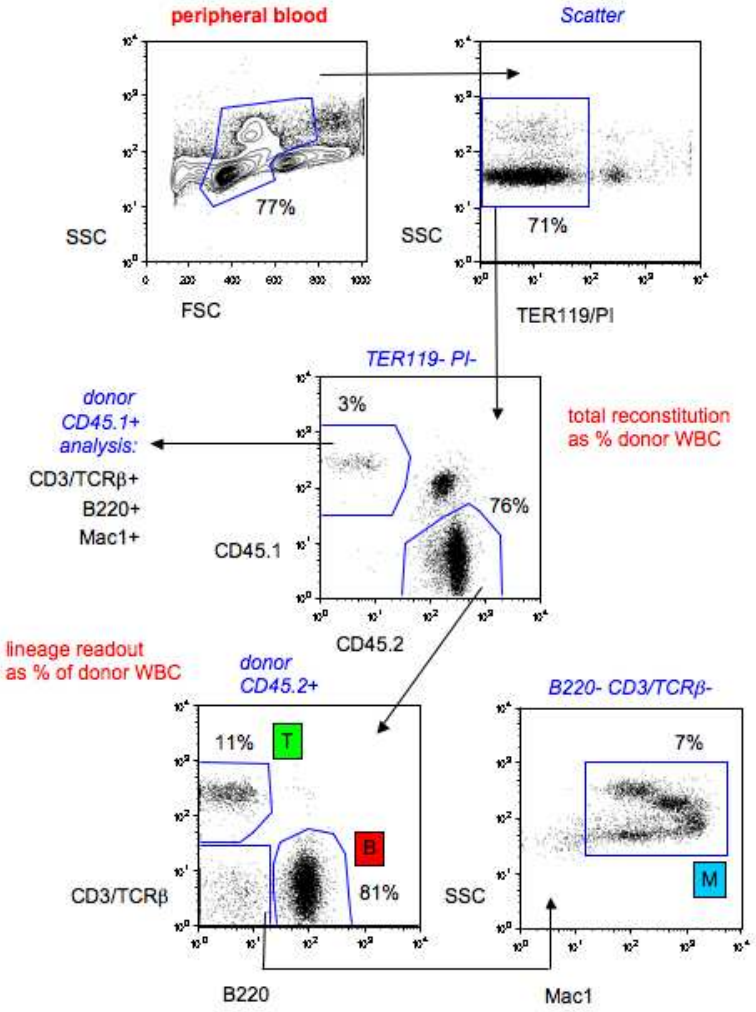
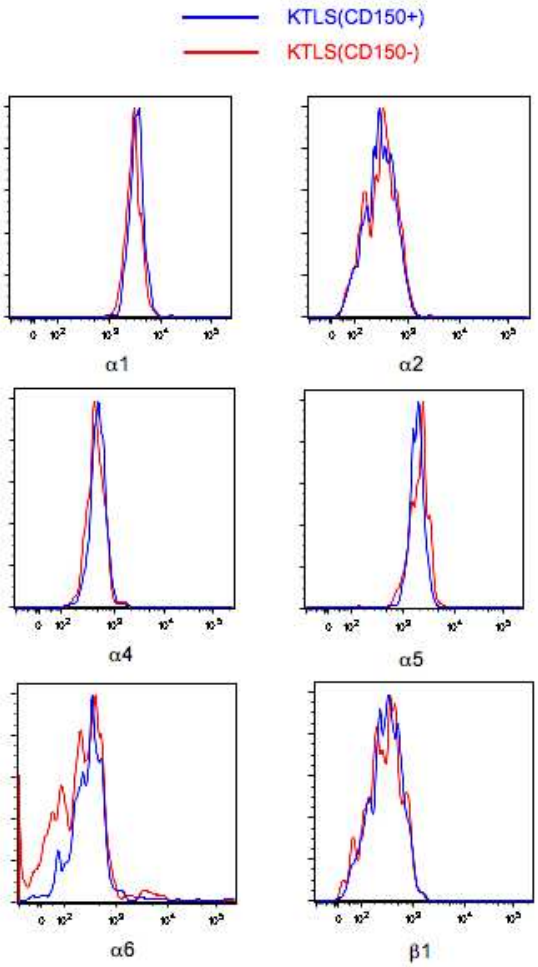


Supplementary Figure 1. Papathanasiou et al.



Supplementary Figure 2. Papathanasiou et al.

Supplementary Table 1. Competitive reconstitution of lethally irradiated recipient mice with KTLS donor cells reveals differences in long-term, multi-lineage engraftment with age of donor HSCs

age of donor cells	weeks post transplant	*n*	-ve mice	+ve mice	mice only showing output in lineage M	B	T	M/B	M/T	B/T	M/B/T
E12.5 FL	4	8	6	2	0	0	0	1	0	0	1
	8	8	6	2	0	0	0	1	0	0	1
	12	8	6	2	0	0	0	0	0	0	2
	16	5	4	1	0	0	0	0	0	0	1
E13.5 FL	4	7	2	5	0	0	0	1	0	0	4
	8	7	3	4	0	0	0	0	0	0	4
	12	7	3	4	0	0	0	1	0	0	3
	16	7	4	3	0	0	0	0	0	0	3
E14.5 FL	4	5	0	5	0	0	0	0	0	0	5
	8	5	0	5	0	0	0	0	0	0	5
	12	5	0	5	0	0	0	0	0	0	5
	16	5	1	4	0	0	0	0	0	0	4
E15.5 FL	4	9	1	8	0	0	0	1	0	0	7
	8	9	1	8	0	0	0	0	0	0	8
	12	9	1	8	0	0	0	0	0	0	8
	16	9	1	8	0	0	0	0	0	0	8
E16.5 FL	4	7	0	7	0	0	0	1	0	0	6
	8	6	0	6	0	0	0	0	0	0	6
	12	6	0	6	0	0	0	0	0	0	6
	16	6	0	6	0	0	0	0	0	0	6
E17.5 FL	4	4	0	4	0	0	0	0	0	0	4
	8	4	0	4	0	0	0	0	0	0	4
	12	4	0	4	0	0	0	0	0	0	4
	16	4	0	4	0	0	0	0	0	0	4
E18.5 FL	4	4	0	4	0	0	0	0	0	0	4
	8	4	0	4	0	0	0	0	0	0	4
	12	4	0	4	0	0	0	0	0	0	4
	16	4	0	4	0	0	0	0	0	0	4
6wk BM	4	10	0	10	0	0	0	3	0	0	7
	8	10	2	8	0	0	0	0	0	0	8
	12	10	2	8	0	0	0	0	0	0	8
	16	10	2	8	0	0	0	0	0	0	8

25 donor CD45.2$^+$ KTLS cells were double-sorted as cKit+Lin(-/lo)Sca1+Thy1.1(lo)Flk2- and transplanted into lethally irradiated CD45.1$^+$ recipient animals along with recipient-type $3x10^5$ competitor cells from adult whole BM for radioprotection. The lineage (Lin) cocktail for donor KTLS cells sourced from FL contained the following mature cell markers: B220, CD3, CD4, CD5, CD8, Gr1, TER119. Mac1 was also used in the Lin cocktail for only the 8 week old donor bone marrow. Donor cell reconstitution was assayed via readout in lysed, TER119- peripheral blood cells of recipient mice up to 16 weeks post transplantation. Recipient animals were considered positive for engraftment if they boasted a robust population of large (SSC(med-hi)) myeloid (Mac1+) donor cells above background (>0.3%). Lineage potential was assayed by FACS analysis of donor-derived Mac1+ (myeloid, M, lineage), B220+ (B lineage) and CD3/TCRβ+ (T lineage) cells.

SUPPLEMENTARY FIGURE LEGENDS

Papathanasiou et al.

"Evaluation of the Long-Term Reconstituting Subset of Hematopoietic Stem Cells with CD150"

Supplementary Figure 1. FACS gating strategy used to analyze in vivo reconstitution and lineage distribution of donor CD45.2$^+$CD45.1$^-$ KTLS(CD150+) and CD45.2$^-$ CD45.1$^+$ KTLS(CD150-) cells in CD45.2$^+$CD45.1$^+$ recipient peripheral blood from 4 weeks post-transplant onwards. Cells were first gated according to Scatter, excluding FSC(small) (red cells) and FSC(large) (doublet) cells. Live (Propidium Iodide-negative), TER119- cells were then gated, to compare overall CD45.2 versus CD45.1 donor white blood cell (WBC) reconstitution. Each donor subset, whether CD45.2$^+$CD45.1$^-$ or CD45.2$^-$CD45.1$^+$, was then analyzed according to the expression of B220+, CD3/TCRβ+ and Mac1+SSC(large) to assay the output of the B, T and M lineages, respectively. A similar gating strategy was utilized to assay the in vivo reconstitution and donor lineage distribution where only one CD45.2$^+$ donor subset was transplanted into CD45.1$^+$ recipients.

Supplementary Figure 2. Expression of α1 (Ha31/8), α2 (Hmα2), α4 (R1-2), α5 (5H10-27), α6 (GoH3), and β1 (HMβ1-1) integrins on the cell surface of KTLS(CD150+) (blue) and KTLS(CD150-) (red) bone marrow cells.