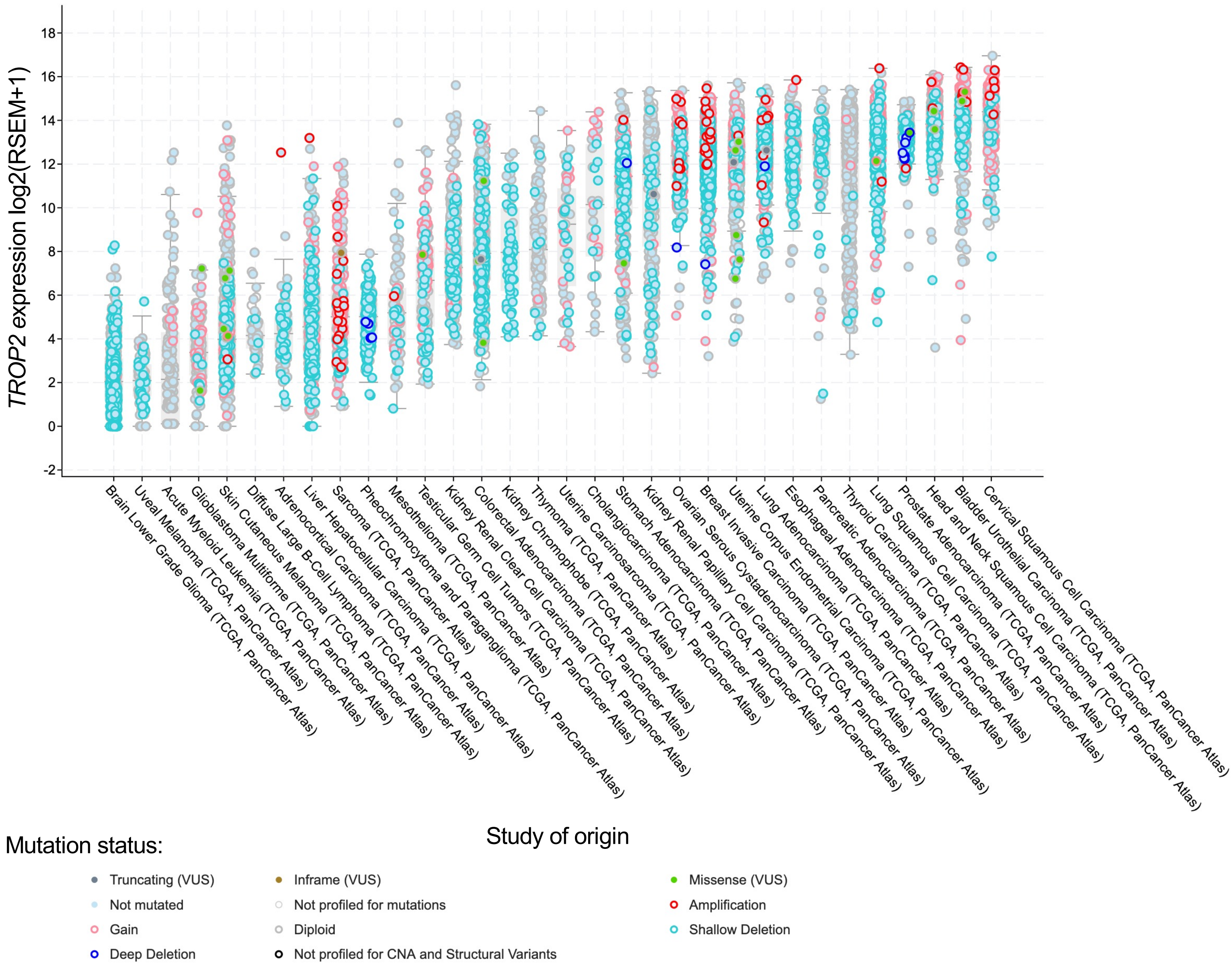
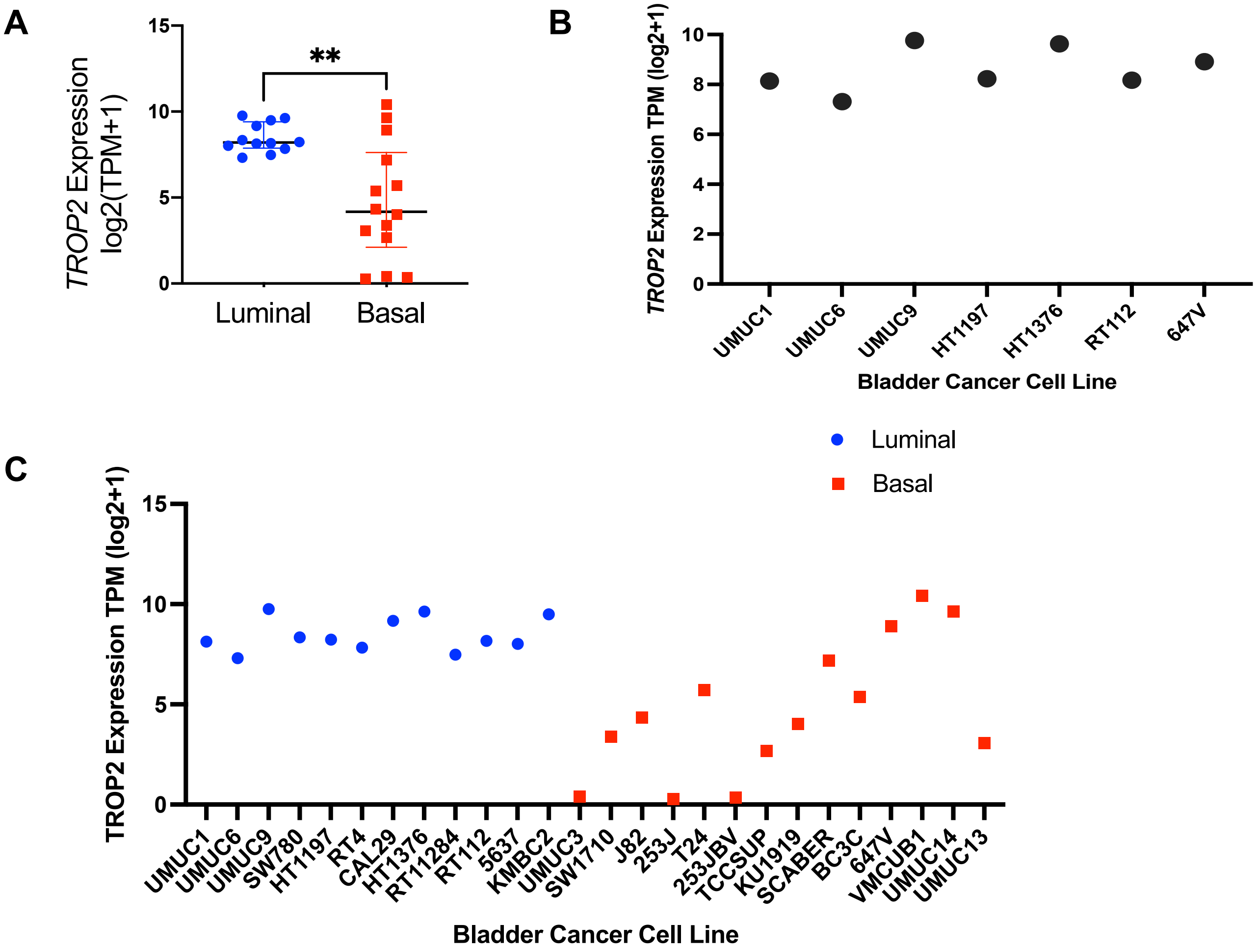
Supplemental Figure 1



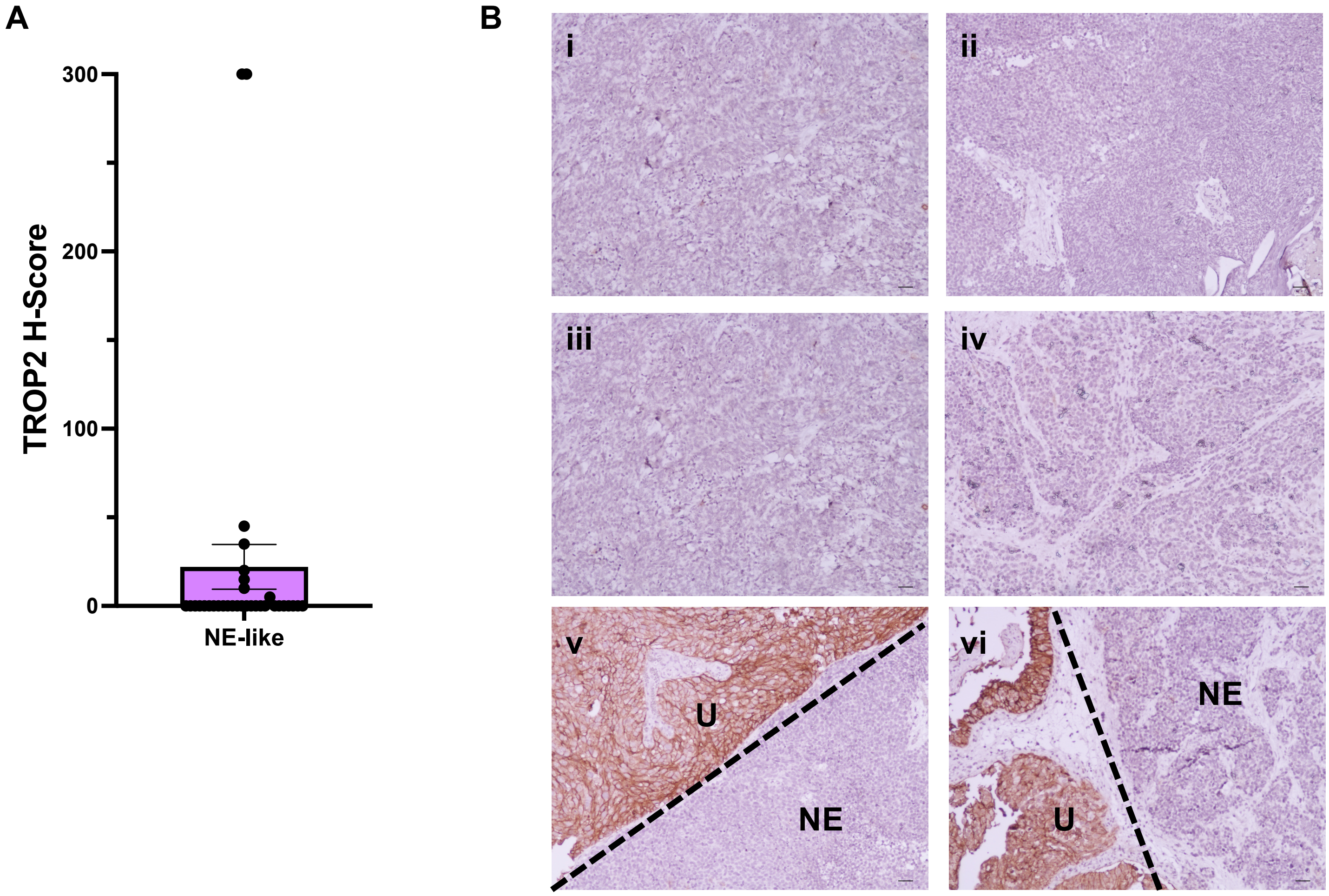
Supplemental Figure 1. ***TROP2* mRNA expression across cancer types.** *TROP2* mRNA is highly expressed across multiple types of cancer in the TCGA database, with the highest expression in urothelial and cervical squamous cell carcinomas. Datapoints are color-coded by presence and type of sequence mutations.

Supplemental Figure 2



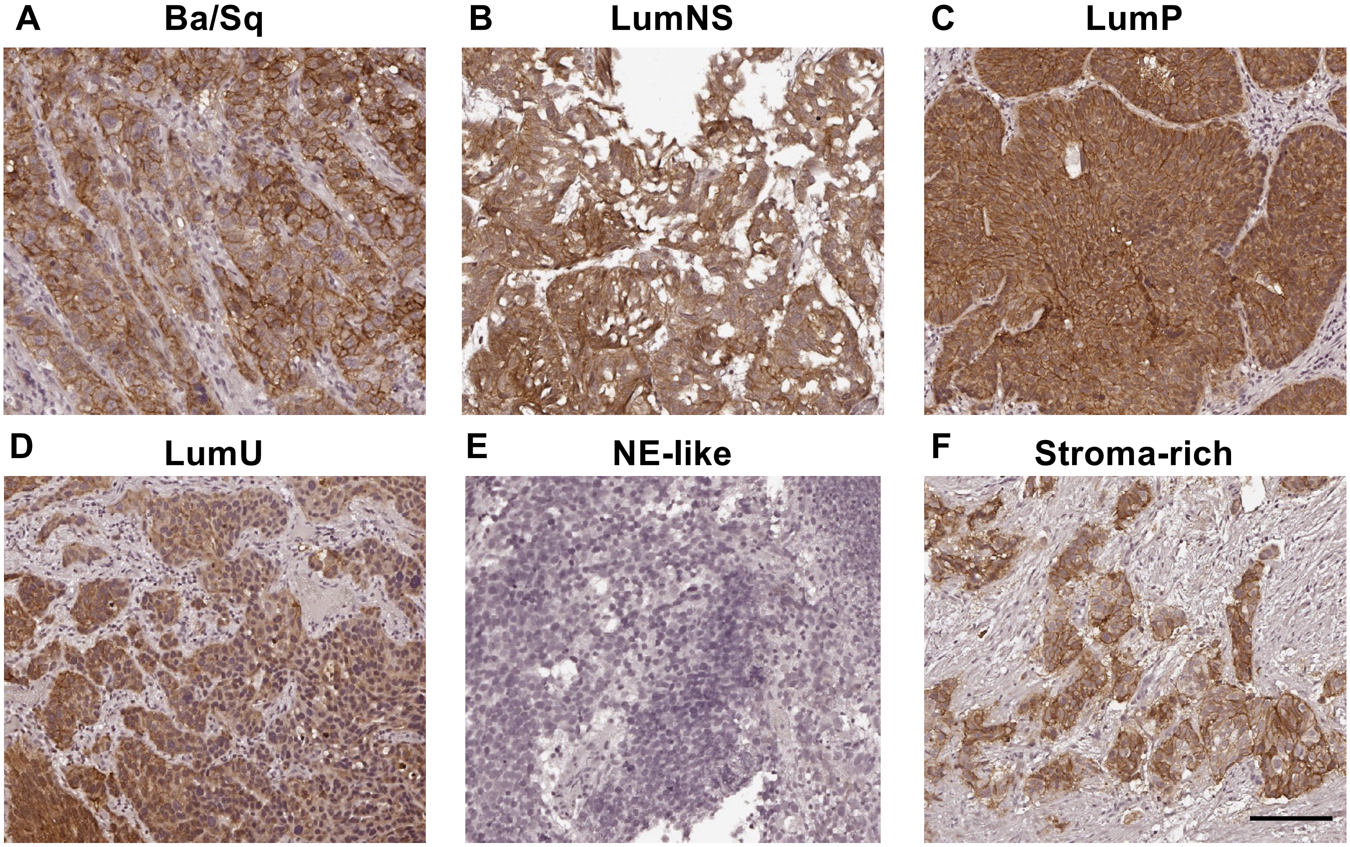
Supplemental Figure 2. *TROP2* mRNA expression in bladder cancer cell lines. (A) *TROP2* mRNA expression in luminal (blue dots, n=12) and basal (red squares, n=14) bladder cancer cell lines. The median and interquartile range are plotted. ** indicates p ≤ 0.005 by the Wilcoxon rank-sum test. (B) *TROP2* mRNA expression in the cell lines utilized in this study, corresponding to the surface protein expression in Fig 2E. (C) *TROP2* mRNA expression in a panel of 26 luminal and basal bladder cancer cell lines. mRNA expression data was obtained from DepMap.

Supplemental Figure 3



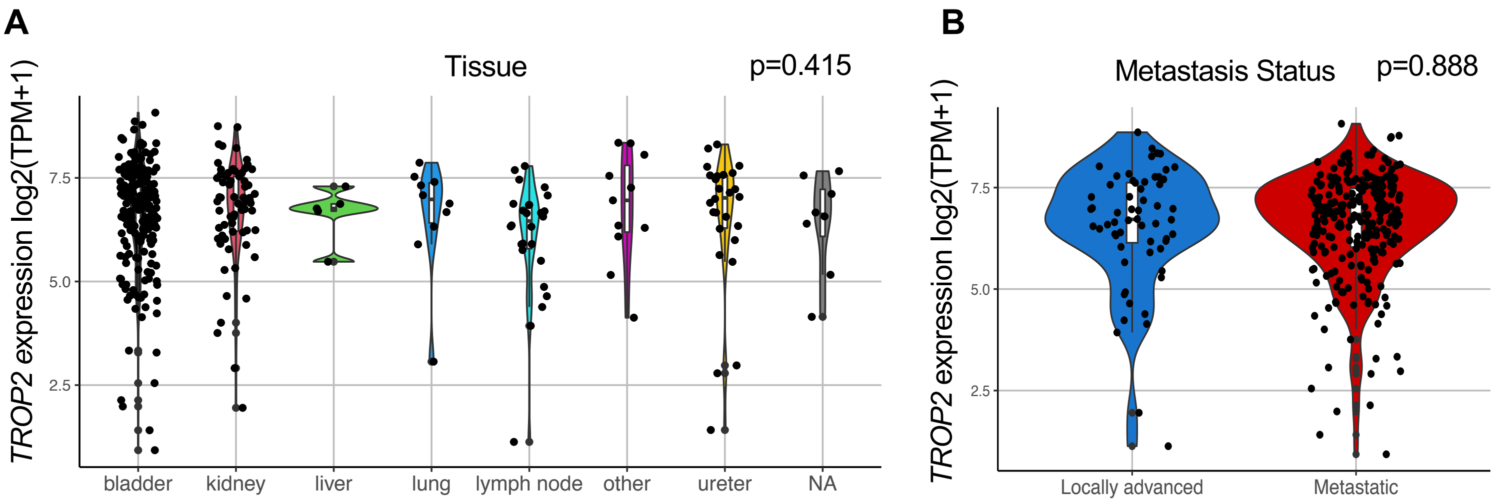
Supplemental Figure 3: TROP2 protein expression in NE/small cell bladder cancer. (A) TROP2 H-scores were determined by immunohistochemistry in NE/small cell bladder cancer (n=17 patient biopsies, in duplicate sections and 2 independent areas per section scored). The average TROP2 H-score +/- SEM is shown, and median H-score was 0. (B) Examples of TROP2 immunohistochemistry are shown for six tumors (i – vi). In panels v and vi, the tumors had mixed components. The urothelial component (labeled U) showed strong staining (H score = 300) for TROP2 while the neuroendocrine component (labeled NE) showed absent staining (H score = 0). The dotted line depicts the border between the urothelial and the NE/small cell areas of the tumor.

Supplemental Figure 4



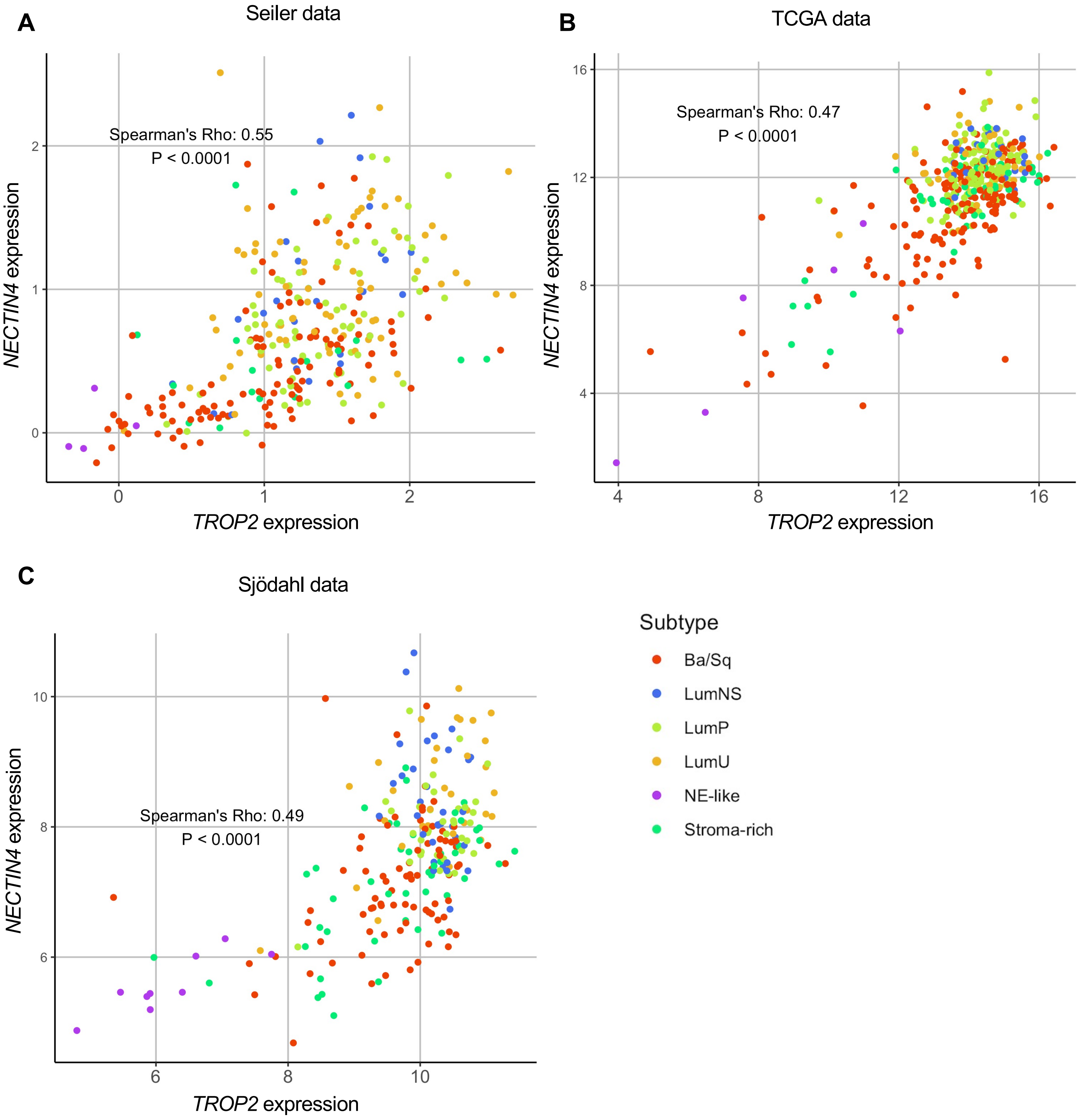
Supplemental Figure 4: TROP2 protein expression in molecular subtypes of bladder cancer. (A-F) Representative immunohistochemistry staining for TROP2 in the Ba/Sq (A), LumNS (B), LumP (C), LumU (D), NE-like (E) and Stroma-rich (F) bladder cancer subtypes using a bladder cancer tissue microarray (TMA). The subtypes were previously determined as described in Seiler et al, 2018. Scale bar denotes 100 μm.

Supplemental Figure 5



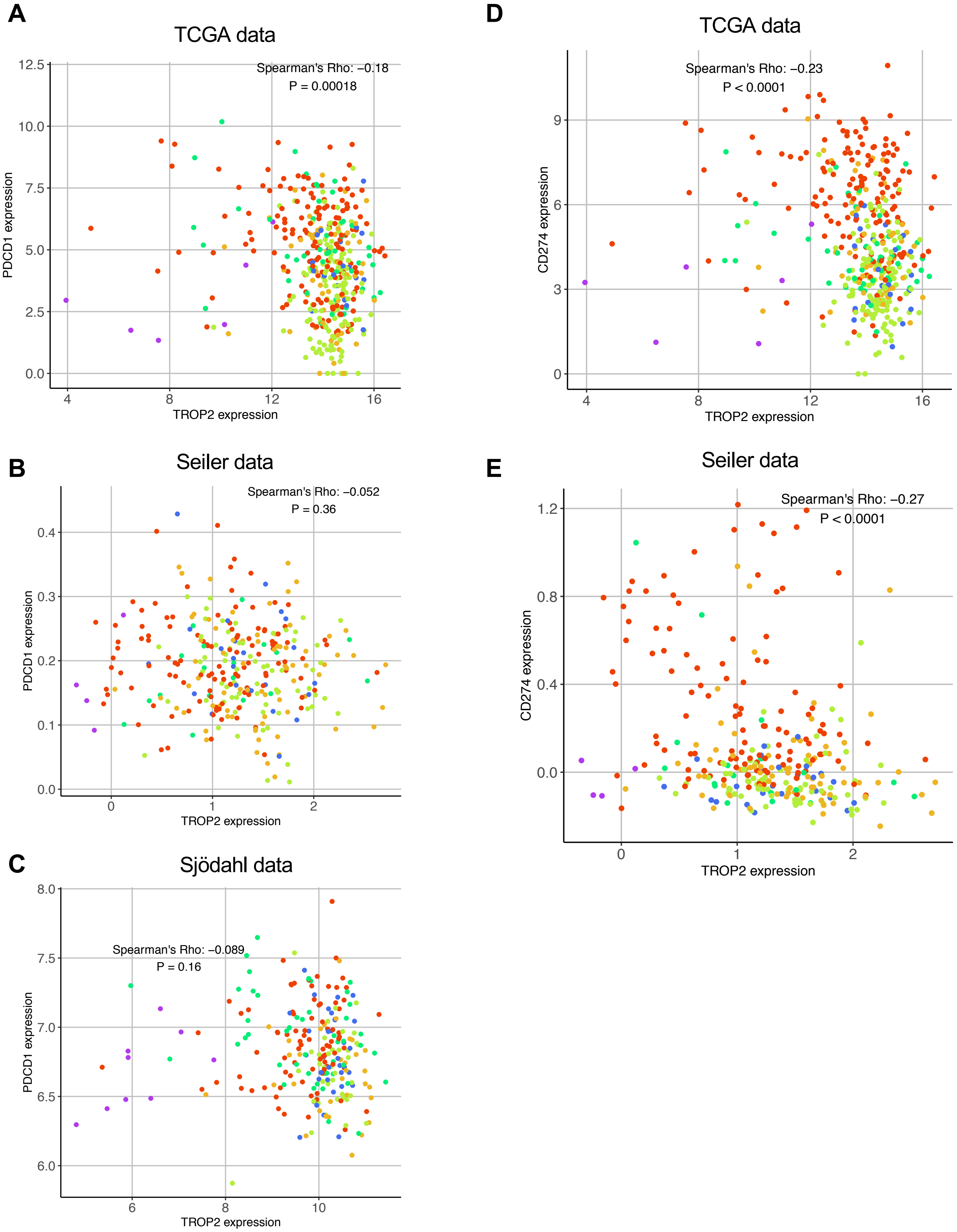
Supplemental Figure 5: *TROP2* mRNA expression in primary and metastatic sites. (A) *TROP2* mRNA expression levels in biopsies performed from various organs, including primary (including bladder, kidney and ureter) and metastatic sites from the IMVigor210 study. There was no difference in *TROP2* expression (p=0.415 by Kruskal-Wallis test, n=348 samples). (B) *TROP2* mRNA expression levels in patients with local/locally advanced and metastatic disease, from the IMVigor210 study. There was no difference in *TROP2* expression (p=0.888 by Wilcoxon test).

Supplemental Figure 6



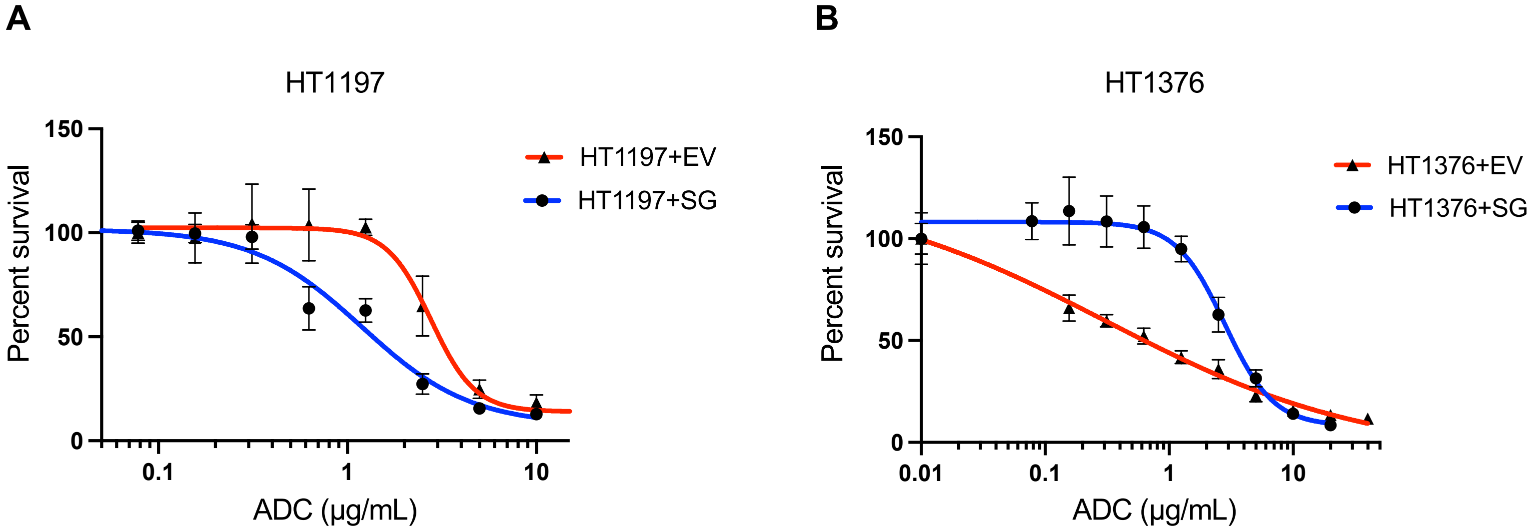
Supplemental Figure 6. *TROP2* and *NECTIN4* mRNA expression correlation. (A-C) Scatter plots showing correlation between *TROP2* and *NECTIN4* mRNA expression levels in the Seiler (A), TCGA (B) and Sjödahl (C) cohorts. The Spearman correlation coefficient and p value are shown for each cohort.

Supplemental Figure 7



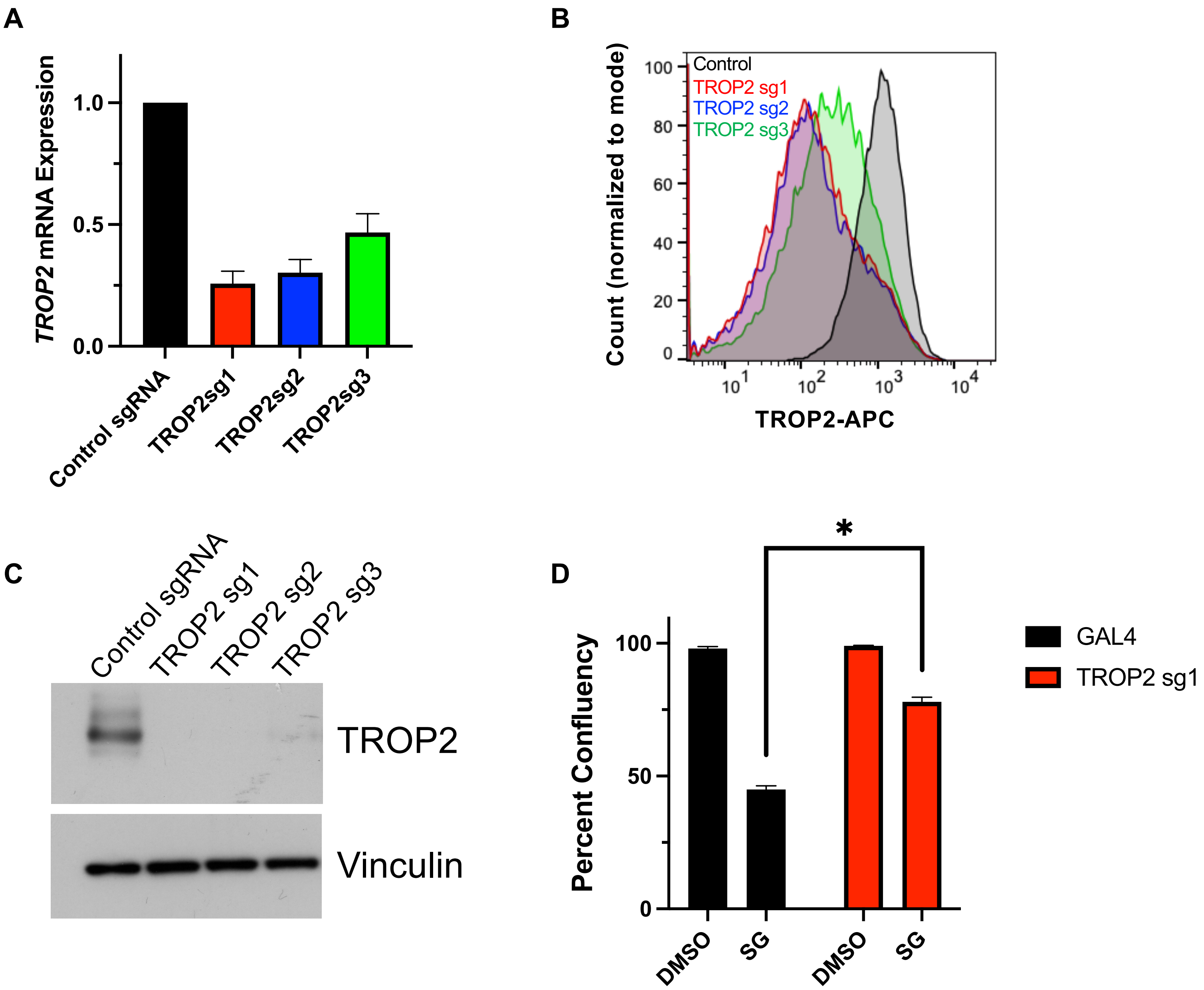
Supplemental Figure 7: *TROP2, PDCD1 (PD1)* and *CD274 (PDL1)* mRNA expression correlation. (A-C) Scatter plots showing correlation between *TROP2* and *PDCD1* (encoding PD1) mRNA expression levels in the TCGA (A), Seiler (B) and Sjodahl (C) cohorts. (D-E) Scatter plots showing correlation between *TROP2* and *CD274* (encoding PD-L1) mRNA expression levels in the TCGA (D) and Seiler (E) cohorts.

Supplemental Figure 8



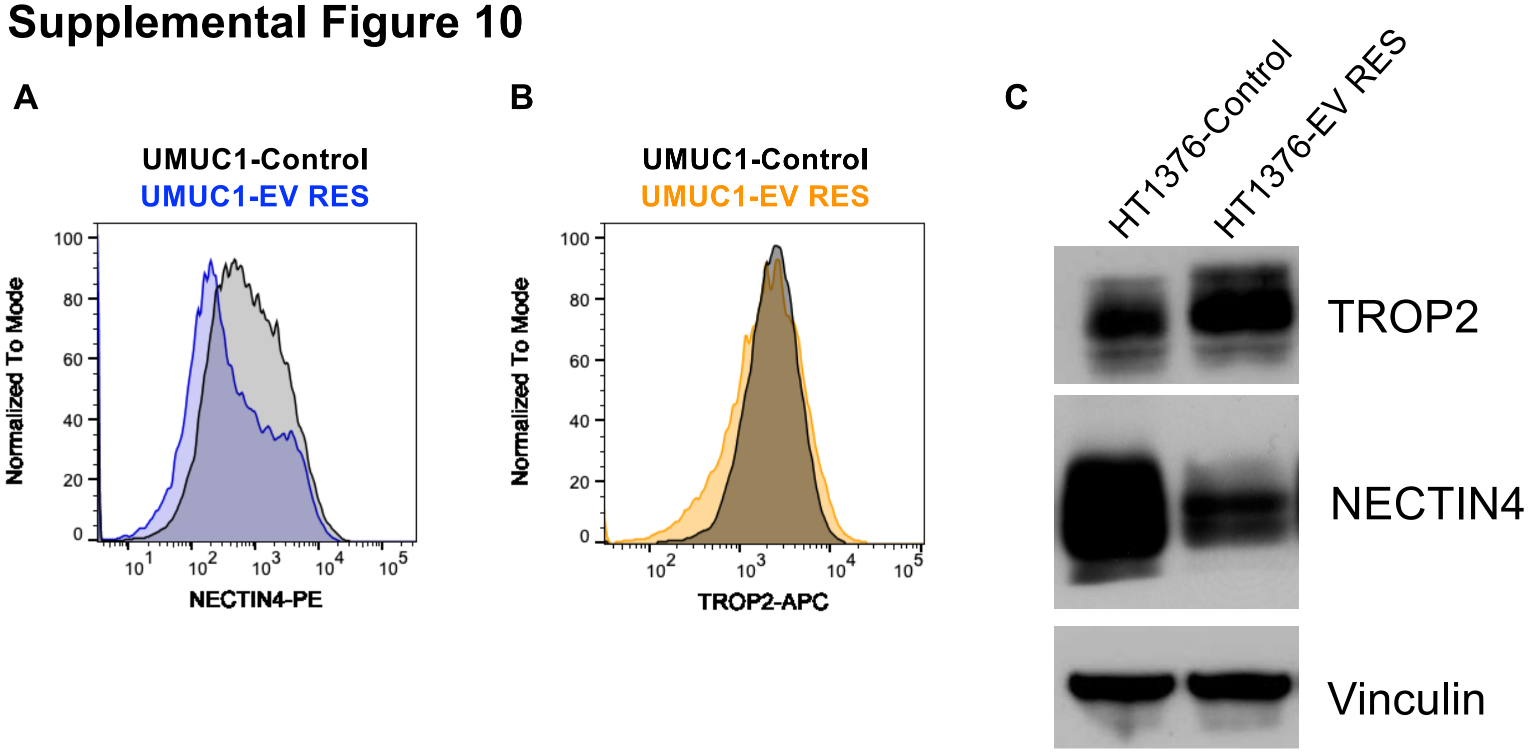
Supplemental Figure 8. Dose response curves to the antibody drug conjugates (ADC) enfortumab vedotin (EV) and sacituzumab govitecan (SG). Dose response curves to EV and SG in the HT-1197 (A) and HT-1376 (B) cell lines.

Supplemental Figure 9



Supplemental Figure 9: TROP2 loss leads to SG resistance. (A) Quantitative PCR (QPCR) for *TROP2* mRNA in 647V cells expressing a Control or 3 unique sgRNAs targeting TROP2 (sg1, sg2 and sg3). (B) Surface TROP2 protein staining in Control and TROP2 knockdown cells. (C) Western blot showing total TROP2 protein in Control and TROP2 knockdown cells. (D) Control (GAL4) or TROP2 knockdown (sg1) cells were treated with DMSO or 30ng/ml of SG and the percent confluency on Day 7 was measured. * $p<0.05$.

Supplemental Figure 10



Supplemental Figure 10: NECTIN4 and TROP2 protein levels in EV-resistant cells. (A-B) Surface NECTIN4 (A) and TROP2 (B) protein staining in UMUC1-Control and UMUC1-EV RES cells. (C) Western blot showing total NECTIN4 and TROP2 protein in HT1376-Control and HT1376-EV RES cells.