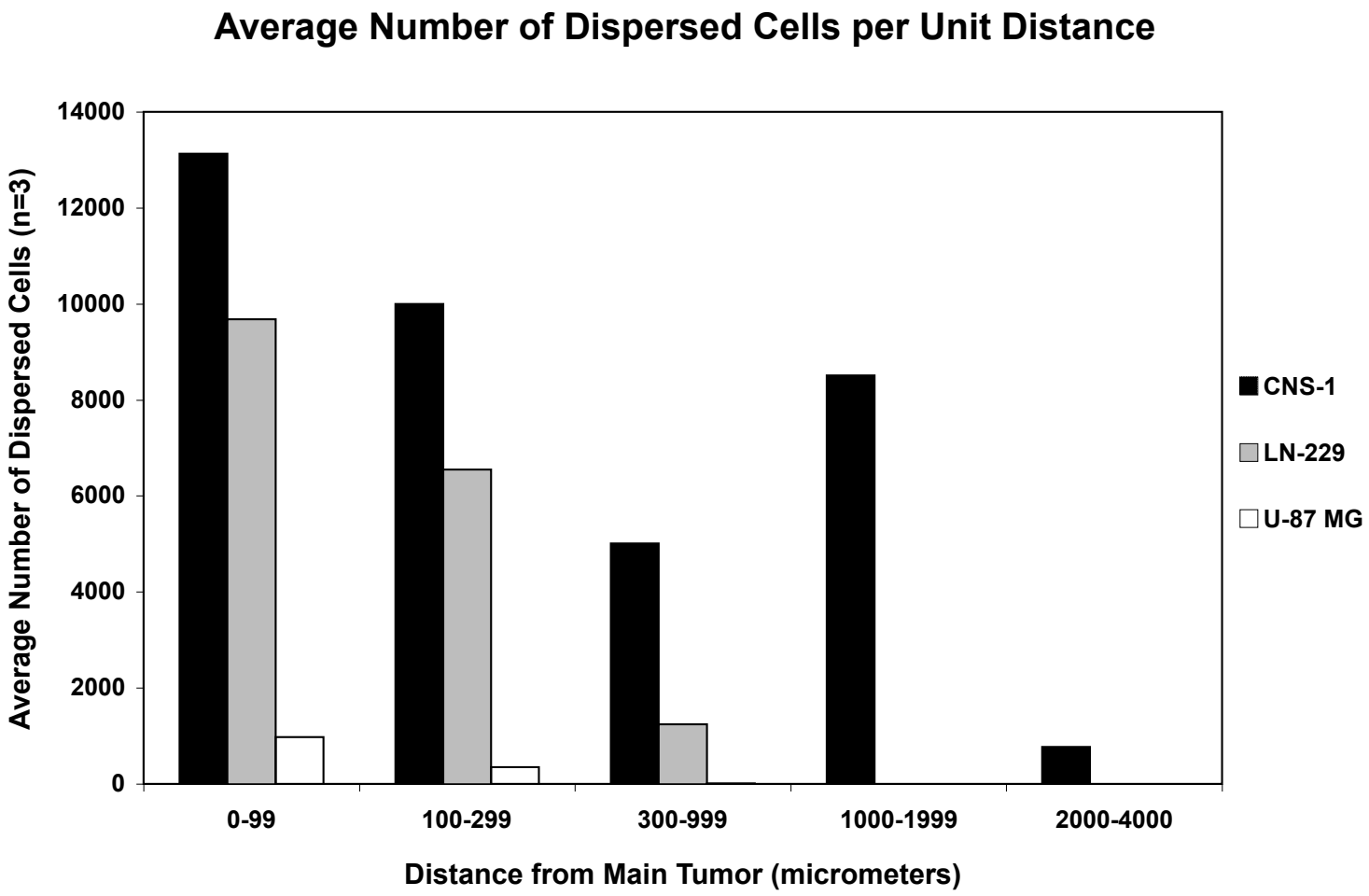

Supplementary Figure 1. Cell dispersal distance measurements.

The average number of dispersed cells is shown for various distance ranges. LN-229 and CNS-1 cells migrate 0.5-4mm away from the main tumor mass.

Tumor Type	Tumor Blood Vessel Volume Average (mm^3)	Control Blood Vessel Volume Average (mm^3)	Std. Dev.	Tumor Blood Vessel Volume Normalized to ROI (mm^3)	Control Blood Vessel Volume Normalized to ROI (mm^3)	% Increase in Tumor Blood Vessel Volume Compared to Control
CNS-1	0.8128	0.4679	0.1224	0.1022	0.0604	**169%**
Gli36Δ5	9.5070	5.9155	0.1132	0.0964	0.0596	**162%**
LN-229	1.4027	1.2541	0.1706	0.0992	0.0921	**108%**
U-87 MG	0.8549	0.5361	0.3223	0.1139	0.0684	**166%**

Supplementary Table 1. Analysis of tumor blood vessel density.

Supplementary Video 1. CNS-1 cell dispersal along blood vessels, related to Figure 5.

The CNS-1-GFP glioma cell line was xenograft into the brain of an athymic nude mouse, and images are shown at seven days post-implantation. Brain brightfield images rendered in 3-D are presented initially, and fade to show the main tumor mass (pseudocolored green) and dispersed cells (pseudocolored yellow). The 3-D blood vessel volume for the entire brain is overlaid (pseudocolored red), and a bounding box is used to focus on the region containing the tumor. Zooming and rotation around the tumor clearly show the details of extensive tumor cell association with and dispersal along blood vessels.

Supplementary Video 2. CNS-1 cell dispersal on white matter, related to Figure 6.

The CNS-1-GFP glioma cell line was xenograft into the brain of an athymic nude mouse, and images are shown at seven days post-implantation. Brain brightfield images rendered in 3-D are presented initially, and fade to show the main tumor mass (pseudocolored green), dispersed cells (pseudocolored yellow) and white matter (pseudocolored gray). Dispersed cells in contact with white matter are pseudocolored magenta. Zooming and rotation of the tumor show a large subset of tumor cells dispersing on white matter.

Supplementary Methods

Distance of dispersed tumor cells

Distance of tumor cell dispersal from the main tumor mass was measured using a 3-D morphological distance algorithm (Qutaish et al., Submitted). Briefly, a series of dilations were applied to the main tumor volume. After each dilation, the dispersed cell volume was checked to determine whether any cell-containing voxels were captured by the dilation. The first two dilations closest to the tumor were discarded to reduce nonspecific error. The data from this analysis was plotted to show dispersal distance and the number of dispersed cells at each particular distance.

Determination of Blood Vessel Density

Blood vessel density within tumors was determined using Amira Software. For each brain, a 3-D region of interest (ROI) was drawn over the main tumor and an identical ROI was placed in a comparable location on the opposite hemisphere of the brain (non-

tumor control). The blood vessel volume (mm^3) was determined within each ROI and normalized to the ROI volume. The value from the tumor ROI was then normalized to the control ROI to result in the percent increase in blood vessel density in the tumor.

Video production

2-D brightfield and fluorescence images from the cryo-imaged tissue block face were segmented and rendered into 3-D data volumes using Amira software (Mercury Computer System Inc., Chelmsford, MA). The resultant 3-D data volumes included brain brightfield, main tumor mass, dispersed tumor cells, blood vessels and white matter. The 3-D volumes were overlaid and registered in Amira. For movie production, the DemoMaker module in Amira was used to define the actions in the movies (rotation, zoom, fade, etc), then the MovieMaker module was used to render the movies using .mpg file format. Movies were produced at maximum quality using 30 fps and a size of 320x240 pixels.