

Effect of Metformin on tau pathology and proteostasis in cellular and a *Drosophila* model of Tauopathy

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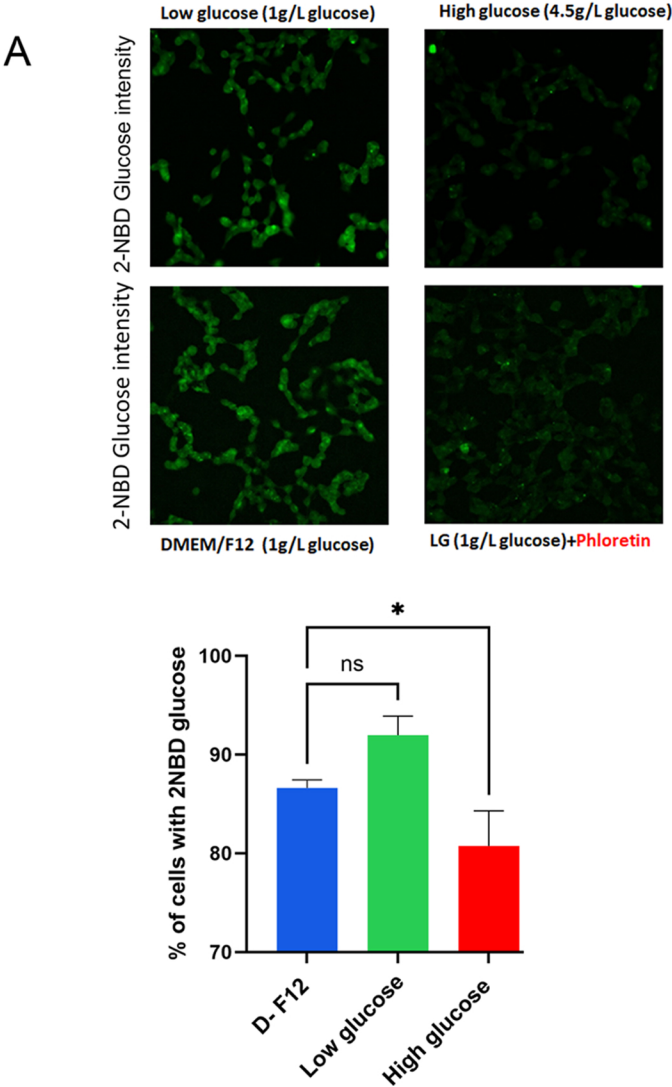
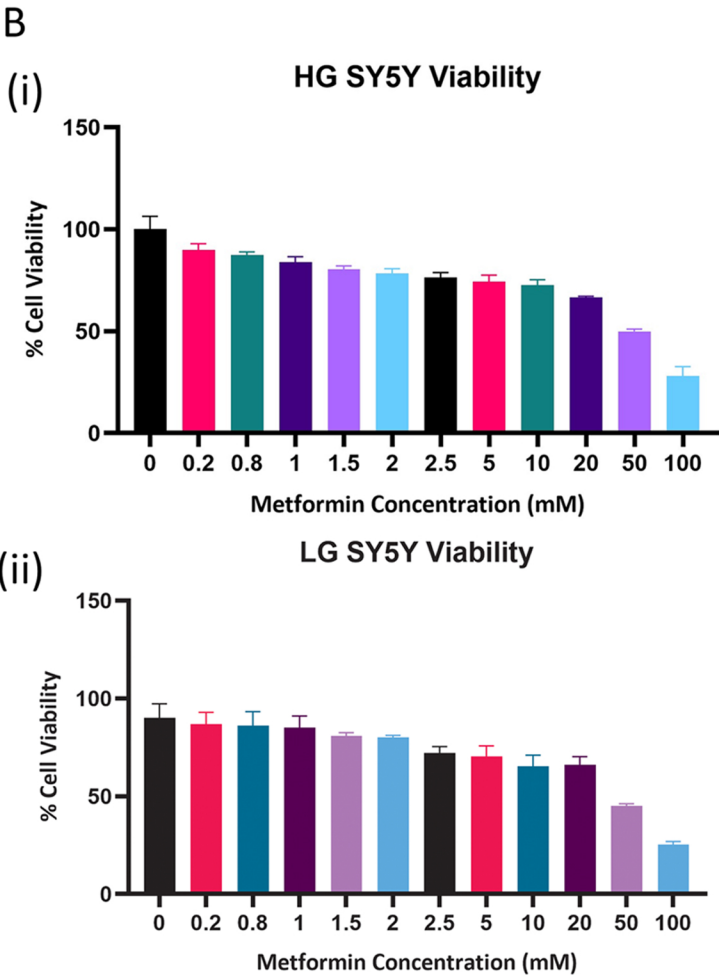
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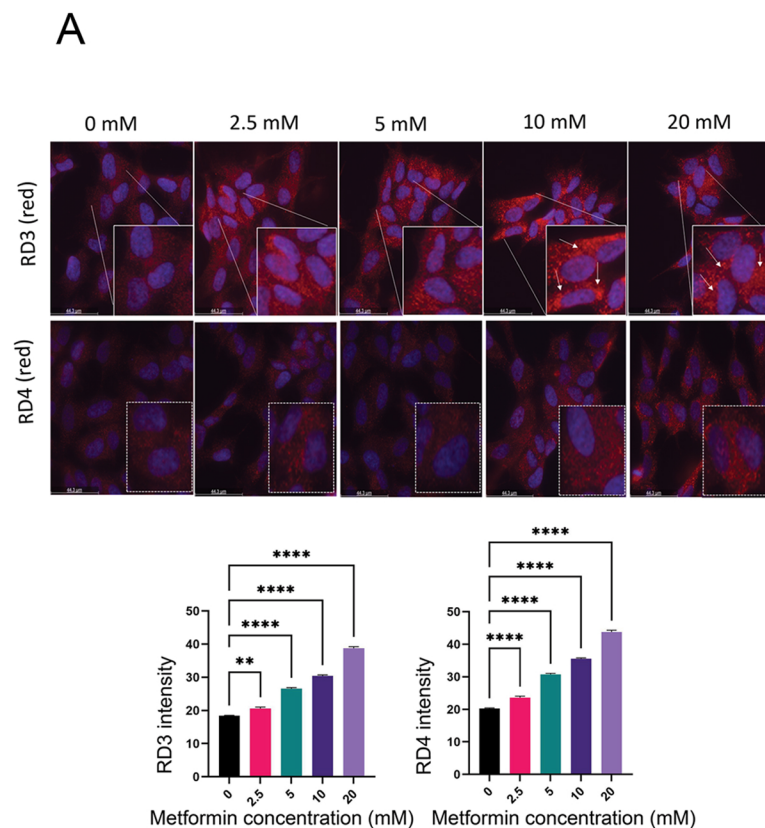
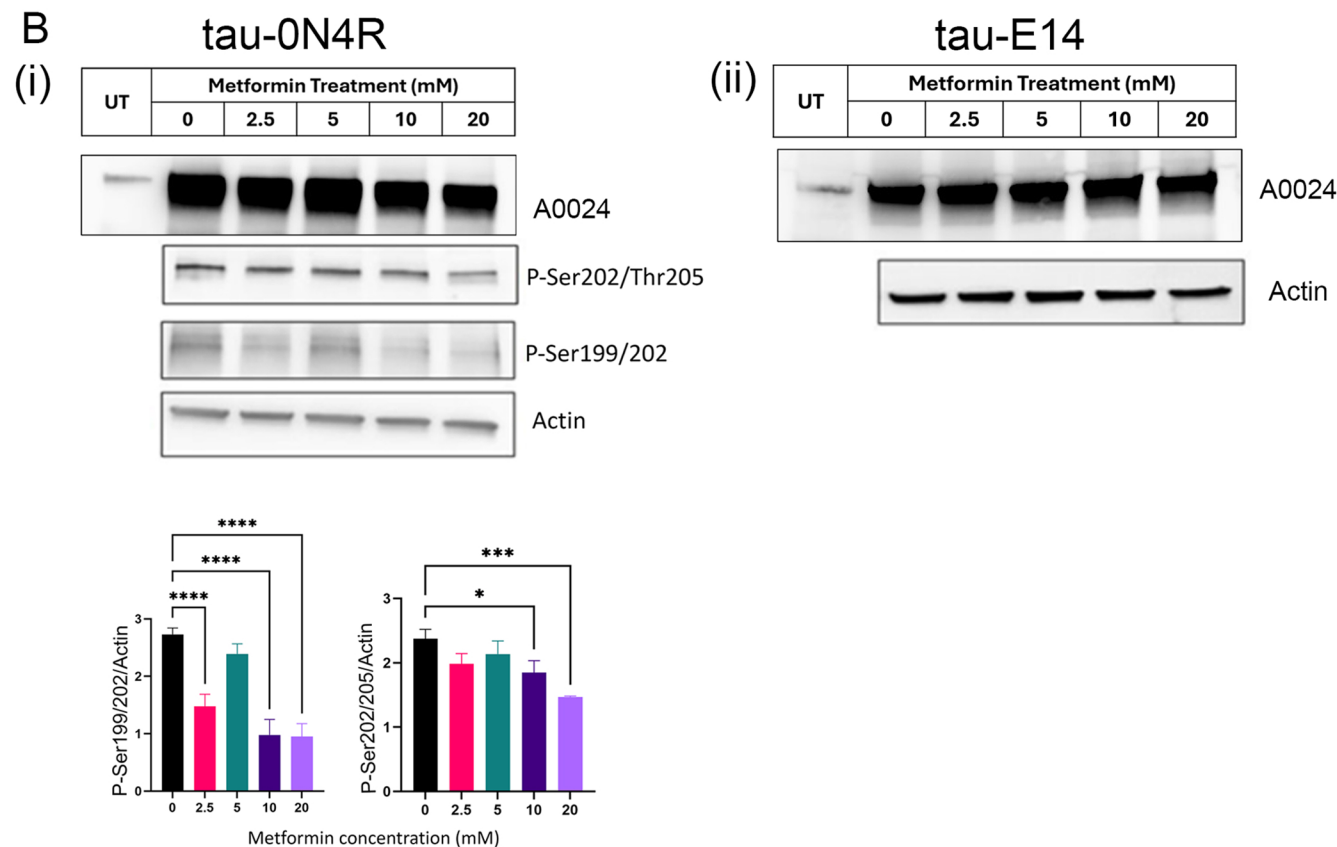
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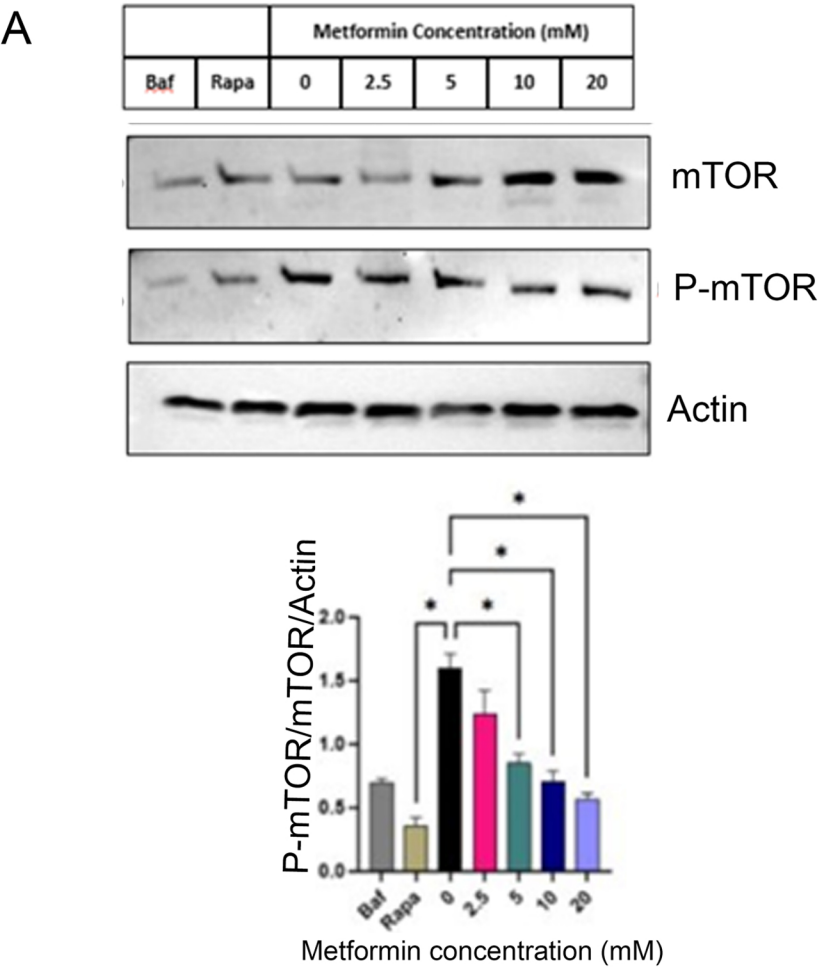
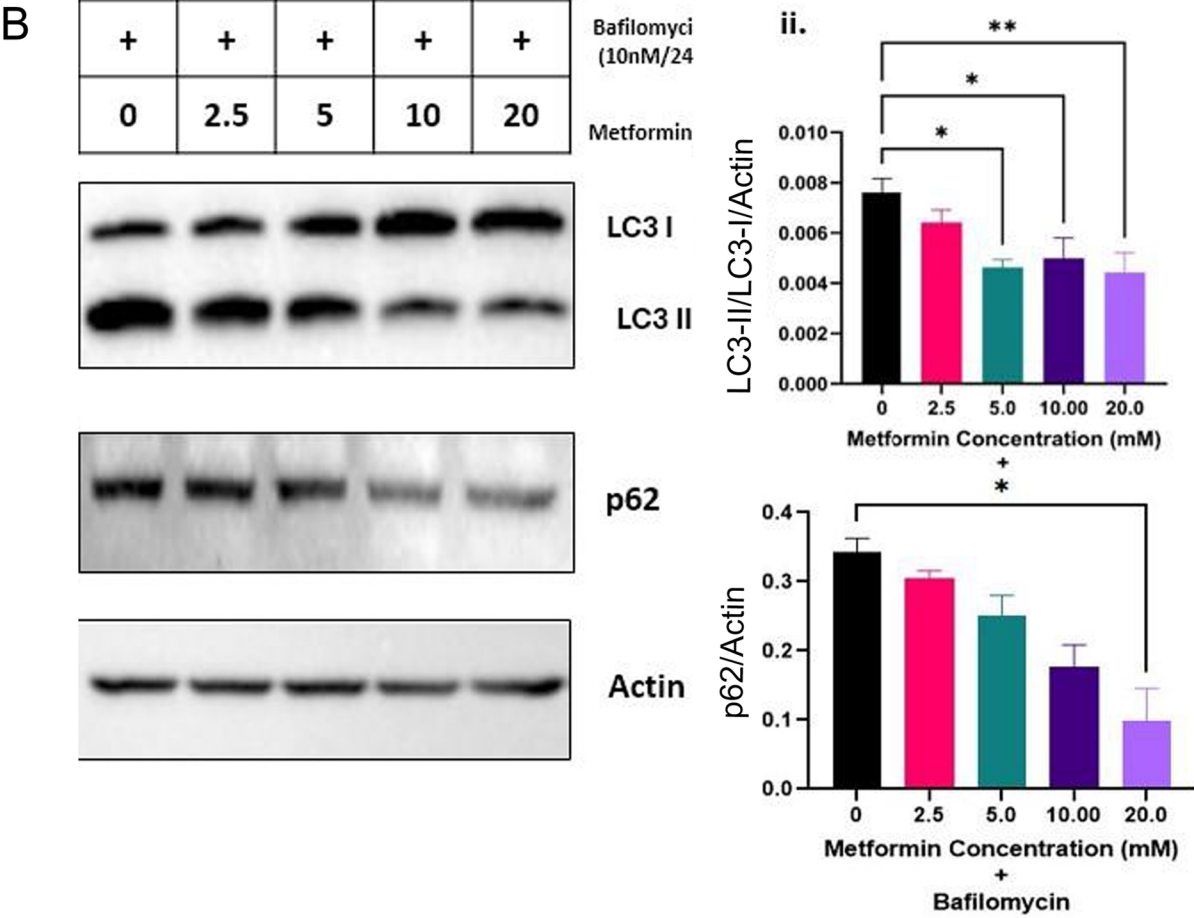
Supplementary Figure 1



Supplementary Figure 2

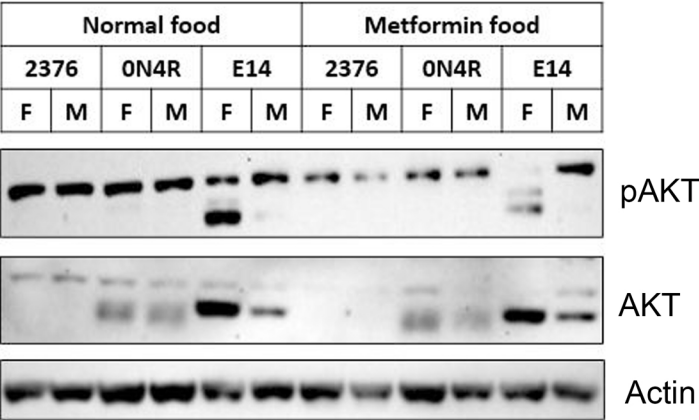


Supplementary Figure 3

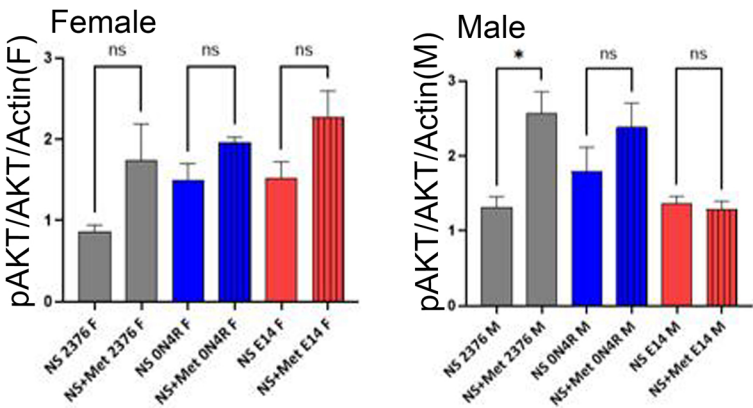


Supplementary Figure 4

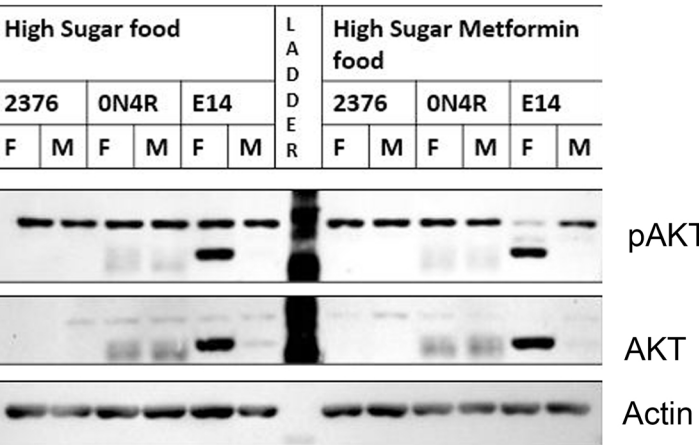
A



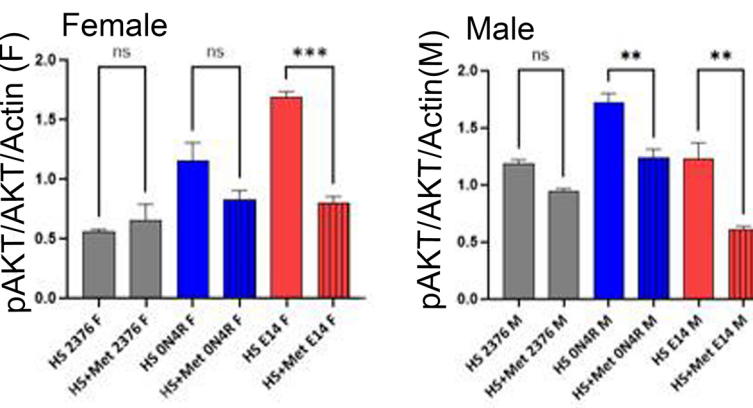
Normal Food +/- Metformin



B



High Sugar Food +/- Metformin



Supplementary Figure 5

Figure 1 – Full Blots of total Tau

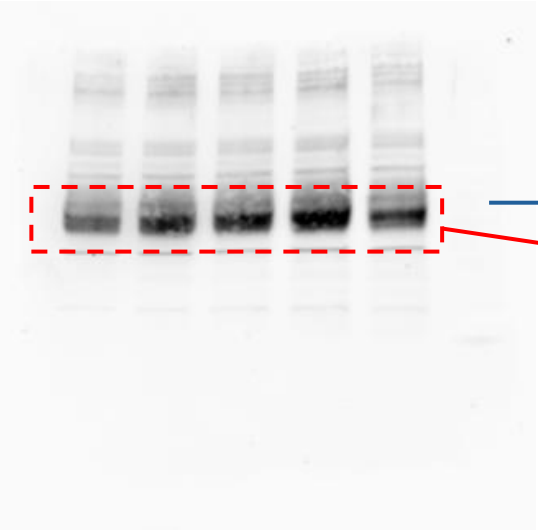
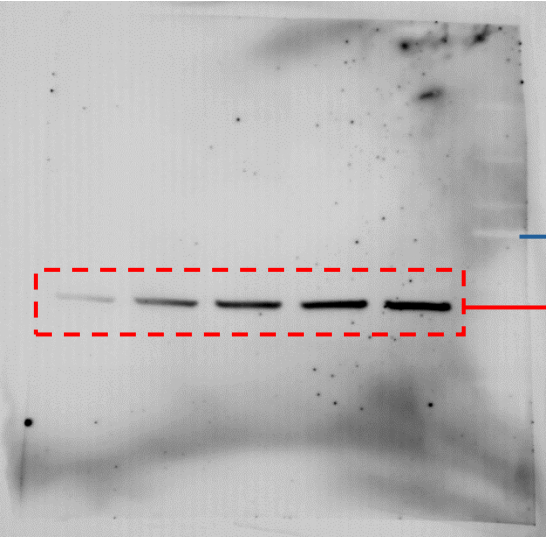
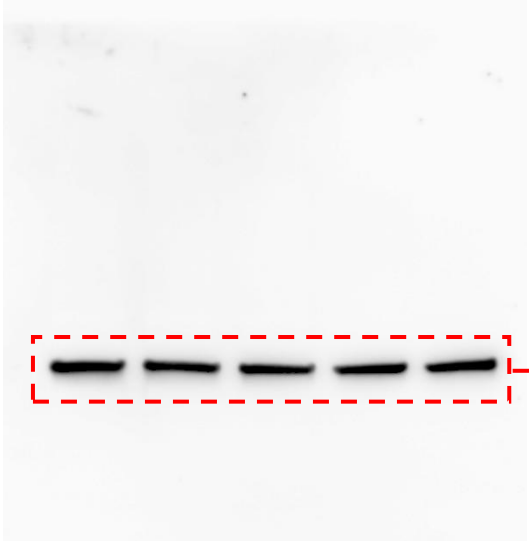
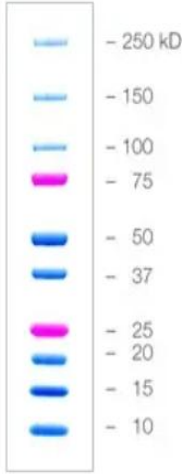
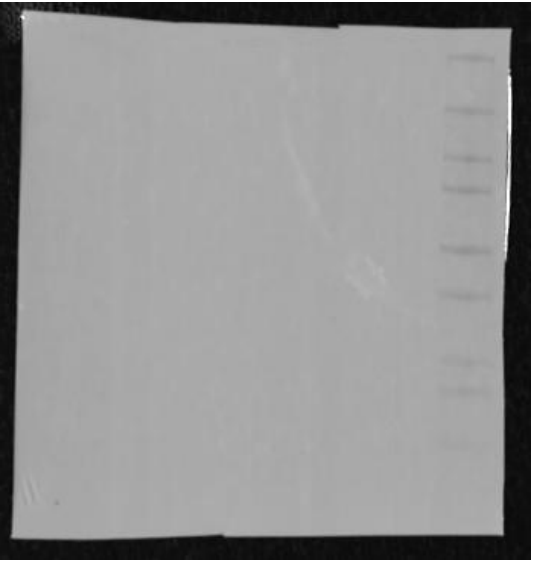
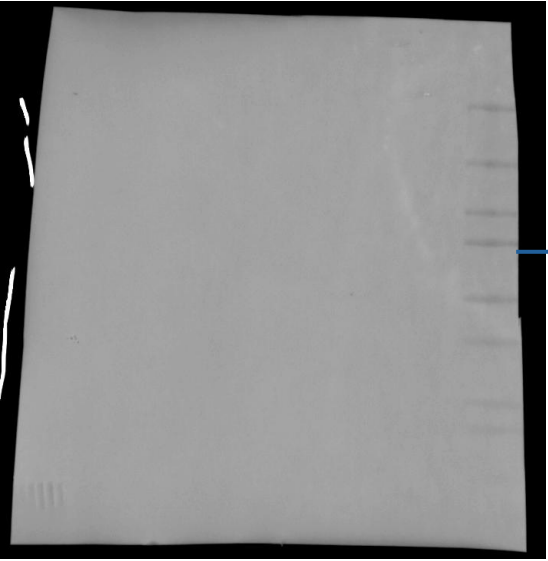
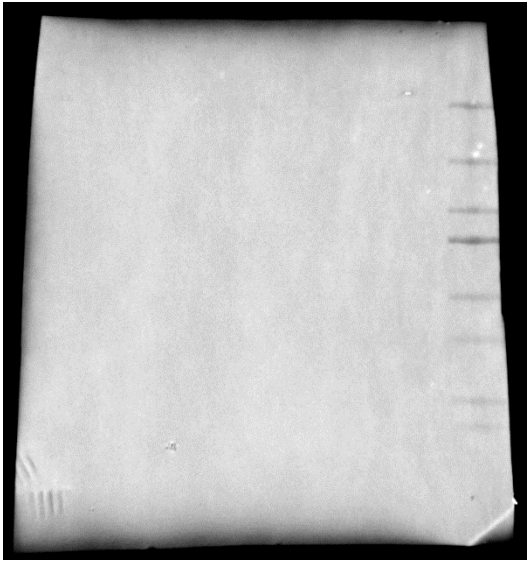


Figure 2 – Full Blots of phospho-Tau

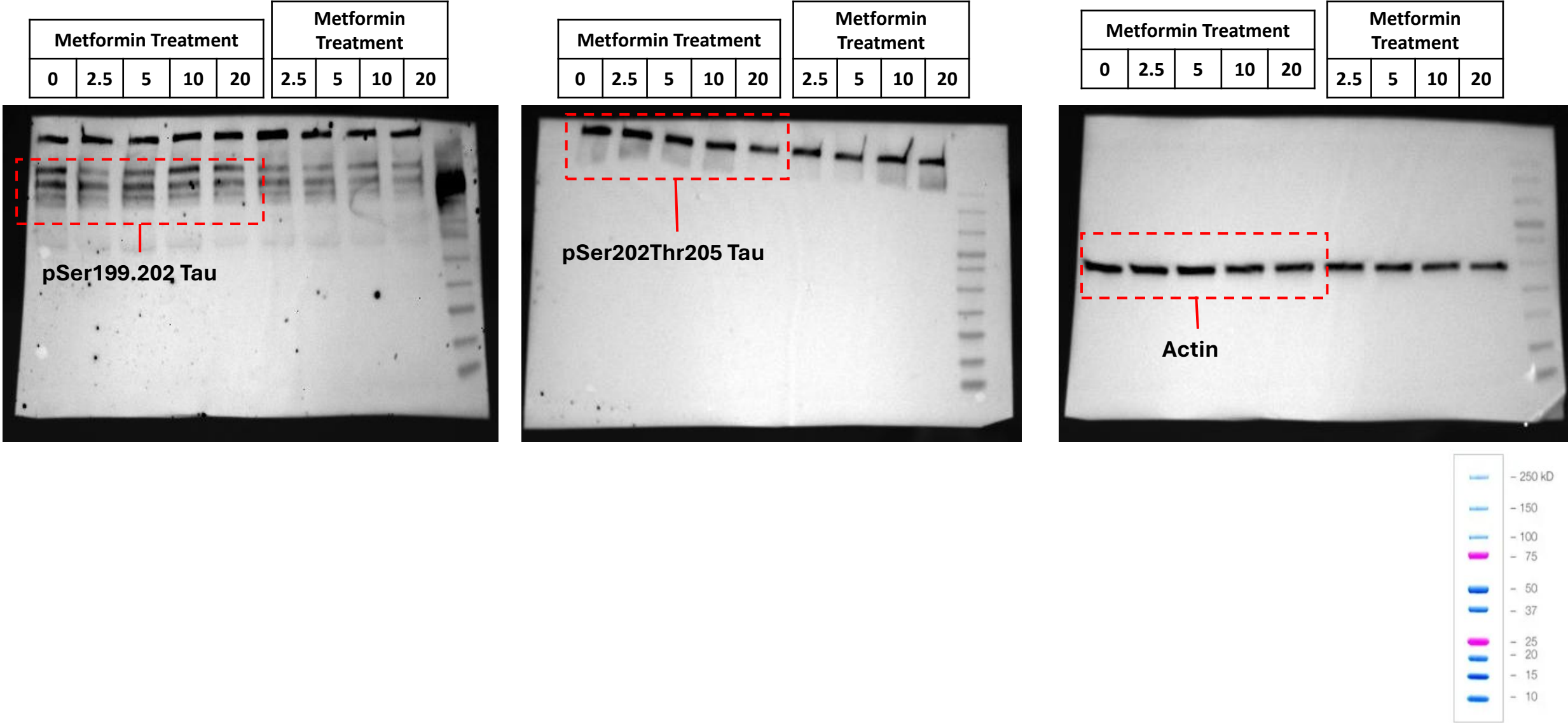
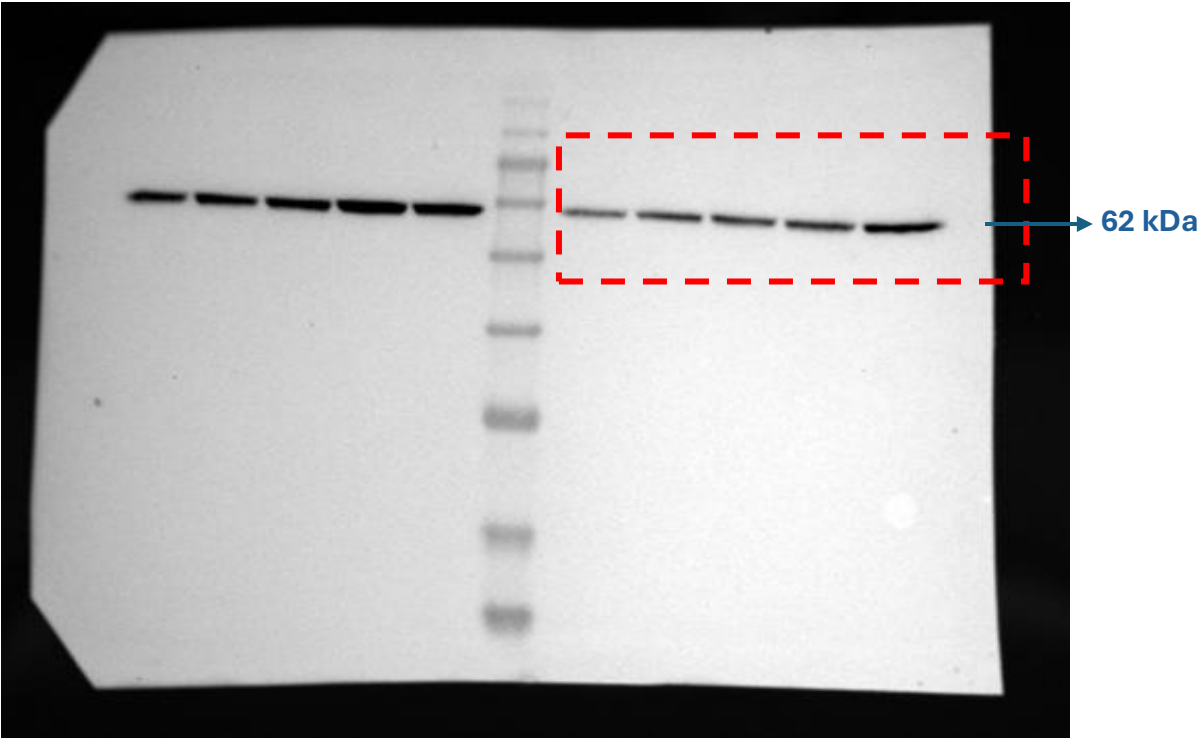


Figure 3 A –Full Blots of pAKT

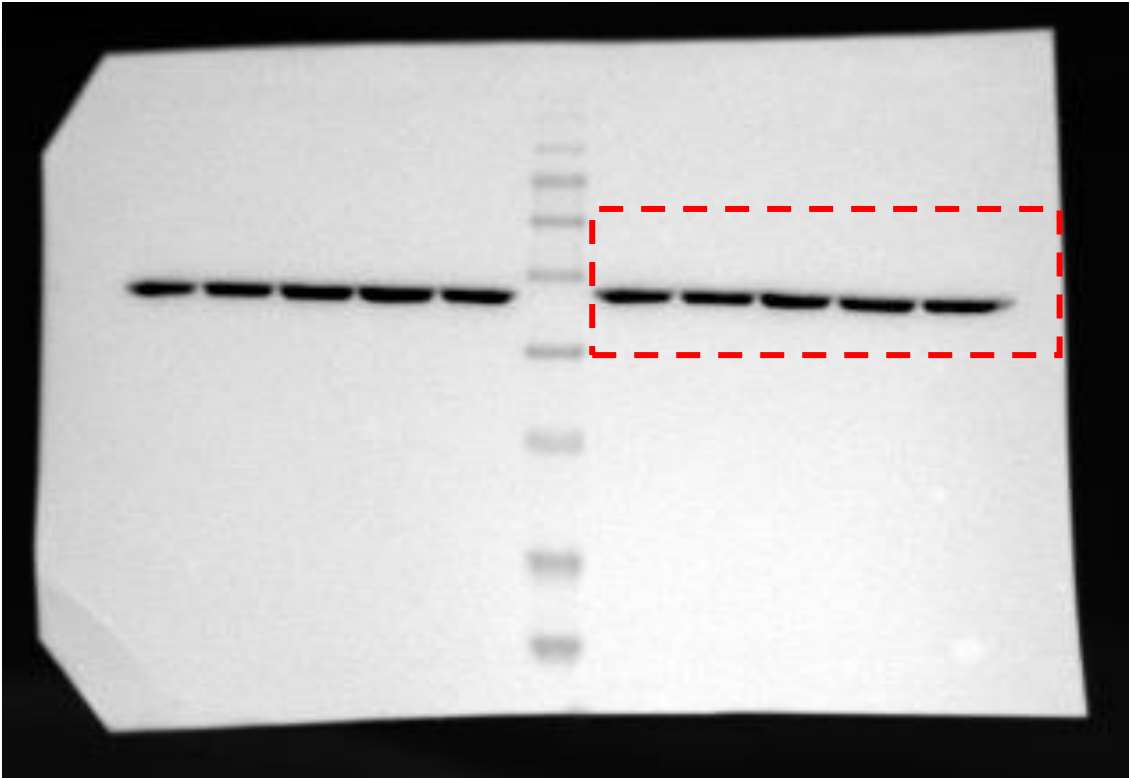
Figure 3A

Metformin Treatment				
0	2.5	5	10	20



pAKT – Low EXPOSURE
Exposure time – 3 seconds

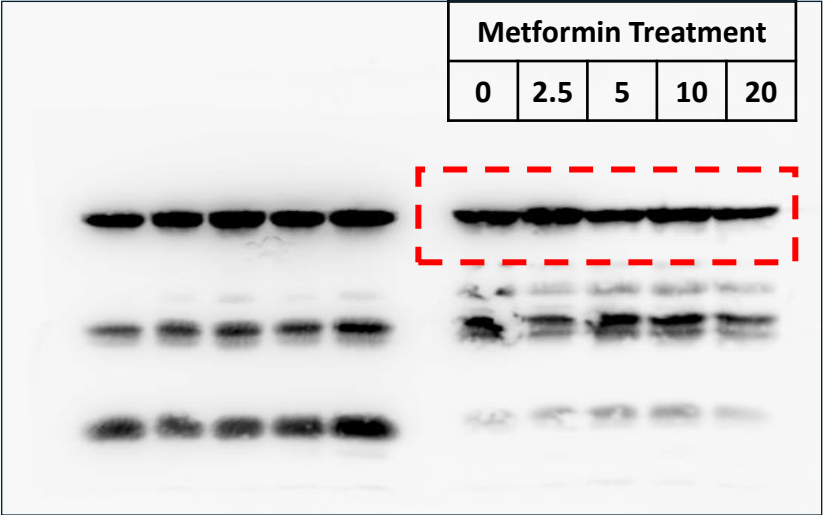
Metformin Treatment				
0	2.5	5	10	20



pAKT – High EXPOSURE
Exposure time – 5 seconds

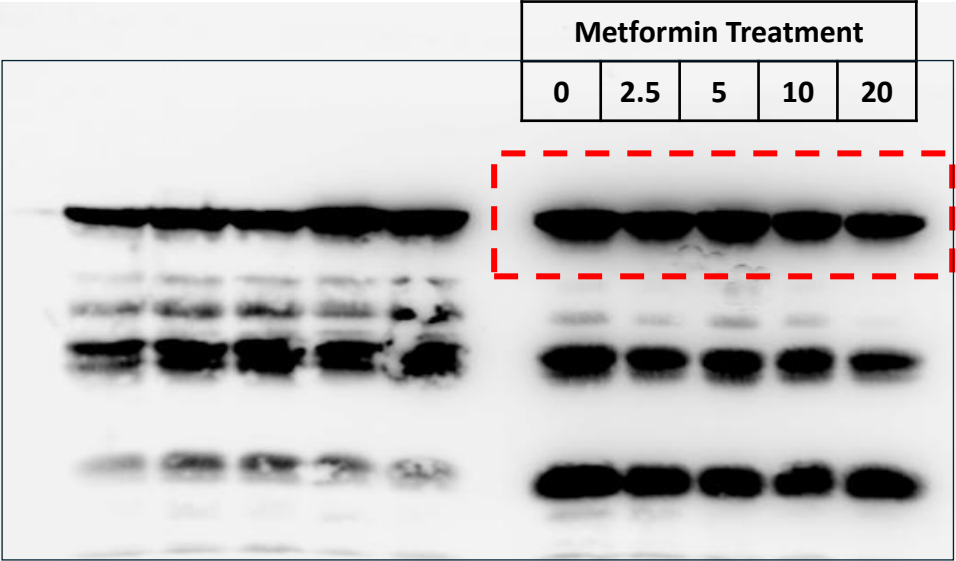
Supplementary Figure 7

Figure 3A- Full blots of AKT



AKT → 60 kDa

Low exposure



AKT

Equipment used to take images - ImageQuant LAS 4000 (GE healthcare)

High exposure

Figure 3 A- Full blot of Actin

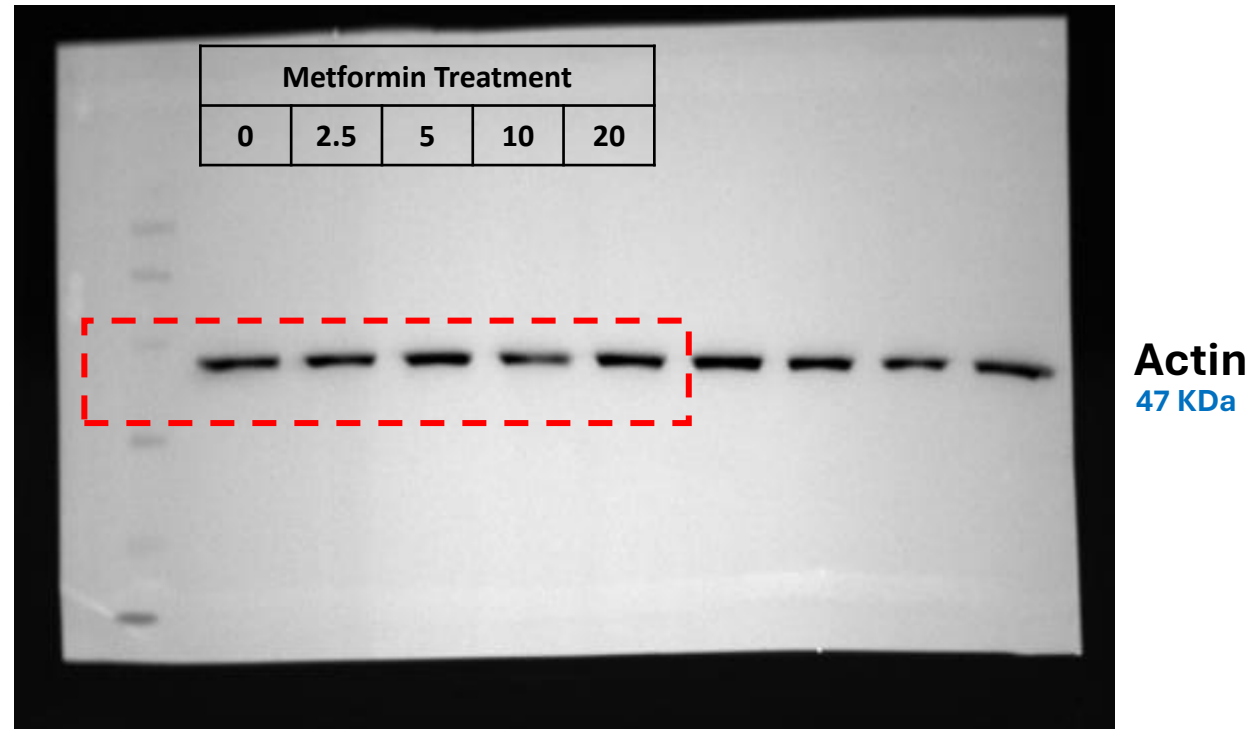


Figure 3B – Full blots of total and p-GSK3β

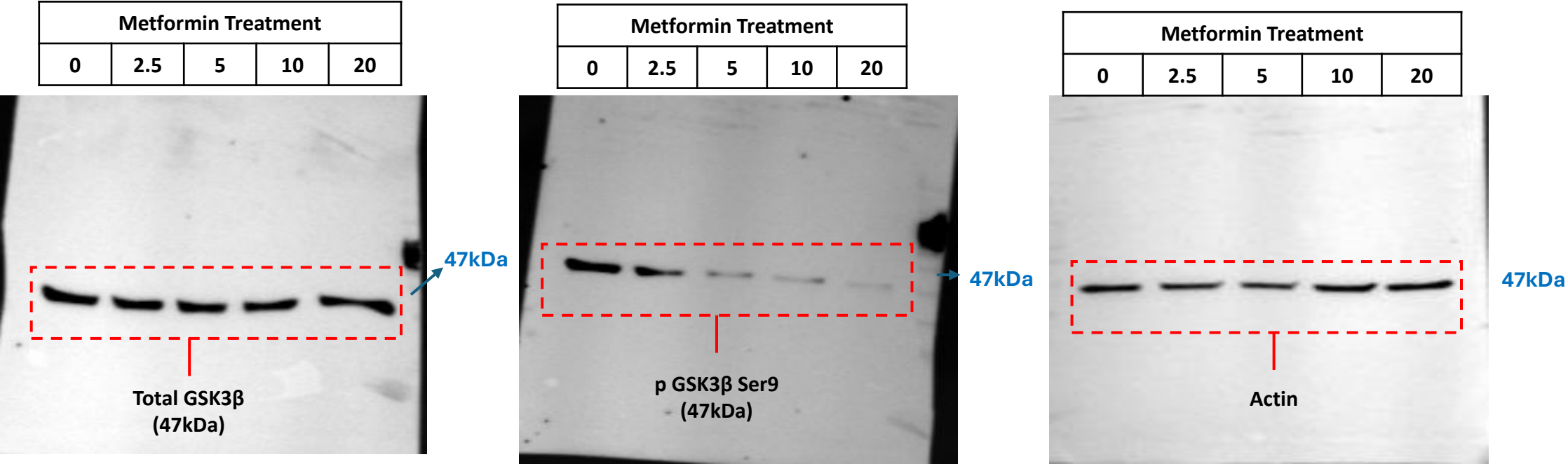
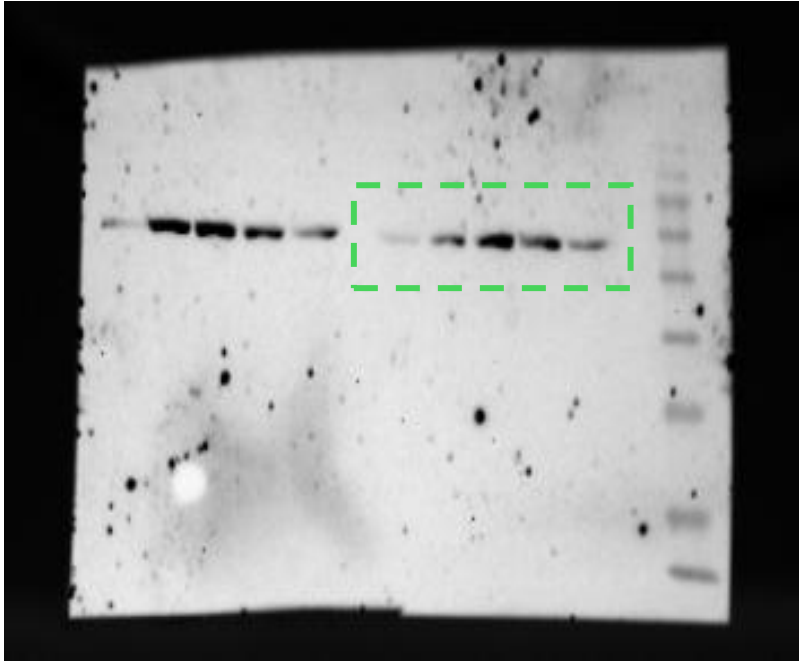


Figure 3C- Full blots of DMe-PP2AC

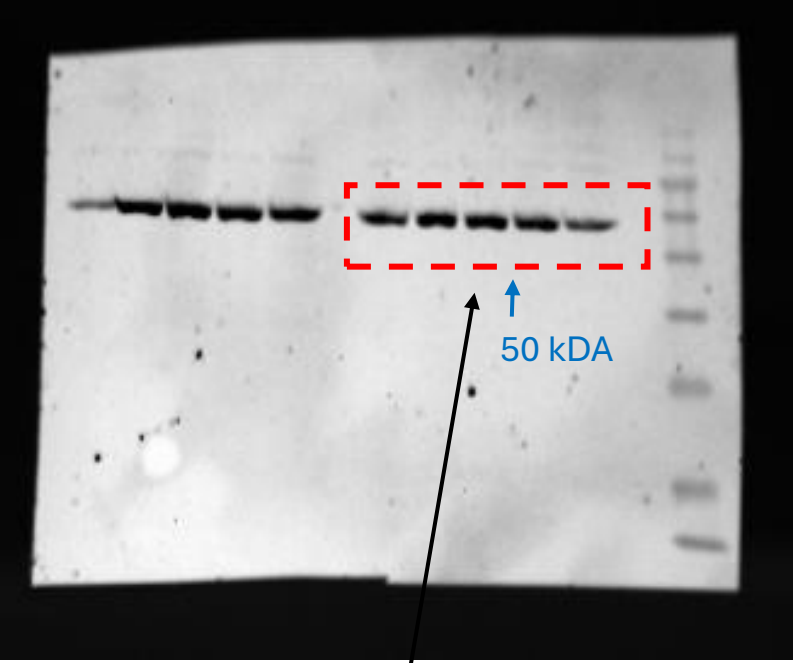
DMe-PP2AC

Metformin Treatment				
0	2.5	5	10	20



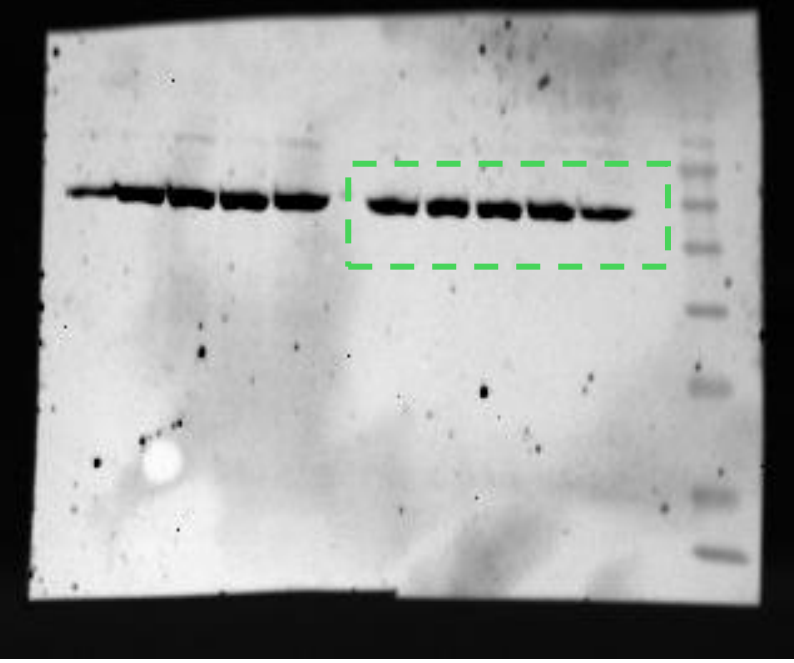
Low exposure

Metformin Treatment				
0	2.5	5	10	20



High Exposure

Metformin Treatment				
0	2.5	5	10	20



Blot used in Figure 3 C

Figure 3C- Full blot of PP2A-C

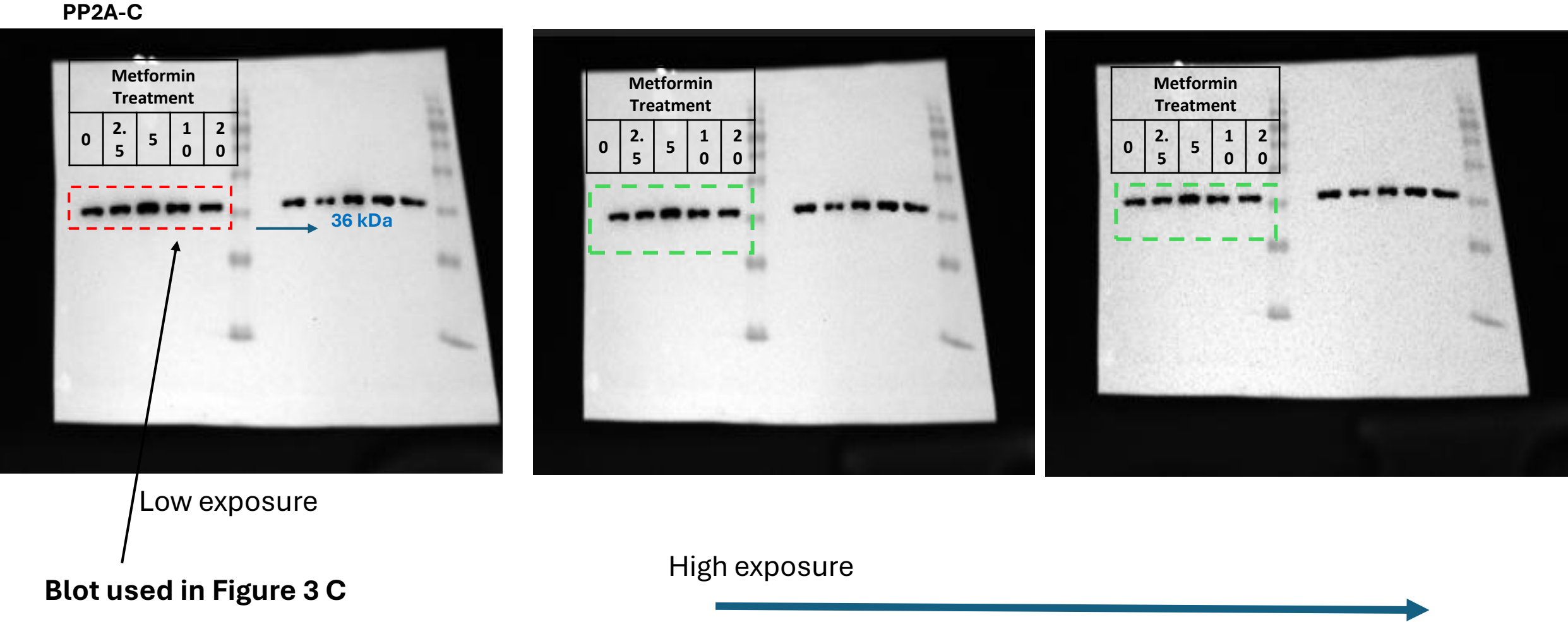
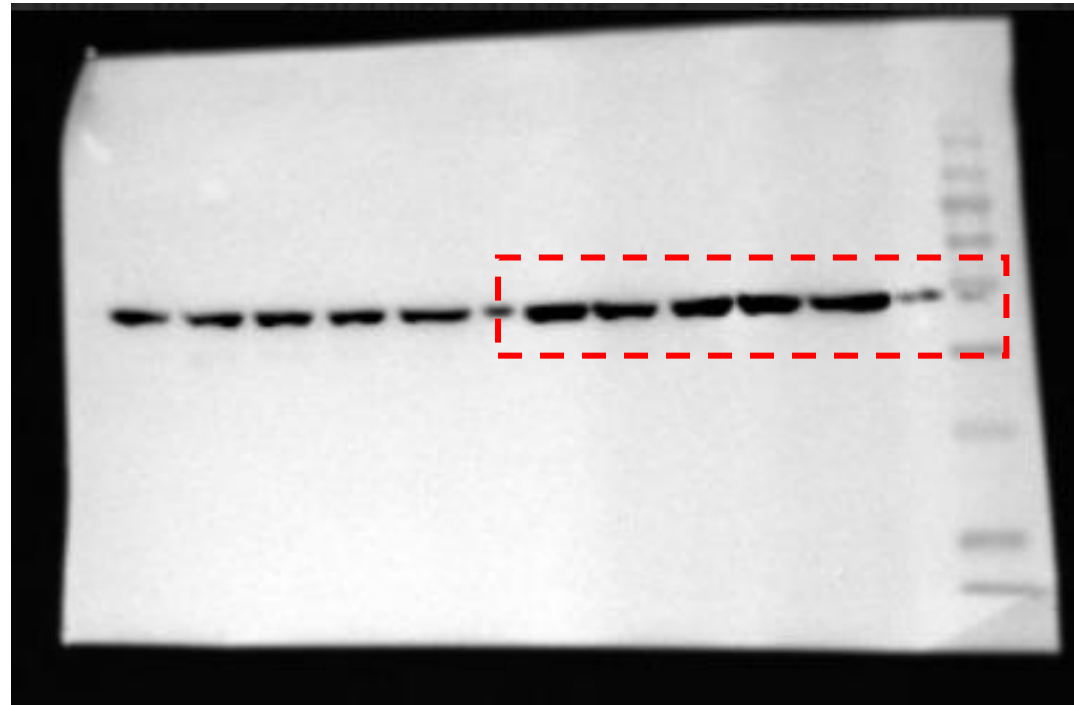


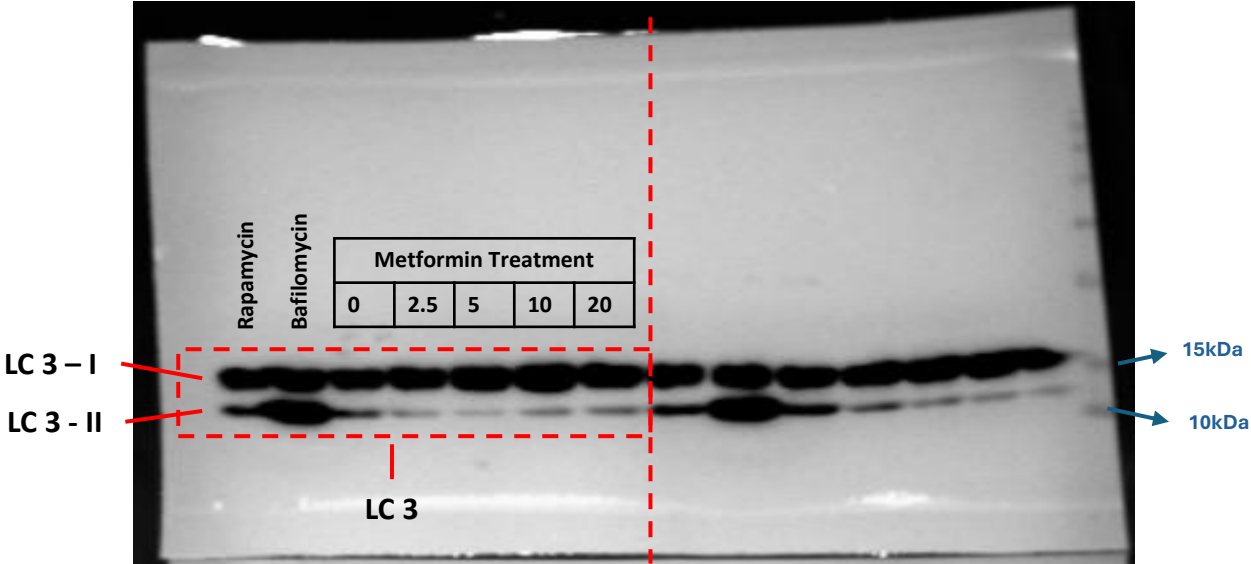
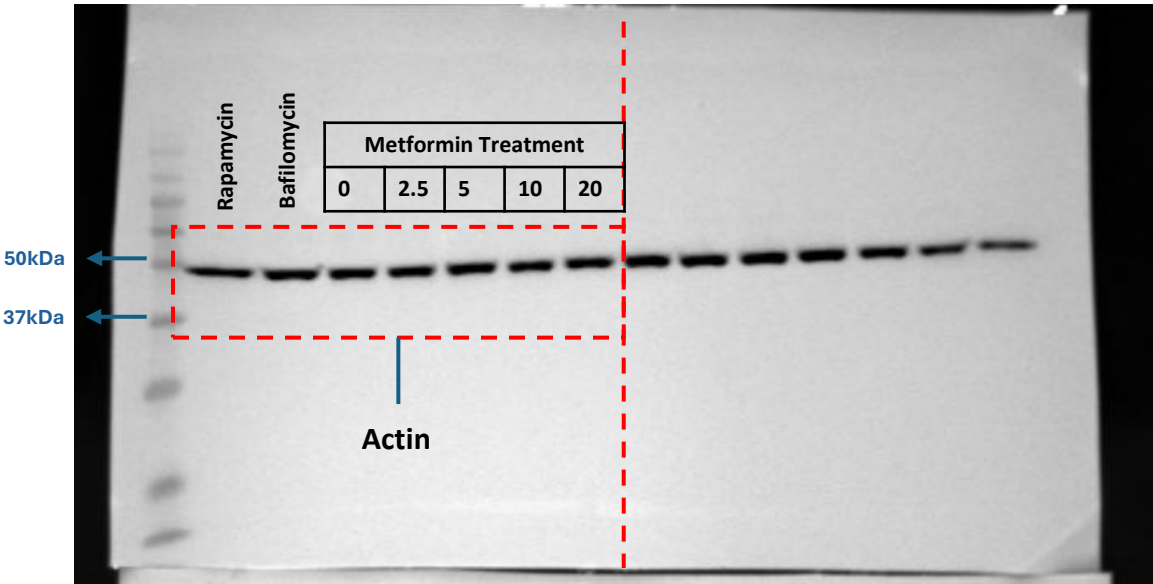
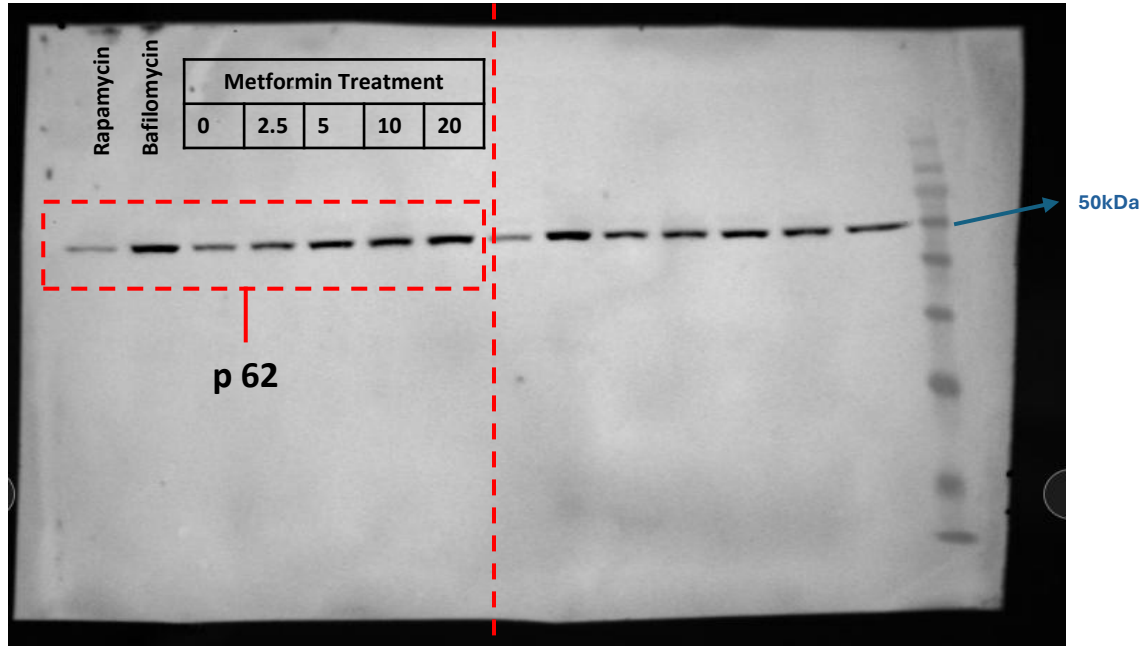
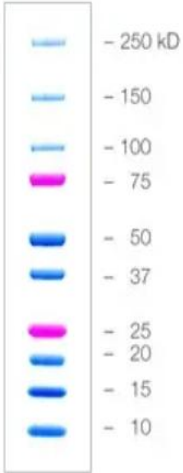
Figure 3C- Full blot of Actin



Actin
47 kDa

Supplementary Figure 8

Figure 5 B – Full Blots of LC3 and p62



Supplementary Figure 9

Figure 7Q Upper Panel full blot total and phospho-Tau

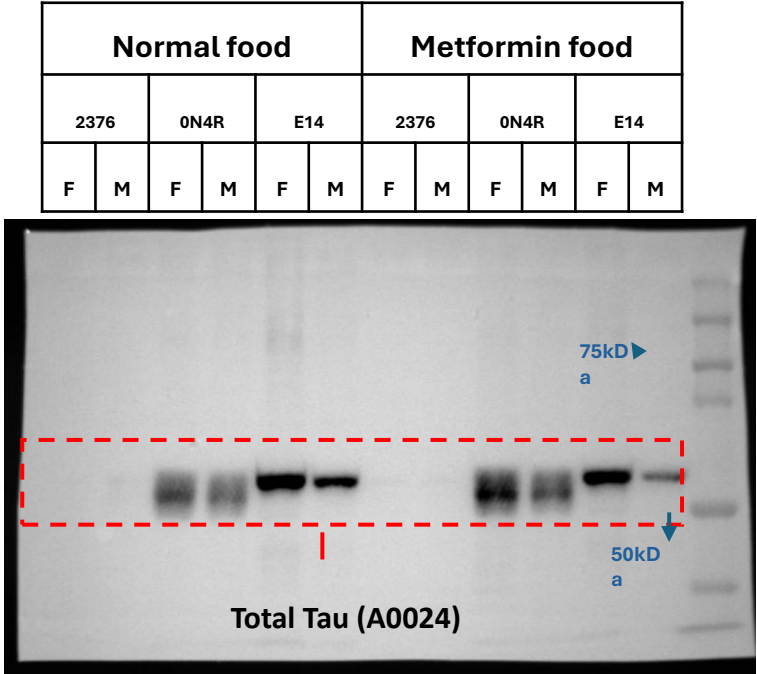
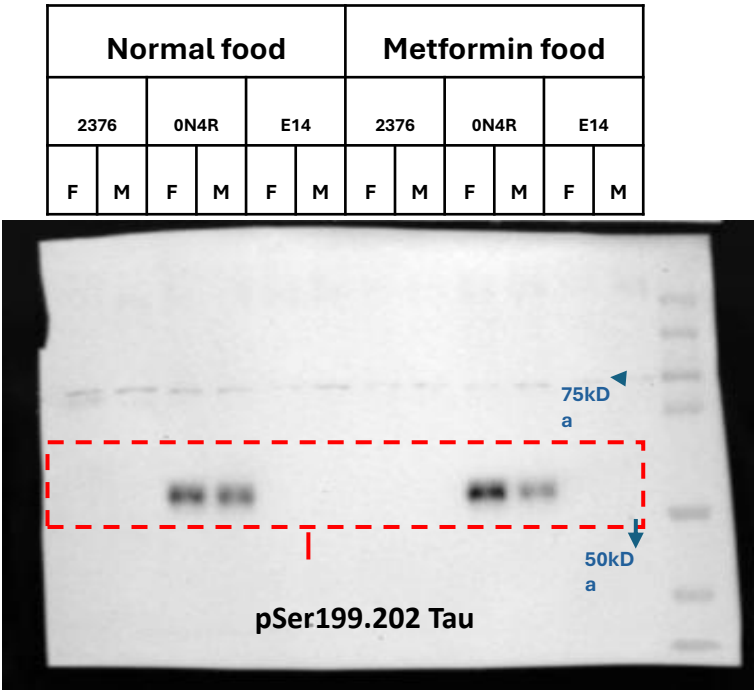
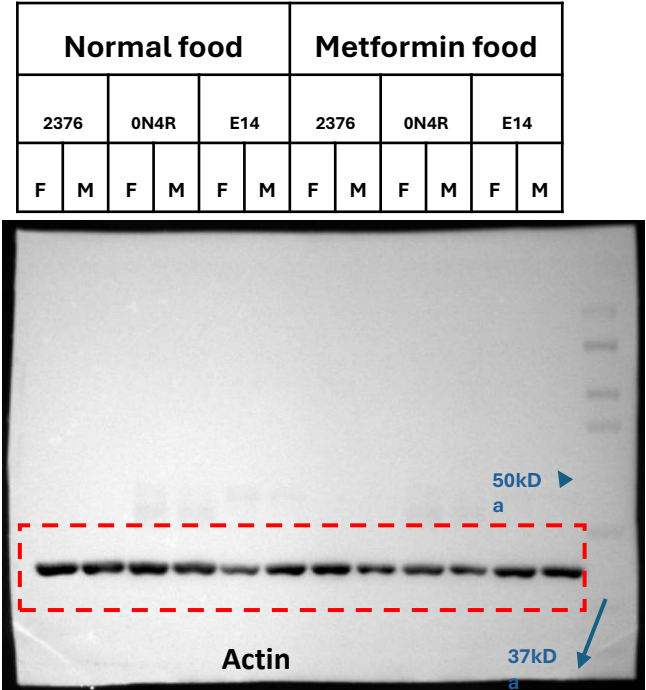
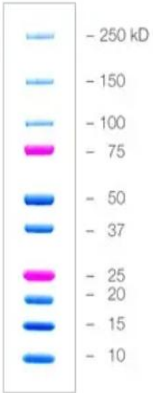


Figure 7Q Lower Panel full blot of total and phospho Tau

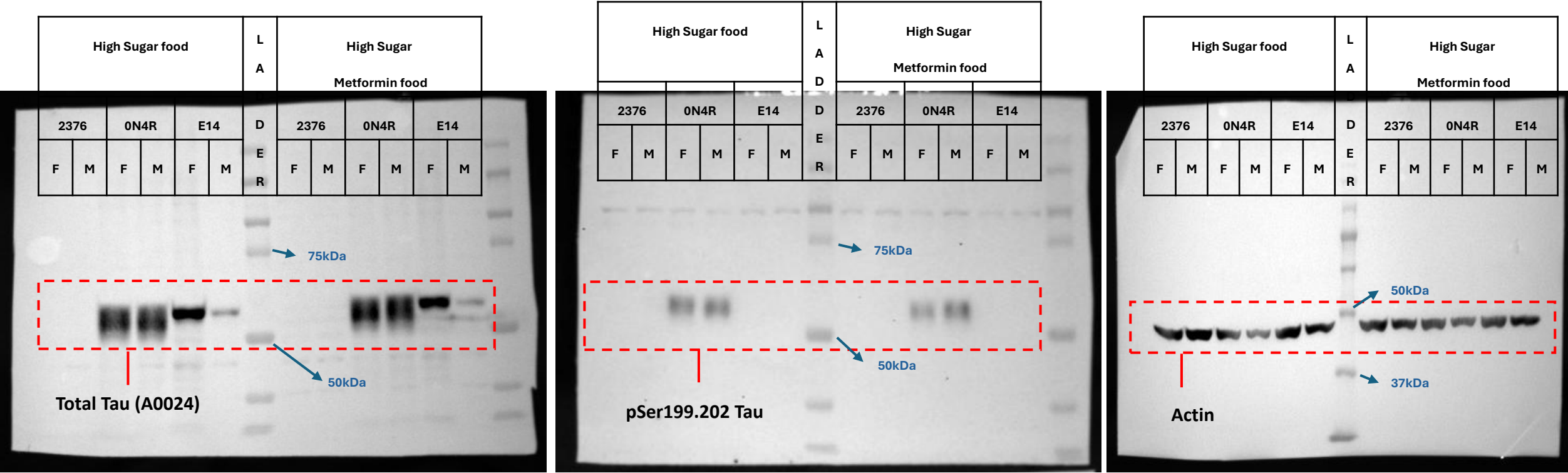
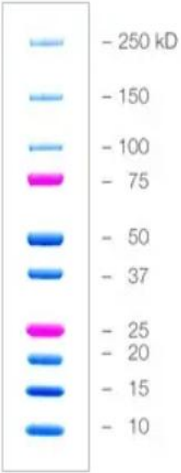
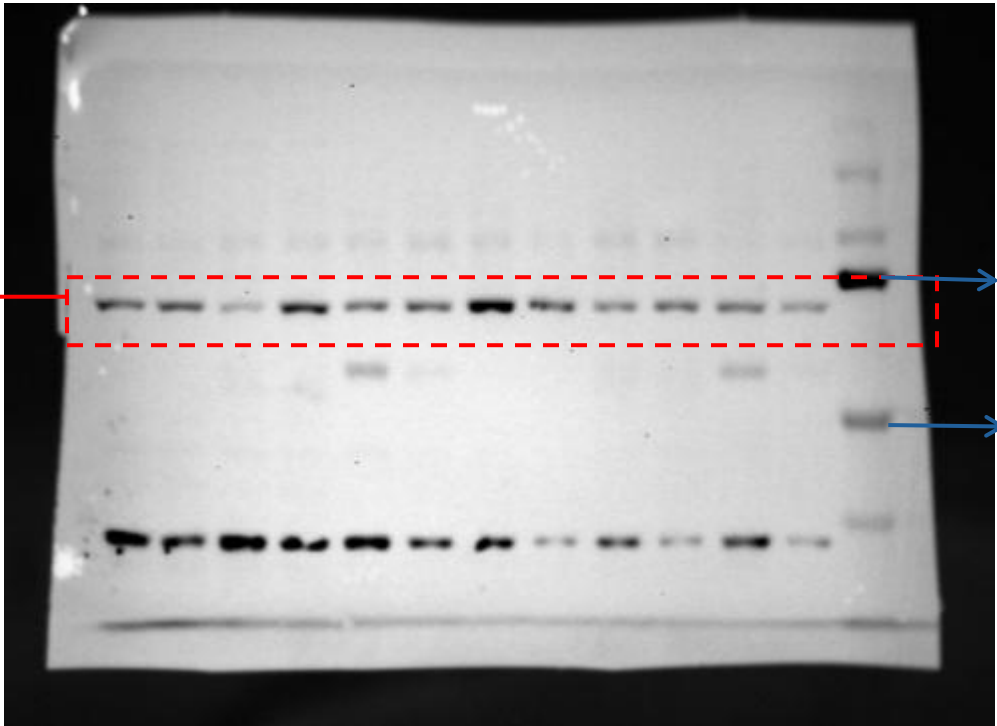


Figure 8 Upper Panel full blot of Ref 2P

Normal food						Metformin food					
2376		0N4R		E14		2376		0N4R		E14	
F	M	F	M	F	M	F	M	F	M	F	M



Normal food						Metformin food					
2376		0N4R		E14		2376		0N4R		E14	
F	M	F	M	F	M	F	M	F	M	F	M

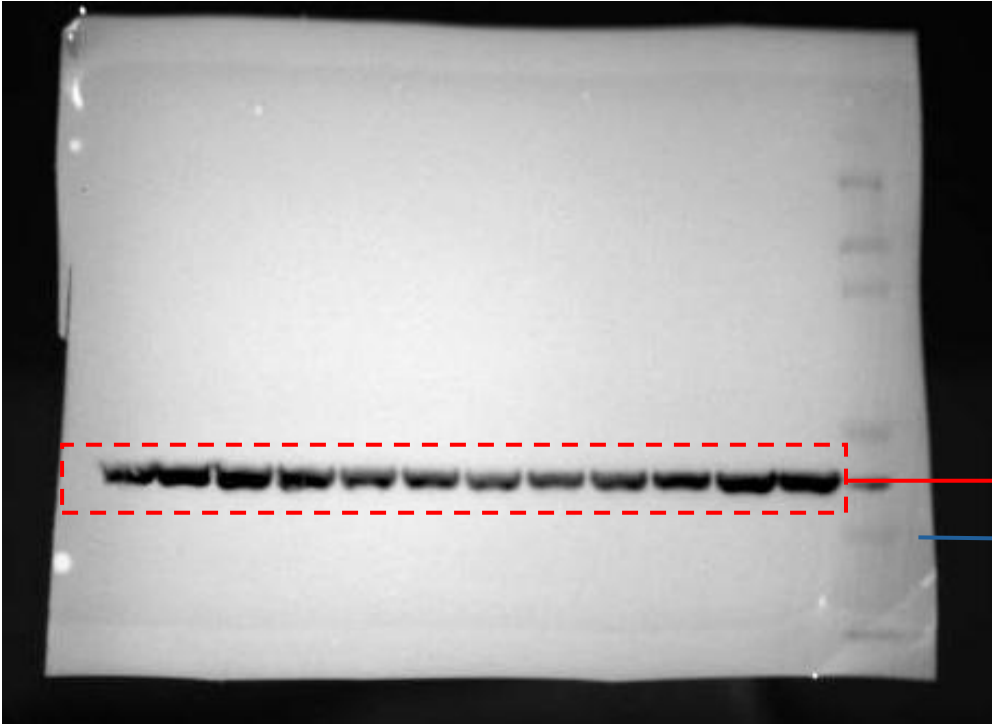
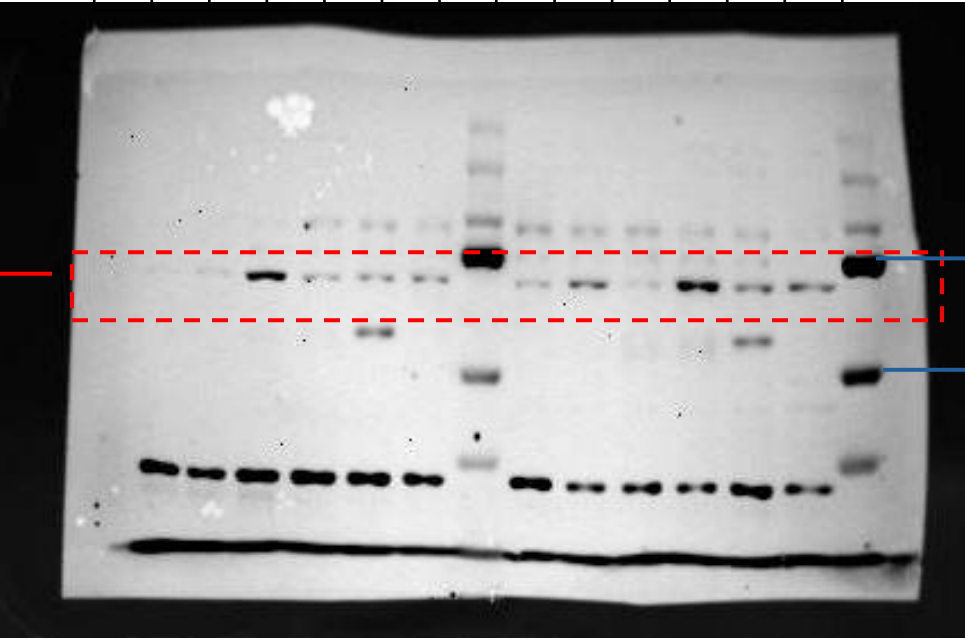
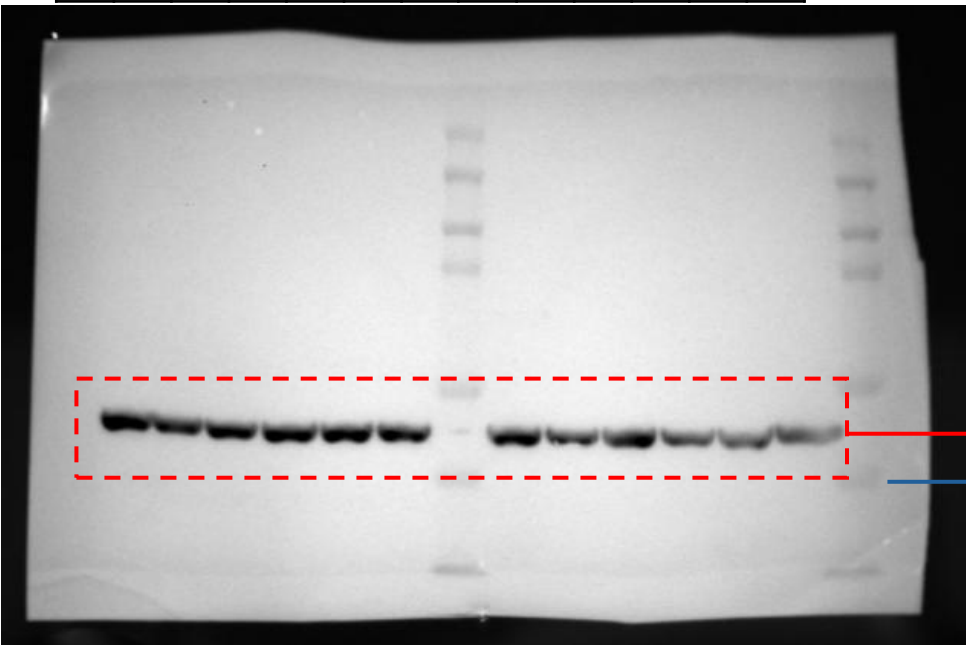


Figure 8 Lower Panel full blot of Ref 2P

High Sugar food						L A D D E R	High Sugar Metformin food					
2376		0N4R		E14			2376		0N4R		E14	
F	M	F	M	F	M		F	M	F	M	F	M



High Sugar food						L A D D E R	High Sugar Metformin food					
2376		0N4R		E14			2376		0N4R		E14	
F	M	F	M	F	M		F	M	F	M	F	M



SUPPLEMENTARY FIGURE LEGENDS

Fig.S1 Glucose uptake is impaired under insulin-resistant conditions. A Representative fluorescence images showing uptake of the fluorescent glucose analogue 2-NBDG in cells cultured in standard DMEM/F12, low-glucose (LG), and high-glucose (HG) media. Cells maintained in low-glucose conditions exhibit enhanced 2-NBDG uptake compared to high-glucose conditions, indicating increased glucose uptake under nutrient-limited environments. Quantification of 2-NBDG-positive cells demonstrates a significant reduction in glucose uptake in high-glucose medium relative to low-glucose conditions. Scale bars, as indicated. Data are presented as mean $\pm$ S.E.M. from independent experiments ($n = 3$). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. ns, not significant; $*p < 0.05$. **Dose-dependent effect of Metformin after cell viability assessed by MTT assay at 24 h time-course.** B SHSY5Y cells cultured in High Glucose (HG) and Low Glucose (LG) with increased concentration of Metformin (0mM to 100 mM) shows 80% viability at 20 mM Metformin, less than 50% viability with 50 mM Metformin and less than 25% viability with 100 mM Metformin respectively. Data shown are representative of 3 separate experiments and values are given as mean $\pm$ SD in (i) Insulin resistant (High glucose medium) and (ii) Insulin sensitive (Low glucose medium).

Fig.S2 3R and 4R tau expressions are significantly increased with Metformin treatment in a dose dependent manner. A Representative immunofluorescence images of 3R tau and 4R tau (red) with nuclear staining (DAPI, blue) in cells treated with increasing concentrations of Metformin (0, 2.5, 5, 10, and 20 mM). Metformin treatment results in a dose-dependent increase in intracellular tau for both 3R and 4R isoforms. Scale bars, 44.3 μ m. Data are presented as mean $\pm$ S.E.M from independent experiments ($n = 5$). Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc test, with $**p < 0.01$, $****p < 0.0001$. **Hyperphosphorylated tau is significantly decreased upon Metformin treatment in 0N4R-Tau-EGFP transfected SHSY5Y cells.** B (i) and (ii) Representative western blots of total and phosphorylated tau (pSer199/202 and pSer202/Thr205) in tau-0N4R-EGFP and total tau in tau-E14-EGFP transfected SH-SY5Y cells respectively. Quantification of band intensities normalised to Actin is shown (bottom). Data are presented as mean $\pm$ S.E.M, $n=3$. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. ns, not significant; $*p < 0.05$; $***p < 0.001$; $****p < 0.0001$.

Fig.S3 Metformin inhibits mTOR signalling and induces autophagy. A Representative western blots phospho-mTOR (p-mTOR), total mTOR and Actin in SH-SY5Y cells treated with Bafilomycin, Rapamycin or Metformin (0–20 mM). Quantification of p-mTOR/mTOR normalised to Actin respectively following treatment with Bafilomycin followed by Metformin (0–20 mM). Data are presented as mean $\pm$ S.E.M, $n=3$. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. $*p < 0.05$. **High-dose Metformin activates autophagic flux in the presence of Bafilomycin.** B Representative western blots of LC3-I/LC3-II and p62 normalised to Actin in SH-SY5Y cells treated with increased Metformin concentrations in the presence of Bafilomycin. Quantification of LC3-II/LC3-I ratio and p62

normalised to Actin respectively following treatment with Bafilomycin followed by Metformin (0–20 mM). Data are presented as mean $\pm$ S.E.M, n=3. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. *p < 0.05; **p < 0.01.

Fig. S4. High sugar diet induces insulin-resistance in tau transgenics A and B, Representative western blots of phospho and total AKT normalised to Actin in controls, tau-0N4R, and tau-E14 flies under normal and high-sugar diets supplemented with and without Metformin. A Quantification of pAKT/AKT in normal food with and without Metformin. In controls and tau-0N4R transgenics, supplementing the normal food with Metformin elevated the pAKT/AKT ratio indicating an insulin-sensitive state although this alteration was not significant. B Quantification of pAKT/AKT in High Sugar Diet (HSD) with and without Metformin. In HSD both tau lines displayed significant reduction in pAKT/AKT levels with Metformin supplementation. Data are mean $\pm$ SEM (n = 3); one-way ANOVA with Tukey's post-hoc test. ns, not significant; *p< 0.05; **p< 0.01; ***p< 0.001; ****p< 0.0001.

Fig. S5: Full Western Blots of Main Figure 1A showing total Tau (A0024 and T46) at 65kDa and β -Actin (47kDa) after stripping the blots. Molecular Weight marker shown in the Nitrocellulose membrane.

Fig. S6: Full Western blots of Main Figure 2A showing hyperphosphorylated Tau (pSer199/202) at 75kDa and pSer202/Thr205 at 150kDa. β -Actin is the loading control.

Fig. S7: Full Western blots of Main Figures 3A, 3B and 3C showing phospho-AKT and total AKT (60 kDa), phospho-GSK3 β and total GSK3 β (47 kDa), DMPP2A-C and total PP2A-C (37kDa) bands. β -Actin is the loading control.

Fig. S8: Full Western blots of Main Figure 5B showing LC3-II/LC3-1 (15kDa and 10kDa), p62 (50kDa) bands. β -Actin is the loading control.

Fig. S9: Full Western Blots of Main Figure 7Q (Upper and Lower panels) showing total Tau (A0024) and hyperphosphorylated tau (pSer199/202) at 65kDa and β -Actin loading controls in *Drosophila* Tau lines in Normal and High Sugar diet with and without Metformin in female and male flies respectively.

Fig. S10: Full Western Blots of Main Figure 8 (Upper and Lower panels) showing Ref2(P) (65kDa) and β -Actin loading controls in *Drosophila* Controls and Tau lines in Normal and High Sugar diet with and without Metformin in female and male flies respectively.