Supplementary Information

NANOG regulates glioma stem cells and is essential in vivo acting in a cross-functional network with GLI1 and p53

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Supplementary Figures

Figure S1. NANOG expression as revealed by YAb and KAb.

A) Confocal indirect immunofluorescence localization of NANOG protein in peripheral nuclei (green, arrows) of large GBM-8 gliomaspheres using YAb. Nuclei are counterstained with DAPI (blue). Note that weaker expression levels are detected in cells other than those with strong expression. Bottom panel shows a control staining without primary antibody.

B) Confocal localization of NANOG with KAb in GBM-8 gliomasphere cells. Lower panel shows only the DAPI stain to show that all cells in the upper panel express NANOG.

C) NANOG staining in U87 cells using KAb and confocal microscopy. All cells express NANOG. Bottom: No-primary antibody control.

D) Double confocal indirect immunoflourescence images showing of the lack of co-localization of exogenous FLAG-tagged NANOG (detected with anti-FLAG epitope or KAb), endogenous NANOG (using KAb) and PML (top) or 53BP1 (bottom) foci as indicated.

Scale bar = 20μm (A top, B), 10μm (A bottom, C), 2μm (D).

Figure S2. Requirement of NANOG in vivo in cells previously grown in adherent substrates.

Representative images of dissected whole brains with developed 'red/green' brain tumors after orthotopic xenotransplantation of adherent GBM-8 (A) and GBM-12 (B) cells transduced with control red (RFP^+) plus either control green (GFP^+) or GFP^+/*shNANOG*-expressing lentivectors as indicated. The same samples are shown in each row under visible and fluorescent light, the latter with filters to selectively detect green or red fluorescence. Far right panels show green/red ratios.

Scale bar = 3.5mm (A,B).

Figure S3. NANOG function is epistatic to an active HH-GLI pathway in GBM-12.

Representative images of dissected whole brains with developed 'red/green' brain tumors after orthotopic xenotransplantation of GBM-12 gliomaspheres transduced with control red (RFP^+) plus green (GFP^+) lentivectors expressing different shRNAs as indicated. Each row shows the images of dorsal brains with anterior to the left under visible, green or red fluorescence. Green/red FACS ratios are given in the right column.

Sc ale bar = 3.5mm.