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Supplemental Information

The ZGRF1 Helicase Promotes Recombinational Repair of Replication-Blocking DNA Damage in Human Cells

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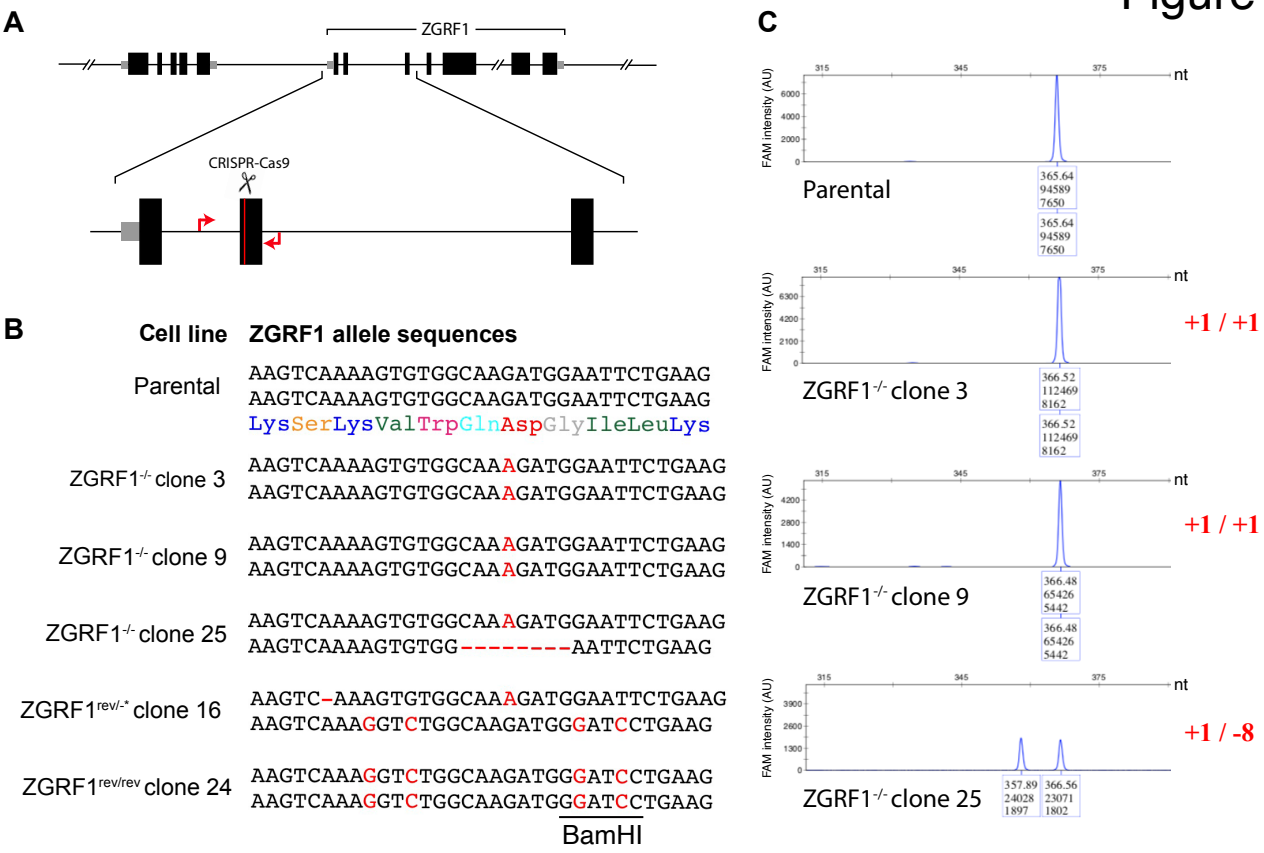
Figure S1



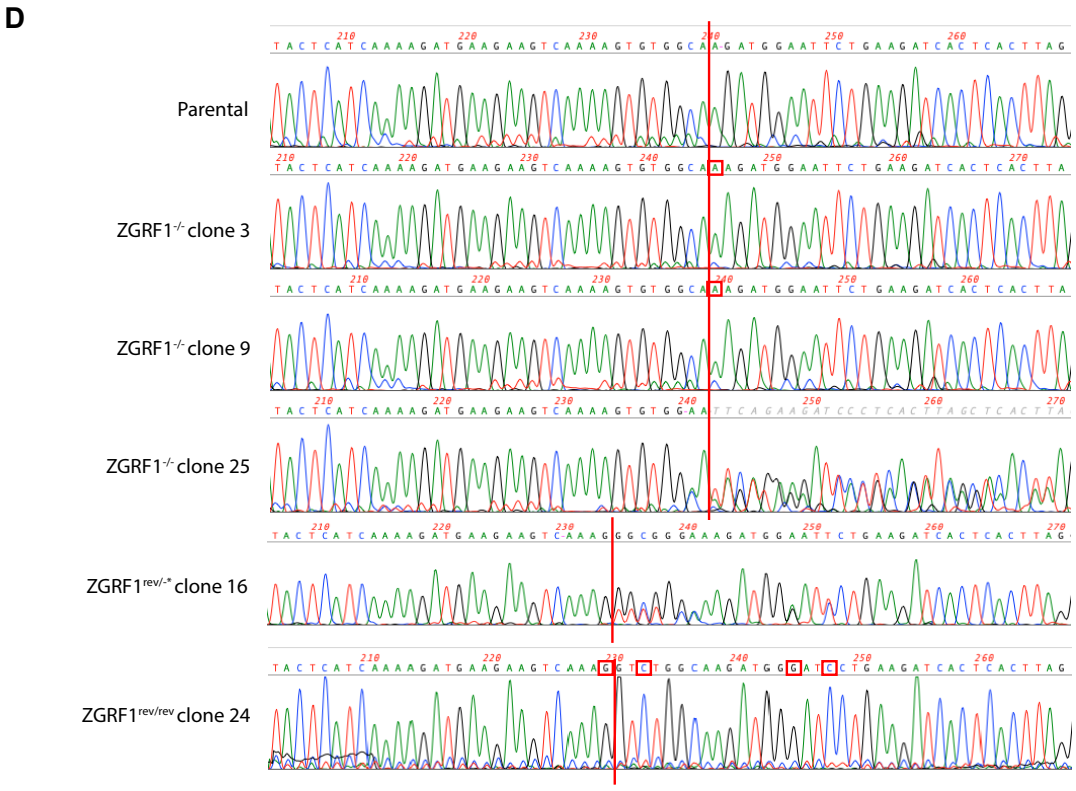
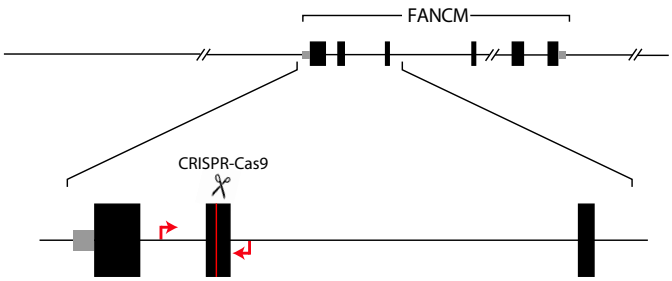


Figure S1 related to Figure 1. CRISPR-Cas9 editing of ZGRF1. (A) Strategy for genotyping HCT116 clones. Cas9 is directed to cleave within the second exon of ZGRF1. PCR amplification of the targeted region using the primers in red followed by sequencing enabled identification of indels by the Tracking Indels by DEcomposition (TIDE) program. (B) Sequences of CRISPR-Cas9-generated ZGRF1 alleles. ZGRF1rev/-* clone 16 was generated from ZGRF1-/- clone 25. The +1 allele of ZGRF1rev/-* clone 16 obtained a -1bp deletion after the 1st round of Cas9 editing. ZGRF1rev/rev clone 24 was generated from ZGRF1rev/-* clone 16. Red highlighting indicates indels. Diagnostic BamHI site indicated. (C) Fragment size analysis by capillary electrophoresis of 5'-FAM-labelled PCR products spanning the Cas9 targeted region in ZGFR1-/- and parental cell lines. Numbers in the blue boxes indicate the estimated size of the PCR products based on the peak(s). Numbers in red indicate the number of nucleotides inserted or deleted on each allele. AU, arbitrary units. (D) Chromatograms of PCR products spanning the Cas9 targeted region in ZGRF1 edited cell lines. Red vertical lines indicate Cas9 cut sites. Red boxes indicate inserted or modified nucleotides (not shown for ZGRF1-/- clone 25 and ZGRF1rev/-*).

Figure S2

A



B

Cell line	FANCM allele sequences
Parental	GTCATGGTAAATGACCTTTCTAGAGGAGCTTGTCCCGCTGCTGAAATAAAGTGTTTAGTTATTGATGAAGCTCATAAAGCTCTCGGA
	GTCATGGTAAATGACCTTTCTAGAGGAGCTTGTCCCGCTGCTGAAATAAAGTGTTTAGTTATTGATGAAGCTCATAAAGCTCTCGGA
	ValMetValAsnAspLeuSerArgGlyAlaCysProAlaAlaGluIleLysCysLeuValIleAspGluAlaHisLysAlaLeuGly
FANCM $^{-/-}$ clone 77 +1/+1	GTCATGGTAAATGACCTTTCT**T**AGAGGAGCTTGTCCCGCTGC**TGA**AATAAAGTGTTTAGTTAT**TGATGA**AGCTCA**TAA**AGCTCTCGGA
	GTCATGGTAAATGACCTTTCT**T**AGAGGAGCTTGTCCCGCTGC**TGA**AATAAAGTGTTTAGTTAT**TGATGA**AGCTCA**TAA**AGCTCTCGGA

ZGRF1$^{-/-}$ clone 25 Background

2xDel clone 78 +1/+1	GTCATGGTAAATGACCTTTCT**T**AGAGGAGCTTGTCCCGCTGC**TGA**AATAAAGTGTTTAGTTAT**TGATGA**AGCTCA**TAA**AGCTCTCGGA
	GTCATGGTAAATGACCTTTCT**T**AGAGGAGCTTGTCCCGCTGC**TGA**AATAAAGTGTTTAGTTAT**TGATGA**AGCTCA**TAA**AGCTCTCGGA
2xDel clone 106 +1/+1	GTCATGGTAAATGACCTTTCT**T**AGAGGAGCTTGTCCCGCTGC**TGA**AATAAAGTGTTTAGTTAT**TGATGA**AGCTCA**TAA**AGCTCTCGGA
	GTCATGGTAAATGACCTTTCT**T**AGAGGAGCTTGTCCCGCTGC**TGA**AATAAAGTGTTTAGTTAT**TGATGA**AGCTCA**TAA**AGCTCTCGGA

C

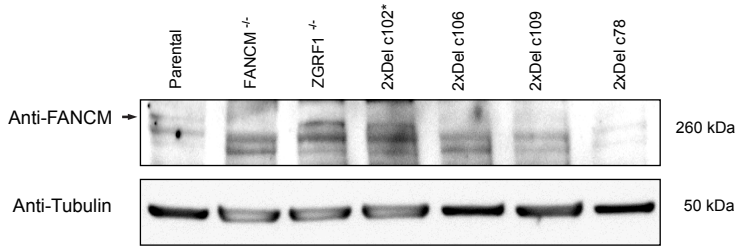


Figure S2 related to Figure 2. Construction of FANCM-/- cell lines. (A) Strategy for genotyping HCT116 FANCM edited clones. Cas9 was directed to cleave within the second exon of FANCM. PCR amplification of the targeted region using the primers in red followed by sequencing enabled identification of indels by the Tracking Indels by DEcomposition (TIDE) program. (B) Sequences of CRISPR-Cas9-generated FANCM alleles. FANCM-/- clone 77 was generated from the parental cell line, while 2xDel clone 78 and 2xDel clone 106 were generated from ZGRF1-/- clone 25. Black letters: coding sequence in parental cell line; Red letters: insertion; Bold letters underlined with red: stop codons. (C) Western blot of FANCM in parental, FANCM-/-, ZGRF1-/-, 2xDel c102* (contains an in-frame deletion of FANCM on one allele), 2xDel c106, 2xDel c109 and 2xDel c78 cell lines. Arrow indicates position of FANCM.

Figure S3

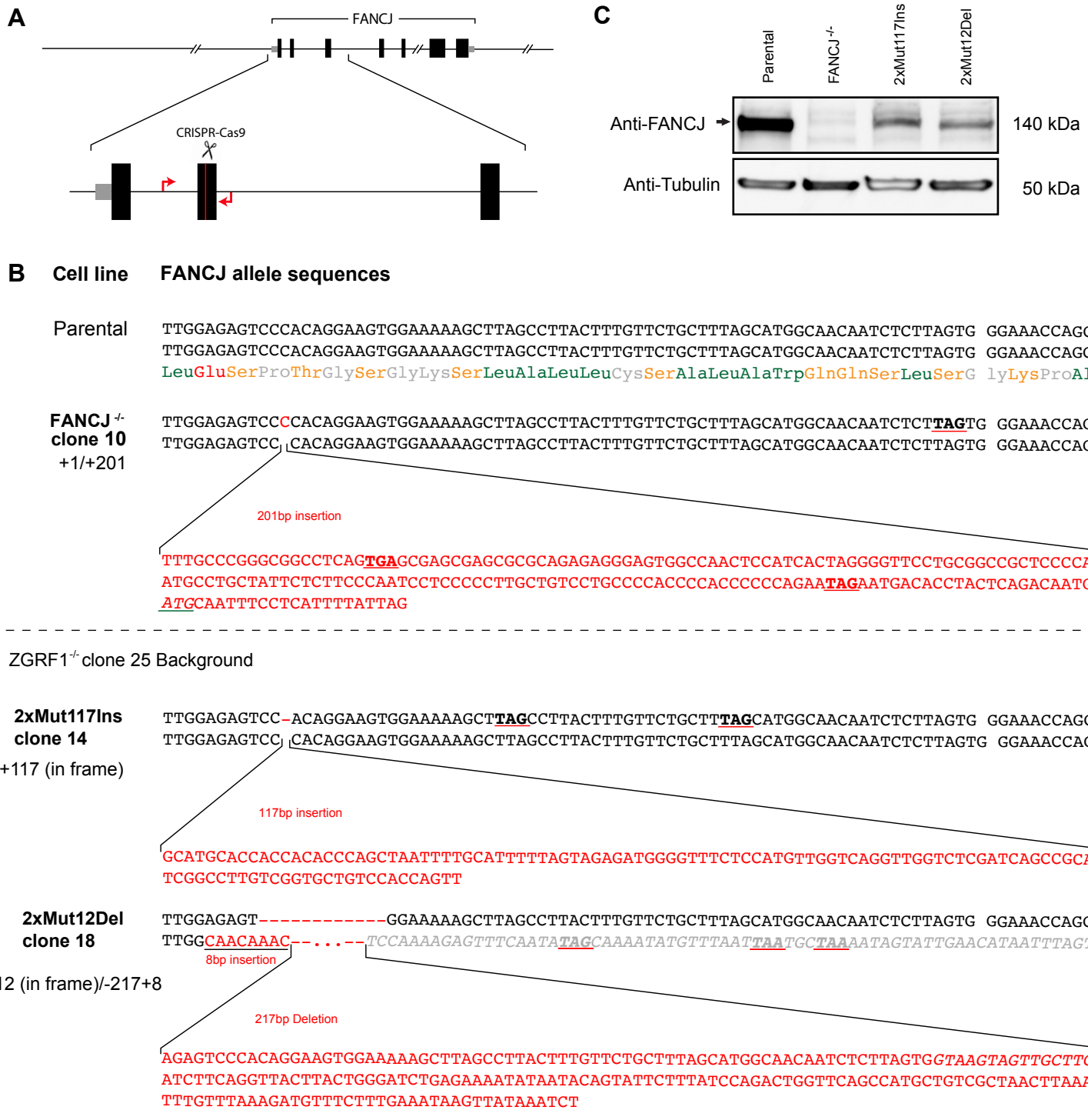


Figure S3 related to Figure 2. Construction of FANCJ-/- cell lines. (A) Strategy for genotyping HCT116 FANCJ edited clones. Cas9 was directed to cleave within the second exon of FANCJ. PCR amplification of the targeted region using the primers in red followed by sequencing enabled identification of indels by the Tracking Indels by DEcomposition (TIDE) program. (B) Sequences of CRISPR-Cas9-generated FANCJ alleles. FANCJ-/- clone 10 was generated from the parental cell line, while 2xMut117Ins clone 14 and 2xMut12Del clone 18 were generated from ZGRF1-/- clone 25. Black letters: coding sequence in parental cell line; Red letters or dashes: indels; Gray italic letters: sequence of the second intron of FANCJ; Bold letters underlined with red: stop codons; Letters underlined with green: potential start codon. (C) Western blot of FANCJ in parental, FANCJ-/-, and ZGRF1 FANCJ double mutant (2xMut117Ins and 2xMut12Del) cell lines. Arrow indicates position of FANCJ.

Figure S4

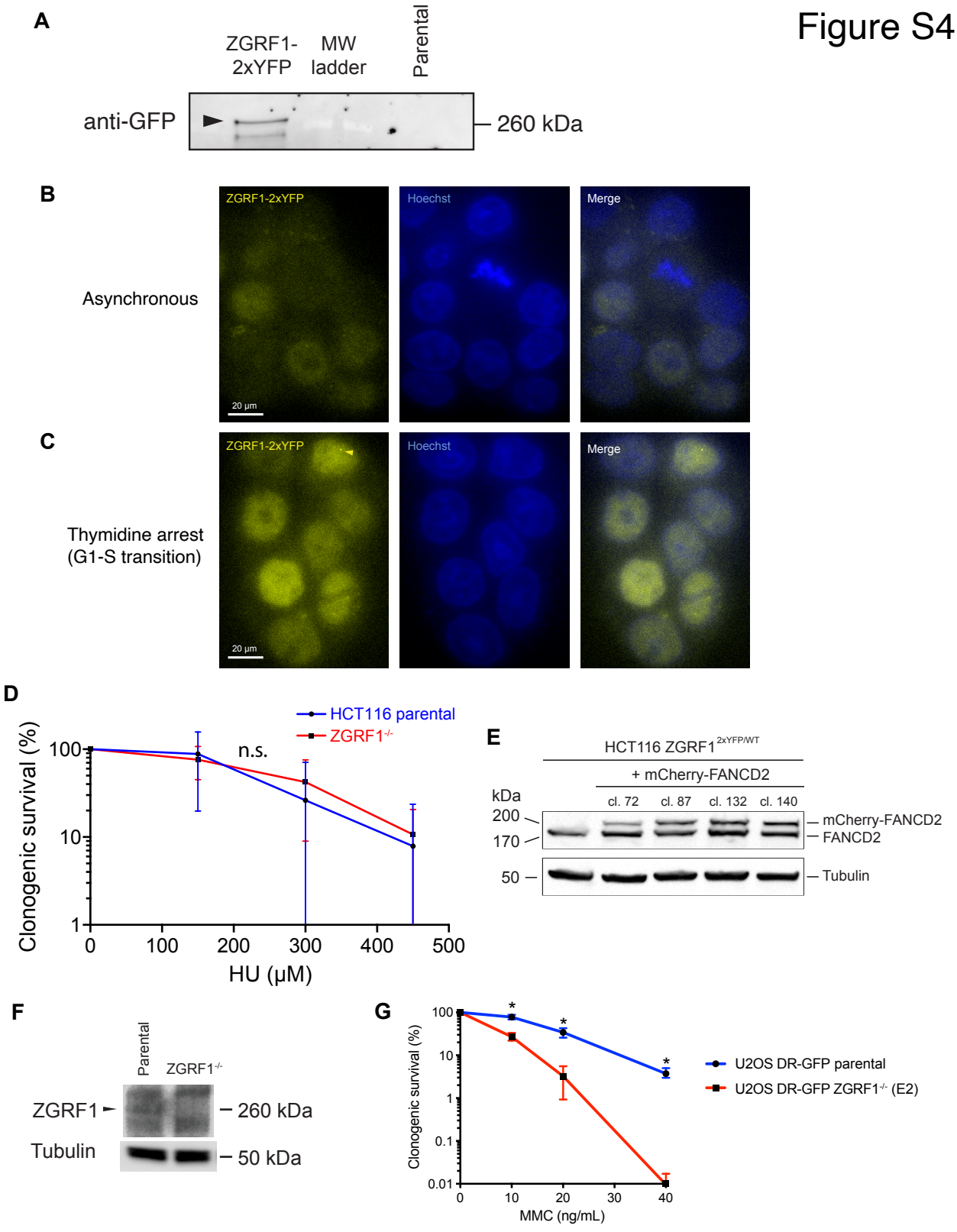


Figure S4 related to Figures 3 and 4. YFP-tagging of ZGRF1 and its localization. (A) Western blot of ZGRF1-2xYFP. Arrow indicates position of ZGRF1-2xYFP. MW, molecular weight. (B) ZGRF1-2xYFP localization in asynchronously growing cells. Cells expressing ZGRF1-2xYFP from the endogenous promoter were stained with 0.4 µM Hoechst 33258 for 30 min and imaged by fluorescence microscopy. (C) ZGRF1-2xYFP localization in cells arrested at the G1-S transition. Cells expressing ZGRF1-2xYFP from the endogenous promoter were synchronized at the G1-S transition by treatment with 2 mM thymidine for 18 h before staining with 0.4 µM Hoechst 33258 for 30 min and imaged by fluorescence microscopy. Arrow indicates a ZGRF1 focus. (D) ZGRF1-/- cells exhibit parental levels of HU sensitivity. Clonogenic survival of HCT116 parental and ZGRF1-/- cells after continuous growth in the indicated concentrations of HU for 8 days. n.s, not significant. (E) Western blot of FANCD2 in ZGRF1-2xYFP cells and four individual clones where mCherry-FANCD2 is stably expressed after random integration of the mCherry-FANCD2 fusion gene. (F) Western blot of U2OS DR-GFP parental and ZGRF1-/- cell lines. Arrow indicates position of ZGRF1. (G) Colony formation assay of U2OS DR-GFP parental and ZGRF1-/- cell lines treated with the indicated doses of MMC for 24 h (N = 3). The graph shows the mean with 95% confidence interval. Statistical significance was calculated using unpaired t-tests without assuming consistent standard deviation. *, $P < 0.05$.

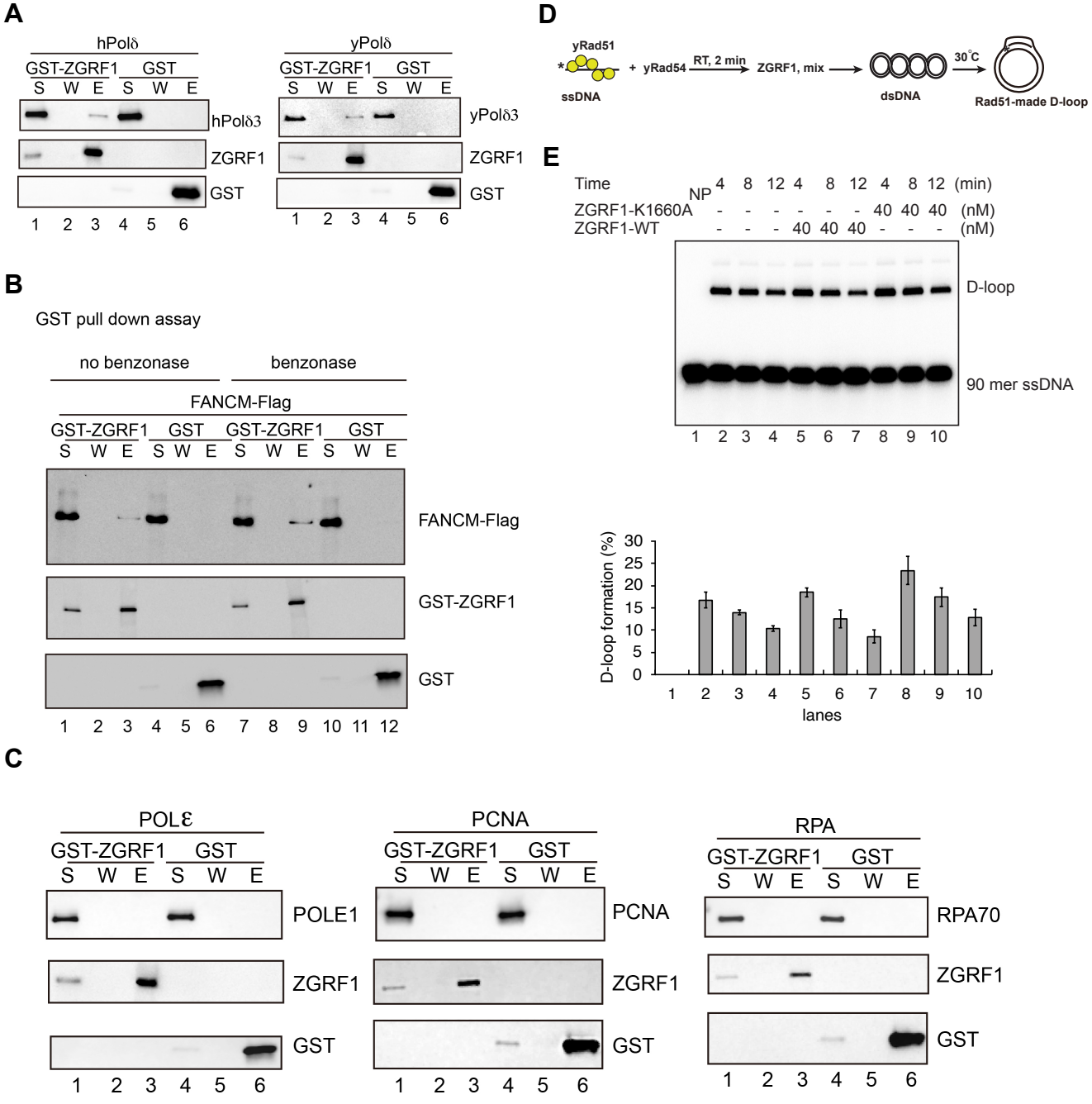


Figure S5 related to Figure 5. ZGRF1 interacts with Polδ and FANCM, but not with Polε, PCNA or RPA. (A) Co-affinity precipitation of Polδ with ZGRF1. GST-ZGRF1 or GST (0.3 μg) was incubated with human or budding yeast Polδ complex (0.5 μg) on Glutathione Sepharose 4B resin. The Supernatant (S), Wash (W) and SDS eluate (E) were resolved by SDS-PAGE followed by western blotting. GST-ZGRF1 or GST was detected using an α-GST antibody (same in (B) and (C)), while the human Myc-Polδ3 subunit was detected using an α-Myc antibody and the budding yeast Flag-Polδ3 subunit was detected using an α-Flag antibody. (B) Co-affinity precipitation of FANCM with ZGRF1. GST-ZGRF1 or GST (0.3 μg) was incubated with FANCM-Flag (0.25 μg) on Glutathione Sepharose 4B resin, in the presence or absence of benzonase. The Supernatant, Wash and SDS eluate were resolved by SDS-PAGE followed by western blotting. FANCM-Flag was detected using an α-Flag antibody. (C) No co-affinity precipitation of Polε, PCNA or RPA with ZGRF1. GST-ZGRF1 or GST (0.3 μg) was incubated with human Polε (0.5 μg), PCNA (0.5 μg) or RPA (0.5 μg) on Glutathione Sepharose 4B resin. The Supernatant, Wash and SDS eluate were resolved by SDS-PAGE followed by western blotting. Human HA-Polε1 was detected using an α-HA antibody, human PCNA was detected using an α-PCNA antibody and human RPA was detected using an α-RPA70 antibody. (D) Experimental set-up for examining yeast Rad51-catalyzed strand-exchange. *, 5'-^{32}P radiolabel. (E) ZGRF1 does not stimulate strand-exchange by budding yeast Rad51-Rad54. Time course analysis of strand exchange catalyzed by budding yeast Rad51 and Rad54, in the absence or presence of ZGRF1 or its helicase dead mutant ZGRF1-K1660A was shown. NP, no protein. The D-loop product was quantified, and data are means ± s.d., n = 3.

Figure S6

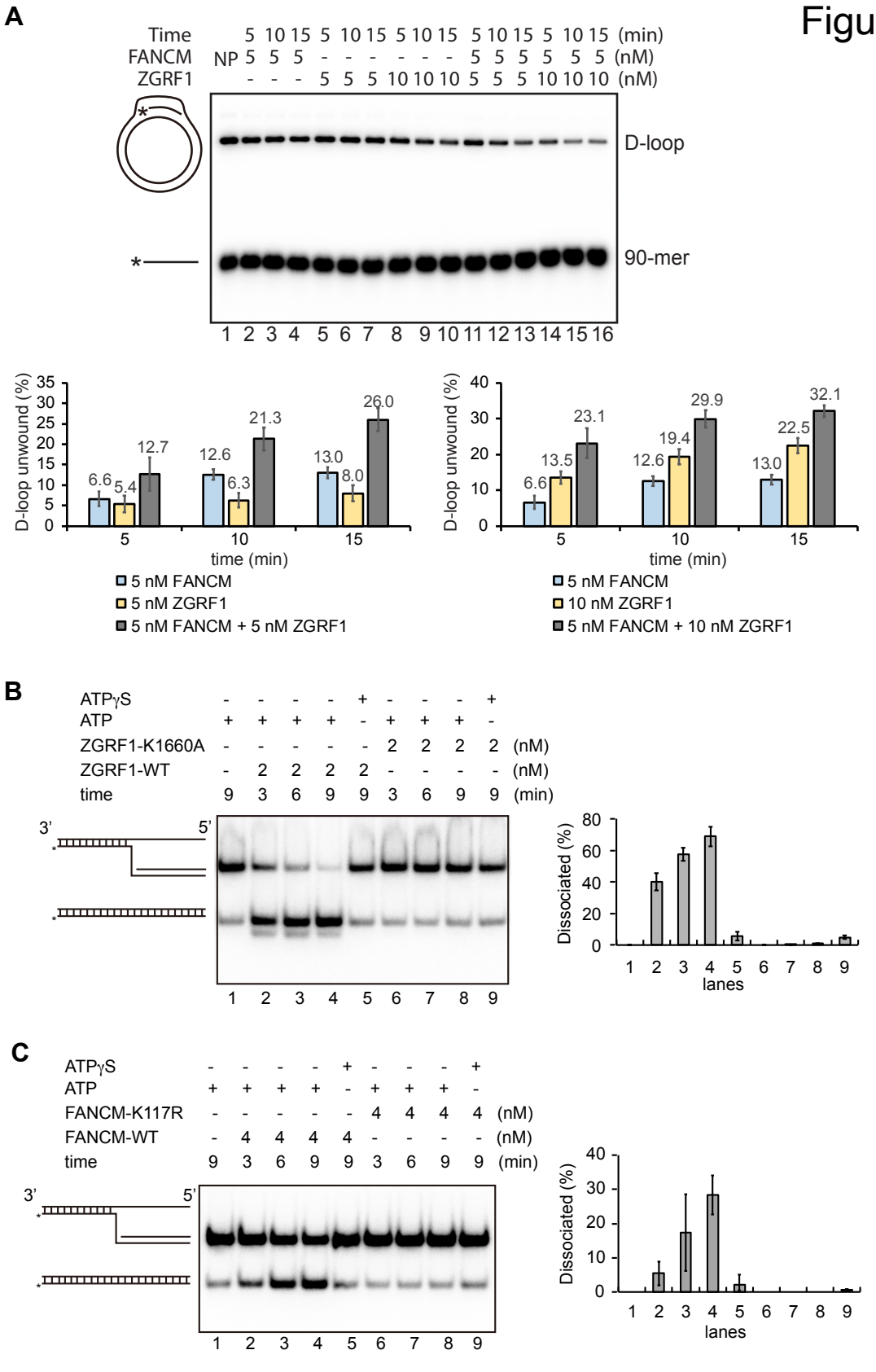


Figure S6 related to Figure 6. Comparison of ZGRF1 and FANCM. (A) ZGRF1 and FANCM dissociate D-loops additively. The deproteinized Rad51-made D-loops (~2.2 nM) were incubated with either FANCM alone (5 nM), ZGRF1 alone (5 nM or 10 nM), or their combination at 37°C for 5, 10, or 15 min. The reaction products were resolved in 0.9% agarose gels. NP, no protein. The percentage D-loop unwound was quantified and displayed as means ± s.d., n = 3. (B) ZGRF1 or ZGRF1-K1660A was incubated with the indicated DNA flap structure at 30°C for the indicated time. Quantification is shown on the right. Data are mean of two experiments. Error bars indicate variance. (C) FANCM or FANCM-K117R was incubated with the indicated DNA flap structure at 30°C for the indicated time. Quantification is shown on the right. Data are mean of two experiments. Error bars indicate variance.

Table S1 related to METHODS. Cell lines used in this study.

Name	Genotype	Parent	Reference
HCT116		HCT116	(Brattain et al., 1981)
ZGRF1 KO col. 25	ZGRF1$^{+1/-8}$	HCT116	This study
ZGRF1 KO col. 3	ZGRF1$^{+1/+1}$	HCT116	This study
ZGRF1 KO col. 9	ZGRF1$^{+1/+1}$	HCT116	This study
FANCM KO col. 77	FANCM$^{+1/+1}$	HCT116	This study
ZGRF1 FANCM KO col. 78	ZGRF1$^{+1/-8}$ FANCM$^{+1/+1}$	HCT116	This study
ZGRF1 FANCM KO col. 106	ZGRF1$^{+1/-8}$ FANCM$^{+1/+1}$	HCT116	This study
FANCJ KO col. 10	FANCM$^{+1/+201}$	HCT116	This study
ZGRF1 FANCJ KO col. 14	ZGRF1$^{+1/-8}$ FANCJ$^{-1/+117}$	HCT116	This study
ZGRF1 FANCJ KO col. 18	ZGRF1$^{+1/-8}$ FANCJ$^{-12/-217+8}$	HCT116	This study
U2OS	Flp-In T-REx	U2OS	(Ponten and Saksela, 1967)
ZGRF1 KO col. 36	ZGRF1$^{+1/-7}$ Flp-In T-REx	U2OS	This study
ZGRF1 2xYFP	ZGRF1$^{2xYFP/+}$	HCT116	This study
ZGRF1-2xYFP mCherry-FANCD2 col. 72, 87, 132 and 140	ZGRF1$^{2xYFP/+}$ mCherry-FANCD2-PuroR	HCT116	This study
RPE-1	hTERT	RPE-1	(Jiang et al., 1999)
ZGRF1 KO col. 41	hTERT ZGRF1$^{+1/-8}$	RPE-1	This study
U2OS	DR-GFP	U2OS	(Pierce et al., 2001)
ZGRF1 KO col. E2	DR-GFP ZGRF1$^{+1/-8}$	U2OS	This study

Table S2 related to METHODS. Plasmids used in this study.

Plasmid	Genotype	Source
pX458	*Amp^R Cas9-2A-EGFP*	(Ran et al., 2013)
pBluescript SK+	*Amp^R*	(Short et al., 1988)
pAB_KO1	*Amp^R Cas9-2A-EGFP ZGRF1-KO-gRNA*	This study
pAB_KO_rev	*Amp^R Cas9-2A-EGFP ZGRF1-KO_rev-gRNA*	This study
pAB_KO_rev3	*Amp^R Cas9-2A-EGFP ZGRF1-KO_rev-gRNA3*	This study
pX461	*Amp^R Cas9n-2A-EGFP*	(Ran et al., 2013)
pAB_2xYFP_A	*Amp^R Cas9n-2A-EGFP ZGRF1-2xYFP-gRNA-A*	This study
pAB_2xYFP_B	*Amp^R Cas9n-2A-EGFP ZGRF1-2xYFP-gRNA-B*	This study
pAK_ZGRF1-2xYFP	*Amp^R BSR ZGRF1_cDNA*	This study
pCre-GFP	*Amp^R Cre-GFP*	(Williams et al., 2015)
pKSV1	*Amp^R Cas9-2A-EGFP FANCJ-KO-gRNA*	This study
pKSV2	*Amp^R Cas9-2A-EGFP FANCM-KO- gRNA*	This study
pKSV15	*Amp^R mCherry-FANCD2-PuroR*	(Motnenko et al., 2018)
pGEX-3X-ZGRF1-N	*Amp^R GST-DUF2439*	This study
pCS2-mRFP	*Amp^R* m*RFP*	(Sartori et al., 2007)
pCBA-I-SceI	*Amp^R I-SceI*	(Richardson et al., 1998)

Table S3 related to METHODS. DNA oligoes used in this study. =, phosphorothioated bonds; *, 5'-phosphorylated bases; FAM, fluorescein amidite.

Oligo Name	Length	Sequence (5' to 3')
O1	60	GCACCAGATTCAGCAATTAAGCTCTAAGCCGC
		TGACGGCTCGATGCTGATCGTAGCATCG
O2	15	CGATGCTACGATCAG
O3	15	TGCTGAATCTGGTGC
D1	90	CATTGCATATTTAAAACATGTTGGATCCCACG
		TTGCATGCTGATAGCCTACTAGAGCTGCATGA
		ATTCAAATGACCTCTTATCAAGTGAC
D2	90	GTCACTTGATAAGAGGTCATTTGAATTCATGG
		CTTAGAGCTTAATTGCTGAATCTGGTGCTGGG
		ATCCAACATGTTTTAAATATGCAATG
D5'	60	GTGCTACGATGCTAGTCGTAGCTCGGGAGTG
		CACCAGATTCAGCAATTAAGCTCTAAGCC
D3'	60	GCACCAGATTCAGCAATTAAGCTCTAAGCCGC
		TGACGGCTCGATGCTGATCGTAGCATCG
XX1	60	ACGCTGCCGAATTCTACCAGTGCCTTGCTAG
		GACATCTTTGCCCACCTGCAGGTTCACCC
XX2	60	GGGTGAACCTGCAGGTGGGCAAAGATGTCCC
		AGCAAGGCACTGGTAGAATTCGGCAGCGT
D5'F	30	GGGTGAACCTGCAGGTGGGCAAAGATGTCC
ZGRF1_KO_IDAA_FW		TCTCTCTCCCTTCCTTCCCTTATT
ZGRF1_KO_IDAA_RV		TTGCTCTTGCACCCTATAGTCAAC
ZGRF1_KO_guide1_FW		CACCGAAGTCAAAAGTGTGGCAAGA
ZGRF1_KO_guide1_RV		AAACTCTTGCCACACTTTTGACTT
ZGRF1_rev_gRNA_FW		CACCGAGATGAAGAAGTCAAAAGTG
ZGRF1_rev_gRNA_RV		AAACCACTTTTGACTTCTTCATCTC
ZGRF1_rev_gRNA3_FW		CACCGAAGATGAAGAAGTCAAAGTG
ZGRF1_rev_gRNA3_RV		AAACCACTTTGACTTCTTCATCTTC
ZGRF1_rev_ssDNA_templat		A=T=AGTCAACTGCTTTGTACTCACTTTGTTTC
e_2		CTAAGTGAGTGATCTTCAGGATCCCATCTTGC
		CAGACCTTTGACTTCTTCATCTTTTGATGAGTA
		TATAGAACCTTATTTCCAAAAA=T=A

ZGRF1_KO_IDAA_FAMFW	AGCTGACCGGCAGCAAAATTGTCTCTCTCCCT TCCTTCCCTTATT
FamF	FAM-AGCTGACCGGCAGCAAAATTG
ZGRF1_KO_IDAA_RV	TTGCTCTTGCACCCTATAGTCAAC
ZGRF1-N-F	GGGATCCCCATGGAAAGCCAAGAATTTATTG
ZGRF1-N-R	ATTCCCGGGGCCAGAGGATATAAATGTCC
GuideA_ZGRF1_FW	CACCGCACTTGTTATTGAAGTATAC
GuideA_ZGRF1_RV	AAACGTATACTTCAATAACAAGTGC
GuideB_ZGRF1_FW	CACCGTGTCTCCAGAACTCATCTGT
GuideB_ZGRF1_RV	AAACACAGATGAGTTCTGGAGACAC
3’arm_FW	GGATCCGCAGTGTTCAACATCTGACCATGG
3’arm_RV	GCGGCCGCATAGGGAGGTCTGGGCAGCA
5’arm_FW	GGTACCAGCTGTTGCGGCAAAAATGCA
5’arm_RV	GTCGACTGAATGAGATTTATCTTTAGATTT CTCTTTTTCACTCTT
ZGRF1_outsite_FW2	CTGGTCTGGCTTTTTTTCTGTATAATTAAATG
ZGRF1_YFP_RV	CACGCTGAACTTGTGGCCGTTTA
FANCJ_KO_guide1_FW	CACCGTGTTTGTTGGAGAGTCCCAC
FANCJ_KO_guide1_RV	AAACGTGGGACTCTCCAACAAACAC
FANCM_KO_guide1_FW	*CACCGCGGGACAAGCTCCTCTAGAA
FANCM_KO_guide1_RV	*AAACTTCTAGAGGAGCTTGTCCCGC
FANCJ_KO_700_FW	CCTCTTTTCATTTCTTCTCTTGTGCTTGAT
FANCJ_KO_700_RV	GTTTGTTTGTTTTCTTTCACCCAGGCT
FANCM_KO_700_FW	CAAGCCCAAAACTGATGAATAGGGTT
FANCM_KO_700_RV	TCTGTTCTGAGATAATAGACGTAAAGGCT