

Fig. S8. HSP2 and CycT do not affect iron metabolism and hypoxia induction of HIF1. (A) Iron stain did not detect any difference between control and treated tumor tissues. Shown are montages, 10X, and 40X images of control, HSP2 and CycT-treated tumor tissue sections stained with Iron Stain. The blue rectangles in montages, 10X images the regions shown in 10X and 40X images, respectively. Scale bar: montage, 1 mm for controls and 500 µm for HSP2 and CycT treated tissues; 10X, 200 µm, 40X 50 µm. (B) Representative IHC images of H1299 NSCLC tumor tissue sections and graphs showing the levels of transferrin receptor TFRC in control, HSP2, and CycT-treated tumors. (C) Representative IHC images of H1299 tumor tissue sections and graphs showing the levels of ferroportin 1, SLC40A1, in control, HSP2, and CycT-treated tumors. Shown are montages and 10X images of control, HSP2 and CycT-treated tumor tissue sections stained with DAPI or antibodies against the indicated protein. The white rectangles in DAPI images denote the regions shown in 10X images. Scale bar: montage, 1 mm (for control tumor sections) and 500 µm (for HSP2 and CycT treated tumor sections); 10X, 20 µm. Protein levels were quantified, and data are plotted as mean ± SEM. The values shown in the graphs are averages of signals quantified from three independent IHC experiments. For statistical analysis, the levels in treated tumors were compared to the levels in control tumors with a Welch 2-sample t-test. P \* <0.05, \*\* < 0.005. (D) Representative images of cells stained with DAPI or antibodies to HIF1. H1299 cells pretreated with no reagent, HSP2, or CycT were subjected to hypoxia for 24 hours, and then cells were subjected to immunofluorescence staining with anti-HIF1 antibodies. HIF1 levels were estimated by quantifying fluorescence intensities and plotted here. Normoxic cells are also shown for comparison. Scale bar: 40X, 100 µm. For statistical analysis, the levels in hypoxic cells were compared to the levels in normoxic cells with a Welch 2-sample t-test. P \* <0.05, \*\* < 0.005.