Interplay between acetylation and ubiquitination controls PSAT1 protein stability in lung adenocarcinoma

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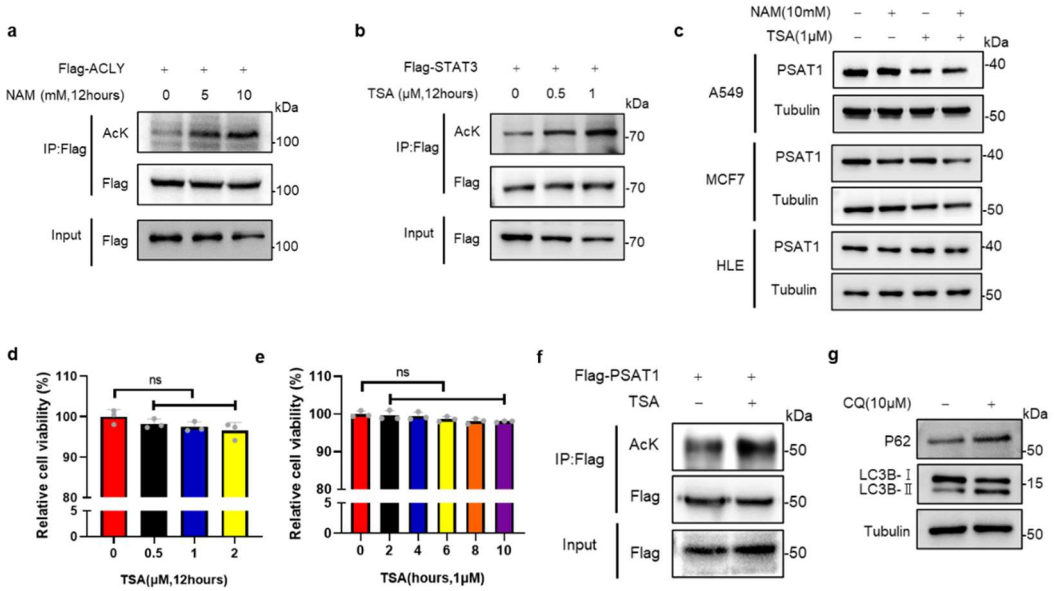
2. Supplementary Tables

Supplementary Table 1: All primer sequence information mentioned in this article

Supplementary Fig. 1

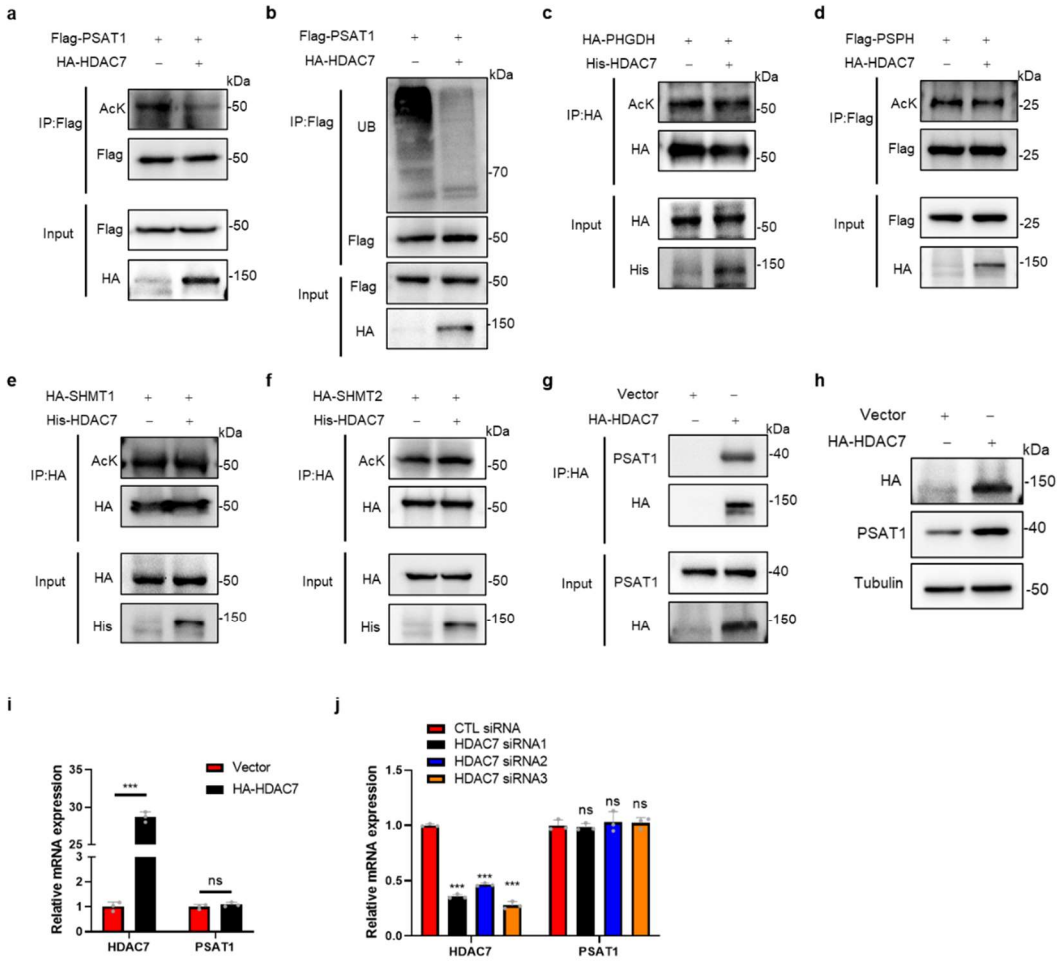


Supplementary Fig. 1: The treatment efficiencies of the inhibitors

(a) H1299 cells was transfected with Flag-ACLY plasmid. Co-IP was used to detect the acetylation level of ACLY after NAM treatment at different concentrations. (b) H1299 cells was transfected with Flag-STAT3 plasmid. Co-IP was used to detect the acetylation level of STAT3 after TSA treatment at different concentrations. (c) Western blot was used to detect PSAT1 protein levels after NAM (10mM) and TSA (1μM) treatment for 12 h in A549, MCF7 or HLE cells. (d, e) The cell viability was determined using CCK8 assay after TSA treatment at different time points or concentrations. Data represented as the average of three independent experiments (mean ± SD), ns: $P > 0.05$. (f) A549 cells were transfected with Flag-PSAT1 plasmid, and TSA (1μM) was added 12 h before sample collection. Co-IP was used to detect the acetylation level of PSAT1. (g) H1299 cells were experimented with CQ and the expressions of autophagic

marker proteins were detected using indicated antibodies by western blot.

Supplementary Fig. 2

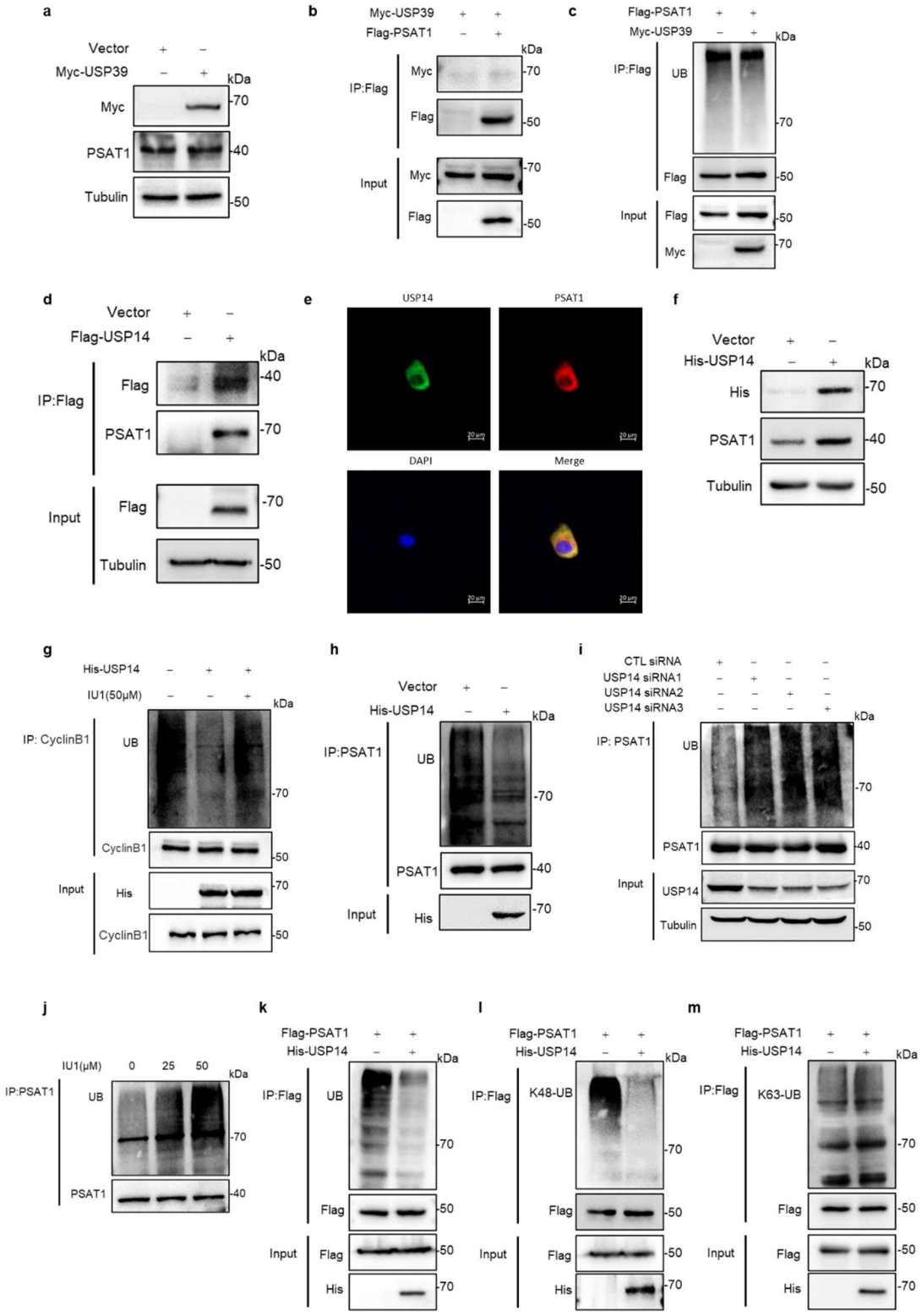


Supplementary Fig. 2: HDAC7 regulates PSAT1 acetylation, ubiquitination and protein levels in A549 cells

(a, b) In A549 cells, Flag-PSAT1 and HA-HDAC7 plasmids were co-transfected. The acetylation and ubiquitination of PSAT1 was detected. (c-f) HDAC7 and HA-PHGDH, Flag-PSPH, HA-SHMT1 or HA-SHMT2 plasmids were co-transfected. The acetylation of every protein was detected. (g) In A549 cells, endogenous interaction between PSAT1 and HDAC7 was tested using anti-HA antibodies for Co-IP. The immunoprecipitants were blotted with anti-PSAT1 antibodies. (h) In A549

cells, HDAC7 was overexpressed to detect PSAT1 protein by Western blot. (I, j) H1299 cells were transfected with control Vector or HA-HDAC7 plasmid or transiently transfected with CTL siRNA or HDAC7 selective siRNAs. The mRNA levels of *PSAT1* and *HDAC7* were detected by qPCR. Data represented as the average of three independent experiments (mean ± SD), ns: $P > 0.05$, ***$P < 0.001$.

Supplementary Fig. 3

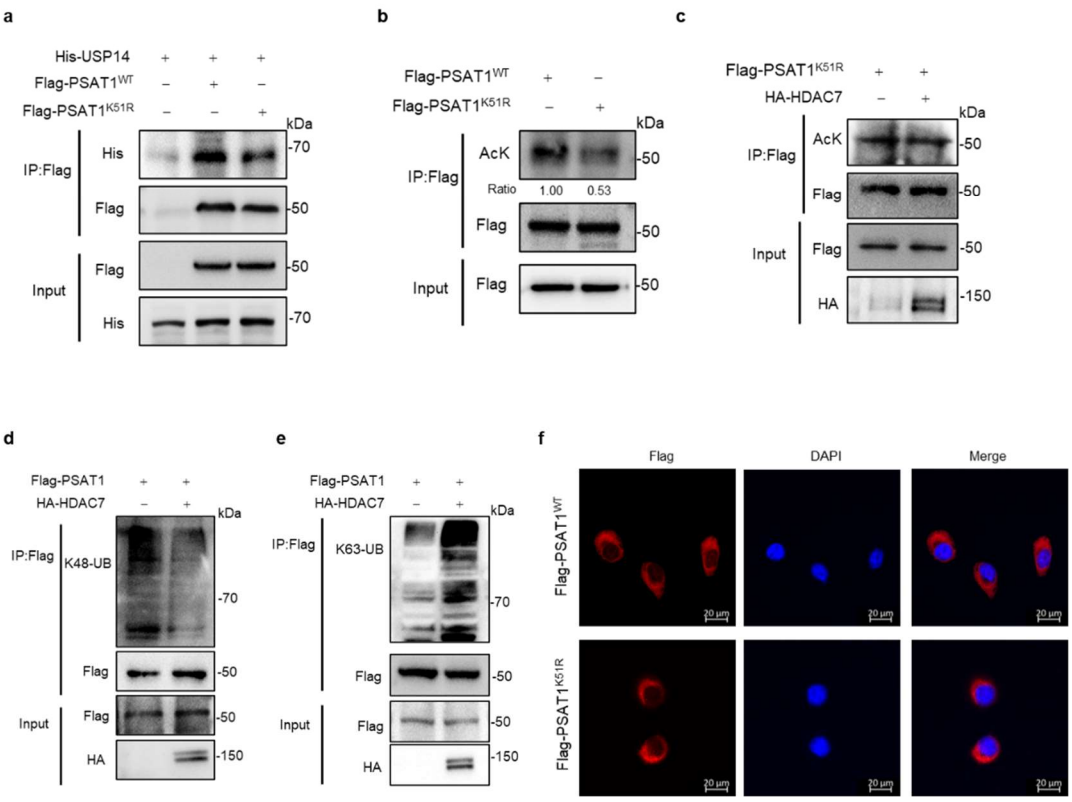


Supplementary Fig. 3: USP14 interacts with PSAT1 and regulates ubiquitination of PSAT1 in H1299 and A549 cells

(a) In H1299 cells, USP39 was overexpressed to detect PSAT1 protein by

Western blot. (b) H1299 cells were co-transfected with Myc-USP39 and Flag-PSAT1. The immunoprecipitants were blotted with anti-Myc or anti-Flag antibodies. (c) In H1299 cells, Flag-PSAT1 and Myc-USP39 plasmids were co-transfected. Co-IP was used to detect the ubiquitination level of PSAT1. (d) In A549 cells, endogenous interaction between PSAT1 and USP14 was tested using anti-Flag antibodies for Co-IP. The immunoprecipitants were blotted with anti-PSAT1 antibodies. (e) The colocalization of USP14 and PSAT1 was detected by immunofluorescence assay. (f) In A549 cells, USP14 was overexpressed to detect PSAT1 protein by Western blot. (g) The treatment efficacies of inhibitor IU1 were detected by Western blot. Co-IP was used to detect the ubiquitination level of CyclinB1. (h, i) In H1299 cells, His-USP14 plasmids or USP14 selective siRNA were transfected. Co-IP was used to detect the ubiquitination level of PSAT1. (j) H1299 cells were treated with different concentrations of IU1 treatment. Co-IP was used to detect the ubiquitination level of PSAT1. (k-m) In A549 cells, Flag-PSAT1 and His-USP14 plasmids were co-transfected. Co-IP was used to detect the total, K48-linked or K63-linked ubiquitination level of PSAT1.

Supplementary Fig. 4

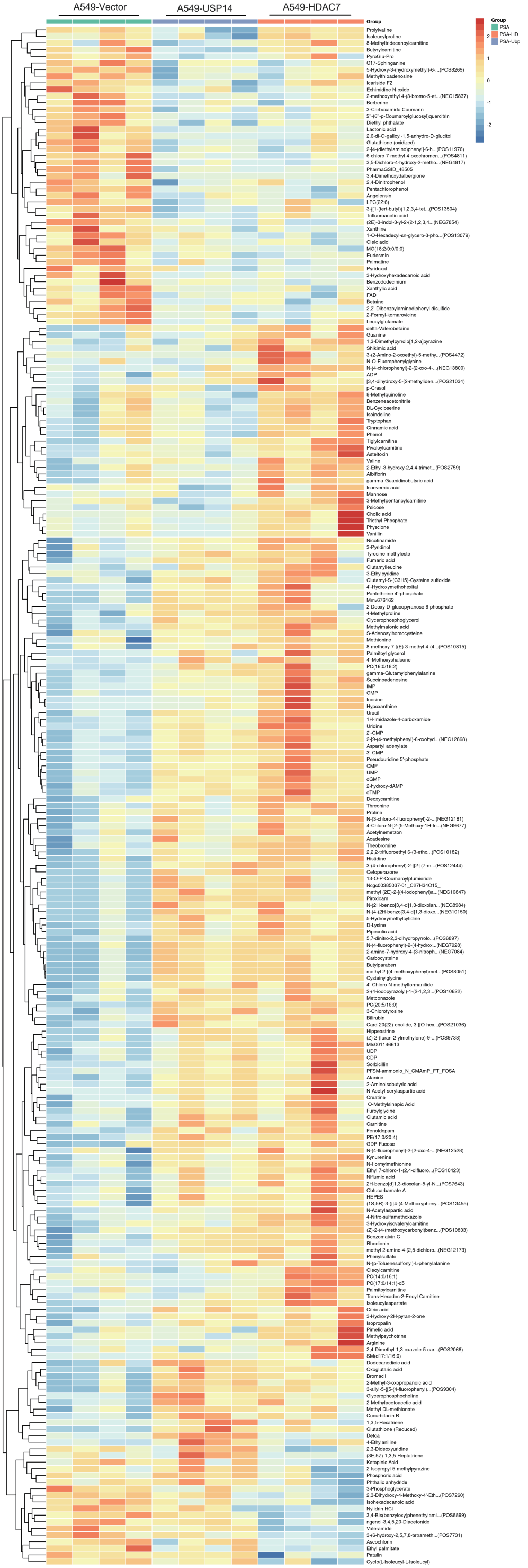


Supplementary Fig. 4: Lysine 51 of PSAT1 is an essential acetylation site in A549 cells

(a) A549 cells were co-transfected with His-USP14, Flag-PSAT1^{WT} or Flag-PSAT1^{K51R}. The immunoprecipitants were blotted with anti-His or anti-Flag antibodies. (b) A549 cells were transfected with Flag-PSAT1^{WT} or Flag-PSAT1^{K51R}. Co-IP was used to detect the acetylation level of PSAT1. The relative AcK expression compared with that of Flag was quantified. (c) A549 cells were transfected with Flag-PSAT1^{K51R} plasmid alone or co-transfected with HA-HDAC7. Co-IP was used to detect the acetylation level of PSAT1. (d, e) In A549 cells, Flag-PSAT1 and HA-HDAC7 plasmids were co-transfected. Co-IP was used to detect the K48-

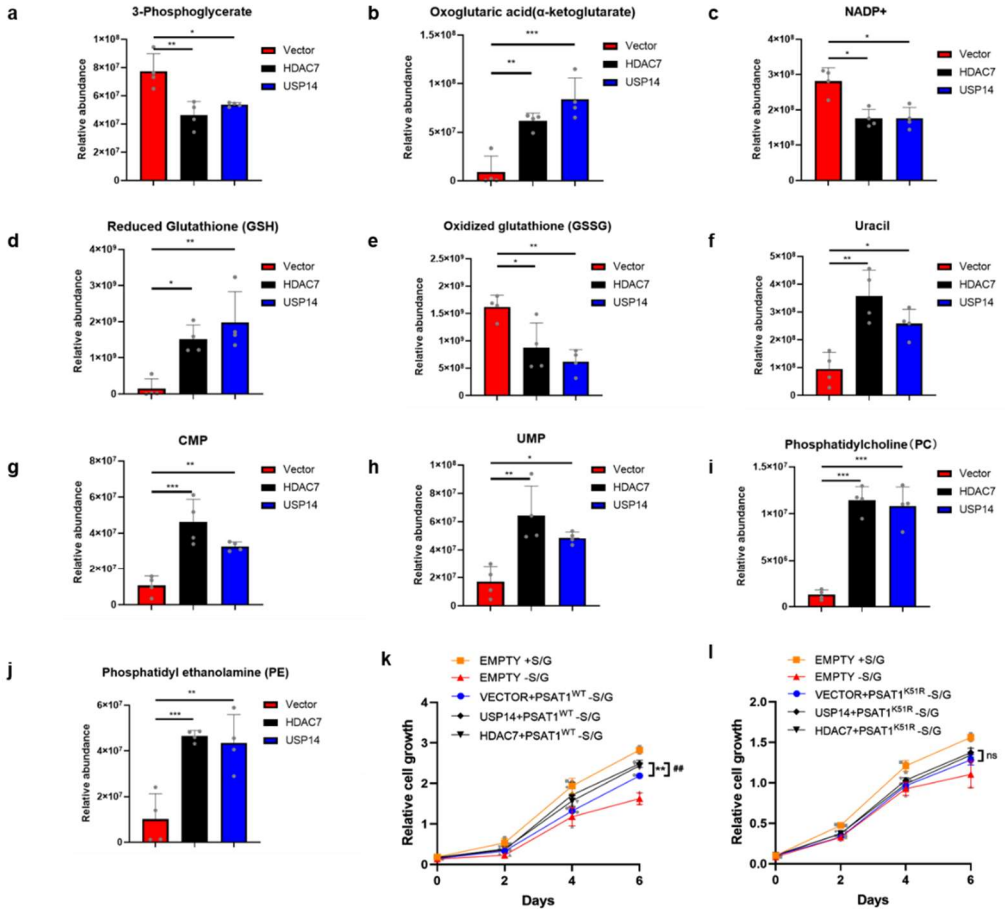
linked or K63-linked ubiquitination level of PSAT1. (f) The localization of $PSAT1^{WT}$ and $PSAT1^{K51R}$ were detected by immunofluorescence assay.

Supplementary Fig. 5



Supplementary Fig. 5: The heat map of the metabolites identified from metabolomics analysis

Supplementary Fig. 6

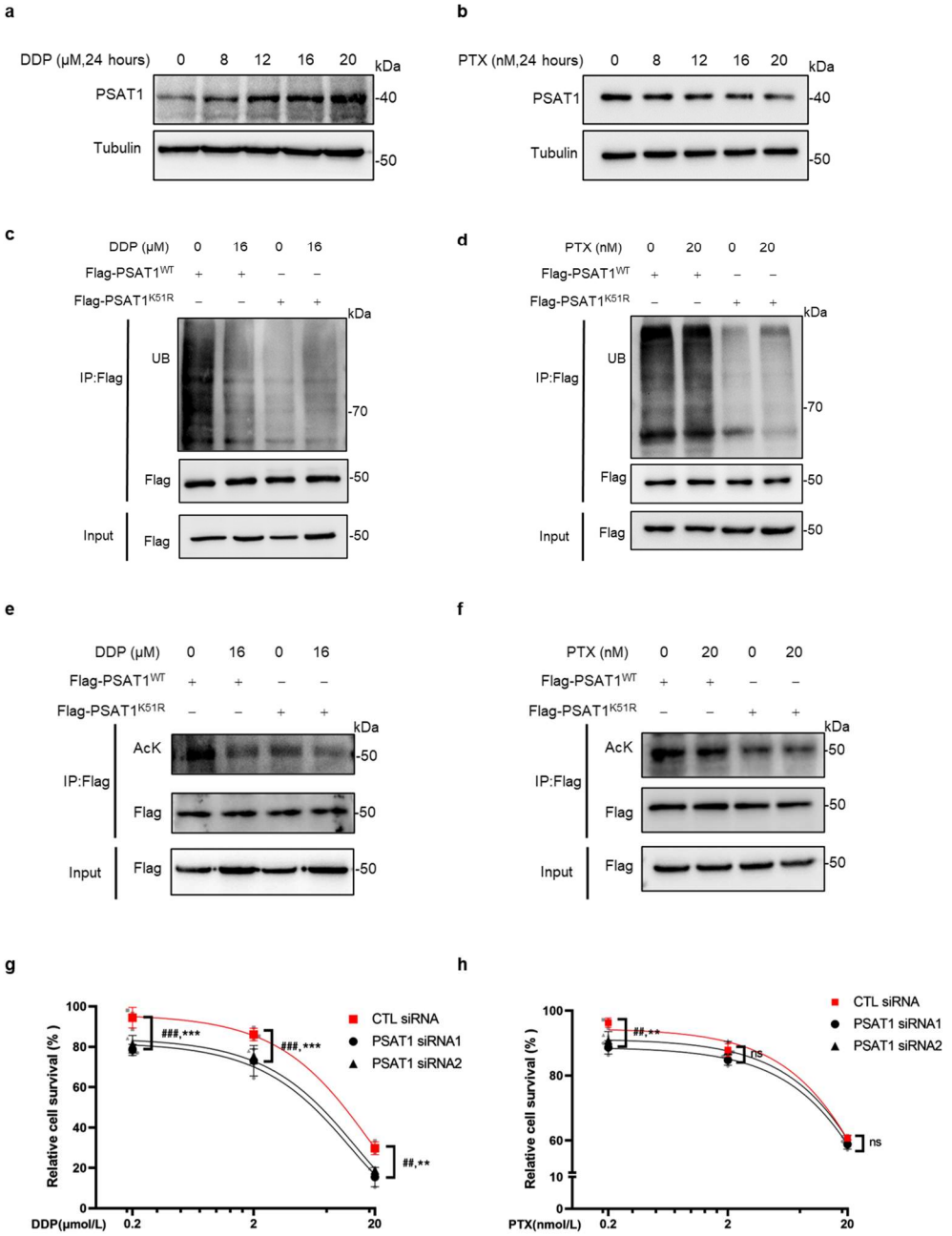


Supplementary Fig. 6: Acetylation of PSAT1 on Lysine 51 regulates serine metabolism and proliferation of lung adenocarcinoma

(a-j) The metabolites levels of 3-phosphoglycerate, α-ketoglutarate, NADP+, GSH, GSSG, uracil, CMP, UMP, PC and PE in A549-Vector, A549-HDAC7 and A549-USP14 cells measured by mass spectrometry. n=4 biologically independent samples (mean ± SD), *P < 0.05, **P < 0.01, ***P< 0.001. (k, l) A549 cells were transfected with Flag-PSAT1^{WT} or Flag-PSAT1^{K51R} plasmid alone or co-transfected with His-USP14 or His-

with His-USP14 or His-HDAC7. Then, cell proliferation experiments were conducted with or without serine and glycine. Data represented as the average of three independent experiments (mean ± SD). *, USP14+PSAT1^{WT}-S/G versus Vector+PSAT1^{WT}-S/G; #, HDAC7+PSAT1^{WT} -S/G versus Vector+PSAT1^{WT} -S/G. ns: $P > 0.05$, ** or ##: $P < 0.01$.

Supplementary Fig. 7

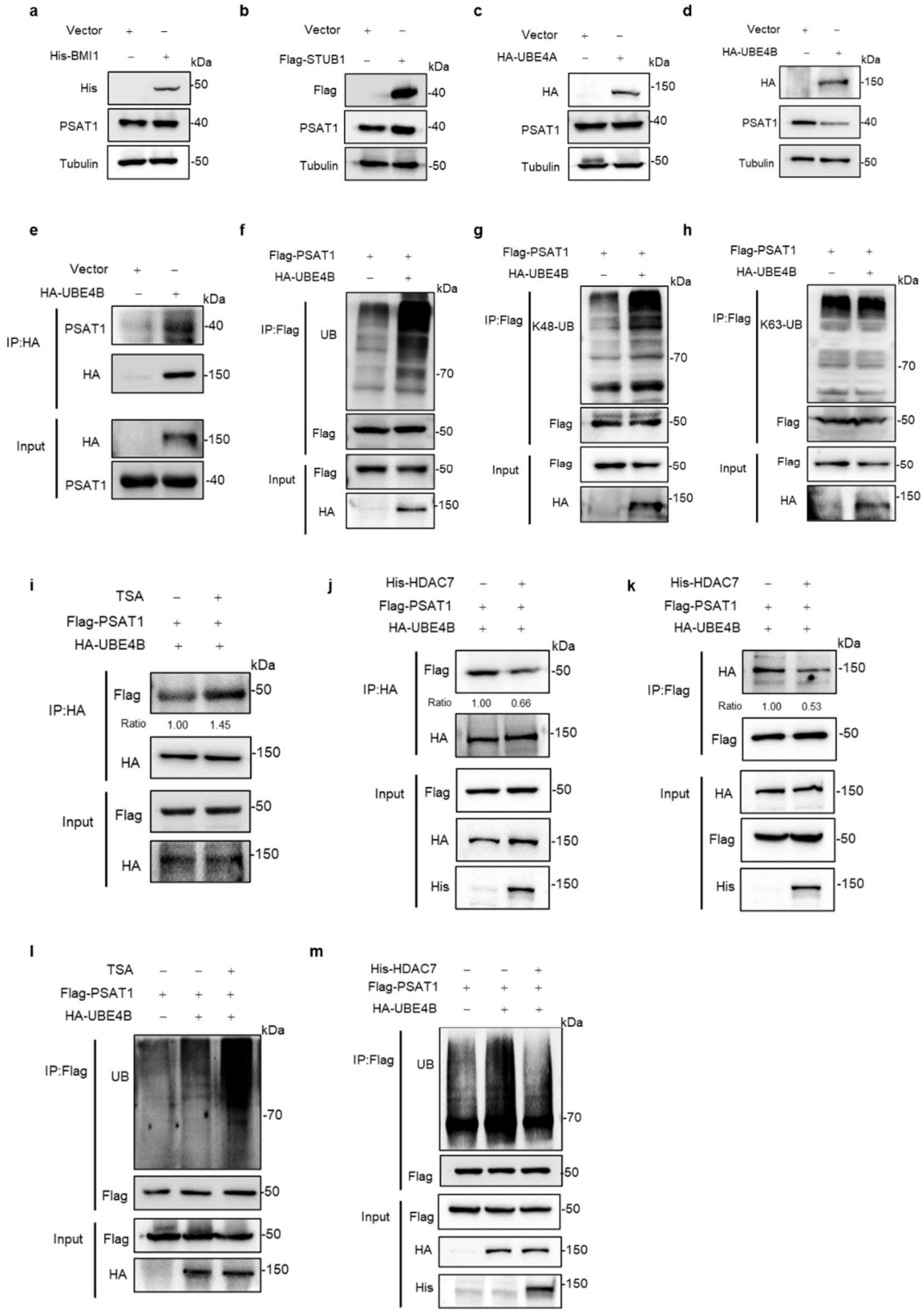


Supplementary Fig. 7: PSAT1 affects the sensitivity of lung adenocarcinoma cells to cisplatin

(a, b) DDP and PTX at different concentrations was added into H1299 cells for 24 h, cells were collected and the protein level of PSAT1 was detected by Western blot. (c-f) In H1299 cells, Flag-PSAT1^{WT} or Flag-PSAT1^{K51R}

plasmids were transfected with or without DDP (16μM) or PTX (20nM) for 24 h. Then, Co-IP was used to detect the ubiquitination and acetylation level of PSAT1. (g, h) H1299 cells were transiently transfected with CTL siRNA or PSAT1 selective siRNAs. Then, the cells were treated with DDP at different concentrations (0.2μM, 2μM and 20μM) or PTX at different concentrations (0.2μM, 2μM and 20nM) for 48 h. After fixation and stained, we measured the relative absorbance at 595 nm and calculated the relative cell survival percentage. Data represented as the average of three independent experiments (mean ± SD). #, CTL siRNA versus PSAT1 siRNA1; *, CTL siRNA versus PSAT1 siRNA2. ns: $P > 0.05$, ** or ##: $P < 0.01$, *** or ###: $P < 0.001$.

Supplementary Fig. 8

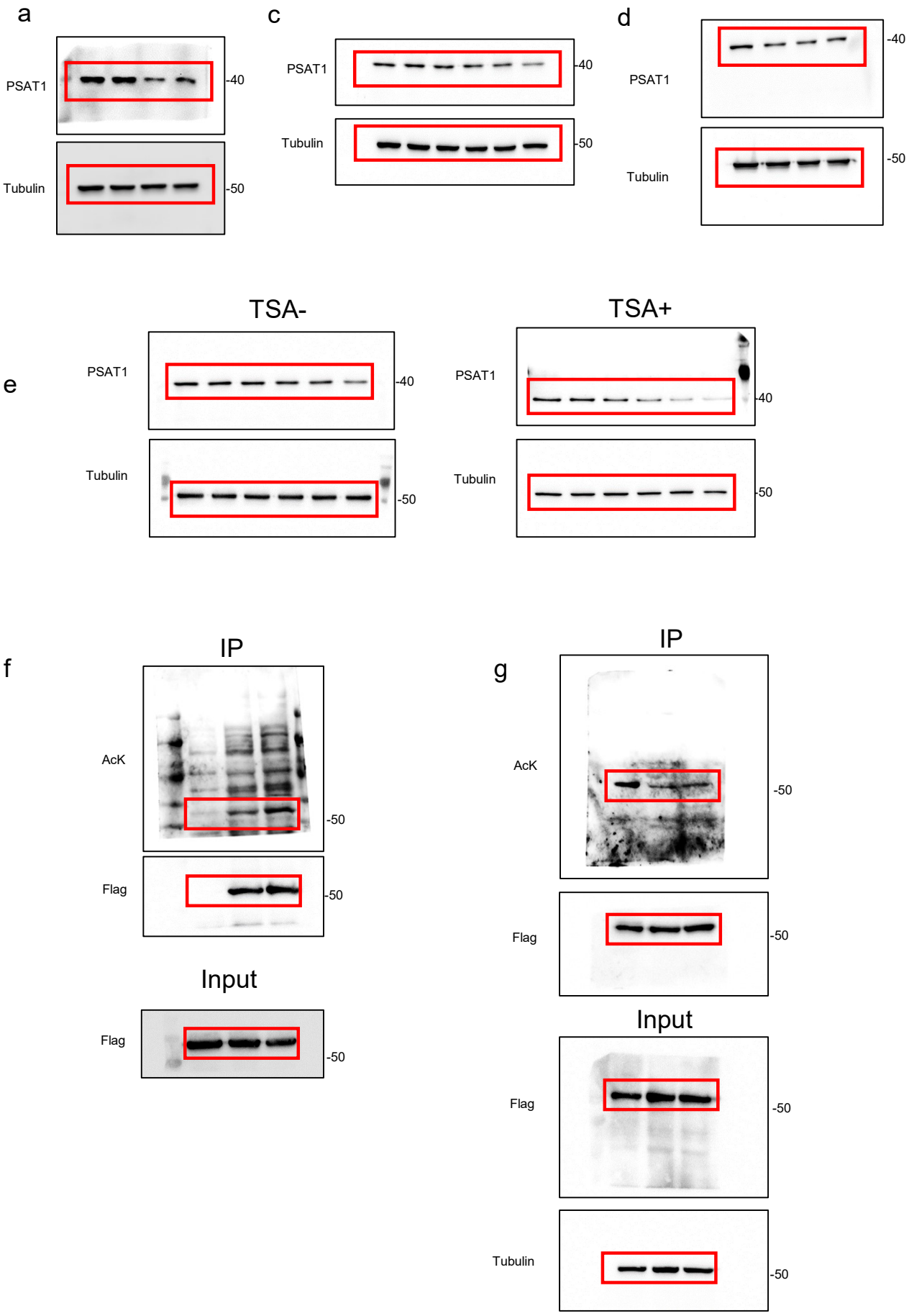


Supplementary Fig. 8: UBE4B ubiquitinates the acetylated PSAT1 for proteasomal degradation

(a-c) BMI1, STUB1 and UBE4A were overexpressed to detect PSAT1 protein by Western blot in H1299 cells. (d) UBE4B was overexpressed to detect PSAT1 protein by Western blot in A549 cells. (e) In A549 cells, endogenous interaction between PSAT1 and UBE4B was tested using anti-HA antibodies for Co-IP. The immunoprecipitants were blotted with anti-PSAT1 and anti-HA antibodies. (f-h) In A549 cells, Flag-PSAT1 and HA-UBE4B plasmids were co-transfected. Co-IP was used to detect the total, K48-linked or K63-linked ubiquitination levels of PSAT1. (i) A549 cells were co-transfected with Flag-PSAT1 and HA-UBE4B.Then TSA (1μM) was added 12 h before sample collection. The immunoprecipitants were blotted with anti-Flag or anti-HA antibodies. The relative Flag expression compared with that of HA was quantified. (j, k) H1299 cells were co-transfected with Flag-PSAT1, HA-UBE4B and His-HDAC7 or Vector. The immunoprecipitants were blotted with anti-Flag or anti-HA antibodies. The relative Flag or HA expression compared with that of another was quantified. (l) A549 cells were transfected with Flag-PSAT1 plasmid alone or co-transfected with HA-UBE4B plasmids, and were treated with or without TSA (1μM). Co-IP was used to detect the ubiquitination level of PSAT1. (m) H1299 cells were co-transfected with Flag-PSAT1, HA-UBE4B and His-HDAC7 or Vector. Co-IP was used to detect the ubiquitination level of PSAT1.

Supplementary Fig. 9. Uncropped western blots

Supplemental Material to Fig. 1



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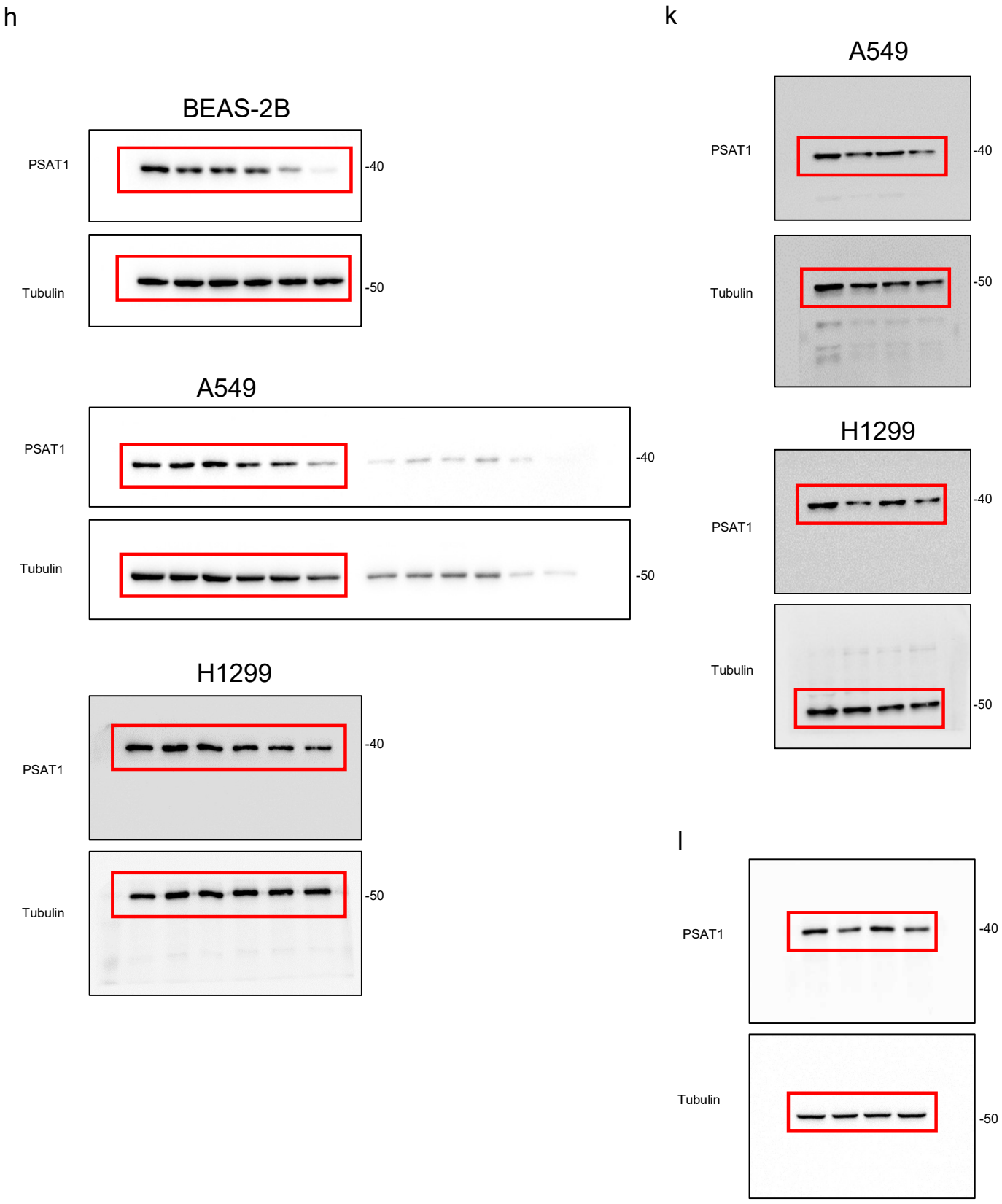

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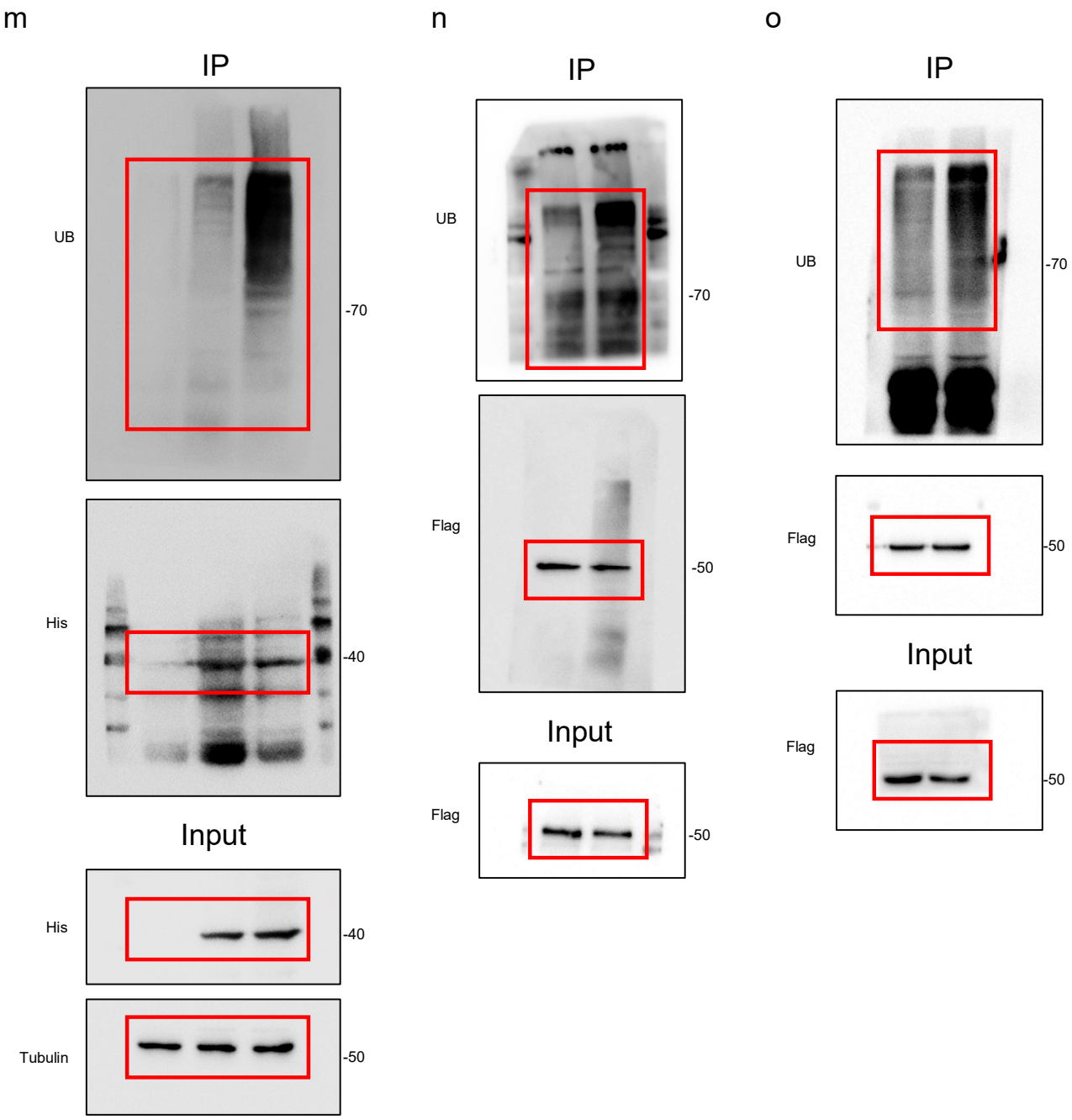

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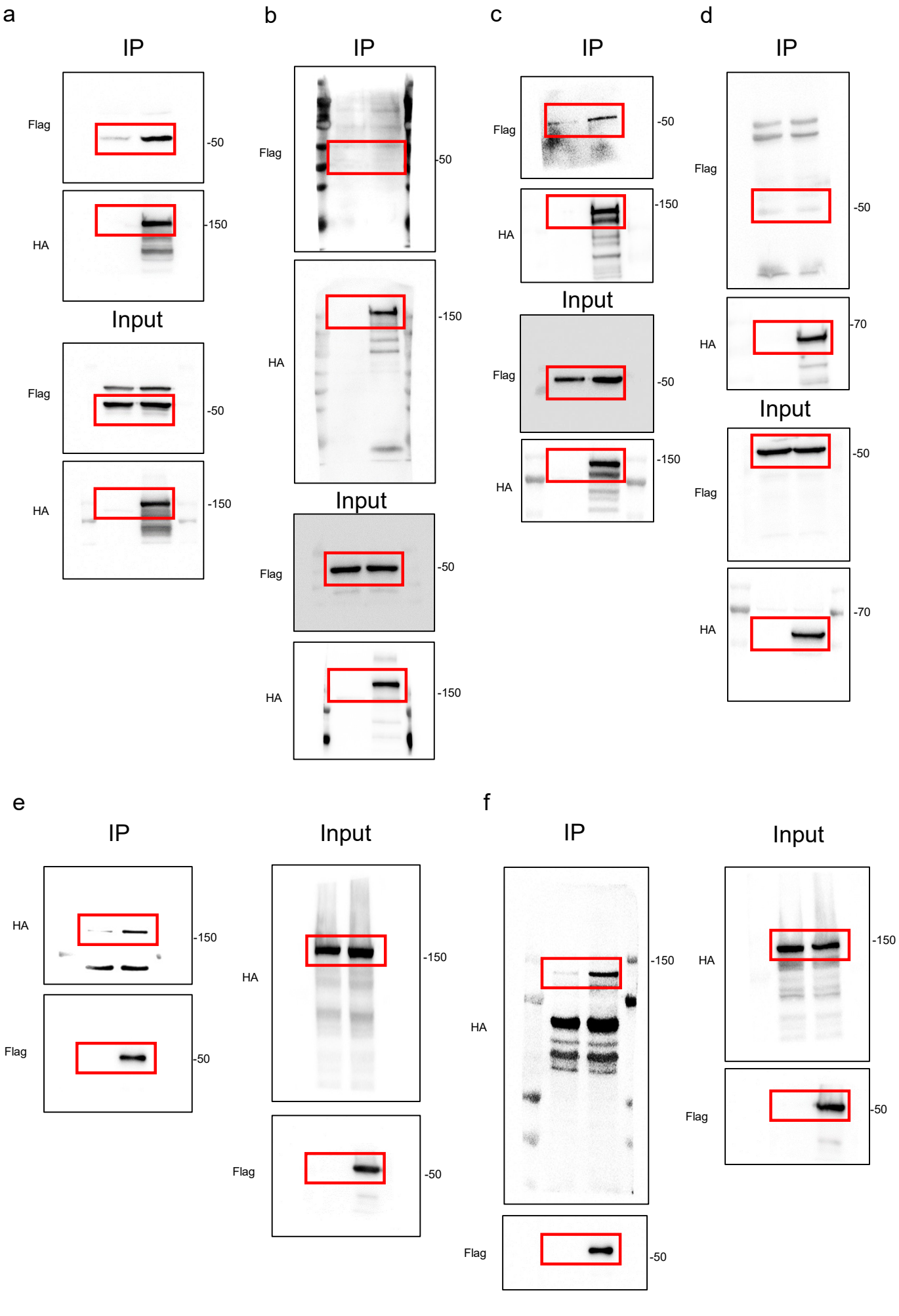

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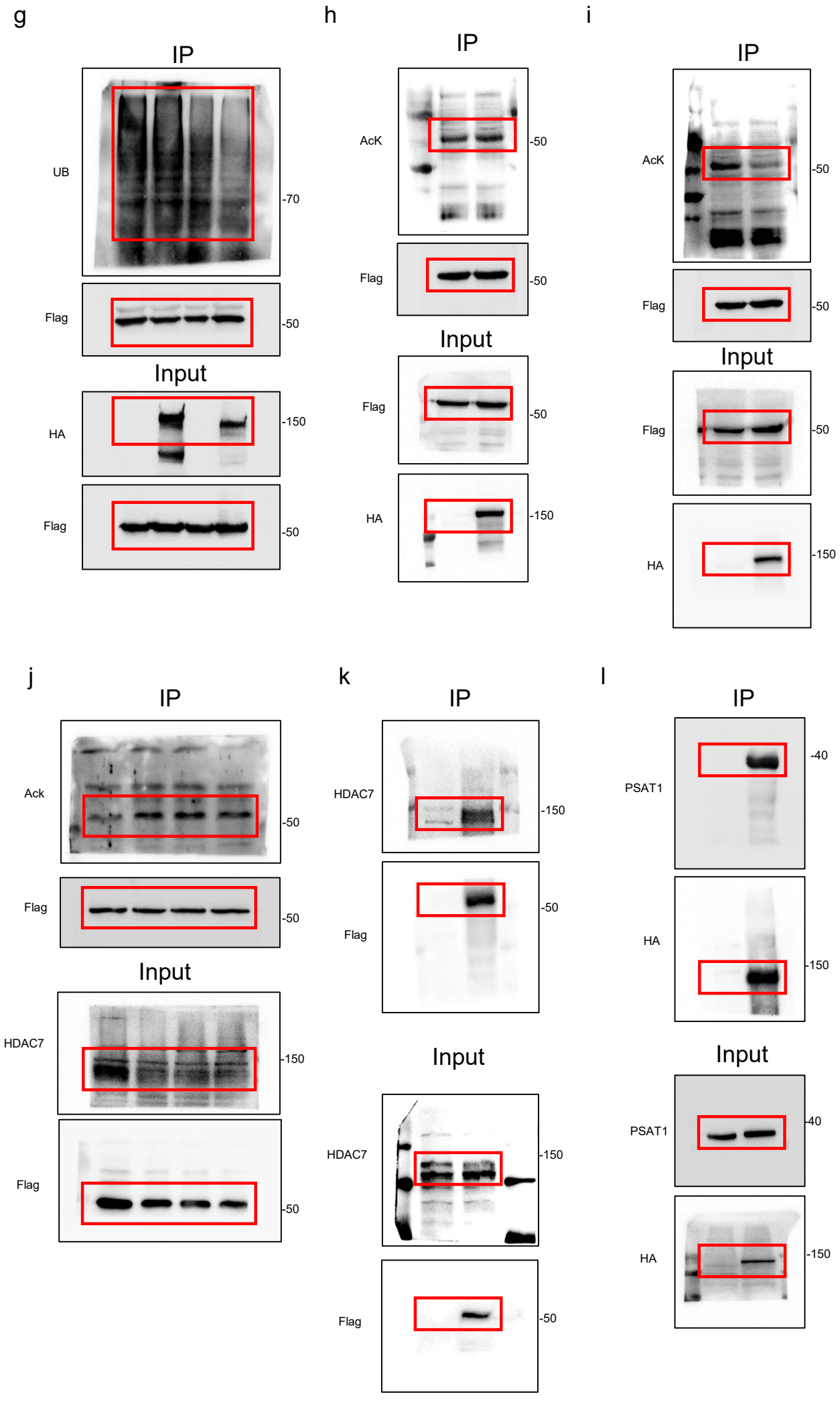

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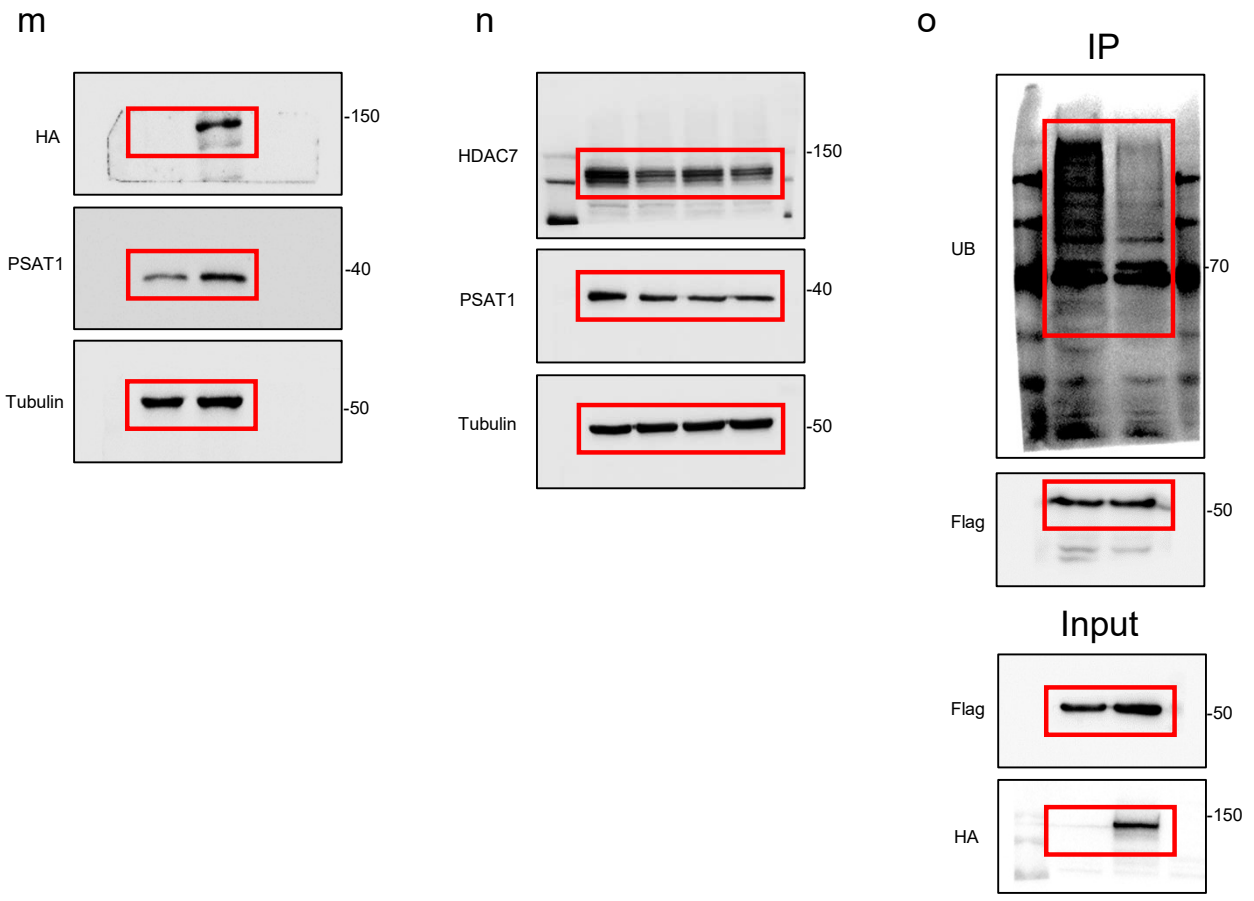

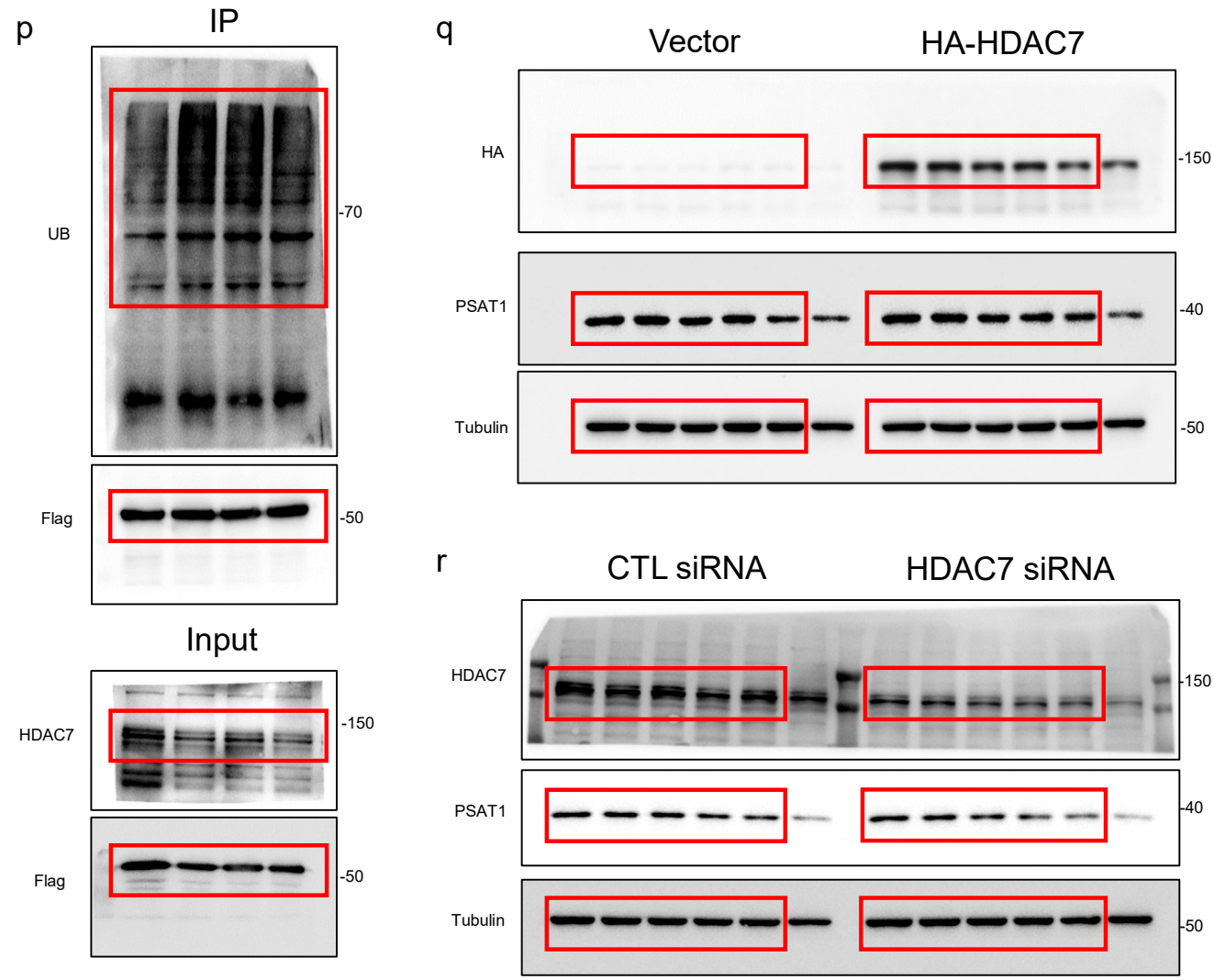

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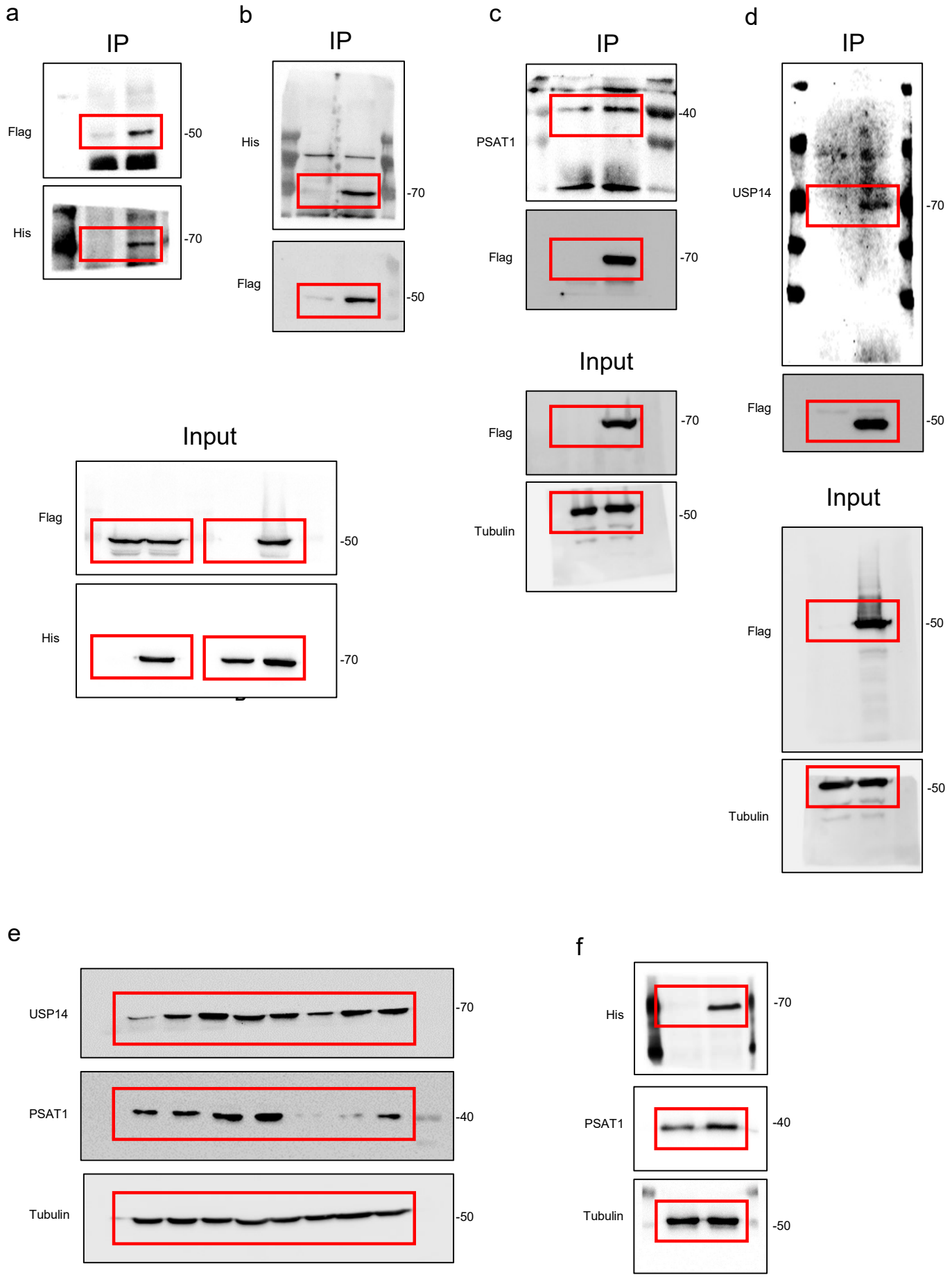

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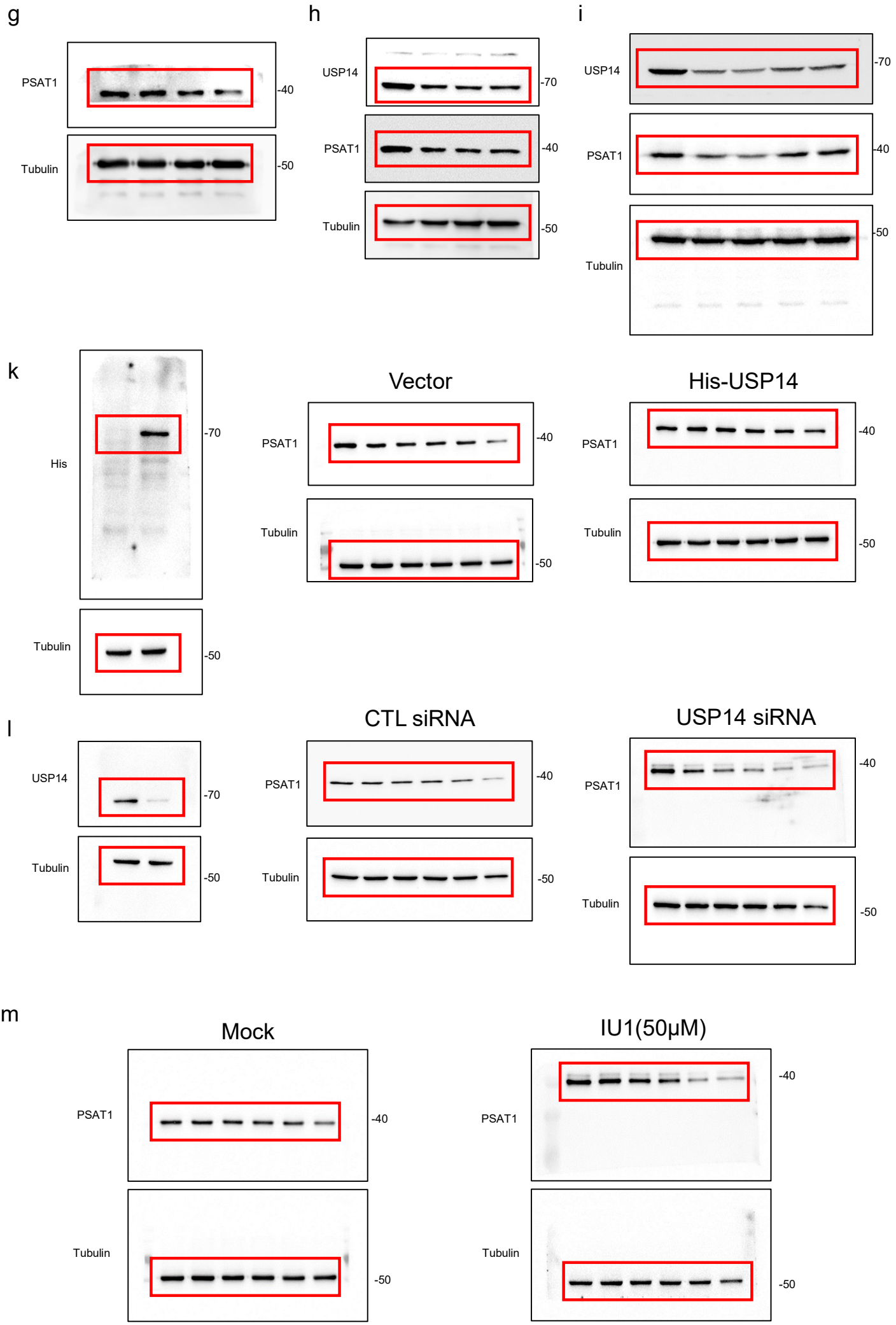

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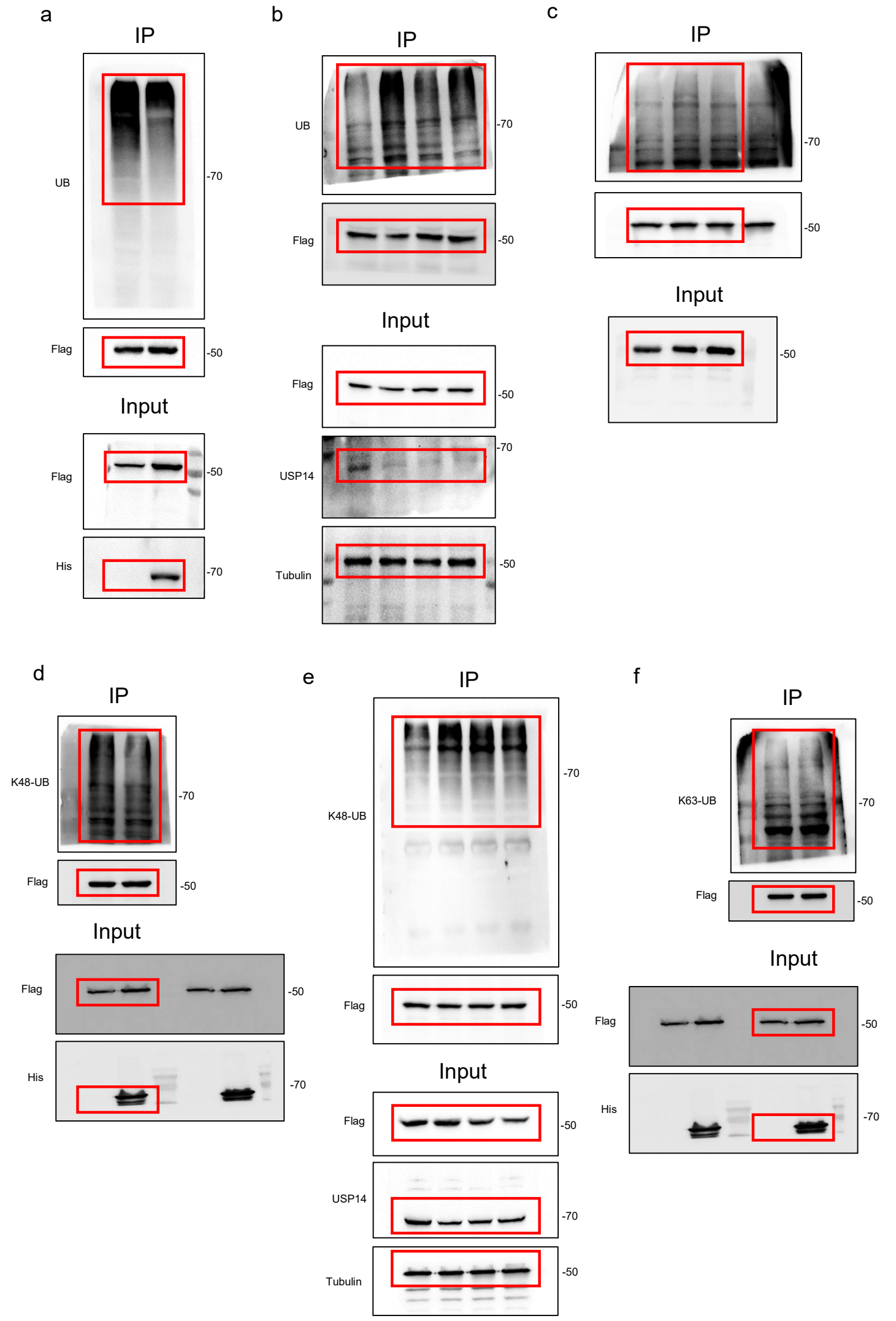

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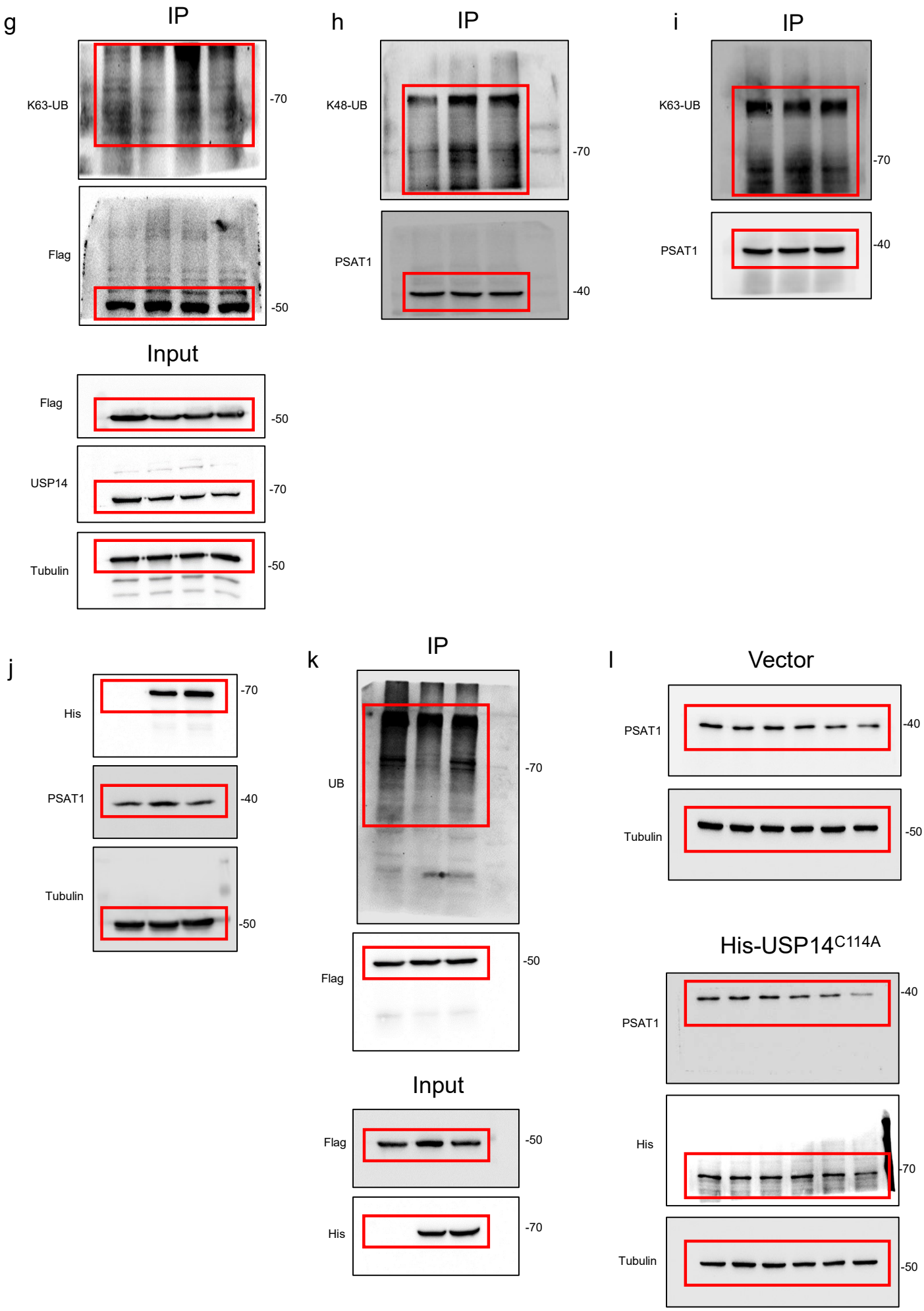

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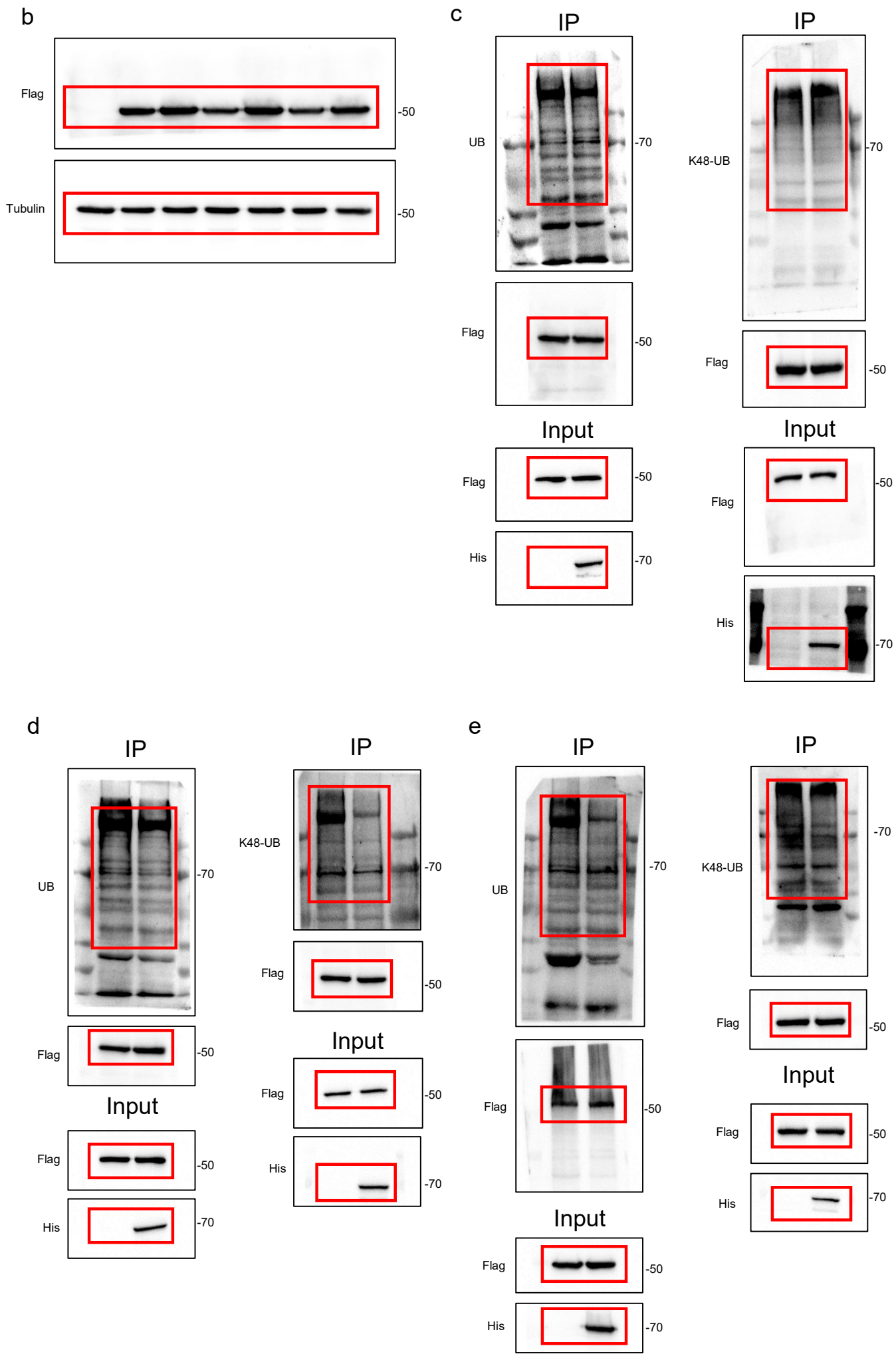

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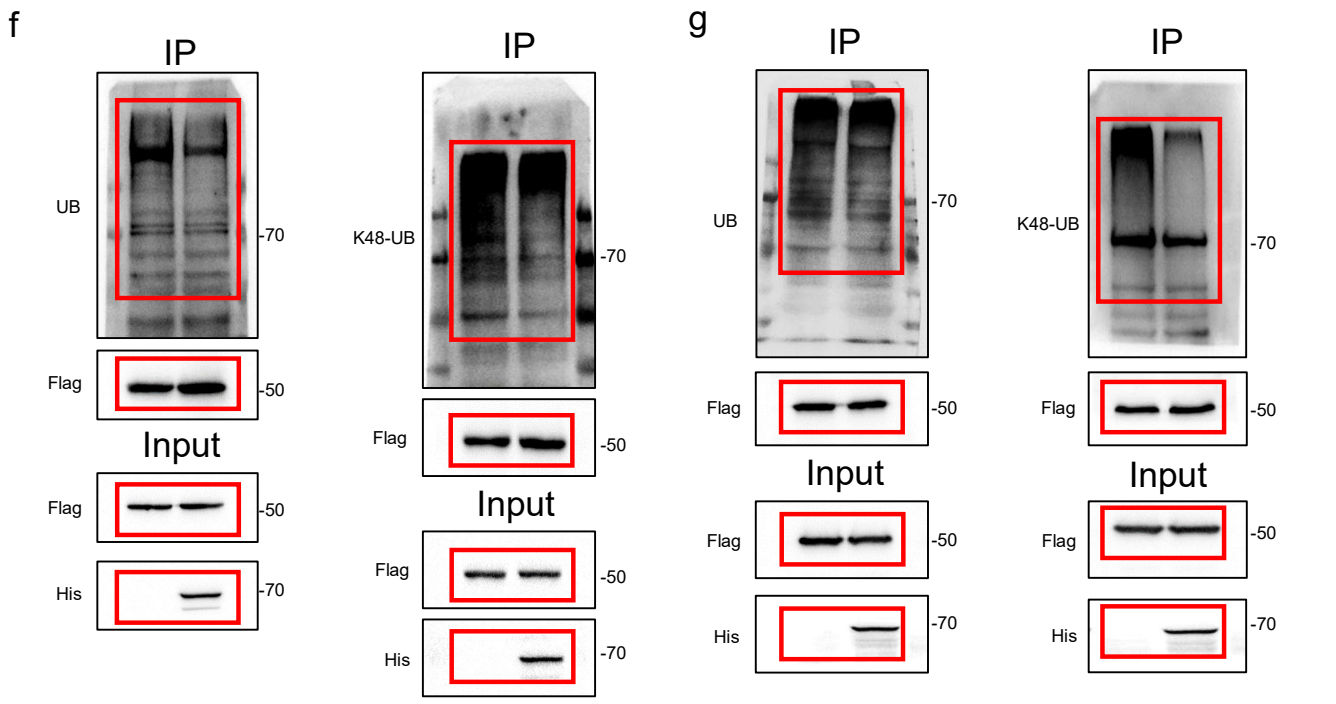

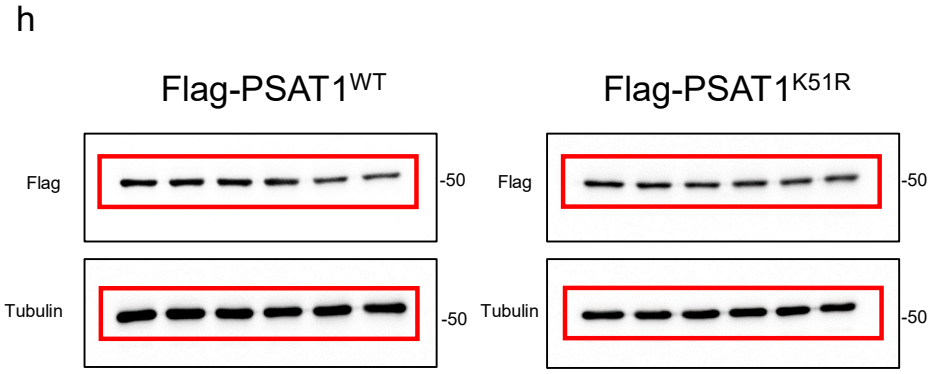

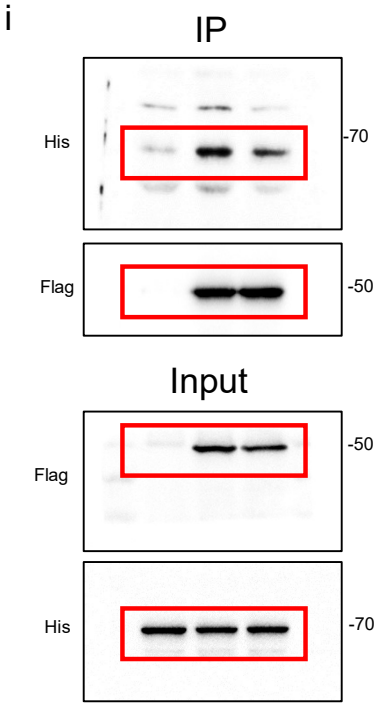

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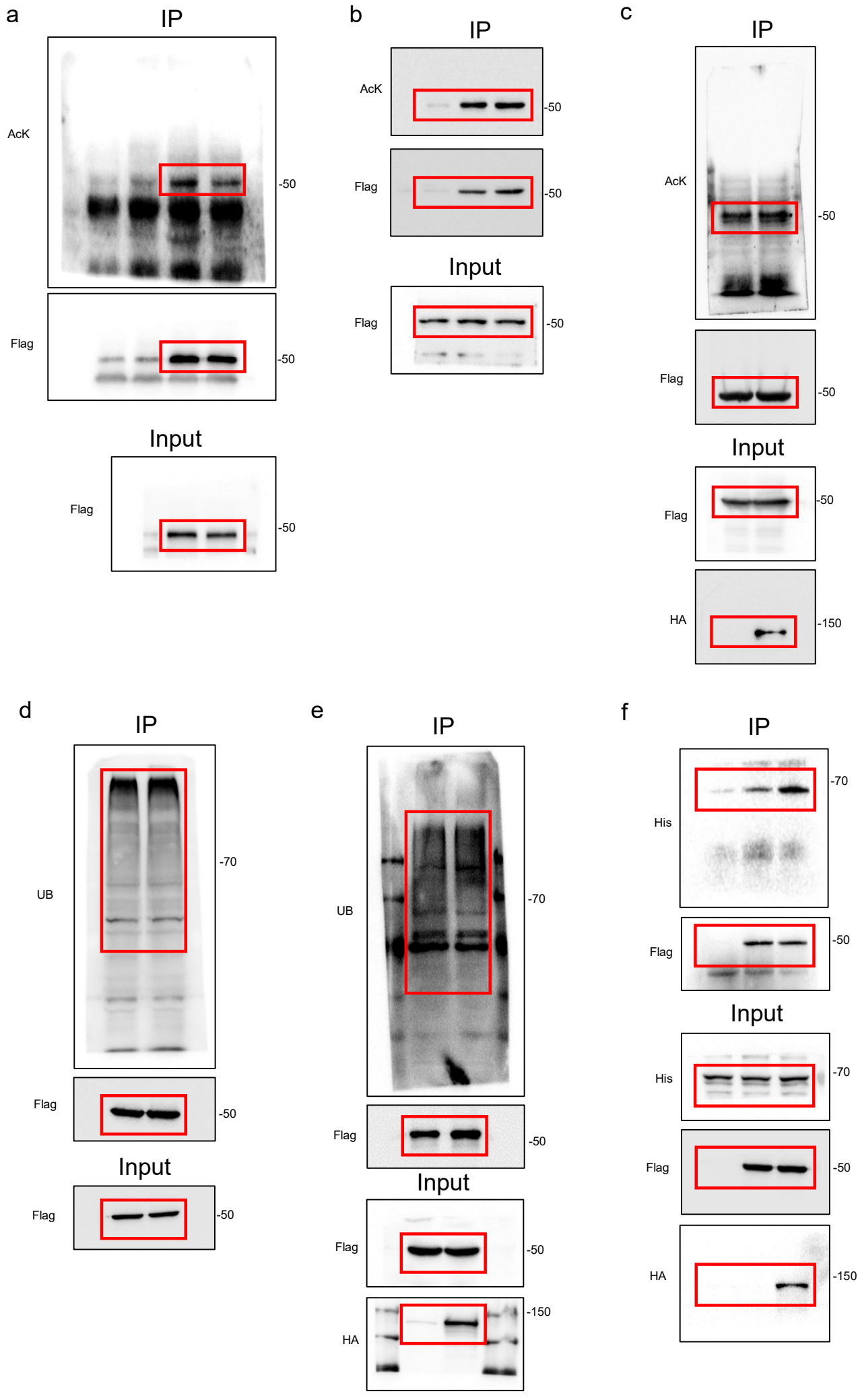

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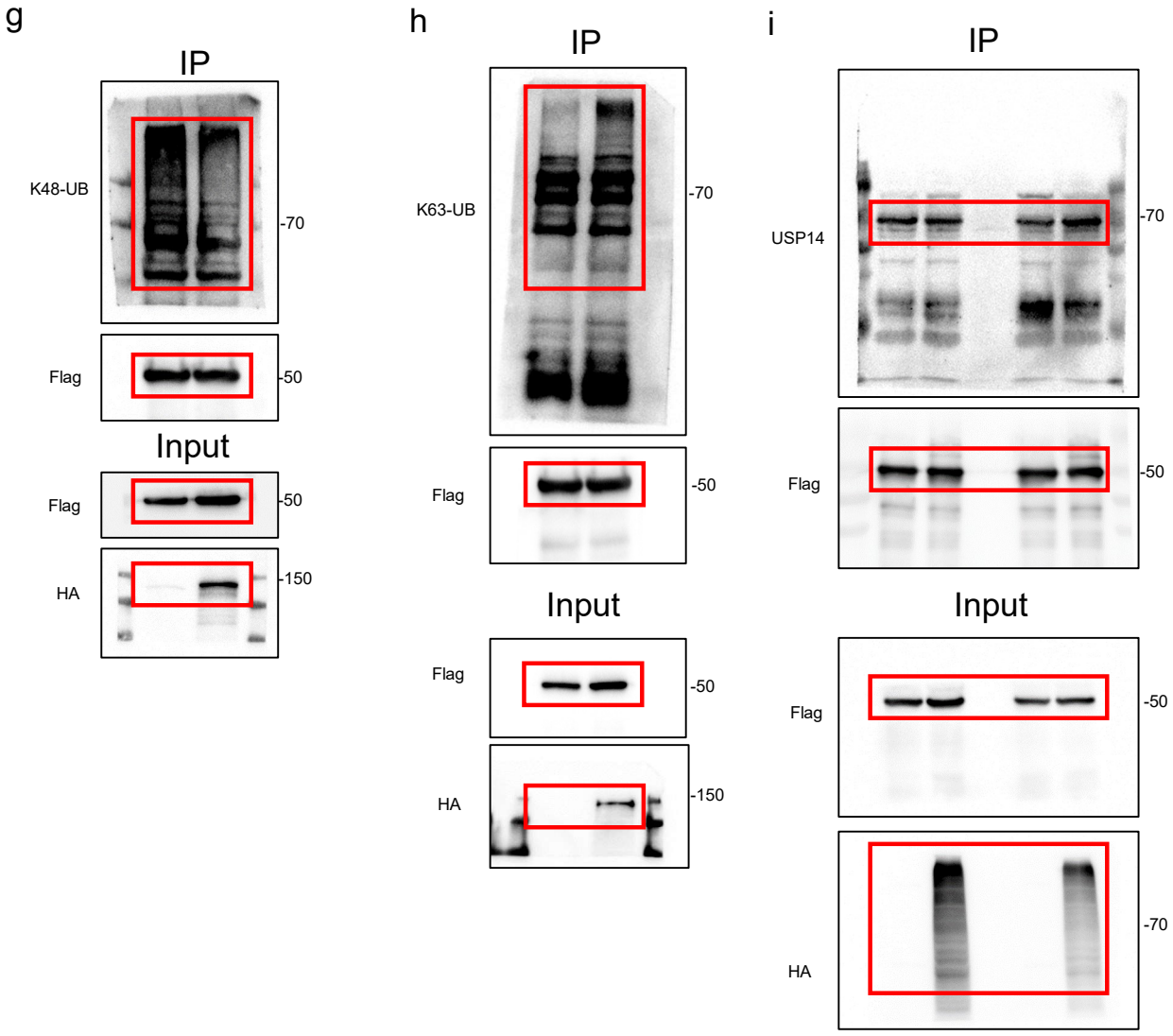

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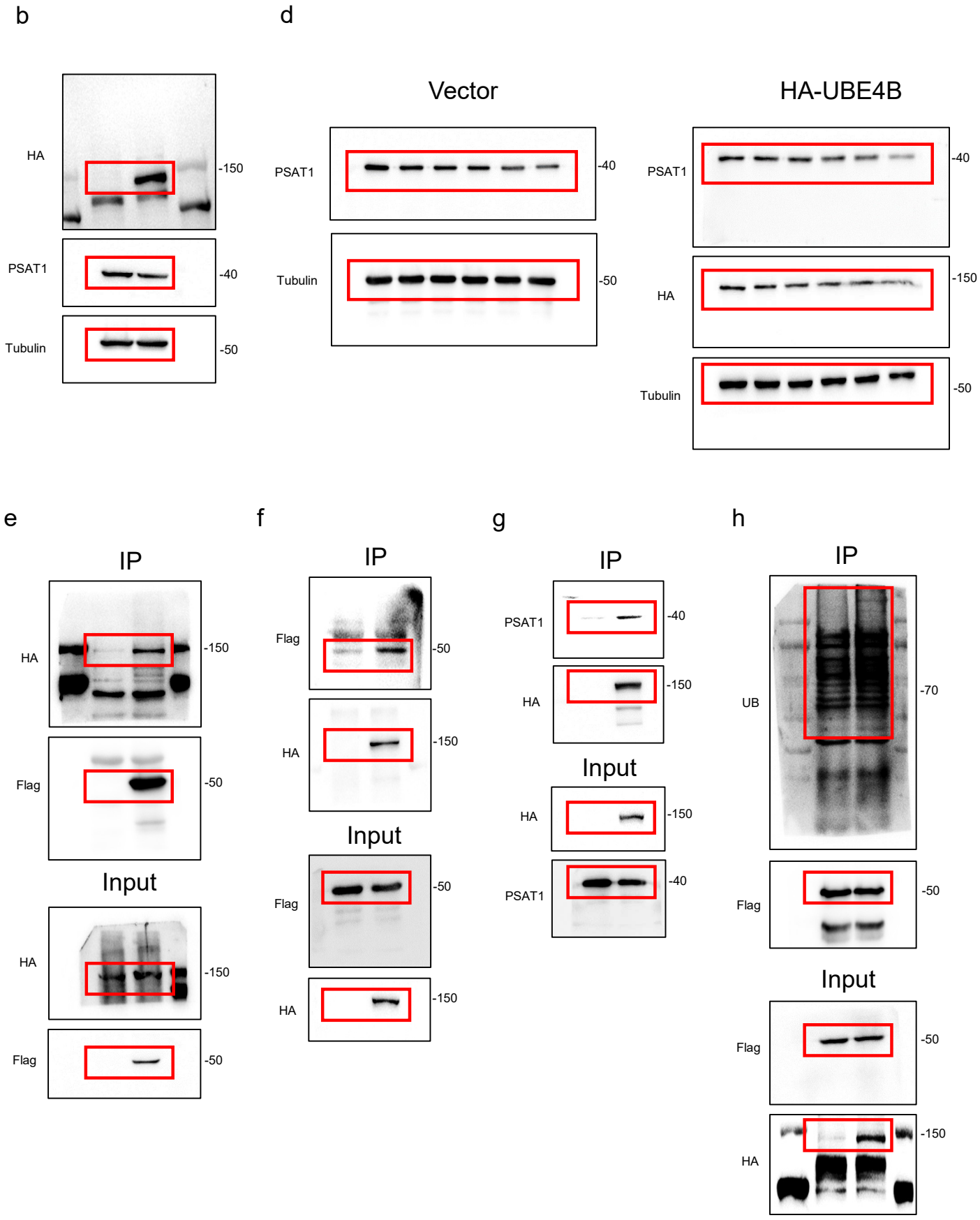

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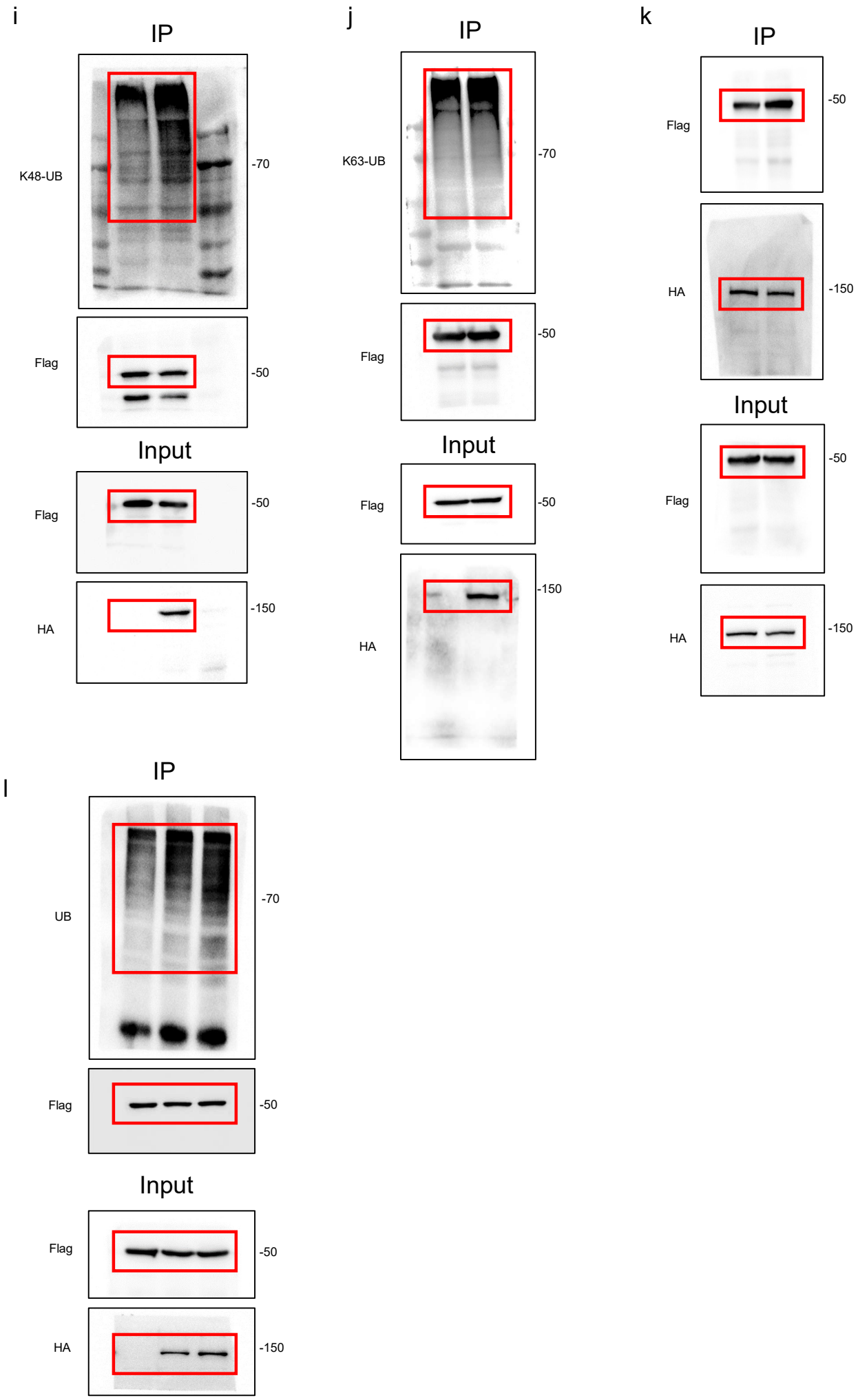

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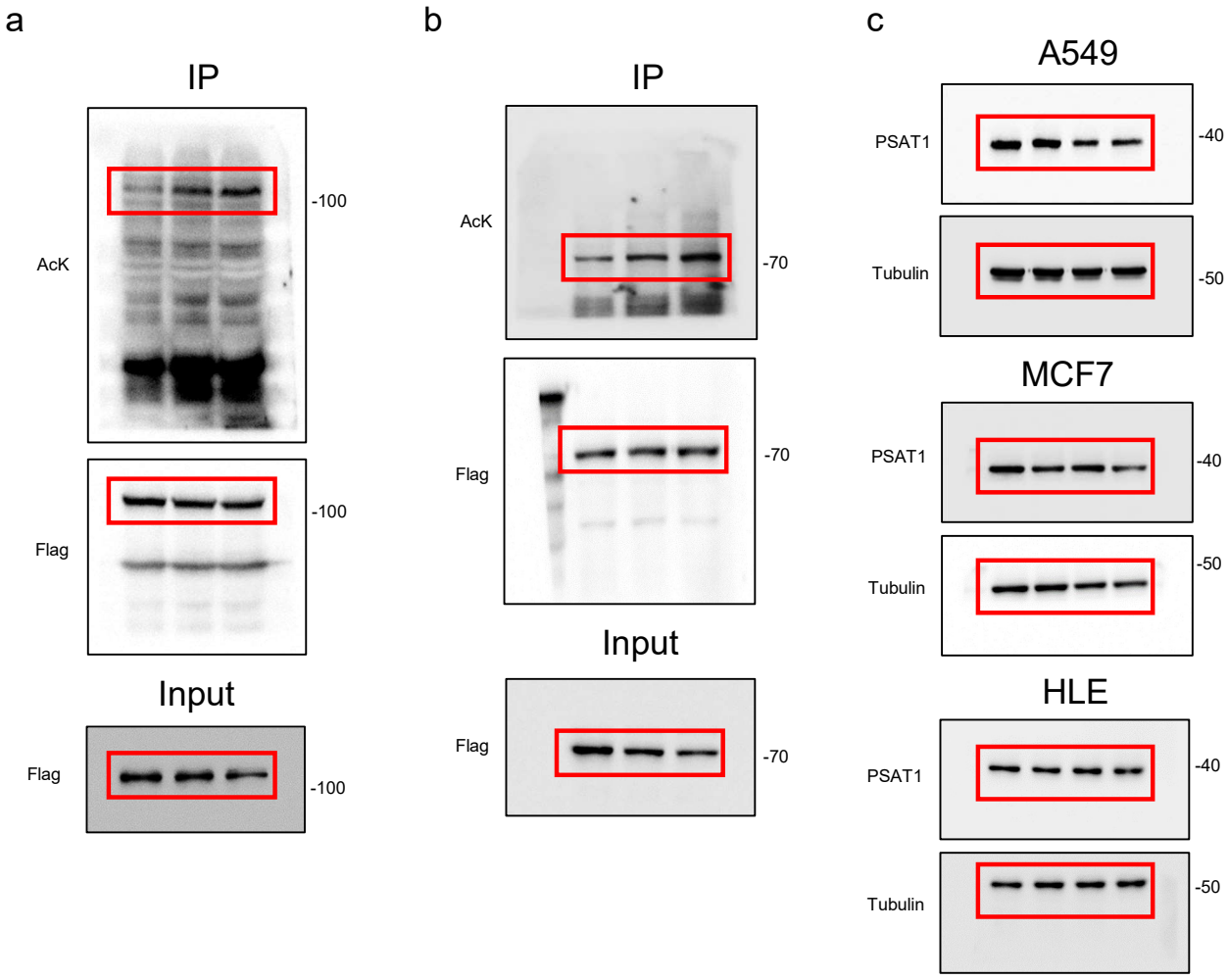

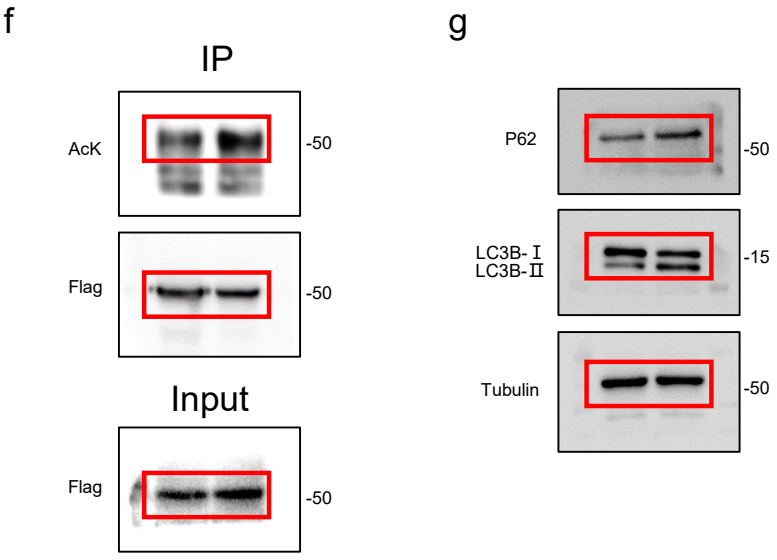

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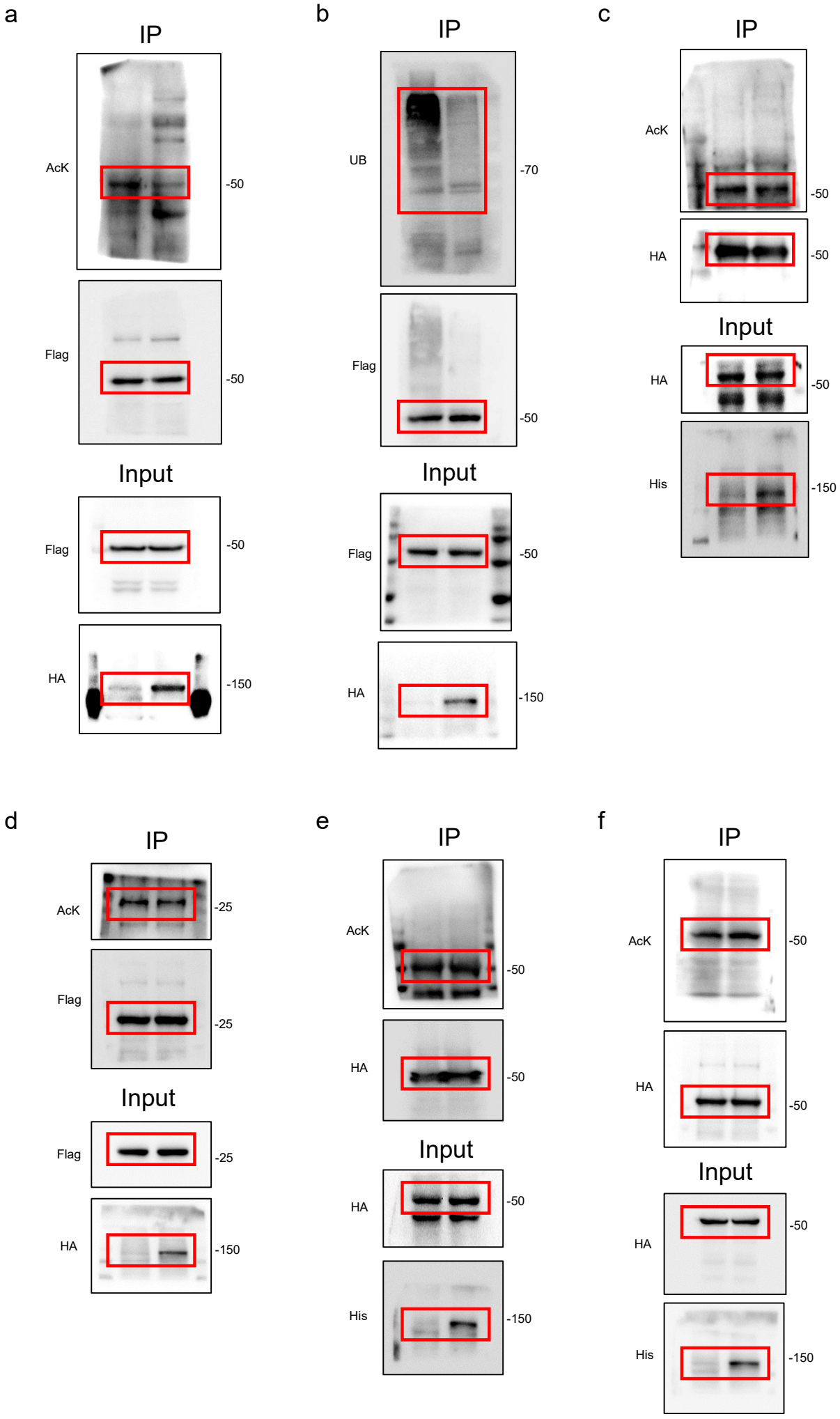

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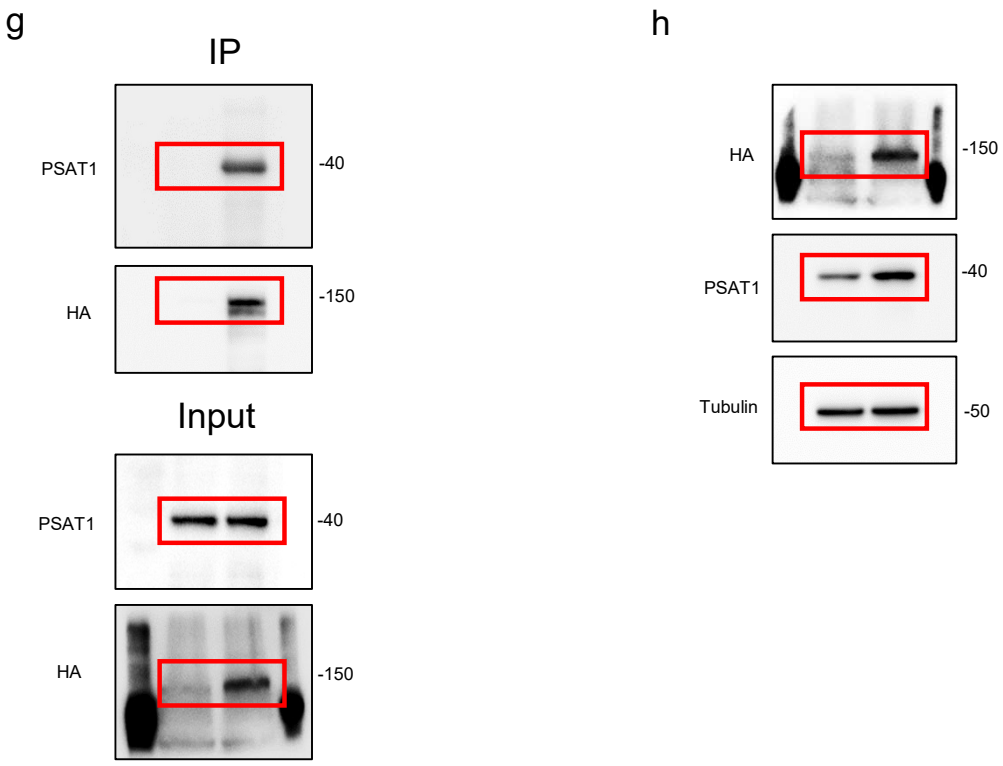

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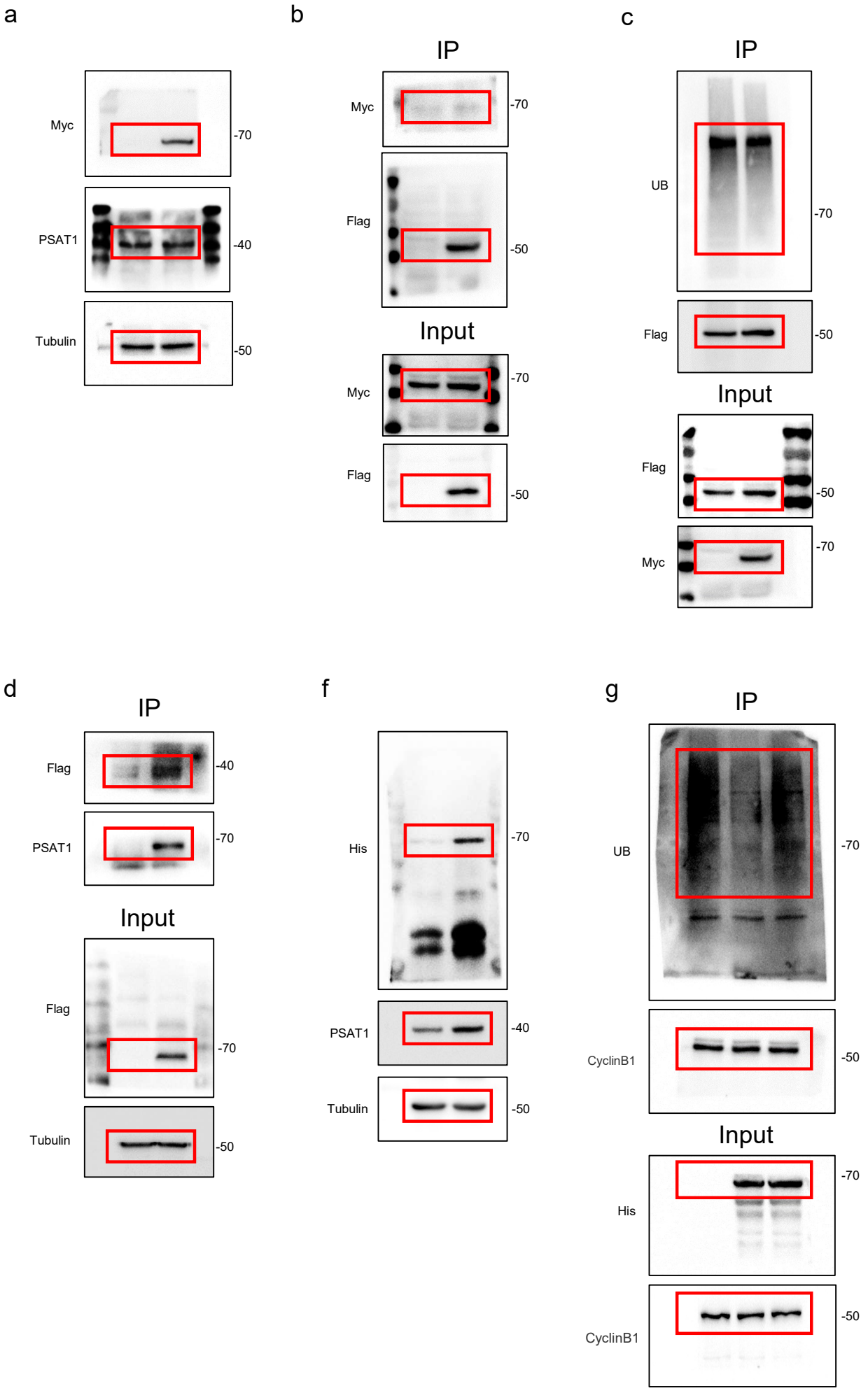

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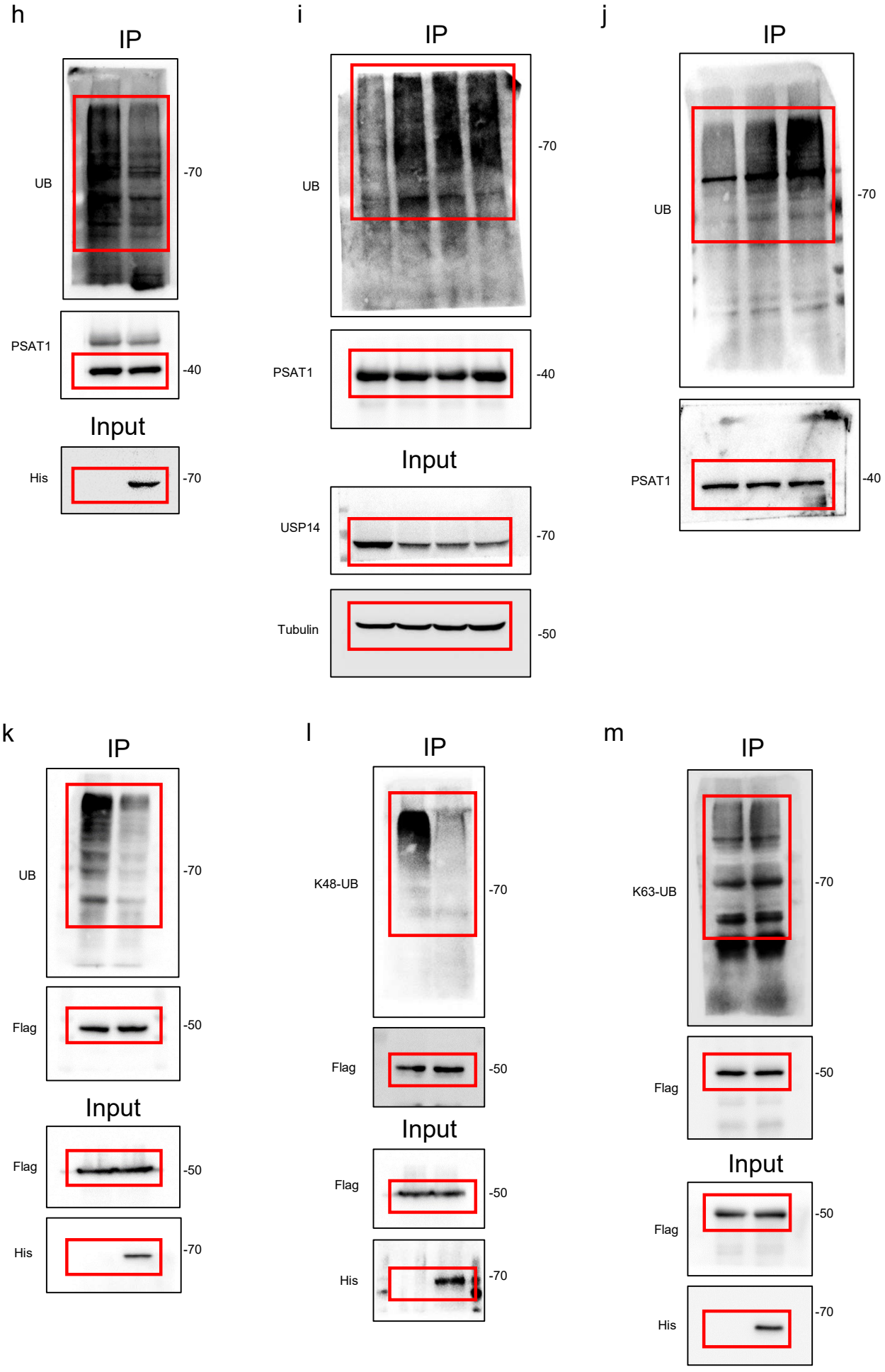

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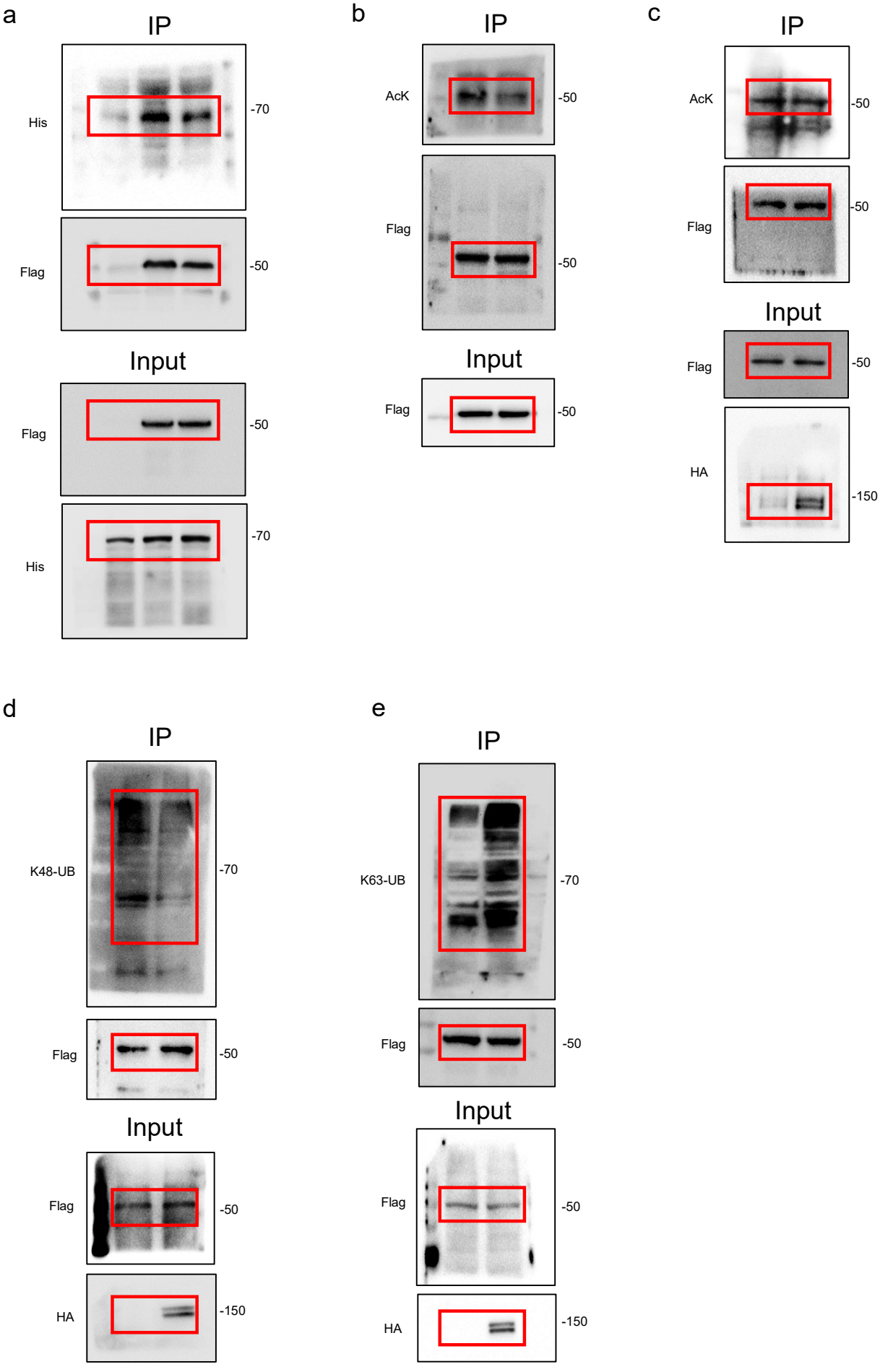

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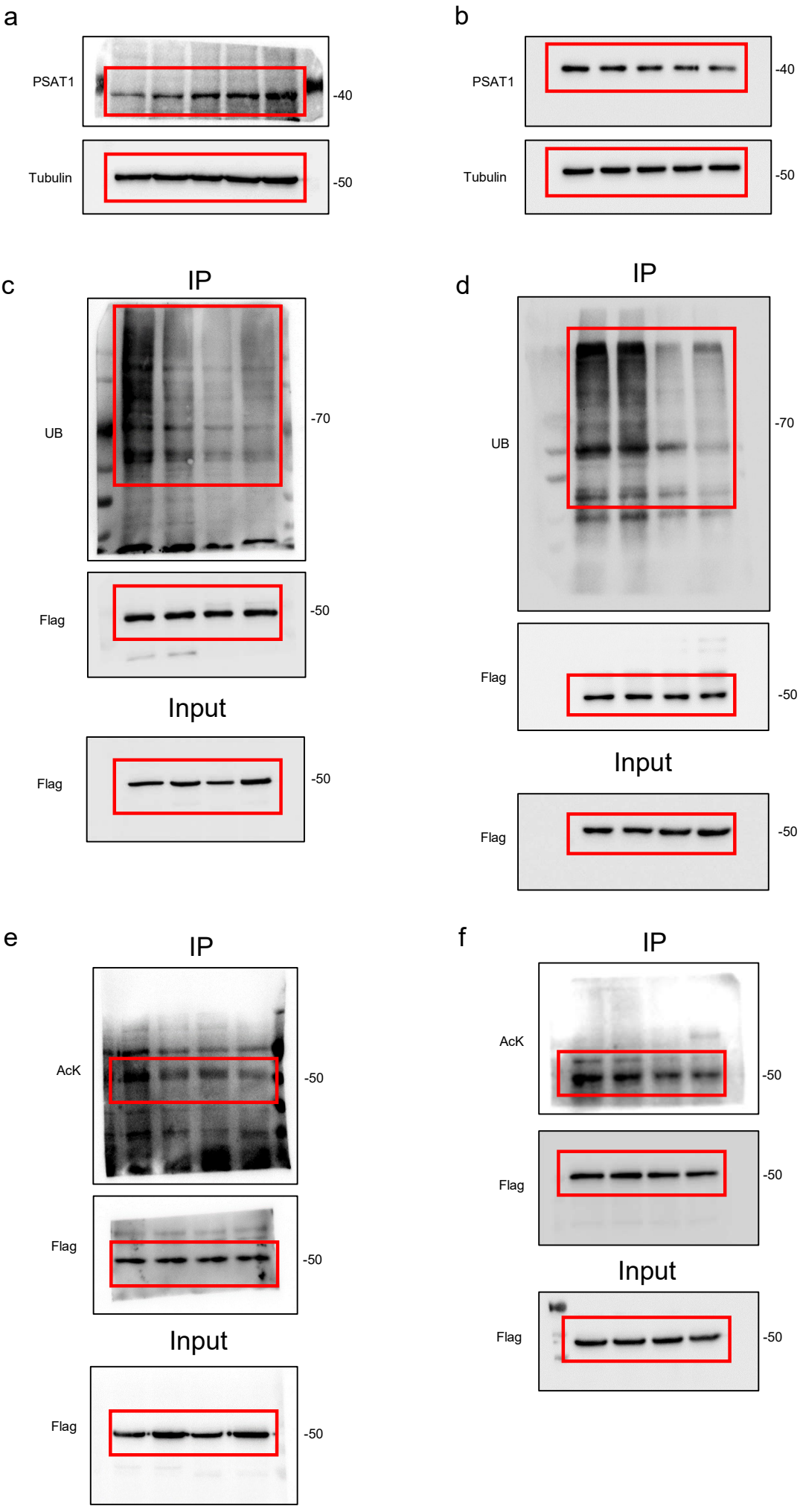
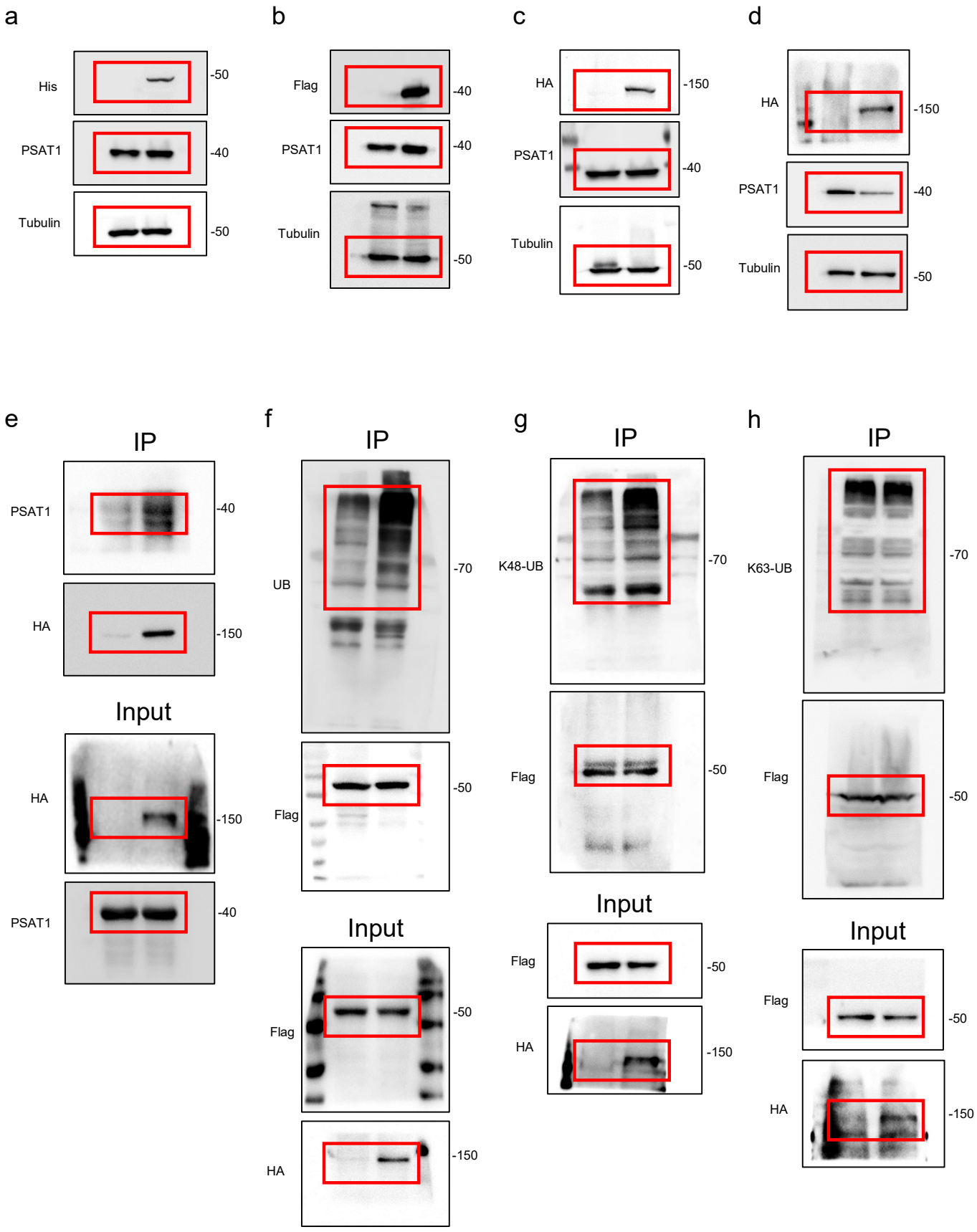
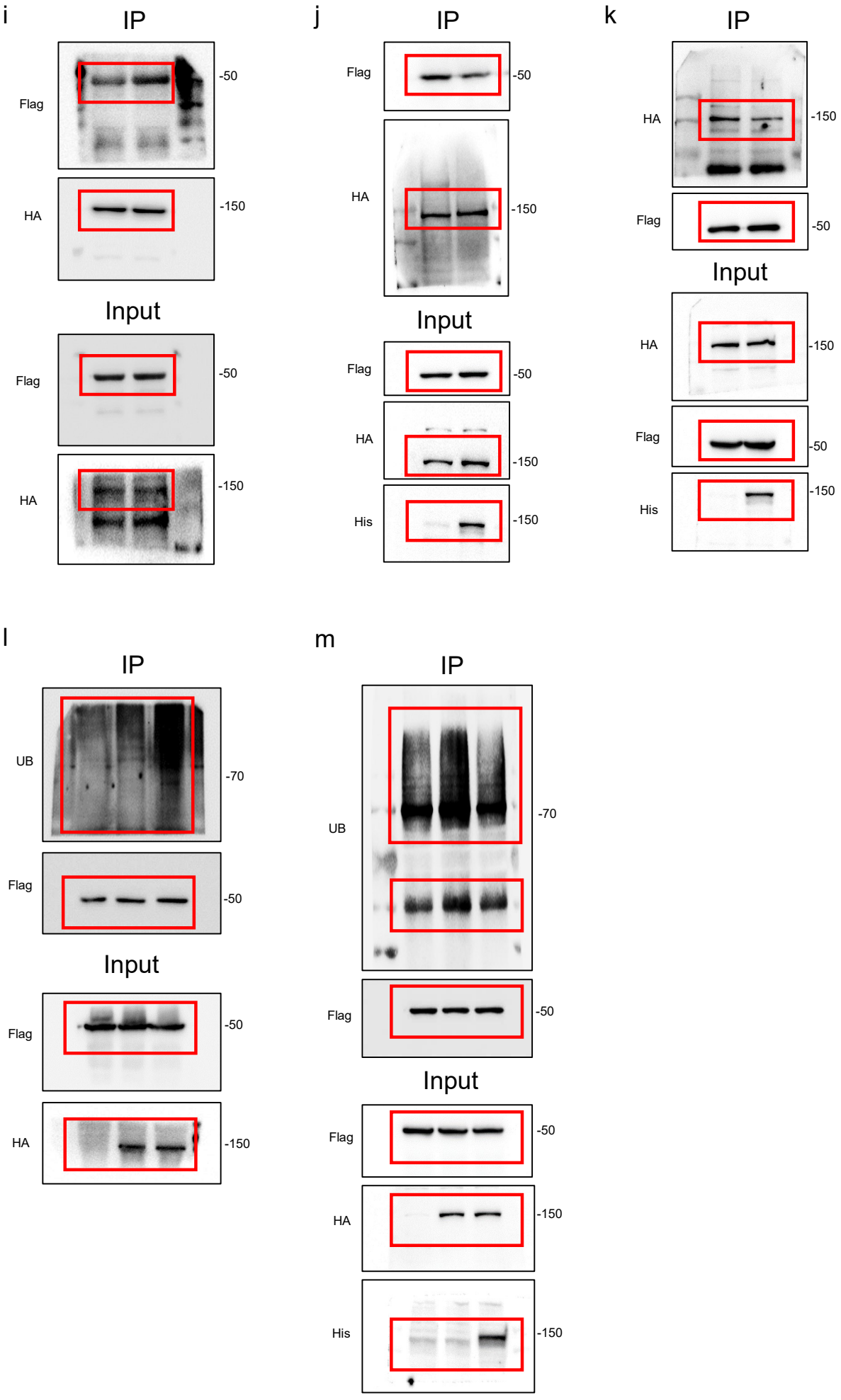


Supplemental Material to Supplementary Fig. 8



Supplemental Material to Supplementary Fig. 8



Supplementary Table 1. All primer sequence information mentioned in this article

Primer sequences for plasmids

Name	Sequence
pcDNA3.1-His-*PSAT1*-F	5’-TGGCTAGTTAAGCTTGGTACCATGGACGCCCCCAGGCA
pcDNA3.1-His-*PSAT1*-R	5’-CGCGGGCCCTCTAGACTCGAGCGTAGCTGATGCATCTCCAAAAATTT
pCMV-Flag-*PSAT1*-WT-F	5’-TCGATAGATCTGATATCGGTACCAATGGACGCCCCCAGGC
pCMV-Flag-*PSAT1*-WT-R	5’-TGAGATGAGTTTTTGTTCGGATCCTAGCTGATGCATCTCCAAAAATTT
PSAT1-K51R-F	5’-GGTCATCAGATTTTGCCAGGATTATTAACAATACAGAGAATCTTGTGCG
PSAT1-K51R-R	5’-CGCACAAGATTCTCTGTATTGTTAATAATCCTGGCAAAATCTGATGACC
PSAT1-K311R-F	5’-TCCGCATTGGCAATGCCAGAGGAGATGATGCTTTAGAAAAAAGATTT
PSAT1-K311R-R	5’-AAATCTTTTTTCTAAAGCATCATCTCCTCTGGCATTGCCAATGCGGA
PSAT1-K323R-F	5’-AAAAAAGATTTCTTGATAGAGCTCTTGAACTCAATATGTTGTCCTT
PSAT1-K323R-R	5’-AAGGACAACATATTGAGTTCAAGAGCTCTATCAAGAAATCTTTTTT
PSAT1-K333R-F	5’-TCAATATGTTGTCCTTGAGAGGGCATAGGTCTGTGGGAGG
PSAT1-K333R-R	5’-CCTCCCACAGACCTATGCCCTCTCAAGGACAACATATTGA
PSAT1-K363R-F	5’-TCGATAGATCTGATATCGGTACCAATGGACGCCCCCAGGC
PSAT1-K363R-R	5’-TGAGATGAGTTTTTGTTCGGATCCTAGCTGATGCATCTCCAAAAATCTT
pcDNA3.1-His-*USP14*-F	5’-TGGCTAGTTAAGCTTGGTACCATGCCGCTCTACTCCGTTACTG
pcDNA3.1-His-*USP14*-R	5’-CGCGGGCCCTCTAGACTCGAGCGCTGTTCACTTTCCTCTTCCATTATTTC
USP14-C114A-F	5’-CAAACCTTGGTAACACTGCTTACATGAATGCCACAGTTCAGTGTA
USP14-C114A-R	5’-TACACTGAACTGTGGCATTCATGTAAGCAGTGTTACCAAGGTTTG
pCMV-HA-*UBE4B*-F	5’-TGGCCATGGAGGCCCGAATTCGGATGGAGGAGCTGAGCGCTG
pCMV-HA-*UBE4B*-R	5’-GATCCCCGCGGCCGCGGTACCTTAGTGATCGCTGTTCTGTTTCTCTC

Primer sequences for RT-PCR

Name	Sequence
GAPDH-F	5’-GGCTGTTGTCATACTTCTCATGG
GAPDH-R	5’-GGAGCGAGATCCCTCCAAAAT
PSAT1-F	5’-TCAGCATCTACGTCATGGGC
PSAT1-R	5’-TTTGGGGCTCCACTGGACAA
HDAC7-F	5’-CAAGGACAAGAGCAAGCGAA
HDAC7-R	5’-CTGCTTCAGAGGTGTGGGGA
USP14-F	5’-CCAGAAGAACCCTCAGCCAAA
USP14-R	5’-CTCAAGGCACCTGCATACCT
UBE4B-F	5’-AGCTTATGATACACAGCACCTG
UBE4B-R	5’-GTCCAAGCTCCTAGGAAATCA

siRNAs sequences

Name	Sequence
CTL siRNA	5’-UUCUCCGAACGUGUCACGU

PSAT1 siRNA1	5’-CUACGUGUAUUAUUGCGCA
PSAT1 siRNA2	5’-GGAAUUAUUAGACUACAA
HDAC7 siRNA1	5’-GCAAGAUCCUCAUUGUAGA
HDAC7 siRNA2	5’-GCUGAUCUAUGACUCGGUCAU
HDAC7 siRNA3	5’-GGUGAGGGCUUCAAUGUCA
USP14 siRNA1	5’- CCUUAGAGAUUUGUUUGAUUCCAUG
USP14 siRNA2	5’- GGAAGCAAUAGAGGAUGAUUCUGUU
USP14 siRNA3	5’- ACGUUGCAAAGAAAUGCCUUGUAUA