

Figure S1. Reverse transcription-quantitative PCR of Ki67. Gene expression levels are shown across the entire cultivation period for 10 independent GBO preparations (n=10) at five time points: Days 0, 3-5, 7-8, 14-15, and 26-28. The x-axis represents days, the left y-axis shows ΔC_T values, and the right y-axis displays the logarithmic scale of expression relative to GAPDH as the housekeeping gene (GAPDH expression=1). Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparisons post hoc test. *P<0.05. GBO, glioblastoma organoid; Ki67, Kiel 67.

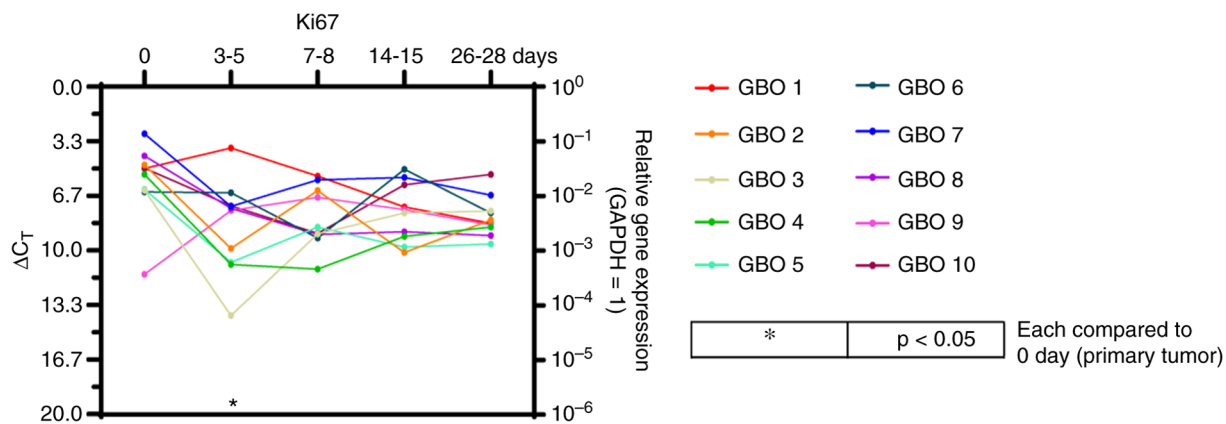


Figure S2. Reverse transcription-quantitative PCR analysis of glial structural proteins, proliferation, stemness, vessel, and immune cell markers. Gene expression levels are shown as box plots across the entire cultivation period for 10 independent glioblastoma organoid preparations (n=10) at five time points: days 0, 3-5, 7-8, 14-15, and 26-28. The x-axis represents days, the left y-axis shows ΔC_T values, and the right y-axis displays the logarithmic scale of expression relative to GAPDH as the housekeeping gene (GAPDH expression=1). (A) Glial structural proteins. (B) Proliferation marker. (C) Stemness markers. (D) Vessel marker. (E) Immune cell markers. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparisons post hoc test. * $P < 0.05$ and ** $P < 0.01$. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein B; Ki67, Kiel 67; OCT4, octamer-binding transcription factor 4; MS11, Musashi RNA-binding protein 1; SOX2, SRY-box transcription factor 2; KLF4, Krüppel-like factor 4; CD133, cluster of differentiation 133; CD31, cluster of differentiation 31; CD11b, cluster of differentiation 11b; Iba1, ionized calcium-binding adapter molecule 1; CD68, cluster of differentiation 68; CD3, cluster of differentiation 3.

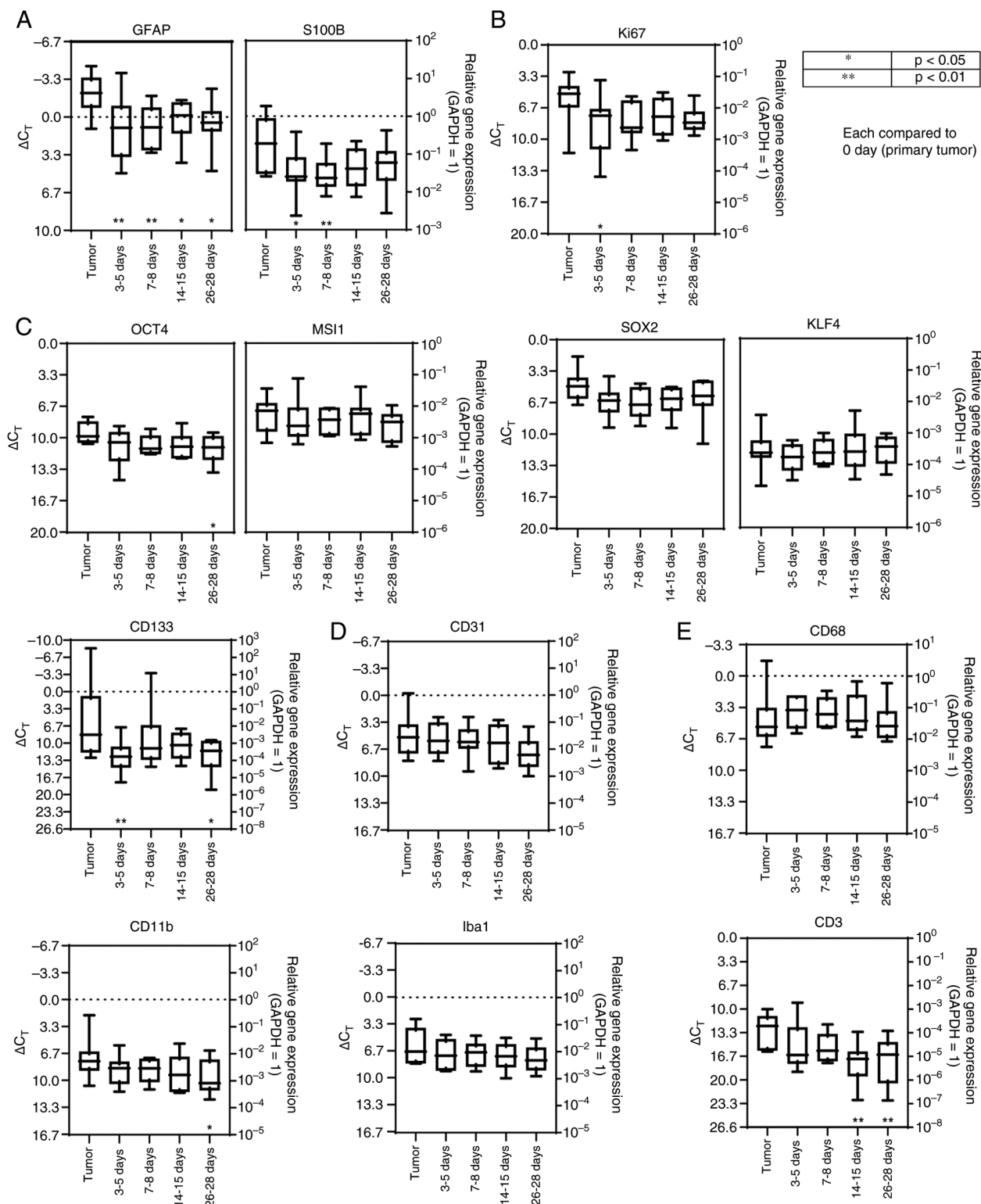


Figure S3. Reverse transcription-quantitative PCR analysis of chemokines and cytokines with their respective receptors. Gene expression levels are shown as box plots across the entire cultivation period for 10 different glioblastoma organoid preparations (n=10) at five time points: days 0, 3-5, 7-8, 14-15, and 26-28. The x-axis represents days, the left y-axis shows ΔC_T values, and the right y-axis displays the logarithmic scale of expression relative to GAPDH as the housekeeping gene (GAPDH expression=1). (A) Chemokines and chemokine receptors. (B) Cytokines and cytokine receptors. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparisons post hoc test. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CX3CL1, C-X3-C motif chemokine ligand 1; CX3CR1, C-X3-C motif chemokine receptor 1; CXCL12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C motif chemokine receptor 4; CXCR7, C-X-C motif chemokine receptor 7; CXCL16, C-X-C motif chemokine ligand 16; CXCR6, C-X-C motif chemokine receptor 6; IL-1 β , interleukin-1 beta; IL-1 β R, IL-1 β receptor; IL-6, interleukin-6; IL-6R, IL-6 receptor.

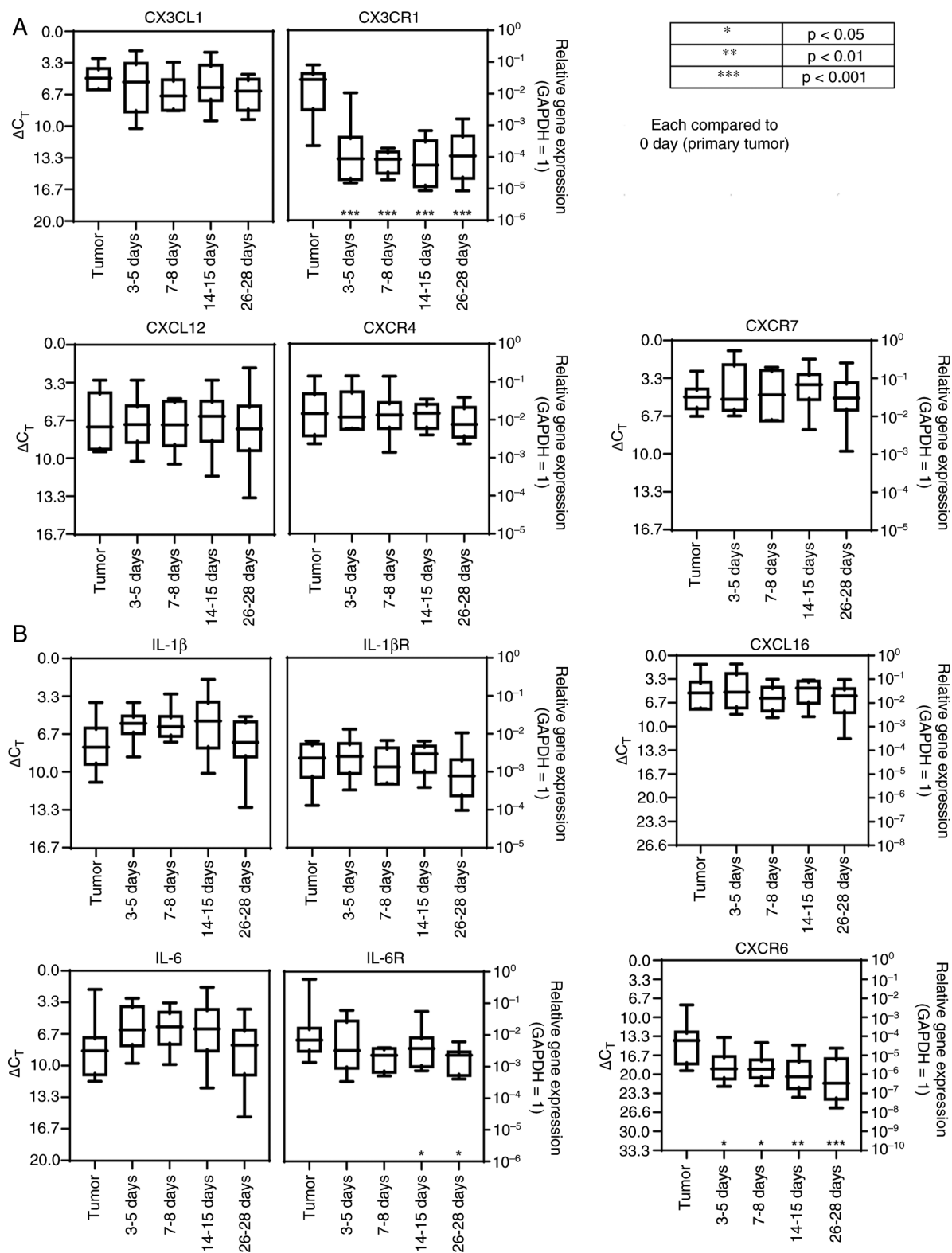


Figure S4. Immunofluorescence double-staining images of KLF4 from an exemplary glioblastoma organoid preparation at five time points: days 0, 4, 7, 14, and 28. GFAP, glial fibrillary acidic protein; KLF4, Krüppel-like factor 4.

