**Akt/mTOR integrate energy metabolism with Wnt signal to influence wound epithelium growth in *Gekko Japonicus***

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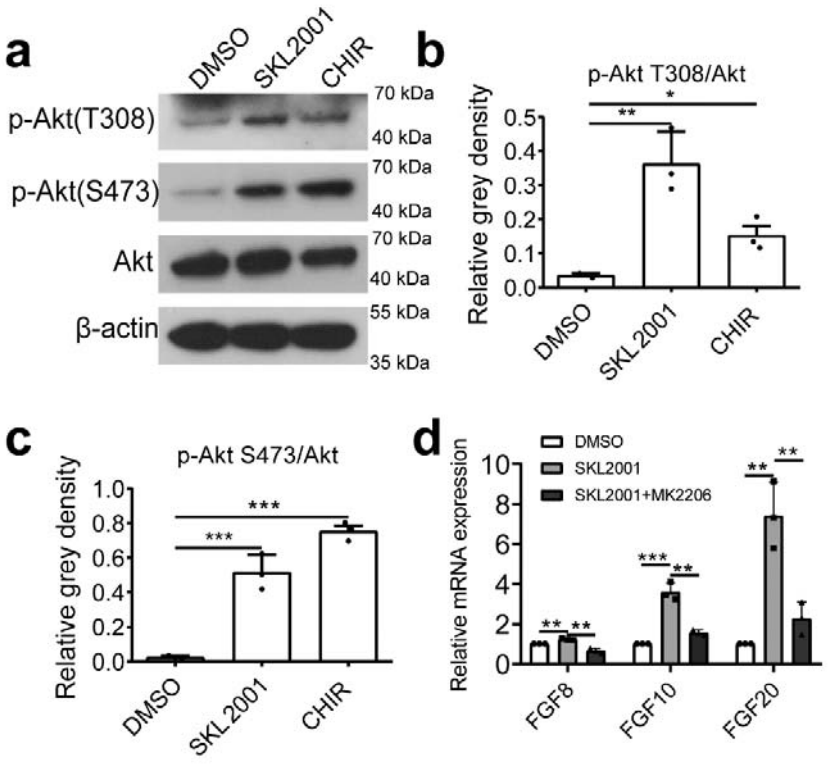
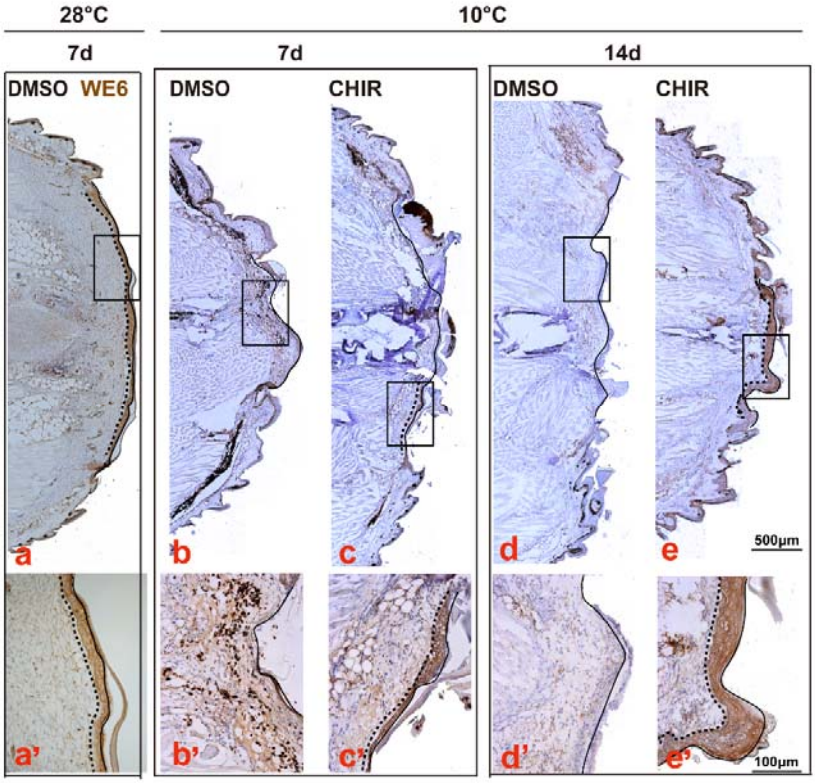
**Supplementary figures**

**Supplementary Figure 1 Wnt pathway promoted Akt activity and enhanced FGFs expression in blastema cells**



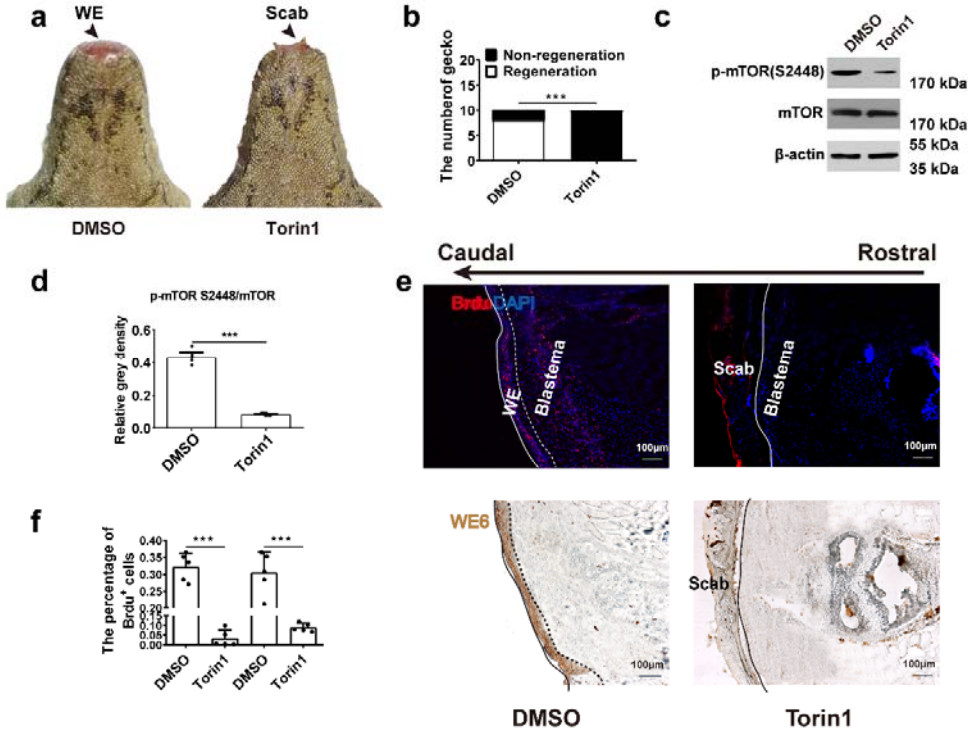

**a**, Wnt agonist (SKL2001 or CHIR) promoted Akt phosphorylation in cultured blastema cells. **b-c**, Relative level of p-Akt (Thr308)/Akt and p-Akt (Ser473)/Akt in **Supplementary Fig. 1a**. **d**, The transcriptional expression of FGF8, FGF10 and FGF20 were enhanced in SKL2001 treated blastema cells, while attenuated by administration of MK-2206 (Akt antagonist), N=3. Values represent mean ± SD, $^{ns}p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Supplementary Figure 2 Wnt agonist partially rescued the deficit of wounding epithelium formation in low temperature during tail regeneration.**




**a**, Wounding epithelium positively stained with WE6 antibody were observed at day 7 post tail amputation in normal temperature. **b-c**, The epithelium formation was inhibited in low temperature, while the treatment of Wnt agonist CHIR gave rise to partial formation of epithelium at day 7 post tail amputation in low temperature. **d-e**, The treatment of CHIR that produced partial epithelium could not proceed and complete regeneration after 14 days post amputation in low temperature condition. **a'-e'** were the enlarged views of boxed areas in **a-e**, respectively. Solid line indicates the caudal border of wound epithelium, dotted line indicates border between wound epithelium and blastema.

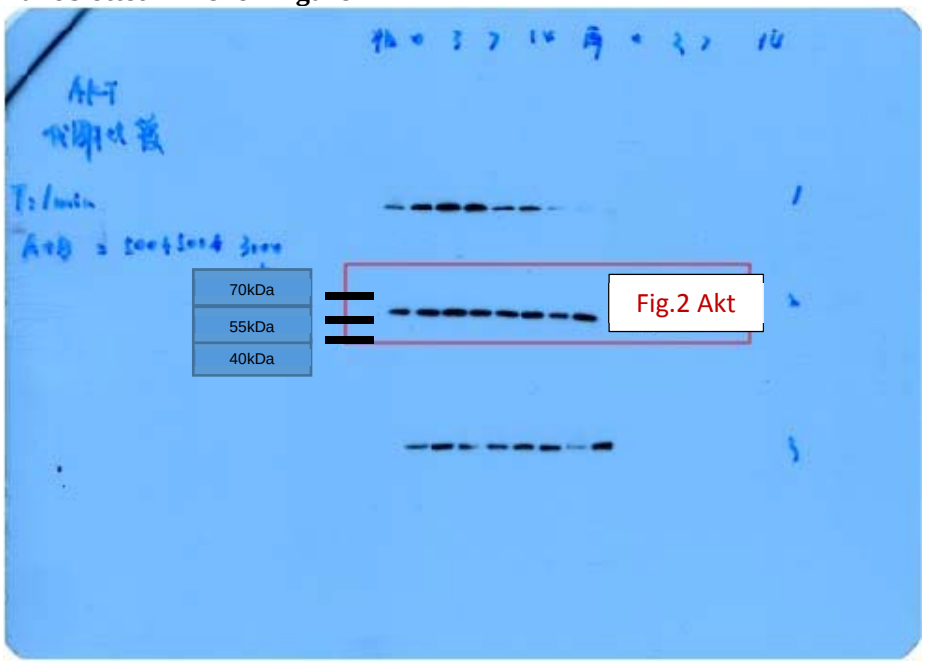
**Supplementary Fig. 3 mTOR inhibition by Torin1 hindered the regeneration wound epithelium**

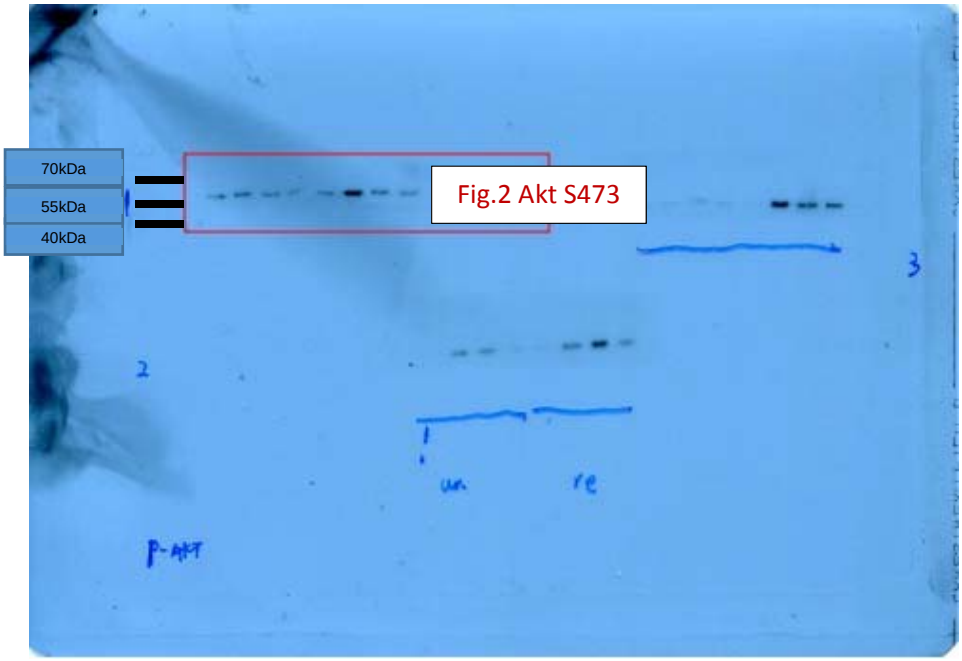


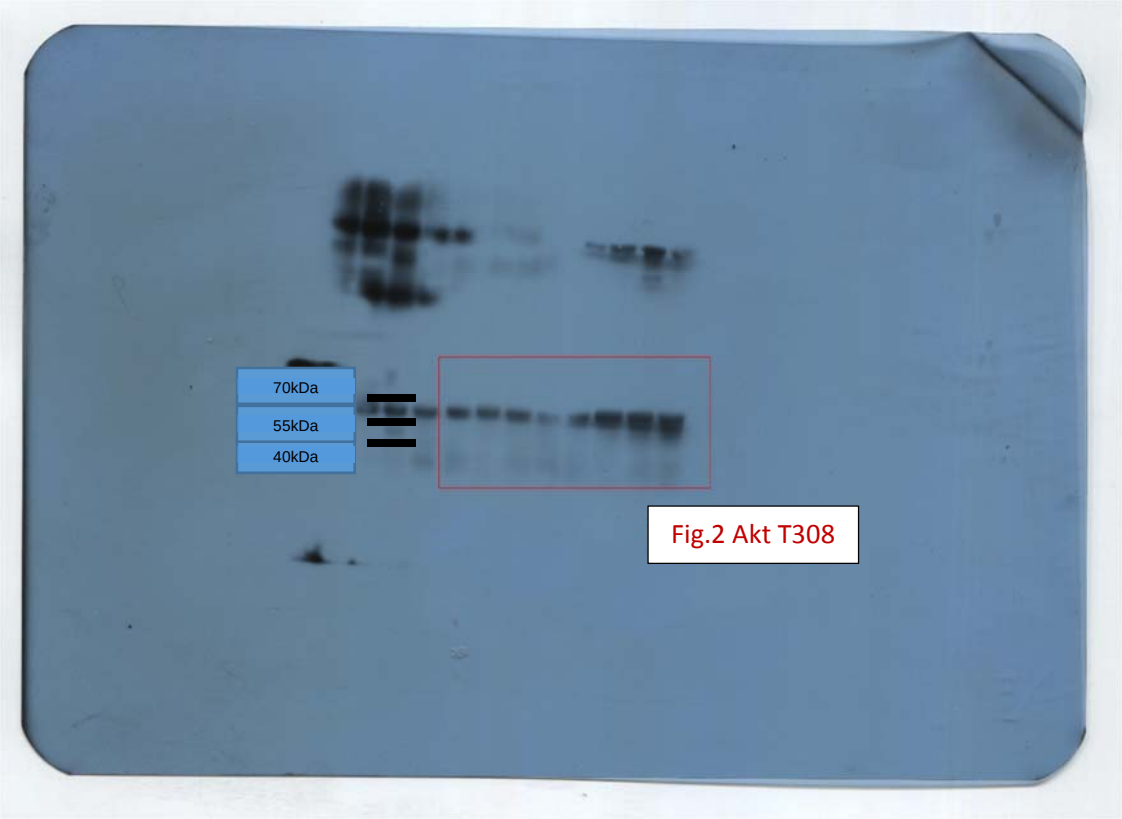

**a**, Formation of wound epithelium (WE) in the control (DMSO) and mTOR inhibition groups (Torin1). **b**, Number of geckos with regeneration in control and Torin1 groups. N=10. **c**, Protein level of p-mTOR (Ser2448), and mTOR in tail tissues. **d**, Relative level of p-mTOR (Ser2448)/mTOR. N=3. **e**, Immunofluorescence analysis of BrdU staining in DMSO and Torin1 group (upper panel). Immunohistochemical analysis of WE6 staining in the same location of the samples (lower panel). Scale bar = 100μm. Solid line indicates border between wound epithelium and scab, and dotted line indicates border between rostral end of wound epithelium and blastema. **f**, Number of BrdU positive cells in control and mTOR inhibition groups. N = 5. Values represent mean ± SD, ns $p > 0.05$, *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Supplementary Fig. 4 Uncropped immunoblots**

**Raw immunoblotted films for Figure 2**




70kDa
55kDa
40kDa
Fig.2 Akt T308

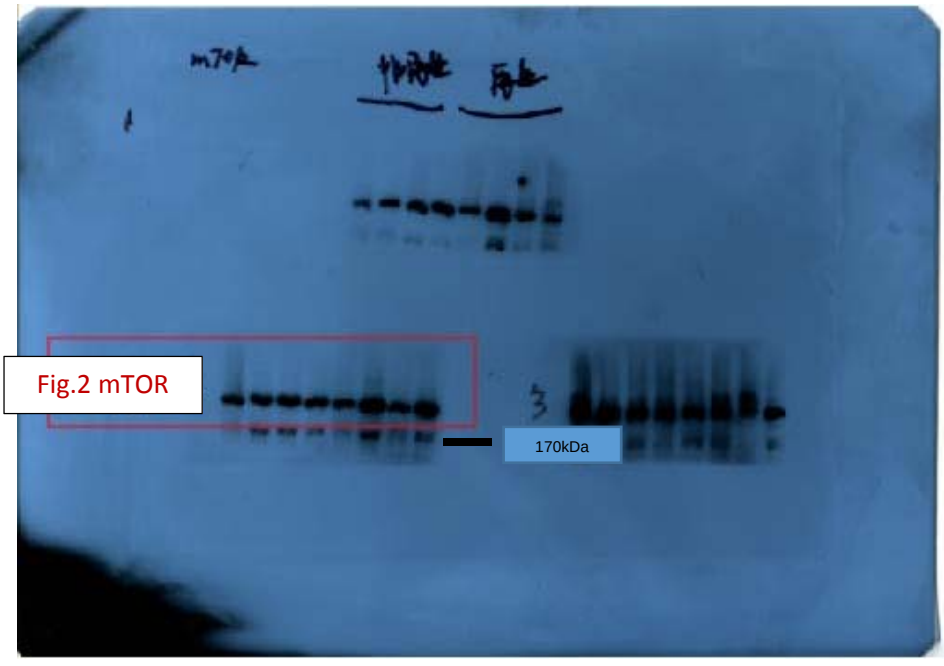


Fig.2 mTOR
170kDa


Fig.2 p-mTOR S2448

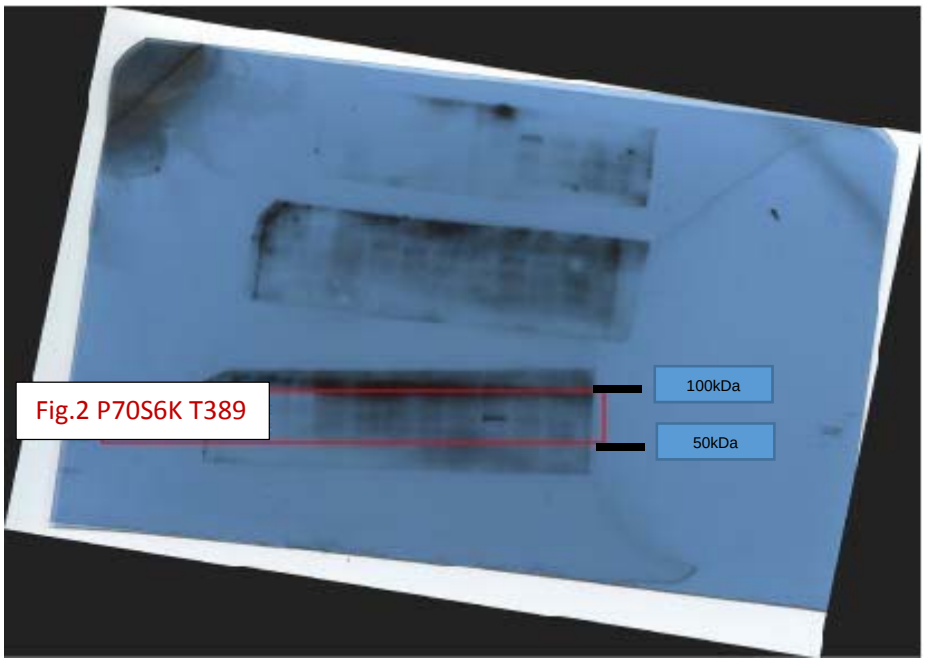
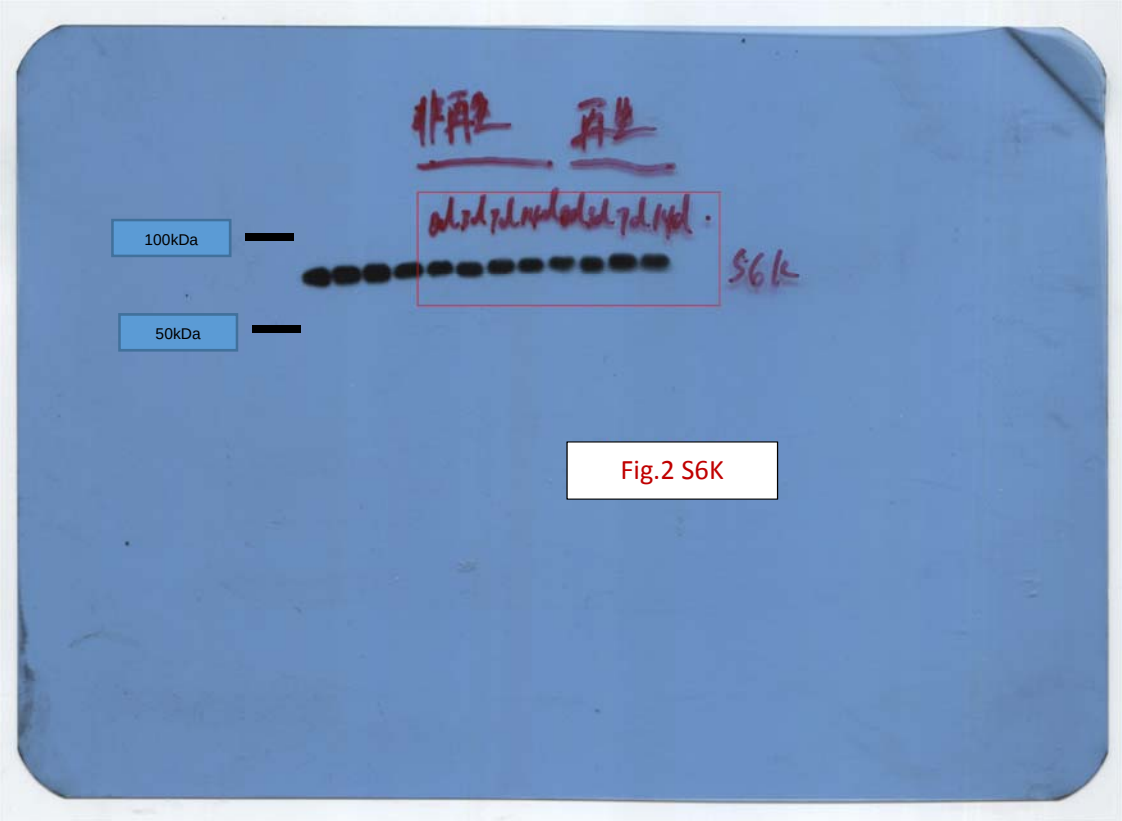
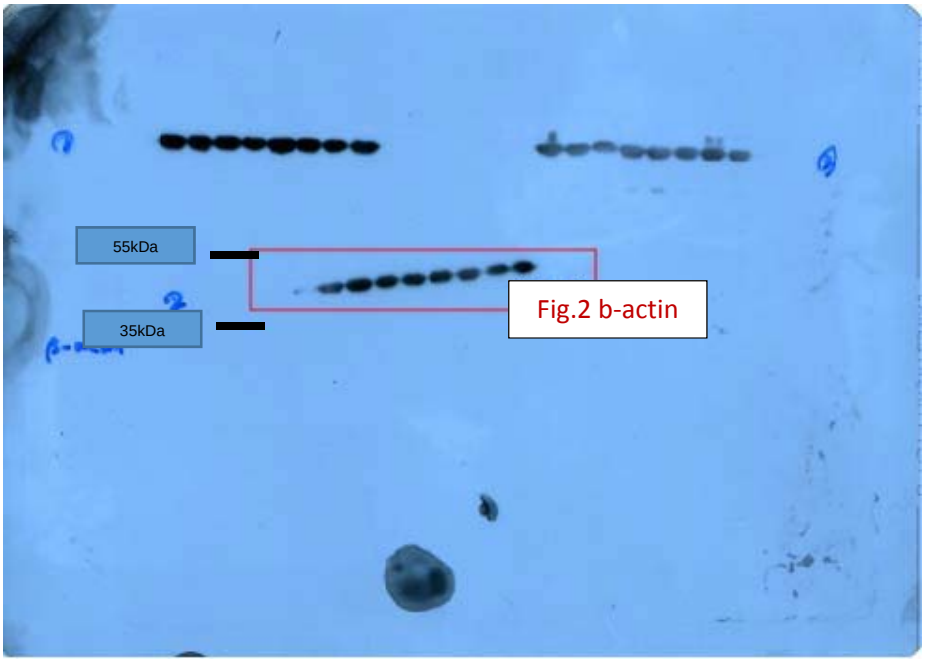
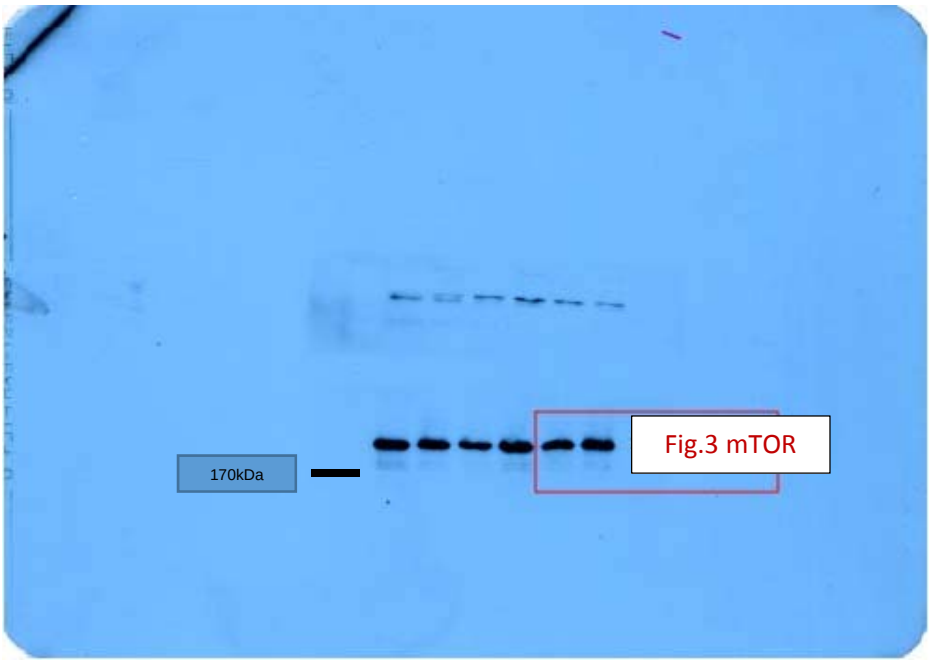



Fig.2 P70S6K T389


100kDa
50kDa
S6K
Fig.2 S6K


55kDa
35kDa
Fig.2 b-actin

**Raw immunoblotted films for Figure 3**

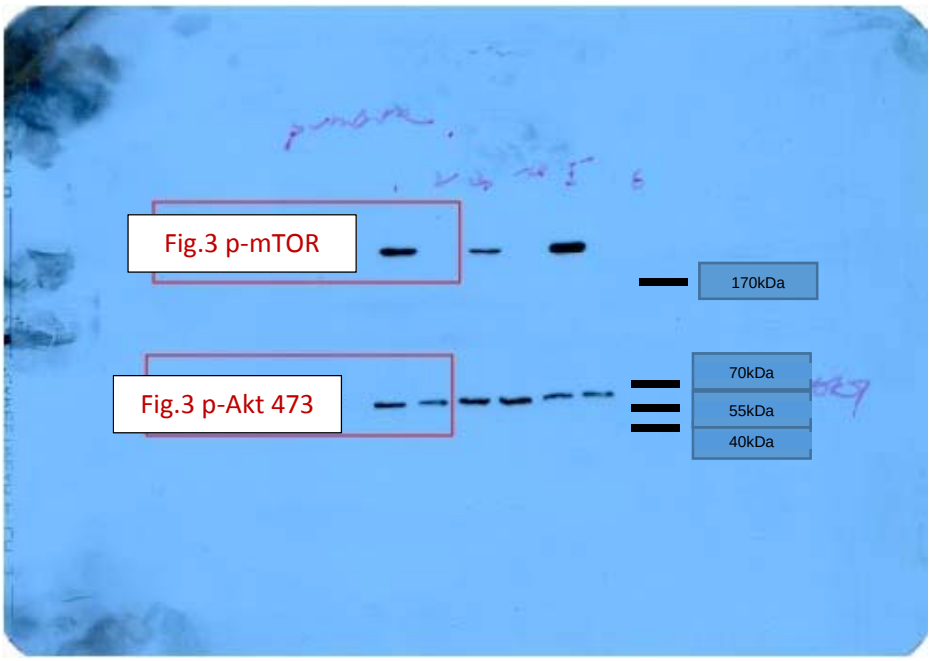
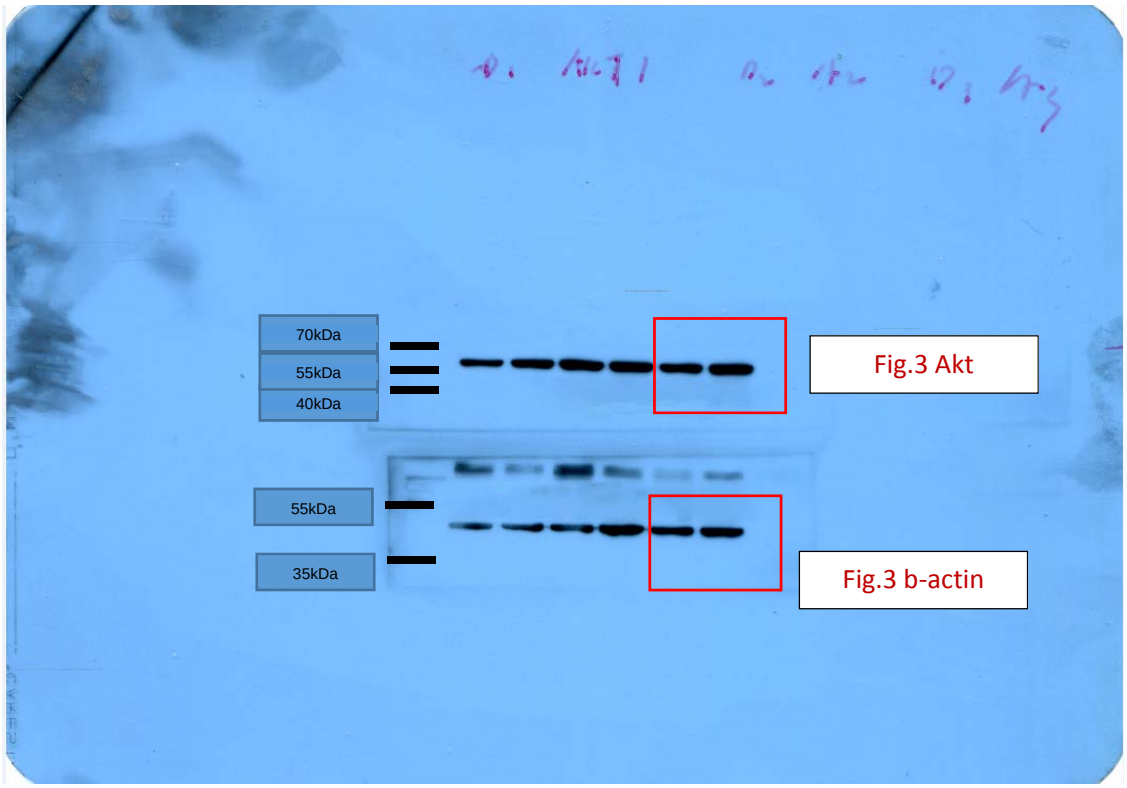
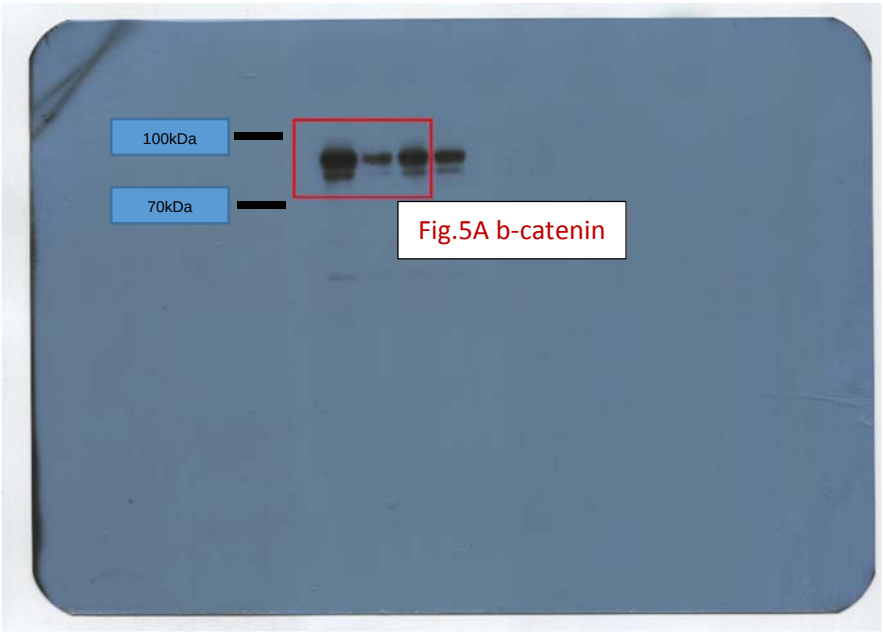


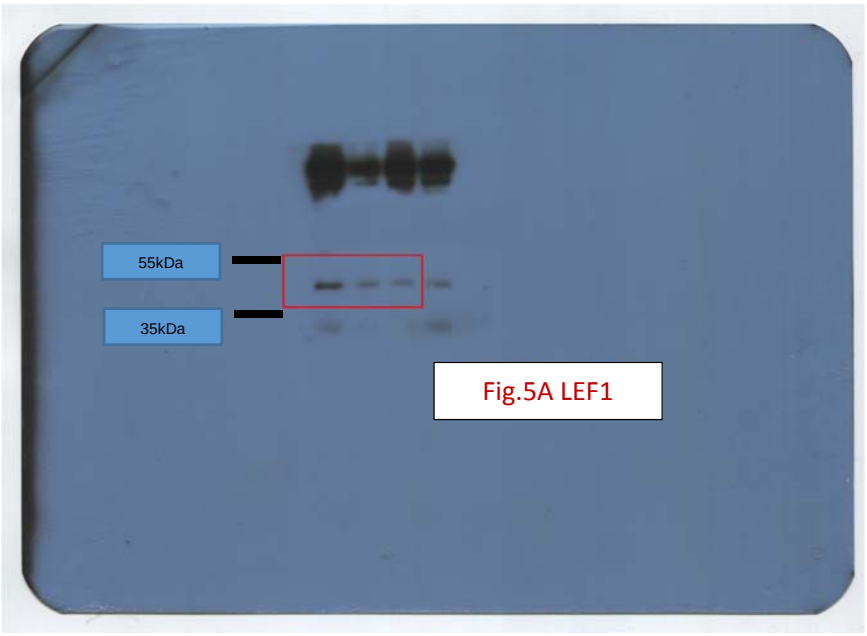
Fig.3 p-mTOR
170kDa
Fig.3 p-Akt 473
70kDa
55kDa
40kDa

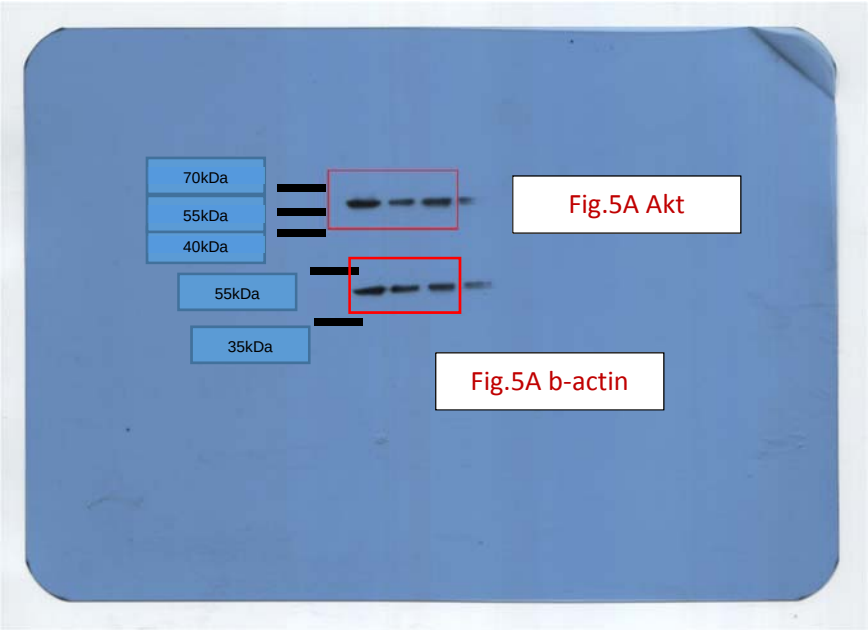

70kDa
55kDa
40kDa
Fig.3 Akt
55kDa
35kDa
Fig.3 b-actin

**Raw immunoblotted films for Figure 5**

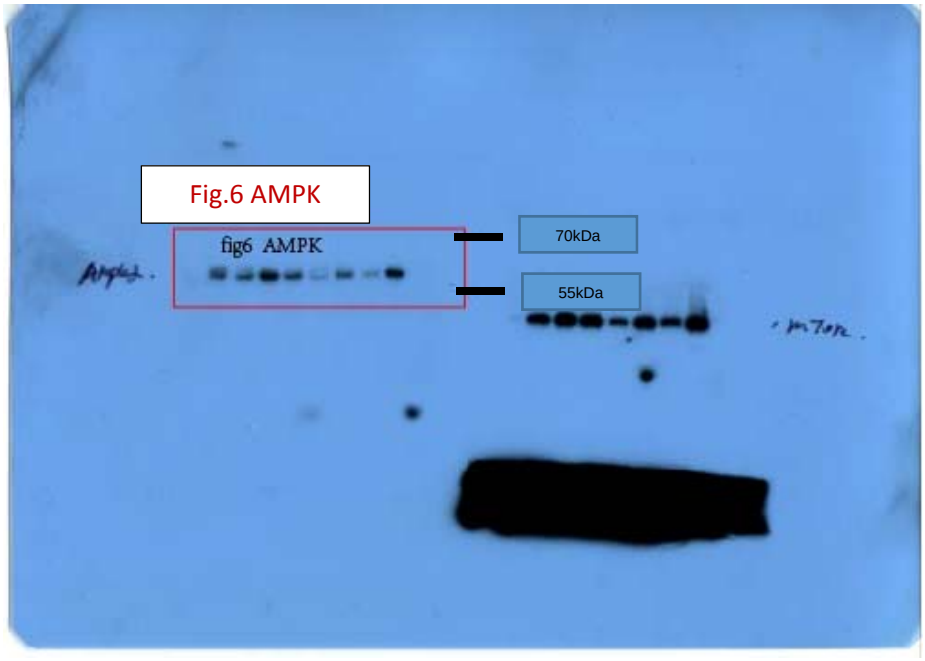



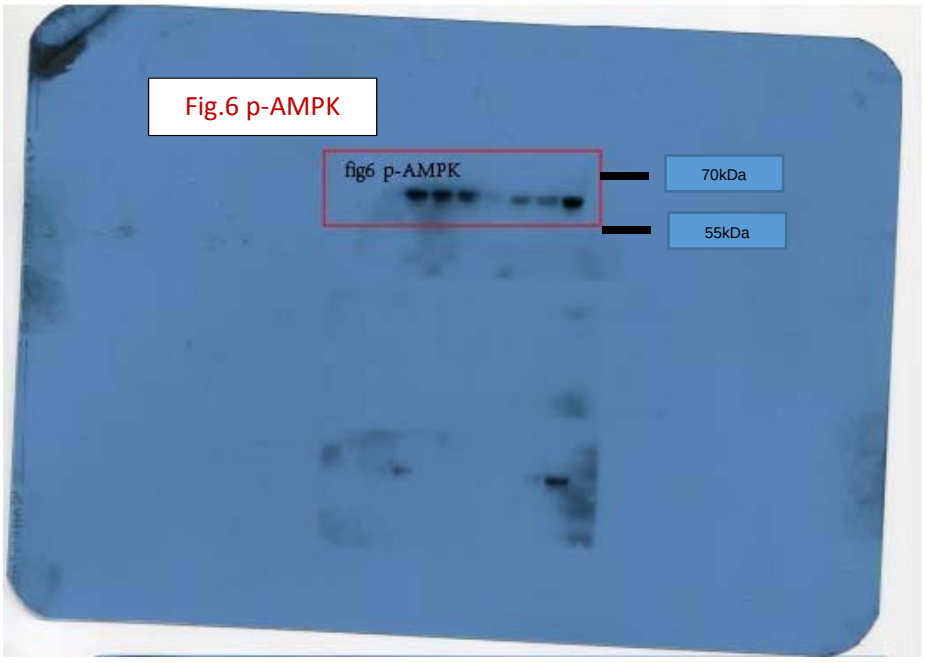

70kDa
55kDa
40kDa
Fig.5A Akt S473


55kDa
35kDa
Fig.5A LEF1


70kDa
55kDa
40kDa
55kDa
35kDa
Fig.5A Akt
Fig.5A b-actin

**Raw immunoblotted films for Figure 6**



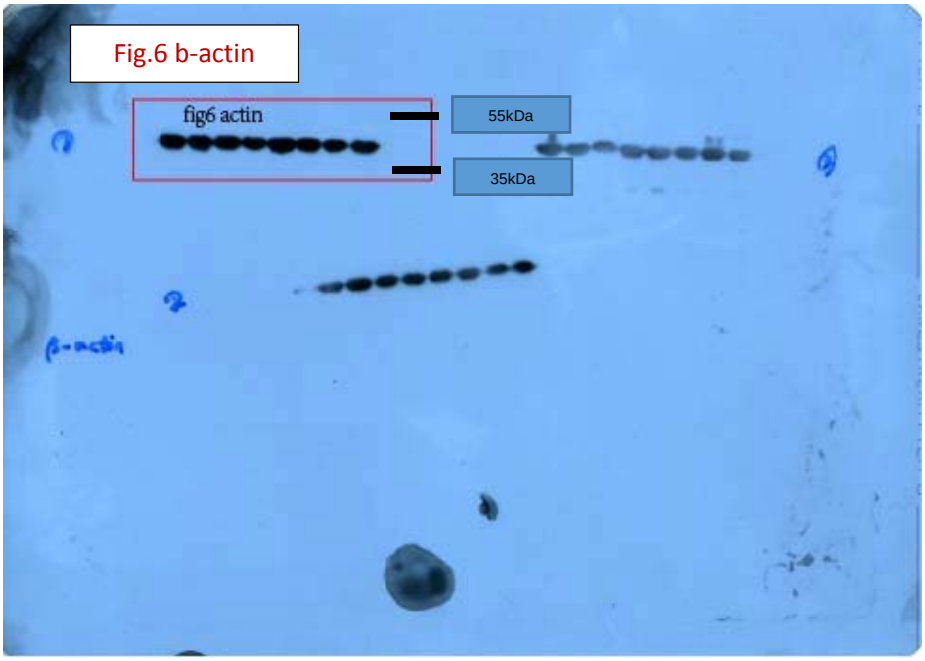
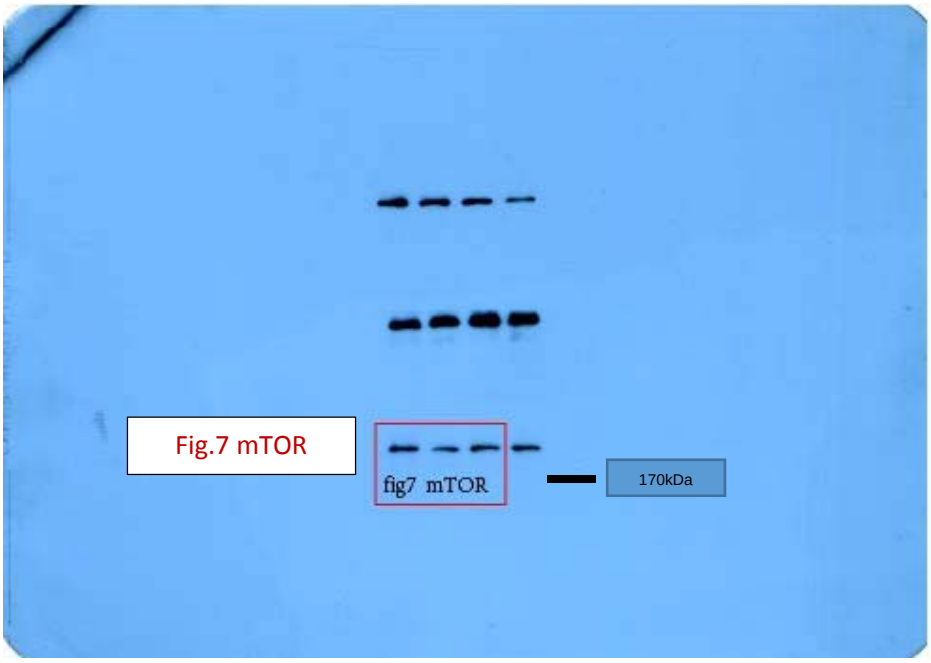
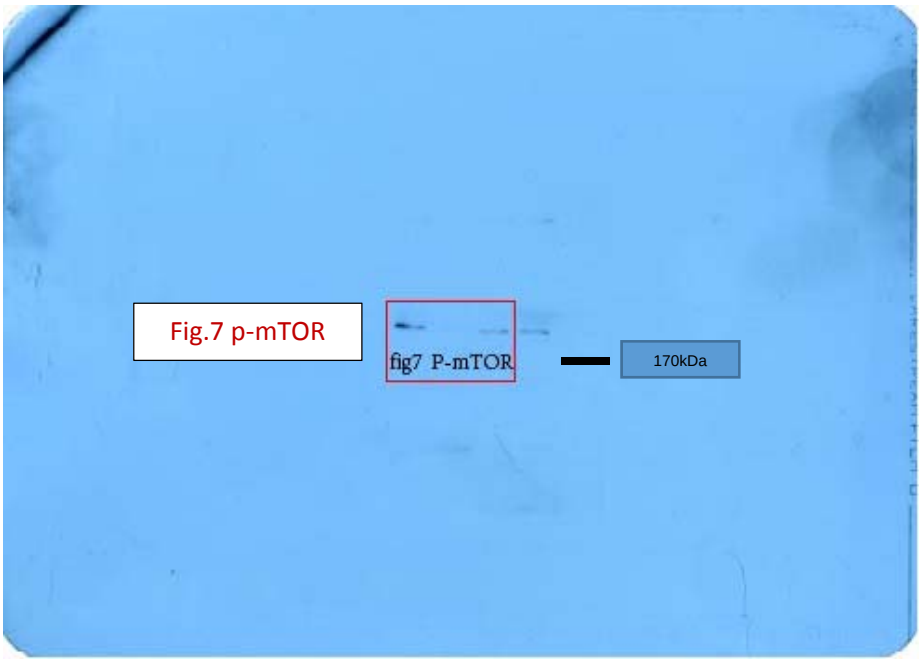


Fig.6 b-actin
fig6 actin
55kDa
35kDa
β-actin

**Raw immunoblotted films for Figure 7**



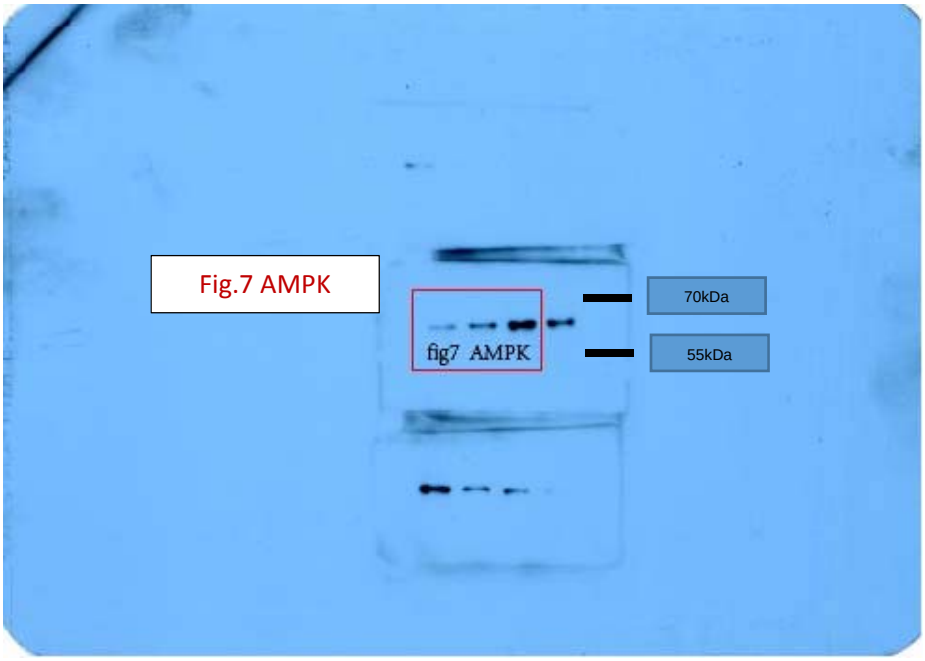


Fig.7 AMPK
fig7 AMPK
70kDa
55kDa

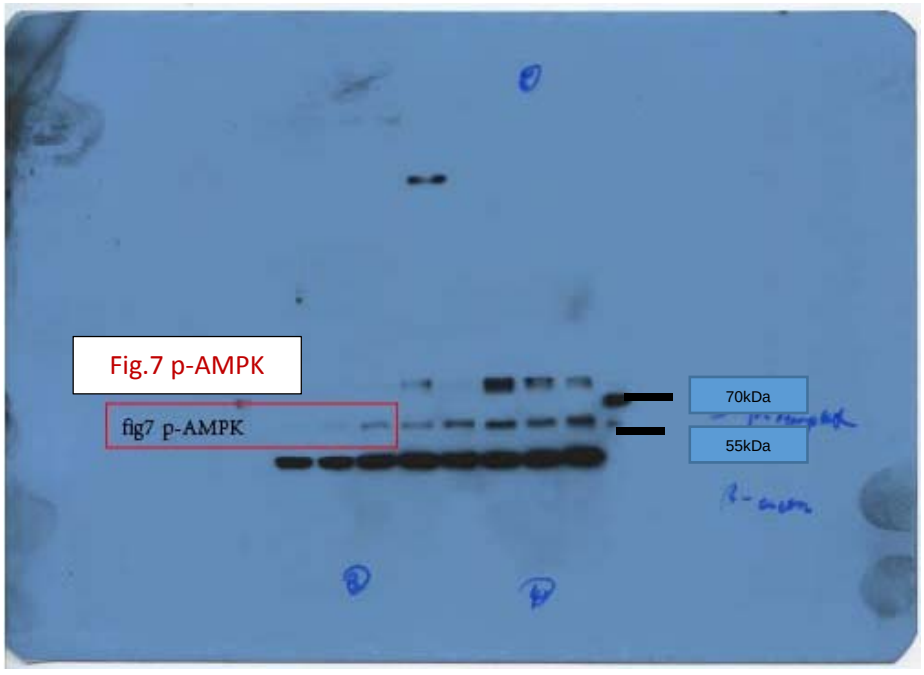


Fig.7 p-AMPK
fig7 p-AMPK
70kDa
55kDa

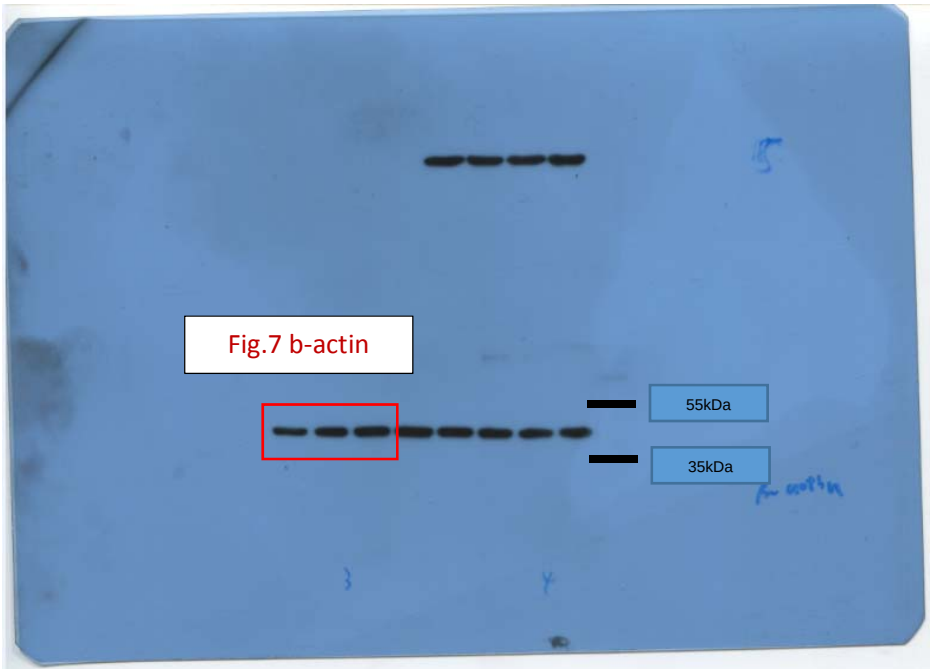
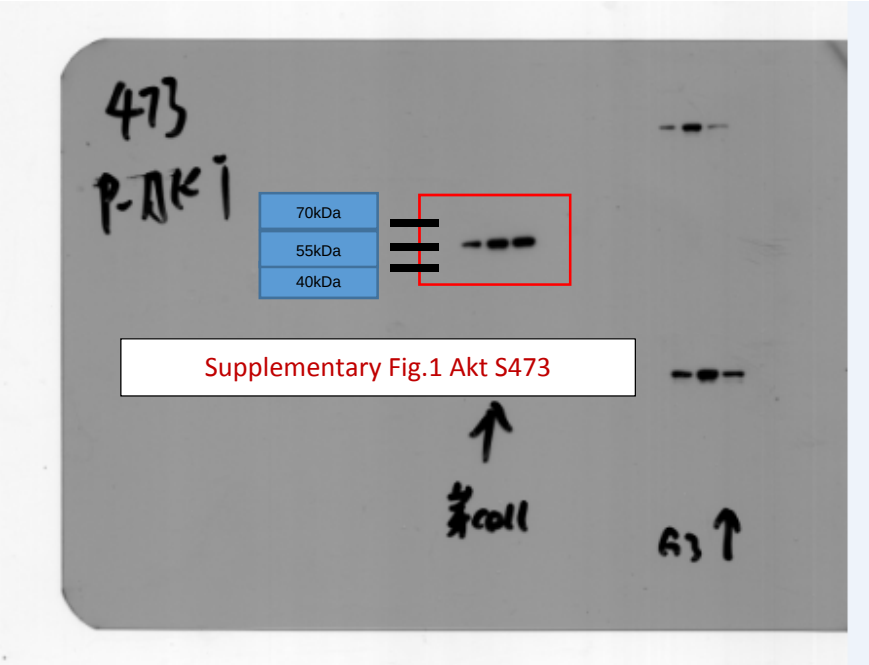
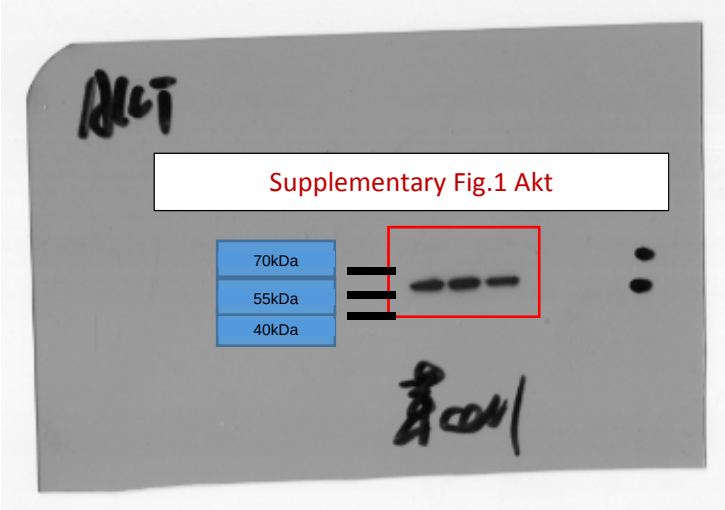
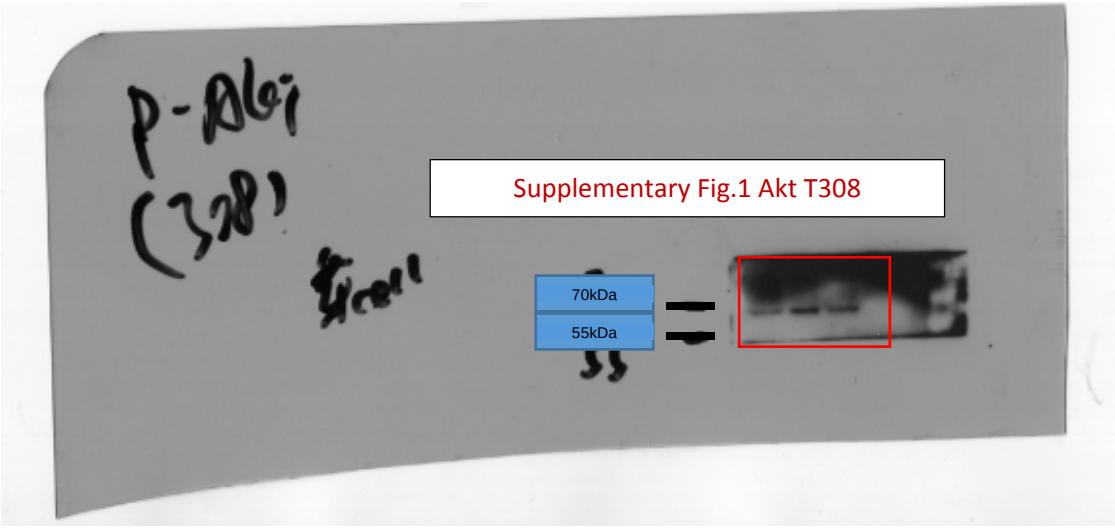


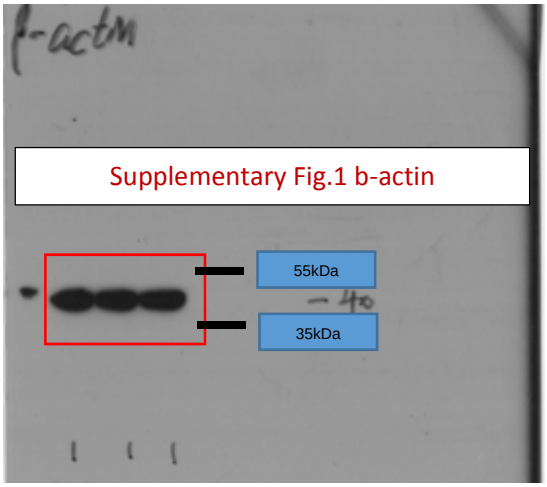
Fig.7 b-actin
55kDa
35kDa

**Raw immunoblotted films for Supplementary Figure 1**

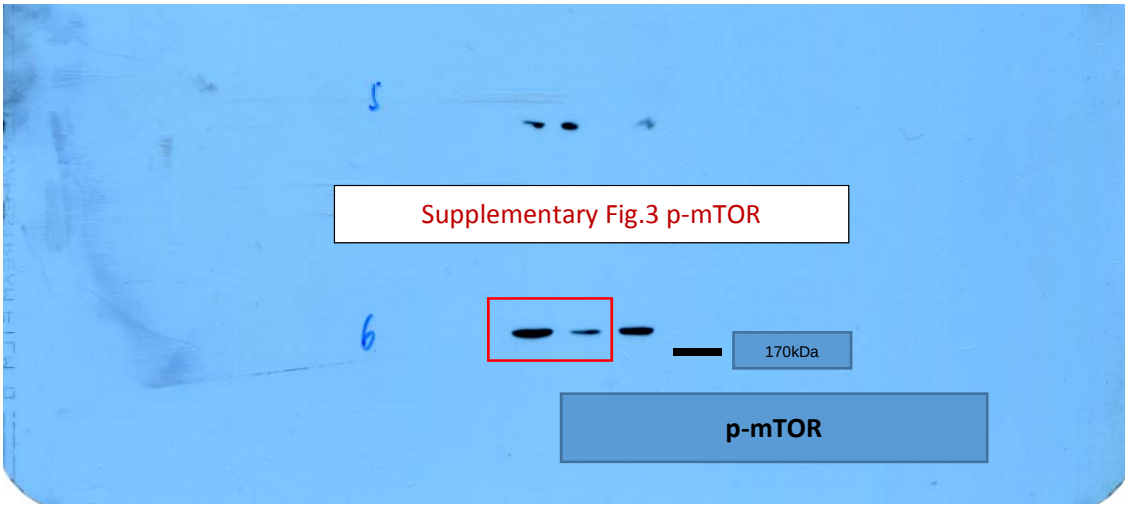


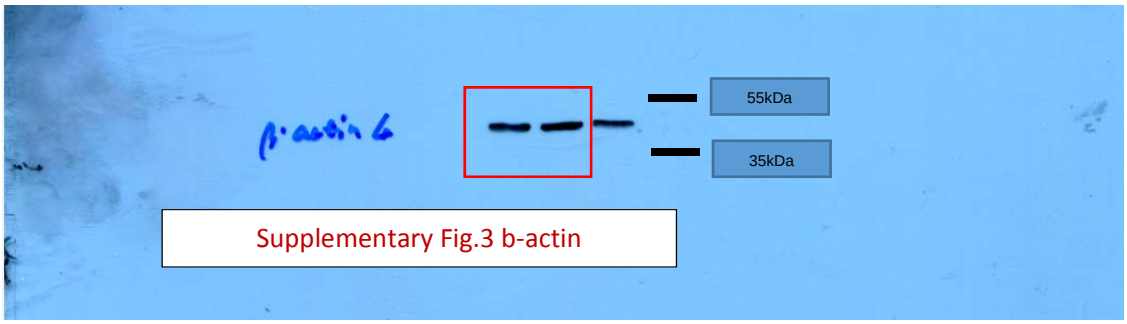



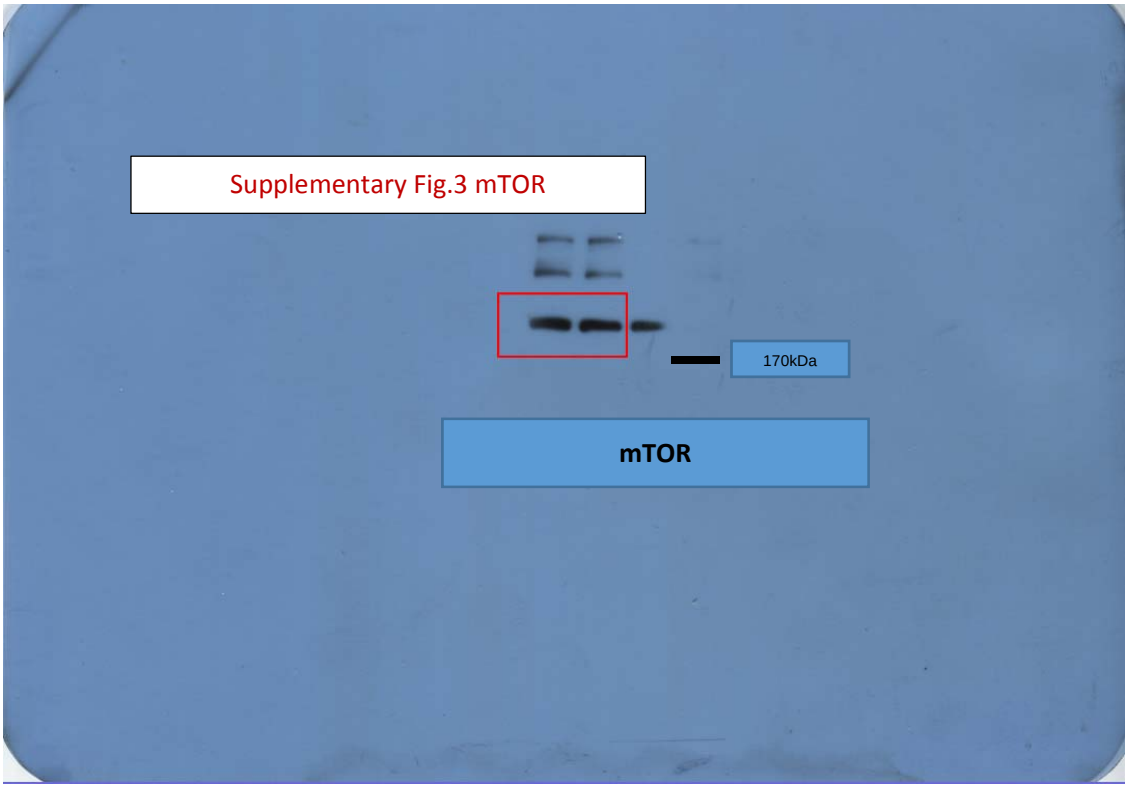

Supplementary Fig.1 Akt T308
70kDa
55kDa


Supplementary Fig.1 b-actin
55kDa
35kDa

**Raw immunoblotted films for Supplementary Figure 3**




Supplementary Fig.3 mTOR
170kDa
mTOR