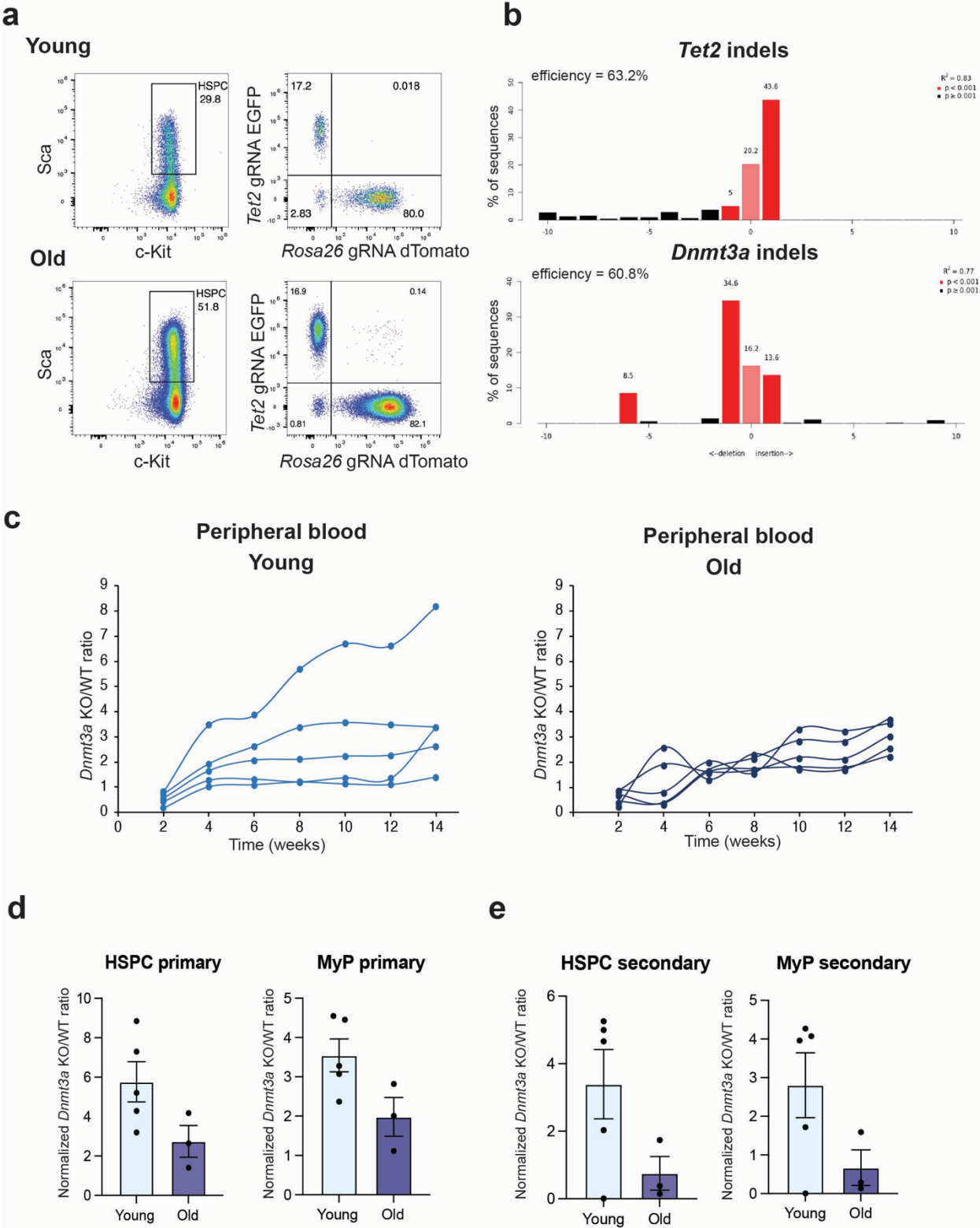


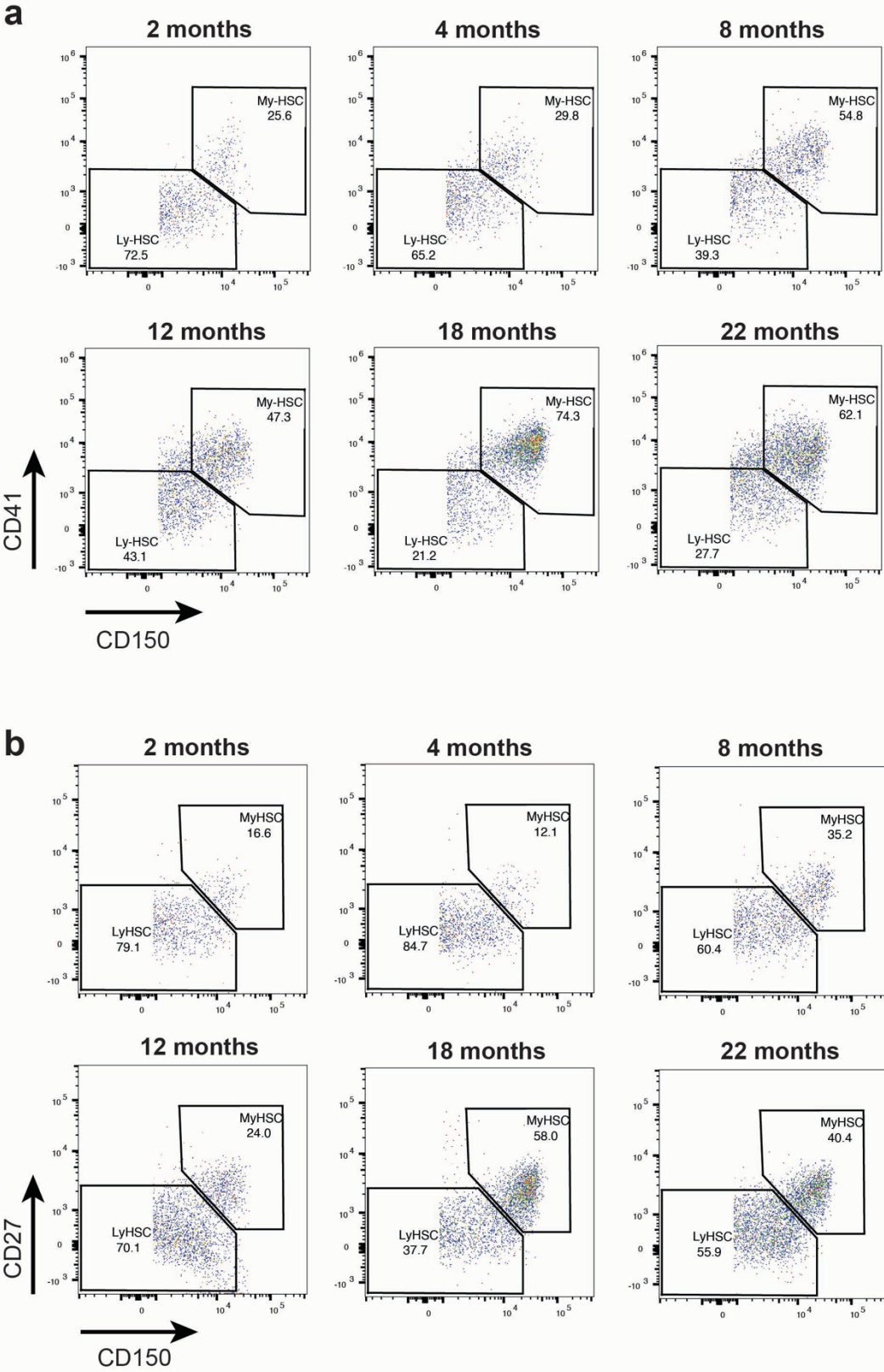
Extended Data Figure 1



**Extended Data Fig. 1 | *Dnmt3a* KO hematopoietic cells are not selected with aging.**

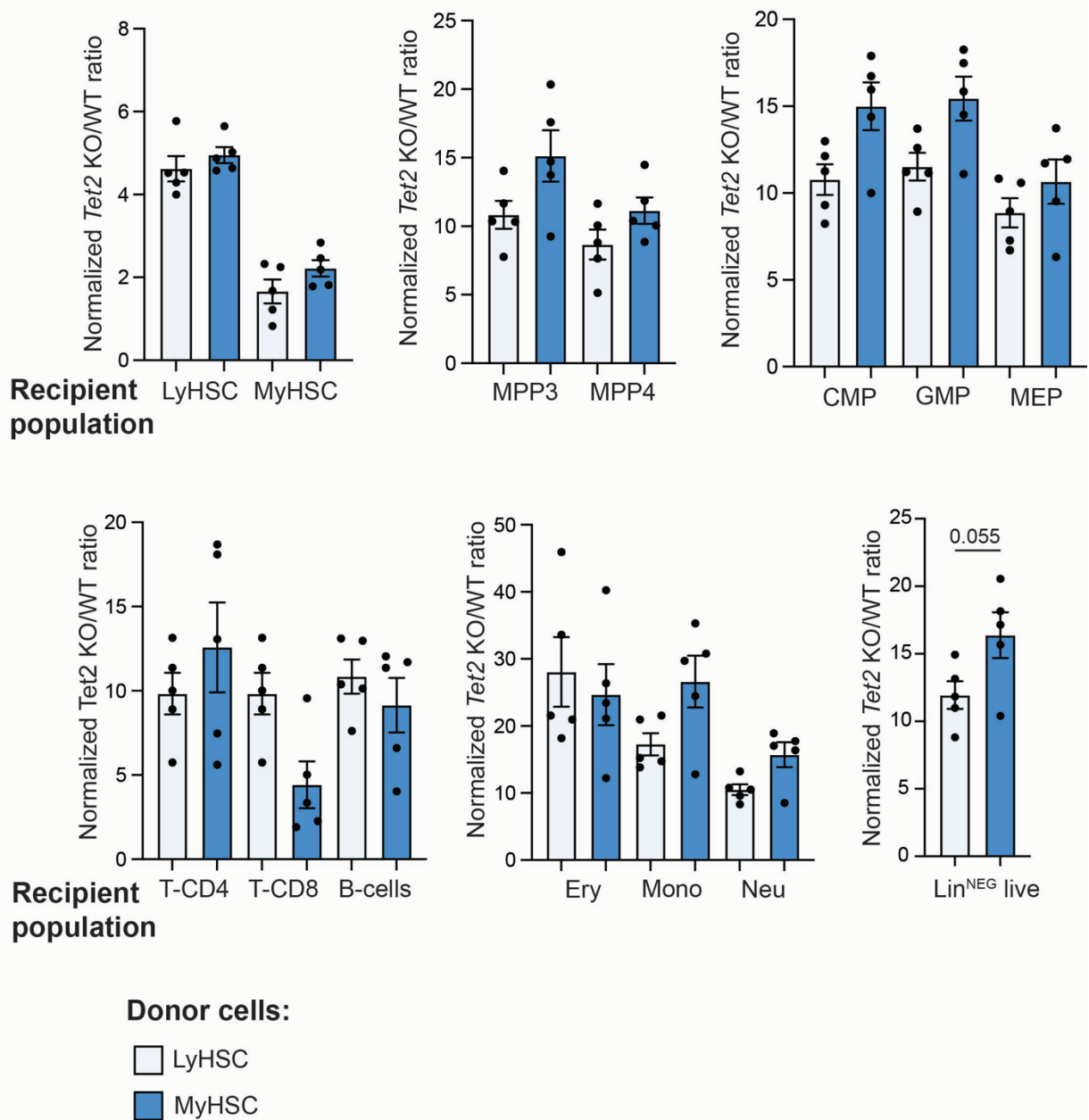
**a)** representative flow cytometry analysis of donor cells at the end of the in vitro CRISPR protocol, at the time of transplantation; left panels are gated on live, lineage<sup>NEG</sup> cells, right panels are gated on HSPC. **b)** results from TIDE analysis of PCR products spanning the genomic target regions of *Tet2* CRISPR (top) or *Dnmt3a* CRISPR (bottom) from cells subjected to CRISPR for the indicated genes. **c)** Ratio of *Dnmt3a* KO/WT cells over time in the peripheral blood of recipient young or old mice. **d-e)** relative *Dnmt3a* KO/WT expansion normalized by the ratio of transplanted cells in the bone marrow MyP or HSC of recipient mice at 16 weeks after primary transplantation (**d**) or 16 weeks after secondary transplantation (**e**).

Extended Data Figure 2



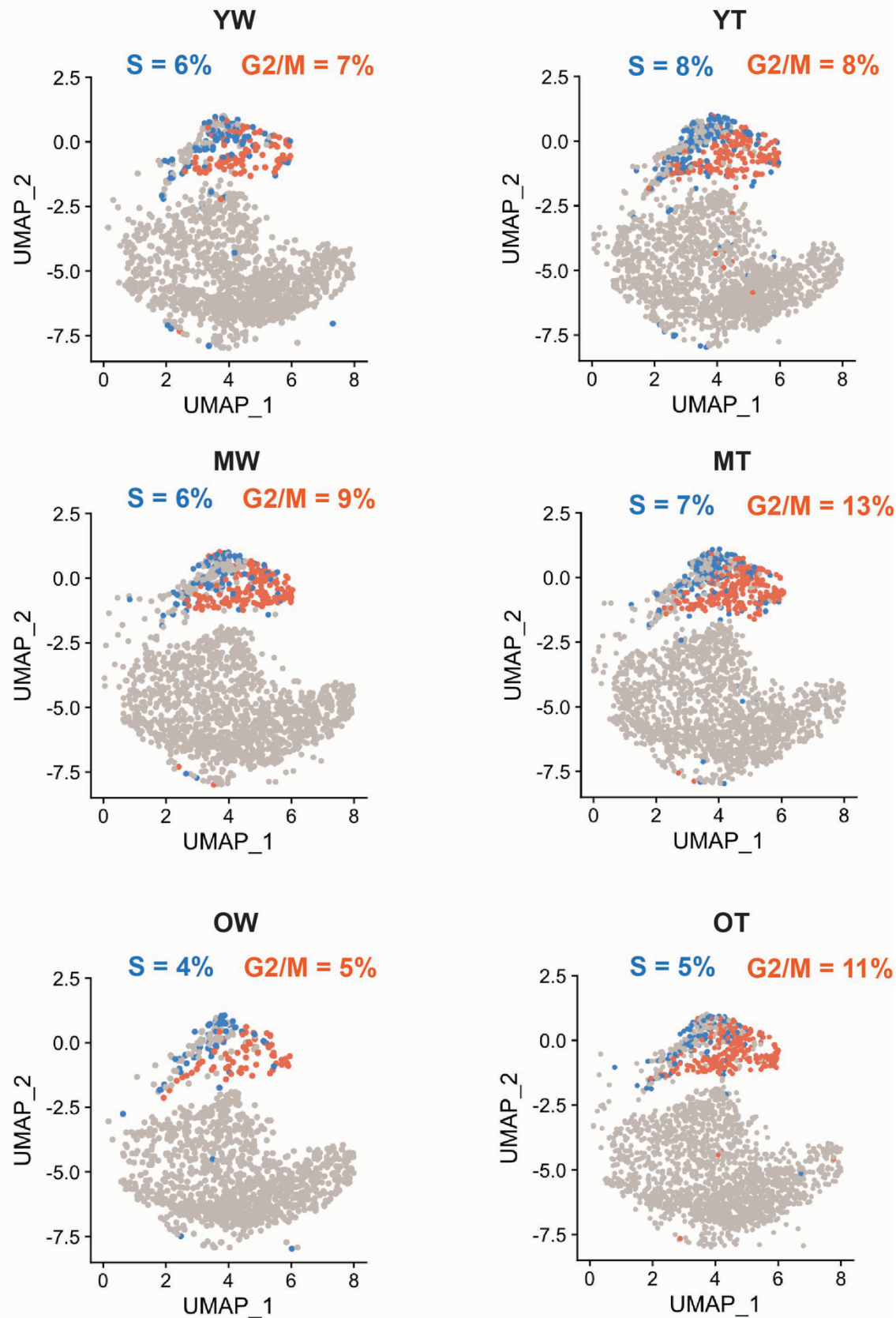
**Extended Data Fig. 2 | Aging is associated with increased flow cytometry detection of myeloid biased HSC expressing CD150, CD41 and CD27.** Flow cytometry analysis of bone marrow HSC from female mice of the indicated ages and MyHSC quantification by CD150-CD41 expression (**a**) or CD150-CD27 expression (**b**).

## Extended data Figure 3



**Extended Data Fig. 3 | *Tet2* KO cells from aged donor MyHSC or LyHSC show similar levels of selection.** Relative *Tet2* KO/WT expansion normalized by the ratio of transplanted cells for the indicated donor LyHSC or MyHSC cells in the different populations in the bone marrow of recipient mice 12 weeks post-transplantation.

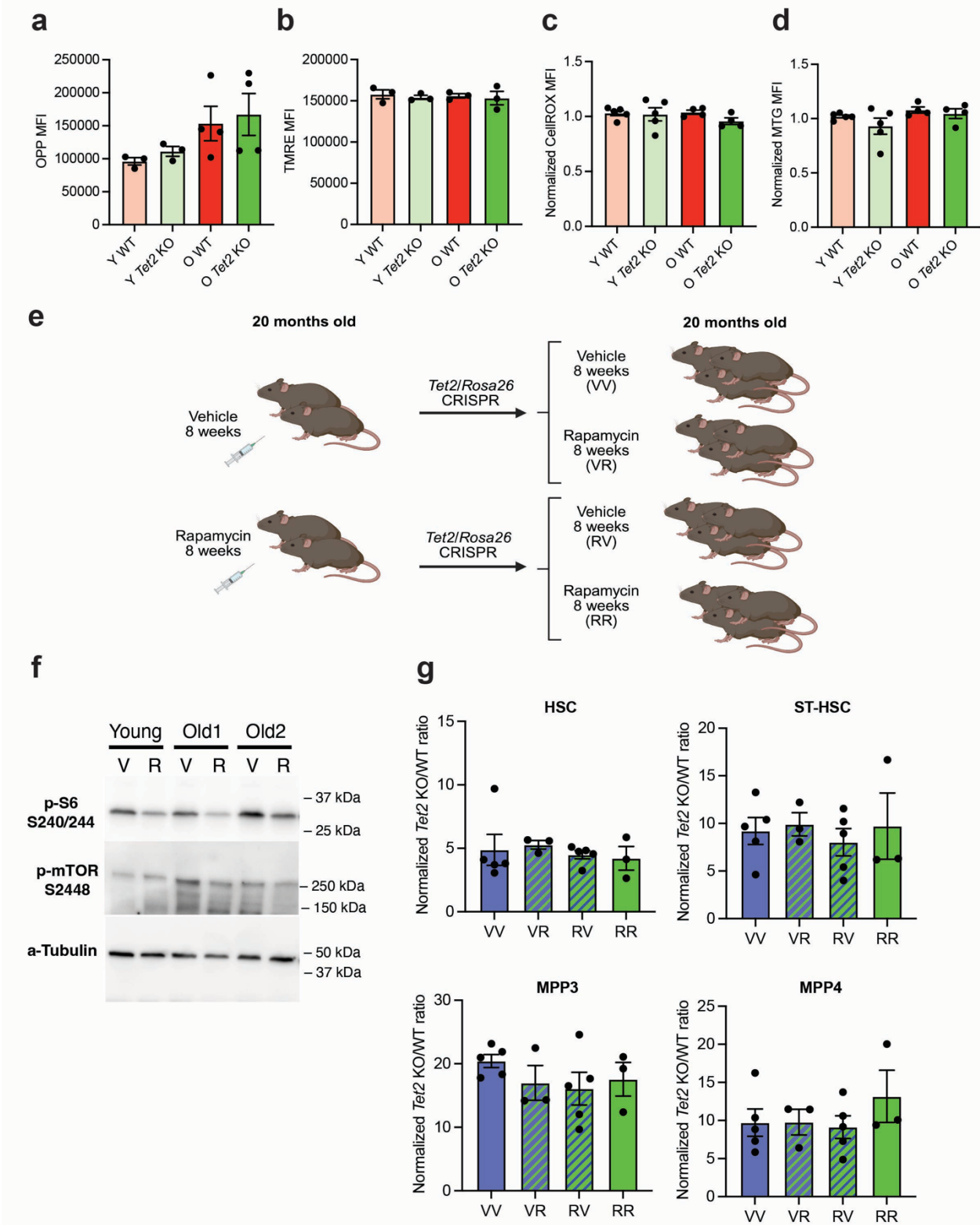
Extended Data Figure 4



**Extended data Fig. 4 | Reduced cycling HSC with aging are rescued by *Tet2* KO.**

Colored UMAP for cell cycle analysis displaying S and G/2M fraction of cells in LT + ST-HSC based on gene expression.

Extended Data Figure 5





**Extended Data Fig. 5 | Selection of *Tet2* KO cells with aging is not dependent on alterations in mitochondrial and ribosomal functions.** **a)** Analysis of translation efficiency by OPP staining of the indicated populations in the bone marrow 12 weeks post competitive transplantation of *Tet2* KO or WT cells. **b)** Analysis of mitochondrial membrane potential by TMRE staining of the indicated populations in the bone marrow 12 weeks post non-competitive transplantation of *Tet2* KO or WT cells (EGFP tagged transplanted in different mice). **c-d)** analysis of ROS abundance by CellROX staining (**c**) or mitochondrial mass by MTG staining (**d**) of the indicated populations in the bone marrow 12 weeks post non-competitive transplantation of *Tet2* KO or WT cells (dTomato tagged transplanted in different mice). **e)** Experimental design of rapamycin intervention to assess the role in *Tet2* KO age-related expansion. **f)** Immunoblot analysis of c-Kit-enriched bone marrow cells from one young or two old non-transplanted mice administered one dose of rapamycin and sacrificed 12 hours later. **g)** Relative *Tet2* KO/WT expansion in the indicated recipient BM populations normalized by the ratio of transplanted cells from mice treated with rapamycin or vehicle as indicated.

**Supplementary Table 1. Ribosomal protein genes modulating p53.**

<b>Ribosomal protein</b>	<b>References</b>
RPL11	Zhang et al. Molecular Cell Biology 2003 <sup>1</sup>
RPL15	Shi et al. Cancer Cell International 2022 <sup>2</sup>
RPL22	Cao et al. Oncotarget 2017 <sup>3</sup> ; Jansen et al. Cell Reports 2024 <sup>4</sup>
RPL23	Dai et al. Molecular Cell Biology 2004 <sup>5</sup>
RPL26	Ofir-Rosenfeld et al. Molecular Cell 2008 <sup>6</sup>
RPL37	Llanos et al. Cell Cycle 2010 <sup>7</sup> , Daftuar et al. PLOS 2013 <sup>8</sup>
RPL4	He et al. Oncotarget 2015 <sup>9</sup>
RPL5	Dai et al. Journal of Biol Chem 2004 <sup>10</sup>
RPL6	Bai et al. Nuclei Acid Research 2014 <sup>11</sup>
RPS14	Zhou et al. Oncogene 2013 <sup>12</sup>
RPS15	Daftuar et al. PLOS 2013 <sup>8</sup>
RPS15A	Chen et al. Intern Journal of Oncology 2016 <sup>13</sup>
RPS2	Cho et al. Biochem and Bioph Res Comm 2020 <sup>14</sup>
RPS20	Daftuar et al. PLOS 2013 <sup>8</sup>
RPS25	Zhang et al. Oncogene 2013 <sup>15</sup>
RPS26	Cui et al Oncogene 2014 <sup>16</sup>
RPS27	Xiong et al. Oncogene 2011 <sup>17</sup>
RPS27A	Sun et al. Journ of Biolog Chem 2011 <sup>18</sup>
RPS27L	Xiong et al. Oncogene 2011 <sup>17</sup>
RPS3	Yadavilli et al. DNA repair 2009 <sup>19</sup>
RPS7	Sun et al. Journ of Biolog Chem 2011 <sup>18</sup>

## Supplementary Methods

### Flow cytometry

All flow cytometry experiments were analyzed with a BD CytoFLEX flow cytometer equipped with violet, blue, yellow and red lasers.

Peripheral blood was stained with the following antibody panel: Per-CP Cyanine 5.5. anti-mouse GR1 1:200, PE-Cyanine7 anti-mouse CD11b 1:200, APC-Cyanine 7 anti-mouse CD4 1:200, APC-Cyanine 7 anti-mouse CD8a 1:200, BV785 anti-mouse CD45R/B220 1:200, TruStain FcX (anti-mouse CD16/32) 1:100. Blood cell populations were defined as follows: Neutrophils (DAPI<sup>NEG</sup>, CD11b<sup>+</sup>, GR1<sup>+</sup>), Monocytes (DAPI<sup>NEG</sup>, CD11b<sup>+</sup>, GR1<sup>NEG</sup>), B-cells (DAPI<sup>NEG</sup>, B220<sup>+</sup>), T-cells (DAPI<sup>NEG</sup>, CD4<sup>+</sup>/CD8a<sup>+</sup>).

Bone marrow “Mature” antibody panel was as follows: APC anti-mouse c-Kit 1:100, PE-Cyanine7 anti-mouse CD11b 1:200, Brilliant Violet 605 anti-mouse CD45R/B220 1:100, Pacific Blue anti-mouse TER-119 1:200, Per-CP Cyanine 5.5 anti-mouse GR1 1:200, Alexa Fluor anti-mouse CD8a 1:200, Brilliant Violet 510 anti-mouse CD4 1:200, Brilliant Violet 785 anti-mouse Ly6C 1:200, Near-IR Dead Cell Stain Kit 1:200, TruStain FcX (anti-mouse CD16/32) 1:100. Cells were stained in 50  $\mu$ L of FACS buffer for 30 minutes, at 4 °C, in the dark. Cell populations were defined as follows: neutrophils (Near-IR<sup>NEG</sup>, c-Kit<sup>NEG</sup>, CD11b<sup>+</sup>, Ly-6C<sup>low</sup>, GR1<sup>+</sup>), monocytes (Near-IR<sup>-</sup>, c-Kit<sup>NEG</sup>, CD11b<sup>+</sup>, Ly-6C<sup>+</sup>, GR1<sup>low</sup>), B-cells (Near-IR<sup>NEG</sup>, c-Kit<sup>NEG</sup>, B220<sup>+</sup>), erythroid cells (Near-IR<sup>NEG</sup>, c-Kit<sup>-</sup>, TER-119<sup>+</sup>), CD4<sup>+</sup> T-cells (Near-IR<sup>NEG</sup>, c-Kit<sup>NEG</sup>, CD8a<sup>-</sup>, CD4<sup>+</sup>), CD8<sup>+</sup> T-cells (Near-IR<sup>NEG</sup>, c-Kit<sup>NEG</sup>, CD4<sup>NEG</sup>, CD8a<sup>+</sup>).

Bone Marrow “Progenitor” antibody panel was as follows: lineage cocktail (APC-Cyanine 7 anti-mouse TER-119 1:100, APC-Cyanine 7 anti-mouse CD3e 1:100, APC-Cyanine 7 anti-mouse CD8a 1:200, APC-Cyanine 7 anti-mouse CD45R/B220 1:200, APC-Cyanine 7 anti-mouse CD11b 1:200, APC-Cyanine 7 anti-mouse GR1 1:200), Brilliant Violet 785 anti-mouse Sca 1:100, PE-Cyanine 7 anti-mouse c-Kit 1:100, eFluor 660 anti-mouse CD34 1:200, APC-R700 anti-mouse CD16/32 1:100, Brilliant Violet 421 anti-mouse CD135 1:100, Brilliant Violet 711 anti-mouse CD127 1:100, Near-IR Dead Cell Stain Kit

1:200, IgG from rat serum 50 µg/mL. Cells were stained in 50 µL of FACS buffer for 30 minutes, at 4 °C, in the dark. Cell populations were defined as follows: myeloid progenitors/MyP (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>NEG</sup>), common lymphoid progenitors/CLP (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>low</sup>, Sca<sup>low</sup>, CD127<sup>+</sup>, CD135<sup>+</sup>) common myeloid progenitors/CMP (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>NEG</sup>, CD34<sup>+</sup>, CD16/32<sup>NEG</sup>), granulocyte-monocyte progenitors (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>NEG</sup>, CD34<sup>+</sup>, CD16/32<sup>+</sup>), megakaryocyte erythrocyte progenitors/MEP (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>NEG</sup>, CD34<sup>NEG</sup>, CD16/32<sup>NEG</sup>).

Bone Marrow “Stem” antibody panel was as follows: lineage cocktail (see above), APC-Anti mouse Sca 1:100, PE-Cyanine 7 anti-mouse c-Kit 1:100, Brilliant Violet 421 anti-mouse CD135, Alexa Fluor 700 anti-mouse CD48 1:100, Brilliant Violet 785 anti-mouse CD150 1:100, Brilliant Violet 711 anti-mouse CD41 1:100, IgG from rat serum 50 µg/mL. Cells were stained in 100 µL of FACS buffer for 30 minutes, at 4 °C, in the dark. Cell population were defined as follows: myeloid progenitors/MP (Near-IR<sup>NEG</sup>, lineage<sup>-</sup>, c-Kit<sup>+</sup>, Sca<sup>NEG</sup>), HSPC (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>+</sup>), MPP4 (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>+</sup>, CD135<sup>+</sup>), MPP3 (Near-IR<sup>NEG</sup>, lineage<sup>-</sup>, c-Kit<sup>+</sup>, Sca<sup>+</sup>, CD135<sup>NEG</sup>, CD48<sup>+</sup>, CD150<sup>NEG</sup>), MPP2 (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>+</sup>, CD135<sup>NEG</sup>, CD48<sup>+</sup>, CD150<sup>+</sup>), short-term HSC/ST-HSC (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>+</sup>, CD135<sup>NEG</sup>, CD48<sup>NEG</sup>, CD150<sup>NEG</sup>), HSC (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>+</sup>, CD135<sup>NEG</sup>, CD48<sup>NEG</sup>, CD150<sup>+</sup>), lymphoid-biased HSC/LyHSC (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>+</sup>, CD135<sup>NEG</sup>, CD48<sup>NEG</sup>, CD150<sup>low</sup>, CD41<sup>NEG</sup>), myeloid biased HSC/MyHSC (Near-IR<sup>NEG</sup>, lineage<sup>NEG</sup>, c-Kit<sup>+</sup>, Sca<sup>+</sup>, CD135<sup>NEG</sup>, CD48<sup>NEG</sup>, CD150<sup>high</sup>, CD41<sup>+</sup>).

For RNA FISH-Flow cells were stained with the following antibody cocktail: APC-Cyanine 7 anti-mouse TER-119 1:100, APC-Cyanine 7 anti-mouse CD3e 1:100, APC-Cyanine 7 anti-mouse CD8a 1:200, APC-Cyanine 7 anti-mouse CD45R/B220 1:200, APC-Cyanine 7 anti-mouse CD11b 1:200, APC-Cyanine 7 anti-mouse GR1 1:200 Brilliant Violet 785 anti-mouse Sca 1:100, Brilliant Violet 711 anti-mouse c-Kit 1:100, Alexa Fluor 700 anti mouse CD48 1:100, Brilliant Violet 605 anti mouse CD150 1:100, IgG from rat serum 50 µg/mL.

For OPP analysis cells were stained with the following antibody cocktail: lineage cocktail (APC-Cyanine 7 anti-mouse TER-119 1:100, APC-Cyanine 7 anti-mouse CD3e 1:100, APC-Cyanine 7 anti-mouse CD8a 1:200, APC-Cyanine 7 anti-mouse CD45R/B220 1:200, APC-Cyanine 7 anti-mouse CD11b 1:200, APC-Cyanine 7 anti-mouse GR1 1:200), Brilliant Violet 421 anti-mouse Sca 1:100, PE-Cyanine 7 anti-mouse c-Kit 1:100, Brilliant Violet 785 anti-mouse CD150 1:100, Alexa Fluor 700 anti mouse CD48 1:100, IgG from rat serum 50 µg/mL.

For TMRE assay cells were stained with the following antibody cocktail: lineage cocktail (APC-Cyanine 7 anti-mouse TER-119 1:100, APC-Cyanine 7 anti-mouse CD3e 1:100, APC-Cyanine 7 anti-mouse CD8a 1:200, APC-Cyanine 7 anti-mouse CD45R/B220 1:200, APC-Cyanine 7 anti-mouse CD11b 1:200, APC-Cyanine 7 anti-mouse GR1 1:200), APC anti-mouse Sca 1:100, PE-Cyanine 7 anti-mouse c-Kit 1:100, Brilliant Violet 785 anti-mouse CD150 1:100, Brilliant Violet 421 anti mouse CD135 1:100, Alexa Fluor 700 anti mouse CD48 1:100, Near-IR Dead Cell Stain Kit 1:200, IgG from rat serum 50 µg/mL.

For MTG and CellROX assay cells were stained with the following antibody cocktail: (APC-Cyanine 7 anti-mouse TER-119 1:100, APC-Cyanine 7 anti-mouse CD3e 1:100, APC-Cyanine 7 anti-mouse CD8a 1:200, APC-Cyanine 7 anti-mouse CD45R/B220 1:200, APC-Cyanine 7 anti-mouse CD11b 1:200, APC-Cyanine 7 anti-mouse GR1 1:200), APC anti-mouse Sca 1:100, Brilliant violet 711 anti-mouse c-Kit 1:100, Brilliant Violet 785 anti-mouse CD150 1:100, Brilliant Violet 421 anti mouse CD135 1:100, Alexa Fluor 700 anti mouse CD48 1:100, IgG from rat serum 50 µg/mL.

**Supplementary table 2. Reagents and resources details.**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
APC-Cyanine 7 anti-mouse TER-119	Biolegend	Cat#116223
APC-Cyanine 7 anti-mouse CD3e	Biolegend	Cat#100330
APC-Cyanine 7 anti-mouse CD4	Biolegend	Cat#100526
APC-Cyanine 7 anti-mouse CD8a	Biolegend	Cat#100714
APC-Cyanine 7 anti-mouse CD45RB220	Biolegend	Cat#103224
APC-Cyanine 7 anti-mouse Ly-6G/Ly-6C (GR-1)	Biolegend	Cat#108424
APC Cyanine 7 anti-mouse CD11b	BD	Cat#557657
APC anti-mouse Ly-6A/E (Sca-1)	eBioscience	Cat#17-5981-83
PE-Cyanine 7 anti-mouse CD117 (c-Kit)	eBioscience	Cat#25-1171-82
PE-Cyanine 7 anti-mouse CD11b	eBioscience	Cat#25-0112-82
FITC anti mouse CD48	Biolegend	Cat#103404
Alexa Fluor 700 anti mouse CD48	Biolegend	Cat#103426
Brilliant Violet 421 anti-mouse CD150	Biolegend	Cat#115943
Brilliant Violet 605 anti-mouse CD150	Biolegend	Cat#115937
Brilliant Violet 785 anti-mouse CD150	Biolegend	Cat#115927
Brilliant Violet 711 anti-mouse CD127	Biolegend	Cat#135035
Brilliant Violet 421 anti-mouse CD135	Biolegend	Cat#135314
Brilliant Violet 711 anti-mouse CD41	BD	Cat#740712
Brilliant Violet 785 anti-mouse CD45R/B220	Biolegend	Cat#103246
Brilliant Violet 605 anti-mouse CD45R/B220	Biolegend	Cat#103244
Brilliant Violet 510 anti mouse CD4	Biolegend	Cat#100559
Brilliant Violet 785 anti-mouse Ly-6C	Biolegend	Cat#128041
Brilliant Violet 711 anti-mouse CD117 (c-Kit)	Biolegend	Cat#105835
PE/Dazzle 594 anti-mouse CD41	Biolegend	Cat#133935
PE/Dazzle 594 anti-mouse IL27Ra	Biolegend	Cat#159007
eFluor 660 anti-mouse CD34	eBioscience	Cat#50-0341-82
APC-R700 anti-mouse CD16/CD32	BD	Cat#565502
Alexa Fluor 700 anti-mouse CD8a	Biolegend	Cat#100730
Pacific Blue anti-mouse TER-119	Biolegend	Cat#116208
PE-Cyanine 7 anti-mouse CD11b	eBioscience	Cat#25-0112-82
PE anti-mouse TER-119	Biolegend	Cat#116223
PE anti-mouse CD3e	BD	Cat#553064
PE anti-mouse CD4	Biolegend	Cat#100408
PE anti-mouse CD8a	Biolegend	Cat#162304
PE anti-mouse CD45RB220	BD	Cat#553090
PE anti-mouse Ly-6G/Ly-6C (GR-1)	eBioscience	Cat#12-5931-82
PE anti-mouse CD11b	BD	Cat#557397
PerCP Cyanine 5.5 anti-mouse Ly-6G/Ly-6C (GR-1)	Biolegend	Cat#108428
APC anti-mouse CD201 (EPCR)	Biolegend	Cat#141506
APC anti mouse CD117 (c-Kit)	BD	Cat#555356
TruStain FcX (anti-mouse CD16/32)	Biolegend	Cat#101320
Anti-Mouse CD4 [Clone GK1.5]	Leinoco	Cat#C1333
Anti-Mouse CD8a (Ly 2.2) [Clone 2.43]	Leinoco	Cat#C380

CD117 MicroBeads, mouse	Miltenyi	Cat#130-097-146
IgG from rat serum	Millipore Sigma	Cat#I4131-50MG
Phospho-S6 Ribosomal Protein (Ser240/244)	CST	Cat#2215S
Phospho-mTOR (Ser2448)	CST	Cat#2971S
Anti-TUBA4A (TUBA1) Antibody	Millipore Sigma	Cat#T6199
Goat Anti-Rabbit IgG (H + L)-HRP Conjugate	Biorad	Cat#1706515
Goat Anti-Mouse IgG (H + L)-HRP Conjugate	Biorad	Cat#1706516
<b>Chemicals, peptides, and recombinant proteins</b>		
PEI MAX® - Transfection Grade Linear Polyethylenimine Hydrochloride (MW 40,000)	Polysciences	Cat#25765
Poly(vinyl alcohol) average Mw 146,000-186,000, 87-89% hydrolyzed	Millipore Sigma	Cat#363103
Human Fibronectin	Millipore Sigma	Cat#FC010
SCF, Mouse	Genescript	Cat#Z02997-10
TPO, Mouse	Genescript	Cat#Z03175-50
Insulin-Transferrin-Selenium-Ethanolamine (ITS -X) (100X)	Gibco	Cat#51500056
DMEN, high glucose, pyruvate	Gibco	Cat#11995065
IMDM	Gibco	Cat#12440053
Ham's F12 Nutrient Mix	Gibco	Cat#11765054
Penicillin-Streptomycin (10,000 U/mL)	Gibco	Cat#15140122
HEPES (1M)	Gibco	Cat#15630080
Fisherbrand™ Research Grade Fetal Bovine Serum, Canadian Sourced	Fischer Scientific	Cat#FB12999102
Sucrose	Millipore Sigma	Cat#S0389-5KG
Histopaque®-1119	Millipore Sigma	Cat#11191-6X100ML
DAPI	ThermoFisher	Cat#D3571
Ammonium Chloride	Fischer Scientific	Cat#A661-500
Potassium bicarbonate	Millipore Sigma	Cat#237205-500G
O-propargyl puromycin	Vector Labs	Cat#CCT-1407-25
Rapamycin	MedChemExpress	Cat#AY-22989
Tetramethylrhodamine, Ethyl Ester, Perchlorate (TMRE)	ThermoFisher	Cat#T669
CellROX™ Green Reagent, for oxidative stress detection	ThermoFisher	Cat#C10444
MitoTracker™ Green FM Dye, for flow cytometry	ThermoFisher	Cat#M46750
Sodium dodecyl sulphate	Millipore Sigma	Cat#L5750
Tween-20	ThermoFisher	Cat#BP330-500
Tris Base	ThermoFisher	Cat#BP152-5
Bromophenol Blue	Fisher Scientific	Cat#AC403151000
Sodium chloride	Fisher Scientific	Cat#S-6713
Immobilon Western Chemiluminescent HRP Substrate	Millipore Sigma	Cat#WBKLS0500
Glycerol	Fisher Scientific	Cat#BP229-1
<b>Critical commercial assays</b>		
T7-FlashScribe™ Transcription Kit	Cellscript	Cat#C-ASF3507
ScriptCap™ Cap 1 Capping System	Cellscript	Cat#C-SCCS1710

RNeasy Plus Micro Kit	Qiagen	Cat#74034
DNeasy Blood and Tissue Kit	Qiagen	Cat#69506
Plasmid Plus Maxi Kit	Qiagen	Cat#12943
LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit, for 633 or 635 nm excitation	ThermoFisher	Cat#L10119
UltraComp eBeads™ Compensation Beads	ThermoFisher	Cat#01-2222-42
LS columns	Miltenyi	Cat#130-042-401
Click-&-Go® Plus 647 OPP	Vector labs	Cat#CTT-1496
BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit	BD	Cat#554714
4-20% acrylamide mini protean TGX precast gels	Biorad	Cat#4561095
Phusion™ High-Fidelity DNA Polymerase	NEB	Cat#M0530L
<b>Deposited data</b>		
Aging and <i>Tet2</i> KO mouse scRNAseq data	GEO	GSE310533
<b>Experimental models: Cell lines</b>		
HEK293T cells	Takara	Cat#632180
<b>Experimental models: Organisms/strains</b>		
C57BL6/J	Jackson laboratories	Cat#000664
C57BL/6JN	National Institute on Aging	<a href="https://www.nia.nih.gov/research/dab/aged-rodent-colonies">https://www.nia.nih.gov/research/dab/aged-rodent-colonies</a>
<b>Oligonucleotides</b>		
Mouse <i>Tet2</i> gRNA: GGTATATCGGAGATCGAGTG	IDT (design: this paper)	N/A
Mouse <i>Rosa26</i> gRNA: GCAATACCATGCCAGGTGGA	IDT (design: this paper)	N/A
Mouse <i>Dnmt3a</i> gRNA: CAGGCCGAATTGTGTCTTGG	IDT (design: this paper)	N/A
Mouse <i>Tet2</i> FW TIDE: ATTCAAGCCCCCTCTGGAGAA	IDT (design: this paper)	N/A
Mouse <i>Tet2</i> REV TIDE: CCCACTCTATAGCTGGGCAAT	IDT (design: this paper)	N/A
Mouse <i>Rosa26</i> FW TIDE: CCACATTGGCACCATAGTCA	IDT (design: this paper)	N/A
Mouse <i>Rosa26</i> REV TIDE: AGCCATCTGGGCCTTTTAAC	IDT (design: this paper)	N/A
Mouse <i>Dnmt3a</i> FW TIDE: GCTTGGCTTTGTACCTGAT	IDT (design: this paper)	N/A
Mouse <i>Dnmt3a</i> REV TIDE: CAGCTCAGTGATGGTGGCTA	IDT (design: this paper)	N/A
<b>Recombinant DNA</b>		
pLCv2_U6.(BsmBI).chRNA2_EFS.EGFP_wPRE	Yudovich et al.	N/A
pLCv2_U6.(BsmBI).chRNA2_EFS.dTomato_wPRE	Yudovich et al. (dTomato inserted)	N/A
pMD2.G	Addgene	Cat#12259
psPAX2	Addgene	Cat#12260
pCas9-polyA	Addgene	Cat#79602
<b>Software and algorithms</b>		
FlowJo™ v11	BD	<a href="https://flowjo.com/flowjo/overview">https://flowjo.com/flowjo/overview</a>
GraphPad Prism v10	Dotmatics	<a href="https://www.graphpad.com/features">https://www.graphpad.com/features</a>
Biorender	Biorender	<a href="https://www.biorender.com">https://www.biorender.com</a>



Shinyapps	Anschutz Bioinformatic Core	<a href="https://bioinformatics.cuanschutz.edu">https://bioinformatics.cuanschutz.edu</a>
CRISPick	CRISPick	<a href="https://portals.broadinstitute.org/gppx/crispick/public">https://portals.broadinstitute.org/gppx/crispick/public</a>
Illustrator	Adobe	<a href="https://www.adobe.com/creativecloud/plans.html?plan=edu&amp;product=illustrator">https://www.adobe.com/creativecloud/plans.html?plan=edu&amp;product=illustrator</a>
Primer3	Primer3	<a href="http://frodo.wi.mit.edu/primer3/">http://frodo.wi.mit.edu/primer3/</a>
Heatmapper	Heatmapper	<a href="http://heatmapper.ca">http://heatmapper.ca</a>

## Supplementary references

1. Zhang, Y., Wolf, G.W., Bhat, K., Jin, A., Allio, T., Burkhart, W.A., and Xiong, Y. (2003). Ribosomal protein L11 negatively regulates oncoprotein MDM2 and mediates a p53-dependent ribosomal-stress checkpoint pathway. *Mol Cell Biol* 23, 8902-8912. 10.1128/mcb.23.23.8902-8912.2003.
2. Shi, R., and Liu, Z. (2022). RPL15 promotes hepatocellular carcinoma progression via regulation of RPs-MDM2-p53 signaling pathway. *Cancer Cell Int* 22, 150. 10.1186/s12935-022-02555-5.
3. Cao, B., Fang, Z., Liao, P., Zhou, X., Xiong, J., Zeng, S., and Lu, H. (2017). Cancer-mutated ribosome protein L22 (RPL22/eL22) suppresses cancer cell survival by blocking p53-MDM2 circuit. *Oncotarget* 8, 90651-90661. 10.18632/oncotarget.21544.
4. Jansen, J., Bohnsack, K.E., Böhlken-Fascher, S., Bohnsack, M.T., and Dobbstein, M. (2024). The ribosomal protein L22 binds the MDM4 pre-mRNA and promotes exon skipping to activate p53 upon nucleolar stress. *Cell Rep* 43, 114610. 10.1016/j.celrep.2024.114610.
5. Dai, M.S., Zeng, S.X., Jin, Y., Sun, X.X., David, L., and Lu, H. (2004). Ribosomal protein L23 activates p53 by inhibiting MDM2 function in response to ribosomal perturbation but not to translation inhibition. *Mol Cell Biol* 24, 7654-7668. 10.1128/mcb.24.17.7654-7668.2004.
6. Ofir-Rosenfeld, Y., Boggs, K., Michael, D., Kastan, M.B., and Oren, M. (2008). Mdm2 regulates p53 mRNA translation through inhibitory interactions with ribosomal protein L26. *Mol Cell* 32, 180-189. 10.1016/j.molcel.2008.08.031.
7. Llanos, S., and Serrano, M. (2010). Depletion of ribosomal protein L37 occurs in response to DNA damage and activates p53 through the L11/MDM2 pathway. *Cell Cycle* 9, 4005-4012. 10.4161/cc.9.19.13299.
8. Daftuar, L., Zhu, Y., Jacq, X., and Prives, C. (2013). Ribosomal proteins RPL37, RPS15 and RPS20 regulate the Mdm2-p53-MdmX network. *PLoS One* 8, e68667. 10.1371/journal.pone.0068667.
9. He, X., Li, Y., Dai, M.S., and Sun, X.X. (2016). Ribosomal protein L4 is a novel regulator of the MDM2-p53 loop. *Oncotarget* 7, 16217-16226. 10.18632/oncotarget.7479.
10. Dai, M.S., and Lu, H. (2004). Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. *J Biol Chem* 279, 44475-44482. 10.1074/jbc.M403722200.
11. Bai, D., Zhang, J., Xiao, W., and Zheng, X. (2014). Regulation of the HDM2-p53 pathway by ribosomal protein L6 in response to ribosomal stress. *Nucleic Acids Res* 42, 1799-1811. 10.1093/nar/gkt971.

12. Zhou, X., Hao, Q., Liao, J., Zhang, Q., and Lu, H. (2013). Ribosomal protein S14 unties the MDM2-p53 loop upon ribosomal stress. *Oncogene* 32, 388-396. 10.1038/onc.2012.63.
13. Chen, J., Wei, Y., Feng, Q., Ren, L., He, G., Chang, W., Zhu, D., Yi, T., Lin, Q., Tang, W., et al. (2016). Ribosomal protein S15A promotes malignant transformation and predicts poor outcome in colorectal cancer through misregulation of p53 signaling pathway. *Int J Oncol* 48, 1628-1638. 10.3892/ijo.2016.3366.
14. Cho, J., Park, J., Shin, S.C., Kim, J.H., Kim, E.E., and Song, E.J. (2020). Ribosomal protein S2 interplays with MDM2 to induce p53. *Biochem Biophys Res Commun* 523, 542-547. 10.1016/j.bbrc.2020.01.038.
15. Zhang, X., Wang, W., Wang, H., Wang, M.H., Xu, W., and Zhang, R. (2013). Identification of ribosomal protein S25 (RPS25)-MDM2-p53 regulatory feedback loop. *Oncogene* 32, 2782-2791. 10.1038/onc.2012.289.
16. Cui, D., Li, L., Lou, H., Sun, H., Ngai, S.M., Shao, G., and Tang, J. (2014). The ribosomal protein S26 regulates p53 activity in response to DNA damage. *Oncogene* 33, 2225-2235. 10.1038/onc.2013.170.
17. Xiong, X., Zhao, Y., He, H., and Sun, Y. (2011). Ribosomal protein S27-like and S27 interplay with p53-MDM2 axis as a target, a substrate and a regulator. *Oncogene* 30, 1798-1811. 10.1038/onc.2010.569.
18. Sun, X.X., DeVine, T., Challagundla, K.B., and Dai, M.S. (2011). Interplay between ribosomal protein S27a and MDM2 protein in p53 activation in response to ribosomal stress. *J Biol Chem* 286, 22730-22741. 10.1074/jbc.M111.223651.
19. Yadavilli, S., Mayo, L.D., Higgins, M., Lain, S., Hegde, V., and Deutsch, W.A. (2009). Ribosomal protein S3: A multi-functional protein that interacts with both p53 and MDM2 through its KH domain. *DNA Repair (Amst)* 8, 1215-1224. 10.1016/j.dnarep.2009.07.003.