SUPPLEMENTARY APPENDIX

The lifespan quantitative trait locus gene *Securin* controls hematopoietic progenitor cell function

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Supplementary data

Supplementary figure legends

Suppl Fig 1: SNP analysis for B6, line A, D2 and line K mice from *Fig 1D* showing all chromosomes.

Suppl Fig 2: The chromosome 11 locus controls sensitivity of HSPCs to HU exposure but not HSPC frequency, cell cycle activity, apoptosis and replication fork stalling.

(A) LDBM cells from all four strains were treated with 200 µg/ml HU or its solvent for 1 h and additionally with BrdU for the last 30 m. Thereafter, the number of LK cells which incorporated BrdU was measured by flow cytometry. Left panel: Representative FACS plots. Right panel: Quantification. n=3. **(B)** Total BM cells were isolated from the indicated mouse strains, treated with HU or its solvent following CAFC assay. At day 7 the cobblestone frequency of progenitors sensitive to HU treatment was measured. n=4-5. (**C**) Lin-cKit+ or Lin-cKit- cells freshly isolated from B6 mice were plated in serial dilutions on FBMD-1 feeder layers. At day 7 and 14 cobblestones were counted and the frequency of cells to form colonies was calculated. n=2. (**D**) Telomere length in kMESF of HSPCs isolated from the four mouse strains. n=3. (**E**) HSPCs from all four mouse strains were treated with 200 µg/ml HU or PBS for 1 h following analysis of *p16* expression by RT-PCR. n=3. (**F**) Total (left panel) or S-phase specific (right panel) AnnexinV+ rates of HSCs, LSKs an LK cells within the four mouse strains which were BrdU injected prior to analysis. n=4. (**G**) Upper panel: Schematic illustration of the experiment. LDBM cells from all four mouse strains were treated with either HU for 1 h or the corresponding solvent (PBS). Thereafter HU was removed and all samples were incubated for 15.5 h. Then the second sample was treated with HU whereas the third sample was treated with PBS. All samples were then treated with BrdU for 30 m following analysis of cell cycle

distribution and apoptosis. n=3. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. BM = Bone marrow, HU = Hydroxyurea, LK = Lin-cKit+, LSKs = Lin-cKit+Sca1+ cells, HSCs = Hematopoietic stem cells, HSPCs = Hematopoietic stem and progenitor cell

Suppl Fig 3: Chromosome 11 associated *Pttg1* has an altered promotor sequence in D2/A mice leading to enhanced expression

(**A**) Upper panel: Schematic illustration of the *Pttg1* locus showing its exons, an 1,500 bp part of the corresponding promotor and all D2-specific SNPs. Lower panel: Comparison of the B6 and D2 transcriptional start regions as well as the predicted binding sites of transcription factors. (**B**) Agarose gel electrophoresis comparing *Pttg1* promotor regions from our four mouse strains. (**C**) Sequencing results showing the promotor and the region between the transcriptional and the ORF start of *Pttg1* within B6/K and D2/A. The asterisks indicate the 5' and 3' ends of the promotor fragments used for the luciferase assays. ORF = Opening reading frame

Suppl Fig 4: *Pttg1* promotes HU sensitivity of HSPCs

(**A**) PTTG1 *in silico* protein model showing B6- and D2-PTTG1. The arrow indicates the modest increase in 3_{10} helices within the D2 variant. (**B**) GFP+ chimerism in peripheral blood of mice transplanted with cells expressing a control (*Egfp*) or *Pttg1-Egfp* 4 weeks post transplantation. 12-19 mice per group. (**C**) Representative FACS plots showing GFP+ cells (total LDBM cells vs LK cells) in BM 5 weeks after transplantation isolated from mice transplanted with lentiviruses mediating *Egfp* (control) or *Pttg1-Egfp* (PTTG1 OE) expression. (**D**) Left panel: RT-PCR analysis of *Pttg1* upon knockdown in HSPCs from B6 mice. n=3. Right panel: HU sensitivity rates in HSPCs from D2 and line A mice upon knockdown of *Pttg1* and transplantation. n=3. Cells from line A and D2 mice (each 3 sets of mice) were used and transplanted into the corresponding B6 or D2 recipients. ***P<0.001; ****P<0.0001. HU =

Hydroxyurea, HSPC = Hematopoietic stem and progenitor cell, BM = Bone marrow, LDBM = Low density bone marrow, LK = Lin-cKit+ cells, OE = Overexpression

Suppl Table 1A:

% HU responses and mean life spans of all BXD and parental strains used for QTL mapping

Suppl Table 1B:

WebQTL analysis of the HU responses and mean life spans

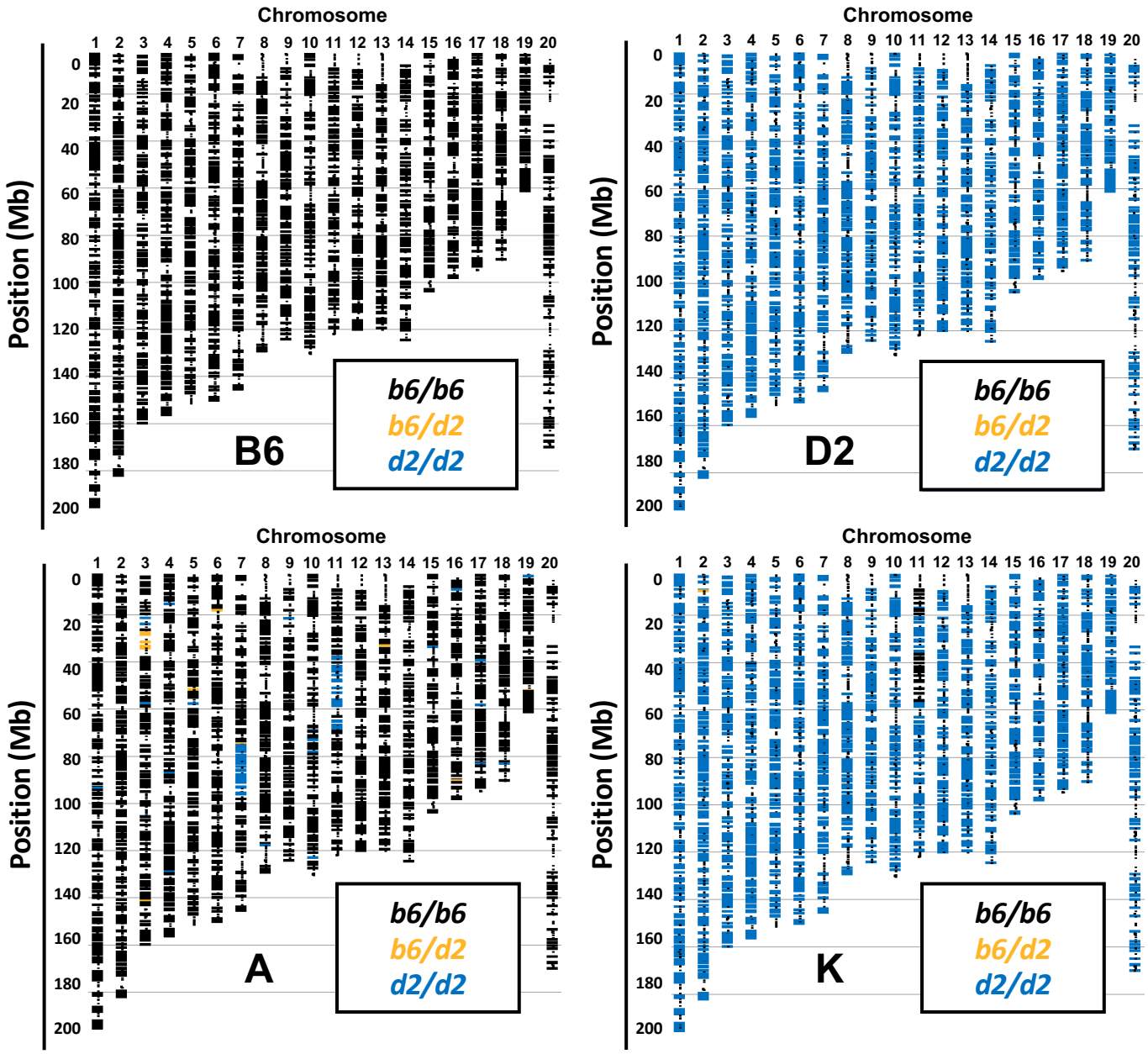
Suppl Table 2:

Complete list of SNP data for all analyzed strains. Indicated is the 18.6 Mb spanning region of chromosome 11.

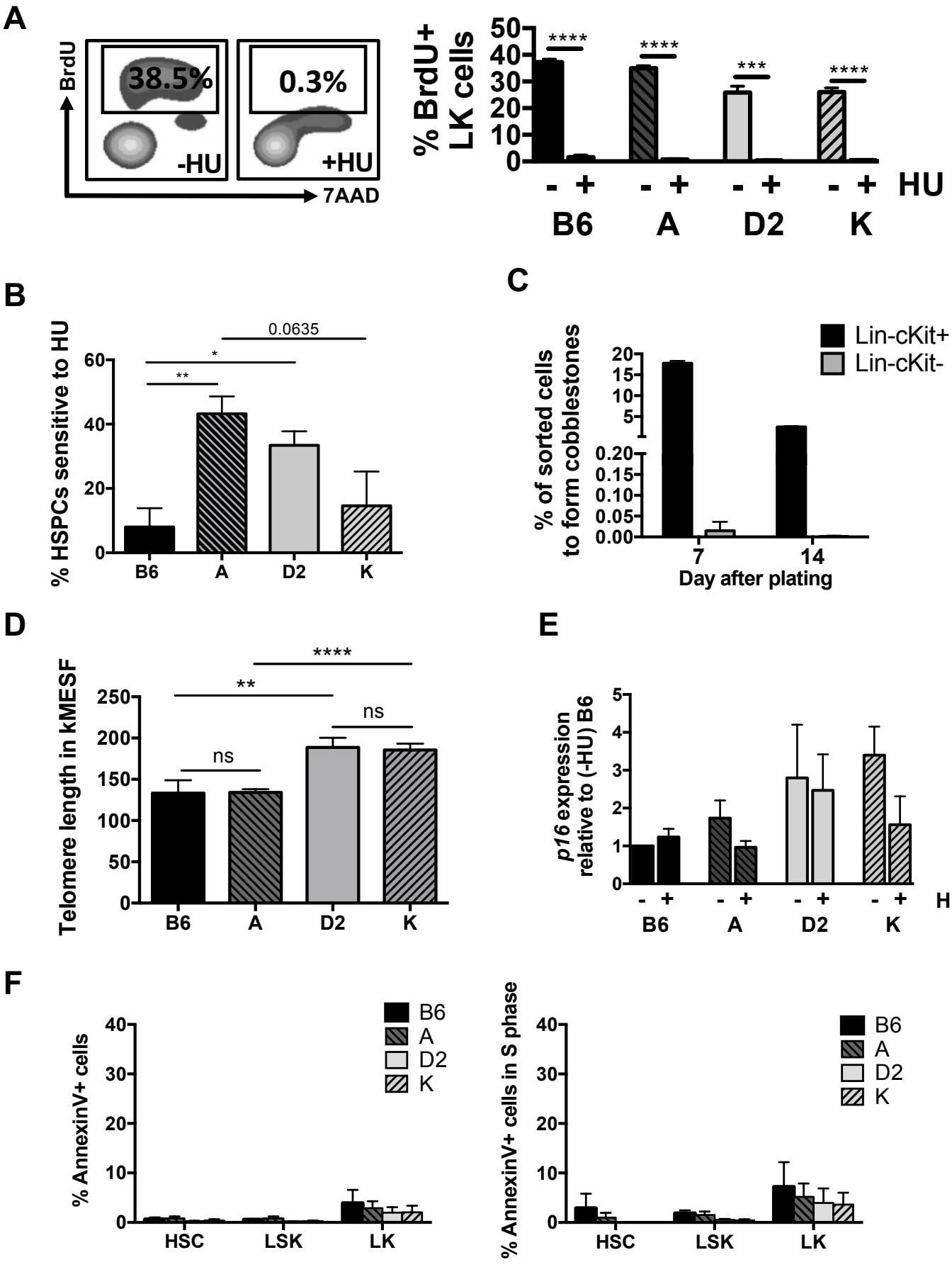
Suppl Table 3:

List of all 130 genes located on the proximal chromosome 11 locus. Indicated in brackets is the reading orientation.

Suppl Fig 1

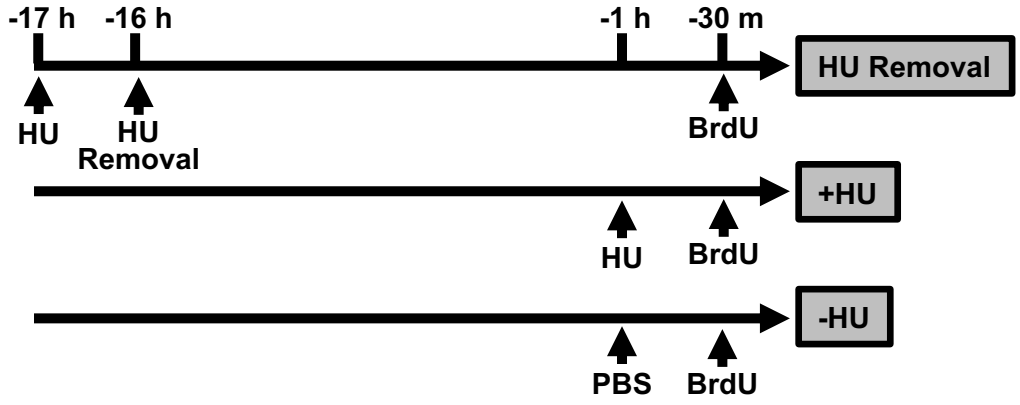

Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Position (Mb)
0 20 40 60 80 100 120 140 160 180 200
B6
b6/b6
b6/d2
d2/d2
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Position (Mb)
0 20 40 60 80 100 120 140 160 180 200
D2
b6/b6
b6/d2
d2/d2
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Position (Mb)
0 20 40 60 80 100 120 140 160 180 200
A
b6/b6
b6/d2
d2/d2
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Position (Mb)
0 20 40 60 80 100 120 140 160 180 200
K
b6/b6
b6/d2
d2/d2

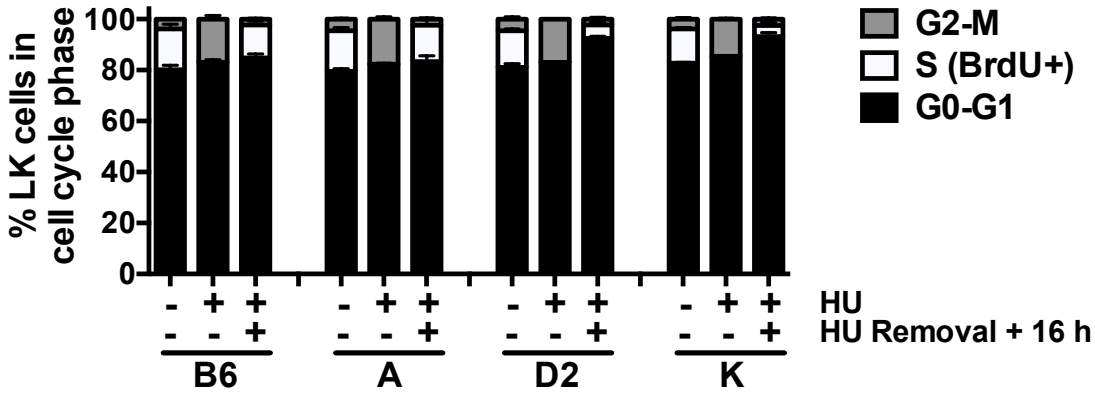
Suppl Fig 2

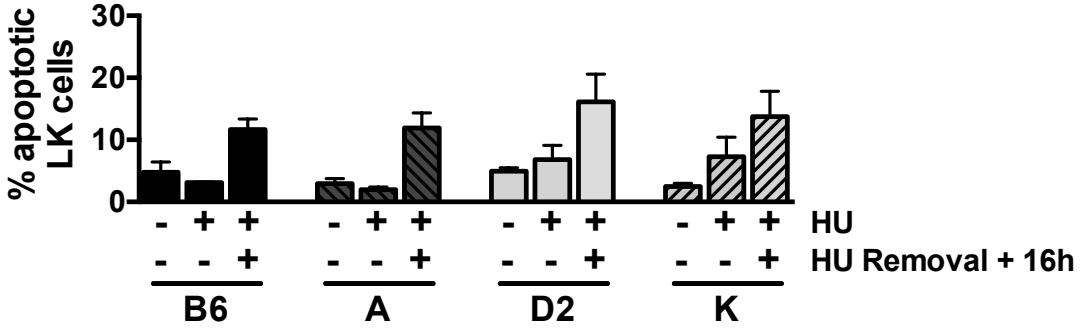

A
BrdU
38.5%
-HU
0.3%
+HU
7AAD
% BrdU+ LK cells
50
40
30
20
10
0
****
****
***
****
- + - + - + - + HU
B6 A D2 K
B
% HSPCs sensitive to HU
60
40
20
0
0.0635
*
**
B6 A D2 K
C
% of sorted cells to form cobblestones
Lin-cKit+
Lin-cKit-
20
15
10
5
0.20
0.15
0.10
0.05
0.00
7 14
Day after plating
D
Telomere length in kMESF
250
200
150
100
50
0
****
**
ns
ns
B6 A D2 K
E
p16 expression relative to (-HU) B6
5
4
3
2
1
0
- + - + - + - + HU
B6 A D2 K
F
% AnnexinV+ cells
40
30
20
10
0
B6
A
D2
K
HSC LSK LK
% AnnexinV+ cells in S phase
40
30
20
10
0
B6
A
D2
K
HSC LSK LK

Suppl Fig 2 (Continued)

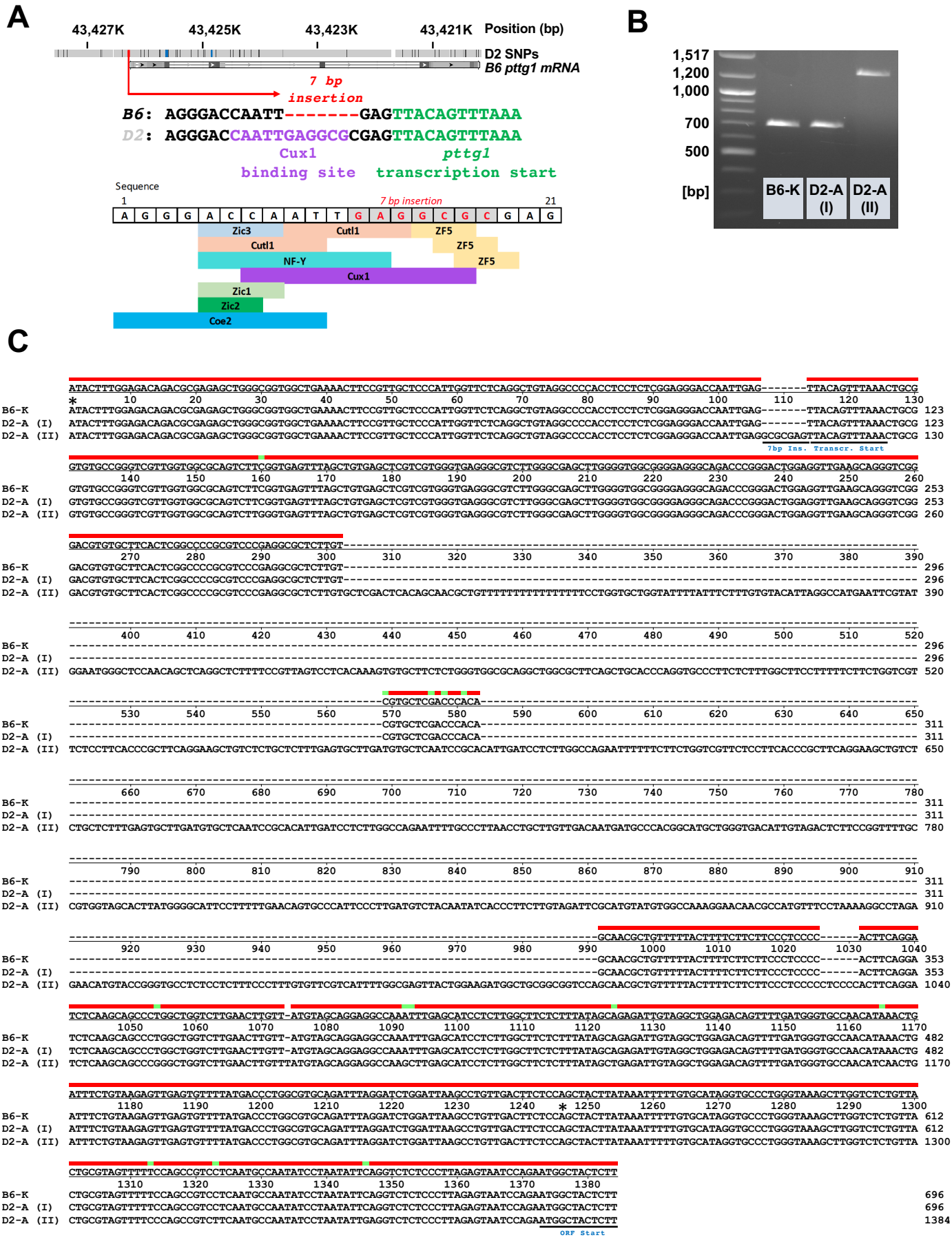
G



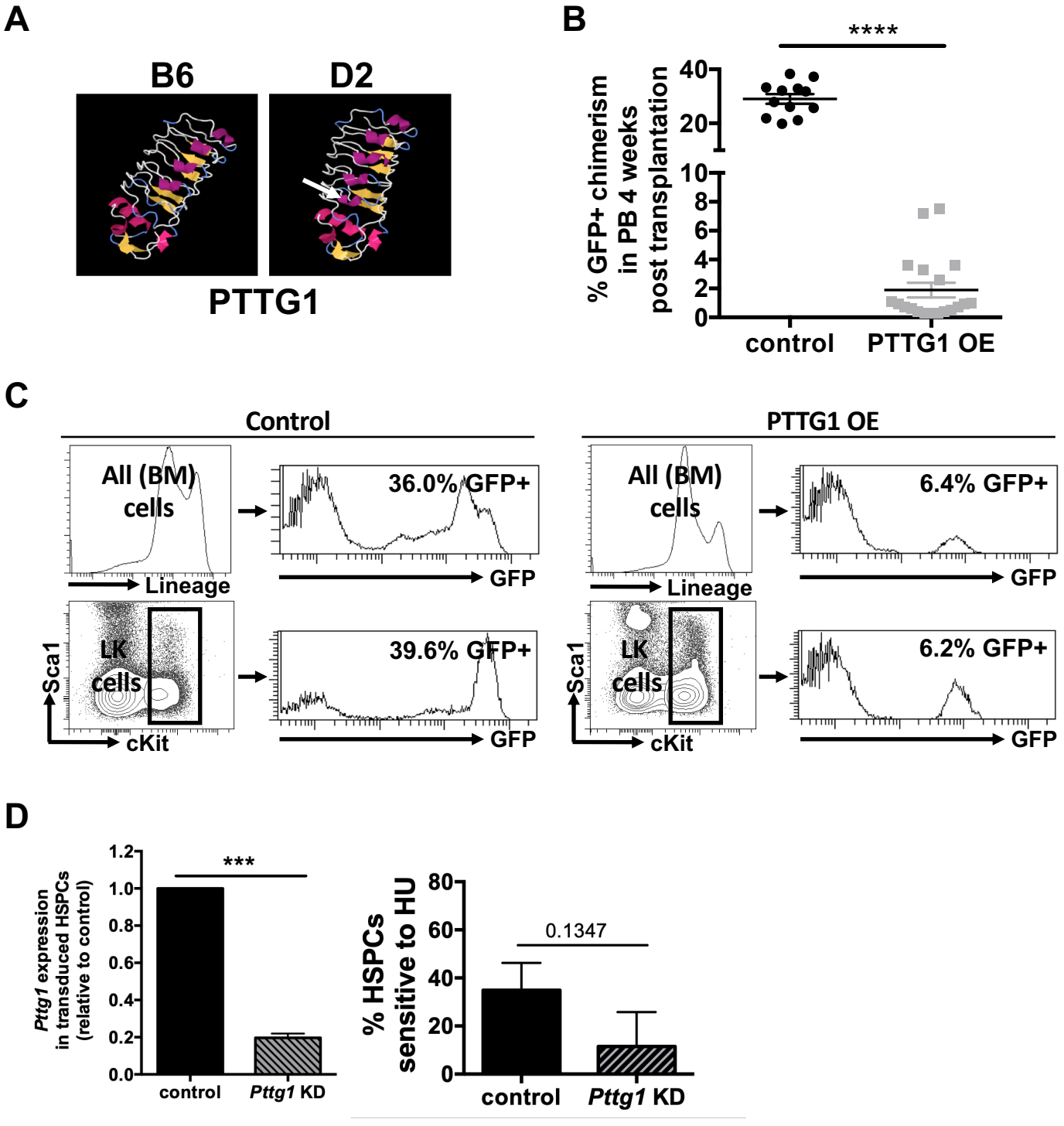




Suppl Fig 3


A
43,427K
43,425K
43,423K
43,421K
Position (bp)
D2 SNPs
B6 pttg1 mRNA
7 bp
insertion
B6: AGGGACCAATT-------GAGTTACAGTTTAAA
D2: AGGGACCAATTGAGGCGCGAGTTACAGTTTAAA
Cux1
binding site
pttg1
transcription start
Sequence
1
7 bp insertion
21
A G G G A C C A A T T G A G G C G A G T T A C A G T T T A A A
Zic3
Cutl1
ZF5
Cutl1
ZF5
NF-Y
ZF5
Cux1
Zic1
Zic2
Coe2
B
1,517
1,200
1,000
700
500
[bp]
B6-K
D2-A (I)
D2-A (II)
C
B6-K
D2-A (I)
D2-A (II)
7bp Ins.
Transcr. Start
ORF Start

Suppl Fig 4


A
B6
D2
PTTG1
B
****
% GFP+ chimerism in PB 4 weeks post transplantation
40
20
10
8
6
4
2
0
control
PTTG1 OE
C
Control
PTTG1 OE
All (BM) cells
Lineage
36.0% GFP+
GFP
LK cells
Sca1
cKit
39.6% GFP+
6.4% GFP+
6.2% GFP+
D
***
Pttg1 expression in transduced HSPCs (relative to control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
control
Pttg1 KD
% HSPCs sensitive to HU
80
60
40
20
0
0.1347

Supplementary methods

Genotyping of congenic mice

Congenic mice were generated as described in the main section. Offspring from line A was PCR analyzed with primers D11MIT177_F (GTAATGGTTATCACAGGAAGTTTGG) and D11MIT177_R (ACCCAGTCTGCAAACAT). The appearance of a 114 bp band indicated B6 mice congenic for the corresponding D2-locus, whereas a 124 bp band was B6-derived. Line K offspring was analyzed with primers D11MIT174_F (GGAAGGCATCCATGTTTGG) and D11MIT174_R (GGTAAGCCATTTGTAAACTGTGG). D2/B6 congenic mice corresponded to the appearance of a band at 147 bp, whereas non-congenic mice showed a band at 165 bp.

Cell culture

NIH/3T3 mouse fibroblasts (ATCC) were cultured in DMEM and HEK-293 cells (ATCC) in IMDM (ThermoFisher) supplemented with 10% FBS and antibiotics (#P11-010, ThermoFisher) at 37 °C and 5 % CO_2. FBMD-1 cells were kindly gifted by José Cancelas and cultured at 33 °C and 5 % CO_2 in IMDM containing L-glutamine (1/100 GlutaMAX, Gibco), 5% horse serum (Sigma Aldrich), 10% fetal bovine serum (sera from Gibco), 10^{-4} mol/L β-mercaptoethanol, 10^{-5} mol/L hydrocortisone (Sigma, St Louis, MO), 80 U/mL penicillin and 80 µg/mL streptomycin (both from Gibco). BM or LDBM cells were incubated in IMDM with 10% FBS and antibiotics.

Cell cycle and apoptosis staining

Mice were injected i.p. with 200 µl 2.5 mg/ml BrdU (#559619, BD Pharmigen) for 45 m prior to analysis. For apoptosis and cell-cycle analyses, $2*10^6$ LDBM cells were stained with a cocktail of biotinylated lineage antibodies (CD5, B220, Mac-1, CD8a, Gr-1, Ter-119, BD) after Fc block (#553142, BD) for 15 m. Cells were washed once and stained with the following

antibodies from eBioscience: Streptavidin APC-Cy7, anti-c-Kit-Alexa 700 (clone ACK2), anti-CD34 APC (RAM34), and anti-Sca1 PE-Cy7 (D7) for 1 h on ice. For identification of apoptotic cells, the antibody stained cells were washed and incubated in Annexin V Binding Buffer (#556454, BD) containing Annexin V (#560506, BD) for 20 m at RT and analyzed by flow cytometry. For cell cycle analysis, antibody stained cells were fixed and permeabilized using Cytofix/Cytoperm buffer (#554722, BD). Cells were again permeabilized the next day using Cytofix Buffer Plus (#561651, BD) and Cytofix/Cytoperm buffer (BD). Cells were then treated with 30 µg/µl DNase (#D4513, Sigma Aldrich) in PBS with Ca^{2+}/Mg^{2+} for 1.5 h at 37 °C and after washing incubated with anti-BrdU antibody (#559619, BD) for 20 m at RT. Directly before analysis with a LSRII flow cytometer (BD), cells were resuspended in PBS and 7AAD (#559925, BD) was added. HSCs were defined as Lin-cKit+Sca-1+CD34-, LSK represented Lin-cKit+Sca1+cells, and hematopoietic progenitor cells were gated Lin-cKit+Sca1-. The data acquisition and analysis were performed using BD FACS DIVA.

Plasmids and cloning of Pttg1

Expression vector SF-LV-cDNA-EGFP and packaging plasmids pMD2.G/pxPAX2 as described previously were kindly provided by Lenhard Rudolph.[1,2] Murine *Pttg1* ORF was PCR-cloned from a B6 cDNA library and transferred into SF-LV-cDNA-EGFP vector with *XhoI* and *NotI* using the following primers:

5'XHOI_MPTTG1: ATAATCTCGAGATGGCTACTCTTATCTTTG

3'NOTI_MPTTG1: GATATGCGGCCGCTTAAATATCTGCATCGTAACAA

Cloning success was confirmed by restriction analysis and DNA sequencing.

Generation of lentiviral particles

Lentiviruses were generated with the calcium phosphate transfection method as described previously[3] with HEK-293 cells (#632180, Clontech) using the Calphos Mammalian

Transfection Kit (#062013, Clontech). The absolute ratio of SF-LV-cDNA-EGFP or shRNA pGFP-C-shLenti, pxPAX2 and pMD2.G was 3:2:1. After 24 h and 48 h, raw viral supernatants were harvested, filtered (0.45 μm), and concentrated for 2 h with 25 000 rpm at 4 °C. Infectious titers were determined on NIH/3T3 or 293T cells performing titration. Multiplicity of infection (MOI) of 1 was set when 50 % of NIH/3T3 cells are GFP+. For generation of *Pttg1* knockdown particles, PTTG1 Mouse shRNA pGFP-C-shLenti (#TL502795, OriGene Technologies) was used.

Transduction and transplantation

Lin- cells isolated from B6 mice as described[4] were seeded on Retronectin (Takara, Japan) coated 24 well plates using IMDM supplemented with 100 ng/ml mG-CSF, mTPO and mSCF (Prospec). After 24 h cells were transduced for 6-8 h with lentiviruses coding for E*gfp* (control), *Pttg1-Egfp* or *Pttg1*-shLenti using a MOI of 15-25. The next day cells were harvested with Cell Dissociation Reagent (#07174, STEMCELL Technologies), washed, resuspended in PBS and transplanted. For transplantation between 2.0 and $5.0*10^5$ B6 Lin- cells were tail-injected into lethally irradiated (7+4 Gy) B6 or D2 recipient mice. After 4 weeks, GFP+ chimerism in peripheral blood was analyzed using anti-CD3e (clone 145-2C11), anti-B220 (clone RA3-6B2), anti-Mac-1 (clone M1/70) and anti-Gr-1 (clone RC57BL/6-8C5) antibodies from eBioscience. 5-6 weeks after transplantation mice were sacrificed and GFP+/APC- cells were sorted using a BD Aria II/III device.

RT-PCR

Total RNA was extracted from cells using the Qiagen Micro RNA Kit and cDNA was synthesized using 500 ng of total RNA and the QuantiTect Reverse Transcription Kit (#205310 , Qiagen) according to the supplier's protocols. Briefly, 2 μl of gDNA Wipeout buffer and 500 ng of total RNA were added to the reaction system which was adjusted to a volume of 14 μl

using RNase-free water and incubated at 42 °C for 2 m. Next, 4 µl 5X Quantiscript Reverse Transcriptase Buffer, 1 µl Quantiscript Reverse Transcriptase and 1 µl dNTP mixture were added to the reaction system and incubated at 42 °C for 15-30 m and then at 95 °C for 3 m. Quantitative PCR (qRT-PCR) was performed using specific primers targeted against *Pttg1, p16 and Gapdh* from Thermo Fisher Scientific (Mm00479224_m1, Mm00494449_m1, Mm99999915_g1). For PCR amplification, we took 2 µl of cDNA, 10 µl 2x TaqMan Universal PCR Master Mix (#4304437, Thermo Fisher Scientific), 1 µl TaqMan primer and added RNAse-free water to a total volume of 20 µl. Using a ABI Prism 7900HT device (Applied Biosystems), the reaction was as follows: Initial heating step by 95 °C for 10 m, followed by 40 cycles of two-step reactions at 95 °C for 15 s and 60 °C for 1 m. Analysis was done with SDS 2.4 and RQ Manager 1.2.1 (Applied Biosystems).

Immunofluorescence imaging

Sorted cells were treated as indicated. Thereafter, cells were harvested and fixed using Cytofix solution (#554655, BD) for 20 m at 4 °C. Following a 20 m permeabilization step in PBS + 0.2% Triton X, cells were blocked for 20 m in PBS containing 10% donkey serum (D9663, Sigma Aldrich). Primary antibody was anti-γH2AX (#05-636, Millipore) at 1:1000. Following an incubation step for 1 h at 37 °C, cells were washed twice and incubated using secondary antibodies (anti-mouse Alexa488, Jackson Immunoresearch) at a dilution of 1:1,000. Before analyzing cells were mounted on glass slides using Antifade with DAPI solution (#P-36931, ThermoFisher). Cells were analyzed using a Zeiss Observer Z.1 microscope. Pictures were taken with a Zeiss LSM 710 laser scanning microscope.

Western Blot

$5*10^6$ LDBM cells were resuspended and incubated for 10 m at 95 °C in 1xSDS sample buffer containing 10 % SDS (Carl Roth), 10 mM β-mercaptoethanol (Carl Roth), 0,2 M Tris-HCl pH

6.8 (Biorad) and 0.05 % bromophenol blue (Sigma) and sonicated for 5 m. Equal amounts of protein were loaded onto a 12 % polyacrylamide gel. After running the gel first 10 m at 95 V and then 80 m at 110 V, the gel was blotted onto a nitrocellulose blotting membrane (Amersham Protran 0,45 µm, GE Healthcare Life Sciences) using a Trans-Blot SD semi-dry Transfer Cell (Biorad). Proteins were visualized by overnight incubation with rabbit-anti-PTTG1 antibody (#ABIN484400, Assay BioTech) and mouse-anti-b-actin (1:1000) antibody (#A1978, Sigma Aldrich) after blocking with PBS containing 5 % milk powder. After washing, the membranes were incubated with rabbit-IgG-HRP or mouse-IgG-HRP antibody (#sc-2077, #sc-2314, Santa Cruz) for 1 h. For detection ECL-reagent from the Super Signal West Femto Kit (#34094 , Thermo Scientific) was used.

Flow-FISH

Analysis of telomere length by flow cytometry (Flow-FISH) was performed as described[5,6] using 10^4 LK cells. Samples were measured on a BD LSR II.

DNA sequencing

Fragments of various sizes with respect to the PTTG1 promotor regions of B6, line A, D2 and line K mice were amplified from the corresponding genomic DNA by PCR with Herculase II (#600675, Agilent) according to their instructions, separated by gel electrophoresis, purified, digested with NheI-HF/EcoRV-HF (#R3131, #R3195 , New England Biolabs) and cloned into digested/dephosphorylated pNL1.1[Nluc], #N1001, Promega) using T4 DNA ligase (#M0202S , New England Biolabs), XL1-Blue competent cells (#200249, Agilent) and the QIAprep Spin Miniprep Kit (#27106, Qiagen). Plasmids were sent to GATC Biotech (Konstanz, Germany) for sequencing. Analysis and alignments were done with Lasergene DNAStar.

Cloning primers:

5'NheI-B6/D2_PTTG1_pr1: ATTAGCTAGCATACTTTGGAGACAGACGCGAG

3’EcoRV-B6/D2_PTTG1_pr2: ATAAGATATCCCAGGGCTGCTTGAGATCCT

3’EcoRV-B6/D2_PTTG1_pr3: AAGCGATATCTGGAGAAGTCAACAGGCTTAATCC

5’NheI-B6/D2_PTTG1_pr4: ATTAGCTAGCGAAGCCAAAACCATAAAAGTGAGC

3’EcoRV-B6/D2_PTTG1_pr5: ATAAGATATCCCCGGGCTGCTTGAGATCC

3'EcoRV-B6/D2_PTTG1_ORF: ATAAGATATCAAGAGTAGCCATTCTGGATTACTC

Sequencing primers:

PNL1[NLUC]_SEQ_FOR: GTGTGAATCGATAGTACTAA

PNL1[NLUC]_SEQ_REV: AAGGACTTGGTCCAGGTTGT

Analysis of SNPs and the pttg1 promotor

The image in *Suppl Fig 3A* showing SNPs in D2 and B6 regions of *Pttg1* was generated using the JAX/MCI database (http://www.informatics.jax.org/snp). Alignment of D2- and B6 promotor regions was done with Lasergene DNAStar. Transcription factor binding site prediction was performed with PROMO:

http://alggen.lsi.upc.es/cgibin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3

3D in silico modelling

Models of B6- and B2-PTTG1 (primary sequences see below, differences are highlighted) were generated using Jmol:

http://jmol.sourceforge.net/index.en.html

B6-PTTG1

MATLIFVDKDNEEPG**R**RLASKDGLKLG**T**GVKALDGKLQVSTPRVGKVFNAPA**V**PKA

SRKALGTVNRVAEKPMKTGKPLQPKQPTLTGKKITEKSTKTQSSVPAPDDAYPEIEKF

FPFNPLDFESFDLPEEHQISLLPLNGVPLMTLNEERGLEKLLHLGPPSPLKTPFLSWESD

PLYSPPSALSTLDVELPPVCYDADI

D2-PTTG1

MATLIFVDKDNEEPGSRLASKDGLKLGSGVKALDGKLQVSTPRVGKVFNAPALPKA
SRKALGTVNRVAEKPMKTGKPLQPKQPTLTGKKITEKSTKTQSSVPAPDDAYPEIEKF
FPFNPLDFESFDLPEEHQISLLPLNGVPLMTLNEERGLEKLLHLGPPSPLKTPFLSWESD
PLYSPPSALSTLDVELPPVCYDADI

Luciferase Assay

B6/K and D2/A promotor regions were cloned as described under the *DNA sequencing section* using 5'NheI-B6/D2_PTTG1_pr1 and 3'EcoRV-B6/D2_PTTG1_pr3. Cloning success was confirmed by restriction analysis and DNA sequencing.

The day before analysis, 30,000 NIH/3T3 cells were seeded onto cell-culture coated 24 well plates (Sarstedt) using IMDM medium with FCS and antibiotics. The next day, medium was removed and 400 µl fresh medium without antibiotics was added. 4 h later, each sample was transfected for 18 h with FuGene 6 transfection reagent (#E2693, Promega) according to their manual using 96 µl OPTI-MEM (#31985-062, Gibco), 1.5 µl FuGene 6 reagent, 100 ng Salmon Sperm DNA (#15632-011, Invitrogen), 75 ng pGL4.54 plasmid (#KM359769, Promega), which codes for the firefly luciferase and 75 ng of the pNL1.1 vectors coding for the various promotor fragments and the Nanoluc luciferase. Assay was done with the Dual-Luciferase Reporter Assay System (#E1910, Promega) in three rounds and triplicates according to the manufacturer's protocol and included a positive (pNL1.1CMV[Nluc/CMV], #N1091, Promega) and a negative (pNL1.1[Nluc], #N1001, Promega) control. For detection of chemiluminescence Nunclon Delta Surface plates (#136101, Thermo Scientific) and a GloMax 96 Microplate Luminometer (#E4861, Promega) were used. The relative luminescence was calculated by dividing the measured Nanoluc luminescence with the Firefly luminescence values.

Supplementary references

1. Wang J, Sun Q, Morita Y, et al. A differentiation checkpoint limits hematopoietic stem cell self-renewal in response to DNA damage. Cell. 2012;148(5):1001-1014.
2. Cribbs AP, Kennedy A, Gregory B, Brennan FM. Simplified production and concentration of lentiviral vectors to achieve high transduction in primary human T cells. BMC Biotechnol. 2013;13:98.
3. Moehrle BM, Nattamai K, Brown A, et al. Stem Cell-Specific Mechanisms Ensure Genomic Fidelity within HSCs and upon Aging of HSCs. Cell Rep. 2015;13(11):2412-2424.
4. Brown A, Pospiech J, Eiwen K, et al. The Spindle Assembly Checkpoint Is Required for Hematopoietic Progenitor Cell Engraftment. Stem Cell Reports 2017;9(5):1359–1368.
5. Rudolph KL, Chang S, Lee HW, et al. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell. 1999;96(5):701-712.
6. Hultdin M, Grönlund E, Norrback K, Eriksson-lindström E, Just T, Roos G. Telomere analysis by fluorescence in situ hybridization and flow cytometry. Nucleic Acids Res. 1998;26(16):3651-3656.