Figure S10. Expression of Six1 in Primary Glioma and TPC Lines and Validation of Six1-Induced Knockdown, Related to Figure 6

(A)Relative quantification of Six1 gene expression using quantitative RT-PCR in $A2B5^+$ glioma tumor progenitor cells (TPCs) derived from primary low- (LG)(n= 4) and high-grade(HG) gliomas (n = 8) and glioma-derived TPC lines (CL) (n= 5),relative to A2B5+cells isolated from non-tumor (NT) adult human brain (n = 4). Gene expression levels were normalized to GAPDH and presented as log2-transformed fold change values ± SEM.
(B)Western blot analysis of Six1 and GAPDH protein expression in nuclear extracts derived from primary gliomas and GBM-derived TPCs lines compared to non-tumor adult human brain (AHB). *AST: Astrocytoma; aAST: anaplastic astrocytoma; OLG: Oligodendroglioma; aOLG: anaplastic oligodendroglioma; GBM: Glioblastoma; GBM-CL: GBM cell lines.*
(C and D) Validation of Six1 silencing on gene (C) and protein (D) levels using, respectively, quantitative RT-PCR and Western blot, in GBM-derived TPCs lines. Gene expression levels were normalized to GAPDH and presented as relative expression to scrambled controls (mean ± SEM). *Cyt: Cytoplasmic; Nuc: Nuclear extracts; KD: Knock-down.* TPCs lines were derived from primary GBM and maintained in serum-free media supplemented with FGF, EGF (20ng/ml) and PDGF (10ng/ml) for less than 10 passages. Values indicated means ± SEM. P-values were calculated using a one-way ANOVA with repeated-measures followed by Tukey post-hoc comparisons with *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
(E) Representative illustration of the number of HNA^+ cells (red) following orthotopic transplantation of untransduced control A2B5+TPCs, and A2B5+ TPCs transduced with control scrambled and SIX1 knock-down (KD) lentiviruses, 6 weeks post-surgery.