

Dual intracellular targeting by ruxolitinib and the Mcl-1 inhibitor S63845 in interleukin-6-dependent myeloma cells blocks *in vivo* tumor growth

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Supplementary figures

Figure S1

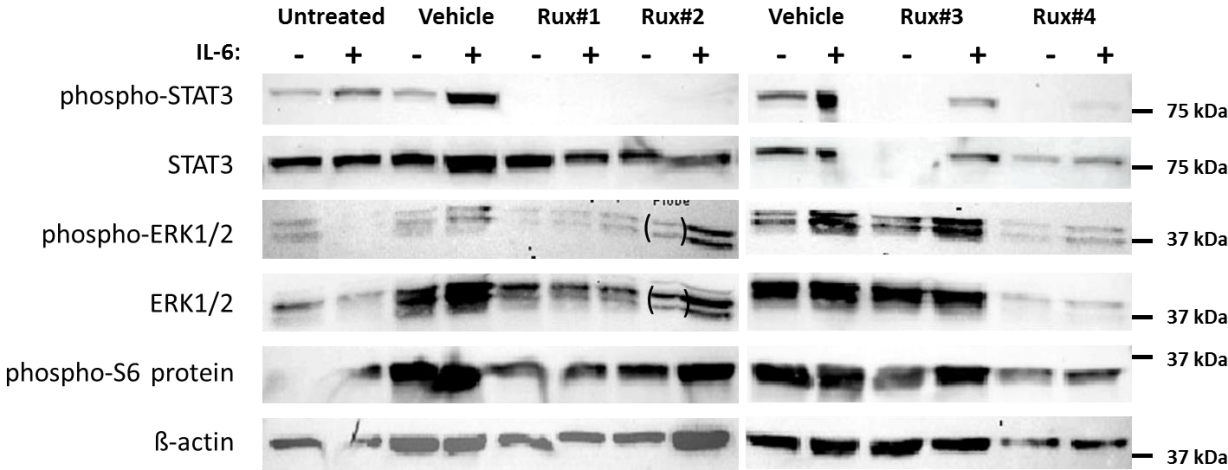


Figure S1. Ruxolitinib selectively blocks STAT3 activation in INA-6 plasmacytomas *in vivo*. Mice xenografted with INA-6 plasmacytomas received one oral dosing (60 mg/kg body weight) of ruxolitinib (Rux) (n=4). One control mouse was left untreated, one mouse was treated with vehicle only. After two hours and euthanasia, tumors were explanted and single cell suspensions in serumfree medium prepared. One part of the cells was stimulated with IL-6 (10 ng/ml, 10 min). Whole cell lysates were prepared and subjected to SDS-PAGE and Western blot analysis for phosphorylated and total STAT3, ERK1/2 and S6 ribosomal protein as described (shown are cropped blots)⁸. β-actin was used as loading control.

Figure S2

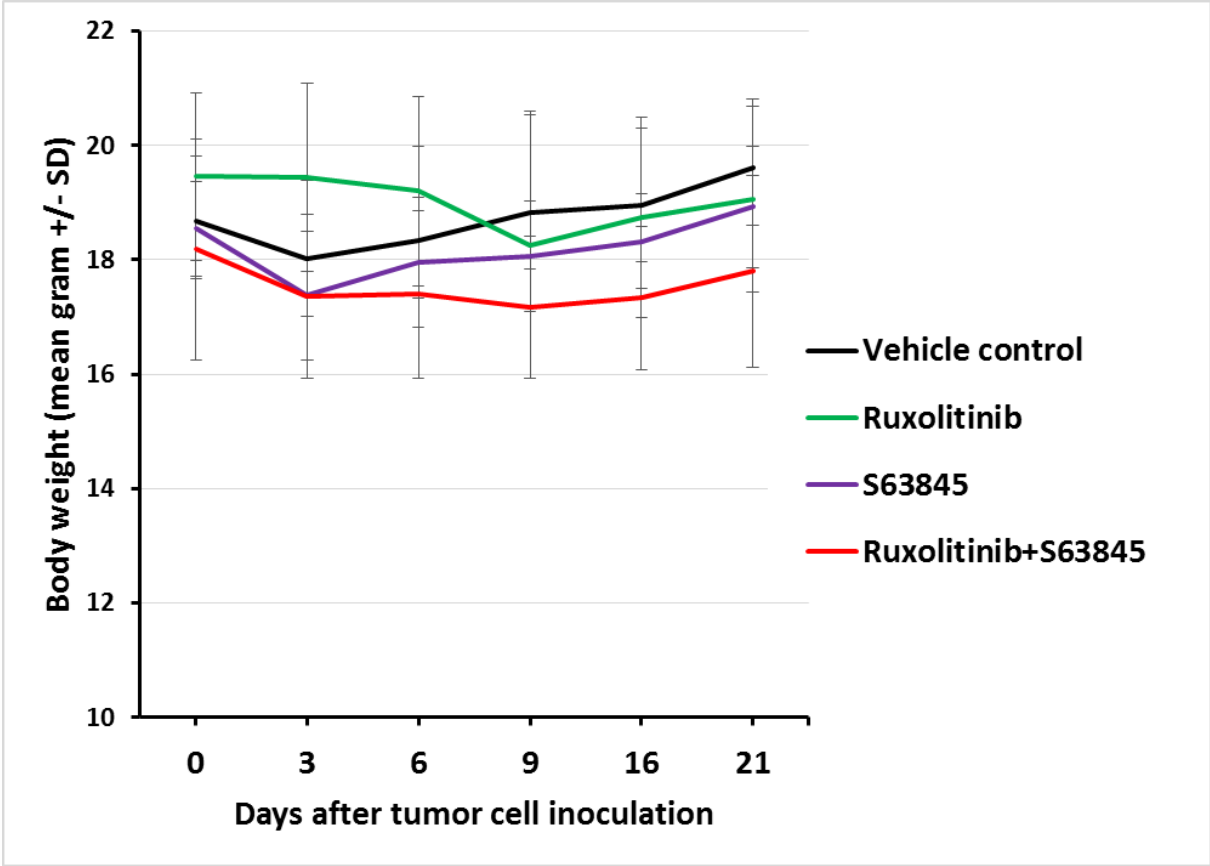


Figure S2. Body weights of mice by treatment groups during the first 20 days after tumor cell inoculation. Treatment started at day 1 and lasted for 10 consecutive days. Shown are the mean body weight values in gram. Error bars, standard deviation. Vehicle group: n=8, ruxolitinib group: n=7, S63845 group: n=8, combination group: n=6.

Figure S3

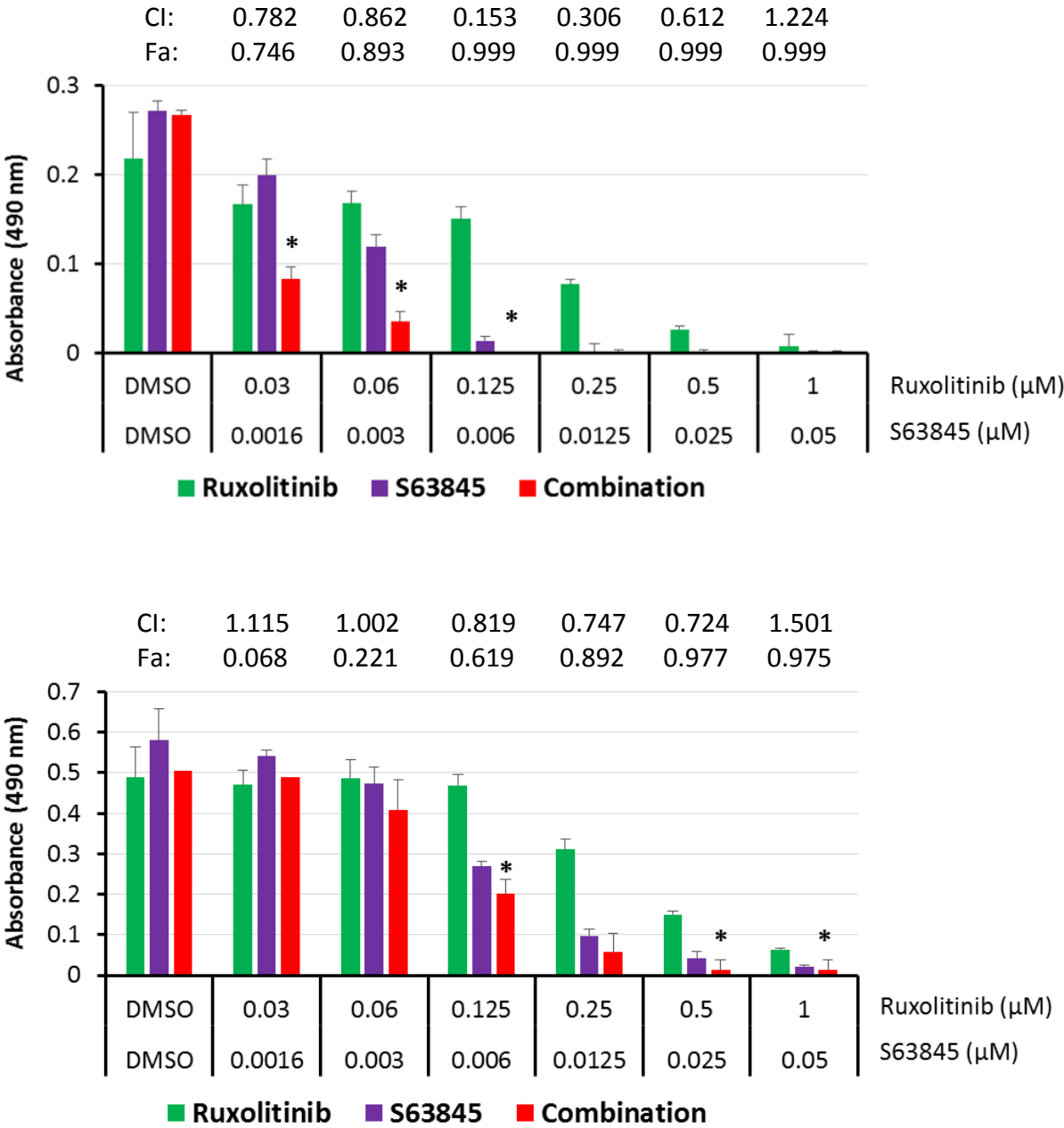


Fig. S3. Drug sensitivity of INA-6 tumors from mice treated with ruxolitinib or S63845. Upper panel: cells from a tumor grown in a mouse after treatment with ruxolitinib (tumor explanted at day 56 after cell inoculation), lower panel: cells from a tumor grown in a mouse treated with S63845 (tumor explanted at day 84 after cell inoculation). Cell growth was measured by an MTS-based colorimetric assay. Drugs were added at the indicated concentrations. Shown are the mean values of quadruplicate cultures \pm standard deviation (error bars). *) indicates a significant difference of the combination to either single agent (p-value < 0.05 by unpaired

two-tailed t-test). The drug combination indices (CI) for experimental values were calculated with the method of Chou and Talalay (Pharmacol Rev 2006; 58: 621-681) proposing a constant ratio drug combination (CalcuSyn v2.0 software, Biosoft, UK). $CI < 1$, synergistic; $CI = 1$, additive; $CI > 1$, antagonistic. Fa, affected fraction.