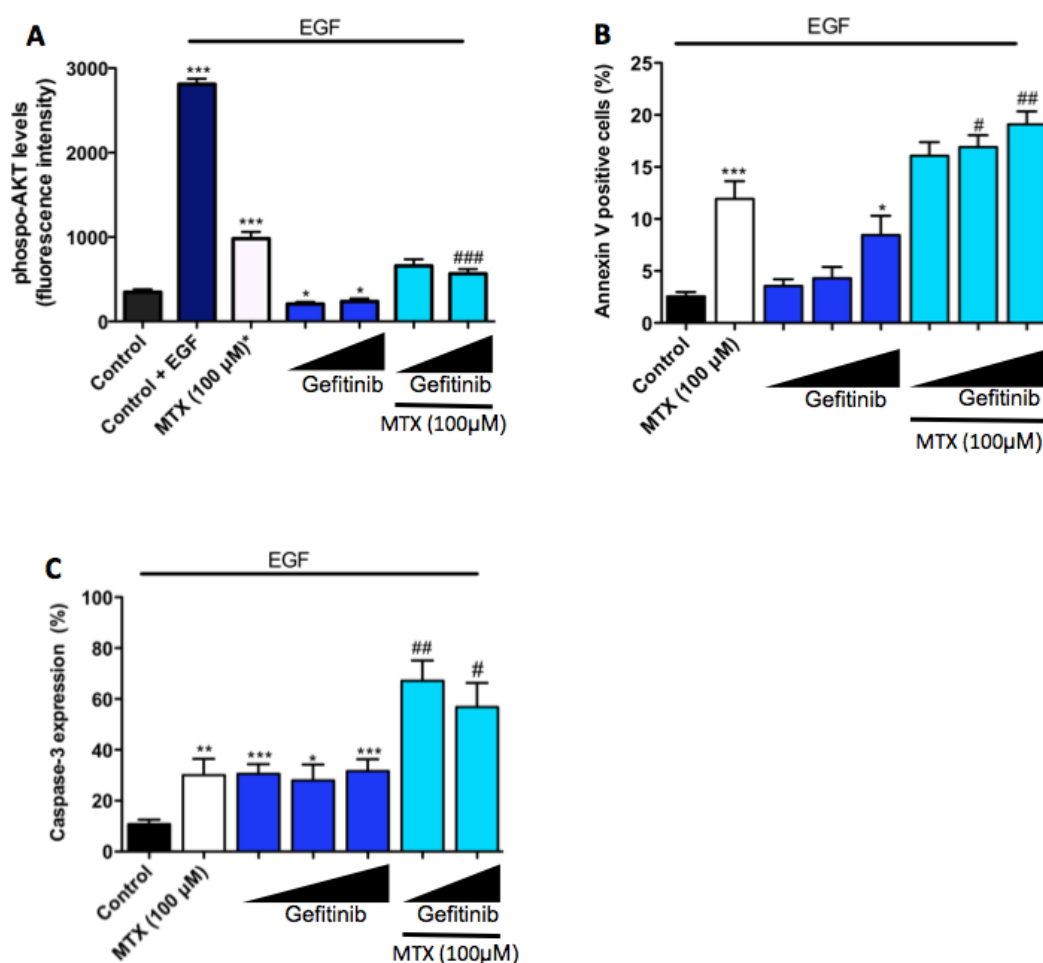


S2



Supplementary Figure 2: Combination treatment with methotrexate (MTX) and gefitinib is more potent in blocking EGFR and Akt phosphorylation, and enhancing apoptosis compared to either agent alone. **(A)** Phosphorylated Akt levels after treatment of JEG3 cells with MTX ± gefitinib (at 1 or 10 µM). EGF was added to all groups, except untreated. **(B)** Annexin V staining (Marker of apoptosis) by fluorescence-activated cell sorting. Graphed are results from four independent experiments. Gefitinib was added at 2,4 or 8 µM. **(C)** Caspase 3 (Marker of apoptosis) staining of JEG3 cells by fluorescence-activated cell sorting. Results for Caspase 3 are combined data from two independent experiments. Gefitinib was added at 2,4 or 8 µM. For all graphs Mean ± S.E.M. shown. Comparisons were done using one-way ANOVA with post-hoc T-Tests: * $P \leq 0.05$ vs. control, ** $P \leq 0.01$ vs. control, *** $P \leq 0.001$ vs. control, # $P \leq 0.05$ vs. MTX, ## $P \leq 0.01$ vs. MTX.