**Supplementary Figure Legends**

**Supplementary Figure S1.** Organoid formation by human 4121 CSCs and genetically engineered mouse RCAS-GFAP-tva/PDGFB glioma cells. Organoids were seeded with either human- (A, B) or mouse-derived (C, D) glioma cells and imaged by phase-contrast microscopy after two weeks.

**Supplementary Figure S2.** Growth of a brain metastasis sample in organoid format. A, Cell viability (alamarBlue) of CCF3108 esophageal adenocarcinoma brain metastasis specimen. 6 replicate cultures were followed longitudinally for 3 weeks following initial isolation from patient biopsy. B, Growth of CCF3108 in organoid format over 5 weeks following patient isolation, scale bar = 400 µm.

**Supplementary Figure S3.** Organoid stem cell gradients are stable over time.A-C, Immunofluorescence imaging of IN528 organoids at the indicated time points. Scale bars = 200 µm.

**Supplementary Figure S4.** SOX2 and OLIG2 expression in tumorspheres and organoids.A-D,Immunofluorescence imaging of 387 tumorspheres for SOX2 and OLIG2 proteins. Scale bars = 50 µm. E,Wide-field mosaic image of SOX2 and OLIG2 immunofluorescence of a 387 organoid. Scale bar = 100 µm.

**Supplementary Figure S5.** Wide-field mosaic image of SOX2 and OLIG2 immunofluorescence in an IN528 organoid. Scale bars = 200 µm.