Supplemental Figures Legends

Figure S1. Example of gating of spleen, BM, and Lung PMN neutrophils ($CD11b^+Ly6C^{lo}Ly6G^+$) and monocytes ($CD11b^+Ly6C^{hi}Ly6G^-$).

Figure S2. SNPH deficiency doesn't affect the presence of myeloid cells. (A). The proportion of PMN and MON in peripheral blood of WT and SNPH-KO mice. N=5. **(B).** PMN were isolated from BM, spleen, or lung of WT and SNPH-KO mice and were cultured for 18 hours in the complete media without cytokines. The proportion of recovered cells from input is shown (n=5). **(C)**. Flow cytometric quantification of $CD11b^+Ly6G^+Ly6C^{low}$ PMN **(C)** and $CD11b^+Ly6G^-Ly6C^+$ MON **(D)** in BM or spleen of WT and SNPH-KO tumor-bearing mice (n=4 per group). Mean and SD are shown. Each symbol represents an individual mouse. N=4 in all experiments. *P* values (not shown due to lack of significance) were calculated in two-sided unpaired Student's t-test.

Figure S3. SNPH deficiency doesn't affect the presence of T cells. (A) The absolute number and percentage of $CD8^+$ T cells in spleen of WT and SNPH-KO mice. **(B)** The absolute number and percentage of $CD4^+FOXP3^+$ Tregs in spleen of WT and SNPH-KO mice. (**C**) The absolute number and percentage of $CD4^+Foxp3^-$ T cells in spleen of WT and SNPH-KO mice. **(D)** The percentage of proliferating $CD8^+$ T cells from WT and SNPH-KO mice after stimulation with CD3/CD28 antibodies. **(E)** Geometric MFI of IFN-γ staining in $CD8^+$ T cells from WT and SNPH-KO mice in response to PMA/Ionomycin stimulation. Each symbol represents an individual mouse. N=4 in all experiments. *P* values (not shown due to lack of significance) were calculated in two-sided unpaired Student's t-test.

Figure S4. Effect of SNPH deficiency on mRNA gene expression in PMN. (A) Expression heatmap for top genes (FDR < 5%) affected by SNPH deficiency. The list of networks **(B)** and pathways **(C)** were determined by Ingenuity Pathway Analysis (IPA) as significantly enriched by SNPH depletion.

Figure S5. Effect of SNPH deletion of mitochondria. (A) Mitochondrial mass (Mito Tracker green staining) of BM PMN from WT and SNPH-KO mice. **(B-C)** Spontaneous and stimulated (fMLP) cytoplasmic ROS level **(B)** and mitochondrial ROS level (Mitosox staining) **(C)** in BM PMN from WT and SNPH mice. **(D)** Expression of indicated genes by qPCR in BM PMN from WT and SNPH mice. Each symbol represents an individual mouse. Data are shown as mean ± SD. N=3 in all experiments. *P* values (not shown due to lack of significance) were calculated in two-sided unpaired Student's t-test.

Figure S6. Glucose metabolism in BM PMN from WT and SNPH-KO mice.

Flow of $^{13}C_6$-glucose metabolism in BM PMN from WT and SNPH-KO mice (n=3 per group). Data are shown as mean ± SD. *P* values were calculated in two-sided unpaired Student's t-test.

Supplemental Video 1.

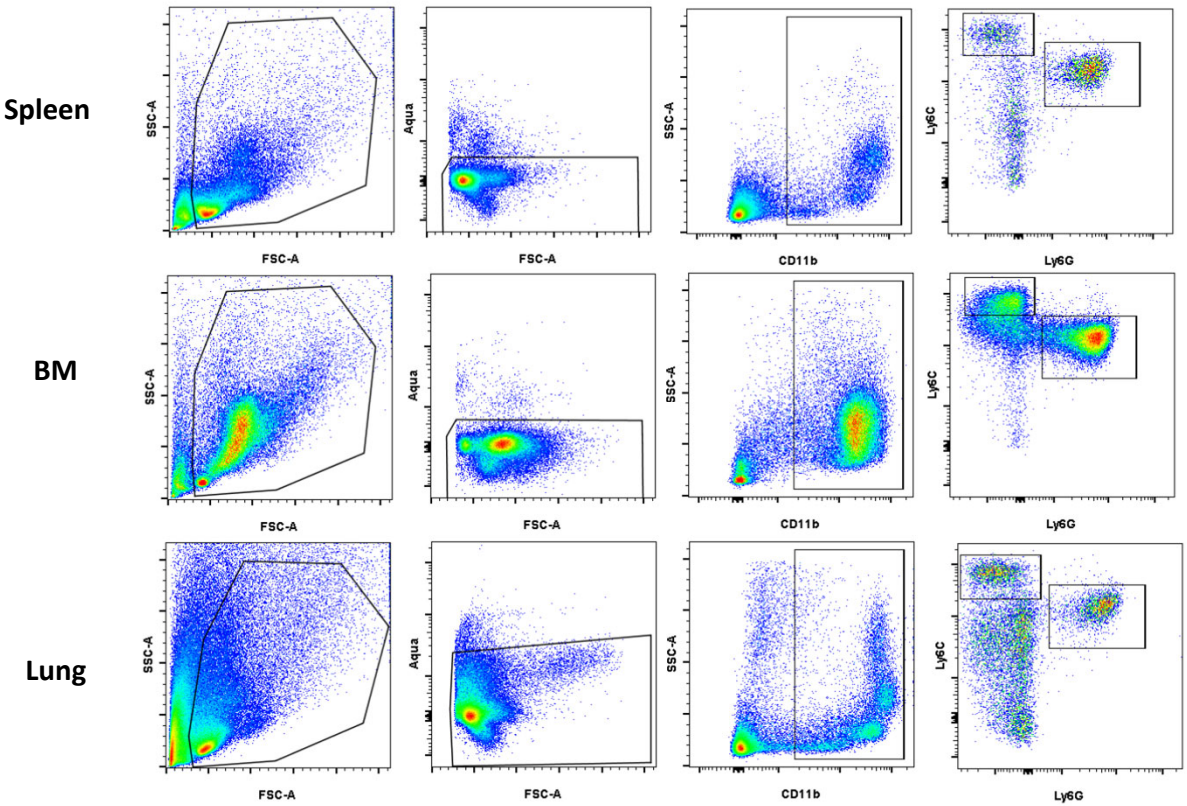


Figure S1

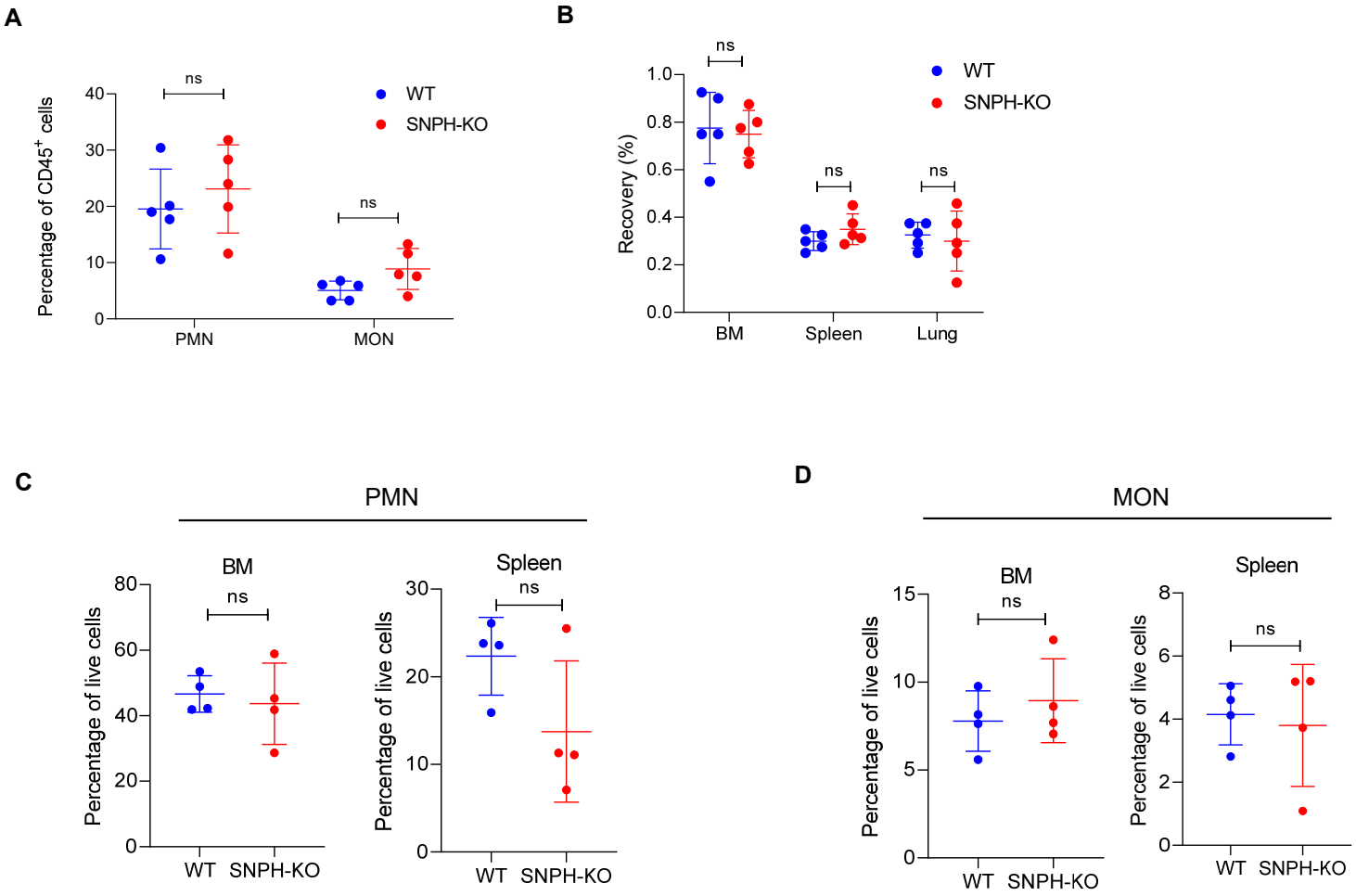

A
Percentage of CD45+ cells
40
30
20
10
0
ns
ns
WT
SNPH-KO
PMN
MON
B
Recovery (%)
1.0
0.8
0.6
0.4
0.2
0.0
ns
ns
ns
WT
SNPH-KO
BM
Spleen
Lung
C
PMN
BM
Spleen
Percentage of live cells
80
60
40
20
0
30
20
10
0
ns
ns
WT
SNPH-KO
WT
SNPH-KO
D
MON
BM
Spleen
Percentage of live cells
15
10
5
0
8
6
4
2
0
ns
ns
WT
SNPH-KO
WT
SNPH-KO

Figure S2

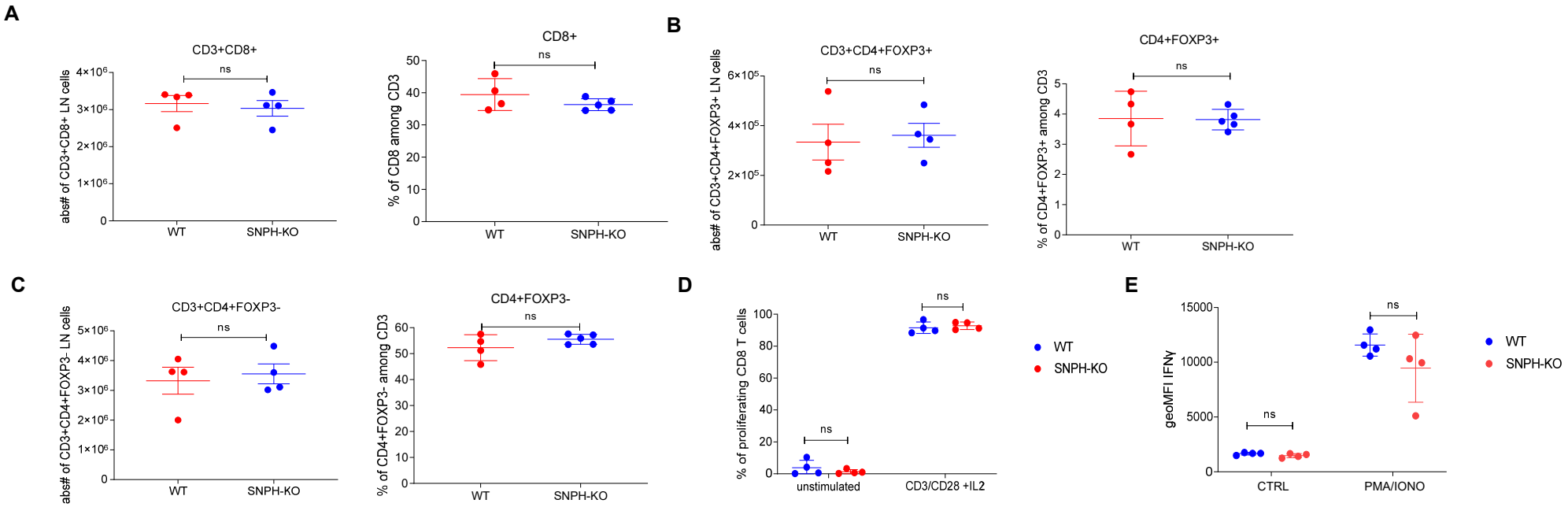

A
CD3+CD8+
abs# of CD3+CD8+ LN cells
4×10⁶
3×10⁶
2×10⁶
1×10⁶
0
ns
WT
SNPH-KO
CD8+
% of CD8 among CD3
50
40
30
20
10
0
ns
WT
SNPH-KO
B
CD3+CD4+FOXP3+
abs# of CD3+CD4+FOXP3+ LN cells
6×10⁵
4×10⁵
2×10⁵
0
ns
WT
SNPH-KO
CD4+FOXP3+
% of CD4+FOXP3+ among CD3
5
4
3
2
1
0
ns
WT
SNPH-KO
C
CD3+CD4+FOXP3-
abs# of CD3+CD4+FOXP3- LN cells
5×10⁶
4×10⁶
3×10⁶
2×10⁶
1×10⁶
0
ns
WT
SNPH-KO
CD4+FOXP3-
% of CD4+FOXP3- among CD3
60
50
40
30
20
10
0
ns
WT
SNPH-KO
D
% of proliferating CD8 T cells
100
80
60
40
20
0
ns
unstimulated
CD3/CD28 +IL2
WT
SNPH-KO
E
geoMFI IFNγ
15000
10000
5000
0
ns
CTRL
PMA/IONO
WT
SNPH-KO

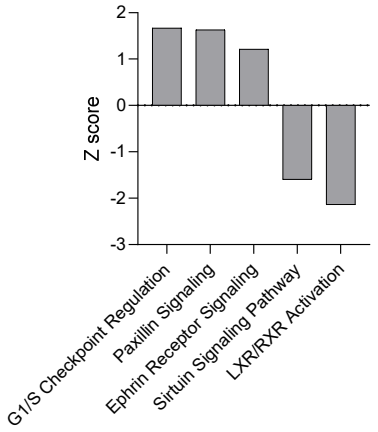
Figure S3

A

FC	p	fdr	Heatmap wt 1	wt 2	wt 3	KO 1	KO 2	KO 3	Gene Name
5.08	9E-06	0%							Gm24924
4.52	5E-05	1%							Erdr1
3.71	1E-08	0%							Ighg1
2.35	3E-06	0%							Jchain
2.26	1E-06	0%							Stfa2
2.17	5E-17	0%							BC100530
1.92	9E-13	0%							Stfa1
1.87	2E-23	0%							Gm5483
1.81	1E-04	3%							Gm13822
1.71	4E-06	0%							Tmem45a2
1.70	2E-07	0%							Stfa2l1
1.66	2E-06	0%							Socs3
1.59	1E-04	2%							Agpat4
1.51	1E-05	0%							Upp1
1.51	3E-05	1%							Sertad3
1.48	2E-07	0%							Sirpb1c
1.48	2E-06	0%							1810013L24Rik
1.40	1E-09	0%							Picalm
1.34	8E-06	0%							Ifitm2
1.33	1E-04	3%							Sirpb1b
1.31	2E-04	4%							Sfxn5
1.31	2E-04	3%							Emilin2
1.31	2E-05	1%							Clec4d
1.31	2E-04	3%							Pla2g7
1.30	4E-09	0%							Ccl6

FC	p	fdr	Heatmap wt 1	wt 2	wt 3	KO 1	KO 2	KO 3	Gene Name
-3.43	1E-06	0%							Timd4
-2.73	2E-13	0%							Mafb
-2.69	1E-04	3%							Slc12a7
-2.66	8E-13	0%							Cd163
-2.66	1E-04	3%							Tmem26
-2.65	6E-09	0%							Lgmn
-2.59	3E-06	0%							Nr1h3
-2.52	3E-06	0%							Itgad
-2.50	1E-07	0%							Myo10
-2.49	3E-06	0%							Nrgn
-2.45	6E-05	2%							Fkbp1a
-2.41	3E-08	0%							Frmd4b
-2.34	1E-04	2%							Fcgr1
-2.33	2E-04	4%							Cln8
-2.32	3E-11	0%							Mrc1
-2.30	1E-05	0%							Slc1a3
-2.29	1E-06	0%							Ccr3
-2.26	8E-08	0%							Spic
-2.24	3E-16	0%							Axl
-2.21	4E-24	0%							Cd5l
-2.21	7E-08	0%							Fcgrt
-2.20	9E-05	2%							Lap3
-2.19	3E-05	1%							Kcnj10
-2.14	2E-25	0%							Fcna
-2.13	2E-04	4%							Ms4a7

C



B

Network	Score	N
Cellular Assembly and Organization, Cellular Function and Maintenance, Energy Production	43	30
Cellular Function and Maintenance, Free Radical Scavenging, Small Molecule Biochemistry	40	29
Cell Signaling, Cellular Assembly and Organization, Post-Translational Modification	34	26
Cellular Function and Maintenance, Molecular Transport, Small Molecule Biochemistry	34	26

Figure S4

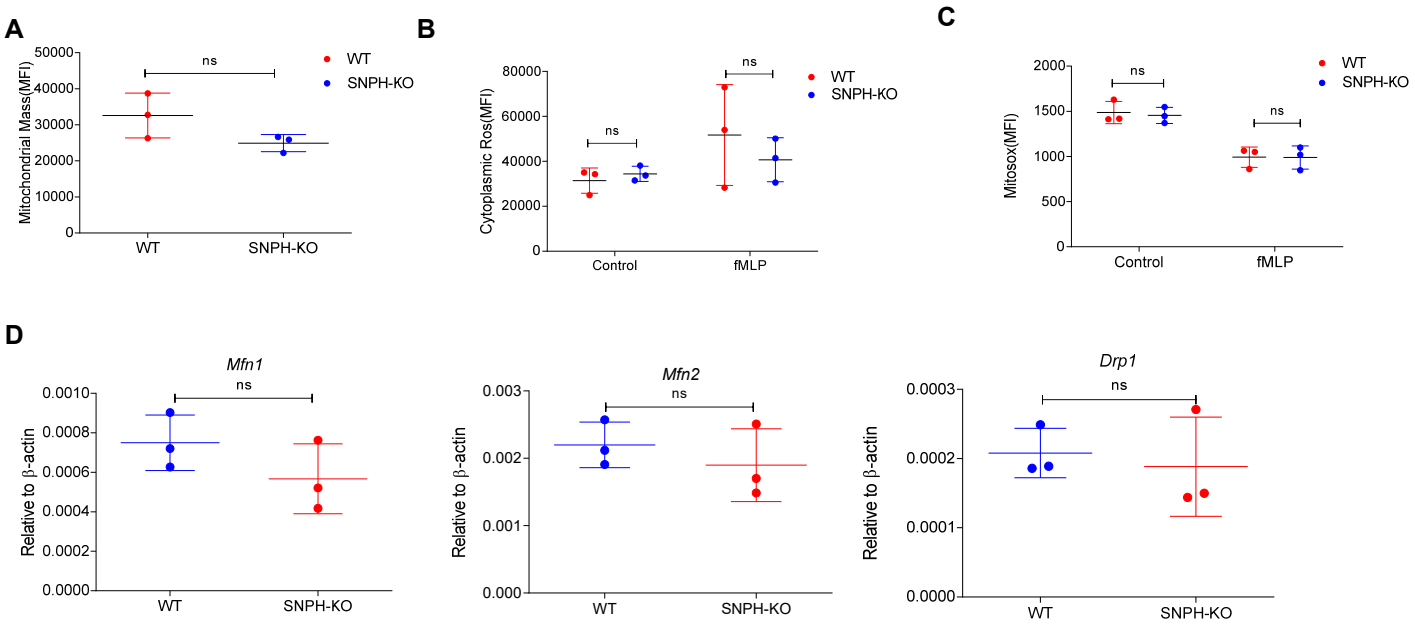

A
Mitochondrial Mass(MFI)
50000
40000
30000
20000
10000
0
ns
WT
SNPH-KO
WT
SNPH-KO
B
Cytoplasmic Ros(MFI)
80000
60000
40000
20000
0
ns
ns
WT
SNPH-KO
Control
fMLP
C
Mitosox(MFI)
2000
1500
1000
500
0
ns
ns
WT
SNPH-KO
Control
fMLP
D
Mfn1
Relative to β-actin
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
ns
WT
SNPH-KO
Mfn2
Relative to β-actin
0.003
0.002
0.001
0.000
ns
WT
SNPH-KO
Drp1
Relative to β-actin
0.0003
0.0002
0.0001
0.0000
ns
WT
SNPH-KO

Figure S5

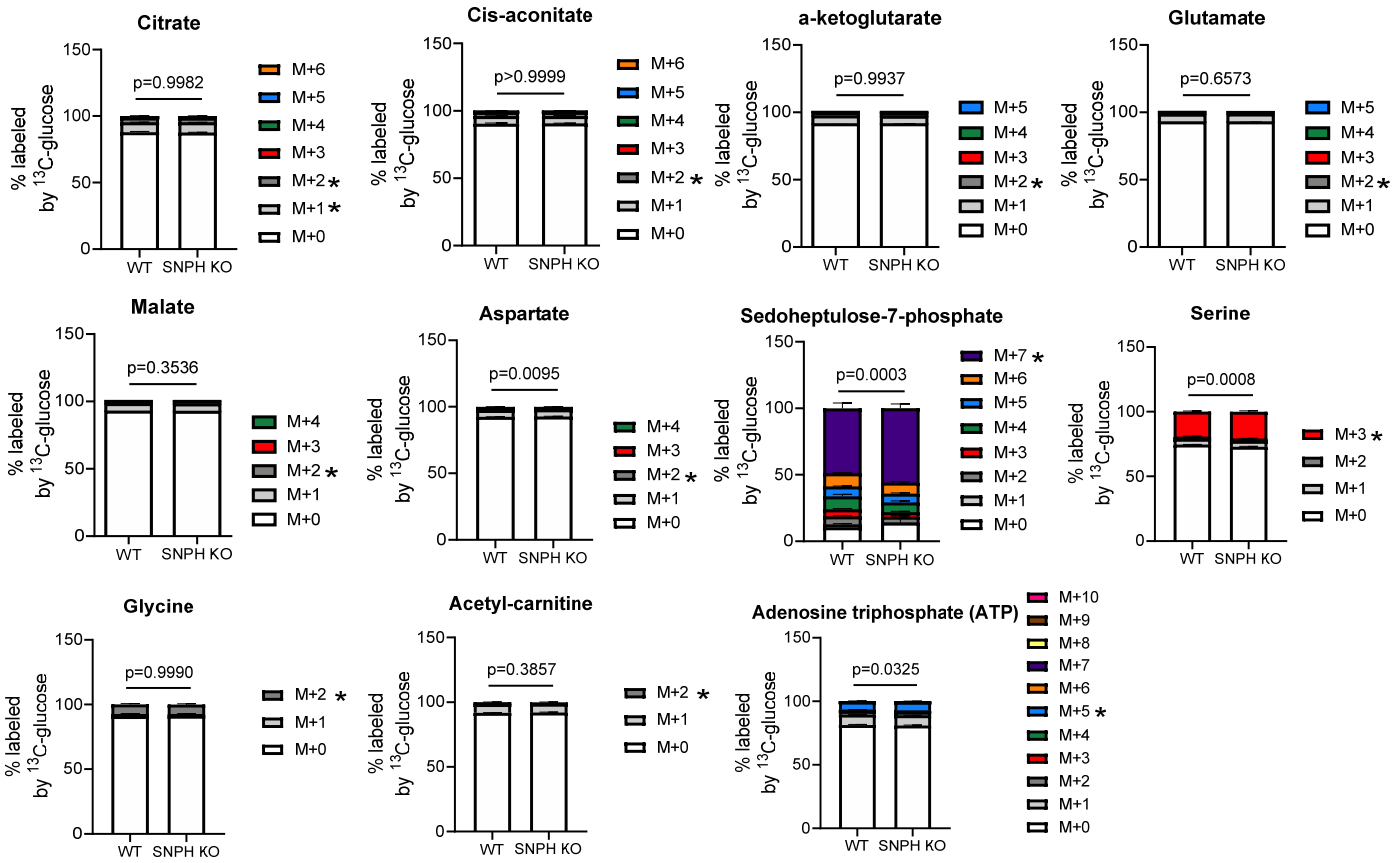

Citrate
p=0.9982
M+6
M+5
M+4
M+3
M+2 *
M+1 *
M+0
Cis-aconitate
p>0.9999
M+6
M+5
M+4
M+3
M+2 *
M+1
M+0
a-ketoglutarate
p=0.9937
M+5
M+4
M+3
M+2 *
M+1
M+0
Glutamate
p=0.6573
M+5
M+4
M+3
M+2 *
M+1
M+0
Malate
p=0.3536
M+4
M+3
M+2 *
M+1
M+0
Aspartate
p=0.0095
M+4
M+3
M+2 *
M+1
M+0
Sedoheptulose-7-phosphate
p=0.0003
M+7 *
M+6
M+5
M+4
M+3
M+2
M+1
M+0
Serine
p=0.0008
M+3 *
M+2
M+1
M+0
Glycine
p=0.9990
M+2 *
M+1
M+0
Acetyl-carnitine
p=0.3857
M+2 *
M+1
M+0
Adenosine triphosphate (ATP)
p=0.0325
M+10
M+9
M+8
M+7
M+6
M+5 *
M+4
M+3
M+2
M+1
M+0
% labeled by 13C-glucose
WT
SNPH KO

Figure S6

Supplemental Table 1: List of reagents

Company	Antibody name	Catalog number	Clone	RRID
BD Bioscience	CD45.1 FITC	553775	A20	AB_395043
	CD45.2 APC-Cy7	560694	104	AB_1727492
	Ly6G PE	551461	1A8	AB_394208
	CXCR2-FITC	551126	6C6	AB_394060
	Cytofix/Cytoperm Solution	554722	N/A	AB_2869010
	Perm/Wash Buffer	323020	N/A	
Biolegend	CD11b BV421	101236	M1/70	AB_11203704
ThermoFisher Scientific	Phalloidin-AF488	A12379	N/A	
	Aqua Live/Dead Fixable 405	L34966		
	MitoTrackerTM Green FM	M7514		
	MitoSOX	M36008		
	CM-H2DCFDA	C6827		
Cell Signaling Technology	DRP1	5391	D8H5	AB_11178938
	P-DRP1 (S616)	4494	D9A1	AB_11178659
	Myosin Light Chain 2	8505S	D18E2	AB_2728760
	Phospho-Myosin Light Chain 2 (Ser19)	3671S		AB_330248
Abcam	SDHA	14715	2E3GC12FB2AE2	AB_301433
	SDHB	14714	21A11AE7	AB_301432
	Anti-NDUFB8 antibody	ab110242	20E9DH10C12	AB_10859122

Supplemental Table 2: Primers sequence

Primers (Mouse)	Sequences
Snph	F: CGGTTCAGTCCTCAAAGACAG
	R: GCCTCAAGCTCGTCACCTAC
18s	F: GGACCAGAGCGAAAGCATTTGCC
	R: TCAATCTCGGGTGGCTGAACGC
Mfn1	F: CCTACTGCTCCTTCTAACCCA
	R: AGGGACGCCAATCCTGTGA
Mfn2	F: AGAACTGGACCCGGTTACCA
	R: CACTTCGCTGATACCCCTGA
Drp1	F: CAGGAATTGTTACGGTTCCCTAA
	R: CCTGAATTAACTTGTCCCGTGA

Primers (Human)	Sequences
SNPH	F: CAACTCAGCAGTGGTGGTGACAG
	R: GCAGCCTCCTCCTCCTCTTCC
GAPDH	F: GAAGGTCGGAGTCAACGGAT
	R: TGAAACACCGTCTGGCCC