Title	TRIM29 regulates the assembly of DNA repair proteins into damaged chromatin
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Citation	Nature communications, 6, 7299 https://doi.org/10.1038/ncomms8299
Issue Date	2015-06
Doc URL	http://hdl.handle.net/2115/59741
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Type	article
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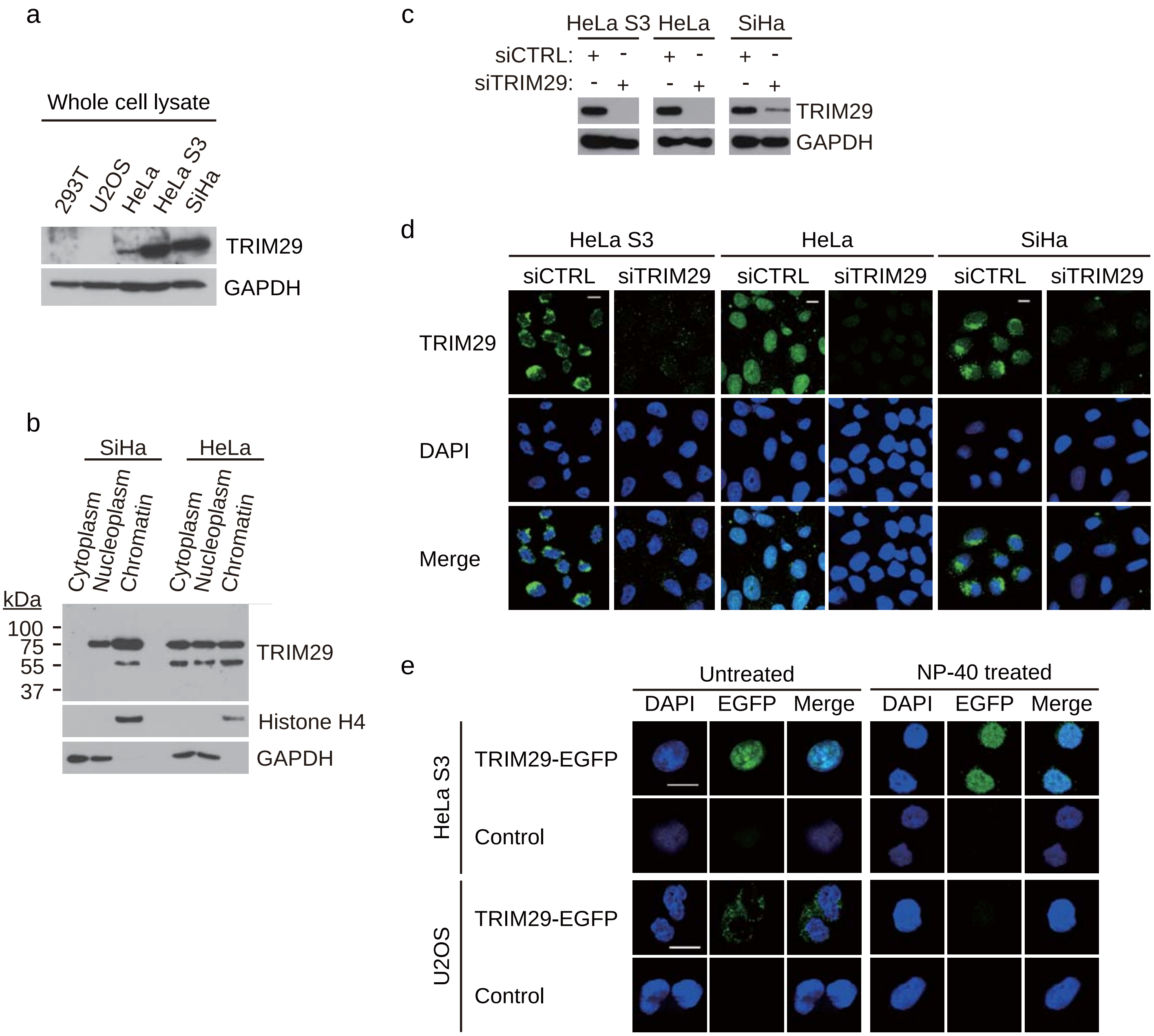
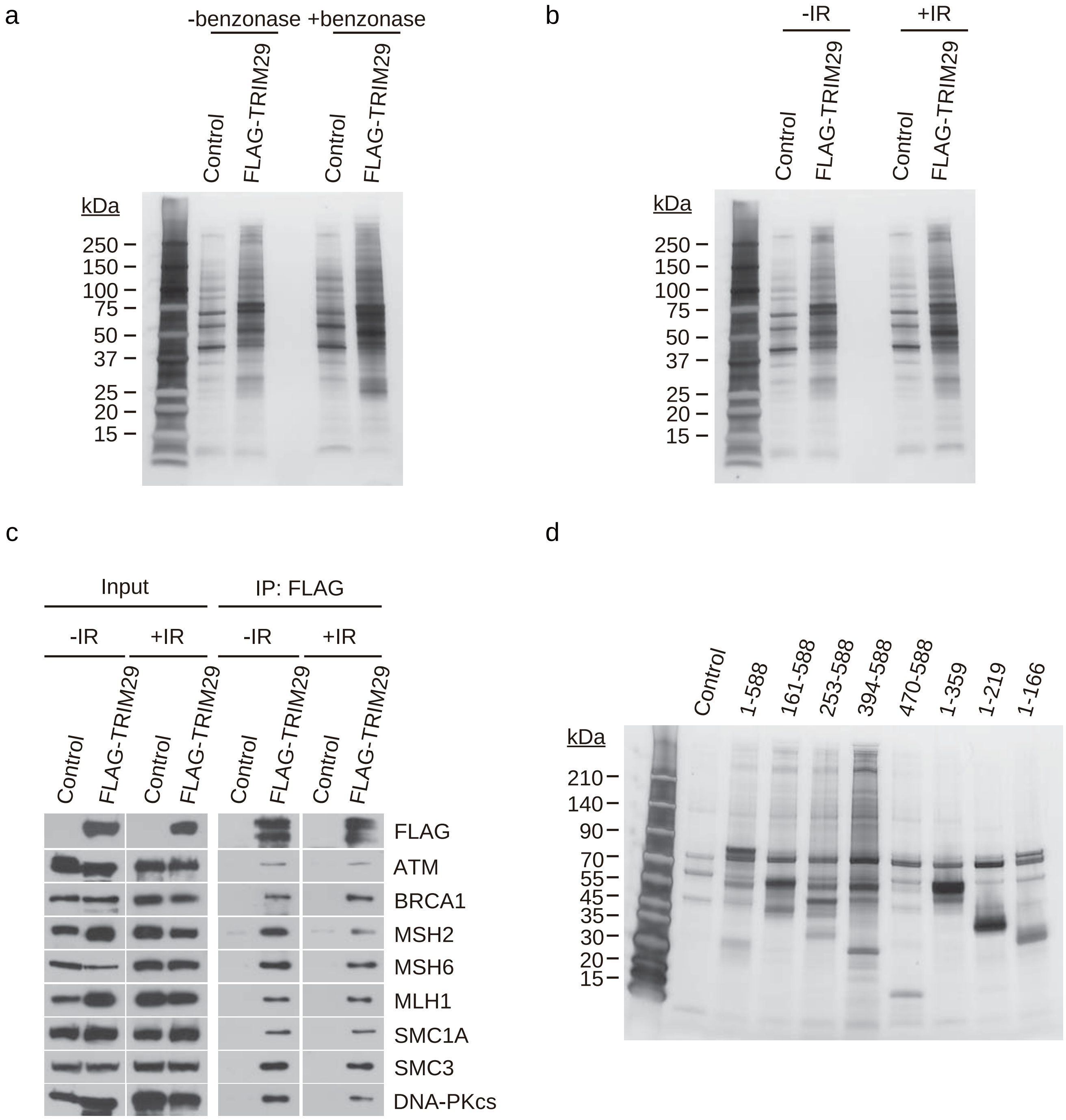
File Information	ncomms8299-s1.pdf (Supplementary Figures 1-20 and Supplementary Tables 1-4)

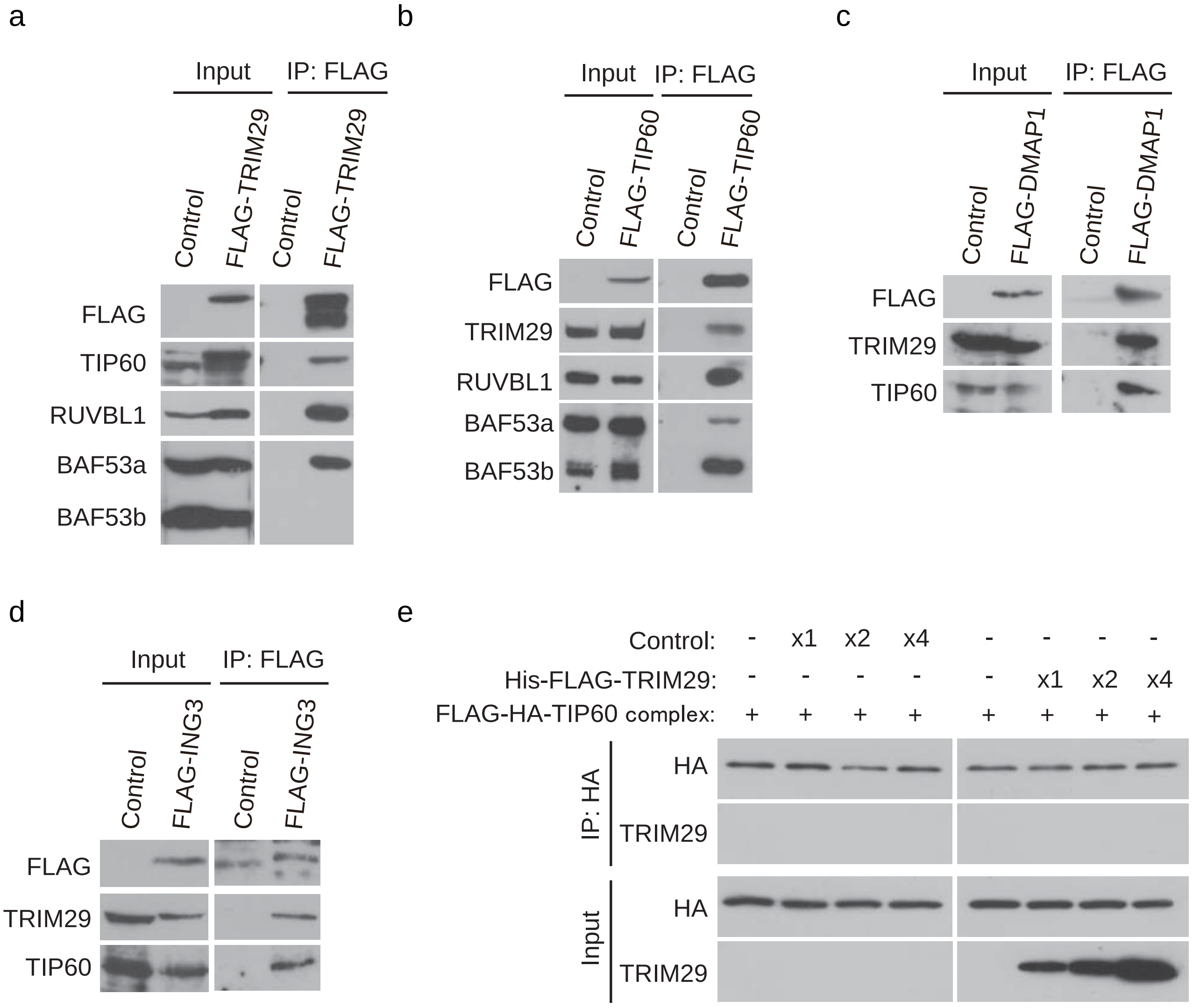


Supplementary Figure 1. Expression of endogenous and exogenous TRIM29.
(a) Expression pattern of endogenous TRIM29 in 293T, U2OS, HeLa, HeLa S3 and SiHa cells. Total proteins (10 μg) from whole cell lysates of 293T, U2OS, HeLa, HeLa S3 and SiHa cells were subjected to SDS-PAGE followed by Western blotting. **(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

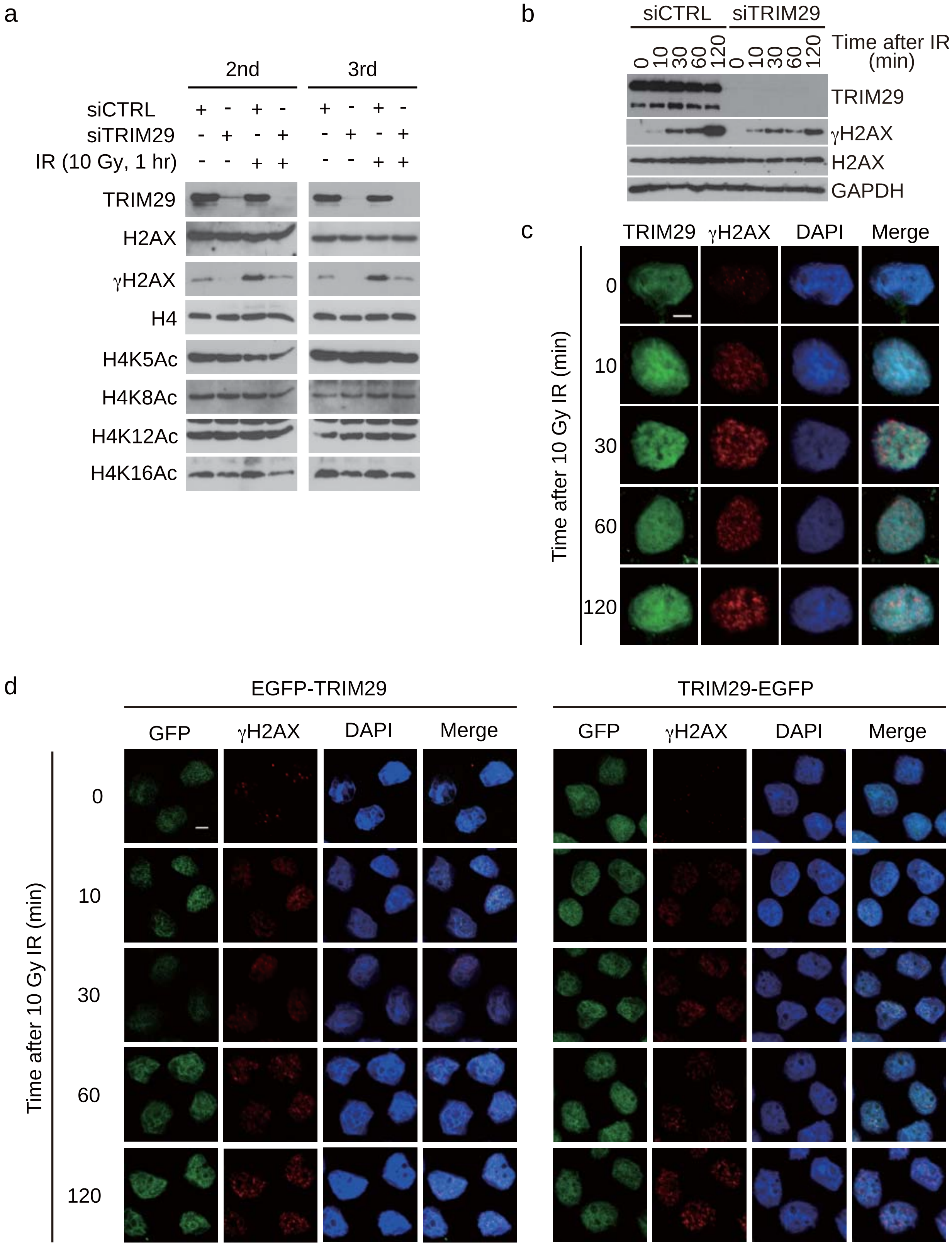


Supplementary Figure 2. Analysis of proteins associated with FLAG-tagged TRIM29.
(a) Silver staining of FLAG-tagged immunoprecipitates from benzonase-treated nuclear extracts of cells expressing FLAG-tagged TRIM29 on 4-20% gradient SDS-PAGE gel. **(b)** Silver staining of FLAG-tagged immunoprecipitates 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.



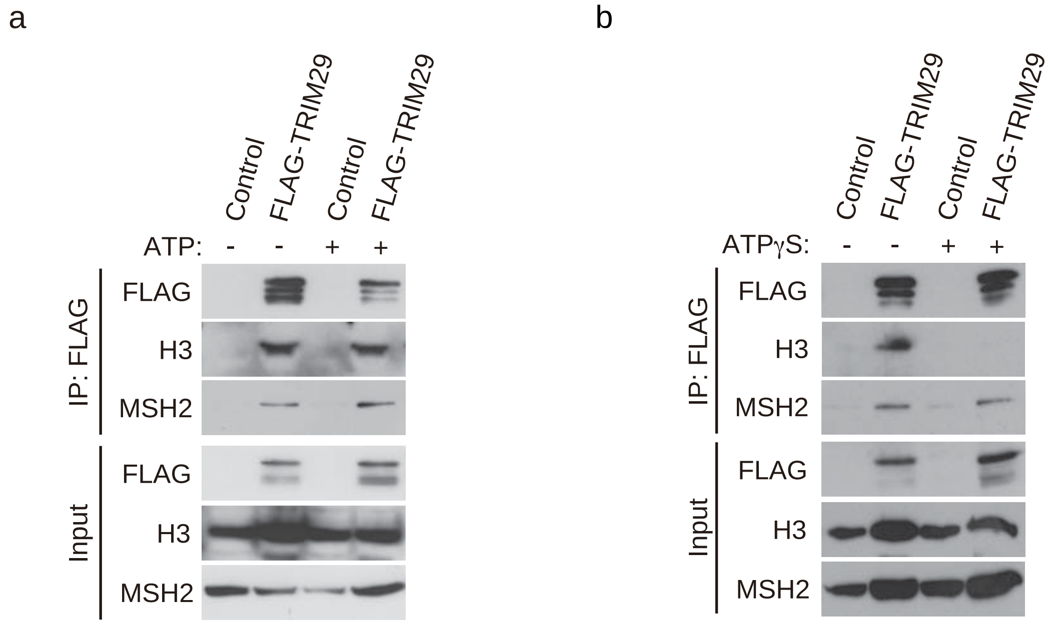
Supplementary Figure 3. Interaction of TRIM29 with subunits of TIP60 complex.
(a) Binding of TRIM29 to the components of TIP60 complex. **(b)** Binding of TIP60 to TRIM29 and other TIP60 complex proteins. **(c)** Binding of DMAP1 to TRIM29 and TIP60. **(d)** Binding of ING3 to TRIM29 and TIP60. **(e)** 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.

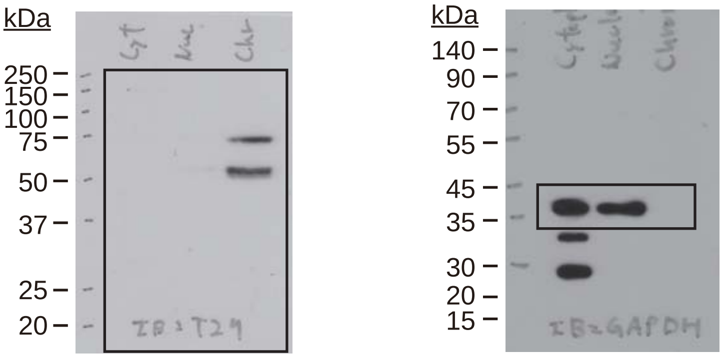
(a) Effect of TRIM29 knockdown on histone modifications induced by IR treatment. HeLa S3 cells were transfected with siControl or siTRIM29. After 48 h, the cells were treated with 10 Gy IR and collected after 1 h. Histones were analyzed by Western blotting using the indicated antibodies. **(b)** Effects 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 γH2AX. 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 γH2AX antibodies. DNA was counterstained with DAPI. Scale bar, 5 μm. **(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 μm.



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

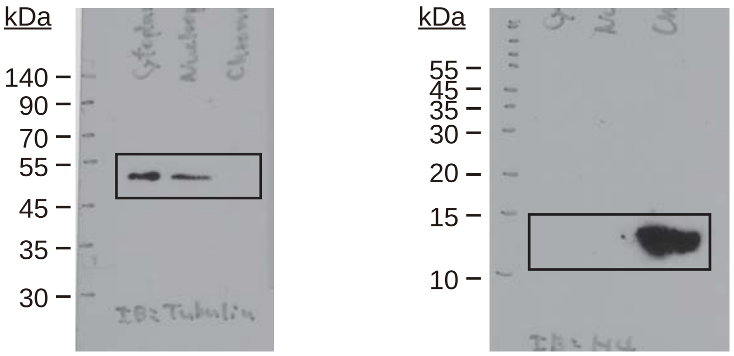
Western blot analysis of anti-FLAG immunoprecipitates from nucleosome-enriched nuclear extracts of HeLa S3 cells expressing FLAG-tagged TRIM29. These assays were performed in the presence or absence of 1 mM ATP **(a)** and 50 μM ATPγS **(b)**.

Figure S5



IB: TRIM29

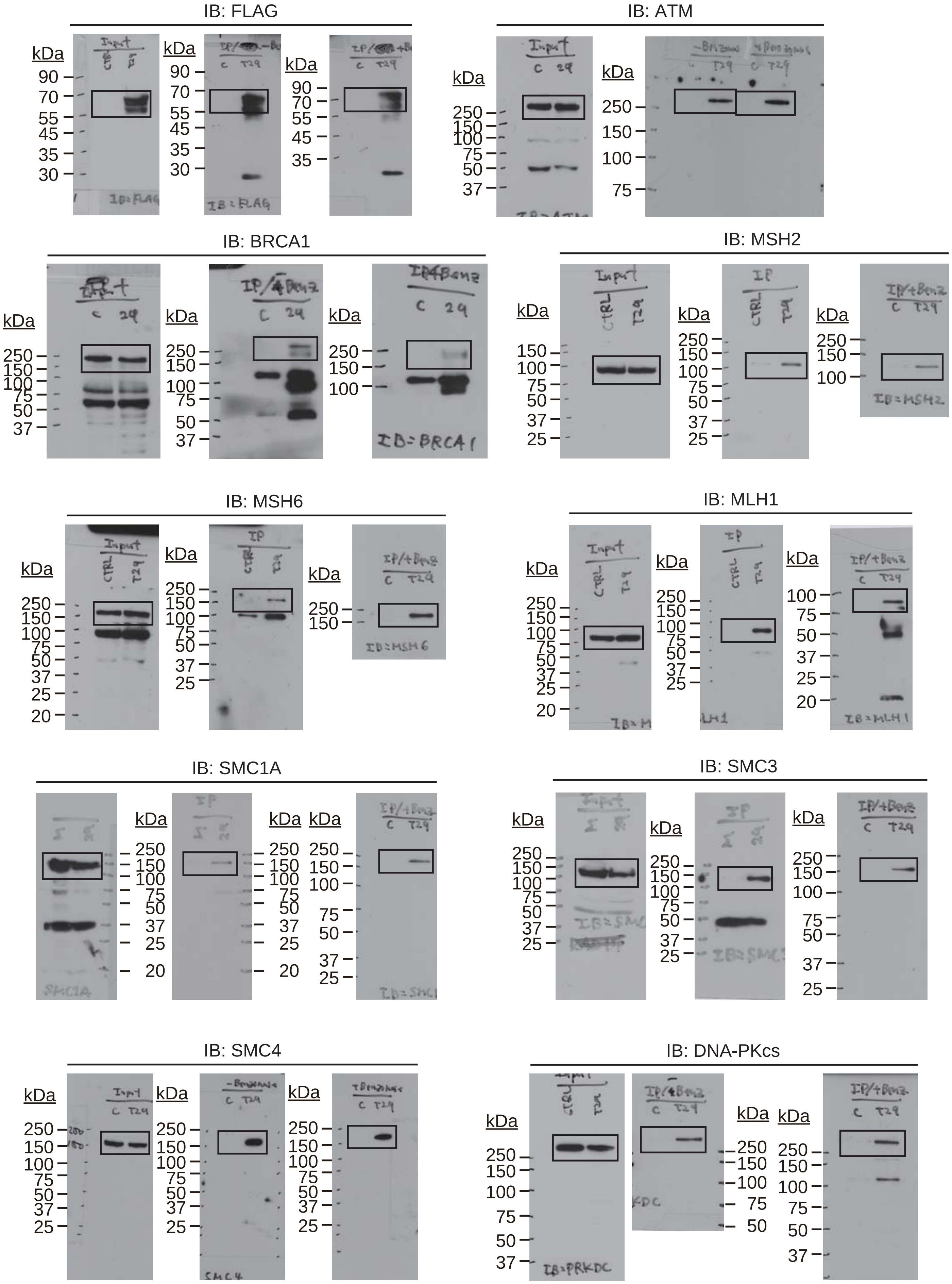
IB: GAPDH



IB: Tublin

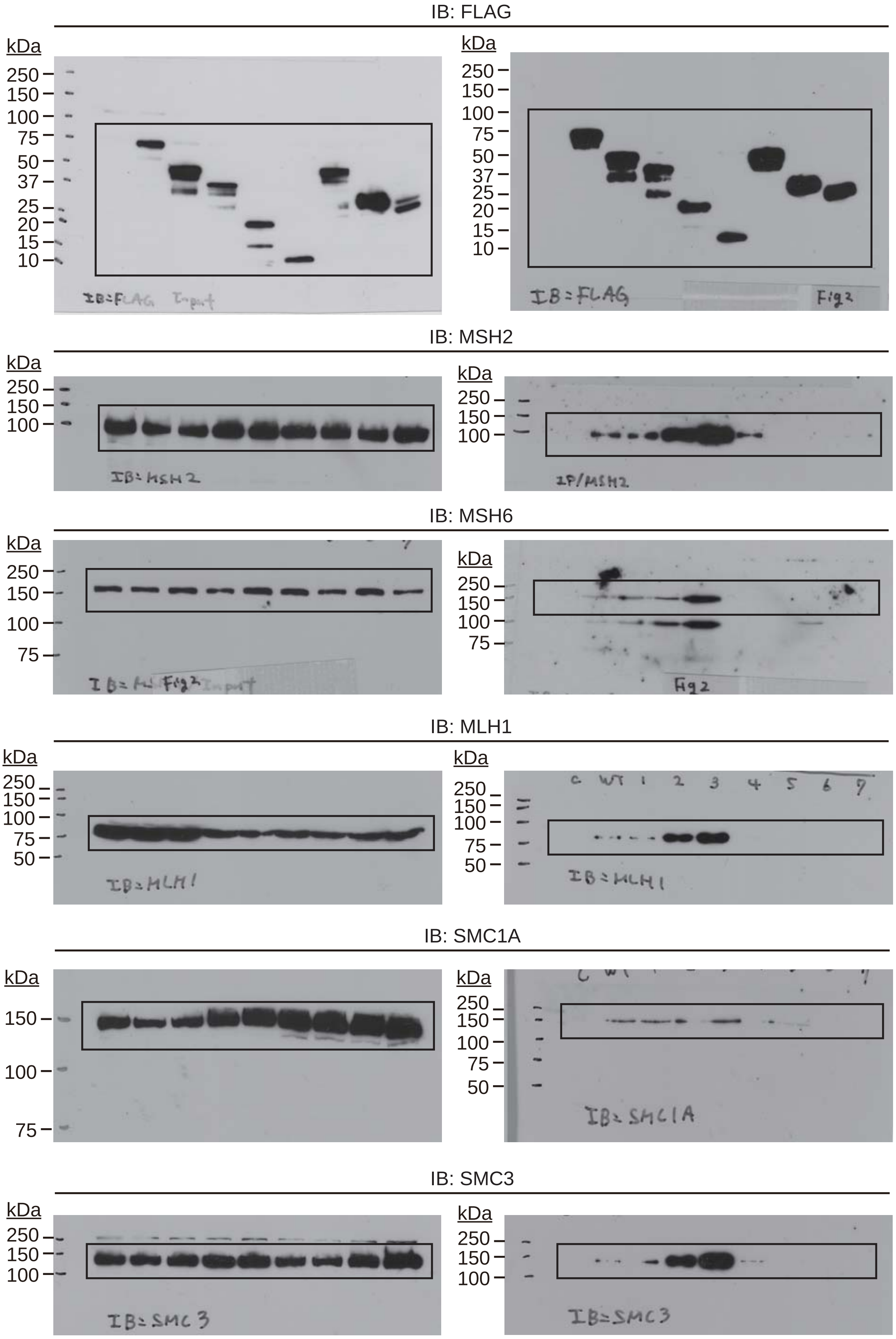
IB: H4

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



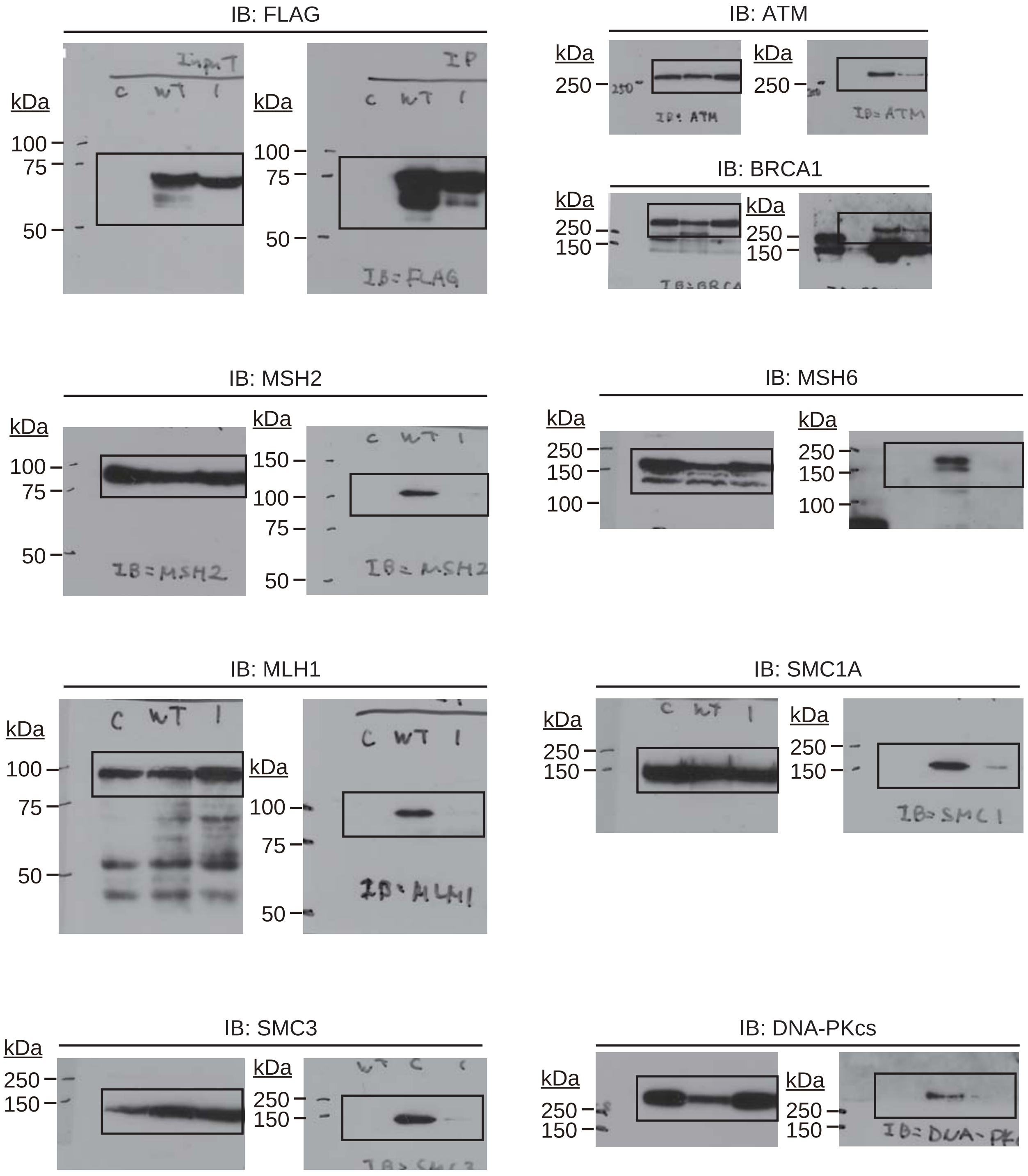
Supplementary Figure 7: Original immunoblot data for Fig. 1g

Figure S7



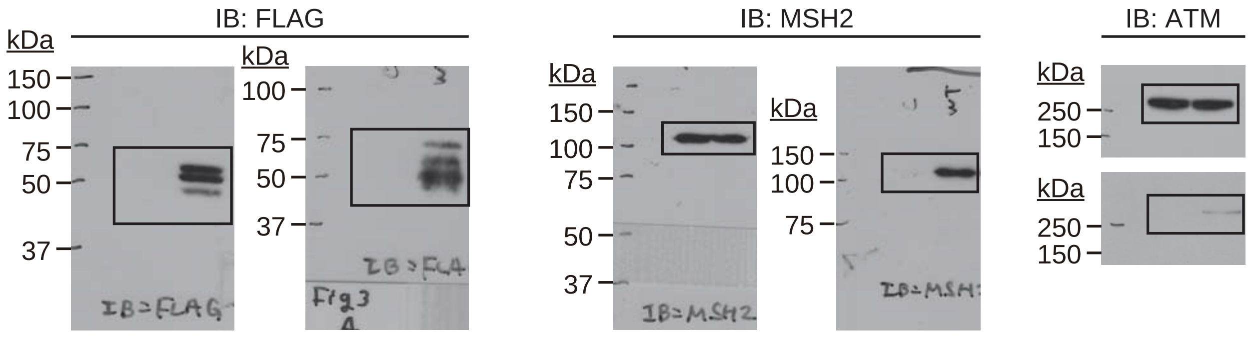
Supplementary Figure 8: Original immunoblot data for Fig. 2b

Figure S8

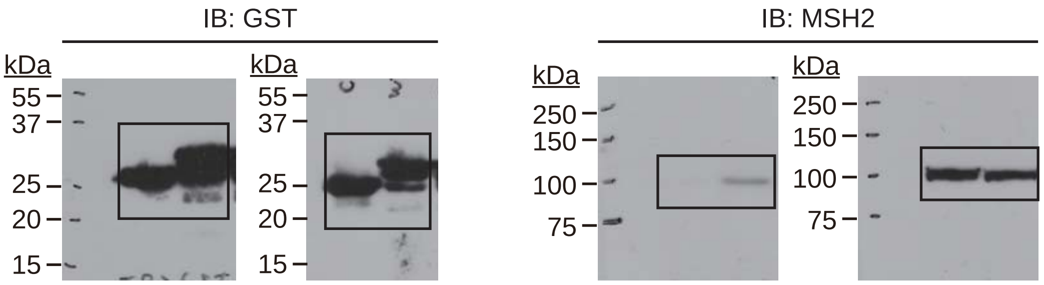


Supplementary Figure 9: Original immunoblot data for Fig. 2e

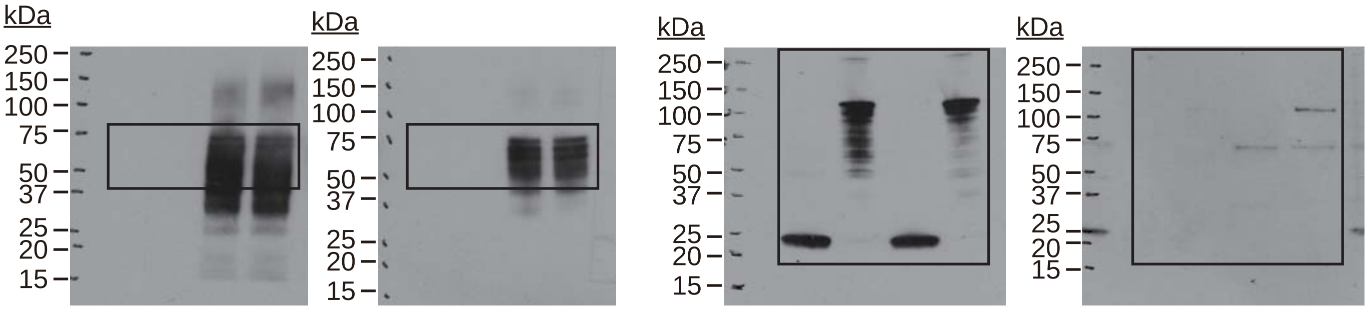
Original immunoblot data for Fig. 3a



Original immunoblot data for Fig. 3b

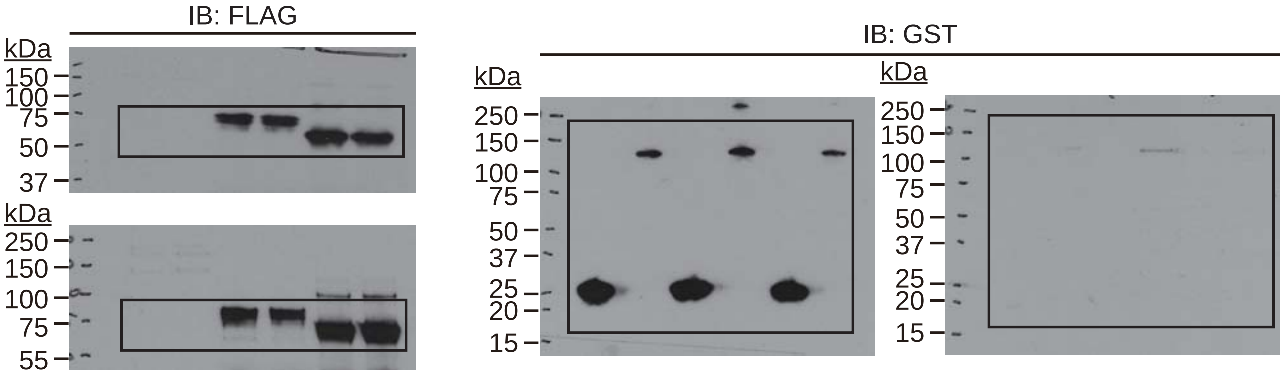


Original immunoblot data for Fig. 3c

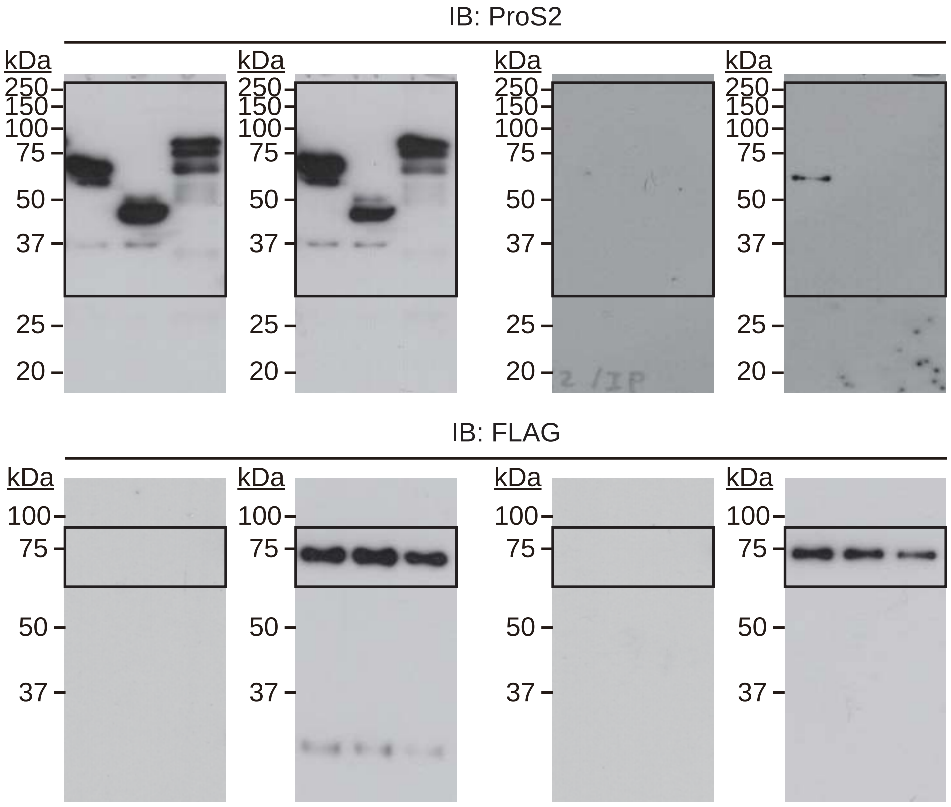


Supplementary Figure 10: Original immunoblot data for Fig. 3a, b and c

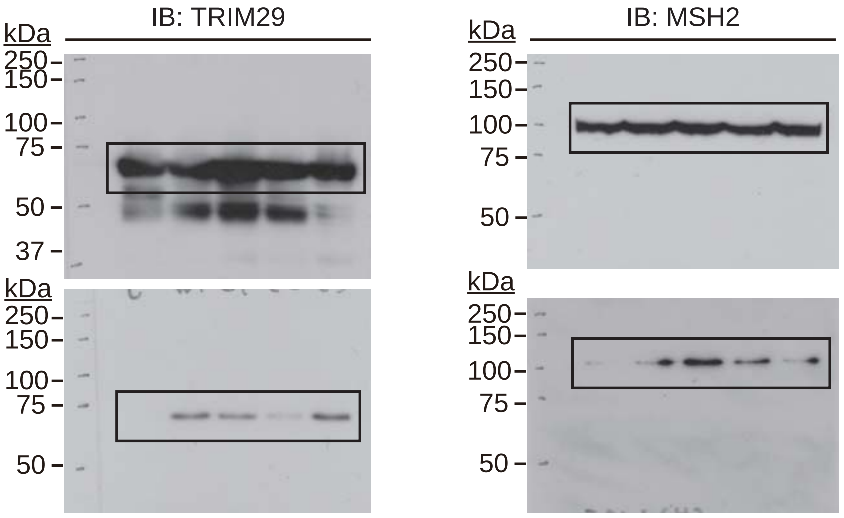
Original immunoblot data for Fig. 3d



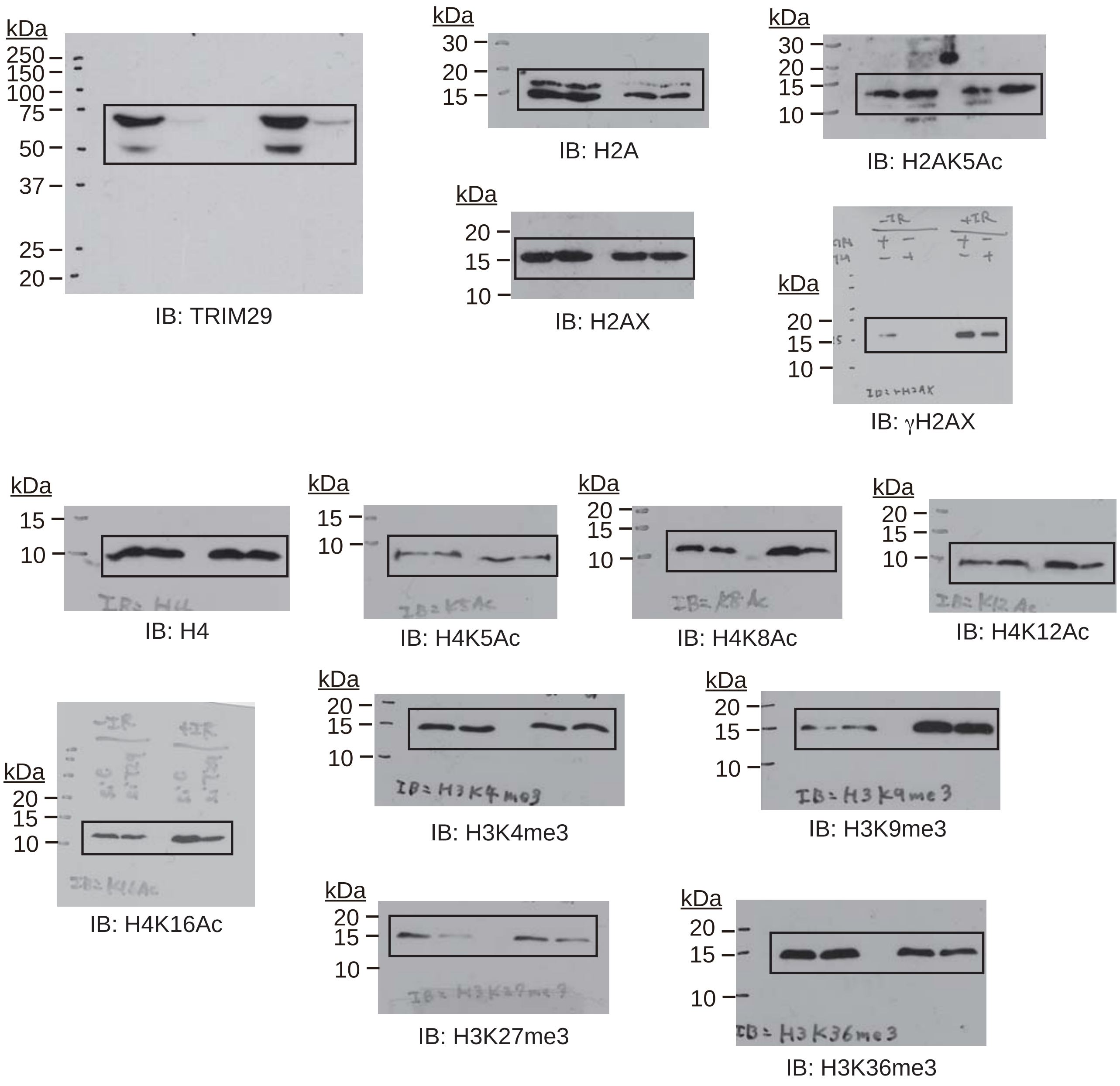
Original immunoblot data for Fig. 3f



Original immunoblot data for Fig. 3g

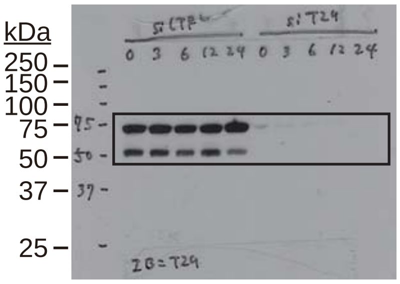


Supplementary Figure 11: Original immunoblot data for Fig. 3d, f and g

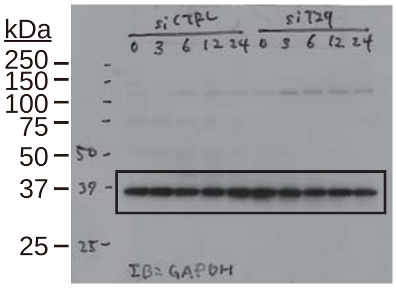


Supplementary Figure 12: Original immunoblot data for Fig. 4a

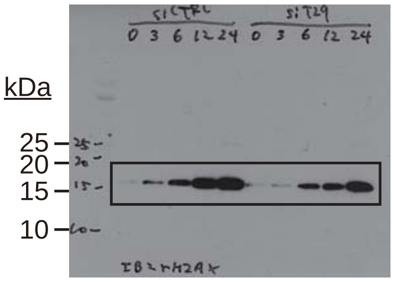
Original immunoblot data for Fig. 4d



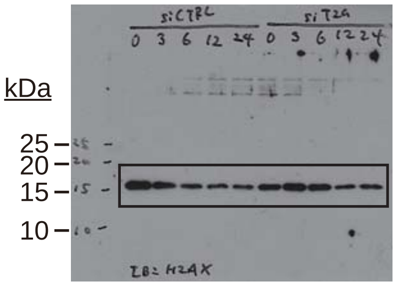
IB: TRIM29



IB: GAPDH

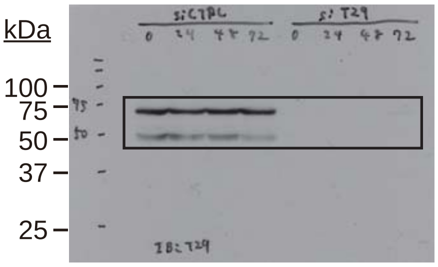


IB: γH2AX

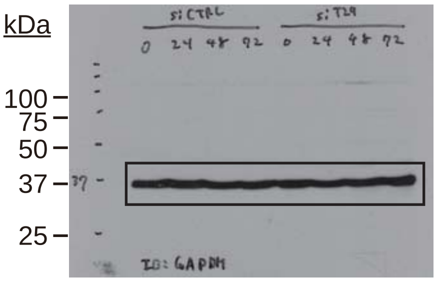


IB: H2AX

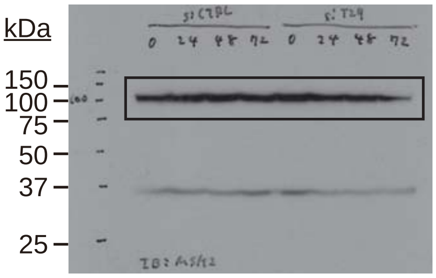
Original immunoblot data for Fig. 4i



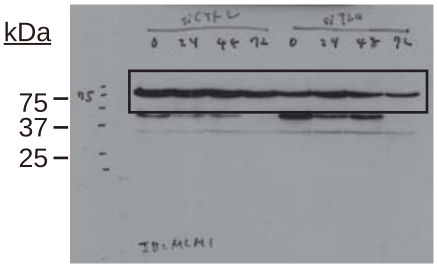
IB: TRIM29



IB: GAPDH

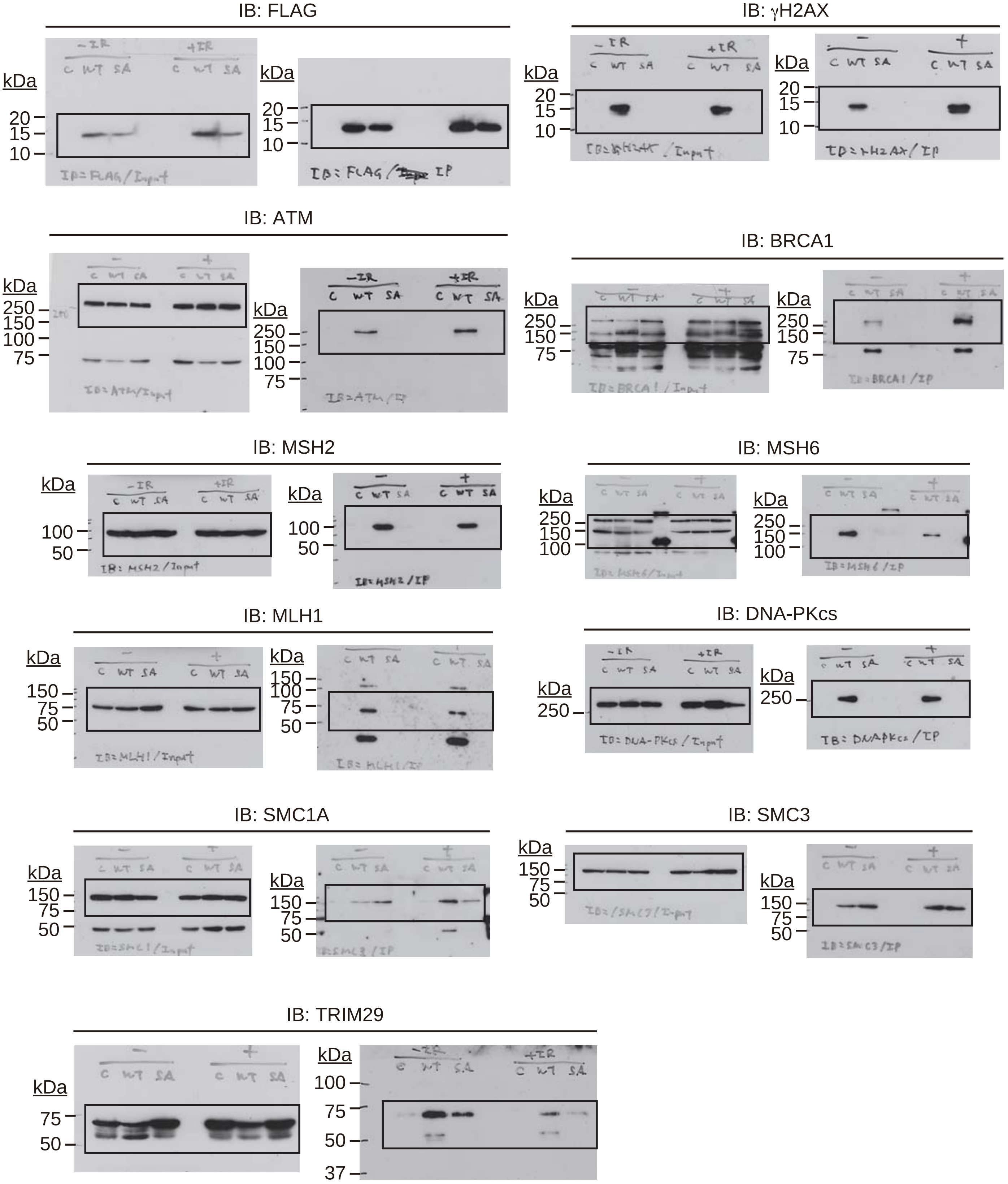


IB: MSH2

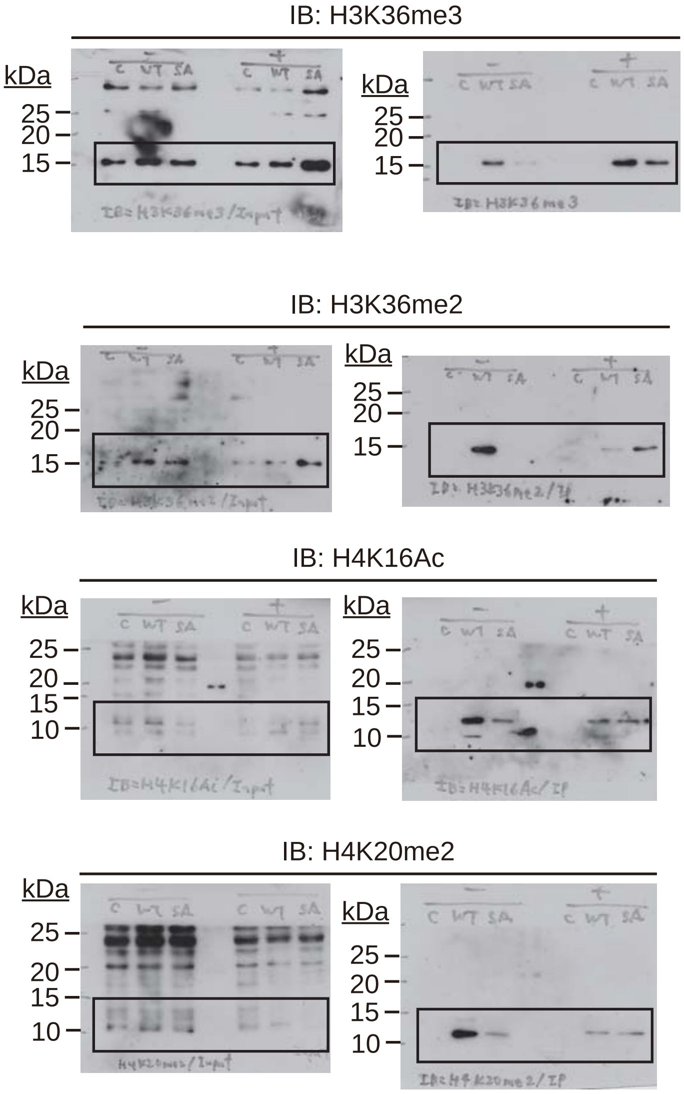


IB: MLH1

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

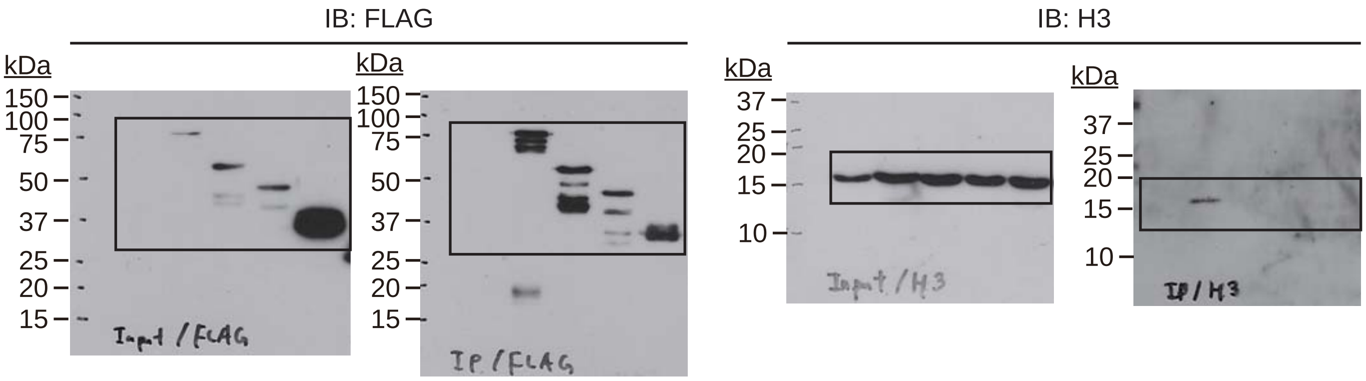


Supplementary Figure 14: Original immunoblot data for Fig. 5b

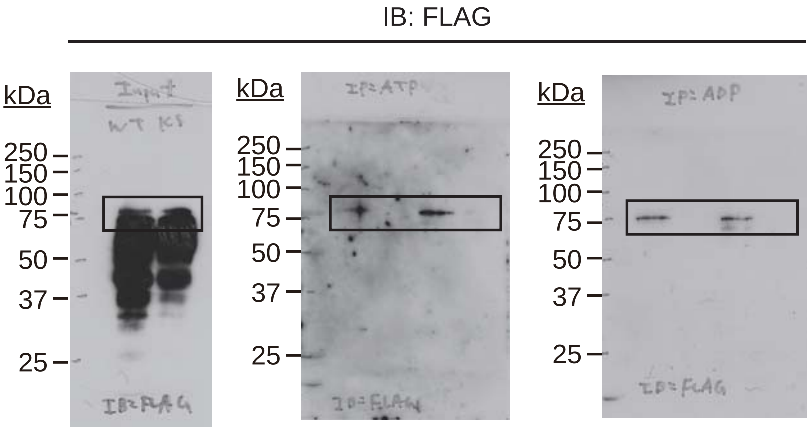


Supplementary Figure 15: Original immunoblot data for Fig. 5b

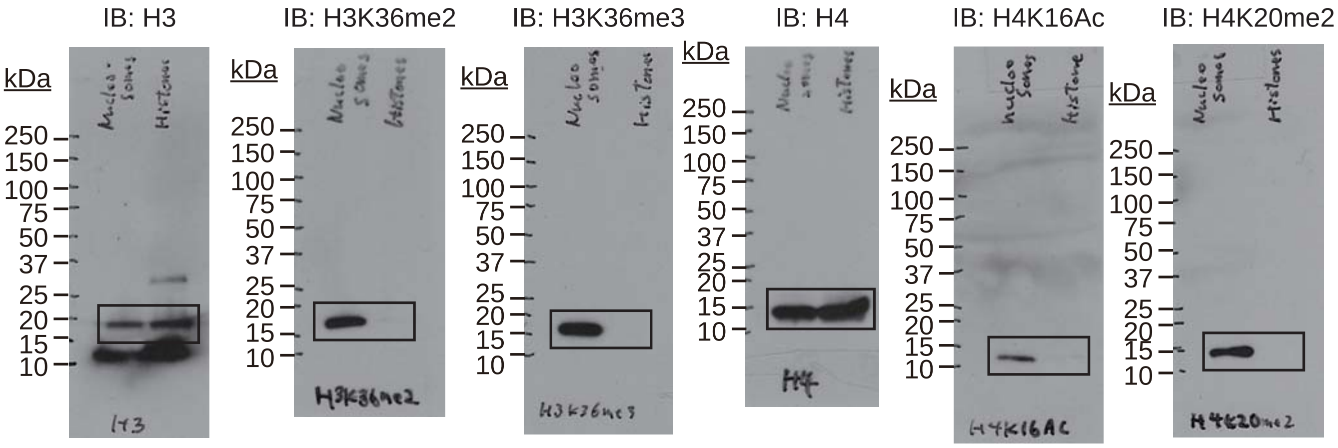
Original immunoblot data for Fig. 6a



Original immunoblot data for Fig. 6c

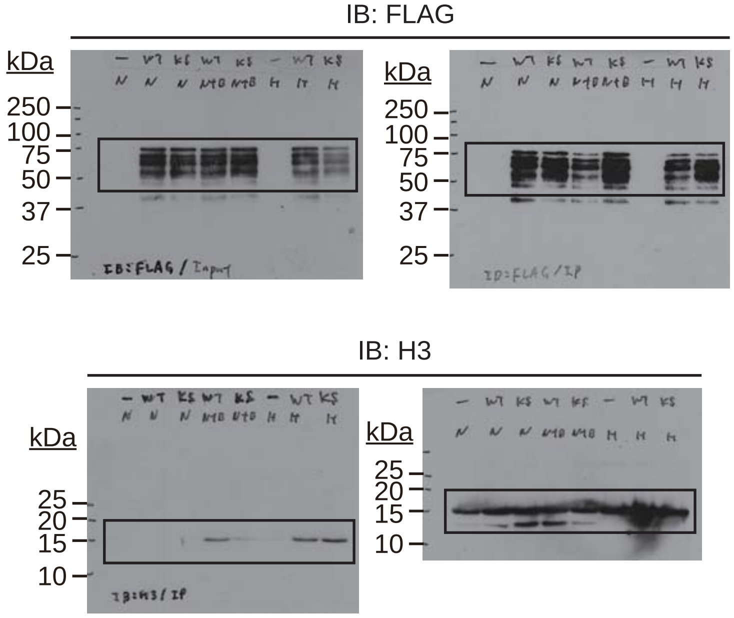


Original immunoblot data for Fig. 6d

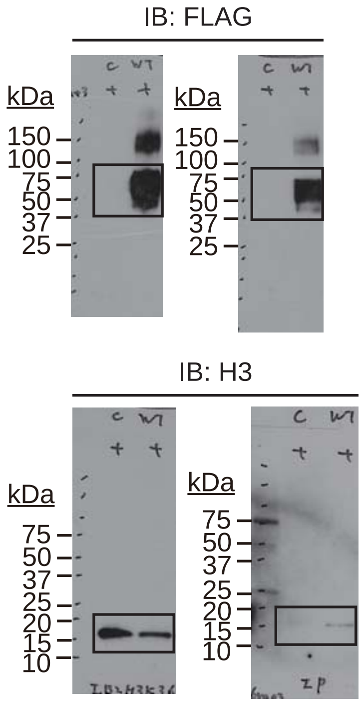


Supplementary Figure 16: Original immunoblot data for Fig. 6a, c and d

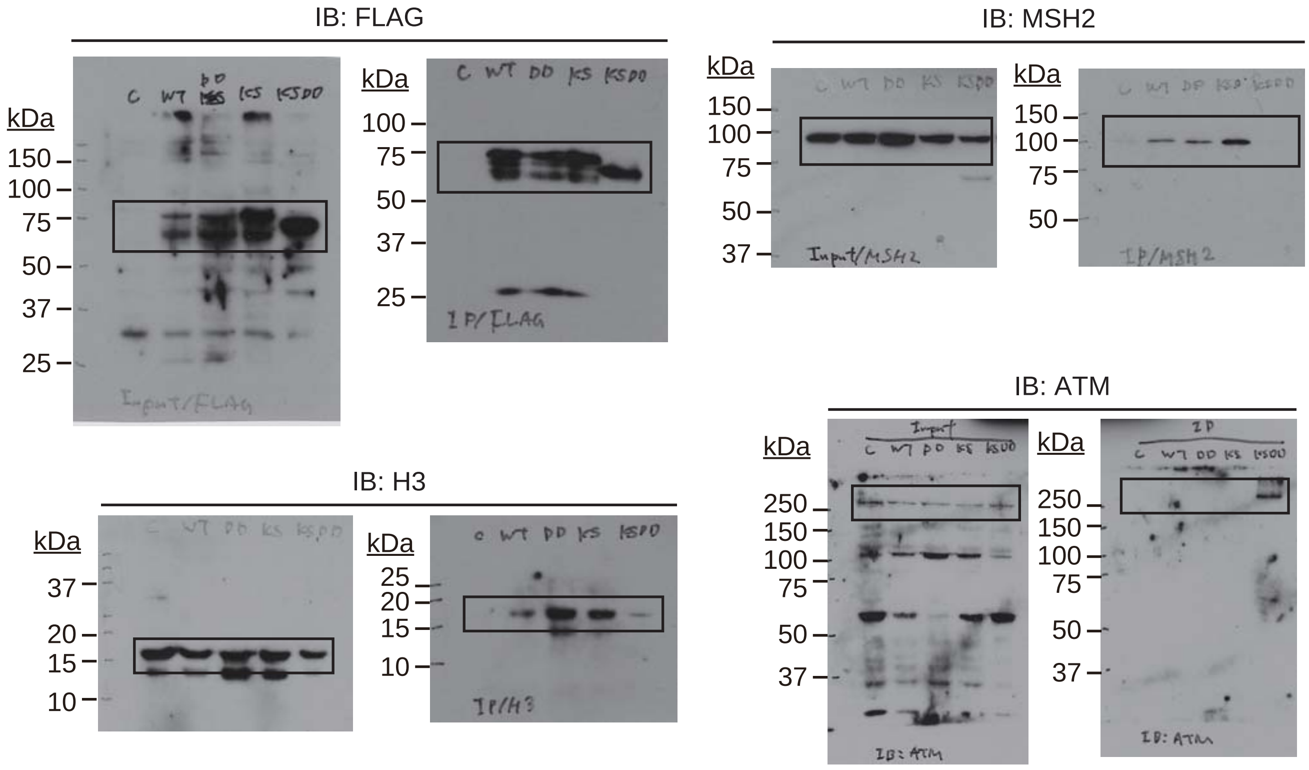
Original immunoblot data for Fig. 6e



Original immunoblot data for Fig. 6g

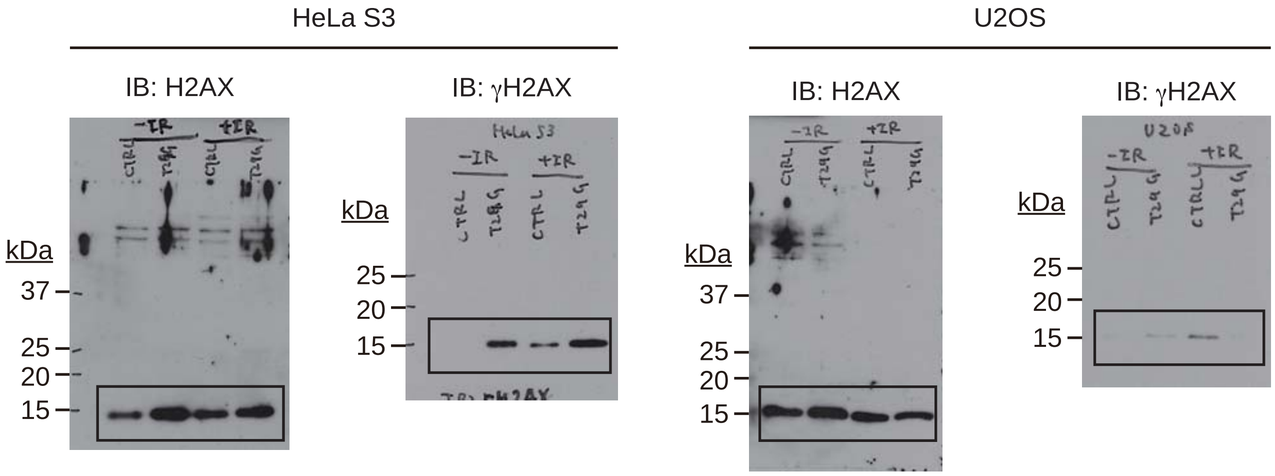


Original immunoblot data for Fig. 6h

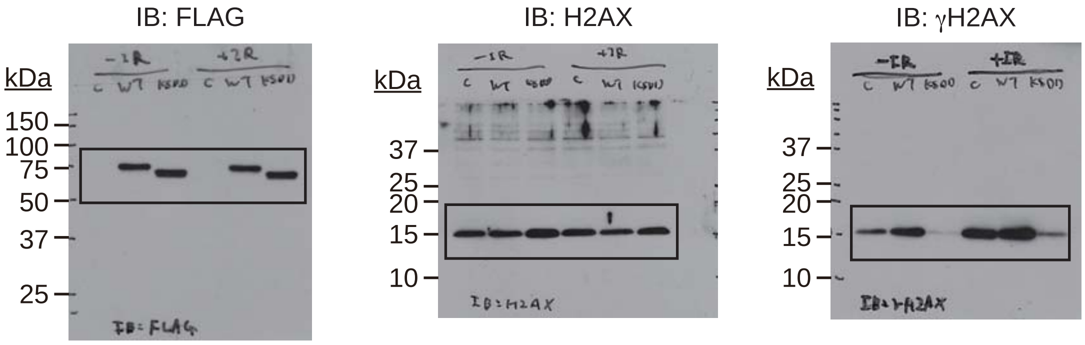


Supplementary Figure 17: Original immunoblot data for Fig. 6e, g and h

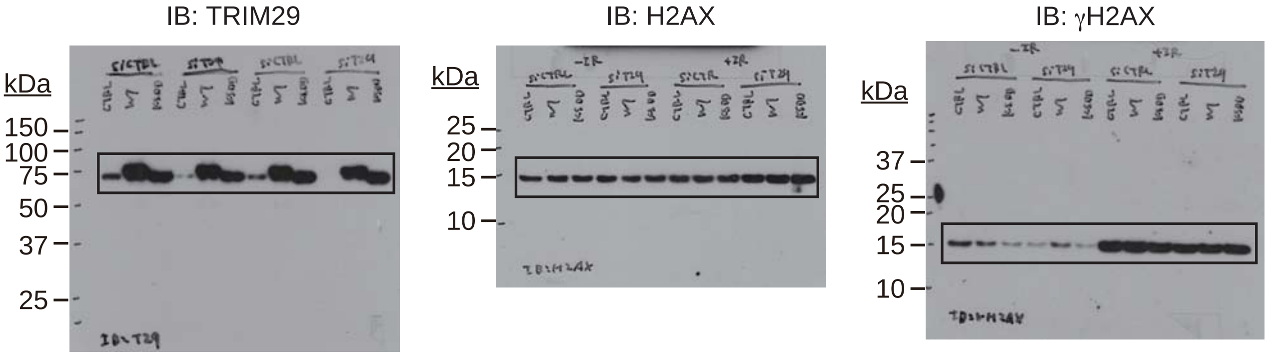
Original immunoblot data for Fig. 7b



Original immunoblot data for Fig. 7d

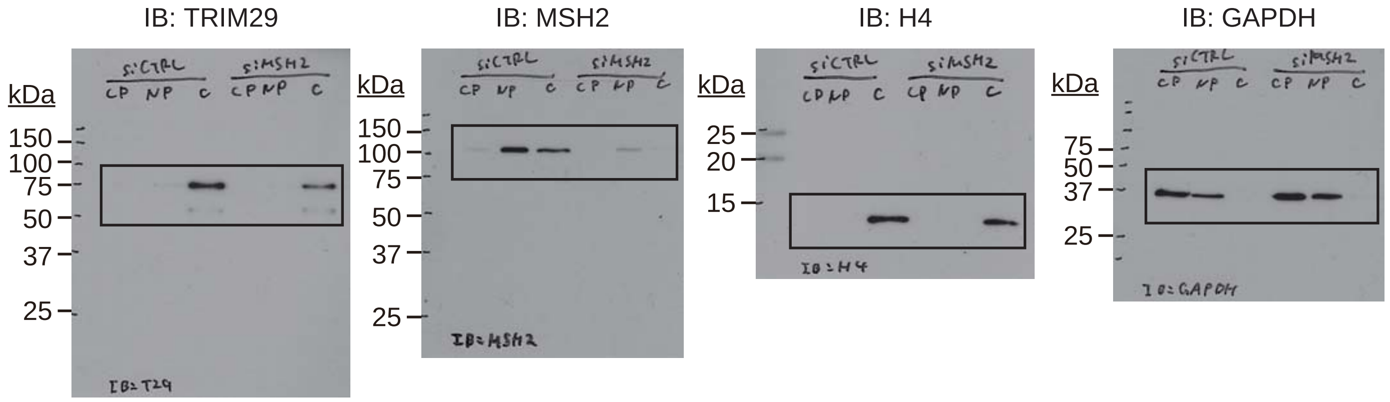


Original immunoblot data for Fig. 7g

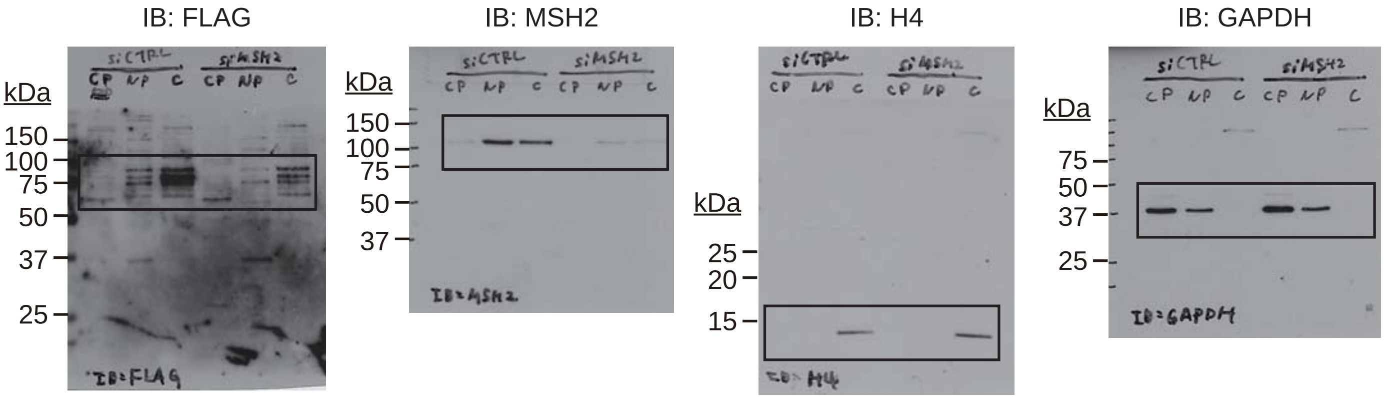


Supplementary Figure 18: Original immunoblot data for Fig. 7b, d and g

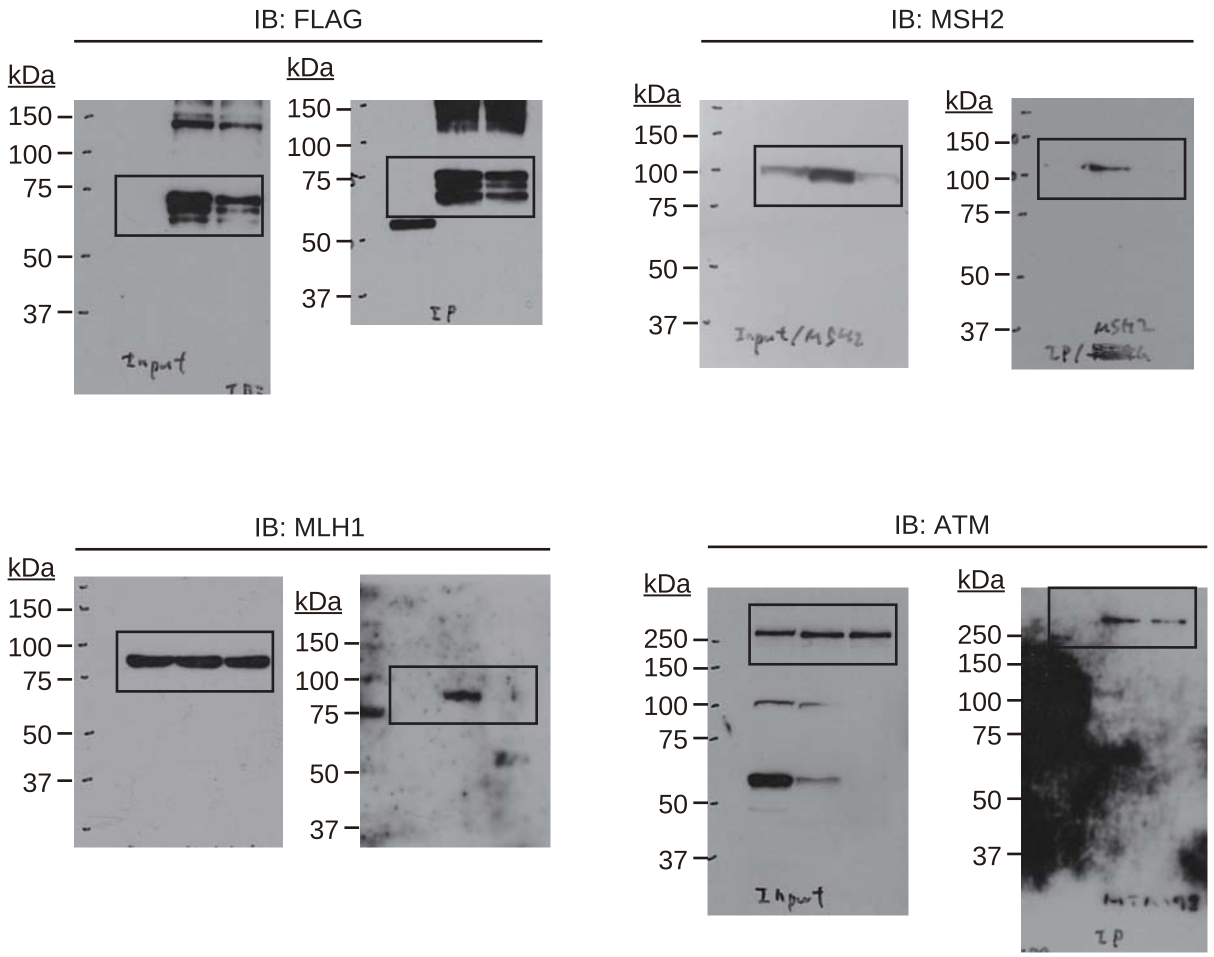
Original immunoblot data for Fig. 8a



Original immunoblot data for Fig. 8b



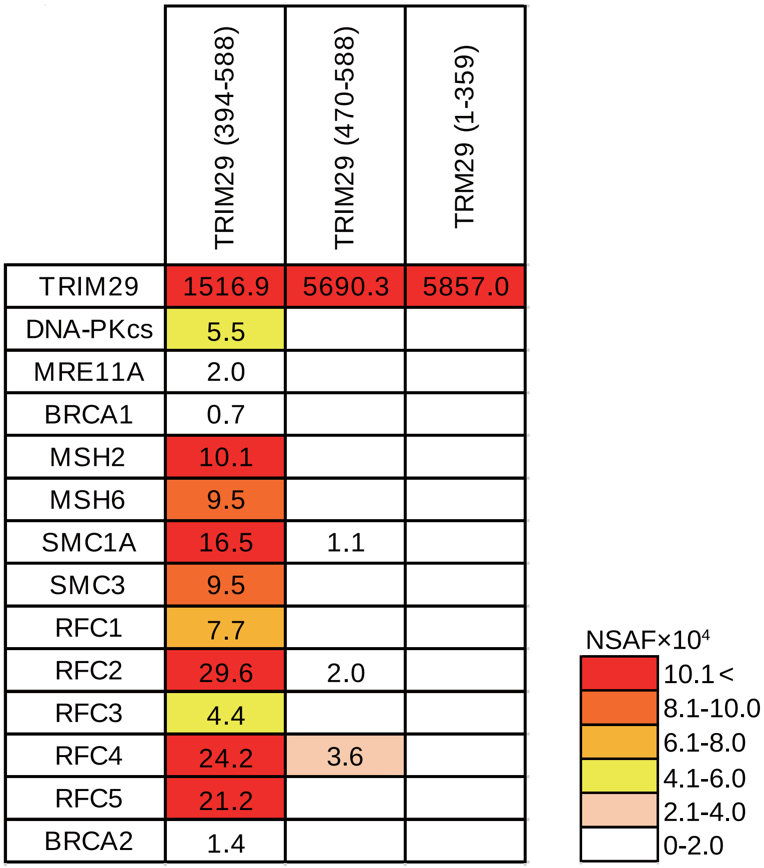
Supplementary Figure 19: Original immunoblot data for Fig. 8a and b



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

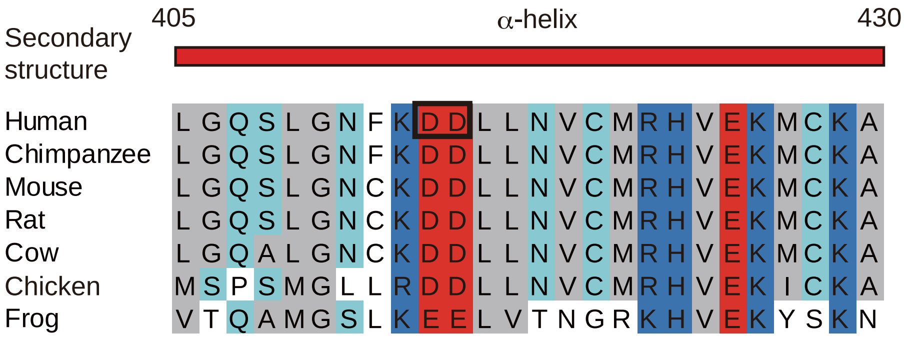


	TRIM29 (394-588)	TRIM29 (470-588)	TRM29 (1-359)
TRIM29	1516.9	5690.3	5857.0
DNA-PKcs	5.5		
MRE11A	2.0		
BRCA1	0.7		
MSH2	10.1		
MSH6	9.5		
SMC1A	16.5	1.1	
SMC3	9.5		
RFC1	7.7		
RFC2	29.6	2.0	
RFC3	4.4		
RFC4	24.2	3.6	
RFC5	21.2		
BRCA2	1.4		

NSAF×10^4

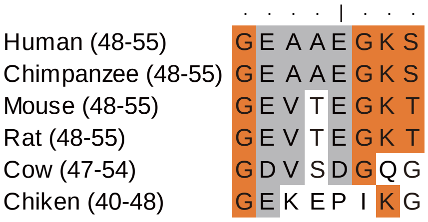
🟥 10.1<
🟧 8.1-10.0
🟧 6.1-8.0
🟨 4.1-6.0
▨ 2.1-4.0
□ 0-2.0

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



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
Chiken (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