

Supplementary Figures

TUBB4A interacts with MYH9 to protect the nucleus during cell migration and promotes prostate cancer via GSK3 β / β -catenin signalling

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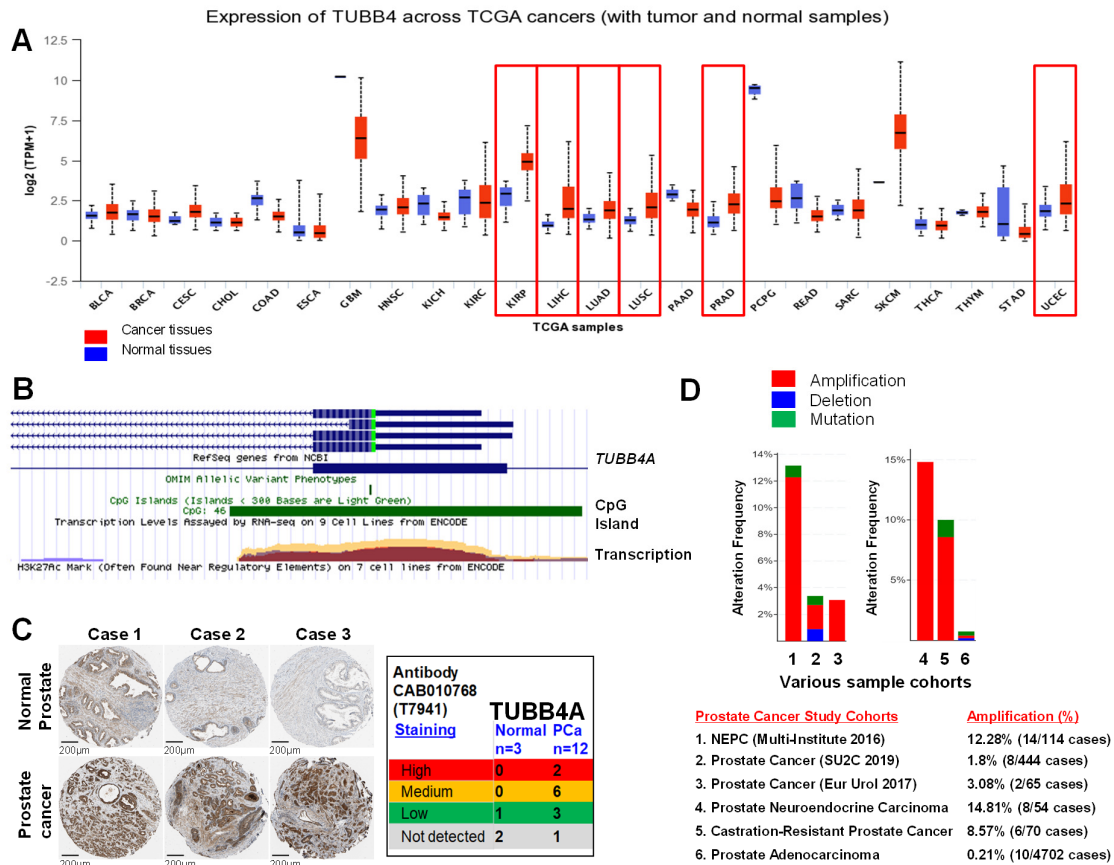


Figure S1. Characterization of TUBB4A expression profiling and genetic alteration in human primary prostate cancer. **A**, Expression of TUBB4A across tumors with normal control samples from TCGA dataset was analyzed using UALCAN (ualcan.path.uab.edu). Data are presented as the medians and interquartile ranges. Red frames indicate high expression of TUBB4A in tumors as compared to normal controls. **B**, CpG Island in promoter and transcription of human TUBB4A gene. Screenshot from the UCSC genome browser showing location of the TUBB4A promoter, CpG island, exon1, intron1, and transcription (Broad Institute Histone data). **C**, Representative IHC staining and summary of TUBB4A intensity patterns in normal prostate and prostate cancer tissues (Uhlén M *et al.*, Mol Cell Proteomics, 2005

PubMed: 16127175) from The Human Protein Atlas (THPA, www.proteinatlas.org). Anti-human TUBB4A rabbit Ab (Millipore-Sigma, T7941) was used for IHC staining. **D**, Genetic alterations of *TUBB4A* in primary prostate cancers. Public dataset-based analysis and summary of genetic alterations (amplification, deletion, and mutation) of *TUBB4A* in primary prostate cancers from 6 studies using cBioPortal (www.cbioportal.org) with various public datasets. BLCA, bladder carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma.

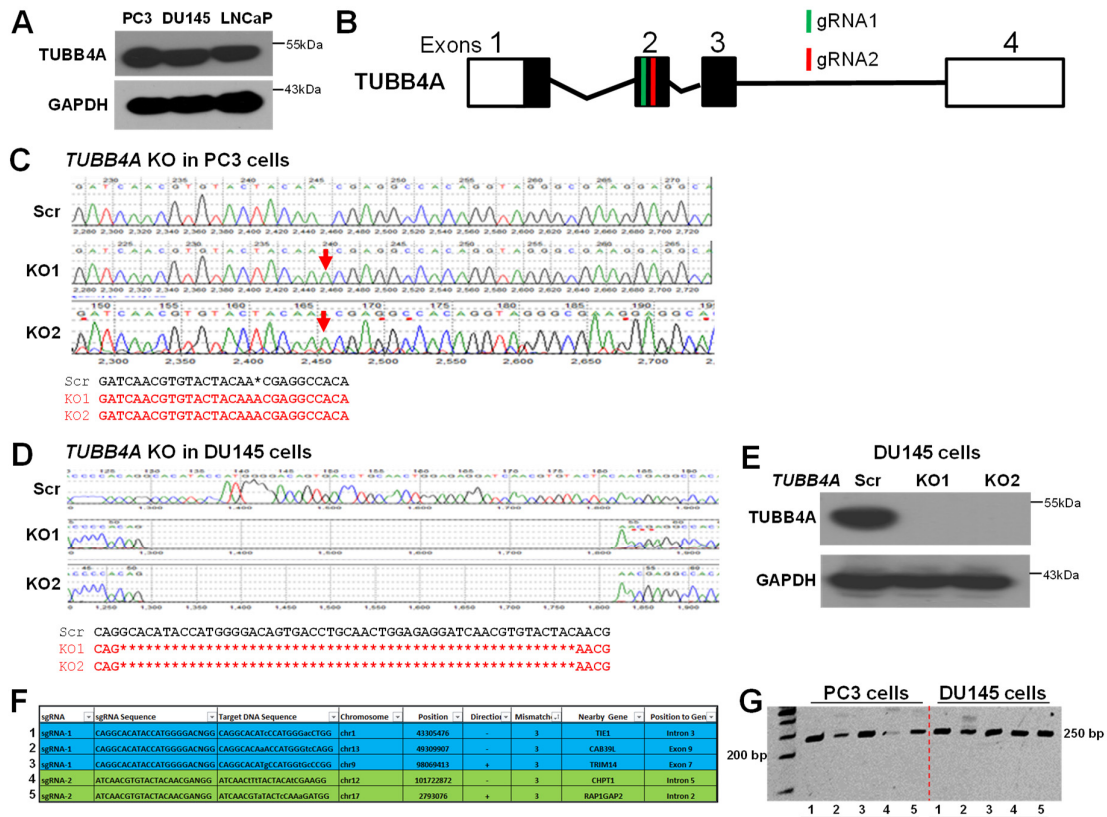


Figure S2. CRISPR-Cas9 *TUBB4A* knockout (KO) in prostate cancer cell lines. A, *TUBB4A* expression in three prostate cancer cell lines. **B**, Schematic diagram of guide RNA (gRNA) design at human *TUBB4A* gene. **C**, Sanger DNA sequencing of scrambled (Scr) and *TUBB4A* KO PC3 cells (red arrow indicates the base A insertion). **D**, **E**, Sanger DNA sequencing and Western blot confirmation of scrambled (Scr) and *TUBB4A* KO DU145 cells. **F**, The potential off-target regions as predicted by the Cas-OFFinder tool. **G**, PCR analysis of potential off-target genes in *TUBB4A* KO PC3 and DU145 cells. Source data are provided as a Source Data file.

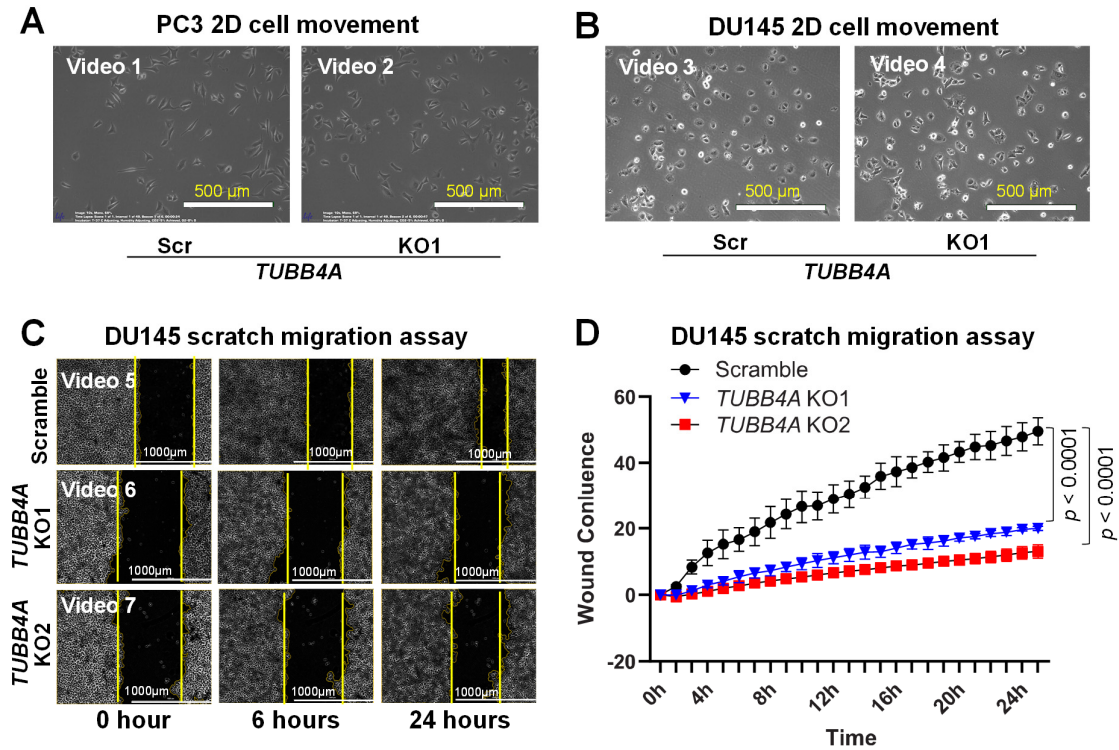


Figure S3. Cell movement and migration of scrambled and *TUBB4A* KO prostate cancer cell lines. **A**, Automated live-cell 2D video movement assay of PC3 cells (also see [Supplementary Videos 1 and 2](#)). Scale bar, 500 μm . **B**, Automated live-cell 2D video movement assay of DU145 cells (also see [Supplementary Videos 3 and 4](#)). Scale bar, 500 μm . **C**, **D**, Automated live-cell 2D video scratch migration assay and wound healing curves of DU145 cells (also see [Supplementary Videos 5-7](#)). Data are replicated 3 times (D). Data are presented as the means and SD with a repeated-measures ANOVA test. Source data are provided as a Source Data file. Scr, scramble; KO, knockout.

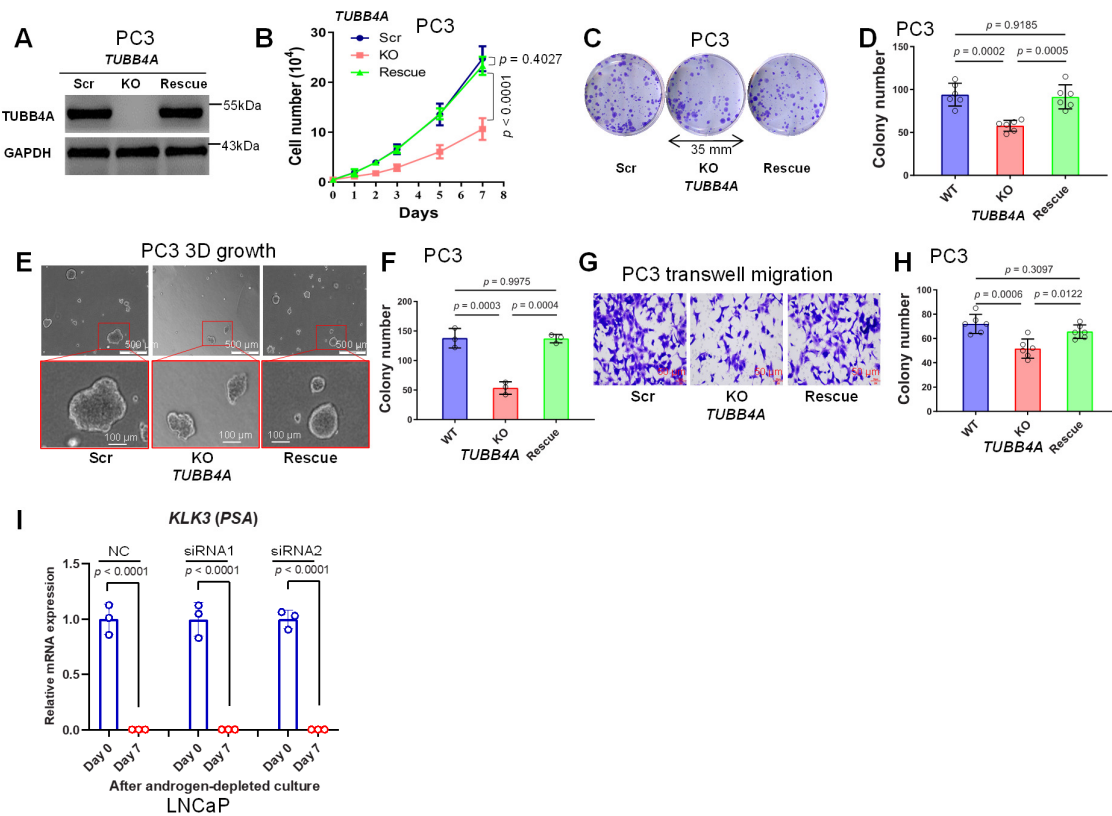


Figure S4. Ectopic expression of TUBB4A rescues the *TUBB4A* KO-reduced proliferation and migration of prostate cancer cells. **A-D**, Confirmation of *TUBB4A* KO and rescue, cell growth, clone formation, and statistical analysis of PC3 cells. A colony was considered to be 50 cells or more under a microscope. **E, F**, 3D soft-agar colony formation and statistical analysis of PC3 cells. A 3D colony was counted based on the capacity of single cells to grow to colonies consisting of at least 50 cells. Scale bar, 500 μ m. **G, H**, Transwell assay and statistical analysis of PC3 cells. Scale bar, 50 μ m. **I**, Relative mRNA levels of *KLK3* (*PSA*) as a percentage of *GAPDH* expression as determined by qPCR in LNCaP cells transfected with negative control siRNA or *TUBB4A* siRNAs in androgen-depleted culture medium (charcoal-stripped growth medium). Data are replicated 3 (B, F, I) and 6 (D, H) times. Data are presented as the means and SD with a two-tailed *t* test (I), a repeated measures ANOVA test (B), or an ANOVA followed by Tukey's post hoc *t* test (D, F, H). Source data are provided as a Source Data file. Scr, scramble; KO, knockout, NC, negative control siRNA; siRNA, small interfering RNA.

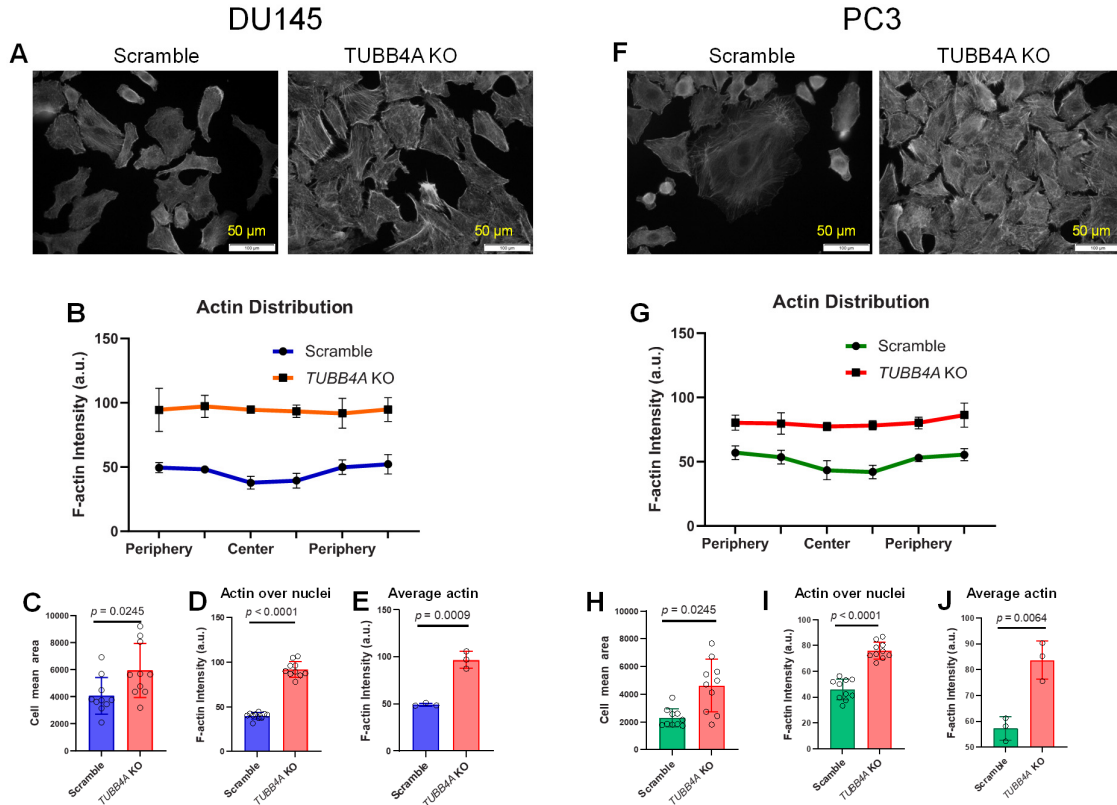


Figure S5. *TUBB4A* KO increases the cell area and the F-actin intensity in prostate cancer cells. **A**, Expression levels and localization of F-actin by immunofluorescence imaging in scrambled control and KO DU145 cells. **B**, Quantification of the distribution of F-actin intensity in scrambled control and KO DU145 cells. **C-E**, Quantification of the cell area, F-actin intensity over nuclei, and average F-actin intensity in scrambled control and KO DU145 cells. **F-J**, Expression levels, localization, distribution, and intensity of F-actin in scrambled control and KO PC3 cells. Data are replicated 3 (E, J) and 10 (C-D and H-I) times. Data are presented as the means and SD with a two-tailed *t* test (C-E and H-J). Source data are provided as a Source Data file. Scr, scrambled control; KO, knockout.

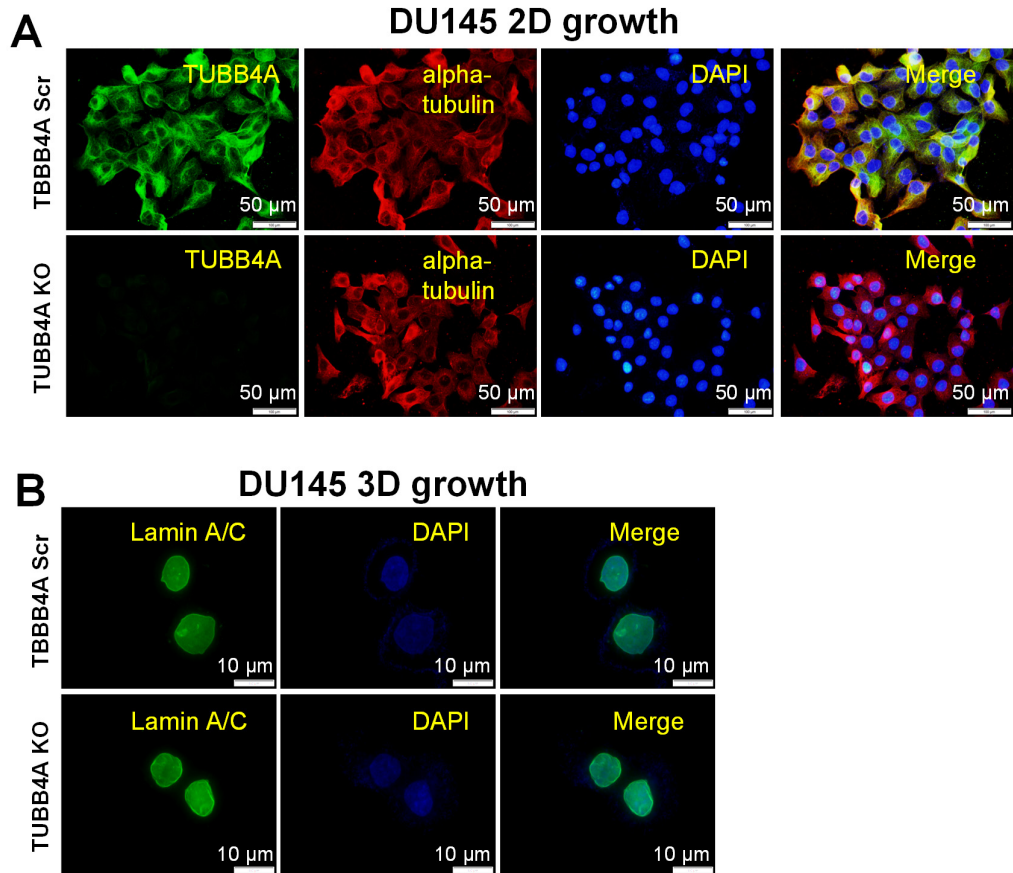
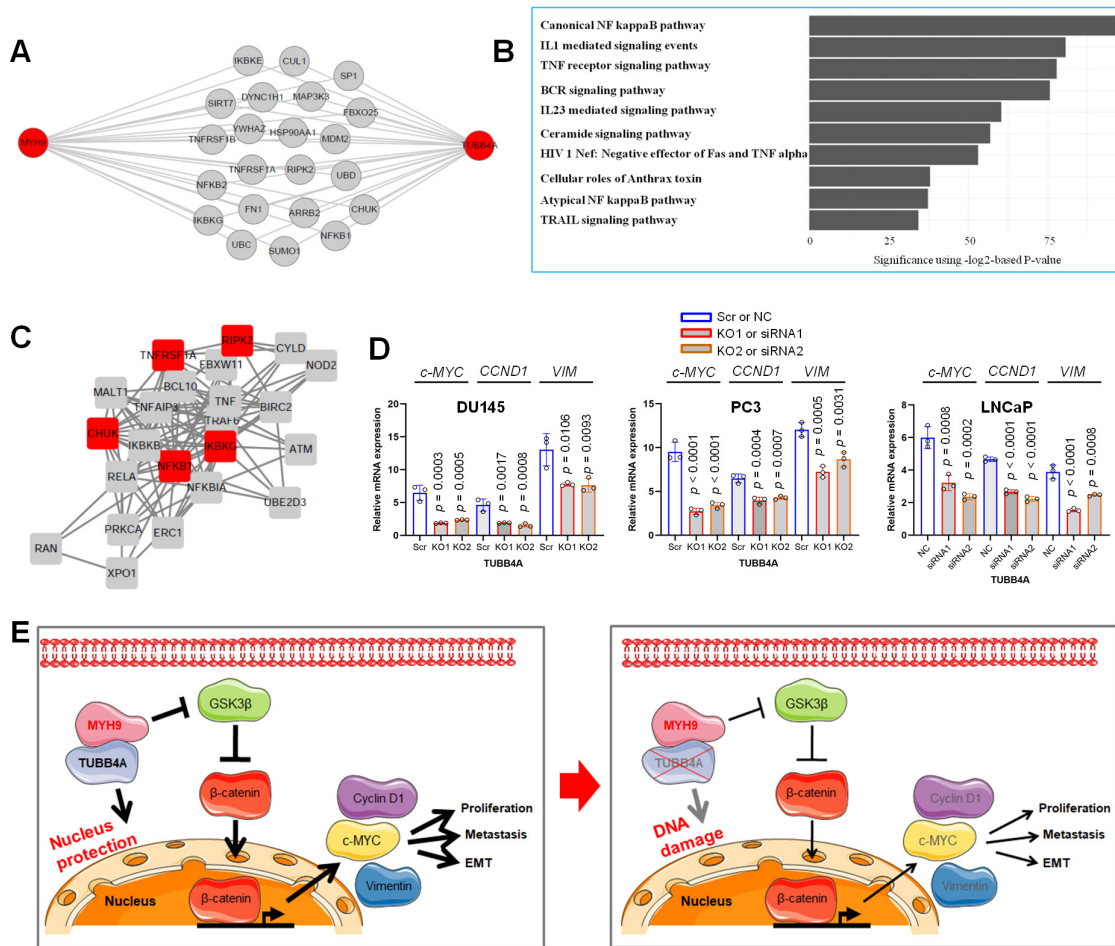


Figure S6. *TUBB4A* KO does not change α -tubulin or lamin. **A**, IF staining of TUBB4A and α -tubulin in DU145 cells in 2D collagen gels. **B**, IF staining of TUBB4A and lamin A/C in DU145 cells after migration in 3D collagen gels.



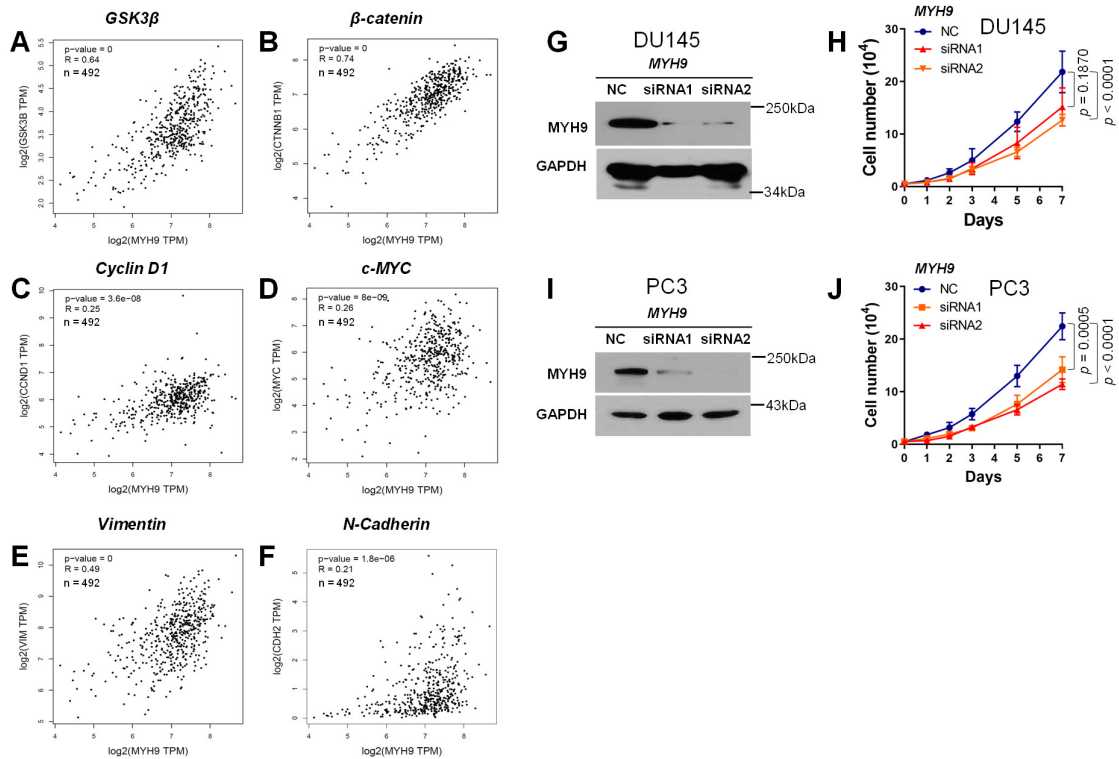


Figure S8. MYH9-related GSK3β/β-catenin signaling gene network and the effect of MYH9 knockdown on growth of prostate cancer cells. **A-F**, Relationship between mRNA expression levels of *MYH9* with *GSK3β*, *β-catenin*, *cyclin D1*, *c-MYC*, *vimentin*, and *N-cadherin* in human primary prostate cancer tissues from TCGA dataset was analyzed using Gene Expression Profiling Interactive Analysis (GEPIA, gepia.cancer-pku.cn). **G-J**, Confirmation of *MYH9* knockdown by Western blots and effect of *MYH9* knockdown on proliferation of DU145 and PC3 cells. Data are replicated 3 times. Data are presented as the means and SD with a repeated measures ANOVA test. Source data are provided as a Source Data file. NC, negative control siRNA; siRNA, small interfering RNA.

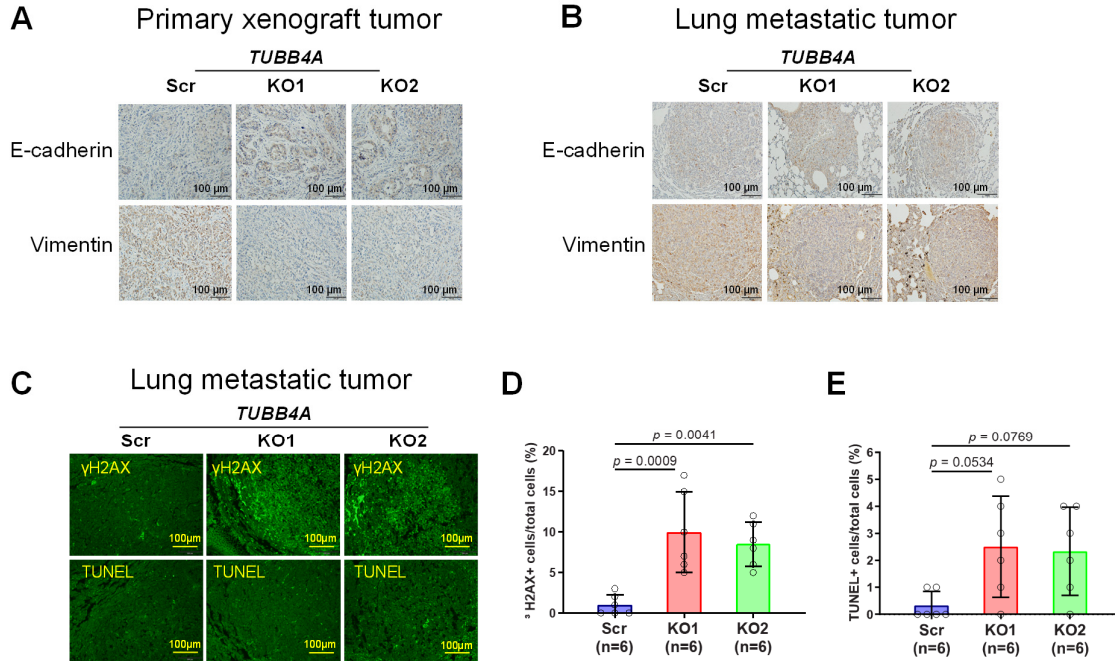


Figure S9. Expression of EMT, DNA damage response and apoptotic markers in scrambled and *TUBB4A* KO DU145 xenograft tumors of NSG mice. A, B, IHC staining of primary and metastatic tumors by specific antibodies to E-cadherin and vimentin for scrambled and KO groups. Scale bar, 100 μm. C-E, IF staining and statistical analysis of lung metastases by DNA damage response maker γH2AX and apoptotic marker TUNEL for scrambled and KO groups. Scale bar, 100 μm. Data are replicated with 6 times. Data are presented as the means and SD with an ANOVA followed by Tukey's post hoc *t* test (*TUBB4A* KO vs. scramble control). Source data are provided as a Source Data file. Scr, scramble; KO, knockout.

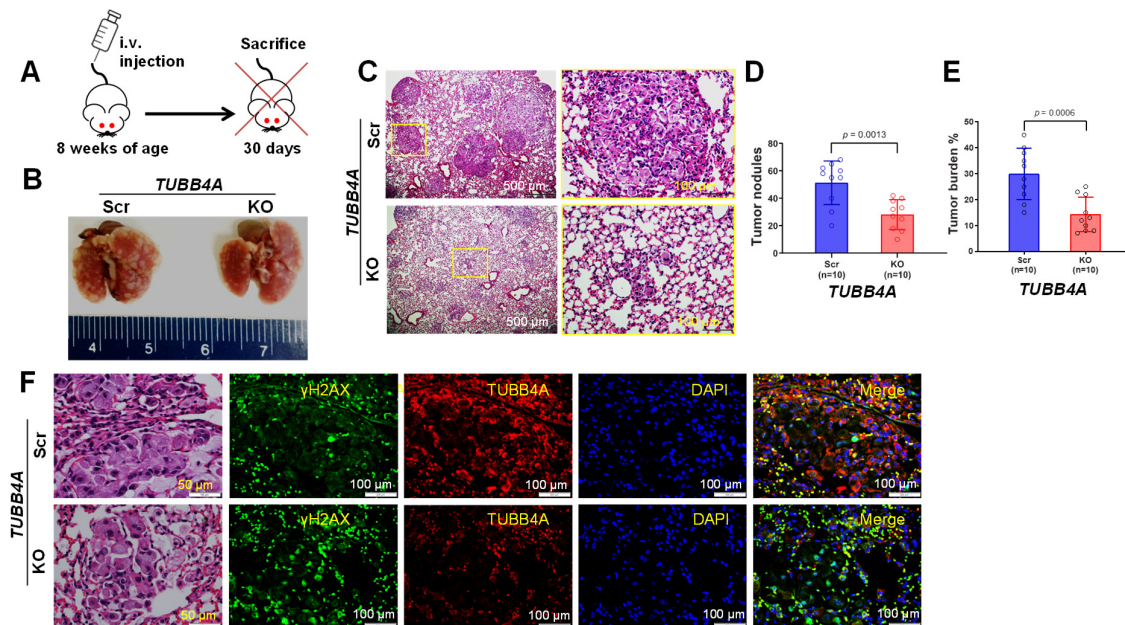


Figure S10. *TUBB4A* KO reduces tumor colonization in the lungs of NSG mice.

A, Schematic diagram of IV xenograft tumors followed for up to 30 days in NSG mice. **B**, **C**, Representative tumor metastases in the lung. **D**, **E**, Statistical analysis of tumor nodules and burdens of lung metastasis at day 30. Data are replicated 10 times. Data are presented as the means and SD with a two-tailed *t* test. Source data are provided as a Source Data file. **F**, H/E and IF staining of γ H2A and TUBB4A in lung metastatic tumors.

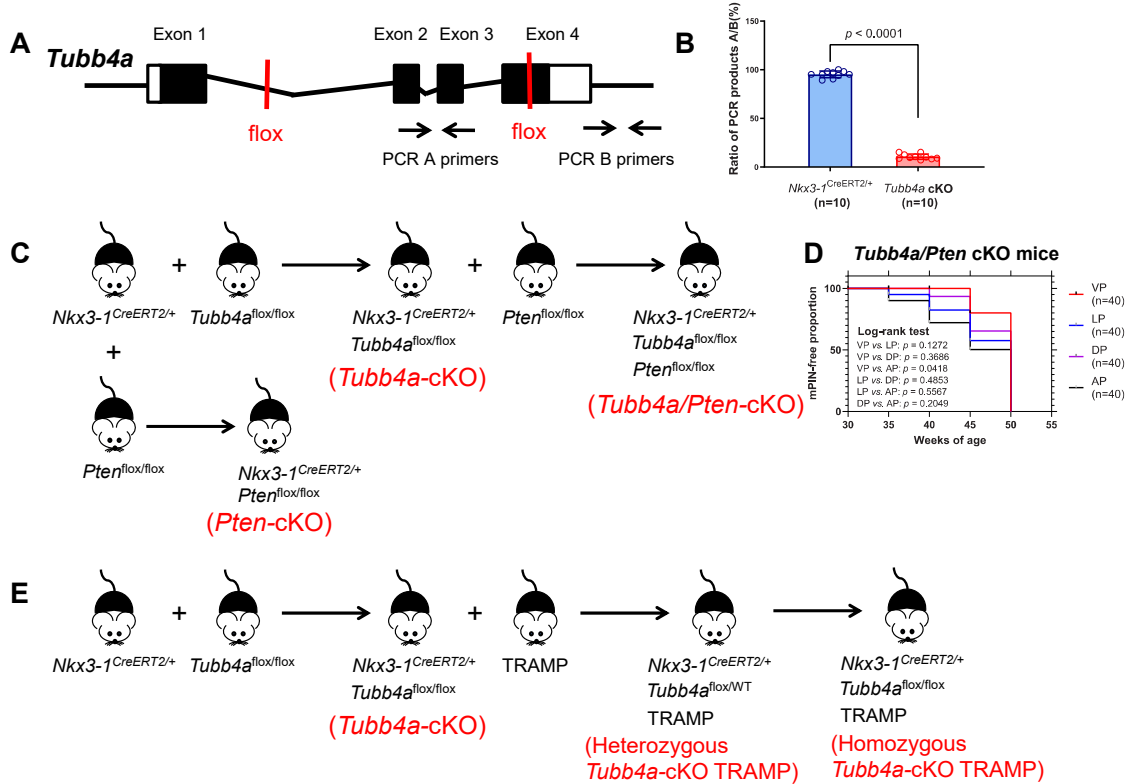


Figure S11. Establishment of genetically engineered spontaneous prostate cancer mouse models. **A**, Diagram of the floxed *Tubb4a* locus and position of the primers used to measure the ratio of deleted versus undeleted alleles. **B**, Efficient DNA deletion of the *Tubb4a* locus in the *Tubb4a* cKO but not the *Nkx3-1^{CreERT2/+}* control mice. Data are replicated 10 times. Data are presented as the means and SD with a two-tailed *t* test. **C**, Diagram of the development of *Nkx3-1^{CreERT2/+} Tubb4a^{flox/flox}* (*Tubb4a* cKO), *Nkx3-1^{CreERT2/+} Pten^{flox/flox}* (*Pten* cKO), and *Nkx3-1^{CreERT2/+} Tubb4a^{flox/flox} Pten^{flox/flox}* (*Tubb4a/Pten* cKO) models. **D**, Kaplan–Meier curves of mPIN incidences for up to 50 weeks of age in AP, DP, LP, and VP lobes of *Tubb4a/Pten* cKO mice, respectively. Data are replicated 40 times. **E**, Diagram of the development of *Nkx3-1^{CreERT2/+} Tubb4a^{flox/flox}* (*Tubb4a* cKO) TRAMP models. AP, anterior prostate; DP, dorsal prostate; LP, lateral prostate; VP, ventral prostate; cKO, prostate conditional knockout; mPIN, mouse prostatic intraepithelial neoplasia. Source data are provided as a Source Data file.

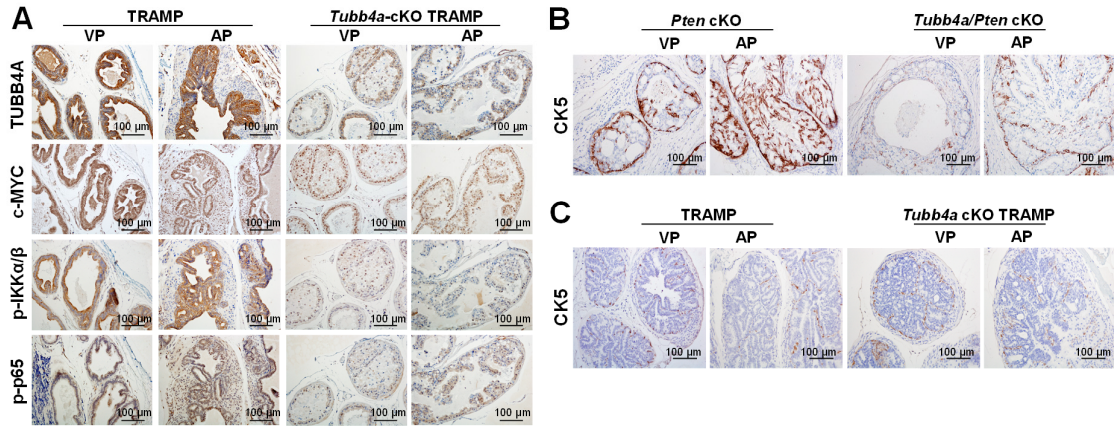


Figure S12. Representative immunostaining of genetically engineered spontaneous prostate cancer mouse models. A, Immunostaining for TUBB4A, AR, Ki67, c-MYC, p-IKK, p-p65 and in the prostates of wild-type TRAMP and *Tubb4a* cKO TRAMP mice at 12 weeks of age. **B, C,** Immunostaining for CK5 in the prostates of *Pten* cKO and *Tubb4a/Pten* cKO mice at 35 weeks of age and wild-type TRAMP and *Tubb4a* cKO TRAMP mice at 30 weeks of age. Scale bar, 100 μm.

Supplementary Video 1. Automated live-cell two-dimensional random movement assay for scramble control PC3 cells. Images were taken several times throughout 24 hours in a 6-well cell culture plate at 37°C under 5% CO₂. This video is related to Fig. S3A, left panel.

Supplementary Video 2. Automated live-cell two-dimensional random movement assay for *TUBB4A* KO PC3 cells. Images were taken several times throughout 24 hours in a 6-well cell culture plate at 37°C under 5% CO₂. This video is related to Fig. S3A, right panel.

Supplementary Video 3. Automated live-cell two-dimensional random movement assay for scramble control DU145 cells. Images were taken several times throughout 24 hours in a 6-well cell culture plate at 37°C under 5% CO₂. This video is related to Fig. S3B, left panel.

Supplementary Video 4. Automated live-cell two-dimensional random movement assay for *TUBB4A* KO DU145 cells. Images were taken several times throughout 24 hours in a 6-well cell culture plate at 37°C under 5% CO₂. This video is related to Fig. S3B, right panel.

Supplementary Video 5. Automated live-cell two-dimensional scratch migration assay in scramble control DU145 cells. Images were taken several times throughout 24 hours in a 6-well cell culture plate at 37°C under 5% CO₂. This video is related to Fig. S3C, left panel.

Supplementary Video 6. Automated live-cell two-dimensional scratch migration assay in *TUBB4A* KO1 DU145 cells. Images were taken several times throughout 24 hours in a 6-well cell culture plate at 37°C under 5% CO₂. This video is related to Fig. S3C, right panel.

Supplementary Video 7. Automated live-cell two-dimensional scratch migration assay for *TUBB4A* KO2 DU145 cells. Images were taken several times throughout 24 hours in a 6-well cell culture plate at 37°C under 5% CO₂. This video is related to Fig. S3C, right panel.

Table S1. Human specimen characteristics

Groups	Normal prostate^a n=50	Prostate cancer n=136
Age y, median (range)	64 (47–76)	63 (46–76)
PSA (ng/ml), median (range)		5.9 (1.9–46.8)
Race		
European-Americans (EA)	27	75
African-Americans (AA)	23	61
Tumor stage (TNM)		
T1		0
T2		62
T3		51
T4 or N+ or M+		23
Gleason score		
G6		14
G7		92
G8		10
G9		16
G10		4

^a Histologically normal prostate tissue adjacent to the prostate cancer tissue

Table S2. Mass spectrometry analysis for TUBB4A-interacting proteins

Ranking	%Cov(95) ^a	Accession	Name	Species	Peptides(95%)
1	41.12	sp P35579 MYH9_HUMAN	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4	HUMAN	77
2	34.75	sp Q00610 CLH1_HUMAN	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5	HUMAN	59
3	22.63	sp P46940 IQGA1_HUMAN	Ras GTPase-activating-like protein IQGAP1 OS=Homo sapiens GN=IQGAP1 PE=1 SV=1	HUMAN	36
4	46.07	sp P68371 TBB4B_HUMAN	Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1	HUMAN	21
5	9.31	sp P35580 MYH10_HUMAN	Myosin-10 OS=Homo sapiens GN=MYH10 PE=1 SV=3	HUMAN	20
6	38.96	sp P07437 TBB5_HUMAN	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2	HUMAN	19
7	12.89	sp O00159 MYO1C_HUMAN	Unconventional myosin-1c OS=Homo sapiens GN=MYO1C PE=1 SV=4	HUMAN	14
8	5.86	sp P21333 FLNA_HUMAN	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4	HUMAN	12
9	19.29	sp P68363 TBA1B_HUMAN	Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1	HUMAN	8
10	19.47	sp P63261 ACTG_HUMAN	Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1	HUMAN	7
11	19.47	sp P60709 ACTB_HUMAN	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1	HUMAN	7
12	16.48	sp Q9BQE3 TBA1C_HUMAN	Tubulin alpha-1C chain OS=Homo sapiens GN=TUBA1C PE=1 SV=1	HUMAN	7
13	16.41	sp Q71U36 TBA1A_HUMAN	Tubulin alpha-1A chain OS=Homo sapiens GN=TUBA1A PE=1 SV=1	HUMAN	7
14	11.68	sp P14618 KP YM_HUMAN	Pyruvate kinase PKM OS=Homo sapiens GN=PKM PE=1 SV=4	HUMAN	7
15	5.72	sp Q9UM54 MYO6_HUMAN	Unconventional myosin-VI OS=Homo sapiens GN=MYO6 PE=1 SV=4	HUMAN	7
16	10.96	sp Q9Y608 LRRF2_HUMAN	Leucine-rich repeat flightless-interacting protein 2 OS=Homo sapiens GN=LRRFIP2 PE=1 SV=1	HUMAN	6
17	16.23	sp Q5VTE0 EF1A3_HUMAN	Putative elongation factor 1-alpha-like 3 OS=Homo sapiens GN=EEF1A1P5 PE=5 SV=1	HUMAN	6
18	16.23	sp P68104 EF1A1_HUMAN	Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1	HUMAN	6
19	8.73	sp P10809 CH60_HUMAN	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2	HUMAN	5
20	11.80	sp P07355 ANXA2_HUMAN	Annexin A2 OS=Homo sapiens GN=ANXA2 PE=1 SV=2	HUMAN	4
21	11.80	sp A6NMY6 AXA2L_HUMAN	Putative annexin A2-like protein OS=Homo sapiens GN=ANXA2P2 PE=5 SV=2	HUMAN	4
22	6.22	sp P08238 HS90B_HUMAN	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4	HUMAN	4
23	2.22	sp P41252 SYIC_HUMAN	Isoleucine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=IARS PE=1 SV=2	HUMAN	3
24	3.82	sp Q09666 AHNK_HUMAN	Neuroblast differentiation-associated protein AHNK OS=Homo sapiens GN=AHNK PE=1 SV=2	HUMAN	3
25	14.57	sp Q06830 PRDX1_HUMAN	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1	HUMAN	3
26	1.86	sp P01860 IGHG3_HUMAN	Ig gamma-3 chain C region OS=Homo sapiens GN=IGHG3 PE=1 SV=2	HUMAN	3
27	2.12	sp P01857 IGHG1_HUMAN	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1	HUMAN	3
28	2.15	sp P01859 IGHG2_HUMAN	Ig gamma-2 chain C region OS=Homo sapiens GN=IGHG2 PE=1 SV=2	HUMAN	3
29	2.14	sp P01861 IGHG4_HUMAN	Ig gamma-4 chain C region OS=Homo sapiens GN=IGHG4 PE=1 SV=1	HUMAN	3
30	3.86	sp P14625 ENPL_HUMAN	Endoplasmic OS=Homo sapiens GN=HSP90B1 PE=1 SV=1	HUMAN	3
31	15.05	sp P05109 S10A8_HUMAN	Protein S100-A8 OS=Homo sapiens GN=S100A8 PE=1 SV=1	HUMAN	3
32	24.56	sp P06702 S10A9_HUMAN	Protein S100-A9 OS=Homo sapiens GN=S100A9 PE=1 SV=1	HUMAN	2
33	19.53	sp P62987 RL40_HUMAN	Ubiquitin-60S ribosomal protein L40 OS=Homo sapiens GN=UBA52 PE=1 SV=2	HUMAN	2
34	16.03	sp P62979 RS27A_HUMAN	Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens GN=RPS27A PE=1 SV=2	HUMAN	2
35	32.85	sp P0CG48 UBC_HUMAN	Polyubiquitin-C OS=Homo sapiens GN=UBC PE=1 SV=3	HUMAN	2

36	32.75	sp P0CG47 UBB_HUMAN	Polyubiquitin-B OS=Homo sapiens GN=UBB PE=1 SV=1	HUMAN	2
37	1.66	sp Q9UPQ0 LIMC1_HUMAN	LIM and calponin homology domains-containing protein 1 OS=Homo sapiens GN=LIMC1 PE=1 SV=4	HUMAN	2
38	1.40	sp P31327 CPSM_HUMAN	Carbamoyl-phosphate synthase [ammonia], mitochondrial OS=Homo sapiens GN=CPS1 PE=1 SV=2	HUMAN	2
39	1.09	sp Q14152 EIF3A_HUMAN	Eukaryotic translation initiation factor 3 subunit A OS=Homo sapiens GN=EIF3A PE=1 SV=1	HUMAN	2
40	13.91	sp P60660 MYL6_HUMAN	Myosin light polypeptide 6 OS=Homo sapiens GN=MYL6 PE=1 SV=2	HUMAN	2
41	4.08	sp P11413 G6PD_HUMAN	Glucose-6-phosphate 1-dehydrogenase OS=Homo sapiens GN=G6PD PE=1 SV=4	HUMAN	2
42	2.20	sp P00352 AL1A1_HUMAN	Retinal dehydrogenase 1 OS=Homo sapiens GN=ALDH1A1 PE=1 SV=2	HUMAN	1
43	1.91	sp P08195 4F2_HUMAN	4F2 cell-surface antigen heavy chain OS=Homo sapiens GN=SLC3A2 PE=1 SV=3	HUMAN	1
44	0.60	sp P07814 SYEP_HUMAN	Bifunctional glutamate/proline--tRNA ligase OS=Homo sapiens GN=EPRS PE=1 SV=5	HUMAN	1
45	0.83	sp P00533 EGFR_HUMAN	Epidermal growth factor receptor OS=Homo sapiens GN=EGFR PE=1 SV=2	HUMAN	1
46	0.57	sp P42704 LRPPRC_HUMAN	Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens GN=LRPPRC PE=1 SV=3	HUMAN	1
47	1.05	sp Q9Y5B9 SP16H_HUMAN	FACT complex subunit SPT16 OS=Homo sapiens GN=SUPT16H PE=1 SV=1	HUMAN	1
48	0.93	sp P17301 ITA2_HUMAN	Integrin alpha-2 OS=Homo sapiens GN=ITGA2 PE=1 SV=1	HUMAN	1
49	3.74	sp B9A064 IGLL5_HUMAN	Immunoglobulin lambda-like polypeptide 5 OS=Homo sapiens GN=IGLL5 PE=2 SV=2	HUMAN	1
50	8.11	sp P61626 LYSC_HUMAN	Lysozyme C OS=Homo sapiens GN=LYZ PE=1 SV=1	HUMAN	1
51	1.07	sp P63010 AP2B1_HUMAN	AP-2 complex subunit beta OS=Homo sapiens GN=AP2B1 PE=1 SV=1	HUMAN	1
52	1.99	sp P39023 RL3_HUMAN	60S ribosomal protein L3 OS=Homo sapiens GN=RPL3 PE=1 SV=2	HUMAN	1
53	3.53	sp A6NCN2 KR87P_HUMAN	Putative keratin-87 protein OS=Homo sapiens GN=KRT87P PE=5 SV=4	HUMAN	1
54	0.45	sp Q8TF72 SHRM3_HUMAN	Protein Shroom3 OS=Homo sapiens GN=SHROOM3 PE=1 SV=2	HUMAN	1
55	1.76	sp Q14677 EPN4_HUMAN	Claathrin interactor 1 OS=Homo sapiens GN=CLINT1 PE=1 SV=1	HUMAN	1
56	2.46	sp P02768 ALBU_HUMAN	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	HUMAN	1
57	2.58	sp P31997 CEAM8_HUMAN	Carcinoembryonic antigen-related cell adhesion molecule 8 OS=Homo sapiens GN=CEACAM8 PE=1 SV=2	HUMAN	1
58	1.71	sp P13688 CEAM1_HUMAN	Carcinoembryonic antigen-related cell adhesion molecule 1 OS=Homo sapiens GN=CEACAM1 PE=1 SV=2	HUMAN	1
59	1.28	sp P06731 CEAM5_HUMAN	Carcinoembryonic antigen-related cell adhesion molecule 5 OS=Homo sapiens GN=CEACAM5 PE=1 SV=3	HUMAN	1
60	3.24	sp P61981 1433G_HUMAN	14-3-3 protein gamma OS=Homo sapiens GN=YWHAG PE=1 SV=2	HUMAN	1
61	3.25	sp Q04917 1433F_HUMAN	14-3-3 protein eta OS=Homo sapiens GN=YWHAH PE=1 SV=4	HUMAN	1
62	3.27	sp P63104 1433Z_HUMAN	14-3-3 protein zeta/delta OS=Homo sapiens GN=YWHAZ PE=1 SV=1	HUMAN	1
63	3.14	sp P62258 1433E_HUMAN	14-3-3 protein epsilon OS=Homo sapiens GN=YWHA E PE=1 SV=1	HUMAN	1
64	3.23	sp P31947 1433S_HUMAN	14-3-3 protein sigma OS=Homo sapiens GN=SFN PE=1 SV=1	HUMAN	1
65	3.25	sp P31946 1433B_HUMAN	14-3-3 protein beta/alpha OS=Homo sapiens GN=YWHAB PE=1 SV=3	HUMAN	1
66	3.27	sp P27348 1433T_HUMAN	14-3-3 protein theta OS=Homo sapiens GN=YWHAQ PE=1 SV=1	HUMAN	1
67	2.48	sp P42330 AK1C3_HUMAN	Aldo-keto reductase family 1 member C3 OS=Homo sapiens GN=AKR1C3 PE=1 SV=4	HUMAN	1
68	2.48	sp Q04828 AK1C1_HUMAN	Aldo-keto reductase family 1 member C1 OS=Homo sapiens GN=AKR1C1 PE=1 SV=1	HUMAN	1
69	2.48	sp P52895 AK1C2_HUMAN	Aldo-keto reductase family 1 member C2 OS=Homo sapiens GN=AKR1C2 PE=1 SV=3	HUMAN	1
70	2.48	sp P17516 AK1C4_HUMAN	Aldo-keto reductase family 1 member C4 OS=Homo sapiens GN=AKR1C4 PE=1 SV=3	HUMAN	1
71	0.63	sp P54289 CA2D1_HUMAN	Voltage-dependent calcium channel subunit alpha-2/delta-1 OS=Homo sapiens GN=CACNA2D1 PE=1 SV=3	HUMAN	1

^a %Cov (95): percentage of cover 95% peptides

Table S3 Specific primary antibodies used in this study

	Antigen	Supplier	Cat #	Clone	Dilution
Western blotting	TUBB4A	Abcam	ab179509	EPR16776	3000
	MYH9	Proteintech Rosemont	14844-1-AP		1000
	E-cadherin	Abcam	ab76055	M168	3000
	N-cadherin	Abcam,	ab18203		2000
	Vimentin	Abcam	ab92547	EPR3776	2000
	c-MYC	Abcam,	ab32072	Y69	3000
	CyclinD1	Abcam	ab134175	EPR2241	2000
	p44/42 MAPK (Erk1/2)	Cell Signaling Technology	4695	137F5	3000
	p-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	Cell Signaling Technology	4376	20G11	2000
	p38 MAPK	Cell Signaling Technology	9212		3000
	p-p38 MAPK (Thr180/Tyr182)	Cell Signaling Technology	9211		2000
	TRAF6	Cell Signaling Technology	8028	D21G3	1000
	IRAK1	Abcam	ab238		2000
	IKK α	Cell Signaling Technology	2682		2000
	p-IKK α / β (Ser176/180)	Cell Signaling Technology	2697	16A6	1000
	IKK γ	Cell Signaling Technology	2685		2000
	p-IKK γ (Ser376)	Cell Signaling Technology	2689		1000
	NF- κ B p65	Santa Cruz Biotechnology	sc-514451	A-12	2000
	NF- κ B p-p65	Abcam	ab86299	S536	1000
	ATM	Santa Cruz Biotechnology	sc-377293	G12	1000
	p-ATM (Ser1981)	Abcam	ab81292	EP1890Y	1000
	BCL-2	Santa Cruz Biotechnology	sc-7382	C-2	1000
	BCL-XL	Cell Signaling Technology	2764P	54H6	1000
	Caspase3	Santa Cruz Biotechnology	sc-56053	31A1067	500
	MEK1/2	Proteintech Rosemont	11049-1-AP		1000
	p-MEK1/2	Cell Signaling Technology	9121	Ser217/221	1000
	TAK1	Proteintech Rosemont	12330-2-AP		1000
	p-TAK1	Proteintech Rosemont	28958-1-AP		1000
	GSK3 β	Cell Signaling Technology	9315	27C10	2000
	p-GSK3 β	Cell Signaling Technology	5558S	S9	2000
	β -catenin	BD Biotechnology	610154		2000
	Lamin B	Santa Cruz Biotechnology	sc-374015	B-10	250
	GAPDH	Cell Signaling Technology	5174	D16H11	5000
IF	TUBB4A	Abcam	ab11315	ONS.1A6	200

	MYH9	Proteintech Rosemont	60233-1-Ig		200
	γ H2AX	Cell Signaling Technology	9718	20E3 Rb	200
	53BP1	Cell Signaling Technology	4937		200
	alpha-tubulin	Sigma-Aldrich	T6199	DM1A	500
	Lamin A/C	Santa Cruz Biotechnology	sc-376248	E-1	
	Goat-anti mouse IgG H&L	Abcam	ab150117	Alex 488	500
	Goat-anti mouse IgG H&L	Abcam	ab175473	Alex 568	500
	Alexa Fluor® 568 phalloidin (for F-actin)	Thermo Fisher Scientific	A12380		500
IHC	TUBB4A	Abcam	ab179509	EPR16776	500
	Androgen Receptor	Santa Cruz Biotechnology	sc-7305	441	200
	Ki67	Abcam	ab15580		500
	γ H2AX	Cell Signaling Technology	9718	20E3 Rb	200
	TUNEL	ABP Biosciences	A050		
	E-cadherin	Abcam	ab76055	M168	500
	Vimentin	Abcam	ab92547	EPR3776	200
	CK5	Abcam	ab52635	EP1601Y	500
	CyclinD1	Abcam	ab134175	EPR2241	200
	c-MYC	Abcam	ab32072	Y69	200
	NF- κ B p-p65	Abcam	ab86299	S536	100
	NF- κ B p-p66	Sigma-Aldrich	SAB5700363		100
	p-IKK α / β (Ser176/180)	Cell Signaling Technology	2697		100

Table S4 The sequences of primer, sgRNA, siRNA, and ORF used in this study

Primer Name	Sequence
Gene KO testing PCR primers	
Mouse Tubb4a A primer-F	ATCAGTGATGAGCACGGCAT
Mouse Tubb4a A primer-R	TACAAAGTTGTCTGGCCGAA
Mouse Tubb4a B primer-F	AGTGGTGGGGTACAGGGATT
Mouse Tubb4a B primer-R	GAAGATGCATTTCAGTGTGGC
CRISP/Cas9 sgRNAs	
Human TUBB4A gRNA1	CACCGTCCCCATGGTATGTGCCTG
Human TUBB4A gRNA2	AAACCAGGCACATACCATGGGGAC
RT-PCR primers	
Mouse c-Myc-F	CTAGTGCTGCATGAGGAGACA
Mouse c-Myc-R	TGTGCGGAGGTTTGTCTGT
Mouse Ccnd1-F	TGCGTGCAGAAGGAGATTGT
Mouse Ccnd1-R	CTTCTTCAAGGGCTCCAGGG
Mouse Vim-F	CTCCAGAGAGAGGAAGCCGA
Mouse Vim-R	CGTTCAAGGTCAAGACGTGC
Mouse Hprt- realtime-F	CAGGCCAGACTTTGTTGGAT
Mouse Hprt- realtime-R	GCGTCTCATCTTAGGCTTTGT
Human KLK3 (PSA)-F	CGTGACGTGGATTGGTGCT
Human KLK3 (PSA)-R	ACCCAGCAAGATCACGCTTT
Human GAPDH-F	CCCCTTCATTGACCTCAACTACAT
Human GAPDH-R	CGCTCCTGGAAGATGGTGA
CRISP/Cas9 off-target PCR primers	
TIE-off-target-F	CCATGGGACCCTCAGCTTTG
TIE-off-target-R	CCAGGTGGGCTATGTGACAG
CAB39L-off-target-F	CTAGAGAACTGTGAACATGTGGC
CAB39L-off-target-R	ACACATGAATCATTCTCCTTCCA
TRIM14-off-target-F	CGTGACAGCCTCCAGAATGTCATG
TRIM14-off-target-R	GTGTGATCTCGGCTCACTGCAAC
CHPT1-off-target-F	ATAAGCTGGGTGTGGTGGCTCTC
CHPT1-off-target-R	CACACACGTTTTAAACTGGCTA
RAP1GAP2-off-target-F	GGCCGGAGTCTGTCCCGGCTCTG
RAP1GAP2-off-target-R	CACGCCACTGCACTCCAGCCTG
Mouse genotyping primers	
Tubb4a-21673	ATGGGGTCCTAGGGAATGAG
Tubb4a-23806	AGTTAGTGGATGCCGTCCTG
Tubb4a-23808	TCTCATCAGTGTCTCCACCA
Tubb4a-oIMR7202	CGGTCGCTACCATTACCAGT
Pten-oIMR9554	CAAGCACTCTGCGAACTGAG
Pten-oIMR9555	AAGTTTTTGAAGGCAAGATGC
TRAMP-10363	TAC AAC TGC CAA CTG GGA TG
TRAMP-10364	CAG GCA CTC CTT TCA AGA CC
TRAMP-21238	CTG TCC CTG TAT GCC TCT GG
TRAMP-21239	AGA TGG AGA AAG GAC TAG GCT ACA
Nkx3-1CreWT-F	CTCCGCTACCCTAAGCATCC
Nkx3-1CreWT-R	GCACTGTTCATATTACTTGGACC
Nkx3-1CreMut-F	CAGATGGCGCGGCAACACC
Nkx3-1CreMut-R	GCGCGGTCTGGCAGTAAAAAC

siRNA sequences

Human TUBB4A siRNA-1-SEQ1
Human TUBB4A siRNA-1-SEQ2
Human TUBB4A siRNA-2-SEQ1
Human TUBB4A siRNA-2-SEQ2
Human MYH9 siRNA13.1-SEQ1
Human MYH9 siRNA13.1-SEQ2
Human MYH9 siRNA13.2-SEQ1
Human MYH9 siRNA13.2-SEQ2

GCAACAUGAAUGACCUUGUAUCUGA
UCAGAUACCAGGUCAUUCAUGUUGCUC
CAACAUGAAUGACCUUGUAUCUGAG
CUCAGAUACCAGGUCAUUCAUGUUGCU
AUGUUUUCACUGAGCAUCAACAATA
UAUUGUGAUGCUCAGUGGAAAACAUCU
GACCGAGAAGAUCAAUCCAUCUUGT
ACAAGAUGGAUUGAUCUUCUCGGUCUU

TUBB4A open reading frame (ORF) sequence

CGGCCAGTGCGGCAACCAGATCGGGGCCAAGTTTTGGG
AGGTTATCAGTGACGAACATGGCATCGACCCACAGGCA
CATACCATGGGGACAGTGACCTGCAACTGGAGAGGATCA
ACGTGTACTACAACGAGGCCACAGGAGGAAATTATGTCC
CCAGAGCGGTGCTGGTGGACCTGGAACCCGGCACCATG
GACTCTGTCCGTTCTGGCCCTTCGGTCAGATCTTTCCG
CCGGACAACCTTCGTGTTTGGCCAATCCGGAGCCGGCAAC
AACTGGGCAAAGGGGCACTACACGGAGGGCGCAGAGCT
GGTGGACGCTGTCTGGACGTAGTCCGGAAGGAGGCCG
AGAGCTGCGACTGCCTTCAGGGCTTCCAGCTGACCCACT
CGCTGGGGGGTGGCACGGGGTCCGGAATGGGCACGCT
GCTCATCAGTAAGATCCGCGAGGAGTTCCCAGACCGCAT
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CAAGCTGACCACCCACCTACGGGGACCTCAACCACCT
GGTGTCCGGCCACCATGAGCGGGGTCAACCACCTGCCTGC
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GCGCCACGGCCGCTACCTGACCGTGGCCGCGTGTTC
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CTGAGCGTGCAGAGCAAGAACAGCAGCTACTTCGTGGAG
TGGATCCCCAACACGTGAAGACGGCCGTGTGCGACATC
CCGCCCCGCGGCCTGAAGATGGCCGCGACCTTCATCGG
CAACAGCACGGCCATCCAGGAGCTGTTCAAGCGCATCTC
CGAGCAGTTCACGGCCATGTTCCGGCGCAAGGCCCTTCTT
GCACTGGTACACGGGCGAGGGCATGGACGAGATGGAGT
TCACCGAGGCCGAGAGCAACATGAATGACCTGGTATCTG
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Fig. 2A, 2E, 2I

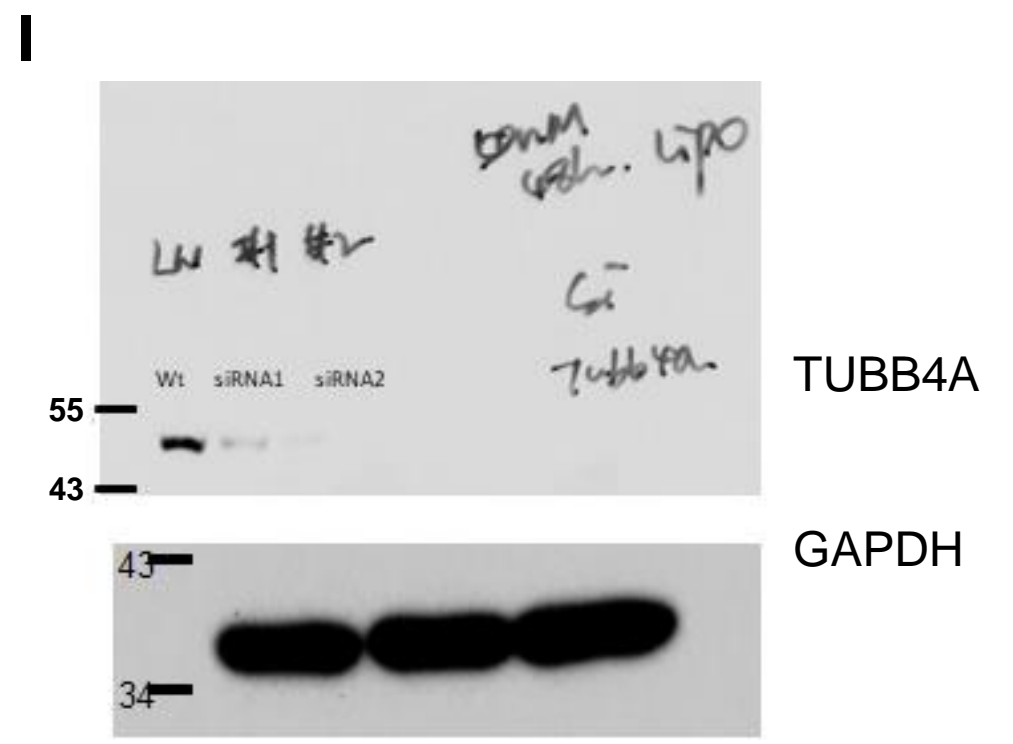
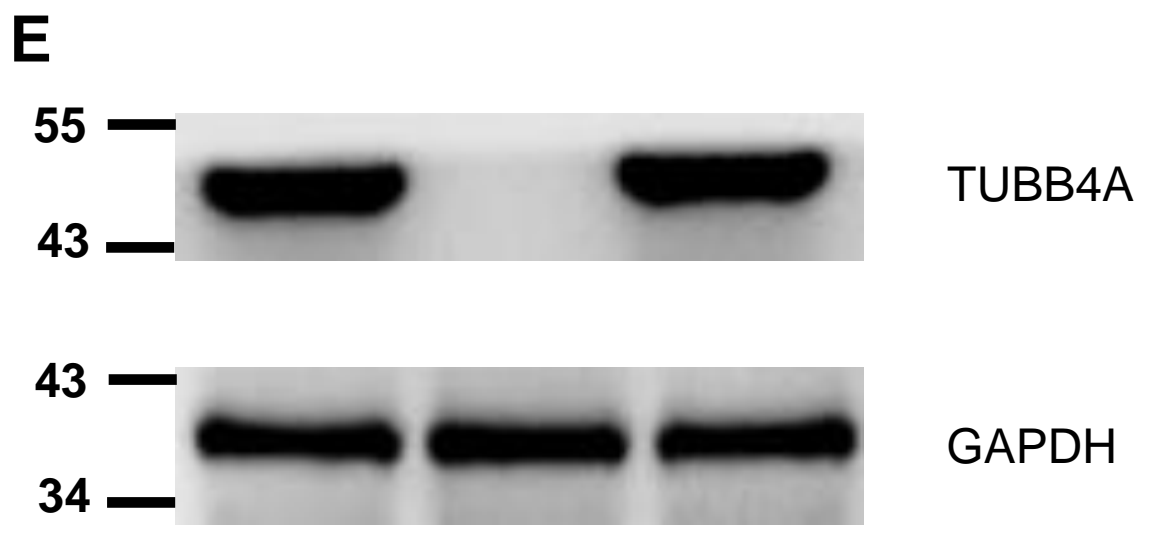
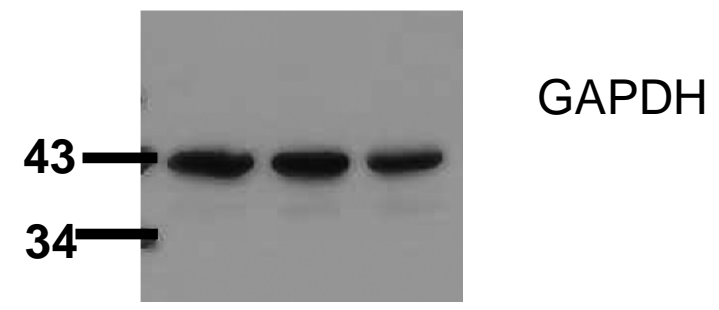


Fig. 4C

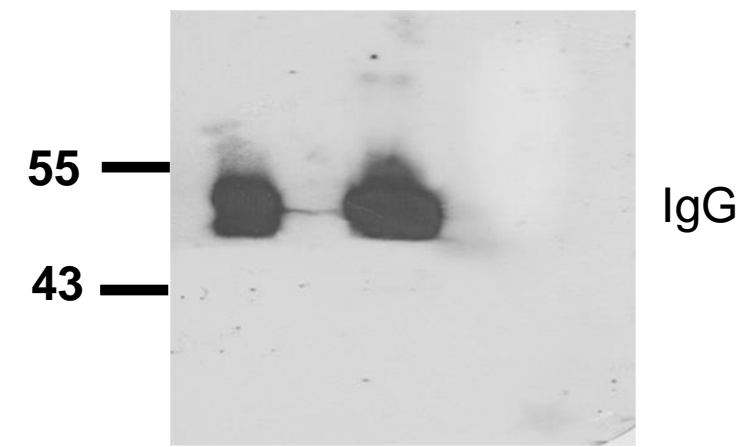
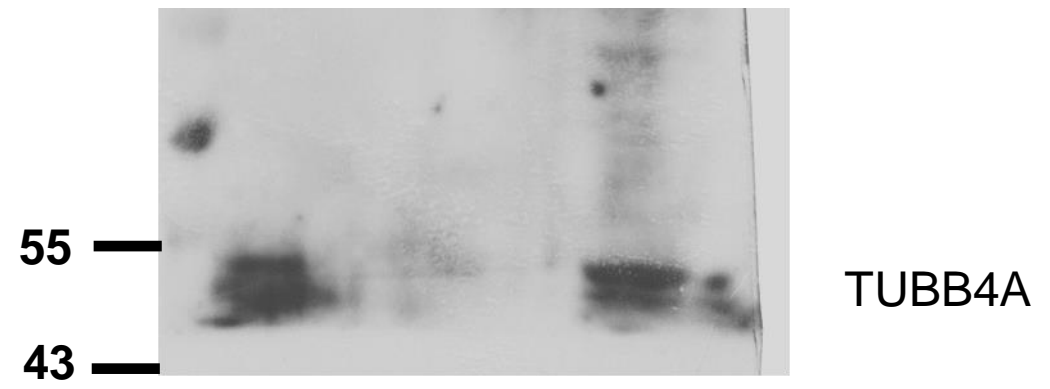
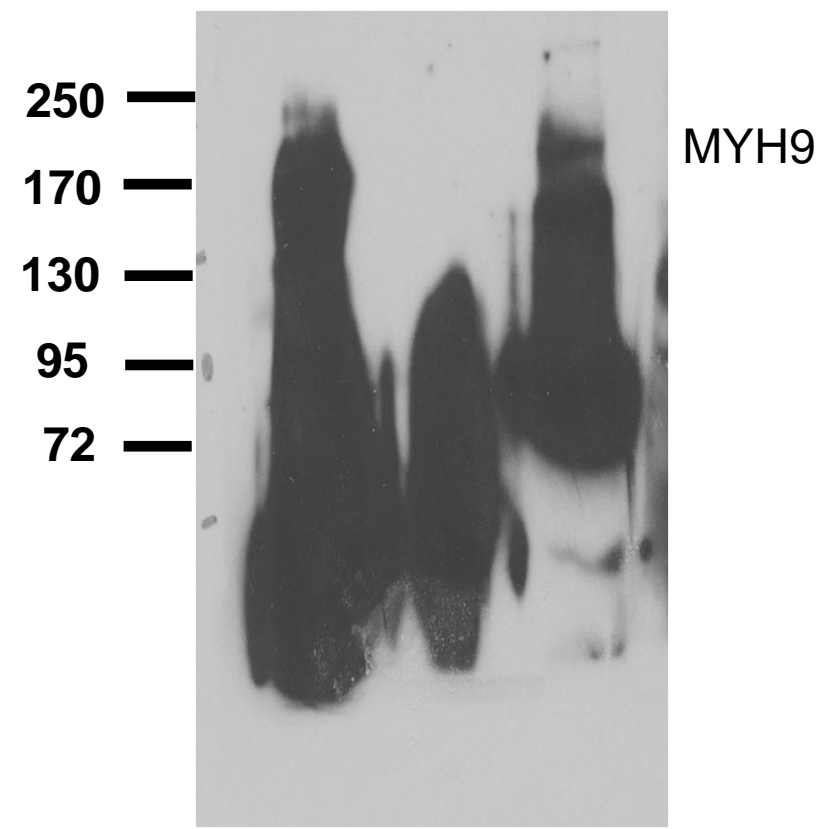
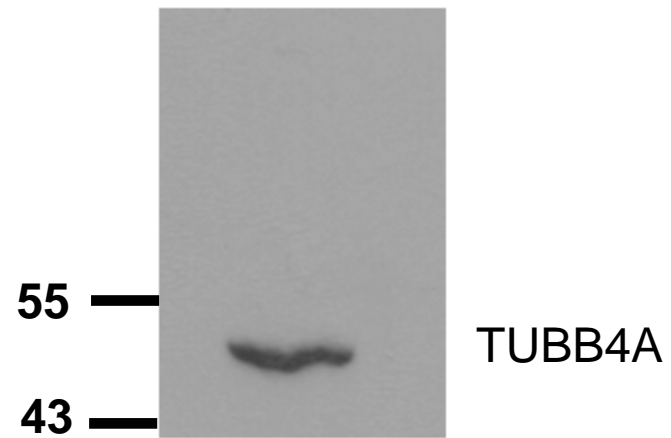
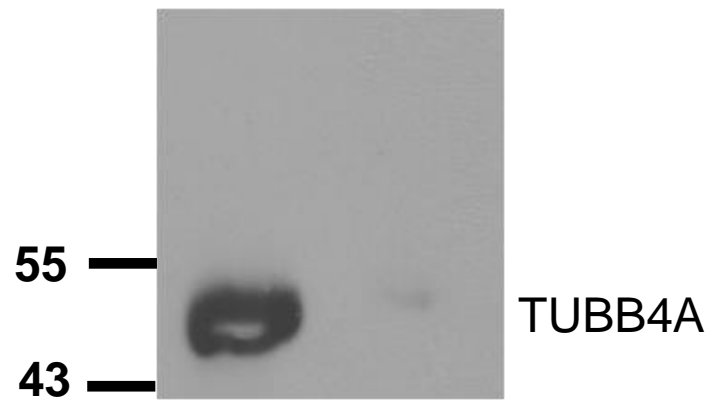
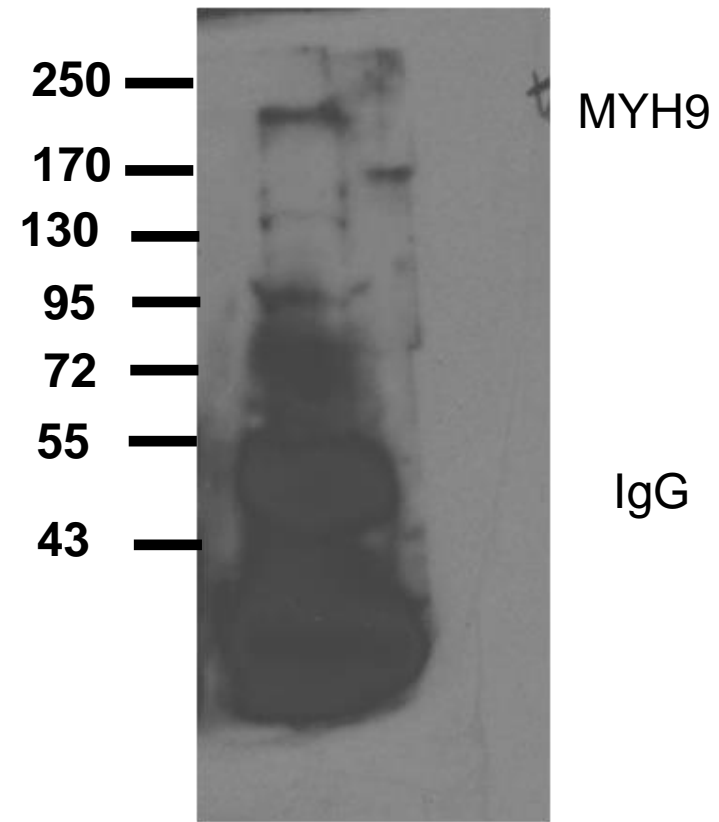
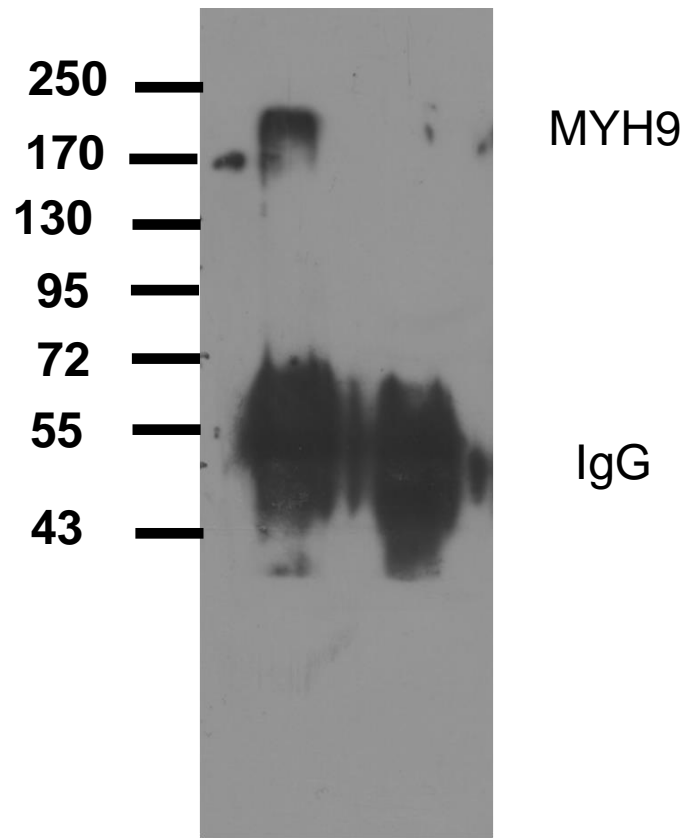


Fig. 4D



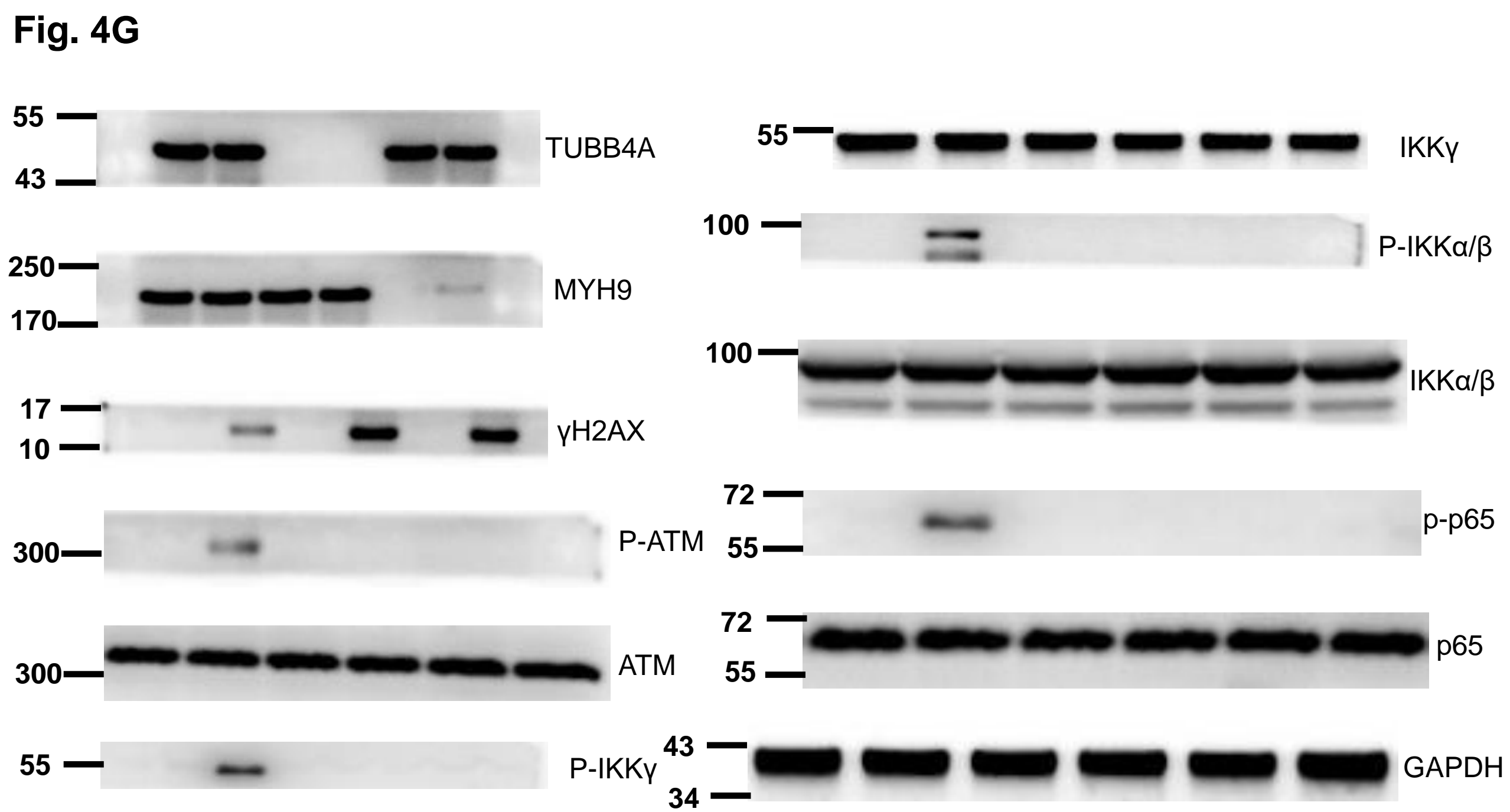


Fig. 4H

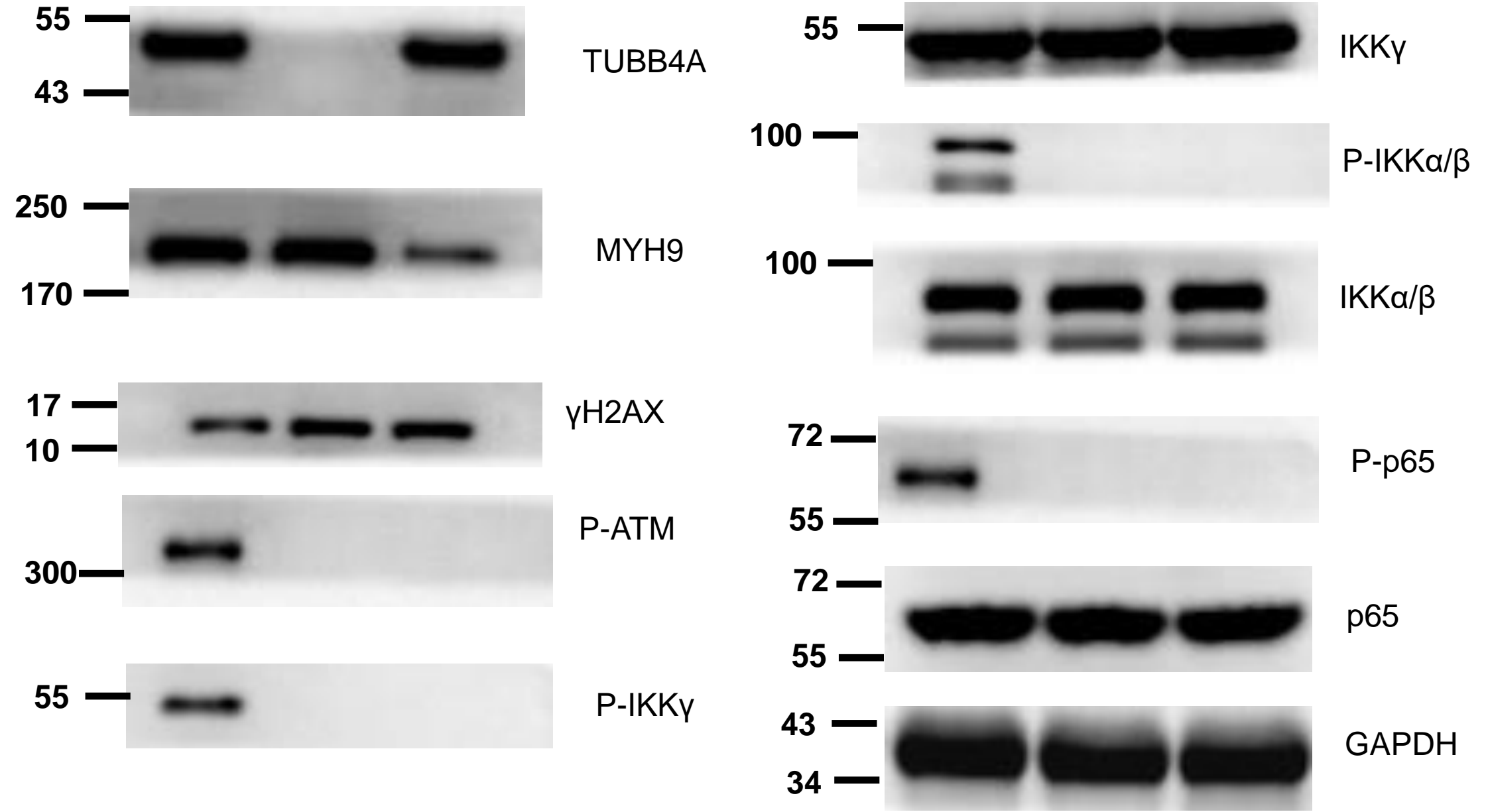


Fig. 4I

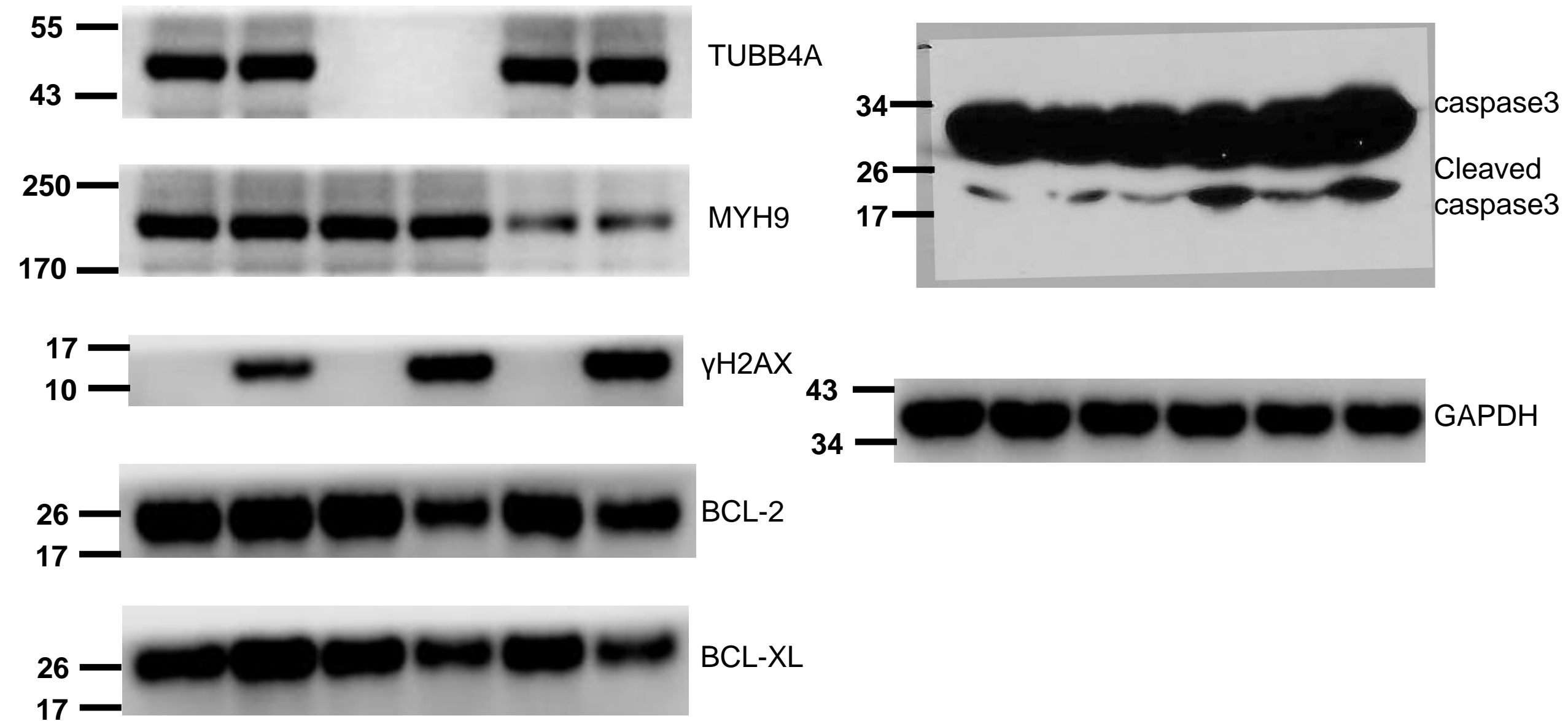


Fig. 4J

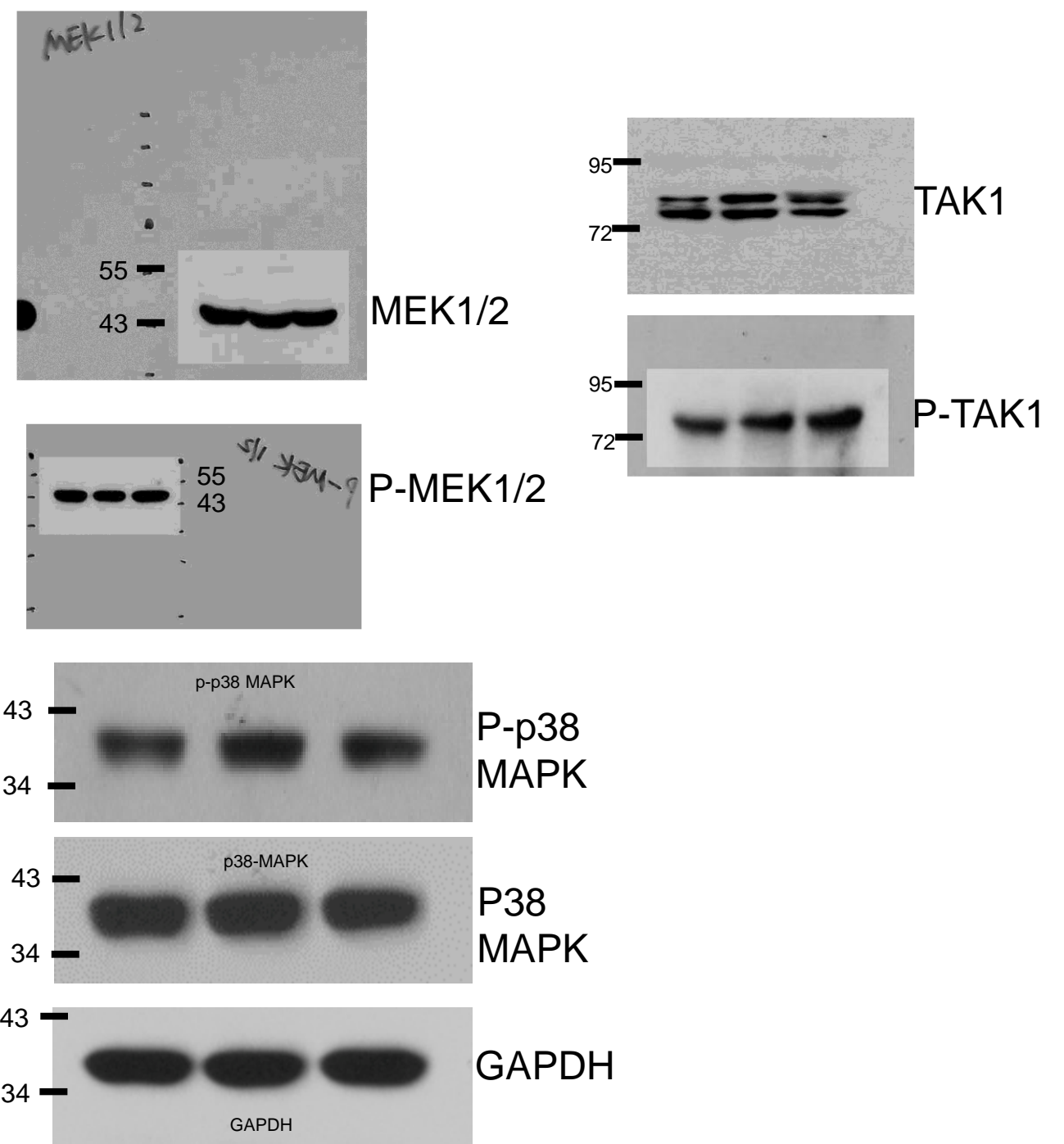
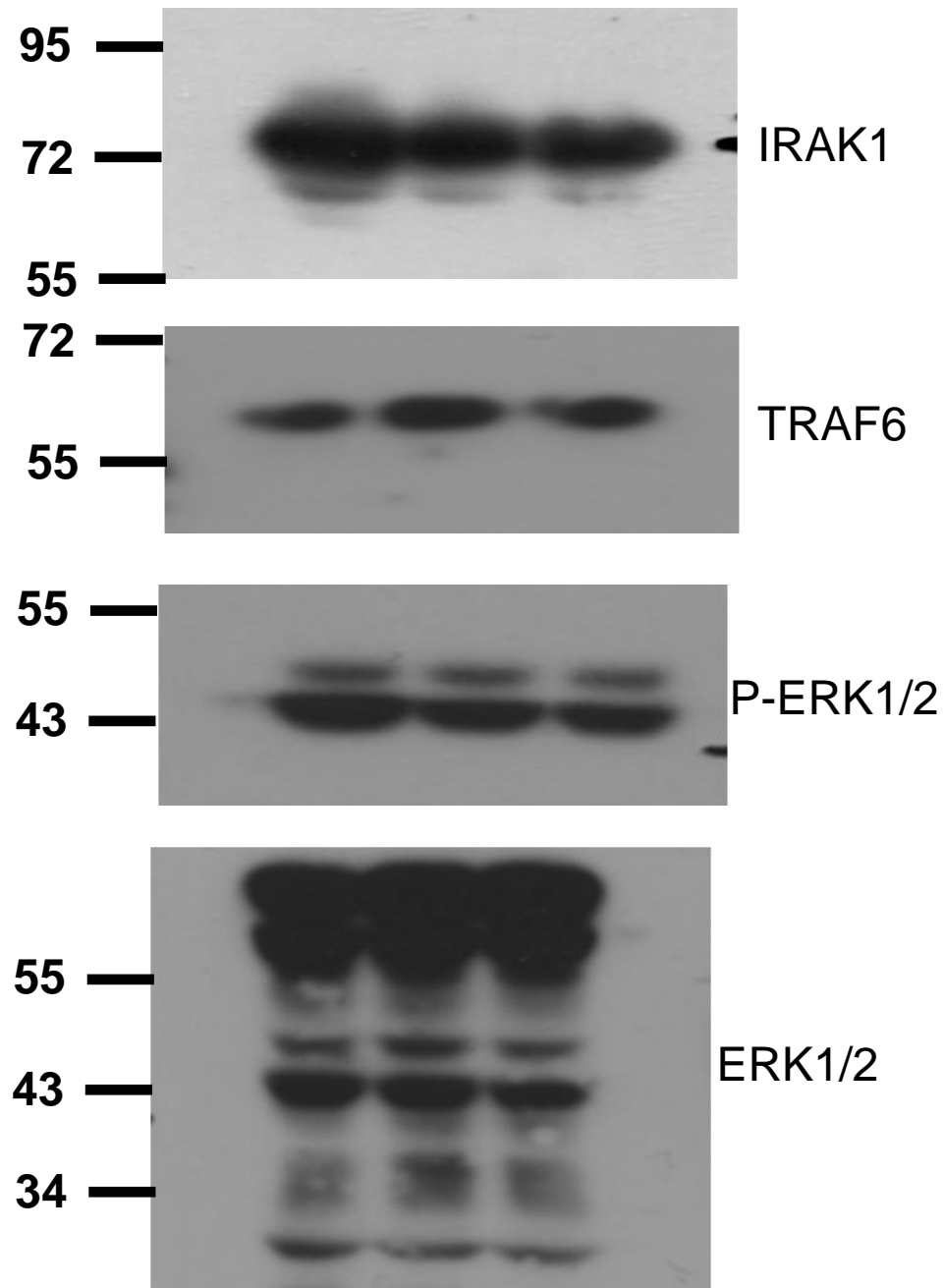


Fig. 5A

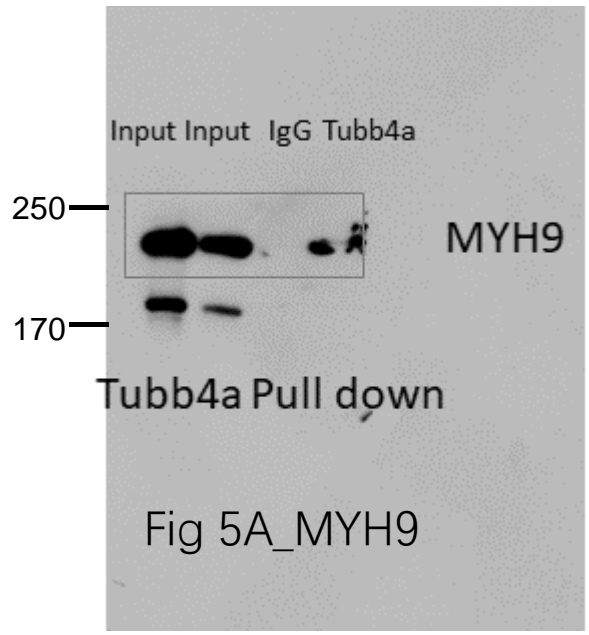
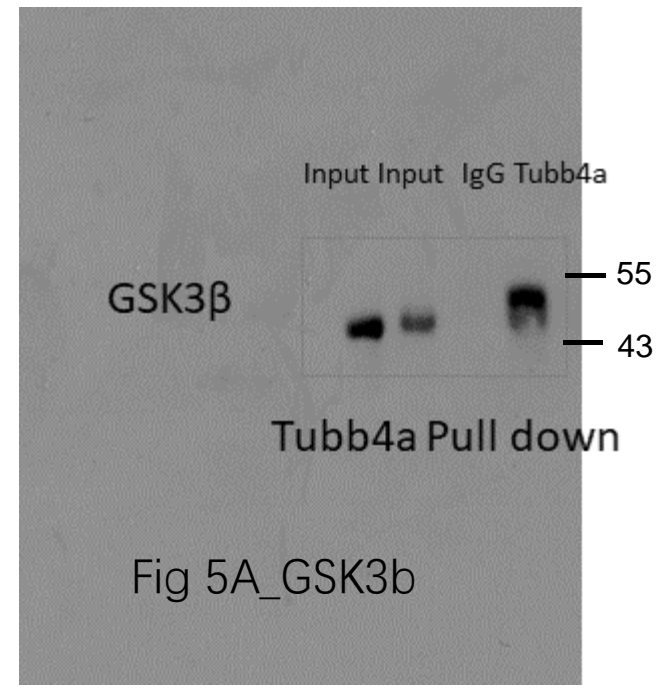
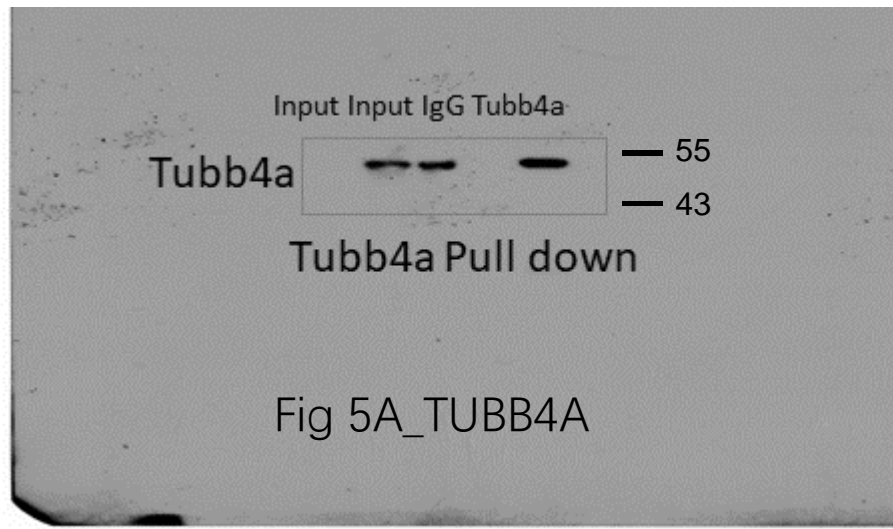


Fig. 5B

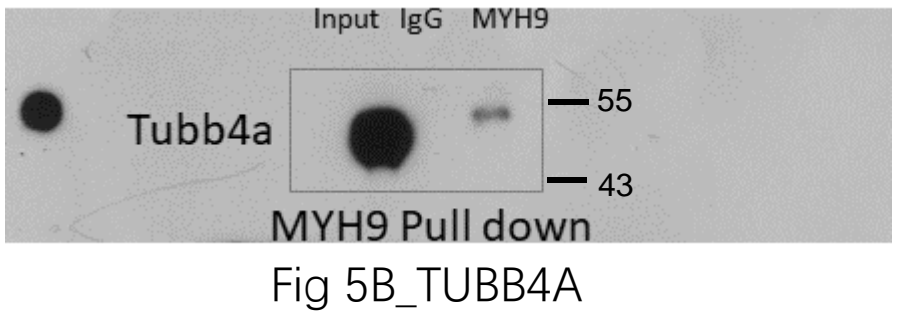
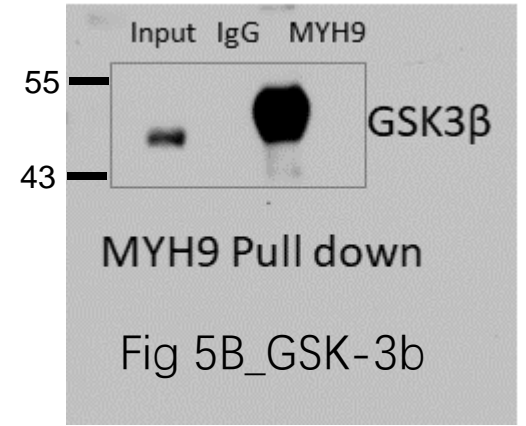
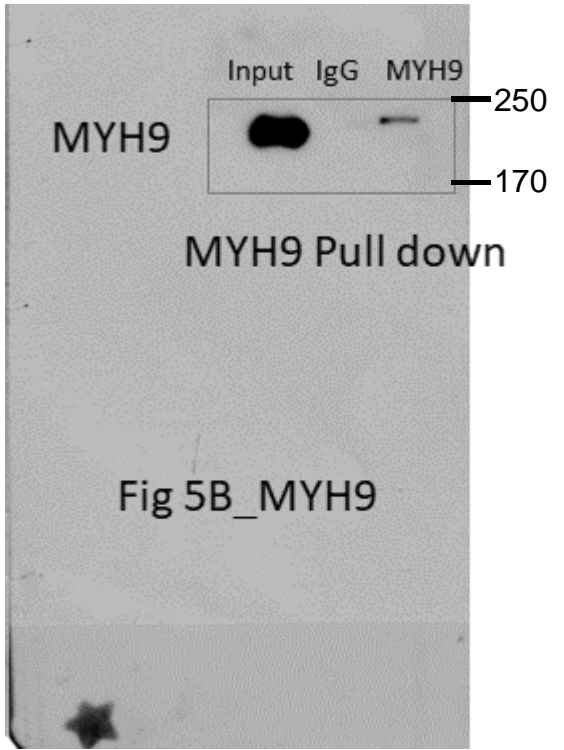
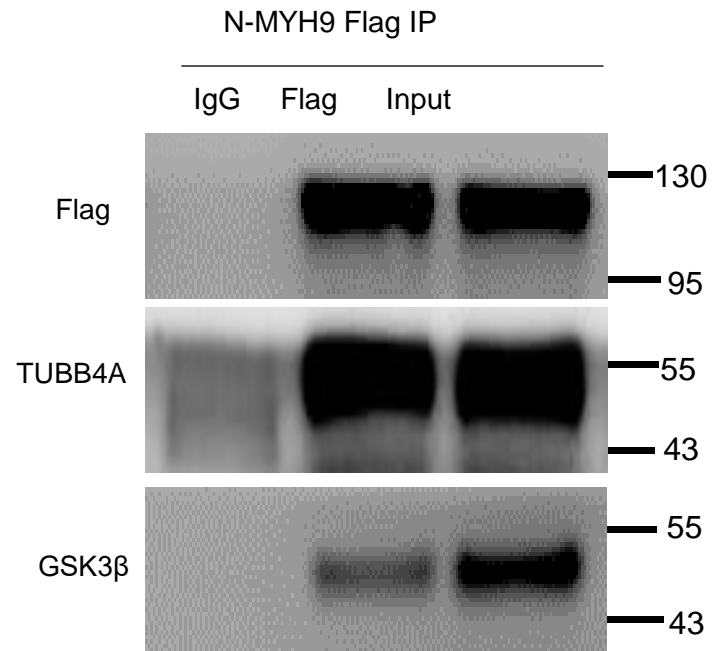
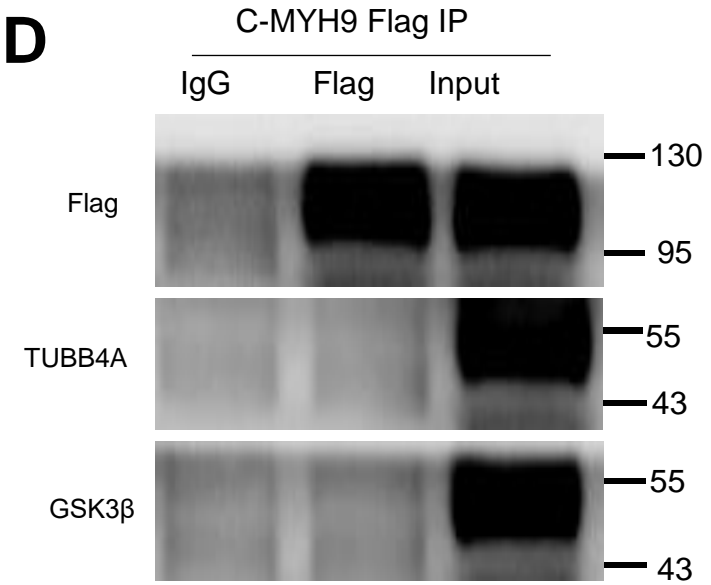


Fig. 5C-D

C



D



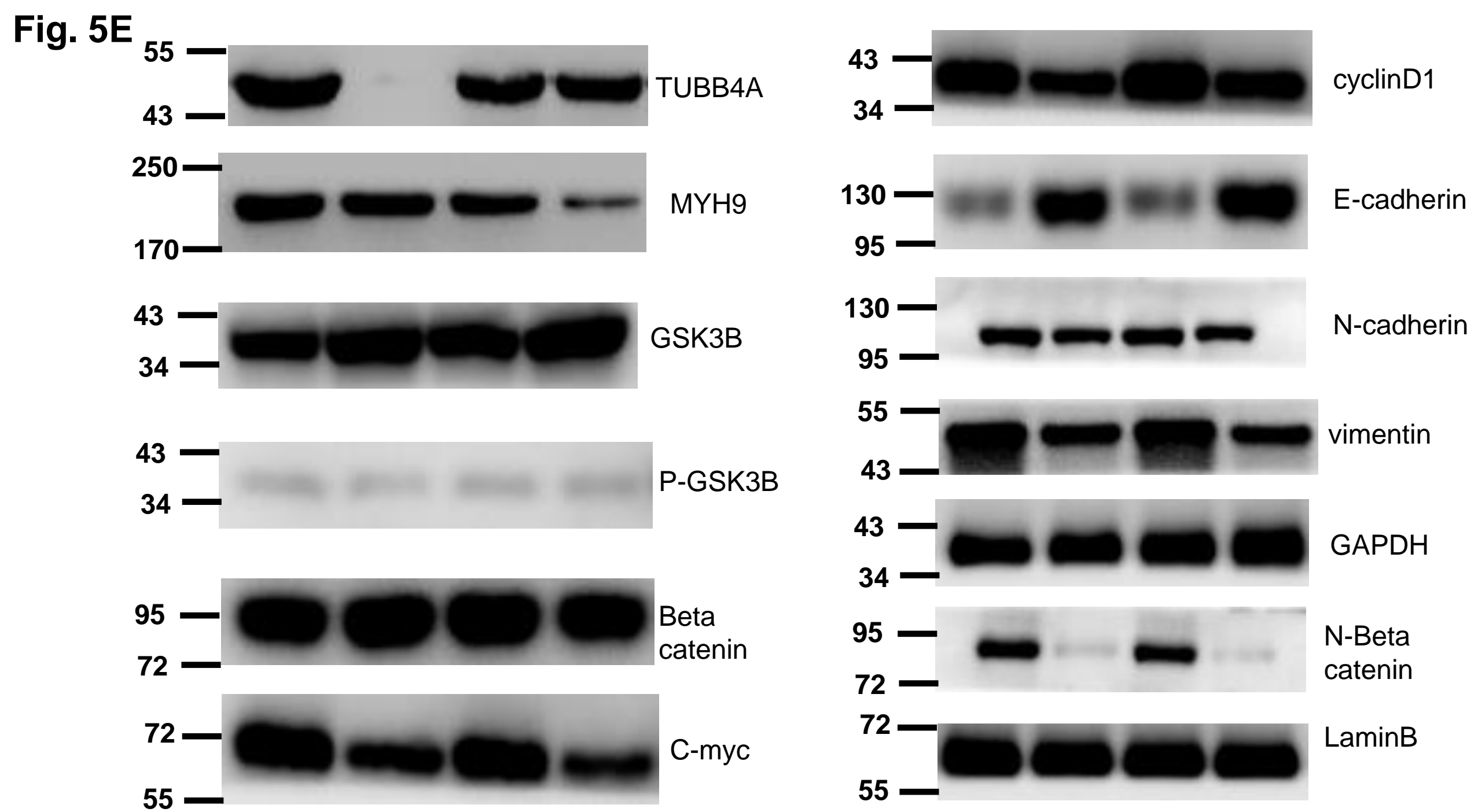


Fig. 5F

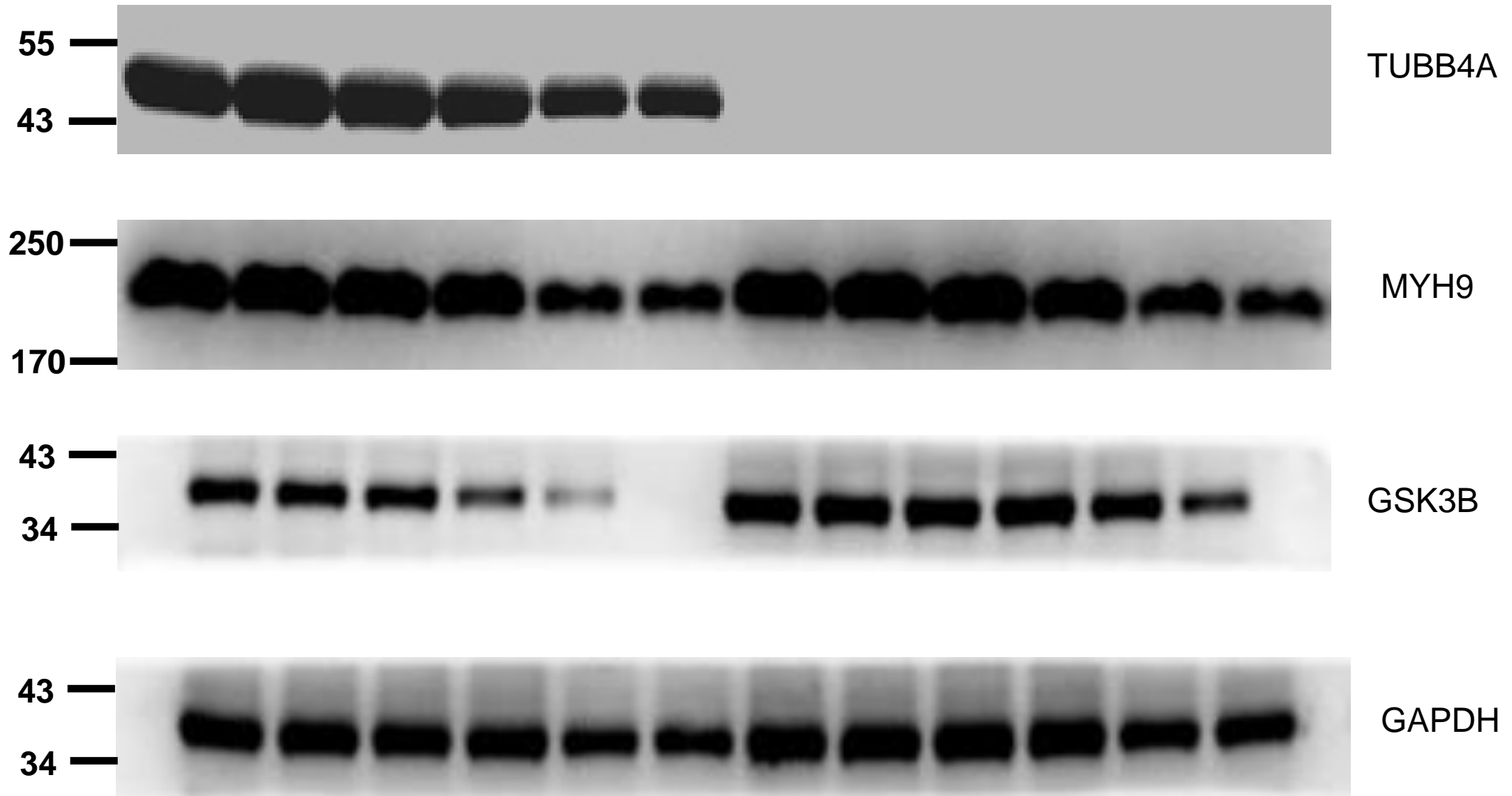


Fig. 5G

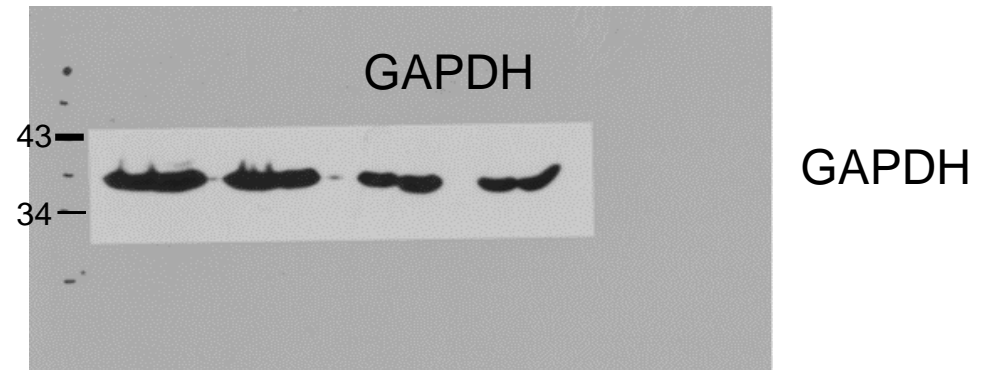
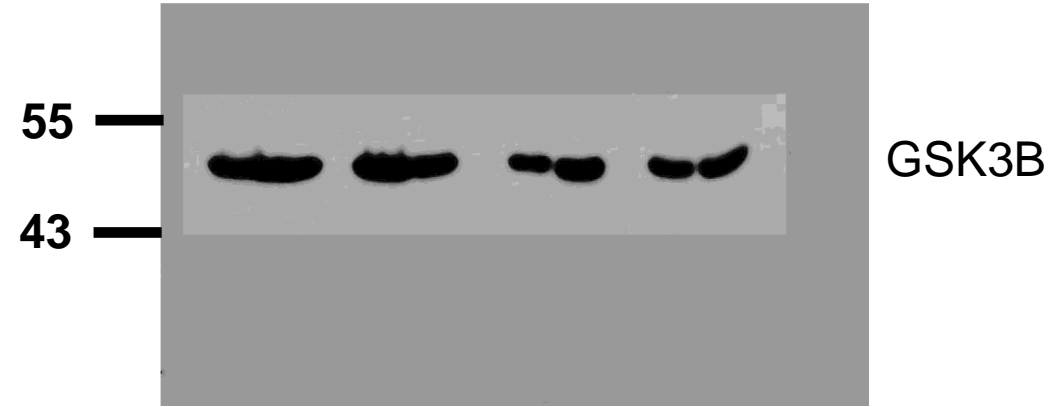
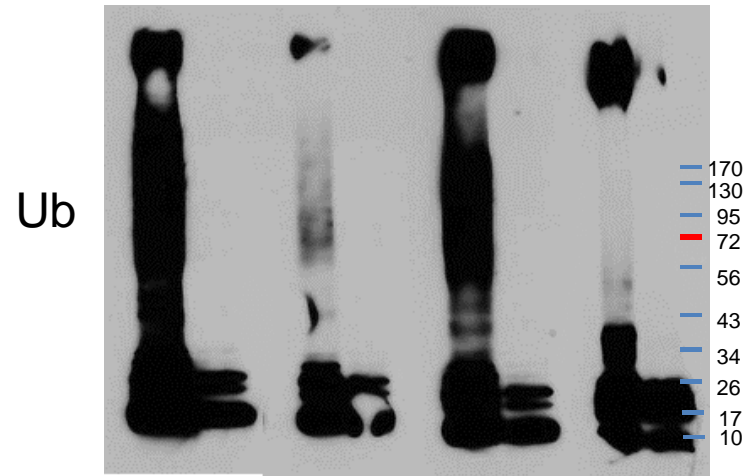


Fig. S2A, 2E, 4A, 8G, 8I

Fig S2A

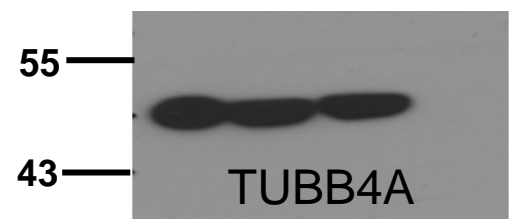


Fig S2E

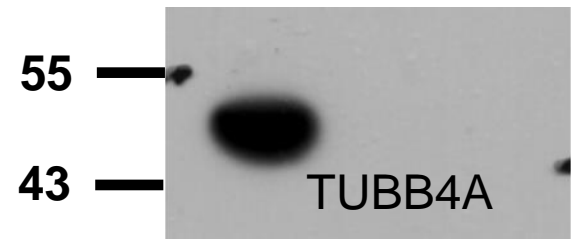


Fig S8G

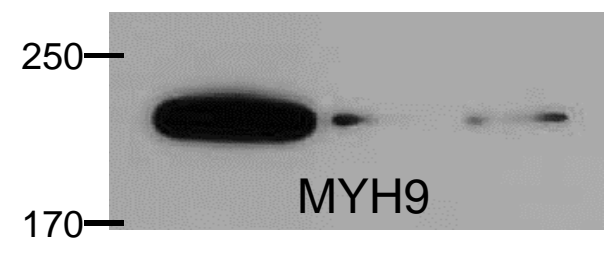


Fig S8I

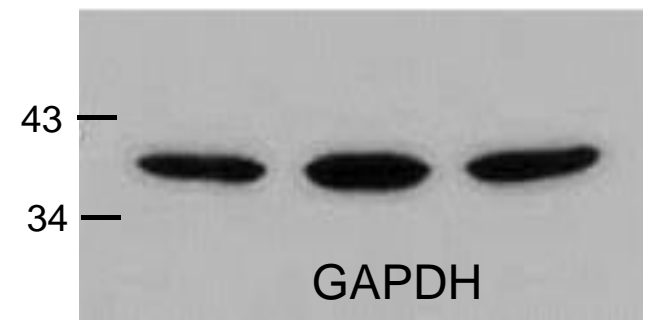
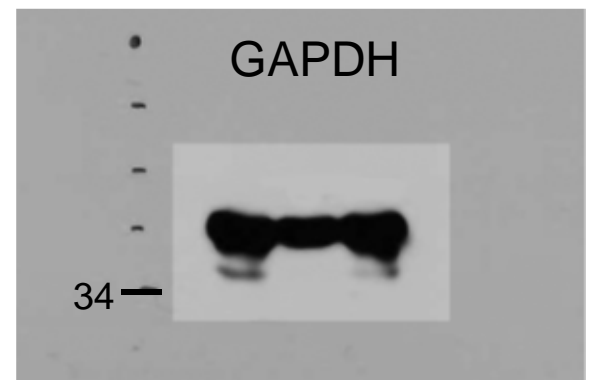
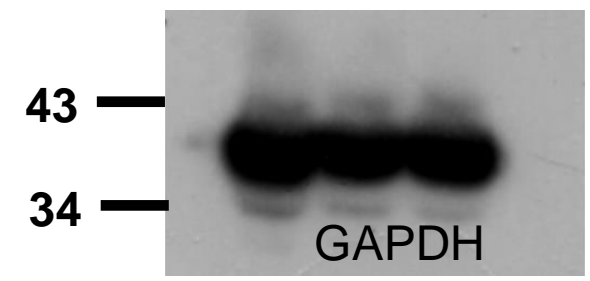
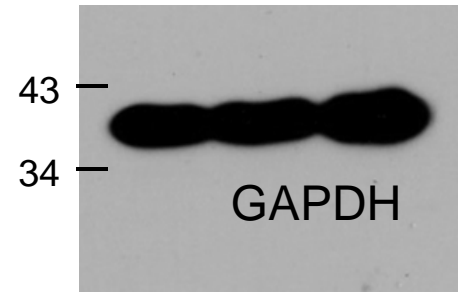
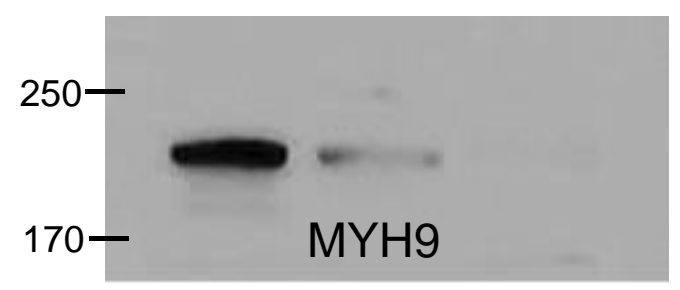


Fig S4A



Fig. S2G

