Suppl. Figure 1. DSG2 immunohistochemistry on sections of human breast cancer biopsies. DSG2 staining appears in brown. Representative sections of different clinical stages are shown. The scale bars are 20μm.
Suppl. Figure 2. JO-1 improves efficacy of chemotherapy agents. Immunodeficient CB17-SCID/beige mice were injected with 3x10⁶ MDA-MB-231 cells, a Her2/neu negative human breast cancer cell line (Day 0). Treatment was started when tumors reached a volume of ~ 65 mm³ on day 15. Treatment was then initiated. JO-1 was injected intravenously at a dose of 2mg/kg followed by an intravenous injection of paclitaxel (PAC) at a dose of 5 mg/kg one hour later. Animals received a total of 6 doses of JO-1 and PAC co-therapy (marked by arrows). n=5. PAC vs. JO-1 + PAC: P<0.05
Suppl. Figure 3. Effect of JO-1 on Doxil and cisplatin in an ovarian cancer model. Immunodeficient CB17-SCID/beige mice were injected with $3 \times 10^6$ ovc316 cells, a primary cell line that has been used in studies on cancer stem cells. Treatment was started when tumors reached a volume of $\sim 65 \text{ mm}^3$ on day 7. JO-1 was injected intravenously at a dose of 2mg/kg followed by an intravenous injection of 1.5mg/kg Doxil (A) or 2mg/kg cisplatin (B). Injection was repeated 7 days later. The day when tumors reached a volume of 600mm$^3$ was taken as an endpoint in Kaplan-Meier survival studies. n=5. The difference between “Doxil” and “JO-1+Doxil” is significant (p<0.05).
Suppl. Figure 4. JO-1 in liver and spleen. hDSG2 transgenic mice were injected with JO-1 (2mg/kg). Organs were harvested 6 hours later. A) Staining of liver sections for JO-1 (red) and the Kupffer cell marker F4/80 (green). B) Staining of spleen sections for JO-1 (red) and the adherens junction marker claudin 7 (green). Representative sections are shown. The scale bars are 20μm.
Suppl. Figure 5. Serum clearance of JO-1. hDSG2 transgenic mice (hDSG2-pos) or nontransgenic littermates (nDSG2-neg) were intravenously injected with JO-1 (2mg/kg) and serum samples were analyzed for JO-1 by ELISA. N=3.
Suppl. Figure 6. JO-1 safety studies in hDSG2 transgenic mice. hDSG2 transgenic mice were intravenously injected with JO-1 at 2mg/kg and 10mg/kg. Twenty four hours later, mice were sacrificed. A) Analysis of White blood cells (WBC), neutrophils (NE), lymphocytes (LY) and platelets (PLT). N=3. B) Analysis of blood chemistry. SGPT=ALT. n=3. C) H&E staining of liver sections. Fat droplets are indicated by arrows. The scale bar is 20μm.
Suppl. Figure 7. Circulating tumor cells. A total of 4x10^6 Her2/neu-positive HCC1954 cells, were injected into the mammary fat pad of CB17-SCID/beige mice. When tumors reached a volume of ~300 mm³, mice received an intravenous injection of 50μg of JO-1 (2 mg/kg) or PBS. Twenty-four hours later blood was collected, red cell were lysed and the remaining cells were subjected to flow cytometry with antibodies specific to Her2/neu. n=3. The difference between PBS and JO-1 injected mice is not significant (p=0.16).