Supplemental Material

SLFN11 promotes stalled fork degradation that underlies the phenotype in Fanconi anemia cells

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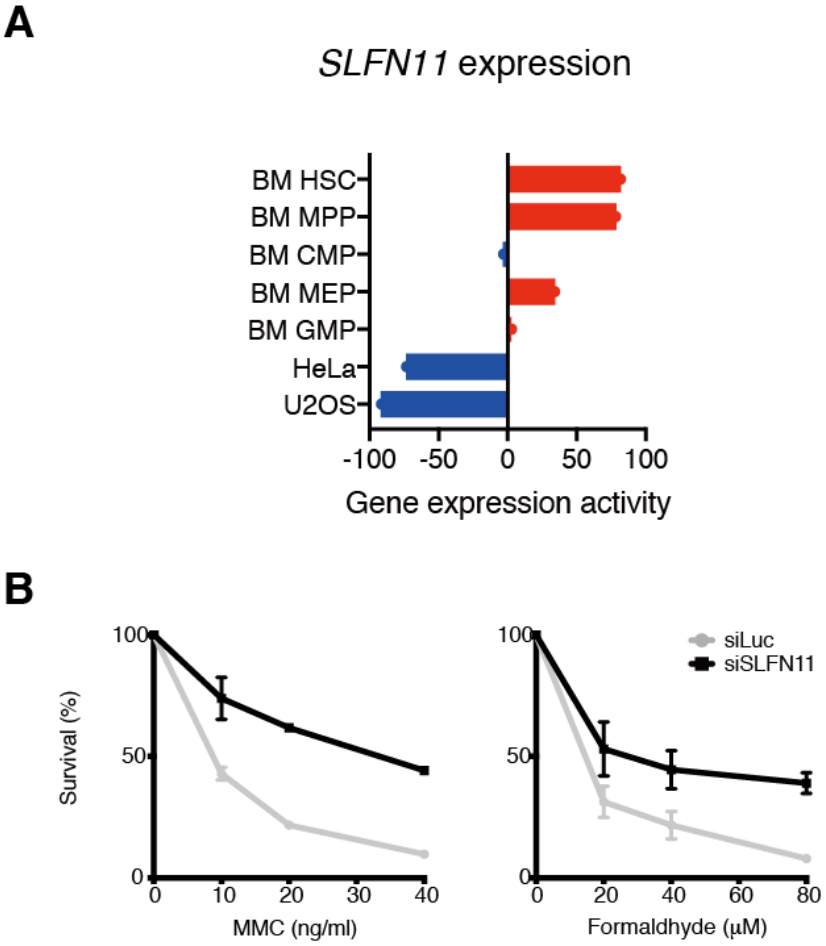
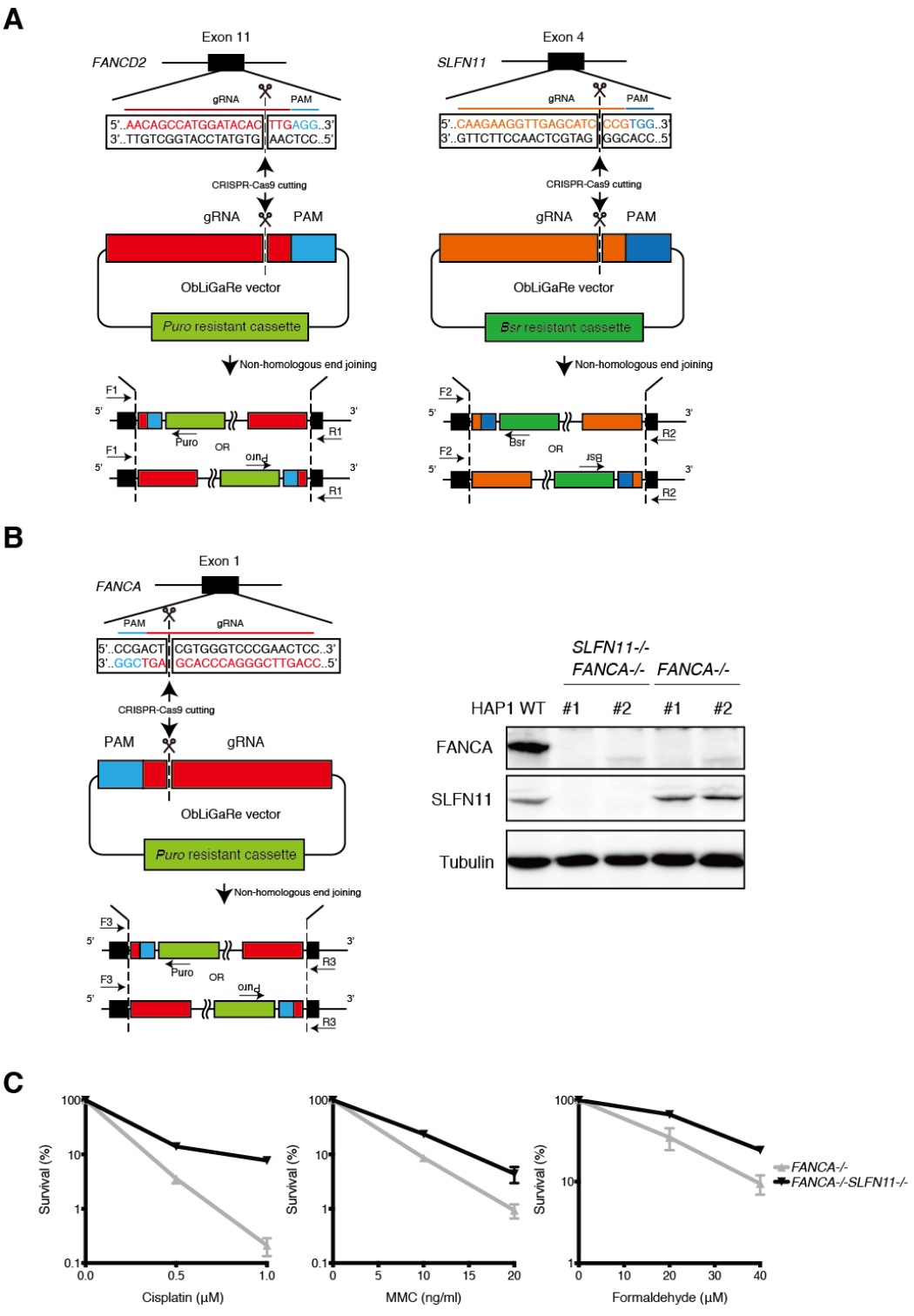
supplemental Table 1. Primer sequences used in this study

SLFN11 expression		
SLFN11 cloning into pENTR	KD17-1	GCAGGCTCCGCGGCCGCCACCATGGAGGCAAATCAGTGCCCCCTGG
	KD17-3	GCCCAAGCTTGAATTC ATGGCCACCCCACGGAAAAATATAC
CRISPR for HAP1		
FANCD2 (gRNA+PAM)	KD17-116	CACCGAACAGCCATGGATACACTTG
	KD17-117	AAACCAAGTGTATCCATGGCTGTTC
SLFN11 (gRNA+PAM)	KD16-570	CAAGAAGGTTGAGCATCCCGGTTTTAGAGCTAGAAATAGCAAGTT
	KD16-571	CGGGATGCTCAACCTTCTTGCGGTGTTTCGTCCTTTCCACAAGAT
FANCA (gRNA+PAM)	KD14-17	GGAGTTCGGGACCCACGAGTGTTTT
	KD14-18	ACTCGTGGGTCCCGAACTCCCGGTG
ObLiGaRe vectors		
FANCD2 (gRNA+PAM)	KD17-118	GGCCAACAGCCATGGATACACTTGAGGT
	KD17-119	GATCACCTCAAGTGTATCCATGGCTGTT
SLFN11 (gRNA+PAM)	KD17-77	GGCCCAAGAAGGTTGAGCATCCCGTGGT
	KD17-78	GATCACCACGGGATGCTCAACCTTCTTG
FANCA (gRNA+PAM)	KD17-67	GGCCGGAGTTCGGGACCCACGAGTCGGA
	KD17-68	GATCTCCGACTCGTGGGTCCCGAACTCC
Check for HAP1 gene disruption		
FANCD2	F1 (KD17-153)	GCTGCAGATCCAACTTCTCCCGAAGCTCAG
	R1 (KD17-154)	AAGTGGGAAGATGGAGTAAGAGAAGTGATT
	Puro-primer	CAGCGCCCGACCGAAAGGAGCGCACGACC
SLFN11	F2 (KD17-583)	ATCAGTGCCCCCTGGTTGTGGAACCATCTT
	R2 (KD17-584)	CTGCAAAGGCGGGGCTTGACAGAGCGATCT
	Bsr-primer	TTGAAGAACTCATTCCACTCAAATATACCC
FANCA	F3 (KD13-225)	GCAGCCAATAGGAAGGCAGCGCGGGCTCGG
	R3 (KD14-172)	GATCCGACCGGCGGAGGCTCTGGCGGGAAG
	Puro-primer	CAGCGCCCGACCGAAAGGAGCGCACGACC
CRISPR for hTERT RPE-1		
FANCD2 (gRNA+PAM)	KD17-116	CACCGAACAGCCATGGATACACTTG
	KD17-117	AAACCAAGTGTATCCATGGCTGTTC
Check for hTERT RPE-1 gene disruption		
FANCD2	Forward	GGAAGATGGAGTAAGAGAAGT
	Reverse	TGCTCATTCATAGTGGGTAG
	sequencing	TGAGGTAGTGACATGAAAACC

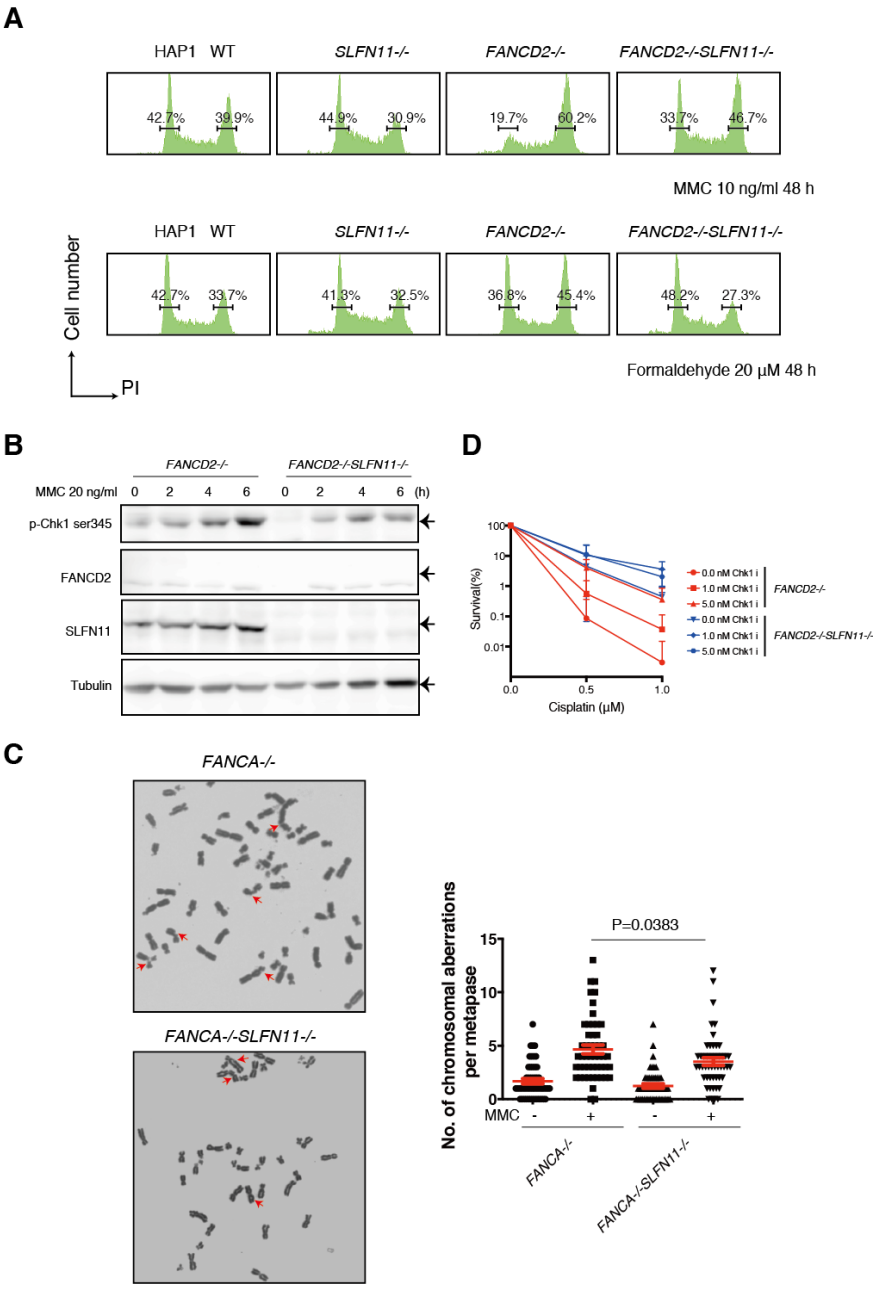


Supplemental Figure 1. SLFN11 expression in hematopoietic stem cells and its effects on ICL sensitivity in PD20 cells. (A) Gene expression activity of SLFN11 in human bone marrow cells as well as HeLa and U2OS cells (taken from Gene expression Commons. https://gexc.riken.jp)[1]. Absolute gene expression profiling is achieved by mapping sample data against a common reference obtained via meta-analysis of a large number of public microarray data sets. BM HSC, bone marrow hematopoietic stem cells (Lin- CD34+ CD38- CD90+ CD45RA-); BM MPP, multi-potent progenitor cells (Lin- CD34+ CD38- CD90- CD45RA-); BM CMP, common myeloid progenitor cells (Lin- CD34+ CD38+ CD123+ CD45RA-); BM MEP, megakaryocyte erythroid progenitor cells (Lin- CD34+ CD38+ CD123- CD45RA-); BM GMP, granulocyte monocyte progenitor cells (Lin- CD34+ CD38+ CD123+ CD45RA+). (B) Cell survival assay of PD20 cells treated with siSLFN11. Means ± s.e.m. of three independent experiments are shown.



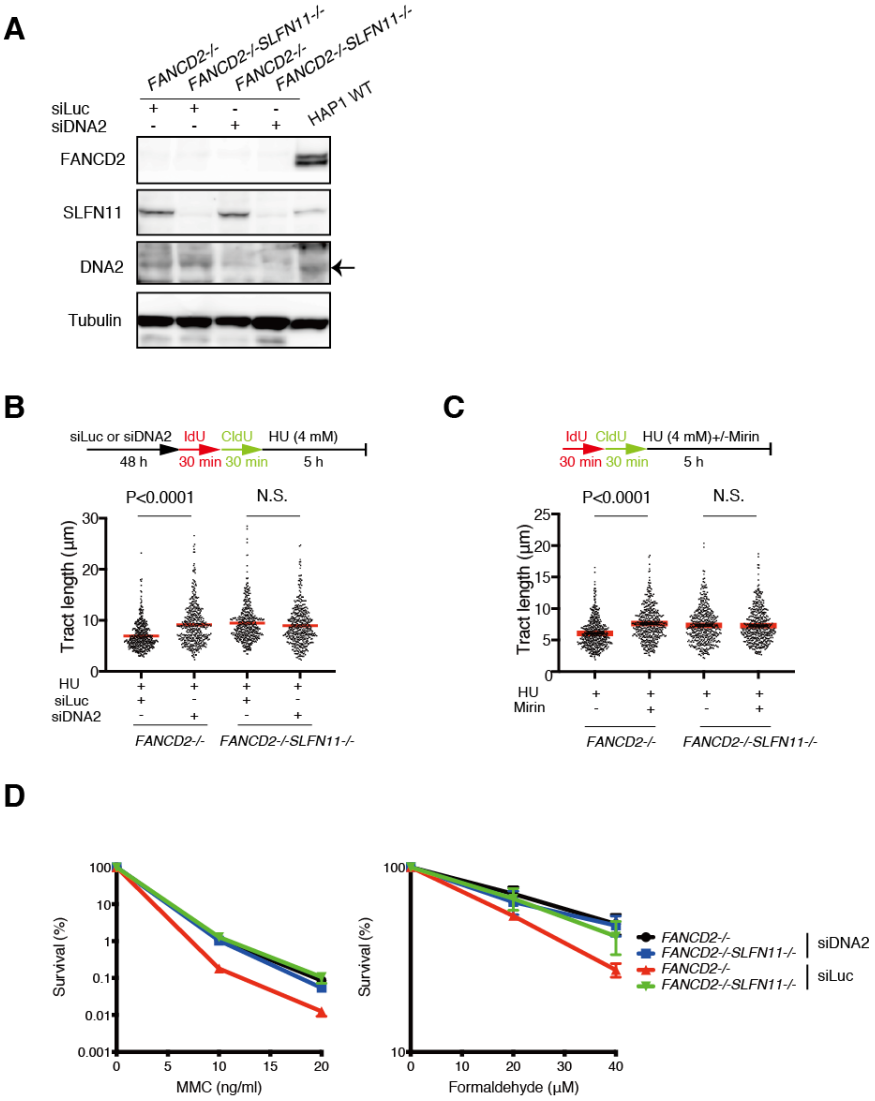
Supplemental Figure 2. Generation of *SLFN11, FANCD2*, and *FANCA* disruptions in HAP1 cells. (A) A schematic of *FANCD2* or *SLFN11* gene disruption by the ObLiGaRe methods as described [2]. (B) A schematic of *FANCA* disruption by the ObLiGaRe method. *FANCA* and *SLFN11* gene disruptions were confirmed by western

blotting analysis. (C) Clonogenic cell survival assay of *FANCA-/-* and *FANCA-/-SLFN11-/-* HAP1 cells. Cells were exposed to the indicated doses of cisplatin, MMC, or formaldehyde. Means ± s.e.m. of three independent experiments are shown.

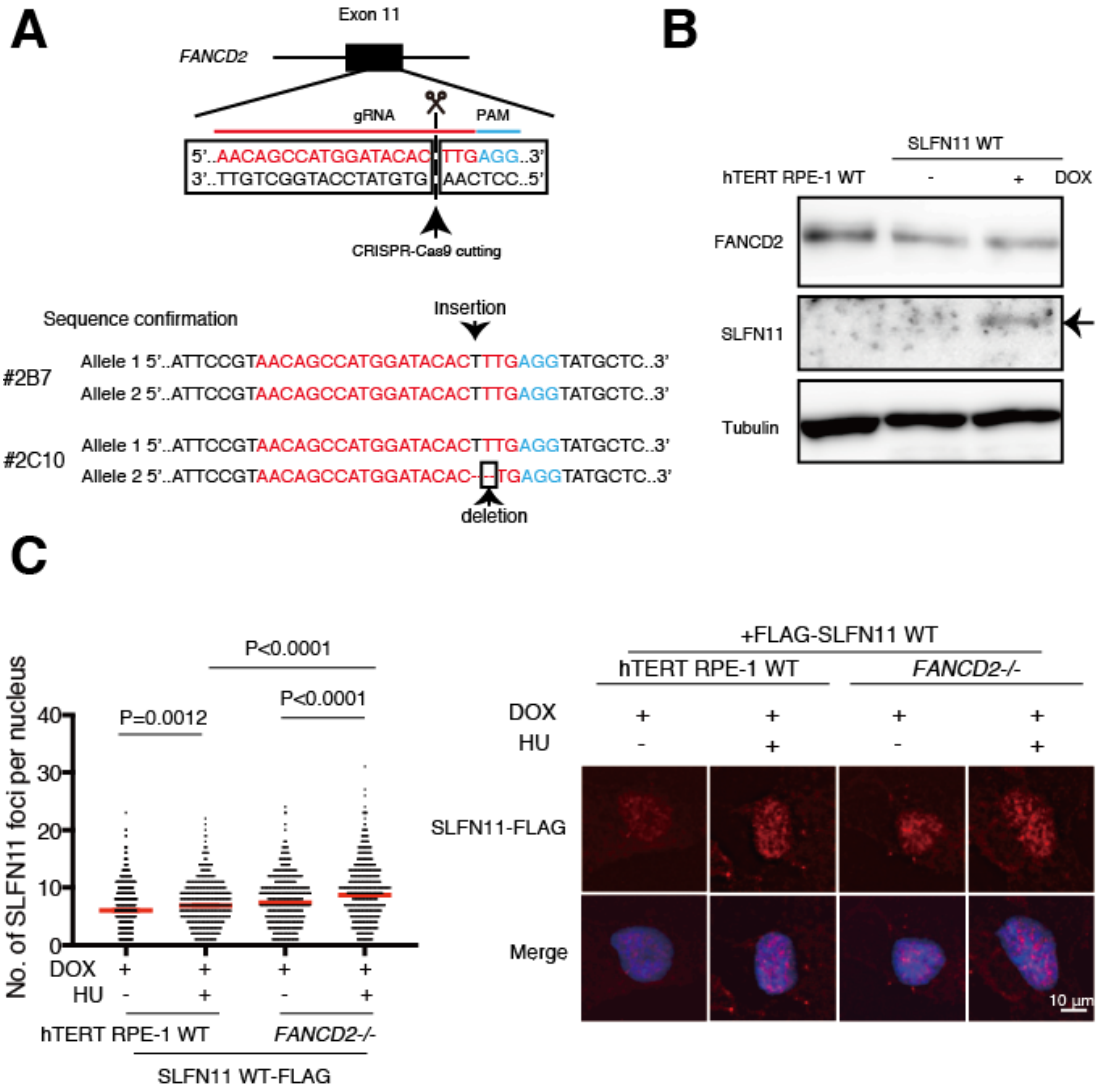


Supplemental Figure 3. *SLFN11* disruption causes partial phenotypic correction in *FANCD2*-/- or *FANCA*-/- cells. (A) Cell cycle profile. Cells were treated with MMC (10 ng/ml, 48 h) or formaldehyde (20 µM, 48 h) and then analyzed using a FACS Canto II flow cytometer (Becton-Dickinson). (B) Analysis of CHK1 activation by western blotting. CHK1 phosphorylation on Serine 345 was examined at the indicated time points during 20 ng/ml MMC exposure in *FANCD2*-/- and *FANCD2-/-SLFN11*-/- HAP1 cells. (C) Chromosome breakage analysis. Cells were subjected to 40 ng/ml MMC exposure for 24 h. Each dot represents the number of chromosomal breaks in a single

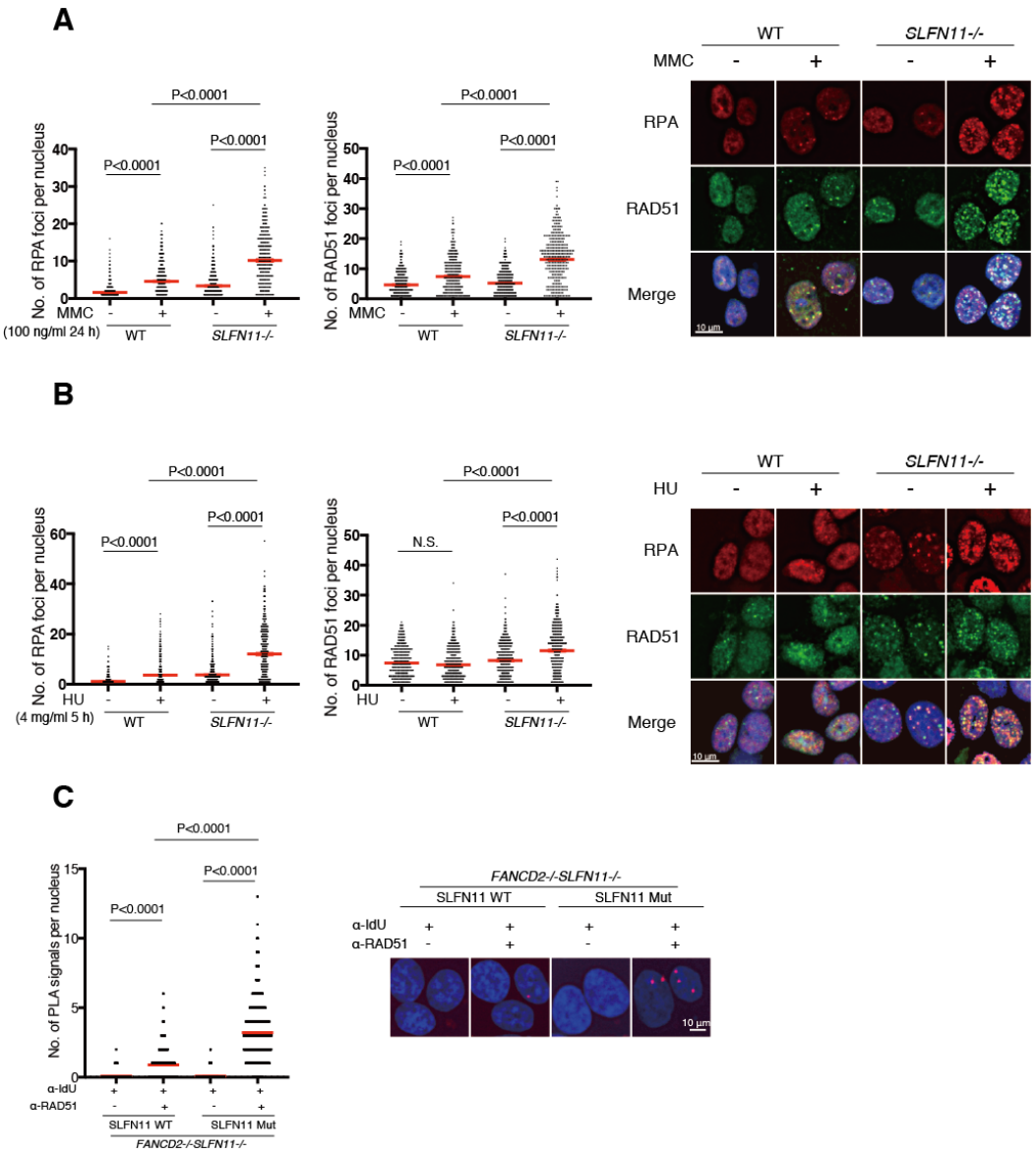
metaphase, and means ± s.e.m. are shown. More than 50 metaphases were scored in each condition. Representative images of metaphases after MMC treatment are shown on the left. P-values were calculated by unpaired, two-tailed Student's *t*-test. (D) Clonogenic cell survival assay of *FANCD2-/-* or *FANCD2-/-SLFN11-/-* HAP1 cells treated with the indicated dosage of CHK1 inhibitor. Cells were exposed to indicated doses of CDDP for 24 h, and the number of colonies was counted 5-7 days later. Means ± s.e.m. of three independent experiments are shown.



Supplemental Figure 4. MRE11 inhibitor or DNA2 depletion by siRNA reverses the FA phenotype. (A) DNA2 depletion by siRNA was confirmed by western blotting analysis in the HAP1 mutant cell lines. (B) DNA fiber assays in *FANCD2-/-* or *FANCD2-/-SLFN11-/-* cells treated with control or siRNA targeting DNA2. (C) DNA fiber analysis to quantify fork degradation/protection. Cells were treated with or without 50 μM Mirin together with HU (4 mM, 5 h). Means ± s.e.m. of tract lengths are shown. The tract lengths of more than 450 fibers were quantified from three independent experiments. P-values were calculated by unpaired, two-tailed Student's *t*-test. (D) Clonogenic cell survival assay. Cells were transfected with control or siRNA targeting DNA2; then, 48 h later, cells were exposed to the indicated doses of MMC or formaldehyde for 24 h. Colonies were counted 5-7 days later. Means ± s.e.m. of three independent experiments are shown.



Supplemental Figure 5. Disruption of *FANCD2* gene or expression of SLFN11 in hTERT RPE-1 cells. (A) Generation of *FANCD2*-/- hTERT RPE-1 cells. hTERT RPE-1 cells were transfected with the CRISPR/Cas9 construct targeting exon 11 of the *FANCD2* locus, as described in Methods. Two clones (#2B7 and #2C10) were confirmed to have biallelic frameshifting mutations using primers described in supplemental Table 1. (B) DOX-induced expression of wildt ype (WT) SLFN11-FLAG as detected by anti-SLFN11 western blotting. Lentivirally-transduced WT hTERT RPE-1 cells were stimulated or not with doxycycline (2 μg/ml) for 48 h. (C) SLFN11-FLAG foci formation in WT and *FANCD2*-/- hTERT RPE-1 cells upon HU treatment (4 mM, 5 h). Means ± s.e.m. of SLFN11 foci in more than 500 nuclei with representative images from two independent experiments are shown. P-values were calculated by unpaired, two-tailed Student's *t*-tests.



Supplemental Figure 6. SLFN11 regulates RPA/RAD51 foci formation and prevents RAD51 accumulation via its ATPase activity. (A and B) RPA and RAD51 foci formation in WT and *SLFN11-/-* HAP1 cells treated or not with MMC (100 ng/ml, 24 h) or HU (4mM, 5 h). Means ±s.e.m. are shown. More than 300 nuclei were examined in two independent experiments (left). Representative images are shown (right). (C) PLA between nascent DNA (IdU tract) and RAD51. Cells were pulsed for 30 min with IdU, then stimulated with HU (4 mM, 5 h). PLA was performed in a native condition as previously described[3]. PLA performed with anti-IdU alone served as a negative control. Means ± s.e.m. with representative images from two independent experiments are shown. P-values were calculated by unpaired, two-tailed Student's *t*-tests.

References

1. Seita J, Sahoo D, Rossi DJ, et al. Gene Expression Commons: an open platform for absolute gene expression profiling. *PLoS ONE*. 2012;7(7):e40321.
2. Maresca M, Lin VG, Guo N, Yang Y. Obligate ligation-gated recombination (ObLiGaRe): custom-designed nuclease-mediated targeted integration through nonhomologous end joining. *Genome Research*. 2013;23(3):539–546.
3. Malacaria E, Pugliese GM, Honda M, et al. Rad52 prevents excessive replication fork reversal and protects from nascent strand degradation. *Nat Commun*. 2019;10(1):1412.