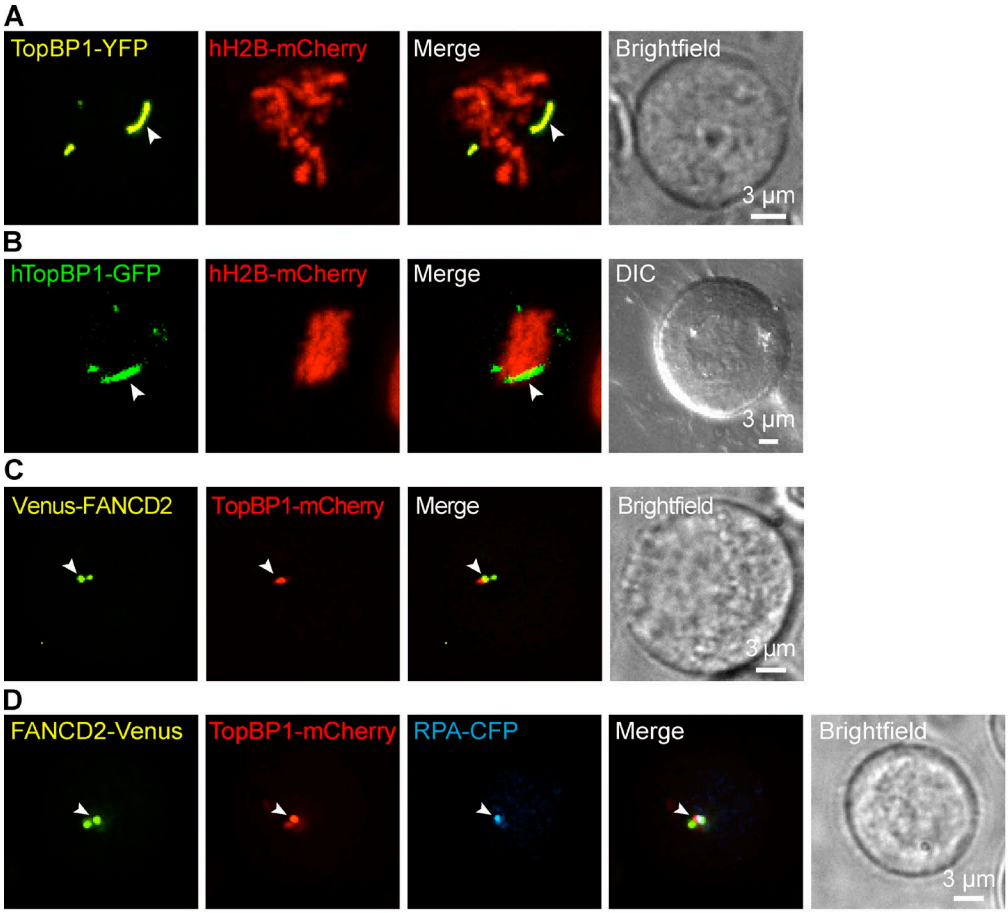
Pedersen et al., http://www.jcb.org/cgi/content/full/jcb.201502107/DC1



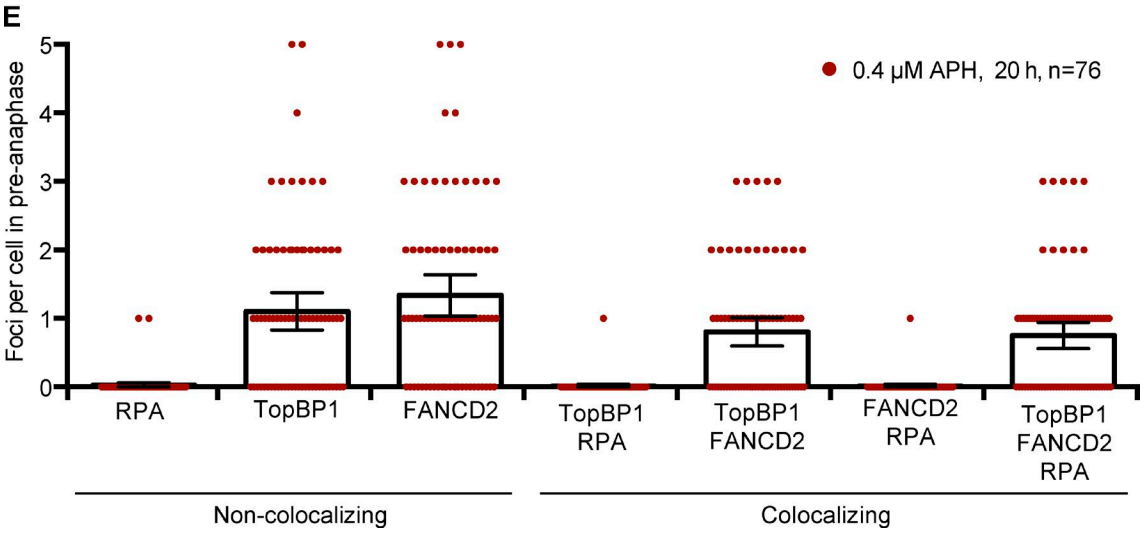


Figure S1. **TopBP1 thread-like structures in mitosis.** (A) Representative images of the DT40 cell line RTP217 displaying a TopBP1 thread-like structure in mitosis. Arrowheads indicate TopBP1 thread-like structures. (B) Representative images of an untreated HeLa cell transiently transfected with constructs expressing GFP-hTopBP1 and hH2B-mCherry displaying a TopBP1 thread-like structure in mitosis. Arrowheads indicate TopBP1 thread-like structures. (C) Representative images of APH-treated DT40 cell line RTP284. Arrowheads indicate TopBP1 colocalization with FANCD2 sister foci. (D) Representative images of APH-treated DT40 cell line, RTP356 (TopBP1$^{mCherry/WT}$/RPA$^{CFP/WT}$/FANCD2$^{Venus/WT}$). Arrowheads indicate TopBP1 and RPA colocalization with a FANCD2 sister focus. (E) Quantification of RPA, FANCD2, and TopBP1 foci in live cell images. RTP356 cells were treated with 0.4 µM APH for 20 h before imaging. RPA, FANCD2, and TopBP1 foci in preanaphase cells were quantified. Error bars represent 95% confidence intervals. The number of cells analyzed is indicated in the graph (*n*).

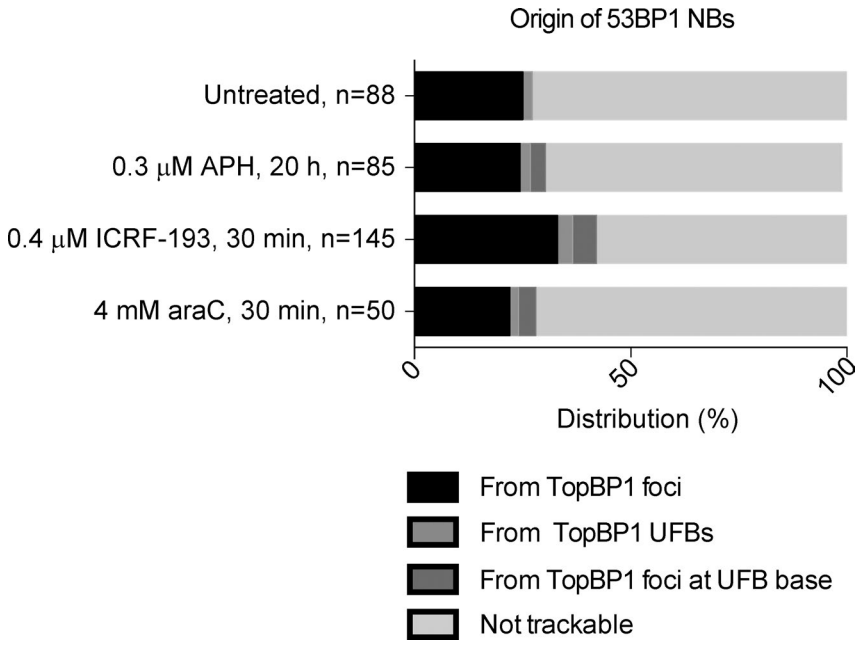


Figure S2. **Bar chart of the anaphase/telophase TopBP1 structures leading to 53BP1 NBs in G1.** Distribution of mitotic structures that transition into 53BP1 NBs. Time-lapse microscopy of the DT40 cell line RTP252 with an imaging frequency of 2 min for 30 min is quantified. Cells were monitored from anaphase to early G1, which was defined as the first 10 min after chromatin decondensation. TopBP1 was tracked as far back as possible from G1 into anaphase/telophase. The number of foci analyzed is indicated (*n*). Cells were treated with 0.4 µM APH for 20 h, 0.4 µM ICRF-193 for 30 min, or 0.0125% DMSO (vol/vol, untreated) for 20 h before imaging.

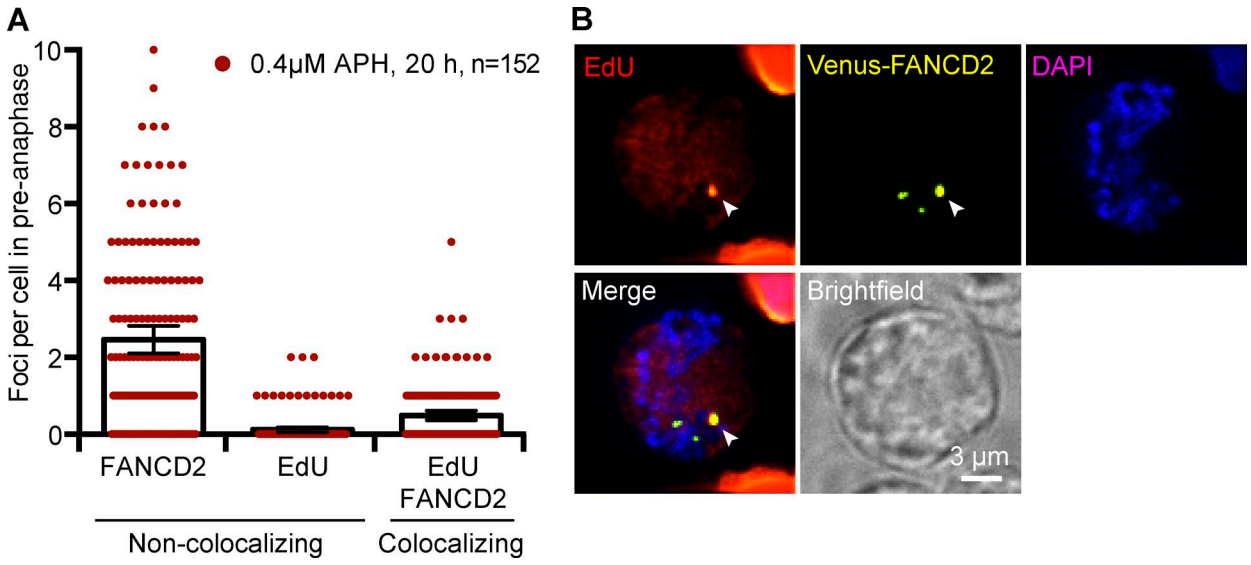


Figure S3. **FANCD2 localizes to unscheduled DNA synthesis in mitosis.** (A) Quantification of EdU and FANCD2 foci in prometaphase and metaphase cells in the DT40 cell line VH2 (FANCD2$^{Venus/WT}$). Before fixation, cells were treated with 0.4 µM APH for 20 h and pulse labeled with 20 µM EdU for the last 20 min. FANCD2 foci and EdU foci were quantified in prometaphase and metaphase. Phases were identified based on chromatin condensation. Error bars represent 95% confidence intervals. The number of cells analyzed is indicated in the graph (*n*). (B) Representative images of fixed cells treated with 0.4 µM APH for 20 h and pulse labeled with 20 µM EdU for 20 min. White arrowheads indicate FANCD2 foci colocalizing with EdU.

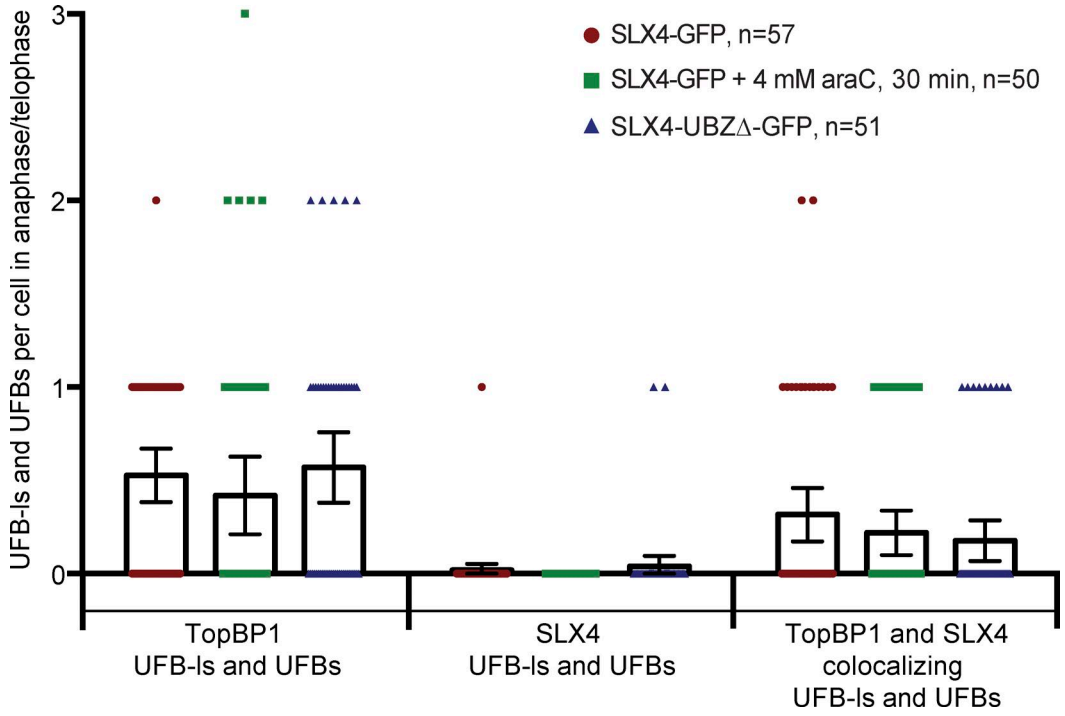


Figure S4. **TopBP1 and SLX4 colocalize on UFB-Is**. Quantification of SLX4 and TopBP1 UFBs or UFB-Is in the cell lines RTP302 and RTP305. Cells were treated with or without 4 mM araC for 30 min before the beginning of the time-lapse experiments, and monitored from anaphase to telophase. The maximum number of structures visible at one time point was noted as representative for anaphase of a given cell. Error bars represent 95% confidence intervals. The number of cells analyzed is indicated in the graph (*n*).

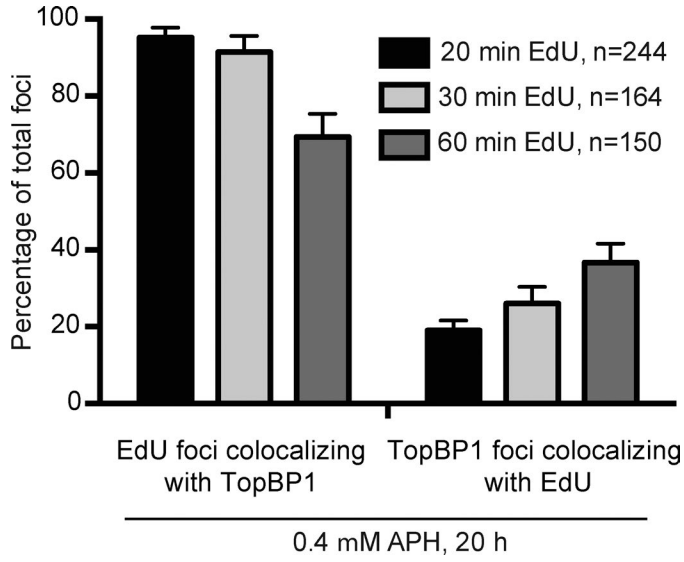


Figure S5. **Prolonged EdU pulse increases the colocalization with TopBP1.** A quantification of the relationship between the length of the EdU pulse and colocalization of EdU and TopBP1 foci is shown. The experimental setup was identical to Fig. 5 C, except that 30- and 60-min EdU pulses were applied as indicated. Error bars represent 95% confidence intervals. The number of foci analyzed is indicated in the graph (*n*).

Table S1. **DT40 cell lines used in this study**

Cell line	Genotype	Source
DT40	Wild type	Buerstedde et al., 1990
VH1	TopBP1$^{YFP/WT/WT}$ (BSR)	Germann et al., 2011
VH2	FANCD2$^{Venus/WT}$ (loxed)	This study
X1	FANCD2$^{-/-}$ (HIS/BSR)	Yamamoto et al., 2005
RTP156	TopBP1$^{YFP/WT/WT}$ (BSR), RPA$^{CFP/WT}$ (BSR), hH2B-mCherry (BSR)	Germann et al., 2014
RTP164	TopBP1$^{YFP\text{-}AID/YFP\text{-}AID/YFP\text{-}AID}$ (loxed), osTIR (NEO)	This study
RTP217	TopBP1$^{YFP\text{-}AID/YFP\text{-}AID/YFP\text{-}AID}$ (loxed), 53BP1$^{TFP/WT}$ (BSR), osTIR (loxed), hH2B-mCherry (PAC)	This study
RTP252	PICH$^{YFP/YFP}$ (loxed), 53BP1$^{TFP/WT}$ (BSR), TopBP1$^{mCherry/WT/WT}$ (PAC)	This study
RTP284	PICH$^{TFP/WT}$ (loxed), FANCD2$^{Venus/WT}$ (loxed), TopBP1$^{mCherry/WT/WT}$ (PAC)	This study
RTP292	hH2B-mCherry (PAC)	This study
RTP302	TopBP1$^{mCherry/WT}$ (BSR), SLX4-GFP (NEO)	This study
RTP305	TopBP1$^{mCherry/WT}$ (BSR), SLX4-ΔUBZ-GFP (NEO)	This study
RTP315	TopBP1$^{YFP\text{-}AID/YFP\text{-}AID/YFP\text{-}AID}$ (loxed), TopoIIα$^{TFP/WT}$ (PAC), osTIR (NEO)	This study
RTP317	TopBP1$^{YFP\text{-}AID/YFP\text{-}AID/YFP\text{-}AID}$ (loxed), GFP-hTopBP1 (BSR), osTIR (NEO)	This study
RTP319	TopBP1$^{YFP\text{-}AID/YFP\text{-}AID/YFP\text{-}AID}$ (loxed), SLX4-GFP (BSR), osTIR (NEO)	This study
RTP328	TopBP1$^{mCherry/WT}$ (BSR), TopoIIβ$^{GFP/WT}$ (PAC)	This study
RTP335	PICH$^{mCherry/mCherry}$ (loxed), SLX4-GFP (BSR)	This study
RTP349	SLX4-GFP (NEO), FANCD2$^{-/-}$ (HIS/BSR)	This study
RTP356	TopBP1$^{mCherry/WT}$ (loxed), RPA$^{CFP/WT}$ (loxed), FANCD2$^{Venus/WT}$ (loxed)	This study
RTP368	TopBP1$^{YFP\text{-}AID/YFP\text{-}AID/YFP\text{-}AID}$ (loxed), GFP-hTopBP1-ΔNLS (PAC), osTIR (NEO)	This study

DT40 cell lines in this study are derivatives of DT40 wild type (Buerstedde et al., 1990).

Table S2. **Plasmids used in this study**

Plasmid	Relevant markers	Source
pRTP5	AMP^r PICH-YFP BSR	Germann et al., 2014
pRTP7	AMP^r 53BP1-YFP BSR	Oestergaard et al., 2012
pRTP9	AMP^r PICH-YFP PAC	Germann et al., 2014
pRTP14	AMP^r TopBP1-YFP-AID NEO	Germann et al., 2014
pRTP15	AMP^r TopBP1-YFP-AID BSR	Germann et al., 2014
pRTP16	AMP^r TopBP1-YFP-AID PAC	Germann et al., 2014
pRTP17	AMP^r PICH-TFP BSR	Germann et al., 2014
pRTP23	*AMP^r Human H2B-mCherry* (chicken *β-actin* promoter) *PAC*	Germann et al., 2014
pRTP24	*AMP^r osTIR* (chicken *β-actin* promoter) *NEO*	This study
pRTP27	AMP^r TopBP1-mCherry BSR	This study
pRTP28	AMP^r TopBP1-mCherry PAC	This study
pRTP31	AMP^r 53BP1-TFP BSR	This study
pRTP32	AMP^r PICH-mCherry BSR	This study
pRTP33	AMP^r PICH-mCherry PAC	This study
pRTP37	AMP^r TopoIIα-TFP PAC	This study
pRTP38	*AMP^r* Human *TopBP1-GFP* (chicken *β-actin* promoter) *BSR*	This study
pRTP40	*AMP^r* Chicken *SLX4-GFP* (chicken *β-actin* promoter) *BSR*	This study
pRTP46	*AMP^r Human TopBP1-ΔNLS-GFP* (chicken *β-actin* promoter) *PAC*	This study
pVHO2	AMP^r RPA-CFP BSR	Germann et al., 2011
pVHO3	AMP^r TopBP1-YFP BSR	Germann et al., 2011
pVHO4	AMP^r Venus-FANCD2 PAC	This study
pX33	AMP^r TopoIIα -FLAG PAC	Johnson et al., 2009
pX34	AMP^r TopoIIβ-GFP PAC	Johnson et al., 2009
pX45	*Kan^r* Chicken *SLX4-GFP NEO*	Yamamoto et al., 2011
pX46	*Kan^r* Chicken *SLX4-UBZΔ-GFP NEO*	Yamamoto et al., 2011
pExpress	AMP^r chicken β-actin promoter	Arakawa et al., 2001
pLOX-BSR	AMP^r BSR	Arakawa et al., 2001
pLOX-PURO	AMP^r PAC	Arakawa et al., 2001
pLOX-NEO	AMP^r NEO	Arakawa et al., 2001
pPGK-CRE	AMP^r CRE	Arakawa et al., 2001
pmTurquoise2-N1	Kan^r mTurquoise2	Goedhart et al., 2010
pmCherry-C1	Kan^r mCherry	Takara Bio Inc.
pNHK65	Kan^r osTIR1 NEO	Nishimura et al., 2009

Table S3. **Primer list**

Primer	Sequence (5′ to 3′)	Use
VO128	TCTAGAATGGTGAGCAAGGGCGAG	Forward primer for mCherry tag for pRTP27
VO129	AGATCTGCTTTACTTGTACAGCTCGTCC	Reverse primer for mCherry tag for pRTP27
RTP59	GTCGACGGTATGGTGAGCAAGGGCGAGGAG	Forward primer for mCherry tag for pRTP32-33
RTP60	GAATTCTTACTTGTACAGCTCGTCC	Reverse primer for mCherry tag for pRTP32-33
RTP68	GCTAGCTTATTGTGATAATCCAGTCCCAAG	Reverse primer for *GFP-TopBP1-ΔNLS* for pRTP46
RTP72	ATGTCGTAACAACTCCGCC	Forward primer for *GFP-TopBP1-ΔNLS* for pRTP46
VO67	AGGGATCCACCATG GTGAGCAAGGGCGAGGAG	Forward primer for Venus tag
VO252	ACT AGT CTTGTACAGCTCGTCC	Reverse primer for Venus tag
VO300	ACTAGTGTTTCGAAAAGGAAGTTGTCT	Forward primer for FANCD2 5′ arm
VO301	GCGGCCGCTATCCAGCAATATATTTATCTGCCAGT	Reverse primer for FANCD2 5′ arm
VO302	GTCGACATTCAGCAATGAAACCTCTGAGGAAAG	Forward primer for FANCD2 3′ arm
VO303	GGATCCTTTGTCACTGCCTGTAAGAAC	Reverse primer for FANCD2 3′ arm

References

Arakawa, H., D. Lodygin, and J.M. Buerstedde. 2001. Mutant loxP vectors for selectable marker recycle and conditional knock-outs. *BMC Biotechnol.* 1:7. http://dx.doi.org/10.1186/1472-6750-1-7

Buerstedde, J.M., C.A. Reynaud, E.H. Humphries, W. Olson, D.L. Ewert, and J.C. Weill. 1990. Light chain gene conversion continues at high rate in an ALV-induced cell line. *EMBO J.* 9:921–927.

Germann, S.M., V.H. Oestergaard, C. Haas, P. Salis, A. Motegi, and M. Lisby. 2011. Dpb11/TopBP1 plays distinct roles in DNA replication, checkpoint response and homologous recombination. *DNA Repair (Amst.).* 10:210–224. http://dx.doi.org/10.1016/j.dnarep.2010.11.001

Germann, S.M., V. Schramke, R.T. Pedersen, I. Gallina, N. Eckert-Boulet, V.H. Oestergaard, and M. Lisby. 2014. TopBP1/Dpb11 binds DNA anaphase bridges to prevent genome instability. *J. Cell Biol.* 204:45–59.

Goedhart, J., L. van Weeren, M.A. Hink, N.O. Vischer, K. Jalink, and T.W. Gadella Jr. 2010. Bright cyan fluorescent protein variants identified by fluorescence lifetime screening. *Nat. Methods.* 7:137–139. http://dx.doi.org/10.1038/nmeth.1415

Johnson, M., H.H. Phua, S.C. Bennett, J.M. Spence, and C.J. Farr. 2009. Studying vertebrate topoisomerase 2 function using a conditional knockdown system in DT40 cells. *Nucleic Acids Res.* 37:e98. http://dx.doi.org/10.1093/nar/gkp480

Nishimura, K., T. Fukagawa, H. Takisawa, T. Kakimoto, and M. Kanemaki. 2009. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. *Nat. Methods.* 6:917–922. http://dx.doi.org/10.1038/nmeth.1401

Oestergaard, V.H., C. Pentzold, R.T. Pedersen, S. Iosif, A. Alpi, S. Bekker-Jensen, N. Mailand, and M. Lisby. 2012. RNF8 and RNF168 but not HERC2 are required for DNA damage-induced ubiquitylation in chicken DT40 cells. *DNA Repair (Amst.).* 11:892–905. http://dx.doi.org/10.1016/j.dnarep.2012.08.005

Yamamoto, K., S. Hirano, M. Ishiai, K. Morishima, H. Kitao, K. Namikoshi, M. Kimura, N. Matsushita, H. Arakawa, J.M. Buerstedde, et al. 2005. Fanconi anemia protein FANCD2 promotes immunoglobulin gene conversion and DNA repair through a mechanism related to homologous recombination. *Mol. Cell. Biol.* 25:34–43. http://dx.doi.org/10.1128/MCB.25.1.34-43.2005

Yamamoto, K.N., S. Kobayashi, M. Tsuda, H. Kurumizaka, M. Takata, K. Kono, J. Jiricny, S. Takeda, and K. Hirota. 2011. Involvement of SLX4 in interstrand cross-link repair is regulated by the Fanconi anemia pathway. *Proc. Natl. Acad. Sci. USA.* 108:6492–6496. http://dx.doi.org/10.1073/pnas.1018487108