

Supplementary Information

A proximity-labeling proteomic approach to investigate invadopodia molecular landscape in breast cancer cells

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Supplementary Table S1: List of proteins biotinylated by Tks5-BirA* fusion protein. Protein list results from the comparison of proteins biotinylated by Tks5-BirA* fusion protein with proteins biotinylated by BirA* control protein, after their isolation by affinity capture and identification by mass spectrometry. Proteins listed here correspond to proteins above the full line indicative of protein hits obtained at a pFDR of 1% on Fig.3A volcano plot. For each protein their link to invadosome, their implication in actin cytoskeleton organization and their link to cancer invasion and metastasis have been analyzed, by comparison to the list of proteins obtained for "invadosome" query by GLAD4U literature mining and manual literature searching. The presence of Proline-rich regions and SH3 domains determined by InterPro site is also referenced. Data result from two different experiments processed three times.

Supplementary Table S2: List of proteins biotinylated by Δ PX-Tks5-BirA* fusion protein. Protein list results from the comparison of proteins biotinylated by Δ PX-Tks5-BirA* fusion protein with proteins biotinylated by BirA* control protein, after their isolation by affinity capture and identification by mass spectrometry. Proteins listed here correspond to proteins hits obtained at a pFDR of 1%. Data result from two different experiments processed three times.

Supplementary Table S3: Tables listing proteins biotinylated by both Tks5-BirA* and Δ PX-Tks5-BirA* (left), only by Tks5-BirA* (middle) and only by Δ PX-Tks5-BirA* (right) fusion proteins. Proteins listed here correspond to proteins highlighted in red, blue and green, respectively, on Fig.4A volcano plot. Tables result from the intersection of Tks5-BirA* (Supplementary Table S1) and Δ PX-Tks5-BirA* (Supplementary Table S2) close neighbors. Proteins biotinylated by both Tks5-BirA* fusion proteins correspond to proteins identified as different (pFDR of 1%) in each of the fusion protein relative to BirA* control condition. Proteins designed as Tks5-BirA* or Δ PX-Tks5-BirA* only correspond to proteins different (pFDR of 1%) only in the fusion protein addressed relative to BirA* control condition. For each protein, its difference of abundance in Tks5-BirA* fusion proteins *versus* BirA* conditions is indicated and its difference of abundance in Tks5-BirA* *versus* Δ PX-Tks5-BirA* conditions has been calculated and is reported in the third column. ⁽¹⁾ Grey-colored columns correspond to proteins with no significant differential of abundance in the Tks5-BirA* fusion protein analyzed relative to BirA* control condition.

Supplementary Table S4: Primers pairs used for amplifying Tks5 and Δ PX-Tks5 sequences.

Supplementary Figure 1: (A) Analysis of the efficiency of Tks5 knockdown by western blotting. MDA-MB-231 cells stably expressing BirA*, Tks5-BirA* or Δ PX-Tks5-BirA* were transfected with a siRNA directed against the 3'UTR of Tks5 mRNA (siTks5) or with a control siRNA directed against LacZ mRNA (siLacZ) for 36 h. An anti-Tks5 antibody was used to analyze Tks5 levels. α -Tubulin was used as a loading control. The efficiency of Tks5 knockdown is clearly observed in BirA* expressing cells. In cells expressing Tks5-BirA* fusion proteins, degradation products coincided with the size of endogenous Tks5 making it difficult to visualize the efficiency of Tks5 knock-down in these cells. **(B) Analysis of the biotinylation of newly identified Tks5 neighbors in presence or absence of endogenous Tks5.** MDA-MB-231 cells stably expressing BirA*, Tks5-BirA* or Δ PX-Tks5-BirA* were transfected with a siRNA against the 3'UTR of Tks5 mRNA (siTks5) or with a siRNA control directed against LacZ mRNA (siLacZ) for 36 h and then seeded on gelatin in presence of biotin for 16 h. After cell lysis, biotinylated proteins were isolated by affinity capture using avidin-coated beads. The presence of proteins of interest was assessed in whole cell extract (WCE) or after avidin-based pulldown by western blotting with specific antibodies. Knock-down of endogenous Tks5 had no impact on the efficiency of Tks5-BirA fusion proteins to biotinylate newly identified Tks5 close neighbors. Full-length blots are presented in Supplementary Figure 2.

Supplementary Figure 2: Full-size blots/gels from Figures 1C, 2B-D, 4B and Supplementary Figure 1.

Supplementary Table S2: List of proteins biotinylated by ΔPX-Tks5-BirA* fusion protein.

Protein list results from the comparison of proteins biotinylated by ΔPX-Tks5-BirA* fusion protein with proteins biotinylated by BirA* control protein, after their isolation by affinity capture and identification by mass spectrometry. Proteins listed here correspond to proteins hits obtained at a pFDR of 1%. Data result from two different experiments processed three times.

Protein IDs	Protein names	Gene names	Peptides	Sequence coverage [%]	Mol. weight [kDa]	p-value (-Log10)	Differential (Log2) ΔPX-Tks5-BirA* versus BirA*
Q5TCZ1;A1X283	SH3 and PX domain-containing protein 2A	SH3PXD2A	120	81	125.29	14.00469377	16.79133638
Q14247	Src substrate cortactin	CTTN	28	50.5	61.585	10.5672796	7.658259074
P49959	Double-strand break repair protein MRE11A	MRE11A	18	35.9	80.592	7.46094941	7.174569766
Q9Y5K6	CD2-associated protein	CD2AP	13	24.3	71.45	7.966618016	6.196915785
Q96RU3	Formin-binding protein 1	FNBP1	6	12.5	71.306	4.670416997	5.608518759
O60732	Melanoma-associated antigen C1	MAGEC1	6	8.5	123.64	5.166906572	5.325631301
Q14195	Dihydropyrimidinase-related protein 3	DPYSL3	12	30.4	61.963	4.583939755	5.090364933
Q86T24	Transcriptional regulator Kaiso	ZBTB33	3	8.8	74.484	7.226418622	4.578668594
Q969G3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1	SMARCE1	2	8	46.649	2.205633135	4.180418491
Q15652	Probable JmjC domain-containing histone demethylation protein 2C	JMJD1C	3	1.8	284.52	5.743897208	4.147589048
Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2	IGF2BP2	3	8.5	66.121	5.538307203	3.866715272
Q96EB1	Elongator complex protein 4	ELP4	6	24.1	46.587	8.216873652	3.530961037
O00151	PDZ and LIM domain protein 1	PDLIM1	4	27.7	36.071	3.974932048	3.492241383
Q9UKK9	ADP-sugar pyrophosphatase	NUDT5	4	23.3	24.327	4.395425637	3.363261541
Q8TE02	Elongator complex protein 5	ELP5	5	24.1	34.841	3.549250869	3.296580474
P50479	PDZ and LIM domain protein 4	PDLIM4	2	10.6	35.398	4.918884984	3.1442132
Q726K5	Arpin	ARPIN	1	5.3	24.943	5.78745232	3.105995337
P31946	14-3-3 protein beta/alpha;14-3-3 protein beta/alpha, N-terminally processed	YWHAH	6	40.7	28.082	3.102797521	2.770226796
Q5T0N5	Formin-binding protein 1-like	FNBP1L	3	8.6	70.065	3.163598993	2.732745806
P61981	14-3-3 protein gamma;14-3-3 protein gamma, N-terminally processed	YWHAG	5	32.4	28.302	3.841400861	2.722501755
P27816	Microtubule-associated protein 4	MAP4	16	20.4	121	6.926183764	2.682752927
Q9NS25	Sperm protein associated with the nucleus on the X chromosome B/F	SPANXB1	2	27.2	11.84	5.73212081	2.642450651
Q9NS26	Sperm protein associated with the nucleus on the X chromosome A	SPANXA1					
Q07157	Tight junction protein ZO-1	TJP1	24	20.7	195.46	8.285232815	2.545808792
Q0PNE2	Elongator complex protein 6	ELP6	2	9.8	29.793	2.754979228	2.469645182
Q9Y4E8	Ubiquitin carboxyl-terminal hydrolase 15	USP15	1	2.1	112.42	3.096935139	2.253142675
O43426	Synaptojanin-1	SYNJ1	2	3.1	173.1	4.567370702	2.237295151
P29401	Transketolase	TKT	4	11.1	67.877	2.172138733	2.136732419
P55209	Nucleosome assembly protein 1-like 1	NAP1L1	2	5.4	45.374	3.424767194	2.123708725
Q99733	Nucleosome assembly protein 1-like 4	NAP1L4					
Q9HCN4	GNP-loop GTPase 1	GPN1	1	4.3	41.74	3.390386248	2.03527228
P23588	Eukaryotic translation initiation factor 4B	EIF4B	2	6.2	69.15	2.576545034	1.942392349
O75815	Breast cancer anti-estrogen resistance protein 3	BCAR3	2	3.3	92.565	2.435325241	1.941552321
P62258	14-3-3 protein epsilon	YWHAH	9	37.6	29.174	7.019493727	1.934883118
Q9UJU6	Drebrin-like protein	DBNL	1	2.6	48.207	1.841725873	1.888232867
Q9UDY2	Tight junction protein ZO-2	TJP2	14	17.2	133.96	8.786069397	1.886053403
Q9UPQ0	LIM and calponin homology domains-containing protein 1	LIMCH1	2	2.7	121.87	4.156091474	1.855589549
P40763	Signal transducer and activator of transcription 3	STAT3	1	3.4	88.067	2.973856904	1.832819303
Q13162	Peroxisome oxidin-4	PRDX4	2	8.1	30.54	2.654100435	1.831843694
Q9UHB6	LIM domain and actin-binding protein 1	LIMA1	4	7.4	85.225	3.11598462	1.791516304
P0DMV9	Heat shock 70 kDa protein 1B	HSPA1B	37	70.2	70.051	8.983589311	1.561729113
P0DMV8	Heat shock 70 kDa protein 1A	HSPA1A					
P49023	Paxillin	PXN	8	23.2	64.505	5.453557229	1.528698921
P63104	14-3-3 protein zeta/delta	YWHAZ	10	45.3	27.745	4.353593263	1.456914902
P34931	Heat shock 70 kDa protein 1-like	HSPA1L	15	29.8	70.374	4.714809086	1.392109235
O60711	Leupaxin	LPXN	1	5.7	43.332	4.515592353	1.385031064
P49321	Nuclear autoantigenic sperm protein	NASP	4	11.4	85.237	6.546653084	1.354332606
Q09666	Neuroblast differentiation-associated protein AHNAK	AHNAK	104	39.7	629.09	6.490538917	1.249121666

Supplementary Table S3: Tables listing proteins biotinylated by both Tks5-BirA* and ΔPX-Tks5-BirA* (left), only by Tks5-BirA* (middle) and only by ΔPX-Tks5-BirA* (right) fusion proteins.

Proteins listed here correspond to proteins highlighted in red, blue and green, respectively, on Fig.4A volcano plot

Tables result from the intersection of Tks5-BirA* (Supplementary Table S1) and ΔPX-Tks5-BirA* (Supplementary Table S2) close neighbors.

Proteins biotinylated by both Tks5-BirA* fusion proteins correspond to proteins identified as different (pFDR < 1%) in each of the fusion protein relative to BirA* control condition.

Proteins designed as Tks5-BirA* or ΔPX-Tks5-BirA* only correspond to proteins different (pFDR < 1%) only in the fusion protein addressed relative to BirA* control condition.

For each protein, its difference of abundance in Tks5-BirA* fusion proteins versus BirA* conditions is indicated, and its difference of abundance in Tks5-BirA* versus ΔPX-Tks5-BirA* conditions has been calculated and is reported in the third column.

⁽¹⁾ Grey-colored columns correspond to proteins with no significant differential of abundance in the Tks5-BirA* fusion protein analyzed relative to BirA* control condition.

In both (27)	Differential (Log2) Tks5-	Differential (Log2) ΔPX-	Differential (Log2) Tks5-
	BirA* versus BirA*	Tks5-BirA* versus BirA*	BirA* versus ΔPX-Tks5-BirA*
MAP4	4.93	2.68	2.25
CD2AP	7.23	6.20	1.03
MRE11A	7.61	7.17	0.44
MAGEC1	5.63	5.33	0.31
SPANXA1/SPANXB1	2.94	2.64	0.30
DBNL	2.13	1.89	0.24
PRDX4	1.87	1.83	0.04
NASP	1.39	1.35	0.04
NAP1L1/NAP1L4	2.08	2.12	-0.05
BCAR3	1.87	1.94	-0.07
CTTN	7.52	7.66	-0.14
SYNJ1	2.07	2.24	-0.17
HSPA1A/HSPA1B	1.29	1.56	-0.28
LIMCH1	1.58	1.86	-0.28
ELP5	2.90	3.30	-0.39
TJP1	2.10	2.55	-0.44
TJP2	1.40	1.89	-0.49
USP15	1.75	2.25	-0.50
NUDT5	2.82	3.36	-0.55
ELP6	1.78	2.47	-0.69
ELP4	2.81	3.53	-0.72
ARPN	2.35	3.11	-0.76
PDLIM1	2.70	3.49	-0.79
IGF2BP2	2.99	3.87	-0.88
FNBP1	4.44	5.61	-1.17
PDLIM4	1.87	3.14	-1.28
DPYSL3	3.64	5.09	-1.45

In Tks5-BirA* only (13)	Differential (Log2) Tks5-	Differential (Log2) ΔPX-	Differential (Log2) Tks5-
	BirA* versus BirA*	Tks5-BirA* versus BirA* ⁽¹⁾	BirA* versus ΔPX-Tks5-BirA*
RTN4	7.34	1.74	5.60
FGD1	4.93	0.00	4.93
SHC1	3.88	1.47	2.40
MIB1	2.92	2.64	0.27
ANTXR1	2.78	1.15	1.64
ADAM15	2.63	-0.38	3.01
RAPH1	2.17	1.44	0.72
KIF2C	2.00	1.11	0.88
ARAF	1.83	1.22	0.61
PRPS2	1.75	1.30	0.45
EIF4A3	1.74	-0.47	2.21
EIF2S3/EIF2S3L	1.52	1.16	0.36
RPIA	1.41	1.10	0.31

In ΔPX-Tks5-BirA* only (17)	Differential (Log2) Tks5-	Differential (Log2) ΔPX-	Differential (Log2) Tks5-
	BirA* versus BirA* ⁽¹⁾	Tks5-BirA* versus BirA*	BirA* versus ΔPX-Tks5-BirA*
ZBTB33	0.86	4.58	-3.72
SMARCE1	0.00	4.18	-4.18
JMJD1C	1.13	4.15	-3.02
YWHAB	0.22	2.77	-2.55
FNBP1L	1.36	2.73	-1.38
YWHAG	0.22	2.72	-2.50
TKT	1.54	2.14	-0.59
GPN1	0.64	2.04	-1.39
EIF4B	1.51	1.94	-0.43
YWHAE	0.64	1.93	-1.29
STAT3	1.15	1.83	-0.68
LIMA1	1.31	1.79	-0.48
PXN	1.16	1.53	-0.37
YWHAZ	0.35	1.46	-1.11
HSPA1L	1.06	1.39	-0.33
LPXN	0.77	1.39	-0.62
AHNAK	1.18	1.25	-0.07

Supplementary Table S4 : Primer pairs used for amplifying Tks5 and Δ PX-Tks5 sequences

Name	Sequence
attB1-Nter Tks5	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGCTCGC CTA CTGCGTGCAGGATG-3'
attB1-delPX Tks5	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGGAGGC TCGACCCGAGGATGTC-3'
attB2-Cter Tks5	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCGTTCTTTTCTCAAGGTAGTTGGAAG-3'

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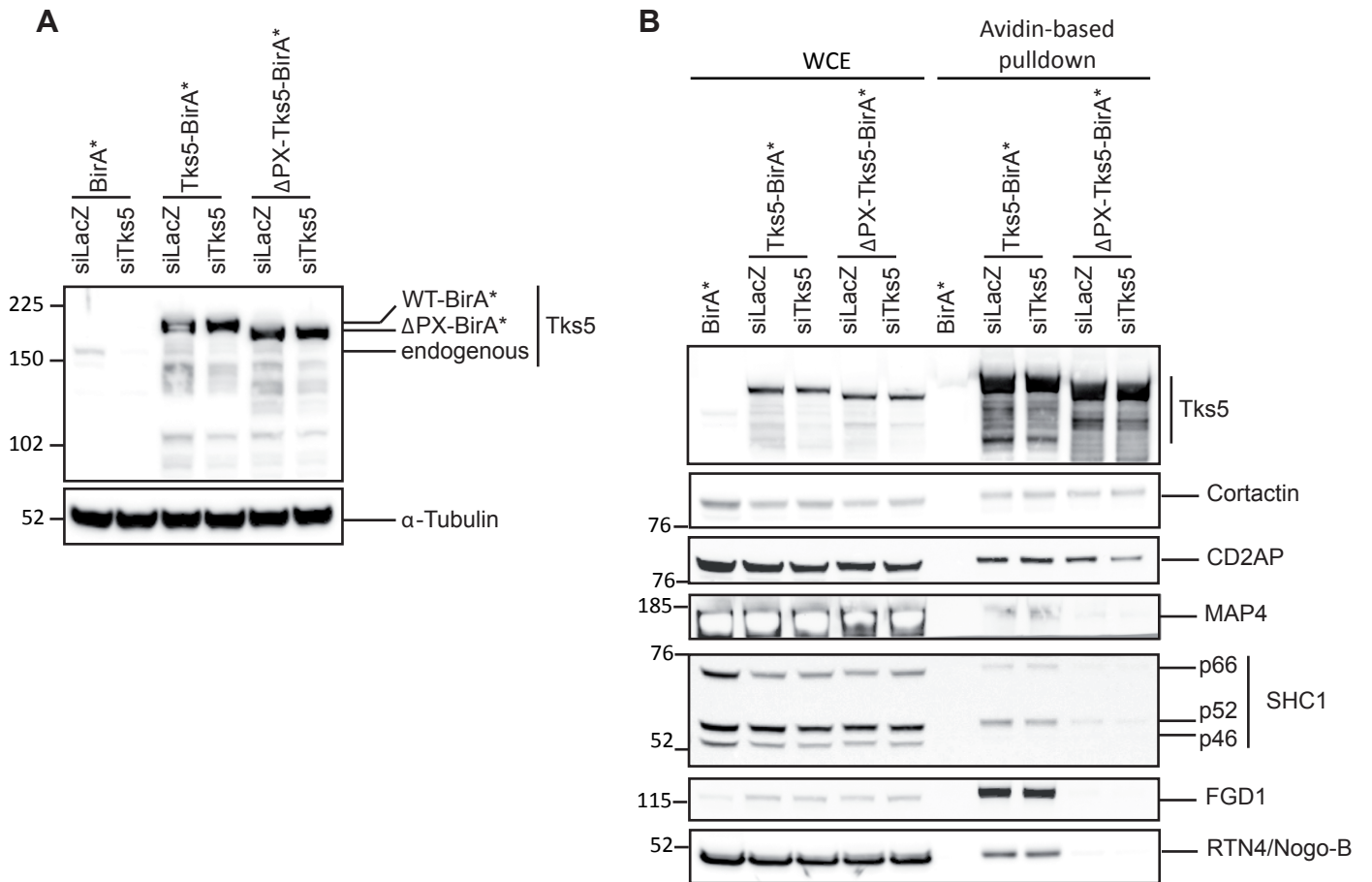


Figure 1C

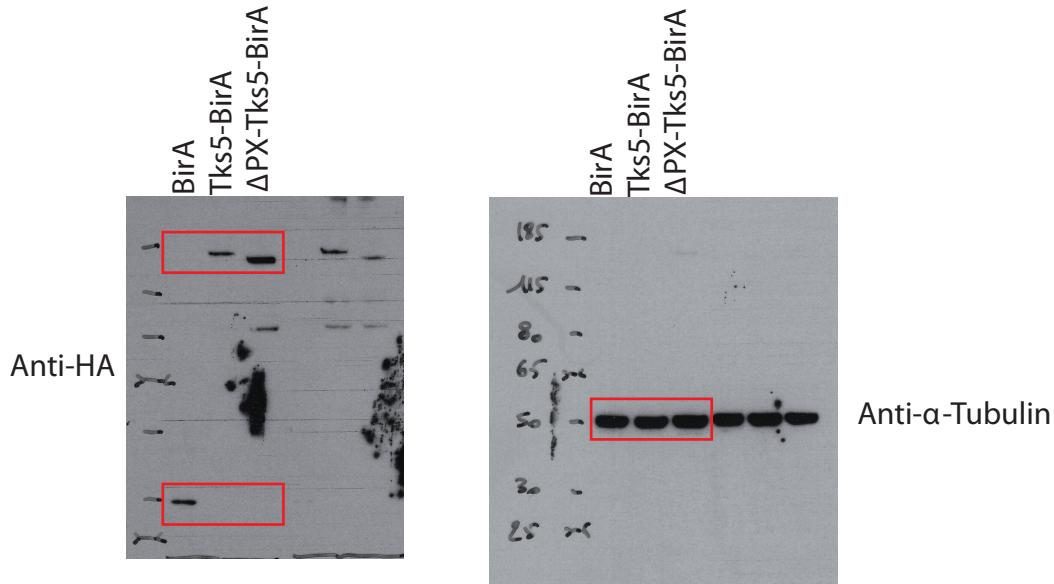


Figure 2B

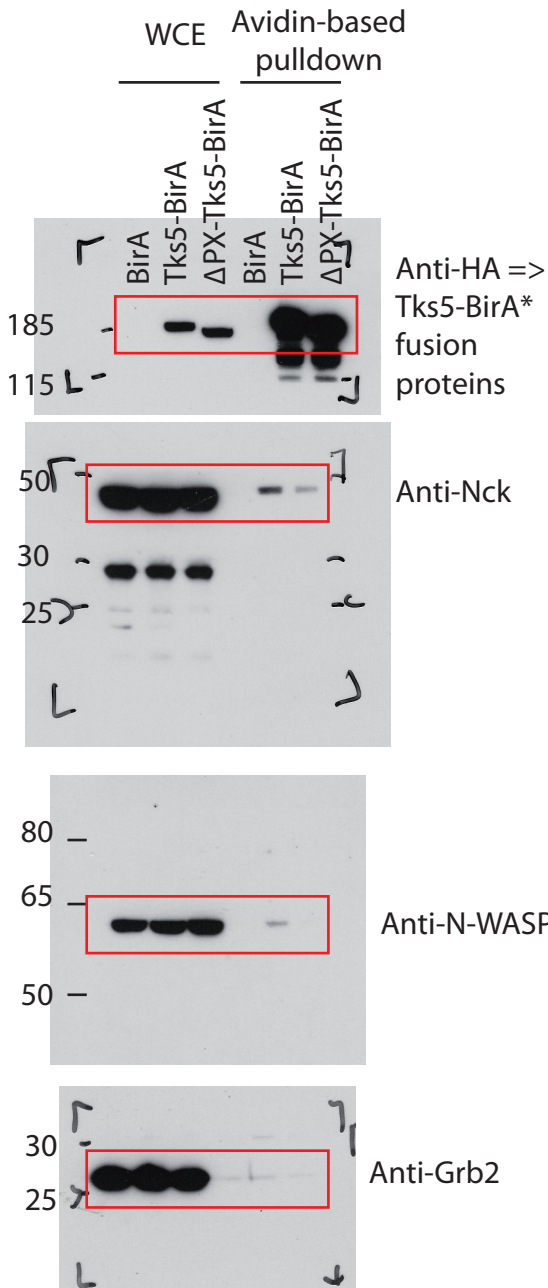


Figure 2C

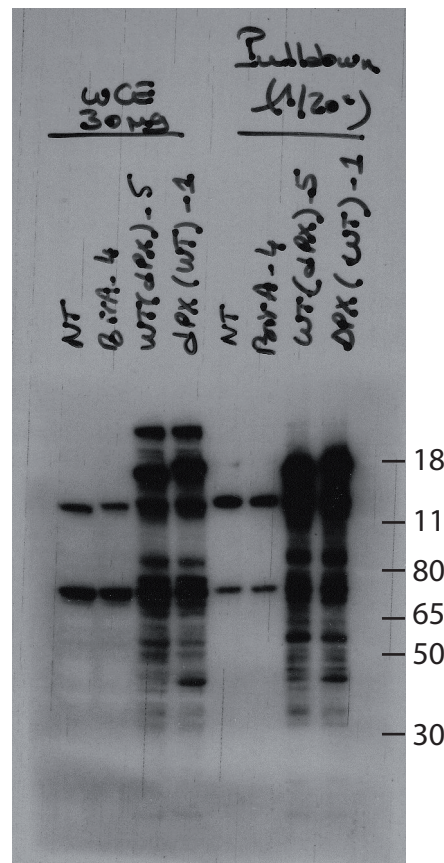


Figure 2D

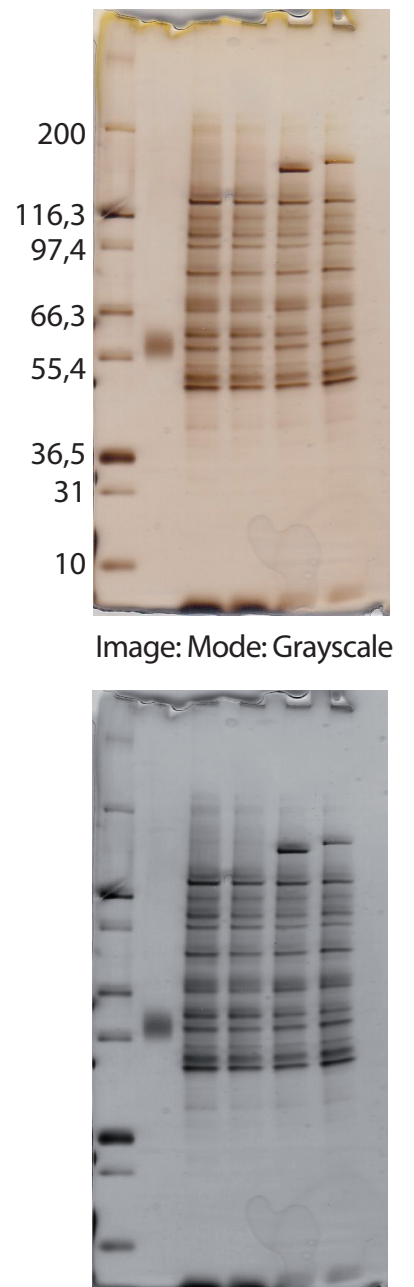
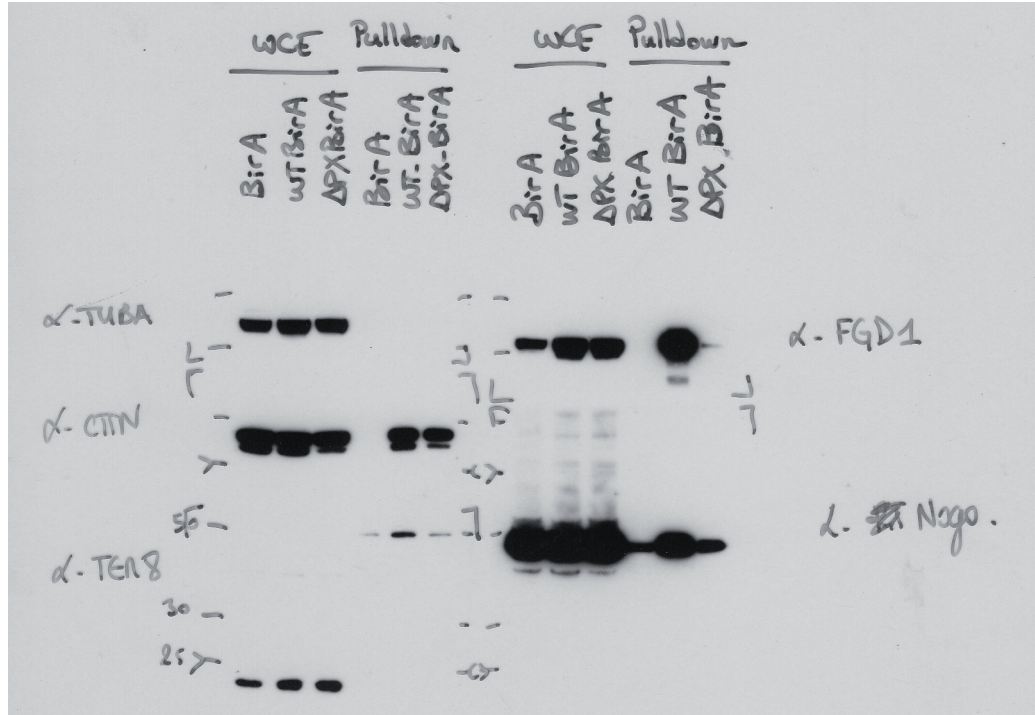
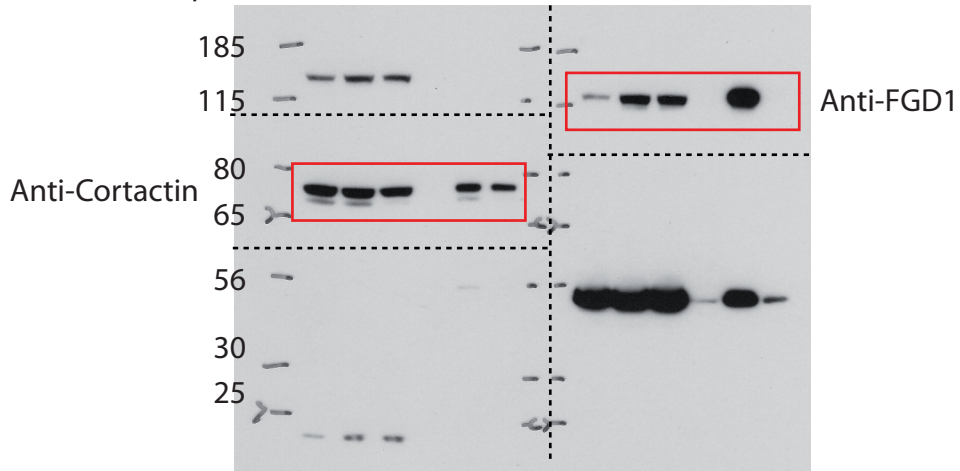


Figure 4B

5min exposition



10sec exposition



1sec exposition

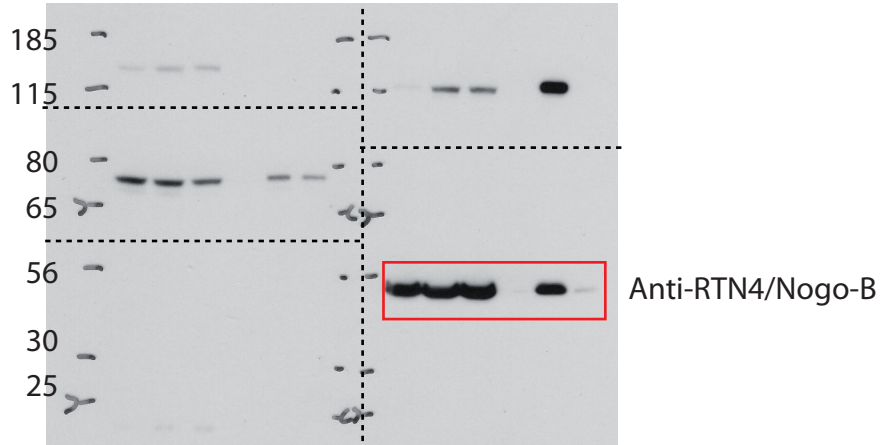
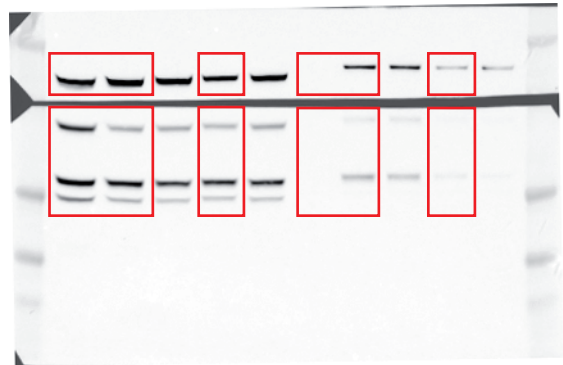
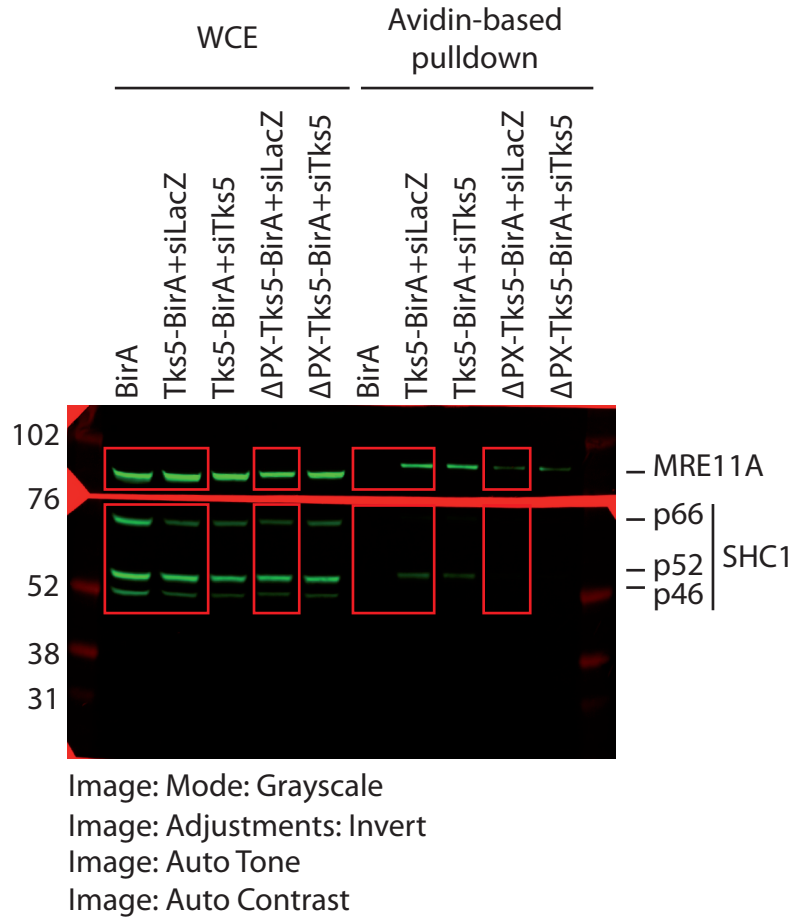
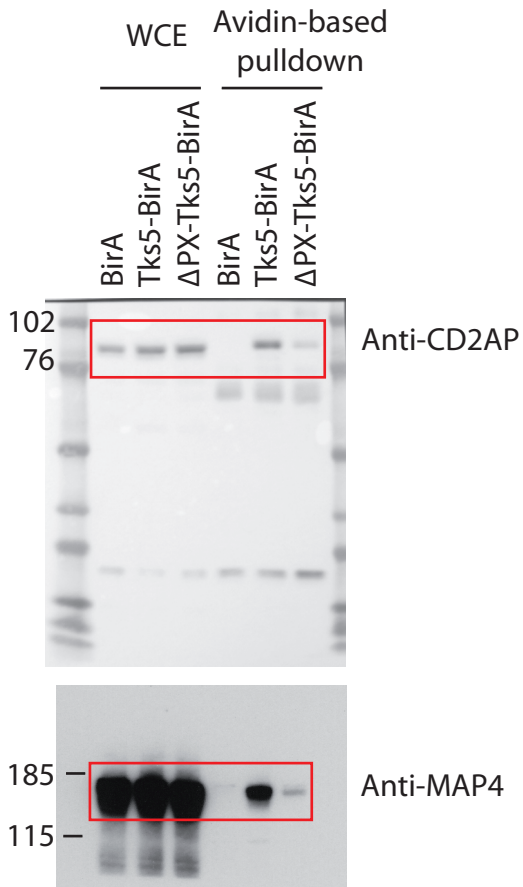
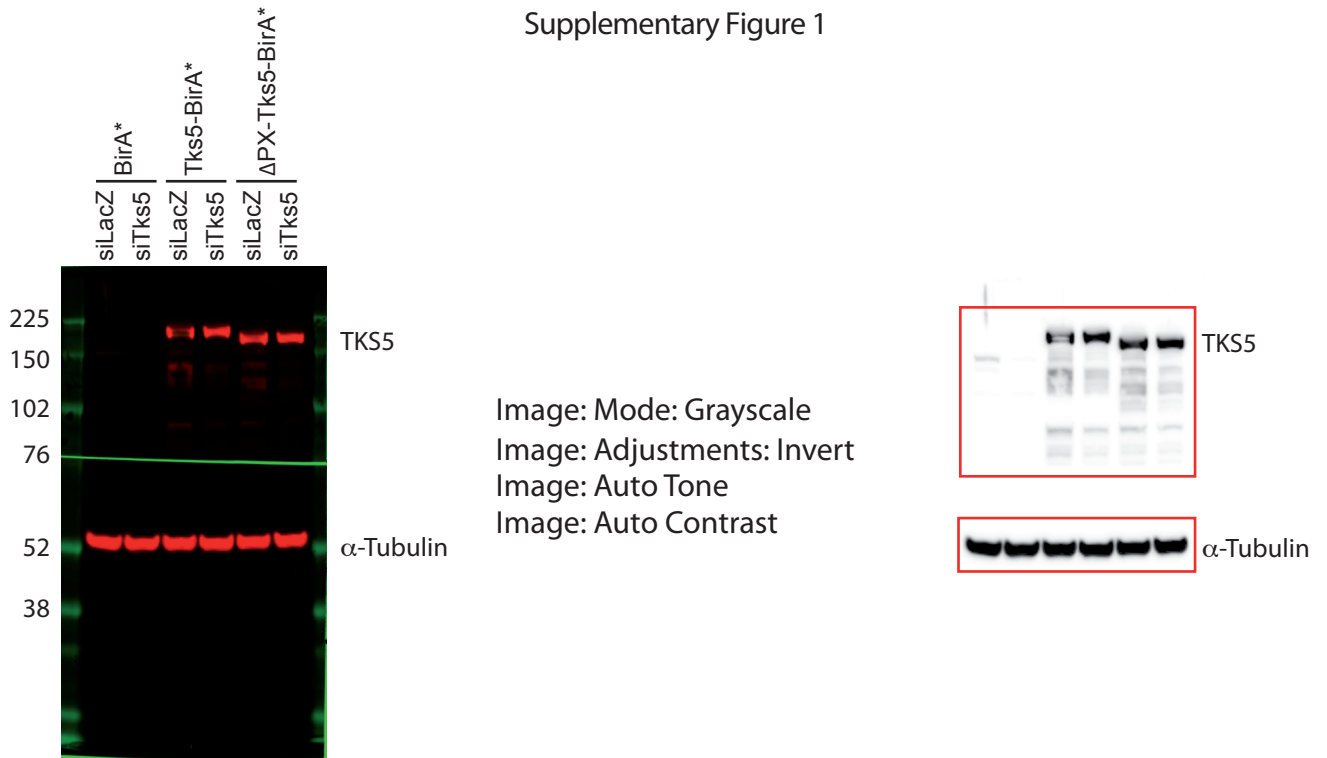


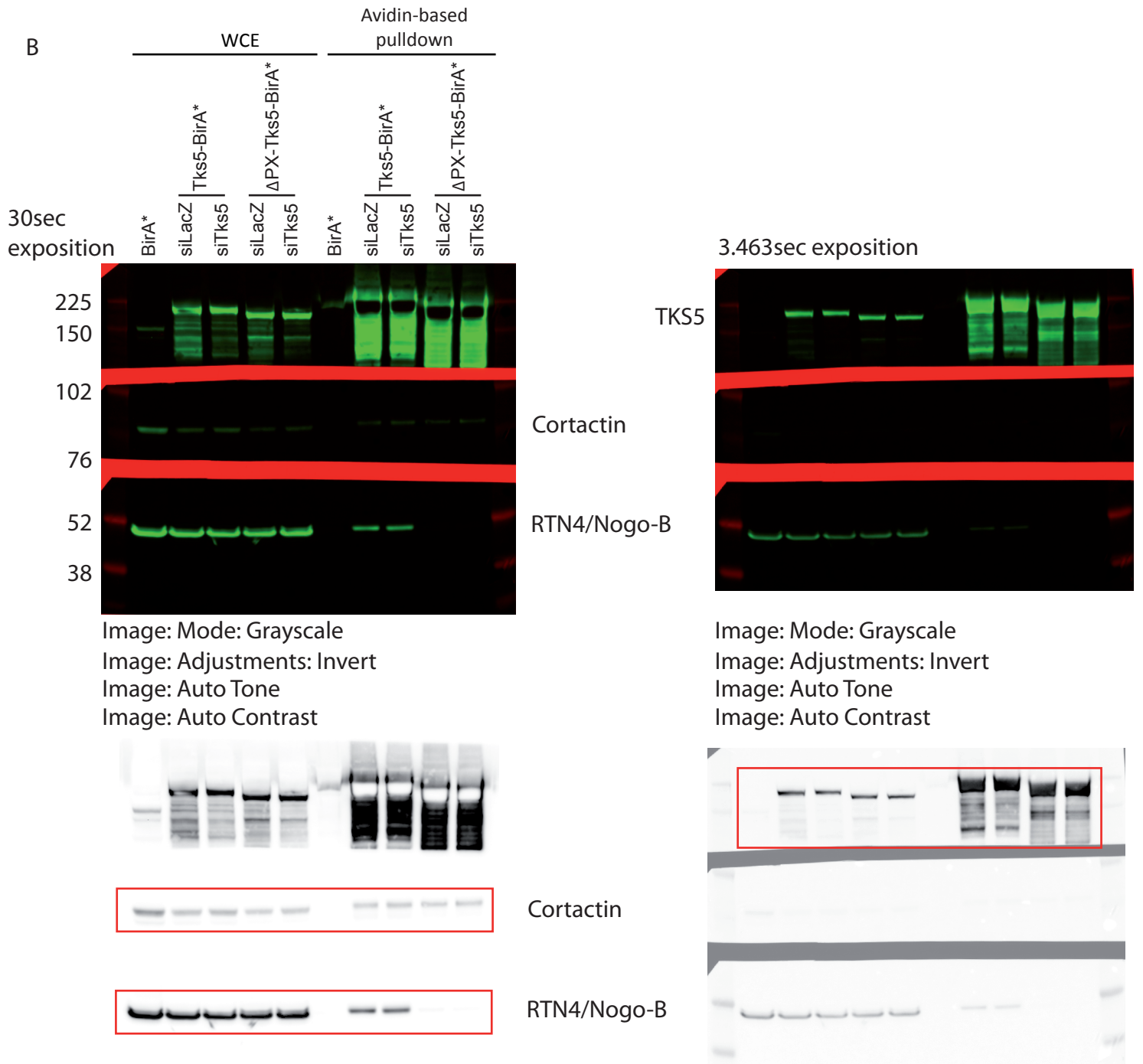
Figure 4B (continuation)



A



B



Supplementary Figure 1 (continuation)

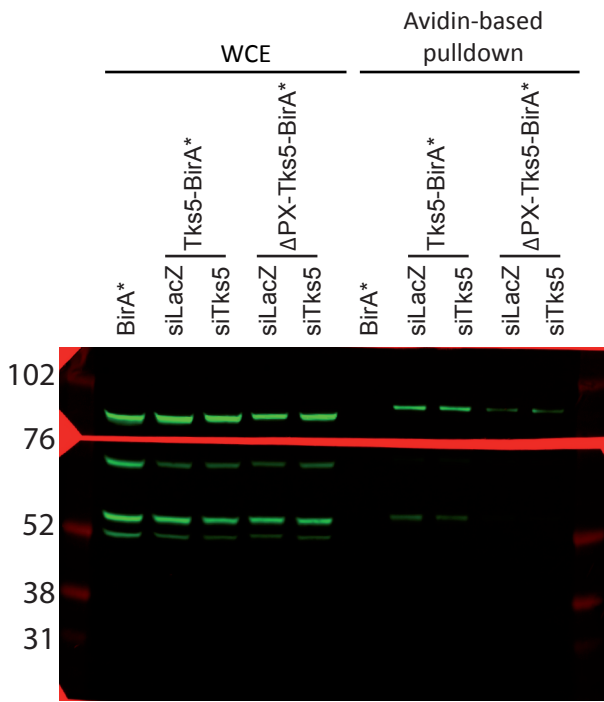


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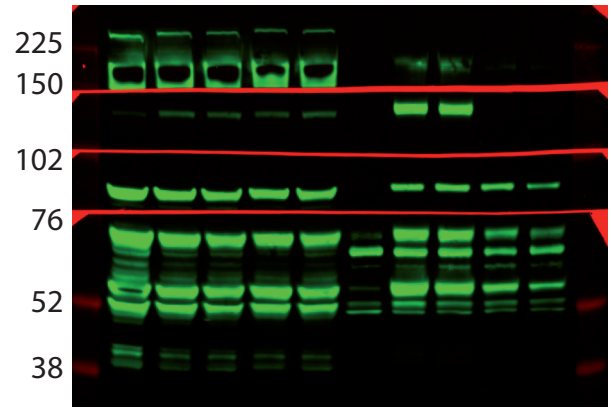


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