Figure S1. Generation and characterization of NPCs carrying oncogenic gene alterations. (A) Expression levels of *PTEN* were confirmed by qPCR in ESC-shPTEN cells. shPTEN1 (shRNA#1) was used for further experiments. (B) Morphology of Ctrl, KRAS^{G13D}-shPTEN, BRAF-shPTEN and BRAF-EGFR-shPTEN ESCs. (C) Expression levels of *PTEN*, *KRAS*, *BRAF* and *EGFR* were confirmed by qPCR in Ctrl, KRAS^{G13D} (transfected with pSIN-EF2-Pur-FLAG-ENNSPC-KRAS), BRAF-shPTEN, KRAS^{G13D}-shPTEN and BRAF-EGFR-shPTEN ESCs. (D) Expression levels of *NANOG*, *OCT4*, *SOX2* were detected by qPCR in ESCs carrying oncogenic gene alterations. Data are presented as the mean \pm SD (n=3). (E) Morphology of Ctrl, KRAS^{G13D}-shPTEN, BRAF-shPTEN, BRAF-EGFR-shPTEN NPCs. (F) Immunofluorescence staining of neural progenitor-specific markers in NPCs carrying oncogenic gene alterations. Scale bar, 50 μ m. *P<0.05, **P<0.01, ***P<0.001 vs. ESC. NPC, neural progenitor cell; q, quantitative; ESC, embryonic stem cell; sh, short hairpin; Ctrl, control; OCT4, octamer-binding transcription factor; NC, negative control; PAX6, paired box 6; NS, not significant.



Figure S2. Migration, clonal expansion and proliferation of NPCs carrying oncogenic gene alterations. (A) Cell Counting Kit-8 assay was performed to detect the cell proliferation ability. (B) Migration of NPCs carrying oncogenic gene alterations was evaluated by Transwell assay. (C) Relative cell migration efficiency was determined in (B). Data are presented as the mean \pm SD (n=3). (D) Clonal expansion analysis in NPCs carrying oncogenic gene alterations. (E) Crystal violet-positive cells were calculated (n=4). Scale bar, 50 μ m. **P<0.01, ***P<0.001 compared with NPC-Ctrl (A, C, E). NPC, neural progenitor cell; OD, optical density; Ctrl, control; sh, short hairpin; NS, not significant.



Figure S3. NPC-KRAS^{G13D} cells are passaged steadily. (A) Expression of *KRAS* was confirmed by qPCR in ESC-KRAS^{G13D}. Expression of (B) *NANOG* and *OCT4* and (C) *PAX6* and *SOX1* genes was detected by qPCR in ESC-KRAS^{G13D}, NPC-KRAS^{G13D}, P1 and NPC-KRAS^{G13D} P6 cells. Data are presented as the mean \pm SD (n=3). ***P<0.001. NPC, neural progenitor cell; q, quantitative; OCT4, octamer-binding transcription factor; PAX6, paired box 6; NS, not significant; P, passage.



Figure S4. Isolation and characterization of GSCs from human glioma cell lines. (A) Sorting of CD133⁺ cells in human glioma cell lines U118MG and U251 by flow cytometry. (B) Morphology of U118MG-GSC and U251-GSC cells. Scale bar, 100 μ m. (C) Expression of *NANOG*, *Nestin*, *CD133* and *SOX2* was detected by quantitative PCR in human glioma cells. Data are presented as the mean \pm SD (n=3). (D) Western blotting detected expression of Nestin and CD133 protein in human glioma cells. GAPDH was used as the loading control. **P<0.01, ***P<0.001. GSC, glioma stem cell.

