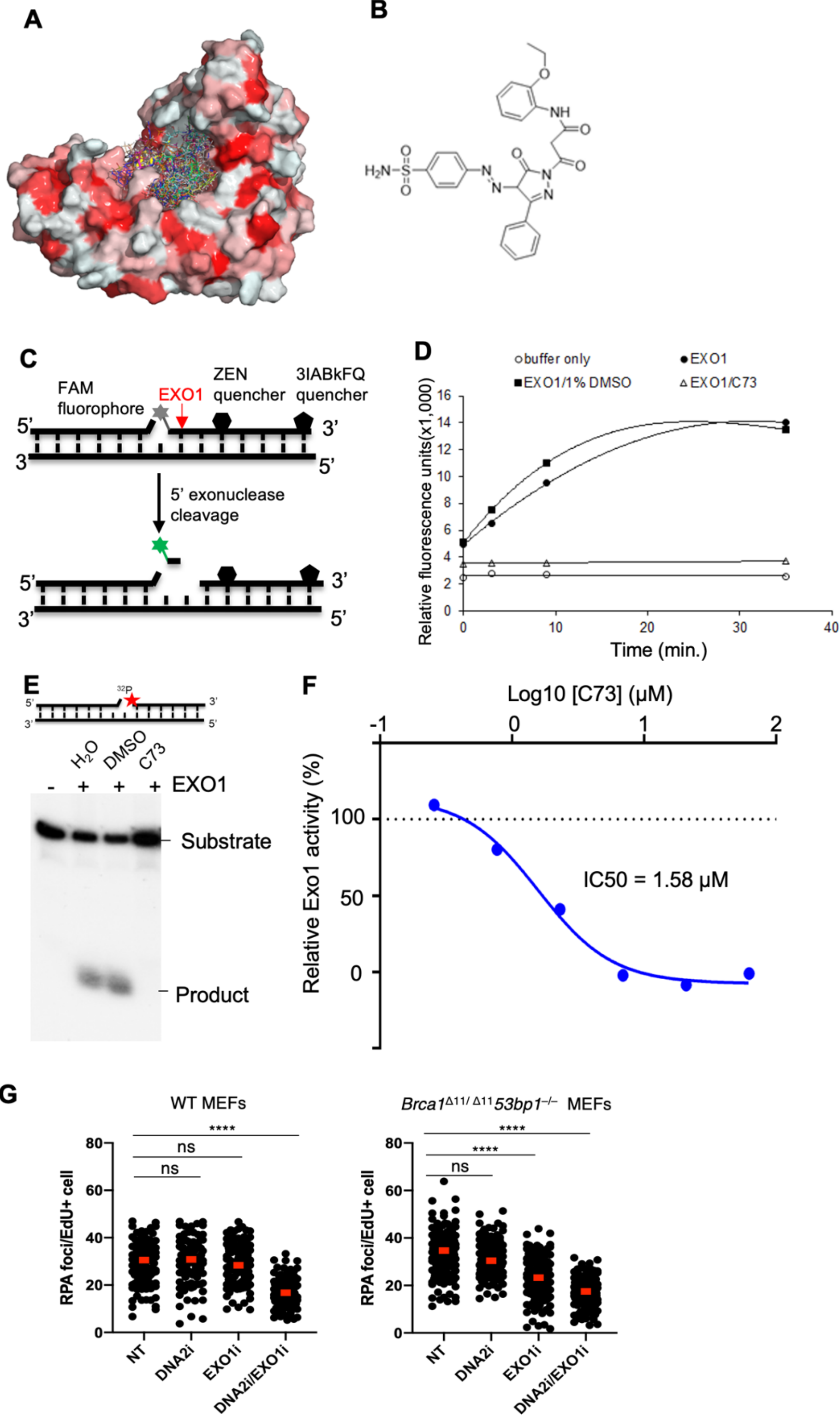
Paiano_Supp_FigS7



Supplemental Fig. S7. Discovery and validation of the novel EXO1 inhibitor C73.

A) A model for hEXO1 N-terminal domain was generated based on the X-ray structures of hEXO1 (PDB ID 5v08). The modeled hEXO1 structure was used as a target to perform molecular docking screening against NCI/DTP library which contains over 260,071 compounds. Metal-coordination constraint was applied to adopt the ligand atom to chelate the metal coordination site of the target. Top 115 hit compounds based on the docking scores were assayed for their inhibition of hEXO1 by the nuclease assay. **B)** Chemical structure of hEXO1 inhibitor C73. **C)** Schematic graph of the fluorescence resonance energy transfer (FRET)-based exonuclease assay. The nick substrate is labeled with FAM fluorophore at the 5' end of the downstream oligo, which also labeled with a ZEN quencher and a 3IABkFQ quencher internally and at the 3' end, respectively. Cleavage of the nick substrate by EXO1 will light up FAM to give green fluorescence. In the presence of EXO1 small inhibitors, complete suppression of cleavage will produce no fluorescence, while partial suppression will lead to a decrease in the fluorescence intensity. **D)** Time-dependent relative fluorescence signal (RFU) of EXO1 activity assays. FRET-DNA substrate (200 nM) was incubated with buffer only or EXO1 (0.25 nM) in the absence or presence of 1% DMSO or 10 µM C73. **E)** Assay C73 inhibiting EXO1 by 32P-based exonuclease assay. Top panel: Diagram of 5' 32P-labeled DNA substrate for EXO1 nuclease activity assay. Bottom panel: representative image of EXO1 nuclease activity assay. 32P-DNA substrate (40 nM) was incubated with buffer only or EXO1 (0.25 nM) in the absence or presence of 1% DMSO or 10 µM C73. DNA substrate and product were resolved in 15% denaturing PAGE and visualized by

autoradiography. **F)** Inhibition of EXO1 by varying concentrations of C73 were assayed by the FRET-based exonuclease assay. The RFU in the blank was subtracted from that in each reaction. The relative activity was calculated by setting the subtracted RFU in the reaction with EXO1 and 1% DMSO (control) was as 100% and comparing the subtracted RFU values in reactions in the presence varying concentrations of C73 with the control RFU. IC50 of C73 in inhibiting EXO1 was calculated. **G)** Validation of DNA2 inhibitor (DNA2i) and C73/EXO1 inhibitor (EXO1i) in WT and *Brca1*$^{\Delta 11/\Delta 11}$53bp1$^{-/-}$ mouse embryonic fibroblasts (MEFs). Cells were given 10 Gy gamma irradiation in the presence of 2 µM DNA2i, 20 µM EXO1i, or both and allowed to recover for 3 hr. Cells were incubated for 30 min with EdU, fixed, immunostained for RPA, and EdU visualized via Click-iT chemistry. RPA foci were quantified in EdU+ S phase cells on a Lionheart LX automated microscope.