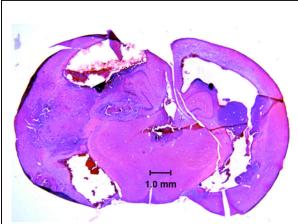
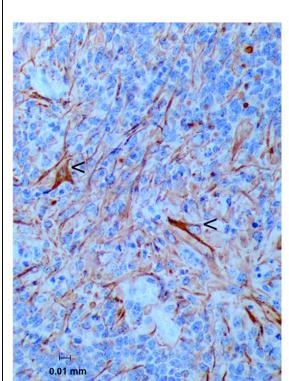
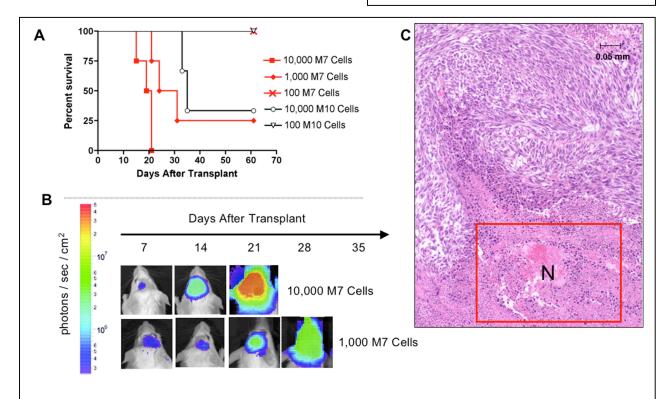
I. Supplementary Figures



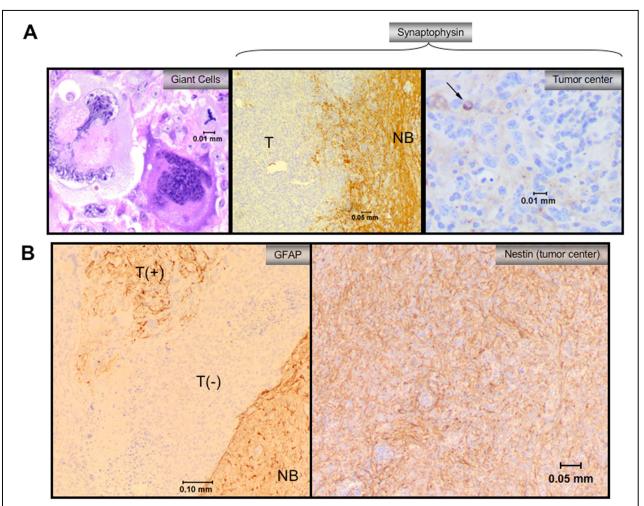
Supplementary Figure 1. H&E stained coronal section of brain from experiment two exhibiting an enlarged right lateral ventricle filled with tumor cells. The left ventricle is also full of tumor.



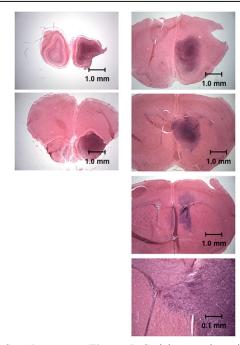
Supplementary Figure 2. 40X magnification of GFAP immunohistochemistry from experiment two. A mixture of well differentiated (empty arrowheads), less differentiated, and undifferentiated cells were often observed in the same tumor mass.



Supplementary Figure 3. (a) The indicated number of M7 and M10 cells were intracerebrally injected into SCID mice (n=3-4/group). Survival for all transplants is shown. (b) Bioluminescence imaging of M7-transplanted mice shown. (c) H&E stain of transplanted M7 cells in the SCID mouse brain. The tumor was histologically indistinguishable from other SV40-LgT/NRAS/Fluc-induced tumors exhibiting some areas of necrosis (red box).



Supplementary Figure 4. (a) Representative images of giant cells from tumors in experiment 14 are shown in the left panel. Other panels show that the bulk tumor mass (T) is essentially negative for the neuronal marker synaptophysin but the normal brain (NB) is positive. However, rarely positive cells were observed in the tumor (arrow). (b) Image in left panel depicts the typical heterogeneity of GFAP expression observed in these tumors. Normal brain can be seen in lower right of image, with very organized GFAP positive astrocytes next to a well demarcated portion of the tumor that is negative for GFAP "T (-)" on the periphery but positive for GFAP in the tumor center "T (+)". In contrast, the entire tumor mass is uniformly positive for nestin.



Supplementary Figure 5. Serial coronal sections of brains from different mice four weeks post transfection. In some animals, focal luminescence was noted between the eyes when viewed caudally (data not shown). Tumor was present in the olfactory bulbs (Top, left column) of these mice and extended into the parenchyma (bottom, left column). Note the contralateral olfactory bulb is unaffected. Tissues harvested four weeks post transfection that demonstrated detectable in vivo bioluminescence always had detectable tumor by H&E stain that typically originated around or near the lateral ventricle (right column). Tumor was present throughout the majority of the cerebrum and occasionally entered the contralateral hemisphere via the corpus callosum (Bottom, right column).



Supplementary Figure 6. Sagittal section of affected brain harvested four weeks post transfection from a different animal that demonstrated focal bioluminescence located between the eyes when viewed caudally. Olfactory bulb is completely replaced by tumor cells. Several other foci are evident (arrowheads), notably in or near the subventricular zone of the lateral ventricle.

II. Supplementary Tables

Supplementary Table I. Grade of GEM Astrocytoma Induced by Oncogene Transfer

Oncogenes	Grade 3	Grade 4	^a Experiment(s) #
SV40-LgT + NRAS GV12	10/13 (77%)	3/13 (23%)	1, 2, 3, 20
AKT + NRAS GV12 + shp53	3/5 (60%)	2/5 (40%)	13
NRAS GV12 + shp53	1/3 (33%)	2/3 (66%)	17
NRAS GV12 +EGFRvIII+shP53	4/7 (57%)	3/7 (43%)	14

^a Refers to mice identified by the experiment number shown in left column of Table I

Supplementary Table II. Map Positions of SB-mediated Integrations

Cell Line	Chromosome	Gene	Location Relative to Gene	Distance (bp)	Transposon Orientation Relative to Gene
M2	1	Cep170	downstream	55585	+
M2	3	Nhedc1	exon 5	NA	-
M2	4	6230409E13Rik	upstream	8579	+
M2	5	Glt1d1	intron 1	NA	-
M2	11	Adra1b	upstream	36848	+
M2	12	Serpina5	upstream	8326	+
M2	14	9030625A04Rik	downstream	101371	-
M2	18	ENSMUSG00000069391	downstream	52477	+
M2	5	Gm444	upstream	2720	+
M7	1	2700049P18Rik	downstream	31879	+
M7	1	Zfp281	upstream	1552	+
M7	1	ENSMUSG00000064860	upstream	46145	-
M7	3	S100a11	intron 2	NA	+
M7	3	Atp11b	Introns 9	NA	+
M7	4	Tie1	downstream	238406	+
M7	4	ENSMUSG00000077214	downstream	141025	-
M7	4	ENSMUSG00000068029	upstream	119035	+
M7	4	4933407E24Rik	upstream	38850	-
M7	5	ENSMUSG00000070220	upstream	379132	-
M7	5	Tmem132d	intron 2	NA	-
M7	6	Grm7	intron 4	NA	+
M7	6	Tnpo3	upstream	25517	+
M7	6	Pde3a	intron 2	NA	-
M7	8	Gm1698	downstream	72588	-
M7	11	Fxr2	upstream	3660	-
M7	11	Olfr1395	upstream	4328	-
M7	12	Ctage5	intron1	NA	+
M7	12	ENSMUSG00000077292	upstream	40219	-
M7	12	Akap6	upstream	11445	-
M7	13	Calm4	upstream	16382	-
M7	14	ENSMUSG00000077636	downstream	197194	+
M7	15	L3mbtl2	intron 3	5758	+
M7	17	ENSMUSG00000071033	upstream	61977	-
M7	19	Prune2	upstream	24641	-
M7	19	E030010A14Rik	intron 1	3463	-
M7	19	Sorbs1	intron 20	NA	+
M7	X	4930447F04Rik	downstream	55143	+
M10	1	ENSMUSG00000077207	downstream	13892	-
M10	2	Ehf	downstream	15459	+
M10	2	Phf21a	intron 4	NA	+
M10	2	A2AJX4_MOUSE	intron 4	NA	+
M10	2	Chac1	upstream	2794	+
M10	3	Trpc3	upstream	103101	-
M10	4	4930473A06Rik	intron 6	NA	+
M10	4	Ppap2b	downstream	120325	+
					-
M10	16	Zbtb20	intron 10	NA	-