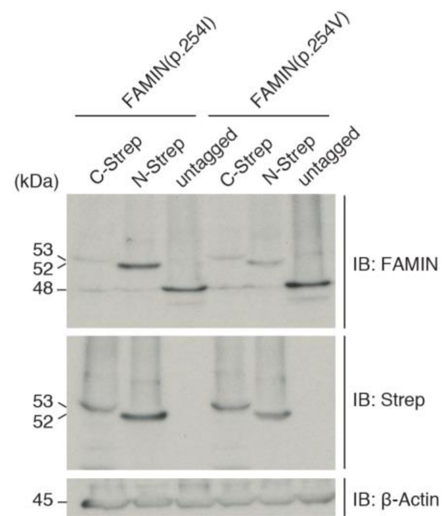
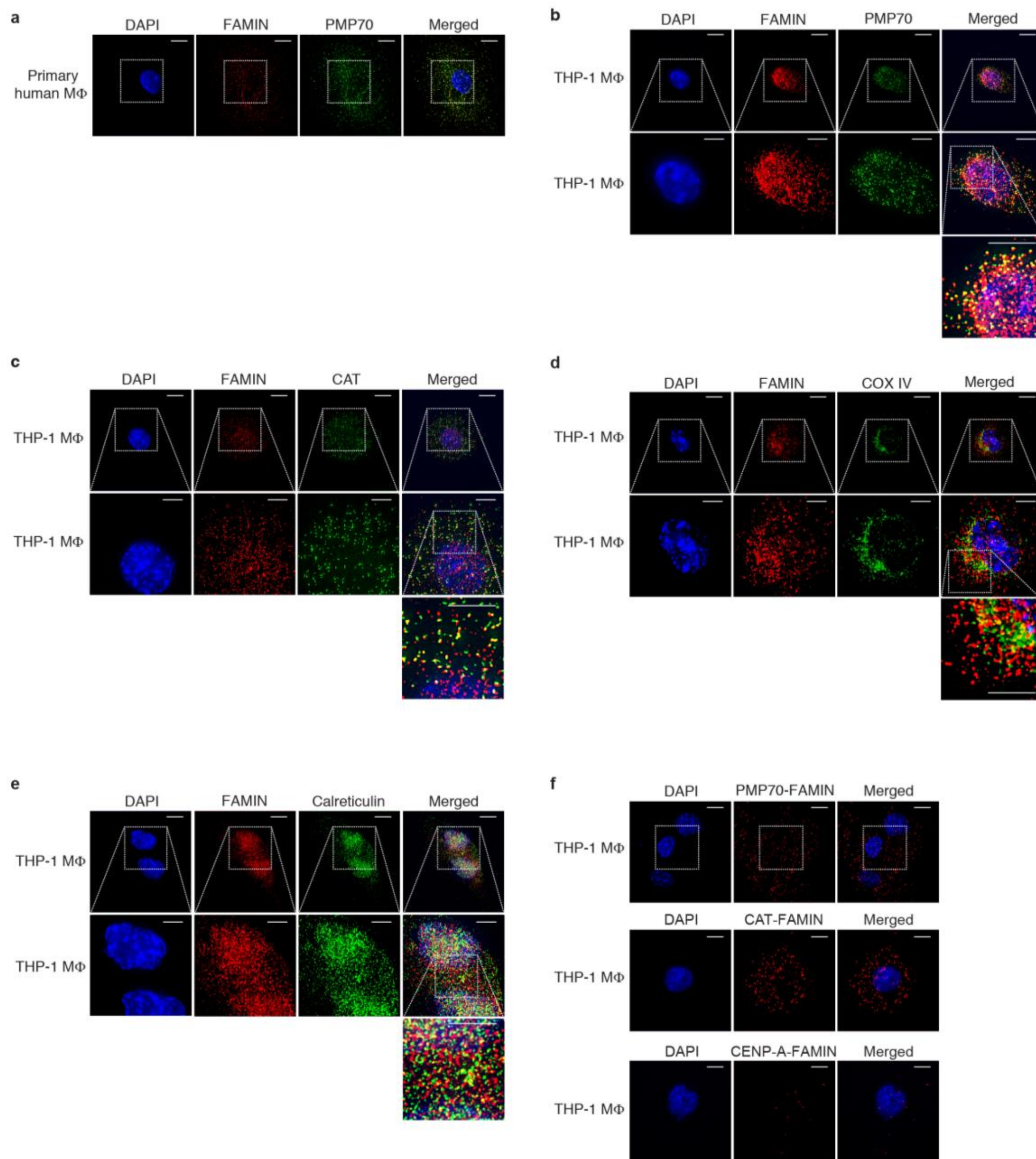


Supplementary Figure 1

Expression of Strep-tagged FAMIN in HEK293T cells.

Immunoblots (IB) of HEK293T lysates expressing N- and C-terminally Strep-tagged FAMIN(p.254I) and FAMIN(p.254V) variants for FAMIN and Strep-tag; β-actin loading control. Data are representative of three independent experiments.

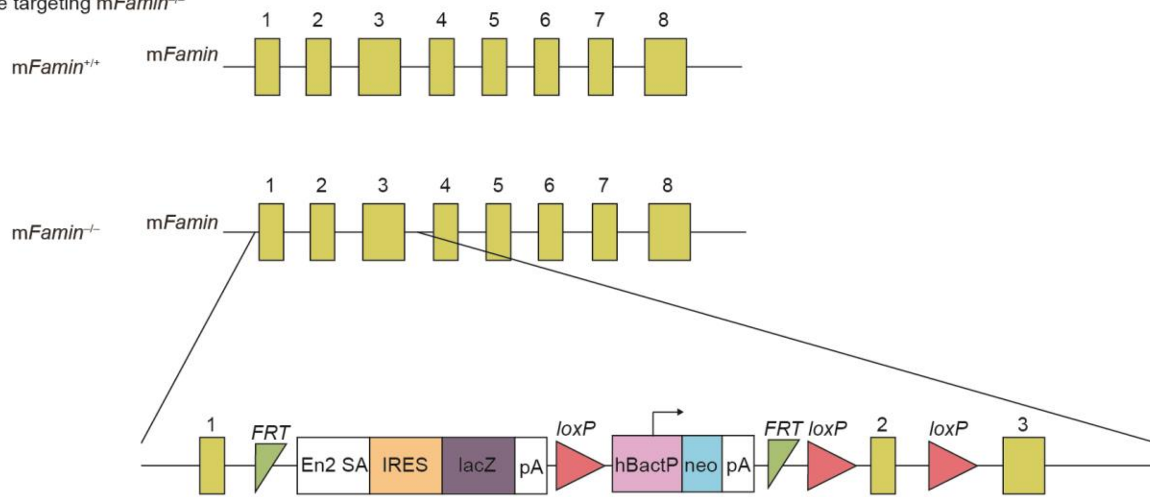


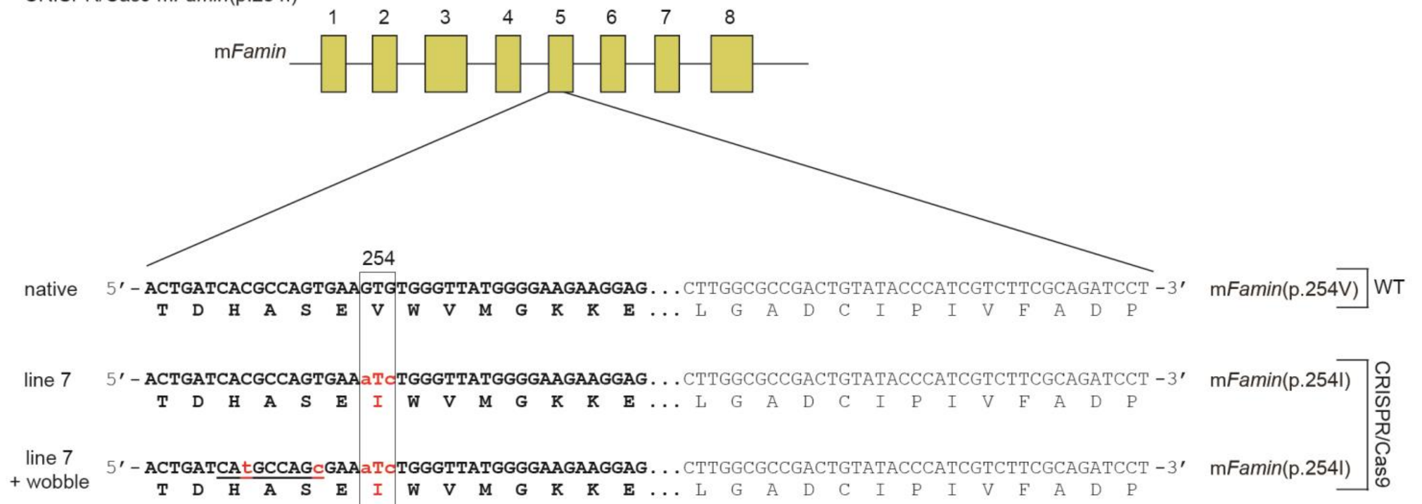
Supplementary Figure 2

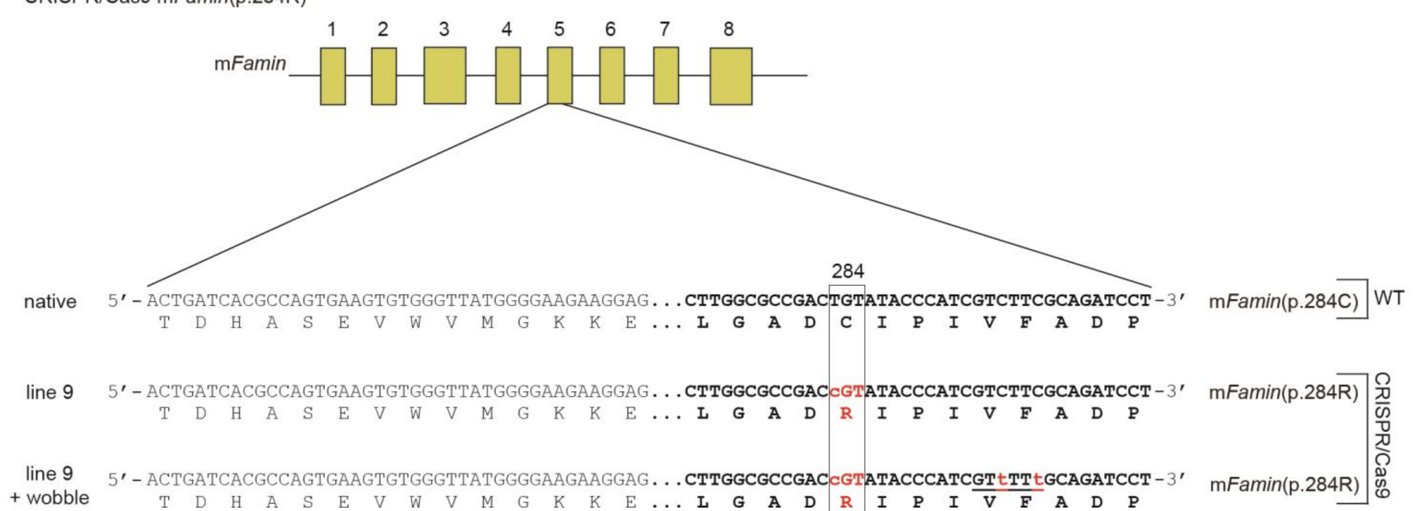
FAMIN localizes to peroxisomes.

(**a**) Co-localization by immunofluorescence (IF) of FAMIN (red) with PMP70 (green) in primary human macrophages (MΦ). DAPI nuclear staining, blue; scale bar = 10 μm. White box corresponds to enlarged images shown in **Fig. 1d**. (**b–e**) Co-localization by IF of FAMIN (red) with PMP70 (green), catalase (green), cytochrome oxidase IV (green) and calreticulin (green) in THP-1 macrophages. DAPI, blue; scale bar = 10 μm for original images and 5 μm for enlarged images. *Upper* panels: original image, *middle* panels: enlargement of the area shown in the white box, *lower* panels: further enlargement as indicated. No significant co-localization of FAMIN was detected with CENP-A, centromere; caveolin-2,

cholesterol/sphingolipid enriched plasma membrane; EEA1 and RAB5, early endosome; LAMP1, lysosomes; NUP98, nuclear envelope; fibrillin, nucleolus and syntaxin 6, trans-Golgi network (data not shown). (**f**) Proximity-ligation assay (PLA) of FAMIN in combination with PMP70, Catalase (red) or centromere protein A (red) as negative control in THP-1 macrophages. DAPI, blue; scale bar = 10 µm. White box corresponds to enlarged images shown in **Fig. 1e**. Data are representative of three independent experiments.


Gene targeting mFamin–/–
mFamin+/+
mFamin
1 2 3 4 5 6 7 8
mFamin–/–
mFamin

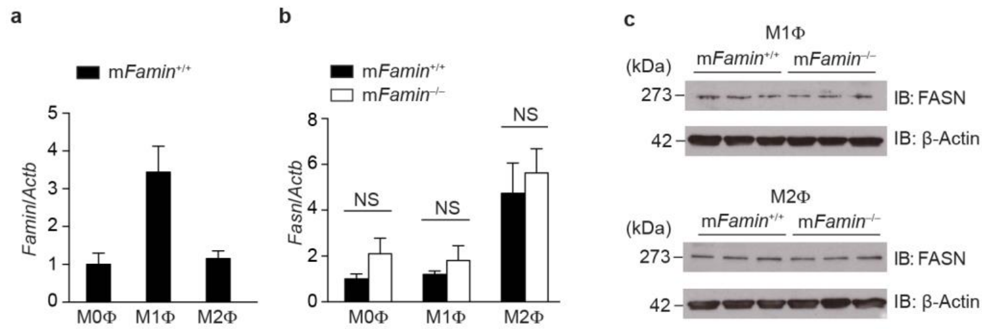

1
FRT
En2 SA
IRES
lacZ
pA
loxP
hBactP
neo
pA
FRT
loxP
2
loxP
3
CRISPR/Cas9 mFamin(p.254I)
1 2 3 4 5 6 7 8
mFamin
254
native 5′-ACTGATCACGCCAGTGAAGTGTGGGTTATGGGGAAGAAGGAG...CTTGGCGCCGACTGTATACCCATCGTCTTCGCAGATCCT-3′
T D H A S E V W V M G K K E ... L G A D C I P I V F A D P
mFamin(p.254V)
WT
line 7 5′-ACTGATCACGCCAGTGAAaTcTGGGTTATGGGGAAGAAGGAG...CTTGGCGCCGACTGTATACCCATCGTCTTCGCAGATCCT-3′
T D H A S E I W V M G K K E ... L G A D C I P I V F A D P
mFamin(p.254I)
line 7 + wobble 5′-ACTGATCAtGCCAGcGAAaTcTGGGTTATGGGGAAGAAGGAG...CTTGGCGCCGACTGTATACCCATCGTCTTCGCAGATCCT-3′
T D H A S E I W V M G K K E ... L G A D C I P I V F A D P
mFamin(p.254I)
CRISPR/Cas9


CRISPR/Cas9 mFamin(p.284R)
1 2 3 4 5 6 7 8
mFamin
284
native 5′-ACTGATCACGCCAGTGAAGTGTGGGTTATGGGGAAGAAGGAG...CTTGGCGCCGACTGTATACCCATCGTCTTCGCAGATCCT-3′
T D H A S E V W V M G K K E ... L G A D C I P I V F A D P
mFamin(p.284C)
WT
line 9 5′-ACTGATCACGCCAGTGAAGTGTGGGTTATGGGGAAGAAGGAG...CTTGGCGCCGACcGTATACCCATCGTCTTCGCAGATCCT-3′
T D H A S E V W V M G K K E ... L G A D R I P I V F A D P
mFamin(p.284R)
line 9 + wobble 5′-ACTGATCACGCCAGTGAAGTGTGGGTTATGGGGAAGAAGGAG...CTTGGCGCCGACcGTATACCCATCGTtTTtGCAGATCCT-3′
T D H A S E V W V M G K K E ... L G A D R I P I V F A D P
mFamin(p.284R)
CRISPR/Cas9

Supplementary Figure 3

Generation of m*Famin*$^{-/-}$, m*Famin*^{p254I} and m*Famin*^{p284R} mice.

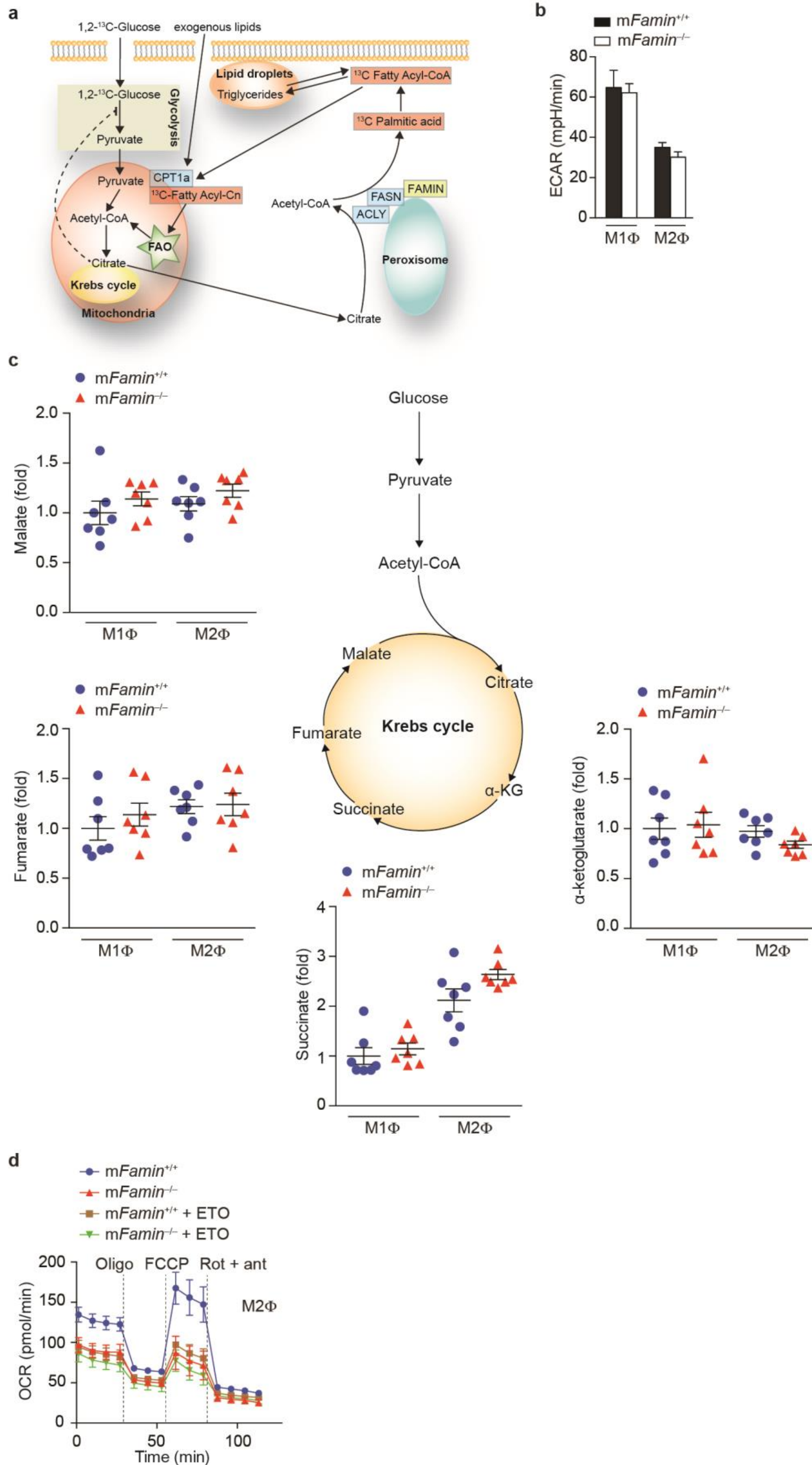
Schematic representation of the targeting strategy for creating a m*Famin*$^{-/-}$ allele in murine ES cells. The targeting vector, L1L2_Bact_P (International Mouse Knockout Consortium), contains a reading frame-independent *LacZ* gene trap cassette consisting of an *En2* splice acceptor (SA), an internal ribosomal entry site (IRES), *LacZ* gene and a polyadenylation site (PA). The vector also contains a selectable marker consisting of a human β-actin promoter (hBactP), neomycin resistance gene (neo) and a PA site. The vector is flanked by flippase recognition target (FRT) sites to allow removal of the targeting cassette and conditional m*Famin* deletion upon *Cre*-mediated recombination of the *lox*P sites. m*Famin*^{p254I} and m*Famin*^{p284R} mice were generated by CRISPR/Cas9 genome editing to introduce nucleotide changes to encode p.254I or p.284R amino acids, respectively, at indicated positions. Nucleotides in m*Famin* exon 5 were targeted using guide RNAs, 'line' 7 and 'line' 9, respectively as outlined in the methods. Two different targeting oligodeoxynucleotides were used for each: one containing only the nucleotide changes leading to the amino acid substitutions, the other containing 2 additional synonymous nucleotide changes ('wobble') in the underlined region.



Supplementary Figure 4

***Famin* expression is highest in M1 macrophages, and FAMIN deficiency does not affect FASN expression.**

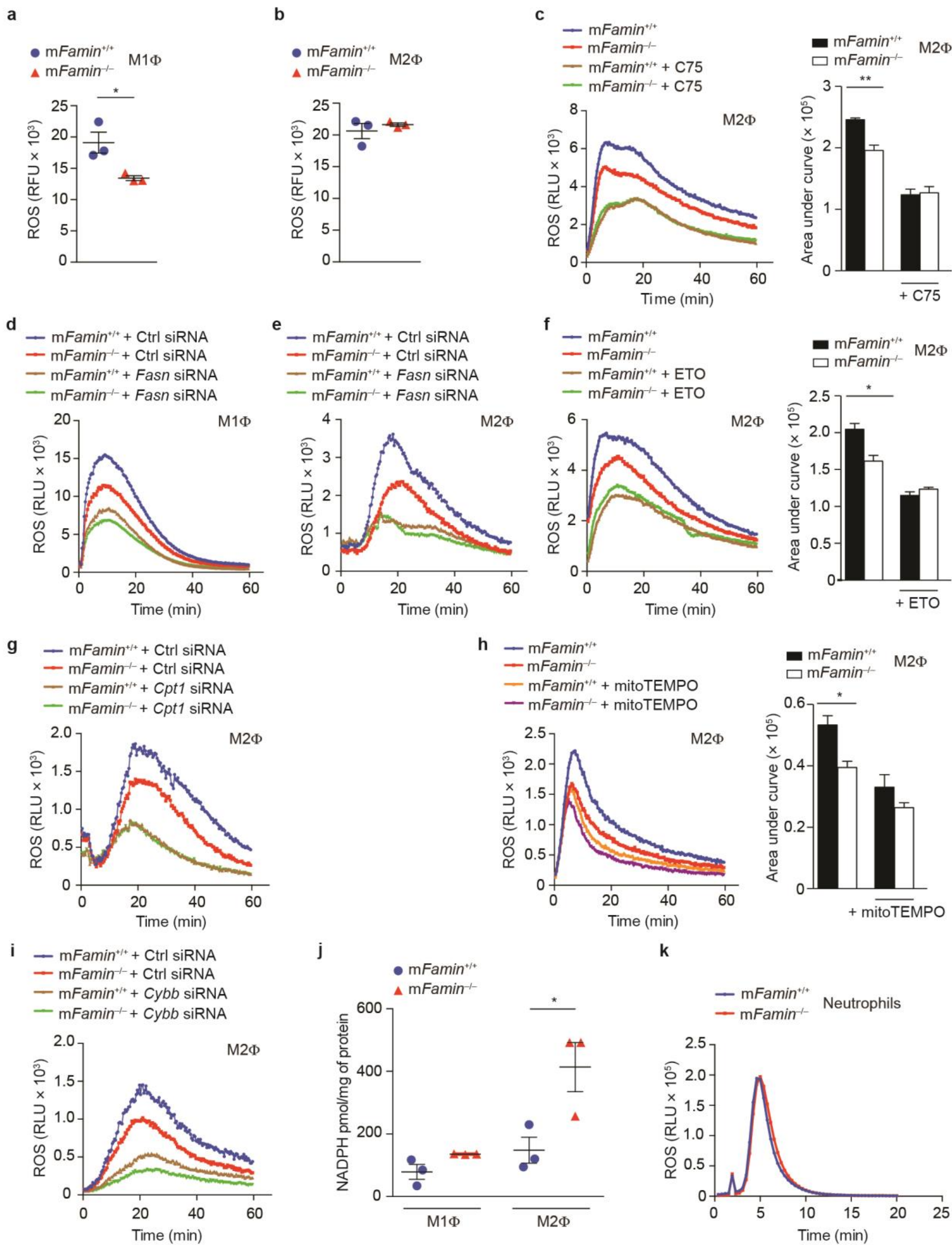
(**a**) mRNA expression of *Famin* in M0, M1 and M2 macrophages (MΦ). (**b**) mRNA expression of *Fasn* in M0, M1 and M2 macrophages. (**c**) Immunoblots (IB) of FASN in M1 and M2 macrophage cell lysates; β-actin loading control. Data are from one experiment with three mice representative of two (**b**,**c**; mean ± S.E.M.) or three independent experiments (**c**).


a
1,2-13C-Glucose
exogenous lipids
Glycolysis
Pyruvate
Lipid droplets
Triglycerides
13C Fatty Acyl-CoA
13C Palmitic acid
CPT1a
13C-Fatty Acyl-Cn
Acetyl-CoA
FAO
Citrate
Krebs cycle
Mitochondria
FASN
FAMIN
ACLY
Peroxisome
b
mFamin+/+
mFamin−/−
ECAR (mpH/min)
M1Φ
M2Φ
c
Malate (fold)
Glucose
Acetyl-CoA
Malate
Citrate
Fumarate
Krebs cycle
Succinate
α-KG
Fumarate (fold)
α-ketoglutarate (fold)
Succinate (fold)
d
mFamin+/+ + ETO
mFamin−/− + ETO
Oligo
FCCP
Rot + ant
OCR (pmol/min)
Time (min)

Supplementary Figure 5

FAMIN does not directly affect the Krebs cycle.

(**a**) Schematic representation of 1,2-^{13}C-glucose incorporation, via citrate, into fatty acyl species. (**b**) Basal extracellular acidification rate (ECAR) of m*Famin*$^{-/-}$ and m*Famin*$^{+/+}$ M1 and M2 macrophages (MΦ) in the presence of exogenous pyruvate (n = 5/14). (**c**) Relative levels of malate, fumarate, succinate and α-ketoglutarate in M1 and M2 macrophages. (**d**) Oxygen consumption rate (OCR) of m*Famin*$^{-/-}$ and m*Famin*$^{+/+}$ M2 macrophages treated as indicated with 40 µM etomoxir (ETO) 1 h prior to OCR measurement and followed by sequential treatment (dotted vertical lines) with oligomycin (Oligo), FCCP, and rotenone plus antimycin (Rot + ant). *$P < 0.05$, **$P < 0.01$ (Unpaired, two-tailed Student's *t*-test). Data are pooled from three independent experiments (**b**; mean ± S.E.M.), from one experiment with seven mice per group (**c**; mean ± S.E.M.) or from one experiment with three mice representative of two independent experiments (**d**; mean ± S.E.M.).

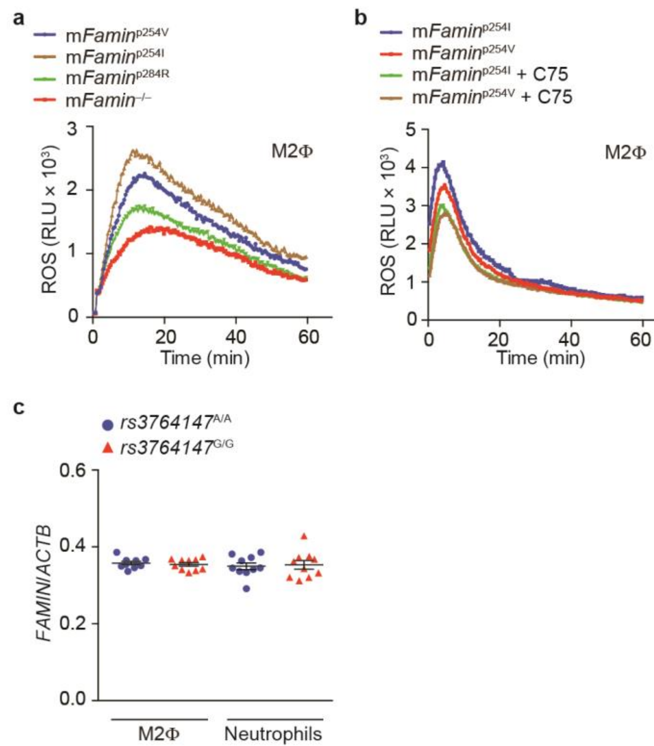


Supplementary Figure 6

Nature Immunology: doi:10.1038/ni.3532

FAMIN-deficient M2 macrophages exhibit impaired mitochondrial ROS- and FAO-dependent production of extracellular ROS.

(**a**,**b**) Intracellular ROS measurement in unstimulated M1 and M2 m*Famin*$^{-/-}$ and m*Famin*$^{+/+}$ macrophages (MΦ) stained with the cytosolic ROS indicator, 5-(and-6)-chloromethyl-2-7-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA) and measured in relative fluorescence units, RFU. (**c**–**i**) Zymosan induced eROS production in M1 and M2 macrophages treated as indicated for 16 h with 20 μM C75; or 1 h 40 μM etomoxir (ETO) or 500 μM mitoTEMPO prior to stimulation; or silenced for *Fasn* (*Fasn* siRNA), *Cpt1a* (*Cpt* siRNA) or *Cybb* (*Cybb* siRNA) or scrambled siRNA (Ctrl siRNA); *Left*, eROS kinetic plots measured in relative light units, RLU and *right*, area under curve, AUC. (**j**) NADPH quantification in M1 and M2 macrophages cell lysates. (**k**) PMA-induced eROS production in m*Famin*$^{-/-}$ and m*Famin*$^{+/+}$ neutrophils. *$P < 0.05$, **$P < 0.01$ (Unpaired, two-tailed Student's *t*-test). Data are from one experiment with three mice representative of two independent experiments (**a**–**k**; mean ± S.E.M.).



Supplementary Figure 7

FAMIN p.I254V and p.C284R M2 macrophage have impaired eROS production.

(**a**) Zymosan-stimulated eROS production in m*Famin*$^{-/-}$, m*Famin*^{p254V}, m*Famin*^{p254I} and m*Famin*^{p284R} murine M2 macrophages (MΦ). (**b**) Zymosan induced eROS production in m*Famin*^{p254I} and m*Famin*^{p254V} M2 macrophages treated as indicated for 16 h with 20 μM C75. (**c**) *FAMIN* mRNA expression in M2 macrophages and neutrophils from healthy donors homozygous for the Crohn's disease and leprosy risk ('*rs3764147*$^{G/G}$') and non-risk ('*rs3764147*$^{A/A}$') haplotypes. Data are from one experiment with three mice representative of three independent experiments (**a**,**b**; mean ± S.E.M.) or pooled from ten independent experiments (**c**; mean ± S.E.M.).

Nature Immunology: doi:10.1038/ni.3532

Supplementary Table 1. FAMIN interacting proteins

UniProt ID	Protein name	No. peptides (Strep-C13orf31)	No. peptides (C13orf31-Strep)	pep_cover (Strep-C13orf31)	pep_cover (C13orf31-Strep)
P49327	**Fatty acid synthase**	**115**	**37**	**26.6**	**10.1**
Q5EFE5	Anti-RhD monoclonal T125 gamma1 heavy chain	58	37	25.9	10.5
B4DKR1	cDNA FLJ51884, highly similar to Mitochondrial inner membrane protein	28	39	19	29.5
E9PG19	FCH and double SH3 domains protein 2	26	31	2.5	3.4
Q96ST3	Paired amphipathic helix protein Sin3a	40	16	19.7	9.3
Q6FHQ0	RBBP7 protein	31	13	39.1	13.4
Q16531	DNA damage-binding protein 1	30	12	14	9.4
B7Z3V1	cDNA FLJ60077, highly similar to Sodium/potassium-transporting ATPase alpha-1 chain	27	19	15.7	13.8
K7EIG1	Clustered mitochondria protein homolog	24	17	13.1	7.2
A4D1U5	Multiple substrate lipid kinase, isoform CRA_a	23	14	32.9	20.4
Q13263	Transcription intermediary factor 1-beta	76	0	42.6	0
P36952	Serpin B5	45	0	48	0
Q5I6Y6	Lamin A/C transcript variant 1	38	0	34.6	0
P07476	Involucrin	32	0	44.3	0
Q6N093	Putative uncharacterised protein DKFZp686I04196	32	0	16.5	0
P43487	Ran-specific GTPase-activating protein	32	9	71.1	20.9
L0R5A1	Alternative protein CSF2RB	30	0	7.4	0
A6NMY3	Alstrom syndrome protein 1	29	7	7.4	2.1
P16152	Carbonyl reductase [NADPH] 1	29	0	50.2	0
Q13228	Selenium-binding protein 1	29	0	43	0
E7DVW5	**Fatty acid binding protein 5 (Psoriasis-associated)**	**28**	**0**	**35.6**	**0**
P00338-3	Isoform 3 of L-lactate dehydrogenase A chain	28	0	31.3	0
Q9HAW4-2	Isoform 2 of Claspin	25	0	13.7	0
B3KWI4	cDNA FLJ43122 fis, clone CTONG3003737, highly similar to Leucine-rich repeat-containing protein 15	24	0	20.7	0
P12277	Creatine kinase B-type	23	0	30.7	0
Q9Y2I1	Nischarin	23	0	10.2	0
B4DDF7	cDNA FLJ53296, highly similar to Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	22	10	21.1	12.8
O14929	Histone acetyltransferase type B catalytic subunit	22	8	25.1	10.7
Q9UI33-3	Isoform 3 of Sodium channel protein type 11 subunit alpha	22	0	1.5	0
Q8IWZ8	SURP and G-patch domain-containing protein 1	22	0	28.7	0
Q15007	Pre-mRNA-splicing regulator WTAP	21	0	31.3	0
I3L2N2	Segment polarity protein dishevelled homolog DVL-2	21	0	17.9	0
Q8IV20	FAMIN	1157	584	80.5	68.4

Supplementary Table 2: Free fatty acid quantification in M1 and M2 macrophages

Lipid species (ng per sample)	*mFAMIN*$^{+/+}$		*mFAMIN*$^{-/-}$		*mFAMIN*$^{+/+}$ vs *mFAMIN*$^{-/-}$	
	Mean	S.E.M	Mean	S.E.M	Change	*P*-value
M1 total free fatty acid	38314.72	807.94	38651.61	467.48	336.88	0.7257
M1 C16:0 fatty acid	17893.60	208.84	17999.37	281.72	105.77	0.7691
M2 total free fatty acid	43177.34	651.22	43245.70	869.66	68.36	0.9511
M2 C16:0 fatty acid	22393.77	569.99	21739.00	567.95	-654.77	0.4347

Supplementary Table 3: Genotyping primers

Primer	Sequence
mFAMIN common	AAATTGATGAAGAAAATCTGAGCTG
mFAMIN$^{+/+}$	GTGGGACTGCTGCTCTCTTC
mFAMIN$^{-/-}$	TGTACAAACTTGTTGATATCGTGGT
mFAMIN$^{p.I254V\text{-}C284R}$ Forward*	GCCTCATCCTACCACAAAGCA
mFAMIN$^{p.I254V\text{-}C284R}$ Reverse*	TTCCCTAGGAACCACATCTGC

* PCR product submitted for Sanger sequencing as described in the Online methods

Supplementary Table 4: CRISPR/Cas9 guide RNAs and targeting oligonucleotides

a. Guide RNAs

gRNA	Sequence[1]
tyrex2R	GCTCCCATCTTCAGCAGATG**TGG**
m*Famin* Line 7	TGATCACGCCAGTGAAGTGT**GGG**
m*Famin* Line 9	GAAGACGATGGGTATACAGT**CGG**

[1] PAM sites in bold

b. Targeting oligonucleotides

oligo	Sequence (5' to 3')[2]
Oligo7	TCCTCTCTGATTTGTGACGATCCCATCGTAAGATTCAGGCTCCTTCTTCCCC ATAACCCA**gAt**TTCACTGGCGTGATCAGTCTAACACGAAACACAGAGCAAA ATGTCTGGAAAACGAAACAT
Oligo7 wobble	TCCTCTCTGATTTGTGACGATCCCATCGTAAGATTCAGGCTCCTTCTTCCCC ATAACCCA**gAt**TTCgCTGGCaTGATCAGTCTAACACGAAACACAGAGCAAA ATGTCTGGAAAACGAAACAT
Oligo9	CCTACCGGAGTGAGCAACCCCACATGCCTTTTTCACAGGATCTGCGAAGA CGATGGGTAT**ACg**GTCGGCGCCAAGAGCAGTGATTGTGACTCCTCTCTGAT TTGTGACGATCCCATCGTAAGA
Oligo9 wobble	CCTACCGGAGTGAGCAACCCCACATGCCTTTTTCACAGGATCTGCaAAaAC GATGGGTAT**ACg**GTCGGCGCCAAGAGCAGTGATTGTGACTCCTCTCTGATT TGTGACGATCCCATCGTAAGA

[2] SNPs in red, with additional sSNPs ('wobble') introduced in two of the lines underlined

Supplementary Table 5: Quantitative PCR primers

Gene	Forward primer	Reverse primer
m*Famin*	TGGGGTTGCTCACTCCGGCTG	GGAGACTGCTGATTCTTTGGGAAGA
Fasn	CCCCGGAGTCGCTTGAGTAT	GGATCTCAGGGTTGGGGTTG
Cpt1a	TGGCATCATCACTGGTGTGTT	GTCTAGGGTCCGATTGATCTTTG
Cybb	TGTGGTTGGGGCTGAATGTC	CTGAGAAAGGAGAGCAGATTTCG