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Supplementary Information for

FEN1 endonuclease as a therapeutic target for human cancers with defects in homologous recombination

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Legends for Dataset S1

Other supplementary materials for this manuscript include the following:

Datasets S1

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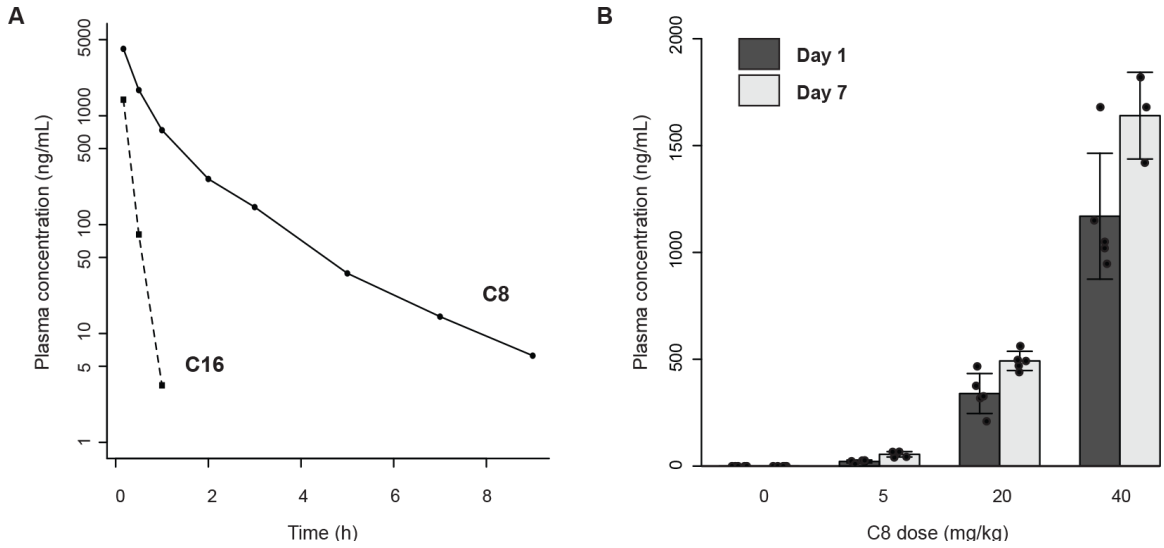

Fig. S1. Mouse pharmacokinetics and maximum tolerated dose. A. C8 and C16 were administered to groups of 3 male CD-1 mice IP at a dose of 5 mg/kg, plasma samples were obtained at the indicated time points, and the concentration of C8 and C16 determined by LC-MS/MS. **B.** C8 was administered to groups of 5 female athymic nude mice once per day for 7 days and the plasma concentrations of C8 were determined by LC-MS/MS on day 1 and day 7 at 4 hours post dose. Error bars indicate the standard deviation. Weights were recorded every day. Starting and ending mouse weights: 0 mg/kg dose weights - day 0 = 21.8 +/- 0.5 (SD) gm, day 8 = 22.1 +/- 0.1 (SD) gm; 5 mg/kg dose weights - day 0 = 21.5 +/- 0.6 (SD) gm, day 8 = 22.5 +/- 0.6 (SD) gm; 20 mg/kg dose weights - day 0 = 20.6 +/- 0.7 (SD) gm, day 8 = 22.0 +/- 0.6 (SD) gm; 40 mg/kg dose weights - day 0 = 21.3 +/- 0.5 (SD) gm, day 8 = 20.1 +/- 0.8 (SD) gm.

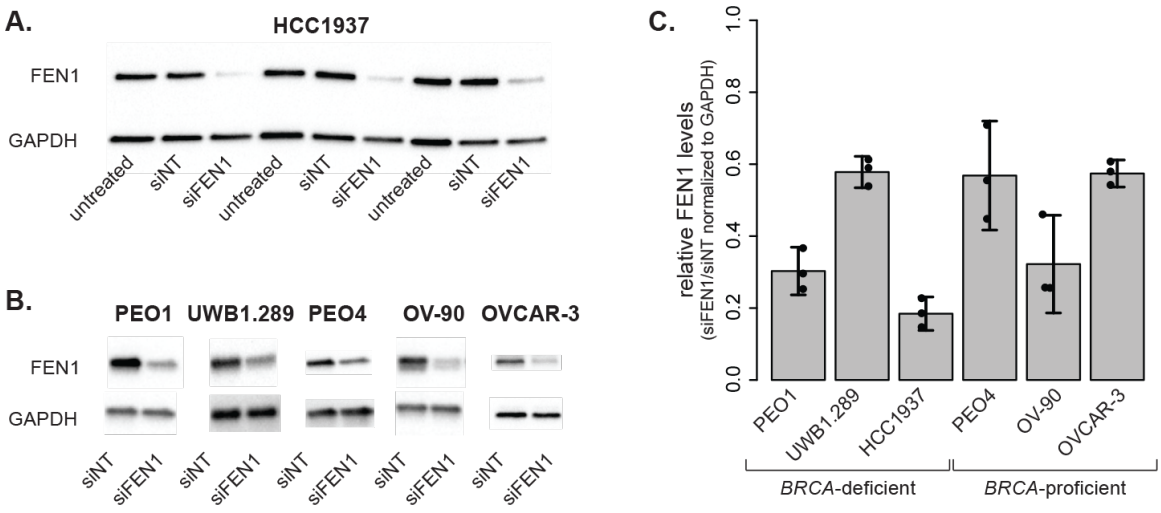

Fig. S2. siFEN1 reduces the expression of FEN1 in both *BRCA*-deficient and *BRCA*-proficient cell lines. A. FEN1 and GAPDH levels in HCC1937 after no treatment, siNT treatment, or siFEN1 treatment. Three technical replicates are shown. **B.** The average FEN1 levels in the siFEN1 treated cells relative to siNT treated cells after normalization to GAPDH levels are displayed as bars. Individual measurements are shown as overlaid points. Error bars correspond to 2 times the standard error of the mean.

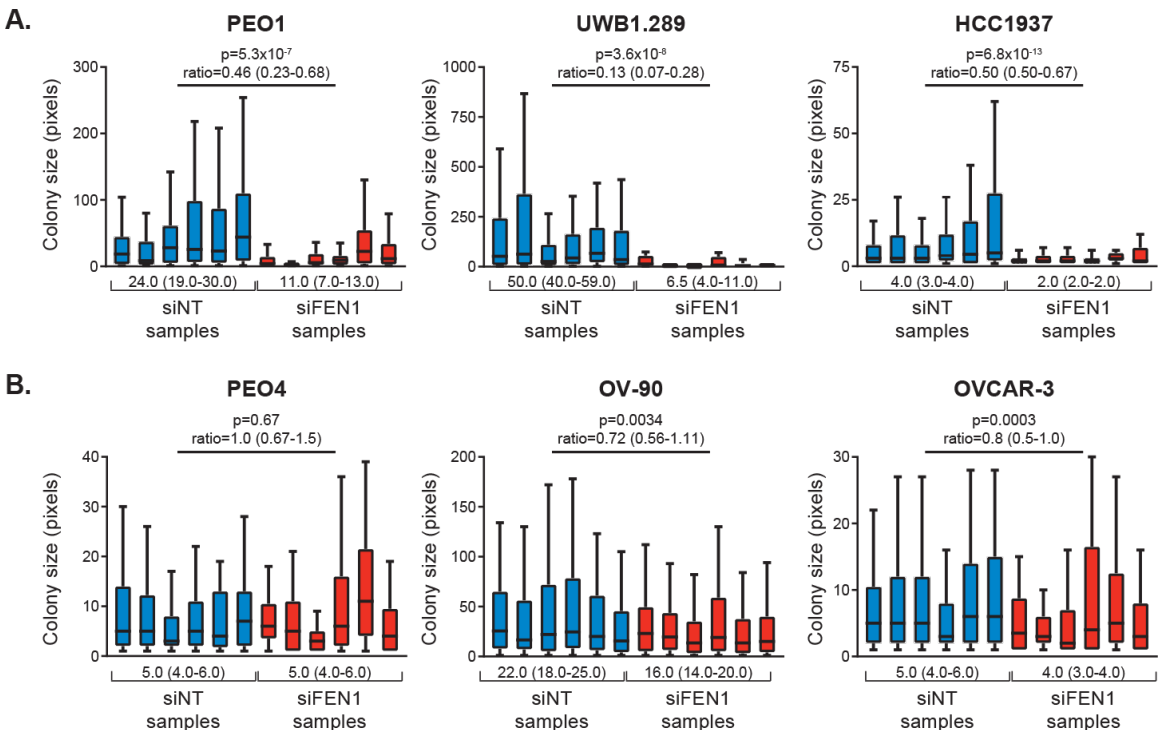

Fig. S3. FEN1 inhibition reduces the size of colonies in clonogenic survival assays in *BRCA*-deficient cell lines. The sizes of the colonies arising after treatment with siNT (blue) or siFEN1 (red) were determined for *BRCA*-deficient (**panel A**) and *BRCA*-proficient (**panel B**) cell lines for 6 independent biological replicates each (individual bars). The central bar corresponds to the median, the edges of the box are the 25% and 75% quartiles, and the whiskers show the entire data range. Reduced colony sizes were observed for the *BRCA*-deficient and two *BRCA*-proficient cell lines (OV-90 and OVCAR-3); however, the reduction in colony size was much greater for the *BRCA*-deficient cell lines. The median of the colony size and 95% confidence interval for the median (below x-axis) were determined from all replicates. This 95% confidence interval was determined by a bootstrap approach in which random samples (n=20,000) were taken with replacement from the data, and the 95% confidence interval was calculated from the 2.5% and 97.5% percentiles of the medians of the randomized samples. The ratio of the colony sizes and range of the ratio were calculated from the medians and the 95% confidence intervals of the medians. The p-values for the difference between colony sizes of the siNT and siFEN1 treated samples were calculated using a two-sided Kolmogorov-Smirnov test.

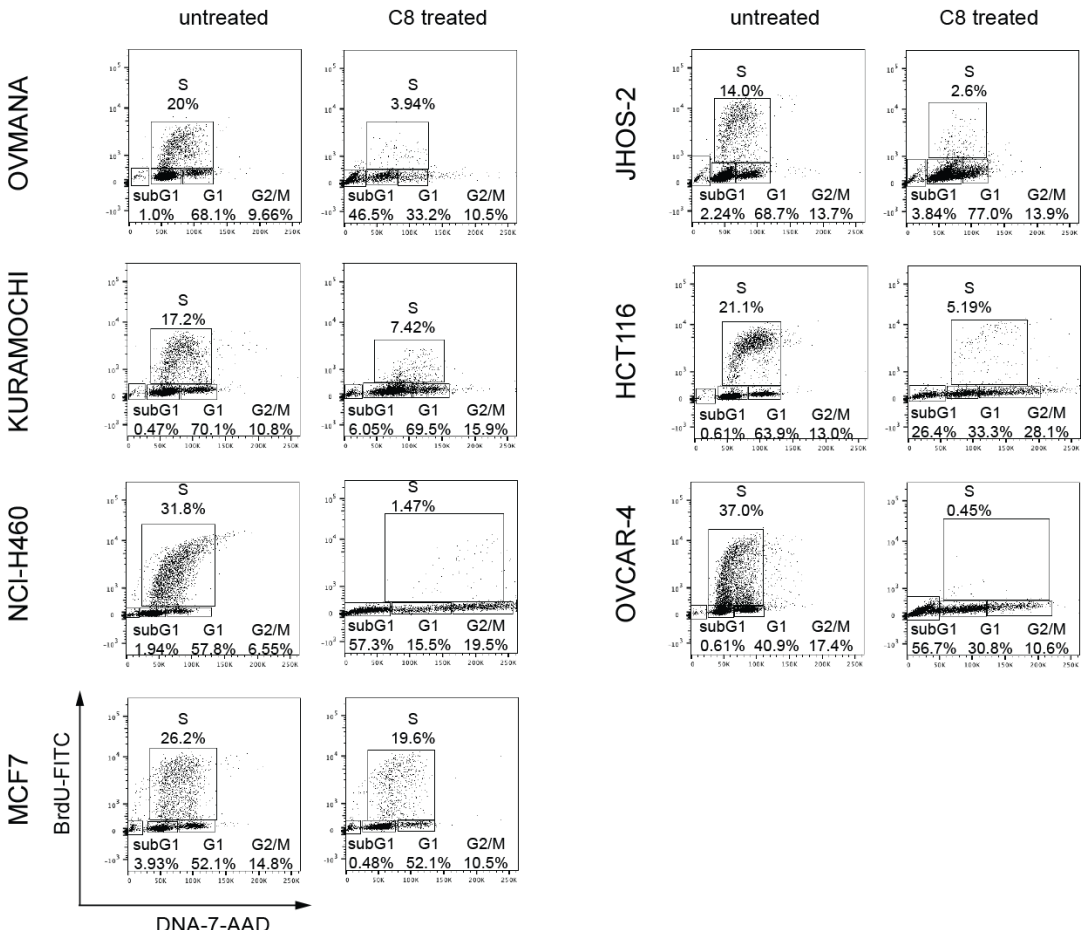

Fig. S4. Treatment of BRCA-deficient cell lines with C8 inhibits DNA replication. FACS profiles of total DNA (x-axis) and incorporated BrdU (y-axis) of cells treated with 25 μM C8 for 3 days and then pulse-labeled with BrdU for 45 min. Percentages of the number of cells in different phases of the cell cycle are shown. Rectangles depict the gates used to determine percentages.

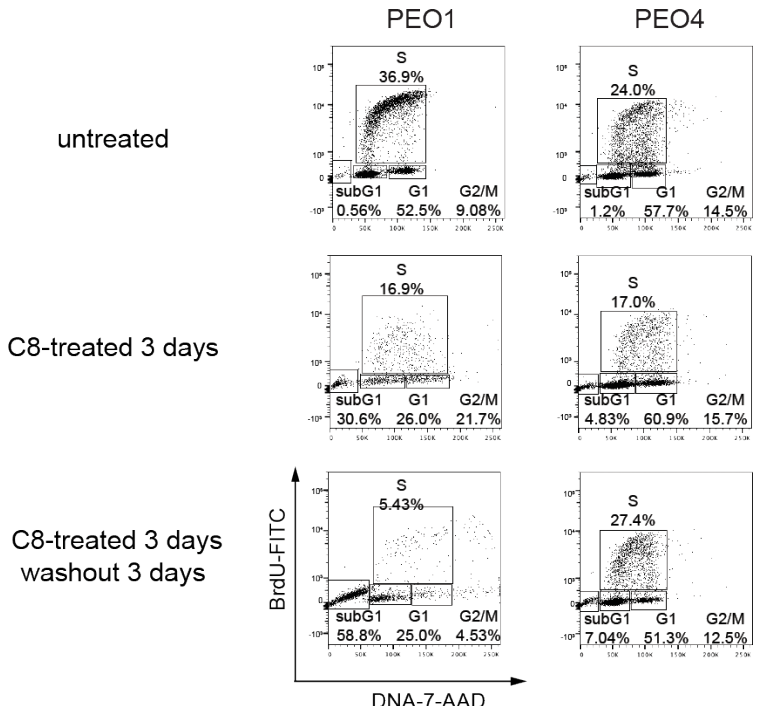

Fig. S5. PEO1 cells do not recover from C8 treatment. Both PEO1 and PEO4 cells were treated for 3 days with C8 and then shifted to medium either lacking or containing C8 for an additional three days. At each time point, cells were shifted into BrdU-containing medium for 45 minutes, and BrdU incorporation was then analyzed by FACS. FACS profiles are displayed with total DNA along the x-axis and incorporated BrdU along the y-axis.

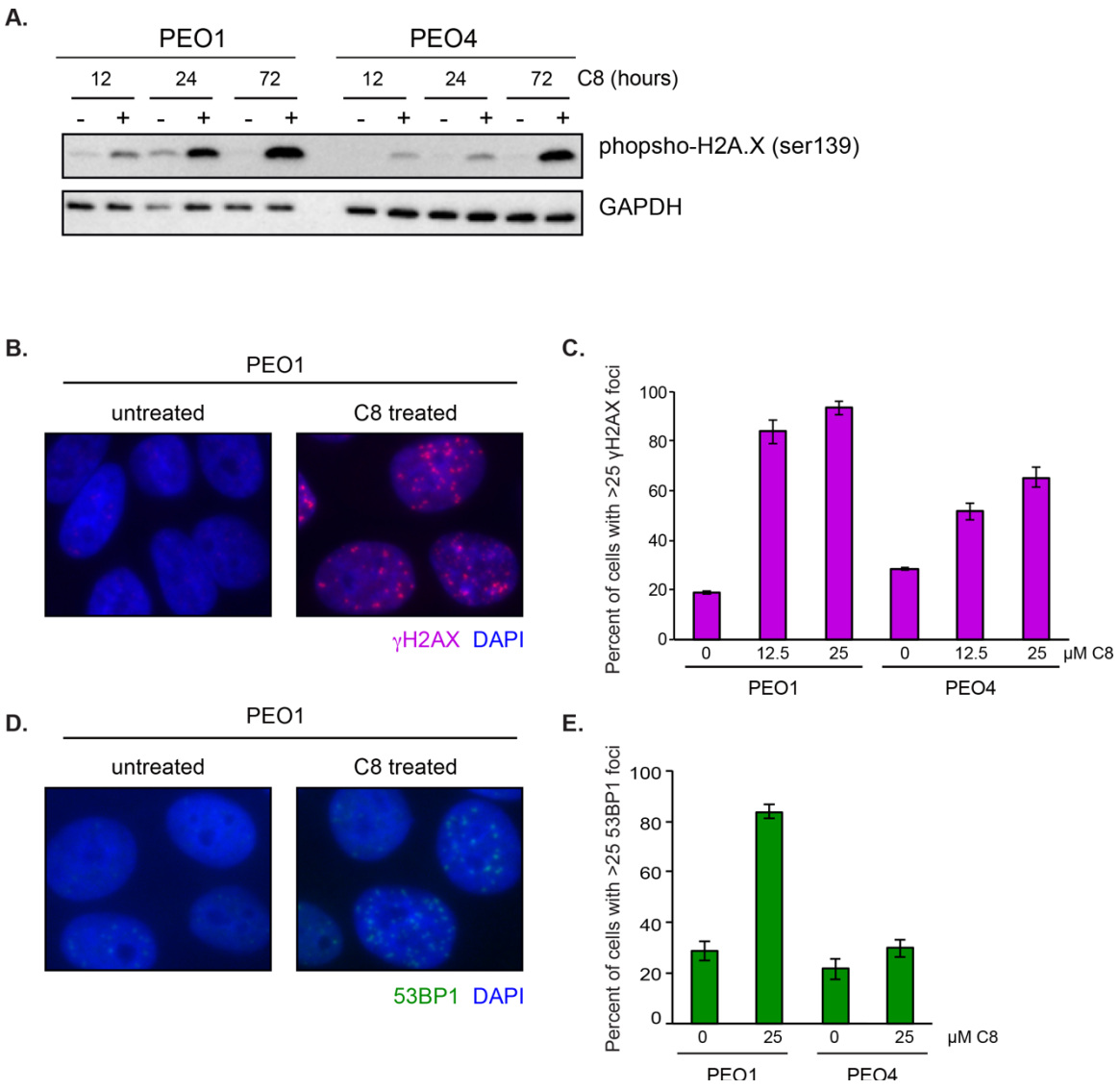

Fig. S6. C8 treatment induces DNA damage markers in both PEO1 and PEO4 cells. A. PEO1 and PEO4 cells were treated for 12, 24, and 72 hours with medium containing 25 μM C8. Cells were then lysed, and the protein extracts were analyzed by western blot using a phospho-specific γH2AX antibody and a GAPDH antibody (loading control). Both PEO1 and PEO4 showed induction of γH2AX formation upon prolonged exposure to C8. **B.** Representative images of PEO1 cells treated for 2 days with vehicle or 25 μM C8, then stained with DAPI (blue) and an antibody against γH2AX (pink). **C.** Histogram of the percent of PEO1 or PEO4 cells containing greater than 25 nuclear γH2AX foci after being treated for 2 days with 0, 12.5, or 25 μM C8. Error bars indicate the standard deviation. **D.** Representative images of PEO1 cells treated for 1 day with vehicle or 25 μM C8, then stained with DAPI (blue) and an antibody against 53BP1 (green). **E.** Histogram of the percent of PEO1 or PEO4 cells containing greater than 25 nuclear 53BP1 foci after being treated for 1 day with vehicle or 25 μM C8. Error bars indicate the standard deviation.

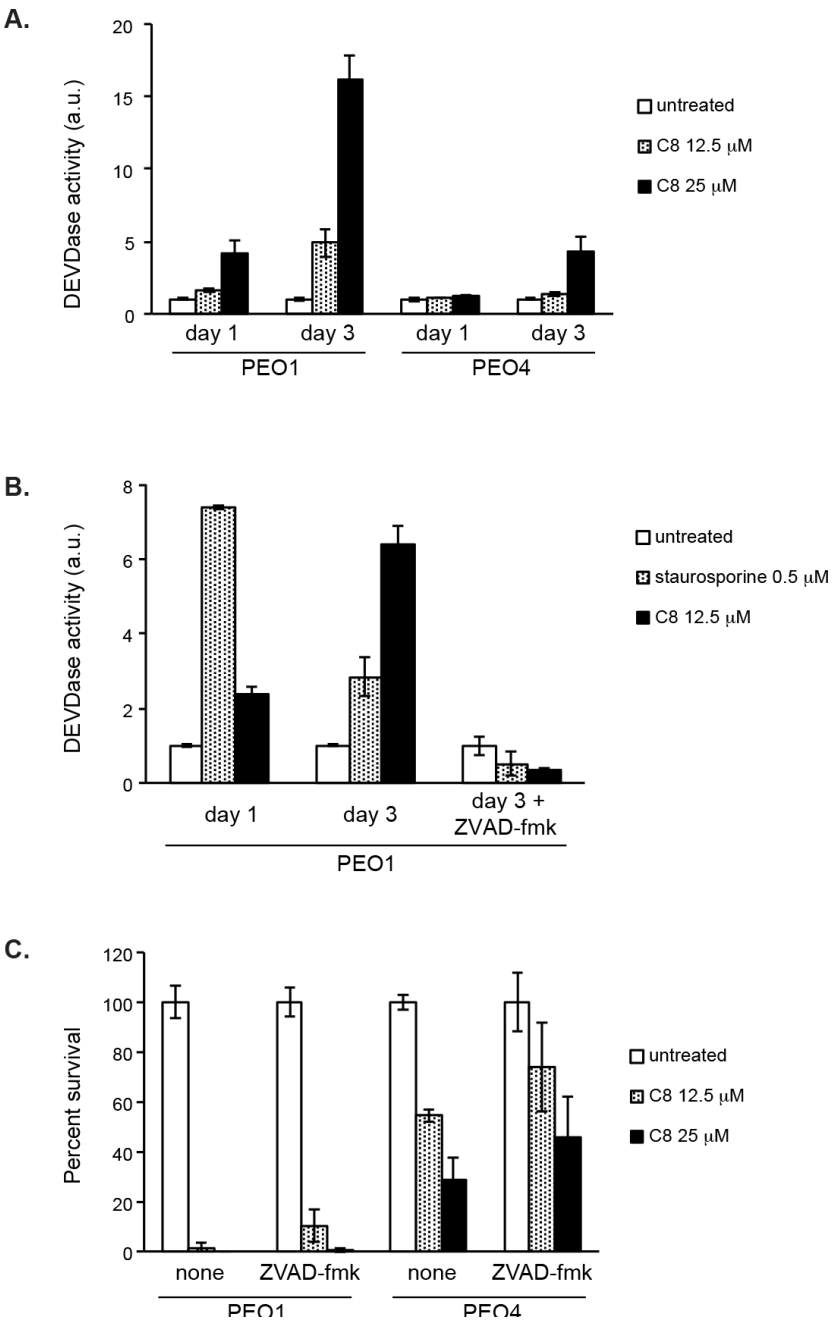

Fig. S7. C8 treatment induces caspase activity in PEO1 cells. A. Caspase activity was measured for PEO1 and PEO4 cells at 1 day and 3 days in 0, 12.5, or 25 μM C8. Higher caspase induction was observed with PEO1 cells than PEO4 cells and after 3 days of treatment. Error bars indicate the standard deviation. **B.** Caspase activity was measured in PEO1 cells after 1 day or 3 days when untreated, treated with 0.5 μM staurosporine, or treated with 12.5 μM C8. Caspase activity was highest in day 1 with staurosporine, whereas it was highest in day 3 with C8. Caspase activity at day 3 was suppressed by addition of 20 μM ZVAD-fmk caspase inhibitor. Error bars indicate the standard deviation. **C.** Clonogenic survival of PEO1 and PEO4 cells was measured after treatment with 0, 12.5, or 25 μM C8 with and without 20 μM ZVAD-fmk for 3 days followed by substitution of the media to media lacking C8 and ZVAD-fmk**.** Addition of the ZVAD-fmk caspase inhibitor only modestly increased the survival of C8-treated PEO1 and PEO4 cells. Error bars indicate the standard deviation.

Dataset S1 (separate file). *Saccharomyces cerevisiae* genes that have synthetic lethal interactions with GIS genes. Genes are identified by both the “Systematic ORF” and “Locus Name” columns. Genes that have SL interactions with GIS genes that are themselves GIS genes have a “yes” in the “Is GIS gene” column, otherwise the column contains “no”. The number of GIS genes that the gene has a SL interaction with are reported in the “Number of GIS genes with Synthetic Lethal Interactions” column.