Cell Metabolism, Volume *33*

Supplemental information

Mitochondrial cristae-remodeling protein OPA1 in POMC neurons couples Ca^{2+} homeostasis with adipose tissue lipolysis

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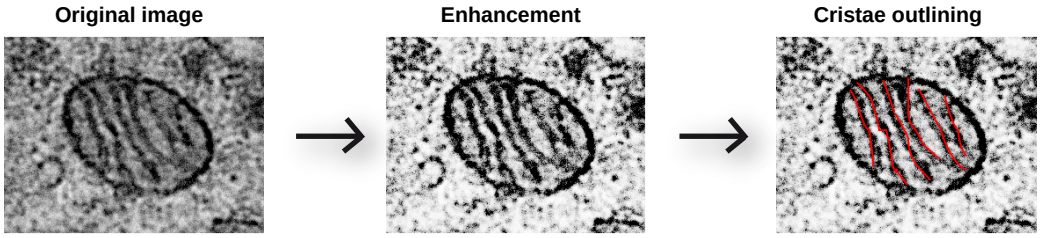
Supplemental Figures

Figure S1

A



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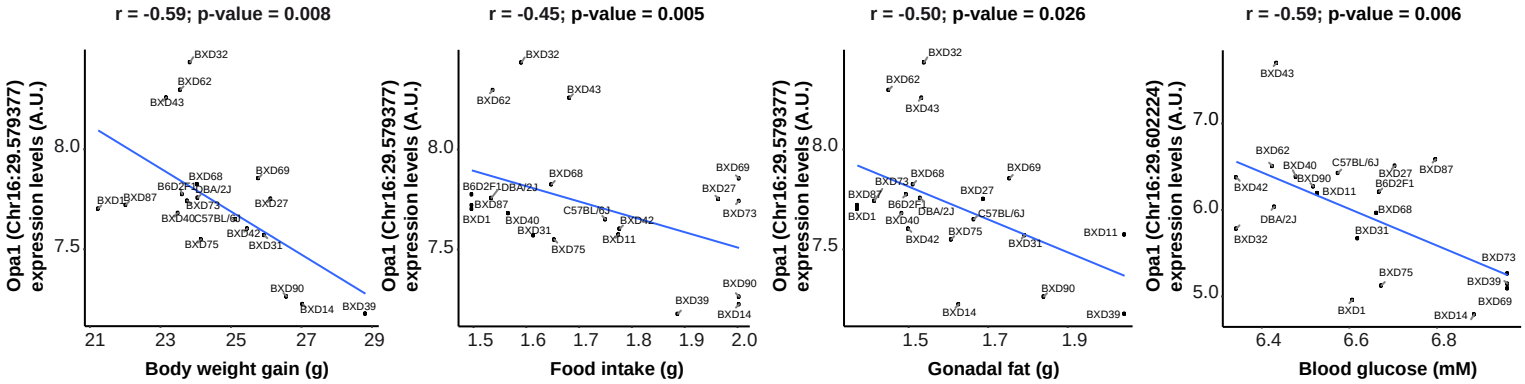

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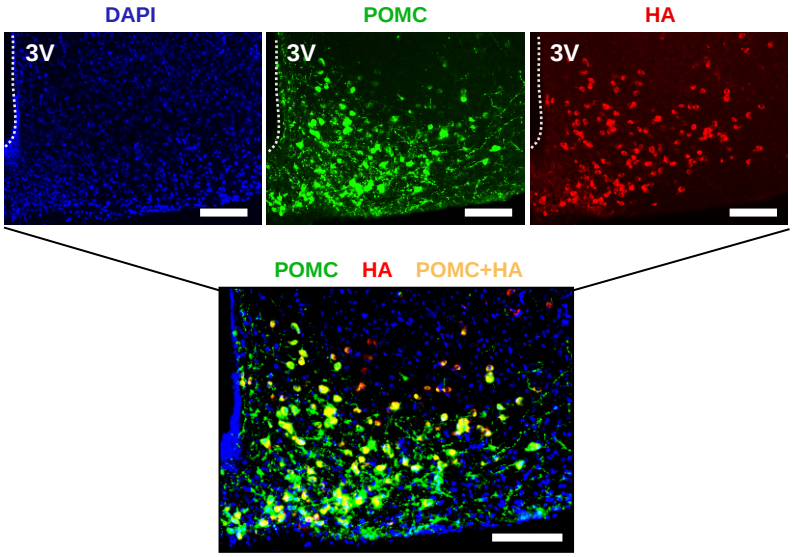

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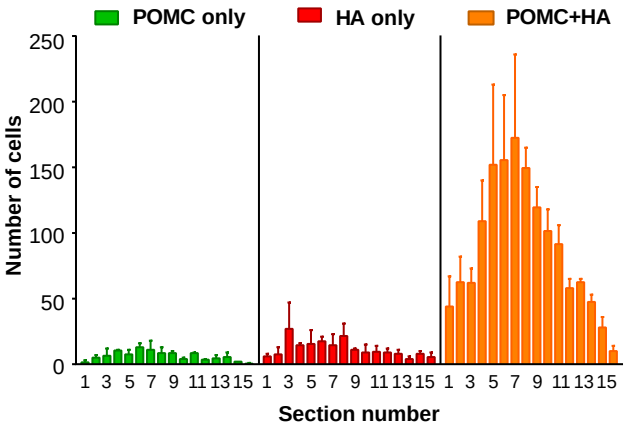

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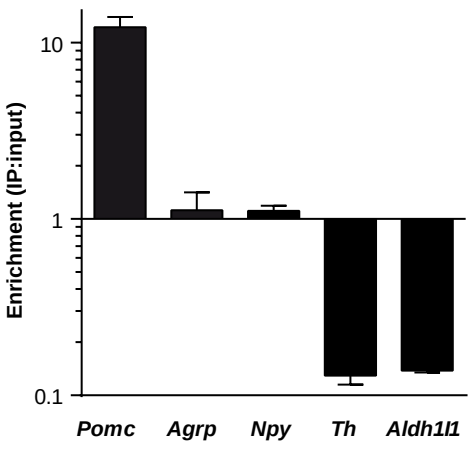

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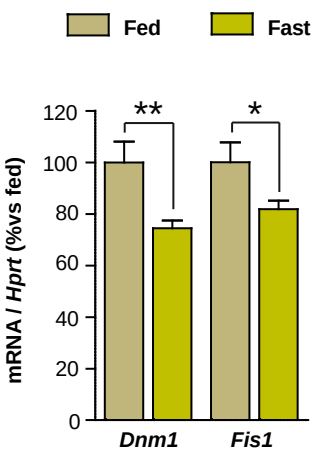

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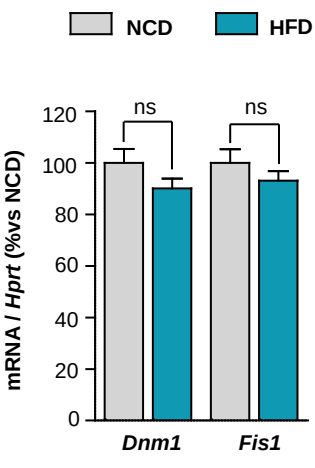


Supplementary Figure 1. Mitochondrial cristae outlining, *Opa1* correlation analysis in BXD strains and validation of neuronal specificity in the *POMCRiboTag* mouse model. Related to Figure 1.

(A) Schematic representation of mitochondria cristae analysis. Original mitochondria image (step 1, left), image enhancement (step2, middle panel) and manual mitochondria outlining (Step 3, right panel) are shown.

(B) Pearson's correlations between *Opa1* expression in the hypothalami and body weight gain (females and males between 4 and 20 weeks of age), food intake, adiposity and glycaemia (at 17-20 weeks of age) across 20 BXD mouse strains fed with high-fat diet. Data and correlation analysis was performed using GeneNetwork website. Pearson's correlation index (r) and p-value are indicated.

(C) Representative ARC sections showing immunofluorescence colocalization for POMC (green) and HA (red). Nuclei were labeled in blue (DAPI). Two brains were processed with equivalent results. 3V: third ventricle. Scale bar represents 100µm.

(D) Average cell count and distribution throughout the ARC of positive cells for POMC but negative for HA (green bars), positive cells for HA but negative for POMC (red bars) and double positive cells for POMC and HA (orange bars). Two brains were processed with equivalent results.

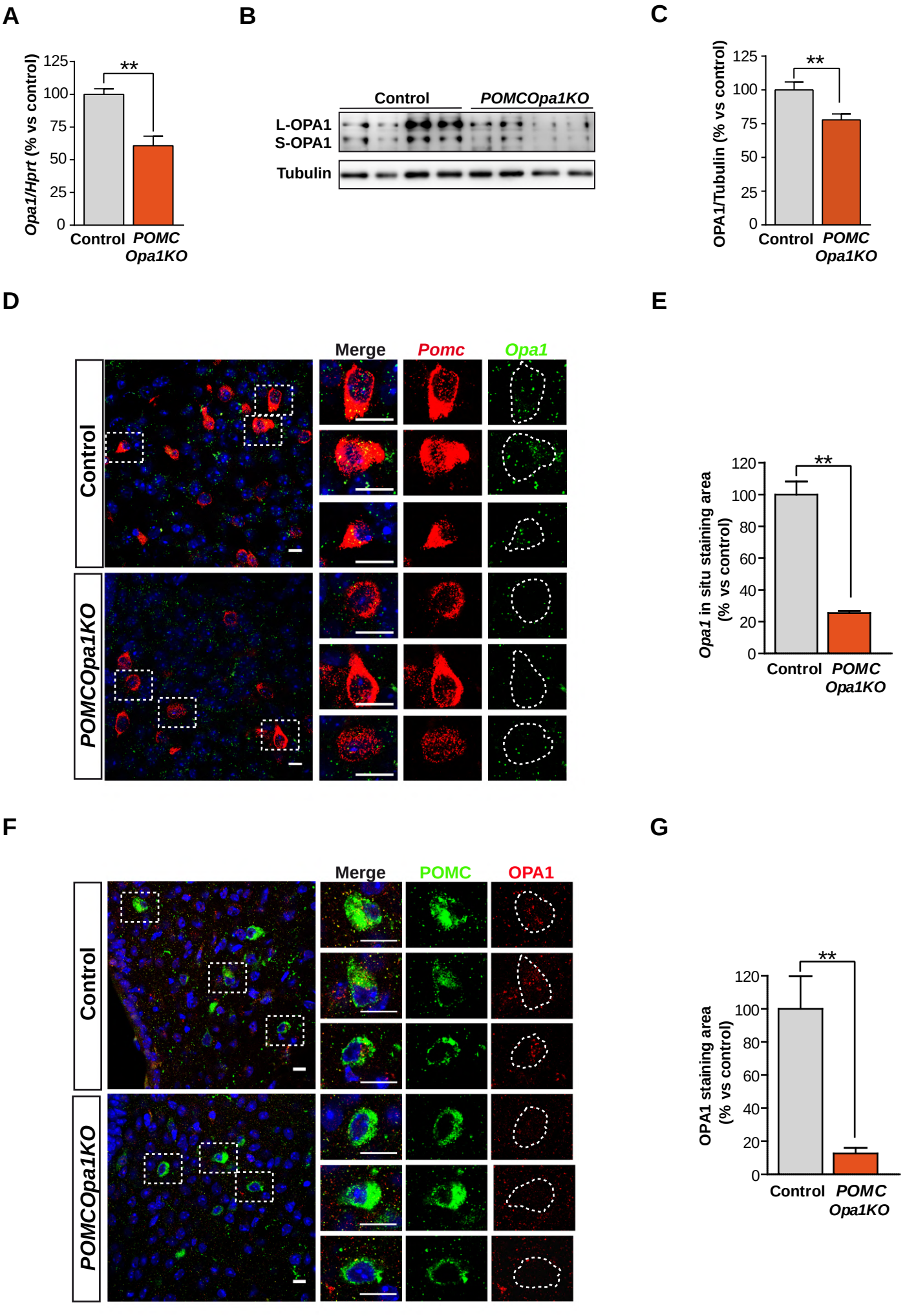
(E) Enrichment expression analysis of specific neuropeptides and negative control markers (*Th* and *Aldh1L1*) in POMC neuron-enriched mRNA samples from *POMCRiboTag* mice. The average of two independent immunoprecipitation (IP) experiments is represented.

(F) Expression of mitochondrial fission genes *Dnm1* and *Fis1* in POMC neurons from random fed or fasted *POMCRiboTag* mice (n=6-8/ group).

(G) Expression of mitochondrial fission *Dnm1* and *Fis1* genes in POMC neurons from *POMCRiboTag* mice fed with NCD or HFD (n=5-7/ group).

All studies, unless otherwise stated, were conducted in 12-14 week-old male *POMCRiboTag* mice. Data are expressed as mean ± SEM. *p<0.05; **p<0.01; ns: not significant.

Figure S2


A
Opa1/Hprt (% vs control)
125
100
75
50
25
0
**
Control
POMC Opa1KO
B
Control
POMCOpa1KO
L-OPA1
S-OPA1
Tubulin
C
OPA1/Tubulin (% vs control)
125
100
75
50
25
0
**
Control
POMC Opa1KO
D
Merge
Pomc
Opa1
Control
POMCOpa1KO
E
Opa1 in situ staining area (% vs control)
120
100
80
60
40
20
0
**
Control
POMC Opa1KO
F
Merge
POMC
OPA1
Control
POMCOpa1KO
G
OPA1 staining area (% vs control)
120
100
80
60
40
20
0
**
Control
POMC Opa1KO

Supplementary Figure 2. Validation of *POMCOpa1KO* mouse model: *Opa1* deletion specifically in POMC neurons. Related to Figure 2.

(A) Gene expression analysis of *Opa1* in ARC-enriched microdissections from control (n=5) and *POMCOpa1KO* (n=11) mice.

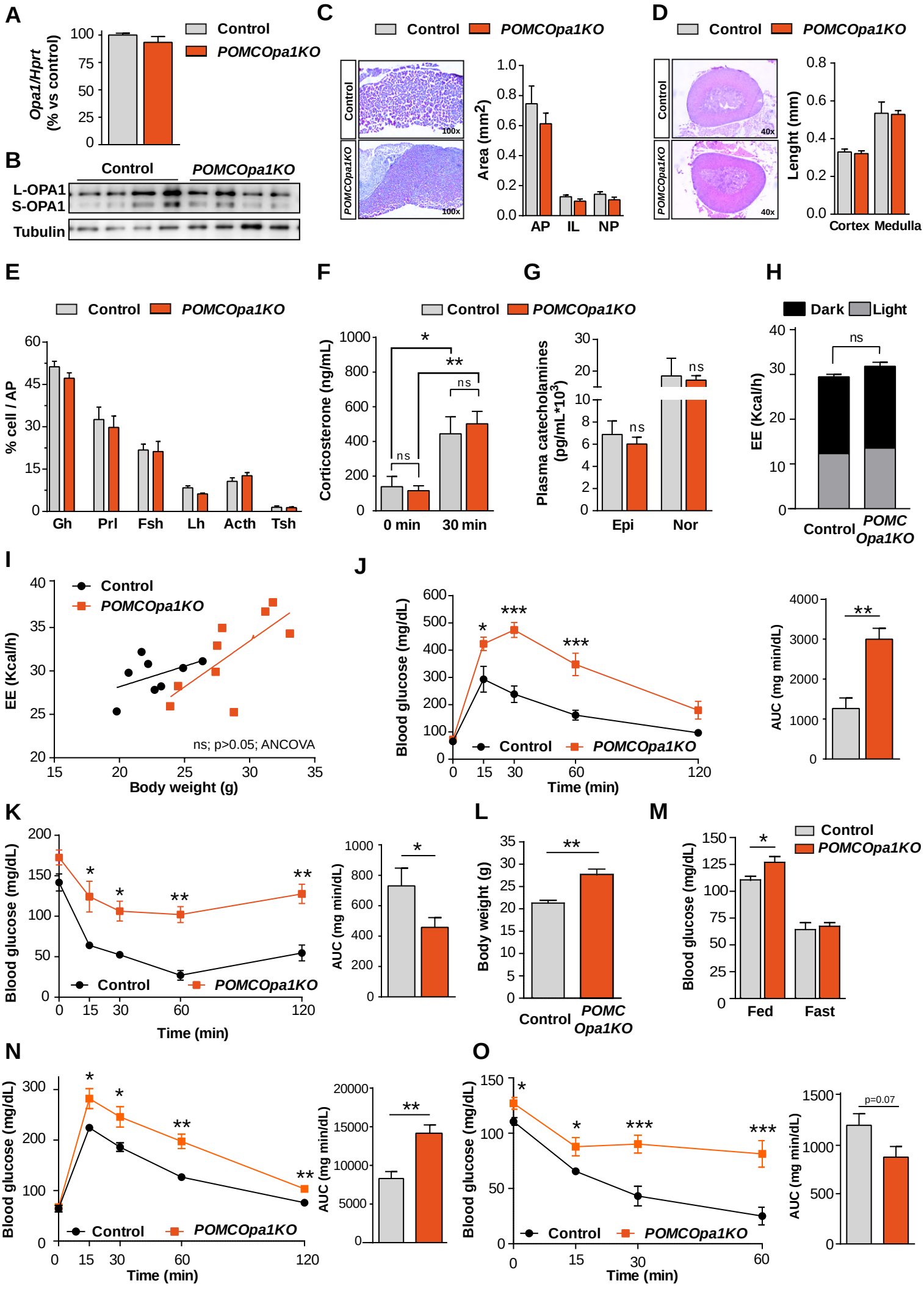
(B) Representative immunoblot images of long (L-OPA1) and short (S-OPA1) isoforms in ARC-enriched microdissections and **(C)** its densitometric quantification of total OPA1 protein content (L-OPA1+ S-OPA1 isoforms). Results from two independent experiments are pooled (control n=8 and *POMCOpa1KO* n=9). Tubulin was used as loading control.

(D) Representative confocal images and **(E)** quantification of *in situ* hybridization of *Opa1* (green) and *Pomc* (red) mRNA and nuclear counterstaining (blue) in coronal sections of the arcuate nucleus of the hypothalamus of control (n=4) and *POMCOpa1KO* (n=4) mice. Scale bar: 25µm.

(F) Representative confocal images and **(G)** quantification of immunofluorescence detection of OPA1 (red) and POMC (green) and nuclear counterstaining (blue) in coronal sections of the arcuate nucleus of the hypothalamus of control (n=3) and *POMCOpa1KO* (n=3) mice. Scale bar: 25µm.

All studies were conducted in 12-14 week-old male control and *POMCOpa1KO* mice. Data are expressed as mean ± SEM. **p<0.01.

Figure S3


A
Opa1/Hprt (% vs control)
Control
POMCOpa1KO
B
Control
POMCOpa1KO
L-OPA1
S-OPA1
Tubulin
C
Control
POMCOpa1KO
100x
Area (mm2)
AP
IL
NP
D
Control
POMCOpa1KO
40x
Lenght (mm)
Cortex
Medulla
E
% cell / AP
Gh
Prl
Fsh
Lh
Acth
Tsh
F
Corticosterone (ng/mL)
0 min
30 min
ns
G
Plasma catecholamines (pg/mL*10^3)
Epi
Nor
H
Dark
Light
EE (Kcal/h)
Control
POMC Opa1KO
I
EE (Kcal/h)
Body weight (g)
ns; p>0.05; ANCOVA
J
Blood glucose (mg/dL)
Time (min)
AUC (mg min/dL)
K
Blood glucose (mg/dL)
Time (min)
AUC (mg min/dL)
L
Body weight (g)
M
Blood glucose (mg/dL)
Fed
Fast
N
Blood glucose (mg/dL)
Time (min)
AUC (mg min/dL)
O
Blood glucose (mg/dL)
Time (min)
AUC (mg min/dL)
p=0.07

Supplementary Figure 3. Validation of *POMCOpa1KO* mouse model: unaltered pituitary-adrenal axis and metabolic phenotyping. Related to Figure 2.

(a) Gene expression analysis of *Opa1* in pituitaries from control (n=6) and *POMCOpa1KO* (n=5) mice.

(B) Representative immunoblot images of long (L-OPA1) and short (S-OPA1) isoforms in pituitaries from control (n=6) and *POMCOpa1KO* (n=5) mice. Representative lanes are shown.

(C) Representative images of hematoxylin and eosin staining of the biggest sections of pituitary glands and its corresponding area quantification for anterior pituitary (AP), intermediate pituitary (IP) and posterior or nervous pituitary (NP) from weight matched control (n=4) and *POMCOpa1KO* (n=3) mice.

(D) Representative images of hematoxylin and eosin staining of central sections of adrenal glands and its corresponding length quantification for adrenal cortex and medulla from weight matched control (n=4) and *POMCOpa1KO* (n=3) mice.

(E) Number of positive cells for growth hormone (Gh), prolactin (Prl), follicle-stimulating hormone (Fsh), luteinizing hormone (Lh), adrenocorticotropic hormone (Acth) and thyroid-stimulating hormone (Tsh) in the anterior pituitary (AP) from weight matched control (n=4) and *POMCOpa1KO* (n=3).

(F) Plasma corticosterone levels on basal (0 min) and after restrain-induced stress (30 min) conditions in control (n=7) and *POMCOpa1KO* (n=6) mice.

(G) Circulating levels of epinephrine (Epi) and norepinephrine (Nor) in weight-matched control (n=4) and *POMCOpa1KO* (n=4) mice.

(H) Total energy expenditure (EE) and (**I**) EE adjusted for body weight using multiple linear regression (ANCOVA) in control (n=8) and *POMCOpa1KO* (n=9) mice.

(J) Glucose tolerance and (**K**) insulin sensitivity test in control (n=5) and *POMCOpa1KO* mice (n=11). Area under the curve (AUC) is shown.

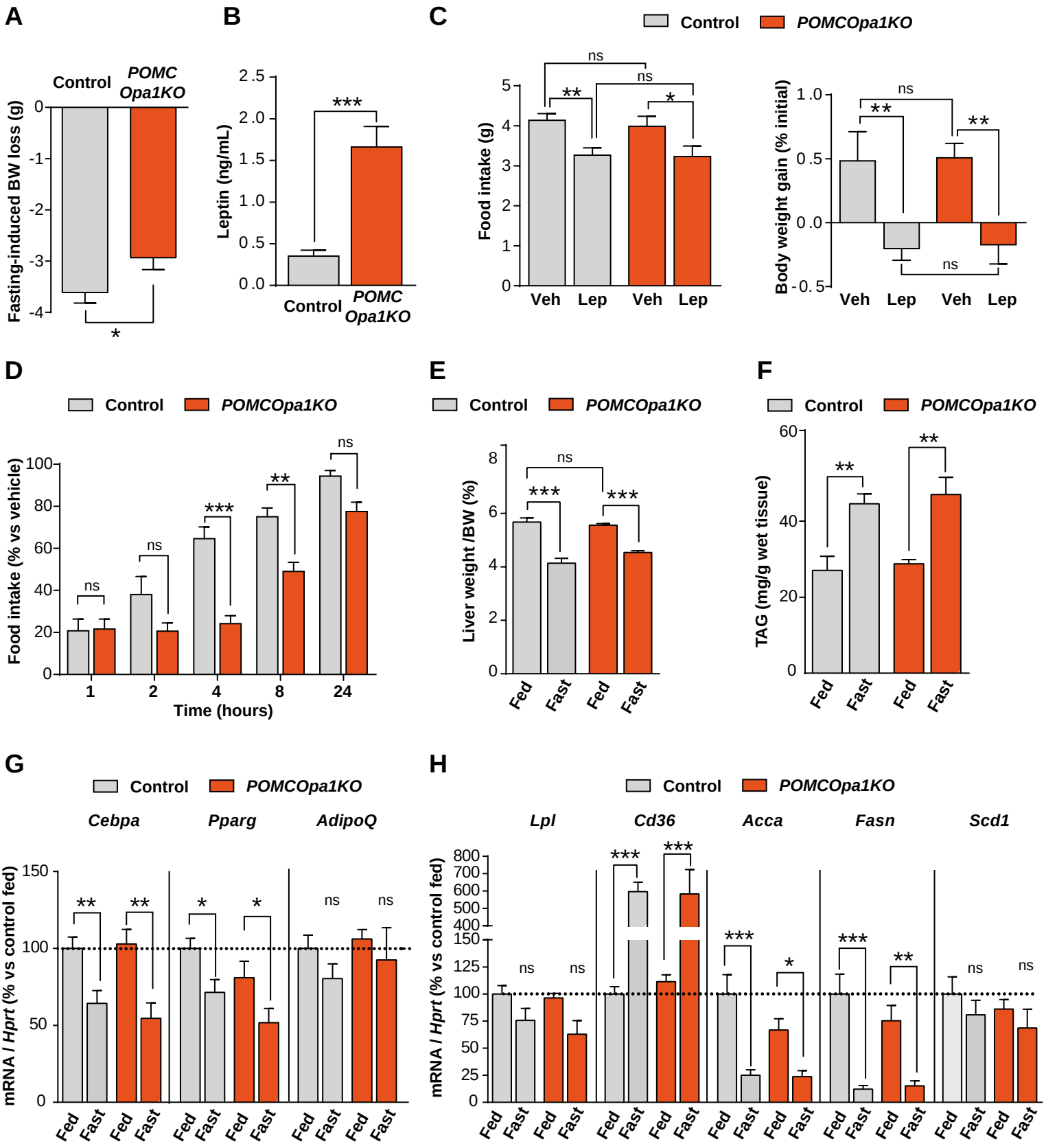
(L) Body weight at 12 weeks of age in control (n=5) and *POMCOpa1KO* (n=7) female mice.

(M) Blood glucose levels in control (n=5) and *POMCOpa1KO* (n=7) female mice.

(N) Glucose tolerance test and **(O)** insulin sensitivity test in control (n=6) and *POMCOpa1KO* (n=7) female mice. Area under the curve (AUC) is shown.

Studies were conducted in 12-14 week-old male control and *POMCOpa1KO* mice or otherwise stated. Data are expressed as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001. ns: not significant.

Figure S4


A
Control
POMC Opa1KO
Fasting-induced BW loss (g)
B
Leptin (ng/mL)
Control
POMC Opa1KO
C
Control
POMCOpa1KO
Food intake (g)
Veh
Lep
Body weight gain (% initial)
D
Food intake (% vs vehicle)
Time (hours)
E
Liver weight /BW (%)
Fed
Fast
F
TAG (mg/g wet tissue)
G
Cebpa
Pparg
AdipoQ
mRNA / Hprt (% vs control fed)
H
Lpl
Cd36
Acca
Fasn
Scd1

Supplementary Figure 4. Phenotypical characterization of young weight-matched *POMCOpa1KO* mice. Related to Figure 3.

(A) Body weight loss after an overnight (16 h) fast in 10-12 weeks old control (n=12) and *POMCOpa1KO* (n=8) mice.

(B) Plasma leptin levels in fasting conditions in control (n=6) and *POMCOpa1KO* (n=7) mice.

(C) Leptin sensitivity test. Food intake and body weight gain after vehicle (Veh) or leptin (Lep) injection is shown. N=9-13 genotype/treatment.

(D) Food intake of control (n=10) and *POMCOpa1KO* (n=7) mice after MT-II administration. Data are normalized by the vehicle-injected groups.

(E) Liver weight, relative to total body weight, in control and *POMCOpa1KO* mice under fed or fasting conditions. N=4-6/genotype/nutritional status.

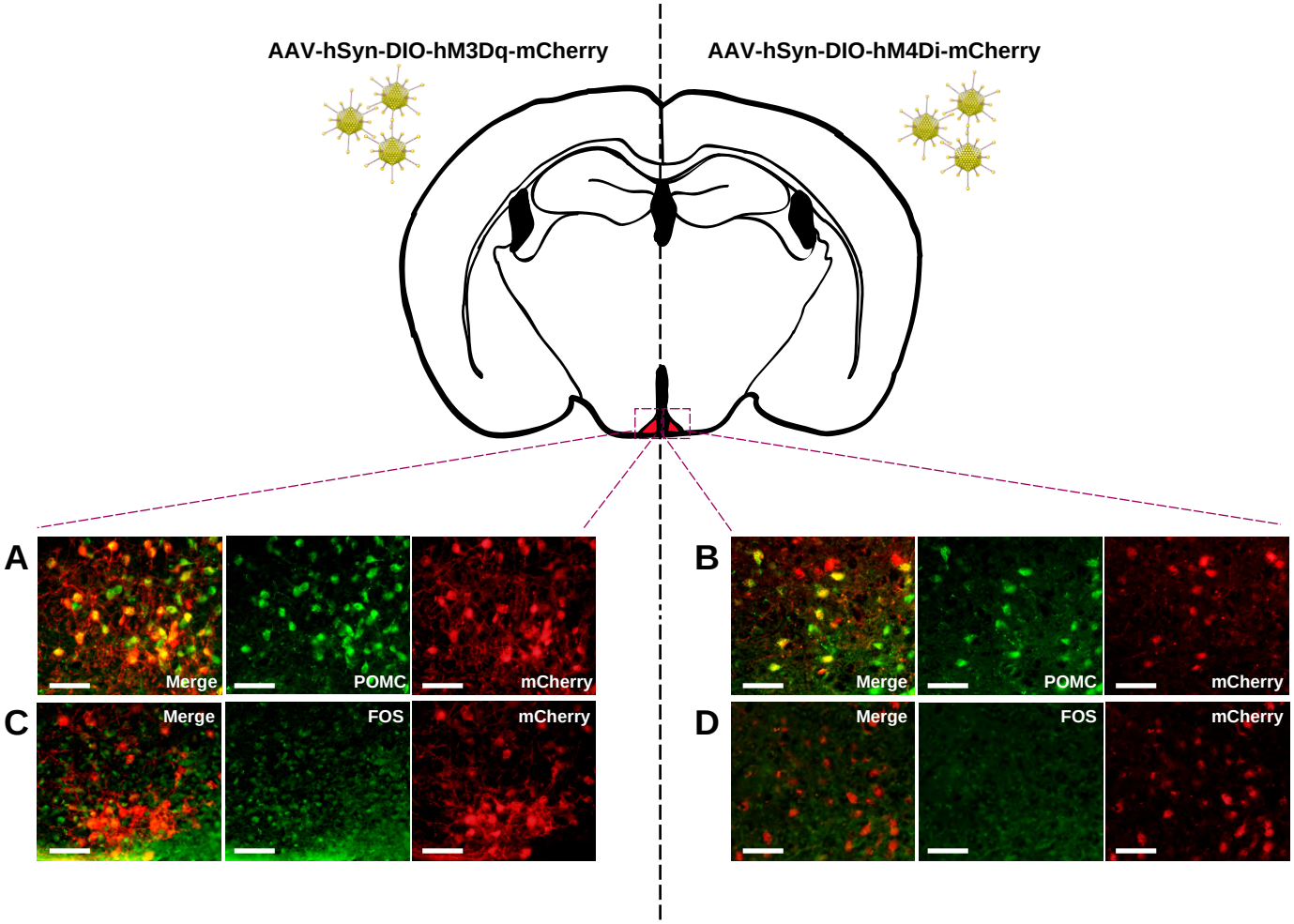
(F) Hepatic triglyceride (TAG) content in control and *POMCOpa1KO* mice under fed or fasting conditions. N=4-6/genotype/nutritional status.

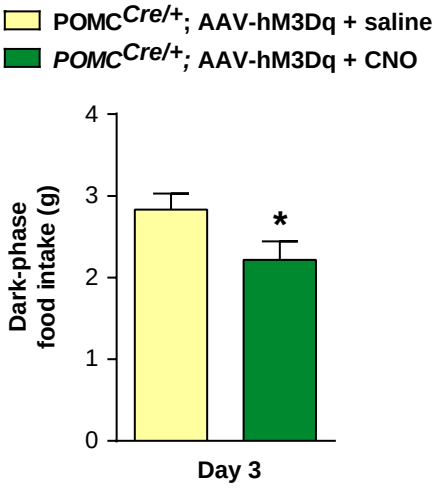
(G) Gene expression analysis of representative adipocyte marker genes (*Cebpa*: CCAAT/enhancer-binding protein alpha; *Pparg*: peroxisome proliferator-activated receptor gamma; *Adipoq*: adiponectin) in pgWAT from control and *POMCOpa1KO* mice. N=6-8/genotype/nutritional status.

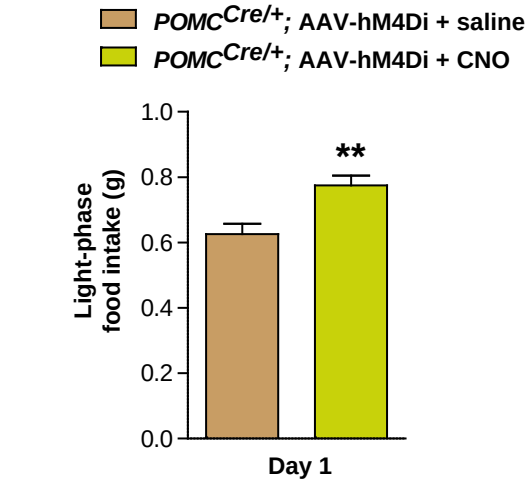
(H) Gene expression analysis of key genes involved lipid uptake (*Lpl*: lipoprotein lipase; *Cd36:* cluster of differentiation 36) and *de novo* lipogenesis (*Acca:* acetyl-CoA carboxylase A; *Fasn:* fatty acid synthase*; Scd1:* stearoyl-CoA desaturase) in pgWAT from control and *POMCOpa1KO* mice. N=6-8/genotype/nutritional status.

All studies were conducted in 5-6 week-old male control and *POMCOpa1KO* mice or otherwise stated. Data are expressed as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001. ns: not significant.

Figure S5


AAV-hSyn-DIO-hM3Dq-mCherry
AAV-hSyn-DIO-hM4Di-mCherry
A
Merge
POMC
mCherry
B
Merge
POMC
mCherry
C
Merge
FOS
mCherry
D
Merge
FOS
mCherry


E
POMC$^{Cre/+}$; AAV-hM3Dq + saline
POMC$^{Cre/+}$; AAV-hM3Dq + CNO
Dark-phase food intake (g)
0
1
2
3
4
*
Day 3


F
POMC$^{Cre/+}$; AAV-hM4Di + saline
POMC$^{Cre/+}$; AAV-hM4Di + CNO
Light-phase food intake (g)
0.0
0.2
0.4
0.6
0.8
1.0
**
Day 1

Supplementary Figure 5. Validation of DREADD-mediated POMC neuron activity modulation. Related to Figure 4.

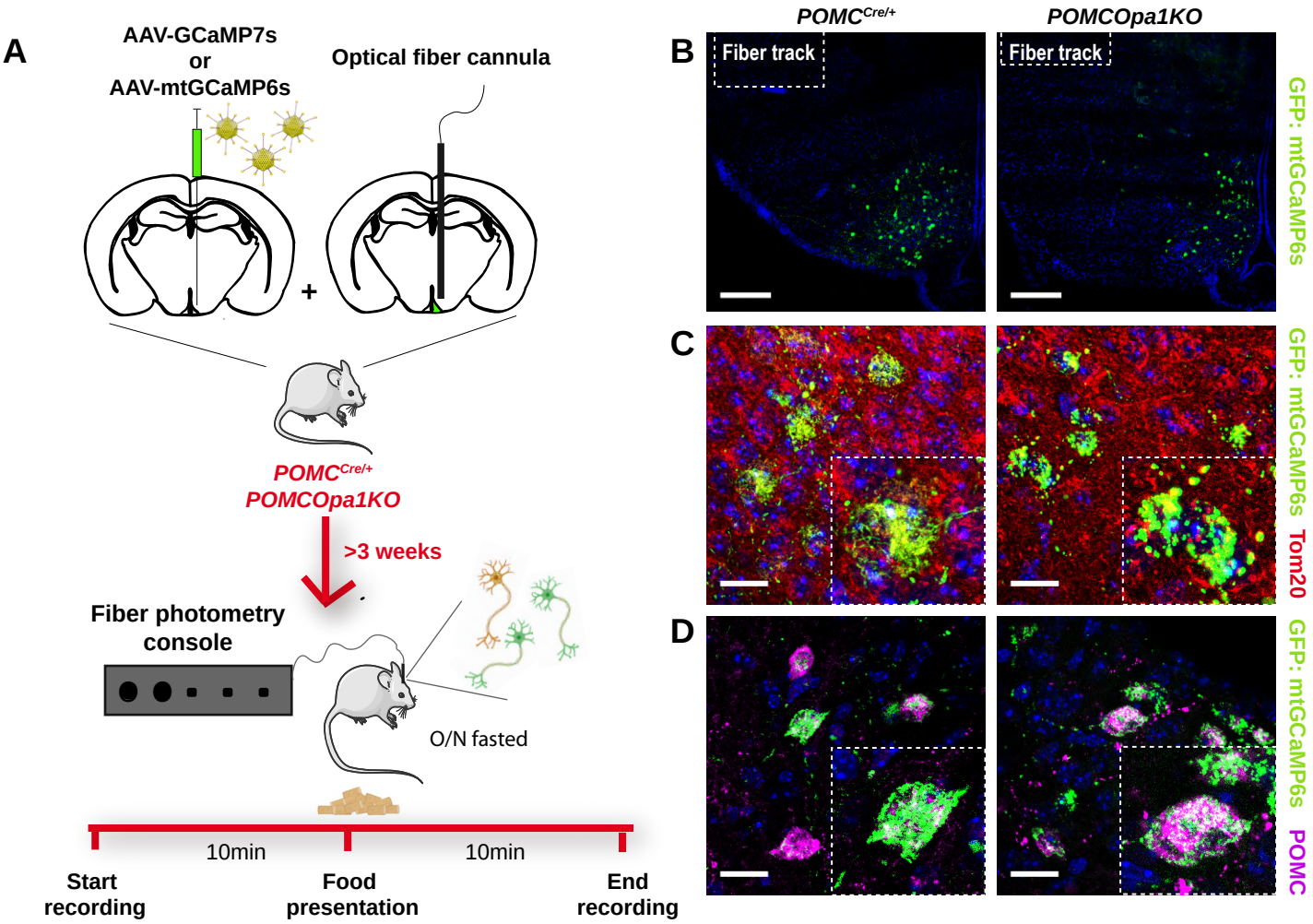
(A-D) Representative images of mCherry, FOS and POMC staining in the ARC of *POMC*$^{Cre/+}$ mice transduced with activatory (**A**, **C**) and inhibitory (**B**, **D**) DREADDs. Scale bar represents 100µm.

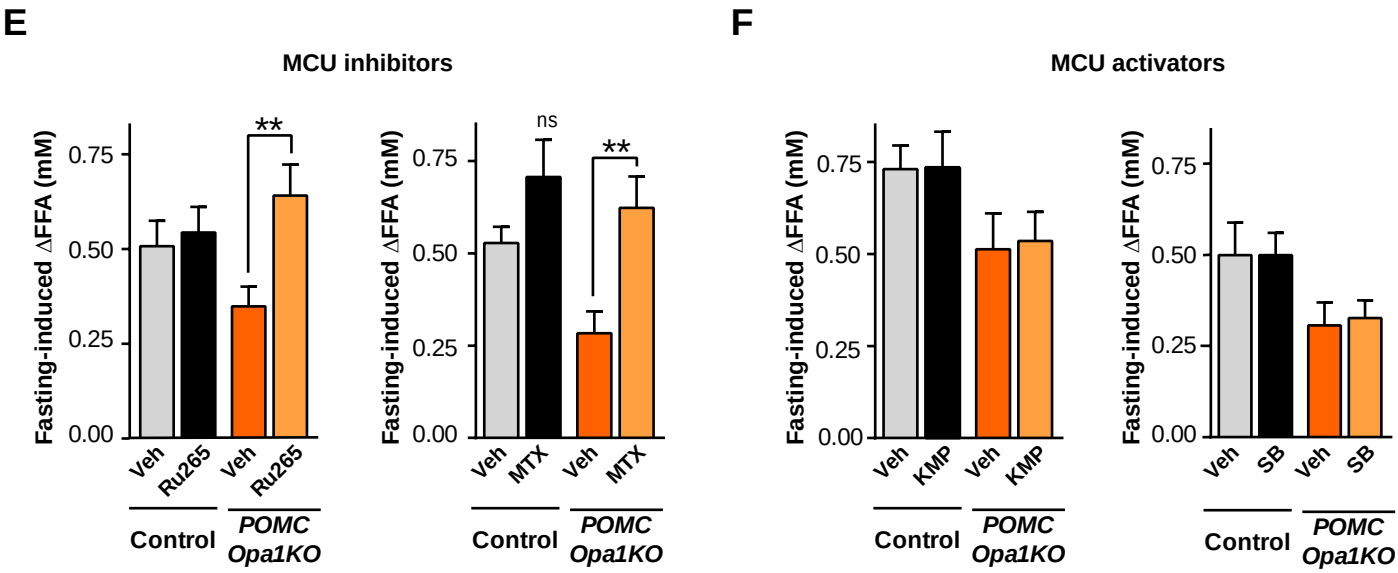
(E) Food intake after CNO-mediated activation of POMC neuronal activity (n=15/group).

(F) Food intake after CNO-mediated inhibition of POMC neuronal activity (n=9/group).

All studies were conducted in 14-16 week-old male *POMC*$^{Cre/+}$ mice transduced with activatory or inhibitory DREADDs injected with saline or CNO. Data are expressed as mean ± SEM. *p<0.05; **p<0.01.

Figure S6


A
AAV-GCaMP7s
or
AAV-mtGCaMP6s
Optical fiber cannula
+
POMC Cre/+
POMCOpa1KO
>3 weeks
Fiber photometry
console
O/N fasted
10min
10min
Start
recording
Food
presentation
End
recording
B
POMC Cre/+
POMCOpa1KO
Fiber track
Fiber track
GFP: mtGCaMP6s
C
GFP: mtGCaMP6s Tom20
D
GFP: mtGCaMP6s POMC


E
MCU inhibitors
Fasting-induced ΔFFA (mM)
0.00
0.25
0.50
0.75
**
Veh
Ru265
Veh
Ru265
Control
POMC Opa1KO
ns
**
Veh
MTX
Veh
MTX
Control
POMC Opa1KO
F
MCU activators
Veh
KMP
Veh
KMP
Control
POMC Opa1KO
Veh
SB
Veh
SB
Control
POMC Opa1KO

Supplementary Figure 6. Assessment of mitochondrial Ca^{2+} dynamics in POMC neurons. Related to Figure 6.

(A) Brain schematic of viral injection of GCaMP Ca^{2+} sensors, optical fiber cannula implantation and fiber photometry setup.

(B-D) Representative confocal images showing *Cre*-dependent expression of mtGCaMP6s by GFP immunofluorescence in ARC sections together with: **(B)** Fiber placement marked by a dotted line. Scale bar: 200µm; **(C)** TOM20 co-localization. Scale bar: 20µm; **(D)** POMC co-localization. Scale bar: 20µm. Representative sections from control *POMC*$^{Cre+/-}$ and *POMCOpa1KO* mice are shown to show equivalent expression.

(E-F) Fasting-induced increase of plasma FFA levels in control and *POMCOpa1KO* mice after i.c.v. injection of vehicle (Veh) and either **(E)** MCU inhibitors (Ru265 and Mitoxantrone; MTX) or **(F)** activators (Kaempferol; KMP or SB202190; SB). N=6-12 genotype/treatment.

All studies were conducted in 12-16 week-old male mice. Data are expressed as mean ± SEM. **p<0.01; ns: not significant.