SUPPLEMENTARY APPENDIX

CD150^{high} CD4 T cells and CD150^{high} regulatory T cells regulate hematopoietic stem cell quiescence via CD73

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 doi:10.3324/haematol.2018.198283

Received: May 23, 2018.

Accepted: December 10, 2018.

Pre-published: December 13, 2018.

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Supplemental Figure 1. CD73 regulates HSC quiescence and abundance.

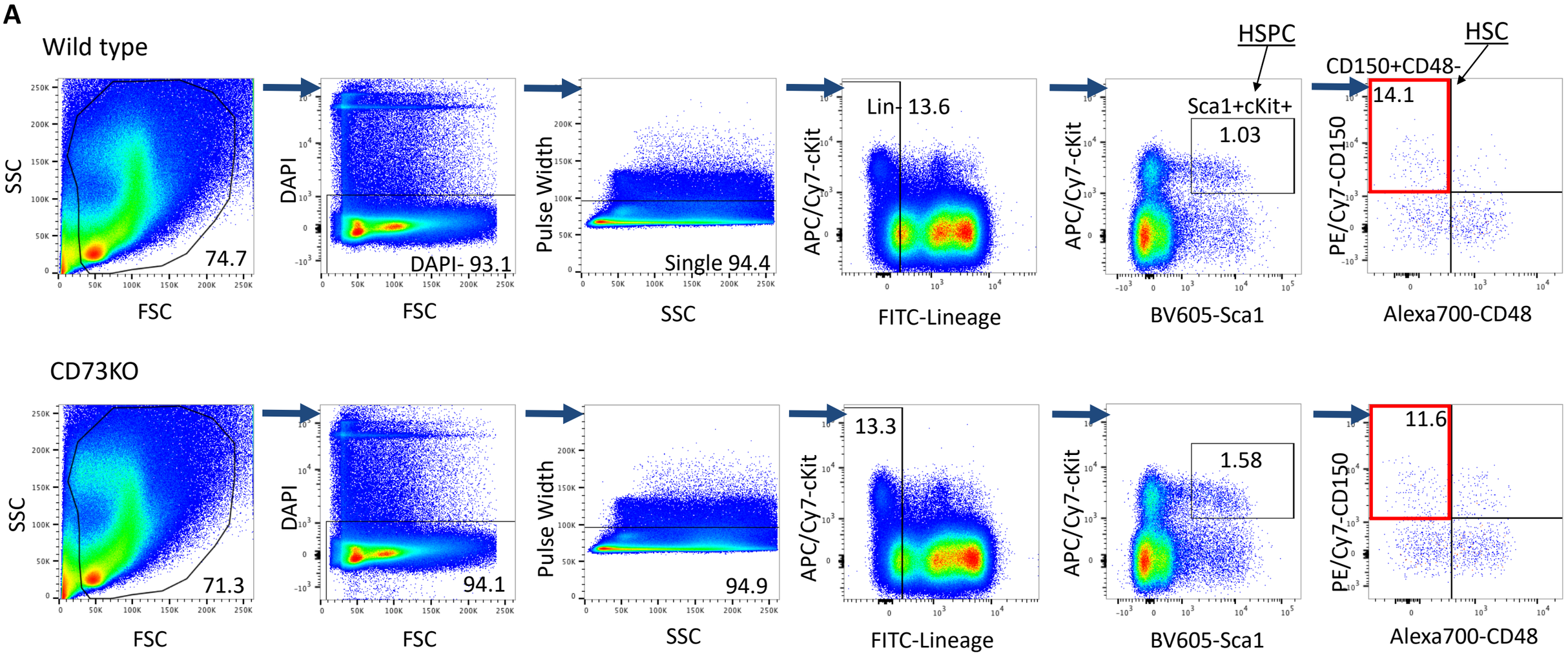
A. Gating strategy in flow cytometric analysis of HSCs and HSPCs in CD73 KO mice and wild type mice.

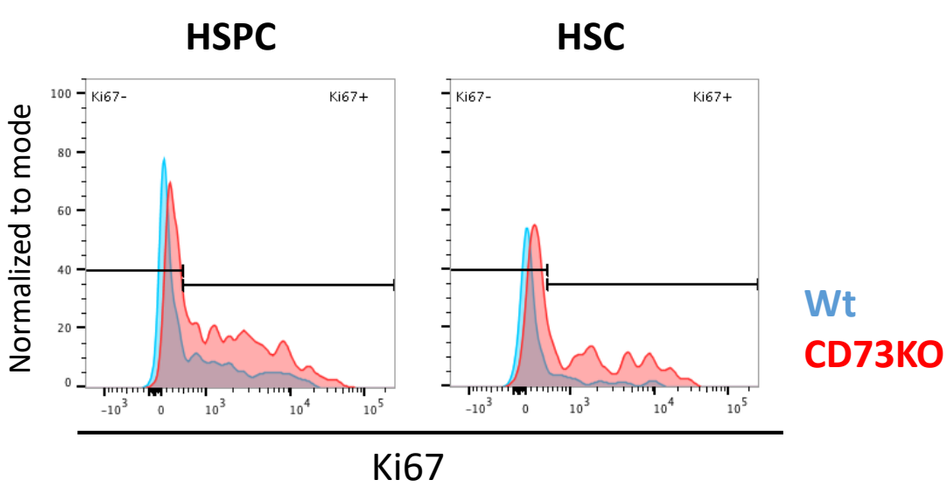
B. Representative histogram showing Ki67 staining levels in HSPCs and HSCs of CD73 KO mice and wild type mice.

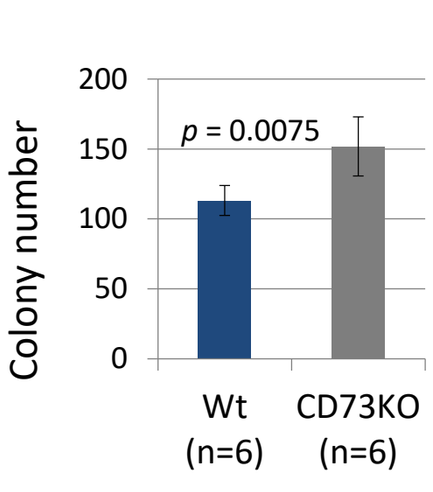
C. Numbers of colony formed following in vitro culture of BM cells isolated from CD73 KO or control mice.

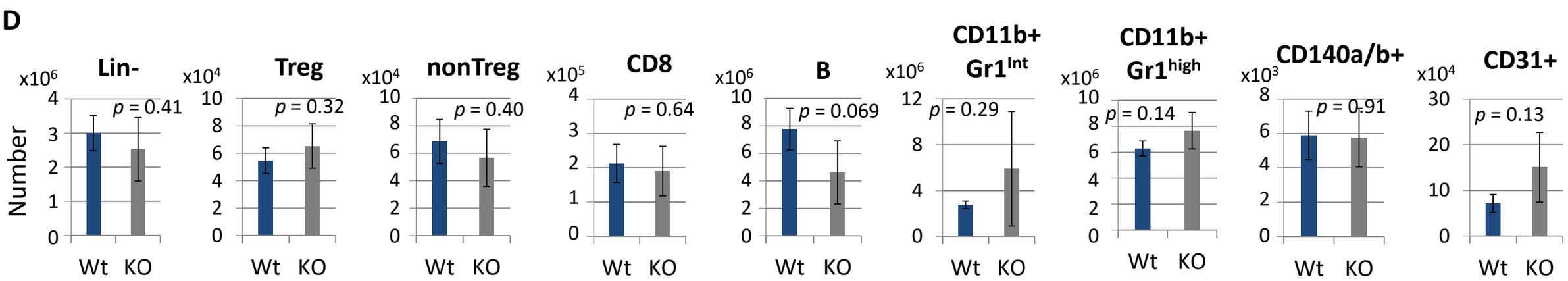
D. Numbers of various BM cell populations in CD73 KO and wild type mice. CD140a/CD140b+: CD45-CD140a+CD140b+CD31-. CD31+: CD45-CD31+. Refer to Figure 3 and Supplemental Figure 3.

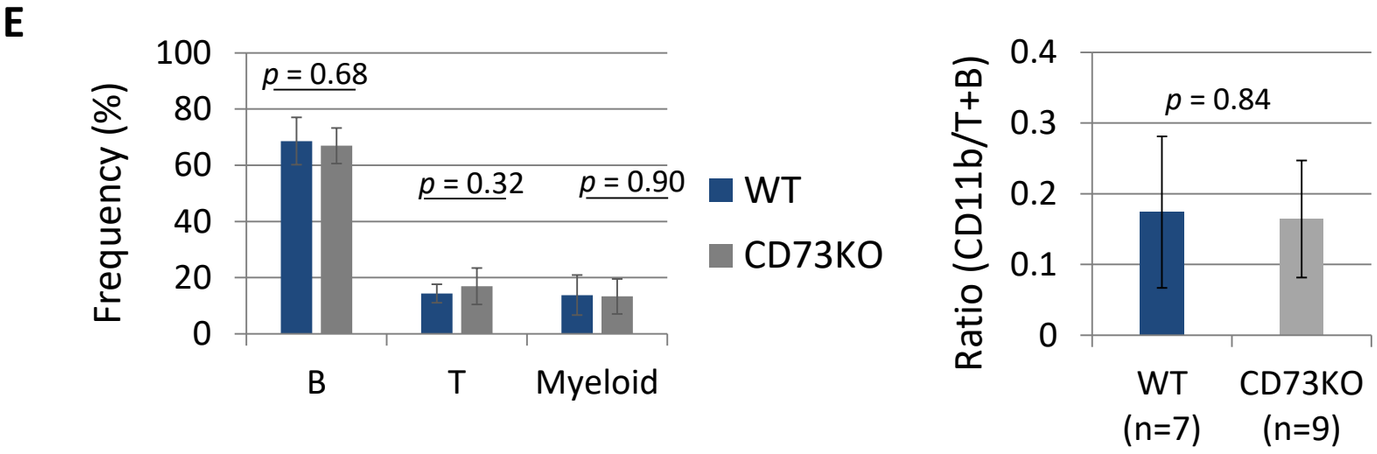
E. Frequencies of myeloid (CD11b+), T (TCRb), and B cells (B220+) in donor-derived hematopoietic cells four months after transplantation of BM cells from CD73 KO or control mice into lethally irradiated B6 SJL mice.







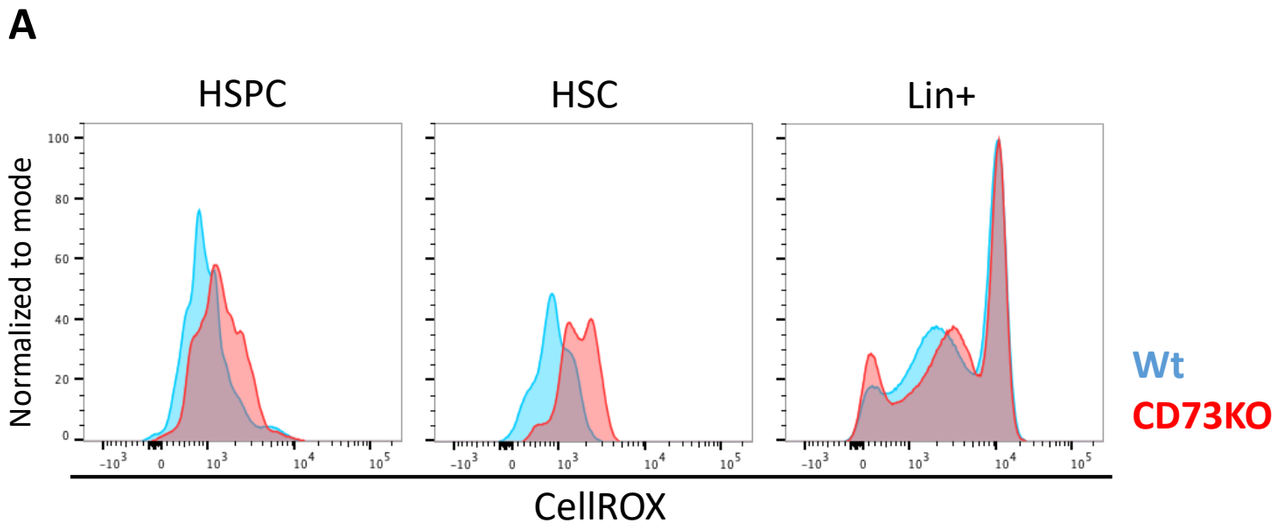


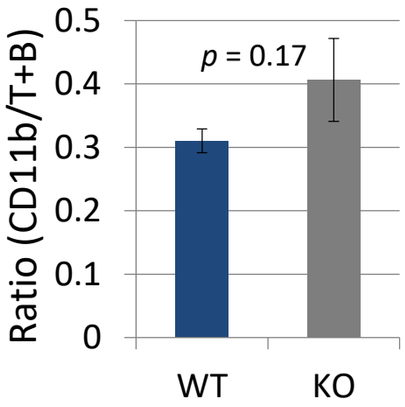


Supplemental Figure 2. Flow cytometric analysis of HSCs and peripheral blood in CD73 KO mice.

A. Representative histograms showing ROS levels in HSCs, HSPCs, and Lin- cells in CD73 KO mice and wild type mice. Flow cytometric analysis was performed following incubation of BM cells with 2uM CellROX Deep Red (Invitrogen) for 30 minutes.

B. Ratios of myeloid to lymphoid (B and T) cells in the peripheral blood of CD73 KO mice (N=3).





Supplemental Figure 3. CD39 and CD73 expression levels in various BM cell populations.

A. Flow cytometric analysis of CD39 expression levels on different BM cell populations. Treg: CD4+CD3+NK1.1-FoxP3YFP+ cells. nonTreg: CD4+CD3+NK1.1-FoxP3YFP- cells. HSC: CD150+CD48-cKit+Sca1+Lin-. HSPC: cKit+Sca1+Lin-. CD8: CD8+CD3+NK1.1-T cells. NK: NK1.1+ CD3- cells. CD4NKT: NK1.1+CD3+CD4+. B cell: B220+ cells.

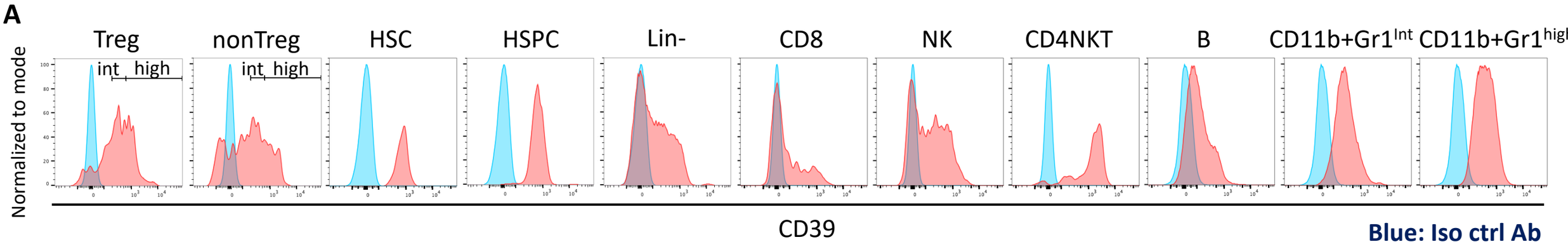
B. Gating strategy to define Tregs and CD4+ nonTregs.

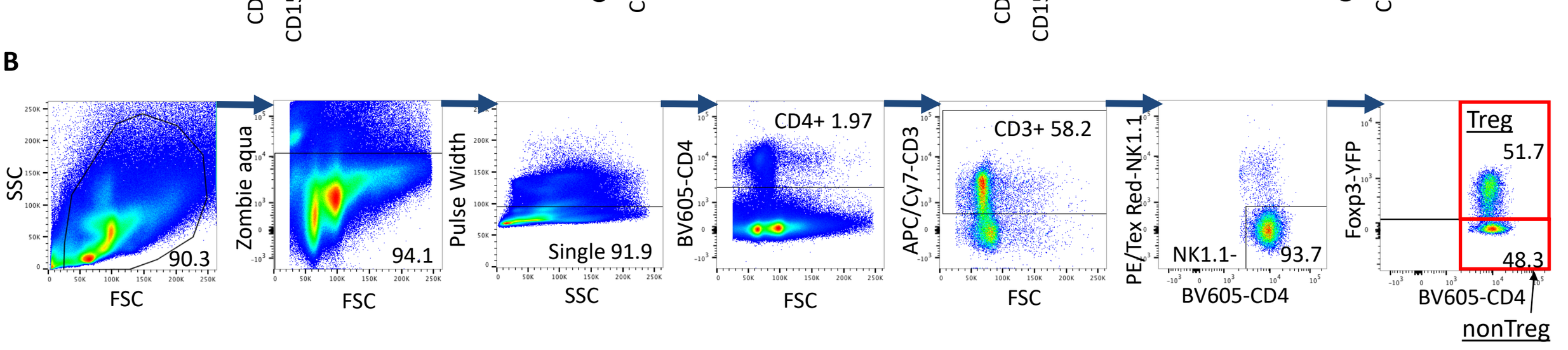
C. Frequencies of effector memory ($CD44^{high}CD62L^{low}$), central memory ($CD44^{high}CD62L^{high}$), naive ($CD44^{low}CD62L^{low}$) T cells within CD4+ nonTregs.

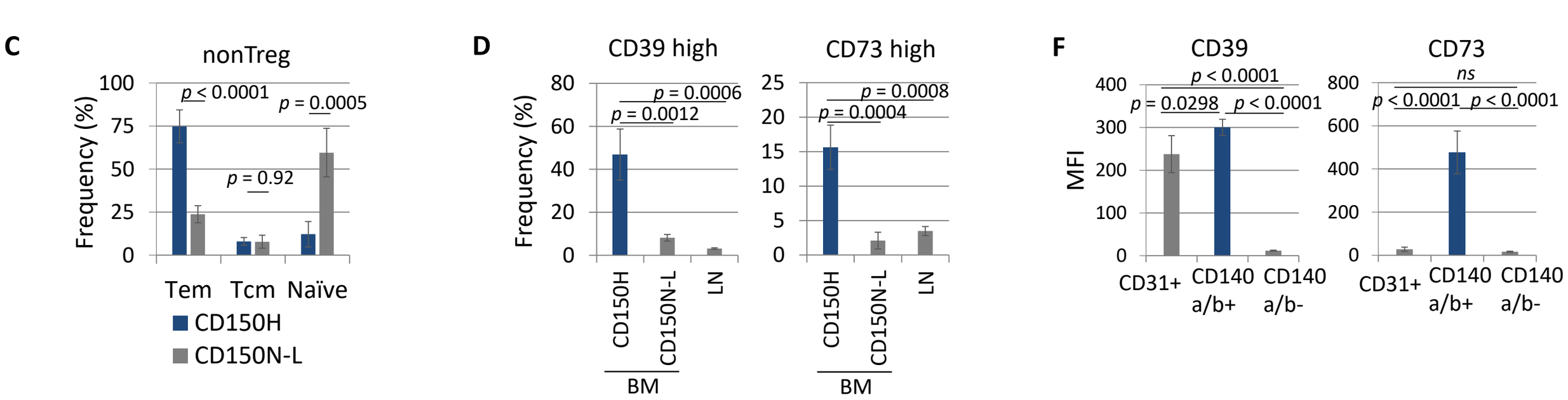
D. Frequencies of $CD39^{high}$ or $CD73^{high}$ cells in BM $CD150^{high}$ nonTregs, BM $CD150^{neg\text{-}low}$ nonTregs, and LN CD4+ nonTregs.

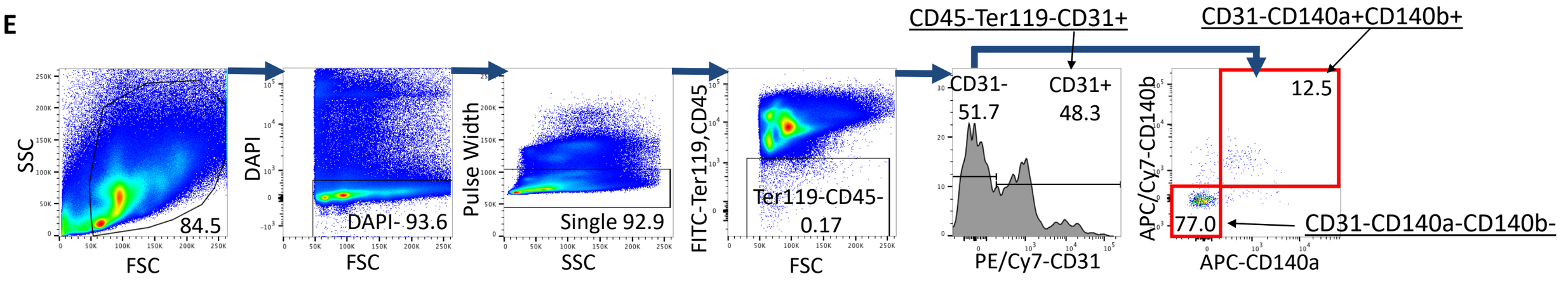
E. Gating strategy to define the following three mesenchymal cell populations: CD140a+CD140b+CD31-CD45- cells; CD140a-CD140b-CD31-CD45- cells; and CD31+CD45- cells.

F. Mean fluorescence intensity (MFI) of CD39 and CD73 expression levels by CD140a+CD140b+CD45- cells (CD140a/b+); CD140a-CD140b-CD45- cells (CD140a/b-); and CD31+CD45- cells (CD31+).







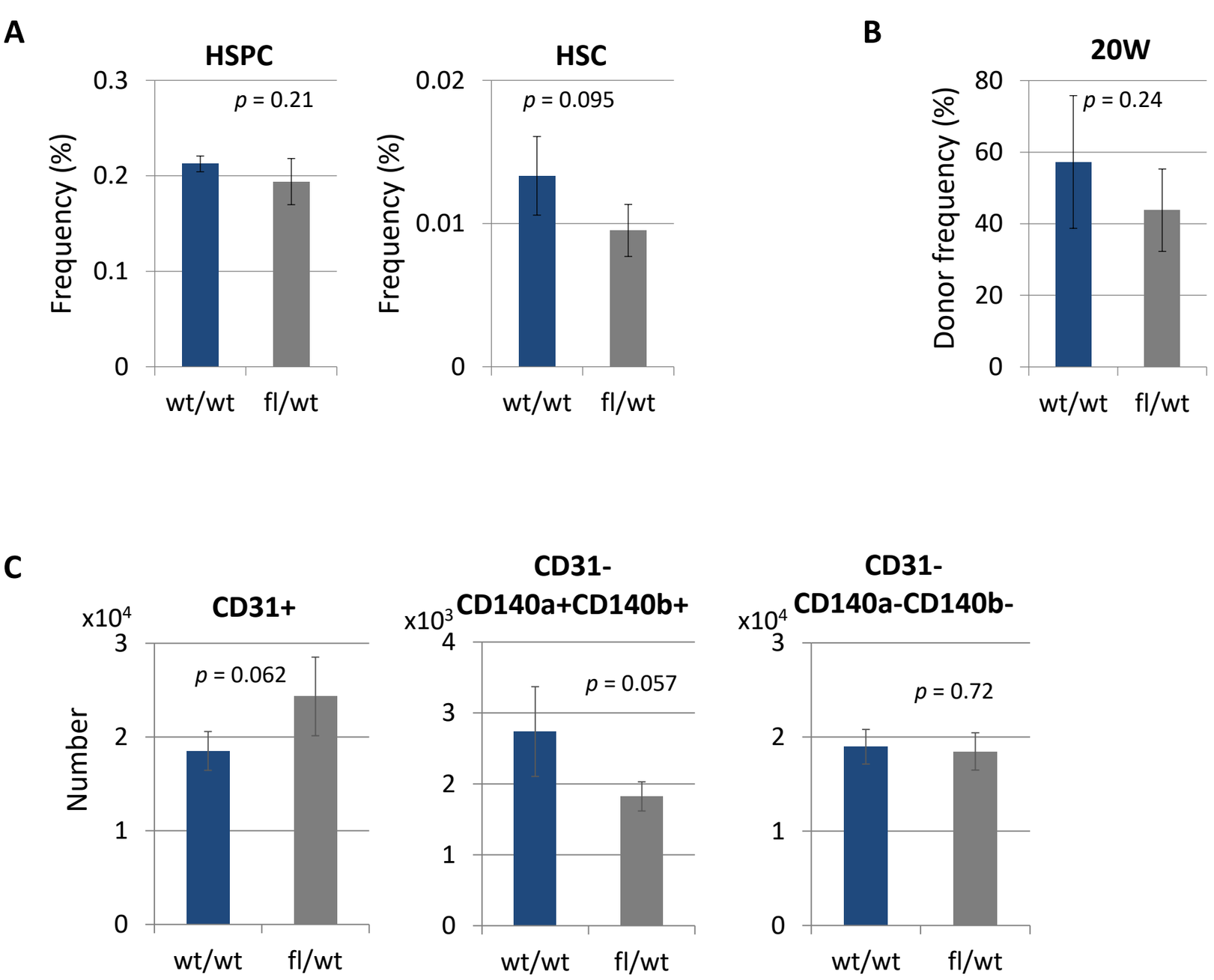


Supplemental Figure 4. Conditional deletion of CD39 in lepr+ cells did not influence HSC pool size.

A. Flow cytometric analysis of HSC frequencies in $lepr^{cre}$ $CD39^{fl/wt}$ mice. Experiments were performed using four to five mice per group. Data are presented as mean ± SD. Data analyzed by 2-tailed t-test.

B. Donor chimerism frequencies in the peripheral blood of lethally irradiated mice receiving wild-type SJL BM cells (CD45.1) together with BM cells of $lepr^{cre}$ $CD39^{fl/wt}$ or control mice (CD45.2). BM cells from each donor mouse (four to five) were transplanted into one to two recipient. Data are presented as mean ± SD. Data analyzed by 2-tailed t-test.

C. Numbers of mesenchymal cell populations (CD140a+CD140b+CD45- cells, CD140a-CD140b-CD45- cells, and CD31+CD45- cells) in $lepr^{cre}$ $CD39^{fl/wt}$ mice. Data are presented as mean ± SD. Data analyzed by 2-tailed t-test.



Supplemental Table 1. HSPC frequencies and numbers and BM cellularity of CD73 KO or B6 control mice.
We analyzed the numbers of total BM cells isolated from one tibia and one femur by crushing. Data from the second experiment are shown in Figure 1C.

				Frequency (%)		Cell Number	
		Mouse	BM cell number	KSL	CD150+CD48-KSL	KSL	CD150+CD48-KSL
Wt	1st experiment	#1	24960000	0.19	0.019	47424	4742
		#2	26240000	0.15	0.018	39360	4723
		#3	23360000	0.14	0.017	32704	3971
	2nd experiment	#4	23040000	0.16	0.018	36864	4147
		#5	23040000	0.16	0.022	36864	5069
		#6	22400000	0.16	0.019	35840	4256
	3rd experiment	#7	23040000	0.16	0.018	36864	4147
		#8	22320000	0.16	0.019	35712	4241
		#9	22680000	0.13	0.019	29484	4309
73KO	1st experiment	#1	33600000	0.32	0.027	107520	9072
		#2	34240000	0.28	0.023	95872	7875
		#3	33280000	0.32	0.026	106496	8653
	2nd experiment	#4	27200000	0.35	0.026	95200	7072
		#5	28160000	0.26	0.025	73216	7040
		#6	28480000	0.26	0.024	74048	6835
	3rd experiment	#7	28080000	0.21	0.021	58968	5897
		#8	27360000	0.21	0.023	57456	6293
		#9	29520000	0.22	0.02	64944	5904