

Supplementary Material: Endocrine Therapy-Resistant Breast Cancer Cells Are More Sensitive to Ceramide Kinase Inhibition and Elevated Ceramide Levels than Therapy-Sensitive Breast Cancer Cells

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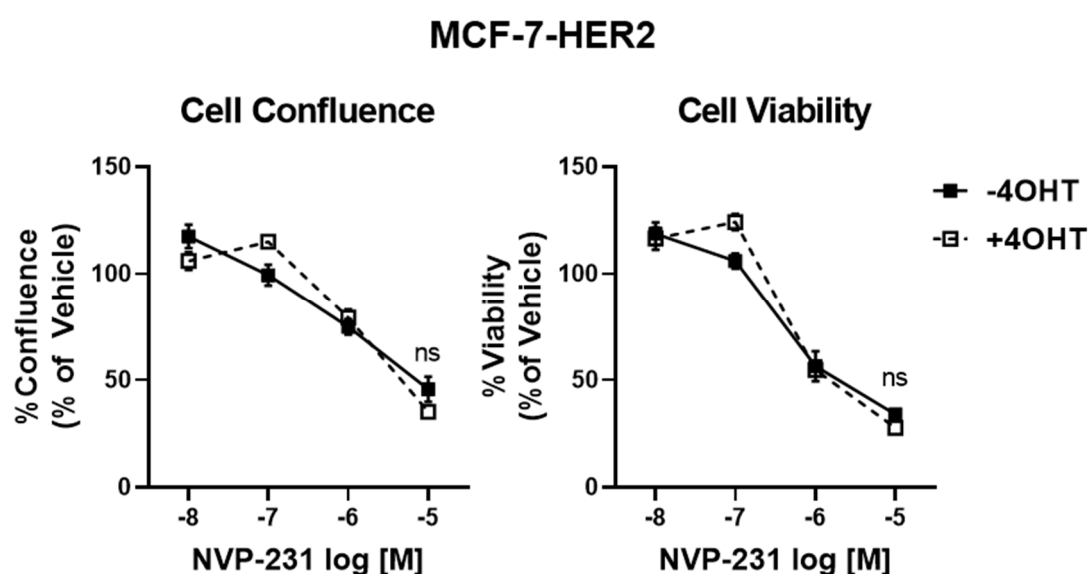


Figure S1. MCF-7-HER2 cells were treated with an increasing dose of NVP-231 with or without 1 μ M of 4-hydroxytamoxifen (4-OHT). After 48-h of treatment, cell confluence (brightfield) and viability (PI-negative Hoechst-positive) were estimated from Hoechst-PI endpoint assay. ns: not significant.

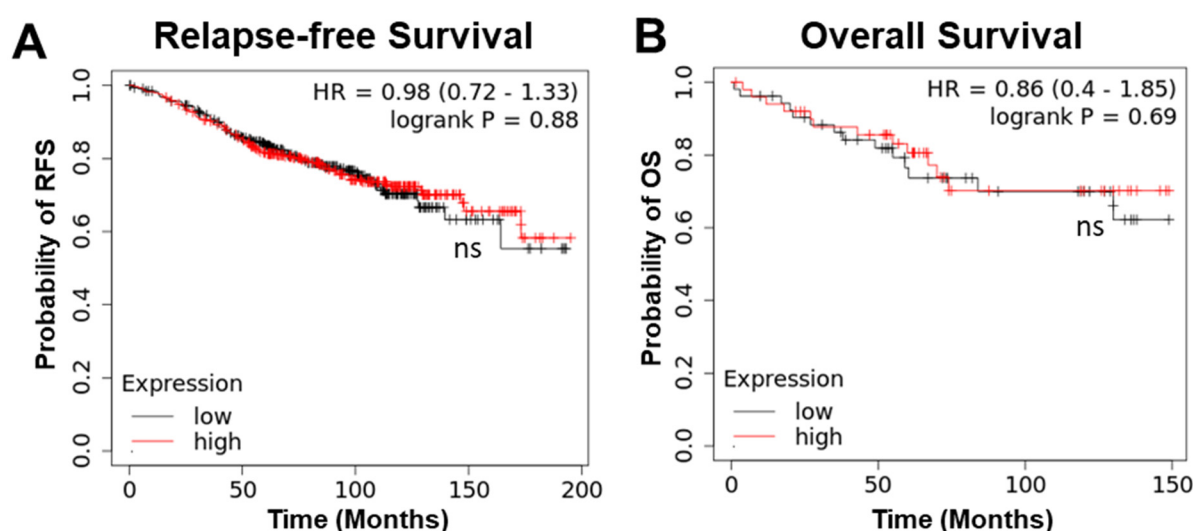


Figure S2. ER+ breast tumors treated with tamoxifen from publicly available datasets were analyzed for association between CERK mRNA expression and (A) relapse-free ($n = 662$) or (B) overall patient survival ($n = 104$). Statistical significance was determined using log-rank test. ns: not significant.

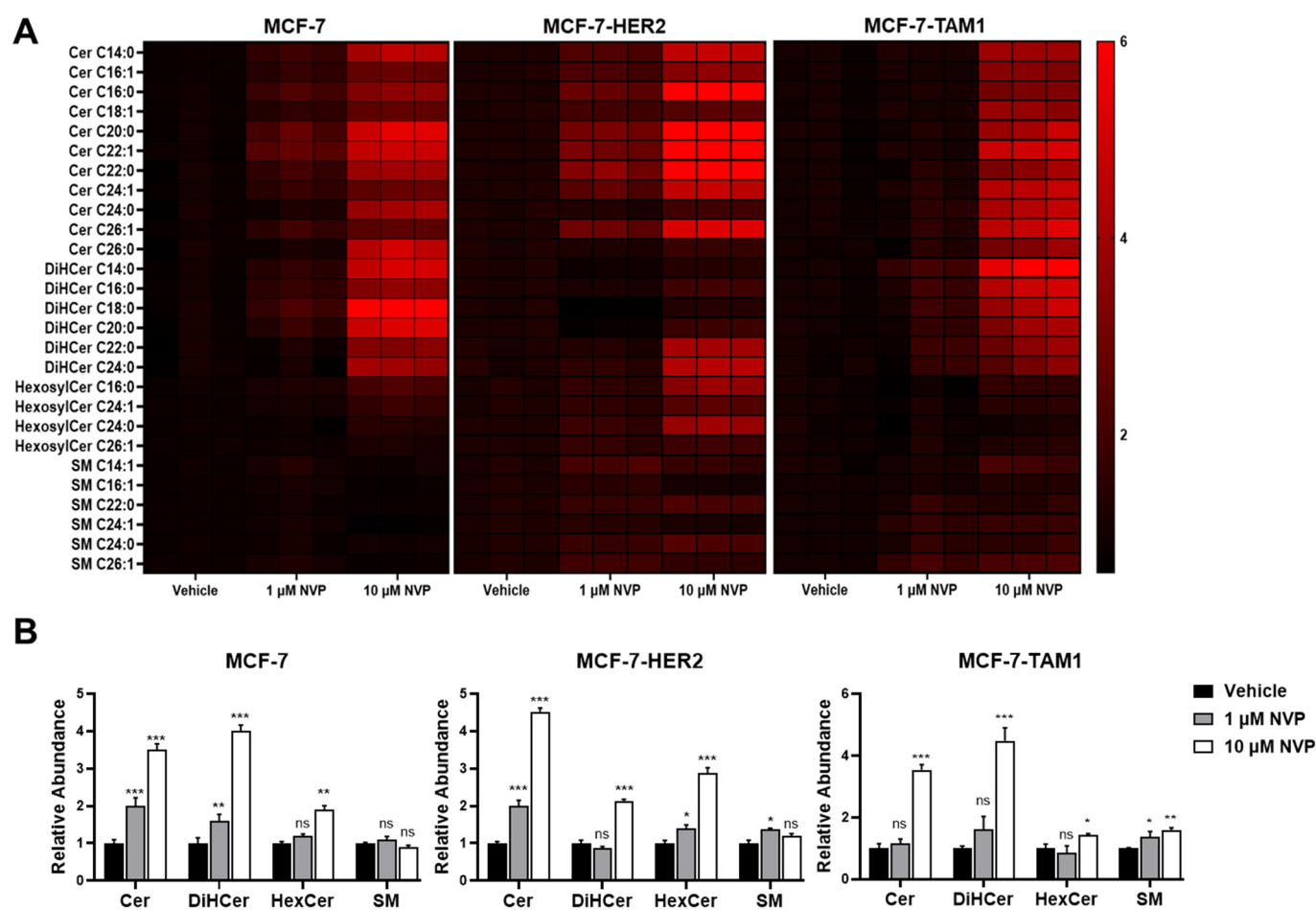
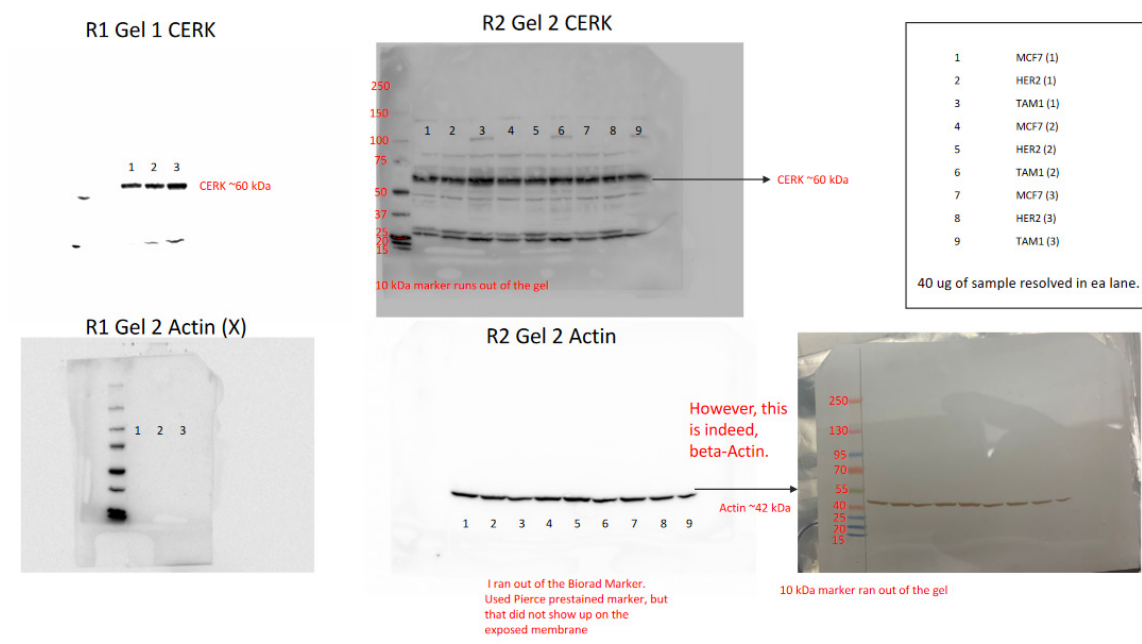


Figure S3. (A) MCF-7, MCF-7-HER2, MCF-7-TAM1 cells were treated with 1 and 10 μ M of NVP-231 for 48-h and relative abundance of different sphingolipid species were measured relative to respective sphingolipid species levels in vehicle-treated (DMSO) control for each cell line and presented as heatmap. (B) Abundances of ceramides, dihydroceramides, hexosylceramides, and sphingomyelins in the NVP-231 treated groups relative to vehicle-treated controls were calculated by dividing the abundance of a certain sphingolipid species by the average abundance of that species in vehicle-treated controls for each cell line. ns: not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Calculated Gray Area for each set, CERK and Actin.

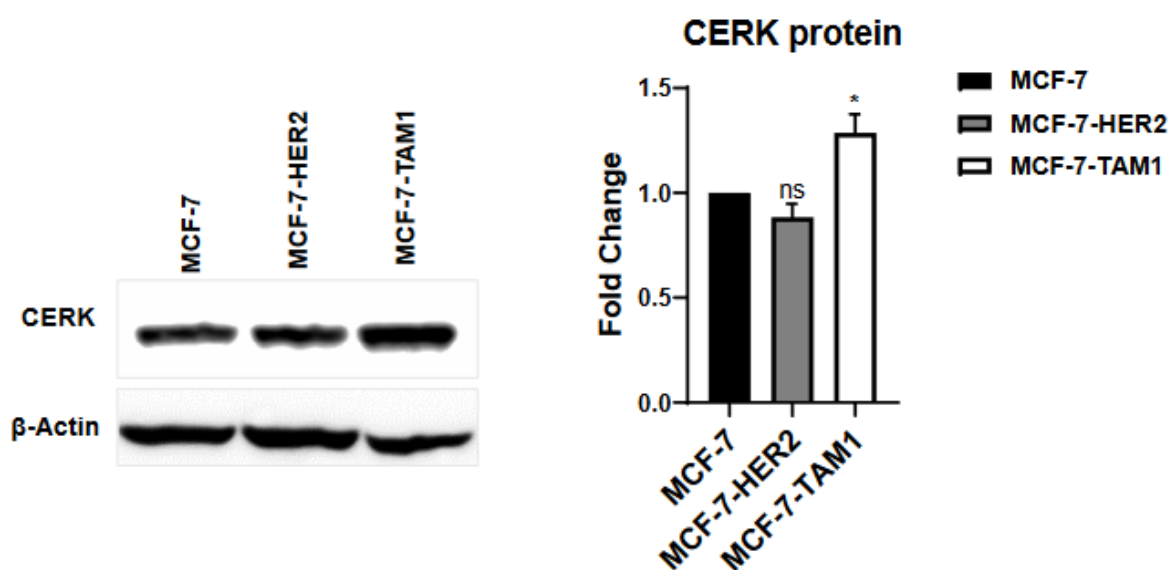
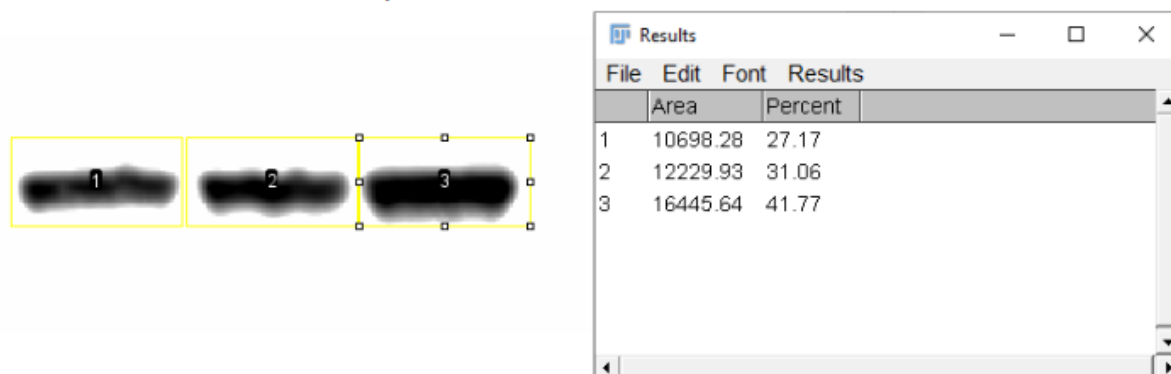


Figure S4. The uncropped Western blots.