Supplementary Data

Supplementary Methods

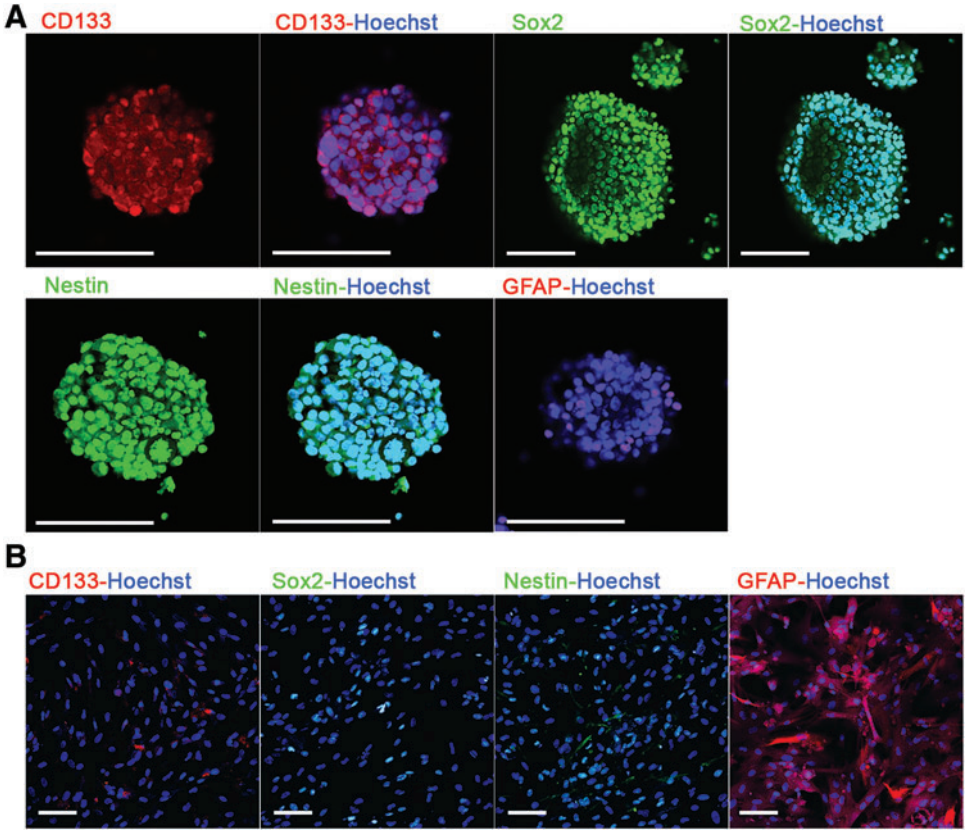
Immunofluorescence staining

To corroborate the expression of stem markers, GBAM1 cells were cultured in liquid media and collected after 4 days. Human astrocytes (ScienCell, Carlsbad, CA) were used as negative control. Cells were washed and fixed with formaldehyde 4%, permeabilized with Triton X-100 0.1%, and incubated with Sox2 Alexa Fluor-488®, Nestin Alexa Fluor-488® (eBioscience, Santa Clara, CA), human CD133-APC (Miltenyi Biotech, Inc., San Diego, CA), and GFAP-Cy3® (Sigma-Aldrich, St. Louis, MO) at dilutions of 1:100, 1:100, 1:10, and 1:100, respectively. Counterstaining for nuclei was performed with Hoechst 33342 (Life Technologies, Carlsbad, CA).

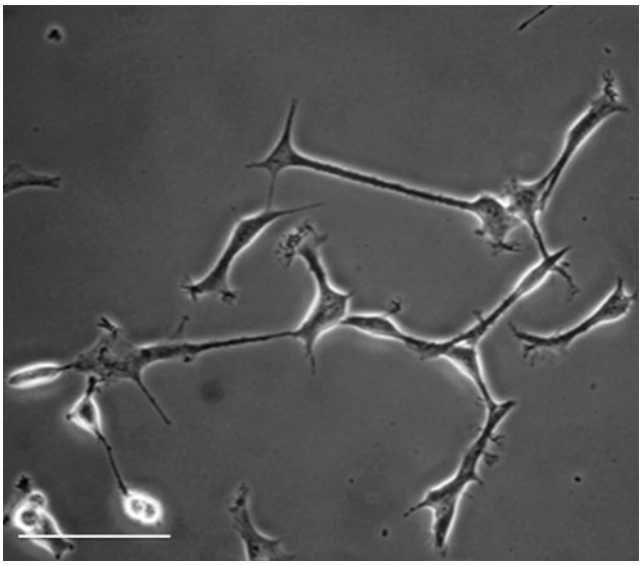
To analyze glioblastoma stem cell (GSC) collective protrusions in Matrigel 50%, neurospheres were fixed after 24 h of culture with formaldehyde 4%, permeabilized with Triton X-100 0.1%, and incubated with Alexa Fluor 488 Phalloidin (Life Technologies) (dilution 1:100) at 4°C for 6 h.

Differentiation of GSCs

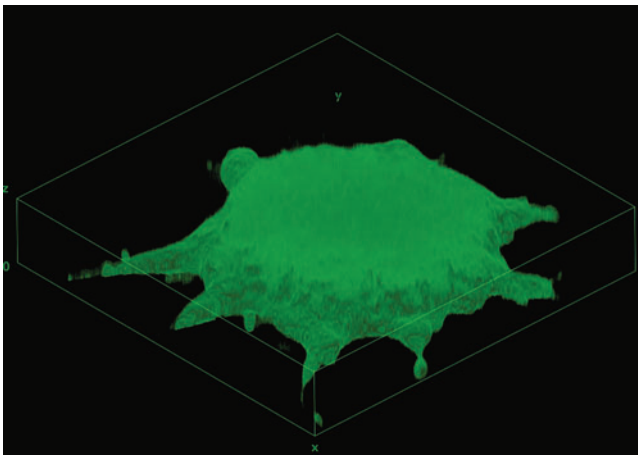
Differentiation media included DMEM/F12 and 10% FBS (Life Technologies); cells were fed with media and FBS every other day for at least 12 days.



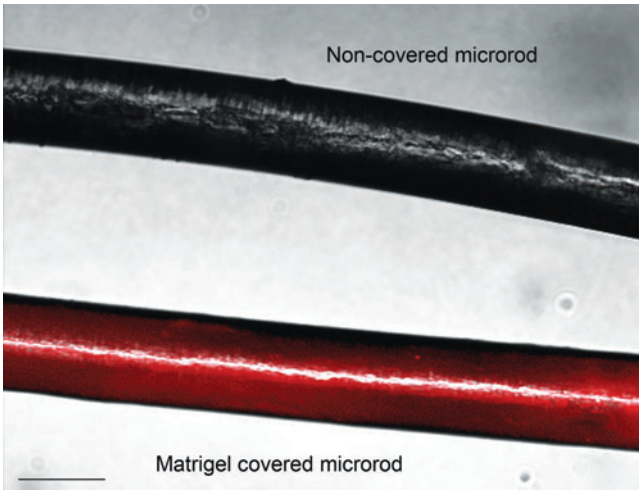
SUPPLEMENTARY FIG. S1. **(A)** GBAM1 neurospheres (passages 17–23) exhibit markers of glioblastoma cancer stem-like cells (CD133, Sox2, and Nestin) after 5 days of liquid culture. GFAP marker for differentiated astrocytes is not expressed. **(B)** Human primary astrocytes do not express stem-like markers (CD133, Sox2, and Nestin), but are positive for GFAP expression. Scale bars 100 μm.



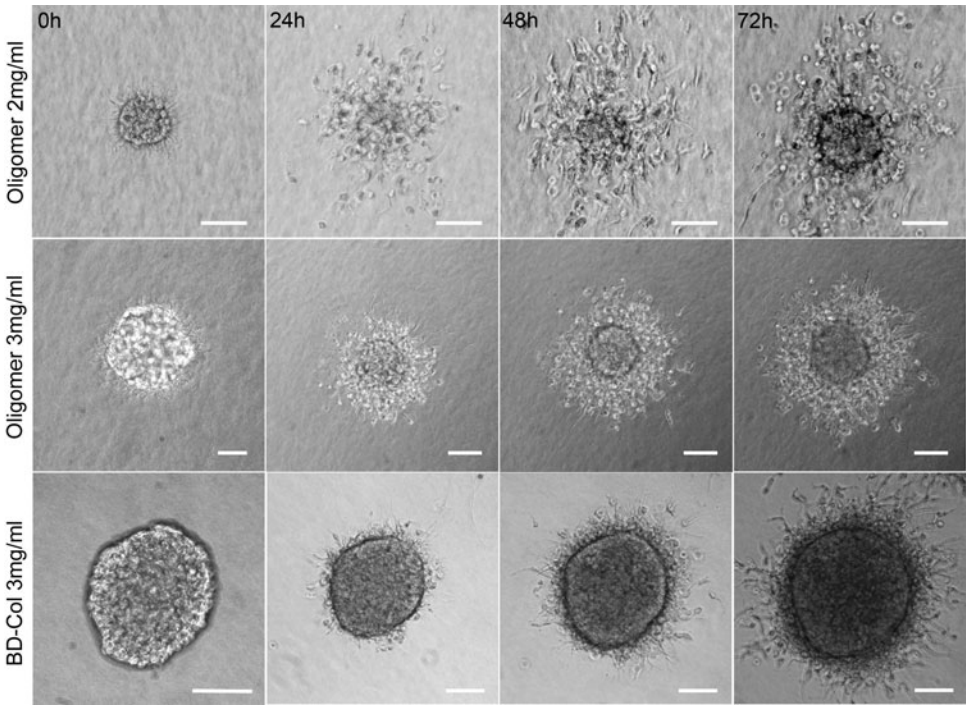
SUPPLEMENTARY FIG. S2. Glioblastoma stem cells (GSCs) do not form neurospheres and differentiate into astrocytes after 15 days of culture in the differentiation medium. Scale bars 100 μm.



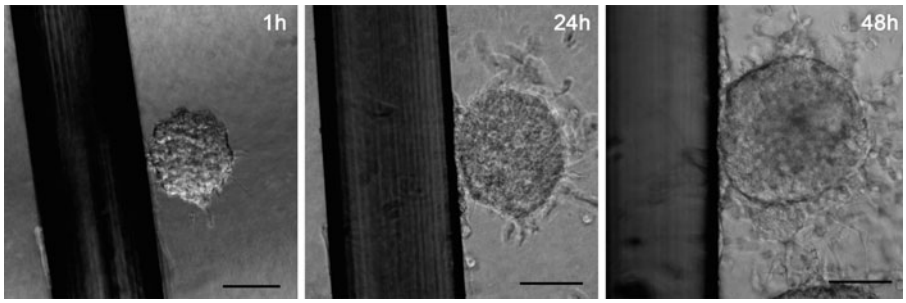
SUPPLEMENTARY FIG. S4. Z-reconstruction of GSC migrating in Matrigel 50% after 24 h shows that collective migrating extensions are located in the same focal plane.



SUPPLEMENTARY FIG. S3. Microrods covered and noncovered with Matrigel. Immunostaining with anti-Collagen type IV eFluor® 660 (red) was used to corroborate the presence of Matrigel deposited on the surface of the microrods. Scale bars 100 μm.



SUPPLEMENTARY FIG. S5. GSC (GBAM1) neurosphere migratory behavior over time when cultured in matrices of Oligomer 3 mg/mL, Oligomer 2 mg/mL, and BD-Col 3 mg/mL. The neurospheres show expansive growth accompanied by cell detachment and mesenchymal-like single migration. Scale bars 100 μm.



SUPPLEMENTARY FIG. S6. GSCs (GBAM1) present multiple migration modes in a HA:10-Col:2 mg/mL matrix with embedded noncoated rods, single-cell migration toward the matrix, and collective strand migration along the microrods. Scale bars 100 μm.

Supplementary Table S1. Viscoelastic Properties of the Composite Matrices of Oligomer Collagen and Hyaluronan ($N=3$, Mean ± SEM)

	Viscoelastic properties		
Matrix	*G′ (Pa)*	*G″ (Pa)*	*δ (degrees)*
HA:2-Col:2 mg/mL	126.2 ± 14.6	15.8 ± 0.4	7.2 ± 0.1
HA:5-Col:2 mg/mL	107.9 ± 19.6	13.4 ± 2.2	7.1 ± 0.1
HA:10-Col:2 mg/mL	36.1 ± 0.6	3.7 ± 0.1	5.9 ± 0.0

HA, hyaluronan.