Supplementary Data for

GnRH-R targeted lytic peptide sensitizes BRCA wild-type ovarian cancer to PARP inhibition

Running title: EP-100 sensitizes ovarian cancer cells to olaparib

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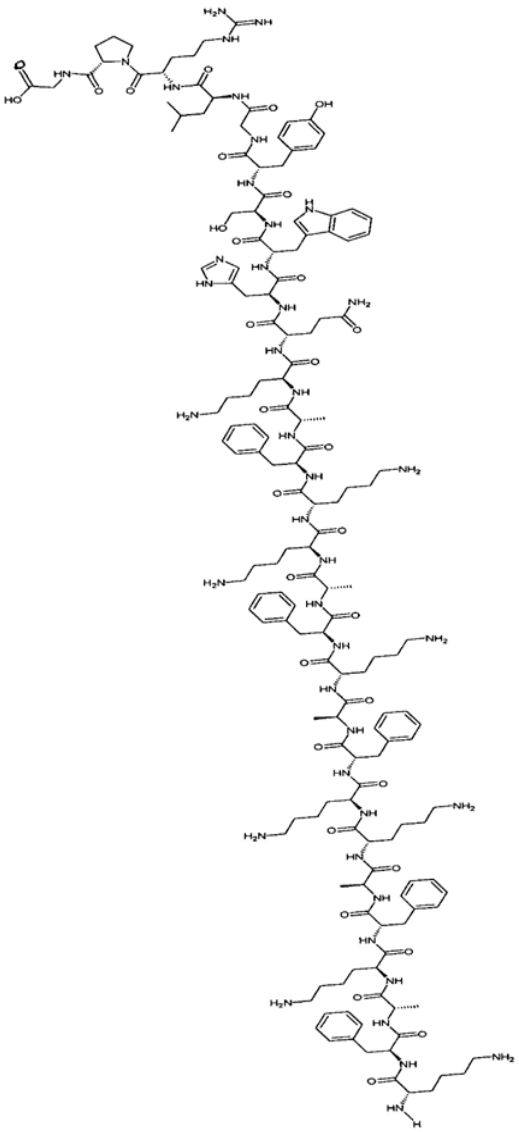
Supplementary Materials

Chemical structure and amino acid sequence of EP-100:



The complete amino acid sequence: Lys - Phe - Ala - Lys - Phe - Ala - Lys - Lys - Phe - Ala - Lys - Phe - Ala - Lys - Lys - Phe - Ala - Lys - Gln -His - Trp - Ser - Tyr - Gly - Leu - Arg - Pro – Gly.

Figures and Figure Legends

Supplementary Figure S1. Expression and localization of GnRH-R in ovarian cancer cells. (**A**) Normal human ovary tissues (Normal#1 and Normal#2) were used as negative controls. Two de-identified normal samples were collected under the IRB-approved lab 02-188 protocol. (**B**) Cells were fixed without permeabilization and images were acquired by confocal microscope (TCS SP5 MP; Leica Microsystems, Buffalo Grove, IL). (**C**) Cells were fixed and permeabilized. Images were acquired by Leica DM4000 B LED microscope. Scale bar: 50 µM.

Supplementary Figure S2. The effects of GnRH-R loss on the cytotoxicity of EP-100. (**A**) Confirmation of the knockdown efficiency of GnRH-R according to Western blot analysis 48 h after transfection of HeyA8 and A2780ip2 cells with shGnRH-R or scrambled plasmid. (**B**) Viability of shGnRH-R cells after treatment with different concentrations of EP-100 after 4 hrs. (**C**) Viability of shGnRH-R cells after treatment with different concentrations of EP-100 after 72 hrs. Error bars are standard deviations. (**D**) Cell viability of shGnRH-R cells treated with the combination of EP-100 and olaparib and Fa-CI plots in shGnRH-R cells. Dose-response curves for cell viability were representative of three experiments. The Fa-CI plots represent the mean ± standard deviation values calculated from three independent experiments. A CI less than 1.0 indicates a synergistic effect and a CI greater than 1.0 indicates antagonism.

Supplementary Figure S3. Effects of chemotherapeutic agents on the viability of ovarian cancer cells. The representative cell viability curves for (**A**) doxorubicin, (**B**) paclitaxel, (**C**) olaparib, (**D**) topotecan, and (**E**) cisplatin against ovarian cancer cells. IC_{50} values are presented as mean ± standard deviation from three independent experiments. Dose-response curves for cell viability were representative of three experiments.

Supplementary Figure S4. Combined effects of EP-100 with doxorubicin, paclitaxel, topotecan and cisplatin on ovarian cancer cells. HeyA8, HeyA8-MDR, A2780ip2 and A2780CP20 cell viabilities after the combination treatment of EP-100 with (**A**) doxorubicin, (**B**) paclitaxel, (**C**) topotecan, or (**D**) cisplatin. Dose-response curves for cell viability were

representative of three experiments. Fa-CI plots for different combinations of EP-100 and each drug at different doses required to achieve the desired effect (vary from 5% to 97%) in the ovarian cancer cells. CI values were calculated from three independent experiments. Error bars are standard deviations.

Supplementary Figure S5. Protein expressions as detected by RPPA assay. We normalized the data sets according to the process provided by MD Anderson RPPA core facility. (**A**) Activated or inhibited signaling pathways in EP-100 treatment compared to control group were analyzed by ingenuity pathway analysis. (**B**) A heatmap of protein expressions in all four treatments after normalization.

Supplementary Figure S6. The combined effect of treatment with EP-100 and olaparib in a subcutaneous mice model. Photographs of (**A**) harvested tumors in mice inoculated with HeyA8 cells that received a vehicle (control), 0.2 mg/kg EP-100, 50 mg/kg olaparib, or a combination of EP-100 and olaparib (olaparib was given 1 h after EP-100 administration) (n=5 per group). (**B**) The tumor volume and (**C**) tumor weights of each group at the end of the experiment. The formula we used to calculate the tumor volume is: $V = \frac{A \times B^2}{2}$. A and B represents the length and width of tumors, respectively.

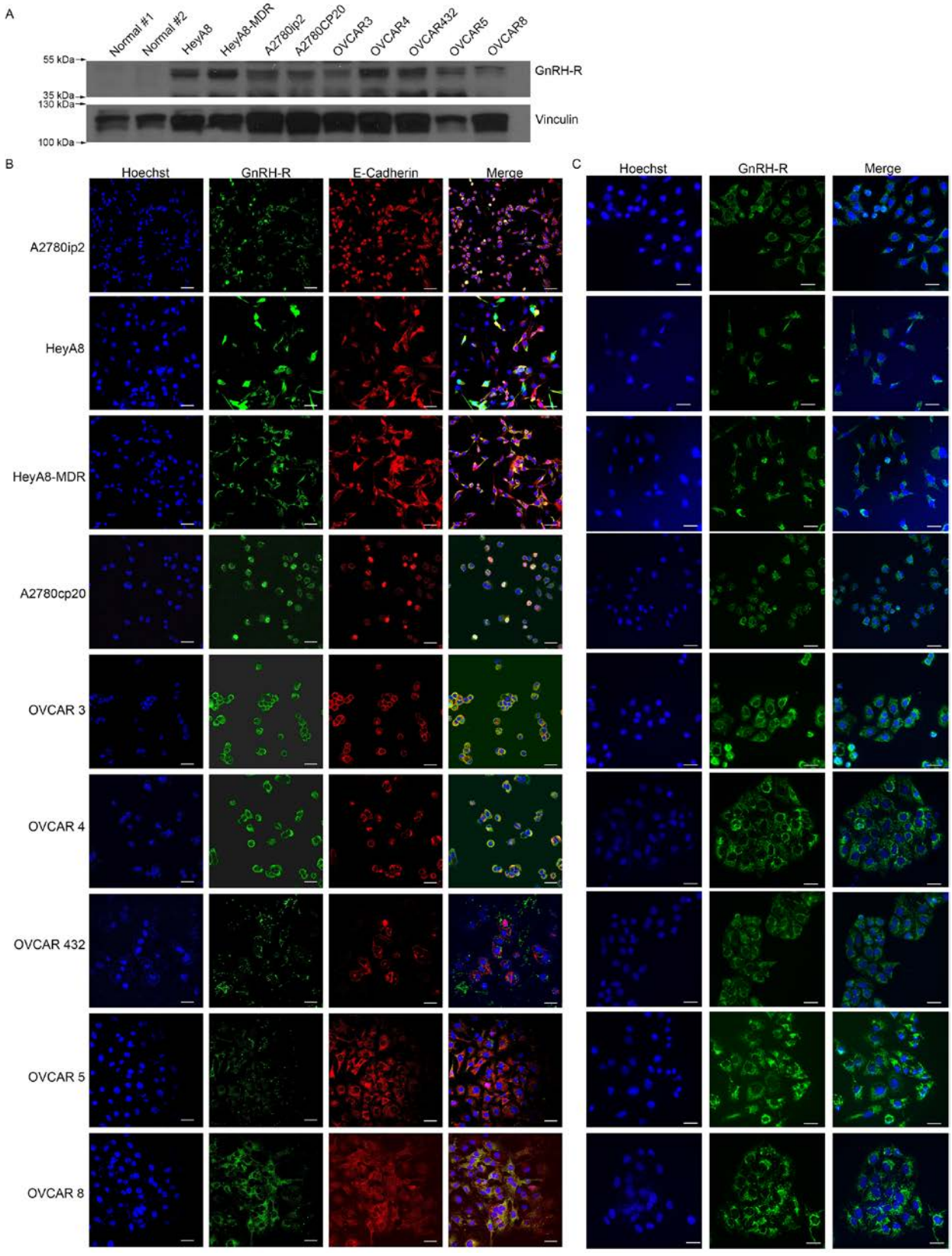
Supplementary Figure S7 (related to Supplementary Figure S1 and Supplementary Figure S2). The original figures of the Western Blotting bands for GnRH-R expression. (**A**) The Western Blotting bands for Vinculin expression under short and long-time exposure. (**B**) The Western Blotting bands for GnRH-R expression under short and long-time exposure. (**C**) The original bands for GnRH-R expression in knockdown experiment from Supplementary Figure S2.

Supplementary Figure S8 (related to Figure 4A). Representative comets from comet assays. Images of HeyA8 and A2780ip2 cells treated with a vehicle (control), EP-100 (1 μm),

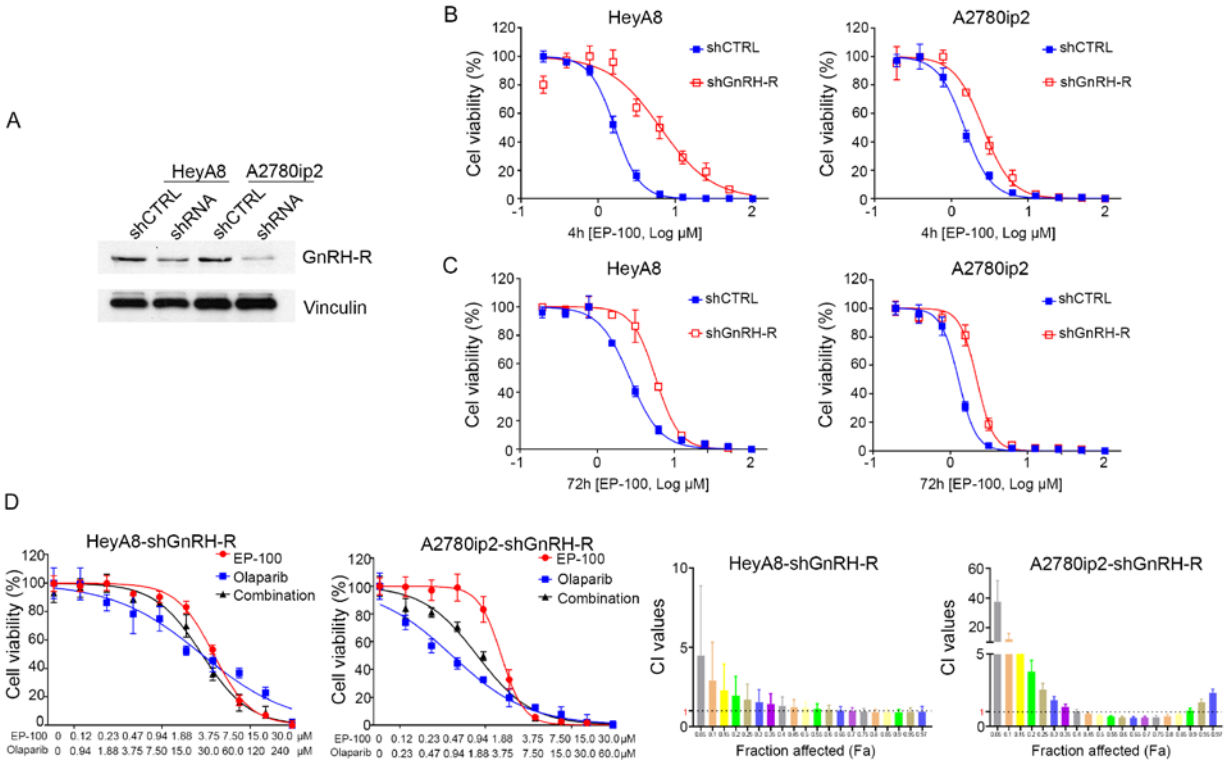
olaparib (10 μM), or EP-100 and olaparib. H_2O_2 (0.5 mM) is used as positive control. Scale bar: 50 μM.

Supplementary Figure S9 (related to Figure 4C and D). Representative images from immunofluorescence assay. (**A**) Immunofluorescence stains for γH2AX foci formation in HeyA8 and A2780ip2 cells. (**B**) The stains for RAD 51 foci formation. Scale bar: 50 μM.

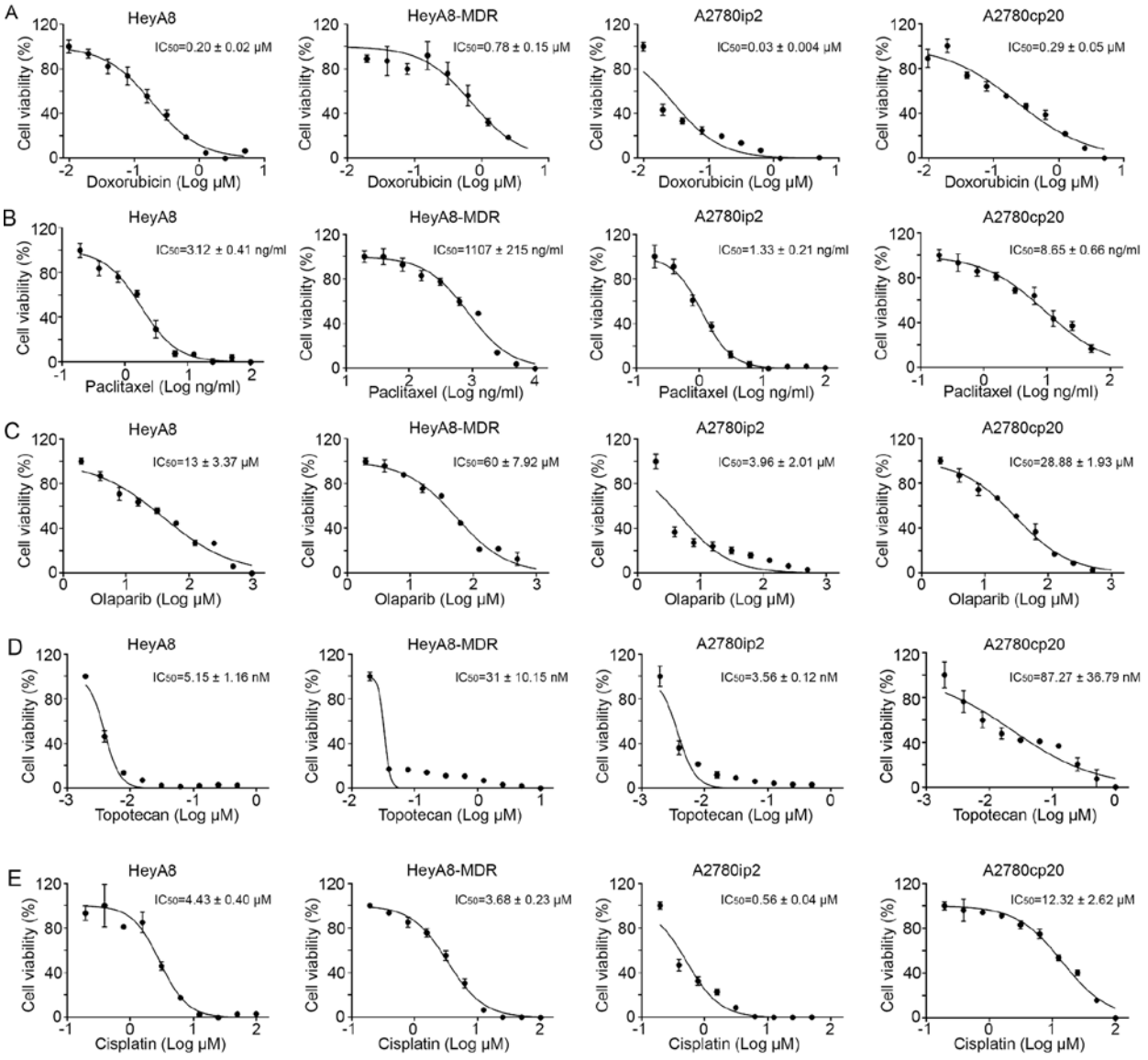
Supplementary Figure S1



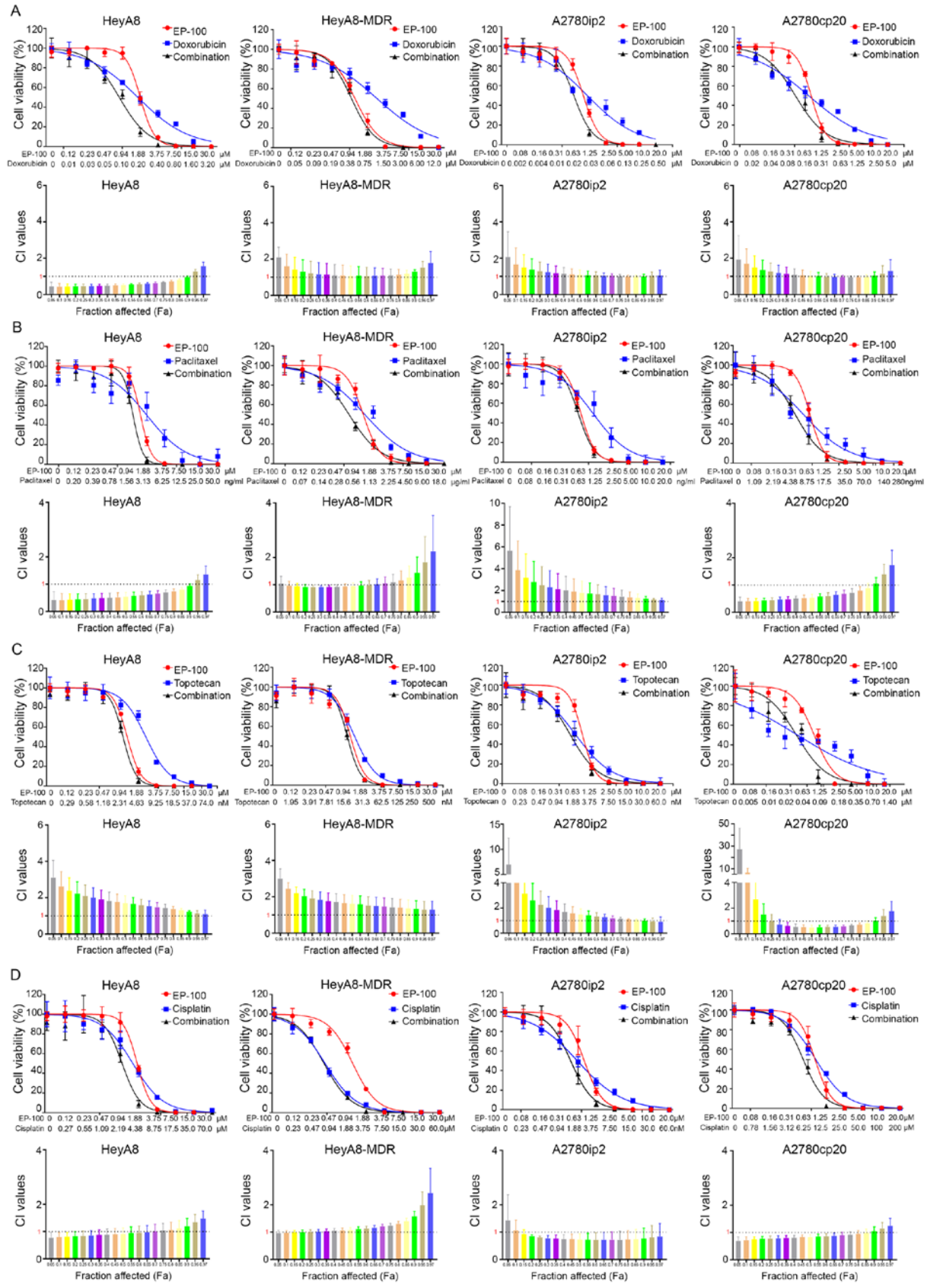
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4

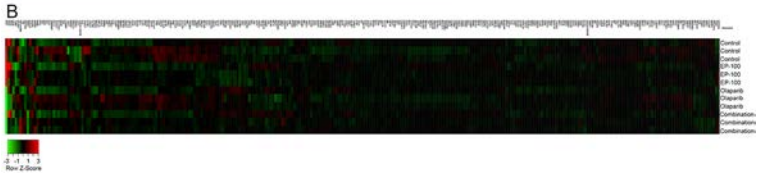


Supplementary Figure S5

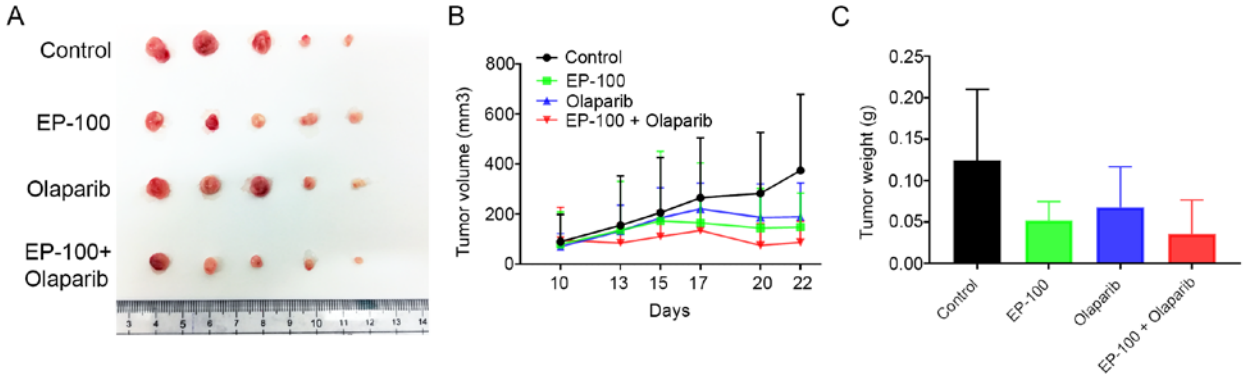
A

Top Canonical Pathways	p-Value	Status
PI3K/AKT Signaling	1.22E-29	Inhibited
Pancreatic Adenocarcinoma Signaling	1.05E-25	Activated
Ovarian Cancer Signaling	5.53E-25	No activated or inhibited
ERK/MAPK Signaling	6.46E-25	Activated

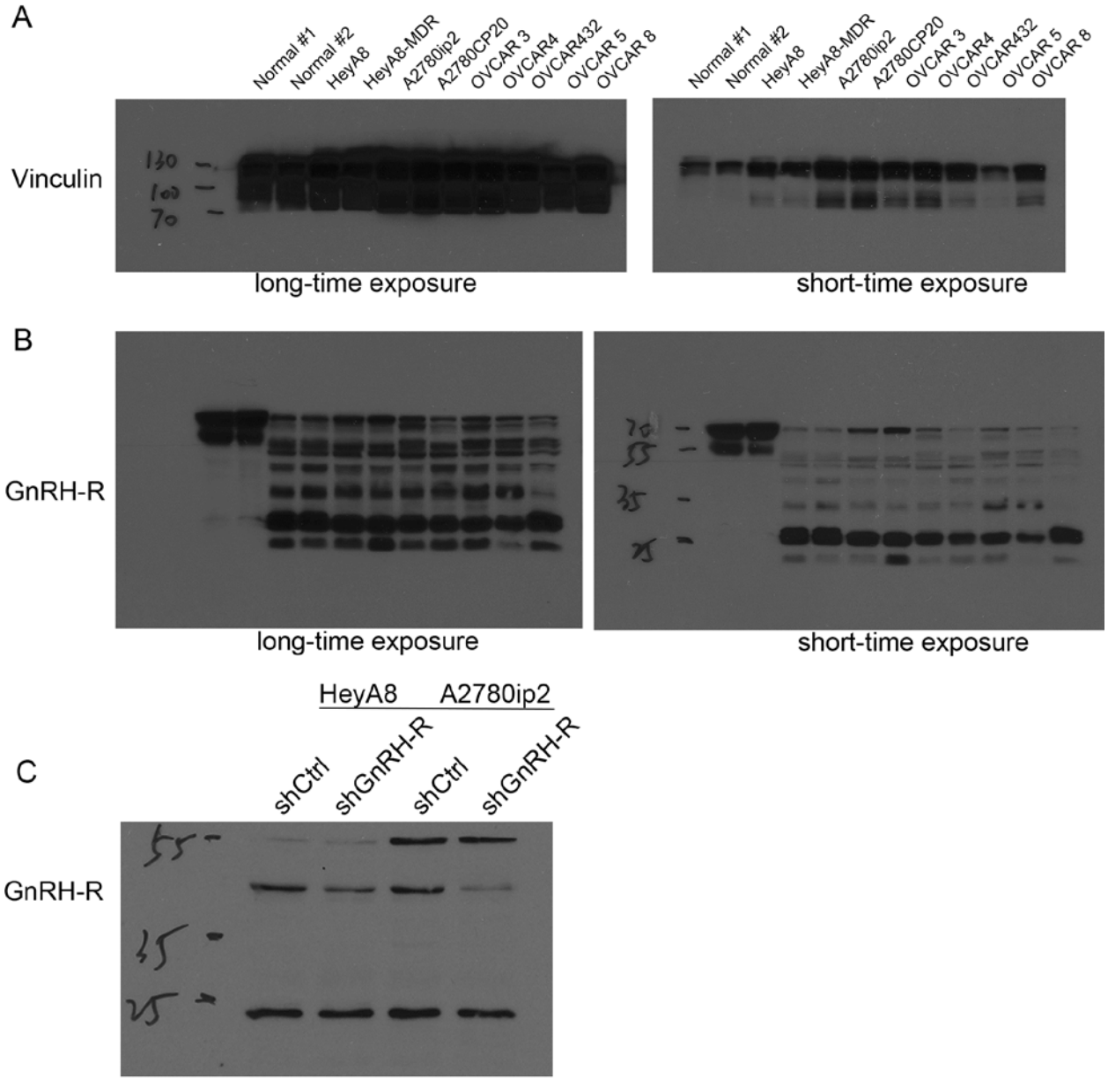
B



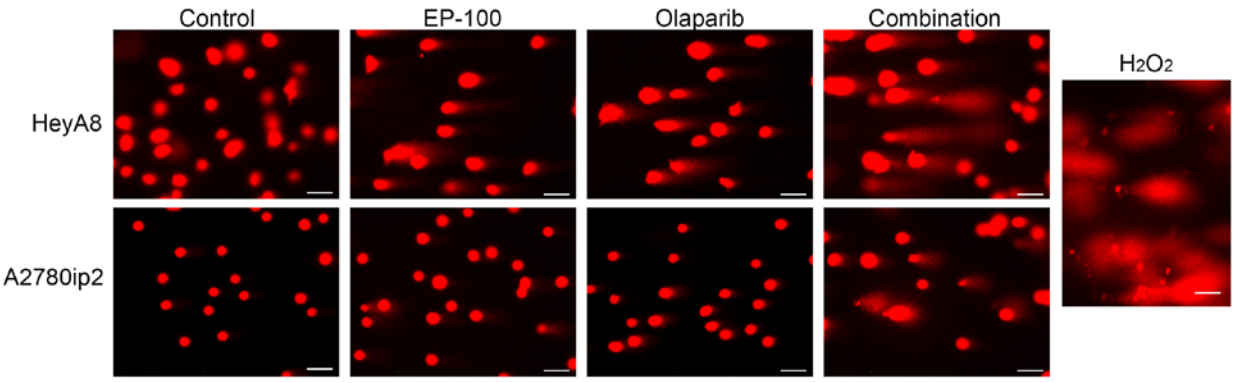
Supplementary Figure S6



Supplementary Figure S7



Supplementary Figure S8



Supplementary Figure S9