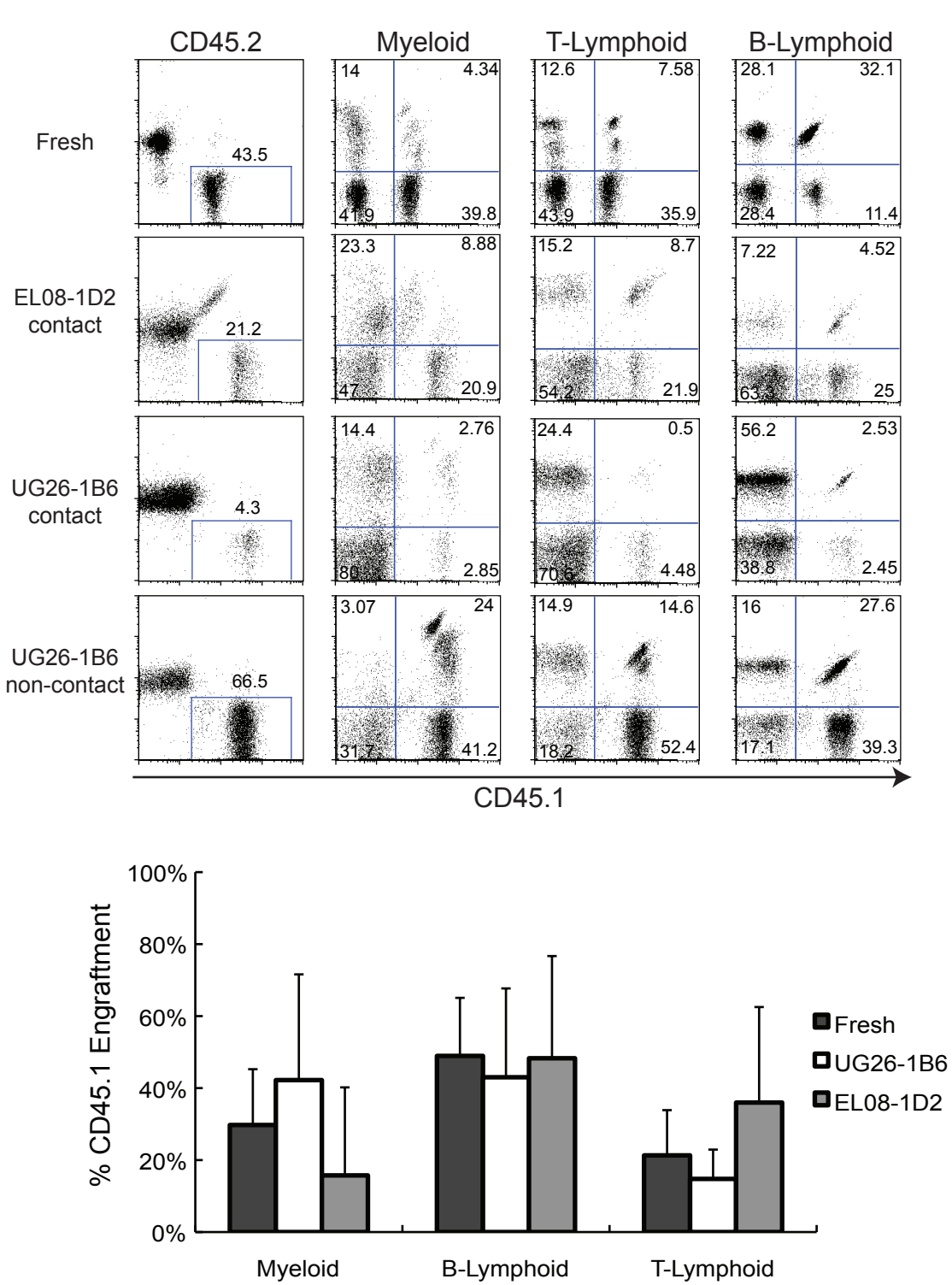
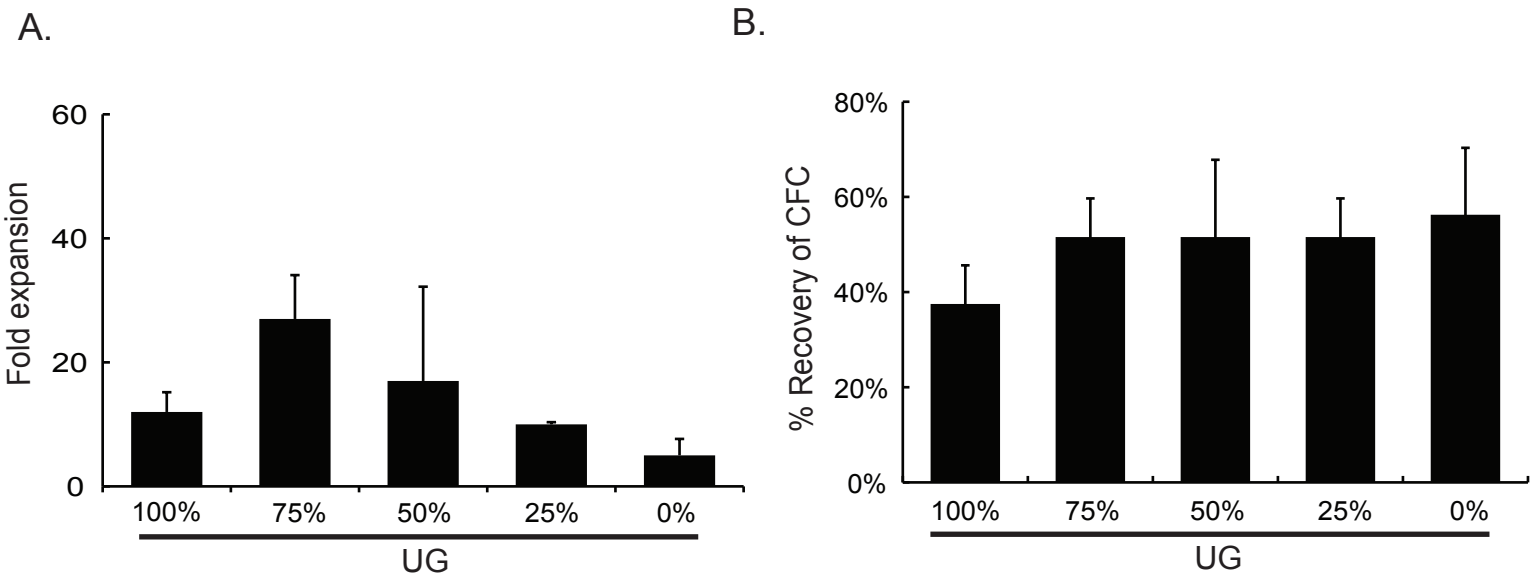


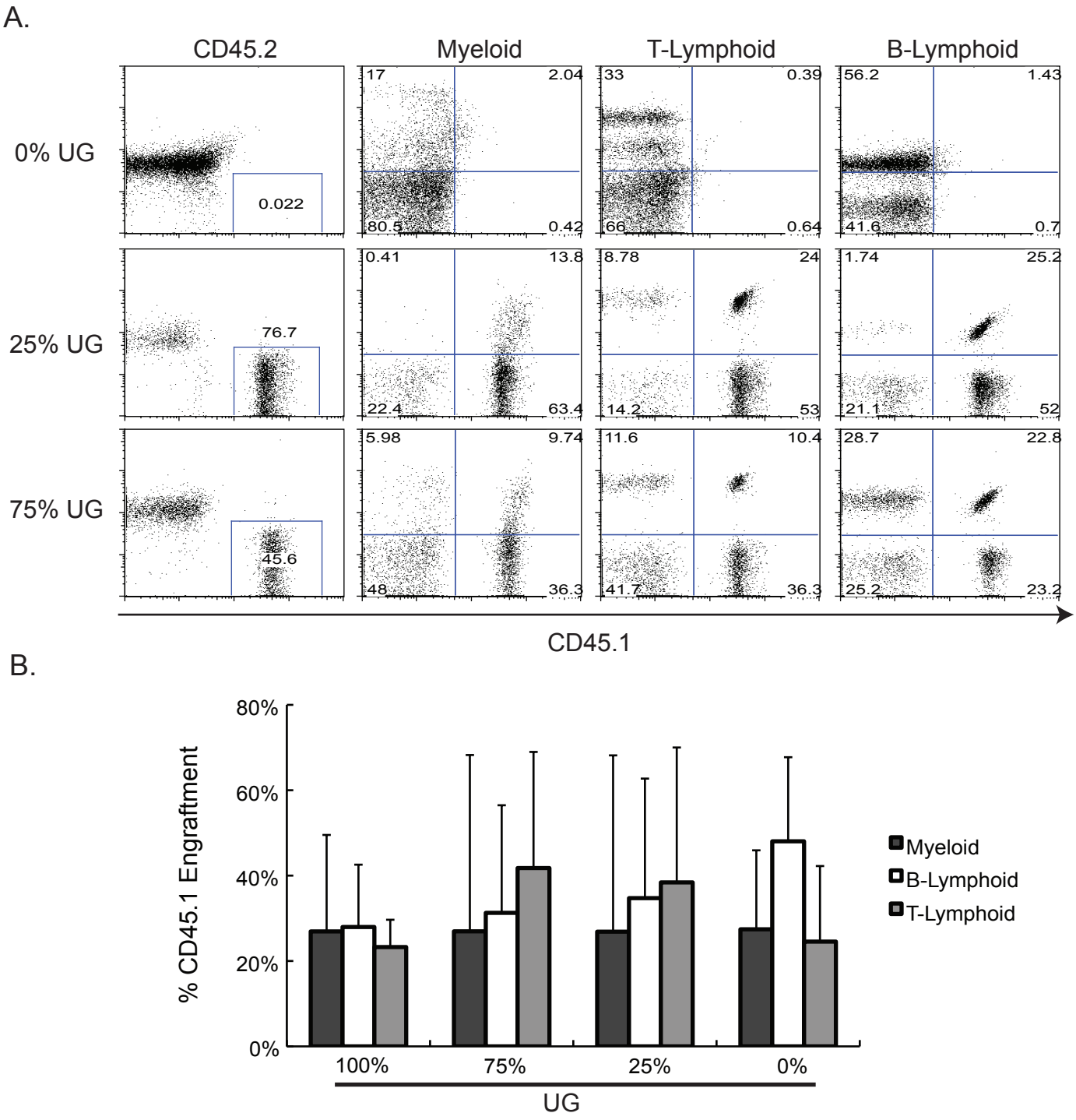
Supplementary Figure 1. Lin- BM cells co-cultured in contact with all feeders and in transwells above UG26-1B6 cells have multi-lineage potential in competitive repopulation assays; however repopulation by progeny from EL08-1D2-contact cultures is skewed towards the T-lymphoid lineage.

A combination of 2x105 CD45.2+ competitor BM and 104 fresh CD45.1+ Lin- BM cells, or progeny from 104 CD45.1+ Lin- BM cells cultured for 3 weeks in contact with or in transwells above UG26-1B6 or EL08-1D2 cells were transplanted in CD45.2+ recipients. Twelve to sixteen weeks after transplantation,PB was collected and analyzed by FACS for presence of CD45.2+ and CD45.1+ cells, and for CD45.1+ cells co-labeling with anti-Gr-1/Mac-1+, anti-B220+, or anti-CD4/CD8+. A) The FACS plots demonstrate multi-lineage engraftment (Gr-1/Mac-1+, B220+, and CD4/CD8+ cells) of one representative mouse from each group transplanted with cells co-cultured with stromal cells. Analysis was done on PB 3 months after transplantation. B) The figure demonstrates multilineage repopulation of all transplanted mice that were engrafted with donor-derived cells. Bars represent percent ± standard deviation for myeloid, B-lymphoid and T-lymphoid cells within the CD45.1+ population. Statistical significance in all experiments was determined by two-tail t-test.



Supplementary Figure 2. No significant differences in cell expansion and recovery of CFC when Lin- BM cells are cultured on feeders consisting of different ratios of UG26-1B6 and EL08-1D2.

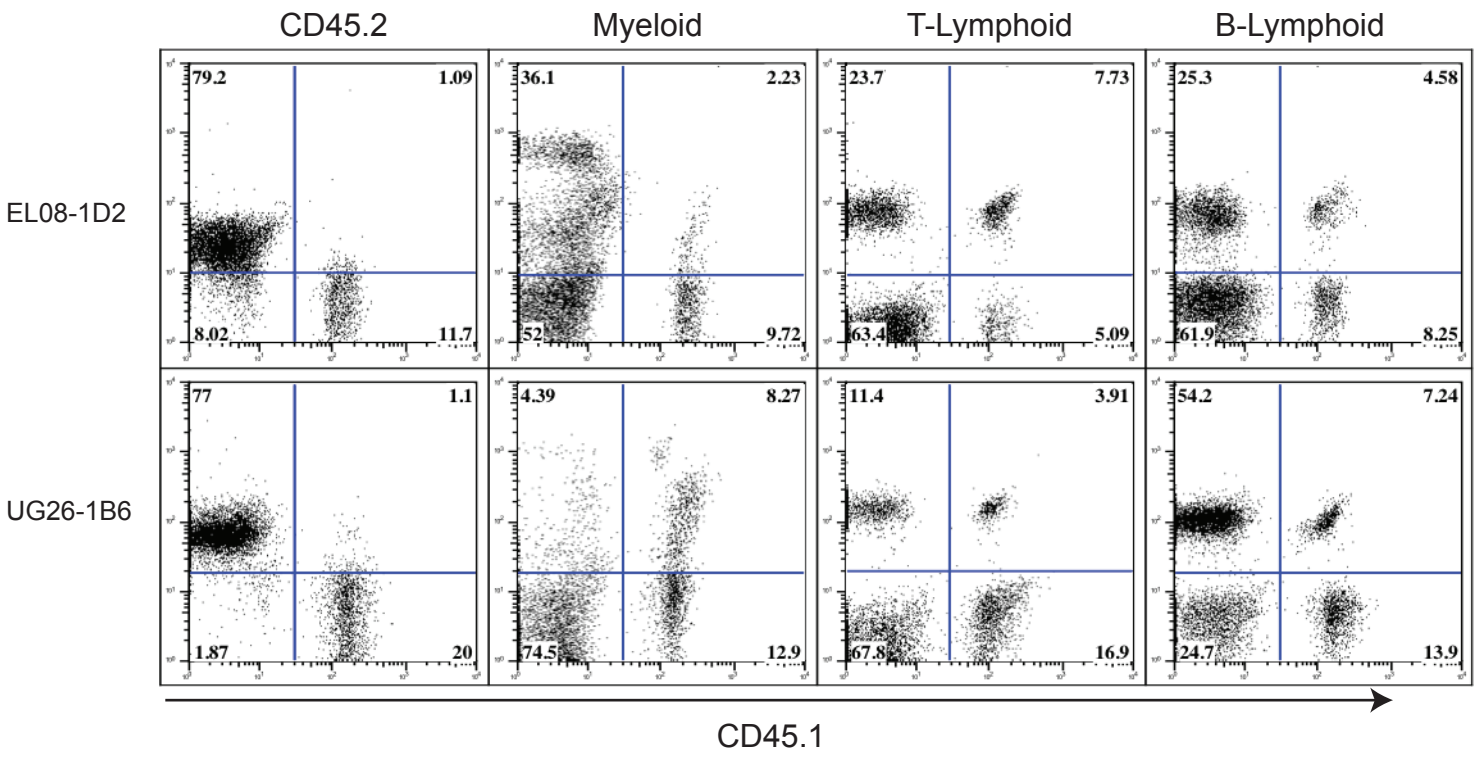
104 CD45.1+ Lin- BM cells were cultured in transwells above mixed feeders containing variable percentages of UG26-1B6 and EL08-1D2 cells, shown in the X-axis. A) After 3 weeks of culture nucleated cells were enumerated using trypan blue to assess cell expansion. B) After 3 weeks of culture the frequency of CFC was determined. CFCs were enumerated at day 12. % CFC recovery = (#CFC generated by the progeny of 104 Lin- BM cells recovered after 3 weeks of culture /#CFC/ 104 fresh Lin- BM cells) x 100. Experiments were repeated three times with separate isolations of Lin- BM cells. Statistical significance in all experiments was determined by two-tail t-test.



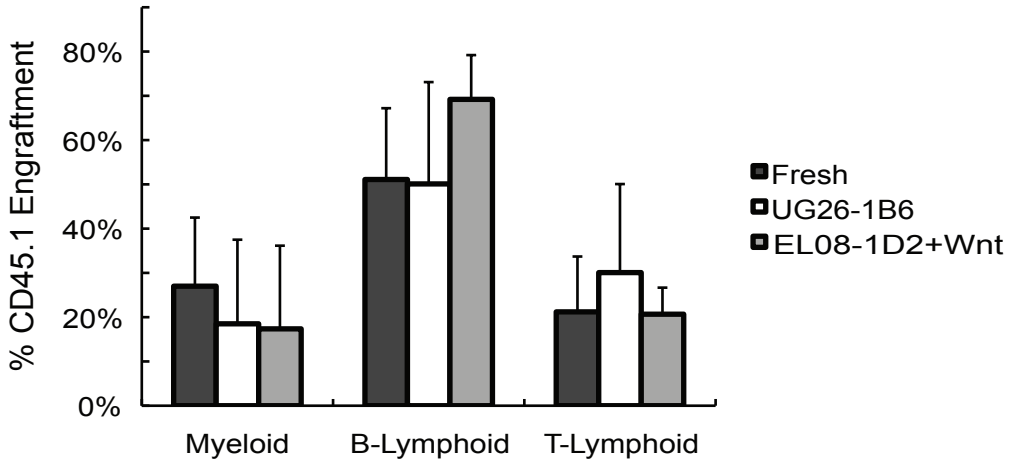
Supplementary Figure 3. Lymphoid skewing is not found in animals repopulated with progeny from cultures of Lin- BM cells cultured above mixed feeders with 25% or more UG26-1B6 cells, combined with EL08-1D2 cells.

A combination of 2x105 CD45.2+ competitor BM and 104 fresh CD45.1+ Lin- BM cells, or progeny from 104 CD45.1+ Lin- BM cells cultured for 3 weeks in contact with or in transwells above different mixtures of UG26-1B6 or EL08-1D2 cells were transplanted in CD45.2+ recipients. Twelve to sixteen weeks after transplantation, PB was collected and analyzed by FACS for presence of CD45.2+ and CD45.1+ cells, and for CD45.1+ cells co-labeling with anti-Gr-1/Mac-1+, anti-B220+, or anti-CD4/CD8+. A) The FACS profiles demonstrate multi-lineage engraftment (Gr-1/Mac-1+, B220+, and CD4/CD8+ cells) of one representative mouse from each group that was transplanted with cells co-cultured with mixed stromal cells. PB of animals was analyzed at 12 weeks post transplantation. B) Bars represent percent of myeloid, B-lymphoid and T-lymphoid cells within the CD45.1+ population. Statistical significance was determined by two-tail t-test.

A.



B.



Supplementary Figure 4. Addition of Wnt5a to EL08-1D2 non-contact cultures enables EL08-1D2 cells to support multilineage repopulating LTR-HSC in transwells above the feeder.

Lin- BM cells were cultured in transwells above irradiated UG26-1B6 and EL08-1D2 cells with 10ng/ml Wnt5a. After 3 weeks, progeny of 104 CD45.1+ Lin- BM cells were transplanted together with 105 CD45.2+ fresh BM in lethally irradiated CD45.2+ recipients. After 4 months, PB of recipient animals was analyzed for CD45.1+ cells. A) The FACS plots demonstrate multi-lineage engraftment (Gr-1/Mac-1+, B220+, and CD4/CD8+ cells) of one representative mouse grafted with cells cultured above irradiated UG26-1B6 and EL08-1D2 cells with 10ng/ml Wnt5a. B) Bars represent percent of myeloid, B-lymphoid and T-lymphoid cells within the CD45.1+ population. Statistical significance was determined by two-tail t-test.

(n=4)	%Chimerism in primary recipient PB	Percent of donor derived cells in secondary recipients			
		Total	Myeloid	B Lymphoid	T Lymphoid
EL08-1D2 contact#1	31.8%	2.5±1.7	11.6±7.7	71.2±19.6	17.2±15.2
EL08-1D3 contact#2	86.7%	43.7±7.1	10.3±4.2	13.5±15.5	79.3±12.4
UG26-1B6 non-contact#1	7.1%	68.9±6.3	53.6±15.5	5.7±1.4	40.7±14.3
UG26-1B6 non-contact#2	5.7%	7.8±14.9	64.9±10.6	21.6±13.5	13.5±15.7

Supplementary Table 1. Culture of Lin- BM cell in UG26-1B6 non-contact cultures preserves LTR-HSC.

1x104 Lin- CD45.1+ BM cells were cultured in contact with or transwells above confluent irradiated UG26-1B6 or ELO8-1D2 cells. After 3 weeks, progeny from the 1x104 Lin- CD45.1+ BM cells were transplanted with 2x105 fresh CD45.2+ TBM IV into C57BL/6J (CD45.2+) mice. After 3-4 months, 106 TBM cells from two separate primary recipients were injected into groups of four CD45.2+ secondary recipients for each primary recipient. The two separate primary recipients that were used for secondary transplantations are labeled #1 and #2. Peripheral blood was collected 3 months after transplantation, and analyzed by FACS for CD45.1+ derived cells. The total contribution of donor CD45.1+ cells or donor myeloid, B- or T-lymphoid cells was determined as described in the Materials and Methods section.