Supplemental tables:

Super Pathway	Sub Pathway		Biochemical Name	Fold change
			Two-Way ANOVA Contrasts	Welch's Two-Sample *t*-Test
			MEF *Nf2*$^{-/-}$ / MEF *Nf2*$^{f/f}$	**FH912 / FC912**
Vitamins and Cofactors	Nicotinate and Nicotinamide Metabolism	nicotinamide adenine dinucleotide (NAD+)	**1.61**	**4.55**
		nicotinamide adenine dinucleotide reduced (NADH)	**1.34**	**3.79**
		nicotinamide adenine dinucleotide phosphate (NADP+)	1.22	**6.09**
		adenosine 5'diphosphoribose	0.29	1.08
	Riboflavin Metabolism	riboflavin (Vitamin B2)	0.95	**1.3**
		flavin adenine dinucleotide	**0.81**	**0.76**
	Pantothenate and CoA Metabolism	pantothenate	**1.11**	**2.1**
		phosphopantetheine	**2.01**	**5.81**
		coenzyme A	**3**	**0.54**
	Ascorbate Metabolism	gulono-1,4-lactone	**2.04**	**5.06**
	Tocopherol Metabolism	alpha-tocopherol	**0.65**	1
	Folate Metabolism	5-methyltetrahydrofolate	1.03	**1.59**
	Hemoglobin Metabolism	bilirubin (Z,Z)	1	1.46
	Thiamine Metabolism	thiamin (Vitamin B1)	**1.54**	0.91
		thiamin diphosphate	**0.41**	0.74
	Vitamin B6 Metabolism	pyridoxine (Vitamin B6)	1	**0.5**
		pyridoxal	**0.68**	1.16

Table S1. Elevated levels of vitamins and cofactors in *Nf2*-deficient cell lines.

Samples were processed and analyzed as in Table 1.

Super Pathway	Sub Pathway	Biochemical Name	Two-Way ANOVA Contrasts	Welch's Two-Sample *t*-Test
			MEF *Nf2*$^{-/-}$ MEF *Nf2*$^{f/f}$	**FH912 FC912**
Aminoacids	Glycine, Serine and Threonine Metabolism	glycine	0.92	**0.86**
		serine	**0.75**	0.93
		threonine	**0.82**	**0.77**
		N-acetylthreonine	**0.87**	1.12
	Alanine and Aspartate Metabolism	aspartate	1.01	**0.77**
		asparagine	**0.49**	1.4
	Glutamate Metabolism	glutamate	**1.4**	0.98
		glutamine	**0.37**	**0.85**
		N-acetylglutamate	0.72	**4.8**
		glutamate, gamma-methyl ester	**1.57**	**0.65**
	Histidine Metabolism	histidine	**0.75**	1.08
	Lysine Metabolism	lysine	**0.8**	**0.64**
		N6-acetyllysine	**0.34**	0.96
		2-aminoadipate	**0.56**	**0.65**
	Phenylalanine and Tyrosine Metabolism	phenylalanine	**0.76**	0.99
		phenylacetylglycine	**0.23**	1
		tyrosine	**0.67**	1.18
	Tryptophan Metabolism	tryptophan	**0.69**	0.97
		C-glycosyltryptophan*	**0.79**	**0.75**
	Leucine, Isoleucine and Valine Metabolism	leucine	**0.83**	**0.81**
		4-methyl-2-oxopentanoate	0.95	**0.65**
		isovalerylcarnitine	**0.28**	1
		isoleucine	**0.74**	1.12
		valine	**0.6**	1.09
	Methionine, Cysteine, SAM and Taurine Metabolism	methionine	**0.75**	0.93
		N-acetylmethionine	**0.77**	0.98
		hypotaurine	**1.46**	**0.38**

Table S2. Reduced amino acids levels in *Nf2*-deficient cell lines. Samples were processed and analyzed as in Table 1.

Super Pathway	Sub Pathway	Biochemical Name	Fold change		
			Two-Way ANOVA Contrasts		
			MEF *Nf2*$^{-/-}$ / MEF *Nf2*$^{f/f}$	MEF *Nf2*$^{f/f}$Ceru / MEF *Nf2*$^{f/f}$	MEF *Nf2*$^{-/-}$Ceru / MEF *Nf2*$^{-/-}$
Energy	TCA Cycle	citrate	**1.47**	**1.34**	0.93
		succinate	**2.62**	**1.91**	0.75
		fumarate	**1.23**	**1.44**	**1.17**
		malate	**1.94**	**1.8**	1.03
	Oxidative Phosphorylation	acetylphosphate	**1.8**	**1.67**	**0.33**
		pyrophosphate	0.81	1.25	0.63
		phosphate	1.01	**1.2**	0.92

Table S3. Different response to cerulenin treatment in *Nf2*-deficient and control cell lines. MEF *Nf2*$^{f/f}$Ceru – WT MEFs treated with 5 μM cerulenin for 24 hours prior to sample preparation, MEF *Nf2*$^{-/-}$Ceru – *Nf2*-knockout MEFs treated with 5 μM cerulenin for 24 hours prior to sample preparation. Samples were processed and analyzed as in Table 1.

Supplemental Materials and Methods

List of qPCR primers:

Actb (potential normalizer) Fwd: CCAGCAGATTGTGATCAGCA;

Rev: CTTGCGGTGCACGATGG

Acaca Fwd: TGACAGACTGATCGCAGAGAAAG;

Rev: TGGAGAGCCCCACACACA

Acacb Mm01204683_m1

Acl Fwd: TGACAGACTGATCGCAGAGAAAG;

Rev: TGGAGAGCCCCACACACA

Acsl1 Fwd: GTACATAGCACCAGAAAAGATTGAAAAT;

Rev: GGTACCACAACTATGAGAAAGG

Acss2 Fwd: AGTCCCCACCAGTTAAGAGGC;

Rev: GTGTACAAGATGAAGAGTGGGTCCT

Cpt1c Fwd: GTGTTCCGCCCAGTATGAGAG;

Rev: GAGAGTGAGTCCCTACACGGAAG

Cpt2 Fwd: ATATGATGGCTGAGTGCTCCA; Rev: AGAGCTCAGGCAGGGTGAC

Fasn (Taqman set) Mm00662319_m1

Lpin1 Mm00550511_m1

Mlycd Fwd: CCGCTGCCATCTTCTACTCC; Rev: GATAGGCGACAGGCTTGAAAA

Srebp1 Fwd: GGCCGAGATGTGCGAACT; Rev: TTGTTGATGAGCTGGAGCATGT

Acyl-CoA quantification. Acetyl-CoA, malonyl-CoA, and propionyl-CoA standards were individually prepared as 1 mM stock in water and stored at -80°C until use. All acyl-CoA standards were diluted in 50 mM ammonium formate:acetonitrile (30:70, v/v) to prepare a set of calibration standards. In addition, propionyl-CoA was chosen as the recovery standard (RS) [2].

Cells were grown in 30 cm tissue culture dishes in complete media until confluent. 5 μM cerulenin was added to part of the cells 12 hours after plating and incubated for 24 hours. Cells were tripsynized, and cell pellets snap-frozen (dry ice – ethanol). Thawed pellets with RS added, ~1g per sample type, were pulse sonicated in 10% perchloric acid and neutralized with neutralization buffer (2 M KOH, 0.4 M KCl and 0.4 M imidazole),

and then centrifuged at 8,000 x *g* at 4°C for 20 min, in preparation for solid phase extraction (SPE).

SPE was conducted with a Waters Oasis HLB 3 cc extraction cartridge (WAT094226, Milford, MA). The acidified cell extracts (2% formic acid) from each sample were loaded on a SPE cartridge and eluted with 0.2 mL water, 0.2 mL methanol, and 3 mL methanol sequentially. The last 3 mL portion of eluates was collected and then was evaporated using a SpeedVac concentrator. The residuals were re-dissolved in 50 mM ammonium formate:acetonitrile (30:70, v/v).

A Waters Acquity H-class ultra-high performance liquid chromatography (UPLC, Milford, MA) was used to inject 10 μL of the processed samples or the calibration standards to an Acquity HSS T3 column (2.1 x 50 mm; particle size 1.8 μm) and to elute with the isocratic mobile phase, 50 mM ammonium formate:acetonitrile (30:70, v/v). All injected samples were eluted over 10 min at 0.4 mL/min at 30°C, and the eluates were directly introduced into the coupled mass spectrometer (MS) for quantitative analysis.

Quantitation was conducted by a Thermo triple quadrupole mass spectrometer (TSQ Quantum Access, Waltham, MA). The mass spectrometric conditions were set as the followings: electrospray voltage: +4 kV; sheath gas pressure: 45 psi; auxiliary gas pressure: 20 psi; ion sweep gas pressure: 2 psi; collision gas pressure: 1.5 mTorr; and capillary temperature: 380°C. Unit resolution was selected for ion scans [3]. Multiple reaction monitoring (MRM) was used to detect the elution of the target acyl-CoAs. The mass transitions for acetyl-CoA, malonyl-CoA, and propionyl-CoA are 810→303, 854→347, and 824→317, respectively [1]. The areas under individual resolved peaks were integrated using Xcalibur software (version 2.1, Thermo).

Supplemental figure legends

Figure S1A. Immunoblot detection of reexpressed Merlin level in mouse schwannoma cells. SC4-9 cells were transfected with pBabe-Merlin-puro and pBabe-puro plasmids. Levels of Merlin were detected 24 hours after transfection. β-Actin was used for loading control.

Figure S1B. Immunoblot quantification of Casp3 cleavage caused by Fasn inhibition. Quantification performed using ImageJ software. Mean values and CI95 are shown on graphs. **** – $p \leq 0.0001$.

Figure S1C. Increased Casp3 cleavage in *Nf2*$^{-/-}$ MEFs transfected with anti-*Fasn* siRNA and treated with GSK2194069. Cells were transfected by electroporation with anti-*Fasn* and scrambled (negative control) siRNAs, final concentration 100 nM, and plated into 6-well plates (250,000 cells/well). To compare the GSK2194069 effects to *Fasn* knockdown, cells transfected with non-silencing siRNA were treated with 0.5 μM GSK2194069 for 12 hours before lysis. Lysates were analyzed by immunoblotting using rabbit anti-FASN and anti-cleaved Casp3 antibodies. Typical blots are shown. 1 – vehicle, 2 – GSK2194069, 3 – anti-*Fasn* siRNA *Fasn177858*, 4 – anti-*Fasn* siRNA *Fasn177854*

Figure S1D.

Figure S2. Modulation of the effect of FASN inhibitors by genetic knockdown of acetyl-CoA carboxylase (ACC1) and malonyl-CoA (MCD) decarboxylase activity. **A)** Effect of *Acaca* knockdown on cerulenin toxicity. Cells were transfected by electroporation with anti-*Acaca* and scrambled (negative control) siRNAs. Indicated amounts of cerulenin were added in 24 hours and incubated for 48 hours. Parallel transfections for immunoblotting knockdown control were done in a 6-well format. Each

lysate type was run in duplicate for transfer consistency control. Experiments were done in quadruplicates and repeated 3 times. Mean values and 95% confidence intervals are shown on graphs. **B)** Effect of *Acaca* knockdown on GSK2194069 toxicity. Cells were transfected by electroporation with anti-*Acaca* and scrambled (negative control) siRNAs. Indicated amounts of cerulenin were added in 24 hours and incubated for 48 hours. Parallel transfections for immunoblotting knockdown control were done in a 6-well format. **C)** Effect of *Mlycd* knockdown on GSK2194069 toxicity. Cells were transfected by electroporation with anti-*Mlycd* and scrambled (negative control) siRNAs. Indicated amounts of cerulenin were added in 24 hours and incubated for 48 hours. Parallel transfections for knockdown control were done in a 6-well format. Experiments were done in quadruplicates and repeated 4 times. Mean values and 95% confidence intervals are shown on graphs.

Figure S3. Quantification of immunoblot detection of levels of expression and phosphorylation of lipogenesis-related proteins in *Nf2*$^{-/-}$ and *Nf2*$^{f/f}$ MEFs.
Experiments were repeated 4 times. ImageJ software was used for the band intensity quantification. Band intensities were normalized to beta-Actin band intensity. Mean values and 95% confidence intervals are shown on graphs. Statistical significance *p* was determined by Holm-Sidak *t*-test. * – $p \leq 0.05$, ** – $p \leq 0.01$, *** – $p \leq 0.001$, **** – $p \leq 0.0001$

Figure S4. Quantification of immunoblot detection of levels of expression and phosphorylation of lipogenesis-related proteins in SC4-9 Babe and SC4-9 Merlin cells. Data was processed as and analyzed as in Figure S1.

Figure S5. Role of TORC1 in lipogenesis upregulation in *Nf2*$^{-/-}$ MEFs. A) *Mtor* knockdown effect on FASN inhibition. Cells were transfected by electroporation with anti-*Mtor* and scrambled (negative control) siRNAs. Indicated amounts of GSK2194069 were added in 24 hours and incubated for 48 hours. **B)** *Rptor* knockdown effect on FASN inhibition. Cells were transfected by electroporation with *Rptor* and scrambled (negative control) siRNAs. Indicated amounts of GSK2194069 were added in 24 hours and incubated for 48 hours. All experiments were repeated 2 times, dose response curves were done in quadruplicates, and for WBs each lysate type was run in duplicates for transfer consistency control. Mean values and 95% confidence intervals are shown on graphs.

Figure S6. Increased mitochondrial respiration in *Nf2*-deficient cells. Oxygen consumption rate (OCR) was determined in *Nf2*$^{-/-}$ and *Nf2*$^{f/f}$ MEFs in a 96-well format on a Seahorse XF Metabolic Flux analyser using the Seahorse Mito Stress Kit (Seahorse Bioscience, N. Billerica, USA) according to the manufacturer's protocol. Initial cell density was four thousand cells/well. At time points a (20 min), b (50 min), and c (80 min), oligomycin (5 µM), FCCP (1 µM), and antimycin were added.

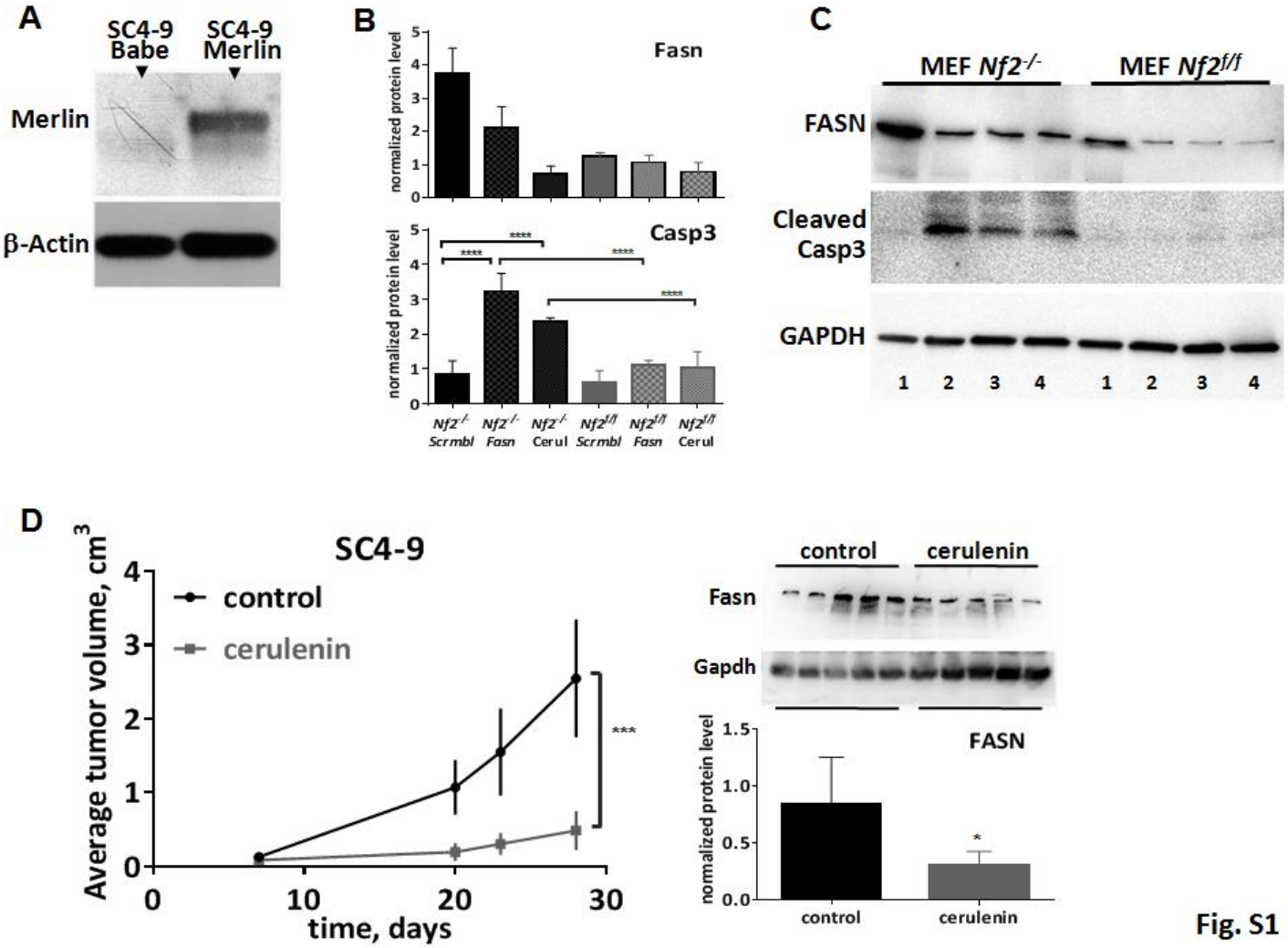
Figure S7. Decreased levels of fatty acid beta-oxidation gene expression in *Nf2*-deficient cell lines. RNA quantification was performed on *Nf2*$^{-/-}$ and *Nf2*$^{f/f}$ MEFs; SC4-9 Babe (SC4-9 cells transiently transfected with empty pBabe-puro plasmid); SC4-9 Merlin (SC4-9 cells transiently transfected with pBabe-Merlin plasmid); and *Nf2*$^{-/-}$ (FH912) and *Nf2*$^{f/f}$ (FC912) mouse Schwann cells. All experiments were repeated 2 times. Mean and 95% CI are shown on graphs. *Acadl* = acyl-CoA dehydrogenase, long chain; *Acadm* = acyl-CoA dehydrogenase, medium chain; *Acadls* = acyl-CoA dehydrogenase, short chain; *Acox1* = acyl-CoA oxydase 1; *Acox3* = acyl-CoA oxydase 3; *Acaa1* = acetyl-CoA acyltransferase 1; *Echs1* = enoyl CoA hydratase, short chain, 1, mitochondrial; *Hadh* = hydroxyacyl-CoA dehydrogenase.

References

1 Gilibili RR, Kandaswamy M, Sharma K, Giri S, Rajagopal S, Mullangi R. Development and validation of a highly sensitive LC-MS/MS method for simultaneous quantitation of acetyl-CoA and malonyl-CoA in animal tissues. Biomed Chromatogr 2011; 25: 1352-1359.

2 Hayashi O, Satoh K. Determination of acetyl-CoA and malonyl-CoA in germinating rice seeds using the LC-MS/MS technique. Biosci Biotechnol Biochem 2006; 70: 2676-2681.

3 Kuo YM, Andrews AJ. Quantitating the specificity and selectivity of Gcn5-mediated acetylation of histone H3. PLoS One 2013; 8.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SC4-9 Merlin
Merlin
β-Actin
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Fasn
normalized protein level
Casp3
****
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Nf2-/- Fasn
Nf2-/- Cerul
Nf2f/f Scrmbl
Nf2f/f Fasn
Nf2f/f Cerul
C
MEF Nf2-/-
MEF Nf2f/f
FASN
Cleaved Casp3
GAPDH
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D
SC4-9
control
cerulenin
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time, days
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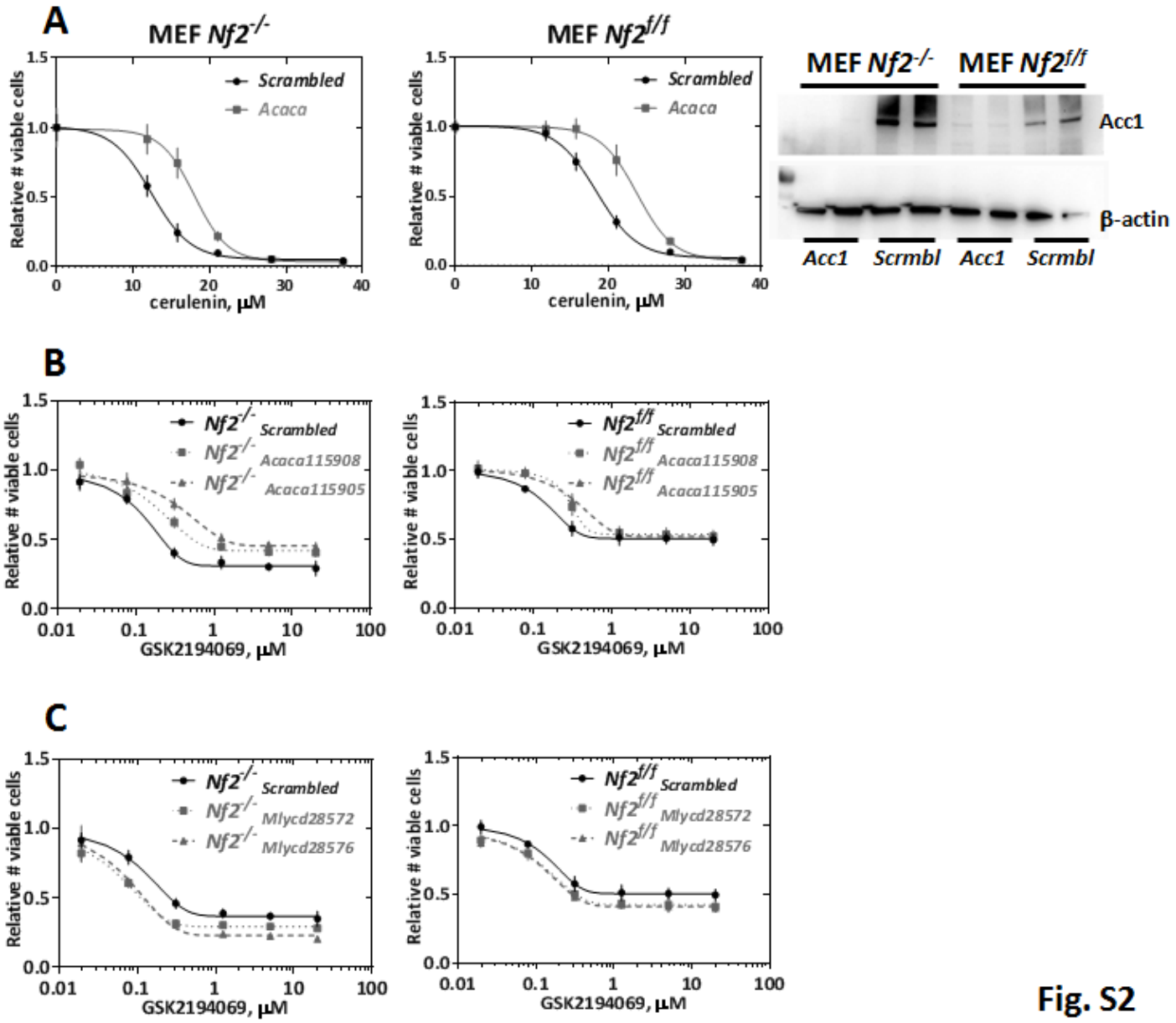
control
cerulenin
Fasn
Gapdh
FASN
normalized protein level
*

Fig. S1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MEF Nf2f/f
Scrambled
Acaca
Relative # viable cells
cerulenin, μM
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Acc1
β-actin
Acc1 Scrmbl Acc1 Scrmbl
B
Nf2-/- Scrambled
Nf2-/- Acaca115908
Nf2-/- Acaca115905
Nf2f/f Scrambled
Nf2f/f Acaca115908
Nf2f/f Acaca115905
GSK2194069, μM
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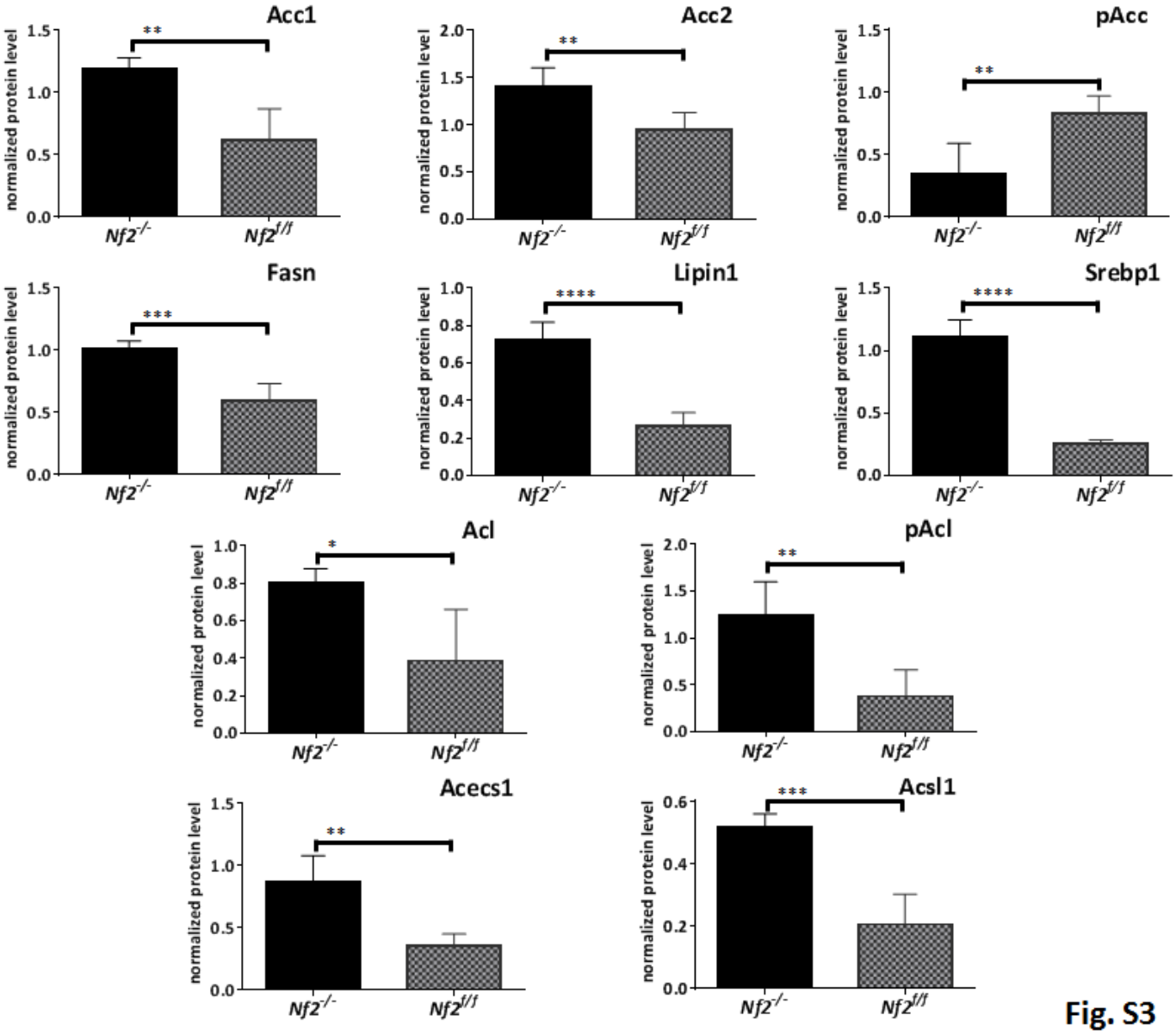
Fig. S2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Acc2
pAcc
Fasn
Lipin1
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Acecs1
Acsl1
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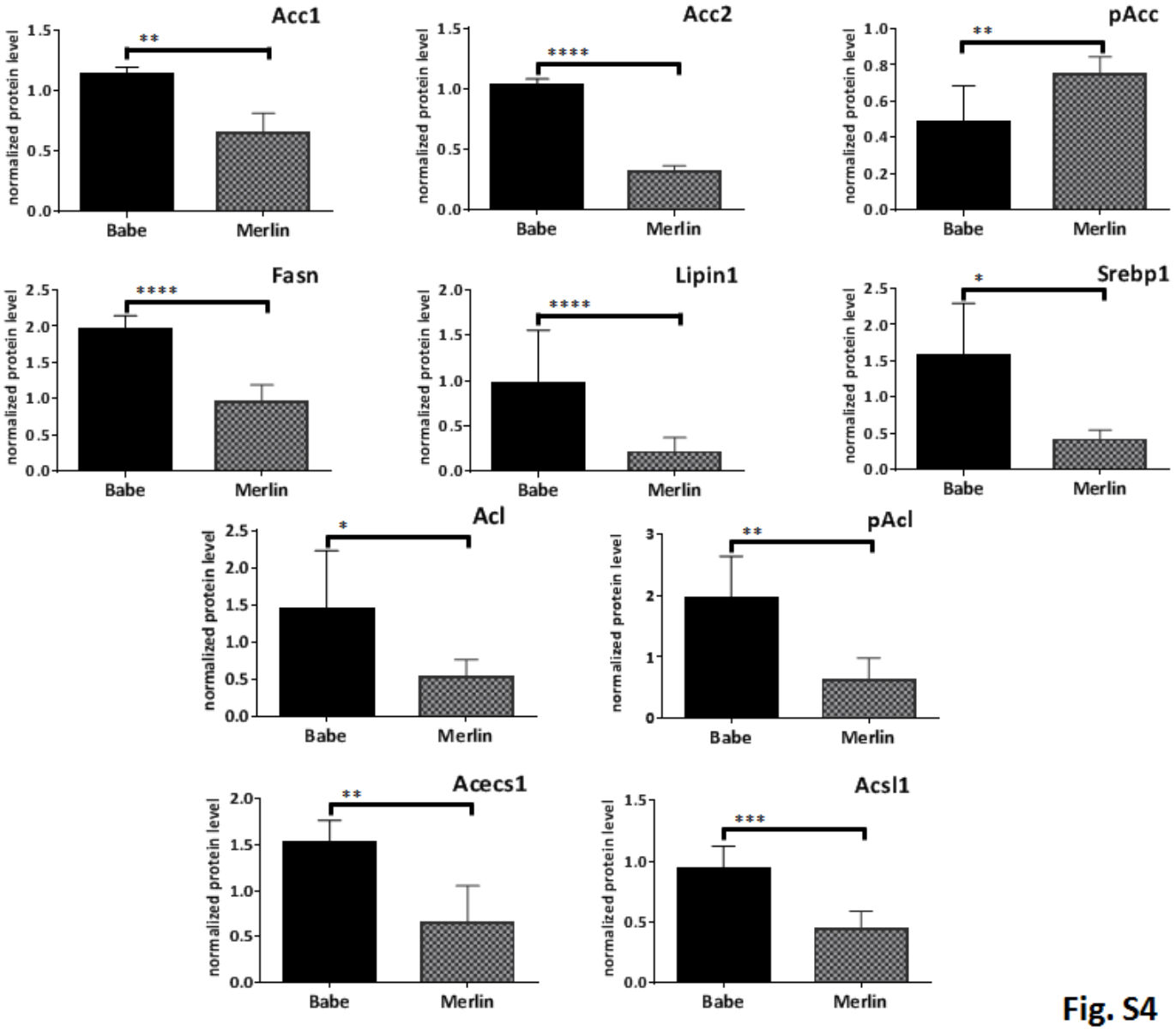
Nf2-/-
Nf2f/f

Fig. S3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Babe
Merlin
Acc2
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0.0
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Babe
Merlin
pAcc
normalized protein level
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Merlin
Srebp1
normalized protein level
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2.0
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1.0
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*
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Merlin
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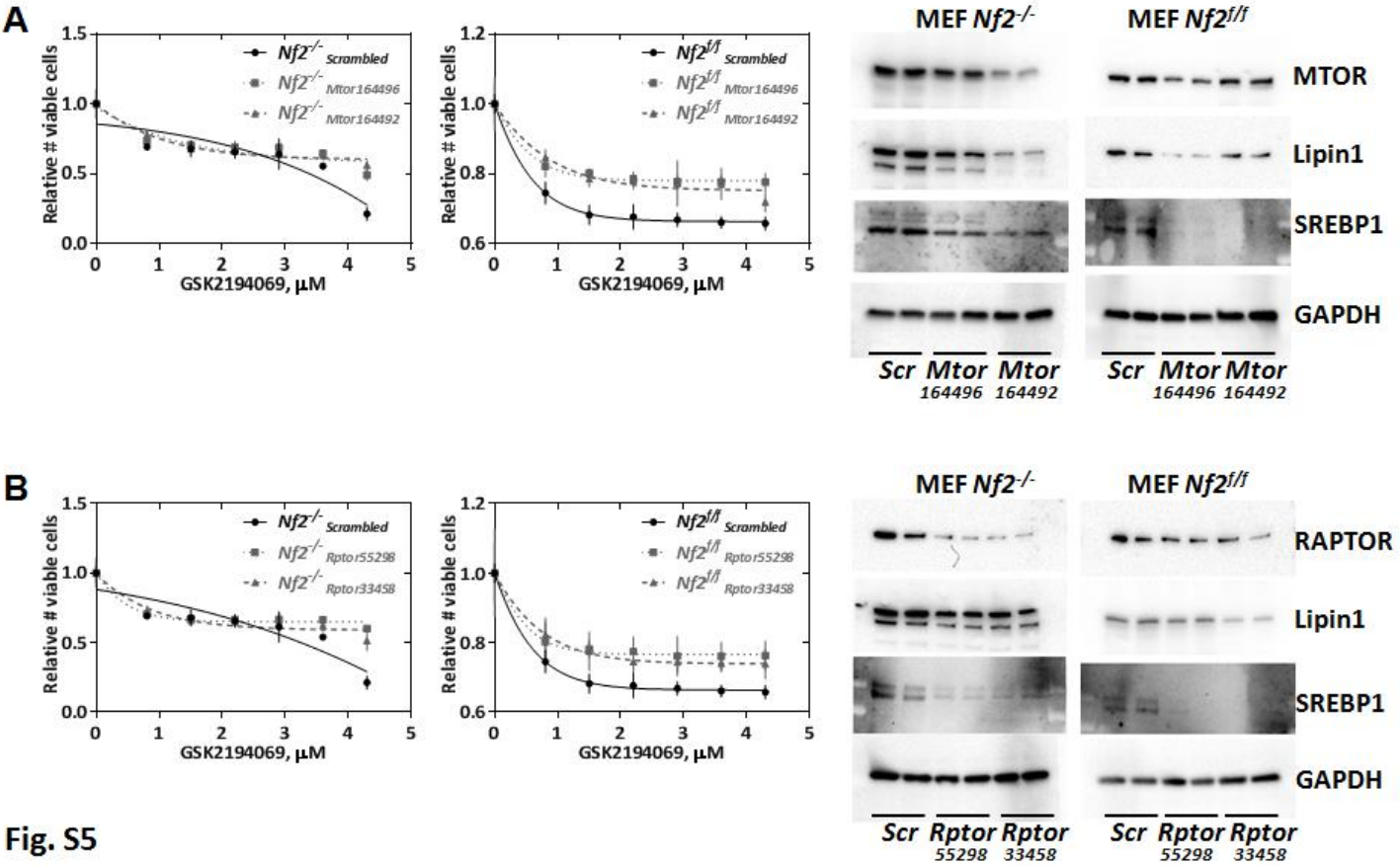
***
Babe
Merlin

Fig. S4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Nf2f/f Mtor164492
MEF Nf2-/-
MEF Nf2f/f
MTOR
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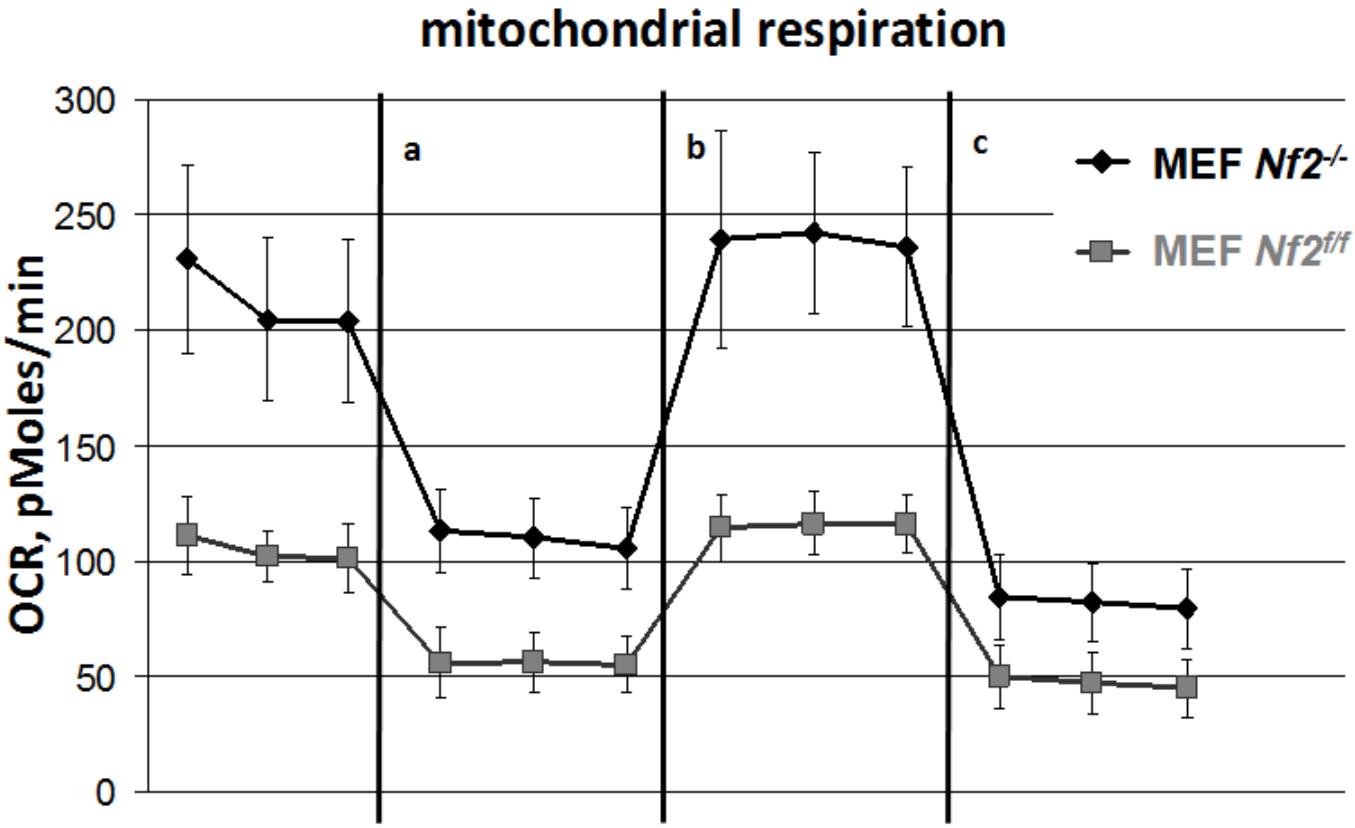
Fig. S5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100
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0
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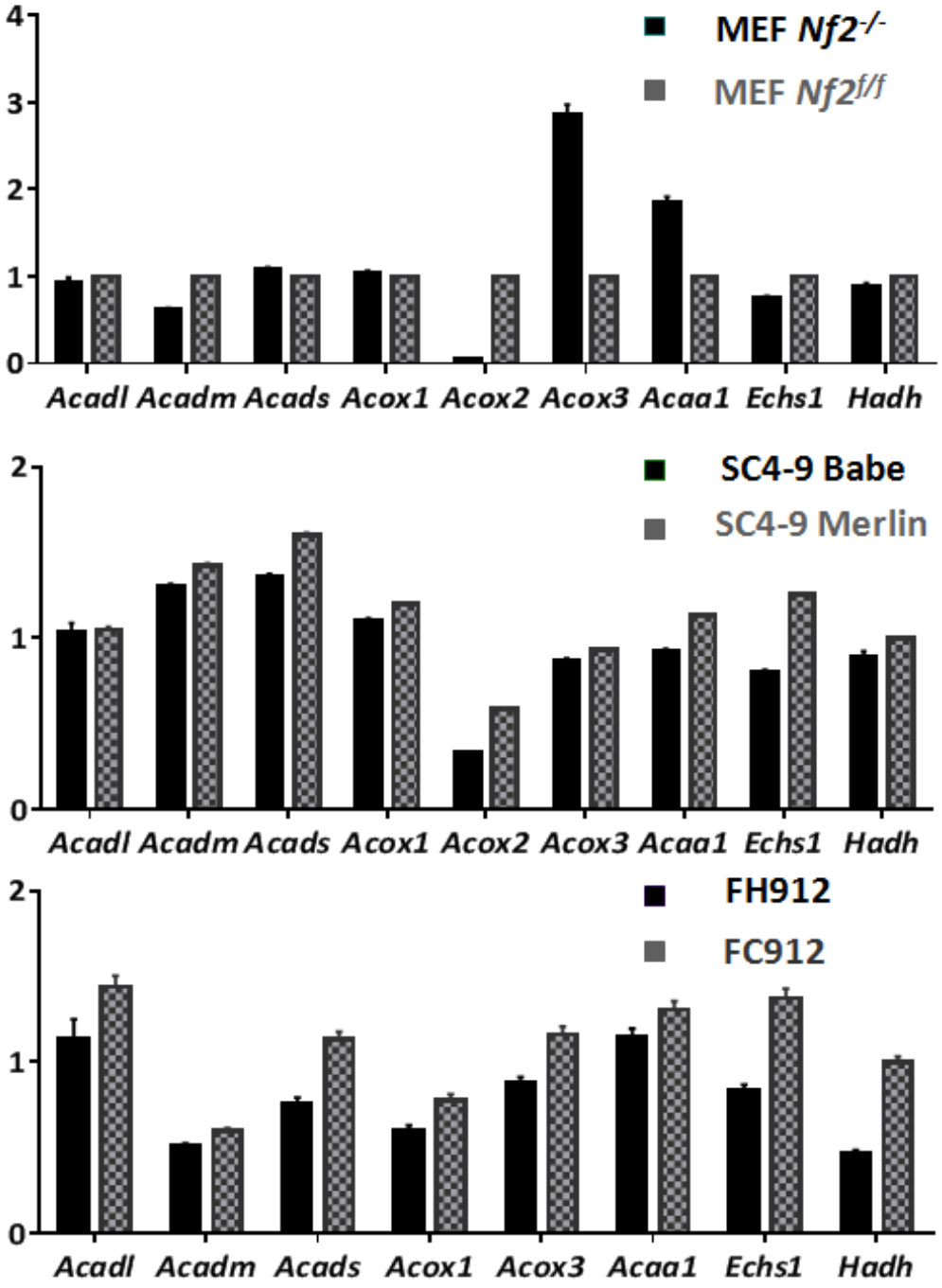
b
c
MEF Nf2-/-
MEF Nf2f/f

Fig. S6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FH912
FC912
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Fig. S7