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Supplemental information

FANCM regulates repair pathway choice at stalled replication forks

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Supplemental Figure Legends

Figure S1. Genotype of *Fancm*$^{\Delta 85/\Delta}$ *Ter*-HR reporter clone #39. Related to **Figure 1**.

A. PCR primers for detection of 85 bp frame-shift *Fancm*$^{\Delta 85}$ allele. Red half-arrow heads: genotyping primers. Gel: PCR products from *Fancm*$^{+/+}$ and *Fancm*$^{\Delta 85/\Delta}$ gDNA. **B.** Primary DNA sequencing chromatogram of PCR product from a *Fancm*$^{\Delta 85/\Delta}$ clone harboring the *Fancm*$^{\Delta 85}$ allele. **C.** Whole genome sequencing reads spanning *Fancm* exons 1 and 2 in *Fancm*$^{\Delta 85/\Delta}$ clone #39. Note how 85 bp deletion within exon 2 has zero coverage. Red gapped reads in alignment track identify 2544 bp deletion in second (*Fancm*$^{\Delta}$) allele, overlapping the 85 bp deletion of the *Fancm*$^{\Delta 85}$ allele. *Fancm* exons are shown as blue bars below alignment track. Red bars below exons identify exact chromosome positions of the two deletions. **D.** RT qPCR analysis of *Fancm* mRNA normalized to *Gapdh* mRNA using the $2^{-\Delta CT}$ method. Data shows mean ± standard deviation (SD) from three independent experiments (n=3). ****: $P < 0.0001$ by one-way ANOVA. **E.** Cell cycle analysis of Fancm$^{+/+}$ and *Fancm*$^{\Delta 85/\Delta}$ clones showing modest enrichment of G2/M fraction in *Fancm*$^{\Delta 85/\Delta}$ cells.

Figure S2. *Fancm* hemizygous cells retain wild type stalled fork repair phenotypes. Related to **Figure 2**.

A. RT qPCR analysis of *Brca1* mRNA in *Fancm*$^{+/+}$ or *Fancm*$^{\Delta 85/\Delta}$ clones treated with siRNAs shown. Data shows mean ± SD, normalized to *Gapdh* mRNA using the $2^{-\Delta CT}$ method and analyzed by Student's *t*-test (n=3). **: $P<0.01$. **B.** Gene modification strategy to generate hemizygous *Fancm*$^{+/-}$ clones using Cas9 with dual sgRNAs targeting exons 2 and 23. The *Fancm*$^{-}$ allele harbors the expected loss of the 53.2 kb *Fancm* gene. Red half arrowheads: PCR primers specific to Exons 2 and 23. Predicted PCR product size for *Fancm*$^{+}$ wild type allele shown. **C.** Upper left: Gel showing PCR products from *Fancm*$^{+/+}$ and *Fancm*$^{+/-}$ gDNA. Lower left: Primary DNA sequencing chromatogram of *Fancm*$^{-}$ allele PCR product verifies exon2 (green) to exon 23 (red) breakpoint. Right: Primary DNA sequencing chromatograms of *Fancm*$^{+}$ allelic PCR products from the same clone verify wild type sequence of *Fancm*$^{+}$ allele at sgRNA target sites. **D.** RT qPCR analysis of *Fancm* mRNA in *Fancm*$^{+/+}$ or *Fancm*$^{+/-}$ clones. Data shows mean ± SD, normalized to *Gapdh* using the $2^{-\Delta CT}$ method. Analysis by Student's *t*-test (n=3). ns: not significant. **E.** Tus/*Ter*- and I-SceI-induced repair in *Fancm*$^{+/+}$, *Fancm*$^{+/-}$, and

Fancm$^{\Delta 85/\Delta}$ *Ter*-HR reporter clones co-transfected with Tus or I-SceI expression plasmids and siRNAs as shown. Data shows mean ± SEM. Analysis by Student's *t*-test (n=4). *Fancm*$^{\Delta 85/\Delta}$ cells serve as control for *Fancm* mutant phenotype. All repair outcomes for *Fancm*$^{+/+}$ *vs*. *Fancm*$^{+/-}$ are not significantly different.

Figure S3. Characterization of *Fancm*$^{\Delta MM1/-}$ cells. Related to **Figure 3**. **A.** *Fancm*$^{\Delta MM1}$ allele. Red half-arrow heads: genotyping primers as shown. Gel: PCR products from *Fancm*$^{+/-}$ and *Fancm*$^{\Delta MM1/-}$ gDNA. **B.** Primary DNA sequencing chromatogram from a representative *Fancm*$^{\Delta MM1/-}$ clone indicates in-frame 366 bp deletion of MM1 coding sequence within the residual *Fancm* allele. **C.** Cell cycle analysis of *Fancm*$^{+/-}$ and *Fancm*$^{\Delta MM1/-}$ clones. **D.** I-SceI-induced HR in *Fancm*$^{+/-}$ (white bars) clones *vs.* *Fancm*$^{\Delta MM1/-}$ (gray) clones. Data obtained from same experiments as in **Figure 3G**. Data shows mean ± SEM. **: $P < 0.01$, ***: $P < 0.001$ by one-way ANOVA (n=5). ns: not significant. **E.** I-SceI-induced HR in *Fancm*$^{+/-}$ *vs*. *Fancm*$^{\Delta MM1/-}$ clones co-transfected with I-SceI expression plasmid and siRNAs as shown. Data obtained from same experiments as in **Figure 3H**. Data shows mean ± SEM. Analysis by Student's *t*-test (n=6). *: $P < 0.05$, ns: not significant. **F.** Tus/*Ter*-induced repair in *Fancm*$^{+/-}$ *vs*. *Fancm*$^{\Delta MM1/-}$ clones co-transfected with Tus expression plasmid and siRNAs as shown. Data shows mean ± SEM. Analysis by Student's *t*-test (n=4). *: $P < 0.05$, ns: not significant. **G.** RT qPCR analysis of *FANCA* and *FANCF* mRNA in *Fancm*$^{+/-}$ or *Fancm*$^{\Delta MM1/-}$ clones treated with siRNAs shown. Data shows mean ± SD, normalized to *Gapdh* mRNA using the $2^{-\Delta CT}$ method. Analysis by Student's *t*-test (n=3). **: $P<0.01$.

Figure S4. Characterization of *Fancm*$^{\Delta MM2/-}$ cells. Related to **Figure 4**. **A.** *Fancm*$^{\Delta MM2}$ allele. Red half-arrow heads: genotyping primers as shown. Gel: PCR products of *Fancm*$^{+/-}$ and *Fancm*$^{\Delta MM2/-}$ gDNA. **B.** Primary DNA sequencing chromatogram from a representative *Fancm*$^{\Delta MM2/-}$ clone indicates in-frame 114 bp deletion of MM2 coding sequence within the residual *Fancm* allele. **C.** Proliferative competition assay in presence of Mitomycin C (MMC), measuring enrichment of GFP^{+} *Fancm*$^{+/-}$ *vs*. GFP^{-} *Fancm*$^{\Delta MM2/-}$ cells. Data, normalized to 0 μg/mL MMC, shows mean value (n=3). Error bars: standard deviation. **D.** Cell cycle analysis of *Fancm*$^{+/-}$ and *Fancm*$^{\Delta MM2/-}$ clones. **E.** ChIP analysis of FANCM and FANCA at Tus/*Ter* RFB in *Fancm*$^{+/-}$ cells (n=3)

co-transfected with Tus expression plasmid and siRNAs as shown. Elements and data analysis as in Figure 1G. ***: P < 0.001 by one-way ANOVA. **F.** I-SceI-induced HR in three *Fancm*$^{+/-}$ (white) clones *vs.* three *Fancm*$^{\Delta MM2/-}$ (gray) clones. Data obtained from same experiments as in Figure 4F. Data shows mean ± SEM. Analysis by one-way ANOVA (n=4). ns: not significant. **G.** I-SceI-induced HR in *Fancm*$^{+/-}$ *vs*. *Fancm*$^{\Delta MM2/-}$ clones co-transfected with I-SceI expression plasmid and siRNAs as shown. Data obtained from same experiments as in Figure 4G. Data shows mean ± SEM. Analysis by Student's *t*-test (n=5).*: P < 0.05, **: P < 0.01, ns: not significant.

Figure S5. Characterization of ATP hydrolysis -defective *Fancm*$^{\Delta DEAH/-}$ and point mutant *Fancm*$^{D202A/\Delta 85}$ cells. Related to **Figure 5**. **A.** *Fancm*$^{\Delta DEAH/-}$ allele. Red half-arrow heads: genotyping primers as shown. Gel: PCR products from *Fancm*$^{+/-}$ and *Fancm*$^{\Delta DEAH/-}$ gDNA. **B.** Primary DNA sequencing chromatogram from a representative *Fancm*$^{\Delta DEAH/-}$ clone indicates in-frame 66 bp deletion of sequence encoding the DEAH motif within the residual *Fancm* allele. **C.** Cell cycle analysis of *Fancm*$^{+/-}$ and *Fancm*$^{\Delta DEAH/-}$ cells. **D.** I-SceI-induced HR in *Fancm*$^{+/-}$ (white) clones *vs. Fancm*$^{\Delta DEAH/-}$ (gray) clones. Data shows mean ± SEM values from same experiments as in **Figure 5I**. Analysis by one-way ANOVA (n=5). ****: P < 0.0001. ns: not significant. **E.** I-SceI-induced HR in *Fancm*$^{+/-}$ *vs*. *Fancm*$^{\Delta DEAH/-}$ clones co-transfected with I-SceI expression plasmid and siRNAs as shown. Data shows mean ± SEM values from same experiments as in **Figure 5J**. Analysis by Student's *t*-test (n=5). *: P < 0.05, **: P < 0.01, ***: P < 0.001, ns: not significant. **F.** Primary DNA sequencing chromatogram from a *Fancm*^{D202A} clone indicates D202A point mutation sequence. **G.** Immunoblot of chromatin-extracted FANCM in *Fancm*$^{+/-}$ and *Fancm*$^{D202A/\Delta 85}$ clones. *: non-specific band. **H.** Immunoblot showing FANCD2 ubiquitination in *Fancm*$^{+/-}$ and *Fancm*$^{D202A/\Delta 85}$ cells. **I.** Proliferative competition assay in presence of MMC, measuring enrichment of GFP^{+} *Fancm*$^{+/-}$ *vs*. GFP^{-} *Fancm*$^{D202A/\Delta 85}$ cells. Data, normalized to 0 μg/mL MMC, shows mean ± SD (n=3). **J.** ChIP analysis of FANCM and FANCA at Tus/*Ter* in *Fancm*$^{+/-}$ and *Fancm*$^{D202A/\Delta 85}$ cells. Elements and data analysis as in **Figure 1G**. ***: P < 0.001 by one-way ANOVA (n=3). **K.** Tus/*Ter*-induced repair in *Fancm*$^{+/-}$ *vs*. *Fancm*$^{D202A/\Delta 85}$ clones co-transfected with Tus expression plasmid

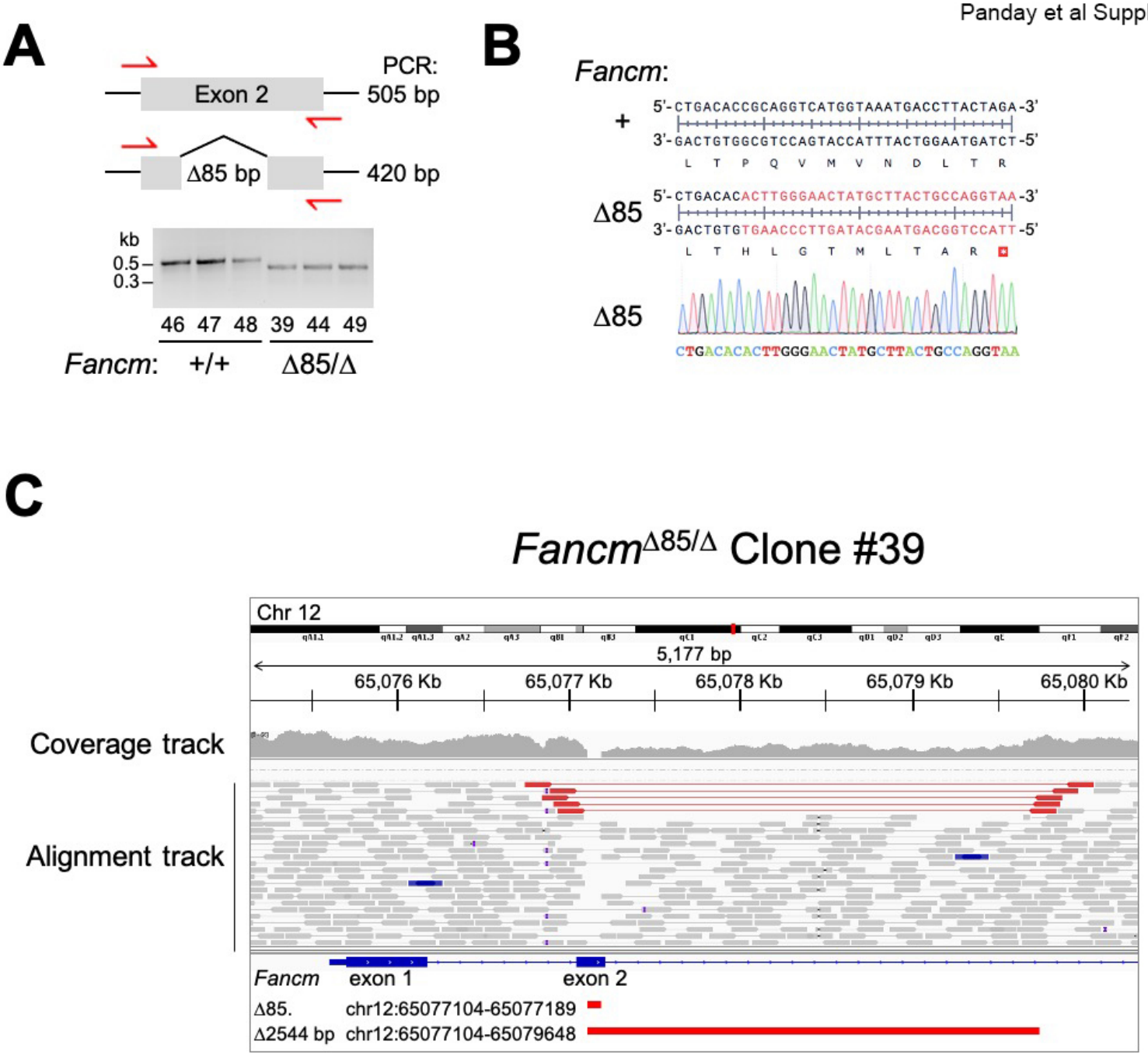
and siRNAs as shown. Data shows mean ± SEM. Analysis by Student's *t*-test (n=5). *: P < 0.05, **: P < 0.01, ***: P < 0.001, ****: P < 0.0001, ns: not significant.

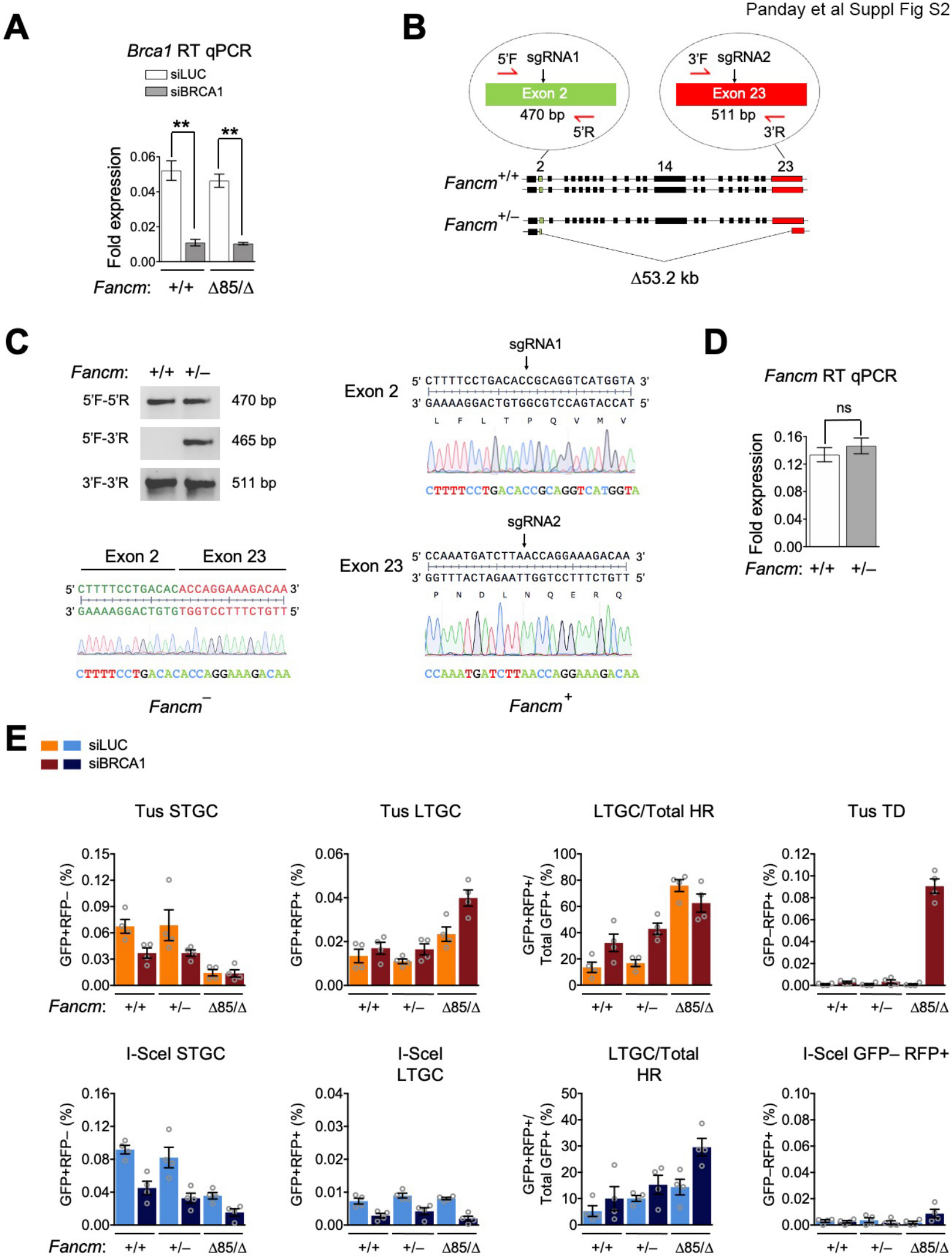
Figure S6. BLM can act independently of *Fancm* in stalled fork repair. Related to **Figure 5**. **A.** Upper: Cartoon of degron tagged BLM, indicating position of SMASh protease cleavage. Note 8x HA tag is positioned at the very C terminus of the protease-cleaved protein. Lower: Anti-HA immunoblot of degron-tagged BLM 24 hours after addition of degron-activating drugs 5-IAA and/or Asunaprevir, *vs*. DMSO control. H3: Histone H3 loading control. **B.** Anti-HA ChIP analysis of BLM-HA at Tus/*Ter* in $Blm^{deg/-}$ cells treated with 5-IAA+Asunaprevir or with DMSO control (n=3). Data shows mean ± SD. Analysis by ANOVA (n=3). ****: P < 0.0001. **C.** Tus/*Ter*-induced repair in $Blm^{deg/-}$ $Fancm^{+/+}$ and $Blm^{deg/-}$ $Fancm^{\Delta 85/\Delta}$ clones co-transfected with Tus expression plasmid and siRNAs as indicated. Cells were treated with IAA+Asunaprevir *vs*. DMSO control beginning 6 hours after transfection with replenishment with fresh drug 24 hours after transfection. Data shows mean ± SEM. Analysis by Student's *t*-test (n=5). *: P<0.05, **: P < 0.01. ns: not significant. **D.** RT qPCR analysis of *BRCA1* mRNA in $Fancm^{+/+}$ or $Fancm^{\Delta 85/\Delta}$ clones treated with siRNAs shown. Data shows mean ± SD, normalized to *Gapdh* mRNA using the $2^{-\Delta CT}$ method. Analysis by Student's *t*-test (n=3). **: P<0.01 **E.** Tus/*Ter*-induced repair in $Fancm^{+/+}$, $Fancm^{\Delta MM2/-}$, $Fancm^{\Delta 85/\Delta}$ and $Fancm^{\Delta DEAH/-}$ clones co-transfected with Tus expression plasmid and siRNAs as indicated. Data shows mean ± SEM. Analysis by Student's *t*-test (n=5). *: P<0.05. **F.** RT qPCR analysis of *Blm* mRNA in $Fancm^{+/+}$ or $Fancm^{\Delta 85/\Delta}$ clones treated with siRNAs shown. Data shows mean ± SD, normalized to *Gapdh* mRNA using the $2^{-\Delta CT}$ method. Analysis by Student's *t*-test (n=3). ***: P < 0.001 ,**: P<0.01. **G.** RT qPCR analysis of *Brca1* or *Blm* mRNA in $Fancm^{+/-}$, $Fancm^{\Delta 85/\Delta}$, clones treated with siRNAs as shown. Data shows mean ± SD, normalized to *Gapdh* mRNA using the $2^{-\Delta CT}$ method. Analysis by Student's *t*-test (n=3). **: P < 0.01, ***: P < 0.001, ns: not significant.

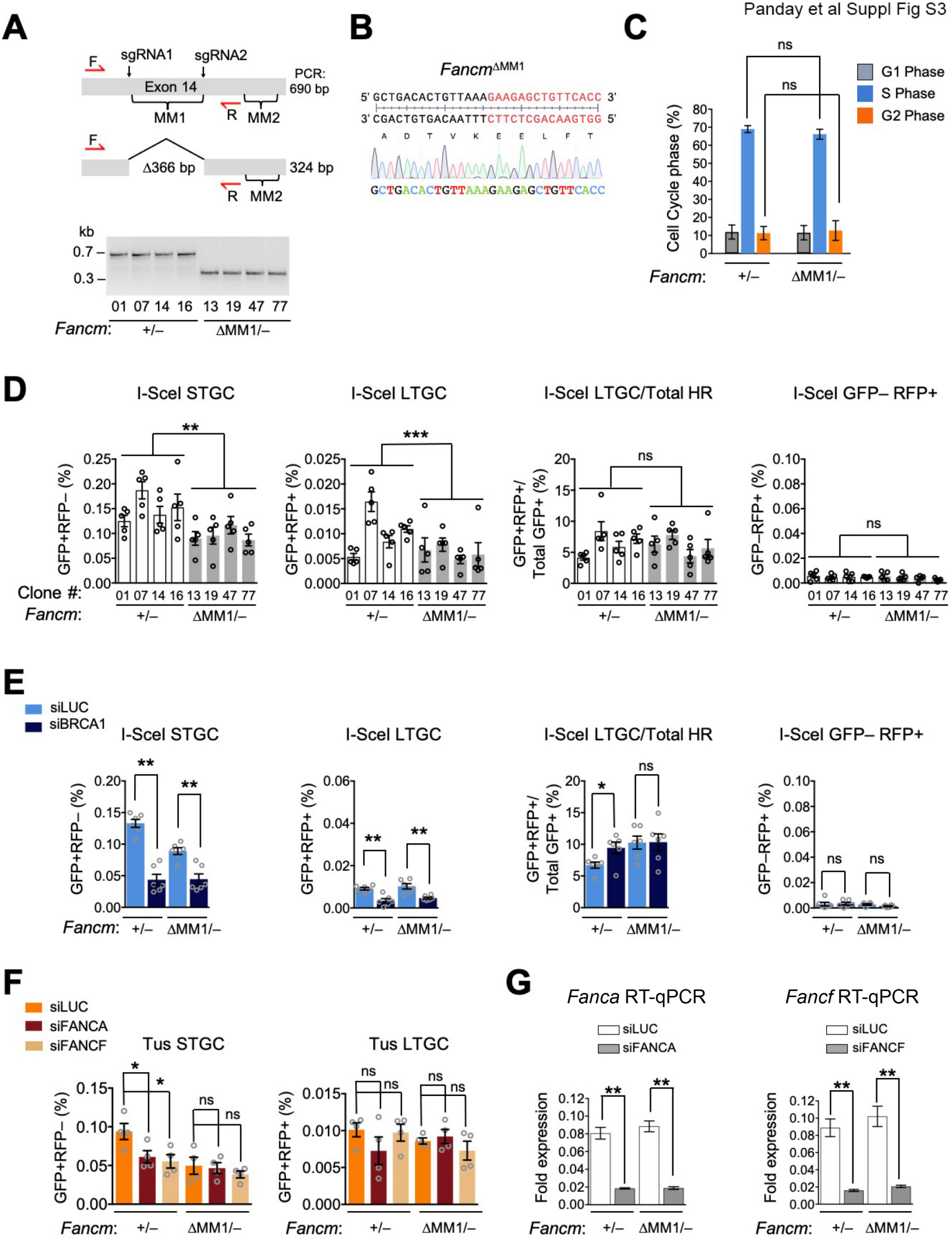
Figure S7. Analysis of viable $Fancm^{\Delta 85/\Delta}$ $Brca1^{\Delta/11}$ clone #68. Related to **Figure 6**.

A. Whole genome sequencing reads spanning *Brca1* exons 10, 11 and 12 in the viable $Fancm^{\Delta 85/\Delta}$ $Brca1^{\Delta/11}$ clone #68. *Brca1* exons shown in blue bars beneath alignment track. *Fancm* exons are shown as blue bars below

alignment track. Red bars below exons identify positions of the two deletions within exon 11. **B.** Whole genome sequencing reads spanning *Fancm* exons 1 and 2 in $Fancm^{\Delta 85/\Delta}$ clone #39. Elements as in Figure S1C. **C.** Tus/*Ter*-induced repair in $Fancm^{+/+}$ or Cre-transduced $Fancm^{\Delta 85/\Delta}$ clones having retained or deleted (clone #68) *Brca1* exon 11, co-transfected with Tus-expression plasmid and siRNAs as shown. Note induction of TDs in $Fancm^{\Delta 85/\Delta}$ $Brca1^{\Delta/11}$ clone in absence of BRCA1 depletion. Data shows mean ± SEM. Analysis by Student's *t*-test (n=3). *: $P<0.05$, **: $P < 0.01$. ns: not significant. **D.** I-SceI-induced repair in the same experiments as in panel C.


A
PCR:
Exon 2
505 bp
Δ85 bp
420 bp
kb
0.5
0.3
46 47 48 39 44 49
Fancm: +/+ Δ85/Δ
B
Fancm:
+
5'-CTGACACCGCAGGTCATGGTAAATGACCTTACTAGA-3'
3'-GACTGTGGCGTCCAGTACCATTTACTGGAATGATCT-5'
L T P Q V M V N D L T R
Δ85
5'-CTGACACACTTGGGAACTATGCTTACTGCCAGGTAA-3'
3'-GACTGTGTGAACCCTTGATACGAATGACGGTCCATT-5'
L T H L G T M L T A R
Δ85
CTGACACACTTGGGAACTATGCTTACTGCCAGGTAA
C
Fancm^Δ85/Δ Clone #39
Chr 12
5,177 bp
65,076 Kb
65,077 Kb
65,078 Kb
65,079 Kb
65,080 Kb
Coverage track
Alignment track
Fancm
exon 1
exon 2
Δ85.
chr12:65077104-65077189
Δ2544 bp
chr12:65077104-65079648
D
Fancm RT qPCR
****
Fold expression
0.16
0.12
0.08
0.04
0.00
Clone #: 46 47 48 39 44 49
Fancm: +/+ Δ85/Δ
E
****
**
Cell Cycle phase (%)
70
60
50
40
30
20
10
0
G1 Phase
S Phase
G2 Phase
Fancm: +/+ Δ85/ Δ


A
Brca1 RT qPCR
siLUC
siBRCA1
Fold expression
0.06
0.04
0.02
0.00
**
**
Fancm: +/+ Δ85/Δ
B
5'F sgRNA1
Exon 2
470 bp
5'R
3'F sgRNA2
Exon 23
511 bp
3'R
2
14
23
Fancm+/+
Fancm+/–
Δ53.2 kb
C
Fancm: +/+ +/–
5'F-5'R
470 bp
5'F-3'R
465 bp
3'F-3'R
511 bp
Exon 2 Exon 23
5' CTTTTCCTGACACACCAGGAAAGACAA 3'
3' GAAAAGGACTGTGTGGTCCTTTCTGTT 5'
CTTTTCCTGACACACCAGGAAAGACAA
Fancm–
sgRNA1
Exon 2
5' CTTTTCCTGACACCGCAGGTCATGGTA 3'
3' GAAAAGGACTGTGGCGTCCAGTACCAT 5'
L F L T P Q V M V
CTTTTCCTGACACCGCAGGTCATGGTA
sgRNA2
Exon 23
5' CCAAATGATCTTAACCAGGAAAGACAA 3'
3' GGTTTACTAGAATTGGTCCTTTCTGTT 5'
P N D L N Q E R Q
CCAAATGATCTTAACCAGGAAAGACAA
Fancm+
D
Fancm RT qPCR
ns
Fold expression
0.16
0.12
0.08
0.04
0.00
Fancm: +/+ +/–
E
siLUC
siBRCA1
Tus STGC
GFP+RFP– (%)
0.15
0.12
0.09
0.06
0.03
0.00
Fancm: +/+ +/– Δ85/Δ
Tus LTGC
GFP+RFP+ (%)
0.06
0.04
0.02
0.00
+/+ +/– Δ85/Δ
LTGC/Total HR
GFP+RFP+/ Total GFP+ (%)
100
80
60
40
20
0
+/+ +/– Δ85/Δ
Tus TD
GFP–RFP+ (%)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
+/+ +/– Δ85/Δ
I-SceI STGC
GFP+RFP– (%)
0.16
0.12
0.08
0.04
0.00
Fancm: +/+ +/– Δ85/Δ
I-SceI LTGC
GFP+RFP+ (%)
0.04
0.03
0.02
0.01
0.00
+/+ +/– Δ85/Δ
LTGC/Total HR
GFP+RFP+/ Total GFP+ (%)
50
40
30
20
10
0
+/+ +/– Δ85/Δ
I-SceI GFP– RFP+
GFP–RFP+ (%)
0.10
0.08
0.06
0.04
0.02
0.00
+/+ +/– Δ85/Δ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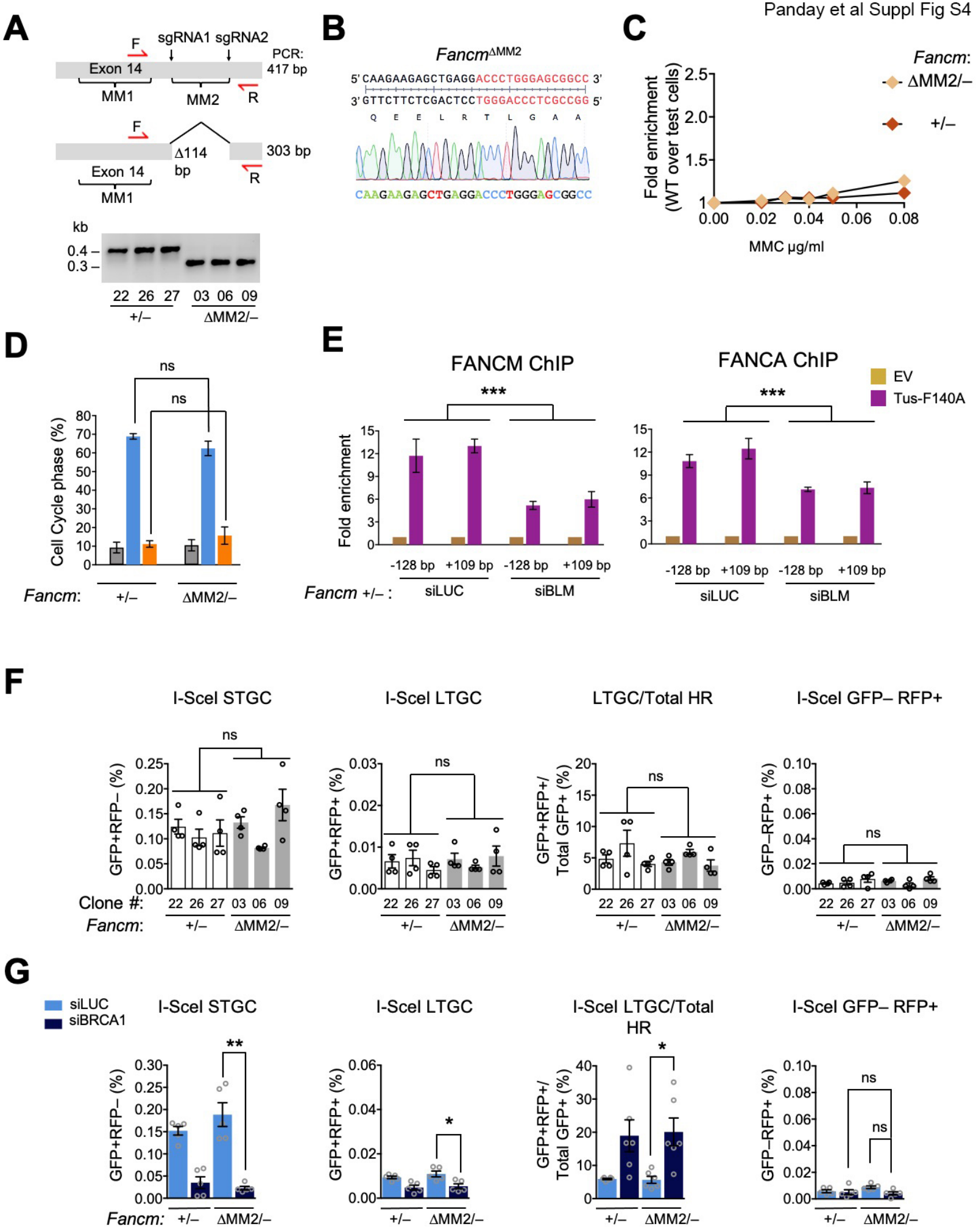
F
sgRNA1
sgRNA2
Exon 14
PCR:
690 bp
MM1
R
MM2
F
Δ366 bp
324 bp
R
MM2
kb
0.7
0.3
01 07 14 16 13 19 47 77
Fancm: +/– ΔMM1/–
B
Fancm^ΔMM1
5' GCTGACACTGTTAAAGAAGAGCTGTTCACC 3'
3' CGACTGTGACAATTTCTTCTCGACAAGTGG 5'
A D T V K E E L F T
GCTGACACTGTTAAAGAAGAGCTGTTCACC
C
ns
ns
G1 Phase
S Phase
G2 Phase
Cell Cycle phase (%)
Fancm: +/– ΔMM1/–
D
I-SceI STGC
I-SceI LTGC
I-SceI LTGC/Total HR
I-SceI GFP– RFP+
GFP+RFP– (%)
GFP+RFP+ (%)
GFP+RFP+/ Total GFP+ (%)
GFP–RFP+ (%)
**
***
ns
Clone #: 01 07 14 16 13 19 47 77
Fancm: +/– ΔMM1/–
E
siLUC
siBRCA1
I-SceI STGC
I-SceI LTGC
I-SceI LTGC/Total HR
I-SceI GFP– RFP+
GFP+RFP– (%)
GFP+RFP+ (%)
GFP+RFP+/ Total GFP+ (%)
GFP–RFP+ (%)
*
**
ns
Fancm: +/– ΔMM1/–
F
siLUC
siFANCA
siFANCF
Tus STGC
Tus LTGC
GFP+RFP– (%)
GFP+RFP+ (%)
*
ns
Fancm: +/– ΔMM1/–
G
Fanca RT-qPCR
Fancf RT-qPCR
siLUC
siFANCA
siFANCF
Fold expression
**
Fancm: +/– ΔMM1/–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F
sgRNA1 sgRNA2
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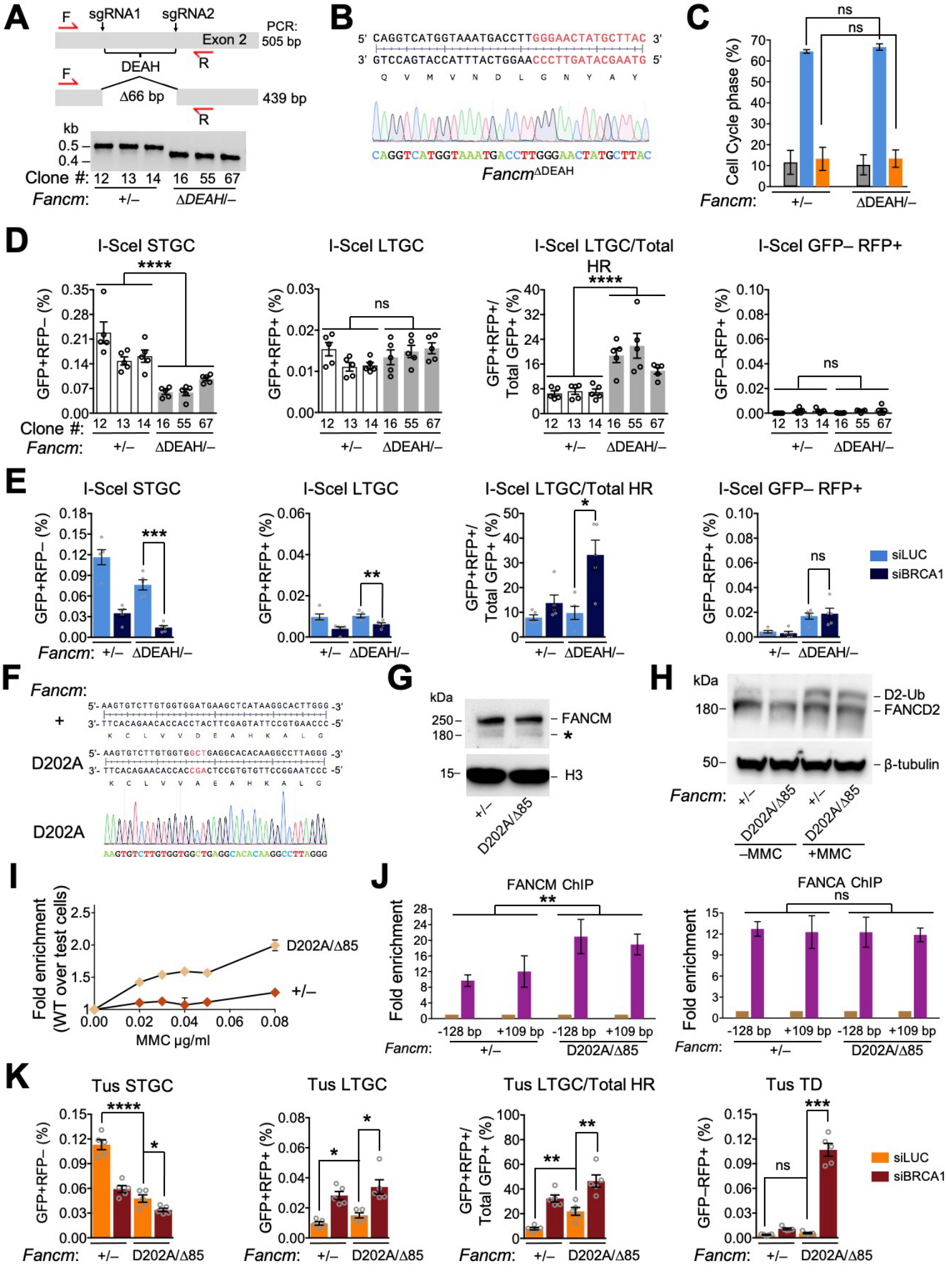
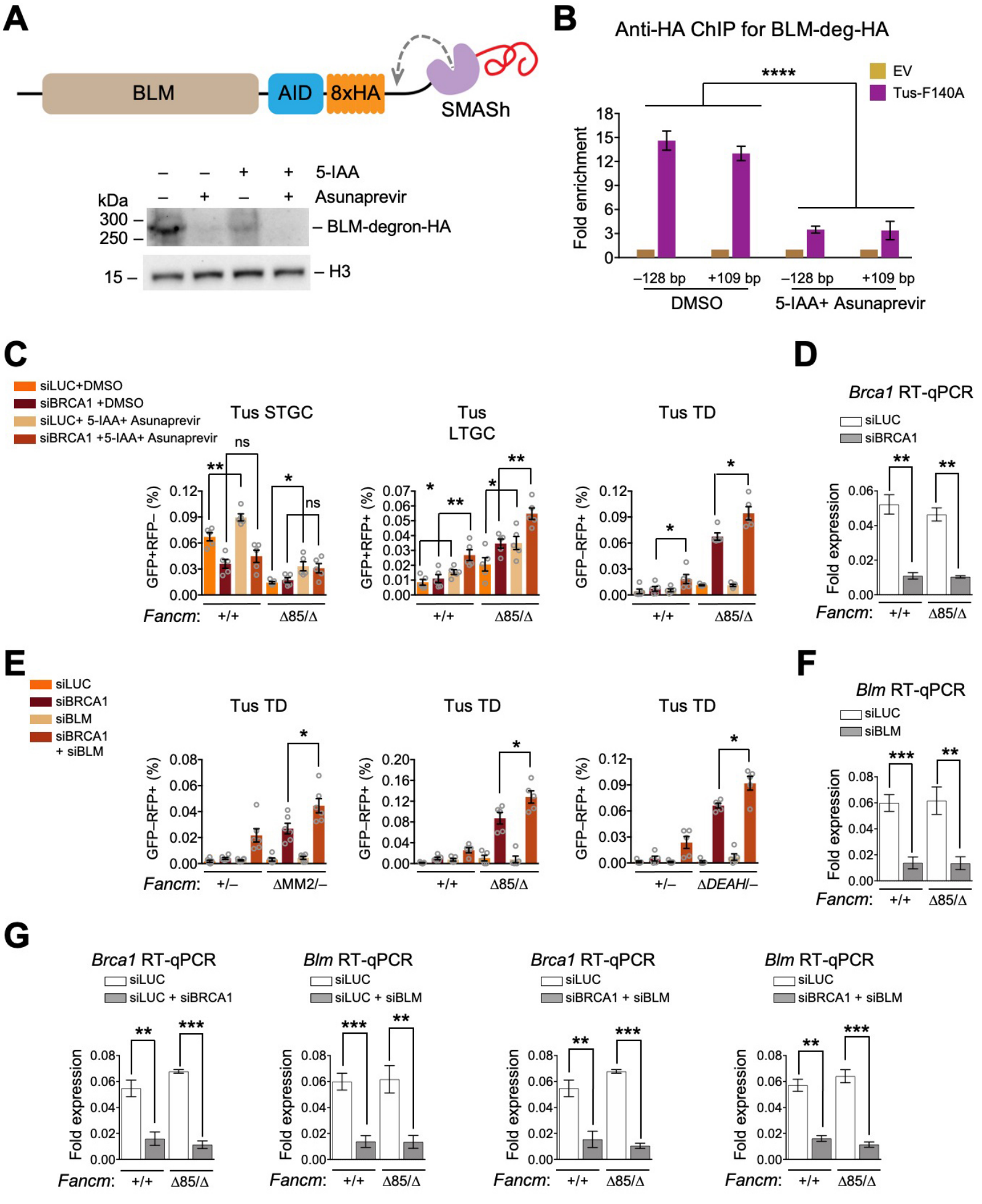
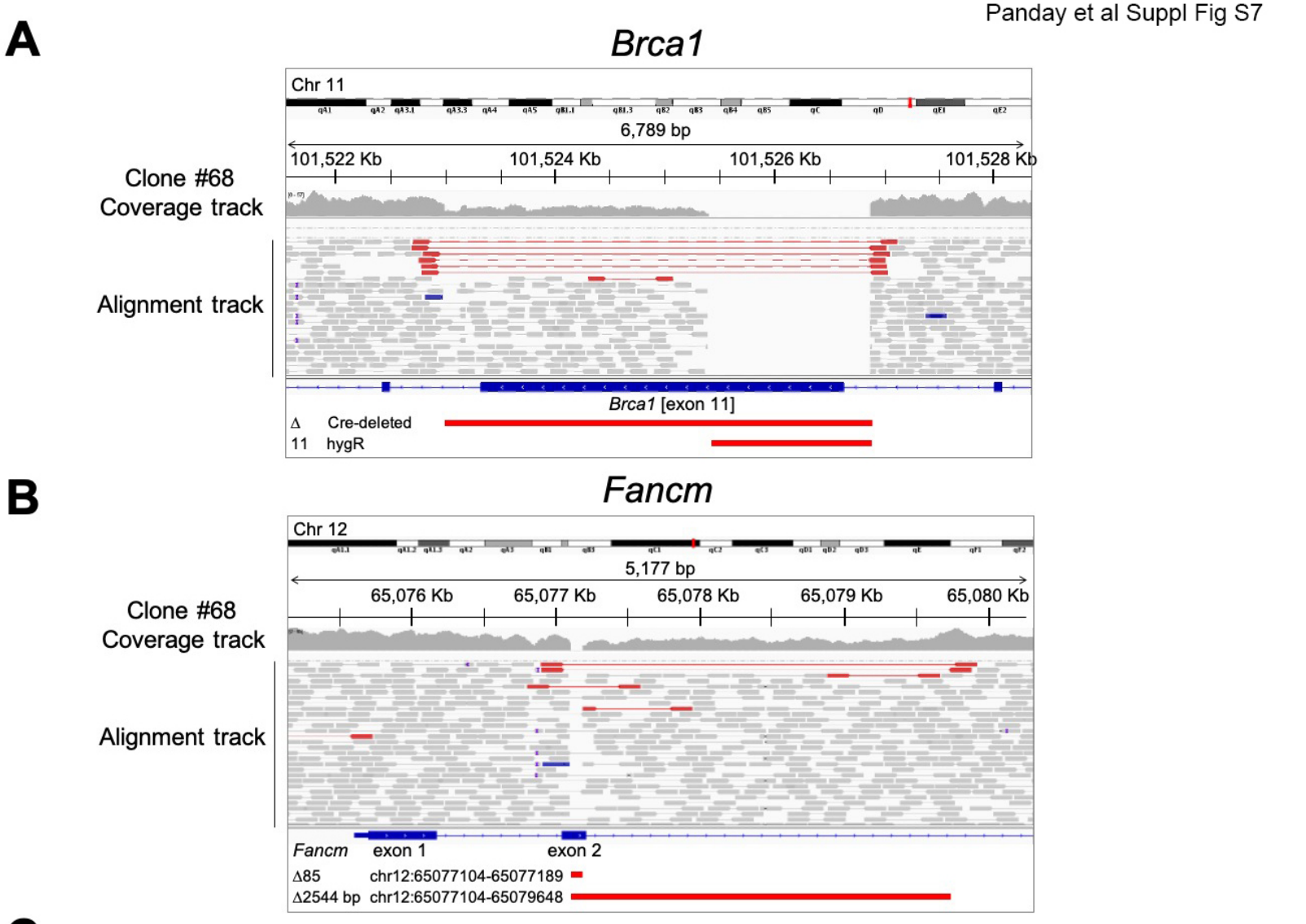
417 bp
Exon 14
MM1
MM2
R
F
Δ114
bp
303 bp
Exon 14
MM1
R
kb
0.4 –
0.3 –
22 26 27 03 06 09
+/–
ΔMM2/–
B
Fancm^ΔMM2
5' CAAGAAGAGCTGAGGACCCTGGGAGCGGCC 3'
3' GTTCTTCTCGACTCCTGGGACCCTCGCCGG 5'
Q E E L R T L G A A
CAAGAAGAGCTGAGGACCCTGGGAGCGGCC
C
Fancm:
ΔMM2/–
+/–
Fold enrichment (WT over test cells)
2.5
2.0
1.5
1
0.00 0.02 0.04 0.06 0.08
MMC μg/ml
D
ns
ns
Cell Cycle phase (%)
80
70
60
50
40
30
20
10
0
Fancm: +/– ΔMM2/–
E
FANCM ChIP
FANCA ChIP
EV
Tus-F140A
***
***
Fold enrichment
15
12
9
6
3
0
-128 bp +109 bp -128 bp +109 bp
Fancm +/– : siLUC siBLM
-128 bp +109 bp -128 bp +109 bp
siLUC siBLM
F
I-SceI STGC
I-SceI LTGC
LTGC/Total HR
I-SceI GFP– RFP+
ns
GFP+RFP– (%)
GFP+RFP+ (%)
GFP+RFP+/ Total GFP+ (%)
GFP–RFP+ (%)
Clone #: 22 26 27 03 06 09
Fancm: +/– ΔMM2/–
G
siLUC
siBRCA1
I-SceI STGC
I-SceI LTGC
I-SceI LTGC/Total HR
I-SceI GFP– RFP+
**
*
*
ns
ns
GFP+RFP– (%)
GFP+RFP+ (%)
GFP+RFP+/ Total GFP+ (%)
GFP–RFP+ (%)
Fancm: +/– ΔMM2/–


A
F
sgRNA1
sgRNA2
PCR:
Exon 2
505 bp
DEAH
R
F
Δ66 bp
439 bp
R
kb
0.5
0.4
Clone #: 12 13 14 16 55 67
Fancm: +/– ΔDEAH/–
B
5' CAGGTCATGGTAAATGACCTTGGGAACTATGCTTAC 3'
3' GTCCAGTACCATTTACTGGAACCCTTGATACGAATG 5'
Q V M V N D L G N Y A Y
CAGGTCATGGTAAATGACCTTGGGAACTATGCTTAC
FancmΔDEAH
C
ns
ns
Cell Cycle phase (%)
70
60
50
40
30
20
10
0
Fancm: +/– ΔDEAH/–
D
I-SceI STGC
I-SceI LTGC
I-SceI LTGC/Total HR
I-SceI GFP– RFP+
****
ns
****
ns
GFP+RFP– (%)
GFP+RFP+ (%)
GFP+RFP+/ Total GFP+ (%)
GFP–RFP+ (%)
0.35
0.28
0.21
0.14
0.07
0.00
0.03
0.02
0.01
0.00
40
32
24
16
8
0
0.10
0.08
0.06
0.04
0.02
0.00
Clone #: 12 13 14 16 55 67
Fancm: +/– ΔDEAH/–
E
I-SceI STGC
I-SceI LTGC
I-SceI LTGC/Total HR
I-SceI GFP– RFP+
***
**
*
ns
GFP+RFP– (%)
GFP+RFP+ (%)
GFP+RFP+/ Total GFP+ (%)
GFP–RFP+ (%)
0.18
0.15
0.12
0.09
0.06
0.03
0.00
0.06
0.04
0.02
0.00
50
40
30
20
10
0
0.10
0.08
0.06
0.04
0.02
0.00
siLUC
siBRCA1
Fancm: +/– ΔDEAH/–
F
Fancm:
+
5'- AAGTGTCTTGTGGTGGATGAAGCTCATAAGGCACTTGGG -3'
3'- TTCACAGAACACCACCTACTTCGAGTATTCCGTGAACCC -5'
K C L V V D E A H K A L G
D202A
5'- AAGTGTCTTGTGGTGGCTGAGGCACACAAGGCCTTAGGG -3'
3'- TTCACAGAACACCACCGACTCCGTGTGTTCCGGAATCCC -5'
K C L V V A E A H K A L G
D202A
AAGTGTCTTGTGGTGGCTGAGGCACACAAGGCCTTAGGG
G
kDa
250
180
FANCM
*
15
H3
+/–
D202A/Δ85
H
kDa
180
D2-Ub
FANCD2
50
β-tubulin
Fancm: +/– D202A/Δ85 +/– D202A/Δ85
–MMC +MMC
I
Fold enrichment (WT over test cells)
2.5
2.0
1.5
1
0.00 0.02 0.04 0.06 0.08
MMC μg/ml
D202A/Δ85
+/–
J
FANCM ChIP
**
FANCA ChIP
ns
Fold enrichment
28
24
20
16
12
8
4
0
15
12
9
6
3
0
-128 bp +109 bp -128 bp +109 bp
Fancm: +/– D202A/Δ85
K
Tus STGC
Tus LTGC
Tus LTGC/Total HR
Tus TD
****
*
*
*
**
**
***
ns
GFP+RFP– (%)
GFP+RFP+ (%)
GFP+RFP+/ Total GFP+ (%)
GFP–RFP+ (%)
0.15
0.12
0.09
0.06
0.03
0.00
0.08
0.06
0.04
0.02
0.00
100
80
60
40
20
0
0.15
0.12
0.09
0.06
0.03
0.00
siLUC
siBRCA1
Fancm: +/– D202A/Δ85


A
BLM
AID
8xHA
SMASh
5-IAA
Asunaprevir
kDa
300
250
BLM-degron-HA
15
H3
B
Anti-HA ChIP for BLM-deg-HA
EV
Tus-F140A
****
Fold enrichment
18
15
12
9
6
3
0
–128 bp
+109 bp
–128 bp
+109 bp
DMSO
5-IAA+ Asunaprevir
C
siLUC+DMSO
siBRCA1 +DMSO
siLUC+ 5-IAA+ Asunaprevir
siBRCA1 +5-IAA+ Asunaprevir
Tus STGC
Tus LTGC
Tus TD
GFP+RFP– (%)
GFP+RFP+ (%)
GFP–RFP+ (%)
ns
Fancm: +/+ Δ85/Δ
D
Brca1 RT-qPCR
siLUC
siBRCA1
Fold expression
Fancm: +/+ Δ85/Δ
E
siLUC
siBRCA1
siBLM
siBRCA1 + siBLM
Tus TD
GFP–RFP+ (%)
Fancm: +/– ΔMM2/–
Fancm: +/+ Δ85/Δ
Fancm: +/– ΔDEAH/–
F
Blm RT-qPCR
siLUC
siBLM
Fold expression
Fancm: +/+ Δ85/Δ
G
Brca1 RT-qPCR
siLUC
siLUC + siBRCA1
Blm RT-qPCR
siLUC
siLUC + siBLM
Brca1 RT-qPCR
siLUC
siBRCA1 + siBLM
Blm RT-qPCR
siLUC
siBRCA1 + siBLM
Fold expression
Fancm: +/+ Δ85/Δ


A
Brca1
Chr 11
6,789 bp
101,522 Kb
101,524 Kb
101,526 Kb
101,528 Kb
Clone #68
Coverage track
Alignment track
Brca1 [exon 11]
Δ Cre-deleted
11 hygR
B
Fancm
Chr 12
5,177 bp
65,076 Kb
65,077 Kb
65,078 Kb
65,079 Kb
65,080 Kb
Clone #68
Coverage track
Alignment track
Fancm exon 1 exon 2
Δ85 chr12:65077104-65077189
Δ2544 bp chr12:65077104-65079648
C
siLUC
siBRCA1
Tus STGC
Tus LTGC
Tus LTGC/Total HR
Tus TD
GFP+RFP– (%)
GFP+RFP+ (%)
GFP+RFP+/ Total GFP+ (%)
GFP–RFP+ (%)
ns
*
**
Fancm: +/+ Δ85/Δ85 Δ85/Δ
Brca1: fl/11 Δ/11 #68
D
siLUC
siBRCA1
I-SceI STGC
I-SceI LTGC
I-SceI LTGC/Total HR
I-SceI GFP– RFP+
GFP+RFP– (%)
GFP+RFP+ (%)
GFP+RFP+/ Total GFP+ (%)
GFP–RFP+ (%)
ns
*
Fancm: +/+ Δ85/Δ
Brca1: fl/11 Δ/11 #68

Supplemental Table S1. Oligonucleotides used in this study. Related to STAR Methods.

Oligonucleotides	SOURCE	IDENTIFIER
Primer: *Fancm*$^{\Delta 85/\Delta}$ 5' exon2 breaksite sgRNA transcription, 5'-GGTTCTTTTCCTGACACCGCAGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm*$^{\Delta 85/\Delta}$ 3' exon2 breaksite sgRNA transcription, 5'-GATGAAGCTCATAAGGCACTTGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* exon23 breaksite sgRNA transcription, 5'-GTTACCAAATGATCTTAACCAGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* exon2 sense PCR and sequencing, 5'-CTACCTCAAGCTCCAGAGTCCTGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* exon2 antisense PCR and sequencing, 5'-AGTTCCCATCACTGAGACTTATTCC-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* exon23 sense PCR and sequencing, 5'-CTACCTCAAGCTCCAGAGTCCTGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* exon23 antisense PCR and sequencing, 5'-AGGGATGACCTGAGGTTGTC-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* MM1 5' breaksite sgRNA transcription, 5'-ATGCTGACACTGTTAAACAAAGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* MM1 3' breaksite sgRNA transcription, 5'-GTGAACAGCTCTTCTTCCAATGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* MM2 5' breaksite sgRNA transcription, 5'-CAAGAAGAGCTGAGGACTGACGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* MM2 3' breaksite sgRNA transcription, 5'-TCTGATAGGACTCGCACCCTGGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* DEAH 5' breaksite sgRNA transcription, 5'-TGGTAAATGACCTTACTAGAGGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* DEAH 3' breaksite sgRNA transcription, 5'-ATGAAGCTCATAAGGCACTTGGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* MM1 sense PCR and sequencing, 5'-CTTGTTTGGTAGGGTGAATGCA-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* MM1 antisense PCR and sequencing, 5'-GGGAGAACGGGATAAAAATCTCT-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* MM2 sense PCR and sequencing, 5'-TAGATGATGATTCTGAACCTGAAGAC-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* MM2 antisense PCR and sequencing, 5'-TGTGCTCCTGACTCTCTGCT-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* DEAH sense PCR and sequencing, 5'-CTACCTCAAGCTCCAGAGTCCTGG-3'	ThermoFisher Scientific	N/A
Primer: *Fancm* DEAH antisense PCR and sequencing, 5'-AGTTCCCATCACTGAGACTTATTCC-3'	ThermoFisher Scientific	N/A
ultramer: ssODN D202A mutation within exon 2, 5'-TGGTCCAGCAGGAGGGTTCTTTTCCTGACACCGCAGGTCA TGGTAAATGACCTTACTAGAGGGGCTGTTCCTGCCACCCAC GTAAAGTGTCTTGTGGTGGCTGAGGCACACAAGGCCTTAG GGAACTATGCTTACTGCCAGGTAAGCTCTTTTTTCAAATGCT AGTTTGTAGAGGATGCATAAATTCTTAACGGGCTTGG-3'	Integrated DNA Technologies	N/A
Primer: *Fancm* exon2 D202A targeting sgRNA transcription, 5'-GATGAAGCTCATAAGGCACTTGG-3'	ThermoFisher Scientific	N/A
Primer: *Blm* gene 5' breaksite sgRNA transcription, 5'-ATCCACCCCAGGAAATCCCGAGG-3'	ThermoFisher Scientific	N/A

Primer: *Blm* gene 3' breaksite sgRNA transcription, 5'-GCACGCTCTGCGCGGCAAGAAGG-3'	ThermoFisher Scientific	N/A
Primer: *Blm* gene exon22 targeting sgRNA transcription, 5'-CGCAGAGAACTGTTAGGAGAAGG-3'	ThermoFisher Scientific	N/A
Primer: *Blm* gene 5' breaksite sense PCR and sequencing, 5'-CATTCGGATTGGGCTTTAGTAGTACG-3'	ThermoFisher Scientific	N/A
Primer: *Blm* gene 5' breaksite antisense PCR and sequencing, 5'-TTTCTGGGTCACCAGGTCTCTACTC-3'	ThermoFisher Scientific	N/A
Primer: *Blm* gene 3' breaksite sense PCR and sequencing, 5'-CAAGGAAAATCATGTTTGTTCTCCTGG-3'	ThermoFisher Scientific	N/A
Primer: *Blm* gene 3' breaksite antisense PCR and sequencing, 5'-TGTTTCCTCTGTCATTTGTCAAGGC-3'	ThermoFisher Scientific	N/A
Primer: *Blm* exon22 sense PCR and sequencing, 5'-ATGTCTTCATGCCAGGCAGTG-3'	ThermoFisher Scientific	N/A
Primer: AiD antisense PCR and sequencing, 5'-GGAGGTTTGGCTGGATCTTTA-3'	ThermoFisher Scientific	N/A
Primer: Neomycin sense PCR and sequencing, 5'-CTATCGCCTTCTTGACGAGT-3'	ThermoFisher Scientific	N/A
Primer: *Blm* gene exon22 antisense PCR and sequencing, 5'-GCATTACACAAAGGGCAAACTAGG-3'	ThermoFisher Scientific	N/A
Primer: SMASh antisense PCR and sequencing, 5'-AGGAACCCTTATCGTCATCGTCC-3'	ThermoFisher Scientific	N/A
SMASh antisense2 PCR and sequencing, 5'-GGTTCTCCACAGGGATGAAGTCC-3'	ThermoFisher Scientific	N/A
Blm 3' locus sense PCR and sequencing, 5'-TGCTTCTCAGGCAACATCATCAGC-3'	ThermoFisher Scientific	N/A
Neomycin antisense PCR and sequencing, 5'-GCCCAGTCATAGCCGAATAG-3'	ThermoFisher Scientific	N/A
AiD antisense PCR and sequencing, 5'-CTCCGTCCATTGATACCTTCAC-3'	ThermoFisher Scientific	N/A
ChIP Primer: +109 bp sense 5'-TCCGGATAGGGATAACAGGGTA-3'	ThermoFisher Scientific	N/A
ChIP Primer: +109 bp antisense 5'-GTCGGCCATGATATAGACGTTG-3'	ThermoFisher Scientific	N/A
ChIP Primer: +309 bp sense 5'-AGCTCGCCGACCACTAC-3'	ThermoFisher Scientific	N/A
ChIP Primer: +309 bp antisense 5'-TCCAGCAGGACCATGTGAT-3'	ThermoFisher Scientific	N/A
ChIP Primer: +921 bp sense 5'-GGACAAGACTTCCCACAGATT-3'	ThermoFisher Scientific	N/A
ChIP Primer: +921 bp antisense 5'-GAGGCGGATCACAAGCAATAAT-3'	ThermoFisher Scientific	N/A
ChIP Primer: +1.6 kb sense 5'-TCCACATTTGGGCCTATTCTC-3'	ThermoFisher Scientific	N/A
ChIP Primer: +1.6 kb antisense 5'-CAATAATGAAATATACCTTTTAATGTCT-3'	ThermoFisher Scientific	N/A
ChIP Primer: 128 bp sense 5'-GAGCGCACCATCTTCTTCA-3'	ThermoFisher Scientific	N/A
ChIP Primer: 128 bp antisense 5'-TCCCTACGATGCCCTTCA-3'	ThermoFisher Scientific	N/A
ChIP Primer: 350 bp sense 5'-CTGGACGGCGACGTAAAC-3'	ThermoFisher Scientific	N/A
ChIP Primer: 350 bp antisense 5'-CGGTGGTGCAGATGAACTT-3'	ThermoFisher Scientific	N/A

ChIP Primer: 900 bp-sense 5'-TCTGGAGCATGCGCTTTAG-3'	ThermoFisher Scientific	N/A
ChIP Primer: 900 bp antisense 5'-CTAAAGCGCATGCTCCAGA-3'	ThermoFisher Scientific	N/A
ChIP Primer: 1.4kb sense 5'-CCACTGCCCTTGTGACTAAA-3'	ThermoFisher Scientific	N/A
ChIP Primer: 1.4kb antisense 5'-AGGCTACACCAACGTCAATC-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Gapdh* sense 5'-CGTCCCGTAGACAAAATGGT-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Gapdh* antisense 5'-TCGTTGATGGCAACAATCTC-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancm* sense 5'-GTCGTTATCCTCGCTGAAGG-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancm* antisense 5'-TTTGTTGGACTGACTCTGATTATATGT-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancm* MM1 sense 5'-CTGTTAAACAAAGGGATTCTAAAT-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancm* MM1 antisense 5'-GATACAGATTTCTCATCACTG A-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancm* MM2 sense 5'-TCGTTGTAGTTCGGGTTCAGA-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancm* MM2 antisense 5'-AGTGTTCAACTTCAGTGCGCC-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancm* DEAH sense 5'-TGGCTGAAATGACAGGTTCAACT-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancm* DEAH antisense 5'-GCCTTATGAGCTTCATCCACC-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Brca1* sense 5'-ATGAGCTGGAGAGGATGCTG-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Brca1* antisense 5'-CTGGGCAGTTGCTGTCTTCT-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fanca* sense 5'-GGCAGCCCTGTACAACTGAT-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fanca* antisense 5'-GCCAGCAGCTCTGTCATGTT-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancf* sense 5'-TATGATCTGCAAAAGGGTGCCTGG-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Fancf* antisense 5'-CTCTTCCTGCAGAAATGGCTGGGC-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Blm* sense 5'-CGCGACGTAAGCCTGAGT-3'	ThermoFisher Scientific	N/A
RT qPCR Primer: *Blm* antisense 5'-TGGCTGAGTGTCGCTGTAGT-3'	ThermoFisher Scientific	N/A
Genotyping Primer: *Brca1* intron10 sense 5'-CTGGGTAGTTTGTAAGCATCC-3'	ThermoFisher Scientific	N/A
Genotyping Primer: *Brca1* exon11 antisense 5'-CAATAAACTGCTGGTCTCAGGC-3'	ThermoFisher Scientific	N/A
Genotyping Primer: *Brca1* exon12 antisense 5'-CTGCGAGCAGTCTTCAGAAAG-3'	ThermoFisher Scientific	N/A