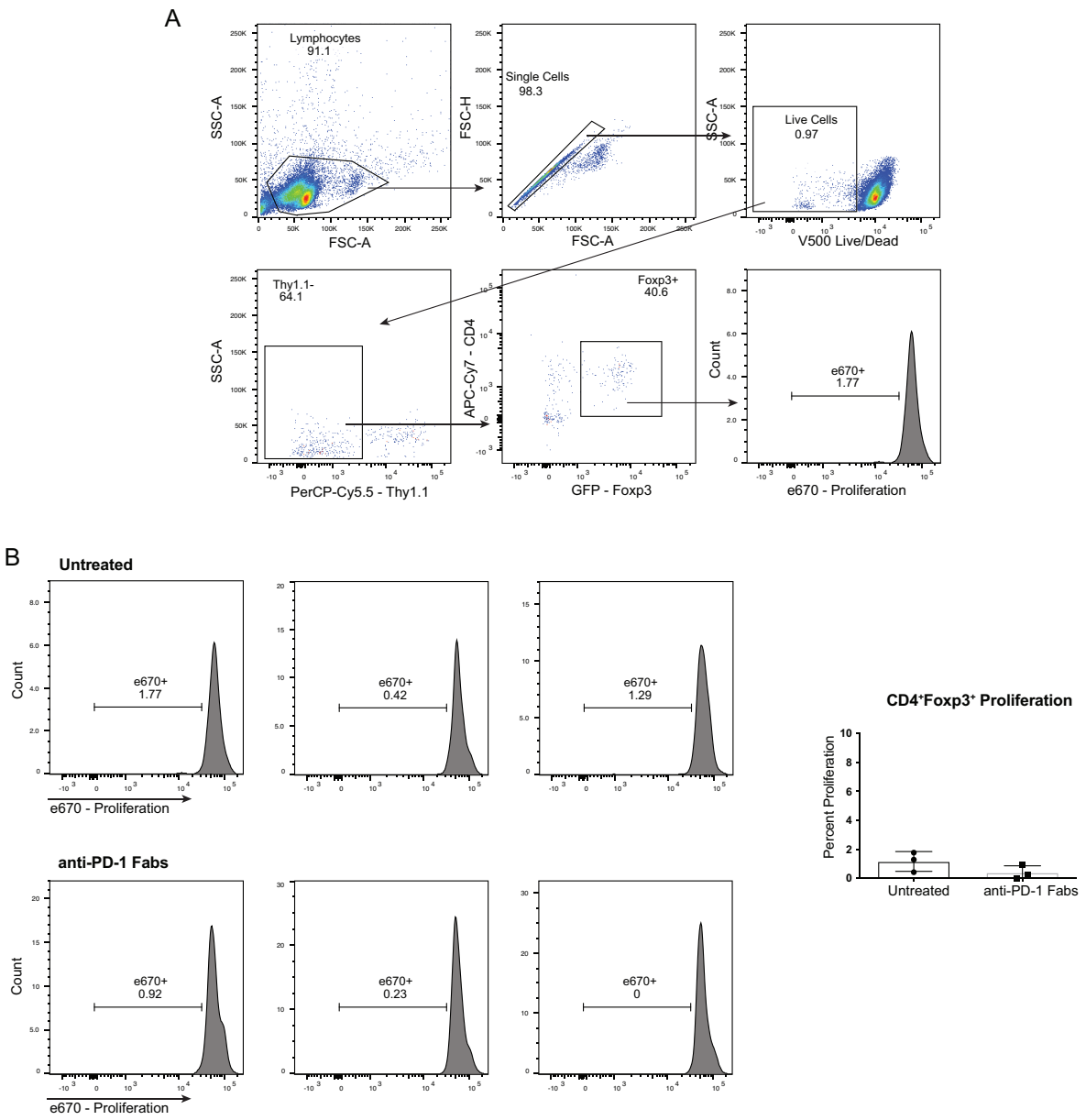
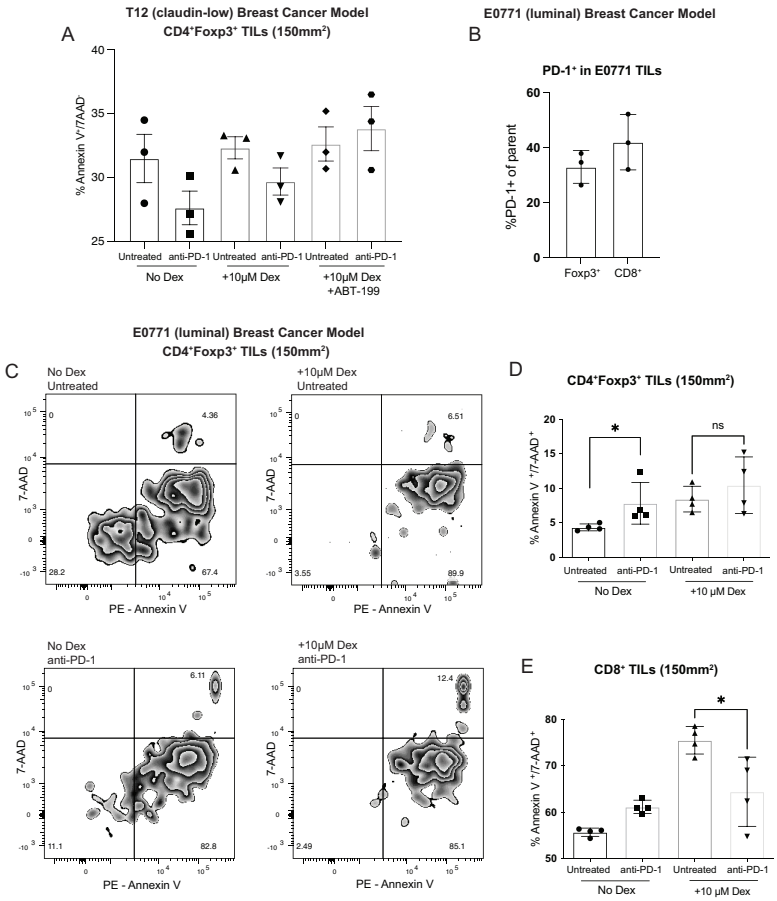
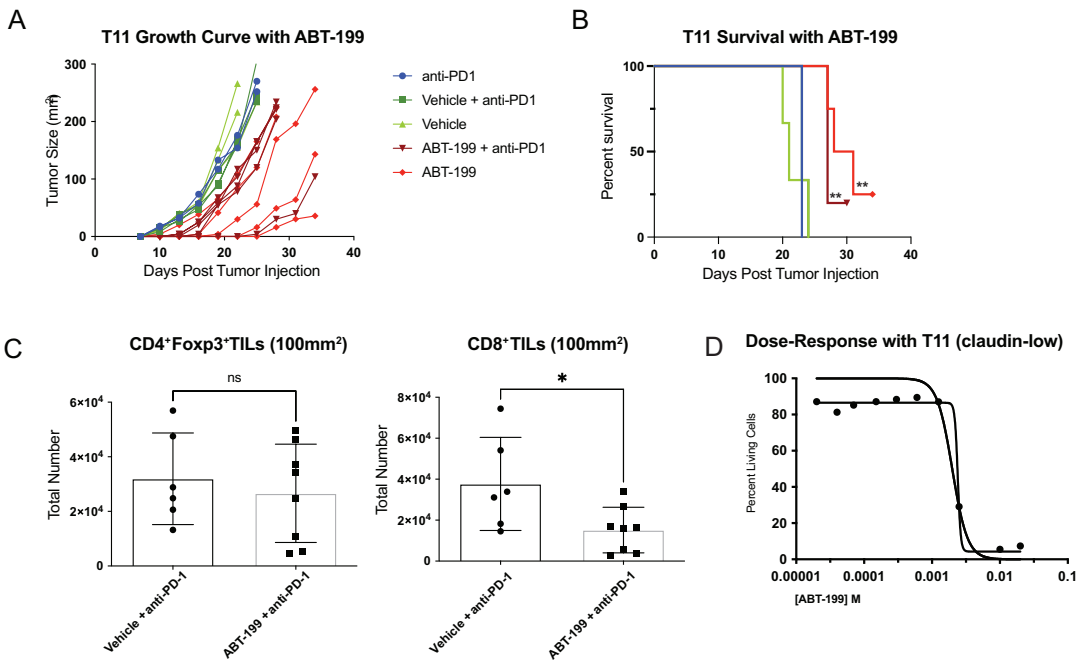


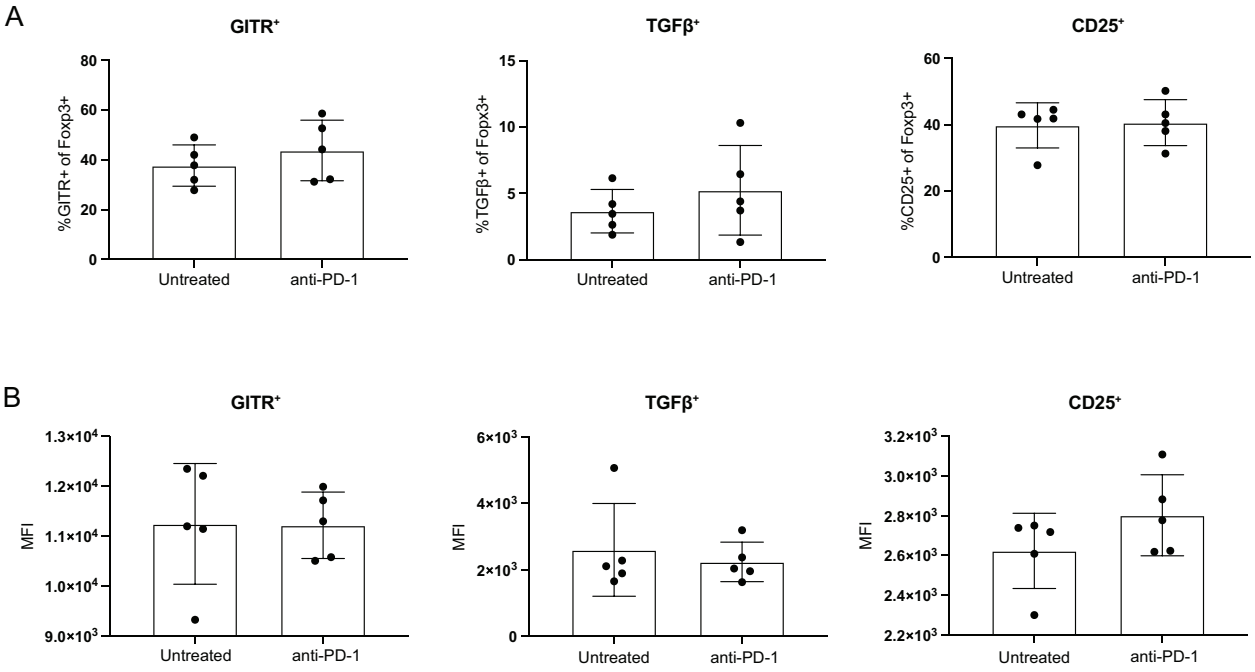
Supplementary Figure S1: PD-1 antibody alone does not result in T_{reg} proliferation. Mice were injected with 1 x 10^4 T11 (claudin-low) tumor cells. (**A-B**) Tumors were harvested at 150mm^2, digested, enriched for lymphocytes, and GFP+ T_{regs} were sorted using MoFlo-XDP cell sorter. T_{regs} stained with proliferation dye were incubated with or without α-PD-1 Fabs and irradiated APCs without α-CD3 in culture for 72 hours. (**A**) Flow cytometry gating strategy for proliferation of T_{regs} cultured without or with α-PD-1 Fabs. (n=3) (**B**) Percent proliferating $CD4^+Foxp3^+$ T_{regs} from in vitro culture.



Supplementary Figure S2. Apoptosis of T_{regs} after PD-1 blockade in T12 (claudin-low) and E0771 (luminal) breast cancer models. **(A)** BALB/c Foxp3-GFP mice were injected with 1 x 10^5 T12 (claudin-low) tumor cells (n=3) or **(B-E)** B6 Foxp3-GFP mice were injected with 2.5x10^5 E0771 cells (n=4). Mice were untreated or treated with 200 μg α-PD-1 antibody (J43) injected IP twice a week for the duration of the experiment. Isolated total T cells were cultured in 96 well plate in complete media or complete media + 10μM Dexamethasone + 20μM ABT-199. Apoptosis was measured using Annexin V and 7-AAD staining. **(A)** Percent $CD4^+Foxp3^+$7-AAD/Annexin V^+ T_{regs}. **(B)** Percent PD-1^+ of T_{regs} or CD8 T cells among E0771 TILs. (**C**) Representative flow plots gated on GFP^+ T_{regs}. (**D**) Percent $CD4^+Foxp3^+$7-AAD/Annexin V^+ T_{regs} from $CD45^+$ parent population. (**E**) Percent $CD8^+$/7-AAD/Annexin V^+ T cells from $CD45^+$ parent population. Statistical significance determined by Mann-Whitney test. * denotes $p < 0.05$.



Supplementary Figure S3: Inhibition of Bcl-2 leads to delay of tumor growth and increase in survival. BALB/c mice were injected with 1 x 10^4 T11 (claudin-low) tumor cells. Mice were untreated or treated with 200µg α-PD-1 antibody (J43) injected IP twice a week for the duration of the experiment. Mice were also given ABT-199 (100mg/kg) daily, or vehicle daily by oral gavage from day +1 for the duration of the experiment. (**A**) Individual replicates of tumor growth curves. (**B**) Mice receiving Bcl-2 inhibitor ABT-199 and ABT-199 + α-PD-1 (n = 3) have a significant survival benefit compared to mice receiving vehicle (n=3) or α-PD-1 alone (n = 3) (p = 0.0046; log-rank test for Vehicle + α-PD-1 vs ABT-199 + α-PD-1) (p = 0.0042; log-rank test for Vehicle vs ABT-199). (**C**) Tumors were harvested at 100mm^2, digested, enriched for lymphocytes, and analyzed by FACS. (Vehicle + α-PD-1 n=6, ABT-199 + α-PD-1 n=8) (**C**) Total number of $CD4^+Foxp3^+$ T_{regs}. (**D**) T11 (claudin-low) tumor cells were plated in a 96 well plate at 1.5x10^4 cells/well and incubated for 24 hours at 37°C. ABT-199 was added at a starting concentration of 20µM and serially diluted. T11 cells plus ABT-199 were incubated at 37°C 5% CO2 for 48 hours. Cell death was measured using Sigma MTT Cell Growth Assay.



Supplementary Figure S4: Characterization of T_{regs} from T12 (claudin-low) tumor model. Mice were injected with 1 x 10^5 T12 (claudin-low) tumor cells in Matrigel low-growth factor. Mice were untreated or treated with 200 μg α-PD-1 antibody (J43) injected IP twice a week for the duration of the experiment. (**A-B**) Tumors were harvested at 150mm^2, digested, enriched for lymphocytes, and analyzed by FACS. (**A**) Percent $CD4^+Foxp3^+$ T_{regs} expressing suppressive molecules; GITR, TGFβ, and CD25 from mice treated with α-PD-1 versus untreated (n=5). (**B**) Geometric Mean Fluorescence Intensity of suppressive molecules in $CD4^+Foxp3^+$ cells (n=5). Statistical significance determined by Mann-Whitney test.