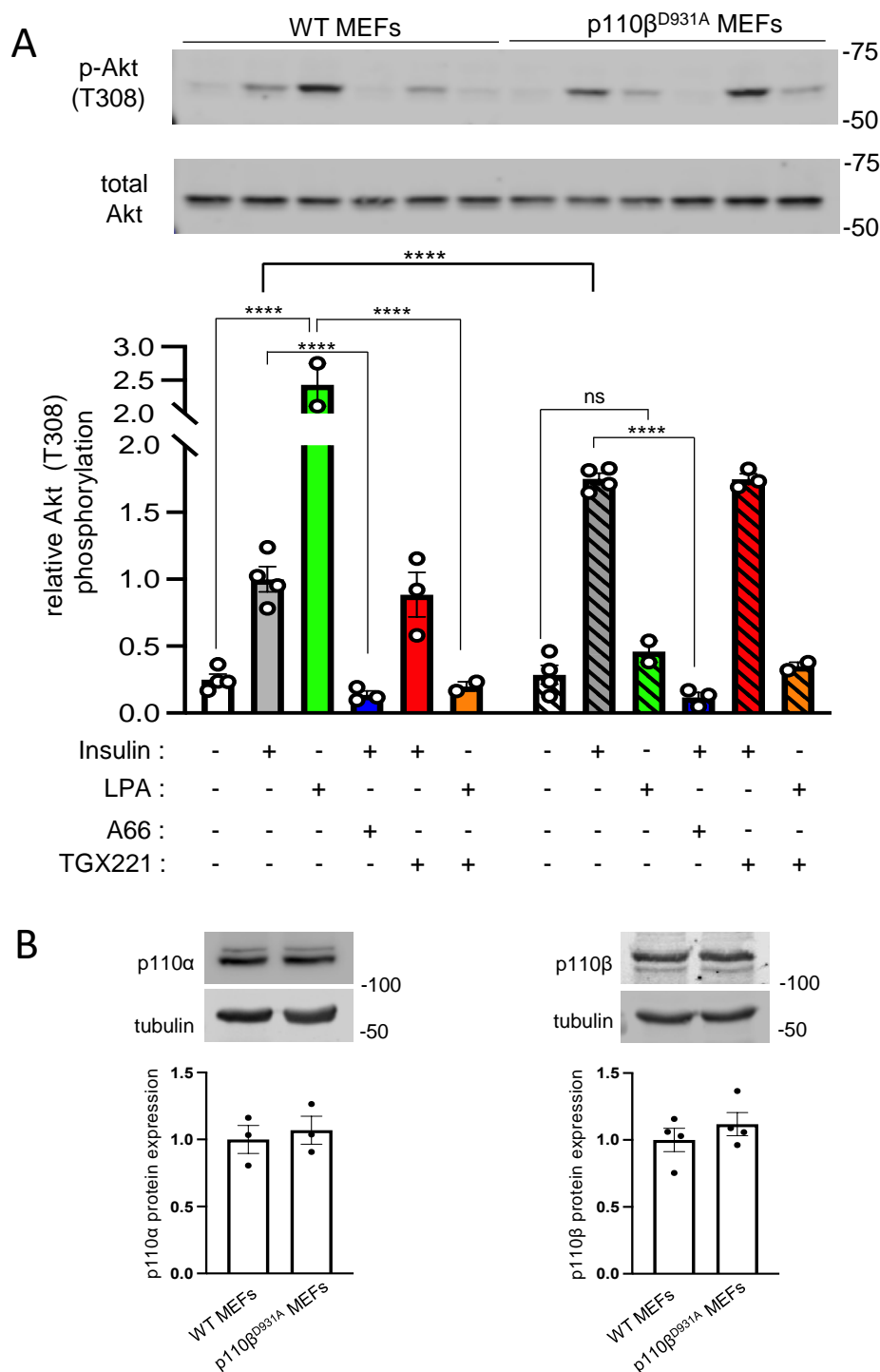


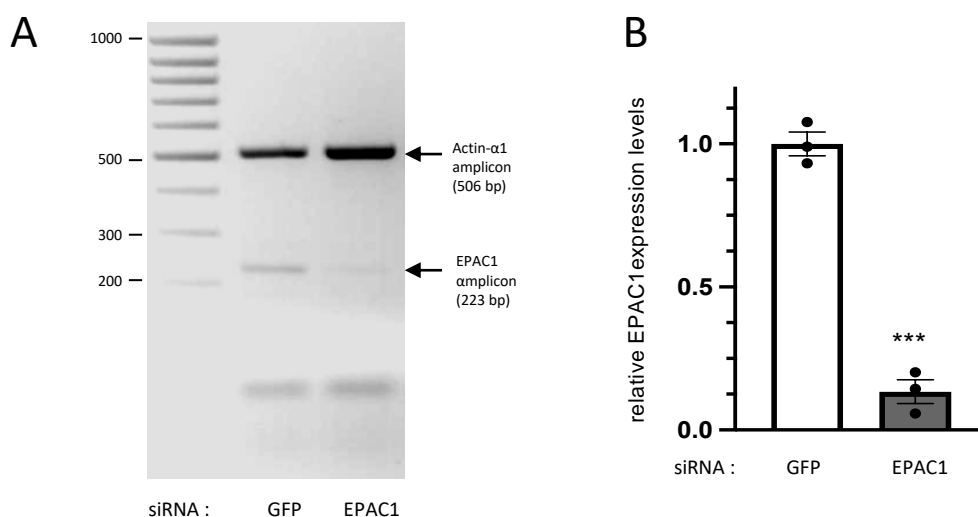
# Supporting Information

Zhang et al. 'Dominant role of PI3K p110 $\alpha$  over p110 $\beta$  in insulin and  $\beta$ -adrenergic receptor signalling'

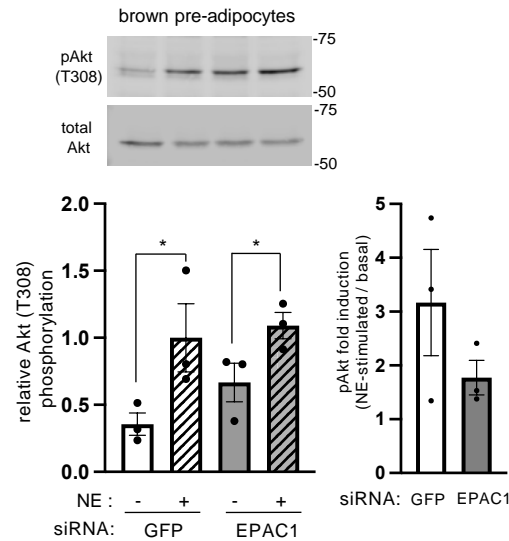
This file contains Supplementary Figures S1-S3 and original, uncropped blots for Figures 1-5 and S1-S3.



**Figure S1 p110 $\alpha$  is the principal PI3K isoform engaged in insulin signalling in MEFs.** Wild-type (WT) and p110 $\beta$ <sup>D931A</sup> MEFs were treated with A66 (1  $\mu$ M) or TGX221 (0.5  $\mu$ M) or a combination of both inhibitors followed by stimulation with 10 nM of insulin for 15 min at 37°C. **(A)** Levels of Akt (T308) phosphorylation were determined by immunoblot analysis. Phosphorylation levels were normalised to total Akt levels detected in a second blot performed in parallel using the same lysates. Treatment with LPA (10  $\mu$ M), which is known to activate Akt via p110 $\beta$ , was used as a control of lack of p110 $\beta$  activity in p110 $\beta$ <sup>D931A</sup> MEFs **(B)** The levels of expression of p110 $\alpha$  and p110 $\beta$  in WT and p110 $\beta$ <sup>D931A</sup> MEFs were also determined by immunoblot analysis. p110 $\alpha$  and p110 $\beta$  signal intensities were normalised to tubulin. Representative immunoblots and corresponding bar graphs with data from three or four (n=3-4) independent experiments are shown. Data are presented as mean  $\pm$  SEM. Statistical analysis was performed by two-way ANOVA with Sidak's multiple comparisons test **(A)** or by unpaired two-tailed t-test **(B)**.



**Figure S2 siRNA-mediated EPAC1 knock-down in brown pre-adipocytes.** Levels of EPAC1 mRNA were determined 72 h following transfection of siRNAs targeting either GFP (control) or EPAC1 by RT-PCR and densitometric quantification of the EPAC1 amplicon's intensity following agarose gel electrophoresis. Actin- $\alpha$ 1 amplification was used as a loading control (A). (B) Average EPAC1 mRNA expression upon knock-down with EPAC1 siRNA was 13% of the control (GFP siRNA). Data are derived from three independent experiments and presented as mean  $\pm$  SEM. Statistical analysis was performed by unpaired two-tailed t-test.



**Figure S3 EPAC1 siRNA-mediated knockdown reduces NE-stimulated Akt fold activation.**

Brown pre-adipocytes were transfected with the EPAC1 siRNA or GFP esiRNA control. 72 h later, cells were serum-deprived for 3h followed by stimulation with 1  $\mu$ M norepinephrine (NE) for 15 min at 37°C. Levels of Akt T308 phosphorylation were determined by immunoblot analysis.

Phosphorylation levels were normalised to total Akt levels detected in a second blot performed in parallel using the same lysates. A representative immunoblot and a bar graph with data from three (n=3) independent experiments are shown. Values from the bar graph have been used for calculation of NE-stimulated Akt phosphorylation fold induction shown in the right-hand bar graph. Data are presented as mean  $\pm$  SEM. Statistical analysis was performed by two-way ANOVA with Tukey's multiple comparisons test.

Figure 1A: Brown pre-adipocytes pAkt (T308) blot

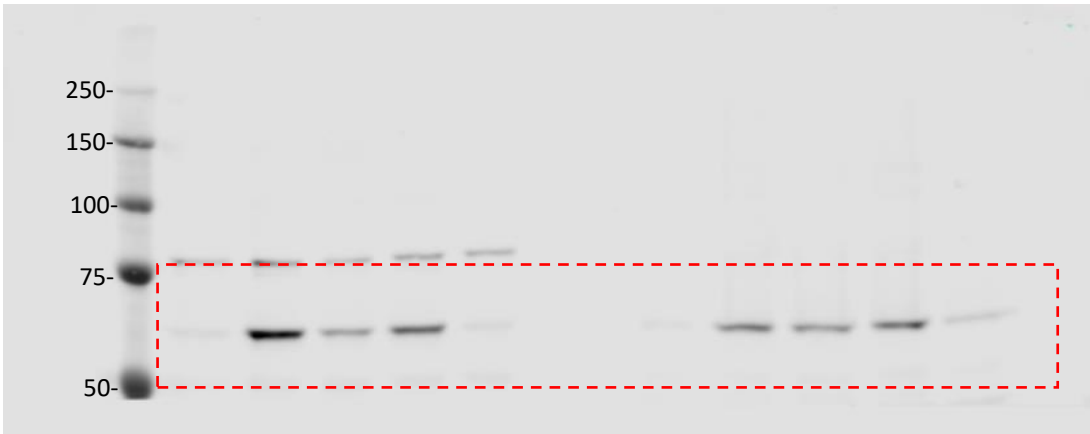


Figure 1A: Brown pre-adipocytes total Akt blot

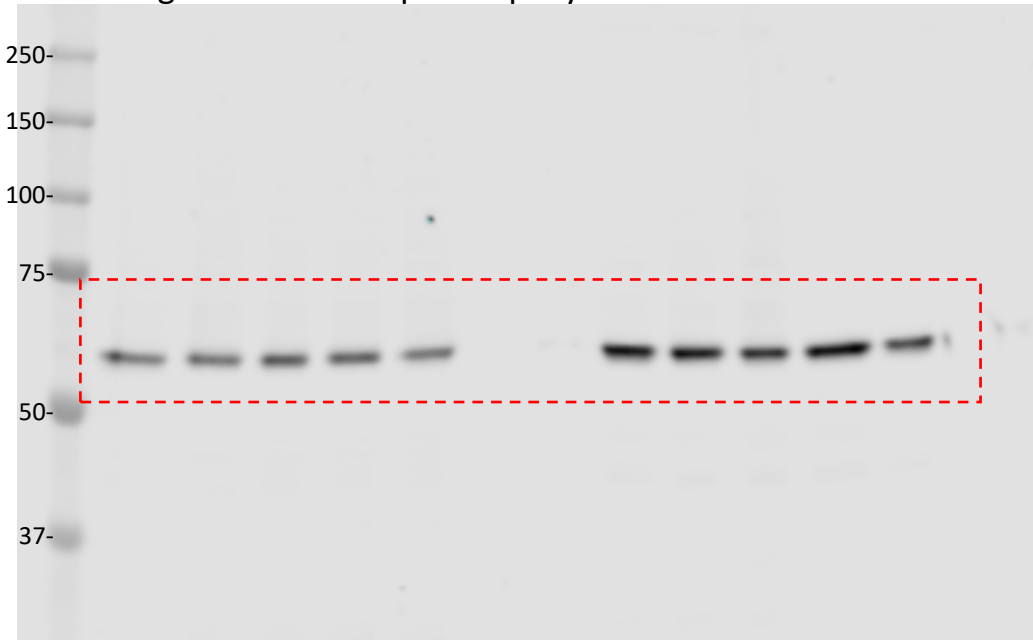


Figure 1B: Brown pre-adipocytes p-rpS6 (S240/244) blot

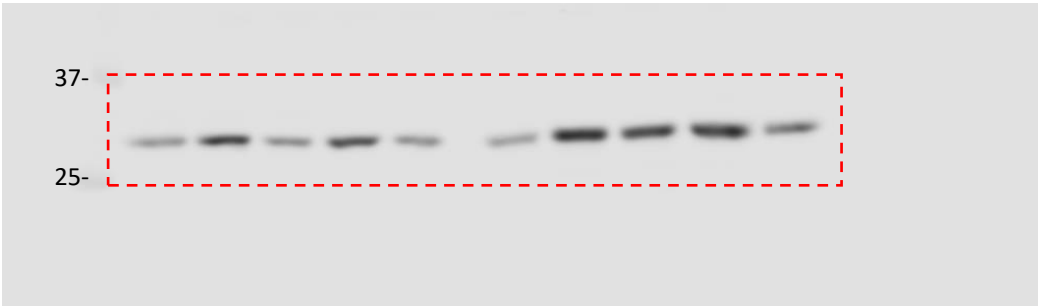


Figure 1B: Brown pre-adipocytes total rpS6 blot

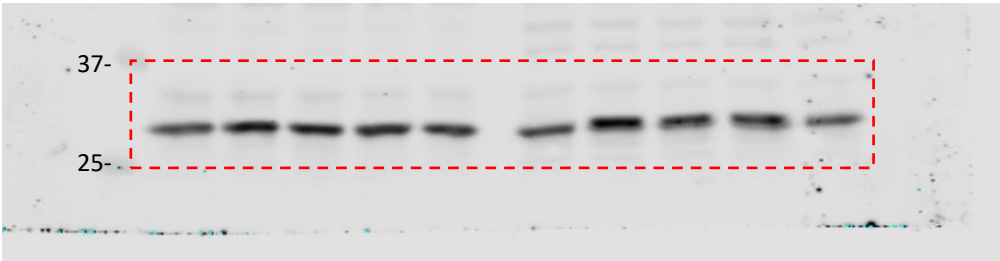
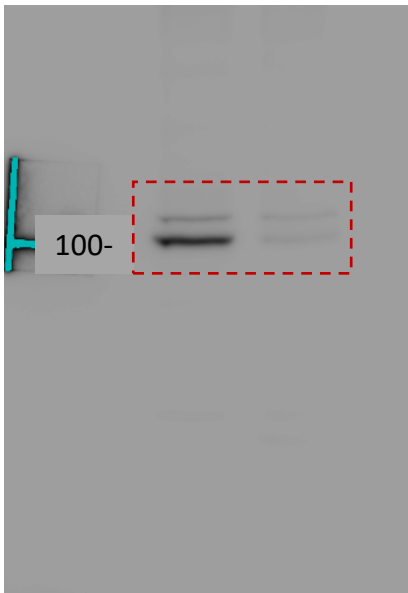


Figure 1C: Brown pre-adipocytes  
p110 $\alpha$  blot



Brown pre-adipocytes  
vinculin blot for p110 $\alpha$

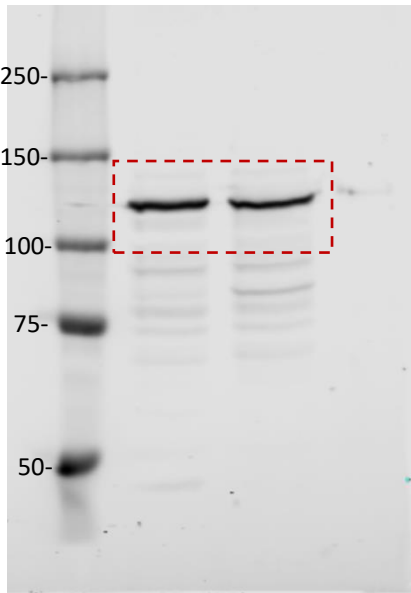
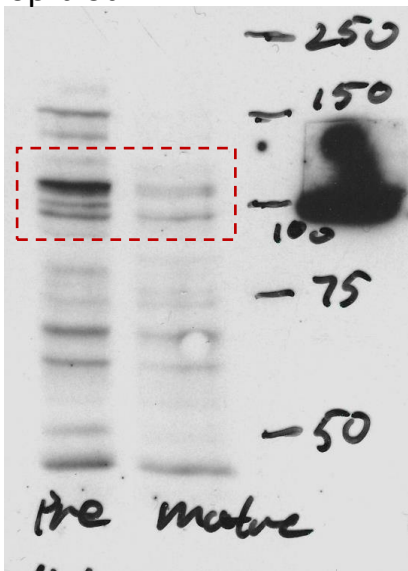


Figure 1C: Brown pre-adipocytes  
p110 $\beta$  blot



Brown pre-adipocytes  
vinculin blot for p110 $\beta$

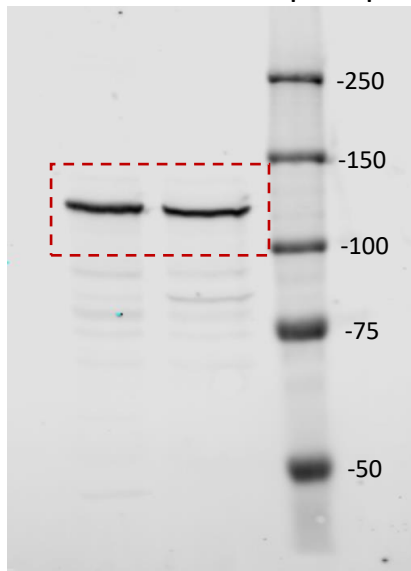


Figure 1D: Brown adipocytes pAkt blot

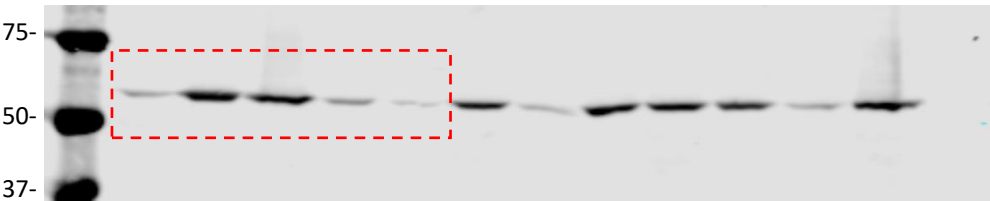


Figure 1D: Brown adipocytes total Akt blot

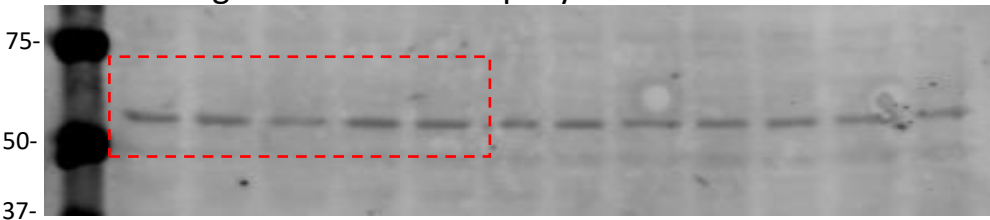


Figure 2A: 3T3-L1 pre-adipocytes pAkt (T308) blot

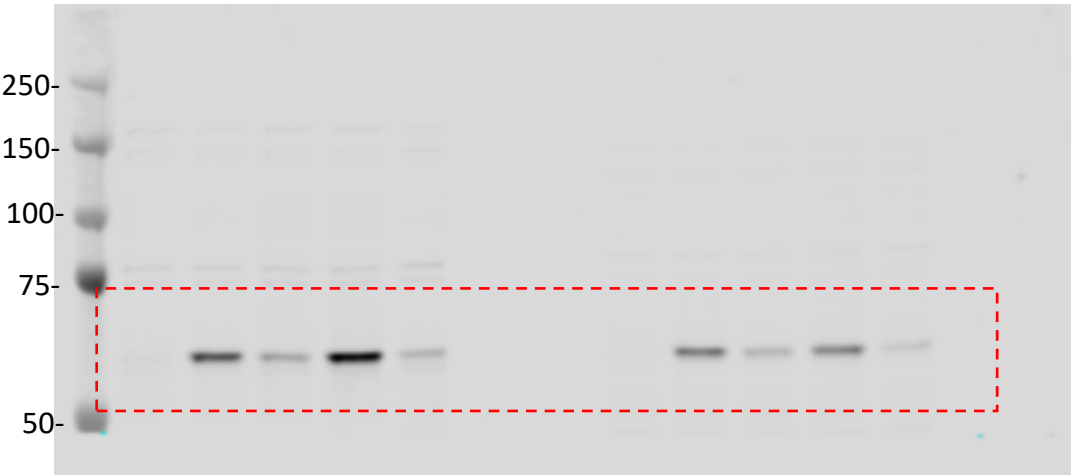


Figure 2A: 3T3-L1 pre-adipocytes total Akt blot

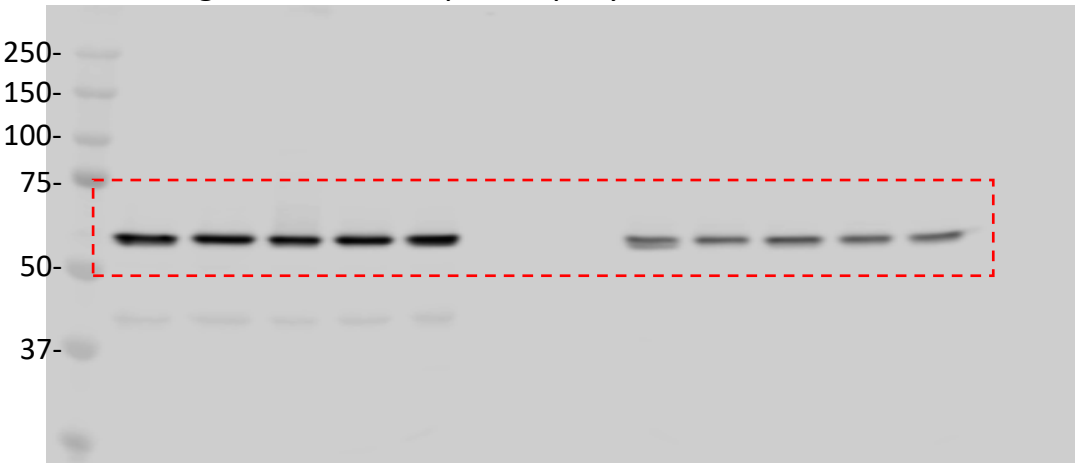


Figure 2B: 3T3-L1 pre-adipocytes p-rpS6 (S240/244) blot

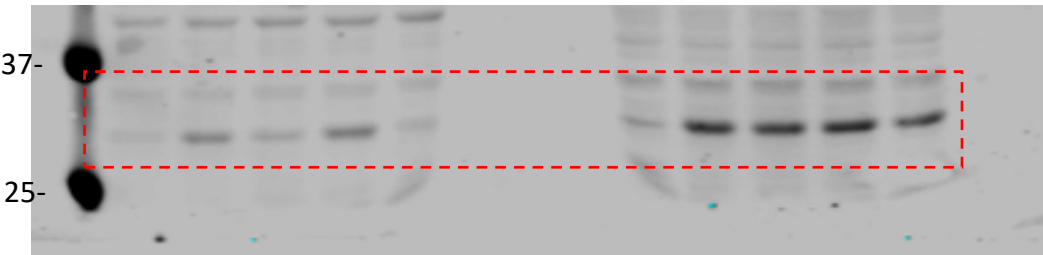


Figure 2B: 3T3-L1 pre-adipocytes total rpS6 blot

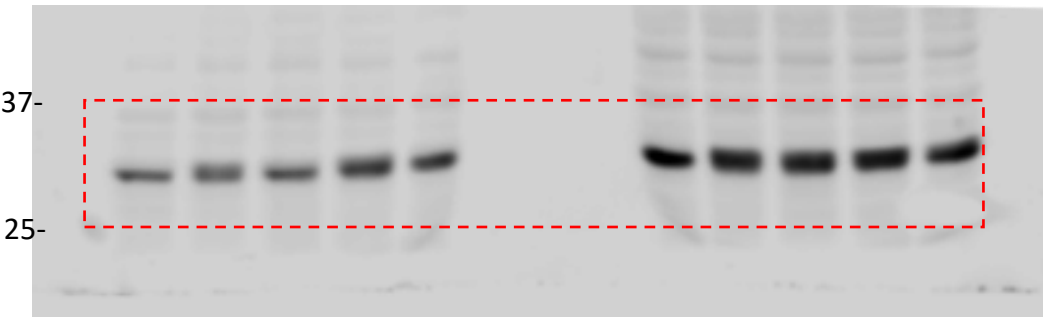
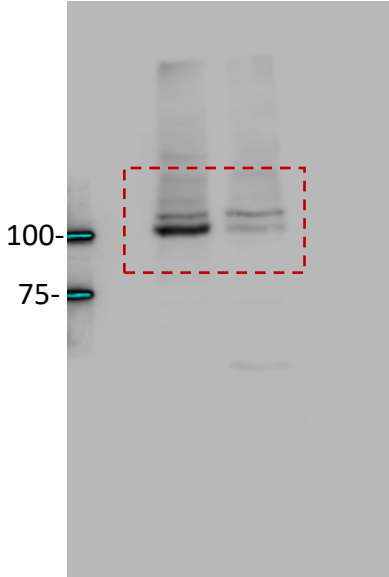


Figure 2C: 3T3-L1 pre-adipocytes  
p110 $\alpha$  blot



3T3-L1 pre-adipocytes  
vinculin blot for p110 $\alpha$

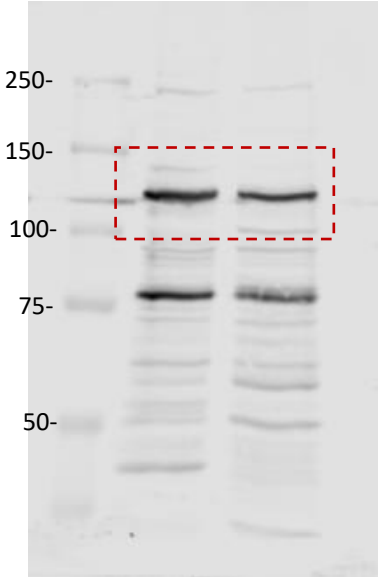
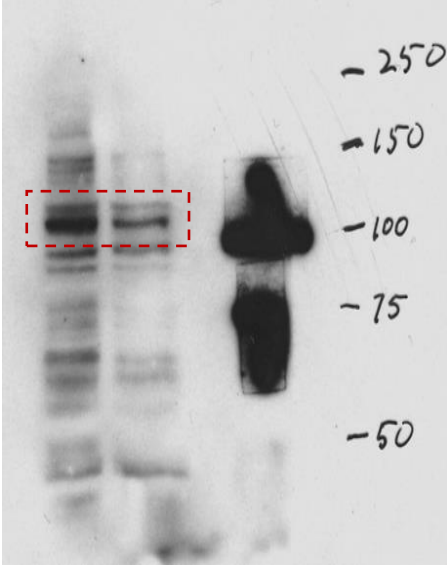


Figure 2C: 3T3-L1 pre-adipocytes  
p110 $\beta$  blot



3T3-L1 pre-adipocytes  
vinculin blot for p110 $\beta$

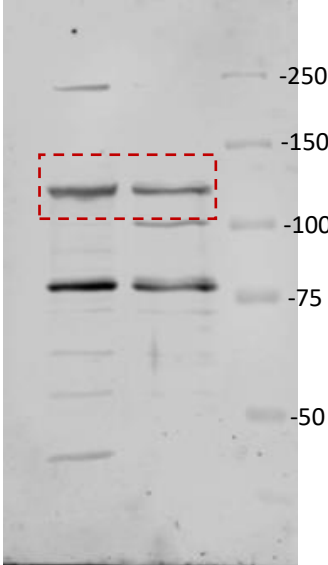




Figure2D: Hepa 1-6 pAkt blot

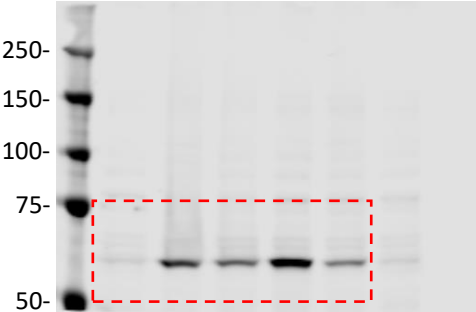


Figure2D: Hepa 1-6 p-rpS6 blot

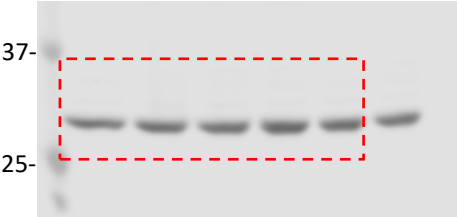


Figure2D: Hepa 1-6 total Akt blot

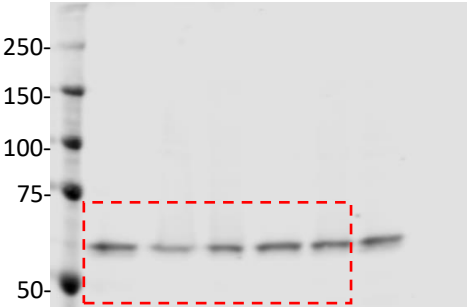


Figure2D: Hepa 1-6 total rpS6 blot

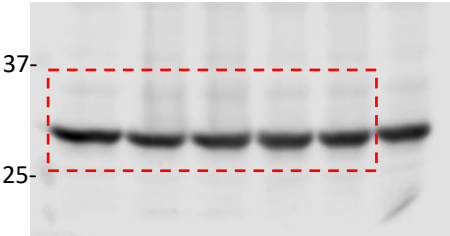


Figure 3A: p110 $\alpha$ -RBD-MEFs pAkt blot



Figure 3A: p110 $\alpha$ -RBD-MEFs vinculin blot



Figure 3B: p110 $\alpha$ -RBD-MEFs p-rpS6 blot

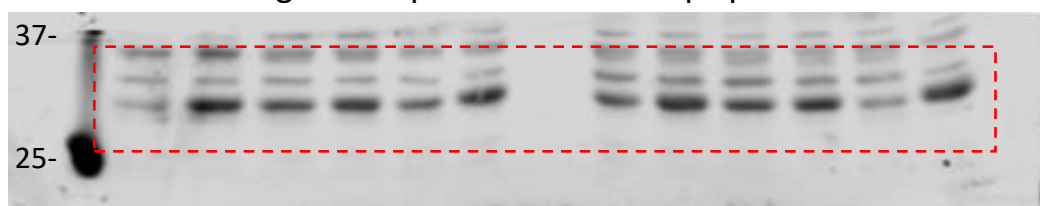


Figure 3B: p110 $\alpha$ -RBD-MEFs total rpS6 blot

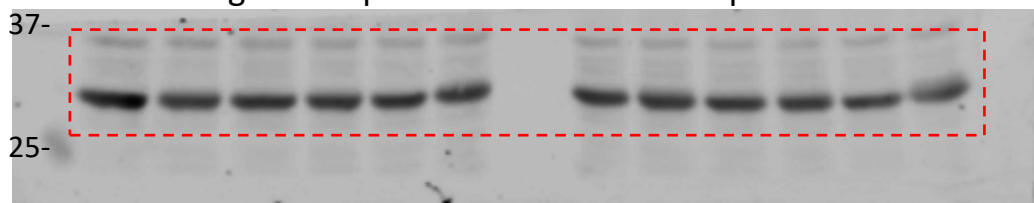


Figure 3C: p110 $\alpha$ -RBD-MEFs pERK1/2 blot

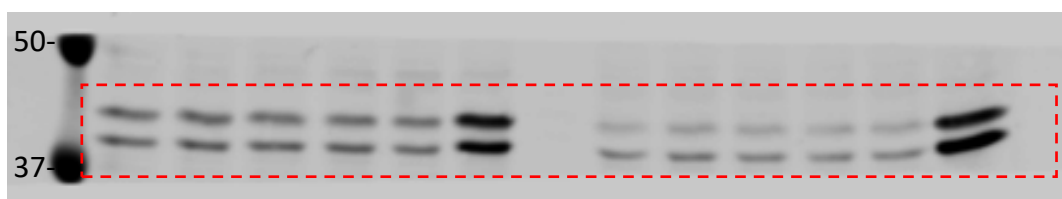


Figure 3C: p110 $\alpha$ -RBD-MEFs total ERK1/2 blot

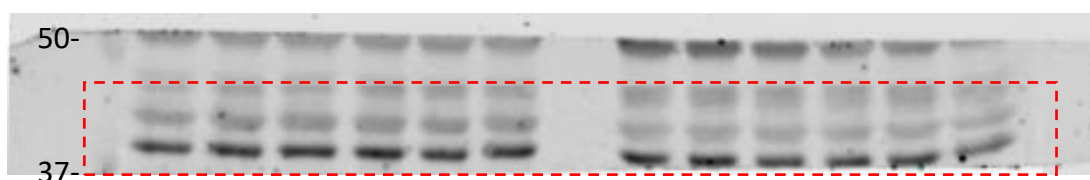


Figure 4A: Brown pre-adipocytes pAkt blot

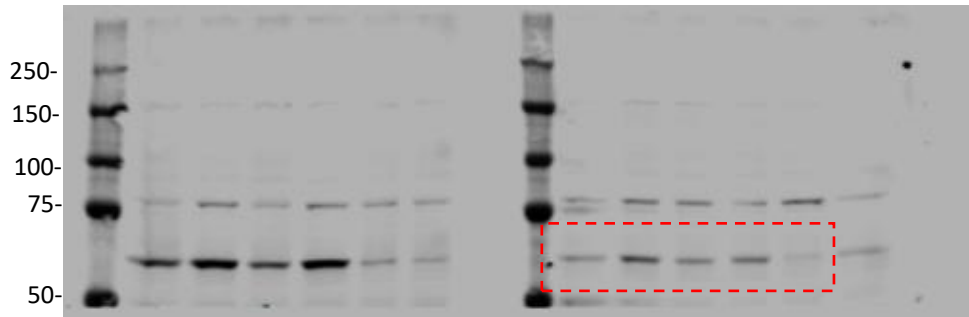


Figure 4A: Brown pre-adipocytes total Akt blot

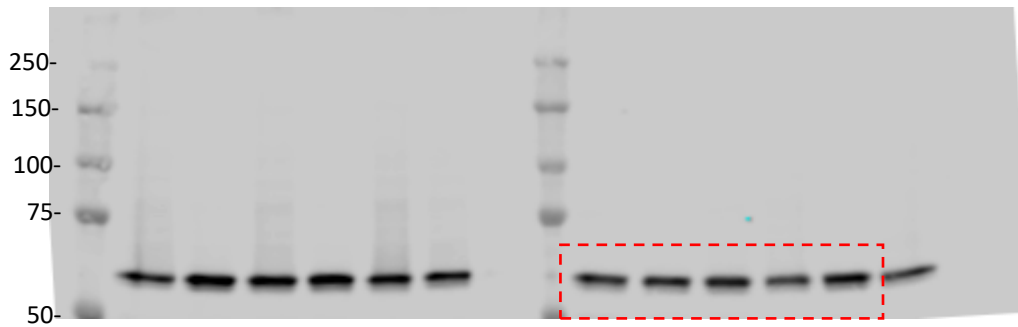


Figure 4B: Brown adipocytes pAkt blot

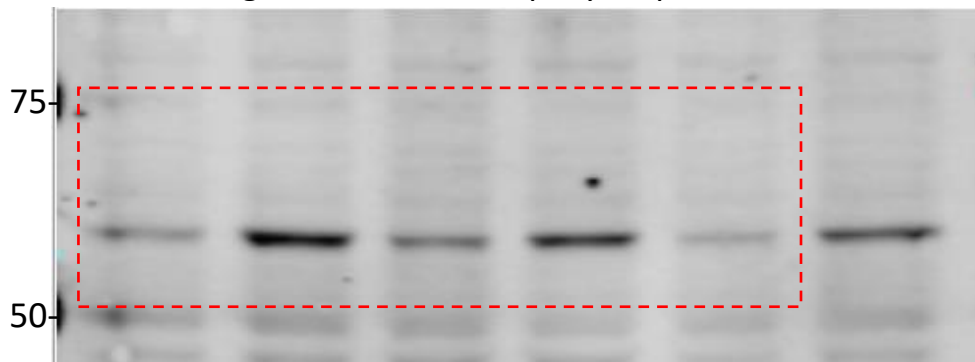


Figure 4B: Brown adipocytes total Akt blot

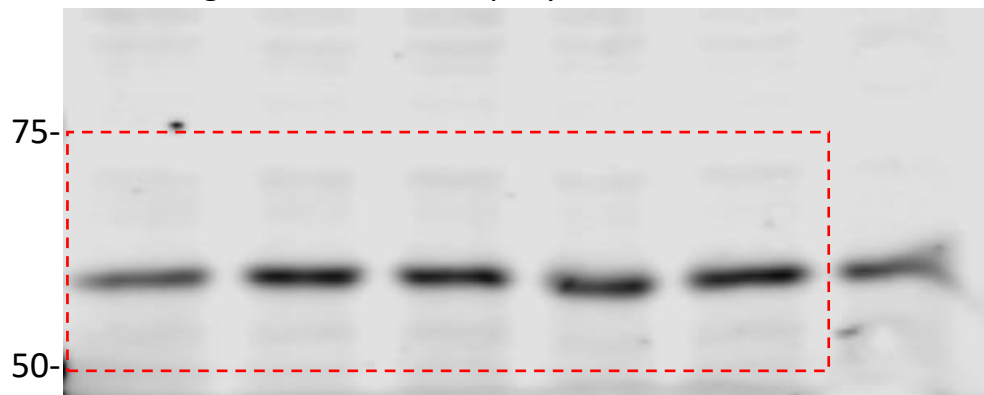


Figure 4C: 3T3-L1 adipocytes pAkt blot



Figure 4C: 3T3-L1 adipocytes total Akt blot

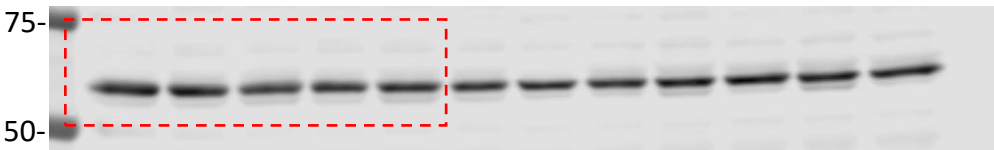


Figure 5A: Brown pre-adipocytes pAkt (T308) blot

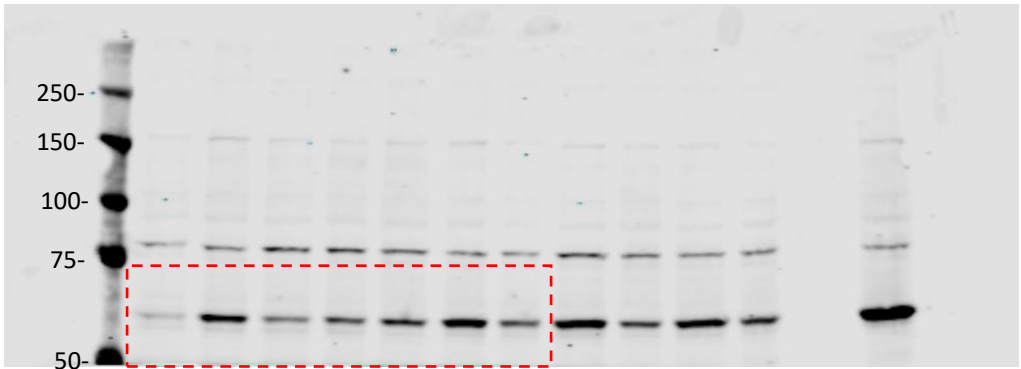


Figure 5A: Brown pre-adipocytes total Akt blot

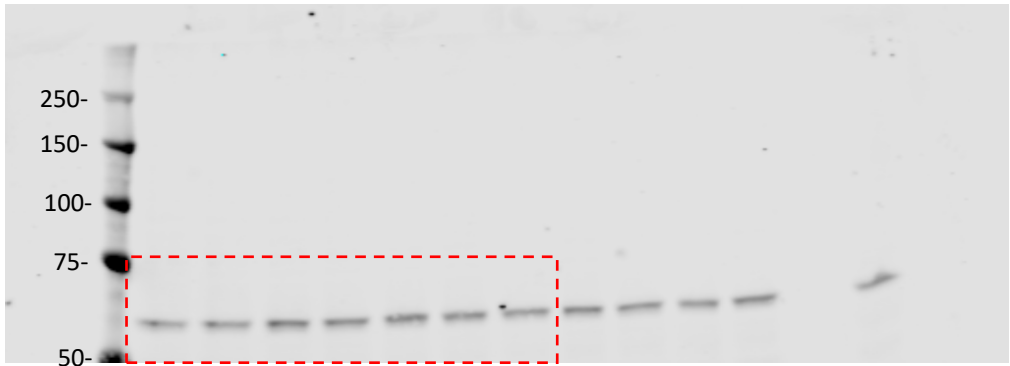


Figure 5B: Brown pre-adipocytes pAkt (T308) blot

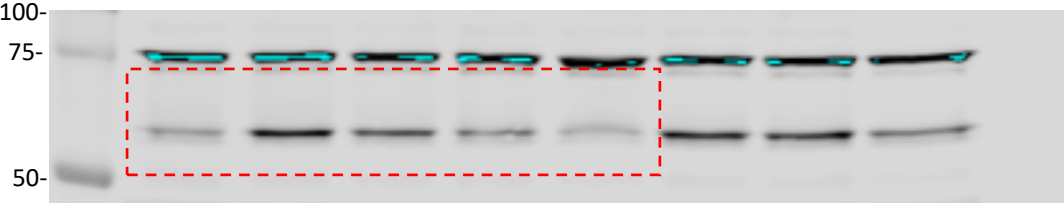


Figure 5B: Brown pre-adipocytes total Akt blot

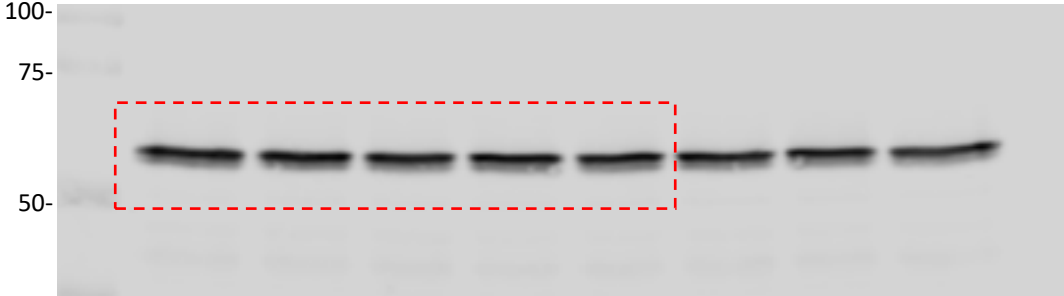


Figure S1: p110 $\beta$ <sup>D931A</sup> MEFs pAkt (T308) blot

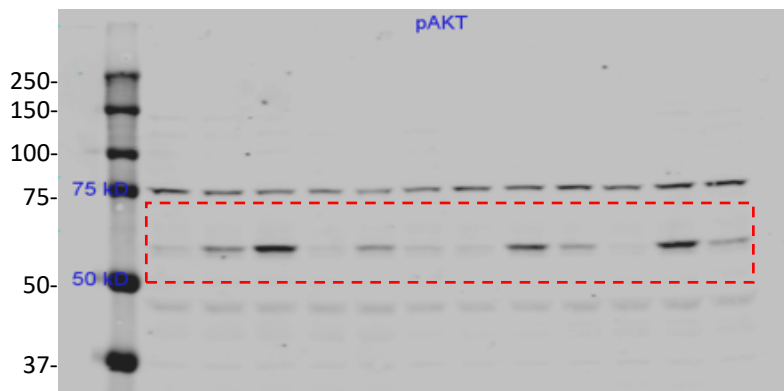


Figure S1: p110 $\beta$ <sup>D931A</sup> MEFs total Akt blot

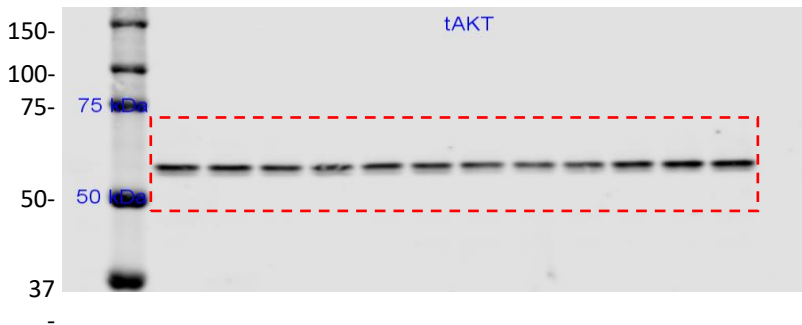


Figure S1: p110 $\beta$ <sup>D931A</sup> MEFs p110 $\alpha$  blot

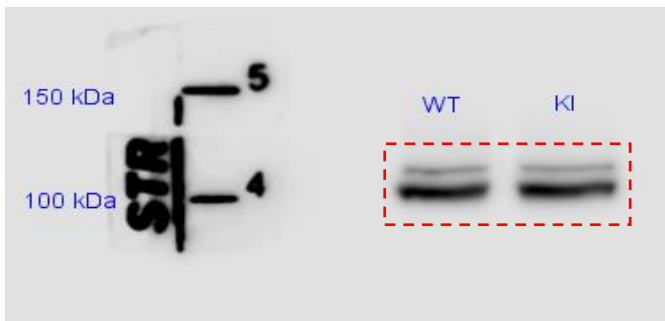


Figure S1: tubulin probing for p110 $\alpha$  blot

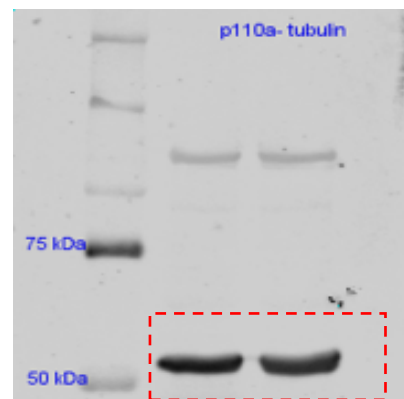


Figure S1: p110 $\beta$ <sup>D931A</sup> MEFs p110 $\beta$  blot

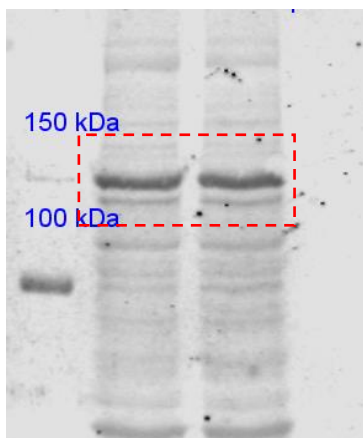


Figure S1: tubulin probing for p110 $\beta$  blot

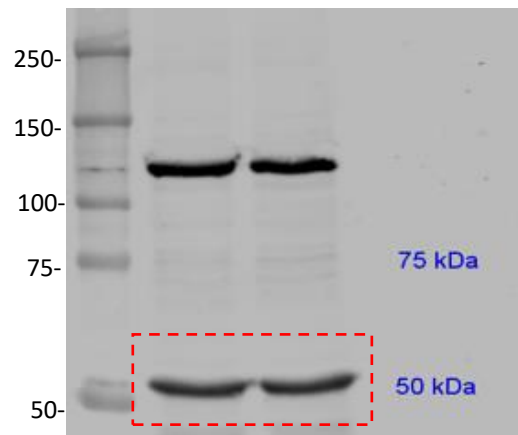


Figure S3: Brown pre-adipocytes pAkt (T308) blot

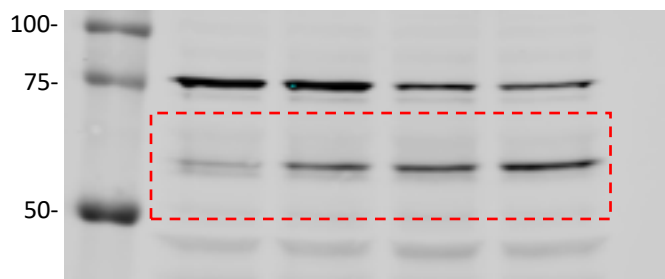


Figure S3: Brown pre-adipocytes total Akt blot

