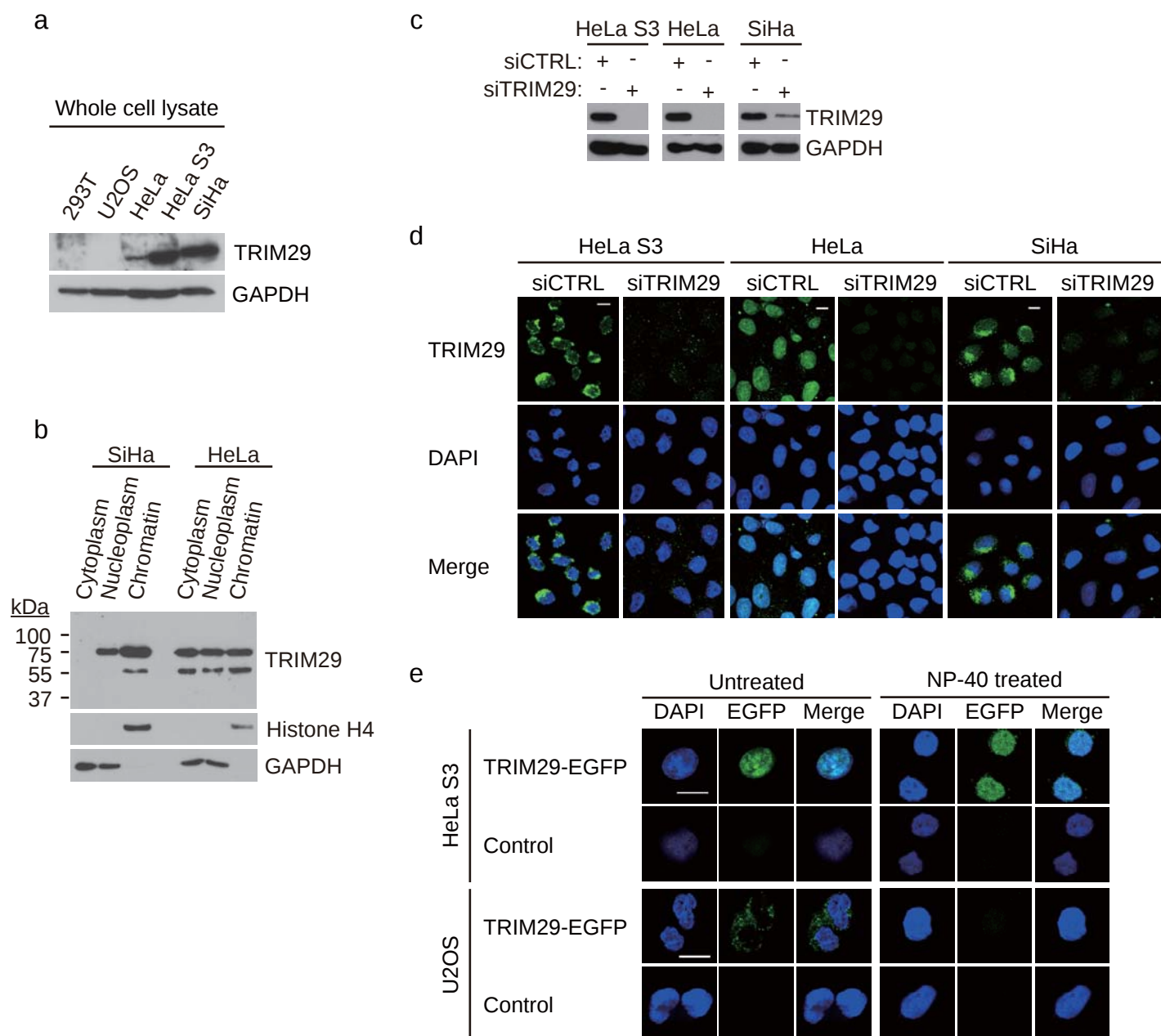




HOKKAIDO UNIVERSITY

Title	TRIM29 regulates the assembly of DNA repair proteins into damaged chromatin
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Type	journal article
File Information	ncomms8299-s1.pdf, Supplementary Figures 1-20 and Supplementary Tables 1-4

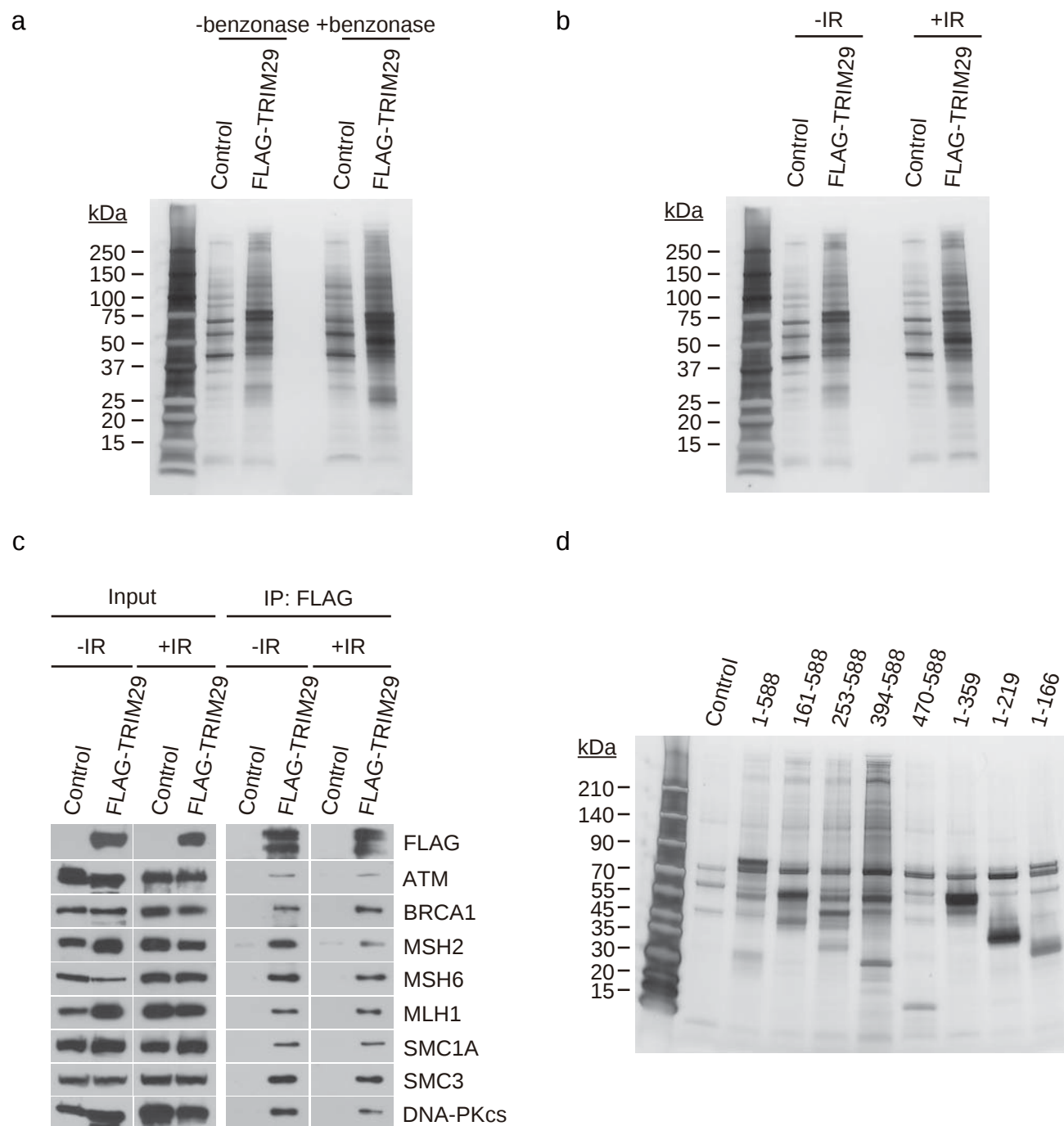




Supplementary Figure 1. Expression of endogenous and exogenous TRIM29.

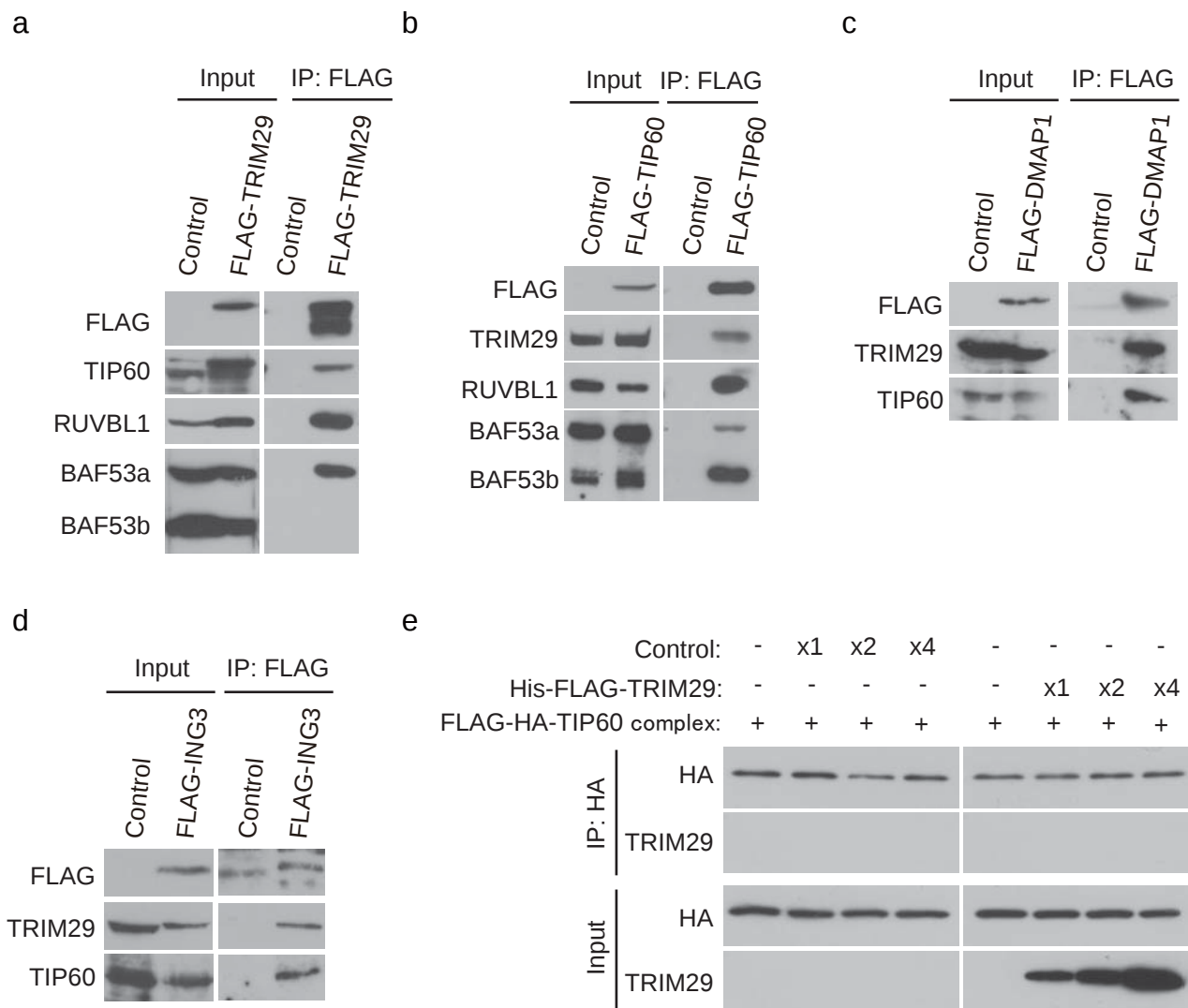
(a) Expression pattern of endogenous TRIM29 in 293T, U2OS, HeLa, HeLa S3 and SiHa cells. Total proteins (10 µg) from whole cell lysates of 293T, U2OS, HeLa, HeLa S3 and SiHa cells were subjected to SDS-PAGE followed by Western blotting. **(b)** Subcellular localization of endogenous TRIM29 in HeLa and SiHa cells. Total proteins (10 µg) from cytoplasm, nucleoplasm and chromatin fractions were subjected to SDS-PAGE followed by Western blotting. **(c)** Evaluation of TRIM29 knockdown in each cell line. HeLa S3, HeLa and SiHa cells were transfected with siControl or siTRIM29. After 48 h, cells were collected and whole cell lysates were subjected to SDS-PAGE followed by Western blotting. **(d)** Effect of TRIM29 siRNA. HeLa S3, HeLa and SiHa cells were transfected with siControl or siTRIM29. After 48 h, cells were fixed and stained with anti-TRIM29 antibody. DNA was counterstained with DAPI. Scale bar, 10 µm. **(e)** Confocal immunofluorescence images showing the localization of TRIM29-EGFP in HeLa S3 and U2OS cells. Cells expressing TRIM29-EGFP were treated with or without a detergent in order to reveal the subcellular localization of TRIM29-EGFP. DNA was counterstained with DAPI. Scale bar, 10 µm.

Figure S1



Supplementary Figure 2. Analysis of proteins associated with FLAG-tagged TRIM29.

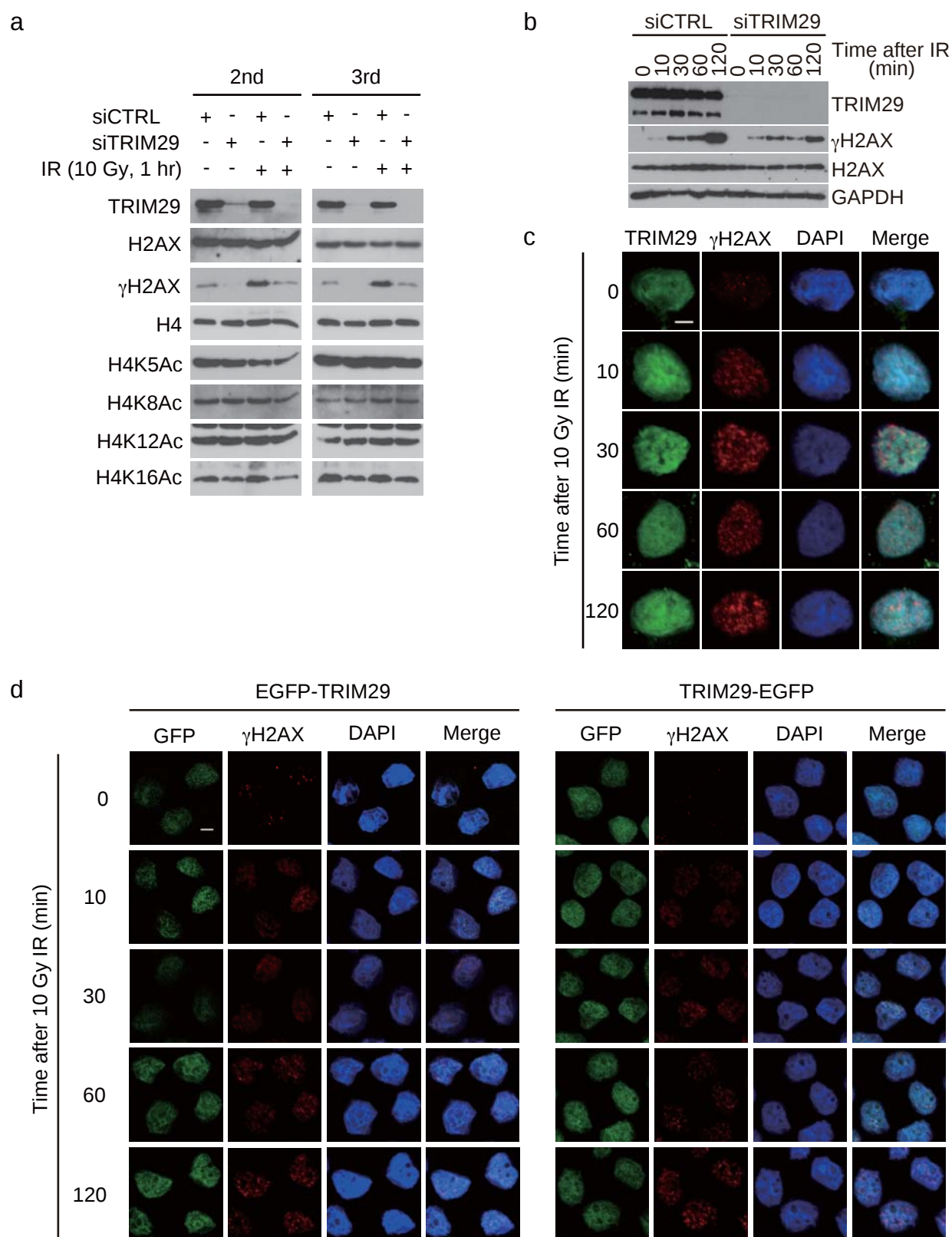
(a) Silver staining of FLAG-tagged immunoprecipitates from benzonase-treated nuclear extracts of cells expressing FLAG-tagged TRIM29 on 4-20% gradient SDS-PAGE gel. **(b)** Silver staining of FLAG-tagged immunoprecipitates from nuclear extracts of IR-treated HeLa S3 cells expressing FLAG-tagged TRIM29 on 4-20% gradient SDS-PAGE gel. **(c)** Effect of IR treatment on binding of TRIM29 to DNA repair proteins. HeLa S3 cells expressing FLAG-tagged TRIM29 treated with 10 Gy IR and collected after 1 h. M2-agarose was incubated with nuclear extracts of HeLa S3 cells. The bound proteins were washed, eluted by 3×FLAG peptides, and analyzed by Western blotting. **(d)** Silver staining of FLAG-tagged immunoprecipitates from nuclear extracts of cells expressing FLAG-tagged wild-type TRIM29 and mutants (shown in Fig. 2a) on 4-20% gradient SDS-PAGE gel.



Supplementary Figure 3. Interaction of TRIM29 with subunits of TIP60 complex.

(a) Binding of TRIM29 to the components of TIP60 complex. **(b)** Binding of TIP60 to TRIM29 and other TIP60 complex proteins. **(c)** Binding of DMAP1 to TRIM29 and TIP60. **(d)** Binding of ING3 to TRIM29 and TIP60. **(e)** Binding of TRIM29 to TIP60 complex. FLAG-HA-TIP60 complex was purified from HeLa S3 cells stably expressing TIP60. FLAG-HA-TIP60 complex attached to anti-HA agarose was incubated with increasing amounts of His-FLAG-TRIM29. The bound proteins were washed, eluted by HA peptides, and analyzed by Western blotting.

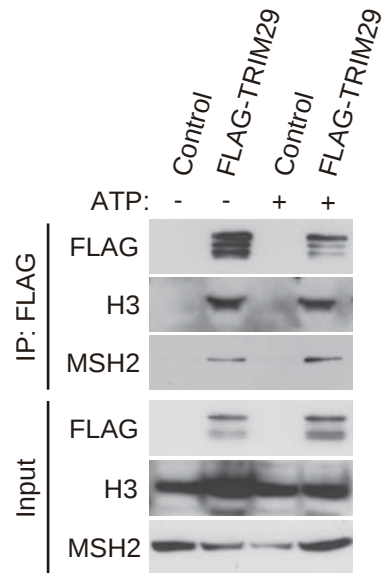
Figure S3



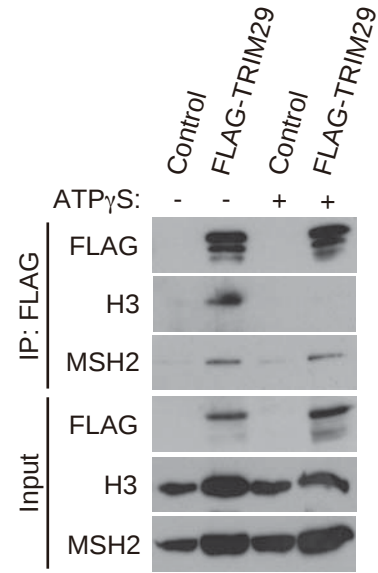
Supplementary Figure 4. Immunofluorescence images of ectopically expressed EGFP-TRIM29 and TRIM29-EGFP.

(a) Effect of TRIM29 knockdown on histone modifications induced by IR treatment. HeLa S3 cells were transfected with siControl or siTRIM29. After 48 h, the cells were treated with 10 Gy IR and collected after 1 h. Histones were analyzed by Western blotting using the indicated antibodies. **(b)** Effects of TRIM29 knockdown on the phosphorylation of H2AX. HeLa S3 cells were transfected with siControl or siTRIM29. After 48 h, cells were treated with 10 Gy IR and collected at the indicated time points from 0 to 120 min after IR treatment. Whole cell lysates were analyzed by Western blotting using the indicated antibodies. **(c)** Immunofluorescence images showing the localization of TRIM29 and γ H2AX. HeLa S3 cells were treated with 10 Gy IR, fixed at the indicated time points from 0 to 120 min after IR treatment, and stained with anti-TRIM29 and γ H2AX antibodies. DNA was counterstained with DAPI. Scale bar, 5 μ m. **(d)** HeLa S3 cells transfected with EGFP-TRIM29 (N-terminal tag) and TRIM29-EGFP (C-terminal tag) were treated with 10 Gy IR and fixed at the indicated time points from 0 to 120 min after IR treatment. DNA was counterstained with DAPI. Scale bar, 5 μ m.

a



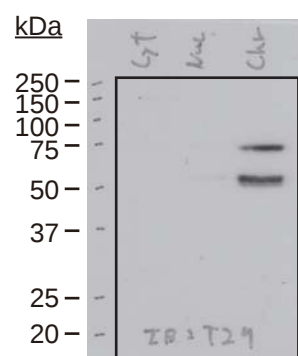
b



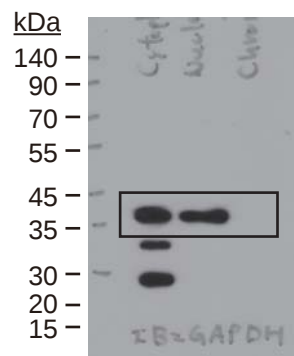
Supplementary Figure 5. Effect of ATP and ATPγS on the interaction of TRIM29-MSH2 complex with nucleosomes.

Western blot analysis of anti-FLAG immunoprecipitates from nucleosome-enriched nuclear extracts of HeLa S3 cells expressing FLAG-tagged TRIM29. These assays were performed in the presence or absence of 1 mM ATP (**a**) and 50 μM ATPγS (**b**).

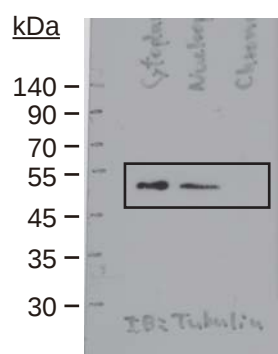
Figure S5



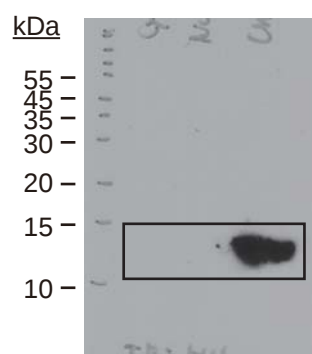
IB: TRIM29



IB: GAPDH



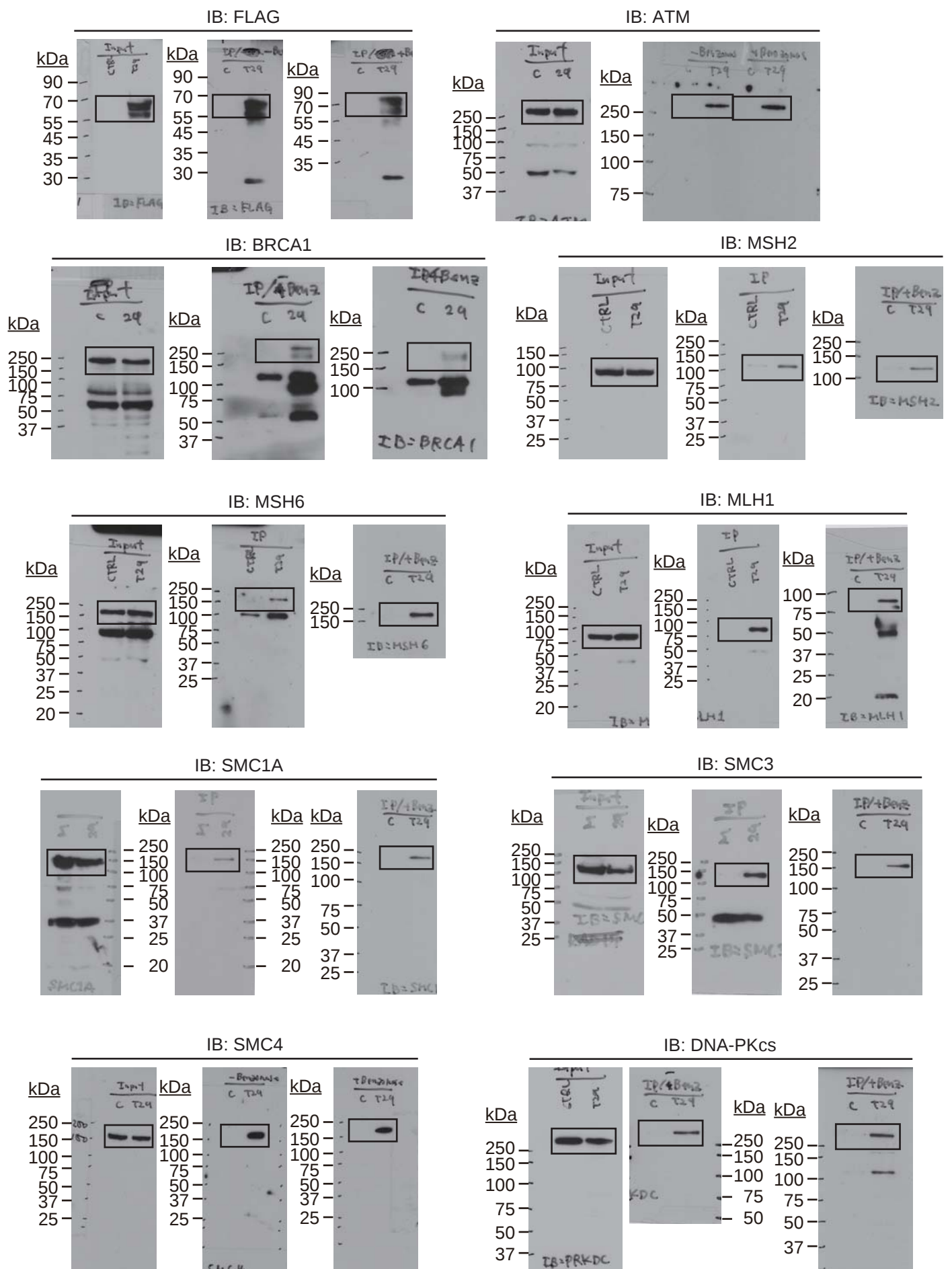
IB: Tublin



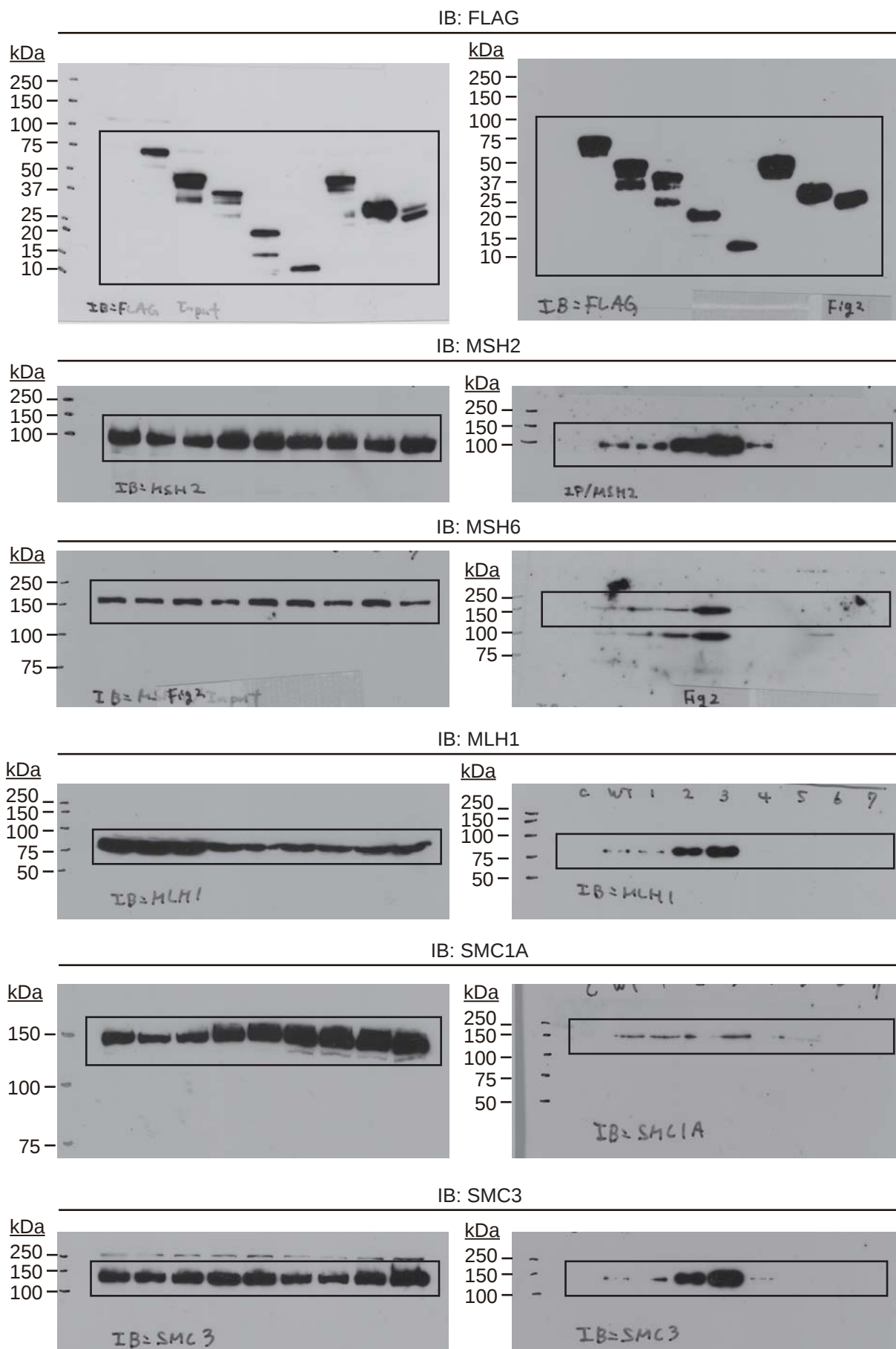
IB: H4

Supplementary Figure 6. Original immunoblot data for Fig. 1b

Figure S6

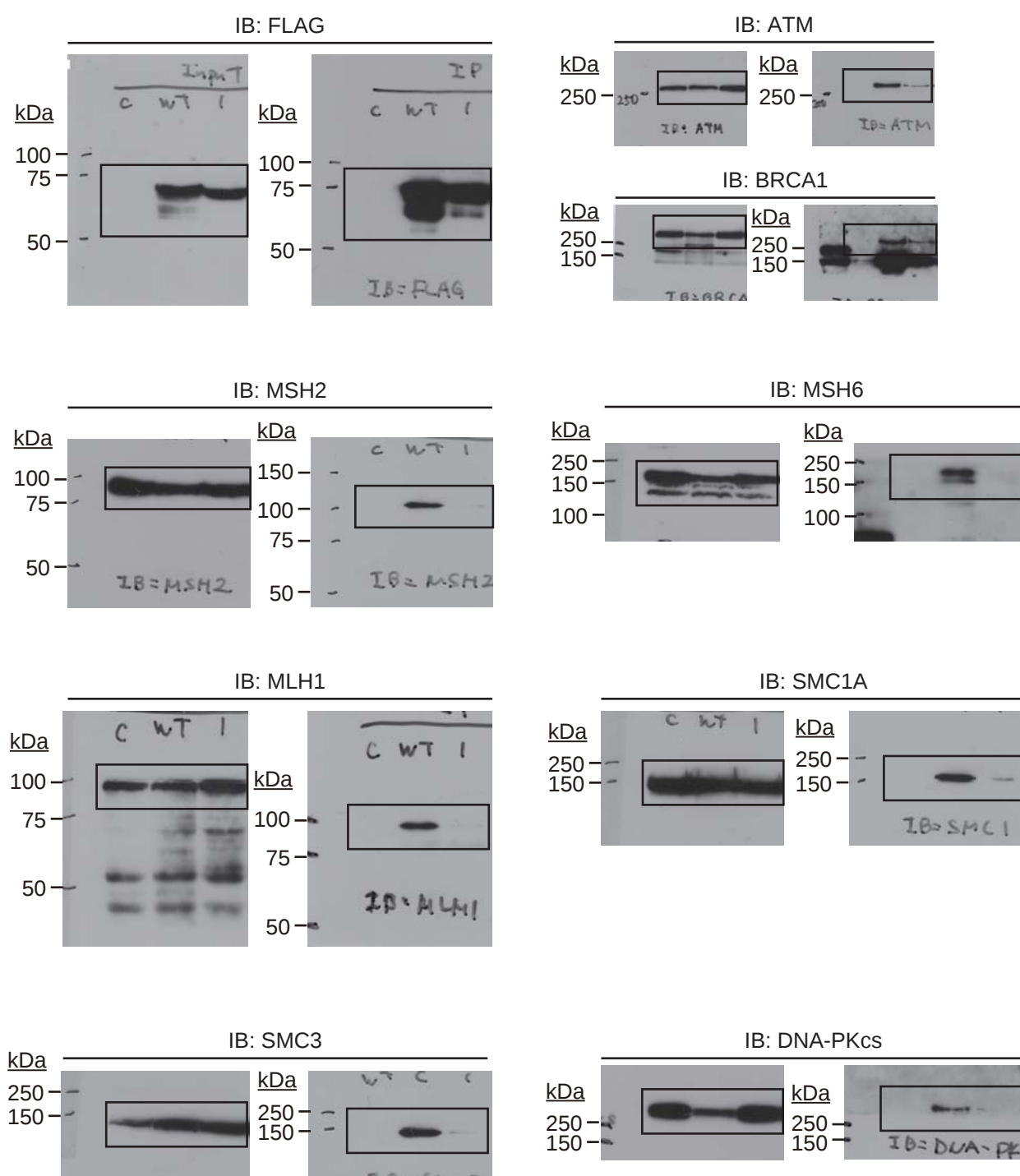


Supplementary Figure 7: Original immunoblot data for Fig. 1g



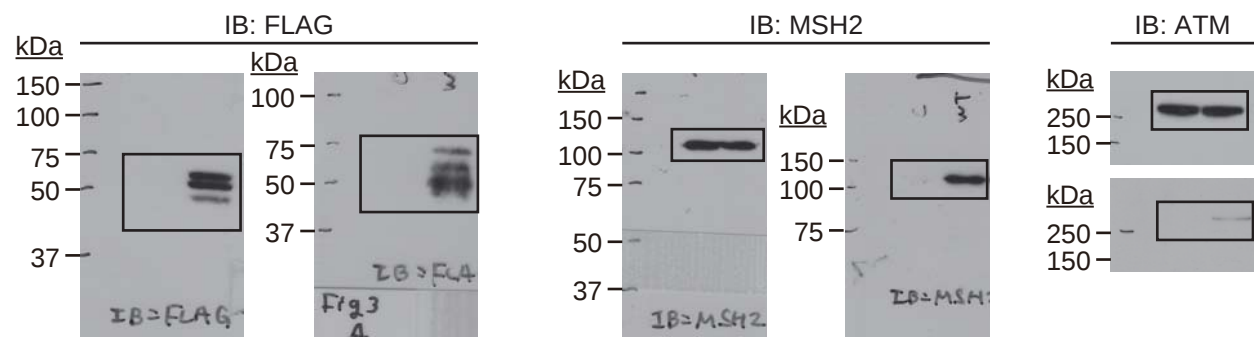
Supplementary Figure 8: Original immunoblot data for Fig. 2b

Figure S8

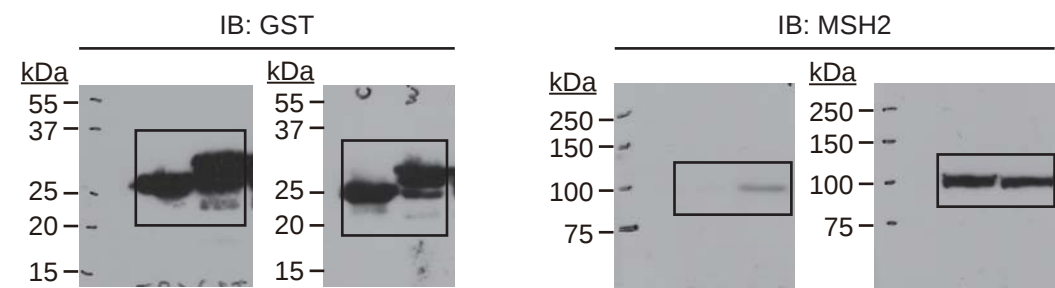


Supplementary Figure 9: Original immunoblot data for Fig. 2e

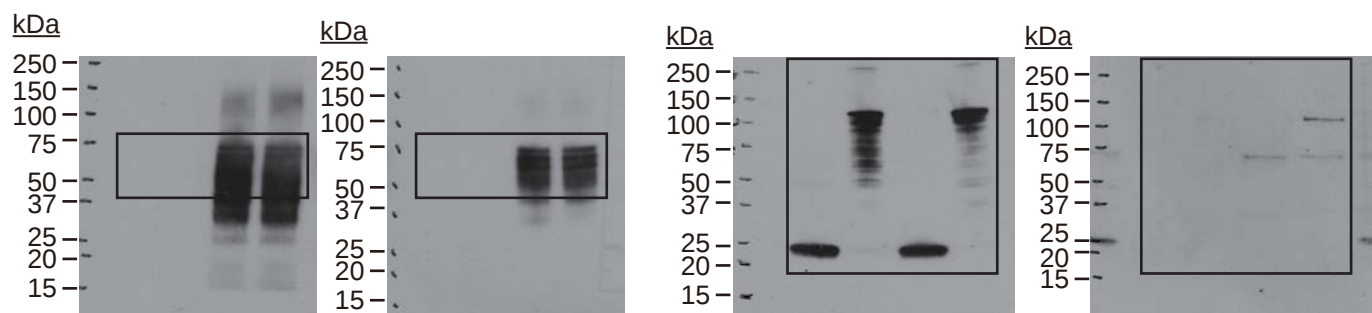
Original immunoblot data for Fig. 3a



Original immunoblot data for Fig. 3b



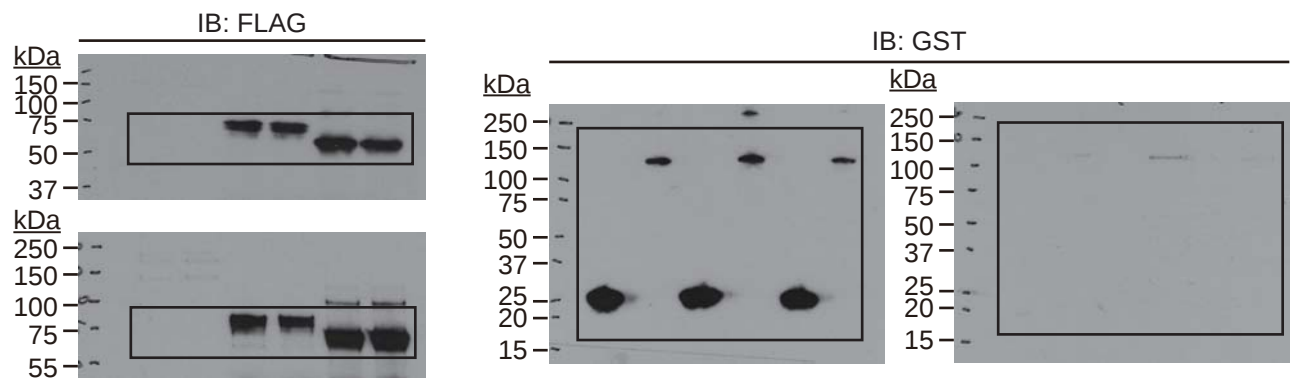
Original immunoblot data for Fig. 3c



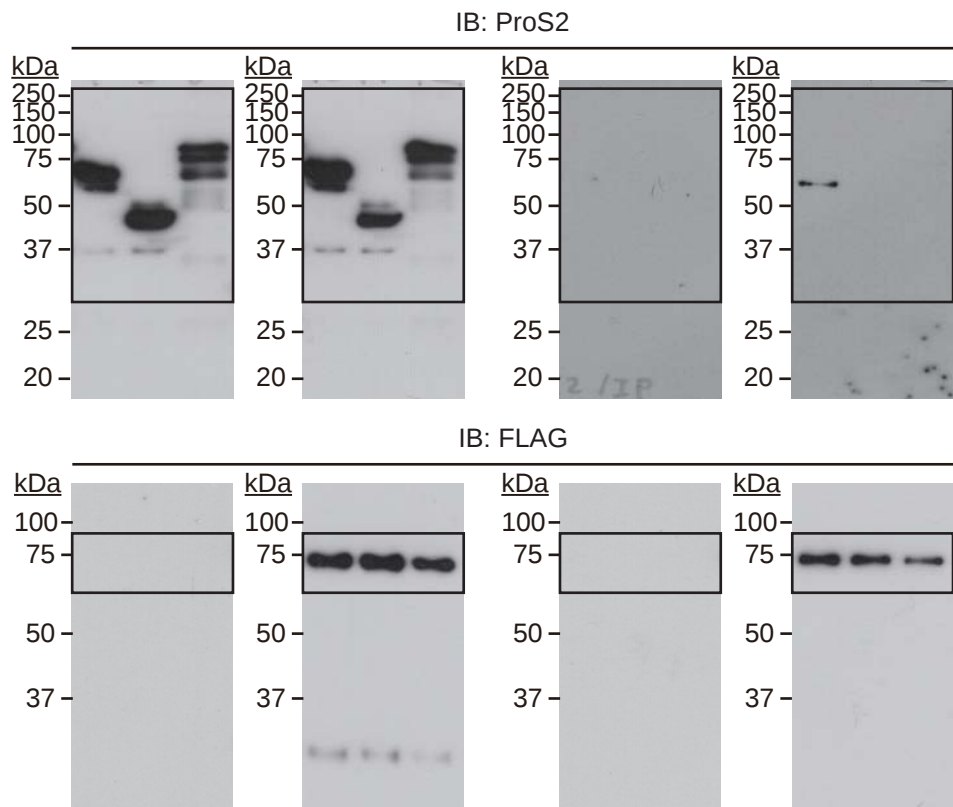
Supplementary Figure 10: Original immunoblot data for Fig. 3a, b and c

Figure S10

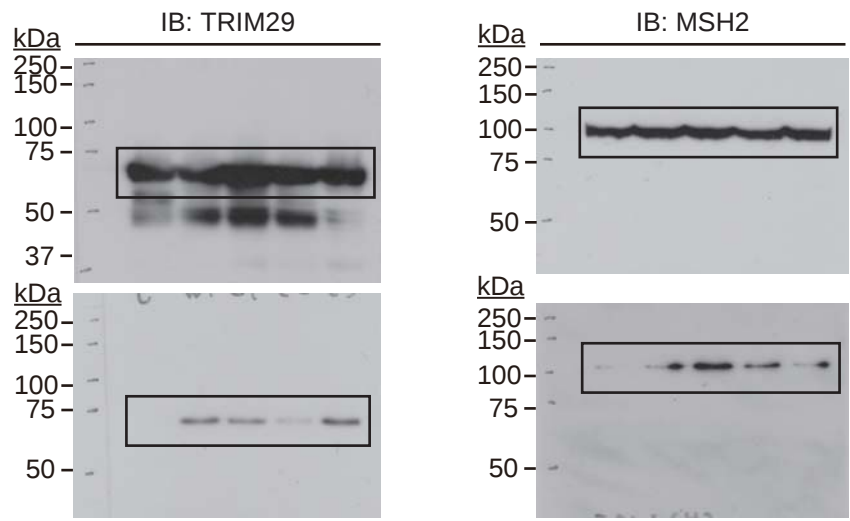
Original immunoblot data for Fig. 3d



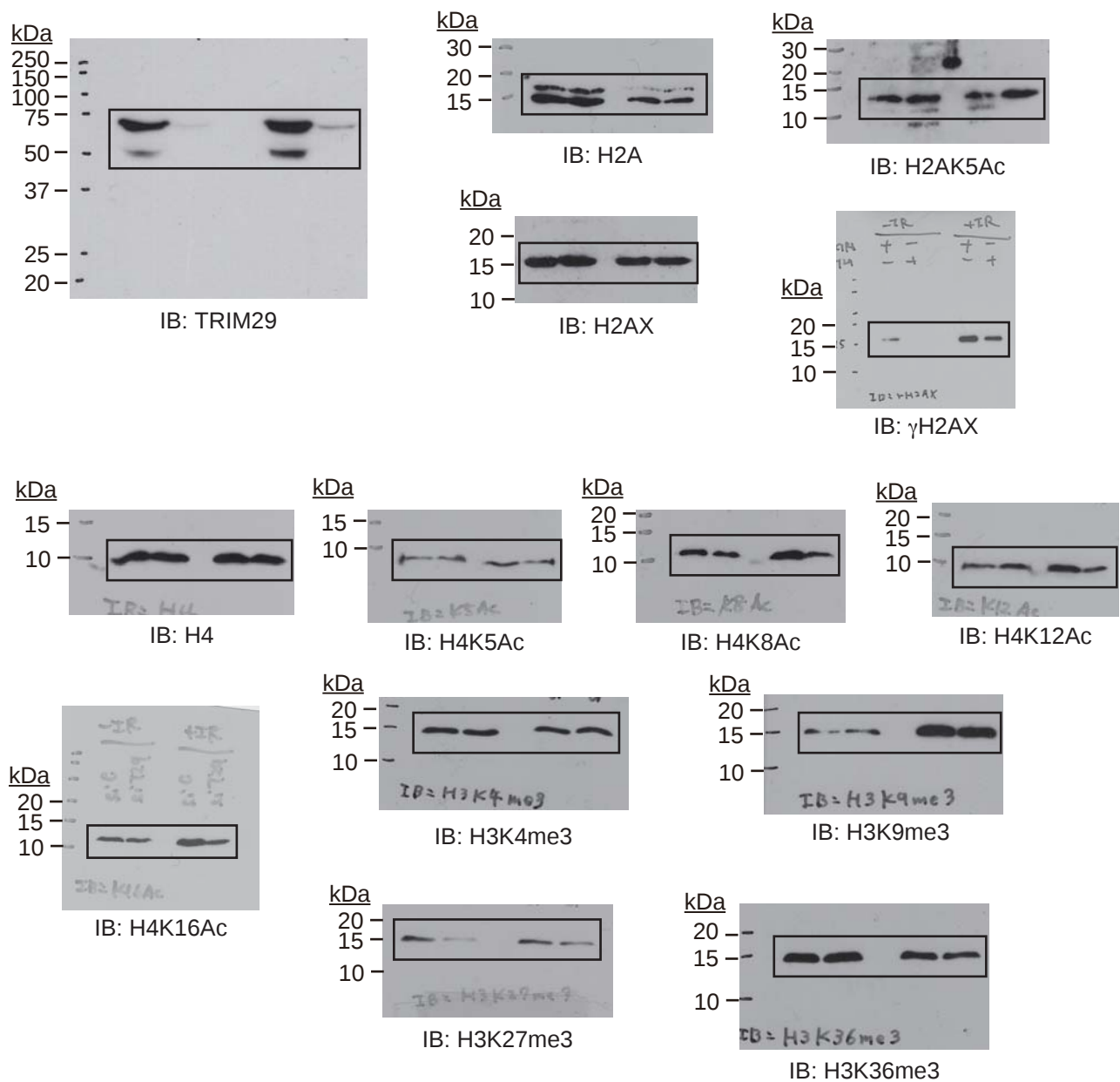
Original immunoblot data for Fig. 3f



Original immunoblot data for Fig. 3g

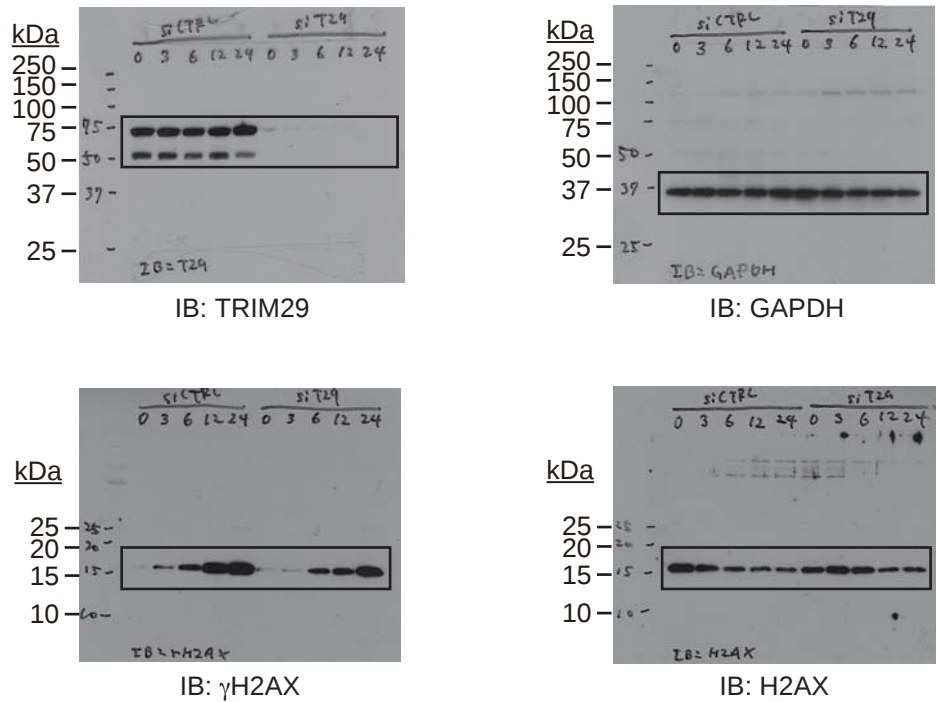


Supplementary Figure 11: Original immunoblot data for Fig. 3d, f and g

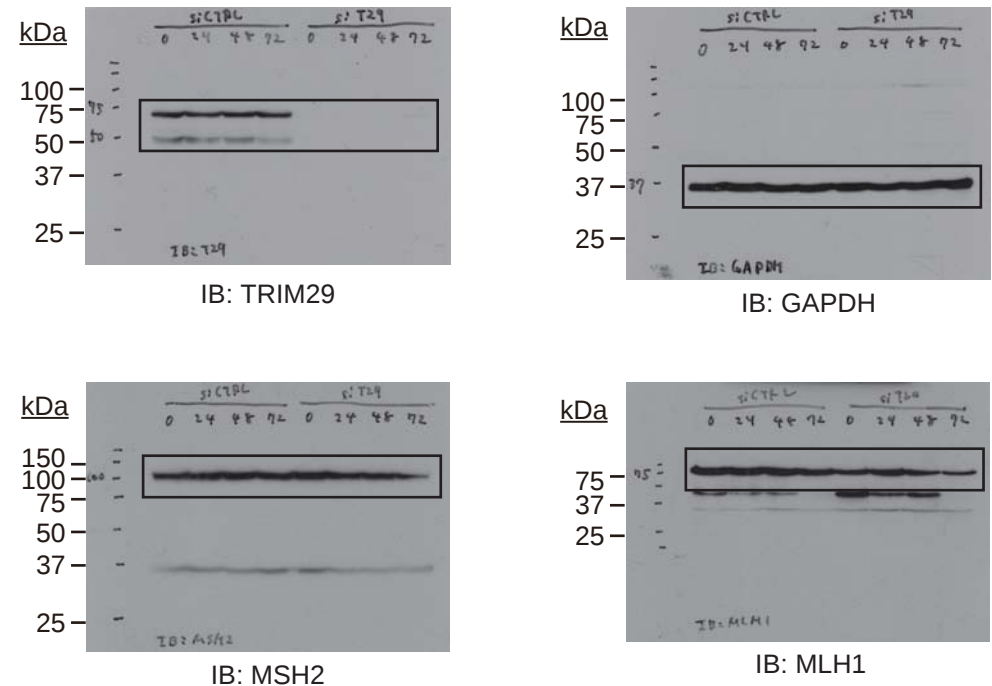


Supplementary Figure 12: Original immunoblot data for Fig. 4a

Original immunoblot data for Fig. 4d

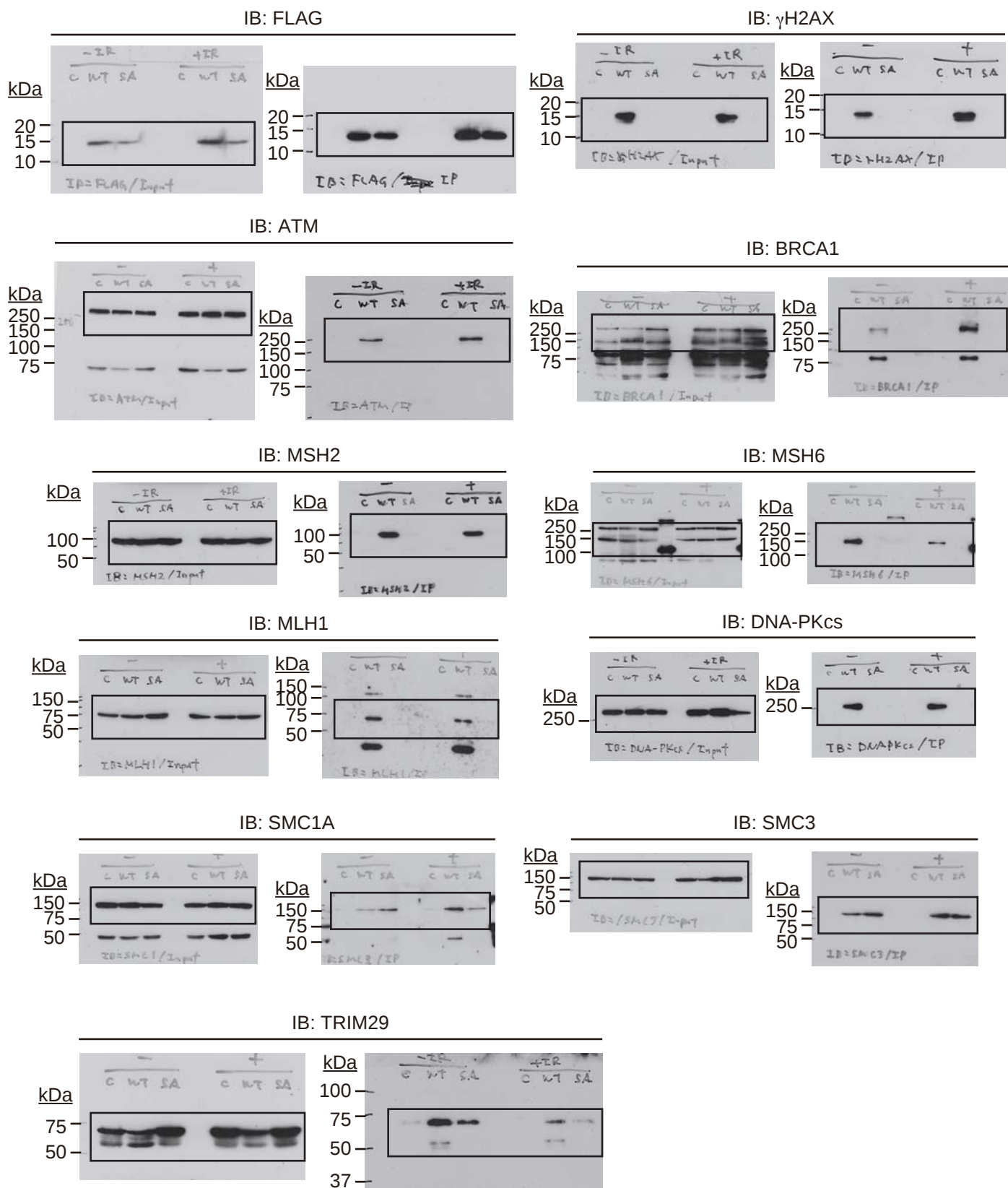


Original immunoblot data for Fig. 4i

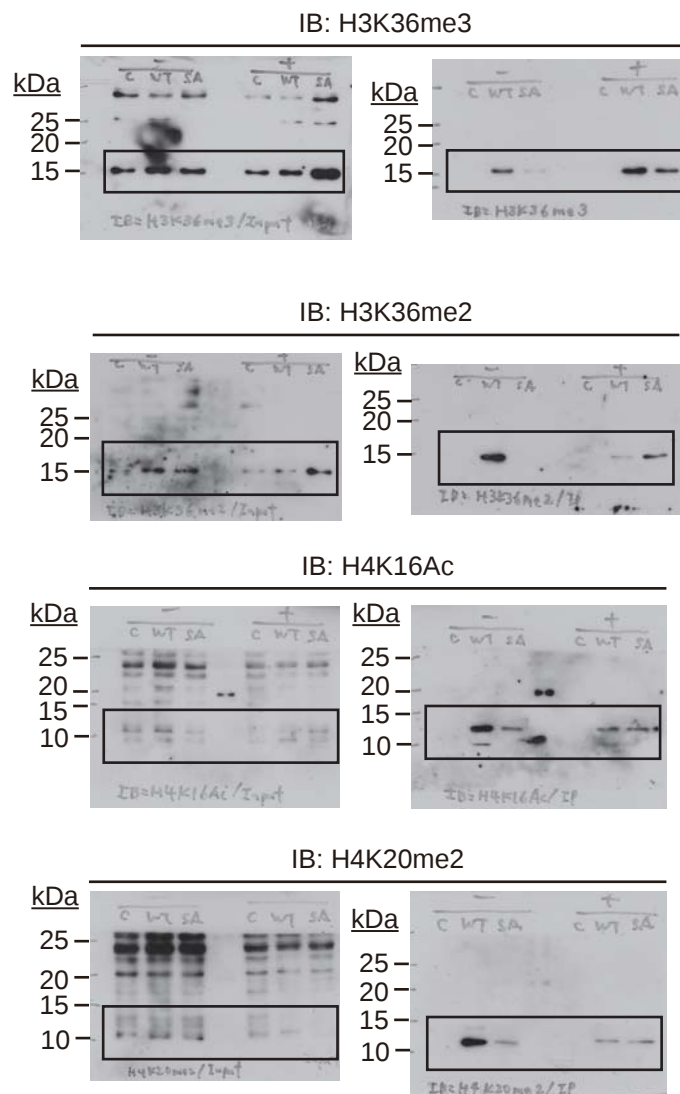


Supplementary Figure 13: Original immunoblot data for Fig. 4d and i

Figure S13



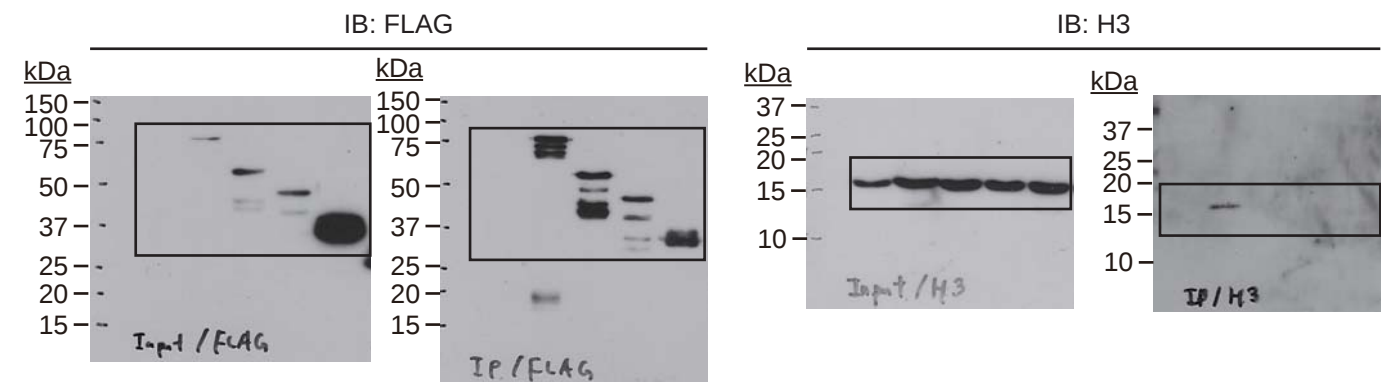
Supplementary Figure 14: Original immunoblot data for Fig. 5b



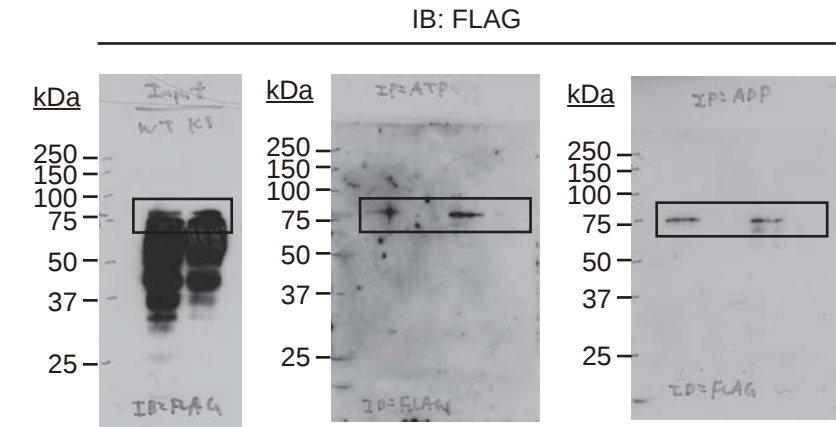
Supplementary Figure 15: Original immunoblot data for Fig. 5b

Figure S15

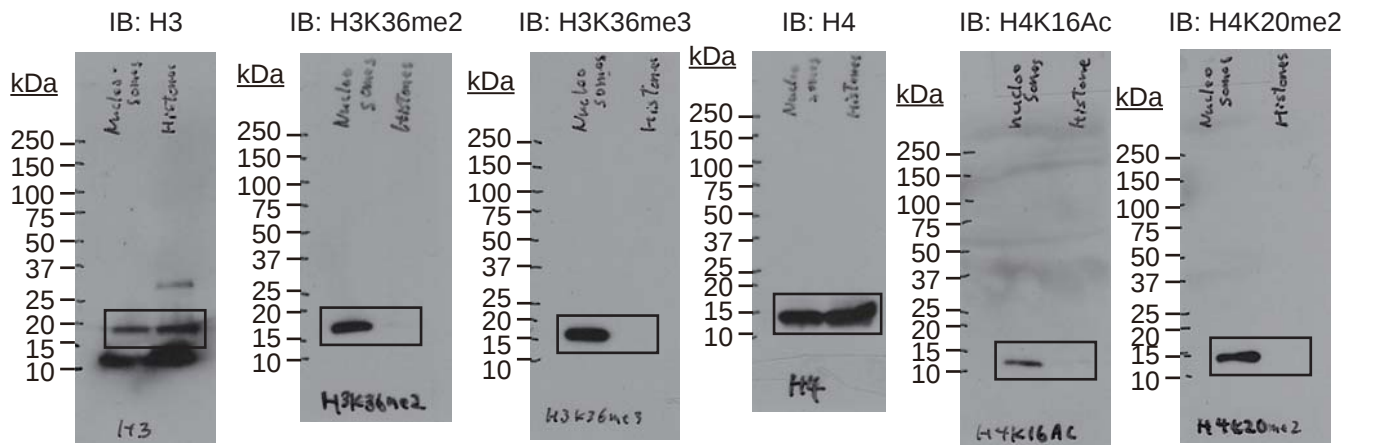
Original immunoblot data for Fig. 6a



Original immunoblot data for Fig. 6c



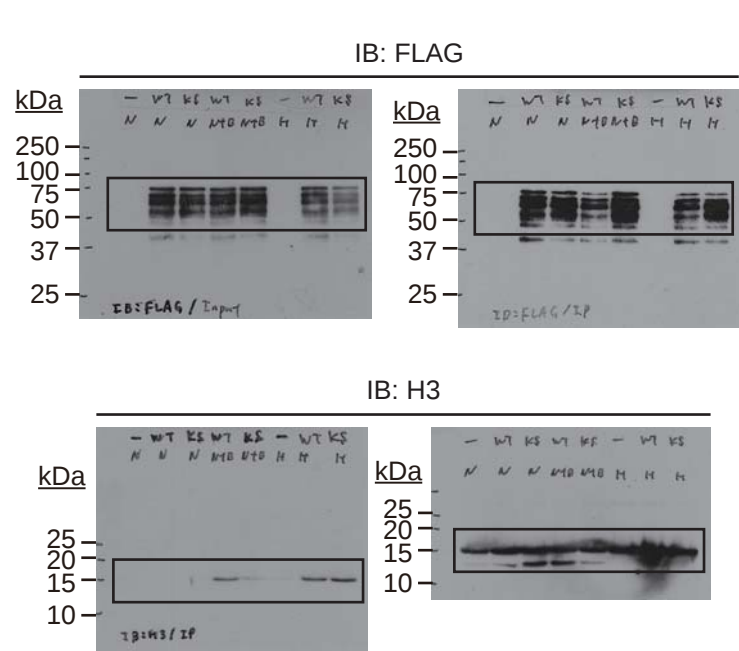
Original immunoblot data for Fig. 6d



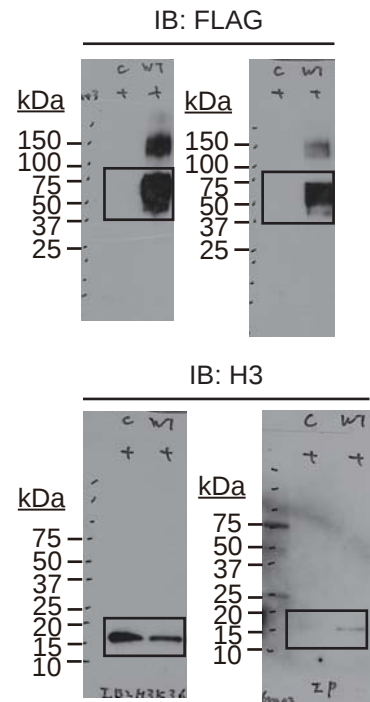
Supplementary Figure 16: Original immunoblot data for Fig. 6a, c and d

Figure S16

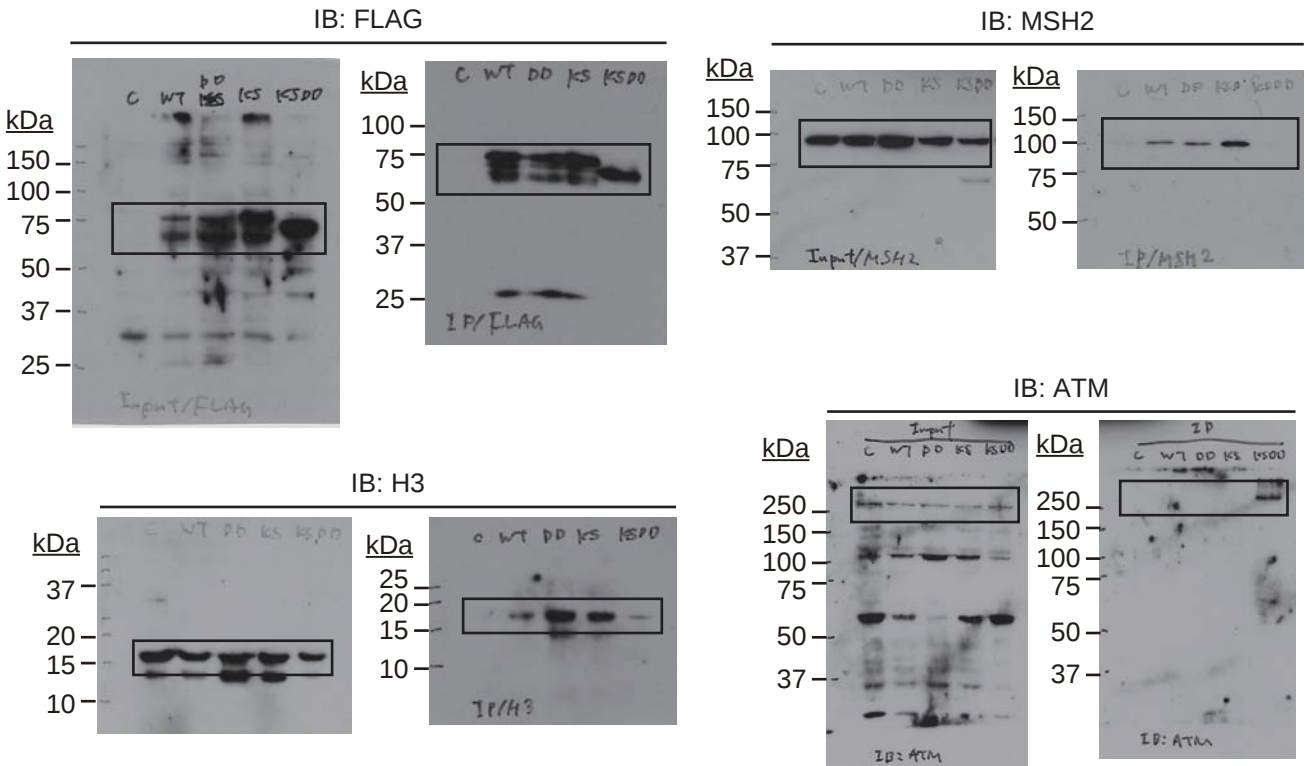
Original immunoblot data for Fig. 6e



Original immunoblot data for Fig. 6g



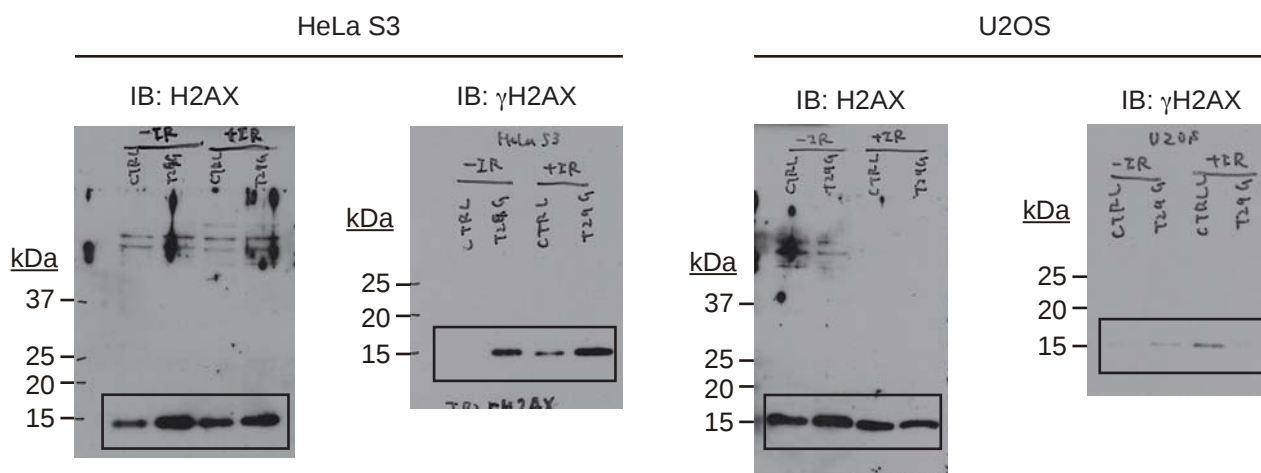
Original immunoblot data for Fig. 6h



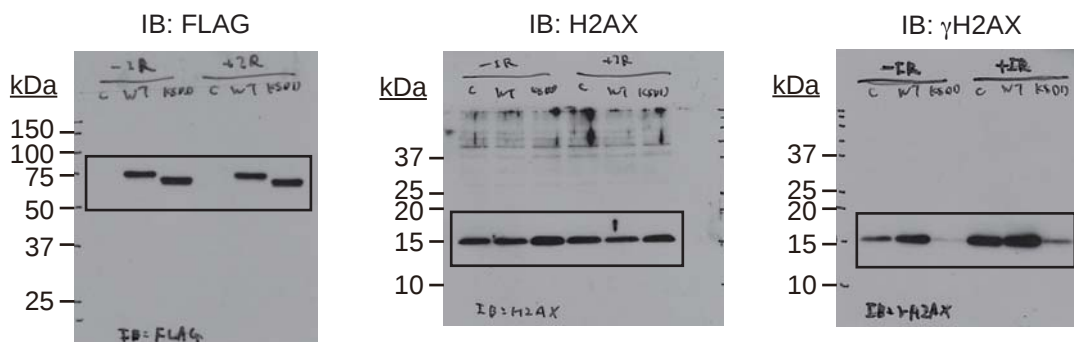
Supplementary Figure 17: Original immunoblot data for Fig. 6e, g and h

Figure S17

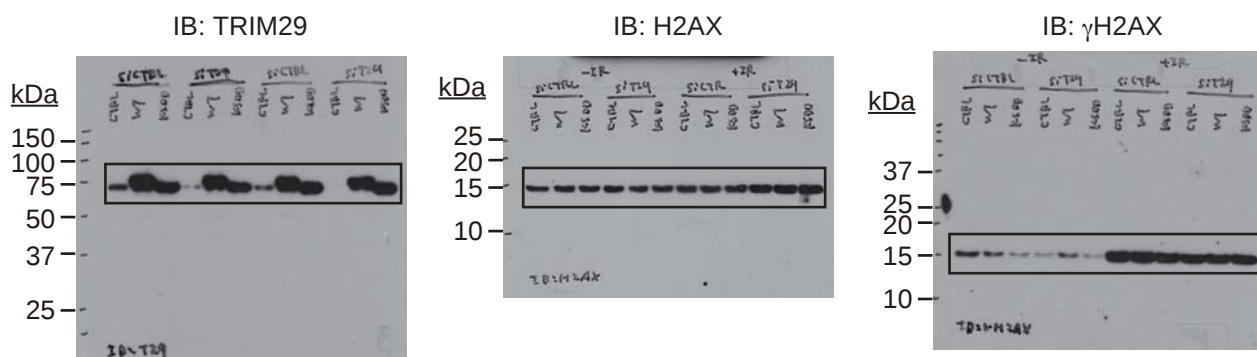
Original immunoblot data for Fig. 7b



Original immunoblot data for Fig. 7d

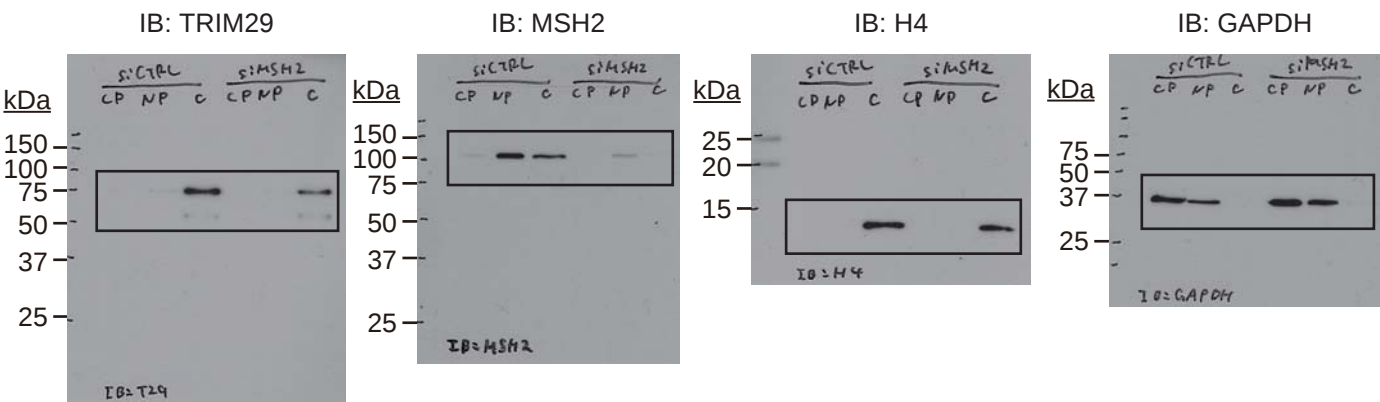


Original immunoblot data for Fig. 7g

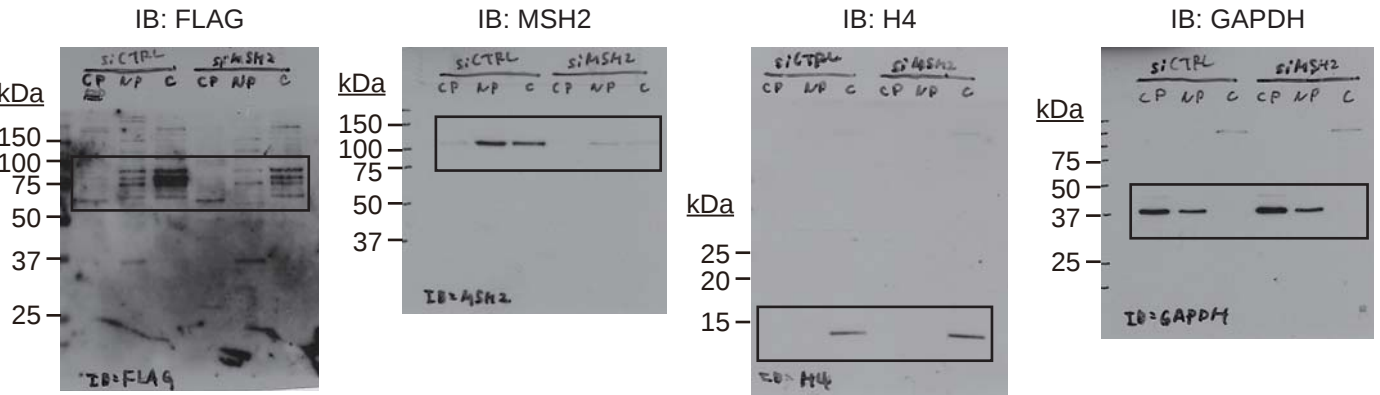


Supplementary Figure 18: Original immunoblot data for Fig. 7b, d and g

Original immunoblot data for Fig. 8a

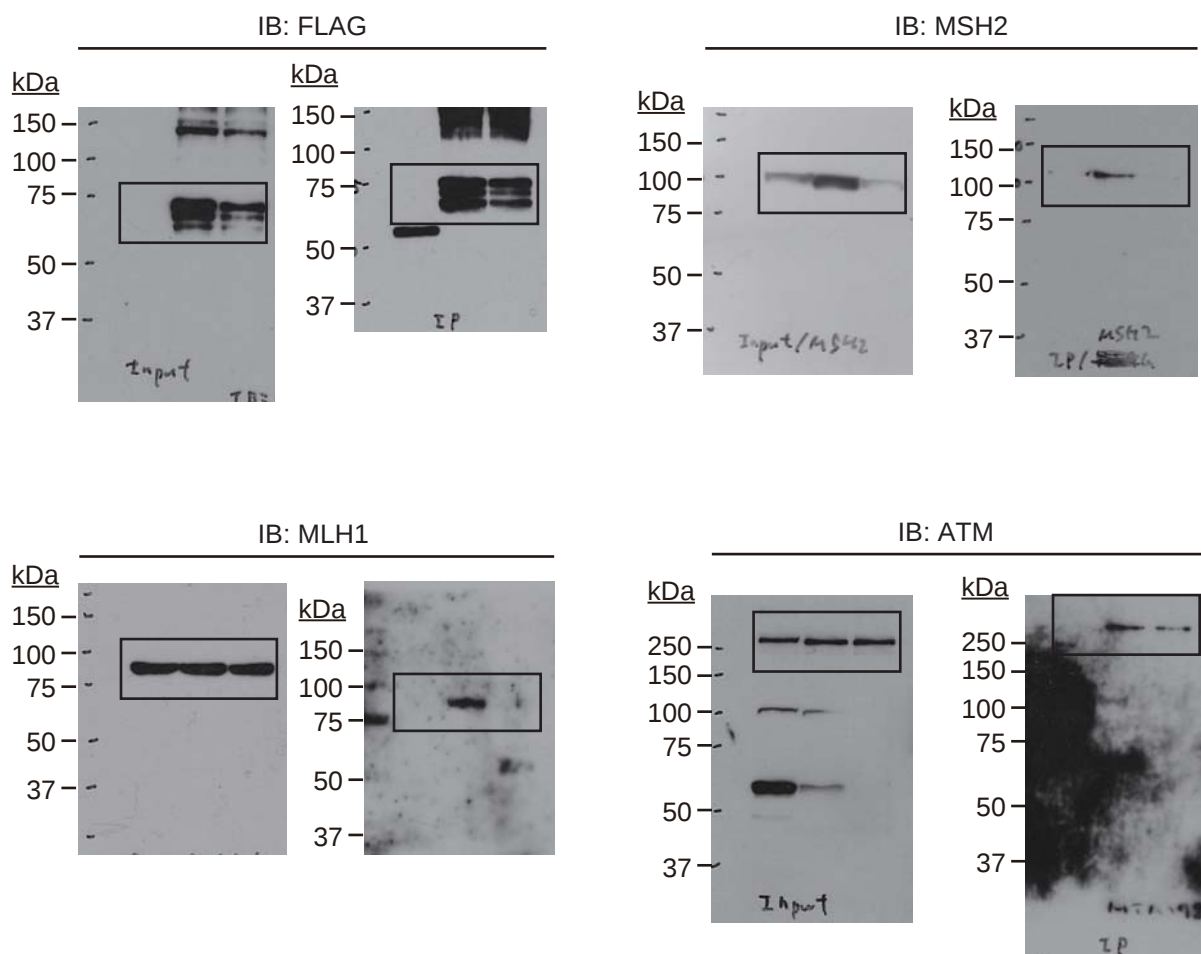


Original immunoblot data for Fig. 8b



Supplementary Figure 19: Original immunoblot data for Fig. 8a and b

Figure S19



Supplementary Figure 20: Original immunoblot data for Fig. 8d

Accession Numbers	Description	Gene	WT-T29_HeLa-NE_FLAG-IP_Ti_L4_2_dNSAF	T29-394-588_HeLa-NE_FLAG-IP_Ti_L4_2_dNSAF	T29-470-588_HeLa-NE_FLAG-IP_Ti_L5_1_dNSAF	T29-1-359_HeLa-NE_FLAG-IP_Ti_L5_1_dNSAF
	tripartite motif-containing protein 29 [Homo sapiens] ;	TRIM29	0.2053872	0	0	0
	chs_TRIM29-394-588		0	0.15169004	0	0
	chs_TRIM29-470-588		0	0	0.56902721	0
	chs_TRIM29-1-359		0	0	0	0.58569764
gi 126032350 ref NP_001075109.1	DNA-dependent protein kinase catalytic subunit isoform 2 [Homo sapiens]	DNA-PKcs	0.00023046	0.0005452	0	0
gi 4503841 ref NP_001460.1	X-ray repair cross-complementing protein 6 [Homo sapiens]	Ku70	0.00034454	0	0	0
gi 132626688 ref NP_055456.2	mediator of DNA damage checkpoint protein 1 [Homo sapiens]	MDC1	0.0000287	0	0	0
gi 24234690 ref NP_005581.2	double-strand break repair protein MRE11A isoform 2 [Homo sapiens]	MRE11A	0.00017632	0.00019908	0	0
gi 19924129 ref NP_005723.2	DNA repair protein RAD50 [Homo sapiens]	RAD50	0.00004569	0	0	0
gi 33356172 ref NP_002476.2	nibrin [Homo sapiens]	NBS1	0.00003975	0	0	0
gi 237681119 ref NP_009231.2	breast cancer type 1 susceptibility protein isoform 2 [Homo sapiens]	BRCA1	0.00000825	0.00007186	0	0
gi 119395734 ref NP_000050.2	breast cancer type 2 susceptibility protein [Homo sapiens]	BRCA2	0.00007016	0.00013862	0	0
gi 384871702 ref NP_001245210.1	DNA mismatch repair protein Msh2 isoform 1 [Homo sapiens]	MSH2	0.0008665	0.00101376	0	0
gi 4504191 ref NP_000170.1	DNA mismatch repair protein Msh6 [Homo sapiens]	MSH6	0.00057304	0.00094564	0	0
gi 4505913 ref NP_000526.1	mismatch repair endonuclease PMS2 isoform a [Homo sapiens]	PMS2	0.00041728	0	0	0
gi 384871676 ref NP_001245200.1	DNA mismatch repair protein Mlh1 isoform 4 [Homo sapiens]	MLH1	0.00113441	0	0	0
gi 284813531 ref NP_002430.3	DNA mismatch repair protein Msh3 [Homo sapiens]	MSH3	0.00010545	0	0	0
gi 30581135 ref NP_006297.2	structural maintenance of chromosomes protein 1A [Homo sapiens]	SMC1A	0.00104534	0.0016469	0.00010612	0
gi 4885399 ref NP_005436.1	structural maintenance of chromosomes protein 3 [Homo sapiens]	SMC3	0.00125613	0.00094552	0	0
gi 388240808 ref NP_001252531.1	structural maintenance of chromosomes protein 2 [Homo sapiens]	SMC2	0.00020033	0	0	0
gi 50658065 ref NP_005487.3	structural maintenance of chromosomes protein 4 [Homo sapiens]	SMC4	0.00020945	0	0	0
gi 32528306 ref NP_002904.3	replication factor C subunit 1 isoform 1 [Homo sapiens]	RFC1	0.00057493	0.00076717	0	0
gi 28882049 ref NP_002905.2	replication factor C subunit 2 isoform 1 [Homo sapiens]	RFC2	0.00135478	0.00296134	0.00020445	0
gi 108773789 ref NP_853536.2	replication factor C subunit 3 isoform 1 [Homo sapiens]	RFC3	0.0002105	0.00044385	0	0
gi 31881687 ref NP_853551.1	replication factor C subunit 4 [Homo sapiens]	RFC4	0.00123862	0.00242408	0.00036046	0
gi 194306567 ref NP_853556.2	replication factor C subunit 5 isoform 2 [Homo sapiens]	RFC5	0.00084568	0.00212187	0	0
gi 331284125 ref NP_056224.3	E1A-binding protein p400 [Homo sapiens]	EP400	0.00013437	0	0	0
gi 78000213 ref NP_001029196.1	DNA methyltransferase 1-associated protein 1 [Homo sapiens]	DMAP1	0.00006419	0.00028988	0	0
gi 4506753 ref NP_003698.1	ruvB-like 1 [Homo sapiens]	RUVBL1	0.00571884	0.00519533	0	0.00018904
gi 5730023 ref NP_006657.1	ruvB-like 2 [Homo sapiens]	RUVBL2	0.00213642	0.00073097	0.00014131	0
gi 30089999 ref NP_829888.1	actin-like protein 6A isoform 2 [Homo sapiens]	BAF53a	0.00030982	0	0	0
gi 197313748 ref NP_054878.5	histone-lysine N-methyltransferase SETD2 [Homo sapiens]	SETD2	0.00014029	0.0000792	0	0

Supplementary Table 1. Detailed data of MudPIT analysis of proteins bound to FLAG-TRIM29 wild-type and mutants

	TRIM29 (394-588)	TRIM29 (470-588)	TRIM29 (1-359)
TRIM29	1516.9	5690.3	5857.0
DNA-PKcs	5.5		
MRE11A	2.0		
BRCA1	0.7		
MSH2	10.1		
MSH6	9.5		
SMC1A	16.5	1.1	
SMC3	9.5		
RFC1	7.7		
RFC2	29.6	2.0	
RFC3	4.4		
RFC4	24.2	3.6	
RFC5	21.2		
BRCA2	1.4		

NSAF×10⁴

10.1<

8.1-10.0

6.1-8.0

4.1-6.0

2.1-4.0

0-2.0

Supplementary Table 2. List of TRIM29 (wild type and mutants)-associated proteins identified by MudPIT.

Secondary structure	405 α-helix 430																									
Human	L	G	Q	S	L	G	N	F	K	D	D	L	L	N	V	C	M	R	H	V	E	K	M	C	K	A
Chimpanzee	L	G	Q	S	L	G	N	F	K	D	D	L	L	N	V	C	M	R	H	V	E	K	M	C	K	A
Mouse	L	G	Q	S	L	G	N	C	K	D	D	L	L	N	V	C	M	R	H	V	E	K	M	C	K	A
Rat	L	G	Q	S	L	G	N	C	K	D	D	L	L	N	V	C	M	R	H	V	E	K	M	C	K	A
Cow	L	G	Q	A	L	G	N	C	K	D	D	L	L	N	V	C	M	R	H	V	E	K	M	C	K	A
Chicken	M	S	P	S	M	G	L	L	R	D	D	L	L	N	V	C	M	R	H	V	E	K	I	C	K	A
Frog	V	T	Q	A	M	G	S	L	K	E	E	L	V	T	N	G	R	K	H	V	E	K	Y	S	K	N

Supplementary Table 3. Multiple sequence alignment of residues 405-430 in TRIM29 among several species

	.	.	.	.		.	.	.
Human (48-55)	G	E	A	A	E	G	K	S
Chimpanzee (48-55)	G	E	A	A	E	G	K	S
Mouse (48-55)	G	E	V	T	E	G	K	T
Rat (48-55)	G	E	V	T	E	G	K	T
Cow (47-54)	G	D	V	S	D	G	Q	G
Chicken (40-48)	G	E	K	E	P	I	K	G

Supplementary Table 4. Multiple sequence alignment of Walker A motif in TRIM29 in several species