Supplemental Information

Supplemental Figure 1. (A) The indicated cell lines were passaged in culture for 8 d and counted to determine cumulative population doublings (PDs) at an early (3 d) and late (8 d) time point. Error bars reflect the variability of biological triplicates. **(B)** All *BRCA2* mutations from METABRIC breast cancer study were mapped onto their location along the gene body of *BRCA2*. The type of each mutation is indicated by color and shape, and the location of each mutation in *BRCA2* is depicted graphically. The functional impact of missense mutations (damaging versus benign) was assessed using Polyphen-2. **(C)** Information about the screens performed in this study is summarized, including the background of the relevant isogenic cell line pair and the number of genes targeted by each sublibrary.

Supplemental Figure 2. (A) Volcano plot for the secondary shRNA screen, performed with the colonic *BRCA2* isogenic cell line pair. Significance ($-\log_{10}$FDR) is plotted against average log2 fold-change for each gene in *BRCA2* MUT versus *BRCA2* WT cells. Genes that met a significance threshold of $-\log_{10}$FDR>1 are color-coded as green for relative dropout or red for relative enrichment in *BRCA2* MUT vs *BRCA2* WT cells. **(B)** Results from the secondary shRNA screen in the colonic *BRCA2* isogenic background, plotted as the log2 fold-change in *BRCA2* MUT cells against the log2 fold-change in *BRCA2* WT cells. **(C)** Distribution of log2-fold changes (Log2FC) for each shRNA in the primary shRNA screen, performed in colonic *BRCA2* isogenic cell line background. **(D)** Distribution of log2-fold changes (Log2FC) for each shRNA in the secondary shRNA screen, performed in colonic *BRCA2* isogenic cell line background. **(E)** Distribution of log2-fold changes (Log2FC) for each sgRNA in the CRISPR colonic B2SL screen. **(F)** Distribution of log2-fold changes (Log2FC) for each sgRNA in the CRISPR Ovarian B2SL screen. **(G)** Schematic depicting the multicolor competition assay (MCA). GFP-labeled BRCA2 MUT cells are mixed with E2-Crimson-labeled BRCA2 WT cells. The cell mixture is passaged in the presence of a drug or transduced with a virus expressing a gRNA and Cas9. The change in percent in GFP^+ cells measured by FACS indicates the relative growth of BRCA2 MUT versus BRCA2 WT cells in the presence of the treatment. **(H)** MCA assays were performed after infection with the indicated gRNAs in the indicated isogenic cell line pairs. GFP-labeled *BRCA2* MUT cells were mixed with E2-Crimson-labeled BRCA2 WT cells and infected with lentivirus expressing Cas9 and the indicated gRNAs. Relative growth was measured as the change in GFP^+ cells by FACS after 12 d and normalized to negative control gRNA-expressing cells. The B2MUT line of one isogenic cell line pair (colonic) possesses a *BRCA2* truncation mutant that terminates after BRC repeat 5, while the *BRCA2* MUT line of a second isogenic cell line pair (pancreatic) possesses a longer mutant protein that terminates in BRC repeat 8.

Supplemental Figure 3. (A) MCA assays were performed in which ovarian GFP-labeled B2MUT cells and E2-Crimson-labeled B2WT cells were mixed and co-infected with Cas9 and negative control gRNAs.

Several negative control gRNAs were tested including gRNAs that cut intergenic regions with low predicted off-target cutting (cutting), and gRNAs that do not induce cutting by Cas9 in the human genome, targeting *E. coli* sequences (non-cutting). The relative change in percent GFP cells after 7 d of culture was quantified by FACS and plotted. In later experiments below and in the text, experimental gRNAs were normalized to the average of all negative control gRNAs. Error bars reflect the variability of biological triplicates. **(B)** Ovarian B2MUT cells were transduced with Cas9 and the indicated gRNAs, and the corresponding extracts were isolated and immunoblotted with antibodies to Ape1 and vinculin**. (C)** RNA was isolated from ovarian B2MUT cells transduced with Cas9 and the indicated gRNAs, and transcript levels of *APEX2* were quantified relative to GAPDH by qPCR. **(D)** MCA assay as described in (A) in which colonic GFP-labeled B2MUT cells were mixed with E2-Crimson-labeled B2WT cells and co-infected with the indicated cutting negative control gRNAs. **(E)** Extracts from the indicated cell lines were isolated and immunoblotted with antibodies to BRCA1, p53, or vinculin. P indicates the corresponding parental cell line while c1, c2, and c3 indicate three individual genetically modified subclones. **(F-H)** To determine synthetic lethality with BRCA1, clones c1 and c3 from (E) were compared in MCA assays as described in (A). GFP-labeled BRCA1 MUT cells and E2-Crimson-labeled BRCA1 WT cells were mixed and co-infected with Cas9 and three individual gRNAs to the indicated genes. Error bars reflect the variability of biological triplicates, and the number of asterisks indicates the statistical significance for the corresponding experiment (*=$p<0.05$,**=$p<0.01$,***=$p<0.001$).

Supplemental Figure 4. (A) Extracts from ovarian B2MUT cells, treated or untreated with an *APEX2* gRNA, were transduced with constructs constitutively expressing the indicated gRNA-resistant or negative control (NC) ORF, as described in Figure 4G. Extracts from each cell population were isolated and immunoblotted with the indicated antibodies. **(B)** Extracts from ovarian B2MUT cells, treated or untreated with an *APEX2* gRNA, were transduced with constructs constitutively expressing the indicated gRNA-resistant or negative control (NC) ORF, as described in Figure 4I. Extracts from each cell population were isolated and immunoblotted with the indicated antibodies. **(C-I)** MCA assays were performed in which the indicated GFP-labeled mutant cells and corresponding E2-Crimson-labeled wild-type cells were mixed and co-infected with Cas9 and negative control gRNAs or gRNAs to the genes indicated below. The change in percent GFP cells after 7 d of culture was quantified by FACS, and experimental gRNAs were normalized to the average of all negative control gRNAs. Error bars reflect the variability of biological triplicates, and the number of asterisks indicates the statistical significance for the corresponding experiment (*=$p<0.05$,**=$p<0.01$,***=$p<0.001$).

Supplemental Figure 5. (A) MCA assay in which GFP-labeled *BRCA1* MUT cells were mixed with E2-Crimson *BRCA1* WT cells. The cell mixture was transduced with the indicated gRNAs and Cas9. The change in percent in GFP^+ cells was quantified by FACS before and after 7 d of culture, and normalized to negative control gRNAs. Error bars reflect the variability of biological triplicates, and the number of asterisks indicates the statistical significance for the corresponding experiment (*=p<0.05,**=p<0.01,***=p<0.001). **(B)** Ovarian B2MUT cells were transduced with Cas9 and a negative control gRNA. The gRNA-resistant or negative control ORFs listed in figure 5F were subsequently transduced, and their effect on viability was quantified after 8 d of culture. Error bars indicate the variability of biological triplicates. **(C)** Effect of siRNA-mediated depletion of the indicated genes or treatment with the indicated drugs on the cell cycle distribution of ovarian BRCA2 WT cells.

Supplemental Table 1. Characterization of BRCA2 mutations found in breast tumors in the METABRIC Study. All *BRCA2* mutations found across 2,433 breast tumors in the METABRIC breast cancer mutation profiling study (Pereira et al., 2016) were downloaded from cBioPortal (Cerami et al., 2012). Each mutation was classified according to mutation type, and the functional impact of each *BRCA2* mutation was assessed by batch querying Polyphen-2 (Adzhubei et al., 2010).

Supplemental Table 2. edgeR Camera Analysis of shRNA and CRISPR screen results. Read counts were analyzed by edgeR Camera gene set analysis to determine p-values and false discovery rates (FDRs) for each gene's performance. Each of four screens was analyzed separately: (1) the primary shRNA screen performed in the colonic background, (2) a secondary shRNA screen performed in the colonic background, (3) the CRISPR colonic screen, and (4) the CRISPR ovarian screen. Entries marked as "NIL" indicate that the corresponding gene was not included in the indicated library.

Supplemental Table 3. Analysis of screen results by MAGeCK mle module. MAGeCK-VISPR analysis was performed for each screen. The beta score (see Li *et. al* 2015 for a description of beta score), p-value, and false discovery rate (FDR) was determined for every gene included in each of the four screens performed: (1) the primary shRNA screen performed in the colonic background, (2) a secondary shRNA screen performed in the colonic background, (3) the CRISPR colonic screen, and (4) the CRISPR ovarian screen. Entries marked as "NIL" indicate that the corresponding gene was not included in the indicated library.

Supplemental Table 4. MAGeCK Analysis of shRNA and CRISPR screen results. Read counts were analyzed by MAGeCK to determine the p-values and false discovery rates (FDRs) for each gene's

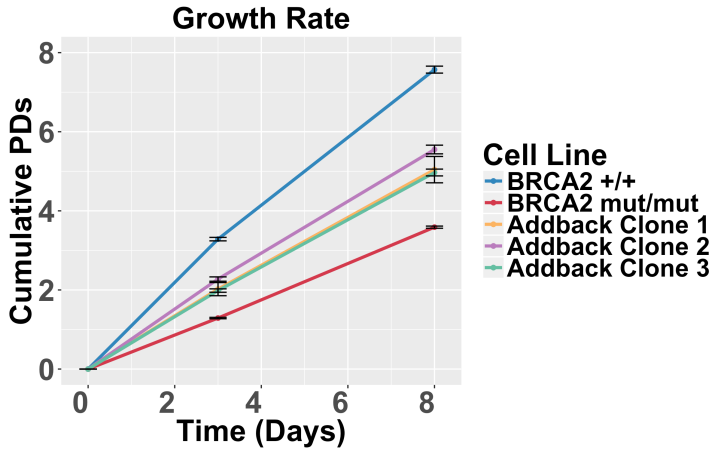
performance. Each of four screens was analyzed separately: (1) the primary shRNA screen performed in the colonic background, (2) a secondary shRNA screen performed in the colonic background, (3) the CRISPR colonic screen, and (4) the CRISPR ovarian screen. Entries marked as "NIL" indicate that the corresponding gene was not included in the indicated library.

Supplemental Table 5. ATARiS Solutions for primary shRNA screen. The ATARiS computational method (Shao et al., 2013) was employed to calculate the phenotypic score for each gene based on the read counts for 50 shRNAs per gene in the primary shRNA screen. Phenotypic scores are listed for each gene.

Supplemental Table 6. Gene Ranks for shRNA and CRISPR Screens. The ranking of each gene is listed for each of the four screens performed, as determined by edgeR camera gene set analysis and MAGeCK analysis. Overall edgeR and MAGeCK ranks from both CRISPR screens are also listed for each gene, as well as an overall CRISPR rank (see STAR Methods for details).

Supplemental Figure 1

A



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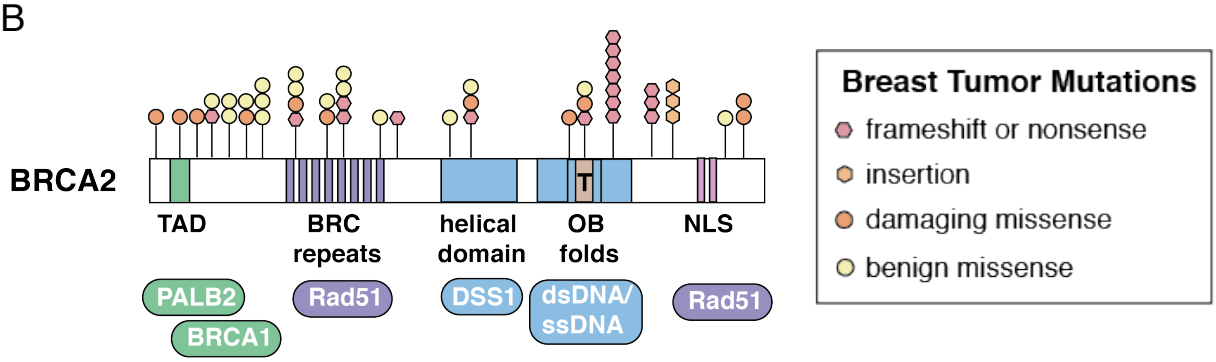
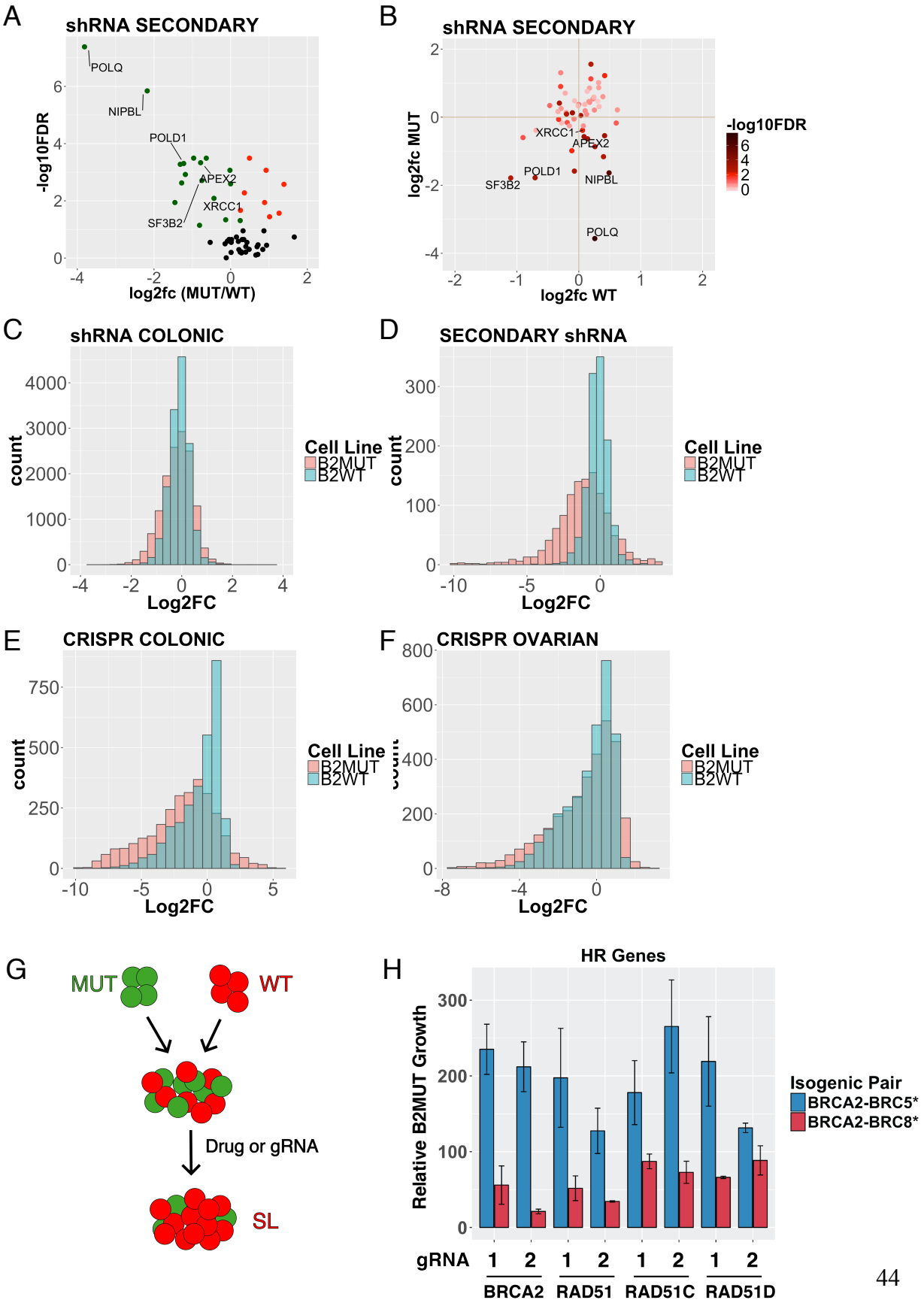


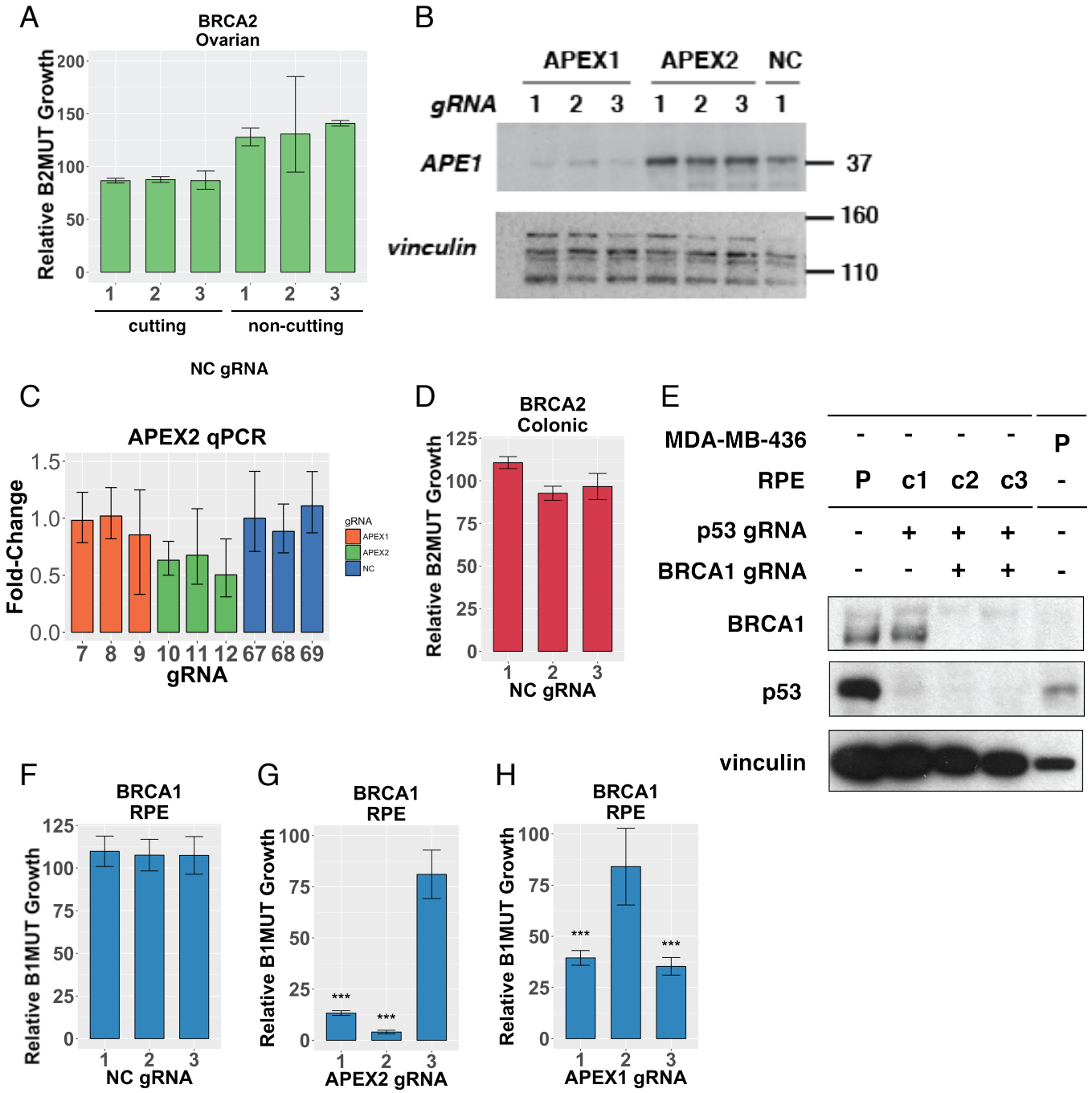
C

#	Screen	Background	# Genes	# Reagents/Gene	Reagent Type
1	Primary shRNA	Colonic	380	50	shRNA
2	Secondary shRNA	Colonic	50	10	shRNA
3	CRISPR Colonic	Colonic	357	10	sgRNA
4	CRISPR Ovarian	Ovarian	357	10	sgRNA

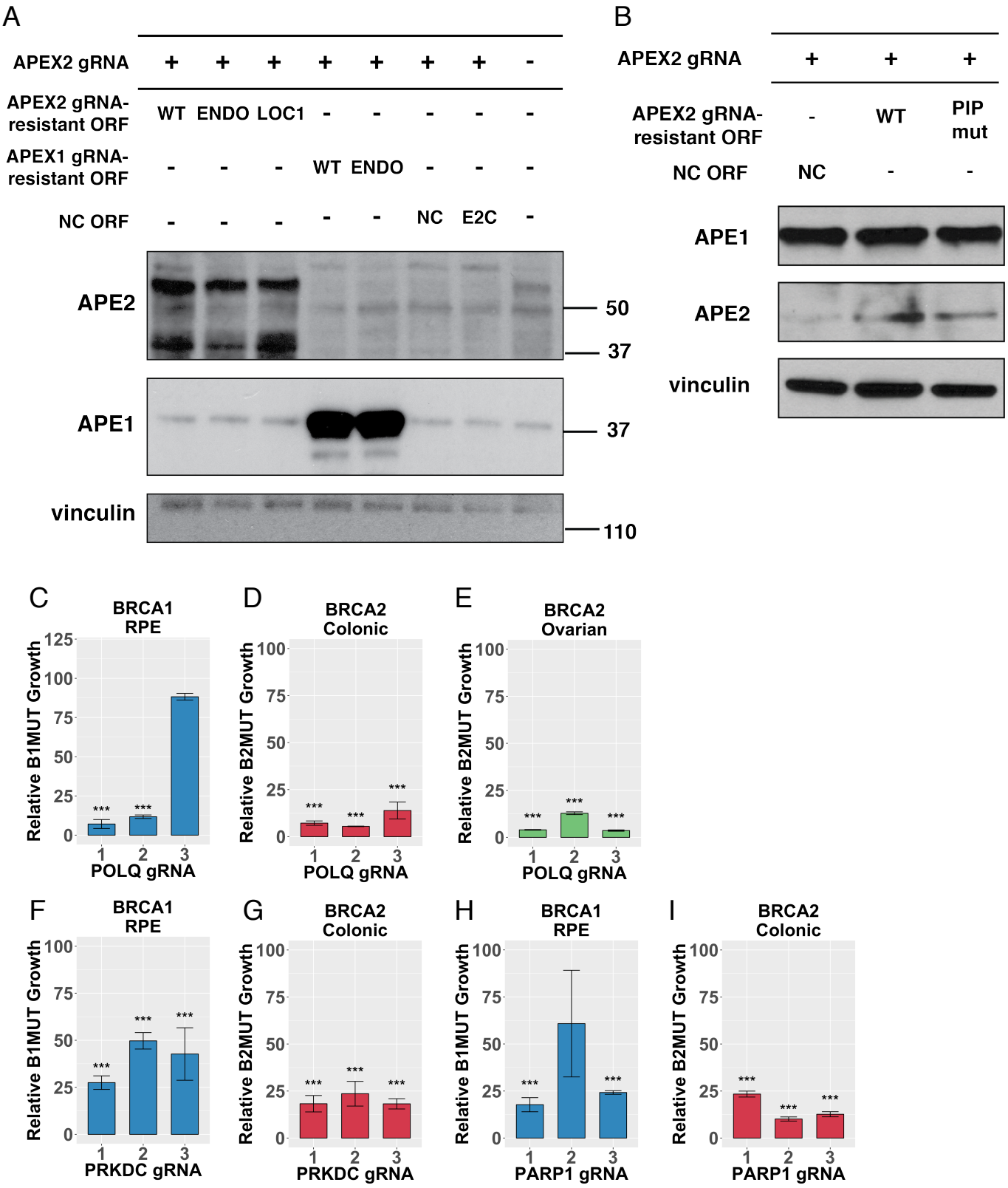
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5