**Title:**

AMP-activated protein kinase modulates tau phosphorylation and tau pathology *in vivo*

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**Figure S1. p-Thr$^{172}$AMPK staining specificity**

**a.** Primary neurons at 15 DIV were treated with the AMPK activator AICAR (1mM, 6 h) and labeled with p-Thr$^{172}$AMPK (pAMPK) antibody using identical acquisition settings. **b.** Immunofluorescence staining of p-Thr$^{172}$AMPK without (top) or with (bottom) p-Thr$^{172}$AMPK blocking peptide in primary neurons at DIV 15 using identical acquisition settings. Scale bars = 50 µm.

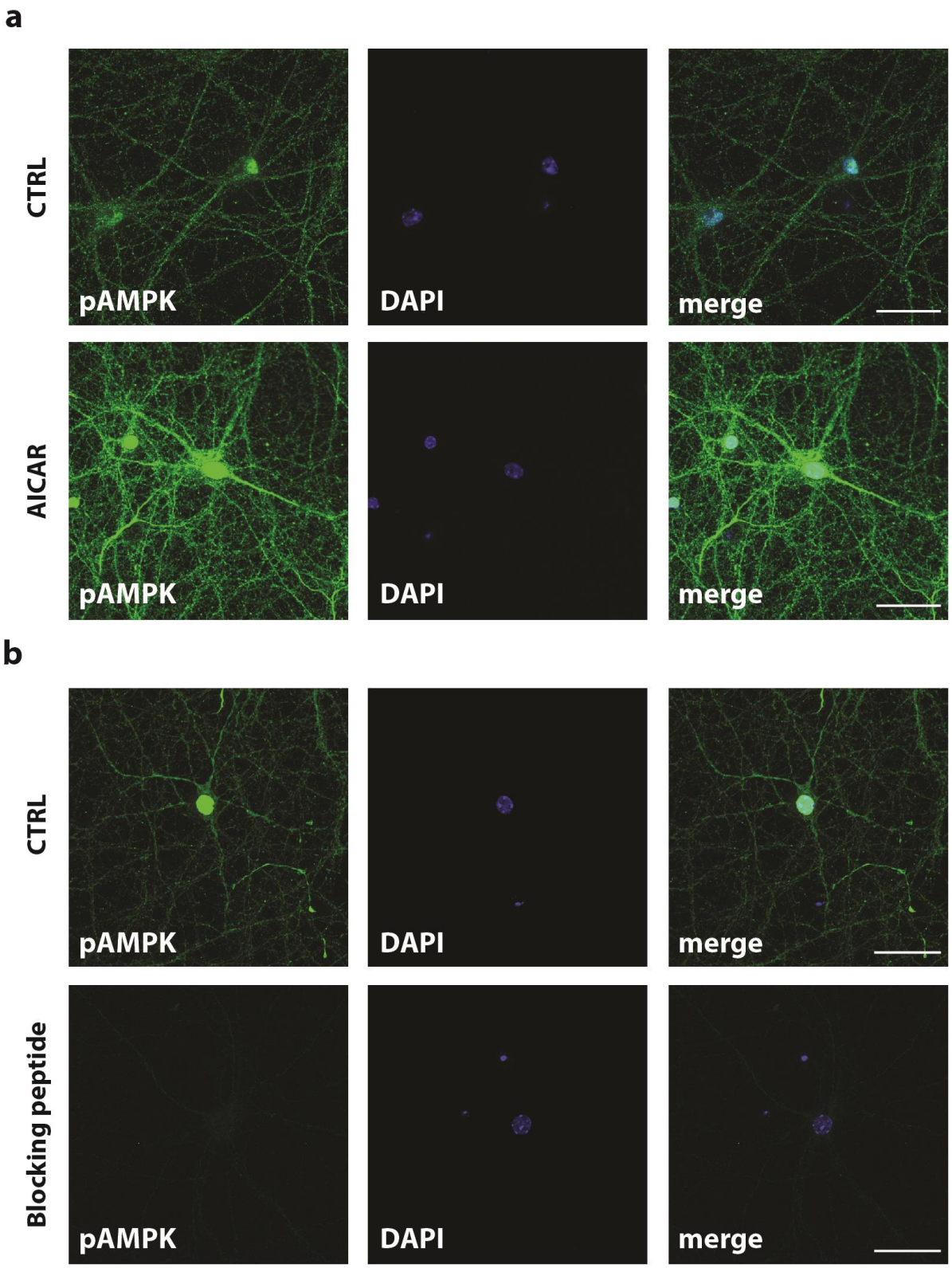
**Figure S2. Metformin-mediated AMPK activation induces tau phosphorylation in primary neurons**

Primary neurons at 15 DIV were treated for the indicated times with metformin (2.5 mM). AMPK and ACC expression and activation were monitored by WB using antibodies against AMPK, p-Thr$^{172}$AMPK (pAMPK), ACC, p-Ser$^{79}$ACC (pACC) and actin (**a**). Quantifications of the ratios AMPK/actin (**b**) pAMPK/AMPK (**c**) and pACC/ACC (**d**). WB analysis (**e**) and quantification of total tau (**f**) and phosphorylated tau at epitopes Ser$^{262/356}$ (**g**), Thr$^{231}$ (**h**), Ser$^{396/404}$ (**i**) and Ser$^{202}$ (**j**) expressed in ratios. LDH cytotoxicity assay following 6 h and 24 h metformin treatments (**k**). Results represent mean ± SD, n=4-6. a.u., arbitrary units. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to Ctrl, One way ANOVA with Bonferroni's post hoc test.

**Figure S3. Western-blot analysis of tau in heat-stable, soluble and insoluble fractions**

WB analysis of total tau and phosphorylated tau at epitopes Thr$^{231}$, Ser$^{396/404}$ and Ser$^{202}$ in heat stable (**a**), soluble (**b**), and insoluble (**c**) fractions of 8-months old AMPK$\alpha2^{+/+}$:tau$^{P301S}$ (Tg) and AMPK$\alpha2^{-/-}$:tau$^{P301S}$ (Tg x $\alpha$2 KO) mouse brains.

## Figure S1


a
CTRL
pAMPK
DAPI
merge
AICAR
pAMPK
DAPI
merge
b
CTRL
pAMPK
DAPI
merge
Blocking peptide
pAMPK
DAPI
merge

## Figure S2

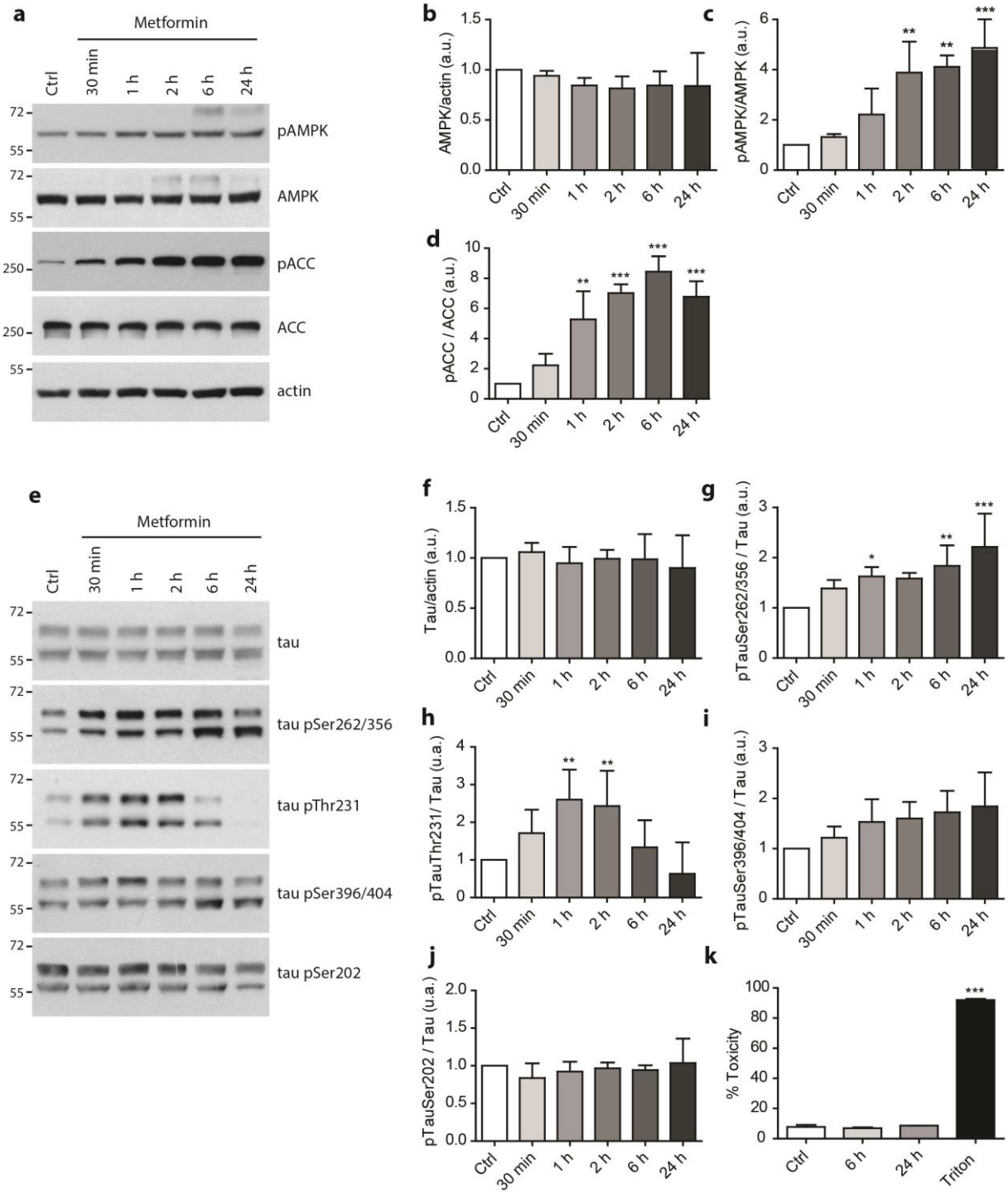
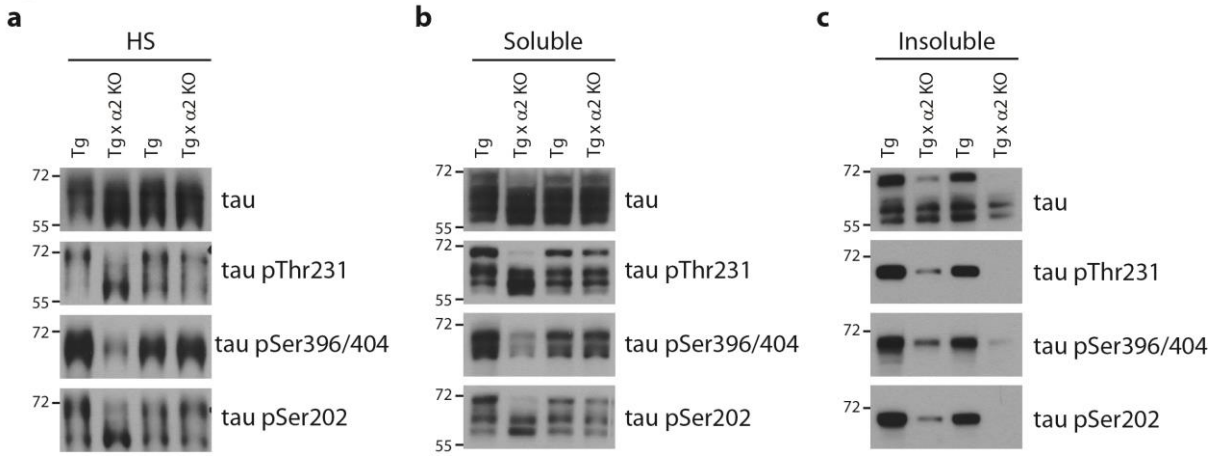

a
Metformin
Ctrl
30 min
1 h
2 h
6 h
24 h
72
55
pAMPK
AMPK
250
pACC
ACC
actin
b
AMPK/actin (a.u.)
c
pAMPK/AMPK (a.u.)
d
pACC / ACC (a.u.)
e
tau
tau pSer262/356
tau pThr231
tau pSer396/404
tau pSer202
f
Tau/actin (a.u.)
g
pTauSer262/356 / Tau (a.u.)
h
pTauThr231/ Tau (u.a.)
i
pTauSer396/404 / Tau (a.u.)
j
pTauSer202 / Tau (u.a.)
k
% Toxicity
Triton

**Figure S3**


a
HS
Tg
Tg x α2 KO
Tg
Tg x α2 KO
72
55
tau
tau pThr231
tau pSer396/404
tau pSer202
b
Soluble
c
Insoluble