	1:200
Mac-1	M1/70	APC-Cy7	BD Biosciences	AB_396772	1:200
Mac-1	M1/70	eFluor450	Thermo Fisher	AB_1582236	1:200
Nk1.1	PK136	APC	Biolegend	AB_313397	1:200

Rat IgG2a, $\kappa$ Isotype Ctrl Antibody	RTK2758	PE	Biolegend	AB_326530	1:200
RUNX1	EPR3099	Unconjugated	Abcam	AB_2049267	1:500
Sca-1	D7	PerCP-Cy5.5	Thermo Fisher	AB_914372	1:200
Streptavidin		PerCP-Cy5.5	Biolegend	AB_2716577	1:200
Ter119	TER-119	APC	Biolegend	AB_313713	1:200
Ter119	TER-119	eFluor450	Thermo Fisher	AB_1518808	1:200
Ter119	TER-119	PerCP-Cy5.5	Thermo Fisher	AB_925766	1:200
TNF-alpha	MP6-XT22	Pacific Blue	Biolegend	AB_893639	1:200
Goat Anti- Rabbit IgG		Alexa Fluor 488	Abcam	AB_2630356	1:1000
Goat Anti- Rabbit IgG		Alexa Fluor 647	Thermo Fisher	AB_2535813	1:1000
Goat Anti-Rat IgG		Alexa Fluor 555	Abcam	Cat. No: ab150158	1:1000
Goat Anti-Rat IgG		Alexa Fluor 647	Abcam	AB_2864291	1:500