

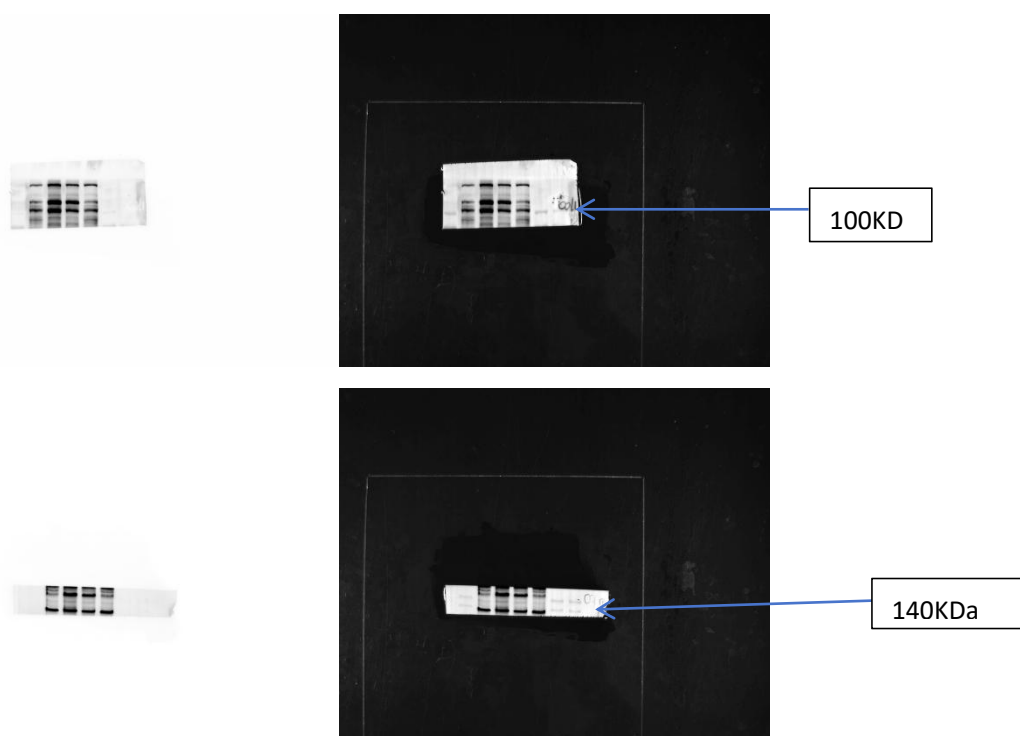
Geniposide Alleviates Kidney Fibrosis by Targeting STAT3-HK2 Mediated Glycolysis

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², Rui-feng Wang^{1,2}, De-guang Wang^{1,2*}

**Supplementary figures (the whole uncropped images of the original western
blots)**

Figure 2 GP alleviates UUO mice kidney fibrosis.

Figure 2A Collagen I



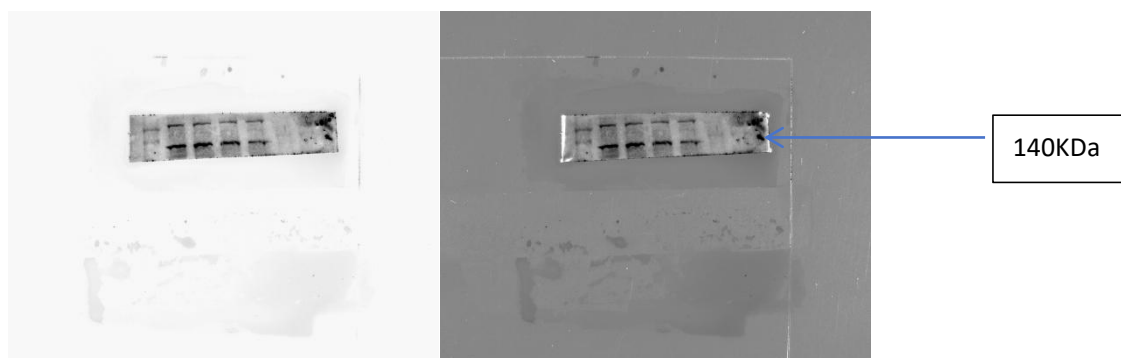


Figure 2A α -SMA

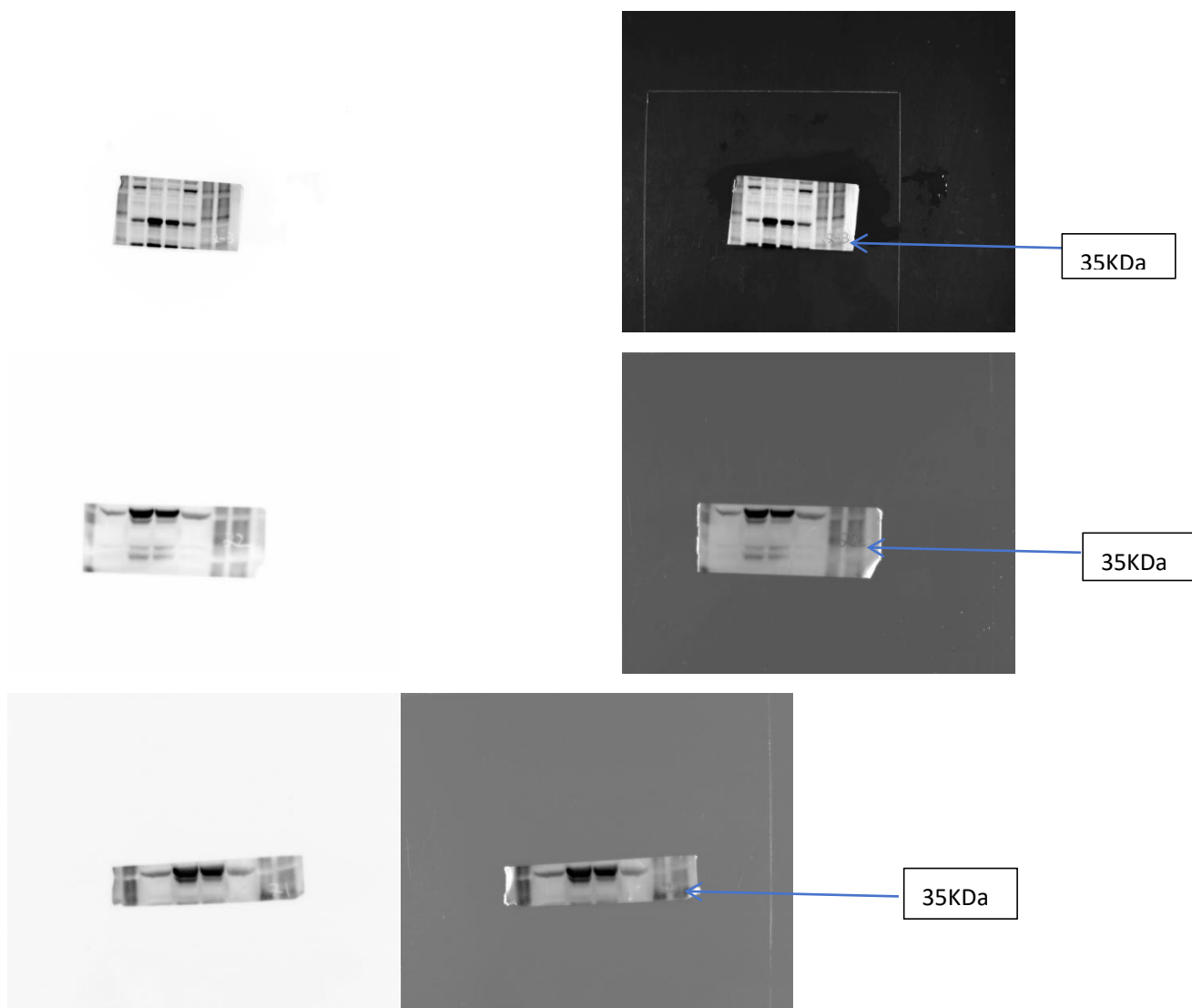


Figure 2A α -Tubulin

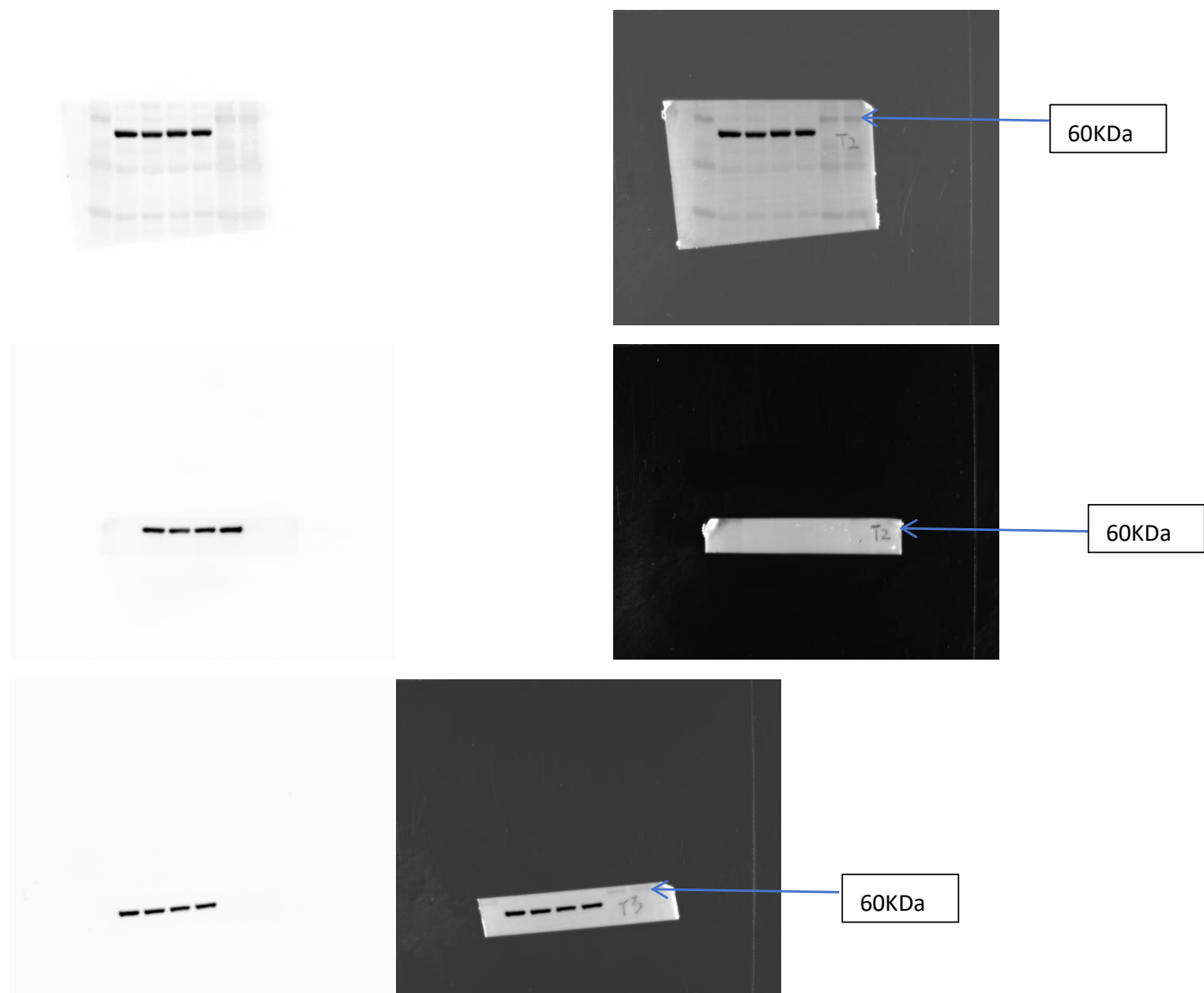
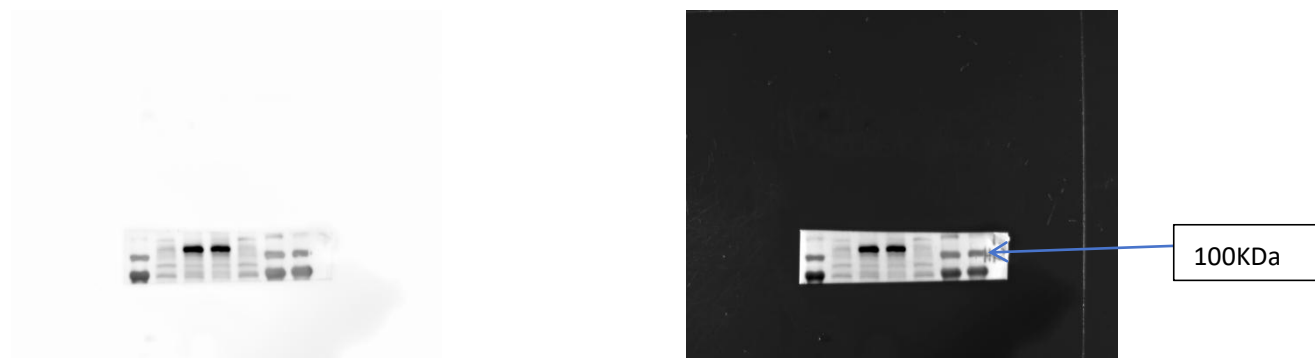


Figure 3 GP ameliorates renal fibrosis by meditating glycolysis
Figure 3F HK2



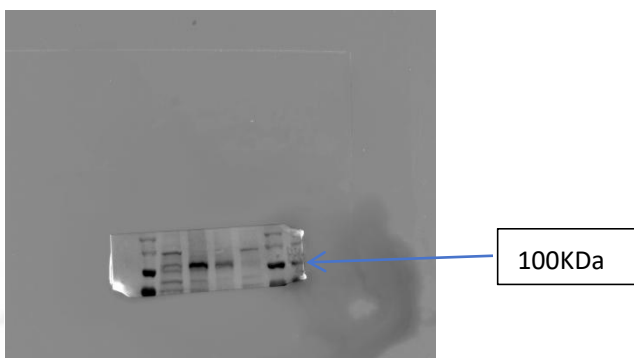
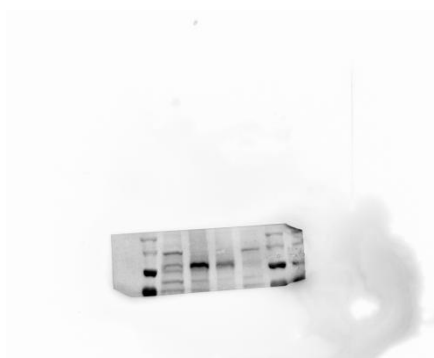
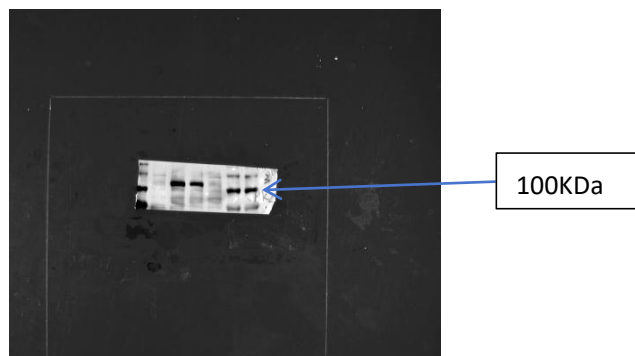
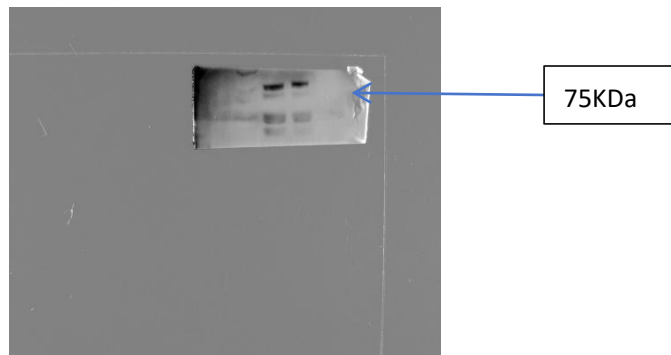
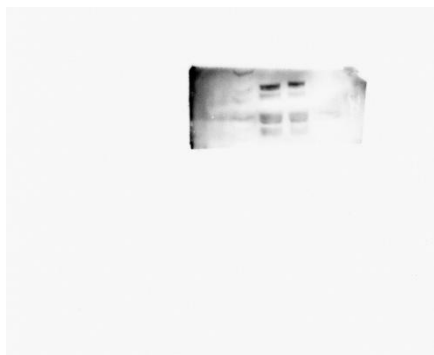
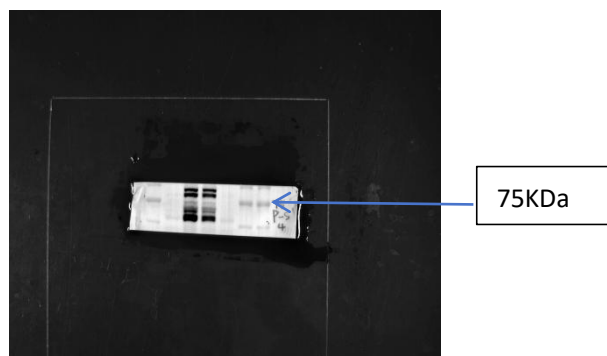


Figure 3F p-STAT3



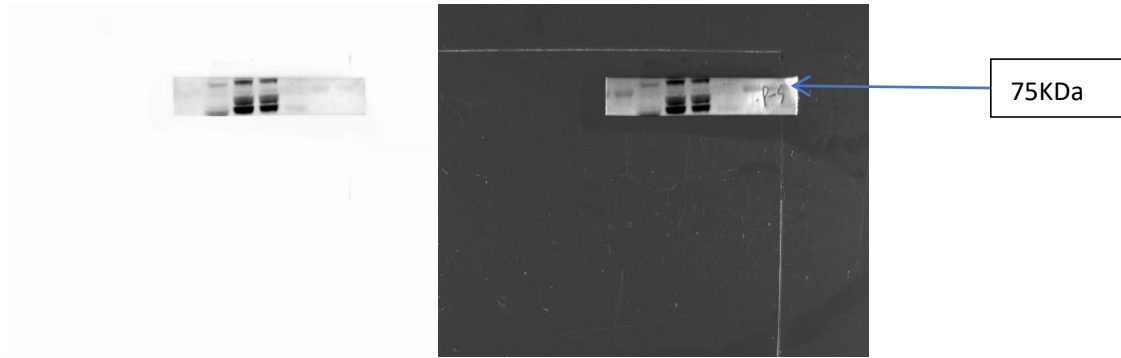


Figure 3F STAT3

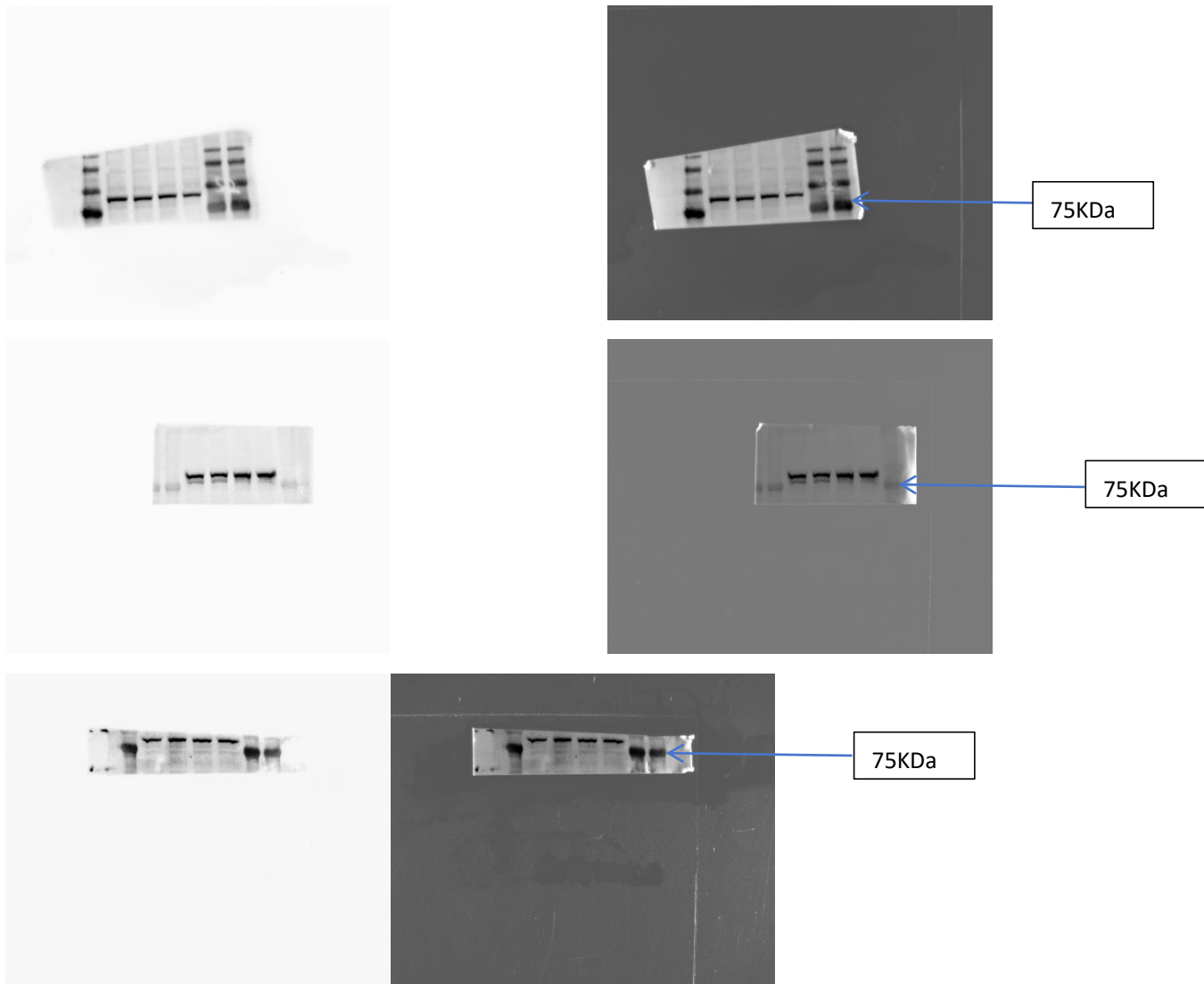


Figure 3F α -Tubulin

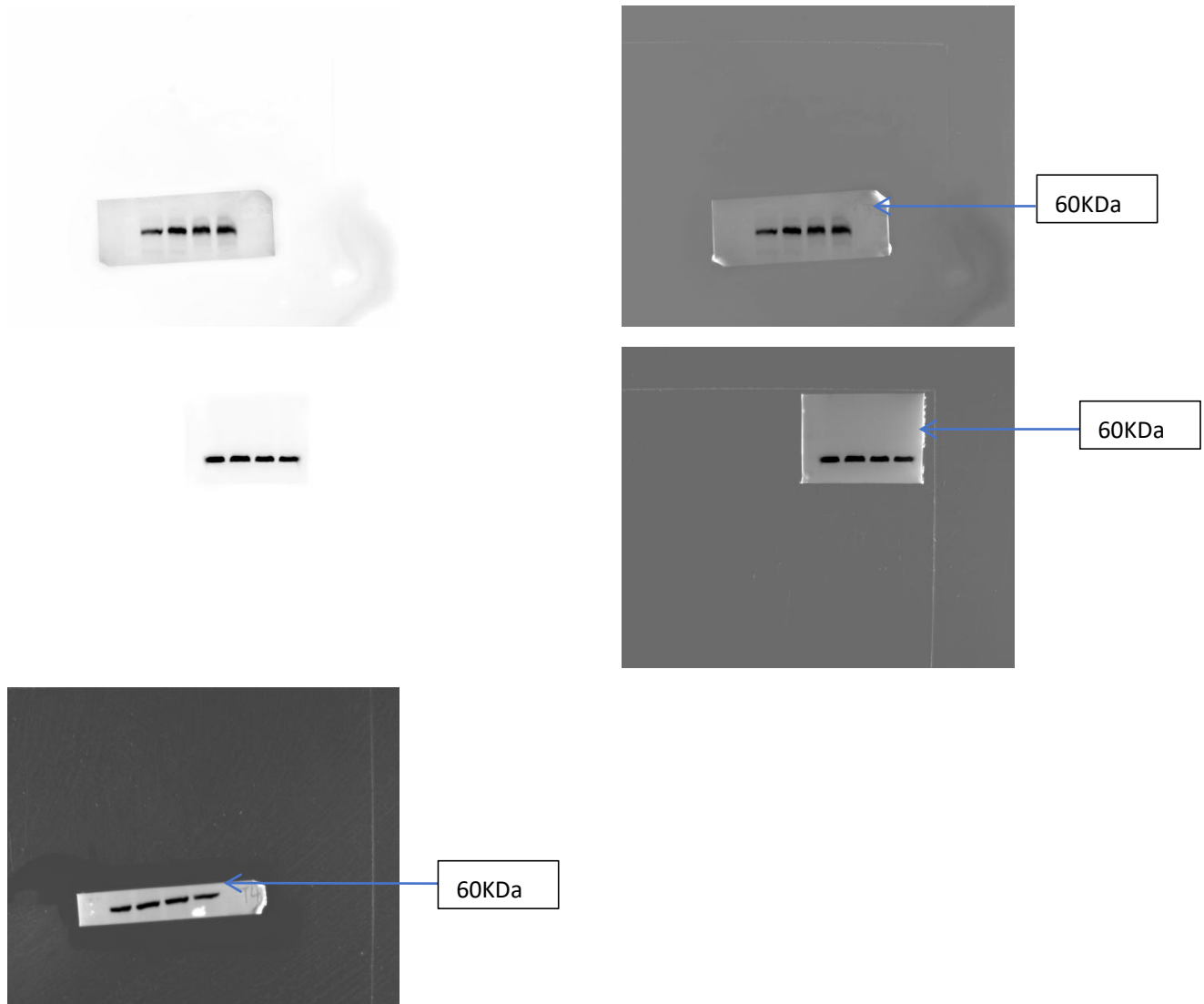


Figure 4 Effects of GP on EMT in TGF- β 1-treated HK-2 cells
Figure 4D Collagen I

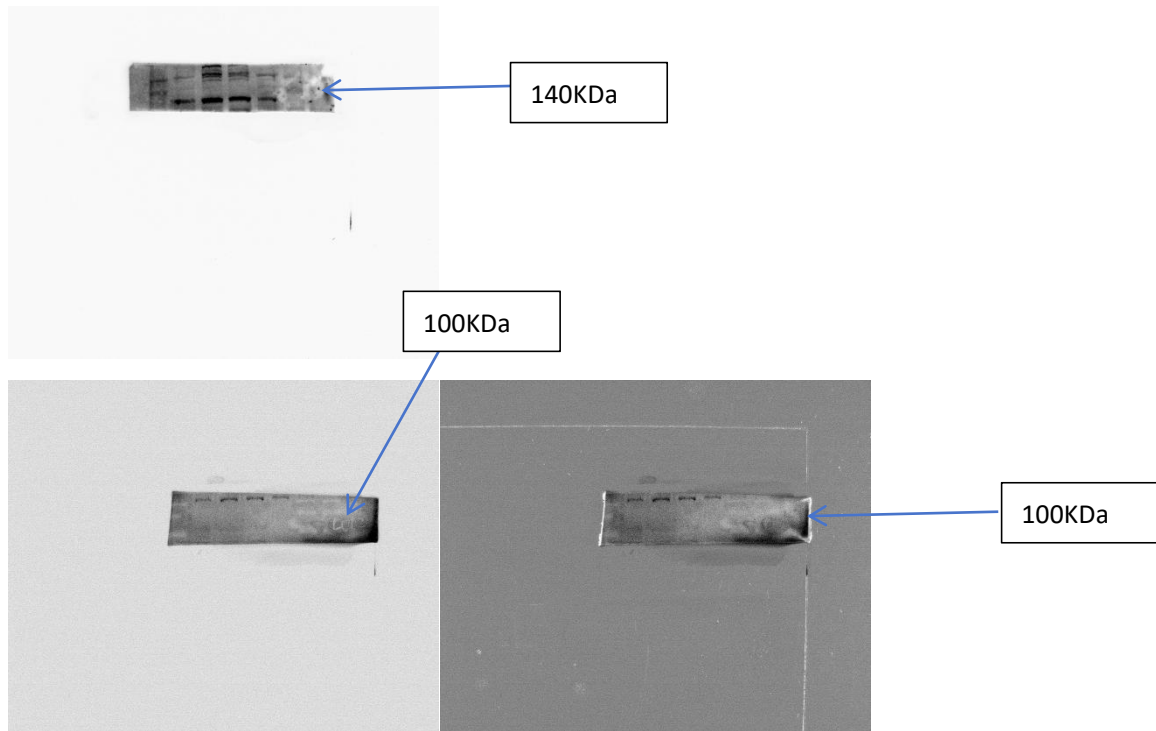
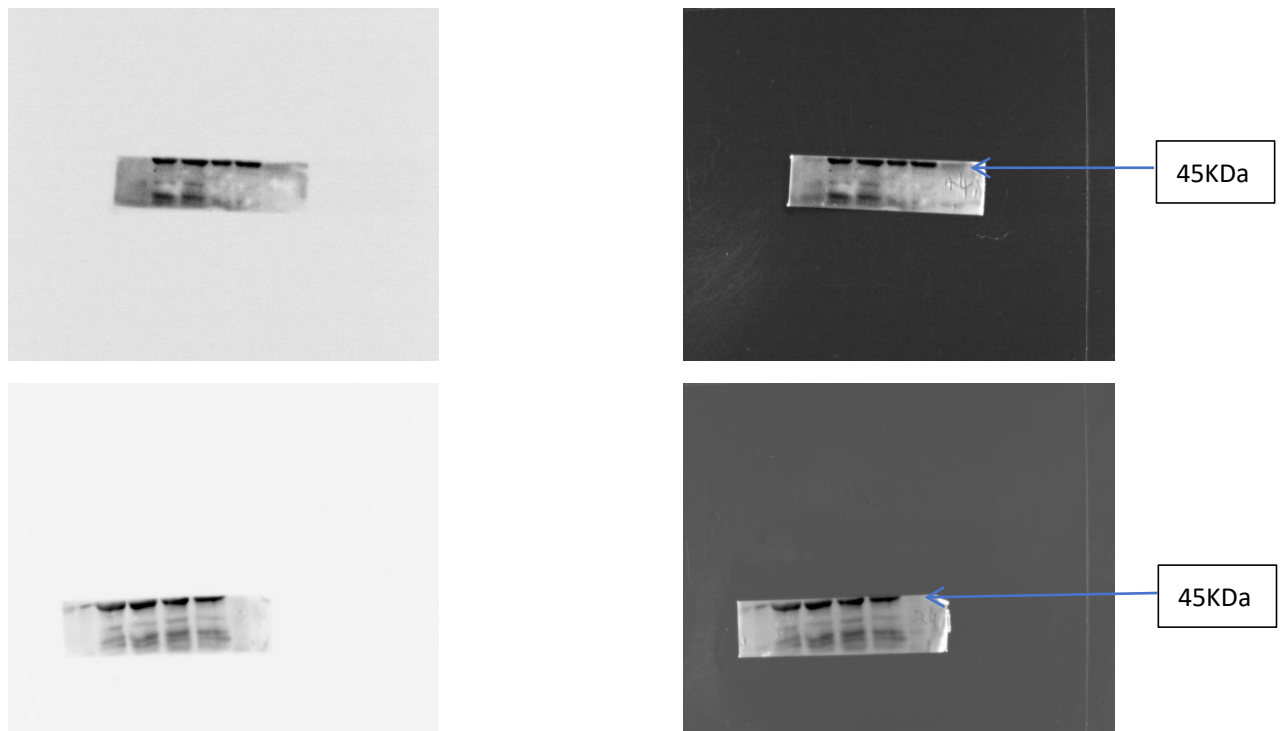


Figure 4D α -SMA



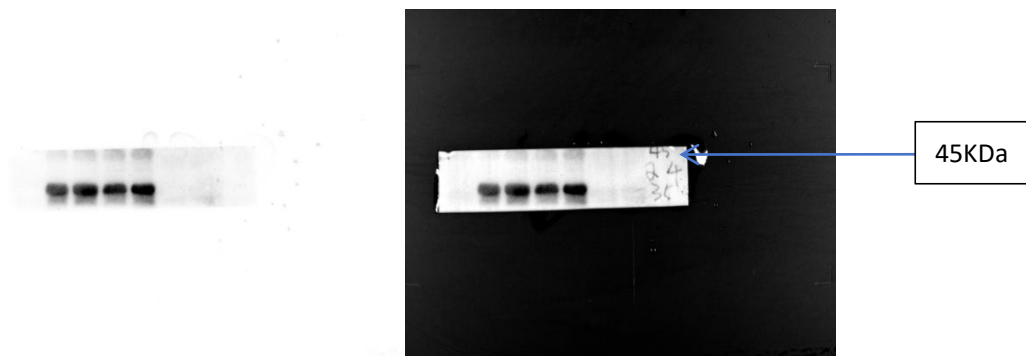


Figure 4D α -Tubulin

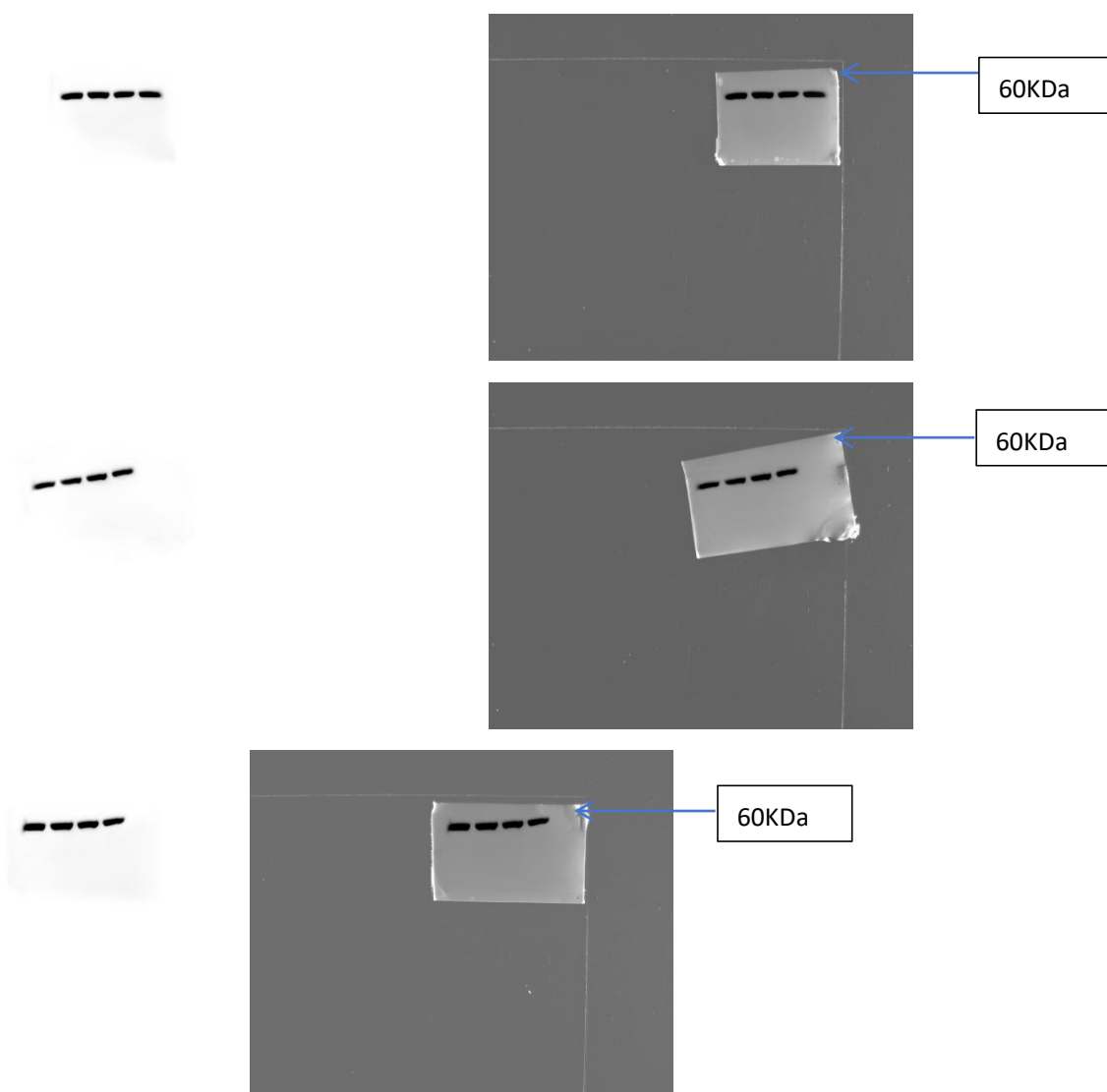


Figure 4E E-cadherin

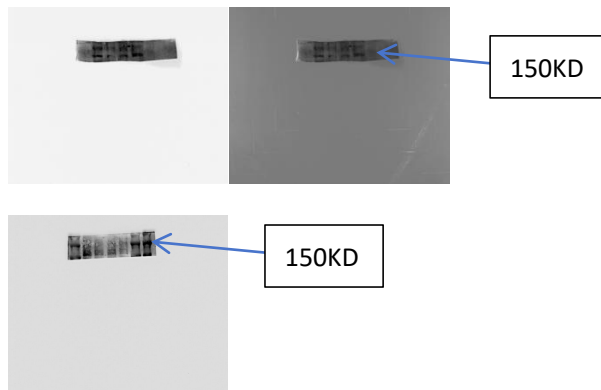


Figure 4E Vimentin

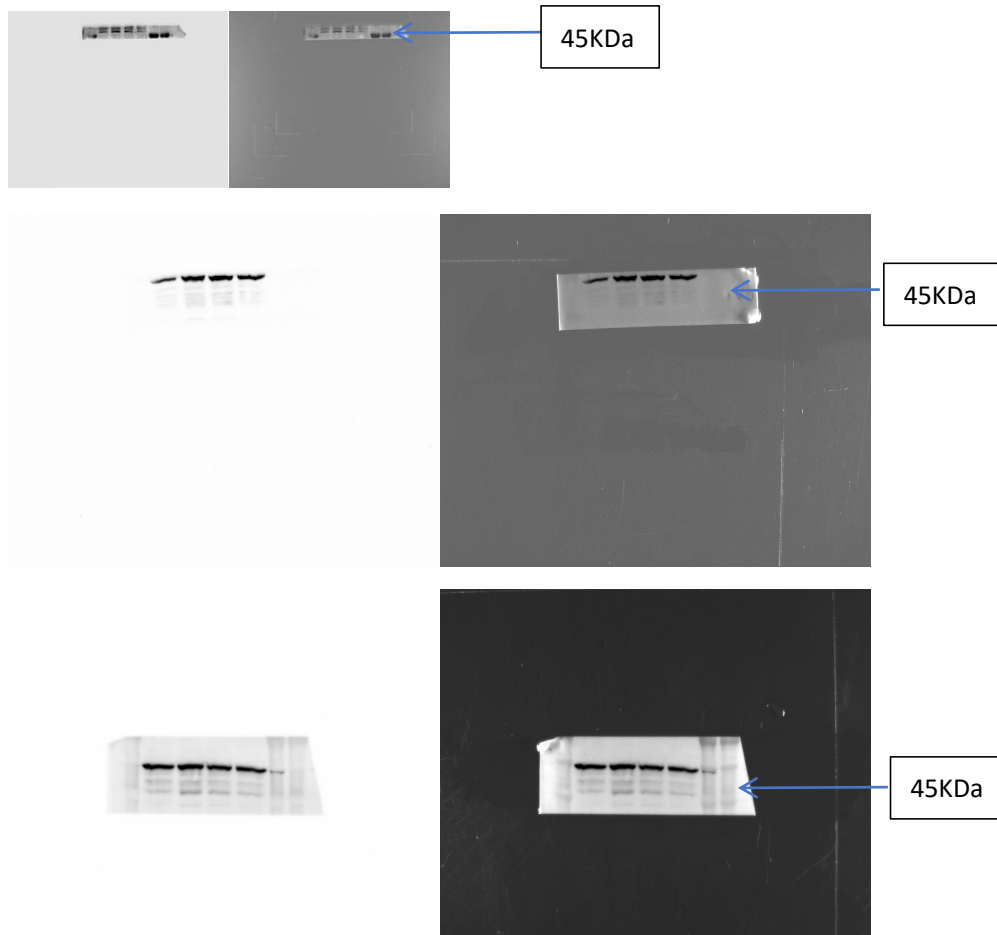


Figure 4E β -actin



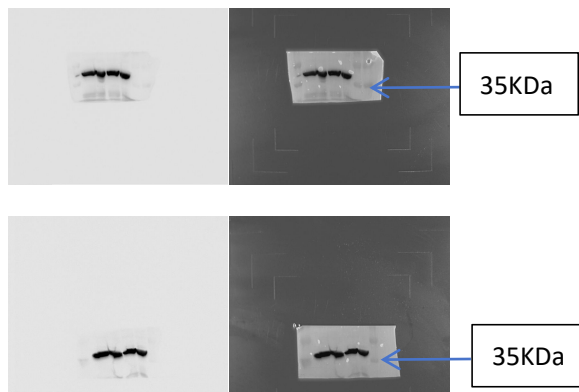


Figure 5 GP blocked the glycolysis and enhances FAO in vitro

Figure5C HK2

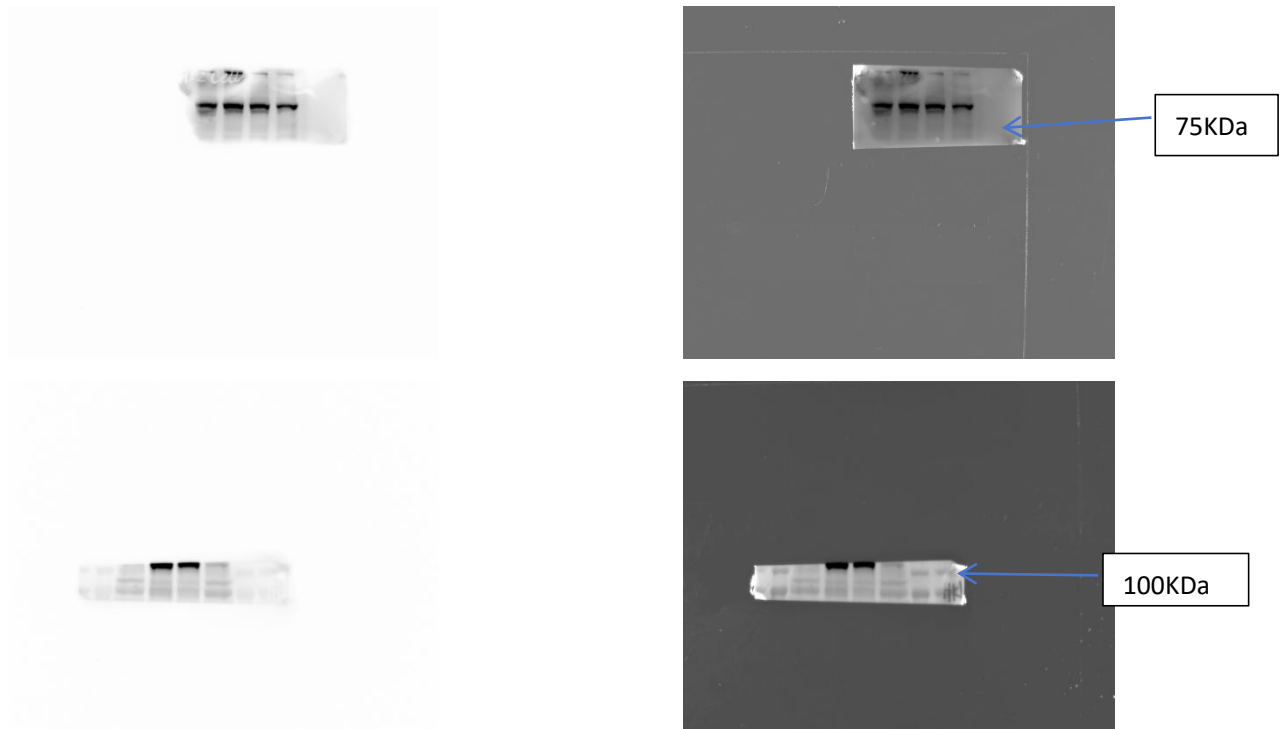




Figure5C P-STAT3

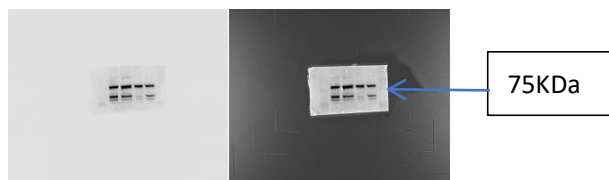
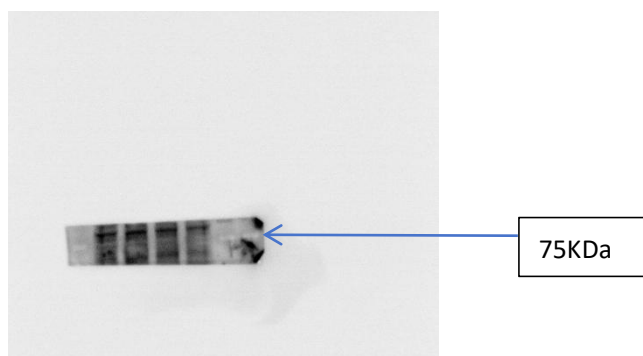
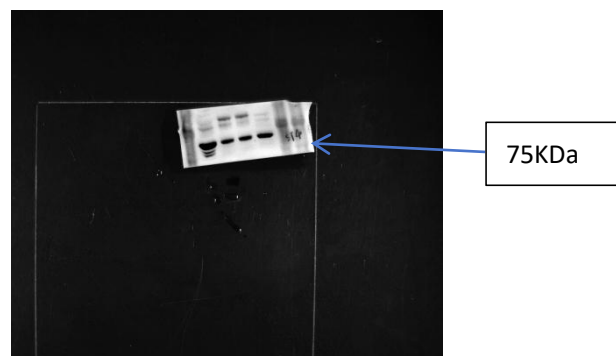


Figure5C STAT3



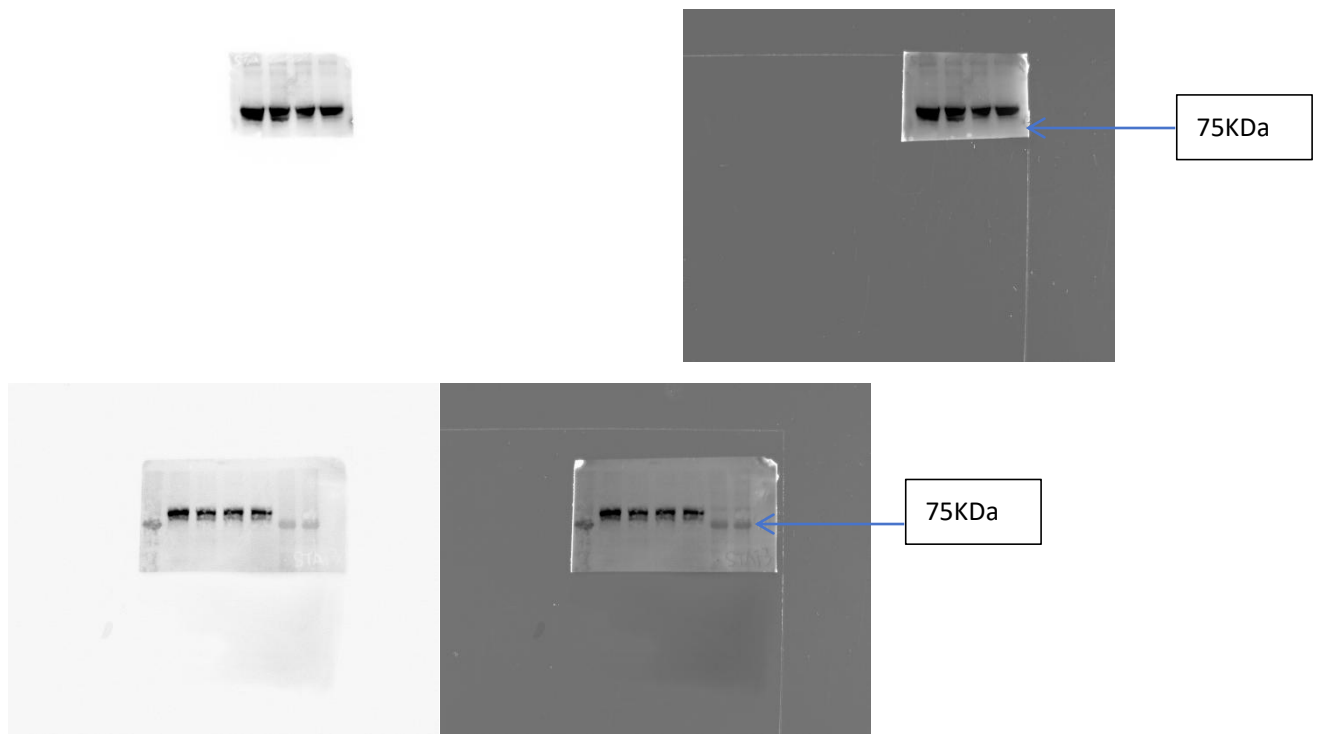


Figure5C α -Tubulin

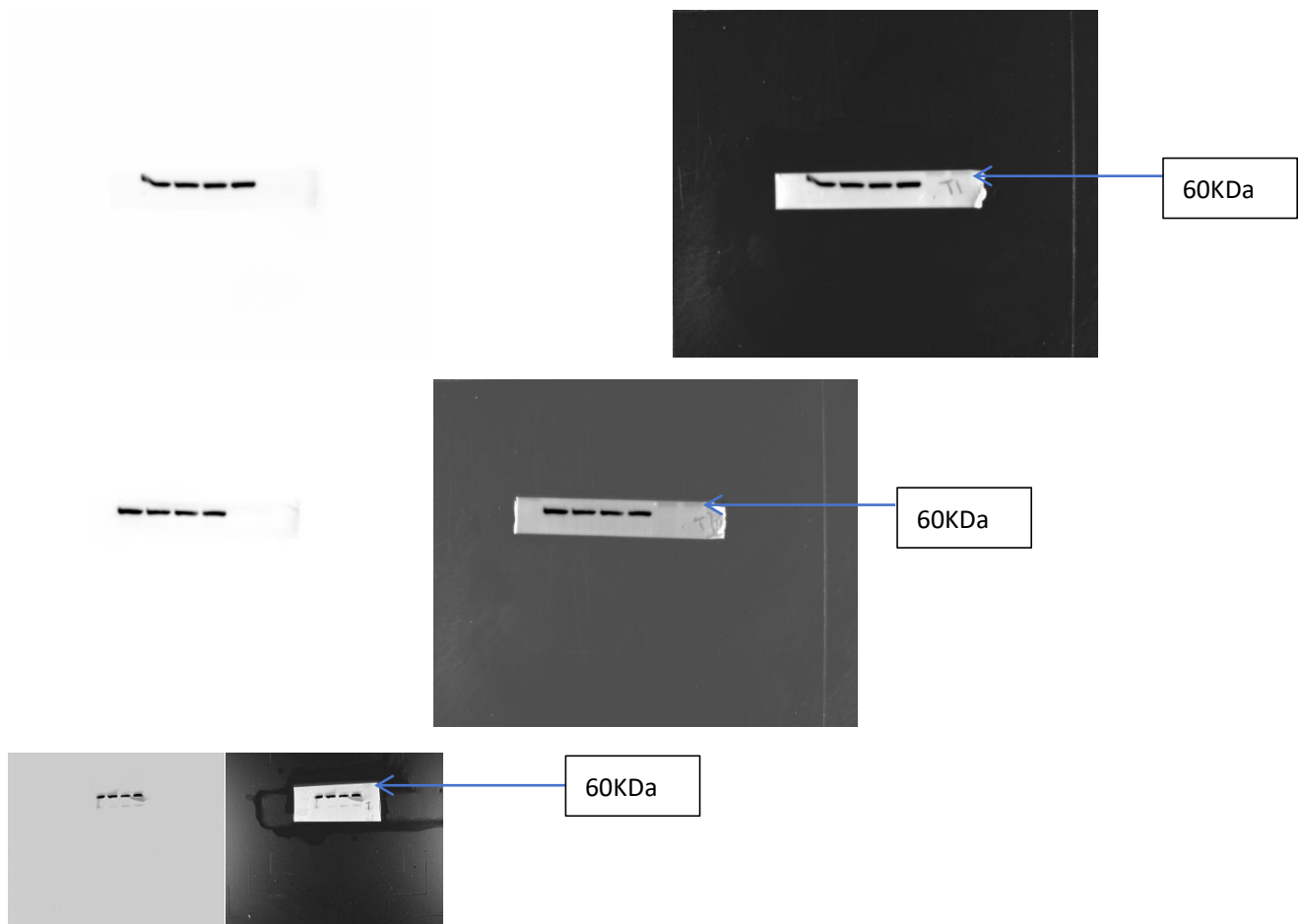


Figure 5D ACOX1

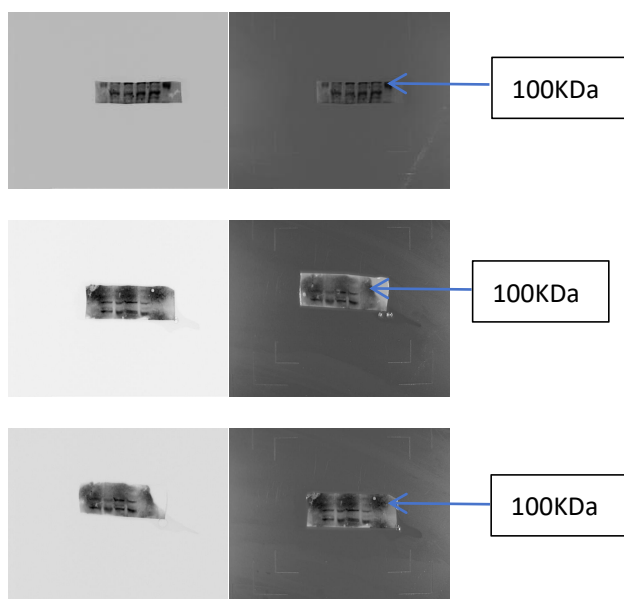


Figure 5D CPT1

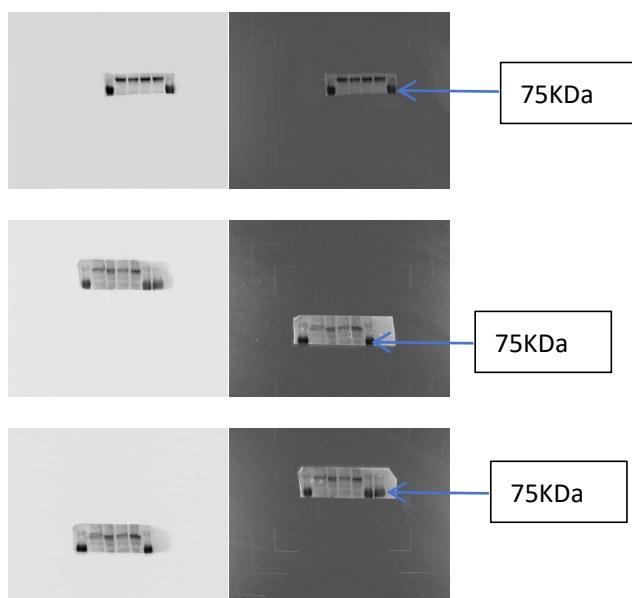
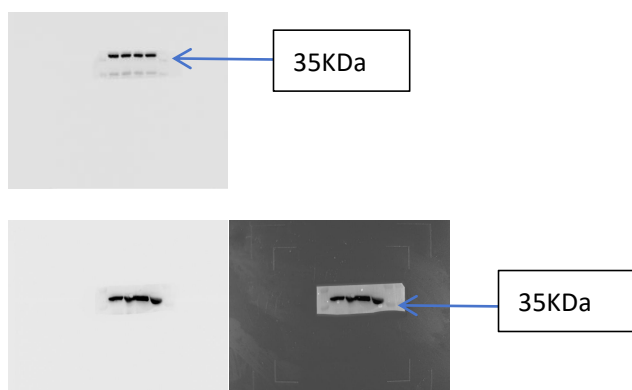


Figure 5D β -Actin



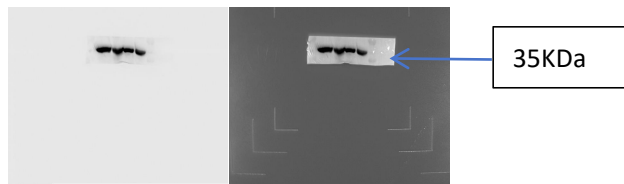


Figure 6 GP ameliorates renal fibrosis by meditating glycolysis through the STAT3-HK2 pathway

Figure 6E Collagen I

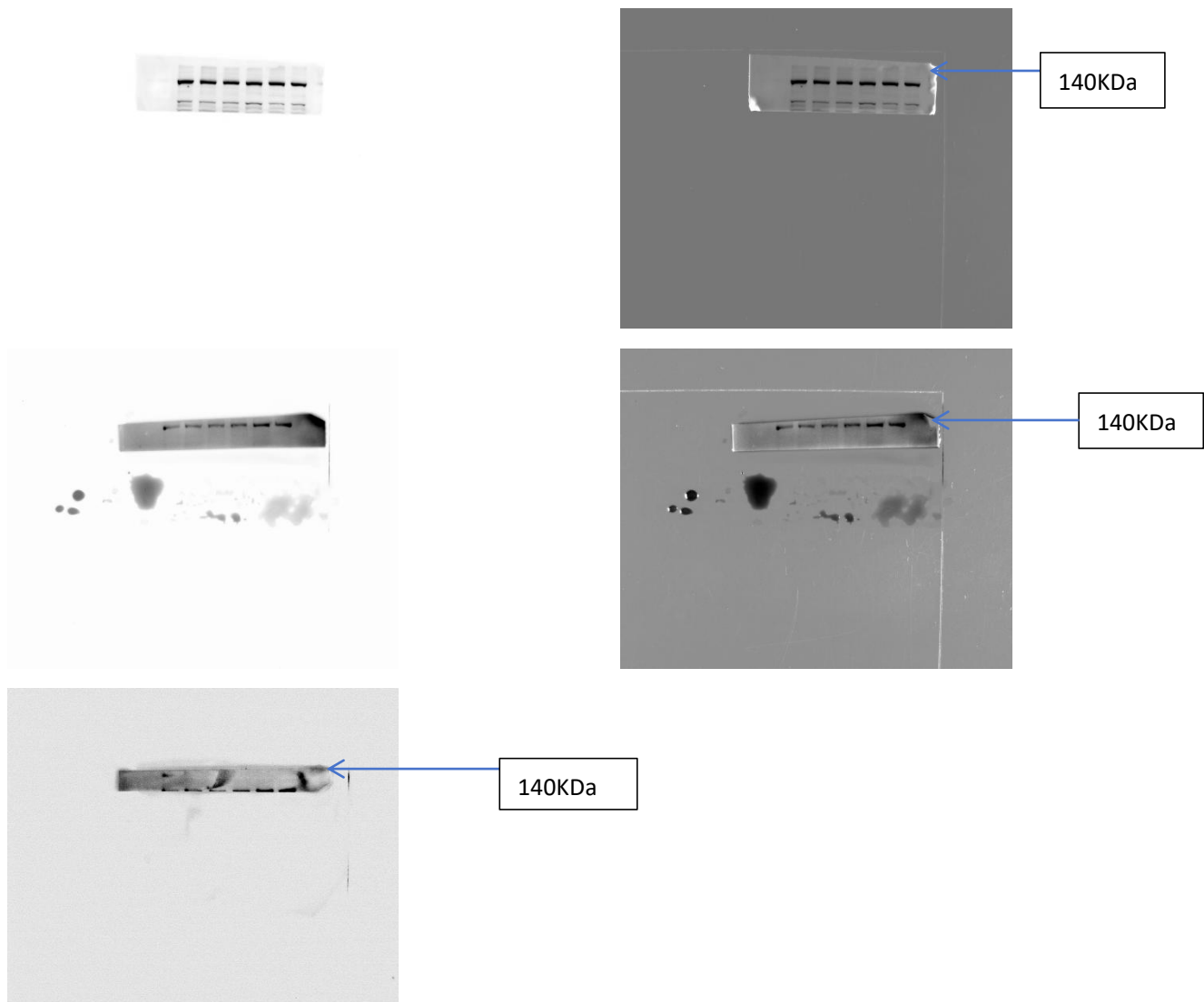
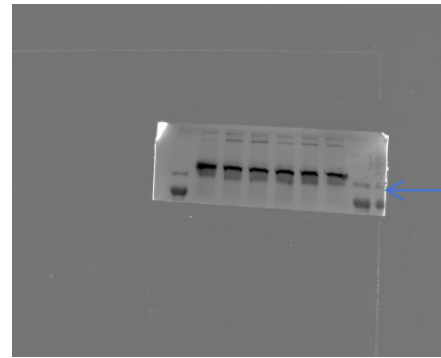
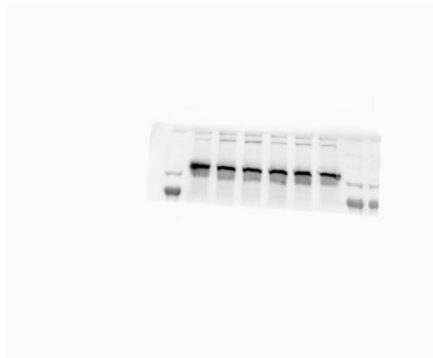
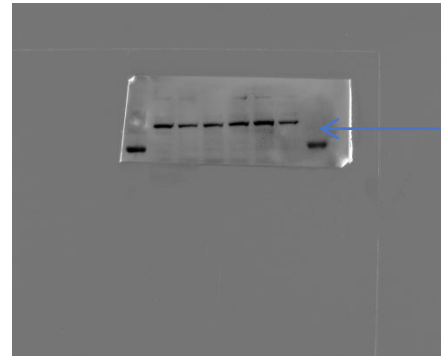
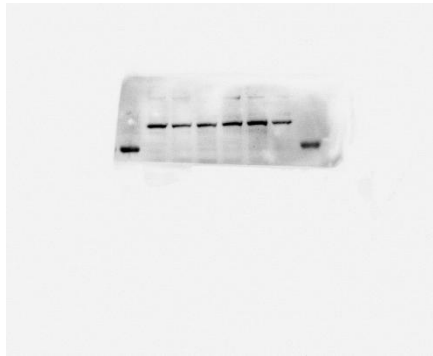


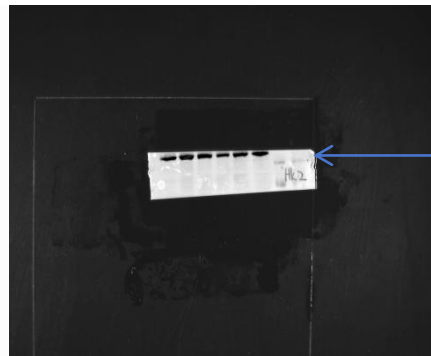
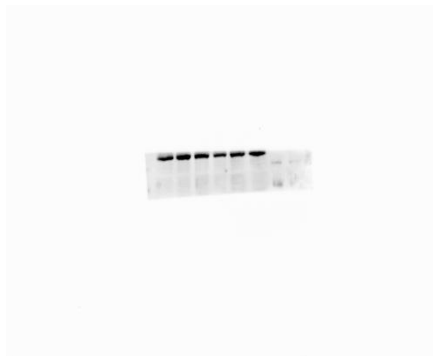
Figure 6E HK2



100KDa

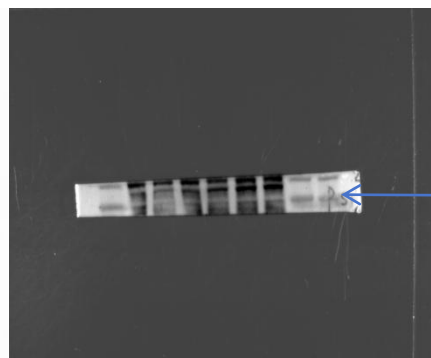
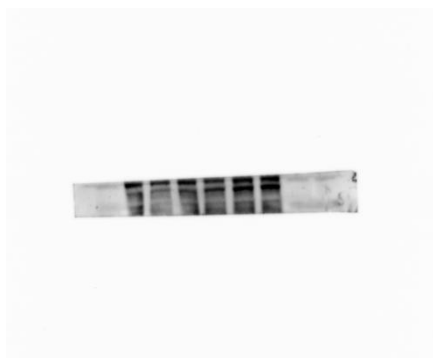


100KDa

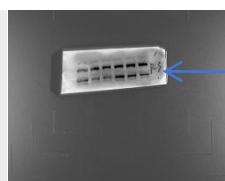


100KDa

Figure 6E P-STAT3



75KDa



75KDa

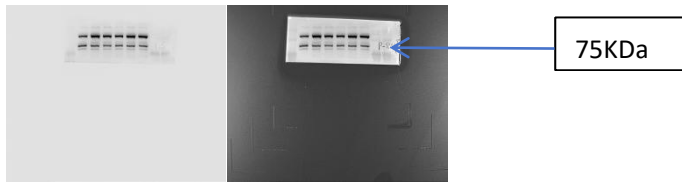


Figure 6E STAT3

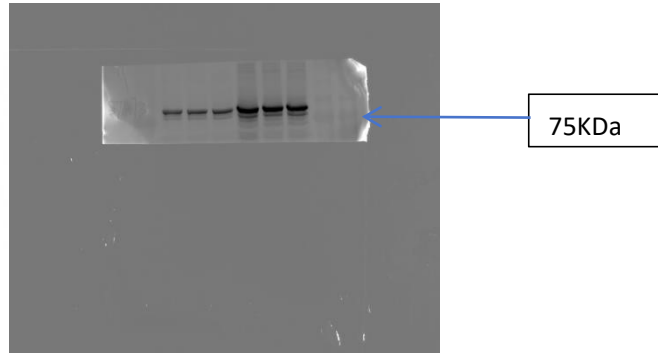
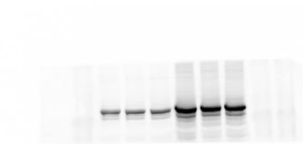
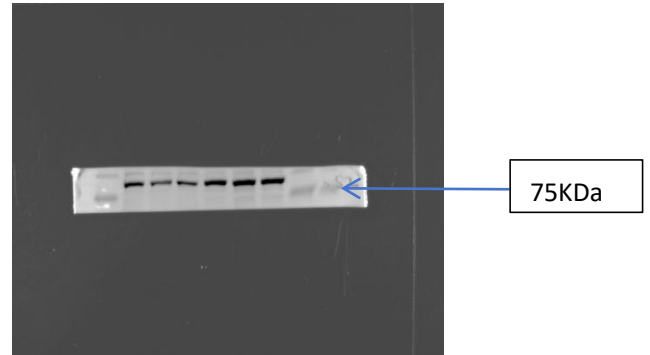
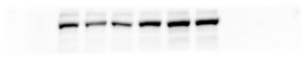
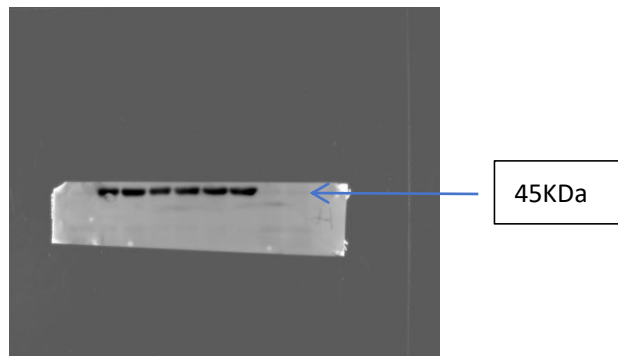
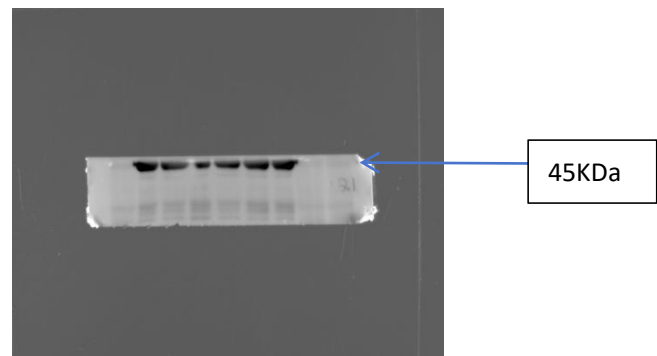
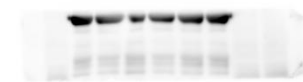
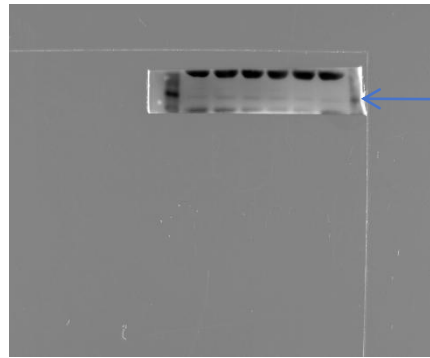
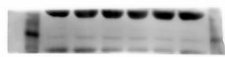


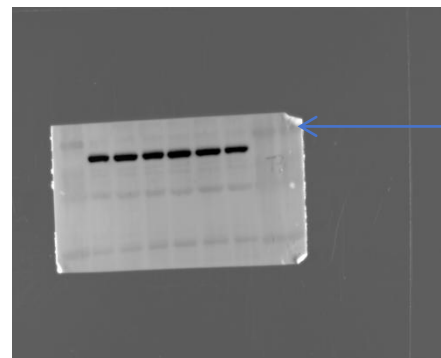
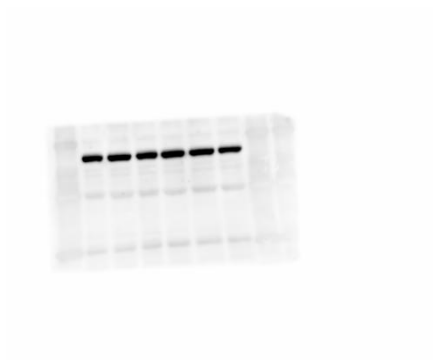
Figure 6E Sma



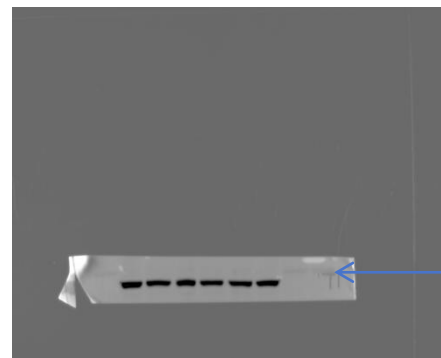


35KDa

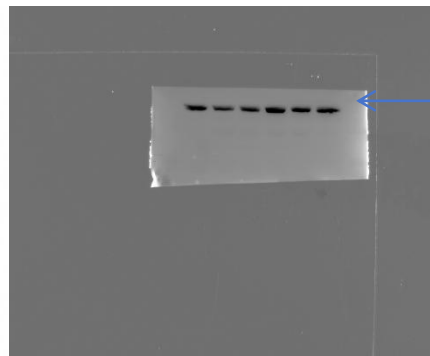
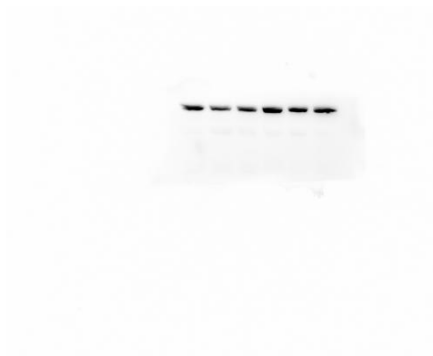
Figure 6E α -Tubulin



60KDa



60KDa



60KDa

Supplementary table1

Table 1 The primer sequences used for RT-qPCR			
Gene	Species	Primer sequence 5'-3' (Up: Forward, Down: Reverse)	
Collagen I	Mouse	GACGCCATCAAGGTCTACTG ACGGGAATCCATCGGTCA	
α -SMA	Mouse	ACTGGGACGACATGGAAAAG GTTCAAGTGGTGCCTCTGTCA	
Fibronectin	Mouse	ATCATAGTGGAGGCACTGCAGAA GGTCAAAGCATGAGTCATCTGTAGG	
HK2	Mouse	TACCACACACCCTACAGCAG CTCGGGAATGGCGTAGATCT	
PKM2	Mouse	GCTCTAGGTATCGCAGCAGG GTAAGCGTTGTCCAGGGTGA	
PFKM	Mouse	CATCGCCGTGTTGACCTCT CCCGTGAAGATACCAACTCGG	
β -actin	Mouse	CATTGCGACAGGATGCAGAAGG TGCTGGAAGGTGGACAGTGAGG	
Collagen I	Human	TGACCTCAAGATGTGCCACT ACCAGACATGCCTCTTGTCC	
α -SMA	Human	CTTGTTTGGGAAGCAAGTGGG GATTCCTGACAGTGCTTGGC	
STAT3	Human	GAGAATCGTGGAGCTGTTTAG GACCAGCAACCTGACTTTAG	
HK2	Human	GTGAATCGGAGAGGTCCCAC GCTAACTTCGGCCACAGGAT	
β -actin	Human	TCACCATTGGCAATGAGCGG AGTTTCGTGGATGCCACAG	

Supplementary table2 KEGG pathway

KEGG_A_class	KEGG_B_class	Pathway	UO-vs-Geni poside (521)	All (922 1)	Pvalue	Qvalue	Pathway ID	Genes
Organismal Systems	Immune system	Antigen processing and presentation	28	90	3.25E-14	1.04E-11	ko04612	ENSMUSG00000021270(Hsp90);ENSMUSG00000037548(H2-Eb1);ENSMUSG00000023274(NSMUSG00000056116(H2-Q10
Metabolism	Global and overview	Metabolic pathways	152	1637	2.85E-11	4.54E-09	ko01100	ENSMUSG00000058927(Cycs);NSMUSG00000062070(Pgk1);E

Category	System	Pathway	Gene Count	Protein Count	Log10 P-value	Log10 Q-value	KEGG ID	Pathway Description
Immune Systems	Immune system	Antigen presentation	10	10	1.2E-05	1.2E-05	ko0466	Antigen presentation via MHC class II
Human Diseases	Infectious disease: viral	Epstein-Barr virus infection	44	361	9.52E-07	3.04E-05	ko05169	Epstein-Barr virus infection
Human Diseases	Infectious disease: viral	Human T-cell leukemia virus 1 infection	34	252	1.78E-06	5.17E-05	ko05166	Human T-cell leukemia virus 1 infection
Cellular Processes	Transport and catabolism	Phagosome	38	301	2.30E-06	6.04E-05	ko04145	Phagosome
Organismal Systems	Immune system	T cell receptor signaling pathway	20	109	2.46E-06	6.04E-05	ko04660	T cell receptor signaling pathway
Metabolism	Global and overview maps	Carbon metabolism	21	121	3.51E-06	8.00E-05	ko01200	Carbon metabolism
Metabolism	Carbohydrate metabolism	Propanoate metabolism	10	31	4.59E-06	9.76E-05	ko00640	Propanoate metabolism
Organismal Systems	Endocrine system	PPAR signaling pathway	17	92	1.27E-05	2.38E-04	ko03320	PPAR signaling pathway
Metabolism	Amino acid metabolism	Valine, leucine and isoleucine degradation	13	57	1.27E-05	2.38E-04	ko00280	Valine, leucine and isoleucine degradation
Human Diseases	Immune disease	Inflammatory bowel disease	14	66	1.44E-05	2.56E-04	ko05321	Inflammatory bowel disease
Organismal Systems	Immune system	Hematopoietic cell lineage	28	217	3.38E-05	5.42E-04	ko04640	Hematopoietic cell lineage
Human Diseases	Immune disease	Allograft rejection	25	183	3.40E-05	5.42E-04	ko05330	Allograft rejection

Genetic diseases							
Disease		Gene		Frequency		Prevalence	
Disease		Gene		Frequency		Prevalence	
Human Diseases	Cardiovascular disease	Viral myocarditis	27	207	3.84E-05	5.83E-04	ko05416
Human Diseases	Infectious disease: viral	Human immunodeficiency virus 1 infection	30	248	6.12E-05	8.87E-04	ko05170
Human Diseases	Infectious disease: viral	Measles	22	158	7.47E-05	9.95E-04	ko05162
Human Diseases	Immune disease	Rheumatoid arthritis	27	215	7.48E-05	9.95E-04	ko05323
Human Diseases	Infectious disease: parasitic	Malaria	12	59	9.10E-05	1.16E-03	ko05144
Metabolism	Xenobiotics biodegradation and metabolism	Drug metabolism - other enzymes	16	99	0.000122926	1.51E-03	ko00983
Metabolism	Global and overview maps	Fatty acid metabolism	12	61	0.00012769	1.51E-03	ko01212
Metabolism	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	8	31	0.000244178	2.78E-03	ko00630
Organismal Systems	Excretory system	Aldosterone-regulated sodium reabsorption	9	39	0.00025257	2.78E-03	ko04960
Human Diseases	Immune disease	Autoimmune thyroid disease	24	199	0.0003441	3.66E-03	ko05320
Metabolism	Carbohydrate metabolism	Ascorbate and aldarate metabolism	8	33	0.00038856	4.00E-03	ko00053
Metabolism	Carbohydrate	Glycolysis / Gluconeogenesis	12	69	0.000427753	4.26E-03	ko00010

									0000014956(PPP1CB);ENSMUSG000000032047(Acat1);ENSMUSG000000026687(Aldh9a1);ENSMUSG000000028603(Scp2);ENSMUSG000000058927(Cycs);ENSMUSG000000071715(Ncf4);ENSMUSG000000052914(Cyp2j6);ENSMUSG000000035042(Ccl5);ENSMUSG000000029868(Trpv6);ENSMUSG000000040907(Atp1a1);ENSMUSG000000026664(Phyh);ENSMUSG000000021751(Acox2);ENSMUSG000000076490(TRBC94(H2-Aa);ENSMUSG00000006094(H2-Aa);ENSMUSG000000035775(Ighm);ENSMUSG00000000290(Itgb2);ENSMUSG000000076490(TRBC94(H2-Aa);ENSMUSG00000006094(H2-Aa);ENSMUSG000000024401(Ighm);ENSMUSG000000058927(Cycs);ENSMUSG000000025393(Atp5f1);ENSMUSG00000006094(H2-Aa);ENSMUSG000000037649(H2-DP1);ENSMUSG000000015947(Fcgr1);ENSMUSG000000011179(Odc1);ENSMUSG000000030004(Nat8);ENSMUSG000000038224(Serpinc1);ENSMUSG000000011034(Slc5a1);ENSMUSG000000040061(Plcb2);ENSMUSG000000027070(Lrp2);ENSMUSG000000017754(Pltp)
Metabolism	Amino acid metabolism	Tryptophan metabolism	9	52	0.002279318	1.58E-02	ko00380		
Metabolism	Lipid metabolism	Primary bile acid biosynthesis	5	18	0.00261683	1.78E-02	ko00120		
Human Diseases	Infectious disease: parasitic	Chagas disease	14	108	0.002939609	1.95E-02	ko05142		
Human Diseases	Cardiovascular disease	Lipid and atherosclerosis	23	221	0.003318928	2.14E-02	ko05417		
Organismal Systems	Digestive system	Mineral absorption	9	55	0.003389922	2.14E-02	ko04978		
Cellular Processes	Transport and catabolism	Peroxisome	12	87	0.003419407	2.14E-02	ko04146		
Metabolism	Carbohydrate metabolism	Pentose and glucuronate interconversions	7	37	0.004132012	2.53E-02	ko00040		
Human Diseases	Infectious disease: bacterial	Staphylococcus aureus infection	25	252	0.004256688	2.56E-02	ko05150		
Human Diseases	Immune disease	Asthma	17	149	0.004347904	2.57E-02	ko05310		
Metabolism	Energy metabolism	Oxidative phosphorylation	16	139	0.005117034	2.97E-02	ko00190		
Human Diseases	Infectious disease: parasitic	Leishmaniasis	20	192	0.005899215	3.36E-02	ko05140		
Metabolism	Metabolism of other amino acids	Glutathione metabolism	10	71	0.006254693	3.43E-02	ko00480		
Organismal Systems	Immune system	Complement and coagulation cascades	12	94	0.006442905	3.43E-02	ko04610		
Organismal Systems	Digestive system	Carbohydrate digestion and absorption	8	50	0.006466937	3.43E-02	ko04973		
Organismal Systems	Digestive system	Cholesterol metabolism	8	50	0.006466937	3.43E-02	ko04979		

	Metabolism								
Metabolism	of cofactors and vitamins	Pantothenate and CoA biosynthesis	5	22	0.00667 022	3.43E- 02	ko00 770	ENSMUSG000000033308(Dpyd)	
Organisma l Systems	Excretory system	Proximal tubule bicarbonate reclamation	5	22	0.00667 022	3.43E- 02	ko04 964	ENSMUSG000000033161(Atp1a)	
Environm ental Informatio n Processing	Signal transduction	TNF signaling pathway	14	120	0.00759 4102	3.69E- 02	ko04 668	ENSMUSG000000054072(Iigp1) G000000022414(Tab1);ENSMUS	
Organisma l Systems	Immune system	Leukocyte transendothelial migration	14	120	0.00759 4102	3.69E- 02	ko04 670	ENSMUSG000000056917(Sipa1) 000000071715(Ncf4);ENSMUSG	
Metabolism	Xenobiotics biodegradation and metabolism	Drug metabolism - cytochrome P450	10	73	0.00761 2231	3.69E- 02	ko00 982	ENSMUSG000000032348(Gsta4) NSMUSG000000040170(Fmo2);	
Organisma l Systems	Excretory system	Endocrine and other factor-regulated calcium reabsorption	9	62	0.00762 7964	3.69E- 02	ko04 961	ENSMUSG000000036899(Trpv5) SG000000028222(Calb1);ENSMU	
Environm ental Informatio n Processing	Signaling molecules and interaction	Viral protein interaction with cytokine and cytokine receptor	12	97	0.00825 6688	3.92E- 02	ko04 061	ENSMUSG000000024401(Tnf);E SG000000034855(Cxcl10);ENSM	
Human Diseases	Infectious disease: viral	Hepatitis C	18	172	0.00834 6493	3.92E- 02	ko05 160	ENSMUSG000000058927(Cycs); 0000021116(EIF2S1);ENSMUS 066861(Oas1a);ENSMUSG0000	
Human Diseases	Immune disease	Primary immunodeficiency	17	160	0.00882 0799	4.08E- 02	ko05 340	ENSMUSG00000000409(Lck);E G000000076613(Igh-3);ENSMUS 2094(Cd3d);ENSMUSG0000000	
Metabolism	Lipid metabolism	Steroid hormone biosynthesis	12	99	0.00967 2296	4.41E- 02	ko00 140	ENSMUSG000000068086(Cyp2c la5);ENSMUSG000000024087(C	
Environm ental Informatio n Processing	Membrane transport	ABC transporters	8	54	0.01029 408	4.63E- 02	ko02 010	ENSMUSG000000031333(Abcb7) MUSG000000044749(Abca6)	
Metabolism	Metabolism of cofactors	Porphyrin metabolism	7	44	0.01094 449	4.85E- 02	ko00 860	ENSMUSG000000025270(Alas2)	

	and vitamins								
Human Diseases	Cancer: overview	Chemical carcinogenesis - reactive oxygen species	22	232	0.01174 986	5.13E -02	ko05 208	ENSMUSG00000000171(Sdhd), USG00000038462(Uqcrfs1);ENSMUSG00000025428(Atp5f1a); ;ENSMUSG00000020828(Pld2)	
Organismal Systems	Digestive system	Bile secretion	12	104	0.01403 736	6.04E -02	ko04 976	ENSMUSG00000079262(Slco1a7c);ENSMUSG00000089943(Ug	
Metabolism	Global and overview maps	Biosynthesis of amino acids	10	80	0.01420 876	6.04E -02	ko01 230	ENSMUSG00000062070(Pgk1), 00032294(Pkm);ENSMUSG000	
Metabolism	Carbohydrate metabolism	Starch and sucrose metabolism	6	36	0.01447 154	6.07E -02	ko00 500	ENSMUSG00000030244(Gys2)	
Human Diseases	Drug resistance: antineoplastic	Platinum drug resistance	10	83	0.01807 264	7.49E -02	ko01 524	ENSMUSG00000058927(Cycs); USG00000000817(Faslg);ENSM	
Human Diseases	Infectious disease: viral	Human papillomavirus infection	30	359	0.01996 485	8.17E -02	ko05 165	ENSMUSG00000020566(Atp6v NSMUSG00000000386(Mx1);ENSMUSG00000040033(Stat2);ENSMUSG00000029561(Oasl2);ENSMUSG00000000142(Axin2);ENSM	
Metabolism	Energy metabolism	Sulfur metabolism	3	11	0.02104 987	8.44E -02	ko00 920	ENSMUSG00000068874(Selenb	
Organismal Systems	Endocrine system	Thyroid hormone synthesis	9	73	0.02116 91	8.44E -02	ko04 918	ENSMUSG00000061808(Ttr);ENSMUSG00000040907(Atp1a3);ENSM	
Organismal Systems	Immune system	C-type lectin receptor signaling pathway	12	113	0.02547 335	1.00E -01	ko04 625	ENSMUSG00000039936(Pik3cc USG00000018899(Irf1);ENSM	
Organismal Systems	Immune system	Chemokine signaling pathway	18	194	0.02595 93	1.01E -01	ko04 062	ENSMUSG00000039936(Pik3cc G00000033220(Rac2);ENSMUSG00000060216(Arrb2);ENSMUSG	
Organismal Systems	Digestive system	Gastric acid secretion	9	76	0.02679 883	1.03E -01	ko04 971	ENSMUSG00000041248(Kcnj16);ENSMUSG00000051497(Kcnj16)	
Organismal Systems	Immune system	Intestinal immune network for IgA production	16	168	0.02792 612	1.05E -01	ko04 672	ENSMUSG00000076490(TRBC 94(H2-Aa);ENSMUSG00000060000(Ighm);ENSMUSG00000076928	
Organismal Systems	Immune system	Natural killer cell mediated cytotoxicity	21	238	0.02805 098	1.05E -01	ko04 650	ENSMUSG00000000409(Lck);ENSMUSG00000076615(Igh-1a);ENSMUSG00000015437(Gzmb);ENSMUSG00000	
Metabolism	Amino acid metabolism	Lysine degradation	8	65	0.02917 818	1.08E -01	ko00 310	ENSMUSG00000032047(Acat1);ENSMUSG00000029455(Aldh2)	
Organismal Systems	Immune system	Toll-like receptor	11	103	0.03048	1.12E	ko04	ENSMUSG00000029304(Spp1).	

l Systems	system	signaling pathway			748	-01	620	G00000060183(Cxcl11);ENSMUSG000000032047(Acat1)
Organisma l Systems	Digestive system	Fat digestion and absorption	6	44	0.03595 977	1.30E -01	ko04 975	ENSMUSG000000032047(Acat1)
Human Diseases	Cancer: overview	Chemical carcinogenesis - DNA adducts	10	93	0.03654 056	1.30E -01	ko05 204	ENSMUSG000000032348(Gsta4);ENSMUSG000000024087(Cyp1b1)
Human Diseases	Infectious disease: viral	Coronavirus disease - COVID-19	30	378	0.03676 106	1.30E -01	ko05 171	ENSMUSG000000095079(IGHA1);ENSMUSG000000033831(Fgb);ENSMUSG000000009(F13a1);ENSMUSG000000036(Rpl10a);ENSMUSG000000025794(Rpl13a);ENSMUSG000000078193(Rpl35)
Human Diseases	Infectious disease: bacterial	Yersinia infection	22	262	0.04028 679	1.41E -01	ko05 135	ENSMUSG000000076490(TRBC1);ENSMUSG000000095079(IGHA2);ENSMUSG0000000039936(Pik3cd);ENSMUSG000000000(HOG)
Organisma l Systems	Endocrine system Metabolism	Ovarian steroidogenesis	8	70	0.04283 106	1.49E -01	ko04 913	ENSMUSG000000033715(Akr1c1);ENSMUSG000000052520(Cyp2c19)
Metabolis m	of terpenoids and polyketides	Terpenoid backbone biosynthesis	4	24	0.04353 272	1.49E -01	ko00 900	ENSMUSG000000032047(Acat1)
Human Diseases	Infectious disease: viral	Herpes simplex virus 1 infection	41	556	0.04650 833	1.58E -01	ko05 168	ENSMUSG000000058927(Cyca3);ENSMUSG000000037548(H2-D1);ENSMUSG000000039936(Irf1);ENSMUSG000000060550(H2-Q7);ENSMUSG000000022414(Tab1);ENSMUSG000000029605(Oas1b);ENSMUSG000000069307(H2bc7)
Human Diseases	Immune disease	Systemic lupus erythematosus	22	267	0.04793 812	1.61E -01	ko05 322	ENSMUSG00000002-DMb1);ENSMUSG0000000734(Ighg1);ENSMUSG0000000766(Irf1);ENSMUSG000000059089(Fcgr4)
Metabolis m	Metabolism of other amino acids	Selenocompound metabolism	3	15	0.04910 577	1.63E -01	ko00 450	ENSMUSG000000024899(Papss2)

Supplementary table3 the expression data of HK2, PFKM, and PKM2 genes in the transcriptome sequencing results of 6 mice

	UUO1_fpk	UUO2_fpk	UUO3_fpk	Geniposide1	Geniposide1	Geniposide1
HK2	19.26	12.27	5.95	6.75	2.96	0.87
PKM 2	272.65	175.19	268.54	155.46	167.48	139.66
PFKM	8.84	7.88	7.01	5.12	5.85	4.3

Figure Captions

Fig. S1 Cell viability of HK-2 cells treated with different concentrations of GP, as detected by MTT.

Fig. S2 Immunofluorescence image showing the expression of vimentin and E-cadherin (scale bar = 20um).