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Spatial proximity of M2 macrophages to glioma cells identifies an immune niche associated with unfavorable prognosis

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Abstract

Background: The spatial organization of immune cells may provide prognostic information beyond cell abundance in glioma. We examined whether Toll-like receptor 2 (TLR2) identifies a prognostic tumor-associated macrophage (TAM)–tumor spatial niche.

Methods: TCGA, CGGA, GTEx, and single-cell RNA-sequencing datasets were analyzed to define TLR2 expression, prognostic and immune associations, and its cellular source. Multiplex immunohistochemistry of a 141-case glioma tissue microarray confirmed macrophage localization and quantified TAM infiltration and 20-μm proximity to S100⁺ or TLR2⁺S100⁺ tumor cells in relation to survival. TLR2 silencing or activation in M2-like macrophages assessed polarization; co-culture models evaluated effects of silencing on glioma-cell proliferation, migration, and invasion.

Results: TLR2 was upregulated, associated with malignancy and poor prognosis, and expressed mainly by macrophages. TLR2⁺ tumors showed higher infiltration densities of M0 and M2-like TAMs. Spatially, TLR2⁺ M2-like TAMs were closest to glioma cells (mean distance, 2.82 μm), and greater proximity was associated with shorter overall and disease-free survival. Comparison with TLR2⁺ tumor-cell centers suggested that TLR2 expression in macrophages was the more informative spatial feature. Co-culture models showed that TLR2 silencing in M2-like macrophages significantly reduced glioma-cell migration and invasion but did not affect proliferation. Silencing also reduced M2 markers, whereas activation produced opposite changes.

Conclusion: Close positioning of TLR2⁺ M2-like TAMs around glioma cells defines an unfavorable spatial immune feature whose prognostic pattern varies by index tumor cell. These findings support further evaluation of TLR2 expression in macrophages and associated M2-like polarization as spatial prognostic markers and candidate targets for TAM-directed intervention.

40 1 Introduction

41 Glioma frequently recurs despite surgery, radiotherapy, and chemotherapy, with the poorest
42 outcomes occurring in glioblastoma (GBM) (1–3). Immune-checkpoint inhibitors, chimeric antigen
43 receptor T cells, vaccines, natural killer cells, and oncolytic viruses have all been explored in this
44 setting (4–8). Their activity is nevertheless limited by a strongly immunosuppressive tumor
45 microenvironment (TME) that is actively shaped by glioma cells (9–11). A clearer account of this
46 local immune organization is therefore needed to improve the use of immunotherapy in glioma (12,
47 13).

48 Within the glioma TME, tumor-associated macrophages (TAMs) constitute a major non-neoplastic
49 population (14). The conventional M1-like/M2-like distinction describes antitumor and protumor
50 functional tendencies, respectively, but does not represent fixed cell states; TAM phenotypes span a
51 context-dependent continuum (15). Glioma-associated macrophages often acquire M2-like features
52 and participate in immune suppression, tumor progression, and treatment resistance (16, 20, 21).
53 Although TAM abundance has repeatedly been linked to poor outcome (17–19), therapies directed
54 broadly at these cells have shown limited clinical benefit. Their diverse origins, functional states, and
55 tissue locations are likely to contribute to that limitation (22, 23).

56 Cell counts alone cannot describe how immune cells are arranged around tumor cells. Their
57 neighborhood membership and intercellular distances determine opportunities for contact and local
58 communication (24, 25). Mouse studies have resolved important features of TAM origin and state
59 (26, 27), but bulk-tissue measurements and conventional histology provide limited information about
60 cell-to-cell positioning in heterogeneous human tumors (28–30). By detecting several antigens in the
61 same section, multiplex immunohistochemistry (mIHC) permits single-cell phenotyping together
62 with spatial localization (31). The organization of specific TAM subsets around glioma cells,
63 however, has not been sufficiently connected to patient outcome.

64 Toll-like receptor 2 (TLR2) contributes to immune regulation and has also been implicated in tumor
65 development (32–34). Its prognostic signal in glioma could arise from tumor cells, from infiltrating
66 myeloid cells, or from both compartments. Whether TLR2⁺ M2-like TAMs form a clinically relevant
67 spatial niche around glioma cells is likewise unresolved. We addressed these questions by combining
68 bulk and single-cell transcriptomic datasets with mIHC, multispectral imaging, and distance
69 measurements in a 141-case glioma tissue microarray. Immune neighborhoods and their associations
70 with OS and DFS were analyzed separately using all S100⁺ tumor cells or only TLR2⁺S100⁺ tumor
71 cells as index populations. Conditioned-medium and Transwell experiments then tested whether
72 manipulating TLR2 in M2-like macrophages altered macrophage polarization or the migratory and
73 invasive behavior of glioma cells. This design allowed tissue-level expression, cellular source, spatial
74 context, and in vitro function to be assessed within one analytical framework.

75 2 Materials and methods

76 2.1 Patients and tissue samples

77 The commercial glioma tissue microarray HBraG144Su01 was obtained from Shanghai Outdo
78 Biotech Co., Ltd. (Shanghai, China). It initially represented 144 surgically treated patients (94 male
79 and 50 female; 3–80 years of age) whose operations were performed from January 2008 through
80 December 2011. After heat-induced antigen retrieval, cores lacking tissue or containing insufficient
81 material were removed from the analysis. The final study set comprised 141 cases.

82 **2.2 Data acquisition**

83 The TCGA dataset assembled for this study comprised 704 RNA-sequencing sample profiles from
84 the TCGA glioblastoma multiforme (TCGA-GBM; n=171) and brain lower-grade glioma (TCGA-
85 LGG; n=533) projects; matched expression and clinical data were available for 667 cases. Two
86 CGGA RNA-sequencing cohorts, mRNAseq_325 (n=325; BioProject PRJCA001746) and
87 mRNAseq_693 (n=693; BioProject PRJCA001747), were also included. Analyses and visualizations
88 were performed in R version 4.1.2. The two CGGA expression matrices were merged, and batch-
89 associated variation was corrected using limma (35) and sva (36) before downstream analysis. Within
90 each cohort, cases were divided into TLR2-high and TLR2-low groups according to the cohort-
91 specific median TLR2 expression value.

92 **2.3 TLR2 expression in glioma**

93 Tissue-level TLR2-positive cell counts were obtained by mIHC for all 141 evaluable TMA cases.
94 Using the median count as the cutoff, 70 cases were assigned to the TLR2-negative group and 71
95 cases to the TLR2-positive group. For comparisons with non-neoplastic brain tissue, GEPIA2 was
96 used because normal brain samples are sparse in TCGA. GEPIA2 integrates uniformly processed
97 TCGA and GTEx RNA-sequencing data (37), enabling comparison of TLR2 expression among
98 LGG, GBM, and normal brain tissues.

99 **2.4 Clinicopathological analyses**

100 Using limma and ggpubr in R, we tested the relationships of TLR2 expression with patient age,
101 WHO grade, recurrence, histological classification, IDH mutation, and 1p/19q codeletion.

102 **2.5 Survival analyses**

103 Survival distributions according to TLR2 expression were displayed as Kaplan–Meier curves with
104 the survival package, and group differences in OS and DFS were tested by the log-rank method. Cox
105 proportional hazards models were subsequently fitted in univariate and multivariate form to examine
106 TLR2, immune-cell infiltration, and spatial-distance variables in relation to each survival endpoint.

107 **2.6 Functional enrichment analyses**

108 Within each cohort, limma was used to contrast the TLR2-high and TLR2-low groups. Genes with an
109 adjusted false-discovery-rate P value below 0.05 and an absolute log2 fold change greater than 1
110 were retained as differentially expressed genes (DEGs). GO and KEGG terms were evaluated with
111 clusterProfiler and enrichplot, using $P < 0.05$ and $q < 0.05$ as the enrichment criteria; ggplot2 was
112 used to display the results. GSEA provided a complementary gene-set-level comparison of the two
113 TLR2 expression groups.

114 **2.7 Single-cell analysis of TLR2 expression**

115 We first surveyed TLR2 expression across annotated cell populations in the 17 human glioma single-
116 cell RNA-sequencing datasets curated in Tumor Immune Single-cell Hub 2 (TISCH2) (32) and
117 visualized its cross-dataset cell-type distribution as a heatmap. The independent glioblastoma single-
118 cell RNA-sequencing dataset GSE273274 (BioProject PRJNA1141154) was then reprocessed using
119 Seurat. Cells with fewer than 200 detected genes and genes detected in fewer than three cells were
120 excluded. Cells in which mitochondrial or ribosomal transcripts accounted for more than 20% of total
121 counts were also removed. Harmony was used to reduce batch effects. The number of principal

122 components was selected on the basis of the elbow plot; Seurat clustering was performed at a
123 resolution of 0.8, followed by dimensionality-reduction visualization.

124 **2.8 TLR2 expression and immune infiltration**

125 Bulk immune content was characterized in several complementary ways. ESTIMATE generated
126 ImmuneScore, StromalScore, and ESTIMATEScore for each transcriptomic profile, whereas
127 CIBERSORT estimated the relative fractions of 22 immune-cell populations (38). TIMER was used
128 to relate immune infiltration to TLR2 expression and survival in the LGG and GBM cohorts (39).
129 Macrophage abundance was cross-checked with estimates from TIMER, EPIC, and xCell.

130 **2.9 Multiplex immunohistochemistry**

131 The Opal 7-Color Manual IHC Kit (NEL801001KT, PerkinElmer) was used to examine TLR2, CD4,
132 FOXP3, CD68, CD163, and S100 on the glioma TMA. Slides were heated at 63°C for 1 h, cleared
133 twice in xylene for 15 min, and passed successively through 100%, 90%, 80%, and 70% ethanol. For
134 antigen retrieval, 1× retrieval solution was brought to high microwave power for 3 min and then kept
135 at low power for 15–20 min with the sections fully immersed. Once cooled, sections were rinsed in
136 TBST and blocked for 10 min. The following primary antibodies were applied for 1 h at room
137 temperature: TLR2 (1:100, ab213676, Abcam), CD4 (1:1,600, ET1609-52, HUABIO), FOXP3
138 (1:100, ab20034, Abcam), CD68 (1:500, ZM0060, ZSGB-BIO), CD163 (1:200, ZM0428, ZSGB-
139 BIO), and S100 (1:200, GZ031129, Gene Tech). Each staining cycle included a 10-min secondary-
140 antibody step and a 10-min incubation with Opal reagent diluted 1:100; microwave treatment
141 removed the antibody complex before the next marker was introduced. After the sequential cycles,
142 nuclei were labeled with DAPI (Thermo Fisher Scientific) for 10 min and coverslips were applied
143 with antifade medium (Vector Laboratories).

144 **2.10 Image acquisition, cell phenotyping, and spatial-neighborhood analysis**

145 Images were collected on the Mantra Quantitative Pathology Imaging System (PerkinElmer) and
146 handled in Phenochart and inForm 2.4. Spectra from individually stained controls formed the
147 reference library, while unstained sections supplied the autofluorescence profile. After spectral
148 unmixing, representative images were used to train inForm for tissue and nuclear segmentation and
149 for supervised assignment of cellular phenotypes. Nuclear detection incorporated DAPI intensity and
150 morphology. Within each annotated compartment, the density of a phenotype was calculated as its
151 cell count divided by the total number of cells analyzed in that region.

152 Phenotype labels also determined the analytical role of each cell. S100⁺ tumor cells and, in a separate
153 analysis, TLR2⁺S100⁺ tumor cells were treated as index cells; the specified immune phenotypes were
154 treated as neighbors. For every index cell, we counted neighboring-cell centroids falling inside a 20-
155 μm circle. ES corresponded to the average number of such neighbors per index tumor cell. EP
156 represented the fraction of index tumor cells for which at least one eligible neighbor was present
157 within 20 μm (40).

$$158 \quad \text{Effective score} = \frac{\text{number of edges}}{\text{number of central tumor cells}}$$

$$159 \quad \text{Effective percent} = \frac{\text{number of central tumors cells with edges}}{\text{number of central tumor cells}}$$

2.11 Spatial-distance analysis

The x–y coordinates of segmented cells were exported from the inForm cell_seg_data files. To set marker-positivity thresholds, intensity values from regions annotated by a pathologist as positive or negative were modeled in R. The stats density function, with automatic bandwidth selection, supplied Gaussian-kernel estimates of the corresponding intensity distributions. Image-calibration factors converted pixels to micrometers. We then computed two-dimensional Euclidean distances between the centroids of eligible index and neighboring cells and averaged the distances for pairs located within the prespecified 20-μm neighborhood.

$$d(x,y):=\sqrt{(x_1-y_1)^2+(x_2-y_2)^2+\cdots+(x_n-y_n)^2}=\sqrt{\sum_{i=1}^n(x_i-y_i)^2}$$

2.12 Western blotting and reverse transcription-quantitative PCR

Cells were lysed in X-RIPA UltraMix buffer (X-Blot, Suzhou, China), and protein content was measured by bicinchoninic acid assay (Bioss). One volume of 5× loading buffer was combined with four volumes of sample before heating at 100°C for 10 min. Equal protein loads were resolved by SDS–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. After a 20-min block at room temperature, the membranes were exposed overnight to antibodies against TLR2 (1:1,000, DF7002, Affinity), CD11b (1:1,000, ab133357, Abcam), CD204 (1:1,000, ab271070, Abcam), CD206 (1:1,000, DF4149, Affinity), CD163 (1:1,000, ab182422, Abcam), CD86 (1:1,000, ab239075, Abcam), or β-actin (1:5,000, AF7018, Affinity). Horseradish-peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (H⁺L; 1:5,000, Affinity) was added for 2 h. SuperSignal West Pico PLUS substrate (Thermo Fisher Scientific) was used for detection, and chemiluminescence was recorded with a GelView 6000Plus workstation. Full, uncropped western blot images are provided in Supplementary File 2. For RT-qPCR, RNA prepared with the SevenFast Total RNA Extraction Kit was converted to cDNA using the SevenClever First-Strand cDNA Synthesis Kit. Reactions were run with 2× SYBR Green qPCR Master Mix II; Table 1 lists all primer sequences.

Table 1 | Primer sequences used for RT-qPCR.

Gene name	Primer sequence (5'–3')
<i>GAPDH</i> forward	GATGTTTCGTCATGGGTGTGAA
<i>GAPDH</i> reverse	CGCATGGACTGTGGTCATGAG
<i>TLR2</i> forward	CTTCACTCAGGAGCAGCAAGCA
<i>TLR2</i> reverse	ACACCAGTGCTGTCTCTGTGACA
<i>CD163</i> forward	CCAGAAGGAACTTGTAGCCACAG
<i>CD163</i> reverse	CAGGCACCAAGCGTTTTGAGCT
CD206 forward	AGCCAACACCAGCTCCTCAAGA
CD206 reverse	CAAAACGCTCGCGCATTGTCCA
<i>CD86</i> forward	CCATCAGCTTGTCTGTTTCATTCC

Gene name	Primer sequence (5'–3')
<i>CD86</i> reverse	GCTGTAATCCAAGGAATGTGGTC
CD204 forward	TGCACAAGGCAGCTCACTTTGG
CD204 reverse	GTGCAAGTGACTCCAGCATCTTC
CD11B forward	GGAACGCCATTGTCTGCTTTTCG
CD11B reverse	ATGCTGAGGTCATCCTGGCAGA

2.13 Cell culture, macrophage polarization, and siRNA transfection

U251 and LN229 glioma cells were obtained from Cellverse Co., Ltd. (Shanghai, China); THP-1 monocytes were obtained from HySigen Biosciences Co., Ltd. (Suzhou, China). The glioma lines were maintained in DMEM and THP-1 cells in RPMI 1640, with both media containing 10% fetal bovine serum (Gibco) and 1% penicillin–streptomycin. THP-1 cells were plated at 1×10^6 cells per well in six-well plates and exposed to PMA (100 ng/mL, MedChemExpress) for 24 h to generate M0 macrophages. Fresh medium containing PMA (100 ng/mL), interferon- γ (20 ng/mL, PeproTech), and lipopolysaccharide (100 ng/mL, Solarbio) was used for M1-like polarization. M2-like cells were generated with PMA (100 ng/mL), interleukin-4 (20 ng/mL, PeproTech), and interleukin-13 (20 ng/mL, PeproTech). The M2-like cultures were subsequently exposed to Pam3CSK4 (100 ng/mL, MedChemExpress) or transfected with 50 nM control siRNA or TLR2-directed siRNA using Lipofectamine 3000 (Thermo Fisher Scientific). Protein and RNA were collected 72 h later. KeyGen Biotechnology (Suzhou, China) synthesized the TLR2 siRNA (5'-UUAACGUUCCACUUUACCTG-3').

2.14 Preparation of conditioned medium

For conditioned-medium (CM) preparation, M2-like macrophages were plated at 2×10^6 cells in each 6-cm dish and transfected for 72 h with control or TLR2-targeting siRNA. The medium was then recovered, clarified at $500 \times g$ for 15 min at 4°C, passed through a 0.2- μ m filter, and stored in aliquots at –80°C. These macrophage supernatants were used in the indicated glioma-cell and ELISA experiments.

2.15 Wound-healing and Transwell invasion assays

For the wound-healing experiment, U251 or LN229 cells were plated at 5×10^5 cells per well in six-well plates containing DMEM supplemented with 30% macrophage CM. A 200- μ L tip was drawn vertically through each monolayer, detached cells were removed by two or three PBS washes, and the same fields were photographed immediately and after 24 h. Invasion was tested in 24-well Transwell inserts (Corning) using the assay configuration described in (39). M2-like macrophages were placed in the lower wells at 2×10^5 cells per well and transfected with control or TLR2-targeting siRNA for 72 h. Matrigel-coated upper inserts then received 2×10^4 U251 or LN229 cells in serum-free medium. Co-culture lasted 24 h for U251 and 48 h for LN229 at 37°C. Invading cells on the underside of the membrane were fixed in 4% paraformaldehyde, stained with 0.1% crystal violet, and counted in at least three random fields. Three independent experiments were performed.

2.16 EdU assay

DNA synthesis was measured after U251 and LN229 cells had been exposed to the corresponding CM for 72 h. The cells received a 2-h EdU pulse from the EdU-488 Cell Proliferation Detection Kit

220 (C0071S, Beyotime), were washed with PBS, and were counterstained with DAPI. Images were
221 recorded by fluorescence microscopy.

222 **2.17 Enzyme-linked immunosorbent assay**

223 Culture supernatants were assayed for TNF- α , IL-6, TGF- β , and IL-10 with commercial ELISA kits
224 (Mlbio, Shanghai, China). Samples were obtained from M2-like macrophages after control or TLR2-
225 targeting siRNA transfection and from parallel control or Pam3CSK4-treated cultures. The cytokine
226 measurements were considered supporting indicators of polarization-associated change.

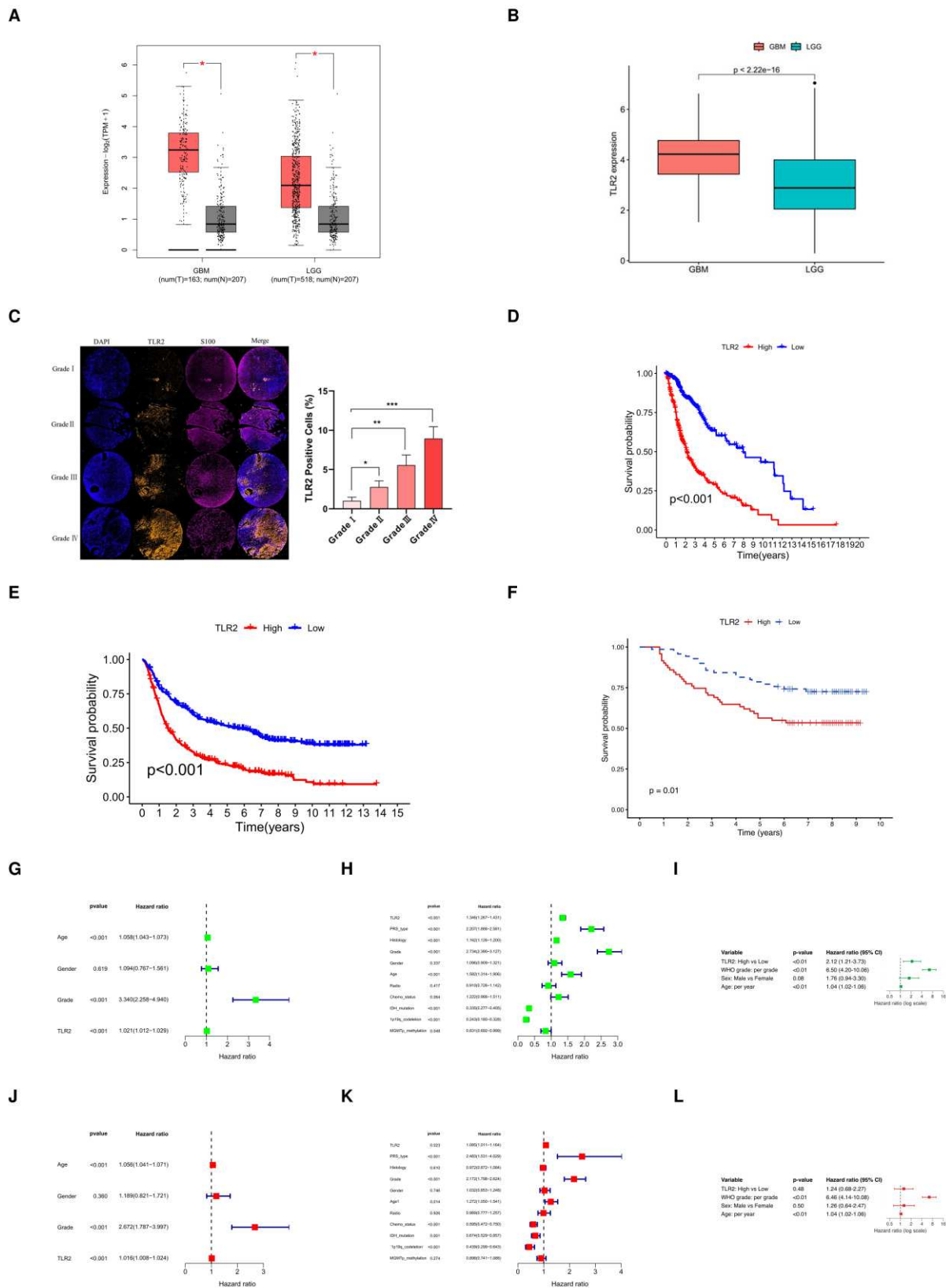
227 **2.18 Statistical analysis**

228 GraphPad Prism 8 and R 4.1.2 were used for all statistical calculations. Survival curves were
229 constructed by the Kaplan–Meier method and compared with log-rank tests; prognosis-related
230 variables were examined in univariate and multivariate Cox proportional hazards models. The
231 Wilcoxon signed-rank test was used for paired observations, whereas Student's t-test was used when
232 comparing the means of two independent groups. Statistical significance was defined as $P < 0.05$.

233 **3 Results**

234 **3.1 TLR2 is highly expressed in glioma and is associated with unfavorable prognosis**

235 TLR2 mRNA was more abundant in LGG and GBM than in normal brain in the combined
236 TCGA/GTEX comparison (Figure 1A), and the CGGA data showed a further increase from LGG to
237 GBM (Figure 1B). We next examined the 141-case TMA together with the available data on grade,
238 sex, age, and OS. The percentage of TLR2-positive cells varied across WHO grades ($P < 0.05$;
239 Figure 1C): grade IV tumors had the highest values, followed by grades III and II, whereas grade I
240 had the lowest. Patients with high TLR2 expression had poorer survival in Kaplan–Meier analyses (P
241 < 0.05 ; Figure 1D–F). Increased risk was also evident in the univariate Cox models ($P < 0.01$; Figure
242 1G–I). TLR2 remained independently associated with survival in the TCGA and CGGA cohorts, but
243 not in the TMA cohort after adjustment for age, sex, and WHO grade (Figure 1J–L). Within CGGA,
244 TLR2 varied significantly with age, WHO grade, histological type, IDH mutation, and 1p/19q
245 codeletion, but not with recurrence (Supplementary Figure 1).



246

247 Figure 1 | TLR2 is highly expressed in glioma and is associated with unfavorable prognosis. (A)
248 Comparison of TLR2 expression among normal brain tissue, glioblastoma (GBM), and lower-grade

glioma (LGG) using TCGA and GTEx data. (B) Comparison of TLR2 expression between GBM and LGG in the CGGA cohort. (C) Representative mIHC images of TLR2, S100, and DAPI in gliomas of different WHO grades, together with quantification of the proportion of TLR2-positive cells. (D–F) Kaplan–Meier OS curves for patients with high and low TLR2 expression in the TCGA, CGGA, and TMA cohorts, respectively. (G–I) Univariate Cox regression analyses of TLR2 and clinicopathological variables in the three cohorts. (J–L) Multivariate Cox regression analyses in the corresponding cohorts. Kaplan–Meier curves were compared using the log-rank test; forest plots show HRs and 95% confidence intervals (CIs). TCGA, The Cancer Genome Atlas; CGGA, Chinese Glioma Genome Atlas; TMA, tissue microarray. *P < 0.05, **P < 0.01, ***P < 0.001.

3.2 TLR2 is associated with immune-related functions in glioma

After dividing the TCGA and CGGA cohorts by TLR2 expression, the 40 leading upregulated and downregulated genes in each comparison were displayed in clustered heatmaps (Figure 2A, B). The GO results emphasized leukocyte-mediated immunity, regulation of immune effector activity, immune-receptor function, and cell-surface receptor signaling involved in immune responses (Figure 2C, D). Phagocytosis and cytokine–receptor interaction pathways were prominent in the KEGG analysis (Figure 2E, F). GSEA also identified the MONOCYTE VS PDC signature (Figure 2G, H). Across these analyses, high TLR2 expression consistently mapped to monocyte/macrophage-related biology in the glioma microenvironment.

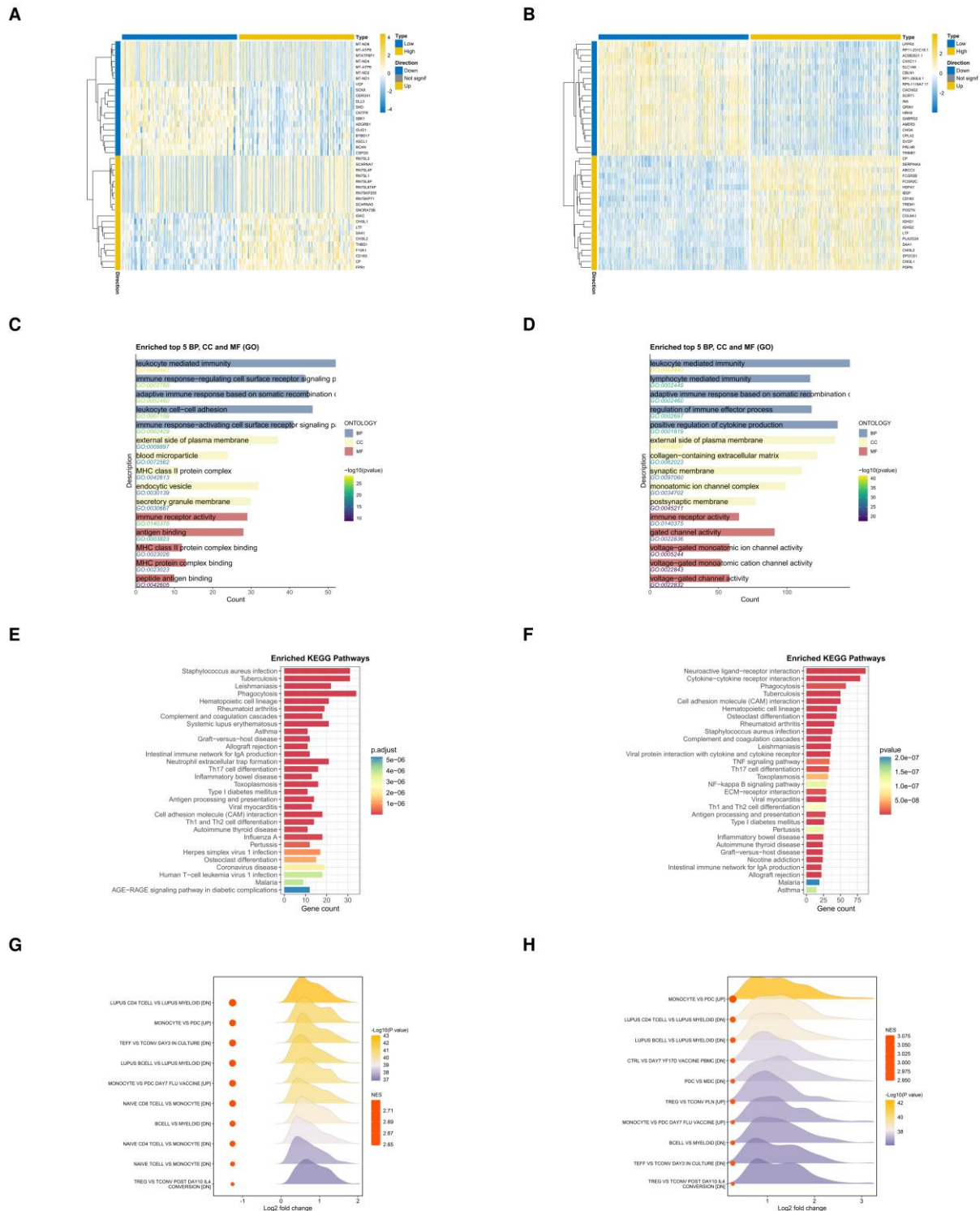


Figure 2 | TLR2-associated differentially expressed genes are enriched primarily in immune-related biological processes. (A, B) Clustered heatmaps of the most significantly differentially expressed genes between the TLR2-high and TLR2-low groups in the TCGA and CGGA cohorts, respectively. (C, D) GO enrichment analyses of the DEGs in the two cohorts, showing the leading terms in biological process (BP), cellular component (CC), and molecular function (MF). (E, F) KEGG

pathway enrichment analyses of the DEGs in the TCGA and CGGA cohorts. (G, H) GSEA of immune-cell states and immune-response gene sets associated with TLR2 expression in the two cohorts. Heatmap colors indicate normalized gene-expression levels, and colors in the enrichment plots indicate enrichment significance. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, gene set enrichment analysis.

3.3 TLR2 expression is associated with TAM infiltration

All three ESTIMATE-derived scores were higher in the TLR2-high group than in the TLR2-low group ($P < 0.001$; Supplementary Figure 2A–F), and higher values were linked to worse outcomes (Supplementary Figure 2G–L). Correlation analyses further connected TLR2 with several immune populations, including CD4⁺ T cells, M2-like macrophages, and Tregs (Figure 3A, B). At single-cell resolution, macrophages carried the strongest TLR2 signal (Figure 3C–E). Concordantly, macrophage estimates from TIMER, xCell, and EPIC each correlated positively with TLR2 expression (Figure 3F–H). The TMA cases were divided at the median number of TLR2-positive cells (70 negative and 71 positive); mIHC demonstrated greater CD68⁺CD163⁺ M2-like TAM abundance in the positive group (Figure 3I). TIMER2.0 additionally linked B cells, CD8⁺ T cells, CD4⁺ T cells, macrophages, neutrophils, and dendritic cells to survival in LGG and to TLR2 expression. In GBM, only dendritic cells retained a significant survival association ($P < 0.05$; Supplementary Figure 3).

correlation coefficients and P values are shown. (I) Representative mIHC images of TLR2, CD68⁺CD163⁺ M2-like TAMs, and DAPI in TLR2-negative and TLR2-positive gliomas, together with quantitative comparison of CD68⁺CD163⁺ cells. TAM, tumor-associated macrophage; mIHC, multiplex immunohistochemistry. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

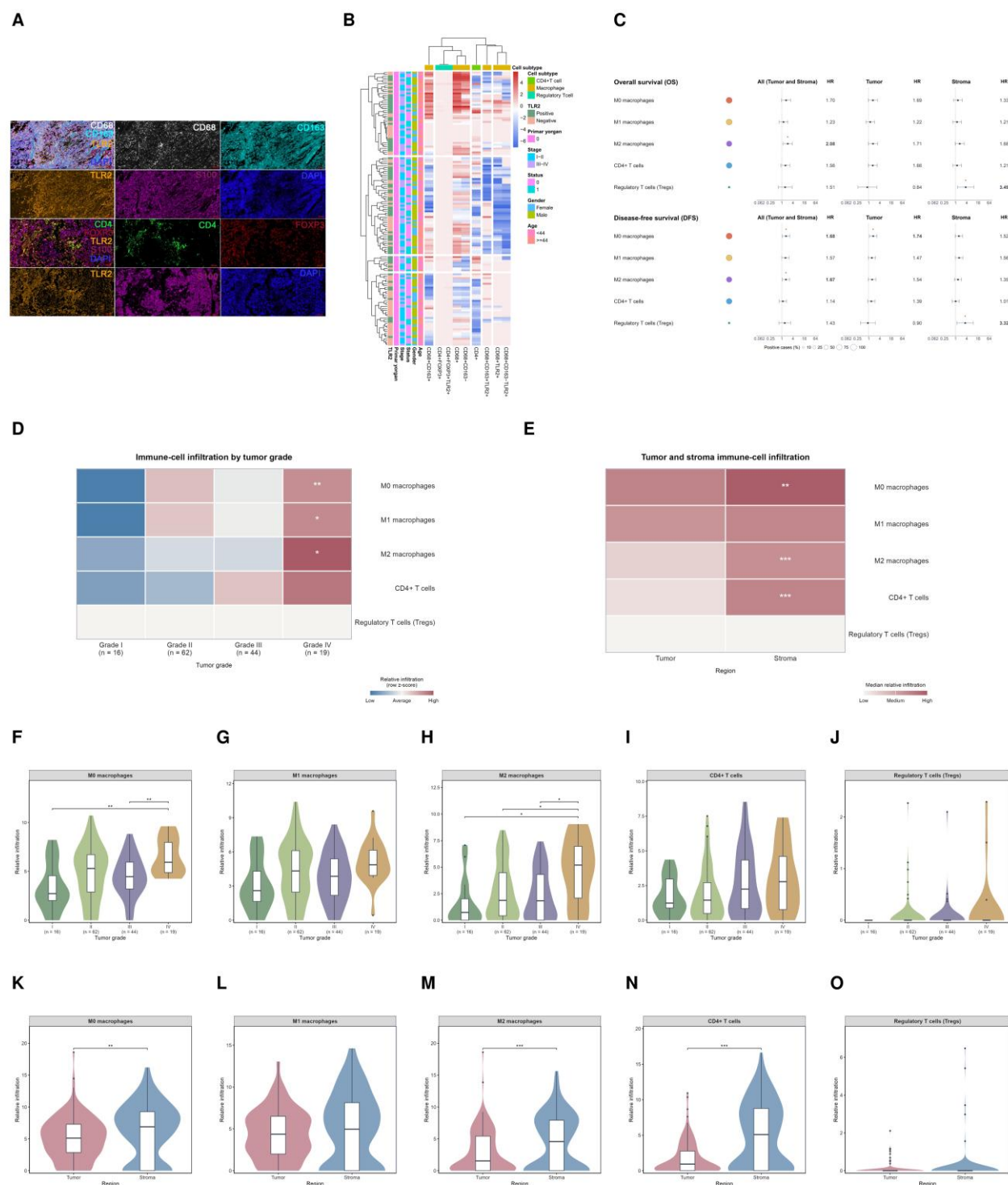
3.4 Immune-cell infiltration and its prognostic significance in glioma

mIHC was used to quantify immune subsets in tumor and stromal regions of the 141-case TMA (Figure 4A, B). Relative infiltration was greater in TLR2-positive than in TLR2-negative tumors for CD68⁺ cells designated as M0 [1.915 vs. 1.239, *P* = 0.004], CD68⁺CD163⁺ M2-like TAMs [1.041 vs. 0.529, *P* = 0.009], CD4⁺ T cells [0.807 vs. 0.244, *P* < 0.0001], and CD4⁺FOXP3⁺ Tregs [0.015 vs. 0.002, *P* = 0.049]. CD68⁺CD163⁻ M1-like TAMs also increased numerically (1.388 vs. 1.017), but the comparison was not significant (*P* = 0.062). Thus, TLR2 status was associated with increases in selected immune populations rather than a uniform rise across all subsets.

We then related these infiltration measurements to clinical outcome. Considering tumor and stroma together, high M2-like TAM infiltration corresponded to greater hazards for both death (HR = 2.08, *P* < 0.05) and a DFS event (HR = 1.67, *P* < 0.05). High M0 infiltration was associated only with DFS (HR = 1.68, *P* < 0.05). When compartments were examined separately, tumor-region M0 cells remained associated with DFS events (HR = 1.74, *P* < 0.05), whereas stromal Tregs were associated with OS (HR = 3.49, *P* < 0.05) and DFS (HR = 3.32, *P* < 0.05). No significant relationship with either endpoint was detected for the remaining subset–compartment combinations (Figure 4C).

The abundance of immune cells also differed by pathological grade. Heatmap visualization showed that M0, M1-like, and M2-like macrophages were generally enriched in grade IV tumors, whereas Tregs lacked a consistent grade trend (Figure 4D). M0 values in grade IV exceeded those in grades I and III (both *P* < 0.01; Figure 4F). M2-like TAMs were likewise more abundant in grade IV than in each of grades I–III (all *P* < 0.05; Figure 4H). Figure 4G, I, and J show the corresponding grade comparisons for M1-like TAMs, CD4⁺ T cells, and Tregs.

Compartment analysis revealed higher relative levels of M0 cells, M2-like TAMs, and CD4⁺ T cells in stroma than in tumor regions (*P* < 0.01, *P* < 0.001, and *P* < 0.001, respectively). Neither M1-like TAMs nor Tregs differed between the two locations (Figure 4E, K–O). Taken together, the tissue data showed that immune infiltration varies with TLR2 status, grade, anatomical compartment, and outcome. The recurrent adverse association of M2-like TAMs provided the basis for examining their local position relative to tumor cells.



330

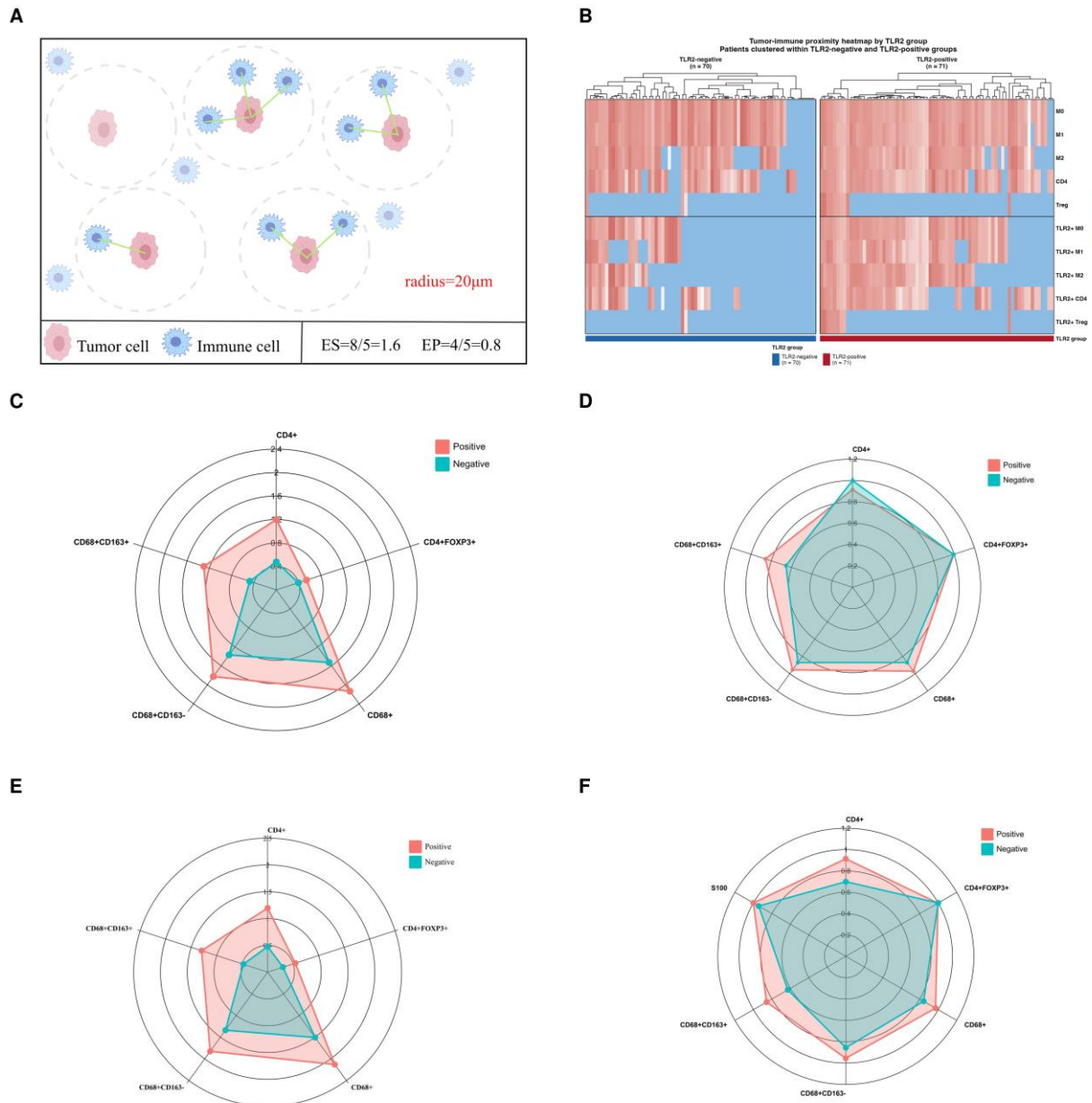
331 Figure 4 | Immune-cell infiltration in glioma: prognostic significance and distributions by
 332 pathological grade and tissue compartment. (A) Representative merged and single-channel mIHC
 333 images used to identify TAM subsets, CD4+ T cells, Tregs, and glioma cells; scale bar, 100 μ m. (B)
 334 Hierarchical-clustering heatmap of immune-cell infiltration and TLR2-related phenotypes in 141
 335 glioma specimens, annotated by TLR2 status, WHO grade, clinical status, sex, and age. (C) Cox
 336 regression analyses of the associations of M0, M1-like, and M2-like TAMs, CD4+ T cells, and Tregs

in whole tissue and in the tumor and stromal compartments with OS and DFS. Forest plots show HRs and 95% CIs; dot size indicates the proportion of positive cases for the corresponding subset. (D) Heatmap of relative immune-cell infiltration across WHO grades. (E) Heatmap of median relative infiltration in the tumor and stromal compartments. (F–J) Relative infiltration of M0, M1-like, and M2-like TAMs, CD4⁺ T cells, and Tregs across WHO grades I–IV, respectively. (K–O) Relative infiltration of the same five subsets in the tumor and stromal compartments, respectively. M0, M1-like, and M2-like TAMs were operationally defined as CD68⁺, CD68⁺CD163[–], and CD68⁺CD163⁺ cells, respectively; Tregs were defined as CD4⁺FOXP3⁺ cells. OS, overall survival; DFS, disease-free survival. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

3.5 TLR2 status is associated with tumor–immune spatial-neighborhood features

Local immune organization was summarized within a 20-μm radius of each tumor-cell centroid using ES and EP (Figure 5A), and the resulting profiles were displayed by TLR2 group in heatmaps (Figure 5B). With all S100⁺ tumor cells as the index population, TLR2-positive gliomas had higher ES values for M0 cells [1.980 vs. 1.524, *P* = 0.001], M1-like TAMs [1.695 vs. 1.406, *P* = 0.035], M2-like TAMs [1.321 vs. 0.774, *P* = 0.0004], and CD4⁺ T cells [1.199 vs. 0.615, *P* < 0.0001] (Figure 5C). EP was also increased for M0 cells [0.972 vs. 0.871, *P* = 0.026], M2-like TAMs [0.859 vs. 0.657, *P* = 0.005], and CD4⁺ T cells [0.915 vs. 0.714, *P* = 0.002] (Figure 5D). No group difference was found for M1-like TAM EP or for either Treg measure.

Repeating the analysis with TLR2⁺S100⁺ tumor cells as the index population produced a similar but not identical pattern. ES was greater in TLR2-positive tumors for M0 cells [1.979 vs. 1.456, *P* = 0.0003], M1-like TAMs [1.977 vs. 1.385, *P* < 0.0001], M2-like TAMs [1.320 vs. 0.723, *P* = 0.0001], CD4⁺ T cells [1.198 vs. 0.295, *P* < 0.0001], and adjacent S100⁺ tumor cells [1.000 vs. 0.943, *P* = 0.041] (Figure 5E). The corresponding EP comparisons were 0.971 vs. 0.842 (*P* = 0.008), 0.955 vs. 0.857 (*P* = 0.043), 0.858 vs. 0.628 (*P* = 0.002), 0.914 vs. 0.699 (*P* = 0.001), and 1.000 vs. 0.943 (*P* = 0.041), respectively (Figure 5F). Treg ES and EP again showed no significant difference.



362

363 Figure 5 | Tumor-immune spatial-neighborhood features associated with TLR2 status. (A) Schematic
 364 of the 20-μm neighborhood analysis. Tumor cells served as index cells, and neighboring immune
 365 cells within a 20-μm radius were counted. The effective score (ES) is the mean number of
 366 neighboring cells per index tumor cell; the effective proportion (EP) is the proportion of index tumor
 367 cells with at least one neighboring cell. (B) Heatmaps of tumor-neighborhood measures for immune-
 368 cell subsets and their TLR2-positive counterparts in TLR2-negative (n = 70) and TLR2-positive (n =
 369 71) gliomas, with cases hierarchically clustered within each TLR2 group. (C, D) ES and EP of
 370 immune-cell subsets in the two TLR2 groups when all S100+ tumor cells were used as index cells. (E,
 371 F) ES and EP of neighboring cells when TLR2+S100+ tumor cells were used as index cells; the EP
 372 of neighboring S100+ cells is also shown in panel F. In the radar plots, Positive and Negative denote
 373 TLR2-positive and TLR2-negative gliomas, respectively.

3.6 Spatial proximity between tumor cells and TAMs and its prognostic significance

We next measured centroid distances within the same 20- μ m neighborhoods. When all S100⁺ tumor cells served as index cells, mean distances were 5.29 μ m for M0 cells, 5.25 μ m for M1-like TAMs, 4.48 μ m for M2-like TAMs, 4.89 μ m for CD4⁺ T cells, and 5.69 μ m for Tregs. Their TLR2-positive counterparts were closer: 3.64, 3.16, 2.82, 3.54, and 4.72 μ m, respectively. The minimum mean distance was therefore observed between TLR2⁺ M2-like TAMs and S100⁺ tumor cells (Figure 6A). M2-like macrophages were also the nearest subset when the index population was limited to TLR2⁺S100⁺ tumor cells (Figure 6B). These measurements indicate spatial opportunity for contact but do not by themselves demonstrate a cellular interaction.

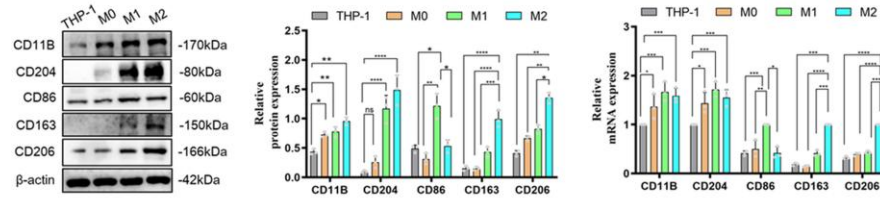
Survival associations depended on which tumor cells defined the neighborhood. Greater proximity of TLR2⁺ M2-like TAMs predicted shorter OS and DFS when all S100⁺ cells were used as index cells (Figure 6C, E). After restricting the index population to TLR2⁺S100⁺ cells, M2-like TAM distance was no longer significantly associated with OS or DFS, although the directions remained unfavorable ($P = 0.054$ and $P = 0.073$; Figure 6D, F). This constitutes an index-definition-related pattern rather than proof that the two effect estimates differ; larger cohorts and a direct comparison are required. Kaplan–Meier curves further showed shorter OS for high M0 and M2-like TAM proximity ($P = 0.0088$ and $P = 0.0091$) but longer OS for high M1-like TAM proximity ($P = 0.013$; Figure 6G–I). DFS followed the same subtype-specific direction: $P = 0.0061$ for M0, $P = 0.005$ for M2-like TAMs, and $P = 0.018$ for M1-like TAMs (Figure 6J–L). The unfavorable niche defined by TLR2⁺ M2-like TAMs close to glioma cells motivated the subsequent functional experiments.

Figure 6 | Tumor cell–TAM spatial distance and prognostic associations under different index-cell definitions. (A) Mean centroid-to-centroid distances within 20 μm between all S100+ index tumor cells and immune-cell subsets or their TLR2-positive counterparts. (B) Mean distances between TLR2+S100+ index tumor cells and neighboring immune-cell subsets or S100+ cells. (C, E) Univariate Cox regression analyses of the associations of mean distances with OS and DFS when all S100+ tumor cells were used as index cells. (D, F) Corresponding analyses when TLR2+S100+ tumor cells were used as index cells. Forest plots show HRs and 95% CIs. (G–I) OS curves for high- and low-proximity groups of M0, M1-like, and M2-like TAMs. (J–L) DFS curves for the corresponding TAM subsets. High proximity denotes a shorter mean distance between TAMs and tumor cells. Survival distributions were compared using the Kaplan–Meier method and log-rank test; shading indicates 95% CIs.

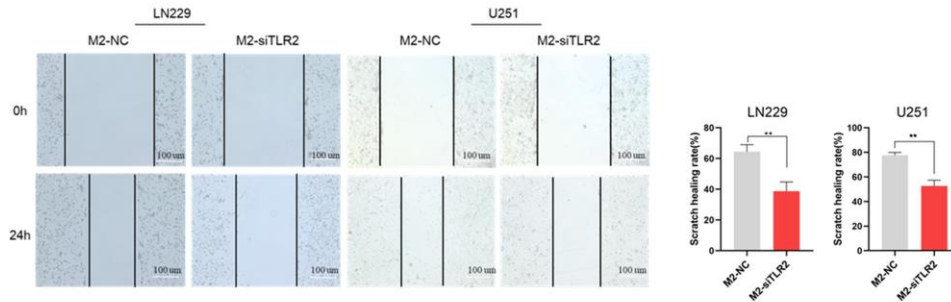
3.7 TLR2 in M2-like macrophages promotes glioma-cell migration and invasion

We tested whether lowering TLR2 in M2-like macrophages altered glioma-cell behavior in a manner compatible with the spatial findings. THP-1 cells were first differentiated into M0, M1-like, and M2-like states. Western blotting and RT-qPCR confirmed higher CD86 in the M1-like cells and higher CD163 and CD206 in the M2-like cells (Figure 7A). At 24 h, wound closure by LN229 and U251 cells was reduced in cultures receiving CM from M2-siTLR2 macrophages compared with CM from M2-NC macrophages (Figure 7B). In the Transwell system, both glioma lines also showed fewer invading cells when co-cultured with M2-siTLR2 rather than M2-NC macrophages (Figure 7C). EdU fluorescence was comparable between groups for both cell lines (Figure 7D). Thus, under these assay conditions, macrophage TLR2 silencing selectively reduced the migratory and invasive phenotypes measured in the glioma cells.

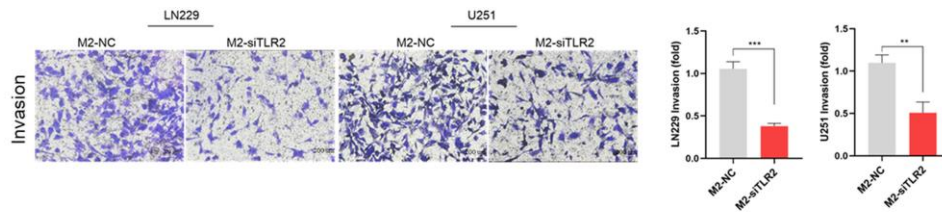
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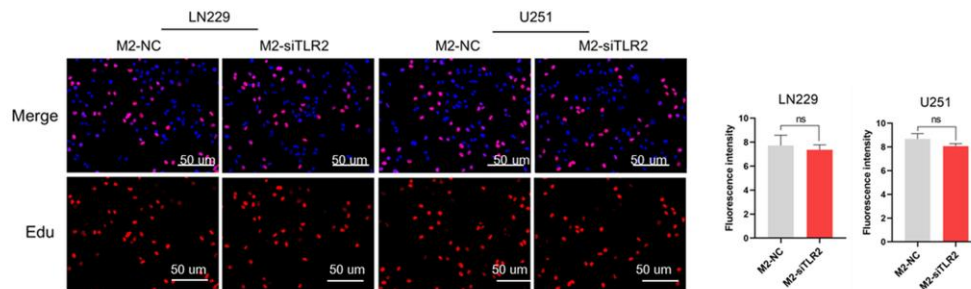
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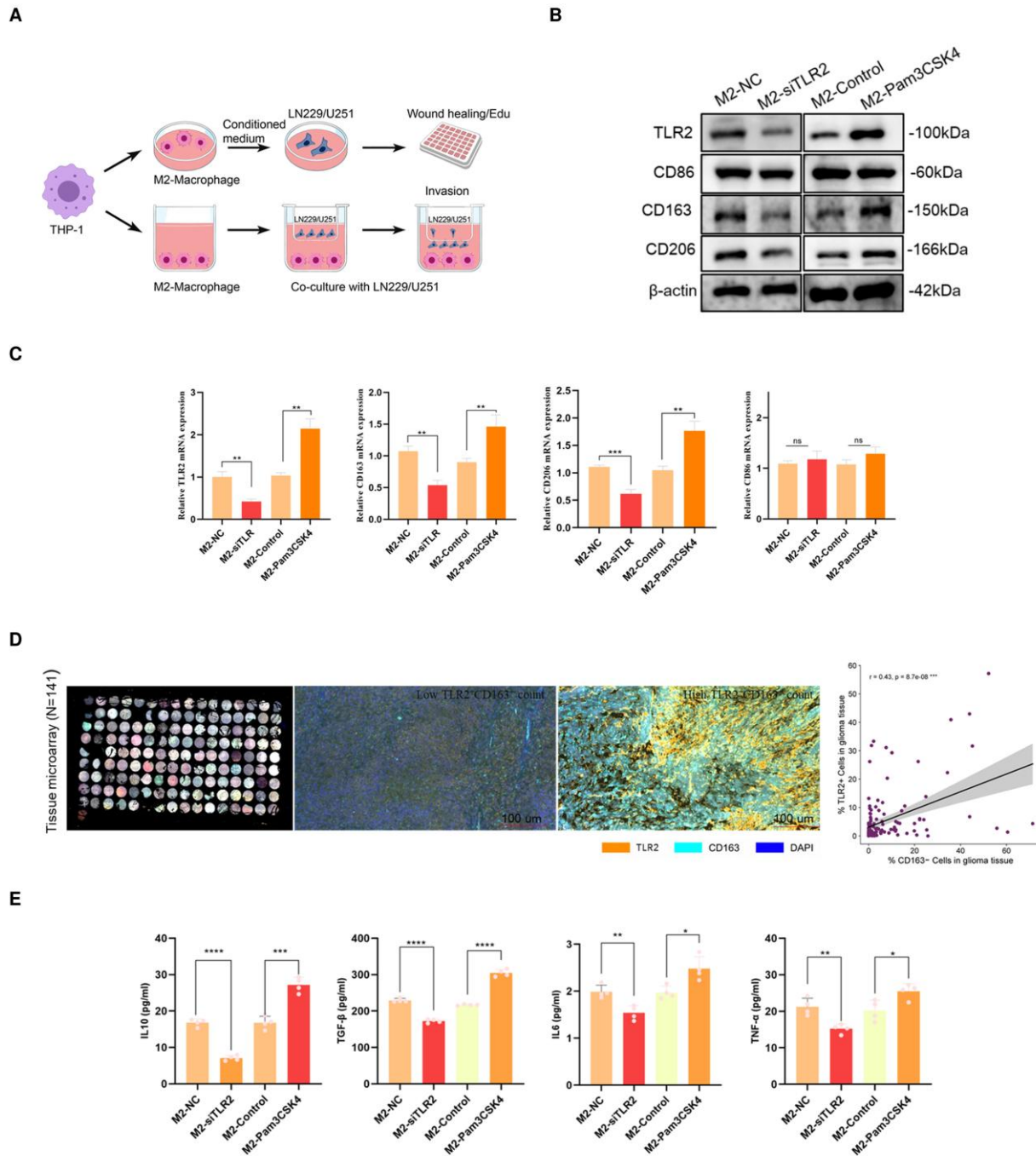


417

418 Figure 7 | TLR2 in M2-like macrophages promotes glioma-cell migration and invasion. (A) Western
 419 blotting and RT-qPCR analysis of CD11B, CD204, CD86, CD163, and CD206 in THP-1 cells and
 420 induced M0, M1-like, and M2-like macrophages to verify macrophage differentiation and
 421 polarization. (B) Representative wound-healing images and wound-closure rates of LN229 and U251
 422 cells treated with CM from M2-NC or M2-siTLR2 macrophages; scale bar, 100 μ m. (C)
 423 Representative Transwell images and quantification of LN229 and U251 invasion after co-culture
 424 with M2-NC or M2-siTLR2 macrophages; scale bar, 100 μ m. (D) EdU staining and quantitative
 425 analysis of LN229 and U251 cells treated with the corresponding CM; EdU, red; DAPI, blue; scale
 426 bar, 50 μ m. M2-NC, M2-like macrophages transfected with negative-control siRNA; M2-siTLR2,
 427 M2-like macrophages transfected with TLR2-targeting siRNA. ns, not significant; * P < 0.05, ** P <
 428 0.01, *** P < 0.001, **** P < 0.0001.

429 **3.8 Silencing TLR2 inhibits M2-like macrophage polarization in vitro**

430 To assess its contribution to the M2-like state, TLR2 was reduced by siRNA in one set of
431 macrophage cultures and activated with Pam3CSK4 in parallel cultures. Figure 8A summarizes the in
432 vitro workflow, including CM-based assays and Transwell co-culture. Relative to M2-NC cells, M2-
433 siTLR2 macrophages contained less TLR2, CD163, and CD206 protein, while CD86 changed little.
434 Pam3CSK4 increased TLR2, CD163, and CD206 relative to its control without a marked CD86
435 response (Figure 8B). The RT-qPCR results followed the same direction: TLR2, CD163, and CD206
436 transcripts decreased after silencing and increased after agonist treatment, whereas CD86 remained
437 statistically unchanged (Figure 8C). In the 141 glioma specimens, mIHC detected TLR2/CD163
438 double-positive cells, and the percentages of TLR2-positive and CD163-positive cells were positively
439 correlated (Spearman $r = 0.43$, $P = 8.7 \times 10^{-8}$; Figure 8D). IL-10, TGF- β , IL-6, and TNF- α
440 concentrations all decreased in M2-siTLR2 supernatants relative to M2-NC and increased after
441 Pam3CSK4 relative to the corresponding control (Figure 8E). Considered as complementary
442 polarization readouts, these ELISA changes were concordant with the CD163 and CD206 results.
443 Overall, the opposing effects of silencing and agonist stimulation support a role for TLR2 in
444 maintaining the M2-related phenotype in vitro.



445

446 Figure 8 | TLR2 silencing inhibits M2-like macrophage polarization in vitro. (A) Schematic of the in
 447 vitro functional assays. Conditioned medium was used for wound-healing and EdU assays, and a
 448 Transwell co-culture system was used for invasion assays. (B) Western blot analysis of TLR2, CD86,
 449 CD163, and CD206 protein expression in the M2-NC, M2-siTLR2, M2-Control, and M2-Pam3CSK4
 450 groups; β -actin served as the loading control. (C) RT-qPCR analysis of TLR2, CD163, CD206, and
 451 CD86 mRNA levels in the four groups. (D) Representative mIHC images of glioma TMA regions
 452 with low or high TLR2 and CD163 expression, together with Spearman correlation analysis of the
 453 proportions of TLR2-positive and CD163-positive cells; TLR2, orange; CD163, cyan; DAPI, blue;
 454 scale bar, 100 μ m ($r = 0.43$, $P = 8.7 \times 10^{-8}$). (E) ELISA measurements of IL-10, TGF- β , IL-6, and

TNF- α in culture supernatants from the four groups, used as complementary polarization-associated readouts. M2-Control denotes the corresponding control for Pam3CSK4 treatment; Pam3CSK4 is a TLR2 agonist. ns, not significant; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

4 Discussion

This study places TLR2 within a cell-resolved spatial context rather than treating it only as a bulk glioma marker. Across transcriptomic datasets, a 141-case mIHC cohort, and in vitro assays, the TLR2-associated prognostic signal was linked to macrophage enrichment, local tumor-immune arrangement, and the state of M2-like macrophages. TLR2⁺ M2-like TAMs located close to S100⁺ tumor cells identified patients with shorter OS and DFS. In contrast, M2-like TAM proximity was not significantly prognostic when the analysis was confined to TLR2⁺S100⁺ index tumor cells. The effects of manipulating TLR2 in M2-like macrophages further indicate that macrophage-associated TLR2 is a plausible biological component of this spatial feature, while not excluding tumor-cell-intrinsic roles.

Higher tissue-level TLR2 accompanied advanced pathological features and unfavorable outcome, consistent with earlier data-mining studies (41). A bulk transcript measurement, however, cannot assign expression to malignant or infiltrating cells. Here, single-cell datasets localized TLR2 mainly to macrophages, and mIHC showed a positive relationship between the TLR2-positive and CD163-positive fractions. Part of the bulk signal may therefore reflect the abundance or state of myeloid cells. This interpretation complements reports that tumor-cell TLR2 promotes glioma stem-cell invasion and autophagy (42, 43); the two cellular sources need not be mutually exclusive. Associations with WHO grade, IDH status, and 1p/19q codeletion also suggest that TLR2 abundance is partly conditioned by the molecular composition of the tumors being compared.

The mIHC data did not indicate generalized immune accumulation in TLR2-positive tumors. Instead, increases were selective for M0 cells, M2-like TAMs, CD4⁺ T cells, and Tregs, and M2-like TAM abundance was associated with shorter OS and DFS. This myeloid-predominant but heterogeneous pattern agrees with the functional diversity of macrophages described in glioma (20–22, 44–47). Macrophage infiltration was most evident in grade IV tumors. M0 cells, M2-like TAMs, and CD4⁺ T cells were also more abundant in stromal than in tumor regions. Such compartmental enrichment describes distribution only; it does not reveal the direction of recruitment or migration. More importantly, it shows why whole-region density and immediate tumor-cell proximity must be evaluated as separate features.

The neighborhood measurements made that distinction explicit. ES quantified how many eligible immune neighbors surrounded an average index tumor cell, whereas EP quantified how frequently index cells had at least one such neighbor. TLR2-positive tumors had higher ES for several TAM populations and higher EP for M0 and M2-like cells. Yet tissue density and local topology did not always move together: Treg density increased without a change in Treg ES or EP, while M1-like TAM ES increased despite no significant density difference. Spatial mIHC work in other cancers has similarly shown that cell arrangement can add information beyond abundance (29, 30, 48–51). In our cohort, M0 and M2-like TAM proximity was unfavorable, whereas M1-like TAM proximity was favorable, indicating that the meaning of proximity depends on the neighboring phenotype. ES, EP, and distance remain sensitive to cell density, sampled area, and tissue architecture; without a spatial null model or density adjustment, they should not be interpreted as evidence of active recruitment or physical contact.

The most distinctive result emerged from changing the index-cell definition. Using all S100⁺ tumor cells asked which immune phenotypes preferentially occupied general tumor neighborhoods; in that analysis, TLR2⁺ M2-like TAMs were the nearest subset, and greater proximity predicted shorter OS and DFS. Using only TLR2⁺S100⁺ tumor cells instead tested whether TLR2-expressing malignant cells had a specifically prognostic M2-like neighborhood. Those survival associations did not reach significance, although the P values of 0.054 and 0.073 retained an unfavorable direction. The available data therefore suggest that the distribution of TLR2⁺ M2-like TAMs may carry more prognostic information than tumor-cell TLR2 alone, consistent with the context-dependent specialization of glioma myeloid cells (20, 21, 26, 27). This remains an index-definition-related observation; a formal interaction test, larger samples, and independent spatial cohorts are required before concluding that the two effects differ.

The cell experiments offer functional plausibility for the tissue observations. Reducing TLR2 in M2-like macrophages decreased U251 and LN229 migration in the CM assay and invasion in Transwell co-culture, while EdU incorporation was unchanged under the tested conditions. The same manipulation lowered CD163 and CD206, whereas Pam3CSK4 shifted these markers in the opposite direction and had little effect on CD86. Cytokine measurements were concordant with this polarization change but do not identify a specific soluble mediator. These data fit with reciprocal macrophage–glioma signaling (20) and extend the tumor-cell-centered literature on TLR2-dependent invasion and autophagy (42, 43). The relevant ligand and downstream pathway were not established, despite known roles of TLR2 in innate immune activation (52–56). Moreover, neither CM treatment nor Transwell co-culture experimentally altered intercellular distance. They support the biological relevance of the TLR2-associated niche but cannot show that proximity causes progression or establish the direction of signaling in tissue.

Clinically, a spatial measure based on TLR2⁺ M2-like TAM proximity could prove more informative than TLR2 expression considered alone. Its incremental value should be tested in models that also include TAM density, ES, EP, WHO grade, IDH status, and established clinical variables. The therapeutic implication is similarly selective: the results favor investigating TLR2⁺ M2-like TAMs or their polarization state rather than depleting all macrophages. TAM-directed approaches have translated poorly in part because macrophage origin, state, and location can produce different or opposing effects (44–46). Cell-restricted modulation of TLR2, macrophage reprogramming, and local delivery are testable possibilities, but their feasibility must first be demonstrated in patient-derived and in vivo systems.

Several limitations define the interpretation of these results. A retrospective TMA with limited cores cannot fully represent intratumoral or intertumoral heterogeneity, and multiregional whole-section cohorts are needed for validation. The marker panel supplied operational cell definitions, but the M0/M1/M2 labels cannot capture the continuum or plasticity of TAM states. Spatial measurements were two-dimensional centroid distances within a fixed 20- μ m radius; they do not demonstrate membrane contact, three-dimensional adjacency, or signal exchange and may vary with cell density and tissue architecture. Testing multiple subsets, index populations, and outcomes also raises issues of multiplicity, cutoff choice, and statistical power. Finally, THP-1-derived macrophages, established glioma lines, CM exposure, and Transwell co-culture do not reproduce the full interaction among patient-derived microglia, monocyte-derived macrophages, and malignant cells. We did not perform bidirectional cell-type-specific TLR2 manipulation, rescue experiments, or in vivo validation. Future work should combine higher-dimensional spatial profiling with patient-derived models and cell-restricted genetic approaches to identify the relevant ligands, downstream pathways, and causal direction.

544 In conclusion, our findings extend the study of glioma TLR2 from bulk abundance to cellular source
545 and spatial organization. Close apposition of TLR2⁺ M2-like TAMs to glioma cells was associated
546 with unfavorable prognosis, and macrophage TLR2 supported an M2-related state together with
547 glioma-cell migration and invasion in vitro. This combination provides a basis for testing spatial
548 immune risk measures and more selective TAM-directed strategies. Independent prognostic value
549 and therapeutic relevance still require confirmation in higher-resolution spatial datasets, separate
550 clinical cohorts, and in vivo models.

551 **5 Conflict of interest**

552 No commercial or financial relationship that could be interpreted as a potential conflict of interest
553 was present during this research.

554 **6 Author contributions**

555 ZG and XZ contributed equally to this work and share first authorship. WZ and DJ conceived and
556 supervised the study. ZG, XZ, SZ, CZ, PS, CY, TY, and SL performed the experiments and collected
557 the data. ZZ, HG, HYG, JX, and JY contributed to data curation, validation, and visualization. ZG,
558 XZ, SZ, and CY analyzed the data and interpreted the results. ZG and XZ drafted the manuscript.
559 WZ and DJ critically revised the manuscript and acquired funding. All authors reviewed, edited, and
560 approved the final version of the manuscript.

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566 Medical University (QYZDXK-003).

567 **8 Ethics approval and accordance statement**

568 The use of archived human glioma tissue specimens in this study was reviewed and approved by the
569 Ethics Committee of Shanghai Outdo Biotech Co., Ltd. (project No. HBraG144Su01; ethics review
570 document No. SHYJS-CP-1801021; approval date: 8 January 2018). The study was conducted in
571 accordance with the Declaration of Helsinki and applicable institutional guidelines.

572 **9 Consent to participate**

573 The requirement for informed consent for the use of the archived tissue specimens was waived by the
574 Ethics Committee of Taizhou Hospital of Zhejiang Province (approval No. K20200506) as part of the
575 biobank public service platform project conducted in collaboration with Shanghai Outdo Biotech Co.,
576 Ltd.

577 **10 Consent to publish**

578 Not applicable. This manuscript contains no identifiable personal information, individual-level
579 identifying details, or identifiable patient images or videos.

580 **11 Clinical trial number**

581 Not applicable. This study was not a clinical trial and did not prospectively assign human participants
582 to any health-related intervention.

583 12 Data availability statement

584 All publicly available datasets analyzed in this study are accessible as follows. TCGA RNA-
585 sequencing and clinical data were obtained from the NCI Genomic Data Commons projects TCGA-
586 GBM (<https://portal.gdc.cancer.gov/projects/TCGA-GBM>) and TCGA-LGG
587 (<https://portal.gdc.cancer.gov/projects/TCGA-LGG>). The CGGA mRNAseq_325 and mRNAseq_693
588 expression and clinical datasets are available from the CGGA download portal
589 (<https://www.cgga.org.cn/download.jsp>); the corresponding raw sequencing data are deposited under
590 BioProject accessions PRJCA001746 (<https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA001746>) and
591 PRJCA001747 (<https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA001747>), respectively.
592 Comparisons with normal brain tissue were performed using GEPIA2 ([http://gepia2.cancer-](http://gepia2.cancer-pku.cn/#analysis)
593 [pku.cn/#analysis](http://gepia2.cancer-pku.cn/#analysis)), which integrates uniformly processed TCGA and GTEx data. Information and
594 open-access data for the GTEx project (dbGaP study accession phs000424) are available from the
595 GTEx Portal (<https://gtexportal.org/home/downloads/adult-gtex/overview>). TLR2 expression across
596 cell populations was examined using the collection of 17 human glioma single-cell datasets curated
597 in Tumor Immune Single-cell Hub 2 (TISCH2; <https://tisch.compbio.cn/gallery/>). Dataset-level
598 identifiers and metadata are available through the TISCH2 dataset browser. The single-cell RNA-
599 sequencing dataset independently reanalyzed using Seurat is available from the Gene Expression
600 Omnibus under accession GSE273274
601 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE273274>) and is associated with
602 BioProject PRJNA1141154 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1141154>). Additional
603 data supporting the findings of this study are available from the corresponding authors upon
604 reasonable request.

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