Supplementary Figure S1. Complementation of Fancd2/Mlh1-deficient immortalized fibroblasts. Isogenic cell lines were generated in which FANCD2 and/or MLH1 expression was restored by stable retroviral transduction. Cells that are deficient in expression of FANCD2 and/or MLH1 were transduced with an equivalent retrovirus encoding the puromycin or hygromycin selection marker only. Human FANCD2 is readily detectable in cells that were stably transduced with *FANCD2* cDNA retrovirus. Note that our FANCD2 antibody does not recognize the mouse Fancd2 protein in 3T3 cells. The deficiency of mouse Fancd2 in the immortalized fibroblasts line is concluded from genotyping results and based on MMC hypersensitivity.

Supplementary Figure S2. Complementation of Fancd2/Mlh1-deficient immortalized fibroblasts. Isogenic cell lines were generated in which FANCD2 and/or MLH1 expression was restored by stable retroviral transduction. Cells that are deficient in expression of FANCD2 and/or MLH1 were transduced with an equivalent retrovirus encoding the puromycin or hygromycin selection marker only. Human MLH1 is readily detectable in cells that were stably transduced with *Mlh1* cDNA retrovirus. Human HeLa and mouse NIH 3T3 cells were used as a positive control cells to detect expression of FANCD2 or MLH1 and make a comparison to endogenous protein levels.

Supplementary Figure S3. MlH1 increases chromosomal breakage frequencies in immortal Fancd2-deficient fibroblasts after exposure to MMC or DEB. A, B) Average number of cells displaying indicated numbers of chromosomal breaks after 48 hr of

continuous exposure to 15 ng/ml MMC or 100 ng/ml DEB. Cells with chromosomal interchanges are subtracted before displaying breakage data.

Supplementary Figure S4. Mlh1 increases chromosomal breakage frequencies in primary Fancd2-deficient fibroblasts after exposure to MMC or DEB. A, B) Average number of cells displaying indicated numbers of chromosomal breaks after 48 hr of continuous exposure to 15 ng/ml MMC or 100 ng/ml DEB. Cells with chromosomal interchanges are subtracted before displaying breakage data.