

Supplementary Figure 1. AICAR induced autophagy, inhibited senescence and promoted anabolism process and inhibited catabolism process.

(a) Western blot analysis showing the protein expression of COL2A1, ACAN, ADAMTS5, and MMP3 in SD rat NP cells stimulated with TNF- α and AICAR. (b) Semiquantitative analysis and statistical analysis of the Western blots in (a). (c) Western blot analysis showing the protein expression of p21 in SD rat NP cells stimulated with TNF- α and AICAR. (d) Semiquantitative analysis and statistical analysis of the Western blots in (c). (e) Western blot analysis showing the protein expression of p62 in SD rat NP cells stimulated with TNF- α and AICAR. (f) Semiquantitative analysis and statistical analysis of the Western blots in (e). (g) Western blot analysis showing the protein expression of LC3 II/I in SD rat NP cells stimulated with TNF- α and AICAR. (h) Semiquantitative analysis and statistical analysis of the Western blots in (g). (i) β -gal staining showing the level of senescence in SD rat NP cells in response to stimulation with TNF- α and AICAR. Scale bar =100 μ m. (j) Western blot analysis showing the protein expression of p-mTOR and mTOR in SD rat NP cells stimulated with TNF- α and glutamine. (k) Semiquantitative analysis and statistical analysis of the Western blots in (j).

Supplementary Figure 2. KEGG Enrichment Analysis Showing the Different Pathways in Human NP cells in Response to TNF- α and Glutamine stimulation (TNF- α and TNF- α +glutamine).

Supplementary Figure 3. Intracellular glutaminolysis of NP cells under the stimulation of TNF- α and glutamine of metabolomics analysis.

Supplementary Figure 4. Changes in key glycolysis enzymes in human NP cells in response to TNF- α and glutamine stimulation of transcriptomics analysis.

Supplementary Figure 5. Changes in the intermediate products of glycolysis in human NP cells stimulated with TNF- α and glutamine of metabolomics analysis.

Supplementary Figure 6. The identified metabolites in positive ionization mode of metabolomics

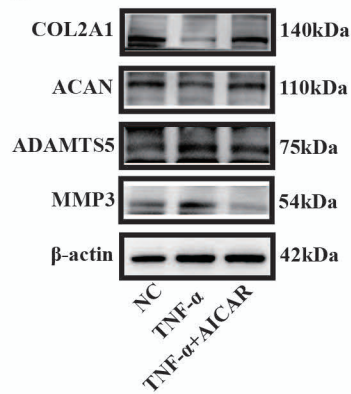
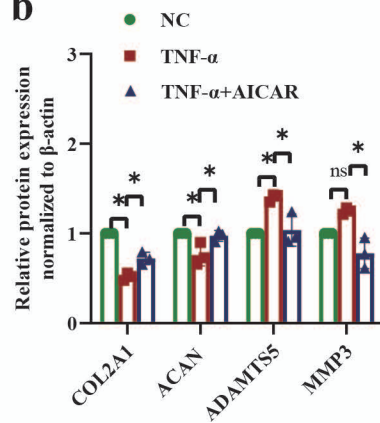
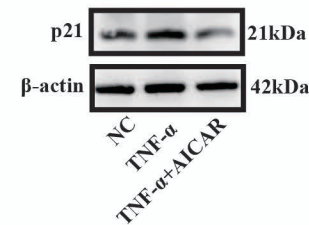
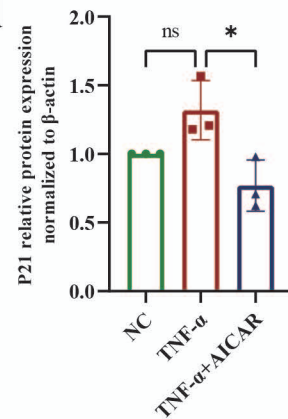
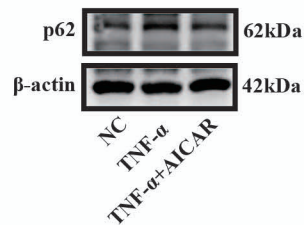
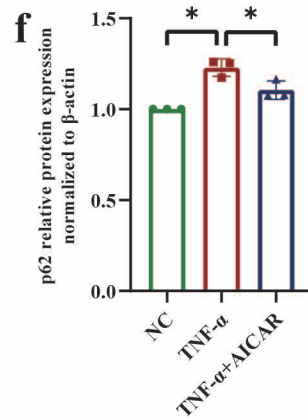
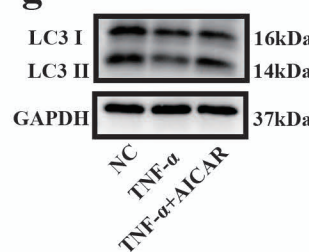
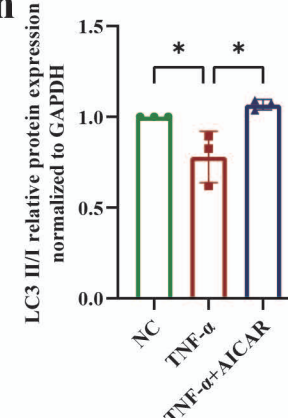
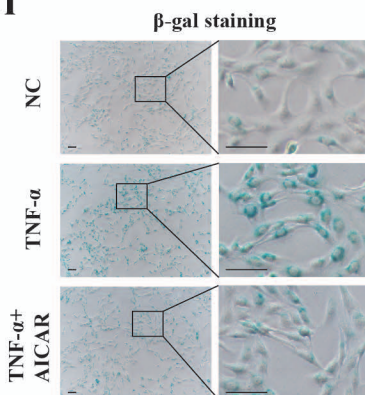
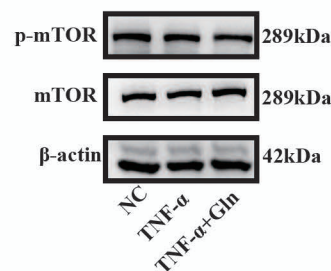
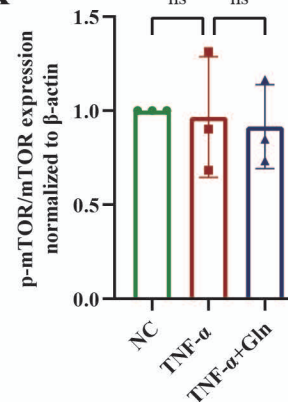
analysis.

(a)The proportion of identified metabolites in each chemical classification. (b)The positive ionization mode of hierarchical clustering analysis showing other changes in metabolites. (c) The positive ionization mode of the volcano plot showing other changes in metabolites in response to TNF- α stimulation and control conditions. (d) The positive ionization mode of the volcano plot showing other changes in metabolites in response to stimulation with glutamine and TNF- α .

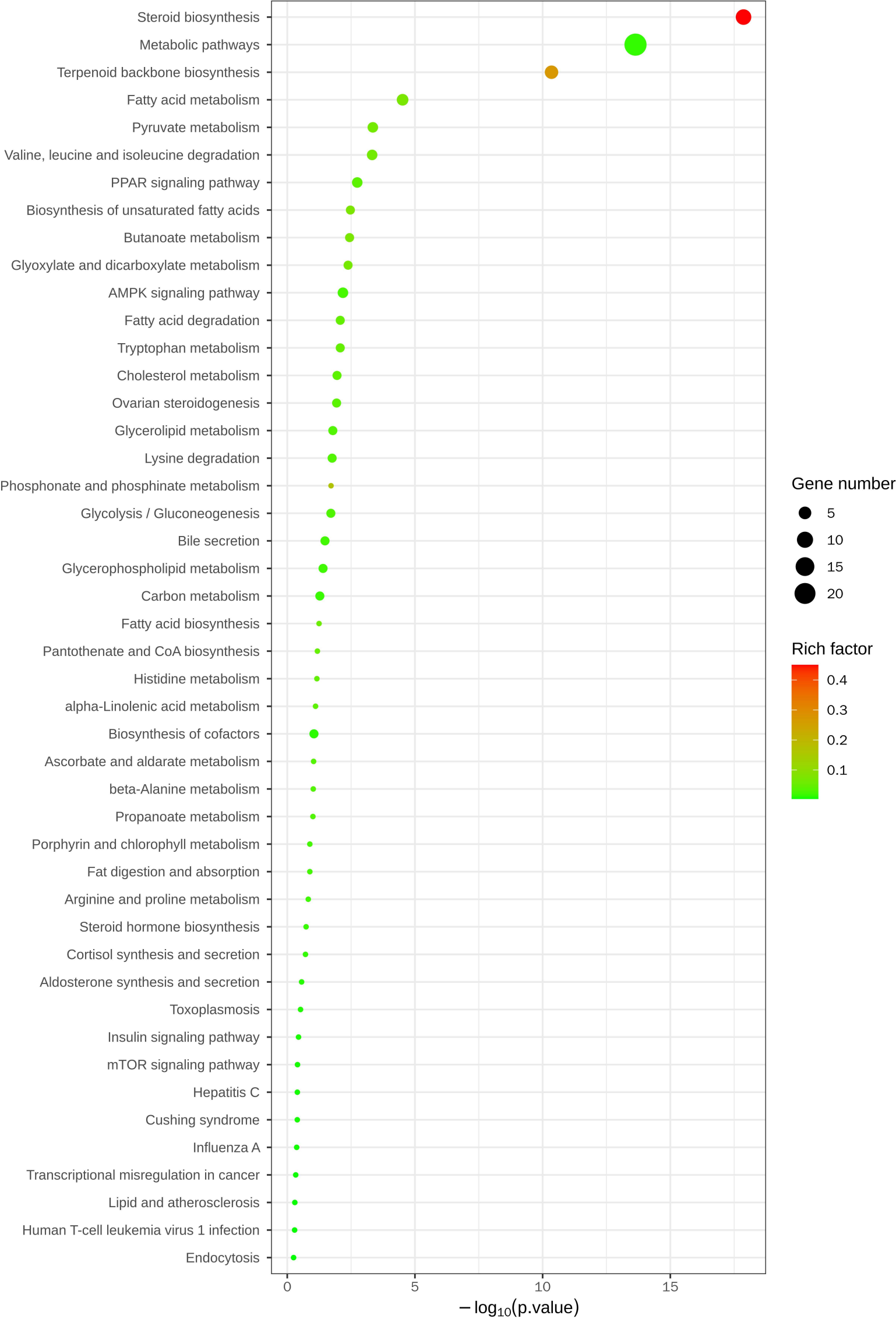
Supplementary Figure 7. A significant change in metabolites of negative ionization mode of hierarchical clustering analysis.

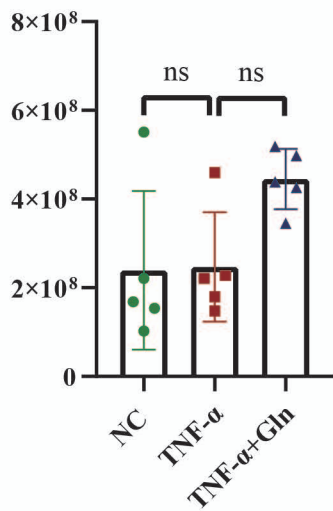
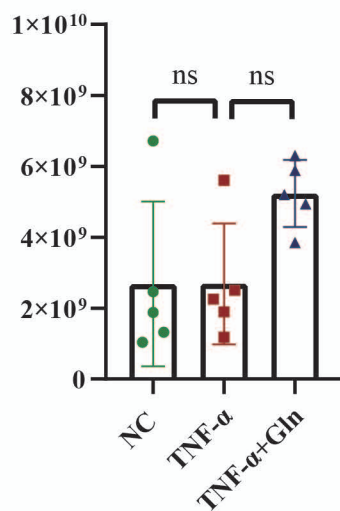
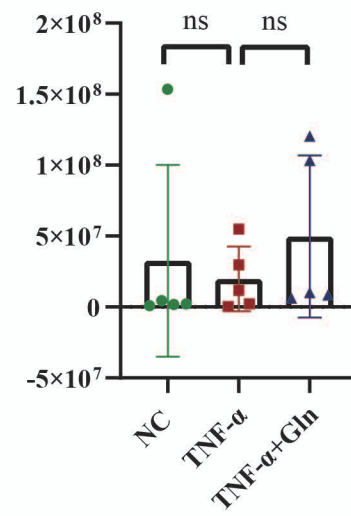
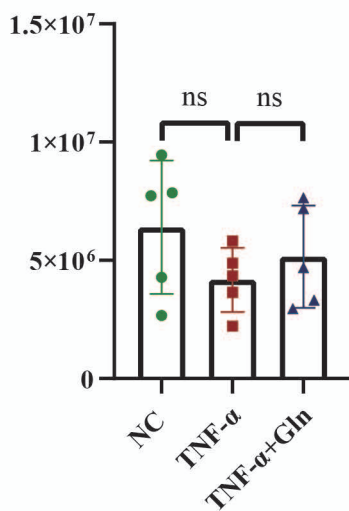
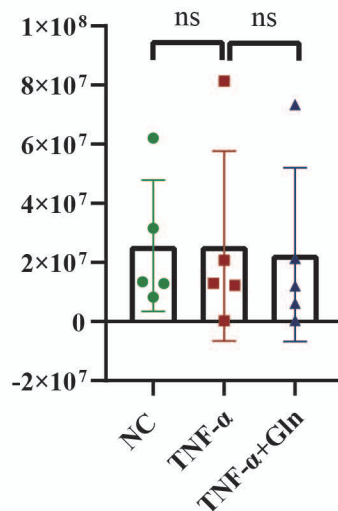
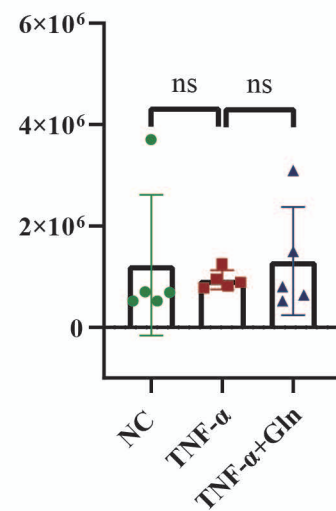
Supplementary Figure 8. A significant change in metabolites of positive ionization mode of hierarchical clustering analysis.

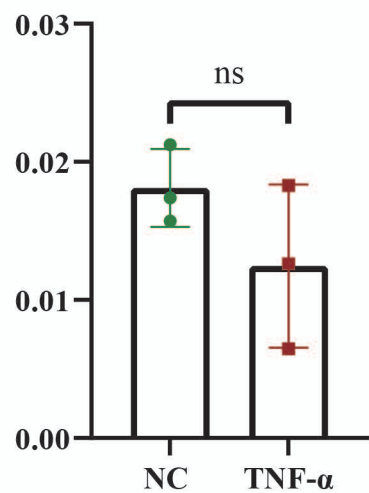
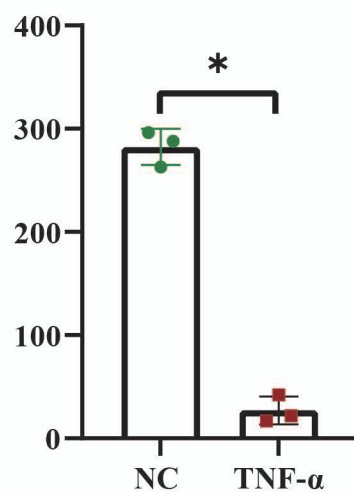
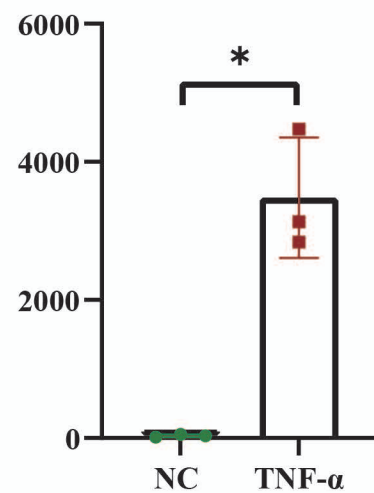
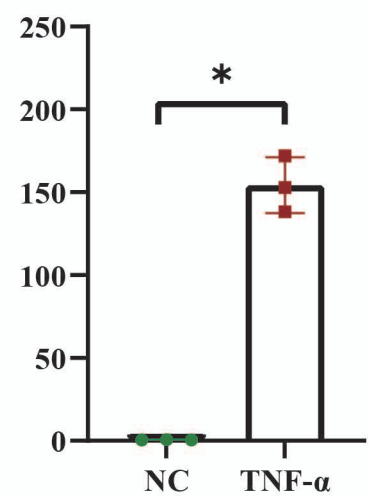
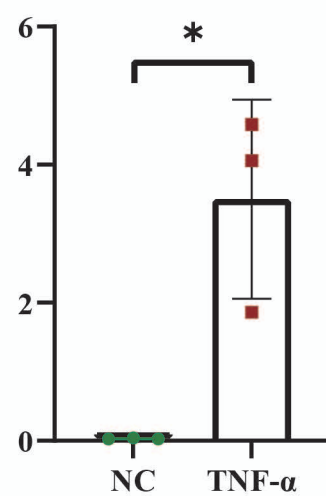
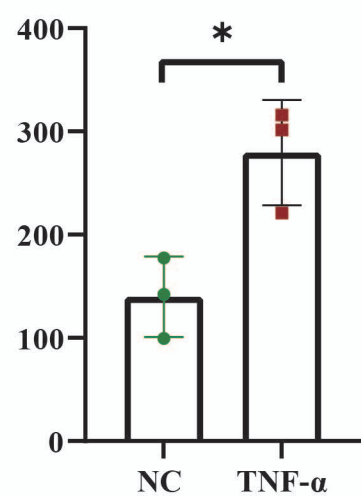
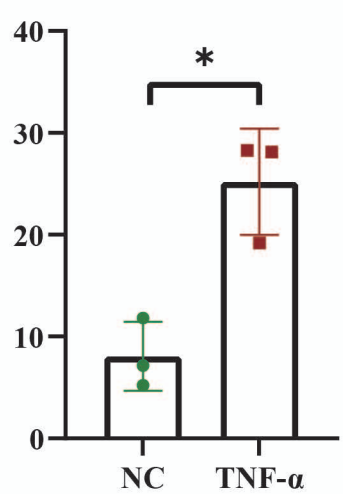
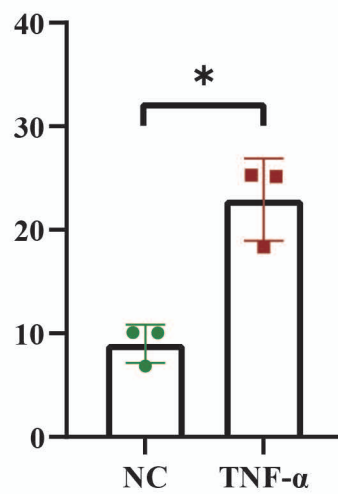
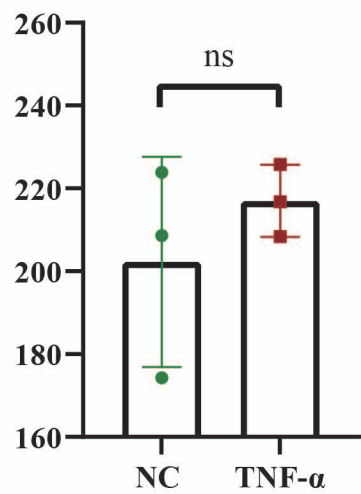
Supplementary Figure 9. The full, unedited blots in Figures.

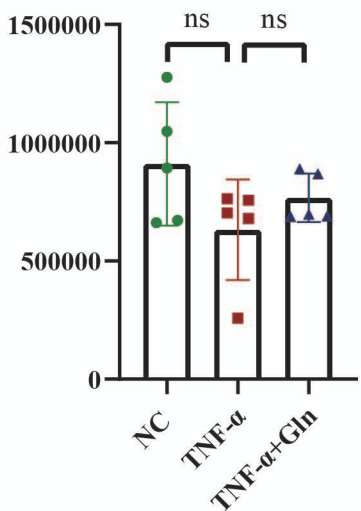
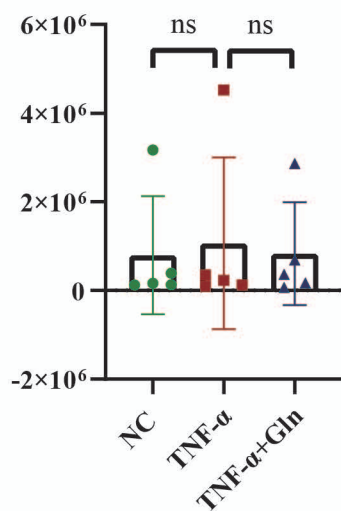
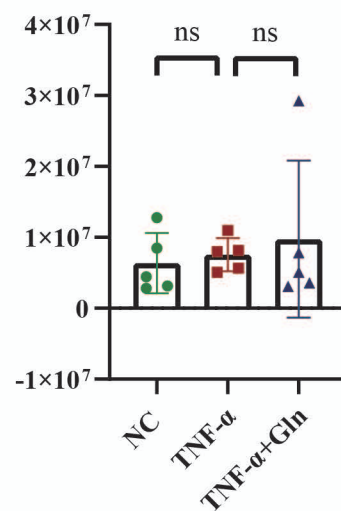
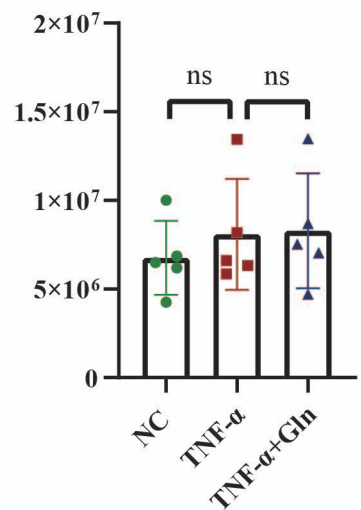
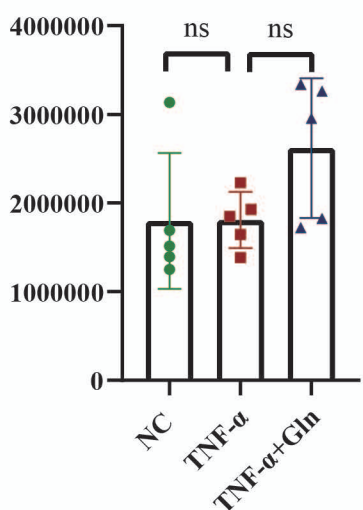
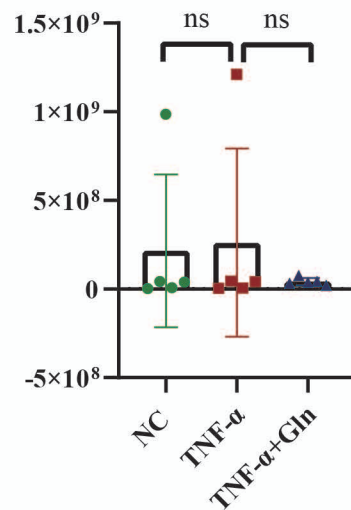
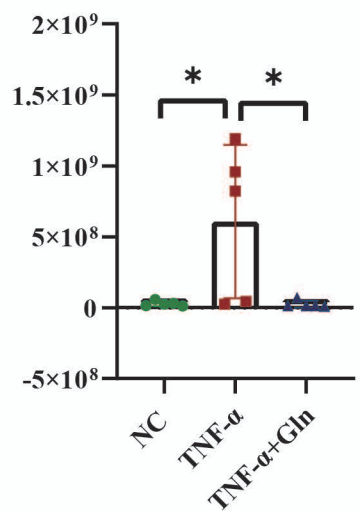
a**b****c****d****e****f****g****h****i****j****k**

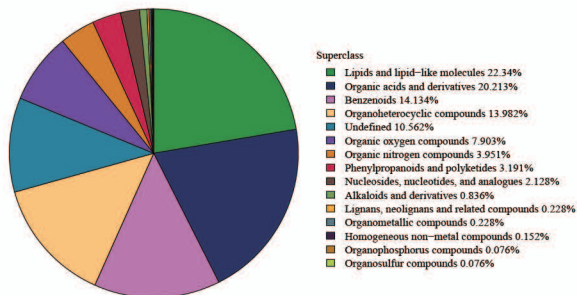
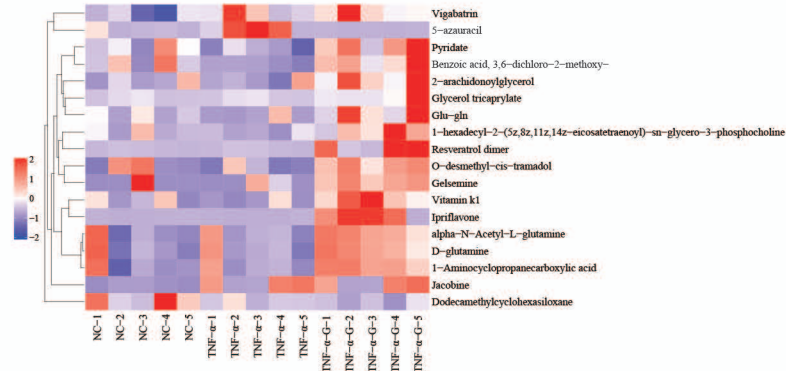
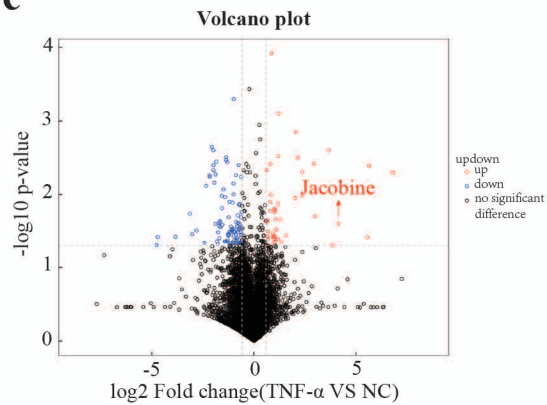
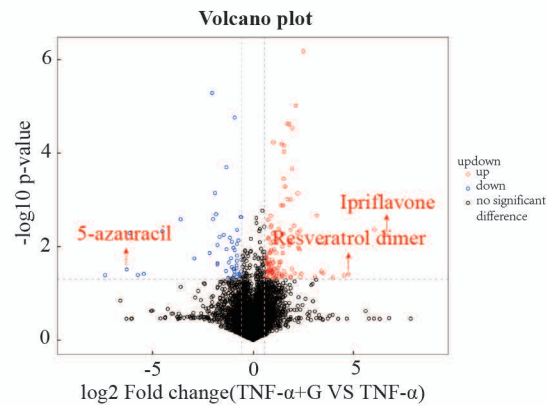
KEGG Pathways Enrichment

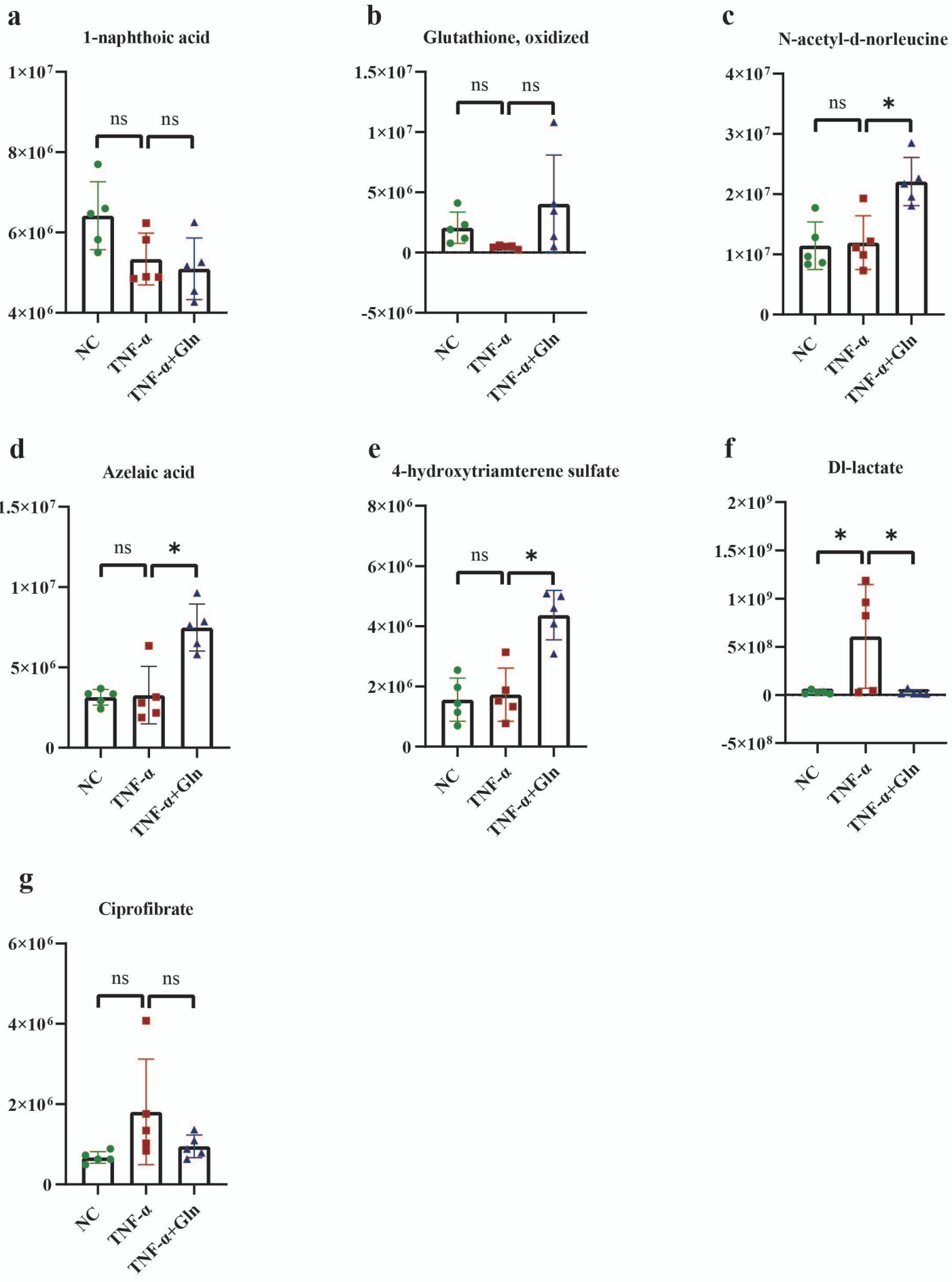


a**alpha-N-Acetyl-L-glutamine****b****D-glutamine****c****L-Glutamine****d****L-Glutamate****e****N-acetyl-L-glutamate****f****N-fructosyl pyroglutamate**

a**COL2A1****b****ACAN****c****MMP3****d****MMP13****e****ADAMTS4****f****LDHA****g****HK2****h****PFKP****i****PKM**

a**D-glucose 6-phosphate****b****Fructose 1,6-diphosphate****c****Glyceraldehyde, 3-(dihydrogen phosphate)****d****3-phosphoglyceric acid****e****D-2-phosphoglyceric acid****f****Pyruvate****g****DL-lactate**

a**b****c****d**



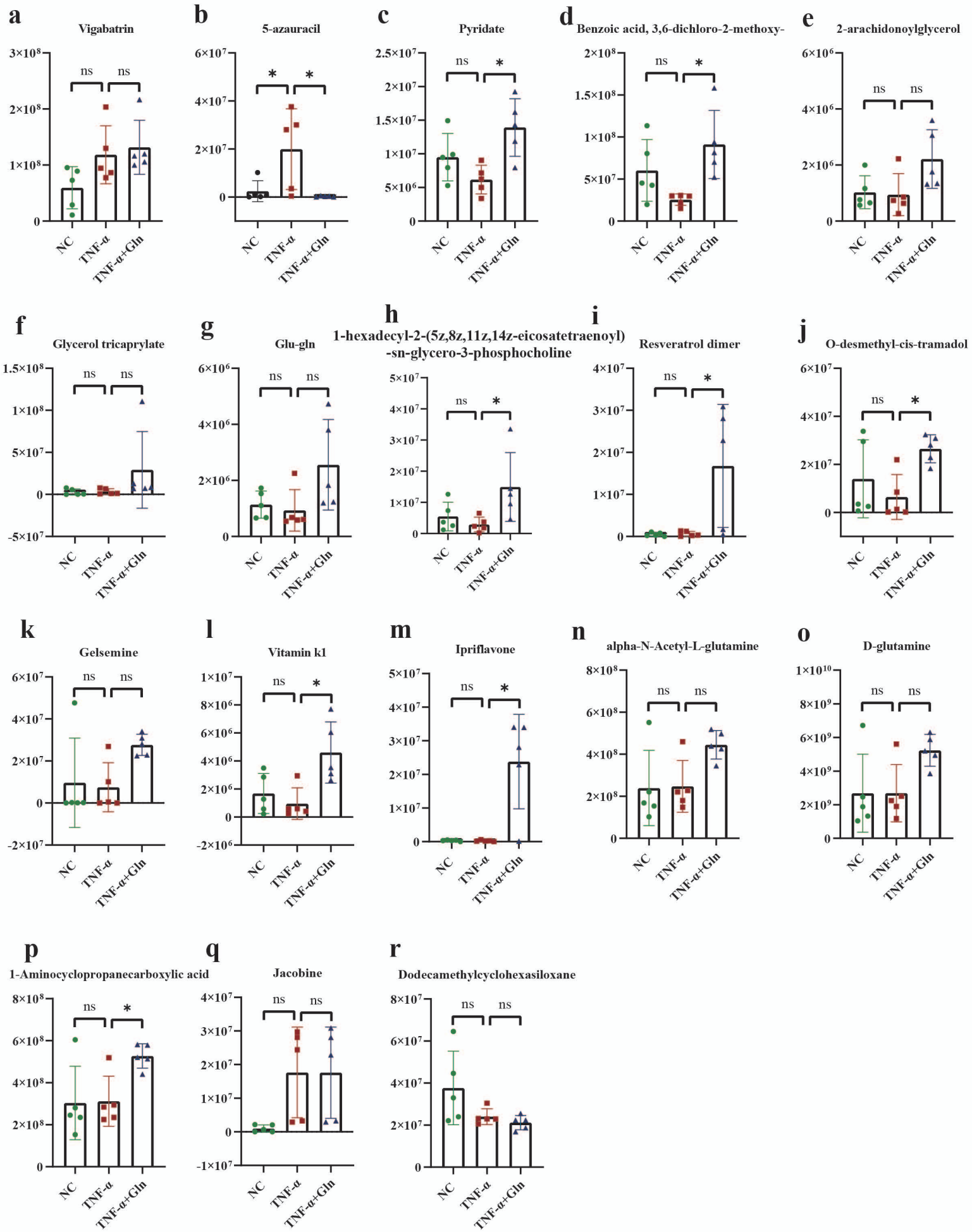


Fig 1h

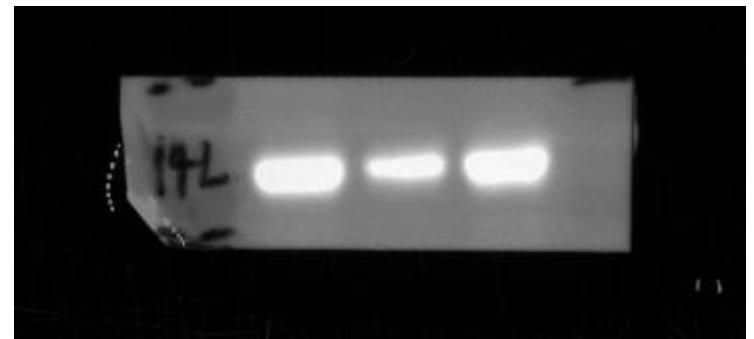
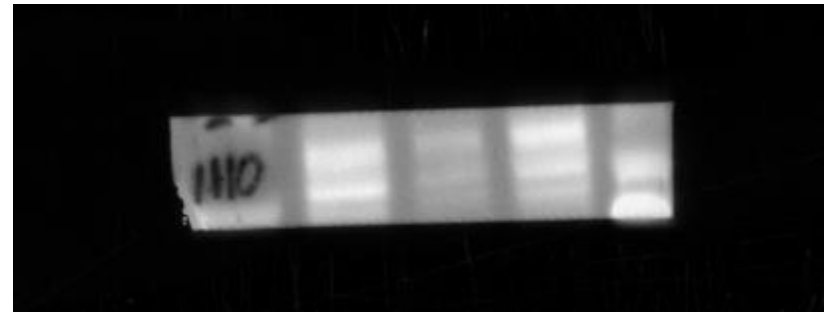
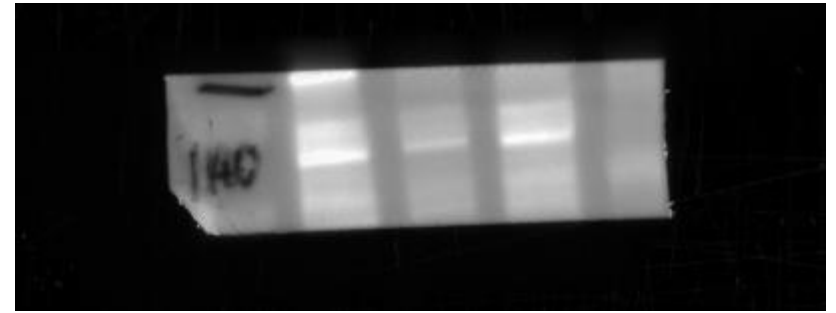
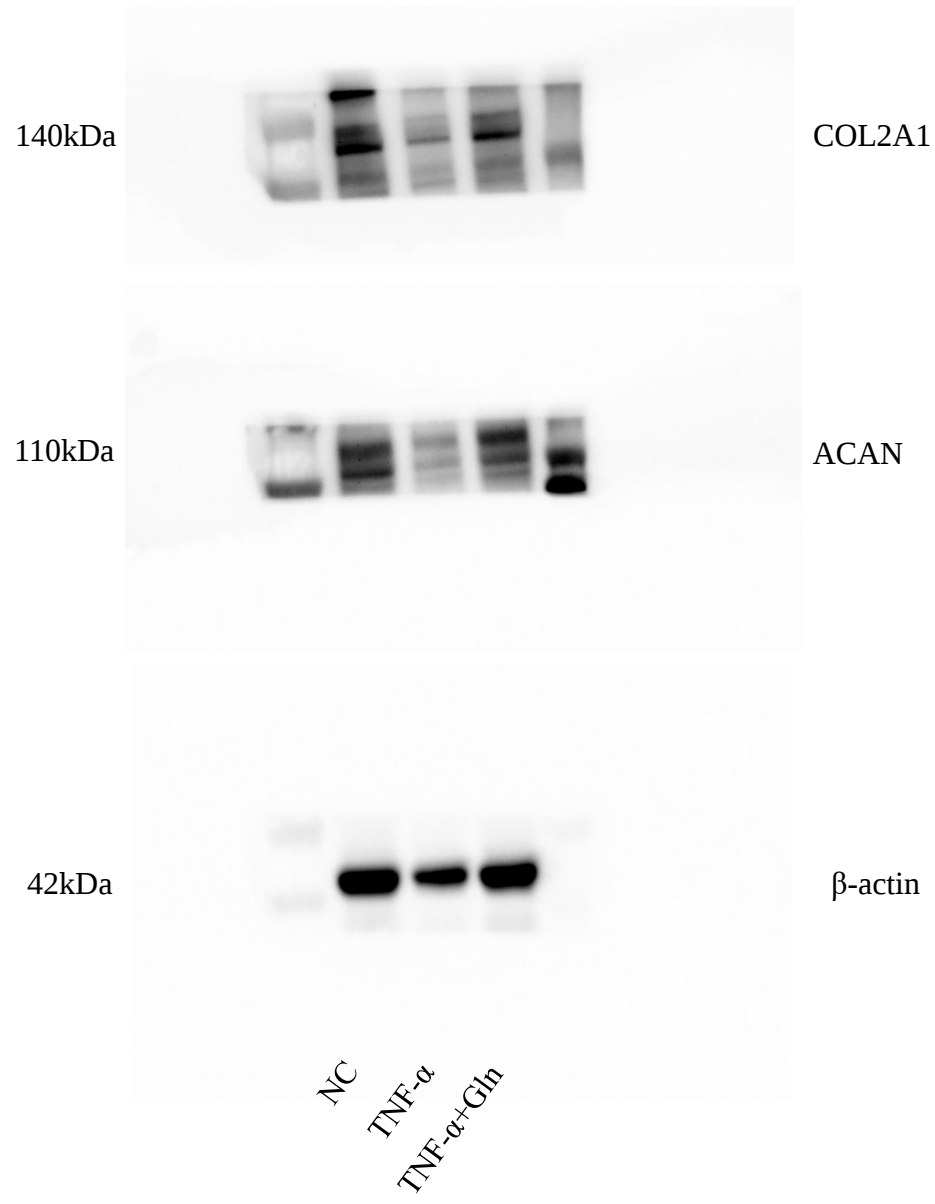


Fig 1h

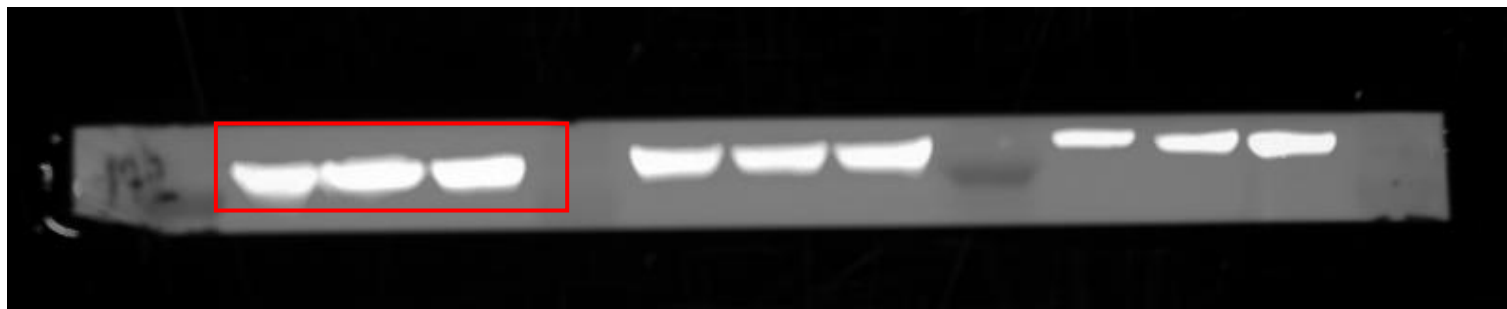
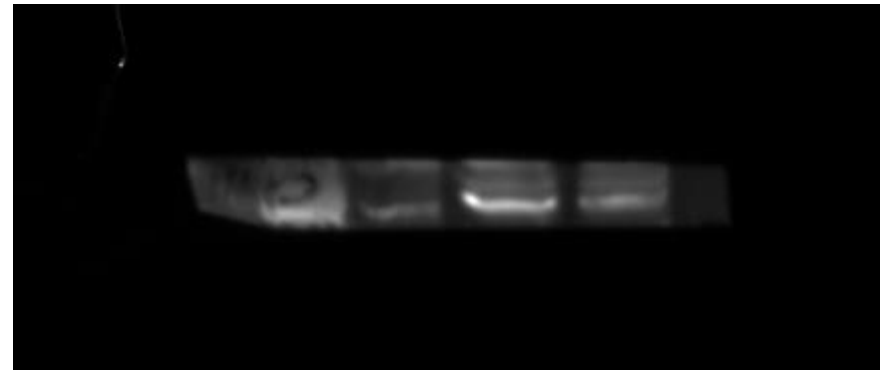
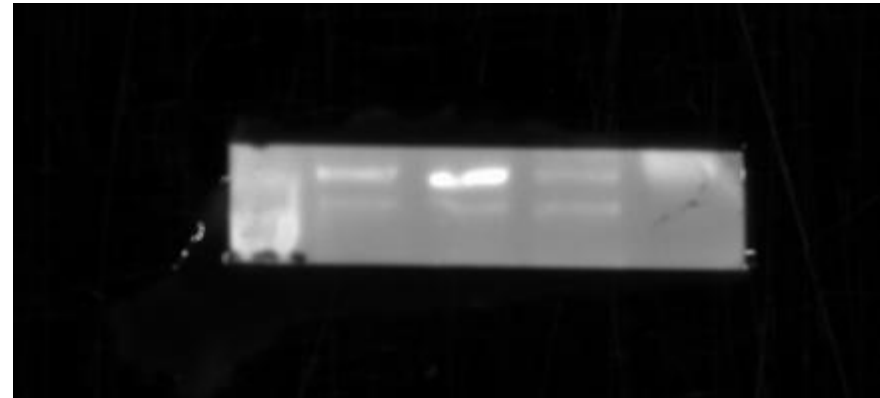
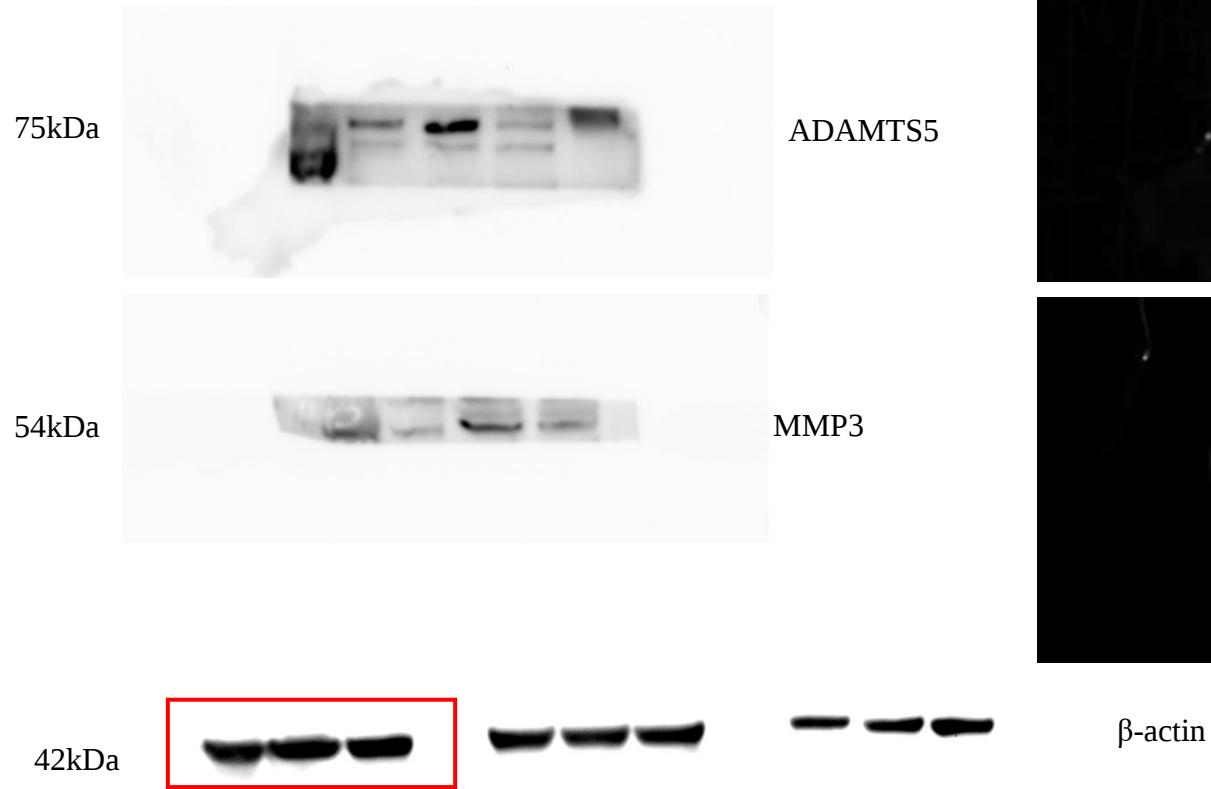


Fig 1j

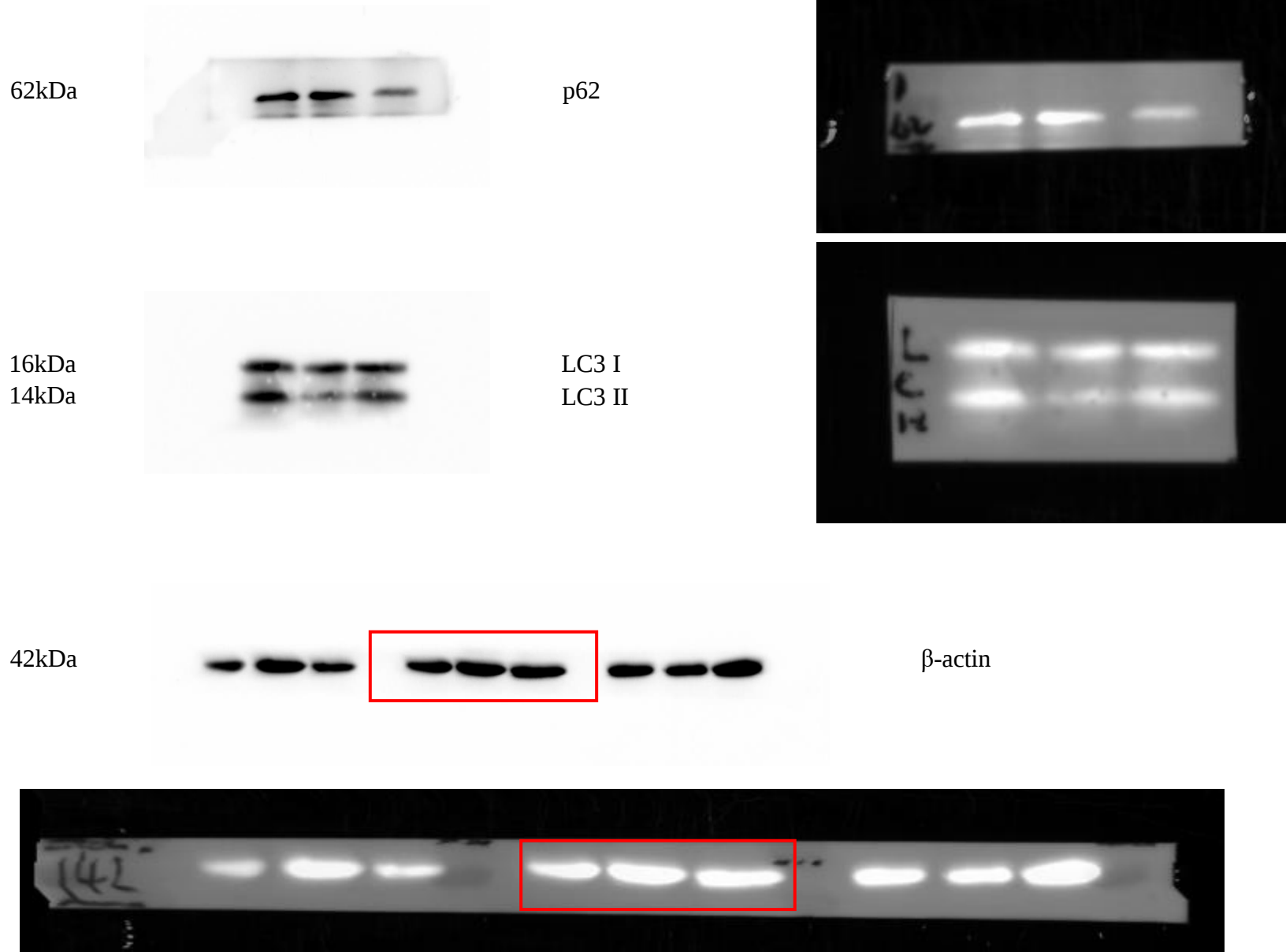


Fig 1j

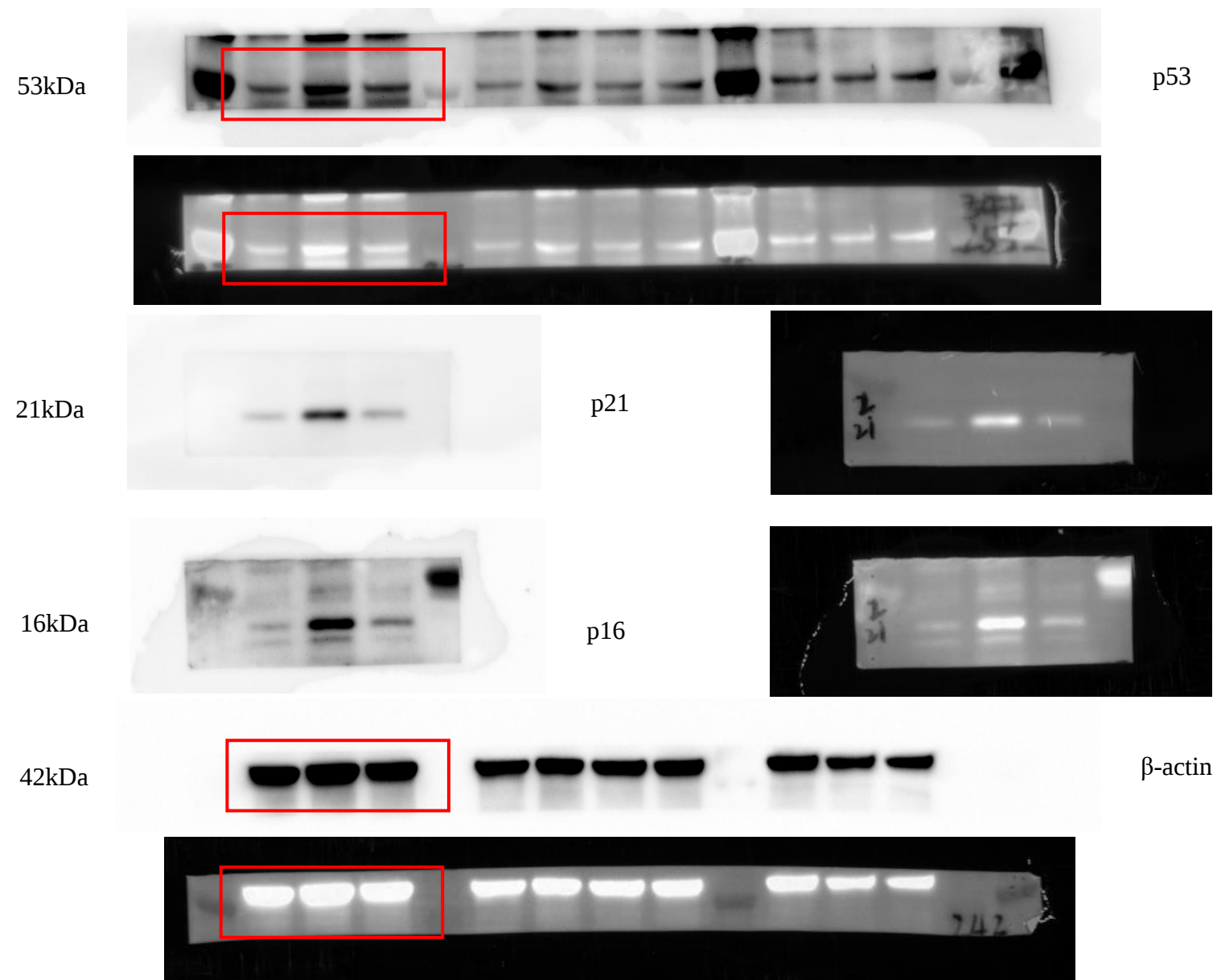


Fig 2c

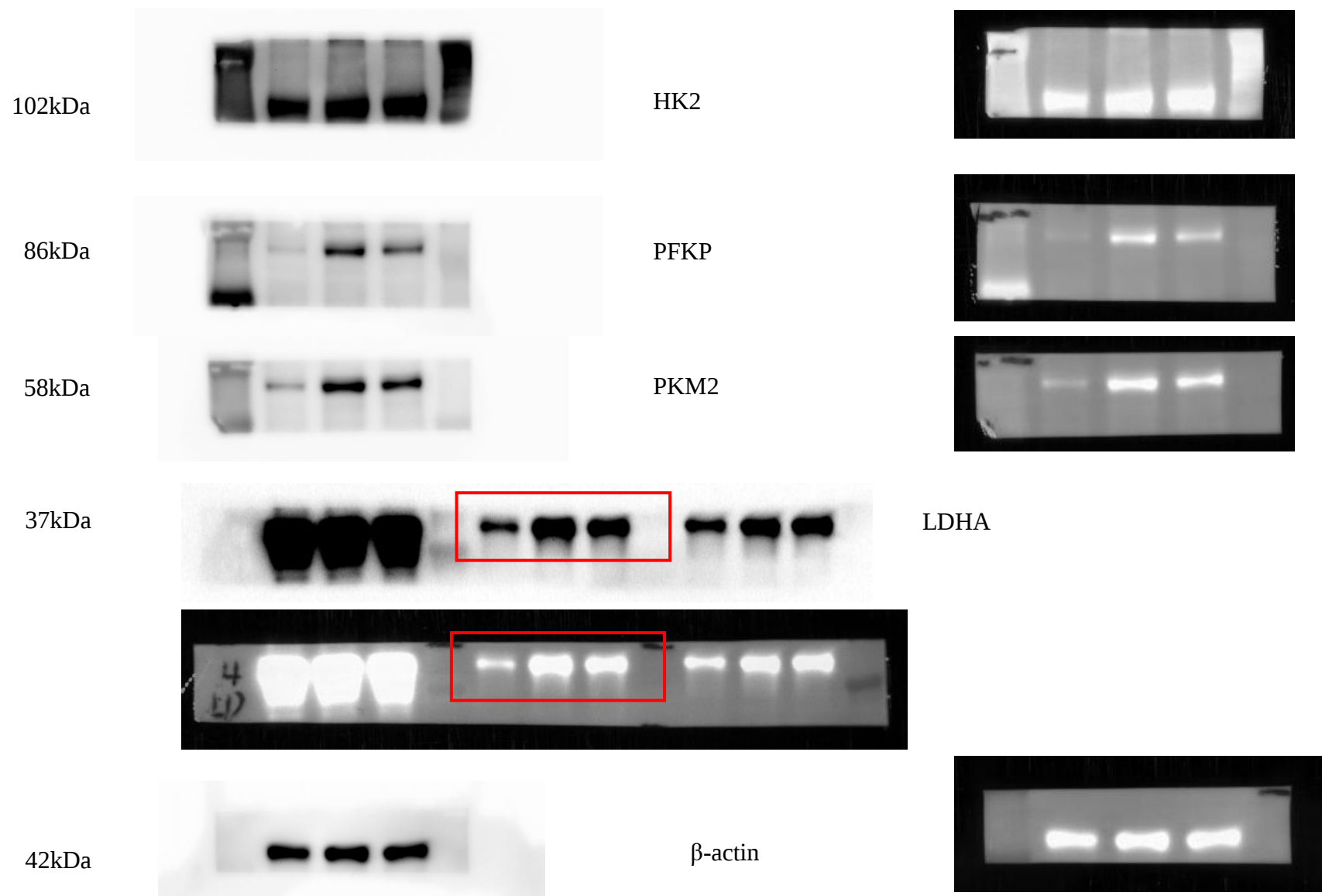
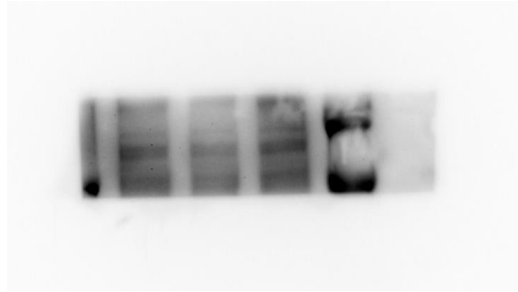


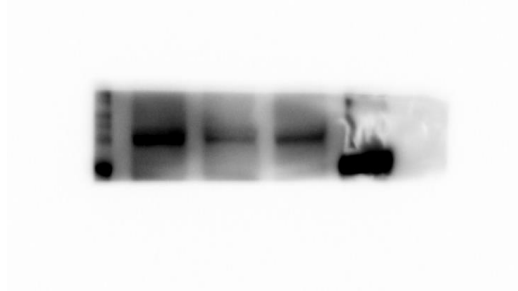
Fig 2f

140kDa



COL2A1

110kDa



ACAN

42kDa



β-actin

NC
TNF-α
TNF-α+2-DG

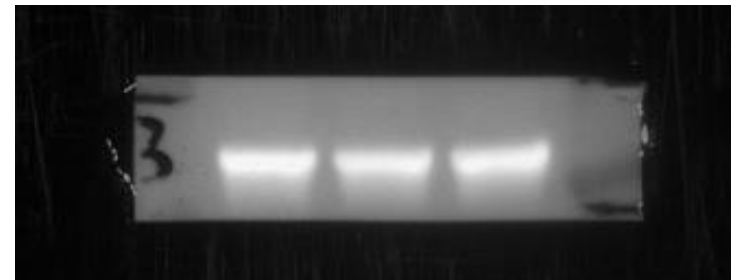


Fig 2f

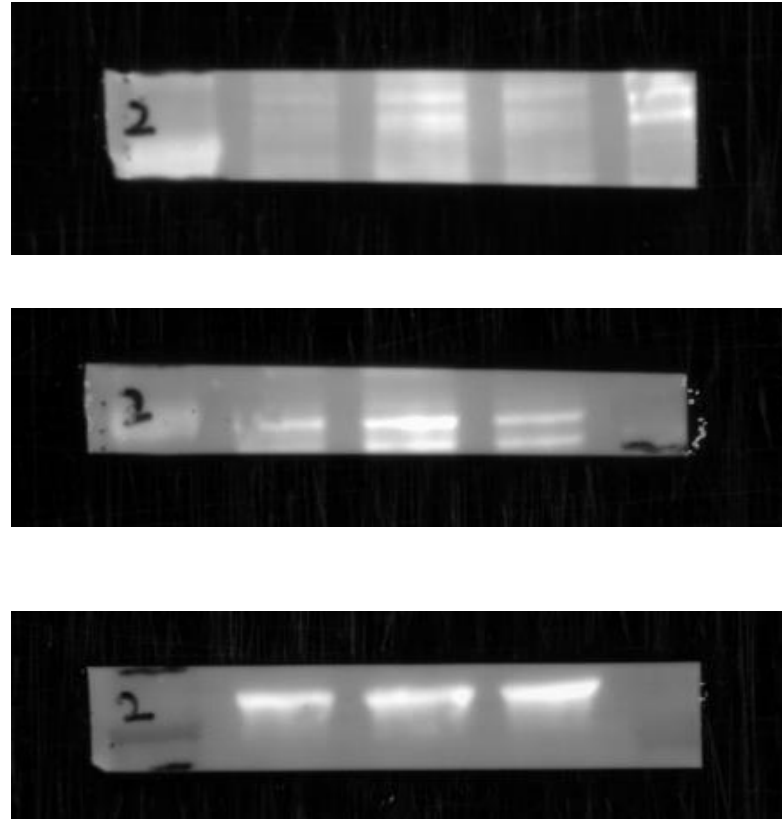
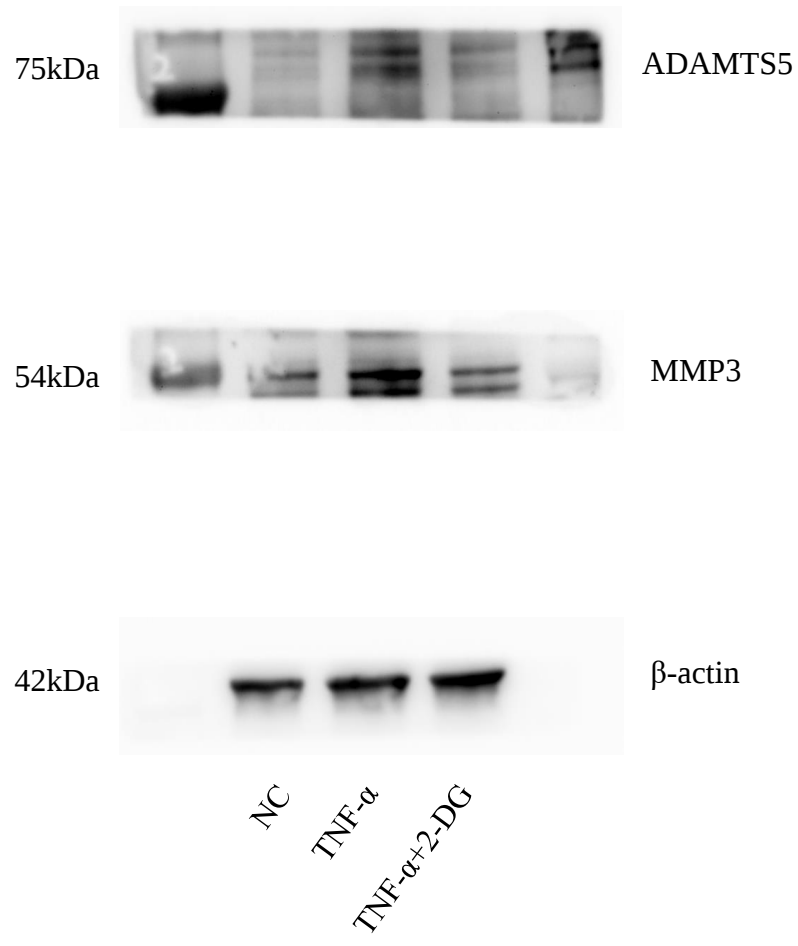


Fig 2g

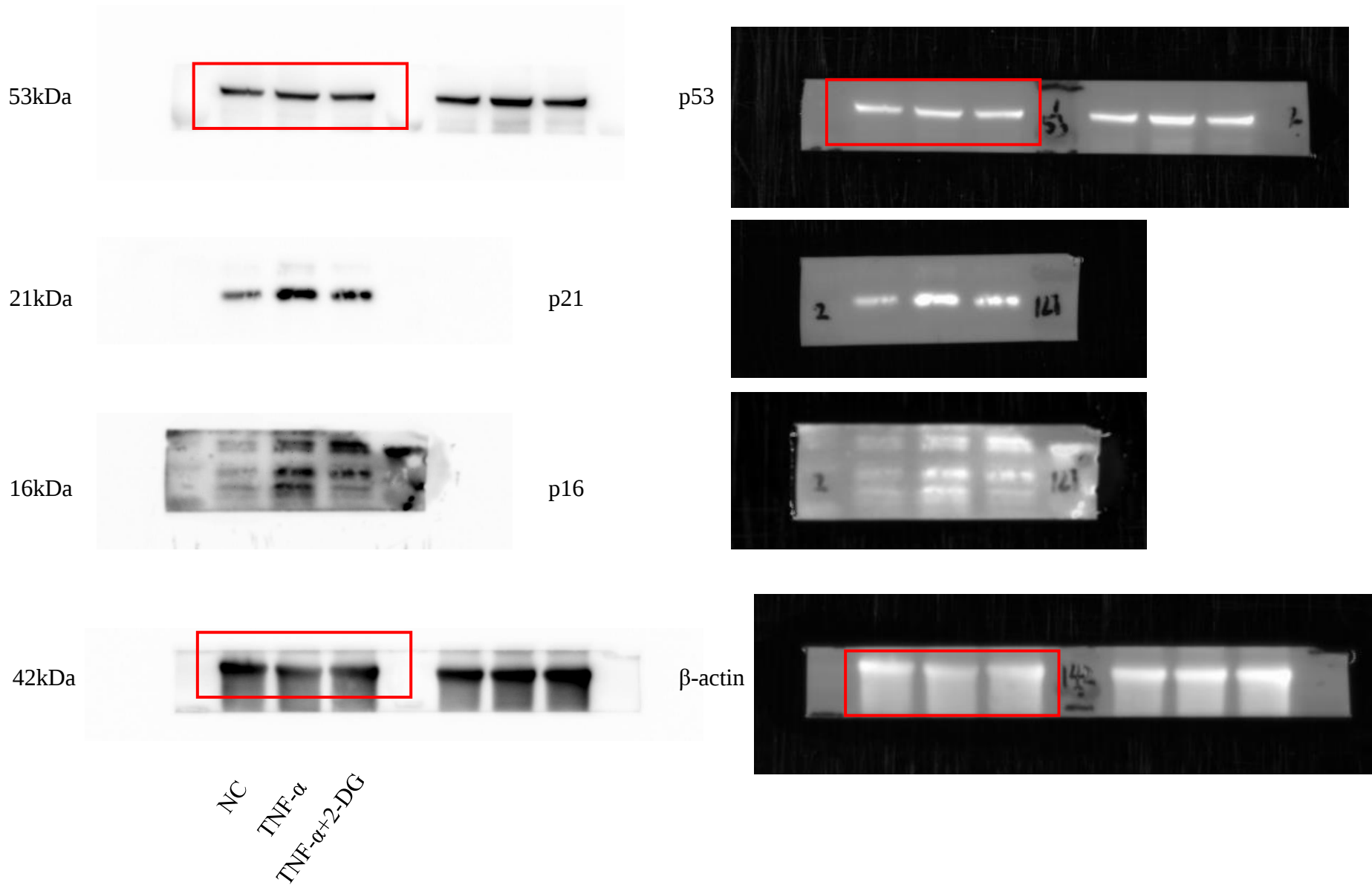


Fig 2i

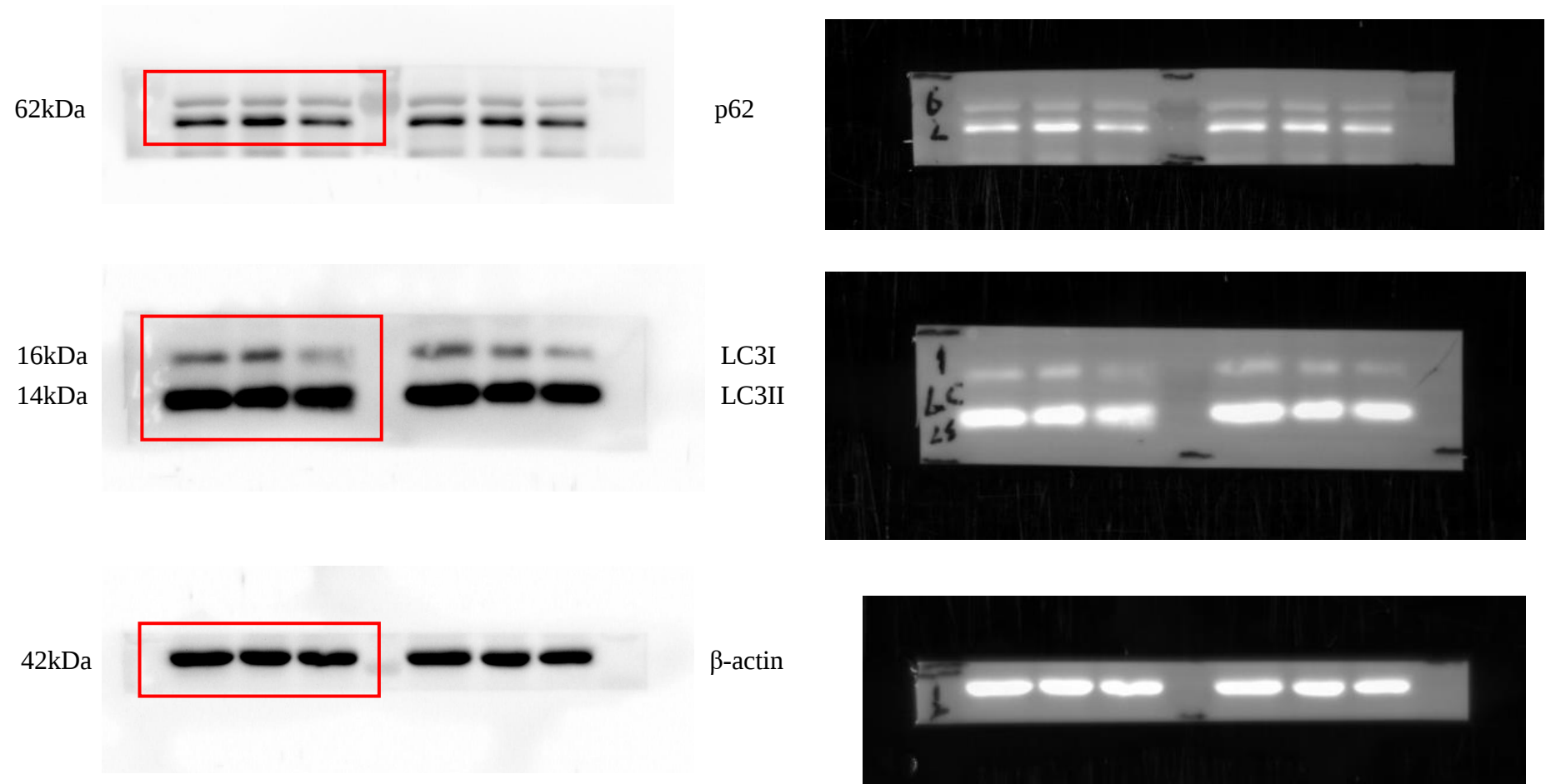
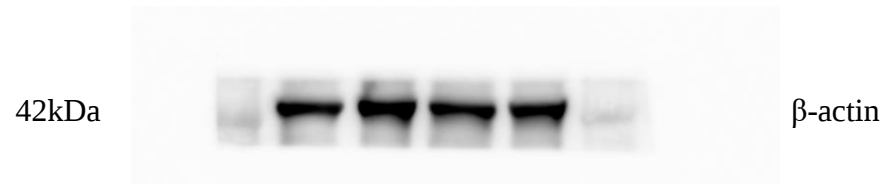
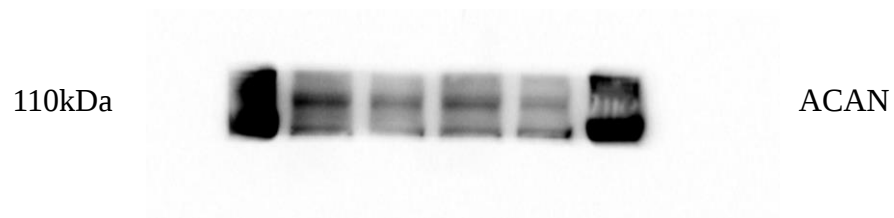
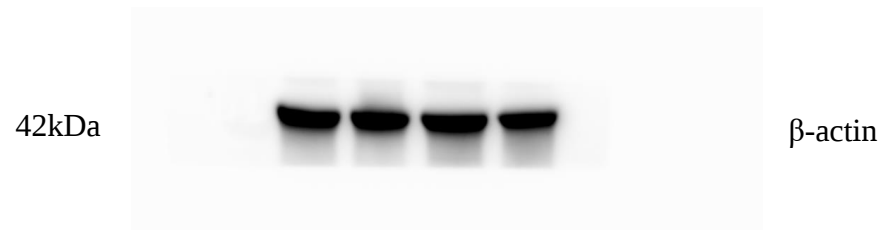


Fig 3g



NC
TNF- α
TNF- α +Gln
TNF- α +Gln+La

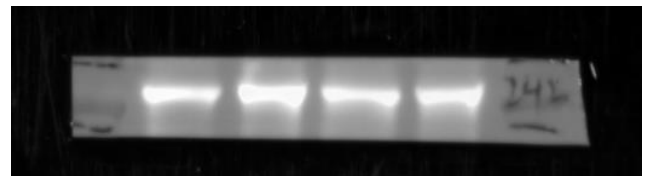
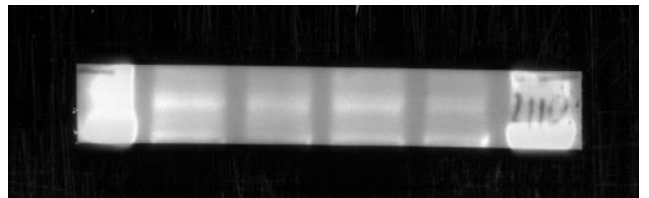
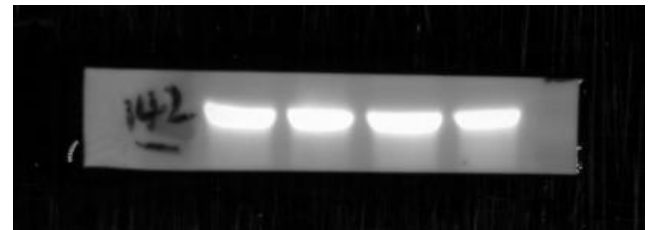
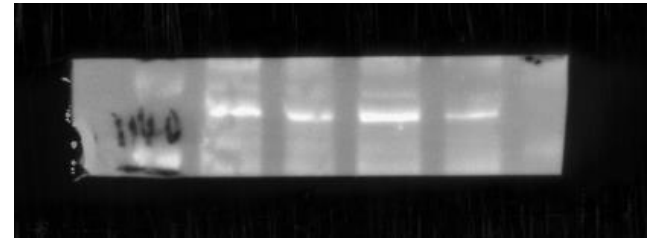
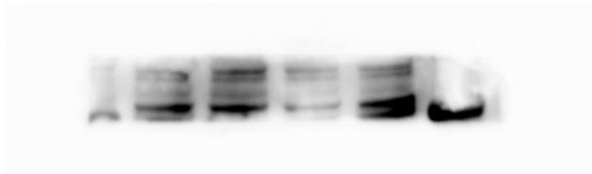
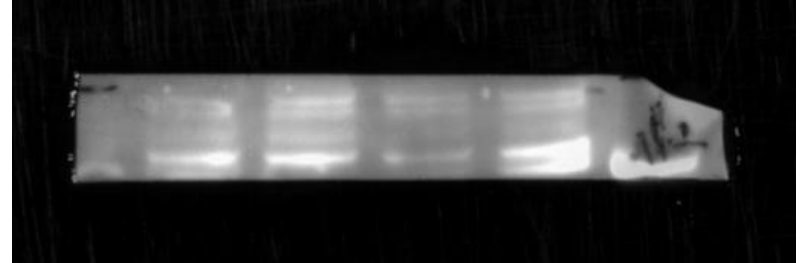


Fig 3h

75kDa



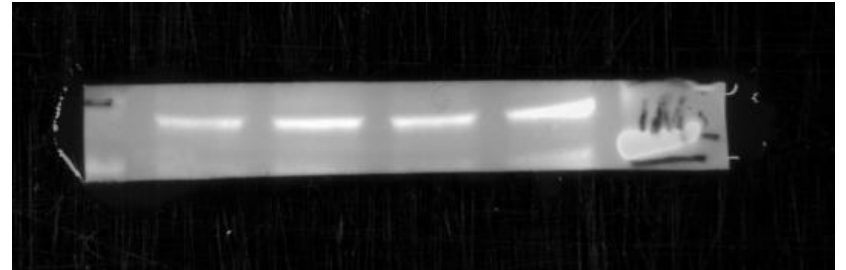
ADAMTS5



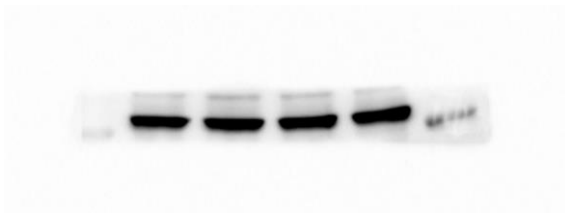
54kDa



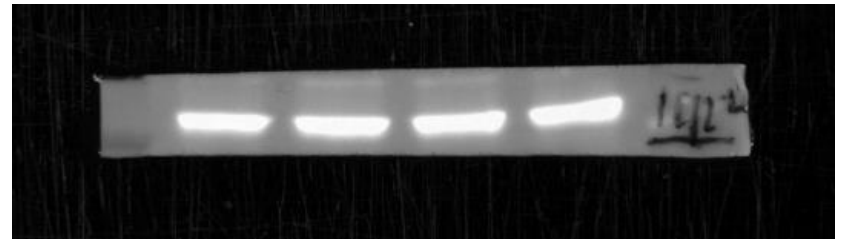
MMP3



42kDa



β -actin



NC
TNF- α
TNF- α +Gln
TNF- α +Gln+La

Fig 3j

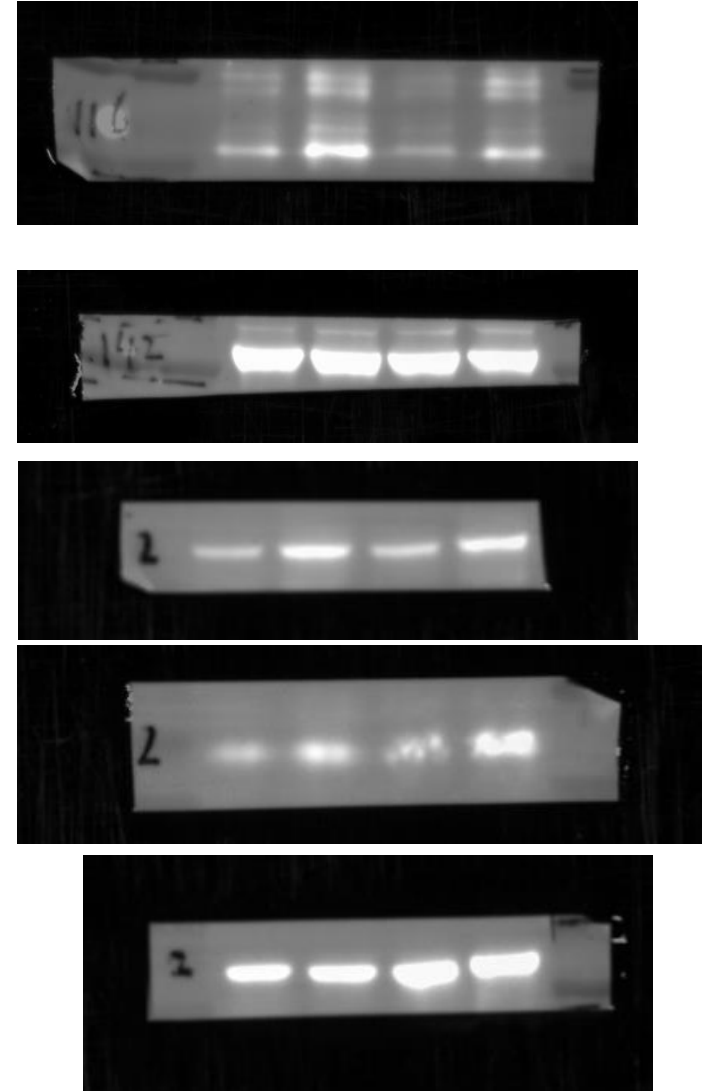
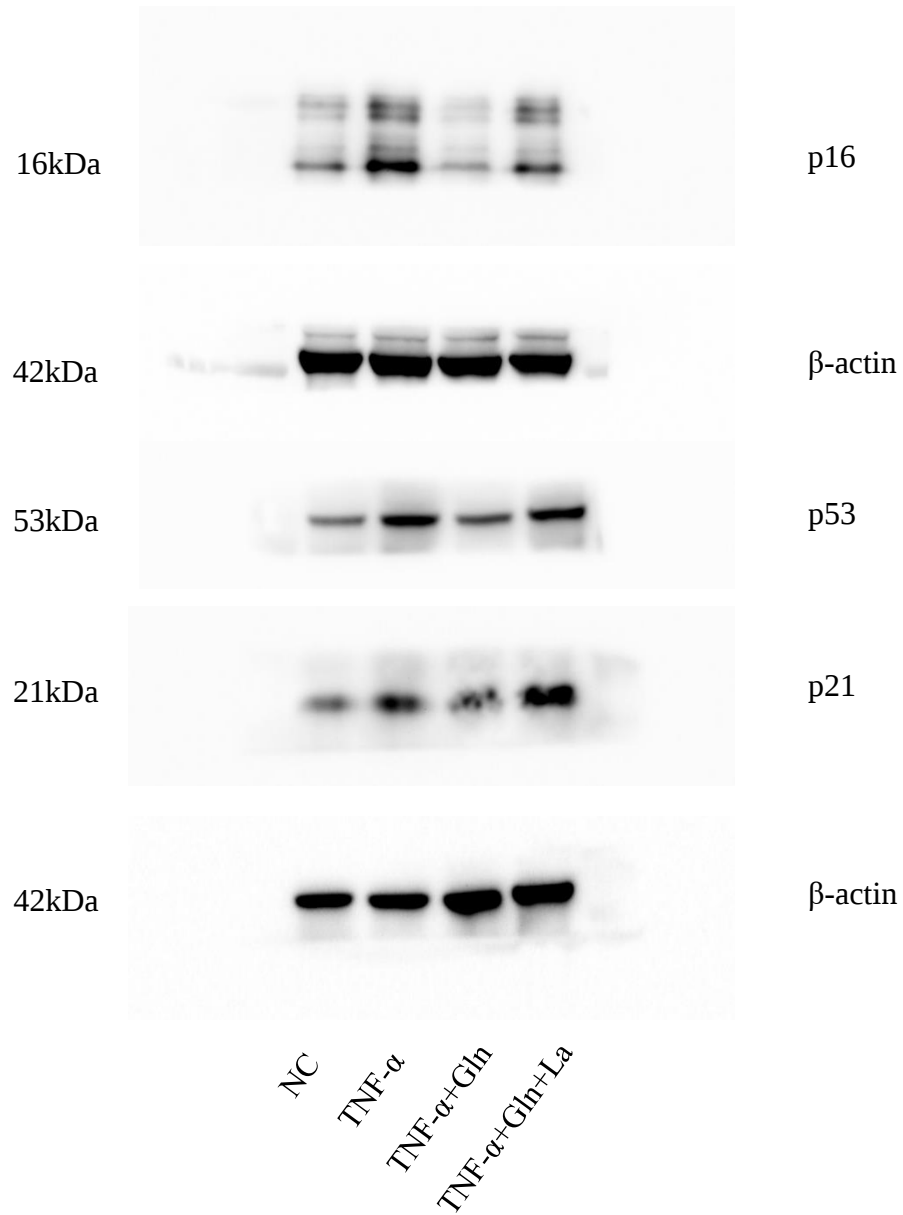


Fig 3k

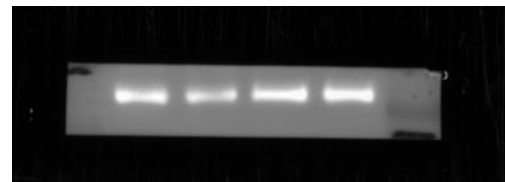
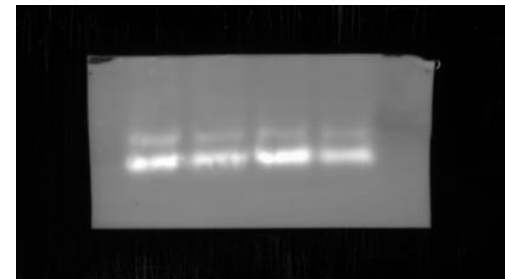
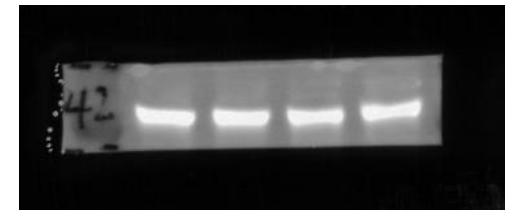
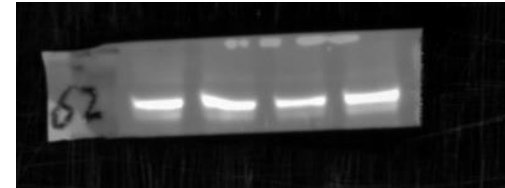
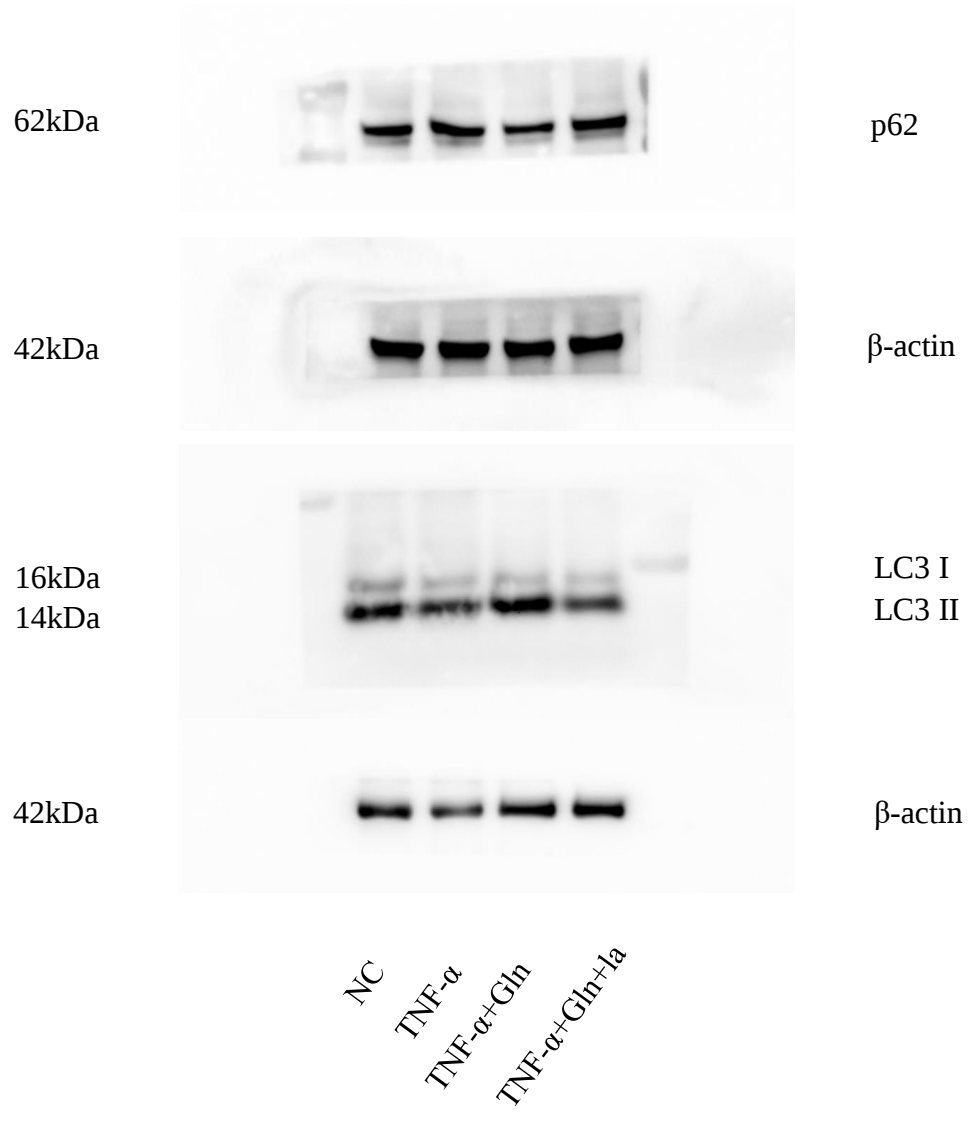


Fig 4a

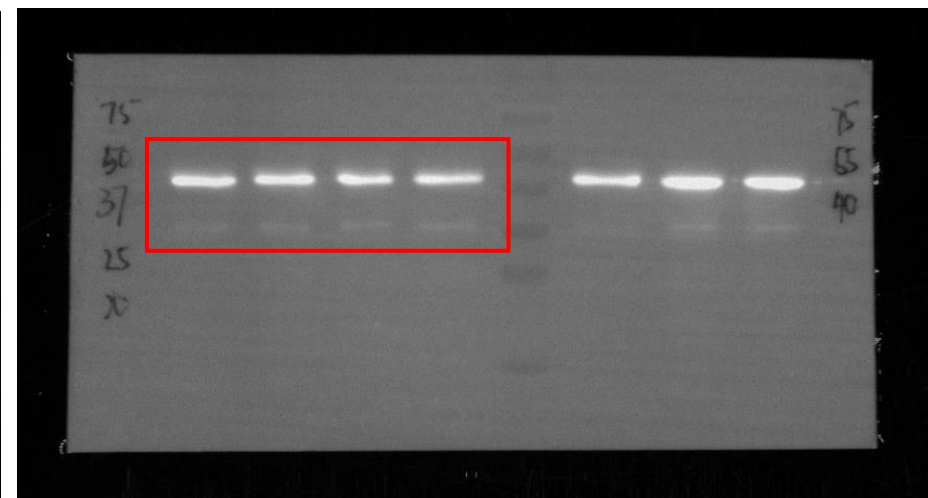
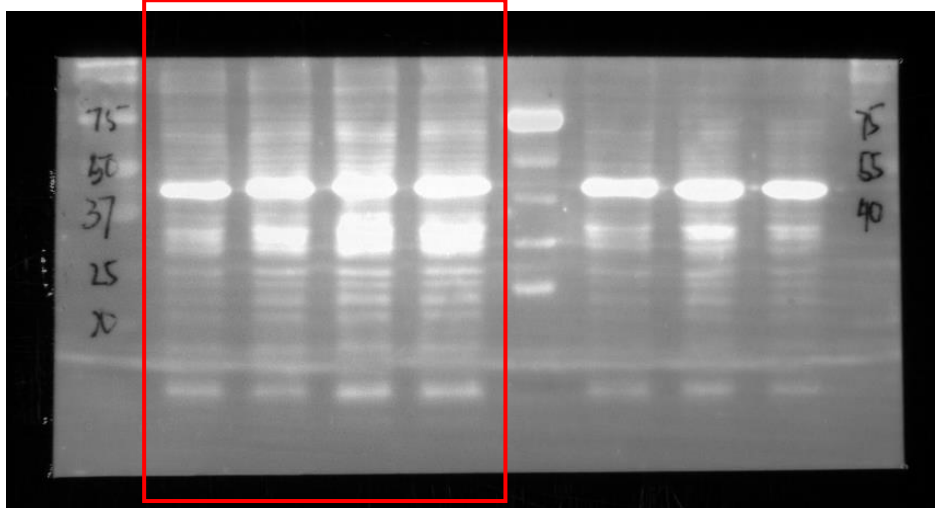
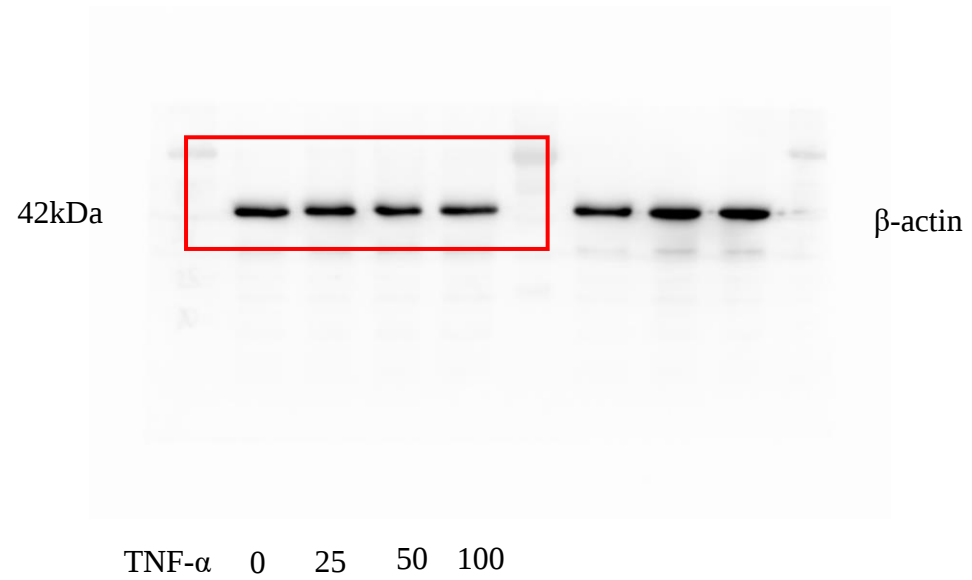
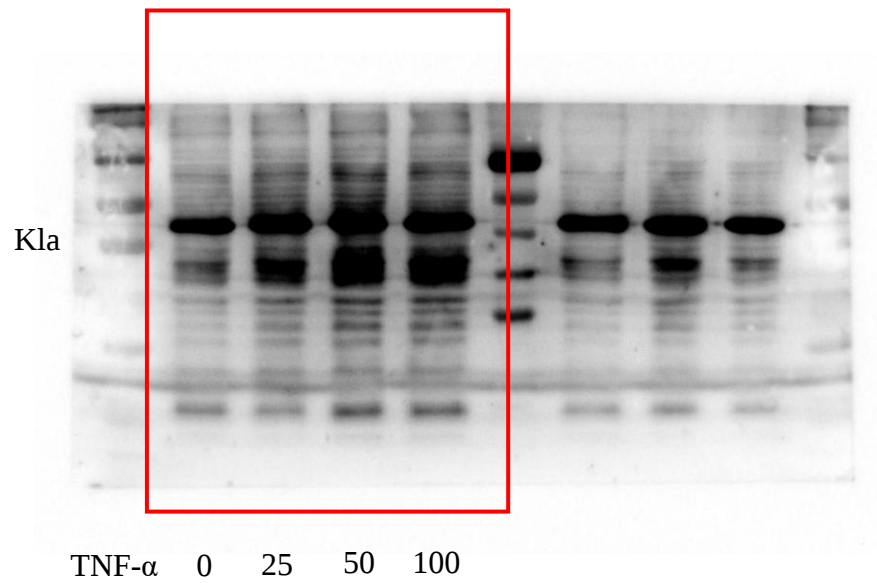
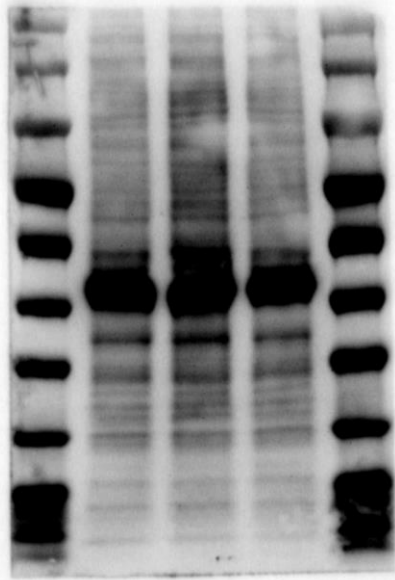
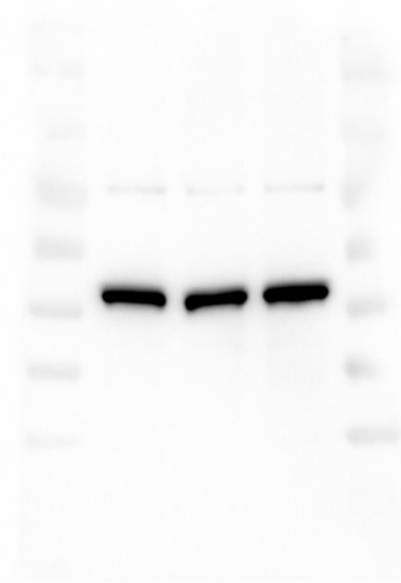


Fig 4b



Kla

42kDa



β -actin

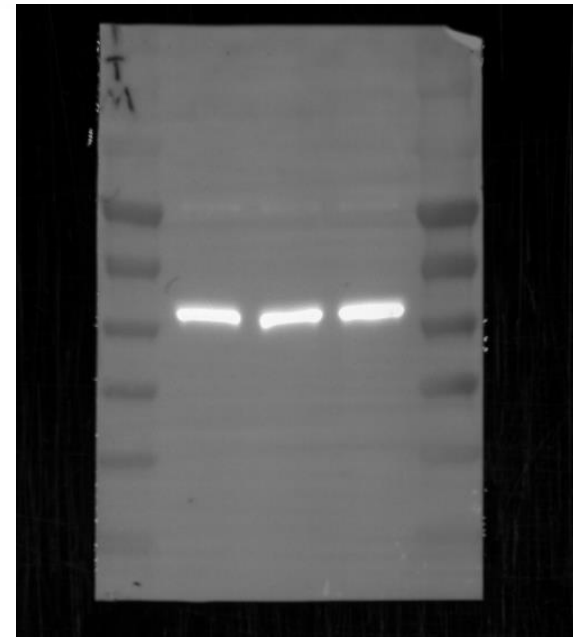
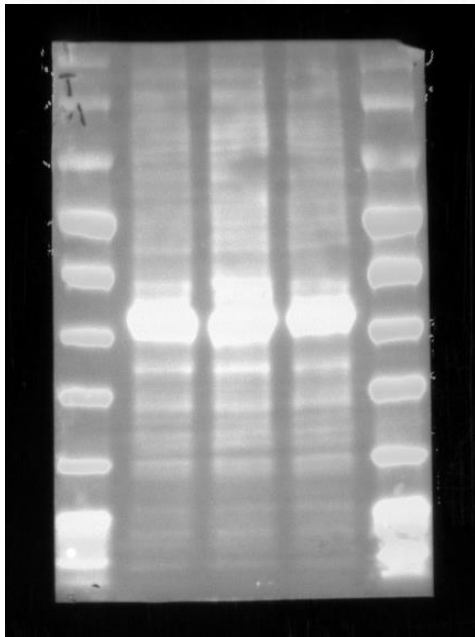
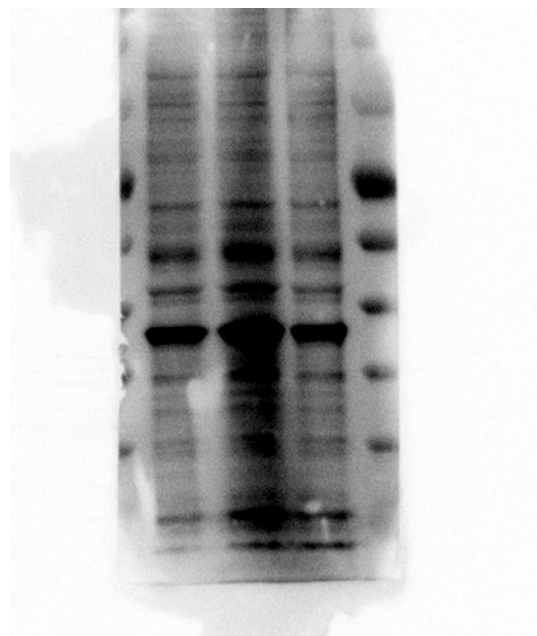
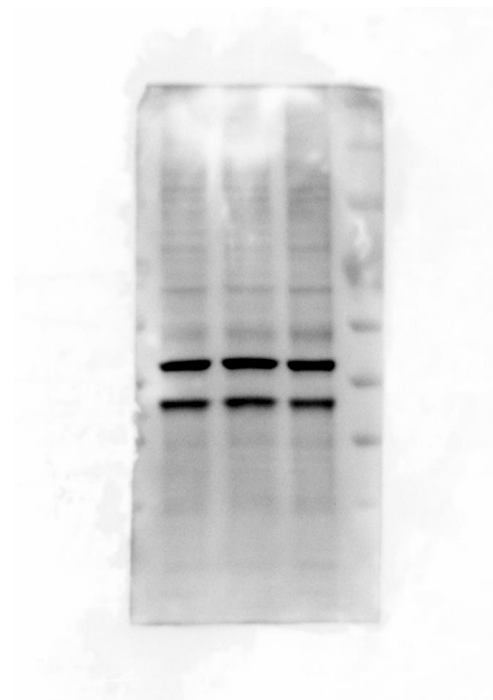


Fig 4c



Kla

42kDa



β -actin

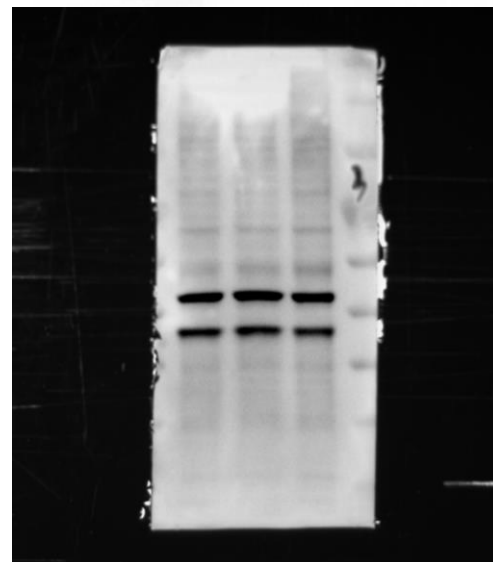
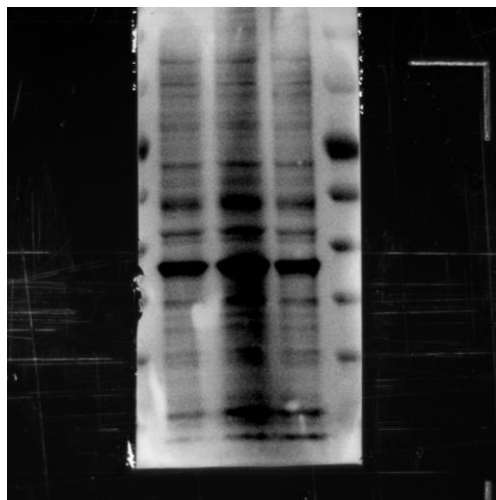


Fig 4d

Kla

42kDa

β -actin

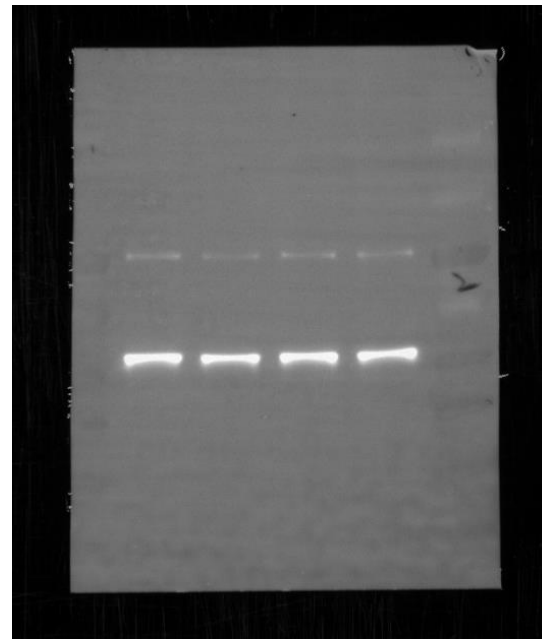
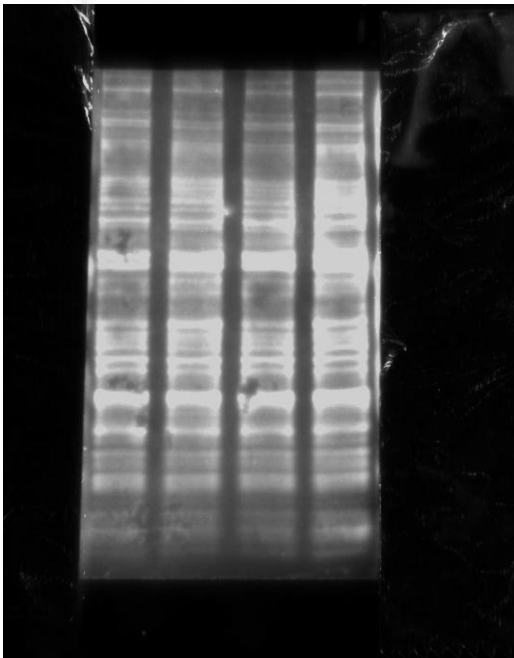
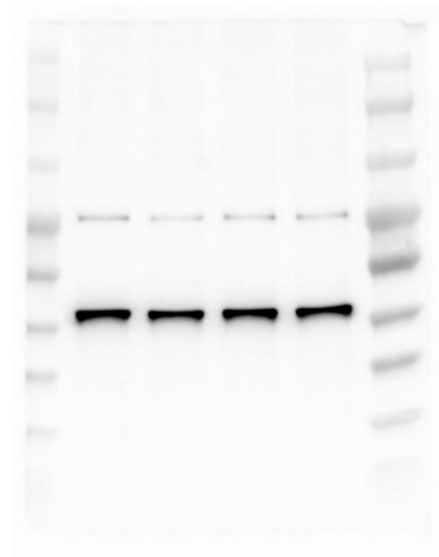
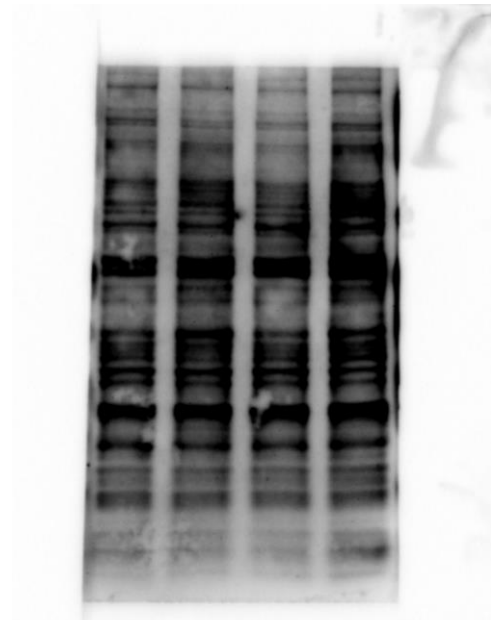
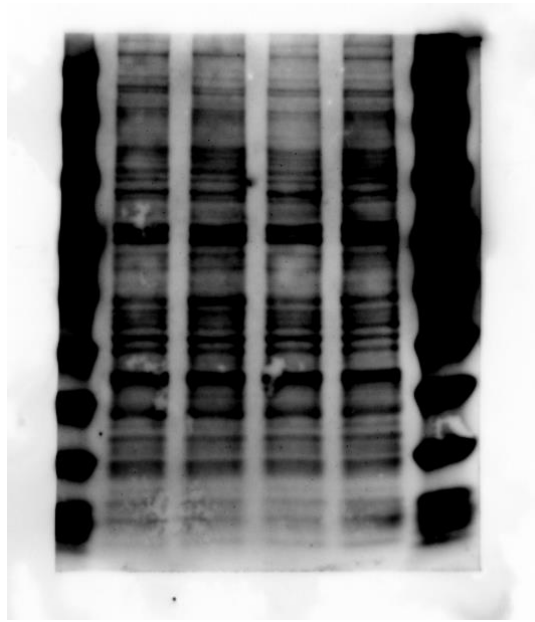
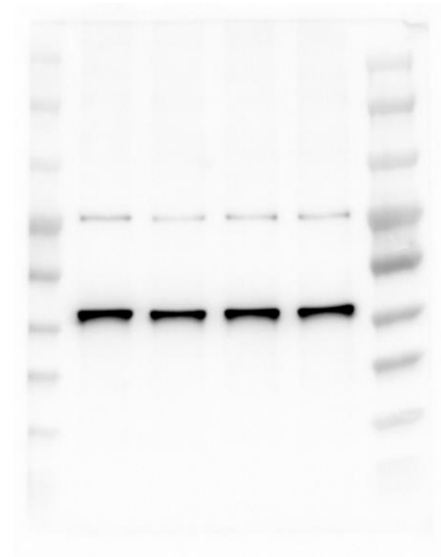


Fig 4d



Kla



42kDa

β-actin

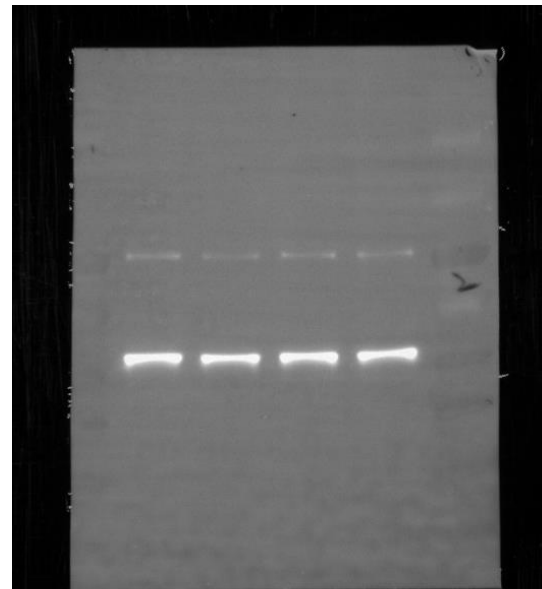
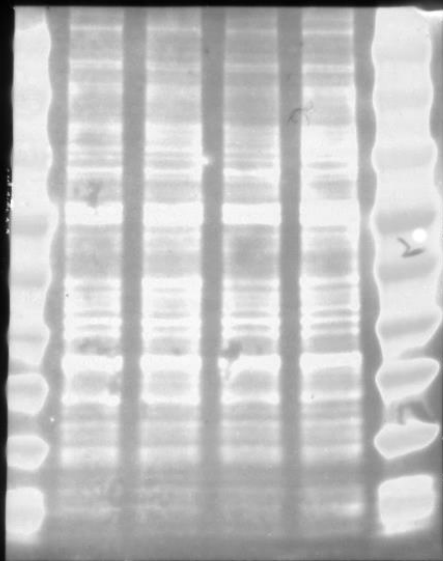


Fig 4e

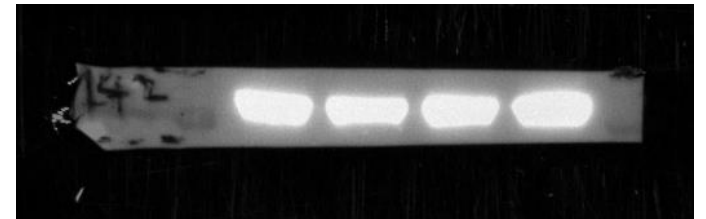
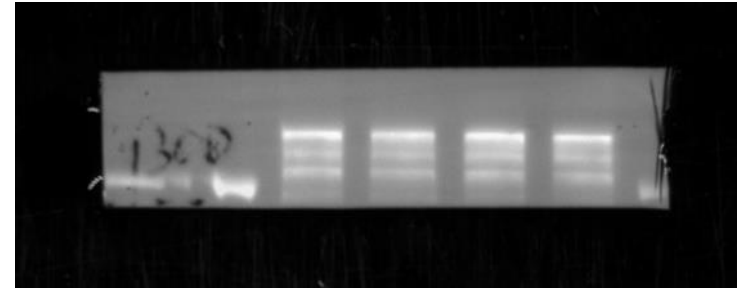
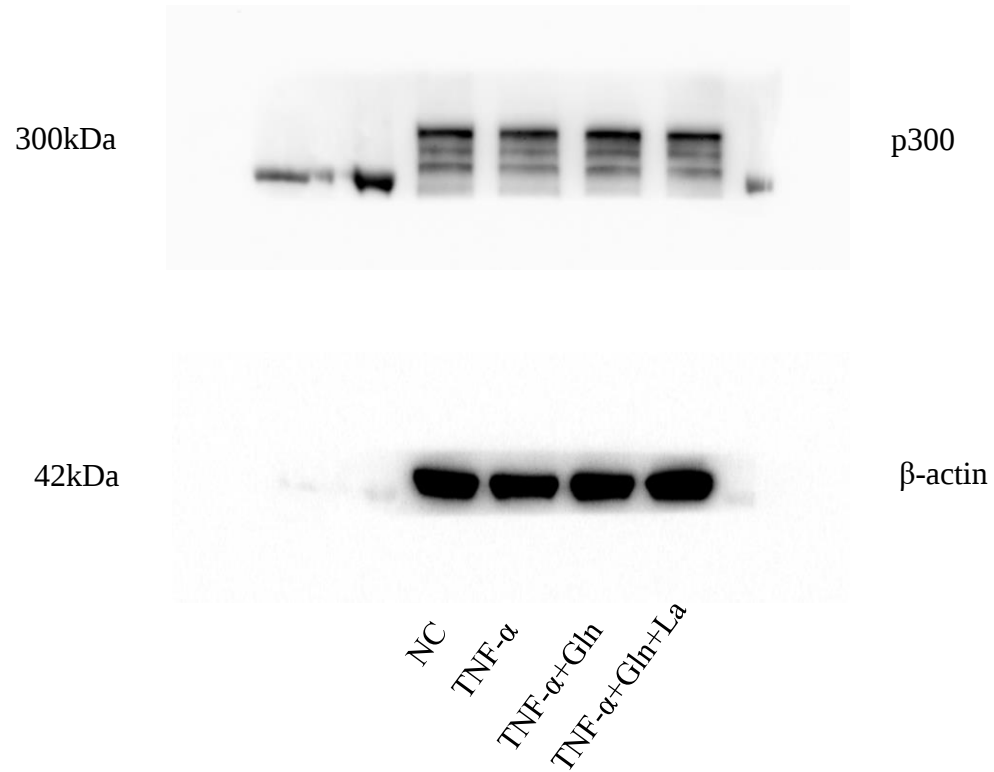


Fig 5a

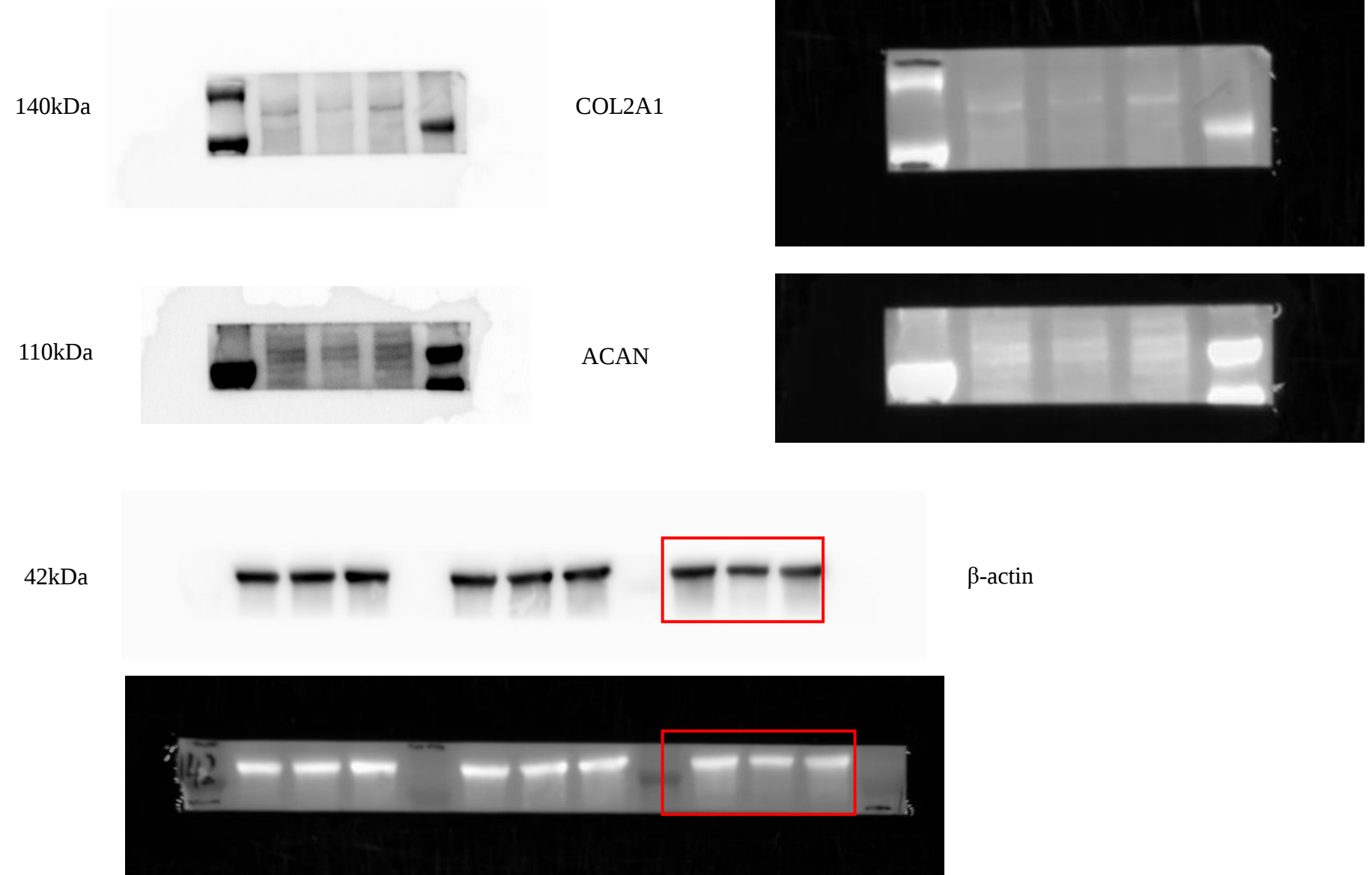


Fig 5a

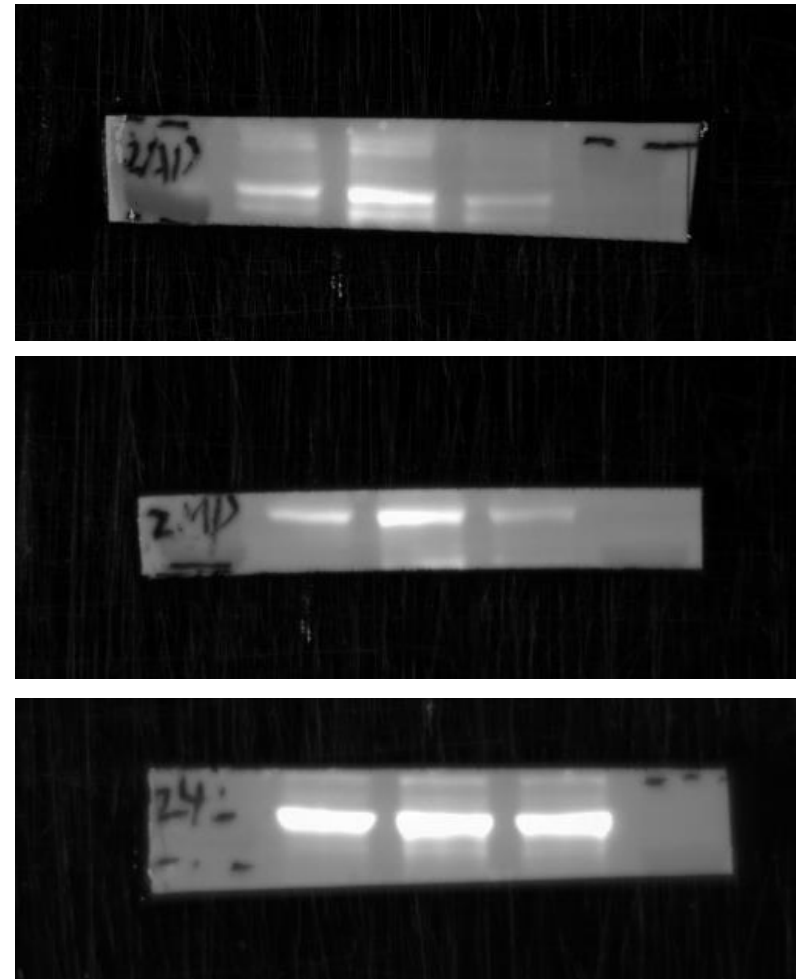
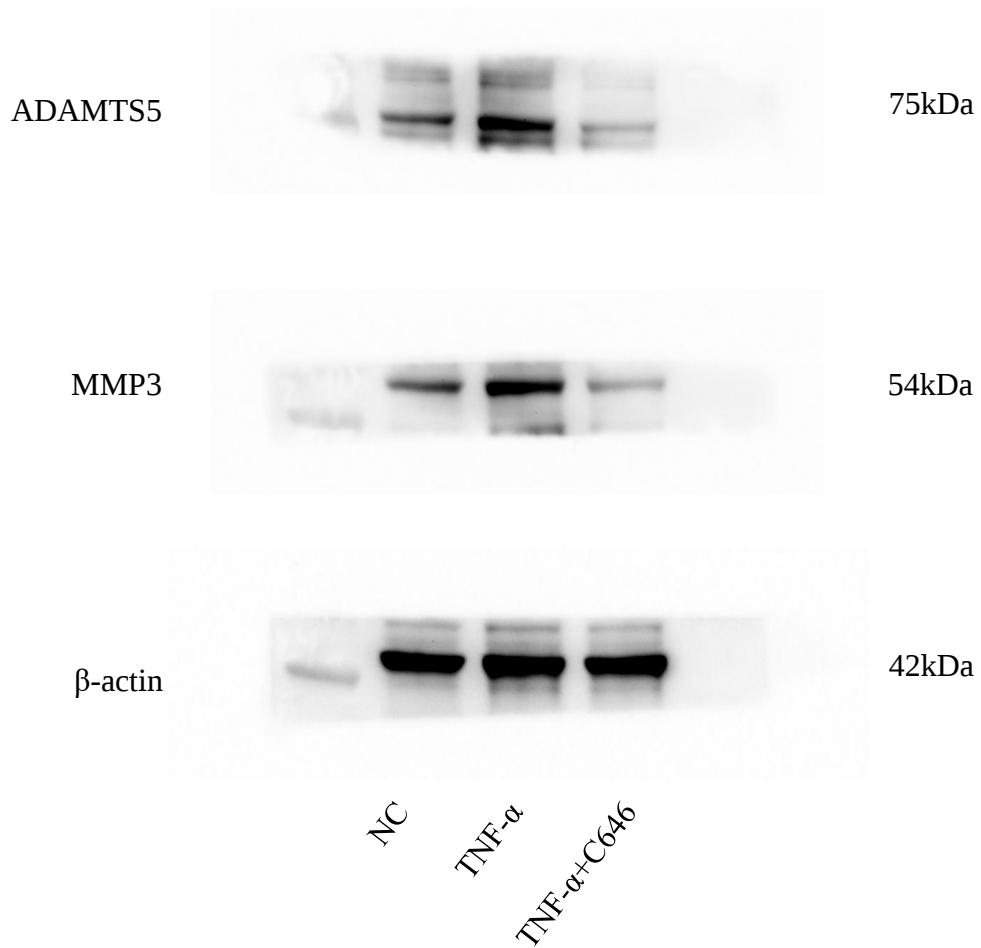
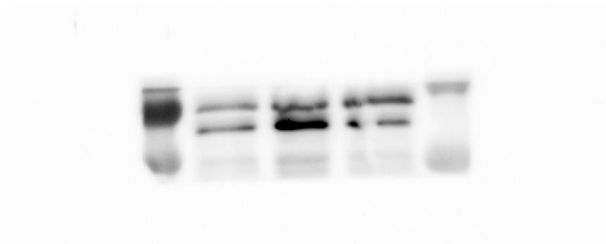
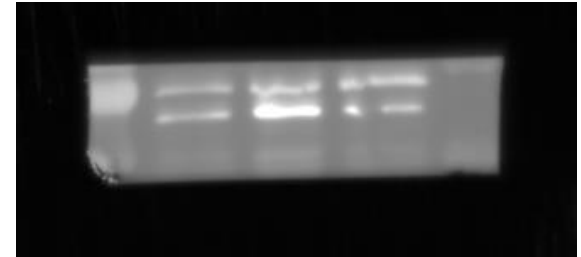


Fig 5c

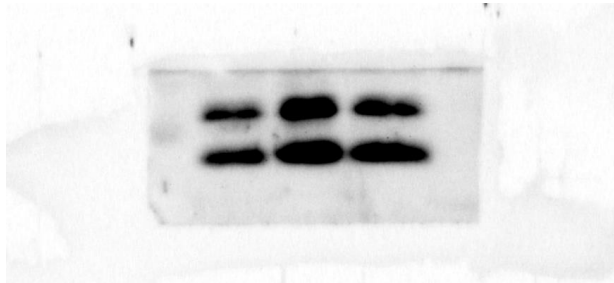
62kDa



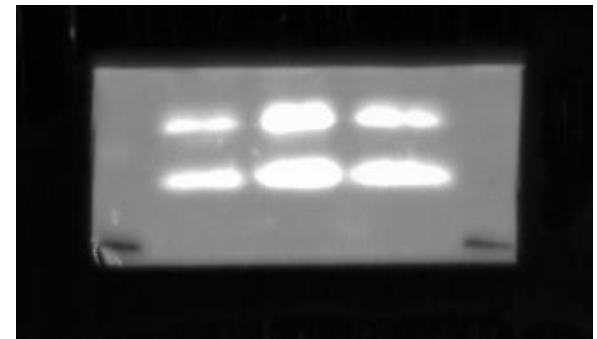
p62



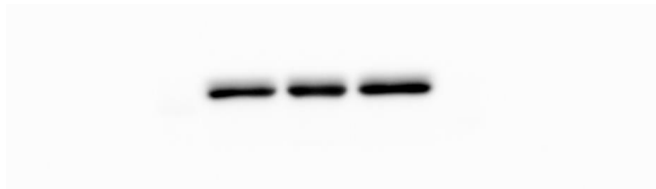
16kDa
14kDa



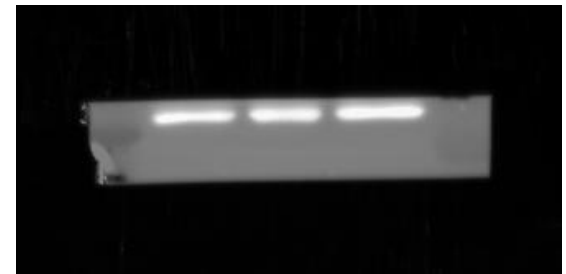
LC3 I
LC3 II



42kDa



β-actin



NC
TNF- α
TNF- α +C646

Fig 5e

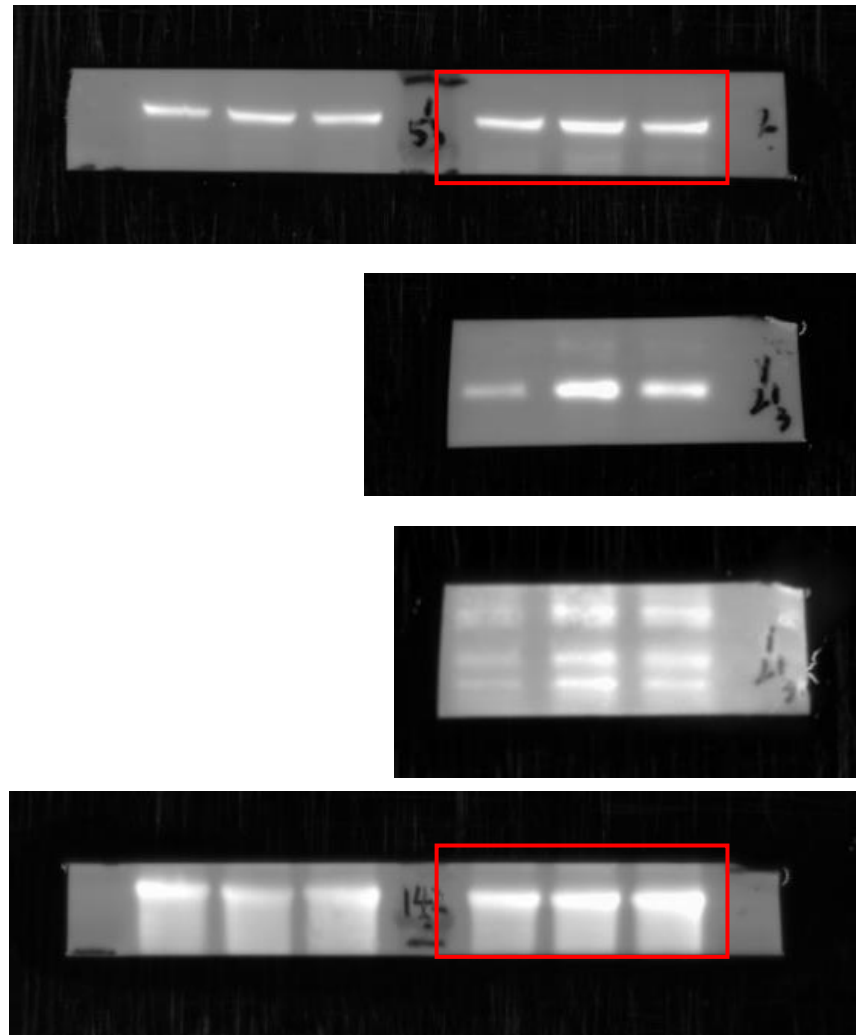
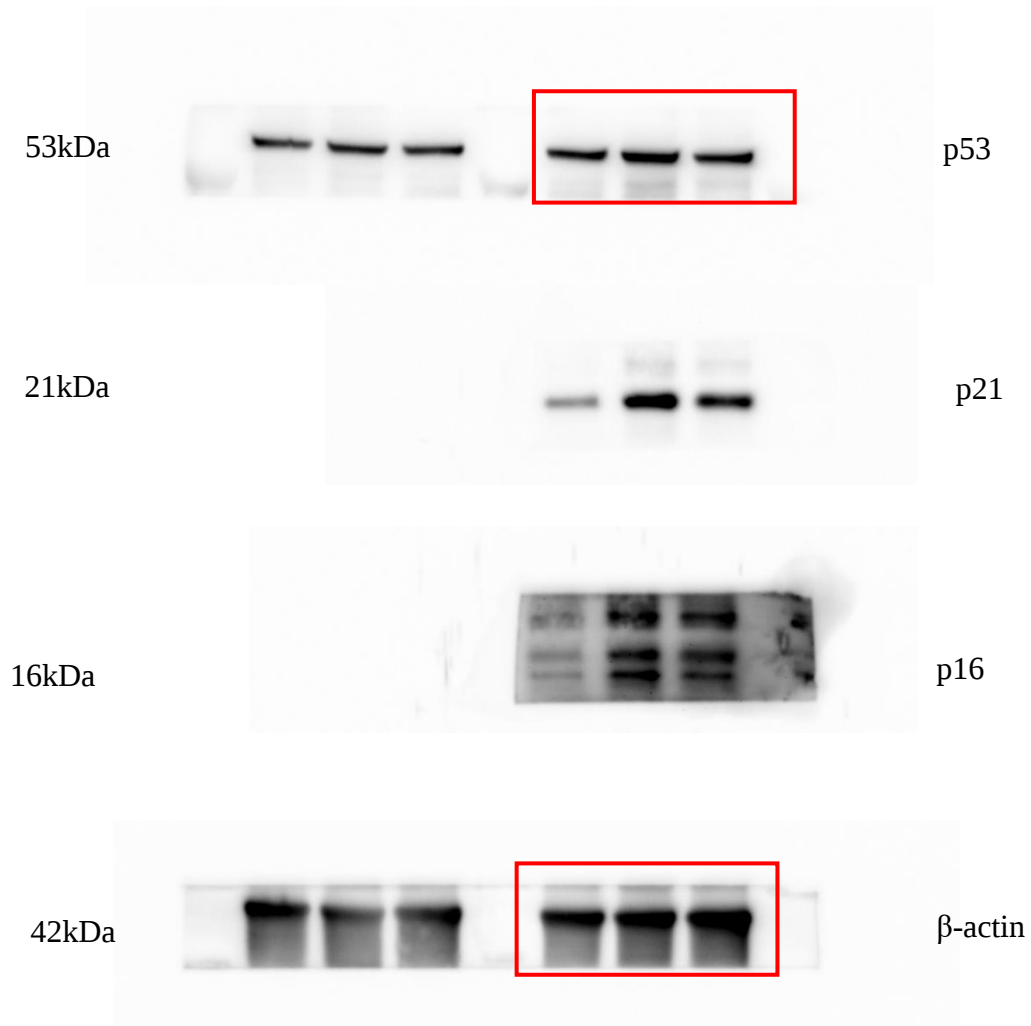
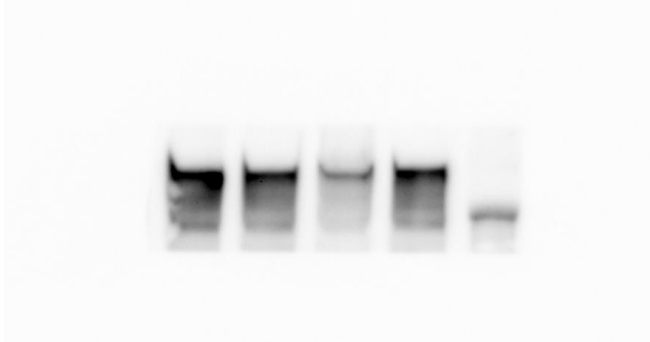
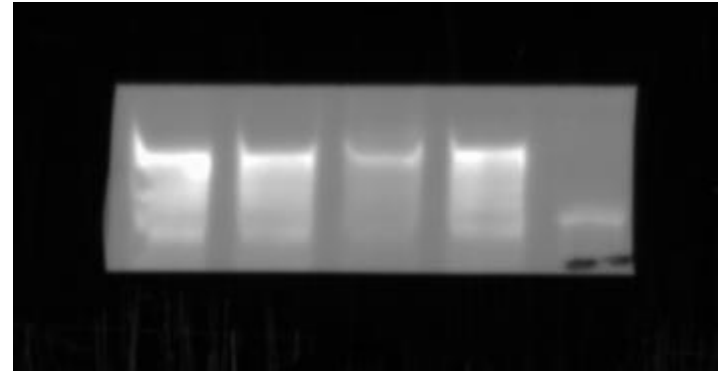


Fig 5h

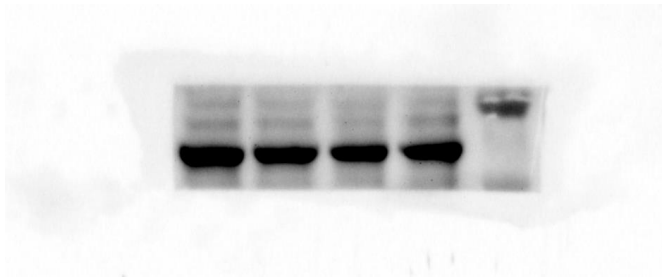
300kDa



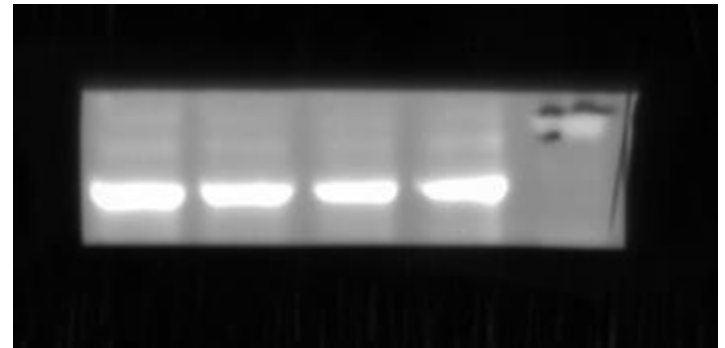
p300



42kDa



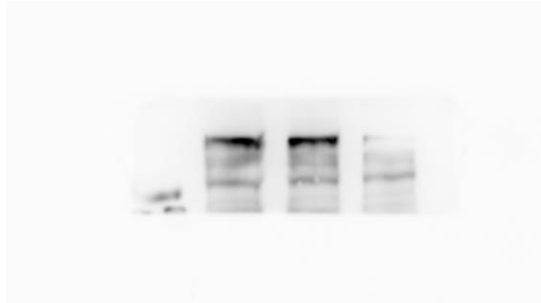
β-actin



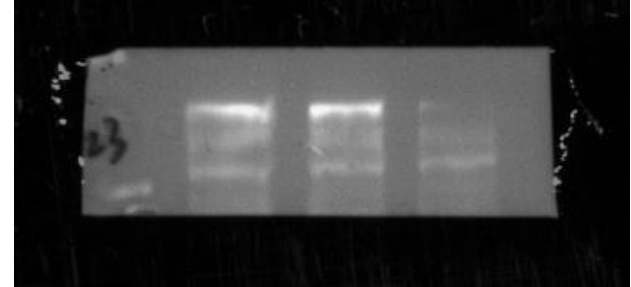
siNC
sip300-1
sip300-2
sip300-3

Fig 5i

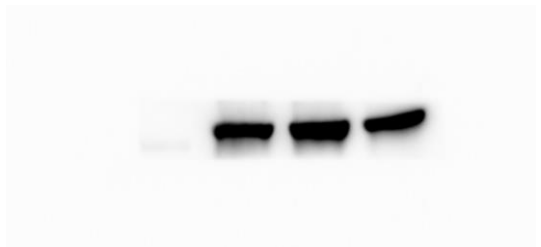
300kDa



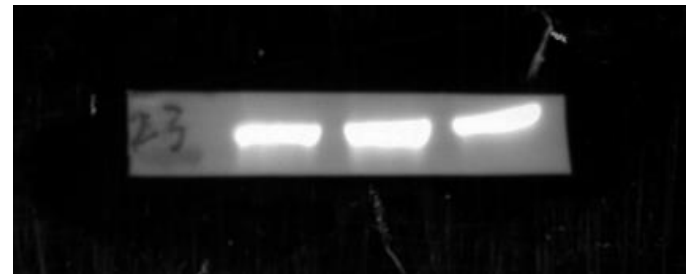
p300



42kDa



β -actin



siNC
TNF- α +siNC
TNF- α +sip300

Fig 5i

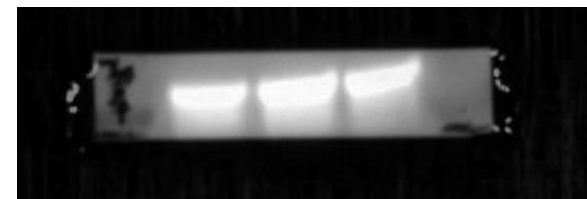
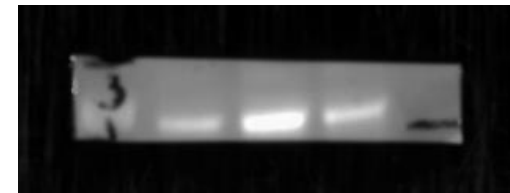
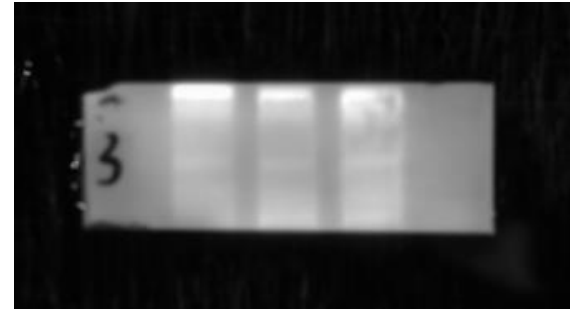
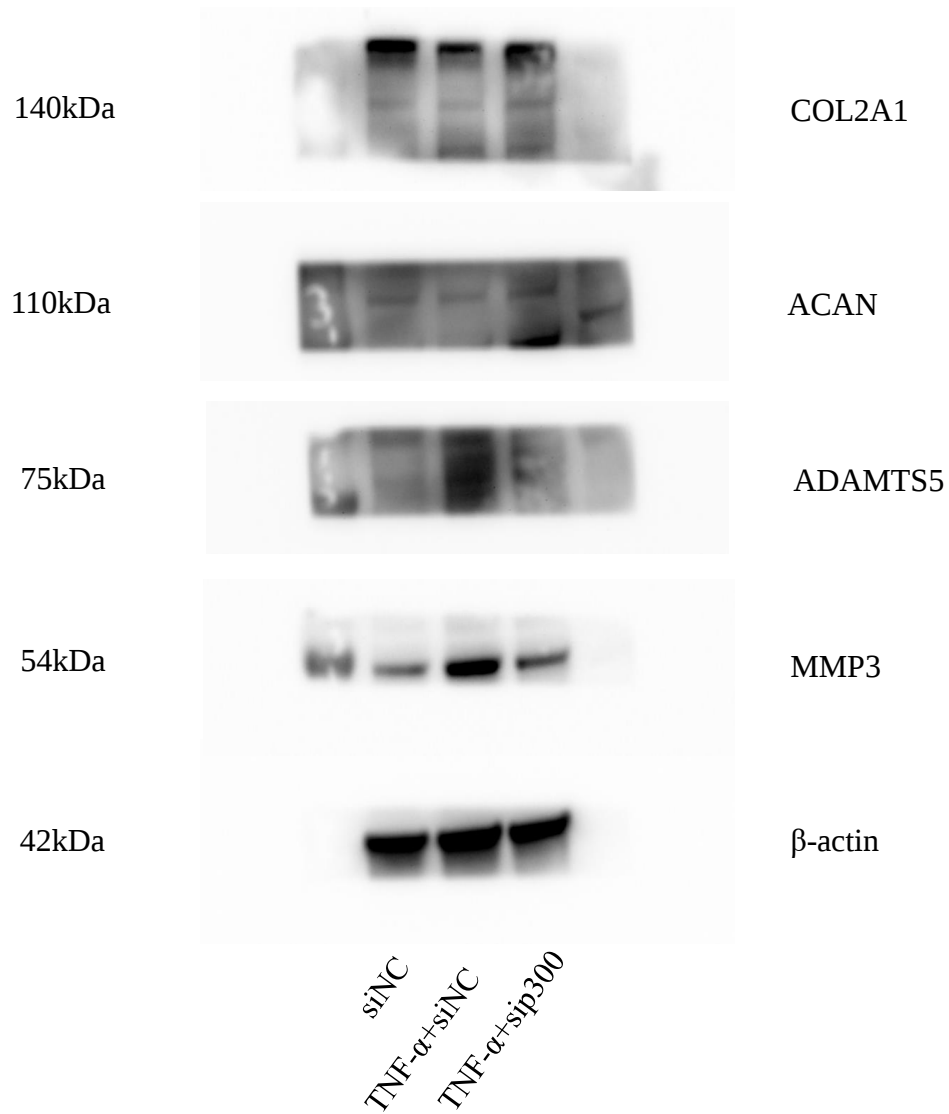
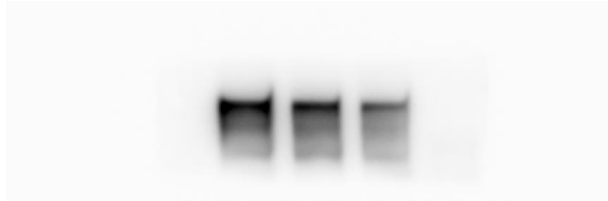
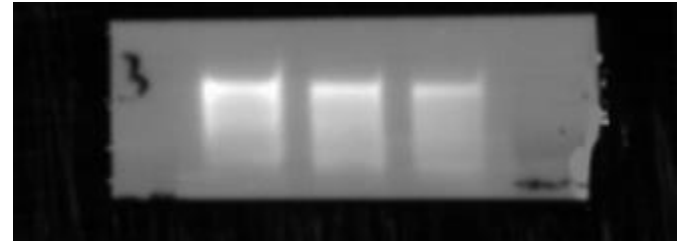


Fig 5j

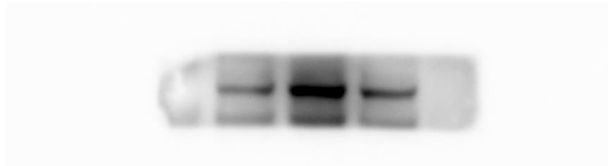
300kDa



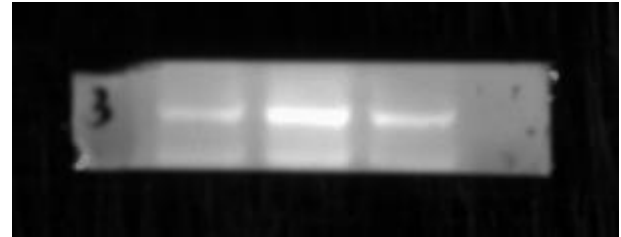
p300



53kDa



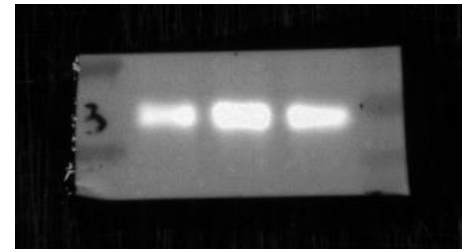
p53



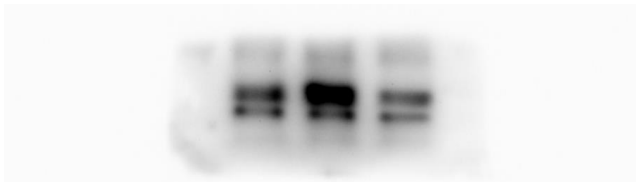
21kDa



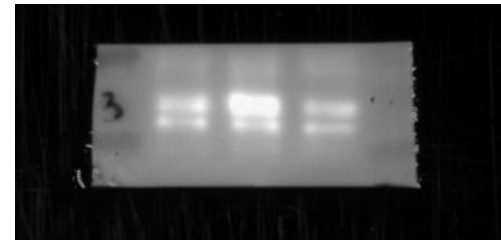
p21



16kDa



p16



42kDa



β -actin

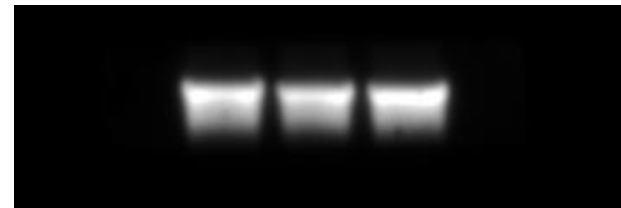


Fig 5k

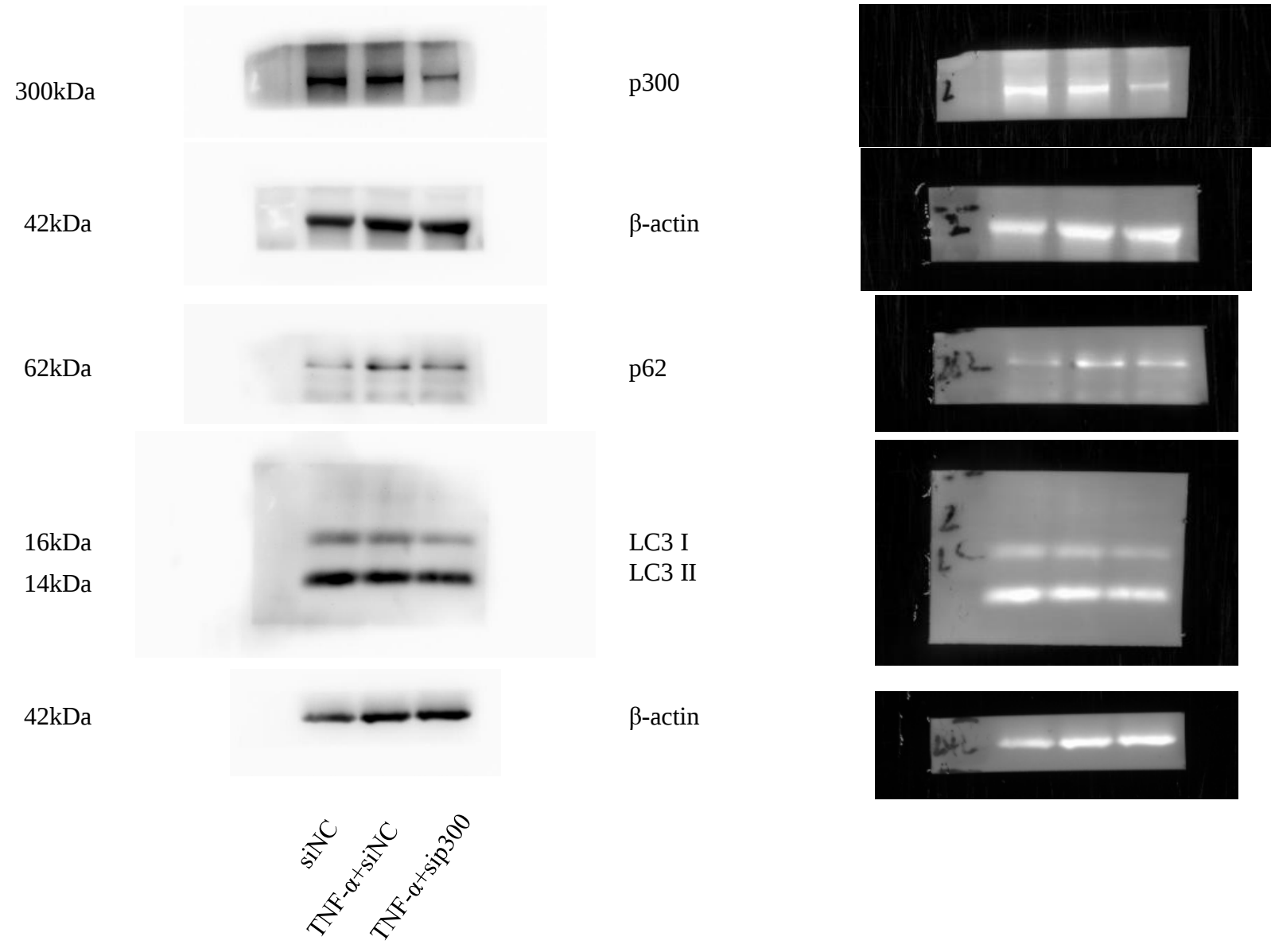
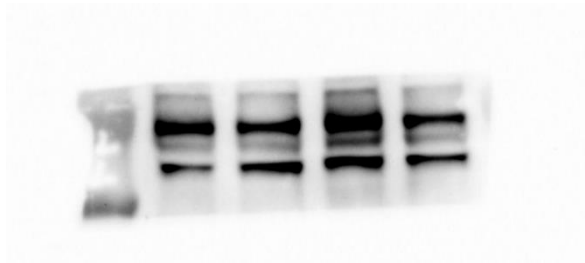
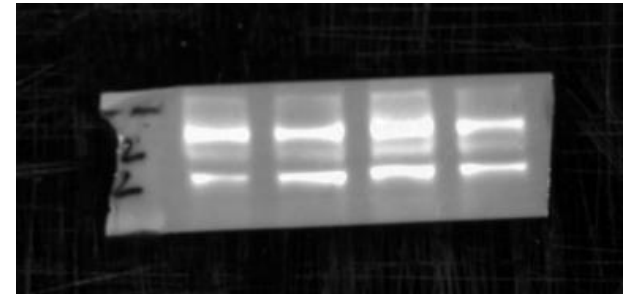


Fig 6b

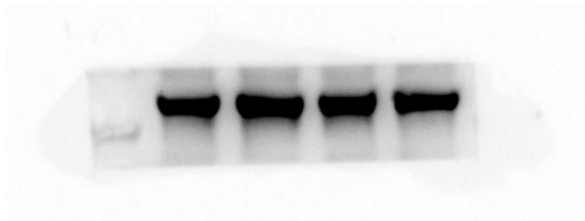
65kDa



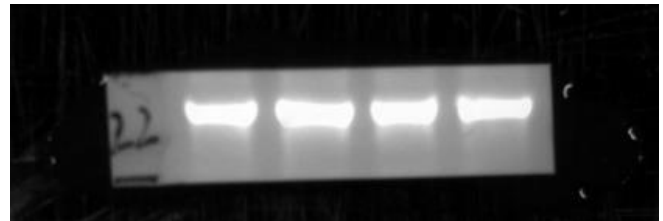
p-AMPK α



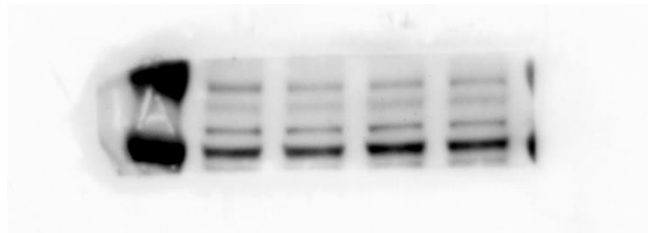
42kDa



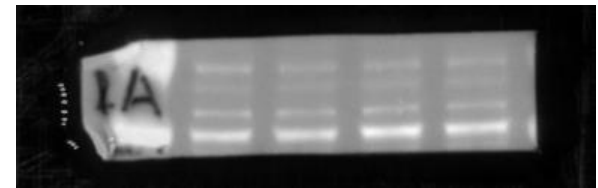
β-actin



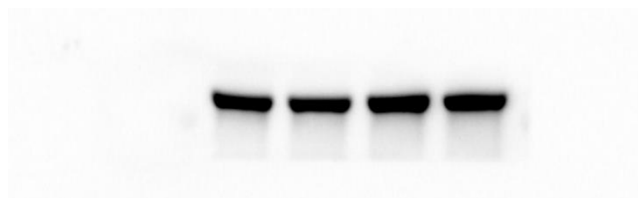
65kDa



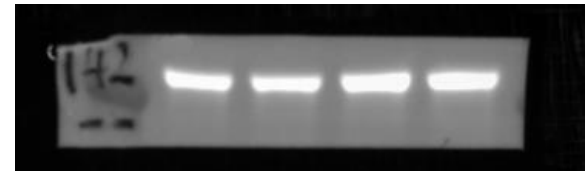
AMPK α



42kDa



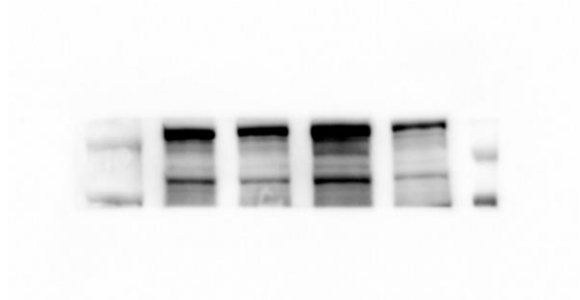
β-actin



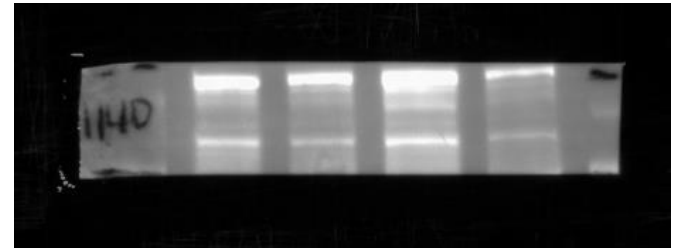
NC
TNF- α
TNF- α +Gln
TNF- α +Gln+La

Fig 7a

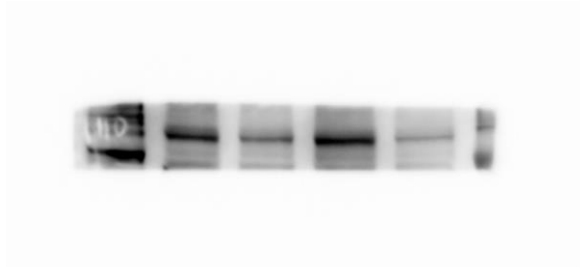
140kDa



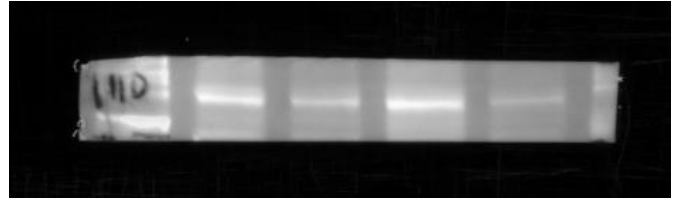
COL2A1



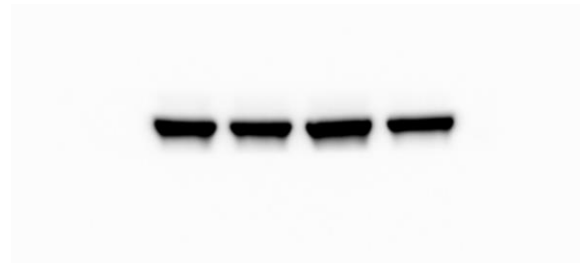
110kDa



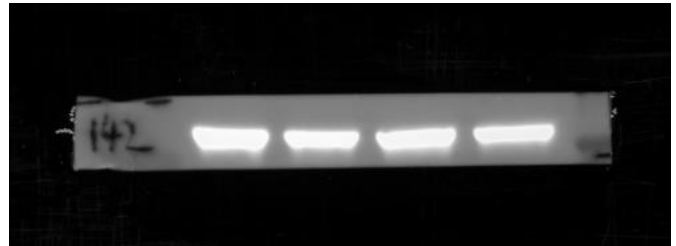
ACAN



42kDa



β-actin



NC
TNF-α
TNF-α+Gln
TNF-α+Gln+ComC

Fig 7a

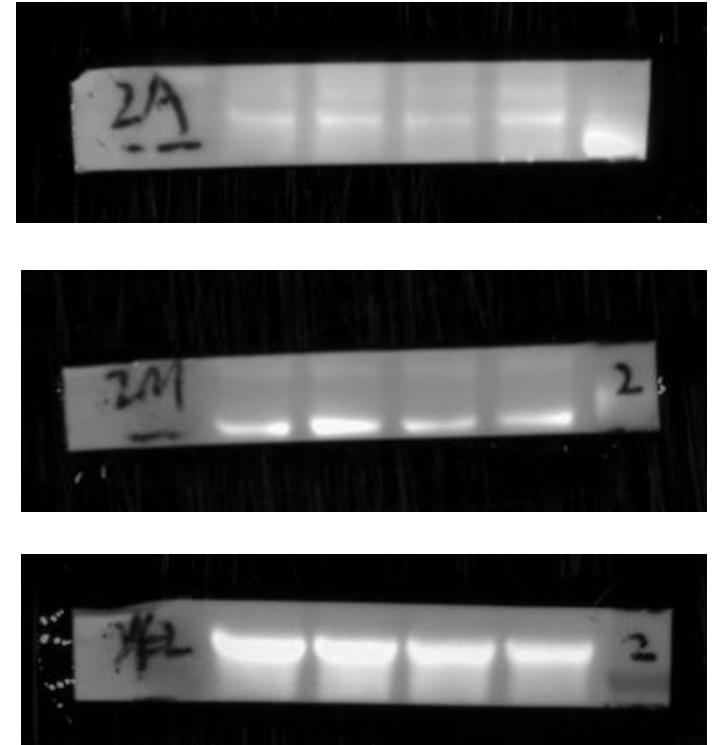
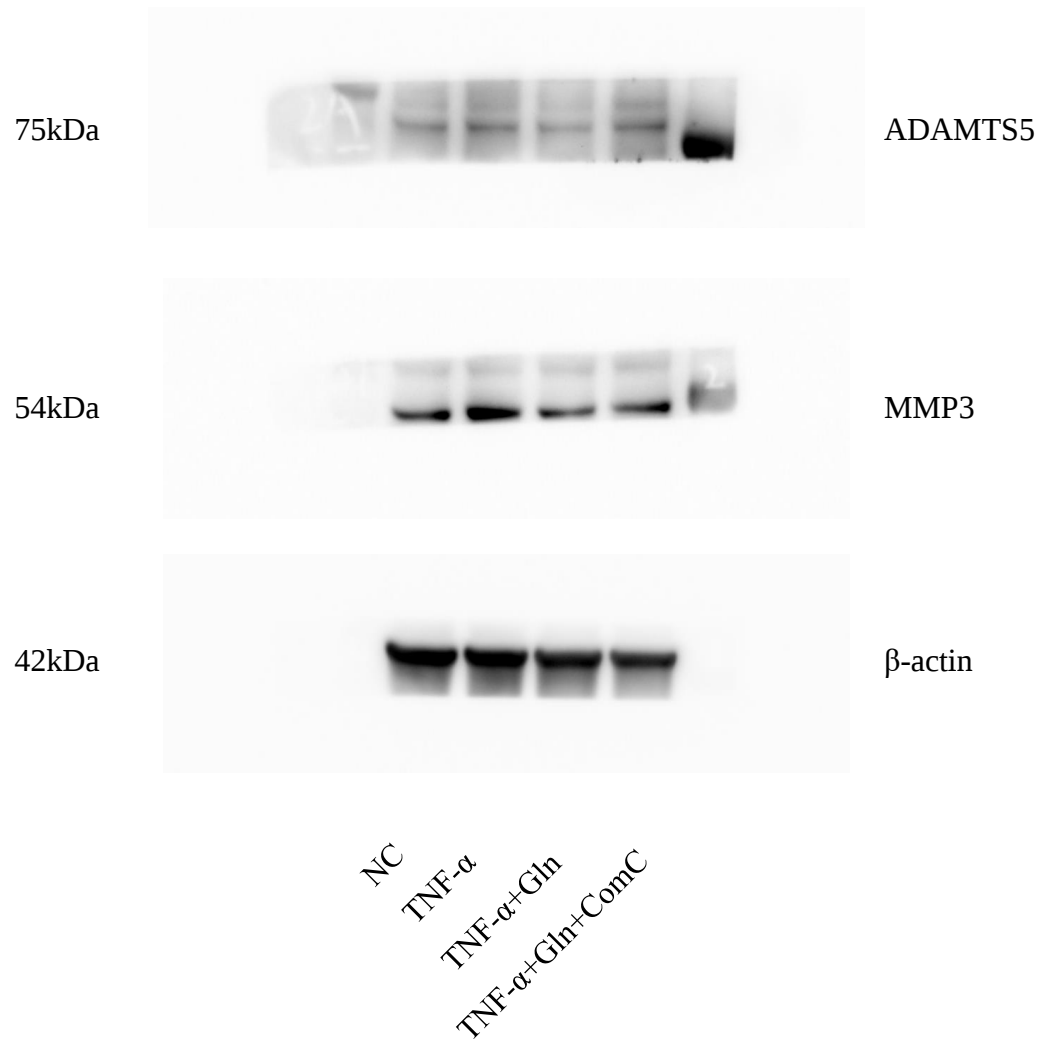


Fig 7c

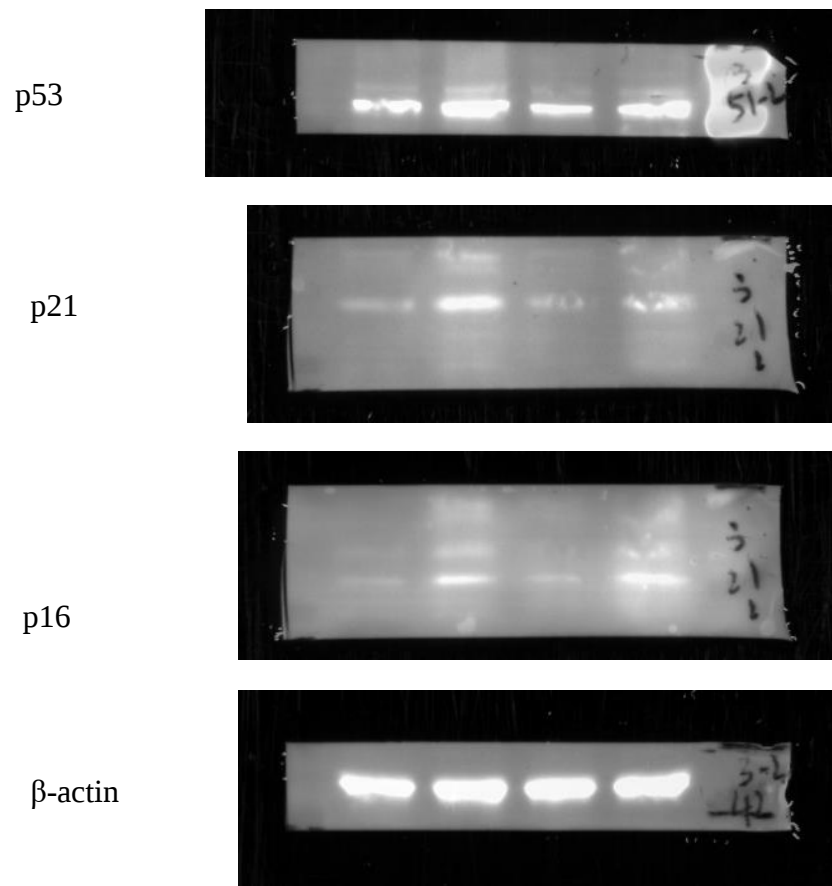
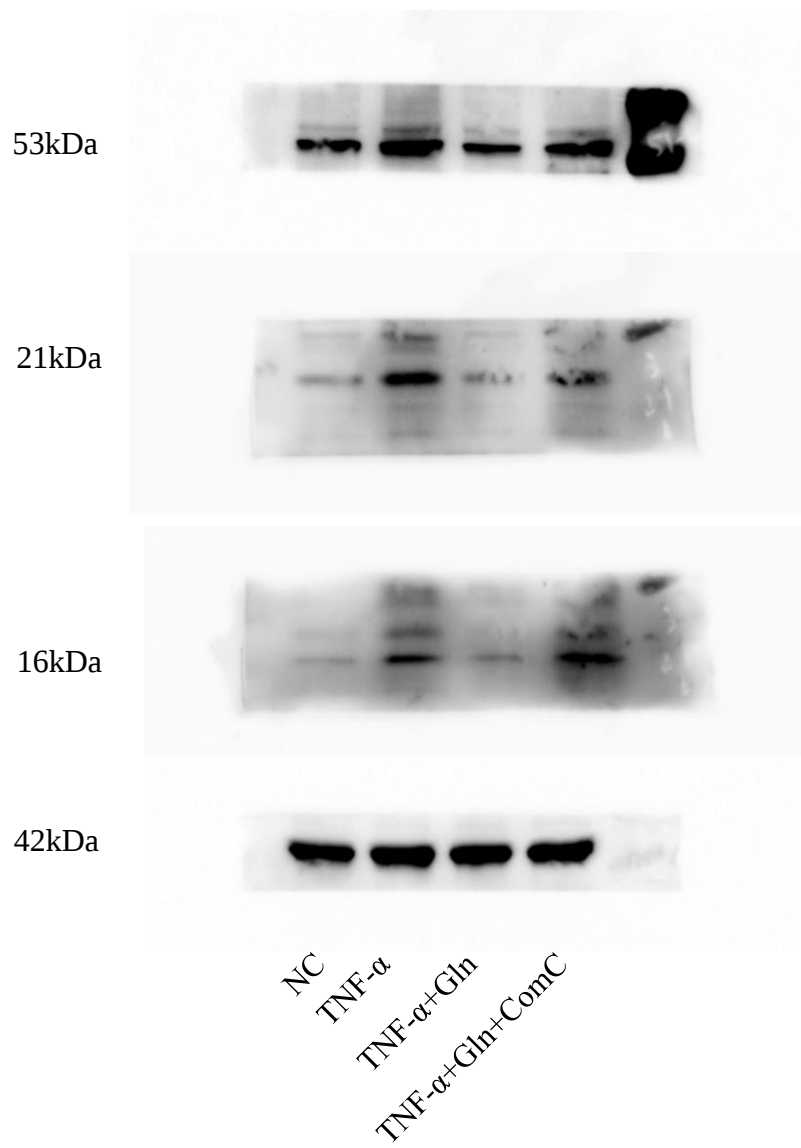
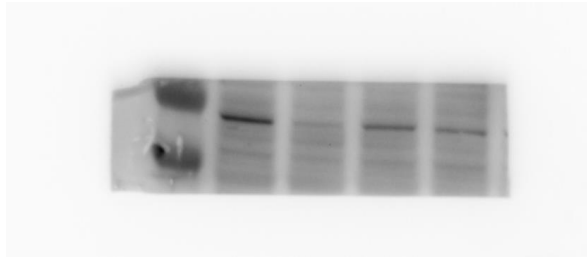


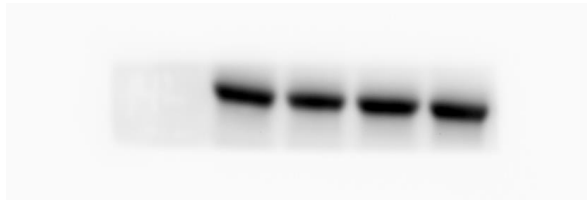
Fig 7h

62kDa



AMPK α 1

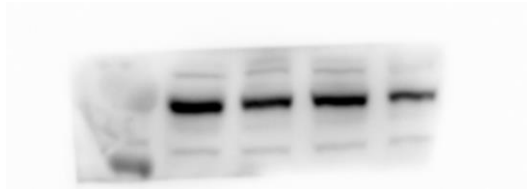
42kDa



β-actin

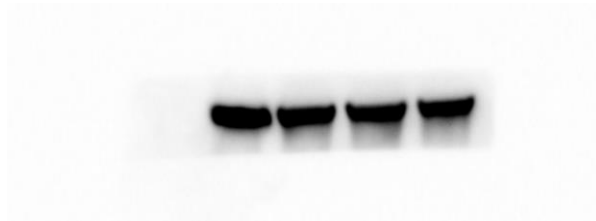
si-NC
si-AMPK α 1-1
si-AMPK α 1-2
si-AMPK α 1-3

64kDa



AMPK α 2

42kDa



β-actin

si-NC
si-AMPK α 2-1
si-AMPK α 2-2
si-AMPK α 2-3

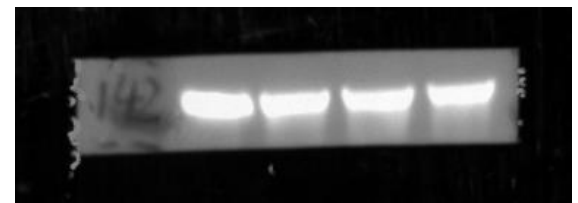
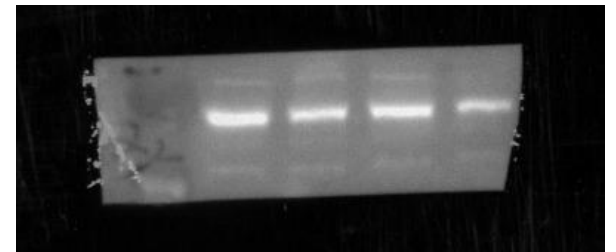
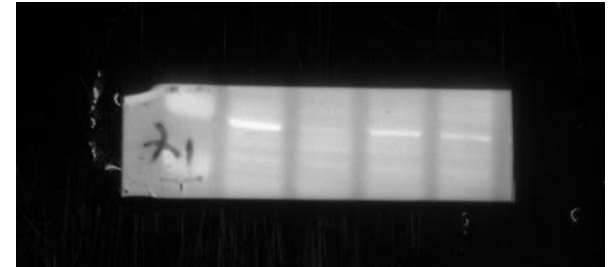
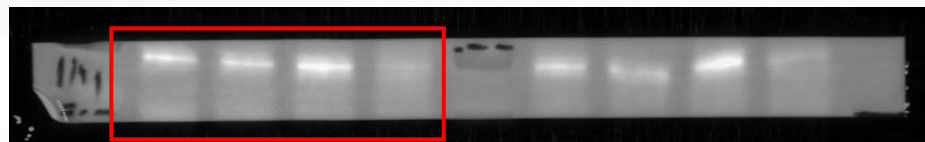


Fig 7i

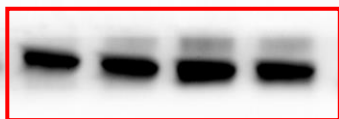
64kDa



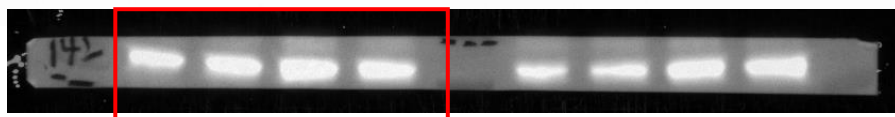
AMPKα1



42kDa



β-actin



62kDa

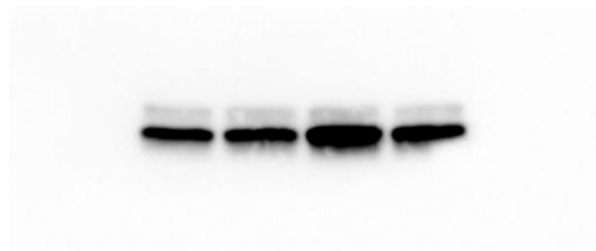


p62

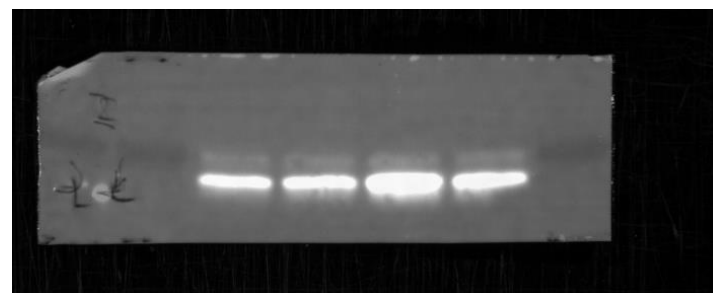


16kDa

14kDa



LC3 I
LC3 II



42kDa



β-actin

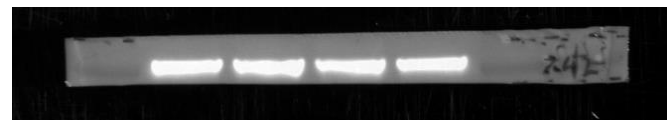
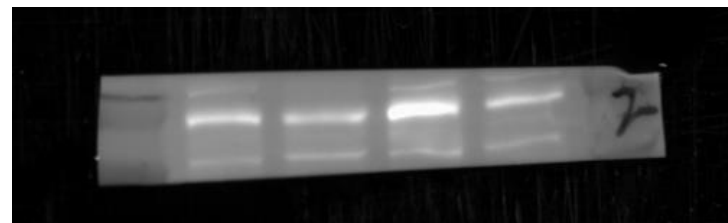


Fig 7j

64kDa



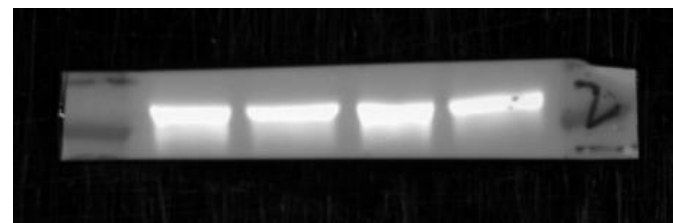
AMPK α 2



42kDa



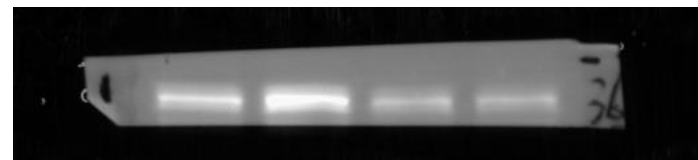
β-actin



62kDa

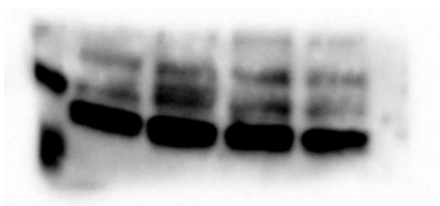


p62



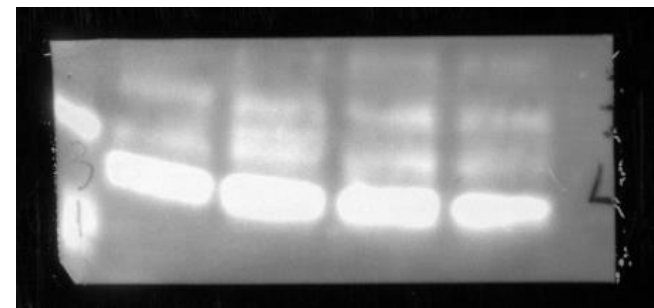
16kDa

14kDa



LC3 I

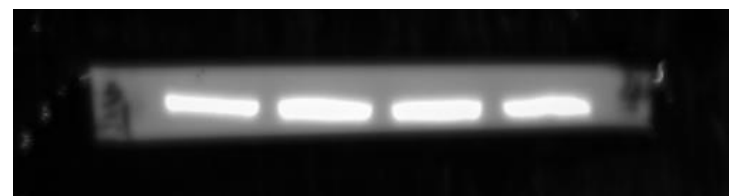
LC3 II



42kDa

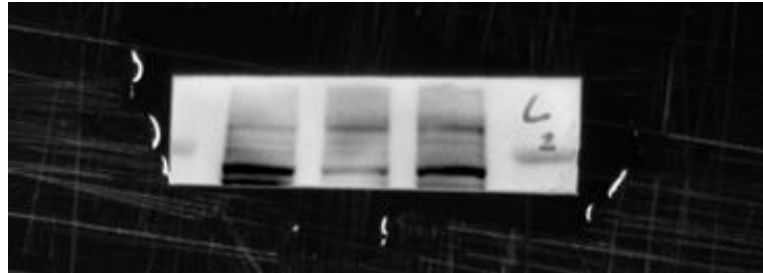


β-actin



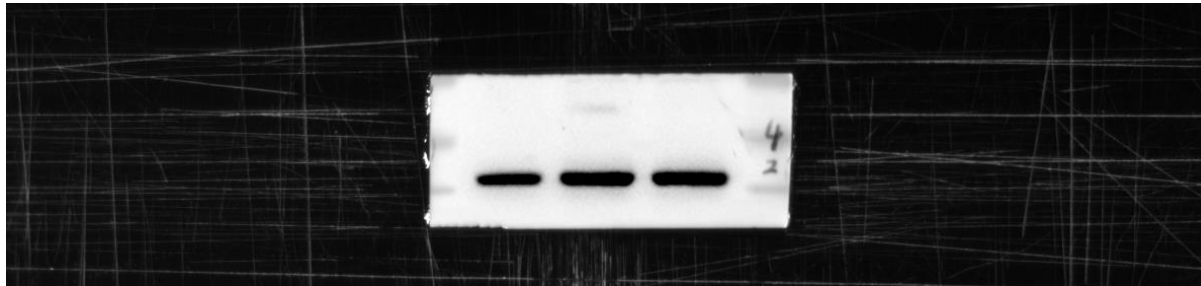
Supplementary Figure 1a

140kDa



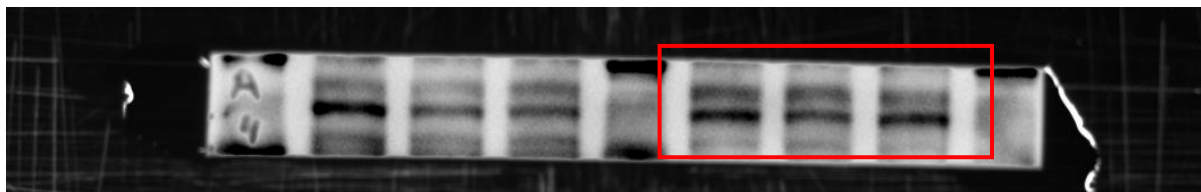
COL2A1

42kDa



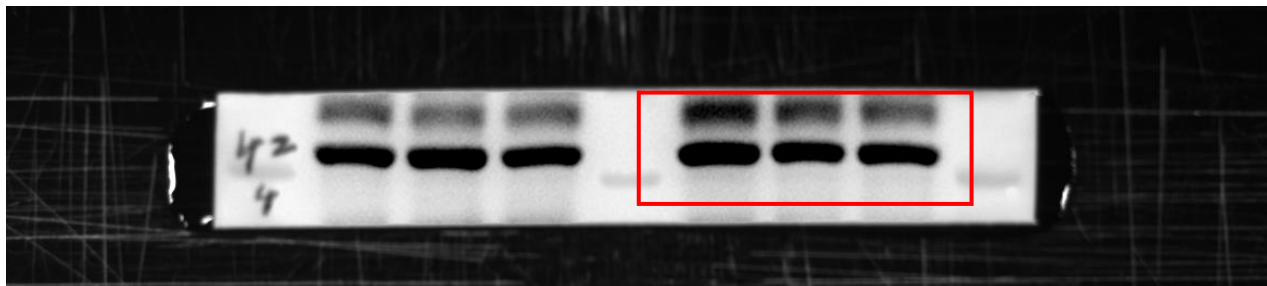
ACTB

110kDa



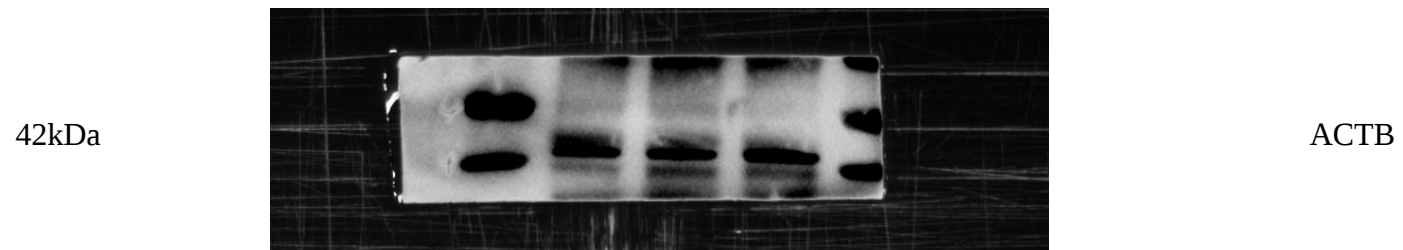
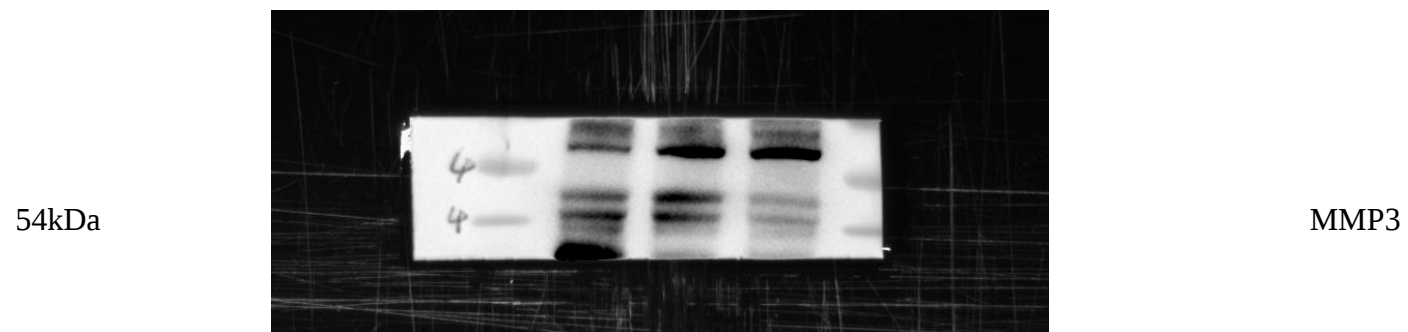
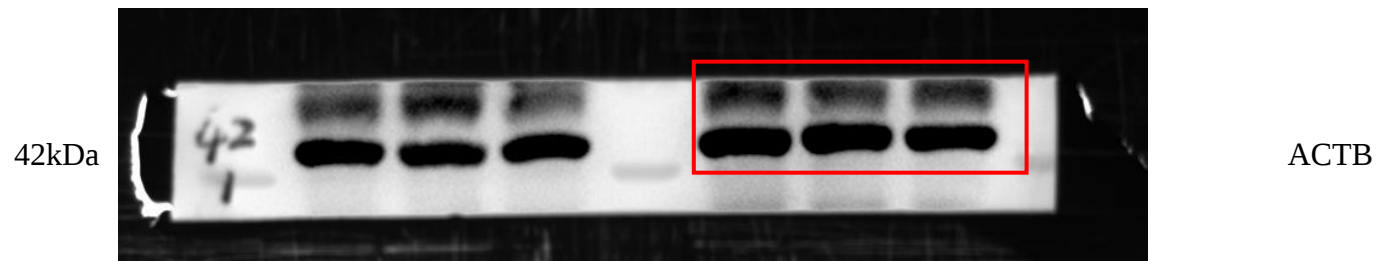
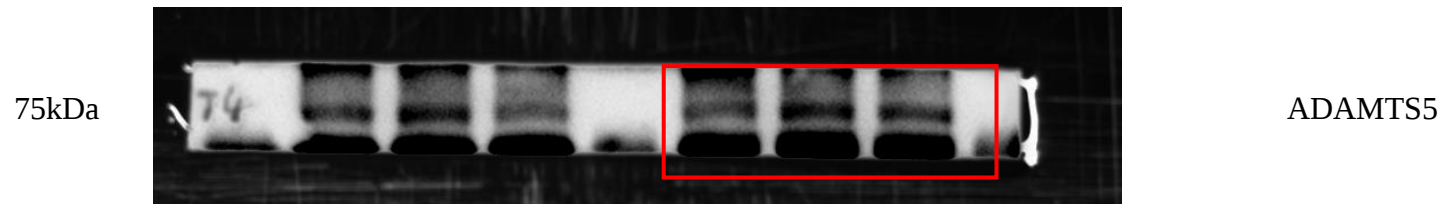
ACAN

42kDa



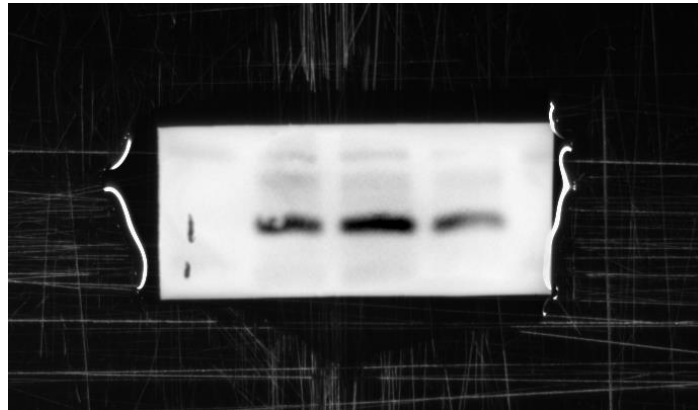
ACTB

Supplementary Figure 1a



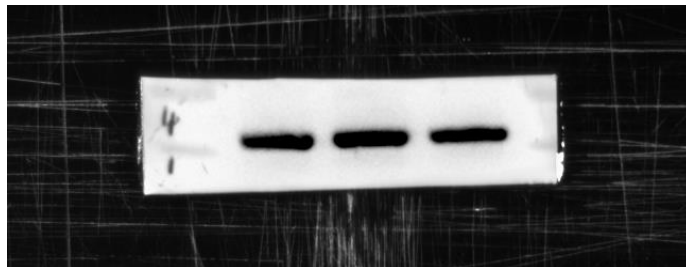
Supplementary Figure 1c

21kDa



P21

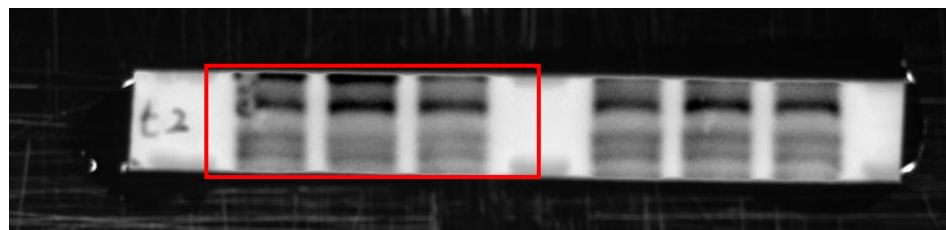
42kDa



ACTB

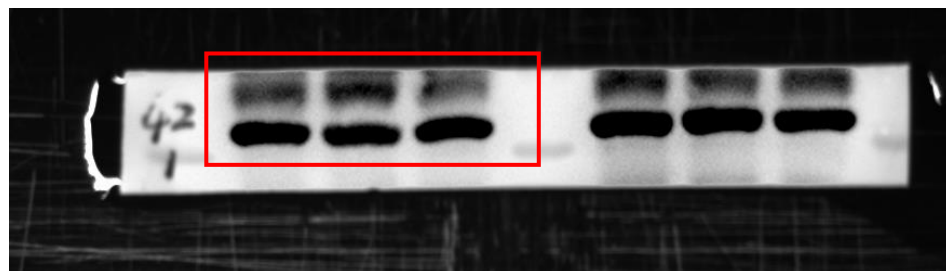
Supplementary Figure 1e and 1g

62kDa



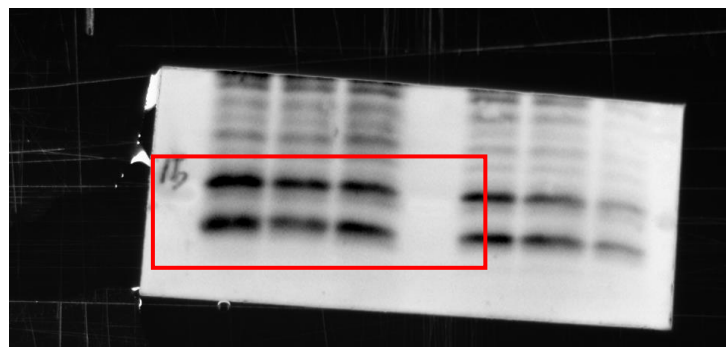
P62

42kDa



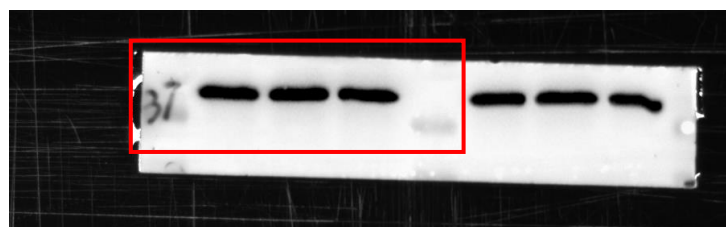
ACTB

16kDa
14kDa



LC3 I
LC3 II

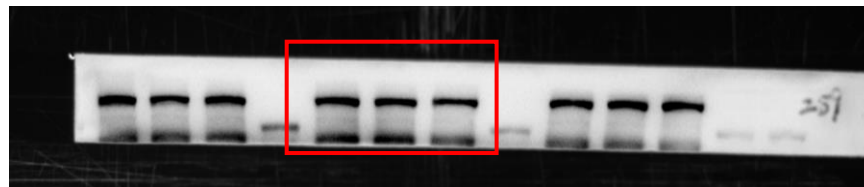
37kDa



GAPDH

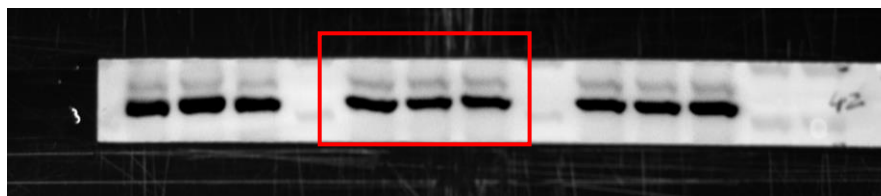
Supplementary Figure 1j

289kDa



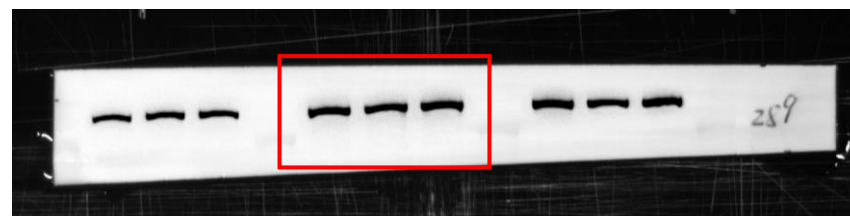
p-mTOR

42kDa



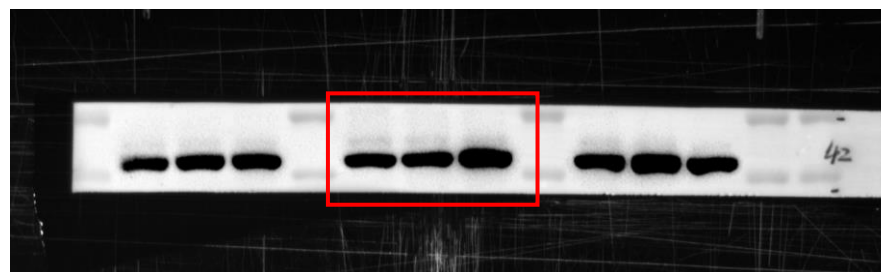
ACTB

289kDa



mTOR

42kDa



ACTB

Supplementary Table. 1. The primary antibodies for western blotting

Name	Dilution	Lot Number	Company	Country
COL2A1	1:500	YT1022	Immunoway	USA
ACAN	1:500	ab36861	Abcam	USA
ADAMTS5	1:1000	Ab41037	Abcam	USA
MMP3	1:1000	YT4465	Immunoway	USA
β -actin	1:1000	AC026	Abclonal	USA
P53	1:500	60283-2-Ig	Proteintech	USA
P21	1:1000	Ab109199	Abcam	USA
P16	1:1000	Ab51243	Abcam	USA
P62	1:1000	23214	CST	USA
LC3	1:1000	2775S	CST	USA
HK2	1:1000	22029-1-AP	Proteintech	USA
PFKP	1:1000	13389-1-AP	Proteintech	USA
PKM2	1:1000	15822-1-AP	Proteintech	USA
LDHA	1:1000	19987-1-AP	Proteintech	USA
P300	1:1000	ab275378	Abcam	USA
Kla	1:1000	PTM1401	PTM BIO	China
p-AMPK α	1:1000	2535T	CST	USA
AMPK α	1:1000	2532S	CST	USA
AMPK α 1	1:1000	ET1608-40	HUABIO	China
AMPK α 2	1:1000	HA600078	HUABIO	China
p-mTOR	1:1000	2971S	CST	USA
mTOR	1:1000	2972S	CST	USA
GAPDH	1:1000	YN5585	Immunoway	USA
Rabbit IgG	-	2729s	CST	USA

Supplementary Table. 2. The sequence of siRNA for knockdown

siRNA		Sequence (5'-3')
siRNA-NC	Forward strand	UUCUCCGAACGUGUCACGUdTdT
	Reverse strand	ACGUGACACGUUCGGAGAAdTdT
siRNA-p300	Forward strand	GGACUACCCUAUCAAGUAAACdTdT
	Reverse strand	GUUUACUUGAUAGGGUAGUCCdTdT
siRNA-AMPK α 1	Forward strand	CGAGUUGACUGGACAUAAAdTdT
	Reverse strand	UUUAUGUCCAGUCAACUCGdTdT
siRNA-AMPK α 2	Forward strand	CCAGAUGAAUGCUAAGAUAdTdT
	Reverse strand	UAUCUUAGCAUUCAUCUGGdTdT

Supplementary Table. 3. The primary antibodies for immunohistochemical staining

Name	Dilution	Lot Number	Company	Country
ACAN	1:100	A8536	Abclonal	USA
MMP3	1:100	YT4465	Immunoway	USA
P16	1:100	ab241543	Abcam	USA
LC3	1:100	14600-1-AP	Proteintech	USA
HK2	1:100	22029-1-AP	Proteintech	USA
PFKP	1:100	13389-1-AP	Proteintech	USA
PKM2	1:100	15822-1-AP	Proteintech	USA
LDHA	1:100	19987-1-AP	Proteintech	USA
P300	1:100	A13016	Abclonal	USA
Kla	1:100	PTM1401	PTM BIO	China
p-AMPK α	1:100	YP0575	Immunoway	USA
AMPK α	1:100	YT0216	Immunoway	USA

Supplementary Table. 4. The list of DEGs for all pathways

symbol	pvalue	padj	pathway_id	pathway_name
INSIG1	3.80E-13	1.43E-08		
MVD	1.85E-11	3.48E-07	hsa00900;hsa01100	Terpenoid backbone biosynthesis ;Metabolic pathways
ACAT2	6.09E-11	5.73E-07	hsa00071;hsa00280;hsa00310;hsa00380;hsa00620;hsa00630;hsa00650;hsa00900;hsa01100;hsa01200;hsa01212;hsa04975	Fatty acid degradation ;Valine, leucine and isoleucine degradation ;Lysine degradation ;Tryptophan metabolism ;Pyruvate metabolism ;Glyoxylate and dicarboxylate metabolism ;Butanoate metabolism ;Terpenoid backbone biosynthesis ;Metabolic pathways ;Carbon metabolism ;Fatty acid metabolism ;Fat digestion and absorption
TMEM97	5.29E-11	5.73E-07		
FDPS	8.01E-11	6.04E-07	hsa00900;hsa01100;hsa05164;hsa05166	Terpenoid backbone biosynthesis ;Metabolic pathways ;Influenza A ;Human T-cell leukemia virus 1 infection
MSMO1	4.00E-10	2.51E-06	hsa00100;hsa01100	Steroid biosynthesis ;Metabolic pathways
DHCR7	2.42E-09	1.30E-05	hsa00100;hsa01100	Steroid biosynthesis ;Metabolic pathways
FDFT1	1.02E-08	4.82E-05	hsa00100;hsa01100	Steroid biosynthesis ;Metabolic pathways
IDI1	2.16E-08	9.04E-05	hsa00900;hsa01100	Terpenoid backbone biosynthesis ;Metabolic pathways
LPIN1	4.05E-08	0.000139	hsa00561;hsa00564;hsa01100;hsa04150	Glycerolipid metabolism ;Glycerophospholipid metabolism ;Metabolic pathways ;mTOR signaling pathway
ACSS2	3.75E-08	0.000139	hsa00010;hsa00620;hsa00630;hsa00640;hsa01100;hsa01200	Glycolysis / Gluconeogenesis ;Pyruvate metabolism ;Glyoxylate and dicarboxylate metabolism ;Propanoate metabolism ;Metabolic pathways ;Carbon metabolism
SCD	5.07E-08	0.000159	hsa01040;hsa01100;hsa01212;hsa03320;hsa04152	Biosynthesis of unsaturated fatty acids ;Metabolic pathways ;Fatty acid metabolism ;PPAR signaling pathway ;AMPK signaling pathway
SQLE	7.94E-08	0.00023	hsa00100;hsa01100	Steroid biosynthesis ;Metabolic pathways
HMGCS1	1.68E-07	0.000428	hsa00280;hsa00650;hsa00900;hsa01100;hsa03320	Valine, leucine and isoleucine degradation ;Butanoate metabolism ;Terpenoid backbone biosynthesis ;Metabolic pathways ;PPAR signaling pathway

FADS2	1.71E-07	0.000428	hsa00592;hsa01040;hsa01100;hsa01212;hsa03320	alpha-Linolenic acid metabolism ;Biosynthesis of unsaturated fatty acids ;Metabolic pathways ;Fatty acid metabolism ;PPAR signaling pathway
LSS	4.00E-07	0.000941	hsa00100;hsa01100	Steroid biosynthesis ;Metabolic pathways
PCSK9	5.46E-07	0.00121	hsa04979	Cholesterol metabolism
NUPR1	8.43E-07	0.001764	hsa05202	Transcriptional misregulation in cancer
ERG28	1.01E-06	0.001997		
DHCR24	1.66E-06	0.003123	hsa00100;hsa01100	Steroid biosynthesis ;Metabolic pathways
LDLR	3.86E-06	0.006924	hsa04144;hsa04913;hsa04925;hsa04927;hsa04934;hsa04976;hsa04979;hsa05145;hsa05160;hsa05417	Endocytosis ;Ovarian steroidogenesis ;Aldosterone synthesis and secretion ;Cortisol synthesis and secretion ;Cushing syndrome ;Bile secretion ;Cholesterol metabolism ;Toxoplasmosis ;Hepatitis C ;Lipid and atherosclerosis
HSD17B7	4.52E-06	0.007421	hsa00100;hsa00140;hsa01100;hsa04913	Steroid biosynthesis ;Steroid hormone biosynthesis ;Metabolic pathways ;Ovarian steroidogenesis
HMGCR	4.53E-06	0.007421	hsa00900;hsa01100;hsa04152;hsa04976	Terpenoid backbone biosynthesis ;Metabolic pathways ;AMPK signaling pathway ;Bile secretion
MMAB	5.36E-06	0.008407	hsa00860;hsa01100;hsa01240	Porphyrin and chlorophyll metabolism ;Metabolic pathways ;Biosynthesis of cofactors
SNRPGP10	5.67E-06	0.008539		
CLCN6	8.91E-06	0.012907		
FASN	1.74E-05	0.024287	hsa00061;hsa01100;hsa01212;hsa04152;hsa04910	Fatty acid biosynthesis ;Metabolic pathways ;Fatty acid metabolism ;AMPK signaling pathway ;Insulin signaling pathway
CCRL2	2.42E-05	0.032021		
POM121L9P	2.47E-05	0.032021		
PCYT2	3.03E-05	0.038067	hsa00440;hsa00564;hsa01100	Phosphonate and phosphinate metabolism ;Glycerophospholipid metabolism ;Metabolic pathways

ALDH1B 1	3.70E-05	0.044954	hsa00010;hsa00053;hsa00071;hsa00280;hsa00310;hsa00330;hsa00340;hsa00380;hsa00410;hsa00561;hsa00620;hsa00770;hsa01100;hsa01240	Glycolysis / Gluconeogenesis ;Ascorbate and aldarate metabolism ;Fatty acid degradation ;Valine, leucine and isoleucine degradation ;Lysine degradation ;Arginine and proline metabolism ;Histidine metabolism ;Tryptophan metabolism ;beta-Alanine metabolism ;Glycerolipid metabolism ;Pyruvate metabolism ;Pantothenate and CoA biosynthesis ;Metabolic pathways ;Biosynthesis of cofactors
SC5D	3.86E-05	0.045408	hsa00100;hsa01100	Steroid biosynthesis ;Metabolic pathways
CYP51A 1	4.36E-05	0.04974	hsa00100;hsa01100	Steroid biosynthesis ;Metabolic pathways