Supplemental Information

Combination treatment with orlistat-containing nanoparticles and taxanes is synergistic and enhances microtubule stability in taxane-resistant prostate cancer cells

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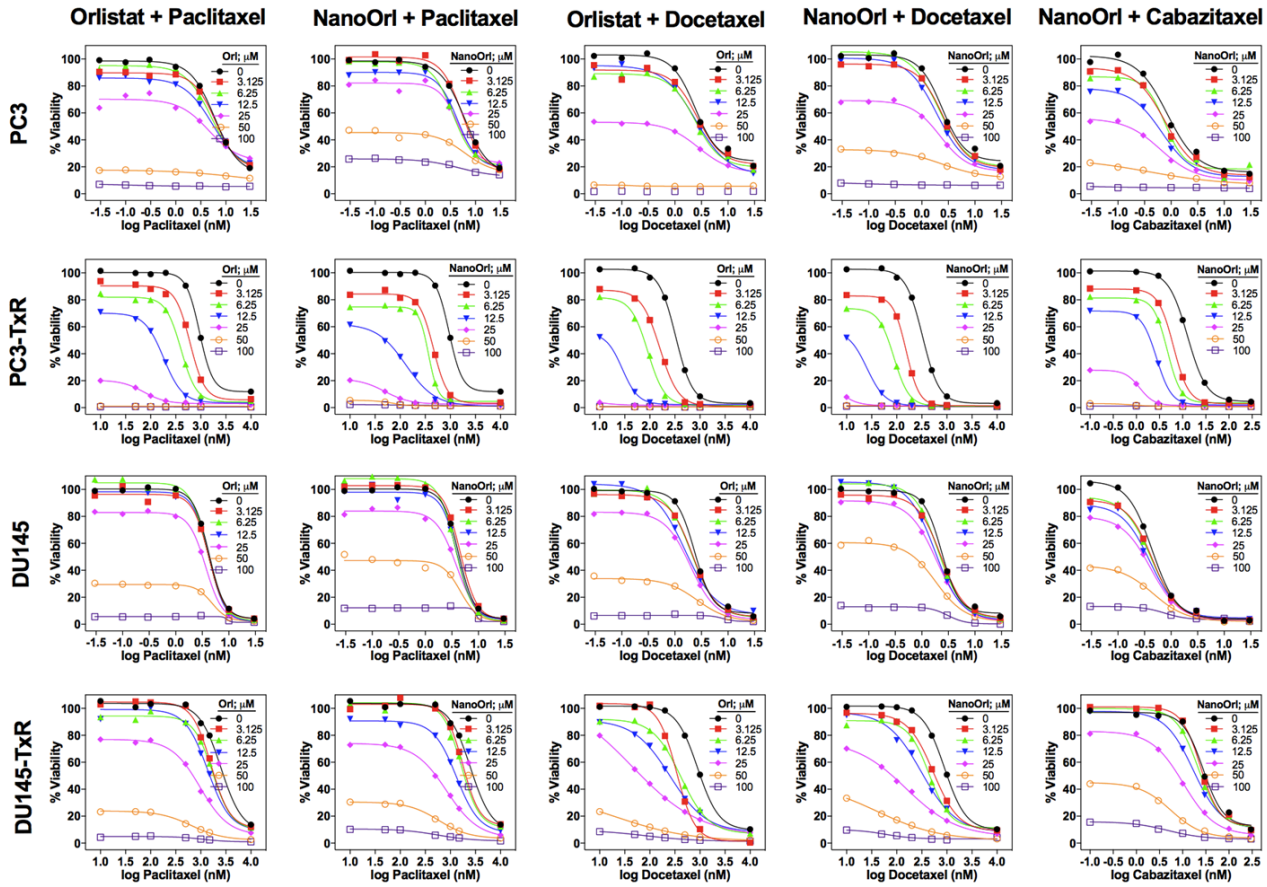
Running title: *NanoOrl synergizes with taxanes in resistant cancer*

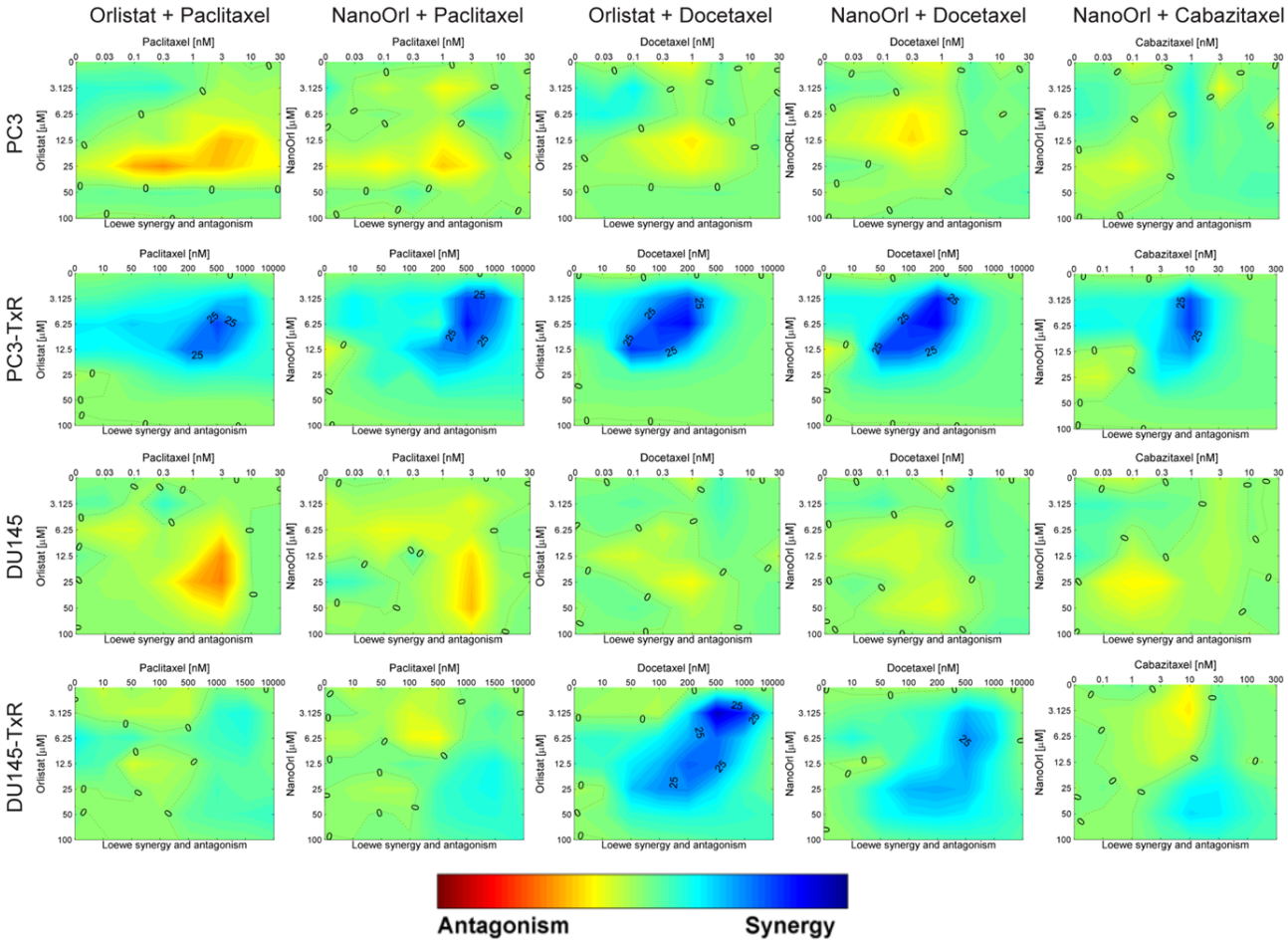
SUPPLEMENTARY FIGURES

Supplemental Figure S1.



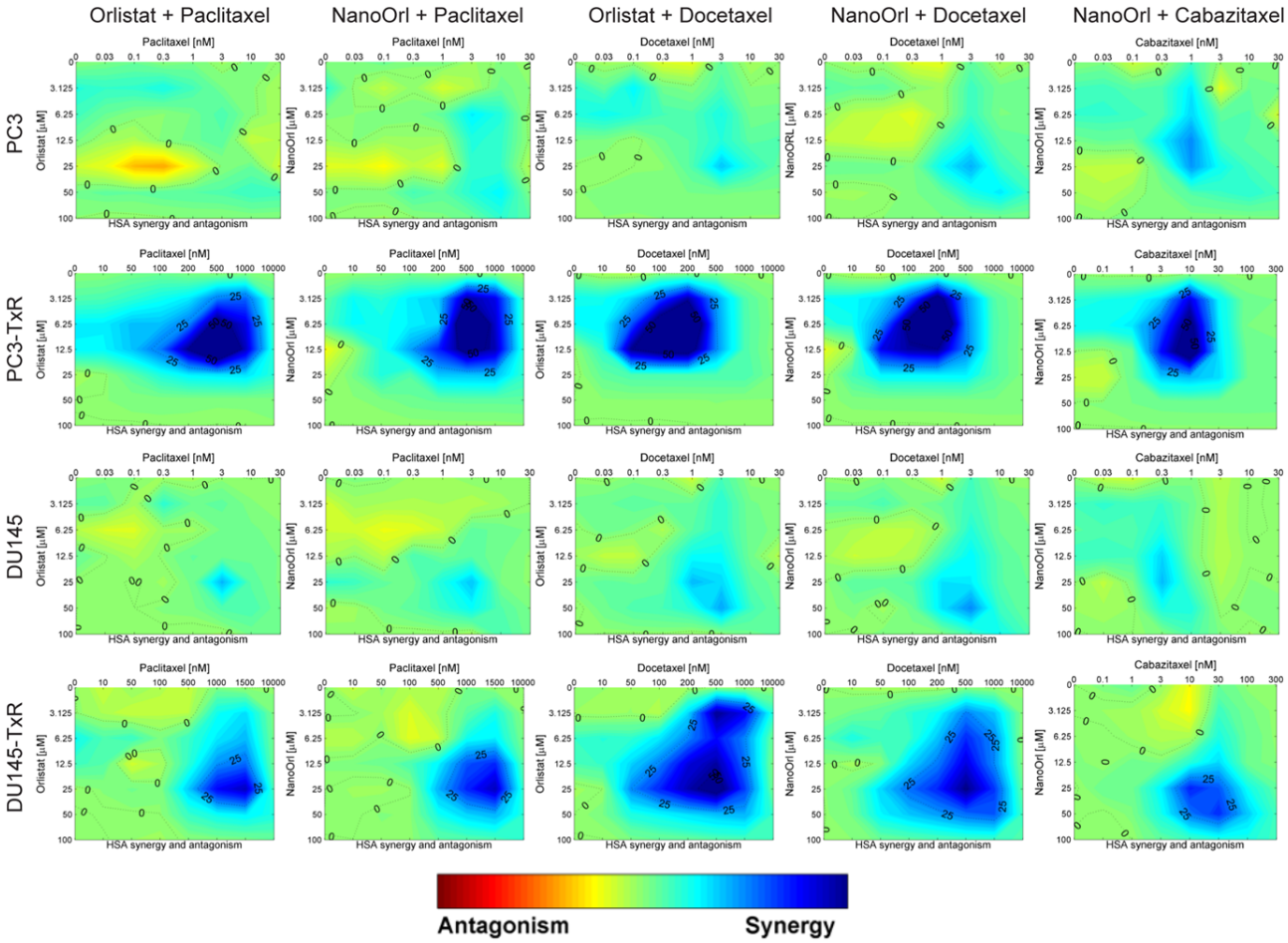
Supplementary Figure S1. Dose-response curves of combinations of orlistat or NanoOrl with taxanes. Parent PC3 and DU145 or taxane-resistant PC3-TxR and DU145-TxR cells were treated 8 concentrations of the indicated taxane in combination with 7 concentrations of Nano-Orlistat (NanoOrl) as indicated (n = 6 replicates per combination). After 72 hours, cell viability was assessed with the CCK-8 assay. Cell viability data was normalized to untreated control wells on each plate. Dose response curves were plotted as log (inhibitor) vs. response and fitted with a variable slope (four parameter) nonlinear regression using GraphPad Prism.

Supplemental Figure S2.



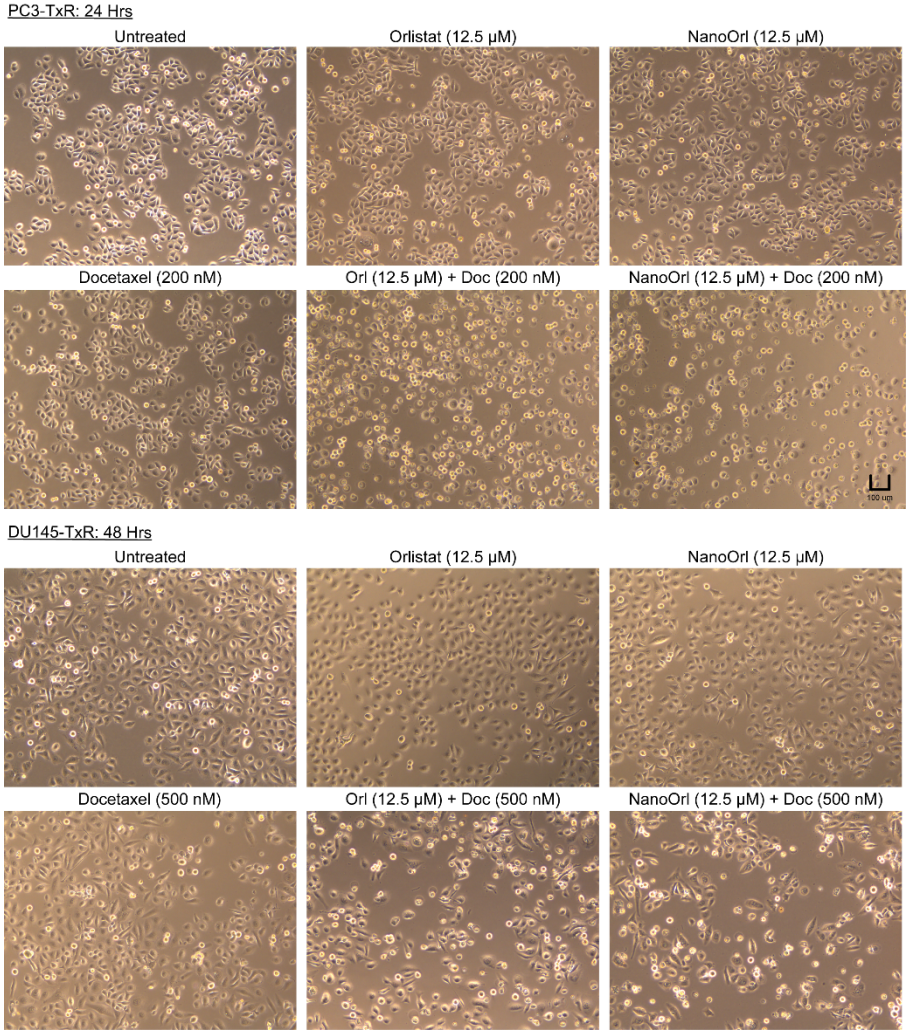
Supplementary Figure S2. Synergy analysis with Combenefit (Loewe model). Cells were treated as in Figure 1 with the indicated concentrations (taxane concentration above the x-axis, orlistat or NanoOrl concentration on the y-axis) and assessed by CCK-8 assay. Cell viability data was normalized to untreated control wells on each plate. Synergy was analyzed using Combenefit software. Results show the "Contour" view of synergy/antagonism calculations of synergy scores (the difference between the predicted additivity and the observed viability) for the Loewe model. Significant synergy is denoted by dark blue areas, and areas with synergy scores above 25 are marked.

Supplemental Figure S3.



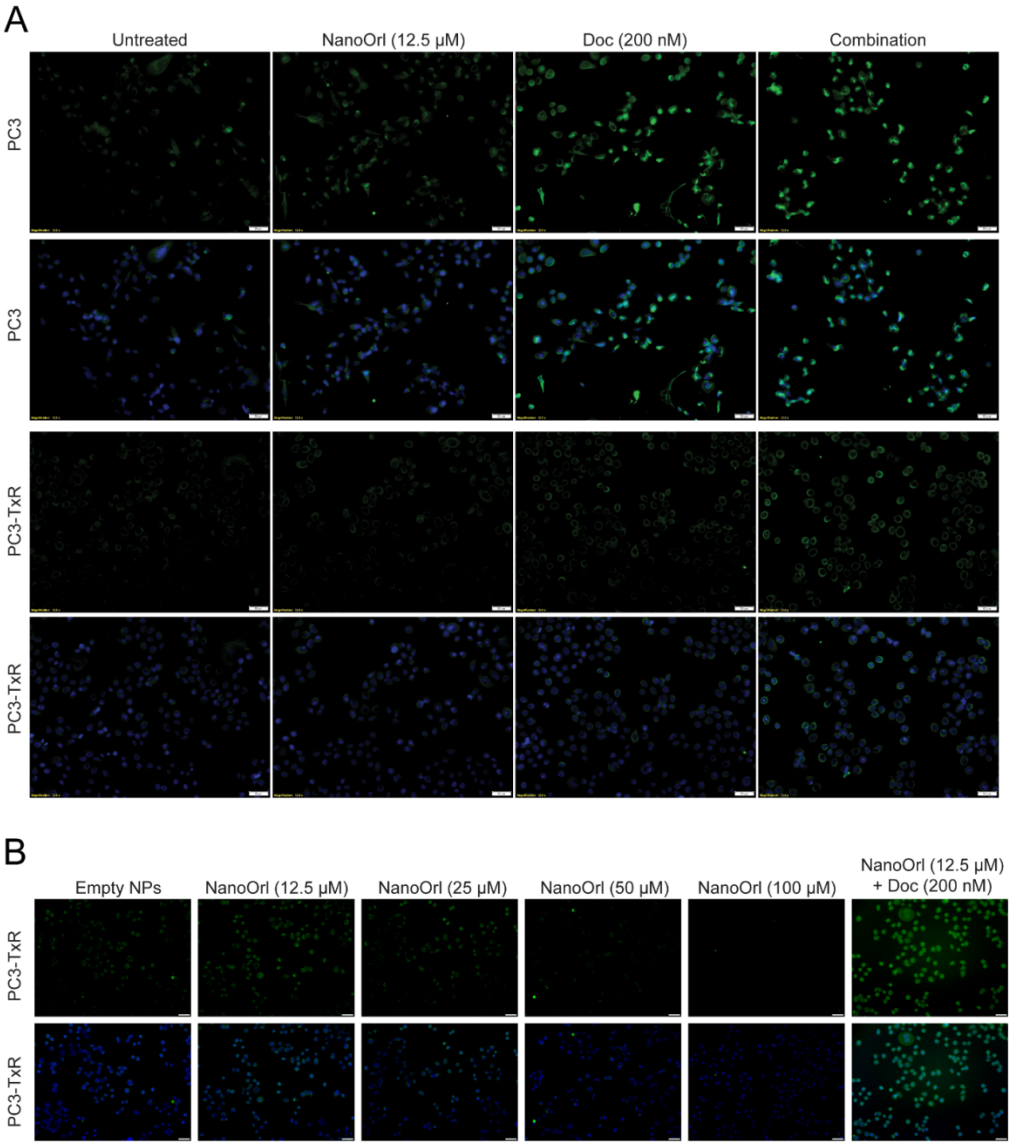
Supplementary Figure S3. Synergy analysis with Combenefit [Highest Single Agent (HSA) model]. Cells were treated as in Figure 1 with the indicated concentrations (taxane concentration above the x-axis, orlistat or NanoOrl concentration on the y-axis) and assessed by CCK-8 assay. Cell viability data was normalized to untreated control wells on each plate. Synergy was analyzed using Combenefit software. Results show the "Contour" view of synergy/antagonism calculations of synergy scores (the difference between the predicted additivity and the observed viability) for the Highest Single Agent (HAS) model. Significant synergy is denoted by dark blue areas, and areas with synergy scores above 25 are marked.

Supplementary Figure. S4.



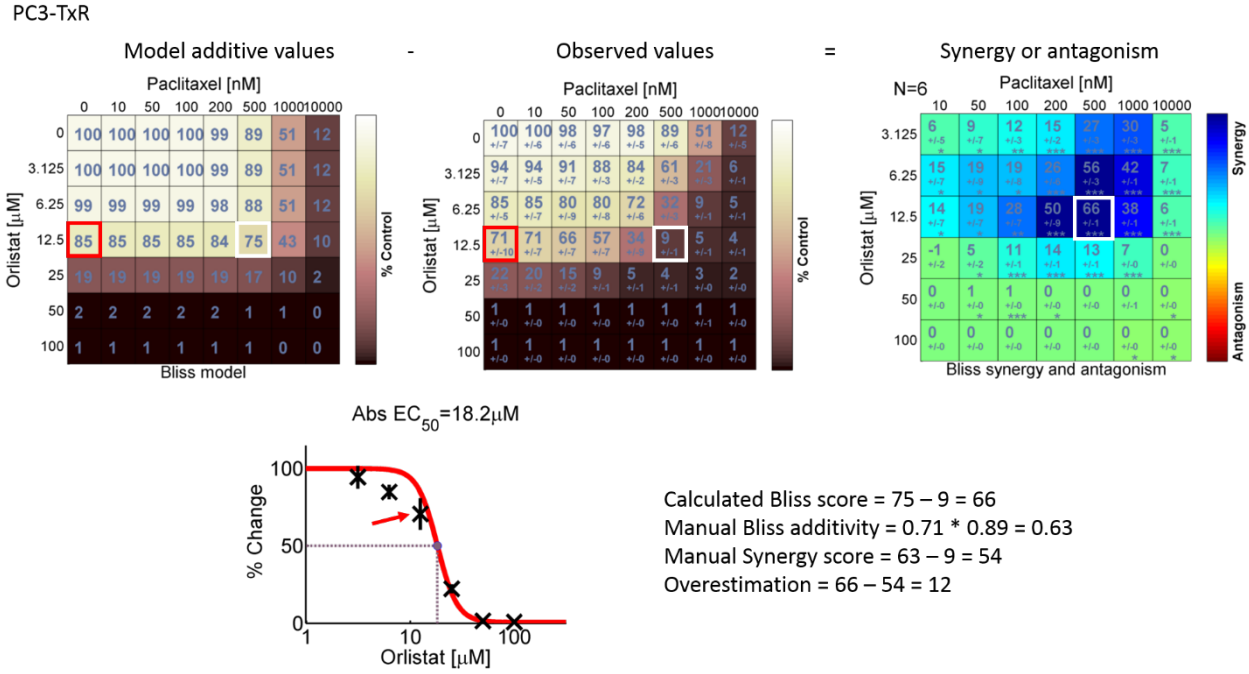
Supplementary Figure. S4. Morphological changes associated with combination treatment. PC3-TxR or DU145-TxR cells were treated with indicated concentrations of drugs for indicated time. Cell morphology was examined by light microscopy, and pictures were taken with an Infinity 1 camera (Lumenera Corporation) using Infinity Analyze software. Scale bar = 100 µm.

Supplemental Figure S5.



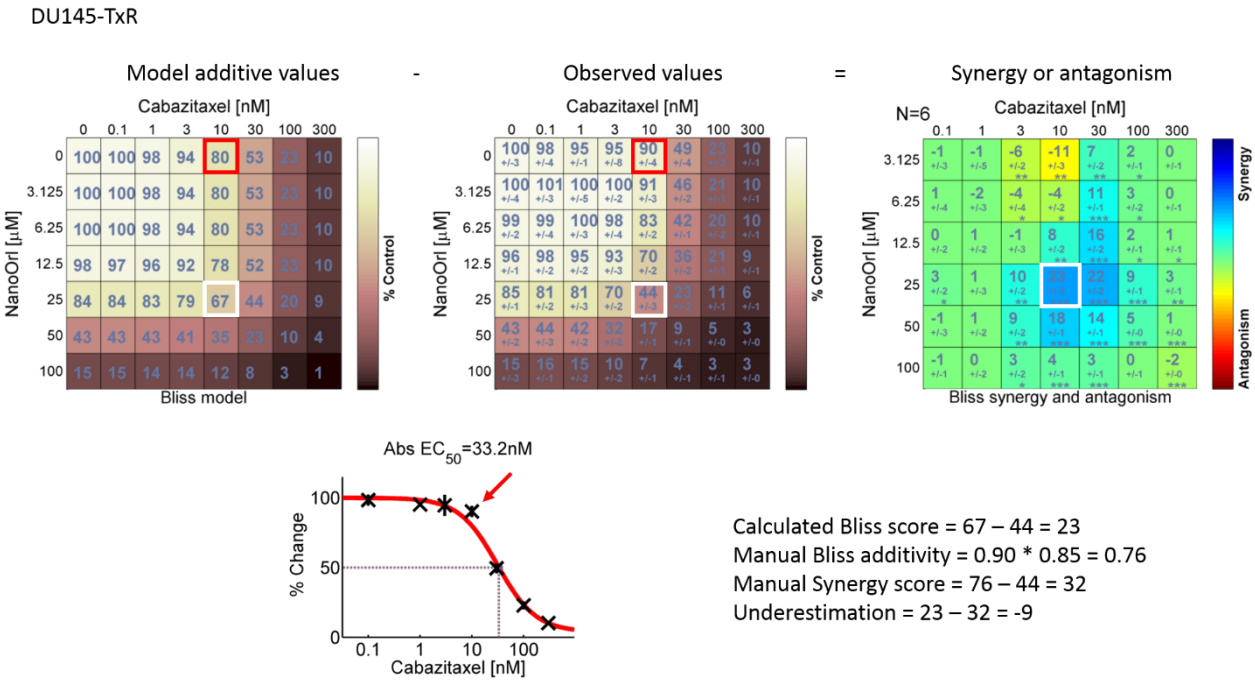
Supplementary Figure S5. Combination of NanoOrl plus docetaxel stabilizes microtubules in taxane-resistant cells. (A) PC3 or PC3-TxR cells were treated with 200 nM docetaxel (Doc), 12.5 uM NanoOrl, or the combination for 2.5 hours. Cells were co-stained with anti-detyrosinated tubulin antibody (green), a marker of stabilized microtubules, and DAPI (blue). Scale bar = 50 μm. **(B)** PC3-TxR cells were treated with NanoOrl as indicated for 2.5 hours and then stained as in **(A)**. Treatment was done on a separate date from **(A)**. Scale bar = 50 μm.

Supplemental Figure S6.



Supplementary Fig. S6. Example of overestimation of synergy.

Supplemental Figure S7.



Supplementary Fig. S7. Example of underestimation of synergy.