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Supplementary Materials for

Astrocytic lipid metabolism determines susceptibility to diet-induced obesity

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Figs. S1 to S5

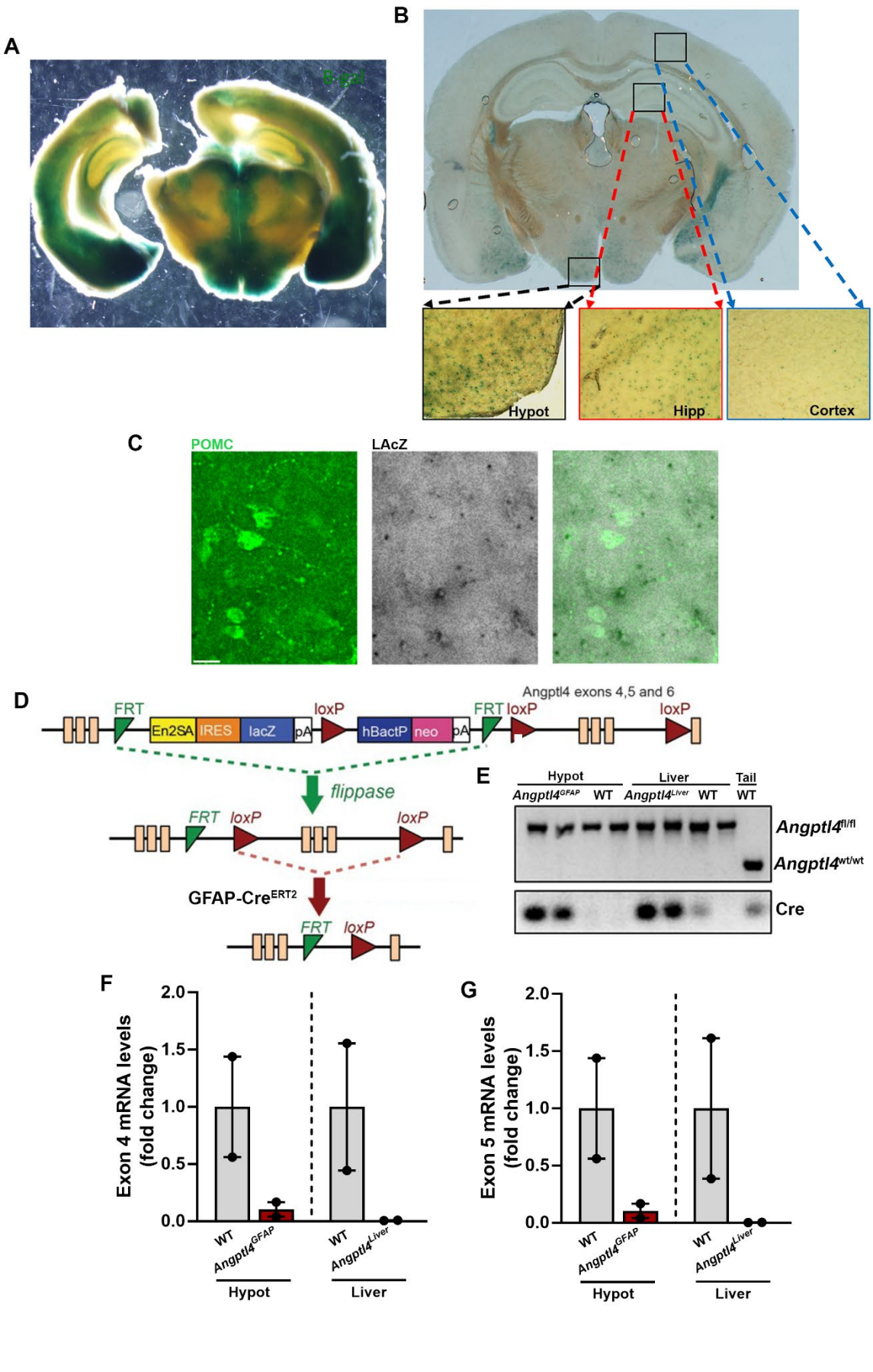
A
B-gal
B
Hypot
Hipp
Cortex
C
POMC
LAcZ
D
FRT
En2SA
IRES
lacZ
pA
loxP
hBactP
neo
pA
FRT
loxP
loxP
Angptl4 exons 4,5 and 6
flippase
FRT
loxP
loxP
GFAP-Cre^ERT2
FRT
loxP
E
Hypot
Liver
Tail
Angptl4^GFAP
WT
Angptl4^Liver
WT
WT
Angptl4^fl/fl
Angptl4^wt/wt
Cre
F
Exon 4 mRNA levels (fold change)
2.0
1.5
1.0
0.5
0.0
WT
Angptl4^GFAP
WT
Angptl4^Liver
Hypot
Liver
G
Exon 5 mRNA levels (fold change)
2.0
1.5
1.0
0.5
0.0
WT
Angptl4^GFAP
WT
Angptl4^Liver
Hypot
Liver

Fig. S1. *Angptl4* expression is downregulated in *Angptl4*^{GFAP} mice. (**A**) Low magnification image of LAcZ staining in the brain (coronal section = 300 μm). (**B**) High resolution image of LAcZ expression in the brain (coronal section=50 μm). Inserts show high magnification of three different areas of the brains (Hypot=hypothalamus, Hipp =Hippocampus). (**C**) Representative images showing lack of colocalization of POMC neurons and LacZ (scale bar = 20μm). (**D**) Schematic diagram illustrating the generation of astrocyte-specific ANGPTL4 (*Angptl4*^{GFAP}) mice. (**E**) PCR amplification of *Angptl4*$^{fl/fl}$ mice displaying bands from both or none of the alleles floxed. As control, we show the PCR amplification bands from the liver of our previously reported mice loosing *Angptl4* specifically in liver (*Angptl4*^{Liver}). mRNA expression of *Angptl4* (**F**) exon 4 and (**G**) exon 5 from hypothalamus of WT and *Angptl4*^{GFAP}. As a positive control, we also show the mRNA expression in livers from our previously described *Angptl4*^{Liver} mice.

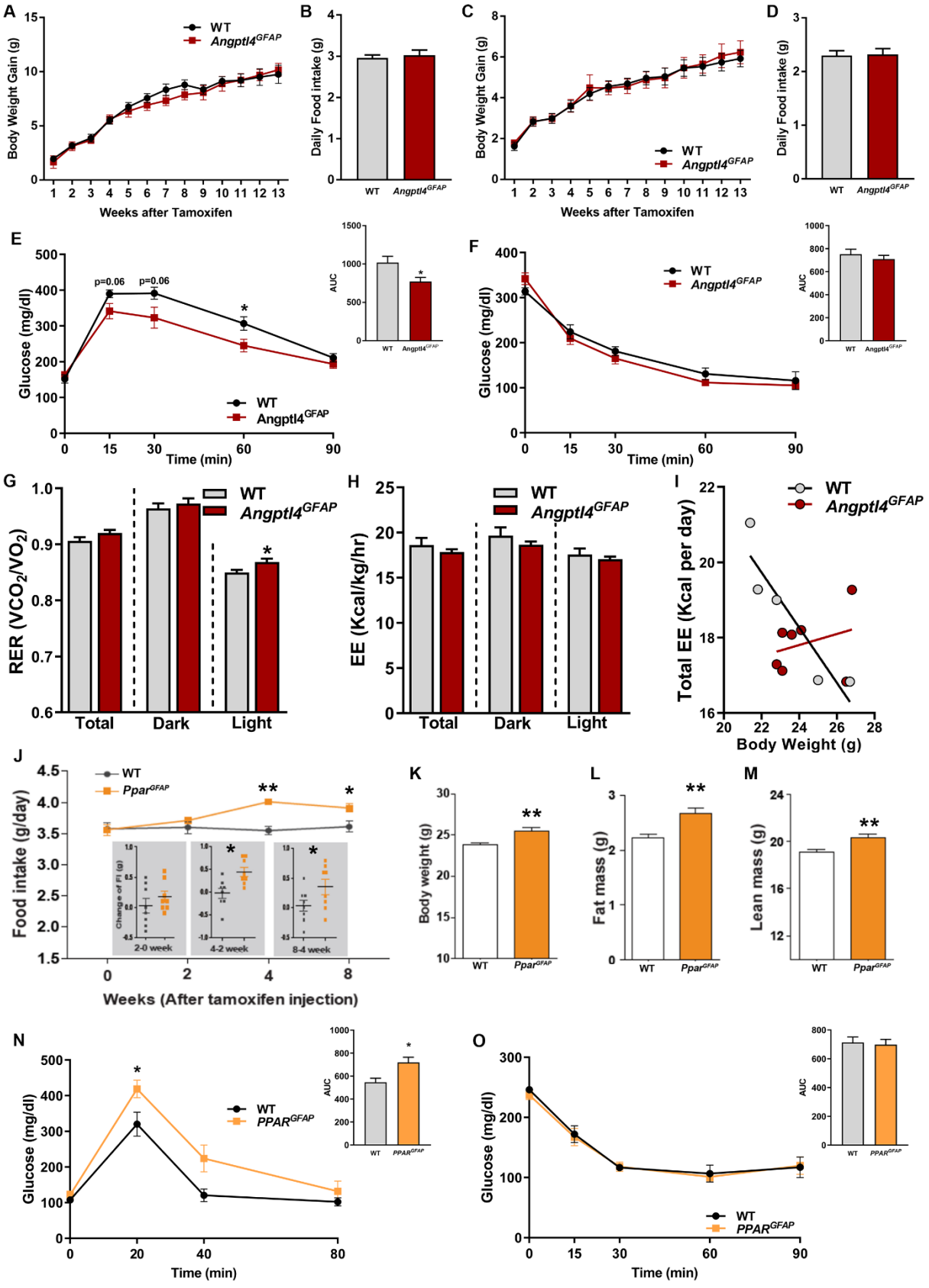
A
Body Weight Gain (g)
WT
Angptl4GFAP
Weeks after Tamoxifen
B
Daily Food intake (g)
C
Body Weight Gain (g)
D
Daily Food intake (g)
E
Glucose (mg/dl)
p=0.06
p=0.06
Time (min)
AUC
F
Glucose (mg/dl)
Time (min)
AUC
G
RER (VCO2/VO2)
Total
Dark
Light
H
EE (Kcal/kg/hr)
I
Total EE (Kcal per day)
Body Weight (g)
J
Food intake (g/day)
PparGFAP
2-0 week
4-2 week
8-4 week
Weeks (After tamoxifen injection)
K
Body weight (g)
L
Fat mass (g)
M
Lean mass (g)
N
Glucose (mg/dl)
PPARGFAP
Time (min)
AUC
O
Glucose (mg/dl)
Time (min)
AUC

Fig. S2. Metabolic characterization of *Angptl4*^{GFAP} and *Ppar*^{GFAP} mice on chow diet. Body weight gain of *Angptl4*^{GFAP} (**A**) male and (**C**) female mice and their control littermates eating a chow diet (CD). Daily food intake of *Angptl4*^{GFAP} (**B**) male and (**D**) female mice and their controls eating a CD (males: n = 10/9 mice per group; females: 10/9 mice per group). (**E**) Glucose tolerance test (GTT) and AUC, and (**F**) Insulin tolerance test (ITT) and AUC in *Angptl4*^{GFAP} mice and their control littermates on CD (n = 9 mice per group). (**G**) RER and (**H** and **I**) Energy Expenditure of *Angptl4*^{GFAP} mice and their control littermates on CD (n = 5/7 mice per group). (**J**) Weekly food intake of *Ppar*^{GFAP} mice and their control littermates fed a CD. (**K**) Body weight, (**L**) Fat mass and (**M**) Lean mass of *Ppar*^{GFAP} mice and their control littermates fed a CD (n = 8 mice per group). (**N**) Glucose tolerance test and AUC, and (**O**) Insulin tolerance test and AUC in *Ppar*^{GFAP} mice and their control littermates fed a CD (n = 4/6 mice per group respectively). Data are presented as mean ± SEM. *$p \leq 0.05$ and **$p \leq 0.01$ as determined by two-tailed t-test.

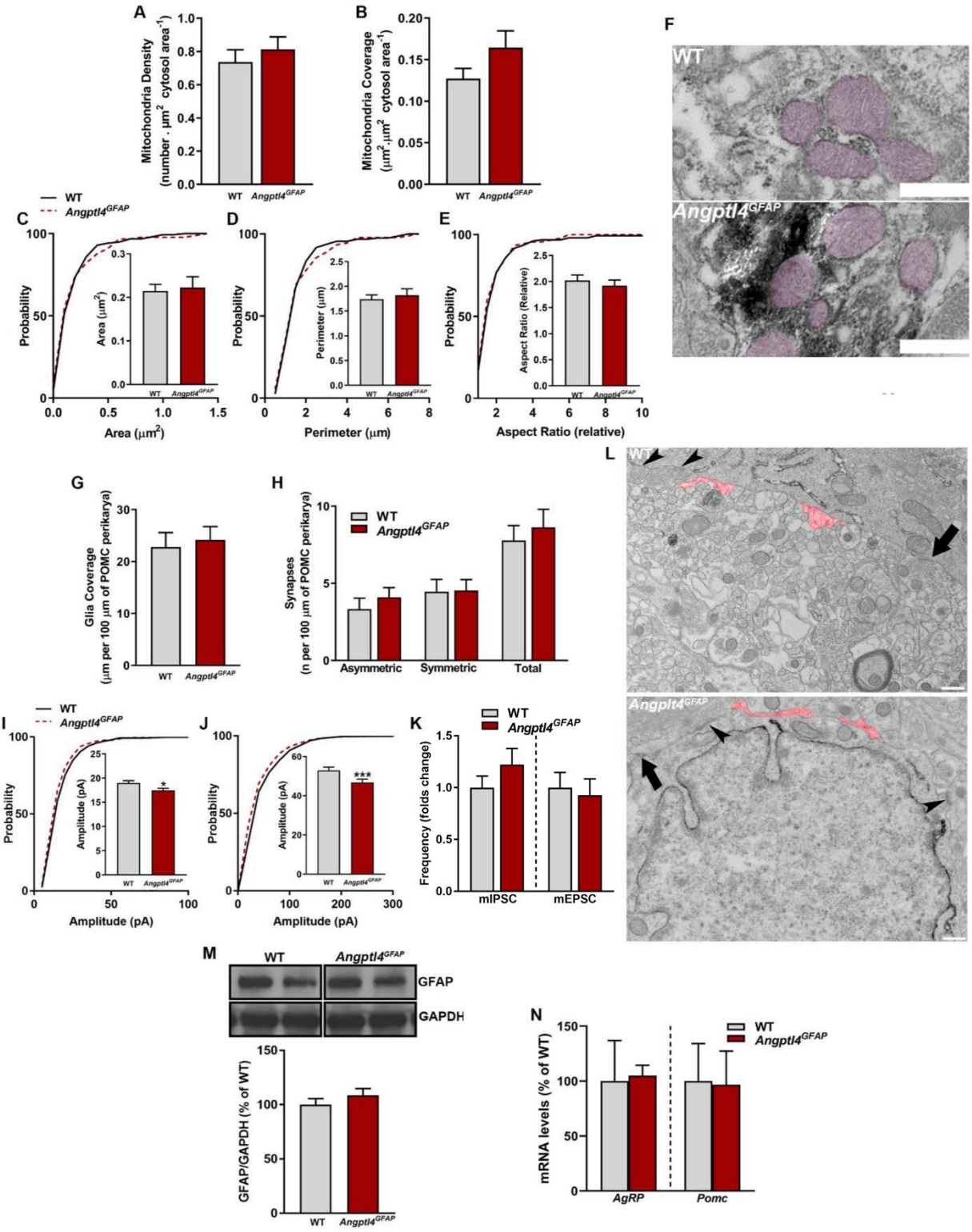
A
Mitochondria Density (number . µm2 cytosol area-1)
WT
Angptl4GFAP
B
Mitochondria Coverage (µm2.µm2 cytosol area-1)
F
WT
Angptl4GFAP
C
D
E
Probability
Area (µm2)
Perimeter (µm)
Aspect Ratio (Relative)
G
Glia Coverage (µm per 100 µm of POMC perikarya)
H
Synapses (n per 100 µm of POMC perikarya)
Asymmetric
Symmetric
Total
L
I
J
K
Amplitude (pA)
Frequency (folds change)
mIPSC
mEPSC
M
GFAP
GAPDH
GFAP/GAPDH (% of WT)
N
mRNA levels (% of WT)
AgRP
Pomc

Fig. S3. Astrocytic Mitochondria morphology and synaptic events onto POMC neurons on *Angptl4^{GFAP}* mice on chow diet. Mitochondria (**A**) density and (**B**) coverage from MBH astrocytes of *Angptl4^{GFAP}* mice and their control littermates fed a chow diet (CD). Cumulative Distribution and mean of mitochondria (**C**) area, (**D**) perimeter and (**E**) aspect ratio from MBH astrocytes of *Angptl4^{GFAP}* mice and their control littermates fed a CD (n >90 mitochondria from >12 astrocytes from 4/5 mice per group respectively). (**F**) Representative electron micrographs from MBH astrocytes of *Angptl4^{GFAP}* mice and their control littermates fed a CD (scale bar=500nm). (**G**) Glia Coverage and (**H**) Number of synapses per 100µm of POMC perikarya of *Angptl4^{GFAP}* mice and their controls fed a CD (n = 15/21 neurons per group). Electrophysiological recordings showing the amplitude of (**I**) mIPSC and (**J**) mEPSC, and (**K**) frequency of mIPSC and mEPSC from POMC neurons of ANGPTL4^{GFAP} mice and their control littermates fed a chow diet (n = 18 cells from 4 mice per group). (**L**) Representative electron micrographs showing glia coverage and synaptic events onto the POMC perikarya of *Angptl4^{GFAP}* mice and their control littermates fed a chow diet. Red trace illustrates glia coverage. Arrowheads depict symmetric synapses. Arrows depict asymmetric synapses. (Scale bar=500nm). (**M**) Quantification and representative blots (from the same film) showing the levels of GFAP levels in the MBH of *Angptl4^{GFAP}* mice and their controls on chow diet (n = 5 mice per group respectively). (**N**) *AgRP* and *Pomc* mRNa levels in MBH of *Angptl4^{GFAP}* mice and their controls on CD. Data are presented as mean ± SEM. ***p≤ 0.001 as determined by two-tailed t-test or Kolmogorov-Smirnov test for analyses of cumulative distribution.

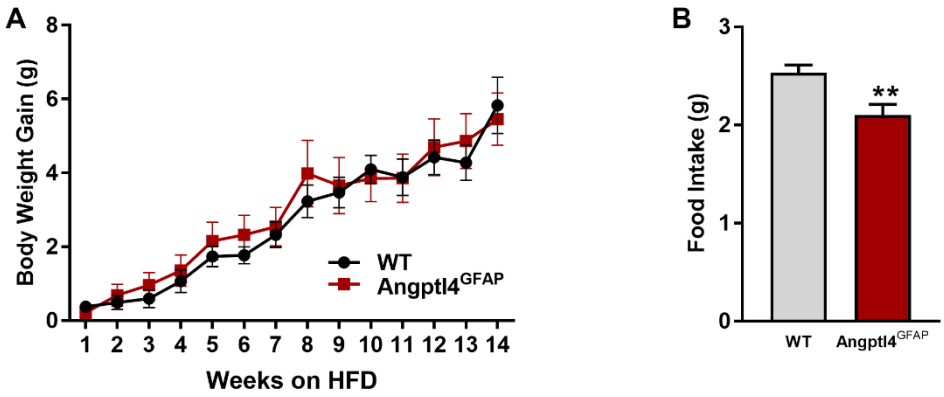

Fig S4. Metabolic phenotype of *Angptl4*^{GFAP} female mice during HFD treatment. (**A**) Weekly Body weight gain and (**B**) daily food intake of *Angptl4*^{GFAP} female mice and their control littermates during chronic exposure to HFD. Data are presented as mean ± SEM. **p≤ 0.01 as determined by two-tailed t-test

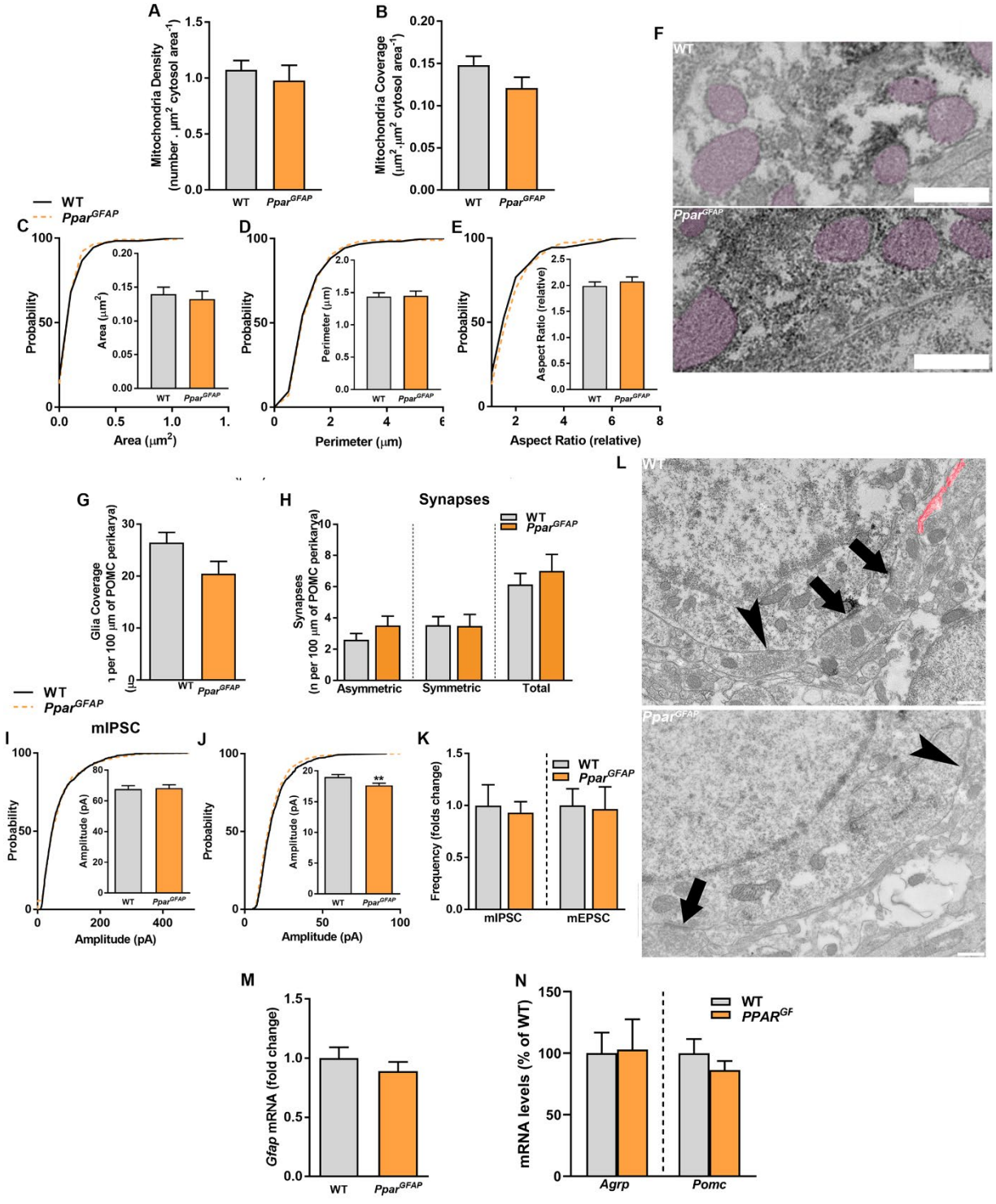
A
Mitochondria Density (number . µm² cytosol area⁻¹)
WT
PparGFAP
B
Mitochondria Coverage (µm² . µm² cytosol area⁻¹)
F
WT
PparGFAP
C
Probability
Area (µm²)
D
Perimeter (µm)
E
Aspect Ratio (relative)
G
Glia Coverage per 100 µm of POMC perikarya
H
Synapses
Synapses (n per 100 µm of POMC perikarya)
Asymmetric
Symmetric
Total
L
I
mIPSC
Amplitude (pA)
J
K
Frequency (folds change)
mIPSC
mEPSC
M
Gfap mRNA (fold change)
N
mRNA levels (% of WT)
Agrp
Pomc
PPARGF

Fig. S5. Astrocytic Mitochondria morphology and synaptic events onto POMC neurons on *Ppar*^{GFAP} mice on chow diet. Mitochondria (**A**) density and (**B**) coverage from MBH astrocytes of *Ppar*^{GFAP} mice and their control littermates fed a chow diet (CD). Cumulative Distribution and mean of mitochondria (**C**) area, (**D**) perimeter and (**E**) aspect ratio from MBH astrocytes of *Ppar*^{GFAP} mice and their control littermates fed a CD (n > 114 mitochondria from >13 astrocytes from 4/3 mice per group respectively). (**F**) Representative electron micrographs from MBH astrocytes of *Ppar*^{GFAP} mice and their control littermates fed a CD (scale bar=500nm). (**G**) Glia Coverage and (**H**) Number of synapses per 100µm of POMC perikarya of *Ppar*^{GFAP} mice and their controls fed a CD (n = 24/18 neurons from 5/4 mice per group respectively). Electrophysiological recordings showing the amplitude of (**I**) mIPSC and (**J**) mEPSC, and (**K**) frequency of mIPSC and mEPSC from POMC neurons of *Ppar*^{GFAP} mice and their control littermates fed a chow diet (n = 16/15 neurons from 3 mice per group). (**L**) Representative electron micrographs showing glia coverage and synaptic events onto the POMC perikarya of *Ppar*^{GFAP} mice and their control littermates fed a chow diet. Red trace illustrates glia coverage. Arrowheads depict symmetric synapses. Arrows depict asymmetric synapses. (Scale bar=500nm). (**M**) *Gfap* mRNA levels in the MBH of *Ppar*^{GFAP} mice and their controls on CD (n = 4/5 mice per group respectively). (**N**) *AgRP* and *Pomc* mRNA levels in MBH of *Ppar*^{GFAP} mice and their controls on CD. Data are presented as mean ± SEM. **p≤ 0.01 as determined by two-tailed t-test or Kolmogorov-Smirnov test for analyses of cumulative distribution.