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Supplemental Information

Improved Human Erythropoiesis and Platelet Formation in Humanized NSGW41 Mice

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Figure S1:

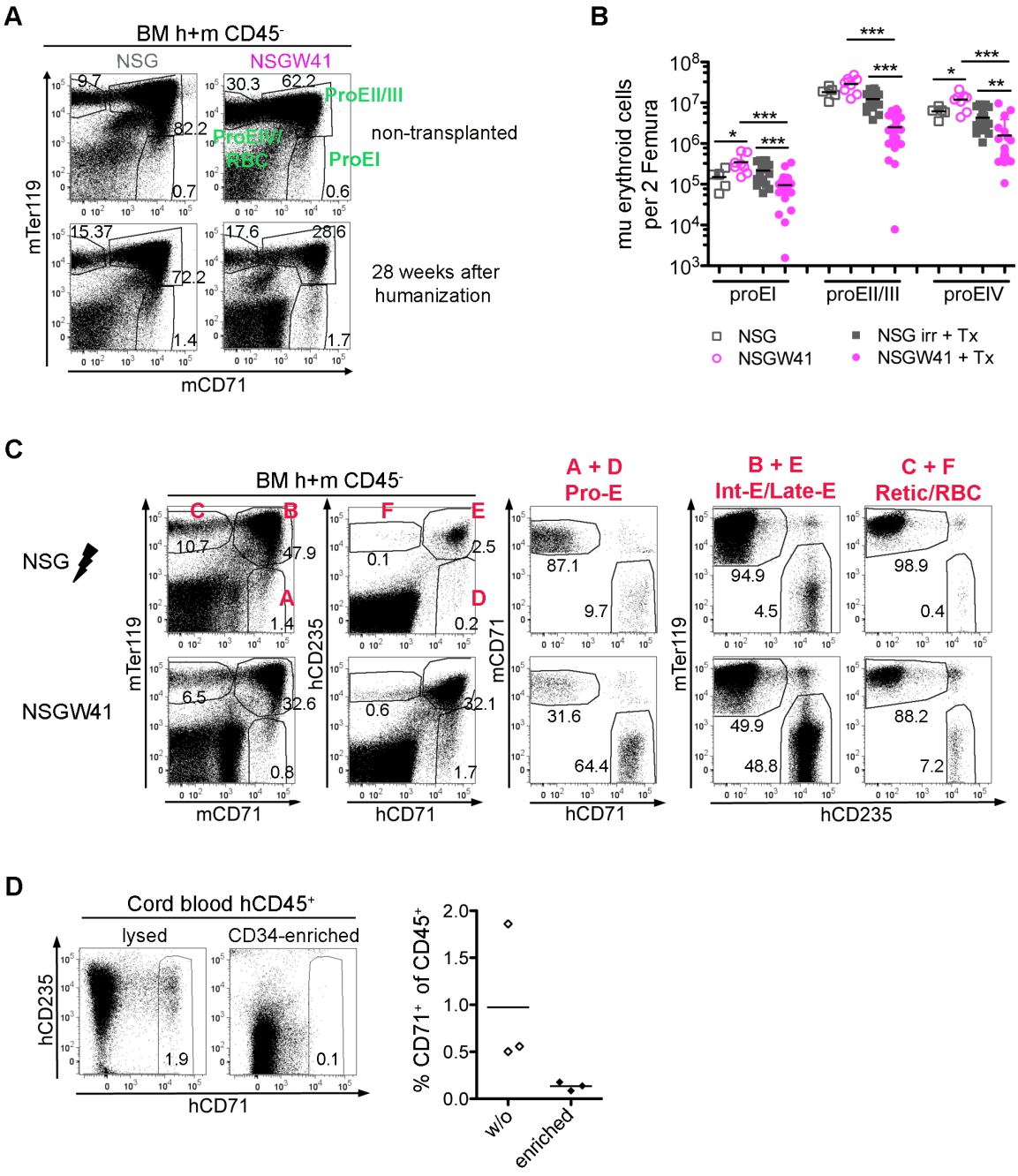
A
BM h+m CD45-
NSG
NSGW41
9.7
82.2
0.7
30.3
62.2
0.6
ProEII/III
ProEIV/ RBC
ProEI
non-transplanted
15.37
72.2
1.4
17.6
28.6
1.7
28 weeks after humanization
mTer119
mCD71
B
mu erythroid cells per 2 Femura
proEI
proEII/III
proEIV
NSG
NSGW41
NSG irr + Tx
NSGW41 + Tx
C
BM h+m CD45-
A + D Pro-E
B + E Int-E/Late-E
C + F Retic/RBC
NSG
NSGW41
C
B
A
F
E
D
10.7
47.9
1.4
0.1
2.5
0.2
6.5
32.6
0.8
0.6
32.1
1.7
87.1
9.7
31.6
64.4
94.9
4.5
49.9
48.8
98.9
0.4
88.2
7.2
mTer119
hCD235
mCD71
mCD71
hCD71
hCD71
mTer119
hCD235
D
Cord blood hCD45+
lysed
CD34-enriched
1.9
0.1
hCD235
hCD71
% CD71+ of CD45+
2.0
1.5
1.0
0.5
0.0
w/o
enriched

Figure S2:

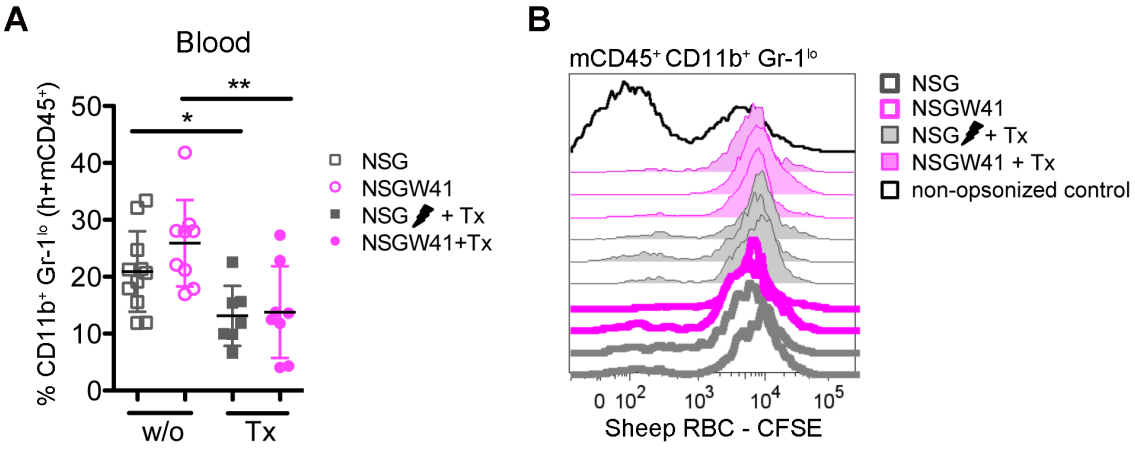
A
Blood
% CD11b+ Gr-1lo (h+mCD45+)
50
40
30
20
10
0
*
**
w/o
Tx
NSG
NSGW41
NSG + Tx
NSGW41+Tx
B
mCD45+ CD11b+ Gr-1lo
0 10^2 10^3 10^4 10^5
Sheep RBC - CFSE
NSG
NSGW41
NSG + Tx
NSGW41 + Tx
non-opsonized control

Figure S3:

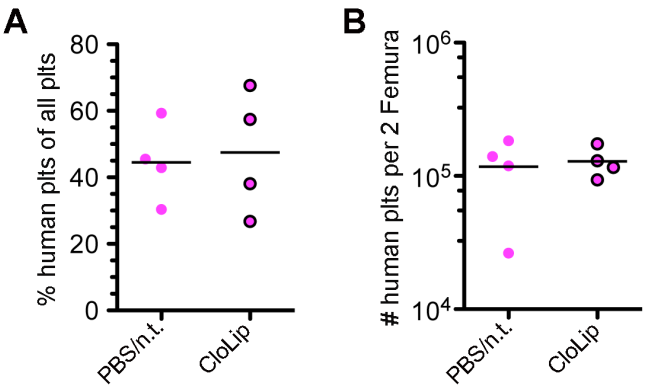
A
% human plts of all plts
80
60
40
20
0
PBS/n.t.
ClodLip
B
human plts per 2 Femura
10^6
10^5
10^4
PBS/n.t.
ClodLip

Figure S1. Analysis of murine and human erythroid progenitors in humanized mice by flow cytometry, Related to Figure 1

(A) Dot plots show the expression of Ter119 and mCD71 on $CD45^-$ (human and mouse) bone marrow cells of humanized NSG and NSGW41 mice. (B) Total numbers of murine RBC differentiation stages in 2 femura of non-transplanted and humanized NSG and NSGW41 mice. 6 expts, 2-9 mice each per expt. Depicted are mean ± SD. (C) Gating strategies to determine the relative contribution of human donor cells to the indicated erythroid progenitor compartments. (D) Dot plots (left) and graph (right) show the presence of $CD71^+$ erythroid progenitors within ACK-lysed or CD34-enriched human cord blood (2 expts).

Figure S2. Murine macrophages in humanized NSG and NSGW41 mice, Related to Figure 3

(A) Plot shows the frequency of $CD11b^+$ $Gr\text{-}1^{lo}$ macrophages in the blood of humanized or non-humanized NSG or NSGW41 mice. Mice had received human CD34-enriched CB cells 11-21 weeks before. 3 expts, 2-4 mice each per expt. Depicted are mean ± SD. (B) Phagocytic activity of murine blood macrophages from indicated mouse strains before and after humanization.

Figure S3. Human thrombocytes in bone marrow after CloLip treatment, Related to Figure 4

NSGW41 mice were injected with 200µl clodronate liposomes (CloLip) to deplete macrophages 11 weeks after humanization. (A) Frequency of human platelets within all platelets in bone marrow of CloLip- or PBS-treated NSGW41 mice. (B) Total numbers of human platelets in 2 femura of CloLip- or PBS-treated NSGW41 mice. 1 expt, 4 mice each. Depicted are mean ± SD.

Supplemental Experimental Procedures

Genotyping of NSGW41 mice

Genotyping of *Kit*^{W41} allele was performed by PCR on tail-snip DNA (forward: 5'- AAGGAAGGTTAGAACCCCTGG; reverse: 5'- AGCTCCCAGAGGAAAATCCC). The products were sequenced using the forward primer. The *Kit*^{W41} allele contains a point mutation at position 2519 (G / A) (Nocka et al., 1990).

Antibodies

Human cells were stained using antibodies specific for (clone names given in brackets): CD3 (OKT3), CD19 (HIB19), CD33 (WM-53), CD42a (GR-P), CD45 (HI30), CD235 (HIR2) from eBioscience, CD45 (HI30), CD16 (3G8), CD41 (HIP8), CD61 (VI-PL2) from Biolegend, CD14 (M5E2) (BD Biosciences) and CD71 (MEM-75) from ImmunoTools. Murine cells were stained with the following antibodies: CD11b (M1/70), CD41 (eBioMWReg30), CD45 (30-F11), CD115 (AFS98), Gr-1 (RB6-8C5), Ly-6C (HK1.4), Ter119 (TER-119) (all from eBioscience), CD41 (MWReg30) and CD71 (C2) from BD Biosciences. Blocking reagents used were ChromPure mouse IgG (Jackson ImmunoResearch) for human cells and rat IgG (Dianova) as well as anti-mouse CD16/CD32 (93) (eBioscience) for murine cells. For immunohistochemistry sections were blocked with rat Ig (500 µg/ml, Jackson ImmunoResearch Laboratories) and Streptavidin/Biotin blocking kit (Vector Laboratories). Antibodies used for immunostaining were hCD45 (HI30), mCD45 (30-F11), hCD42a (GR-P) and mCD41 (MWReg30) (eBioscience). Anti-FITC-Alexa488 (Molecular Probes) and streptavidin-Cy3 (Jackson ImmunoResearch Laboratories) were used for secondary steps. Dapi (2 µg/ml) was used to stain nuclei.

EPO ELISA

Samples were collected into EDTA containing micro tubes (plasma) or non-coated tubes (serum) and centrifuged at 2000g for 15 minutes at RT. Plasma samples from NSGW41 h*EPO*-tg mice were diluted 2- to 10-fold. All serum samples were diluted 1:2 before use. The optical density was measured on a Sunrise™ absorbance reader (Tecan). A standard curve was generated using nonlinear regression.

May-Gruenwald-Giemsa staining

May-Gruenwald-Giemsa staining was modified from Waskow et al., 2008. In brief, cytospins were stained in 100% and 50% May-Gruenwald solution (Merck, Darmstadt, Germany) for 3 minutes each. After rinsing in water (pH6.8) for 2 minutes the slides were stained in Giemsa (Merck, Darmstadt, Germany) for 15 minutes. Samples were then washed in water (pH6.8) for 3 minutes and quickly rinsed with ethanol and water to remove excessive dye. Slides were air-dried before microscopy.

Primers for globin chain expression

α-globin (NM_000558.4): f: 5'- CTGGAGAGGATGTTCCTGTCCTTG, r: 5'- CAGCTTAACGG TATTTG GAGGTCAT (t_A=59 °C; 24/26/28/30 cycles)

β-globin (NM_000518.4): f: 5'- TCCTGAGGAGAAGTCTGCCGTTAT, r: 5'- GAAATTGGACAG CAAGA AAGCGGA (t_A=53 °C; 26/28/30/32 cycles)

γ-globin (NM_000559.2): f: 5'- ACTCGCTTCTGGAACGTCTGA, r: 5'- GTATCTGGAGGAC AGGGCA CT (t_A=57 °C; 24/26/28/30 cycles)

δ-globin (NM_000519.3): f: 5'-GCAGATTACTGGTGGTCTACCCTTG, r: 5'- GGAAACAGTCC AGGAT CTCAAT GG (t_A=55 °C; 26/28/30/32 cycles)

β-actin (NM_001101.3): f: 5'- AGCGAGCATCCCCCAAAGTT, r: 5'- GGGCACGAAGGCTCAT CATT (t_A=59 °C; 28/30/32/34 cycles)

Phagocytosis assay

3ml defibrinated sheep red blood cells (sRBC, Thermo Scientific) were opsonized in 10ml PBS/1% BSA containing 100µg/ml rabbit IgG specific for sRBC (MP Biomedicals) for 30' at 37°C and 30' on ice and subsequently labeled ($4*10^7$ sRBCs/ml) with CFSE at a final concentration of 5µM. WBCs from lysed blood of non-transplanted and humanized (21 weeks post Tx) NSG and NSGW41 mice were incubated with opsonized CFSE-labeled sheep RBCs in a ratio of 1:100 for 4h at 37°C. Subsequently, sheep RBCs were lysed in ACK buffer for 1min, cells were stained and analyzed by

flow cytometry. Non-opsonized sRBCs were used as control, and non-engulfed sheep RBCs sticking to WBCs were excluded by gating on PE anti-rabbit IgG negative cells.

Supplemental References

Nocka, K., Tan, J.C., Chiu, E., Chu, T.Y., Ray, P., Traktman, P., and Besmer, P. (1990). Molecular bases of dominant negative and loss of function mutations at the murine c-kit/white spotting locus: W37, Wv, W41 and W. The EMBO journal *9*, 1805-1813.

Waskow, C., Liu, K., Darrasse-Jeze, G., Guermonprez, P., Ginhoux, F., Merad, M., Shengelia, T., Yao, K., and Nussenzweig, M. (2008). The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. Nature immunology *9*, 676-683.