Cell Metabolism, *Volume 16*

Supplemental Information

p70S6 Kinase Phosphorylates AMPK on Serine 491 to Mediate Leptin's Effect on Food Intake

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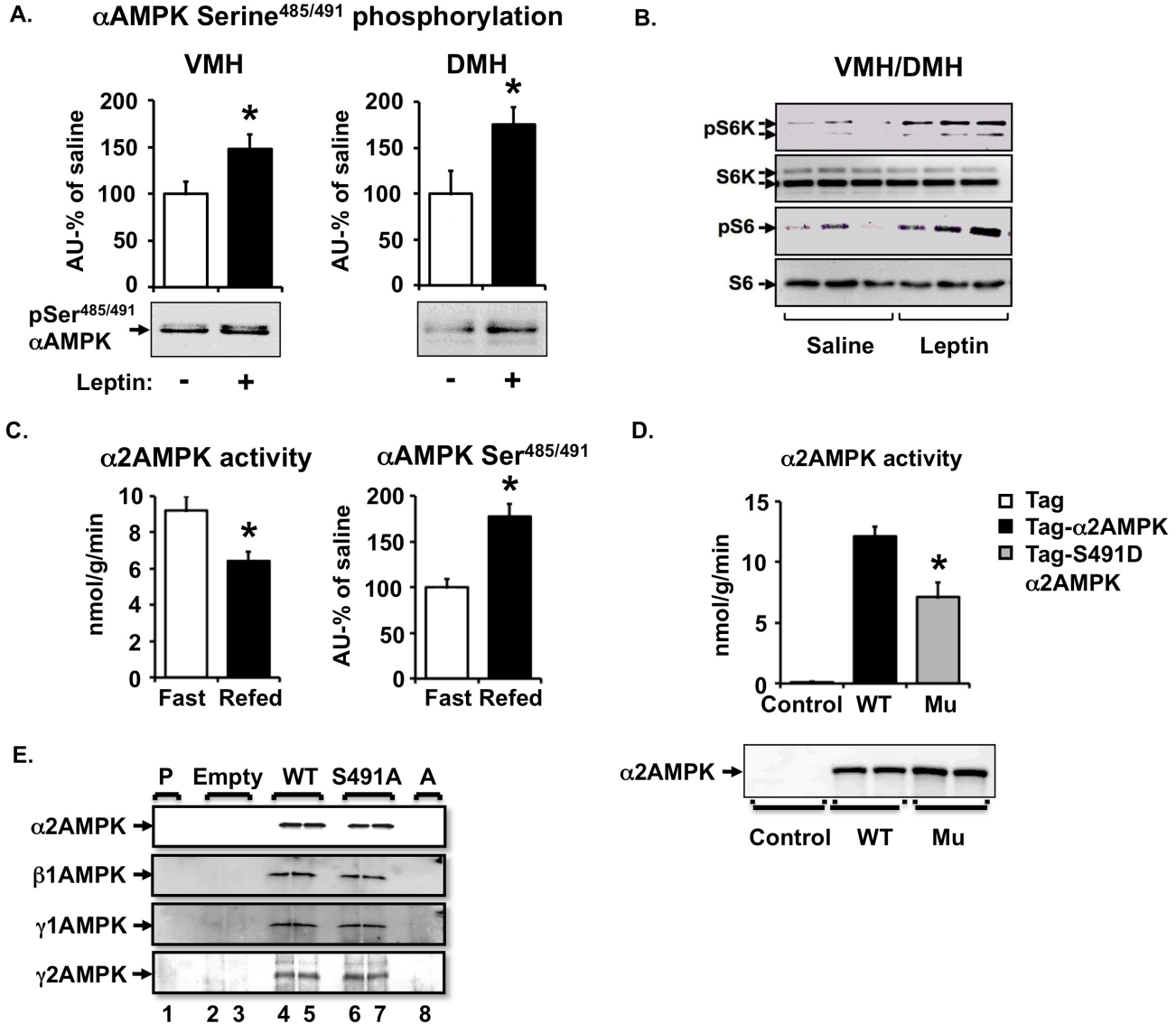

Figure S1. Related to Figure 1

A. αAMPK serine$^{485/491}$/α2AMPK was analyzed in VMH and DMH after saline or leptin injection (20 ng i.c.v.; 3h) in overnight fasted mice (n=6/group). *p<0.05 versus saline.

B. p70S6K and S6 phosphorylation in VMH/DMH after saline or leptin injection (20 ng i.c.v.; 3h) in overnight fasted mice used in Fig. 1A (n=3/group).
C. Mice were fasted overnight (white bars) and refed for 6 hours (black bars). ARC extracts were analyzed for αAMPK serine$^{485/491}$/α2AMPK and α2AMPK activity (n=6-8/group). *p<0.05 versus saline.
D. GT1-7 neurons were transfected with Flag-empty (Tag), Flag-WT α2AMPK (Tag-α2AMPK) or Flag-S491D-α2AMPK (Tag-S491D α2AMPK) for 24 hours. Serum was removed overnight followed by glucose removal for 6 hours. Cell extracts were first analyzed for α2AMPK expression (bottom panel), then immunoprecipitated with Flag antibody and analyzed for α2AMPK activity corrected for α2AMPK protein levels (n=6/group). *p<0.05 versus all other groups.
E. GT1-7 neurons were transfected with Flag-empty (lanes 2-3), Flag-WT (lanes 4-5) or Flag-S491A-α2AMPK (lanes 6-7) for 24 hours. Cell extracts were immunoprecipitated with Flag antibody. Controls have: (P) protein only, with no antibody (lane 1) or (A) antibody only, no cell extract (lane 8). α2AMPK, β1AMPK, γ1AMPK and γ2AMPK subunits were detected.
Data in all bat graphs are shown as means +/- SEM.

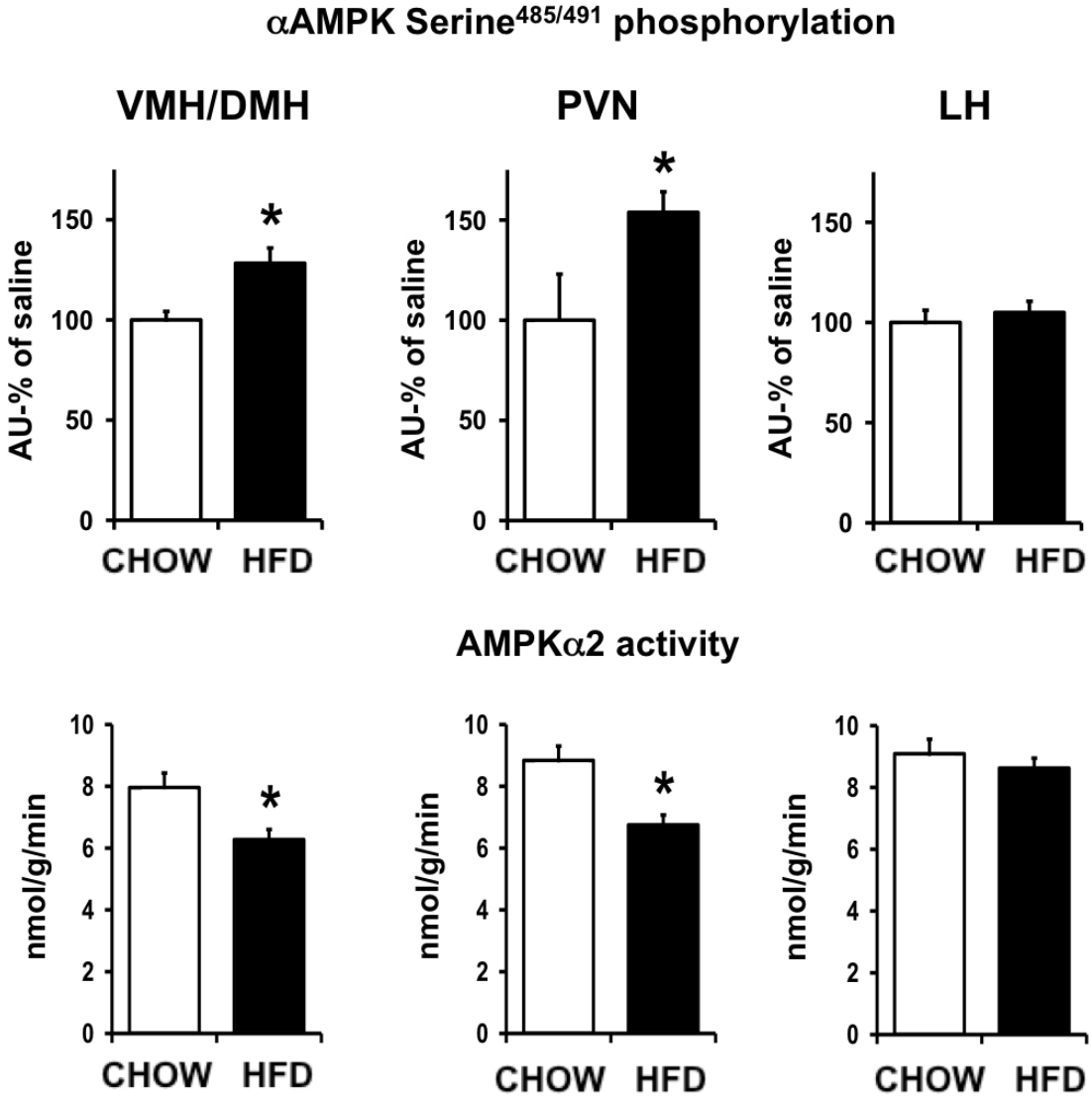

Figure S2. Related to Figure 2

Mice were fed chow (white bars) or a high fat diet (HFD, black bars) for 6 months and analyzed for αAMPK serine$^{485/491}$/α2AMPK and α2AMPK activity in the hypothalamus (n=7-9/group). *p<0.05 versus saline.

Data are shown as means +/- SEM.

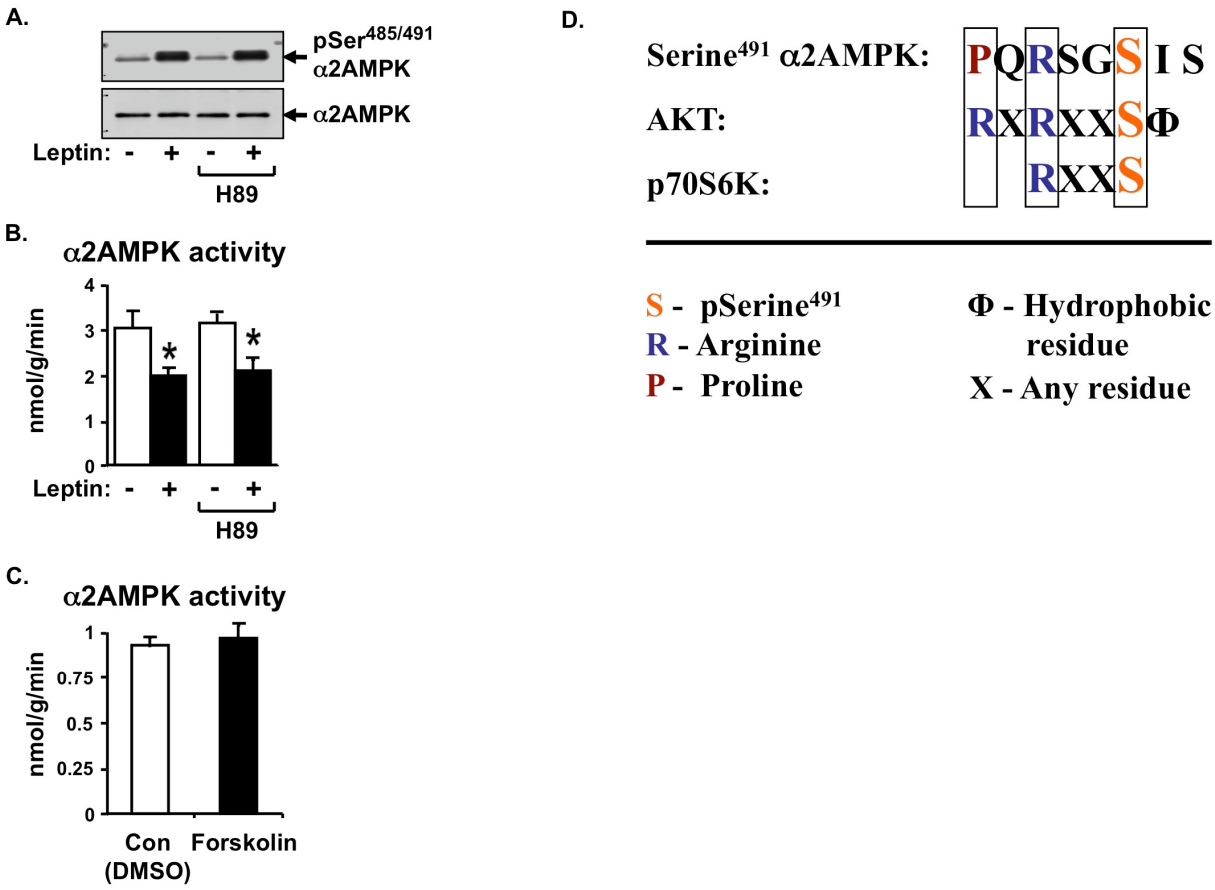

Figure S3. Related to Figure 3

PKA is not involved in leptin dependent or independent regulation of AMPK serine$^{485/491}$ phosphorylation and inhibition of α2AMPK activity in GT1-7 neurons.

GT1-7 neurons were treated with vehicle (H_20) or PKA inhibitor H89 (1h) followed by 0.5 μg/ml leptin (+) or vehicle (-) for 2h in DMEM medium with 5 mM glucose. Cell extracts were (A) immunoblotted for pSerine$^{485/491}$ and total α2AMPK or (B) analyzed for α2AMPK activity. *p<0.05 versus vehicle. C. GT1-7 neurons were treated with vehicle (DMSO) or forskolin (10 μM) for 1h in DMEM medium with 5 mM glucose and analyzed for α2AMPK activity. Data in all panels are shown as means +/- SEM.

D. Lineup of Serine^{491} α2AMPK phosphorylation site with the minimal motifs required for phosphorylation by AKT and p70S6K.

Proline residue in position -5 renders Serine^{491} α2AMPK phosphorylation site unfavorable for direct phosphorylation by AKT but acceptable for p70S6K.

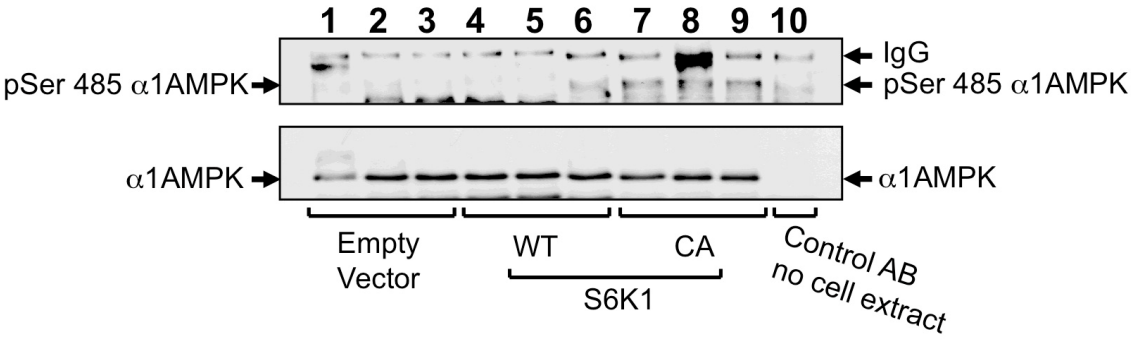

Figure S4. Related to Figure 4
GT1-7 neurons were transfected with Empty (lanes 1-3), WT-S6K1 (lanes 4-6) or CA-S6K1 (7-9) vectors for 24h and serum starved overnight in DMEM medium with 5 mM glucose. Cell extracts were immunoprecipitated with α1AMPK antibody (Upstate Biotechnology) and analyzed for pSerine^{485} and α1AMPK.