**Supplemental Data**

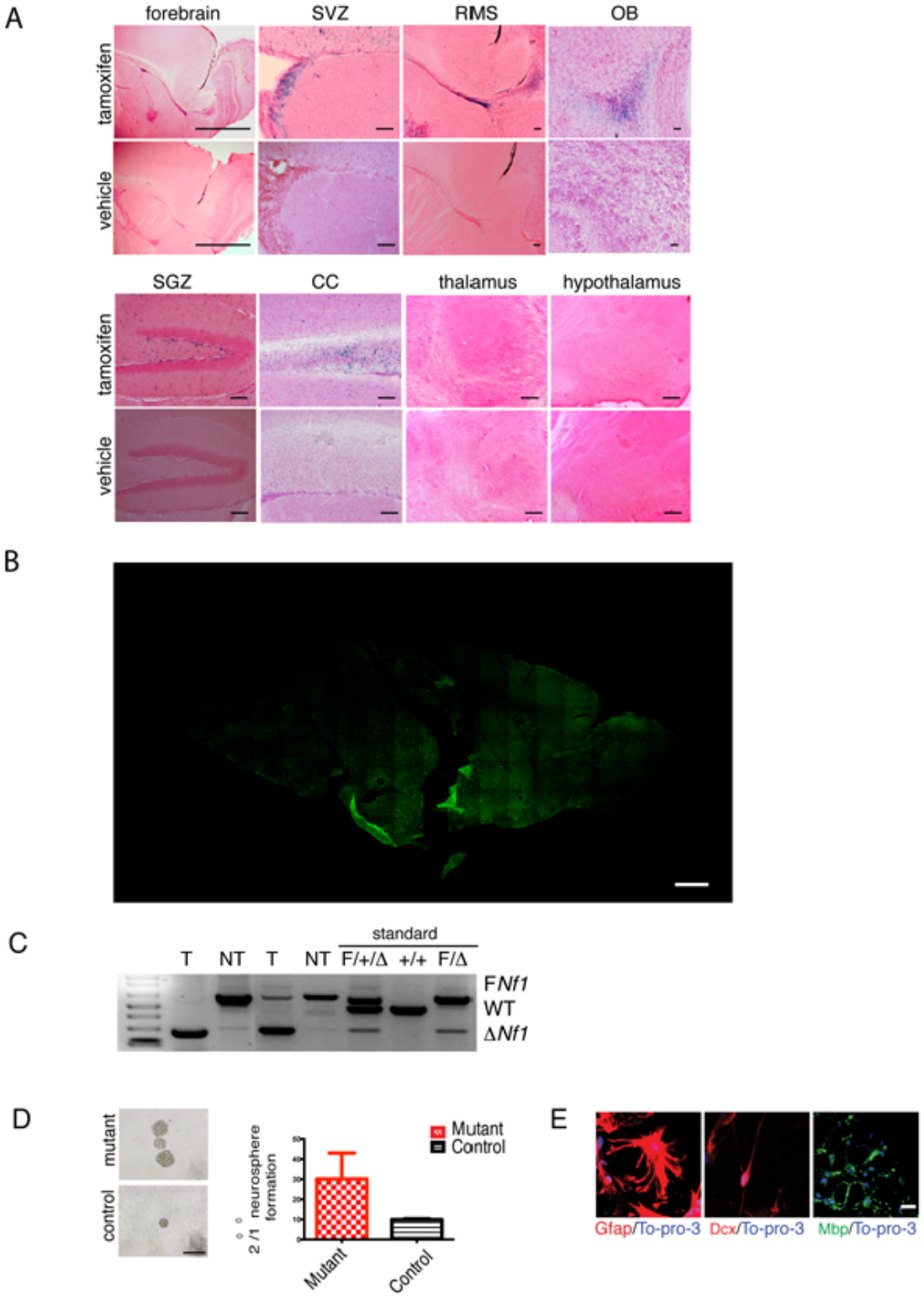

**Figure S1, related to Figure 1. *Ascl1-creER$^{TM}$* reporter expression and AscNP tumor genotypes and behavior in culture.** (**A**) X-gal staining of *Ascl1-creER$^{TM}$;R26-stop-lacZ* mouse brains either treated with tamoxifen or vehicle two weeks post-induction showing the pattern of recombination in the SVZ-RMS-OB, SGZ of the dentate gyrus, corpus callosum (CC), thalamus and hypothalamus. Scale bars: forebrain, 2 mm; all other sub-panels, 200 μm. (**B**) Whole brain immunostaining for YFP of an *Ascl1-creER$^{TM}$;R26-stop-YFP* reporter mouse induced at 4 weeks of age and analyzed 1 month post-induction. Scale bar: 1 mm. (**C**) Genotyping of AscNP tumor (T) and non-tumor (NT) tissue for *Nf1* alleles (F=floxed, Δ=recombined, and +=WT=wild type alleles). (**D**) Tumors from symptomatic mice (n=3 mutants, 3 controls) were dissected, cultured in serum-free suspension conditions, and grown as neurospheres. AscNP tumorspheres and SVZ control neurospheres were assayed for self-renewal. Scale bar: 1 mm. p= 0.0461. Error bar denotes mean ± SEM. (**E**) Tumor sphere cells differentiate into Glial fibrillary acidic protein-expressing astrocytes, Doublecortin-expressing neurons and Myelin basic protein-expressing oligodendrocytes. Scale bar: 20 μm.

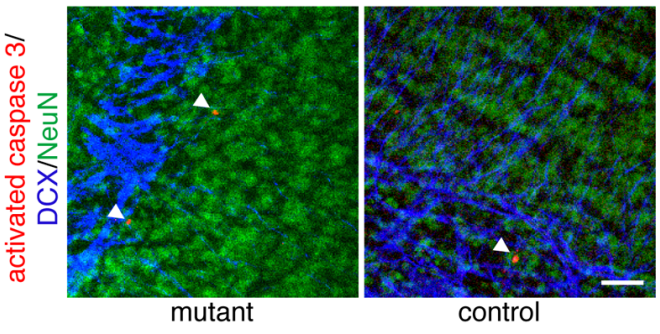


**Figure S2, related to Figure 2. Apoptosis in Pre-tumorigenic AscNP Olfactory Bulb.** Ascl1 mutants and controls were analyzed at 4 months post-induction for apoptosis in the olfactory bulb by immunostaining for activated caspase 3. Scale bar: 50 μm.

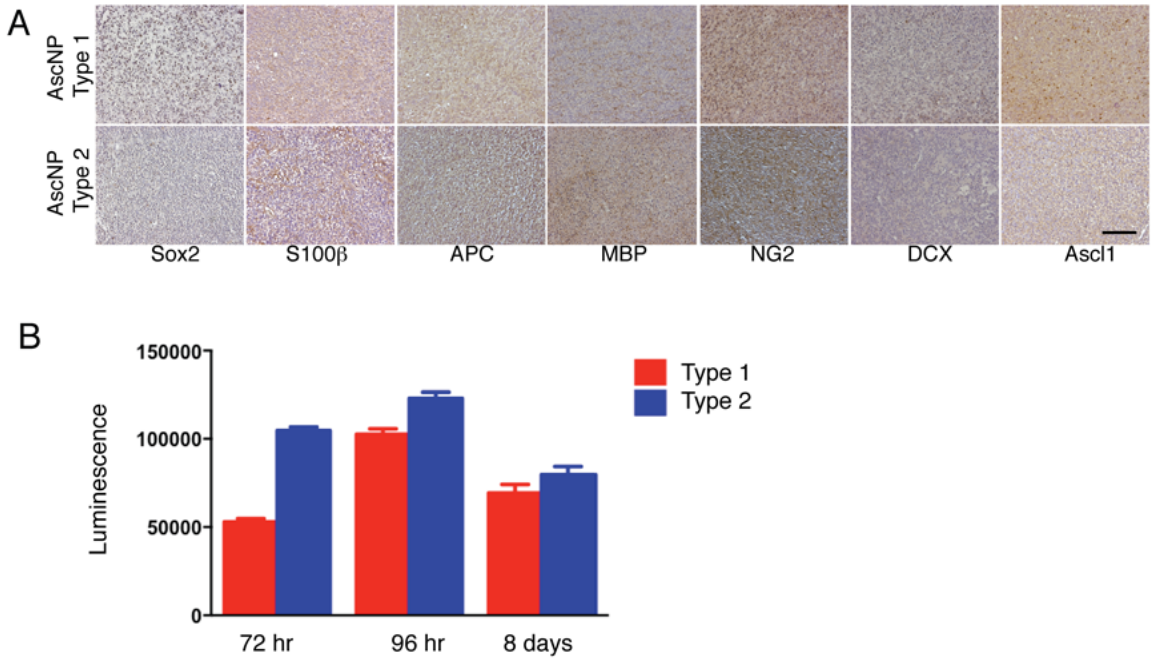


**Figure S3, related to Figure 3. Characterization of Ascl1-creER Type 1 and Type 2 tumors and Growth in Culture. (A)** Immunohistochemical analysis of AscNP Type 1 and Type 2 tumors using lineage markers. Scale bar: 200 μm. (**B**) ATP luminescence assay was used to determine ATP content (a surrogate marker of cell activity) in AscNP Type 1 vs. Type 2 tumor cells in culture derived from AscNP mice. n=2 independent Type 1 and Type 2 tumor cell lines each. 72 hr, $p<.0001$; 96 hr, $p<0.0001$; 8 days, $p=0.1306$). All error bars denote mean $\pm$ SEM.

Table S1, related to Figure 5. Differentially Expressed Genes between AscNP Model Tumors vs. Controls. Provided as an Excel File.

Table S2, related to Figure 5. Differentially Expressed Genes between AscNP Type 1 vs. Type 2 Tumors. Provided as an Excel File.

Table S3, related to Figure 5. Gene Ontology Analysis of Differentially Expressed Genes between AscNP Type 1 vs. Type 2 Tumors. Provided as an Excel File.

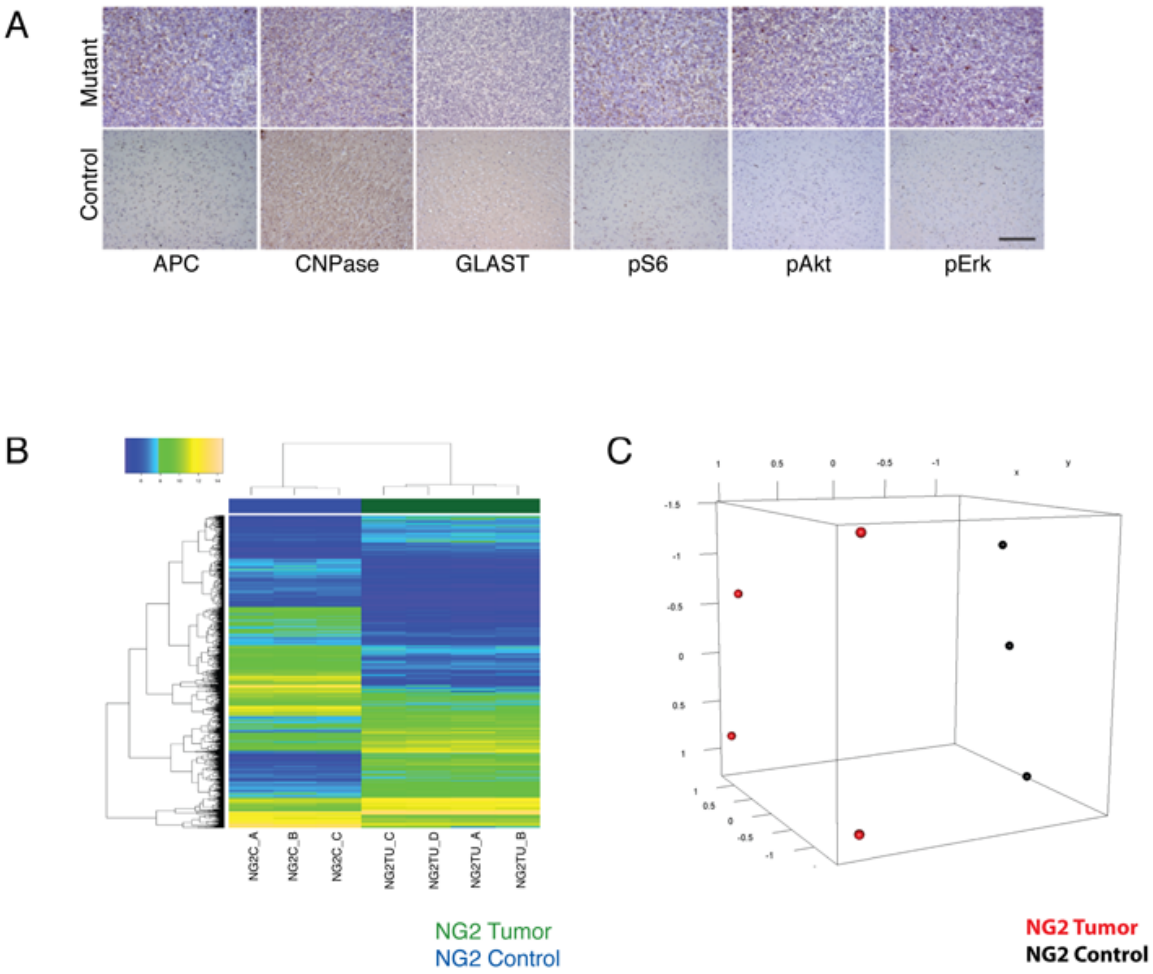


**Figure S4, related to Figure 6. Characterization of NG2NPP tumors.** (**A**) Immunohistochemisty of NG2NPP tumors using different markers. Scale bar: 200 μm. (**B**) Heat map showing differentially expressed genes between NG2NPP tumors and corresponding controls. (**C**) Locally Linear Embedding (LLE) dimension reduction analysis showing differentially expressed genes between NG2NPP tumors and controls projected in 3D space.

Table S4, related to Figure 6. Gene Set Enrichment Analysis (GSEA) of NG2NPP Model Tumors Compared to AscNP Type 1 and Type 2 tumors. Provided as an Excel File.

Table S5, related to Figure 6. AscNP Type 1 and Type 2 Gene Sets for GSEA. Provided as an Excel File.

Table S6, related to Figure 7. Gene Set Enrichment Analysis of Tumor Suppressor Mouse Models. Provided as an Excel File.

Table S7, related to Figure 7. Differentially Expressed Genes between SVZ NSCs vs. OPCs (Beckervordersandforth et al., 2010). Provided as an Excel File.

Table S8, related to Figure 7. Top 310 Genes With Highest Differential Gene Expression. Provided as an Excel File.

## Supplemental Experimental Procedures

### *Mouse Experiments*

All mouse models were maintained on a mixed 129Svj/C57Bl6/B6CBA background. Tumor suppressor floxed mice containing the *Ascl1-creER$^{TM}$*, *NG2-creER$^{TM}$* or *Nestin-creER$^{T2}$* transgenes were administered with tamoxifen (Sigma) or vehicle (9:1, sunflower oil: ethanol mixture) by oral gavage at a working concentration of 50 mg/mL. Four-week-old AscNP mutant mice (*Ascl1-creER$^{TM}$;Nf1$^{flox/flox}$;Trp53$^{flox/flox}$* or *Ascl1-creER$^{TM}$;Nf1$^{flox/flox}$;Trp53$^{flox/-}$*) were administered with 500 mg/kg tamoxifen once a day as a single dose or for 2 consecutive days. An additional cohort of AscNPP (*Ascl1-creER$^{TM}$;Nf1$^{flox/flox}$;Trp53$^{flox/flox}$;Pten$^{flox/+}$*) mice were induced using 83.8 mg/kg tamoxifen twice a day for 5 consecutive days. NG2NPP (*NG2-creER$^{TM}$;Nf1$^{flox/flox}$;Trp53$^{flox/flox}$;Pten$^{flox/+}$* or *NG2-creER$^{TM}$;Nf1$^{flox/+}$;Trp53$^{flox/flox}$;Pten$^{flox/+}$*) or NesNPP (*Nestin-creER$^{T2}$;Nf1$^{flox/+}$;Trp53$^{flox/flox}$;Pten$^{flox/+}$*) model mice were administered with tamoxifen using the double dose regimen. Cre-negative conditional mice given tamoxifen, or mutant mice given vehicle were used as controls. BrdU (5-bromo-2'-deoxyuridine) pulsing was done at a dose of 100 mg/kg using 10 mg/mL working BrdU solution injected intraperitoneally every 2 hours for 5 doses done 24 hours prior to perfusion.

### *Histology and Tumor Analysis*

Mice were perfused and fixed with 4% paraformaldehyde. Five-µm paraffin-embedded sections were cut and every fifth slide was stained with H&E. Brains used for X-gal staining were post-fixed in 2% PFA overnight. Fifty-µm vibratome sections were stained in X-gal solution, and sections were counterstained by nuclear fast red, as described previously (Kwon et al., 2008).

***Immunohistochemistry***

Paraffin sections were deparaffinized, rehydrated, and subjected to citrate-based antigen retrieval. In other cases, 50-mm vibratome sections were used for staining. Primary antibodies against the following were used as follows: Gfap (DAKO, 1:1000; BD Biosciences, 1:200), Ki67 (Novocastra, 1:1000), Nestin (BD Biosciences, 1:100), Olig2 (Chemicon, 1:1000), Sox2 (Santa Cruz, 1:200), β-gal (ICN, 1:1000), Doublecortin (Santa Cruz, 1:200), NeuN (Chemicon, 1:500), Mbp (Sternberger, 1:200), GFP (Chemicon, 1:1000), Pdgfrα (Santa Cruz, 1:100), NG2 (Millipore, 1:1000), CNPase (Millipore, 1:1000), APC (Calbiochem, 1:200), Ascl1 (Kim et al., 2007;1:100), pAkt (Cell Signaling, 1:100), pErk (Cell Signaling, 1:100) and pS6 (Cell Signaling, 1:100). We used both immunofluorescence staining using Cy2, Cy3, or Cy5 (Jackson Labs, 1:400) and biotin-streptavidin-Alexa Fluor-conjugated secondary antibodies (Molecular Probes, 1:1000), as well as horseradish peroxidase-based Vectastain ABC Kit (Vector Lab). Sections were examined using optical, fluorescence and confocal microscopy (Olympus and Zeiss).

***Tissue Culture and Transplantations***

Harvested tissues were minced into single cell suspension and plated onto low attachment plates (Corning), where they were grown as neurospheres in serum free media with basic fibroblast growth factor, epidermal growth factor and B27 without Vitamin A (Gibco) in low O2 conditions. Glioma stem cells were maintained using low attachment plates or polylysine/laminin-coated plates.

For transplantation experiments, 4 to 8-week-old female *nu/nu* mice (Jackson Labs) were anesthetized using Dormitor/Ketamine cocktail injected intraperitoneally and mounted onto a stereotactic frame. Craniotomy was performed and 2 x $10^5$ cells/5 uL were directly injected into

the dorsal striatum (coordinates: 0 AP, 1.4 ML, 2.3 DV with respect to the bregma). Mice were aged until symptomatic.

***Gene Expression Analysis***

Primary tumor tissue and respective control brains were harvested from symptomatic AscNP, NG2NPP and NesNPP mice and corresponding controls. Tissue samples were flash frozen in liquid nitrogen and RNA extracted using Qiagen Lipid Tissue RNeasy Mini Kit and performed according to manufacturer's instructions. Total RNA was submitted to the UT Southwestern Microarray Core for cDNA synthesis and purification, cRNA synthesis and purification, hybridization, and scanning using IlluminaWG-6 V2 beadchips.

The Illumina BeadArray microarray data was summarized using the Illumina Beadstudio software and then processed in R statistical environment by beadarray package (Dunning et al., 2007). Differentially expressed genes were identified using the limma package. Unsupervised hierarchical clustering and gene expression heatmap were created in R by package gplots (Warnes, 2012). Locally Linear embedding (LLE) Dimension Reduction Analysis was processed by package RDRToolbox (Bartenhagen, 2010). In this study, genes with adjusted p values less than 0.01 and more than 2-fold change were considered as differentially expressed. Color keys in heat maps indicate normalized intensity.

Gene expression profiles of different cell types in mouse brain (GSE9566) were retrieved from the Gene Expression Omnibus (GEO). Gene expression on Affymetix platform was normalized by package gcrma. Batch effects among data from different sources were removed by distance weighted discrimination and fulfilled by package inSilicoMerging. Mouse and human gene expression profiles were merged using official gene symbols by package virtualArray (Heider and Alt, 2013). Gene Set Enrichment Analysis was performed in GSEA software

(Subramanian et al., 2005). To calculate enrichment scores of NG2NPP model tumors, gene sets were defined by differentially expressed genes derived from comparing AscNP model Type 1 and Type 2 tumors to their respective controls. Gene expression profiles of NG2NPP tumors and their respective controls were used as gene list. AscNP Type 1 vs. Type 2 glioma gene profiles were also analyzed for enrichment of human glioblastoma molecular subtype gene sets (Verhaak et al., 2010).

For GSEA of mouse models compared to human cancers, we used the following human genomic data sets: breast cancer (GSE3744), Colon Cancer (GSE41285), Melanoma (GSE3189), Liver cancer (GSE62232), Pancreatic cancer (GSE16515), Medulloblastoma (GSE62600), Ovarian cancer (GSE18520), Prostate cancer (GSE55945), PNET (GSE14295), Astrocytoma Grade II (GSE4290), Astrocytoma Grade III (GSE4290), GBM (GSE4290), Oligodendroglioma Grade II (GSE4290), and Oligodendroglioma Grade III (GSE4290). Gene lists were derived from gene expression profiles of different human tumor types compared to their corresponding controls. Differentially expressed genes defined by comparing *Ascl1-creER*$^{TM}$, *Nestin-creER*$^{T2}$ and *NG2-creER*$^{TM}$ model tumors with normal mouse brain tissue were used as gene sets.

For genomic comparison of the three mouse models, we first analyzed published data from the Gotz group (Beckervordersandforth et al., 2010), comparing gene expression data from SVZ-derived neural stem cells (NSCs) vs. OPCs after removing the batch effect. Using the 1249 differentially expressed genes from this data set, we used unsupervised hierarchical clustering analysis to determine the relationships of AscNP, NesNPP and NG2NPP mice. The heat map was generated using the top 310 genes showing the greatest differential levels with MAD>0.7 among mouse tumors. Gene expression intensity was scaled by subtracting the median intensity

to sample intensity, and subsequently divided by the distance between the $75^{th}$ and $25^{th}$ percentile.

**Supplemental References**

Bartenhagen, C. (2010). RDRToolbox: A package for nonlinear dimension reduction with Isomap and LLE.
Dunning, M. J., Smith, M. L., Ritchie, M. E., and Tavare, S. (2007). beadarray: R classes and methods for Illumina bead-based data. Bioinformatics *23*, 2183-2184.
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