Supplemental material

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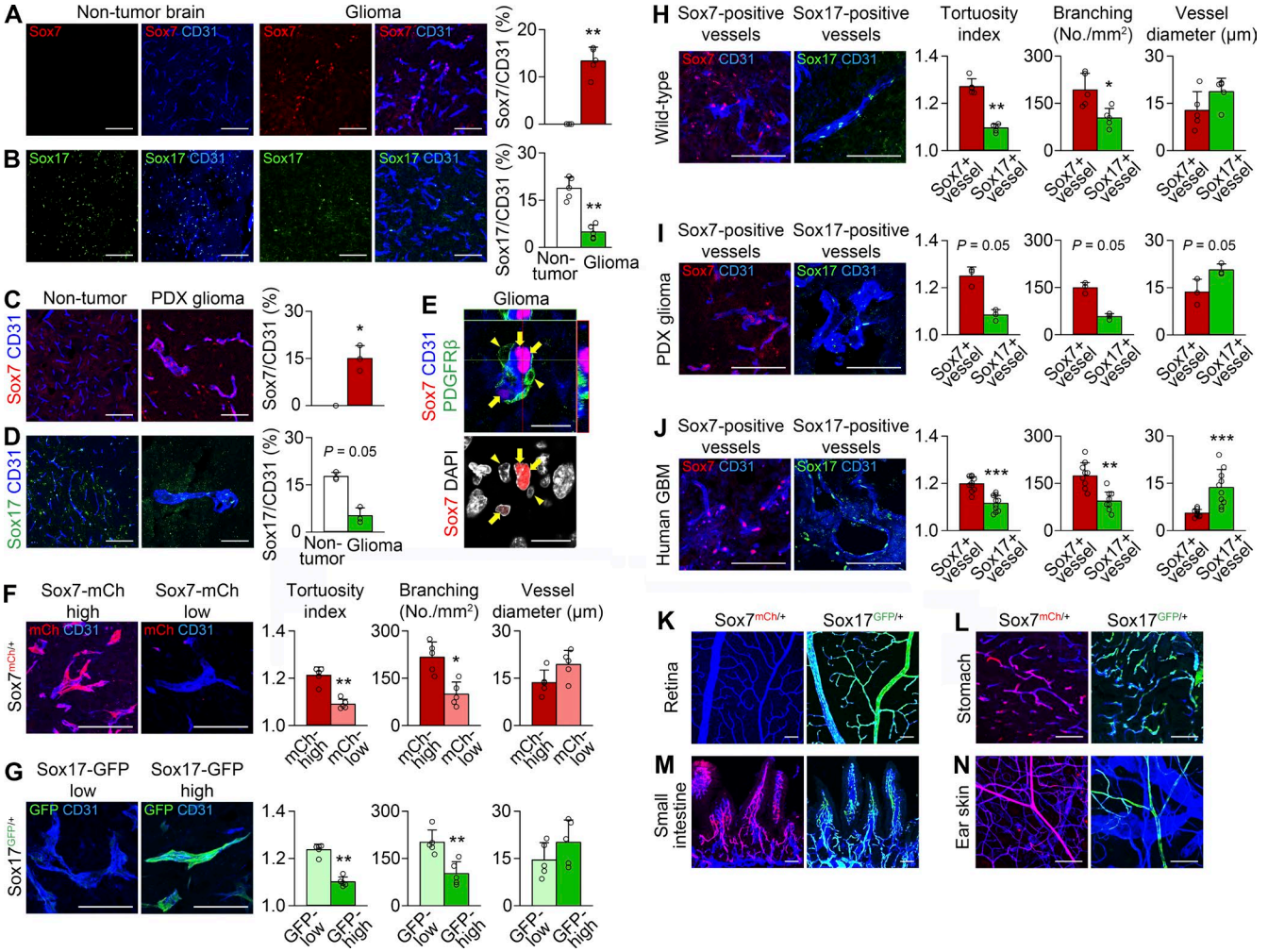

Figure S1. **Sox7 and Sox17 levels with an inverse relationship categorize HGG vessels by morphology. (A–E)** CD31-positive vessels in nontumor brain and orthotopic HGG. (A–D) Increased Sox7 and repressed Sox17 immunostaining in the vessels of GL261 HGG grown in wild-type mice (A and B) and patient-derived xenograft (PDX) HGG grown in nude mice (C and D). (E) Sox7 immunostaining specific to endothelial nuclei but not pericytes in GL261 HGG vessels. Horizontal green and vertical red lines indicate the corresponding locations of each z-stack views in the orthogonal plane image. The thickness of the single layer was 0.5 µm, and z-axis stacking was 4.5 µm. Arrows and arrowheads indicate nuclei of ECs and pericytes, respectively. **(F–J)** Categorization of HGG vessels and quantification of vessel tortuosity, branching, and diameter for each category. (F) Sox7-mCherry–high and –low tumor vessels in *Sox7*$^{mCh/+}$ mice. (G) Sox17-GFP–low and –high tumor vessels in *Sox17*$^{GFP/+}$ mice. (H–J) Sox7- and Sox17-positive vessels in GL261 HGG grown in wild-type mice (H), in patient-derived xenograft HGG from 83NS glioblastoma stem cells (I), and in HGG patients (J). **(K–N)** Heterogeneous Sox7 and Sox17 expression in adult vessels. Sox7-mCherry and Sox17-GFP fluorescence in the retina (K), stomach (L), small intestine (M), and skin vessels (N) of 10-wk-old *Sox7*$^{mCh/+}$ and *Sox17*$^{GFP/+}$ mice, respectively. Values are presented as mean ± SD (n = 5 in A–H; n = 3 in I; n = 10 in J). *, P < 0.05; **, P < 0.01; ***, P < 0.001. Bars: 20 µm (E); 100 µm (A–D and F–N).

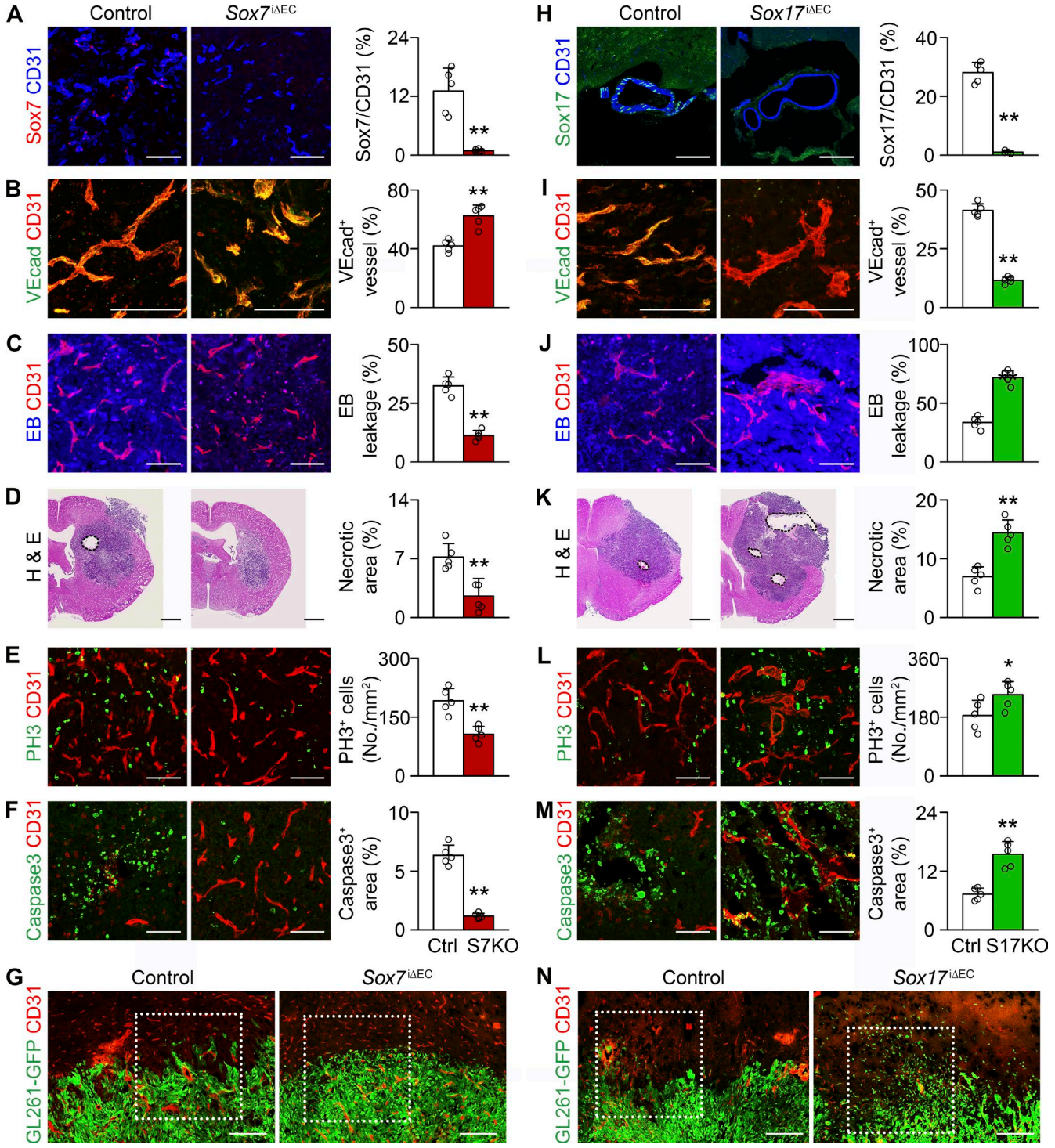

Figure S2. ***Sox7* and *Sox17* deletion induce opposite changes on HGG and its vessels. (A–N)** CD31-positive HGG vessels in control (Ctrl), *Sox7*$^{i\Delta EC}$ (S7KO; A–G), or *Sox17*$^{i\Delta EC}$ (S17KO; H–N) mice. (A and H) Efficient excision of *Sox7* (A) and *Sox17* (H) genes. (B and I) VE-cadherin (VEcad) distribution and quantification. (C and J) Evans blue (EB) and quantification of leakage. (D and K) H&E staining and quantification of the necrotic area. (E and L) PH3-positive cells and quantification of proliferating cells. (F and M) Caspase 3–positive cells and quantification of apoptotic cells. (G and N) Wide views showing the invasive margin of HGG. The area marked by dashed boxes of G and N is magnified in Fig. 3 I and Fig. 4 K, respectively. Values are presented as mean ± SD (n = 5). *, P < 0.05; **, P < 0.01. Bars: 100 µm (A–C, E–J, and L–N); 1 mm (D and K).

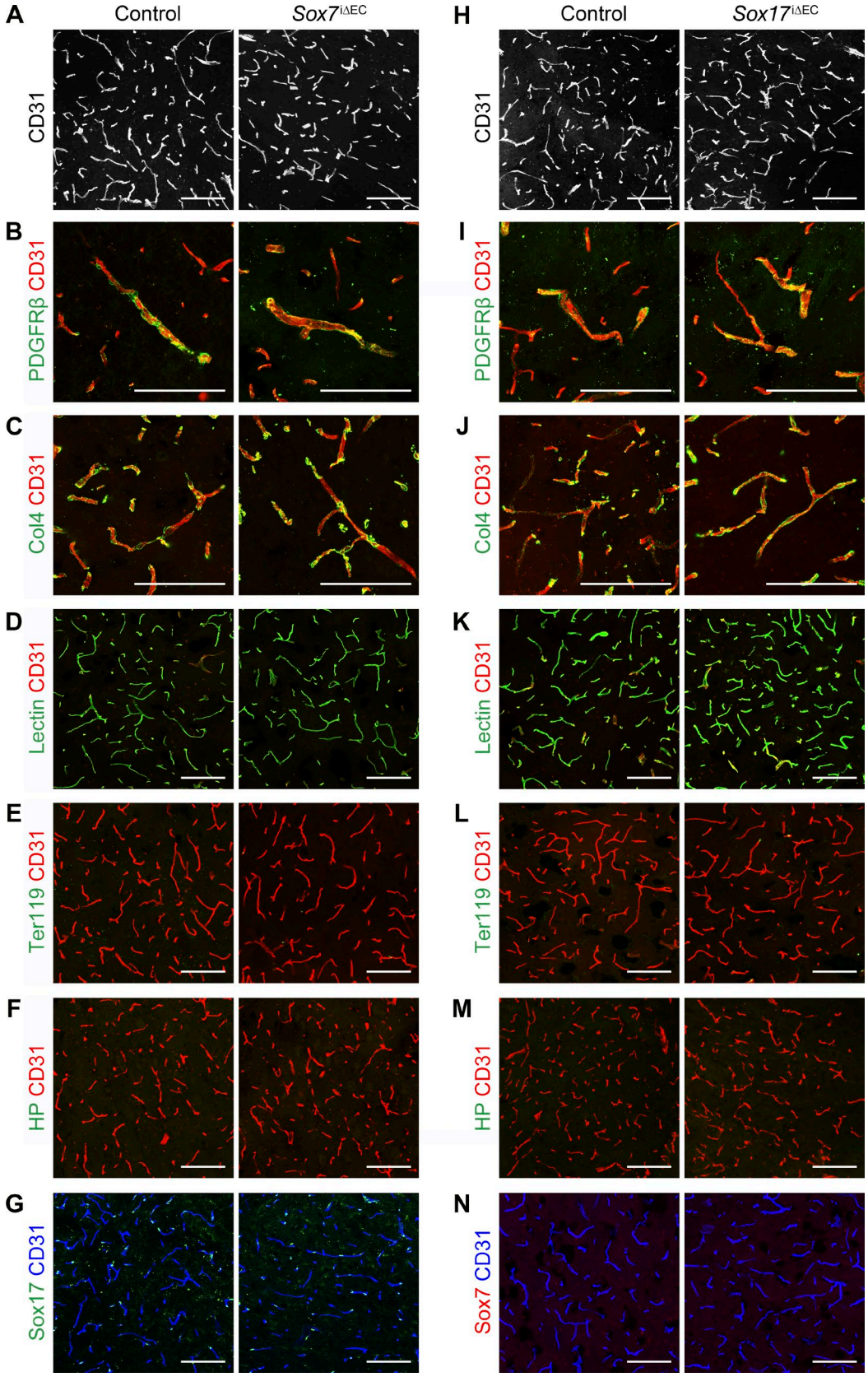

Figure S3. ***Sox7* and *Sox17* deletion have no effect on nontumor brain vessels. (A–N)** CD31-immunostained nontumor brain vessels in control, *Sox7*$^{\Delta EC}$ (A–G), or *Sox17*$^{\Delta EC}$ mice (H–N). (A and H) Morphology of the vascular network. (B and I) PDGFRβ-positive pericyte coverage. (C and J) Col IV–positive basement membrane coverage. (D and K) Efficient lectin perfusion. (E and L) No Ter119-positive erythrocyte leakage. (F and M) Rare immunostaining of pimonidazole adduct (hypoxyprobe, HP). (G and N) Sox17 (G) and Sox7 (N) immunostaining. Bars, 100 µm.

A

	Sox7 (+) group (n = 62)	Sox7 (-) group (n = 127)	P-value
Sex ratio	1.38 (M:F = 36:26)	1.44 (M:F = 75:52)	> 0.05
Age (years) min~max	55.79 31 ~ 78	54.28 16 ~ 80	> 0.05
Tumor volume (mm^3) min~max	44,529 ± 26,218 5,082 ~ 128,944	40,058 ± 34,025 3,200 ~ 236,600	> 0.05
Treatment	Surgery + CCRT STUPP's protocol	Surgery + CCRT STUPP's protocol	
Overall Survival			
Median (d)	552	742	< 0.05
1-y OS rate	69.3 %	84.3 %	
2-y OS rate	24.1 %	44.1 %	
3-y OS rate	8.1 %	23.6 %	
4-y OS rate	4.8 %	15.7 %	
5-y OS rate	3.2 %	8.7 %	
Progression-Free Survival			
Median (d)	306	475	< 0.01
1-y PFS rate	35.4 %	51.9 %	
2-y PFS rate	8.1 %	20.4 %	
3-y PFS rate	6.4 %	12.6 %	
4-y PFS rate	4.8 %	8.6 %	
5-y PFS rate	3.2 %	6.3 %	

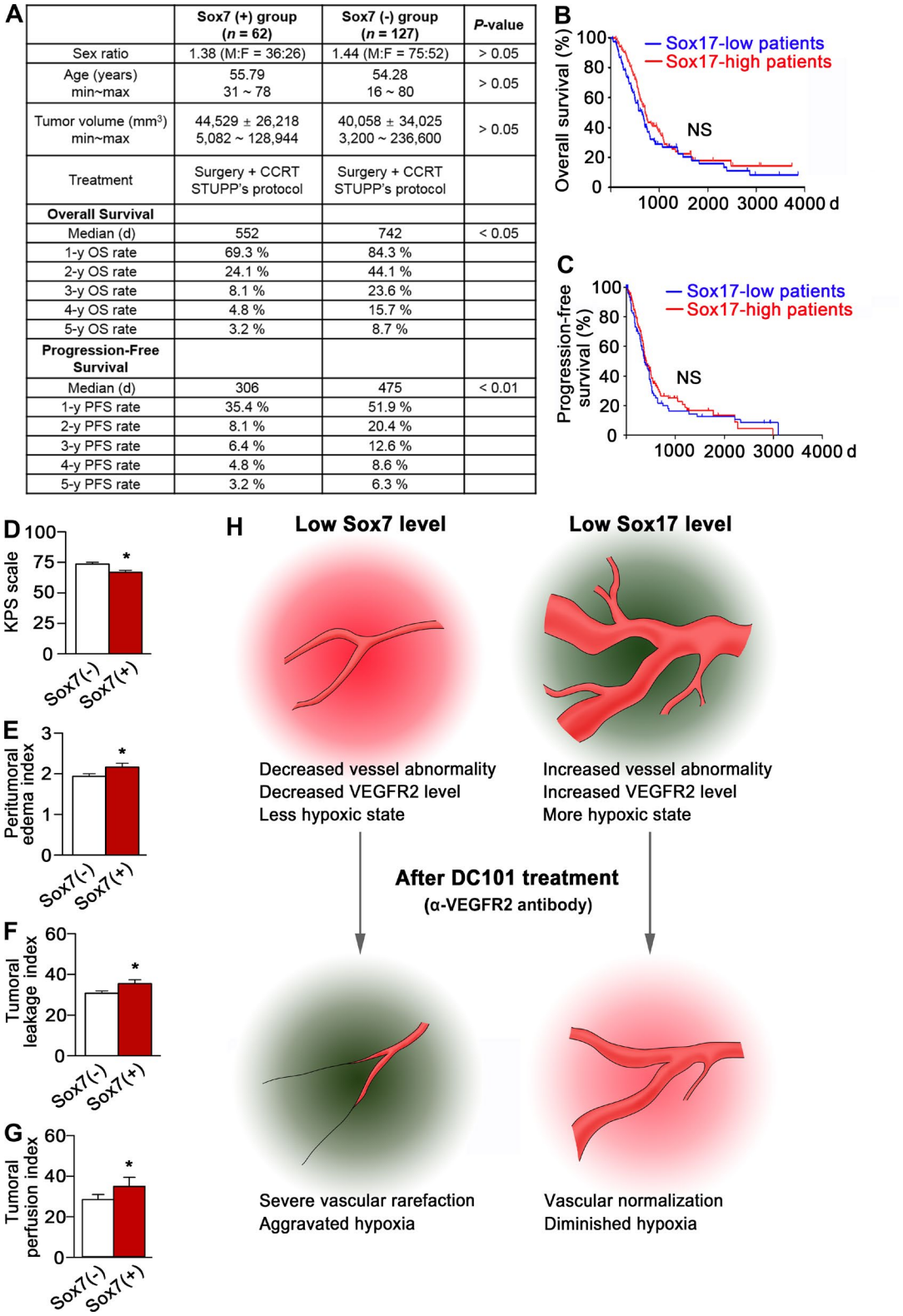

Figure S4. **Sox7 expression correlates with poor symptoms and a higher degree of tumor leakage of grade IV glioma patients. (A)** Clinical characteristics of the Sox7-positive and Sox7-negative grade IV glioma patient group. CCRT, concurrent chemoradiation therapy; OS, overall survival; PFS, progression-free survival. **(B and C)** Sox17 levels independent from prognosis of patients. Overall survival (B) and progression-free survival (C) of Sox17-high (n = 104) and Sox17-low (n = 85) grade IV glioma populations. **(D)** Scoring of initial performance by Karnofsky performance status (KPS). **(E)** Peritumoral edema index at initial diagnosis as measured by MR fluid-attenuated inversion recovery (FLAIR) imaging. **(F)** Quantitation of intratumoral leakage index as measured by DCE MRI in Fig. 10 (F, H, J, and L). **(G)** Quantitation of tumor perfusion as measured by perfusion MRI in Fig. 10 (G and K). Values are presented as mean ± SE (n = 62 for Sox7-positive and n = 127 for Sox7-negative grade IV glioma populations). **(H)** Diagram depicting biological behaviors of HGG and therapeutic response to α-VEGFR2 Ab affected by Sox7 and Sox17 levels. *, P < 0.05; Student's t test. NS, not significant.

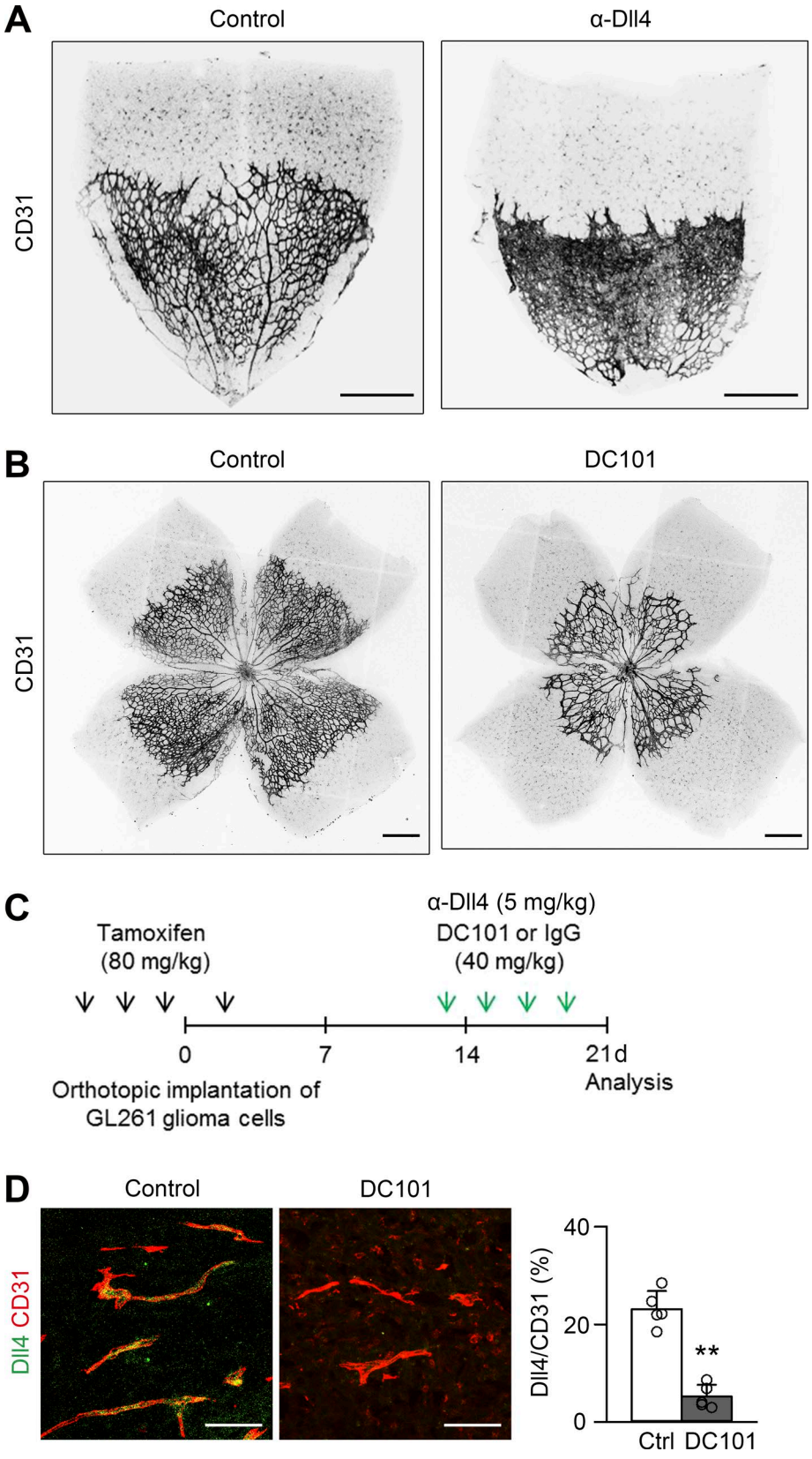

Figure S5. **Validation of α-Dll4 and α-VEGFR2 Abs. (A)** Increased vessel area and reduced outgrowth of postnatal retina vessel by 5 mg/kg α-Dll4 Ab compared with control (IgG) Ab. **(B)** Inhibited postnatal retina angiogenesis by 40 mg/kg α-VEGFR2 (DC101) Ab. **(C)** Schedule of glioma implantation, tamoxifen injection, and administration of control, α-Dll4, or DC101 Ab for experiments in Figs. 6 and 8. **(D)** Decreased Dll4 immunostaining in tumor vessels after DC101 treatment. Values are presented as mean ± SD (n = 5). **, P < 0.01. Bars: 200 µm (A and B); 100 µm (D).