Tumor cells rely on the thiol oxidoreductase PDI for PERK signaling in order to survive ER stress

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Supplementary data

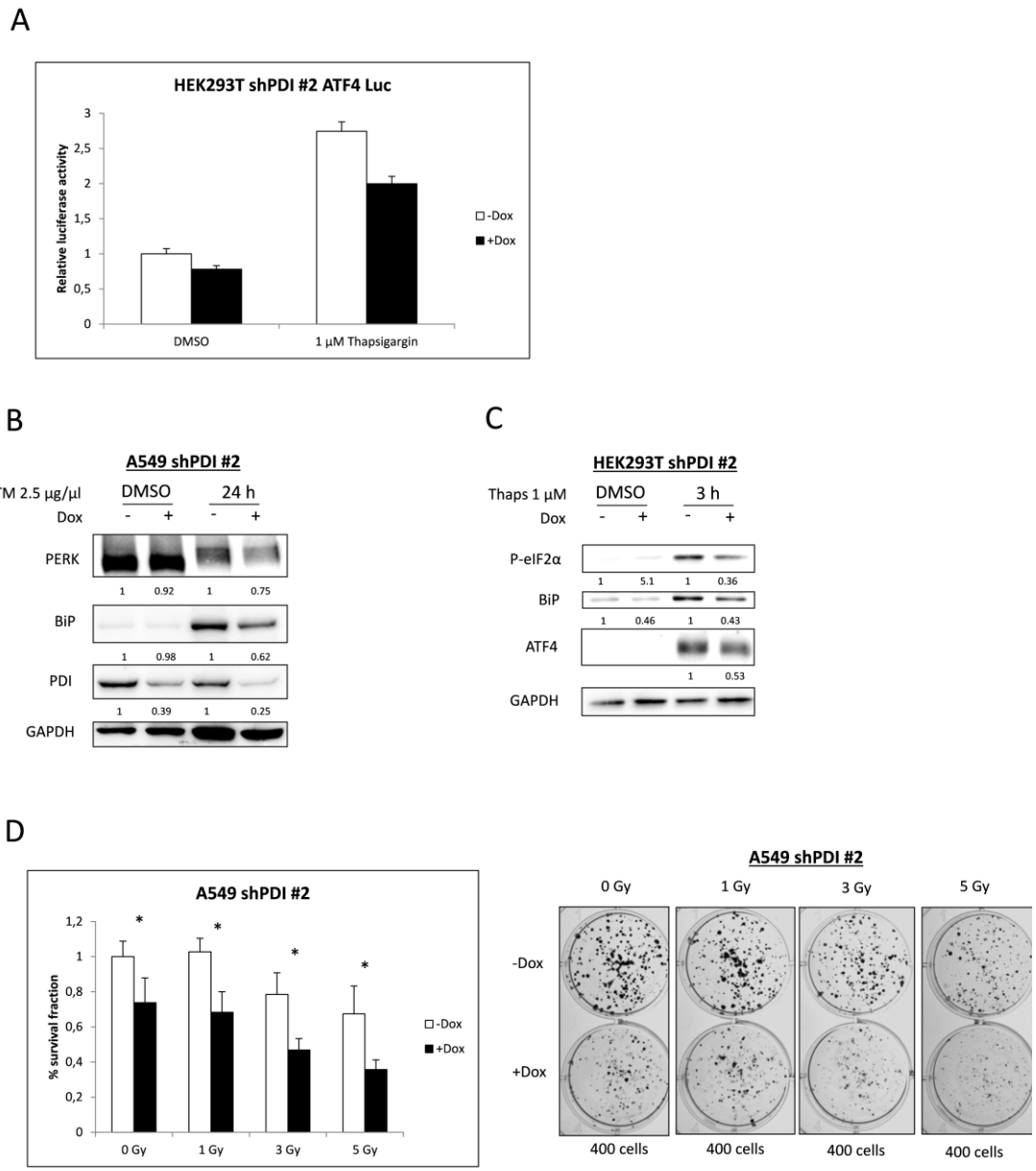

Figure S1: Key experiments with second shRNA (A) ATF4 luciferase assay in HEK293T shPDI #2 cells after 96 h of KD induction and 20 h treatment with 1 µM thapsigargin. **(B)** A549 shPDI #2 cells were treated with 2.5 µg/ml tunicamycin (TM) for 24 h after 48 h of KD induction and expression of PERK, BiP, PDI and GAPDH were tested by western blotting. **(C)** HEK293T shPDI #2 cells were treated with DMSO or 1 µM thapsigargin (Thaps.) for 3 h after 72 h of KD induction. Phosphorylation of eIF2α and expression of BiP, ATF4 and GAPDH were tested by western blotting. **(D)** Colony formation assay of A549 shPDI #2 cells irradiated with 0, 1, 3 and 5 Gy. Survival fraction is presented in bar graphs and representative images of colonies formed in 6-Well plates are shown. In subfigures B and C, densitometry was used to quantify protein band intensity. After *normalization to GAPDH,* the induced KD sample (+Dox) was compared to its control sample (-Dox, set to 1).