**SUPPLEMENTARY DATA**

**SUPPLEMENTARY FIGURE 1**

**Vector for supplemental.tif**

**SUPPLEMENTARY FIGURE 2**

**D:\Shayanne\UPenn Research Fellowship\Dropbox\Synthetic Lethality\Data\Figures\Supplementary\Suppl Fig 1A.tifD:\Shayanne\UPenn Research Fellowship\Dropbox\Synthetic Lethality\Data\Figures\Supplementary\Suppl Fig 1C.tifD:\Shayanne\UPenn Research Fellowship\Dropbox\Synthetic Lethality\Data\Figures\Supplementary\Suppl Fig 1B.tifSUPPLEMENTARY FIGURE 3**

**Telomere instability quantif B 006.tifTelomere instability A repres.tifTelomere instability quantif C 012.tif**

**GFP expression in vivo A 006.tifGFP expression in vivo B 006.tifSUPPLEMENTARY FIGURE 4**

**SUPPLEMENTARY FIGURE 5**

**Apoptosis representative both.tif**

**FIGURE LEGENDS**

**Supplementary Figure 1. Construction of Ad-Nbs1 vector and its disruptive effect on wild-type Nbs1 expression.** The constructed adenoviral vector contains the coding for GFP protein as a reporter and for the last 300 amino acids of the Nbs1 gene, leading to the expression of a truncated protein, much like the product in Nijmegen Breakage Syndrome, which lacks the FHA/BRCT domain, but contains the Mre11 binding domain.

**Supplementary Figure 2. Baseline BRCA and hTERT protein expression, and ALT status.** (A) Western blot analysis shows wild-type expression of BRCA and hTERT in both JHU006 and JHU012 cell lines. (B) Intact hTERT activity in JHU006, JHU012, as well as in the positive control lanes as demonstrated by the presence of a ladder of amplification products in 6 base pair increments. In contrast, heat-inactivated control lanes do not show the characteristic ladder. (C) Dot blot image of the C-circle assay demonstrates nearly undetectable telomeric signal in JHU006, JHU012, and HeLa (negative control) when incubated with or without Φ29 DNA polymerase. Conversely, the well known ALT positive Saos-2 cell line demonstrated more than 20-fold increase in telomeric signal when incubated with Φ29 DNA polymerase. A representative image and quantification of C-circles (A.U.) from two independent experiments are shown. Error bars represent SD.

**Supplementary Figure 3. Telomere instability.** (A) Representative picture of the loss of telomere signal at the ends of chromosomes in JHU006 as an indicator of telomeric genomic instability. For better contrast, the chromatin (DAPI) channel was pseudocolored red, and the Telomere (Cy3) channel was pseudocolored green. The white arrows indicate absence of telomeric signal. (Original magnification, X100). (B and C) Quantified data shows significant telomeric loss 48h after PARPi monotherapy, or after combination of Ad-Nbs1 with PARPi, compared with Control and Ad-Nbs1 treatment groups, respectively, in both JHU006 and JHU012 cell lines. Percentage ± SEM is shown. At least 500 chromosomes were evaluated per sample (\*\*P<0.01; \*\*\*P<0.001).

**Supplementary Figure 4. In vivo GFP expression in tumor xenografts.** (A) Merged molecular imaging pictures, black & white (BW)+GFP, or XRAY+GFP, at day 3 after tumor injection with Ad-Nbs1 demonstrate efficient infection and transduction of the dominant-negative vector of JHU006 tumor xenografts *in vivo*. (B) Seven days later, the Ad-Nbs1-treated JHU006 tumor xenograft was harvested and subjected to flow-cytometry analysis which demonstrated 22.5% of cells were still expressing GFP.

**Supplementary Figure 5. Apoptosis in tumor xenografts.** (A) TUNEL staining was performed on JHU006 and JHU012 HNSCC xenograft tumor samples after harvest, 7-10 days post-treatment. Representative high-powered field images from each treatment group for both cell lines are shown. Cells stained with brown (DAB) are considered positive for apoptosis. The cells nuclei were counterstained with methyl-green (blue-green color). (Scale Bar = 100µm; Original magnification, X20)