

SUPPLEMENTAL MATERIAL AND METHOD

Peripheral blood analysis

Blood samples were analyzed by the Pathology Core Laboratory at the University of Texas MD Anderson Cancer Center. Routine hematology fluorescence flow cytometry of whole blood samples were carried out using an XN-10 automated analyzer (Sysmex Corporation). Data on healthy individuals ($n = 57$), glioblastoma patients ($n = 60$), grade III brain tumor patients ($n = 30$ [anaplastic astrocytoma, $n = 17$; anaplastic oligodendroglioma, $n = 13$]), low-grade (I + II) brain tumor patients ($n = 33$ [grade I juvenile pilocytic astrocytoma, $n = 17$; grade II diffuse glioma, $n = 3$; grade II oligodendroglioma, $n = 13$]) was collected.

Glioblastoma tissue microarray

Glioblastoma-specific tissue microarrays (TMAs) were constructed under protocol number PA12-0136 and contained 137 glioblastoma samples described previously (1). For TMA construction, two 1-mm cores per tumor sample were obtained from different areas of the tumors. The rationale for using TMAs was to facilitate analysis of the largest number of tumor samples possible. The study neuropathologist (G.N. Fuller) gathered the tissue sections from archived paraffin-embedded blocks and confirmed the pathologic tumor type. The time from resection to fixation was less than 20 minutes in all cases, in accordance with the Clinical Laboratory Improvement Amendments standard.

Glioblastoma volumetric analysis

Image analysis and software

A total of 85 patient images were available for analysis. The 3D-Slicer software (version 4.3.1; www.slicer.org), an open-source image analytics platform, was used for all image processing and segmentation (2-4). The segmented images were reviewed by two experienced neuroradiologists (R.R. Collen and A. Elakkad).

Image registration

The contrast-enhanced T1-weighted imaging (T1WI) and Fluid-Attenuated Inversion Recovery (FLAIR) sequences were used in the study. Prior to segmentation, each FLAIR image was registered to the contrast-enhanced T1WI, and they were rigidly aligned with each other. Resampling was performed, when differences in voxel size between the FLAIR and post-contrast T1WI were observed. Adequate registration was designated as an error of 2-mm or less.

Image segmentation and volume extraction

The contrast-enhanced T1WI was used for segmentation of the enhancing portion (defined as active tumor) and non-enhancing central T1-hypointense portion (defined as necrosis) of the tumor. The perilesional non-enhancing FLAIR hyperintense portion of the tumor, defined as the edema/invasion component, was segmented using the FLAIR sequence. Hyperintensity on pre-contrast T1WI was used to assess for hemorrhage. Images were segmented from the peripheral (edema/invasion) to central (necrosis). Three main distinct imaging phenotypes were segmented, and the edema/invasion, tumor and necrosis volumes were obtained.

Immunofluorescence study

Staining with an anti-Iba1 antibody was used to detect the microglia/macrophages in the glioblastoma TMA samples. Five-micrometer paraffin-embedded TMA sections were deparaffinized and subjected to graded rehydration prior to staining. Standard antigen retrieval (10 mM citrate buffer, pH 6.0) and blocking with 10% serum were performed, followed by incubation with an anti-Iba1 antibody (diluted 1:1000; Wako Pure Chemical Industries, Ltd.) overnight at 4°C. Afterward, slides were incubated with an Alexa Fluor 555-conjugated antibody (diluted 1:1000; Invitrogen). Fluorescently-labeled slides were stained with 4',6-diamidino-2-phenylindole (Sigma) and mounted with a fluorescence mounting medium (Dako). Imaging was performed using the Vectra Automated Multispectral Imaging System with a Vectra 2 microscope (Perkin Elmer). Iba1-positive cells were quantified using the InForm 2.1 software (Perkin Elmer) and manually by an independent scientist.

Antibodies

Human immune cells were stained with the following antibodies and matched IgG controls: CD11b-PE, CD33-APC, CD45-APC-H7, CD86-PerCP-Cy5.5, CD163-Brilliant Violet 421, and HLA-DR-DP-DQ-FITC (BD Biosciences); CD206-APC and CD14-PE-Vio770 (Miltenyi Biotec); CD15-PerCP-Cy5.5, CD33-Brilliant Violet 570, CD33-PerCP-Cy5.5, CD80-PE-Cy7, and colony-stimulating factor 1 receptor (CSF-1R)/CD115-APC (Biolegend); and CD204-PE (R&D Systems). For intracellular staining, IL10-APC, pSTAT1-Alexa Fluor 488, and pSTAT3-PerCP-Cy5.5 (BD Biosciences) and TGF- β 1 (LAP)-PE-Cy7 and TNF- α -Brilliant Violet 421 (Biolegend) were used.

Surface marker and intracellular cytokine staining

The most commonly used criterion to distinguish CNS microglia from macrophages is differential CD45 expression (CD45^{low} for microglia and CD45^{high} for macrophages) in CD11b⁺/CD11c⁺ cells (5-7). Human MDSCs express the cell surface markers CD11b, CD45, and CD33 (8). CD11b⁺ cells were incubated with fluorescence-labeled primary antibodies or matched isotype IgG controls for 15 minutes at 4°C. For intracellular cytokine analysis, CD11b⁺ cells were fixed with Fix/Perm (BD Biosciences), washed in PermWash (Transcription Factor Buffer Set; BD Biosciences) according to manufacturer's protocol, and stained with fluorescence-labeled monoclonal antibodies or isotype controls for 50 minutes at 4° C. Before acquisition, cells were re-suspended in a 0.1% paraformaldehyde solution (Santa Cruz Biotechnology) in PBS. Approximately 1x10⁵ live gated events based on forward scatter and side scatter were assessed using a Gallios 561 Flow Cytometer (Beckman Coulter), and analyzed using the FlowJo software (Tree Star Inc.).

RNA isolation and comparative analysis

After extraction using a mirVana kit (Ambion), RNA samples from glioblastoma-infiltrating CD14⁺ cells ($n = 4$) with matched CD14⁺ blood cells and CD14⁺ blood cells from healthy donors ($n = 4$) were checked for purity and quality using an Agilent Bioanalyzer before being submitted to Human Whole Genome OneArray Version 5.1 (30,275 human genes) analysis provided by the Phalanx Biotech Group (Belmont). This microarray data were submitted to the Gene Expression Omnibus database (National Cancer for Biotechnology Information) under accession number GSE77043.

Identification of glioblastoma subtypes

RNA isolation and sequencing

For RNA-sequencing, total RNA from 27 glioblastoma samples was isolated using a Masterpure Complete DNA and RNA Purification Kit (Epicentre) after proteinase K digestion according to the manufacturer's instructions. Paired-end sequencing of the RNA was performed using an Illumina HiSeq3000 at the MD Anderson Sequencing and Microarray Core Facility. An average of 55 million paired-ends was generated for each sample (range 43-71 million). Each end was 75 bp in size. Short transcript reads were mapped to human protein-coding genes in an Ensembl reference transcriptome (version 64), and reads per kilobase per million reads and values were generated using Pipeline for RNA-sequencing Data Analysis (9). The Cancer Genome Atlas glioblastoma transcriptional subtypes (Proneural, Neural, Classical and Mesenchymal) (10, 11) were assigned to the 27 samples using single-sample gene set enrichment analysis (GSEA) (12) and previously defined subtype signature genes (10, 11). The accession number is GSE77530.

nCounter miRGE assay

Using a mirVana miRNA isolation kit (Life Technologies) according to the manufacturer's protocol, total RNA from healthy-donor CD14⁺ blood cells ($n = 11$) and glioblastoma-patient CD14⁺ blood cells ($n = 11$) were extracted and used in a Nanostring analysis, and glioblastoma-infiltrating

CD14⁺ cells ($n = 11$) with matched CD14⁺ blood cells ($n = 11$) were extracted and used for a second Nanostring analysis. RNA concentration was measured using a Nanodrop 2000c spectrophotometer (Thermo Scientific). RNA (200 ng) at a concentration of 40 ng/μl in a total volume of 5 μl was prepared for an nCounter miRGE assay. Our custom-made CodeSet detects 99 mRNAs (Table 1), 24 miRNAs, and 5 long noncoding RNAs (lncRNAs) (Table 2). The nCounter miRGE assay is a highly sensitive, multiplexed assay designed to detect both mRNAs and miRNAs in a single tube from total RNA. Sample preparation and hybridization were performed for the miRGE assay according to the manufacturer's instructions (NanoString Technologies, Inc.). Briefly, RNA samples were prepared by ligating a specific DNA tag (miR-tag) onto the 3' end of each mature miRNA according to the manufacturer's instructions, and excess tags were removed via restriction digestion at 37°C. After processing using an miRNA sample preparation kit, the entire 10-μl reaction volume containing mRNA and tagged miRNAs was hybridized with a 10-μl reporter CodeSet, 10 μl of hybridization buffer, and a 5-μl capture ProbeSet (for a total reaction volume of 35 μl) at 65°C for 16-20 hours. Excess probes were removed using two-step magnetic bead-based purification with an nCounter Prep Station. The specific target molecules were quantified using an nCounter Digital Analyzer by counting the individual fluorescent barcodes and assessing target molecules. The data were collected using the nCounter Digital Analyzer after obtaining images of the immobilized fluorescent reporters in the sample cartridge using a charge-coupled device camera.

Analysis of Nanostring and microarray data

Nanostring

Nanostring data were analyzed using the NanoStringNorm R package (version 1.1.17). The following normalization parameters were used: 1) CodeCount (positive controls): geometric mean; 2) Background (negative controls): mean plus two standard deviations; and 3) SampleContent (RNA content): top geometric mean (top 75% expressed). Given the technical differences in sample preparation, miRNA expression data were analyzed independently of mRNA and lncRNA expression data (13). Differential expression between groups was assessed using a two-sample, two-sided *t*-test. Multiple

testing corrections were performed using the Benjamini-Hochberg method. The maximum false discovery rate threshold level (q -value) was 0.05.

Microarray

Normalized RNA expression matrices were obtained from Phalanx Biotech Group. Differential expression analysis was performed using the limma R package (version 3.22.7).

Functional enrichment analysis

Pathways and ontologies enriched in global expression were investigated by two complementary strategies. 1) Pre-ranked GSEA was performed using limma differential expression gene expression t -test statistics. 2) Ingenuity pathway analysis (IPA; summer 2015 release; QIAGEN) was performed using the threshold \log_2 expression ratio (absolute) > 1 and q -value < 0.01 .

Data visualization and unsupervised analyses

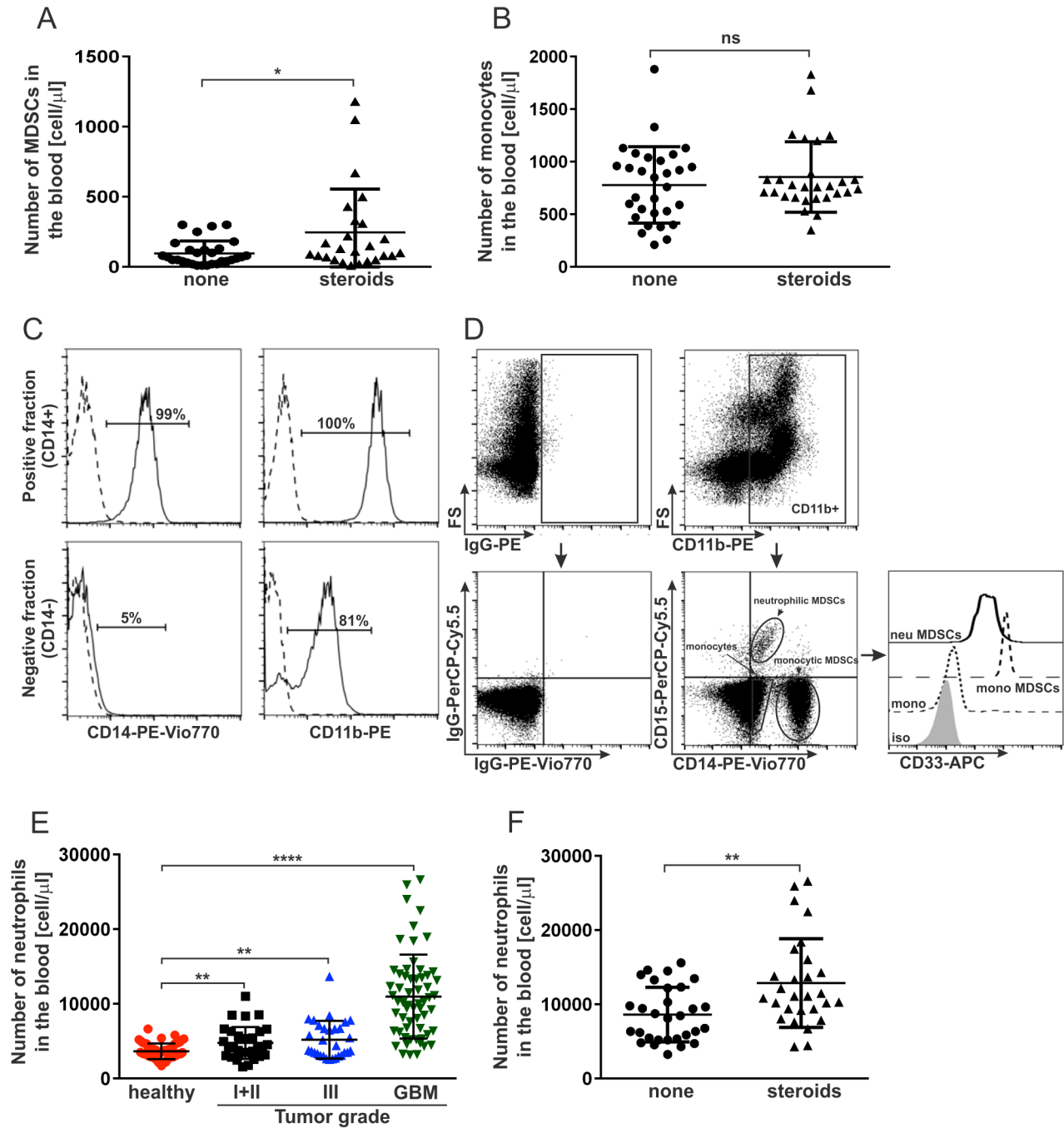
Both microarray expression array and Nanostring data were visualized using the ggplot2 graphing package in heat maps of \log_2 expression values scaled for each gene across samples. Plots of enriched gene sets or pathways were also generated with ggplot2. Hierarchical clustering of the data was performed in the R language using Ward's method with the square root of the Jensen-Shannon divergence as the distance metric. Plots illustrating differentially expressed genes detected by Nanostring or microarray reflect the statistical thresholds described above. Hierarchical clustering and principal coordinate analysis of Nanostring expression profiles in Figure 7 considered all genes with a mean \log_2 expression value > 1 .

References

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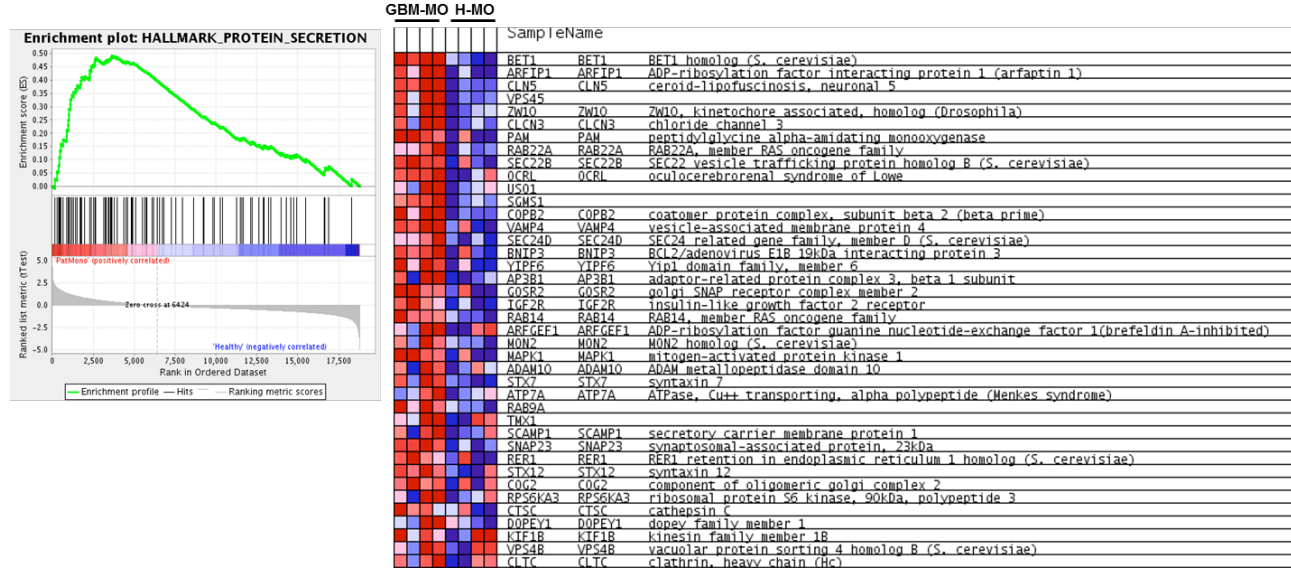
Supplemental Figure 1



Supplemental Figure 1. (A) Numbers of MDSCs in the blood of glioblastoma patients not given ($n = 29$) or given the steroid dexamethasone ($n = 25$) before tumor resection. The data are presented as the mean \pm SD. An unpaired t -test with Welch's correction was used to calculate P values. $*P < 0.05$. (B) Numbers

of monocytes in the blood of glioblastoma patients not given ($n = 30$) or given the steroid dexamethasone ($n = 28$) before tumor resection. The data are presented as the mean \pm SD. An unpaired t -test with Welch's correction was used to calculate P values. ns, not significant. **(C)** Representative histograms for CD14-positively (top) and -negatively (bottom) selected cell fractions from glioblastoma-patient blood cells stained with anti-CD14 (left) and CD11b (right) antibodies. **(D)** Gating strategy for glioblastoma-patient peripheral blood mononuclear cells is shown. CD11b⁺ cells were gated based on dot plots for FS/IgG (top left) and FS/CD11b (top right). Monocytes (CD11b⁺/CD14⁺), monocytic MDSCs (CD11b⁺/CD14^{high}/CD15⁻), and neutrophilic MDSCs (CD11b⁺/CD14^{low}/CD15⁺) were distinguished based on dot plots for IgG/IgG (bottom left) and CD15/CD14 (bottom middle). A representative histogram overlay for CD33 expression in monocytes, monocytic MDSCs, and neutrophilic MDSCs relative to isotype control (gray histogram) is shown. **(E)** Numbers of neutrophils in blood obtained from healthy donors (red circles, $n = 57$), low-grade (I + II) brain tumor patients (black squares, $n = 33$), grade III brain tumor patients (blue triangles, $n = 30$), and glioblastoma (GBM) patients (green triangles, $n = 60$). The data are presented as the mean \pm SD. An unpaired t -test with Welch's correction was used to calculate P values. $**P < 0.01$; $****P < 0.0001$. **(F)** Numbers of neutrophils in the blood of glioblastoma patients not given ($n = 30$) or given the steroid dexamethasone ($n = 28$) before tumor resection. The data are presented as the mean \pm SD. An unpaired t -test with Welch's correction was used to calculate P values. $**P < 0.01$.

Supplemental Figure 2



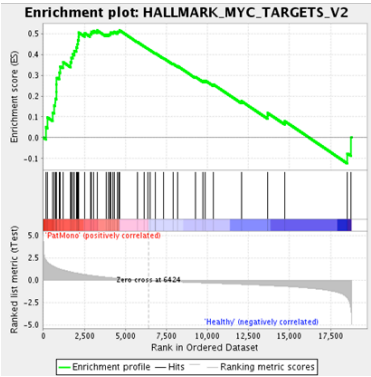
Supplemental Figure 2. GSEA enrichment plots and expression heat maps for glioblastoma-patient CD14⁺ blood cells (GBM-MO, $n = 4$) and healthy-donor CD14⁺ blood cells (H-MO, $n = 4$).



		SampleName	
		ATP6V1C1 ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1
		IOH1 IOH1	isocitrate dehydrogenase 1 (NADP+), soluble
		ETFDH ETFDH	electron-transferring-flavoprotein dehydrogenase
		SUC1A2 SUC1A2	succinate-CoA ligase, ADP-forming, beta subunit
		ATP6V1D ATP6V1D	ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D
		COX5A COX5A	cytochrome c oxidase subunit Va
		MTX2 MTX2	metaxin 2
		PDH8 PDH8	pyruvate dehydrogenase (lipoamide) beta
		CYC5 CYC5	cytochrome c, somatic
		ACADM ACDM	acyl-coenzyme A dehydrogenase, C-4 to C-12 straight chain
		PDHX PDHX	pyruvate dehydrogenase complex, component X
		COX15 COX15	COX15 homolog, cytochrome c oxidase assembly protein (yeast)
		ATFH1	
		TOM70A TOM70A	translocase of outer mitochondrial membrane 70 homolog A (S. cerevisiae)
		FH FH	fumarate hydratase
		NDH1 NDH1	malate dehydrogenase 1, NAD (soluble)
		COX11 COX11	Cox11 homolog, cytochrome c oxidase assembly protein (yeast)
		PDK4 PDK4	pyruvate dehydrogenase kinase, sorcy 4
		NDUFA8 NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa
		MTRF1 MTRF1	mitochondrial translational release factor 1
		PRDX3 PRDX3	peroxiredoxin 3
		SLC25A20 SLC25A20	solute carrier family 25 (carnitine/acylcarnitine translocase), member 20
		ACAT1 ACAT1	acyl-coenzyme A acetyltransferase 1 (acetoacetyl Coenzyme A thiolase)
		HTRA2 HTRA2	HTRA serine peptidase 2
		VOAC3 VOAC3	voltage-dependent anion channel 3
		OGDH OGDH	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)
		NDUFB6 NDUFB6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6, 17kDa
		NDUFB3 NDUFB3	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa
		SDHC SDHC	succinate dehydrogenase complex, subunit C, integral membrane protein, 15kDa
		NMT NMT	nicotinamide nucleotide transhydrogenase
		ETFA ETFA	electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)
		IMMT IMMT	inner membrane protein, mitochondrial (mitofilin)
		CV85A CV85A	cytochrome b5 type A (microsomal)
		DC1R1 DC1R1	2,4-dienoyl CoA reductase 1, mitochondrial
		LRPPRC LRPPRC	leucine-rich PPR-motif containing
		UCRFS1 UCRFS1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
		RHO1T RHO1T	ras homolog gene family, member T1
		TM1010 TM1010	translocase of inner mitochondrial membrane 10 homolog (yeast)
		PDHA1 PDHA1	pyruvate dehydrogenase (lipoamide) alpha 1
		MRP115 MRP115	mitochondrial ribosomal protein L15
		MRP135 MRP135	mitochondrial ribosomal protein L35
		TM10B8 TM10B8	translocase of inner mitochondrial membrane 8 homolog B (yeast)
		TM10A7A TM10A7A	translocase of inner mitochondrial membrane 17 homolog A (yeast)
		ACO2 ACO2	aconitase 2, mitochondrial
		SLC25A11 SLC25A11	solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11
		ATP6V1E1 ATP6V1E1	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E1
		OPF1 OPF1	optic atrophy 1 (autosomal dominant)
		SUC1G1 SUC1G1	succinate-CoA ligase, GDP-forming, alpha subunit
		POLR2F POLR2F	polymerase (RNA) II (DNA directed) polypeptide F
		FXN FXN	frataxin
		AF3L2 AF3L2	AF3 ATPase family gene 3-like 2 (yeast)
		MRP511 MRP511	mitochondrial ribosomal protein S11
		UCRC2 UCRC2	ubiquinol-cytochrome c reductase core protein II
		RETSAT RETSAT	retinol saturase (all-trans-retinol 13,14-reductase)
		NDUFA1 NDUFA1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1, 8kDa
		NDUFA2 NDUFA2	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)
		MRP512 MRP512	mitochondrial ribosomal protein S12
		ACAD5B ACAD5B	acyl-coenzyme A dehydrogenase, short/branched chain
		NDUFC1 NDUFC1	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6kDa
		LDBH LDBH	lactate dehydrogenase B
		NDUFA2 NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa
		MTRR MTRR	5-methyltetrahydrofolate homocysteine methyltransferase reductase
		COX10 COX10	Cox10 homolog, cytochrome c oxidase assembly protein, heme A-Tarnesyltransferase (yeast)
		NDUFA5 NDUFA5	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)
		ATP5B ATP5B	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b

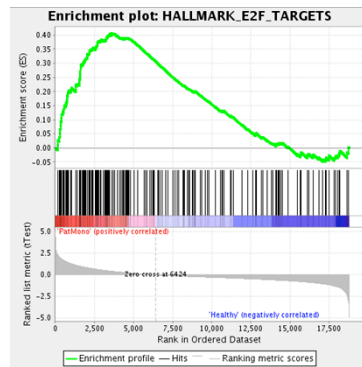


		SampleName	
		G3BP1	
		MYC	MYC v-myc myelocytomatosis viral oncogene homolog (avian)
		PCNA	PCNA proliferating cell nuclear antigen
		ORC2	
		SRF1	
		ARCE1	ARCE1 ATP-binding cassette, sub-family F (OABP), member 1
		COXA5	COXA5 cytochrome c oxidase subunit Va
		NOLC1	NOLC1 nucleolar and coiled-body phosphoprotein 1
		NOP16	
		NUP56	
		NCBP2	NCBP2 nuclear cap binding protein subunit 2, 20kDa
		XP01	XP01 exportin 1 (CRM1 homolog, yeast)
		EIF251	EIF251 eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa
		EPBS	EPBS glutamyl-prolyl-tRNA synthetase
		URE2L3	URE2L3 ubiquitin-conjugating enzyme E2L 3
		TMEM70A	TMEM70A translocase of outer mitochondrial membrane 70 homolog A (S. cerevisiae)
		VBPI	VBPI von Hippel-Lindau binding protein 1
		CSTF2	CSTF2 cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa
		DDX18	DDX18 DEAD (Asp-Glu-Ala-Asp) box polypeptide 18
		CTC2	CTC2 chaperonin containing TCP1, subunit 2, (beta)
		CLN3	CLN3 quinine nucleotide binding protein-like 3 (nucleolar)
		CAD	CAD carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase
		PRDX3	PRDX3 peroxiredoxin 3
		USP1	USP1 ubiquitin specific peptidase 1
		SF3B3	SF3B3 splicing factor 3b, subunit 3, 130kDa
		CTCS	CTCS chaperonin containing TCP1, subunit 5 (epsilon)
		EIF252	EIF252 eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa
		RPL6	RPL6 ribosomal protein L6
		RRP9	
		COF55	COF55 constitutive photomorphogenic homolog subunit 5 (Arabidopsis)
		VDAC3	VDAC3 voltage-dependent anion channel 3
		PHB	PHB prohibitin
		SERPBP1	SERPBP1 SERPINE1 mRNA binding protein 1
		SNRPD1	SNRPD1 small nuclear ribonucleoprotein D1 polypeptide 16kDa
		CTPS	CTPS CTP synthase
		REFC4	REFC4 replication factor C (activator 1) 4, 37kDa
		PSM01	PSM01 proteasome (prosome, macropain) 26S subunit, non-ATPase, 1
		HSPD1	HSPD1 heat shock 60kDa protein 1 (chaperonin)
		DUT	DUT dUTP pyrophosphatase
		HSP61	HSP61 heat shock 10kDa protein 1 (chaperonin 10)
		BUB3	BUB3 budding uninhibited by benzimidazoles 3 homolog (yeast)
		GL01	GL01 glyoxalase 1
		CNA2	CNA2 cyclin A2
		C10BP	C10BP complement component 1, g subcomponent binding protein
		EEF1B2	EEF1B2 eukaryotic translation elongation factor 1 beta 2
		PWF1	PWF1 homolog (S. cerevisiae)
		PSM014	PSM014 proteasome (prosome, macropain) 26S subunit, non-ATPase, 14
		EIF1AX	EIF1AX eukaryotic translation initiation factor 1A, X-linked
		WAD2L1	WAD2L1 WAD2 mitotic arrest deficient-like 1 (yeast)
		PSMG6	PSMG6 proteasome (prosome, macropain) 26S subunit, ATPase, 6
		SNRPD3	SNRPD3 small nuclear ribonucleoprotein D3 polypeptide 18kDa
		AIH2P	
		NME1	NME1 non-metastatic cells 1, protein (NM23A) expressed in
		GSPT1	GSPT1 G1 to S phase transition 1
		DDX21	DDX21 DEAD (Asp-Glu-Ala-Asp) box polypeptide 21
		HDAC2	HDAC2 histone deacetylase 2
		SRPK1	SRPK1 SFRS protein kinase 1
		HNENPA3	
		RRM1	RRM1 ribonucleotide reductase M1 polypeptide
		PSMA4	PSMA4 proteasome (prosome, macropain) subunit, alpha type, 4
		E1F3B	
		PSM07	PSM07 proteasome (prosome, macropain) 26S subunit, non-ATPase, 7 (Moy34 homolog)
		HNENPA2B1	
		RAN	RAN RAN, member RAS oncogene family
		KPN2A	KPN2A karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
		XPOT	XPOT exportin, RNA (nuclear export receptor for RNAs)
		S8	S8 Sjogren syndrome antigen 8 (autoantigen Ia)
		HPRT1	HPRT1 hypoxanthine phosphoribosyltransferase 1 (Lesch-Myhan syndrome)
		CLNS1A	CLNS1A chloride channel, nucleotide-sensitive, 1A
		HDHC2	HDHC2 HD domain containing 2
		CBX3	CBX3 chromobox homolog 3 (HP1 gamma homolog, Drosophila)
		PSMB7	PSMB7 proteasome (prosome, macropain) subunit, beta type, 7



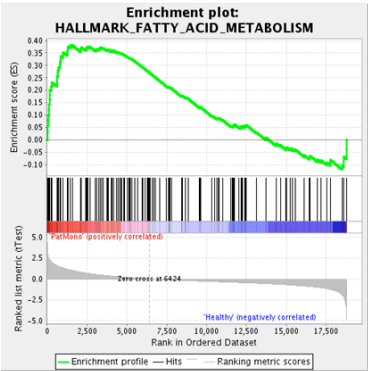
GBM-MO H-MO

		SampleName
	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)
	PRMT3	protein arginine methyltransferase 3
	NOL1	nucleolar and coiled-body phosphoprotein 1
	NOP56	
	NOP56	
	MPHOSPH10	MPHOSPH10 M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein)
	NDUFA4	
	FARSA	
	BYS1	bystin-like
	DDX18	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18
	GNL3	guanine nucleotide binding protein-like 3 (nucleolar)
	PPAN	peter pan homolog (Drosophila)
	EXOSC5	exosome component 5
	RRP9	
	PHB	prohibitin
	HSPD1	heat shock 60kDa protein 1 (chaperonin)
	HSPA1	heat shock 10kDa protein 1 (chaperonin 10)
	WAP3K6	mitogen-activated protein kinase kinase kinase 6
	UNG	uracil-DNA glycosylase
	RABEPK	Rab9 effector protein with kelch motifs
	TFB2M	transcription factor B2, mitochondrial
	AIWP2	
	WDR43	WD repeat domain 43
	GRWD1	glutamate-rich WD repeat containing 1
	TBRG4	transforming growth factor beta regulator 4



GBM-MO H-MO

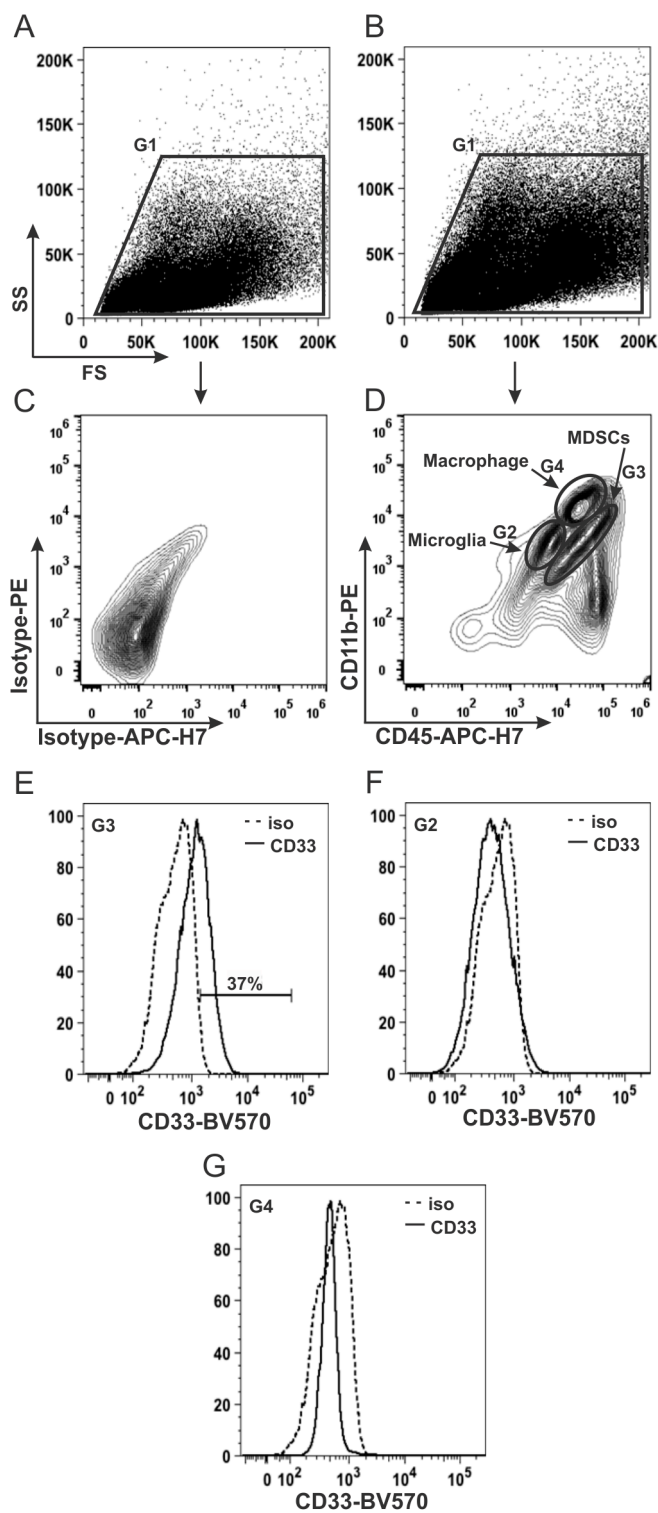
SampleName			
MYC	MYC	Y-myc myelocytomatosis viral oncogene homolog (avian)	
ASF1A	ASF1A	ASF1 anti-silencing function 1 homolog A (S. cerevisiae)	
SHMT1	SHMT1	serine hydroxymethyltransferase 1 (soluble)	
PCNA	PCNA	proliferating cell nuclear antigen	
ORC3	ORC3		
GIN53	GIN53	GIN5 complex subunit 3 (Psf3 homolog)	
DCLRE1B	DCLRE1B	DNA cross-link repair 18 (F502 homolog, S. cerevisiae)	
DKC	DKC	deoxycytidine kinase	
SRSF1	SRSF1		
ZW10	ZW10	ZW10, kinetochore associated, homolog (Drosophila)	
MCB3	MCB3	MCB3 minichromosome maintenance deficient 3 (S. cerevisiae)	
NOLC1	NOLC1	nucleolar and coiled-body phosphoprotein 1	
NUP205	NUP205	nucleoporin 205kDa	
NUP55	NUP55		
BRC1A	BRC1A	breast cancer 1, early onset	
XP01	XP01	exportin 1 (CRM1 homolog, yeast)	
EIF251	EIF251	eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa	
LYAR	LYAR		
RACGAP1	RACGAP1	Rac GTPase activating protein 1	
PDL03	PDL03	polymerase (DNA-directed), delta 3, accessory subunit	
PPP1R8	PPP1R8	protein phosphatase 1, regulatory (inhibitor) subunit 8	
USP1	USP1	ubiquitin specific peptidase 1	
STAG1	STAG1	stromal antigen 1	
HW02	HW02	high-mobility group box 2	
NCAPD2	NCAPD2	non-SMC condensin I complex, subunit D2	
NASP	NASP	nuclear autoantigenic sperm protein (histone-binding)	
CSE1L	CSE1L	CSE1 chromosome segregation 1-like (yeast)	
MTFHD2	MTFHD2	methylene-tetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	
LUC13	LUC13		
PBYNC	PBYNC	protein kinase, RNA-activated, catalytic polypeptide	
HUS1	HUS1	HUS1 checkpoint homolog (S. pombe)	
MSH2	MSH2	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	
RAO51C	RAO51C	RAO51 homolog C (S. cerevisiae)	
PAIC5	PAIC5	phosphoribosylaminimidazole carboxylase, phosphoribosylaminimidazole succinocarboxamide synthetase	
RNA5EH2A	RNA5EH2A	ribonuclease H2, subunit A	
RPA1	RPA1	replication protein A1, 70kDa	
NUP107	NUP107	nucleoporin 107kDa	
TP53	TP53	tumor protein p53 (Li-Fraumeni syndrome)	
CKS2	CKS2	CKS2 protein kinase regulatory subunit 2	
CTPS	CTPS	CTP synthase	
LMBN1	LMBN1	lamin B1	
DUT	DUT	dUTP pyrophosphatase	
PAN2	PAN2		
UNG	UNG	uracil-DNA glycosylase	
RPA2	RPA2	replication protein A2, 32kDa	
RDCD1	RDCD1	RDC1 required for cell differentiation1 homolog (S. pombe)	
CHK2	CHK2	CHK2 checkpoint homolog (S. pombe)	
RFC3	RFC3	replication factor C (activator 1) 3, 38kDa	
AURKA	AURKA	aurora kinase A	
RAO50	RAO50	RAO50 homolog (S. cerevisiae)	
NBN	NBN	nibrin	
UBE2T	UBE2T	ubiquitin-conjugating enzyme E2T (putative)	
MRE11A	MRE11A	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	
SMC1A	SMC1A	structural maintenance of chromosomes 1A	
MLH1	MLH1	mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)	
REC2	REC2	replication factor C (activator 1) 2, 40kDa	
PSTP1	PSTP1	Pc4 and SPB1 interacting protein 1	
MAD2L1	MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	
AK2	AK2	adenylate kinase 2	
WWE1	WWE1	non-metastatic cells 1, protein (NM23A) expressed in	
CTCF	CTCF	CTCF-binding factor (zinc finger protein)	
GSP11	GSP11	G1 to S phase transition 1	
PPH10	PPH10	protein phosphatase 10 magnesium-dependent, delta isoform	
BIRC5	BIRC5	baculoviral IAP repeat-containing 5 (survivin)	
ING3	ING3	inhibitor of growth family, member 3	
SMC6	SMC6	structural maintenance of chromosomes 6	
DNMT1	DNMT1	DNA (cytosine-5-)-methyltransferase 1	
EXOSC8	EXOSC8	exosome component 8	
TBRG4	TBRG4	transforming growth factor beta regulator 4	
EEO	EEO	embryonic ectoderm development	
RAN	RAN	RAN, member RAS oncogene family	
TACC3	TACC3	transforming, acidic coiled-coil containing protein 3	
EZH2	EZH2	enhancer of zeste homolog 2 (Drosophila)	
UBE25	UBE25	ubiquitin-conjugating enzyme E25	
KPNA2	KPNA2	karyopherin alpha 2 (RAN cohort 1, importin alpha 1)	
CDKN2A	CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	
SMC4	SMC4	structural maintenance of chromosomes 4	
TUBG1	TUBG1	tubulin, gamma 1	



GBM-MO H-MO

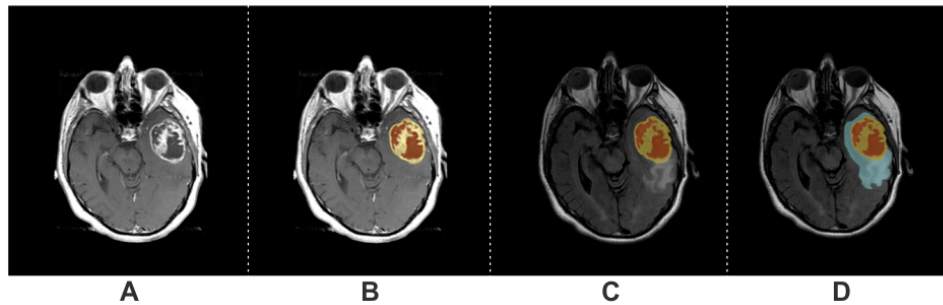
		SampleName
	MAOA	MAOA monoamine oxidase A
	VNH1	VNH1 vanin 1
	IDH1	IDH1 isocitrate dehydrogenase 1 (NADP+), soluble
	EHHA0H	EHHA0H enoyl-Coenzyme A hydratase/3-hydroxyacyl Coenzyme A dehydrogenase
	HSD17B7	HSD17B7 hydroxysteroid (17-beta) dehydrogenase 7
	CPDX	CPDX coproporphyrinogen oxidase
	ETFDH	ETFDH electron-transferring-flavoprotein dehydrogenase
	HT6CH	HT6CH 3-hydroxyisobutyryl-Coenzyme A hydrolase
	HSD17B4	HSD17B4 hydroxysteroid (17-beta) dehydrogenase 4
	SUCLA2	SUCLA2 succinate-CoA ligase, ADP-forming, beta subunit
	HMGL	HMGL 3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria)
	MLYCD	MLYCD malonyl-CoA decarboxylase
	ACOX1	ACOX1 acyl-Coenzyme A oxidase 1, palmitoyl
	CRY2	CRY2 crystallin, zeta (quinone reductase)
	PDHB	PDHB pyruvate dehydrogenase (lipoamide) beta
	HPGD	HPGD hydroxyprostaglandin dehydrogenase 15-(NAD)
	ACADN	ACADN acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain
	AUH	AUH AU RNA binding protein/enoyl-Coenzyme A hydratase
	UCDH	UCDH UDP-glucose dehydrogenase
	ALDH3A2	ALDH3A2 aldehyde dehydrogenase 3 family, member A2
	MCEE	MCEE methylmalonyl CoA epimerase
	FH	FH fumarate hydratase
	MDH1	MDH1 malate dehydrogenase 1, NAD (soluble)
	CBR1	CBR1 carbonyl reductase 1
	DHCR24	DHCR24 24-dehydrocholesterol reductase
	ACSL5	ACSL5 acyl-CoA synthetase long-chain family member 5
	ALDH1A1	ALDH1A1 aldehyde dehydrogenase 1 family, member A1
	HSD12	HSD12 hydroxysteroid dehydrogenase like 2
	PPARA	PPARA peroxisome proliferative activated receptor, alpha

Supplemental Figure 3



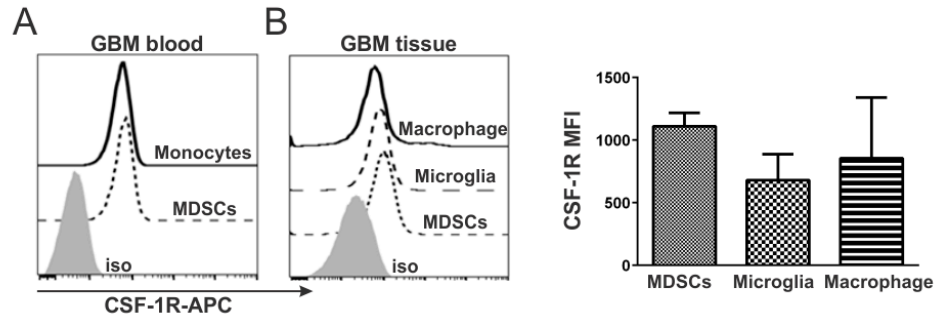
Supplemental Figure 3. Gating strategy for analysis of immune cells isolated from glioblastomas. Brain tumor samples were enzymatically digested, and the myelin was separated by Percoll gradient centrifugation. CD11b⁺ cells were separated using a magnetic-bead-conjugated anti-CD11b antibody and stained with IgG-PE, IgG-APC, IgG-BV570 (isotype controls) or CD11b-PE, CD45-APC-H7, and CD33-BV570 (stained) to identify myeloid cells. Glioblastoma-infiltrated immune cells were analyzed using side-scatter versus forward-scatter dot plots for both the isotype **(A)** and stained cells **(B)**. **(C)** and **(D)** analysis of 1 x 10⁵ cells of CD11b⁺ cells from the G1 gate. G2, G3, G4 gates were drawn to indicate microglia (CD11b⁺/CD45^{low}), MDSCs (CD11b⁺/CD45^{med}) and macrophage (CD11b⁺/CD45^{high}) populations, respectively. Additional histograms for CD33 expression in MDSCs **(E)**, microglia **(F)**, and macrophages **(G)** are shown.

Supplemental Figure 4



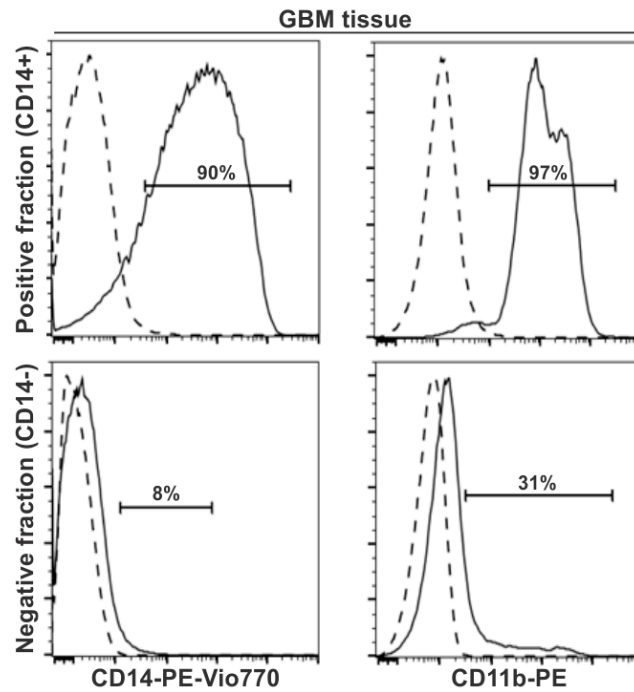
Supplemental Figure 4. Representative volumetric analysis of left temporal glioblastoma in a 63-year-old man. **(A)** T1 postcontrast image. **(B)** Image of segmented tumor label overlaid on the postcontrast image. Of note is the segmented enhancement/tumor in yellow and necrosis in red. **(C)** Image of the segmented tumor label overlaid on a FLAIR image registered on the T1 postcontrast image as the baseline. **(D)** Segmented label depicting edema/invasion (blue), tumor (yellow) and necrosis (red).

Supplemental Figure 5



Supplemental Figure 5. Expression of CSF-1R in a glioblastoma myeloid-lineage compartment. **(A)** Representative histogram overlay for CSF-1R expression in monocytes and MDSCs from glioblastoma-patient blood sample compared to isotype control (gray histogram). **(B)** Representative histogram overlay for CSF-1R expression in MDSCs, microglia, and macrophages isolated from resected glioblastoma tissue relative to isotype control (gray histogram). MFI of CSF-1R expression in MDSCs, microglia, and macrophages isolated from four glioblastomas. The data are presented as the mean \pm SD.

Supplemental Figure 6

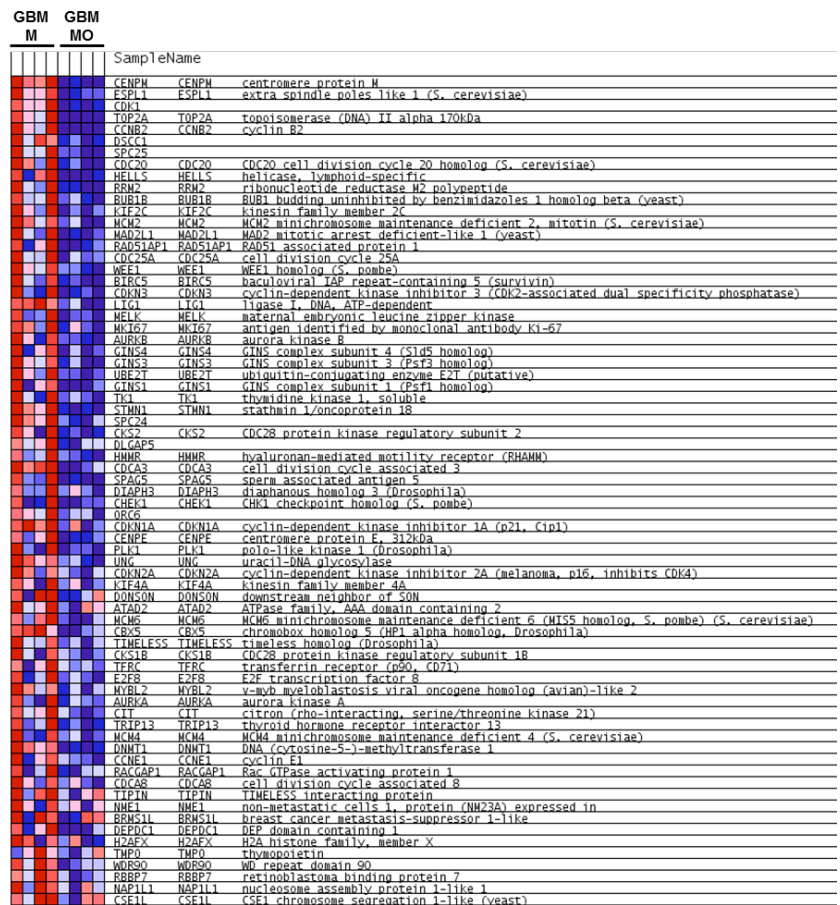


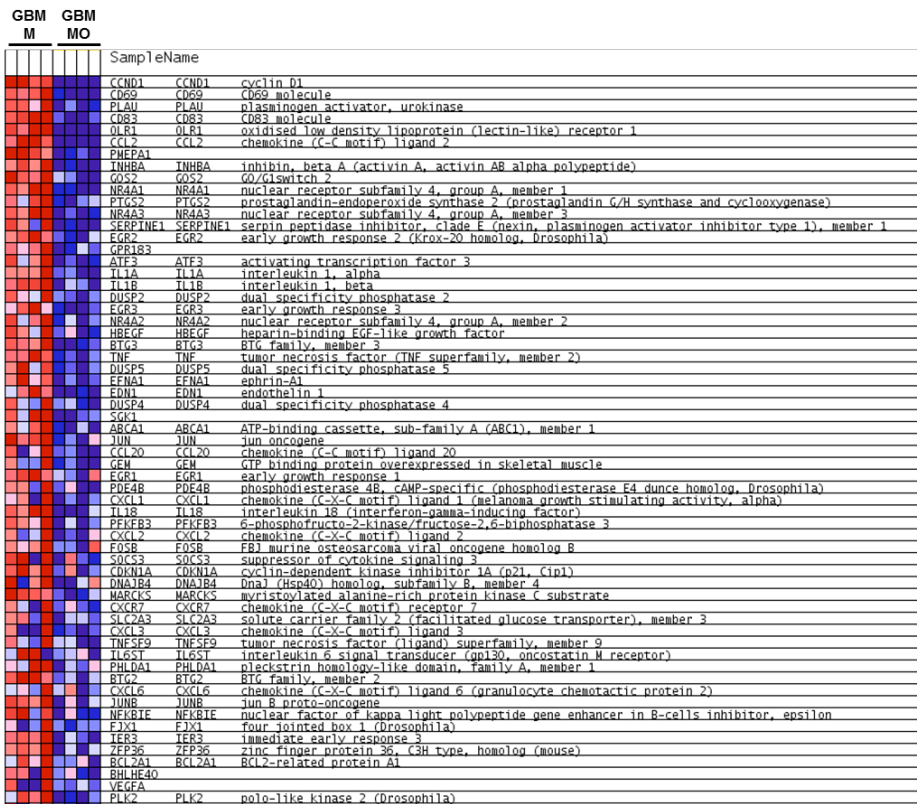
Supplemental Figure 6. Representative histograms for CD14-positively (top) and -negatively (bottom) selected cell fractions from resected glioblastoma (GBM) tissue stained with anti-CD14 (left) and -CD11b (right) antibodies.

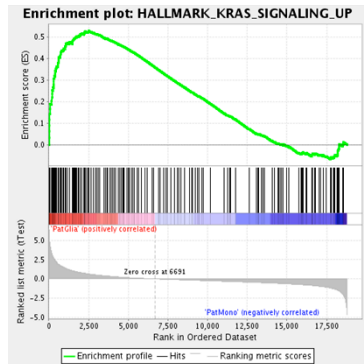
Supplemental Figure 7



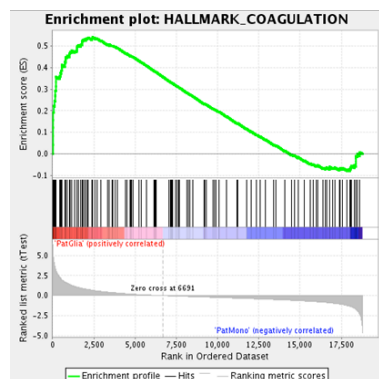
Supplemental Figure 7. GSEA enrichment plots and expression heat maps for glioblastoma-infiltrating CD14⁺ cells (GBM-M, $n = 4$) and matched CD14⁺ blood cells (GBM-MO, $n = 4$).



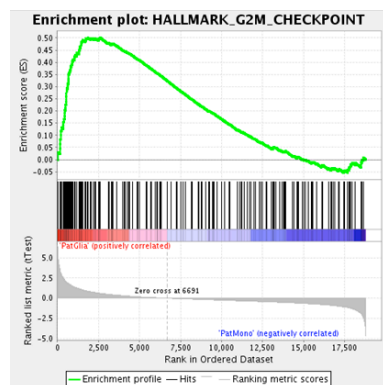




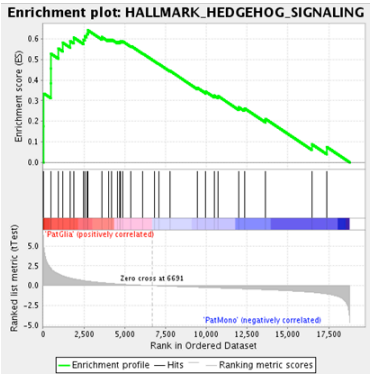
GBM M	GBM MO	SampleName	
		SPPI	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
		RG516	regulator of G-protein signalling 16
		PLAU	plasminogen activator, urokinase
		SPRY2	serouty homolog 2 (Drosophila)
		NRP1	neuropilin 1
		GFNHR	glycoprotein (transmembrane) nhb
		TNHR8	inhibin, beta A (activin A, activin AR alpha polypeptide)
		GOS2	G0/G1switch 2
		SCN1B	sodium channel, voltage-gated, type I, beta
		PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
		ETS5	ets variant gene 5 (ets-related molecule)
		SPARC1	SPARC-like 1 (masto, hevyn)
		ENG	endoglin (Osler-Rendu-Weber syndrome 1)
		IL18	interleukin 1, beta
		MMD	monocyte to macrophage differentiation-associated
		IGFBP3	insulin-like growth factor binding protein 3
		HBE6	heparin-binding EGF-like growth factor
		DCBLD2	discoidin, CUB and ICD domain containing 2
		ST6GAL1	ST6 beta-galactosamide alpha-2,6-sialyltransferase 1
		ANKH	anklyosis, progressive homolog (mouse)
		PRDM1	PR domain containing 1, with ZNF domain
		CFB	complement factor B
		SERPINA3	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
		TFPI	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)
		ALDH1A2	aldehyde dehydrogenase 1 family, member A2
		CCL20	chemokine (C-C motif) ligand 20
		TSPAN13	tetraspanin 13
		GADD45G	growth arrest and DNA-damage-inducible, gamma
		PLVAP	plasmalemma vesicle associated protein
		ANGPTL4	angiotensin-like 4
		TSPAN7	tetraspanin 7
		SODCAG8	serologically defined colon cancer antigen 8
		AKAP12	A kinase (PKA) anchor protein (gravin) 12
		PLEK2	pleckstrin 2
		WMP11	matrix metalloproteinase 11 (stromelysin 3)
		SLPI	secretory leukocyte peptidase inhibitor
		HSD11B1	hydroxysteroid (11-beta) dehydrogenase 1
		ARG1	arginase, liver
		CBOT	carnitine 0-octanoyltransferase
		ANO1	
		CFH	complement factor H
		CCND2	cyclin D2
		KIF5C	kinesin family member 5C
		PIGB	polymeric immunoglobulin receptor
		ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)
		CPE	carboxypeptidase E
		USH1C	usher syndrome 1C (autosomal recessive, severe)
		PLAT	plasminogen activator, tissue
		AP00	apolipoprotein D
		SPON1	spondin 1, extracellular matrix protein
		THEM158	transmembrane protein 158
		CMUR1	chemokine-like receptor 1
		GNCYA3	guanylate cyclase 1, soluble, alpha 3
		SNAP25	synaptosomal-associated protein, 25kDa
		IL7R	interleukin 7 receptor
		CYPK	glycophorin C (kerbich blood group)
		PRKG2	protein kinase, cAMP-dependent, type II
		GNGL1	guanine nucleotide binding protein (G protein), gamma 11
		EPHB2	EPH receptor B2
		PRCDLG2	programmed cell death 1 ligand 2
		TRIB1	tribbles homolog 1 (Drosophila)
		THEM176B	transmembrane protein 176B
		ADAMDEC1	ADAM-like, decysin 1



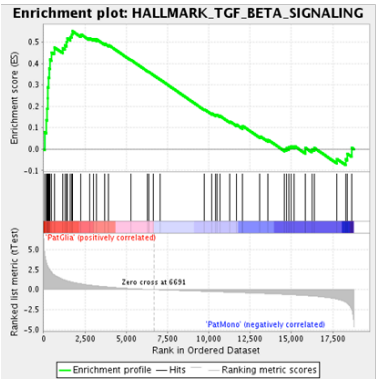
GBM M	GBM MO	SampleName
		AP0C1 AP0C1 apolipoprotein C-I
		C3 C3 complement component 3
		A2M A2M alpha-2-macroglobulin
		PLAU PLAU plasminogen activator, urokinase
		OLR1 OLR1 oxidised low density lipoprotein (lectin-like) receptor 1
		LGMN LGMN legumain
		PROS1 PROS1 protein S (alpha)
		TIMP3 TIMP3 TIMP metalloproteinase inhibitor 3 (Sorsby fundus dystrophy, pseudoinflammatory)
		C10A C10A complement component 1, q subcomponent, A chain
		SERPINE1 SERPINE1 serpin peptidase inhibitor, clade F (nexin, plasminogen activator inhibitor type 1), member 1
		AP0C2 AP0C2 apolipoprotein C-II
		HTRA1 HTRA1 HTRA serine peptidase 1
		PDGFR PDGFR platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)
		MEP1A MEP1A meprin A, alpha (PABA peptide hydrolase)
		TF TF transferrin
		MMP14 MMP14 matrix metalloproteinase 14 (membrane-inserted)
		CFB CFB complement factor B
		SERPINE1 SERPINE1 serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)
		MMP7 MMP7 matrix metalloproteinase 7 (matrilysin, uterine)
		KLKB1 KLKB1 kallikrein B, plasma (Fletcher factor) 1
		CFI CFI complement factor I
		PROC PROC protein C (inactivator of coagulation factors Va and VIIIa)
		C2 C2 complement component 2
		C1R C1R complement component 1, r subcomponent
		MMP11 MMP11 matrix metalloproteinase 11 (stromelysin 3)
		CD9 CD9 CD9 molecule
		S100A13 S100A13 S100 calcium binding protein A13
		AP0A1 AP0A1 apolipoprotein A-I
		MMP8 MMP8 matrix metalloproteinase 8 (neutrophil collagenase)
		DPP4 DPP4 dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)
		CFH CFH complement factor H
		USP11 USP11 ubiquitin specific peptidase 11
		DUSP14 DUSP14 dual specificity phosphatase 14
		FBN1 FBN1 fibrillin 1
		MMP1 MMP1 matrix metalloproteinase 1 (interstitial collagenase)
		VWF VWF von Willebrand factor
		RGN RGN regucalcin (senescence marker protein-30)
		PLAT PLAT plasminogen activator, tissue
		ADAM9 ADAM9 ADAM metalloproteinase domain 9 (matrin gamma)
		RAPGEF3 RAPGEF3 Ras guanine nucleotide exchange factor (GEF) 3
		GSN GSN gelsolin (amyloidosis, Finnish type)
		ITGB3 ITGB3 integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
		FN1 FN1 fibronectin 1
		PRSS23 PRSS23 protease, serine, 23
		MAFF MAFF v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)
		THBD THBD thrombomodulin



GBM M	GBM MO	SampleName
		CCND1 CCND1 cyclin D1
		ESPL1 ESPL1 extra spindle poles like 1 (S. cerevisiae)
		SLC7A5 SLC7A5 solute carrier family 7 (cationic amino acid transporter, y+ system), member 5
		CDK1 CDK1
		TOP2A TOP2A topoisomerase (DNA) II alpha 170kDa
		TPX2 TPX2 TPX2, microtubule-associated, homolog (Xenopus laevis)
		CCNB2 CCNB2 cyclin B2
		KIF20B KIF20B
		CDK20 CDK20 CDK20 cell division cycle 20 homolog (S. cerevisiae)
		KIF2C KIF2C kinesin family member 2C
		PRC1 PRC1 protein regulator of cytokinesis 1
		WDCB0 WDCB0
		MCH2 MCH2 MCH2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae)
		MAD2L1 MAD2L1 MAD2 mitotic arrest deficient-like 1 (yeast)
		UBE2C UBE2C ubiquitin-conjugating enzyme E2C
		CENPA CENPA centromere protein A
		TTK TTK TTK protein kinase
		CDK25A CDK25A cell division cycle 25A
		TRRAP TRRAP tropomyosin associated protein (tastin)
		CTNS2 CTNS2 CTNS complex subunit 2 (Psf2 homolog)
		BTRC5 BTRC5 baculoviral IAP repeat-containing 5 (survivin)
		CDKN3 CDKN3 cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
		MK167 MK167 antigen identified by monoclonal antibody K1-67
		CCNB2 CCNB2 cyclin B2
		BUB1 BUB1 BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)
		CDK6 CDK6 CDK6 cell division cycle 6 homolog (S. cerevisiae)
		AURKB AURKB aurora kinase B
		KIF11 KIF11 kinesin family member 11
		PRK PRK PDZ binding kinase
		NEK2 NEK2 NIMA (never in mitosis gene a)-related kinase 2
		STMN1 STMN1 stathmin 1/oncoprotein 18
		KIF15 KIF15 kinesin family member 15
		CKS2 CKS2 CDK28 protein kinase regulatory subunit 2
		PTTG3P PTTG3P
		HMMR HMMR hyaluronan-mediated motility receptor (RHAMM)
		CHEK1 CHEK1 CHEK1 checkpoint homolog (S. pombe)
		ORC6 ORC6
		CENPE CENPE centromere protein E, 312kDa
		PLK1 PLK1 polo-like kinase 1 (Drosophila)
		SMC2 SMC2 structural maintenance of chromosomes 2
		WARRCS WARRCS wavy-striated alanine-rich protein kinase C substrate
		TNP02 TNP02 transportin 2 (importin 3, karyopherin beta 2b)
		HIF1A HIF1A hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
		EXO1 EXO1 exonuclease 1
		KIF4A KIF4A kinesin family member 4A
		CCNT1 CCNT1 cyclin T1
		WRN WRN Werner syndrome
		MCM6 MCM6 MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)
		ATF5 ATF5 activating transcription factor 5
		CENPE CENPE centromere protein E, 350/400kDa (mitotin)
		CKS1B CKS1B CDK28 protein kinase regulatory subunit 1B
		NUSAP1 NUSAP1 nucleolar and spindle associated protein 1
		MYBL2 MYBL2 v-myb myeloblastosis viral oncogene homolog (avian)-like 2
		AURKA AURKA aurora kinase A
		KPNB1 KPNB1 karyopherin (importin) beta 1
		SETD8 SETD8 SET domain containing (lysine methyltransferase) 8
		RACGAP1 RACGAP1 Rac GTPase activating protein 1
		CC45 CC45
		CASC5 CASC5 cancer susceptibility candidate 5
		CASP8AP2 CASP8AP2 CASP8 associated protein 2
		CNE CNE cyclin F
		H2AFX H2AFX H2A histone family, member X
		RAD54L RAD54L RAD54-like (S. cerevisiae)
		TMP0 TMP0 thymopoietin
		STIL STIL SCL/TAL1 interrupting locus
		DMD DMD dystrophin (muscular dystrophy, Duchenne and Becker types)



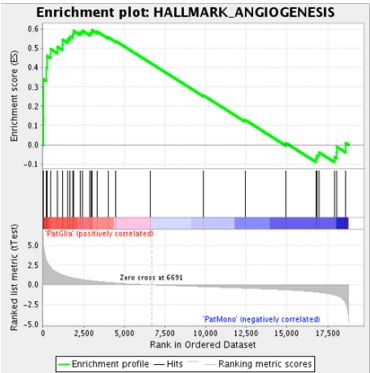
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				GPR56 GPR56 G protein-coupled receptor 56
				NRP1 NRP1 neuropilin 1
				NRP2 NRP2 neuropilin 2
				NRCAM NRCAM neuronal cell adhesion molecule
				VLDLR VLDLR very low density lipoprotein receptor
				HEY1 HEY1 hairy/enhancer-of-split related with YRPW motif 1
				CDK6 CDK6 cyclin-dependent kinase 6
				GLI1 GLI1 glioma-associated oncogene homolog 1 (zinc finger protein)
				VEGFA
				NF1 NF1 neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)
				SHH SHH sonic hedgehog homolog (Drosophila)
				CELSR1 CELSR1 cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, Drosophila)
				CRMP1 CRMP1 collapsin response mediator protein 1



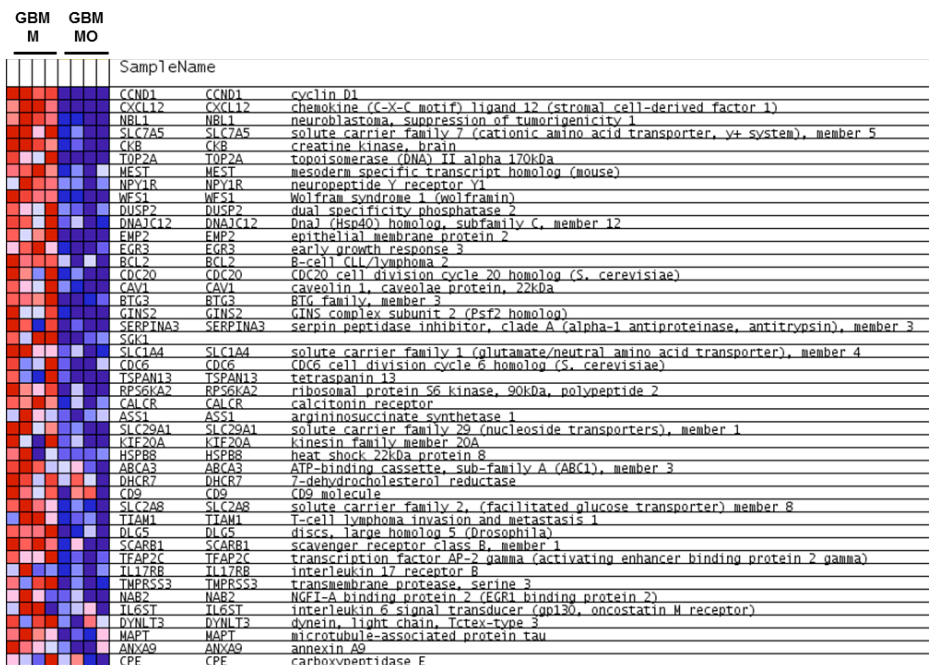
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M

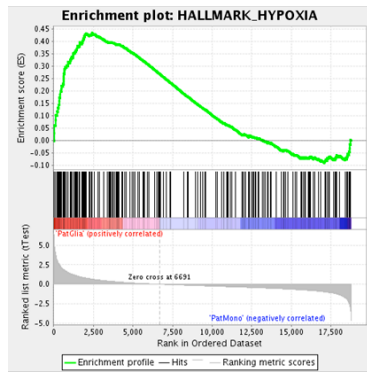
GBM
MO

SampleName		
PHEPA1		
SHAD6	SHAD6	SHAD, mothers against DPP homolog 6 (Drosophila)
SERPINE1	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
TJP1	TJP1	tight junction protein 1 (zona occludens 1)
BCAR3	BCAR3	breast cancer anti-estrogen resistance 3
ENG	ENG	endoglin (Osler-Rendu-Weber syndrome 1)
SKIL	SKIL	SKI-like
SHAD7	SHAD7	SHAD, mothers against DPP homolog 7 (Drosophila)
IO3	IO3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
LTBP2	LTBP2	latent transforming growth factor beta binding protein 2
BMPR2	BMPR2	bone morphogenetic protein receptor, type II (serine/threonine kinase)
WMTR1	WMTR1	WW domain containing transcription regulator 1
IO1	IO1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein
TGFBRI	TGFBRI	transforming growth factor, beta receptor 1 (activin A receptor type II-like kinase, 53kDa)
CDKN1C	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)
SKI	SKI	v-ski sarcoma viral oncogene homolog (avian)
JUNB	JUNB	jun B proto-oncogene

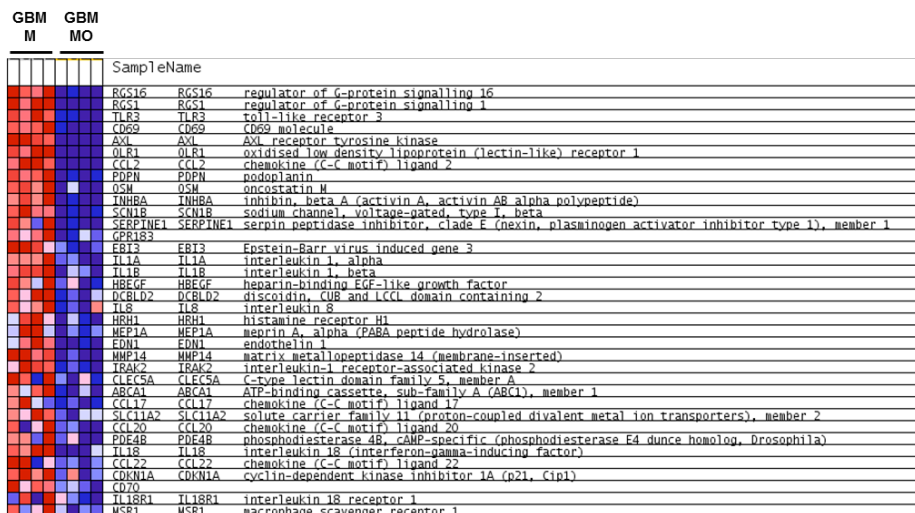


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				OLR1	OLR1	oxidised low density lipoprotein (lectin-like) receptor 1
				NRP1	NRP1	neuropilin 1
				TNFRSF21	TNFRSF21	tumor necrosis factor receptor superfamily, member 21
				JAG2	JAG2	jagged 2
				ITGAV	ITGAV	integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
				PSIL1	PSIL1	folistatin-like 1
				COL3A1	COL3A1	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)
				FGFR1	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)
				CCND2	CCND2	cyclin D2
				CXCL6	CXCL6	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
				PTK2	PTK2	PTK2 protein tyrosine kinase 2
				VEGFA	VEGFA	platelet-derived growth factor alpha polypeptide
				PDGFA	PDGFA	platelet-derived growth factor alpha polypeptide
				THBD	THBD	thrombomodulin
				LPL	LPL	lipoprotein lipase
				KCNJ8	KCNJ8	potassium inwardly-rectifying channel, subfamily J, member 8
				PRG2	PRG2	proteoglycan 2, bone marrow (natural killer cell activator, eosinophil granule major basic protein)

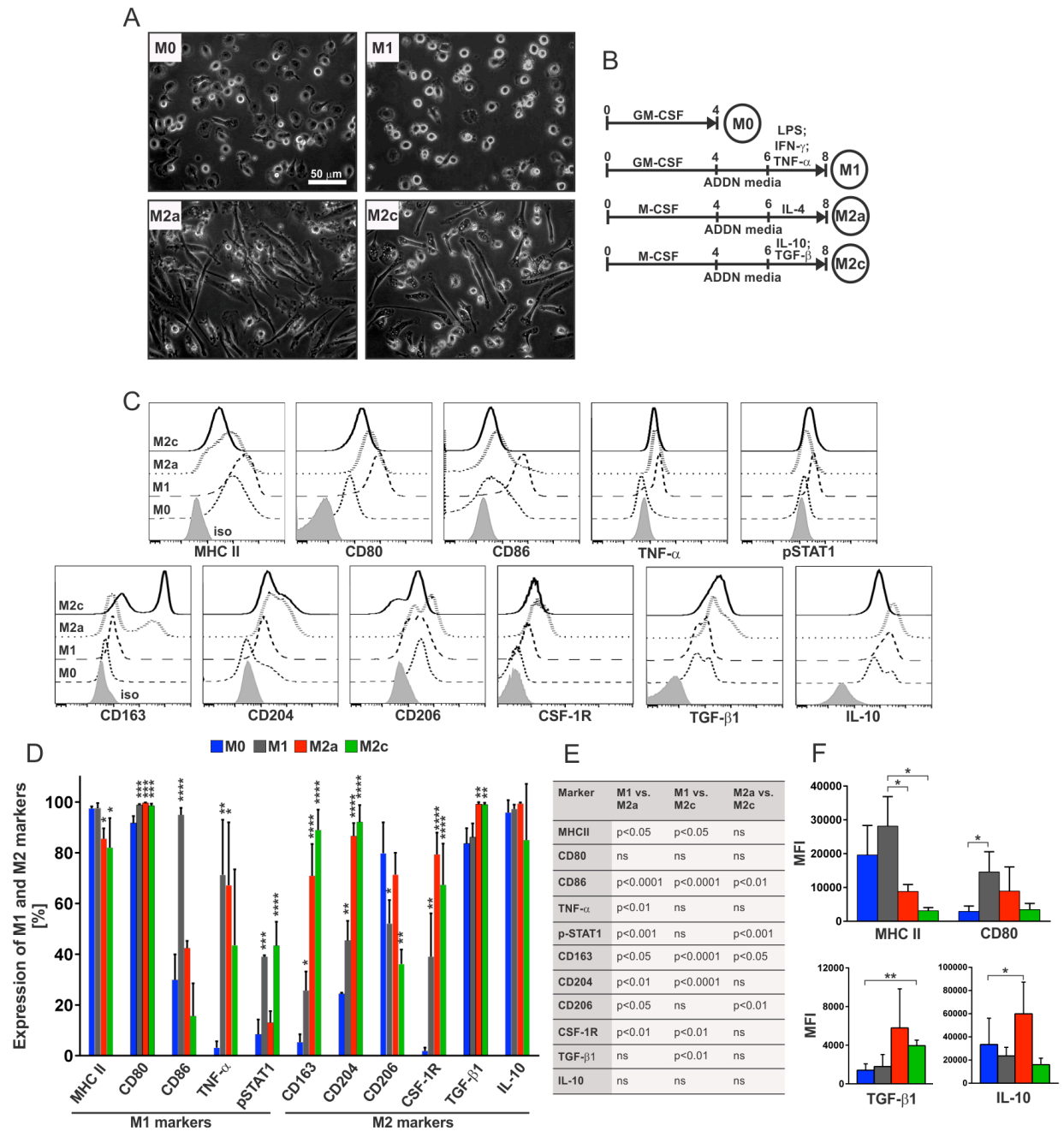




GBM M	GBM MO	SampleName
		HS3ST1 HS3ST1 heparan sulfate (glucosamine) 3-O-sulfotransferase 1
		SLC2A5 SLC2A5 solute carrier family 2 (facilitated glucose/fructose transporter), member 5
		CTGF CTGF connective tissue growth factor
		PPP1R3C PPP1R3C protein phosphatase 1, regulatory (inhibitor) subunit 3C
		SEPPIN1 SEPPIN1 serpin peptidase inhibitor, clade F (nexin, plasminogen activator inhibitor type 1), member 1
		ATF3 ATF3 activating transcription factor 3
		IGFBP3 IGFBP3 insulin-like growth factor binding protein 3
		C58P2 C58P2 cysteine and glycine-rich protein 2
		BCL2 BCL2 B-cell CLL/lymphoma 2
		CAV1 CAV1 caveolin 1, caveolae protein, 22kDa
		PDGFR PDGFR platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)
		EFNA1 EFNA1 ephrin-A1
		VLDR VLDR very low density lipoprotein receptor
		HAS1 HAS1 hyaluronan synthase 1
		ERBBF1 ERBBF1 ERBB receptor feedback inhibitor 1
		NEDD4L NEDD4L neural precursor cell expressed, developmentally down-regulated 4-like
		LUN LUN lun oncogene
		DCN DCN decorin
		SLC37A4 SLC37A4 solute carrier family 37 (glycerol-6-phosphate transporter), member 4
		GPC1 GPC1 glypican 1
		DTNA DTNA dystrobrevin, alpha
		GAPDH5 GAPDH5 glyceraldehyde-3-phosphate dehydrogenase, spermatogenic
		ANGPTL4 ANGPTL4 angiopoietin-like 4
		PFKFB3 PFKFB3 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3
		AKAP12 AKAP12 A kinase (PRKA) anchor protein (gravin) 12
		CKN1A CKN1A cyclin-dependent kinase inhibitor 1A (p21, Cip1)
		VDLR3 VDLR3 VDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3
		ATP7A ATP7A ATPase, Cu++ transporting, alpha polypeptide (Menkes syndrome)
		MTIE MTIE metallothionein 1E (functional)
		SDC3 SDC3 syndecan 3 (N-syndecan)
		ROSA ROSA ROSA-related orphan receptor A
		CXCR7 CXCR7 chemokine (C-X-C motif) receptor 7
		SLC2A3 SLC2A3 solute carrier family 2 (facilitated glucose transporter), member 3
		SCARB1 SCARB1 scavenger receptor class B, member 1
		PHKG1 PHKG1 phosphorylase kinase, gamma 1 (muscle)
		ENO3 ENO3 enolase 3 (beta, muscle)
		PRKCA PRKCA protein kinase C, alpha
		SDC2 SDC2 syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)
		TPD52 TPD52 tumor protein 52
		CKN1C CKN1C cyclin-dependent kinase inhibitor 1C (p57, Kip2)
		ETS1 ETS1 v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)
		TER3 TER3 immediate early response 3
		ZFP36 ZFP36 zinc finger protein 36, C3H type, homolog (mouse)
		GPC4 GPC4 glypican 4
		DHLHE40 DHLHE40
		VEGFA VEGFA
		PTRF PTRF polymerase I and transcript release factor
		LARGE LARGE like-glycosyltransferase
		DDIT4 DDIT4 DNA-damage-inducible transcript 4
		SELENBP1 SELENBP1 selenium binding protein 1
		ADW ADW adrenomedullin
		ILVBL ILVBL ilvB (bacterial acetolactate synthase)-like
		HK2 HK2 hexokinase 2
		EGFR EGFR epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
		SLC2A1 SLC2A1 solute carrier family 2 (facilitated glucose transporter), member 1
		PKLR PKLR pyruvate kinase, liver and RBC
		MAFF MAFF v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)



Supplemental Figure 8



Supplemental Figure 8. In vitro model of monocyte-to-macrophage differentiation and polarization toward the M1, M2a, and M2c phenotypes. **(A)** Morphology of non-polarized M0 and M1-, M2a-, and M2c-polarized macrophages. Scale = 50 μ m. **(B)** Scheme of macrophage polarization in vitro. **(C)** Representative histogram overlay for MHC class II, CD80, CD86, TNF- α , p-STAT1, CD163, CD204,

CD206, CSF-1R, TGF- β 1, and IL-10 expression in M0, M1, M2a, and M2c macrophages relative to isotype control (gray histogram). **(D)** Expression of M1 and M2 markers in M0, M1, M2a, and M2c macrophages is shown. The data were derived from three independent experiments and are presented as the mean \pm SD. A linear mixed-effects model was used to calculate *P* values (M1, M2a, and M2c vs. M0). **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. **(E)** Results of statistical analysis of each marker. ns, not significant. A random effects model was used to calculate *P* values. **(F)** MFI of MHC class II, CD80, TGF- β 1, and IL-10 expression is shown. The data are presented as the mean \pm SD. A two-sided paired *t*-test was used to calculate *P* values. **P* < 0.05; ***P* < 0.01.