Supplementary Figure 1. Effects of cetuximab in combination with the selective MEK1/2 inhibitor BAY 86-9766 in CRC cells growth in vitro. A-B The indicated CRC cell lines were treated every day with different concentrations of cetuximab (range, 0.01 to 20 μg/ml) and/or BAY-86-9766 (range, 0.01 to 5 μM) for 96 hours at a fixed drug ratio of 1:1 and cell proliferation was evaluated by MTT assay. Combination Index (CI) values were calculated according to the Chou and Talalay mathematical model for drug interactions using the CalcuSyn software, as described in Materials and Methods. For each cell line, it is shown in the left panel the percent of cell growth inhibition determined by treatment with cetuximab, with BAY 86-9766 or with their combination. For each cell line, in the right panel it is shown the combination index for the combination of cetuximab plus BAY 86 9766. Results represent the median of three separate experiments, each performed in quadruplicate.

Supplementary Figure 2. Effects of BAY 86-9766 on intracellular signaling pathways in HCT15, HCT116, GEO-CR and SW48-CR CRC cells. Analysis of intracellular signaling pathways by Western blotting in the indicated CRC cell lines treated with with BAY 86-9766 at the indicated doses for 24 hrs. Total cell protein extracts (25 μg) were subjected to immunoblotting with the indicated antibodies, as described in Materials and Methods. Anti-tubulin antibody was used for normalization of protein extract content.

Supplementary Figure 3. Effects of cetuximab in combination with the selective MEK1/2 inhibitor BAY 86-9766 on mice body weight. Nude Mice injected subcutaneously with HCT15, HCT116, GEO-CR and SW48-CR cancer cells and treated with cetuximab and or BAY 86-9766 were weighed twice weekly for the entire period of treatment.