

Cellular and inflammatory characteristics of patient-derived glioblastoma organoids during *in vitro* cultivation

JESSICA NOJSZEWSKI*, NILS OLIVER SCHRÖDER*, JACQUELINE CLÜVER, JONNA HOLLER,
HAJRULLAH AHMETI, MICHAEL SYNOWITZ and JANKA HELD-FEINDT

Department of Neurosurgery, University Medical Center Schleswig-Holstein (UKSH), Campus Kiel, D-24105 Kiel, Germany

Received November 25, 2025; Accepted February 10, 2026

DOI: 10.3892/or.2026.9090

Abstract. Glioblastomas are characterized by extensive intra- and intertumoral heterogeneity, which drives their rapid invasive growth and frequent recurrence. As a result, prognosis remains poor, and developing effective treatments continues to be a major challenge. The survival and growth of glioblastomas are supported by a specific, protective tumor microenvironment (TME) that influences tumor morphology, growth rate, therapy resistance and recurrence. To better represent the complex TME with its immune cells and inflammatory mediators and the tumor heterogeneity, including glioma stem-like cells (GSCs), realistic *in vitro* preclinical models are required. Patient-derived glioblastoma organoids (GBOs), directly derived from tumor tissue obtained after surgical resection, were generated in the present study. Preserving the original tumor state in organoids, particularly regarding the TME and heterogeneity, is crucial. The present study evaluates the long-term preservation of key tumor properties in cultivated GBOs, including glial structural proteins, GSCs, immune cells, endothelial cells, proliferation marker, and inflammatory mediators such as cytokines and chemokines. Expression levels of these components were measured at five defined time points in 10 independent GBO preparations, at both the mRNA and protein level, and statistical correlation analyses were performed. The findings of the present study indicate that the key components were generally maintained over a one-month cultivation period. However, some markers showed reduced expression at later time points or recovered after an initial drop at early cultivation time points. Therefore, the optimal time window for employing patient-derived organoids as an

advanced preclinical model for glioblastoma research appears to be between 7-14 days of cultivation.

Introduction

Glioblastomas are among the most aggressive malignant brain tumors in adults, characterized by rapid and invasive growth and a high recurrence rate, making effective treatment a major challenge and contributing to their poor prognosis (1). The current standard therapy combines surgical resection and adjuvant radio-chemotherapy (2). However, median survival remains low at 14.6 months (3). Despite numerous clinical trials since the introduction of temozolomide, no new effective drugs have been developed, highlighting the urgent need for further research (2).

One of the main challenges in targeting glioblastomas is tumor heterogeneity (4). Based on transcriptional profiles, as well as genetic and epigenetic alterations, glioblastomas are highly diverse tumors that can be classified into distinct subtypes, even among patients with the same tumor grade (5). Differences in tumor composition between patients define intertumoral heterogeneity, whereas the presence of multiple subtypes within a single tumor in the same patient is referred to as intratumoral heterogeneity (6). A key contributor to intratumoral heterogeneity is the presence of glioma stem-like cells (GSCs), a subpopulation of cells with self-renewal capacity and tumor-initiating potential (4,7). GSCs not only drive tumor progression and recurrence but also interact dynamically with the tumor microenvironment (TME), which further supports their maintenance and resistance to therapy (4,7). This dynamic interaction between GSCs and the TME underscores the critical role of the TME, which acts as a protective niche for tumor cells (8). The TME comprises, for example, immune cells (microglia, T cells and macrophages), modulatory factors such as cytokines and chemokines, as well as structural elements including fibroblasts, pericytes, endothelial cells, and the extracellular matrix (8-10). Microglia and peripheral macrophages, collectively referred to as glioma-associated microglia/macrophages, infiltrate tumor regions and secrete inflammatory mediators, including cytokines and chemokines (9). These mediators bind to specific receptors, activating intracellular signaling pathways through second messengers and triggering diverse cellular responses that promote tumor growth and invasiveness (11-14). Chemokines

Correspondence to: Dr Jessica Nojszewski, Department of Neurosurgery, University Medical Center Schleswig-Holstein (UKSH), Campus Kiel, 3 Arnold-Heller-Street, D-24105 Kiel, Germany
E-mail: jessicaecem.nojszewski@uksh.de

*Contributed equally

Key words: glioblastoma, long-term cultivation, organoids, tumor heterogeneity, tumor microenvironment, tumoroids

further induce chemotaxis within the TME and play a key role in tumor metabolism, including cell proliferation, resistance to apoptosis, and the regulation of genes involved in these processes (10,11,15).

Given that glioblastoma is a highly heterogeneous tumor, developing research models that accurately preserve its complex TME remains a major challenge. Traditional models, including cell lines and animal models, have significant limitations (16,17). Cell lines often undergo spontaneous mutations and, in long-term cultures, also lack oxygen, nutrients, pH gradients, physiological inputs, and interactions between tumor cells and TME (18,19). Animal models are more time-consuming and high-cost models with ethical limitations (17,20). Furthermore, interspecies genetic differences complicate the representation of human pathophysiology and make the translation of data to human outcomes complex (16). Considering the significant limitations of traditional cell lines and animal models in accurately representing the complexity of glioblastoma, novel models are required. In this context, glioblastoma organoids (GBOs) have recently gained attention, as they preserve tumor heterogeneity by exhibiting structural and functional characteristics similar to those of human organs and tissues (17,21). Organoids can be derived either from stem cells or directly from tumor tissue (19,21). These models offer a high success rate in culture, short formation times, relatively low cost, and reproducible tumor traits (22). Moreover, organoids serve as valuable tools to study tumor biology, including the TME, and test radiotherapy, chemotherapy and immunotherapy (18,20–22). Human brain organoid technology was first introduced by Lancaster *et al* (2013) (23), marking the beginning of rapid advancements in this field. Since then, numerous brain tumor organoids have been developed and optimized (24). For example, Hubert *et al* (2016) (25) cultured patient-derived GBOs within two months using Matrigel and EGF/bFGF. Jakob *et al* (2020) (26) further optimized this method by eliminating Matrigel and growth factors, reducing the formation time of 70 organoid strains from 53 patients to just 1–2 weeks after tumor resection.

However, despite these advancements in cultivation speed and methodology, it remains unclear whether GBOs maintain the characteristics of the original tumor over a long-term cultivation period. This gap raises questions about the suitability of GBOs as reliable models for studying glioblastoma. The present study aimed to investigate the long-term preservation of key tumor properties, including cellular components such as GSCs, immune cells, endothelial cells, proliferation marker, and inflammatory mediators such as cytokines and chemokines in long-term cultivated GBOs.

Materials and methods

Cultivation of patient-derived GBOs. Tumor tissues were obtained directly via surgical resection (from a total of 10 patients: five women and five men aged between 53 and 75 years and with a median age of 61 years) by the Department of Neurosurgery (University Medical Center Schleswig-Holstein, UKSH Kiel, Germany) after written informed patient consent in accordance with the Helsinki Declaration of 1975, revised in 2013. Approval was granted by the Ethics Committee of the University of Kiel (approval no.

D524/17; Kiel, Germany). Histological analysis was performed by the Department of Pathology at the University Medical Center Hamburg-Eppendorf (UKE, Hamburg, Germany).

The obtained fresh tumor samples were prepared as previously described by Jacob *et al* (2020) (26). The preparation did not involve classical enzymatic or mechanical tissue dissociation. Instead, the tumor samples were only roughly cut and placed directly into culture. In detail, tumor samples were transferred to a sterile dish within H+GPSA wash medium containing Hibernate A, 1X GlutaMax, 1X PenStrep and 1X Amphotericin B (all from Thermo Fisher Scientific, Inc.), and then cut into smaller pieces excluding areas of cell death and blood vessels. Several wash steps were performed to remove tissue debris. Cutting was continued under a stereomicroscope Stemi 305 KMA (Carl Zeiss AG) to identify vessels and areas of cell death in detail, and to finally get pieces of 1–3 mm size. The medium was then removed, and RBC-lysis buffer (Thermo Fisher Scientific, Inc.) was added to selectively destroy red blood cells. After shaking at 2 x g at room temperature for 10 min, the buffer was removed, and another wash step was performed. GBO medium was prepared as previously described by Hellmold *et al* (2025) (27) and added to tissue pieces. A total of 2 ml of the medium was added to each well of an ultra-low attachment 6-well plate (Corning, Inc.). A total of ~10 tissue pieces in GBO medium were transferred into each well, resulting in a total volume of 4 ml. The plates were incubated on an orbital shaker (cat. no. 8012-1771; Binder GmbH) at 120 U/min at 37°C and 5% CO₂. The GBOs were observed regularly, and medium exchange was performed every second day (Fig. 1).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). To analyze gene expression levels of glial structural proteins, proliferation marker, specific markers for GSCs, immune and endothelial cells, as well as cytokines and chemokines during the *in vitro* cultivation of 10 different GBO preparations over time, RT-qPCR was employed. For each GBO preparation, RNA isolation was performed at five different time points using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.), and cDNA was then synthesized as previously described by Hellmold *et al* (2025) (27). Each cDNA sample was also analyzed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), serving as an internal standard. qPCR was performed using TaqMan primer probes (Applied Biosystems; Thermo Fisher Scientific, Inc.). Double determination, including positive and negative controls, was applied. The plate was run in a real-time PCR cycler (QuantStudio5; Applied Biosystems; Thermo Fisher Scientific, Inc.) using the following conditions: 50 cycles, volume of 20 µl, enzyme activation at 95°C for 10 min, denaturation at 95°C for 15 sec, and annealing at 60°C for 1 min. Cycles of threshold (C_T) were detected, and the ΔC_T values were calculated as $C_{T \text{ analyzed gene}} - C_{T \text{ GAPDH}}$. The applied gene-specific primers and probes are listed in Table SI.

Hematoxylin-eosin (H&E) staining and immunofluorescence double-staining. H&E staining was employed to assess the long-term preservation of the cellular structure of GBOs over cultivation time using the H&E Staining Kit (cat. no. 245880; Abcam).

Due to the limited material, a simple qualitative visualization of cellular markers was performed over time in different GBO preparations ($n=4$) on the protein level, using immunofluorescence double-staining with cryosections. The first primary antibody was added and incubated overnight at 4°C. The first secondary antibody, labeled with Alexa Fluor 488 or Alexa Fluor 555 (1:1,000; Thermo Fisher Scientific, Inc.), was incubated for 1 h at 37°C the next day. The second primary antibody was then incubated overnight at 4°C, followed by the second secondary antibody labeled with Alexa Fluor 488 or Alexa Fluor 555 (1:1,000; Thermo Fisher Scientific, Inc.) the next day. The applied primary antibodies are listed in Table SII. For negative controls, primary antibodies were omitted. Nuclei staining was subsequently accomplished with 4',6-diamidino-2-phenylindole (DAPI) for 30 min at room temperature. The embedded slides were analyzed using the fluorescence microscope (AxioObserver.Z1; Carl Zeiss AG) with the ZEN 3.5 (Blue Edition) software (Carl Zeiss AG).

Statistical analysis. Statistical analyses were accomplished using GraphPad Prism 8.4[®] software (GraphPad Software, Inc. Dotmatics) with a one-way ANOVA with Dunnett's multiple comparisons post hoc test as indicated for each experiment in the figure captions. Microsoft Excel was used to perform correlation analyses by calculating the Pearson correlation index. Statistical significance in both is outlined according to the P-value: $P<0.05$ was considered to indicate a statistically significant difference. The sample size is stated in the figure captions.

Results

GBO cellular characteristics generally persist over the course of one month. After obtaining tissue pieces of 1-3 mm in size (Fig. 1), the cellular GBO characteristics were addressed in a total of 10 different GBO preparations during long-term cultivation. For this purpose, the quantitative gene expression levels of structural proteins, proliferation, stemness, immune cells, and vessel markers were analyzed using RT-qPCR, as shown in Figs. 2 and S1, with the respective findings for each individual GBO preparation traceable. In addition, all RT-qPCR results were presented as box plots as an alternative representation to provide an improved overview (Fig. S2).

First, the Ki67 mRNA expression, a well-established proliferation marker in glioblastoma (28), was analyzed. Although the expression level decreased at 3-5 days in most GBO preparations, a recovery was observed (Figs. S1 and S2B), and expression remained detectable for up to 28 days, confirming the viability of the GBOs.

Glial fibrillary acidic protein (GFAP) and S100 calcium-binding protein B (S100B) were used as markers for structural proteins. GFAP is involved in glial differentiation and serves as a classical marker for glial tumor cells (29), whereas S100B is overexpressed in malignant gliomas and modulates microglia/macrophage activation (30). In the data of the present study, GFAP mRNA expression also exhibited a drop at 3-5 days in most GBO preparations, reaching its lowest point, as reflected by a negative correlation when comparing the GFAP expression data between the primary tumor tissue and GBOs cultured for 3-5 days. This drop was followed by

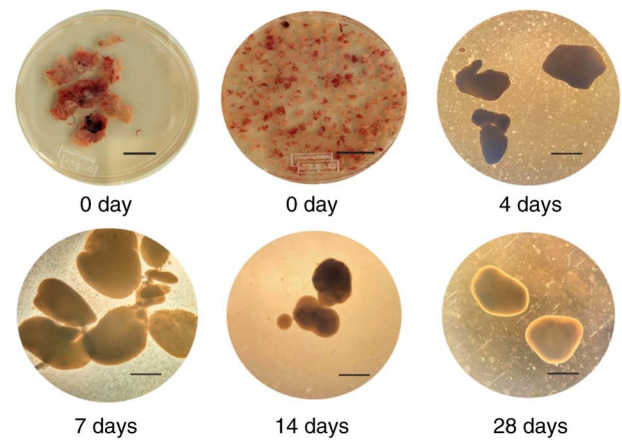


Figure 1. GBO preparation based on Jakob *et al* (26). The process is shown from day 0 to day 28. At day 0, the resected tumor tissue is displayed on the left, with smaller dissected pieces in the center of the image. GBO morphology was observed on days 4, 7, 14, and 28 using light microscopy. The scale bar at day 0 represents 1 cm; scale bars for days 4, 7, 14, and 28 represent 1 mm. GBO, glioblastoma organoid.

a gradual recovery with a positive correlation between the primary tumor tissue and GBOs cultured for 26-28 days (Figs. 2A and E, and S2A). However, the overall GFAP expression level remained lower at the end of the observation period compared with the beginning. S100B showed a similar drop at 3-5 days, followed by a slight recovery. However, in the case of S100B, no negative correlation was observed between the primary tumor tissue and GBOs cultured for 3-5 days. In general, the baseline expression of S100B was lower than that of GFAP.

Stemness markers such as octamer-binding transcription factor 4 (OCT4), Musashi RNA-binding protein 1 (MSI1), SRY-box transcription factor 2 (SOX2), Krüppel-like factor 4 (KLF4) and cluster of differentiation 133 (CD133) are associated with tumor growth, invasiveness and poor prognosis (31-33). Therefore, these markers were selected to investigate their presence in the different GBO preparations over cultivation time. The expression of OCT4, SOX2 and KLF4 was relatively homogeneous in the analyzed GBO preparations, resulting in no significant correlation between the primary tumor tissues and GBOs cultured for all investigated time points (Figs. 2B and E, and S2C). MSI1 showed a similar pattern but tended to display a mild positive correlation around GBO cultivation days 3-5 and 14-15, forming a subtle V-shaped trend. However, when considering the overall MSI1-positive population, which was more heterogeneous than OCT4, SOX2 and KLF4, no significant differences were detected. CD133 expression was highly heterogeneous, showing initially high values that subsequently declined to lower levels, becoming undetectable in one GBO preparation. This expression pattern led to a slight, insignificant negative correlation between the primary tumor tissues and GBOs cultured for most investigated time points. However, toward the end, a slight positive correlation emerged, with more homogeneous expression patterns approaching a plateau. Regarding baseline expression of stemness markers, SOX2 showed the highest level. OCT4 and MSI1 exhibited similar profiles, while KLF4 showed lower expression, and CD133 displayed the lowest baseline expression.

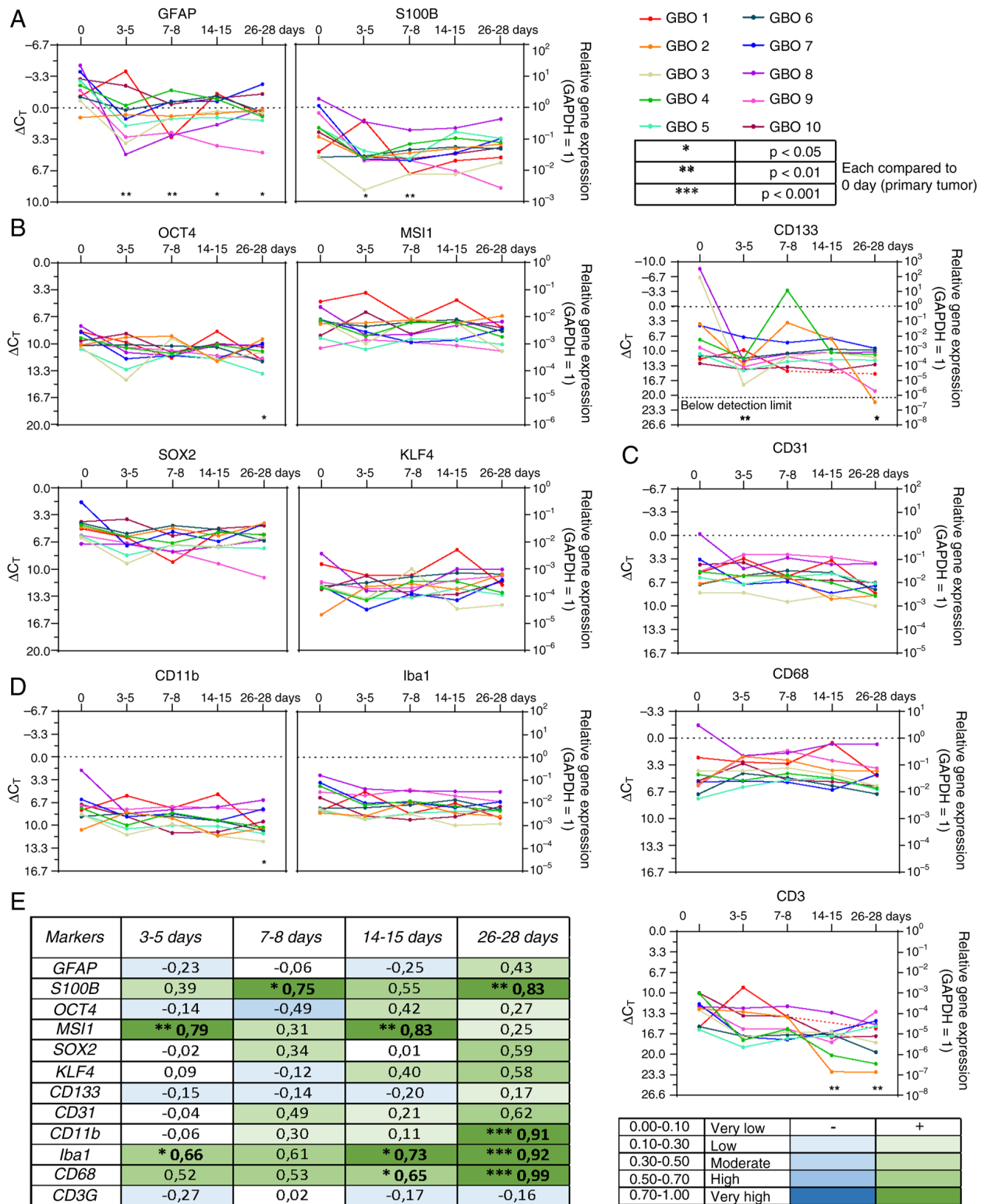


Figure 2. Reverse transcription-quantitative PCR and correlation analysis of glial structural proteins, stemness, vessel, and immune cell markers. Gene expression levels are shown across the entire cultivation period for 10 independent GBO preparations (n=10) at five time points: days 0, 3-5, 7-8, 14-15, 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). (A) Glial structural proteins. (B) Stemness markers. (C) Vessel marker. (D) Immune cell markers. (E) Correlation analysis. Correlations are indicated by a color gradient: Dark green represents a high positive correlation, light green a low positive correlation, dark blue a high negative correlation, and light blue a low negative correlation. 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. GBO, glioblastoma organoid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein B; OCT4, octamer-binding transcription factor 4; MSI1, 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.

Immune cells such as microglia, macrophages and microglia-associated cells represent a key component of the TME and play a critical role in tumor infiltration (9). Ionized calcium-binding adapter molecule 1 (Iba1) was analyzed as a microglia marker, which is upregulated in glioblastomas (33), cluster of differentiation 3 (CD3) as a marker for T lymphocytes and NK cells (34), as well as cluster of differentiation 68 (CD68) and cluster of differentiation 11b (CD11b), which are expressed in macrophages and microglia (35). In the current analysis, Iba1, CD68 and CD11b exhibited relatively homogeneous mRNA expression patterns with significant positive correlations between the primary tumor tissues and GBOs cultured for up to 28 days (Figs. 2D and E, S2E). By contrast, CD3 expression displayed greater heterogeneity, with some GBO preparations showing lower expression levels. Overall, CD3 predominantly revealed a negative correlation between the primary tumor tissues and GBOs, characterized by a downward trend. Iba1 and CD68 exhibited the highest baseline expression, followed by CD11b, whereas CD3 showed the lowest.

To address the presence of tumor vessels in GBOs, cluster of differentiation 31 (CD31) was used (36). Depending on the analyzed GBO preparations, CD31 expression was generally similar in magnitude with slight variations (Figs. 2C and E, and S2D). With some exceptions, the baseline expression level was comparable to that of the immune cell marker CD68, with no significant correlations when comparing the expression between primary tumor tissues and GBOs cultured for all investigated time points.

To illustrate the persistence of the cellular structure of the investigated GBO preparations over cultivation time, exemplary H&E staining was performed as shown in Fig. 3A with whole-organism and corresponding high-magnification inset images. The GBOs presented no significant visual differences, indicating that cellular structures remained unchanged once a GBO had been formed. Additionally, blood vessels were observed up to 28 days, indicating that the vascular structure of organoids was preserved during a one-month cultivation period.

After the evaluation of the markers at the RNA level to ensure precise scientific quantification, immunofluorescence double-staining was performed to visualize stable marker expression and typical co-staining of cell-type-specific markers at the protein level throughout the culture period. Exemplary images are demonstrated in Figs. 3 and 4, highlighting typical constellations. Due to the limited materials for valid quantitative analyses, these images were included to support the validity of the RNA-based findings using a total of three GBO preparations without suggesting any quantitative assessment.

CD3/Iba1 were stained to label T cells and microglia, CD11b/CD68 to assess macrophages, and vWF/S100B to visualize vasculature and glial tissue (Fig. 3B-D). The images revealed that all markers remained detectable at the protein level over a cultivation period of one month. In some cases, co-staining was observed as expected, for example, with CD11b and CD68 (Fig. 3C).

To identify the presence of GSCs during the GBO cultivation period, typical stemness markers were combined, including SOX2, OCT4, MSI1, CD133 and KLF4, with

typical glial structural markers such as GFAP and S100B (Fig. 4A-E). The staining results revealed that stem-like cells persist over a cultivation period of one month alongside glial cells. Co-staining of stemness and glial structural markers was rarely observed; in most cases, cells positive for stemness markers were found as single cells or small groups near cells positive for GFAP or S100B during the whole GBO cultivation period. Especially, SOX2 and KLF4 (Fig. 4C, day 14 and E) were also observed in the nucleus, in addition to their cytoplasmic localization, illustrating their role as transcription factors. This is also highlighted by the apotome images of KLF4 (Fig. 4E, whole visualization of the marker is provided in Fig. S4).

Notably, some GBO preparations exhibited a progressive cell death over time (exemplary data shown in Fig. 3E), highlighting the heterogeneity of organoids and probably suggesting that the presence of certain cellular markers may vary depending on the microenvironmental conditions.

Inflammatory features are retained in GBOs for up to one month. Inflammation plays a significant role in the pathogenesis of glioblastoma, contributing to tumor proliferation, progression, invasion and therapeutic resistance (11,15). Therefore, it is of interest to determine whether the previously mentioned existing cells in GBOs exhibited their characteristic features during cultivation time, such as the expression of inflammatory mediators and their receptors. Focus was addressed on the chemokines C-X-C motif chemokine ligand 12 (CXCL12), C-X-C motif chemokine ligand 16 (CXCL16) and C-X3-C motif chemokine ligand 1 (CX3CL1), as well as interleukin-6 (IL-6) and interleukin-1 beta (IL-1 β), together with their respective receptors. C-X-C motif chemokine receptor 4 (CXCR4), one of the receptors for CXCL12, is overexpressed in gliomas and, together with its ligand, promotes migration and invasion (12). Moreover, the CXCL12-CXCR4 axis facilitates temozolomide resistance, highlighting its central role in glioblastoma treatment (37). C-X-C motif chemokine receptor 7 (CXCR7), another receptor for CXCL12, is known for its anti-apoptotic effects contributing to enhanced tumor cell survival (38). It can serve as a prognostic marker, correlating with patient outcomes (39). The CXCL16-C-X-C motif chemokine receptor 6 (CXCR6) axis enhances migration via reverse signaling mechanisms (40) and modulates T cell activity within the glioblastoma microenvironment, suggesting a dual role in tumor progression and immune regulation (41). Finally, the CX3CL1-C-X3-C motif chemokine receptor 1 (CX3CR1) axis participates in tumor-immune interactions by accumulating tumor-associated microglia/macrophages, thereby influencing tumor growth and progression through modulation of the TME (42).

The results are demonstrated in Fig. 5, with the respective findings for each individual GBO preparation traceable. In addition, all qPCR results were presented as box plots as an alternative representation to provide an improved overview (Fig. S3).

In the experiments of the present study, CXCL12 mRNA expression showed some heterogeneity in the investigated GBO preparations but exhibited, over time, a highly significant positive correlation from day 7 onwards when comparing the expression between the primary tumor

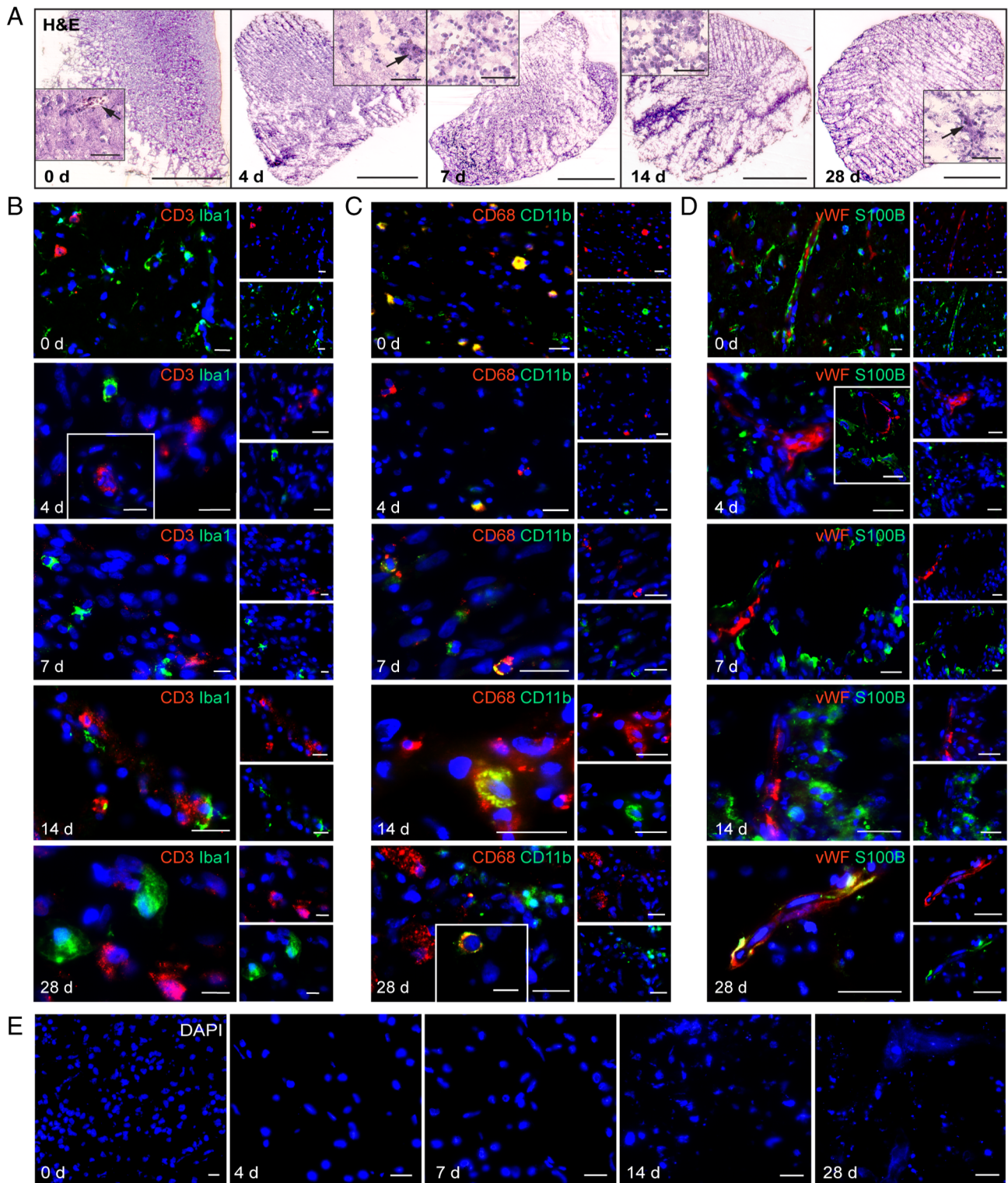


Figure 3. H&E staining and immunofluorescence double-staining images of an exemplary GBO preparation at five time points: days 0, 4, 7, 14, and 28. Selected insets were taken from two additional GBO preparations (total $n=3$). (A) H&E staining images with low (x2,5)- and high (x40)-magnification; black arrows indicate blood vessels. (B and C) Immune cell markers. (D) Vessel marker. (E) Areas with cell death in one GBO preparation during the cultivation period. Scale bars: 1 mm for whole-organism H&E images, 50 μm for the corresponding H&E-insets, 20 μm for immunofluorescence images. GBO, glioblastoma organoid; DAPI, 4',6-diamidino-2-phenylindole; vWF, von Willebrand factor; CD11b, cluster of differentiation 11b; Iba1, ionized calcium-binding adapter molecule 1; CD68, cluster of differentiation 68; CD3, cluster of differentiation 3; S100B, S100 calcium-binding protein B.

tissues and GBOs cultured for the respective time points (Figs. 5A and C, and S3A). CXCL16 expression was more homogeneous in the investigated GBO preparations and consistent over time, resulting in no significant differences,

while CX3CL1 displayed a very homogeneous mRNA expression at the beginning of the cultivation procedure and then developed a certain heterogeneity, resulting in a negative correlation between the primary tumor tissues and GBOs

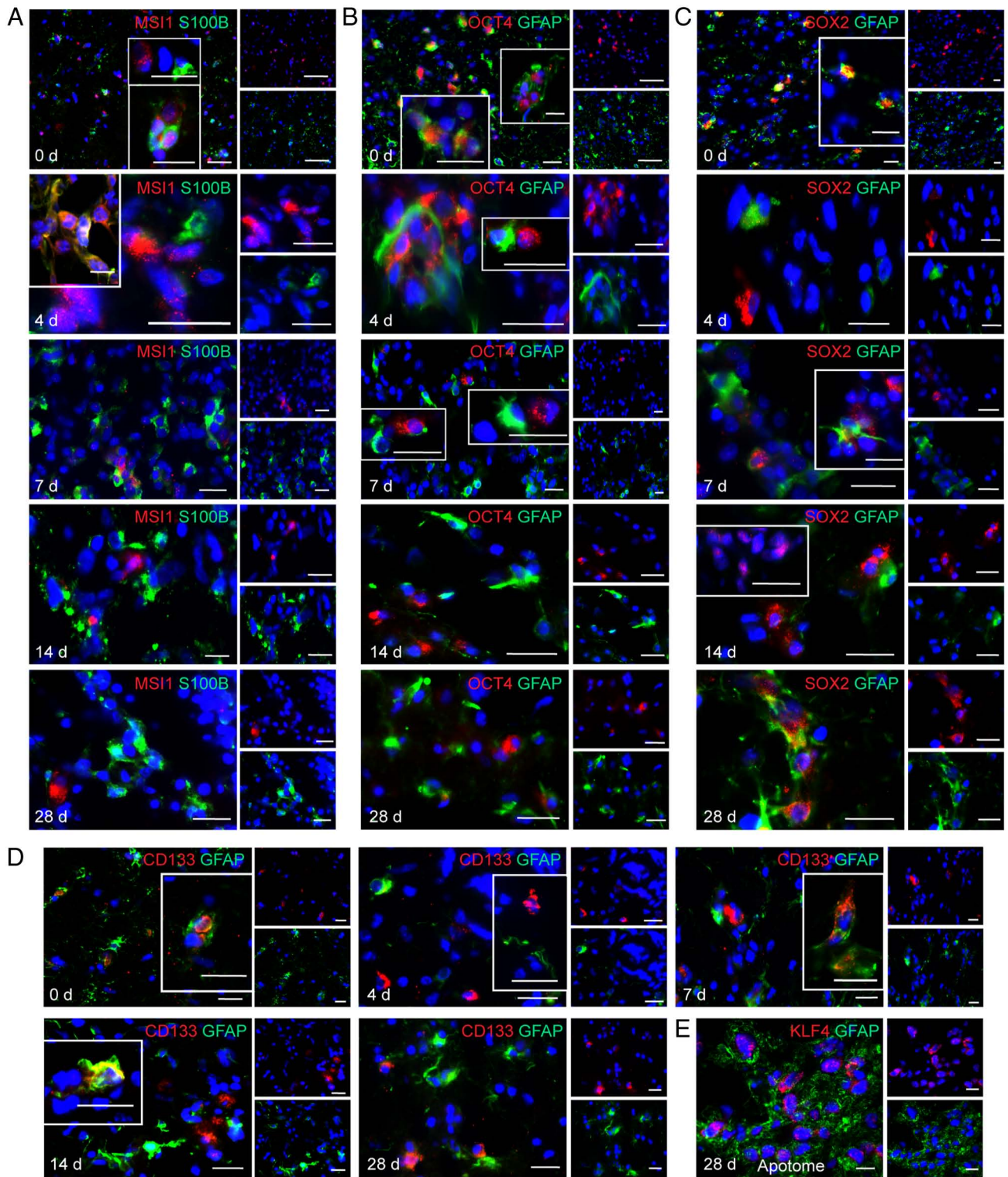


Figure 4. Immunofluorescence double-staining images of an exemplary GBO preparation at five time points: Days 0, 4, 7, 14, and 28. Selected insets were taken from two additional GBO preparations (total n=3). (A-D) Stemness markers. (E) Exemplary Apotome image of KLF4 at day 28. GBO, glioblastoma organoid; GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein B; OCT4, octamer-binding transcription factor 4; MSI1, Musashi RNA-binding protein 1; SOX2, SRY-box transcription factor 2; CD133, cluster of differentiation 133.

cultured for 28 days (Figs. 5A and C, and S3A). The baseline expression levels were similar in magnitude for all three ligands (Figs. 5A and S3A). The mRNA expression of the chemokine receptor CXCR4 still revealed a statistically significant positive correlation on days 7-8 and remained unchanged at other time points (Figs. 5A and C, and S3A). CXCR7

displayed similar patterns with some exceptions, showing relatively consistent expression, exhibiting a positive correlation between the primary tumor tissues and GBOs cultured for days 14-15. CXCR6 presented the lowest baseline expression of all investigated chemokine receptors and exhibited a positive correlation between the primary tumor tissues and GBOs

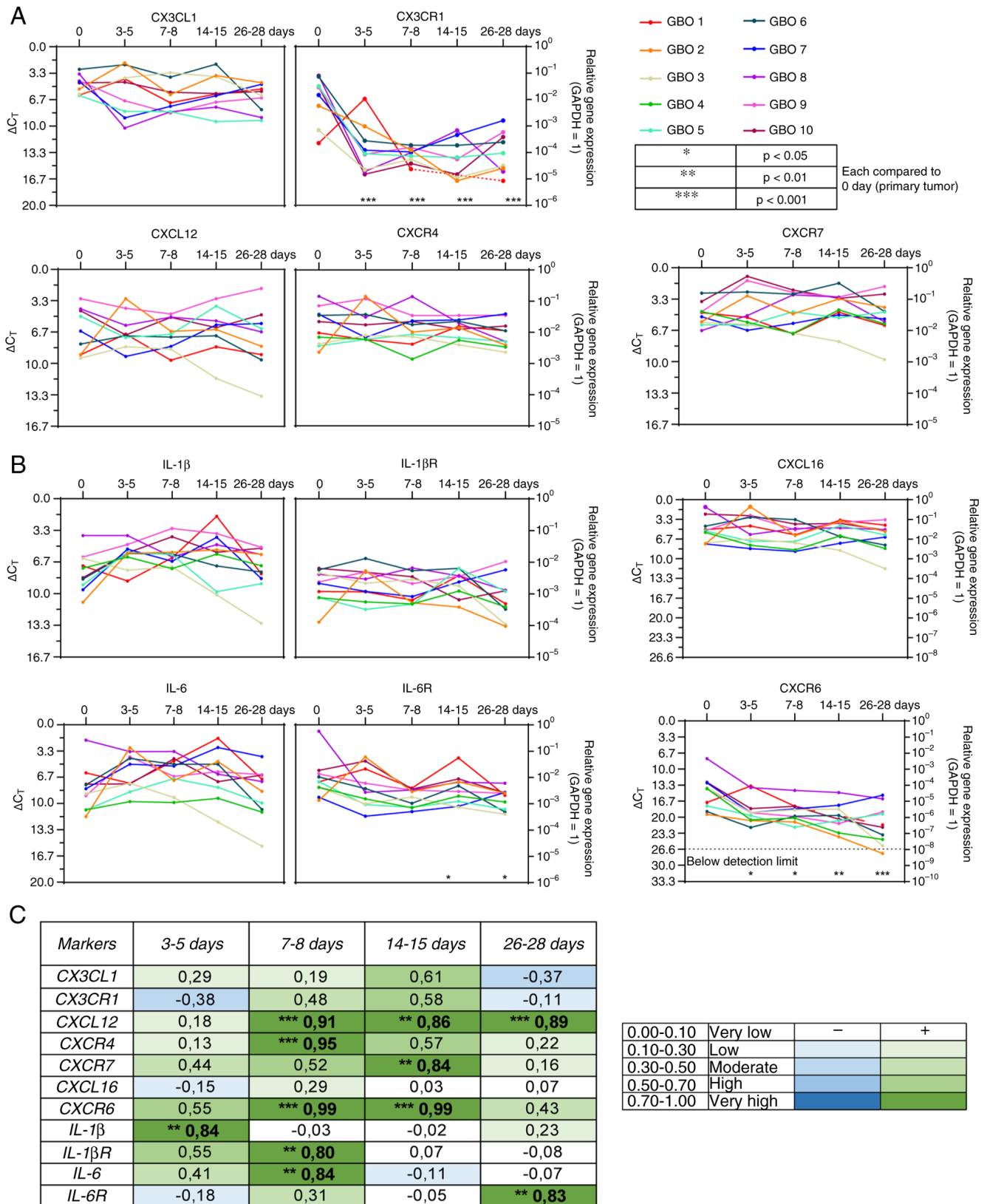


Figure 5. Reverse transcription-quantitative PCR and correlation analysis of chemokines and cytokines with their respective receptors. Gene expression levels are shown across the entire cultivation period for 10 different glioblastoma organoid preparations (n=10) at five time points: days 0, 3-5, 7-8, 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. (C) Correlation analysis. Correlations are indicated by a color gradient: Dark green represents a high positive correlation, light green a low positive correlation, dark blue a high negative correlation, and light blue a low negative correlation. 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, interleukin-1 beta receptor; IL-6, interleukin-6; IL-6R, interleukin-6 receptor.

at two time points. There was a tendency toward downregulation over time, and one GBO preparation became undetectable at the latest time point; however, this was not clearly reflected in the correlation analysis due to the heterogeneity of the expression patterns in the different GBO preparations (Figs. 5A and C, S3A). By contrast, CX3CR1 exhibited a highly heterogeneous mRNA expression with a significant decline at the end of the GBO cultivation period, resulting in a negative correlation between the primary tumor tissues and GBOs cultured for 28 days (Figs. 5A and C, and S3A).

Proinflammatory cytokines such as IL-6 and IL-1 β , along with their receptors, promote tumor cell proliferation, invasiveness, and survival in glioblastoma (13,14). In the present study, the interleukins presented some heterogeneity within the various GBO preparations, exhibiting similar baseline mRNA expression levels (Figs. 5B and C, and S3B). Moreover, the interleukins, particularly IL-6, showed a downward trend in the correlation analysis during cultivation time when comparing primary tumor tissues and GBOs. IL-1 β initially displayed a positive correlation, which shifted to negative or weakly positive values over time before becoming slightly more positive at the end (Figs. 5B and C and S3B). In general, IL-6 receptor (IL-6R) showed a higher baseline mRNA expression than IL-1 β receptor (IL-1 β R). IL-1 β R also exhibited a positive correlation at the beginning, which then became slightly negative. IL-6R displayed a relatively homogeneous expression pattern across the different time points, with a positive correlation observed at the end of the GBO cultivation period (Figs. 5B and C, and S3B).

Cytokines and chemokines, along with their respective cellular sources, were also visualized qualitatively at the protein level using immunofluorescence double-staining. Representative images are shown in Figs. 6 and 7. The stemness markers SOX2 and MSI1 were combined with CXCR4 and CXCR6 (Fig. 6B and E), as both receptors are known to be expressed in glioblastoma progenitor cells (43,44). S100B was stained together with CXCR7 and CX3CL1, which are reported to be expressed in glioma cells (38,45). CX3CR1 was combined with Iba1, reflecting its expression in microglia (46). Finally, CXCL12 and CXCL16 were stained in combination with GFAP, as both chemokines are expressed in glial cells (38,47). In numerous staining constellations, co-staining was observed during the whole GBO cultivation period, for example, CXCL12 and CXCL16 with GFAP (Fig. 6A and D), CX3CL1 with S100B (Fig. 6F), and CXCR4 with MSI1 (Fig. 6B). Similarly, co-staining of CX3CR1 and Iba1 was detected (Fig. 6G), consistent with known findings aforementioned.

To assess cytokine secretion in immune cells, IL-1 β and its receptor were stained in combination with Iba1 (Fig. 7A and B) to visualize both the secreted cytokine and its receptor on macrophages and microglia (48). Additionally, CD11b was stained with IL-6 and IL-6R (Fig. 7C and D), given the positive correlation between cytokine expression and immune cell infiltration reported in glioblastoma (49). Similar to the chemokines, the cytokines remained detectable at the protein level during 1 month of cultivation. Moreover, co-staining of, for example, Iba1 and IL-1 β , and CD11b and IL-6R was detectable in GBOs up to 28 days of cultivation time (Fig. 6B and D). Collectively, all fluorescence images

demonstrated that inflammatory features persisted throughout the 1-month cultivation period.

In summary, structural proteins, proliferation, stemness and inflammatory markers remain preserved at the RNA level during the cultivation method over the course of one month, with some exceptions (for example, reduction of CXCR6, CX3CR1, and CD3 expression during the cultivation period, and presence of a GFAP and S100B mRNA expression drop after a 3-5-day cultivation period). All fluorescence images confirmed that structural proteins, stemness, and inflammatory markers remained present and detectable at the protein level even after 28 days of GBO cultivation time.

Discussion

The present study aimed to analyze the cellular characteristics and inflammatory features of patient-derived GBOs during a 1-month cultivation period. The present findings demonstrated that glial structural proteins, GSCs, immune cells, vessels, proliferation marker, and inflammatory mediators remained detectable up to the final time point in most cases. H&E staining revealed no major structural alterations over time, and immunofluorescence confirmed the continued presence of nearly all analyzed markers at the protein level after 28 days. GSCs were distinct from glial cells, occasionally forming small clusters near GFAP/S100B-positive cells. Co-staining could be observed as expected, such as CXCL12/CXCL16 with GFAP, CXCR4 with MSI1, and CX3CR1 with Iba1, highlighting the preservation of cellular diversity and functional integrity over time. Both RNA and protein levels confirmed long-term stability of the GBO preparations, as most markers remained largely preserved with minor fluctuations in most cases.

The maintenance of cellular architecture and characteristic features, including blood vessels, immune cells, and inflammatory mediators, is essential for preserving an intact TME in glioblastoma. Jacob *et al* (2020) (26) successfully optimized patient-derived GBOs with a particular investigation of cellular characteristics. They established a biobank and documented the viability of organoids following cryopreservation. However, at that time, the long-term stability of the GBOs and the maintenance of key TME features, including inflammatory mediators, remained uninvestigated, potentially limiting the translation of data into patient care. The present study aimed to address this gap by conducting quantitative analyses of different cell components and inflammatory mediators over a 1-month cultivation. Despite some exceptions over a prolonged cultivation period, the observed stability in the current experiments supports the reliability of GBOs as a physiologically relevant preclinical model. The persistence of GSCs within GBO preparations also aligns with previous findings suggesting that GSCs contribute to tumor recurrence and therapy resistance (4,7). Their continued presence alongside glial structural proteins indicates that GBOs can, in principle, recapitulate the intratumoral heterogeneity typical of primary glioblastoma tissue.

Nevertheless, although most markers in patient-derived GBOs showed stable expression, not all remained consistent throughout the cultivation period. In the present study, the proliferation marker Ki67 and the glial structural proteins GFAP and S100B displayed an early, transient drop between

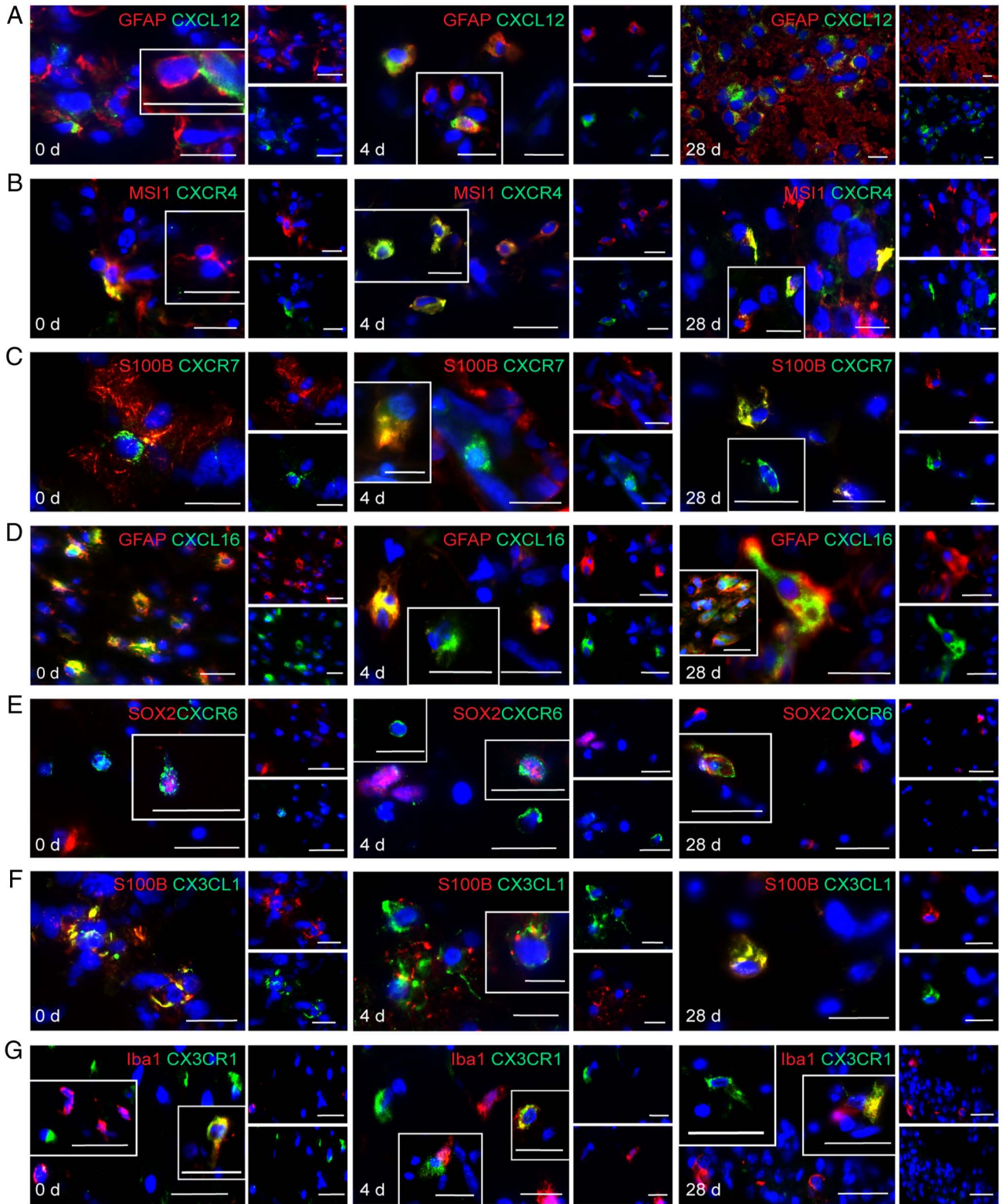


Figure 6. Immunofluorescence double-staining images of three glioblastoma organoid preparations at three time points (days 0, 4, and 28). (A-G) Chemokines and chemokine receptors. (A-C) CXCL12-CXCR4-CXCR7 axis. (D and E) CXCL16-CXCR6 axis. (F and G) CX3CL1-CX3CR1 axis. Scale bars: 20 μ m. CXCL12, C-X-C 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; CX3CL1, C-X3-C motif chemokine ligand 1; CX3CR1, C-X3-C motif chemokine receptor 1; GFAP, glial fibrillary acidic protein; S100B, S100 calcium-binding protein B; MSI1, Musashi RNA-binding protein 1; SOX2, SRY-box transcription factor 2; Iba1, ionized calcium-binding adapter molecule 1.

days 3-5, followed by gradual recovery. This may be due to glial tumor cells experiencing stress in the cultured GBOs, temporarily reducing marker expression. The stemness marker

CD133, however, exhibited high heterogeneity and declined over time in some GBO preparations. This heterogeneity can be attributed to the coexistence of CD133-positive and

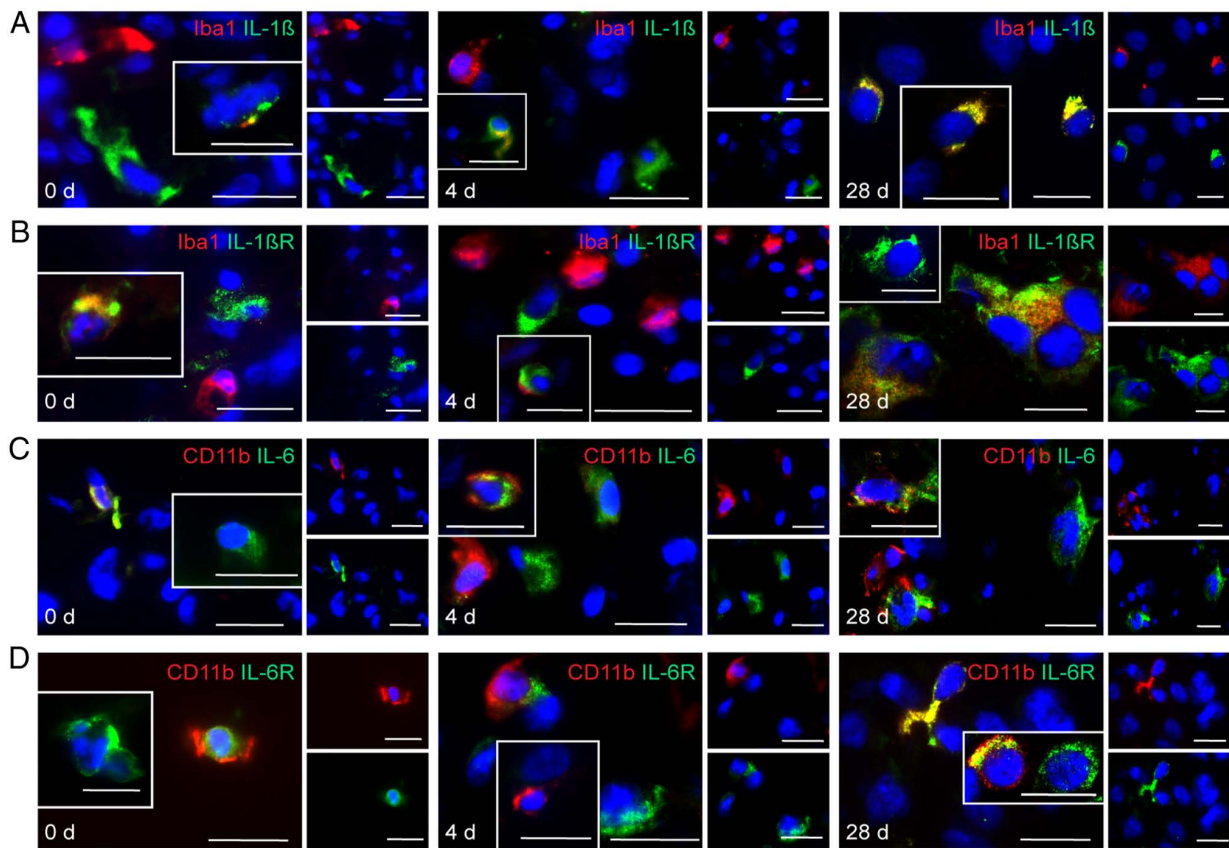


Figure 7. Cytokines and cytokine receptors were visualized using immunofluorescence double-staining at three time points (days 0, 4, and 28), exemplified for one glioblastoma organoid preparation. (A and B) IL-1 β and IL-1 β R. (C and D) IL-6 and IL-6R. Scale bars, 20 μ m. IL-1 β , interleukin-1 beta; IL-1 β R, IL-1 β receptor; IL-6, interleukin-6; IL-6R, IL-6 receptor; Iba1, ionized calcium-binding adapter molecule 1; CD11b, cluster of differentiation 11b.

CD133-negative GSCs (50) and may also be due to population shifts favoring CD133-negative cells over time. Similarly, CXCR6, expressed in glioblastoma progenitor cells (43,44), showed low expression and declined at later time points. This may result from transcriptional downregulation or epigenetic silencing, or from loss of the CXCR6-positive subset, while overall stemness markers remained. These findings align with Hattermann *et al* (2013) (43), where CXCR6 was restricted to a small subset of proliferating glioblastoma cells co-expressing MSI1, SOX2 and OCT4. A decline in CD3, a marker for T cells and NK cells (34), was observed after 14-15 days, likely due to the absence of survival or activation signals in the organoid environment. CX3CR1, expressed in microglia (46), decreased significantly over time, whereas Iba1 expression as a microglia marker remained stable, suggesting that the CX3CR1 decline reflects reduced microenvironmental signaling rather than microglial loss. Notably, CXCR6 and CD133 became undetectable at day 28 in one GBO preparation, and CD3 decline was most substantial in one GBO preparation. Comparing these results, all changes were pronounced in GBO 2, highlighting heterogeneity between GBO preparations.

In summary, the observations of the present study indicate that key components of the TME fluctuate depending on the marker, tumor material, and cultivation time. The current results suggest that the optimal time window for studying patient-derived GBOs is likely between 7-14 days, when cellular composition is most stable. Over time, some GBOs developed progressive cell death, indicating that GBO quality depends

on the original tumor material and reflects both heterogeneity and microenvironment-dependent variation. Therefore, careful evaluation of GBOs for structural abnormalities is essential before experimental use to ensure reproducibility and reliability.

Based on the present findings, future research on the GBO model should aim to further optimize its application as a preclinical model. Future studies could routinely implement intraoperative diagnostics, such as frozen section or detailed sequencing analyses. This would enable the cultivation of organoids separately according to their specific genetic and epigenetic profiles, which could be crucial for testing individualized patient treatments. Furthermore, correlations between treatment responses observed in patient-derived GBOs and those of the corresponding patients could be systematically evaluated.

The scientific significance of the GBOs lies in their ability to preserve tumor heterogeneity by exhibiting structural and functional characteristics similar to those of human organs and tissues (17,21). GBOs offer a high success rate in culture, short formation times, relatively low cost, and reproducible tumor traits (22). Moreover, organoids serve as valuable tools for studying tumor biology, including the TME, and for testing radiotherapy, chemotherapy and immunotherapy (18,20-22). However, several limitations should be considered: First, the key TME components can fluctuate across different markers, depending on the marker and tumor material (batch-to-batch variability). Second, during long-term cultivation, some

characteristics changed (reduced expression of specific immune markers and loss of chemokine receptors in some GBO preparations), as also observed in the present study. Third, due to the limited material, the fluorescence images in the present study were included only to visualize stable marker expression and typical co-staining of cell-type-specific markers at the protein level throughout the culture period, without suggesting quantitative assessment. Nevertheless, while the patient-derived GBO model of the present study preserves tumor heterogeneity and TME in general, and can therefore serve as a valuable preclinical model, it should be used for exploratory and hypothesis-generating studies. Therefore, its application may be limited to providing biological and mechanistic insights and should be used to a limited extent to determine clinical efficacy or therapeutic decision-making without independent *in vivo* or clinical validation.

In summary, the long-term preservation of cellular and inflammatory characteristics underscores the robustness of patient-derived GBOs as glioblastoma models. By maintaining the key components of the TME and the heterogeneity of the original tumor, GBOs provide a powerful platform for experimental studies and preclinical drug testing. Their ability to bridge the gap between experimental research and clinical application highlights their value in accelerating translational glioblastoma research and advancing the development of effective, individualized treatment strategies. However, its application may be limited to providing biological and mechanistic insights and should be used to a limited extent to determine clinical efficacy or therapeutic decision-making, without independent *in vivo* or clinical validation. Moreover, time-dependent variations in some marker expression determine an optimum time window with these organoids, between 7-14 days, which may limit their use for certain experiments.

Acknowledgements

The authors would like to thank Mrs Corinna Keller [Department of Neurosurgery, University Medical Center Schleswig-Holstein (UKSH), Campus Kiel, 24105 Kiel, Germany] for her expert technical assistance.

Funding

The present study was supported by the Department of Neurosurgery of Kiel (grant no. NCH 2025).

Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

JHF conceptualized the study. JHF, NOS, JH, JC and JN developed methodology. NOS, JN and JC performed software analysis and investigation. NOS and JN validated data. NOS and JN confirm the authenticity of all the raw data. HA and MS provided resources. NOS, JN, JC, HA, MS and JHF curated data. JN prepared the original draft of the manuscript.

All authors wrote, reviewed and edited the manuscript. JN visualized data. JHF supervised the study and conducted project administration. MS acquired funding. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was performed in accordance with the Declaration of Helsinki and received approval from the Ethics Committee of the University of Kiel (approval no D524/17; Kiel, Germany). Written informed consent was obtained from all participants involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

References

1. Schaff LR and Mellinghoff IK: Glioblastoma and other primary brain malignancies in adults: A review. *JAMA* 329: 574, 2023.
2. Tan AC, Ashley DM, López GY, Malinzak M, Friedman HS and Khasraw M: Management of glioblastoma: State of the art and future directions. *CA Cancer J Clin* 70: 299-312, 2020.
3. Stupp R, Weller M, Belanger K, Bogdahn U, Ludwin SK, Lacombe D and Mirimanoff RO: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352: 987-996, 2005.
4. Alves ALV, Gomes INF, Carloni AC, Rosa MN, da Silva LS, Evangelista AF, Reis RM and Silva VAO: Role of glioblastoma stem cells in cancer therapeutic resistance: A perspective on anti-neoplastic agents from natural sources and chemical derivatives. *Stem Cell Res Ther* 12: 206, 2021.
5. Zhang P, Xia Q, Liu L, Li S and Dong L: Current opinion on molecular characterization for GBM classification in guiding clinical diagnosis, prognosis, and therapy. *Front Mol Biosci* 7: 562798, 2020.
6. Sottoriva A, Spiteri I, Piccirillo SG, Touloumis A, Collins VP, Marioni JC, Curtis C, Watts C and Tavaré S: Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. *Proc Natl Acad Sci* 110: 4009-4014, 2013.
7. Dirkse A, Golebiewska A, Buder T, Nazarov PV, Muller A, Poovathingal S, Brons NHC, Leite S, Sauvageot N, Sarkisjan D, *et al*: Stem cell-associated heterogeneity in Glioblastoma results from intrinsic tumor plasticity shaped by the microenvironment. *Nat Commun* 10: 1787, 2019.
8. Barthel L, Hadamitzky M, Dammann P, Schedlowski M, Sure U, Thakur BK and Hetze S: Glioma: Molecular signature and cross-roads with tumor microenvironment. *Cancer Metastasis Rev* 41: 53-75, 2022.
9. Giering A, Pszczolkowska D, Walentynowicz KA, Rajan WD and Kaminska B: Immune microenvironment of gliomas. *Lab Invest* 97: 498-518, 2017.
10. Nagarsheth N, Wicha MS and Zou W: Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol* 17: 559-572, 2017.

11. Corsaro A, Tremonti B, Bajetto A, Barbieri F, Thellung S and Florio T: Chemokine signaling in tumors: Potential role of CXC chemokines and their receptors as glioblastoma therapeutic targets. *Expert Opin Ther Targets* 28: 937-952, 2024.
12. Dai Y, Yu C, Zhou L, Cheng L, Ni H and Liang W: Chemokine receptor CXCR4 interacts with nuclear receptor Nur77 and promote glioma invasion and progression. *Brain Res* 1822: 148647, 2024.
13. Detchou D and Barrie U: Interleukin 6 and cancer resistance in glioblastoma multiforme. *Neurosurg Rev* 47: 541, 2024.
14. Narasimhappagari J, Liu L, Balasubramaniam M, Ayyadevara S, Aboud O and Griffin WST: The seminal role of the proinflammatory cytokine IL-1 β and its signaling cascade in glioblastoma pathogenesis and the therapeutic effect of Interleukin-1 β receptor antagonist (IL-1RA) and tolcapone. *Int J Mol Sci* 26: 6893, 2025.
15. Mentlein R, Hattermann K and Held-Feindt J: Migration, metastasis, and more: The role of chemokines in the proliferation, spreading, and metastasis of tumors. In: *Trends in Stem Cell Proliferation and Cancer Research*. Resende RR and Ulrich H (eds). Springer Netherlands, Dordrecht, pp339-358, 2013.
16. Klein E, Hau AC, Oudin A, Golebiewska A and Niclou SP: Glioblastoma organoids: Pre-Clinical applications and challenges in the context of immunotherapy. *Front Oncol* 10: 604121, 2020.
17. Pernik MN, Bird CE, Traylor JI, Shi DD, Richardson TE, McBrayer SK and Abdullah KG: Patient-derived cancer organoids for precision oncology treatment. *J Pers Med* 11: 423, 2021.
18. Zhang C, Jin M, Zhao J, Chen J and Jin W: Organoid models of glioblastoma: Advances, applications and challenges. *Am J Cancer Res* 10: 2242-2257, 2020.
19. Xu X, Li L, Luo L, Shu L, Si X, Chen Z, Xia W, Huang J, Liu Y, Shao A and Ke Y: Opportunities and challenges of glioma organoids. *Cell Commun Signal* 19: 102, 2021.
20. Pawlowski KD, Duffy JT, Babak MV and Balyasnikova IV: Modeling glioblastoma complexity with organoids for personalized treatments. *Trends Mol Med* 29: 282-296, 2023.
21. Wang X, Sun Y, Zhang DY, Ming G and Song H: Glioblastoma modeling with 3D organoids: Progress and challenges. *Oxf Open Neurosci* 2: kvad008, 2023.
22. Xu C, Yuan X, Hou P, Li Z, Wang C, Fang C and Tan Y: Development of glioblastoma organoids and their applications in personalized therapy. *Cancer Biol Med* 20: 353-368, 2023.
23. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP and Knoblich JA: Cerebral organoids model human brain development and microcephaly. *Nature* 501: 373-379, 2013.
24. Soubéran A and Tchoghandjian A: Practical review on preclinical human 3D glioblastoma models: Advances and challenges for clinical translation. *Cancers* 12: 2347, 2020.
25. Hubert CG, Rivera M, Spangler LC, Wu Q, Mack SC, Prager BC, Couce M, McLendon RE, Sloan AE and Rich JN: A Three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found in vivo. *Cancer Res* 76: 2465-2477, 2016.
26. Jacob F, Salinas RD, Zhang DY, Nguyen PTT, Schnoll JG, Wong SZH, Thokala R, Sheikh S, Saxena D, Prokop S, *et al*: A Patient-derived glioblastoma organoid model and biobank recapitulates Inter- and Intra-tumoral heterogeneity. *Cell* 180: 188-204.e22, 2020.
27. Hellmold D, Johanning L, Clüver J, Holler J, Schröder NO, Bayler F, Ahmeti H, Kubelt-Kwamin C, Wieker S, Helmers AK, *et al*: Effect of focused ultrasound-induced mechanical ablation on stemness and dormancy properties of residual/peri-focally localized glioblastoma cells. *Neurooncol Adv* 7: vdaf184, 2025.
28. Qiang J, Wei Z, Xiao-guang Q and Wei Y: Gene expression profiling reveals Ki-67 associated proliferation signature in human glioblastoma. *Chin Med J (Engl)* 124: 2584-2588, 2022.
29. Jung CS, Foerch C, Schanzer A, Heck A, Plate KH, Seifert V, Steinmetz H, Raabe A and Sitzer M: Serum GFAP is a diagnostic marker for glioblastoma multiforme. *Brain* 130: 3336-3341, 2007.
30. Wang H, Zhang L, Zhang IY, Chen X, Da Fonseca A, Wu S, Ren H, Badie S, Sadeghi S, Ouyang M, *et al*: S100B promotes glioma growth through chemoattraction of Myeloid-derived macrophages. *Clin Cancer Res* 19: 3764-3775, 2013.
31. Wang P, Zhao L, Gong S, Xiong S, Wang J, Zou D, Pan J, Deng Y, Yan Q, Wu N and Liao B: HIF1 α /HIF2 α -Sox2/Klf4 promotes the malignant progression of glioblastoma via the EGFR-P13K/AKT signalling pathway with positive feedback under hypoxia. *Cell Death Dis* 12: 312, 2021.
32. Polat B, Wohlleben G, Kosmala R, Lisowski D, Mantel F, Lewitzki V, Löhr M, Blum R, Herud P, Flentje M and Monoranu CM: Differences in stem cell marker and osteopontin expression in primary and recurrent glioblastoma. *Cancer Cell Int* 22: 87, 2022.
33. Noorani I, Petty G, Grundy PL, Sharpe G, Willaime-Morawek S, Harris S, Thomas GJ, Nicoll JA and Boche D: Novel association between microglia and stem cells in human gliomas: A contributor to tumour proliferation? *J Pathol Clin Res* 1: 67-75, 2015.
34. Kmiecik J, Poli A, Brons NH, Waha A, Eide GE, Enger PØ, Zimmer J and Chekenya M: Elevated CD3+ and CD8+ tumor-infiltrating immune cells correlate with prolonged survival in glioblastoma patients despite integrated immunosuppressive mechanisms in the tumor microenvironment and at the systemic level. *J Neuroimmunol* 264: 71-83, 2013.
35. Lu-Emerson C, Snuderl M, Kirkpatrick ND, Goveia J, Davidson C, Huang Y, Riedemann L, Taylor J, Ivy P, Duda DG, *et al*: Increase in tumor-associated macrophages after antiangiogenic therapy is associated with poor survival among patients with recurrent glioblastoma. *Neuro Oncol* 15: 1079-1087, 2013.
36. Mei X, Chen YS, Chen FR, Xi SY and Chen ZP: Glioblastoma stem cell differentiation into endothelial cells evidenced through live-cell imaging. *Neuro-Oncol* 19: 1109-1118, 2017.
37. Wang S, Chen C, Li J, Xu X, Chen W and Li F: The CXCL12/CXCR4 axis confers temozolomide resistance to human glioblastoma cells via up-regulation of FOXM1. *J Neurol Sci* 414: 116837, 2020.
38. Hattermann K, Held-Feindt J, Lucius R, Mürköster SS, Penfold MET, Schall TJ and Mentlein R: The chemokine receptor CXCR7 is highly expressed in human glioma cells and mediates antiapoptotic effects. *Cancer Res* 70: 3299-3308, 2010.
39. Deng L, Zheng W, Dong X, Liu J, Zhu C, Lu D, Zhang J, Song L, Wang Y and Deng D: Chemokine receptor CXCR7 is an independent prognostic biomarker in glioblastoma. *Cancer Biomark* 20: 1-6, 2017.
40. Adamski V, Mentlein R, Lucius R, Synowitz M, Held-Feindt J and Hattermann K: The chemokine receptor CXCR6 evokes reverse signaling via the transmembrane chemokine CXCL16. *Int J Mol Sci* 18: 1468, 2017.
41. Chia TY, Billingham LK, Boland L, Katz JL, Arrieta VA, Shireman J, Rosas AL, DeLay SL, Zillinger K, Geng Y, *et al*: The CXCL16-CXCR6 axis in glioblastoma modulates T-cell activity in a spatiotemporal context. *Front Immunol* 14: 1331287, 2024.
42. Lee S, Latha K, Manyam G, Yang Y, Rao A and Rao G: Role of CX3CR1 signaling in malignant transformation of gliomas. *Neuro Oncol* 22: 1463-1473, 2020.
43. Hattermann K, Held-Feindt J, Ludwig A and Mentlein R: The CXCL16-CXCR6 chemokine axis in glial tumors. *J Neuroimmunol* 260: 47-54, 2013.
44. Ehtesham M, Mapara KY, Stevenson CB and Thompson RC: CXCR4 mediates the proliferation of glioblastoma progenitor cells. *Cancer Lett* 274: 305-312, 2009.
45. Sciumè G, Soriani A, Piccoli M, Frati L, Santoni A and Bernardini G: CX3CR1/CX3CL1 axis negatively controls glioma cell invasion and is modulated by transforming growth factor-beta1. *Neuro Oncol* 12: 701-710, 2010.
46. Hattermann K, Sebens S, Helm O, Schmitt AD, Mentlein R, Mehdorn HM and Held-Feindt J: Chemokine expression profile of freshly isolated human glioblastoma-associated macrophages/microglia. *Oncol Rep* 32: 270-276, 2014.
47. Ludwig A, Schulte A, Schnack C, Hundhausen C, Reiss K, Brodway N, Held-Feindt J and Mentlein R: Enhanced expression and shedding of the transmembrane chemokine CXCL16 by reactive astrocytes and glioma cells. *J Neurochem* 93: 1293-1303, 2005.
48. Kai K, Komohara Y, Esumi S, Fujiwara Y, Yamamoto T, Uekawa K, Ohta K, Takezaki T, Kuroda J, Shinojima N, *et al*: Macrophage/microglia-derived IL-1 β induces glioblastoma growth via the STAT3/NF- κ B pathway. *Hum Cell* 35: 226-237, 2022.
49. Kim S, Kim KH, Jung H, Jeong EO, Lee HJ, Kwon J, Kwon HJ, Choi SW, Koh HS and Kim SH: Elevated serum IL-6 as a negative prognostic biomarker in glioblastoma: Integrating bioinformatics and clinical validation. *J Cancer* 16: 802-811, 2025.
50. Chen R, Nishimura MC, Bumbaca SM, Kharbada S, Forrest WF, Kasman IM, Greve JM, Soriano RH, Gilmour LL and Rivers CS: A hierarchy of Self-renewing Tumor-initiating cell types in glioblastoma. *Cancer Cell* 17: 362-375, 2010.

