# CD27, CD201, FLT3, CD48, and CD150 cell surface staining identifies long-term mouse hematopoietic stem cells in immunodeficient non-obese diabetic severe combined immune deficient-derived strains

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# Complementarity of CD27, CD201, FLT3, CD48, and CD150 cell surface staining to identify long-term reconstituting mouse hematopoietic stem cells in immunodeficient non-obese diabetic severe combined immune deficient derived strains

**Short Title:** CD27, CD201, FLT3, CD48, and CD150 identify HSC in mice

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## Supplementary Methods

### Flow cytometry

HSC stain was performed on $5x10^6$ BM cells from a femur flushed with 1 mL of PBS + 2% FBS. Cells were stained in PBS+ 2% FBS containing 0.1 µg/mL purified rat anti-mouse CD16/CD32 antibody (Fc Block) (BD Bioscience), lineage (CD5, CD3ε, Ter119, CD11b, Ly6G/Ly6C, CD41)-FITC, anti-SCA1-PE, CD150-Brilliant Violet (BV)-605, c-KIT-APC-Cy7, CD48-Pacific Blue, FLT3-PE-CF594, CD27-PECy7 and CD201-biotin. Control stain with isotype-matched antibody controls instead of CD27, CD201, and CD48 antibodies were used to position gates correctly. Cells were incubated for 40 minutes and washed before a secondary stain of streptavidin-APC in PBS+ 0.5% bovine serum albumin (BSA) for 30 minutes. Samples were then washed and resuspended in PBS with 2% FBS containing 2 µg/mL dead cell discriminator dye 7-amino-actinomycin D (7-AAD) (Invitrogen) and analyzed on a CyAn flow cytometer (Beckman Coulter). Full gating strategy for HSC is provided in Supplementary Figure S1. All antibodies used in the experiments are listed in Supplementary Table S1.

Lineage stain was performed on $10^6$ BM or spleen cells from each mouse. Cells were stained in PBS+ 2% FBS containing Fc Block, CD11b-PECy7, B220-APC-Cy7, CD3ε-Pacific Blue, NK1.1-PE, F4/80-AlexaFluor647, CD169-FITC, and CD244-biotin. Control stain with isotype-matched antibody controls instead of CD244 were used to position gates correctly. Cells were incubated for 40 minutes and washed before a secondary stain of streptavidin-BV-605 in PBS plus 5% BSA for 30 minutes. Samples were then washed and resuspended in PBS plus 2% FBS containing 7-AAD and analyzed on a CyAn flow cytometer (Beckman Coulter). Gating strategy for lineage stain is in Supplementary Figures S3-4.

All collected endosteal cells depleted of hematopoietic cells by magnetic enrichment as per manufactures protocol and were stained with PBS + 2% FBS containing Fc Block, CD45-BV785, Ter119-FITC, CD31-BV421, PDGFRα-APC, SCA1-PE-Cy7 and CD51-PE for 40 minutes. Control stain with isotype-matched antibody controls instead of PDGFRα, CD51, and SCA1 to were used to position gates correctly. Samples were washed and resuspended in PBS + 2% FBS containing 7-AAD and analyzed on a Fortessa flow cytometer (Beckman Dickson).

For the transplant sort experiments, 'full minus one' (FMO) color controls were used to determine correct gating strategy (Supplementary Figure S5).

**Measurement of male cell engraftment**

DNA was extracted using a modification of the whole blood protocol with a Genomic DNA spin column kit (Bioline BIO-52066). Blood samples were made up to 200 µL with PBS with 4 mM $CaCl_2$ for extraction with the kit. An aliquot of BM flush and splenocyte preparation equaling a fifth of one femur or a fifth of a whole spleen was used for genomic DNA preparation. Samples were digested at 56 ˚C for 3 hours and then centrifuged of 10, 000 rpm to exclude all cell debris and DNA-containing supernatants collected. 10 ng DNA was used in a quantitative PCR reaction using the SYBR green master mix (Thermofisher 4309155) with 4 nM of primers for the *sry* gene versus the *il6* gene. The level of male engraftment was determined by correlating the signal of the *sry* primers (2) (forward: 5'-TTATGGTGTGGTCCCGTGGT-3' and reverse: 5'-GGCCTTTTTTCGGCTTCTGT) compared to total genomic expression of *il6* gene (forward: 5' GAACCAAGACCATCCAATTCATCTTGAAA and reverse: 3' GACCACAGTGAGGAATGTCCACAAA: 50 cycles at 95°C for 30 seconds followed by a minute at 60°C. A calibration and validation of male genomic DNA were made by mixing male and female BM cells in known ratios before extraction to confirm the linearity of Y chromosome

DNA amplicons relative to the proportion of male cells (Supplementary Figure S6). The proportion of male cells in test samples was calculated by linear regression extrapolation from the calibration regression. Mice which were counted as positively reconstituted when more than 1% were of male origin (Supplementary Table 4).

Competitive reconstitution units (CRU) frequencies were calculated by Poisson's statistic using the L-Calc software (Stem Cell Technologies). The number of CRU contained in each gate was then calculated by multiplying for each individual mouse the number of cells in each sort gate by the frequency of CRU in each gate (Supplementary Table S5).

**Isolation culture and staining of Mesenchymal Stromal Cells**

Mouse MSC were isolated from male NSG mice based on plastic adherence as previously reported (1). Briefly femurs and tibias were collected and gently crushed in a mortar with pestle to remove the BM. Bone fragments were washed repeatedly with PBS+ 2% FBS. Bone chips were then incubated in 3 mg/ml collagenase type 1 (Worthington) made in Iscove's Modified Dulbecco's Media (IMDM) for 20 minutes at 37 ˚C with shaking. Cells were then filtered with 70 µm cell strainer and placed into culture with α-Minimum Essential Medium (MEM) supplemented with 10% FBS, 1% penicillin-streptomycin and 100 µM phospho-ascorbic acid in atmosphere with 5% $CO_2$ at 37 ˚C. Medium was changed twice a week and passages were counted at each expansion step. Tissue culture reagents were purchased from Thermofisher unless mentioned otherwise.

After first passage, mouse MSC were depleted of mouse leukocytes by sorting the $CD45^-$ population with a Beckman Coulter Astrios cell sorter. At passage 2, mouse MSC were stained with anti-SCA1 antibody or isotype-matched control to measure its expression on the Cytoflex (Beckman Coulter) flow cytometer.

**Statistics**

Statistics were performed with Graph Pad Prism 7 (La Jolla, CA) using one-way ANOVA analysis with Tukey Correction. The frequencies of competitive repopulation units (CRU) in the transplantation assays were calculated by Poisson's distribution statistics using the L-Calc software (Stem Cell Technologies, Vancouver, Canada).

## Supplementary References


1. Cook MM, Futrega K, Osiecki M, Kabiri M, Kul B, Rice A, et al. Micromarrows--three-dimensional coculture of hematopoietic stem cells and mesenchymal stromal cells. Tissue Eng Part C Methods. 2012;18(5):319-28.
2. Franco C, Britto K, Wong E, Hou G, Zhu S-N, Chen M, et al. Discoidin domain receptor 1 on bone marrow–derived cells promotes macrophage accumulation during atherogenesis. Circulation Research. 2009;105(11):1141.

Supplementary Table S1. Flow Antibodies used in experiment separated into each test

| **HSC stain** | **antibody** | **company** | **clone** | **catalogue number** | Antibody concentration |
|---|---|---|---|---|---|
| | CD5 FITC | Biolegend | 53-7.3 | 100606 | 2.5 µg/mL |
| | CD3ε FITC | Biolegend | 17A2 | 100204 | 2.5 µg/mL |
| | B220 FITC | Biolegend | RA3-6B2 | 103206 | 2.5 µg/mL |
| | Ter119 FITC | Biolegend | TER-119 | 116206 | 2.5 µg/mL |
| | CD11b FITC | Biolegend | M1/70 | 101206 | 2.5 µg/mL |
| | Ly6G/Ly6C (Gr-1) FITC | Biolegend | RB6-8C5 | 108406 | 2.5 µg/mL |
| | CD41 FITC | Biolegend | MWReg30 | 133904 | 2.5 µg/mL |
| | Sca1 PE | Biolegend | D7 | 108108 | 2 µg/mL |
| | CD150 Brilliant Violet 605 | Biolegend | TC15-12F12.2 | 115927 | 1 µg/mL |
| | CD117 (c-KIT) APC-Cy7 | Biolegend | 2B8 | 105826 | 1 µg/mL |
| | CD48 Pacific Blue | Biolegend | HM48-1 | 103418 | 2.5 µg/mL |
| | CD201 (EPCR) Biotin | Biolegend | RCR-16 | 141508 | 2.5 µg/mL |
| | CD27 PE-Cy7 | Biolegend | LG.3A10 | 124216 | 1 µg/mL |
| | CD135 (Flt3) PE-CF594 | BD Bioscience | A2F10.1 | 562537 | 2 µg/mL |
| | Streptavidin APC | Biolegend | | 405207 | 1 µg/mL |
| Lineage stain | antibody | company | clone | catalogue number | Antibody concentration |
| | CD11b PE-Cy7 | Biolegend | M1/70 | 101216 | 1 µg/mL |
| | B220 APC-Cy7 | Biolegend | RA3-6B2 | 103224 | 1 µg/mL |
| | CD3ε Pacific Blue | Biolegend | 17A2 | 100214 | 2.5 µg/mL |
| | NK1.1 PE | Biolegend | PK136 | 108708 | 2 µg/mL |
| | F4/80 Alexa Fluor 647 | Serotec | A3-1 | MCA497A647 | 2.5 µg/mL |
| | CD169 FITC | Biolegend | 3D6-112 | 142406 | 2.5 µg/mL |
| | CD244 Biotin | Biolegend | m2B4 (B6)458.1 | 133505 | 2.5 µg/mL |
| | Streptavidin Brilliant Violet 605 | Biolegend | | 405232 | 0.5 µg/mL |
| Endosteal stain | antibody | company | clone | catalogue number | Antibody concentration |
| | CD45 BV785 | Biolegend | 30-F11 | 109839 | 2 µg/mL |
| | Terr-119 FITC | Biolegend | TER-119 | 116206 | 2.5 µg/mL |
| | CD31 BV421 | Biolegend | 390 | 102423 | 2 µg/mL |
| | CD140 (PDGFRα) APC | Biolegend | APA5 | 135908 | 2 µg/mL |
| | Sca1 PE-Cy7 | Biolegend | D7 | 108114 | 2 µg/mL |
| | CD51 PE | Biolegend | RMV-7 | 104106 | 2 µg/mL |
| Tissue Culture MSC stain | antibody | company | clone | catalogue number | Antibody concentration |
| | Sca1 PE-Cy7 | Biolegend | D7 | 108129 | 2 µg/mL |
| | Rat IgG1, κ | Biolegend | | 400416 | 2 µg/mL |
| | CD45 APC | Biolegend | 30-F11 | 103112 | 2 µg/mL |

Supplementary Table S2. Cell frequencies and total cells per femur between mouse strains

| | C57bl/6 | | NOD-*scid* | | NSG | |
|---|---|---|---|---|---|---|
| | Frequency | Cells/Femur | Frequency | Cells/Femur | Frequency | Cells/Femur |
| **$L^-K^+CD27^+CD201^+$** | 0.019% ± 0.007% | 5,872 ± 2,365 | 0.100%± 0.012% | 23,077 ± 5,495 | 0.041%± 0.014% | 8,744 ±3,520 |
| **$L^-K^+CD27^+CD201^+FLT3^-$ $CD48^-$ $CD150^+$** | 0.0012% ± 0.0004% | 362 ±125 | 0.0015%± 0.0003% | 332 ± 83 | 0.0007% ± 0.0005% | 145 ± 99 |

Average ± standard deviation – 5 mice per group.

Supplementary Table S3. CD244 prevalence in bone marrow and spleen of mice strains

| | | C57BL/6 | | NOD-*scid* | | NSG | |
|---|---|---|---|---|---|---|---|
| | | CD244+ Percent of parent | CD244+ Cells/ femur | CD244+ Percent of parent | CD244+ Cells/ femur | CD244+ Percent of parent | CD244+ Cells/ femur |
| BONE MARROW | Monocytes ($CD11b^+$ $F4/80^+$ $CD169^-$) | 10.85% ± 2.05% | 263,677 ± 58,364 | 0.35% ± 0.05% | 3,588 ± 865 | 0.54% ± 0.22% | 2,614 ± 738 |
| | Macrophages ($CD11b^+$ $F4/80^+$ $CD169^+$) | 2.79% ± 0.31% | 65,609 ± 9,949 | 0.6% ± 0.08% | 10,656 ± 2,349 | 0.73% ± 0.14% | 18,056 ± 1,578 |
| | Neutrophils and myeloid progenitors ($CD11b^+$ $F4/80^-$ $CD169^-$) | 2.01% ± 0.35% | 88,499 ± 11,917 | 0.12% ± 0.04% | 4,934 ± 2,132 | 0.12% ± 0.06% | 2,210 ± 1,264 |
| | B cells ($CD11b^-$ $B220^+$) | 1.55% ± 0.41% | 74,026 ± 30,276 | 0.73% ± 0.26% | 3,570 ± 1,510 | 1.36% ± 0.47% | 2,621 ± 969 |
| | NK cells ($CD11b^+$ $Nk1.1^+$) | 78.44% ± 2.04% | 128,456 ± 20,757 | 62.31% ± 12.27% | 4,858 ± 593 | 84.59% ± 6.04% | 7,687 ± 1,139 |
| | T cell ($CD11b^-$ $B220^-$ $CD3^+$) | 4.04% ± 1.77% | 5,318 ± 2,027 | 2.21% ± 0.59% | 586 ± 171 | 7.61% ± 5.74% | 1,094 ± 404 |
| SPLEEN | Monocytes ($CD11b^+$ $F4/80^+$ $CD169^-$) | 39.36% ± 4.36% | 110,051 ± 27,242 | 3.13% ± 1.4% | 1,797 ± 1,303 | 2.08% ± 0.83% | 955 ± 467 |
| | Macrophages ($CD11b^+$ $F4/80^+$ $CD169^+$) | 31.06% ± 2.09% | 6,075 ± 1,480 | 10.51% ± 8.21% | 1,431 ± 1,113 | 6.97% ± 4.66% | 633 ± 336 |
| | Neutrophils and myeloid progenitors ($CD11b^+$ $F4/80^-$ $CD169^-$) | 23.86% ± 2.38% | 696,150 ± 9,656 | 0.27% ± 0.08% | 140 ± 73 | 0.29% ± 0.04% | 161 ± 35 |
| | B cells ($CD11b^-$ $B220^+$) | 0.5% ± 0.07% | 13,815 ± 1,792 | 5.06% ± 1.18% | 1,183 ± 616 | 3.66% ± 0.4% | 1,051 ± 435 |
| | NK cells ($CD11b^+$ $Nk1.1^+$) | 76.83% ± 2.89% | 105,262 ± 32,778 | 91.46% ± 3.68% | 2,660 ± 1,715 | 90.11% ± 2.94% | 1,545 ± 858 |
| | T cell ($CD11b^-$ $B220^-$ $CD3^+$) | 0.65% ± 0.04% | 6,623 ± 568 | 12.5% ± 5.16% | 113 ± 78 | 10.79% ± 9.33% | 63 ± 41 |

Average ± standard deviation – 5 mice per group

Supplementary Table S4: Transplant results from mice including 12 and 16 week bleeds and 18 week harvest of blood, spleen and bone marrow.

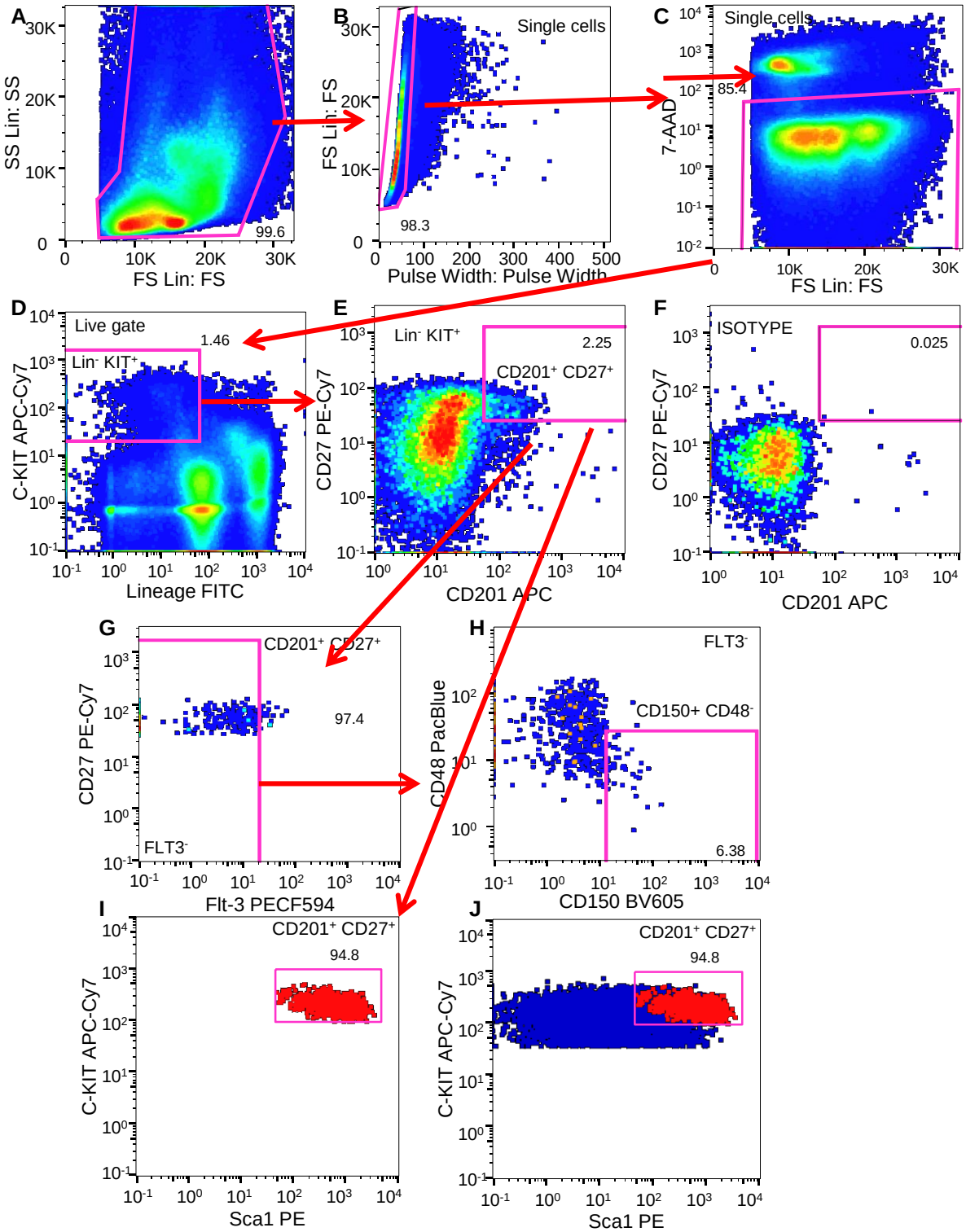
| Transplant results | | | Bleed (Weeks) | | Harvest 18 weeks | | |
|---|---|---|---|---|---|---|---|
| **Group** | Cells tx | mouse # | **12** | **16** | Blood | Bone Marrow | Spleen |
| **LT-HSC** | 10 | H1 | 0.01% | 0.01% | 0.01% | 0.06% | 0.03% |
| | | H2 | 0.02% | 0.05% | 0.08% | 0.03% | 0.03% |
| | | H3 | 0.01% | 0.11% | 0.00% | 0.05% | 0.03% |
| | | H4 | 0.05% | 0.38% | 0.00% | 0.02% | 0.03% |
| | | H5 | 0.41% | 0.04% | 0.00% | 0.10% | 0.11% |
| | | H6 | 0.10% | 0.35% | 0.07% | 0.05% | 0.09% |
| | 50 | H7 | 0.01% | 0.14% | 0.12% | 0.05% | 0.03% |
| | | H8 | **7.42%** | **10.80%** | **13.26%** | **2.71%** | **5.95%** |
| | | H9 | **3.67%** | **1.91%** | **1.17%** | **2.50%** | 0.48% |
| | | H10 | 0.06% | 0.35% | 0.01% | 0.04% | 0.07% |
| | | H11 | **20.32%** | **10.49%** | **9.16%** | 0.07% | 0.27% |
| | | H12 | 0.03% | 0.07% | 0.00% | 0.03% | 0.08% |
| | | B1 | **5.46%** | **3.05%** | **2.07%** | 0.17% | **1.46**% |
| | | B2 | 0.03% | 0.00% | 0.00% | 0.06% | 0.09% |
| | | B3 | 0.15% | 0.00% | 0.01% | 0.03% | 0.05% |
| | | B4 | 0.68% | 0.00% | 0.00% | 0.01% | 0.24% |
| | | B5 | 0.03% | 0.29% | 0.01% | 0.08% | 0.05% |
| | | B6 | 0.04% | 0.00% | 0.01% | 0.16% | 0.10% |
| | | B7 | 0.09% | 0.00% | 0.00% | 0.05% | 0.10% |
| | 150 | C1 | 0.22% | 0.01% | 0.02% | 0.06% | 0.17% |
| | | C2 | **54.98%** | **59.43%** | **23.36%** | **2.21%** | **11.69%** |
| | | C3 | **49.46%** | **50.53%** | **40.41%** | **5.36%** | **25.60%** |
| | | C4 | **33.42%** | **12.03%** | **19.56%** | **1.40%** | **1.37%** |
| | | C5 | **6.50%** | 0.00% | 0.56% | 0.30% | 0.97% |
| | | C6 | **2.24%** | 0.36% | 0.00% | 0.00% | 0.08% |
| **NOT GATE** | 10 | N1 | 0.00% | 0.05% | 0.00% | 0.04% | 0.03% |
| | | N2 | 0.01% | 0.25% | 0.00% | 0.05% | 0.04% |
| | | N3 | 0.01% | 0.10% | 0.06% | 0.04% | 0.06% |
| | | N4 | 0.09% | 0.06% | 0.10% | 0.08% | 0.07% |
| | | N5 | 0.00% | 0.17% | 0.00% | 0.06% | 0.09% |
| | 50 | N6 | 0.01% | 0.03% | 0.00% | 0.09% | 0.06% |
| | | N7 | 0.00% | 0.02% | 0.07% | 0.07% | 0.10% |
| | | N8 | 0.00% | 0.05% | 0.08% | 0.06% | 0.05% |
| | | N9 | 0.00% | 0.03% | 0.01% | 0.07% | 0.04% |
| | | N10 | 0.01% | 0.25% | 0.00% | 0.02% | 0.04% |
| | | D1 | 0.06% | 0.00% | 0.02% | 0.00% | 0.05% |
| | | D3 | 0.06% | 0.00% | 0.01% | 0.06% | 0.05% |
| | | D4 | 0.07% | 0.00% | 0.00% | 0.04% | 0.03% |
| | | D5 | 0.14% | 0.03% | 0.05% | 0.18% | 0.18% |
| | 150 | E1 | 0.04% | 0.00% | 0.01% | 0.07% | 0.18% |
| | | E2 | 0.22% | 0.00% | 0.00% | 0.06% | 0.11% |
| | | E3 | 0.04% | 0.00% | 0.00% | 0.12% | 0.20% |
| | | E4 | 0.12% | 0.00% | 0.07% | 0.01% | 1.01% |
| | | E5 | 0.12% | 0.00% | 0.10% | 0.07% | 0.10% |
| | 1000 | F1 | 0.67% | 0.02% | 0.29% | 0.15% | 0.47% |
| | | F2 | **12.14**% | 0.46% | **1.38%** | **2.22%** | 0.95% |
| | | F3 | 0.77% | 0.02% | 0.04% | 0.06% | 0.27% |
| | | F4 | 0.04% | 0.00% | 0.10% | 0.11% | 0.24% |
| | | F5 | 0.23% | 0.00% | 0.07% | 0.22% | 0.97% |

Percent male (*sry*$^+$) engraftment of female mice at each bleed or harvest. Mice >1% in bold to indicate successful engraftment. Tx= transplanted

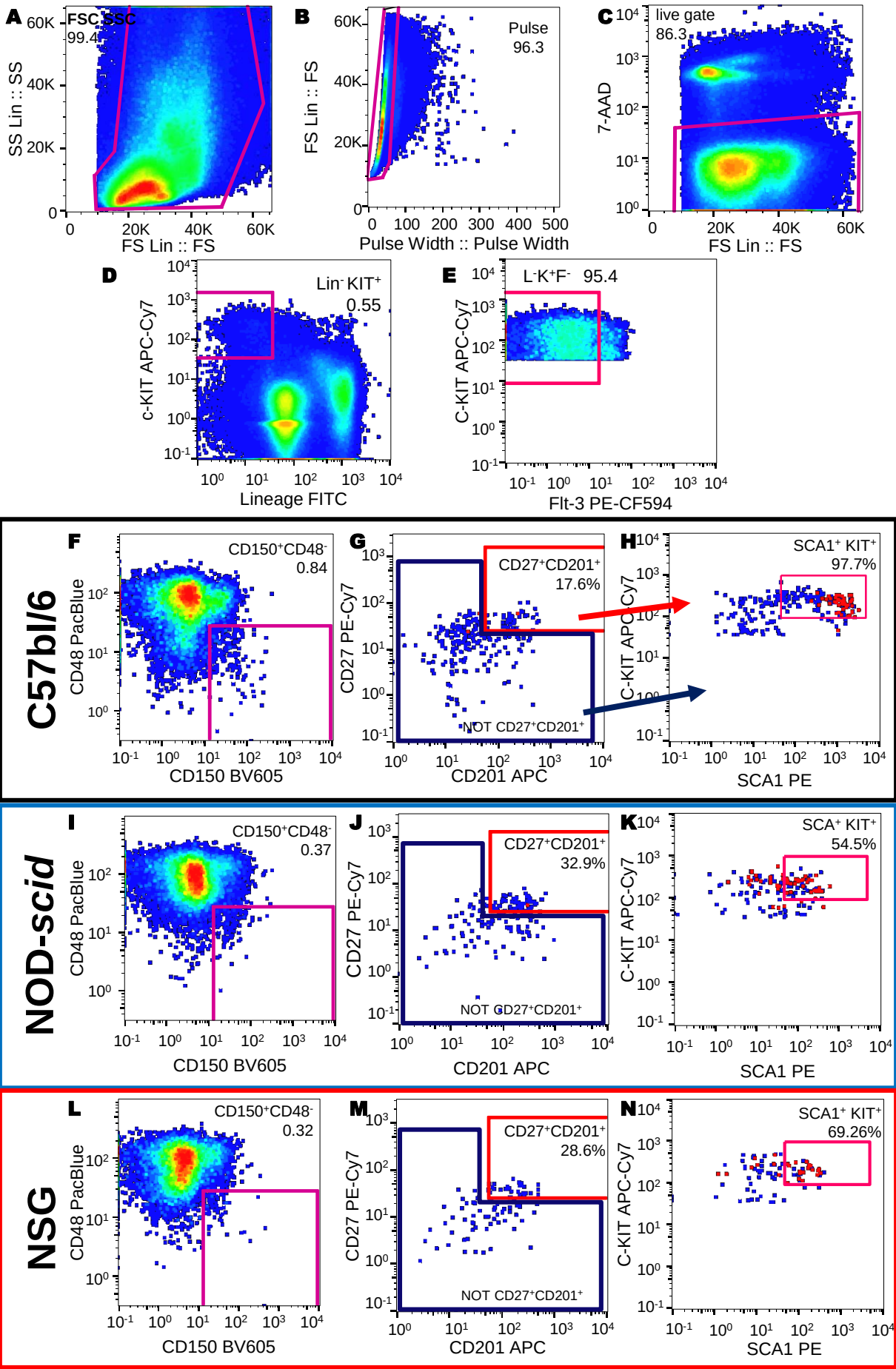
Supplementary Table S5. Competitive reconstitution units per sort gate based on LT-HSC frequencies determined by serial dilution transplants

| Mouse | LK CD27$^+$ CD201$^+$ FLT3$^-$ | CD48$^-$ CD150$^+$ | CRU Frequency | CRU/Gate | NOT GATE | CRU Frequency | CRU/Gate | Proportion CRU in CD48$^-$CD150$^+$ gate versus parent LK CD201$^+$ CD27$^+$ FLTt3$^-$ gate |
|---|---|---|---|---|---|---|---|---|
| 1 | 10,407 | 560 | 1/179 | 3.1 | 9,847 | 1/5,786 | 1.7 | 64.6% |
| 2 | 11,912 | 784 | | 4.4 | 11,128 | | 1.9 | 69.8% |
| 3 | 5,014 | 374 | | 2.1 | 4,640 | | 0.8 | 72.4% |
| 4 | 9,492 | 892 | | 5 | 8,600 | | 1.5 | 76.9% |
| | | | | | | | Mean | 70.9% |
| | | | | | | | SD | 5.2% |

Abbreviations: LK= lineage$^-$ C-KIT$^+$; CRU= competitive reconstitution units; SD= standard deviation.




Supplementary Figure S1. Gating Strategy to identify phenotypic HSC in C57BL/6 mice. $5x10^6$ BM cells were stained with HSC antibody cocktail and analyzed by flow cytometry. (A-C) Viable single cells were gated as (D) Lineage-negative, c-KIT$^+$ and (E) CD201 and CD27 positive. F) Isotype control for CD27 and CD201. Lin$^-$ Kit$^+$ CD27$^+$CD201$^+$ cells were further gated on Flt-3 (G). Lin$^-$ KIT$^+$ CD27$^+$CD201$^+$ cells gated to define the primitive FLT3$^-$ HSPC and subsequently CD48$^-$ CD150$^+$ phenotypic HSC (H). (I) Lin$^-$ KIT$^+$ CD27$^+$ CD201$^+$ cells (red) were examined for SCA1 and KIT expression and (J) overlaid with Lin$^-$ KIT$^+$ CD27$^+$CD201$^-$ cells (blue).The position of the SCA1+ KIT+ gate is highlighted in red.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B
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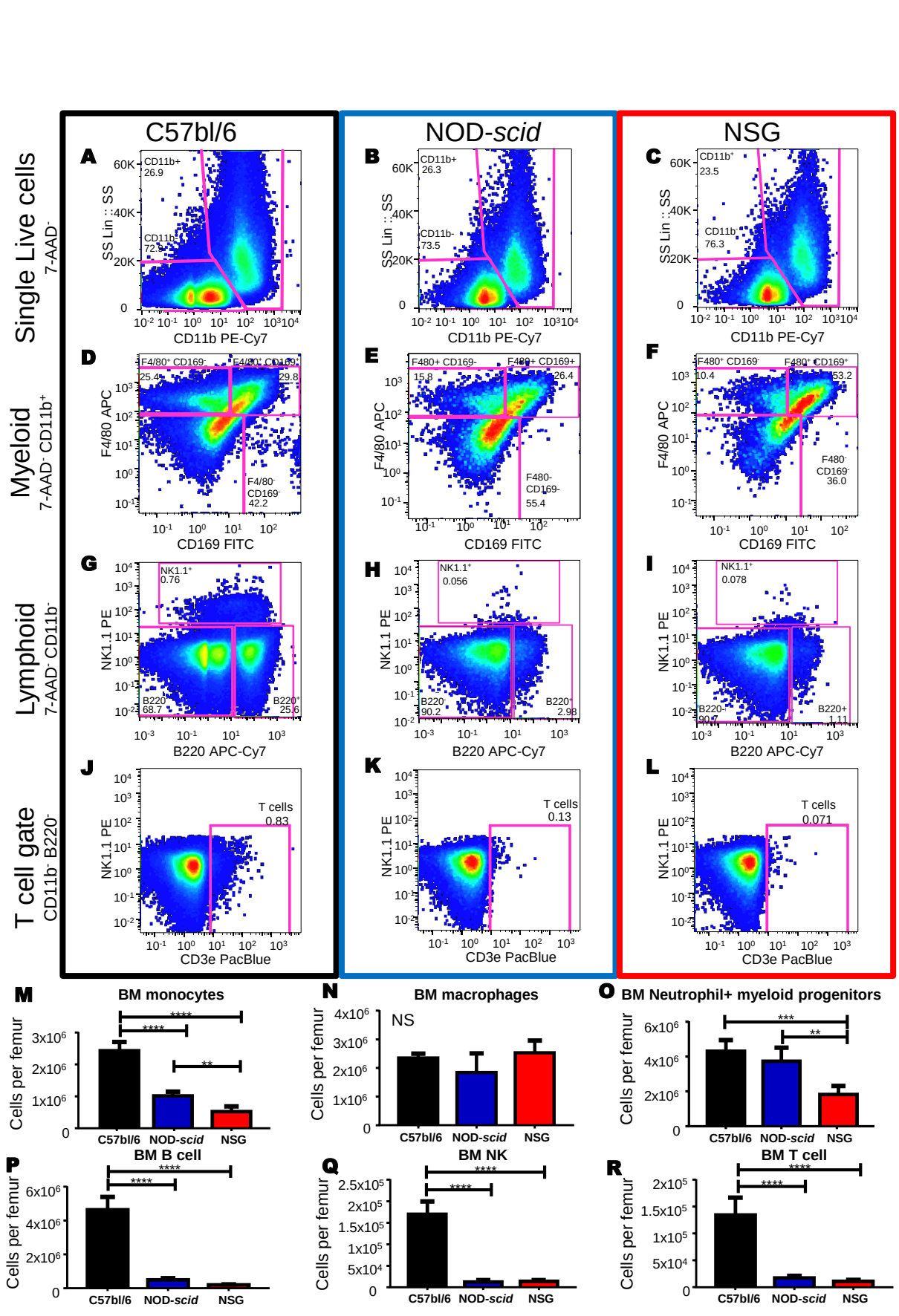
7-AAD
FS Lin :: FS
D
Lin⁻ KIT⁺
0.55
c-KIT APC-Cy7
Lineage FITC
E
L⁻K⁺F⁻ 95.4
C-KIT APC-Cy7
Flt-3 PE-CF594
C57bl/6
F
CD150⁺CD48⁻
0.84
CD48 PacBlue
CD150 BV605
G
CD27⁺CD201⁺
17.6%
NOT CD27⁺CD201⁺
CD27 PE-Cy7
CD201 APC
H
SCA1⁺ KIT⁺
97.7%
C-KIT APC-Cy7
SCA1 PE
NOD-scid
I
CD150⁺CD48⁻
0.37
CD48 PacBlue
CD150 BV605
J
CD27⁺CD201⁺
32.9%
NOT CD27⁺CD201⁺
CD27 PE-Cy7
CD201 APC
K
SCA⁺ KIT⁺
54.5%
C-KIT APC-Cy7
SCA1 PE
NSG
L
CD150⁺CD48⁻
0.32
CD48 PacBlue
CD150 BV605
M
CD27⁺CD201⁺
28.6%
NOT CD27⁺CD201⁺
CD27 PE-Cy7
CD201 APC
N
SCA1⁺ KIT⁺
69.26%
C-KIT APC-Cy7
SCA1 PE

Supplementary Figure S2. Combination of $CD48^-$ $CD150^+$ gating and $CD27^+$ $CD201^+$ gating is necessary to identify phenotypic HSCs. $5x10^6$ BM cells from each mouse strain were stained with HSC antibody cocktail and analyzed on flow cytometry. (A-C) Viable single cells were gated as (D) Lineage negative, c-$KIT^+$ and (E) FLT3 negative, and then examined for CD48 and CD150 for the mice stains, C57BL/6 in black box (F), NOD-*scid* in blue box (I) and NSG in red box (L). $L^-K^+FLT3^-CD48^-CD150^+$ cells were gated to CD27 and CD201 expression in C57bl/6 (G), NOD-*scid* (J) and NSG (M). Only a minority of $Lin^-$ $KIT^+$ $FLT3^-$ $CD48^-$ $CD150^+$ cells are also $CD27^+$ $CD201^+$. SCA1 expression heterogeneity was demonstrated at this level by taking the $CD27^+$ $CD201^+$ gate (red) versus the NOT GATE (blue) for the three mouse strains (H, K, and N).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NSG
Single Live cells
7-AAD-
Myeloid
7-AAD- CD11b+
Lymphoid
7-AAD- CD11b-
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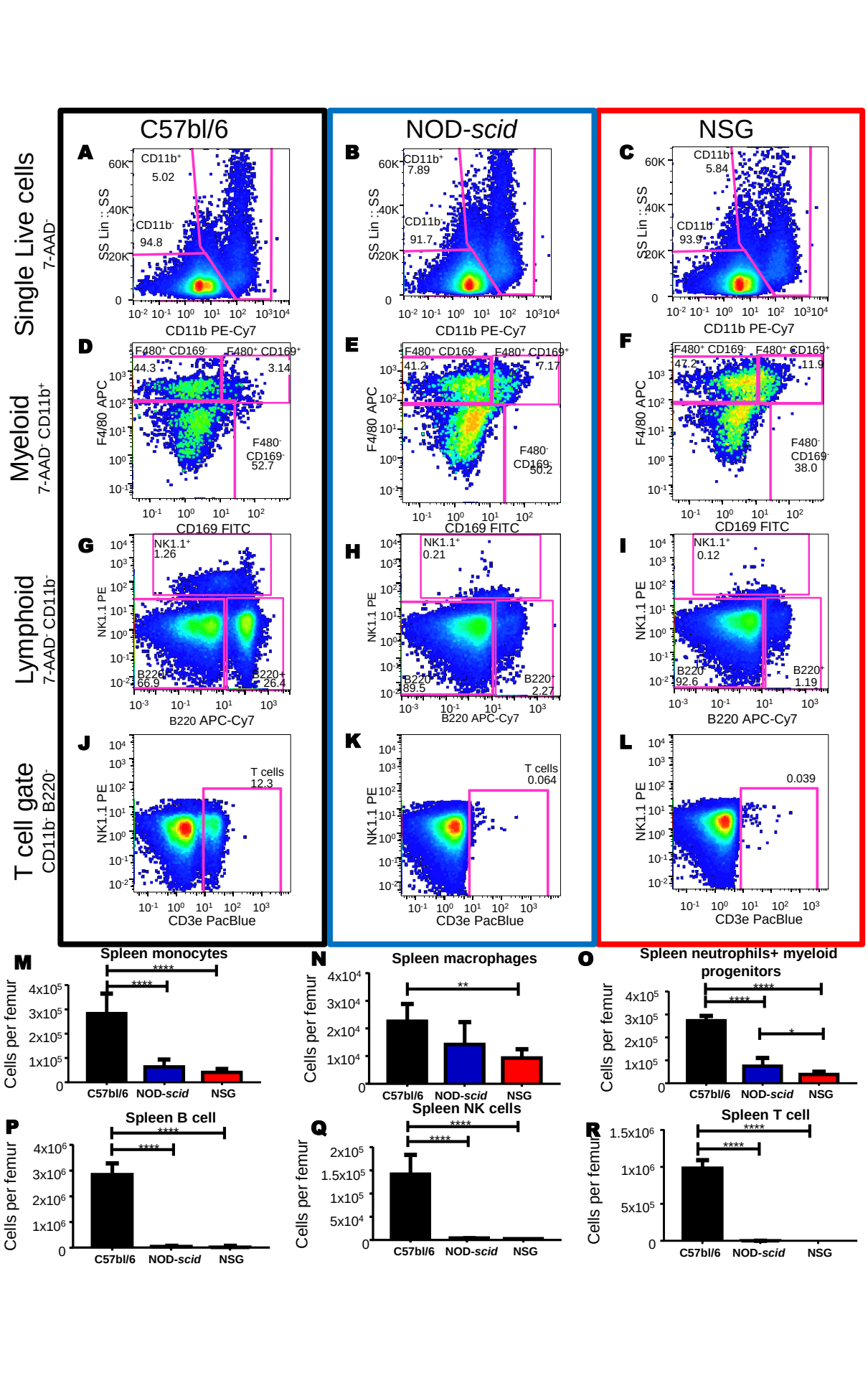
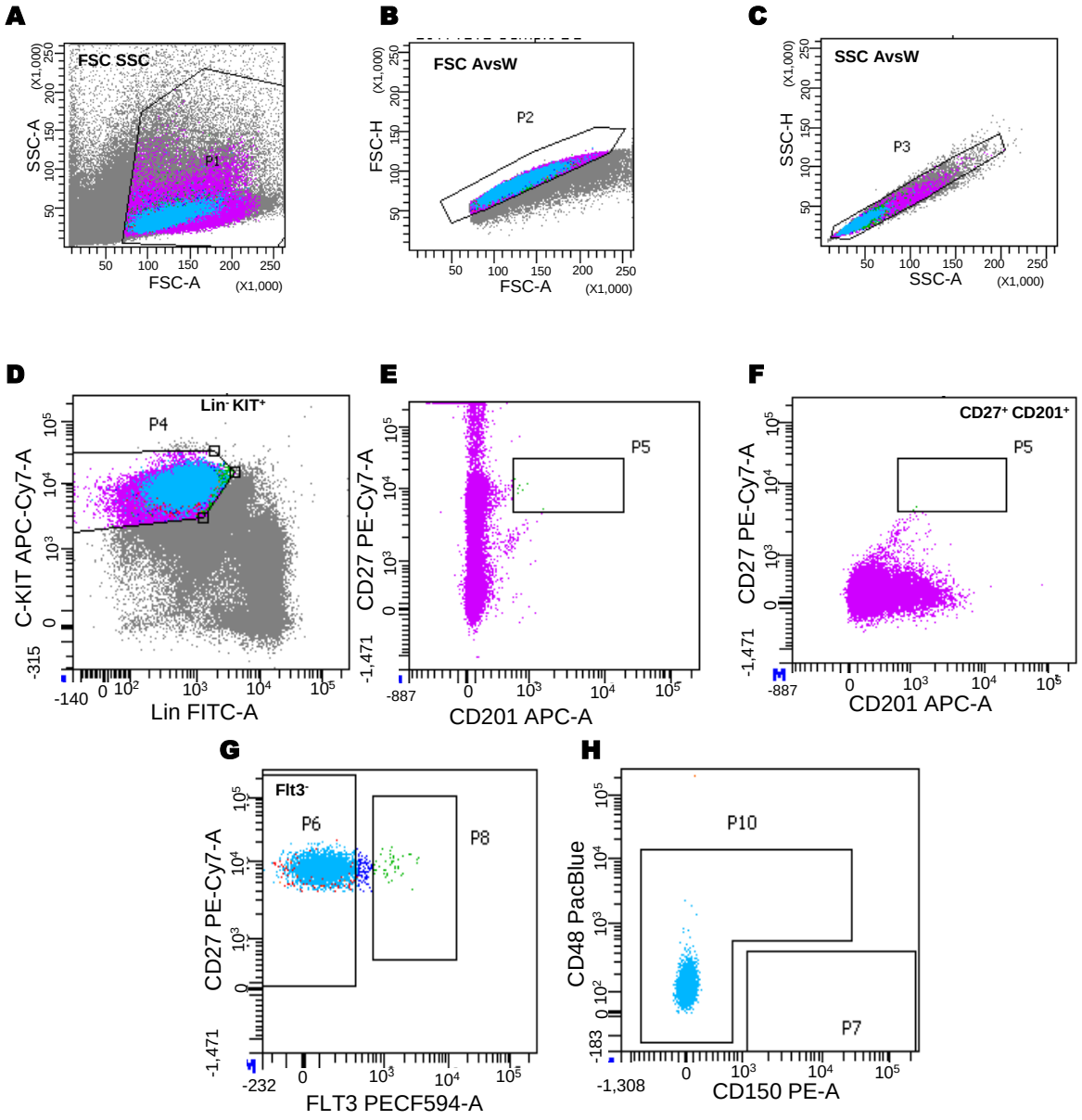
CD11b- B220-
A
B
C
SS Lin :: SS
CD11b+
26.9
CD11b-
72.9
CD11b+
26.3
CD11b-
73.5
CD11b+
23.5
CD11b-
76.3
CD11b PE-Cy7
D
E
F
F4/80 APC
F4/80+ CD169-
25.4
F4/80+ CD169+
29.8
F4/80- CD169- 42.2
F480+ CD169-
15.8
F480+ CD169+
26.4
F480- CD169- 55.4
F480+ CD169-
10.4
F480+ CD169+
53.2
F480- CD169- 36.0
CD169 FITC
G
H
I
NK1.1 PE
NK1.1+
0.76
B220- 68.7
B220+ 25.6
NK1.1+
0.056
B220- 90.2
B220+ 2.98
NK1.1+
0.078
B220- 90.7
B220+ 1.11
B220 APC-Cy7
J
K
L
T cells
0.83
T cells
0.13
T cells
0.071
CD3e PacBlue
M
BM monocytes
N
BM macrophages
NS
O
BM Neutrophil+ myeloid progenitors
Cells per femur
P
BM B cell
Q
BM NK
R
BM T cell
C57bl/6
NOD-scid
NSG

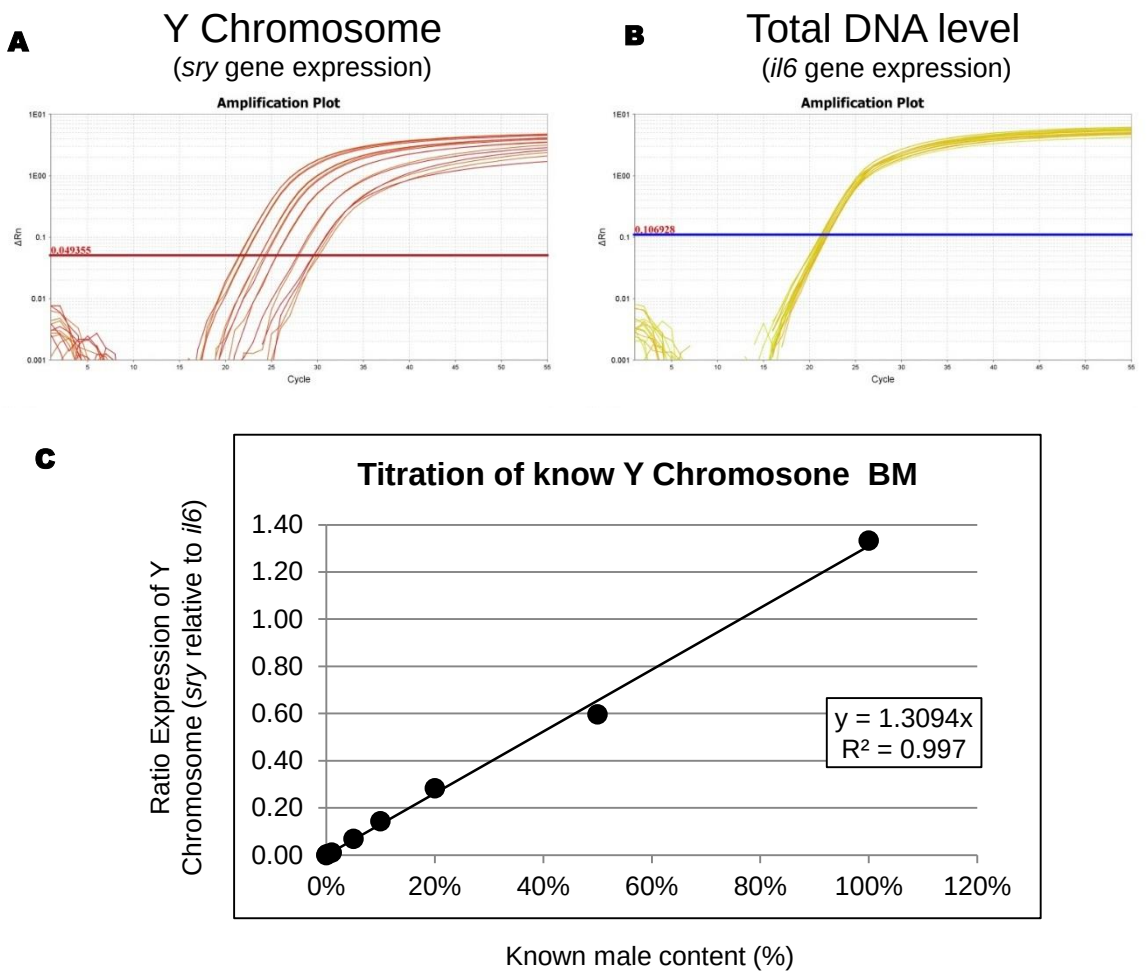
Supplementary Figure S3. Quantification of lineage-positive leukocytes in the bone marrow of different mouse strains. Each mouse strain appears in a different coloured column box: Viable single cells were gated into lymphoid ($CD11b^-$ low side scatter) and myeloid ($CD11b^+$) gates for each mouse strain (A-C). Myeloid cells were further separated using CD169 and F4/80 (D-F) as $CD11b^+$ $F4/80^+$ $CD169^-$ monocytes, $CD11b^+$ $F4/80^+$ $CD169^+$ macrophages and $CD11b^+$ $F4/80^-$ $CD169^-$ gate which includes granulocytes and immature myeloid cells. Lymphoid cells (G-I) were plotted with B220 against NK1.1 to identify B ($CD11b^-$ $B220^+$ $NK1.1^-$) and NK ($CD11b^-$ NK1.1) cells. The $B220^-$ $NK1.1^-$ gate was plotted for CD3ε expression to identify $CD3\varepsilon^+$ T cells (J-L). Total cells per femur for each cell type in histograms (M-R). Data are average ± SD of n=5 mice per group. P-values were calculated by ANOVA with Tukey corrections with multiple comparisons, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.


C57bl/6
NOD-scid
NSG
Single Live cells
7-AAD-
Myeloid
7-AAD- CD11b+
Lymphoid
7-AAD- CD11b-
T cell gate
CD11b- B220-
A
B
C
CD11b+
5.02
CD11b-
94.8
CD11b+
7.89
CD11b-
91.7
CD11b+
5.84
CD11b-
93.9
SS Lin :: SS
CD11b PE-Cy7
D
E
F
F480+ CD169-
44.3
F480+ CD169+
3.14
F480-
CD169-
52.7
F480+ CD169-
41.2
F480+ CD169+
7.17
F480-
CD169-
50.2
F480+ CD169-
47.2
F480+ CD169+
11.9
F480-
CD169-
38.0
F4/80 APC
CD169 FITC
G
H
I
NK1.1+
1.26
B220-
66.9
B220+
26.4
NK1.1+
0.21
B220-
89.5
B220+
2.27
NK1.1+
0.12
B220-
92.6
B220+
1.19
NK1.1 PE
B220 APC-Cy7
J
K
L
T cells
12.3
T cells
0.064
0.039
NK1.1 PE
CD3e PacBlue
M
Spleen monocytes
N
Spleen macrophages
O
Spleen neutrophils+ myeloid progenitors
P
Spleen B cell
Q
Spleen NK cells
R
Spleen T cell
Cells per femur
C57bl/6
NOD-scid
NSG

Supplementary Figure S4. quantification of lineage positive leukocytes in the spleen of different mouse strains. Each mouse strain appears in a different coloured column box: Viable single cells were gated into lymphoid ($CD11b^-$ low side scatter) and myeloid ($CD11b^+$) gates for each mouse strain (A-C). Myeloid cells were further separated using CD169 and F4/80 (D-F) as $CD11b^+$ $F4/80^+$ $CD169^-$ monocytes, $CD11b^+$ $F4/80^+$ $CD169^+$ macrophages and $CD11b^+$ $F4/80^-$ $CD169^-$ gate which includes granulocytes and immature myeloid cells. Lymphoid cells (G-I) were plotted with B220 against NK1.1 to identify B ($CD11b^-$ $B220^+$ $NK1.1^-$) and NK ($CD11b^-$ NK1.1) cells. The $B220^-$ $NK1.1^-$ gate was plotted for CD3ε expression to identify $CD3\varepsilon^+$ T cells (J-L). Total cells per spleen for each cell type in histograms (M-R). Data are average ± SD of n=5 mice per group. P-values were calculated by ANOVA with Tukey corrections with multiple comparisons, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.




Supplementary Figure S5. CD27, CD201, FLT3, CD48, and CD150 sort controls. A-C) single cell sorting. (D) Lineage negative, KIT$^+$ gate to determine the full minus one controls for setting up sort parameters and gates. (E) is the minus CD201-APC control, (F) the minus CD27-PECy7 control. (G) shows the FLT3-PECF594 minus control and (H) shows the CD48-Pacblue and CD150-PE minus controls.




Supplementary Figure S6. qPCR titration of male donor genomic DNA versus female DNA for chimerism quantification. Amplification curves of *sry* (A) and *il6* gene (B) in different mixtures with different proportions of male and female leukocytes. (C) The linear relationship between the proportion of male cells and intensity of *sry* genomic DNA relative to *il6* genomic DNA.