SUPPLEMENTARY APPENDIX

Inhibition of the anti-apoptotic protein MCL-1 severely suppresses human hematopoiesis

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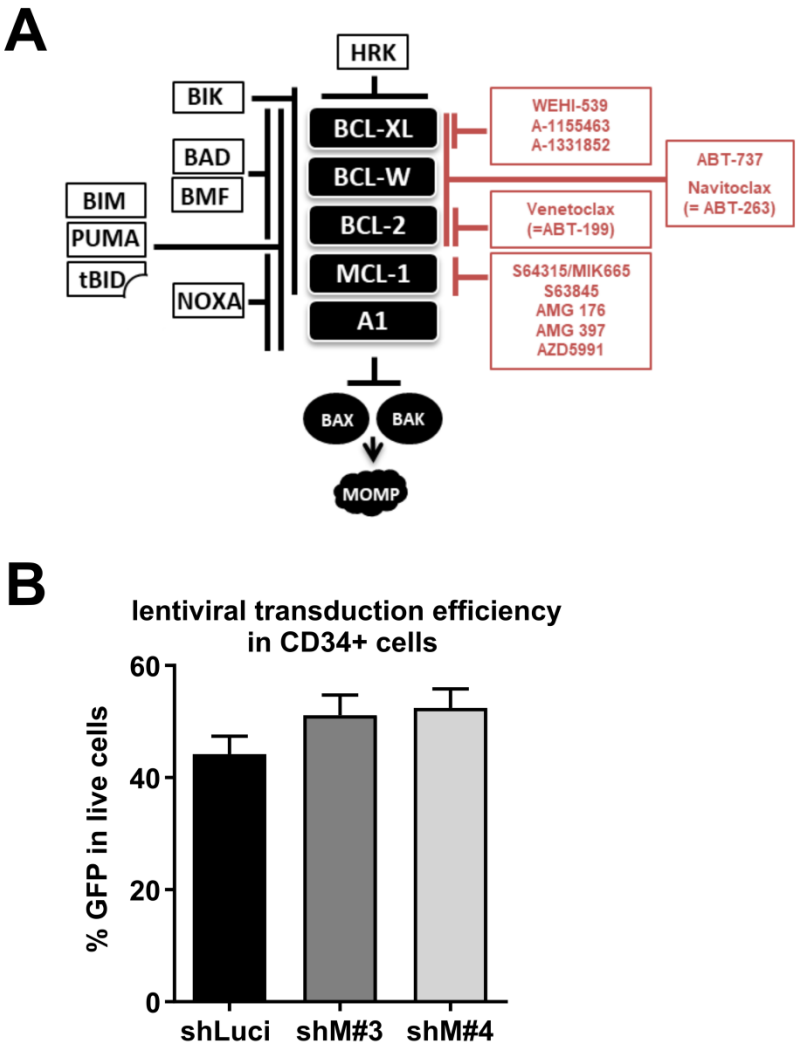
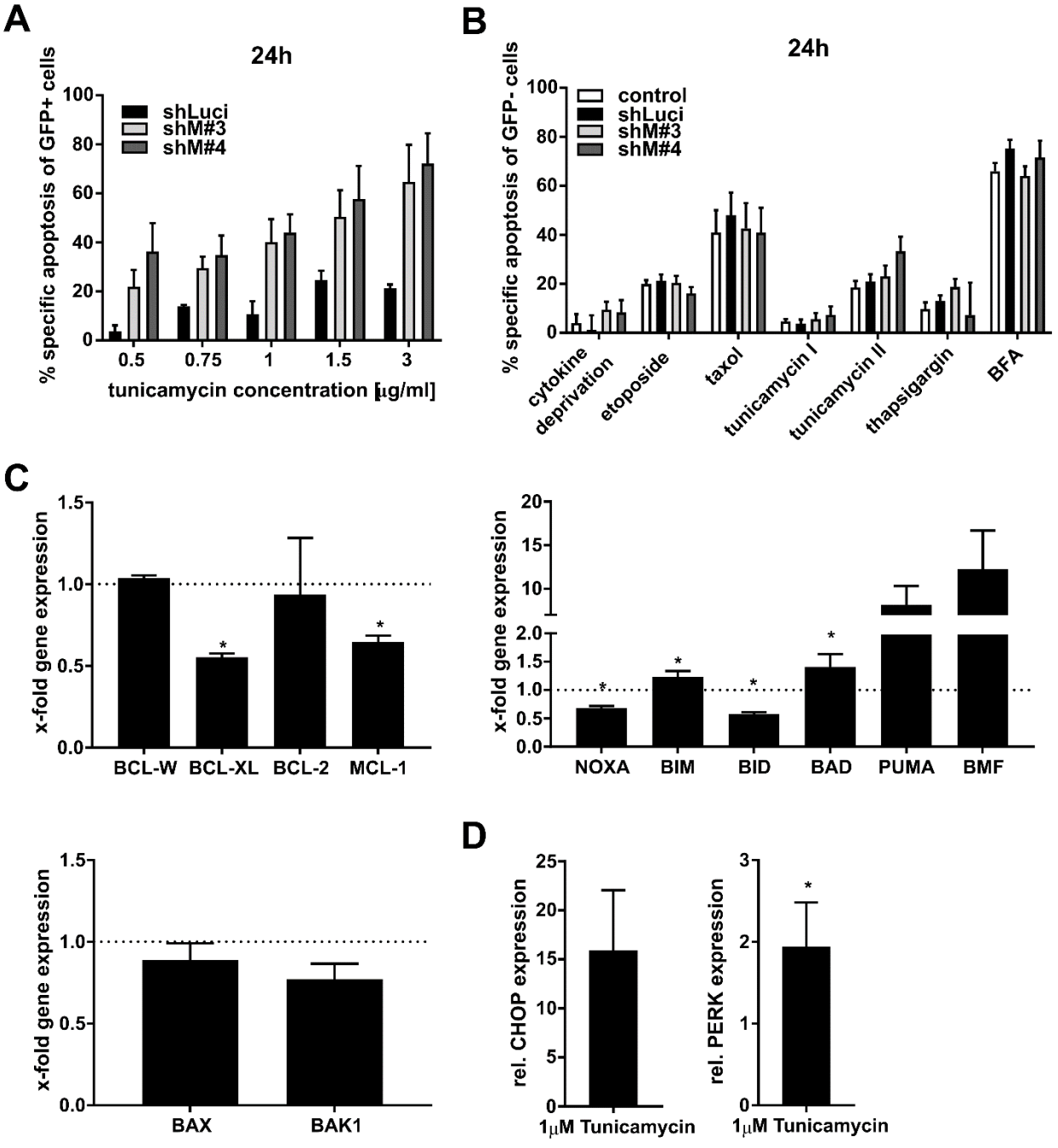
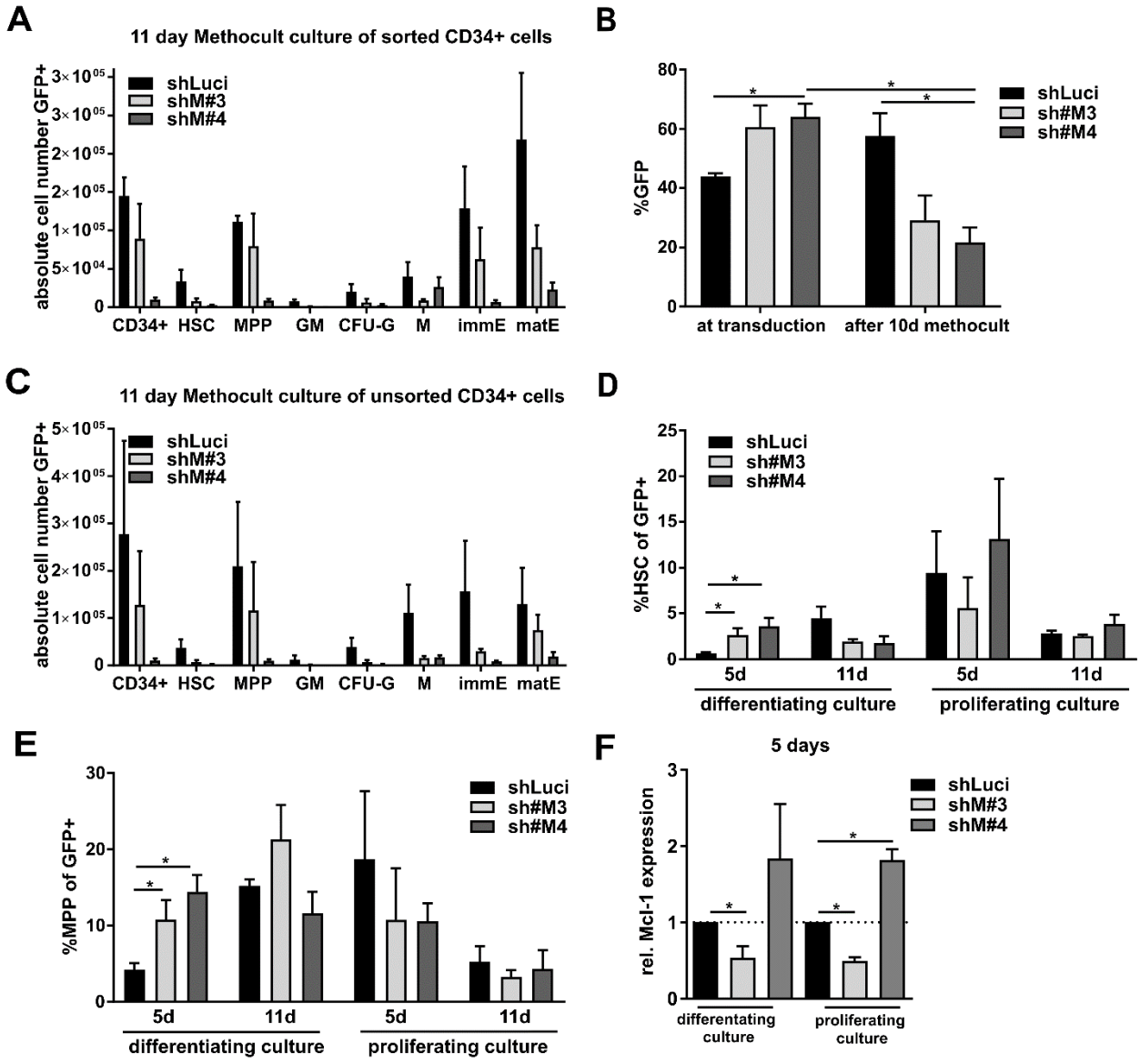


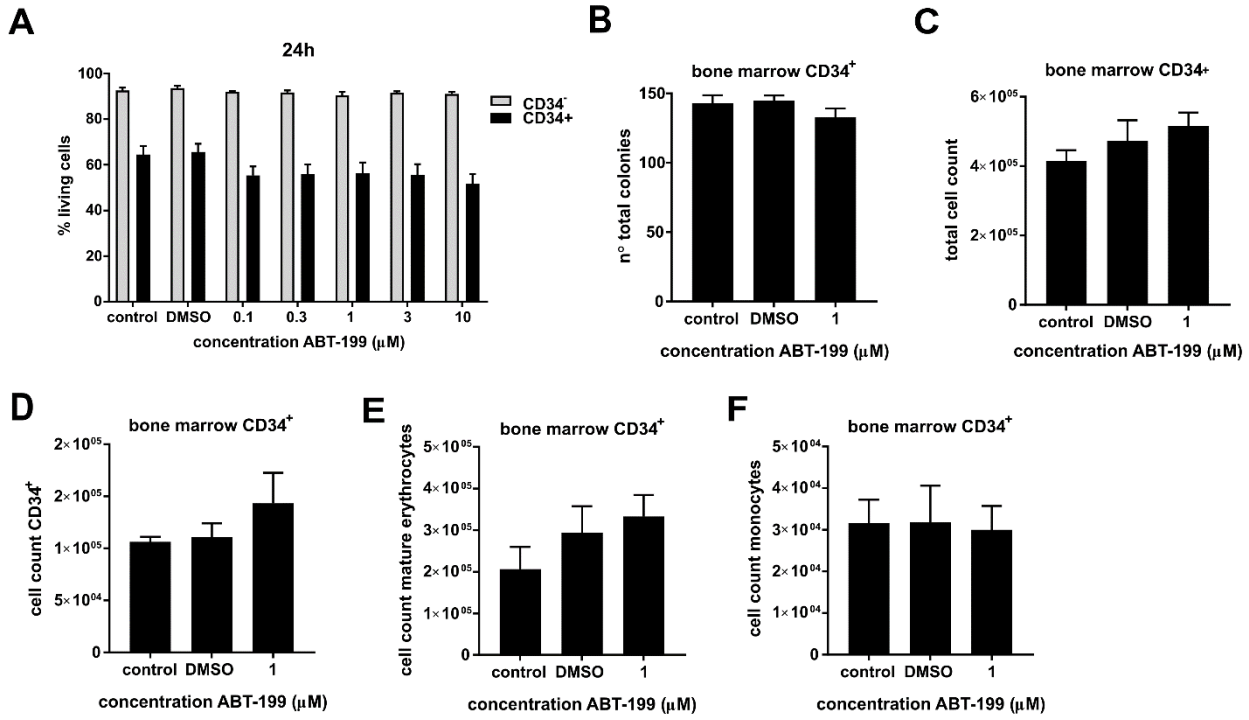
Suppl. Figure 1:
(A) BH3-mimetics (red) inhibit anti-apoptotic BCL-2 proteins (black, rectangular) with specific binding activities, thereby acting like BH3-only proteins (white). As a consequence, BAX and BAK are activated and mitochondrial outer membrane permeabilization (MOMP) is initiated.
(B) Human cord blood-derived $CD34^+$ cells were transduced with the according lentiviruses. Transduction efficiency was measured by GFP expression 24h after the end of viral transduction via flow cytometry. Bars represent mean ±SEM; n=31 from 31 independent experiments.


A
24h
% specific apoptosis of GFP+ cells
shLuci
shM#3
shM#4
tunicamycin concentration [µg/ml]
B
24h
% specific apoptosis of GFP- cells
control
shLuci
shM#3
shM#4
cytokine deprivation
etoposide
taxol
tunicamycin I
tunicamycin II
thapsigargin
BFA
C
x-fold gene expression
BCL-W
BCL-XL
BCL-2
MCL-1
NOXA
BIM
BID
BAD
PUMA
BMF
BAX
BAK1
D
rel. CHOP expression
1µM Tunicamycin
rel. PERK expression
1µM Tunicamycin

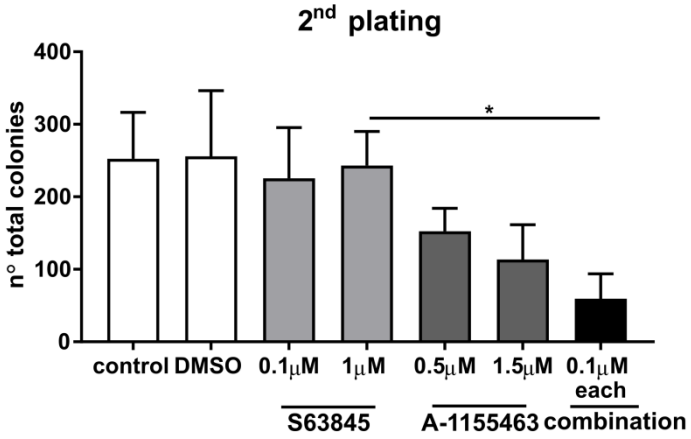
Suppl. Figure 2:
(A) Transduced $CD34^+$ cells were treated with increasing doses of the ER-stress inducer tunicamycin ranging between 0.5 and 3 µg/ml. 24 hours later, apoptosis and GFP expression were detected by flow cytometry. Specific apoptosis of the GFP^+ cells was determined. Bars represent mean ± SEM (n=2)
(B) Transduced $CD34^+$ cells were either cultured under optimal conditions (cytokines and 10% serum) or under conditions of stress; in the presence of serum but deprived of cytokines, etoposide (0.5 µg/ml), taxol (0.125 µg/ml), tunicamycin (0.5 (I) and 1 µg/ml (II)), thapsigargin (3 µM) or brefeldin A (BFA; 0.5 µg/ml). Apoptosis in GFP^- (untransduced) cells was determined by flow cytometry using Annexin V and 7-AAD staining 24 hours later. Bars represent mean ± SEM; n=3-8 from 8 independent experiments.
(C) $CD34^+$ cells were subjected to 1µM tunicamycin and harvested after 12 hours. mRNA was used for RT-MLPA designed to determine levels of apoptosis genes. Anti-apoptotic BCL-2 proteins (first panel), BH3-only proteins (second panel) and pro-apoptotic effector proteins (third panel) are shown. Untreated $CD34^+$ cells were used as controls. Bars represent mean ± SEM from n=3-4 from 4 independent experiments.
(D) $CD34^+$ cells were subjected to 1µM tunicamycin and harvested after 12 hours. mRNA was used for qRT-PCR of ER-stress related genes. *CHOP* and *PERK* mRNA expression was normalized to 18S. Bars represent mean ± SEM; n=4 from 4 independent experiments.


A
11 day Methocult culture of sorted CD34+ cells
absolute cell number GFP+
shLuci
shM#3
shM#4
CD34+ HSC MPP GM CFU-G M immE matE
B
%GFP
shLuci
sh#M3
sh#M4
at transduction
after 10d methocult
C
11 day Methocult culture of unsorted CD34+ cells
absolute cell number GFP+
shLuci
shM#3
shM#4
CD34+ HSC MPP GM CFU-G M immE matE
D
%HSC of GFP+
shLuci
sh#M3
sh#M4
5d
11d
5d
11d
differentiating culture
proliferating culture
E
%MPP of GFP+
shLuci
sh#M3
sh#M4
5d
11d
5d
11d
differentiating culture
proliferating culture
F
5 days
rel. Mcl-1 expression
shLuci
shM#3
shM#4
differentating culture
proliferating culture

Suppl. Figure 3:
(A) Lentivirally transduced human $CD34^+$ cells were sorted for GFP expression and GFP^+CD34^+ cells were seeded in MethoCult medium (1000 cells each).
(B-C) Alternatively, unsorted cells were plated. Different immature cell types were determined by flow cytometry, and their absolute cell numbers were calculated. Bars represent mean ± SEM, n=6 **(A)** from 6 independent experiments and n=4 **(B-C)** from 4 independent experiments. Mann–Whitney test was performed; *p<0.05. The following cell types were determined; HSC: hematopoietic stem cells (CD34+CD38-CD45RA-CD90+), MPP: multipotent progenitors (CD34+38-CD45RA-CD90-), GM: granulocytic–monocytic progenitors (CD34+CD33+CD115+), CFU-G: colony forming unit-granulocytes (CD34-CD33+CD15+CD115), M: monocytes (CD34-CD33+CD14+CD115-), immE: immature erythrocytes (CD71hiCD235a-), matE: mature erythrocytes (CD71+CD235a+)
(D-F) Lentivirally transduced human $CD34^+$ cells were either subjected to differentiating or proliferating culture conditions. To induce differentiation, cells were cultured in semisolid MethoCult plates for 5 days (10.000 cells seeded per plate). To foster proliferation, cells were cultured in stem cell medium containing 10% serum and SCF, FLT3L (200 ng/ml each), TPO (100 ng/ml) and IL-3 (20 ng/ml). After 5 and 10 days, percentages of HSC **(D)** and MPP **(E)** were determined by flow cytometry. After 5 days, knockdown efficiency of MCL-1 was determined in sorted GFP^+ cells by qRT-PCR. mRNA expression was normalized to 18S.**(F)** Bars represent mean ±SEM; n=4 from 4 independent experiments. Mann–Whitney test was performed. *p<0.05



Suppl. Figure 4:
(A) Freshly isolated bone marrow was subjected to density gradient centrifugation and mononuclear cells were divided into $CD34^+$ and $CD34^-$ cells using MACS technology. Both cell fractions were treated with the indicated concentrations of the BCL-2 inhibitor ABT-199. After 24 hours, %living cells were determined by flow cytometry using Annexin V/7-AAD. Bars represent mean ± SEM; n=3-4 from 4 independent experiments.
(B-F) Human bone marrow derived $CD34^+$ cells were differentiated in MethoCult culture containing 1µM of ABT-199. As controls, untreated and DMSO-treated cells were used (n=3 from 3 independent experiments). After 11 days, total colony numbers **(B)** and total cell numbers **(C)** were determined using light microscopy and hemocytometry, respectively. Different cell types were determined by flow cytometry, and their absolute cell numbers were calculated **(D-F).** The following cell types were determined; immature $CD34^+$ cells, monocytes (CD34-CD33+CD14+CD115-) and mature erythrocytes (CD71+CD235a+).



Suppl. Figure 5:
Human CD34$^+$ were differentiated in MethoCult culture (1000 cells seeded per plate) in the presence of the MCL-1 inhibitor S63845 (0.1µM or 1µM), the BCL-XL inhibitor A-1155463 (0.5µM or 1.5µM) or a combination of both inhibitors (0.1µM or 1µM each). After 10 days, cells were isolated from the MethoCult medium and washed. 10,000 cells were replated into fresh MethoCult medium. No inhibitors were added to this culture. After a 10 days culture, colony numbers were determined by light microscopy. Bars represent mean ± SEM, n =5 from 5 independent experiments. Mann–Whitney test was performed. *$p<0.05$

Supplementary Table 1: Oligonucleotides and Primers

Oligonucleotides (Eurofins/Apara)	
Luci shRNA	Top: 5′-AACCCCCGCTGAGTACTTCGAAATGTCTTCAAGAGA GACATTTCGAAGTACTCAGCGTTTTTTC-3′ Bottom: 5′-TCGAGAAAAAACGCTGAGTACTTCGAAATGTC TCTCTTGAAGACATTTCGAAGTACTCAGCGGGGGTT-3′
MCL-1#3 shRNA	Top: 5′ AACCCCGCAAGAGGATTATGGCTAATTCAAGAGA TTAGCCATAATCCTCTTGCTTTTTTC-3′ Bottom: 5′-TCGAGAAAAAAGCAAGAGGATTATGGCTAA TCTCTTGAATTAGCCATAATCCTCTTGCGGGGTT-3′
MCL-1#4 shRNA	Top: 5′-AACCCCGCTGTGTTAAACCTCAGAGTTTTCAAGAGA AACTCTGAGGTTTAACACAGCTTTTTTC-3′ Bottom: 5′-TCGAGAAAAAAGCTGTGTTAAACCTCAGAGTT TCTCTTGAAAACTCTGAGGTTTAACACAGCGGGGTT-3′
qRT-PCR Human Primers (Eurofins/Apara/biomers)	
18S	Forward: 5′-TCAAGAACGAAAGTCGGAGG-3′ Reverse: 5′-GGACATCTAAGGGCATCACA-3′
36B4	Forward: 5′-CAGCAAGTGGGAAGGTGTAATCC-3′ Reverse: 5′-CCCATTCTATCATCAACGGGTACAA-3′
MCL-1	Forward: 5′-AGTTAAACAAAGAGGCTGGGATGGGTT-3′ Reverse: 5′-GCCAAACCAGCTCCTACTCCAGC-3′
PERK	Forward: 5′-AATGCCTGGGACGTGGTGGC-3′ Reverse: 5′-TGGTGGTGCTTCGAGCCAGG-3′
CHOP	Forward: 5′-GGAGCATCAGTCCCCCCACTT-3′ Reverse: 5′-TGTGGGATTGAGGGTCACATC-3′
Sequencing Primers (Eurofins)	
pLeGOhU6	Reverse: 5′-TGGCCCAACGTTAGCTATTTTCAT-3′

Supplementary Table 2: Hematopoietic population definitions

Population	Abbreviation	Characterization
Hematopoietic Stem Cells	HSC	$CD34^{+}$ $CD38^{-}$ $CD45RA^{-}$ $CD90^{+}$
Mixed Lymphoid Progenitor	MLP	CD34+ CD38- $CD45RA^{+}$ $CD10^{+}$
Multipotent Progenitors	MPP	$CD34^{+}$ $CD38^{-}$ $CD45RA^{-}$ $CD90^{-}$
Colony Forming Unit Granulocytes	CFU-G	$CD34^{-}$ $CD33^{+}$ $CD15^{+}$ $CD115^{-}$
Granulocytic Monocytic precursors	GM	$CD34^{+}$ $CD33^{+}$ $CD115^{+}$
Monocytes/Macrophage precursors	M	$CD34^{-}$ $CD33^{+}$ $CD14^{+}$ $CD115^{-}$
Immature erythroid cells	-	$CD71^{high}$ $CD235a^{-}$
Mature erythroid cells	-	$CD71^{+}$ $CD235a^{+}$