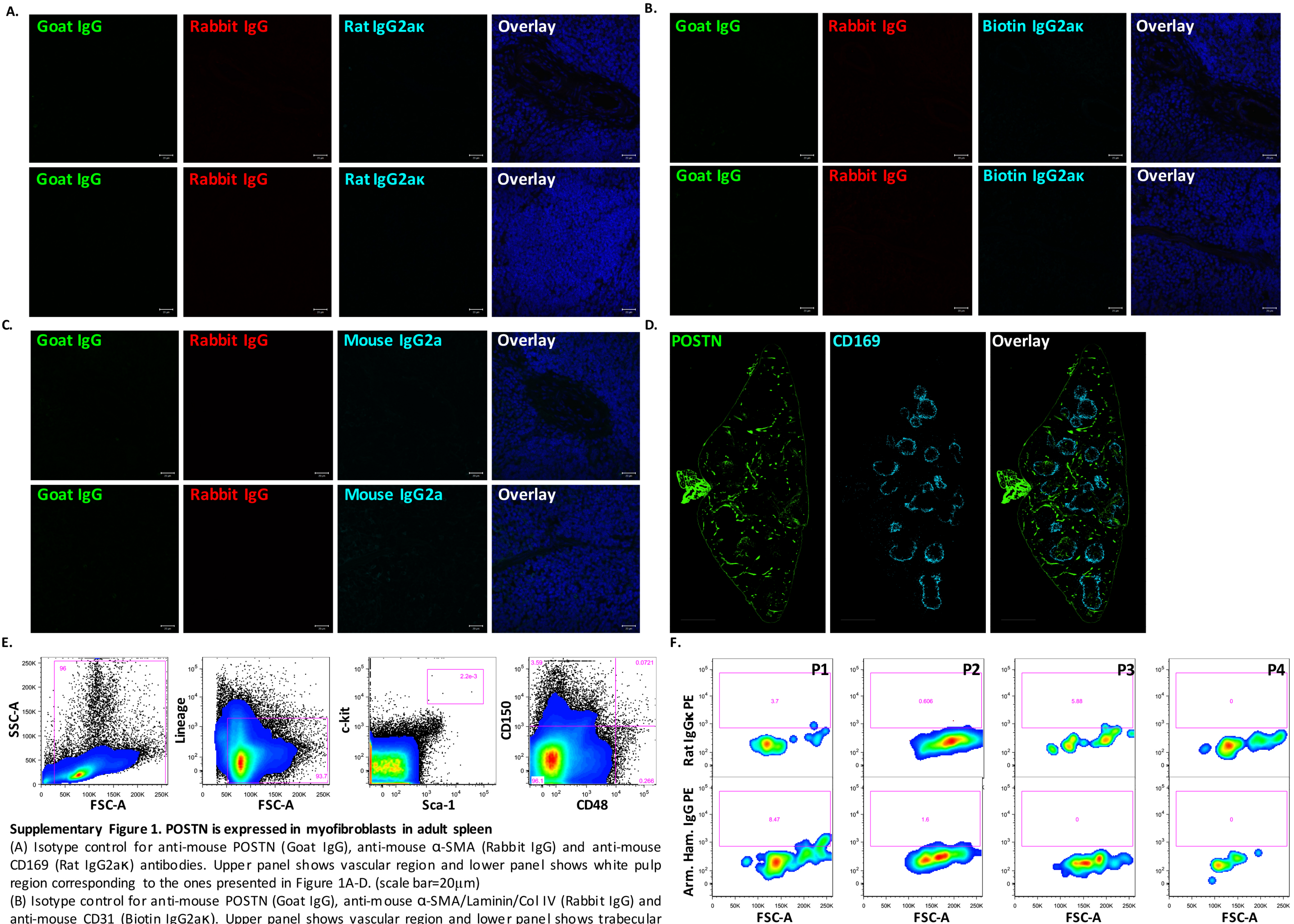
Supplementary table					
List of antibodies used					
Sr. No.	**Antibody**	**Clone**	**Isotype**	**Company**	**Catalog no.**
1	Anti-mouse Periostin	Polyclonal	Goat IgG	R&D	AF2955
2	Anti-mouse α-SMA	Polyclonal	Rabbit IgG	Abcam	ab5694
3	Anti-mouse CD31	Polyclonal	Goat IgG	R&D	AF3628
4	Biotin anti-mouse CD31	MEC13.3	Rat IgG2a,κ	Biolegend	102504
5	Anti-Collagen-IV	Polyclonal	Rabbit IgG	abcam	ab6586
6	Anti-Laminin	Polyclonal	Rabbit IgG	abcam	ab11575
7	Anti-CD169	3D6.112	Rat IgG2a,κ	Biolegend	142419
8	Anti-α-smooth muscle actin	1A4	Mouse IgG2a	R and D	MAB1420
9	Fab Fragment	Polyclonal	Goat IgG	Jackson Imm.	115-007-003
10	Anti-mouse Gr-1 FITC	RB6-8C5	Rat IgG2b,κ	eBioscience	11-5931-85
11	Anti-mouse CD3e FITC	145-2C11	Arm. Ham. IgG	eBioscience	11-0031-85
12	Anti-mouse B220 FITC	RA3-6B2	Rat IgG2a,κ	eBioscience	11-0452-82
13	Anti-mouse Ter119 FITC	TER-119	Rat IgG2b,κ	BD Biosciences	557915
14	Anti-mouse CD11b FITC	M1/70	Rat IgG2b,κ	BD Biosciences	557396
15	Anti-mouse CD48 FITC	HM48-1	Arm. Ham. IgG	eBioscience	11-0481-85
16	Anti-mouse CD48 APC-Cy7	HM48-1	Arm. Ham. IgG	BioLegand	103432
17	Anti-mouse c-kit APC	2B8	Rat IgG2b,κ	BioLegand	105812
18	Anti-mouse c-kit APC-Cy7	2B8	Rat IgG2b, κ	BioLegand	105826
19	Anti-mouse CD51 PE	RMV-7	Rat IgG1,κ	BD Biosciences	551187
20	Anti-mouse CD61 PE	2C9.G3	Arm. Ham. IgG	eBioscience	12-0611-83
21	Anti-mouse CD61 AF647	2C9.G2 (HMβ3-1)	Arm. Ham. IgG	BioLegand	104314
22	Anti-mouse Sca-1 BB700	D7	Rat IgG2a,κ	BD Biosciences	742089
23	Anti-CD150 PE-Cy7	TC15-12F12.2	Rat IgG2a,λ	BioLegand	115914
List of Isotype control antibodies used					
Sr. No.	**Antibody**	**Clone**	**Company**	**Catalog no.**	
1	FITC Rat IgG2b,κ	eB149/10H5	eBioscience	11-4031-82	
2	FITC Arm Ham. IgG	eBio299Arm	eBioscience	11-4888-81	
3	FITC Rat IgG2a,κ	eBR2a	eBioscience	11-4321-82	
4	APC Rat IgG 2b,κ	eB149/10H5	eBioscience	17-4031-82	
5	PerCP-Cy5.5 Rat IgG2a, κ	eBR2a	eBioscience	45-4321-80	
6	PECy7 Rat IgG2a	2A3	Tonbo biosci.	60-4321-U100	
7	APC Arm Ham. IgG	eBio299Arm	eBioscience	17-4888-82	
8	APCCy7 Rat IgG 2b,κ	eB149/10H5	eBioscience	17-4031-82	
List of primers used					
1	Genotyping Postn mice (F)	GGTGCTTCTGTAAGGCCATC			
2	Genotyping Postn mice (R)	GTGAGCCAGGACCTTGTCATA			
3	Genotyping Postn mice (Int-as)	AGCACTGACTGCGTTAGCAA			
4	Genotyping Itgav mice (F)	GGTGACTCAATCTGTGACCTTCAGC			
5	Genotyping Itgav mice (R)	CACAAATCAAGGATGACCAAACTGAG			
6	Genotyping Vav-iCre mice (F)	CCATGGCACCCAAGAAGAAG			
7	Genotyping Vav-iCre mice (R)	GCTTAGTTTTCCTGCAGCGG			
8	Mm Postn (F)	AGTTTGTTCGTGGCAGCACCTT			
9	Mm Postn (R)	TCGTCATTGCAGGTCCTTCCGT			

Suppl. Fig. 1



Supplementary Figure 1. POSTN is expressed in myofibroblasts in adult spleen

(A) Isotype control for anti-mouse POSTN (Goat IgG), anti-mouse α-SMA (Rabbit IgG) and anti-mouse CD169 (Rat IgG2aκ) antibodies. Upper panel shows vascular region and lower panel shows white pulp region corresponding to the ones presented in Figure 1A-D. (scale bar=20μm)

(B) Isotype control for anti-mouse POSTN (Goat IgG), anti-mouse α-SMA/Laminin/Col IV (Rabbit IgG) and anti-mouse CD31 (Biotin IgG2aκ). Upper panel shows vascular region and lower panel shows trabecular region corresponding to the ones presented in Figure 1E and 2A. (scale bar=20μm)

(C) Isotype control for anti-mouse POSTN (Goat IgG), anti-mouse Laminin/Col IV (Rabbit IgG) and anti-mouse α-SMA (Mouse IgG2a). Upper panel shows vascular region and lower panel shows trabecular region corresponding to the ones presented in Figure 2B,C. (scale bar=20μm)

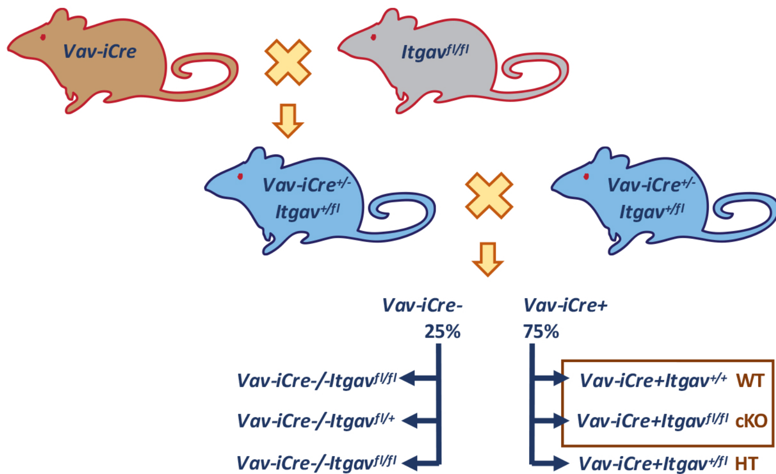
(D) POSTN expression was examined in the adult spleen tissues using immunohistochemistry on 10μm cryo-sections followed by fluorescence imaging using tile scan. Specific antibodies were used to identify the cells expressing POSTN, macrophages lining the WP areas were identified by using CD169 staining, nuclear counterstaining was done using Hoechst 33342. (n=2, N=8, scale bar=0.5mm).

(E) Scheme followed to gate the four sub-populations within the LSK cell population on the basis of isotype controls.

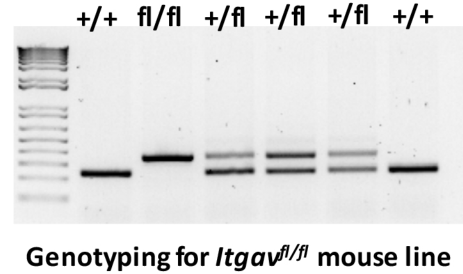
(F) Fluorescence minus one (FMO) controls to examine the proportion of HSPC sub-populations expressing ITGAV (upper panel) and ITGB3 (lower panel). The sub-populations were identified based on the expression of CD150 and CD48 within LSK fraction. Isotype control antibodies for ITGAV and ITGB3 were used to set the gates.

Suppl. Fig. 2

A.



B.



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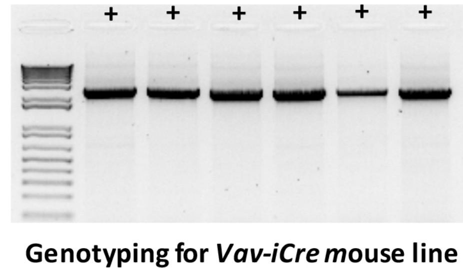
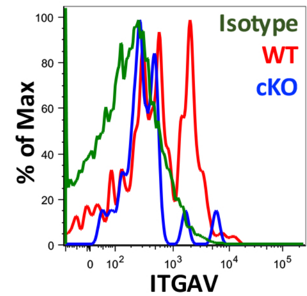
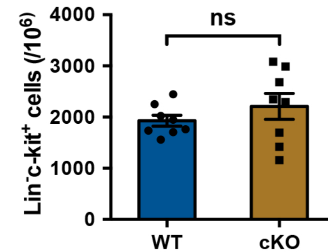


D.



E.



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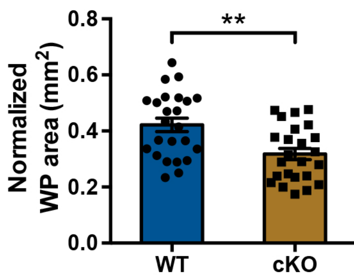
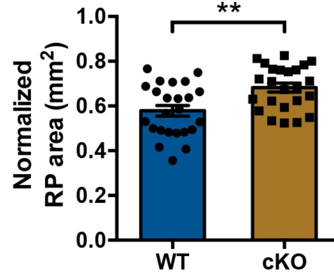
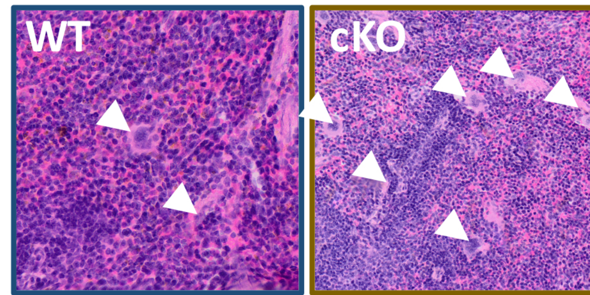


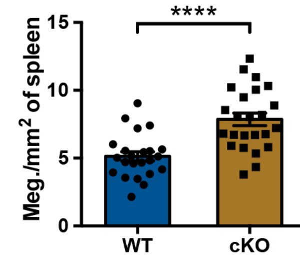
G.



H.



I.



Supplemental Figure 2. *Itgav* deletion leads to poorer lymphopoietic function

(A) Schematic representation of breeding scheme used to generate *Vav-iCre*$^{+}$*;Itgav*$^{+/+}$ (WT) and *Vav-iCre*$^{+}$*;Itgav*$^{-/-}$ (cKO) mice. *Vav*-iCre and *Itgav*$^{fl/fl}$ mice were crossed to delete *Itgav* in the hematopoietic cells.

(B,C) Genotyping PCR performed on tail-tip DNA to identify *Vav-iCre*$^{+}$*;Itgav*$^{+/+}$ (WT), *Vav-iCre*$^{+}$*;Itgav*$^{fl/+}$ (HT) and *Vav-iCre*$^{+}$*;Itgav*$^{-/-}$ (cKO) mice. PCR was performed to identify Itgav^{fl}/Itgav^{+} (B) and *Vav-iCre* (C) alleles, separately.

(D) Confirmation of *Itgav* deletion in HSPC population by flow cytometry. Spleen derived MNCs were immunostained to detect ITGAV expression on HSPCs (LSK cells). Cell surface expression of ITGAV in LSK population was compared between *Vav-Itgav*$^{+/+}$ and *Vav-Itgav*$^{-/-}$ samples.

(E) Mononuclear cells from *Vav-Itgav*$^{+/+}$ (WT), *Vav-Itgav*$^{-/-}$ (cKO) spleen tissues were used for the analysis of HSPC (lin^{-}c-kit^{+} cells) frequency by flow cytometry (n=8).

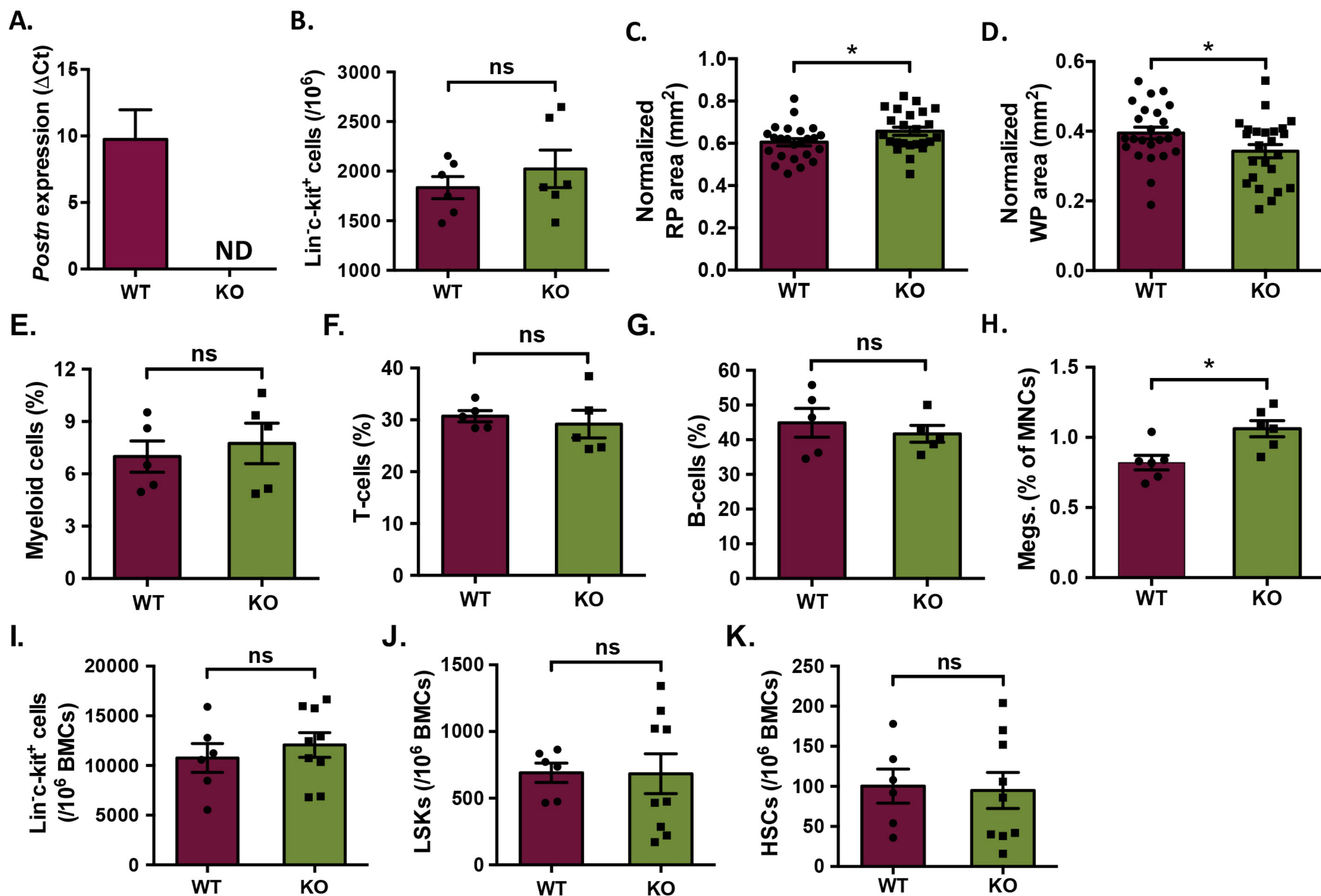
(F,G) Spleen tissues isolated from WT and cKO animals were harvested. The formalin-fixed, paraffin-embedded tissues were used to cut 10 µm sections that were used for H&E staining. (F) The total cross-sectional area under white pulp (WP), and (G) red pulp (RP) was compared between WT and cKO spleen tissues (n=4, N=24).

(H) Histological examination of the WT and cKO spleen sections to identify megakaryocytes (white arrow heads) on the basis of morphological features.

(I) Comparison of megakaryocyte frequency in spleen sections per mm^2 of the total cross-sectional area. (n=4, N=24)

Unpaired two tailed Student's t-test was performed. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, ns indicates not significant.

Suppl. Fig. 3



Supplemental Figure 3. *Postn* deletion leads to poorer lymphopoietic function

(A) Confirmation of lack of *Postn* expression in spleen tissue. Splenocytes were magnetically sorted for $lin^{-}CD45^{-}$ fraction and quantitative RT-PCR was performed to quantify *Postn* expression.

(B) Comparison between the WT and KO spleen tissues for the frequency of hematopoietic stem and progenitor cell population ($lin^{-}c\text{-}kit^{+}$ cells) by flow cytometry.

(C,D) Spleen tissues isolated from WT and KO animals were harvested. The formalin-fixed, paraffin-embedded tissues were used to cut 10 µm sections that were used for H&E staining. (C) The total cross-sectional area under red pulp (RP), and (D) white pulp (WP) per mm^2 of the total spleen cross-sectional area was compared between WT and KO spleen tissues (n=4, N=24).

(E-G) Flow cytometry analysis of the spleen mononuclear cells for the proportion of (E) myeloid cells (CD11b+/Gr-1+), (F) T cells (CD4+/CD8+), (G) B-cells (B220+).

(H) Flow cytometry analysis of the spleen mononuclear cells (MNCs) for the frequency of megakaryocytes identified as $CD41^{+}$ cells using specific antibodies.

(I-K) CFU-S_{12} assay was performed to assess the HSC support potential of spleen. *Postn*$^{+/+}$ (WT) and *Postn*$^{-/-}$ (KO) mice received WT BM cells following lethal dose of irradiation. After 12 days of grafting, spleen colonies were enumerated along with BM analysis for the stem cell sub-populations by flow cytometry. Quantification of frequency of various hematopoietic stem and progenitor cell populations; (I) $lin^{-}c\text{-}kit^{+}$ cells, (J) LSK cells, (K) primitive HSCs.

Unpaired two tailed Student's t-test was performed. n=3, N=9, t test: ns indicates not significant with $p \geq 0.05$.