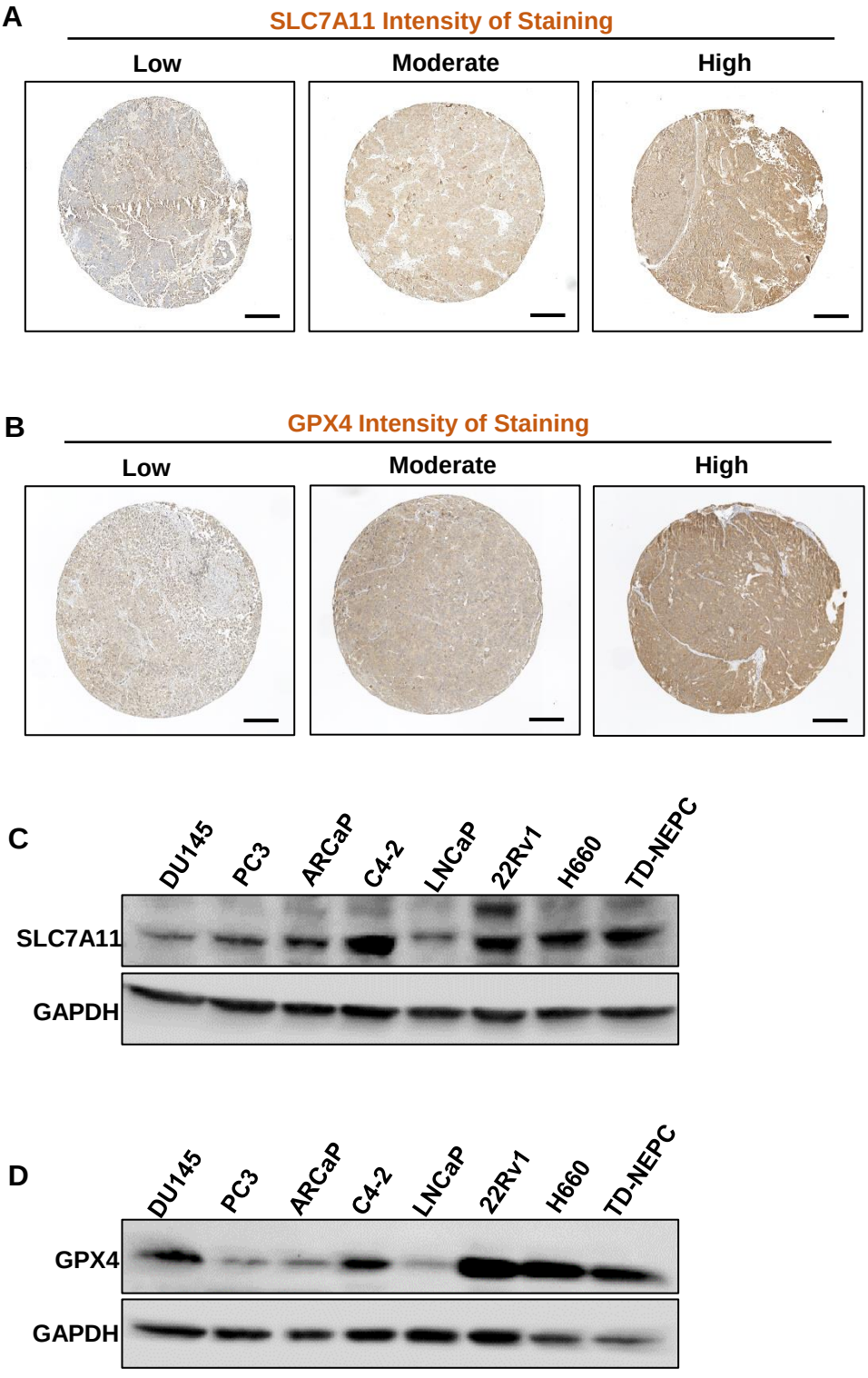
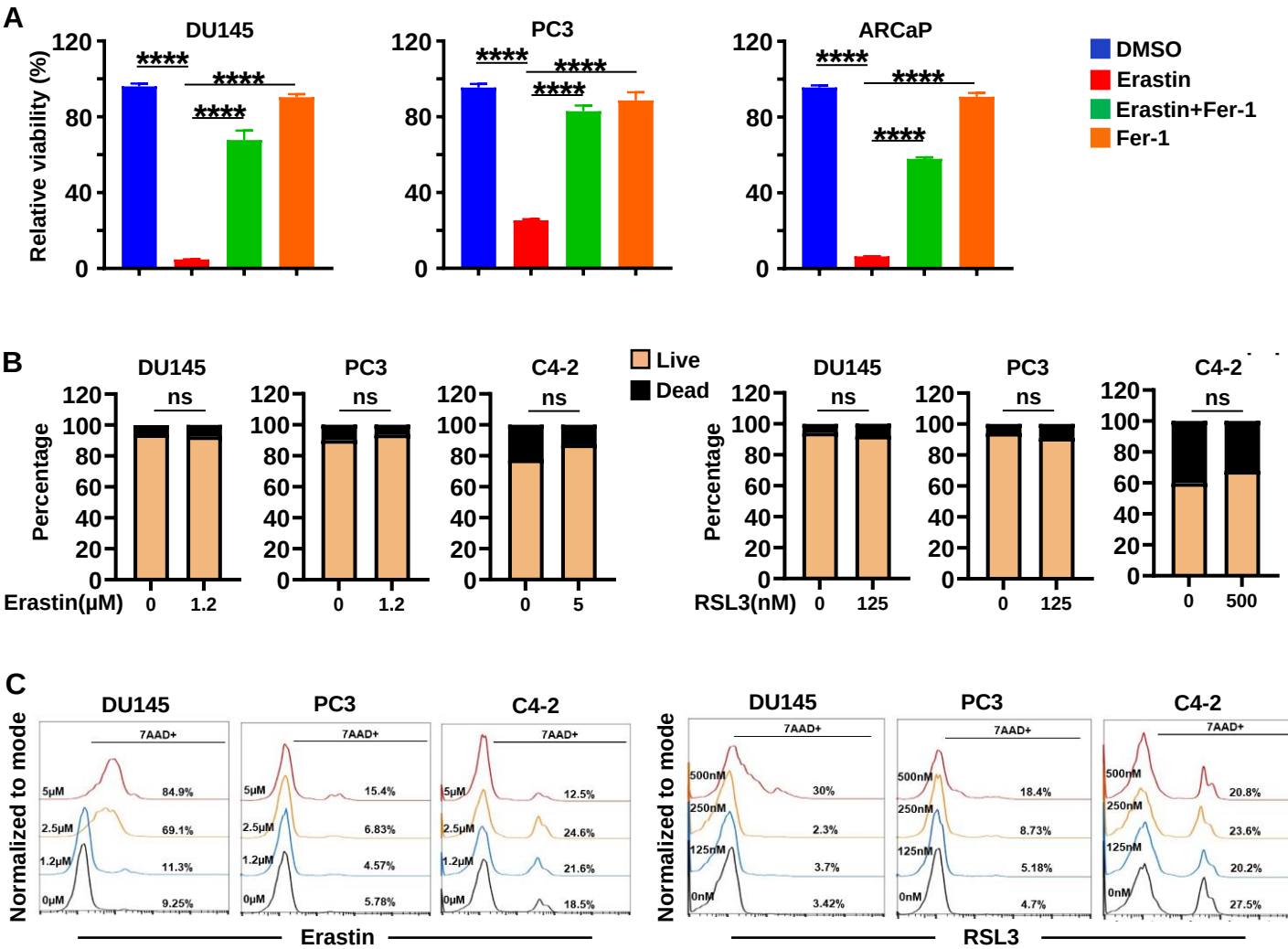
Supplementary Figure S1



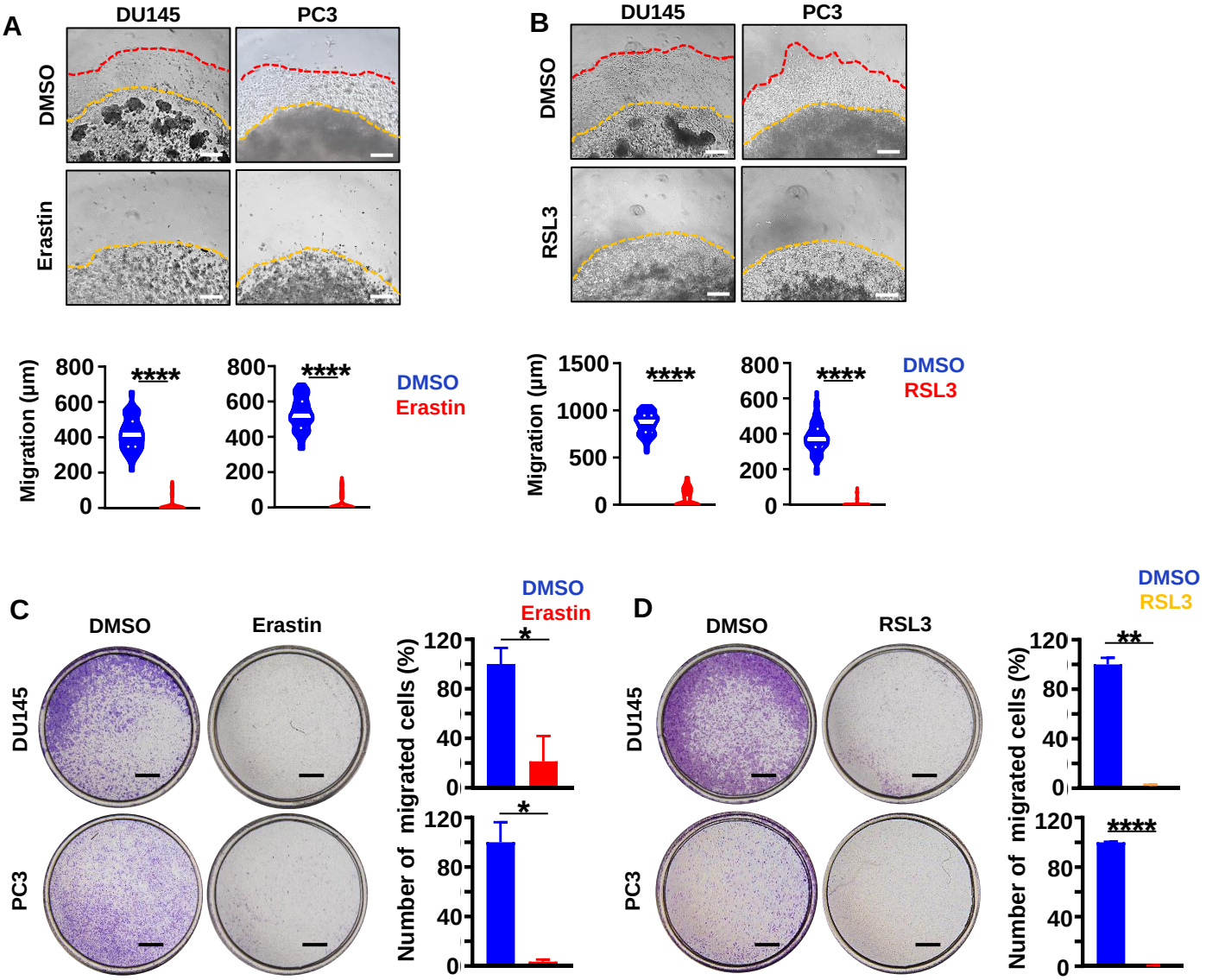
Supplementary Figure S1. Scoring of SLC7A11 and GPX4 intensity of staining and SLC7A11 and GPX4 expression in prostate cancer cell lines *in vitro*. (A) PDX TMA in Figure 1A was subjected to manual blinded scoring for intensity of SLC7A11 staining as low, moderate and high. Representative images of SLC7A11 low, moderate and high intensities are presented. Scale bars represent 200 microns. (B) PDX TMA in Figure 1B was blind scored for intensity of GPX4 staining as low, moderate and high. Representative images of GPX4 low, moderate and high intensities are shown. Scale bars signify 200 microns. (C, D) Expression levels of SLC7A11 (C) and GPX4 (D) proteins in different prostate cancer cells evaluated by western blot analysis. GAPDH was used for loading control.

Supplementary Figure S2



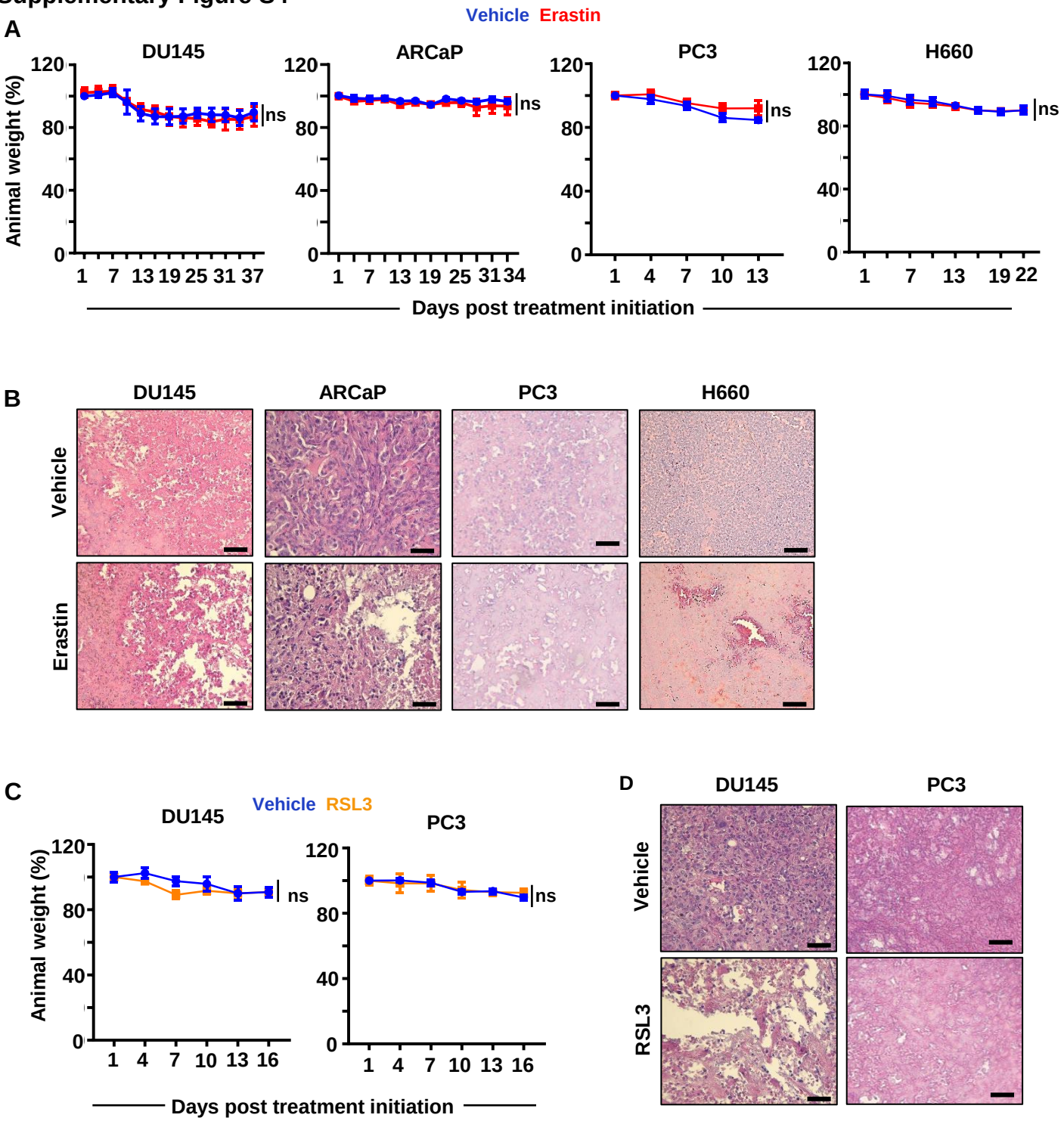
Supplementary Figure S2. Erastin and RSL3 decrease prostate cancer cell viability *in vitro*. (A) Cell viability assay. DU145, PC3, and ARCaP cells were treated with erastin (5 μM), erastin (5 μM) plus Fer-1 or Fer-1 (1 μM) alone for 48 hrs and graphed as relative viability compared to vehicle control. (B) Analysis of live-dead cells by trypan blue after erastin or RSL3 treatment for 72 hrs. (C) 7AAD assay to measure cell death induction after erastin or RSL3 treatment for 72 hrs. **** P<0.0001, ns=not significant, Student's t-test and error bars represent mean ± SEM.

Supplementary Figure S3



Supplementary Figure S3. Treatment with erastin and RSL3 inhibits prostate cancer cell invasion and migration *in vitro*. (A, B) 3D Matrigel drop invasion assay with DU145 or PC3 cells following erastin or RSL3 treatment. Cells were treated with erastin (5 µM) (A) or RSL3 (500 nM) (B) for six days. Media and treatment were exchanged every three days. The distance (µm) cells migrated from the edge of the matrigel drops was measured as on Day 6. DMSO was used as a control. Scale bars=200µm. Representative experiments are shown. (C, D) Transwell migration assay with DU145 and PC3 cells. $5x10^4$ viable cells were plated into transwell chambers for 20 hours after erastin (5 µM) or RSL3 (500 nM) treatment then fixed and stained with methanol and 0.01% crystal violet solution. Scale bars=1 mm. Experiments were performed in duplicate with duplicate wells. Representative experiments are shown. * P<0.05, **P<0.01, *** P<0.001, and **** P<0.0001, Student's t-test and error bars represent mean ± SEM.

Supplementary Figure S4



Supplementary Figure S4. Treatment with erastin and RSL3 have no measurable toxicity *in vivo*. (A) Animal weights were measured every three days for erastin treatment compared to vehicle group from Figure 4 and graphed as percent weight. (B) H&E staining for vehicle or erastin-treated tumors. (C) Animal weight (%) with RSL3 treatment compared to vehicle group from Figure 4. (D) H&E staining for vehicle or RSL3-treated tumors. Scale bars=50μm. Statistical analysis was performed with Student's t-test at each experimental timepoint (ns represents not significant differences) and error bars represent mean ± SEM.