SUPPLEMENTAL METHODS

Detection of human chimerism

Mice were tail-bled every other week after transplantation and PBMCs were isolated by Ficoll (Histopaque-1077, Sigma-Aldrich) separation. Plasma samples were collected and stored at -20^{0}C for immunoglobulin (Ig) measurement. At the time of euthanasia, single-cell suspensions were prepared from BM (crushed femur and tibia), thymus graft and spleen of each animal.

Samples were stained with fluorochrome-labeled mAbs against mouse and human cell surface antigens. The following anti-human Abs were used: CD45 (HI30), CD27 (M-T271), CD20 (L27), IgM (G20-127) from BD Pharmingen; CD19 (HIB19), CD3 (OKT3), CD38 (HIT2), CD10 (HI10A), CD24 (ML5), CD138 (DL-101) from BioLegend; CD34 (QBEnd10) from Beckman Coulter. Mouse cells were stained with anti-murine CD45 (30-F11) and Ter119 (TER-119) from BioLegend. Samples were acquired on the LSRII (BD Biosciences) cytometer and analysis was performed by FlowJo software (TreeStar). Relative CD19 median fluorescent intensity (MFI) was calculated as follows: CD19 MFI of the sample / CD19 MFI of human PBMC run on the flow cytometer in the same experiment. Murine erythroid cells were excluded by gating out mouse Ter119$^+$ cells.

Human immunoglobulin measurement

Human total IgM and IgG were measured in the plasma collected from the mice by Enzyme-linked immunosorbent assay (ELISA). 96-well plates (Costar) were coated overnight at 4ºC with 1ng/ml of purified α-human IgM (MHM-88, BioLegend) or α-human IgG (HP6017, BioLegend). After washing and blocking with PBS containing 1% bovine serum albumin (BSA,

Sigma-Aldrich) appropriate dilutions of the samples were added for 2 hours at room temperature (RT). Plates were washed and incubated for 1 hour at RT with 1ng/ml of biotinylated α-human IgM (G20-127, BD Pharmingen) or α-human IgG (G18-145, BD Pharmingen) followed by Streptavidin-HRP (Thermo Scientific). After a final wash the enzyme activity was determined using the TMB substrate (Thermo Scientific) solution followed by stop solution (0.16M Sulfuric Acid, Sigma-Aldrich). The absorbance was measured at 450nm. A sample of human serum with known concentration from Bethyl was used as reference.

HLA Typing

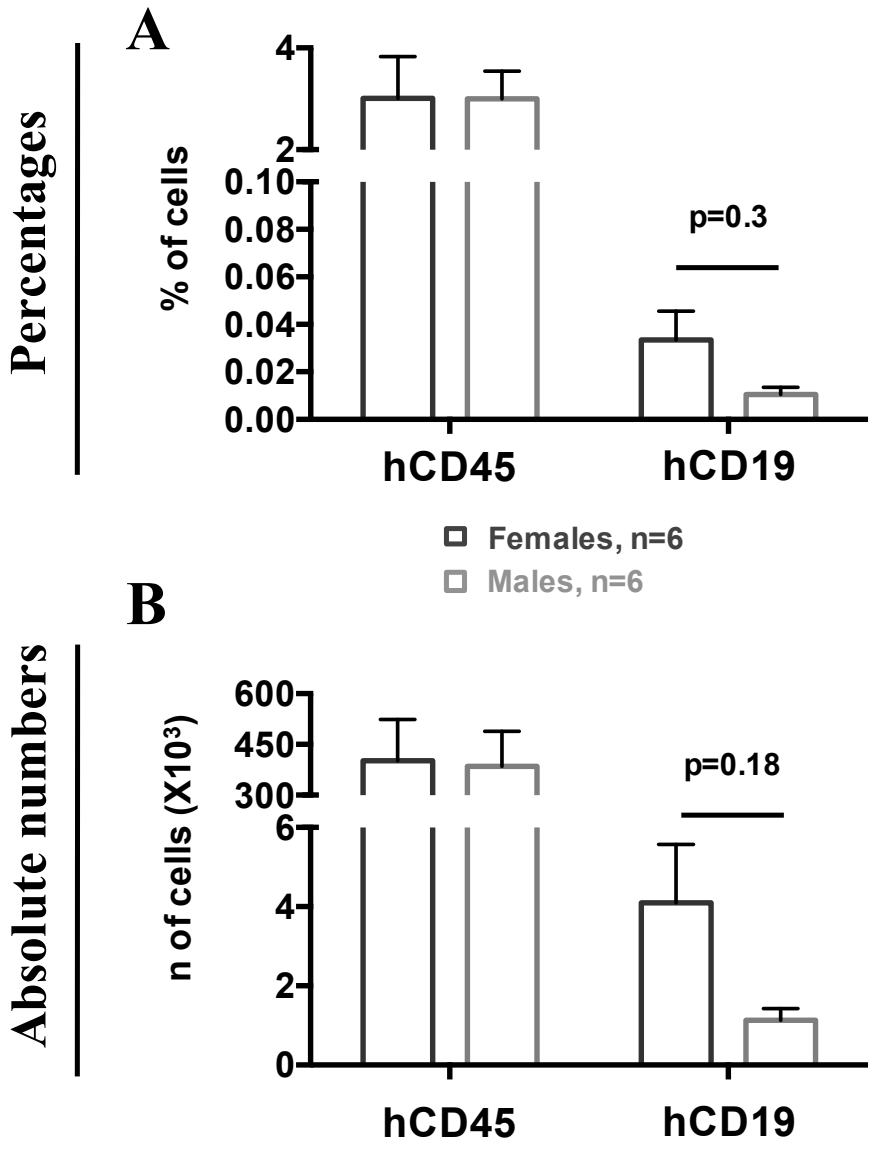
DNA was extracted from blood (BM donors) using Paxgene Blood DNA kit (Qiagen) or phenol chloroform extraction from fetal tissues and was used to perform HLA typing. Single nucleotide polymorphism (SNP) typing (HLA Typing Facility, University of Colorado) was used for experiment 1 and 2 (supplemental Table 1) to determine the molecular genotype at HLA-A, and DQB and DQA loci. Presence or absence of disease associated HLA DR4 or DR3 was inferred. HLA was determined by high resolution direct DNA sequencing in experiments 3-5 and 9-10.

Supplemental Table 1. Degree of HLA matching between BM and fetal thymus used for generating PI humanized mice.

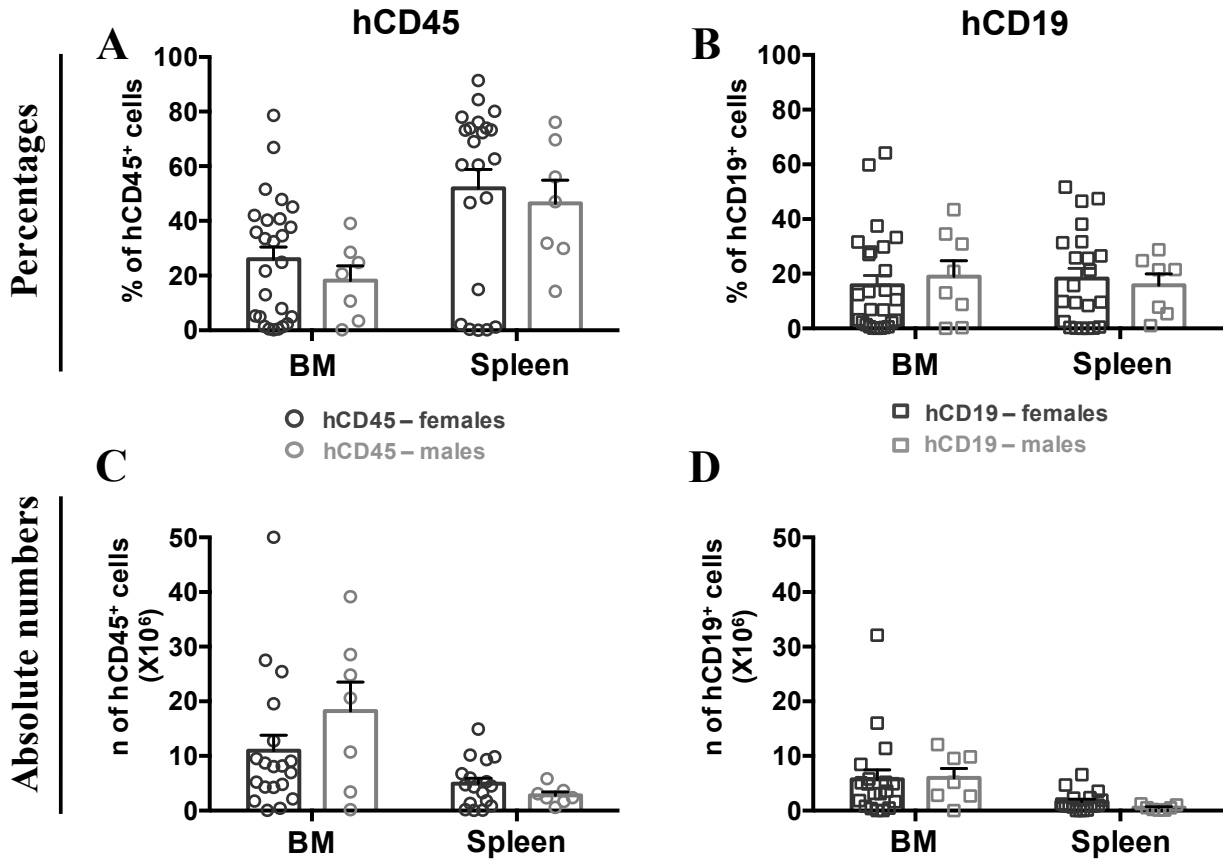
		Gene Locus							
		HLA-A		HLA-B		HLA-DRB		HLA-DQB	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Exp 1	HC#1	*02 (A*02)*	26 (A26)	---	---	*04 (DR4)*	unkn	*03:02 (DQ8)*	unkn
	T1D#1	01 (A1)	11 (A11)	---	---	*04 (DR4)*	*03 (DR3)*	*03:02 (DQ8)*	unkn
	Fetal Thymus#1	*02 (A*02)*	68 (A68)	---	---	*04 (DR4)*	*03 (DR3)*	*03:02 (DQ8)*	unkn
Exp 2	HC#2	*02 (A*02)*	01 (A1)	---	---	unkn	*03 (DR3)*	unkn	unkn
	T1D#2	*02 (A*02)*	01 (A1)	---	---	*04 (DR4)*	*03 (DR3)*	*03:02 (DQ8)*	unkn
	Fetal Thymus#2	*02 (A*02)*	68 (A68)	---	---	*04 (DR4)*	*03 (DR3)*	*03:02 (DQ8)*	unkn
Exp 3	HC#3	*02:01 (A*02)*	*02:01 (A*02)*	40 (B40)	44 (B44)	*04:01 (DR4)*	13 (DR13)	*03:01 (DQ7)*	06:04 (DQ6)
	RA#1	03:01 (A3)	29:02 (A29)	---	---	*04:01 (DR4)*	*04:01 (DR4)*	unkn	unkn
	Fetal Thymus#3	*02:01 (A*02)*	01:01 (A1)	07:02 (B7)	07:02 (B7)	*04:01 (DR4)*	15:01 (DR15)	*03:01 (DQ7)*	06:02 (DQ5)
	T1D#3	24:02 (A24)	74:01 (A74)	40:01 (B40)	49:01 (B49)	*04:04 (DR4)*	08:01 (DR8)	*03:02 (DQ8)*	06:04 (DQ6)
	Fetal Thymus#4	34:01 (A34)	69:01 (A69)	40:35 (B45)	15:01 (B15)	*04:01 (DR4)*	15:02 (DR15)	*03:02 (DQ8)*	06:01 (DQ5)
Exp 4	HC#4	*02:01 (A*02)*	23:01 (A23)	15:01 (B15)	unkn	*04:04 (DR4)*	07:01 (DR7	*03:02 (DQ8)*	06 (DQ6)
	RA#2	*02:02 (A*02)*	31:01 (A31)	---	---	*04:04 (DR4)*	01:01 (DR1)	---	---
	RA#3	*02:05 (A*02)*	11:01 (A11)	---	---	*04:05 (DR4)*	03:01 (DR3)	---	---
	T1D#4	*02:01 (A*02)*	*02:01 (A*02)*	27:05 (B27)	44:02 (B44)	*04:04 (DR4)*	*04:04 (DR4)*	*03:02 (DQ8)*	*03:02 (DQ8)*
	Fetal Thymus#5	*02:01 (A*02)*	31:01 (A31)	07:02 (B7)	40:02 (B40)	*04:04 (DR4)*	14:02 (DR14)	*03:02 (DQ8)*	05:01 (DQ4)
Exp 5	HC#5	11 (A11)	68 (A68)	---	---	---	---	*03:01 (DQ7)*	02:02 (DQ2)
	Fetal Thymus#6	31:01 (A31)	69:01 (A69)	58:01 (B58)	40:01 (B40)	04:04 (DR4)	11:01(DR11)	*03:01 (DQ7)*	03:02 (DQ8)
Exp 6	HC#6	02 (A*02)	24 (A24)	---	---	*04 (DR4)*	unkn	*03:02 (DQ8)*	unkn
	T1D#5	02 (A*02)	02 (A*02)	---	---	*04 (DR4)*	unkn	*03:02 (DQ8)*	06:02 (DQ5)
	Fetal Thymus#7	33 (A33)	68 (A68)	---	---	*04 (DR4)*	unkn	*03:02 (DQ8)*	unkn
Exp 7	HC#7	*01 (A1)*	26 (A26)	---	---	*03 (DR3)*	unkn	*02:01 (DQ2)*	unkn
	T1D#6	*01 (A1)*	02 (A*02)	---	---	*03 (DR3)*	unkn	*02:01 (DQ2)*	unkn
	Fetal Thymus#8	*01 (A1)*	32 (A32)	---	---	*03 (DR3)*	unkn	*02:01 (DQ2)*	06:02 (DQ5)
Exp 8	HC#8	*02 (A*02)*	29 (A29)	44 (B44)	61 (B61)	*04 (DR4)*	unkn	*03:02 (DQ8)*	02:01 (DQ2)
	T1D#6	29 (A29)	*24 (A24)*	---	---	*04 (DR4)*	unkn	*03:02 (DQ8)*	unkn
	Fetal Thymus#9	*02 (A*02)*	*24 (A24)*	---	---	*04 (DR4)*	unkn	*03:02 (DQ8)*	unkn
Exp 9	HC#9	*02 (A*02)*	24 (A24)	---	---	---	---	*02:01 (DQ2)*	03:01 (DQ7)
	Fetal Thymus #10	*02 (A*02)*	29 (A29)	---	---	---	---	*02:01 (DQ2)*	*02:01 (DQ2)*
Exp 10	HC#10	*02 (A*02)*	30 (A30)	---	---	*04 (DR4)*	unkn	06:02 (DQ5)	unkn
	T1D#7	*02 (A*02)*	26 (A26)	---	---	*04 (DR4)*	*03 (DR3)*	02:01 (DQ2)	05:01 (DQ4)
	Fetal Thymus#11	*02 (A*02)*	11 (A11)	---	---	*04 (DR4)*	*03 (DR3)*	---	---

* Molecular typing and serologic interpretation of molecular genotype at each HLA locus and allele are shown. Matching alleles between BM and fetal thymus tissue are in italics. **---** = not done, unkn = unknown (lacked sufficient resolution to determine molecular sequence).

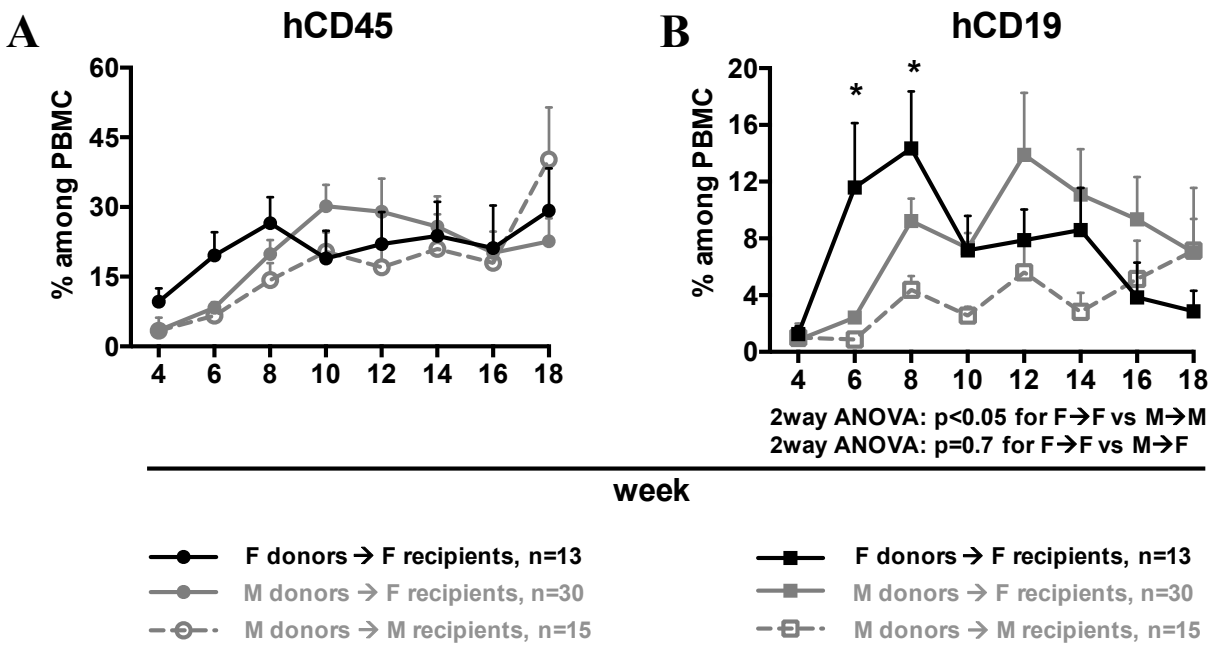
SUPPLEMENTAL FIGURES



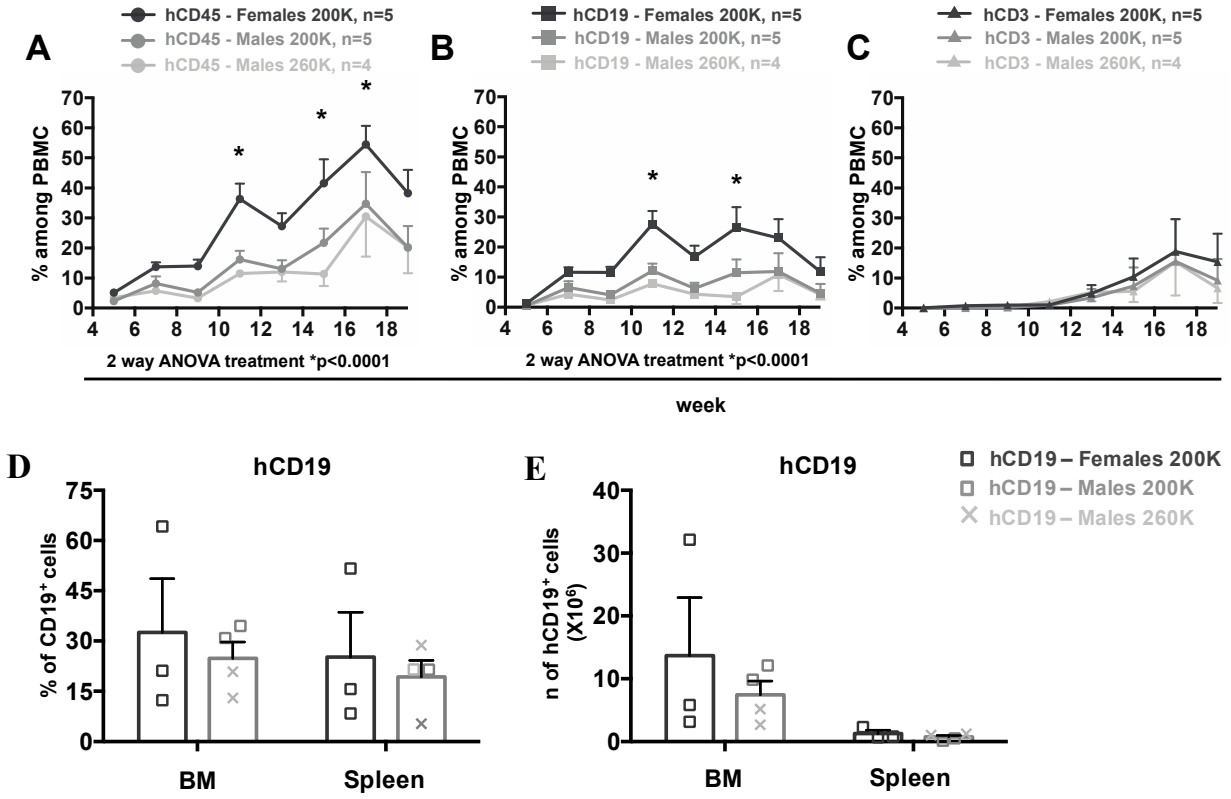
Supplemental Figure 1. Human splenic CD45 and CD19 reconstitution in NSG mice 3 weeks after injection with BM CD34$^+$ cells. Sublethally irradiated NSG mice were injected with 2x10^5 BM CD34$^+$ cells from one healthy BM donor and grafted under the kidney capsule with a partially HLA-matched human fetal thymus. (A-B) Mice were euthanized 3 weeks after transplantation and splenocyte suspensions were collected and analyzed by flow cytometry. Means +SEM of human CD45 and CD19 percentages and numbers are shown.



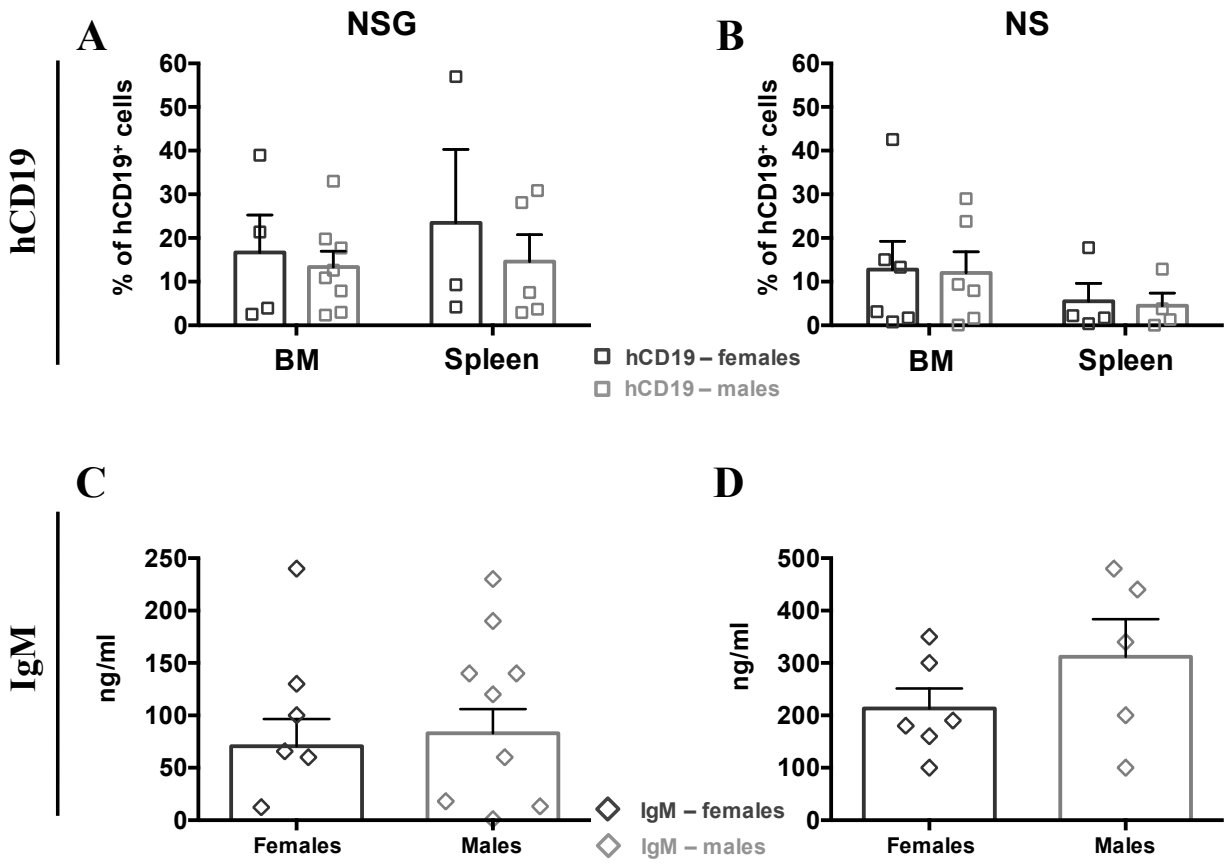
Supplemental Figure 2. Human CD45 and CD19 reconstitution in BM and spleen of NSG mice injected with BM CD34$^+$ cells at week 14-18. Sublethally irradiated NSG mice were injected with 1.5-2x10^5 BM CD34$^+$ cells (7 HC donors for female and 3 HC donors for males) and grafted under the kidney capsule with a partially HLA-matched human fetal thymus. (A-D) Mice were euthanized between week 14-18 after transplantation and BM and spleen cell suspensions were analyzed. Means +SEM of human CD45 and CD19 percentages and numbers are shown. Each circle represents a single mouse.



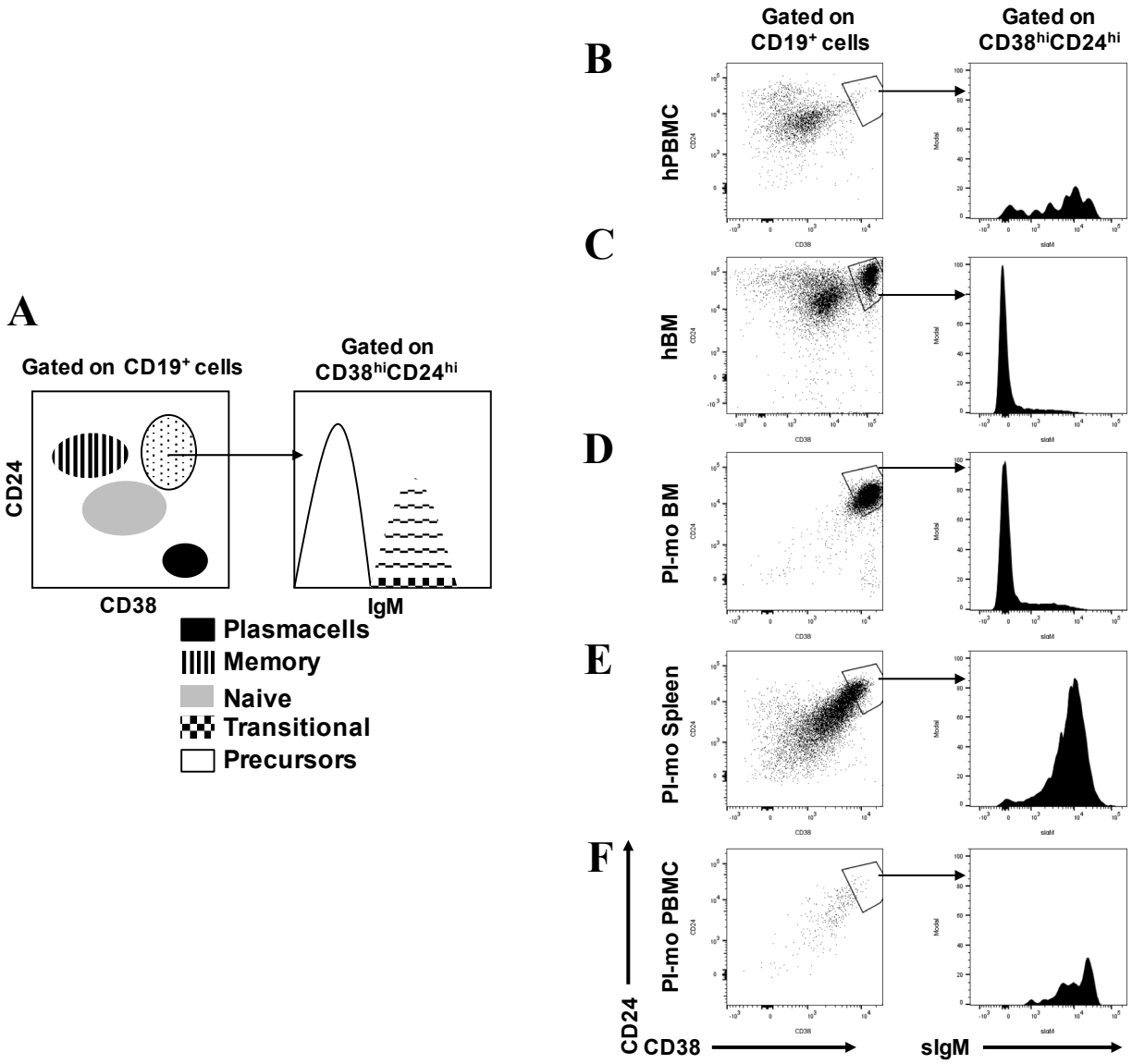
Supplemental Figure 3. Peripheral human CD45 and CD19 kinetics in NSG mice injected with BM CD34$^+$ cells from male vs female donors. Sublethally irradiated NSG mice were injected with 1.5-2x10^5 BM CD34$^+$ cells and grafted under the kidney capsule with a partially HLA-matched human fetal thymus. (A-B) Human peripheral reconstitution was measured by flow cytometry in PB every other week (7 HC male and 3 HC female donors). Means of multilineage chimerism among total (mouse plus human) PBMCs are shown over time. Bars represent +SEM. * indicates statistically significance calculated by Sidak's multiple comparison test.



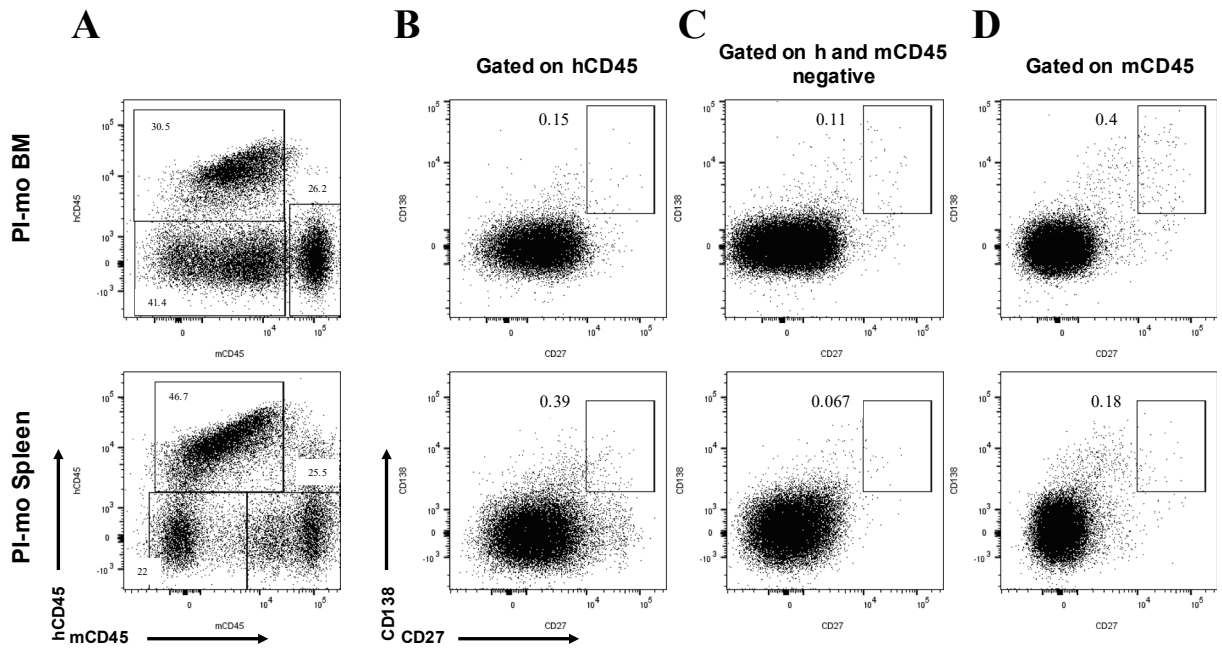
Supplemental Figure 4. Increased weight of males does not explain the difference in peripheral B cell reconstitution between female and male NSG mice. Sublethally irradiated NSG mice were injected with 2-2.6x10^5 BM $CD34^+$ cells and engrafted under the kidney capsule with a partially HLA-matched human fetal thymus. (A-C) Human peripheral chimerism was measured by flow cytometry in PB every other week (1 HC donor, n=4-5 NSG per group). Mean percentages of human $CD45^+$, $CD19^+$ and $CD3^+$ cells among total (mouse plus human) PBMC are shown. Bars represent + or - SEM. (D-E) Mice were euthanized at week 20 after transplantation and BM and Spleen were collected and analyzed by flow cytometry. Means +SEM of CD19 percentage and number are shown. Each square represents a single mouse. * indicates statistically significant $p<0.05$ calculated by Sidak's multiple comparison test.



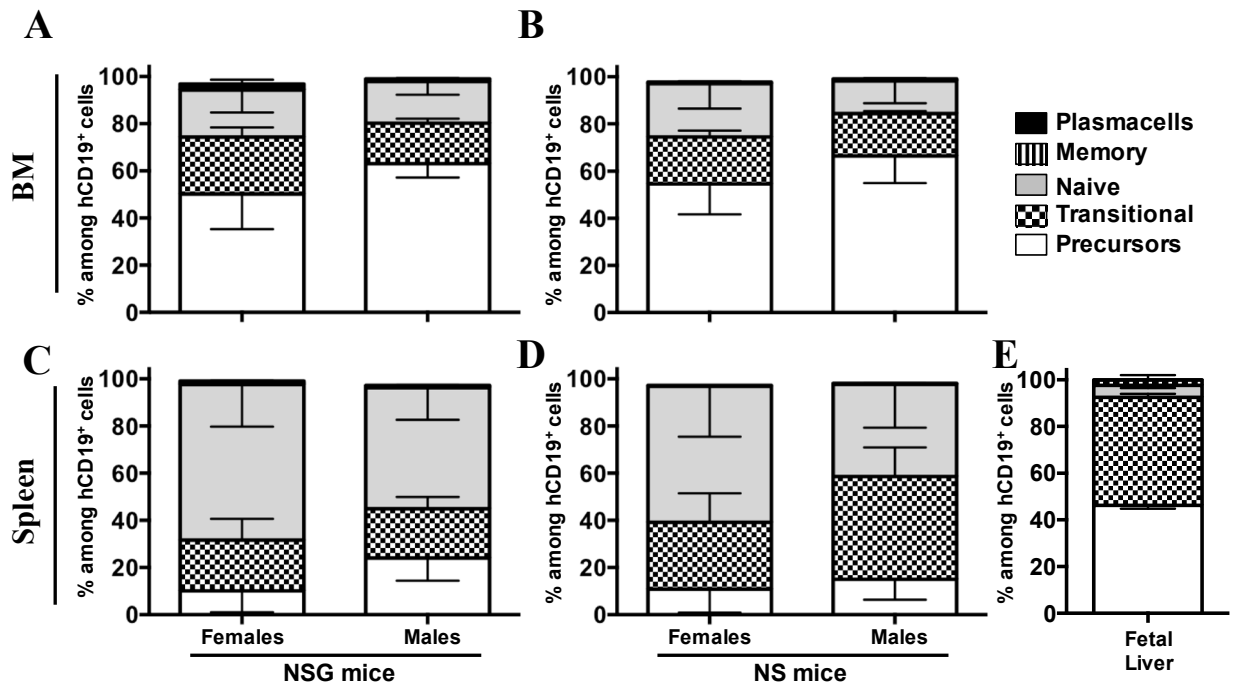
Supplemental Figure 5. Human CD19 percentage and plasma IgM in NSG and NS mice injected with FL CD34$^+$ cells. Sublethally irradiated NSG or NS mice were injected with 1.5-2.5x10^5 FL CD34$^+$ cells and grafted under the kidney capsule with an autologous human fetal thymus. Mice were euthanized between week 20-22 after transplantation and BM, spleen and plasma were collected and analyzed by flow cytometry. (A-B) Means +SEM of CD19 percentage are shown. Each square represents a single animal. (C-D) Means +SEM of IgM level are shown. Each diamond represents a single animal.



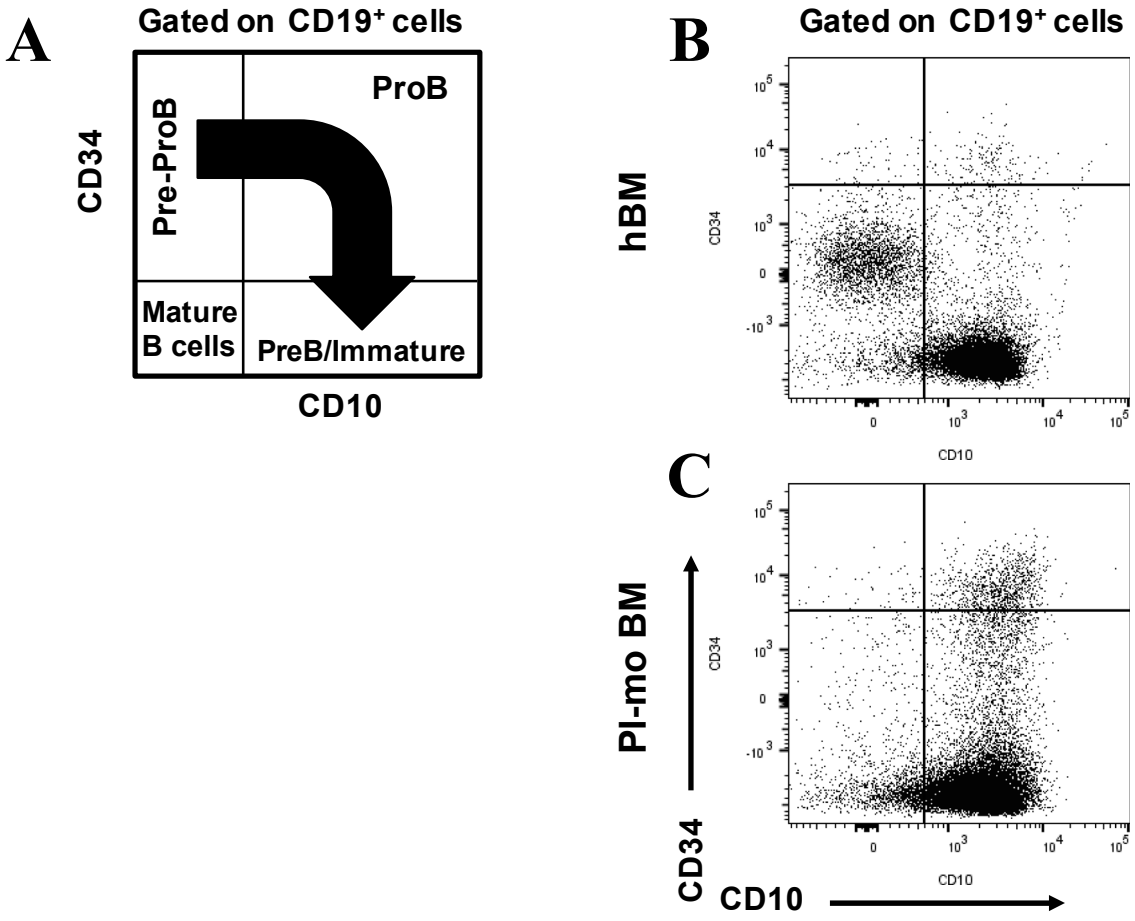
Supplemental Figure 6. Gating strategy for B cell subpopulation analysis. Schematic drawing (A) and representative FACS gating strategy for B cell subpopulations in human PBMC (B), human BM (C), PI-humanized mouse BM (D), Spleen (E) and PBMC (F).



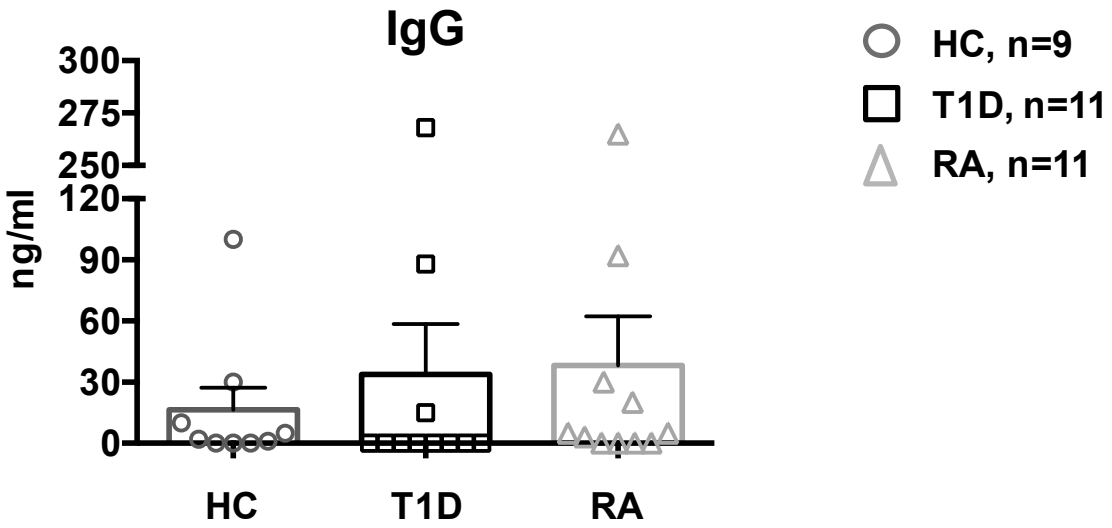
Supplemental Figure 7. Plasma cell evaluation by CD138 and CD27 markers in NSG mice injected with BM CD34$^+$ cells. Sublethally irradiated NSG mice were injected with 2x10^5 BM CD34$^+$ cells and grafted under the kidney capsule with a partially HLA-matched human fetal thymus. Mice were euthanized at week 16 after transplantation and BM and Spleen were collected and analyzed by flow cytometry. Human plasma cell marker CD138 was assessed in combination with CD27 on human CD45$^+$ (B), human and mouse CD45 negative (C) and mouse CD45$^+$ (D) cells. Representative dot plots are shown for BM and Spleen.



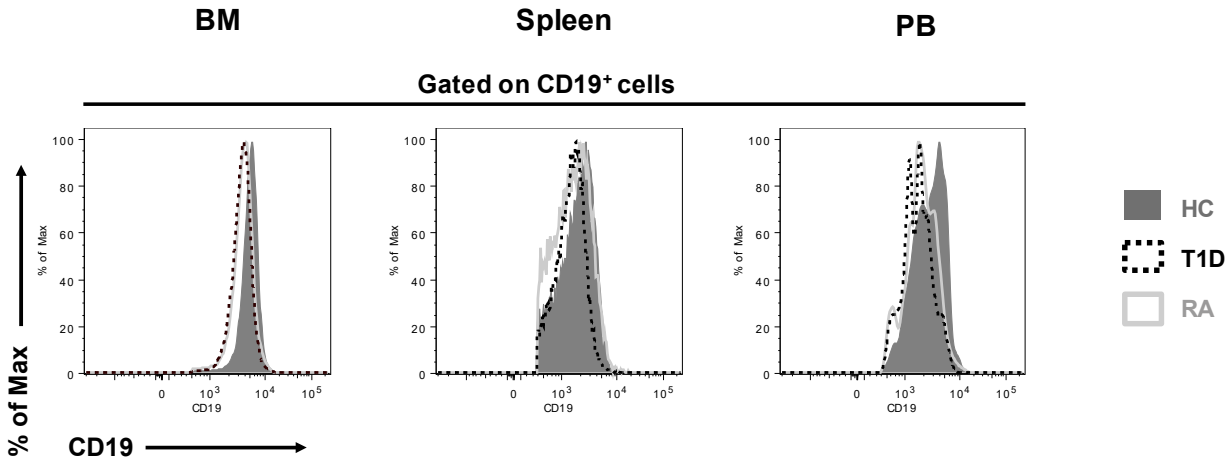
Supplemental Figure 8. B cell subpopulations in BM and spleen of NSG and NS mice injected with FL CD34$^+$ cells. Sublethally irradiated NSG or NS mice were injected with 1.5-2.5x10^5 FL CD34$^+$ cells and grafted under the kidney capsule with an autologous human fetal thymus. Mice were euthanized between week 20-22 after transplantation, and BM (n=4-5 females and 5-8 males) and spleen (n=3 females and 3-5 males) cell suspensions were prepared and analyzed by flow cytometry. (A-D) Means + or - SEM of indicated B cell subpopulation percentage among total CD19$^+$ cells are shown. Animals with less of 1% CD19$^+$ cells were excluded from the analysis. E) Three fetal liver samples were plotted for comparison.



Supplemental Figure 9. Gating strategy for B cell progenitor analysis. (A) Schematic drawing and (B) representative dot plot of B cell progenitors in human BM (B) and PI-humanized mouse BM (C).



Supplemental Figure 10. Plasma IgG levels in NSG mice injected with BM CD34$^+$ cells at week 16. Sublethally irradiated NSG mice were injected with 1.5-2.7x10^5 BM CD34$^+$ cells and grafted under the kidney capsule with a partially HLA-matched human fetal thymus. Mice were euthanized 16 weeks after transplantation and plasma was collected and analyzed by ELISA. Means +SEM of IgG level are shown.



Supplemental Figure 11. Representative histogram overlays of human CD19 expression in NSG mice injected with BM CD34$^+$ cells. Sublethally irradiated NSG mice were injected with 1.5-2.7x10^5 BM CD34$^+$ cells and grafted under the kidney capsule with a partially HLA-matched human fetal thymus. Mice were euthanized between week 14-18 after transplantation and BM, Spleen and PB were collected. Representative histogram overlays of CD19 expression are shown.