Cancer Cell, Volume *22*

Supplemental Information

A Distinct Replication Fork Protection Pathway Connects Fanconi Anemia Tumor Suppressors to RAD51-BRCA1/2

Katharina Schlacher, Hong Wu, and Maria Jasin

Inventory of Supplementary Information

Figure S1. FANCD1 Protects Stalled Replication Forks, Related to Figure 1

Figure S2. Variant FA Sensitivities to Mitomycin C and Camptothecin, Related to Figure 2

Figure S3. BLM, but Not FA/BRCA1, is Required for Replication Restart, Related to Figure 3.

Figure S4. Directionality of Degradation in FANCD2 Defective Cells, Related to Figure 4.

Figure S5. Sporadic Breast Cancer Cells MCF7 are Defective in Fork Protection, Related to Figure 6

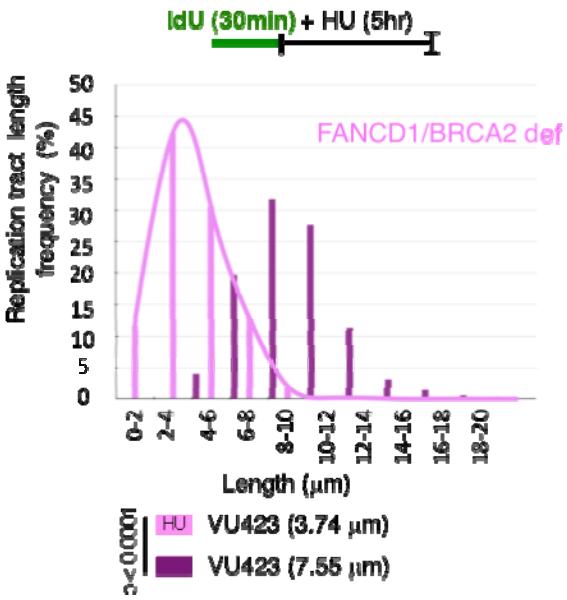

Figure S1, related to Figure 1. FANCD1 (BRCA2) defective cells show replication fork instability.

Nascent IdU tract length distribution curve measured by DNA spreading in patient-derived FANCD1 (BRCA2) defective VU423 cells show nascent strand shortening with with hydroxyurea (HU). See sketch for experimental design. Median IdU tract lengths are given in parentheses. p-value is derived from a two-tailed Mann-Whitney test.

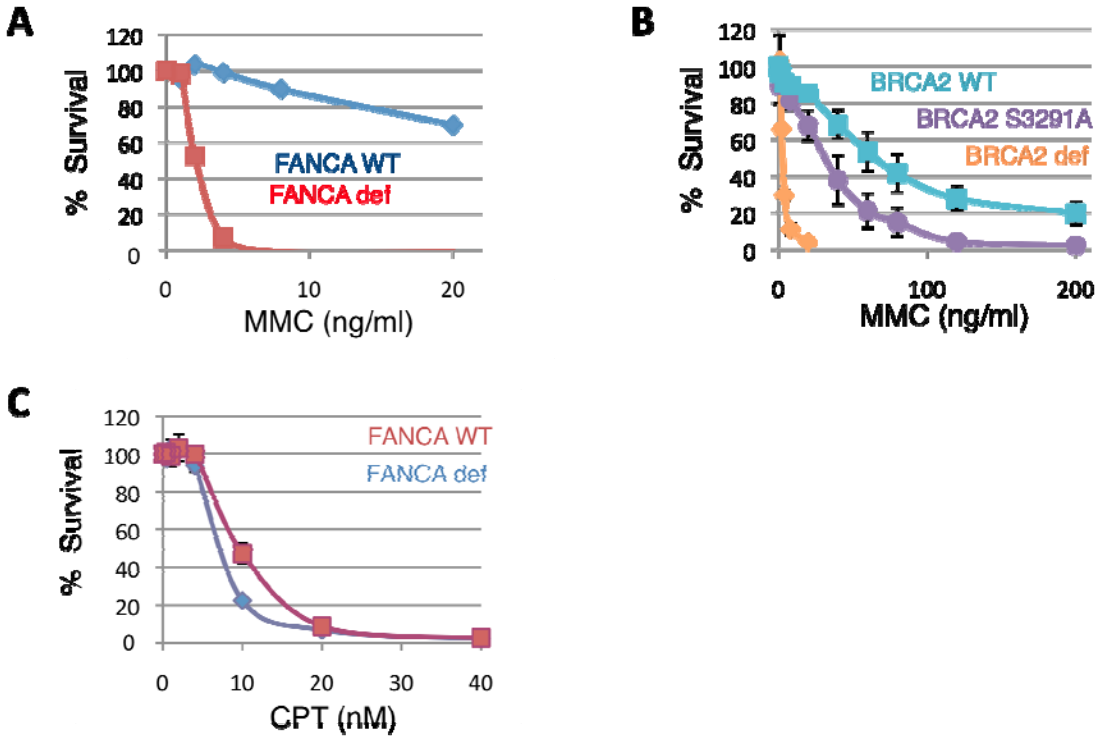

Figure S2, related to Figure 2. FA/BRCA variants show distinct cellular sensitivities to treatments with chemotherapeutic agents.

(A) Cell survival analysis of FANCA-defective, patient-derived GM6914 cells and cells complemented with FANCA upon continuous treatment with mitomycin C (MMC) (+/–SEM, n=2).

(B) Cell survival analysis of FANCD1/BRCA2-defective V-C8 hamster cells, cells complemented wild-type FANCD1/BRCA2 and V-C8 cells with separation function mutant FANCD1/BRCA2 S3291A, which is defective in fork protection but not in DSB repair, upon continuous treatment with mitomycin C (MMC) (+/–SEM, n=6).

(C) Cell survival analysis of FANCA-defective, patient-derived GM6914 cells and cells complemented with FANCA upon continuous treatment with camptothecin (CPT) (+/– SEM, n=3).

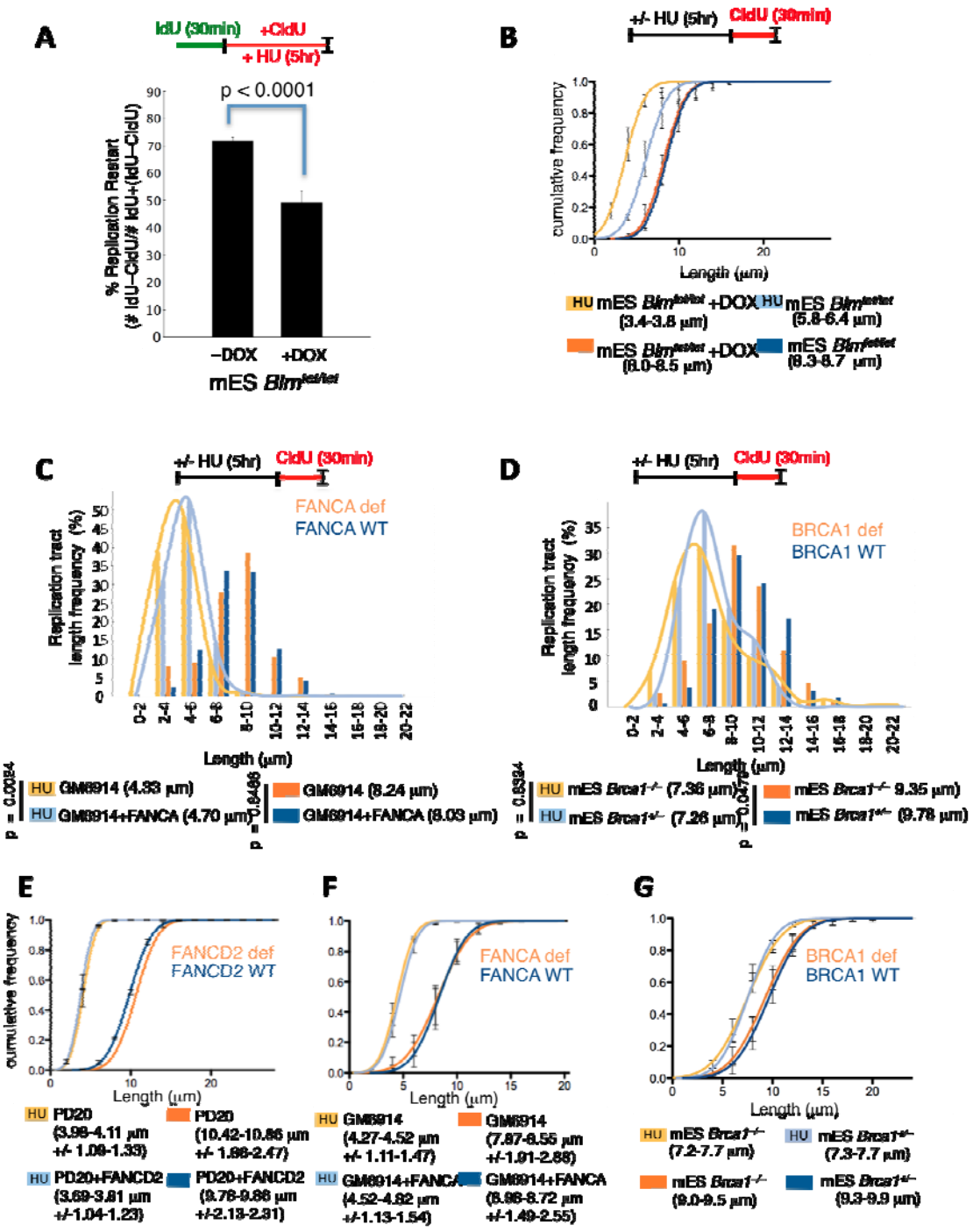

Figure S3, related to Figure 3. Replication recovery is compromised in BLM-, but not FA-deficient cells.

(A) Efficiency of replication restart by DNA spreading is measured as a fraction of the number of replication forks that restart after replication stalling with HU (IdU+CldU tracts) compared to the total number of replication forks (stalled forks (IdU tracts only) and restarted forks (IdU+CldU)) in ES cells depleted for BLM helicase (mES $Blm^{tet/tet}$ +DOX) and ES cells with wild-type BLM (mES $Blm^{tet/tet}$) (+/– SEM, n=8, 401 and 547 DNA fibres, respectively). See sketch above graph for experimental set-up. p-values are derived from Student t-test.

(B) Efficiency of replication recovery by DNA spreading is measured and cumulative frequencies for CldU tract lengths in wild-type (mES $Blm^{tet/tet}$; +/–SEM, n=2) and BLM depleted (mES $Blm^{tet/tet}$ +DOX; +/–SEM, n=3) ES cells after replication stalling with HU are presented (see sketch above graph for experimental set-up). 95% confidence interval of the mean is given in parenthesis.

(C) Cumulative frequencies for CldU tracts measuring replication recovery by DNA spreading in FANCA defective GM6914 cells and FANCA complemented GM6914 cells after replication stalling with HU. Median CldU tract lengths are given in parenthesis. p-value is derived from a two-tailed Mann-Whitney test.

(D) Efficiency of replication recovery by DNA spreading in BRCA1 defective ES cells (mES $Brca1^{-/-}$) and BRCA1 proficient ES cells (mES $Brca1^{+/-}$) after replication stalling with HU. p-value is derived from a two-tailed Mann-Whitney test.

(E)-(G). Cumulative frequencies for CldU tracts in (E) PD20 and PD20+FANCD2 cells corresponding to Figure 2D (+/–SEM, n=2), (F) GM6914 and GM6914+FANCA cells corresponding to Figure S1C (+/–SEM, n=3), and (G) BRCA1 defective and proficient

ES cells corresponding to Figure S1D (+/–SEM, n=3) are given. 95% confidence interval of the mean is given in parenthesis.

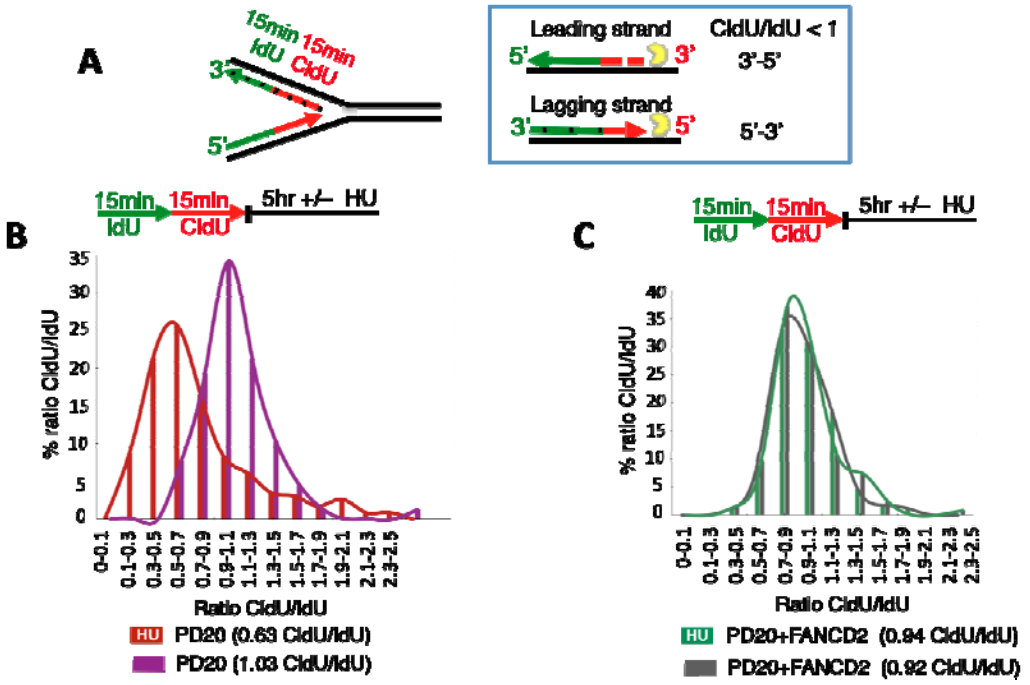

Figure S4, related to Figure 4. Nascent strand degradation occurs with distinct directionality.

(A) Sketch of design and expected outcome of double-label nuclease directionality test; Due to semi-conservative nature of DNA replication, CldU degradation only would result in 3'-5' degradation on the leading strand and 5'-3' degradation on the lagging strand. Tick marks delineate lagging strands.

(B) Distribution curves of the ratio of CldU/IdU tract lengths with or without HU in FANCD2 defective PD20 cells.

(C) Distribution curves of the ratio of CldU/IdU tract lengths with or without HU in proficient PD20 cells complemented with FANCD2.

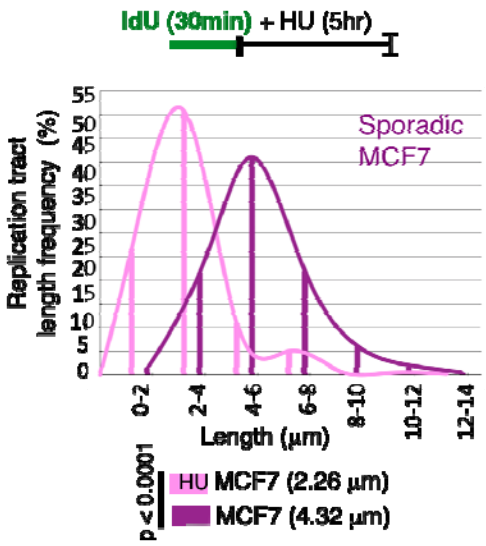

Figure S5, related to Figure 6. Sporadic breast cancer MCF7 cells show replication fork instability.

Nascent IdU tract length distribution curve measured by DNA spreading in the sporadic breast cancer cell line MCF7 show nascent strand shortening with hydroxyurea (HU). See sketch for experimental design. Median IdU tract lengths are given in parentheses. p-value is derived from a two-tailed Mann-Whitney test.