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Supplementary Materials for

FIRRM/C1orf112 mediates resolution of homologous recombination intermediates in response to DNA interstrand crosslinks

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Other Supplementary Material for this manuscript includes the following:

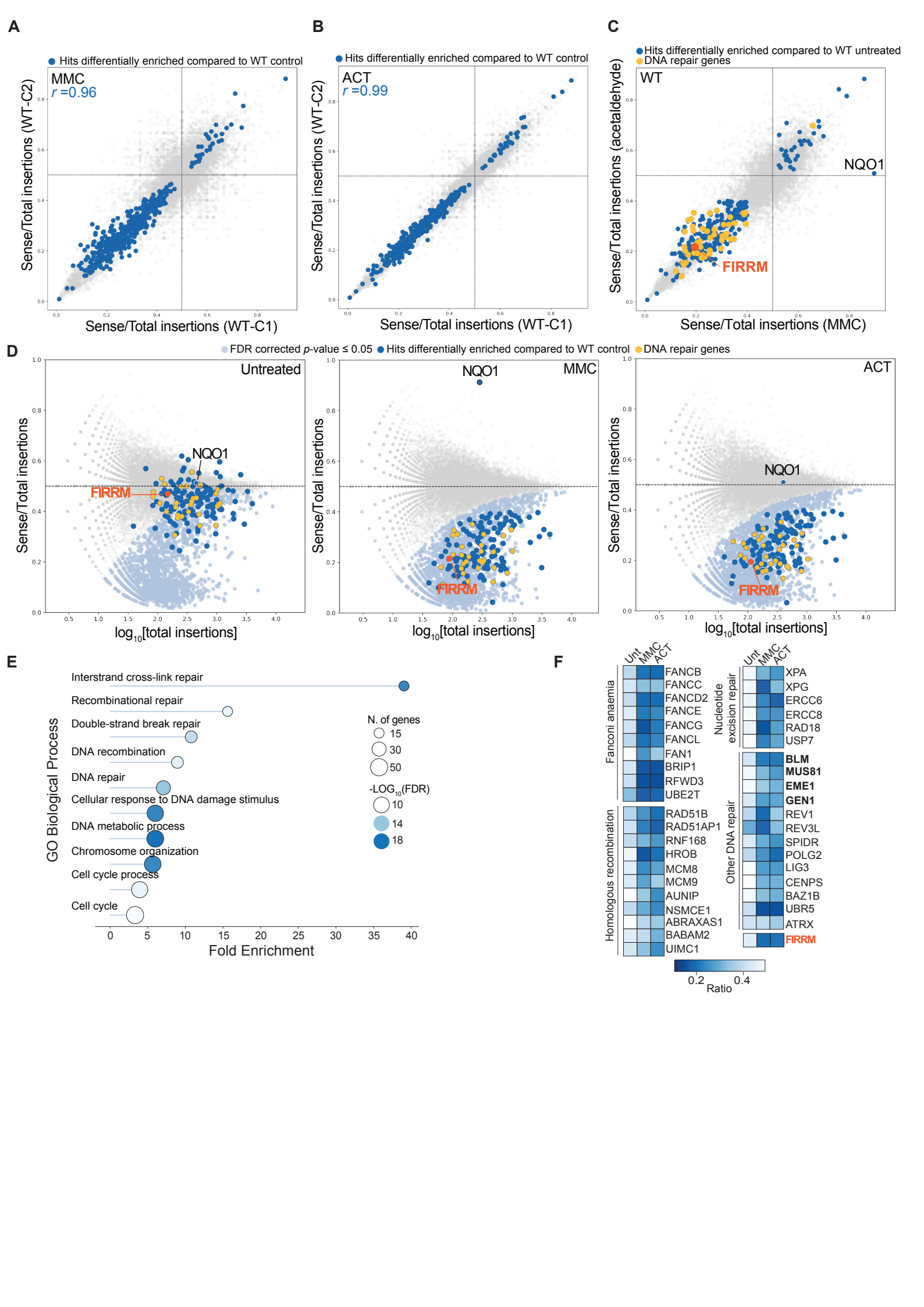

A
Hits differentially enriched compared to WT control
MMC
r =0.96
Sense/Total insertions (WT-C2)
Sense/Total insertions (WT-C1)
B
Hits differentially enriched compared to WT control
ACT
r =0.99
Sense/Total insertions (WT-C2)
Sense/Total insertions (WT-C1)
C
Hits differentially enriched compared to WT untreated
DNA repair genes
WT
NQO1
FIRRM
Sense/Total insertions (acetaldehyde)
Sense/Total insertions (MMC)
D
FDR corrected p-value ≤ 0.05
Hits differentially enriched compared to WT control
DNA repair genes
Untreated
MMC
ACT
NQO1
FIRRM
Sense/Total insertions
log10[total insertions]
E
Interstrand cross-link repair
Recombinational repair
Double-strand break repair
DNA recombination
DNA repair
Cellular response to DNA damage stimulus
DNA metabolic process
Chromosome organization
Cell cycle process
Cell cycle
GO Biological Process
Fold Enrichment
N. of genes
15
30
50
-LOG10(FDR)
10
14
18
F
Unt
MMC
ACT
Fanconi anaemia
FANCB
FANCC
FANCD2
FANCE
FANCG
FANCL
FAN1
BRIP1
RFWD3
UBE2T
Homologous recombination
RAD51B
RAD51AP1
RNF168
HROB
MCM8
MCM9
AUNIP
NSMCE1
ABRAXAS1
BABAM2
UIMC1
Nucleotide excision repair
XPA
XPG
ERCC6
ERCC8
RAD18
USP7
Other DNA repair
BLM
MUS81
EME1
GEN1
REV1
REV3L
SPIDR
POLG2
LIG3
CENPS
BAZ1B
UBR5
ATRX
FIRRM
Ratio

Fig. S1. Synthetic lethality screens reveal important determinants of ICL sensitivity. (**A**, **B**). Scatter plots of the ratios (sense/total insertions) for each gene upon MMC (A) or acetaldehyde (ACT) (B) treatment for wild-type clone 1 compared to clone 2. (**C**) Scatter plot of the ratios (sense/total insertions) for each gene upon mitomycin C (MMC) treatment compared to acetaldehyde (ACT). Genes that are differentially regulated compared to the wild-type untreated are depicted in dark blue and the DNA repair genes are highlighted in orange. (**D**) Fishtail plot depicting the ratios (sense/total insertions) for each gene in untreated, MMC and acetaldehyde treatment in wild-type HAP1 cells (y-axis) and the total number of insertions in the respective gene (x-axis). (**E**) Gene Ontology (GO) terms of the commonly identified genes in MMC and acetaldehyde survival screens. (**F**) Heatmap showing the DNA repair genes that were identified in (C, D) (labelled in orange).

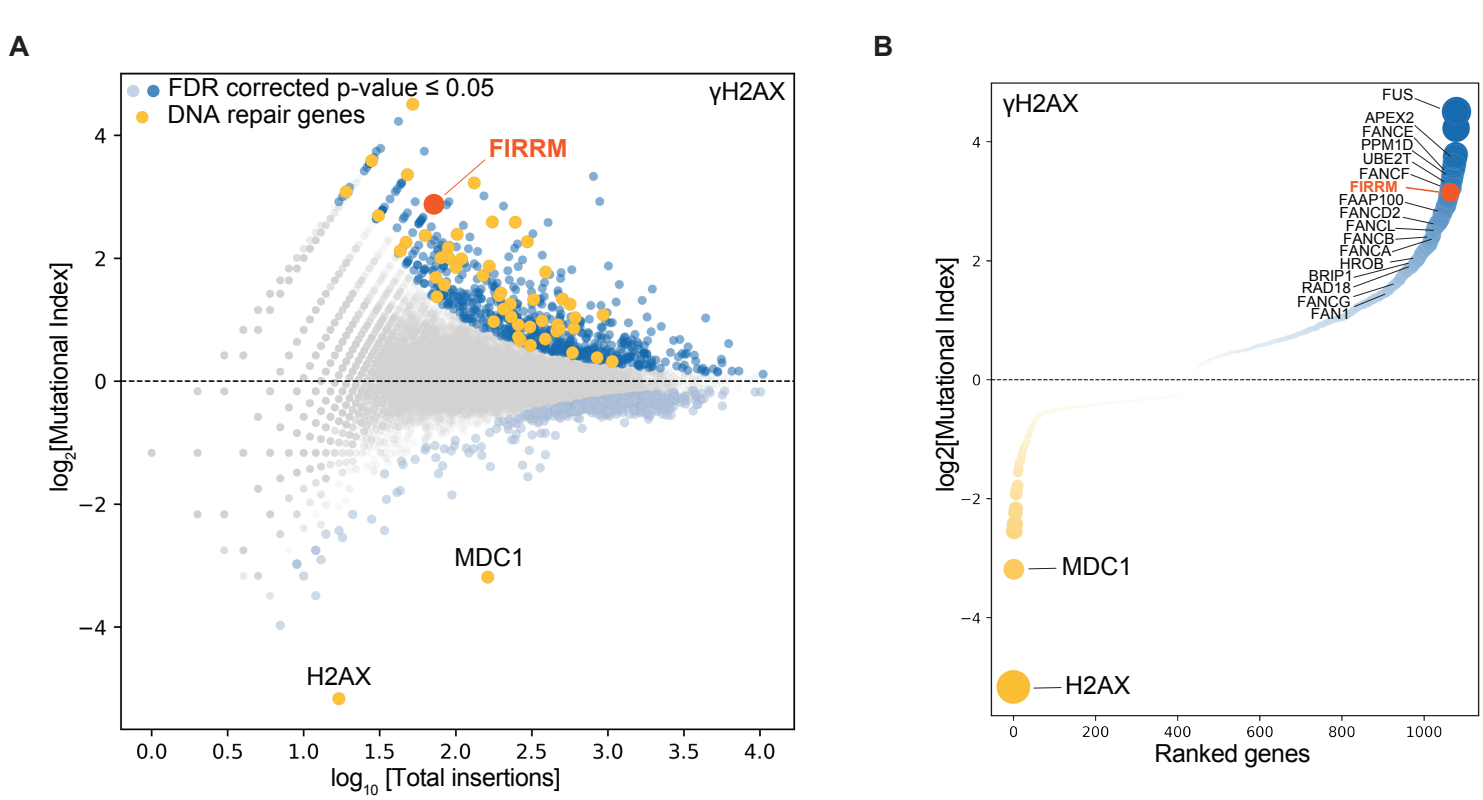
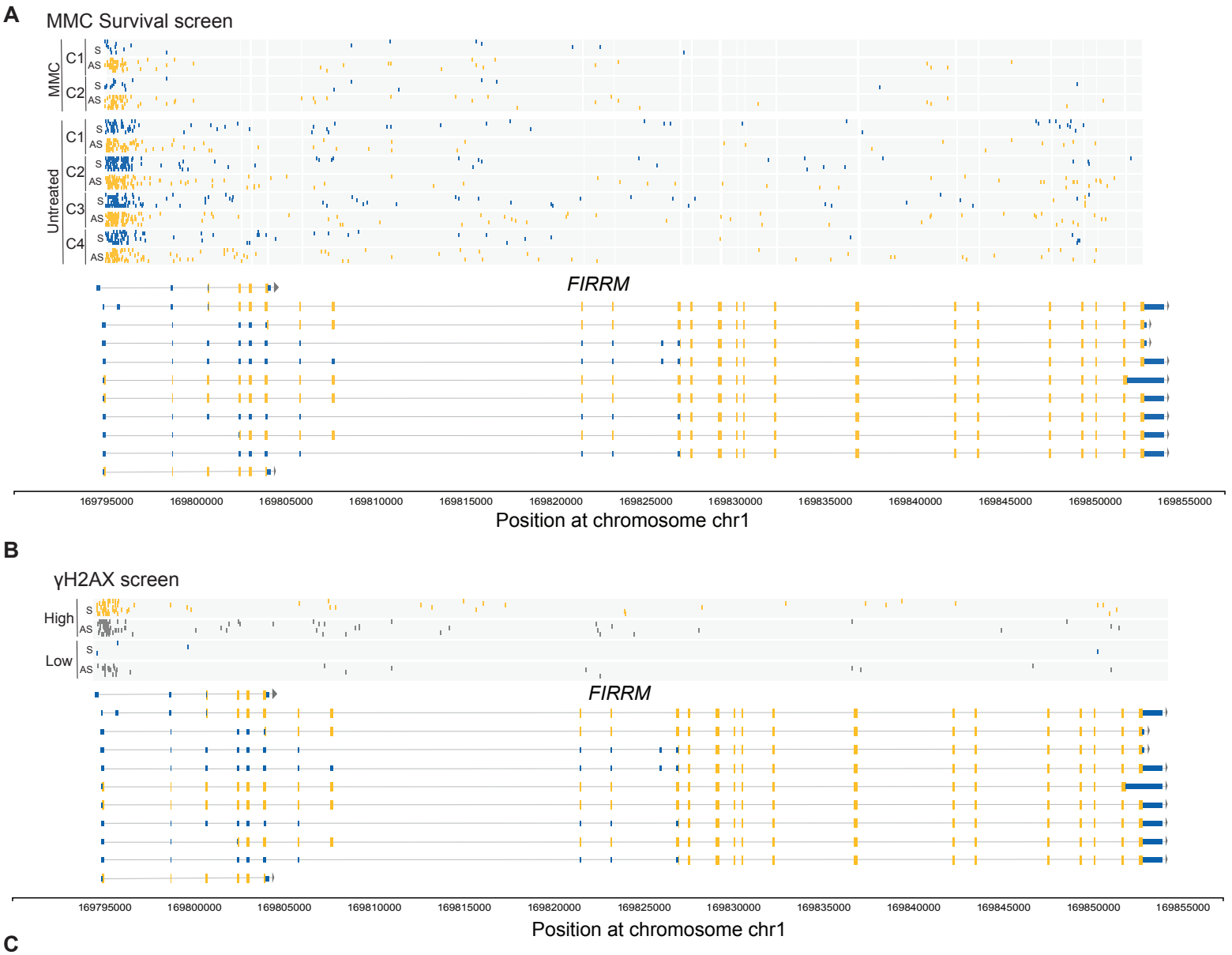

A
FDR corrected p-value ≤ 0.05
DNA repair genes
γH2AX
FIRRM
MDC1
H2AX
log2[Mutational Index]
log10 [Total insertions]
B
γH2AX
FUS
APEX2
FANCE
PPM1D
UBE2T
FANCF
FIRRM
FAAP100
FANCD2
FANCL
FANCB
FANCA
HROB
BRIP1
RAD18
FANCG
FAN1
MDC1
H2AX
log2[Mutational Index]
Ranked genes

Fig. S2. FACS-based genetic screen for γH2AX identifies several key ICL repair factors including *FIRRM*. (**A**) Fishtail plot of the FACS-based haploid genetic screen in S phase cells with low or high levels of γH2AX. Y-axis displays the mutational index (MI) for each gene and x-axis shows the total number of insertions in the respective gene. Negative regulators are highlighted in dark blue and positive regulators are depicted in light blue. DNA repair genes are highlighted in orange. (**B**) Ranking plot depicting the genes significantly enriched or depleted in the γH2AX populations, positive regulators are colored in orange and negative regulators are depicted in blue and ranked according to the mutational index (MI) (FDR corrected $p \leq 0.05$).


A
MMC Survival screen
MMC
C1
C2
S
AS
Untreated
C3
C4
FIRRM
169795000
169800000
169805000
169810000
169815000
169820000
169825000
169830000
169835000
169840000
169845000
169850000
169855000
Position at chromosome chr1

B
γH2AX screen
High
Low
S
AS
FIRRM
169795000
169800000
169805000
169810000
169815000
169820000
169825000
169830000
169835000
169840000
169845000
169850000
169855000
Position at chromosome chr1

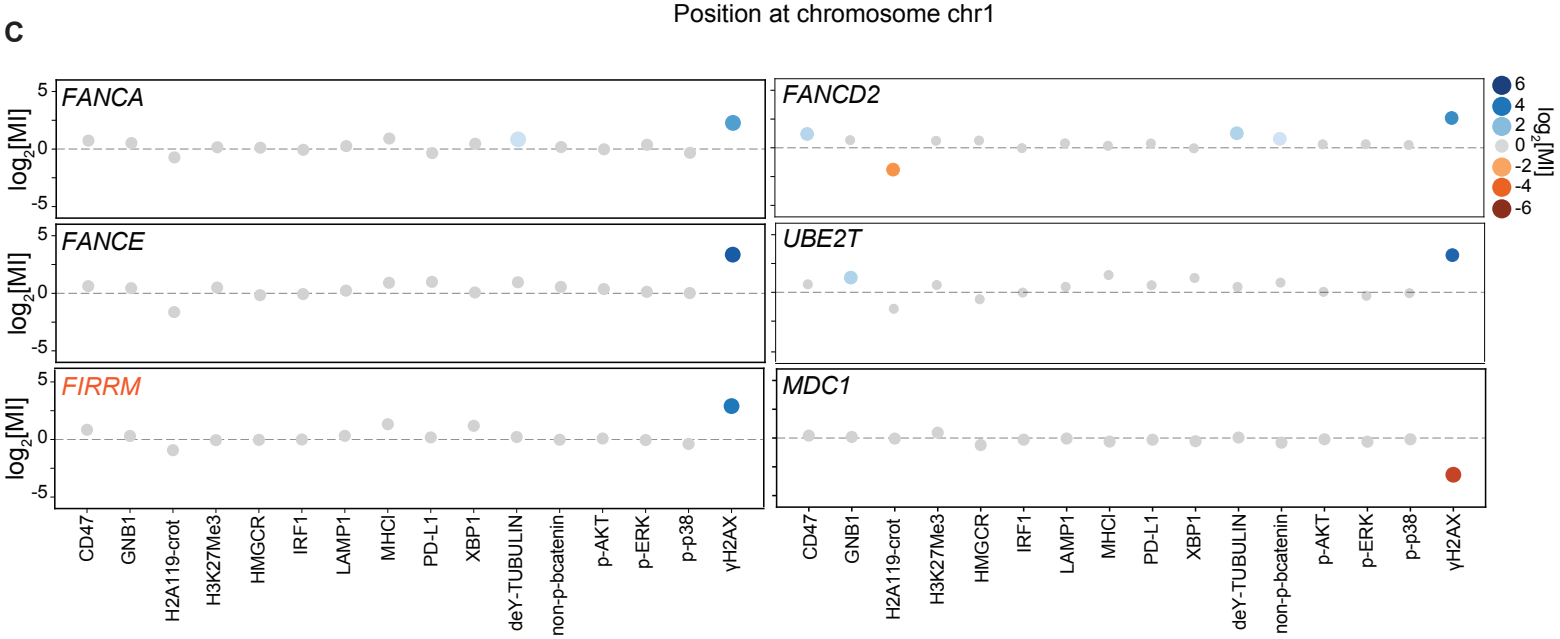

C
FANCA
FANCD2
FANCE
UBE2T
FIRRM
MDC1
log2[MI]
5
0
-5
6
4
2
0
-2
-4
-6
CD47
GNB1
H2A119-crot
H3K27Me3
HMGCR
IRF1
LAMP1
MHCI
PD-L1
XBP1
deY-TUBULIN
non-p-bcatenin
p-AKT
p-ERK
p-p38
γH2AX

Fig. S3. Insertional mutagenesis patterns for *FIRRM* in the performed genetic screens. (A, **B**), Gene-trap insertions identified within the *FIRRM* gene in the sense (S, blue) or antisense orientation (AS, orange) for the survival screens (Untreated *vs* MMC in 2 independent clones) (A) and the FACS-based genetic screen for γH2AX (low *vs* high channel) (B). (**C**) Mutational index (MI) of *FIRRM*, examples of Fanconi anemia genes and *MDC1* plotted across γH2AX and 16 other screens that were previously performed using haploid genetic screens (blue, negative regulator; orange, positive regulator; grey, not significant).

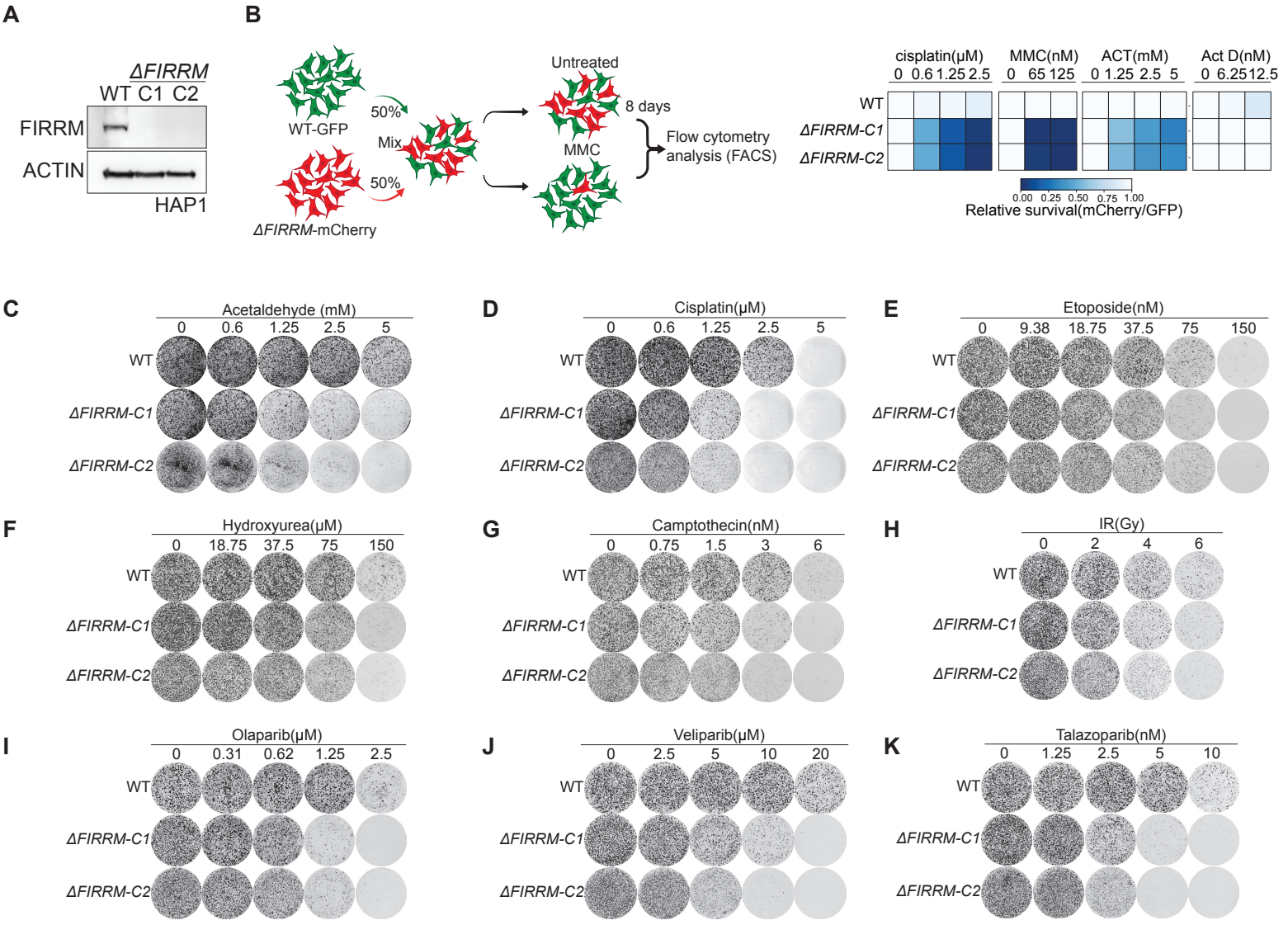

A
ΔFIRRM
WT C1 C2
FIRRM
ACTIN
HAP1
B
WT-GFP
50%
Mix
50%
ΔFIRRM-mCherry
Untreated
8 days
MMC
Flow cytometry analysis (FACS)
cisplatin(μM)
0 0.6 1.25 2.5
MMC(nM)
0 65 125
ACT(mM)
0 1.25 2.5 5
Act D(nM)
0 6.25 12.5
WT
ΔFIRRM-C1
ΔFIRRM-C2
0.00 0.25 0.50 0.75 1.00
Relative survival(mCherry/GFP)
C
Acetaldehyde (mM)
0 0.6 1.25 2.5 5
D
Cisplatin(μM)
0 0.6 1.25 2.5 5
E
Etoposide(nM)
0 9.38 18.75 37.5 75 150
F
Hydroxyurea(μM)
0 18.75 37.5 75 150
G
Camptothecin(nM)
0 0.75 1.5 3 6
H
IR(Gy)
0 2 4 6
I
Olaparib(μM)
0 0.31 0.62 1.25 2.5
J
Veliparib(μM)
0 2.5 5 10 20
K
Talazoparib(nM)
0 1.25 2.5 5 10

Fig. S4. FIRRM deficiency causes hypersensitivity to ICL-inducing agents and PARP inhibitors. (**A**) Immunoblot using an antibody against FIRRM in wild-type or *FIRRM* knockout HAP1 cells generated by CRISPR-mediated gene editing. ACTIN is used as a loading control. The FIRRM antibody recognizes a C terminal peptide located between the residues 694-774. (**B**) (left panel) Schematic representation of the competition growth assays performed in Fig. 1D. (right panel) Heatmap displaying the mean ratios (mCherry/GFP), normalized to untreated (n=3) of competition growth assays in wild-type (GFP) and *ΔFIRRM* (mCherry) HAP1 cells treated with different DNA damaging agents for 8 days. Mitomycin C (MMC), acetaldehyde (ACT), actinomycin D (Act D). (**C**-**K)** Clonogenic survival of wild-type or *FIRRM* knockout HAP1 cell lines in response to different DNA damaging agents for 8 days. Representative scans of three independent replicates are displayed.

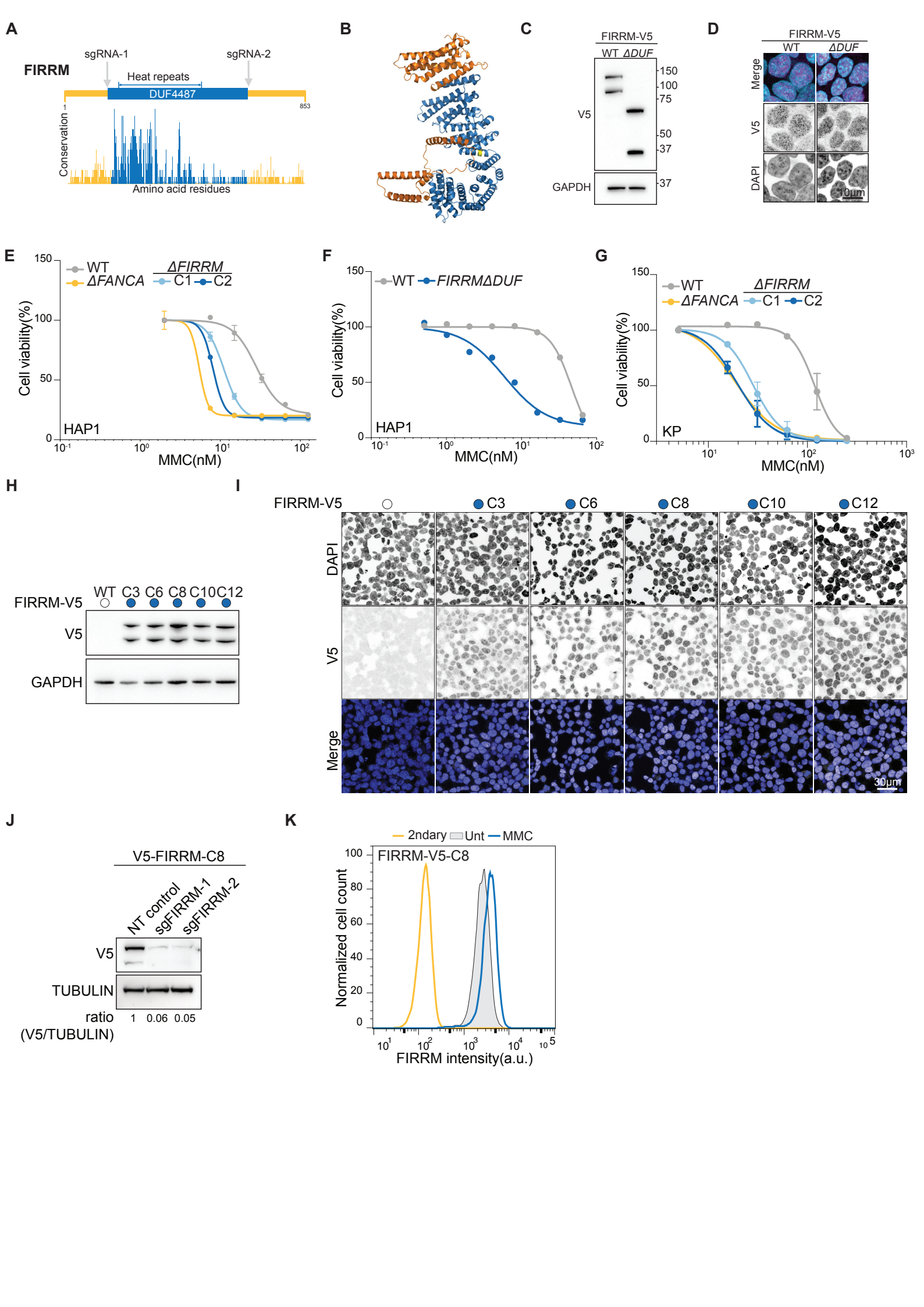

A
FIRRM
sgRNA-1
sgRNA-2
Heat repeats
DUF4487
1
853
Conservation
Amino acid residues
B
C
FIRRM-V5
WT ΔDUF
V5
GAPDH
150
100
75
50
37
37
D
FIRRM-V5
WT
ΔDUF
Merge
V5
DAPI
10μm
E
WT
ΔFANCA
ΔFIRRM
C1
C2
Cell viability(%)
HAP1
MMC(nM)
F
WT
FIRRMΔDUF
Cell viability(%)
HAP1
MMC(nM)
G
WT
ΔFANCA
ΔFIRRM
C1
C2
Cell viability(%)
KP
MMC(nM)
H
FIRRM-V5
WT C3 C6 C8 C10 C12
V5
GAPDH
I
FIRRM-V5
C3
C6
C8
C10
C12
DAPI
V5
Merge
30μm
J
V5-FIRRM-C8
NT control
sgFIRRM-1
sgFIRRM-2
V5
TUBULIN
ratio (V5/TUBULIN)
1
0.06
0.05
K
2ndary
Unt
MMC
FIRRM-V5-C8
Normalized cell count
FIRRM intensity(a.u.)

Fig. S5. FIRRM deficient cells are exquisitely sensitive to MMC. (**A**) Schematic depiction of the FIRRM domains and the degree of conservation. Arrows indicate the positions of the sgRNAs used to generate *FIRRMΔDUF* HAP1 cells. (**B**) Predicted structure of FIRRM using Alphafold2. N and C terminus is colored in orange, DUF domain is marked in blue. (**C**) Immunoblot depicting full length V5-tagged FIRRM and FIRRMΔDUF cells. (**D**) Immunofluorescent staining determining the localization of full-length and FIRRMΔDUF protein in HAP1 cells. (**E**) Quantification of clonogenic survival assays in HAP1 cells depicted in Fig. 1E. Data are mean ± s.e.m, normalized to untreated, *n=3*. (**F**) Quantification of a colony formation assay in HAP1 cells carrying an endogenous V5-tag at the C-terminus of FIRRM (WT) or HAP1 cells lacking the DUF of FIRRM exposed to indicated concentrations of MMC for 8 days. Data are normalized to untreated and a representative of three biological replicates is shown. (**G**) Quantification of clonogenic survival assays in KP cells depicted in Fig. 1F. Data are mean ± s.e.m, normalized to untreated, *n=3*. (**H**, **I**) Analysis of different clones with an endogenous V5-tag at the C-terminus of FIRRM in HAP1 cells. Clones were analyzed by immunoblotting (H) and immunofluorescence (I). (**J**) Immunoblotting using a V5 antibody against FIRRM-V5 in FIRRM-V5 tagged HAP1 cells infected with lentiviral vectors containing a non-targeting (NT) or a *FIRRM*-targeting sgRNA. TUBULIN is used as a loading control. (**K**) HAP1 cells with an endogenous V5-tag at the C-terminus of FIRRM were infected with a lentiviral vector containing a non-targeting sgRNA. These cells were then left untreated or treated with 50nM MMC before FACS analysis for FIRRM-V5 intensity was performed. This serves as a control for Fig.1G.

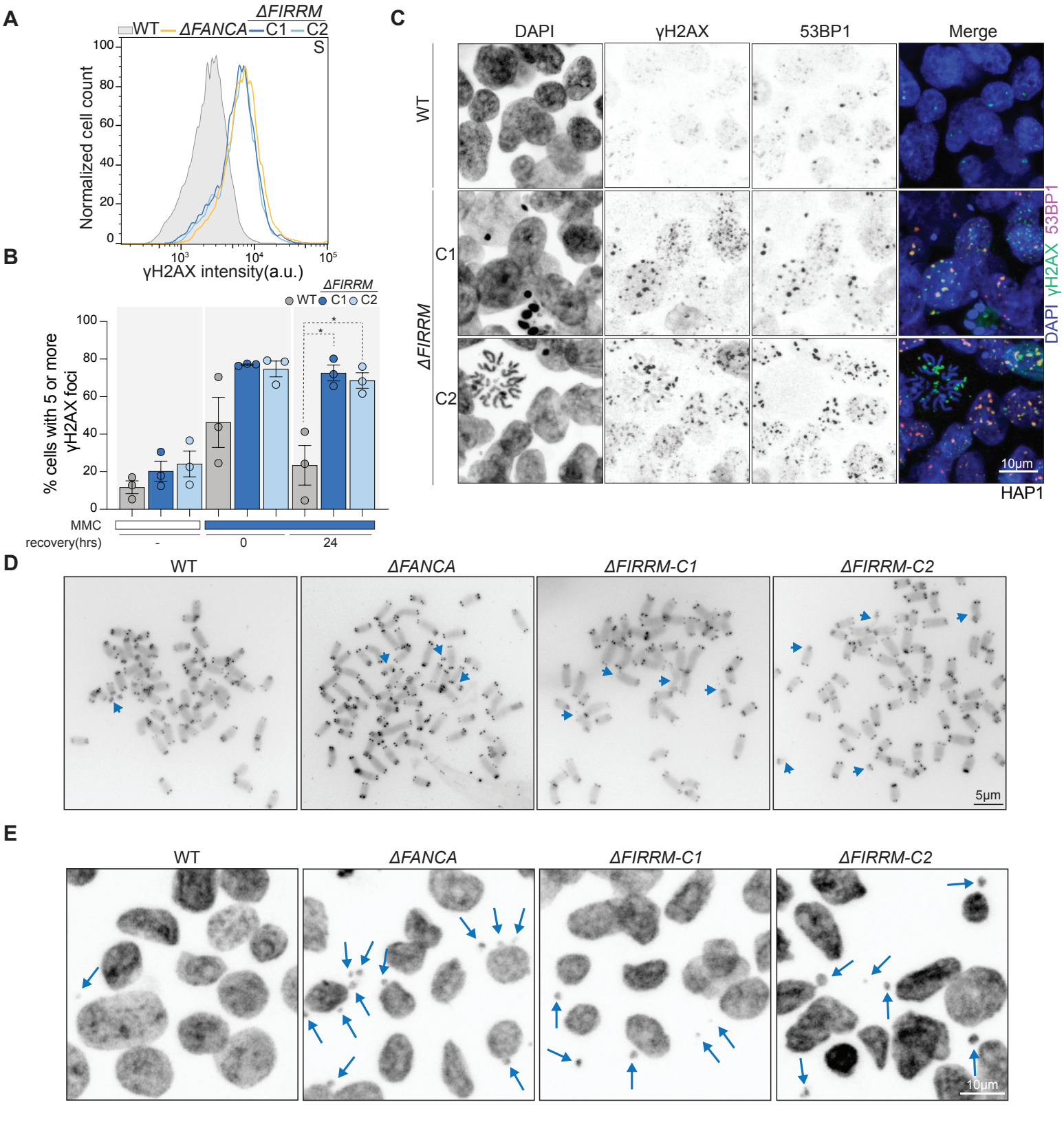

A
ΔFIRRM
WT ΔFANCA C1 C2
S
Normalized cell count
γH2AX intensity(a.u.)
B
ΔFIRRM
WT C1 C2
% cells with 5 or more γH2AX foci
MMC
recovery(hrs)
C
DAPI
γH2AX
53BP1
Merge
WT
ΔFIRRM
C1
C2
DAPI γH2AX 53BP1
10μm
HAP1
D
WT
ΔFANCA
ΔFIRRM-C1
ΔFIRRM-C2
5μm
E
WT
ΔFANCA
ΔFIRRM-C1
ΔFIRRM-C2
10μm

Fig. S6. *FIRRM* knockout cells display elevated levels of γH2AX. (**A**) γH2AX intensities of S phase cells derived from Fig. 2B. (**B**, **C**) Quantification (B) of γH2AX foci in wild-type or *FIRRM* knockout HAP1 cells treated with MMC and recovered for the indicated time points. Values represent percentage of cells with 5 or more foci (mean of 3 biologically independent experiments). Representative images (C) depict cells at the 24 hour recovery time point. At least 200 cells were analyzed per condition in every experiment. *p* value was calculated by a two-tailed *t*-test, * $p<0.05$. (**D**) Representative images of KP cells of indicated genotypes treated with 100nM MMC for 24 hours. Metaphase chromosomes were stained with DAPI and a telomeric FISH probe. Arrows depict chromosomal aberrations detected in individual spreads. At least 30 metaphase spreads were analyzed per condition in a blinded fashion. Quantification is depicted in Fig. 2C. (**E**) Images show micronuclei formation in HAP1 cells treated with 50nM MMC for 24 hours and released for an additional 24 hours. Quantification is presented in Fig. 2D. (**D**, **E**) Images are representative for three biological replicates.

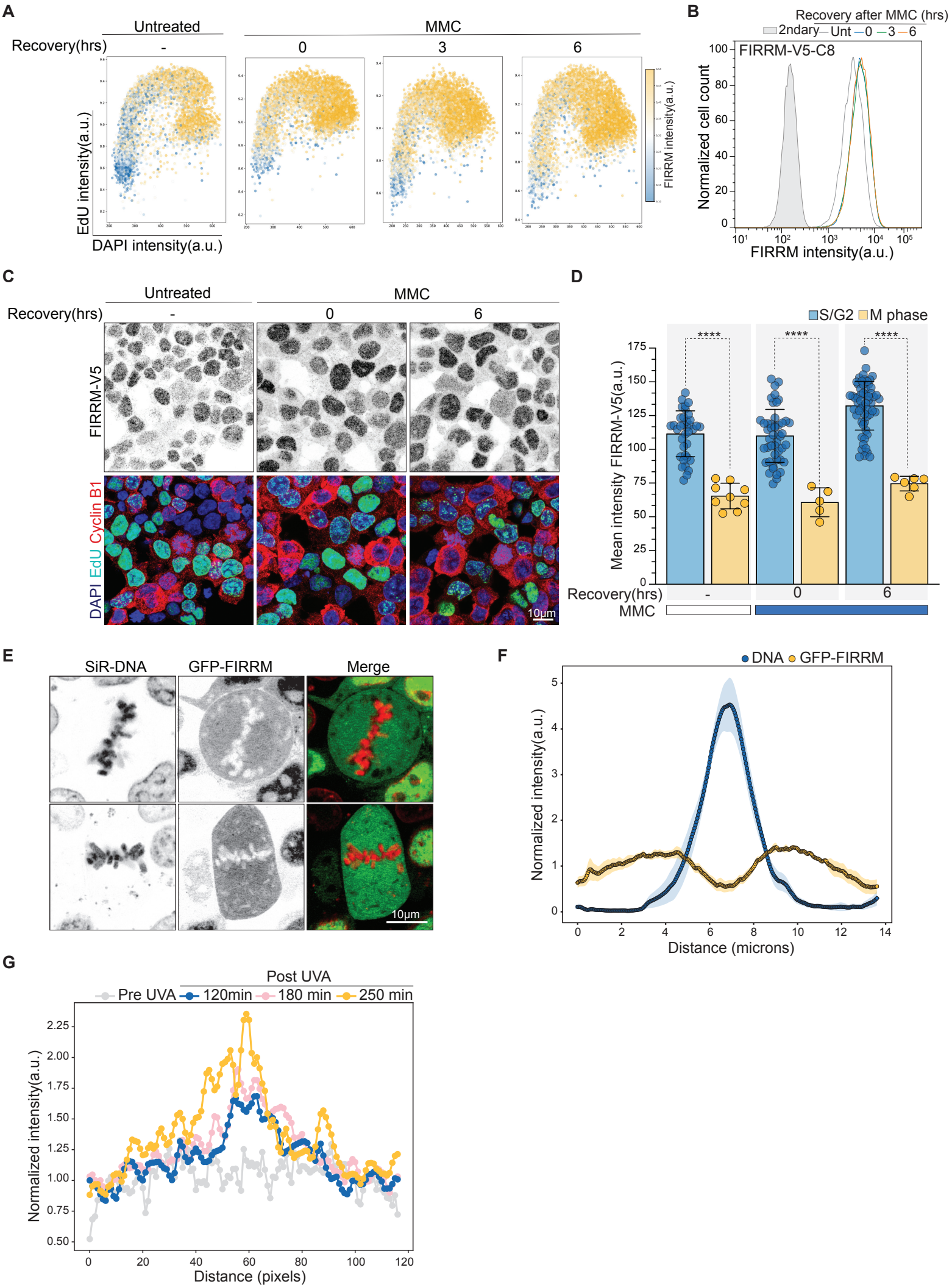

A
Untreated
MMC
Recovery(hrs)
-
0
3
6
EdU intensity(a.u.)
DAPI intensity(a.u.)
FIRRM intensity(a.u.)
B
Recovery after MMC (hrs)
2ndary
Unt
0
3
6
FIRRM-V5-C8
Normalized cell count
FIRRM intensity(a.u.)
C
Untreated
MMC
Recovery(hrs)
-
0
6
FIRRM-V5
DAPI EdU Cyclin B1
10μm
D
S/G2
M phase
Mean intensity FIRRM-V5(a.u.)
****
Recovery(hrs)
MMC
E
SiR-DNA
GFP-FIRRM
Merge
10μm
F
DNA
GFP-FIRRM
Normalized intensity(a.u.)
Distance (microns)
G
Post UVA
Pre UVA
120min
180 min
250 min
Normalized intensity(a.u.)
Distance (pixels)

Fig. S7. FIRRM expression and localization is cell-cycle regulated. (**A**, **B**) Wild-type HAP1 cells with an endogenous V5-tag at the C-terminus of FIRRM were treated with 50nM MMC for 24 hours and left to recover for indicated times before FACS analysis for EdU, DAPI and FIRRM-V5 intensities was performed. FIRRM intensity during S phase is plotted in (B). (**C**, **D**) Representative images (C) and quantification (D) of FIRRM-V5 cells that were left untreated or exposed to 50nM MMC for 24 hours. After 0 or 6 hours of recovery, cells were stained for DAPI, EdU, Cyclin B1 and V5-FIRRM. Data are depicted as mean ± s.d. significance was calculated by a two-tailed t-test. **** $p<0.0001$. (**E**, **F**) Representative images (E) and quantification (F, n=8) of GFP-FIRRM localization to mitotic cells. DNA is labelled by SiR-DNA (red). Data are depicted as mean ± sem. (**G**) Quantification of GFP-FIRRM recruitment to laser UVA micro-irradiation sites after psoralen treatment at the indicated time points in HAP1 cells, shown in Fig. 2F. The intensities are measured by drawing a line across the cells.

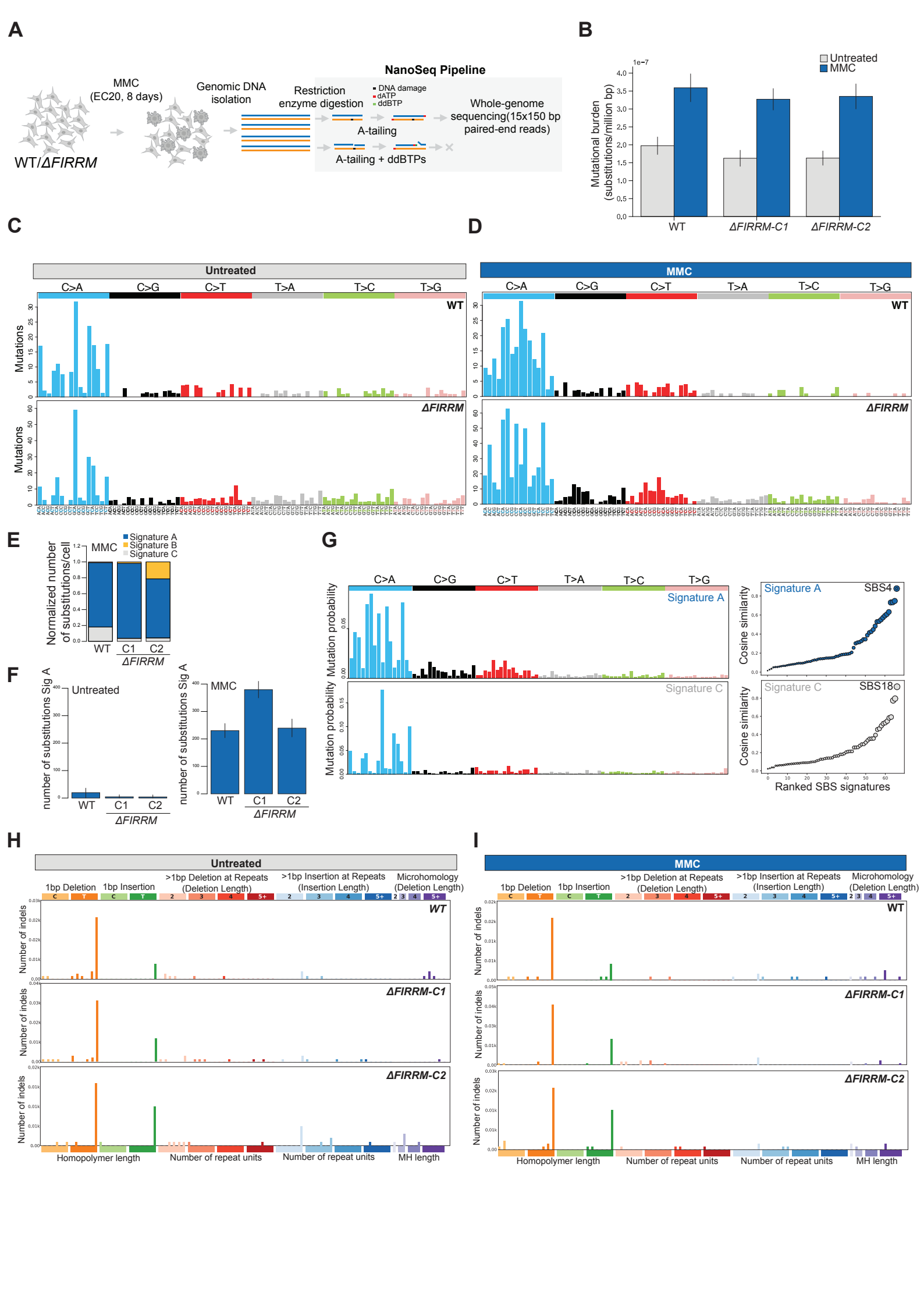

A
MMC
(EC20, 8 days)
WT/ΔFIRRM
Genomic DNA
isolation
NanoSeq Pipeline
Restriction
enzyme digestion
DNA damage
dATP
ddBTP
A-tailing
A-tailing + ddBTPs
Whole-genome
sequencing(15x150 bp
paired-end reads)
B
Untreated
MMC
Mutational burden
(substitutions/million bp)
1e−7
WT
ΔFIRRM-C1
ΔFIRRM-C2
C
D
Untreated
MMC
C>A
C>G
C>T
T>A
T>C
T>G
WT
ΔFIRRM
Mutations
E
Normalized number
of substitutions/cell
MMC
Signature A
Signature B
Signature C
WT
C1
C2
ΔFIRRM
F
Untreated
MMC
number of substitutions Sig A
G
Mutation probability
Signature A
Signature C
Cosine similarity
SBS4
SBS18
Ranked SBS signatures
H
I
Untreated
MMC
1bp Deletion
1bp Insertion
>1bp Deletion at Repeats
(Deletion Length)
>1bp Insertion at Repeats
(Insertion Length)
Microhomology
(Deletion Length)
WT
ΔFIRRM-C1
ΔFIRRM-C2
Number of indels
Homopolymer length
Number of repeat units
Number of repeat units
MH length

Fig. S8. Mutational profiles of *FIRRM* knockout cells. (**A**) Schematic overview of the NanoSeq workflow to determine the mutational signatures of *ΔFIRRM* versus wild-type HAP1 cells. The detailed workflow is described in the Materials and Methods section. Briefly, HAP1 cells of indicated genotypes were passaged in the presence of an EC20 dose of MMC for 8 days or left untreated. Subsequently, genomic DNA was isolated and libraries were prepared as previously described by Abascal et al. (27). (**B**) Estimated mutational burden based on the number of substitutions detected in untreated and MMC-treated HAP1 cell lines. (**C, D**) Substitution profiles for wild-type and *ΔFIRRM* cells in unchallenged (C) or MMC-treated conditions (D). (**E**) Contribution of signatures in wild-type and *ΔFIRRM* cells upon 50nM MMC treatment for 7 days. (**F**) Contribution of MMC-related signature A in unchallenged or MMC treated conditions. (**G**) Signature decomposition of signatures A and C and cosine similarities comparing the identified patterns with existing signatures. (**H, I**) Indel spectra of WT and *ΔFIRRM* HAP1 cells in unchallenged conditions (H) or upon MMC treatment (I).

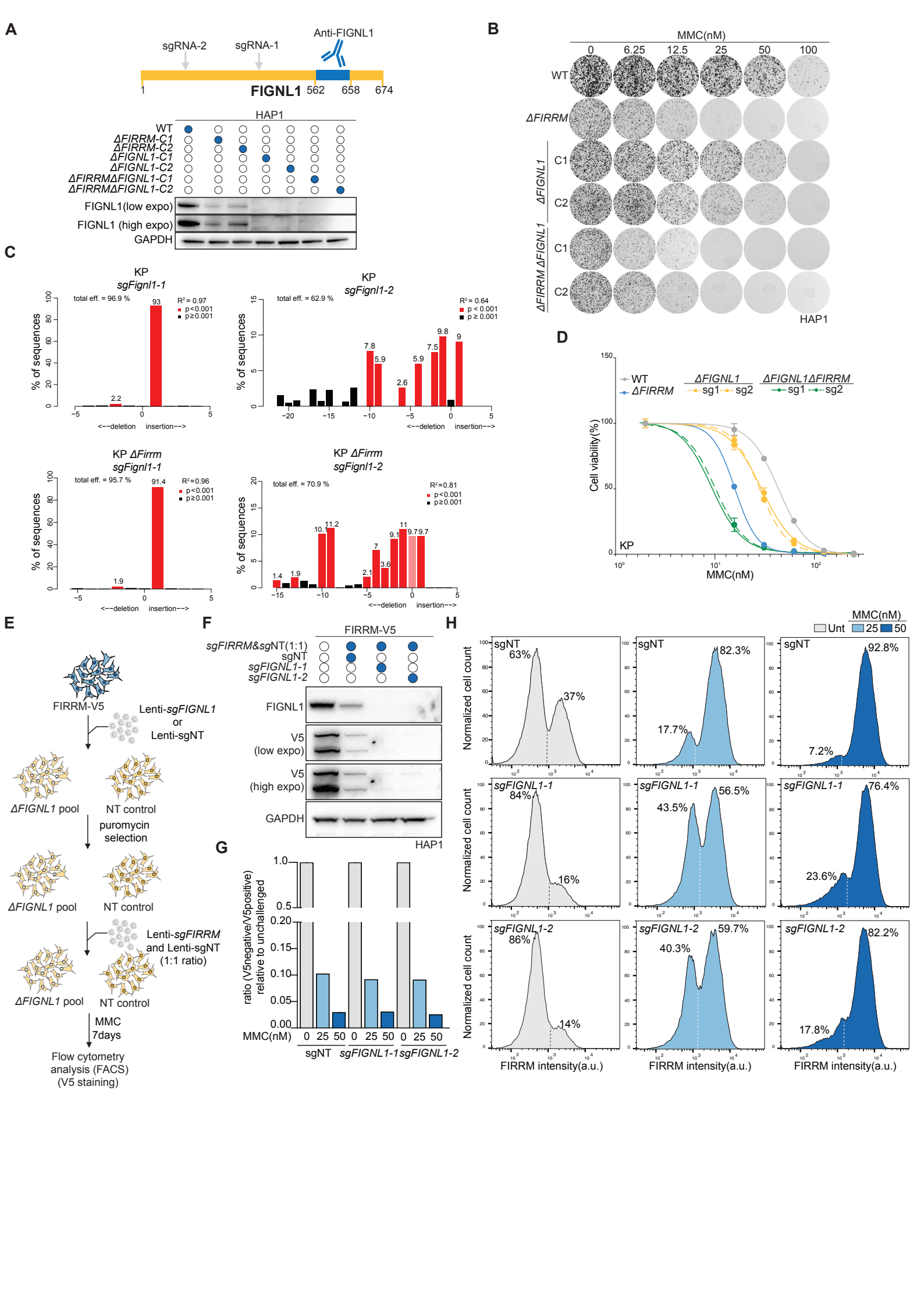
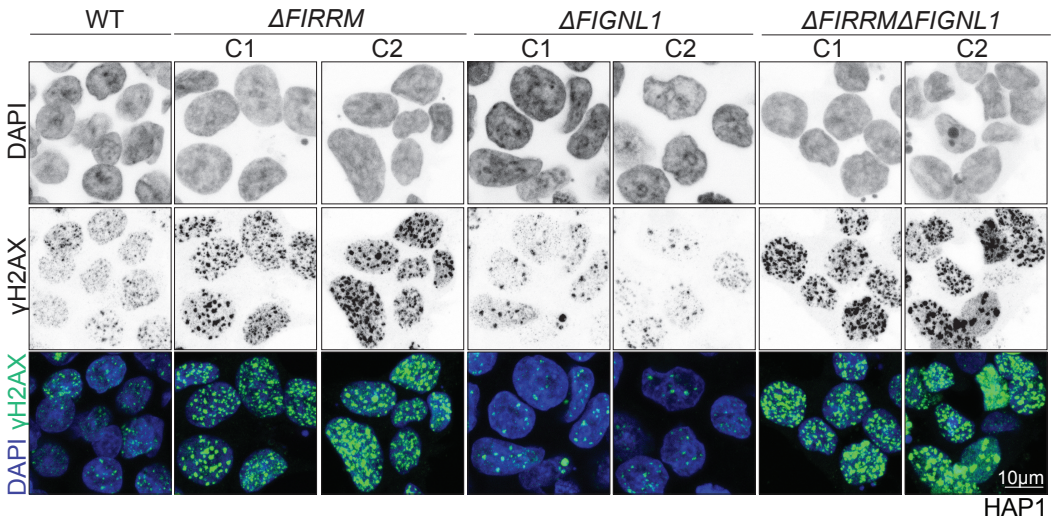

A
sgRNA-2
sgRNA-1
Anti-FIGNL1
1
FIGNL1
562
658
674
HAP1
WT
ΔFIRRM-C1
ΔFIRRM-C2
ΔFIGNL1-C1
ΔFIGNL1-C2
ΔFIRRMΔFIGNL1-C1
ΔFIRRMΔFIGNL1-C2
FIGNL1(low expo)
FIGNL1 (high expo)
GAPDH
B
MMC(nM)
0
6.25
12.5
25
50
100
WT
ΔFIRRM
ΔFIGNL1
C1
C2
ΔFIRRM ΔFIGNL1
C1
C2
HAP1
C
KP
sgFignl1-1
total eff. = 96.9 %
R² = 0.97
p<0.001
p≥0.001
% of sequences
<--deletion
insertion-->
KP
sgFignl1-2
total eff. = 62.9 %
R² = 0.64
p < 0.001
p ≥ 0.001
KP ΔFirrm
sgFignl1-1
total eff. = 95.7 %
R²=0.96
KP ΔFirrm
sgFignl1-2
total eff. = 70.9 %
R²=0.81
D
WT
ΔFIRRM
ΔFIGNL1
sg1
sg2
ΔFIGNL1ΔFIRRM
sg1
sg2
Cell viability(%)
KP
MMC(nM)
E
FIRRM-V5
Lenti-sgFIGNL1
or
Lenti-sgNT
ΔFIGNL1 pool
NT control
puromycin
selection
ΔFIGNL1 pool
NT control
Lenti-sgFIRRM
and Lenti-sgNT
(1:1 ratio)
ΔFIGNL1 pool
NT control
MMC
7days
Flow cytometry
analysis (FACS)
(V5 staining)
F
FIRRM-V5
sgFIRRM&sgNT(1:1)
sgNT
sgFIGNL1-1
sgFIGNL1-2
FIGNL1
V5
(low expo)
V5
(high expo)
GAPDH
HAP1
G
ratio (V5negative/V5positive)
relative to unchallenged
MMC(nM)
0
25
50
sgNT
sgFIGNL1-1
sgFIGNL1-2
H
MMC(nM)
Unt
25
50
Normalized cell count
sgNT
63%
37%
82.3%
17.7%
92.8%
7.2%
sgFIGNL1-1
84%
16%
56.5%
43.5%
76.4%
23.6%
sgFIGNL1-2
86%
14%
59.7%
40.3%
82.2%
17.8%
FIRRM intensity(a.u.)

Fig. S9. FIRRM functions independently of FIGNL1 during ICL repair. (**A**) Schematic depiction of the sgRNAs and antibody targeting sites in FIGNL1 (top panel). Western blot in HAP1 cells of indicated genotypes to assess FIGNL1 protein levels. GAPDH was used as a loading control (bottom panel). (**B**) Clonogenic survival assay in response to different concentrations of MMC for wild-type, *FIRRM*, *FIGNL1* single or double knockouts in HAP1 cells. Quantification is shown in Fig. 4A. (**C**) Analysis of insertions and deletions in KP wildtype or *FIRRM* knockout cells transduced with non-targeting or sgRNAs targeting *Fignl1* using the Tracking of Indels by DEcomposition (TIDE) algorithm (44) . (**D**) Quantification of a colony formation assay performed in KP cells of indicated genotypes after various concentrations of MMC (Fig. 4B). Data are normalized to untreated, mean ± s.e.m, *n=2* (**E**) Schematic representation of the experiment performed in fig. S9F to H. Briefly, FIRRM-V5-tagged cells were transduced with sgRNAs targeting FIGNL1 or a non-targeting (NT) control guide. After antibiotic selection, cells were transduced with a 1:1 mix of NT and *FIRRM* sgRNAs, exposed to indicated concentrations of MMC for 7 days and FIRRM-V5 levels were analyzed using flow cytometry. (**F**) Western blot of the cells generated in (E). (**G** to **H**) Quantification and representative FACS plots of the experiment described in (E), *n=2.*

A


WT
ΔFIRRM
ΔFIGNL1
ΔFIRRMΔFIGNL1
C1
C2
C1
C2
C1
C2
DAPI
γH2AX
DAPI γH2AX
10μm
HAP1

B

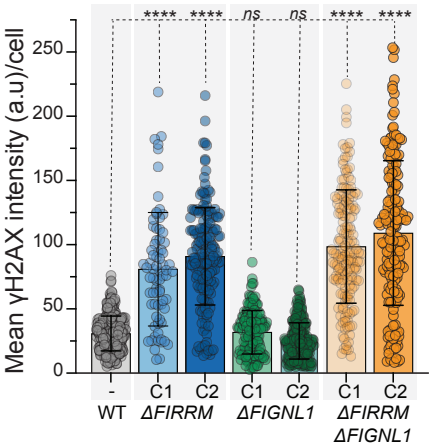

Mean γH2AX intensity (a.u)/cell
0
50
100
150
200
250
****
****
ns
ns
****
****
-
C1
C2
C1
C2
C1
C2
WT
ΔFIRRM
ΔFIGNL1
ΔFIRRM
ΔFIGNL1

Fig. S10: Loss of FIGNL1 does not increase DNA damage upon MMC exposure. (**A, B**) Representative pictures (A) and quantification (B) of HAP1 wild type, two FIRRM knockout clones, two FIGNL1 knockout clones and two double knockout clones. Cells were treated with 50nM MMC for 24 hours, recovered for an additional 24 hours and were fixed and stained for γH2AX and DAPI. Data are depicted as mean ± s.d. significance was calculated by a two-tailed t-test**.** ns not significant, **** $p<0.0001$**.**

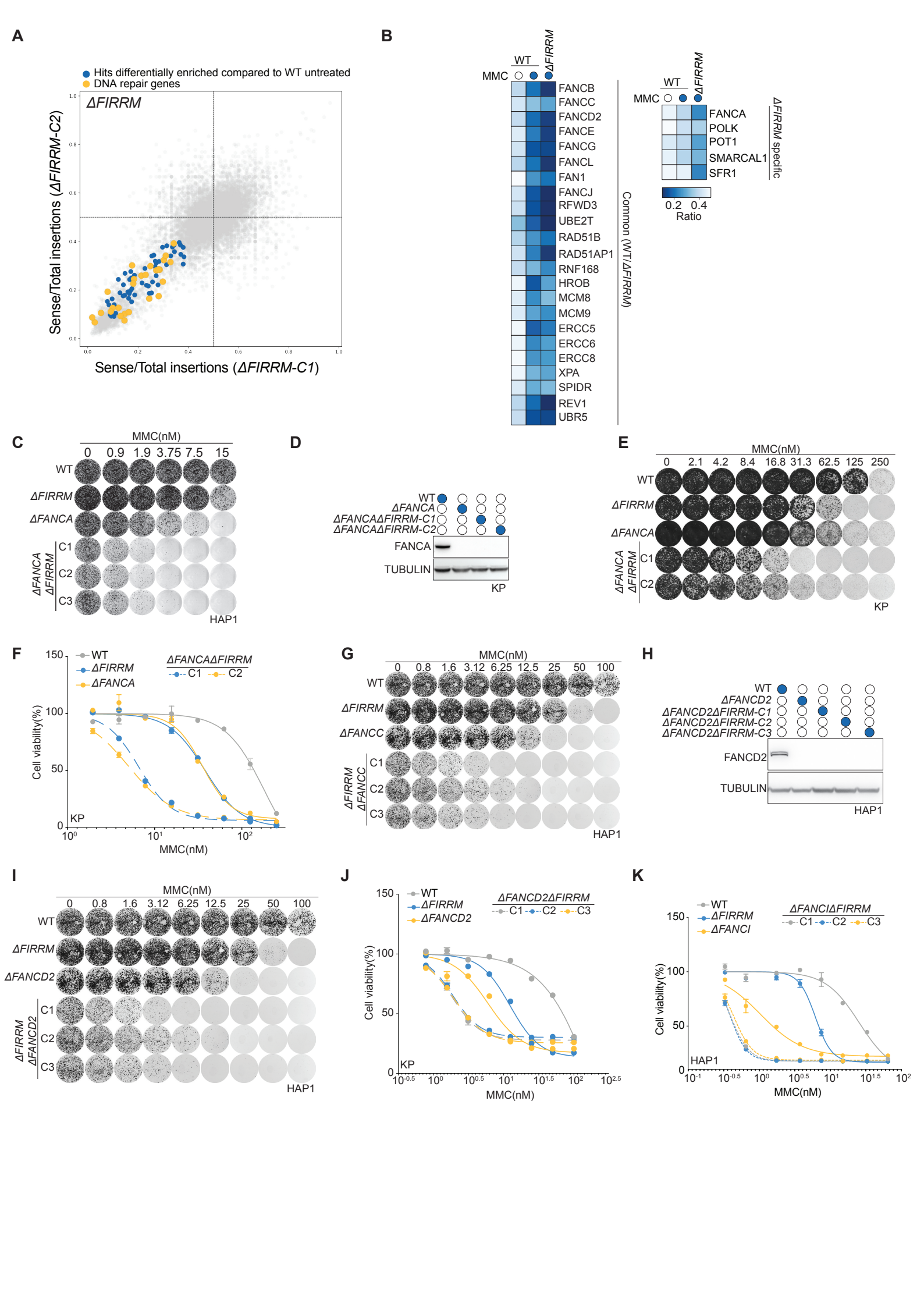

A
Hits differentially enriched compared to WT untreated
DNA repair genes
ΔFIRRM
Sense/Total insertions (ΔFIRRM-C2)
Sense/Total insertions (ΔFIRRM-C1)
B
WT
ΔFIRRM
MMC
FANCB
FANCC
FANCD2
FANCE
FANCG
FANCL
FAN1
FANCJ
RFWD3
UBE2T
RAD51B
RAD51AP1
RNF168
HROB
MCM8
MCM9
ERCC5
ERCC6
ERCC8
XPA
SPIDR
REV1
UBR5
Common (WT/ΔFIRRM)
FANCA
POLK
POT1
SMARCAL1
SFR1
ΔFIRRM specific
0.2 0.4
Ratio
C
MMC(nM)
0 0.9 1.9 3.75 7.5 15
WT
ΔFIRRM
ΔFANCA
ΔFANCA ΔFIRRM
C1
C2
C3
HAP1
D
WT
ΔFANCA
ΔFANCAΔFIRRM-C1
ΔFANCAΔFIRRM-C2
FANCA
TUBULIN
KP
E
MMC(nM)
0 2.1 4.2 8.4 16.8 31.3 62.5 125 250
WT
ΔFIRRM
ΔFANCA
ΔFANCA ΔFIRRM
C1
C2
KP
F
WT
ΔFIRRM
ΔFANCA
ΔFANCAΔFIRRM
C1
C2
Cell viability(%)
KP
MMC(nM)
G
MMC(nM)
0 0.8 1.6 3.12 6.25 12.5 25 50 100
WT
ΔFIRRM
ΔFANCC
ΔFIRRM ΔFANCC
C1
C2
C3
HAP1
H
WT
ΔFANCD2
ΔFANCD2ΔFIRRM-C1
ΔFANCD2ΔFIRRM-C2
ΔFANCD2ΔFIRRM-C3
FANCD2
TUBULIN
HAP1
I
MMC(nM)
0 0.8 1.6 3.12 6.25 12.5 25 50 100
WT
ΔFIRRM
ΔFANCD2
ΔFIRRM ΔFANCD2
C1
C2
C3
HAP1
J
WT
ΔFIRRM
ΔFANCD2
ΔFANCD2ΔFIRRM
C1
C2
C3
Cell viability(%)
KP
MMC(nM)
K
WT
ΔFIRRM
ΔFANCI
ΔFANCIΔFIRRM
C1
C2
C3
Cell viability(%)
HAP1
MMC(nM)

Fig. S11. Loss of the FA pathway further exacerbates MMC sensitivity of FIRRM knockout cells. (**A**) Scatter plot of the haploid genetic screen depicting the ratios (sense/total insertions) for each gene upon MMC treatment in two independent *FIRRM* knockout HAP1 clones. Genes that are differentially regulated compared to the wild-type untreated are depicted in dark blue and the DNA repair genes are highlighted in orange. (**B**) Heatmap showing the DNA repair genes that were identified in both, WT and *FIRRM* knockout cells and only in *FIRRM* knockout cells. (**C**) Clonogenic survival of HAP1 cells of indicated genotypes, quantification displayed in Fig. 5B. (**D**) Immunoblots confirming the loss of *FANCA* in single or *FIRRM-FANCA* double knockouts in KP cells. (**E**, **F**) Clonogenic survival and quantification of KP cells of indicated genotypes exposed to different doses of MMC. (**G**) Colony formation assay in *FANCC*, *FIRRM* single or double knockout HAP1 cells. Quantification is depicted in Fig. 5C. (**H**) Immunoblot confirming the loss of FANCD2 in single and *FIRRM-FANCD2* double knockouts in HAP1 cells. (**I**) Clonogenic survival of HAP1 cells of indicated genotypes, quantification displayed in Fig 5D. (**J**) Quantification of KP cells depleted for *FANCD2* and/or *FIRRM* and exposed to various concentrations of MMC. (**K**) Clonogenic survival in HAP1 cells of indicated genotypes. All data are depicted as mean ± s.e.m, normalized to untreated conditions. All images are representative of at least three independent biological replicates.

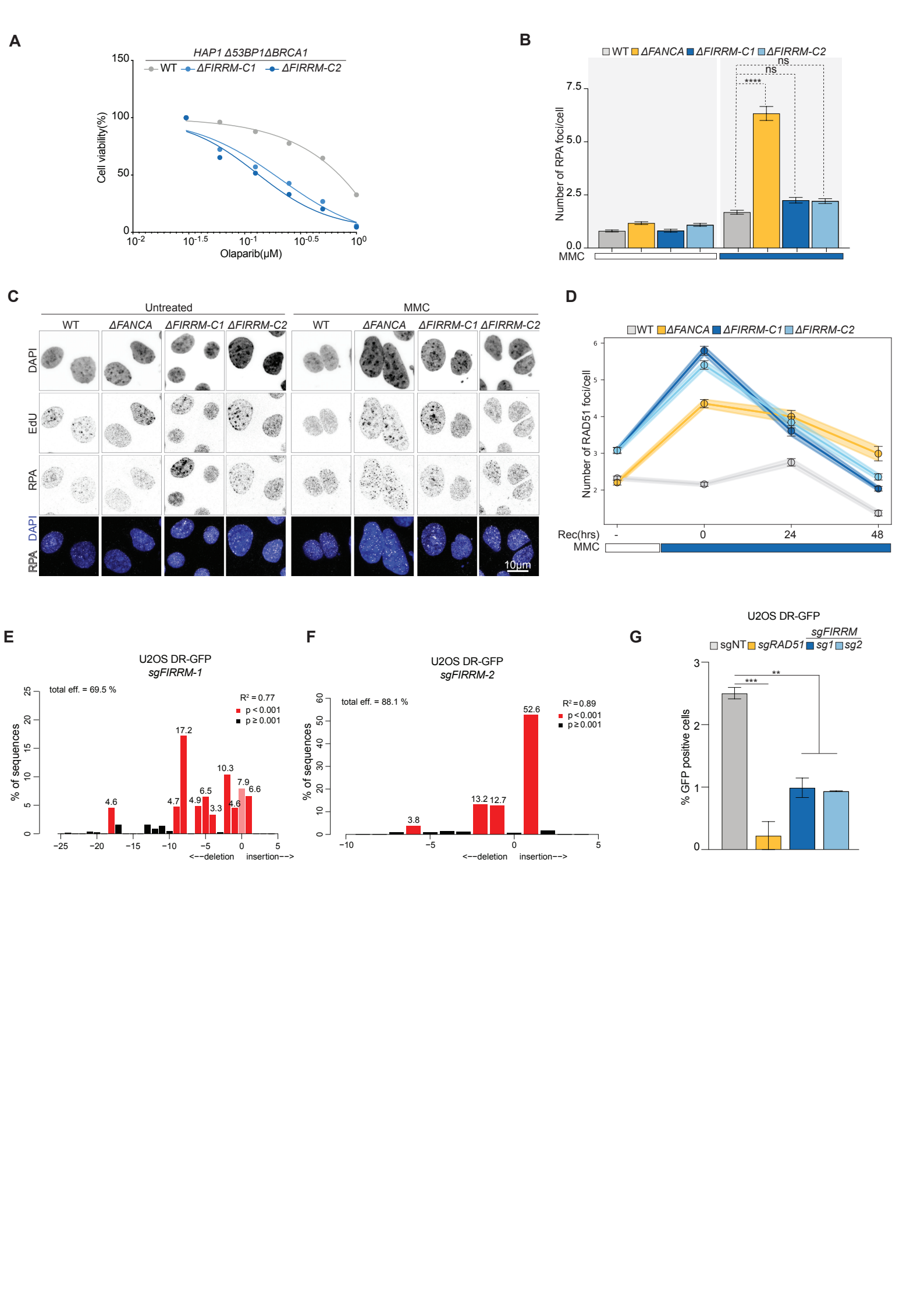

A
HAP1 Δ53BP1ΔBRCA1
WT ΔFIRRM-C1 ΔFIRRM-C2
Cell viability(%)
Olaparib(μM)
B
WT ΔFANCA ΔFIRRM-C1 ΔFIRRM-C2
Number of RPA foci/cell
ns
ns
****
MMC
C
Untreated
MMC
WT ΔFANCA ΔFIRRM-C1 ΔFIRRM-C2
DAPI
EdU
RPA
DAPI
RPA
10μm
D
WT ΔFANCA ΔFIRRM-C1 ΔFIRRM-C2
Number of RAD51 foci/cell
Rec(hrs)
MMC
E
U2OS DR-GFP
sgFIRRM-1
total eff. = 69.5 %
R² = 0.77
p < 0.001
p ≥ 0.001
% of sequences
<--deletion insertion-->
F
U2OS DR-GFP
sgFIRRM-2
total eff. = 88.1 %
R² = 0.89
p < 0.001
p ≥ 0.001
% of sequences
<--deletion insertion-->
G
U2OS DR-GFP
sgFIRRM
sgNT sgRAD51 sg1 sg2
% GFP positive cells

Fig. S12. Loss of FIRRM does not impair initial steps of the HR pathway. (**A**) *Δ53BP1 ΔBRCA1* HAP1 cells were exposed to indicated concentrations of olaparib and survival was quantified after 8 days. (**B**) Quantification of RPA foci in unchallenged conditions or after 24 hours of MMC treatment in KP cells. Data are displayed as mean ± s.e.m. *p* value was calculated by the Mann-Whitney U test. **** $p<0.0001$, ns not significant. *n=3* (**C**) Representative images of KP cells of indicated genotypes unchallenged or treated with 100nM MMC and stained for RPA, EdU and DAPI. (**D**) Quantification of an immunofluorescent staining for RAD51 in HAP1 cells after treatment with 50nM MMC and subsequent recovery of indicated times. Data are displayed as mean ± s.e.m. n=2 (**E**, **F**) Analysis of insertions and deletions in U2OS DR-GFP cells transduced with sgRNAs targeting *FIRRM* using the TIDE algorithm (44). (**G**) U2OS cells carrying the DR-GFP reporter construct were transduced with a non-targeting sgRNA or sgRNAs targeting *RAD51* or *FIRRM* and HR efficiency was assessed by flow cytometry. Data are depicted as mean ± s.e.m. and significance was calculated by a two-tailed t-test, ** $p<0.01$, *** $p<0.001$, *n=2*.

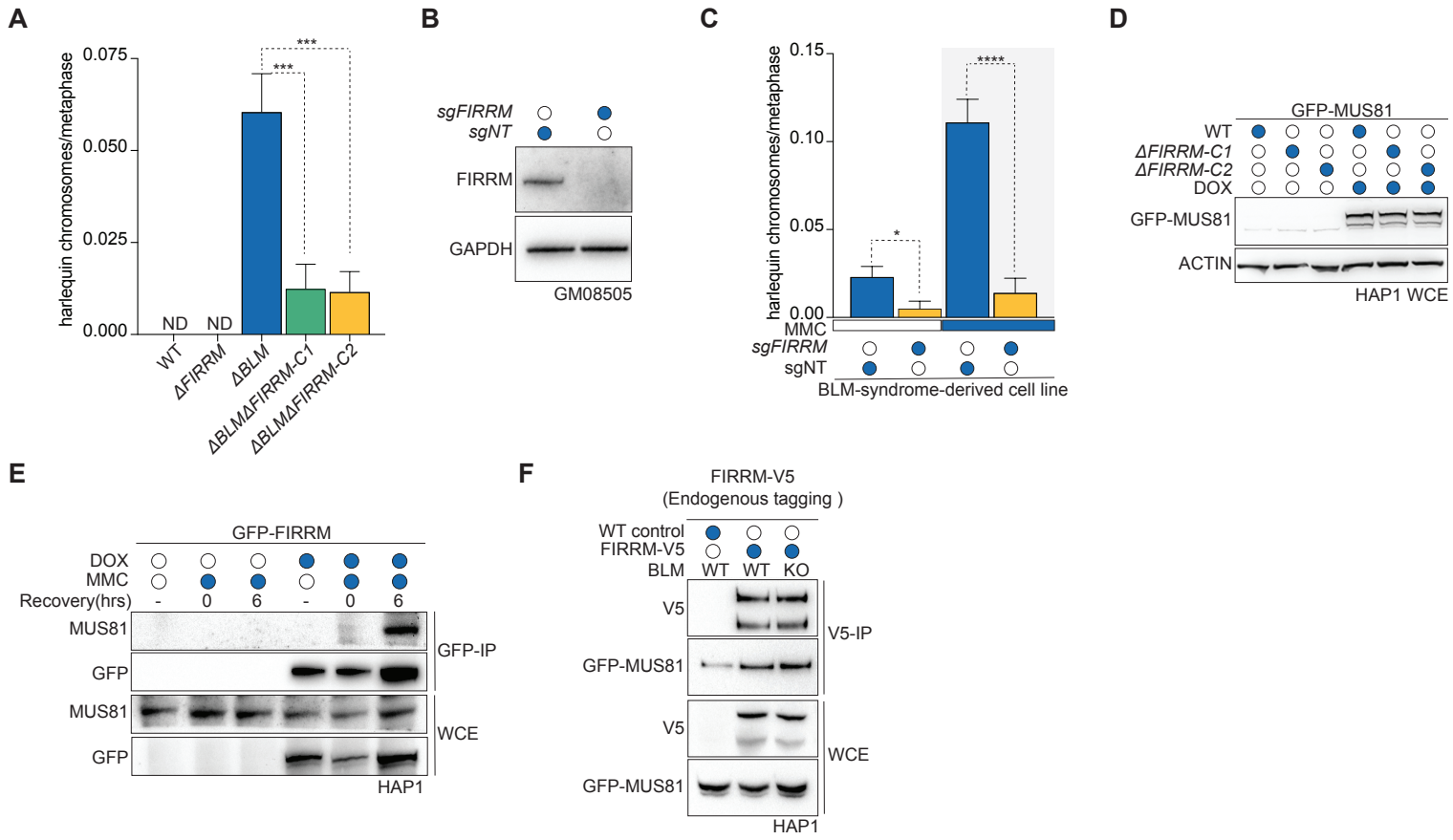

A
harlequin chromosomes/metaphase
0.075
0.050
0.025
0.000
***
***
ND
ND
WT
ΔFIRRM
ΔBLM
ΔBLMΔFIRRM-C1
ΔBLMΔFIRRM-C2
B
sgFIRRM
sgNT
FIRRM
GAPDH
GM08505
C
harlequin chromosomes/metaphase
0.15
0.10
0.05
0.00
****
*
MMC
sgFIRRM
sgNT
BLM-syndrome-derived cell line
D
GFP-MUS81
WT
ΔFIRRM-C1
ΔFIRRM-C2
DOX
GFP-MUS81
ACTIN
HAP1 WCE
E
GFP-FIRRM
DOX
MMC
Recovery(hrs)
- 0 6 - 0 6
MUS81
GFP
GFP-IP
MUS81
GFP
WCE
HAP1
F
FIRRM-V5
(Endogenous tagging)
WT control
FIRRM-V5
BLM WT WT KO
V5
GFP-MUS81
V5-IP
V5
GFP-MUS81
WCE
HAP1

Fig. S13. FIRRM depletion suppresses SCE formation. (**A**) Number of harlequin chromosomes per metaphase (>5 SCEs per chromosome) in HAP1 cells of indicated genotypes upon 50nM MMC treatment for 24 hours. Data are depicted as mean ± s.e.m., *p*-values were calculated using a two-tailed t-test. *** $p<0.001$, $n=3$ (**B**) Immunoblot confirming the depletion of FIRRM in GM08505 cells used in Fig. 6H and fig. S6C. (**C**) Number of harlequin chromosomes per metaphase in GM08505 cells transduced with the indicated sgRNAs and left untreated or exposed to 50nM MMC for 24 hours. Data are depicted as mean ± s.e.m., *p*-values were calculated using a two-tailed t-test. * $p<0.05$, **** $p<0.0001$, $n=3$ (**D**) Immunoblot of whole cell extracts (WCE) from wild-type or *FIRRM* knockout cells carrying doxycycline (DOX)-inducible GFP-tagged MUS81 used in Fig. 7E-G. (**E**) Doxycycline-inducible GFP-tagged FIRRM co-immunoprecipitation with endogenous MUS81 in untreated HAP1 cells or samples treated with 50nM MMC for 24 hours and allowed to recover for indicated times. (**F**) Co-immunoprecipitation of endogenously tagged FIRRM (C terminal V5 tag) with GFP-MUS81 in wild-type or BLM knockout cells treated with 50nM MMC for 24 hours and allowed to recover for an additional 6 hours.

A

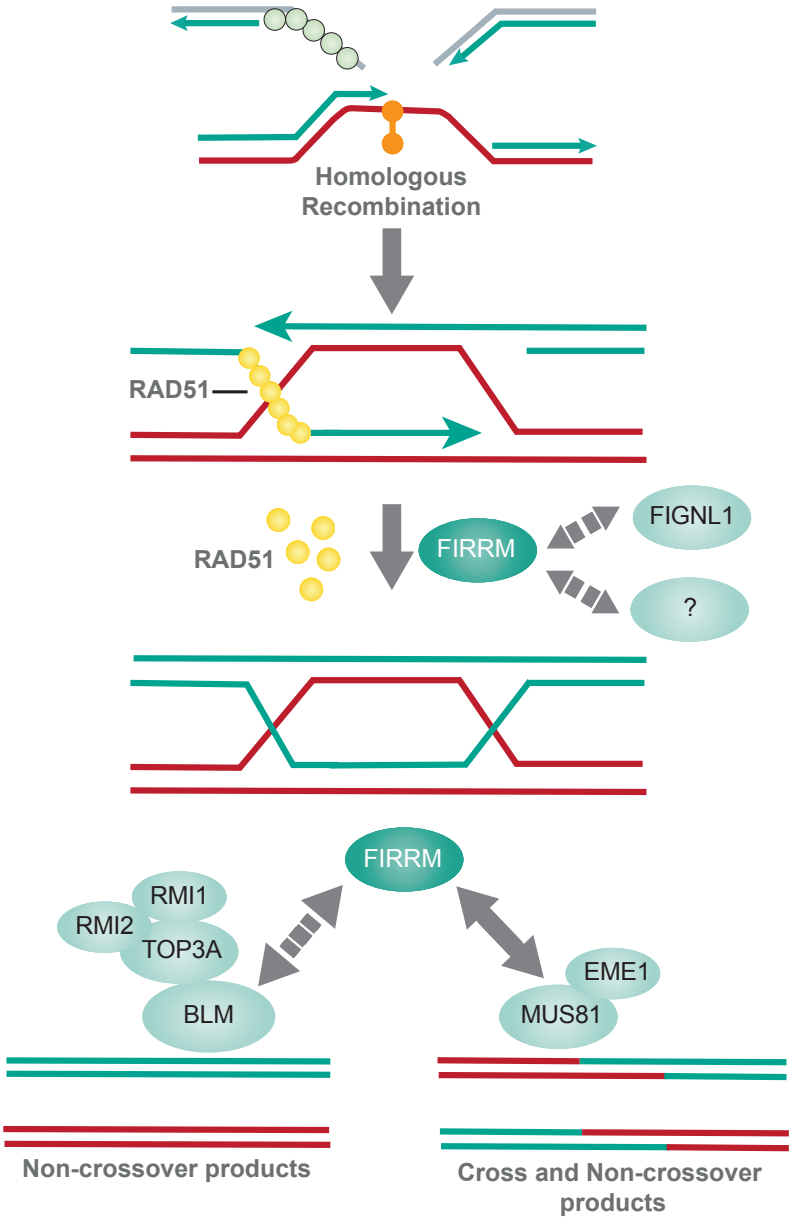

Homologous
Recombination
RAD51
RAD51
FIRRM
FIGNL1
?
FIRRM
RMI1
RMI2
TOP3A
BLM
EME1
MUS81
Non-crossover products
Cross and Non-crossover
products

Fig. S14. Proposed model for FIRRM functions in ICL response. We suggest that FIRRM is involved at two steps during the ICL response. After initial processing of the ICL, RAD51 filaments might be dismantled by FIRRM either by interacting with FIGNL1 and/or other unknown factors. Further downstream, FIRRM may then act to recruit structure-specific endonucleases, such as MUS81, to modulate HR intermediate processing.

Table S1. (separate file)
List of 171 genes that were identified to cause sensitization to both MMC and ACT upon their inactivation.

Table S2. (separate file)
List of the 44 genes identified by intersecting ICL survival genes (MMC and ACT) with the γH2AX negative regulators.

Table S3. Single guide RNAs used in this study

Target genes - human	Sequence (5'-3')
FIRRM guide 1	AACTGTATCTTCAGATACAC
FIRRM guide 2	AGGTCTCAGAAACGACAACC
FIRRM guide for V5-tag insert	AACGTTACATACATACTCTA
FIRRM ΔDUF-1	CAAGTATTGGCATGAAATGCA
FIRRM ΔDUF-2	AGAGTCCCAGAGGATCCTCAA
53BP1	CAGAATCATCCTCTAGAACC
BLM	CCAACACCACAAATCAGCAA
BRCA1	TGTTACAAATCACCCCTCA
FANCA	CGGGATGGTTGCCTCTAGCG
FANCC	GCCAACAGTTGACCAATTGT
FANCD2 guide 1	GGATTCCCAGCACGCTGATG
FANCD2 guide 2	GACAGAAGATGCCTCCAGTA
FANCI	GTATCCAGTTGGTGGAATCG
FIGNL1 guide 1	GAAGACCCTGATGCACGCTG
FIGNL1 guide 2	ATCAGTGGTAATCCATAAGG
MUS81	AGAACAGTCCAGCCCCGCAG
MUS81 for Nt-dTAG knock-in	AGGACCCGCCCTCGGGCTCA
RAD51	CTATAGCTTCCCATTGACCG

Target genes - mouse	Sequence (5'-3')
Firrm	GGAGCACAGAGGATATGACT
Fanca	CAGCTGCAGCAAGAAAACAC
Fancd2 guide 1	TGAGCGAACACATAAGCGAA
Fancd2 guide 2	ATTCCCAGCATGCTAATGTG
Fignl1 guide 1	TCTTCAGACAATCATCAGTG
Fignl1 guide 2	TAGTAGTGCACGGTATGCAT

Table S4. PCR primers used to verify editing of loci targeted by the guide RNAs.

Human targets

Target gene	forward	reverse
FIRRM guide 1	AGGGGTCACATTCAGGATTTT	TGCCATTGGATTTTATCACCTACT
FIRRM guide 2	AAGACCTGCCATGTGAACTG	GGACTACGGTGTTGGCAAGG
FIRRM guide 3	GTTCTGTGAAAAGAGGAAGAGACT	AGGATCCCTGTGTCTGTCTCA
FIRRM ΔDUF	CTCACCCCCATGACATTTGGA	AGAGTCCCAGAGGATCCTCAA
BLM	AAATTAGTTTTGTAGAGTTGGGGGG	TTGCTCGCTTTCAGAGGAGG
FANCA	GGCATTTTAAACAGCAAGTCTTTGG	ATACTGAGCAAACTCTAACAGGGAA
FANCC	ACTAAACAAGAAGCATTCACGTTCC	CAAACCTACACACACATACATGGAC
FANCI	CTTTTTCAAAGCCCTTAACCATTGC	CCCTCAACAAATTACAAACCCTCAA
FIGNL1 guide 1	TCTGGCACCATTGATGCACT	ATGTCTGGCCTCAACATGGG
FIGNL1 guide 2	TCTGACAATGTTGAATCTGGGT	GCAGCAGGAAAACAAGACTGG
MUS81	GCCCTTGGCTTTTAAGCTCC	TCACTTCTACCATGCGCTGC

Mouse targets

Target gene	forward	reverse
FIRRM guide 1	ACTTTTACACATAAACAAGAGAGCA	ATAGCAGCGGGGGATTACTT
Fanca	GCGGTGGGCAGTCTATTACA	TTGCTCGGCAGTTGTGACTT
Fancd2 guide 1	AGGAGCTAGGACCCGTGTT	CAAGCAGGGGCCTGTAGCAA
Fancd2 guide 2	ATAATGTTCTCCCTGCAGGACC	GTGTCCCAGCCTTTATGTTCC
Fignl1 guide 1	GGCCATCAATCCCAGCATACA	TCCCTCACCTACCCACTTAGA

Fignl1 guide 2	TTGACATGGAGACGTCCAGC	GCGTCTTCAGAACCTCCACA

Table S5. Antibodies used in this study

Antibody	Species	Company	Catalog number	Technique	Dilution
β-actin	mouse	Sigma	A5441	Western blot	1:2000
β-catenin	mouse	BD Biosciences	610154	Western blot	1:2000
β-tubulin	rabbit	Cell Signaling	2148S	Western blot	1:1000
γH2AX	mouse	Merck	05-636	Western blot IF FACS	1:1000 1:500 1:500
53BP1	rabbit	Bethyl	A300-272A	IF	1:1000
BLM	rabbit	abcam	ab2179	Western blot	1:1000
FIRRM	rabbit	Thermo Fisher	PA5-54884	Western blot	1:500
Cyclin B1	mouse	Santa Cruz	sc-245	IF	1:400
FANCA	rabbit	abcam	ab272392	Western blot	1:1000
FANCD2	rabbit	Novus	NB100-182	Western blot IF	1:1000 1:500
FIGNL1	rabbit	Atlas antibodies	HPA055542	Western blot	1:1000
GAPDH	mouse	GeneTex	GTX627408	Western blot	1:2000
GFP	mouse	Santa Cruz	sc-9996	Western blot	1:1000
GFP	goat	abcam	ab6673	Western blot	1:1000
HA	mouse	Biolegend	901513	Western blot	1:1000
H3	rabbit	Thermo Fisher	PA5-31954	Western blot	1:2000
MUS81	mouse	abcam	ab14387	Western blot	1:1000
PCNA	mouse	Santa Cruz	sc-56	Western blot	1:1000
RAD51	rabbit	abcam	ab133534	Western blot	1:1000
RAD51	mouse	Genetex	GTX70230	IF	1:250
RPA32	mouse	abcam	ab2175	IF	1:500
V5-Tag	mouse	ThermoFisher eBioscience™	14-6796-82	Western blot IF	1:1000 1:400
V5-Tag	rabbit	Cell Signaling	13202S	Western blot IF	1:1000 1:500

Table S6. Gene editing events in HAP1 knockout clones (sgRNAs are highlighted in bold).

Gene	Indel(bp)	Target sequence
FANCA (WT sequence)		TTCTCTCAGC**CGGGATGGTTGCCTCTAGCG**TGGGACAGAT
ΔFANCA	-7	TTCTCTCAGCCGGGATGGTTGC - - - - - -GTGGGACAGAT
ΔFIRRM ΔFANCA-C1	-11	TTCTCTCAGCCGGGATGGTTG - - - - - - - - - - GGACAGAT
ΔFIRRM ΔFANCA-C2	-7	TTCTCTCAGCCGGGATGGTTGC - - - - - - - GTGGGACAGAT
ΔFIRRM ΔFANCA-C3	-16	TTCTCTCAGCCGGGATGG - - - - - - - - - - - - - - - - ACAGAT
FANCC (WT sequence)		AAGATTCCCC**ACAATTGGTCAACTGTTGGC**AAAAGCTTGT
ΔFANCC	+1	AAGATTCCCC**ACAA**A**TTGGTCAACTGTTGGC**AAAAGCTTG T
ΔFIRRM ΔFANCC-C1	-2093, -53 in exon	AAGATTCCCCAC – (2093bp) - AGGGTCTTGGA
ΔFIRRM ΔFANCC-C2	+6, -23	AAGA**CAACAA** -- GCAAAAGCTTGT
ΔFIRRM ΔFANCC-C3	+29	AAGATTCCCCACA**TCAGAAAAAAGCCAAGACCCCATATCT CC**ATTGGTCAACTGTTGGCAAAAGCTTGT
FANCI (WT sequence)		CACTTGTT**GTATCCAGTTGGTGGAATCG**GGGGATTTGCAG
ΔFANCI	-13	CACTTGTTGTATCC - - - - - -- - - - - - - GGGGGATTTGCAG
ΔFIRRM ΔFANCI-C1	+158	CACTTGTTGTATCCAGTTGGTGGAA ATCGGAGGATTTGCAGAAAGAGTTACTGTCTGAGATCATAA TGATTACTGATGCTGCGAAGTAATATGTGCACCACTGGAATT TTTATATGATCATAGATTTTCGAGGGTTAGCTTTGGATTGAG GGAACGAGTTGTTAGACAGGCAGAATACAAAGATCGGGGG ATTTGCAG
ΔFIRRM ΔFANCI-C2	-7	CACTTGTTGTATCCAGTTGGTGG - - - - - - GGATTTGCAG

ΔFIRRM ΔFANCI-C3	-13	CACTTGTTGTAT - - - - - - - - - - - - - - - TCGGGGGATTTGCAG
FIGNL1 (WT sequence)		CCACCAACCT**CAGCGTGCATCAGGGTCTTC**ATATGGTGGT
ΔFIGNL1-1	-2	CCACCAACCTC - - CGTGCATCAGGGTCTTCATATGGTGGT
ΔFIGNL1-2	-5	CCACCAACC - - GTGCATCAGGGTCTTCATATGGT
ΔFIRRM ΔFIGNL1-C1	-13	CCACCA - - - - - - - - - - - - - TCAGGGTCTTCATATGGTGGT
ΔFIRRM ΔFIGNL1-C2	-11	CCACCAACCTCAG - - - - - - - - - - - GGTCTTCATATGGTGGT
ΔFIRRM ΔFIGNL1-C3	-25	GCAAAAAAAG- GGTCTTCATATGGTGGT

Table S7. Gene editing events in KP knockout clones.

FIRRM	Guide 1	AGTAGATGAG**AATAACGCTTTGATAATGGT**AGGAGGTGAG
ΔFIRRM-1	+116/+7	AGTAGATGAGAATAACGCTTTGATAATG – (+116)- GTGAG AGTAGATGAGAATAACGCTTTGATAATG**TGAGCAA**GTGAG
ΔFIRRM-2	-154/-80	– (-154)- AGGAGGTGAG - (-80) - AGGAGGTGAG
ΔFIRRM ΔFANCD2 -C1	+1/+1	CTAAAGACCGTTCG**G**CTTATGTGTTCGCTCACATTCCTAAC CTAAAGACCGTTCG**G**CTTATGTGTTCGCTCACATTCCTAAC
ΔFIRRM ΔFANCD2 -C2	+1/-2/-36	ATCCTAGGGGATTCCCAGCATGCTAAT**G**GTGGGGAAAGAGC ATCCTAGGGGATTCCCAGCATGCTA - - GTGGGGAAAGAGC ATCCTAGGG – (-36) - TGGGTAAACAC
ΔFIRRM ΔFANCD2 -C3	+1/-2	ATCCTAGGGGATTCCCAGCATGCTAAT**G**GTGGGGAAAGAGC ATCCTAGGGGATTCCCAGCATGCTA - - GTGGGGAAAGAGC