Figures and figure supplements

Correlated magnetic resonance imaging and ultramicroscopy (MR-UM) is a tool kit to assess the dynamics of glioma angiogenesis

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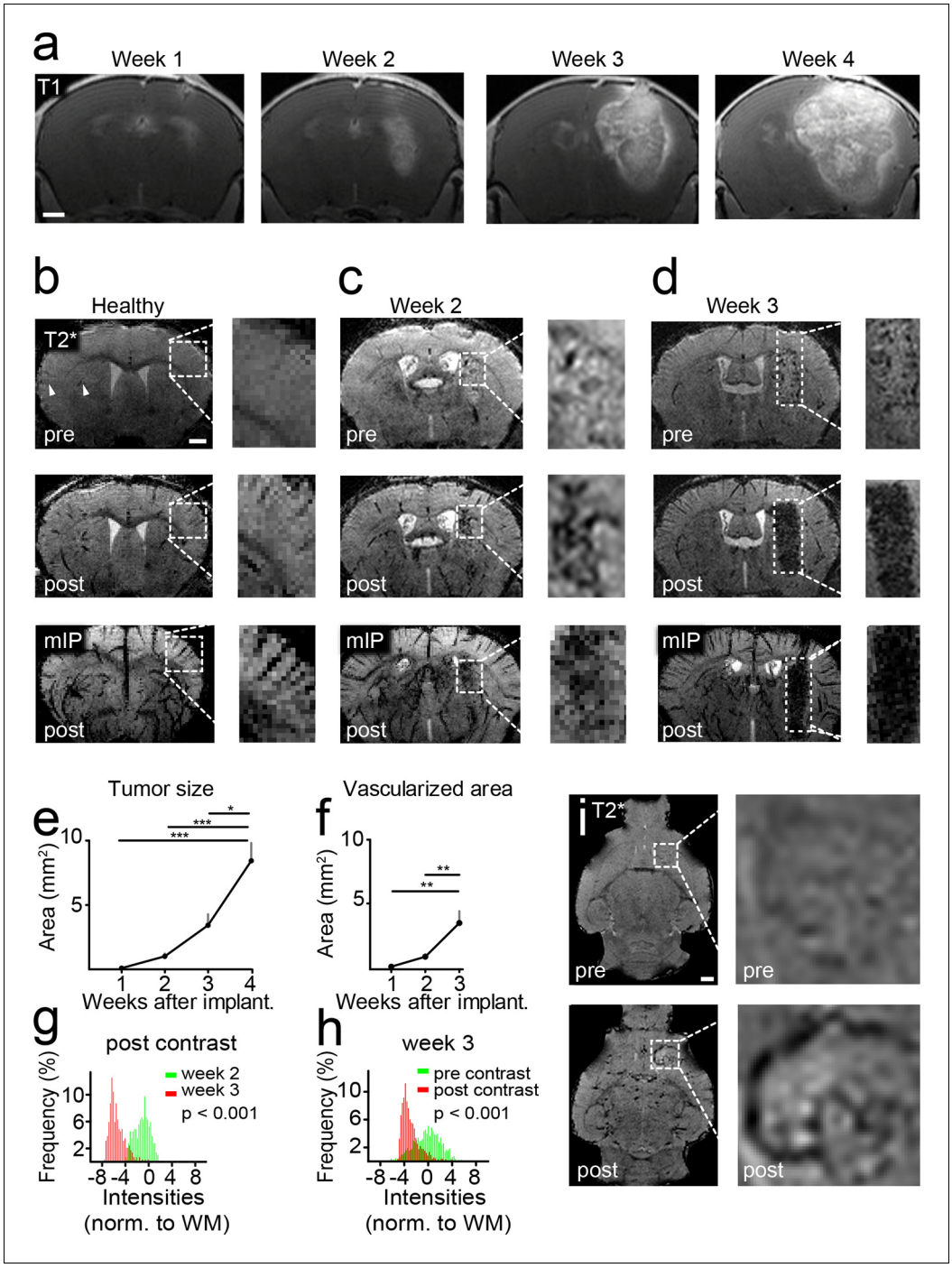

Figure 1. Imaging tumor vessel development with T2*-w sequences. Time course of tumor development on T1-w post Gd-contrast images (**a**). T2*-w images (80 μm resolution). Hypointense tubular structures, most likely venules (arrowheads), are visible due to the BOLD effect on pre-contrast images (upper row). Post-contrast administration arterioles and venules can be visualized (middle row). A minimum intensity projection (mIP) is shown in the bottom row (**b**). T2*-w images two and 3 weeks post-tumor implantation (**c**,**d**). Quantification of tumor sizes on T1-w post-contrast images (**e**). Quantification of the vascularized area on T2* images (**f**). Histogram analysis of the tumor region on post-contrast images 2 (green distribution) and 3 weeks (red) after tumor implantation. A significant signal drop (red distribution) within the tumor relative to the healthy white matter (WM) occurs within 1 week ($p<0.001$, **g**). This signal drop is only visible post-contrast administration (red distribution, $p<0.001$, **h**). Single plane T2*-w images pre- and post-contrast 2 weeks post-tumor implantation. The tubular vessel is only visible after contrast injection (**i**). Note the tortuous appearance and the multiple branches of the vessel. Scale bars are 1 mm.

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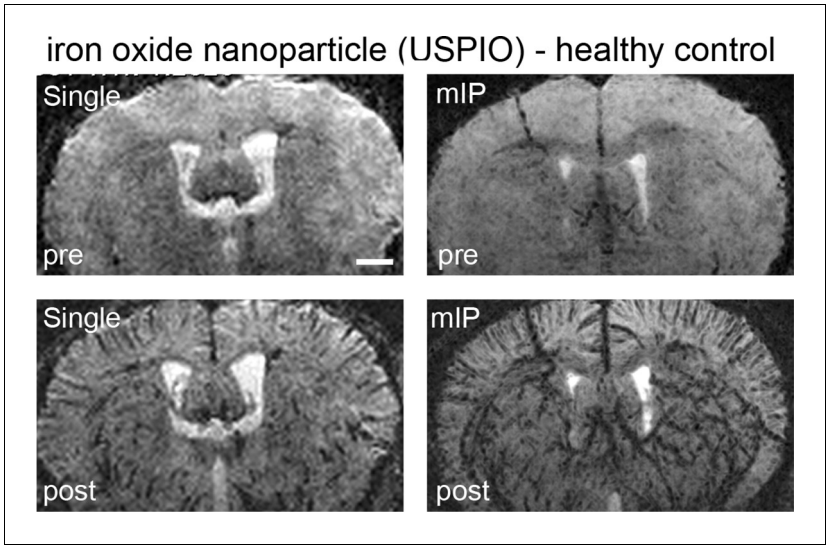

Figure 1—figure supplement 1. Monitoring vascularization with USPIOs in healthy mice. T2*-w images pre- and post iron oxide nanoparticle (USPIO) administration. Post-contrast images were acquired directly after iv injection of USPIO. mIP: minimum intensity projection. Scale bar 1 mm.

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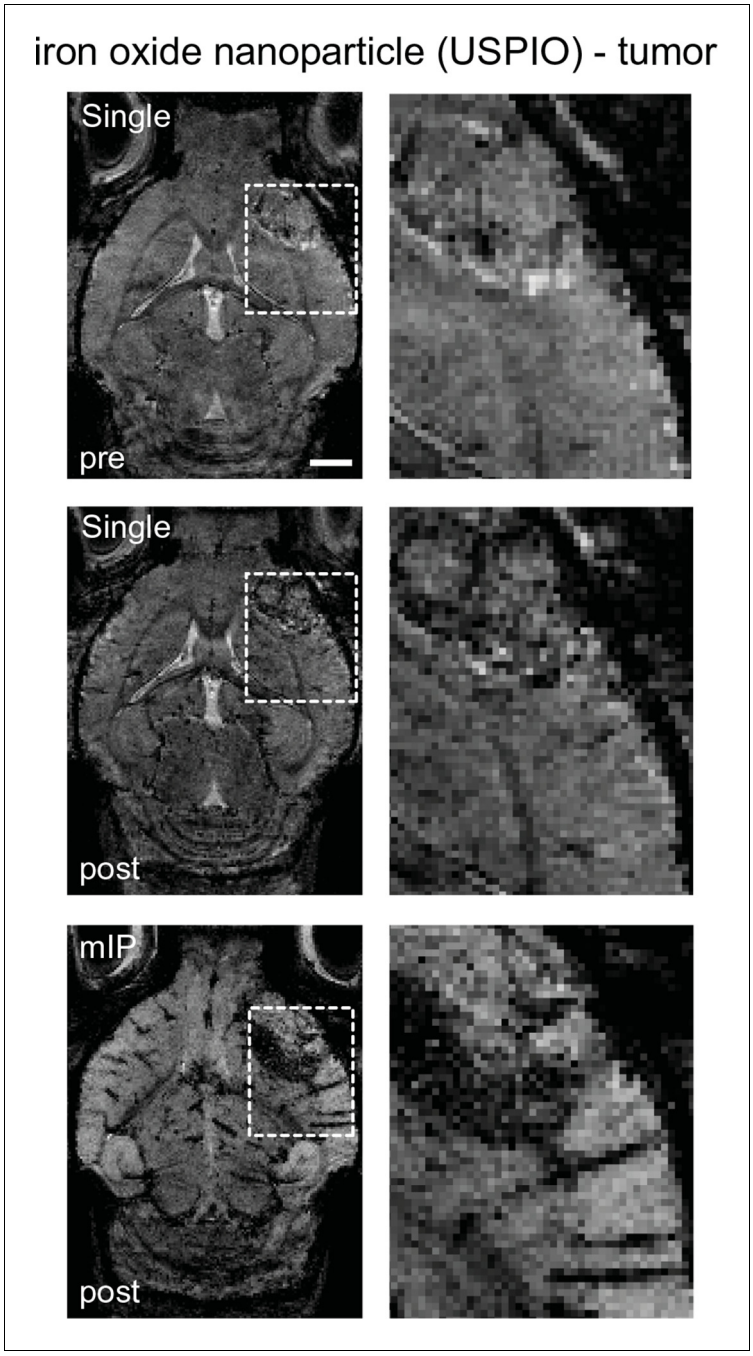

Figure 1—figure supplement 2. Imaging tumor vasculature with USPIOs. T2*-w images pre- and post USPIO administration in a GL261 tumor. Post-contrast images were acquired directly after USPIO injection. Dashed boxes indicate magnified areas (right side). mIP: minimum intensity projection. Scale bar is 1 mm.
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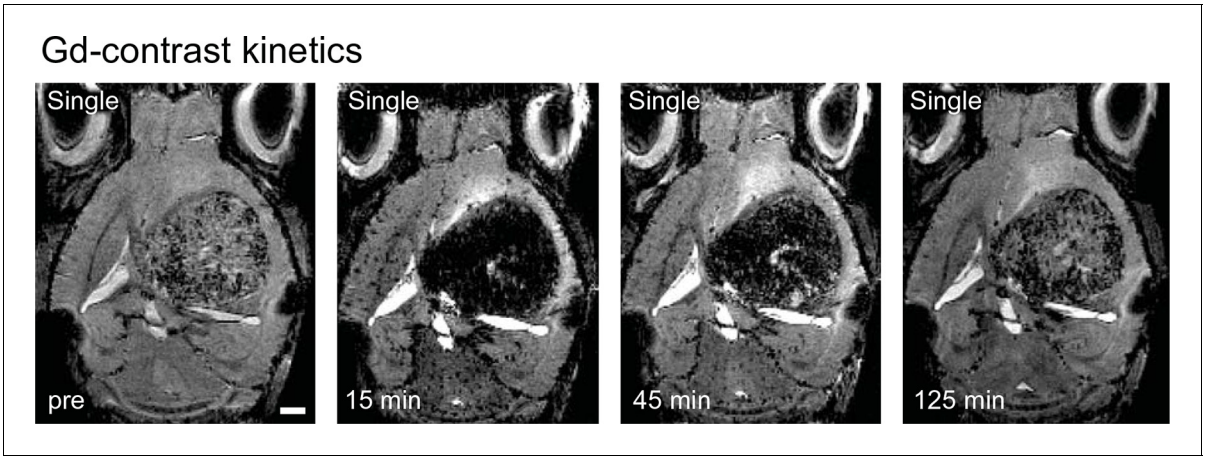

Figure 1—figure supplement 3. Gd contrast kinetics in T2*-w time series Sequential. T2*-w images following Gd-administration. Post-contrast images 15 min after Gd-administration show strong tubular susceptibility changes within the tumor. The contrast agent is subsequently cleared from the circulation. 125 min post-contrast administration the vascular signals have returned to pre levels. Scale bar = 1 mm.
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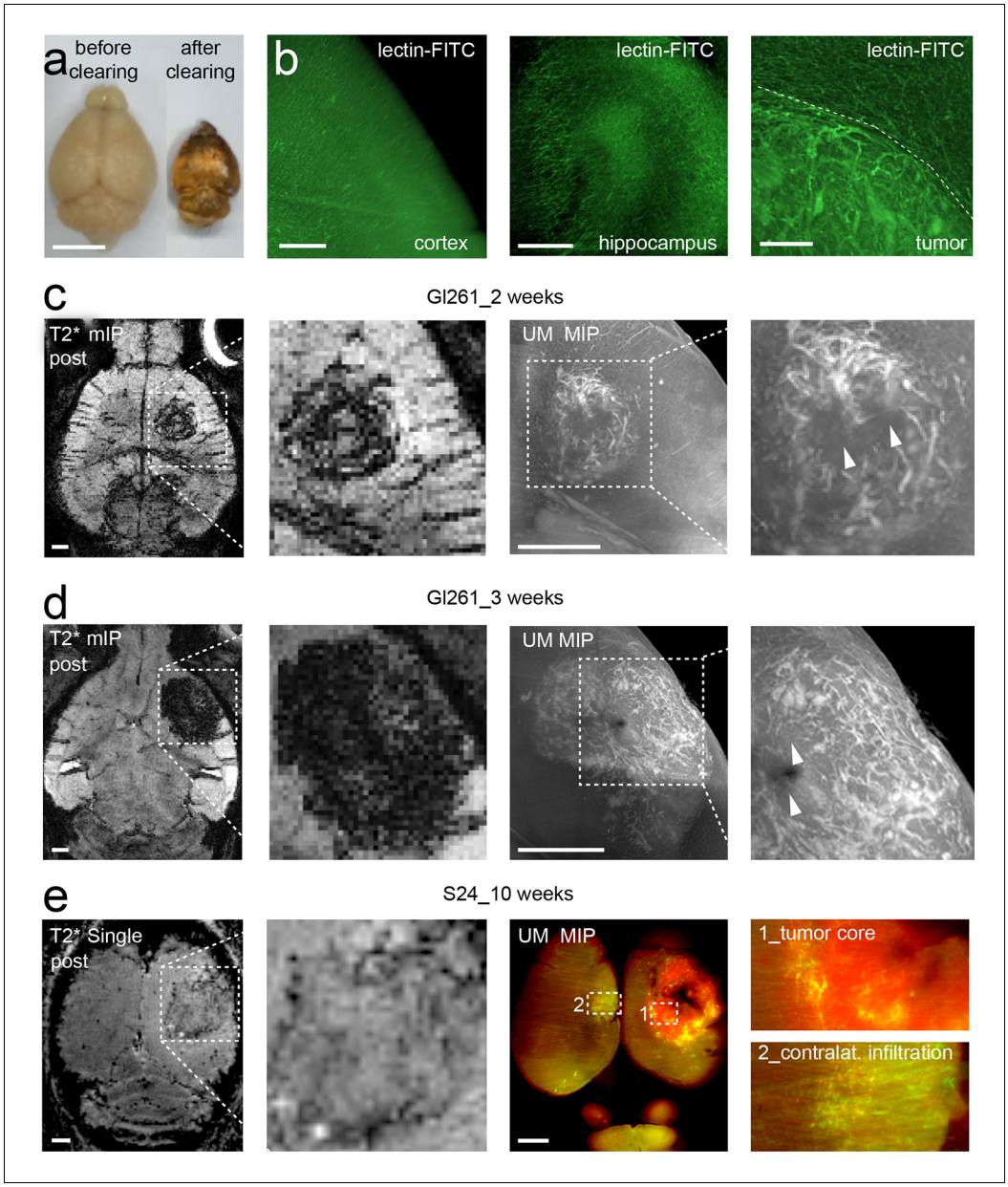

Figure 2. Correlated MR-UM provides complementary information of the tumor vascular architecture. Illustration of the mouse brain before and after clearing using the 3DISCO protocol. The brain shrinks by ~40% in size during the clearing protocol (**a**). Cleared UM images of lectin-FITC stained microvessels. Images show the healthy cortex (left) and hippocampus (middle). The glioma-stroma border (dotted line) is depicted on the right image (**b**). T2*-w images and correlative UM images 2 weeks, (**c**) and 3 weeks (**d**) after GL261 tumor implantation. Arrowheads indicate areas of necrosis. T2*-w image and correlative UM images 10 weeks after S24:td-tomato implantation. Inner necrotic tumor areas around the injection track lack fluorescent signal (**e**). The microvasculature is stained with lectin-FITC. MIP: maximum intensity projection, mIP: minimum intensity projection. Scale bar in (a) is 5 mm and 1 mm for MR images. For UM scale bars are 250 μm in (b) and 1 mm in (c-e).

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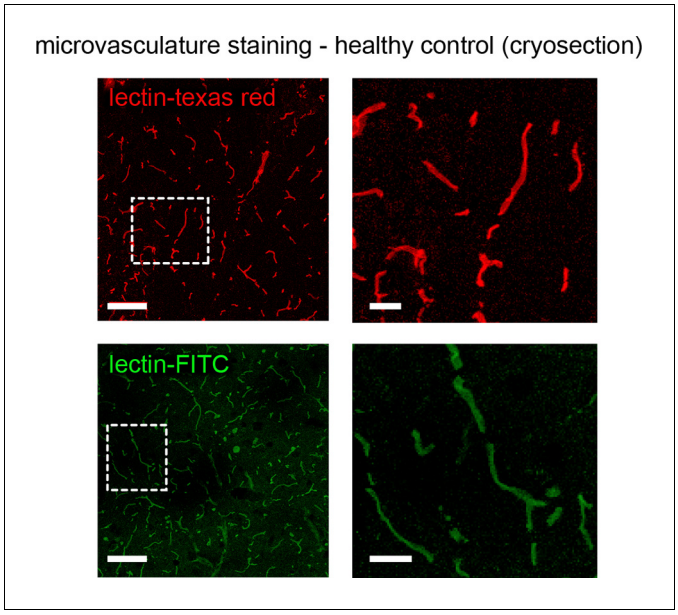

Figure 2—figure supplement 1. Lectin-FITC and lectin-texas red staining in healthy mice. Representative cryosections (10 µm thickness) of microvessels stained with iv lectin-FITC or lectin-texas red. Dashed boxes indicate magnified area (right side). Scale bars are 100 µm on overview images and 25 µm on magnified images.
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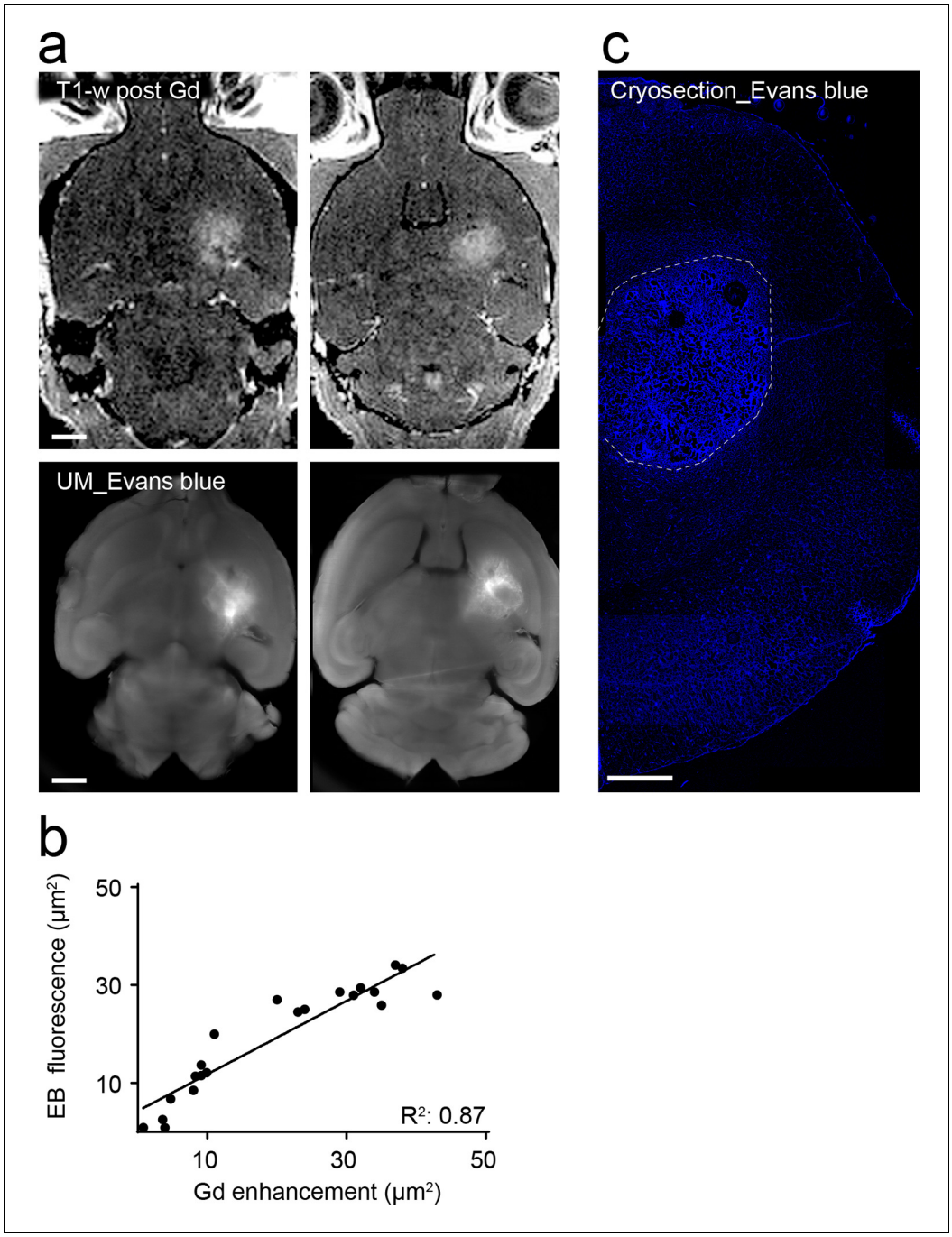

Figure 2—figure supplement 2. Correlated permeability imaging using MR-UM. Two levels of MRI (upper row) and UM images (lower row) after Gd or evans blue injection. The permeability and disrupted BBB can be compared side-by-side (**a**). Correlation analysis of BBB-D on MR and UM (**b**). Evans blue extravasation on cryosection, as assessed by confocal microscopy. The image shows a tile scan of the right hemisphere 2 weeks after GL261 tumor implantation (**c**). Dashed line indicates the tumor-stroma border. Scale bars = 1 mm in (a) and 500 µm in (c).

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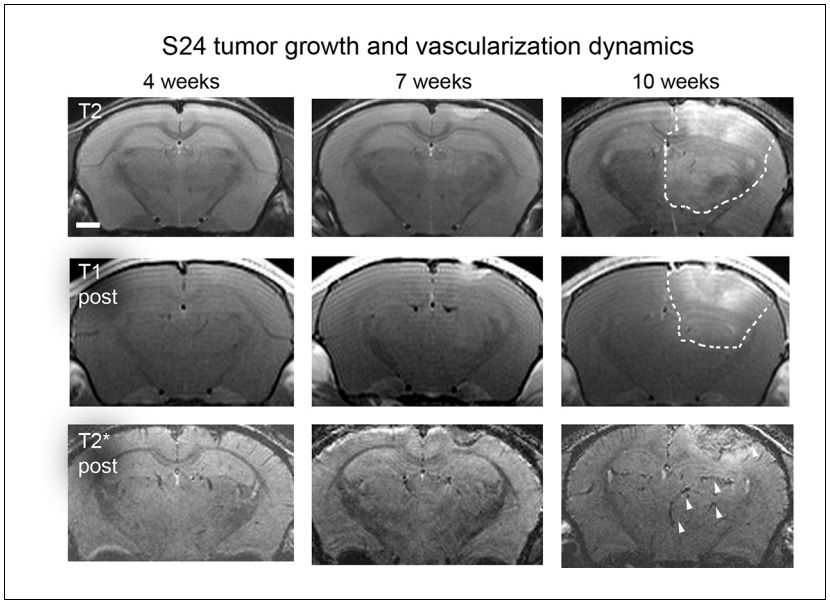

Figure 2—figure supplement 3. Time course of S24 tumor development. T2-w (upper row), T1-w post Gd-contrast (middle row) and T2*-w post-contrast images of S24 tumors 4, 7, and 10 weeks post-tumor implantation. Dashed outline depicts hyperintense areas. Note the heterogeneous tumor mass/edema (T2 signal) and BBB-D (contrast enhancement on T1-w). Arrowheads indicate vascular susceptibility signals. Scale bar = 1 mm.
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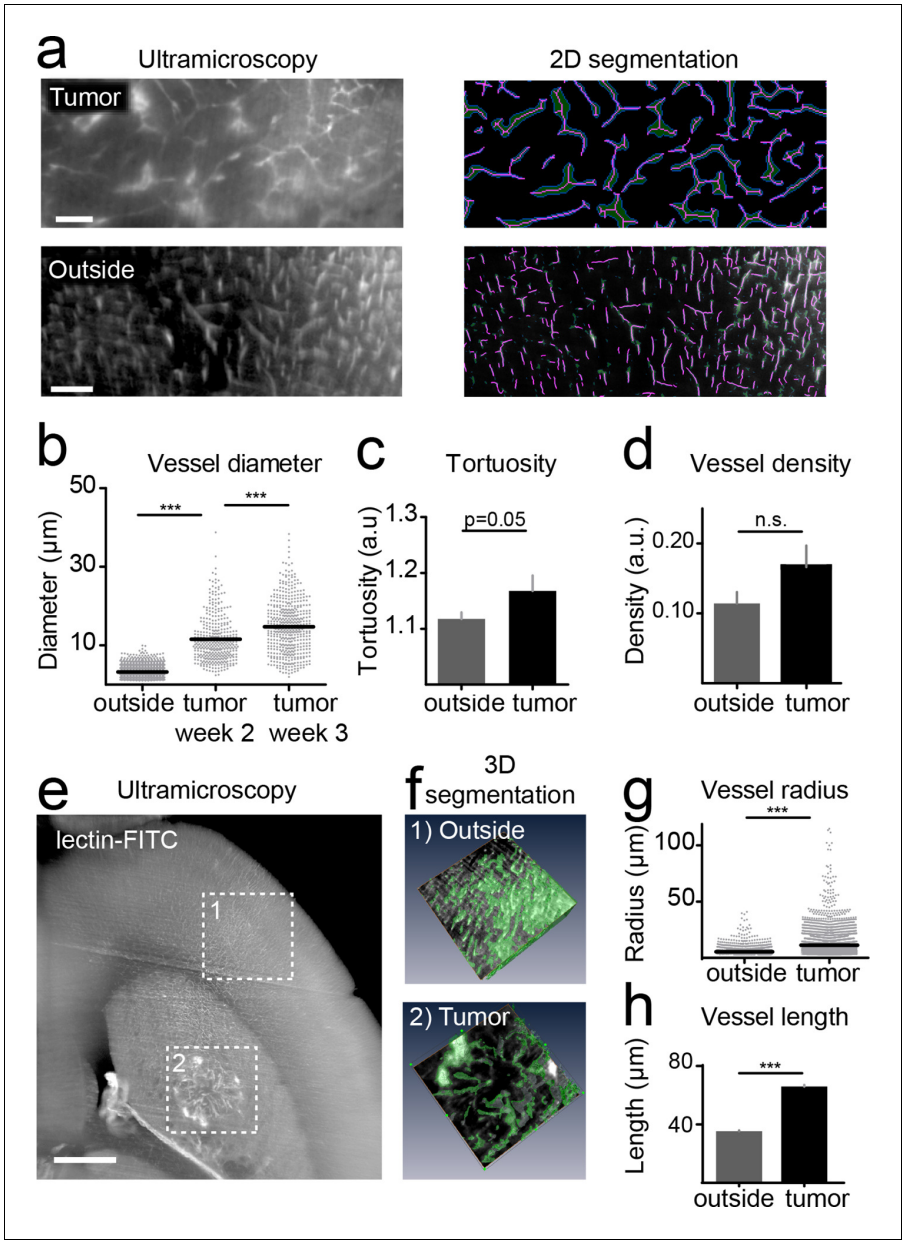

Figure 3. Quantification of neoangiogenesis by ultramicroscopy. Representative single plane image of a cleared brain in the tumor and an 'outside' region. Illustration of the vessel segmentation using the 'tubeness' plugin. Vessel segments (magenta) and vessel outline (green) are used to determine the vascular diameter (**a**). Quantification of the vessel diameter, tortuosity and vessel density (**b–d**). Illustration of vessel segmentation in 3D (**e**,**f**). Vessel radii (**g**) and pathlength (**h**) are shown. Scale bars = 100 µm in (a) and 500 µm in (e).

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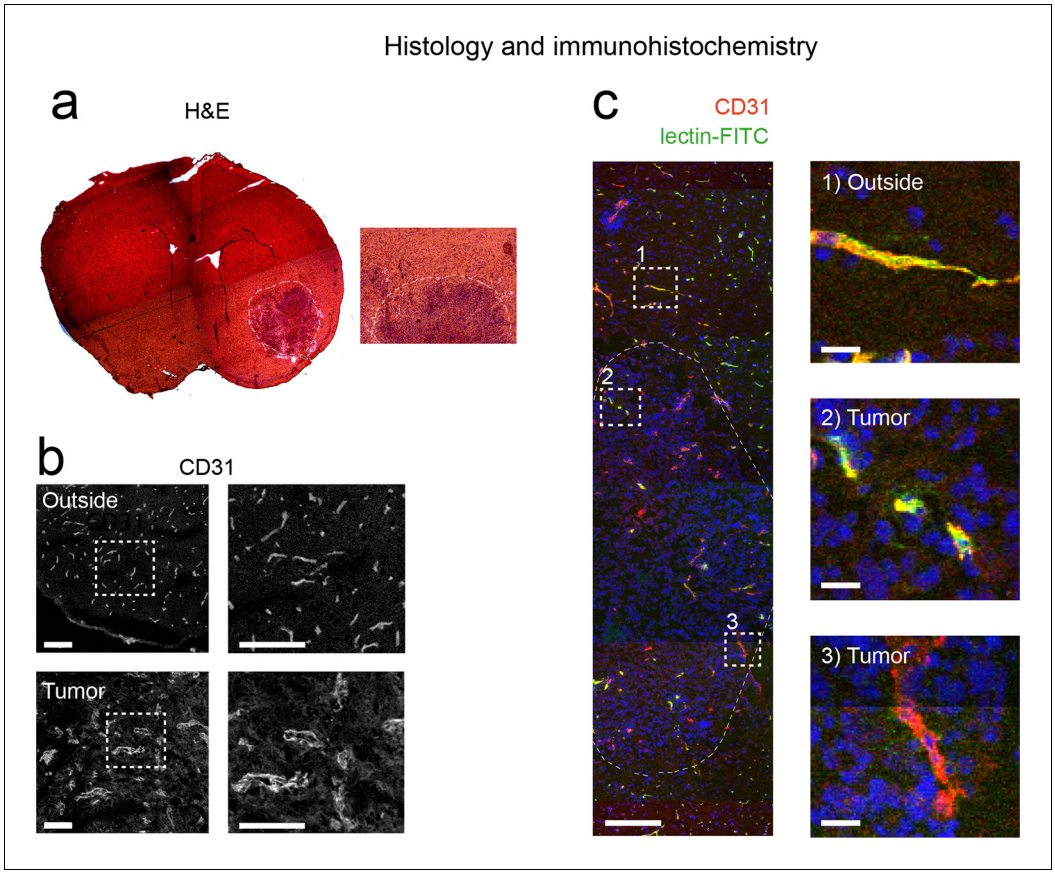

Figure 3—figure supplement 1. Histological assessment. Hematoxylin and eosin staining shows the tumor in the right hemisphere. Magnified image depicts the tumor-stroma border (**a**). Immunohistochemistry for CD31 shows vascular staining patterns in the tumor and an outside region (**b**). Representative immunohistochemistry image, stained for CD31 (red) from a mouse injected with lectin-FITC (green), (**c**). Magnified images show the co-localization of CD31 and lectin outside of the tumor and two tumor vessels (one double positive vessel, middle row; one vessel only positive for CD31, lower row). n=3 mice. Dashed boxes indicate magnified area and dashed line shows the tumor-stroma border. Scale bars = 100 μm in a,b; 200 μm in c and 20 μm in magnified images.
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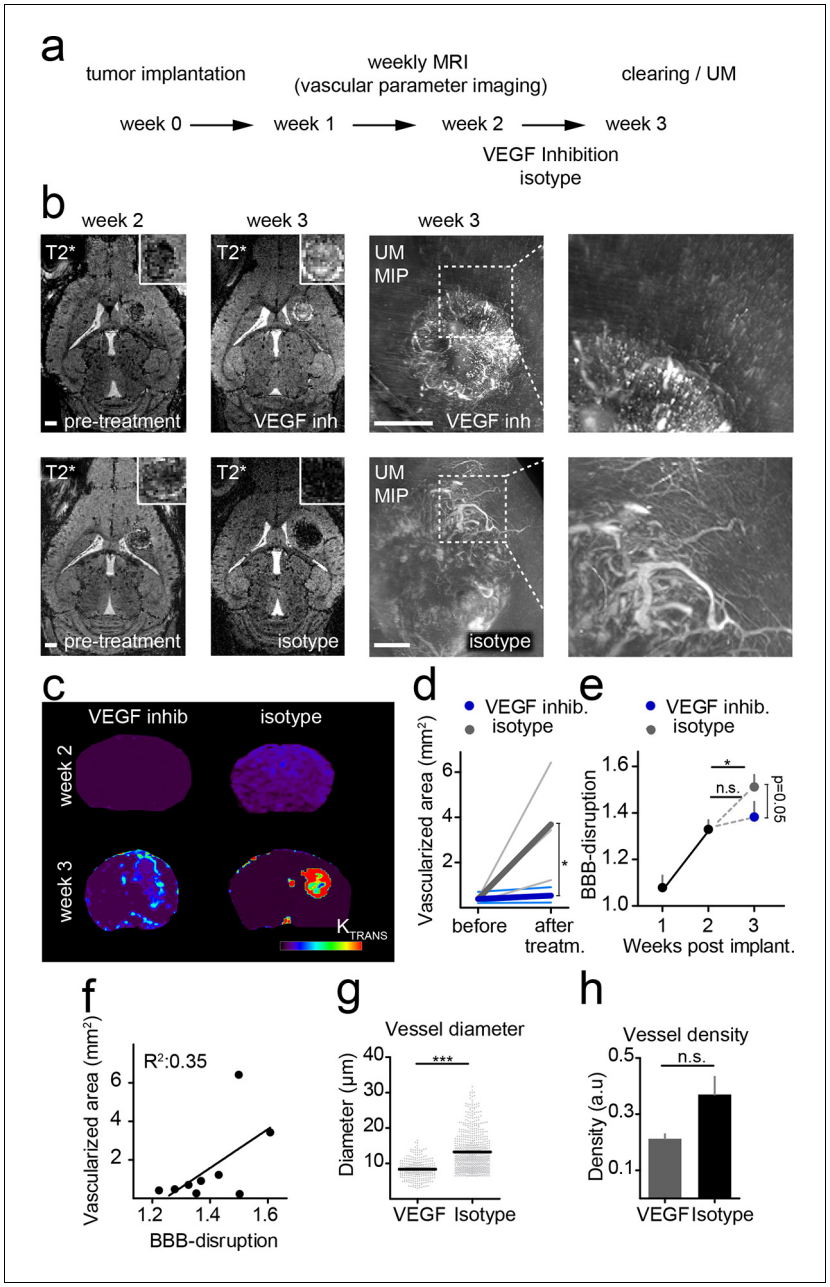

Figure 4. Monitoring treatment effects of VEGF inhibition on glioma vessels using MR-UM. Experimental outline (**a**). Single plane, T2*-w images before (week 2) and after VEGF or isotype control treatment (week 3). Treatment was initiated 2 weeks after tumor implantation when a solid tumor component had formed as confirmed on MRI. Correlative UM is shown of the same animal (**b**). Permeability (K_{trans}) maps, calculated from DCE MRI are depicted in (**c**). Quantification of the vascularized area on T2*-w images (**d**). Quantification of the blood-brain barrier disruption (BBB-D) on DCE images (**e**). Correlation of BBB-D and the vascularized area (**f**). Quantification of vessel diameter and vessel density on UM images (**g**,**h**). MIP: maximum intensity projection. Scale bars are 1 mm on MR images and 500 µm on UM images.

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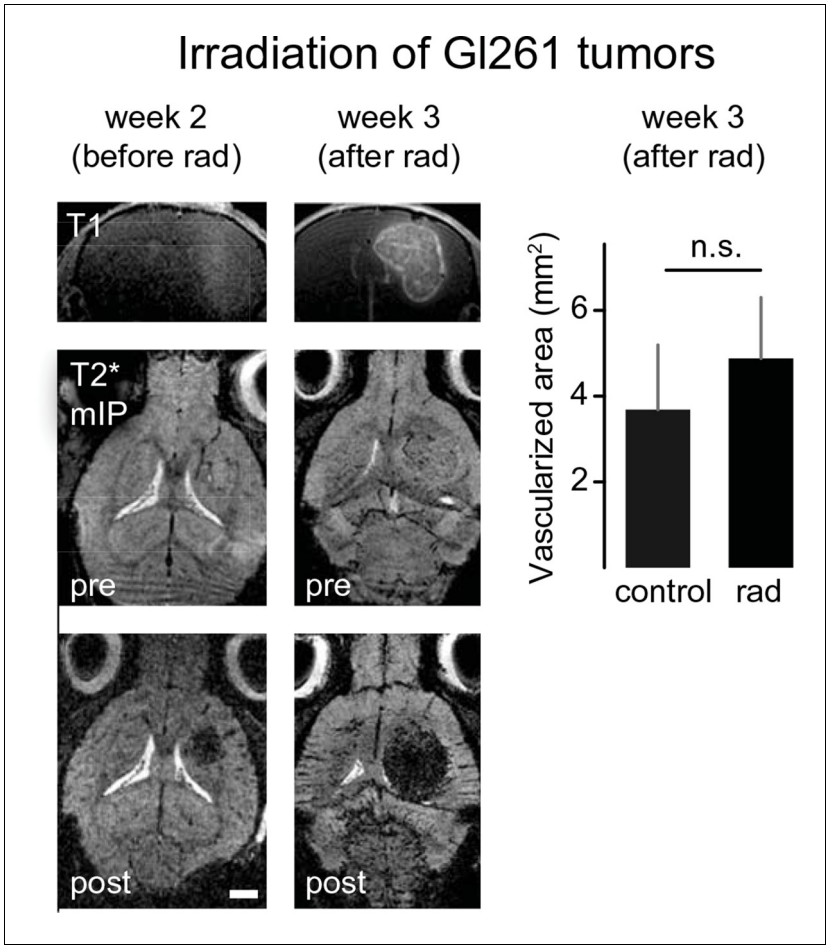

Figure 4—figure supplement 1. Irradiation does not reduce tumor vascularization. T1-w post contrast images show tumor growth in irradiated mice (upper row). T2*-w images post-contrast (middle and lower row) 2 (before treatment) and 3 weeks post-tumor implantation (after treatment). Irradiation with two gray was performed on 4 consecutive days starting 2 weeks post-tumor implantation. Tumor sizes and vascularization status were assessed 3 weeks post-tumor implantation. mIP: minimum intensity projection. Scale bar = 1 mm.
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