

SUPPLEMENTAL INFORMATION

Title: Herpesviruses assimilate kinesin to produce motorized viral particles

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BALB/c mouse body weight following intranasal challenge with HSV-1 strain McKrae (5 µl/nostri)

HSV-1 McKrae produced on WT RPE cells (2.2x10e7 PFU/ml stock)						HSV-1 McKrae produced on ΔKIF5B RPE cells (3.7x10e7 PFU/ml stock)					
	animal #1	animal #2	animal #3	animal #4	animal #5		animal #6	animal #7	animal #8	animal #9	animal #10
day 0*	20.24	22.14	18.06	17.46	18.83	day 0*	19.96	19.90	19.38	20.41	21.41
day 1	20.77	22.70	19.07	17.96	19.75	day 1	20.79	19.99	20.14	20.98	21.53
day 2	20.51	22.49	19.17	18.19	19.69	day 2	20.87	19.99	20.31	21.65	21.69
day 3	20.34	22.55	18.56	18.20	19.80	day 3	21.28	20.40	20.89	21.76	21.79
day 4	19.89	22.36	18.36	17.44	18.06	day 4	21.30	20.54	21.00	22.14	22.13
day 5	17.72	19.88	15.74	15.17	15.85	day 5	21.37	20.37	20.88	22.31	22.27
day 6	16.59	17.50	x	13.86	x	day 6	21.70	20.80	21.15	22.37	22.34
day 7	16.10	16.64		x		day 7	21.95	21.18	21.25	22.72	22.73
day 8	x	16.03				day 8	22.04	21.44	21.36	22.85	23.07
day 9		x				day 9	22.12	21.55	21.29	23.10	23.06
day 10						day 10	22.05	21.49	21.02	23.23	23.02
day 11						day 11	22.16	21.46	20.98	23.35	23.14
day 12						day 12	22.40	21.76	21.31	23.61	23.26
day 13						day 13	22.14	21.75	21.14	23.55	23.31
day 14						day 14	22.34	21.91	21.41	23.87	23.58
day 15						day 15	22.34	22.05	21.53	23.84	23.48
day 16						day 16	22.29	21.95	21.40	23.50	23.11
day 17						day 17	22.82	22.30	21.61	24.10	23.28
day 18						day 18	23.06	22.45	21.70	24.19	23.75
day 19						day 19	22.78	22.62	21.61	24.09	23.72
day 20						day 20	22.91	22.57	21.39	23.32	23.01
day 21						day 21	23.13	23.10	21.69	24.58	23.90
TOD:	171 h	192 h	120 h	157 h	123 h	TOD:	-	-	-	-	-

Supplemental Data 1 Data supporting Figure 2C. (*Mice were 53 days old on day 0)

Identified Protein	Gene name	MW	Spectral Count	Peptide Count	Sequence Coverage	NSAF	empAI	PE	SV	Accession Numbers
Alpha-actinin-4	ACTN4	105	171	54	52.9%	0.02509	2.381	1	2	Q43707
Vinculin	VCL	124	162	47	47.7%	0.02031	1.999	1	4	P18206-2 P18206
Alpha-actinin-1	ACTN1	103	148	44	52.8%	0.02230	2.373	1	2	P12814-2 P12814 P12814-4 P12814-3
Importin-5	IPOS	123	56	28	32.9%	0.00671	1.133	1	4	O00410-3 O00410
Integrin beta-1	ITGB1	86	54	19	27.2%	0.00904	0.871	1	2	P05556
ATP-citrate synthase	ACLY	121	52	26	29.8%	0.00637	0.986	1	3	P53396-2 P53396
Kinesin-1 heavy chain	KIF5B	110	50	20	25.8%	0.00694	0.811	1	1	P33176
Heterogeneous nuclear ribonucleoprotein U	HNRNPU	90	43	13	24.4%	0.00713	0.754	1	6	Q00839-2 Q00839
Heat shock 70 kDa protein 4	HSPA4	94	42	21	39.0%	0.00668	1.455	1	4	P34932
C-1-tetrahydrofolate synthase, cytoplasmic	MTHFD1	102	40	16	20.1%	0.00572	0.589	1	3	P11586
Heat shock protein 105 kDa	HSPH1	97	40	20	25.9%	0.00657	0.816	2	1	Q92598-2 B4DYH1 Q92598 Q92598-3
Neutral alpha-glucosidase AB	GANAB	104	36	18	26.9%	0.00498	0.858	1	3	Q14697-2 Q14697
Transcription intermediary factor 1-beta	TRIM28	88	35	18	42.6%	0.00560	1.667	1	5	Q13263
Exportin-1	XPO1	123	33	16	21.6%	0.00412	0.644	1	1	O14980
Endoplasmic	HSP90B1	90	32	16	23.8%	0.00533	0.730	1	1	P14625
Unconventional myosin-1c	MYO1C	122	31	17	22.4%	0.00397	0.675	2	1	O00159-3 F5H6E2 O00159
Alanine-tRNA ligase, cytoplasmic	AARS	107	30	16	19.6%	0.00414	0.570	1	2	P49588
Staphylococcal nuclease domain-containing protein 1	SND1	102	30	14	21.0%	0.00441	0.622	1	1	Q7KZ4
Exportin-2	CSE1L	110	26	17	25.5%	0.00368	0.799	1	3	P55060-3 P55060
Elongation factor 2	EEF2	95	25	14	27.0%	0.00389	0.862	1	4	P13639
Methionine-tRNA ligase, cytoplasmic	MARS	101	24	11	19.7%	0.00356	0.574	1	2	P56192
Hexokinase-1	HK1	102	22	15	18.2%	0.00321	0.521	2	1	P19367-2 E7ENR4 P19367 P19367-4 P19367-3
Heat shock protein HSP90-alpha	HSP90AA1	85	21	10	15.1%	0.00329	0.416	1	5	P07900-2 P07900
Matrin-3	MATR3	94	21	12	18.5%	0.00331	0.531	1	2	P43243 A8MXP9
Protein transport protein Sec24C	SEC24C	118	20	11	15.1%	0.00244	0.416	1	3	P53992 G5EA31 E7EP00
Major vault protein	MVP	99	20	13	22.2%	0.00299	0.667	1	4	Q14764
Trifunctional purine biosynthetic protein adenosine-3	GART	108	18	11	19.6%	0.00238	0.570	1	1	P22102
Poly [ADP-ribose] polymerase 1	PARP1	113	17	11	17.8%	0.00224	0.507	1	4	P09874
Interleukin enhancer-binding factor 3	ILF3	95	17	9	19.3%	0.00253	0.560	1	3	Q12906-7 Q12906
Transitional endoplasmic reticulum ATPase	VCP	89	15	9	14.4%	0.00249	0.393	1	4	P55072
116 kDa U5 small nuclear ribonucleoprotein component	EFTUD2	109	15	10	16.0%	0.00214	0.445	2	1	Q15029-2 K7EJ81 Q15029
Eukaryotic translation initiation factor 3 subunit C-like protein	EIF3CL	105	14	10	11.7%	0.00205	0.309	3	1	B5ME19 H3BRV0 Q99613
Importin-7	IPO7	120	14	10	12.6%	0.00180	0.337	1	1	O95373
Puromycin-sensitive aminopeptidase	NPEPPS	103	14	6	7.4%	0.00204	0.186	1	2	P55786 E9PLK3
AP-2 complex subunit beta	AP2B1	104	14	11	13.5%	0.00197	0.365	2	1	P63010-2 K7EJ8 P63010 P63010-3
Hepatocyte growth factor-regulated tyrosine kinase substrate	HGS	86	12	5	11.6%	0.00232	0.306	1	1	O14964-2 Q14964
Catenin alpha-1	CTNNA1	100	12	9	14.5%	0.00177	0.396	1	1	P35221 F8W845
AP-1 complex subunit beta-1	AP1B1	105	12	9	10.6%	0.00170	0.276	1	2	Q10567-2 Q10567 Q10567-4 Q10567-3
26S proteasome non-ATPase regulatory subunit 1	PSMD1	106	12	8	15.6%	0.00174	0.432	1	2	Q99460-2 Q99460
AP-2 complex subunit alpha-1	AP2A1	108	11	11	15.7%	0.00154	0.435	1	3	Q95782-2 Q95782
Lon protease homolog, mitochondrial	LONP1	99	11	8	15.3%	0.00167	0.422	1	2	P36776 Q8N8X8 K7EKE6 K7EJE8 F5G227
Splicing factor 3A subunit 1	SF3A1	89	11	7	10.2%	0.00185	0.265	1	1	Q15459
Heterogeneous nuclear ribonucleoprotein U-like protein 2	HNRNPUL2	85	11	6	12.0%	0.00197	0.318	1	1	Q1KMD3
LIM domain and actin-binding protein 1	LIMA1	85	11	4	8.0%	0.00245	0.202	2	1	Q9UH86-2 F8V507 F8VQE1 Q9UH86 Q9UH86-4 Q9UH86-3
Presequence protease, mitochondrial	PITRM1	114	10	6	10.6%	0.00129	0.276	1	3	Q5JRX3-2 Q5JRX3 Q5JRX3-3
General vesicular transport factor p115	USO1	108	9	7	7.1%	0.00124	0.178	2	1	O60763-2 F5H4X1 F5GYR8 O60763
Protein transport protein Sec24D	SEC24D	113	9	7	8.1%	0.00116	0.205	1	2	O94855-2 O94855
DNA replication licensing factor MCM3	MCM3	91	9	7	9.3%	0.00149	0.239	1	3	P25205 J3KQ69 B4DWWW4
Ephrin type-A receptor 2	EPHA2	106	9	6	10.8%	0.00123	0.282	1	2	P29317
Ubiquitin carboxyl-terminal hydrolase 5	USP5	96	9	5	9.7%	0.00144	0.250	1	2	P45974-2 P45974
Neurabin-2	PPP1R9B	89	9	3	9.6%	0.00148	0.247	1	2	Q96583
Ankyrin-2	ANKK1	110	9	9	11.0%	0.00122	0.288	2	1	Q9P0K7-2 E7EMX7 Q9P0K7 Q9P0K7-4 Q9P0K7-3
Heat shock 70 kDa protein 4L	HSPA4L	95	8	3	5.8%	0.00127	0.143	1	3	Q95757 E9PDE8 E7ES43
DNA replication licensing factor MCM2	MCM2	102	8	4	5.3%	0.00118	0.130	1	4	P497336 H0Y8E6
Serrate RNA effector molecule homolog	SRRT	101	8	5	7.8%	0.00123	0.197	2	1	Q9BXP5-2 H7C3A1 Q9BXP5 Q9BXP5-5 Q9BXP5-4 Q9BXP5-3
Endothelin-converting enzyme 1	ECE1	87	7	5	13.0%	0.00124	0.349	2	1	P42892-3 B4DKB2 P42892 P42892-4
Smoothelin	SMTN	99	7	5	9.1%	0.00102	0.233	1	7	P53814-5 P53814 P53814-6
Eukaryotic translation initiation factor 3 subunit B	EIF3B	92	7	5	11.6%	0.00107	0.306	1	3	P55884-2 P55884
MAP7 domain-containing protein 1	MAP7D1	93	7	4	6.8%	0.00117	0.169	1	1	Q3KQU3-2 Q3KQU3 Q3KQU3-4
Pre-mRNA-processing factor 6	PRPF6	107	6	6	8.2%	0.00089	0.208	1	1	Q94906-2 Q94906
POTE ankyrin domain family member J	POTEJ	117	6	3	3.2%	0.00077	0.076	3	1	P0CG39
Integrin alpha-3	ITGA3	114	6	5	5.9%	0.00075	0.146	1	5	P26006-1 P26006
Aspartyl/asparaginyl beta-hydroxylase	ASPH	86	6	3	7.0%	0.00110	0.175	1	3	Q12797-10 Q12797
KN motif and ankyrin repeat domain-containing protein 2	KANK2	91	6	6	10.5%	0.00095	0.274	1	1	Q63Z73-3 Q63Z73
ATP-binding cassette sub-family F member 1	ABCF1	96	6	4	8.7%	0.00099	0.222	3	1	Q8NE71-2 H0YGV7 Q8NE71
Condensin complex subunit 3	NCAPG	114	6	4	5.5%	0.00079	0.135	1	1	Q9BPX3
Extended synaptotagmin-1	ESYT1	123	6	4	5.2%	0.00072	0.127	1	1	Q9BSJ8-2 Q9BSJ8
DNA mismatch repair protein Msh2	MSH2	105	5	5	7.1%	0.00077	0.178	2	1	P43246-2 E9PHA6 P43246
Putative ribosomal RNA methyltransferase NOP2	NOP2	89	5	4	6.3%	0.00083	0.156	1	2	P46087-2 P46087 P46087-4
Unconventional myosin-1e	MYO1E	127	5	4	5.2%	0.00060	0.127	1	2	Q12965
Importin-4	IPO4	119	5	3	5.5%	0.00062	0.135	1	2	Q8TEX9-2 Q8TEX9
Importin-9	IPO9	116	5	5	6.0%	0.00064	0.148	1	3	Q96P70
Nck-associated protein 1	NCKAP1	129	5	5	7.7%	0.00059	0.194	1	1	Q9Y2A7-2 Q9Y2A7

Supplemental Data 2

Kinesin-1 is a structural component of HSV-1 extracellular particles. Although microtubule motors were not previously detected in preparations of HSV-1 and PRV (references 20,21), the presence of kinesin-1 was indicated by the Kif5B-Bla reporter fusion in this study (Fig. 3). Shown here is a tandem mass spectrometry analysis of extracellular wild-type HSV-1 particles enriched for proteins in the range of approximately 100-150 kDa by SDS-PAGE extraction. The results were filtered for proteins with > 4 spectral counts and that were in the expected mass range. The presence of kinesin-1 is highlighted in blue. The lack of kinesin-1 detection in earlier studies may be accounted for by a low virion copy number. The current analysis benefited from advances in mass spectrometry and by enriching for proteins by mass prior to analysis.

Supplemental Data 3 Data derived from automated analysis of capsid localization that supports Figures 1C. The spreadsheet includes all formula for post-analysis of automated data pipeline.

****Data is provided as a separate Excel file**

Supplemental Data 4 Data derived from automated analysis of capsid localization that supports Figures 2D. These spreadsheet includes all formula for post-analysis of automated data pipeline.

****Data is provided as a separate Excel file**

Supplemental Video 1 Motility of transiently expressed pUL36 in Vero and hTERT-RPE cells. The gene encoding pUL36 was cloned from PRV strain Becker as a GFP fusion and truncated for the C-terminal 115aa to prevent autoinhibition (pGS3384 in Extended Data Table 1). Recordings at left document representative cells displaying curvilinear transport. Recording at right exemplifies protein accumulation at the cell periphery and juxtannuclear. Images were captured at 10 frames/s.

****Movie is provided as a separate .mov file**

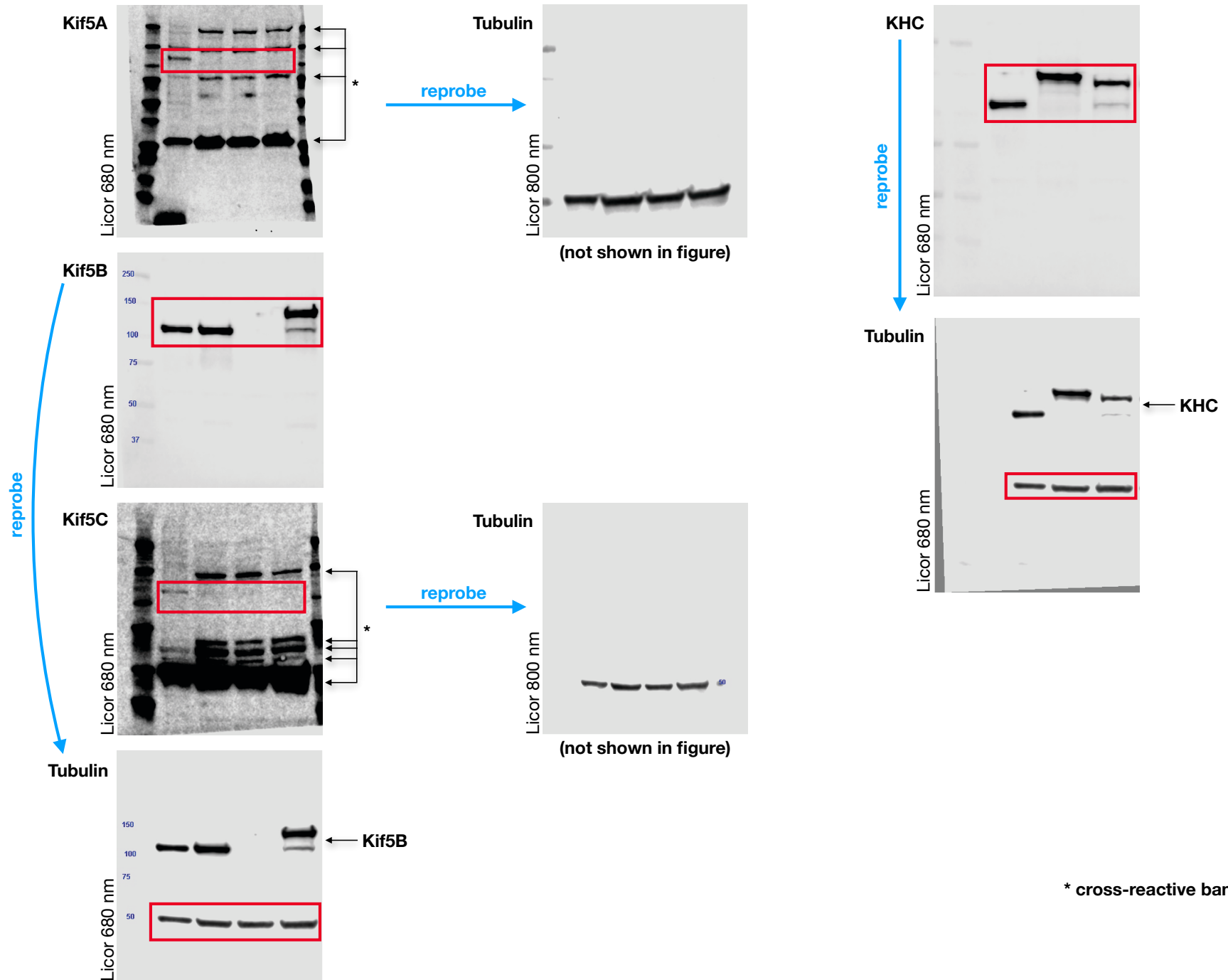
Supplemental Video 2 Capsid motility in the vicinity of neuronal nuclei. Explanted chick dorsal root ganglia were infected with PRV-GS4284 (encodes a red-fluorescent capsid tag; pUL25/mCherry). Images were captured at 10 frames/s between 1-2 hpi.

****Movie is provided as a separate .mov file**

Supplemental Figure 1 Compilation of unprocessed western blots used in the production of the figures. These images immediately follow in the remainder of this document.

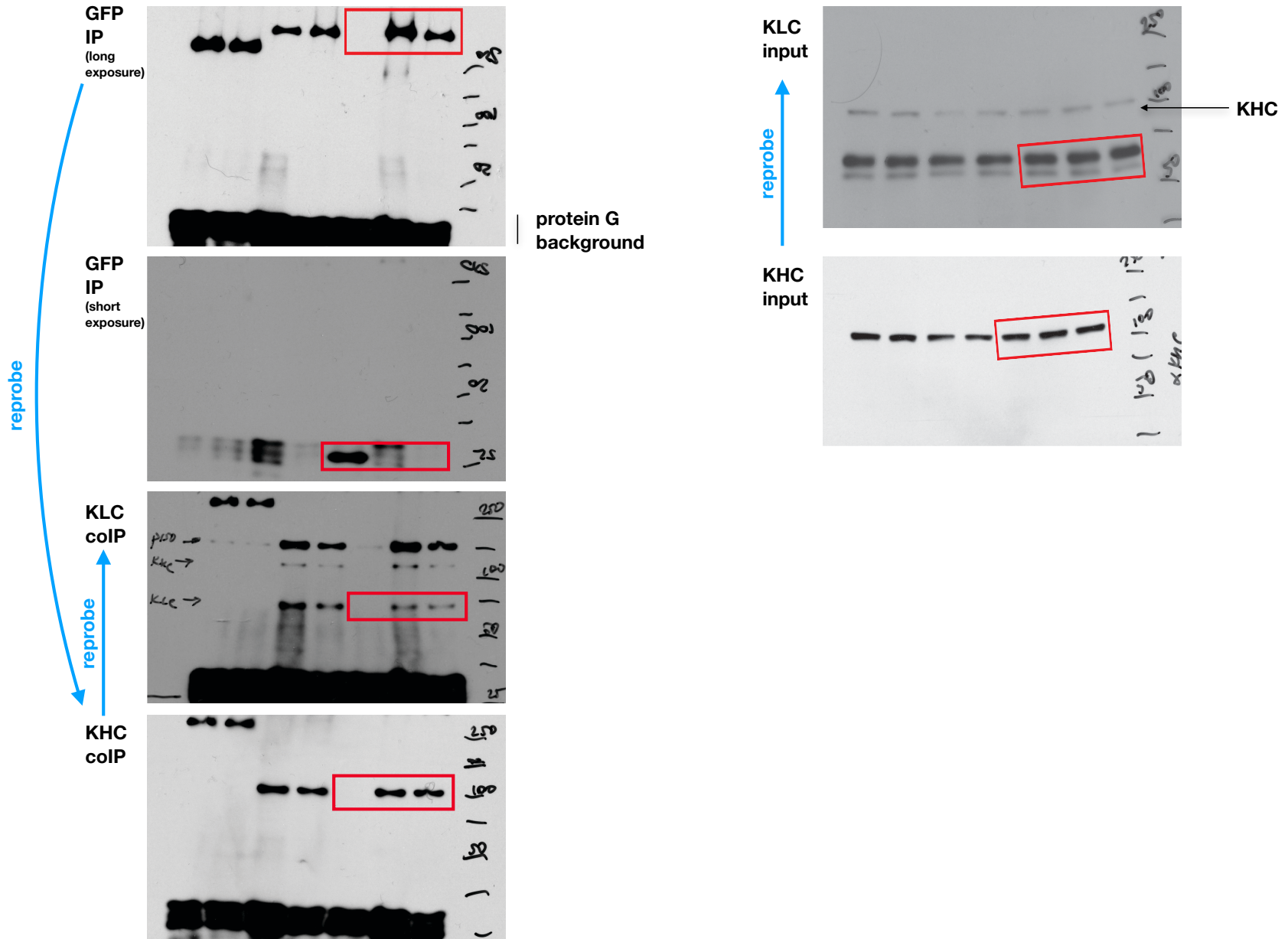
Sup Figure 1

Related to Fig. 1a

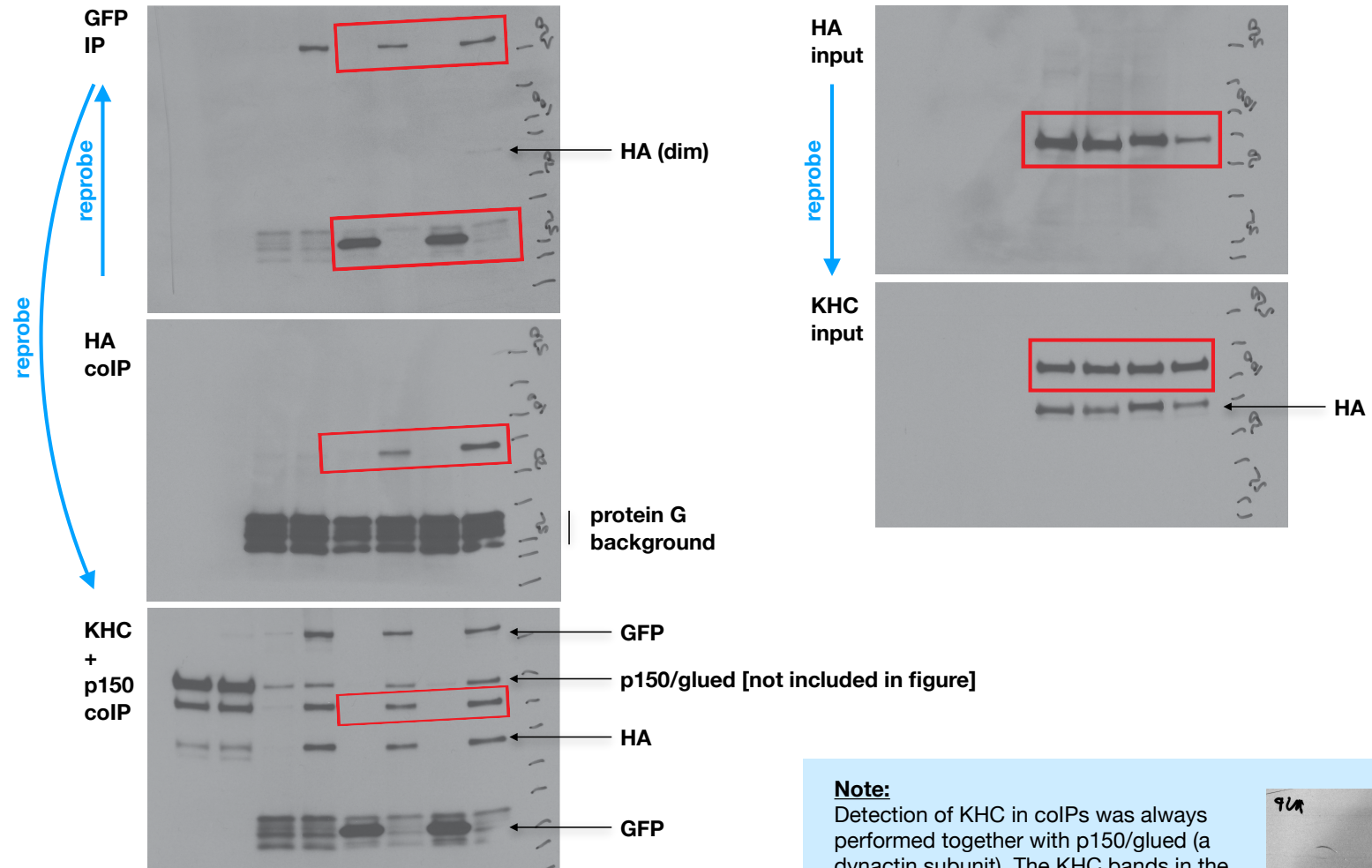


* cross-reactive bands

Related to Extended Data Fig. 2a

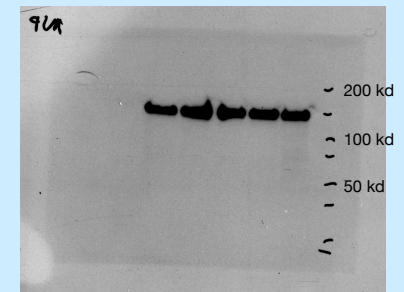


Related to Extended Data Fig. 2c

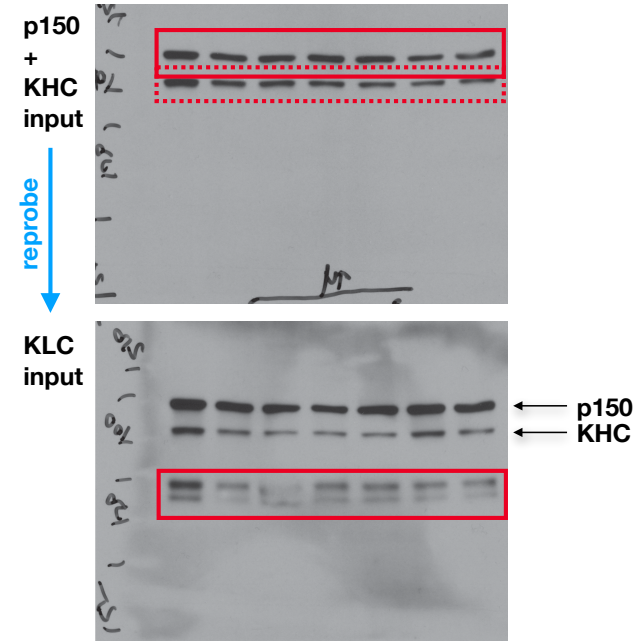
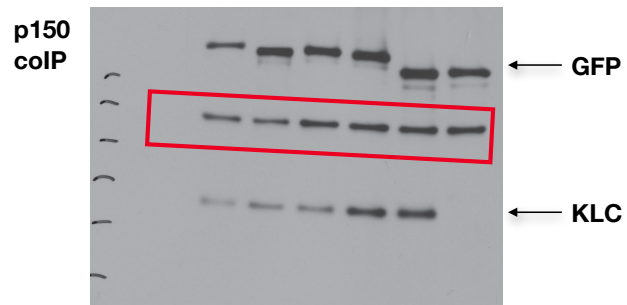
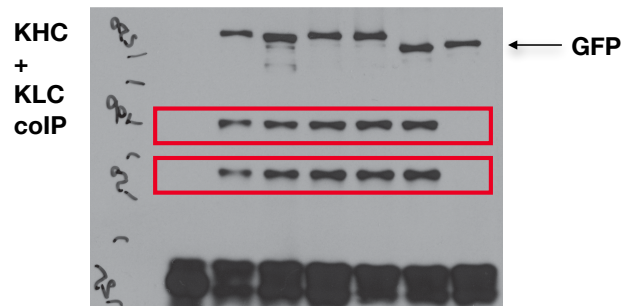
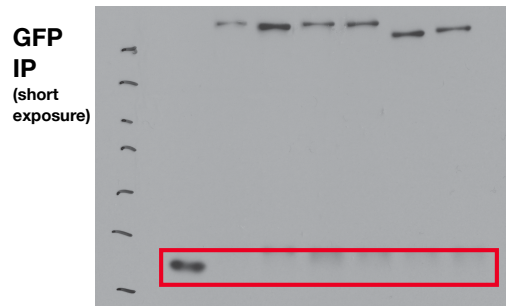
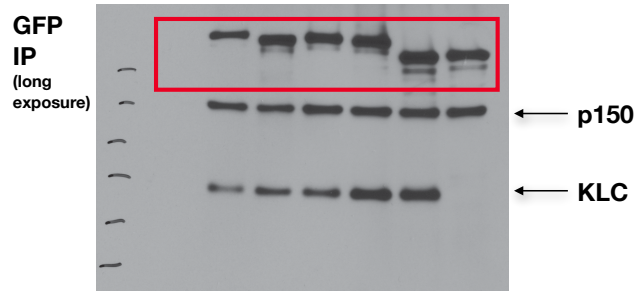


Note:

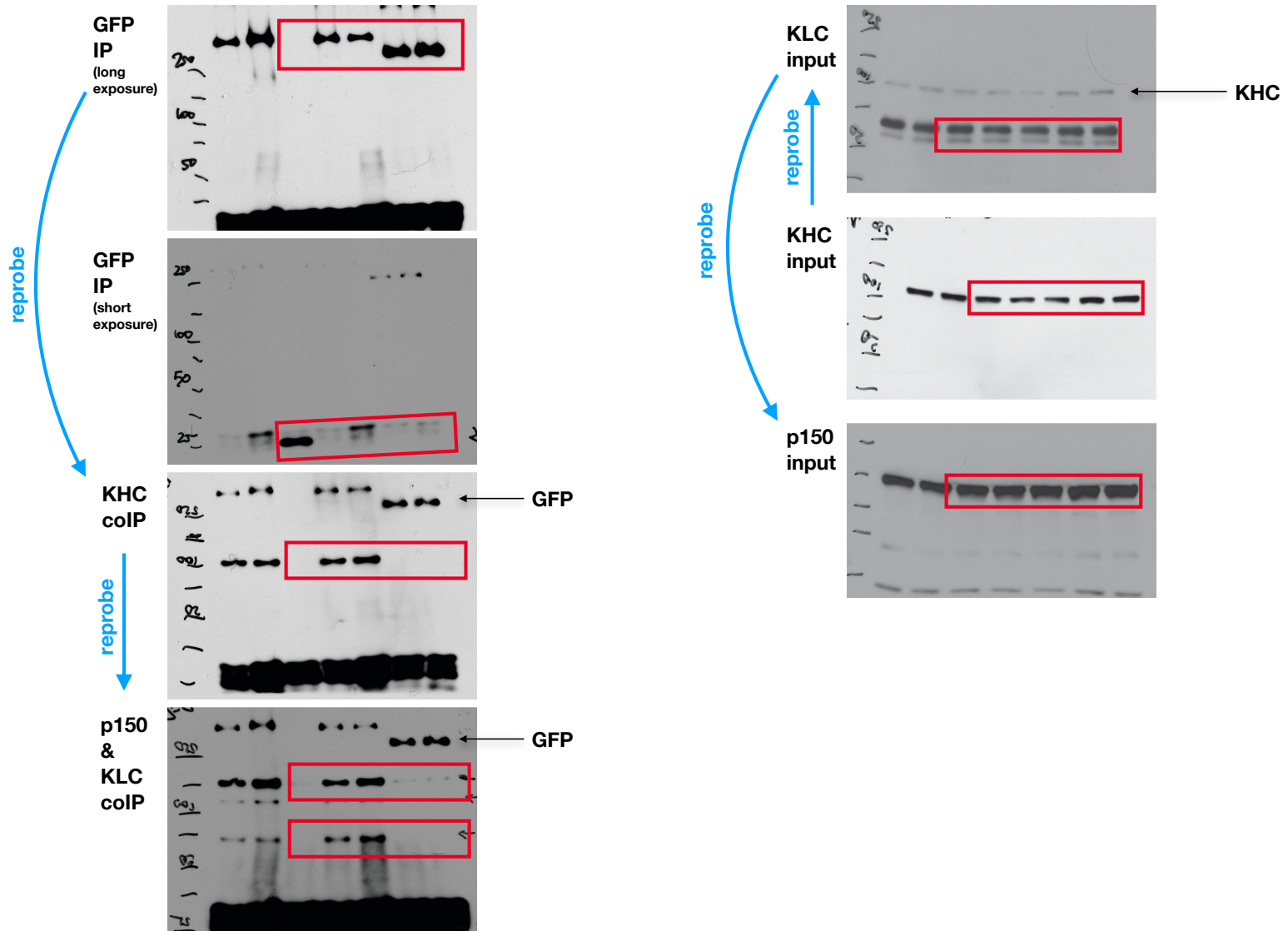
Detection of KHC in colPs was always performed together with p150/glued (a dynactin subunit). The KHC bands in the colP correspond in MW to those observed in the KHC input blot. In further support that the bands identified as KHC are not a p150/glued product, we include a blot of p150/glued using the same cells (HEK293) to document that the Ab detects a single 150 kD species (right).



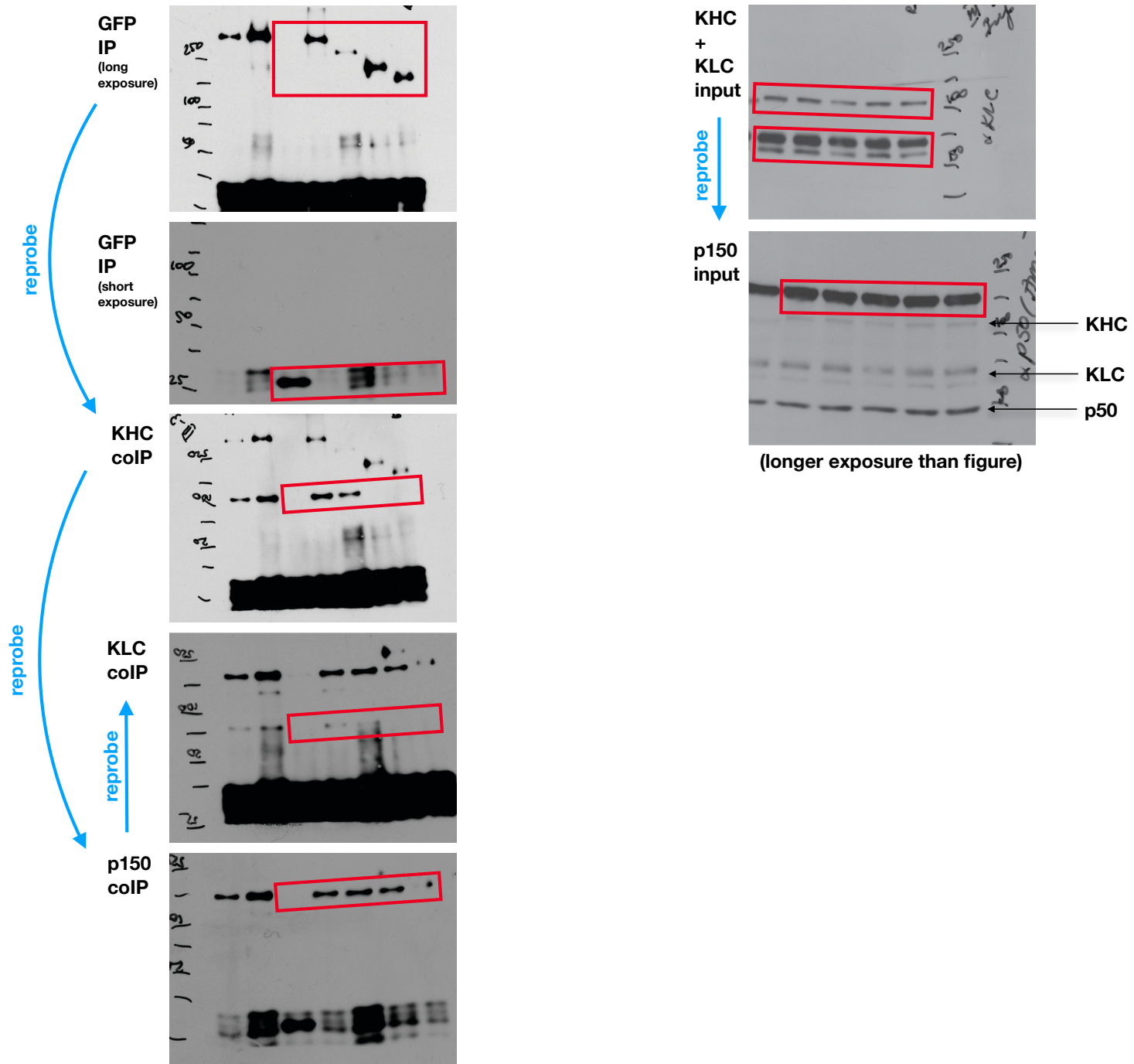
Related to Extended Data Fig. 3b



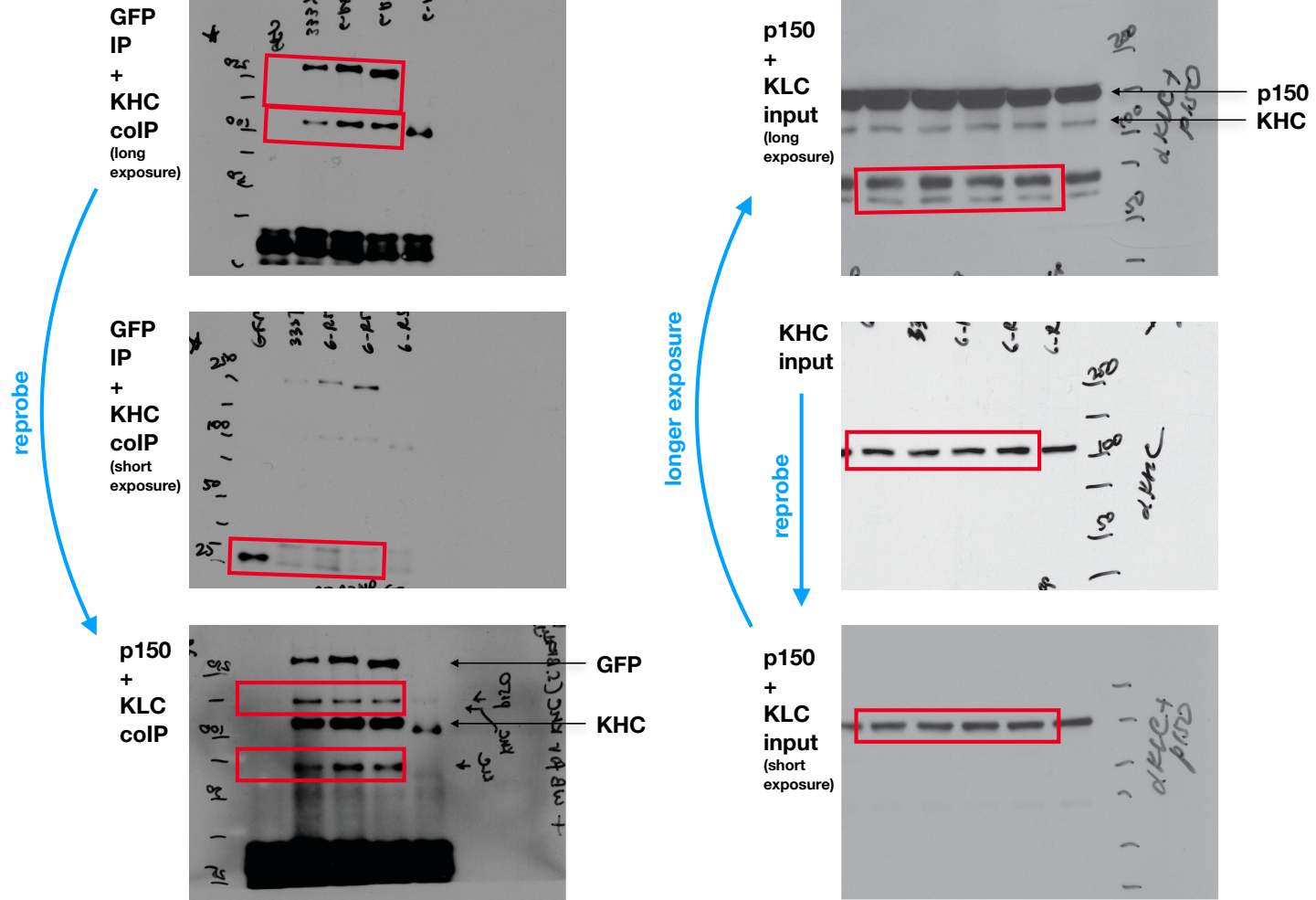
Related to Extended Data Fig. 3c



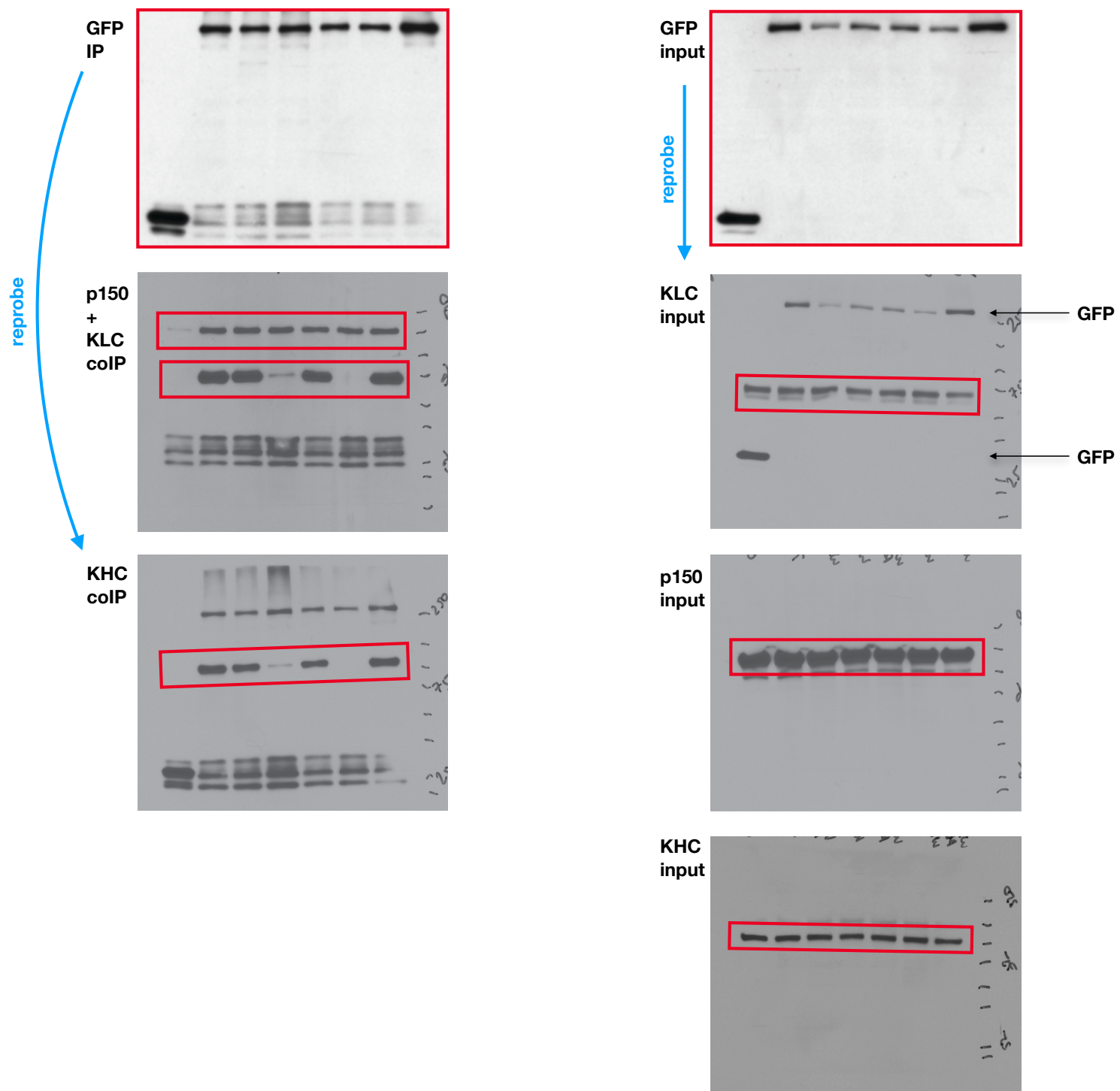
Related to Extended Data Fig. 3d



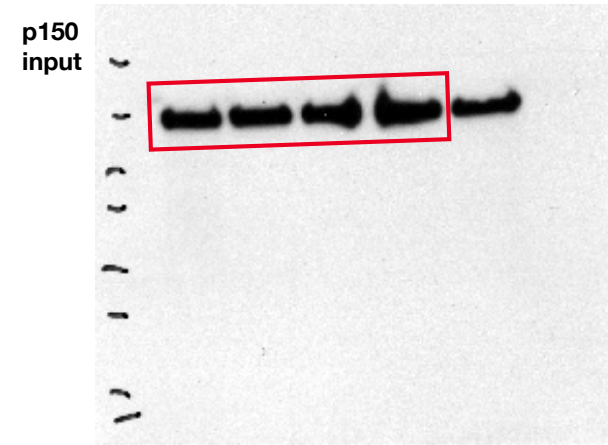
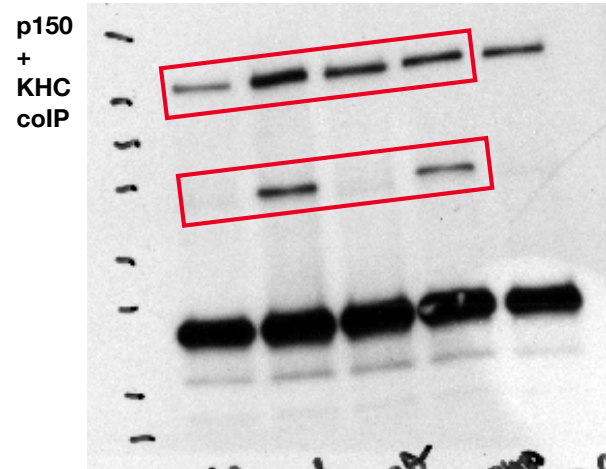
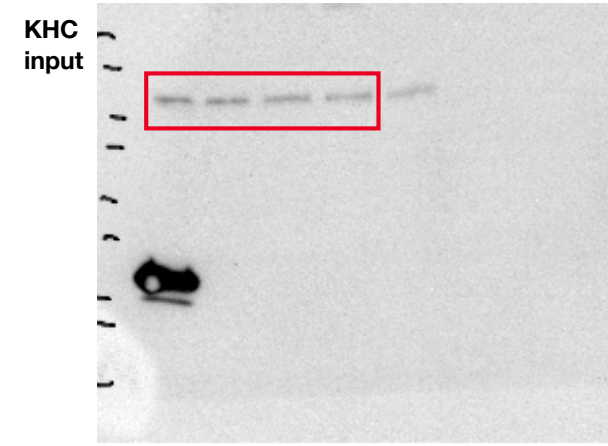
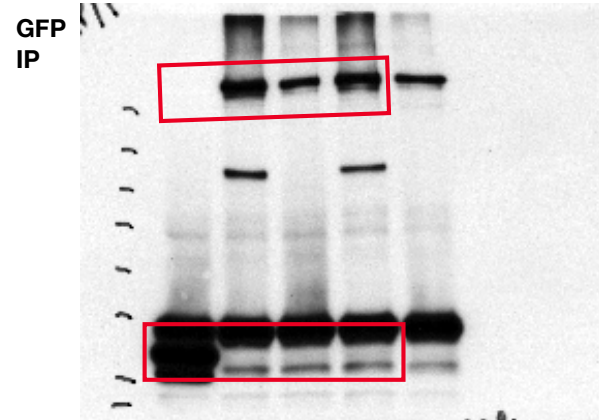
Related to Extended Data Fig. 3e



Related to Extended Data Fig. 4b

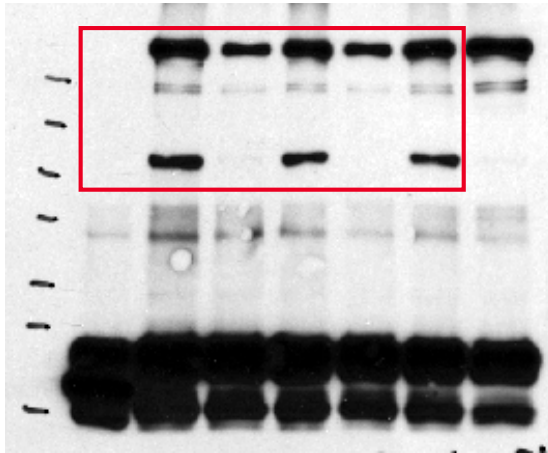


Related to Extended Data Fig. 4c

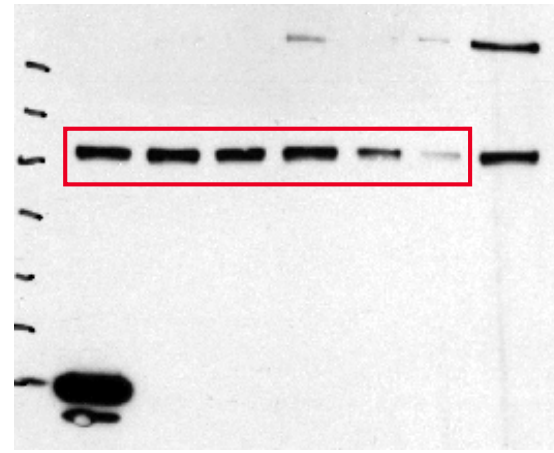


Related to Extended Data Fig. 4d

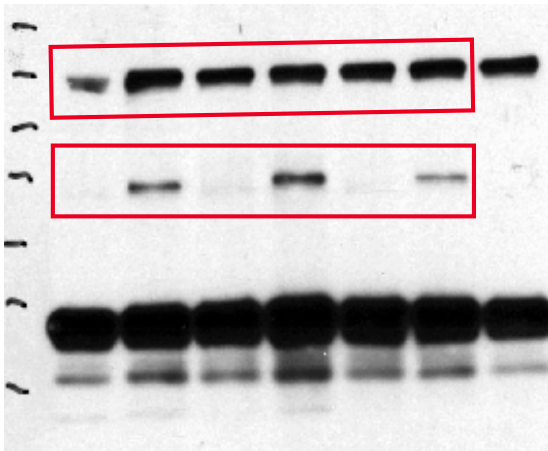
GFP
IP
+
KHC
coIP



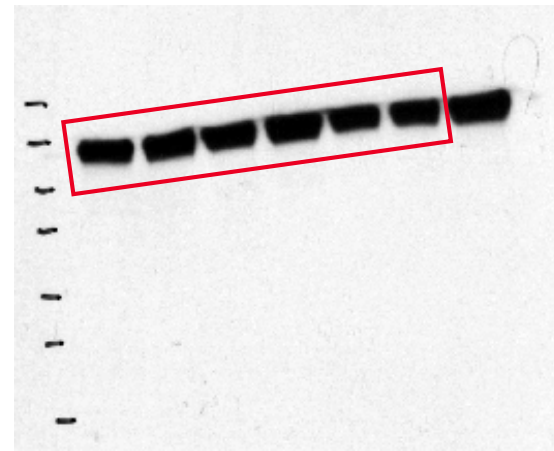
KHC
input



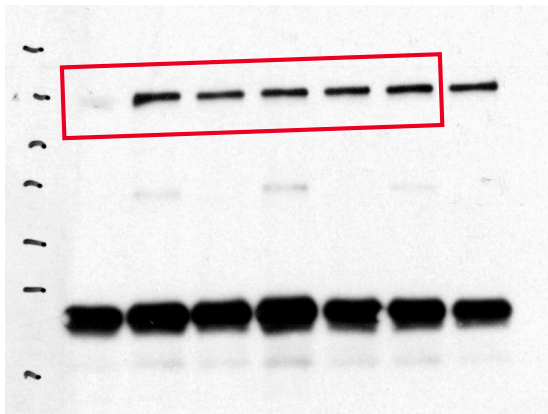
p150
+
KLC
coIP
(long
exposure)



p150
input



p150
+
KLC
coIP
(short
exposure)



Related to Extended Data Fig. 7a

