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 ≤ 0.05 vs. control, **P ≤ 0.01 vs. control, ***P ≤ 0.001 vs. control, #P ≤ 0.05 vs. MTX, ##P ≤ 0.01 vs. MTX.