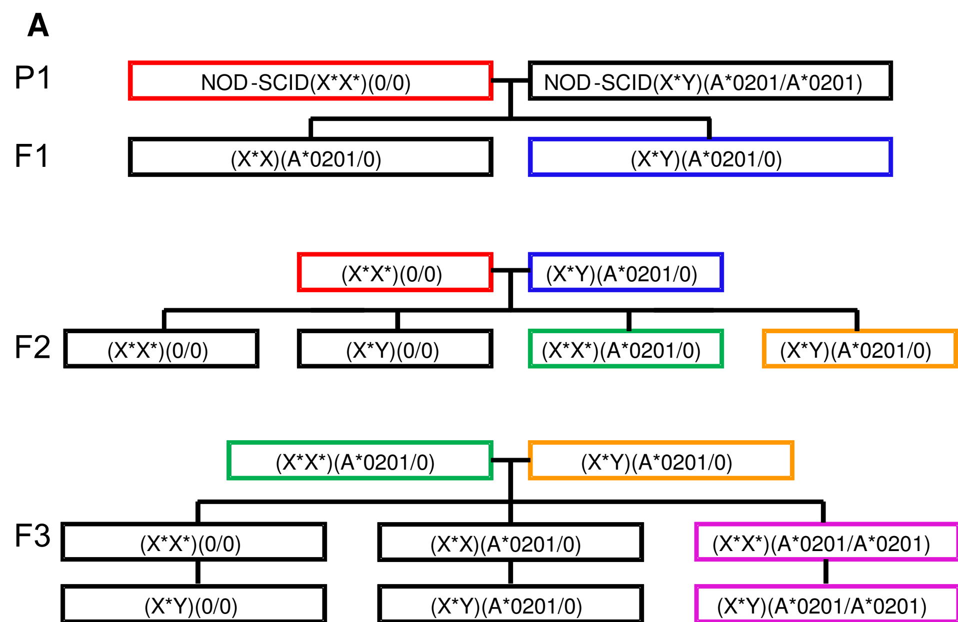
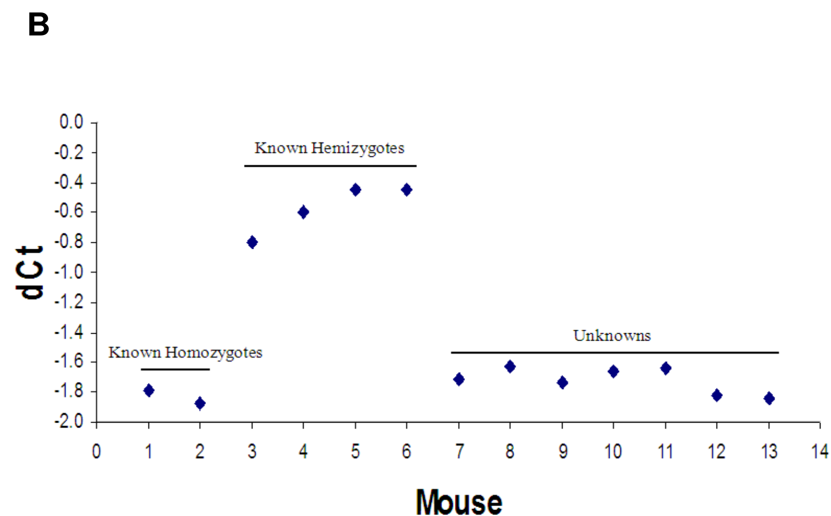
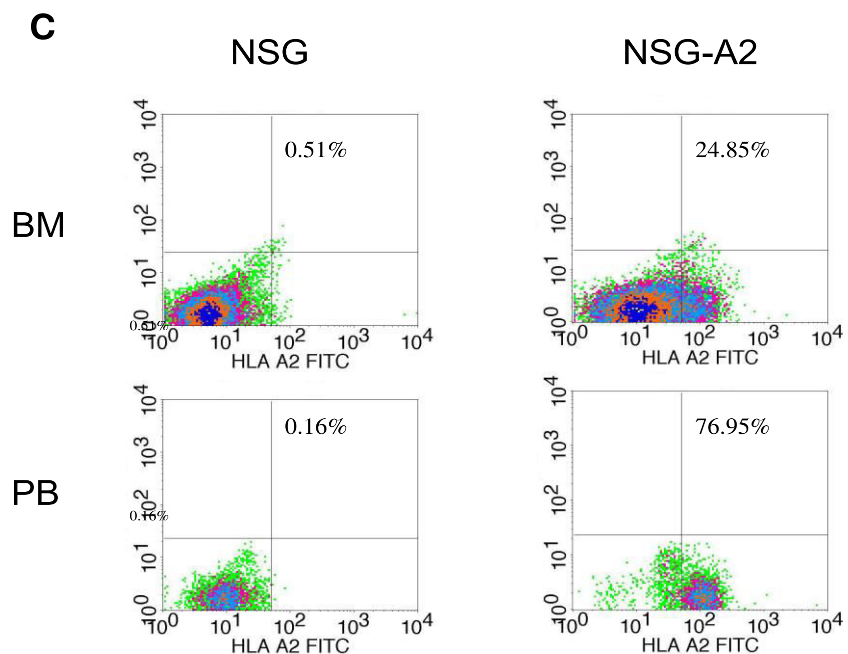
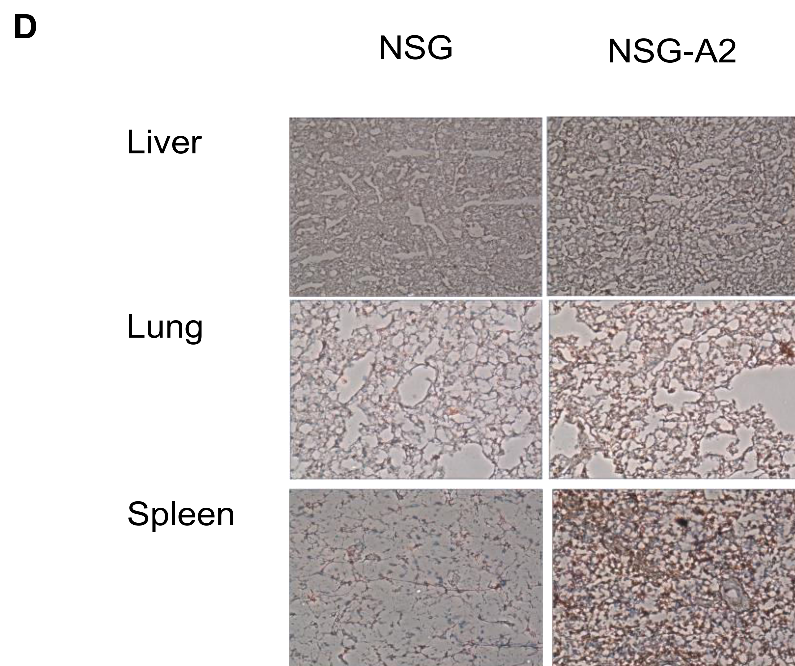
Supplemental Figure 1

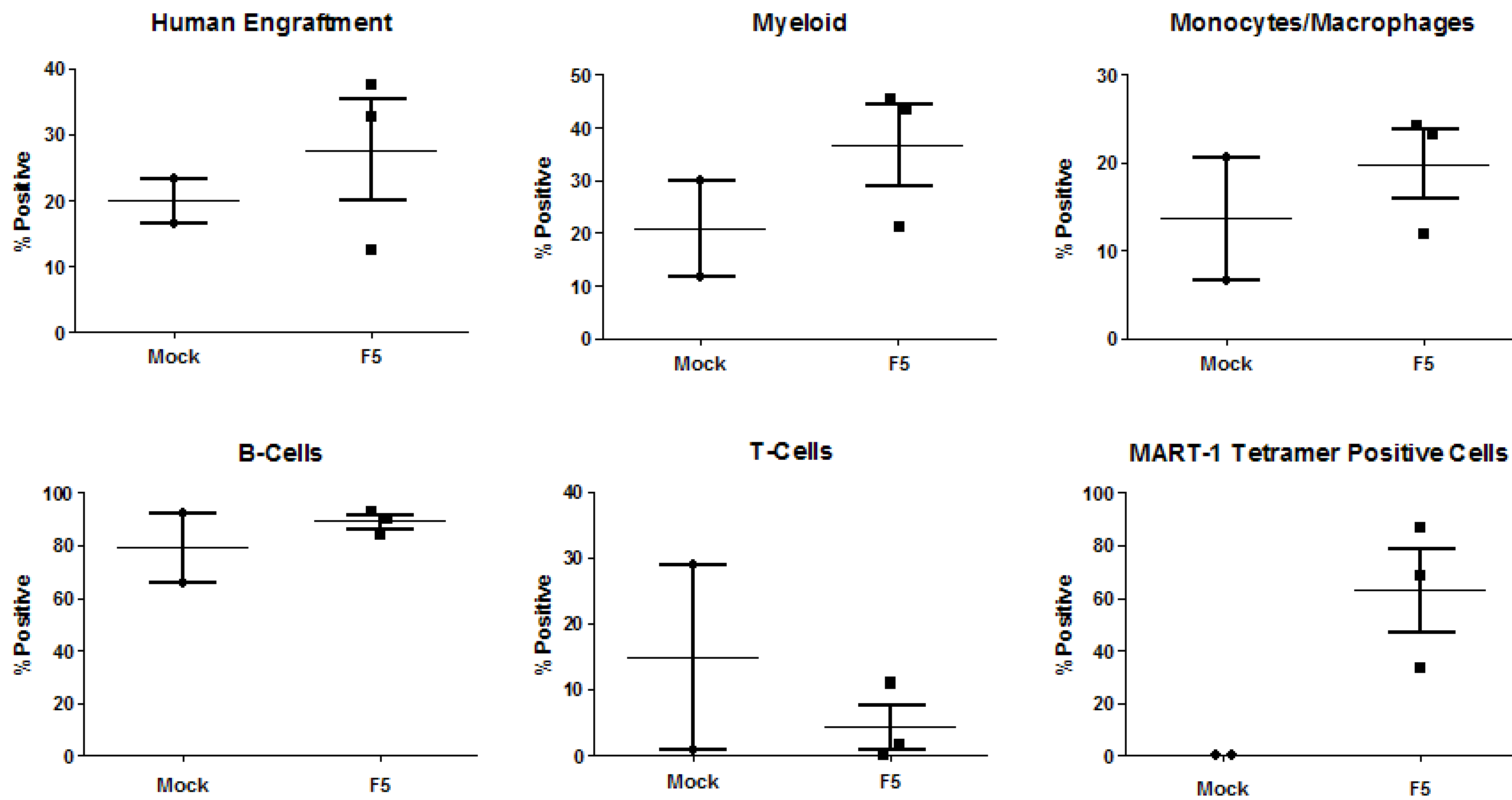








Supplemental Figure 2



SUPPLEMENTAL METHODS

Quantitative PCR for Vector Copy Number (VCN): Proviral vector copy numbers per cell (VCN) were assayed using primers that target the HIV-1 “psi” packaging region of the CCLc-MND-F5 lentivirus: (forward)-AACTGAAAGCGAAAGGGAAAC; (reverse)-CGCACCCATCTCTCTCCTTCT) and (probe)-AGCTCTCTCGACGCAGGACTCGGC with 5’ 6-carboxyfluorescein (FAM) and 3’ 6-carboxy-tetramethyl-rhodamine (TAMRA) labels. Samples assayed for vector proviral VCN content were compared to a known two copy of vector per cell standard and the data analyzed with the Applied Biosystems 7500 Software v. 2.0.4 (Life Technologies Corp., Carlsbad, CA).

Quantitative PCR for Human HLA A*0201 Transgene in Mice: To evaluate mice for the human HLA A*0201 transgene during derivation of the NSG-A2 mice, murine DNA samples were screened for the presence of the human HLA A*0201 allele using the HLA A2 primers (forward)-TCCTCGTCCCCAGGCTCT; (reverse)-TGTCGTCCACGTAGCCCACT) and (probe)-ACGGATGTGAAGAAAT with 5’ FAM and 3’ minor groove binder non-fluorescent quencher (MGBNFQ) labels. Primers to the endogenous murine DMa gene (forward)-CATCCATCTCAGCCGTCGAT; (reverse)-CAAAGGGTTCCGGTGTGAAGT and (probe)-6FAM-TTCCAGGCTTTCTCTTATT-MGBNFQ were used as an internal control. Control

samples included DNA from A2 homozygous mice, DNA from obligate A2 hemizygous mice and DNA from normal mice. Reactions were run in parallel and not multi-plexed.

Data were analyzed with the 7500 Software v. 2.0.4 (Life Technologies Corp.) to determine cycle threshold (Ct) for each replicate. The change in Ct value compared to the DMa gene control was calculated as an average of the replicates for A2 subtracted from the average of replicates for DMa. The delta Ct values were graphed and gauged visually to determine the appropriate cut off for homozygosity (**Supplemental Figure 1**).

Quantitative PCR for Human A2 Haplotype: Human DNA samples that were typed for the presence of HLA A2 used the HLA A2 primers described above. Primers to the endogenous human Syndecan-4 (SDC4) gene (forward)-CAGGGTCTGGGAGCCAAGT; (reverse)-GCACAGTGCTGGACATTGACA and (probe)-VIC-CCCACCGAACCCAAGAAACTAGAGGAGAAT-TAMRA were used as an internal control. Control samples included DNA from a healthy A2 positive donor, DNA from A2 negative K562 cells and murine DNA from A2 positive and negative mice. Reactions were run in parallel and not multi-plexed. Data were analyzed and scored depending upon whether amplification of the sample with the A2 primer set was observed. A2 positive, SDC4 positive samples were scored as “A2 positive.” A2 negative, SDC4 positive samples were scored as “A2 negative.”

Supplemental Table 1: Sequence Analysis of TCR Vβ CDR3 V·D·J Sequences Human CD3+ Cells from NSG Spleens.

F5 Mouse - huCD3(+)/MART Tetramer (+)

Nucleotide	Amino Acid	Copy	Cdr3	VFamily	VGene	DGene	JGene
TGTGCCAGCAGCCTAAGTTTCGGCACTGAAGC	CASSLSFGTE	153178	33	6	TRBV6-4	TRBD1-2	TRBJ1-1
TGTGCCAGCAGCTCCCGACAGAATTACGAGCA	CASSSRQNYE	621	33	7	TRBV7-9	TRBD1-1	TRBJ2-7
TGTGCCAGCAGCTCCCCCGAGACAGGGCCCGCTACGAGCA		567	41	7	TRBV7-6	TRBD1-1	TRBJ2-7
TGTGCCAGCAGCTTAGAGGGGTCGTGGGGATATTACTATGGCTA	CASSLEGSWGYYYG	494	45	11	(undefined)	TRBD1-1	TRBJ1-2
TGTGCCAGCACCTCCAATAATTCACCCCT	CASTSNNSP	340	30	7	TRBV7-2	TRBD1-1	TRBJ1-6
TGTGCCAGCAGTTACTCGACAGGACAGCTTTACTACGAGCA	CASSYSTGQLYYE	162	42	6	(undefined)	TRBD1-1	TRBJ2-7
TGTGCCAGCACATCCCGGGACGGCTCCTACAATGAGCA	CASTSRDGSYNE	159	39	6	(undefined)	TRBD1-1	TRBJ2-1
TGTGCCAGTAGTATATGGGGTGACAACGTCCT	CASSIWGDNV	157	33	19	TRBV19	TRBD1-1	TRBJ2-6
TGTGCCAGCAGCTTAAACGGGGGGCCTCACACTGAAGC	CASSLNGGPHTE	148	39	7	TRBV7-3	TRBD1-2	TRBJ1-1
TGCGCCAGCAGTCAACCTATGACAGGGTGACTATGGCTA		97	40	23	TRBV23-1	TRBD1-1	TRBJ1-2
TGCAGCGTTATGTCGACATACGAGCA	CSVMSTYE	94	27	29	TRBV29-1	TRBD1-1	TRBJ2-7
TGTGCCAGTAGTCGAGTGAATAGCAATCAGCCCCA	CASSRVNSNQP	73	36	19	TRBV19	TRBD1-1	TRBJ1-5
TGTGCCCGGGGGCGGGGGACCAACACTGAAGC	CARGRGTNTE	64	33	30	TRBV30	TRBD1-2	TRBJ1-1
TGCGCCAGCAGCTTAACCCAGACAGCGCGATACTATGGCTA	CASSLTQTARYYG	35	42	5	TRBV5-1	TRBD1-1	TRBJ1-2
TGTGCCAGCAGCTTAAGGCCCTTACTGAAGC		30	32	7	TRBV7-3	TRBD1-1	TRBJ1-1
TGTGCCAGCAGCCGTAGACTAGCGGGGGGGAGCTCCTACGAGCA	CASSRRLAGGSSYE	21	45	4	TRBV4-2	TRBD1-2	TRBJ2-7
TGCAGTGCTAGAGATGAGGGGGGCGCATGGATGGAGGC	CSARDEGGAWME	20	39	20	TRBV20-1	TRBD1-1	TRBJ1-1
TGCAGCGCTGACTGGTACGGGCCCACTGAAGC	CSADWYGPTE	20	33	29	TRBV29-1	TRBD1-1	TRBJ1-1
TGTGCCAGCAGTTTCACAGGGAACTATGGCTA	CASSFTGNYG	17	33	28	TRBV28	TRBD1-1	TRBJ1-2
TGTGCCAGCAGTTTCGGGCGGAAAGAGACCCA	CASSFGRKET	17	33	27	TRBV27	TRBD1-1	TRBJ2-5

Vβ Dβ Jβ

D Gene

Non-Template Insertions

Total:
156,420 reads
38 unique

SUPPLEMENTAL LEGENDS FOR SUPPLEMENTAL FIGURES

Supplemental Figure 1. Generation of HLA A*0201–NSG (NSG-A2) mice. A. Breeding schema for the derivation of the NSG-A2 strain. The matching colored boxes denote mice of the same genotype. Briefly, parent (P) NSG females (indicated as NOD-SCID (X*X*)(00) where X* is the X chromosome with the IL2Rgamma gene disrupted -red) were crossed with NOD-SCID-HLA*A0201 males (indicated as NOD-SCID (XY)(A*0201/A*0201 – black). Male offspring in the F1 generation (X*Y)(A*0201/0 - blue) were backcrossed with NSG females (red) to yield the F2 generation. IL2Rgamma null, A2 hemizygous females (green) and IL2Rgamma null, A2 hemizygous males (yellow) were identified by quantitative PCR for the human HLA*A0201 allele (see below, B) and crossed to yield the F3 generation. Male and female F3 offspring that were both IL2Rgamma null and A2 homozygous were then cross-bred for several generations to create the target strain, NSG-A2.

B. Represenative results from the PCR-based genotyping assay that distinguishes between HLA A*0201 (A2) hemizygous and homozygous mice. Mice 1-2 are known A2 homozygotes (avg. deltaCt = -1.82), mice 3-6 are known A2 hemizygotes (avg. deltaCt = -0.57) and mice 7-13 (avg. deltaCt = -1.72) are test mice of an unknown genotype. The difference in deltaCt between A2 homozygous and hemizygous mice was -1.17, which represents the difference of one amplification cycle.

C. Flow cytometric analysis of cells stained with an anti-human HLA-A2 antibody. The panels on the left are from the bone marrow (BM - top) and peripheral blood (PB - bottom) of a control NSG mouse while the panels on the right are from the BM and PB of an NSG-A2 mouse.

D. Immunohistochemical analysis of tissues for human HLA A2 expression. The sections were stained with anti-hu HLA A2 FITC followed by an anti-FITC secondary antibody conjugated to horseradish peroxidase with diaminobenzidine substrate yielding brown color in A2-positive samples. The panels on the left are from the lung (top) and spleen (bottom) of an NSG control mouse while the panels on the right are from the lung and spleen of an NSG-A2 mouse.

Supplemental Figure 2. Engraftment of NSG-A2 mice was determined by multichromatic flow cytometry analysis of peripheral blood (obtained 8 weeks after transplant. Human engraftment was determined as %hCD45/%totalCD45 (human and murine) in the lymphocyte gate. Subsequent populations were defined as the percentage of the hCD45+ cells that were: myeloid (hCD33+), monocytes/macrophages (hCD33+/hCD14+), B-cells (hCD19+), T-cells (hCD3+). F5 TCR+ T-cells were determined as the percentage of hCD3+ T-cells binding to HLA-2.1 tetramers loaded with the MART-1 peptide (ELAGIGILTV).