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Supplemental Information

Nonirradiated NOD,B6.SCID *Il2rγ*$^{-/-}$ *Kit*$^{W41/W41}$ (NBSGW) Mice Support Multilineage Engraftment of Human Hematopoietic Cells

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SUPPLEMENTAL EXPERIMENTAL PROCEEDURES:

Genotyping— Oligonucleotides (Table S1) were synthesized by Integrated DNA Technologies (IDT, Coralville, IA). To confirm the *Kit*^{W41} allele, DNA was amplified by PCR using ol43 and ol44. The amplicon was sequenced using ol44. Mice were genotyped for a single G/A nucleotide polymorphism at nucleotide 75,652,562 in the *Mus musculus Kit* gene (NC_000071.6). To confirm the *Prkdc*^{SCID} allele, DNA was amplified by PCR using ol49 and ol50. The amplicon was sequenced using ol50. Mice were genotyped for a single T/A nucleotide polymorphism at nucleotide 15,839,180 in the *Mus musculus Prkdc* gene (NC_000082.6). The *IL2R*γ^{-} genotyping protocol is previously published (Shultz et al., 2005). To confirm the *Sirpa* allele, DNA was amplified by PCR using ol113 and ol114. The amplicon was sequenced using both ol113 and ol114 in separate reactions. Sequences were aligned to the C57BL/6J genome (NT_039207.8). Sequences were then analyzed at seven bases between nucleotides 70,488,250 and 70,488,290 for homozygosity and for the NOD specific polymorphisms (Strowig et al., 2011). To confirm *Tyr* allele, tail snip DNA was amplified by PCR using ol111 and ol112. The amplicon was sequenced using ol112. Mice were genotyped for a G/C nucleotide polymorphism at nucleotide 5,241,720 in the *Mus musculus Tyr* gene (NT_039433.8) (Shibahara et al., 1990). All sequence analysis was performed using DNASTAR SeqMan Pro software (Madison, WI).

Human xenograft analysis– Each blood sample was transferred into an eppendorf tube containing 150µL of 2%-dextran (Sigma) in PBS and 150µL of

0.5%-Heparin solution (Sigma). Blood was allowed to settle 20 minutes. The translucent upper layer (containing leukocytes) was transferred to 1 mL of red blood cell lysis buffer (RBCLB; 140mM Ammonia Chloride, 2mM Tris, pH7.6 [Sigma]). After 10 minutes, each tube's volume was increased to 3-mL with cold Flow Cytometry Staining Buffer (FCSB; HBSS, 2% fetal bovine serum [Hyclone], 10mM HEPES [Life Technologies, Corp.], 0.1% Sodium Azide [Sigma]), and the tubes underwent centrifugation to pellet the cells. A second wash with 1 mL of cold FCSB was performed and the tubes were again centrifuged. The femurs were flushed with 1 mL of FCSB. The spleens were homogenized using an 18-gauge needle, mashed with the blunt end of a 3 mL syringe, and filtered through a 70-micrometer mesh. Both were lysed with RBCLB (as outlined above) and washed.

Samples were stained in 100 uL FCSB with the following antibodies: anti-mouse CD45-fluorescein isothiocyanate (FITC; BD 553080), anti-human (α-hu) CD45-allophycocyanin (APC; BD 555485, 340943), α-huCD3-PE (BD 555340, 555333), α-huCD11b-PE (BD 555388), α-huCD15-PE (BD 555402), α-huCD19-PE (BD 349209, 555413), α-huCD33-PE (BD 347787), α-huCD34-PE (BD 348057), α-huCD56-PE (BD 555516), α-huCD66b-PE (BD 561650), α-huGlyA-PE (BD 340947), and α-huIgM-V450 (BD 561286). Viability was assessed using propidium iodide or DAPI (Life Technologies).

Flow cytometry was performed on a BD AriaIII equipped with 405-, 488-, 535-, and 633-nm lasers and appropriate filters. When possible, 2000 huCD45+ events

were recorded; otherwise, between 50,000 and 100,000 total events were acquired. Positive engraftment was defined using both mouse and human peripheral blood stained with the above antibodies for lineage markers. Cell analysis was performed using FlowJo Version 9.5.2 (TreeStar).

Histology and Immunohistochemistry– Primary antibodies used: α-huCD45 [HI30], α-huCD1a [HI149], α-huCD3 [UCHT1], α-huCD19 [LC1], α-huMHC Class II antibody [EPR11226], or isotype IgG1-kappa [eBiosciences].

SUPPLEMENTAL TABLE:

Name	Sequence (5'->3')
ol7	GTGGGTAGCCAGCTCTTCAG
ol8	CCTGGAGCTGGACAACAAAT
ol9	GCCAGAGGCCACTTGTGTAG
ol43	AGAGAGGTGGCAAATCAGTGTCCA
ol44	CCCTGGACTTCTCTGCTCTTAGTT
ol49	TAAAGCCGCCCTAAGAGTCA
ol50	CCCTTAGAGTTTTGAGCAGACA
ol111	ATCCTTCTGTCCAGTGCACCATCT
ol112	CTCGCTTCTCTGTACAATTTGGGC
ol113	CCTGCAGGATCCCTTAAGGTTAGT
ol114	CCCTACTCCTCTGTACCACCTAAT

Table S1: Oligonucleotides used in this study.

SUPPLEMENTAL REFERENCES:

Shibahara, S., Okinaga, S., Tomita, Y., Takeda, A., Yamamoto, H., Sato, M., and Takeuchi, T. (1990). A point mutation in the tyrosinase gene of BALB/c albino mouse causing the cysteine----serine substitution at position 85. Eur J Biochem *189*, 455-461.

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Strowig, T., Rongvaux, A., Rathinam, C., Takizawa, H., Borsotti, C., Philbrick, W., Eynon, E.E., Manz, M.G., and Flavell, R.A. (2011). Transgenic expression of human signal regulatory protein alpha in Rag2-/-gamma(c)-/- mice improves

engraftment of human hematopoietic cells in humanized mice. Proc Natl Acad Sci U S A *108*, 13218-13223.