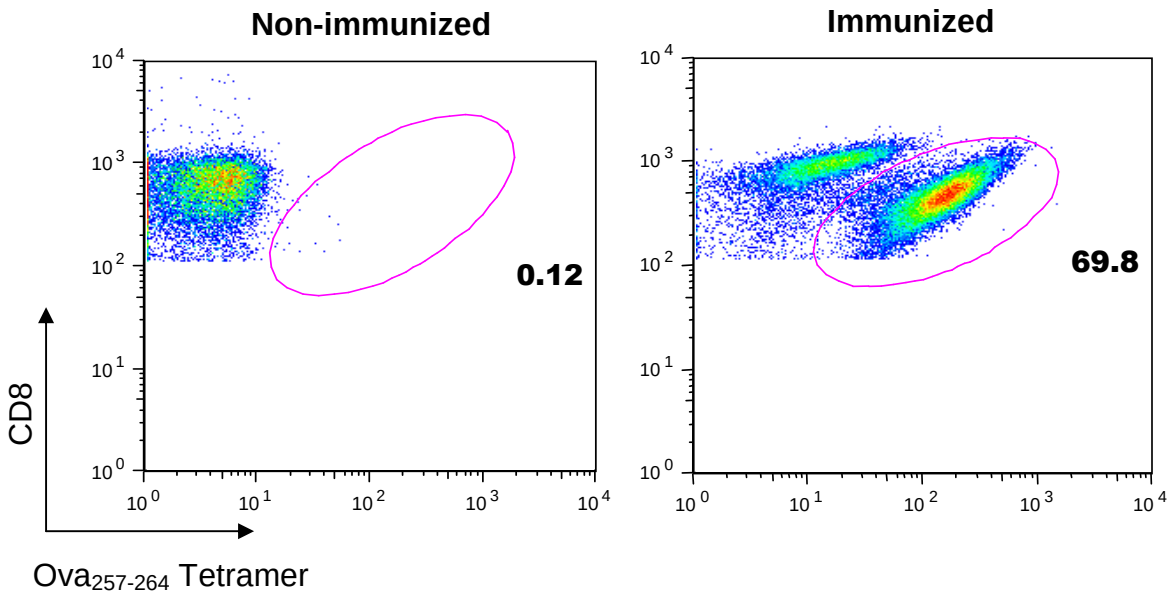


Figure S1. The presence of antigen-specific CD8$^+$ T cells in mice immunized with OVA-derived peptide. C57BL/6 mice were vaccinated with ovalbumin plasmid, followed by a booster immunization with $Ova_{257-264}$–peptide TriVax vaccine 2 week later. Antigen-specific CD8$^+$ T-cells in spleen were evaluated 7 days after the boost using $Ova_{257-264}$–specific tetramers. CD8$^+$ T cells were gated.

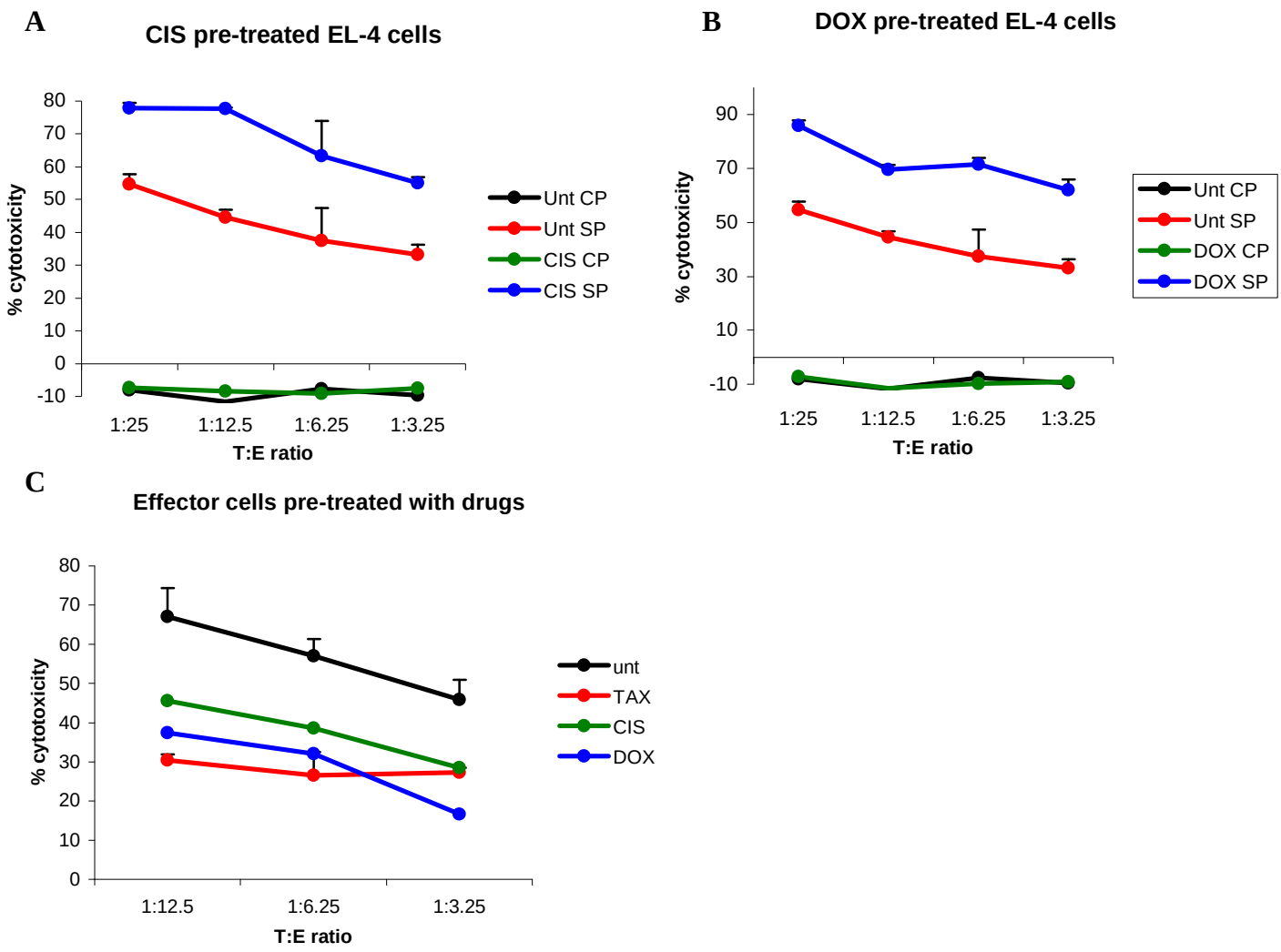


Figure S2. Chemotherapeutic drugs sensitize tumor cells to the cytotoxic effect of CTLs.

A, B. Splenocytes from OT-1 transgenic mice were cultured with 10 µg/ml of the specific peptide for 72 hr. T cells were isolated and used as effectors against either untreated (Unt) EL-4 target cells or cells pre-treated for 18 hr with 12.5 ng/ml CIS (**A**) or 12.5 ng/ml DOX **(B)**. Target cells were loaded with 0.1 µg/ml of either control (CP) or specific peptides (SP). Standard CTL assay was performed in duplicates. Two experiments with similar results were performed. **C.** Splenocytes from OT-1 mice were incubated with 10 µg/ml of the specific peptide for 72 hr. During last 18 hr of incubation 12.5 nM of TAX, 12.5 ng/ml DOX, or 12.5 ng/ml CIS were added. T cells were isolated and used as effector cells against untreated EL-4 tumor cells loaded with specific peptide. Two experiments with the same results were performed. Specific cytotoxicity against target cells loaded with control peptide was lower than 3% in all experiments (not shown).

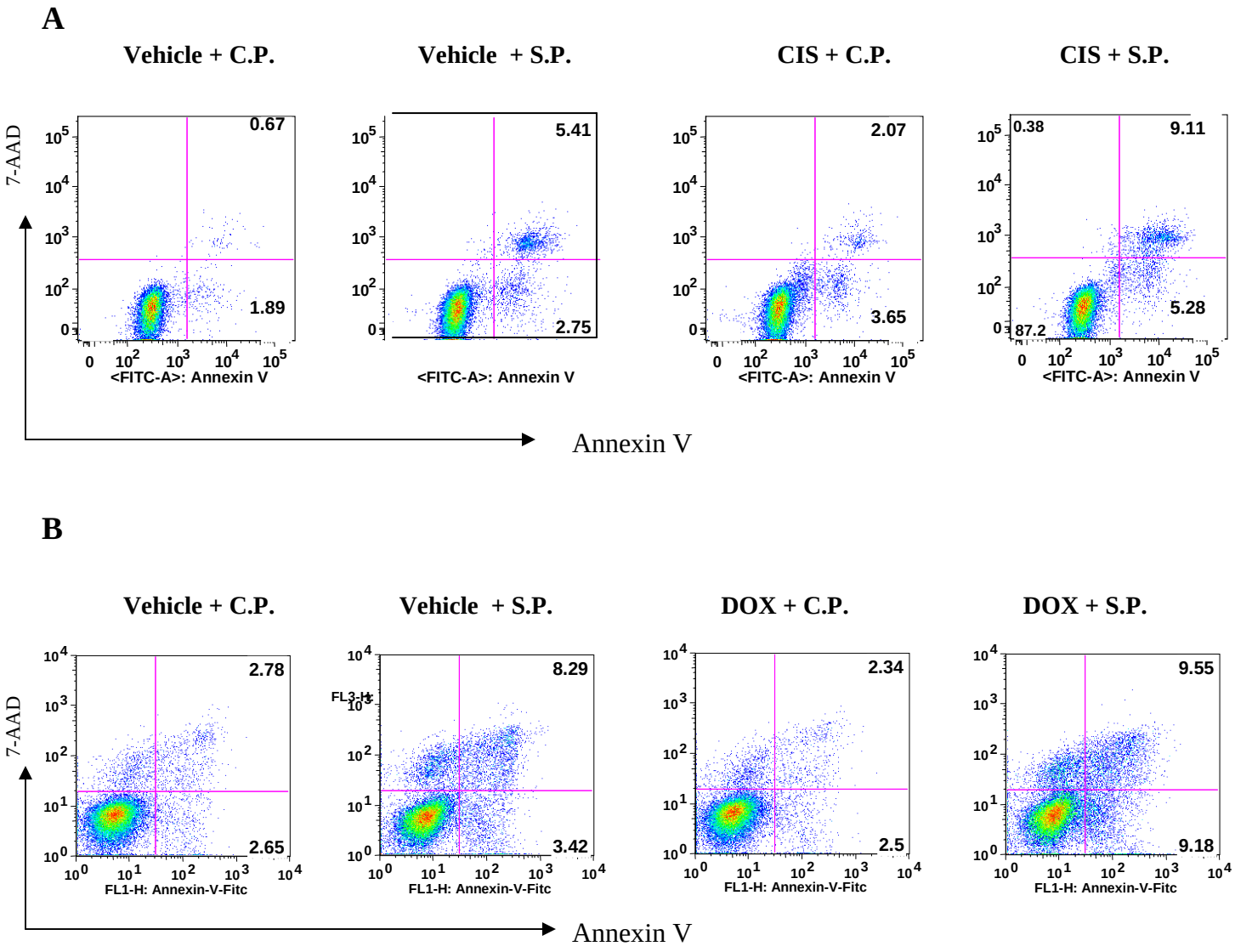
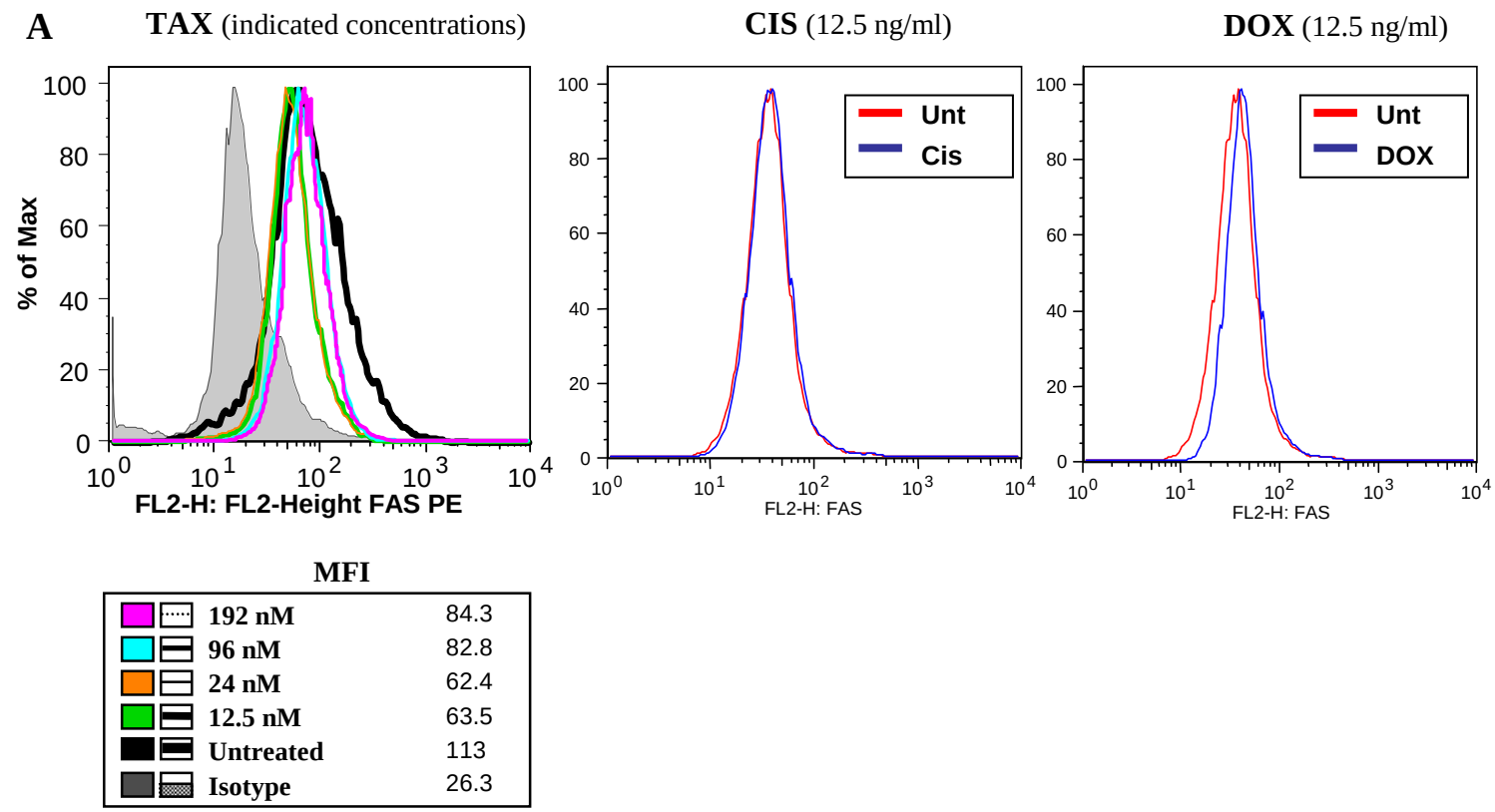


Figure S3. Pre-treatment of tumor cells with cisplatin and doxorubicin enhances cytotoxicity of CTLs. Early apoptosis in EL-4 target cells pre-treated with 12.5 ng/ml cisplatin (CIS) (**A)** or 12.5 ng/ml doxorubicin (DOX) **(B)** was evaluated using Annexin-V-FITC/7-AAD staining. Activated OT-1 T cells were labeled with DDAO-SE and incubated at 30:1 ratio with EL-4 cells loaded with control (C.P.) or specific (S.P.) peptides. Thirty minutes later cells were then labeled with Annexin-V-FITC and 7-AAD and target cells were analyzed after gating out the effector cells. Three experiments with the same results were performed.



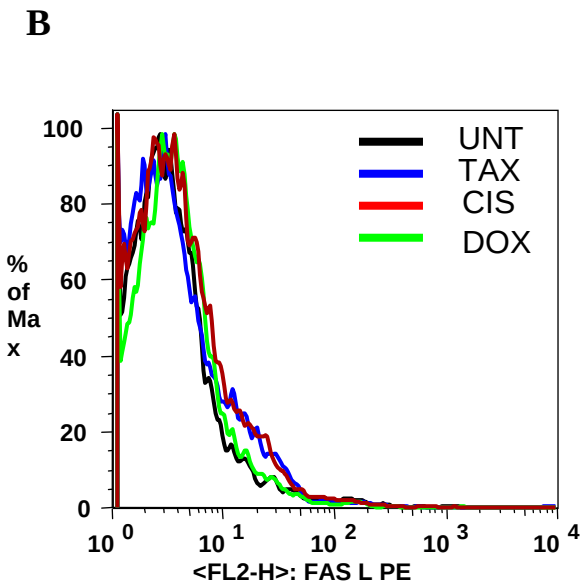


Figure S4. Effect of paclitaxel (TAX), cisplatin (CIS), and doxorubicin (DOX) on the expression of Fas on tumor cells and splenocytes. **A.** EL-4 tumor cells were treated with different drugs at indicated concentrations overnight followed by staining with PE-conjugated Fas antibody (BD Pharmingen).

B. Splenocytes were treated overnight with 12.5 nM of TAX, 12.5 ng/ml CIS, or 12.5 ng/ml of DOX and expression of FasL was evaluated by flow cytometry using PE conjugated FasL antibody (BD Pharmingen)

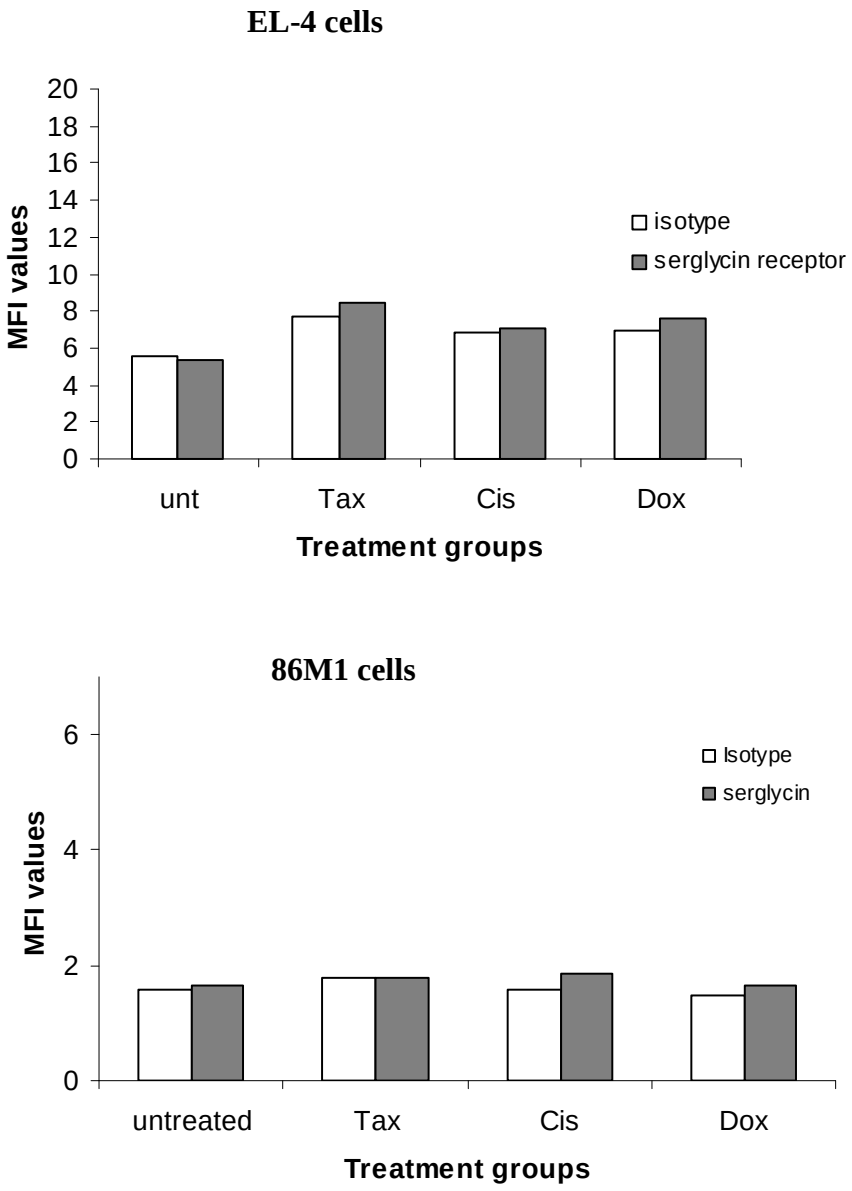


Figure S5. Serglycin expression on EL-4 and 86M1 cells after overnight treatment with Tax, Cis, Dox. EL-4 or 86M1 target cells were treated with either12.5nM TAX, 25ng/ml CIS or 25ng/ml DOX for 16 hr prior to the assay. The cells from both untreated and treated groups were washed, blocked with either 10% mouse or human sera for 20 min at 4^{o}C. The cells were incubated with 2µl/10^{6} cells of serglycin antibody (Santa Cruz) for 30 min at 4^{o}C followed by staining with donkey anti-goat IgG-PE. The cells were washed and acquired on a FACS Calibur. The mean fluorescence intensity (MFI) is shown. Two experiments with the same results were performed.

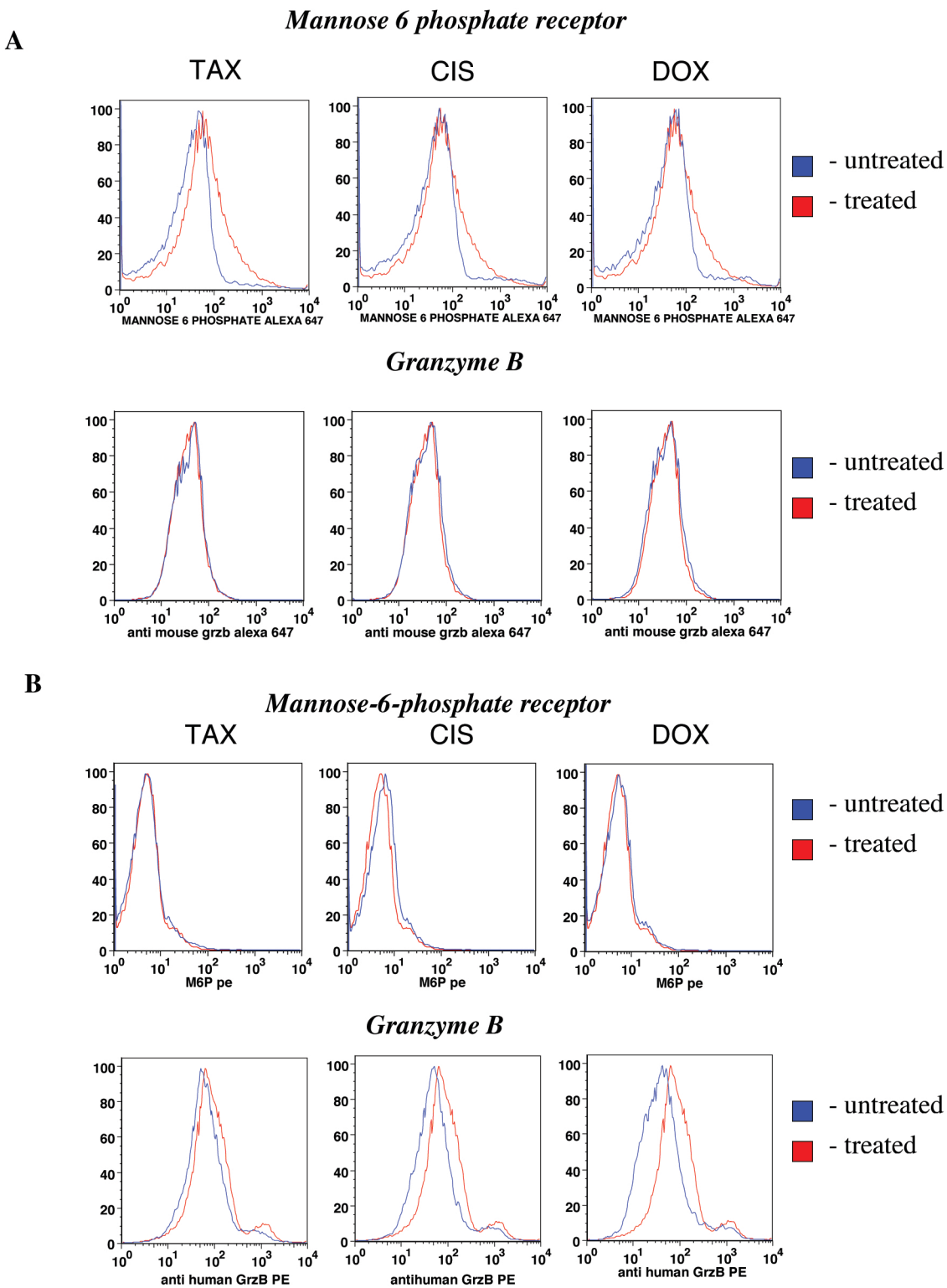


Figure S6. Effect of chemotherapeutic drugs on normal cells. Splenocytes isolated from naïve C57BL/6 mice **(A)** or mononuclear cells isolated from healthy volunteer **(B)** were incubated with 12.5nM TAX, 25ng/ml CIS or 25ng/ml DOX for 16 hr. After that time cells were either stained with anti-MPR antibody to detect the expression of mannose 6 phosphate receptor or were incubated with 1 μg/ml recombinant mouse GrzB for 30 min followed by detection of GrzB with anti-mouse GrzB antibody. Histogram overlays represent untreated cells (blue line) and cells treated with indicated drug (red line). Three experiments with the same results were performed.

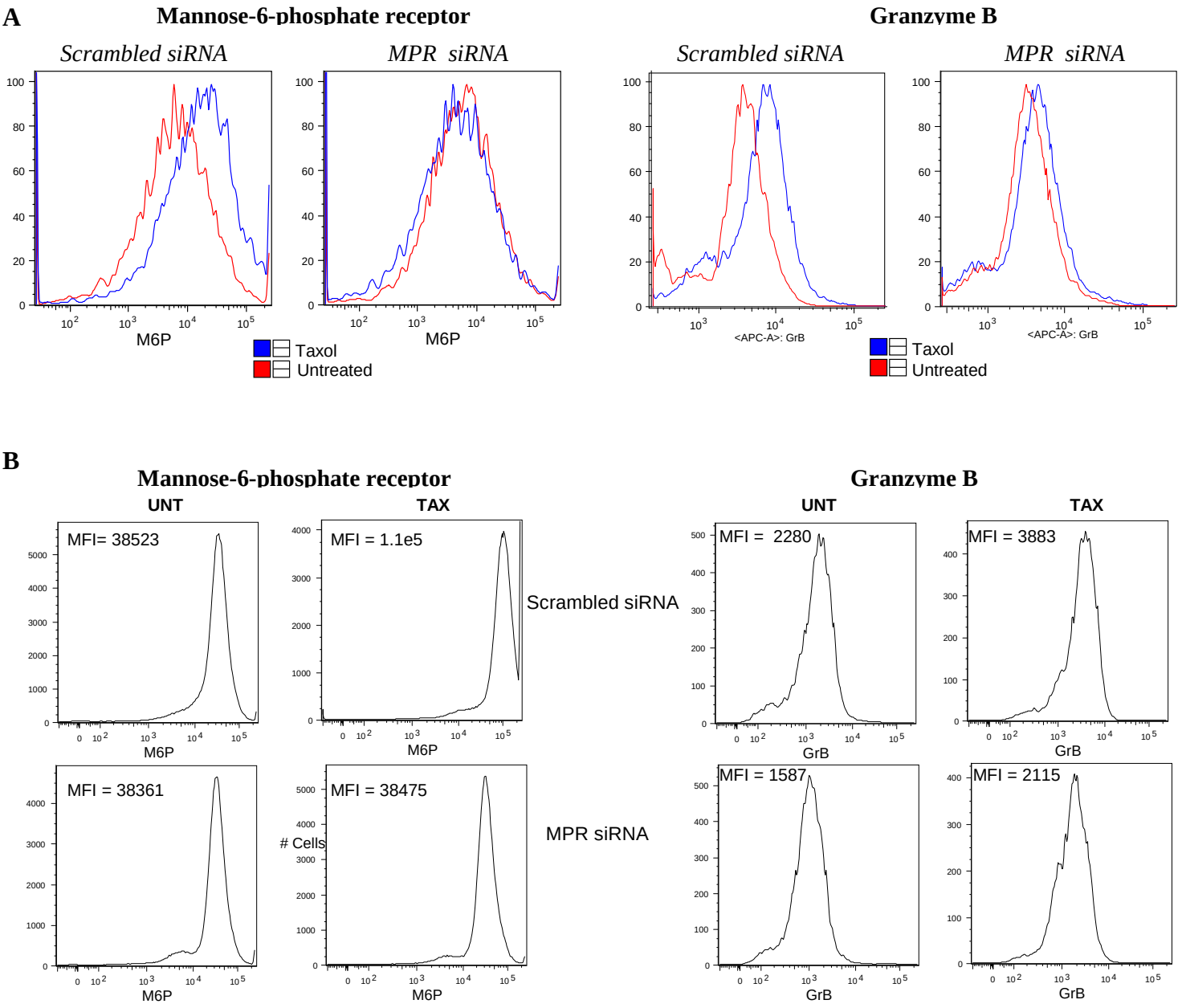


Figure S7. Down-regulation of mannose 6 phosphate receptor in tumor cells prevents GrzB uptake. MPR siRNA (SMARTpool) was obtained from Thermo Scientific and 150 nM was incorporated into 5 x 10^6 tumor cells using electroporation (Amaxa nucleofector 1). For 4T1 cells, mouse macrophage nucleofector kit (Program-Y-01) was used. For EL4 cells mouse cell transfection kit (Program-C-09) was used. As controls, scrambled siRNA was used at the same concentration. After transfection cells were incubated for 48h in complete medium. Half the cells from each group were treated with TAX (12.5nM) overnight. The cells were then washed and labeled with anti-MPR antibody or incubated with GrzB followed by detection of intracellular GrzB as described in Figure S7. The cells were acquired on LSR-II flow cytometer. Dead cells were discriminated from the live population by either DAPI stain or Live/Dead Fixable Dead cell stain kit (Invitrogen). The experiments were repeated three times with the same results. **A –** 4T1 tumor cells. **B** – EL-4 tumor cells.

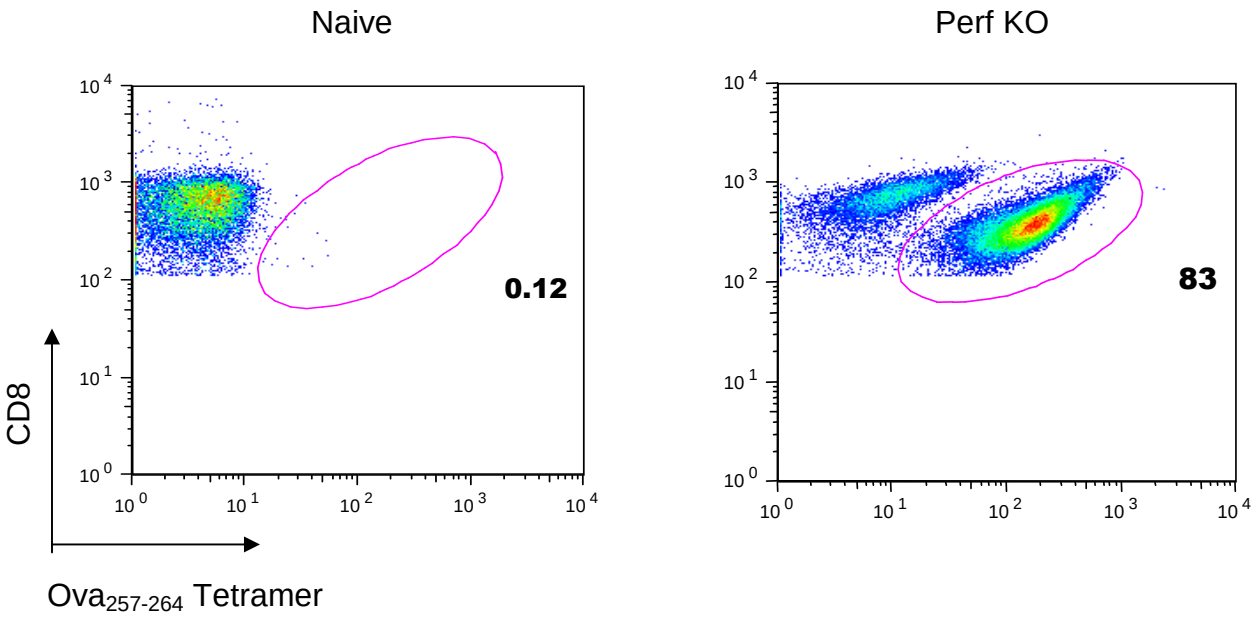


Figure S8. Generation of antigen-specific T cells in perforin knockout mice

Perforin KO mice (C57BL/6 background) were vaccinated with ovalbumin plasmid followed by a booster immunization with $Ova_{257-264}$–peptide TriVax vaccine 2 week later. Antigen-specific $CD8^+$ T-cells in spleen were evaluated 7 days after the boost using $Ova_{257-264}$–specific tetramers. $CD8^+$ T cells were gated.

Video S1. Live cell imaging of the kinetic of interaction of tumor cells with CTLs.

Splenocytes from OT-1 mice were stimulated *in vitro* for 72 hr with SIINFEKL peptide and T cells were isolated. The targets EL-4 cells were treated with 12.5nM TAX overnight prior to loading with specific peptide. T cells were purified, washed and labeled using Vybrant CFDA SE Cell Tracer Kit (Invitrogen, Carlsbad, CA). The target cells were left unlabeled. The cells were mixed together at targets : effectors ratio 1:10 in the presence of 30 μM propidium iodide in a glass bottomed Petri-dish and observed immediately under optimal CO_2 and temperature conditions using an incubation chamber attached to a live cell imaging microscope. Cells were observed for 60 min. Tumor cells are large unlabeled cells that acquire PI staining during culture (arrows). This effect was not observed when tumor cells were loaded with control peptide or were not treated with TAX.