marker Olig2. **(g-h and o-p)** Expression of the neural progenitor cell marker Nestin. Scale bars represent 200 µm.

Figure 6. All gliomas demonstrate MAPK pathway activation and high levels of proliferation. (A-D) Confirmation of MAPK activation. IHC for P-Erk confirmed MAPK activation in all gliomas induced with either KRas<sup>G12D</sup> or BRAF<sup>V600E</sup>. (e-h) Analysis of tumor vasculature. IHC for the endothelial cell marker endomucin highlights the highly abnormal vasculature of Akt/KRas<sup>G12D</sup> gliomas compared with the other tumor samples. (i-l) Assessment of mitotic activity. IHC for the cell division marker Ki67 demonstrates that all of the tumors are highly proliferative. Scale bars represent 200 μm.

Figure S1. Schematic representation of the viral vector used in this study. This vector is Gateway compatible to allow for the easy insertion of experimental sequences. LTR, long terminal repeat; Ψ, packaging signal; SD, splice donor; SA, splice acceptor.

Figure S2. BRAF expression in GBM cell lines and mouse Astrocytes. Analysis of BRAF expression in Human GBM cell lines and mouse astrocyte cell lines infected with RCASBP(A)BRAF<sup>V600E</sup> and Cre revealed BRAF expression to be at lower or similar levels to those seen in the 9 Human GBM cell lines analyzed. Lane 1) Mouse astrocytes infected with RCASBP(A)BRAF<sup>V600E</sup> and Cre; Lane 2) Mouse astrocytes infected with RCASBP(A)GFP; Lanes 3-11) Human GBM cell lines. Lane 3) U87; Lane 4) LN18; Lane 5) SNB19; Lane 6) TG98; Lane 7) SF295; Lane 8) SF268; Lane 9) LN229: Lane 10) U251; and Lane 11) U373.

Figure S3. KRas or BRAF activation induces anchorage-independent growth of astrocytes in vitro when combined with Ink4a/Arf loss or Akt activation. Astrocytes from N-TVA/Ink4a/Arflox/lox mice were infected with viruses containing the indicated genes to evaluate

the ability of  $KRas^{G12D}$  and  $BRAF^{V600E}$  to induce anchorage independent growth in soft agar in combination with Cre or Akt. The astrocytes were also infected with RCASBP(A)GFP as control. Colony formation was assessed after 7 days by counting ten fields at 100 x magnification. Each condition was performed in triplicate and the data represent mean  $\pm$  SE. Representative images are shown.

Figure S4. BRAFV600E tumors express the glial marker S100 in the context of Akt activation but not in the context of *Ink4a/Arf* loss. (a) BRAF $^{V600E}$  + Akt (b) BRAF $^{V600E}$  + Cre. Scale bars represent 200  $\mu$ m.

Figure S5. BRAFV600E tumors do not express the neuroendocrine cell marker Synaptophysin. (a) BRAF $^{V600E}$  + Akt (b) BRAF $^{V600E}$  + Cre. Scale bars represent 200  $\mu$ m.