Supplement Materials: High Antitumor Activity of the Dual Topoisomerase Inhibitor P8-D6 in Breast Cancer

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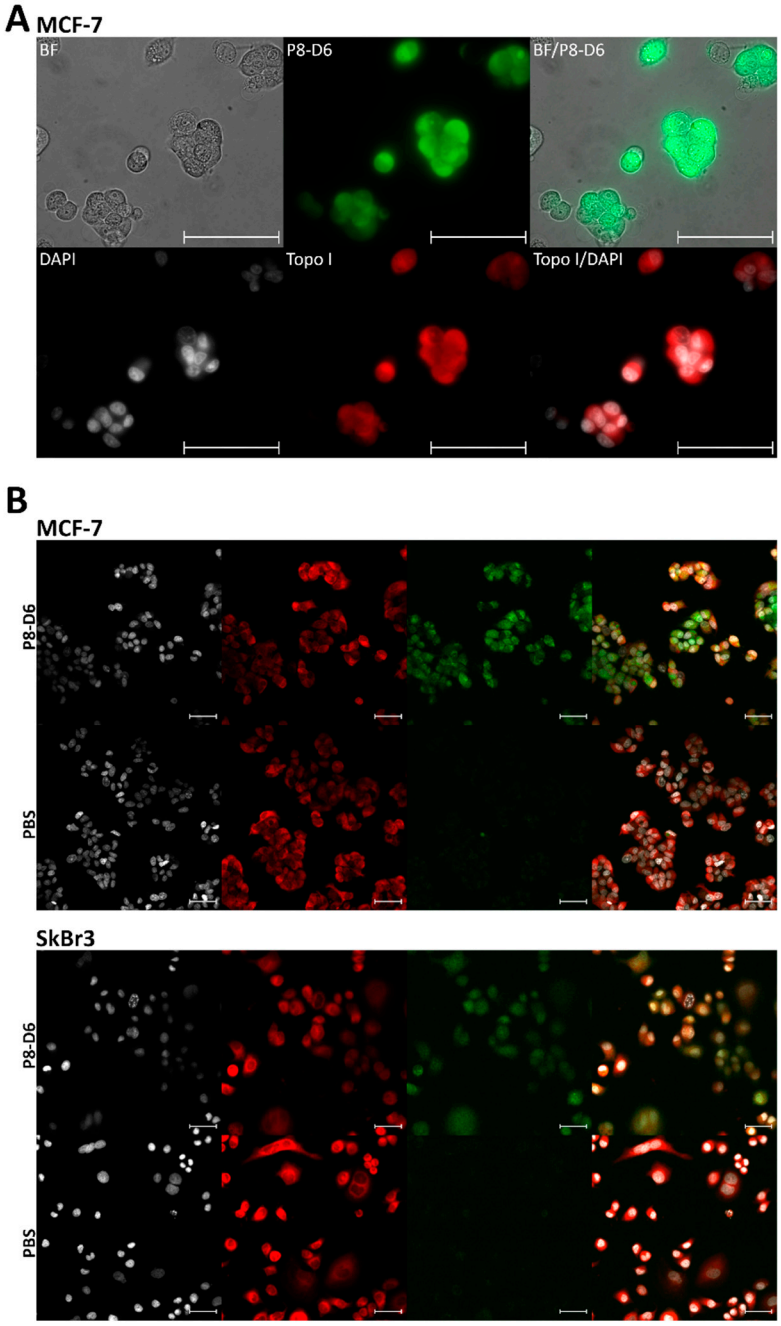

Figure S1. Localization of P8-D6. MCF-7 (**A, B**) and SkBr3 (**C**) cells were treated with 10 µM P8-D6 (fluorophore: 462Ex/530Em) or control (PBS) for 10 h. P8-D6 was localized *in vitro*. (**A**) After fixation topoisomerase I was stained using anti-Topo I antibody (Santa Cruz#sc-271285) and imaged (63x) using LSM 880 and software ZEN 2.5 (blue edition). (**B, C**): After treatment cells were stained with CellTracker™ Deep Red Dye and hoechst 33342 (25x). Fluorescence intensity of P8-D6 was quantified in the nucleus. Fluorescence images show the fluorophore P8-D6 in green, membrane staining (**B, C**) or topoisomerase expression (**A**) in red and nucleus staining in white. Scale bars, 50 µm.

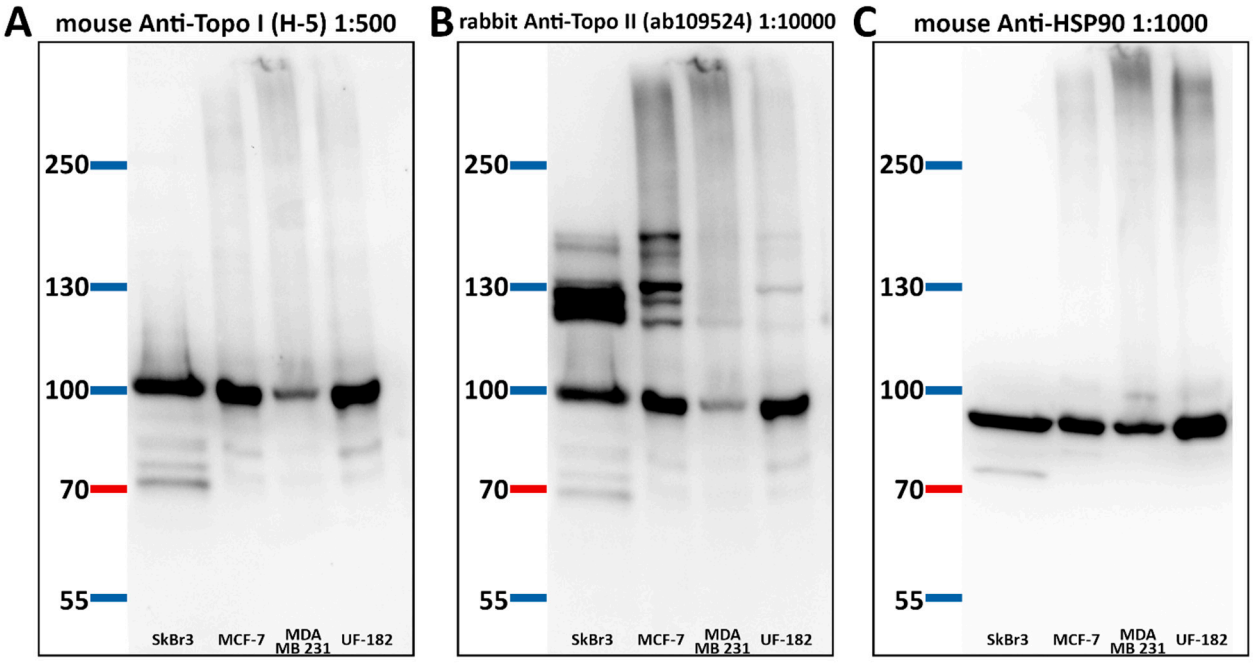

Figure S2. Protein expression of Topoisomerase I/II. Lysates of ovarian cancer cells were analysed by western blot to validate Topo I and Topo II protein expressions. In cell lysates Topo I (**A**) at 100 kDa, Topo II α (**B**) at 174 kDa, Topo II β (**B**) at 180 kDa were detected. HSP 90 (**C**) at 90kDa was used as loading control. After Topo II and before HSP90 detection, the blot was stripped.