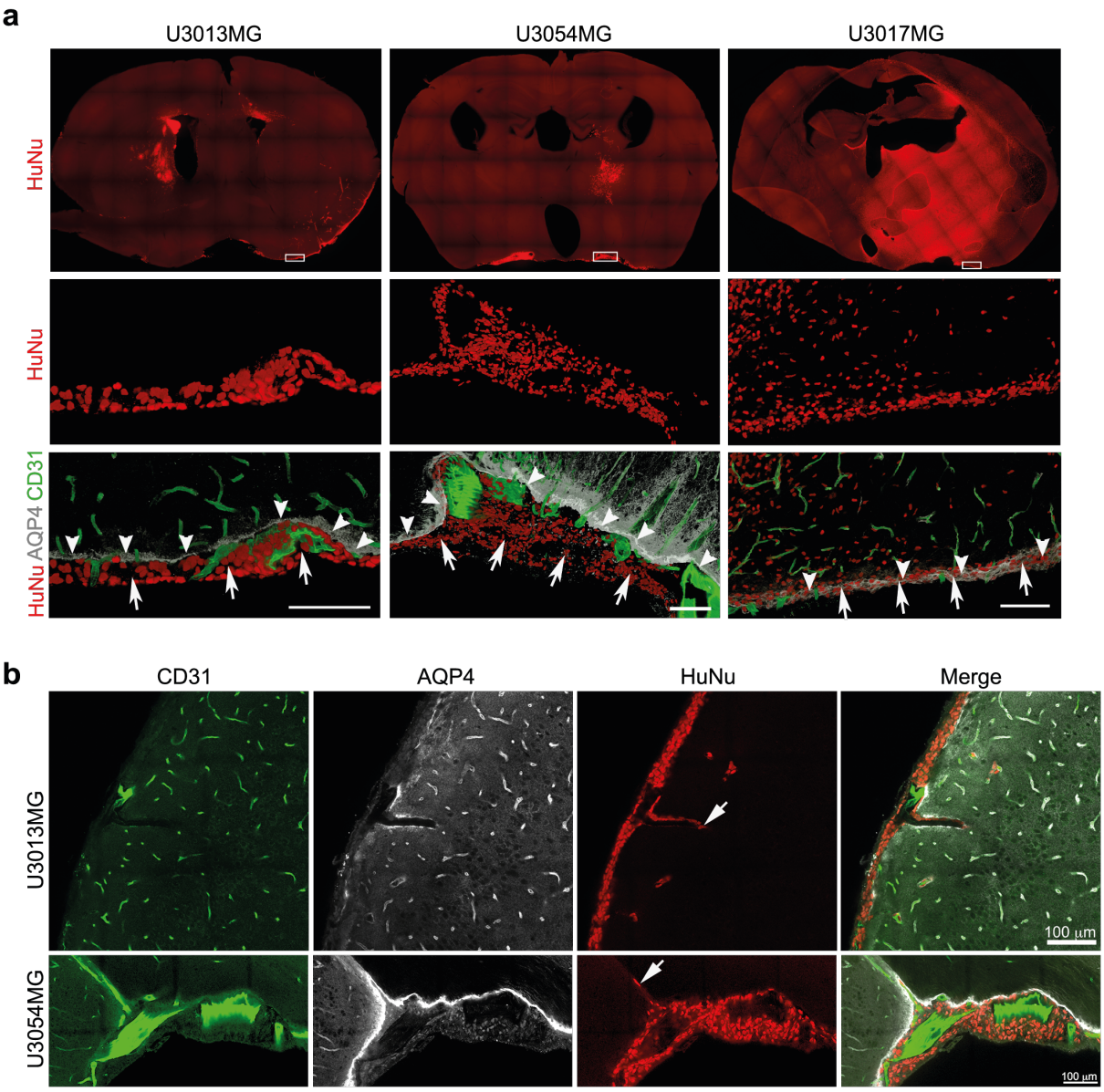
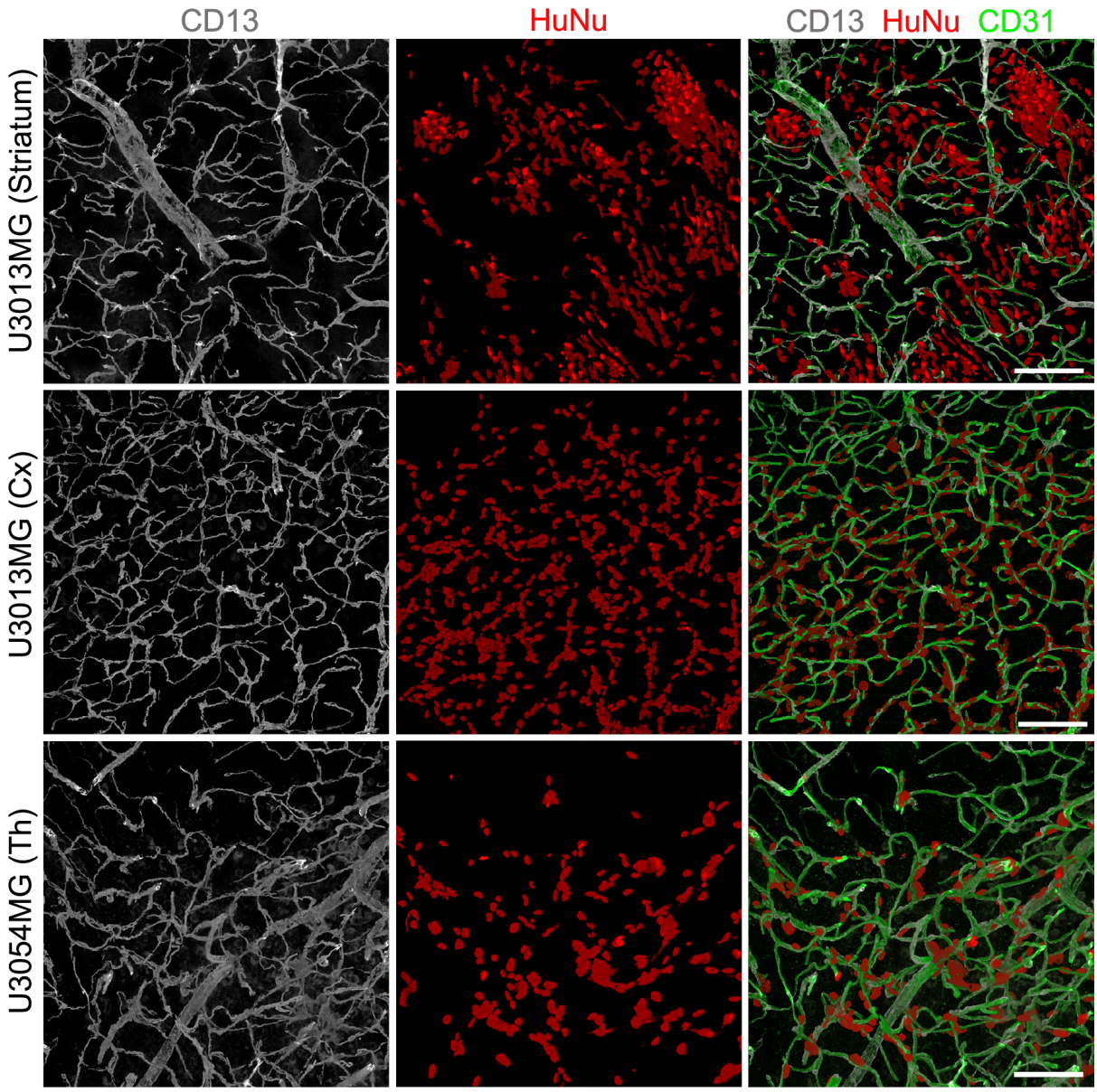
Supplementary Tables and Figures

Supplementary Table 1

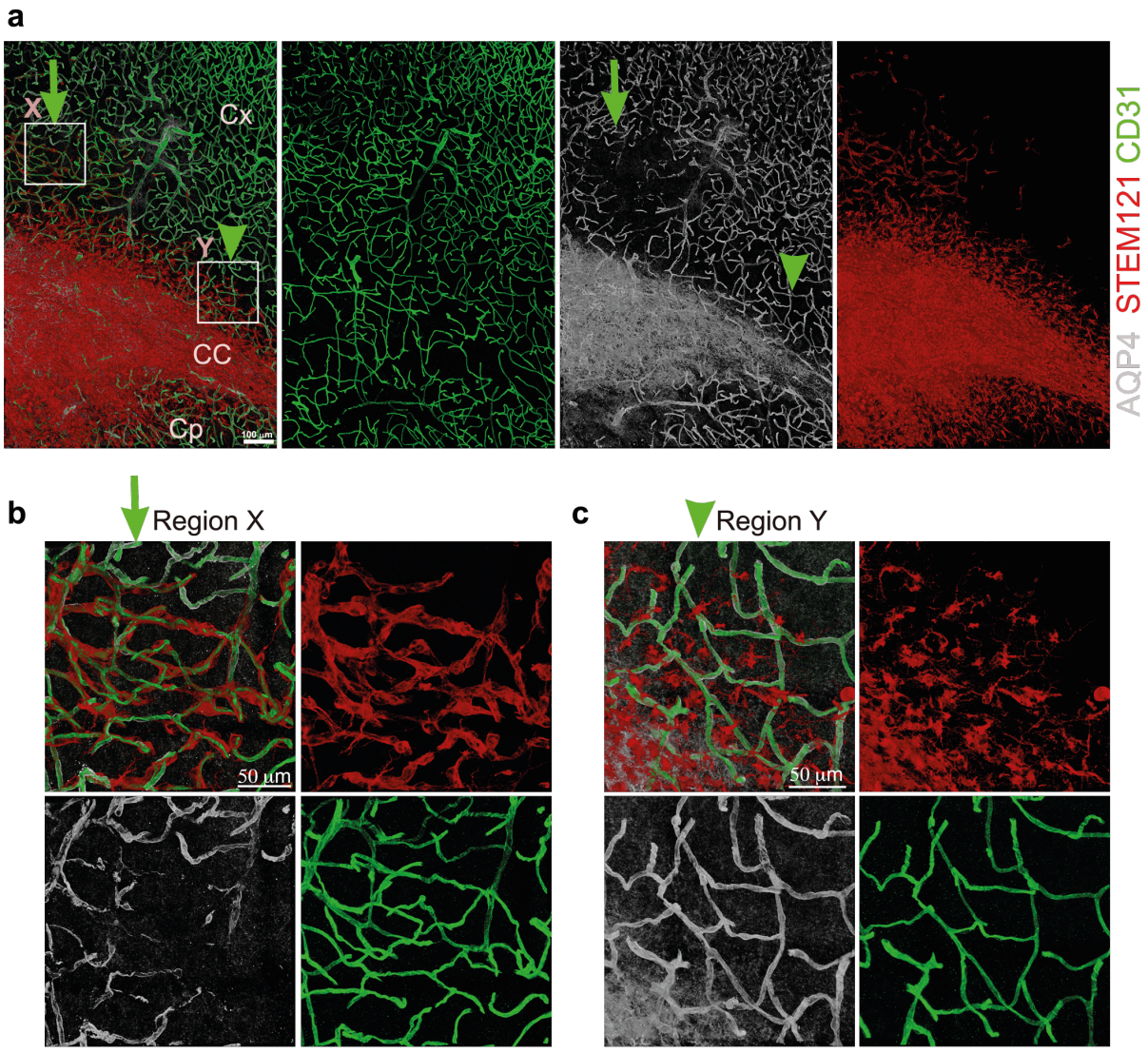
Cell line	Animal ID	Survival time	Tumor size
U3009MG Classical Passage 17	185	59 days	Small
	186	128 days	No tumor
	187	Died during surgery	NA
	188	74 days	Small
	189	91 days	Intermediate
U3013MG Proneural Passage 12	180	85 days	Large
	181	70 days	Intermediate
	182	58 days	Small
	183	70 days	Small
	184	70 days	Intermediate
U3017MG Classical Passage 9	220	58 days	Intermediate
	221	71 days	Large
	222	73 days	Large
	223	76 days	Large
	224	76 days	Large
U3024MG Mesenchymal Passage 9	205	67 days	Small
	206	111 days	Intermediate
	207	69 days	Small
	208	59 days	Small
	209	22 days	Very small
U3047MG Proneural Passage 9	195	67 days	Intermediate
	196	67 days	Large
	197	70 days	Large
	198	59 days	Intermediate
	199	67 days	Large
U3054MG Mesenchymal Passage 14	215	71 days	Small
	216	70 days	Small
	217	Died during surgery	NA
	218	70 days	Small
	219	58 days	Small
U3065MG Mesenchymal Passage 12	210	134 days	Intermediate
	211	58 days	Small
	212	Died during surgery	NA
	213	92 days	Intermediate
	214	76 days	Small
U3082MG Proneural Passage 9	200	67 days	Intermediate
	201	58 days	Intermediate
	202	67 days	Intermediate
	203	67 days	Intermediate
	204	132 days	Large



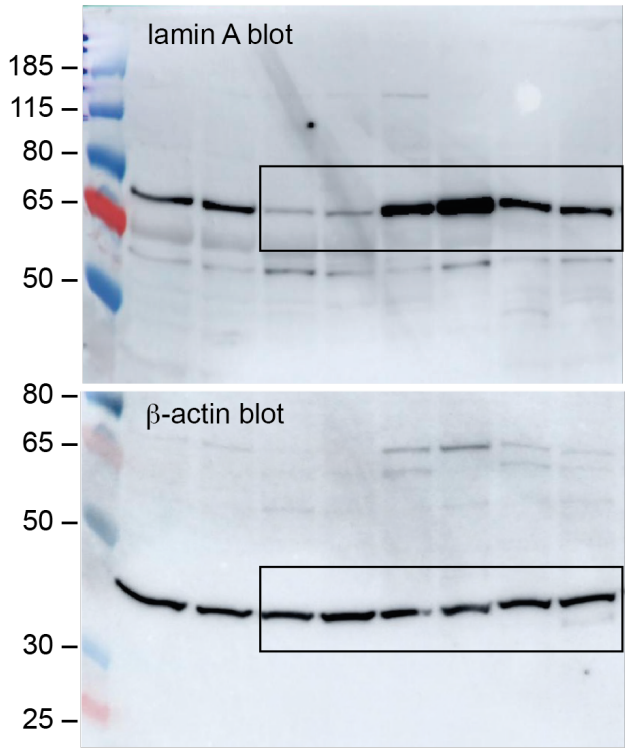
Supplementary Fig. 1 a-b Tumor cell accumulation in the subarachnoid space and penetration into the brain parenchyma. Immunofluorescent staining using antibodies against HuNu, aquaporin 4 (AQP4, astrocyte end-feet) and CD31 (endothelial cells). z-stacks were taken using a Leica confocal microscope, 63X objective. Scale bars, 100 µM. **a** U3013MG and U3054MG tumor cells accumulate and move along subarachnoid space, compared to other GSC lines. Upper panel, tumor growth pattern; lower and middle panels, 3D image snapshots of limited adjacent z-stacks of the selected areas. Arrows indicate glioma cells and arrowheads indicate glia limitans superficialis. U3017MG tumor shown as an example where accumulation of tumor cells in the subarachnoid space is not observed. **b** U3013MG and U3054MG tumor cells in perivascular spaces of vessels penetrating the brain from the subarachnoid space. Arrows indicates entry of tumor cells in perivascular space from subarachnoid space. 100 µm thick coronal sections. Each image represents maximum intensity projection of few z-stacks. **See related Movie 1.**



Supplementary Fig. 2 No detectable loss of pericytes from the vessels occupied by migrating glioma cells. Coronal sections were stained with HuNu (red, human glioma cells), CD13 (white, pericytes), and CD31 (green, blood vessels) antibodies. z-stacks were taken by Leica confocal microscope using 63x objective in tile scan mode. Each image represents a snapshot of a 3D image. Cortex (Cx), Thalamus (Th). Scale bar, 100 μm.



Supplementary Fig. 3 U3013MG tumor cells which enter the cortex from the corpus callosum do not displace astrocyte end-feet from the vessels. **a** Coronal sections (100 µm) were stained with STEM121 (red, human glioma cells), anti-AQP4 (white, astrocyte end-feet), and anti-CD31 (green, blood vessels) antibodies and z-stacks were taken by Leica confocal microscope using tile scan function. Boxes indicates glioma cells moving from corpus callosum to cortex near the lateral ventricle (Box X) or near the tumor invasive front at corpus callosum (Box Y). **b-c** Zoom of Box X and Box Y, respectively. Each image represents a snapshot of a 3D image of selected z-stacks. Abbreviations: CC (corpus callosum), Cx (cortex), Cp (Caudoputamen).



Supplementary Fig. 4 Full-length blots (lamin A, 74 kDa; b-actin, 42 kDa) of cropped western blots shown in Fig. 6e. Framed lanes indicate samples used in this study.

Supplementary Movie 1: U3013MG tumor cells in perivascular spaces of vessels penetrating the brain parenchyma from the subarachnoid space. Immunofluorescent staining of 100 µm thick coronal sections using HuNu, aquaporin 4 (AQP4, astrocyte end-feet) and CD31 (endothelial cells) antibodies. z-stacks were taken using a Leica confocal microscope in tile scan mode, 63X objective. Stitched images from one surface to another surface of the coronal section are played as a movie. **Related to Supplementary Fig. 1b**.

Supplementary Movie 2: Nuclear plasticity and fragmentation (micronuclei) in tumor cells migrating in the corpus callosum. 100 µm thick coronal sections were stained with HuNu (red) antibody to visualize human U3065MG glioma cells. z-stacks were taken by a Leica confocal microscope using 63x objective. The movie represents a rotating 3D image at the x-axis. **Related to Fig. 6b.**