

Supplementary Information

Turning attention to tumor-host interface and focus on the peritumoral heterogeneity of glioblastoma

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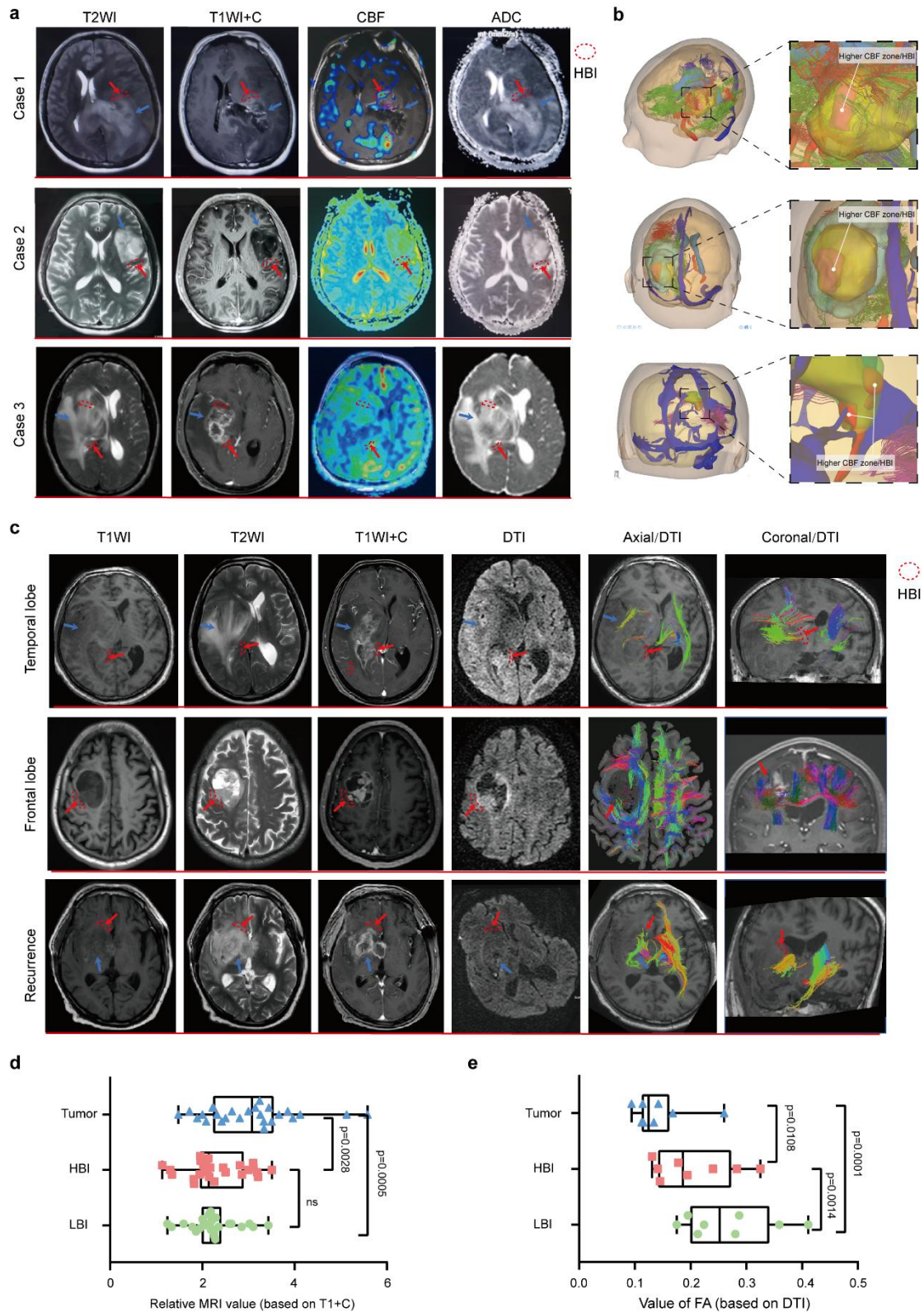
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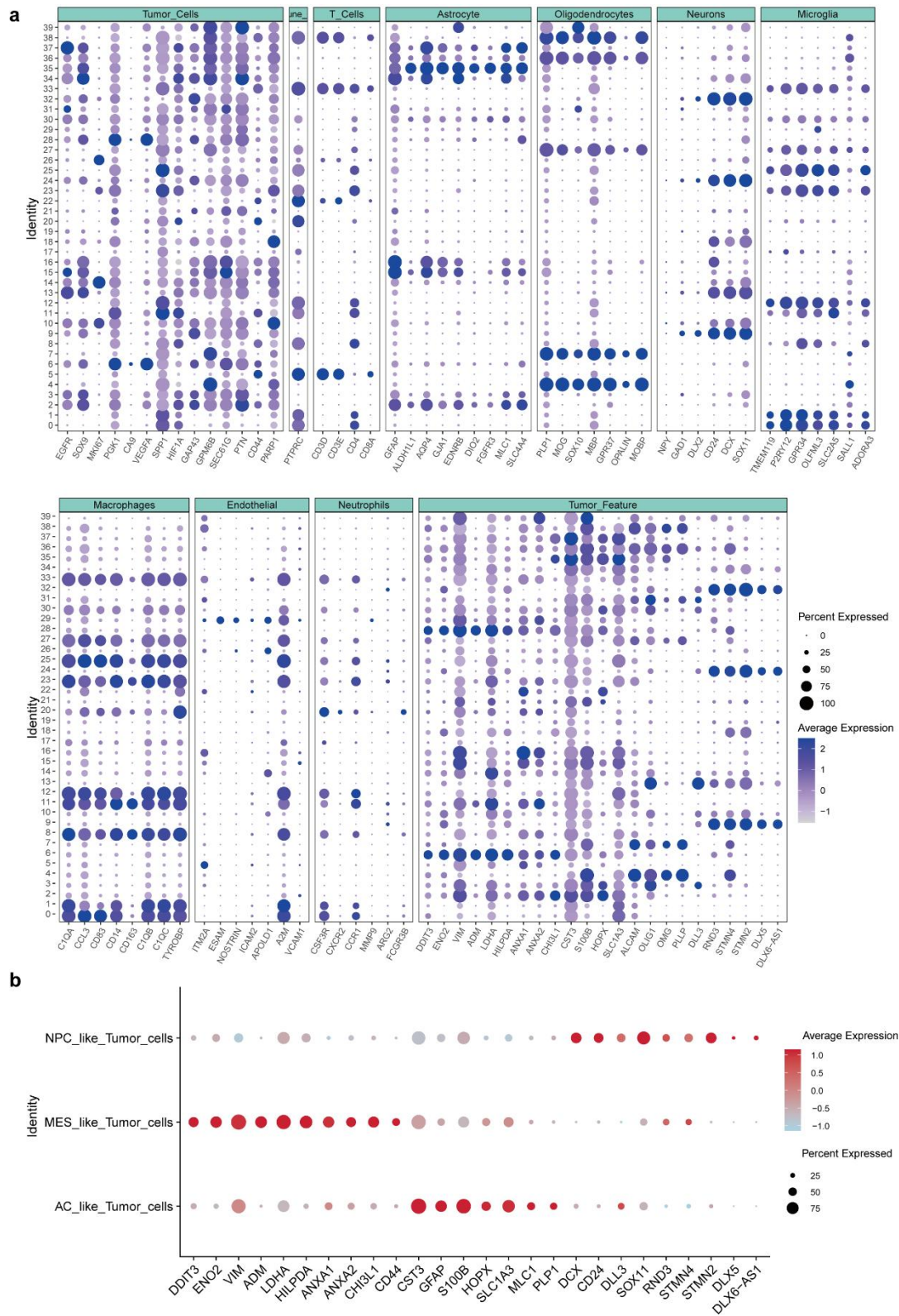
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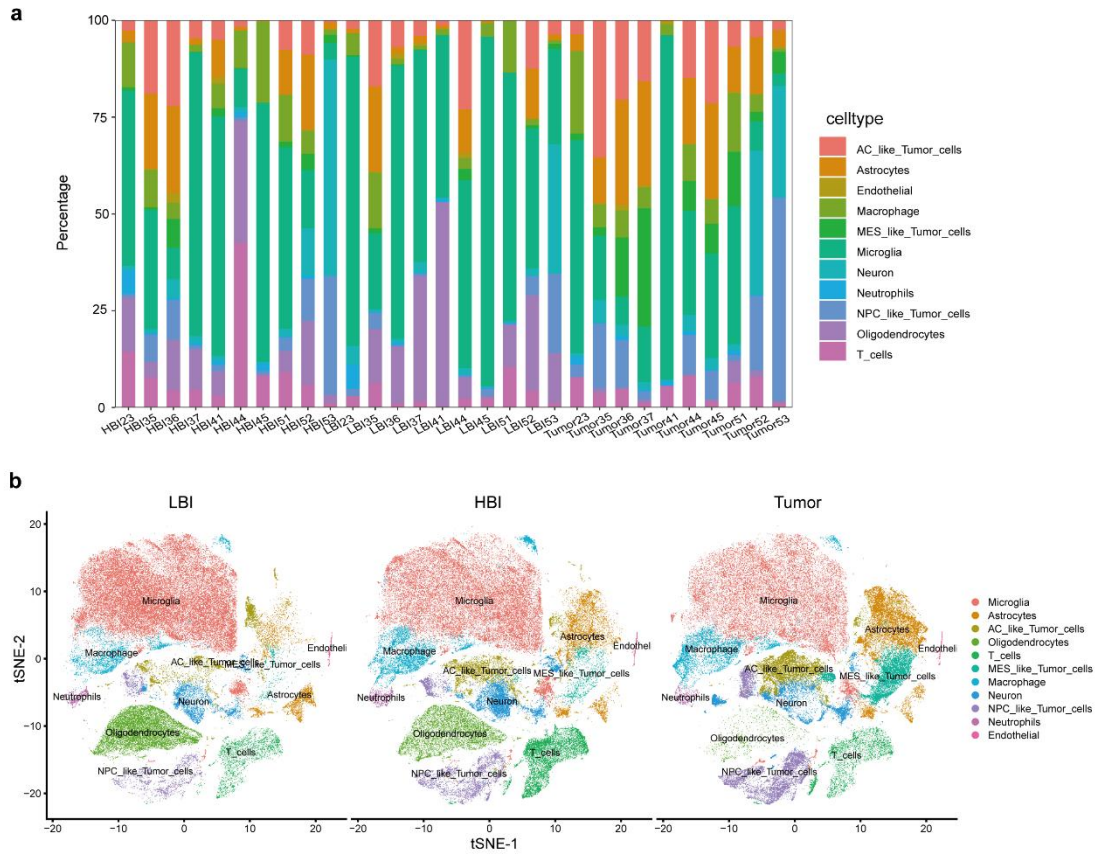


Supplementary fig 1. Segmentation and features of LBI and HBI regions on MRI and 3D reconstruction images. a Multimodal-MRI characteristics of HBI and LBI in different brain regions of GBM (n=26 independent subjects). The red dashed circles

denote the HBI areas. Red arrows point to HBI sampling sites and blue arrows to LBI sampling sites, respectively. **b** Representative 3D reconstruction images of the high CBF zone (HBI) around the tumor (n=26 independent subjects). **c** The nerve fiber bundles of the two regions (HBI and LBI). The red dashed circles denote the HBI areas (n=8 independent subjects). Red arrows point to HBI sampling sites and blue arrows to LBI sampling sites, respectively. **d** Statistical analysis of relative MRI value from T1WI+C images in various regions from patients (n = 26, image data from 26 subjects). **e** Statistical analysis of the FA value in various regions from patients (n = 8, image data from 8 subjects). Statistical analysis is performed using two-tailed Student's t-test in (d, e); ns=no significance. All box plots show median \pm 25/75 percentiles with whiskers showing minimum and maximum values. Source data are provided as a Source Data file.

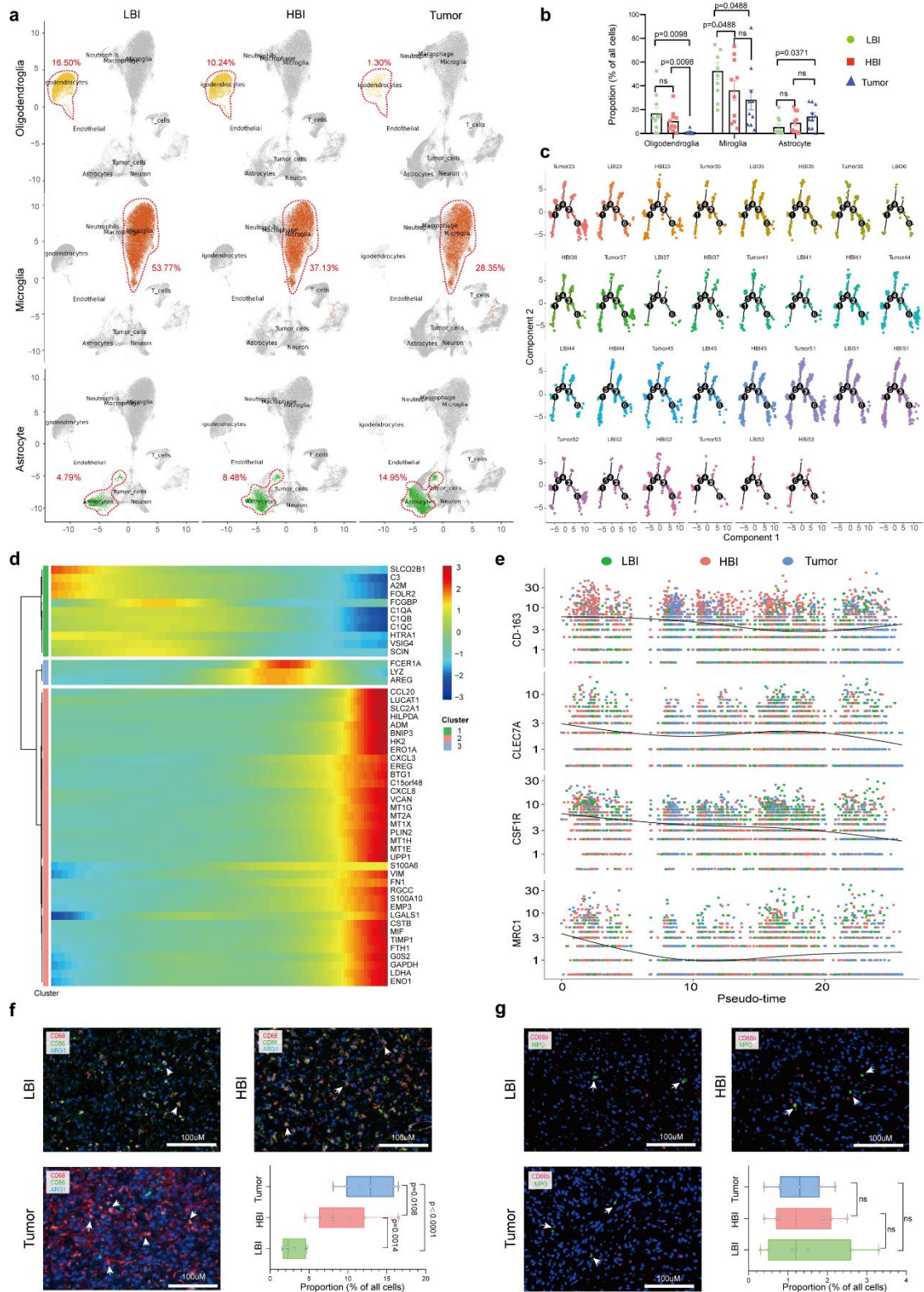


Supplementary fig 2. The marker genes for identifying cell types. a Cell type marker gene bubble plot. **b** The marker genes for identifying tumor cells. Source data are provided as a Source Data file.



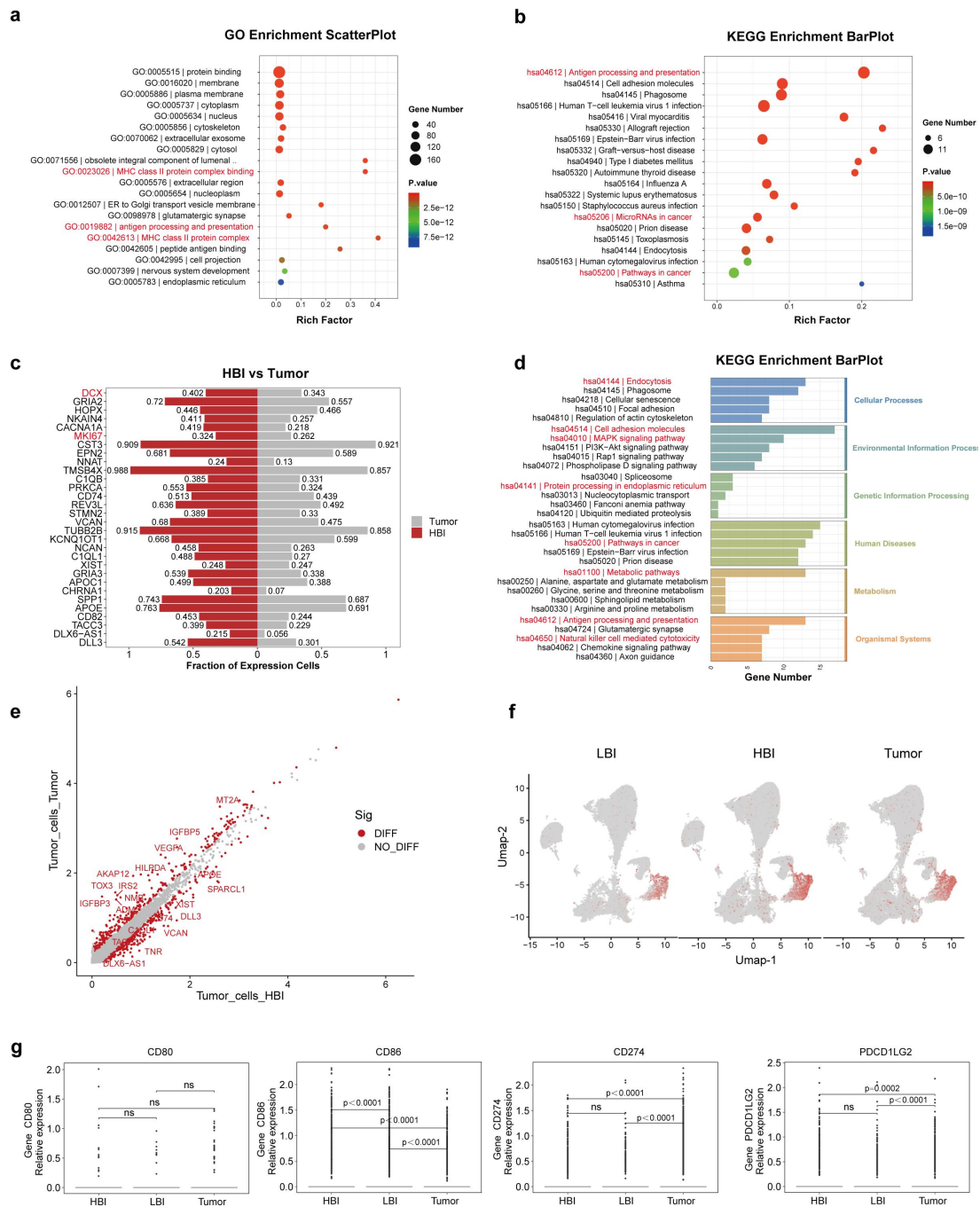
Supplementary fig 3. The distribution of individual cell types in each sample and tSNE plots. **a** The proportions of individual cell types in each sample. **b** tSNE plots of 3 regions based on annotated cell types (LBI: 68,326 cells, HBI: 68,521 cells, Tumor: 72,915 cells) ($n = 10/\text{group}$, tissue samples from 10 independent subjects). Source data are provided as a Source Data file.

times independently with similar results). **d** Quantitative analysis of relative expression of MMP9 protein (Data are presented as mean \pm s.e.m.), (n = 7 biological repeats with three technical replicates per biological repeat). **e** The different cell types hypoxia scores (single cell as a unit in the Box plots, total 209,762 cell, the detail samples size could be available from Source Data file). **f** The hypoxia scores of different cell types from 3 regions (single cell as a unit in the Box plots, total 209,762 cell, the detail samples size could be available from Source Data file). Statistical analysis is performed using two-tailed Student's t-test in (a, d); ns=no significance. Box plots in (e, f) represent the median (center line), the 25th and 75th percentiles (bounds of the box). Source data are provided as a Source Data file.



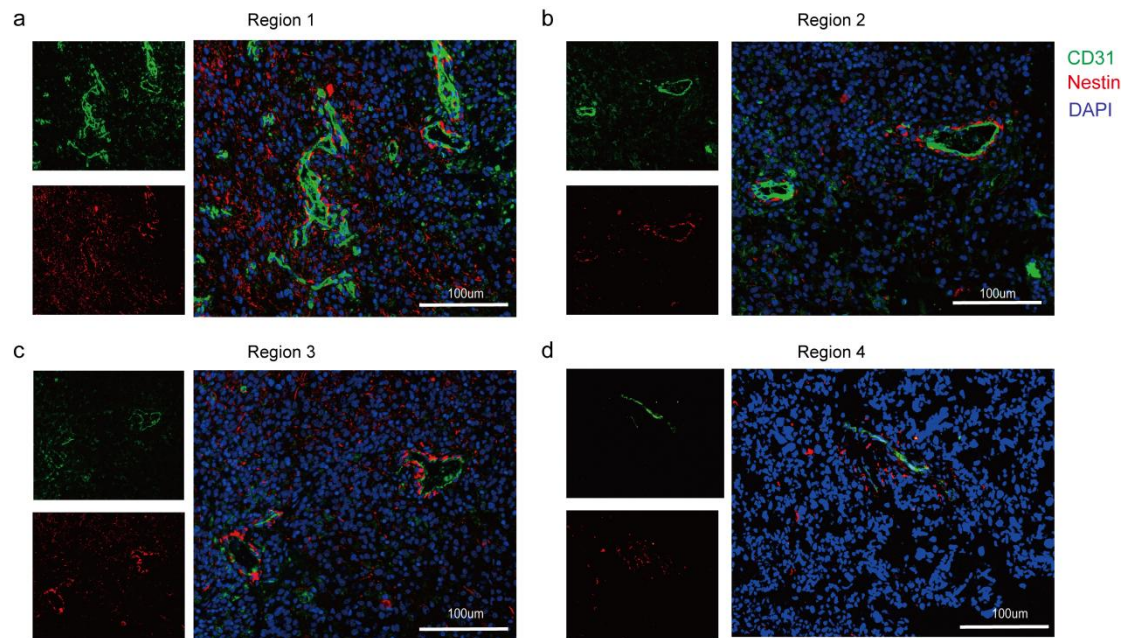
Supplementary fig 5. Analysis of immune microenvironment signature in 3 regions. **a, b** The proportion and quantitative analysis of glial cell in different regions based on single cell data (LBI: 68,326 cells, HBI: 68,521 cells, Tumor: 72,915 cells) ($n = 10/\text{group}$, tissue samples from 10 subjects) (Data are presented as mean \pm

s.e.m.). **c** Pseudo-time trajectory of macrophages in each sample, respectively (total 13,761 cells; n=3,077 cells in LBI, n= 5,498 cells in HBI, n=5,186 cells in Tumor). **d** Heat map shows the expression of top 50 genes across pseudo-time in three clusters. **e** The expression transition of four M2 signature-genes (*CD163*, *MRC1/CD206*, *CSF1R*, *CLEC7A*) along the pseudo-time of macrophages in 3 regions (n= 13,761 cells). **f, g** Multicolor IHC staining and statistical analysis of anti-inflammatory macrophages (f) and activated neutrophils (g) in three regions, (n = 7 biological repeats with three technical replicates per biological repeat) (Scale bar, 100 μ m). Wilcoxon signed rank test were performed in (b); two-tailed Student's t-test were performed in (f, g); ns=no significance. All box plots show median \pm 25/75 percentiles with whiskers showing minimum and maximum values. Source data are provided as a Source Data file.



Supplementary fig 6. The characteristics and distribution of tumor-cells in different regions. **a** GO Enriched scatterplot of differentially expressed genes of neoplastic cells (HBI vs. LBI). **b** KEGG Enriched scatterplot of differentially expressed genes of neoplastic cells (HBI vs. LBI). **c** Differentially expressed genes between neoplastic cells originating from the HBI or Tumor. **d** Enriched KEGG pathways of differentially expressed genes of neoplastic cells (HBI vs. Tumor). **e** Scatter plots of differentially expressed genes of neoplastic cells (HBI vs. Tumor). **f**

uMAP feature plots showing the expression of Ki67 (LBI: 68,326 cells, HBI: 68,521 cells, Tumor: 72,915 cells) (n = 10/group, tissue samples from 10 subjects). **g** Expression of genes coding for ligands of PD1 (*CD274* and *PDCD1LG2*) and CTLA4 (*CD80* and *CD86*) in 3 regions (Single cell as a unit; total 42,863 neoplastic cells in each Box plots; n=7,434 cells in LBI, n= 10,835 cells in HBI, n=24,594 cells in Tumor). Analysis in (a, b, d) was performed using a hypergeometric distribution test; the bimod statistical test was used in (e); Statistical analysis is performed using Wilcoxon signed rank test in (g), ns=no significance. Source data are provided as a Source Data file.



Supplementary fig 7. Multicolor IHC staining images of that Glioma stem-like cells (GSCs) reside within the perivascular niche. a Representative images of GSCs and microvasculature in region 1(a), region 2(b), region 3(c) and region 4(d) (Scale bar, 100 μm) (This experiment was repeated 7 biological times independently with similar results).

Supplementary Table 1. Clinical cohort and metadata

ID	Sex	Age	Location	Primary or Recurrence	IDH status	EGFR	MGMT	ATRX	Ki67	P53	S-100
1	F	45	Left temporal	Primary	WT	NT	NT	NT	80%	Positive	Negative
2	F	47	Right frontal	Recurrence	WT	NT	NT	NT	50%	90%	Positive
3	M	52	Right insula	Primary	WT	Positive	NM	Positive	20%	WT	Positive
4	F	75	Right parietal	Primary	WT	Positive	Positive	Positive	15%	90%	Positive
5	F	67	Right temporal	Primary	WT	NT	NT	NT	30%	NT	Positive
6	F	72	Left insula	Primary	WT	Positive	Positive	Positive	60%	60%	Positive
7	M	57	Left frontal	Primary	WT	Positive	Positive	Positive	35%	Positive	Negative
8	M	25	Left occipital	Primary	WT	NT	NT	Negative	10%	Positive	Positive
9	F	45	Right temporal	Recurrence	WT	NT	NT	Positive	40%	5%	Positive
10	M	48	Right temporal	Primary	WT	NT	NT	Positive	60%	90%	Positive
11	M	68	Right temporal	Primary	WT	Positive	NM	Positive	30%	10%	Positive
12	M	43	Right insula	Primary	WT	Positive	NM	Positive	3%	20%	Positive
13	M	67	Left occipital	Primary	WT	Positive	NT	Positive	60%	60%	Positive
14	M	70	Left frontal	Primary	WT	NT	Positive	Positive	30%	NT	Negative
15	M	54	Left frontal	Primary	WT	Positive	NM	Positive	5%	Positive	Positive
16	F	56	Right frontal	Primary	WT	Positive	Positive	Positive	25%	10%	Negative
17	M	60	Right frontal and parietal	Primary	WT	Positive	NM	Positive	15%	20%	Positive
18	F	74	Right temporal and parietal	Recurrence	WT	Positive	NM	NT	50%	Positive	Positive
19	M	69	Left temporal and occipital	Primary	WT	Positive	NM	NT	10%	Positive	Negative
20	F	73	Left frontal	Primary	WT	Positive	NM	Negative	7%	Positive	Positive
21	M	56	Right temporal	Primary	WT	Positive	Positive	Positive	25%	Positive	Positive
22	M	52	Right temporal and insula	Primary	WT	Positive	Positive	Positive	80%	Positive	Positive
23	M	54	Left temporal	Primary	WT	Positive	Positive	Positive	30%	30%	Positive
24	F	64	Left temporal and parietal	Primary	WT	Positive	Positive	Negative	40%	Positive	Positive
25	M	45	Right frontal temporal	Recurrence	WT	Positive	NM	Negative	50%	WT	Positive
26	M	32	Left frontal	Primary	WT	Positive	Positive	Positive	60%	Positive	Positive

Abbreviation: F = Female; M = Male; WT = Wildtype; NM = Non methylated; NT = Not tested.

Supplementary Table 2. The volume of different regions

No.	Volume (cm ³)					Proportion (%)	
	Volume1 (PBZ + Tumor)	Volume2 (Tumor)	Volume3 (HBI)	Volume4 (PBZ)	Volume5 (LBI)	HBI / PBZ	HBI / Tumor
1	81.75	52.14	2.96	29.61	26.65	10.00	5.68
2	60.73	42.55	3.45	18.18	14.73	18.98	8.11
3	48.75	31.04	0.68	17.71	17.03	3.84	2.19
4	90.81	67.63	1.74	23.18	21.44	7.51	2.57
5	47.04	28.21	0.52	18.83	18.31	2.76	1.84
6	130.78	87.24	0.65	43.54	42.89	1.49	0.75
7	72.62	46.54	1.32	26.08	24.76	5.06	2.84
8	35.43	19.56	0.58	15.87	15.29	3.65	2.97
9	68.32	23.64	1.52	44.68	43.16	3.40	6.43
10	43.77	28.79	0.85	14.98	14.13	5.67	2.95
11	53.4	38.66	0.62	14.74	14.12	4.21	1.60
12	76.28	53.23	0.81	23.05	22.24	3.51	1.52
13	89.38	57.91	1.77	31.47	29.7	5.62	3.06
14	71.48	47.83	2.45	23.65	21.2	10.36	5.12
15	74.82	46.83	1.43	27.99	26.56	5.11	3.05
16	28.93	15.33	0.63	13.6	12.97	4.63	4.11
17	103.2	72.31	4.76	30.89	26.13	15.41	6.58
18	58.17	37.65	1.74	20.52	18.78	8.48	4.62
19	65.39	41.76	0.59	23.63	23.04	2.50	1.41
20	80.49	55.76	3.5	24.73	21.23	14.15	6.28
21	36.71	23.57	0.43	13.14	12.71	3.27	1.82
22	79.24	48.23	1.79	31.01	29.22	5.77	3.71
23	87.64	65.75	2.53	21.89	19.36	11.56	3.85
24	92.43	67.85	3.25	24.58	21.33	13.22	4.79
25	49.38	36.5	0.94	12.88	11.94	7.30	2.58
26	76.89	45.67	2.13	31.22	29.09	6.82	4.66
Average value	57.42	45.47	1.68	23.91	22.23	7.09	3.66
Standard deviation	22.76	17.30	1.13	8.25	7.99	4.39	1.84

Abbreviation: PBZ = peritumoral brain zone; LBI = lower cerebral blood flow interfaces; HBI = higher cerebral blood flow interface.

Supplementary Table 3. The CBF value in different regions

No.	ROI (mm2)			Value of CBF (Mean)		
	LBI	HBI	Tumor	LBI	HBI	Tumor
1	21.81	24.74	21.65	22.82	27.43	11.71
2	21.8	19.82	23.12	45	57	28.71
3	21.21	21.45	18.18	59.29	62.71	88.75
4	22.24	20.38	21.65	80.25	111.29	67.75
5	22.16	24.38	19.94	5.26	18.25	7.64
6	20.88	18.56	15.9	5.33	49	21
7	18.25	20.27	23.36	19.5	25.75	9.75
8	20.27	22.3	24.33	30.25	52	21.5
9	24.33	20.07	20.28	33	101.75	19.25
10	19.94	22.16	24.38	20.64	45	67
11	18.25	20.27	16.22	40.5	57.25	24.8
12	20.28	22.3	19.46	49.75	71.5	27.62
13	21.9	18.25	21.9	19.67	51.06	7.25
14	20.28	22.3	19.47	33.75	69.25	17.57
15	21.28	20.76	15.83	16	64	95
16	15.45	20.08	18.54	27.33	35.6	26.33
17	20.76	20.76	20.76	8.57	51.29	31.57
18	21.28	21.28	17.56	16.5	35.75	59.75
19	19.31	23.17	21.28	17.25	50.25	45.75
20	18.08	21.7	21.7	21.5	35.75	91.5
21	19.31	19.31	19.31	39	62.75	39.5
22	23.17	19.31	23.17	17.5	32.33	3.75
23	21.7	21.1	23.54	14	42.2	40
24	23.37	19.47	19.47	30	50.75	4.25
25	20.87	20.87	20.87	60.29	93.5	39.25
26	22.37	19.47	22.37	10.25	22.25	7.25

Abbreviation: ROI = region of interest; CBF = cerebral blood flow; LBI = lower cerebral blood flow interfaces; HBI = higher

cerebral blood flow interface.

Supplementary Table 4. The information of quality control during sequencing

Sample	Estimated Number of Cells	Mean Reads per Cell	Median Genes per Cell	Reads Mapped to Genome	Reads Mapped Confidently to Genome	Reads Mapped Confidently to Transcriptome	Total Genes Detected	Before filter cell number	After filter cell number
HBI23	3,564	130,091	2,444	96.90%	90.70%	52.30%	34,242	3564	2315
HBI35	5,281	74,990	3,265	98.00%	93.80%	58.00%	36,313	5281	4254
HBI36	9,939	39,951	3,074	97.80%	93.80%	54.30%	36,529	9939	8099
HBI37	10,824	37,226	2,484	97.40%	93.20%	43.00%	36,423	10824	9493
HBI41	9,168	51,884	2,786	97.80%	93.20%	55.70%	37,843	9168	7378
HBI44	9,897	41,478	2,309	96.20%	91.90%	45.50%	38,304	9897	8378
HBI45	9,082	50,983	2,456	96.70%	92.60%	50.50%	35,008	9082	7721
HBI51	10,509	38,734	2,957	96.30%	91.40%	43.00%	39,079	10509	9167
HBI52	6,167	63,029	3,712	97.40%	92.10%	44.10%	40,756	6167	5257
HBI53	8,923	43,126	3,304	95.40%	90.80%	34.80%	38,787	8923	7870
LBI23	8,909	50,140	2,464	97.10%	90.30%	51.80%	35,121	8909	6518
LBI35	6,178	60,536	3,305	98.10%	93.90%	58.10%	36,440	6178	5143
LBI36	8,272	48,401	2,217	97.50%	93.50%	42.40%	34,825	8272	7256
LBI37	7,169	64,610	2,667	97.10%	92.30%	39.20%	36,015	7169	6058
LBI41	8,255	49,911	2,773	97.10%	93.30%	40.80%	35,956	8255	7363
LBI44	8,315	48,813	2,757	94.80%	89.90%	42.10%	38,702	8315	7034
LBI45	9,245	49,459	2,372	96.30%	91.90%	47.60%	33,804	9245	7917
LBI51	9,500	38,941	2,745	95.90%	90.80%	41.90%	37,132	9500	8184
LBI52	7,703	50,778	3,437	97.10%	92.60%	39.50%	40,251	7703	6791
LBI53	8,823	44,636	3,351	96.00%	91.00%	37.90%	39,301	8823	7736
Tumor23	4,754	97,430	3,118	97.60%	91.10%	59.10%	33,326	4754	2936
Tumor35	5,236	76,563	4,106	98.00%	93.20%	54.50%	36,491	5236	4268

Tumor36	7,730	64,701	3,730	97.80%	93.60%	57.40%	36,502	7730	5917
Tumor37	15,473	28,060	2,981	97.70%	93.40%	62.90%	37,077	15473	12484
Tumor41	10,724	36,975	2,038	97.60%	93.40%	42.20%	34,250	10724	9380
Tumor44	7,337	55,776	3,570	96.20%	91.50%	48.30%	38,255	7337	6137
Tumor45	8,414	57,165	3,416	97.10%	92.80%	53.10%	36,861	8414	6327
Tumor51	12,181	35,382	2,891	96.60%	91.40%	46.40%	39,815	12181	10465
Tumor52	9,227	42,688	3,419	96.40%	89.60%	35.80%	40,682	9227	8185
Tumor53	8,635	47,805	3,609	95.40%	89.90%	38.20%	39,406	8635	7529

Abbreviation: LBI = lower cerebral blood flow interfaces; HBI = higher cerebral blood flow interface.

Supplementary Table 5. The antibody used in study

Antibody	Species reactivity	Host species	Supplier	Cat No.	Dilution ratio
IHC					
CD31	Human	Mouse	Abcam	ab9498	1/500
VEGFA	Human	Rabbit	Wanleibio	WL00009b	1/200
EGFR	Human	Rabbit	Wanleibio	WL0682a	1/200
HIF-1 α	Human	Rabbit	proteintech	20960-1-AP	1/500
MMP-9	Human	Rabbit	proteintech	10375-2-AP	1/500
Ki-67	Human	Rabbit	proteintech	27309-1-AP	1/2000
Goat Anti-Rabbit IgG H&L (HRP)	Rabbit	Goat	Abcam	ab6721	1/500
Goat Anti-Mouse IgG H&L (HRP)	Mouse	Goat	Abcam	ab6789	1/500
Western blot					
VEGFA	Human	Rabbit	Wanleibio	WL00009b	1/1000
EGFR	Human	Rabbit	Wanleibio	WL0682a	1/1000
HIF-1 α	Human	Rabbit	proteintech	20960-1-AP	1/2000
MMP-9	Human	Rabbit	proteintech	10375-2-AP	1/1000
β -actin	Human	Mouse	Beyotime	AF0003	1/1000
Goat Anti-Rabbit IgG H&L (HRP)	Rabbit	Goat	Abcam	ab6721	1/5000
Goat Anti-Mouse IgG H&L (HRP)	Mouse	Goat	Abcam	ab6789	1/3000
IF					
Nestin	Human	Mouse	Abcam	ab18102	1/400
CD31	Human	Rabbit	proteintech	11265-1-AP	1/200
CD86	Human	Rabbit	CST	91882S	1/200
Arginase-1	Human	Rabbit	CST	93668T	1/200
CD66b	Human	Rabbit	Affinity	DF10151	1/100
MPO	Human	Rabbit	proteintech	22225-1-AP	1/300
OLIG2	Human	Rabbit	Abcam	ab109186	1/800
SOX2	Human	Rabbit	Abcam	ab92494	1/100
CD68	Human	Rabbit	proteintech	25747-1-AP	1/1000
CD3	Human	Rabbit	proteintech	17617-1-AP	1/1000
SOX9	Human	Rabbit	Servicebio	GB14171-50	1/200
GFAP	Human	Rabbit	Servicebio	GB11096-100	1/1000
TMEM119	Human	Rabbit	Abcam	ab306583	1/1000
iF440-Tyramide	-	-	Servicebio	G1250	1/500
iF488-Tyramide	-	-	Servicebio	G1231	1/500
iF555-Tyramide	-	-	Servicebio	G1233	1/500
iF594-Tyramide	-	-	Servicebio	G1242	1/500
iF647-Tyramide	-	-	Servicebio	G1232	1/500

Abbreviation: IHC = immunohistochemistry; IF =Immunofluorescence.