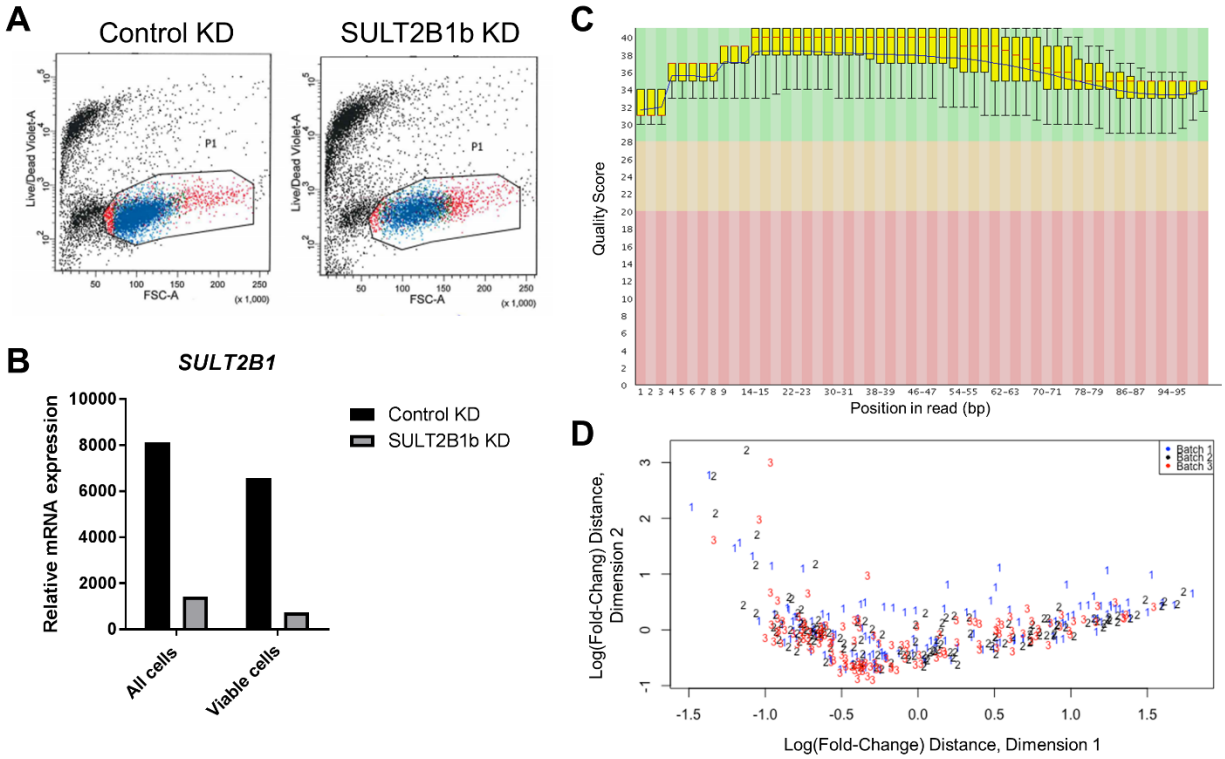
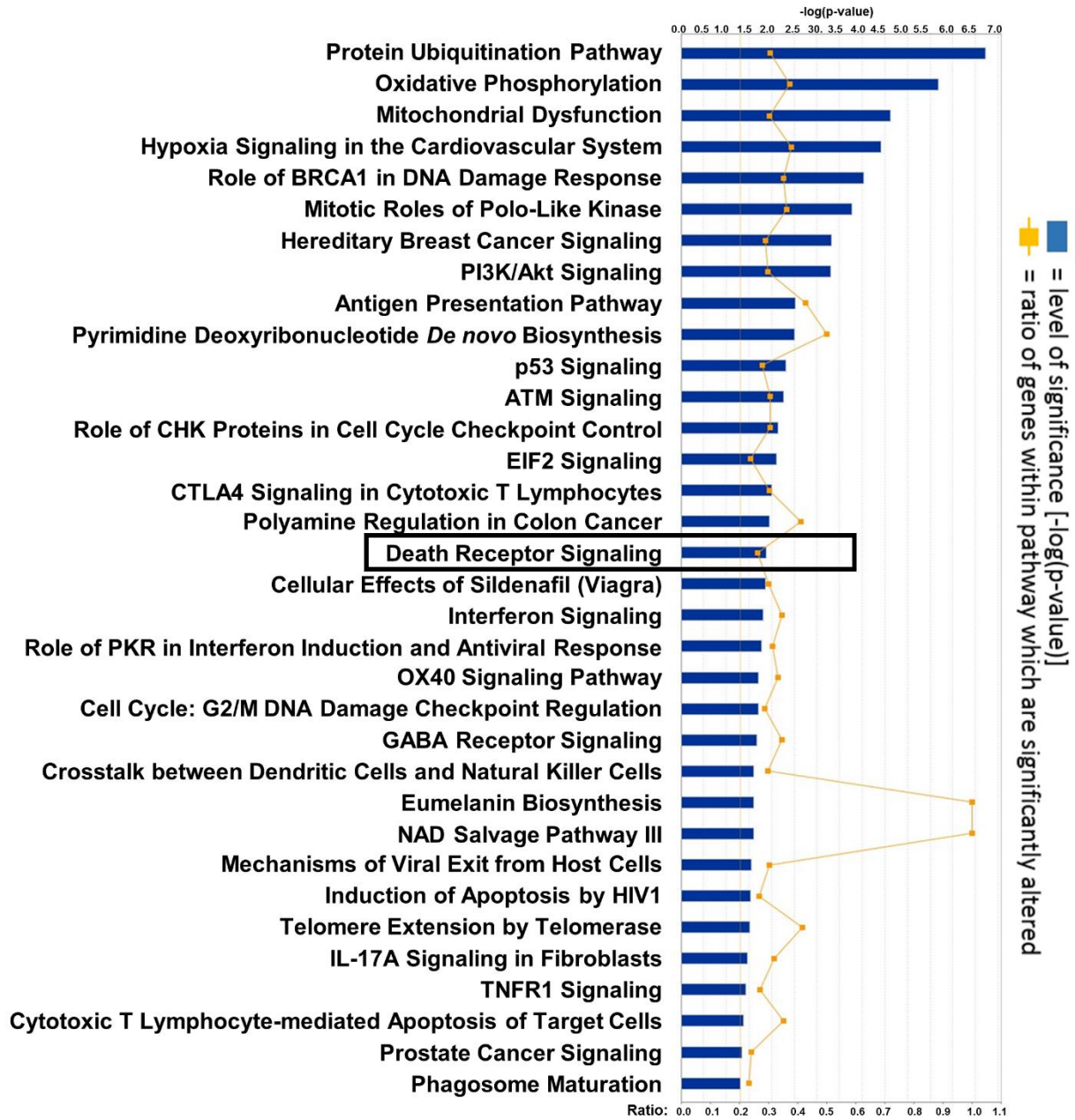
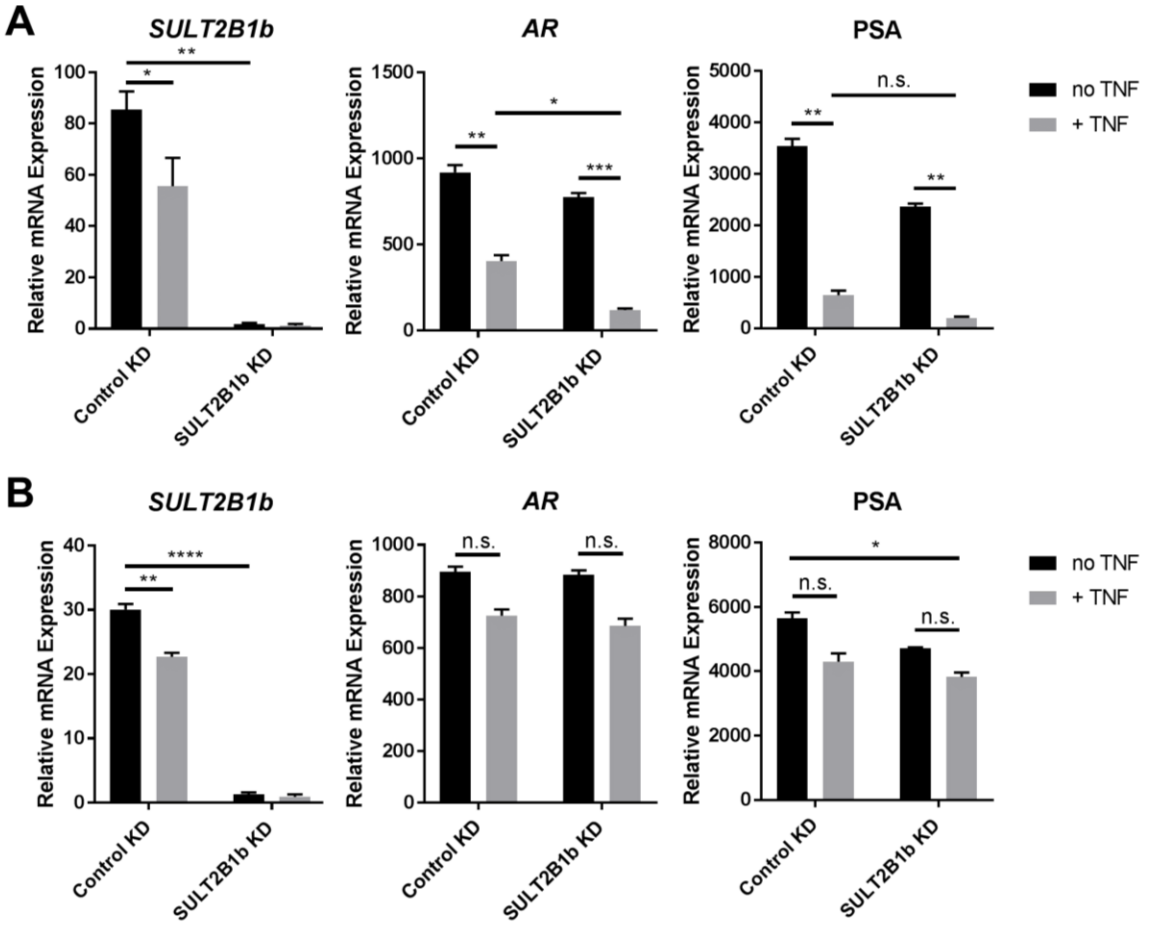


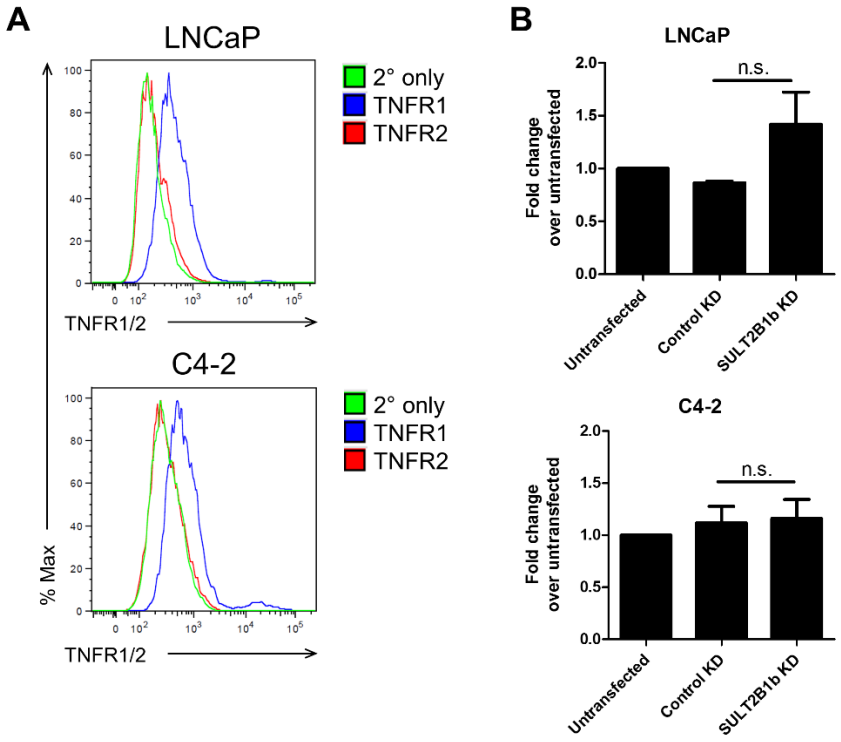
Supplemental Figure 1. Quality control from scRNA-seq analysis. (A) Flow cytometry plots indicating the population from which cells were sorted. LNCaP cells were harvested 48 hours after transfection with Control or SULT2B1b siRNA and stained with Zombie Violet viability dye. Cells were gated on the negative population and viable cells were sorted. Blue cells indicate the sorted population. (B) qRT-PCR analysis of bulk or viable cell sorted Control or SULT2B1b KD cells. Both sets of samples were harvested at 48 hours post-siRNA transfection. (C) Representative read per-base quality score plot observed using FastX-toolkit. (D) Multidimensional scaling plot by treatment highlighting the similarity/difference between single cells of the complete scRNA-seq analysis. Numbers indicate the batch of cells to which the particular sample belongs (1, 2, or 3), and the lack of batch number clustering indicates no confounding batch effect.



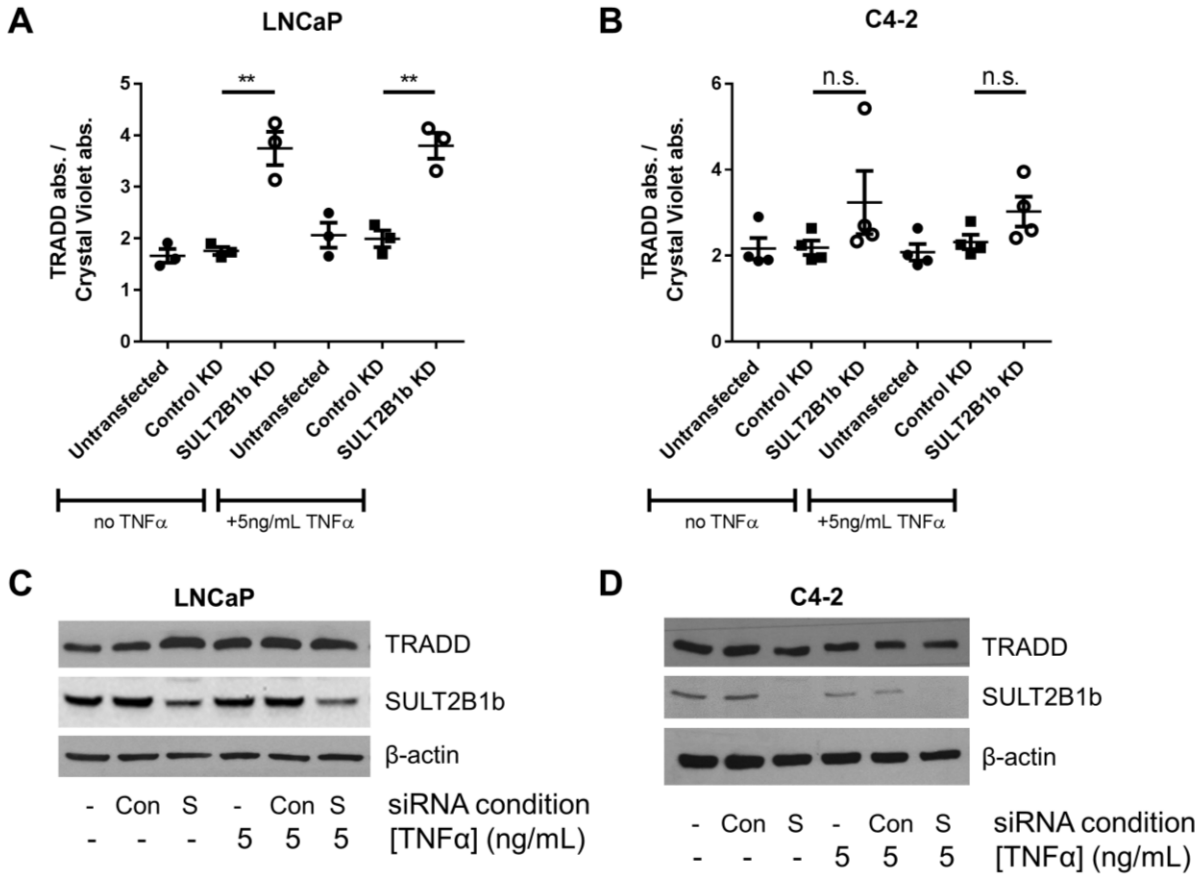
Supplemental Figure 2. Significantly altered pathways in SULT2B1b KD cells compared to Control KD cells. Ingenuity Pathway Analysis (IPA) was used to visualize significantly altered canonical pathways in SULT2B1b KD *versus* Control KD cells. Blue bars indicate the level of significance (-log(p-value)), while the yellow points indicate the ratio of the genes within the pathway which are differentially expressed to the total number of genes in the pathway.



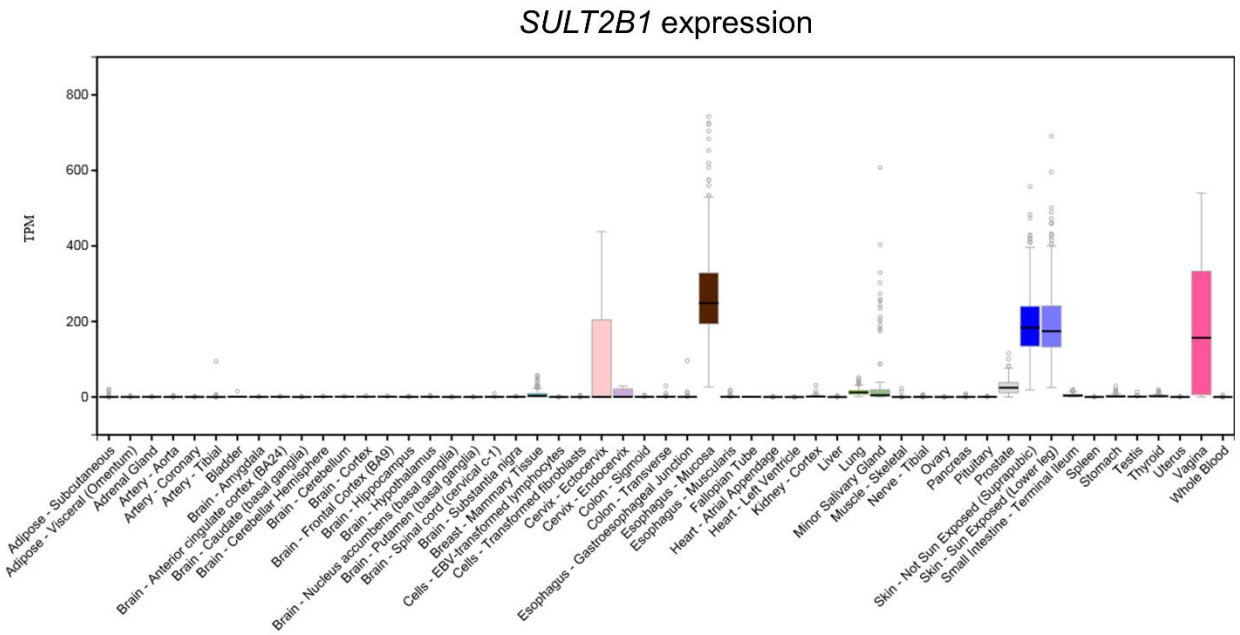
Supplemental Figure 3. TNF and SULT2B1b KD interact to further reduce AR activity in LNCaP, but not C4-2, cells. qRT-PCR indicating *AR* and PSA expression in (A) LNCaP and (B) C4-2 cells after 48 hours Control or SULT2B1b KD with or without addition of 5ng/mL TNF. Samples were analyzed by a two-way ANOVA with Tukey's multiple comparisons test. Bars represent the mean +/- SEM of triplicate samples. LNCaP cells were determined to have significant interaction between SULT2B1b KD and TNF treatment for both *AR* (p=0.0361) and PSA (p=0.0421) expression, by two-way ANOVA. No significant interaction was determined between SULT2B1b KD and TNF treatment in C4-2 cells.



Supplemental Figure 4: SULT2B1b KD does not significantly alter TNF-receptor expression. (A) Flow cytometry analysis indicating TNFR1 and TNFR2 expression in LNCaP and C4-2 cells. (B) Flow cytometry analysis of TNFR1 expression in LNCaP and C4-2 cells after 72 hours Control or SULT2B1b siRNA transfection. Expression level was indicated as the difference in mean fluorescence intensity (MFI) over secondary only controls for each group. Levels were further normalized to the untransfected control group. Bars represent the average fold change +/- SEM over untransfected samples from at least four independent experiments.



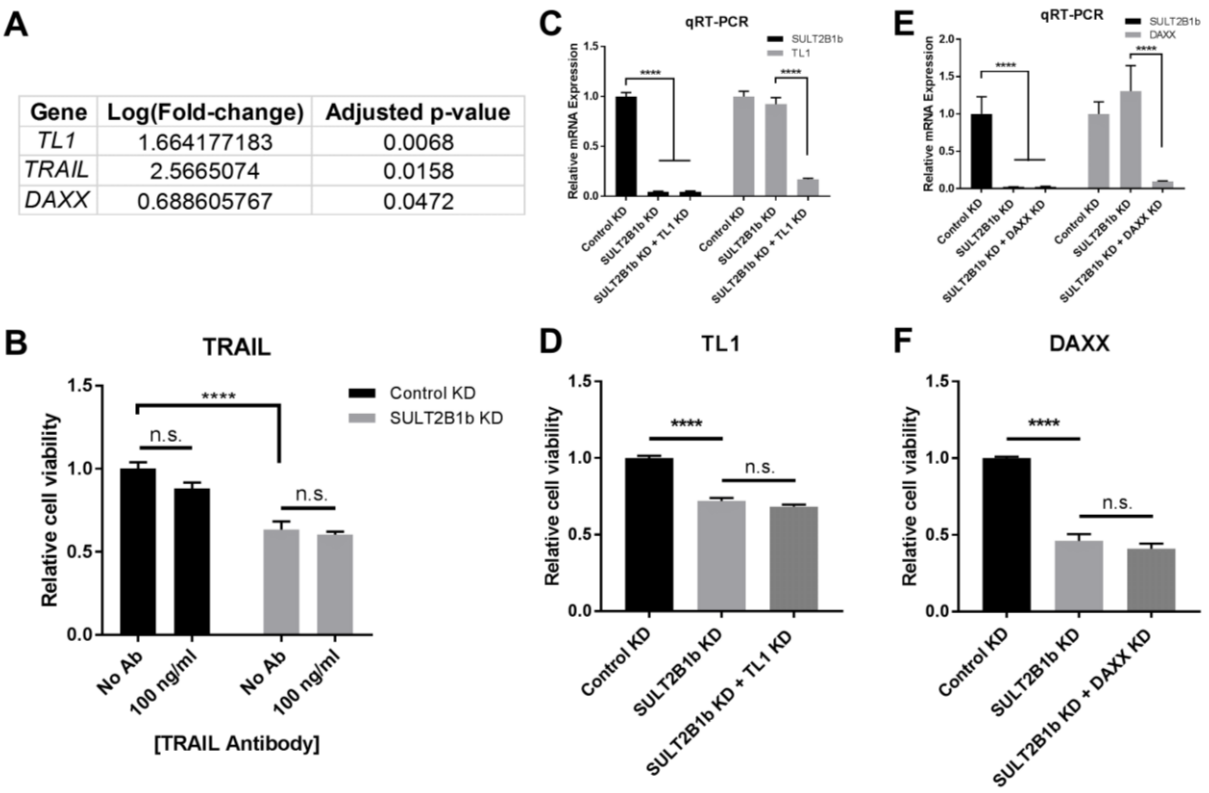
Supplemental Figure 5: SULT2B1b KD induces TRADD expression in LNCaP, but not C4-2 cells. LNCaP and C4-2 cells were treated with Control or SULT2B1b siRNA five hours prior to addition of 5ng/mL TNF to respective wells. (A-B) After 72 hours, cells were fixed to the plate and an ELISA for TRADD expression was completed in (A) LNCaP and (B) C4-2 cells. TRADD absorbance was normalized to crystal violet absorbance for quantification. Data points represent the mean +/- SEM of independent experiments. (C-D) Western blot of indicated proteins in (C) LNCaP or (D) C4-2 cells 72 hours after Control (Con) or SULT2B1b (S) KD.



Supplemental Figure 6. SULT2B1 is a gene with low basal expression in many tissues, including the prostate. ENSG00000088002.7 (*SULT2B1*) gene expression is indicated for many human tissues, as determined by GTEx Portal.

A

Gene	Log(Fold-change)	Adjusted p-value
TL1	1.664177183	0.0068
TRAIL	2.5665074	0.0158
DAXX	0.688605767	0.0472



Supplemental Figure 7. TRAIL, TL1, and DAXX do not mediate SULT2B1b KD-induced apoptosis in LNCaP cells. (A) Examples of genes within the death receptor signaling pathway that are significantly altered in SULT2B1b KD *versus* Control KD cells from the scRNA-seq analysis. (B) LNCaP cells with SULT2B1b KD, followed by treatment with TRAIL neutralizing antibody 24 hours later, were analyzed for cell viability 48 hours after treatment. (C) LNCaP cells consecutively transfected with SULT2B1b siRNA followed by TL1 siRNA after 7 hours. RNA was collected 24 hours after the final transfection and analyzed for SULT2B1b and TL1 expression by qRT-PCR. (D) Cell viability was analyzed 72 hours after consecutive SULT2B1b and TL1 KD. (E) LNCaP cells consecutively transfected with SULT2B1b siRNA followed by DAXX siRNA 12 hours later. RNA was collected 24 hours after the final transfection and analyzed for SULT2B1b and DAXX expression by qRT-PCR. (F) Cell viability was analyzed 72 hours after consecutive SULT2B1b and DAXX KD.

Gene	Correlation Coefficient	P-Value
LTB	**-0.480489465**	**0.0232**
TNFSF4	**-0.455420252**	**0.0424**
TNFSF13B	**-0.514369968**	**0.0164**
TNFAIP8L2	**-0.472903251**	**0.028**
TNFRSF10C	**-0.558005269**	**0.0108**
TNFRSF13C	**-0.445856977**	**0.042**
TNFRSF17	**-0.461122858**	**0.038**
TNFRSF1B	**-0.449093777**	**0.0468**
TNFSF14	**-0.43249588**	**0.0484**

Supplemental Table 1. Significant negative correlations of *SULT2B1* with TNF-related genes in prostate cancer samples from patients with bone metastasis. Genes listed have a significant, negatively correlated relationship with *SULT2B1* in bone-derived metastatic prostate cancer samples from patients with no prior treatments. Pearson correlation coefficients and p-values are indicated for each gene.