iScience, Volume *27*

Supplemental information

Engraftment of adult hematopoietic stem and progenitor cells in a novel model of humanized mice

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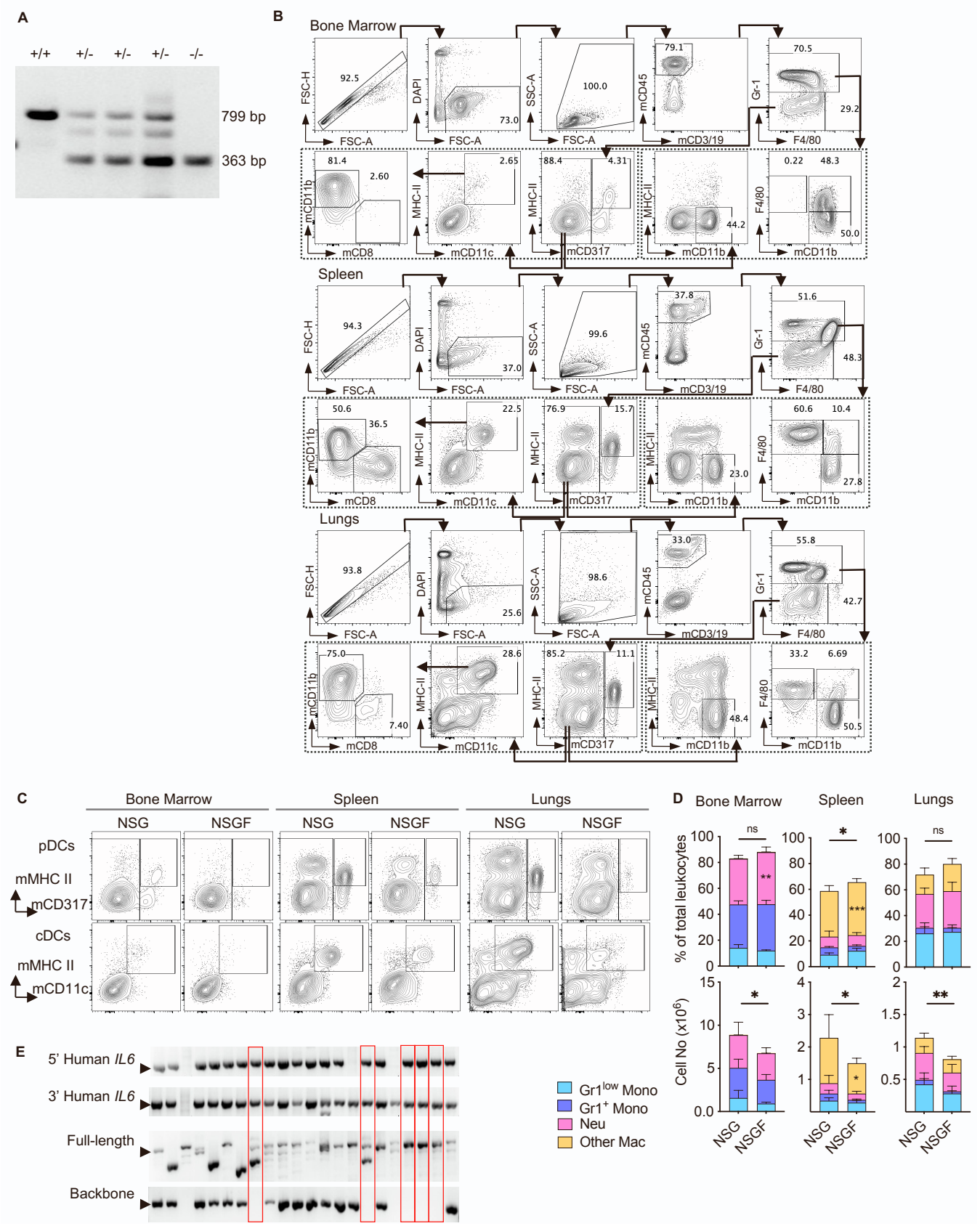
A
+/+
+/-
+/-
+/-
-/-
799 bp
363 bp
B
Bone Marrow
Spleen
Lungs
FSC-H
FSC-A
DAPI
SSC-A
mCD45
mCD3/19
Gr-1
F4/80
mCD11b
mCD8
MHC-II
mCD11c
mCD317
C
Bone Marrow
Spleen
Lungs
NSG
NSGF
pDCs
mMHC II
mCD317
cDCs
mMHC II
mCD11c
D
Bone Marrow
Spleen
Lungs
% of total leukocytes
Cell No (x10^6)
ns
NSG
NSGF
E
5' Human IL6
3' Human IL6
Full-length
Backbone
Gr1low Mono
Gr1+ Mono
Neu
Other Mac

Figure S1. Genetic engineering of novel NSG mice via CRISPR, Related to Figure 1.
(A) F1 littermates from mouse *Flt3* CRISPR KO were screened for *Flt3* mutant allele (363 bp) from wildtype allele (799 bp) by PCR.
(B) The gating strategy for mouse DCs and other myeloid cells on single cell suspension from bone marrow, spleen and lungs of NSG mice by flow cytometry. pDCs were gated as DAPI$^-$, mCD45$^+$, mCD3$^-$, mCD19$^-$, F4/80$^-$ and Gr-1$^-$ with expression of MHC class II and CD317. CD317$^-$ cells were further gated with MHC class II$^+$ and mCD11c$^+$ for cDCs. cDCs were subsequently divided into mCD8$^+$ or mCD103$^+$ cDC1 and mCD11b$^+$ cDC2. Gr1^{low} monocytes (Mono) were gated as DAPI$^-$, mCD45$^+$, mCD3$^-$, mCD19$^-$, F4/80$^-$, Gr-1^{low} , CD317$^-$, MHC class II$^+$ and mCD11b$^+$. Gr1$^+$ monocytes were gated as DAPI$^-$, mCD45$^+$, mCD3$^-$, mCD19$^-$, Gr-1$^+$, F4/80$^+$, and mCD11b$^+$. Neutrophils (Neu) were gated as DAPI$^-$, mCD45$^+$, mCD3$^-$, mCD19$^-$, Gr-1$^+$, F4/80$^-$, and mCD11b$^+$. Other macrophages (Mac) were gated as DAPI$^-$, mCD45$^+$, mCD3$^-$, mCD19$^-$, Gr-1$^+$, F4/80$^+$, and mCD11b^{low}.
(C) Representative FACS plots illustrate mouse pDCs and cDCs from bone marrow, spleen, and lungs of 8-10 wk NSG or NSGF mice (n=7).
(D) Representative FACS plots illustrate other mouse myeloid cells from bone marrow, spleen, and lungs of 8-10 wk NSG or NSGF mice (n=7).
(E) Founder mice from human *IL6* CRISPR KI were screened for mutant allele from wildtype allele by PCR. PCR products obtained with template of genomic DNA from tail tip. Potential founder mice indicated with red box were selected by positive PCR assay targeting 5' and 3' junctions and full length of human *IL6* KI sequence and negative for plasmid backbone.

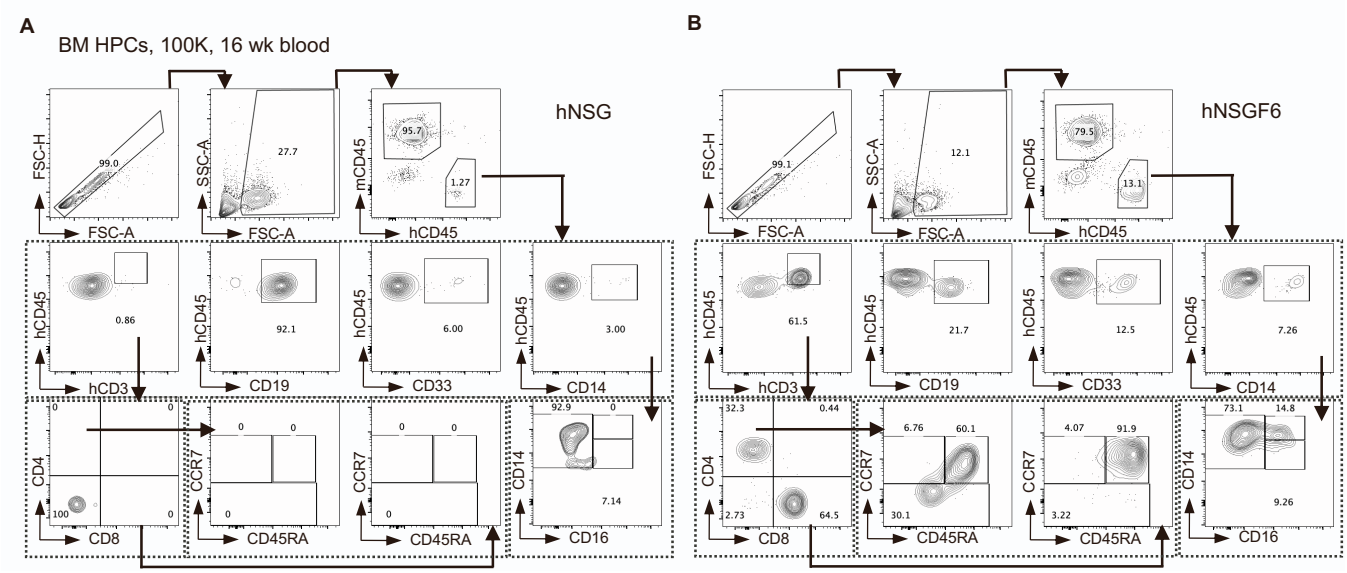

Figure S2. Evaluation of the human engraftment in the blood of humanized mice by flow cytometry, Related to Figure 2 and 4. Humanized mice were generated by engrafting mice with 1x10^5 bone marrow HSPCs. Peripheral blood of mice was collected, stained with specific antibodies, and analyzed by FACS.
(A) The gating strategy for human engraftment in the blood of hNSG at 16 weeks after transplant. hCD45$^+$ cells were gated as mCD45$^-$ with expression of hCD45. hCD45$^+$ cells were further gated into CD14$^+$ monocytes, CD33$^+$ myeloid cells, CD19$^+$ B cells or CD3$^+$ T cells. CD3$^+$ T cells were

subsequently divided into $CD4^+$ or $CD8^+$ T cells with the expression of CD45RA and CCR7 for naïve and memory phenotype. One representative mouse was shown.
(B) Blood of hNSGF6 at 16 weeks after transplant. One representative mouse was shown.

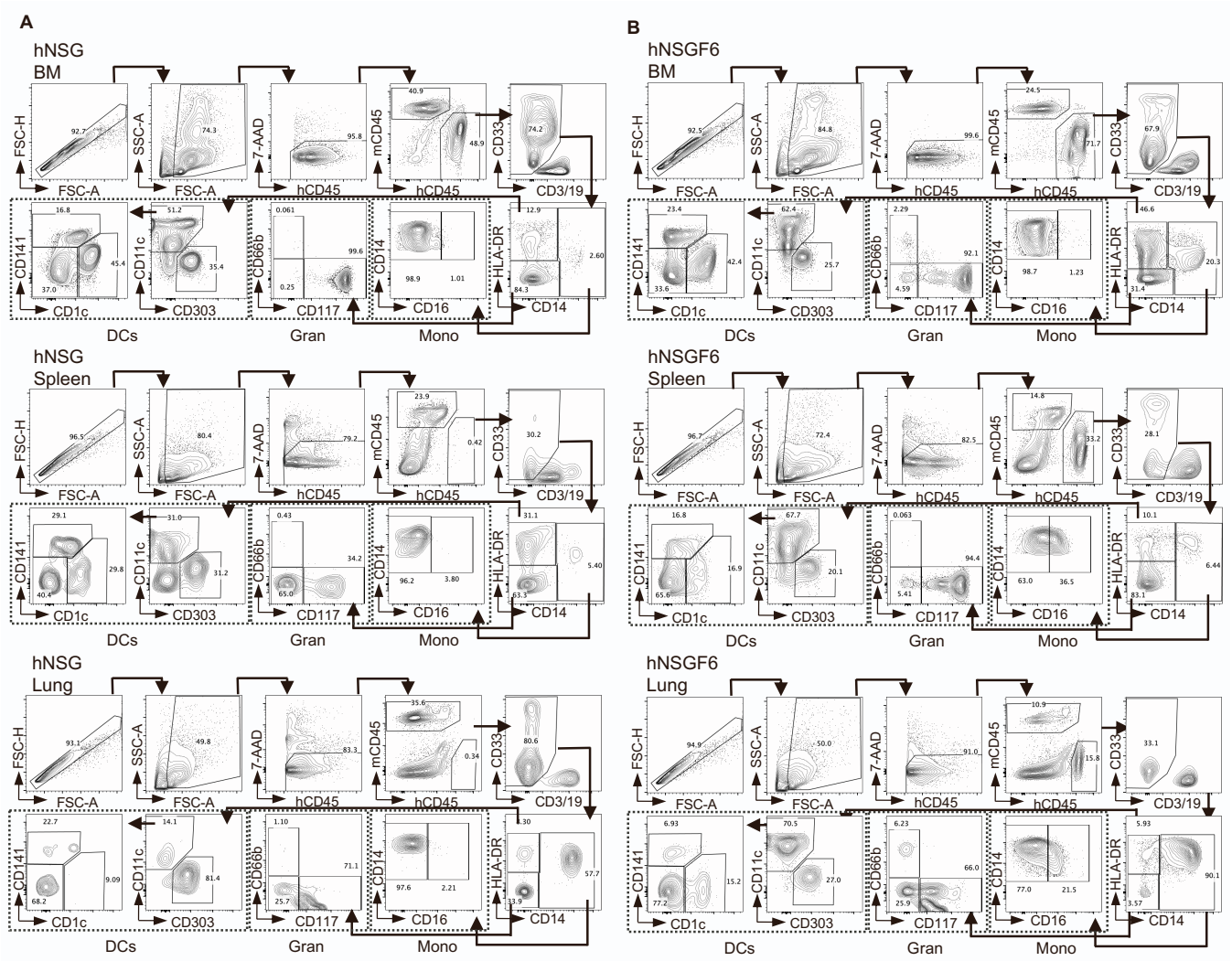

Figure S3. Evaluation of the human myeloid compartment in the tissues of humanized mice by flow cytometry, Related to Figure 3 and 5. Humanized mice were generated by engrafting mice with $1x10^5$ bone marrow HSPCs. Tissues including bone marrow, spleen and lungs of mice were collected. Single cell suspensions were stained with specific antibodies and analyzed by FACS. Human myeloid cells including DCs, monocytes and granulocytes were gated in the sequence as shown in the plots.
(A) Bone marrow, spleen, and lungs of hNSGF6 at 52 weeks after transplant. One representative mouse was shown.
(B) Bone marrow, spleen, and lungs of hNSGF6 at 52 weeks after transplant. One representative mouse was shown.

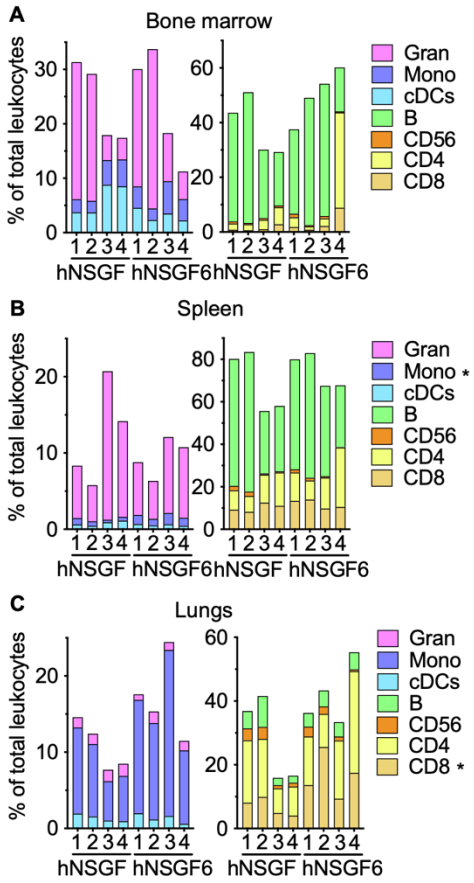

Figure S4. Human myeloid and lymphoid cells in the tissues of hNSGF and hNSGF6 mice, Related to Figure 3. The percentage of human myeloid (left panel) and lymphoid (right panel) cells in total $CD45^+$ leukocytes (mouse and human) from the bone marrow (A), spleen (B) and lungs (C) of hNSGF and hNSGF6 at 20-week post-transplant with cord blood HSPCs. n=4 from two donors. Two-tailed t-test.

Table S1. List of primers for mouse genotypes, Related to STAR Methods.

Target	Primer	Sequence	Product (bp)
Flt3 KO	F	GGTACCAGCAGAGTTGGATAGC	363 (KO)
	R	ATCCCTTACACAGAAGCTGGAG	799 (wt)
IL6 KI 5' junction	F1	CATCTCCTGTGGGACCATTCTTC	4017
	R1	AGTGCAGGTTATCTCACTGTGG	
IL6 KI 3' junction	F2	TTGGAACTGAACCCAAGTGTGC	5163
	R2	GGCTGTCCTCAGACCCAATC	
IL6 KI full-length	F1	CATCTCCTGTGGGACCATTCTTC	8155 (KI)
	R2	GGCTGTCCTCAGACCCAATC	10557 (wt)
IL6 KI	F2	TTGGAACTGAACCCAAGTGTGC	1475
	R3	CACTGGCTGGAGTTGGATGC	
Donor DNA backbone	F4	GAAGTTTGTTGCTATGGAAGGGTC	944
	R4	AGCGCAACGCAATTAATGTG	