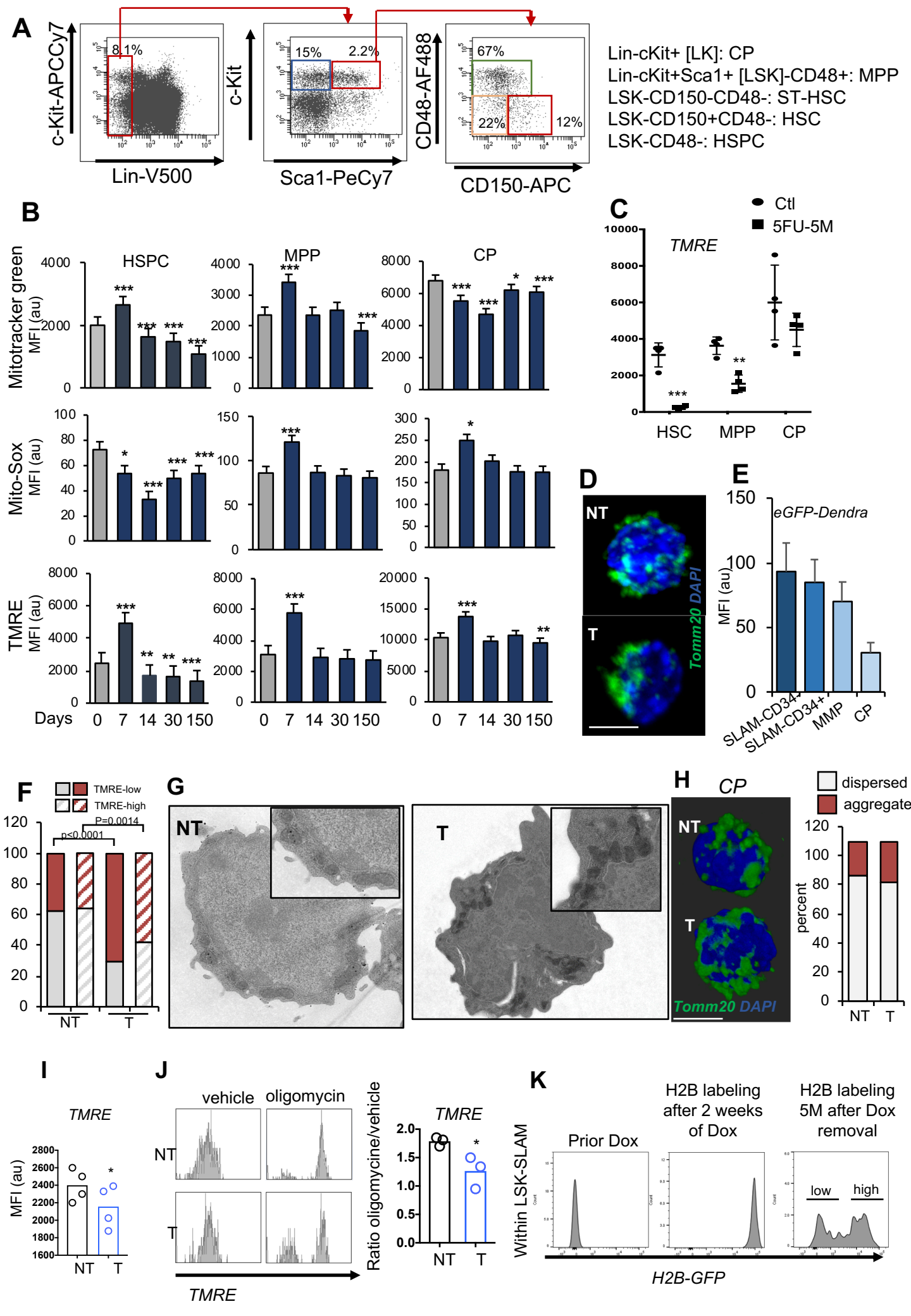

A
c-Kit-APCCy7
Lin-V500
8.1%
c-Kit
Sca1-PeCy7
15%
2.2%
CD48-AF488
CD150-APC
67%
22%
12%
Lin-cKit+ [LK]: CP
Lin-cKit+Sca1+ [LSK]-CD48+: MPP
LSK-CD150-CD48-: ST-HSC
LSK-CD150+CD48-: HSC
LSK-CD48-: HSPC
B
Mitotracker green MFI (au)
Mito-Sox MFI (au)
TMRE MFI (au)
HSPC
MPP
CP
Days 0 7 14 30 150
C
Ctl
5FU-5M
TMRE
HSC MPP CP
D
NT
T
Tomm20 DAPI
E
eGFP-Dendra
MFI (au)
SLAM-CD34-
SLAM-CD34+
MMP
CP
F
TMRE-low
TMRE-high
P=0.0014
p<0.0001
NT T
G
NT
T
H
CP
NT
T
Tomm20 DAPI
dispersed
aggregate
percent
NT T
I
TMRE
MFI (au)
NT T
J
vehicle
oligomycin
NT
T
TMRE
Ratio oligomycine/vehicle
TMRE
NT T
K
Within LSK-SLAM
Prior Dox
H2B labeling after 2 weeks of Dox
H2B labeling 5M after Dox removal
low
high
H2B-GFP

Supplemental Figure 1 . Related to Figure 1

A. Representative flow chart of HSC identification by immunophenotype. HSC: cells are first gated on lineage negative cells (Lin-), then on c-Kit positive and Sca-1 positive cells (LSK), then on CD150 positive and CD48 negative (LSK-SLAM); ST-HSC: short-term HSC: (Lin-), then c-Kit positive and Sca-1 positive cells, then on CD150 negative and CD48 negative. MPP: multipotent progenitors: Lin- gate, then c-Kit positive and Sca-1 positive cells, then CD48 positive and CD150 negative; CP: committed progenitors: Lin-, then c-Kit positive and Sca-1 negative cells; hematopoietic stem and progenitors cells (HSPC): LSK-CD48-, Lin- gate, then c-Kit positive and Sca-1 positive cells, then CD48 negative cells

B. Quantification of mitochondrial parameters in WT mice treated with 5FU (150mg/kg) and analyzed at the indicated time and indicated population, mean±SD, n=6 mice in 2 independent experiments.

C. Quantification of TMRE in WT mice 5 month (5M) post 5FU (150mg/kg) challenge (mean±s.e.m, n=4 mice).

D. Representative images of Tom20 immunostaining in SLAM displayed as maximum intensity projection. Scale bar 5µm.

E. Mean Fluorescence Intensity of mito-Dendra2 levels in various population analyzed by flow cytometry. (mean±SD, n=6 mice in 2 independent experiments)

F. NT and T-SLAM were stained with TMRE and TMRE high vs low fractions were isolated; cells were then for Tom20. quantification of cells with dispersed versus compact mitochondrial organization (n=35 NT; n=36 T LK cells).

G. Representative images of transmission electron microscopy analysis of NT and T LK cells. Insert shows enlargement of one part of each cell. n=30 NT and 15 T cells.

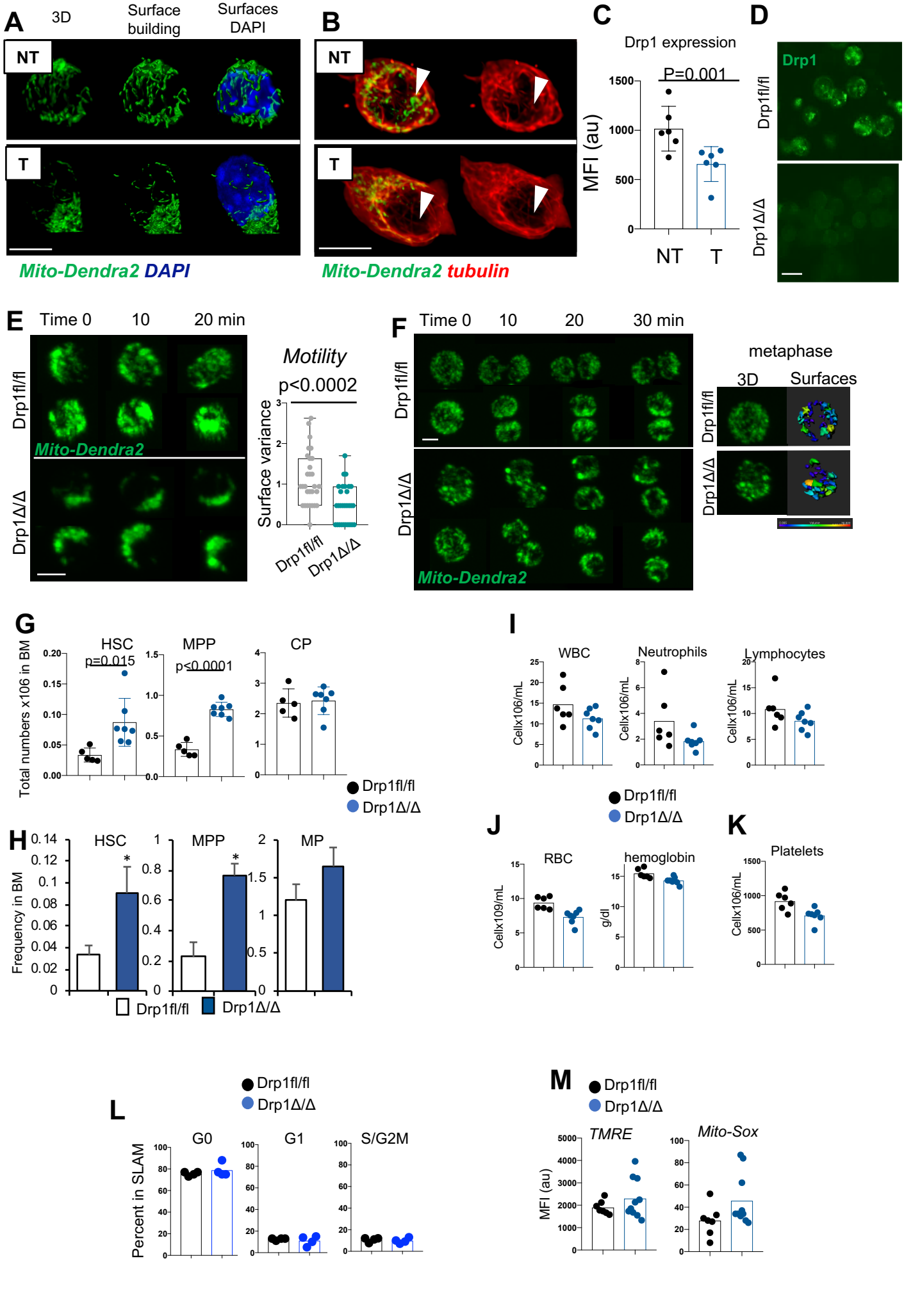
H. Representative images of Tom20 immunostaining in LK displayed as alpha blending and quantification of cells with dispersed versus compact mitochondrial organization (n=76 NT LK cells; n=50 T LK cells). Scale bar 5µm.

I. TMRE quantification in NT and T HSPC after activation in culture for 24h with SCF+TPO; mean±SD, n=4 independent cultures.

J. Representative flow cytometry histogram and TMRE quantification in NT and T SLAM-HSC after oligomycin challenge. mean±SD, n=3 independent samples

K. Representative flow histogram showing levels of GFP labeled H2B within LSK-SLAM before doxycycline (left panel), two weeks after doxycycline treatment (middle panel) and then 5 months after dox removal (right panel). Note, SLAMs are fully labeled two weeks after doxycycline treatment, and the labeled is diluted 5 months after dox removal, indicative of SLAM division within this time frame.

All data are analyzed with unpaired T-Test * $p<0.05$, ** $p<0.01$, *** $p<0.001$.


A
3D
Surface building
Surfaces DAPI
NT
T
Mito-Dendra2 DAPI
B
NT
T
Mito-Dendra2 tubulin
C
Drp1 expression
P=0.001
MFI (au)
1500
1000
500
0
NT
T
D
Drp1
Drp1fl/fl
Drp1Δ/Δ
E
Time 0
10
20 min
Drp1fl/fl
Drp1Δ/Δ
Mito-Dendra2
Motility
p<0.0002
Surface variance
Drp1fl/fl
Drp1Δ/Δ
F
Time 0
10
20
30 min
Drp1fl/fl
Drp1Δ/Δ
Mito-Dendra2
metaphase
3D
Surfaces
Drp1fl/fl
Drp1Δ/Δ
G
HSC
p=0.015
MPP
p≤0.0001
CP
Total numbers x106 in BM
Drp1fl/fl
Drp1Δ/Δ
I
WBC
Neutrophils
Lymphocytes
Cellx106/mL
Drp1fl/fl
Drp1Δ/Δ
H
HSC
MPP
MP
Frequency in BM
Drp1fl/fl
Drp1Δ/Δ
J
RBC
hemoglobin
Cellx109/mL
g/dl
K
Platelets
Cellx106/mL
L
Drp1fl/fl
Drp1Δ/Δ
G0
G1
S/G2M
Percent in SLAM
M
Drp1fl/fl
Drp1Δ/Δ
TMRE
Mito-Sox
MFI (au)

Supplemental Figure 2 A-F: related to figure 2; G-M: related to figure 3

A. Representative IF images of activated mito-Dendra2 NT SLAMs and mito-dendra2 signals identified by surface building algorithm in Imaris. Scale bar 5μm.

B. Representative images of mito-Dendra2 (in green) and tubulin (in red). Arrowhead points to microtubules. (n=25 to 30 cells). Scale bar 5μm.

C. Drp1 expression in NT and T SLAMs analyzed by flow cytometry (mean ± SD, n=6 per group)

D. Representative IF images of Drp1 expression in Drp1fl/fl and Drp1Δ/Δ BM kit+ cells. Scale bar 10μm.

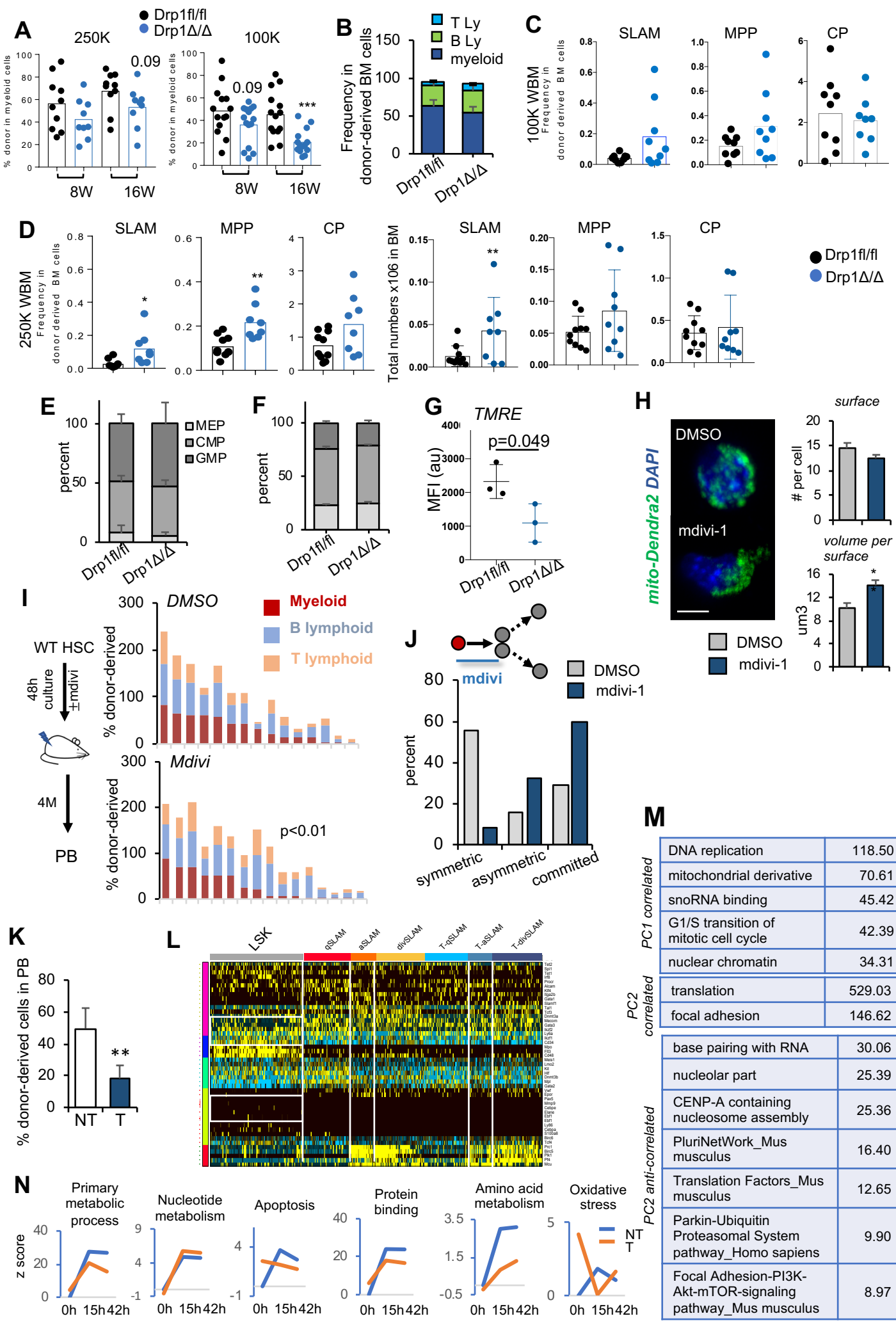
E. Mito-Dendra2-Drp1fl/fl and mito-Dendra2-Drp1Δ/Δ SLAM were imaged live during interphase after in vitro activation. Representative IF images of mitochondrial network during interphase at the indicated time interval. Mitochondria in green. Quantification of mitochondrial motility using variance of surfaces. Numbers of surfaces per cell per time points (3 time points over 30 minutes). were analyzed using Imaris surface building and variance of number of surfaces was calculated. Scale bar 5μm.

F. Mito-Dendra2-Drp1fl/fl and mito-Dendra2-Drp1Δ/Δ SLAM divisions were traced in vitro using live immunofluorescence imaging. Representative IF images of mitochondrial network in mito-Dendra2-Drp1fl/fl and mitoDendra2-Drp1Δ/Δ SLAMs during mitosis, scale bar 5μm. Mitochondria in green.

G-K. Analysis of Drp1Δ/Δ mice 4 months post Drp1 deletion. G-H. Total numbers and frequency of HSC and progenitors in BM (6 bones). n=5 Drp1fl/fl and 7 Drp1Δ/Δ mice. I-K. Blood parameters. n=10 Drp1fl/fl and 9 Drp1Δ/Δ mice

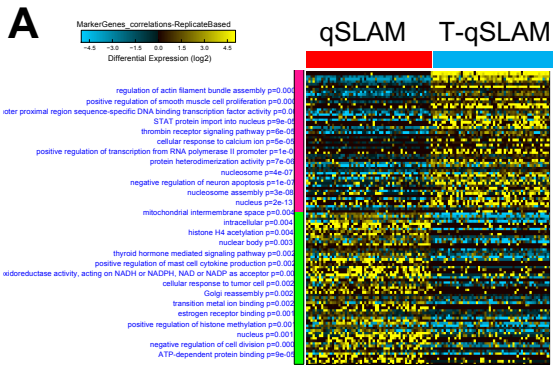
L. Cell cycle analysis using ki67 and Hoechst in Drp1fl/fl and Drp1Δ/Δ SLAMs 4M following Drp1 deletion, mean ± SD, n=4 mice per group.

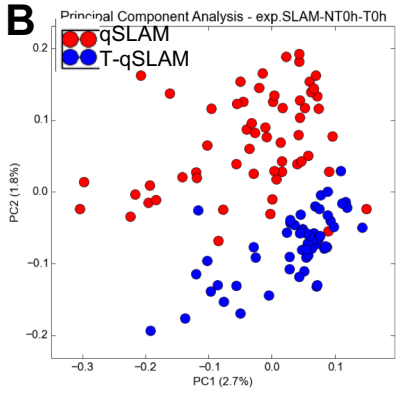
M. Quantification of TMRE and mitoSox in Drp1fl/fl and Drp1Δ/Δ SLAMs 4M following Drp1 deletion


A
Drp1fl/fl
Drp1Δ/Δ
250K
100K
% donor in myeloid cells
0.09
0.09
***
8W
16W
8W
16W
B
T Ly
B Ly
myeloid
Frequency in donor-derived BM cells
Drp1fl/fl
Drp1Δ/Δ
C
100K WBM
Frequency in donor derived BM cells
SLAM
MPP
CP
D
250K WBM
Frequency in donor derived BM cells
SLAM
MPP
CP
Total numbers x106 in BM
SLAM
MPP
CP
Drp1fl/fl
Drp1Δ/Δ
E
percent
MEP
CMP
GMP
Drp1fl/fl
Drp1Δ/Δ
F
percent
Drp1fl/fl
Drp1Δ/Δ
G
TMRE
p=0.049
MFI (au)
Drp1fl/fl
Drp1Δ/Δ
H
mito-Dendra2 DAPI
DMSO
mdivi-1
surface
per cell
volume per surface
um3
DMSO
mdivi-1
I
WT HSC
48h culture
±mdivi
4M
PB
DMSO
Myeloid
B lymphoid
T lymphoid
% donor-derived
Mdivi
% donor-derived
p<0.01
J
mdivi
DMSO
mdivi-1
percent
symmetric
asymmetric
committed
K
% donor-derived cells in PB
**
NT
T
L
LSK
qSLAM
aSLAM
divSLAM
T-qSLAM
T-aSLAM
T-divSLAM
M
PC1 correlated
DNA replication 118.50
mitochondrial derivative 70.61
snoRNA binding 45.42
G1/S transition of mitotic cell cycle 42.39
nuclear chromatin 34.31
PC2 correlated
translation 529.03
focal adhesion 146.62
PC2 anti-correlated
base pairing with RNA 30.06
nucleolar part 25.39
CENP-A containing nucleosome assembly 25.36
PluriNetWork_Mus musculus 16.40
Translation Factors_Mus musculus 12.65
Parkin-Ubiquitin Proteasomal System pathway_Homo sapiens 9.90
Focal Adhesion-PI3K-Akt-mTOR-signaling pathway_Mus musculus 8.97
N
z score
Primary metabolic process
Nucleotide metabolism
Apoptosis
Protein binding
Amino acid metabolism
Oxidative stress
0h 15h 42h
NT
T

Supplemental Figure 3; A-J: related to figure 3; K-N: related to figure 4

A-F. Competitive transplantation of Drp1fl/fl and Drp1Δ/Δ BM cells analyzed at 16 weeks following transplantation. Experiments were performed by transplanting 2 cell doses, 100,000 (100K) or 250,000 (250K) total BM cells into NSG mice. **A** frequency of donor-derived cells in PB; n=10 Drp1fl/fl and 9 Drp1 Δ/Δ recipient mice for 250K; n=14 Drp1fl/fl and 14 Drp1Δ/Δ recipient mice for 100K. **B,** relative frequency of myeloid, B and T cells in BM. n=9 Drp1fl/fl and 8 Drp1Δ/Δ recipient mice for 250K; n=3 Drp1fl/fl and 4 Drp1fl/fl recipient mice for 100K. * p<0.05, **p<0.01, ***p<0.001.**C.** Dot plots show frequency of HSC and progenitors in BM (6 bones). n=9 Drp1fl/fl and 8 Drp1Δ/Δ recipient mice for 100K; **D** Dot plots show frequency and total numbers of SLAM, MPP and CP in BM. Bar graph shows frequency GMP, CMP and MEP within LK gate. n=9 Drp1fl/fl and 9 Drp1 Δ/Δ recipient mice for 250K. * p<0.05, **p<0.01, ***p<0.001. **E**. Frequency GMP, CMP and MEP within LK gate, n=9 Drp1fl/fl and 8 Drp1Δ/Δ recipient mice for 100K. **F**. Frequency GMP, CMP and MEP within LK gate, n=9 Drp1fl/fl and 9 Drp1 Δ/Δ recipient mice for 250K.
G. Quantification of mitochondrial TMRE levels in Drp1fl/fl and Drp1Δ/Δ HSPC after treatment with mitochondrial complex I inhibitor, rotenone (1uM). mean ± SD, n=3 mice per group, unpaired T-Test.
H. NT SLAMs were treated in vitro with mdivi for 24h. Representative images of eGFP-mitoDendra2 HSC. Quantification of number of mitochondrial surfaces and volume per surface using Imaris surface building as in figure 2. mean ± SD, n= 50 cells per group. Scale bar 5μm.
I. NT SLAMs were treated in vitro with or without mdivi for 2 days and then used competitive transplant, stacked bar graphs are donor chimerism (CD45.2+) in each
PB lineages (myeloid, B lymphocytes and T lymphocytes) from individual transplanted mice at 16 weeks post-transplantation. We calculated the ratio of donor contribution to myeloid, B and T cells in each mouse, and classified the reconstitution as myeloid-dominant, balanced or lymphoid-dominant; chi-square test indicates frequency of mice with durable myeloid reconstitution is different between the groups.
J. In vitro paired-daughter cell assay. Single SLAMs from NT mice were treated in vitro with or without mdivi for 48h. Percent of multilineage myeloid differentiation potential of daughter cells after separation was calculated and data show frequency of symmetric division (each daughter cell gave rise to a multipotent clones), asymmetric division (only one daughter cell is multipotent) and committed division (none of the daughter cells are multipotent) (see Supplemental Fig.) n=38-70 single cells; exact fisher test.
K. H2B-eGFP$^+$ LSK-SLAM isolated from non-transplanted or transplanted mice were cultured for the duration of one division in SCF+TPO and used for transplantation. percent donor-derived chimerism in the peripheral blood 4 months after transplantation. Mean SD, n=6 mice **p<0.01.
L. Heatmap of selected genes in each population from scRNA-Seq data (n=251 cells). Columns represent cells. Rows represent genes.
M. Tables are top gene ontology category of differentially expressed genes represented in PC1 and PC2 shown in figure 4 panel C, analyzed using EnrichR.
N. Differential gene expression of each group of cells was compared to qSLAM. Graphs are z-scores of indicated GO category.





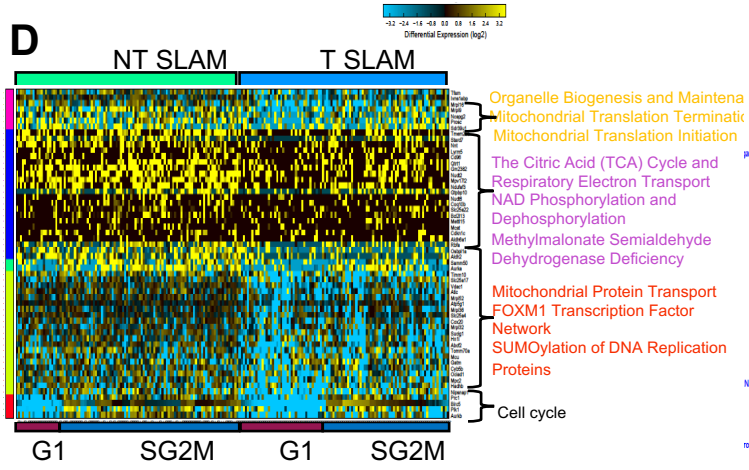
C

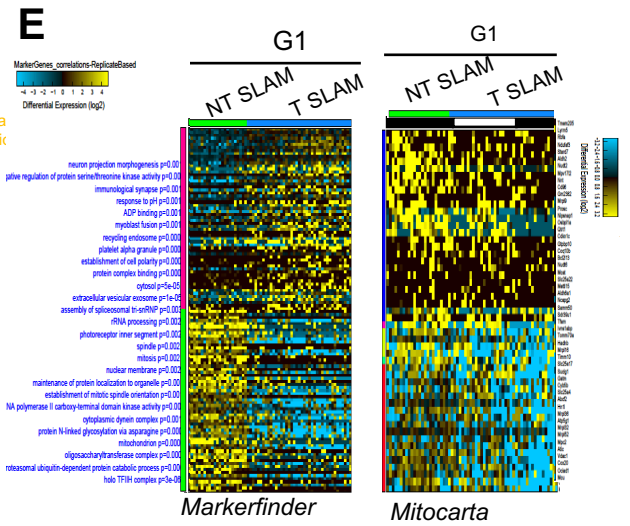
downregulated

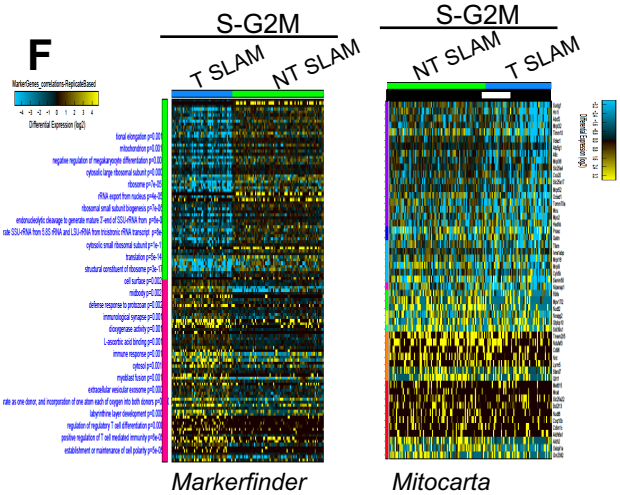
RNA binding	3.862E-12
chromatin binding	2.590E-10
protein-containing complex binding	2.045E-9
zinc ion binding	1.322E-6
DNA-methyltransferase activity	6.346E-6
chromosome organization	7.900E-11
chromatin organization	2.667E-10

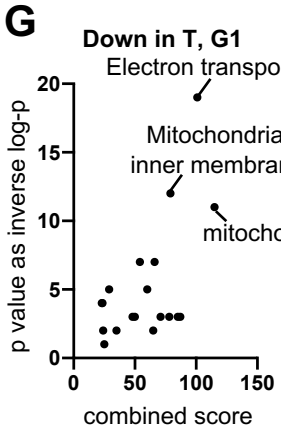
upregulated

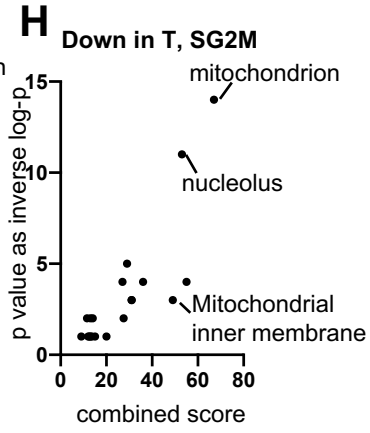
regulation of cell death	8.369E-11
negative regulation of protein modification process	1.767E-9
negative regulation of cell death	2.165E-9
response to organic cyclic compound	7.777E-9
mitochondrion	1.309E-8
regulation of cell proliferation	1.746E-8

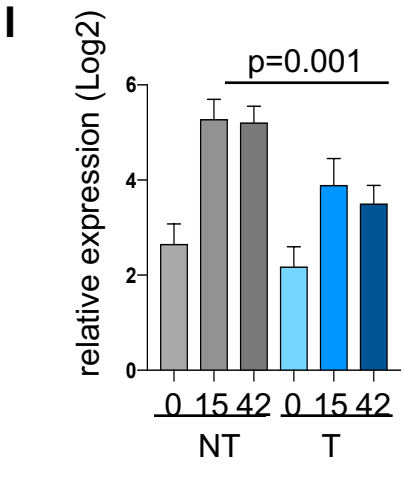


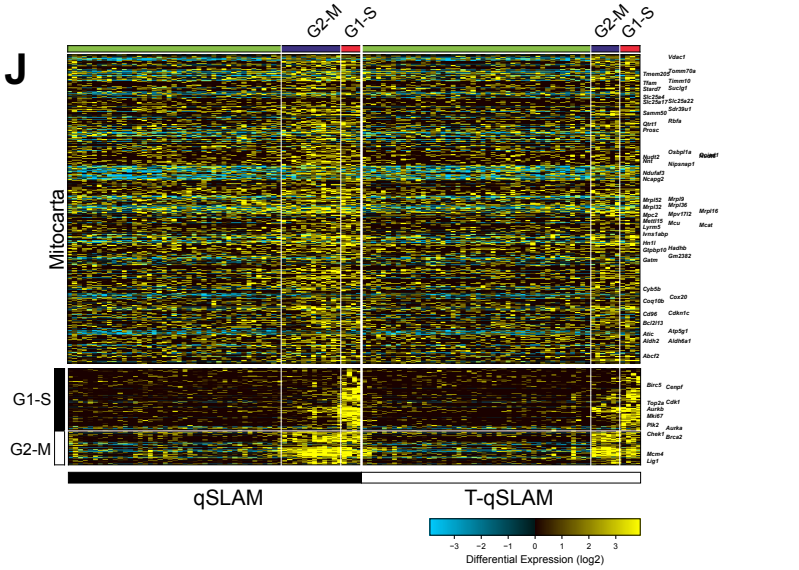












Supplemental Figure 4, related to figure 4

A. Hierarchical clustering of differentially expressed genes in indicated population identified by Markerfinder in Altanalyze.see TableS1

B, PCA visualization of indicated cells.

C. Tables are top gene ontology category of differentially expressed genes represented in PC1 of panel b, analyzed using EnrichR.

D. Hierarchical clustering of differentially expressed cell cycle and mitochondrial genes in activated NT and T SLAMs. Cells were segregated based on their cell cycle status, which was identified in AltAnalyze. Coincident expression of nuclear-encoded genes important for mitochondria are shown.

E. Left panel is Hierarchical clustering of differentially expressed genes in indicated population identified to be in G1 analyzed by Markerfinder in Altanalyze. Right panel is hierarchical clustering of differentially expressed nuclear encoded mitochondrial genes shown as Mitocarta.

F. Left panel is Hierarchical clustering of differentially expressed genes in indicated population identified to be in SG2M analyzed by Markerfinder in Altanalyze. Right panel is hierarchical clustering of differentially expressed nuclear encoded mitochondrial genes shown as Mitocarta.

G. Z score per inverted log-p values of top gene ontology category of downregulated genes in T-SLAMs in G1

H. Z score per inverted log-p values of top gene ontology category of downregulated genes in T-SLAMs in SG2M

I. mRNA expression of Drp1 extracted from scRNA data. (Mean SD, n=30-60 cells per group)

J. Hierarchical clustering of differentially expressed cell cycle and mitochondrial genes in freshly isolated SLAM-HSC from naïve [qSLAM] and transplanted animals [T-qSLAM]. Cells were segregated based on their cell cycle status, which was identified in AltAnalyze using G1-S and G2M cell cycle phase gene expression. Coincident expression of nuclear-encoded genes important for mitochondria are shown as ‘Mitocarta’.see Table S1