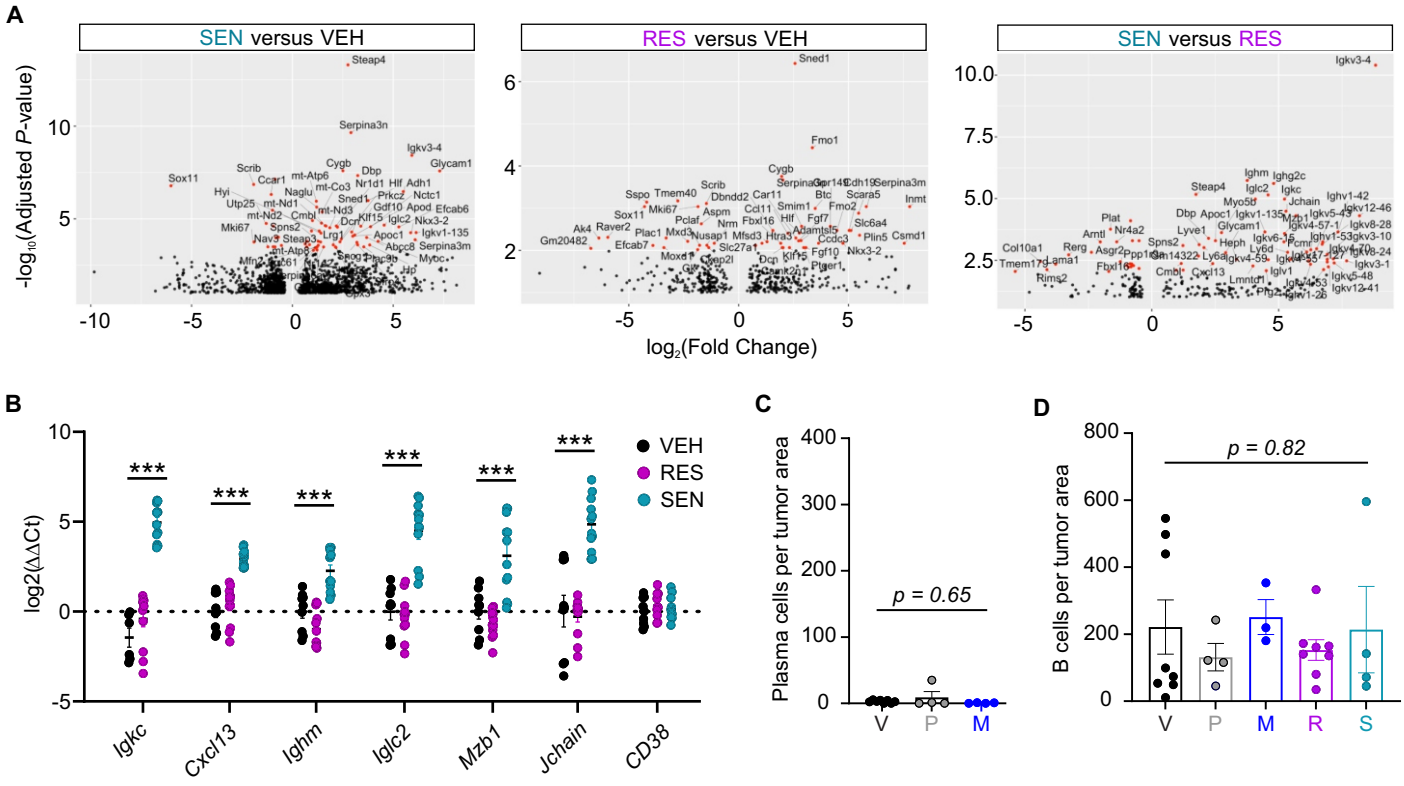
Supplementary Figure S5



Supplementary Figure S5. Evaluation of differentially expressed genes (DEGs) and tumor infiltrating immune cells (plasma cells and B cells) in drug-treated *de novo* MPNSTs. Wild-type mice bearing *de novo* MPNSTs were initiated by CRISPR editing of *Nf1/Ink4a/Arf* in the sciatic nerve. Once tumors reached ~250 mm^3, mice were treated daily with vehicle (V), 100 mg/kg palbociclib (P), 1 mg/kg mirdametinib (M), or the combination of palbociclib plus mirdametinib (R, drug-resistant tumors harvested at endpoint; S, drug-sensitive tumors harvested during response phase). (**A**) Volcano plots comparing significant DEGs in (**left**) drug-sensitive (SEN) MPNSTs treated with the combination versus vehicle (VEH) control treated tumors, (**middle**) drug-resistant (RES) MPNSTs that eventually grew following sustained treatment with the combination versus VEH treated control tumors, and (**right**) SEN versus RES tumors. (**B**) Quantitative RT-PCR analyses performed on tumor samples from each treatment group (VEH, RES, and SEN). Select genes were chosen to validate the "Immune" GO signature displayed in Figure 6C. Relative mRNA expression was calculated using the delta delta CT method. The *CD38* gene was unaltered in the samples by RNAseq analyses and was evaluated in these assays as a comparative control to the other genes, which all displayed upregulation in SEN tumors, as expected. *P* value, One-way ANOVA with Tukey's correction for multiple comparisons. Error Bars, SEM. (***, $P < 0.001$). (**C**) Quantification of plasma cells (stained positive for kappa light chain) per mm^2 of tumor area normalized to V. Note: Quantification for R and S tumors shown in main Figure 5. (**D**) Quantification of B220+ B cells per mm^2 of tumor area for the indicated treatments, including palbociclib or mirdametinib alone. Error bars, SEM. No statistical differences were observed for any comparisons (examined by One-way ANOVA with Tukey's correction for multiple comparisons).