Supplementary File 1 (Figure S1)

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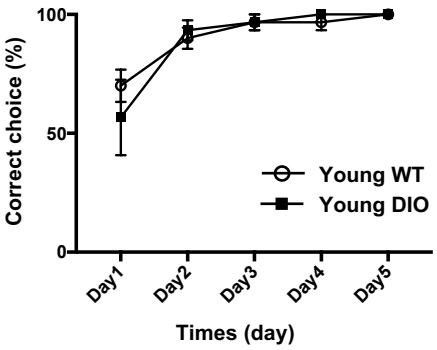
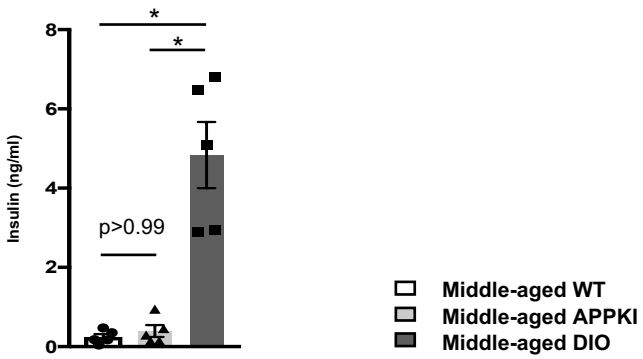


Supplementary File 1 (Figure S1) No difference in memory function between wild-type (WT) and DIO mice at a young age. (A) The water T-maze test for assessing learning and memory function in young WT and DIO mice (12 weeks of age, n = 6 mice per group).

Supplementary File 2 (Figure S2)

A



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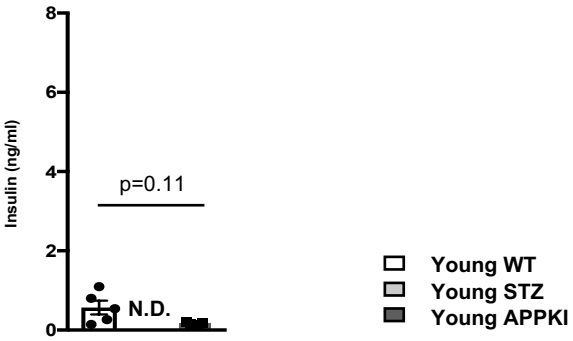

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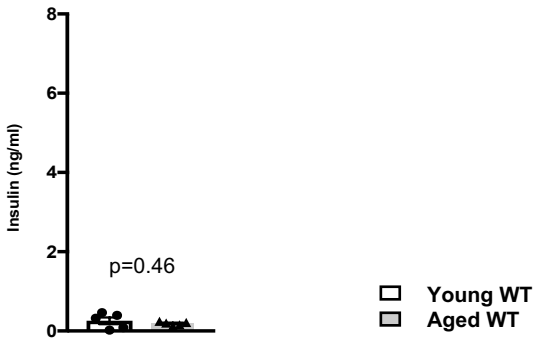
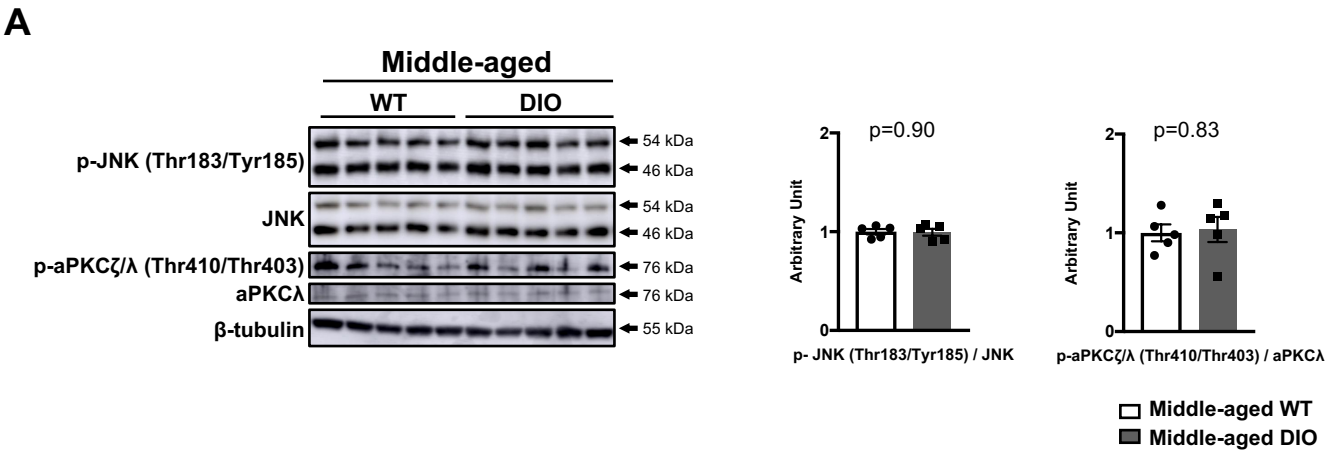
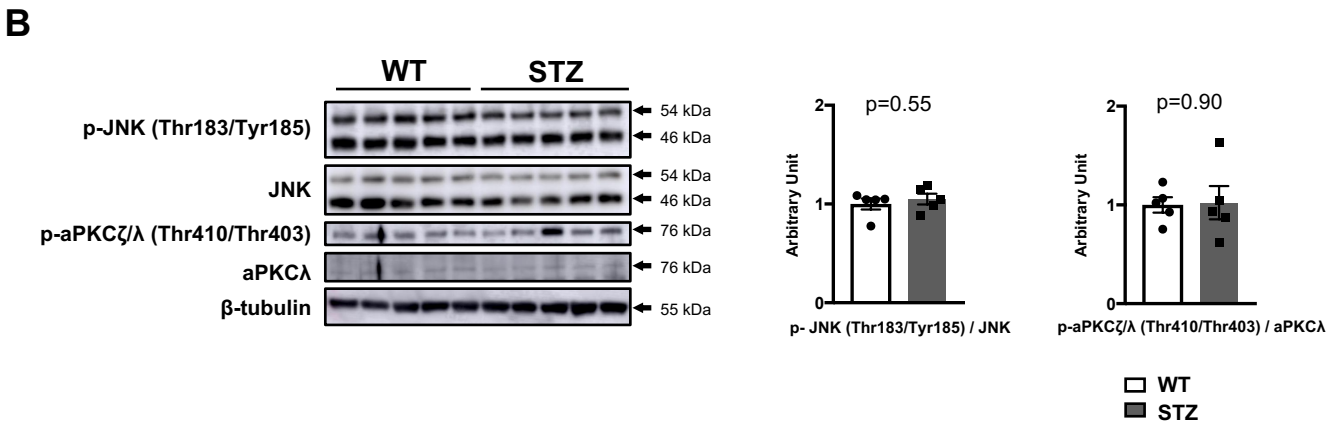
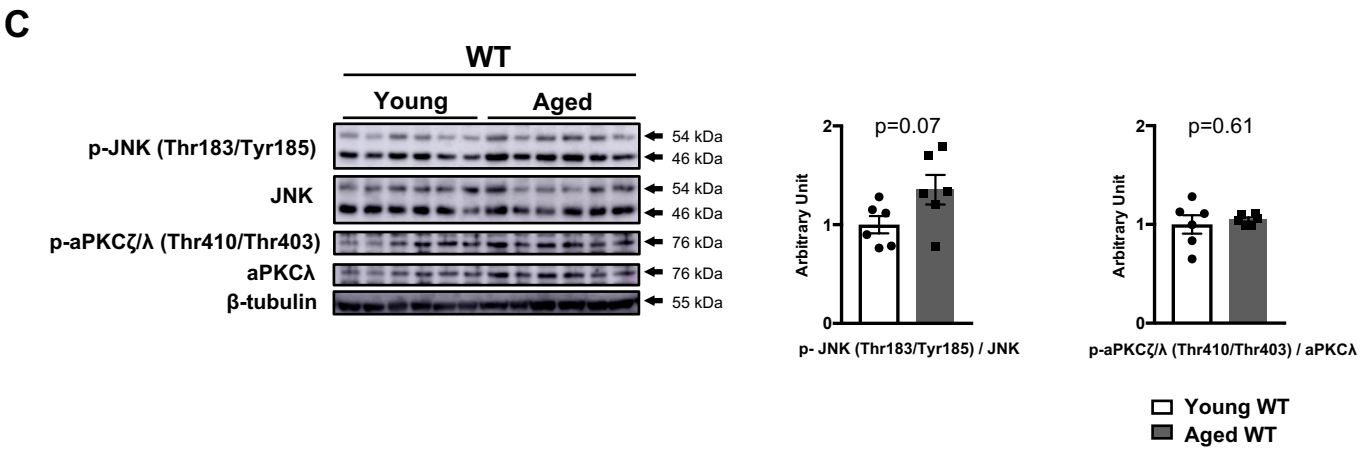


Supplementary File 2 (Figure S2). Levels of blood insulin in the respective mouse models. (A) Quantitative comparison of 3-6 h fasting blood insulin levels of middle-aged wild type (WT) (34–36 weeks of age, n = 5 mice per group), APPKI^{NL-G-F} (34–36 weeks of age, n = 5 mice per group), and DIO mice (34–36 weeks of age, n = 5 mice per group). (B) Quantitative comparison of 3-6 h fasting blood insulin levels of young WT (10 weeks of age, n = 5 mice per group), STZ (10 weeks of age, n = 4 mice per group), and APPKI^{NL-G-F} mice (12 weeks of age, n = 5 mice per group). (C) Quantitative comparison of 3-6 h fasting blood insulin levels of young (12 weeks of age, n = 5 mice per group) and aged (84 weeks of age, n = 5 mice per group) WT mice. Results are presented as mean ± SEM, * $p < 0.05$; ** $p < 0.01$.

Supplementary File 3 (Figure S3)







Supplementary File 3 (Figure S3) (continued)

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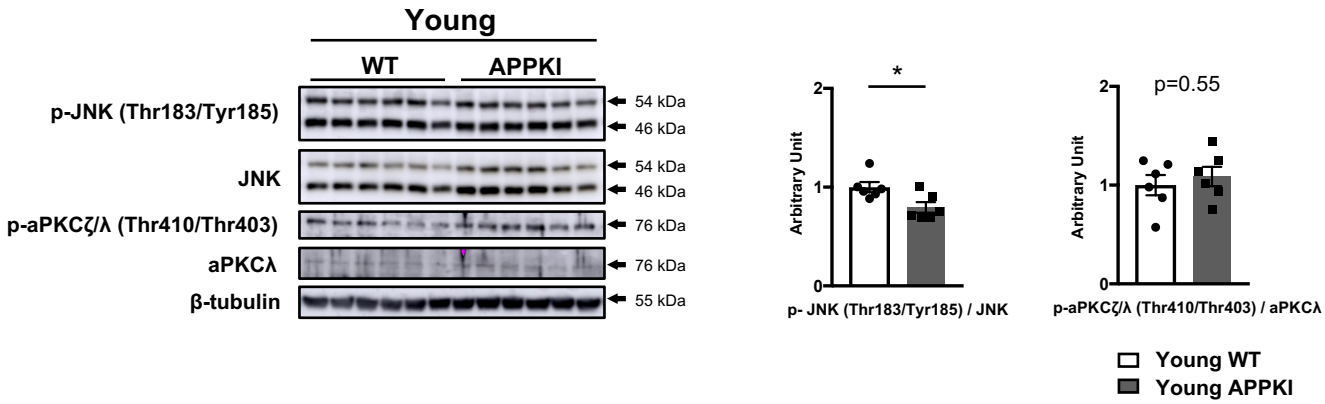

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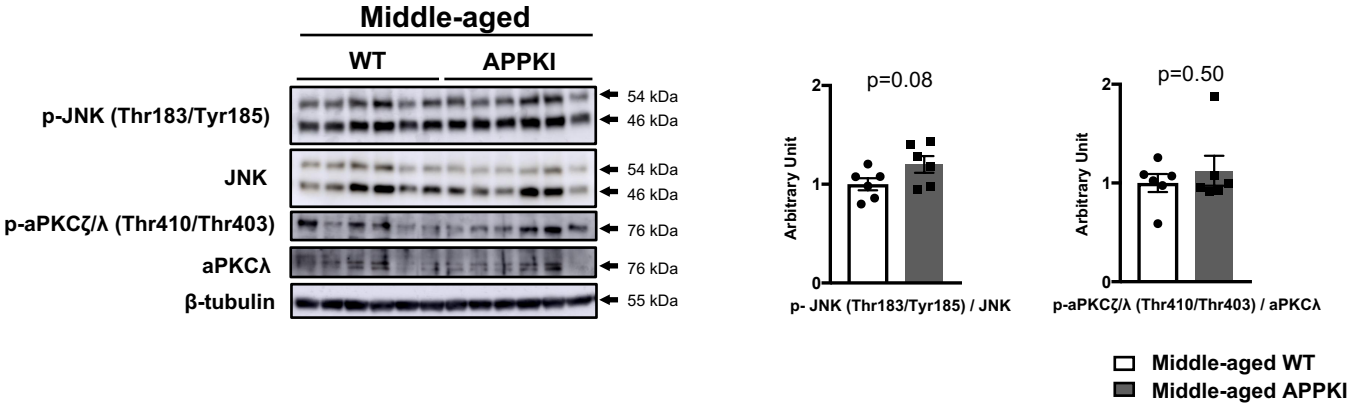
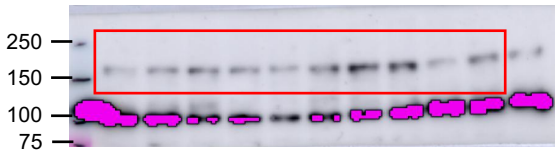


Supplementary File 3 (Figure S3). Evaluation of the other signaling factors associated with IRS1 signaling in the hippocampus. (A-E) Western blot analysis of phosphorylation levels of JNK Thr183/Tyr185, and aPKCζ/λ Thr410/Thr403 as well as total protein levels of JNK, aPKCλ, and β-tubulin in the hippocampi of middle-aged wild-type (WT) and DIO mice (35 weeks of age, n = 5 biologically independent samples per group) [A], WT and STZ mice (10 weeks of age, n = 4 biologically independent samples per group) [B], young (12 weeks of age, n = 6 biologically independent samples) and aged (84 weeks of age, n = 6 biologically independent samples) WT mice [C], young WT and $APPKI^{NL\text{-}G\text{-}F}$ mice (12 weeks of age, n = 6 biologically independent samples per group) [D], and middle-aged WT and $APPKI^{NL\text{-}G\text{-}F}$ mice (34–36 weeks of age, n = 6 biologically independent samples per group) [E]. Quantitative analysis of phosphorylation levels of JNK Thr183/Tyr185, and aPKCζ/λ Thr410/Thr403 normalized to the respective total protein contents in (A-E). Results are presented as mean ± SEM, * $p < 0.05$; ** $p < 0.01$.

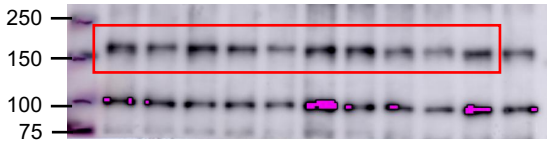
Supplementary File 4 (Figure S4)

A Figure 1E



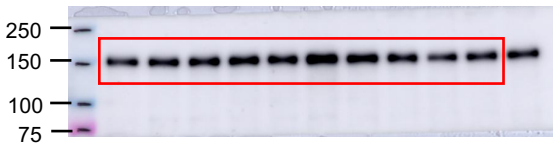
p-IRS1 (Ser307)

p-IRS1 (Ser612)

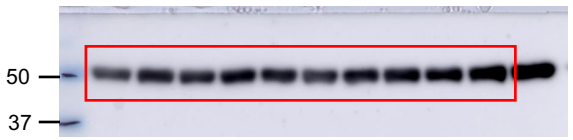


p-IRS1 (Ser632/635)

p-IRS1 (Ser1097)

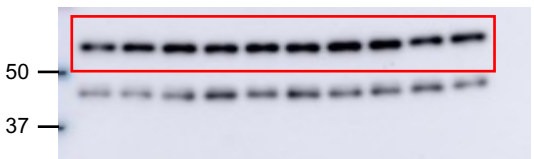


IRS1

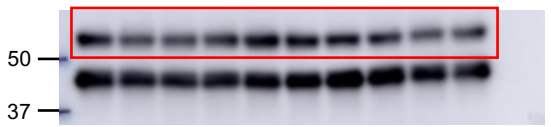


β-tubulin

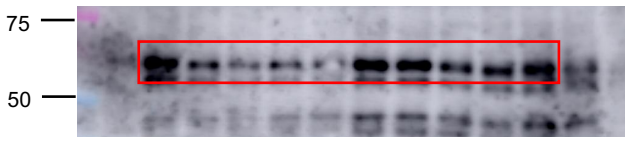
B Figure 1F



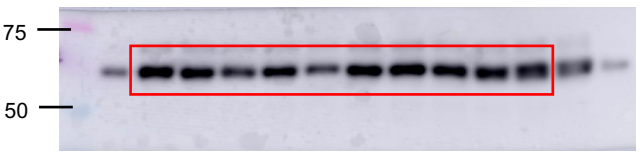
p-Akt (Ser473)



Akt



p-p70S6K (Thr389)



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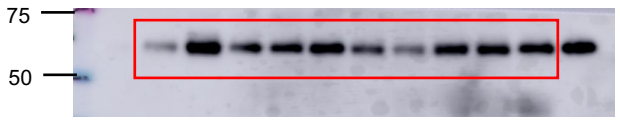
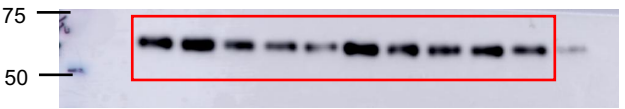
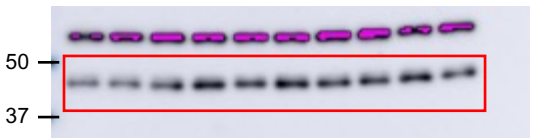


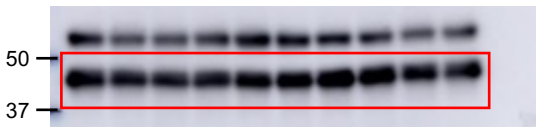
p-AMPK(Thr172)



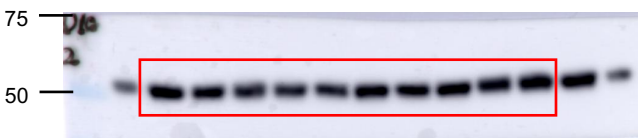
AMPK



p-GSK3β (Ser9)



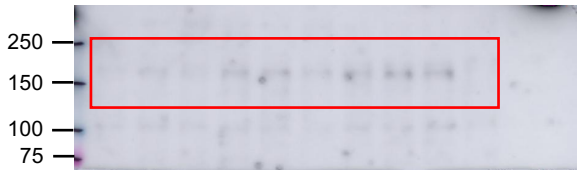
GSK3β



β-tubulin

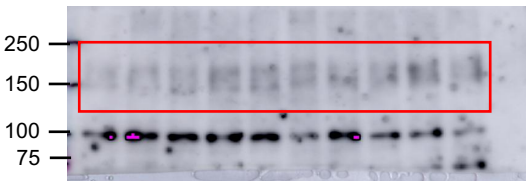
Supplementary File 4 (Figure S4) (continued)

C Figure 2D

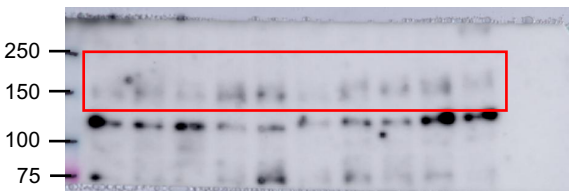


p-IRS1 (Ser307)

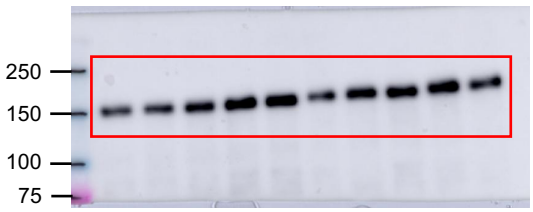
p-IRS1 (Ser612)



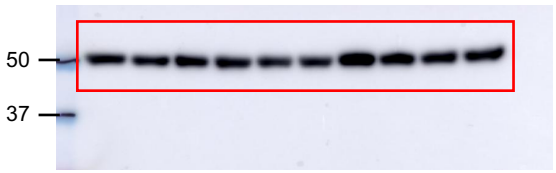
p-IRS1 (Ser632/635)



p-IRS1 (Ser1097)

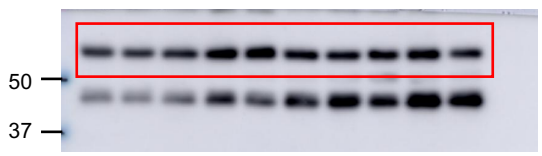


IRS1

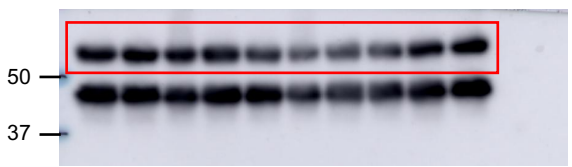


β-tubulin

D Figure 2E



p-Akt (Ser473)



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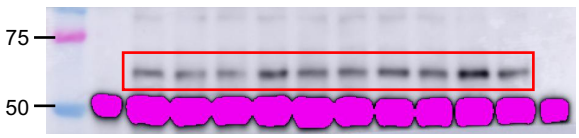
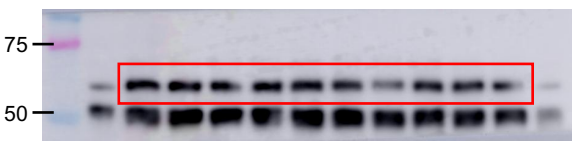
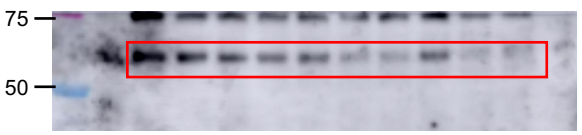


p-p70S6K (Thr389)



p70S6K



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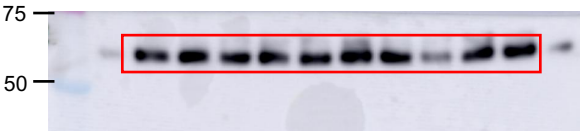
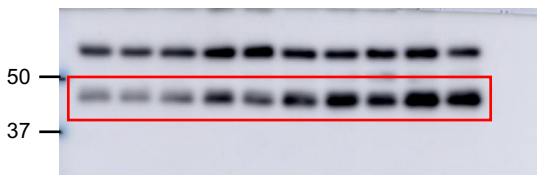
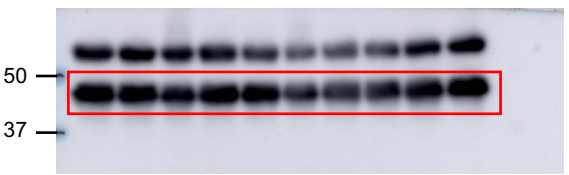


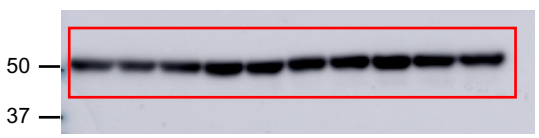
AMPK



p-GSK3β (Ser9)



GSK3β

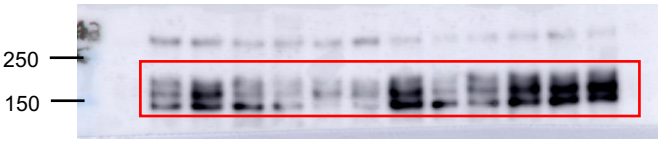


β-tubulin

Supplementary File 4 (Figure S4) (continued)

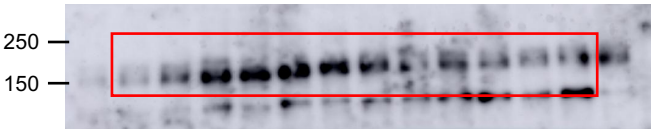
E Figure 3D

p-IRS1 (S307)

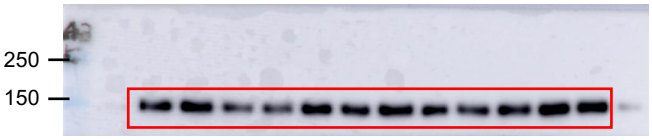


p-IRS1 (S612)

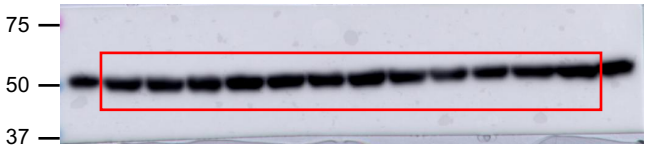
p-IRS1 (S632/S635)



p-IRS1 (S1097)

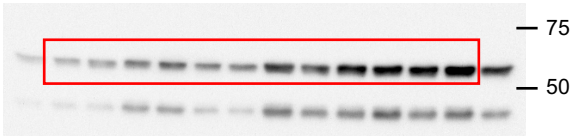


IRS1

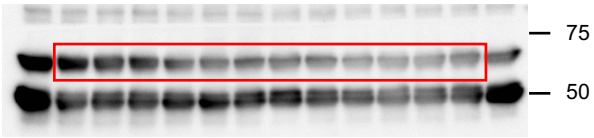


β-tubulin

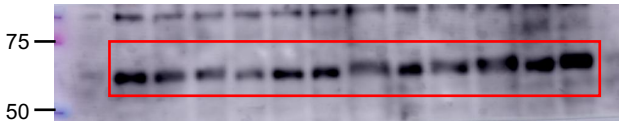
F Figure 3E



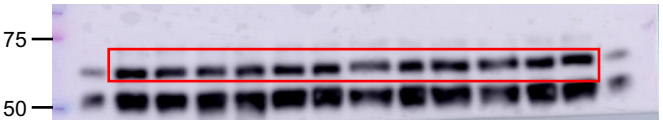
p-Akt (Ser473)



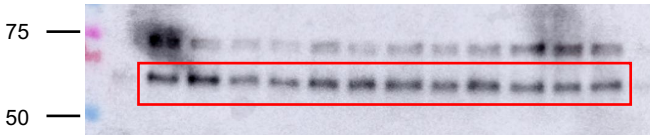
Akt



p-p70S6K (Thr389)

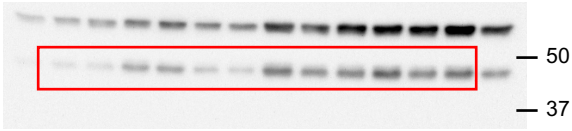


p70S6K

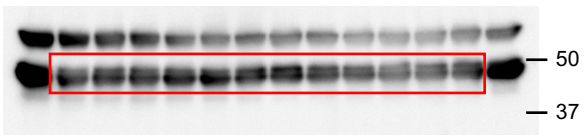


p-AMPK(Thr172)

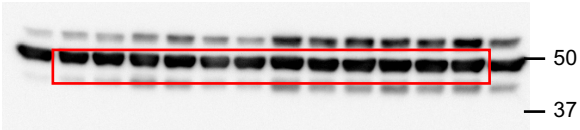
AMPK



p-GSK3β (Ser9)



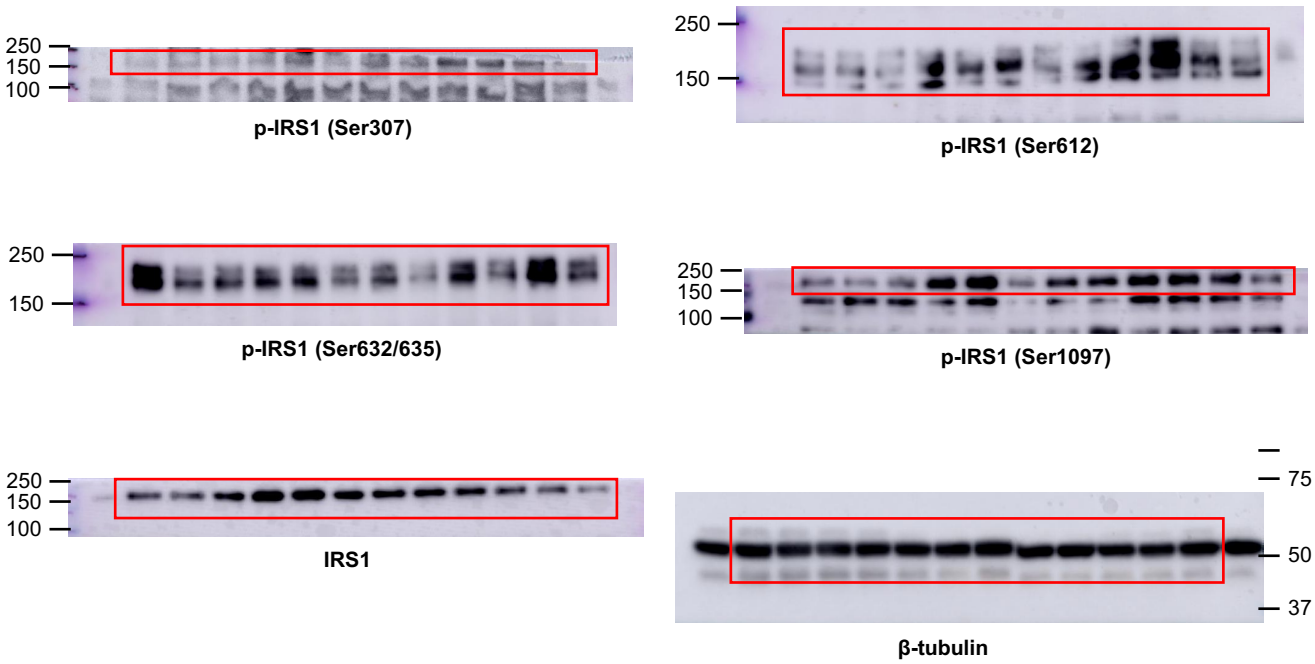
GSK3β



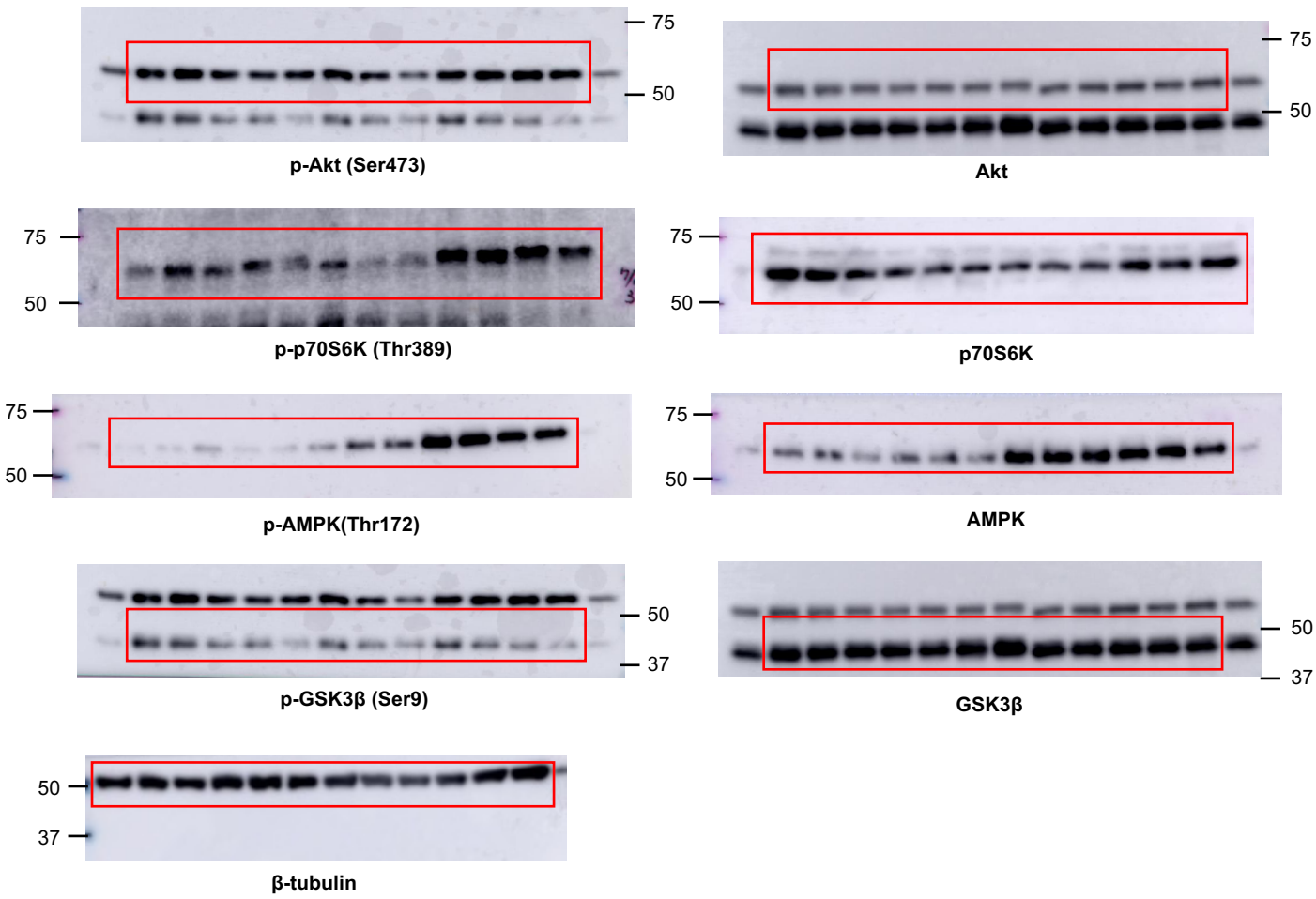
β-tubulin

Supplementary File 4 (Figure S4) (continued)

G Figure 4E

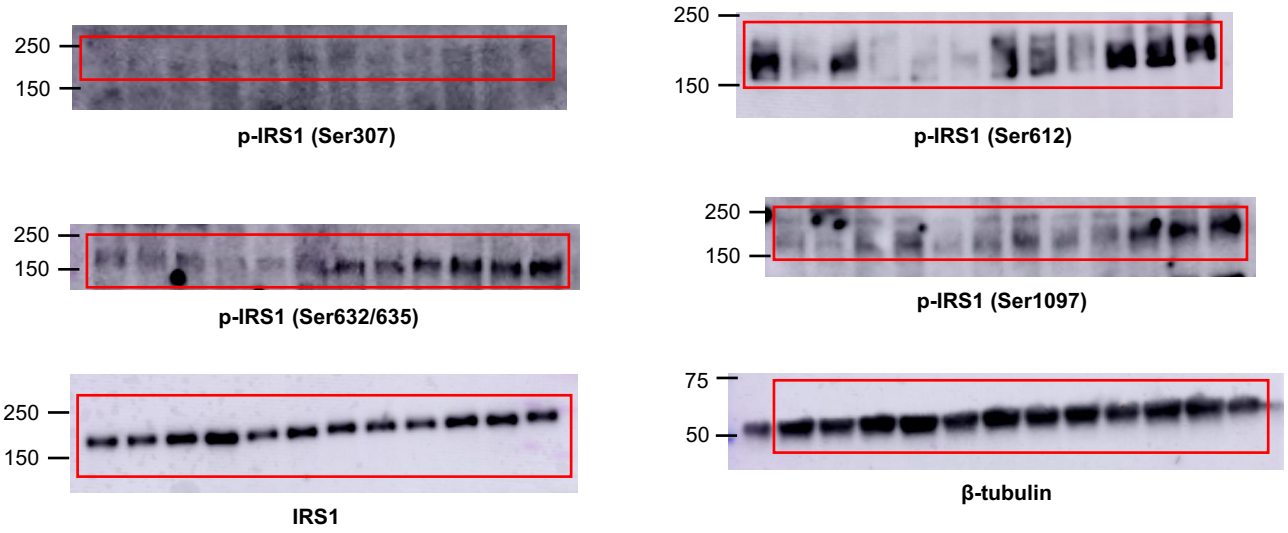


H Figure 4F

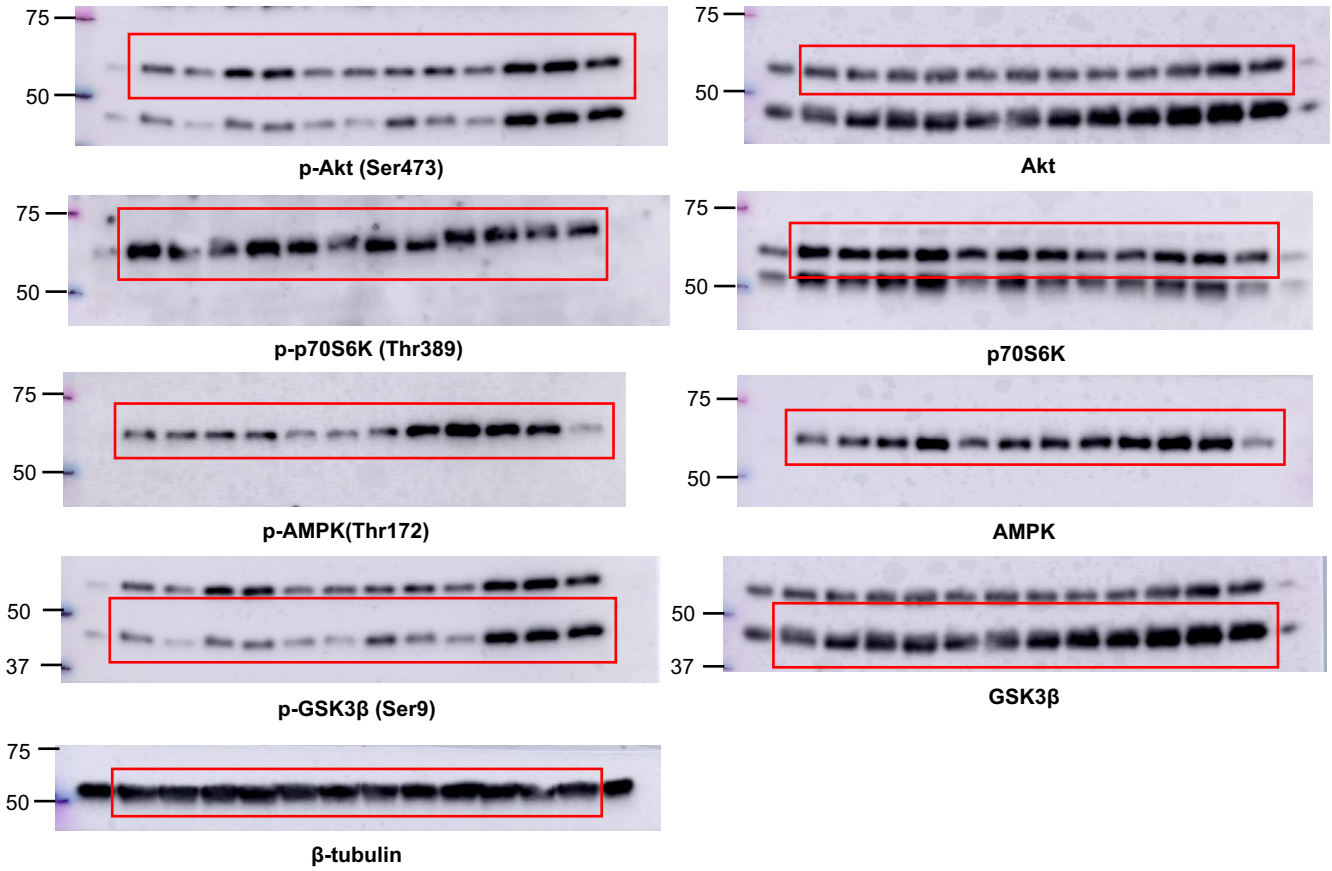


Supplementary File 4 (Figure S4) (continued)

I Figure 5E



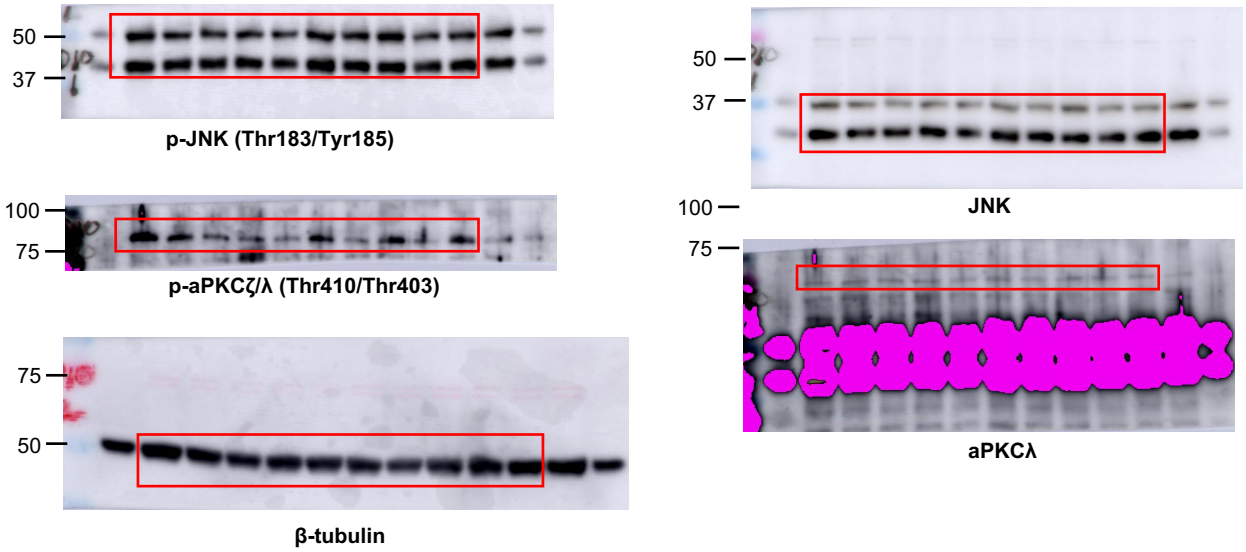
J Figure 5F



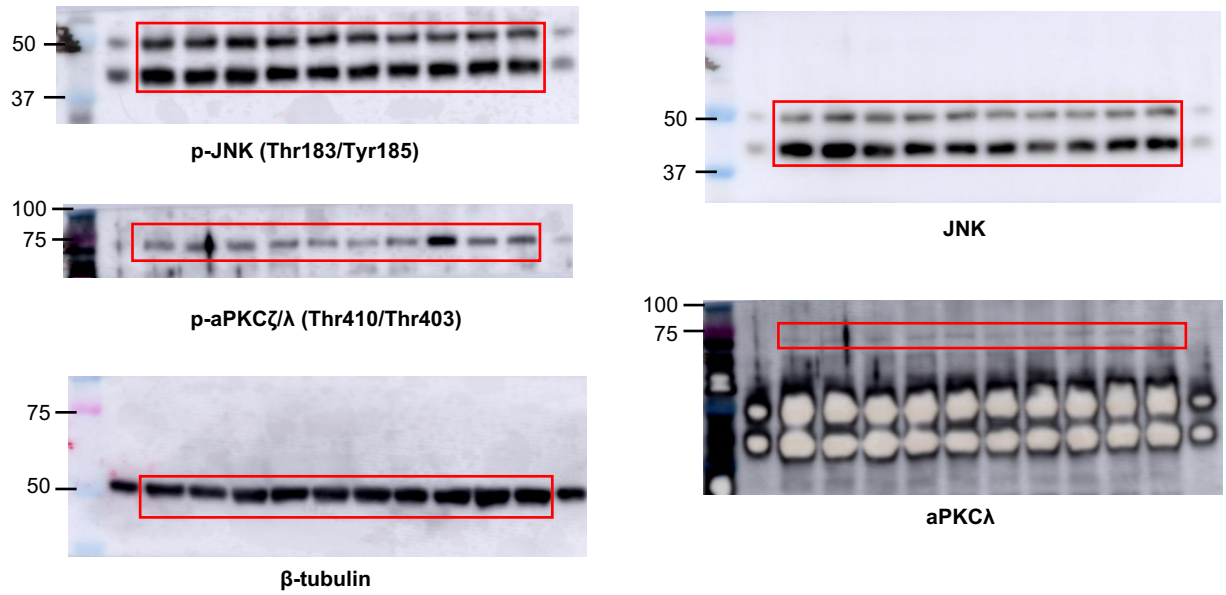
Supplementary File 4 (Figure S4). Full images of western blots. (A) Figure 1E; (C) Figure 2D; (E) Figure 3D; (G) Figure 4E; (I) Figure 5E: phosphorylated-insulin receptor substrates 1 mouse Ser307 [p-IRS1 (mSer307)], p-IRS1 (mSer612), p-IRS1 (mSer632/635), p-IRS1 (mSer1097), IRS1, and β-tubulin. (B) Figure 1F; (D) Figure 2E; (F) Figure 3E; (H) Figure 4F; (J) Figure 5F: phosphorylated and total Akt, p70S6K, AMPK, GSK3β, and β-tubulin.

Supplementary File 5 (Figure S5)

A Figure S3A

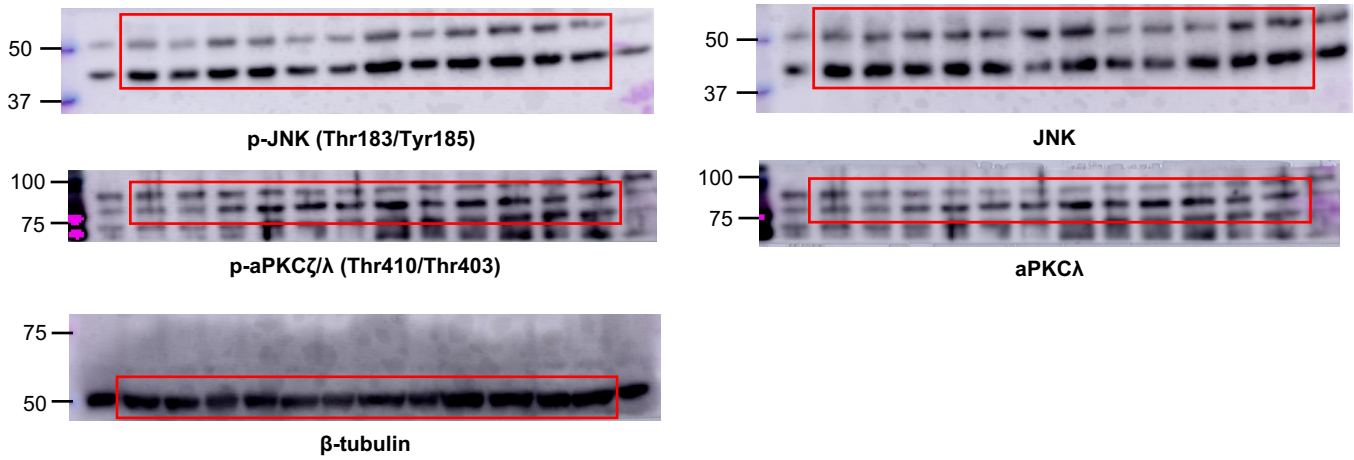


B Figure S3B

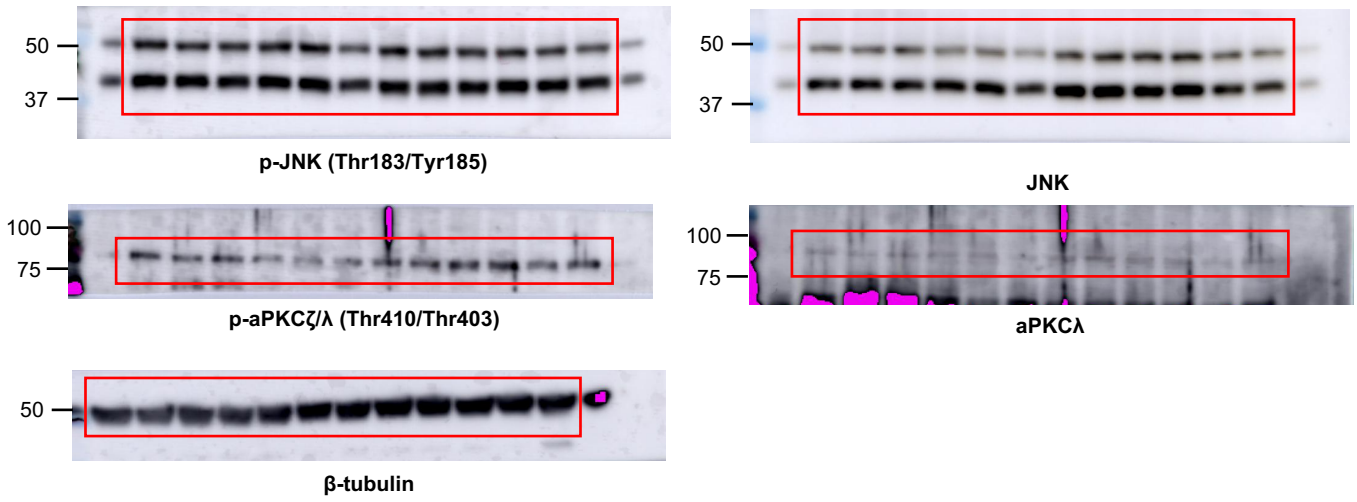


Supplementary File 5 (Figure S5) (continued)

C Figure S3C

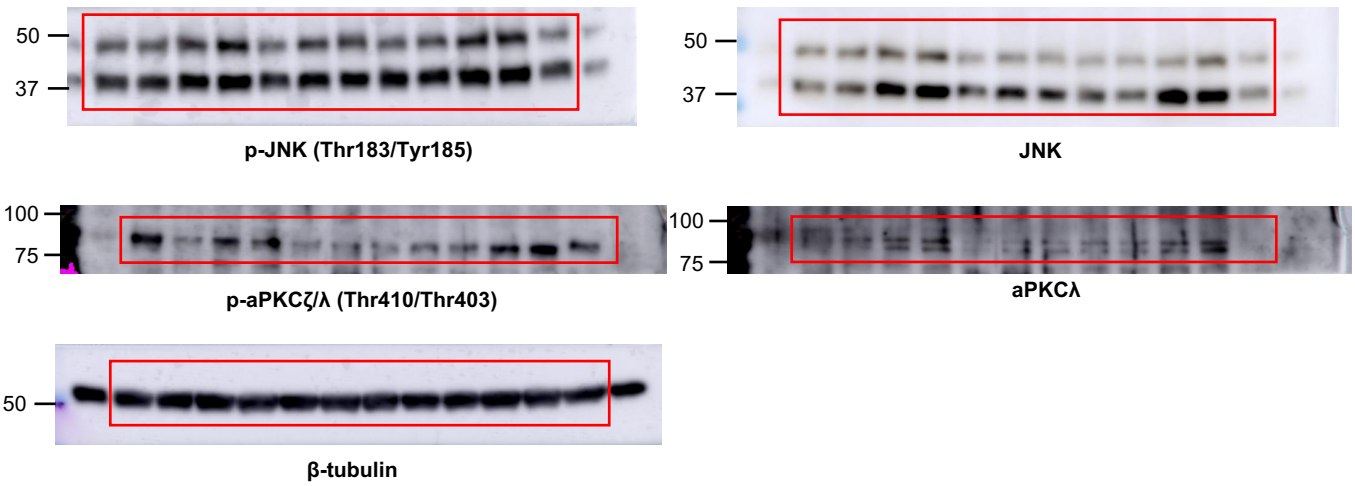


D Figure S3D



Supplementary File 5 (Figure S5) (continued)

E Figure S3E



Supplementary File 5 (Figure S5). Full images of western blots. (A) Figure S3A; (B) Figure S3B; (C) Figure S3C; (D) Figure S3D; (E) Figure S3E: phosphorylated and total JNK, aPKCζ/λ, and β-tubulin.

Table S1. Summary of phosphorylated Ser residues on hippocampal IRS1 in all models

Phosphorylation of hippocampal IRS1 at Ser sites / Types of models	mSer 307	mSer 612	mSer 632/635	mSer 1097
Middle-aged (34-36 weeks old) DIO mice	↑	N.S.	N.S.	↑
STZ (10 weeks old) mice	N.S.	N.S.	N.S.	N.S.
Aged (84 weeks old) mice	↑	↑	↑	N.S.
Young (12 weeks old) $APPKI^{NL-G-F}$ mice	↑	↑	N.S.	↑
Middle-aged (34-36 weeks old) $APPKI^{NL-G-F}$ mice	N.S.	↑	↑	↑

N.S.: no significant difference; ↑ : increased

Table S1. Summary of phosphorylated Ser residues of hippocampal IRS1 in all models.