Supplemental data

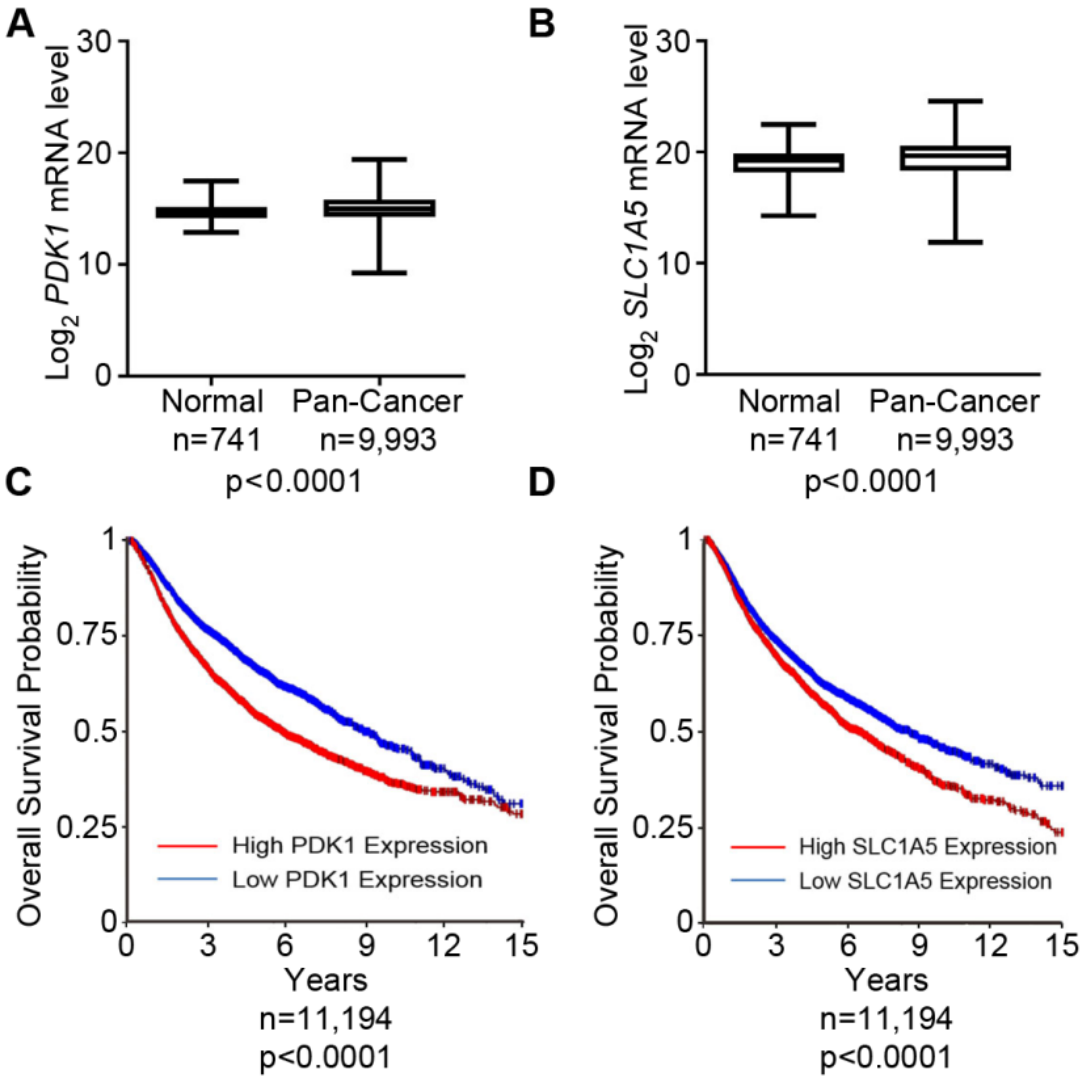
Rational combination with PDK1 inhibition overcomes cetuximab resistance in HNSCC

Haiquan Lu,[1,2,*] Yang Lu,[1] Yangyiran Xie,[3] Songbo Qiu,[1] Xinqun Li,[1] and Zhen Fan[1,2]

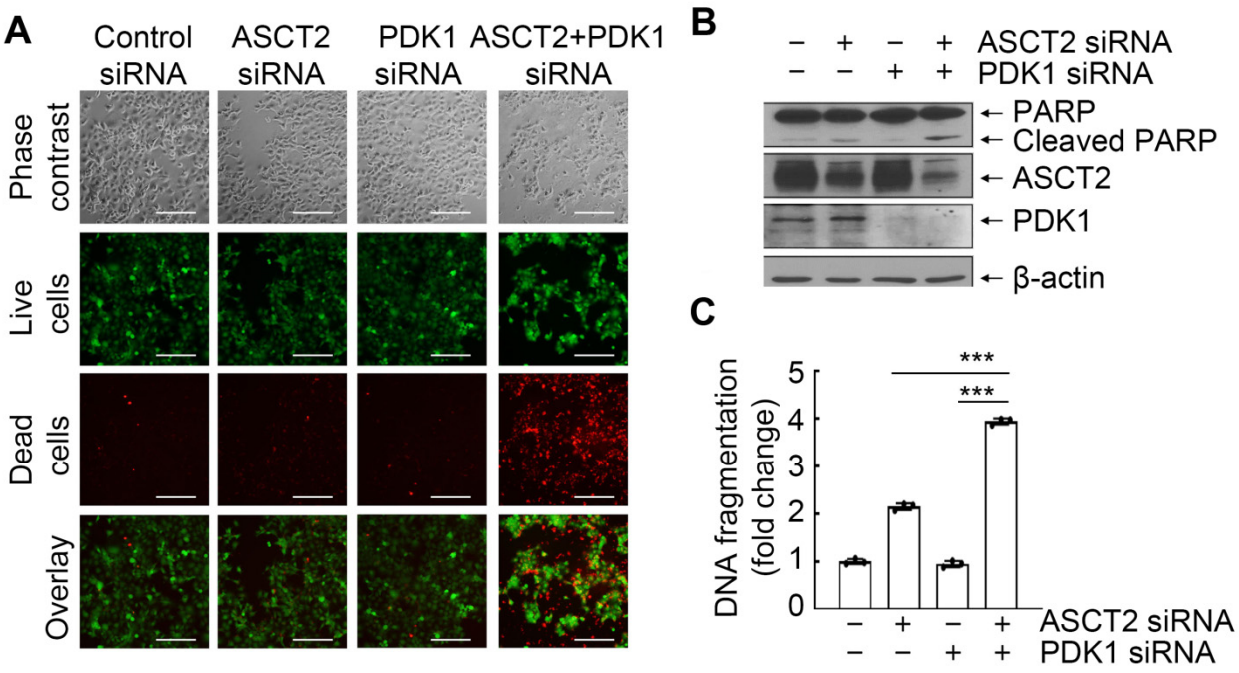
[1]Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. [2]MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX, USA. [3]Program in Neuroscience, Krieger School of Arts and Sciences, Johns Hopkins University, Baltimore, MD, USA.

Address correspondence to: Zhen Fan, Unit 1950, 1515 Holcombe Boulevard, Houston, TX 77030, USA. Phone: 713-745-3560; Email: zfan@mdanderson.org.

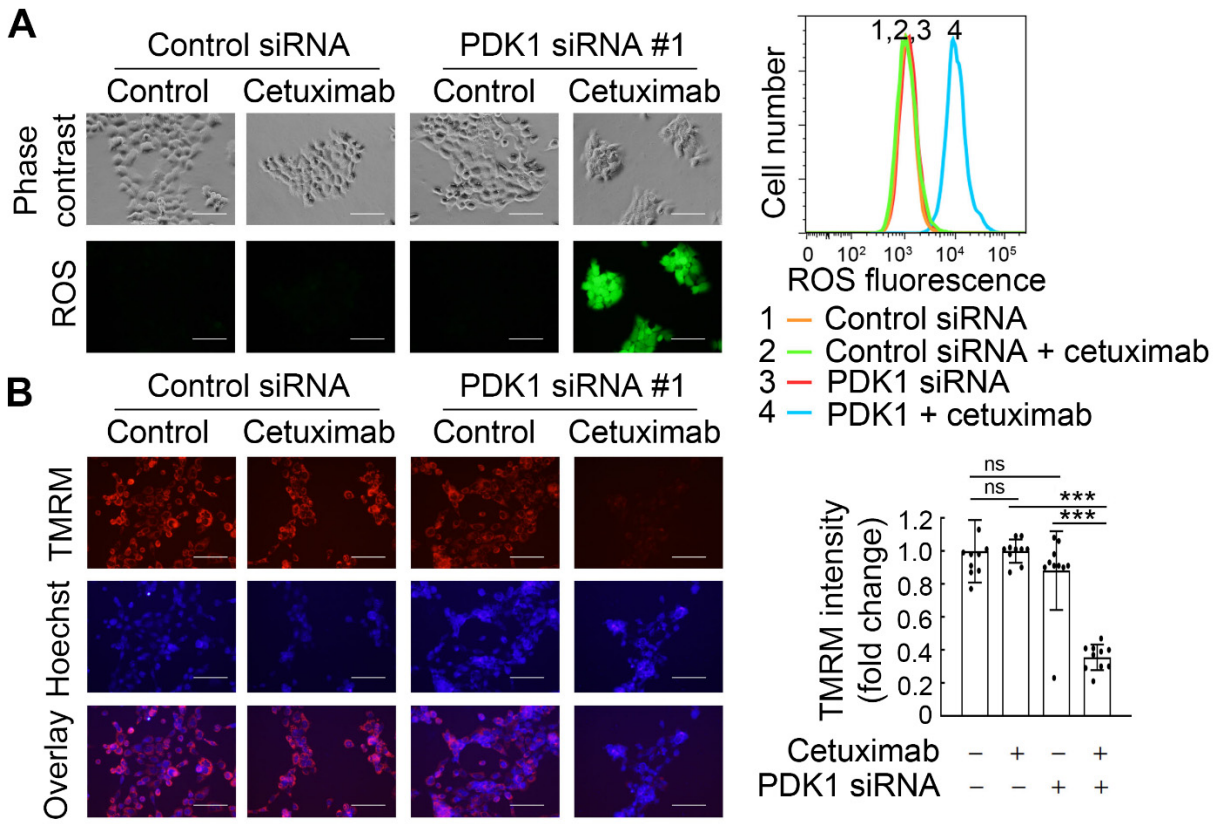
* Current address: Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205



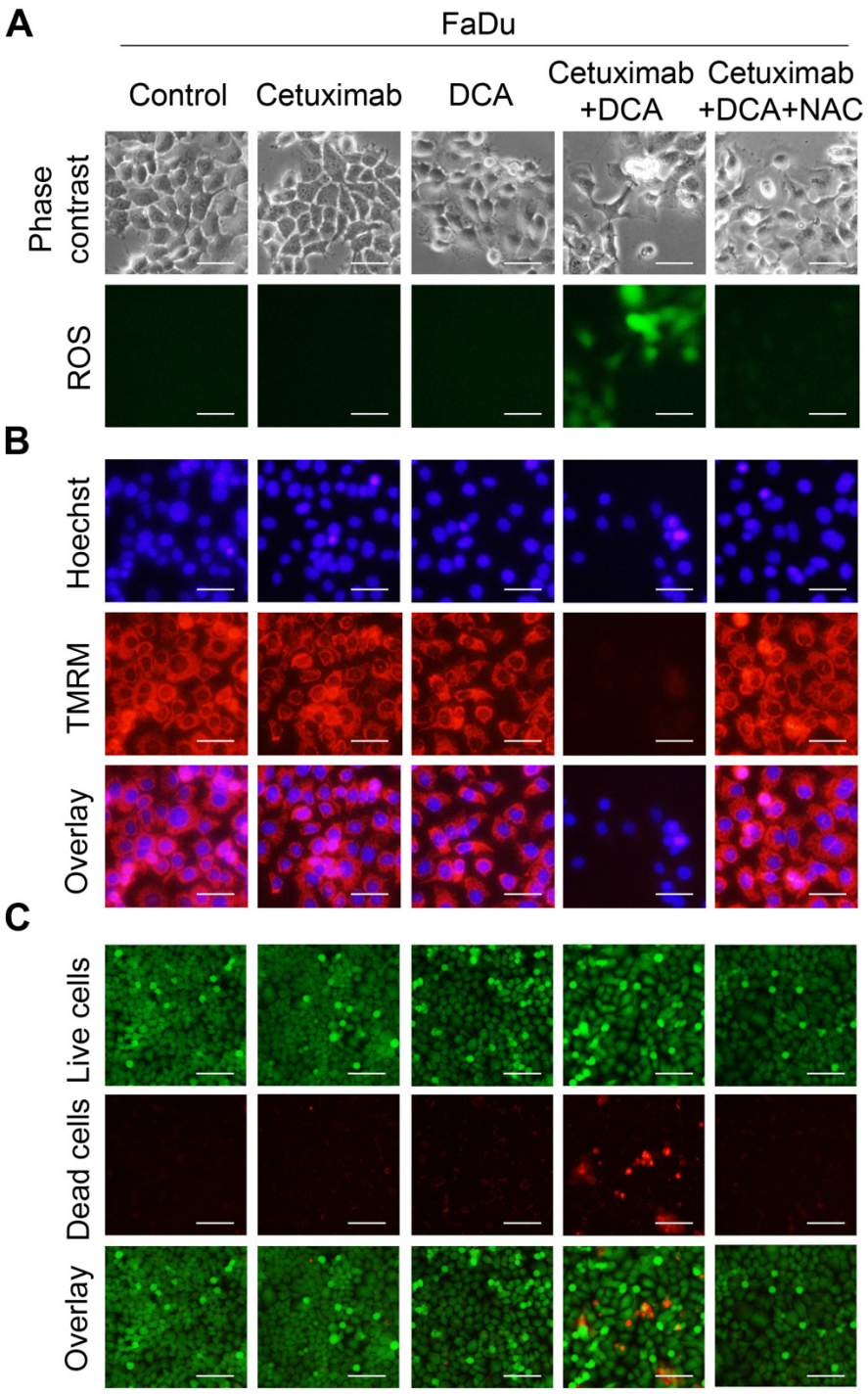
Supplemental Figure 1. *PDK1* and *SLC1A5* are overexpressed in pan-cancer analysis and are associated with patient mortality. (**A** and **B**) The mRNA levels of *PDK1* (**A**) and *SLC1A5* (**B**) in a pan-cancer cohort consisting of 12 datasets of different cancer types (n = 9,993) and corresponding adjacent normal tissues (n = 741) were retrieved from the TCGA database and were plotted and analyzed by Student's t-test. (**C** and **D**) Kaplan-Meier analysis of overall survival according to *PDK1* or *SLC1A5* mRNA levels in the primary tumors was performed based on clinical and molecular data from 11,194 patients. High expression means greater than the median level of *PDK1* or *SLC1A5* expression; low expression means less than the median level of *PDK1* or *SLC1A5* expression. p values are from log-rank test.



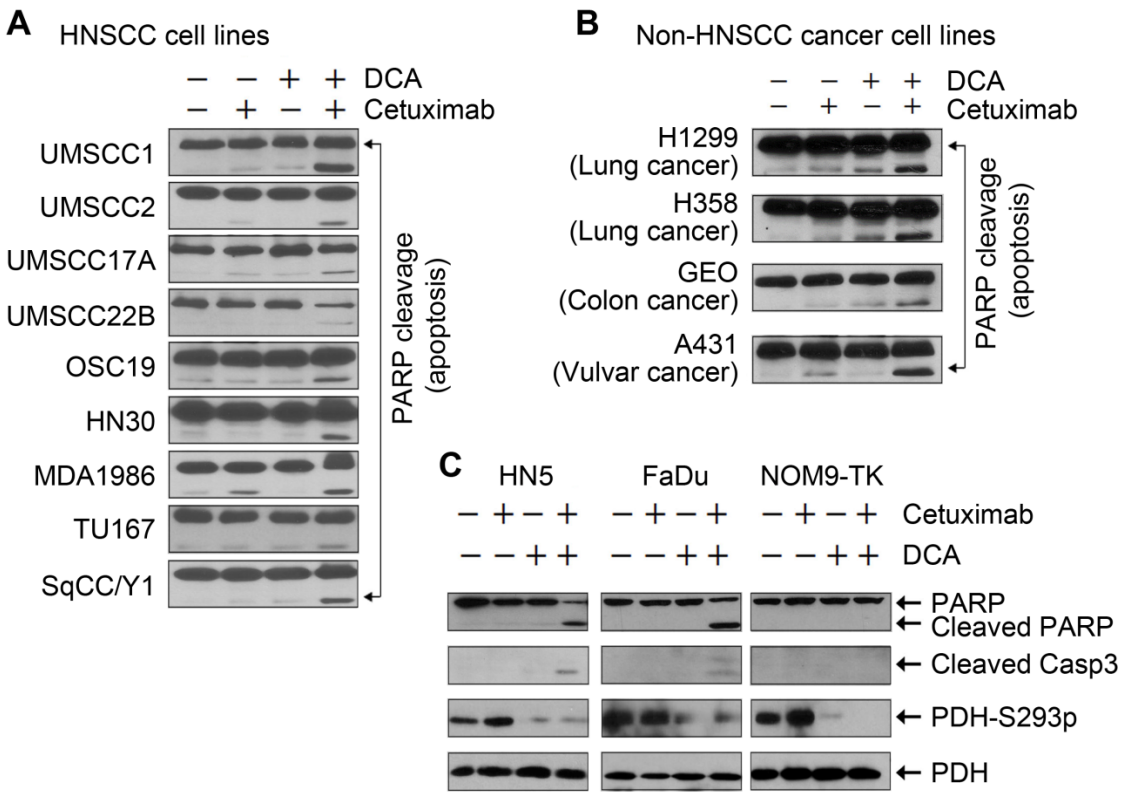
Supplemental Figure 2. Dual silencing of ASCT2 and PDK1 is synthetically lethal to HNSCC cells. FaDu cells were transfected with control siRNA, ASCT2 siRNA, PDK1 siRNA, or ASCT2 siRNA plus PDK1 siRNA for 72 h. (**A**) FaDu cells were subjected to LIVE/DEAD cell viability assay as described in Methods and then observed under a fluorescence microscope (scale bars, 200 μm). (**B**) The cell lysates were subjected to Western blotting with the indicated antibodies. Error bars, SD. *** $p < 0.001$ (ANOVA, n=3). (**C**) The cell lysates were subjected to quantitative apoptosis ELISA as described in Methods.



Supplemental Figure 3. Cetuximab sensitizes HNSCC cells to PDK1-silencing-induced ROS overproduction and mitochondria depolarization. FaDu cells were transfected with control siRNA or PDK1 siRNA for 72 h. Cetuximab (20 nM) was added or not added during the last 24 h of siRNA transfection as indicated. (**A**) The cells were stained with Enzo's ROS detection kit and then observed under a fluorescence microscope (scale bars, 100 μm) (left panel) or subjected to FACS analysis after staining (right panel). (**B**) The cells were stained with mitochondrial membrane potential-sensitive dye tetramethyl rhodamine methyl ester (TMRM) and then observed under a fluorescence microscope (scale bars, 100 μm) (left panel) or analyzed with a fluorescence plate reader (right panel). Error bars, SD. *** $p < 0.001$ (ANOVA, n=10). Similar results were observed with two other PDK1 siRNAs.



Supplemental Figure 4. Cetuximab sensitizes HNSCC cells to DCA -induced ROS overproduction, mitochondria depolarization, and apoptosis. FaDu cells were left untreated or treated with 20 nM cetuximab, 10 mM DCA, or both with or without 10 mM NAC as indicated for 24 h. (**A**) The cells were stained with Enzo's ROS detection kit and then observed under a microscope (scale bars, 50 μm). (**B**) The cells were stained with mitochondrial membrane potential-sensitive dye tetramethyl rhodamine methyl ester (TMRM) and then observed under a microscope (scale bars, 50 μm). (**C**) The cells were subjected to LIVE/DEAD cell viability assay and then observed under a microscope (scale bars, 100 μm).



Supplemental Figure 5. DCA plus cetuximab induces apoptosis in multiple HNSCC and non-HNSCC cell lines but not in nonmalignant head and neck epithelial cells. (**A** and **B**) The indicated HNSCC (**A**) and non-HNSCC cancer cell lines (**B**) were left untreated or treated with 20 nM cetuximab, 10 mM DCA, or both as indicated for 24 h. Cell lysates were then prepared and subjected to Western blotting with the indicated antibodies. (**C**) HN5, FaDu, and NOM9-TK cells were treated as in **A** and **B**. Cell lysates were then prepared and subjected to Western blotting with the indicated antibodies.