Figure S1

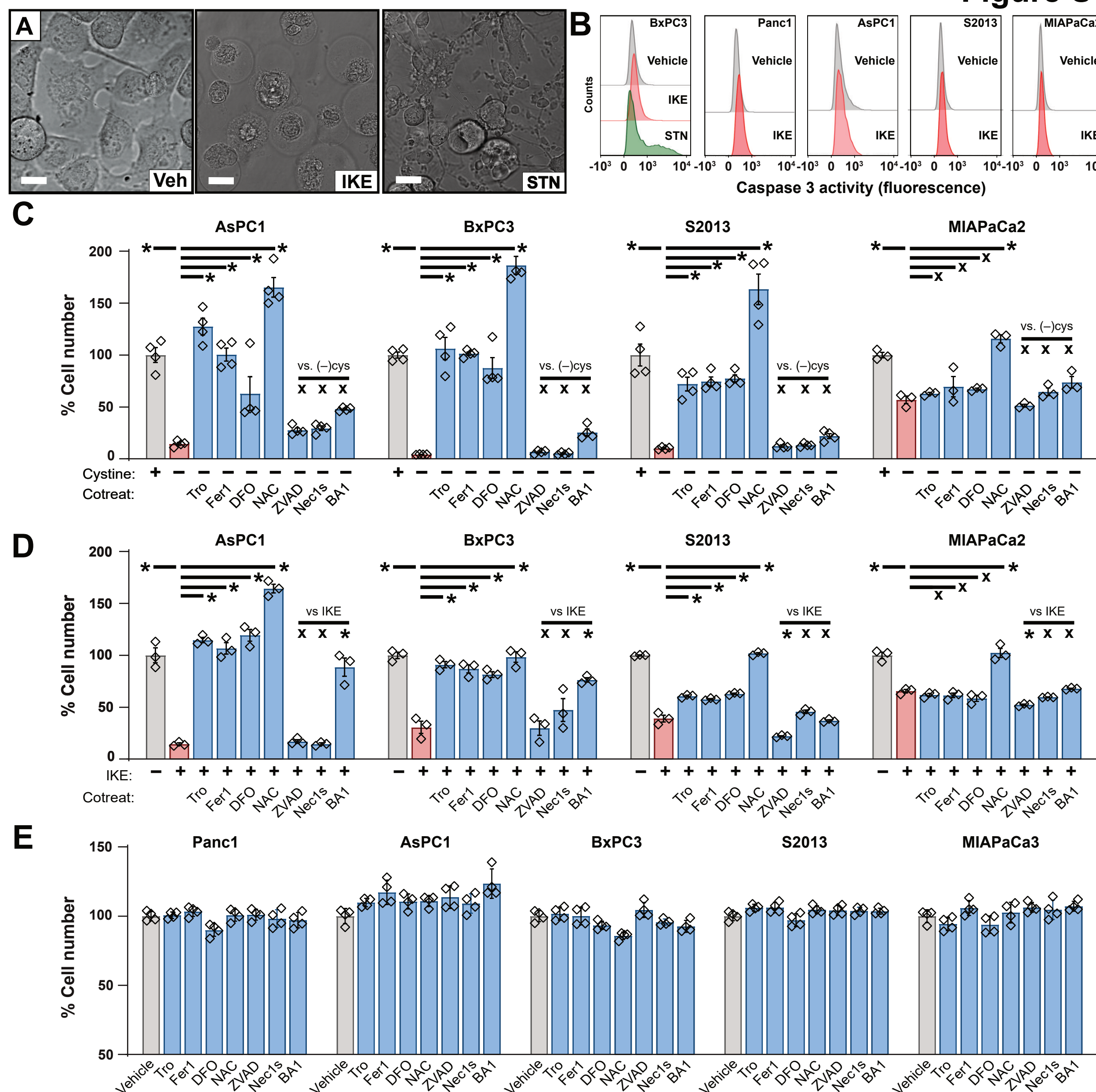

Fig. S1. The mechanism of ferroptosis in PDA cell lines (**A**) High magnification image of PANC-1 cells cultured in the presence of vehicle (0.1% DMSO), 5 µM IKE or 0.2 µM staurosporine (STN), a known inducer of apoptosis, after 16 hours. Bar = 15 µm. (**B**) Flow cytometric analysis of cleaved caspase 3 activation in cell lines stained with a FITC-based active caspase 3 antibody. Cells were treated with vehicle (0.1% DMSO, gray) and IKE (5 µM, red). In BxPC-3 cells, staurosoporine (STN) treatment is used as a positive control (0.2 µM, green). (**C**) Cell viability of a panel of human pancreatic cancer cells cultured in vehicle (0.1% DMSO and 1mM HCl veh, shown in gray), the absence of cystine (no cys, shown in red), and in the absence of cystine but in the presence of 100 µM Trolox (Tro, shown in blue), 500 nM ferrostatin-1 (Fer-1, ferroptosis inhibitor), 100 µM deferoxamine (DFO, iron chelator), 1 mM NAC (NAC), 50 µM ZVAD-FMK (ZVAD, apoptosis inhibitor), 1 nM bafilomycin A1 (BA1, autophagy inhibitor), and 10 µM Necrostatin 1s (Nec1s, necroptosis inhibitor). Viability was assessed after 24 hours of treatment. Error equals +/- SEM. n = 3 biological replicates. * = $p < 0.05$ with Tukey's test. x = no statistically significant difference (**D**) Cell viability of a panel of human pancreatic cancer cells cultured in vehicle (0.2% DMSO, veh), 5 µM IKE, and the aforementioned compounds and the concentrations indicated in Fig. S1C. Viability was assessed at 24 hours after treatment. Error equals +/- SEM. n = 3 biological replicates. * = $p < 0.05$ with Tukey's test. x = no statistically significant difference. (**E**) Single treatment controls for all experiments shown in Fig. 1C, D; Fig. S1C, S1D.