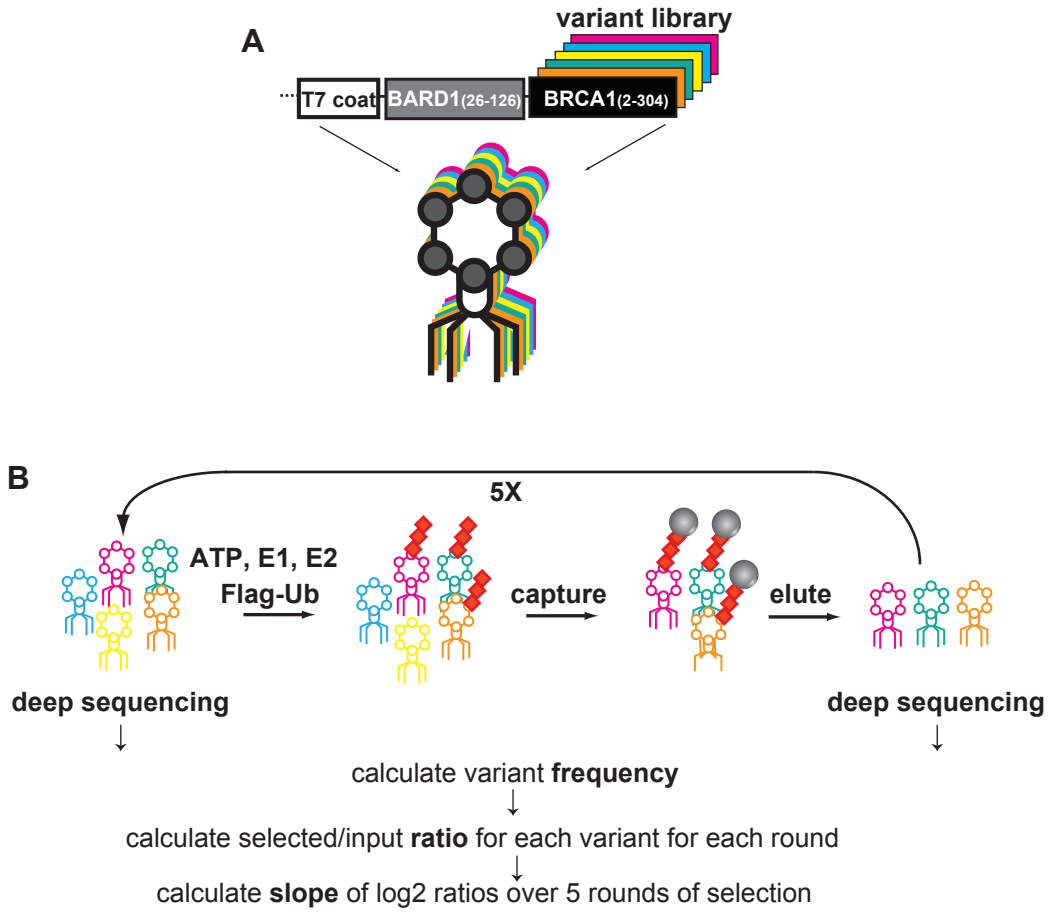

Figure S2 Scoring the effects of missense mutation on the E3 ligase activity of the BRCA1 RING domain.
(A) A fusion protein of BARD1(26-126) and BRCA1(2-304) is an active E3 ligase and capable of autoubiquitination in vitro. The allelic series of BARD1(26-126) - BRCA1(2-304) was expressed at the carboxy-terminus of the coat protein of bacteriophage T7. Residues 2-103 are the structured RING domain and lysine residues within 104-304 are required for autoubiquitination.
(B) A phage population displaying the library of BRCA1 variants was incubated in ubiquitination reactions (purified E1, E2 (UbcH5c), Flag-tagged ubiquitin and ATP), in triplicate in two separate experiments. Phages encoding active variants of BRCA1 became ubiquitinated and were collected on anti-Flag beads. After washing, elution by competition with Flag peptide and re-amplification in E. coli, phages were used in the next round of selection. Phage DNA was extracted after each of five sequential rounds of selection and the barcodes were amplified by PCR and sequenced. Barcodes were tallied by single end Illumina sequencing. After converting the barcodes to BRCA1(2-304) variants, we calculated the frequency of each variant in the input and selected populations. For each of the five rounds of selection, we fit a linear curve to the log ratio of the frequency of each variant divided by its frequency in the input population for each of the six replicates. The functional score for each variant is the slope of the fit curve, normalized by setting stop codons to a score of 0 and the wild-type to a score of 1.