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Supplementary information

Defects of ferroptosis in tumor-associated M2-macrophages during adverse and recurrent glioblastoma

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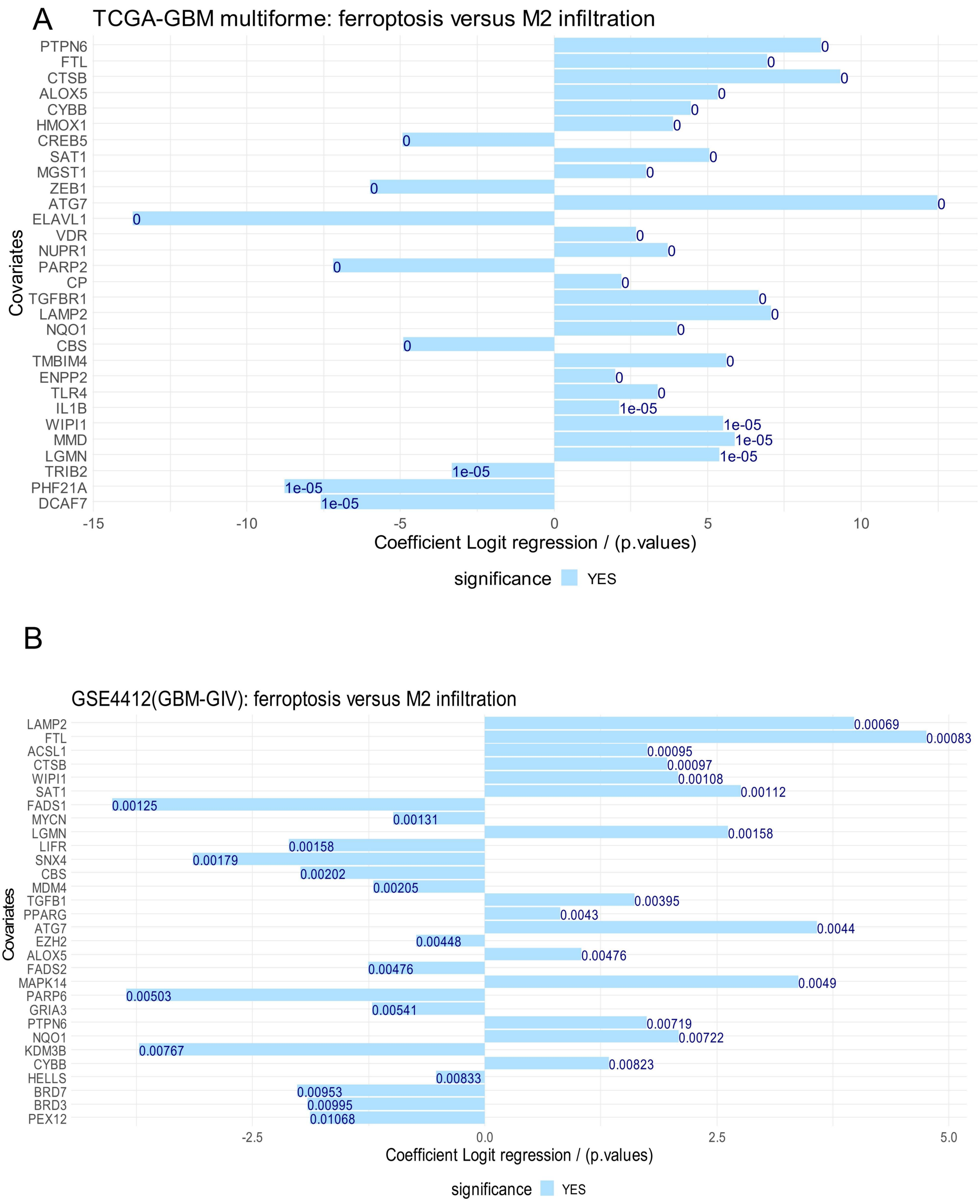


Figure S1. Best ferroptosis related genes associated to M2-macrophage infiltration in Glioblastoma tumors. (A) Logistic beta coefficients of best ferroptosis gene expression according M2-infiltration outcome in TCGA-glioblastoma multiforme training cohort; (B) Logistic beta coefficients of best ferroptosis gene expression according M2-infiltration outcome in GSE4412 glioma grade IV validation cohort.

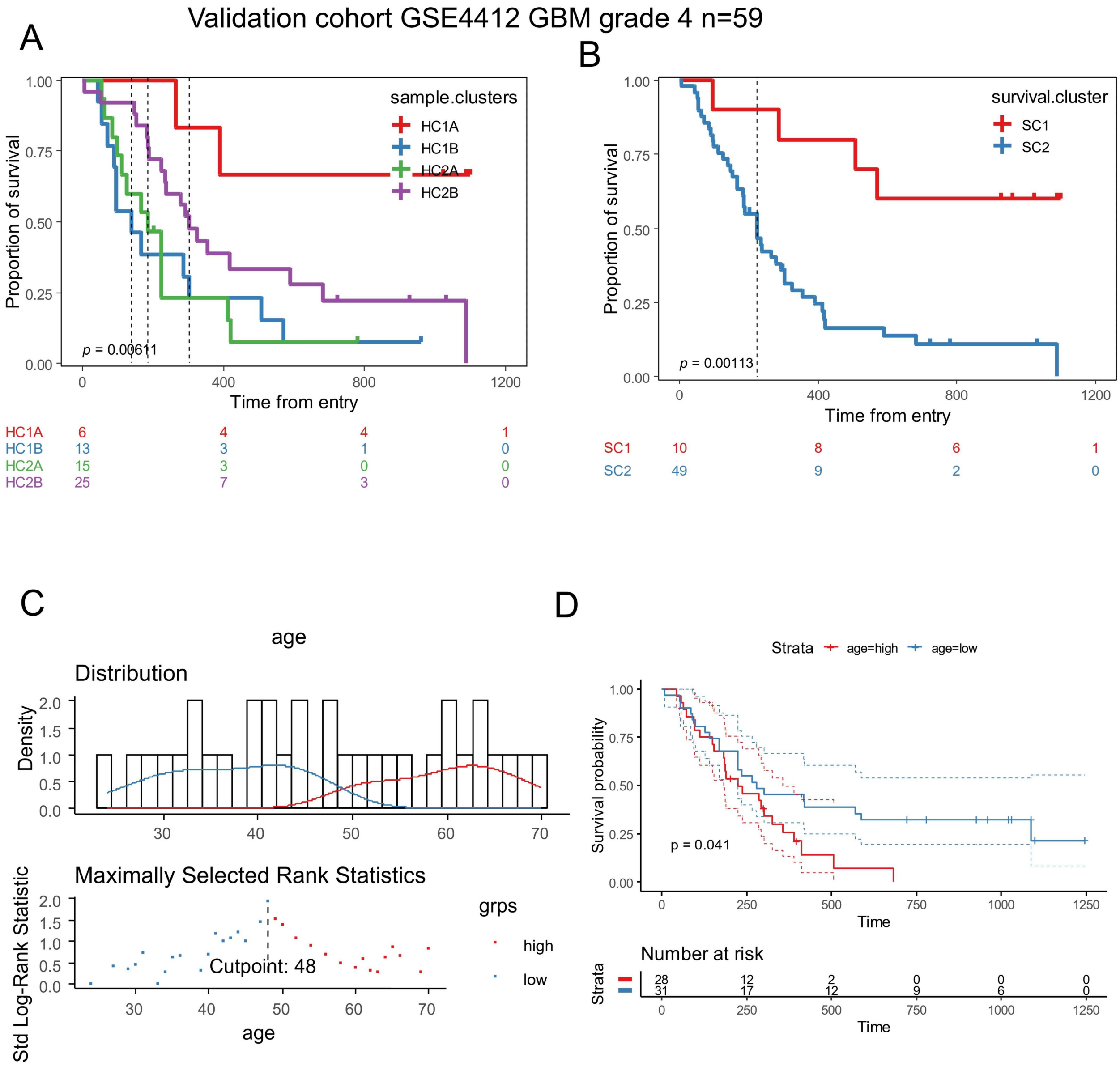


Figure S2. Prognosis markers for stage IV glioma from validation cohort GSE4412. (A) Kaplan Meier and log rank test on overall survival according original expression groups in Stage IV glioma (GSE4412, *n* = 59); (B) Kaplan Meier and log rank test on overall survival according original survival groups in Stage IV glioma (GSE4412, *n* = 59); (C) Optimal threshold determination for age at diagnosis according overall survival of the patients from stage IV; (D) Kaplan Meier and log rank test on overall survival with age categories (low and high: under and over threshold of 48 yo) in glioma stage IV (GSE4412).

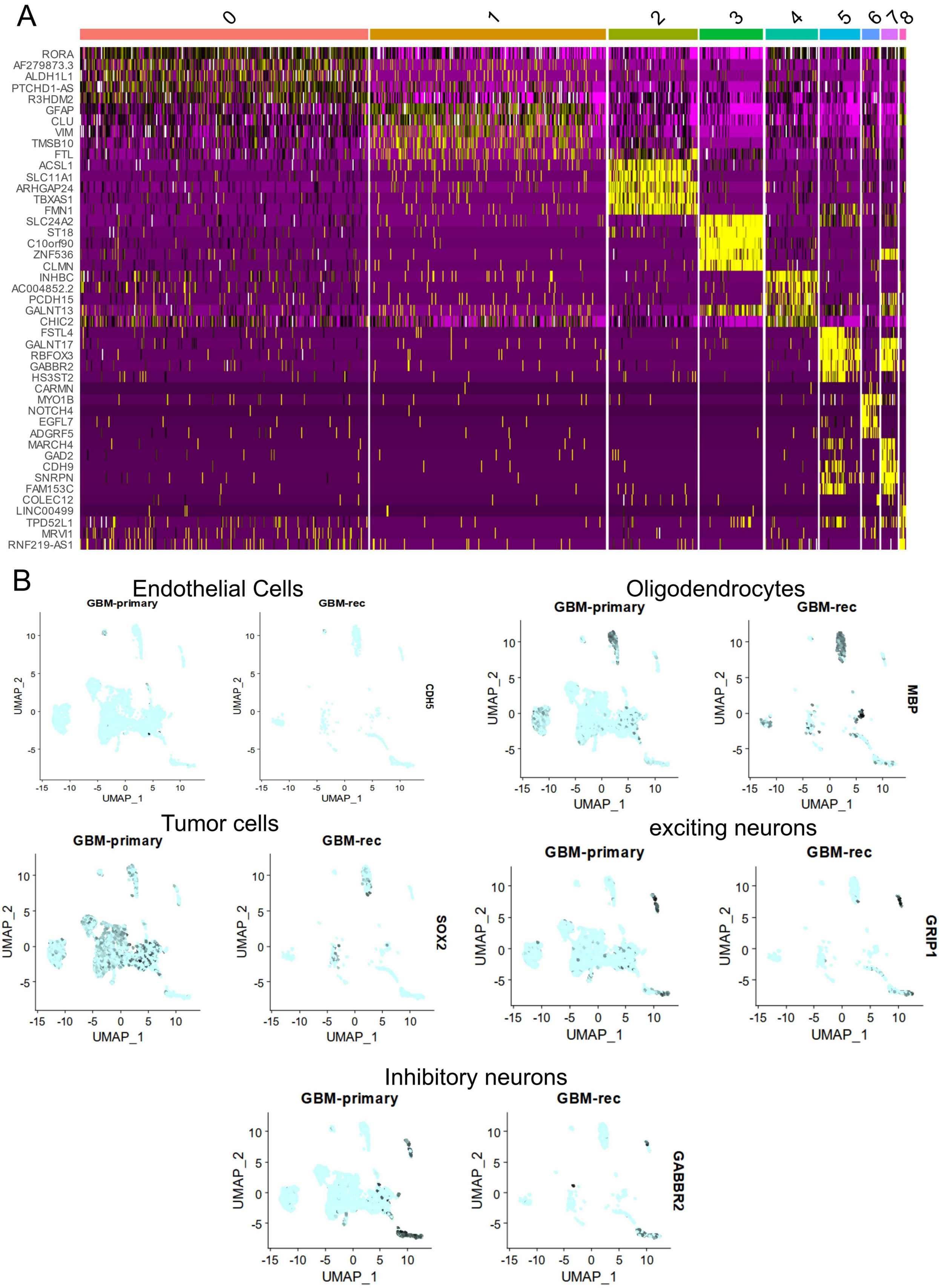


Figure S3. Cell cluster identification in tumor microenvironment of glioblastoma by single cell transcriptome. (A) Expression heatmap of best five markers by single cell clusters in glioblastoma tumors; (B) Feature plot of marker expression by cluster stratified time sampling.

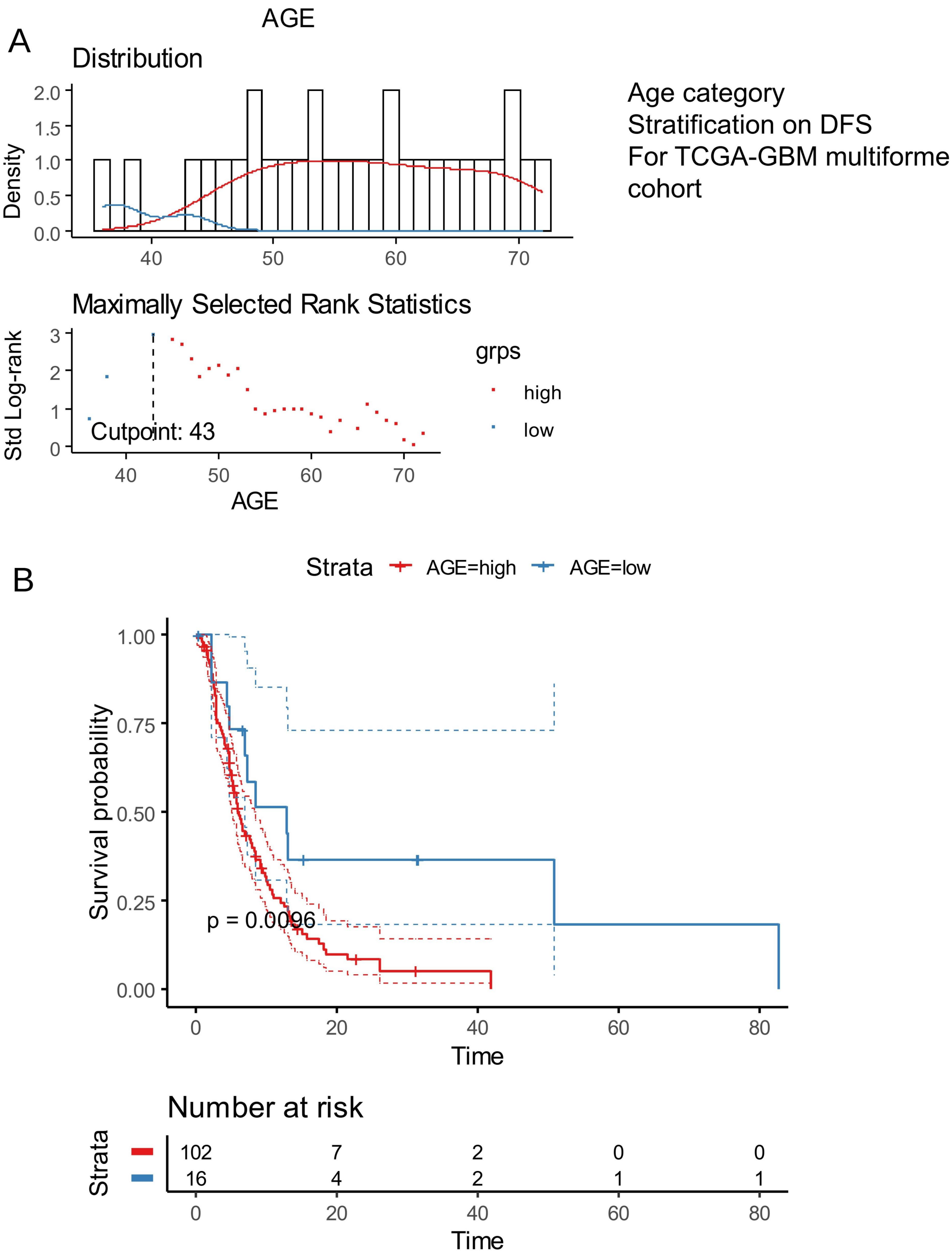


Figure S4. Prognosis age at diagnosis stratification for patients from TCGA-glioblastoma multiforme. (A) Optimal threshold determination for age at diagnosis according disease-free survival (DFS); (B) Kaplan Meier and log rank test on disease free survival according age at diagnosis stratification (threshold 43 yo).

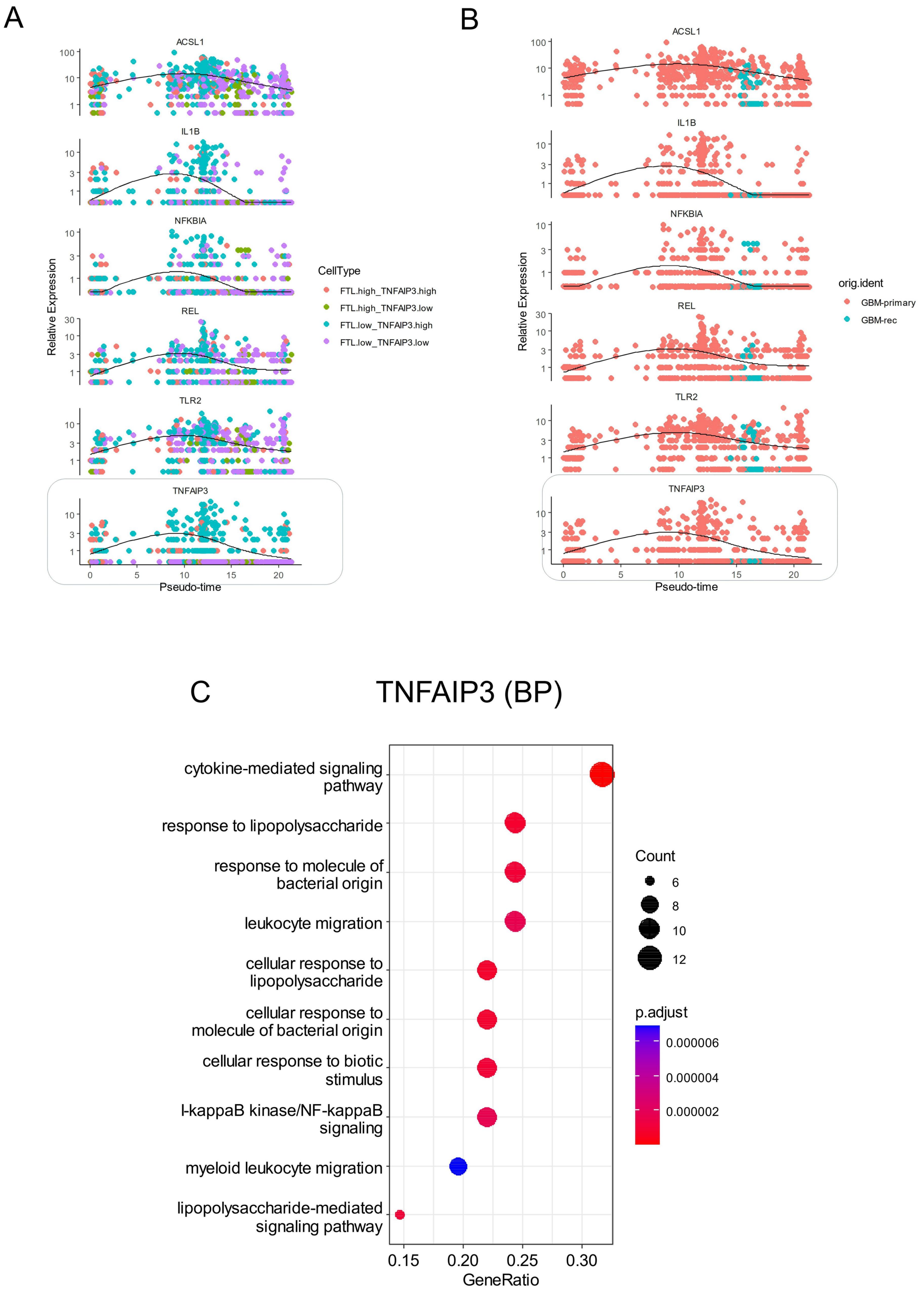


Figure S5. Immune response associated to TNFAIP3 expression in single transcriptome of tumor infiltrated M2-macrophage in glioblastoma. (A) Pseudotime expression plot of markers following TNFAIP3 wave of expression in glioblastoma M2-macrophage: stratification on molecular expression groups; (B) Pseudotime expression plot of markers following TNFAIP3 wave of expression in glioblastoma M2-macrophage: stratification on time of tumor sampling; (C) Functional enrichment performed on Gene Ontology Biological Process (GO) for genes following TNFAIP3 wave of expression in glioblastoma M2-macrophages.

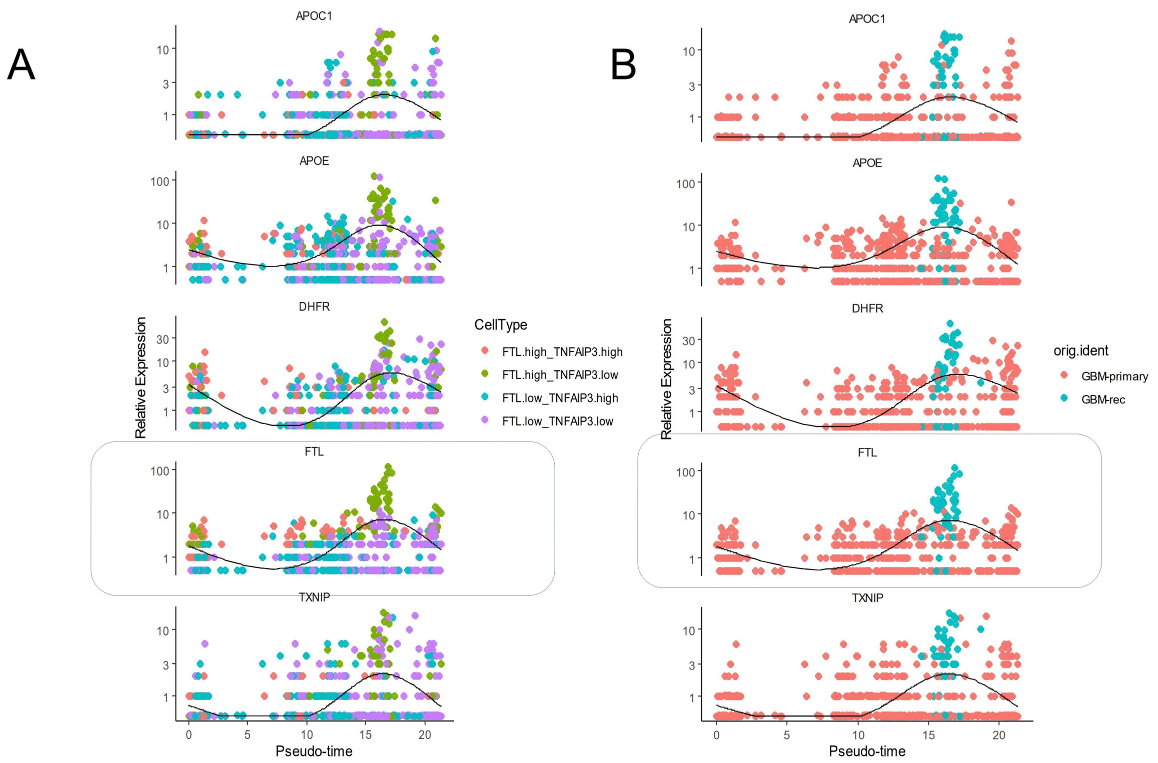
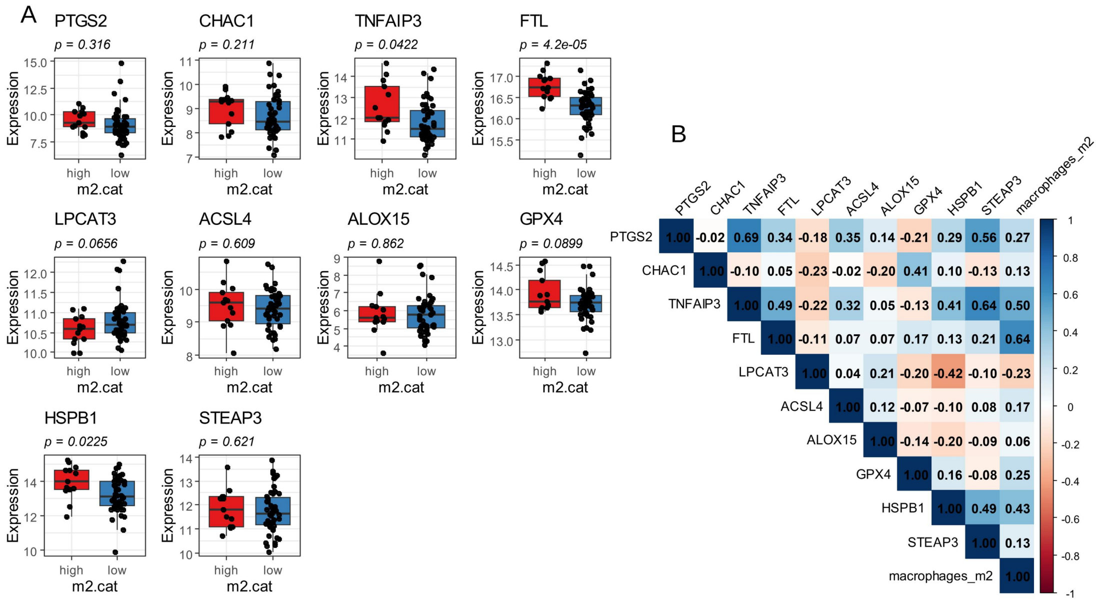


Figure S6. Single cell signature associated to FTL expression in M2-macrophages from recurrent glioblastoma. (A) Pseudotime expression plot of markers following FTL wave of expression in glioblastoma M2-macrophage: stratification on molecular expression groups; (B) Pseudotime expression plot of markers following FTL wave of expression in glioblastoma M2-macrophage: stratification on time of tumor sampling.



	PTGS2	CHAC1	TNFAIP3	FTL	LPCAT3	ACSL4	ALOX15	GPX4	HSPB1	STEAP3	macrophages_m2
PTGS2	1.00	-0.02	0.69	0.34	-0.18	0.35	0.14	-0.21	0.29	0.56	0.27
CHAC1		1.00	-0.10	0.05	-0.23	-0.02	-0.20	0.41	0.10	-0.13	0.13
TNFAIP3			1.00	0.49	-0.22	0.32	0.05	-0.13	0.41	0.64	0.50
FTL				1.00	-0.11	0.07	0.07	0.17	0.13	0.21	0.64
LPCAT3					1.00	0.04	0.21	-0.20	-0.42	-0.10	-0.23
ACSL4						1.00	0.12	-0.07	-0.10	0.08	0.17
ALOX15							1.00	-0.14	-0.20	-0.09	0.06
GPX4								1.00	0.16	-0.08	0.25
HSPB1									1.00	0.49	0.43
STEAP3										1.00	0.13
macrophages_m2											1.00

Figure S7. Association between ferroptosis-related genes and M2 macrophage polarization. (A) Expression levels of selected ferroptosis-related genes (*PTGS2*, *CHAC1*, *TNFAIP3*, *FTL*, *LPCAT3*, *ACSL4*, *ALOX15*, *GPX4*, *HSPB1*, *STEAP3*) stratified by M2 macrophage category (high vs. low). Boxplots display group distributions, and t test p-values indicate the significance of differential expression. TNFAIP3, FTL, and HSPB1 show significant expression differences between M2-high and M2-low groups; (B) Correlation matrix showing pairwise Pearson correlation coefficients between the same ferroptosis-related genes and M2 macrophage abundance. Blue indicates positive correlations and red indicates negative correlations, with color intensity reflecting correlation strength. Notable associations include strong positive correlations between *PTGS2* and *TNFAIP3*, between *FTL* and M2 macrophage levels and between *TNFAIP3* and *STEAP3*.

Table S1. Best significant genes found regulated on pseudotime trajectory of CD16^{+}CD163^{+} cells from glioblastoma according alternative expression of TNFAIP3 and FTL. Respective significant genes are presented in rows with their p-values, adjusted *q*-values and number of positive cells.

gene	**P-values**	**Adjusted q-values**	**Number of positive cells**
TNFAIP3	2.93E-182	5.65E-179	87
MT-ND3	1.66E-173	1.60E-170	268
FTL	9.95E-156	6.38E-153	75
MT-ATP6	4.99E-135	2.40E-132	286
AC105402.3	5.30E-132	2.04E-129	83
MT-ND4	5.98E-126	1.92E-123	293
MT-CO2	1.76E-118	4.83E-116	312
MT-ND2	3.21E-102	7.73E-100	211
MT-CYB	5.53E-92	1.18E-89	239
AP003354.2	2.94E-89	5.66E-87	101
APOE	7.39E-87	1.29E-84	121
MT-ND5	1.67E-63	2.68E-61	132
MT-ND1	2.06E-63	3.05E-61	248
MT-CO3	1.35E-61	1.85E-59	323
DHFR	4.21E-61	5.40E-59	92
NFKB1	9.30E-60	1.12E-57	102
PLEK	2.07E-55	2.34E-53	87
GPNMB	8.42E-52	9.00E-50	17
CD83	1.36E-50	1.38E-48	151
IL1B	2.35E-50	2.26E-48	70
ACSL1	3.92E-49	3.59E-47	338
ICAM1	5.80E-47	5.08E-45	41
MTRNR2L12	7.01E-47	5.87E-45	142
MT-ND4L	4.59E-45	3.69E-43	77
MT-CO1	5.75E-45	4.43E-43	240
NUPR1	5.06E-44	3.75E-42	11
LCP2	4.27E-36	3.05E-34	172
IRAK2	1.11E-33	7.61E-32	73
OLR1	6.82E-32	4.53E-30	103
RNF144B	1.06E-30	6.83E-29	199
APOC1	5.70E-30	3.54E-28	12
LDLRAD4	2.45E-28	1.47E-26	374
CCL3L1	1.72E-27	1.00E-25	22
PSTPIP2	9.80E-27	5.55E-25	38
SLC43A2	1.03E-26	5.68E-25	37
B4GALT1	3.52E-26	1.88E-24	86
MTRNR2L8	6.96E-26	3.62E-24	12
PADI2	2.95E-25	1.49E-23	108
NLRP3	5.58E-25	2.75E-23	29
CCDC26	3.31E-24	1.59E-22	115
RELB	6.83E-23	3.21E-21	27
PDGFB	9.35E-23	4.29E-21	48
NCKAP5	1.03E-21	4.60E-20	154
PSAP	4.97E-21	2.17E-19	105
TXNIP	4.10E-20	1.75E-18	24

ADGRE2	5.30E-20	2.22E-18	54
SUSD6	6.27E-20	2.57E-18	212
CCL3	2.26E-19	9.05E-18	32
EBI3	2.55E-19	1.00E-17	16
HLA-DRB1	9.33E-19	3.59E-17	29
HSPH1	1.66E-18	6.27E-17	32
TMEM163	8.45E-18	3.13E-16	106
NAMPT	1.02E-17	3.70E-16	224
IL1A	1.48E-17	5.28E-16	12
NFKBIA	2.70E-17	9.46E-16	23
PALD1	1.43E-16	4.93E-15	139
GCH1	1.47E-16	4.96E-15	11
REL	2.17E-16	7.21E-15	96
ELMO1	1.50E-15	4.90E-14	262
FRMD4A	1.60E-15	5.14E-14	323
IFNGR2	3.05E-15	9.63E-14	122
SRGN	3.27E-15	1.01E-13	127
B2M	3.93E-15	1.20E-13	88
LIMK2	4.32E-15	1.30E-13	64
SERPINE1	2.29E-14	6.78E-13	12
RNF19B	4.47E-14	1.30E-12	15
MS4A4E	4.93E-14	1.42E-12	17
CHST15	5.49E-14	1.56E-12	76
TLR2	6.27E-14	1.75E-12	196
CST3	8.30E-14	2.28E-12	10
NFKBIZ	9.39E-14	2.55E-12	37
ST6GALNAC3	1.02E-13	2.74E-12	54
C1QB	1.68E-13	4.42E-12	13
HK2	1.86E-13	4.83E-12	43
CD74	2.90E-13	7.39E-12	98