

# **The Tnfaip8-PE complex is a novel upstream effector in the anti-autophagic action of insulin**

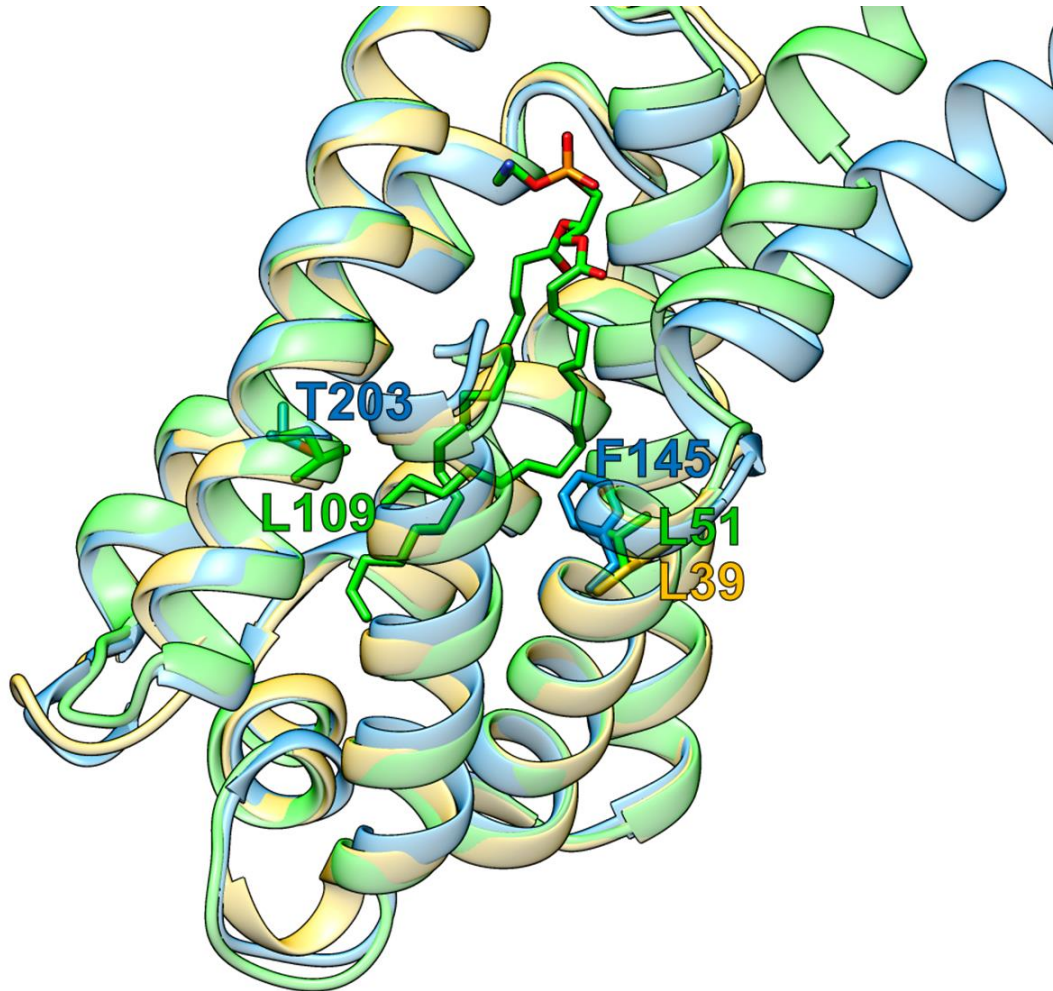
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## Supplementary Fig. 1

**The superimposed cavity structure of mTnfaip8 (green) with that of hTIPE2 (gold; PDB ID: 3F4M) and hTIPE3 (sky blue; 4Q9V)**

Different residues comprising the cavities are drawn with a stick model and labeled according to their ribbon colors.

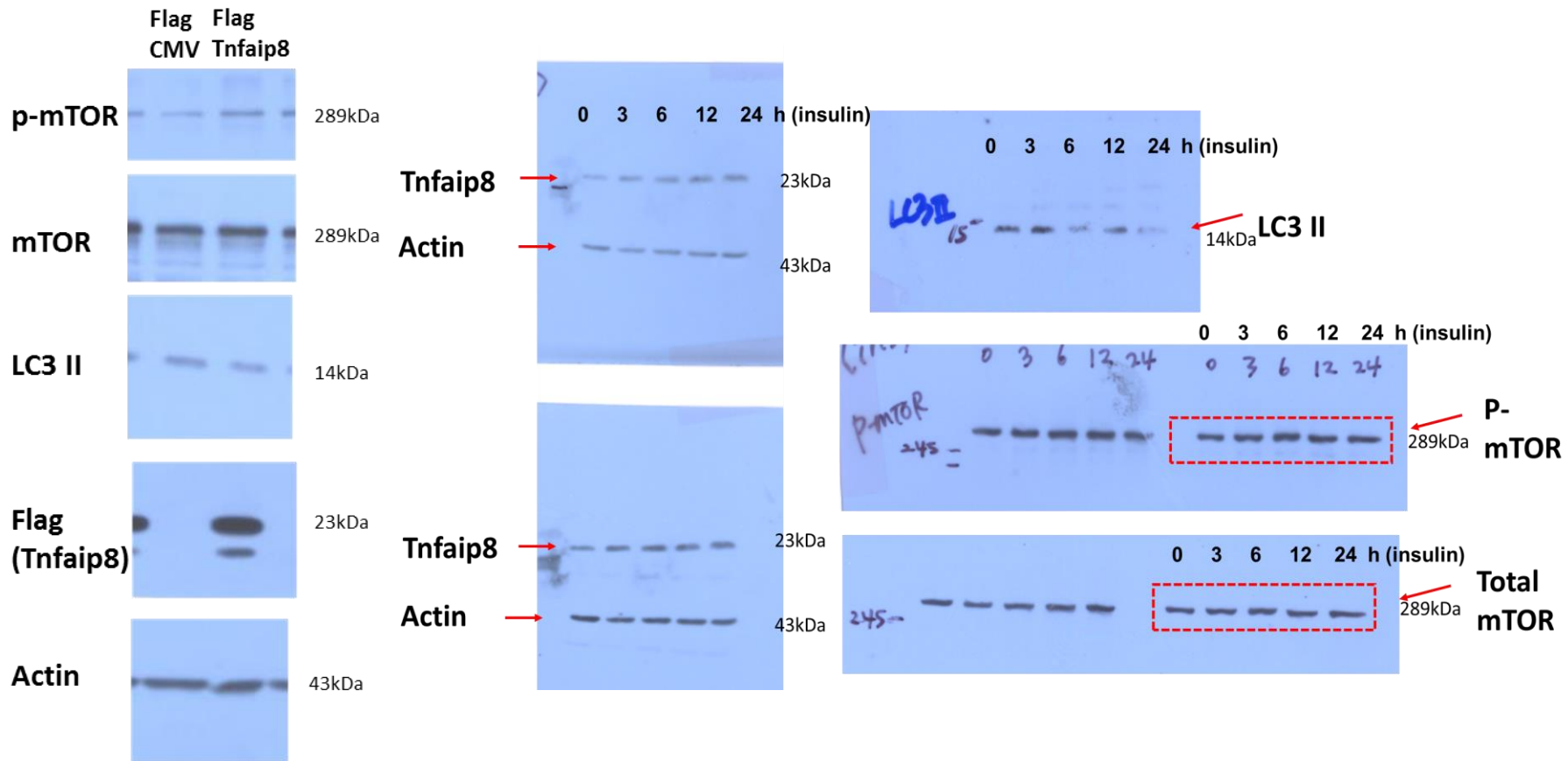


# Supplementary Fig. 2 (For Fig. 1)

## Temporal induction of mTnfaip8 by insulin

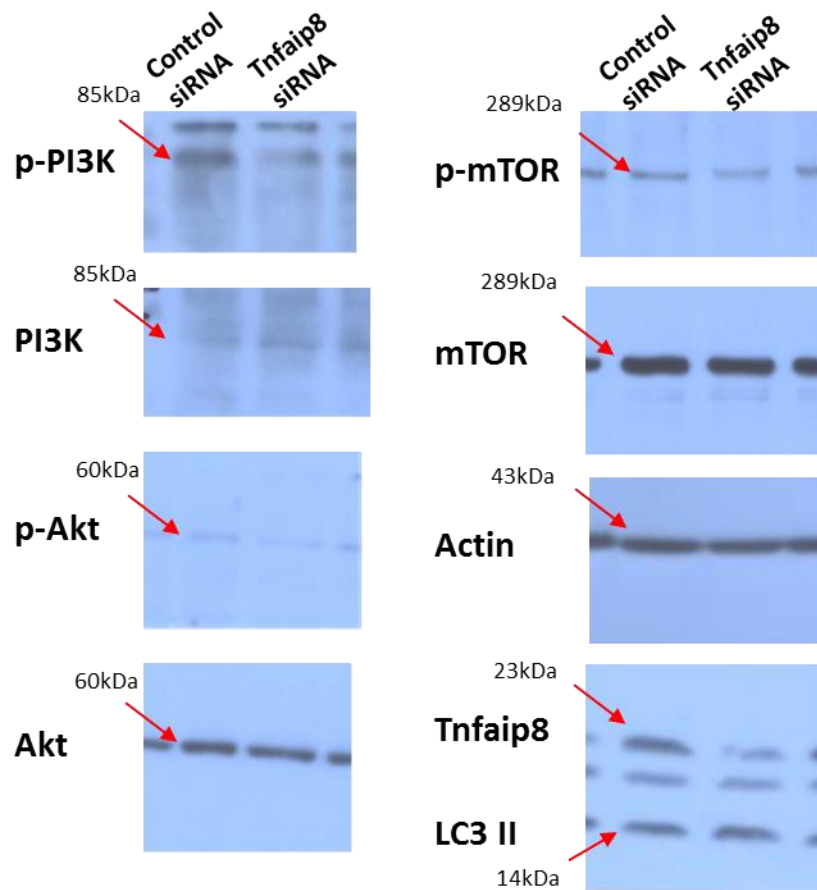
(Fig 1-a) The anti-autophagic activity of mTnfaip8 was confirmed in a hepatic cell line. mTnfaip8 was induced by insulin, followed by phospho-mTOR upregulation and LC3-II downregulation.

(Fig 1-a)

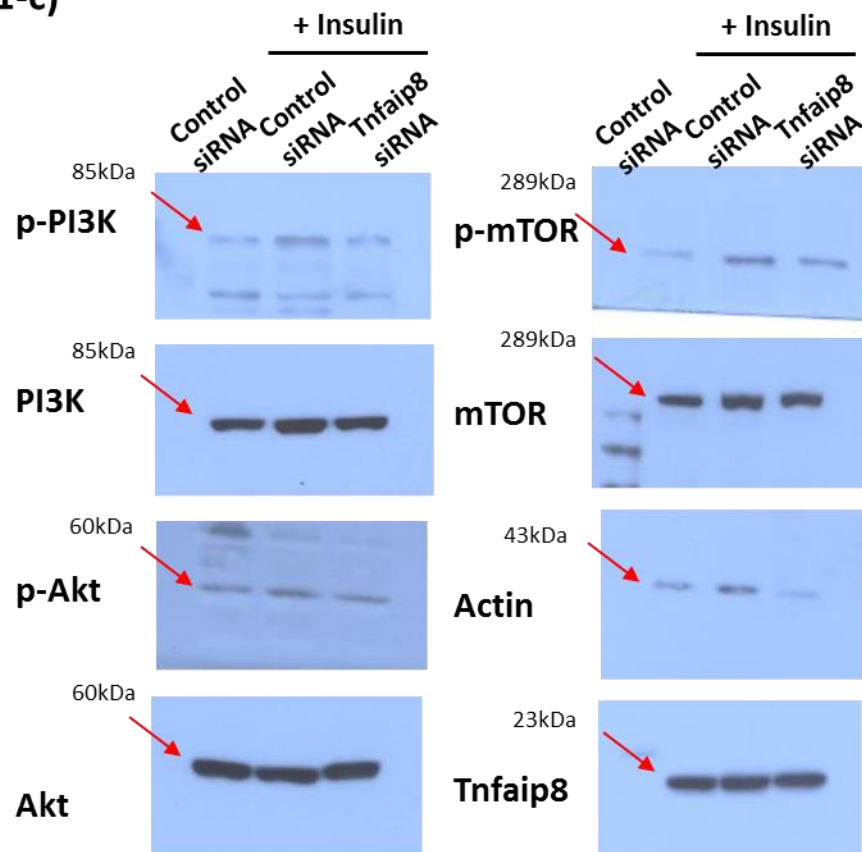


(Fig 1-b/c) The levels of phospho-PI3K, phospho-Akt and phospho-mTOR were decreased and the induction of autophagy was observed in both the absence and presence of insulin

(Fig 1-b)



(Fig 1-c)

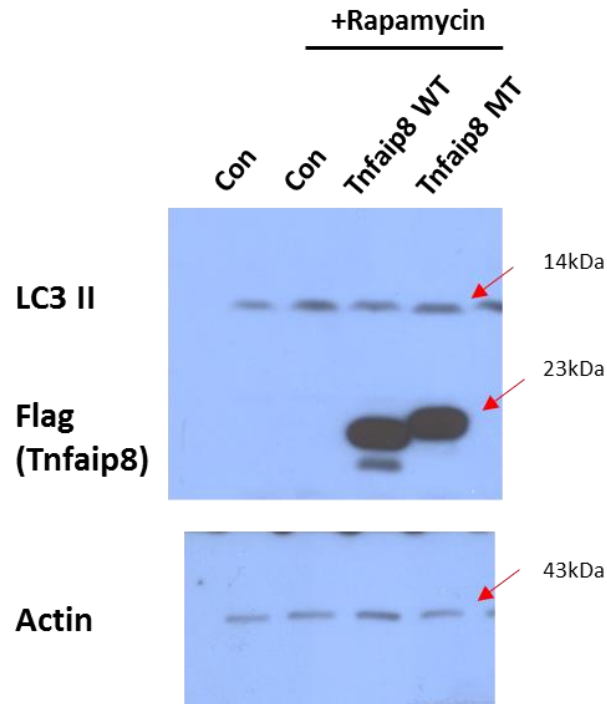


## Supplementary Fig. 3 (For Fig. 6)

### The anti-autophagic action of mTnfaip8

The triple mutant (MT) lost inhibitory regulation of rapamycin-induced autophagy, whereas wild type (WT) demonstrated normal autophagic inhibition

(Fig 6-a)



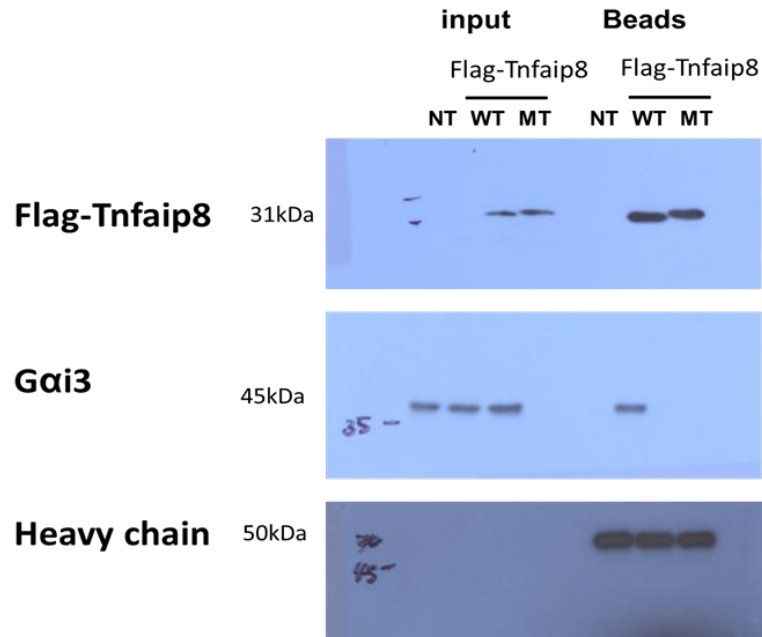
## Supplementary Fig. 4 (For Fig. 7)

### Interaction between wild type mTnfaip8 and the triple mutant with Gai3

(Fig 7-a) The mTnfaip8 triple mutant with PE deficiency barely interacted with Gai3, whereas wild type mTnfaip8 maintained normal interaction with Gai3.

(Fig 7-d) Pertussis toxin (PTX) (100 ng/ml) inhibited insulin-induced anti-autophagy. Anti-autophagic effect of Tnfaip8 was also inhibited by the PTX treatment.

(Fig 7-a)



(Fig 7-d)

