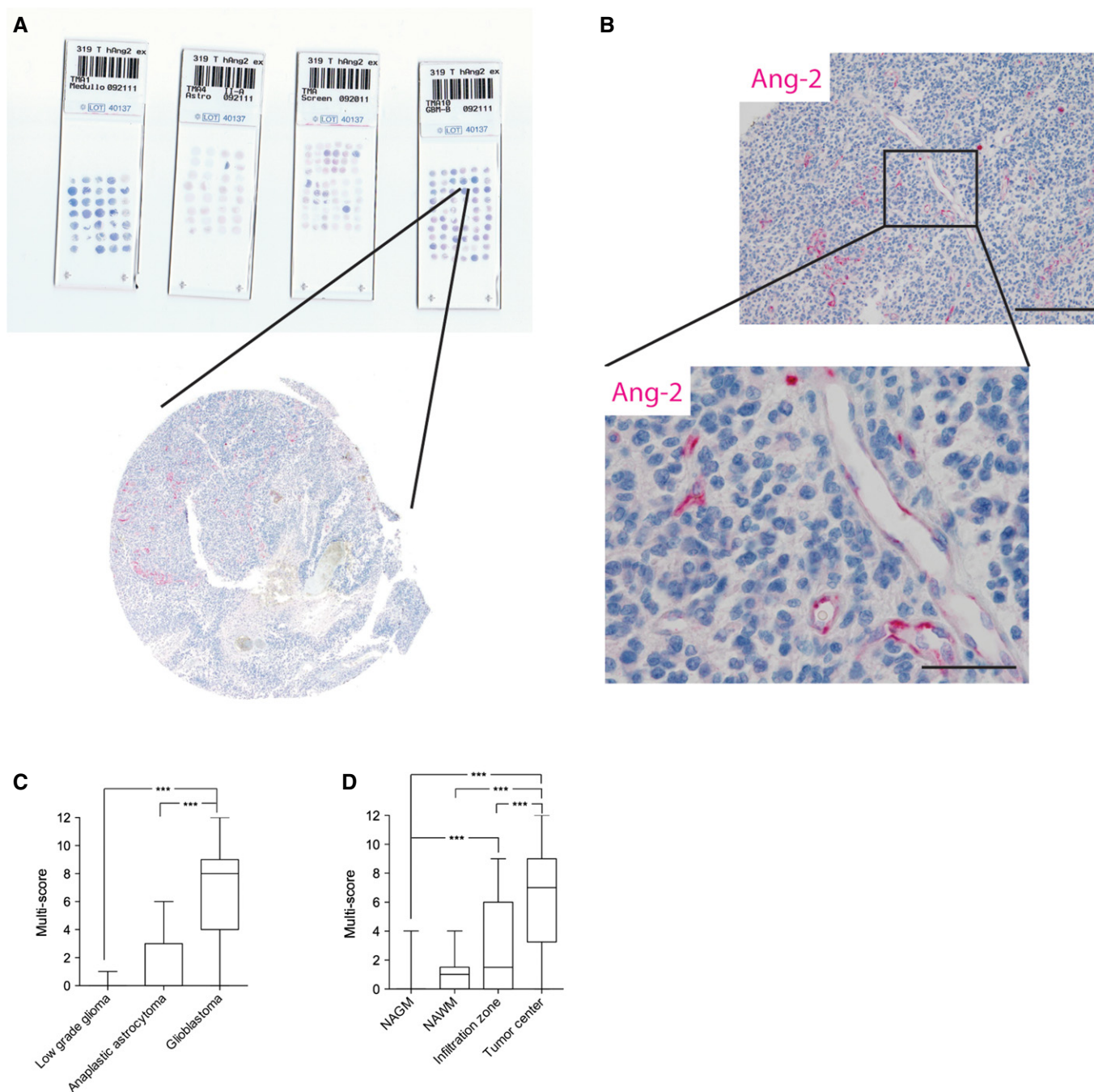# Expanded View Figures



**Figure EV1.  Application of tissue microarrays (TMAs) for the analysis of human glioma samples.**

A, B     Biopsies of 303 human gliomas (WHO grade II, *n* = 16, WHO grade III, *n* = 35, and WHO grade IV, *n* = 252) were spotted on microscope slides (A) and processed for automated anti-Ang-2 immunohistochemistry (Ventana Benchmark platform) (B). Scale bar (B): 200 μm, inset: 50 μm.

C         Ang-2 expression was assessed by applying a semiquantitative scoring system (Harter *et al*, 2010).

D         Spatial expression of Ang-2 in brain specimens from glioblastoma patients was scored in normal-appearing gray matter (NAGM) (*n* = 48), normal-appearing white matter (NAWM) (*n* = 18), infiltration zone (*n* = 39), and tumor center (*n* = 62).

Data information: In (C, D), for statistical analysis, Kruskal–Wallis test (followed by Dunn's post-test) was applied. ***$P$ < 0.005. Whisker Box plots displaying median, 25–75th percentile, upper and lower quartile.
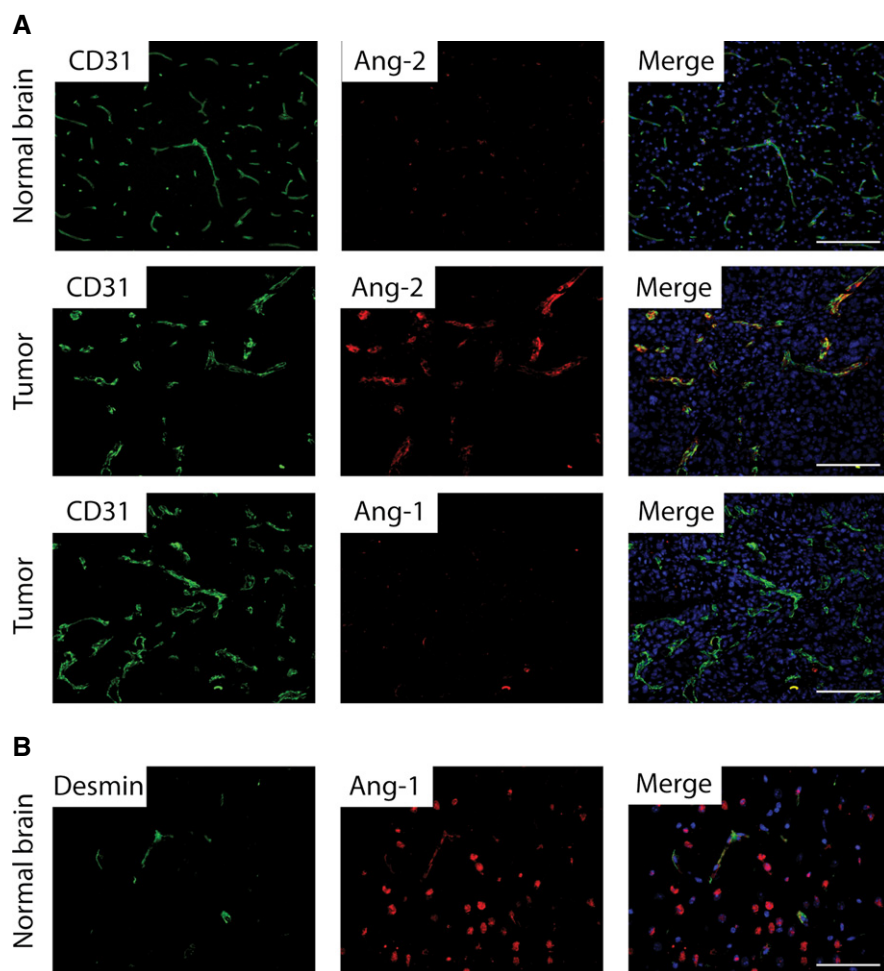
**Figure EV2.   Angiopoietin expression in mouse brain and GL261 tumors.**

A, B   Ang-2 and Ang-1 (red) immunofluorescence co-staining with anti-CD31 (green) was assessed in GL261 brain tumors (A). Ang-2 is not detectable in normal brain (A). Ang-1 (red) and desmin (green) double-immunofluorescence staining in normal mouse brain (B). Scale bars: 100 μm.
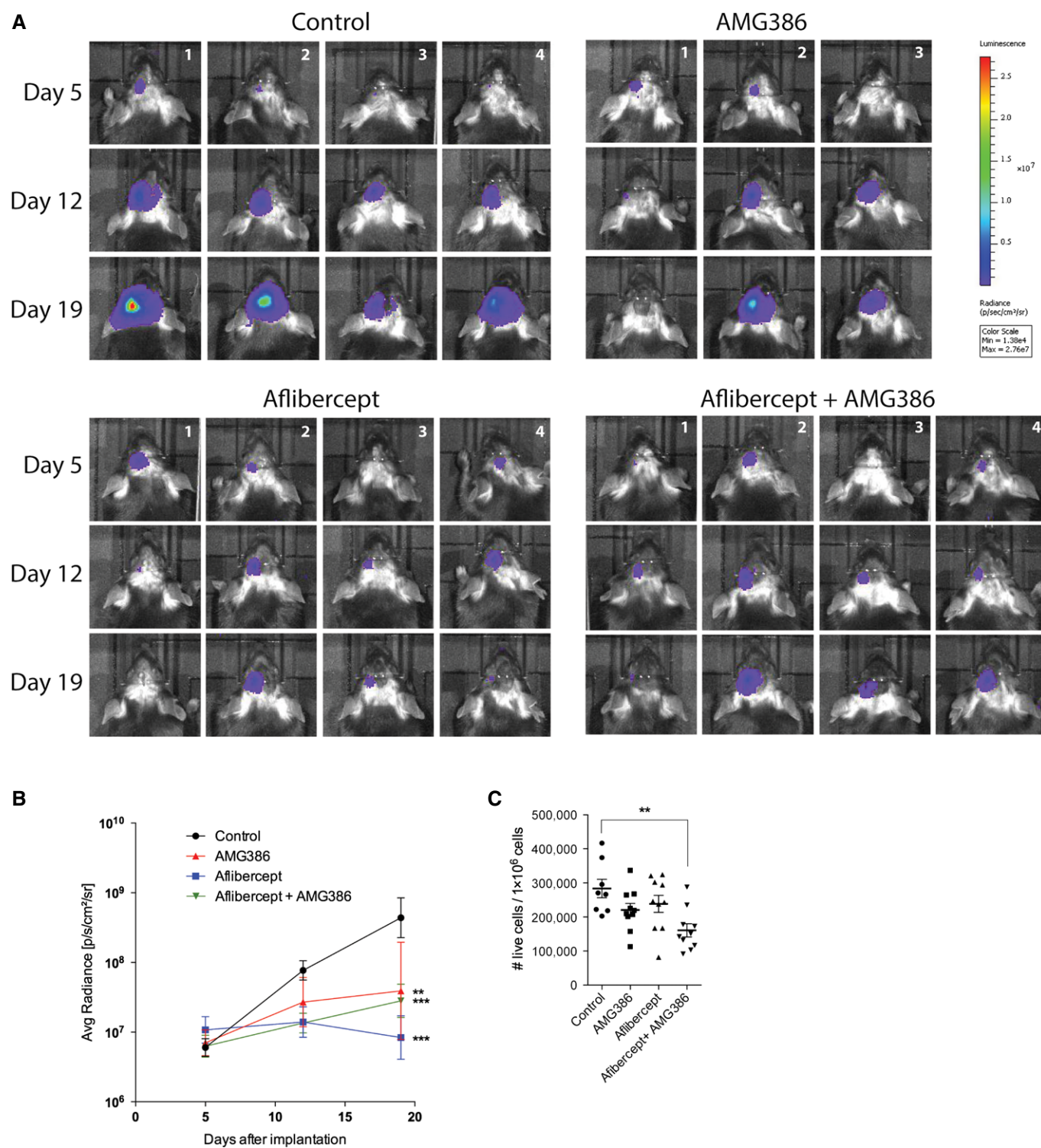
**Figure EV3. Noninvasive imaging of GL261 tumor growth following anti-angiogenic therapy.**

A, B    Bioluminescence imaging of GL261-luc glioma cells on day 5, 12, and 19 after surgical implantation following AMG386, aflibercept, and combination treatment (A), and corresponding quantitative analysis (B). (Control $n = 4$; AMG386 $n = 4$; aflibercept $n = 3$; AMG386 + aflibercept $n = 4$.)

C    Flow cytometric analysis of DAPI-negative live cells in mouse Gl261 brain tumors following anti-angiogenic therapy (control $n = 4$; AMG386 $n = 4$; aflibercept $n = 5$; AMG386 + aflibercept $n = 5$).

Data information: One-way (C) and two-way (B) ANOVA followed by Tukey post-test were performed for statistical analysis, $**P < 0.01$, $***P < 0.005$. Data are mean ± SEM (B), mean ± SD (C).
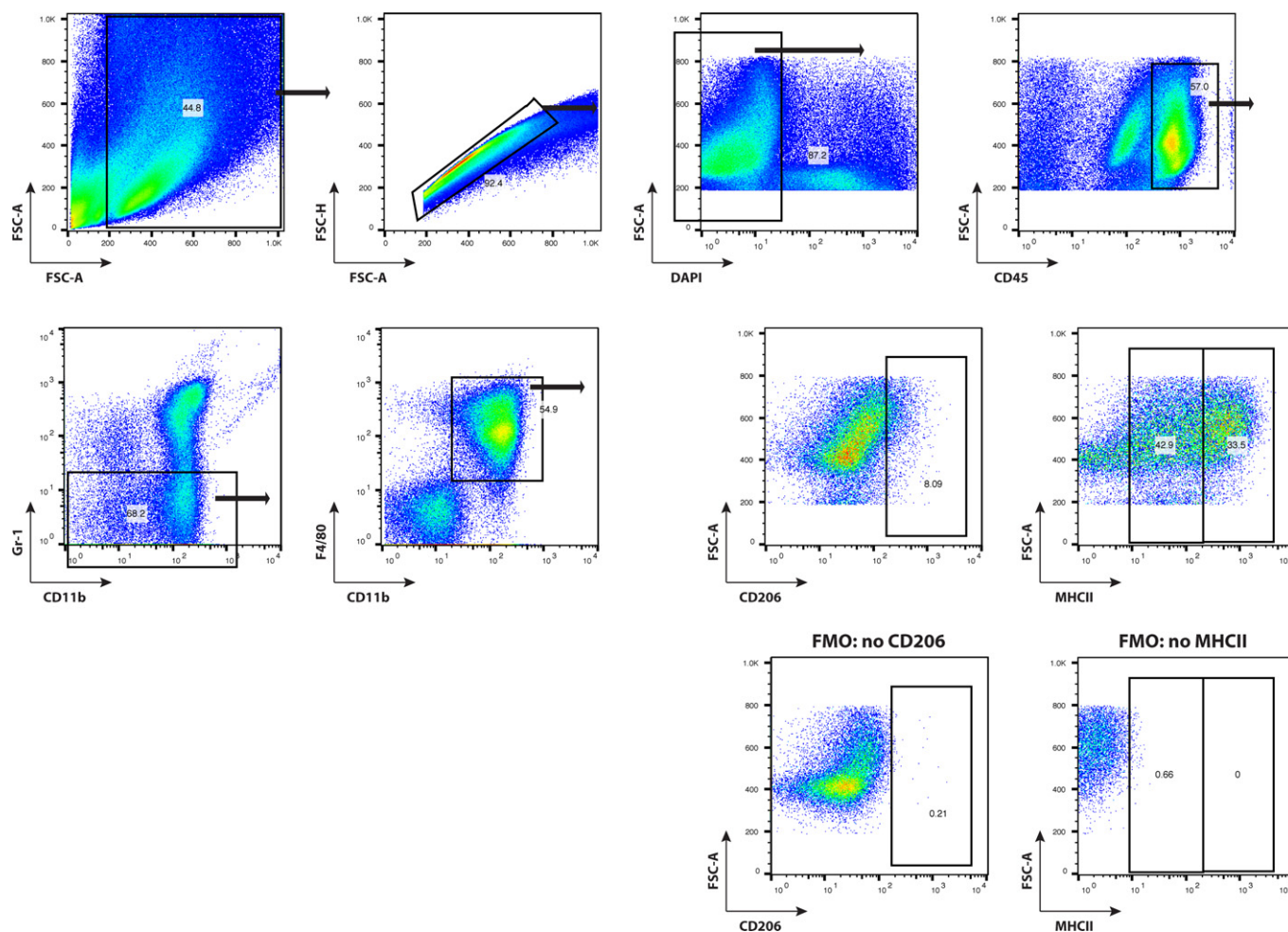
**Figure EV4.  Flow cytometry of brain tumor-infiltrating macrophages.**

Gating strategy to identify brain tumor-infiltrating macrophages is displayed. M2-polarized, pro-angiogenic macrophages are identified as CD45$^{Hi}$CD11b$^+$Gr-1$^-$F4/80$^+$CD206$^+$. M1-polarized macrophages are identified by co-expression of MHC class II (MHCII$^{Hi}$) on CD45$^{Hi}$CD11b$^+$Gr-1$^-$F4/80$^+$ TAMs.