Supplementary Materials: Dual Targeting of Y-Box Binding Protein-1 and Akt Inhibits Proliferation and Enhances the Chemosensitivity of Colorectal Cancer Cells

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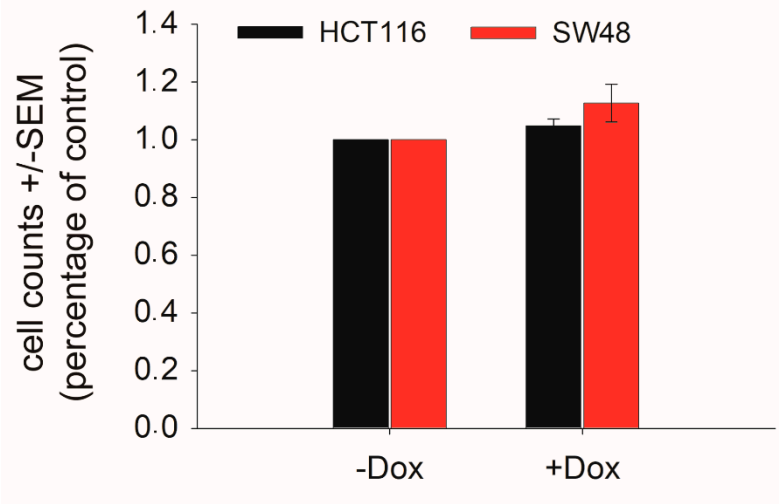

Figure S1. Doxycycline does not block proliferation of indicated CRC cell lines. KRAS(G13D)-mutated HCT116 cells and KRAS wild-type CaCo2 cells were seeded in 60 mm culture dishes and 24 h later were treated with doxycycline (2 μg/mL). Cells were trypsinized and counted using a hemocytometer 72 h after treatment. Date presents mean cell counts +/− SEM from 5 parallel experiments.

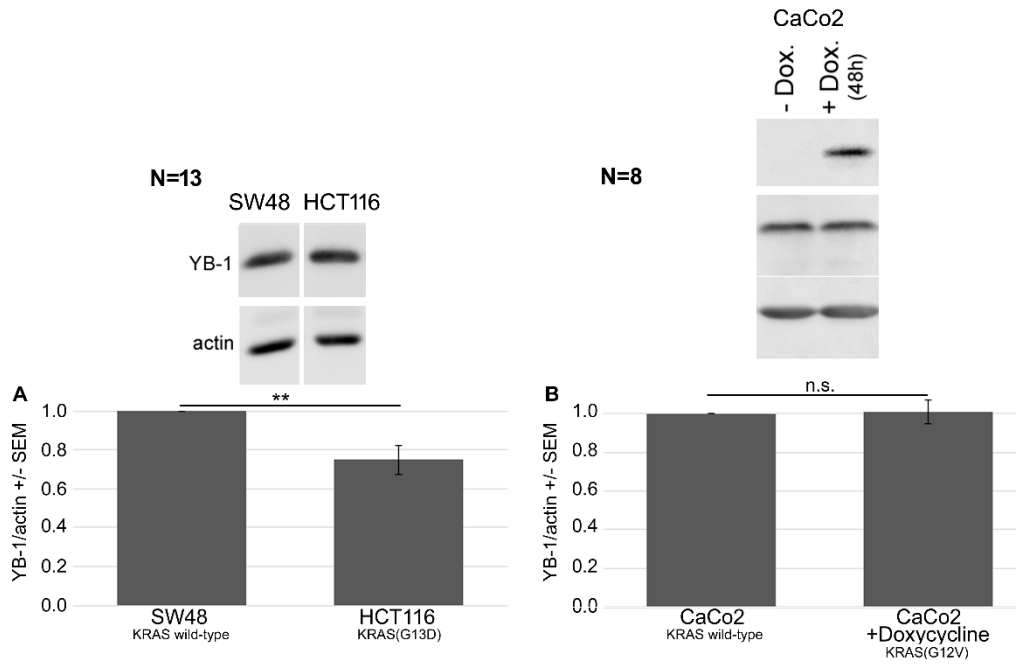

Figure S2. KRAS mutation does not stimulate YB-1 expression in CRC cells. Protein samples were isolated from untreated KRAS wild-type SW48, KRAS(G13D)-mutated HCT116 and KRAS wild-type CaCo2 cells as well as from CaCo2 cells 48 after treatment with doxycycline (2 μg/mL) and subjected

to Western blotting. Date presents ratio of YB-1 to actin normalized to 1 in KRAS wild-type SW48 cells (**A**) and CaCo2 cells (**B**). Asterisks indicate significant difference in expression of YB-1 between SW48 and HCT116 cells between the indicated groups (** $p \leq 0.01$). n.s.: not significant.

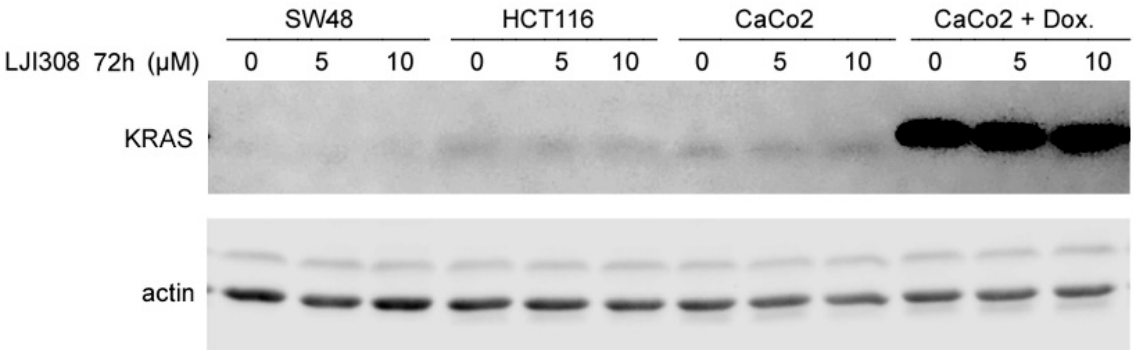

Figure S3. Level of endogenous KRAS in CRC cells. KRAS wild-type SW48 and CaCo2 cells as well as KRAS(G13D)-mutated HCT116 and doxycycline-treated CaCo2 cells were treated with an RSK inhibitor (5 μM and 10 μM) for 72 hours. KRAS was detected by Western blotting. Actin was detected as a loading control.

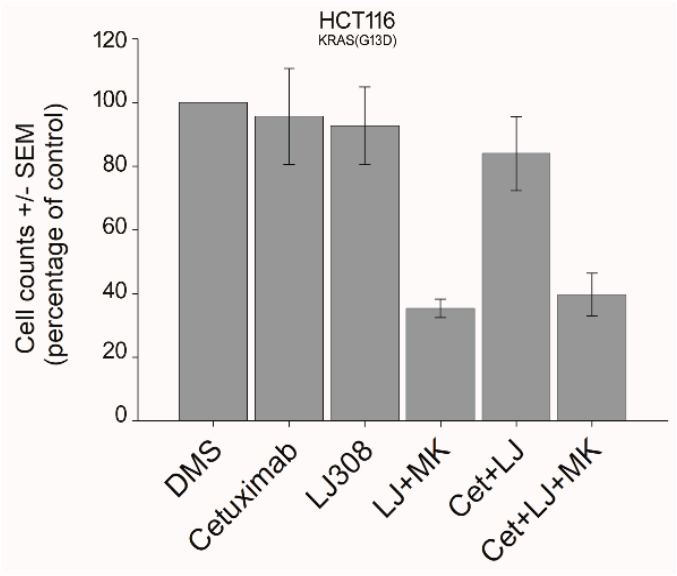

Figure S4. Combination of the RSK (LJ308, 20 μM) and Akt (MK2206, 5 μM) inhibitors with cetuximab (100 ng/mL) does not improve the effect of cetuximab as analyzed by the proliferation assay 72 h after treatment.