

## **SUPPLEMENTAL INFORMATION**

**(Quirós et al. “Loss of mitochondrial protease OMA1 alters processing of the GT-Pase OPA1 and causes obesity and defective thermogenesis in mice”)**

## **SUPPLEMENTAL FIGURES**

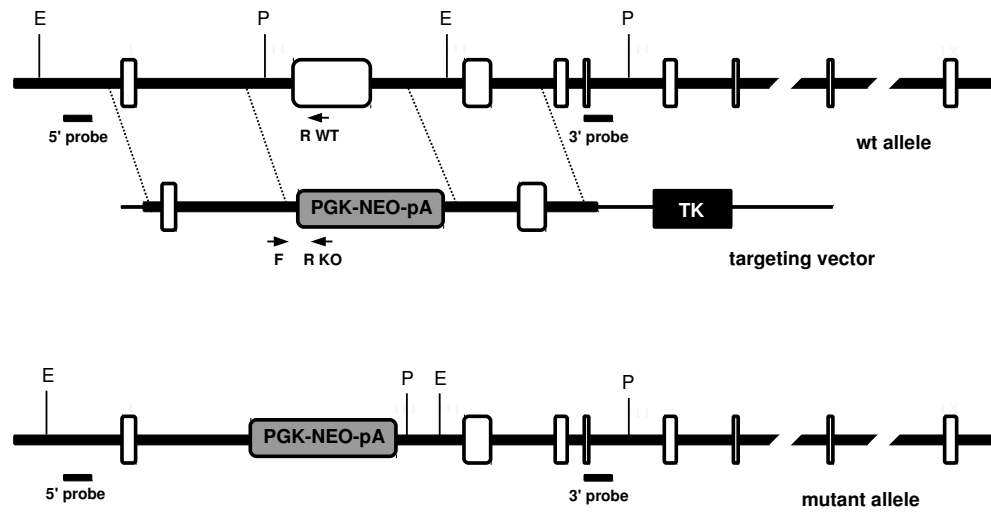
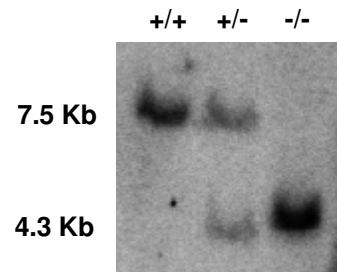
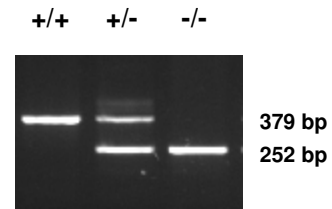
**A****B****C**

Figure S1, Quirós et al.



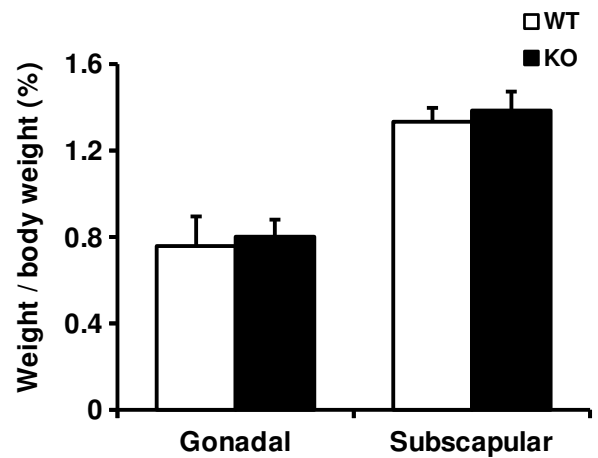
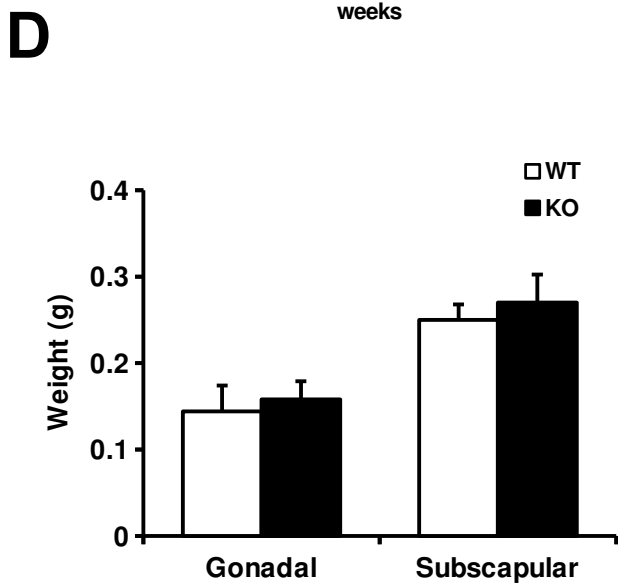
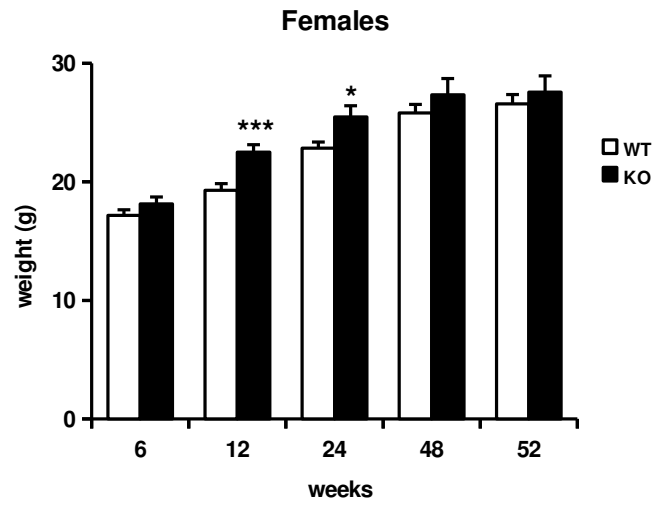
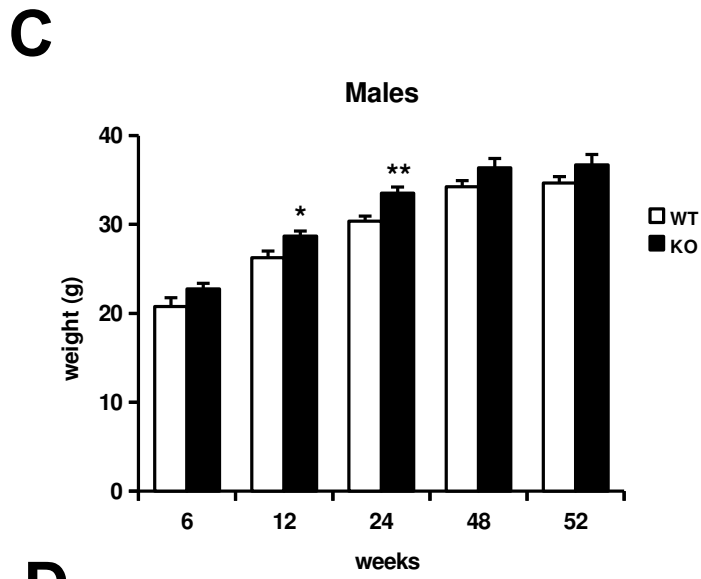
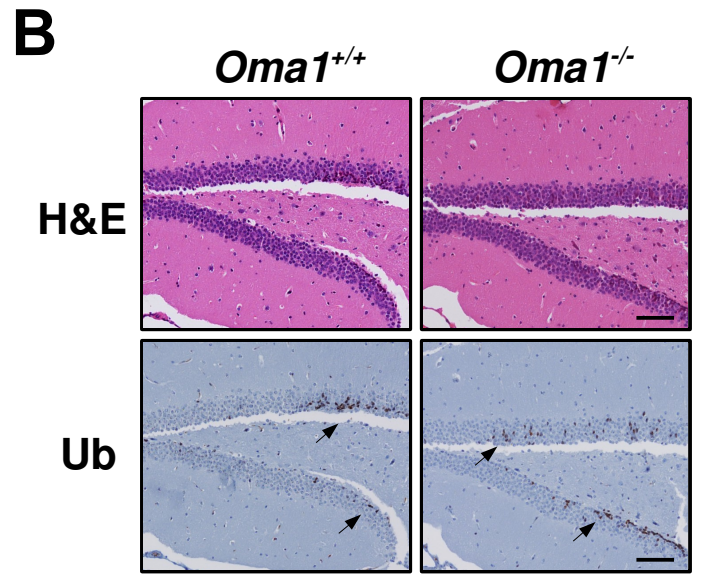
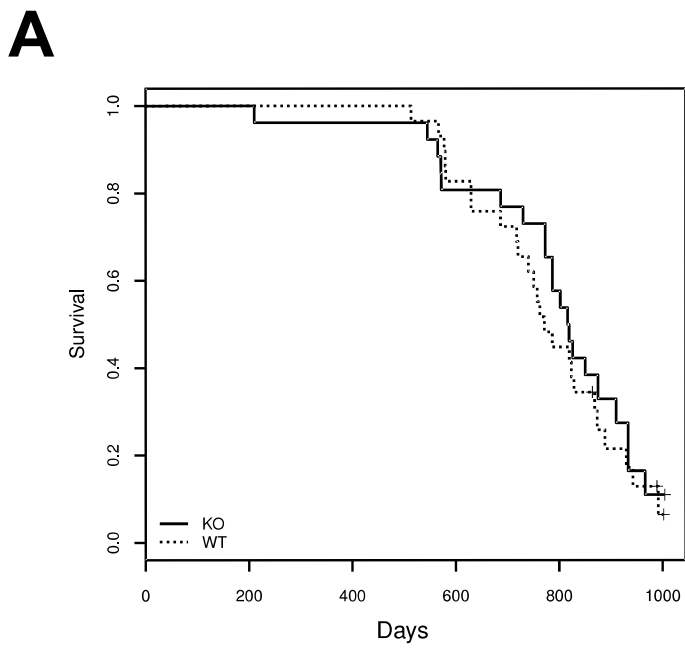
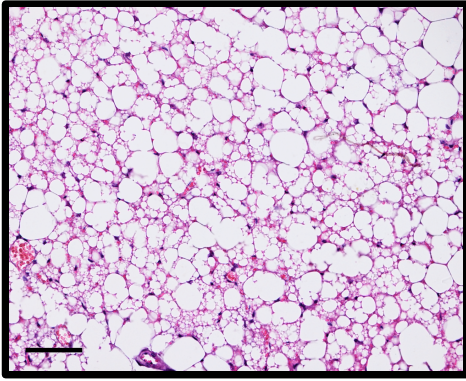


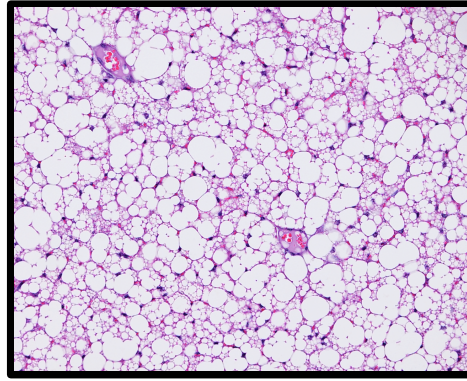
Figure S2, Quirós et al.

**A**

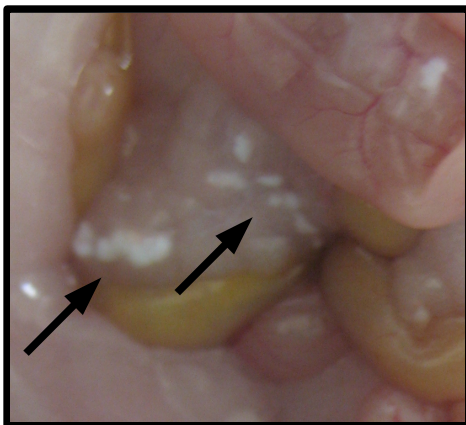
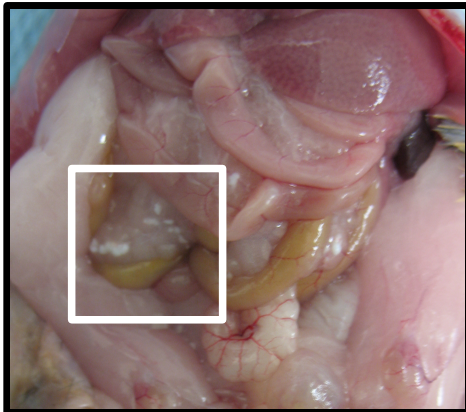
*Oma1*<sup>+/+</sup>



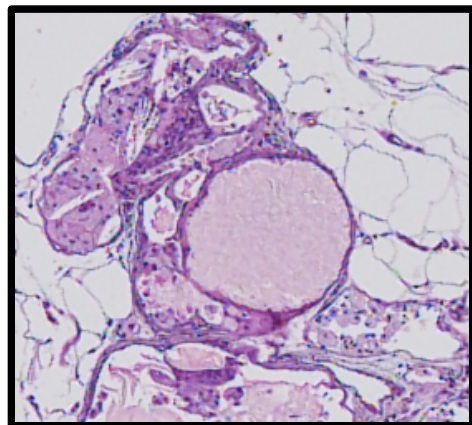
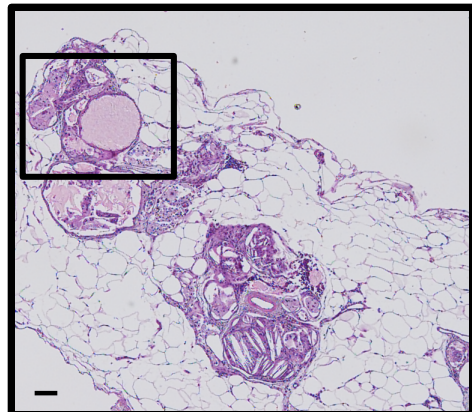
*Oma1*<sup>-/-</sup>



**B**



**C**



**Figure S3, Quirós et al.**



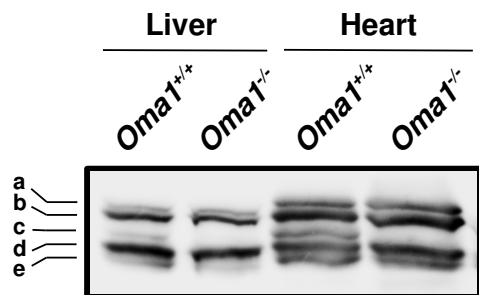
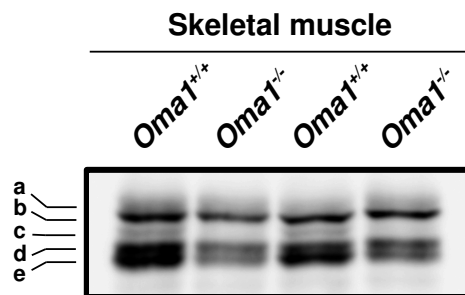
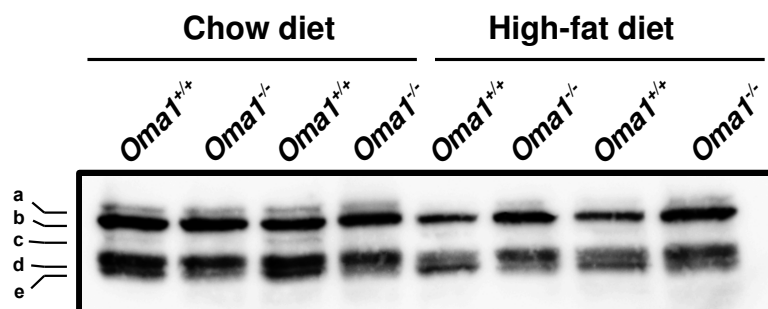
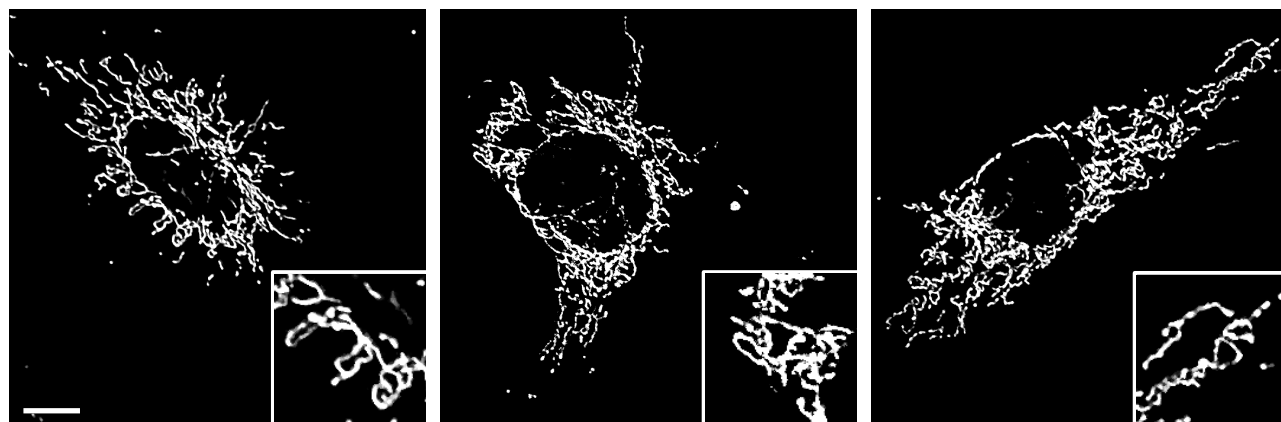
**A****B****C****D**

Figure S5, Quirós et al.

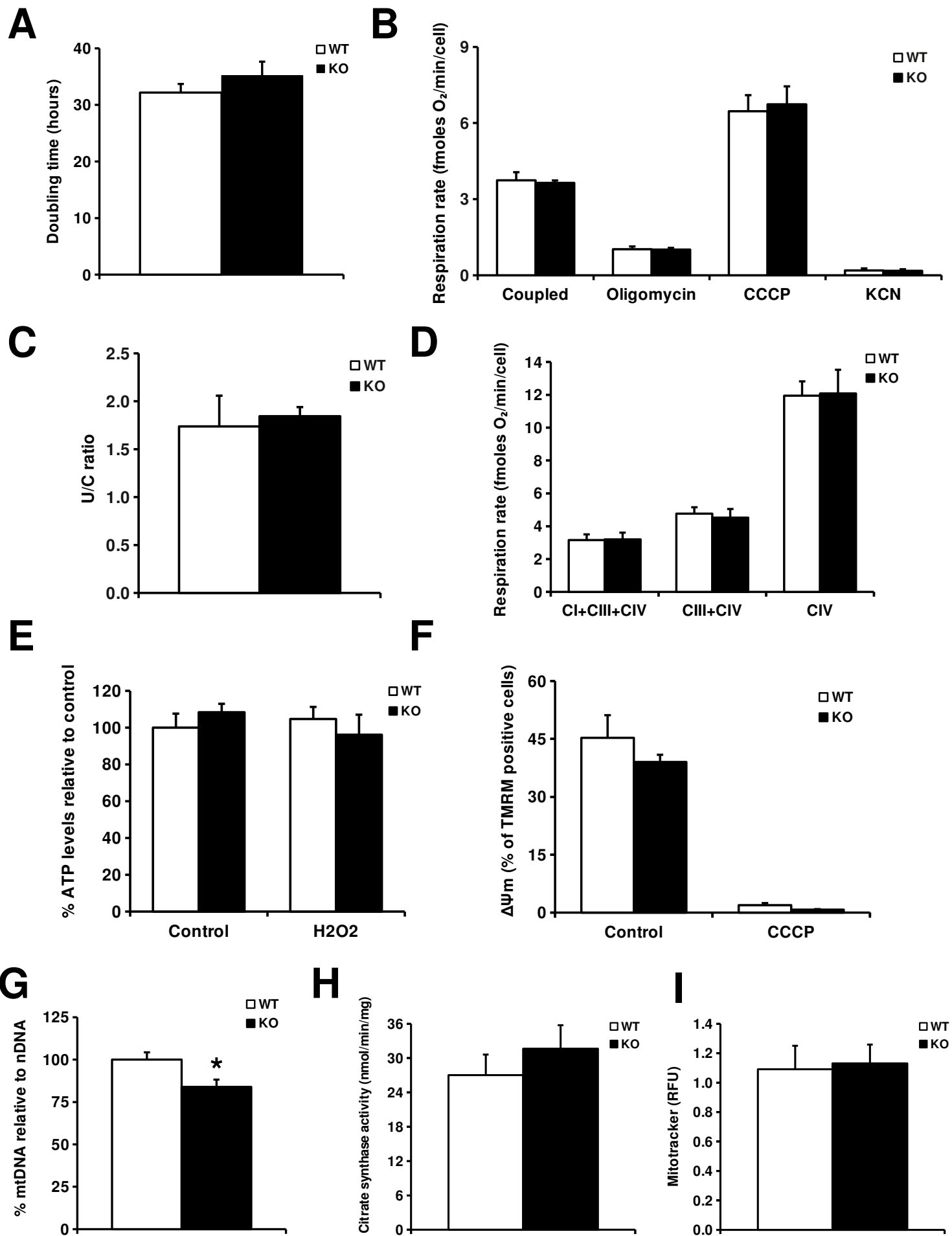


Figure S6, Quirós et al.

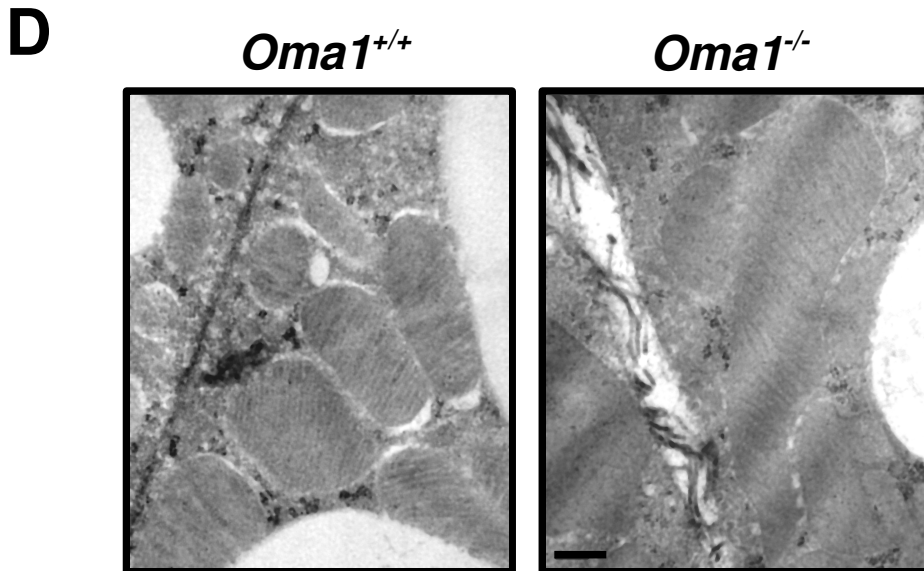
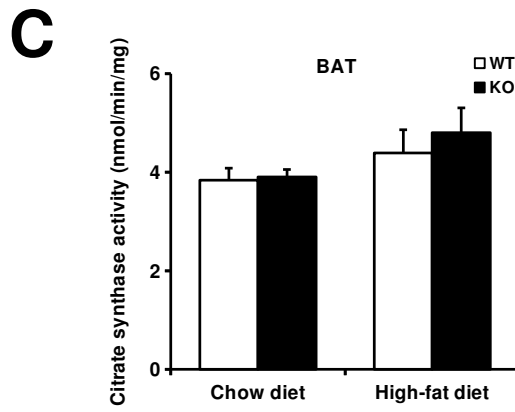
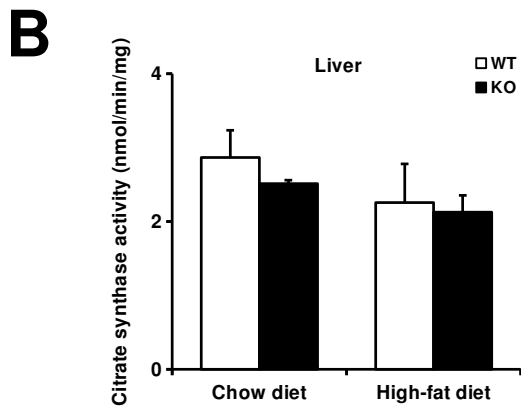
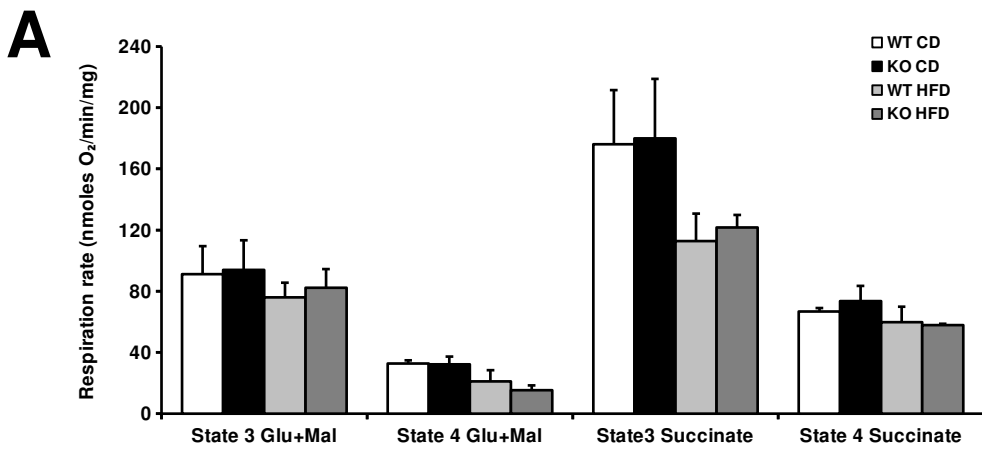


Figure S7, Quirós et al.

## SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure 1. Targeted disruption of the mouse *Omal* gene.** (A) Schematic representation of wild-type and mutant *Omal* alleles, and the targeting vector. The exons are indicated with boxes, the bold solid lines represent introns and the thin solid lines indicate the plasmid backbone in the targeting vector. The positions of probes are indicated with solid bars and the primers with arrows. E, *Eco*RI; P, *Pvu*II; PGK-Neo-pA, neomycin selection cassette; TK, thymidine kinase marker. (B) *Pvu*II Southern blot analysis of *Omal*<sup>+/+</sup>, *Omal*<sup>+/-</sup> and *Omal*<sup>-/-</sup> mice. Substitution of exon 2 in *Omal* with the neomycin cassette changed the restriction pattern obtained after *Pvu*II digestion and hybridization with 3'-probe from 7.5 Kb in the wild-type to 4.3 kb in the knockout mice. (C) PCR genotyping of *Omal* mice with the primers shown in panel A.

**Supplemental Figure 2.** (A) Kaplan-Meier survival plot of *Omal*<sup>+/+</sup> and *Omal*<sup>-/-</sup> mice (n=26-30) (B) H&E and ubiquitin (Ub) histochemistry stain of hippocampus sections from 18-months-old *Omal*<sup>+/+</sup> and *Omal*<sup>-/-</sup> mice. Scale bar: 100  $\mu$ m. (C) Representation of weight of *Omal*<sup>+/+</sup> and *Omal*<sup>-/-</sup> mice males and females, kept on standard chow during 52 weeks. (D) Gonadal and subscapular fat mass and gonadal and subscapular fat mass as a percentage of total body weight of 4-months-old mice. (n=6)

**Supplemental Figure 3.** (A) Interscapular brown adipose tissue (BAT). H&E sections of BAT from *Omal*<sup>+/+</sup> and *Omal*<sup>-/-</sup> mice. Original magnifications 200X. Scale bar: 60  $\mu$ m. (B) Cholesterol granulomas in the *Omal*<sup>-/-</sup> mice after high-fat diet induced obesity. Necropsies of *Omal*<sup>-/-</sup> mice revealed multiple granulomas in the intestinal mesenteric fat of the most severe obese mice. Detail showing the granulomatous lesions indicated by arrows. (C) H&E section of mesenteric fat corresponding to foci of fat necrosis with foamy histiocytes, cholesterol crystals and fibrosis. Original

magnifications 200X. Scale bar: 60  $\mu\text{m}$ .

**Supplemental Figure 4. Microarray analysis of adipose tissue from *Oma1*<sup>-/-</sup> mice after high-fat diet induced obesity.** Representation of network interaction genes upregulated in *Oma1*<sup>-/-</sup> mice implicated in lipid metabolism and molecular transport. Figure was adapted from Ingenuity Pathways Analysis using genes with more than 2-fold (shown in red), and those with less than 2-fold (shown in green).

**Supplemental Figure 5. Alteration of OPA1 processing in tissues derived from *Oma1*-deficient mice.** (A) and (B) Western-blot analysis of OPA1 isoforms in different tissues extracted from *Oma1*<sup>+/+</sup> and *Oma1*<sup>-/-</sup> mice. (C) Analysis of OPA1 processing in white adipose tissue from control and high-fat diet. (D) Confocal images of *Oma1*-deficient MEFs. Scale bar: 10  $\mu\text{m}$ .

**Supplemental Figure 6. Biochemical analyses of mitochondrial function in *Oma1*<sup>-/-</sup> cells.** (A) Doubling time representation of proliferation rates during 72 h of *Oma1*<sup>+/+</sup> and *Oma1*<sup>-/-</sup> fibroblasts. (B) Representation of coupled, oligomycin-, CCCP-uncoupled and KCN-insensitivity respiration rates of *Oma1*<sup>+/+</sup> and *Oma1*<sup>-/-</sup> fibroblasts. (C) Representation of maximum respiration capacity as ratio between uncoupled and coupled respiration rate. (D) Polarography measurements of respiration rates of mitochondrial complex I, III and IV; III and IV; and IV, indicated as fmol O<sub>2</sub>/min/cell, determined in digitonin-permeabilized *Oma1*<sup>+/+</sup> and *Oma1*<sup>-/-</sup> fibroblasts. (E) ATP levels represented as percentage relative to control fibroblasts in basal conditions and after treatment with 100  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub> during 5 h. (F) Mitochondrial membrane potential ( $\Delta\Psi\text{m}$ ), represented as a percentage of positive cells, measured by flow cytometry using FL2, after treatment with 200 nM TMRM for 15 min at 37 °C. For negative control we added 20  $\mu\text{M}$  of CCCP, 30 min before treatment with TMRM. (G) Representation of the percentage levels of mitochondrial DNA (mtDNA) relative to nuclear DNA (nDNA) of



*Omal*<sup>+/+</sup> and *Omal*<sup>-/-</sup> fibroblasts. (H) Citrate synthase activity measurement as a marker of mitochondrial mass. (I) Representation of Mitotracker-Red signal in relative fluorescence units (RFU) as another marker of mitochondrial mass, measured by flow cytometry using FL3 and after treatment with 100 nM of Mitotracker-Red for 15 min at 37 °C. All experiments were carried out in at least two independent experiments with 3 different fibroblast cell lines per genotype. Error bars represent SEM. \*  $P < 0.05$ .

**Supplemental Figure 7.** (A) Respiration rate of state 3 and 4, using glutamate+malate or succinate, measured in mitochondria isolated from liver of *Omal*<sup>+/+</sup> and *Omal*<sup>-/-</sup> mice on control and high-fat diet. Citrate synthase activity of (B) liver and (C) BAT homogenates used for palmitate oxidation measurements. Bars represent means  $\pm$  SEM (n=4). (D) Representative image of mega-mitochondria from EM analysis of BAT samples in control temperature from *Omal*<sup>-/-</sup> mice compared to control. Scale bar 0.4  $\mu$ m.

## **SUPPLEMENTAL TABLES**

**Supplemental Table 1.** Affymetrix Mouse Gene 1.0 ST probes showing a greater than 2-fold increase or decrease in adipose tissue from Oma1-deficient mice were sorted by fold-change.

ID	Gene Symbol	Gene name	WT Signal	KO Signal	Fold
10420616	Sgcg	sarcoglycan, gamma (dystrophin-associated glycoprotein)	32	1042	32.59
10545180	Gm10879	predicted gene 10879	74	818	10.98
10545220	Igk	immunoglobulin kappa chain complex	87	640	7.37
10513497	Mup2	major urinary protein 2	689	3835	5.56
10513521	Mup20	major urinary protein 20	215	1082	5.02
10608377	LOC382133	similar to RIKEN cDNA 1700029H17	29	140	4.80
10513420	Mup7	major urinary protein 7	1258	5923	4.71
10608452	LOC100041256	hypothetical protein LOC100041256	30	139	4.70
10403069	Igh-6	immunoglobulin heavy chain 6 (heavy chain of IgM)	18	79	4.41
10608608	Ssty2	spermiogenesis specific transcript on the Y 2	23	102	4.36
10608327	LOC100040031	hypothetical protein LOC100040031	29	125	4.35
10545249	LOC676175	similar to Ig kappa chain V-V region MPC11 precursor	21	90	4.20
10608477	LOC434960	similar to spermiogenesis specific transcript on the Y 2	23	97	4.20
10608295	LOC100039753	similar to putative	34	143	4.18
10608484	MGC107098	similar to spermiogenesis specific transcript on the Y 2	22	89	4.13
10608480	LOC100039147	similar to spermiogenesis specific transcript on the Y 1	34	138	4.03
10349968	Chi31	chitinase 3-like 1	81	324	4.03
10560744	V1rd15	vomer nasal 1 receptor, D15	21	85	3.99
10513529	Mup3	major urinary protein 3	119	473	3.98
10608506	LOC100039614	hypothetical protein LOC100039614	25	99	3.96
10403034	Igh	immunoglobulin heavy chain complex	198	754	3.81
10560752	V1rd14	vomer nasal 1 receptor, D14	27	101	3.79
10513512	Mup1	major urinary protein 1	357	1349	3.78
10531149	Gc	group specific component	339	1252	3.69
10585005	Apoa1	apolipoprotein A-I	253	904	3.57
10507143	Cyp4a12a	cytochrome P450, family 4, subfamily a, polypeptide 12a	29	103	3.51
10403057	LOC641089	similar to Ig heavy chain V region BCL1 precursor	17	57	3.41
10547633	Gdf3	growth differentiation factor 3	140	460	3.30
10545215	Igk-V28	immunoglobulin kappa chain variable 28 (V28)	105	323	3.09
10349648	Ctse	cathepsin E	105	322	3.07
10585010	Apoa4	apolipoprotein A-IV	200	609	3.04
10560780	V1rd21	vomer nasal 1 receptor, D21	18	54	3.04
10403011	LOC674190	similar to Ig heavy chain V region IR2 precursor	22	65	3.03
10608549	LOC100042428	spermiogenesis specific transcript on the Y 2-like	25	76	3.01
10551293	Cyp2f2	cytochrome P450, family 2, subfamily f, polypeptide 2	138	410	2.98
10608277	LOC100041207	similar to MGC107098 protein	19	56	2.93
10531041	Ugt2b1	UDP glucuronosyltransferase 2 family, polypeptide B1	24	67	2.80
10373330	Rdh7	retinol dehydrogenase 7	58	161	2.76
10509500	Hp1bp3	heterochromatin protein 1, binding protein 3	350	964	2.76
10523062	Alb	albumin	1692	4523	2.67
10571467	Pdgfrl	platelet-derived growth factor receptor-like	57	151	2.67
10583071	Mmp3	matrix metalloproteinase 3	51	131	2.59
10434719	Kng1	kininogen 1	221	568	2.57
10512904	Aldob	aldolase B, fructose-bisphosphate	128	328	2.57
10456392	Cidea	cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	84	213	2.55
10575693	Vat1l	vesicle amine transport protein 1 homolog-like (T. californica)	54	136	2.54
10372652	Lyz1	lysozyme 1	510	1291	2.53
10430297	Pvalb	parvalbumin	44	110	2.50
10545205	Gm1418	predicted gene 1418	109	268	2.47
10566477	Hpx	hemopexin	394	963	2.44
10497451	Cpa3	carboxypeptidase A3, mast cell	282	663	2.35
10531051	Ugt2b36	UDP glucuronosyltransferase 2 family, polypeptide B36	35	82	2.34
10574598	Es31	esterase 31	116	270	2.32
10513630	Ambp	alpha 1 microglobulin/bikunin	97	225	2.32
10608346	LOC665346	similar to spermiogenesis specific transcript on the Y 1	18	42	2.30
10538882	Gm5571	predicted gene 5571	736	1689	2.29
10550778	LOC100043604	V3R8-like protein	23	53	2.29
10535704	Cyp3a11	cytochrome P450, family 3, subfamily a, polypeptide 11	35	80	2.25
10394394	Apob	apolipoprotein B	109	246	2.25
10608212	Sly	Sycp3 like Y-linked	20	45	2.23
10538880	Igk-V1	immunoglobulin kappa chain variable 1 (V1)	143	315	2.21
10507152	Cyp4a12b	cytochrome P450, family 4, subfamily a, polypeptide 12B	32	70	2.21
10608482	LOC665746	similar to spermiogenesis specific transcript on the Y 1	20	45	2.20
10608251	LOC380994	similar to Sycp3 like Y-linked	17	37	2.19

10538965	Fabp1	fatty acid binding protein 1, liver	136	296	2.18
10598225	Gmcl11	germ cell-less homolog 1 (Drosophila)-like	15	33	2.18
10593169	Apoc3	apolipoprotein C-III	252	543	2.15
10548207	Pzp	pregnancy zone protein	187	402	2.15
10387855	Alox15	arachidonate 15-lipoxygenase	125	269	2.15
10608424	Ssty1	spermiogenesis specific transcript on the Y 1	18	38	2.14
10436773	Gm7735	predicted gene 7735	52	111	2.12
10608247	LOC100042196	similar to spermiogenesis specific transcript on the Y 1	20	42	2.11
10545217	LOC100046973	similar to [Human Ig rearranged gamma chain mRNA, V-J-C region and complete cds.], gene product	19	40	2.10
10591135	Fat3	FAT tumor suppressor homolog 3 (Drosophila)	166	349	2.10
10583809	Cnn1	calponin 1	96	201	2.10
10474497	Olf1287	olfactory receptor 1287	14	29	2.10
10608488	LOC665128	similar to spermiogenesis specific transcript on the Y 1	23	48	2.10
10545202	Gm1077	predicted gene 1077	66	138	2.09
10584252	Gm9513	predicted gene 9513	209	437	2.09
10561162	Cyp2a22	cytochrome P450, family 2, subfamily a, polypeptide 22	17	35	2.07
10351546	Apoa2	apolipoprotein A-II	345	715	2.07
10545200	LOC100046894	similar to Igk-C protein	69	143	2.06
10608350	LOC100039552	similar to spermiogenesis specific transcript on the Y 1	19	39	2.04
10507163	Cyp4a10	cytochrome P450, family 4, subfamily a, polypeptide 10	27	55	2.03
10598863	Rgn	regucalcin	25	50	2.03

ID	Gene Symbol	Gene Name	WT Signal	KO Signal	Fold
10600024	Gpr50	G-protein-coupled receptor 50	550	62	-8.85
10415081	EG665955	predicted gene, EG665955	1197	148	-8.11
10504178	4933409K07Rik	RIKEN cDNA 4933409K07 gene	667	101	-6.62
10559796	Peg3	paternally expressed 3	1277	319	-4.01
10584572	Hspa8	heat shock protein 8	7865	2009	-3.92
10538924	LOC100046496	similar to Ig kappa V-region 24B	1597	429	-3.72
10508800	LOC433762	hypothetical gene LOC433762	1712	482	-3.55
10505451	Orm2	orosomuroid 2	342	98	-3.47
10496457	Adh6b	alcohol dehydrogenase 6B (class V)	171	50	-3.43
10602896	Gpr64	G protein-coupled receptor 64	355	103	-3.43
10362440	Trdn	triadin	104	31	-3.36
10553533	Gm6181	predicted gene 6181	221	72	-3.09
10531987	Gbp4	guanylate binding protein 4	729	236	-3.09
10421106	Adam7	a disintegrin and metallopeptidase domain 7	62	21	-3.02
10399428	Snord118	small nucleolar RNA, C/D box 118	320	108	-2.97
10545231	LOC100047053	similar to monoclonal antibody kappa light chain	818	277	-2.96
10585068	Fam55d	family with sequence similarity 55, member D	277	95	-2.93
10506225	Cachd1	cache domain containing 1	985	339	-2.90
10562169	Hamp	hepcidin antimicrobial peptide	100	34	-2.90
10429588	9030619P08Rik	RIKEN cDNA 9030619P08 gene	153	53	-2.89
10606178	Xist	inactive X specific transcripts	59	21	-2.79
10583133	Mmp7	matrix metallopeptidase 7	116	42	-2.76
10398032	Serpina3b	serine (or cysteine) peptidase inhibitor, clade A, member 3B	158	57	-2.76
10474096	Lrrc4c	leucine rich repeat containing 4C	200	75	-2.66
10567518	Dnahc3	dynein, axonemal, heavy chain 3	73	28	-2.58
10564165	Snord116	small nucleolar RNA, C/D box 116 cluster	119	46	-2.58
10501199	Gstm7	glutathione S-transferase, mu 7	903	358	-2.52
10376366	2210415F13Rik	RIKEN cDNA 2210415F13 gene	74	30	-2.47
10568502	Cuzd1	CUB and zona pellucida-like domains 1	79	33	-2.43
10347915	Gm7609	predicted gene 7609	713	295	-2.42
10429029	Adcy8	adenylate cyclase 8	62	26	-2.38
10421186	Gm10002	predicted gene 10002	37	16	-2.36
10566326	Trim12	tripartite motif-containing 12	230	98	-2.35
10500529	Phgdh	3-phosphoglycerate dehydrogenase	711	304	-2.34
10582879	Csprs	component of Sp100-rs	521	228	-2.28
10552594	Klk1b22	kallikrein 1-related peptidase b22	48	21	-2.28
10403043	Igh	immunoglobulin heavy chain complex	1740	766	-2.27
10385518	Tgtp	T-cell specific GTPase	507	224	-2.26
10462313	Slc1a1	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	184	82	-2.25
10374183	Gm4638	predicted gene 4638	129	58	-2.22
10345436	LOC280487	pol polyprotein	129	58	-2.22
10414537	Ang	angiogenin, ribonuclease, RNase A family, 5	1125	508	-2.21
10596279	Dnajc13	DnaJ (Hsp40) homolog, subfamily C, member 13	89	40	-2.20
10505445	Orm3	orosomuroid 3	76	35	-2.18
10551025	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	163	75	-2.16
10359593	Fmo3	flavin containing monooxygenase 3	186	87	-2.14
10419578	Ndrp2	N-myc downstream regulated gene 2	4059	1906	-2.13
10600093	Zfp185	zinc finger protein 185	172	81	-2.11
10403291	Akr1c14	aldo-keto reductase family 1, member C14	161	77	-2.10
10566585	Gm1966	predicted gene 1966	156	75	-2.09
10422635	C6	complement component 6	1718	828	-2.08
10545210	Gm1524	predicted gene 1524	157	76	-2.06
10448079	Gm6686	predicted gene 6686	199	97	-2.06
10555736	Olf558	olfactory receptor 558	316	155	-2.04
10571752	Dctd	dCMP deaminase	224	110	-2.03
10455970	BC023105	cDNA sequence BC023105	116	57	-2.03
10455961	Ilgp1	interferon inducible GTPase 1	319	158	-2.02
10469936	Nrarp	Notch-regulated ankyrin repeat protein	212	105	-2.02
10447649	Fndc1	fibronectin type III domain containing 1	353	175	-2.02
10359689	Atp1b1	ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, beta 1 polypeptide	352	175	-2.01
10586591	Car12	carbonic anhydrase 12	88	44	-2.01
10594802	B230380D07Rik	RIKEN cDNA B230380D07 gene	387	193	-2.01
10556962	Vwa3a	von Willebrand factor A domain containing 3A	101	50	-2.01
10565910	Plekhh1	pleckstrin homology domain containing, family B (evectins) member 1	111	55	-2.01
10559790	Zim1	zinc finger, imprinted 1	68	34	-2.00
10460251	1700055N04Rik	RIKEN cDNA 1700055N04 gene	212	106	-2.00
10500268	Mrps21	mitochondrial ribosomal protein S21	2228	1115	-2.00

**Supplemental Table 2. Expression of mitochondrial genes in skeletal muscle and white adipose tissue of *Oma1<sup>+/+</sup>* and *Oma1<sup>-/-</sup>* under high-fat diet.**

	Skeletal muscle		WAT	
	<i>Oma1<sup>+/+</sup></i>	<i>Oma1<sup>-/-</sup></i>	<i>Oma1<sup>+/+</sup></i>	<i>Oma1<sup>-/-</sup></i>
<b>Mitochondrial dynamics</b>				
<i>Mfn2</i>	1 ± 0.09	0.83 ± 0.04	1 ± 0.11	0.68 ± 0.07 *
<i>Opa1</i>	1 ± 0.08	0.76 ± 0.05 *	1 ± 0.07	0.77 ± 0.10 *
<i>Drp1</i>	1 ± 0.08	0.77 ± 0.06 *	1 ± 0.12	0.83 ± 0.15
<b>OXPPOS genes</b>				
<i>Pgc1a</i>	1 ± 0.07	0.91 ± 0.07		
<i>Ndufa9</i>	1 ± 0.04	0.91 ± 0.07		
<i>Sdha</i>	1 ± 0.06	0.82 ± 0.06 *		
<i>Uqcrc2</i>	1 ± 0.03	0.91 ± 0.09		
<i>CoxIV</i>	1 ± 0.05	0.90 ± 0.09		
<i>Atp5a1</i>	1 ± 0.03	0.82 ± 0.05 *		
<i>Cpt1b</i>	1 ± 0.05	0.91 ± 0.06		

Gene expression relative to beta-actin. Data represent mean ± SEM of at least five mice per group. \* P< 0.05; \*\* P< 0.01