Supplementary Figure S1

Model	*NRG1* alteration	Tissue of origin	Patient-derived/isogenic	Cell Line/xenograft	Source
MDA-MB-175-VII	*PPP6R3-TENM4-NRG1*	Breast	Patient-derived	Cell line	ATCC
MCF7-EV	None	Breast	Isogenic control	Cell line	MSKCC
MCF7-DOC4-NRG1	*DOC4-NRG1*	Breast	Isogenic	Cell line	MSKCC
HCC95	*NRG1* amplification	Lung	Patient-derived	Cell line	ATCC
HBEC	None	Lung	Isogenic control	Cell line	MSKCC
HBEC-CD74-NRG1	*CD74-NRG1*	Lung	Isogenic	Cell line	MSKCC
HBEC-VAMP2-NRG1	*VAMP2-NRG1*	Lung	Isogenic	Cell line	MSKCC
H6c7-EV	None	Pancreas	Isogenic control	Cell line	MSKCC
H6c7-ATP1B1-NRG1	*ATP1B1-NRG1*	Pancreas	Isogenic	Cell line	MSKCC
H6c7-SLC3A2-NRG1	*SLC3A2-NRG1*	Pancreas	Isogenic	Cell line/xenograft	MSKCC
CTG-0943	*APP-NRG1*	Pancreas	Patient-derived	Xenograft	Champions Oncology
LUAD-0061AS3	*SLC3A2-NRG1*	Lung	Patient-derived	Cell line/xenograft	MSKCC
OV-10-0050	*CLU-NRG1*	Ovarian	Patient-derived	Xenograft	Wuxi AppTec
ST3204	*CD74-NRG1*	Lung	Patient-derived	Xenograft	XenoStart
ST2891	*CD74-NRG1*	Lung	Patient-derived	Xenograft	XenoStart

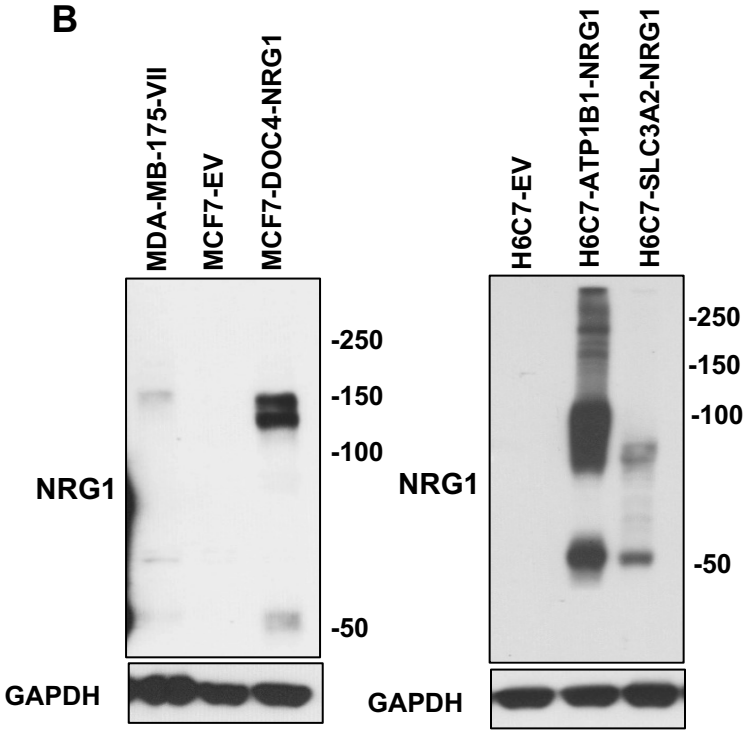
Supplementary Figure S1. Characteristics of cell lines and PDX models used in this study. NRG1 fusions or empty vectors were introduced by lentiviral-mediated overexpression. Cells stably expressing the fusions were selected with antibiotics. HBEC: immortalized (expression of TERT, CDK4, dominant negative p53 mutant) human bronchiolar epithelial cells. H6C7: immortalized (expression of viral E6 and E7 proteins) human pancreatic ductal epithelial cells. MCF7: triple-negative breast cancer cell line. EV: empty vector. ATCC: American Type Culture Collection. MSKCC: Memorial Sloan Kettering Cancer Center

Supplementary Figure S2

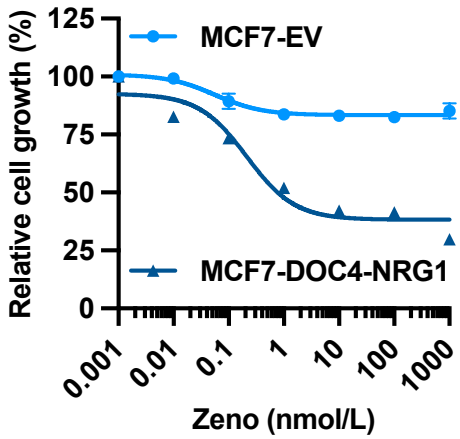
A

Cell Line	IC_{50} For Growth Inhibition (nmol/L)
HBEC-CD74-NRG1	0.05 (0.01-0.22)
HBEC-VAMP2-NRG1	0.11 (0.01-2.1)
HBEC	2995 (847.3-ND)
LUAD-0061AS3	14.2 (2.8-105)
HCC95	0.15 (0.03-1.43)
MCF7-DOC4-NRG1	2.01 (0.36-23.50)
MCF7-EV	4811 (2128-39,251)
MDA-MB-175-VII	0.04 (0.14-1.3)

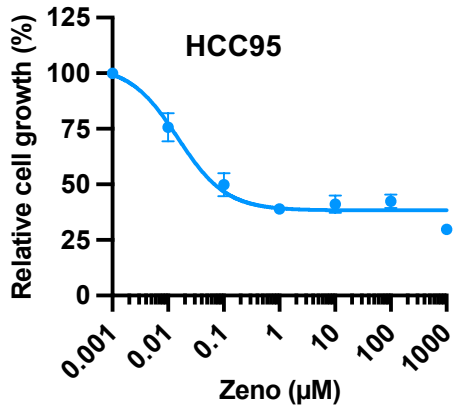
B



C

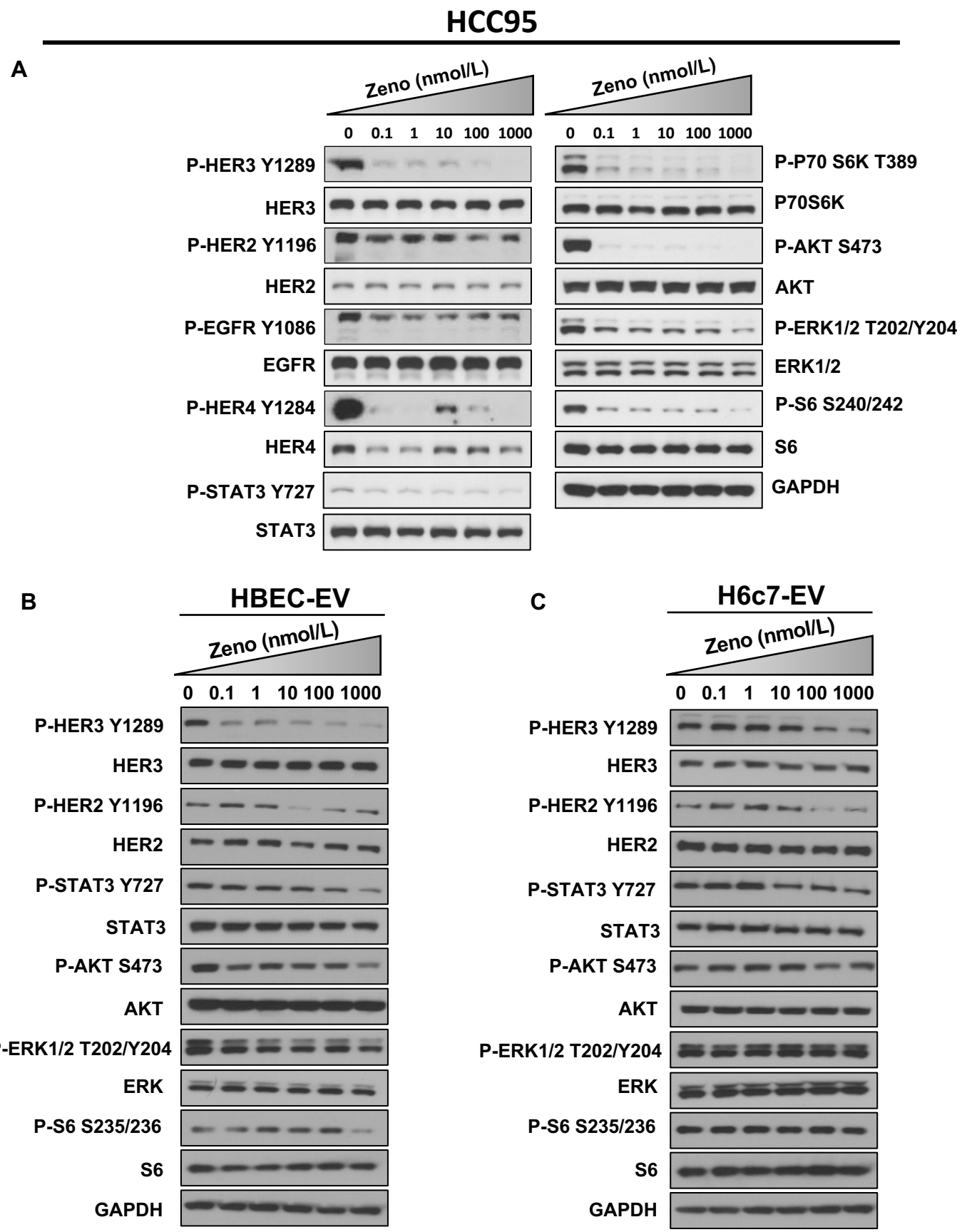


D



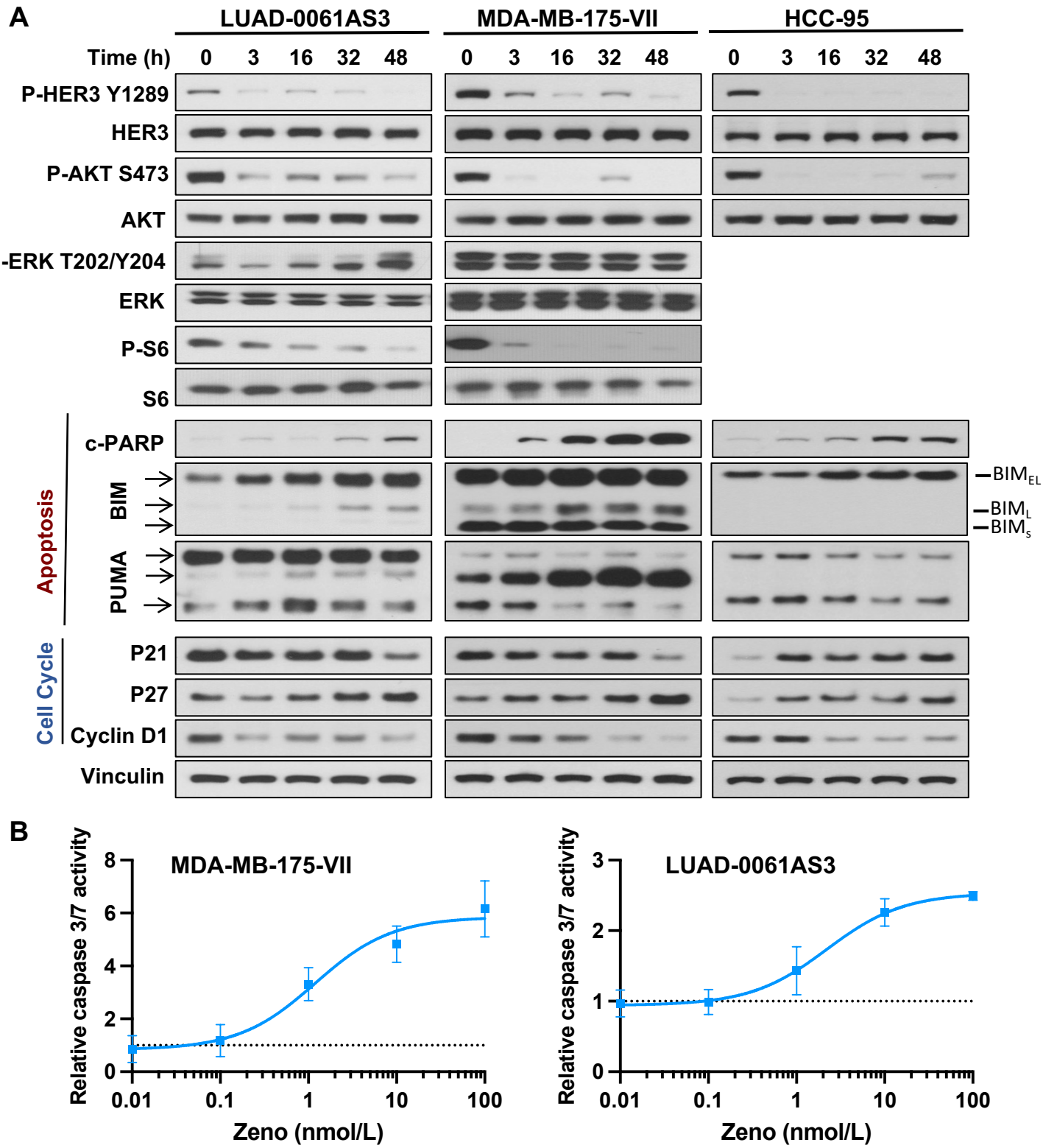
Supplementary Figure S2. IC_{50} values for inhibition of growth by Zeno and expression of NRG1 fusions and . A. Cells were treated with Zeno for 96 hours and then growth determined using AlamarBlue viability dye. Results are expressed relative to the vehicle-treated controls (100%). Data was analyzed by non-linear regression for curve fitting and generation of IC_{50} values. The 95% confidence interval (CI) is given in brackets. Results are from one representative experiment in which each condition was assayed in 3-4 replicates and repeated at least two times. **B.** Expression of NG1 was examined by western blotting. The MDA-MB-175-VII cell line was used as a control for the DOC4-NRG1 fusion. **C** and **D**. Cells were treated with the indicated concentrations of Zeno for 96 h and then viability was determined. Data was analyzed by non-linear regression to determine IC_{50} values for inhibition of growth (see A for IC_{50} values). Results represent the mean ± SD of three replicate determinations in one experiment. ND: Curve could not be fitted to accurately calculate an upper limit.

Supplementary Figure S3



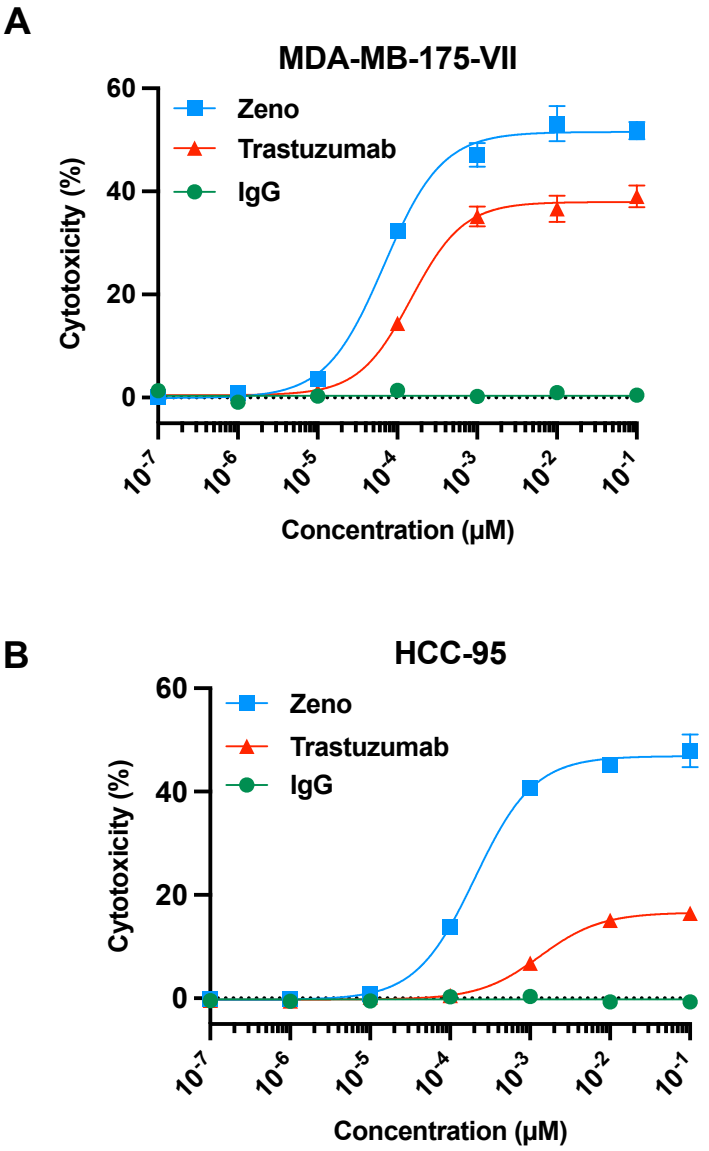
Supplementary Figure S3. Inhibition of signaling by Zeno in HCC95, HBEC-EV and H6c7-EV cells. A-C. Cells were deprived of serum for 24 h and then treated with Zeno for 1.5 hours. Whole-cell extracts were then immunoblotted for the phosphorylated or total proteins indicated. Western blotting was conducted in two independent experiments and representative immunoblots are shown.

Supplementary Figure S4



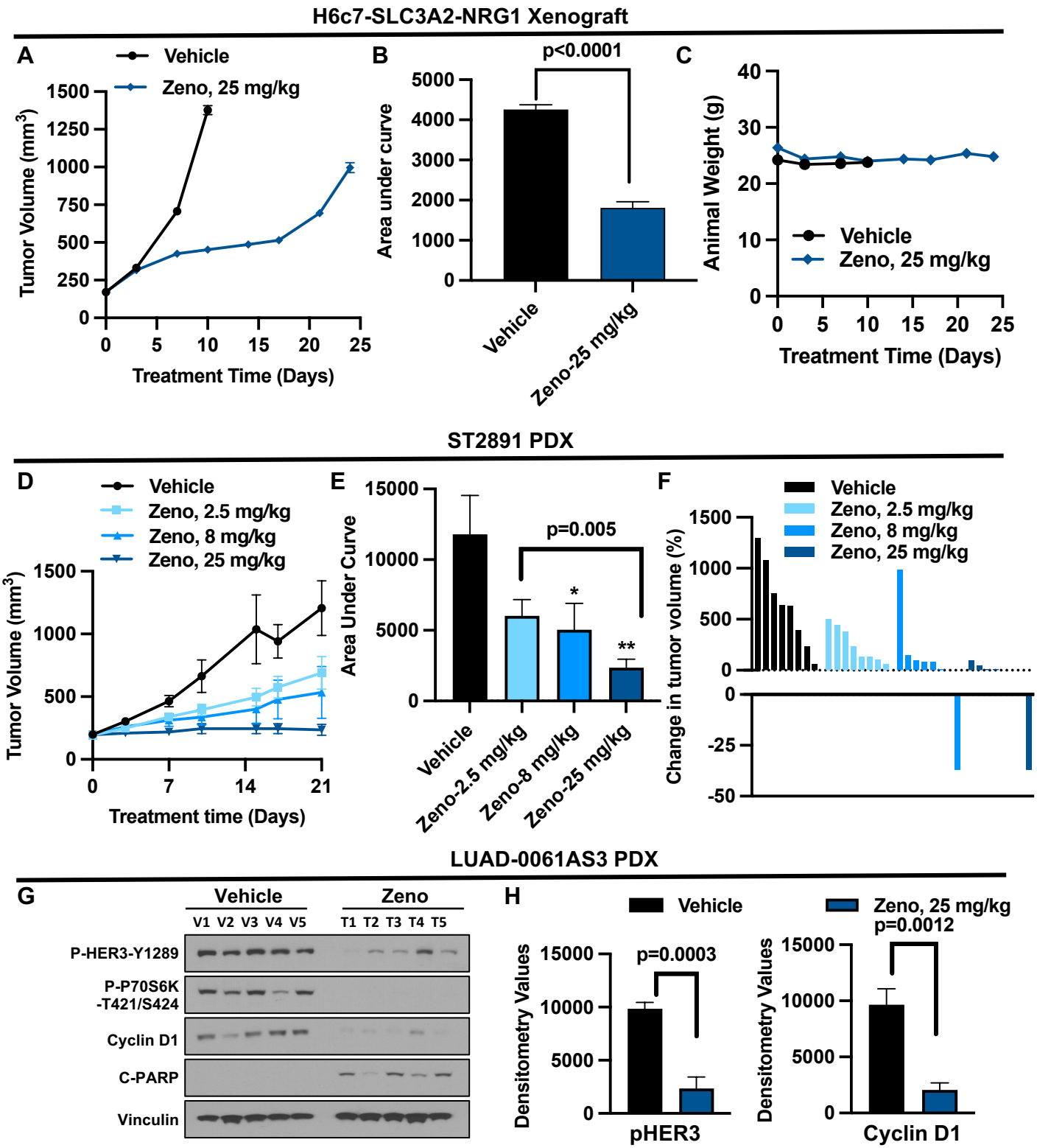
Supplementary Figure S4. Time course of Zeno action and induction of apoptosis. A. Cells were treated with Zeno (50 nmol/L) for up to 48 h and then cell extracts were prepared for western blotting. Representative immunoblots are shown with vinculin expression used as a western blotting loading control. At least two independent experiments were conducted. **B.** MDA-MB-175-VII (**left)** and LUAD-0061AS3 (**right**) cells were treated with the indicated concentrations of Zeno for 72 h then caspase 3/7 enzymatic activity determined in cell homogenates. Results represent the mean ± SD of 6 replicates in one experiment. Caspase activity in DMSO (vehicle)-treated cells was arbitrarily assigned a value of 1 (dotted line) and all other data points are expressed relative to this.

Supplementary Figure S5



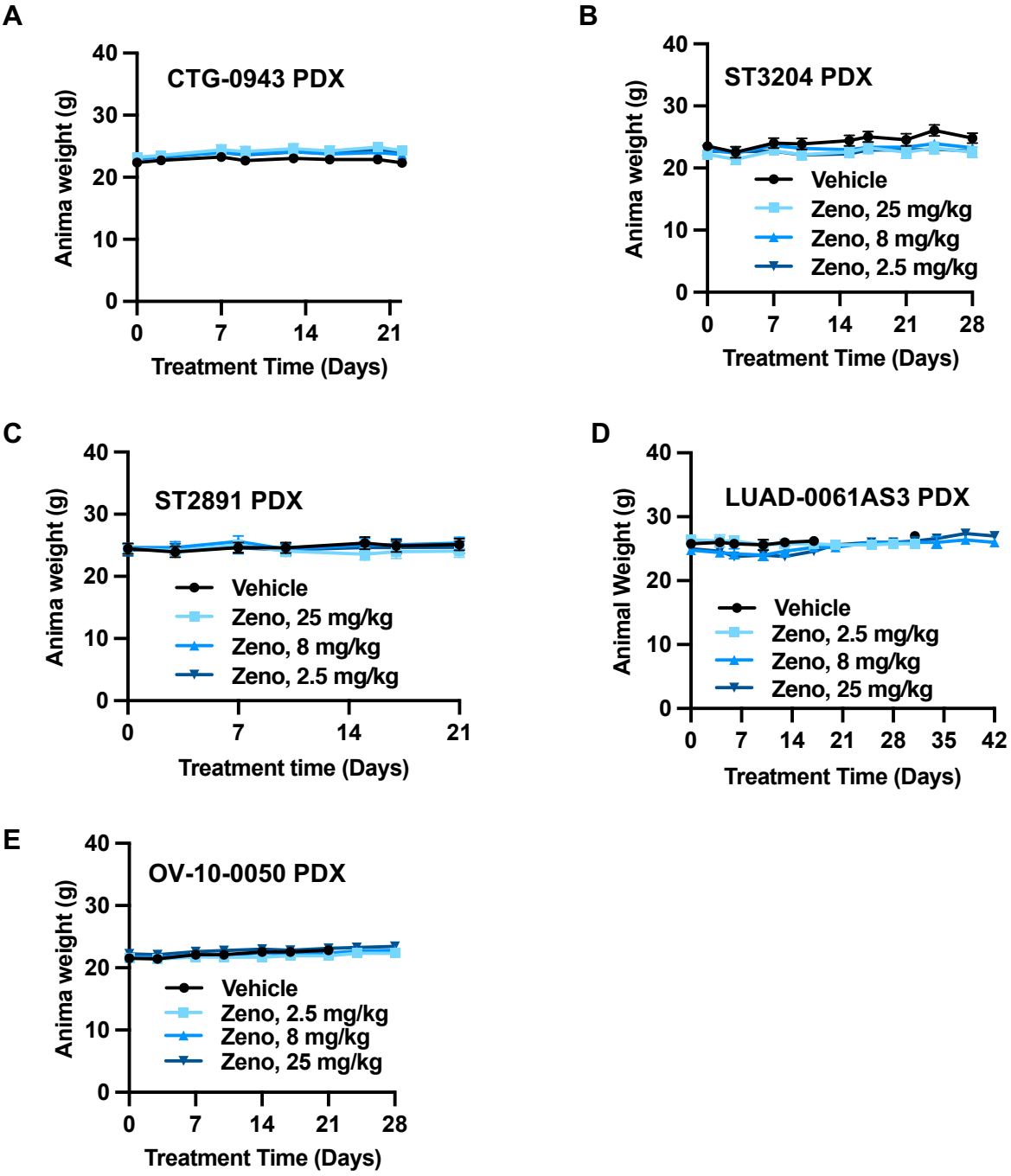
Supplementary Figure S4. Induction of antibody-dependent cellular cytotoxicity (ADCC) by Zeno. Chromium release assay was used to assess ADCC activity of Zeno in comparison to trastuzumab and non-specific IgG1 in MDA-MB-175-VII (**A.**) and HCC-95 (B) cells. Results represent the mean ± SD of three replicates in one experiment.

Supplementary Figure S6



Supplementary Figure S6. Efficacy of Zeno *in vivo*. Mice bearing H6c7-SLC3A2-NRG1 xenograft tumors (**A-C**, 5 mice per group) or ST2891 PDX tumors (**D-F**, eight mice per group) were treated as indicated once weekly. Tumor size and animal weight were measured twice weekly. Tumor volume (**A** and **D**). Animals were weighed twice weekly (**C**). Zeno treatment did not adversely affect weight or animal health during the study. Vehicle-treated tumor in **A** reached the maximum allowable volume by day 10 and animals were sacrificed. Area under cure analysis (**B** and **E**). *P=0.04. **P=0.0014. **F.** Percent change in the volume of individual tumor at the end of the study (day 21). **G**. Mice bearing LUAD-0061AS3 PDX tumors (5 mice per group) were treated twice with vehicle or Zeno, 25 mg/kg, QW. Tumors were harvested 24 after the second dose and processed for western blotting analysis. **H.** Phosho-HER3 (**left**) and cyclin D1 (**right**) immunoblots were quantitated by densitometry. Data represents the mean signals ± SEM of five vehicle- or five Zeno-treated tumors

Supplementary Figure S7



Supplementary Figure S7. Weight of animals bearing PDX tumors. Mice bearing PDX tumors derived from CTG-0943 (A), ST3204 (**B**), ST2891 (**C**), LUAD-0061AS3 (**D**) and OV-10-0050 (**E**) models were treated as indicated with vehicle or Zeno, once weekly. Animals were weighed twice weekly. Zeno treatment did not adversely affect weight ($p>0.05$) or animal health during the study.