

# 1 Immune landscape of isocitrate dehydrogenase stratified human 2 gliomas

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21 **ABSTRACT**

22 The brain tumor immune microenvironment (TIME) continuously evolves during glioma  
23 progression, but only a limited view of a highly complex glioma associated immune contexture  
24 across isocitrate dehydrogenase mutation (IDH) classified gliomas is known. Herein, we present  
25 an unprecedentedly comprehensive view of myeloid and lymphoid cell type diversity based on  
26 our single cell RNA sequencing and spectral cytometry-based interrogation of tumor-associated  
27 leukocytes from fifty-five IDH stratified primary and recurrent human gliomas and three non-  
28 glioma brains. Our analyses revealed twenty-two myeloid and lymphoid cell types within and  
29 across glioma subtypes. Glioma severity correlated with microglial attrition concomitant with a  
30 continuum of invading monocyte-derived microglia-like and macrophages amongst other  
31 infiltrating conventional T and NK lymphocytes and unconventional mucosa associated invariant  
32 T (MAIT) cells. Specifically, certain microglial and monocyte-derived subpopulations were  
33 associated with antigen presentation gene modules, akin to cross-presenting dendritic cells  
34 (DCs). Furthermore, we identified phagocytosis and antigen presentation gene modules  
35 enriched in Triggering receptor expressed on myeloid (TREM)-2<sup>+</sup> cells as a putative anti-glioma  
36 axis. Accelerated glioma growth was observed in *Trem2* deficient mice implanted with CT2A  
37 glioma cells affirming the anti-glioma role of TREM2<sup>+</sup> myeloid cells. In addition to providing a  
38 comprehensive landscape of glioma-specific immune contexture, our investigations discover  
39 TREM2 as a novel immunotherapy target for brain malignancies.

## 40 INTRODUCTION

41 The discovery of meningeal and parenchymal access of immune cells<sup>1</sup> and the presence of  
42 meningeal<sup>2-4</sup> and dural lymphatics<sup>5</sup> in the central nervous system (CNS) has led to redefinition  
43 of the brain being immunologically distinct rather than immune privileged; a notion held for  
44 several decades<sup>6</sup>. In brain pathologies such as traumatic injury or neurodegenerative disorders,  
45 phagocytic cells comprising brain resident microglia and CNS associated  
46 monocytes/macrophages are the first responders to resolve associated inflammation. For  
47 instance, in Alzheimer's disease the protective role of microglia in clearing amyloid plaques has  
48 been established to prevent disease progression<sup>7</sup>. In contrast, tumor associated macrophages  
49 have been linked to poor prognosis in brain neoplasms such as gliomas that arise from  
50 transformed neural progenitor cells<sup>8,9</sup>. Although primarily phagocytic in nature, myeloid cells are  
51 plastic and can undergo functional diversification under the influence of dysregulated cytokine  
52 and chemokine milieu contributed by both infiltrating bone marrow derived leukocytes as well as  
53 tumor cells<sup>10-13</sup>. Myeloid cell functions can also be differentially influenced by tumor necrosis and  
54 inflammation, a defining feature of IDH-wt when compared to IDH-mut gliomas<sup>14</sup>. Standard of  
55 care treatments such as surgical resection followed by temozolamide and ionizing radiation can  
56 unintentionally cause disruption of anatomical barriers, immunomodulation and necrosis, all of  
57 which can skew the properties of myeloid cells and other leukocytes<sup>15,16</sup>.

58 Studies pertaining to immune cell heterogeneity of gliomas at single cell resolution are  
59 emerging, however these studies are either restricted to myeloid cells<sup>13,17-21</sup> or lack in-depth  
60 characterization of low grade in comparison to high grade gliomas<sup>17,18,22-24</sup>. Furthermore, given  
61 that most immunotherapy clinical trials are prioritized in relapsed patients<sup>25</sup>, understanding the  
62 treatment induced changes of brain TIME with unbiased approaches in recurrent tumors of all  
63 glioma subtypes are imperative. Glioma specific TIME studies are broadly focused on microglia  
64 /macrophages collectively referred as glioma-associated macrophages (GAMs), myeloid-  
65 derived suppressor cells (MDSCs), and tumor infiltrating lymphocytes (TILs)<sup>23,26</sup>. However,  
66 oversimplified myeloid cell diversity and M1/M2 functional dichotomization ignores the  
67 phenotypic heterogeneity and plasticity in these cell types<sup>27-29</sup>. Recent cytometry studies have  
68 captured the leukocyte diversity of the brain TIME in primary gliomas and brain metastasis<sup>30,31</sup>  
69 to a certain extent albeit based on *a priori* markers.

70 To address these knowledge gaps and delineate the glioma associated leukocyte  
71 diversity in the TIME, we performed single cell (sc)- and bulk RNA Sequencing (RNA-seq) and  
72 spectral cytometry analyses on tumor-associated leukocytes from fifty-five IDH-stratified primary

73 (treatment naïve) and standard of care treated recurrent glioma subtypes to define their immune  
74 landscape. In addition to corroborating established myeloid dominant glioma characteristics, we  
75 redefine glioma TIME by superimposing our advanced findings across glioma subtypes with  
76 largely IDH-wt glioma restrictive studies<sup>17,18,22-24</sup>. Our major findings include the following: i) We  
77 observed significant attrition of microglia (MG) accompanied by increased infiltration of classical  
78 monocytes (c-Mo), monocyte-derived-microglia-like (Mo-MG or MG-like), -macrophages (MDM)  
79 and conventional dendritic cells (cDC)-2 in recurrent IDH-wild type gliomas relative to other  
80 glioma subtypes; ii) We demonstrate eleven transcriptionally distinct glioma associated MG  
81 states inclusive of tumoricidal, inflammatory and metabolic phenotypes; ii) Infiltration of Tregs,  
82 NK cells and mucosa associated invariant T (MAIT) were significantly abundant in recurrent IDH-  
83 wild type gliomas; and iv) We identified glioma associated myeloid cells with triggering receptor  
84 expressed on myeloid (TREM)-2 cells enriched for phagocytosis and antigen-presentation gene  
85 modules as putative anti-glioma axis and demonstrate their anti-glioma functions using a  
86 xenograft mouse model. In summary, our reverse translational glioma immunophenotyping  
87 investigations reveal an unprecedentedly advanced landscape of glioma TIME that can be  
88 exploited for future immunotherapy applications. We further uncover TREM2 as a novel glioma  
89 specific immunomodulatory target with likely implications in other brain malignancies.

90

## 91 **RESULTS**

### 92 **Transcriptionally defined immune cell diversity in IDH-mutation stratified human gliomas**

93 To discern glioma associated immune cell diversity, we performed single cell RNA sequencing  
94 (scRNA-seq) on flow sorted CD45<sup>+</sup> leukocytes obtained from tumors of eighteen IDH-mutation  
95 classified patients comprising IDH-mutant primary (IMP; n=4), IDH-mutant recurrent (IMR; n=6),  
96 IDH-wild type primary (IWP; n=4), or IDH-wild type recurrent (IWR; n=4) gliomas (hereafter  
97 referred as glioma subtypes). Three quasi-normal, non-glioma brains (NGBs) either from a grade  
98 I meningioma patient or refractory epileptic non-neoplastic patients were used as controls (**Fig.**  
99 **1A and Supplementary Table S1**). Using a previously described immune cell enrichment  
100 protocol<sup>32</sup> and CD45<sup>+</sup> sorting strategy, we consistently obtained highly pure CD45<sup>hi</sup>/CD45<sup>lo</sup>  
101 leukocyte subpopulations in gliomas and NGBs (**Fig. 1A and Supplementary Fig. S1A-C**). This  
102 is in contrast to previous studies using human glioma specimens that were not able to resolve  
103 these two distinct CD45<sup>hi</sup>/CD45<sup>lo</sup> subpopulations<sup>33-35</sup>.

104 Overall scRNA-seq dataset was batch corrected with Harmony using COMBAT software  
105 (**Supplementary Fig. S1D-G**). A low rejection rate in K-BET indicated homogeneous mixing of

106 samples after batch correction (**Supplementary Fig. S1H**) as detailed in methods. Furthermore,  
107 we removed the likely doublets/multiplets, cell debris, and low-quality cells using a multistep  
108 approach (see Methods). Unsupervised clustering followed by Uniform Manifold Approximation  
109 and Projection (UMAP) analyses resolved 144,678 cells into two major immune compartments  
110 predominated by myeloid (n=100,587) and lymphoid cells (n=44,091) within and across glioma  
111 subtypes in line with previous reports<sup>30,31,36,16,17,21-23</sup> with the exception of IWR gliomas, which  
112 had similar proportions of myeloid and lymphoid cell populations (**Fig. 1B**). Glioma associated  
113 myeloid cells exhibited a continuum of overlapping gene features (*SPP1*, *APOE*, *C1QC*) with  
114 MG and macrophage (MAC) subsets yet resolved by core MG gene set including *CX3CR1*,  
115 *GPR34*, *P2RY12*, *P2RY13*, *SALL1*, *TAL1*, and *TMEM119* amongst others (**Fig. 1C**,  
116 **Supplementary Fig. S1J**). Dendritic cells (DC), monocytes (Mo), and neutrophils were clearly  
117 identified based on expression of canonical gene signatures (**Fig. 1C, Supplementary Fig. S1I**  
118 **and J**).

119 Brain resident MG represented the largest myeloid cell type in NGB and IMP (**Fig. 1C**).  
120 In contrast, in response to MG attrition, invading MG-like cells co-expressing MG- (*SORL1*,  
121 *SAMD9L*, *GPR34*) and MAC- (*GLDN*, *MSR1*, *CD163*) signature genes, *VCAN*<sup>+</sup>*FCN1*<sup>+</sup> classical  
122 monocytes (c-Mo), *TCF7L2*<sup>+</sup>*FCGR3A*<sup>+</sup> non-classical monocytes (nc-Mo), *CD163*<sup>+</sup>*MARCO*<sup>+</sup>*FN1*<sup>+</sup>  
123 MAC, *TMEM176A*<sup>+</sup>*SELENOP*<sup>+</sup> MDM proportionately increased in IMR, IWP and IWR glioma  
124 subtype (**Fig. 1C, Supplementary Fig. S1J**). Increased trends with professional antigen  
125 presenting cells (APC) such as *CLEC9A*<sup>+</sup> cDC1, *CD1C*<sup>+</sup> cDC2 and *IL3RA*<sup>+</sup> plasmacytoid DC  
126 (pDC) were observed in IWR gliomas compared to other glioma subtypes. Other notable glioma  
127 associated myeloid cells included indistinguishable cell type with enriched interferon (IFN)  
128 stimulated gene signatures (*IFI44L*, *IFI6*, *ISG15*) defined as MAC/MG\_IFNs, and proliferative  
129 genes (*MKI67*, *PCLAF*) expressing Myeloid\_Proliferative (Myeloid\_prolif) cells and *JMJD1C*<sup>+</sup>  
130 Neutrophils (**Fig. 1C, Supplementary Fig. S1J**). We speculate that the influx of non-MG myeloid  
131 cells is a consequence of depleting niches of MG akin to observations in inflammation associated  
132 conditions where tissue resident macrophage attrition has been reported<sup>37</sup>.

133 Inter- and intratumoral glioma associated lymphoid cell types resolved into T  
134 lymphocytes, *TRDC*<sup>+</sup>  $\gamma\delta$ -T cells, *SLC4A10*<sup>+</sup> Mucosa associated invariant T (MAIT) cells, *NKG7*<sup>+</sup>  
135 *KLRF1*<sup>+</sup> Natural Killer (NK) cells, *CD3D*<sup>+</sup>*NKG7*<sup>+</sup> Natural Killer T (NKT) cells, *CD79A*<sup>+</sup>*MS4A1*<sup>+</sup>B  
136 lymphocytes and *MZB1*<sup>+</sup>*IGHG1*<sup>+</sup> plasma cells (**Fig. 1D, Supplementary Fig. S1K and 1L**).  
137 Notably, amongst lymphoid lineage cells, we identified rare infiltrating populations of MAIT  
138 (0.18%- 4.14%) and NKT (0.35% - 26.4%) in human glioma TIME, which have not been

139 previously reported<sup>38</sup>. We observed glioma subtype specific enrichment patterns, such as  
140 apparently reduced MG and abundance of T-cell and monocyte-derived cells in all patients in  
141 the IWR group (**Supplementary Fig. S1M**). Overall, we report twelve myeloid and ten lymphoid  
142 cell types with transcriptionally defined phylogenetic cellular relationships in the TIME of IDH  
143 stratified primary and recurrent human gliomas (**Supplementary Fig. S1N**).

144

### 145 **Glioma associated transcriptional immune cell phenotypes validated by spectral** 146 **cytometry**

147 In view of transcriptionally redefined immune cell diversity across glioma subtypes, we validated  
148 scRNA-seq inferred cell types with correlative protein markers. A 40-parameter protein marker  
149 panel was designed (**Supplementary Table S2**) to corroborate majority of inferred cell types  
150 (**Supplementary Fig. 1N**). A comprehensive spectral cytometry-based phenotyping  
151 immunoassay was performed across fifty-five patients covering all glioma subtypes and three  
152 refractory epileptic non-neoplastic patients (detailed in **Supplementary Table S1**). We  
153 confirmed P2RY12<sup>+</sup>CX3CR1<sup>+</sup>MG as the most abundant cells across glioma subtypes with  
154 highest proportions evident in NGB and IMP (**Fig. 2A**). Total P2RY12<sup>+</sup>CX3CR1<sup>+</sup>MG and even  
155 reactive CD11c<sup>+</sup>MG were dramatically reduced with glioma recurrence in IMR and IWR  
156 compared to IMP and IWP glioma subtype respectively. A concomitant significant increase in  
157 CD11c<sup>+</sup>CCR2<sup>+</sup>Mo-MG, CD14<sup>+</sup>CD16<sup>-</sup>c-Mo, CD68<sup>+</sup>CCR2<sup>+</sup>MDM and CD1c<sup>+</sup>cDC2 was observed  
158 in IWR relative to IMP glioma subtype while other myeloid cell types such as CD16<sup>-</sup>CD14<sup>+</sup>nc-  
159 Mo, CD68<sup>+</sup>CCR2<sup>+</sup>MDM, Clec9A<sup>+</sup>cDC1 (**Fig. 2 B-E**) and CD66b<sup>+</sup> neutrophils (not shown).  
160 Amongst lymphoid lineage cells Foxp3<sup>+</sup>Tregs, CD56<sup>hi</sup>NK, CD56<sup>lo</sup>NK cells and  
161 TCRV $\alpha$ 7.2<sup>+</sup>mucosa associated invariant T (MAIT) were significantly abundant in IWR gliomas  
162 with co-presence of CD4, CD8 T, NKT, and  $\gamma\delta$ -T cells across glioma subtypes (**Fig 2F-H**).  
163 Altogether, we confirmed all major cell types and their enrichment patterns across glioma  
164 subtypes.

165

### 166 **Transcriptional heterogeneity and inferred functional states of microglial cells in human** 167 **gliomas**

168 In humans, the CNS associated microglial states have been defined in Alzheimer's<sup>39</sup>, multiple  
169 sclerosis<sup>40</sup> and to a limited extent in primary IDH-wt and IDH-mut gliomas using single cell  
170 transcriptomics<sup>17,18,23,41</sup>. We sub-clustered and delineated MG into eleven distinct states  
171 distributed across glioma subtypes (**Fig. 3A, Supplementary Fig. S2B-E**). These MG clusters

172 were distinguished from the rest of the myeloid cells based on the core MG gene sets inclusive  
173 of MG specific transcription factors; *TAL1* and *SALL1* (**Fig. S1J**).

174 To evaluate functional features, we performed gene ontology (GO) analysis which identified MG  
175 expressing unique markers with subtype specific clustering. This included glioma restricted  
176 antigen presentation associated gene modules (e.g., *CSTD*, *MS4A4A*) in MG\_APC-Like 1 and  
177 MG\_APC-like 2 clusters and a lipid metabolism associated *LPL*<sup>+</sup> MG\_Lipid Metab. Cluster (**Fig.**  
178 **3B, Supplementary Fig. S2C**). We also observed IDH-mut glioma restricted MG clusters that  
179 included *BAG3*<sup>+</sup>MG\_hsp and *ATF* expressing metabolically enriched MG\_OxPhos (**Fig. 3B,**  
180 **Supplementary Fig. S2C**). MG that was predominantly seen in NGB were *P2RY12*<sup>+</sup>  
181 MG\_homeostatic and *CCL4L2* expressing inflammatory MG\_Inflam 1 cluster associated with  
182 response to tumor necrosis factor, Interleukin-1 and lipopolysaccharide GO term (**Fig. 3B,**  
183 **Supplementary Fig. S2C**). Tissue macrophages exhibit multifaceted polarization in response  
184 to microenvironmental cues, hence we evaluated the polarization spectrum of MG with nine  
185 distinct macrophage activation programs as previously described<sup>27</sup>. We assessed polarization  
186 states of MG clusters with a spectral polarization view rather than dichotomous M1/M2  
187 polarization model and identified palmitic acid responsive gene module associated with  
188 MG\_Inflam1 along with IL-4 responsive polarization states in MG\_APC-like 2 and MG\_OxPhos  
189 clusters (**Fig. 3C**). A *GNLY*<sup>+</sup>*TNF*<sup>+</sup> cluster defined as MG\_Tumoricidal was also noted across  
190 glioma subtypes. Furthermore, we subclustered the unidentifiable MAC/MG\_IFNs cluster, which  
191 enabled resolution of this cluster into MAC and MG phenotypes. These *IFN* gene associated  
192 clusters were *CD163*<sup>+</sup>*LYZ*<sup>+</sup>MAC\_IFN, *GNLY*<sup>+</sup>*TMIGD3*<sup>+</sup>MG\_IFN *GNLY*<sup>+</sup>*IL1A*<sup>+</sup>MG Inflam 2 (**Fig.**  
193 **3C, Supplementary Fig. S2D-E**) and associated gene signatures are suggestive of their  
194 contribution to inflammation in gliomas. Overall, we identified transcriptionally heterogeneous  
195 MG with their inherent functional likelihoods across glioma subtypes.

### 196

### 197 **Spectrum of invading non-MG myeloid cells in human gliomas**

198 In order to understand non-MG myeloid cell diversity, which have been a subject of intense  
199 investigation in IDH-wt gliomas<sup>12,13,18,20,21,33</sup>, we performed a comprehensive analyses of  
200 invading non-MG myeloid subpopulations across IDH-classified gliomas and identified sixteen  
201 non-myeloid cell states (**Fig. 3D**). We uncovered six MAC and two MDM clusters. Based on DEG  
202 and GO analysis we annotated glioma associated macrophages as *IL10*<sup>+</sup>MAC\_Anti-Inflam,  
203 multiple metabolic phenotypes such as clusters enriched with hypoxia genes (e.g., *SDS*,  
204 *HMOX1*) MAC\_Metab/Hypoxia 1, MAC\_Metab/Hypoxia 2 and *LIPA*<sup>+</sup>MAC\_Lipid Metab

205 subpopulations. A cluster of *LYVE1* expressing MAC associated with vasculature defined as  
206 MAC\_Perivascular was observed across glioma subtypes (**Fig. 3E, Supplementary Fig. S2F-**  
207 **G**). Amongst the MDMs, *C1QA*<sup>+</sup>MDM\_Phagocytic 1 was associated with receptor mediated  
208 endocytic process and *JUN*<sup>+</sup>*SELENOP*<sup>+</sup>MDM\_Inflam subset correlated with positive regulation  
209 of inflammation GO term (**Fig. 3E, Supplementary Fig. S1M, S2F-G**). Although MDMs showed  
210 marked increase in IWR glioma subtype, abundance of MAC was proportionately similar across  
211 IMP, IMR and IWP gliomas (**Fig. 2D**). Monocytic infiltrates in glioma TIME included significantly  
212 abundant *FCN1*<sup>+</sup>*CD14*<sup>+</sup>c-Mo in IWR compared to IMP gliomas whereas *FCGR3A*<sup>+</sup>*TCF7L2*<sup>+</sup> nc-  
213 Mo did not show any noticeable differences across other glioma subtypes (**Fig. 3D, 2C,**  
214 **Supplementary Fig. S1M, S2F**). Glioma associated inflammation led to DC infiltration in  
215 contrast to negligible DCs in NGB *CD1C*<sup>+</sup>cDC2 was significantly abundant in IWR glioma  
216 subtypes, while a proportionally similar levels of infiltration of *CLEC9A*<sup>+</sup>cDC1 and *IL3RA*<sup>+</sup>pDC  
217 were observed across other glioma subtypes (**Fig. 3D, 2E, Supplementary Fig. S1M, S2F-G**).  
218 Taken together, our data provides advanced insight of infiltrating myeloid cell subsets in the  
219 glioma TIME and highlights their similarities and differences with MG.

220

### 221 **Identification of Trem2 as an anti-glioma modulator**

222 To gain a deeper understanding of pathways altered in MG, we inspected genes that regulate  
223 phagocytosis and antigen presentation in myeloid cells, especially MG\_APC-Like 1 and  
224 MG\_APC-like 2 clusters (**Fig. 4A**). These clusters were enriched for *HLA-DR* (**Fig. 4A**), a major  
225 histocompatibility complex (MHC) class II gene. In addition, we found higher expression of  
226 *TREM2* and its regulator *MS4A6A* (**Fig. 4B**), both of which play crucial roles in microglial  
227 functions and gene risk loci for Alzheimer's disease, where MG play a protective role. Recent  
228 studies have also demonstrated a role for Trem2 in immunosuppression of cancer<sup>42,43</sup>. To  
229 examine the influence of TREM2 on glioma growth, we implanted CT-2A glioma cells in C57BL/6  
230 WT mice and compared survival differences with Trem2<sup>-/-</sup> mice. Genetic ablation of Trem2  
231 promoted tumor growth in mice (bioluminescence data not shown) and showed significantly  
232 reduced survival compared to syngeneic WT mice with intracranial gliomas (**Fig. 4C**). These  
233 data demonstrate that in contrast to previous studies on systemic cancers<sup>44,45</sup>, TREM2<sup>+</sup> myeloid  
234 cells play an anti-tumor role in GBM.

235

### 236 **DISCUSSION**



237 Using single cell transcriptomic profiling, our study uncovers the cellular and molecular  
238 landscape of brain-specific glioma immunity. We observed that both myeloid and lymphoid  
239 subpopulations exhibit remarkable cellular diversity depending on IDH status and disease  
240 severity. Recent high throughput studies have elegantly demonstrated the spatio-temporal  
241 distribution of microglial subsets in humans and mice<sup>46</sup>. These studies established the plasticity  
242 of microglial cell states and reveal mechanisms by which MG contribute to limiting or promoting  
243 neurodegenerative diseases<sup>46</sup>. We observed attrition of tissue-resident microglial cells with a  
244 concomitant increased infiltration of non-MG myeloid cells as a distinct feature in IDH-wt  
245 gliomas, which is consistent with recent reports<sup>30,31</sup>. In our investigation, we even report  
246 reduction in MG in relapsed IDH-mutant gliomas and the highest MG attrition evident in IWR  
247 gliomas. Acute inflammation induces transient loss of embryonically derived tissue-resident  
248 macrophages as a result of necroptosis and concomitant replenishment either through self-  
249 renewal or monocytic input as has been described in murine spleen, liver and lungs<sup>37</sup>. In line  
250 with this, we speculate migration of bone marrow derived myeloid cells as a compensatory  
251 mechanism of homeostatic myelopoiesis to fill depleting brain macrophage niches. Our results  
252 showing increased proportion of Mo-MG like cells in response to dramatic reduced MG in IWR  
253 gliomas provides a likely clue for such replenishment patterns in human gliomas. Our findings  
254 with MG provide evidence for multifaceted inflammatory phenotypes characterized by *IL1A*,  
255 *TNF*, *IL6*, *IL10* and *GNLY* expression on various MG subsets. Although recent bulk mRNA-seq  
256 analysis pointed to cumulative spectral nature of glioma associated MG<sup>31</sup>, we clarify  
257 heterogeneity of polarization states and identify an unreported palmitic acid (PA) responsive and  
258 widely acknowledged IL-4 responsive gene modules in distinct clusters of MG. A Glucocorticoid  
259 induced signature has been reported with SEPP1<sup>high</sup> Mo-TAMS<sup>13</sup>. Together our findings suggest  
260 distinct polarization states amongst the multispectral polarization background in glioma  
261 associated MG rather than M1/M2 dichotomy.

262 We observed that certain MG subsets abundantly expressed genes involved in both MHC  
263 class I (*B2M*) and class II (*HLA-DRB1*) molecules suggestive of their APC-like characteristics.  
264 With virtues of antigen presenting molecules, APC-like MG can potentially direct proliferation  
265 and secretion of cytokines in CD4 and CD8 T cells, a hypothesis that is worthy of further  
266 investigation. Interestingly we found that while MG express both MHC class I and class II gene  
267 modules; MAC or MDM are largely restricted to MHC class II. Although MG can orchestrate APC  
268 like functions, their reduced numbers may likely contribute to tumor immune escape. Recent  
269 studies have shown that MG localization is confined to the tumor border compared to

270 macrophages which are enriched in the tumor core, implying that MG may have distinct spatio-  
271 phenotypic features compared to peripheral myeloid cells<sup>22</sup>. Our study also identified novel  
272 clusters of mo-DCs and MDMs that can also possess APC-like properties. Therefore, the dogma  
273 of DCs as *bona fide* immune sentinels and microglia and GAMs as being mainly tumor supportive  
274 needs to be revisited.

275 Despite the evidence of lymphopenia in glioma patients<sup>47</sup> and minimal responsiveness or  
276 treatment refractoriness to checkpoint blockade interventions (e.g., nivolumab)<sup>48</sup>, GBM  
277 immunotherapy clinical trials are T cell centric. In this study, we report paucity of lymphocytes in  
278 primary gliomas, and therefore exclusive T cell-based therapies, may not be effective for such  
279 patient cohorts. In contrast, myeloid cells represented 50-80% of leukocytes compared to  
280 lymphocytes across gliomas. Recent studies have shown that targeting the innate immunity  
281 using either depletion strategies or inducing tumor phagocytosis as alternative  
282 immunomodulatory therapies in GBM<sup>49</sup>. Here, we show that TREM2 as a putative target with  
283 anti-glioma properties. Although TREM2 was shown to be immunosuppressive in other cancers,  
284 TREM2 genetic ablation induced glioma growth in mice indicating differential functions of  
285 TREM2 in brain tumors. Further investigation of TREM2 expression in brain resident and  
286 infiltrating immune cells is warranted.

287 In summary, our unbiased high dimensional studies have paved the way for an advanced  
288 understanding of immune landscape across gliomas that can be exploited for novel  
289 immunotherapy strategies for these cancer types.

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304

305 **AUTHOR CONTRIBUTIONS**

306 P.G. and K.P.B. conceived and designed the study. P.G., S. O., M.P., N.M., A.A. J.G., and K.B.  
307 acquired and processed glioma specimens. A.G., S.A.S and A.M. acquired epileptic clinical  
308 specimens. C.K.M. assisted in accessing the glioma patients' clinical information. P.G., K.B.,  
309 performed sample preparation for scRNA-seq. P.G. performed FACS and related data analysis.  
310 T.M.T. and J.L. performed scRNA-seq experiments. K.C.D. and PG designed cytometry panel.  
311 P.G, S.O. performed and analyzed spectral cytometry experiments under guidance of KCD. K.C.  
312 S.O. and P.G. acquired the data under guidance of K.C.D. Animal studies were performed by  
313 M.P. M.D. and H.S. performed bioinformatics analysis under the supervision of L.W and inputs  
314 from PG, and K.P.B. F.F.L., N.E.N. and A.B.H. provided constructive comments. P.G., M.D.,  
315 L.W. and K.P.B. interpreted the scRNA-seq data. P.G., K.C.D. and K.P.B. interpreted the  
316 spectral cytometry data. P.G., M.D. L.W. and K.P.B. wrote and proofread the manuscript with  
317 contributions from all other authors.

318

319 **Data and Code Availability**

320 All data and code available upon request

321 **Conflict Statement**

322 All authors declare no competing or financial interests

## 323 **METHODS**

324

### 325 **Human brain tumor and tissue collection**

326 The brain tumor/tissue samples were collected from seventeen- patients post appropriate  
327 informed consent during neurosurgery with detailed information pertaining to gender, age,  
328 glioma grade, subtype and, brain site of tumor extraction etc. mentioned in **Supplementary**  
329 **Table S1**. The brain tumor/tissue samples were collected as per MD Anderson internal review  
330 board (IRB)-approved protocol numbers LAB03-0687, LAB04-0001 and 2012-0441. Non-tumor  
331 brain tissue sample was collected from patient undergoing neurosurgery for epilepsy as per  
332 Baylor College of Medicine IRB-approved protocol number H-13798. All experiments were  
333 compliant with the review board of MD Anderson Cancer Center, USA.

334

### 335 **Preparation of leukocyte single cell suspensions from brain tumor and tissue.**

336 The resected brain tumors and, tissues were either freshly processed or transiently stored  
337 overnight MACS Tissue Storage solution (Cat. #130-100-008, Miltenyi Biotec) at 4 degree  
338 Celsius (in case of delayed surgeries) and processed immediately next morning. The brain  
339 tumor/tissue were finely minced and, enzymatic dissociation was performed in prewarmed  
340 digestion medium containing 100 µg/ml Collagenase D (Cat. #11088866001, Sigma-Aldrich)  
341 and, 2U/ml DNase (Cat. #D9905K/ NC0893386, Fisher Scientific) for 45 minutes at 37 degree  
342 Celsius. The enzymatic reaction was neutralized using 2% serum (Cat. #16140-071, gibco) in  
343 IMDM. The enzyme-digested tissues were homogenized by passing through an 18.5G gauge  
344 needle (Cat. #305196, BD) five times followed by further homogenization using syringe piston  
345 on a 100-µ cell strainer (Cat. #0877119/ Corning 352360) placed on a 50ml Falcon. The residual  
346 tissue on the strainer were mechanically dissociated using the piston of a 3ml syringe (Cat.  
347 #309657, BD). The single cells thus obtained were washed in 2% PBS and, centrifuged at 350g  
348 for 5 minutes at 4 degree Celsius. The resulting pellet was further subjected to 33%  
349 Percoll™ (Cat. #17-0891-01, Sigma-Aldrich) gradient and centrifuged at 800g without brakes for  
350 12 minutes at 4 degree Celsius. The resulting pellet was subjected to the RBC lysis reaction  
351 (Cat. #R7757-100ML, Sigma-Aldrich) for 10 minutes at room temperature (R.T.) and reaction  
352 was stopped with 1X PBS (Cat. #21-040-CV, Corning) and resulting cell pellet obtained by  
353 centrifugation (500g, 5min, 4 degree Celsius). The single cells were filtered and cryopreserved  
354 in 10% DMSO (Cat. #D8418, Sigma-Aldrich) in FBS (Cat. #F4135, Sigma-Aldrich) in liquid

355 nitrogen (-196 degree Celsius) after a transient storage at -80 degree Celsius until further  
356 processing.

357

### 358 **CD45 staining and FACS sorting**

359 The cryopreserved leukocytic cells were briefly thawed at 37 degree Celsius and washed with  
360 complete RPMI 1640 (Cat. #10-040-CV, Corning) containing 10% FBS (Cat. #16140-071,  
361 gibco), 1% PSG (Cat. #P4333, Sigma), 10mM HEPES (Cat. #R5158, Sigma) and, incubated at  
362 37 degree Celsius for 30 min. Post incubation, cells were washed with 2% FBS containing PBS  
363 and incubated with 1:20 FcR blocking reagent (Cat. #130-059-901, Miltenyi Biotec) in 2% PBS  
364 for 10 min at RT. The Fc blocked cells were stained with 1:200 diluted anti-human CD45-APC  
365 (Cat. #130-113-114, Miltenyi Biotec) for 20 min at 4 degree Celsius in dark. The CD45-stained  
366 cells were mixed with lived dead Sytox-Green stain (Cat. #S7020, ThermoFisher Scientific) and  
367 CD45<sup>+</sup> cells were sorted with BD FACS Aria<sup>TM</sup>III.

368

### 369 **Single Cell RNA Sequencing (scRNA-seq) Library Preparation**

370 scRNA-seq was performed using 10x Genomics Chromium Single Cell Controller. Sorted cells  
371 were washed with 1X PBS and suspended in PBS/0.04% BSA. Cells were double-checked for  
372 viability and cell number by using the countess II FL and microscope. All cells were diluted to a  
373 concentration of 500-1000 cell/ $\mu$ l in PBS/0.04% BSA before being used for single cell 10X 3'v3.  
374 Single cells were captured using the 10X genomic controller according to the beads types and  
375 chip used for the experiments. The 10X genomic Chromium Single cell 3' GEM, library and Gel  
376 bead Kit 'v3 (cat. #1000075) and chromium chip B single cell kit (pat. #1000073) were used to  
377 capture cells on the controller; cell recovery targeted was in a range of 5000 - 10000 cells.  
378 Captured cells then undergo a GEM-RT, cDNA amplification, and all purification in accordance  
379 to the 10X protocol. Cleanup cDNA was checked via a tape station (Agilent 42000) HSD5000  
380 (cat# part # 5067-5593) for cDNA traces. 25% of the cDNA was used to generate the library,  
381 and the Chromium i7 multiplex kit (part #120262) was used to identify each sample. Library  
382 cleanup was performed using AMPure beads and QC was done again with tape station D1000  
383 tapes (Part # 5067-5583). Ten libraries of equal amount were pooled to give a final concentration  
384 of 10nM and submitted for sequencing with the NovaSeq6000 S2 sequencer, 28 cycles for  
385 read1, 8 cycles for i7 index, and 91 cycles for read 2 through the ATGC core at MD Anderson.  
386 Sequence data was then put through the 10X genomic cell ranger 3.0 pipeline. QC and Fastq  
387 files were obtained and checked for data quality, and Fastq files were used to do further analysis.

## 388 **RNA Isolation**

389 RNeasy Mini Kit (Cat. #74104, Qiagen) was used to extract sorted cells and achieve efficient  
390 purification of total RNA from small amounts of starting material. The technology simplifies total  
391 RNA isolation by combining the stringency of guanidine-isothiocyanate lysis with the speed and  
392 purity of silica-membrane purification to ensure highest-quality RNA with minimum copurification  
393 of DNA. With the RNeasy Mini Kit, total RNA can be purified from 10 to  $1 \times 10^7$  animal or human  
394 cells, 0.5–30 mg human tissues. Briefly, samples are first lysed and then homogenized. Ethanol  
395 is added to the lysate to provide ideal binding conditions. The lysate is then loaded onto the  
396 RNeasy silica membrane. RNA binds (up to 100  $\mu$ g capacity), and all contaminants are efficiently  
397 washed away. For certain RNA applications that are sensitive to very small amounts of DNA,  
398 the residual amounts of DNA remaining can be removed using a convenient on-column DNase  
399 treatment. Pure, concentrated RNA is eluted in 20–100  $\mu$ l water.

400

## 401 **Low input mRNA sequencing**

402 Illumina Compatible low input mRNA libraries were prepared using the Smart-Seq V4 Ultra Low  
403 Input RNA kit (Takara Bio, USA) and KAPA HyperPlus Library Preparation kit (Roche). Briefly,  
404 full length, double-stranded cDNA was generated from 8ng of total RNA using Takara's SMART  
405 (Switching Mechanism at 5' end of RNA Template) technology. The ds cDNA was amplified by  
406 nine cycles of LD-PCR, then purified using Ampure Beads (Agencort). Following bead elution,  
407 the cDNA was evaluated for size distribution and quantity using the Fragment Analyzer High  
408 Sensitivity NGS Fragment Analysis Kit (Agilent Technologies) and the Qubit dsDNA HS Assay  
409 Kit (ThermoFisher) respectively. The cDNA was enzymatically fragmented, and 20ng of the  
410 fragmented cDNA was used to generate Illumina compatible libraries using the KAPA HyperPlus  
411 Library Preparation kit. The KAPA libraries were purified and enriched with 2 cycles of PCR to  
412 create the final cDNA library. The libraries were quantified using the Qubit™ dsDNA HS Assay  
413 (ThermoFisher), then multiplexed 7 libraries per pool. The pooled libraries were quantified by  
414 qPCR using the KAPA Library Quantification Kit (KAPA Biosystems), and assessed for size  
415 distribution using the TapeStation 4200 (Agilent Technologies). The libraries were then  
416 sequenced, one pool per lane, on the Illumina HiSeq4000 sequencer using the 76bp paired end  
417 format.

418

## 419 **scRNA-seq Data Analysis**

420 **Raw sequencing data processing, quality check, data filtering, doublets removal,**  
421 **batch effect evaluation and data normalization.** The raw scRNA-seq data were pre-  
422 processed (demultiplex cellular barcodes, read alignment, and generation of gene count matrix)  
423 using Cell Ranger Single Cell Software Suite provided by 10x Genomics. Detailed QC metrics  
424 were generated and evaluated. Cells with low complexity libraries or likely cellular debris (in  
425 which detected transcripts are aligned to less than 200 genes) were filtered out and excluded  
426 from subsequent analyses. Low-quality cells where >20% of transcripts derived from the  
427 mitochondria were considered apoptotic and also excluded. Following the initial clustering, likely  
428 cell doublets were removed from all clusters. Doublets were identified using a multi-step  
429 approach: 1) library complexity: cells with high complexity libraries (in which detected transcripts  
430 are aligned to more than 6500 genes) were removed; 2) Cluster distribution: doublets or  
431 multiplets likely form distinct clusters with hybrid expression features and exhibit an aberrantly  
432 high gene count; 3) cluster marker gene expression: cells of a cluster express markers from  
433 distinct lineages (e.g., cells in the T-cell cluster showed expression of myeloid cell markers and  
434 vice versa); 4) doublet detection algorithm: DoubletFinder<sup>50</sup>, an algorithm to predict doublets in  
435 scRNA-seq data, was applied to further identify and clean doublets that could have been missed  
436 by steps 1-3. We carefully reviewed canonical marker genes expression on UMAP plots and  
437 repeated the above steps multiple times to ensure elimination of most barcodes associated with  
438 cell doublets.

439 Following removal of poor-quality cells and doublets, a total of 144,678 cells were retained for  
440 downstream analysis. Library size normalization was performed in Seurat v3 (version 3.1.1)<sup>51</sup>  
441 on the filtered gene-cell matrix to obtain the normalized UMI count as previously described<sup>52</sup>.  
442 Statistical assessment of possible batch effects was performed using the R package k-BET (a  
443 robust and sensitive k-nearest neighbor batch-effect test) (PMID: 30573817). k-BET was run on  
444 cells from all samples, and on major cell types including microglia cells and CD8 T cells  
445 separately with default parameters. Each cell type was down sampled to 500 cells. We chose  
446 the k input value from 1% to 100% of the sample size. In each run, the number of tested  
447 neighborhoods was 10% of the sample size. The mean and maximal rejection rates were then  
448 calculated based on a total of 100 repeated k-BET runs. Following estimation of sample  
449 processing- or sequencing-related batch effects using k-BET, we employed Harmony for actual  
450 batch effect correction<sup>53</sup>. Harmony was run with default parameters to remove batch effects in  
451 the PCA space when clustering of major cell lineages before any clustering analysis or cell type  
452 identification/annotation was performed. We carefully evaluated the performance of Harmony in

453 terms of its ability to integrate batches while maintaining cell type separation. Harmony was run  
454 on all cells to firstly identify major cell types. It was also run on each of the major cell types for  
455 subclustering analysis to further identify different cell states. To quantify the performance of  
456 Harmony, we further used k-BET and compared the rejection rate (reflecting batch effect) before  
457 and after Harmony. The data after Harmony showed a low rejection rate, indicating an excellent  
458 performance of batch effect correction in this study.

459 Moreover, we also applied the local inverse Simpson's Index (LISI) to assess the performance  
460 of Harmony. As described previously<sup>53</sup>, the 'integration LISI' (iLISI) measures the degree of  
461 mixing among datasets (batches), ranging from 1 in an unmixed space to the number of datasets  
462 (batches) in a well-mixed space. And the 'cell-type LISI' (cLISI) measures integration accuracy  
463 using the same formulation but computed on cell-type labels instead. An accurate embedding  
464 has a cLISI close to 1 for every neighborhood, reflecting separation of different cell types. Before  
465 batch correction with Harmony, cells were mainly grouped by dataset (iLISI is around 1) and  
466 cells from different cell types were mixed (cLISI is far from 1). After batch correction with  
467 Harmony, iLISI and cLISI were re-computed in the Harmony embedding. iLISI is around 3.5,  
468 indicating a high degree of mixing among different datasets, and cLISI is very close to 1,  
469 reflecting excellent separation of different cell types while remain the well-mixed space.

470

471 ***Unsupervised cell clustering and dimensionality reduction:*** Seurat v3 (version  
472 3.1.1)<sup>51</sup> was applied to the normalized gene-cell matrix to identify highly variable genes (HVGs)  
473 for unsupervised cell clustering. To identify HVGs, the *vst* method in the Seurat package<sup>51</sup> was  
474 used with default parameters. Principal component analysis (PCA) was performed on the top  
475 2000 HVGs. The elbow plot was generated with the *ElbowPlot* function of Seurat<sup>51</sup> and based  
476 on which, the number of significant principal components (PCs) were determined. The  
477 *FindNeighbors* function of Seurat was used to construct the Shared Nearest Neighbor (SNN)  
478 Graph, based on which the unsupervised clustering was done with *Seurat* function *FindClusters*.  
479 Different resolution parameters for unsupervised clustering were then examined in order to  
480 determine the optimal number of clusters. For visualization, the dimensionality was further  
481 reduced using Uniform Manifold Approximation and Projection (UMAP)<sup>54</sup> method with *Seurat*  
482 function *RunUMAP*. The PCs used to calculate the embedding were as the same as those used  
483 for clustering. Two rounds of clustering were performed to identify major cell types (MG, non-  
484 MG myeloid cell, NK and T cells) and cell transcriptomic states within each major cell type. In  
485 the first round, 30-nearest neighbors of each cell were determined based on 30PCs to construct



486 SNN graph. The clustering was performed with resolution 0.8 and each cluster was annotated  
487 by known markers (see ***Determination of major cell types and cell states***). Two major cell  
488 families comprising lymphoid- and, myeloid- cells, as well as negligible number of neural-like  
489 contamination were identified. The second-round clustering was performed on myeloid- and,  
490 lymphoid- cells respectively to identify cell states within each major cell types. For myeloid cell  
491 clustering, 40-nearest neighbors of each cell were determined based on 40 PCs to construct  
492 SNN graph. The clustering was performed with resolution 2, which resulted the identification of  
493 34-cell states. For lymphoid cell clustering, the SNN graph was constructed based on 20-nearest  
494 neighbors of each cell that were determined by 30 PCs. The clustering was performed with  
495 resolution 2, which resulted the identification of 28-cell states.

496

497 ***Determination of major cell types and cell states:*** To define the major cell type of each  
498 single cell, differentially expressed genes (DEGs) were identified for each cell cluster using the  
499 *FindAllMarkers* analysis in the Seurat package<sup>51</sup> and the top 50 most significant DEGs were  
500 carefully reviewed. The top 50 most significant differentially expressed transcription factors were  
501 also identified and reviewed by performing *FindAllMarkers* only on an aggregated TF list<sup>55</sup>. In  
502 parallel, feature plots were generated for top 20 DEGs and a suggested set of canonical  
503 lymphoid and myeloid cell markers, a similar approach as previously described<sup>56,57</sup> followed by  
504 a careful manual review and annotation. The two approaches are combined to infer major cell  
505 types for each cell cluster according to the enrichment of marker genes and top-ranked DEGs  
506 in each cell cluster, and the global cluster distributions as previously described<sup>57</sup>. We sub-  
507 clustered the major myeloid and lymphoid populations iteratively and rigorously annotated the  
508 resulting cell-clusters using a combination of a) top 50 DEGs, b) top 50 differentially expressed  
509 lineage defining transcription factors and c) canonical immune signature genes.

510 ***Hierarchical relationship analysis:*** To study the hierarchical relationships among cell  
511 types identified in this study, unsupervised cluster analysis was performed. Pairwise Spearman  
512 correlations were calculated from average expression level (*Seurat* function  
513 *AverageExpression*) of each cell type, based on which Euclidean distances between cell types  
514 were calculated. Hierarchical cluster analysis was performed by R function *hclust* and the  
515 dendrogram was drawn using R package *ggtree*<sup>58</sup>.

516

517 ***Gene Ontology Enrichment Analysis:*** Top 100 DEGs from each cluster (MG and non-  
518 MG myeloid cells) were used for Gene Ontology enrichment analysis using Bioconductor

519 package ClusterProfiler<sup>59</sup>. Significantly enriched GO-BP (Gene Ontology-Biological processes)  
520 terms were retrieved by setting the threshold of FDR<0.05 and minimum overlapping gene set  
521 size=3. Enriched terms having >=3 queried genes were manually selected using immunological  
522 keywords. Bubble plots were made using ggplot2 R package.

523

524 **Macrophage Polarization Gene Set Enrichment Analysis:** Top 100 DEGs from each  
525 cluster (MG, MAC and MDMs) were used for gene module enrichment analysis with reference  
526 gene signatures previously defined<sup>27</sup> using Bioconductor package ClusterProfiler<sup>59</sup> and plotted  
527 as circos plots<sup>31</sup> using ggplot2 R package.

528

### 529 **Flow Cytometry staining**

530 The cryopreserved cells were thawed at 37 degree Celsius and washed with 10% FBS  
531 (Catalogue No. F4135, Sigma) in Iscove's DMEM (1X, Catalogue No. 10-016-CV, Corning). The  
532 washed cells were pelleted by centrifugation at 500g for 5 mins. Cells were incubated with the  
533 10% FBS containing Iscove's DMEM media at 37°C for 30 mins before staining. PBS washed  
534 cells were stained with a fixable Live Dead Blue Dead cell stain dye (Catalogue No. L34962,  
535 Invitrogen) for 15 mins at 4°C. The Stained cells were washed with 1X PBS. Fc block was  
536 performed with a combination of Fc block -Human Tru Stain Fc block (Catalogue No. 422301,  
537 Biolegend), Nova Block Solution, (Catalogue No. M071437, Phitonex, Cell Blox Blocking Buffer,  
538 Catalogue No. B001T03F01) for 10 mins at 4°C. After Fc block, cell surface staining was  
539 performed with antibody cocktail (mentioned in **Supplementary Table S2**) diluted in BD Horizon  
540 Brilliant Stain buffer (Catalogue No. 566385, Becton Dickinson) and FACS buffer. For cell  
541 surface staining incubate was done for 30 mins at 4°C in dark. The stained cells after staining  
542 were washed with FACS buffer fixed with 200ul True Nuclear fixation buffer (True Nuclear 4X  
543 Fix Concentrate, Catalogue No. 73158, Biolegend and True Nuclear Fix Diluent, Catalogue No.  
544 73160, Biolegend) overnight at 4°C. Overnight fixed cells were permeabilized with 1X  
545 Permeabilization buffer (True Nuclear 10X Perm, Catalogue No. 73162, Biolegend) for  
546 intracellular staining. Permeabilized cells were stained with the intracellular antibody cocktail  
547 (refer to **Supplementary Table S2**) for 20 mins at 4°C. The stained cells were resuspended in  
548 FACS buffer and data was acquired on Cytex Aurora 5 laser spectral flow cytometer.

549

### 550 **Cytometry Analyses**

551 The acquired data was analyzed by Cytex SpectroFlo and Becton Dickinson FlowJo 10.8.1.

552 **Mouse experiments and survival analysis**

553 Six-week-old WT and TREM2<sup>-/-</sup> mice were obtained from Jackson laboratories. One week after  
554 guide screw implantation, 10,000 CT-2A cells were injected intracranially. Animals were  
555 monitored for tumor growth using bioluminescence imaging. For survival analysis, we used the  
556 log-rank test to calculate *P* values between groups, and the Kaplan-Meier method to plot survival  
557 curves using GraphPad Prism9 version 9.2.0 software.

558 **REFERENCES**

- 559 1 Kipnis, J. Multifaceted interactions between adaptive immunity and the central nervous system.  
560 *Science (New York, N.Y.)* **353**, 766 (2016). [https://doi.org:10.1126/science.aag2638](https://doi.org/10.1126/science.aag2638)
- 561 2 Absinta, M. *et al.* Human and nonhuman primate meninges harbor lymphatic vessels that can be  
562 visualized noninvasively by MRI. *eLife* **6** (2017). [https://doi.org:10.7554/eLife.29738](https://doi.org/10.7554/eLife.29738)
- 563 3 Hu, X. *et al.* Meningeal lymphatic vessels regulate brain tumor drainage and immunity. *Cell*  
564 *research* **30**, 229-243 (2020). [https://doi.org:10.1038/s41422-020-0287-8](https://doi.org/10.1038/s41422-020-0287-8)
- 565 4 Louveau, A. *et al.* Structural and functional features of central nervous system lymphatic vessels.  
566 *Nature* **523**, 337-341 (2015). [https://doi.org:10.1038/nature14432](https://doi.org/10.1038/nature14432)
- 567 5 Aspelund, A. *et al.* A dural lymphatic vascular system that drains brain interstitial fluid and  
568 macromolecules. *The Journal of experimental medicine* **212**, 991-999 (2015).  
569 [https://doi.org:10.1084/jem.20142290](https://doi.org/10.1084/jem.20142290)
- 570 6 Sandrone, S., Moreno-Zambrano, D., Kipnis, J. & van Gijn, J. A (delayed) history of the brain  
571 lymphatic system. *Nature Medicine* **25**, 538-540 (2019). [https://doi.org:10.1038/s41591-019-](https://doi.org/10.1038/s41591-019-0417-3)  
572 [0417-3](https://doi.org/10.1038/s41591-019-0417-3)
- 573 7 Hansen, D. V., Hanson, J. E. & Sheng, M. Microglia in Alzheimer's disease. *J Cell Biol* **217**, 459-  
574 472 (2018). [https://doi.org:10.1083/jcb.201709069](https://doi.org/10.1083/jcb.201709069)
- 575 8 Hambardzumyan, D., Gutmann, D. H. & Kettenmann, H. The role of microglia and macrophages  
576 in glioma maintenance and progression. *Nature Neuroscience* **19**, 20-27 (2016).  
577 [https://doi.org:10.1038/nn.4185](https://doi.org/10.1038/nn.4185)
- 578 9 Sørensen, M. D., Dahlrot, R. H., Boldt, H. B., Hansen, S. & Kristensen, B. W. Tumour-associated  
579 microglia/macrophages predict poor prognosis in high-grade gliomas and correlate with an  
580 aggressive tumour subtype. *Neuropathol Appl Neurobiol* **44**, 185-206 (2018).  
581 [https://doi.org:10.1111/nan.12428](https://doi.org/10.1111/nan.12428)
- 582 10 Szulzewsky, F. *et al.* Glioma-associated microglia/macrophages display an expression profile  
583 different from M1 and M2 polarization and highly express Gpnmb and Spp1. *PloS one* **10**,  
584 e0116644 (2015). [https://doi.org:10.1371/journal.pone.0116644](https://doi.org/10.1371/journal.pone.0116644)
- 585 11 Wei, J. *et al.* Immune biology of glioma associated macrophages and microglia: Functional and  
586 therapeutic implications. *Neuro-oncology*, noz212 (2019). [https://doi.org:10.1093/neuonc/noz212](https://doi.org/10.1093/neuonc/noz212)
- 587 12 Gabrusiewicz, K. *et al.* Glioblastoma-infiltrated innate immune cells resemble M0 macrophage  
588 phenotype. *JCI Insight* **1** (2016). [https://doi.org:10.1172/jci.insight.85841](https://doi.org/10.1172/jci.insight.85841)
- 589 13 Pombo Antunes, A. R. *et al.* Single-cell profiling of myeloid cells in glioblastoma across species  
590 and disease stage reveals macrophage competition and specialization. *Nat Neurosci* **24**, 595-610  
591 (2021). [https://doi.org:10.1038/s41593-020-00789-y](https://doi.org/10.1038/s41593-020-00789-y)
- 592 14 Perry, A. & Wesseling, P. Histologic classification of gliomas. *Handb Clin Neurol* **134**, 71-95  
593 (2016). [https://doi.org:10.1016/b978-0-12-802997-8.00005-0](https://doi.org/10.1016/b978-0-12-802997-8.00005-0)
- 594 15 Rong, Y., Durden, D. L., Van Meir, E. G. & Brat, D. J. 'Pseudopalisading' necrosis in glioblastoma:  
595 a familiar morphologic feature that links vascular pathology, hypoxia, and angiogenesis. *J*  
596 *Neuropathol Exp Neurol* **65**, 529-539 (2006). [https://doi.org:10.1097/00005072-200606000-](https://doi.org/10.1097/00005072-200606000-00001)  
597 [00001](https://doi.org/10.1097/00005072-200606000-00001)
- 598 16 Stupp, R. *et al.* Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma.  
599 *New England Journal of Medicine* **352**, 987-996 (2005). [https://doi.org:10.1056/NEJMoa043330](https://doi.org/10.1056/NEJMoa043330)
- 600 17 Sankowski, R. *et al.* Mapping microglia states in the human brain through the integration of high-  
601 dimensional techniques. *Nat Neurosci* **22**, 2098-2110 (2019). [https://doi.org:10.1038/s41593-019-](https://doi.org/10.1038/s41593-019-0532-y)  
602 [0532-y](https://doi.org/10.1038/s41593-019-0532-y)
- 603 18 Muller, S. *et al.* Single-cell profiling of human gliomas reveals macrophage ontogeny as a basis  
604 for regional differences in macrophage activation in the tumor microenvironment. *Genome biology*  
605 **18**, 234 (2017). [https://doi.org:10.1186/s13059-017-1362-4](https://doi.org/10.1186/s13059-017-1362-4)

- 606 19 Ruiz-Moreno, C. *et al.* Harmonized single-cell landscape, intercellular crosstalk and tumor  
607 architecture of glioblastoma. *bioRxiv*, 2022.2008.2027.505439 (2022).  
608 [https://doi.org:10.1101/2022.08.27.505439](https://doi.org/10.1101/2022.08.27.505439)
- 609 20 Abdelfattah, N. *et al.* Single-cell analysis of human glioma and immune cells identifies S100A4  
610 as an immunotherapy target. *Nat Commun* **13**, 767 (2022). [https://doi.org:10.1038/s41467-022-](https://doi.org/10.1038/s41467-022-28372-y)  
611 [28372-y](https://doi.org/10.1038/s41467-022-28372-y)
- 612 21 Lee, A. H. *et al.* Neoadjuvant PD-1 blockade induces T cell and cDC1 activation but fails to  
613 overcome the immunosuppressive tumor associated macrophages in recurrent glioblastoma.  
614 *Nature Communications* **12**, 6938 (2021). [https://doi.org:10.1038/s41467-021-26940-2](https://doi.org/10.1038/s41467-021-26940-2)
- 615 22 Darmanis, S. *et al.* Single-Cell RNA-Seq Analysis of Infiltrating Neoplastic Cells at the Migrating  
616 Front of Human Glioblastoma. *Cell Rep* **21**, 1399-1410 (2017).  
617 [https://doi.org:10.1016/j.celrep.2017.10.030](https://doi.org/10.1016/j.celrep.2017.10.030)
- 618 23 Poon, C. C. *et al.* Differential microglia and macrophage profiles in human IDH-mutant and -wild  
619 type glioblastoma. *Oncotarget* **10**, 3129-3143 (2019). [https://doi.org:10.18632/oncotarget.26863](https://doi.org/10.18632/oncotarget.26863)
- 620 24 Zamler, D. B. *et al.* Immune landscape of a genetically-engineered murine model of glioma relative  
621 to human glioma by single-cell sequencing. *bioRxiv*, 2020.2005.2011.088708 (2020).  
622 [https://doi.org:10.1101/2020.05.11.088708](https://doi.org/10.1101/2020.05.11.088708)
- 623 25 McGranahan, T., Therkelsen, K. E., Ahmad, S. & Nagpal, S. Current State of Immunotherapy for  
624 Treatment of Glioblastoma. *Curr Treat Options Oncol* **20**, 24-24 (2019).  
625 [https://doi.org:10.1007/s11864-019-0619-4](https://doi.org/10.1007/s11864-019-0619-4)
- 626 26 Goswami, S. *et al.* Immune profiling of human tumors identifies CD73 as a combinatorial target  
627 in glioblastoma. *Nature Medicine* **26**, 39-46 (2020). [https://doi.org:10.1038/s41591-019-0694-x](https://doi.org/10.1038/s41591-019-0694-x)
- 628 27 Xue, J. *et al.* Transcriptome-based network analysis reveals a spectrum model of human  
629 macrophage activation. *Immunity* **40**, 274-288 (2014).  
630 [https://doi.org:10.1016/j.immuni.2014.01.006](https://doi.org/10.1016/j.immuni.2014.01.006)
- 631 28 Hambarzumyan, D., Gutmann, D. H. & Kettenmann, H. The role of microglia and macrophages  
632 in glioma maintenance and progression. *Nature neuroscience* **19**, 20-27 (2016).  
633 [https://doi.org:10.1038/nn.4185](https://doi.org/10.1038/nn.4185)
- 634 29 Murray, P. J. *et al.* Macrophage activation and polarization: nomenclature and experimental  
635 guidelines. *Immunity* **41**, 14-20 (2014). [https://doi.org:10.1016/j.immuni.2014.06.008](https://doi.org/10.1016/j.immuni.2014.06.008)
- 636 30 Friebel, E. *et al.* Single-Cell Mapping of Human Brain Cancer Reveals Tumor-Specific Instruction  
637 of Tissue-Invading Leukocytes. *Cell* (2020). [https://doi.org:10.1016/j.cell.2020.04.055](https://doi.org/10.1016/j.cell.2020.04.055)
- 638 31 Klemm, F. *et al.* Interrogation of the Microenvironmental Landscape in Brain Tumors Reveals  
639 Disease-Specific Alterations of Immune Cells. *Cell* (2020).  
640 [https://doi.org:10.1016/j.cell.2020.05.007](https://doi.org/10.1016/j.cell.2020.05.007)
- 641 32 Sheng, J., Ruedl, C. & Karjalainen, K. Most Tissue-Resident Macrophages Except Microglia Are  
642 Derived from Fetal Hematopoietic Stem Cells. *Immunity* **43**, 382-393 (2015).  
643 [https://doi.org:10.1016/j.immuni.2015.07.016](https://doi.org/10.1016/j.immuni.2015.07.016)
- 644 33 Hussain, S. F. *et al.* The role of human glioma-infiltrating microglia/macrophages in mediating  
645 antitumor immune responses. *Neuro Oncol* **8**, 261-279 (2006). [https://doi.org:10.1215/15228517-](https://doi.org/10.1215/15228517-2006-008)  
646 [2006-008](https://doi.org/10.1215/15228517-2006-008)
- 647 34 Bowman, R. L. *et al.* Macrophage Ontogeny Underlies Differences in Tumor-Specific Education  
648 in Brain Malignancies. *Cell reports* **17**, 2445-2459 (2016).  
649 [https://doi.org:10.1016/j.celrep.2016.10.052](https://doi.org/10.1016/j.celrep.2016.10.052)
- 650 35 Quail, D. F. & Joyce, J. A. The Microenvironmental Landscape of Brain Tumors. *Cancer Cell* **31**,  
651 326-341 (2017). [https://doi.org:10.1016/j.ccell.2017.02.009](https://doi.org/10.1016/j.ccell.2017.02.009)
- 652 36 Fu, W. *et al.* High Dimensional Mass Cytometry Analysis Reveals Characteristics of the  
653 Immunosuppressive Microenvironment in Diffuse Astrocytomas. *Frontiers in Oncology* **10**  
654 (2020). [https://doi.org:10.3389/fonc.2020.00078](https://doi.org/10.3389/fonc.2020.00078)

- 655 37 Lai, S. M. *et al.* Organ-Specific Fate, Recruitment, and Refilling Dynamics of Tissue-Resident  
656 Macrophages during Blood-Stage Malaria. *Cell reports* **25**, 3099-3109.e3093 (2018).  
657 <https://doi.org/10.1016/j.celrep.2018.11.059>
- 658 38 Cui, Y. & Wan, Q. NKT Cells in Neurological Diseases. *Frontiers in cellular neuroscience* **13**,  
659 245 (2019). <https://doi.org/10.3389/fncel.2019.00245>
- 660 39 Mathys, H. *et al.* Single-cell transcriptomic analysis of Alzheimer's disease. *Nature* **570**, 332-337  
661 (2019). <https://doi.org/10.1038/s41586-019-1195-2>
- 662 40 Masuda, T. *et al.* Spatial and temporal heterogeneity of mouse and human microglia at single-cell  
663 resolution. *Nature* **566**, 388-392 (2019). <https://doi.org/10.1038/s41586-019-0924-x>
- 664 41 Venteicher, A. S. *et al.* Decoupling genetics, lineages, and microenvironment in IDH-mutant  
665 gliomas by single-cell RNA-seq. *Science (New York, N.Y.)* **355** (2017).  
666 <https://doi.org/10.1126/science.aai8478>
- 667 42 Deczkowska, A., Weiner, A. & Amit, I. The Physiology, Pathology, and Potential Therapeutic  
668 Applications of the TREM2 Signaling Pathway. *Cell* **181**, 1207-1217 (2020).  
669 <https://doi.org/10.1016/j.cell.2020.05.003>
- 670 43 Deming, Y. *et al.* The MS4A gene cluster is a key modulator of soluble TREM2 and Alzheimer's  
671 disease risk. *Science translational medicine* **11** (2019).  
672 <https://doi.org/10.1126/scitranslmed.aau2291>
- 673 44 Katzenelenbogen, Y. *et al.* Coupled scRNA-Seq and Intracellular Protein Activity Reveal an  
674 Immunosuppressive Role of TREM2 in Cancer. *Cell* **182**, 872-885.e819 (2020).  
675 <https://doi.org/10.1016/j.cell.2020.06.032>
- 676 45 Molgora, M. *et al.* TREM2 Modulation Remodels the Tumor Myeloid Landscape Enhancing Anti-  
677 PD-1 Immunotherapy. *Cell* **182**, 886-900.e817 (2020). <https://doi.org/10.1016/j.cell.2020.07.013>
- 678 46 Masuda, T., Sankowski, R., Staszewski, O. & Prinz, M. Microglia Heterogeneity in the Single-  
679 Cell Era. *Cell reports* **30**, 1271-1281 (2020). <https://doi.org/10.1016/j.celrep.2020.01.010>
- 680 47 Chongsathidkiet, P. *et al.* Sequestration of T cells in bone marrow in the setting of glioblastoma  
681 and other intracranial tumors. *Nat Med* **24**, 1459-1468 (2018). <https://doi.org/10.1038/s41591-018-0135-2>
- 682
- 683 48 Muftuoglu, Y. & Liao, L. M. Results From the CheckMate 143 Clinical Trial: Stalemate or New  
684 Game Strategy for Glioblastoma Immunotherapy? *JAMA Oncology* **6**, 987-989 (2020).  
685 <https://doi.org/10.1001/jamaoncol.2020.0857>
- 686 49 Bejarano, L., Jordão, M. J. C. & Joyce, J. A. Therapeutic Targeting of the Tumor  
687 Microenvironment. *Cancer Discov* **11**, 933-959 (2021). <https://doi.org/10.1158/2159-8290.Cd-20-1808>
- 688
- 689 50 McGinnis, C. S., Murrow, L. M. & Gartner, Z. J. DoubletFinder: Doublet Detection in Single-Cell  
690 RNA Sequencing Data Using Artificial Nearest Neighbors. *Cell systems* **8**, 329-337.e324 (2019).  
691 <https://doi.org/10.1016/j.cels.2019.03.003>
- 692 51 Butler, A., Hoffman, P., Smibert, P., Papalexi, E. & Satija, R. Integrating single-cell transcriptomic  
693 data across different conditions, technologies, and species. *Nature biotechnology* **36**, 411-420  
694 (2018). <https://doi.org/10.1038/nbt.4096>
- 695 52 Savas, P. *et al.* Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset  
696 associated with improved prognosis. *Nat Med* **24**, 986-993 (2018). <https://doi.org/10.1038/s41591-018-0078-7>
- 697
- 698 53 Korsunsky, I. *et al.* Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat*  
699 *Methods* **16**, 1289-1296 (2019). <https://doi.org/10.1038/s41592-019-0619-0>
- 700 54 Becht, E. *et al.* Dimensionality reduction for visualizing single-cell data using UMAP. *Nature*  
701 *biotechnology* **37**, 38-44 (2019). <https://doi.org/10.1038/nbt.4314>
- 702 55 Lambert, S. A. *et al.* The Human Transcription Factors. *Cell* **172**, 650-665 (2018).  
703 <https://doi.org/10.1016/j.cell.2018.01.029>

- 704 56 Lambrechts, D. *et al.* Phenotype molding of stromal cells in the lung tumor microenvironment.  
705 *Nat Med* **24**, 1277-1289 (2018). [https://doi.org:10.1038/s41591-018-0096-5](https://doi.org/10.1038/s41591-018-0096-5)
- 706 57 Sade-Feldman, M. *et al.* Defining T Cell States Associated with Response to Checkpoint  
707 Immunotherapy in Melanoma. *Cell* **175**, 998-1013.e1020 (2018).  
708 <https://doi.org:10.1016/j.cell.2018.10.038>
- 709 58 Yu, G., Smith, D. K., Zhu, H., Guan, Y. & Lam, T. T.-Y. ggtree: an r package for visualization  
710 and annotation of phylogenetic trees with their covariates and other associated data. *Methods in*  
711 *Ecology and Evolution* **8**, 28-36 (2017). <https://doi.org:10.1111/2041-210X.12628>
- 712 59 Yu, G., Wang, L.-G., Han, Y. & He, Q.-Y. clusterProfiler: an R package for comparing biological  
713 themes among gene clusters. *OMICS* **16**, 284-287 (2012). <https://doi.org:10.1089/omi.2011.0118>
- 714

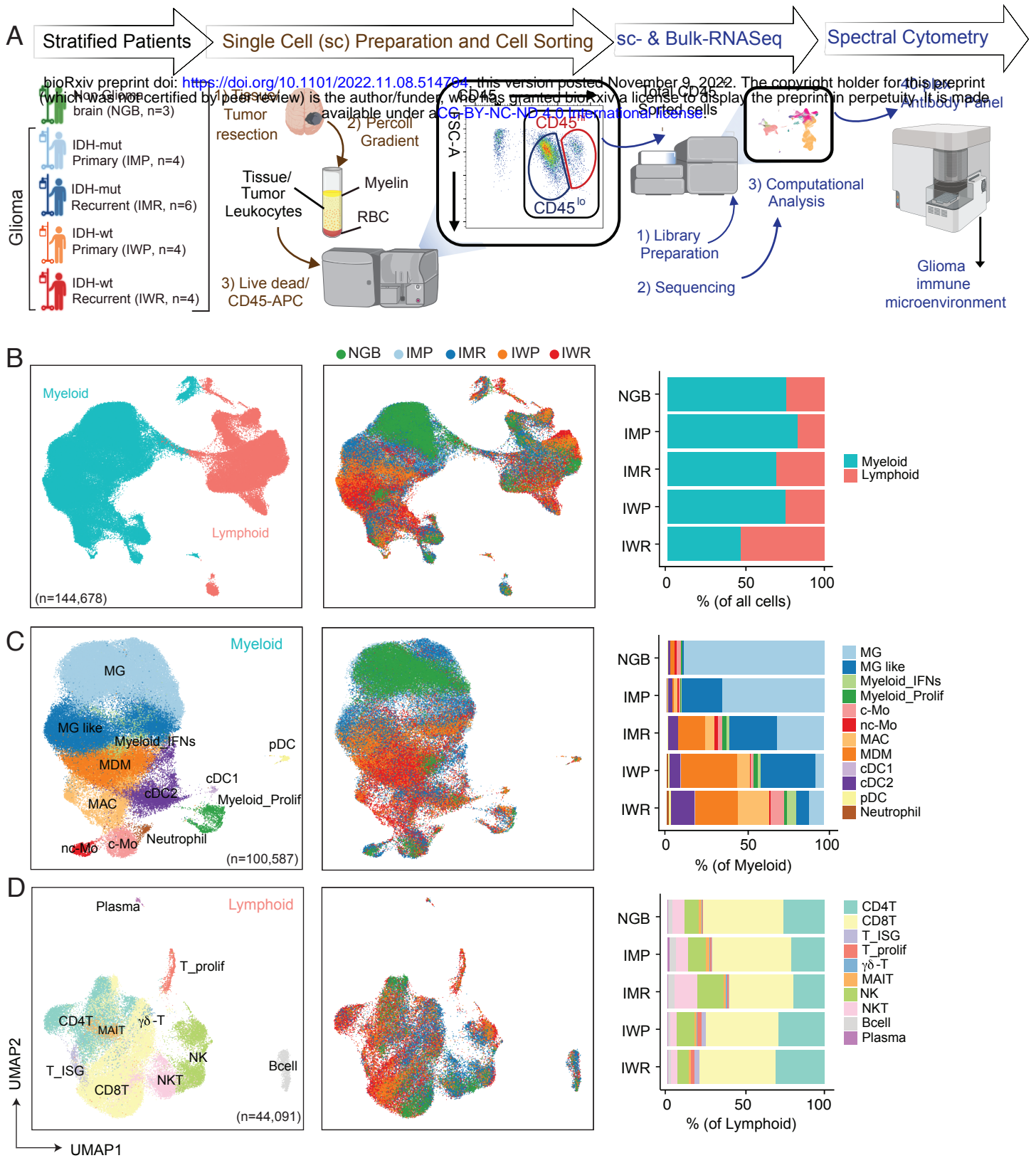


Fig. 1



## Figure Legend

### Fig. 1 | The single cell transcriptional landscape of glioma TIME.

**A)** A schema depicting the experimental workflow from sample preparation (see Methods) of resected brain tissues/tumors to scRNA-seq data generation and spectral flow cytometry validation and computational analysis. Patients were stratified as non-glioma brain (NGB; n = 3) and IDH-mutant primary (IMP; n = 4), IDH-mutant recurrent (IMR; n = 6), IDH-wild type primary (IWP; n = 4), IDH-wild type recurrent (IWR; n = 6) groups; hereafter collectively referred as glioma subtypes (see details in **Supplementary Table 1**). The dissociated CD45-APC-stained cells were FACS sorted to obtain pure CD45<sup>+</sup> glioma associated leukocytes. Subsequently matched sc-RNAseq and bulk RNA-Seq was performed followed by computational analysis. sc-RNA seq inferred cell types were validated by spectral cytometry. **B-D)**, Uniform manifold approximation and projection (UMAP) visualization of unsupervised clustering analysis of **(B)** all immune cells (n = 144,678) that passed quality filtering (see Methods), **(C)** myeloid lineage clusters (n = 100,587), and **(D)** lymphoid lineage clusters (n = 44,091). Cells are color coded for their inferred cell types (left) and the glioma subtypes of their corresponding tumors (middle).

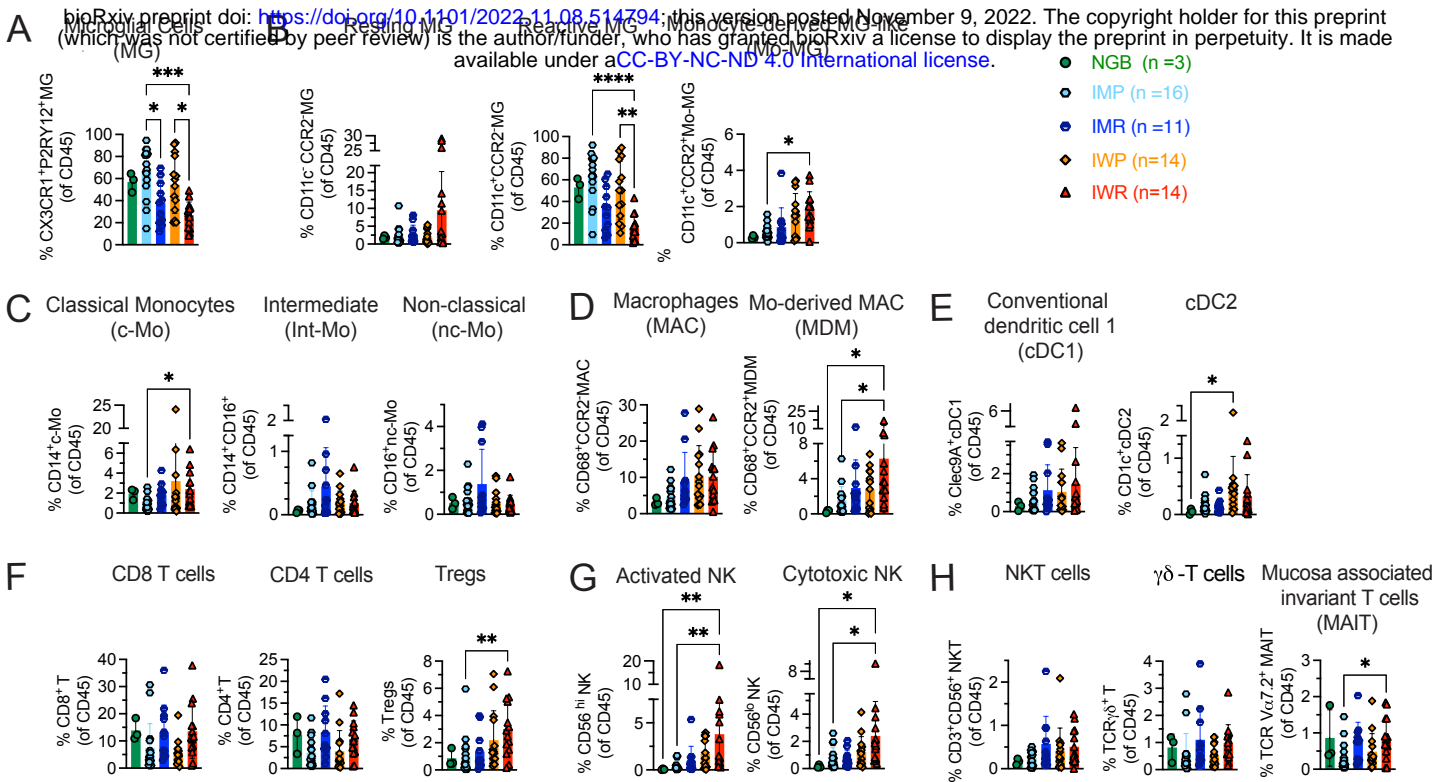


Fig. 2

## **Fig. 2 | Glioma associated leukocyte diversity corroborated with spectral flow cytometric analyses.**

Scatter bar plots represent the proportion of indicated immune cell type (of total CD45<sup>+</sup> leukocytes) across glioma subtypes; NGB (n = 3), IMP (n = 16), IMR (n = 11), IWP (n = 14) and IWR (n = 14) as shown in **(A)** CX3CR1<sup>+</sup>P2RY12<sup>+</sup> Microglial cells (MG); **(B)** Microglial subsets (gated on MG): CD11c<sup>-</sup>CCR2<sup>-</sup> Resting MG (left), CD11c<sup>+</sup>CCR2<sup>-</sup> Reactive Microglia (middle), and CD11c<sup>+</sup>CCR2<sup>+</sup> Monocyte-derived MG-like (Mo-MG); **(C)** Monocyte subsets (gated on CD3<sup>-</sup>CD56<sup>-</sup>): CD14<sup>+</sup>CD16<sup>-</sup> Classical Monocytes (c-Mo), and CD14<sup>+</sup>CD16<sup>+</sup> Intermediate monocytes (Int-Mo) and CD14<sup>-</sup>CD16<sup>+</sup> Non-classical monocytes; **(D)** Macrophages (Gated on CD3<sup>-</sup>CD56<sup>-</sup>): CD68<sup>+</sup>CCR2<sup>-</sup> Macrophages (MAC), and CD68<sup>+</sup>CCR2<sup>+</sup> Monocyte-derived Macrophages (MDM) **(E)** Conventional Dendritic cell (cDC) subsets (gated on CD11b<sup>+</sup>CD11c<sup>+</sup>HLADR<sup>+</sup>): Clec9A<sup>+</sup>CD1c<sup>-</sup> cDC1, and Clec9A<sup>-</sup>CD1c<sup>+</sup> cDC2; **(F)** T cell subsets (Gated on CD3<sup>+</sup>): CD8<sup>+</sup>CD4<sup>-</sup> T cells, CD4<sup>+</sup>Foxp3<sup>-</sup> T cells and CD4<sup>+</sup>Foxp3<sup>+</sup> T regulatory (Tregs) cells; **(G)** Natural Killer cell subsets (gated on CD56<sup>+</sup>): CD56<sup>hi</sup>CD16<sup>lo</sup> Activated NK cells, and CD56<sup>lo</sup>CD16<sup>hi</sup> Cytotoxic NK cells; **(H)** Unconventional T cell subsets: CD3<sup>+</sup>CD56<sup>+</sup> Natural Killer T (NKT) cells, TCR $\gamma\delta$ <sup>+</sup> ( $\gamma\delta$ -T) cells TCRV $\alpha$ 7.2<sup>+</sup> Mucosal associated invariant T (MAIT) cells. Statistical significance was determined using by Kruskal Wallis test at p\* < 0.05, p\*\* < 0.01, p\*\*\* < 0.001.

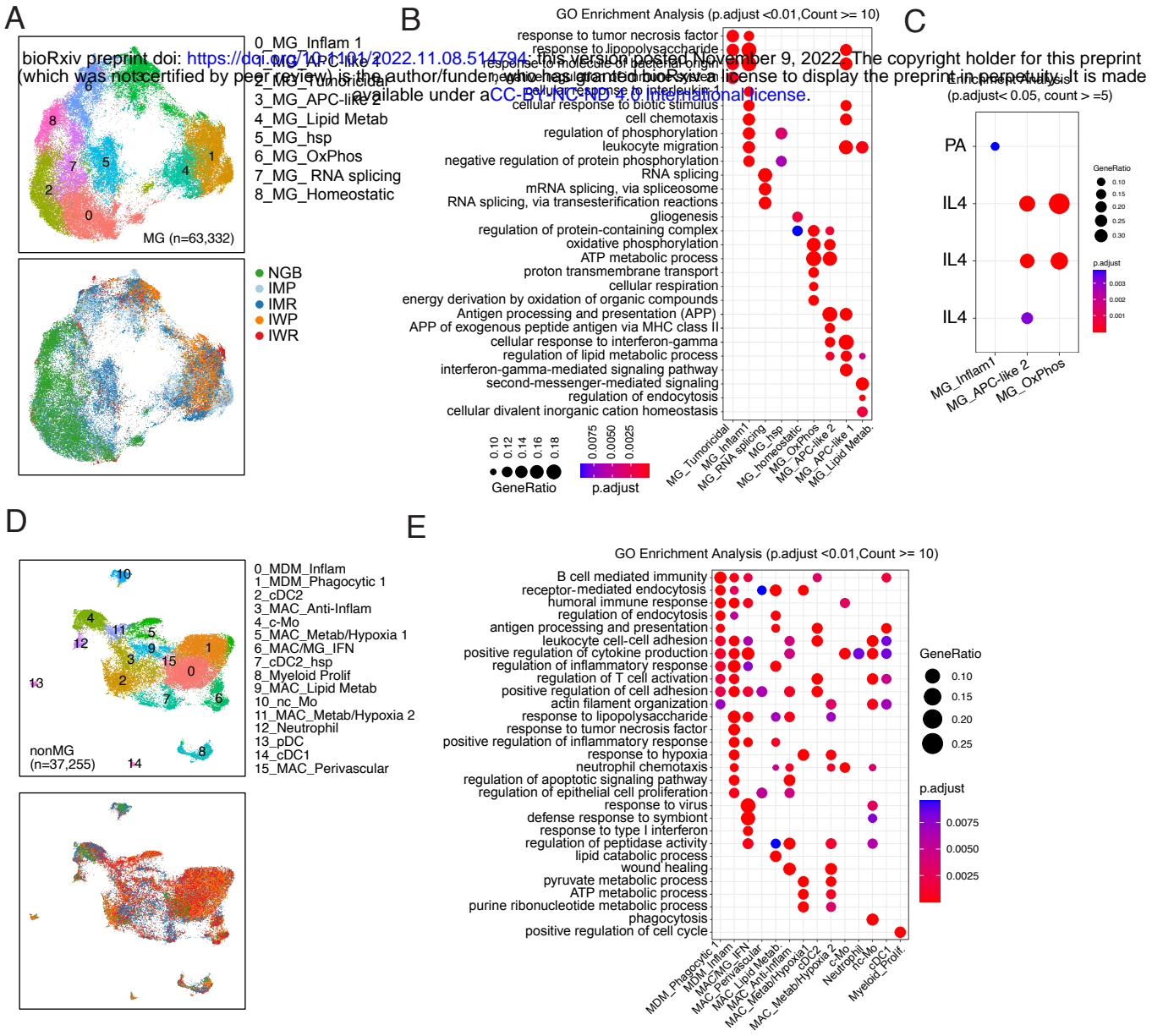
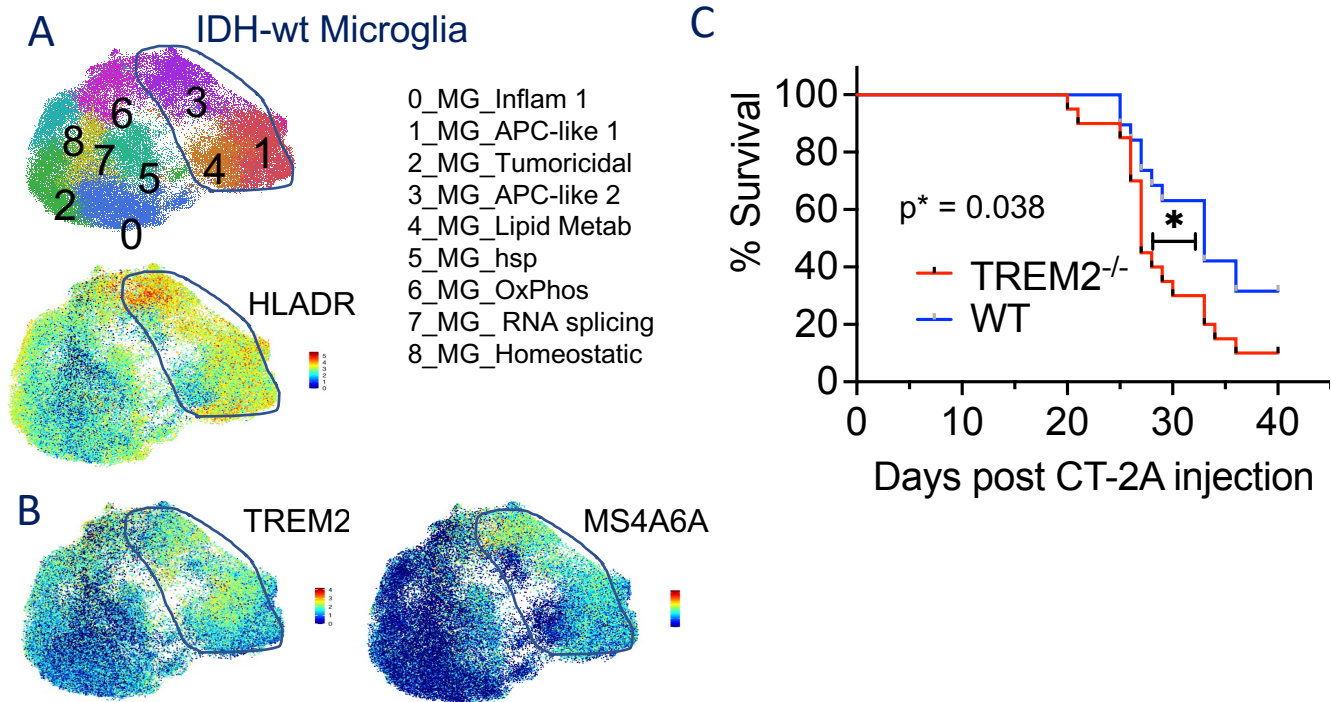


Fig. 3

**Fig. 3 | Glioma associated myeloid cell diversity and their inferred functional states.**

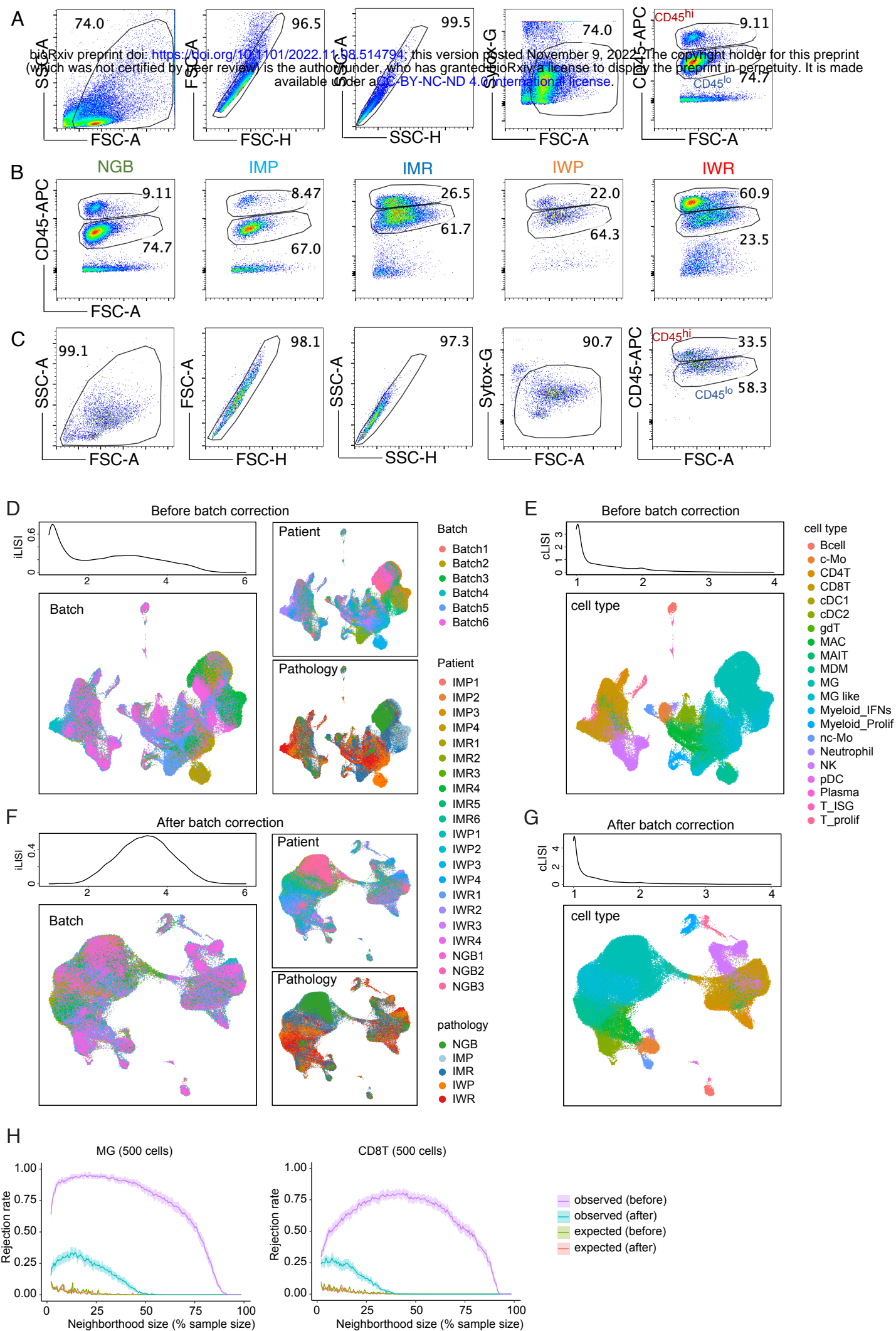
**(A)** UMAP visualization of unsupervised clustering analysis of Microglia (MG,  $n = 63,332$ ), displaying nine distinct cell states (top). Cells are color coded for their inferred cell types (top) and the glioma subtypes of their corresponding tumors (bottom). **(B)** Bubble plot depicting gene ontology (GO) analysis of glioma associated MG states. **(C)** Bubble plot showing overrepresented stimulus-specific Palmitic acid and Interleukin-4 (IL-4) polarization gene expression modules as multispectral polarization (see Methods). **(D)** UMAP visualization of unsupervised clustering analysis of non-MG myeloid cells ( $n = 37,255$ ), displaying sixteen distinct cell states (top). Cells are color coded for their inferred cell types (top) and the glioma subtypes of their corresponding tumors (bottom). **(E)** Bubble plot depicting gene GO analysis of glioma associated non-MG myeloid cell states. **(B, C, E)** In B and E, each bubble represents a GO Term and in C, each bubble represents a polarization module. Bubble size corresponds to gene ratio and the color of the bubble indicates statistical significance. Only polarization modules with 5 or more overlapping genes and an adjusted p value of  $<0.05$  are shown in C.



**Figure 4**

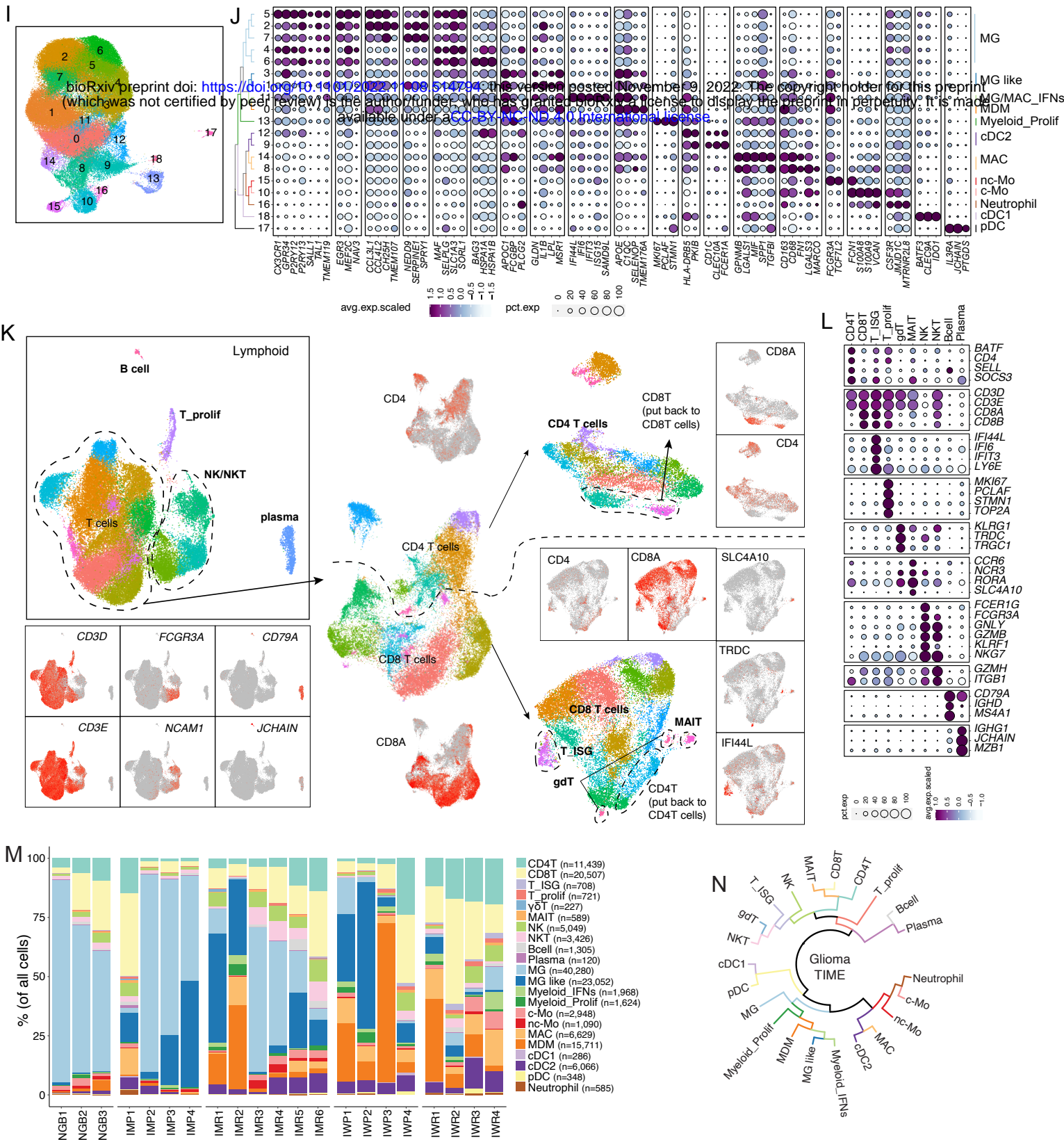
**Fig. 4 | Identification of anti-glioma role of Triggering receptor expressed on myeloid (TREM)-2<sup>+</sup> myeloid cells**

**(A)** UMAP visualization of (top) inferred MG states, expression of *HLADR* (bottom); **(B)** TREM2; **(C)** MS4A6A across MG clusters as shown in (A). **(D)** Representative percentage survival analyses of CT-2A glioma bearing mice TREM2<sup>-/-</sup> (n=20) versus WT mice (n=19) in C57BL/6 genetic background. Statistical significance of survival was determined using Mantel-Cox log-rank test at p\*=0.038.



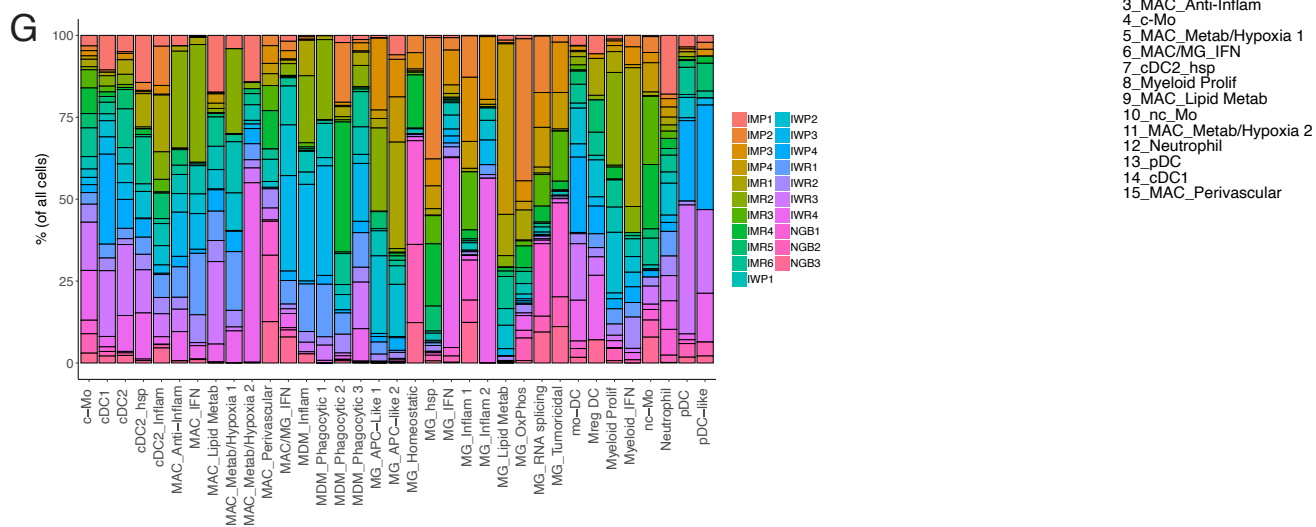
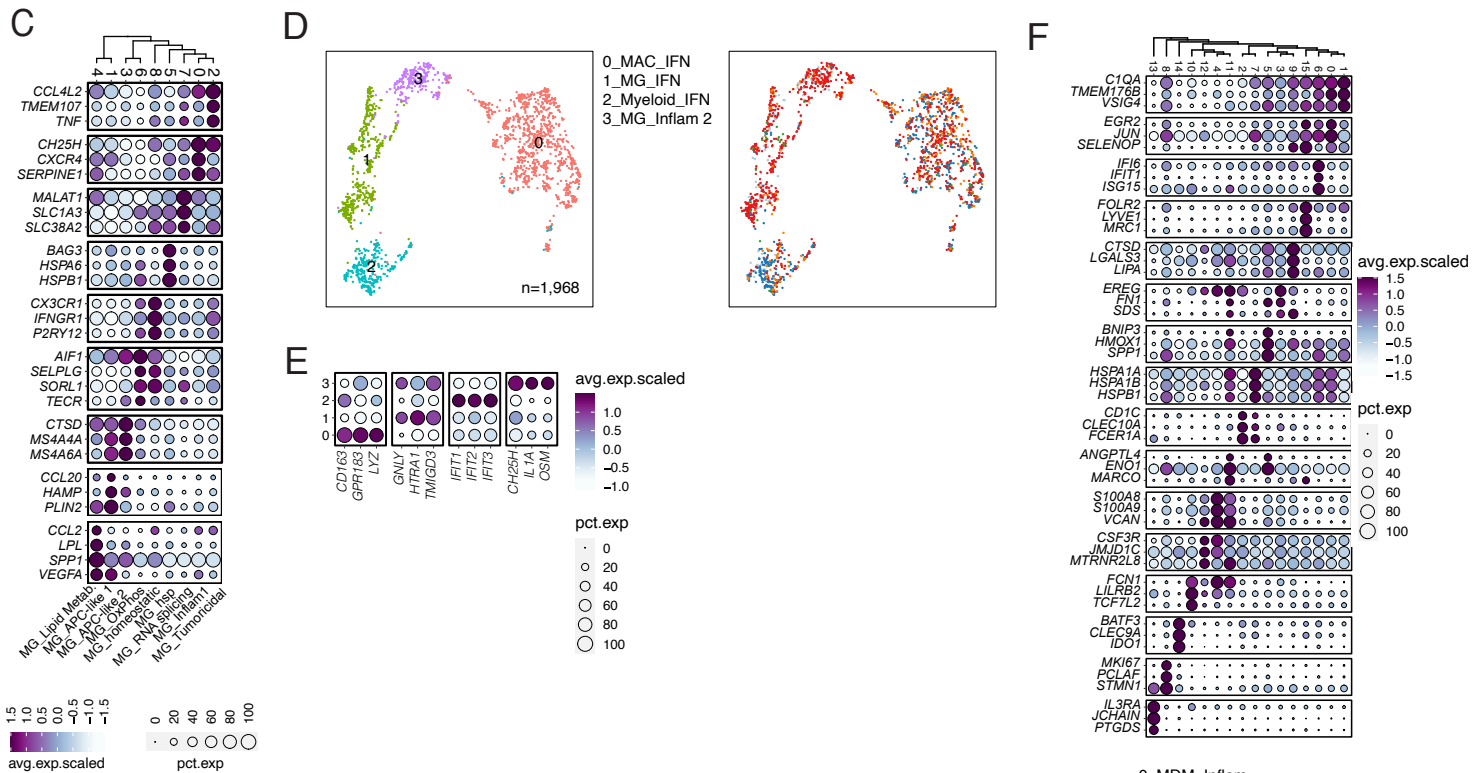
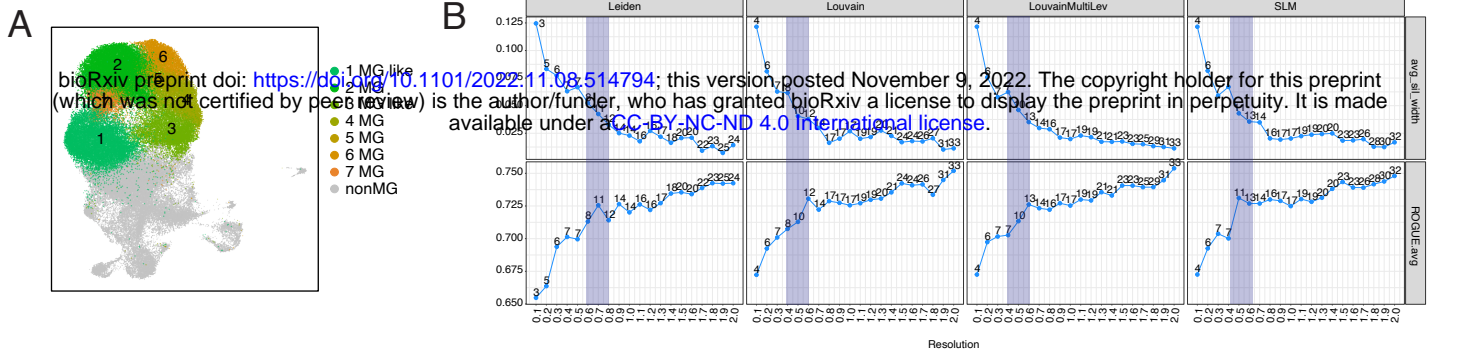
Supplementary Fig. S1





Supplementary Fig. S1 Continued

**Supplementary Fig. S1 | (A):** Flow cytometry pseudo color dot plots showing in (A) gating strategy with SYTOX-G-stained dead cells and CD45 expression on stained tumor associated leukocytes, in (B) differential CD45 expression (CD45<sup>lo</sup> and CD45<sup>hi</sup>) on leukocytes obtained from NGB controls and glioma subtypes as shown and in (C) representative pseudo color plots for purity of sorted CD45 cells. (D) iLISI (local inverse Simpson's Index) computed for every cell's neighborhood and summarized with density plots before batch correction. The UMAP were color coded by batch, patient and pathology. (E) cLISI computed for every cell's neighborhood and summarized with density plots before batch correction. The UMAP were color coded by major cell types. (F) and (G), same as (D) and (E) but after batch correction. (H) Batch effects evaluation using the k-nearest neighbor batch-effect test (k-BET, see Methods). K-BET was run on 500 cells randomly selected from 1 myeloid cell type (MG) and 1 lymphoid cell type (CD8T) separately before and after batch correction. Shaded areas represent the 95th percentile of n = 100 repeated k-BET runs. (I) UMAP visualization of unsupervised clustering analysis of glioma associated myeloid cells. (J) Bubble plot showing the scaled expression (shown by the color of the circle) and percentage of expression (shown by the size of the circle) of different myeloid cell type specific genes. (K) Workflow showing the identification of major lymphoid cell types by multi-steps of sub-clustering. (L) Bubble plot showing the scaled expression (shown by the color of the circle) and percentage of expression (shown by the size of the circle) of different lymphoid cell type specific genes. (M) The stacked bar plot showing relative distribution of glioma associated myeloid and lymphoid cell types across sc-RNAseq sampled patients. (N) Phylogenic relationship between myeloid and lymphoid cell types revealed by hierarchical clustering analysis based on Euclidean distance between cell types (see Methods).



**Supplementary Fig. S2 | (A):** UMAP visualization of unsupervised clustering analysis of glioma associated MG, MG-like (colored) and non-MG myeloid (grey). **(B)** Four different community detection algorithms in Seurat were used to find the best clustering of MG cells. In each algorithm, resolution was tuned from 0.1 to 2 with step 0.1, under which the averaged silhouette width value and ROGUE value were calculated to evaluate the inter-cluster dissimilarity and cluster purity. Shaded region indicated the best trade-off between averaged silhouette width value and ROGUE value. **(C)** Bubble plot showing the scaled expression (shown by the color of the circle) and percentage of expression (shown by the size of the circle) of different lymphoid cell type specific genes. **(D)** UMAP visualization of unsupervised clustering analysis of glioma associated Myeloid interferons (Myeloid\_IFN). Cells are color coded for their inferred cell types (left) and the glioma subtypes of their corresponding tumors (right). **(E-F)** Bubble plot showing the scaled expression (shown by the color of the circle) and percentage of expression (shown by the size of the circle) of different Myeloid interferon clusters and their specific genes in (E) and non-MG myeloid cells in (F). **(G)** Stacked bar plots showing corresponding %myeloid cell type composition across each glioma subtype.