Supplemental Figure Legends

Fig. S1. MRK003 slows growth of HSR-GBM1 XG2 glioblastoma xenografts. In animals treated with MRK003 or vehicle and then sacrificed after 5 doses, mean tumor growth as measured by luciferase imaging was 47% slower in animals treated with oral GSI than in controls ($p<0.05$).

Fig. S2. Changes to vascular factors were assessed in the HSR-GBM1 XG2 xenografts. A, Antibodies for murine PECAM-1(CD31) were used to highlight vascular profiles in treated and untreated tumors, revealing equal or greater numbers of vessels in tumors following Notch blockade (Original Magnification 400X). B, Counting PECAM1 positive vascular profiles in multiple fields of each tumor showed a non-significant ($p = 0.08$) increase in the vascular density in xenografts treated with MRK003. C, Human VEGF mRNA levels were analyzed in the 5 vehicle and 7 MRK003 treated HSR-GBM1 XG2 tumors, and a non-significant 2-fold induction was noted in this key angiogenic factor following Notch blockade.

Fig. S3. A, Cell cycle analysis was performed in triplicate on HSR-GBM1 XG2-derived neurospheres and analyzed on a Guava PCA. No significant differences were detected between cells derived from MRK003 or vehicle treated xenografts, suggesting that changes in tumor clonogenicity and growth were not due to altered cell cycle dynamics. B, To determine if significant numbers of murine cells were present in the HSR-GBM1 XG2 derived neurosphere cultures, cytospins of passage 2 cells were stained with Human specific anti-Nuclei antibody (1:100, MAB1281, Millipore, MA). The HSR-GBM1 parent line was included as an additional control. Almost all of the cells in each of the three cultures were positive for the human specific marker. The small number of negative cells was similar in parental cultures (in which murine contamination is not possible) to those derived from xenografts.