cancers

Supplementary Materials: Combined Targeting of Estrogen Receptor Alpha and XPO1 Prevent Akt Activation, Remodel Metabolic Pathways and Induce Autophagy to Overcome Tamoxifen Resistance

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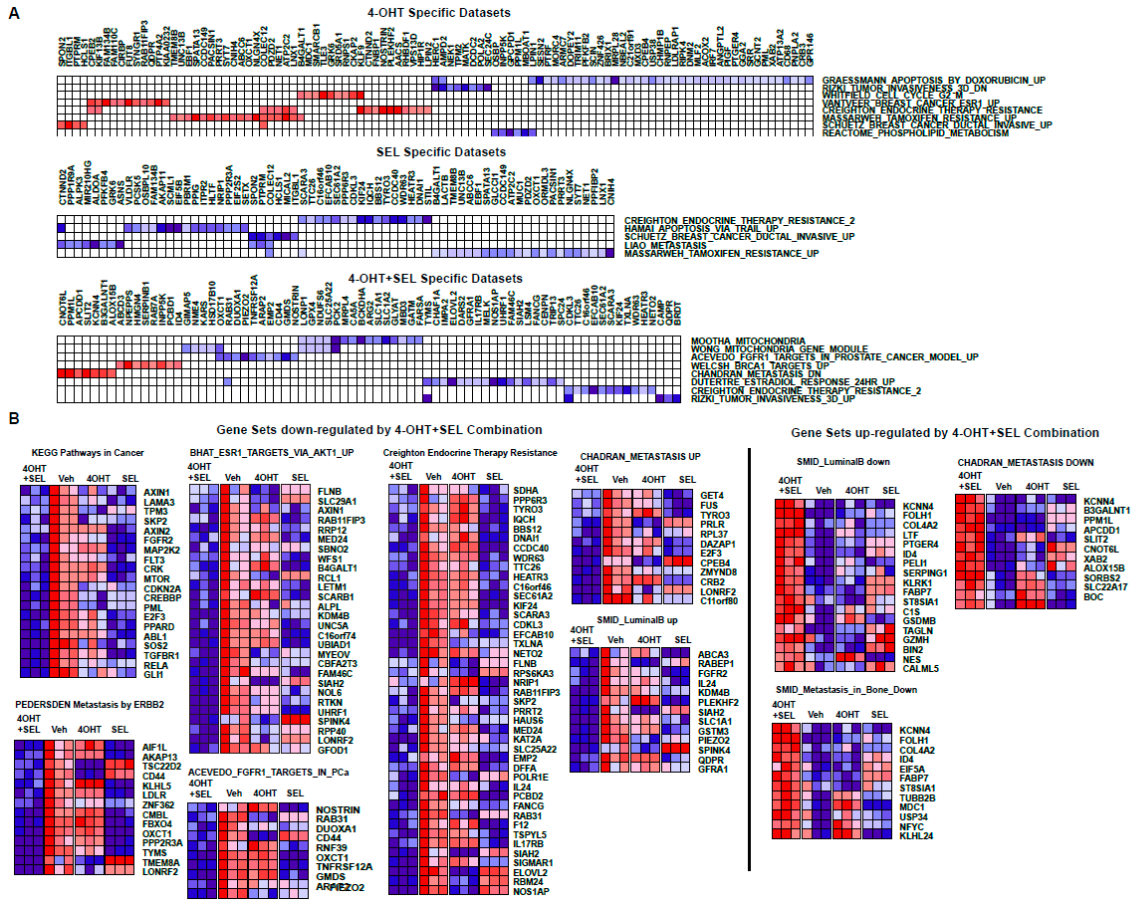

Figure S1. Analysis of gene functional groups associated with different ligand treatments. (**A**) Gene set enrichment analysis of genes that are regulated only in indicated ligand treatment group. (**B**) Gene sets that regulated greater than or equal to 4-OHT+SEL group compared to individual ligand treatments.

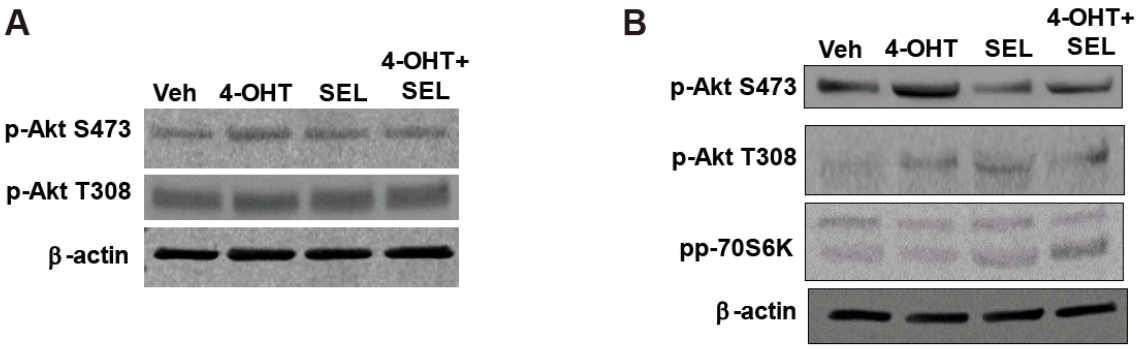

Figure S2. Regulation of signaling pathways by 4-OHT and SEL. (**A**) Western blot analysis of Akt phosphorylation in MCF-7 TAM^R cells. (**B**) Modulation of mTOR signaling by 4-OHT and SEL treatments in BT474 cells.

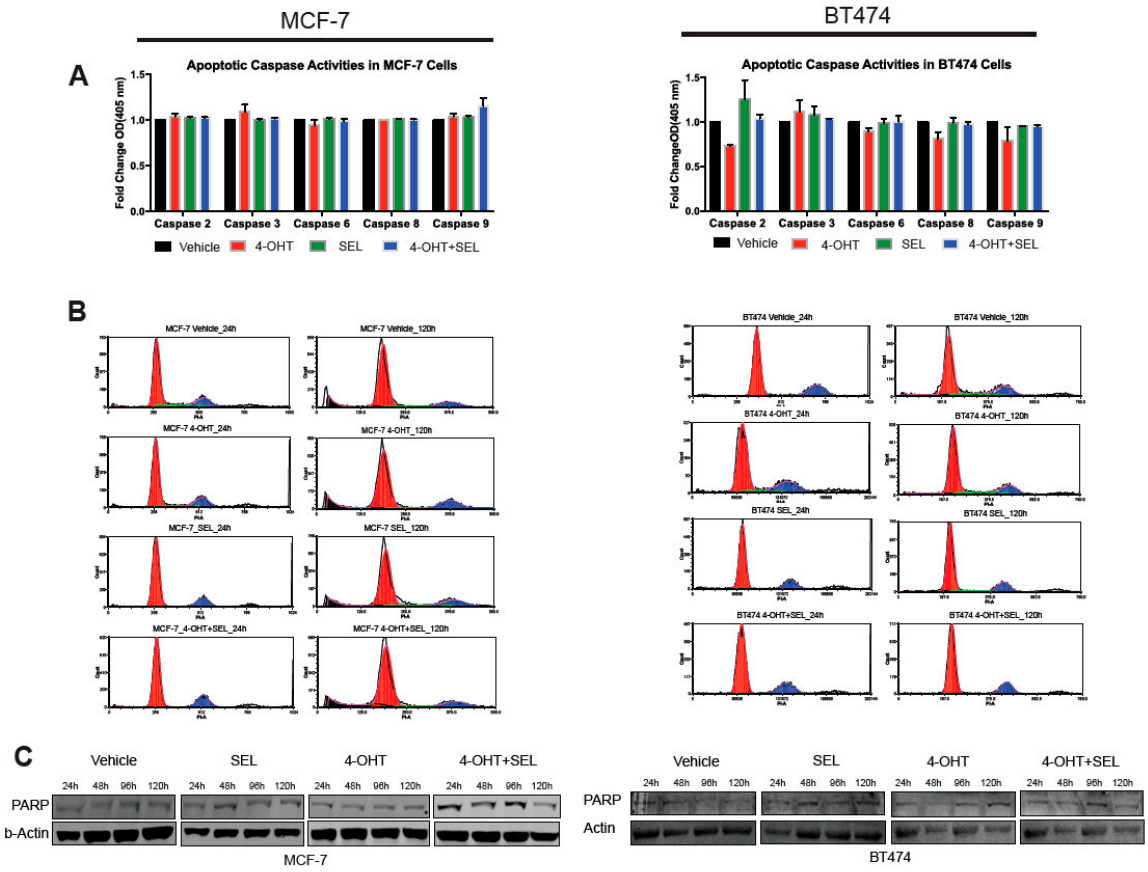

Figure S3. Combined ERα-XPO1 targeting cause apoptosis neither in MCF-7 nor in BT474 cells. (**A**) ERα-XPO1 targeting does not activate caspase protein activities. Apoptotic caspase activities of MCF-7 and BT474 cells were determined by a colorimetric method. Cells were seeded at a density of 2×10^3 cells/well and treated with 4-OHT (10^{-6} M) and SEL (10^{-7} M) alone and in combination for 24 h and different caspase activities were measured at 405 nm. Experiment was performed in triplicates and results were repeated two times. A two-way analysis of variance (ANOVA) model was used for statistical significance of ligand treatments. Values are presented as mean ± SEM from two independent experiments. (**B**) ERα-XPO1 targeting does not cause apoptotic body formation either in MCF-7 or BT474 cells. Detection of apoptosis by flow cytometer analysis. MCF-7 and BT474 cells were seeded at a density of 1×10^6 cells/well and treated with 4-OHT (10^{-6} M) and SEL (10^{-7} M) alone and in combination for 24 h and 120 h. Apoptotic body formation was indicated with cell cycle plots which were created by using FCS Express 6 (Black = Apoptotic bodies, Red = G1, Green = S, Blue = G2). (**C**)

Combined ERα-XPO1 targeting does not kill either MCF-7 or BT474 cells by apoptosis in a 96-h period. Western blot analysis of PARP protein levels in a 120-h time course. Both MCF-7 and BT474 cells were seeded at a density of 1×10^5 cells/well and treated with 4-OHT (10^{-6} M) and SEL (10^{-7} M) alone and in combination for 24, 48, 72, 96 and 120 h (Antibody dilution: 1:1000). Each treatment condition was repeated three times and the bands were quantified and analyzed by using one-way ANOVA method, but no significant different changes was seen between different treatments from 24 to 96 h.