Supplemental Information

Role of reactive oxygen species in regulating 27-hydroxycholesterol-induced apoptosis of hematopoietic progenitor cells and myeloid cell lines

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Supplemental Methods and Materials

7AAD was added before FACS analysis. The following antibody cocktails/antibodies were used:

Mature Cell Lineages:

Antibody	**Company**	**Cat**
PE-Cy7-CD11b (M1/70)	Biolegend	101216
APC-Gr1 (RB6-8C5)	eBioscience	17-5931-82
PE-B220R (RA3-6B2)	BD	553089
APC-Cy7-CD3 (145-2C11)	Biolegend	100330

For HSPC analysis:

Antibody	**Company**	**Cat**
PE-Cy5-CD3 (145-2C11)	BD	555276
PE-Cy5-CD4 (RM4-5)	BD	553050
PE-Cy5-CD8 (53-6.7)	Biolegend	100710
PE-Cy5-CD19 (6D5)	Biolegend	115510
PE-Cy5-B220R (RA3-6B2)	eBioscience	15-0452-82
PE-Cy5-Gr1 (RB6-8C5)	eBioscience	15-5931-82
PE-Cy5-Ter119 (TER119)	eBioscience	15-5921-82
PE-Sca1 (D7)	eBioscience	12-5981-82
APC-cKit (2B8)	Biolegend	105812
PE-Cy7-CD150 (TC15-12F12.2)	Biolegend	115914
APC-Cy7-CD48 (HM48-1)	BD	561242
FITC-CD34 (RAM34)	eBioscience	11-0341-85
PE-Cy7-CD16/32 (93)	eBioscience	25-0161-82
APC-eFluor780-CD127 (A7R34)	eBioscience	47-1271-82

For CD45.2 Analysis:

Antibody	**Company**	**Cat**
APC-CD45.1 (A20)	eBioscience	17-0453-82
FITC-CD45.2 (104)	BD	553772

Supplemental Figure Legends

Supplementary Figure 1. Gene expression levels after administration of 27HC. (A) BM cells are treated with 13 μM Cholesterol or 6.2 μM 27OHChol for 48 h, respectively. *Erα* expression was increased in 27HC-treated $Lin^-Sca1^+cKit^+$ cells (LKS) and HPCs ($Lin^-Sca1^+cKit^+$ $CD48^+$). **(B)** *Bax* expression was increased in 27HC-treated LKS and HPCs. (**p≤ 0.01 and ***p≤ 0.001 vs. control).

Supplementary Figure 2. Exogenous addition of 27HC depletes hematopoietic stem and progenitor cells (HSPCs). (A) BM cells are treated with 13 μM Cholesterol or 6.2 μM 27OHChol for 24 h or 48 h, respectively. The frequencies of $Lin^-Sca1^-cKit^+$ cells (LK), $Lin^-Sca1^+cKit^+$ cells (LKS), HPCs ($Lin^-Sca1^+cKit^+$ $CD48^+$), and HSCs ($Lin^-Sca1^+cKit^+CD150^+CD48^-$, SLAM cells) were decreased in the BM cells after exogenous 6.2 μM 27HC treatment for 24h or 48 h. **(B)** BM cells are treated with 13 μM Cholesterol, 0.62 μM or 6.2 μM 27OHChol for 24 h, respectively. No differences in the numbers of HSPC and mature lineage cells were observed after the 0.62 μM 27HC treatment for 24h. **(C)** BM cells are treated with 13 μM Cholesterol or 6.2 μM 27OHChol for 48 h, respectively. Quantification of the $cKit^+$ cells (Lin^- gate) or $CD48^+$ cells (LKS gate) from BM HSPC after the 6.2 μM 27HC treatment for 48h, respectively. **(D)** The frequencies of $cKit^+$ HSPCs were rescued in the BM cells after 1 mM NAC treatment. (*p≤ 0.01 and ***p≤ 0.001 vs. control; $^{\#}$p≤ 0.01 and $^{\#\#\#}$p≤ 0.001 vs. Chol).

Supplementary Figure 3. Administration of 27HC *in vivo*. Wild-type mice inject 27HC by intraperitoneal administration (20 mg/kg, n=5). **(A-C)** The frequencies of $Lin^-Sca1^+cKit^+$ cells (LKS), HSCs ($Lin^-Sca1^+cKit^+CD150^+CD48^-$, SLAM cells), HPCs ($Lin^-Sca1^+cKit^+$ $CD48^+$), granulocyte-macrophage progenitor (GMP) cells ($Lin^-Sca1^-cKit^+CD34^+CD16/32^+$), common myeloid progenitor (CMP) cells ($Lin^-Sca1^-cKit^+CD34^+CD16/32^-$), common lymphoid progenitor (CLP) cells (Lin^- $Sca1^{low}cKit^{low}$ $CD127^+$), and megakaryocyte-erythroid progenitor (MEP) cells ($Lin^-Sca1^-cKit^+CD34^-CD16/32^-$) were tested in the BM cells after exogenous 27HC Intraperitoneal injection (20 mg/kg). (**p≤ 0.01 and ***p≤ 0.001 vs. control).

Supplementary Figure 4. HL60, KG1a, and K562 myeloid leukemic cells are treated with 13 μM Cholesterol or 6.2 μM 27OHChol for 48 h, respectively. (A) HL60, KG1a, and K562 myeloid leukemic cells are treated with indicated concentrations of 27HC for 48

h. **(B)** FACS plot showing the proportion of apoptotic cells in 27HC-treated HL60, KG1a, and K562 myeloid leukemic cells. **(C)** FACS plot showing ROS production in 27HC-treated HL60, KG1a, and K562 myeloid leukemic cells. **(D)** FACS plot showing the normalized fold change in MFI for peIF2α in 27HC-treated HL60 and K562 myeloid leukemic cells. (**p≤ 0.01 and ***p≤ 0.001 vs. untreated).

Supplementary Figure 5. Comparison of leukemic growth between 27HC and 7aHC in (A) HL60 cells and (B) K562 cells. HL60 and K562 myeloid leukemic cells are treated with 13 µM Cholesterol, 6.2 µM 27OHChol, or 6.2 µM 7aOHChol for 48 h, respectively. (**p≤ 0.01 and ***p≤ 0.001 vs. control).

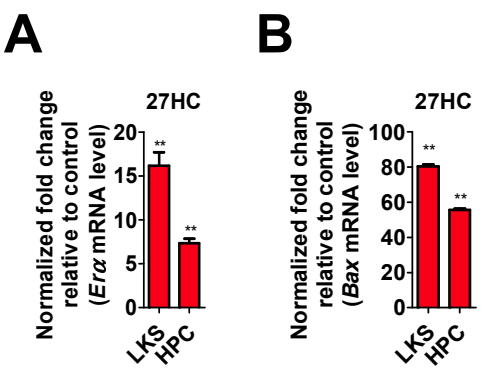
A
27HC
Normalized fold change relative to control (Erα mRNA level)
20
15
10
5
0
**
**
LKS
HPC
B
27HC
Normalized fold change relative to control (Bax mRNA level)
100
80
60
40
20
0
**
**
LKS
HPC

Figure S1

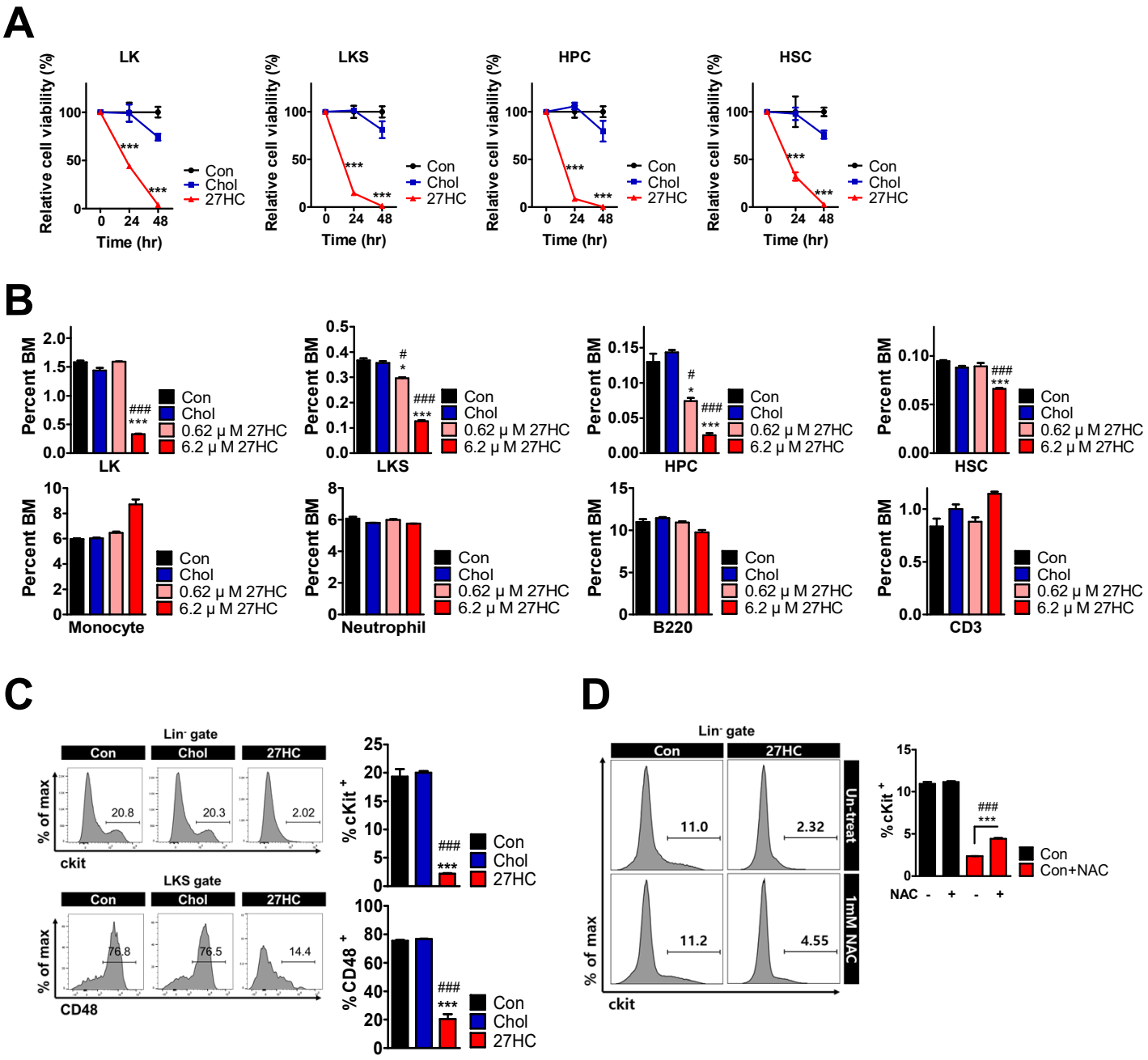
A
LK
LKS
HPC
HSC
Relative cell viability (%)
Time (hr)
Con
Chol
27HC
B
Percent BM
LK
LKS
HPC
HSC
Monocyte
Neutrophil
B220
CD3
Con
Chol
0.62 μ M 27HC
6.2 μ M 27HC
C
Lin- gate
Con
Chol
27HC
% of max
ckit
20.8
20.3
2.02
LKS gate
CD48
76.8
76.5
14.4
% cKit +
% CD48 +
D
Lin- gate
Un-treat
1mM NAC
11.0
2.32
11.2
4.55
Con+NAC
NAC

Figure S2

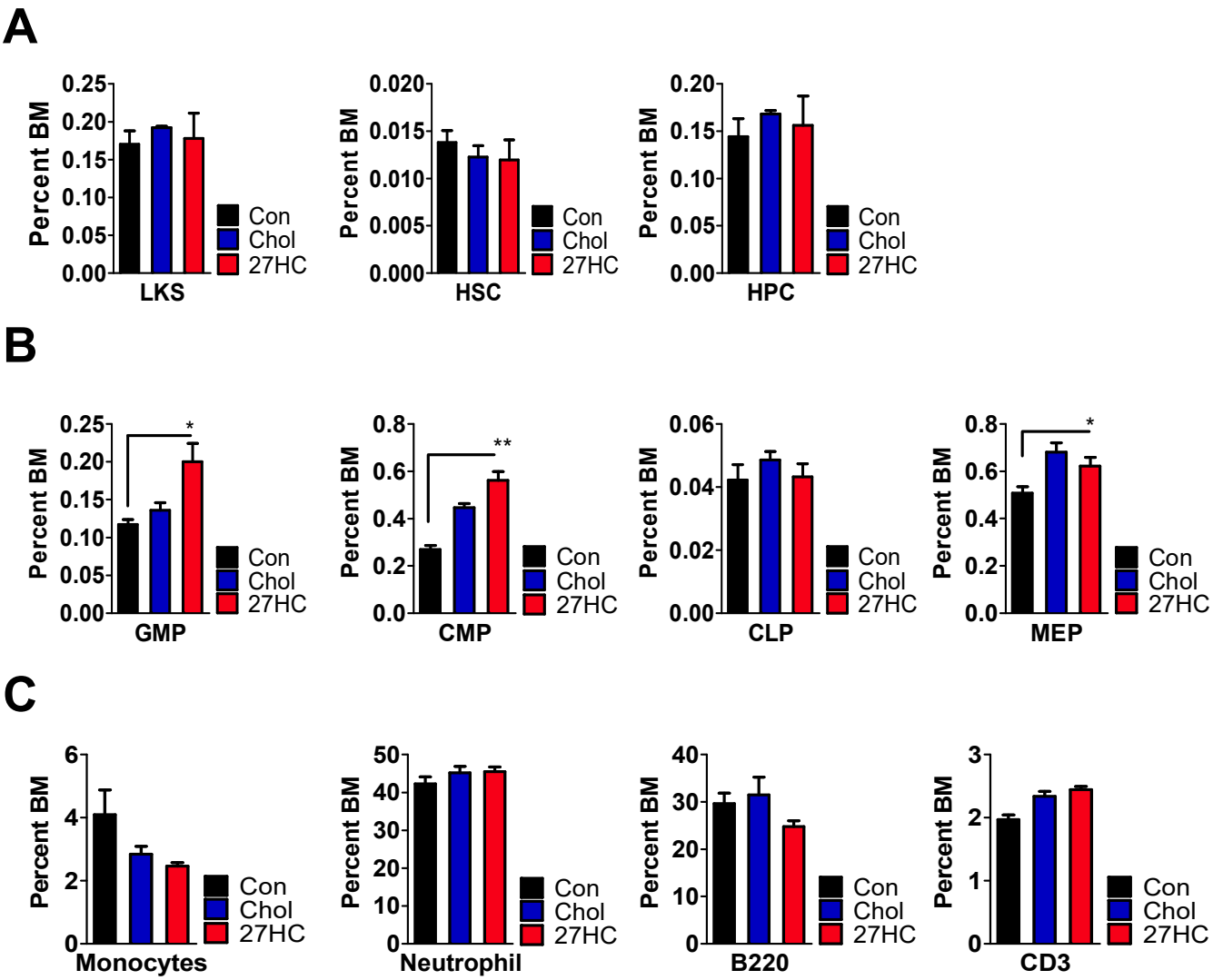
A
Percent BM
0.25
0.20
0.15
0.10
0.05
0.00
LKS
Con
Chol
27HC
0.020
0.015
0.010
0.005
0.000
HSC
0.20
0.15
0.10
0.05
0.00
HPC
B
*
GMP
**
0.8
0.6
0.4
0.2
0.0
CMP
0.06
0.04
0.02
CLP
MEP
C
6
4
2
0
Monocytes
50
40
30
20
10
Neutrophil
B220
3
1
CD3

Figure S3

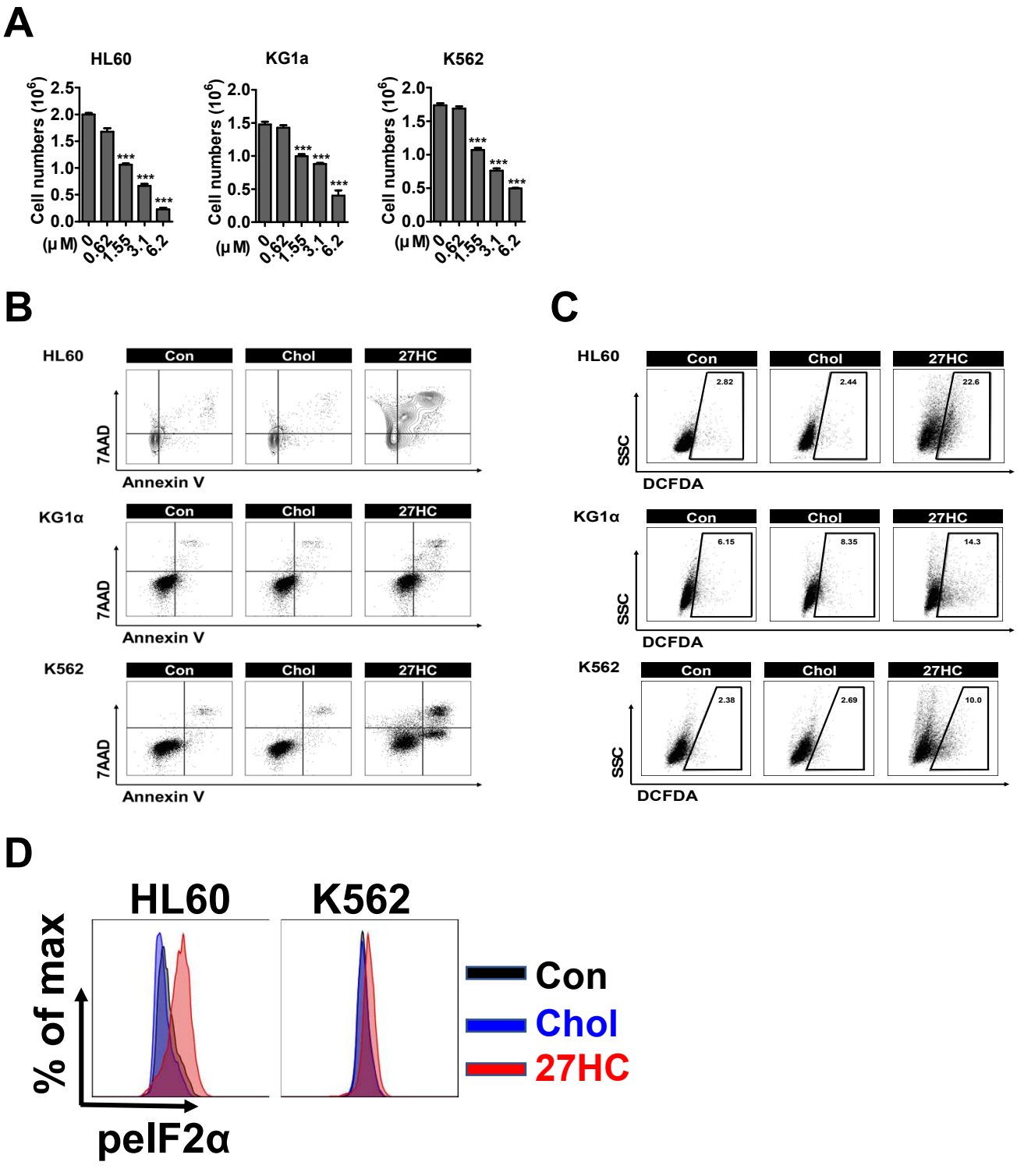
A
HL60
KG1a
K562
Cell numbers (10^6)
(μM) 0 0.62 1.55 3.1 6.2
***
B
HL60
KG1α
K562
Con
Chol
27HC
7AAD
Annexin V
C
HL60
KG1α
K562
Con
Chol
27HC
2.82
2.44
22.6
6.15
8.35
14.3
2.38
2.69
10.0
SSC
DCFDA
D
HL60
K562
% of max
peIF2α
Con
Chol
27HC

Figure S4

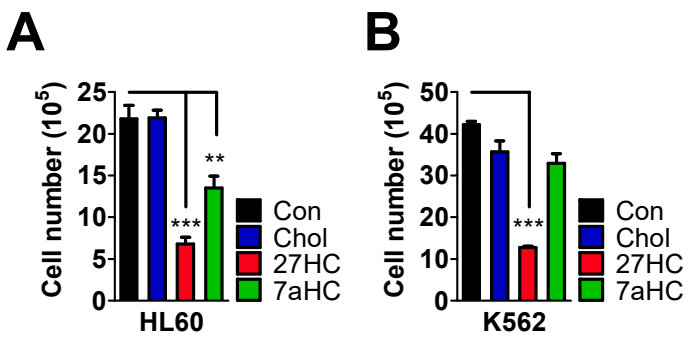
A
B
Cell number (10^5)
25
20
15
10
5
0
**
***
HL60
Con
Chol
27HC
7aHC
Cell number (10^5)
50
40
30
20
10
0
***
K562
Con
Chol
27HC
7aHC

Figure S5