Table S1. Peripheral blood cell parameters and hematopoietic organ cellularity in adult Δ-2.8 mice
Peripheral blood parameters, including FACS identification of white blood cells by lineage and % reticulocytes as assessed by acridine orange staining, as well as spleen and bone marrow cellularity are shown for Δ-2.8 and wild-type littermates. All data was collected on 8–12-week-old mice.

	WT	Δ-2.8	WT	Δ-1.8
WBC (x 10^3 / μl)	4.667 ± 0.3333	4.667 ± 0.6667	7.250 ± 0.8539	6.500 ± 0.6708
% CD3+	2.61 ± 0.805	2.33 ± 0.795	2.300 ± 0.3512	1.663 ± 0.2840
% B220+	0.784 ± 0.1276	0.952 ± 0.0698	3.067 ± 0.4096	3.128 ± 0.3430
% Mac1+ (Gr1-)	0.159 ± 0.0574	0.0939 ± 0.0370	0.1867 ± 0.008819	0.1462 ± 0.03604
% Gr1+/Mac1+	1.597 ± 0.7848	1.824 ± 0.6061	0.7713 ± 0.2009	0.4933 ± 0.08090
RBC (x 10^6 / μl)	7.833 ± 0.4372	8.467 ± 0.1856	8.875 ± 0.4385	8.450 ± 0.2975
Hct (%)	42.25 ± 1.931	41.83 ± 1.815	38.00 ± 2.000	41.50 ± 1.057
Retics (%)	3.950 ± 0.3663	4.300 ± 0.3670	4.033 ± 0.20	4.350 ± 0.43
Platelets (x 10^3 / μl)	907.5 ± 106.2	960.0 ± 34.25	920.0 ± 72.11	783.3 ± 100.9
Spleen (TNC x 10^6)	148.7 ± 7.700	150.2 ± 13.02	195.1 ± 56.47	185.9 ± 22.40
Bone Marrow (TNC x 10^6)	57.30 ± 4.316	50.91 ± 2.975	58.3 ± 7	60.78 ± 8.1

[1] Full blood counts are from 6 ko mice and 3 or 4 wild type littermates for each genotype.
[2] Nucleated marrow cellularity results are from one femur and tibia from 4-7 wt and 4-7 ko mice.
[3] Mean values ± SEM are shown for all hematological parameters. Statistical analysis was performed using the Student's t test.

Figure S1. Generation of mice deficient for the -2.8 GATA switch site of the *Gata2* locus. Region of *Gata2 locus* targeted in generation of mouse line deficient for a 400 bp region 2.8 kb upstream of the *Gata2* transcriptional start site, referred to as the Δ-2.8 allele. The location of the palindromic GATA-binding site deleted in the Δ-1.8 mice is also shown (A). Southern blot strategy outlining the HindIII / SalI digested fragment sizes for the wild-type and targeted alleles, and probe hybridization sites (B). Southern blot of Δ-1.8 germline mice and wild-type (WT) littermates from tail tip genomic DNA (C). PCR genotyping of mice deficient the -2.8 kb GATA-binding region (D).

Figure S2. GATA-2 occupancy is lost at the -2.8 kb GATA-binding region in Δ-2.8 fetal liver progenitor cells. Progenitors from E14.5 fetal livers were enriched using RBC lysis, followed by depletion of Ter119+ cells using the MACS system (Miltenyi Biotec, Auburn, CA) as per manufacturer's instructions. Quantitative ChIP analysis of GATA-2 occupancy at the *Gata2* locus was conducted using an anti-GATA-2 antibody or preimmune serum. GATA-2 occupancy at the -2.8 kb site (measured with the -2.75 kb surrogate primer set), -1.8 kb GATA switch sites, and a negative control +25 kb site is shown. Data are presented as mean ± SEM. Statistical significance was assessed by two-sided Student's t-test and * ,$p \leq 0.05$.

Figure S3. Hematopoietic stem/progenitor cells in the adult Δ-2.8 and Δ-1.8 mice

Gata2 expression in adult stem cells was analyzed by staining whole bone marrow with antibodies to lineage markers, CD34, Sca1, and ckit. RNA was isolated from ST-HSC/MPP (Lin^{dim}c-kit+Sca-1+CD34+) and LT-HSC (Lin^{dim}c-kit+Sca-1+CD34-) populations and subjected to reverse transcriptase reaction and qPCR for *Gata2* expression (A) normalized to β-actin. For

FACS analysis of stem and progenitor subsets, total white cells were enumerated in whole bone marrow, which was treated with Red Blood Cell Lysis buffer to remove red blood cells. Stem cells were analyzed by staining with antibodies to lineage markers, CD34, Sca1, and ckit. Percentages of MPP (Lin^{dim}c-kit+Sca-1+CD34+) and LT-HSC (Lin^{dim}c-kit+Sca-1+CD34-) were obtained and multiplied by marrow white blood cells numbers to obtain absolute numbers of each population (B). Myeloid progenitors were analyzed by staining with antibodies to lineage markers, CD34. FcγR, and, ckit. Percentages of CMP, GMP, and MEP were obtained as described and multiplied by total cells numbers to obtain absolute numbers of each population (C). White blood cell counts were obtained from whole bone marrow from adult mice, and colony-forming assays were performed. Total colony numbers were similar (data not shown) and % colony type is shown (D).

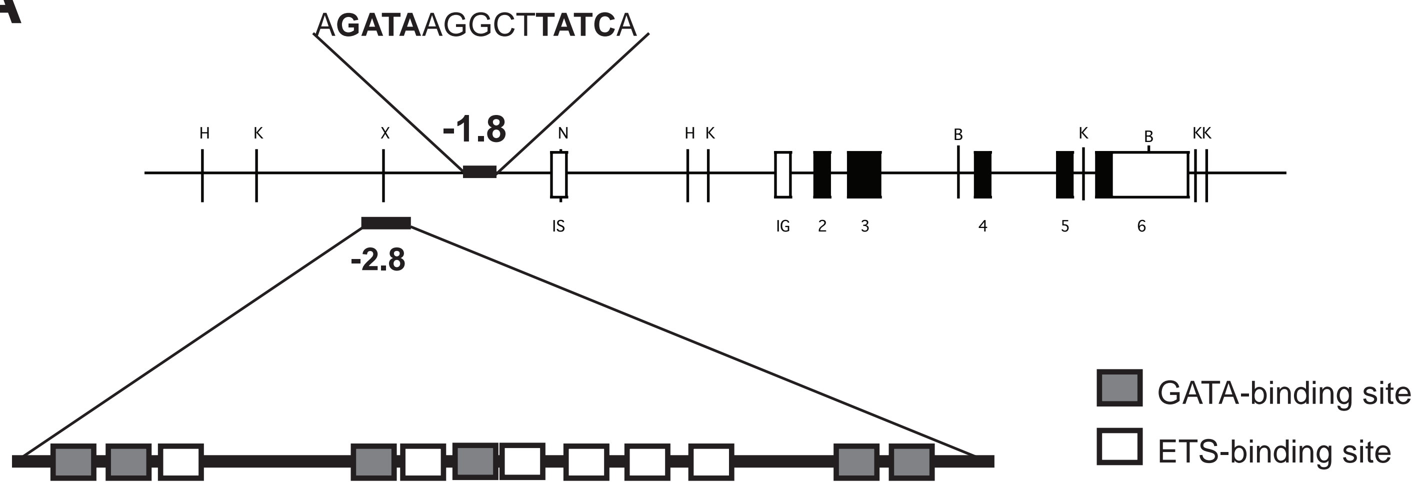
Figure S4. Competitive transplantation ability of HSC in adult Δ-2.8 and Δ-1.8 mice

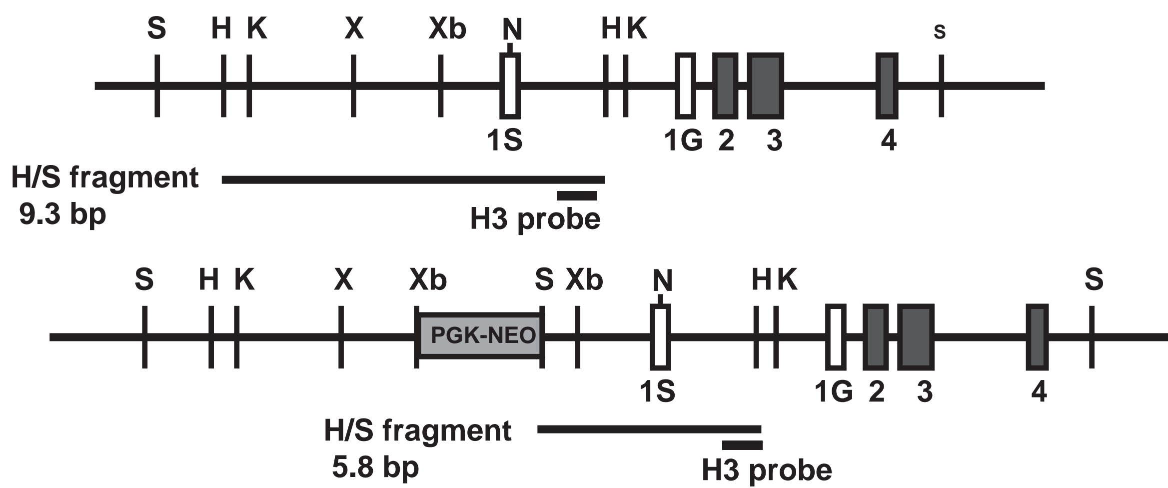
Average PB chimerism, measured at four-week intervals after transplant and represented as % donor, in primary transplant recipients (A, C) receiving $1x10^6$ nucleated bone marrow donor cells from wild-type littermates or mice homozygous for the Δ-2.8 or Δ-1.8 knock-in allele on the CD45.2 background with an equivalent number nucleated bone marrow cells from CD45.1/CD45.2 double positive competitor bone marrow cells. Lineage-specific chimerism of PB from above experiment at 16 weeks post-transplant using positivity to Mac1, CD3, B220, and CD71 to assess lineage identity. Average PB chimerism, measured at four and sixteen weeks after transplant and represented as % donor, in secondary transplant recipients (B, D), in which whole bone marrow was recovered from mice from primary transplant cohorts at 16 weeks post transplant, donor cells were purified based on CD45.2 single positivity, and 2-3 $x10^5$

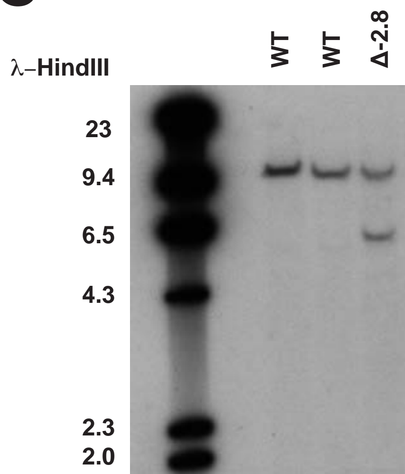
donor cells were pooled and injected with 1 x10^6 freshly isolated competitor cells. Lineage specific chimerism of PB from above experiment at 16 weeks post-transplant (C), using positivity to Mac1, CD3, B220, and CD71 to assess lineage identity. Data are presented as mean ± SEM. Statistical significance was assessed by two-sided Student's t-test and * ,$p \leq 0.05$ and ** ,$p \leq 0.01$.

Figure S5. The -1.8 kb GATA-binding site establishes trimeH3K4 and trimethylH3K27 marks at select sites of the *Gata2* locus. Quantitative ChIP analysis of the *Gata2* locus in E14.5 fetal liver cells using antibodies to dimeH3K4 (A), trimeH3K27 (B), and Total H3 (C) for Δ-1.8 fetal liver cells. Data are presented as mean ± SEM. Statistical significance was assessed by two-sided Student's t-test and * ,$p \leq 0.05$ and ** ,$p \leq 0.01$.

Figure S1







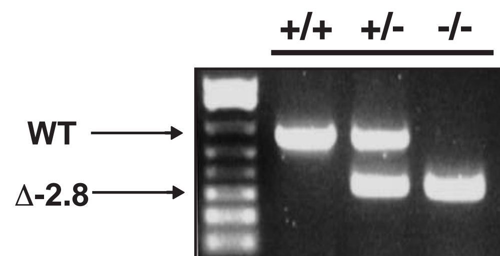


Figure S2

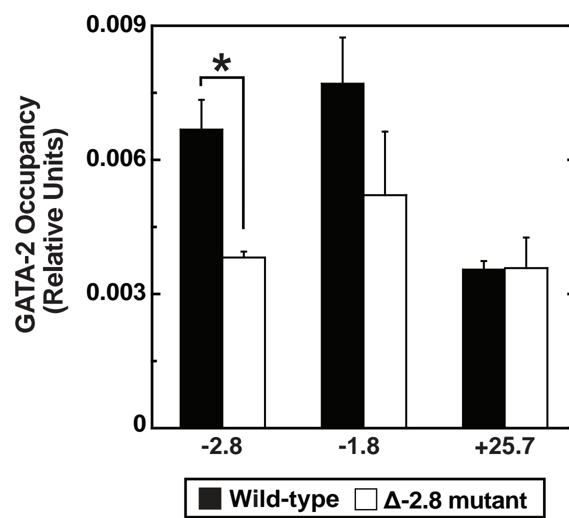


Figure S3

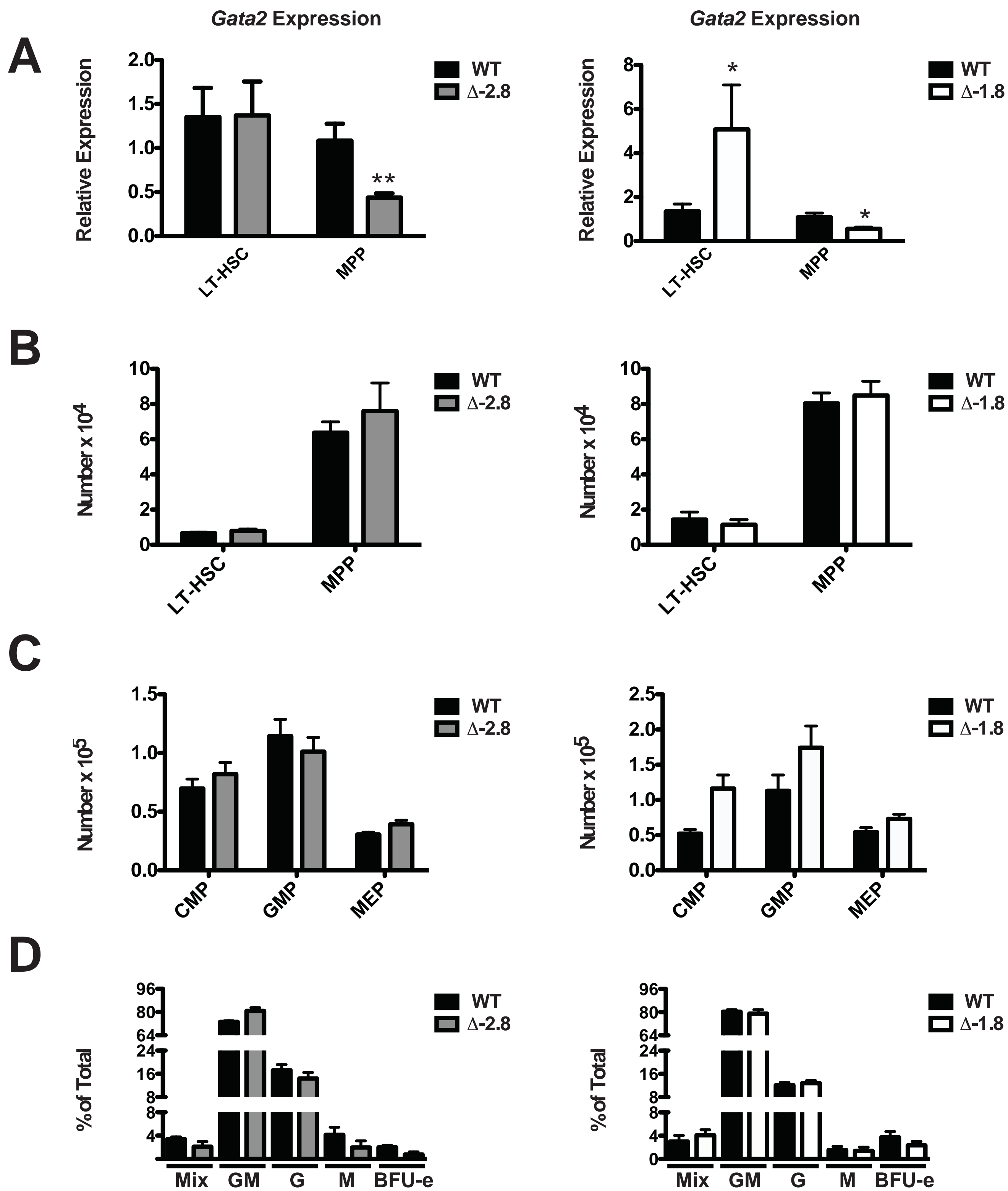
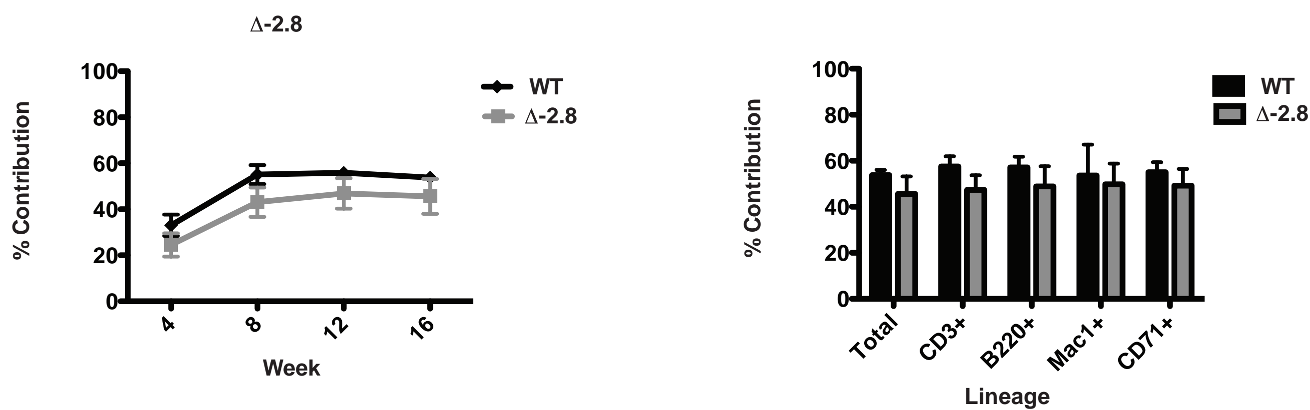


Figure S4

A



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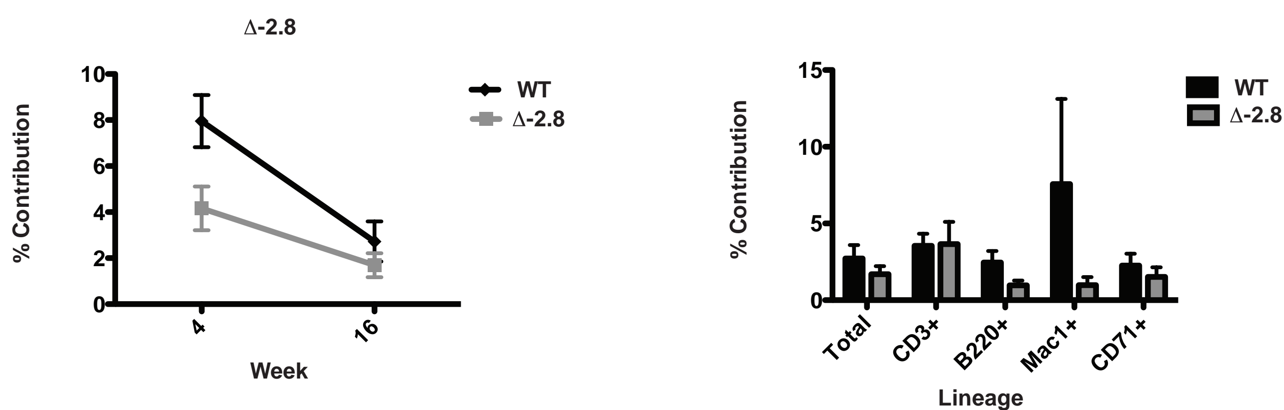

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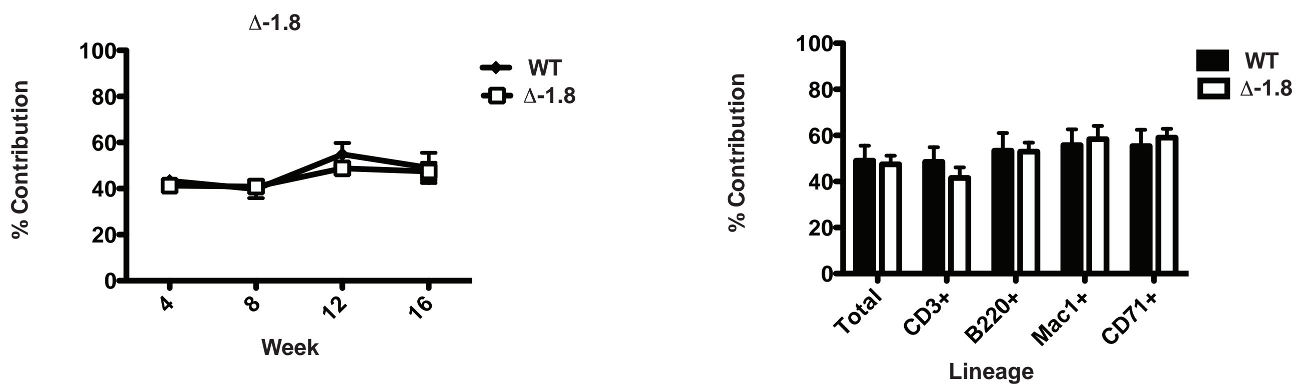

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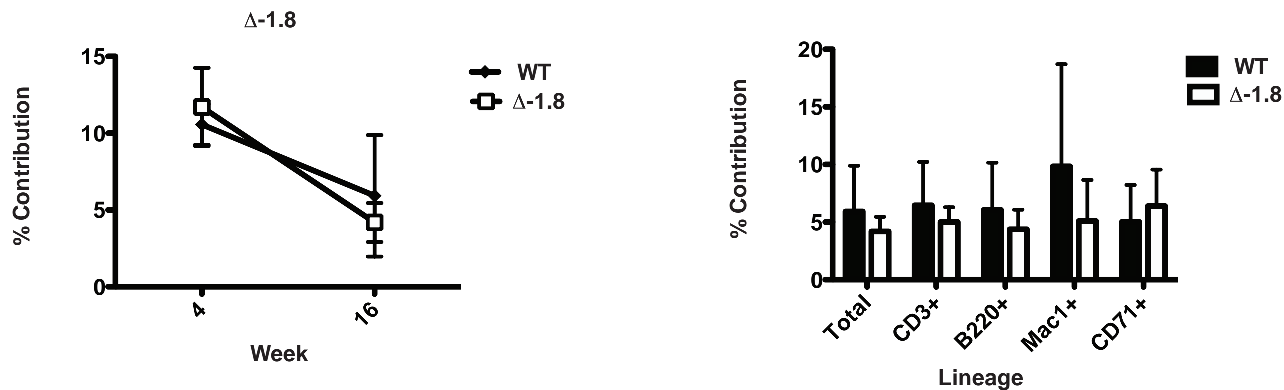

Figure S5