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SUPPLEMENTARY INFORMATION

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Adaptive endoplasmic reticulum stress signalling via IRE1α–XBP1 preserves self-renewal of haematopoietic and pre-leukaemic stem cells

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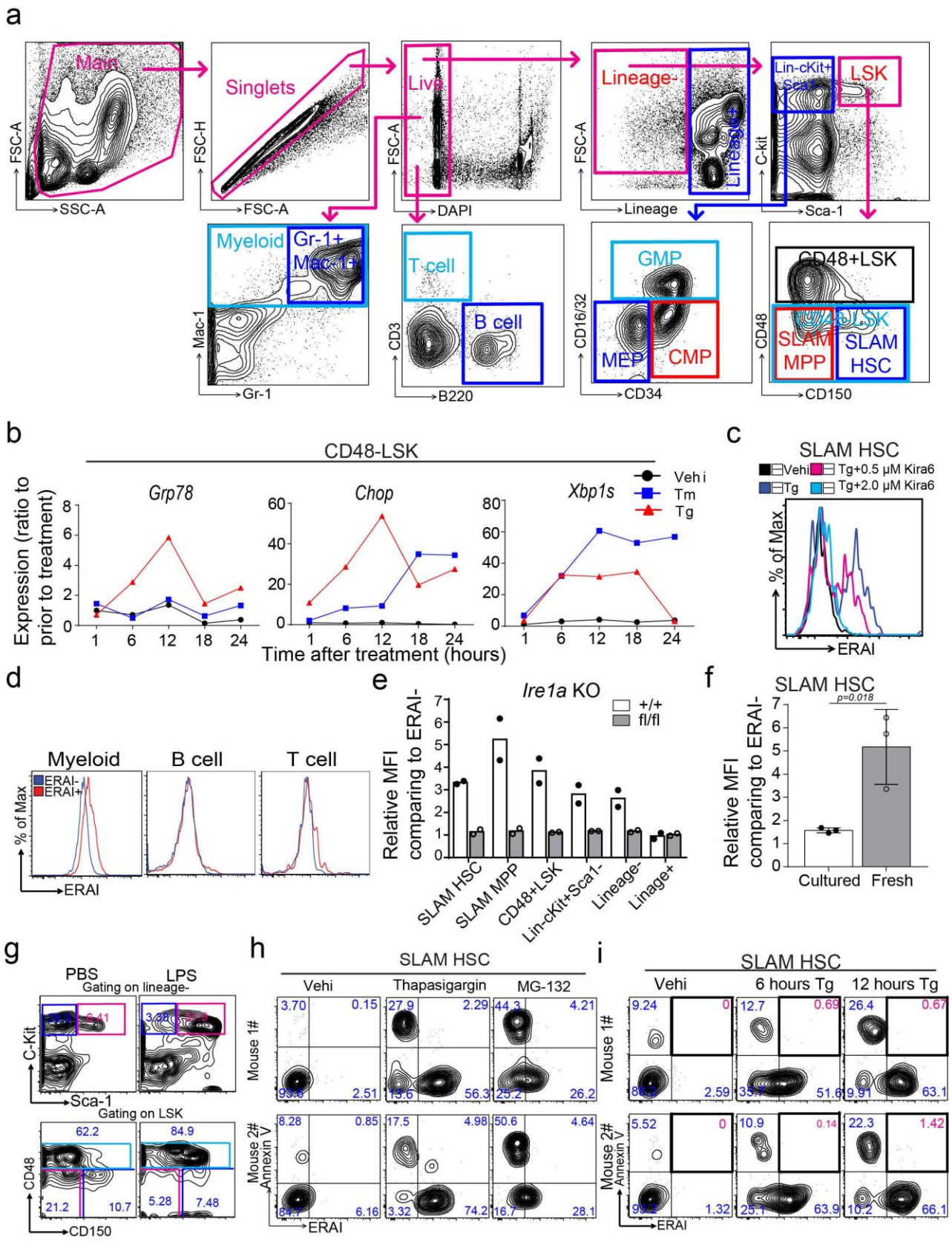
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Main
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Live
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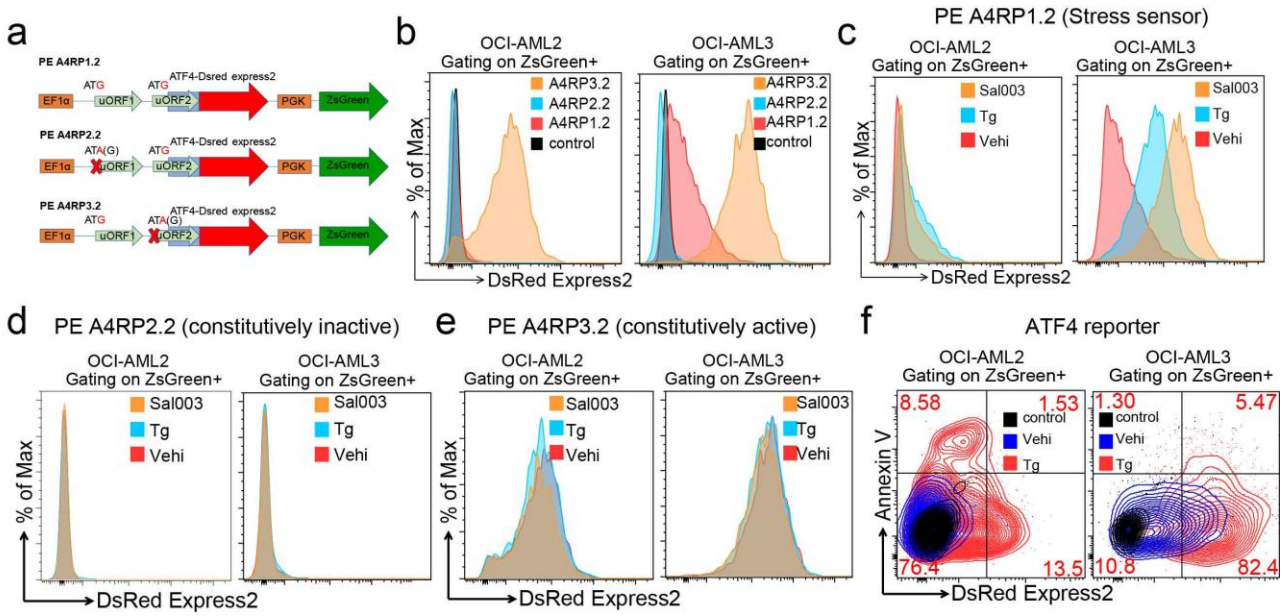
Lin-cKit+ Sca1+
LSK
C-kit
Sca-1
Myeloid
Gr-1+ Mac-1+
Mac-1
Gr-1
T cell
B cell
CD3
B220
GMP
MEP
CMP
CD16/32
CD34
CD48+LSK
CD48-LSK
SLAM MPP
SLAM HSC
CD48
CD150
b
CD48-LSK
Grp78
Chop
Xbp1s
Vehi
Tm
Tg
Expression (ratio to prior to treatment)
Time after treatment (hours)
c
SLAM HSC
Vehi
Tg
Tg+0.5 μM Kira6
Tg+2.0 μM Kira6
% of Max
ERAI
d
Myeloid
B cell
T cell
ERAI-
ERAI+
% of Max
ERAI
e
Ire1a KO
+/+
fl/fl
Relative MFI comparing to ERAI-
SLAM HSC
SLAM MPP
CD48+LSK
Lin-cKit+Sca1-
Lineage-
Linage+
f
SLAM HSC
p=0.018
Relative MFI comparing to ERAI-
Cultured
Fresh
g
PBS
LPS
Gating on lineage-
C-Kit
Sca-1
Gating on LSK
CD48
CD150
h
SLAM HSC
Vehi
Thapasigargin
MG-132
Mouse 1#
Mouse 2# Annexin V
ERAI
i
SLAM HSC
Vehi
6 hours Tg
12 hours Tg
Mouse 1#
Mouse 2# Annexin V
ERAI

Supplementary Figure 1.

ER stress activates UPR in murine long-term HSCs

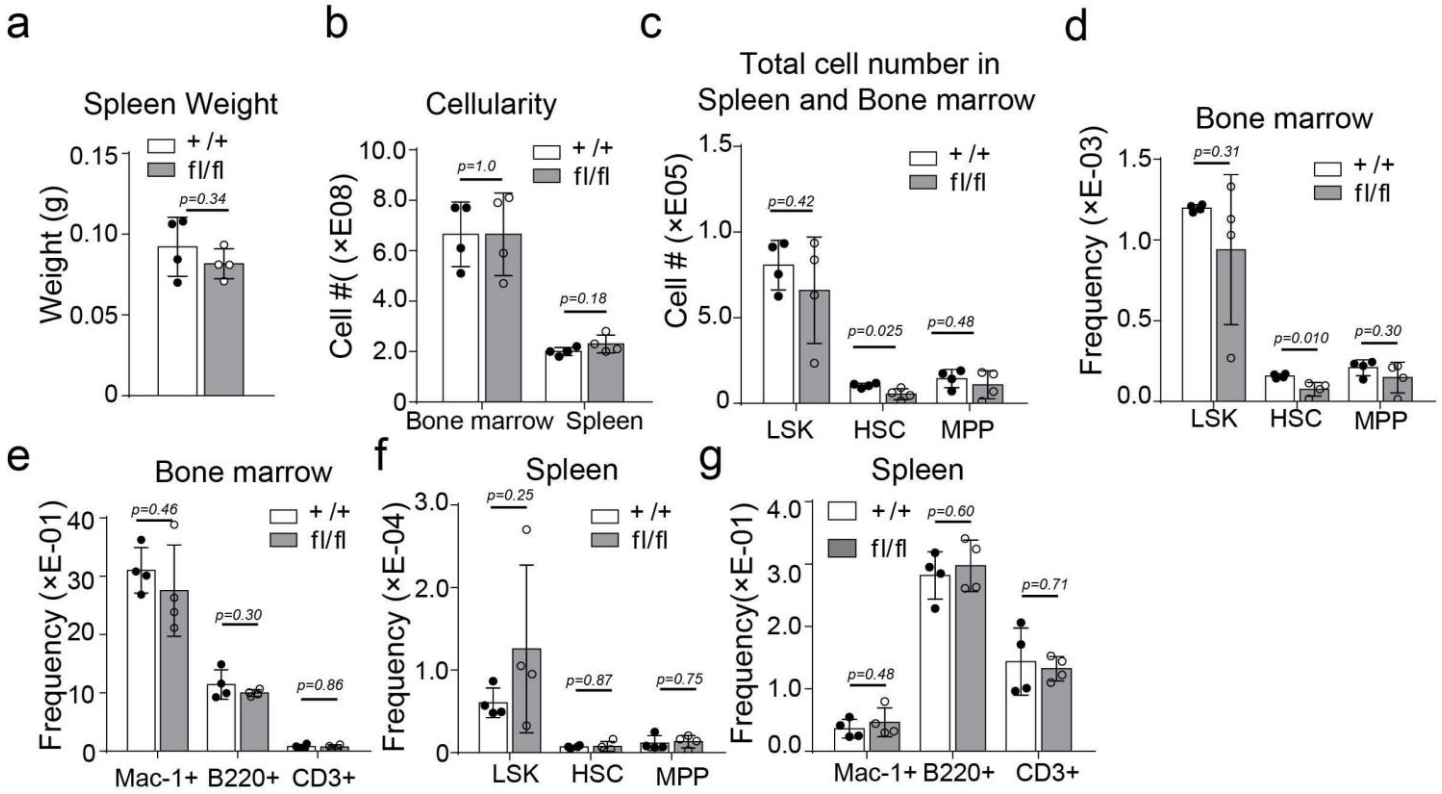
a, Gating strategy for FACS analysis and sorting of haematopoietic populations. **b**, Summary of qRT-PCR of UPR genes in purified $CD48^-$LSKs treated with Tunicamycin or Thpasigargin for 1-24 hours. Levels were normalized to that of cells treated with vehicle for 1 hour. Bone marrow cells were pooled from 3 mice and FACS sorted for different haematopoietic populations. n=2 pools of samples were analyzed by qRT-PCR. **c,** Representative FACS plot showing ERAI signal in wild type HSCs treated with Thapsigargin (Tg) for 18 hours, together with IRE1α kinase inhibitor Kira6 (n=3 biological replicates in 3 independent experiments). **d**, Representative FACS plot showing ERAI signal in Myeloid ($Mac\text{-}1^+$), B cell ($B220^+$), T cell ($CD3^+$) of wild type mice (3 biological replicates in 3 independent experiments). ERAI signal in HSCs isolated from mice without ERAI transgene (ERAI-) was used as negative control. **e,** Relative ERAI signal intensity (normalized to level in ERAI negative cells) in haematopoietic populations from *Mx1-cre*$^+$; *ERAI*$^+$; *Ire1*$\alpha^{fl/fl}$(fl/fl) and *Mx1-cre*$^-$; *ERAI*$^+$; *Ire1*$\alpha^{fl/fl}$ (+/+) mice 2 weeks after pIpC injection (n=2 biological replicates in 2 independent experiments). **f,** ERAI signal intensity of HSCs freshly isolated from wild type mice (Fresh) or cultured for 12 hours (cultured) (n=3 biological replicates in 3 independent experiments). **g,** Representative FACS plot of haematopoietic stem and progenitor populations from wild type mice 24 hours after LPS injection (2 mg/kg body weight) (3 biological replicates in 3 independent experiments). **h**, FACS plot of Annexin V staining and ERAI signal in purified HSCs after 18 hours of Thapsigargin and proteasome inhibitor MG-132 treatment (2 biological replicates in 2 independent experiments). **i**, Representative FACS plot of Annexin V staining and ERAI signal in purified wild type HSC after 6-12 hours of Thapsigargin treatment (2 biological replicates in 2 independent experiments). Data represent mean±s.d. for all panels. Two-sided student t-test was used for statistical analysis.



Supplementary Figure 2

PERK activation as indicated by ATF4 reporter activity didn't predict apoptosis in AML cell lines

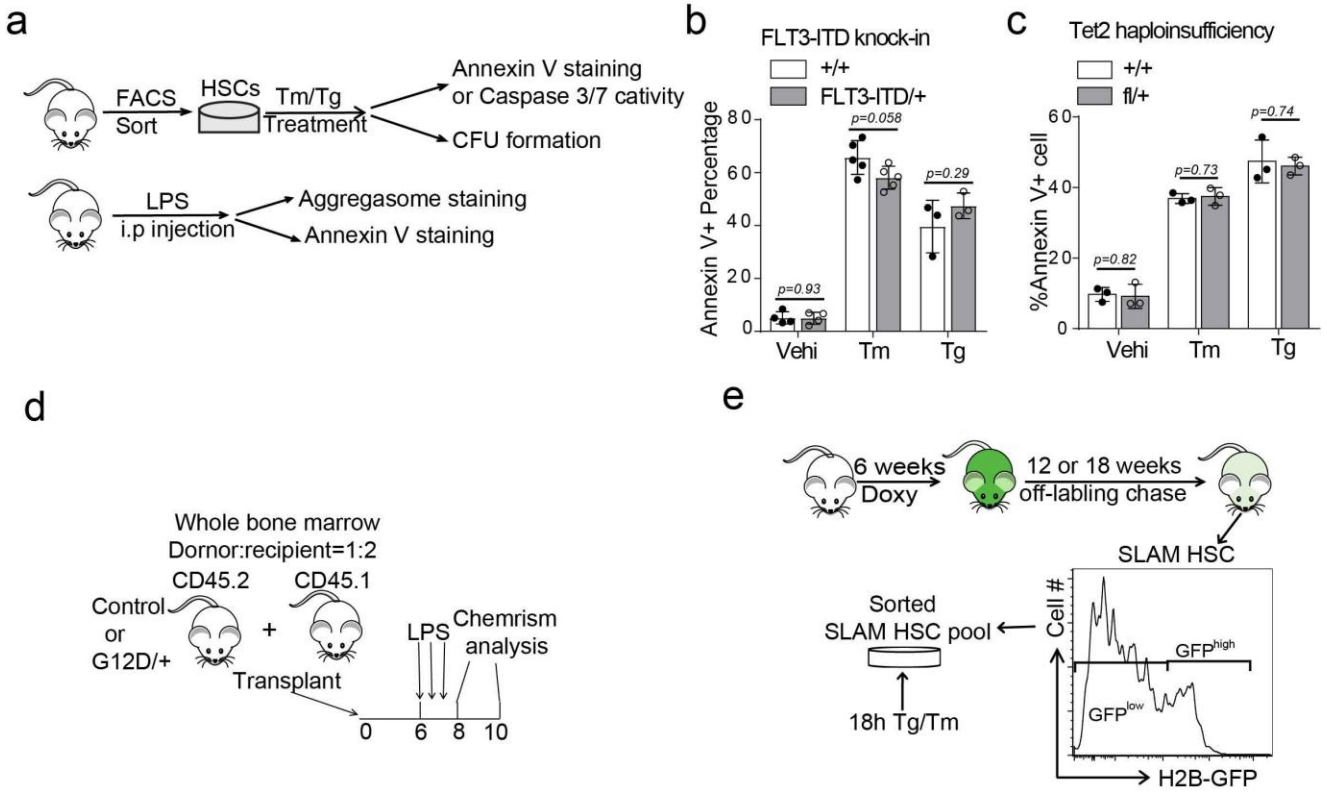
a, Lentiviral construct that expresses reporter gene DsRed express 2 under control of ATF4 uORFs that are activated by eIF2α (stress sensor, PE A4RP1.2), and its controls that express either constitutively active (PE A4RP3.2) or inactive (PE A4RP2.2) DsRed expression. **b**, Representative FACS plot of basal DsRed express 2 level in OCI-AML2 and OCI-AML3 cells transduced with these constructs. **c-e**, Representative FACS plots of DsRed Express 2 level in OCI-AML2 and OCI-AML3 cells transduced with stress sensor (**c**), constitutively inactive (**d**), or constitutively active (**e**) ATF4 reporter constructs and treated with vehicle (vehi), 5 mM Thapsigargin (Tg) or 10 μM Sal003 for 24 hours. Analysis was done on transduced cells (gated on ZsGreen positive cells). **f**, Representative FACS plots of Annexin V staining and ATF4 reporter signal in OCI-AML and OCI-AML3 cells treated with vehicle (vehi), 5 mM Thapsigargin (Tg) or 10 μM Sal003 for 24 hours (2 biological replicates in 2 independent experiments).



Supplementary Figure 3

Effects of IRE1α Knock-out on steady-state haematopoiesis.

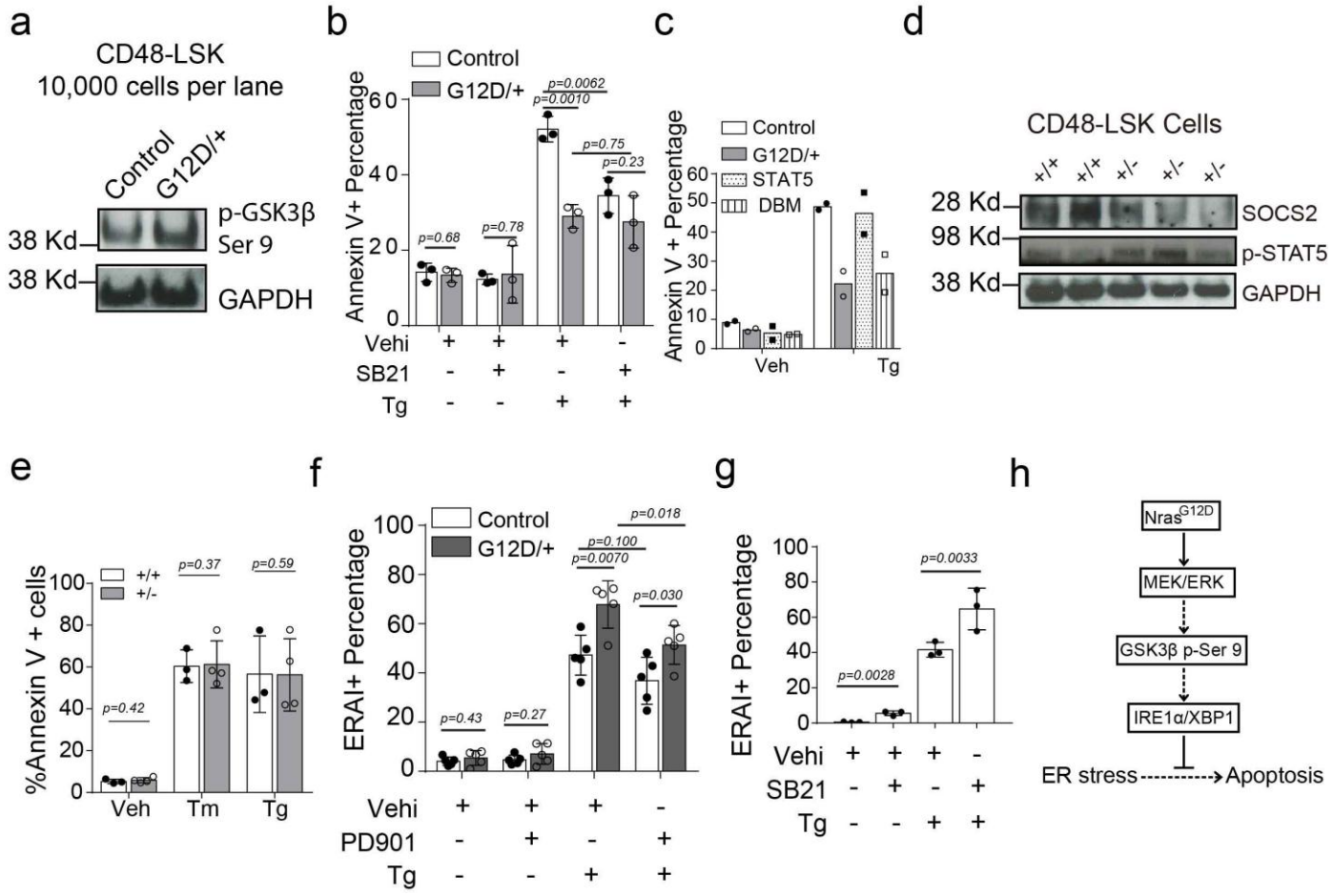
a-g, Analysis of steady-state haematopoiesis in age- and sex-matched *Mx1-cre*$^{+}$; *IRE1α*$^{fl/fl}$ (fl/fl) and *Mx1-cre*$^{-}$; *IRE1α*$^{fl/fl}$ (+/+) mice at least 2 weeks post-pIpC injection (n=4 biological replicates in 4 independent experiments). **a**, Spleen weight. **b**, Cellularity in Bone marrow and spleen. **c**, Total numbers of haematopoietic stem and progenitor cells in the bone marrow and spleen. **d**, Frequency of haematopoietic stem and progenitor cells in bone marrow. **e**, Frequency of myeloid (Mac-1+), B (B220+) and T (CD3+) cells in the bone marrow. **f**, Frequency of haematopoietic stem and progenitor cells in the spleen. **g**, Frequency of myeloid (Mac-1+), B (B220+) and T (CD3+) cells in the spleen. Data represent mean±s.d. Two-sided student t-test was used for statistical analysis.



Supplementary Figure 4

N-Ras^{G12D} but not FLT3-ITD or Tet2 haploinsufficiency leads to HSC protection from ER stress-induced apoptosis

a, Experimental scheme of the assay for results depicted in **Figure 2a-e**, **b,** Summary of the percentage of Annexin V positive cells in HSCs purified from 8-10 weeks old *FLT3-ITD* knock-in mice (FLT3-ITD/+) and sex-matched littermate control mice (+/+) and treated 18 hours of 0.6 μg/ml Tunicamycin(Tm) and 0.2 μM Thapsigargin (Tg) (n=3-5 biological replicates in 3 independent experiments). **c,** Summary of apoptosis analysis of HSCs from *Mx1-cre^{-}; Tet2$^{fl/+}$* (+/+) or *Mx1-cre^{+}; Tet2$^{fl/+}$* (fl/+) mice at least 2 weeks after 6 dose of pIpC injection, and treated with 18 hours of 0.6 μg/ml Tunicamycin(Tm) and 0.2 μM Thapsigargin (Tg) (n=3 biological replicates in 1 independent experiments). **d,** Experimental scheme of chimerism maintenance experiment for **Figure 2f**. 0.5 million bone marrow cells from *Mx1-cre^{+}*; *Nras$^{G12D/+}$* (G12D/+) or control CD45.2 mice (2 weeks after pIpC injection) were transplanted with 0.5 million competitor (CD45.1) bone marrow cells into recipient mice. Six weeks after transplantation, transplant recipients were injected with 2 mg/kg LPS for 3 doses every other day and HSCs chimerism was analyzed 2-4 weeks after LPS injections. **e**, Experimental scheme of analysis in *Col1α1-H2B-GFP; Rosa26-M2-rtTA* mice. Mice were treated with doxycycline for 6 weeks to label HSCs with GFP, after which doxycycline water was removed to allow HSCs to dilute GFP signal upon cell division. GFP^{high} and GFP^{low} HSCs (Gating strategy to separate GFP^{high} and GFP^{low} HSCs is shown on the bottom right) were purified after 18 weeks off doxycycline and were treated with Tg or Tm for 18 hours. Data represent mean±s.d. for panels **b** and **c**. Two-sided student t-test was used for statistical analysis.

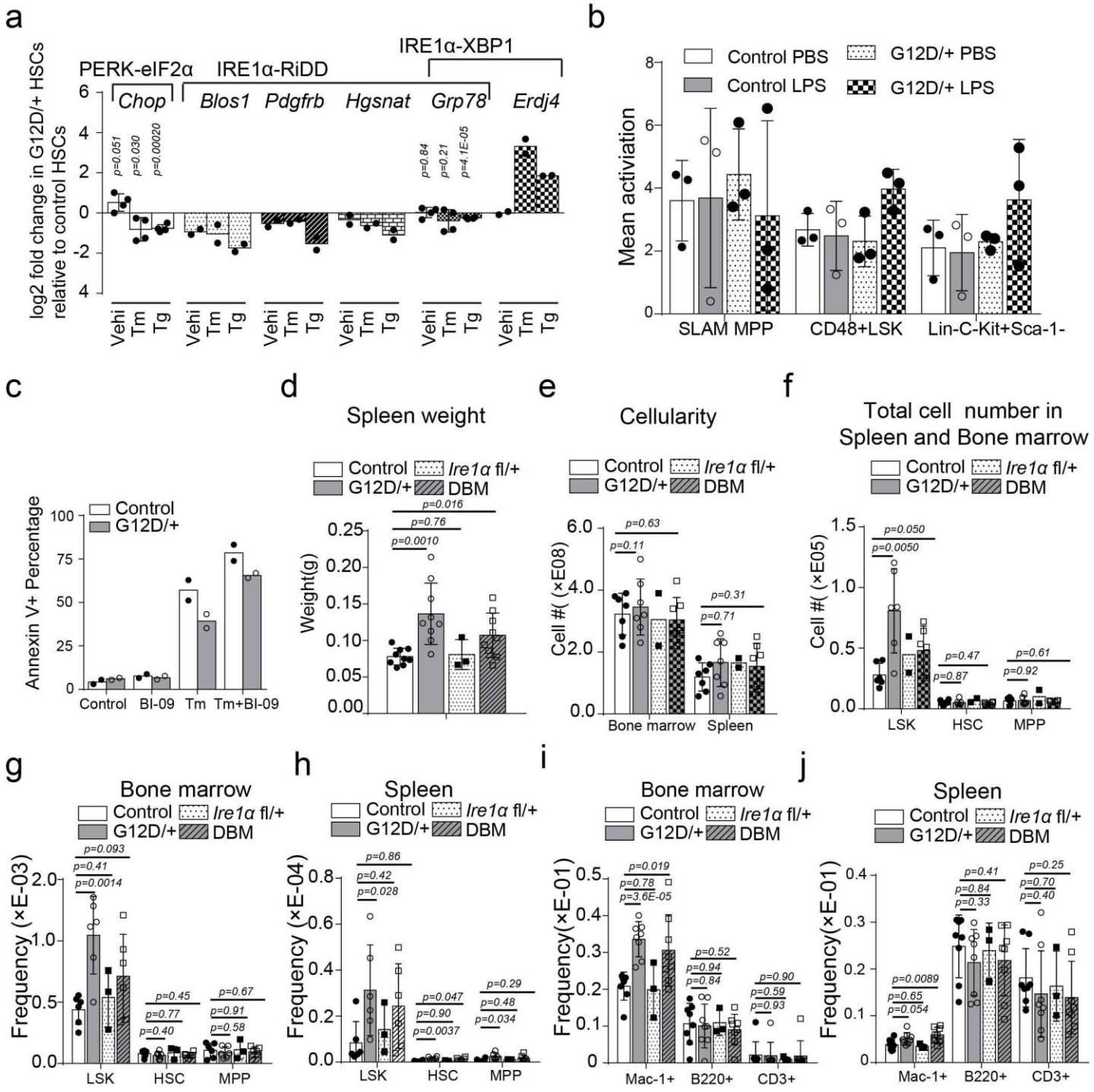


Supplementary Figure 5

MEK/ERK/GSK3β but not STAT5 is required for N-Ras^{G12D} mediated protection under ER stress

a, Representative Western blot detecting phosphorylation of Serine 9 of GSK3β in CD48-LSK purified from *Mx1-cre*$^{-}$; *Nras*$^{G12D/+}$ (Control), or *Mx1-cre*; *Nras*$^{G12D/+}$ (G12D/+), and then cultured for 10 minutes in the presence of 10 ng/ml SCF/TPO (3 biological replicates in 3 independent experiments). Original blots are shown in **Supplementary Figure 7**. **b,** Summary of the percentage of Annexin V positive HSC purified from *Mx1-cre*$^{-}$; *Nras*$^{G12D/+}$ (Control), or *Mx1-cre*$^{+}$; *Nras*$^{G12D/+}$ (G12D/+) mice that were treated with 0.2 μM Thapsigargin (Tg) together with 5 μM GSK3β inhibitor SB216763 (SB21) for 18 hours (n=3 independent experiments). **c,** Summary of the percentage of Annexin V positive HSCs from *Mx1-cre*$^{-}$; *Nras*$^{G12D/+}$ *stat5a/b*$^{fl/+}$ or *Mx1-cre*$^{-}$; *Nras*$^{G12D/+}$ *stat5a/b*$^{+/+}$ (Control), *Mx1-cre*$^{+}$; *Nras*$^{G12D/+}$ (G12D/+), *Mx1-cre*$^{+}$; *stat5a/b*$^{fl/+}$(STAT5), or *Mx1-cre*$^{+}$; *Nras*$^{G12D/+}$ *stat5a/b*$^{fl/+}$ double mutant (DBM) mice after 0.6 μg/ml Tunicamycin (Tm) or 0.2 μM Thapsigargin(Tg) treatment for 18 hours (n=2 biological replicates in 2 independent experiments). **d,** Western blot detecting the level of SOCS2 and phosphorylation of STAT5 in CD48-LSK cells purified from SOCS2$^{+/+}$ (+/+) and

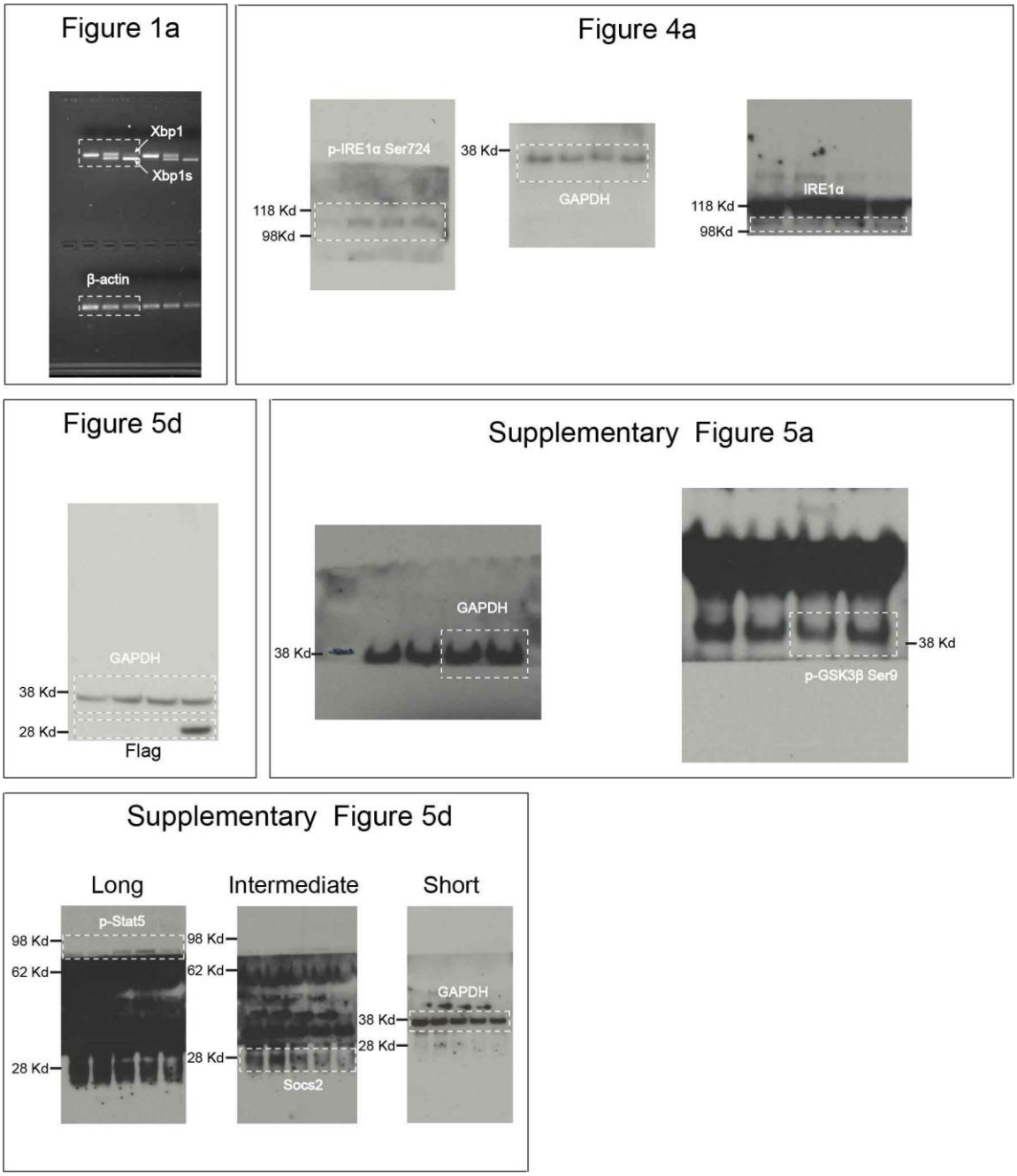
SOCS2$^{+/-}$ (+/-) mice. Original blots are shown in **Supplementary Figure 7**. (2-3 biological replicates in 1 independent experiments) **e,** Summary of the percentage of Annexin V positive CD48-LSK cells purified from 6-12 weeks old SOCS2$^{+/+}$ (+/+) and SOCS2$^{+/-}$(+/-) mice that were treated with 0.6 μg/ml Tunicamycin (Tm) or 0.2 μM Thapsigargin (Tg) for 18 hours (n=3 biological replicates for +/+, n=4 biological replicates for +/-, pooled from 2 independent experiments). **f,** Summary of the percentage of ERAI positive HSC purified from *Mx1-cre*$^{-}$; *ERAI*$^{+}$; *Nras*$^{G12D/+}$ (Control), or *Mx1-cre*$^{+}$; *ERAI*$^{+}$; *Nras*$^{G12D/+}$ (G12D/+) mice that were treated with 0.2 μM Thapsigargin (Tg) together with 100nM MEK inhibitor PD0325901 for 18 hours (n=5 biological replicates in 5 independent experiments). **g,** Summary of the percentage of ERAI positive HSC purified from *ERAI*$^{+}$ mice treated with 0.2 μM Thapsigargin(Tg) together with 5 μM GSK3β inhibitor SB216763 (SB21) for 18 hours (n=3 biological replicates in 3 independent experiments). **h**, Schematic model showing that N-Ras^{G12D}-activated MEK/ERK signaling leads to phosphorylation and inhibition of GSK3β which results in activation of the adaptive IRE1α -XBP1 ER stress signaling to protect HSCs from ER stress-induced apoptosis. Data represent mean±s.d. . Two-sided student t-test was used for statistical analysis.



Supplementary Figure 6

IRE1α is hyper-activated in Nras^{G12D} HSCs and is required for N-Ras^{G12D} mediated HSC protection, LSK and myeloid expansion. **a**, Log ratio of the level of UPR target genes (measured by qRT-PCR) in *Nras* mutant HSCs relative to the level in control HSCs after tunicamycin (Tm) or thpasigargin (Tg) treatment (n=4 biological replicates in 2 independent experiments for *Chop*, *Grp78*, n=2 biological replicates in 2 independent experiments for *Blos1*,*Pdgfrb*,*Hgsnat*,*Erdj4*). **b**, Activation of IRE1α in different haematopoietic

populations from *Mx1-cre*$^{-}$; *ERAI*$^{+}$; *Nras*$^{G12D/+}$ (Control), or *Mx1-cre*$^{+}$; *ERAI*$^{+}$; *Nras*$^{G12D/+}$ (G12D/+) mice injected with LPS was measured by mean induction of ERAI signal (Mean florescent intensity of ERAI in ERAI+ cells relative to ERAI- cells). (n=3 biological replicates in 3 independent experiments)**. c**, Summary of the percentage of Annexin V positive HSC from *Mx1-cre*$^{-}$; *Nras*$^{G12D/+}$ (Control), *Mx1-cre*$^{+}$; *Nras*$^{G12D/+}$ (G12D/+) that were treated with 0.6 μg/ml tunicamycin (Tm) together with 20 μM IRE1α inhibitor BI-09 for 18 hours (n=2 biological replicates in 2 independent experiments). **d-j**, The steady state haematopoiesis in age- and sex-matched *Mx1-cre*$^{-}$; *Nras*$^{G12D/+}$; *Ire1α*$^{fl/+}$ (control) , *Mx1-cre*$^{+}$; *Nras*$^{G12D/+}$; *Ire1α*$^{+/+}$ (G12D/+) , *Mx1-cre*$^{+}$; *Nras*$^{+/+}$; *Ire1α*$^{fl/+}$ (Ire1α fl/+) ,*Mx1-cre*$^{+}$; *Nras*$^{G12D/+}$; *Ire1α*$^{fl/+}$ (DBM) mice was analyzed 2 weeks post-pIpC injection. **d** Spleen weight. n=9 (control, G12D/+ and DBM), and 3 (IRE1α fl/+) biological replicates in 6 independent experiments, **e,** Cellularity in Bone marrow and spleen. .n=7 (control, G12D/+ and DBM), and 2 (IRE1α fl/+) biological replicates in 6 independent experiments, **f,** Total numbers of haematopoietic stem and progenitor cells in bone marrow and spleen. .n=6 (control, G12D/+ and DBM), and 2 (IRE1α fl/+) biological replicates in 6 independent experiments,. **g-h,** Frequency of haematopoietic stem and progenitor cells in bone marrow (**g**) and spleen (h). . n=6 (control, G12D/+ and DBM), and 3 (IRE1α fl/+) biological replicates in 6 independent experiments,. **I-j,** Frequency of lineage cells in bone marrow (i) and spleen (j). . n=8 (control, G12D/+ and DBM), and 3 (IRE1α fl/+) biological replicates in 6 independent experiments, Data represent mean±s.d. Two-sided student t-test was used for statistical analysis.



Supplementary Figure 7

Original images of Western blots and DNA gel.

While boxes indicate the images used for figures. Because of the small number of HSCs (5,000-10,000 cells/mouse) or CD48-LSK (contains HSCs and MPPs; 20,000-30,000 cells/mouse), to ensure adequate protein amount, the entire protein lysates from one set of FACS-sorted samples were used for one SDS-PACE gel. The Western membranes were then sectioned into 3-4 strips based on molecular weight of the standard protein ladder, to allow simultaneous probing for multiple proteins on using the same protein samples. Images for Supplementary Figure 5d represents a Western membrane that was cut into 4 strips, each probed for different antibody. The strips were then aligned together for autoradiography. Three different exposure times were included to show the detection of different proteins.

Supplementary Table 1. List of all antibodies used for experiments.

Supplementary Table 2. Statistics source data.