Supporting information for:

CD11c regulates hematopoietic stem and progenitor cells under stress

Short title: CD11c and HSPCs

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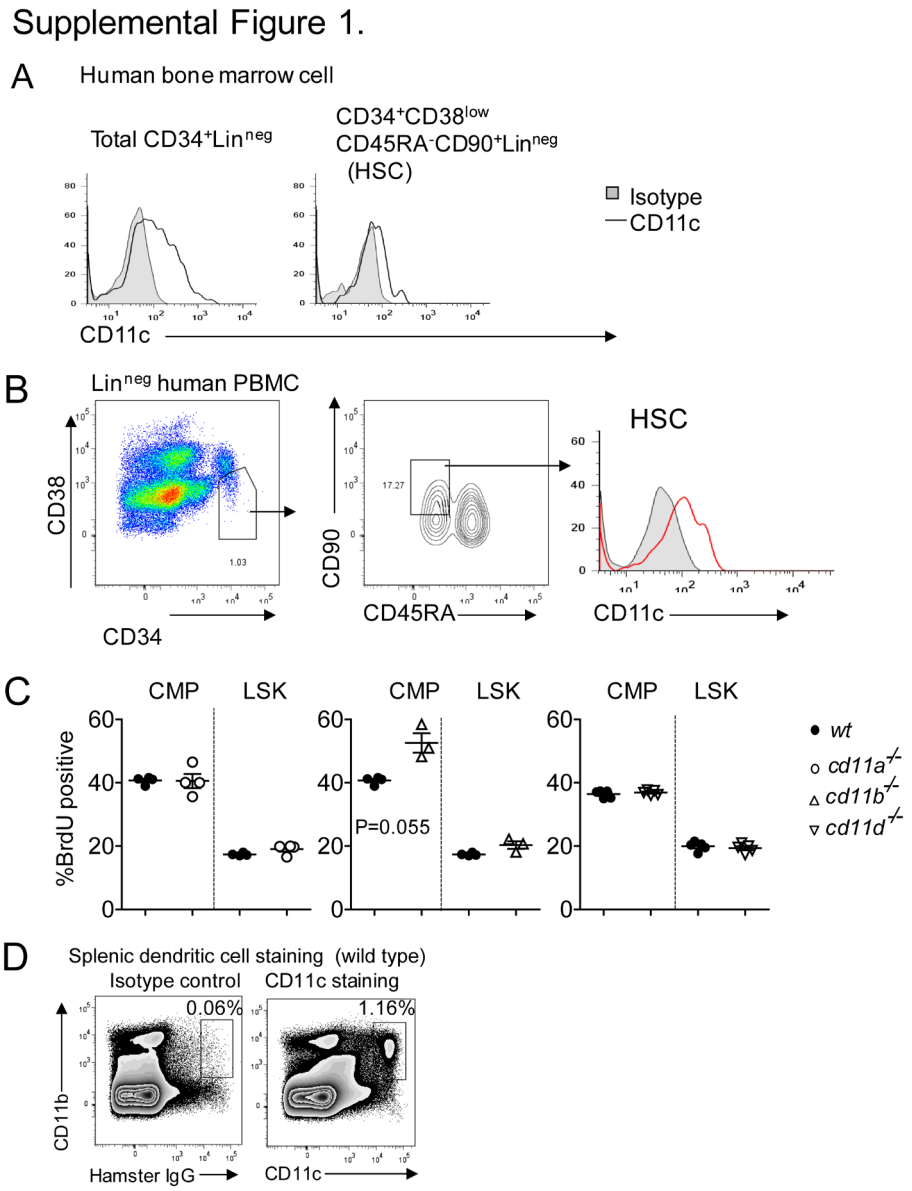
This supplementary material includes the following:

Supplemental Figure 1-3, supplemental Table 1

Supplementary Figures.



Supplemental Figure 1. CD11c expression profiles in human bone marrow cells and proliferation of naïve β2 integrin $^{-/-}$ mice

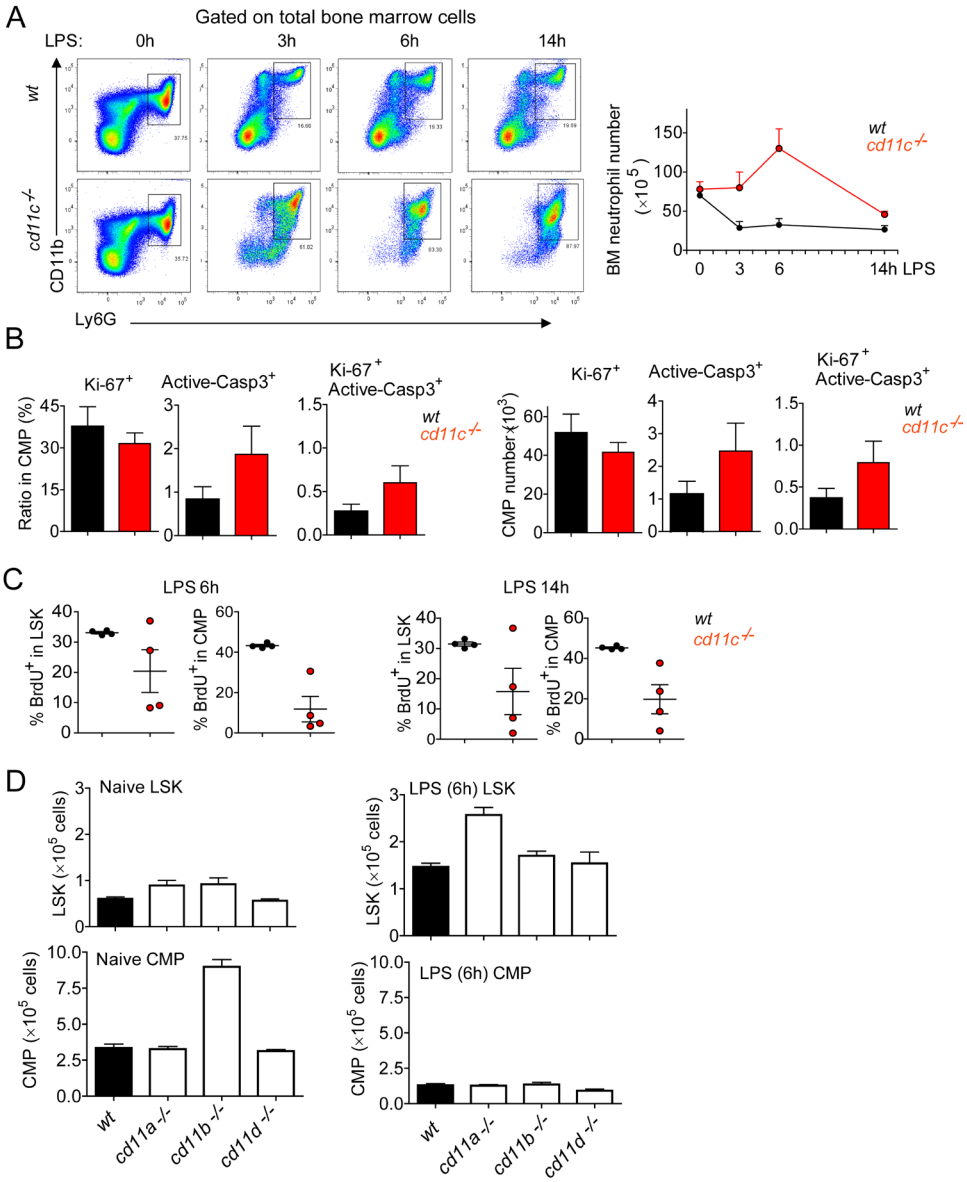
(A) The expression of CD11c on human bone marrow HSC.

(B) The expression of CD11c on human peripheral blood HSC. At the most right, Red line indicates anti-CD11c antibody, and black line indicates isotype control.

(C) BrdU incorporation analysis. All of WT, CD11a$^{-/-}$, CD11b$^{-/-}$, and CD11d$^{-/-}$ mice were pulsed with BrdU for 4 hours, and then were analyzed for BrdU incorporation in the Lineage ^{negative} bone marrow cells. Symbols indicate individual mice. Depicted data are mean ± SEM. Shown is the representative of two experiments with the same pattern. Student's t-test was used for statistical analysis.

(D) CD11c staining of splenic dendritic cells of wild type mice. Shown are representative flow cytometry plots from at least 3 independent experiments with the same pattern.

Supplemental Figure 2.



Supplemental Figure 2. LSK and CMP of β2 integrin member$^{-/-}$ mice subjected to systemic LPS injection

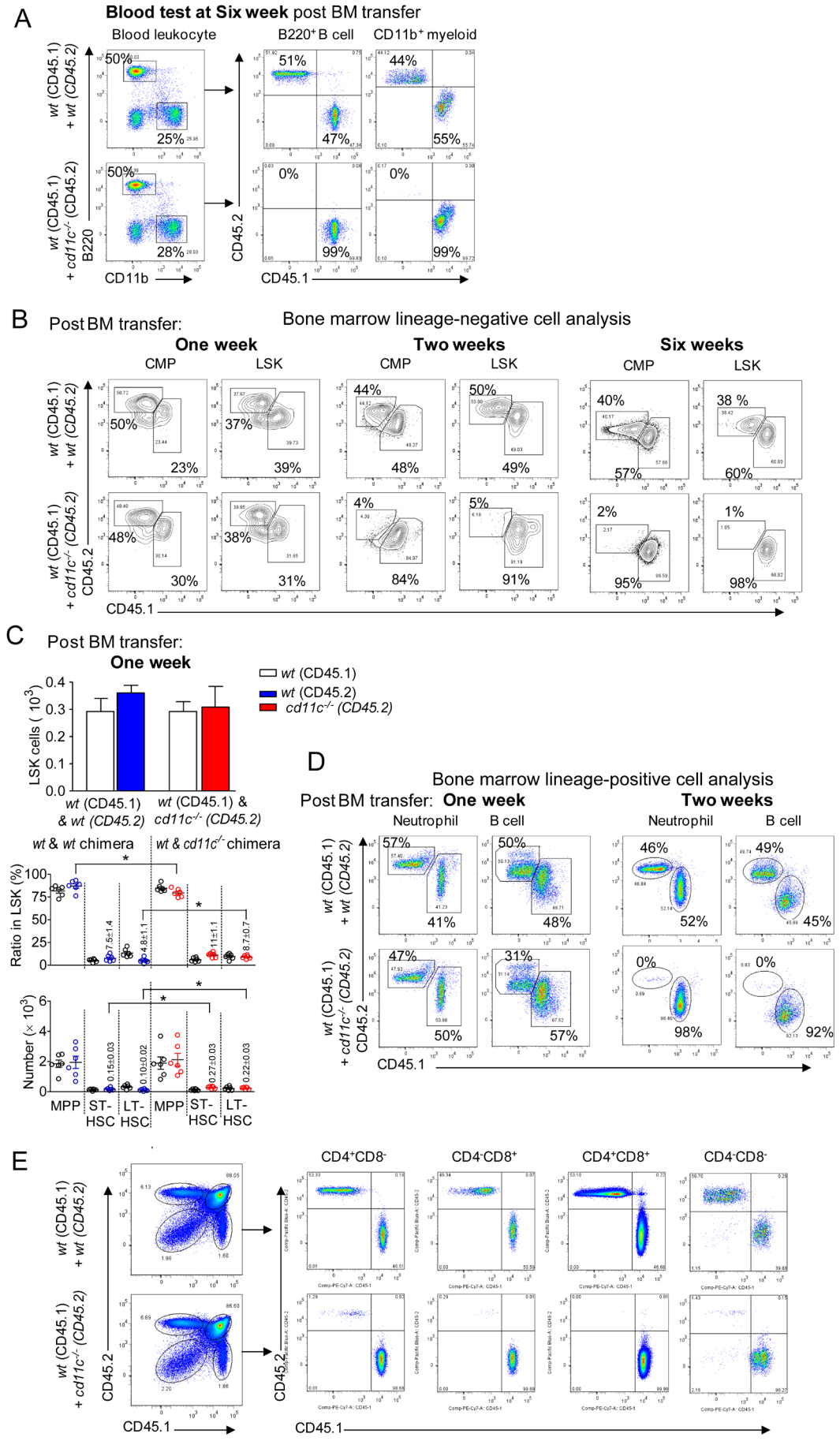
(A) Left: representative flow cytometry plot showing BM neutrophil at different time points post LPS injection (*i.v.* 10 mg/kg). Right: Dynamics of BM neutrophil numbers of WT and CD11c$^{-/-}$ mice after LPS injection. Data were shown as mean ± S.E.M of 3-6 mice per group per time point.

(B) Both WT and CD11c$^{-/-}$ mice were euthanized at 3 hours after LPS injection. Frequency (left-panel) and absolute number (right-panel) of Ki67^{+}, active-caspase3^{+}, and Ki67^{+}/active-caspase 3^{+} in CMPs

(C) Frequency of BrdU^{+} LSK and CMP cells in WT and CD11c$^{-/-}$ mice at 6h and 14h after LPS injection (*i.v.* 10 mg/kg). Data are representative of two experiments. Symbols represent individual mice. Data were shown as mean ± SEM.

(D) The number of LSK and CMP of naïve and LPS injected mice. We examined in WT, CD11a$^{-/-}$, CD11b$^{-/-}$ and CD11d$^{-/-}$ mice. Data were shown as mean ± SEM.

Supplemental Figure 3



Supplemental Figure 3. CD11c does not affect homing of bone marrow cells.
Chimeric mice were made by transplanting the mixture of CD45.1 WT and CD45.2 WT bone marrow (BM) cells or CD45.1 WT and CD45.2 CD11c$^{-/-}$ BM cells at the ratio of 1:1 into lethally irradiated recipient mice.

(A) At 6 weeks post BM transplantation, peripheral blood from recipient mice was collected and subjected to flow cytometry analysis. Shown are representative of 5 mice in both groups.

(B) BM cells from recipient mice at 1, 2, and 6 weeks post BM transplantation were collected and subjected to flow cytometry analysis. Shown are representative flow cytometry plots of LSKs and CMPs from different originations differentiated by congenic markers. N=5 in each time point; experiment was repeated at least three times with exactly the same pattern.

(C) Up panel: Cell number showing that WT(CD45.1)/WT(CD45.2) and WT(CD45.1)/ CD11c$^{-/-}$ (CD45.2) chimeras have the comparable LSK cell constitution at one week after bone marrow transplantation. Experiments were repeated three times with the same pattern. Bottom Panel: LSK cells were further analyzed for MPP, ST-HSC and LT-HSC by staining CD150 and CD48. Symbol indicates individual mouse. Statistical analysis was performed using one-way ANOVA with *post hoc* Bonferroni analysis. *$p<0.05$.

(D) FACS plots of bone marrow neutrophil and B cells in WT(CD45.1)/WT(CD45.2) and WT(CD45.1)/CD11c$^{-/-}$(CD45.2) chimeras at 1 and 2 weeks after BM transplantation. Shown are representative of 5 mice in both groups. Experiments were repeated three times with the same pattern.

(E) FACS plots of thymus of WT(CD45.1)/WT(CD45.2) and WT(CD45.1)/CD11c$^{-/-}$ (CD45.2) chimeras at 12 weeks after bone marrow transplantation. Shown are representative of 5 mice in both groups.

Supplemental Table 1. The list of up-and down-regulated genes in CD11c$^{-/-}$ LSK cells

Naïve LSK		LPS stimulated LSK					
Upregulated	**Downregulated**	**Upregulated**	**Downregulated**				
Efcab7	Ighv6-4	Ighv5-17	F8a	Mfn1	Atp13a1	Lrfn1	Pik3cb
Timp3	Igkv10-95	Ighv1-59	Trp53rka	Sytl2	Med25	Fam208a	Tnfsf11
Fcna	Igkv9-129	Iglv3	Hace1	B3galt6	Ighv5-9-1	Hnrnpl	Med16
Hba-a2	Igkv3-5	Ighv14-4	Slc19a2	Smyd2	Dynll2	Zbtb45	Chn2
C1qc	mt-Nd3	Igkv4-70	Spata5l1	Klhl42	St3gal5	Coro2a	Rala
Ltbp1	Icos	Ighv1-58	Tmem221	Gm15975	1700066M21Rik	Crybg1	Ap2a2
Mrc1	Gm31172	Ighv7-4	Dtwd2	Atp10a	Ccdc102a	Notch2	Pgls
Wfdc21	Ighv3-8	Ighv1-62-2	Gpr180	Pitpna	Ppa2	Fmnl3	Ubr2
Igkv6-20	Stab2	Il17f	Fgfr1	Mpnd	Trp53rkb	Chchd10	Dnttip1
Lhfp	Cd28	Ighv1-22	Nenf	Zfp12	Sh3kbp1	Stk35	Slc25a39
Igkv4-69	Ikzf3	Igkv6-23	Fut11	Thap11	Prr5	Ino80b	Ccni
Treml1	Pxdc1	Igkv4-68	B3gnt2	Brf1	Snx9	Tbc1d20	Ctdnep1
Podxl	Ighv1-5	Ighv7-1	Phf13	Bri3bp	Cnot11	Mdfic	Ttc14
Xcr1	A530040E14Rik	Iglc2	Lrrfip2	Asf1a	Eif5	Susd6	
Dmkn	Cnbd2	Ighv1-82	Cry1	Ubac1	Fam174b	Ptges2	
Igkv4-92	Igkv4-63	Hspb1	Egr1	D130058E05Rik	Malt1	Gm45294	
Rhag	Ighv5-15	C3	2300009A05Rik	Mcoln1	Mpped2	Fbxw2	
Tlr8	Rgs8	Ido1	Nsmce3	Ppm1g	Tef	Ptk7	
Igll1	Hist1h3f	Ighv1-39	C2cd2	Flvcr1	Dnajc1	Snip1	
Fndc7	Gm32554	G530011O06Rik	2-Mar	Tesmin	Edem3	Ip6k1	
Apol7c	Ighv9-4	Cd209a	2410022M11Rik	Prmt3	Ptger4	Lmo2	
Tlr11	Rasgrp3	Clca3a1	Sdccag3	Ighv4-1	Smarca2	Ccna2	
Sptb	Skap1	Saa3	Acot1	E2f4	Jun	Wtap	
Kcnj5	Igkv6-13	Mmp13	Gpt2	Map3k6	Myef2	Gspt1	
Ighv9-2	Gm11342	Steap4	Rab2a	Fam105a	Hgh1	Sema4b	
Siglec1	NA	Ccl12	Clasp2	Pmp22	Gm43672	Tcp11l1	
	Slc7a3	Ighv6-6	Mcu	Kctd13	Usp22	Carmil2	
	Tmtc1	Gm28438	Mybl2	Pgrmc2	Hmgxb3	Gfer	
	Ighv4-2	Ighv1-26	Cdk9	Emilin2	Rbm17	Eid1	
	Ighv1-67	C4b	Gmcl1	Gpam	Samd10	Mfsd12	
	Xlr3a	Hp	Fam13b	Smap2	Igll1	Slc25a25	
	Lrrc27	Epha4	Slc25a1	Hccs	Agpat2	Vamp2	
	Pkdrej	Zbtb32	Bola3	Specc1l	Gm24270	Camsap3	
	Trp53cor1	Cp	Acsl3	Zfp292	Zfp367	Rwdd1	
	D830025C05Rik	Ighv10-1	Atg2b	Arhgap10	Dhcr24	Plekhf2	
	Xlr3b	Ighv1-84	Tiparp	Man1a	Bckdhb	Cep131	
	Rorc	Il33	Ube2j2	Gtf3a	Cog3	Ei24	
	Ighg3		Sipa1	Afg3l2	Rab22a	B4galt1	