Supplementary information

TopBP1 coordinates DNA repair synthesis in mitosis via recruitment of the nuclease scaffold SLX4

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Supplementary Table 1. Cell lines used in this study.

Name	Genotype	Parent	Reference
NCAPH-mEGFP	NCAPH$^{mEGFP/mEGFP}$	HeLa Kyoto WT	(1)
NCAPH-mEGFP TopBP1-Halo	NCAPH$^{mEGFP/mEGFP}$ TopBP1$^{Halo/Halo}$ (loxed)	NCAPH-mEGFP HeLa Kyoto	This study
RPE-1	hTERT	RPE-1	(2)
RPE1 *ROSA26A-TRE*-OsTIR	hTERT *ROSA26A-TRE*-OsTIR	RPE-1	This study
TopBP1-AidSMASh, *ROSA26A-TRE*-OsTIR	hTERT TopBP1$^{mAIDSMASh/mAIDSMASh}$ *ROSA26A-TRE*-OsTIR	RPE-1	This study
HeLa T-REX	T-REX	HeLa	Jakob Nilsson
H2B-TFP	H2B-TFP-NeoR (Random)	HeLa, T-REX	This study
H2B-TFP mCherry-TopBP1	H2B-TFP-NeoR (Random) mCherry-TopBP1 (FRT)	H2B-TFP HeLa, T-REX	This study
H2B-TFP mCherry-TopBP1 K704A	H2B-TFP-NeoR (Random) mCherry-TopBP1-K704A (FRT)	H2B-TFP HeLa, T-REX	This study
H2B-TFP TopBP1-Halo	H2B-TFP-NeoR (Random) TopBP1$^{Halo-PuroR/WT}$	HeLa, T-REX	This study
H2B-TFP TopBP1-Halo mCherry-SLX4	H2B-TFP-NeoR (Random) TopBP1$^{Halo-PuroR/WT}$ mCherry-SLX4 (FRT)	H2B-TFP HeLa, T-REX	This study

H2B-TFP TopBP1-Halo mCherry-SLX4-T1260A	H2B-TFP-NeoR (Random) TopBP1$^{Halo\text{-}PuroR/WT}$ mCherry-SLX4-T1260A (FRT)	H2B-TFP HeLa, T-REX	This study
H2B-TFP TopBP1-Halo mCherry-SLX4-ΔSIM1-2	H2B-TFP-NeoR (Random) TopBP1$^{Halo\text{-}PuroR/WT}$ mCherry-SLX4-ΔSIM1-2 (FRT)	H2B-TFP HeLa, T-REX	This study
H2B-TFP TopBP1-Halo mCherry-SLX4-ΔSIM1-3	H2B-TFP-NeoR (Random) TopBP1$^{Halo\text{-}PuroR/WT}$ mCherry-SLX4-ΔSIM1-3 (FRT)	H2B-TFP HeLa, T-REX	This study
H2B-TFP TopBP1-Halo mCherry-SLX4 DD-SLX4-T1260	H2B-TFP-NeoR (Random) TopBP1$^{Halo\text{-}PuroR/WT}$ mCherry-SLX4 (FRT) CMVp-DD-SLX4-T1260	H2B-TFP-NeoR (Random) TopBP1$^{Halo\text{-}PuroR/WT}$ mCherry-SLX4 (FRT)	This study
H2B-TFP TopBP1-Halo mCherry-SLX4 DD-SLX4-T1260A	H2B-TFP-NeoR (Random) TopBP1$^{Halo\text{-}PuroR/WT}$ mCherry-SLX4 (FRT) CMVp-DD-SLX4-T1260A	H2B-TFP-NeoR (Random) TopBP1$^{Halo\text{-}PuroR/WT}$ mCherry-SLX4 (FRT)	This study

Supplementary Table 2 - Plasmids used in study

Plasmid	Genotype	Source
pBMN-FKBP(DD)-YFP	*Amp^R*, *FKBP(DD)-YFP*	(3) AddGene ID 31763
pmCherry-C1	*Kan^R*, mCherry	Takara Bio Inc.
pEGFP-N1-TetR	*Neo^R/Kan^R, CMVp-TetR-EGFP*	(4)
pJB005	*Amp^R*, hTopBP1-Halo-Puro^R repair template	This study
pJB007	*Amp^R,* hTopBP1 C-terminal gRNA #1 Cas9-D10A-EGFP	This study
pJB008	*Amp^R,* hTopBP1 C-terminal gRNA #2 Cas9-D10A-EGFP	This study
pJB017	*Amp^R,* pEGFP-SLX4	Niels Mailand
pJB030	*Neo^R/Kan^R*, hH2B-TFP	This study
pJB049	*Amp^R*, hTopBP1-Halo-BS^R repair template	This study
pJB064	*Amp^R,* pCDNA5-TO-FRT-mCherry-SLX4	This Study
pJB065	*Amp^R,* pCDNA5-TO-FRT-mCherry-SLX4-T1260A	This Study
pJB066	*Amp^R,* pCDNA5-TO-FRT-mCherry-SLX4-ΔSIM1-2	This Study
pJB067	*Neo^R/Kan^R,* mVenus-C1-SLX4	This Study

pJB068	*Neo^R/Kan^R,* mVenus-C1-SLX4 T1260A	This Study
pJB071	*Amp^R,* pCDNA5-TO-FRT-mCherry-SLX4-ΔSIM1-3	This Study
pJB072	*Neo^R/Kan^R,* mVenus-C1	Addgene, #27794
pJJ2	*Neo^R/Kan^R, CMVp-DD-SLX4-T1260*	This study
pJJ3	*Neo^R/Kan^R, CMVp-DD-SLX4-T1260A*	This study
pOG44	*Amp^R*, CMV-FLP recombinase	Jakob Nilsson
pSNK011	*Amp^R,* pCDNA5-TO-FRT-mCherry	This study
pSNK013	*Amp^R,* pCDNA5-TO-FRT-mCherry-TopBP1	This study
pSNK014	*Amp^R,* pCDNA5-TO-FRT-mCherry-TopBP1-K704A	This study
pTMN4	*Amp^R*, hTopBP1-Halo-Neo^R repair template	This study
pTMN7	*Amp^R,* pCDNA5-TO-FRT	Jakob Nilsson
pX23	*Neo^R/Kan^R,* pEGFP-C1-hTopBP1	Claus Storgaard Sørensen
pX461	*Amp^R,* Cas9-D10A-EGFP	Addgene, #48140

Supplementary Table 3 – Primers used in study

Primer	Sequence (5' → 3')	Used for
gRNA 1 FW	PO_4-CACCGATTTAATGTTTGGTAACTAA	Cloning of JB007
gRNA 1 RV	PO_4-AAACTTAGTTACCAAACATTAAATC	Cloning of JB007
gRNA 2 FW	PO_4-CACCGAATGTGACTGTGATAGATT	Cloning of JB008
gRNA 2 RV	PO_4-AAACAATCTATCACAGTCACATTC	Cloning of JB008
5' Halo FW (+SalI)	GTCGACGAAATCGGTACTGGCTTTCC	Cloning Halo-tag into pJB005
3' Halo RV (+BamHI)	GGATCCTCAACCGGAAATCTCCAGAG	Cloning Halo-tag into pJB005
5' FW Halo-insert validation	AAGTTTATTCCTGGCTGGGCACGGTAGCAC	Validation of Halo-insertion in TopBP1 C-terminus
3' RV Halo-insert validation	CGGGATAGGGCGGATGAACTCCATAAATGC	Validation of Halo-insertion in TopBP1 C-terminus
5' FW PuroR	GTGCTGGTTATTGTGCTGTCTCATCATTTTGGC	Validation of PuroR insertion upstream of TopBP1 3' homology arm
3' RV PuroR	GGTGGGAGGGGGACAGTTTACATCATAATGCTAC	Validation of PuroR insertion upstream of TopBP1 3' homology arm
5' FW mCherry (+ AflIII)	CTTAAGATGGTGAGCAAGGGCGAGGAGGATAAC	Amplification of mCherry
3' RV mCherry (+KpnI)	GGTACCCTTGTACAGCTCGTCCATGCCGCC	Amplification of mCherry

5’ FW SLX4 (+KpnI + ATG)	GGTACCATGAAACTGAGTGTGAATGAGGC	Amplification of SLX4 cDNA
3’ RV SLX4 (+KpnI)	GGTACCTCAGTTCCGCTCCACCTTC	Amplification of SLX4 cDNA
5’ FW TopBP1 (+ KpnI)	GGTACCATGTCCAGAAATGACAAAGAAC	Amplification of TopBP1 cDNA
3’ RV TopBP1 (+ NotI)	GCGGCCGCTTAGTGTACTCTAGGTCGTTTGATTTT	Amplification of TopBP1 cDNA
5’ FW K704A Quikchange	CACTTCTTTGCAGCTTCATATGCAGAGCCACCACGTTCTTTCAG	TopBP1 K704A mutagenesis
3’ RV K704A Quikchange	CTGAAAGAACGTGGTGGCTCTGCATATGAAGCTGCAAAGAAGTG	TopBP1 K704A mutagenesis
5’ FWT1260A Quikchange	GGTGCCCGCCGCCCCGCTGGC	SLX4 T1260A mutagenesis
3’ RV T1260A Quikchange	GCCAGCGGGGCGGCGGGCACC	SLX4 T1260A mutagenesis
DD-HindIII-FW	5’-GCTCAAGCTTCGCCACCATGGGAGTGCAGG-3’	Cloning of pJJ2 and pJJ3
DD-Tpep-RV	5’- AGTCGCGGCCGCCTAAGAACAGTCACGGCTTCTGCTGGCCA GCGGGGTGGCGGGCACCAGCCACGAGAATTCTTCCGGTTTTA GAAGC-3’	Cloning of pJJ2
DD-Apep-RV	5’-AGTCGCGGCCGCCTAAGAACAGTCACGGCTTCTGCTGGCCA GCGGGGCGGCGGGCACCAGCCACGAGAATTCTTCCGGTTTTA GAAGC-3’	Cloning of pJJ3

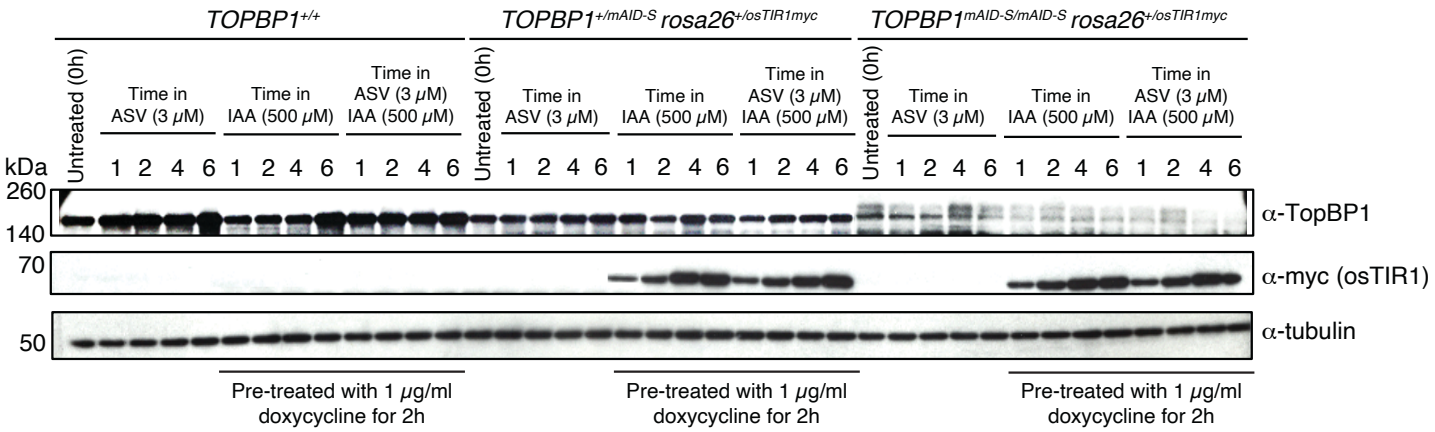
Supplementary Table 4 – siRNA’s used in study

Name	Target	Sequence (5’ → 3’)	Source
siTopBP1	TopBP1 3’ UTR	GUAAAUAUCUGAAGCUGUAUU-TT	Microsynth #4222214
siSLX4 (UTR7062)	SLX4 3’ UTR	GUAAAUAUCUGAAGCUGUAUU-TT	Microsynth #4222212
siSLX4 (UTR87)	SLX4 5’ UTR	GCACAAGGGCCCAGAACAAUU-TT	Microsynth #4222210
siLuciferase	Luciferase	N.A.	Eurofins genomics

Supplementary references

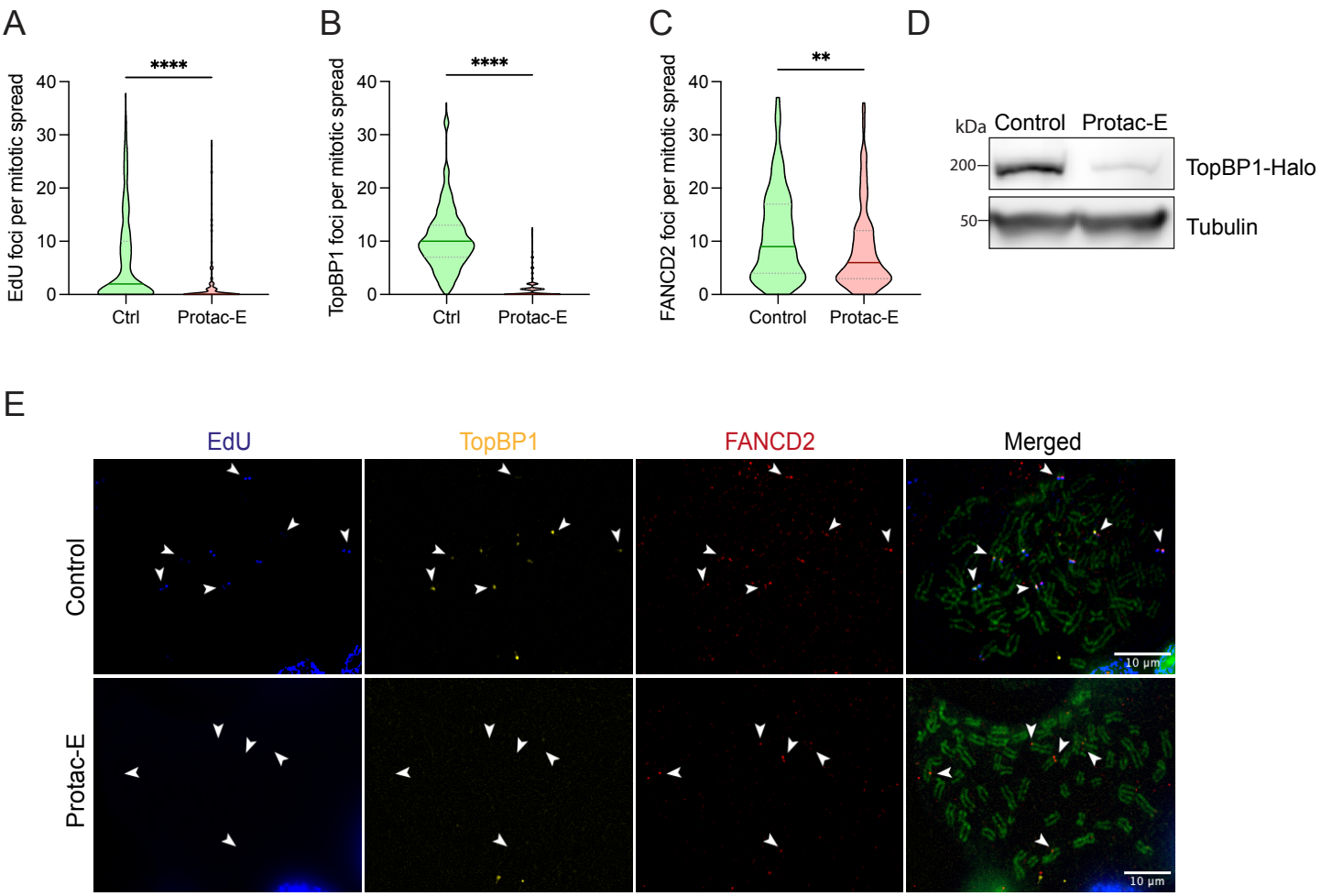
1. Walther, N., Hossain, M.J., Politi, A.Z., Koch, B., Kueblbeck, M., Odegard-Fougner, O., Lampe, M. and Ellenberg, J. (2018) A quantitative map of human Condensins provides new insights into mitotic chromosome architecture. *J Cell Biol*, **217**, 2309-2328.
2. Jiang, X.R., Jimenez, G., Chang, E., Frolkis, M., Kusler, B., Sage, M., Beeche, M., Bodnar, A.G., Wahl, G.M., Tlsty, T.D. *et al.* (1999) Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. *Nat Genet*, **21**, 111-114.
3. Banaszynski, L.A., Chen, L.C., Maynard-Smith, L.A., Ooi, A.G. and Wandless, T.J. (2006) A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. *Cell*, **126**, 995-1004.
4. Rademacher, A., Erdel, F., Trojanowski, J., Schumacher, S. and Rippe, K. (2017) Real-time observation of light-controlled transcription in living cells. *J Cell Sci*, **130**, 4213-4224.

Supplementary Figure 1 - Kinetics of TopBP1 depletion following activation of the miniAID-SMASh system



Detection of TopBP1 and OsTIR1 using anti-TopBP1 and anti-myc antibodies of whole cell extracts of the indicated cell lines run on an SDS-PAGE gel. Cells were treated with either ASV (3 μM) alone to activate the SMASh tag or Dox (1 μg/ml for 2h) and IAA (500 μM) to activate the miniAID tag or a combination of all to make use of the degrading ability of the combined miniAID-SMASh system. Cells were followed for a period of 6h and samples were collected at 0 (untreated), 1, 2, 4 and 6h post drug addition. Note that Dox was added 2h prior to the 0h time point and was not washed off. Beta-tubulin served as the loading control.

Supplementary Figure 2 - MiDAS is dependent on TopBP1

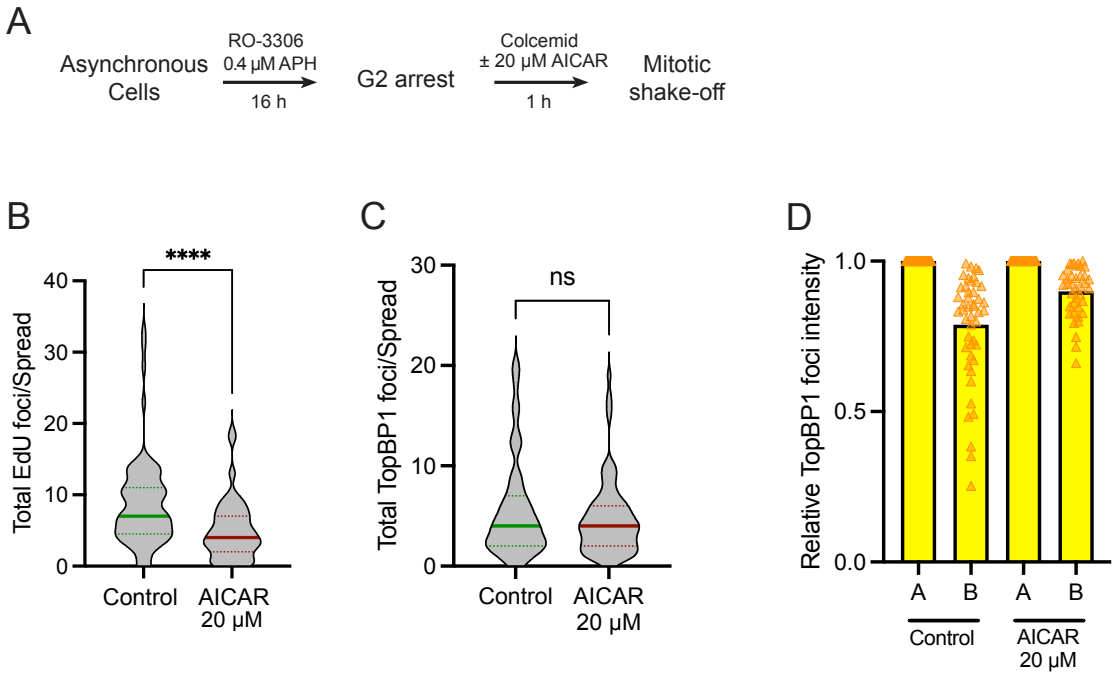


A-C) Quantification of EdU, TopBP1 and FANCD2 foci per mitotic spread in HeLa Kyoto cells (NCAPH-mEGFP, TopBP1-Halo) treated with Protac-E for degradation of endogenouse TopBP1. Statistical significance was evaluated by Student's t-test (n=164 (control) and n=179 (Protac-E). ****, p<0.0001.

D) Western blot showing degradation of TopBP1-Halo by treatment with 500 nM Protac-E for 16 hours.

E) Representative images of native metaphase spreads of cells treated with Protac-E with EdU, TopBP1 and FANCD2 foci.

Supplementary Figure 3 - TopBP1 assymmetry is dependent on MiDAS



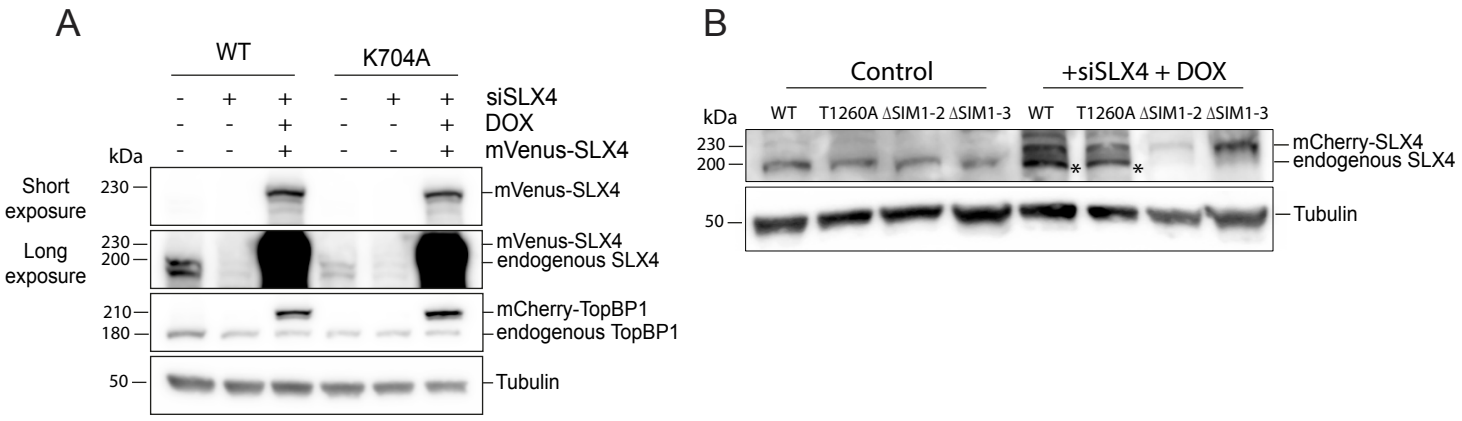
A) Experimental setup for inhibition of MiDAS using the RAD52 inhibitor AICAR.

B) Quantification of EdU foci per metaphase spread with or without the RAD52 inhibitor AICAR (20 μM, 1 h). Statistical significance was evaluated by Student's t-test. ****, $p<0.0001$ (n=77). Horizontal lines in violin plots indicate median and dotted lines indicate quartiles.

C) Quantification of TopBP1 foci per metaphase spread with or without the RAD52 inhibitor AICAR (20 μM, 1 h). Statistical significance was evaluated by Student's t-test (n=77). Horizontal lines in violin plots indicate median and dotted lines indicate quartiles.

D) Quantification of relative intensity of TopBP1 in twin foci under inhibition of MiDAS with or without AICAR. A indicates the sister chromatid containing the brighter TopBP1 focus, while B indicates the sister chromatid with the dimmer TopBP1 focus. Each triangle in the bar plot indicates quantification of a pair of TopBP1 twin-foci. Two biological replicates (n=47 (Control), n=39 (AICAR)).

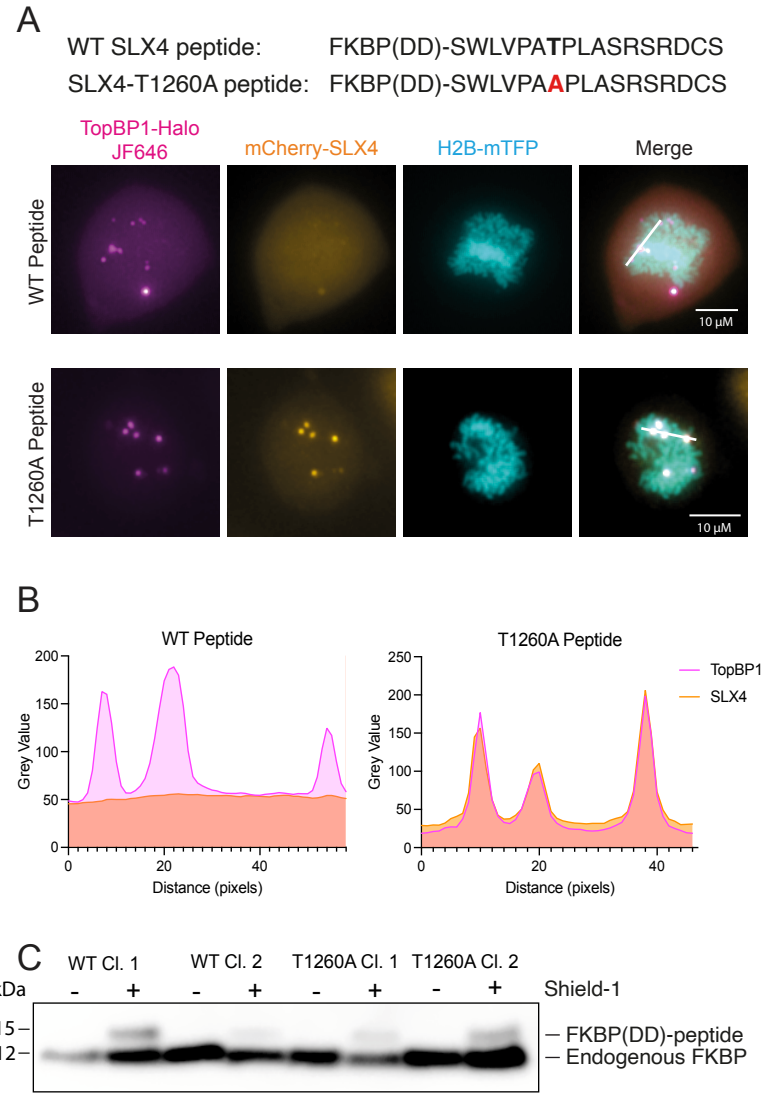
Supplementary Figure 4 - TopBP1 K704, SLX4 T1260A and SIMs contributes to SLX4 recruitment in mitosis



A. Western blot showing protein expression for the experiment in Figure 5.
The western blot shows knockdown of endogenous SLX4, ectopic expression of the mVenus-SLX4 vector and expression of mCherry-TopBP1 induced with doxycycline (DOX) in the HeLa TREX cells containing DOX-inducible TopBP1-WT or TopBP1-K704A.

B. Western blot showing protein expression for DOX-inducible SLX4-WT, SLX4-T1260A, SLX4-ΔSIM1-2 and SLX4-ΔSIM1-3 for the experiment in Figure 6. * unspecific band.

Supplementary Figure 5 - SLX4-T1260/T1260A peptide competition assay

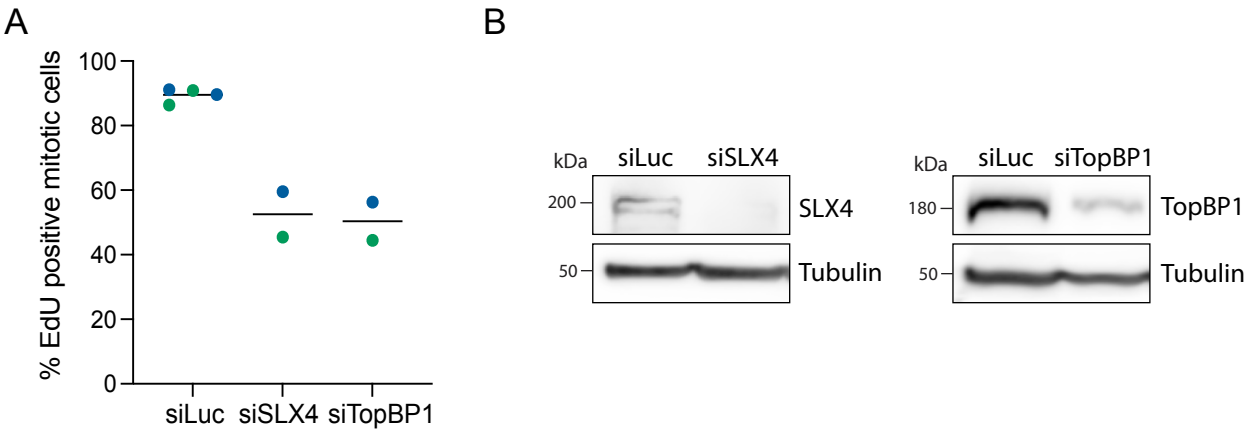


(A) Representative images of mitotic HeLa cells expressing Halo-tagged TopBP1, TFP-tagged H2B, mCherry-tagged SLX4 WT cDNA and FKBP(DD) fused SLX4-WT or T1260A peptides. Cells were treated with APH (0.4 µM), doxycycline (1 µg/mL), and Shield-1 (1 µM) for 16 hours before imaging. TopBP1-Halo was labelled with the Janelia Fluor 646 ligand (JF646). White lines crossing TopBP1-SLX4 foci indicate the line used for quantification of TopBP1-SLX4 intensity values (gray value) plotted in (B).

(B) Plots showing the intensity values of TopBP1 and SLX4 fluorophores along the white lines indicated in (A).

(C) WB against FKBP showing the expression level of the SLX4-WT and SLX4-T1260A peptide in two separate clones with or without Shield-1 treatment (1 µM for 16 hours). Top band shows the expressed FKBP(DD)-peptide. Bottom band shows the endogenous FKBP.

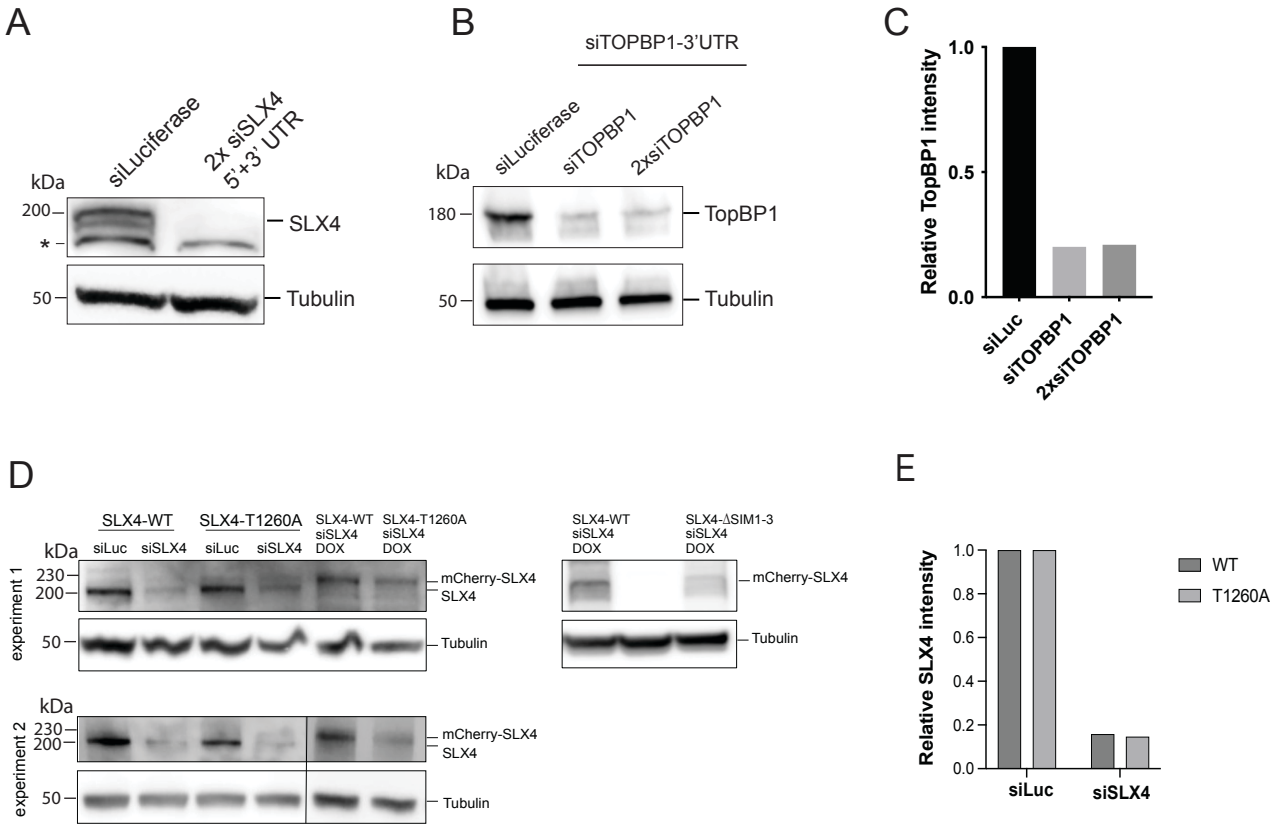
Supplementary Figure 6 - Knockdown of SLX4 and TopBP1 reduces MiDAS



A) Quantification of mitotic cells with EdU foci (in percentage) in parental HeLa TREX cells subjected to mild replication stress after knockdown of endogenous SLX4 and TopBP1. n = 2 (50 cells were quantified per condition for each replicate). The CDK1i (RO-3306) was used for G2-synchronization prior to mitotic release.

B) Western blot showing knockdown efficiency for SLX4 and TopBP1 for the cells used in A.

Supplementary Figure 7 - TopBP1 and SLX4 expression control and knockdown efficiency of siTOPBP1 and siSLX4



(A) Representative Western blot showing knockdown efficiency with two-times knockdown of endogenous SLX4 using siRNAs targeting 5' and 3' UTR of SLX4. Tubulin was used as loading control. *, unspecific band.

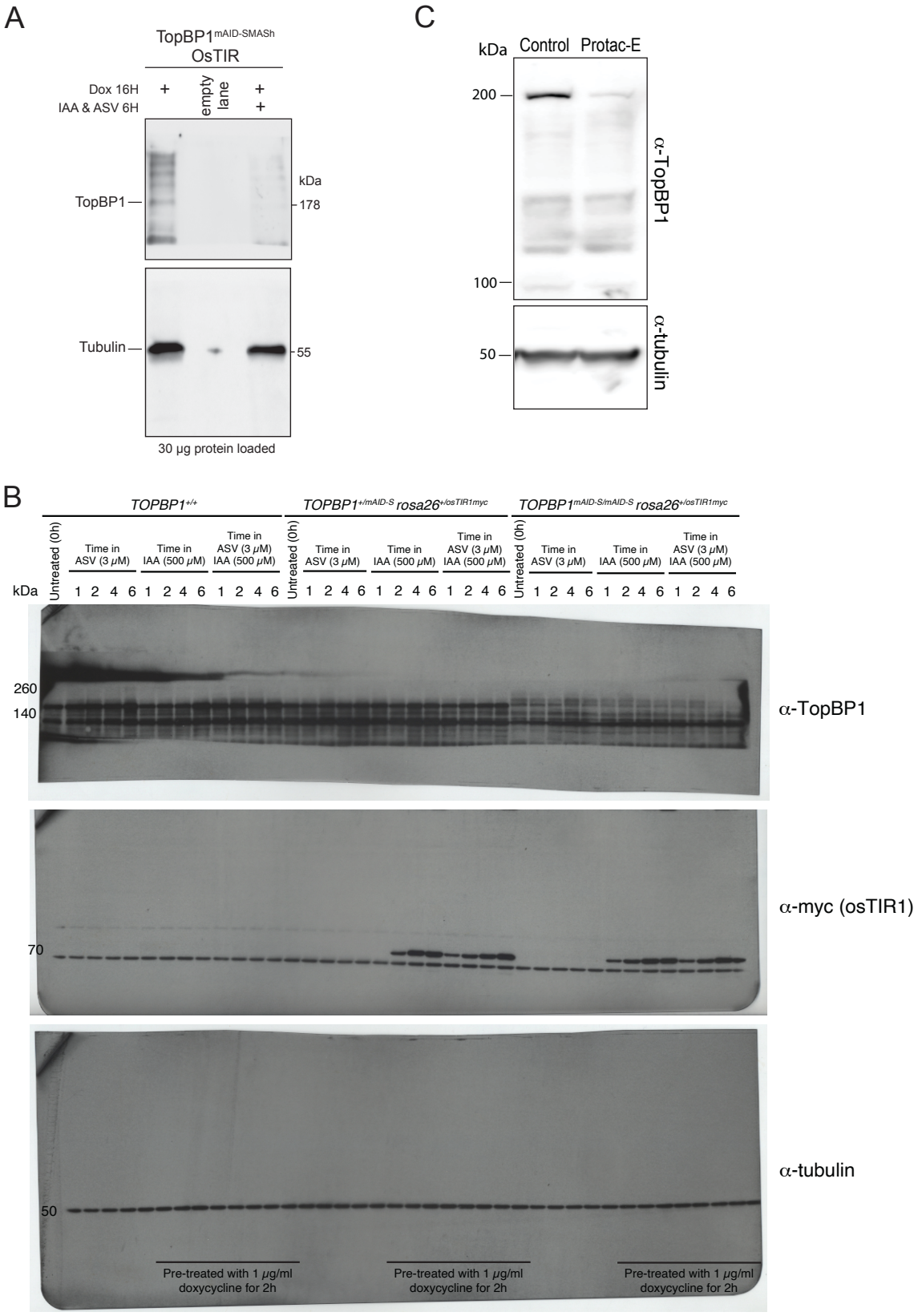
(B) Representative Western blot showing knockdown efficiency with one or two times knockdown of endogenous TopBP1 using siRNA targeting 3' UTR of TopBP1. Tubulin was used as loading control.

(C) Quantification of TopBP1 band signals from (B) relative to TopBP1 band in siLuciferase, normalized to tubulin signal. Tubulin was used as loading control.

(D) Representative Western blot showing knockdown efficiency of endogenous SLX4 using siRNA targeting the 5' and 3' UTRs of SLX4. Tubulin was used as loading control. The panel for experiment 2 is composed of 2 parts, which come from the same experiment, but run on 2 separate gels.

(E) Quantification of SLX4 band signals from (D) relative to SLX4 band in siLuciferase, normalized to tubulin signal. Tubulin was used as loading control. In lanes 5 and 6, mCherry-SLX4 and mCherry-SLX4-T1260A expression, respectively, are induced by doxycycline (n = 2).

Supplementary Figure 8 - Uncropped immunoblots.

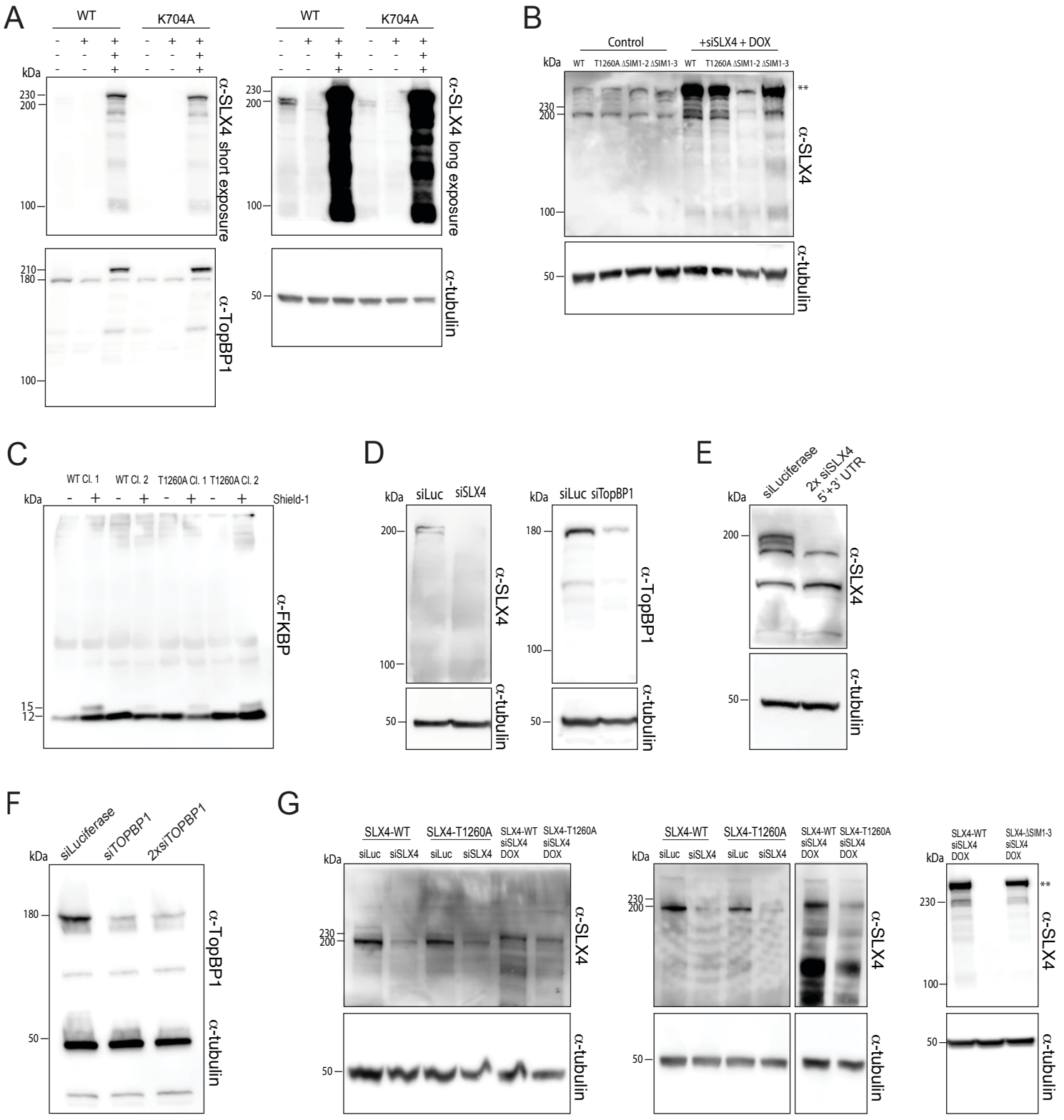


(A) Uncropped blot from Figure 1C.

(B) Uncropped blot from Supplementary Figure 1.

(C) Uncropped blot from Supplementary Figure 2D.

Supplementary Figure 9 - Uncropped immunoblots.



(A) Uncropped blot from Supplementary Figure 4A.

(B) Uncropped blot from Supplementary Figure 4B. **SLX4 dimers

(C) Uncropped blot from Supplementary Figure 5C.

(D) Uncropped blot from Supplementary Figure 6B.

(E) Uncropped blot from Supplementary Figure 7A.

(F) Uncropped blot from Supplementary Figure 7B.

(G) Uncropped blot from Supplementary Figure 7D. **SLX4 dimers