The Fanconi anemia core complex promotes CtIP-dependent end resection to drive homologous recombination at DNA double-strand breaks

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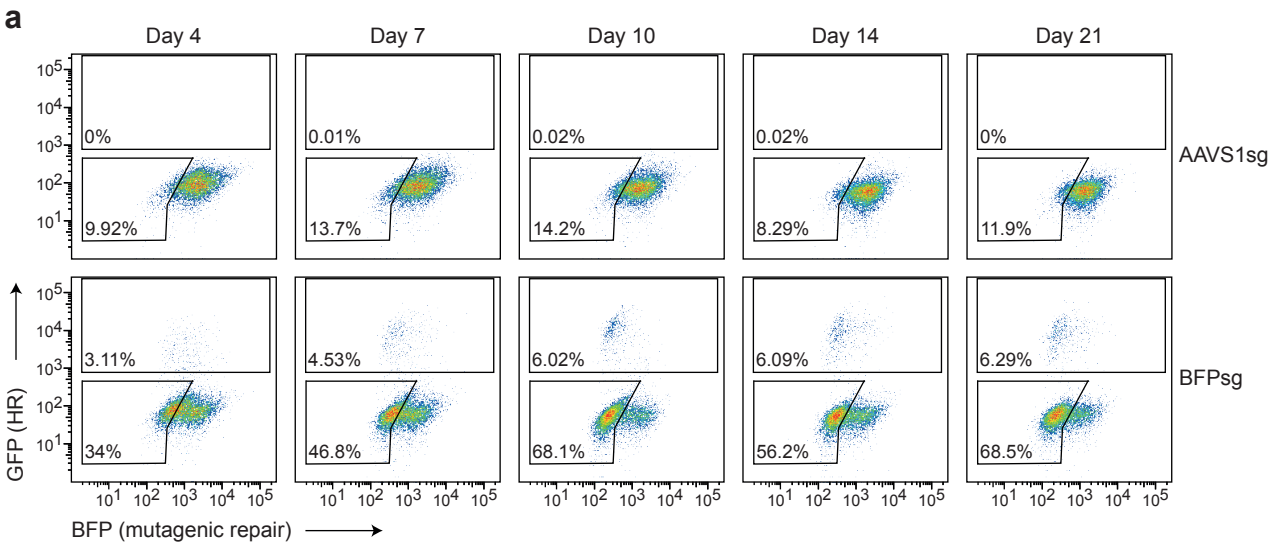
Supplementary Table 1. Oligo table. The NNNNNN stretch in Illumina PCR 2 FWD refers to the indeces used for de-multiplexing (also see Supplementary Data 2).

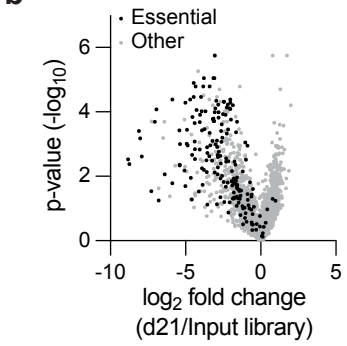
Name	Sequence 5' to 3'
Kinome subpool lib FWD	CATTCTGCACGCTTCAAAAG
Chromatin subpool lib FWD	GACTGGTTCCAATTGACAAGC
Ubiquitin subpool lib FWD	ATCAGTTCGCTTCTCGCTTC
Phosphatase subpool lib FWD	TACCCATACGACGTCCCAGA
U6 FWD for sgRNA library	GTGGAAAGGACGAAACACCG
sgRNA scaffold for library	GTTTTAGAGCTAGAAATAGCAAGTT
Overhang for library	GCGTAGGCTA
sgRNA lib outer REV	GAAATAGCAAGTTGCGTAGGCTA
sgRNA lib inner REV	GTTTTAGAGCTAGAAATAGCAAGTT
Illumina PCR1 FWD	ACACGACGCTCTTCCGATCTYRYRGGAAAGGACGAAACACCG
Illumina PCR1 REV	CGGCATTCCTGCTGAACCGCTCTTCCGATCTGTGGATGAATAC TGCCATTTGTCT
Illumina PCR2 FWD	AATGATACGGCGACCACCGAGATCTACACNNNNNNACACTCTT TCCCTACACGACGCTCTTCCGATCT
Illumina PCR2 REV	AGATCTTGAGACAAATGGCAGTATTCATCCACAGATCGGAAGA GCGGTTCAGCAGGAATGCCGAGACCGGCCACCGGATCTCGTA TGCCGTCTTCTGCTTG
AsiSI DSB1 335_F	GAATCGGATGTATGCGACTGATC
AsiSI DSB1 335_R	TTCCAAAGTTATTCCAACCCGAT
AsiSI DSB 1 1618_F	TGAGGAGGTGACATTAGAACTCAGA
AsiSI DSB 1 1618_R	AGGACTCACTTACACGGCCTTT
AsiSI DSB 2 364_F	GGAGACTTGGAGGAGGGACC
AsiSI DSB 2 364_R	ACAGCACCCAAAACCCCATT
AsiSI DSB 2 1754_F	GAAGCCATCCTACTCTTCTCACCT
AsiSI DSB 1754_R	GCTGGAGATGATGAAGCCCA
FANCL qRT_1 FWD	TGGACACCTCAGAGCTCCTT
FANCL qRT_1 REV	TGCAATTCTGCGTGCTGTTG
B-Actin qRT F	ACTCTTCCAGCCTTCCTTCC
B-Actin qRT R	CAATGCCAGGGTACATGGTG

Supplementary Table 2. Antibody table. WB=western blot, IF=immunofluorescence, FC=flow cytometry.

Target	Species	Company	Cat. No., Clone	Lot No.	Application and dilution
Ube2T	Rabbit	Abcam	ab140611	YJ062105CS	WB (1:1000)
FANCD2	Mouse	Santa Cruz	sc-20022	D1221	IF (1:100) and WB (1:1000)
FANCD2	Rabbit	Novus	NB100-182	L3	WB (1:10000)
HSP90	Mouse	Santa-Cruz	Sc-13119	G2420	WB (1:1000)
Tubulin	Mouse	Sigma Aldrich	T6199, clone DM1A	127611	WB (1:5000)
γH2AX	Mouse	Millipore	#05-636, clone JBW301	4047974	IF (1:2000)
MDC1	Rabbit	Abcam	ab11171-50	GR252252-10	WB (1:1000)
pRPA (S4/S8)	Rabbit	Bethyl	A300-245A	9	IF (1:1000)
Rad51	Mouse	Genetex	GTX70230, Clone 14B4	42590	IF (1:500)
CtIP	Mouse	Sigma Aldrich	MABE1060, Clone 14-1	3780584	WB (1:1000) and IF (1:1000)
CtIP	Mouse	Santa Cruz	sc-271339, Clone D-4	F1510	WB (1:500)
Actin	Mouse	MpBiomedicals	869100, Clone C4	SR05927-1	WB (1:1000)
BrdU	Mouse	Roche	11170376001, Clone BMC9318	10875400	FC (1:200)
Mre11	Rabbit	Cell Signaling	4895	3	WB (1:1000)
GFP	Rabbit	Abcam	Ab290	GR3321575-1	WB (1:5000)

Supplementary Figure 1





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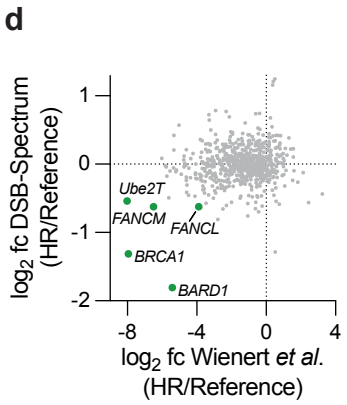
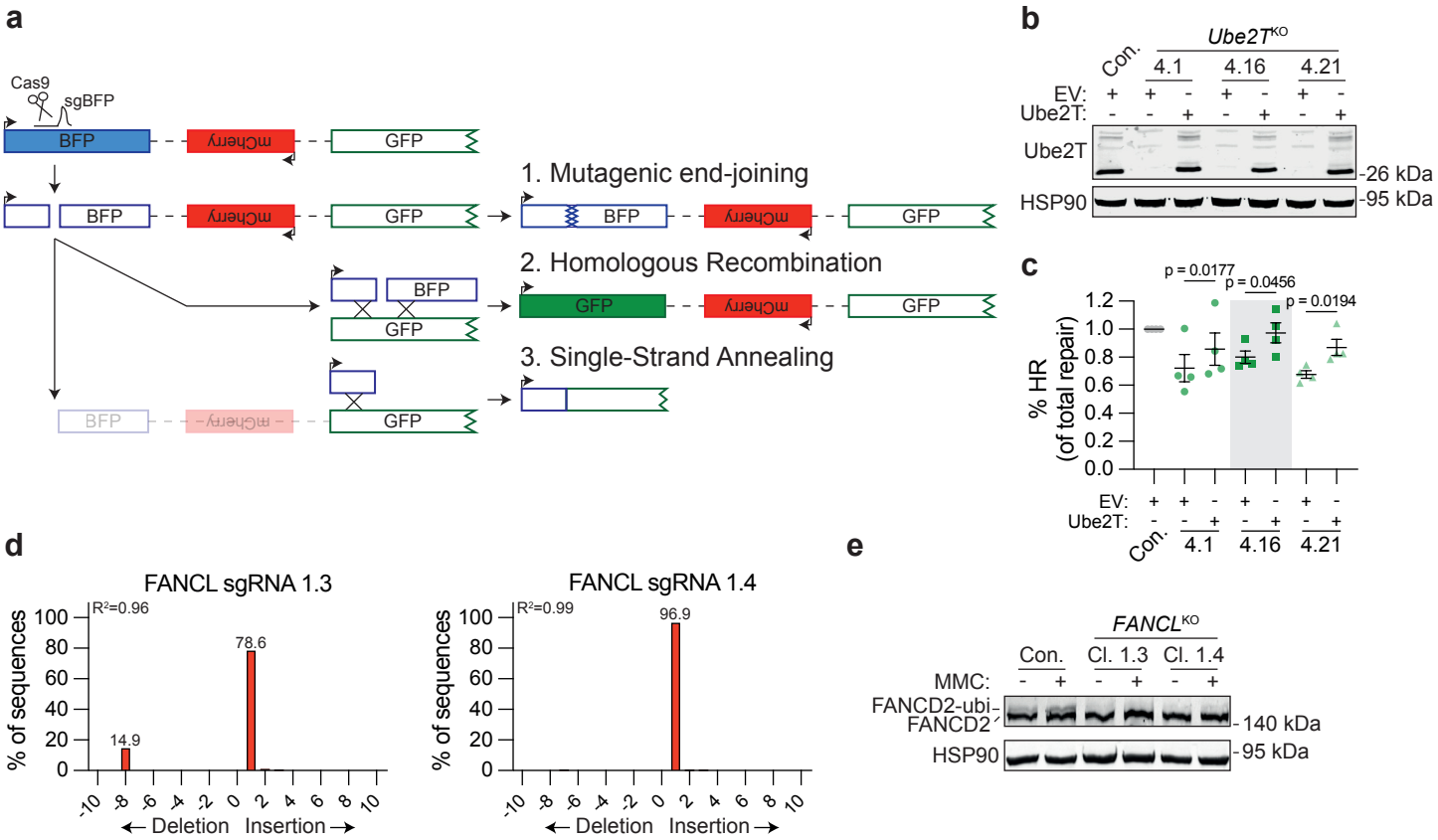
Gene	Fold change	FDR	Notes
BARD1	-1.81	0.04	HR promoting factor
VHL	-1.60	0.00	HR promoting function described
BRCA1	-1.31	0.14	HR promoting factor
FLT1	-0.85	0.14	No HR function described
RNF168	-0.76	0.25	HR promoting factor
FANCM	-0.62	0.14	FA factor, HR promoting factor
GSAP	-0.54	0.25	No HR function described
Ube2T	-0.54	0.09	FA factor
ZNRF3	-0.53	0.14	No HR function described
PAXIP1	-0.46	0.10	anti-HR factor
PHF20	-0.34	0.22	HR promoting function described
USP34	-0.33	0.25	HR promoting function described
ANP32A	-0.31	0.18	No HR function described
WDR13	-0.30	0.14	No HR function described
FAM175A	0.39	0.22	BRCA1-A complex, anti-HR factor
PER1	0.45	0.03	No HR function described
UIMC1	0.51	0.02	BRCA1-A complex, anti-HR factor
ASF1A	0.60	0.03	anti-HR function described
BRCC3	0.69	0.02	BRCA1-A complex, anti-HR factor
BRE	0.72	0.00	BRCA1-A complex, anti-HR factor
POLR3G	0.74	0.00	No HR function described
BABAM1	0.74	0.00	BRCA1-A complex, anti-HR factor
PTK2	0.75	0.06	No HR function described
SIK2	1.10	0.06	No HR function described



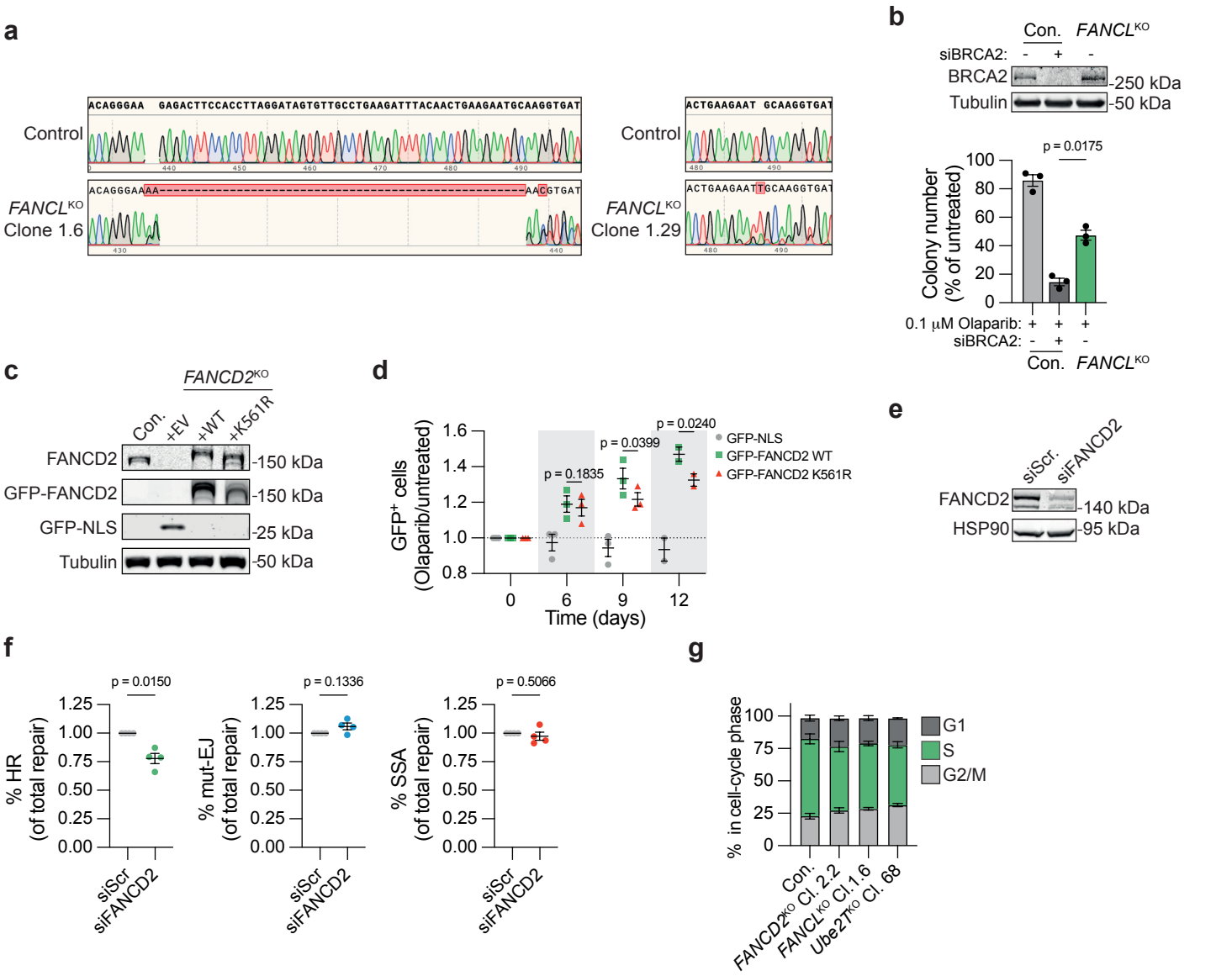
Supplementary Figure 1. Quality control of a targeted CRISPR screen in DSB-Spectrum reporter cells. (a) Representative flow cytometry dot plots of the experiment shown in figure 1b. **(b)** Volcano plot showing the gene targets of sgRNAs that were either enriched or depleted from the total population, as compared to the input sgRNA library, at 21 days after lentiviral introduction of the sgRNA library. Genes were identified as essential based on Wang *et al*., 2015. Enrichment and statistics were determined by MAGeCK. **(c)** Table listing all hits from the HR branch of the screen, defined as genes with cognate sgRNAs enriched or depleted with an False Discovery Rate (FDR) cut-off of ≤0.26. HR promoting factors and anti-HR factors are outlined with a green and red box, respectively. **(d)** Comparison between this study and Wienert *et al*., 2020 of sgRNA depletion and enrichment in the HR population compared to the reference population. HR-promoting factors that were identified by both studies are indicated in green (fc=fold change). Source data are provided as a Source Data file.

Supplementary Figure 2



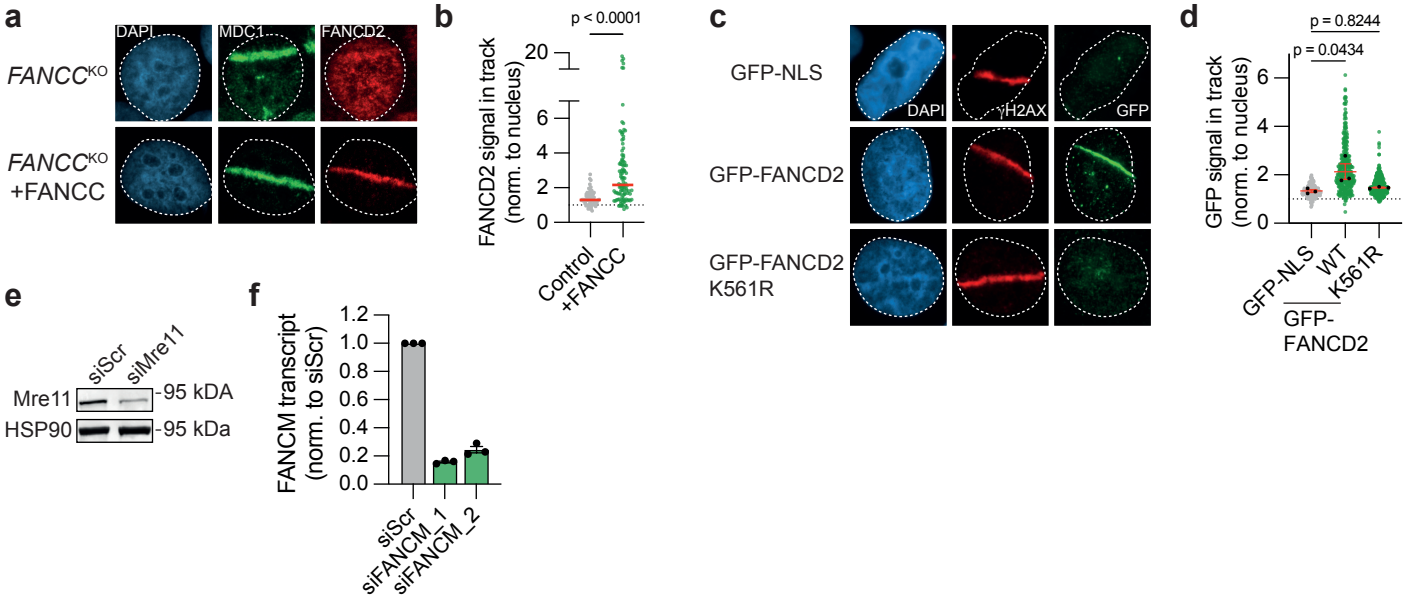
Supplementary Figure 2. Ube2T and FANCL promote DSB-repair by HR. (a) Schematic of the DSB-Spectrum_V3 reporter. Adapted from van de Kooij *et al*., 2022. **(b)** Western blot showing Ube2T expression in the cells analyzed in panel c. **(c)** Indicated HEK 293T+DSB-Spectrum_V3 cell lines were co-transfected with either an empty vector (EV) or Ube2T cDNA, together with the reporter targeting Cas9-sgRNA constructs. Next, cells were analyzed by flow cytometry to determine the frequency of repair by each of the three indicated pathways (n=4 independent biological repeats; mean±SEM; paired t-test). **(d)** InDel formation at the sgRNA target site in the *FANCL* gene was analyzed using TIDE. Exact percentages are indicated as numbers above the bars, the R^2 value indicates the goodness of fit of the modelled InDel spectrum on the composite sequence. **(e)** HEK 293T+DSB-Spectrum_V3 *FANCL*^{KO} clones 1.3 and 1.4, as well as the parental control (Con.) were treated with Mitomycin C (MMC, 300 nM) for 24h. Next, FANCD2 ubiquitination status was analyzed by western blotting. Source data are provided as a Source Data file.

Supplementary Figure 3



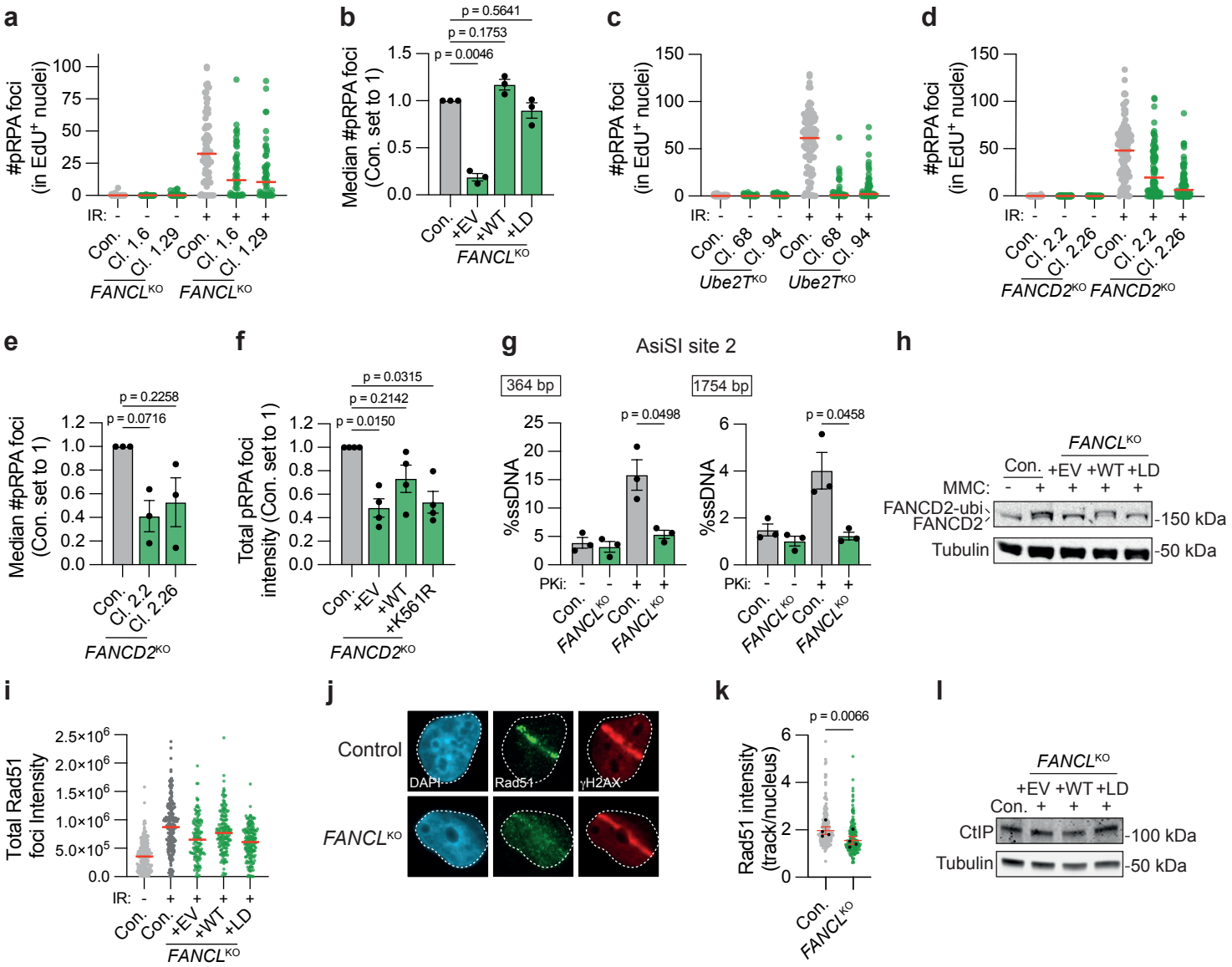
Supplementary Figure 3. Loss of FANCL and FANCD2 ubiquitination causes PARPi sensitivity. (a) DNA sequence alignment of the *FANCL* sgRNA target site in unedited control cells and the U2OS *FANCL*^{KO} clones. Depicted are representative sequence chromatograms, red shaded boxes indicate deviations in the DNA sequence of the *FANCL*^{KO} clone compared to control. **(b)** Wild-type control (Con.) U2OS cells were transfected with a control or BRCA2-targeting siRNA. Next, these cells, together with U2OS *FANCL*^{KO} cells, were treated with olaparib for 14 days. Cell viability was assed by clonogenic survival. Upper panel shows western blot analysis for BRCA2 knock-down, lower panel shows the number of surviving colonies (n=3 independent biological repeats; mean±SEM; Ratio paired t-test). **(c)** U2OS *FANCD2*^{KO} clone 2.2 was lentivirally infected to express GFP-NLS (+EV), GFP-FANCD2 WT, or GFP-FANCD2 K561R. Cells were analyzed by Western blot for FANCD2 expression. **(d)** The cells described in panel c were mixed 1:1 with the GFP-negative *FANCD2*^{KO} parental cells. Next, cells were treated with olaparib (1 μM) for 12 days, and the fraction of GFP-positive cells in the mixed population was monitored by flow cytometry. Plotted is the fraction of GFP-positive cells in the olaparib treated population, normalized for the fraction in the untreated population to correct for olaparib independent growth differences (n=3 iindependent biological repeats, except for day 12 for which n=2; mean±SEM; paired t-test). **(e)** Western blot showing FANCD2 knockdown in FANCD2 siRNA transfected HEK 293T+DSB-Spectrum_V3 cells. **(f)** HEK 293T+DSB-Spectrum_V3 cells were transfected with indicated siRNAs (siScr=siScrambled), followed by a reporter assay as described for figure 2b (n=4 independent biological repeats; mean±SEM; Paired t-test). **(g)** The cell-cycle profile of indicated, asynchronously growing, cell lines was determined by flow cytometry. The differences between each of the knock-out cell lines and the wild-type control cell line were not significant, for neither of the cell-cycle stages (n=3 independent biological repeats; mean±SEM; One-way ANOVA+Dunnett's multiple comparison; p-value ranged from 0.1009 to 0.5659). Source data are provided as a Source Data file.

Supplementary Figure 4



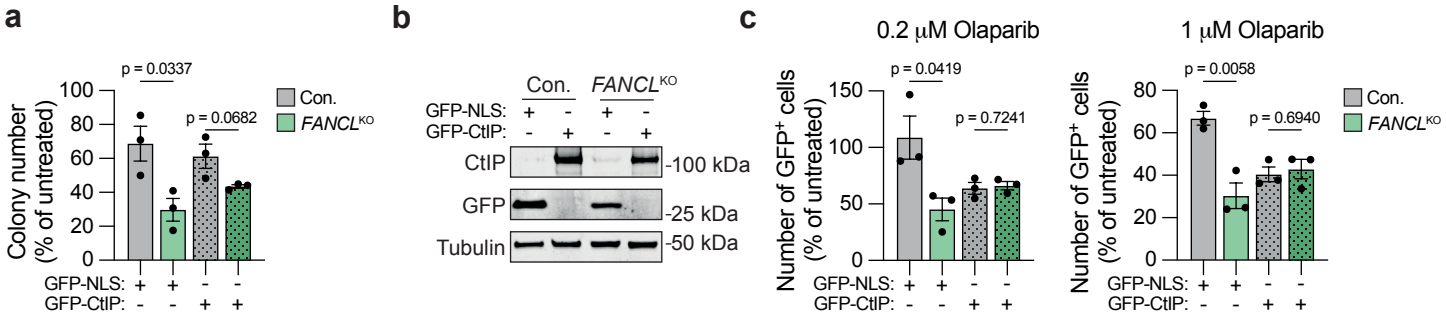
Supplementary Figure 4. FANCL, Ube2T and FANCD2 accumulate at DSBs. (a, b) FANCD2 recruitment to UV-A laser induced DNA damage was analyzed by imunofluorescence microscopy in FANCC-deficient VU1131 fibroblasts, reconstituted or not with FANCC cDNA. Shown are representative images (a) and quantification (b) of one of two independent biological replicates. Dotted line is set at 1 (*i.e.* no recruitment to the track), red lines indicate median (n=63, 106 (Control, +FANCC); Mann-Whitney test). **(c, d)** IF-microscopy of UV-A laser micro-irradiated U2OS *FANCD2*^{KO} cells, stably expressing GFP-NLS, or GFP-tagged FANCD2. Panel c shows representative images, panel d shows the quantification. Plotted are the data from all biological repeats. Each grey or green dot represents an individual track, black dots are the median for each biological repeat (n=3; mean±SEM; ANOVA with post-hoc Dunnetts on the medians). **(e)** Mre11 protein levels in the cells analyzed in figure 4j, k were analyzed by western blot. **(f)** FANCM transcript levels in the cells analyzed in figure 4j, k were analyzed by qPCR (technical triplicate; mean±SEM). Source data are provided as a Source Data file.

Supplementary Figure 5



Supplementary Figure 5. FANCL, Ube2T and FANCD2 promote end resection at DNA double-strand breaks. (a) Depicted is a representative biological repeat of the experiment shown in figure 5a, b. **(b)** For the experiment shown in figure 5c, the number of pRPA foci per S-phase nucleus was plotted (n=3 independent biological repeats; mean±SEM; one-way ANOVA with post-hoc Dunnett's). **(c)** As in figure 5a,b, but for *Ube2T*^{KO} cells. Depicted is a representative experiment of two biological repeats. **(d, e)** As in figure 5a, b, but for *FANCD2*^{KO} cells. Panel d shows the quantification of a representative experiment, panel e shows the quantification of the three bioligical repeats (n=3 independent biological repeats; mean±SEM; one-way ANOVA with post-hoc Dunnett's). **(f)** As in panel e, but for *FANCD2*^{KO} clone 2.2 reconstituted with either GFP-NLS (EV), GFP-FANCD2 WT or GFP-FANCD2 K561R (n=4 independent biological repeats; mean±SEM; one-way ANOVA with post-hoc Dunnett's). **(g)** As in figure 5f, but for a second AsiSI-targeted locus (n=3 independent biological repeats; mean±SEM; paired t-test) **(h)** Western blot analysis of FANCD2 ubiquitination in U2OS AsiSI cells re-expressing FANCL wild-type (WT) or ligase-dead (LD; EV=empty vector, MMC=mitomycin C, 1 μM). **(i)** Depicted is a representative biological repeat of the experiment shown in figure 5j. Each symbol is a single S-phase nucleus. **(j, k)** IF-micropscopy of UV-A laser micro-irradiated cells. Panel i shows representative images, panel k shows the quantification. Plotted are the data from all biological repeats. Each grey or green dot represents an individual track, black dots are the median for each biological repeat (n=4 independent biological repeats; mean±SEM; ratio paired t-test). **(l)** Western blot showing CtIP protein levels in control cells and reconstituted U2OS *FANCL*^{KO} cells lines. Source data are provided as a Source Data file.

Supplementary Figure 6



Supplementary Figure 6. CtIP overexpression rescues the PARPi sensitivity of *FANCL*^{KO} cells. (a) U2OS *FANCL*^{KO} and wild-type control cells were transfected to express GFP alone or GFP-tagged CtIP, followed by FACS to enrich for GFP-positive cells. Next, these cells were plated for clonogenic outgrowth in the presence or absence of olaparib (0.2 μM; n=3 independent biological repeats; mean±SEM; unpaired t-test). **(b)** Western blot showing expression levels of GFP-CtIP after transient transfection. **(c)** U2OS *FANCL*^{KO} and wild-type control cells were transfected to express GFP alone or GFP-tagged CtIP, and treated with olaparib (0.2 μM left panel, 1 μM right panel). The number of live, GFP-positive cells was determined by flow cytometry at four days after the start of the treatment (n=3 independent biological repeats; mean±SEM; unpaired t-test). Source data are provided as a Source Data file.