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Supplemental information

**CD244 expression represents
functional decline of murine hematopoietic
stem cells after *in vitro* culture**

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Supplementary Figure 1

A

Young mice
(10 weeks)



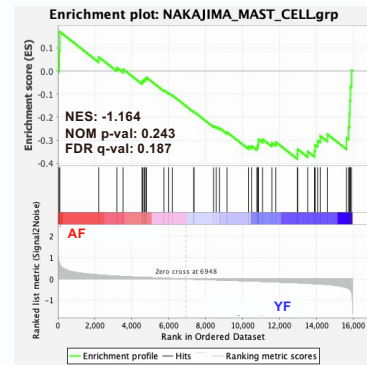
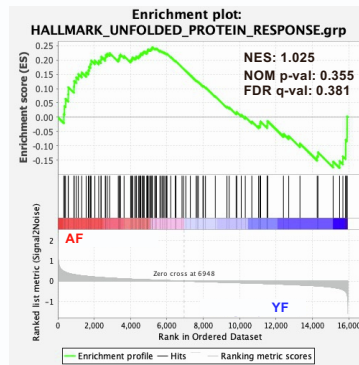
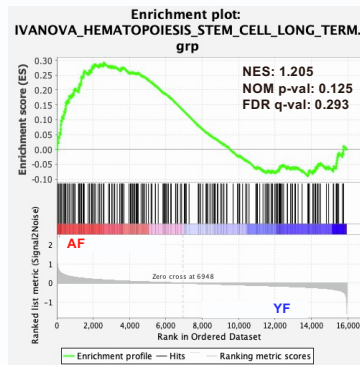
CD48⁺KSL (YF)

Aged mice
(18 months)



CD48⁺KSL (AF)

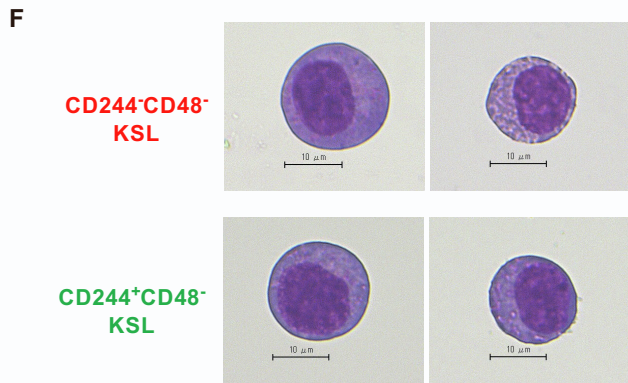
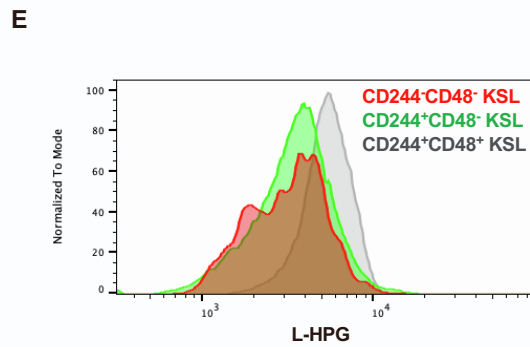
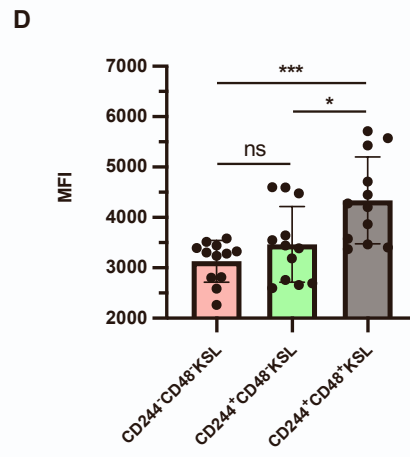
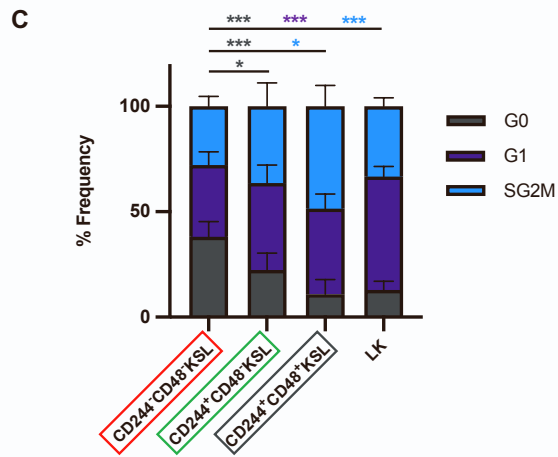
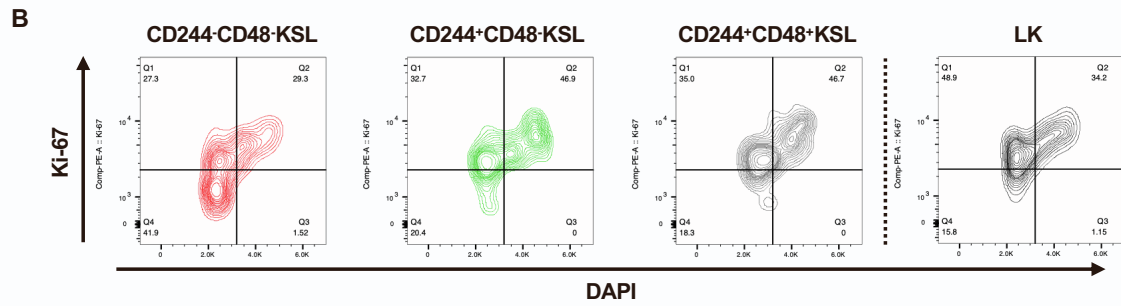
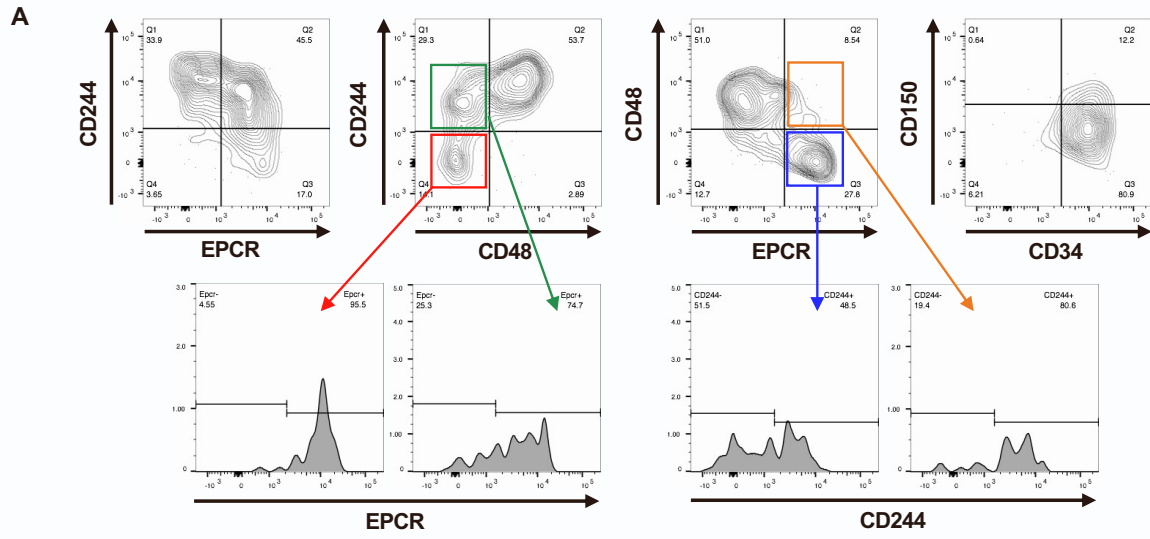
B



Supplementary Figure 1. HSC, UPR and mast cell signatures in aged HSC (Related to Figure 2)

(A and B) GSEA of the microarray data comparing young vs aged HSC. Five-hundred (500) CD48-KSL cells were sorted from BM of young (10 weeks old, YF) or aged (18 months old, AF) mice. Sorted cells were subjected to microarray analysis. NES: normalized enrichment score, NOM p-val: nominal p-value, FDR q-val: false discovery rate q-value.

Supplementary Figure 2



Supplementary Figure 2. Expression patterns of CD244, cell cycle and protein synthesis rate of cultured HSCs (Related to Figure 3)

(A) Expression patterns of CD244, EPCR, CD48, CD150 and CD34 on the cell surface of KSL cells after *in vitro* culture in the conventional condition. Representative FACS plots are shown. (B) Cell cycle analysis of CD244⁺HSC. CD150⁺CD48⁻CD244⁺KSL cells cultured for 7 days were stained with cell surface markers, fixed, and then stained with Ki-67 and DAPI. A representative FACS plots on KSL population are shown. (C) Summary of the cell cycle assay. * $p < 0.05$, *** $p < 0.001$. The color of asterisks represents different cell cycle phase. (D-E) Protein synthesis rate was measured with incorporation of L-HPG in cultured HSCs. Assay was performed on day 7 of culture with standard culture conditions. MFI (D) and representative histogram (E) of L-HPG staining in different populations are shown. * $p < 0.05$, *** $p < 0.001$. ns: not significant. (F) Morphology of CD244⁻CD48⁻KSL cells and CD244⁺CD48⁻KSL cells. The scale indicates 10 μm .

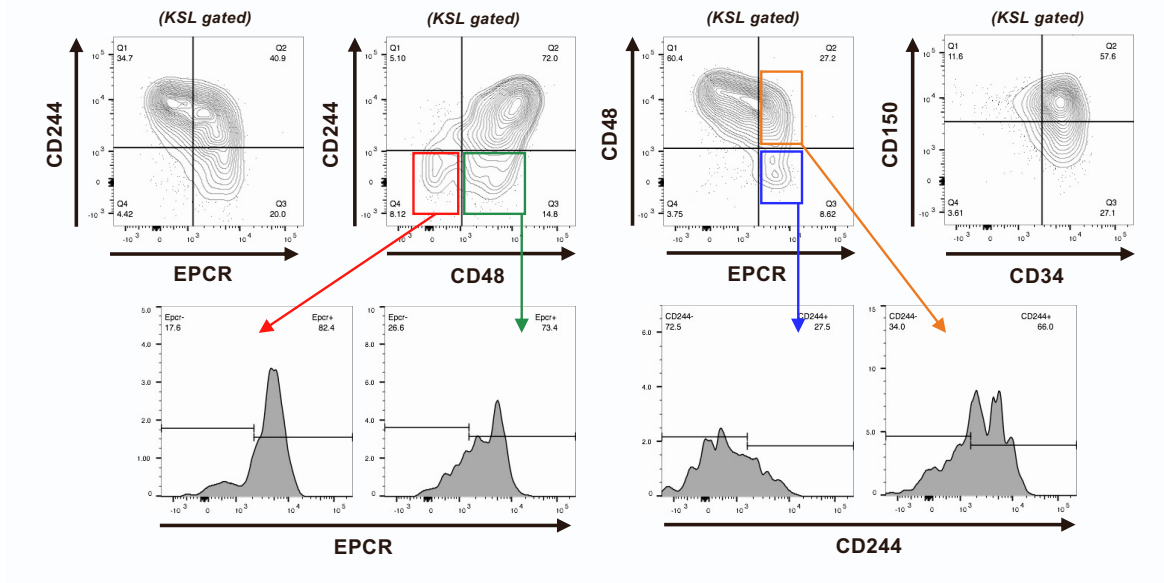
Supplementary Figure 3

Total	KSL	CD48+ CD244+	CD48- CD244+	CD48- CD244-	mSCF (ng/ml)	hTPO (ng/ml)
-0.82	-1.04	-0.74	-0.96	-1.36	1	1
-0.80	-0.97	-0.74	-0.90	-1.25	1	10
-0.72	-0.61	-0.58	-0.68	-0.31	1	50
-0.77	-0.68	-0.59	-0.68	-0.64	1	100
-0.80	-0.99	-0.74	-0.94	-1.19	10	1
-0.68	-0.63	-0.71	-0.69	-0.28	10	10
-0.19	0.23	0.13	-0.03	1.13	10	50
-0.11	0.49	0.64	0.31	0.96	10	100
-0.71	-0.86	-0.74	-0.82	-0.95	50	1
-0.15	-0.22	-0.61	-0.21	-0.01	50	10
0.73	0.93	0.29	0.98	1.01	50	50
1.50	1.71	2.03	1.68	1.49	50	100
-0.70	-0.86	-0.74	-0.80	-1.02	100	1
0.12	0.06	-0.26	0.10	0.06	100	10
1.75	1.32	0.85	1.52	0.76	100	50
2.35	2.10	2.51	2.10	1.61	100	100

Supplementary Figure 3. ER stress induction and cytokine signals affect CD244 expression (Related to Figure 4)

A heatmap showing frequencies of different populations after 7 days culture with various cytokine concentrations. Mean values of z-score (n=3) in each fraction are displayed.

Supplementary Figure 4



Supplementary Figure 4. Expression of HSC markers after *in vitro* culture in PVA medium (Related to Figure 6)

Representative expression patterns of CD244, EPCR, CD48, CD150 and CD34 on the cell surface of KSL cells after *in vitro* culture in the PVA medium. Representative FACS plots are shown.