Cell Reports, Volume *26*

Supplemental Information

Modeling Patient-Derived Glioblastoma with Cerebral Organoids

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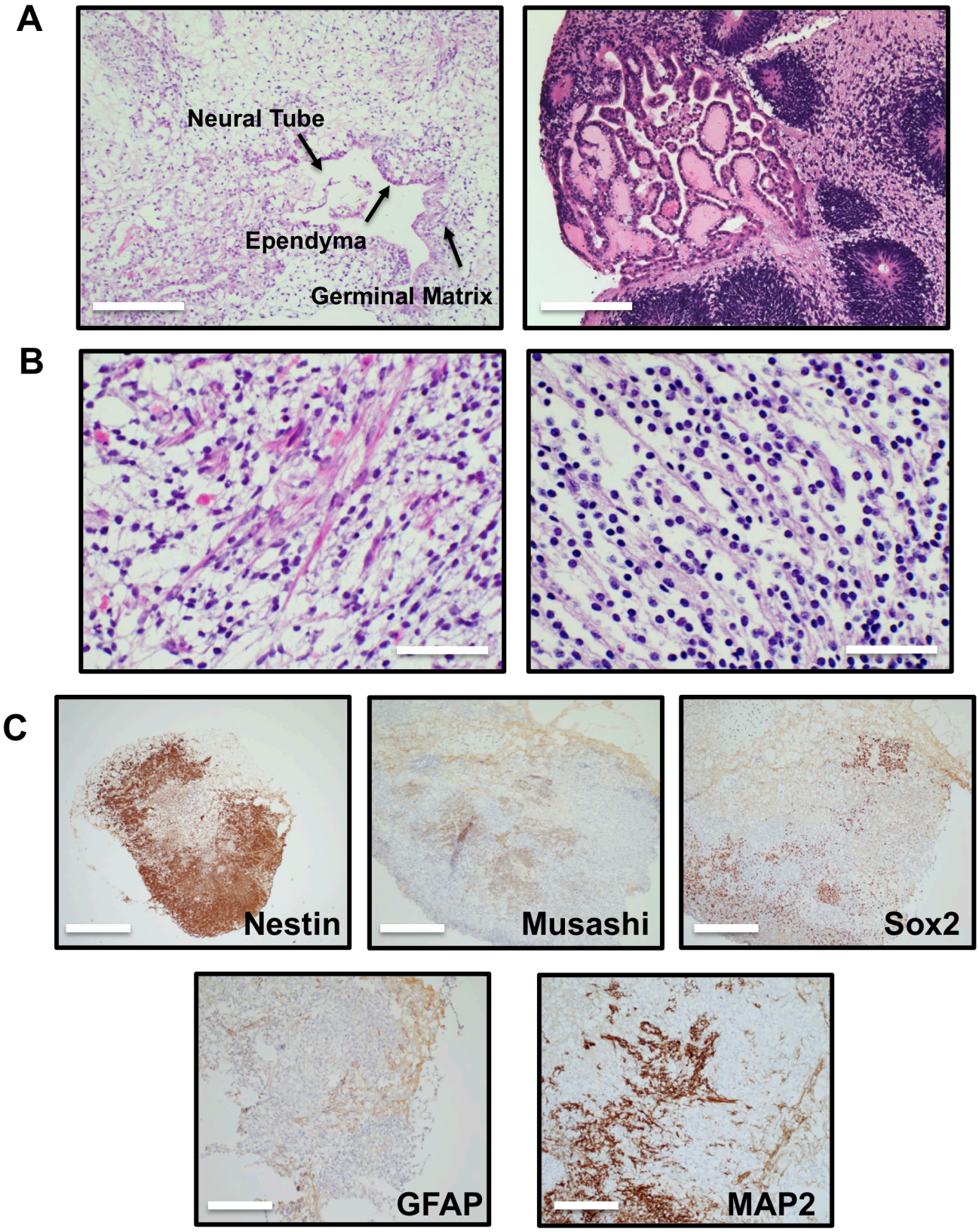

Figure S1: Morphological and Immunohistochemical Analysis Reveals Neural Stem Cells and Lineage-Specific Differentiation in Cerebral Organoids, Related to Figure 1. **(A)** H&E of normal cerebral organoids reveals the presence of a neural tube, ependyma, and germinal matrix (left) and choroid plexus (right); scale bar, 200 μm. **(B)** Normal cerebral organoids (left) are morphologically similar to a fetal brain at 20 weeks of gestation (right); scale bar, 100 μm. **(C)** Immunohistochemical staining of normal cerebral organoids for markers of neural stem cells (Nestin, Musashi, and Sox2), glial cells (GFAP), and neurons (MAP2); scale bar, 1000 μm; n= 4-6 organoids for all experiments.

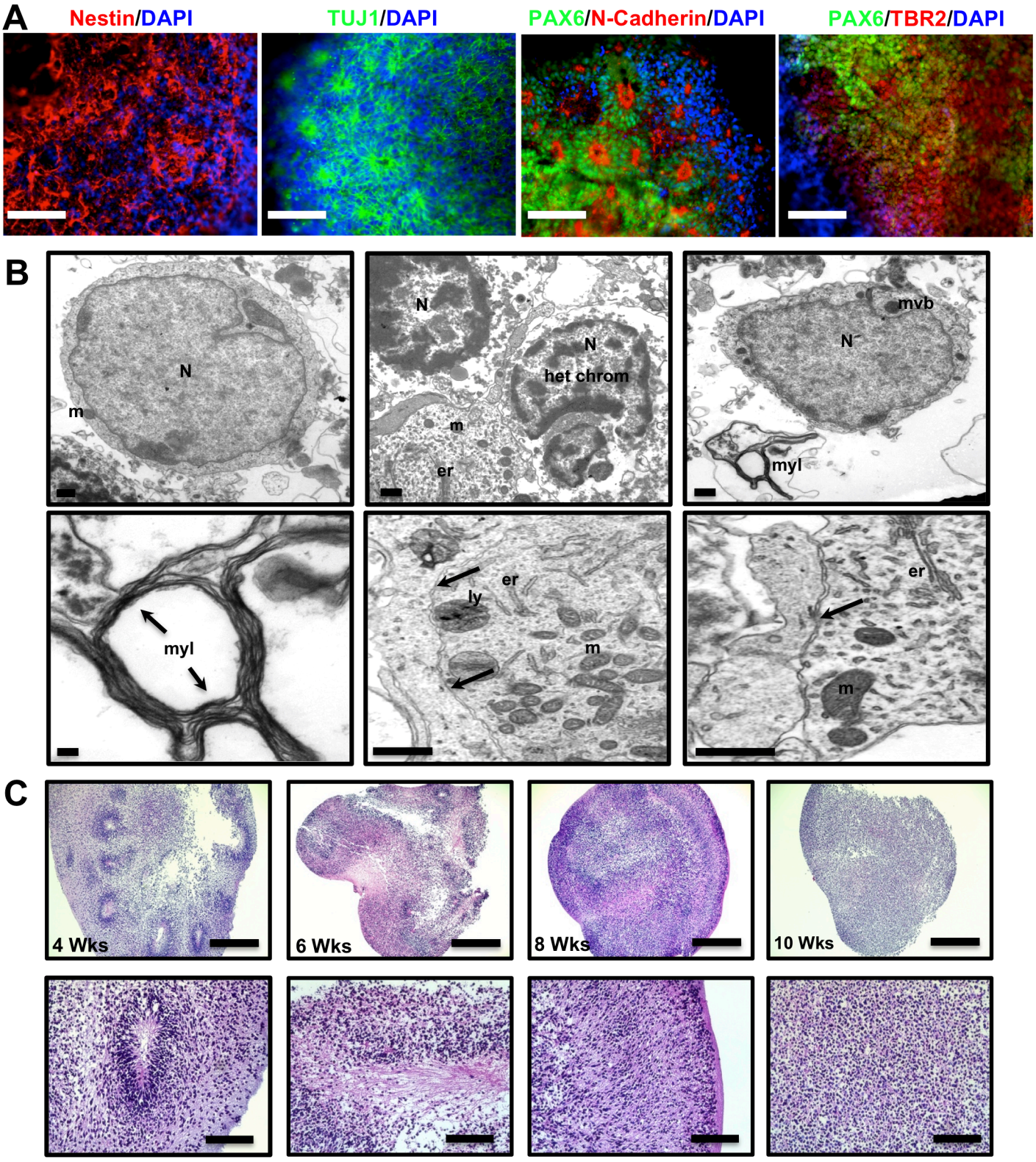

Figure S2: Cerebral Organoids Express Markers for Specific Stages of Neural Development and Demonstrate Myelinated Axons, Dendrodendritic Synapses, Neurons and Glial Cells, Related to Figure 1. (A) Immunofluorescence staining of Nestin, TUJ1, PAX6, N-Cadherin, and TBR2; scale bar, 200 µm. **(B)** Electron microscopy demonstrates neurons (top left; top right), glial cells (top middle, identified by heterochromatin), myelin (top right; bottom left), and dendrodendritic synapses (black arrows indicate synapses between two dendrites; bottom center and right); N, nucleus; m, mitochondria; er, endoplasmic reticulum, mvb, multi-vesicular body; myl, myelin; ly, lysosome; L, Lipid Droplet; G, Golgi apparatus; scale bars for top panels, 500 nm; scale bars for bottom panel, 100 nm (left) and 500 nm (center and right). **(C)** Chronological maturation of cerebral organoids; scale bars (top panel), 400 µm; scale bars (bottom panel), 100 µm; n=4-6 organoids for all experiments.

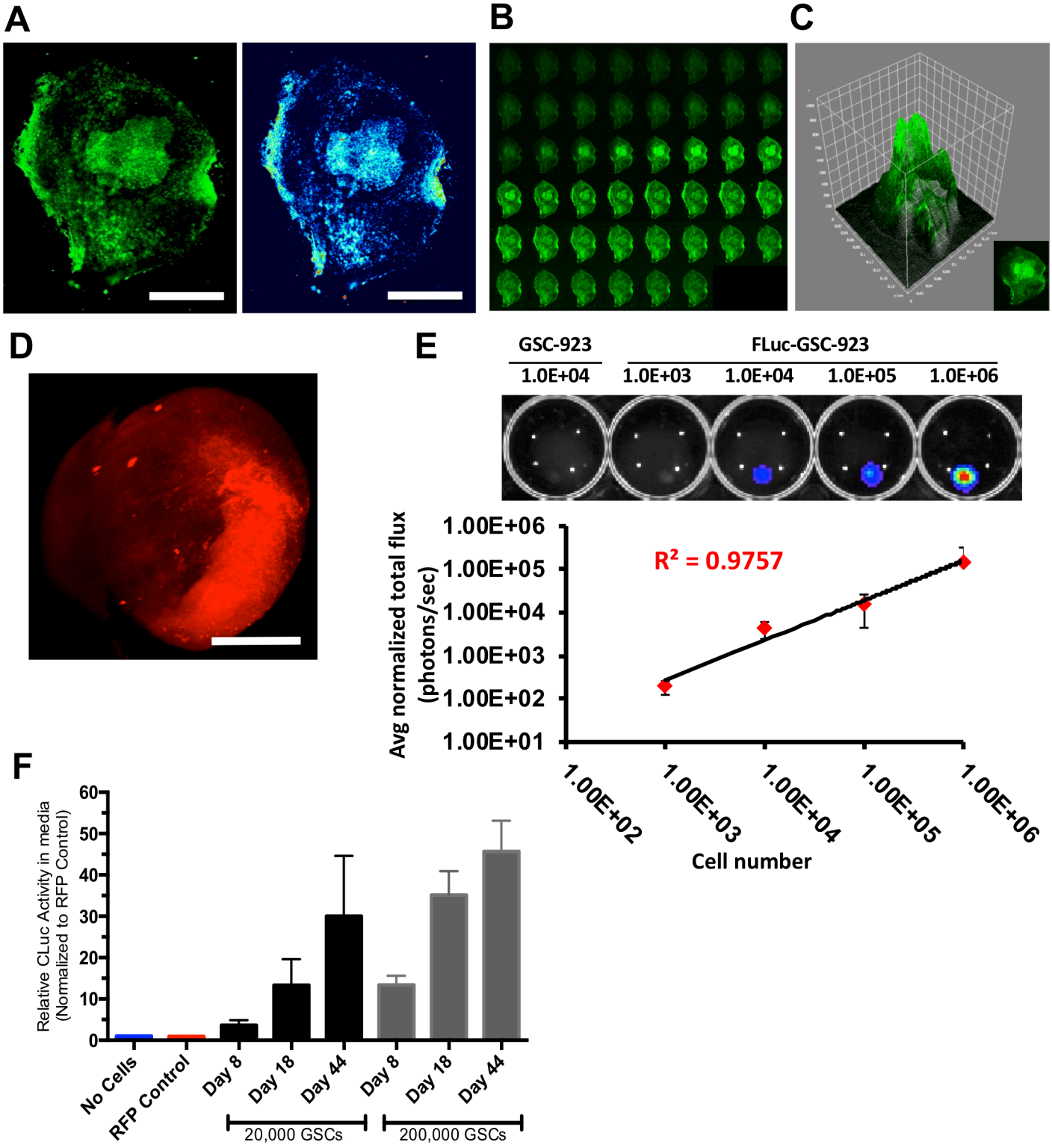

Figure S3: GLICO Tumors are Detectable by Fluorescence and Luciferase-based Imaging, Related to Figure 1. (A) Two-photon volumetric analysis of pixel intensity in GLICO tumor from GFP-positive 923 GSCs at Day 10; scale bars, 400 µm; n=4-6 organoids. **(B)** Multi-photon optical Z-stack image analysis of entire GLICO tumor from GFP-positive 923 GSCs at Day 10; n=4-6 organoids. **(C)** 3D topography of 923 GLICO tumor at Day 10; n=4-6 organoids. **(D)** Light sheet microscopy of GLICO tumor from GFP-positive 923 GSCs at Day 14 (pseudocolored red); scale bar, 500 µm; n=1 organoid. **(E)** Cell density-dependent increase in bioluminescence signal of GLICO tumors from 923 GSCs that stably express firefly luciferase; data were collected from three independent experiments; n=3-5 organoids per group. **(F)** Relative secreted luciferase activity in cerebral organoids that were co-cultured with either 20,000 or 200,000 827 GSCs that stably express a secreted *Cypridina* luciferase. Data were collected in triplicate from three independent experiments; n=3-5 organoids per group.

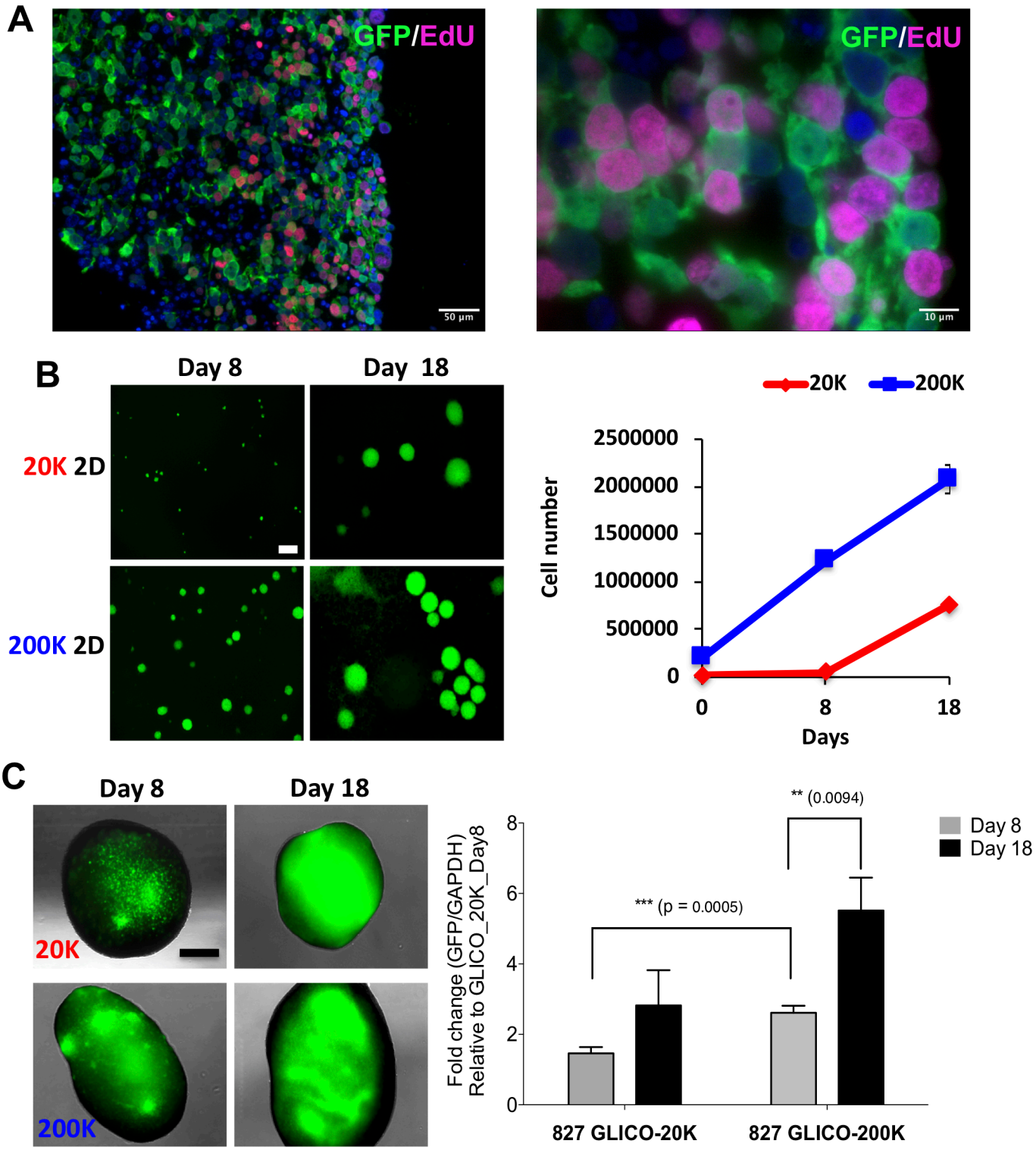

Figure S4: GSCs Proliferate within Cerebral Organoids in a Time-Dependent Manner, Related to Figure 1. (A) GFP-expressing 923 GLICO tumors were grown for 2 weeks and labeled with 5-ethynyl-2'-deoxyuridine (EdU) for 48 hrs. Paraffin-sections of these labeled GLICOs were co-stained for GFP (green) and EdU (magenta); n=3 organoids; scale bar, 50 µm (left) and 10 µm (right). Shown are representative images of proliferating tumor cells (GFP+/EdU+) within the organoid. **(B)** GFP-expressing 827 GSCs were seeded in 2D at either 20,000 or 200,000 cells per well (6-well plate). Cells were counted after 8 or 18 days of growth. Shown are representative 2D images (left) and the corresponding quantification of live cell number (right) at Day 8 and Day 18; scale bar, 400 µm; cells were plated in triplicate. **(C)** 20,000 or 200,000 GFP-expressing 827 GSCs were co-cultured with H1 cerebral organoids. GLICO tumors were harvested at 8 and 18 days post-co-culture and assessed for GFP expression by quantitative PCR (qPCR). Shown are representative images of 827 GLICOs (left) and quantification of GFP expression at corresponding time points (right); scale bar, 500 µm; n=3-5 organoids per group.

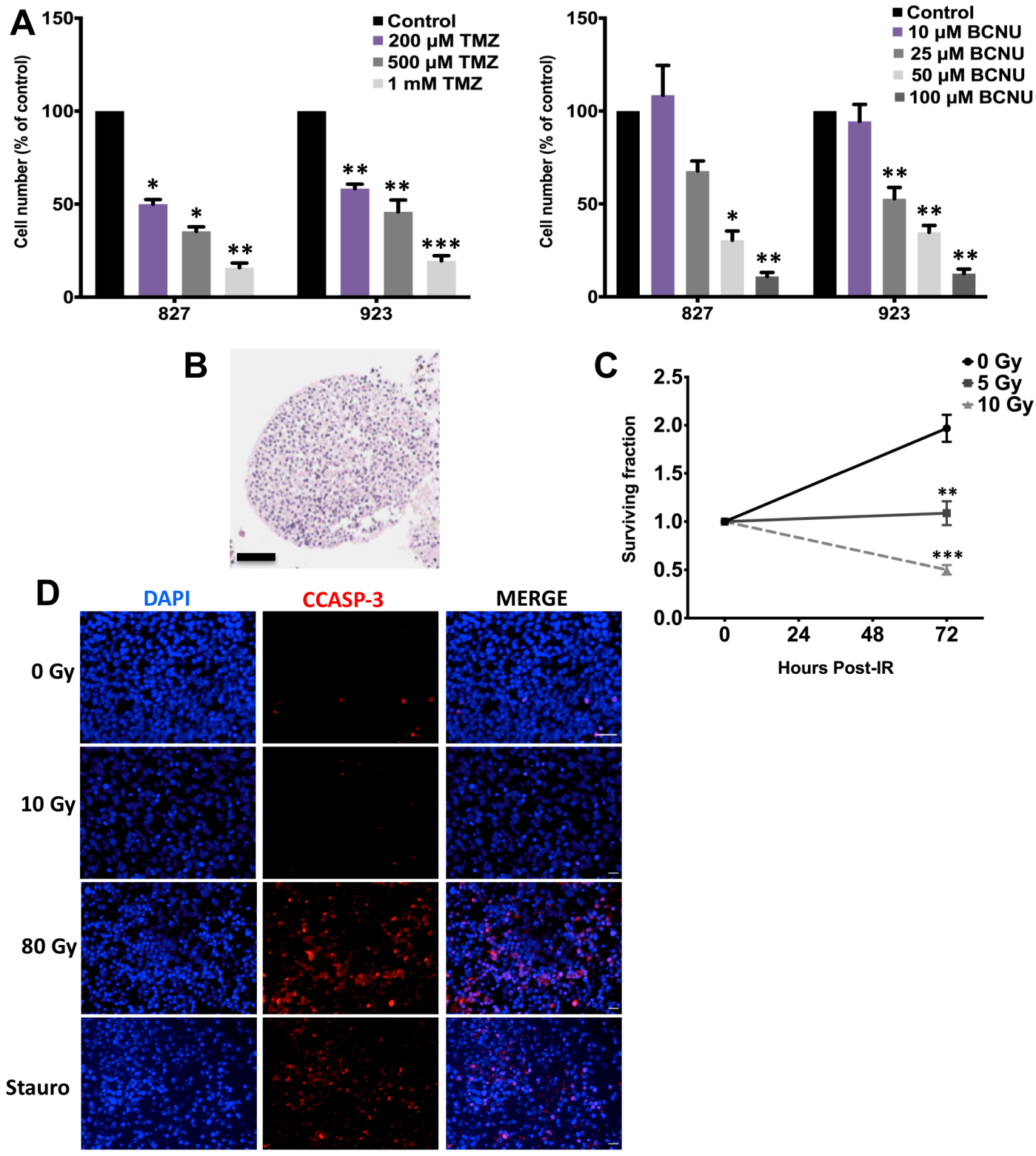

Figure S5: 2D Cultures of GSCs are Significantly More Sensitive to Chemotherapeutic Agents and Ionizing Radiation than their Corresponding GLICO Tumors, Related to Figure 3. **(A)** Shown is TMZ-(left) or BCNU-(right) induced cytoxicity in 2D cultures of 827 or 923 GSCs; cells were plated in triplicate; in comparison to control, *p<0.05; **p<0.01; ***p<0.001. **(B)** H&E immunohistochemical analysis of normal cerebral organoid treated with 1 mM TMZ for 7 days; scale bar, 100 μm; n=3-5 organoids. **(C)** Shown is the cell viability of 2D 923 GSCs at 72 hours post-treatment with a single dose of 0, 5, or 10 Gy of ionizing radiation (IR); in comparison to 0 Gy, **p<0.01; ***p<0.001. (D) Normal organoids were irradiated with 0, 10, or 80 Gy (positive control). Immunofluorescence staining for cleaved caspase-3 (CCASP-3) was used at 72 hrs post-IR to assess apoptosis. Incubation with staurosporine (stauro, 1 μM) for 72 hours was used as an additional positive control for CCASP-3. Shown are representative images; scale bar, 40 μm; n=4 organoids per group.

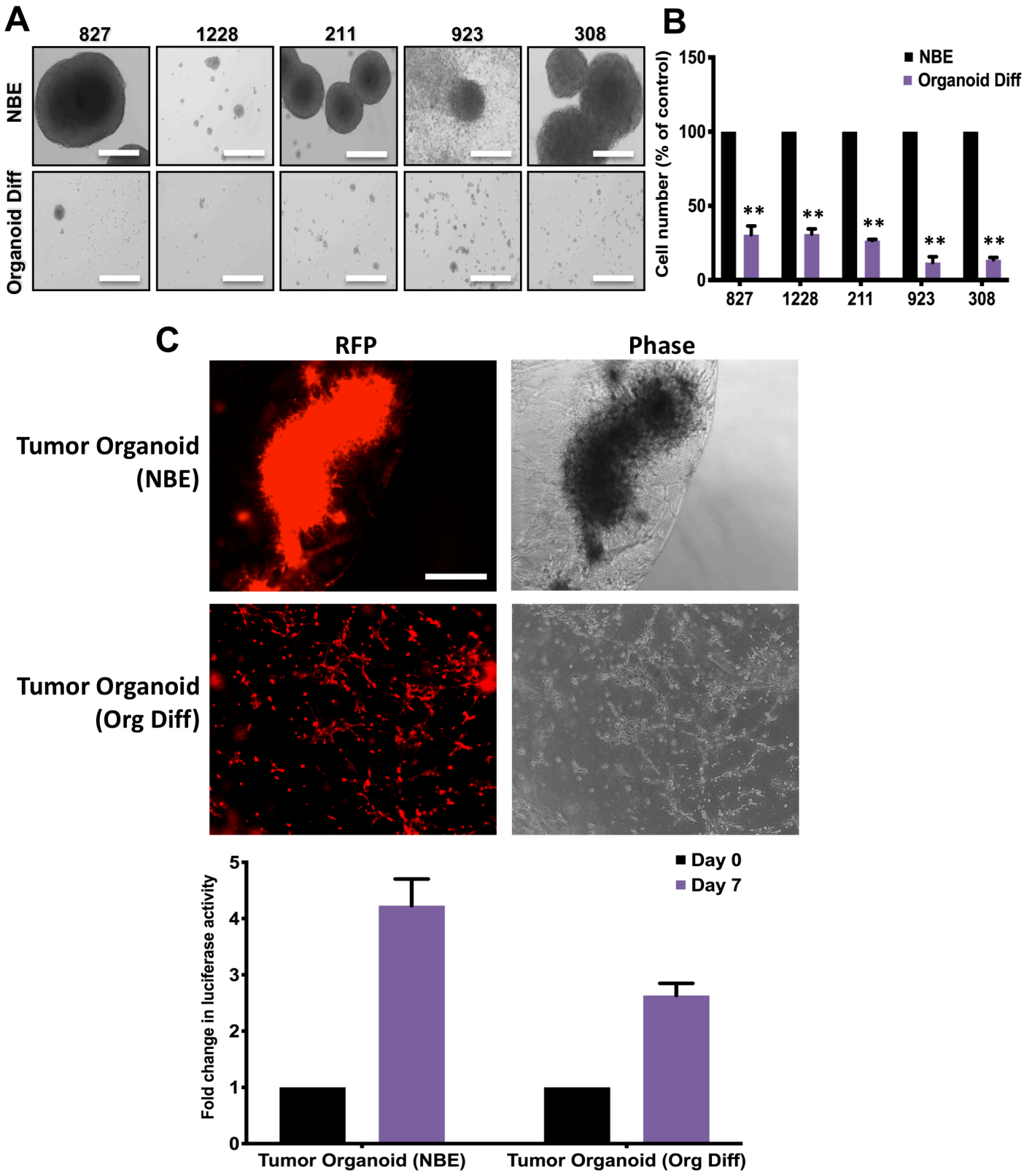

Figure S6: GSC Survival is Attenuated in Organoid Differentiation Medium, Related to Figure 1. **(A)** Representative micrographs of the proliferation of five GSC 2D cultures in NBE vs. Organoid Differentiation Medium; scale bar, 400 μm. **(B)** Bar graph of the average live cell number at Day 7 from three independent experiments; $^{**}p<0.01$. **(C)** Shown are representative images (top) of tumor organoids generated from culturing 923 luciferase-expressing GSCs in Matrigel. Tumor organoids were cultured in either NBE or Organoid Differentiation Medium for 7 days. The bar graph reveals secreted luciferase in each set of conditions 7 days after growth; scale bar, 400 μm; n=4 organoids.

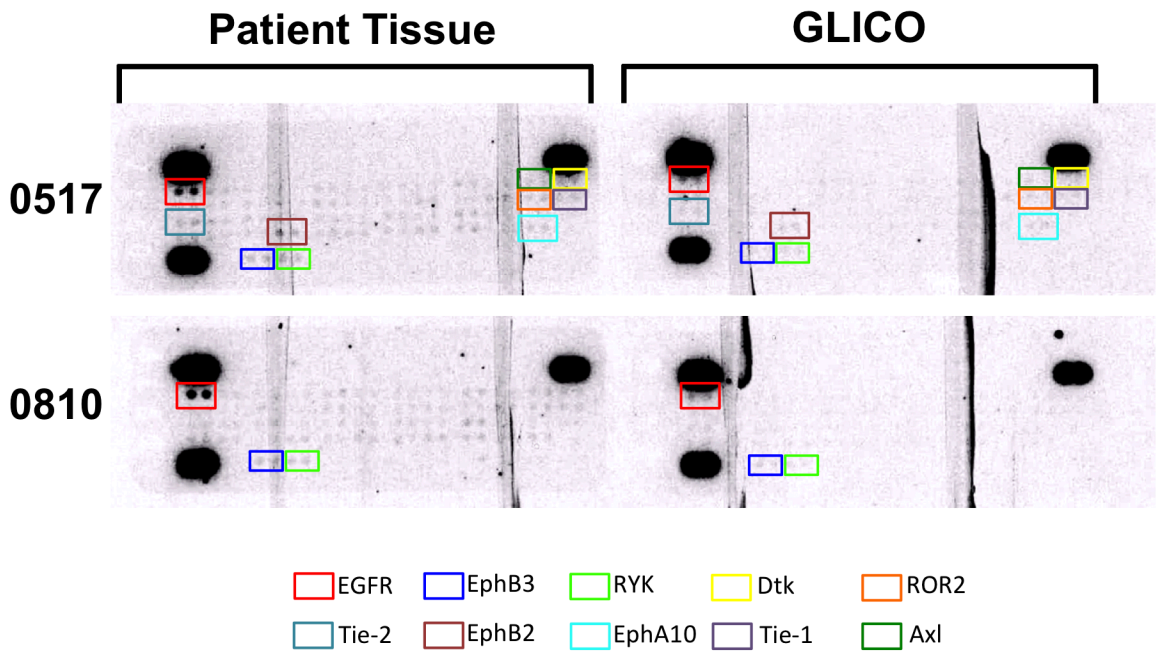

Figure S7: GLICO Tumors Reflect Signaling Networks Found within the Parental Tumor, Related to Figure 1. Phospho-RTK arrays of patient tumor tissue and corresponding GLICOs for GSC lines 0517 and 0810. 50 µg of total protein was used for each array; epidermal growth factor receptor (EGFR), ephrin type-B receptor 2 (EphB2), ephrin type-B receptor 3 (EphB3), ephrin receptor A 10 (EphA10), tyrosine kinase with immunoglobulin-like and EGF-like domain 1 (Tie-1), TEK receptor tyrosine kinase (Tie-2), tyrosine-protein kinase Dtk (Dtk), Axl receptor tyrosine kinase (Axl), related to receptor tyrosine kinase (RYK), and receptor tyrosine kinase-like orphan receptor 2 (ROR2).