Data SI. Supplementary materials and methods

Cell viability. RD or RH30 cells were seeded in 96-well plates at a density of $8x10^3$ cells per well. Following overnight adherence, the cells were treated with a dose dependent treatment of doxorubicin, vincristine, dactinomycin or paclitaxel in the presence or absence of AMD3100 for 24 h. The viability of tumor cells was determined via colorimetric MTT assay measuring the reduction of tetrazolium salts to formazan derivatives by functional mitochondria. Lysis buffer (DMSO, SDS, acid) was added to solubilize the formazan crystals (1). The absorbance of the cell lysates was measured at 560 nm. IC50 values were determined using GraphPad Prism v.8.0 (GraphPad Software, Inc.).

Clonogenic assay. Cells were plated in 6-well plates at $7.5x10^2$ cells per well in 2 ml DMEM supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine and 50 U/ml penicillin and 50 μg/ml streptomycin. Following attachment, the cells were treated with the CXCR4 antagonist AMD3100 in a humidified atmosphere at 37˚C with 5% CO_2. After 72 h, the cells were washed twice with PBS, and fresh media was added. The colonies were cultivated for 7-10 days, fixed in 80% methanol, stained with 0.2% crystal violet and colonies (≥ 50 cells) were counted.

Reference

1. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.

Figure S1. The cytotoxic effects of chemotherapy treatment. Dose-response curves of RMS cell lines RH30 and RD after 24 h treatment with (A) doxorubicin, (B) vincristine, (C) dactinomycin (n=3) or (D) paclitaxel (n=5) compared with the untreated control (100%).

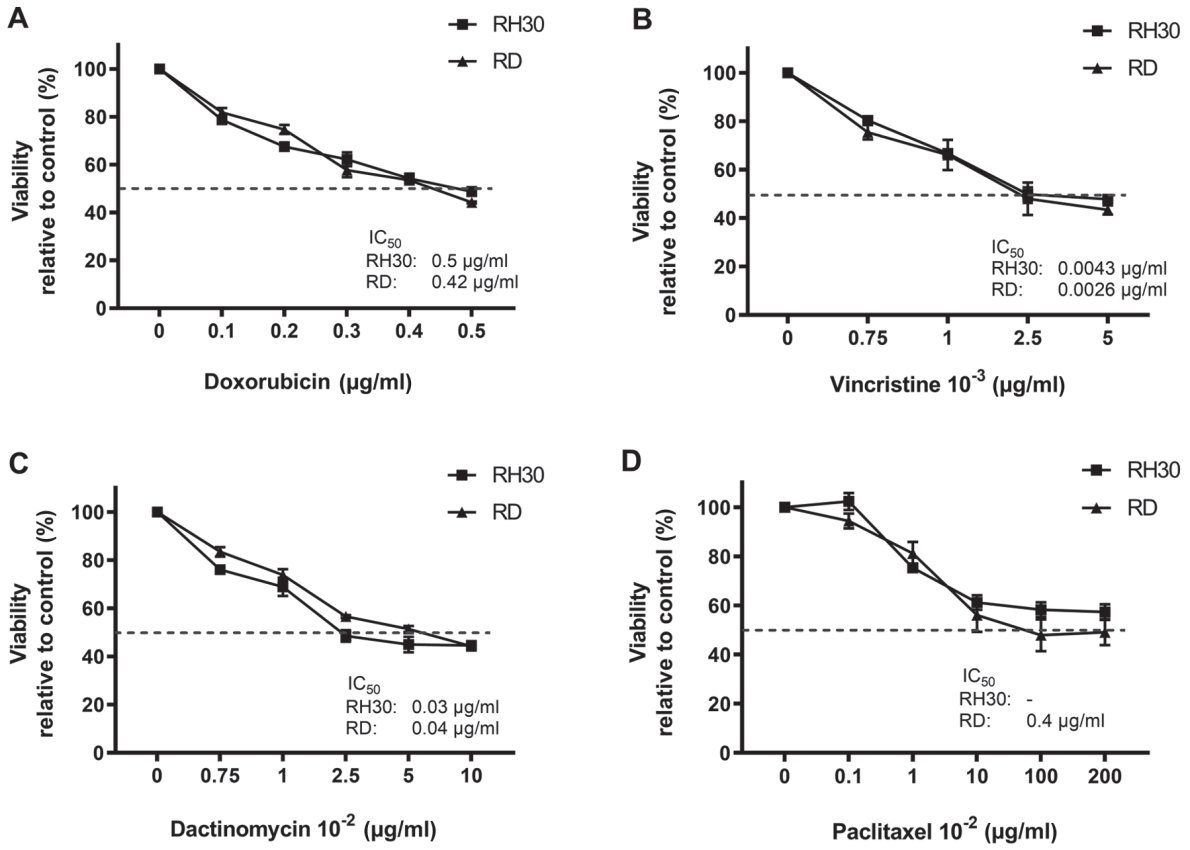

Figure S2. Effects of the CXCR4 antagonist AMD3100 on cytotoxic drug sensitivity of RH30 and RD rhabdomyosarcoma cells. (A-F) Arithmetic means ± SEM (n=3) of the relative numbers of viable RH30 (left) and RD (right) cells following a 24 hours incubation in the absence and presence of AMD3100 (10 μM) with and without (A and B) doxorubicin, (C and D) vincristine and (E and F) dactinomycin. Data were analyzed by one-way ANOVA with the Bonferroni post hoc test. ***P<0.001, ****P<0.0001 vs. control

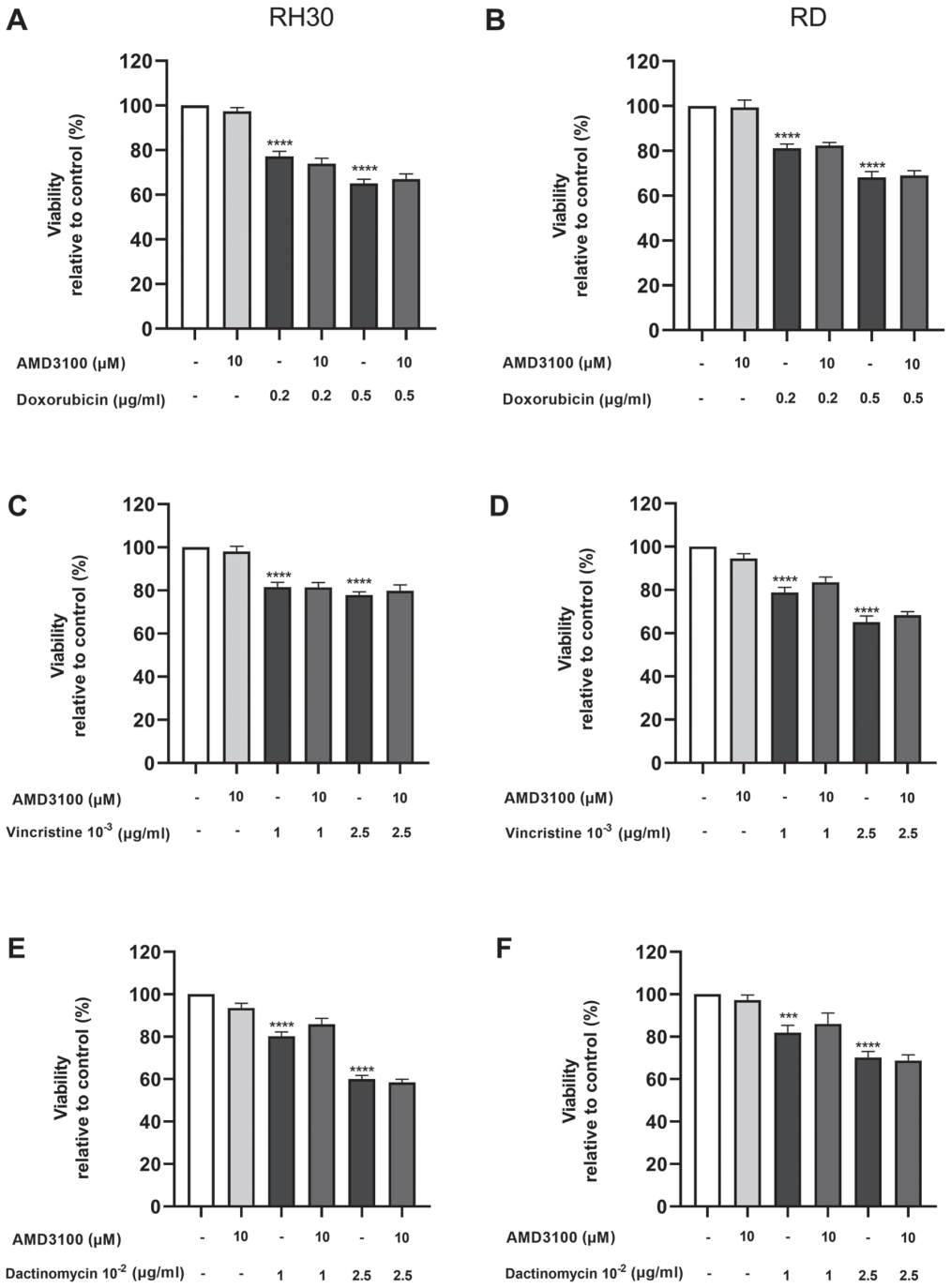

Figure S3. Effects of the CXCR4 antagonist AMD3100 on cell proliferation in a clonogenic assay. (A and B) Arithmetic means ± SEM (n=4) of the number of clones in (A) RH30 and (B) RD cells following a 72-h incubation in the presence of 5, 10 or 15 μM AMD3100 (gray bars) relative to the clones in the absence of AMD3100 (white bars). Data were analyzed by one-way ANOVA with the Dunnett's post hoc test. *P<0.05, **P<0.01 vs. control.