

Thymic precursor cells generate acute myeloid leukemia in NUP98-PHF23/NUP98-HOXD13 double transgenic mice

Subhadip Kundu, Eun Sil Park, Yang Jo Chung, Robert L. Walker, Yuelin J. Zhu, Vijay Negi, Paul S. Meltzer, Peter D. Aplan

Supplementary information included in pdf.

Supplementary Figures 1-16

Supplementary Tables 1-2, 6-12.

Supplementary Tables 3-5 are attached separately as sortable Excel files.

Figure S1

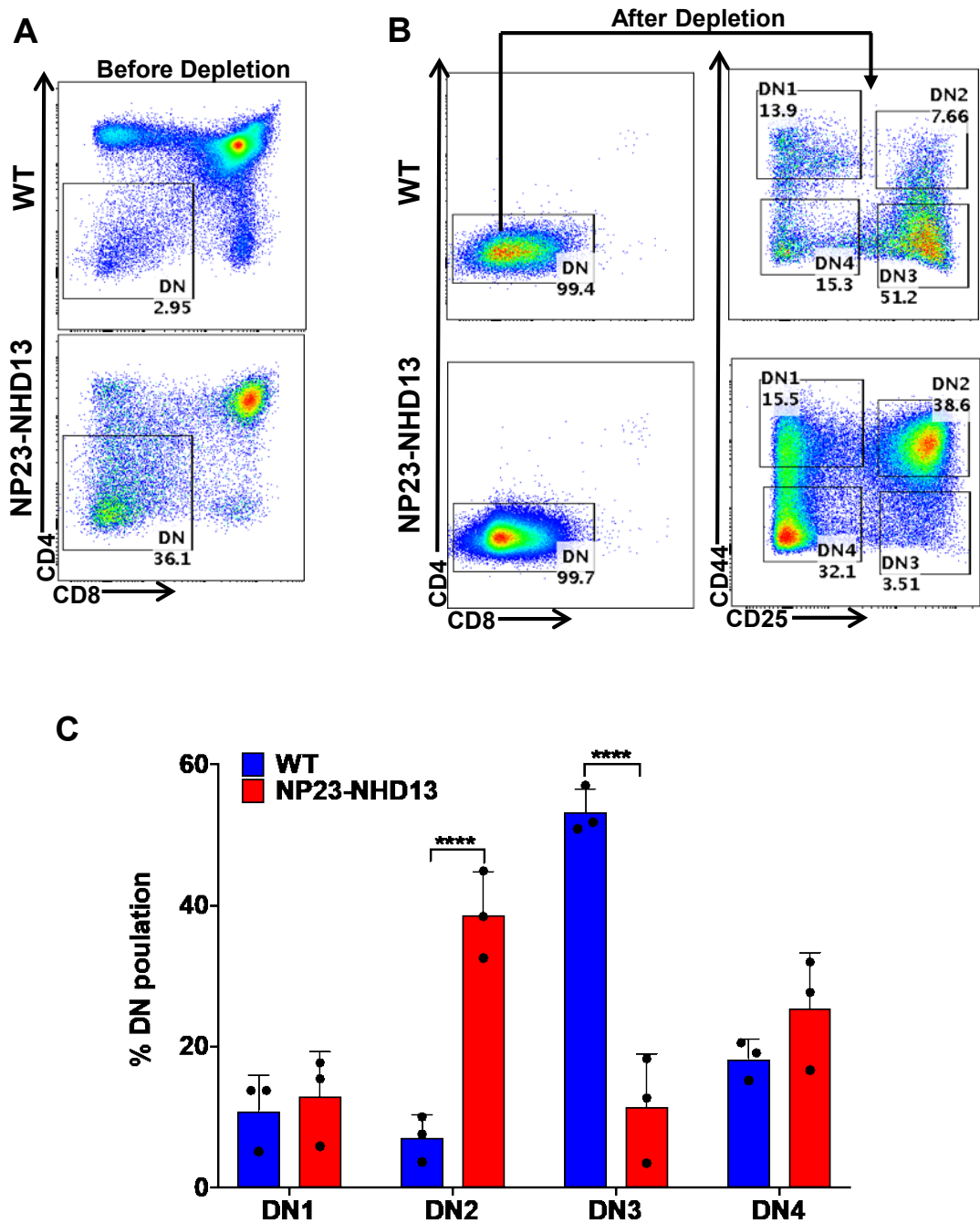
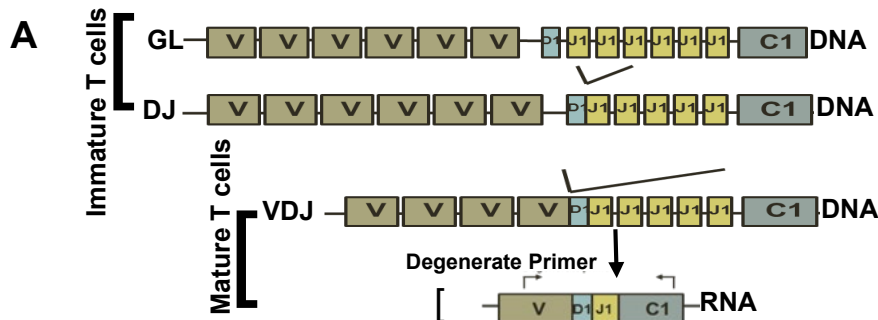
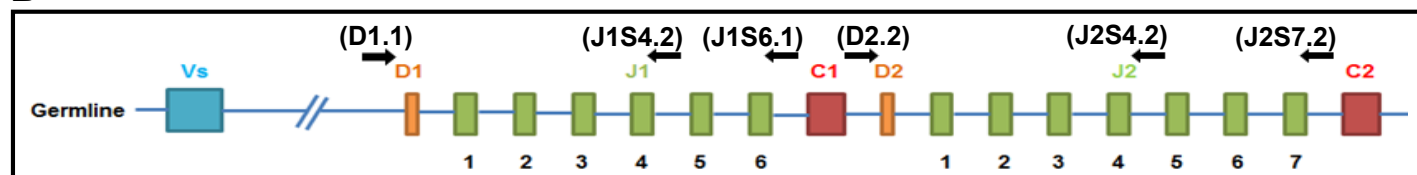


Figure S1. Thymic differentiation is impaired in young, non-leukemic NP23-NHD13 mice. (A) Flow cytometry of young (22-30 days) WT and NP23-NHD13 thymus before depletion of DP and SP CD4 and CD8 cells. **(B)** Flow cytometry of WT and NP23-NHD13 thymus after depletion of DP and SP CD4 and CD8 cells. **(C)** Fractionated DN sub-populations (DN1, DN2, DN3 and DN4). Data are expressed as means \pm SD, $n=3$ mice. **** $P<0.001$, by one tailed student t-test.

Figure S2.



B

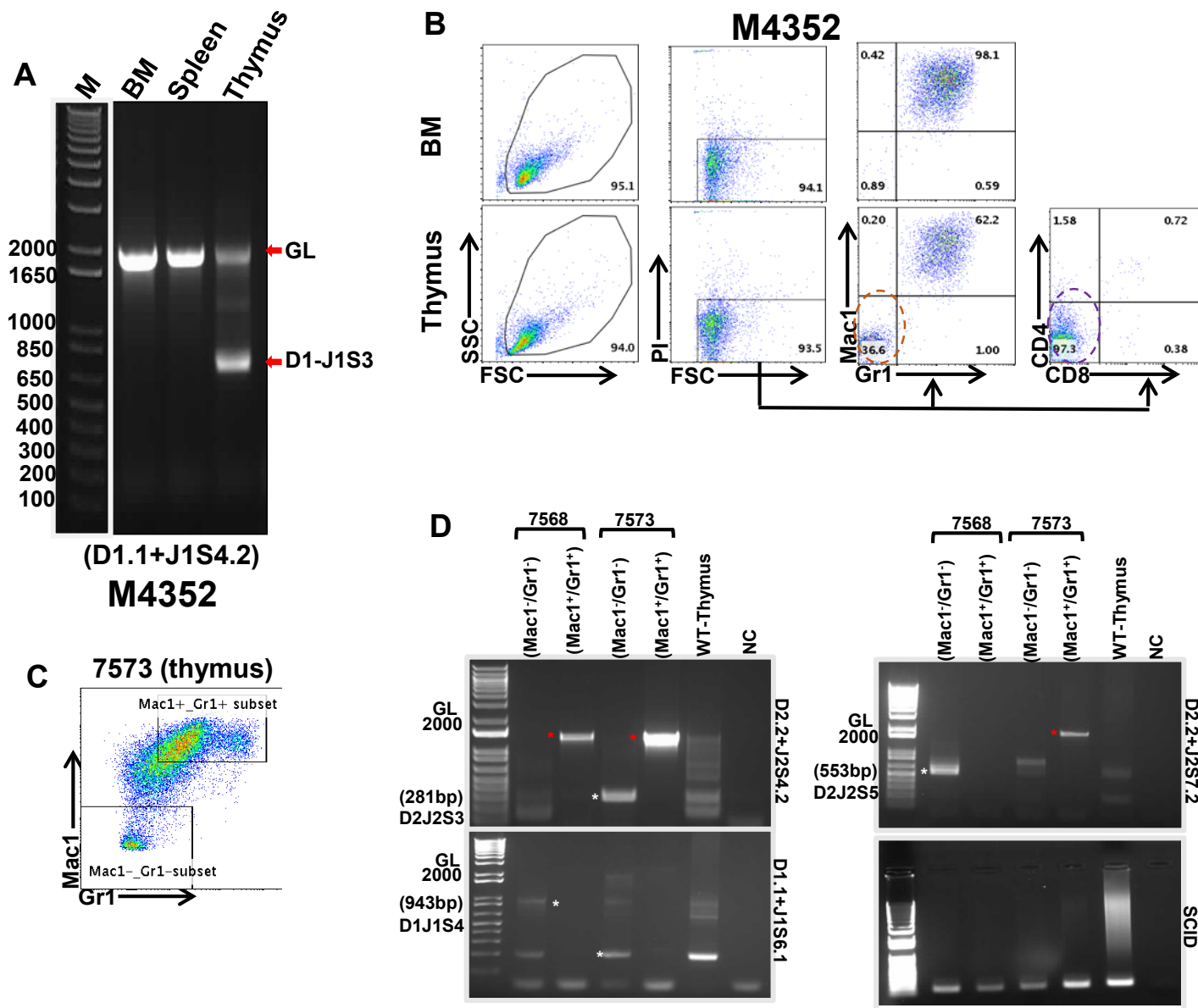


| Rearrangement | D1.1/J1S4.2 Primers (PCR Product Size) | D1.1/J1S6.1 Primers (PCR Product Size) |
|---------------|--|--|
| | No Rearrangement (Germline) | 1750bp |
| D1-J1S1 | 1098bp | 1890bp |
| D1-J1S2 | 960bp | 1753bp |
| D1-J1S3 | 638bp | 1431bp |
| D1-J1S4 | 150bp | 943bp |
| D1-J1S5 | No PCR Product | 671bp |
| D1-J1S6 | No PCR Product | 201bp |
| Rearrangement | D2.2/J2S4.2 Primers (PCR Product Size) | D2.2/J2S7.2 Primers (PCR Product Size) |
| | No Rearrangement (Germline) | 1329bp |
| D2-J2S1 | 751bp | 1253bp |
| D2-J2S2 | 548bp | 1050bp |
| D2-J2S3 | 281bp | 783bp |
| D2-J2S4 | 142bp | 644bp |
| D2-J2S5 | No PCR Product | 553bp |
| D2-J2S6 | No PCR Product | 408bp |
| D2-J2S7 | No PCR Product | 196bp |

C

| Mouse ID | Tcrb-DJ | | | Tcrb-VDJ | | |
|----------|---------|--------|----------------|----------|--------|--------|
| | BM | Spleen | Thymus | BM | Spleen | Thymus |
| M4318 | GL | GL | GL | NO | NO | NO |
| M4324 | GL | GL | GL | NO | NO | NO |
| M4351 | GL | GL | GL | NO | NO | NO |
| M4352 | GL | GL | Clonal(D1J1S3) | NO | NO | NO |
| M4361 | GL | GL | GL | NO | NO | NO |
| M4360 | GL | GL | Clonal(D1J1S4) | NO | NO | NO |
| M4369 | GL | GL | GL | NO | NO | NO |

Figure S2. Schematic representation of Tcrb gene rearrangements and PCR strategies to determine clonal D-J rearrangement. (A) Schematic representation of Tcrb-DJ and VDJ rearrangements during thymocyte development. (B) Figure indicates position of the primers (D1.1, J1S4.2, J1S6.1; D2.2, J2S4.2, J2S7.2) used to amplify regions of the Tcrb locus and potential PCR product sizes. (C) Table indicates specific Tcrb-DJ rearrangements found in different leukemic mice, none of the primary leukemic samples showed Tcrb-VDJ rearrangements (NO=no Tcrb VDJ rearrangements detected).



E

| Mice ID/cell population | D | N | J |
|---|---------------------------------|----------|------------------------------|
| 7568 (Mac1 ⁻ /Gr1 ⁻) | TCRB-D1- <u>GGGACAGGG</u> ggc | AC | ttTCCAACGAAAGATTATTTTCG-J1S4 |
| 7573 (Mac1 ⁻ /Gr1 ⁻) | TCRB-D1- <u>GGGACAGGGGG</u> gc | GAGGGATA | ttccTATAATTCGCCCTCTACTT-J1S6 |
| 7573 (Mac1 ⁻ /Gr1 ⁻) | TCRB-D2- <u>GGGACTGGGGGG</u> GC | AGCG | agtGCAGAAACGCTGTATTTTGG-J2S3 |

Figure S3. Clonal Tcrb-DJ rearrangements in the Mac1⁻/Gr1⁻ fraction of leukemic NP23-NHD13 thymus. (A) A clonal Tcrb-DJ rearrangement (D1J1S3) is shown in NP23-NHD13 (#4352) AML; note that BM shows only the germline DJ fragment, not the clonal D1-J1S3 rearrangement, whereas the thymus shows a clonal D1-J1S3 rearrangement. Unrelated lanes between the size standard marker (M) and samples have been removed. **(B)** Gating strategy for flow cytometry of primary leukemic mouse demonstrating myeloid (BM and thymus) and non-myeloid (thymus) populations in mouse 4352, propidium iodide (PI) is used to discriminate live and dead cells. **(C)** Mac1⁺/Gr1⁺ and Mac1⁻/Gr1⁻ populations, sorted from NP23-NHD13 #7573 for Tcrb-DJ clonality assay. **(D)** PCR amplification demonstrates specific DJ rearrangements (indicated by white asterisk) in Mac1⁻/Gr1⁻ fraction, whereas no rearrangements are found in Mac1⁺/Gr1⁺ fraction. Germline (GL) PCR products are indicated with a red asterisk. Amplification of the *scid* locus is used as a DNA quality control indicator. **(E)** Table of clonal rearrangements in the Mac1⁻/Gr1⁻ fraction from two different NP23-NHD13 thymi.

Figure S4

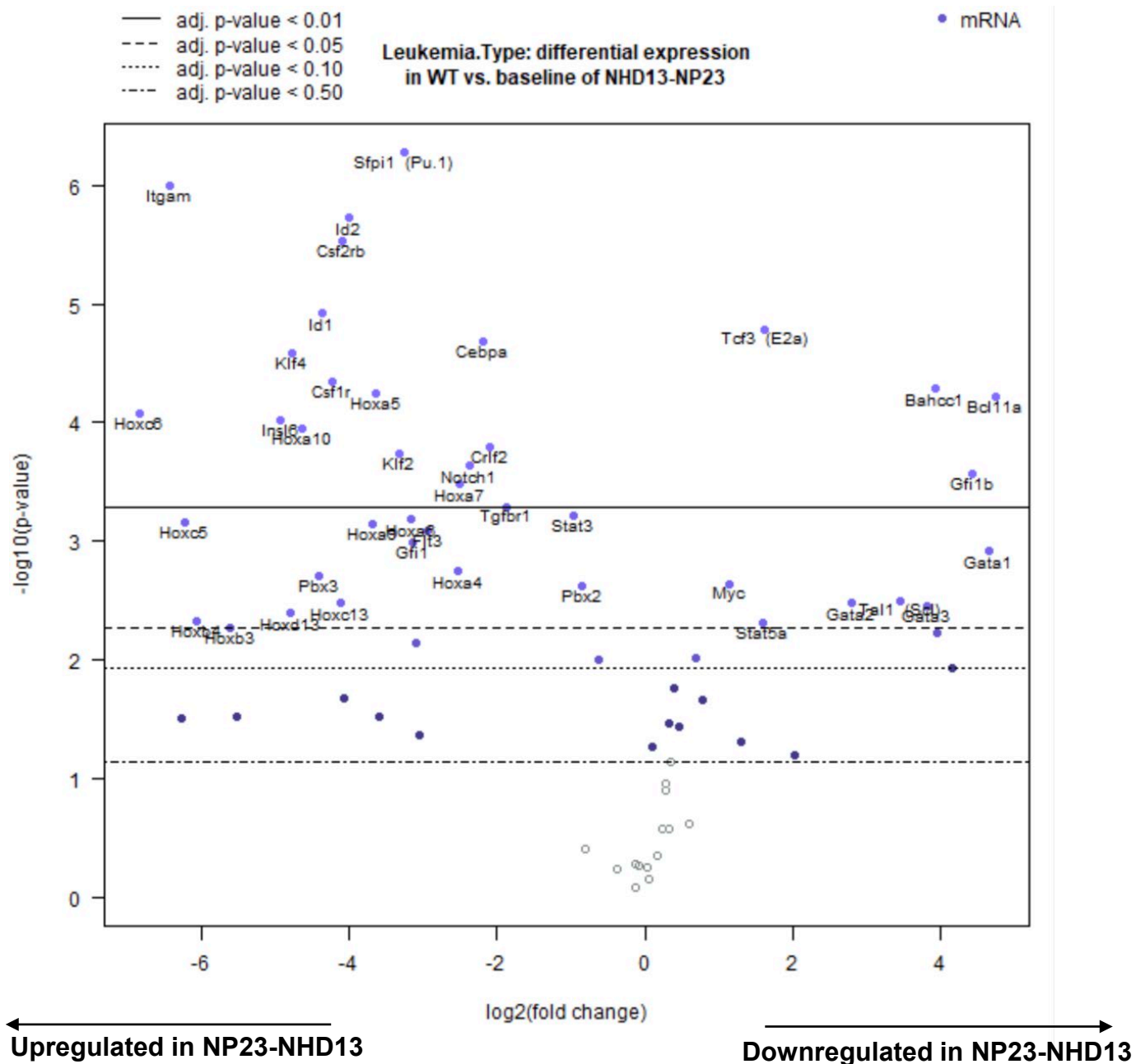


Figure S4. Gene expression assessed by nanostring technology. “Volcano” plot from nanostring assay identifies genes significantly up or down-regulated in NP23-NHD13 AML samples compared with WT Lin⁻ BM. Log₂ (fold change) is on x axis, and -log₁₀ (p value) is on y axis.

Figure S5

Donor cells(CD45.2⁺)

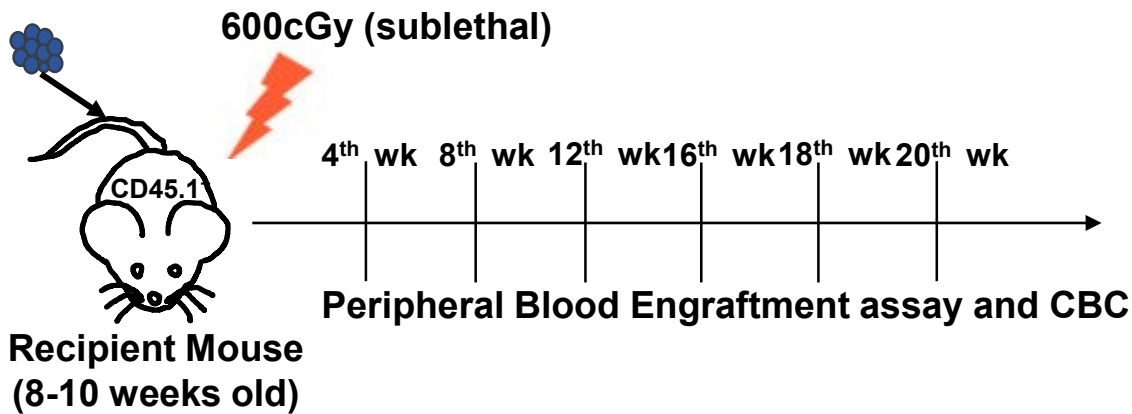


Figure S5. Strategy employed for transplantation experiments.

Figure S6

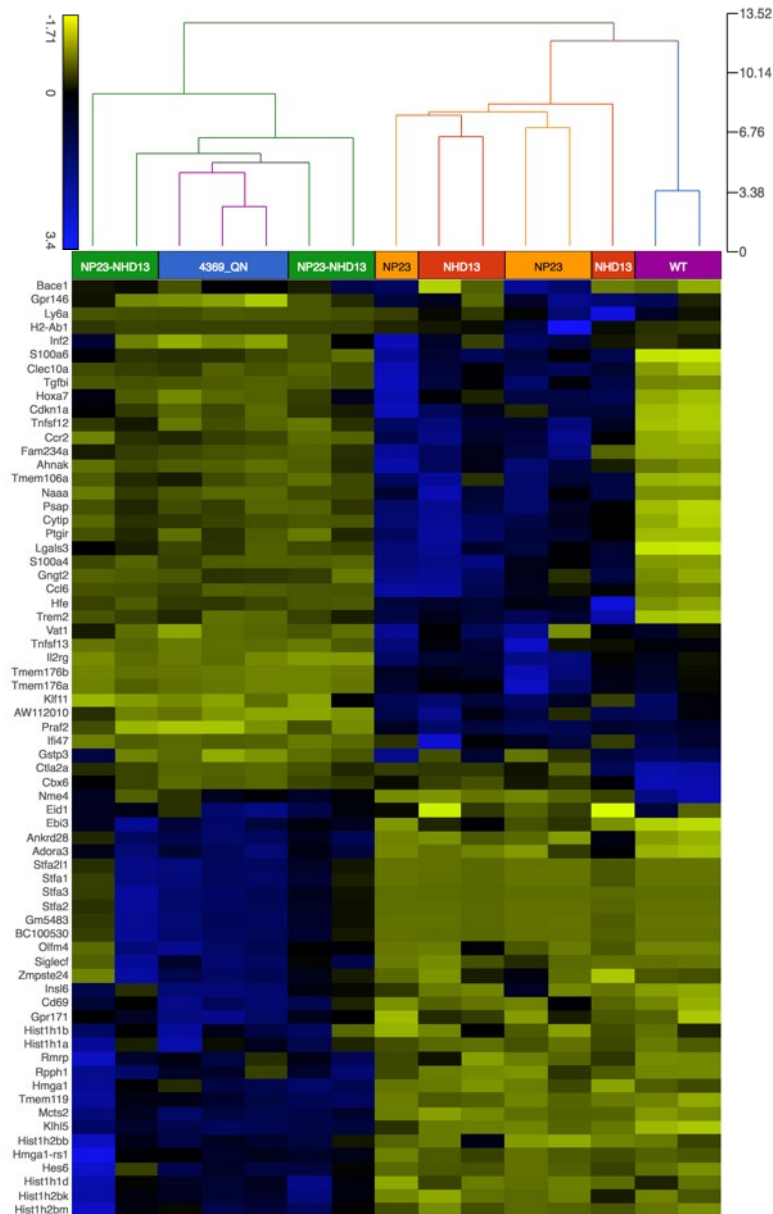


Figure S6. AML samples from QN transplanted recipients cluster with AML samples from primary NP23-NHD13 leukemic mice. Hierarchical clustering of #4369 QN transplant recipients (blue) cluster with primary NP23-NHD13 leukemia samples (green).

Figure S7

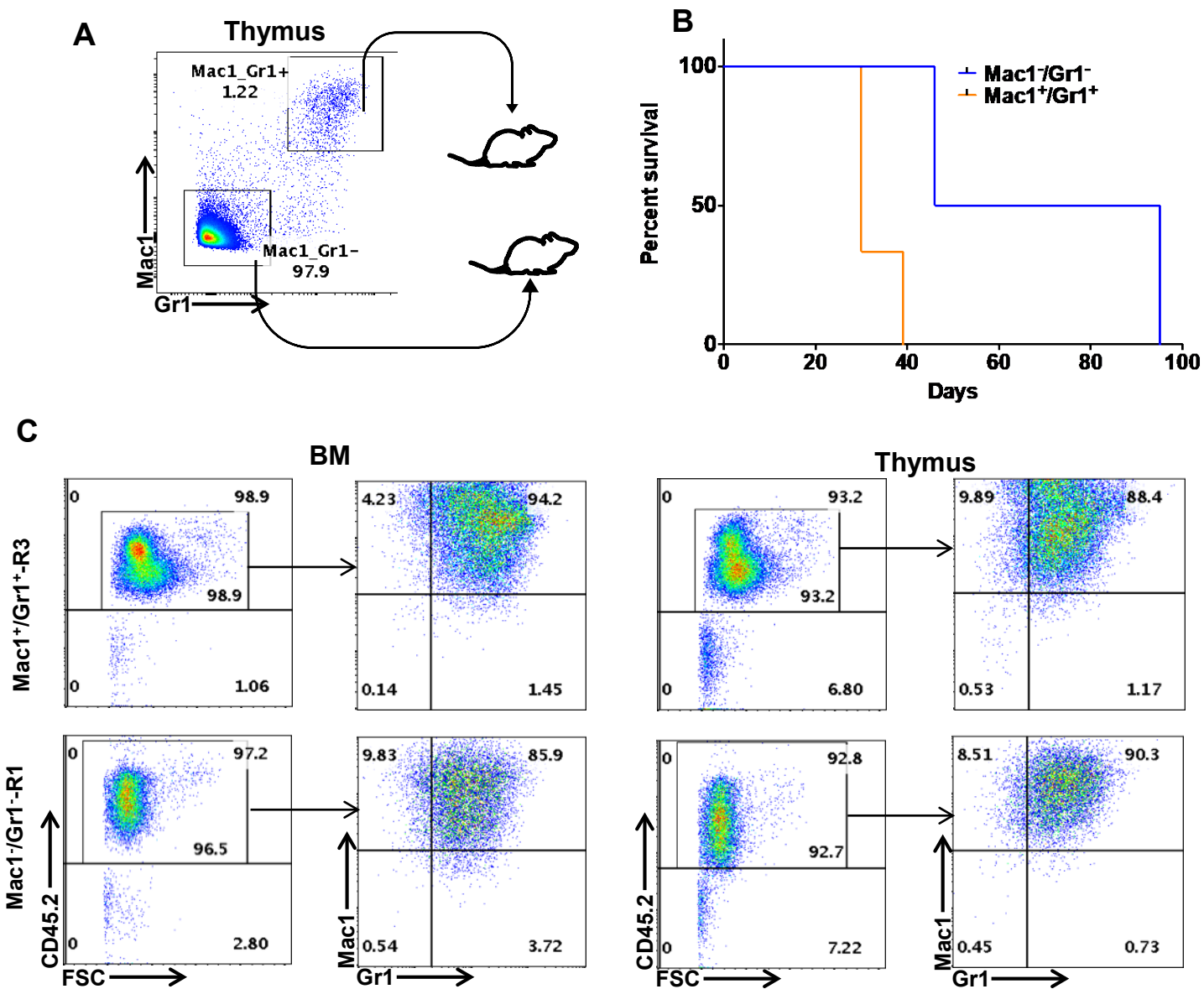


Figure S7. The Mac1⁺/Gr1⁺ population can also transmit AML. (A) FACS sorting of Mac1⁺/Gr1⁺ and Mac1⁻/Gr1⁻ populations from NP23-NHD13 thymus, 1×10^4 cells were transplanted to each recipient, $n=2-3$ mice per group (one Mac1-Gr1⁻ recipient died within 2 weeks of transplant and was excluded from analysis). **(B)** Survival curve; data analyzed by Log-rank (Mantel-Cox) test; $P=0.054$. **(C)** Flow cytometry of recipient indicates that both Mac1⁺/Gr1⁺ and Mac1⁻/Gr1⁻ cells engraft and generate AML(CD45.2⁺/Mac1⁺Gr1⁺).

Figure S8.

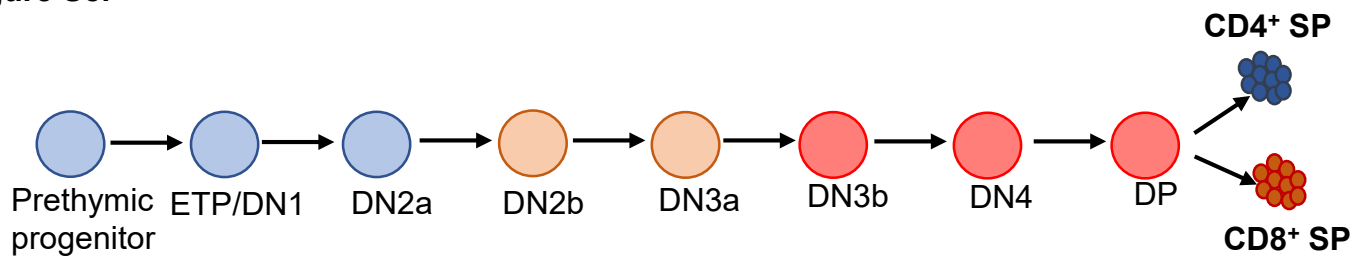


Figure S8. Schematic representation of thymocyte differentiation. Thymocyte differentiation from early T-cell precursors (ETP) to mature CD4⁺ and CD8⁺ thymocytes, adapted from Yui et al., 2014.

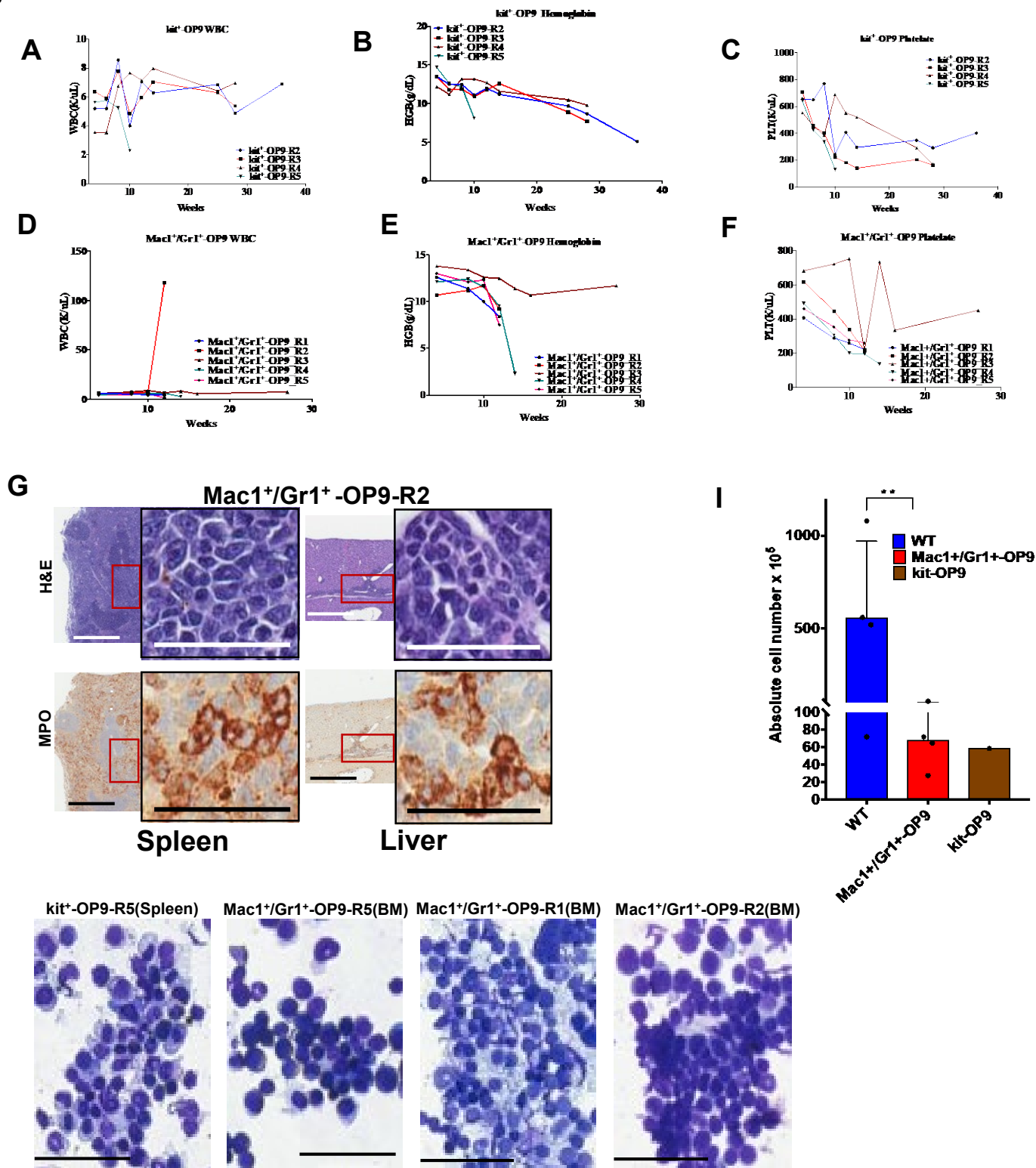
Figure S9

Figure S9. Kit^+ OP9 and $Mac1^+/Gr1^+$ cells from OP9 co-culture develop progressive pancytopenia. (A-F) Serial blood analysis of the Kit^+ OP9 and $Mac1^+/Gr1^+$ cells transplant recipients. **(G)** H & E staining depicts leukemic cell invasion in parenchymal organ (Spleen and liver) of representative $Mac1^+/Gr1^+$ recipient mouse. Myeloperoxidase (MPO) staining confirms the presence of myeloid cell population in the invaded tissues. **(H)** Blast count from ($Mac1^+/Gr1^+$ (BM) and Kit^+ OP9 (Spleen) cells transplanted recipients. **(I)** Total number of BMNC harvested from 2 femora and 2 tibiae. Spleen was used for blast count in Kit^+ OP9 transplant recipient. Scale bars=800 μ m (low power view), 50 μ m (high power view).

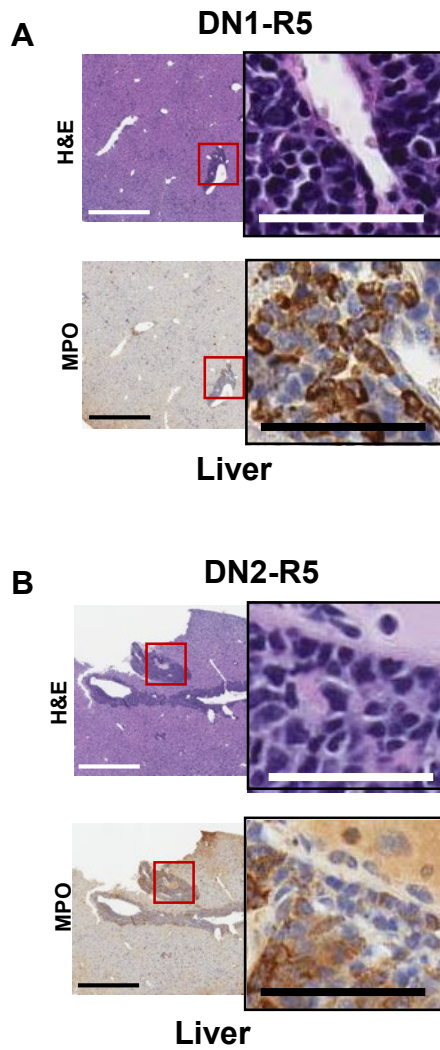


Figure S10. Histology and Immunohistochemistry confirms tissue invasion of leukemic cells. (A and B) H & E staining depicts characteristic perivascular/periportal leukemic cell invasion in liver of representative DN1 and DN2 recipient with AML. Myeloperoxidase staining confirms the presence of myeloid cell population in the invaded tissues. Scale bars=800 μ m (low power view), 50 μ m (high power view).

Figure S11

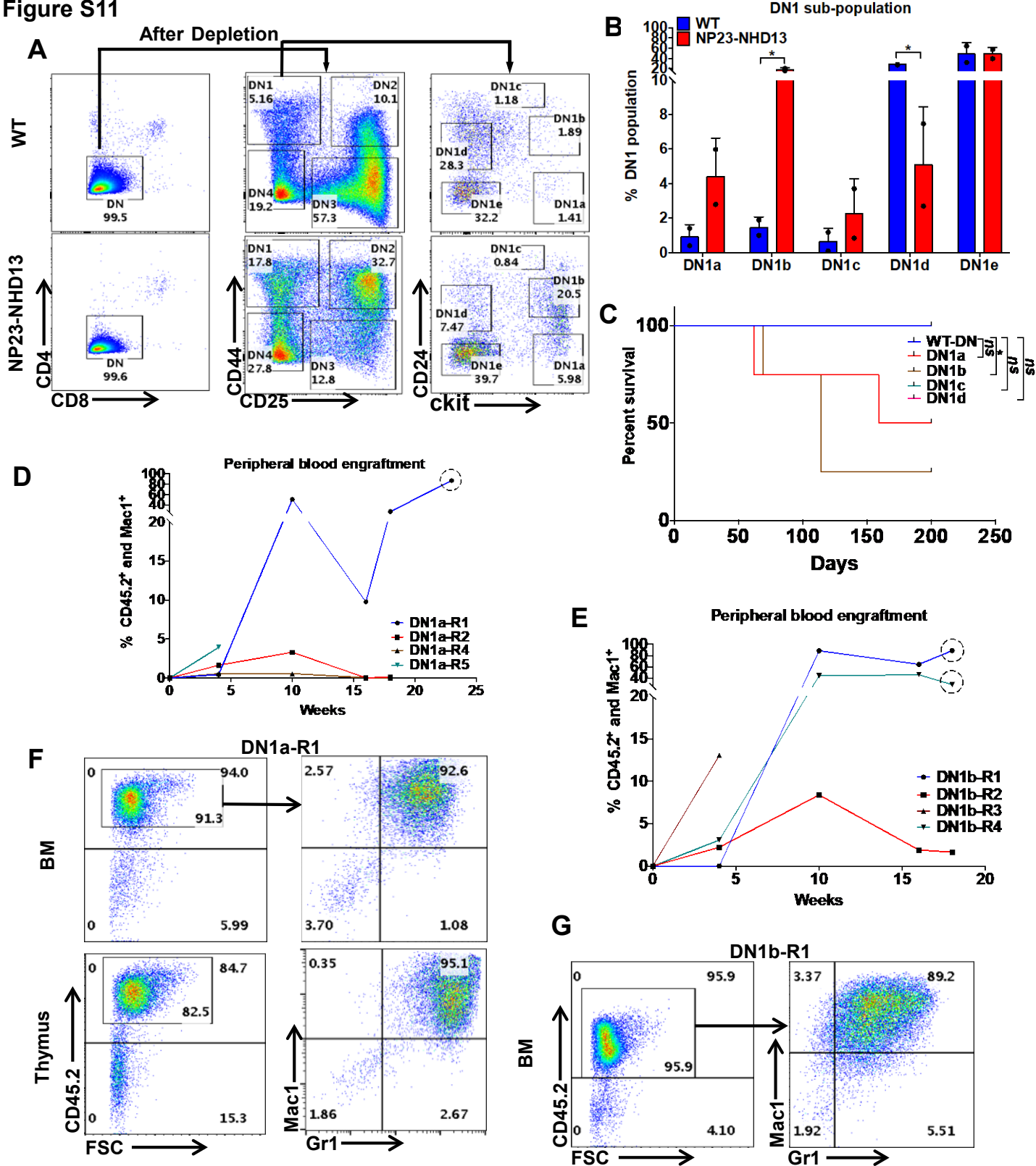


Figure S11. DN1a and DN1b populations are increased in NP23-NHD13 thymocytes and transmit AML. (A) FACS sorting and transplantation of DN1 subpopulation from NP23-NHD13 mice (DN1a=120 cells/mouse, DN1b=400 cells/mouse, DN1c=11cells/mouse, DN1d=114 cells/mouse, (DN1e were also transplanted and did not show engraftment at week 10, (however most of the mice in this group were euthanized because of wounds from penis bites and, therefore, excluded from the study). **(B)** Bar graph indicates increased DN1b and decreased DN1d sub-population, Data are expressed as means \pm SD, $n=2$ mice per group. **(C)** Survival curve indicates DN1a and DN1b recipients develop disease, $n=4-5$ mice per group. Data analyzed by Log-rank (Mantel-Cox) test. Serial engraftment of donor cells (CD45.2⁺) in DN1a **(D)** and DN1b **(E)** recipients (dotted circle indicates engraftment data obtained from BM). **(F and G)** Flow cytometry indicates presence of donor-derived (CD45.2⁺) AML cells in DN1a and DN1b recipients. * $P<0.05$.

Figure S12

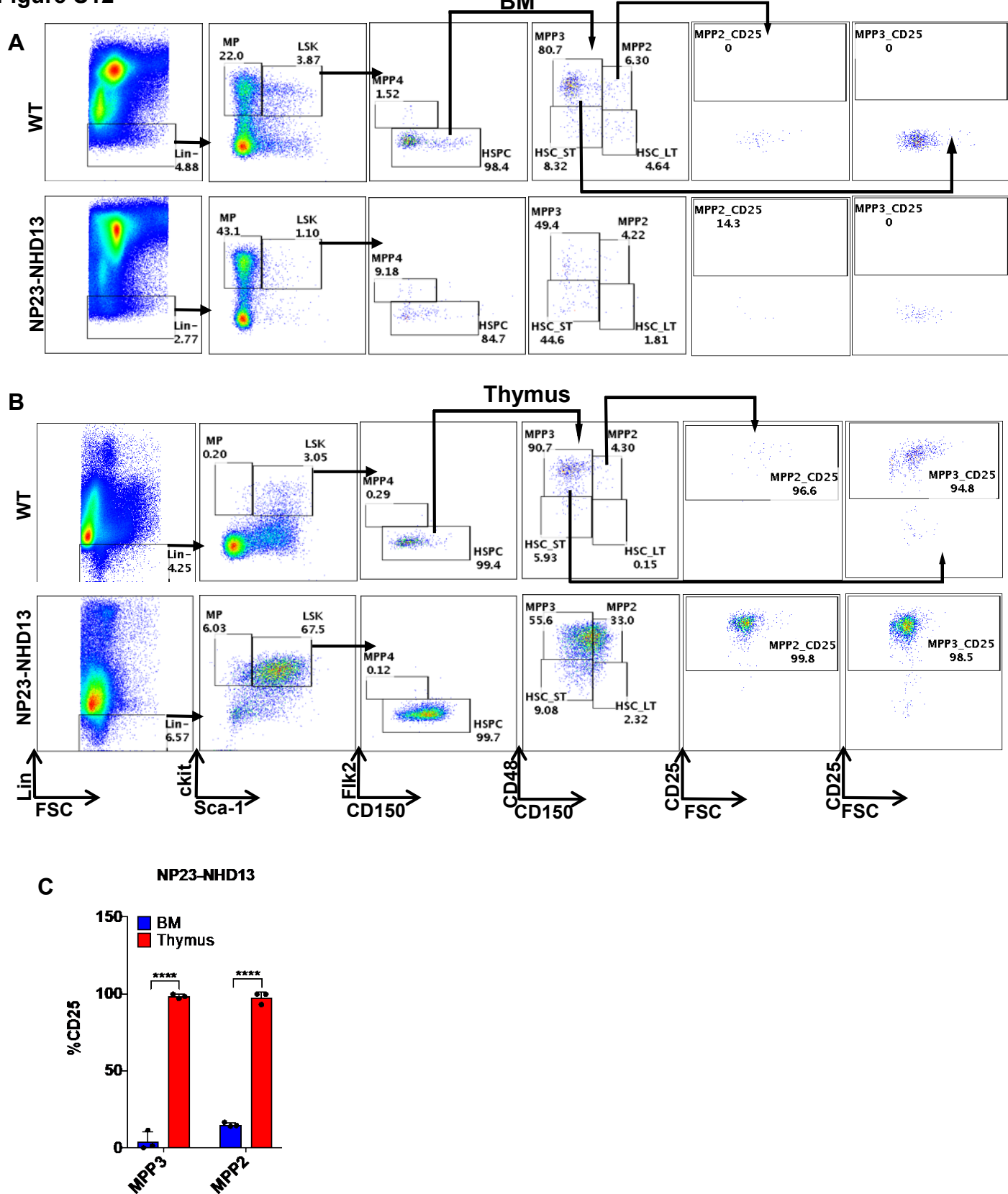


Figure S12. Identification of CD25 positive MPP3 and MPP2 populations in NP23-NHD13 thymus. (A) Analysis of CD25 expression in LSK fractions from NP23-NHD13 and WT BM. **(B)** Analysis of CD25 expression in LSK fraction from NP23-NHD13 and WT thymus. **(C)** Quantification of MPP3 and MPP2 CD25 populations in BM and thymus. Data are expressed as means \pm SD, $n=3$ mice per group, **** $P<0.001$ by one tailed student t-test.

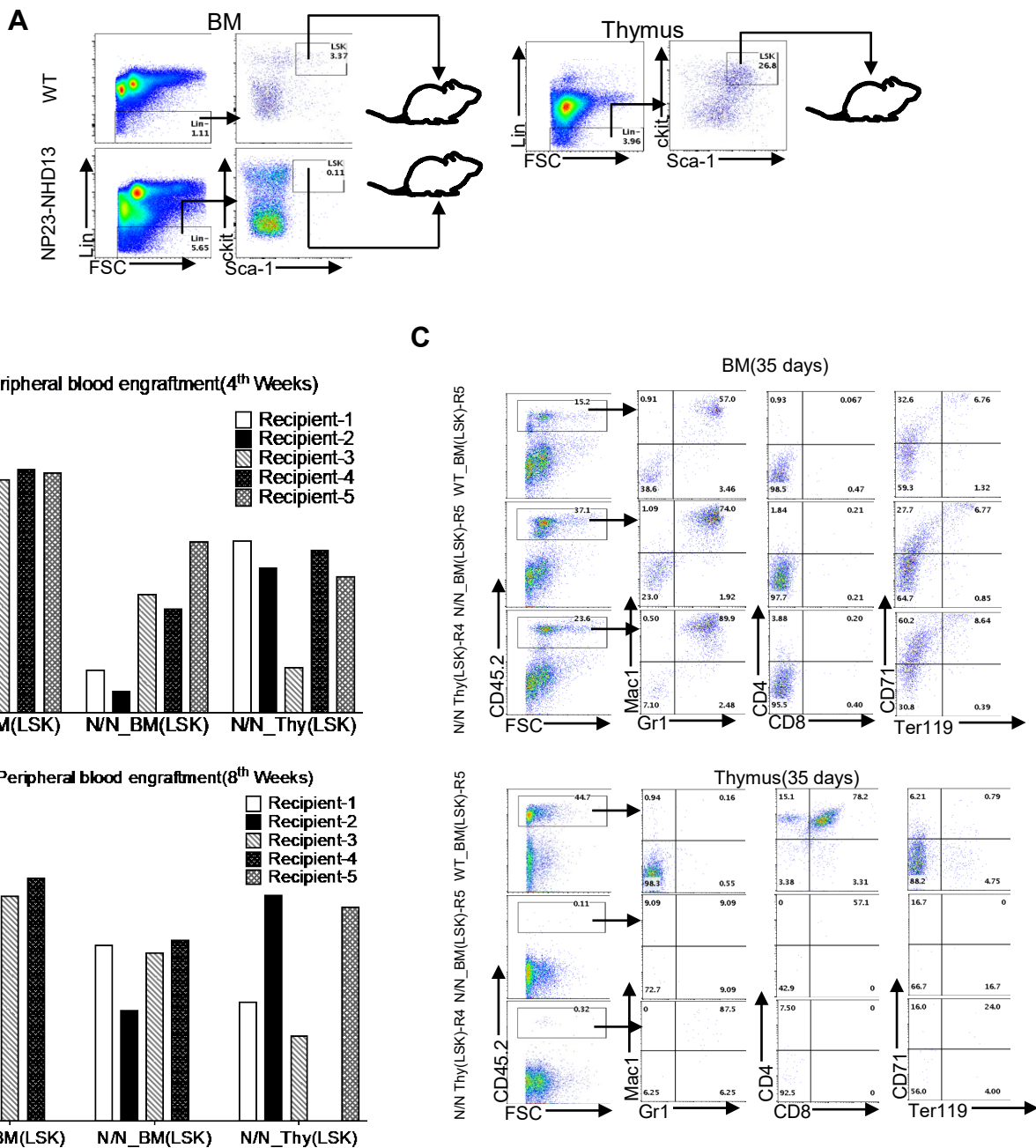
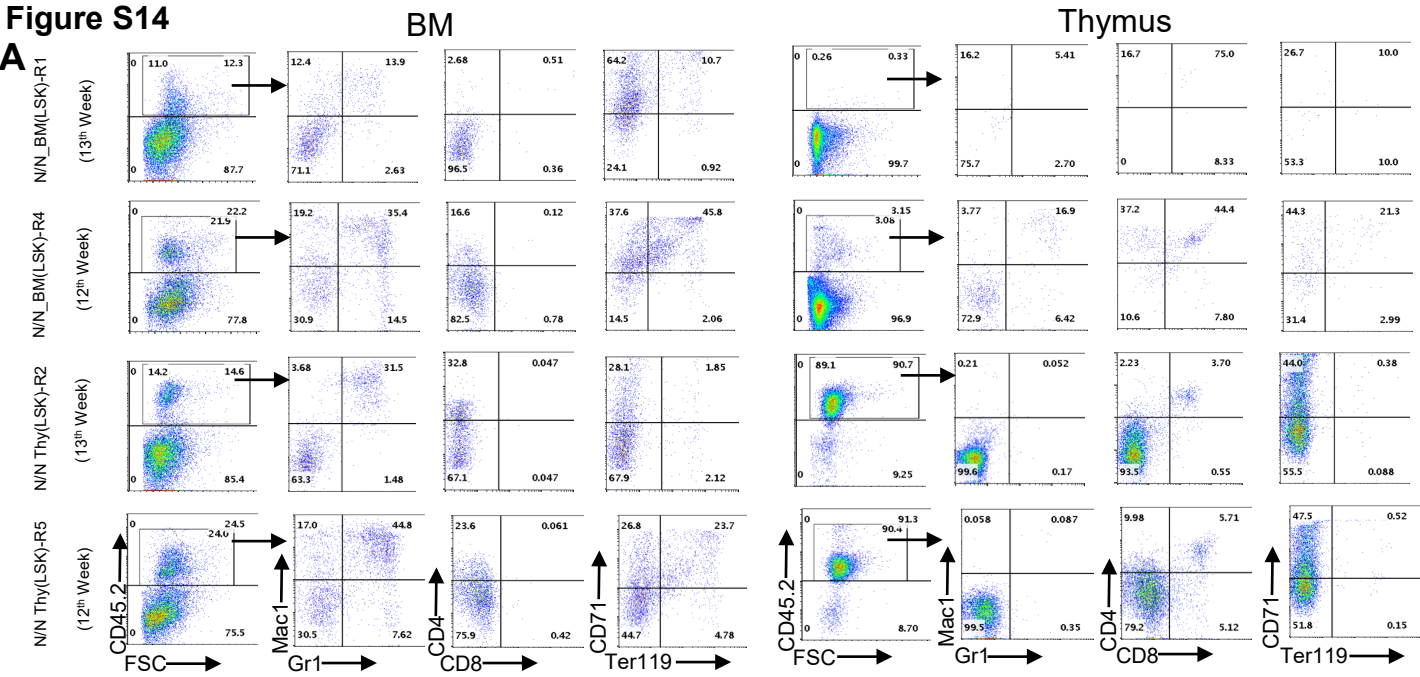
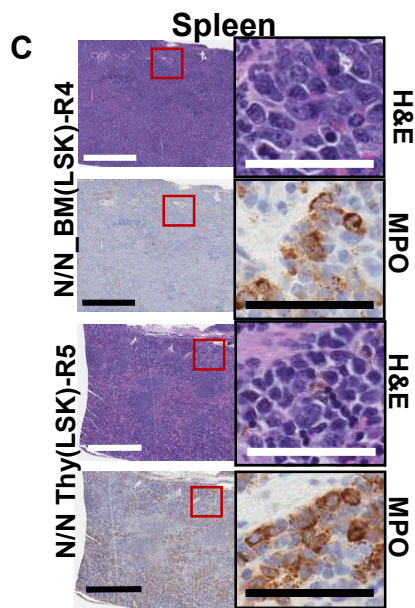
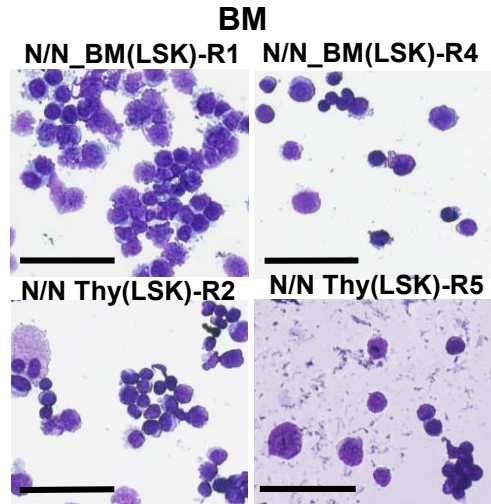


Figure S13. Thymic resident LSK cells engraft WT recipient mice. (A) Gating strategy for cell purification. WT_BM(LSK)(1420 cells/recipient), N/N_BM(LSK)(158 cells/recipient), N/N_Thy(LSK)(5136 cells/recipient). **(B)** Initial engraftment at 4th and 8th week. **(C)** Flow cytometry of BM and thymus from a representative mouse in each group at 5th week.

Figure S14**B****D**

| NP23-NHD13 Donor | Weeks (Post Transplantation) | CBC(at time of death) | | | | |
|-------------------------------------|---------------------------------|-----------------------|------------|-----------|---------------------------|--------|
| | | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | BM Cellularity (2F2T)* | Blast% |
| NP23-NHD13-BM (LSK) Recipient-1 | 13th | 16.58 [†] | 126 | 4.10 | 5.4x10 ⁷ | 98% |
| NP23-NHD13-BM (LSK) Recipient-4 | 12th | ND | ND | ND | 1.55x10 ⁷ | 98% |
| NP23-NHD13-Thy (LSK) Recipient-2 | 13th | 108.48 [†] | 301 | 5.90 | 5.1x10 ⁷ | 98% |
| NP23-NHD13-Thy (LSK) Recipient-5 | 12th | 6.16 | 248 | 8.90 | 1.43x10 ⁷ | 99% |

Figure S14. Thymic resident LSK cells can transmit AML. (A) Flow cytometry profile of leukemic recipients of LSK cells from NP23-NHD13 BM (upper two panels) or thymus (lower two panels). **(B)** Blast morphology in BM of recipients that developed AML **(C)** H &E and myeloperoxidase (MPO) staining demonstrate infiltration of MPO positive cells. **(D)** CBC and blast percentage at the time of death. N/N= NP23-NHD13 mice. Scale bars=800 μ m (low power view), 50 μ m (high power view). ND (Not Determined), [†] WBC for recipients indicate high cell count,* Indicates cell counts from two femur and two tibia.

Figure S15

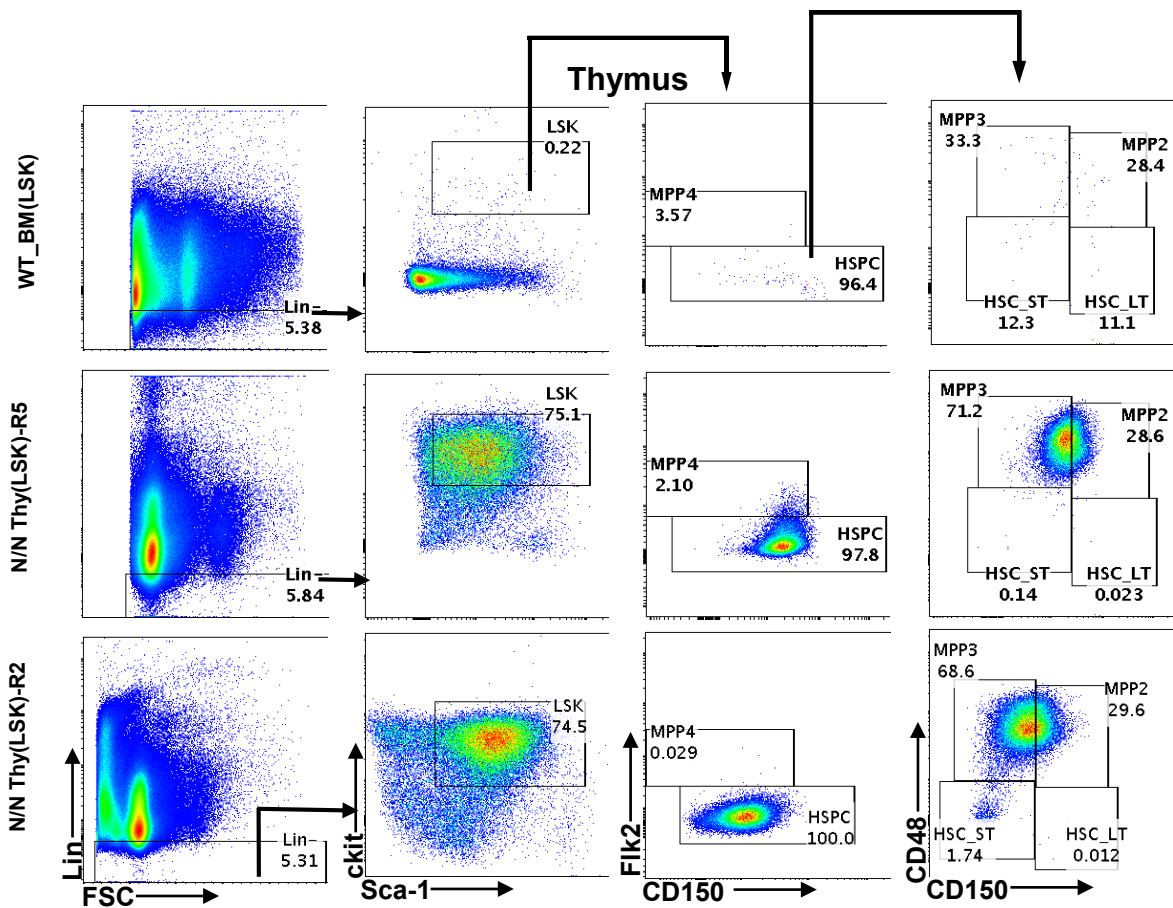
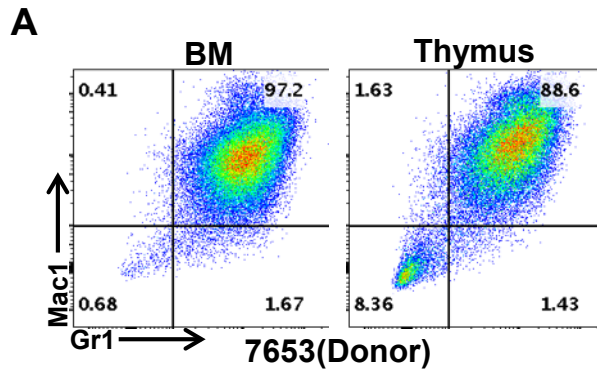


Figure S15. Thymic resident LSK cells from NP23-NHD13 mice migrate to the thymus of transplant recipients. Thymic LSK cells from N/N_Thy (LSK) donor migrates to the thymus of the transplant recipients (N/N_Thy(LSK) recipients R2 and R5) and produce a homogenous MPP2/3 population. N/N= NP23-NHD13 mice.

Figure S16



B

| Cell Transplanted (NP23-NHD13 Leukemic mouse) | Dose (Cell No injected) | Tested (No of Mice) | Response (engraftment) |
|---|-------------------------|---------------------|------------------------|
| Bone Marrow | 40 | 4 | 1 |
| | 100 | 4 | 2 |
| | 1000 | 2 | 1 |
| Thymus | 40 | 3 | 2 |
| | 100 | 2 | 2 |
| | 1000 | 4 | 4 |

Range of LIC from thymus and BM estimated at 1:32(range 10.2-103) and 1:441(range 123-1585) respectively using online tool available at

<http://bioinf.wehi.edu.au/software/elda/>.

Figure S16. LIC are more frequent in thymus than BM. (A) Flow cytometry of BM and thymus from leukemic donor mouse. **(B)** engraftment of 40, 100, or 1000 cells injected into 2-4 sublethally irradiated recipients.

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Supplementary information included in pdf.

Supplementary Figures 1-16

Supplementary Tables 1-2, 6-12.

Supplementary Tables 3-5 are attached separately as sortable Excel files.

Supplementary Table 1. Detailed characterization of NP23-NHD13 leukemic mice

| ID | Sex | Age, Days | CBC | | | | | IHC pathology | | Flow Cytometry(%) | | | | Diagnosis | |
|-------|-----|-----------|-------------------|------------|-----------|-----------|--------------|---------------|-----------------|-------------------|------------|-------|-----------|-----------|-----|
| | | | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) | Myeloperoxidase | | Mac1+/Gr1+ | B220+ | CD4+/CD8+ | | |
| 4318 | F | 69 | High [†] | 765 | 7.5 | 80.46 | High | 98.87 | Spleen | + | BM | 95.83 | 0 | 0.00 | AML |
| | | | | | | | | | Liver | + | Spleen | 46.76 | 0.05 | 0.00 | |
| | | | | | | | | | Thymus | ++ | Thymus | 95.76 | 0.30 | 0.00 | |
| 4324* | M | 67 | 568.32 | 2508 | 10.8 | 256.72 | 204.92 | 78.64 | Spleen | + | BM | 87.84 | 1.53 | 0 | AML |
| | | | | | | | | | Liver | + | Spleen | 42.55 | 1.16 | 0.02 | |
| | | | | | | | | | Kidney | + | Thymus | 98.45 | 1.45 | 0 | |
| 4351 | F | 68 | 118.2 | 658 | 8.10 | 40.52 | 45.80 | 26.90 | Spleen | ++ | BM | 98.36 | ND | 0.30 | AML |
| | | | | | | | | | Liver | + | Spleen | 42.9 | ND | 1.63 | |
| | | | | | | | | | Kidney | + | Thymus | 87.1 | ND | 0.59 | |
| 4352 | F | 40 | 32.82 | 387 | 6.90 | 20.13 | 6.55 | 5.55 | Spleen | ++++ | BM | 98.19 | 0.14 | 0.00 | AML |
| | | | | | | | | | Liver | +++ | Spleen | 81.81 | 2.04 | 0.04 | |
| | | | | | | | | | Thymus | ++ | Thymus | 62.73 | 1.93 | 0.84 | |
| 4361* | F | 51 | High [†] | 1118 | 8.20 | High | High | High | Spleen | ++++ | BM | 56.18 | 1.24 | 0.34 | AML |
| | | | | | | | | | Liver | +++ | Spleen | 10.21 | 0.96 | 0.13 | |
| | | | | | | | | | Thymus | ++++ | Thymus | 85.83 | 0.7 | 0.14 | |
| 4360 | F | 56 | 525.04 | 1994 | 10.4 | 138 | 284.32 | 68.88 | Spleen | ++++ | BM | 83.59 | 0.88 | 0 | AML |
| | | | | | | | | | Liver | +++ | Spleen | 41.74 | 2.55 | 0.28 | |
| | | | | | | | | | Thymus | ++++ | Thymus | 64.05 | 0.02 | 0.14 | |
| 4369* | F | 51 | 171.74 | 643 | 4.2 | 112.82 | 38.09 | 17.48 | Spleen | ++ | BM | 44.91 | 1.51 | 0.57 | AML |
| | | | | | | | | | Liver | +++ | Spleen | 6.81 | 1.14 | 0.02 | |
| | | | | | | | | | - | | Thymus | 84.89 | ND | 0.14 | |
| 1283* | F | 67 | 444.500 | 312 | 3.9 | 324.485 | 45.339 | 1.334 | Spleen | ++ | BM | 85.7 | 0.54 | 0.04 | AML |
| | | | | | | | | | Liver | +++ | Spleen | 31.3 | 0.68 | 0.13 | |
| | | | | | | | | | Kidney | +++ | Thymus | 87.8 | 0.54 | 0.10 | |
| 1286* | F | 50 | ND | ND | ND | ND | ND | ND | Spleen | ++++ | BM | 91.5 | 1.36 | 0.01 | AML |
| | | | | | | | | | Liver | ++ | Spleen | 36.3 | 0.33 | 0.06 | |
| | | | | | | | | | Kidney | +++ | Thymus | 95.3 | 1.00 | 0.13 | |

AML (acute myeloid leukemia); CBC (complete blood count).

+ / + + (moderate expression); + + + / + + + + (high expression).

[†] WBC for samples 4361 and 4318 were out of range for the instrument and reported as “High”.

* Indicates mice in which thymic invasion was greater than BM.

ND (Not Determined).

Supplementary Table 2. CBC and flow cytometry of young WT and NP23-NHD13 mice

| ID | Sex | Age, Days | CBC | | | Flow Cytometry | | | | | |
|------------------|-----|-----------|------------|------------|-----------|--------------------------|--|-------------------------|---|-------------------------|-------------------------|
| | | | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | Mac1 ⁺ (K/uL) | Mac1 ⁺ /Gr1 ⁺ (K/uL) | Gr1 ⁺ (K/uL) | CD19 ⁺ /B220 ⁺ (K/uL) | CD4 ⁺ (K/uL) | CD8 ⁺ (K/uL) |
| 7645(WT) | F | 36 | 9.62 | 757.00 | 12.40 | 0.464646 | 0.204906 | 0.207792 | 5.5796 | 0.706108 | 0.97162 |
| 7659(WT) | M | 30 | 12.68 | 979.00 | 12.70 | 0.4438 | 0.486912 | 0.656824 | 5.05932 | 1.81324 | 1.28068 |
| 7692(WT) | F | 36 | 12.24 | 845.00 | 14.70 | 1.33416 | 0.604656 | 0.454104 | 5.61816 | 1.88496 | 1.7136 |
| 7653(NP23-NHD13) | M | 33 | 4.20 | 571.00 | 11.30 | 0.504 | 1.2558 | 0.02016 | 0.0135 | 0.0693 | 0.0714 |
| 7660(NP23-NHD13) | M | 30 | 5.46 | 1070.00 | 10.30 | 0.11739 | 2.14578 | 0.10374 | 0.020202 | 0.115752 | 0.068796 |
| 7661(NP23-NHD13) | M | 30 | 3.08 | 485.00 | 10.90 | 0.08778 | 1.0318 | 0.051744 | 0.068068 | 0.06622 | 0.018172 |

Supplementary Table. 6 CBC from QN cells transplant recipients

| Leukemic Donor | CBC | | | | | |
|----------------------|-------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| 4369_QN thymocyte_R1 | 190.520 | 469 | 7.7 | 110.54 | 51.40 | 20.8 |
| 4369_QN thymocyte_R2 | High [†] | 597 | 6.7 | 71.71 | 157.40 | High |
| 4369_QN thymocyte_R3 | FD | FD | FD | FD | FD | FD |
| 4369_QN thymocyte_R4 | 208.800 | 598 | 6.1 | 61 | 18 | 20 |
| 4369_QN thymocyte_R5 | 110.7 | 571 | 10.2 | 67.57 | 27.44 | 13.30 |

FD (Found dead).

QN (Quadruple Negative Cells).

[†] *High (out of range of the instrument and reported as “High”).*

Supplementary Table 7. CBC from QN cells transplant recipients

| NP23-NHD13 Donor | CBC | | | | | |
|---------------------------|-----------------------|-----------------------|----------------------|----------------------|-------------------------|------------------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| QN thymocyte_100 cells_R1 | 181.06 | 753 | 10.70 | 27.81 | 114 | 22.25 |
| QN thymocyte_100 cells_R2 | 162.14 | 305 | 5.40 | 28.15 | 96.77 | 26.61 |
| QN thymocyte_100 cells_R3 | 144.62 | 415 | 10.20 | 42.11 | 70.05 | 21.26 |
| QN thymocyte_100 cells_R4 | 161.50 | 346 | 8.60 | 20.70 | 97.51 | 20.70 |

Supplementary Table 8. CBC from kit-OP9 and Mac1⁺/Gr1⁺-OP9 population transplant recipients

| NP23-NHD13 Donor Kit ⁺ -OP9 | CBC(at time of death) | | | | |
|---|-----------------------|---------------|--------------|--------------|-----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) |
| Recipients-2 | 6.97 | 401 | 5.1 | 5.6 | 0.27 |
| Recipients-3 | 5.38 | 161 | 7.70 | 3.93 | 0.95 |
| Recipients-4 | 6.96 | 170 | 9.8 | 5.31 | 1.29 |
| Recipients-5 | 2.28 | 128 | 8.1 | 1.93 | 0.26 |

| NP23-NHD13 Donor Mac-1 ⁺ /Gr-1 ⁺ -OP9 | CBC(at time of death) | | | | |
|--|-----------------------|---------------|--------------|--------------|-----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) |
| Recipients-1 | 4.32 | 224 | 8.40 | 3.17 | 0.78 |
| Recipients-2 | 118.20 | 218 | 9.20 | 20.78 | 72.44 |
| Recipients-3 | 7.44 | 453 | 11.70 | 4.44 | 2.33 |
| Recipients-4 | 2.68 | 137 | 2.30 | 1.06 | 1.45 |
| Recipients-5 | 1.42 | 261 | 7.50 | 1.07 | 0.29 |

Supplementary Table 9. CBC from DN subpopulation transplant recipients

| WT-Donor (DN cells) | CBC (10 th Week) | | | | | |
|------------------------|-----------------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| WT-Recipient-1 | 7.06 | 490 | 11.6 | 5.39 | 0.85 | 0.55 |
| WT-Recipient-2 | 9.12 | 506 | 15.2 | 6.59 | 2.08 | 0.4 |
| WT-Recipient-3 | 8.46 | 639 | 14.10 | 5.85 | 2.09 | 0.42 |
| WT-Recipient-4 | ND | ND | ND | ND | ND | ND |
| WT-Recipient-5 | 7.74 | 665 | 15.00 | 5.29 | 2.06 | 0.37 |

| NP23-NHD13- Donor (DN1 cells) | CBC (10 th Week) | | | | | |
|-------------------------------------|-----------------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN1-Recipient-2 | 248.36 | 469 | 5.10 | 94.56 | 113.10 | 30.82 |
| DN1-Recipient-3 | 7.24 | 747 | 13.50 | 4.81 | 1.92 | 0.40 |
| DN1-Recipient-4 | 6.70 | 147 | 6.80 | 5.36 | 0.87 | 0.38 |
| DN1-Recipient-5 | 9.76 | 128 | 8.80 | 7.59 | 1.54 | 0.39 |

| NP23-NHD13- Donor (DN2 cells) | CBC (10 th Week) | | | | | |
|-------------------------------------|-----------------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN2-Recipient-1 | 3.18 | 295 | 9.60 | 2.43 | 0.51 | 0.23 |
| DN2-Recipient-2 | 4.92 | 401 | 11.10 | 3.19 | 1.41 | 0.27 |
| DN2-Recipient-3 | 6.00 | 309 | 11.50 | 4.08 | 1.60 | 0.28 |
| DN2-Recipient-4 | 5.64 | 761 | 12.90 | 3.85 | 1.32 | 0.15 |
| DN2-Recipient-5 | 15.64 | 163 | 7.40 | 12.40 | 2.10 | 1.00 |

| NP23-NHD13- Donor (DN3 cells) | CBC(10 th Week) | | | | | |
|-------------------------------------|----------------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN3-Recipient-1 | 9.22 | 471 | 12.50 | 6.71 | 2.20 | 0.28 |
| DN3-Recipient-2 | 7.66 | 638 | 12.00 | 5.07 | 2.08 | 0.25 |
| DN3-Recipient-3 | 8.34 | 698 | 13.10 | 6.18 | 1.81 | 0.30 |
| DN3-Recipient-4 | 9.04 | 748 | 14.40 | 6.78 | 1.54 | 0.55 |
| DN3-Recipient-5 | 8.84 | 729 | 13.90 | 6.67 | 1.64 | 0.49 |

| NP23-NHD13- Donor (DN4 cells) | CBC(10 th Week) | | | | | |
|-------------------------------------|----------------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN4-Recipient-1 | 8.54 | 629 | 12.80 | 6.04 | 2.17 | 0.28 |
| DN4-Recipient-2 | 11.24 | 662 | 12.70 | 7.57 | 3.08 | 0.39 |
| DN4-Recipient-3 | 7.06 | 722 | 13.50 | 5.01 | 1.66 | 0.30 |
| DN4-Recipient-4 | 9.76 | 747 | 12.90 | 6.35 | 2.93 | 0.38 |
| DN4-Recipient-5 | 8.06 | 473 | 10.60 | 6.77 | 0.81 | 0.41 |

| NP23- NHD13- Donor (DN1 & DN2) | CBC(at time of death) | | | | | |
|--------------------------------------|-----------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN1-Recipient-2 | 248.36 | 469 | 5.10 | 94.56 | 113.10 | 30.82 |
| DN1-Recipient-5 | 10.56 | 60 | 3.3 | 9.29 | 0.34 | 0.15 |
| DN2-Recipient-2 | 28.6 | 173 | 7.6 | 22.88 | 3.21 | 1.37 |
| DN2-Recipient-3 | 5.06 | 230 | 10.40 | 4.02 | 0.54 | 0.10 |
| DN2-Recipient-5 | 25.56 | 168 | 8.30 | 12.02 | 8.51 | 4.29 |

Not determined is indicated as "ND".

Supplementary Table 10. CBC from DN1 subpopulation transplant recipients

| NP23-NHD13 Donor (DN1a cells) | CBC (10 th Week) | | | | | |
|-------------------------------------|-----------------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN1a-Recipient-1 | 6.40 | 365 | 8.20 | 5.26 | 0.45 | 0.66 |
| DN1a-Recipient-2 | 7.92 | 811 | 11.00 | 4.89 | 2.24 | 0.74 |
| DN1a-Recipient-4 | 10.32 | 970 | 11.30 | 5.15 | 4.58 | 0.42 |
| DN1a-Recipient-5 | FD | FD | FD | FD | FD | FD |

| NP23-NHD13 Donor (DN1b cells) | CBC (10 th Week) | | | | | |
|-------------------------------------|-----------------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN1b-Recipient-1 | 7.16 | 573 | 8.70 | 5.73 | 0.76 | 0.60 |
| DN1b-Recipient-2 | 9.50 | 249 | 10.90 | 6.53 | 2.32 | 0.61 |
| DN1b-Recipient-3 | FD | FD | FD | FD | FD | FD |
| DN1b-Recipient-4 | 4.86 | 158 | 7.50 | 4.00 | 0.42 | 0.39 |

| NP23-NHD13 Donor (DN1c cells) | CBC(10 th Week) | | | | | |
|-------------------------------------|----------------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN1c-Recipient-1 | 9.06 | 623 | 13.60 | 6.27 | 2.01 | 0.66 |
| DN1c-Recipient-2 | 8.76 | 929 | 13.00 | 6.12 | 1.95 | 0.65 |
| DN1c-Recipient-3 | 9.76 | 919 | 11.90 | 6.83 | 1.97 | 0.91 |
| DN1c-Recipient-4 | 10.96 | 937 | 12.70 | 6.42 | 3.61 | 0.80 |
| DN1c-Recipient-5 | 9.26 | 834 | 11.50 | 5.79 | 2.92 | 0.42 |

| NP23-NHD13 Donor (DN1d cells) | CBC(10 th Week) | | | | | |
|-------------------------------------|----------------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN1d-Recipient-1 | 7.86 | 890 | 14.40 | 4.76 | 2.58 | 0.29 |
| DN1d-Recipient-2 | 10.90 | 1060 | 12.00 | 8.10 | 2.13 | 0.59 |
| DN1d-Recipient-3 | 8.08 | 752 | 13.20 | 6.00 | 1.49 | 0.55 |
| DN1d-Recipient-4 | 6.52 | 753 | 12.80 | 4.54 | 1.54 | 0.39 |

| NP23- NHD13- Donor (DN1a & DN1b) | CBC(at time of death) | | | | | |
|--|-----------------------|---------------|--------------|--------------|-----------------|----------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| DN1a-Recipient-1 | 26.86 | 231 | 8.50 | 15.44 | 9.92 | 0.78 |
| DN1b-Recipient-1 | 128.30 | 330 | 3.0 | 91.99 | 8.4 | 4.7 |
| DN1b-Recipient-4 | 15.32 | 98 | 4.10 | 8.24 | 5.57 | 1.12 |

Found dead is indicated as "FD".

Supplementary Table 11. CBC from NP23-NHD13(LSK) transplant recipients

| Donor WT-BM (LSK) | CBC (10th Week) | | | | | |
|------------------------------|-----------------------------------|-----------------------|----------------------|----------------------|-------------------------|------------------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| Recipient-1 | 9.34 | 833 | 14.00 | 5.49 | 3.11 | 0.72 |
| Recipient-2 | 11.00 | 988 | 13.00 | 5.91 | 5.16 | 0.61 |
| Recipient-3 | 13.10 | 803 | 13.10 | 8.39 | 3.86 | 0.79 |

| Donor NP23/NHD13-BM (LSK) | CBC (10th Week) | | | | | |
|--------------------------------------|-----------------------------------|-----------------------|----------------------|----------------------|-------------------------|------------------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| Recipient-1 | 13.58 | 212 | 7.70 | 10.20 | 2.22 | 0.78 |
| Recipient-2 | 7.10 | 250 | 11.70 | 4.59 | 1.96 | 0.53 |
| Recipient-3 | 4.76 | 384 | 10.10 | 3.30 | 1.06 | 0.33 |
| Recipient-4 | ND | ND | ND | ND | ND | ND |

| Donor NP23/NHD13-Thymus(LSK) | CBC (10th Week) | | | | | |
|---|-----------------------------------|-----------------------|----------------------|----------------------|-------------------------|------------------------|
| | WBC (K/uL) | PLT (K/uL) | Hb (g/dL) | LY (K/uL) | Polys (K/uL) | Mono (K/uL) |
| Recipient-1 | 7.64 | 621 | 13.30 | 4.73 | 2.41 | 0.47 |
| Recipient-2 | 7.28 | 231 | 8.50 | 4.93 | 1.86 | 0.39 |
| Recipient-3 | 11.02 | 539 | 12.70 | 6.95 | 3.31 | 0.64 |
| Recipient-5 | 6.16 | 248 | 8.90 | 4.02 | 1.49 | 0.56 |

Supplementary Table 12. Primers used for different experiments

| Primer name | Sequence 5'-3' | Gene |
|-------------------------|---------------------------------------|-----------------|
| NUPPHF(F) | ATTTAATACTACGACAGCCACTTTGG | NUP98 |
| PHF23 (R1) | CATCCAGATCAAAGAGAGAGTCC | PHF23 |
| HOXD13-L1(F) | GGCTTCTAAGCTGTCTGTGGCC | HOXD13 |
| NUP98001(R) | TGGAGGGCCTCTTGGTACAGG | NUP98 |
| 5'TCRBD1.1(F) | CTTATCTGGTGGTTTCTTCCAGC | TCRBD1 |
| 3'TCRBJ1S4.1(R) | CAGACAGCTTGGTTCATGACCG | TCRBJ1S4 |
| 3'TCRBJ1S6.1(R) | AGACCATGGTCATCCAACACAGGC | TCRBJ1S6 |
| 5'TCRBD2.2(F) | TGTATCACGATGTAACATTGTGG | TCRBD2 |
| 3'TCRBJ2S4.2(R) | TACTGGGTGTCTTGGTTCACAGC | TCRBJ2 |
| 3'TCRBJ2S7.2 (R) | TTGAGAGCTGTCTCCTACTATCG | TCRBJ2S7 |
| TCRVB(F) | TAAGCGGCCGCATGKDYTGGTAYMRRRCAG | TCRVB |
| TCRCB(R) | CCCACCAGCTCAGCTCCACGTGG | TCRCB |
| SCIDA(F) | GGAAGAGTTTTGAGCAGACAATG | SCID |
| SCIDB(R) | CATCACAAGTTATAACAGCTGGG | SCID |