

Supplemental Data Figure Legends.

Figure S1. Quenching ROS does not reduce drug –induced Ca^{2+} levels. HEPG2 cells were transfected with empty vector plasmid or with a plasmid to express TRX. Twenty four h after transfection cells were loaded with Fura2 and were treated with vehicle (DMSO) or with sorafenib (S, 3 μM) and Vorinostat (V, 500 nM) in combination. Cytosolic Ca^{2+} levels were measured in octuplicate 5-60 min after drug exposure as per Methods, and plotted as the fluorescence intensity ratio (n = 2, +/- SEM).

Figure S2. Knock down of CD95 does not reduce drug –induced Ca^{2+} levels. HEPG2 and HEP3B cells were transfected with scrambled control siRNA or with an siRNA to knock down CD95. Twenty four h after transfection cells were loaded with Fura2 and were treated with vehicle (DMSO) or with sorafenib (S, 3 μM) and Vorinostat (V, 500 nM) in combination. Cytosolic Ca^{2+} levels were measured in octuplicate 5-60 min after drug exposure as per Methods, and plotted as the fluorescence intensity ratio (n = 2, +/- SEM).

Figure S3. Knock down of CD95 does not reduce Ca^{2+} -induced Ca^{2+} levels. HEPG2 and HEP3B cells were transfected with scrambled control siRNA or with an siRNA to knock down CD95. Twenty four h after transfection cells were loaded with Fura2 and were treated with vehicle (PBS) or with CaCl_2 (25 μM) in combination. Cytosolic Ca^{2+} levels were measured in octuplicate 5-60 min after drug exposure as per Methods, and plotted as the fluorescence intensity ratio (n = 2, +/- SEM).

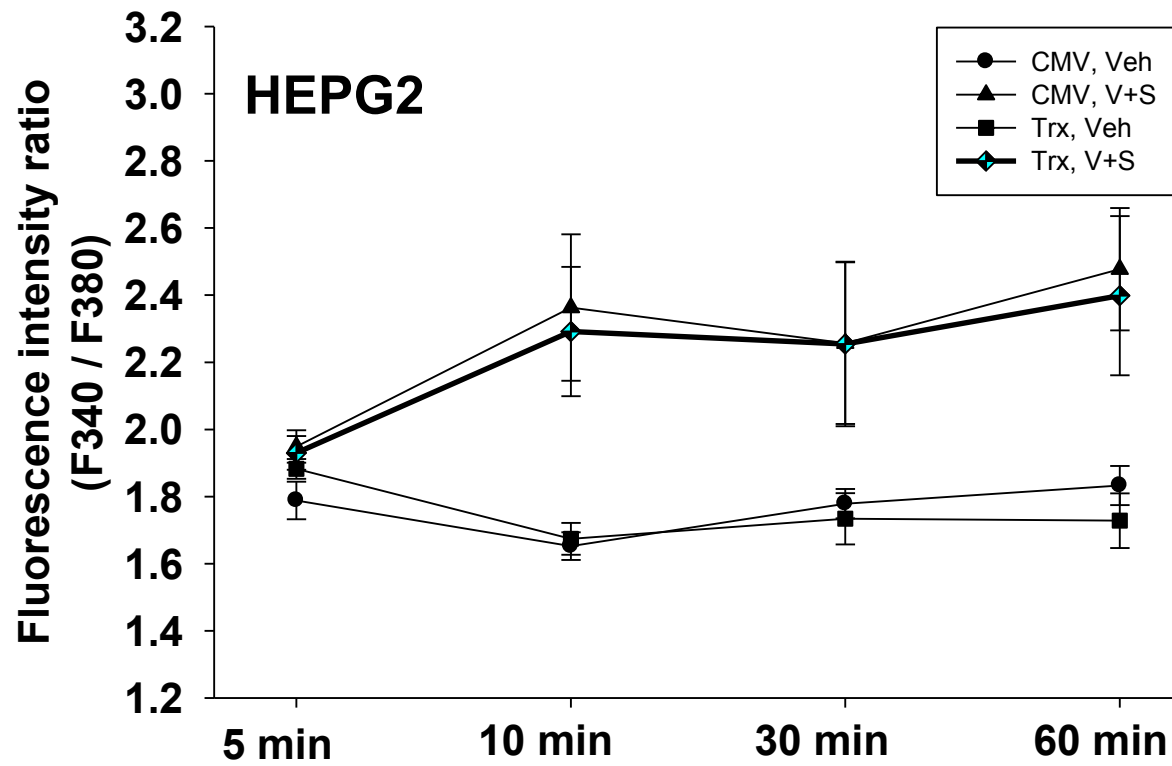
Figure S4. Expression of LASS6 in LASS6 deficient SW620 colon cancer cells facilitates drug –induced ROS generation. SW620 cells were stably transfected with empty vector control (GFP) or with GFP-LASS6 (44, 45). Twenty four h after plating in 96 well plates, cells were treated with vehicle (DMSO) or with sorafenib (Sor, 3 μM) and Vorinostat (Vor, 500 nM) in combination ROS levels were measured in octuplicate 15 min after drug exposure as per Methods, and plotted as the –Fold increase over basal ROS levels (n = 2, +/- SEM).

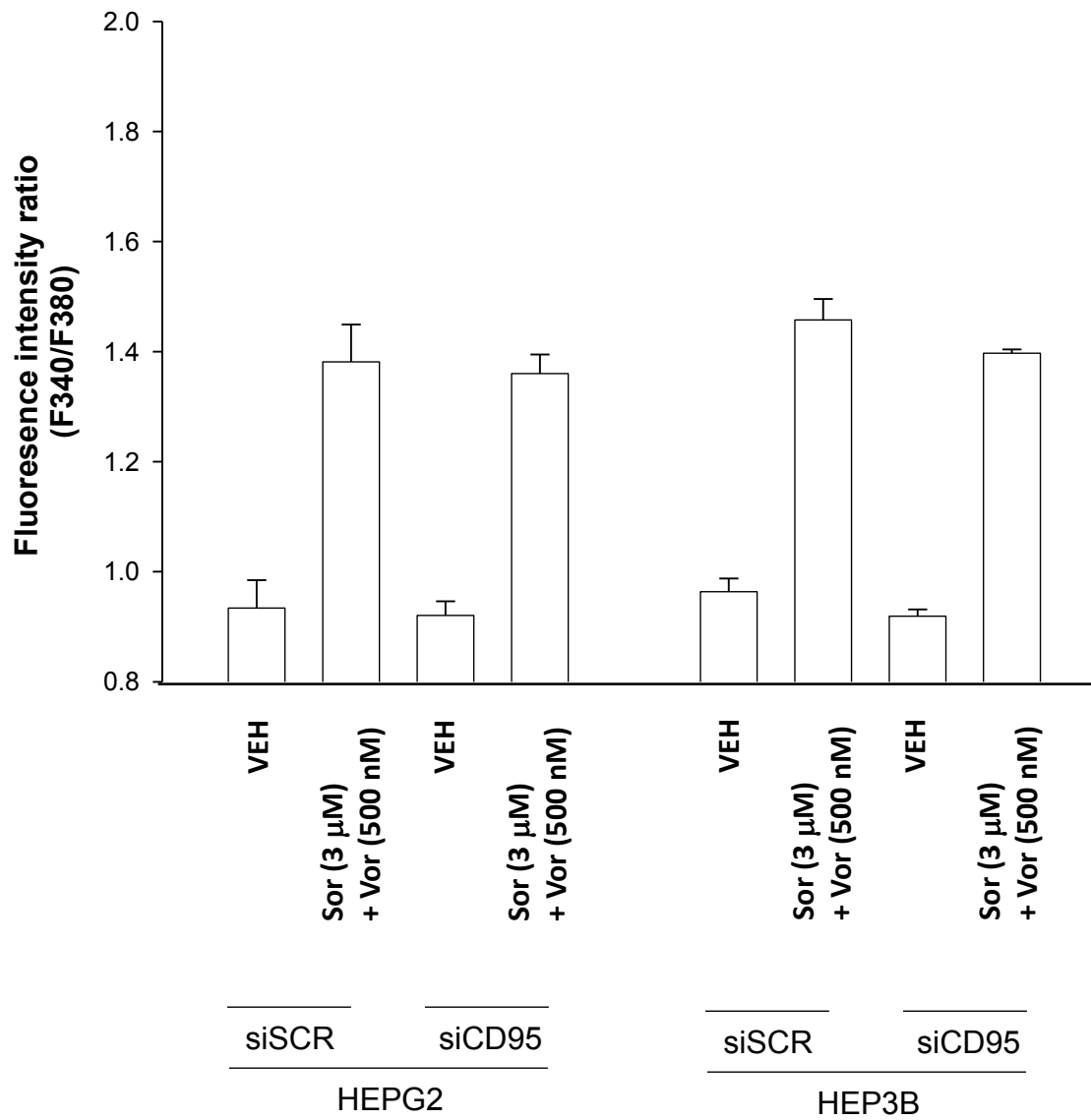
Figure S5. Knock down of LASS6 or quenching of ROS suppresses the radiosensitization of GI tumor cells by sorafenib + vorinostat treatment. MiaPaca2, HEP3B or PAN02 cells in triplicate were transfected as indicated with plasmids to express TRX or to knock down LASS6. Rodent PAN02 cells were treated with myriocin (1 μ M). Twenty four h after transfection cells were treated with vehicle (DMSO) or with sorafenib (Sor, 3 μ M) and Vorinostat (Vor, 500 nM) in combination. Cells were isolated 48h after drug treatment and cell viability determined by trypan blue exclusion assay (n = 2, +/- SEM).

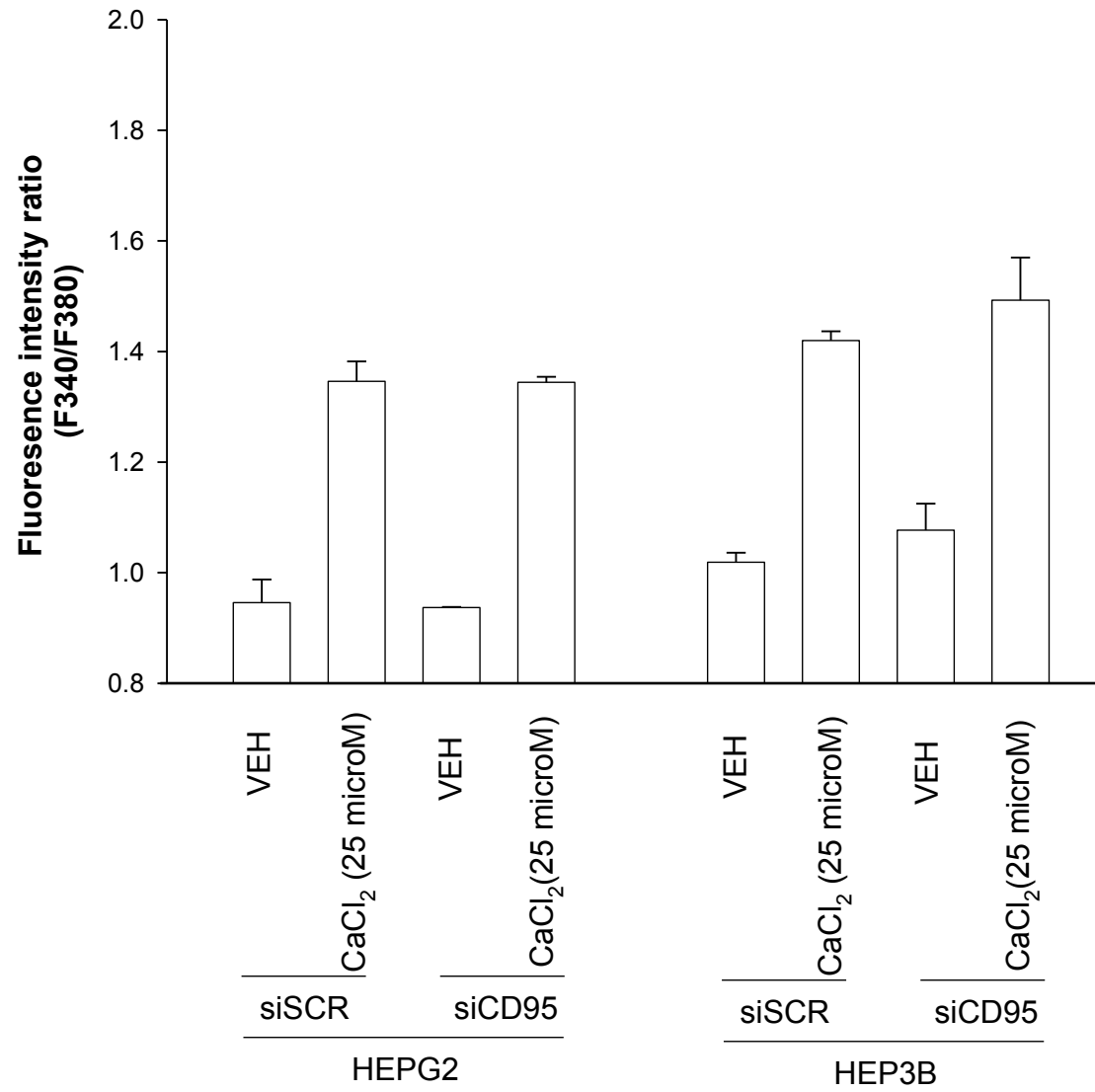
Figure S6. Sorafenib + vorinostat treatment radiosensitizes GI tumor cells. HEP3B and MiaPaca2 cells were plated as single cells in sextuplicate. Twelve h after plating, as indicated cells were treated with sorafenib (0-6 μ M), vorinostat (0-500 nM) or sorafenib + vorinostat (3.0 μ M + 250 nM; 4.5 μ M + 375 nM; 6.0 μ M + 500 nM). Cells were treated with drugs for 48h after which the drugs were removed and colonies permitted to form over the following 10-14 days. For irradiation, cells were either irradiated 30 min after drug exposure or were irradiated 24h after the removal of drugs from the media. Colonies of > 50 cells were counted (n = 2, +/- SEM).

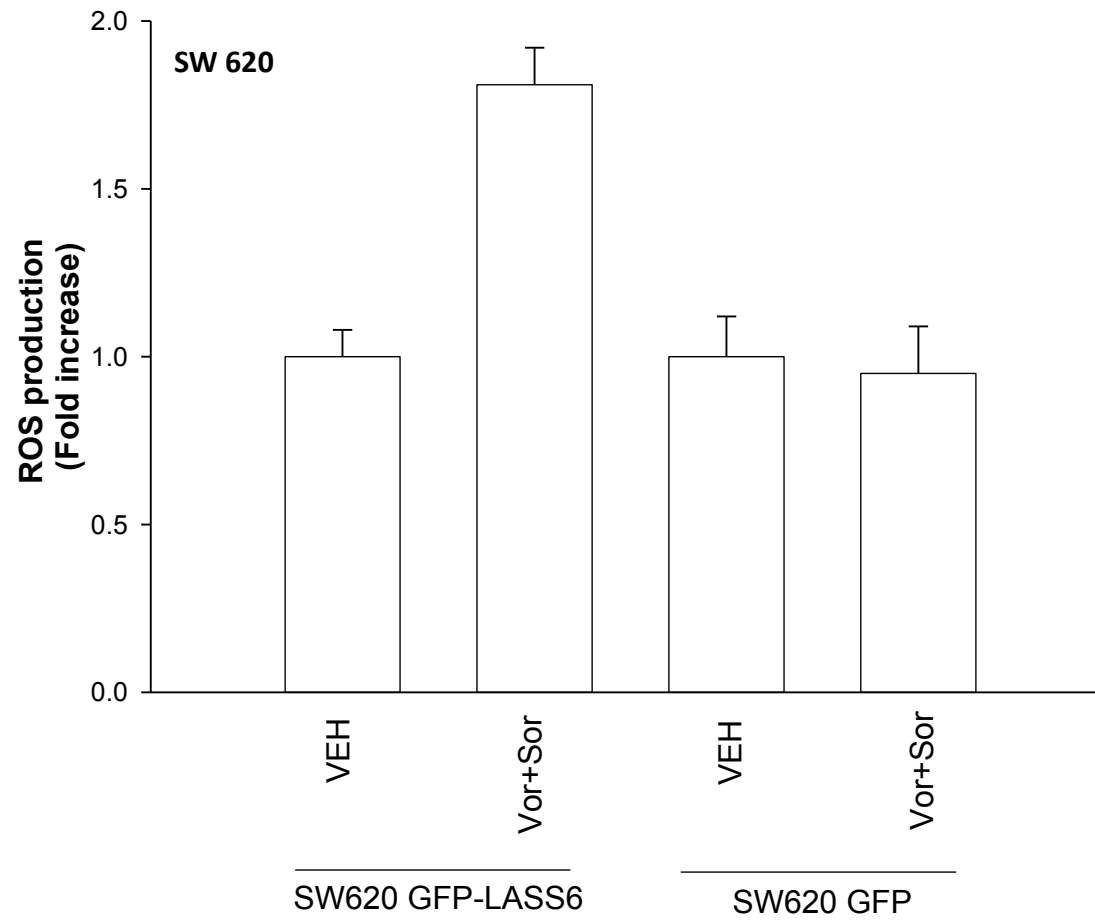
Figure S7. Sorafenib + vorinostat treatment suppresses pancreatic tumor growth in vivo. Athymic mice were implanted S.C. with 1×10^7 Mia Paca2 cells. Fourteen days after implantation tumor volume was determined using calipers. Animals were segregated into four groups of approximate mean tumor volume +/- SEM and then treated by oral gavage with vehicle or sorafenib (20 mg/kg) + vorinostat (20 mg/kg). Animals are treated once per day for 5 consecutive days. Twenty four h after the first drug administration animals are placed in a shielded container and either mock exposed or have their flanks irradiated (2 Gy). Forty eight h after the first irradiation, a second irradiation (2 Gy) is performed. Tumors were treated with two cycles of drugs / radiation. Tumor volume is assessed at least every third day after the initiation of drug treatment (n = 2, +/- SEM; total 8 animals per group).

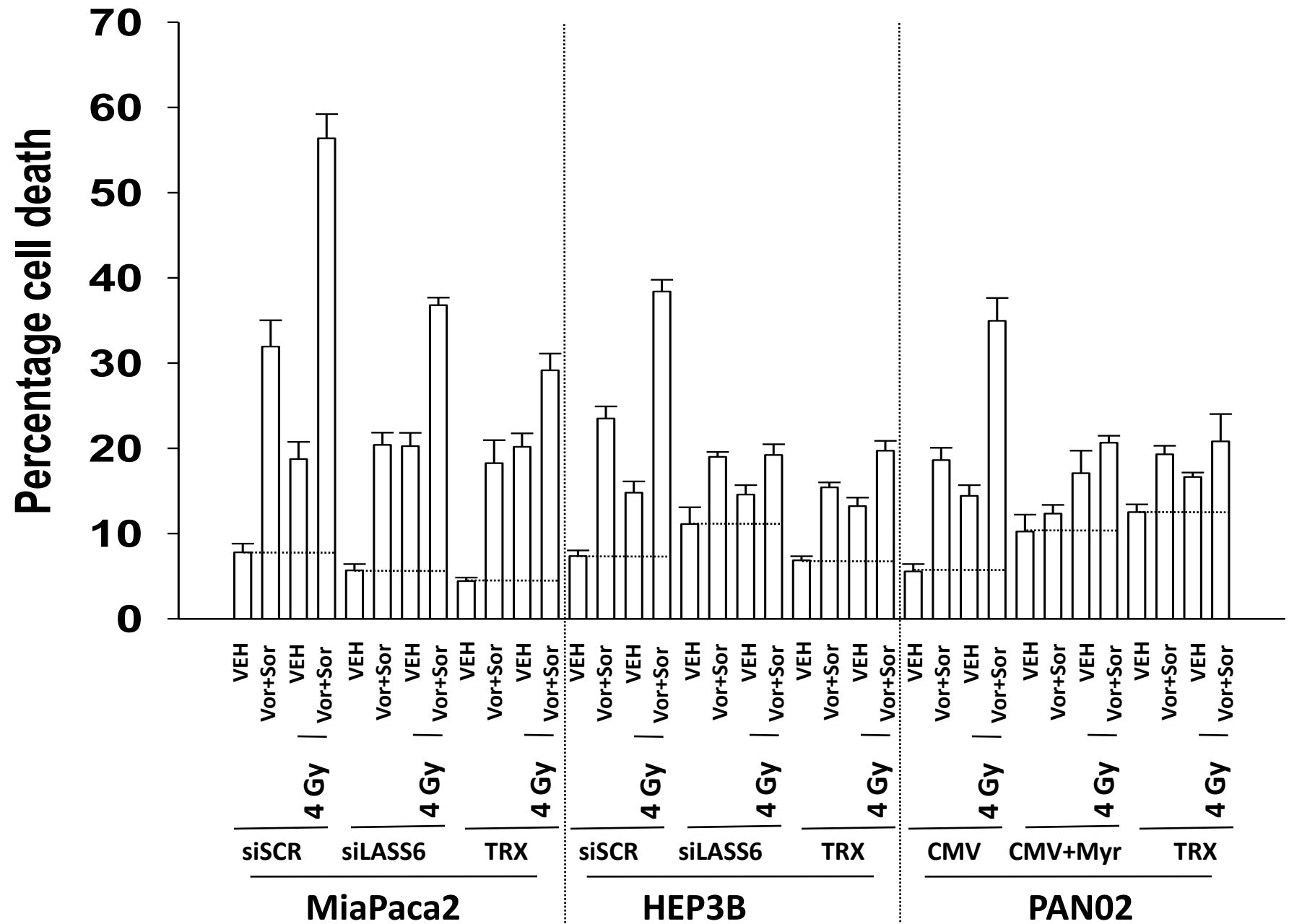
Figure S8. A putative mechanism by which sorafenib and vorinostat combined exposure increases: cytosolic Ca²⁺ levels; ceramide levels; PP2A activity; ROS levels; and CD95 activation.

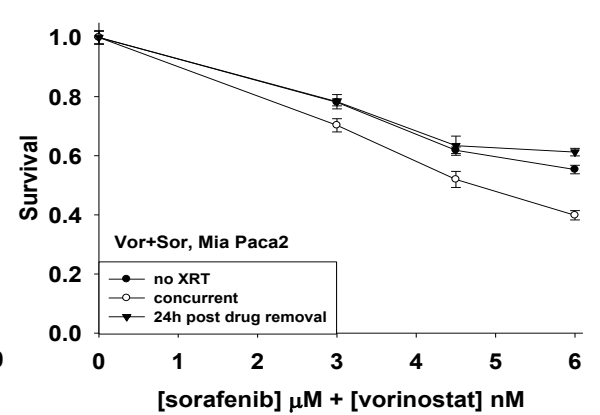
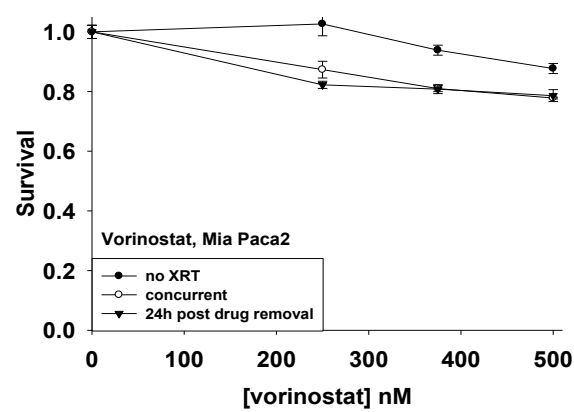
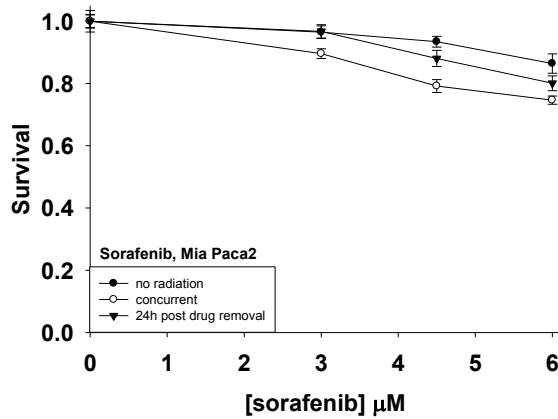
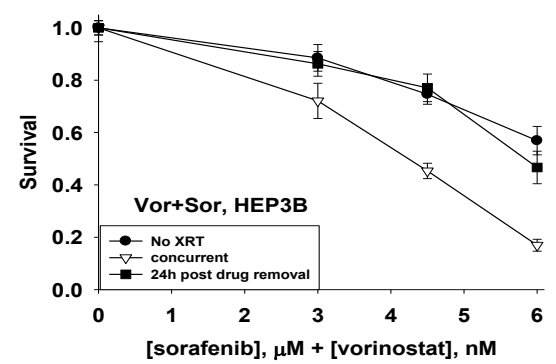
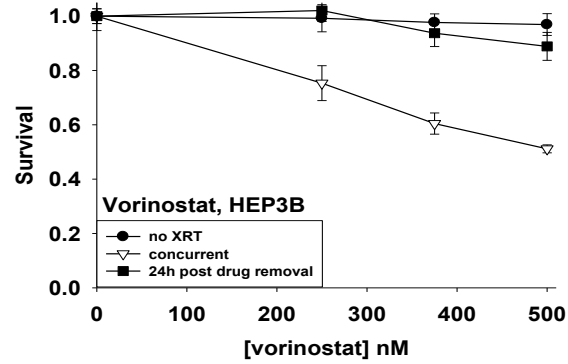
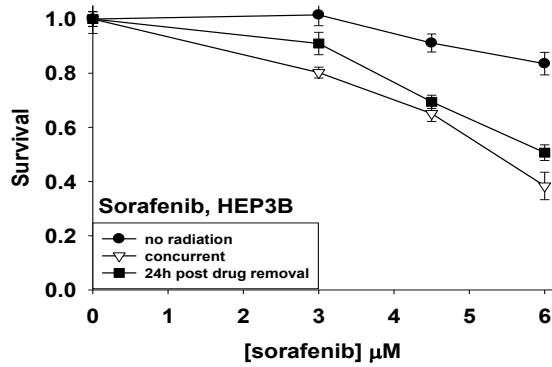


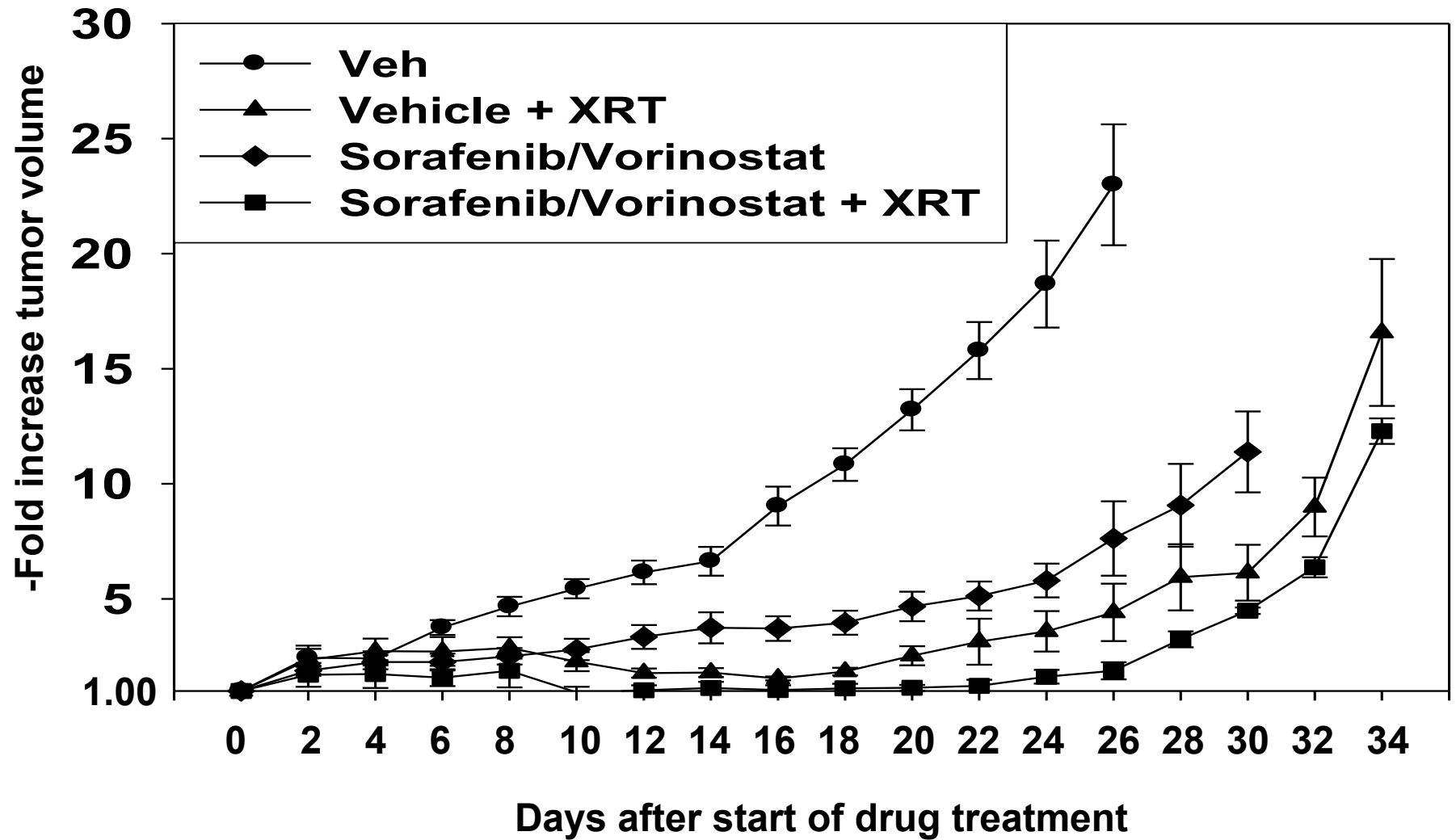












Park et al. Figure S8

