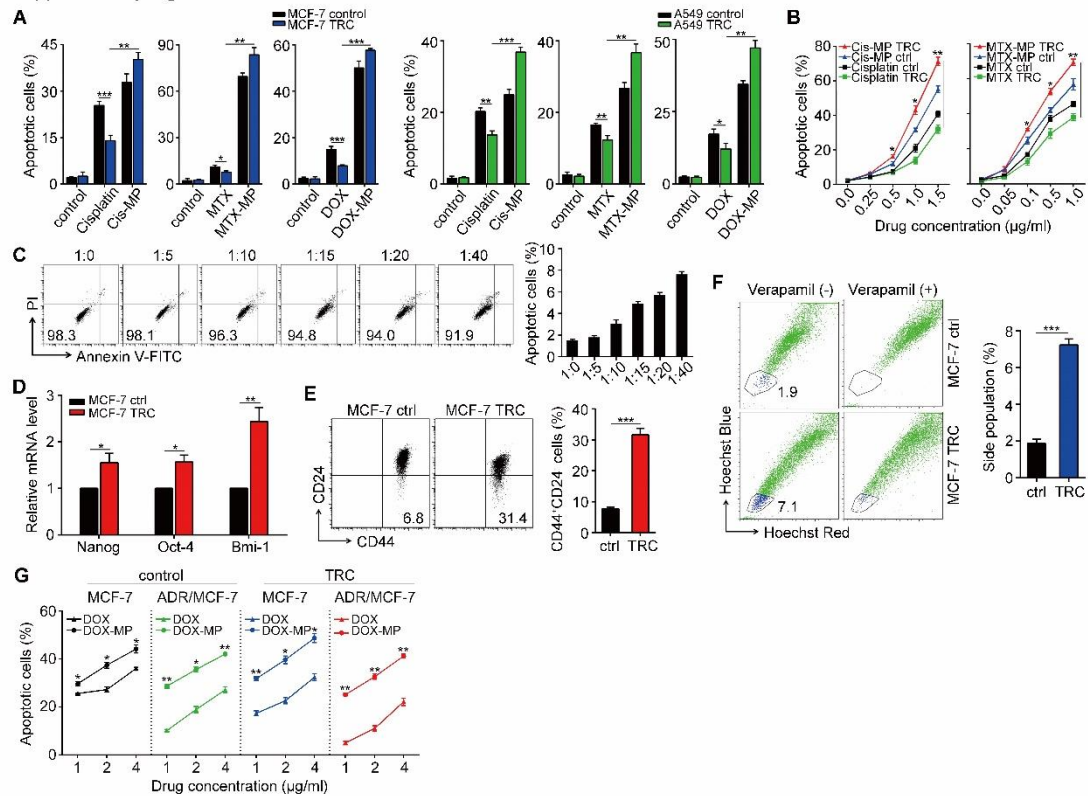


Supplementary Figure 5



Supplementary information, Figure S5. Drug-packaging MPs are capable of partially reversing the drug resistance of TRCs (Related to Figure 2). (A) Drug-packaging MPs reverse drug resistance of MCF-7 and A549 TRCs. MCF-7 and A549 control tumor cells and TRCs were treated with free drugs or drug-MPs, respectively. 36 hours later, cells were stained with APC-Annexin V for flow cytometric analysis. (B) Shaking do not influence the induction of tumor cell apoptosis by drug-packaging MPs. H22 TRCs (5×10^4) were treated with different concentration of cisplatin or Cis-MPs for 36 hours and MTX or MTX-MPs for 24 hours, respectively, under the condition of continuous shaking in the incubator at 37°C . H22 tumor cells cultured on conventional rigid plate were used as control cells. The cells were collected and stained with APC-conjugated Annexin-V for apoptotic detection by flow cytometry. (C) MPs alone do not induce overt apoptosis of tumor cells. Different amounts of bare MPs were used to treat H22 tumor cells. The ratio of H22 cells to MPs was 1:0, 1:5, 1:10, 1:15, 1:20 and 1:40, respectively. 12 hours later, the cells were collected and stained with FITC-conjugated Annexin-V and PI for apoptotic detection by flow cytometry. (D) The expression of stem cell markers (*Nanog*, *Oct-4* and *Bmi-1*) was analyzed by real-time

PCR. (E) High proportion of CD44⁺CD24⁻ cells were present in MCF-7 TRCs versus control cells. MCF-7 TRCs and control cells were stained with FITC-conjugated CD44 and PE-conjugated CD24 antibodies and detected by flow cytometry. (F) MCF-7 TRCs present much higher side population by flow cytometric analysis. 1×10^6 cells were suspended in culture medium containing 1% FBS. Hoechst 33342 dye was added (5 $\mu\text{g}/\text{ml}$) in the presence or absence of 50 μM verapamil, and the cells were incubated at 37°C for 90 min with intermittent shaking every 10 min. The cells were washed twice with PBS and resuspended in 250 μl PBS with 2 $\mu\text{g}/\text{ml}$ PI and kept on ice until FACS analysis. (G) ADR/MCF-7 tumor cells as well as their TRCs were also efficiently targeted by DOX-MP. MCF-7 and ADR/MCF-7 control tumor cells and TRCs were treated with DOX or DOX-MP, respectively. 36 hours later, cells were stained with APC-Annexin V for flow cytometric analysis. For all graphs, error bars indicate mean \pm s.e.m.; n=3 independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Student's *t*-test).