Supplementary Table 1 List of Markers that distinguish the MES-subtype (positive/negative expression), their general function, and significance in GBM

Biomarkers	Subtype	General Function*	Significance in GBM**	References
CD44	MES-marker (upregulated)	A cell-surface and transmembrane glycoprotein	Regulate self-renewal, motility, tumorigenecity. Increased expression is associated with poor survival, radio-resistance and PMT. According to Klank et al, better survival observed with higher CD44 expression levels in MES patients	(Bhat et al., 2013; Phillips et al., 2006; Xu et al., 2010)
CHI3L1/ YKL40	MES-marker (upregulated)	A secreted glycoprotein	Regulate tumor growth, adhesion, invasion and chemo-resistance. Associated with higher glioma grade and shorter survival. Its High expression is associated with worse survival in PN-subtype	(Ku et al., 2011; Phillips et al., 2006; Verhaak et al., 2010)
TGFB1	MES-marker (upregulated)	A cytokine, could be secreted or intracellular stored in the cells	Mediator of tumor invasion, immunosuppression, tumor growth and maintenance of GSCs. High expression is associated with better survival in MES-subtype.	(Bhat et al., 2013; Han et al., 2015; Ikushima et al., 2009; Verhaak et al., 2010; Wesolowska et al., 2008)
MLK4 (mixed lineage kinase 4)	MES-marker (upregulated)	A serine/threonine kinase (Intracellular protein kinase)	Regulates self-renewal, motility, tumorigenesis through activating the DNA binding activity of *NF-kB* that increase radioresistance and PMT in GSCs, and is associated with worse prognosis in MES subtype patients only	(Kim et al., 2016)
S100A4	MES-marker (upregulated)	A small calcium binding protein, localized in the cytoplasm and/or nucleus	Control stemness, tumorigenecity and EMT by acting as upstream regulator of MES transcription factors. High expression tend to have worse survival in MES-subtype	(Behnan et al., 2017; Bhat et al., 2013; Chow et al., 2017; Verhaak et al., 2010)

VIM	MES-marker (upregulated)	Intermediate filaments (cytoplasmic and membranous protein)	Expressed in 84 % of high-grade and 47 % of low-grade glioma, is associated with tumor grade and overall survival	(Lin et al., 2016; Phillips et al., 2006)
MET (proto-Oncogene)	MES-marker (upregulated)	Receptor tyrosine kinase (cytoplasmic and membranous protein)	tyrosine kinase genes altered in 4% of GBM. sustain GSC growth and tumorigenic poetnatial, and increase radio-resistance. High expression have worse survival in PN-subtype	(Bhat et al., 2013; Boccaccio and Comoglio, 2013)
TNF-α receptors **(TNFRSF1A/B)**	MES-marker (upregulated)	A membrane receptor that binds TNF-α and can activate NF-kβ	TNF-α treatment increase CD44 expression, induce PMT, and make GSC more radio-resistant. High expression has worse survival in PN-subtype	(Bhat et al., 2013)
S100A6	MES-marker (upregulated)	A small calcium binding protein (intracellular cytoplasmic and nuclear)	High expression is associated with worse survival in astrocytoma and oligodendroglioma	(Bhat et al., 2013b; Behnan et al., 2017a)
ALDH1A3	MES-marker (upregulated)	Intracellular enzyme (nucleus and cytosol)	Upregulated in GSC-MES. Patient samples that express high level of ALDH1A3 are mainly GBM (72%) and grade III (28%), very low/negative in lower grades. High expression is associated with	(Mao et al., 2013) https://gbm-biodp.nci.nih.gov/
STAT3	MES-marker (upregulated)	master transcription factors (TFs) (protein has cytoplasmic and nuclear expression)	STAT3 alone induce astrocyte differentiation, inhibit neuronal differentiation, together with CEBPB promote MES-differentiation, invasion and tumorigenecity. High expression is associated with worse survival in PN, and double positive tumors STAT3+CEBPB+ is negative predictive marker in general.	(Bhat et al., 2011; Carro et al., 2010; Phillips et al., 2006)

C/EBPb (CEBPB)	MES-marker (upregulated)	Master transcription factors (nuclear expression)	CEBPB alone promotes neurogenesis and opposes gliogenesis, but combine with STAT3 induce MES-differentiation, regulate invasion and tumorigenecity. High expression is associated with worse survival in PN, and double positive tumors STAT3+CEBPB+ is negative predictive marker in general.	(Bhat et al., 2011; Carro et al., 2010; Verhaak et al., 2010)
COL1A1/2	MES-marker (upregulated)	Collagen type I (fibrillar forming collagen)	Among the highest expressed genes in MES subtype by Verhaak et al, Behnan et al, and Wang et al. Act as scaffold providing adhesion cite, promote cell invasion. Correlated with tumor progression and over all patient survival. High expression have better survival in MES-subtype	(Behnan et al., 2017; Pencheva et al., 2017; Verhaak et al., 2010; Wang et al., 2017)
COL5A2	MES-marker (upregulated)	Collagen type V (is a member of Collagen type I group, a fibrillar forming collagen)	Correlated with tumor progression and over all patient survival.	(Bhat et al., 2013b; Pencheva et al., 2017)
COL8A1	MES-marker (upregulated)	A secreted collagen	Correlated with tumor progression and over all patient survival.	(Behnan et al., 2017a; Pencheva et al., 2017)

IL13Rα2 (IL13RA2)	MES-marker (upregulated)	A cell-surface receptor	Expressed by both GSCs and differentiated tumor cells, not normal brain tissue. Its expression is associated with glioma malignancy grade, highest in GBM (58% of GBM patients), positively correlated with ~80% of MES-gene signature in Verhaak and Phillipe's classification, and negatively correlated with 72-93% of PN-genes. It is among the top 1%gene probes that impact GBM patient survival, and is used as target in immunotherapy.	(Brown et al., 2013; Brown et al., 2016; Brown et al., 2018)
RUNX1	MES-marker (upregulated)	A transcription factor (intracellular nuclear protein)	Has role in GBM migration, invasion and angiogenesis. High expression is associated with worse survival in PN	(Phillips et al., 2006; Wang et al., 2017b)
ZBTB18	MES-marker (downregulated)	A transcription factor (Zinc Finger, intracellular protein)	Is downregulated or lost in mouse gliomas and human GBM cell lines, Its loss is associated with poor prognosis and an aggressive tumor phenotype	(Fedele et al., 2017)
NF1	MES-marker (downregulated)	Neurofibromin 1 (negative regulator of RAS pathway, intracellular and membranous)	Its loss induce MES-signature and is used as tumor suppressor target that activate gliomagensis in mouse model. High expression tend to have better survival in PN	(Alcantara Llaguno et al., 2009; Verhaak et al., 2010)
TAZ	MES-marker (Upregulated)	A transcriptional coactivator of HIPPO pathway (intracellular and membrane protein)	Associated with glioma grade, higher expression in short survival GBM-patients, cooperates with PDGF-B to induce MES gliomas in murine model and modulate MES-transition	(Bhat et al., 2011)
CD15	PN-marker (Almost absent in MES)	Cell surface antigene, a carbohydrate molecule expressed by glycoproteins	A GSC marker, high expression is associated with worse survival in GBM	(Bhat et al., 2011)
CD133/PROM1	PN-marker (almost absent in MES)	A cell-surface receptor (membrane and cytoplasmic)	A GSC marker, play role in radio- and chemo-resistance, high expression is associated with worse survival in PN	(Bhat et al., 2013b; Phillips et al., 2006)

OLIG2	PN-marker (almost absent in MES)	A transcription factor (intracellular nuclear protein)	Is expressed in 100%of diffuse glioma irrespective of malignancy grade, GSC marker, is required for inducing glioma in transgenic animal model, and reprogramming cells into GSCs.	(Bhat et al., 2013b; Phillips et al., 2006; Verhaak et al., 2010)
NCAM1	PN-marker (almost absent in MES)	A cell adhesion immunoglobulin protein (membrane and intracellular)	A neural cell adhesion molecule, expressed in two-thirds of GBM patients, and negative predictor for GBM survival	(Bhat et al., 2013b; Phillips et al., 2006; Verhaak et al., 2010)
DLL3	PN-marker (almost absent in MES)	A delta protein ligand family that function as NOTCH ligand (Predicted membrane and secreted protein)	Among top 20-genes expressed in GSCs, not in NSCs, interconnected with EZH2 and KIF18A via NRF1. Inhibitor of NOTCH pathway and downstream target of ASCL1	(Bhat et al., 2013b; Phillips et al., 2006; Verhaak et al., 2010; (Park et al., 2017; Stangeland et al., 2015)
ASCL1	PN-marker (almost absent in MES)	A member of the basic helix-loop-helix (BHLH) family of transcription factors (cytoplasmic)	A chromatic modifier and activator of genes that drive neuronal fate, a downstream inhibitor of NOTCH pathway, has high expression in 54% of GSCs cultures, high expression is associated with better survival. A PN-marker that alone induce neuronl differentiation of GSCs, its overexpression reduce tumorigenic potential	(Bhat et al., 2013b; Phillips et al., 2006; Verhaak et al., 2010; (Park et al., 2017)
SOX2	PN-marker (low in MES)	A transcription factor (intracellular nuclear protein)	Expressed in 90% of GBM samples, its expression is essential for maintaing undifferentiated GSCs, has role in migration and invasion, and required for reprogramming cells into GSCs marker	(Suva et al., 2014; Verhaak et al., 2010)
ERBB3	PN-marker (low in MES)	A receptor tyrosin kinase, a member of EGFR family (membrane and cytoplasmic)	Activated upon targeting EGFR signalling with cetuximab, it maintain GSCs and glioma growth upon deprivation of EGF	(Behnan et al., 2017a; Bhat et al., 2013b; (Clark et al., 2012); Verhaak et al., 2010; Wang et al., 2017b)
SOX10	PN-marker (low in MES)	A transcription factor (intracellular nuclear protein and nucleocytoplasmic shuttle protein)	Expressed in all glioma grade, together with PDGFB induce gliomagenesis, it suppress astrocytic fate and induce oligodendrocyte differentiation. High expression tend to have worse survival in CL and PN	(Behnan et al., 2017a; Ferletta et al., 2007; Verhaak et al., 2010; Wang et al., 2017b)

*Collected from Gene card and The Human Protein Atlas

** the impact on survival of different subtypes was checked in https://gbm-biodp.nci.nih.gov/