

Supplementary Materials: Preferential Response of Basal-Like Head and Neck Squamous Cell Carcinoma Cell Lines to EGFR-Targeted Therapy Depending on *EREG*-Driven Oncogenic Addiction

Sylvie Job, Aurélien de Reyniès, Betty Heller, Amélie Weiss, Eric Guérin, Christine Macabre, Sonia Ledrappier, Cyril Bour, Christine Wasyluk, Nelly Etienne-Selloum, Laurent Brino, Christian Gaidon, Bohdan Wasyluk and Alain C. Jung

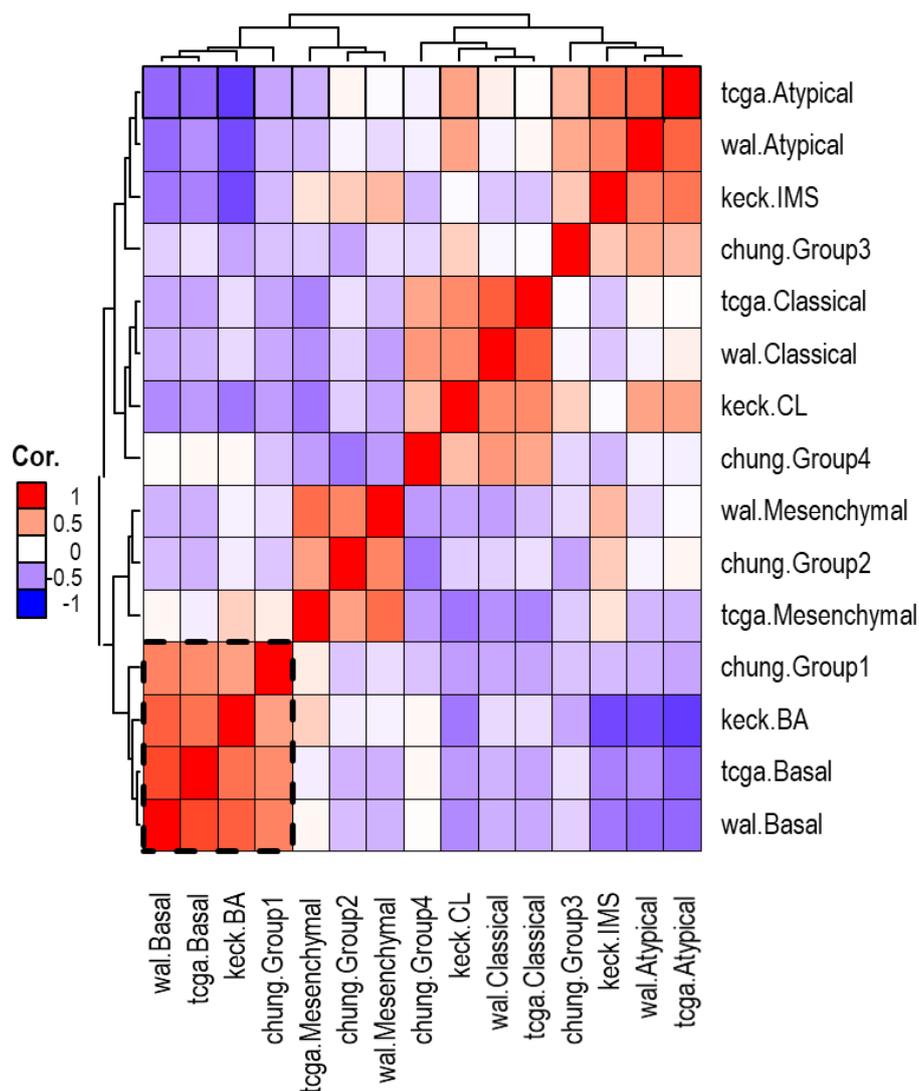


Figure S1. Heatmap of the correlations of the subtype centroids in four public omic datasets: Chung et al. [5], Walter et al. [6], TCGA [7] and Keck et al. [8].

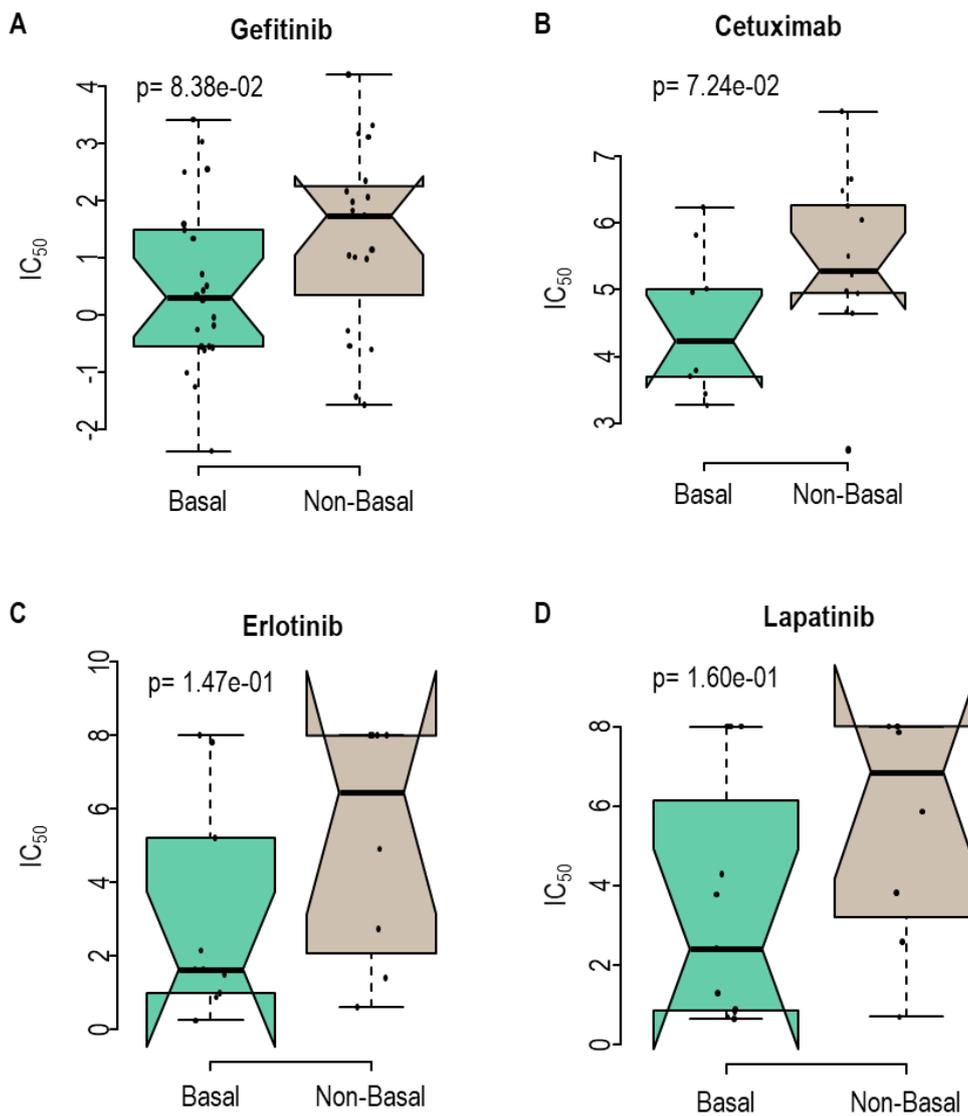


Figure S2. Boxplots of the IC_{50} of Gefitinib (A), Cetuximab (B), Erlotinib (C) and Lapatinib (D) on basal-like and non-basal-like HNSCC cell lines, determined from the Garnett [20] (A,B) and CCLE (C,D) [21] datasets.

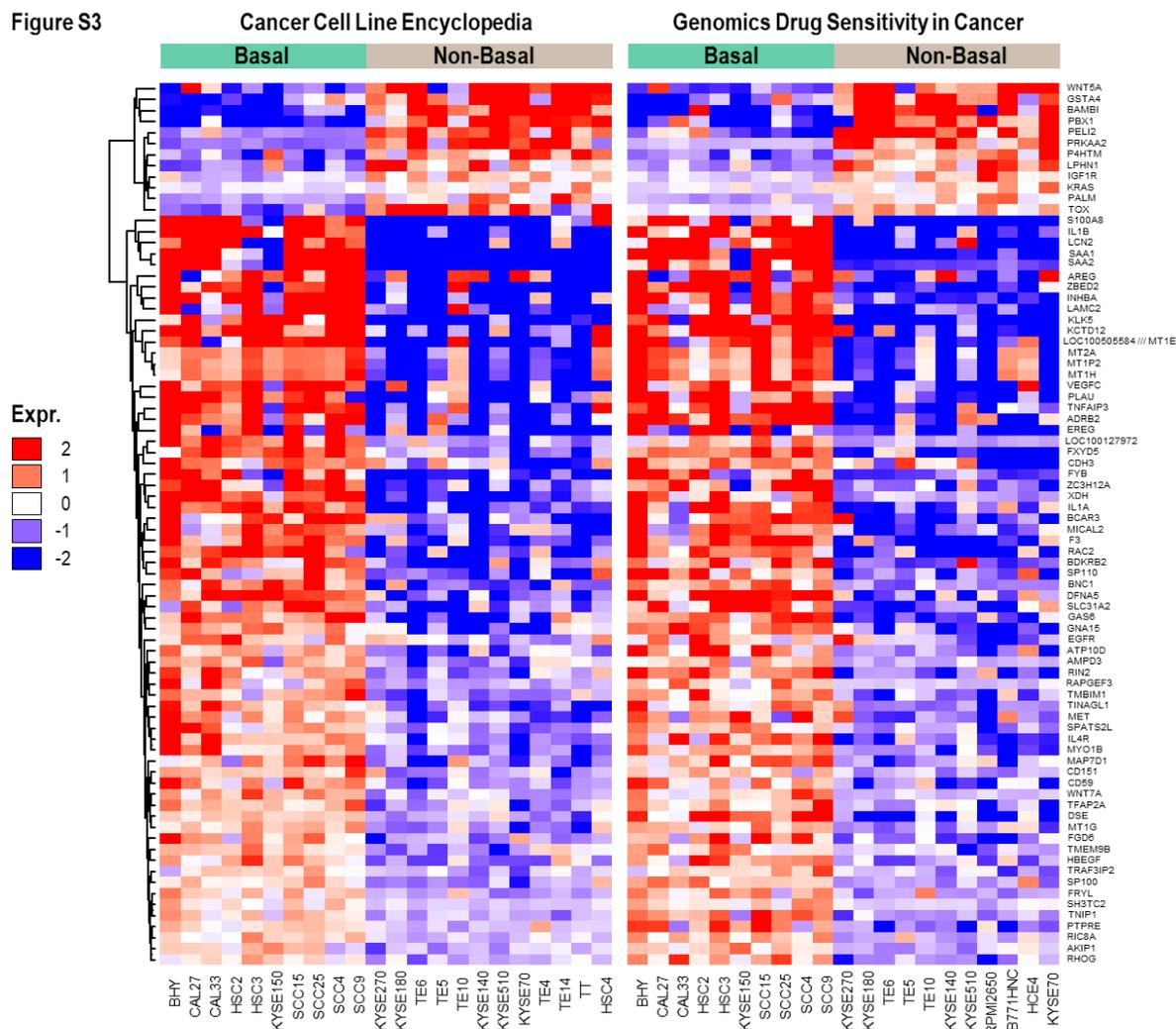


Figure S3. Heatmap of the differentially expressed genes between cell lines of the basal and non-basal subtypes in the CCLL [21] and GDSC [20] public datasets.

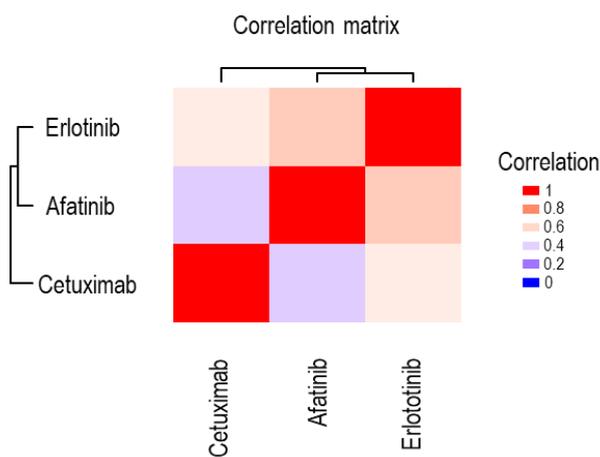


Figure S4. Correlation matrix of the AUC obtained upon treatment with Afatinib, Erlotinib and Cetuximab. A correlation of 1 is shown in red, and a correlation of 0 is shown in blue.

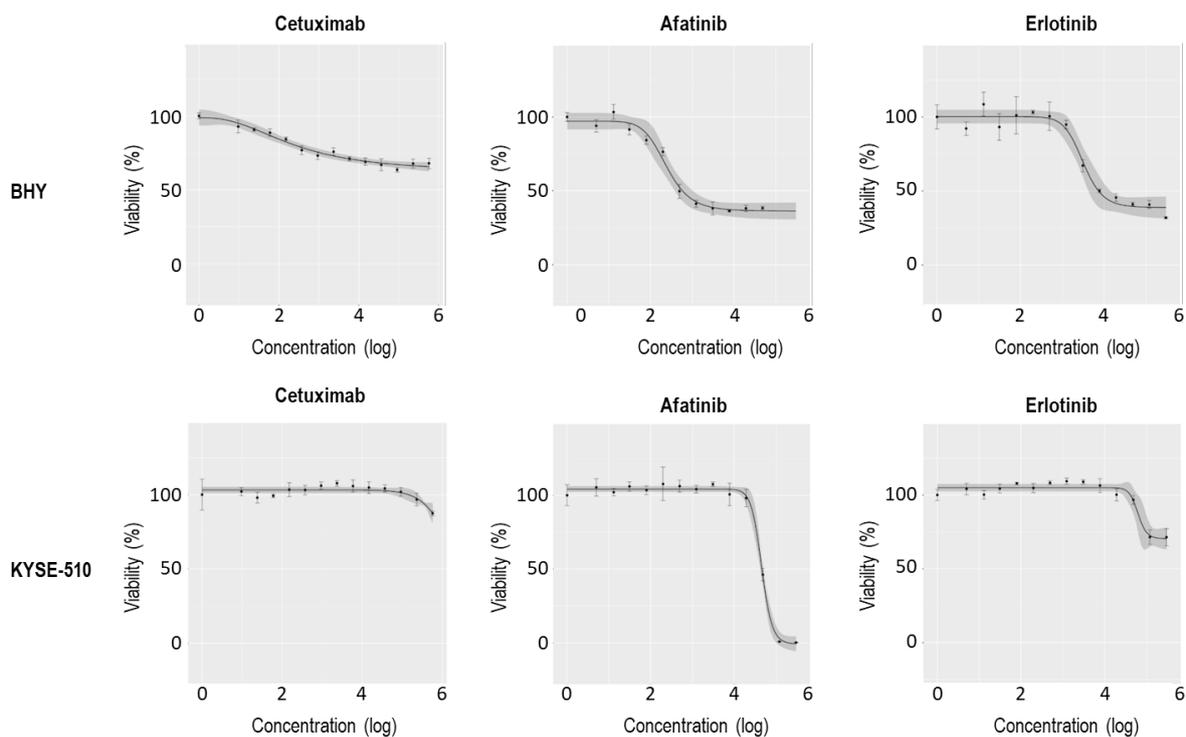


Figure S5. Cell viability dose-response curves obtained on BHY (upper panels) and KYSE-510 (lower panels) cells following 96 h of treatment with Cetuximab (left panels), Afatinib (middle panels) and Erlotinib (right panels).

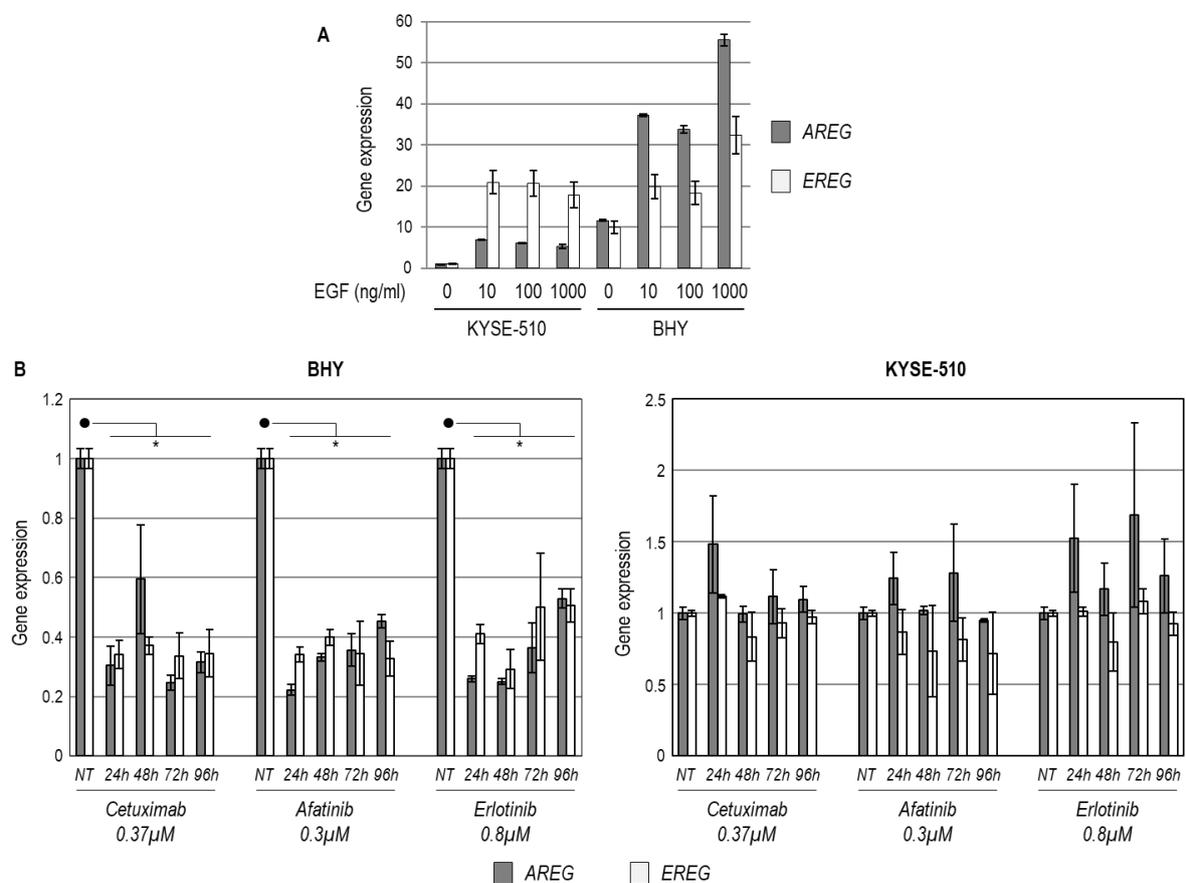


Figure S6. Analysis of *AREG* and *EREG* gene expression in BHY and KYSE-510 cell lines. (A) *AREG* and *EREG* gene expression analysis in BHY and KYSE-510 cells grown in the presence of 10 ng/mL,

100 ng/mL and 1000 ng/mL EGF for 24 h. Non-treated cells were used as a negative control and results were normalized to non-treated KYSE-510 cells. Mean expression levels and standard errors from two independent experiments are represented. **(B)** *AREG* and *EREG* gene expression analysis in BHY and KYSE-510 cells grown in the presence of 0.37 μ M Cetuximab, 0.3 μ M Afatinib and 0.8 μ M Erlotinib for 24 h, 48 h, 72 h and 96 h. Results were normalized to non-treated cells used as negative controls in both cell lines. EGFR-blockade significantly (ANOVA and Student-Newman-Keuls test for pairwise comparison) downregulated the expression of *AREG* and *EREG* in BHY cells.

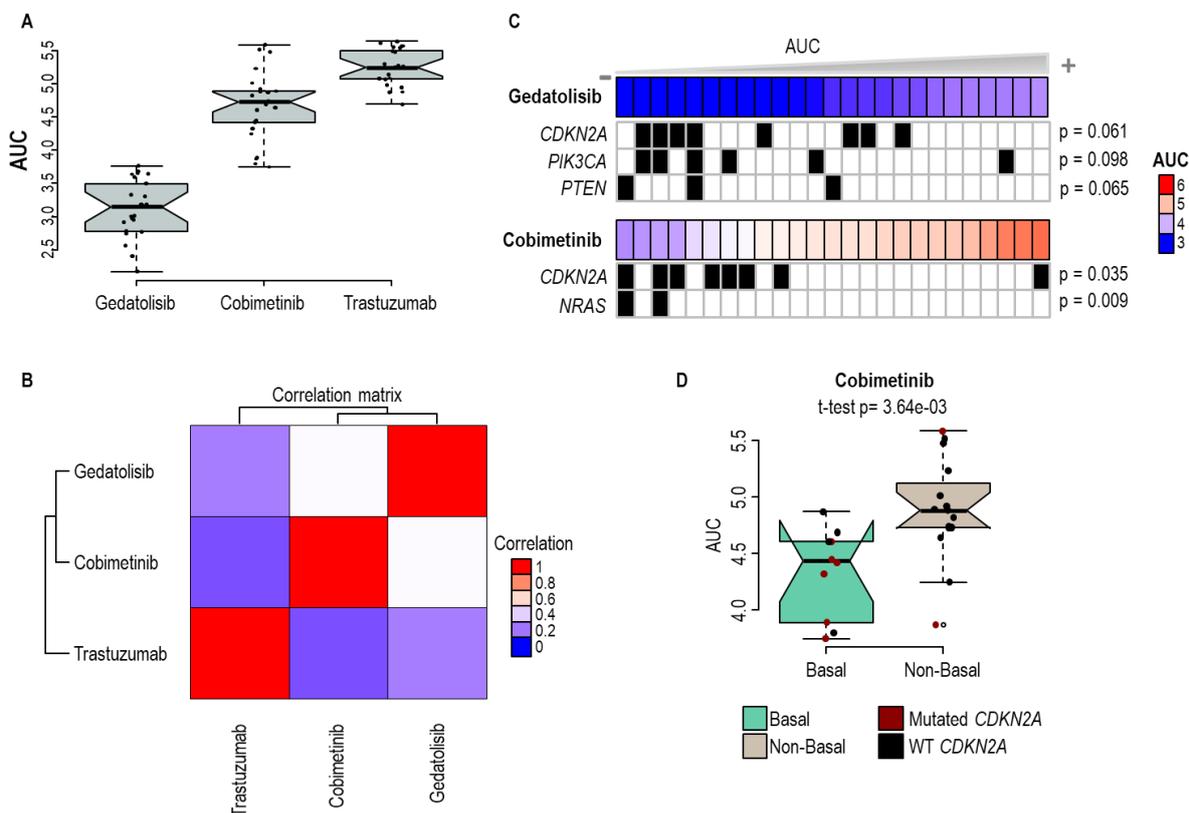


Figure S7. Analysis of the response of the 25 HNSCC cell lines to treatment with Gedatolisib, Trastuzumab and Cobimetinib. **(A)** Boxplot of the AUC for the 25 HNSCC cell lines treated with Gedatolisib, Trastuzumab and Cobimetinib. **(B)** Correlation matrix of the AUC obtained upon treatment with Gedatolisib, Trastuzumab and Cobimetinib. A correlation of 1 is shown in red, and a correlation of 0 is shown in blue. **(C)** Representation of clinically relevant mutations association with response to Gedatolisib and Cobimetinib. For each drug, cell lines are ordered by increasing AUC. *p*-values correspond to moderate *t*-test *p* values comparing AUC between mutated and WT cell lines. **(D)** Boxplot representation of the AUC obtained after Cobimetinib treatment of basal-like and non-basal HNSCC cell lines. The AUC were found to be significantly lower (*t*-test *p*-values are shown) in basal-like cells.

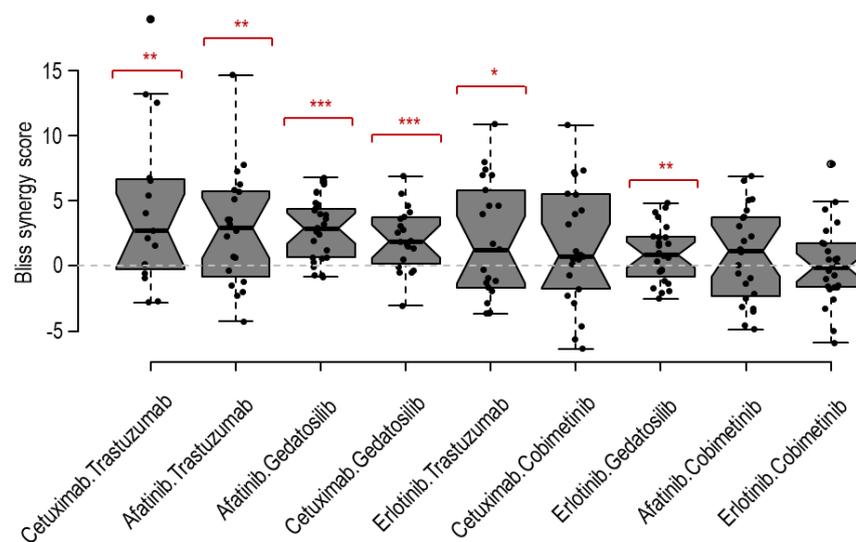


Figure S8. Boxplot of the Bliss synergy scores for the 25 cell lines treated with the nine drug combinations. p values correspond to moderate t -test p values comparing Bliss synergy score to 0: *: p value in $[0.05, 0.1]$, **: p value in $[0.001, 0.05]$ and ***: p value < 0.001 . Red brackets indicate therapeutic combinations that were found to be synergistic on all cell lines.



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