Supplementary Materials

Supplementary Figures

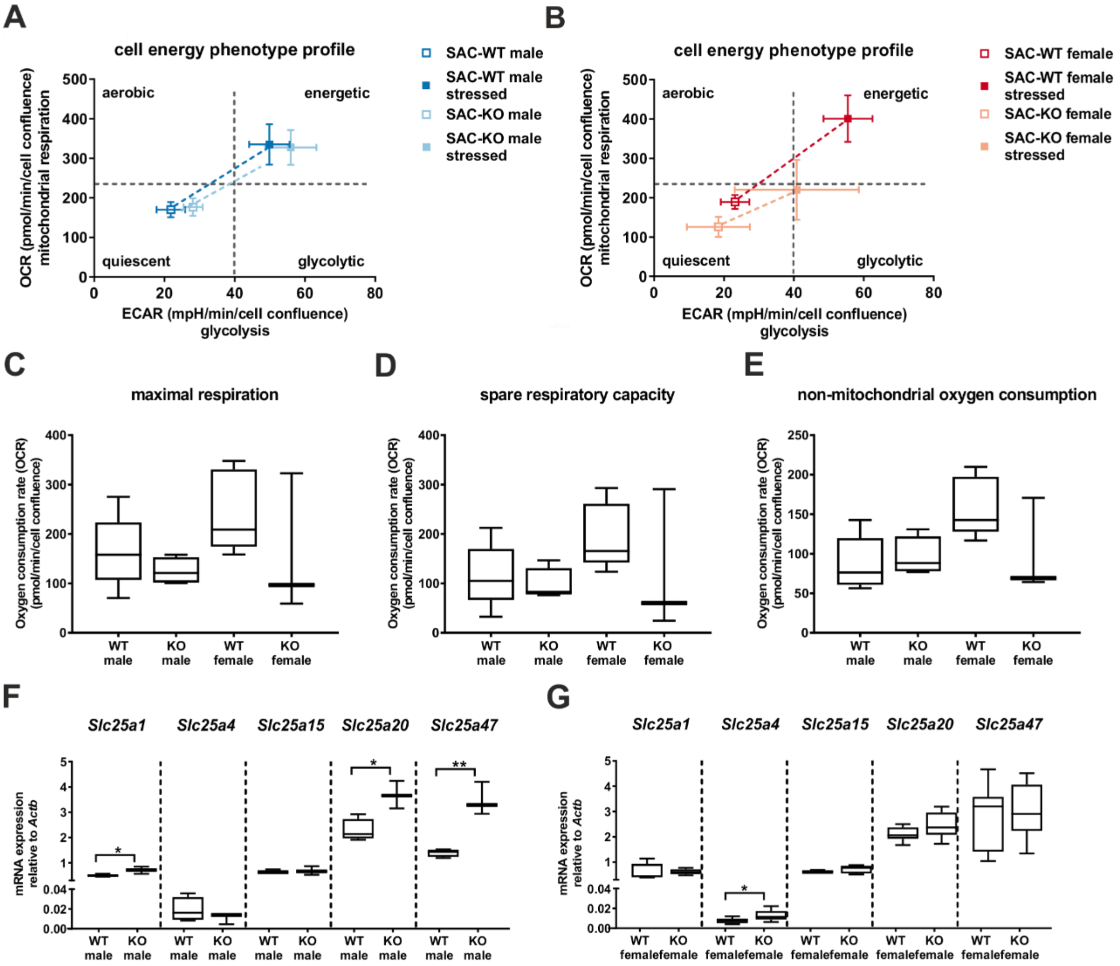


Figure S1: Analysis of the cell energy phenotype and mRNA expression of mitochondrial genes from primary hepatocytes of SAC mice. A-B: To determine the cell energy profile of hepatocytes from male (A) and female (B) SAC mice, hepatocytes were incubated with oligomycin and FCCP (stressed). C-E: Seahorse analysis Mito Stress Test of SAC mice. F-G: qPCR analysis of important genes of mitochondrial metabolism from male (F) and female (G) SAC mice. Error bars in A-B show SEM. Significance was calculated with multiple t-test. Stars show the significance to WT animals. * - $P \leq 0.05$, ** - $P \leq 0.01$, *** - $P \leq 0.001$; N = 3-5.

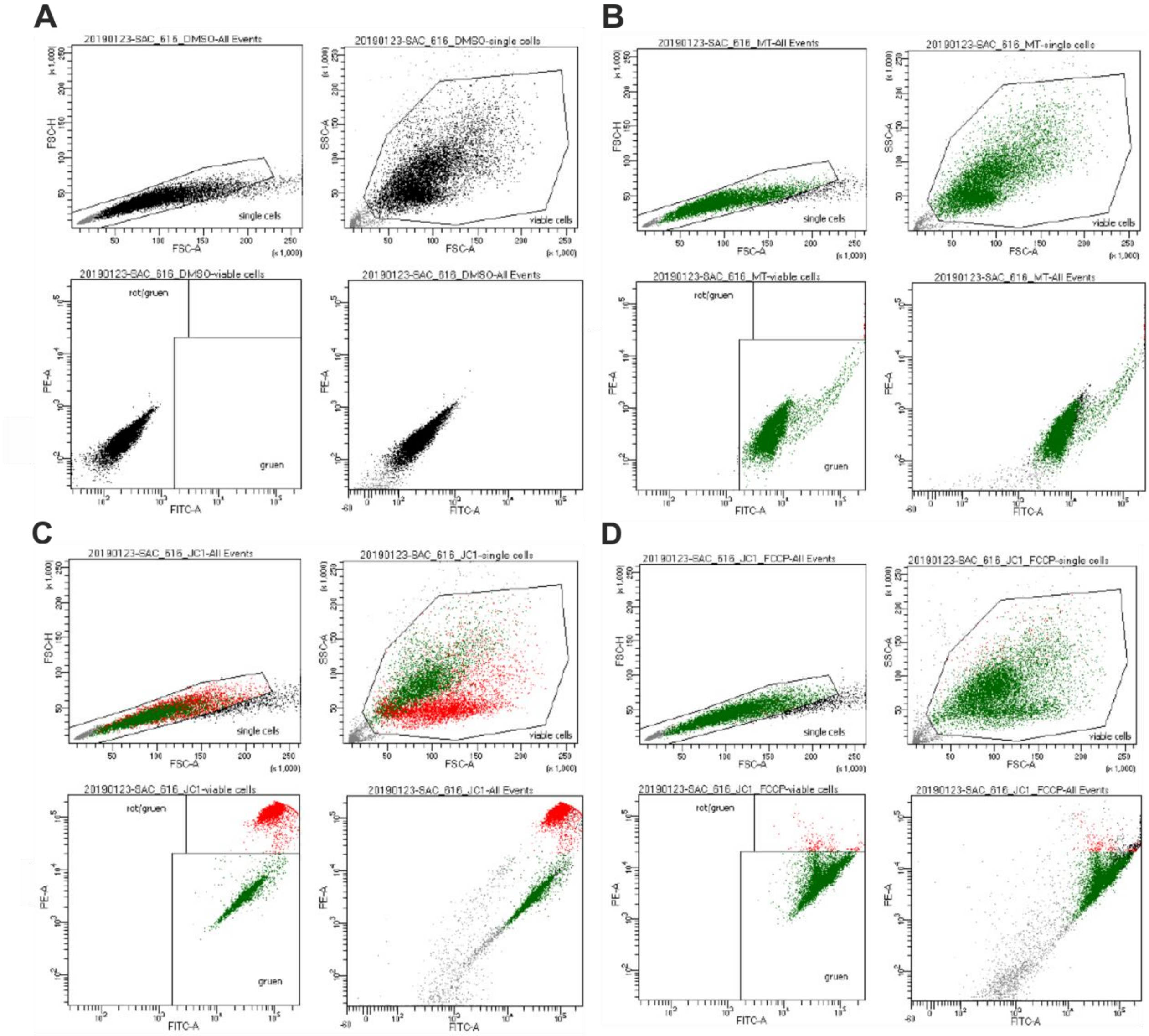
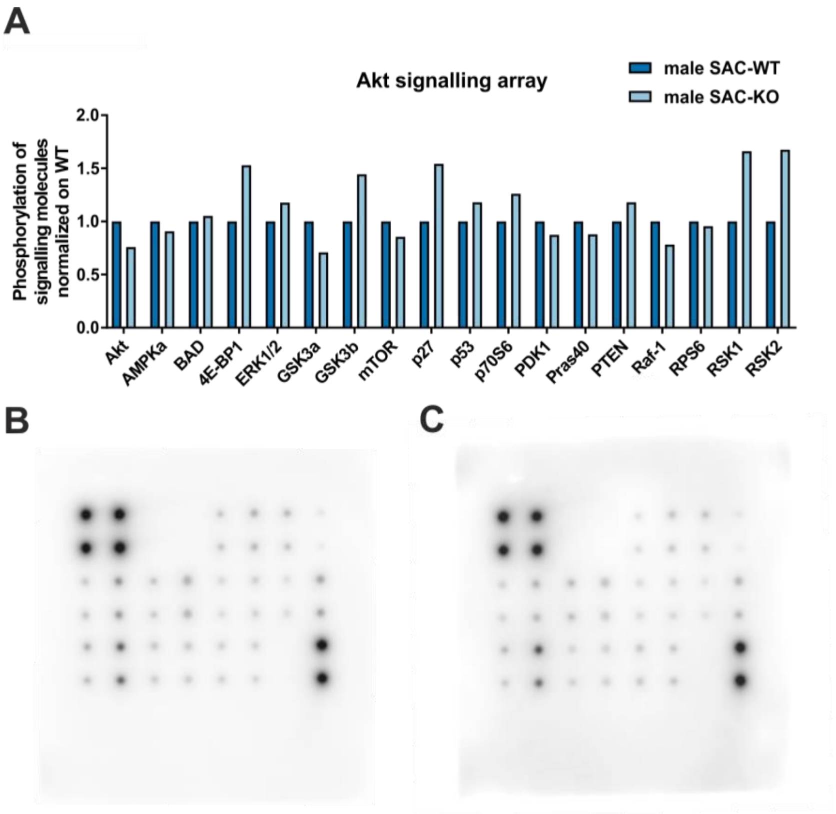


Figure S2: Representative example of flow cytometric analysis of mitochondrial mass and membrane potential. The figure shows representative data from one mouse. A: unstained negative control. B: Mito Tracker green staining for determination of mitochondrial mass. C: JC-10 staining for determination of mitochondrial membrane potential. D: negative control of JC-10 staining (addition of FCCP).



	1	2	3	4	5	6	7	8
1 2	POS	POS	NEG	NEG	Akt (p-Ser473)	AMPKα (p-Thr172)	BAD (p-Ser112)	4E-BP1 (p-Thr36)
3 4	ERK-1 (p-T202/Y204) ERK-2 (p-Y185-Y187)	GSK3α (p-Ser21)	GSK3β (p-Ser9)	mTOR (p-Ser2448)	p27 (p-Thr198)	p53 (p-Ser15)	p70S6 (p-Thr421/Ser 424)	PDK1 (p-Ser241)
5 6	Pras40 (p-Thr246)	PTEN (p-Ser380)	Raf-1 (p-Ser301)	RPS6 (p-Ser235/236)	RSK1 (p-Ser380)	RSK2 (p-Ser386)	NEG	POS

Figure S3: Akt signalling phosphorylation array of hepatocytes from male SAC mice. Primary hepatocytes from a male SAC-WT and -KO mouse were used for an Akt signalling phosphorylation array. A: Densitometric analysis of the spots according to manufacturer's instructions. B: Blot of male SAC-WT mouse. C: Blot of male SAC-KO mouse. POS – positive control, NEG – negative control, Akt - protein kinase B, AMPKα - 5'-AMP-activated protein kinase catalytic subunit alpha, BAD - Bcl2-associated agonist of cell death, 4E-BP1 - Eukaryotic translation initiation factor 4E-binding protein 1, ERK 1/2 - Mitogen-activated protein kinase, GSK3α - Glycogen synthase kinase-3 alpha, GSK3β - Glycogen synthase kinase-3 beta, mTOR – mechanistic target of rapamycin, p27 - Cyclin-dependent kinase inhibitor 1B, p53 - Cellular tumor antigen p53, p70S6 - Ribosomal protein S6 kinase beta-1, PDK1 - 3-phosphoinositide-dependent protein kinase 1, Pras40 - Proline-rich AKT1 substrate 1, PTEN - Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase, Raf-1 - RAF proto-oncogene serine/threonine-protein kinase, RPS6 - 40S ribosomal protein S6, RSK1/2 - Ribosomal protein S6 kinase alpha-1.

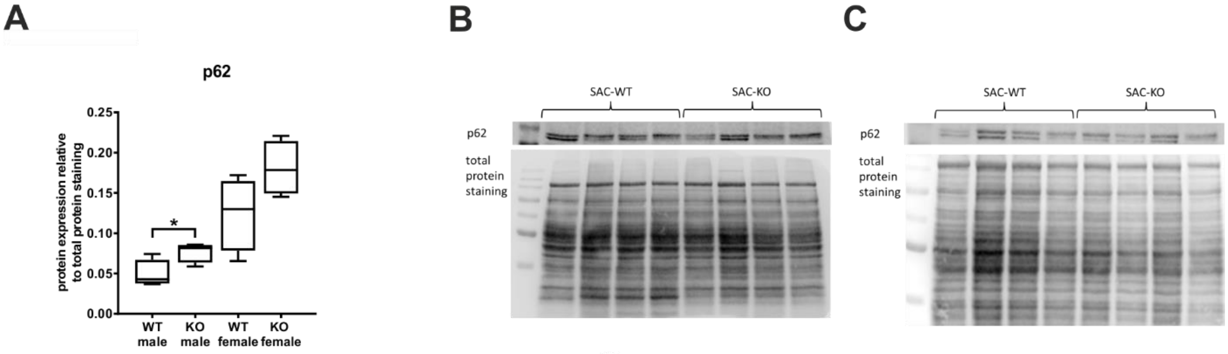


Figure S4: Impact of hepatocyte-specific SAC-KO on autophagy. A: Densitometric analysis of the Western blot of p62 in hepatocytes from male and female SAC mice. B: Western blot of p62 in male SAC mice. C: Western blot of p62 in female SAC mice. Significance was calculated with multiple t-test. Stars show the significance to WT animals. * - P ≤ 0.05, N = 4.

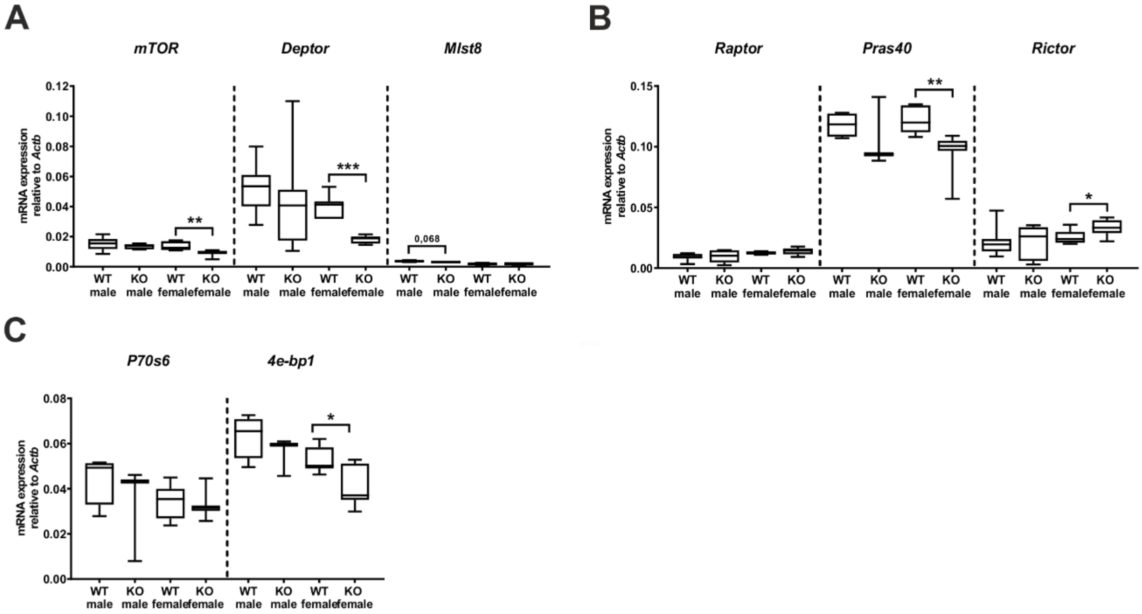


Figure S5: RNA expression of mTOR-related genes in SAC mice. A: mTORC1 and -C2 components *Mtor*, *Deptor* and *Mlst8*. B: mTORC1 components *Raptor* and *Pras40* and mTORC2 component *Rictor*. C: mTOR downstream signalling kinases *P70s6* and *4e-bp1*. Significance was calculated with unpaired t-test. Stars show the significance to WT animals. * - P ≤ 0.05, ** - P ≤ 0.01, *** - P ≤ 0.001; N = 3-8.

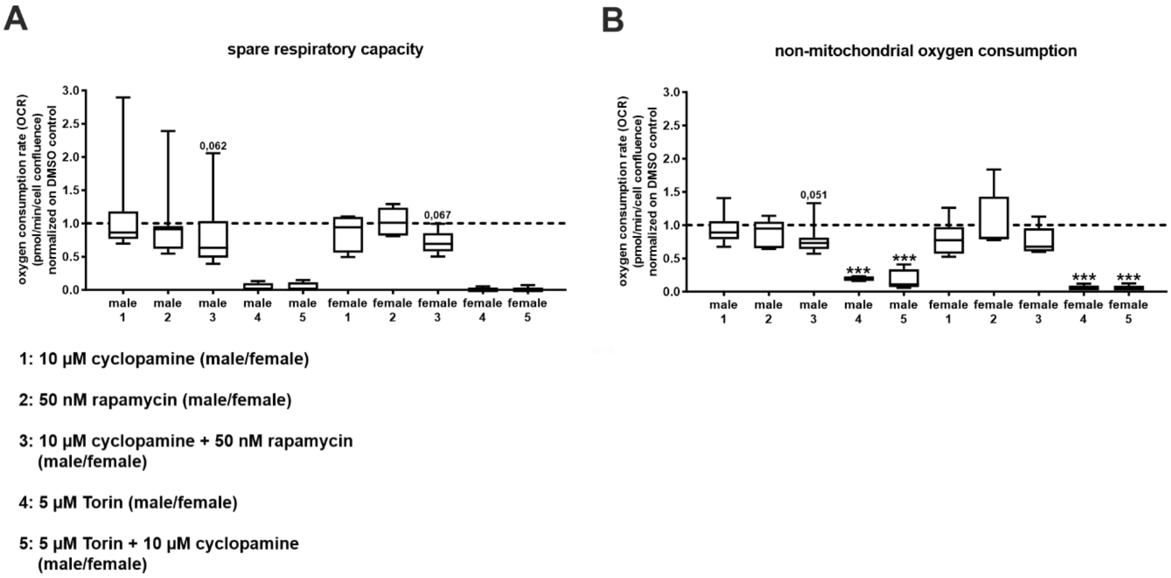


Figure S6: Combinatory effects of Hh and mTOR inhibitors on mitochondrial respiration. Mito stress test and cell energy phenotype analysis of primary hepatocytes from C57BL/6N mice. The oxygen consumption rate (OCR) was measured with the Seahorse analyser. The dashed lines show the DMSO control. A: Spare respiratory capacity. B: Non-mitochondrial oxygen consumption. Significance was calculated with one-way ANOVA for repeated measurements. Stars show the significance to DMSO control. * - P ≤ 0.05, ** - P ≤ 0.01, *** - P ≤ 0.001; N = 3-4.

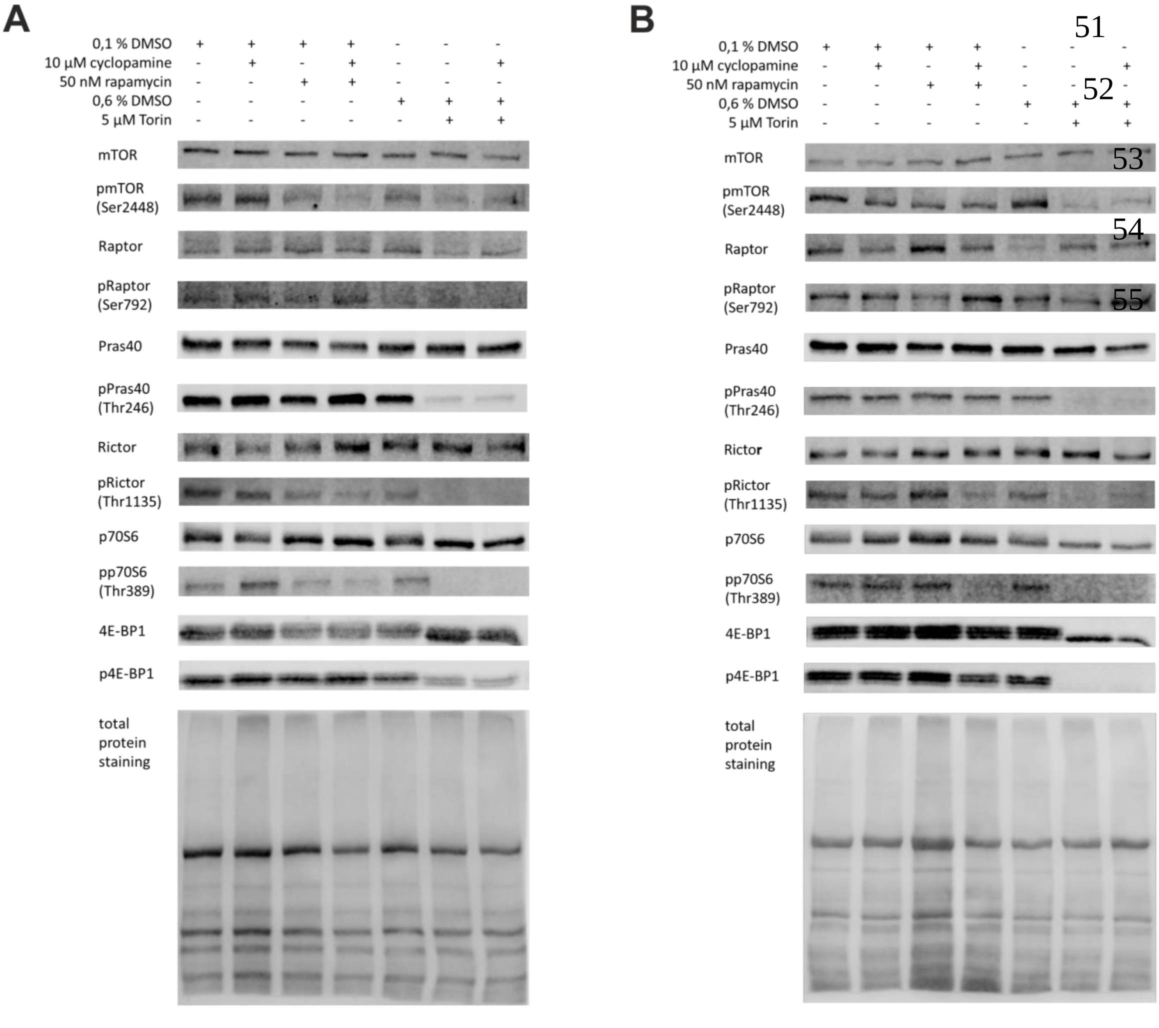


Figure S7: Representative examples of the Western blots of male and female C57BL/6N mice after treatment with cyclopamine, rapamycin and Torin. A: primary hepatocytes from male mice. B: primary hepatocytes from female mice.

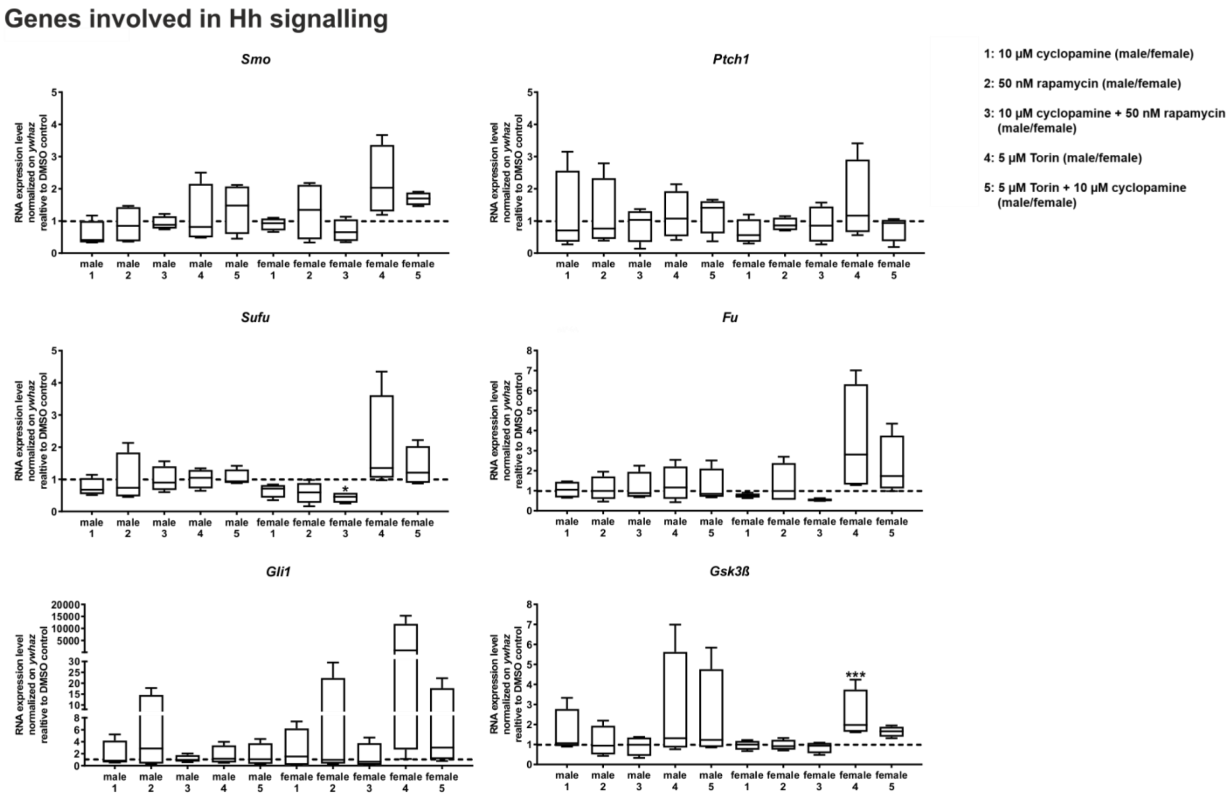


Figure S8: qPCR analysis of male and female hepatocytes treated with cyclopamine, rapamycin and Torin for 24 h. Results are shown relative to DMSO control (dashed line). Significance was calculated with one-way ANOVA for repeated measurements. Stars show the significance to DMSO control. * - P ≤ 0.05, ** - P ≤ 0.01, *** - P ≤ 0.001; N = 4

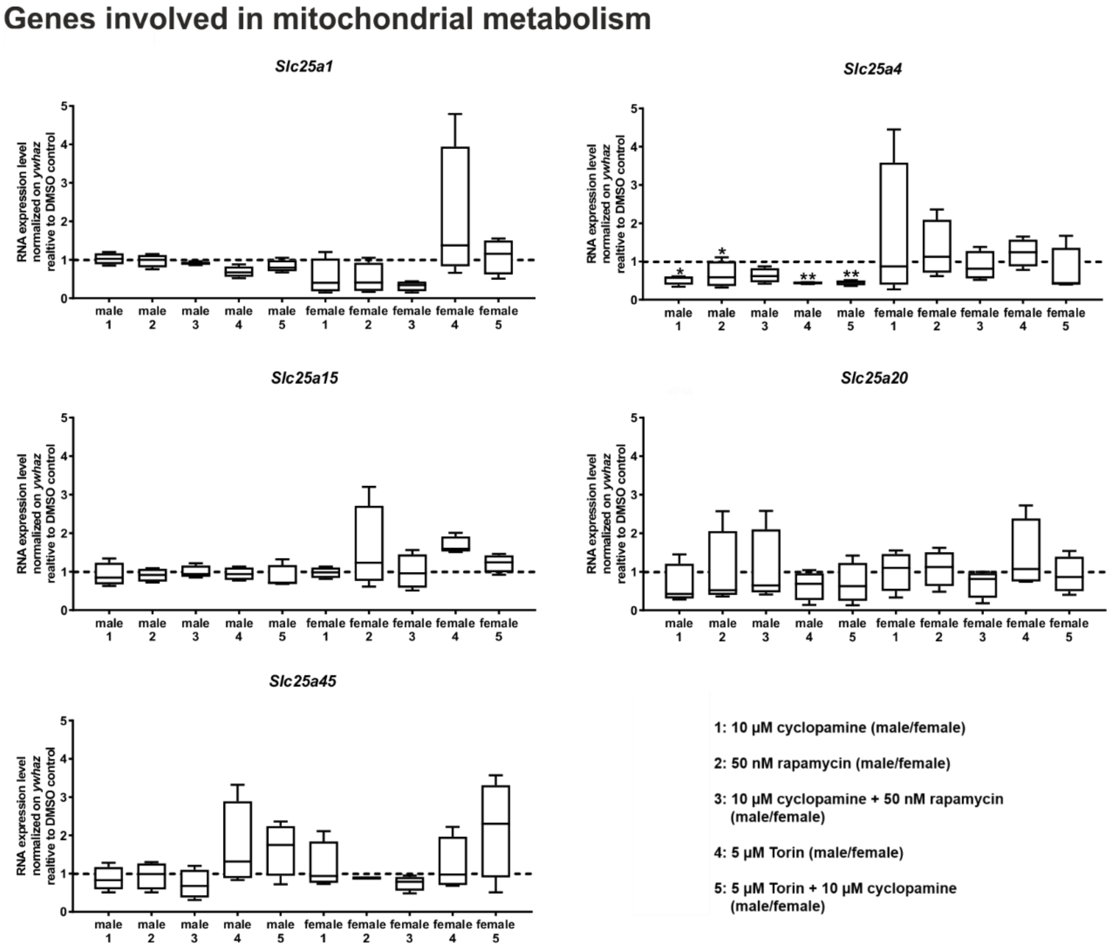


Figure S9: qPCR analysis of male and female hepatocytes treated with cyclopamine, rapamycin and Torin for 24 h. Results are shown relative to DMSO control (dashed line). Significance was calculated with one-way ANOVA for repeated measurements. Stars show the significance to DMSO control. * - $P \leq 0.05$, ** - $P \leq 0.01$, *** - $P \leq 0.001$; N = 4

Supplementary Tables

Table S1: IPA analysis of the oxidative phosphorylation of male SAC mice. Red color shows significantly upregulated proteins compared to WT mice.

Symbol	Entrez Gene Name	UniProt	Expression Fold Change	Expression p-value
ATP5A1	ATP synthase F1 subunit alpha	Q03265	1,058682801	0,212241
ATP5B	ATP synthase F1 subunit beta	P56480	1,022766332	0,640625
ATP5C1	ATP synthase F1 subunit gamma	Q8C2Q8	1,17285	0,0463706
ATP5D	ATP synthase F1 subunit delta	Q4FK74	-1,115838139	0,220541
ATP5F1	ATP synthase peripheral stalk-membrane subunit b	Q5I0W0	1,201342282	0,00826018
ATP5J	ATP synthase peripheral stalk subunit F6	P97450	-1,304911722	0,0944521
ATP5J2	ATP synthase membrane subunit f	P56135	1,042747041	0,83614
ATP5L	ATP synthase membrane subunit g	Q9CPQ8	1,373346649	0,0878789
ATP5O	ATP synthase peripheral stalk subunit OSCP	Q9DB20	1,197819315	0,0218166
COX4I1	cytochrome c oxidase subunit 4I1	A2RSV8	1,253557645	0,00489636
COX5A	cytochrome c oxidase subunit 5A	P12787	1,076923077	0,106636
COX6B1	cytochrome c oxidase subunit 6B1	P56391	1,160487117	0,16575
COX7A2	cytochrome c oxidase subunit 7A2	P48771	1,102265051	0,555348
NDUFA10	NADH:ubiquinone oxidoreductase subunit A10	Q99LC3	1,01743246	0,852348
NDUFA11	NADH:ubiquinone oxidoreductase subunit A11	G5E814	1,410237061	0,0420379
NDUFA12	NADH:ubiquinone oxidoreductase subunit A12	A0A0R4J275	1,487447699	0,00188871
NDUFA13	NADH:ubiquinone oxidoreductase subunit A13	Q9ERS2	1,283015152	0,000532561
NDUFA2	NADH:ubiquinone oxidoreductase subunit A2	Q9CQ75	1,571160291	0,0284502
NDUFA4	NDUFA4, mitochondrial complex associated	Q62425	1,741386456	0,021344
NDUFA5	NADH:ubiquinone oxidoreductase subunit A5	Q9CPP6	1,708982214	0,000554673
NDUFA6	NADH:ubiquinone oxidoreductase subunit A6	Q9CQZ5	1,476046516	0,052486
NDUFA7	NADH:ubiquinone oxidoreductase subunit A7	Q9Z1P6	1,613658743	0,000524843
NDUFA8	NADH:ubiquinone oxidoreductase subunit A8	Q9DCJ5	1,115959238	0,195955
NDUFA9	NADH:ubiquinone oxidoreductase subunit A9	A0A0R3P9C8	1,409242912	0,00416985
NDUFAB1	NADH:ubiquinone oxidoreductase subunit AB1	F6ZFT1	1,629851867	0,0175605
NDUFB10	NADH:ubiquinone oxidoreductase subunit B10	Q9DCS9	1,367406525	0,069593
NDUFB3	NADH:ubiquinone oxidoreductase subunit B3	Q9CQZ6	1,110904366	0,358154
NDUFB4	NADH:ubiquinone oxidoreductase subunit B4	Q9CQC7	1,01435099	0,890547
NDUFB5	NADH:ubiquinone oxidoreductase subunit B5	Q9CQH3	1,301350534	0,117685
NDUFB7	NADH:ubiquinone oxidoreductase subunit B7	Q9CR61	-1,138921732	0,522834
NDUFB8	NADH:ubiquinone oxidoreductase subunit B8	Q9D6J5	1,206208426	0,108491
NDUFB9	NADH:ubiquinone oxidoreductase subunit B9	Q9CQJ8	1,279164179	0,0499908
NDUFS1	NADH:ubiquinone oxidoreductase core subunit S1	Q91VD9	1,435179782	0,00107408
NDUFS2	NADH:ubiquinone oxidoreductase core subunit S2	Q91WD5	1,2595194	0,00122861

NDUFS3	NADH:ubiquinone oxidoreductase core subunit S3	Q9DCT2	1,031561462	0,761362
NDUFS4	NADH:ubiquinone oxidoreductase subunit S4	E9QPX3	1,427370367	0,0131995
NDUFS6	NADH:ubiquinone oxidoreductase subunit S6	P52503	1,409856632	0,0609841
NDUFS7	NADH:ubiquinone oxidoreductase core subunit S7	Q9DC70	1,322733173	0,0140408
NDUFS8	NADH:ubiquinone oxidoreductase core subunit S8	Q5M9P5	1,379349762	0,0302519
NDUFV1	NADH:ubiquinone oxidoreductase core subunit V1	D3YUM1	1,366963099	0,00507041
NDUFV2	NADH:ubiquinone oxidoreductase core subunit V2	Q9D6J6	1,220702055	0,0373881
NDUFV3	NADH:ubiquinone oxidoreductase subunit V3	Q3U422	-1,091105268	0,467469
SDHA	succinate dehydrogenase complex flavoprotein subunit A	Q8K2B3	1,132224774	0,0577188
SDHB	succinate dehydrogenase complex iron sulfur subunit B	Q9CQA3	1,019306565	0,727502
SDHC	succinate dehydrogenase complex subunit C	F8WGB3	1,381577175	0,00263165
SDHD	succinate dehydrogenase complex subunit D	Q9CXV1	1,497884537	0,00199167
UQCR10	ubiquinol-cytochrome c reductase, complex III subunit X	Q5NCJ9	1,084144879	0,628202
UQCRB	ubiquinol-cytochrome c reductase binding protein	Q9CQB4	1,585364739	0,00320201
UQCRC1	ubiquinol-cytochrome c reductase core protein 1	Q9CZ13	1,103466495	0,0302956
UQCRC2	ubiquinol-cytochrome c reductase core protein 2	Q9DB77	1,307964858	0,0023692
UQCRFS1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	Q9CR68	1,164235726	0,225653
UQCRQ	ubiquinol-cytochrome c reductase complex III subunit VII	Q9CQ69	1,421077458	0,0112891

Table S2: IPA analysis of the oxidative phosphorylation of female SAC mice. Blue color shows significantly downregulated proteins compared to WT mice.

Symbol	**Entrez Gene Name**	**UniProt**	**Expression Fold Change**	**Expression p-value**
ATP5B	ATP synthase F1 subunit beta	P56480	-1,0483	0,022013
ATP5L	ATP synthase membrane subunit g	Q9CPQ8	1,043214	0,37549
ATP5O	ATP synthase peripheral stalk subunit OSCP	Q9DB20	1,00571	0,653439
COX5A	cytochrome c oxidase subunit 5A	P12787	1,101418	0,49025
COX7A2	cytochrome c oxidase subunit 7A2	P48771	-1,04944	0,129319
ND1	NADH dehydrogenase, subunit 1 (complex I)	P03888	-1,48769	0,499383
NDUFA10	NADH:ubiquinone oxidoreductase subunit A10	Q99LC3	-1,09567	0,012843
NDUFA13	NADH:ubiquinone oxidoreductase subunit A13	Q9ERS2	-1,16658	0,049629
NDUFA2	NADH:ubiquinone oxidoreductase subunit A2	Q9CQ75	-1,10987	0,017984
NDUFA3	NADH:ubiquinone oxidoreductase subunit A3	Q9CQ91	-1,12179	0,00759
NDUFA6	NADH:ubiquinone oxidoreductase subunit A6	Q9CQZ5	-1,0644	0,048424
NDUFA7	NADH:ubiquinone oxidoreductase subunit A7	Q9Z1P6	-1,26195	0,017791
NDUFA8	NADH:ubiquinone oxidoreductase subunit A8	Q9DCJ5	-1,04613	0,052604
NDUFB11	NADH:ubiquinone oxidoreductase subunit B11	O09111	-1,06108	0,740741
NDUFB3	NADH:ubiquinone oxidoreductase subunit B3	Q9CQZ6	-1,0454	0,388971
NDUFB4	NADH:ubiquinone oxidoreductase subunit B4	Q9CQC7	1,053449	0,382914

NDUFB7	NADH:ubiquinone oxidoreductase subunit B7	Q9CR61	-1,16581	0,001242
NDUFB9	NADH:ubiquinone oxidoreductase subunit B9	Q9CQJ8	-1,07164	0,045184
NDUFS1	NADH:ubiquinone oxidoreductase core subunit S1	Q91VD9	-1,04716	0,041061
NDUFS3	NADH:ubiquinone oxidoreductase core subunit S3	Q9DCT2	-1,02622	0,054845
NDUFS6	NADH:ubiquinone oxidoreductase subunit S6	P52503	-1,12152	0,050392
NDUFV2	NADH:ubiquinone oxidoreductase core subunit V2	Q9D6J6	-1,23939	0,019085
NDUFV3	NADH:ubiquinone oxidoreductase subunit V3	Q3U422	-1,15421	0,059305
SDHA	succinate dehydrogenase complex flavoprotein subunit A	Q8K2B3	1,023128	0,346857
SDHB	succinate dehydrogenase complex iron sulfur subunit B	Q9CQA3	-1,1003	0,015305
SDHD	succinate dehydrogenase complex subunit D	Q9CXV1	-1,20444	0,147099
UQCRC1	ubiquinol-cytochrome c reductase core protein 1	Q9CZ13	-1,06154	0,008637
UQCRC2	ubiquinol-cytochrome c reductase core protein 2	Q9DB77	1,001478	0,120837
UQCRFS1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	Q9CR68	-1,01547	0,61814
UQCRQ	ubiquinol-cytochrome c reductase complex III subunit VII	Q9CQ69	-1,0132	0,310037

Table S3: IPA analysis of the mTOR pathway in male SAC mice. Red colour shows significantly upregulated and blue colour significantly downregulated proteins compared to WT mice.

Symbol	**Entrez Gene Name**	**UniProt**	**Expression Fold Change**	**Expression p-value**
Cdc42	cell division cycle 42	P60766	1,540981	1,94E-05
EIF3A	eukaryotic translation initiation factor 3 subunit A	P23116	-1,2476	0,091012
EIF3B	eukaryotic translation initiation factor 3 subunit B	Q8JZQ9	-1,20117	0,022585
EIF3C	eukaryotic translation initiation factor 3 subunit C	Q8R1B4	-1,23968	0,120117
EIF3D	eukaryotic translation initiation factor 3 subunit D	O70194	1,09707	0,433826
EIF3E	eukaryotic translation initiation factor 3 subunit E	Q3UIG0	-1,07593	0,22065
EIF3F	eukaryotic translation initiation factor 3 subunit F	Q9DCH4	-1,28723	0,04318
EIF3G	eukaryotic translation initiation factor 3 subunit G	Q544H0	-1,46485	0,036465
EIF3H	eukaryotic translation initiation factor 3 subunit H	Q5M9L0	-1,64027	0,036758
EIF3I	eukaryotic translation initiation factor 3 subunit I	Q9QZD9	1,018038	0,877609
EIF3J	eukaryotic translation initiation factor 3 subunit J	Q3UGC7	-1,25137	0,03772
EIF3L	eukaryotic translation initiation factor 3 subunit L	Q8QZY1	-1,13806	0,456783
EIF4A1	Eukaryotic Translation Initiation Factor 4A1	Q5F2A7	-1,35793	0,00517
EIF4A2	Eukaryotic Translation Initiation Factor 4A2	P10630	1,005995	0,941128
EIF4A3	Eukaryotic Translation Initiation Factor 4A3	Q91VC3	1,040051	0,464287
eIF4B	eukaryotic translation initiation factor 4B	Q8BGD9	-1,06853	0,429906
eIF4E	eukaryotic translation initiation factor 4E	A0A0G2JH04	1,224622	0,193666
EIF4G1	Eukaryotic Translation Initiation Factor 4 Gamma 1	E9PVC6	1,009655	0,897905
EIF4G2	Eukaryotic Translation Initiation Factor 4 Gamma 2	F7CBP1	-1,04425	0,395205
FKBP1	Peptidyl-prolyl cis-trans isomerase	P26883	-1,09399	0,723345

MAPK3	mitogen-activated protein kinase 3	D3Z3G6	1,064703	0,777924
PPP2CA	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	P63330	-1,00567	0,968446
PPP2R1A	protein phosphatase 2 scaffold subunit Aalpha	Q76MZ3	1,05696	0,395291
PTPA	Serine/threonine-protein phosphatase 2A activator	Q543N6	-1,10334	0,387378
RAC	RAS-related C3 botulinum substrate 1	K7Q7T7	1,139008	0,371887
RAP1A	Ras-related protein Rap-1A	P62835	1,084862	0,315107
RHOA	Ras family member A	Q4VAE6	-1,00361	0,981248
RHOT1	Mitochondrial Rho GTPase 1	Q8BG51	1,199016	0,177205
RPS10	ribosomal protein S10	Q5M9K7	-1,10841	0,609755
RPS11	ribosomal protein S11	Q3UC02	-1,14374	0,300757
RPS12	ribosomal protein S12	Q6ZWZ6	-1,2036	0,1127
RPS13	ribosomal protein S13	Q5BLJ7	1,123551	0,474207
RPS14	ribosomal protein S14	P62264	-1,33818	0,018767
RPS15	ribosomal protein S15	D3YTQ9	-1,25502	0,142416
RPS15A	ribosomal protein S15A	Q5M9M4	1,011522	0,914649
RPS16	ribosomal protein S16	A4FUS1	-1,24287	0,009313
RPS17	ribosomal protein S17	Q5M9L7	-1,26688	0,101462
RPS18	ribosomal protein S18	Q561N5	-1,08507	0,329736
RPS19	ribosomal protein S19	Q9CZX8	-1,26948	0,021572
RPS2	ribosomal protein S2	Q58EU3	-1,11017	0,383971
RPS20	ribosomal protein S20	Q5BLK2	-1,41691	0,002367
RPS21	ribosomal protein S21	Q9CQR2	-1,43761	0,000159
RPS23	ribosomal protein S23	P62267	1,034375	0,777361
RPS24	ribosomal protein S24	P62849	-1,11807	0,41224
RPS25	ribosomal protein S25	Q58EA6	-1,0557	0,625492
RPS26	ribosomal protein S26	Q497N1	-1,60241	0,000878
RPS27A	ribosomal protein S27A	P62983	1,049891	0,212852
RPS27L	ribosomal protein S27L	Q6ZWY3	-1,22078	0,004931
RPS28	ribosomal protein S28	G3UYV7	-2,46397	0,007698
RPS29	ribosomal protein S29	P62274	-1,21994	0,417447
RPS3	ribosomal protein S3	Q5YLW3	-1,20102	0,133493
RPS4Y1	ribosomal protein S4Y1	Q545X8	-1,07143	0,501606
RPS5	ribosomal protein S5	D3YYM6	-1,17434	0,160589
RPS6KA1	Non-specific serine/threonine protein kinase	G3UZI3	1,25737	0,037294
RPS7	ribosomal protein S7	Q4FZE6	-1,61447	0,039958
RPS8	ribosomal protein S8	Q497E9	-1,26073	0,000361
RPS9	ribosomal protein S9	Q6ZWN5	-1,11871	0,262164
RPSA	ribosomal protein SA	P14206	-1,22301	0,014658
RRAS2	Ras-related protein R-Ras2	P62071	-1,08332	0,505504

Table S4: IPA analysis of the mTOR pathway in female SAC mice. Red colour shows significantly upregulated proteins compared to WT mice.

Symbol	Entrez Gene Name	UniProt	Expression Fold Change	Expression p-value
Cdc42	cell division cycle 42	P60766	1,165059	0,457934
EIF3A	eukaryotic translation initiation factor 3 subunit A	P23116	1,125472	0,007718
EIF3B	eukaryotic translation initiation factor 3 subunit B	Q8JZQ9	1,147002	0,013067
EIF3C	eukaryotic translation initiation factor 3 subunit C	Q8R1B4	1,297426	0,005495
EIF3D	eukaryotic translation initiation factor 3 subunit D	O70194	1,114022	0,163498
EIF3F	eukaryotic translation initiation factor 3 subunit F	Q9DCH4	1,226081	0,000242
EIF3I	eukaryotic translation initiation factor 3 subunit I	Q9QZD9	1,203921	0,009641
EIF3L	eukaryotic translation initiation factor 3 subunit L	Q8QZY1	1,289237	0,009817
eIF4B	eukaryotic translation initiation factor 4B	Q8BGD9	1,075572	0,123038
MAPK1	mitogen-activated protein kinase 1	P63085	1,01818	0,254812
PPP2R1A	protein phosphatase 2 scaffold subunit Aalpha	Q76MZ3	-1,07324	0,177206
RPS21	ribosomal protein S21	Q9CQR2	1,18445	0,000504
RPS23	ribosomal protein S23	P62267	1,18845	0,016624
RPS24	ribosomal protein S24	P62849	1,181007	0,157707
RPS29	ribosomal protein S29	P62274	1,182092	0,001764
RPSA	ribosomal protein SA	P14206	1,232653	0,001462