SUPPLEMENTAL METHODS

Blood counts, flow cytometric analysis and BM sorting

Murine blood samples were obtained by bleeding from the cheek. Blood was analyzed with an Auto Hematology Analyser (BC 5300 Vet, Mindray Bio-Medical Electronics, Shenzhen, China). BM was collected from femur and tibia of mice by grinding the muscle free bones. For flow cytometric analysis, single cell suspensions of peripheral blood (PB), bone marrow (BM) and spleen (SP) from WT and dnTCF4 mice were subjected to red cell lysis. Cells were stained with fluorescence-conjugated antibodies and analyzed using a LSRII or Symphony instrument (BD Biosciences, San Jose, CA, USA).

The antibodies used for the analysis of murine hematopoietic tissues by flow cytometry were: CD45/B220 APC, CD45/B220 PE (RA3-6B2), CD3ε APC (145-2C11), Ly6G PE-Cy7 (1A8), and CD11b APC, CD11b PE, CD11b PB (M1/70), anti-mouse Lineage Cocktail Pacific Blue (including CD3(17A2); Gr1 (RB6-8C5); CD11b (M1/70); CD45R/B220 (RA3-6B2); TER-119 (Ter-119)), c-kit APC, BV711, BV605, PE, PerCP/Cy5.5 (2B8), Sca-1 APC, BV650 (E13-161.7), FcγRII/III Pe-Cy7 (93), CD34 (MEC14.7) biotinylated or conjugated to Alexa-Fluor 700, and streptavidin PE conjugate. BM transplant recipients were analyzed using the following antibodies: Ly5.2 PE/Cy7 (104), Ly5.1 PB (A20), Gr1 APC or PerCP/Cy 5.5 (RB6-8C5), CD11b APC (M1/70), CD45R/B220 APC or PerCP/Cy5.5 (RA3-6B2), CD3 PE (17A2). BM granulocytic development was analyzed as previously described [1,2]. Preparation of cells for sorting of short-term (ST-HSC) and long-term HSCs (LT-HSCs) for transplantation assays was performed in 2 steps. First, Lin^+ cells were labeled with biotinylated lineage markers: CD45/B220 (RA3-6B2), CD3 (145-2c11), Ter119 (TER-119), Gr1 (RB6-8C5), and CD11b (M1/70) and depleted using anti-biotin magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) on MACS separator (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's protocol. Second, Lin- cells were labeled

with different combinations of the following antibodies: c-Kit PerCP/Cy 5.5 (2B8), Sca-1 APC (E13-161.7), CD150 Pe-Cy7 (TC15-12F12.2), CD48 PE (HM48-1), streptavidin-eFluor450. An Influx instrument (BD Biosciences, San Jose, CA, USA) was employed to sort according to the following sorting strategy[3]: LT-HSC were sorted as: Lin^-, $c\text{-}kit^+$, $Sca\text{-}1^+$; $CD150^+$, $CD48^-$. ST-HSC were sorted as: Lin^-, $c\text{-}kit^+$, $Sca\text{-}1^+$; $CD150^-$, $CD48^+$. To exclude dead cells, cell suspensions were stained with Hoechst 33258. All antibodies were purchased from BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA), BioLegend (San Diego, CA, USA), or Exbio (Praha, Vestec, Czech Republic). Data were acquired using Diva software (BD Biosciences, San Jose, CA, USA) and analyzed with the FlowJo software (Tree Star Incorporation, Ashland, OR, USA).

Western blot analysis

BM from WT and dnTCF4 was extracted and 8×10^5 cells were lysed (65mM Tris. pH 6.8, 10% glycerol, 1% SDS, 0.5% DTT). Protein lysates were separated using reducing SDS-PAGE (10% acrylamide gel), transferred to nitrocellulose membrane, and stained with anti Tcf4 (ab185736 – Abcam, Cambridge, UK) and anti β-actin (13E5 - Cell Signaling, Danvers, MA, USA) antibodies following manufacturer's instructions. Of note, the antibody against Tcf4 recognizes both mouse and human Tcf4, including dnTCF4 variant. Immunoblot was developed using Azure Biosystem c300 (Dublin, CA, USA).

Chemical compounds

BIO (6-bromoindirubin-3'-oxime) and CHIR99021 were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). These compounds are GSK3β inhibitors, and thus they inhibit β-catenin phosphorylation favoring its translocation to the nucleus and inducing transactivation

of β-catenin target genes. Cercosporin, an inhibitor of β-catenin interaction with transcription factors of TCF/LEF family, was purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

RNA isolation, cDNA preparation and quantitative RT-PCR

RNA from murine BM cells was extracted with Tri Reagent RT (Molecular Research Center, Cincinnati, OH, USA) and treated with DNaseI (Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions. Briefly, cDNA was prepared using SuperScript II Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA). Quantitative RT-PCR was performed using a LightCycler® 480 SYBR Green I Master mix and samples were run on a LightCycler® 480 Instrument II (both Roche Molecular Systems, Pleasanton, CA, USA). For each sample, transcript levels of tested genes were normalized to *Gapdh* or *Actb*. Primer sequences used for quantitative RT-PCR are listed in below:

Gene	Orientation	Organism	Sequence (5' – 3')
dnTCF4	F	human	ACATGGTCCTGCTGGAGTTC
	R	human	TCGGAGGAGCTGTTTTGATT
Axin2	F	mouse	GATGTCTGGCAGTGGATGTTAG
	R	mouse	GACTCCAATGGGTAGCTCTTTC
Nkd1	F	mouse	AGGACGACTTCCCCCTAGAA
	R	mouse	TGCAGCAAGCTGGTAATGTC
Csf3r	F	mouse	GGACCTCTTCACCTACTACA
	R	mouse	GGAGAACTCATTTCCCACT
Il1r1	F	mouse	GGTGGAGGACTCAGGATATT
	R	mouse	AGCCAGGGTCATTCTCTAA
Irf4	F	mouse	CCCTGACAAGAGAAGACATAC
	R	mouse	AACCTACAGCTCCTCTATCC
Spp1	F	mouse	GGTGATAGCTTGGCTTATGG
	R	mouse	TTCATGTGAGAGGTGAGGT
Ela2	F	mouse	ACTCTGGCTGCCATGCTACT
	R	mouse	GCCACCAACAATCTCTGA
Ctsg	F	mouse	CTGACTAAGCAACGGTTCTGG
	R	mouse	GATTGTAATCAGGATGGCGG
Cebpe	F	mouse	AAGGCCAAGAGGCGCATT
	R	mouse	CGCTCGTTTTCAGCCATGTA
Ltf	F	mouse	TATTTCTTGAGGCCCTTGGA
	R	mouse	TCTCATCTCGTTCTGCCACC

Lef1	F	mouse	GCTGCCTACATCTGAAACATGGTG
	R	mouse	CTGTGGAGACAGTCTGGGG
Tcf7	F	mouse	GCCAGAAGCAAGGAGTTCAC
	R	mouse	TACACCAGATCCCAGCATCA
Tcf7l1	F	mouse	CCCCCTACTTTCCCAGCTAC
	R	mouse	CTTTGTGTTTCCCCCTTCCT
Tcf7l2	F	mouse	CGTAGACCCCAAAACAGGAAT
	R	mouse	TCCTGTCGTGATTGGGTACA
Gapdh	F	mouse	AACTTTGGCATTGTGGAAGG
	R	mouse	ATCCACAGTCTTCTGGGTGG
Actb	F	mouse	GATCTGGCACCACACCTTCT
	R	mouse	GGGGTGTTGAAGGTCTCAAA

The sequences of primers used for ChIP-qPCR investigating LEF1 enrichment in the *CSF3R* gene locus in human K562 cells are listed below:

Region		Sequence (5‘ – 3‘)
R1 (TSS)	F	GGCCCTAAGCCTCTTTTCAG
	R	TGAAGAGGGGAGCAGTGAGT
R2 (-3.3 kb)	F	GGAGTGTCCAGTTTCTTTCTAA
	R	CTGTGTGTGACCTCGATTG
CR (-6.1 kb)	F	GGGAGGCAAAGTGCTTACTG
	R	GAGGGCAGATGAGAGATGGA

In vivo BrdU incorporation assays

Mice were injected with 1 mg of bromodeoxyuridine (BrdU) intraperitoneally and sacrificed 24 hours after the treatment. BM was isolated, stained with fluorescence-conjugated antibodies, and subsequently processed with FITC BrdU Flow Kit (BD Pharmigen™, New Jersey, USA) following manufacturer’s instructions.

Cell cycle analysis

Purified hematopoietic stem and progenitor cells were sorted and suspended in phosphate-citrate buffer solution with 0.02% saponin for permeabilization (pH 4.8) for 20 minutes at room temperature. Next, cells were washed once with PBS and once with cell cycle staining solution (1.5 ug/mL pyronin Y (Sigma-Aldrich, St. Louis, MO, USA) and 2 ug/mL Hoechst 33342 (Invitrogen, CA, USA)). Cells were resuspended in cell cycle staining solution,

incubated 10 minutes on ice, and analyzed using a LSRII instrument (BD Biosciences, San Jose, CA, USA).

Colony culture and re-plating assays

Murine myeloid colony culture assays were performed using Methocult GF M3434 or M3231 (Stemcell Technologies, Vancouver, BC, Canada). M3231 was supplemented with human granulocyte colony-stimulating factor (G-CSF, 50 ng/mL) and murine stem cell factor (SCF, 100 ng/mL). For re-plating assays 1×10^4 whole BM cells were initially plated, cells were harvested after 7 days, and 5×10^3 cells were re-plated. Colony culture assays with human primary cells were performed using Methocult H4545 (Stemcell Technologies, Vancouver, BC, Canada). 500 freshly isolated human cord blood $CD34^+$ cells were plated in semi-solid medium containing 0.01% DMSO, 1µM BIO, or 1µM CHIR99021 (both from Sigma-Aldrich, St. Louis, Missouri, USA). Colonies were scored after 7 days of *in vitro* culture. All cytokines except G-CSF (Neupogen, Amgen, Thousand Oaks, CA, USA) were purchased from Peprotech (Rocky Hill, NJ, USA).

Morphological analysis and differential counting

Morphological analysis and manual leukocyte differential counts of BM cells were performed using May-Grünwald Giemsa stained cytospins. A minimum of 200 cells was analyzed.

BM Histology

BM samples were fixed in 4% formaldehyde for 72 h, then decalcified in Osteosoft (Merck) for 3 weeks, moved into 70% ethanol solution to process using an automated tissue processor (Leica ASP 6025, Leica Microsystems,Germany), and embedded in paraffin blocks using a Leica EG1150H paraffin embedding station (Leica Microsystems, Germany). Longitudinal

sections of 2 μm were cut using a microtome (Leica RM2255, Leica Microsystems, Germany) on standard glass slides (Waldemar Knittel, GmbH, Germany). Sections were stained with haematoxylin–eosin and mounted using Leica ST5020 automated staining instrument in combination with the Leica CV5030 coverslipper (Leica Microsystems, Germany).

Whole BM transplantation assays

Donor ($Ly5.2^+$) BM was isolated from WT and dnTCF4 animals. Two different doses (1×10^5 and 5×10^5) of donor cells were intravenously transplanted along with 5×10^5 competitive BM cells ($Ly5.1^+$). PB and BM were analyzed 4 weeks and 16 weeks after transplantation. Cells were stained with anti-Ly5.1 and Ly5.2 antibodies (to distinguish donor-derived cells), and lineage-specific antibodies (CD11b, Gr1, B220, and CD3).

LT-HSC limiting dilution transplantation assays

C57BL/6NCrl mice ($CD45.1^+$) were employed as recipients and were lethally irradiated (6 Gy). Donor cells were isolated from WT and dnTCF4 ($Ly5.2^+$) murine BM. Three different doses (10, 20, and 40) of LT-HSCs, defined as LKS $CD48^-$ $CD150^+$, were sorted and intravenously transplanted along with 5×10^5 BM cells as a support ($Ly5.1^+$). BM and blood of recipients were analyzed 16 weeks after transplantation. Cells were stained with anti-Ly5.1 and Ly5.2 antibodies to distinguish donor-derived cells from the support cells, as well as with lineage-specific antibodies CD11b, Gr1, B220, and CD3. A recipient mouse was defined as positive when engraftment was >0.1% and presented at least two lineages reconstituted.

Genome-wide transcription factor occupancy and histone modification profiles visualization

The genome-wide transcription factor (TF) occupancy and histone modification profiles presently available were downloaded from the ENCODE database using accession numbers ENCSR000EUY (LEF1 HEK293T cells), ENCSR000EVQ (LEF1 HepG2 cells), ENCSR000EWT (LEF1 MCF-7 cells), ENCSR000EXL (LEF1 PANC-1 cells), ENCSR000EVF (LEF1 Hela S3 cells), ENCSR000AOF (H3K4me3 HelaS3 cells), ENCSR000AOC (H3K27Ac HelaS3 cells) and ENCSR000APV (H3K4me1 HepG2 cells). All TF occupancy and histone modification profiles were viewed in integrative genomics viewer (IGV)[4].

Chromatin immunoprecipitation and data analysis

Formaldehyde cross-linked chromatin from 1×10^7 K562 cells per sample was prepared and used in ChIP assays as previously described[5]. ChIP enrichment for a set of 3 biological replicates assayed in triplicate by qPCR was calculated as previously described[6]. The anti-LEF1 rabbit antibody (D6J2W, Cell Signaling, Danvers, MA, USA) was used. Quantitative RT-PCR was performed using a LightCycler® 480 SYBR Green I Master mix and samples were run on a LightCycler® 480 Instrument II (both Roche Molecular Systems, Pleasanton, CA, USA).

Intracellular analysis of phosphorylated proteins by flow cytometry

BM cells were isolated from WT and dnTCF4 mice and treated with 100 ng/mL G-CSF (Neupogen, Amgen, Thousand Oaks, CA, USA) for the indicated period of time. Next, cells were fixed with 4% formaldehyde (Thermo Scientific, Waltham, Massachusetts, USA), permeabilized with 90% methanol and stained with anti p-Stat3 PE- (Tyr705) (13A3-1,

Biolegend, San Diego, CA, USA), anti p-Stat5 PE- (Tyr694) (C71E5, Cell Signaling, Danvers, MA, USA) conjugated antibodies, or rabbit anti-pJAK2 (Tyr1007,Tyr 1008) (ab3201, Abcam, Cambridge, UK) overnight. Next day samples were stained with secondary goat anti-rabbit IgG Alexa Fluor 647 conjugate (Life Technologies, Carlsbad, CA, USA), lineage specific pacific blue (PB) antibody cocktail, anti c-Kit BV711 or BV605 conjugate, and anti Sca-1 APC or BV650 conjugate for 60 minutes on ice, and measured on FACS Symphony instrument (BD Biosciences, San Jose, CA, USA).

Blood serum extraction and G-CSF level assessment by ELISA

Peripheral blood was collected from cheek of PBS- or LPS-injected mice and left undisturbed for 30 minutes at room temperature to coagulate. Emerging clot was centrifuged (2300×g, 10 minutes, 4˚C) and supernatant transferred to a new tube and centrifuged again (13000×g, 10 minutes, 4˚C). Supernatant (blood serum) was subsequently snap-frozen in liquid nitrogen. G-CSF levels were assessed by Mouse G-CSF Quantikine ELISA Kit (R&D Systems, Minnesota, MN, USA) following manufacturer‘s instructions.

In vivo* infection with *Candida albicans

Candida albicans (18804, American Type Culture Collection) was plated on Sabourand dextrose agar plates and grown for 24 hours at 37 °C. Before injection, a single colony was cultivated in 5 mL of Sabourand dextrose broth (Sigma-Aldrich, St. Louis, MI, USA) at 37 °C overnight. Mice were monitored daily and moribund animals were sacrificed. Number of CFU was confirmed retrospectively by plating different dilutions of yeast suspension.

Neutrophil isolation

Murine BM was flushed with 3 mL of PBS 0.1% FBS from femur and tibia, and red blood cells were lysed with 2.5 mL of ACK for 2 minutes. Neutrophils were isolated through negative selection using a murine neutrophil isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and MACS separator (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's protocol. Cells were kept in cold conditions during whole procedure.

Reactive oxygen species (ROS) detection

ROS production was assessed by luminol-based chemiluminescence *in vitro*. Isolated neutrophils were resuspended in IMDM (antibiotics free, 0.1% FCS, 100µM luminol, 4 °C) to a final concentration of 1×10^6 cells per mL. 90 µl were plated per well into a pre-chilled black 96-well plate (SPL Life Sciences, Naechon-Myeon, Korea), and placed in a 5% CO_2 incubator for 15 minutes at 37 °C. After incubation, 50 µg (10 µL) of Silica diluted in PBS per well were added and samples were immediately measured on an EnVision plate reader (Perkin Elmer, Waltham, MA) at 37 °C. Wells were scanned every minute for 1 hour.

Phagocytosis assay

Isolated neutrophils (1×10^6 cells in 0.5 mL IMDM 0.1% FCS) were plated in untreated flat-bottom 24 well plate (Eppendorf AG, Hamburg, Germany) and kept on ice for 15 minutes. *E. Coli* (TOP10 strain) was heat-inactivated (60 °C, 30 minutes) and stained with carboxyfluorescein succinimidyl ester (CSFE) dye (30 µM, overnight). *E. coli* was added to each well in a final OD_{600} 0.25, and the plate was immediately moved to a 5% CO2 incubator at 37 °C for 20 minutes. Each well was treated with cold EDTA (0.02% in PBS) for 2 minutes on ice to de-attach adhering neutrophils. Neutrophils were then transferred to pre-chilled

micro-centrifugation tubes and immediately centrifuged (800×g, 2 minutes). Cells were stained with PE-Cy7 conjugated anti Ly6C and Pacific blue conjugated anti Ly6G antibodies in the presence of Fc block (2.4g2 supernatant) (Biolegend, San Diego, CA, USA) for 5 minutes in PBS with 0.1% sodium azide. After washing, stained samples were resuspended in 120 uL of 0.02% Trypan blue (in PBS with sodium azide) to quench fluorescence from non-phagocytosed *E. Coli* and measured on FACS Symphony instrument (BD Biosciences, San Jose, CA, USA).

Migration assays

In vitro migration assays were performed using Corning Costar Transwell cell culture inserts (6.5 mm inserts, 3 μm polycarbonate membrane) (Sigma-Aldrich, St. Louis, Missouri, USA) according to manufacturer's instructions. Briefly, upper wells were coated with 50 μg/mL fibronectin overnight. 1×10^6 murine BM cells were placed in the upper well in 100 μL of migration medium (IMDM 0.5% bovine serum albumin). 600 μL of migration medium with or without 100 ng/mL SDF-1 were placed to the lower well. Plates were incubated for 1 h at 37 ºC, 5% CO_2. Migrated cells, present in the lower well, were harvested and stained with pacific blue conjugated CD11b and AlexaFluor647 conjugated anti Ly6G antibody. Total BM was similarly stained to assess the percentage and number of neutrophils in each assay (input). After staining 30 000 beads (AccuCount fluorescent particles, 7.9 μm, Spherotech, IL, ISA) were added to the tubes and the number of migrated neutrophils was determined using a FACS Symphony instrument (BD Biosciences, San Jose, CA, USA). The percentage of migrated neutrophils (% input) was calculated based on the number of neutrophils present in the input.

Human umbilical cord blood CD34$^+$ cell isolation and differentiation

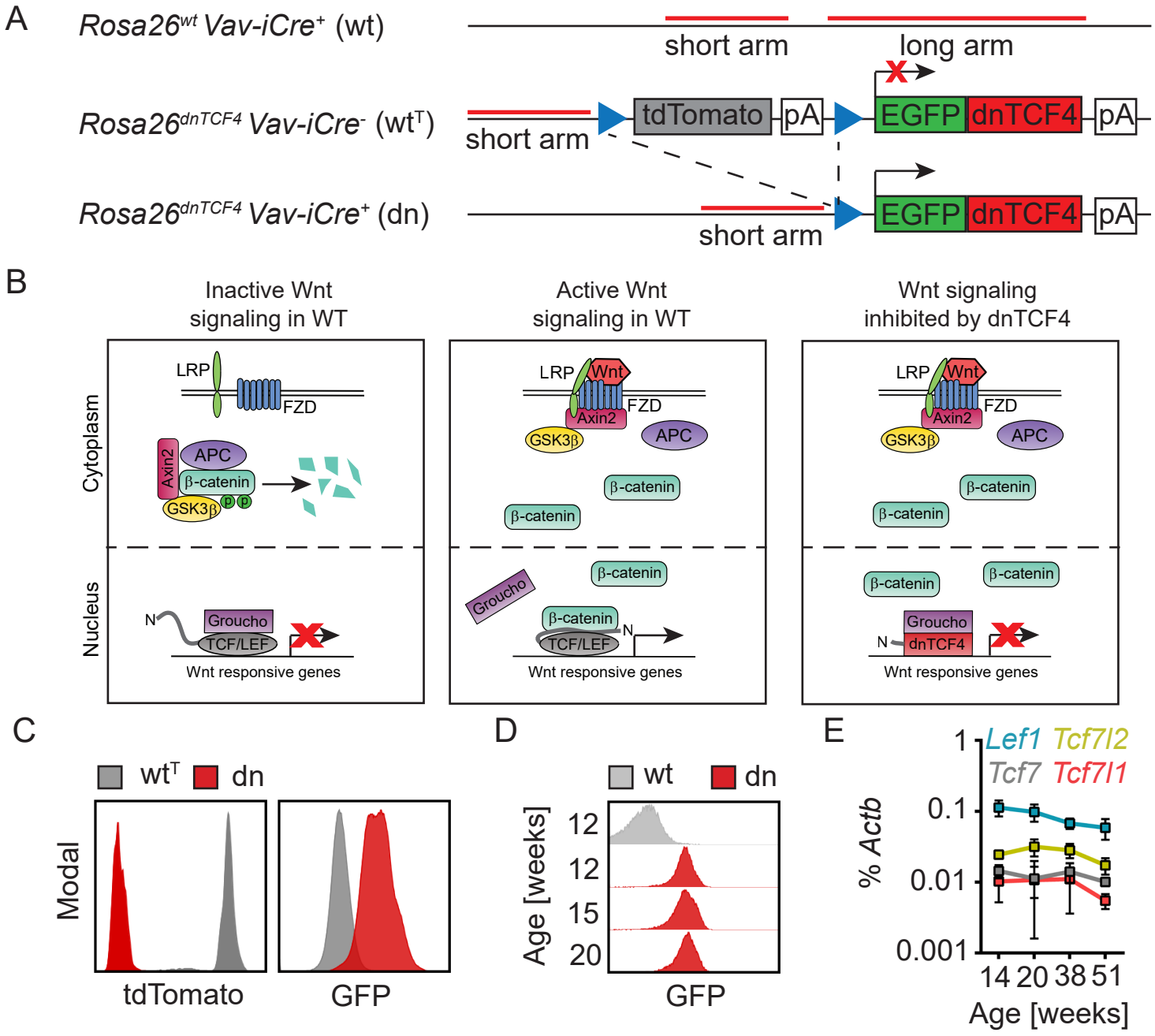
Frozen primary human umbilical cord blood samples were thawed and erythrocytes were lysed with ACK buffer. CD34$^+$ cell fraction was isolated using CD34 Microbead Kit UltraPure (Miltenyi Biotec, Bergisch Gladbach, Germany). Neutrophilic differentiation of human primary CD34$^+$ cells was performed as described previously[7] with minor changes. Briefly, StemSpan SFEM (Stemcell Technologies, Vancouver, Canada) medium was used instead of IMDM with nutrition supplements. *Ex vivo* progenitor expansion medium contained 10% FBS, 100 ng/mL SCF, 50 ng/mL G-CSF, 25 ng/mL IL-3, 100 ng/mL FLT3L, and 20 ng/mL TPO. Neutrophil differentiation medium contained 10% FBS, 100 ng/mL SCF, 100 ng/mL G-CSF, and 100 ng/mL FLT3L. The antibodies used for the analysis of human primary cord blood cells included: CD34 PE-Cy7 (581), CD11b PB (M1/70), CD18 APC (MEM-48), CD66b PE (G10F5), CD16 PB and Alexa Fluor700 (3G8), CD15 V500 (HI98). Antibodies were purchased from BD Biosciences (San Jose, CA, USA), BioLegend (San Diego, CA, USA), and Exbio (Praha, Vestec, Czech Republic). Flow cytometry was performed using a Symphony instrument (BD Biosciences, San Jose, CA, USA). Human samples were collected with written informed consent in accordance to the Declaration of Helsinki and under Ethics committee approval of the Institute of Hematology and Blood Transfusion in Prague, Czech Republic.

Retroviral production and transduction of umbilical cord blood CD34$^+$ cells

Amphotropic retroviral particles (empty control MSCV or dnTCF4-MSCV expressing vectors) were produced with Platinum A cell line (Cell Biolabs, San Diego, CA, USA) and concentrated on Centricon Plus 70 (Milipore, Burlington, MA, USA) columns. 24-well plates were coated with 12 μg/mL human retronectine (Takara BIO, Kusacu, Japan) for 2 hours at

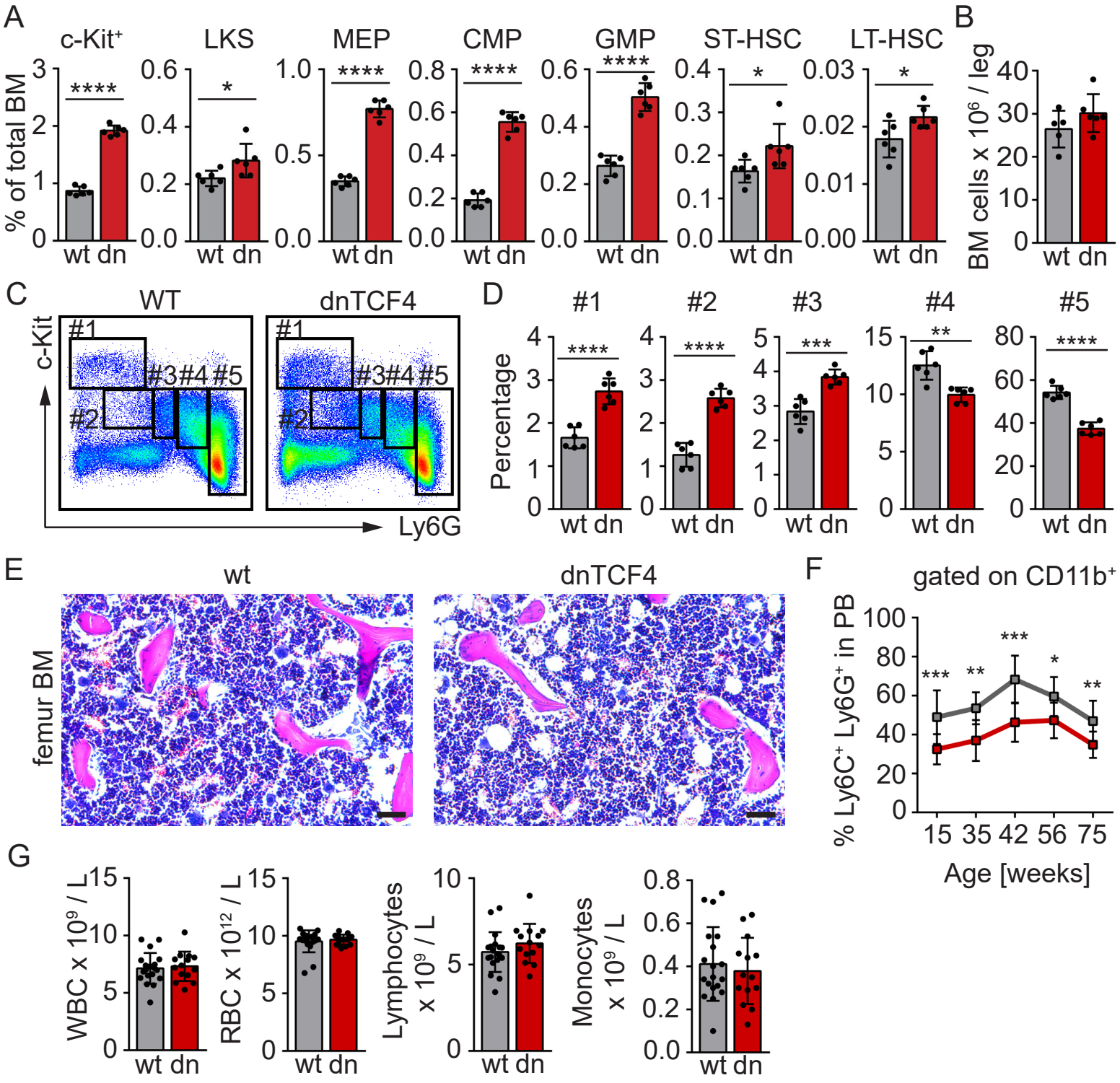
room temperature. Upon retronectine removal, PBS containing 2% BSA was added to the dishes for 30 minutes and removed. Dishes were washes 2x with PBS and coated with retroviral particles for 2 hours at 37 °C in a 5% CO_2 incubator. Upon removal of medium, dishes were carefully washed twice with PBS and purified human $CD34^+$ cells added. Human cells were cultured in StemSpan SFEM medium (Stemcell Technologies, Vancouver, Canada) containing 100 ng/mL SCF, 100 ng/mL TPO, 100 ng/mL FLT3L, 20 ng/mL IL-3, and 20 ng/mL IL-6 (purchased from Peprotech, Rocky Hill, NJ, USA) and subjected to two consecutive infections with MOI (multiplicity of infection) = 20. Transduced cells were expanded for 48 hours and GFP^+ cells were sorted using Influx instrument (BD Biosciences, San Jose, CA, USA).

Supplemental Figure 1



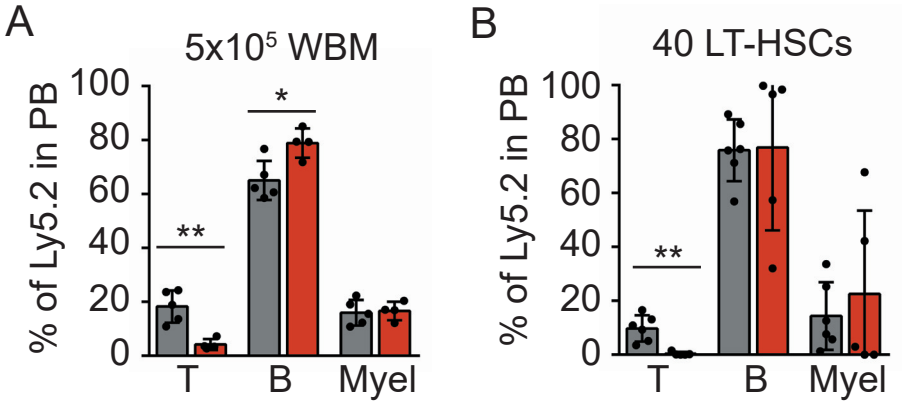
Supplemental Figure 1. Generation and analysis of mice with tissue-specific expression of dnTCF4. (A) Schematic representation of *Rosa26* locus in mouse models employed. *Rosa26*^{wt} *Vav-iCre*$^{+}$ mice (upper panel) and *Rosa26*^{dnTCF4} *Vav-iCre*$^{-}$ mice (middle panel) were used as WT control and referred as WT and WT^{T}, respectively. Blue triangles indicate LoxP sites, flanking the transcriptional blocker which includes a *tdTomato* cDNA and the polyadenylation signal (pA). In the presence of Cre recombinase (*Rosa26*^{dnTCF4} *Vav-iCre*$^{+}$ mice, referred as dn) the floxed region was excised and expression of dnTCF4 permitted (lower panel). (B) Schematic description of the dnTCF4-mediated inhibition of canonical Wnt signaling pathway. When canonical Wnt signaling is not active (left panel), β-catenin docks to β-catenin destruction complex (composed of Axin2, APC, and other proteins), where it receives GSK3β-mediated phosphorylation that targets it for proteasomal degradation. Meanwhile, TCF/LEF transcription factors associate with repressors (Groucho) and prohibit transcription of Wnt responsive genes. Binding of Wnt ligand to Frizzled receptor (FZD) (middle panel) results in disassembly of β-catenin destruction complex and consequent accumulation and nuclear translocation of unphosphorylated (stable) β-catenin. This allows β-catenin to bind to TCF/LEF factors, releasing repressors and activating transcription of Wnt responsive genes. However, the β-catenin-mediated transcription of Wnt responsive genes can be inhibited by dnTCF4 (right panel). dnTCF4 occupies regulatory regions of Wnt responsive genes and associates with transcriptional repressors. Since dnTCF4 lacks N-terminal β-catenin binding domain, even stable nuclear β-catenin can not interact and release repressors from dnTCF4. Thus, the transcription of Wnt responsive genes remains constantly inhibited in dnTCF4-expressing cells. (C) Flow cytometry histograms illustrating the expression levels of tdTomato (left) and GFP (right) in WT^{T} and dnTCF4 expressing BM cells. (D) Flow cytometry histograms illustrating constant EGFP-dnTCF4 levels in dnTCF4 BM during aging. (E) Expression of *Lef1* (Lef1), *Tcf7* (Tcf1), *Tcf7L1* (Tcf3), and *Tcf7L2* (Tcf4) genes relative to *Actb* control in WT BM during aging assessed by RT-PCR (n = 2-4). Data represent mean ±s.d.

Supplemental Figure 2



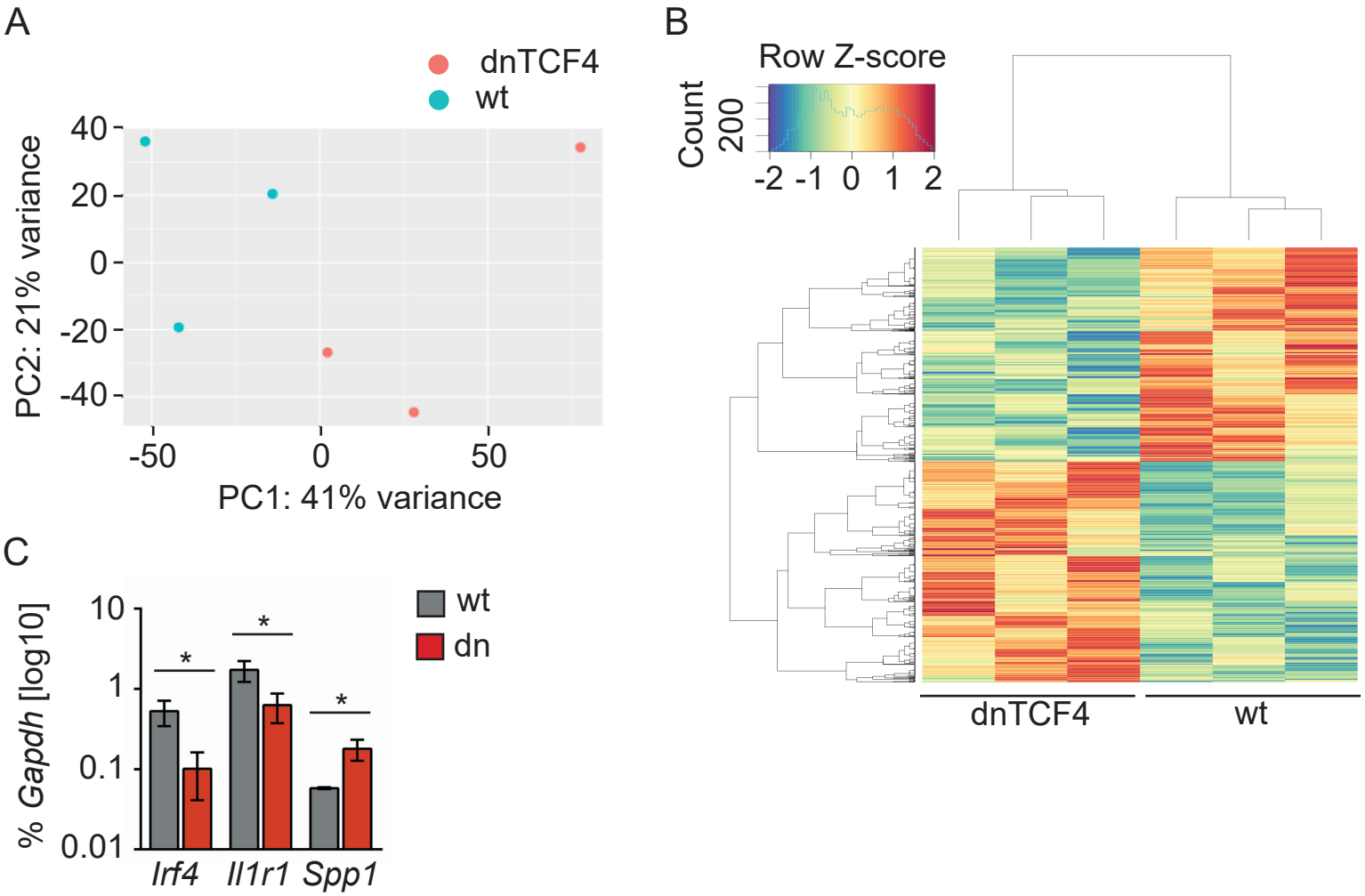
Supplemental Figure 2. Analysis of mice with tissue-specific expression of dnTCF4. (A) Frequencies of c-Kit$^+$ (Lin$^-$ c-Kit$^+$ Sca-1$^-$), LKS (Lin$^-$ c-Kit$^+$ Sca-1$^+$), MEP (Lin$^-$ c-kit$^+$ Sca-1$^-$ CD34$^-$ FcgRII/III$^-$), CMP (Lin$^-$ c-kit$^+$ Sca-1$^-$ CD34$^+$ FcgRII/III^{lo}), GMP (Lin$^-$ c-kit$^+$ Sca-1$^-$ CD34$^+$ FcgRII/III^{hi}), ST-HSC (Lin$^-$ c-kit$^+$ Sca-1$^+$ CD48$^+$ CD150$^-$), and LT-HSC (Lin$^-$ c-kit$^+$ Sca-1$^-$ CD48$^-$ CD150$^+$) in BM of 15-week-old WT and dnTCF4 mice (n = 6). (B) BM cellularity of 15-week-old WT and dnTCF4 animals. Y axis indicates number of cells per hind leg. (C) Representative flow cytometry plots showing neutrophilic development based on c-Kit and Ly6G expression in BM of 15-week-old WT and dnTCF4 mice. Boxes indicate populations at distinct stages of development, ranging from the most immature population (#1) to mature neutrophils (population #5). (D) Quantification of panel C. Y axes indicate percentage of parental gate (CD3$^-$ B220$^-$ Ter119$^-$ SSC^{int}) as described by Satake et al[2]. n=6 mice. (E) BM histology of WT (left picture) and dnTCF4 (right picture) femurs. Sections were stained with hematoxilin-eosin. Scale bar (black) represents 50 µm. (F) Percentage of neutrophils in PB of WT^r (gray line) and dnTCF4 (red line) mice during aging. X axis represents age of mice in weeks. Y axis indicates the percentage of Ly6G$^+$ and Ly6C$^+$ in CD11b$^+$ population. n=6 mice. (G) Number of white blood cells (WBC), red blood cells (RBC), lymphocytes, and monocytes per liter in PB of 15-week old WT and dnTCF4 mice based on Auto Hematology Analyzer. At least 14 animals were included in each group. In this figure, data represent mean ±s.d. Each symbol (dot) represents one animal. Two-tailed Student's t-test was used to assess statistical significance of all data shown (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001).

Supplemental Figure 3



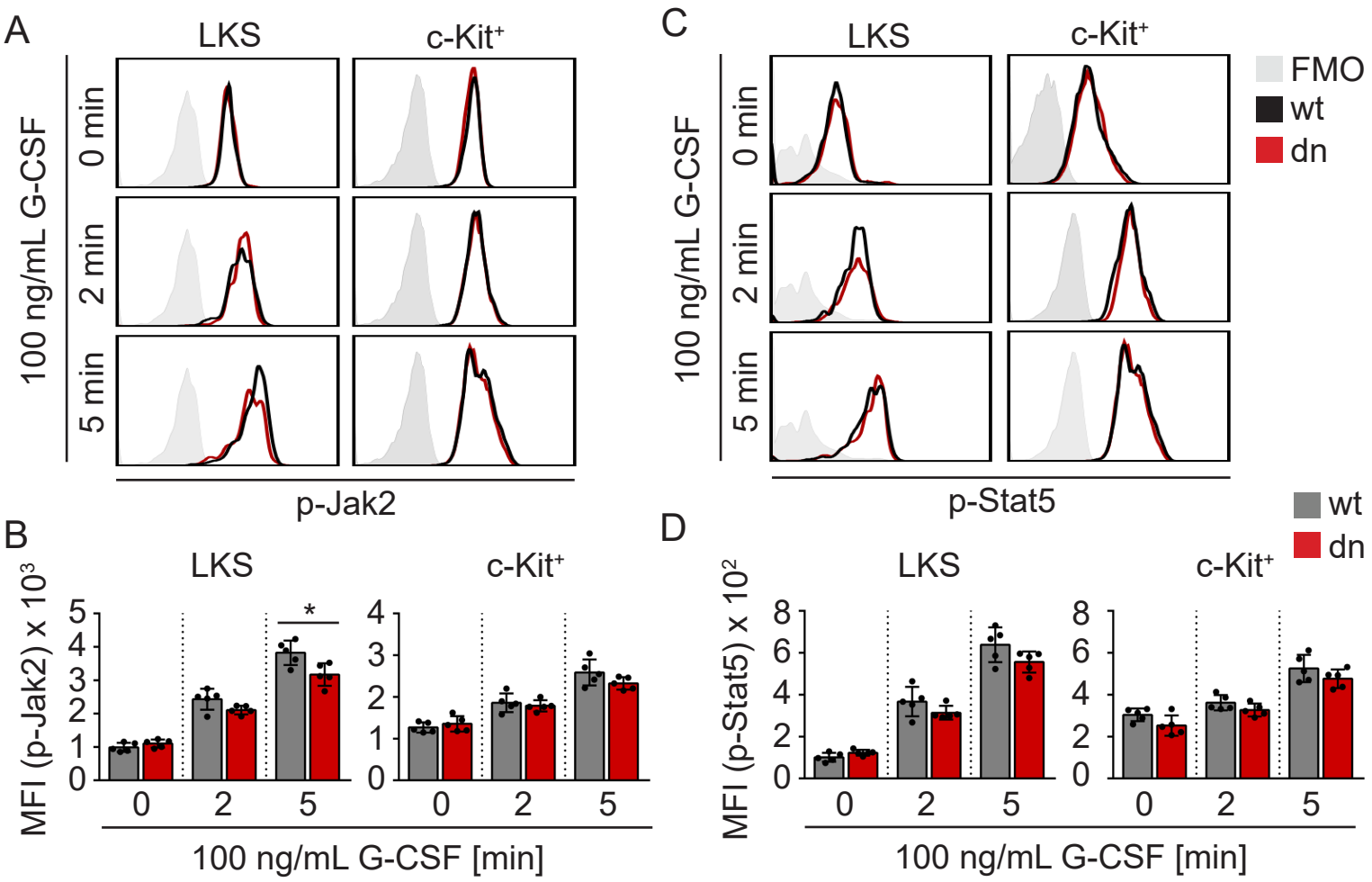
Supplemental Figure 3. Tri-lineage reconstitution upon transplantation. (A) Quantification of flow cytometric data. Y axis indicates the percentage of donor derived Ly5.2$^+$ T cells ('T', defined as CD3$^+$), B cells ('B', defined as B220$^+$), and myeloid cells ('Myel', defined as Gr1$^+$ CD11b$^+$) in BM. Recipient mice received 5×10^5 WT BM (gray) or dnTCF4 (red) BM along with 5×10^5 competitive (Ly5.1$^+$) BM cells. (B) Quantification of flow cytometric data. Y axis indicates the percentage of donor derived Ly5.2$^+$ T cells ('T', defined as CD3$^+$), B cells ('B', defined as B220$^+$), and myeloid cells ('Myel', defined as Gr1$^+$ CD11b$^+$) in BM. Recipient mice received 40 sorted LT-HSC isolated from WT (gray) or dnTCF4 (red) mice.

Supplemental Figure 4



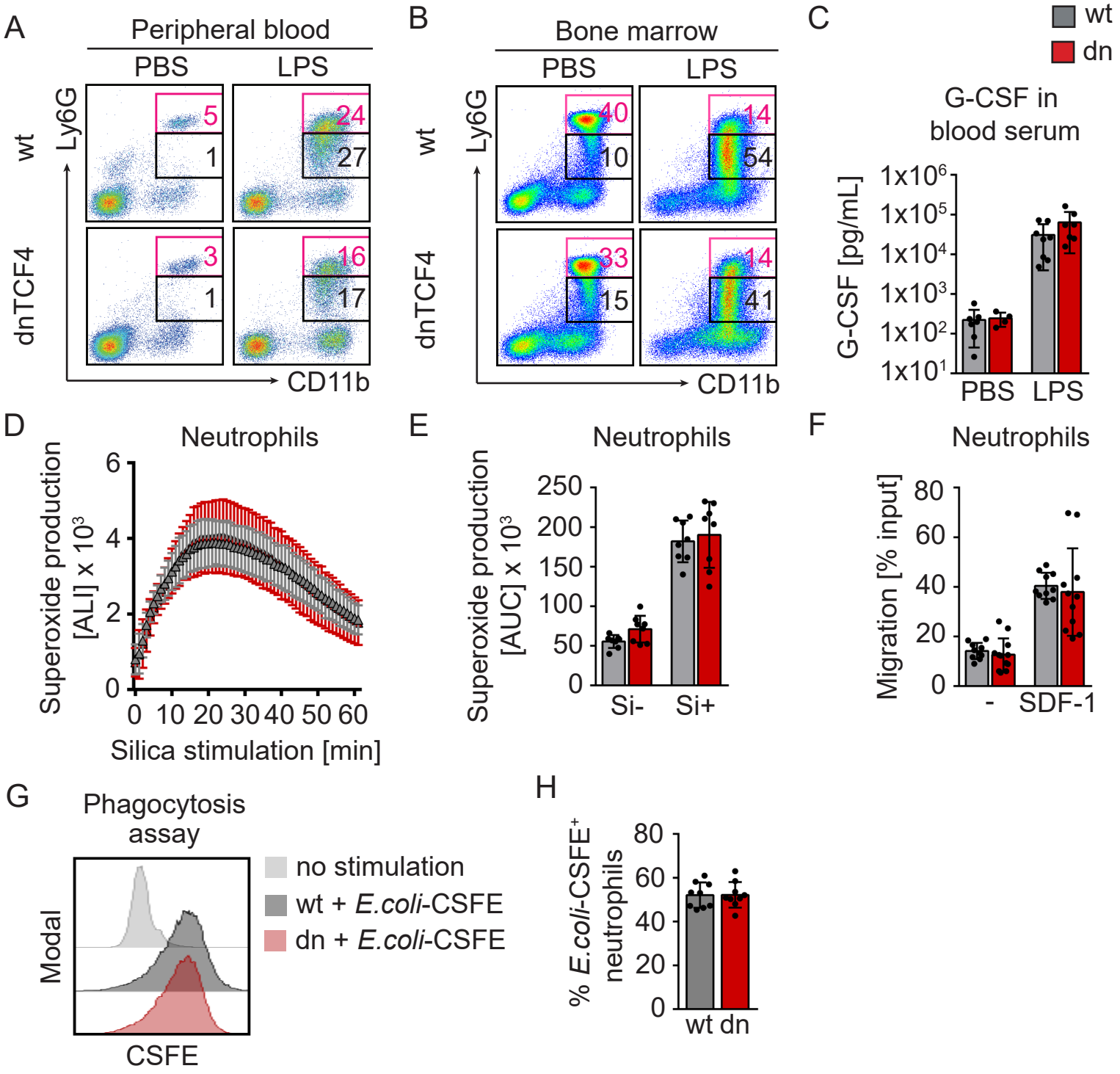
Supplemental Figure 4. RNAseq analysis and quantitative RT-PCR of ST-HSCs isolated from WT and dnTCF4 mice. (A) Principal component analysis (PCA) plot of 3 WT (blue) and 3 dnTCF4 (red) samples. (B) Heat map visualization of unsupervised hierarchical clustering of genes differentially expressed in dnTCF4 versus WT ST-HSCs ($p<0.05$, log2 fold change >0.5). Data were normalized to z-scores for each gene. Red/blue color indicates increase/decrease in gene expression relative to the universal mean for each gene. (C) Quantitative RT-PCR in ST-HSC isolated from WT (gray) and dnTCF4 (red) mice. Y axis indicates expression of *Irf4*, *Il1r1*, and *Spp1* relative to *Gapdh*. Data represent mean ±s.d., n=3 mice. Two-tailed Student's t-test was used to assess statistical significance (*$p<0.05$).

Supplemental Figure 5



Supplemental Figure 5. Analysis of Jak2/Stat5 phosphorylation in WT and dnTCF4 mice. (A) Representative histograms illustrating Jak2 phosphorylation level upon stimulation of WT (black) and dnTCF4 (red) c-Kit$^+$ and LKS cells with G-CSF (100 ng/mL) measured by flow cytometry. (B) Quantification of panel A. X axes represent mean fluorescence intensity (MFI) of phosphorylated Jak2 (p-Jak2). Data represent mean ±s.d., n=5, each black dot symbol represents value for one animal. (C) Representative histograms ilustrating Stat5 phosphorylation level upon stimulation of WT (black) and dnTCF4 (red) c-Kit$^+$ and LKS cells with G-CSF (100 ng/mL) measured by flow cytometry. (D) Quantification of panel C. X axes represent mean fluorescence intensity (MFI) of phosphorylated Stat5 (p-Stat5). Data represent mean ±s.d., n=5, each black dot symbol represents value for one animal. Two-tailed Student's t-test was used to assess statistical significance (*$p<0.05$)

Supplemental Figure 6



Supplemental Figure 6. Impaired emergency granulopoiesis in dnTCF4 mice. (A-B) Representative flow cytometry dot plots of peripheral blood (A) and bone marow (B) isolated from WT and dnTCF4 mice treated with PBS or LPS. Expression of cell surface markers Ly6G and CD11b is shown. Pink box indicates the percentage of $CD11b^{+}$ $Ly6G^{hi}$ mature neutrophils and black boxes indicate the percentage of $CD11b^{+}$ $Ly6G^{lo}$ immature neutrophils. (C) Levels of G-CSF determined by ELISA assay in blood serum of WT and dnTCF4 mice injected intraperitoneally with PBS or LPS. (D) Superoxide production of silica-stimulated WT (gray curve) and dnTCF4 (red curve) purified neutrophils assessed by luminol-based chemiluminiscence assay. X axis represents arbitrary luminiscence intensity units (ALI). X axis indicates the duration of silica stimulation. Graph depicts mean ±s.d. for each minute of the treatment and shows data from 3 independent experiments (n=8). (E) Quantification of panel D. X axis represents area under the curve (AUC) of unstimulated (Si-) or stimulated (Si+) WT (gray bar) and dnTCF4 (red bar) neutrophils. Data represent mean ±s.d. (n=8). (F) In vitro migration of WT (gray bar) and dnTCF4 (red bar) neutrophils ($CD11b^{+}$ $Ly6G^{+}$) towards 100 ng/mL of SDF-1. Y axis indicates migrated neutrophils as percentage of input. Graph shows mean ±s.d. from 3 independent experiments. Each group contains 10-12 samples, each of them indicated by a black dot. (G) Representative histograms showing the ability of WT and dnTCF4 purified neutrophils to phagocytose heat-inactivated CSFE-stained *E.coli* during 20 min of co-culture. X axis represents intensity of CSFE fluorescence in neutrophils. (H) Quantification of panel G. Y axis indicates percentage of $CSFE^{+}$ neutrophils. Graph shows mean ±s.d. from 3 independent experiments, n=9, each animal represented by a black dot symbol. Two-tailed Student's t-test was used to assess statistical significance.

SUPPLEMENTAL REFERENCES

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