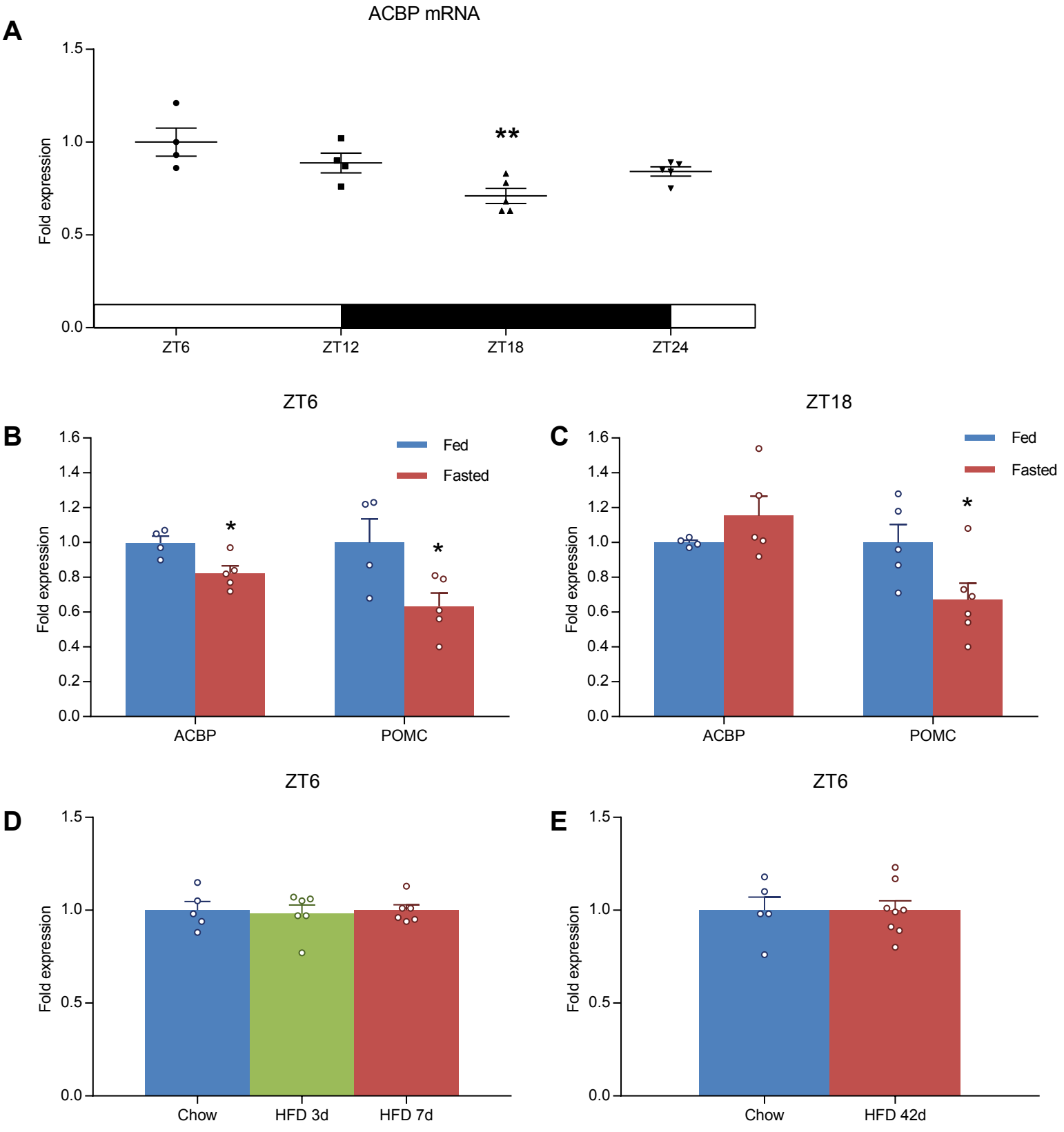
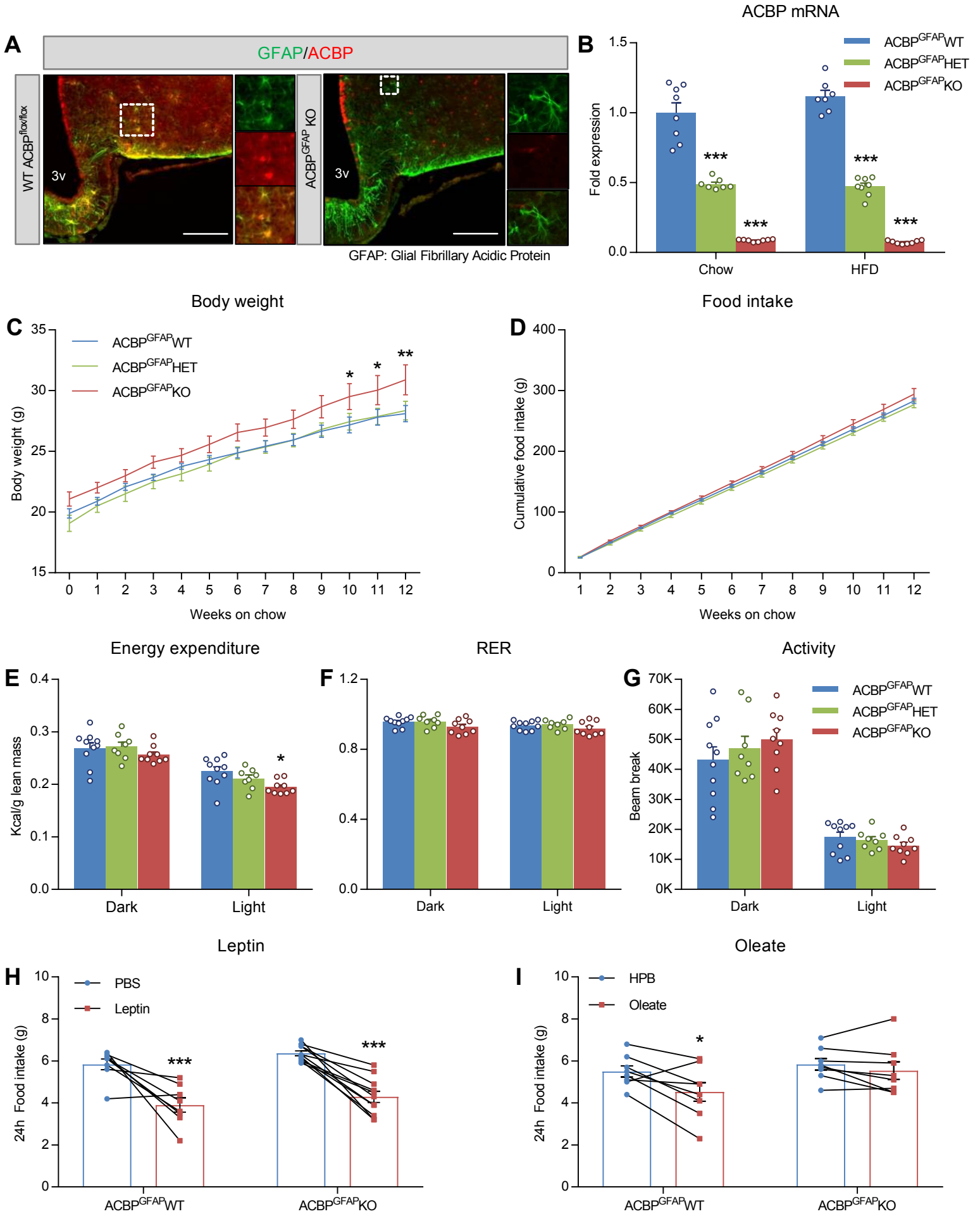
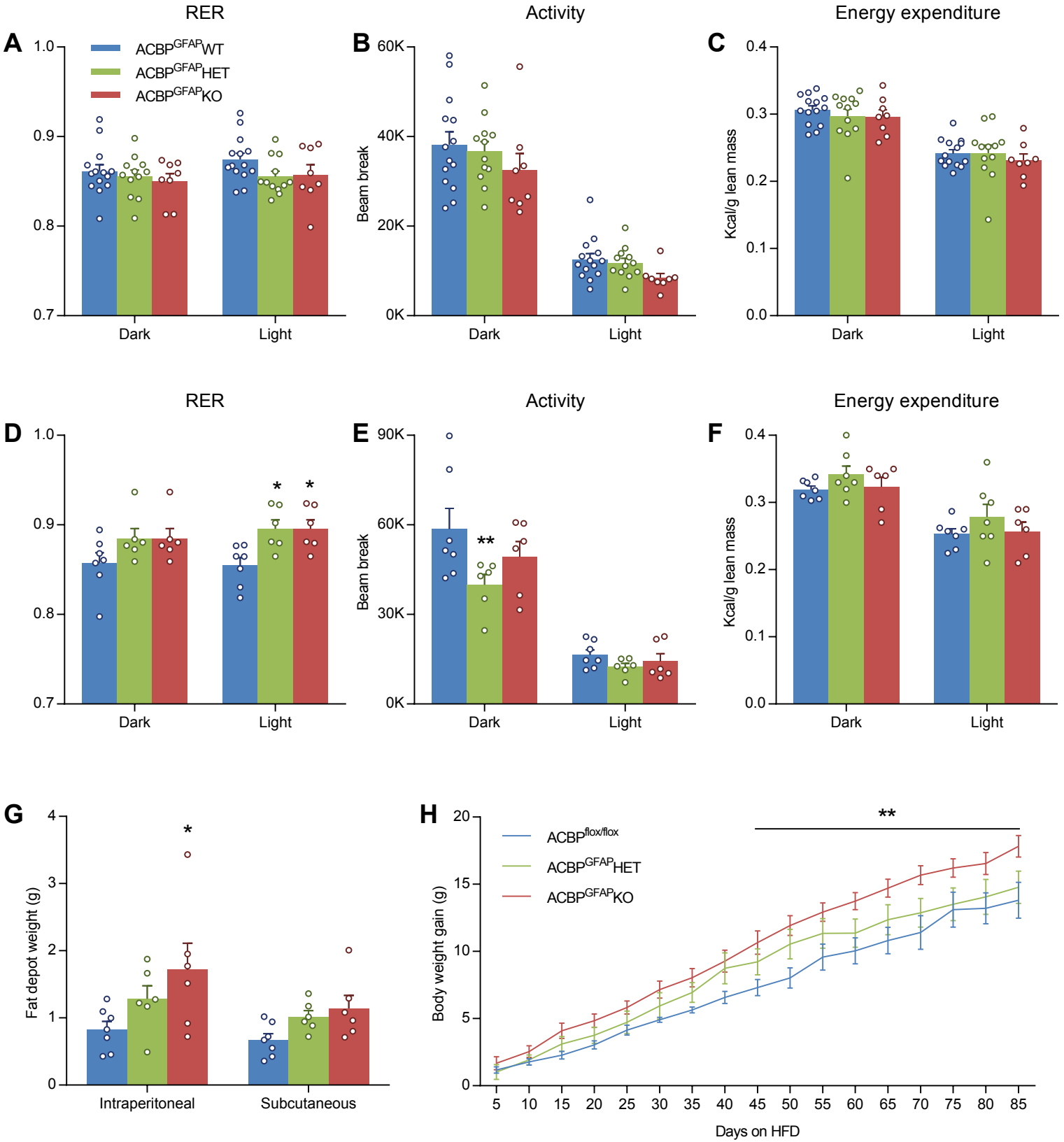
Supplemental Figure 1: *Acbp* gene expression is dependent on the circadian time and nutritional status. A- *Acbp* expression measured by qPCR in ARC microdissections of C57BL/6 WT male mice at different Zeitgeber times (ZT) (ZT0 corresponds to the onset of the light cycle in a 12 h light/dark cycle), ** $p<0.01$ compared to ZT6, One-way ANOVA with Bonferroni post hoc test, N=4-5. *Acbp* and *pomc* expression in ARC microdissections of fed or 16 h-fasted C57BL/6 WT male mice at **B-** ZT6 or **C-** ZT18, * $p<0.05$ Student's *t*-test compared to controls, N=4-5. *Acbp* expression in ARC microdissections (ZT6) of WT male mice fed with a chow or high fat diet during **D-** 3 or 7 days or **E-** 42 days. N=4-8.


A
ACBP mRNA
Fold expression
1.5
1.0
0.5
0.0
**
ZT6
ZT12
ZT18
ZT24
B
ZT6
Fed
Fasted
Fold expression
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
ACBP
POMC
C
ZT18
Fed
Fasted
Fold expression
*
ACBP
POMC
D
ZT6
Fold expression
1.5
1.0
0.5
0.0
Chow
HFD 3d
HFD 7d
E
ZT6
Fold expression
1.5
1.0
0.5
0.0
Chow
HFD 42d

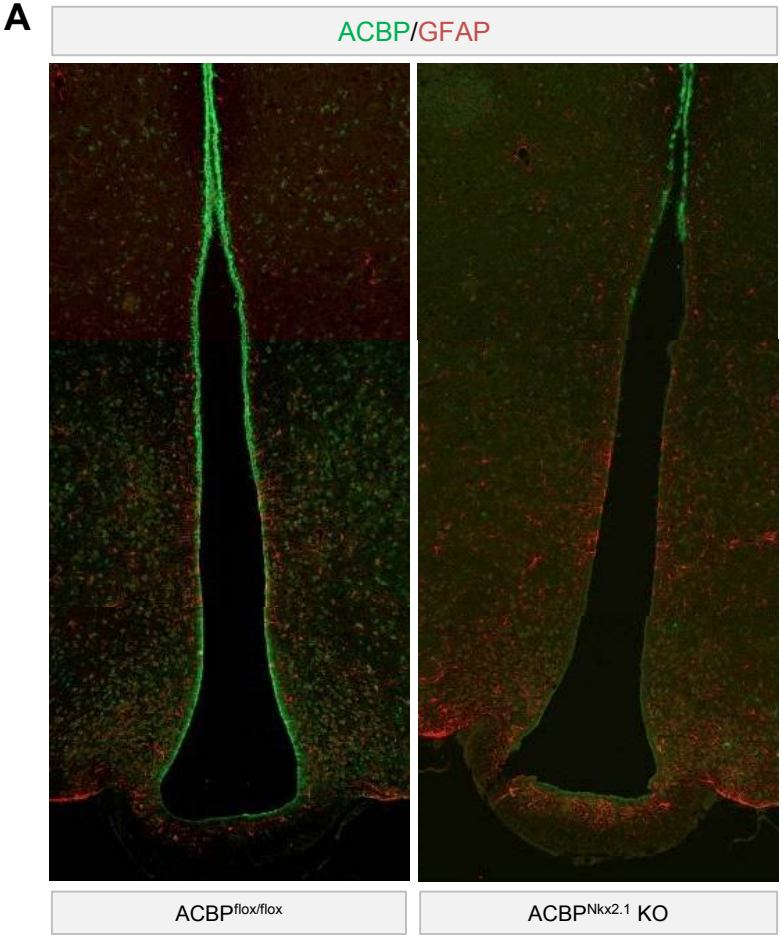
Supplemental Figure 2: Pan-brain astroglial ACBP invalidation in male mice. A- Immunostaining of ACBP (red) and GFAP (green) in *ACBP*$^{flox/flox}$ (WT) and ACBP^{GFAP} KO mice. Scale bar represents 100 µm. **B-** *Acbp* expression measured by qPCR in ARC microdissections from ACBP^{GFAP} WT, HET and KO male mice fed with either chow (12 weeks) or HFD (16 weeks). *** p<0.001 compared to control littermates, One-way ANOVA followed by Bonferroni post hoc test, N=8. **C-** Body weight and **D-** cumulative food intake of ACBP^{GFAP} WT, HET and KO male mice fed with chow. **E-** Energy expenditure (normalized to lean mass), **F-** RER and **G-** Locomotor activity measured in CLAMS metabolic cages during 48 h following 24 h acclimation, * p<0.05, ** p<0.01 compared to controls, Two-way ANOVA with Bonferroni post hoc test, N=8-10. **H-** 24 h food intake after a 16 h fast in ACBP^{GFAP} WT and KO male mice receiving an ICV injection of PBS on day 1 and Leptin (1µg/2µl) on day 10, N=8-11. **I-** 24 h food intake in 2 h-fasted ACBP^{GFAP} WT and KO male mice receiving an ICV injection of 4.5 % cyclodextrin HPB on day 1 and oleate (6 nmol/2µl) on day 10, N=8. * p<0.05, *** p<0.001 compared to vehicle injection, Two-way ANOVA repeated measures with Bonferroni post hoc test (each animal is used as its own control).

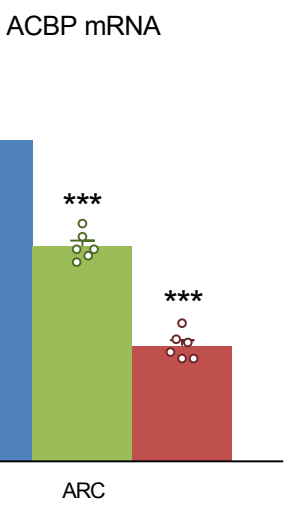

A
GFAP/ACBP
WT ACBP flox/flox
ACBP GFAP KO
3v
3v
GFAP: Glial Fibrillary Acidic Protein
ACBP mRNA
B
ACBP GFAP WT
ACBP GFAP HET
ACBP GFAP KO
Fold expression
1.5
1.0
0.5
0.0
***
***
***
***
Chow
HFD
Body weight
C
ACBP GFAP WT
ACBP GFAP HET
ACBP GFAP KO
Body weight (g)
35
30
25
20
15
*
*
**
0 1 2 3 4 5 6 7 8 9 10 11 12
Weeks on chow
Food intake
D
Cumulative food intake (g)
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12
Weeks on chow
Energy expenditure
E
Kcal/g lean mass
0.4
0.3
0.2
0.1
0.0
*
Dark
Light
RER
F
1.2
0.8
0.4
0.0
Dark
Light
Activity
G
ACBP GFAP WT
ACBP GFAP HET
ACBP GFAP KO
Beam break
70K
60K
50K
40K
30K
20K
10K
0K
Dark
Light
Leptin
H
PBS
Leptin
24h Food intake (g)
10
8
6
4
2
0
***
***
ACBP GFAP WT
ACBP GFAP KO
Oleate
I
HPB
Oleate
24h Food intake (g)
10
8
6
4
2
0
*
ACBP GFAP WT
ACBP GFAP KO

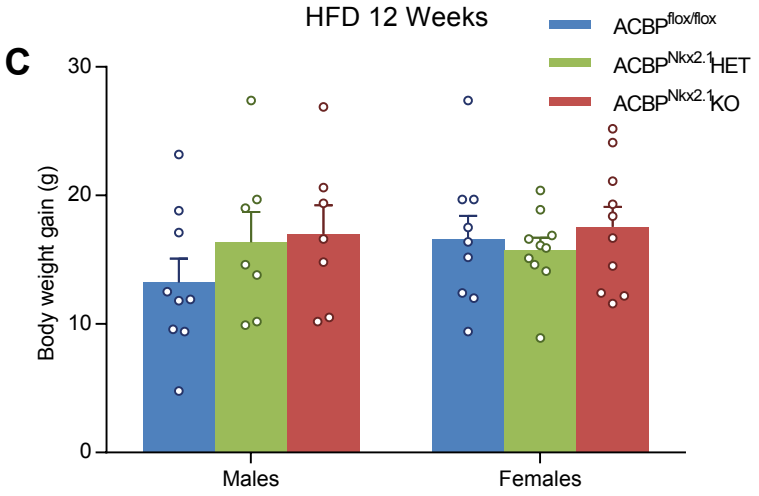
Supplemental Figure 3: Pan-brain astroglial ACBP invalidation in male and female mice on a high-fat diet. A and **D-** RER, **B** and **E-** locomotor activity and, **C** and **F-** energy expenditure (normalized to lean mass) in male (N=8-15) **(A-C)** or female (N=6-7) **(D-F)** $ACBP^{GFAP}$ WT, HET and KO mice (backcrossed BL/6J) fed with a HFD during 16 weeks. **G-** Weight of intraperitoneal and subcutaneous fat pads of $ACBP^{GFAP}$ WT, HET and KO female mice, N=6-7 (backcrossed BL/6J). **H-** Body weight gain of $ACBP^{flox/flox}$, $ACBP^{GFAP}$ HET and KO female mice on a mixed BL/6J-Bom genetic background fed with a HFD during 12 weeks. * $p<0.05$, ** $p<0.01$ compared to control littermates, One-Way ANOVA with Bonferroni post hoc test **(A-G)**. ** $p<0.01$ Two-way ANOVA with Bonferroni post hoc test compared to controls, N=6-9 **(H)**.

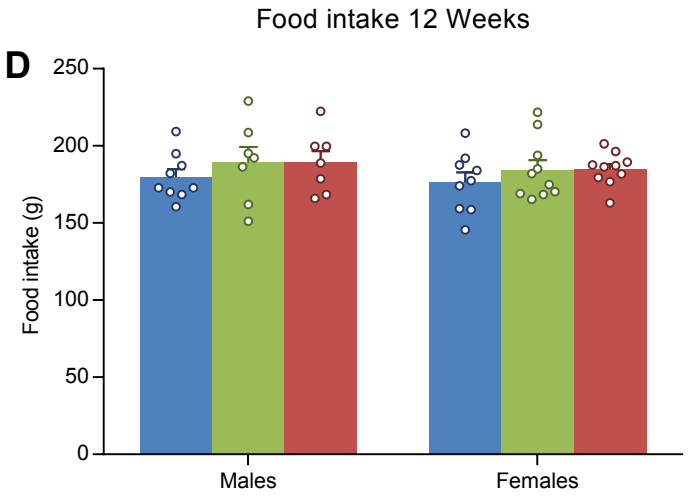

A
RER
ACBP^GFAP^WT
ACBP^GFAP^HET
ACBP^GFAP^KO
1.0
0.9
0.8
0.7
Dark
Light
B
Activity
Beam break
60K
40K
20K
0K
Dark
Light
C
Energy expenditure
Kcal/g lean mass
0.4
0.3
0.2
0.1
0.0
Dark
Light
D
RER
1.0
0.9
0.8
0.7
*
*
Dark
Light
E
Activity
Beam break
90K
60K
30K
0K
**
Dark
Light
F
Energy expenditure
Kcal/g lean mass
0.4
0.3
0.2
0.1
0.0
Dark
Light
G
Fat depot weight (g)
4
3
2
1
0
*
Intraperitoneal
Subcutaneous
H
Body weight gain (g)
20
15
10
5
0
ACBP^flox/flox
ACBP^GFAP^HET
ACBP^GFAP^KO
**
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
Days on HFD

Supplemental Figure 4: ACBP invalidation in Nkx2.1 neural cells. A- Immunostaining of ACBP (green) and GFAP (red) in the hypothalamus of *ACBP*$^{flox/flox}$ (WT) and ACBP$^{Nkx2.1}$ KO male mice. **B-** *Acbp* expression measured by qPCR in ARC microdissections from *ACBP*$^{flox/flox}$, ACBP$^{Nkx2.1}$ HET and KO mice. *** $p<0.001$ compared to controls, One-Way ANOVA with Bonferroni post hoc test, N=6. **C-** Body weight gain and **D-** cumulative food intake in *ACBP*$^{flox/flox}$, ACBP$^{Nkx2.1}$ HET and KO male and female mice (mixed BL/6J-Bom background) fed with a HFD during 12 weeks. N=7-9.


A
ACBP/GFAP
ACBPflox/flox
ACBPNkx2.1 KO

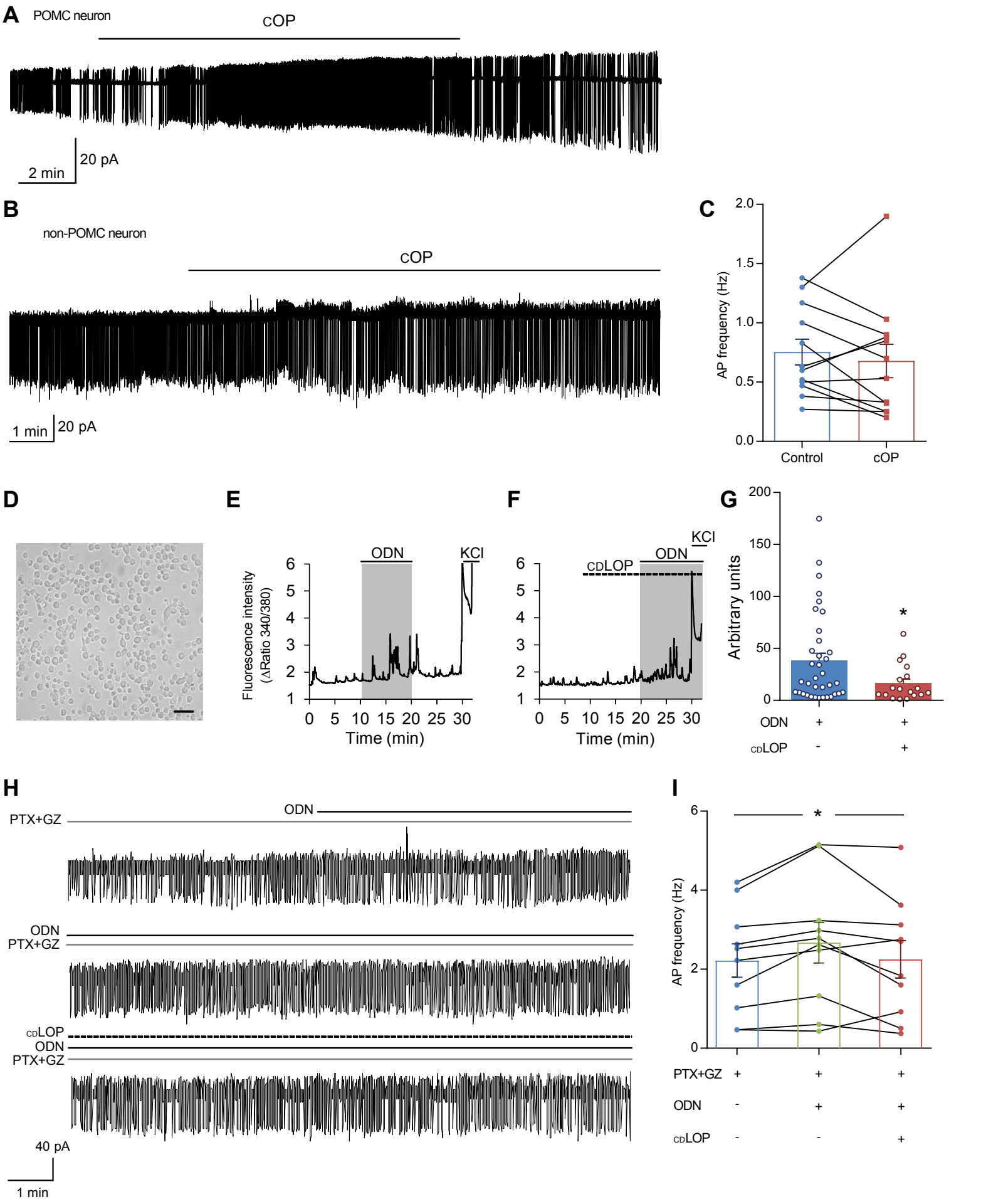

B
ACBP mRNA
Fold expression
1.2
0.8
0.4
0.0
***
***
ARC


C
HFD 12 Weeks
ACBPflox/flox
ACBPNkx2.1 HET
ACBPNkx2.1 KO
Body weight gain (g)
30
20
10
0
Males
Females


D
Food intake 12 Weeks
Food intake (g)
250
200
150
100
50
0
Males
Females

Supplemental Figure 5: Modulation of neuronal activity by ODN and ODN GPCR. Representative cell-attached recording of **A-** POMC or **B-** non-POMC neuron in response to 2 nM $_{C}$OP. **C-** Quantification of AP frequency of non-POMC neurons in response to 2 nM COP, N=12 neurons from 3 mice. **D-** Representative bright-field image of dissociated mediobasal hypothalamic cells in culture, scale bar represents 40 µm. Representative Ca^{2+} imaging traces from dissociated neurons treated with 1 nM ODN in **E-** absence or **F-** presence of $_{CD}$LOP ODN receptor antagonist (10 nM). **G-** Quantification of ODN response intensity (AUC) with or without $_{CD}$LOP (ODN: N=36 ODN responsive / 425 total cells; ODN + $_{CD}$LOP: N=17 ODN responsive/ 455 total cells), 3 independent cultures from 6 animals, * p <0.05, Student's *t*-test. **H-** Representative trace and **I-** quantification of AP frequency of POMC neurons in the presence of $GABA_A$ inhibitors (gabazine 5 µM and picrotoxin 100 µM) in response to ODN application (1 nM) with or without $_{CD}$LOP (10 nM). * p <0.05 compared to control (GZ + PTX) and $_{CD}$LOP, One-way ANOVA repeated measures with Bonferroni post hoc test, N=10 neurons from 3 mice.

Methods: Panel **A**, POMC-neurons were recorded from POMC-eGFP mice as detailed in the method section. Non-POMC (panels **B** and **C**) and POMC neurons (panels **H** and **I**) were recorded in brain slices from 6 weeks old POMC-Cre male mice (Tg(Pomc1-cre)16Lowl/J, JAX Stock #005965, Jackson Laboratory, USA) bilaterally injected in the ARC (stereotaxic coordinates: antero-posterior −1.1 mm from bregma; lateral ± 0.3 mm; dorso-ventral −5.8 mm) with an AAV-DIO-mCherry (350 nL rAVV8-hSyn-DIO-mCherry, $3.7x10^9$ GC/µL, UNC Vector, Lot #AV4981E), 2-3 weeks before performing electrophysiological recordings as described in the method section. To decrease the chance of recording false negative cells (POMC neurons not infected by the AAV), we recorded non-fluorescent neurons (N=12) surrounded by POMC-mCherry positive neurons (panels **B** and **C**).


A
POMC neuron
cOP
2 min
20 pA
B
non-POMC neuron
cOP
1 min
20 pA
C
AP frequency (Hz)
Control
cOP
D
E
ODN
KCl
Fluorescence intensity (ΔRatio 340/380)
Time (min)
F
cDLOP
ODN
KCl
Time (min)
G
Arbitrary units
ODN
cDLOP
H
PTX+GZ
ODN
ODN
PTX+GZ
cDLOP
ODN
PTX+GZ
40 pA
1 min
I
AP frequency (Hz)
PTX+GZ
ODN
cDLOP