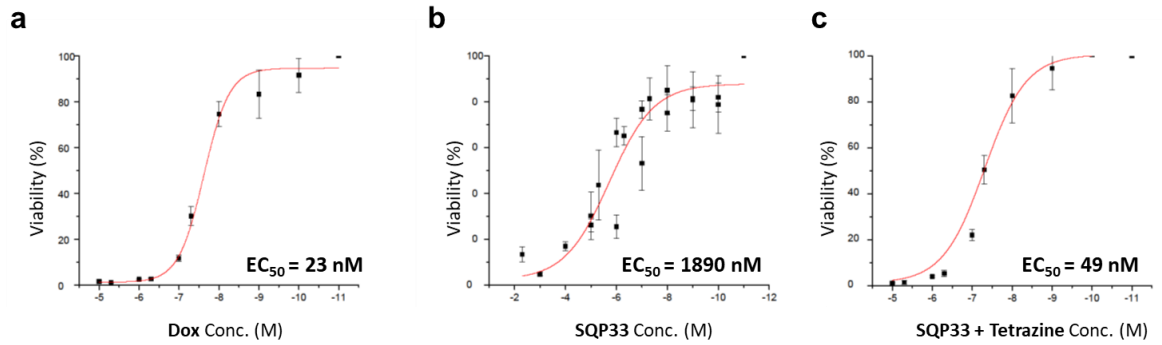
Supporting Information

Figure S1 – MTT data from Max with MC38 cells.

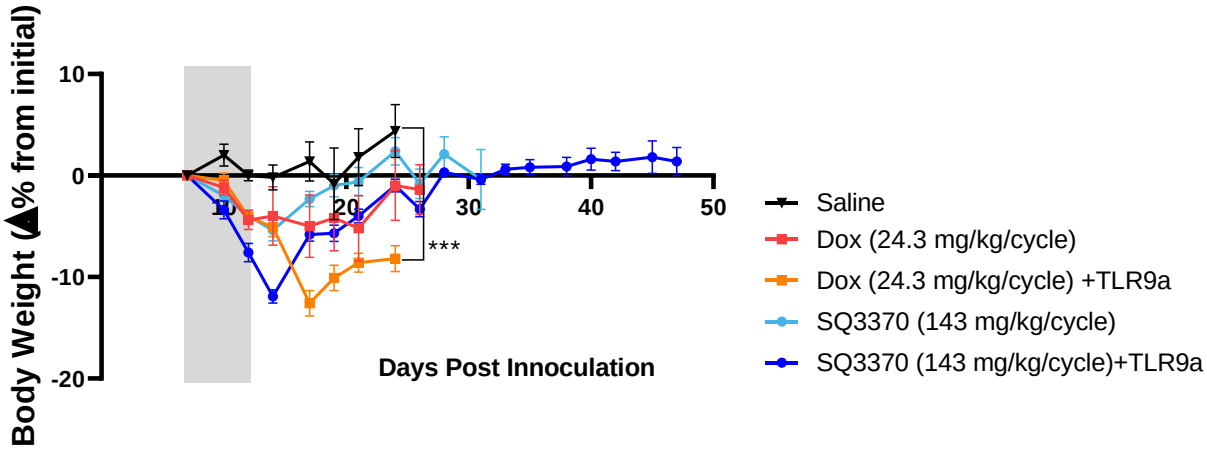
Figure S2 – Body Weight data as a measure of toxicity.

Figure S3 – Rechallenge w/ or w/o TLR



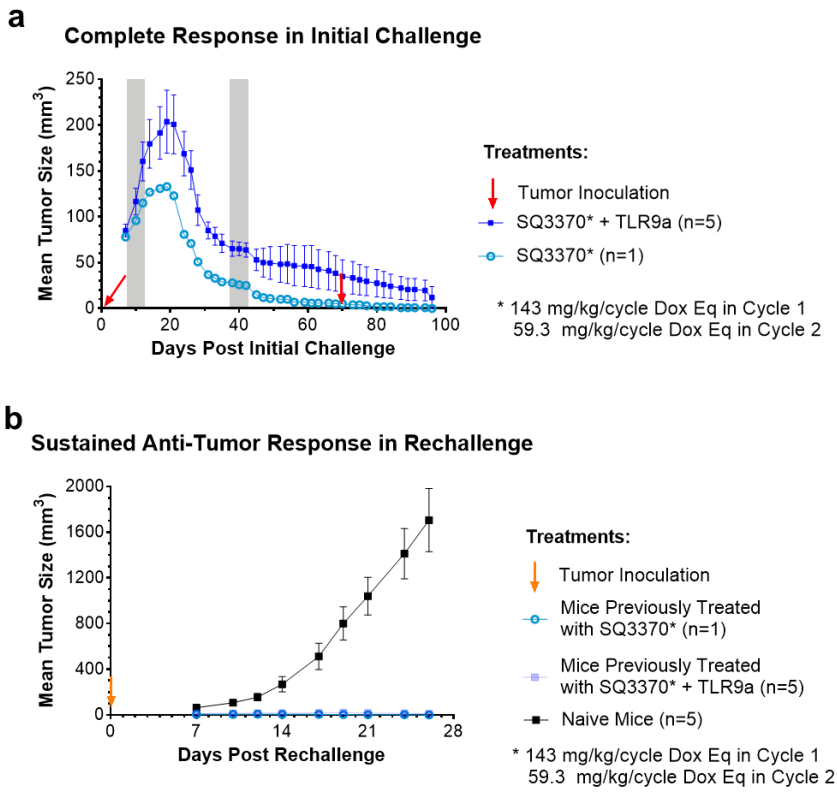
Supplemental Figure S1: Cell Viability of MC38 Tumor Cell-line Was Assessed Using an MTT Assay

MC38 cells were seeded in 96-well microplates and exposed to SQP33 protodrug, Dox for 3 days using the The CellTiter 96® AQueous One Solution Cell Proliferation Assay, a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays. The assay reagent contains a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] and an electron coupling reagent (phenazine ethosulfate) (MTT assay). The quantity of formazan product as measured by absorbance at 490 nm is directly proportional to the number of live cells. Cytotoxic effects of a) Dox, b) SQP33 protodrug, and c) SQP33 protodrug in the presence of tetrazine were compared were compared in an MC38 cell line after incubation for 3 days. Samples were run in triplicate. Percent viability is shown for increasing concentrations of each drug. EC50 is the concentration of a drug that gives half-maximal response. Without activation by tetrazine, the EC50 of SQP33 protodrug was 82-fold lower (b) than with tetrazine (c).



Supplemental Figure S2: Changes in Body Weight in Tumor Bearing Mice after Treatment

Body weights as percent change from initial during the start of treatment shown for saline, Dox, Dox+TLR9a, SQ3370, and SQ3370+TLR9a treated groups, respectively. Gray bars represent the treatment duration. Data points without errors bars occurred when the standard error was smaller than the symbol used to represent the treatment condition. Curves stopped after 1 or more mice in that group died or were sacrificed when tumor volume reached 2000 mm3. Gray bars represent the SQ3370 treatment duration. Statistical significance in curves was determined by comparison to saline group using a one-way ANOVA with Tukey's multiple comparisons for each day. *p<0.05; **p<0.01;***p<0.001.



Supplemental Figure S3: Sustained Immune Response After Tumor Re-challenge in SQ3370-Treated Mice. Immunocompetent male C57BL/6 mice were inoculated SC in the right flank with 5 x 10^5 MC38 tumor cells. These mice received peri-/intra- tumoral biopolymer injections (100 µL/mouse). One hour later, mice receive IV saline control (n=5) or SQP33 protodrug (28.6 mg/kg Dox Eq QD x 5 days; cumulative dose of 143 mg/kg Dox Eq) (n=10) or SQ3370 and TLR9a intratumorally after the last SQP33 dose at 25 µg per mouse (n=10). Mice received a second cycle of SQ3370 at 59.3 mg/kg/cycle Dox Eq, with or without TLR9a. Only the complete responders were re-challenged with MC38 tumors on Day 70 and each treatment group was compared with a control group of naïve mice also inoculated on the same day; a) Initial challenge and b) rechallenge tumor growth curves are shown for the complete responders in SQ3370 and SQ3370+TLR9a groups. Gray bars represent the duration of treatment in each cycle. Tumor growth curves show mean ± SEM.