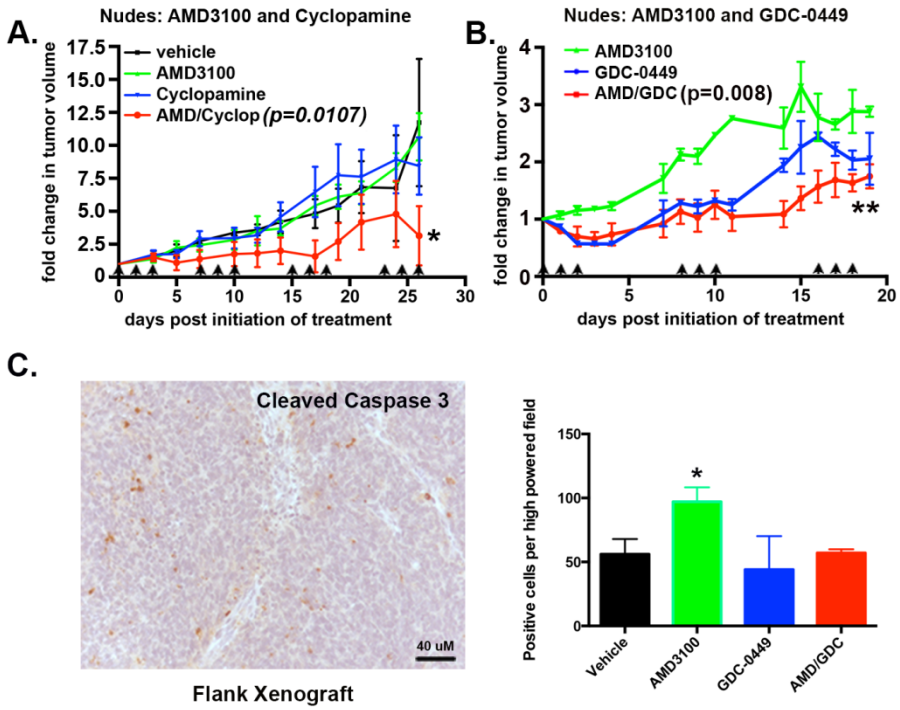
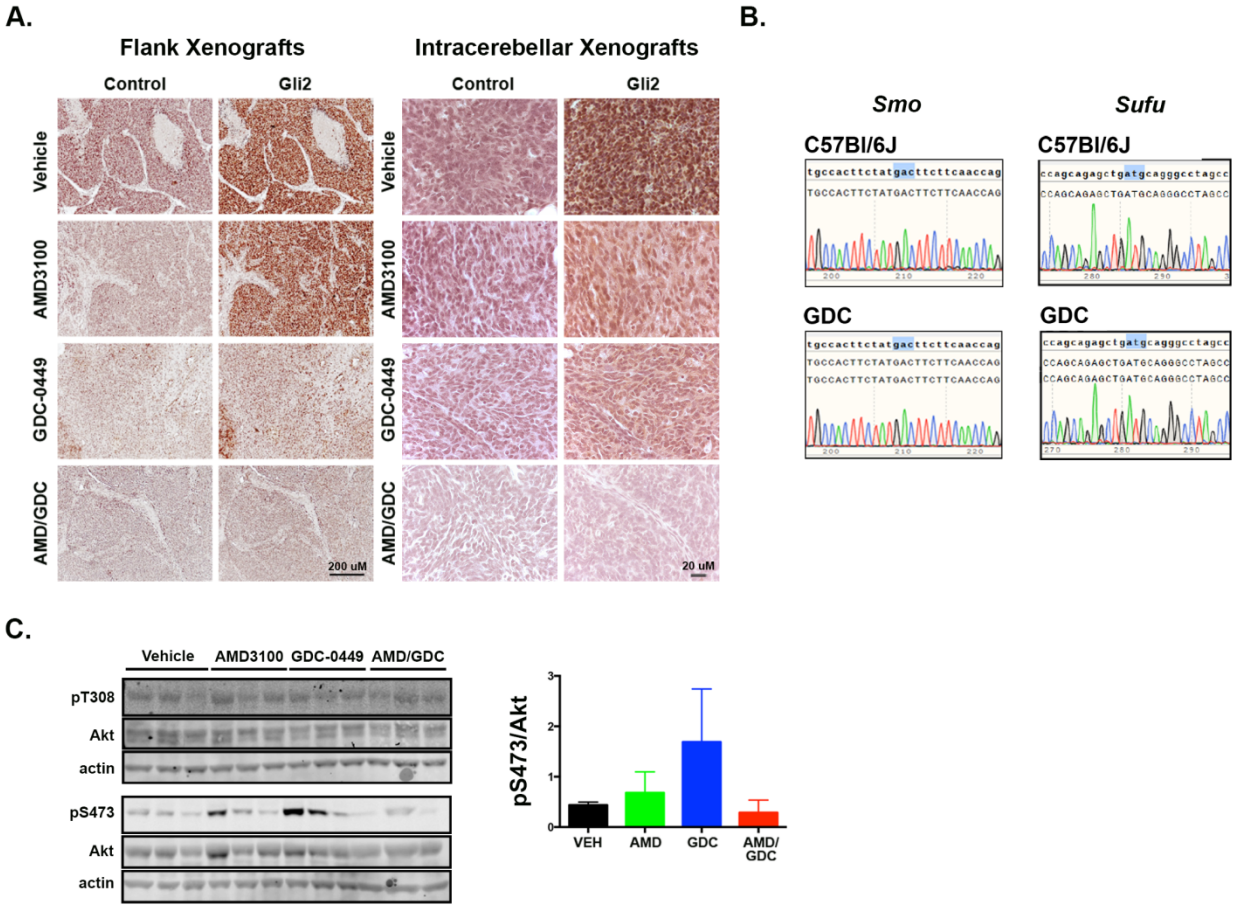
Ward *et al.* Supplemental Figure 1



Supplemental Figure 1: Flank Xenografts in Nude mice. (**A**) SmoA1 cells isolated from three spontaneous tumors were used to generate NCRNU nude mice bearing flank xenografts. Tumors were allowed to grow until at least 10mm by 10mm in size prior to separation into one of four treatment groups (vehicle, AMD3100, cyclopamine, and AMD and cyclopamine combined, n=3 mice/treatment group). Mice were treated daily for three days (arrowheads) followed by five days without treatment, and this schedule was repeated four times prior to tumor harvest. Tumor size was measured daily using digital calipers in three dimensions (width, length and height) and data is represented as fold change from tumor size at day 0. Tumors treated with AMD in combination with cyclopamine were significantly smaller than other groups (p=0.0107). **(B)** Similarly prepared nude mouse SmoA1 xenografts were treated with AMD3100 or GDC-0449 alone or the two drugs in combination for three days followed by a period of no drug. Tumors treated with AMD3100 did not respond to treatment, while tumors treated with GDC-0449 responded to active dosing but regrew when drug was removed. Dual-treated tumors grew significantly less than tumors treated with GDC-0449 alone (p=0.008 comparing GDC-0449 treated tumors and AMD3100/GDC-0449 treated tumors, 2-way ANOVA). (**C**) A representative image of cleaved caspase 3 immunostaining and accompanying quantification of numbers of positive cells per field (n=3 for all treatment groups). *p<0.05 as determined by *t*-test compared to vehicle controls. Scale bar = 40 microns.

Ward *et al.* Supplemental Figure 2



Supplemental Figure 2: Mechanisms of resistance. (**A**) Gli2 expression was examined in treated flank and intracerebellar tumor tissue. GDC-0449 alone reduced Gli2 expression compared to vehicle controls and AMD3100 treated tumors. Combined AMD3100 and GDC-0449 treatment was associated with the greatest reduction in Gli2 expression. Shown are matched no primary control and Gli2 antibody stained sections. Scale bars = 200 microns (flank xenografts) and 20 microns (Intracerebellar xenografts). (**B**) Tumor genomic DNA was analyzed for *Smoothened* (*Smo*) ligand pocket domain mutations by sequencing and *Sufu*. Representative traces for each analysis are shown for the GDC-0449 treated group and the parental mouse strain (C57Bl/6J). (**C**) Phosphorylation of threonine 308 (pT308) and serine 473 (pS473) of Akt was measured by Western blot. Density of bands corresponding to pT308, pS473, total Akt and actin were measured and the ratio of pAkt to Akt was calculated. Presented is the ratio of pS473/total Akt.