Chk1-mediated Phosphorylation of FANCE is Required for the Fanconi Anemia/BRCA Pathway

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Supplementary Materials and Methods

Mutation Analysis

The mutations were analyzed by RT-PCR amplification of total RNA purified from DF1179 cells (FA-E) and U2OS cells (control) using the specific primer pairs, then cDNA of both cell lines were analyzed by DNA sequencing using different primers spanning from exon 1 to exon 10 of *FANCE*.

Supplementary Figure Legends

Figure S1 Mutation analysis of a fibroblast line (DF1179) derived from an FA-E patient **A**. RT-PCR amplification of RNA purified from DF1179 (FA-E) cells and U2OS cells (control) was performed using the specific primer pairs, and cDNA products were analyzed by agarose gel electrophoresis. **B, C**. Mutation of the *FANCE* gene in FA-E fibroblast cell line (DF1179) was confirmed by direct DNA sequencing using

different primers spanning from exon 1 to exon 10 of *FANCE*. The chromatograms shown indicate a C to T point mutation at 1111 of *FANCE* results in a missense mutation (R371W, Arg to Trp). **D.** Complementation of MMC sensitivity of an FA-E fibroblast line, DF1179, with wild-type FANCE, but not with the double mutant of FANCE (TS/AA). MMC sensitivity of an FA-E fibroblast cell line, DF1179, with empty vector (pMMP), pMMP-FLAG-FANCEwt, pMMP-FLAG-TS/AA (the double mutant of T346A, S374A). The indicated retroviral supernatants were generated and used to transduce DF1179 cells. Puromycin-resistant cells were selected, and MMC sensitivity was determined as described in "Materials and Methods". The values shown are the mean ± standard deviation (SD) from four separate experiments.

Figure S2 Phospho-T346-FANCE foci formation in response to DNA damage

A, B. Phospho-T346-FANCE foci formation in response to lower dose of DNA damage. HeLa cells were exposed to lower dose of DNA damage: UV ($10J/m^2$), IR (2Gy) or MMC (40ng/ml) and incubated for different periods of time as indicated before fixation, immunofluorescence was performed using anti-pT346-FANCE antibody. Magnification × 400 (**A**). Cells with more than four distinct pT346-FANCE foci were counted as positive. 200 cells/sample were analyzed. The values shown are the mean ± SD from three separate experiments (**B**). **C, D.** Effects of Chk1 inhibitors on phospho-T346-FANCE foci formation. HeLa cells were pretreated without or with Chk1 inhibitors Gö6976 and SB218078 (5μM) for 30 min, and then were exposed to UV at ($60J/m^2$) and incubated for 3hr before fixation, immunofluorescence was performed using anti-pT346-FANCE antibody. Magnification, × 400 (**C**). Cells with more than four distinct pT346-

FANCE foci were counted as positive. 200 cells/sample were analyzed. The values shown are the mean ± SD from three separate experiments **(D)**.

Figure S3 **A.** FA-E fibroblasts (DF1179) stably expressing empty vector (DF1179 + Vec.), wild-type FANCE (DF1179 + FLAG-FANCEwt) or the double mutant (DF1179 + FLAG-TS/AA) were either untreated or treated with UV (60J/m^2) and incubated for 6hr, whole cell extracts were subjected to immunoprecipitation with anti-FLAG, and the immune complexes were analyzed by SDS-PAGE, followed by Western blot analysis with anti-FANCA, anti-FANCG, anti-FANCC and anti-FLAG antibodies. Heavy chain IgG was used as a loading control. **B.** U2OS, GM0637 or HEK293T cells were either untreated or treated with UV at 60 J/m^2 and incubated for 3hr or 8hr, and whole cell extracts were analyzed for Western blot with indicated antibodies. Anti- β-Tubulin blot was used as a loading control.