Supplemental Data Figure Legends

Supp Fig. 1. Gating strategy for flow cytometry. Tumors cells were dispersed and strained through a 100 μm filter then stained with Aqua Zombie, followed by anti-CD45-Alexa Fluor 700 (clone 30-F11), anti-CD11b-APC/Fire 750 (clone M1/70), anti-CD4-PE (clone RM4-4), anti-CD19-FITC (clone 6D5), anti-CD25-PE/Cy7 (clone PC61.5), anti-CD3ε-Brilliant Violet 421 (clone 145-2C11) and anti-CD8a-Brilliant Violet 605 (clone 53-6.7). Cells were then fixed and stained for intracellular FoxP3 using anti-FoxP3-APC (clone 3G3). One spleen was processed alongside each group of tumors. Forward and side scatter detector voltage settings were selected based on the position of CD3+ or CD45+ T cell staining. Splenocytes were used as a guide for setting up the machine, guiding gating strategy and detector voltages. There was a significant amount of heterogeneity in the tumor samples, thus a logarithmic setting was used for side scatter in order to display the full range of SSC signals. The CD45+ cells were clearly defined. Thus we determined where the position of putative leukocytes were in the tumor cell scatter plot through back gating analysis of CD45+ staining. Once we identified leukocytes in the tumor cell scatter plot we chose an appropriate forward scatter detector voltage. Our gating strategy was anchored on AlexaFluor700-CD45+ and all subpopulation percentages are expressed as a % of CD45+. A) The gating strategy used for the splenocytes. First we applied a size gate to select for lymphocytes, another size gate to select single cells, followed by a viability gate (Aqua Zombie negative) to exclude dead cells. Live (Aqua Zombie-) cells were gated for CD45+ and these were split into CD11b+ and CD11b- cells. The CD11b+ cells were gated for CD19 and the CD19- cells were further gated to be CD3- based on splenocyte CD3 staining/gating.

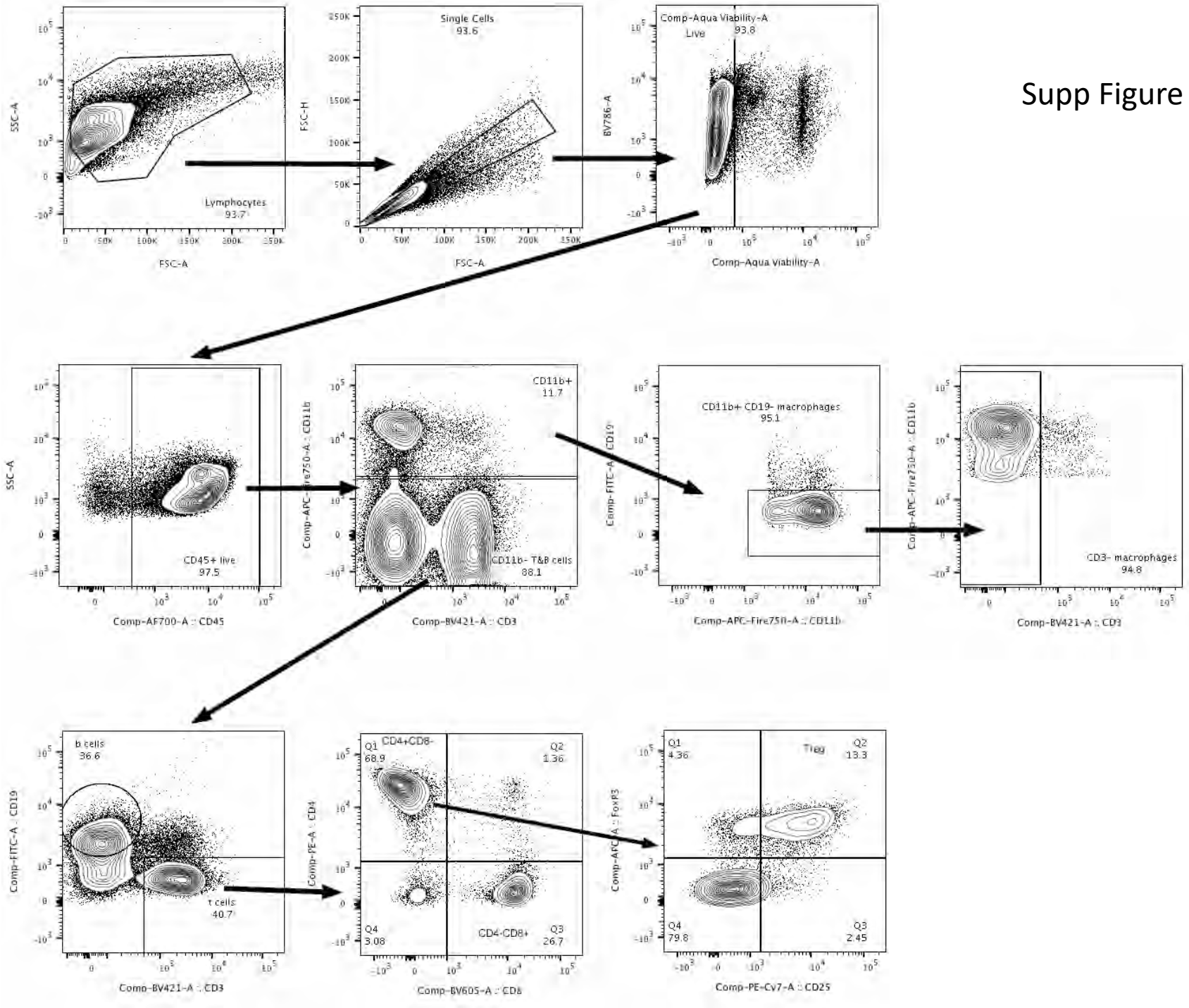
The CD11b- cells (T&B cells) were gated for CD3-CD19+ (b-cells) and CD3+CD19- cells. The CD3+CD11b-CD19- cells were gated into CD4+CD8- and CD4-CD8+ T cells. The CD4+ T cells were then gated for CD25 and FoxP3 to select CD4+CD25+FoxP3+ cells (Tregs). B) The gating strategy used for the splenocytes was applied to the tumor cells with the following differences. A time gate was initially applied to exclude any electronic noise (not shown, not required for all tumors). The color dot plots show the lymphocyte gate on the forward vs side scatter plot with back gating of CD45+ live cells, CD45+CD11b-CD19-CD3+CD4+ live cells, CD45+CD11b-CD19-CD3+CD8+ live cells, CD45+CD11b+CD19- macrophages and CD45+CD11b-CD3-CD19+ b cells. The detection of CD3+ cells in tumor samples had poor resolution and varied between tumors. Thus BV421-CD3^{lo} and BV421-CD3^{hi} cells were all classified as CD3+, and this gate was determined based on splenocyte gating and other markers like CD19.

Supp Fig. 2. RENCA-luc cell viability is reduced by KPT-9274 in a dose-dependent manner. RENCA-luc cells were plated in 96-well plates (1.4x10^3/well), and the next day were treated with varying doses of KPT-9274 for three days then assayed using MTT (viability). Data are means ± SEM (n=8) and representative of three experiments. *P<0.02 compared with 0 μM (Students t-test).

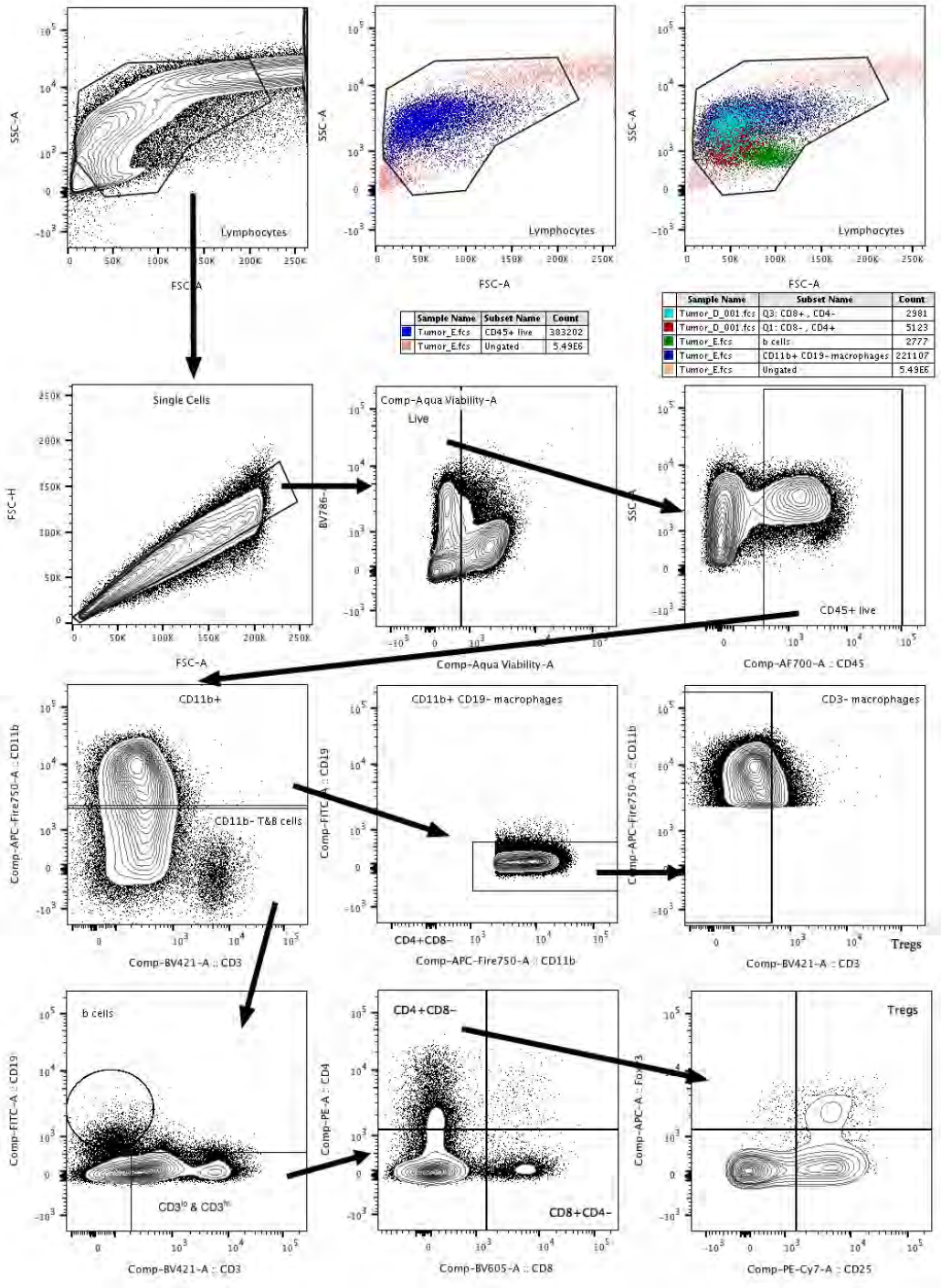
Supp Fig. 3. Subcutaneous measurements of tumor growth of RENCA-luc cells in 10-week-old male Balb/cJ mice over the 21 days of treatment. RENCA-luc cells (250,000) were injected subcutaneously in 30% matrigel and treatments started 10 days after injection. Each line is the growth of one tumor. PD1 = anti-mouse PD1 antibody. Control = isotype control.

Supp Fig. 4. Sections of Balb/c RENCA tumors excised 21 days after treatment with combinations of KPT-9274 and/or anti-PD1 antibody. Tissues were formalin-fixed and parrafin-embedded, sectioned and stained with hematoxylin and eosin. Sections of the tumors demonstrated extensive areas of necrosis admixed with sheets of viable tumor cells.

Supp Fig. 5. KPT-9274 decreased phosphorylation of β-catenin and total PAK4 expression. Protein was extracted from RENCA tumors in mice treated with PD1 antibody (Anti-PD1), KPT-9274 or a combination of both (n=6 per treatment group). Each lane contains the protein from one tumor/mouse. Proteins were immunoblotted for phospho (P)-β-catenin, PAK4 and β-actin. Vehicle, mice received KPT-9274 vehicle.

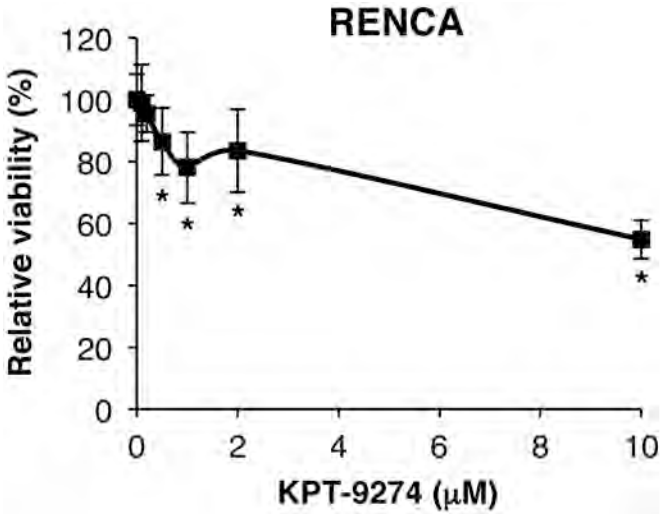


Supp Figure 1A

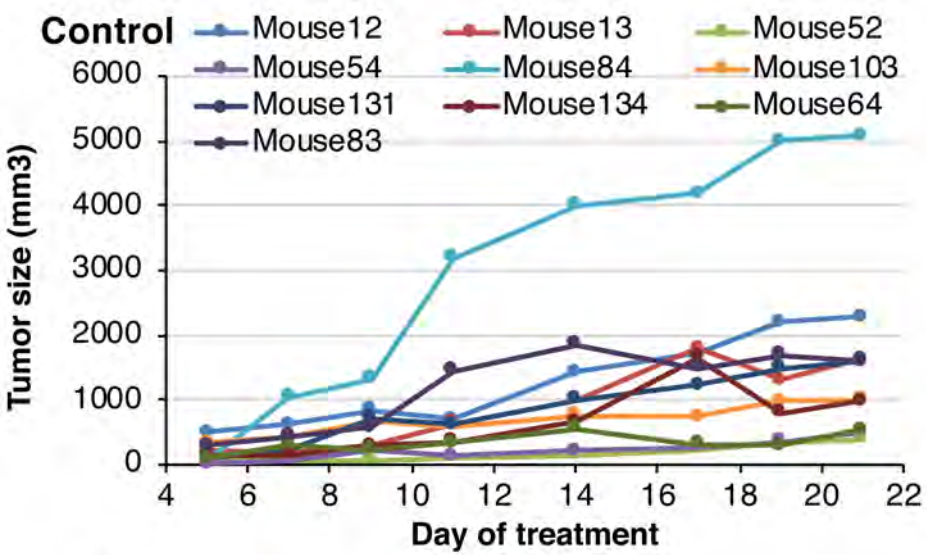


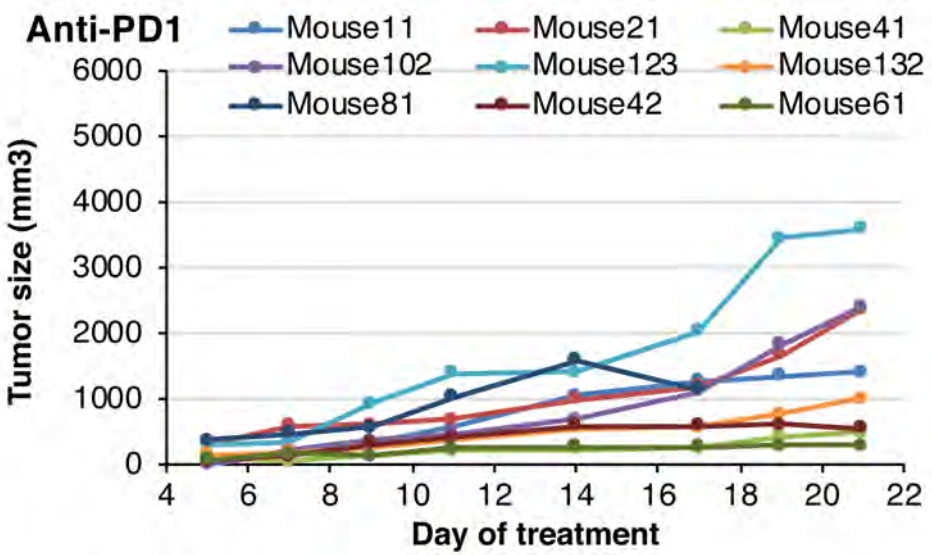
Supp Figure 1B

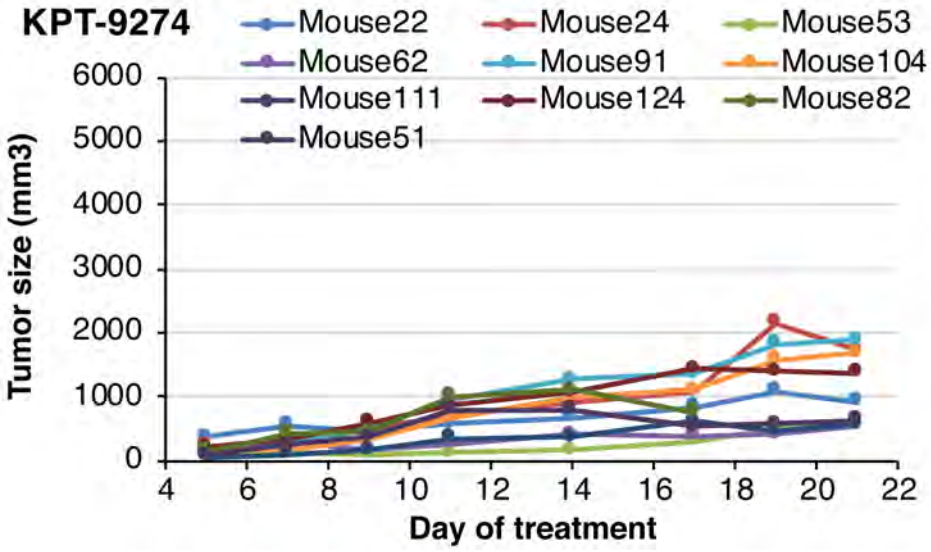
Supp Figure 2

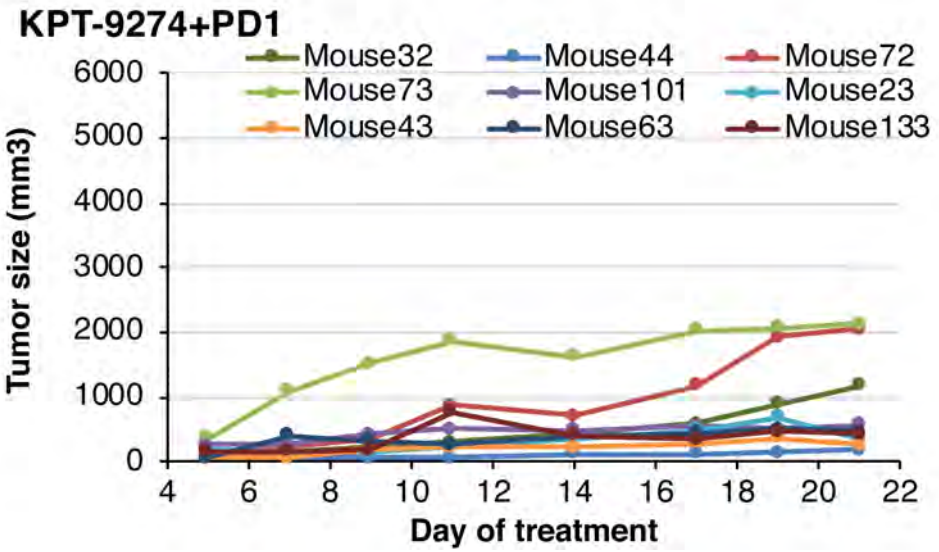


Supp Figure 3

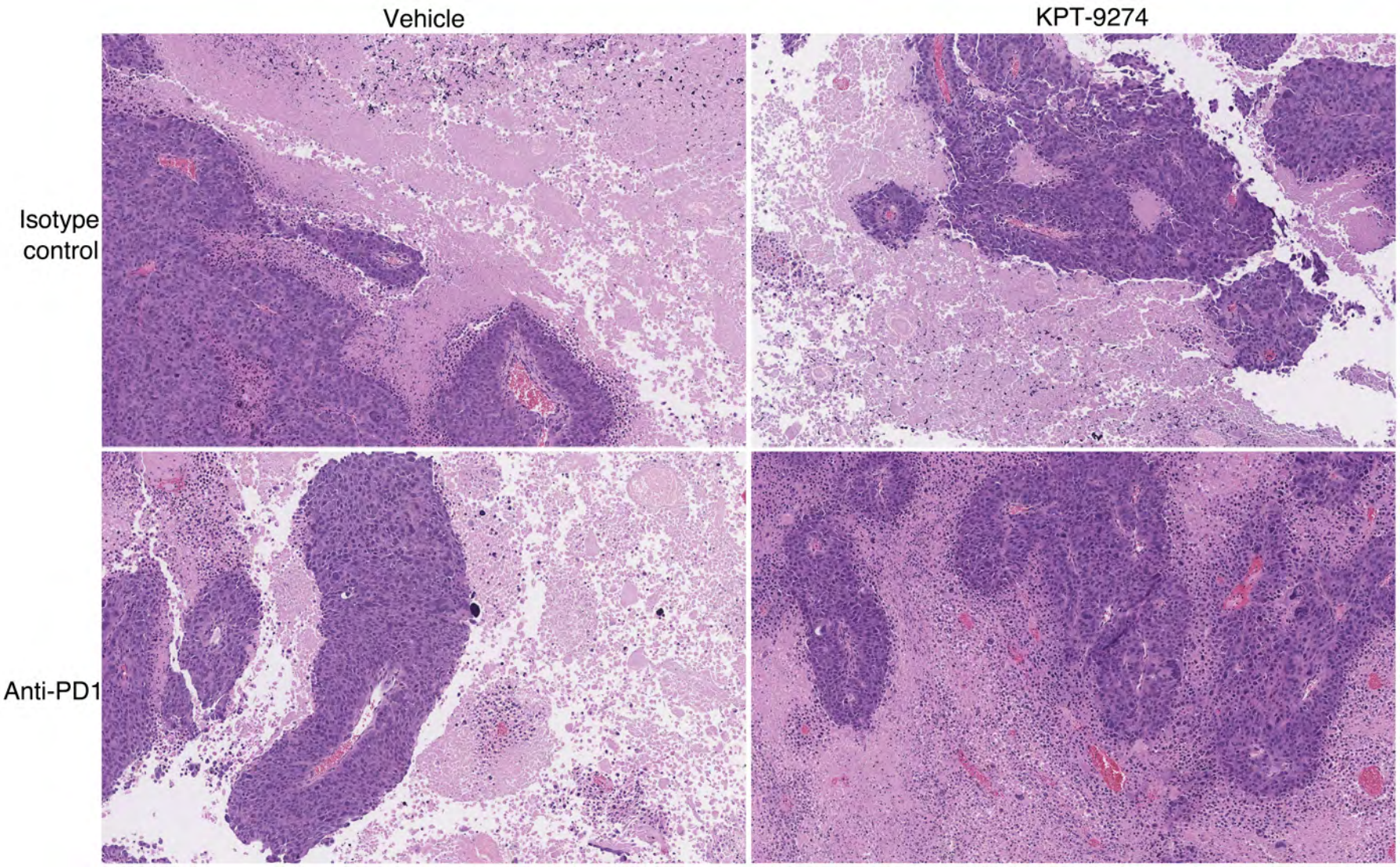








Supp Figure 4



Supp Figure 5