cancers

MDPI

Supplementary Materials

Chemotherapeutic Agents Sensitize Resistant Cancer Cells to the DR5-Specific Variant DR5-B more Efficiently than to TRAIL by Modulating the Surface Expression of Death and Decoy Receptors

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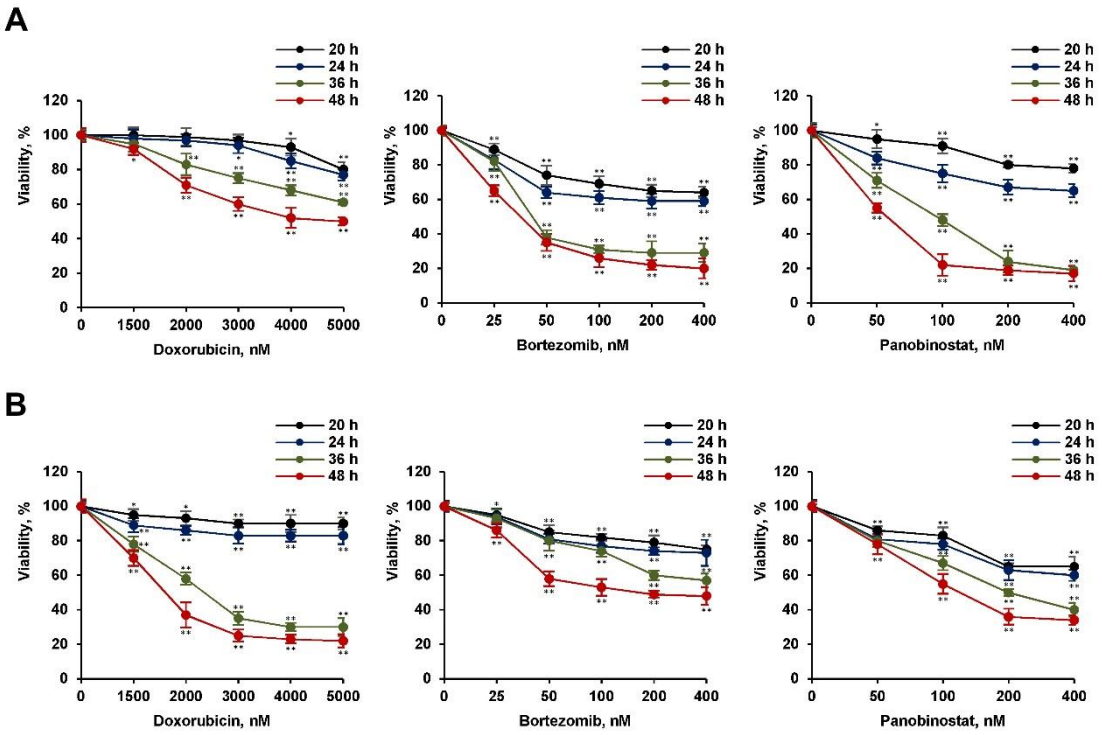

Figure S1. Dose and time-dependent inhibition of cell viability by chemotherapeutic drugs. HT-29 (**A**) and A549 (**B**) cells were incubated with indicated concentrations of doxorubicin (Dox), bortezomib (Btz) and panobinostat (Pst) for 20, 24, 36 and 48 h and Cell viability was determined by WST-1 colorimetric assay. Mean ± Standard Deviation ($n = 4$). The asterisks indicated significance (* $p < 0.05$) and (** $p < 0.001$) relative to control cells.

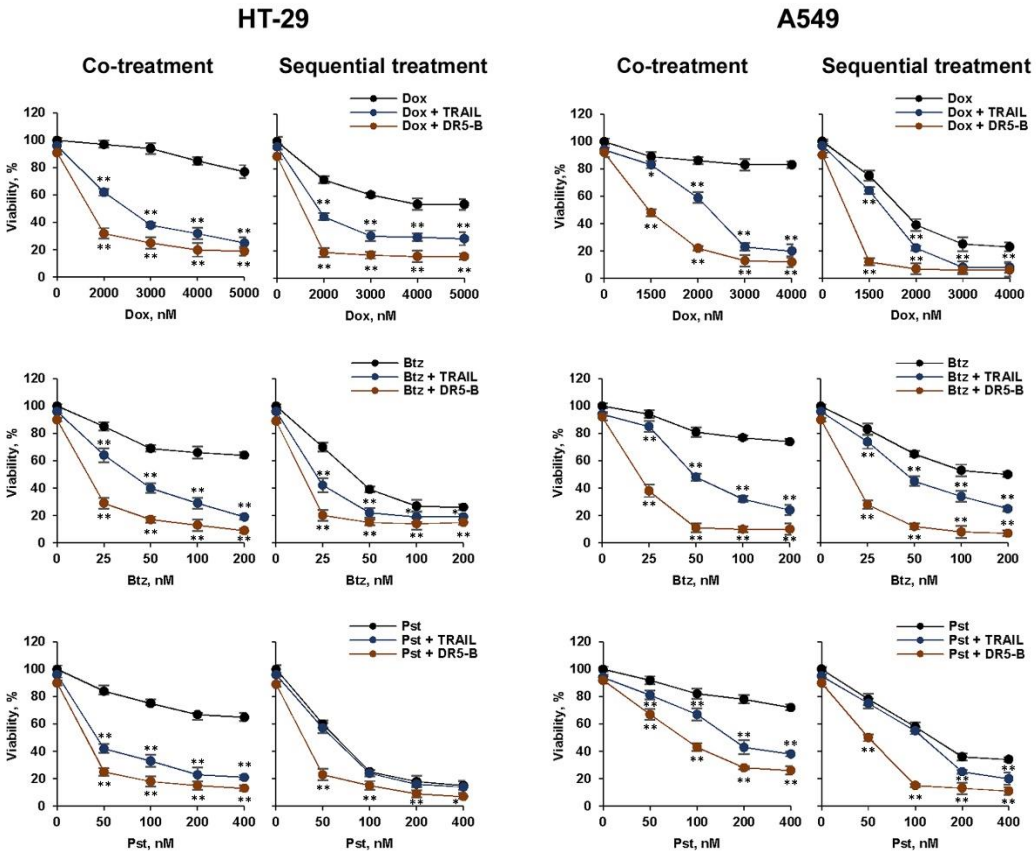

Figure S2. Sensitization of the resistant cancer cells to wild type TRAIL and DR5-selective variant DR5-B by chemotherapeutic agents. In co-treatment experiments HT-29 and A549 cells were incubated with 1000 ng/mL TRAIL or DR5-B and doxorubicin (Dox), bortezomib (Btz) and panobinostat (Pst) at the indicated concentrations for 24 h. In a sequential treatment, cells were pretreated with chemotherapy for 24 h followed by treatment with TRAIL or DR5-B for another 24 h. Cell viability was determined by WST-1 colorimetric assay. Mean ± Standard Deviation (n = 4). The asterisks indicated significance (* $p < 0.05$) and (** $p < 0.001$) relative to cells treated with chemotherapy without ligands.

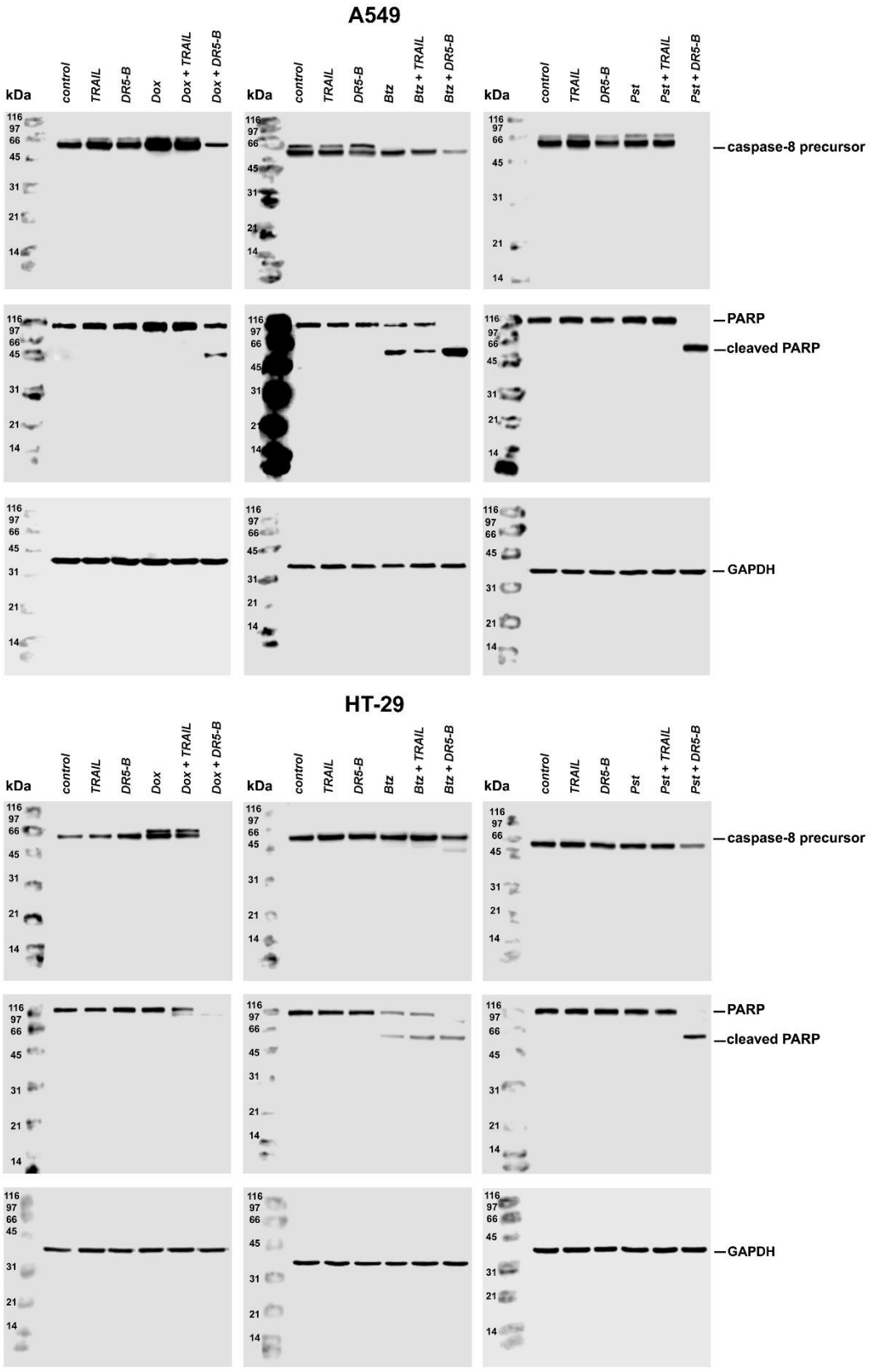

Figure S3. Western blot analysis of caspase-8 and PARP (Poly(ADP-ribose) Polymerase-1) proteins after sequential treatment of cells with chemotherapy and TRAIL or DR5-B. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used for normalization of protein bands. Cells were pre-incubated with 1500 nM and 2500 nM doxorubicin (Dox), 50 nM and 25 nM bortezomib (Btz), 100 nM and 50 nM panobinostat (Pst) for A549 and HT-29 respectively for 16 h, followed by

1000 ng/mL ligands for another 6 h for Dox and Pst and 3 h for Btz. Protein concentration was determined using a Micro BSA Protein Assay Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Samples containing 20 μg protein were heated at 95 °C for 5 min, subjected to reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. To mark the position of molecular markers, polyvinylidene difluoride (PVDF) membranes after trans-blotting were stained with 0.1% Ponceau S in 5% acetic acid and marked with Western Blot Marker chemiluminescence Pen.