Table S1. Markers used for gliosphere characterization by RT-qPCR, flow cytometry or immunocytochemistry

Protein or antigen	Other or complete name	Main function	Localization	Role in GBM	Associated with	Technique used	References
A2B5		Ganglioside epitope	Membrane	Associated with clonogenicity, growth, aggressivity, migration and invasion.	GSC	FC	[34, 47, 48]
ALDH1A3	Aldehyde Dehydrogenase 1 Family Member A3	Enzyme involved in aldehydes detoxification	Cytoplasm	Involved in mesenchymal GSC growth.	MES GSC	RT-qPCR	[8]
Bmi-1	Polycomb complex protein BMI-1	Enzyme involved in gene repression	Cytoplasm-Nucleus	Stem cell self-renewal. Growth and survival in stress condition: therapeutic resistance.	MES GSC	RT-qPCR	[49, 50]
CD133	Prominin-1 (*PROM1*)	Receptor	Membrane	Therapeutic resistance. GSC maintenance.	PN GSC	RT-qPCR/FC	[11, 51-53]
CD36	Platelet glycoprotein 4	Scavenger receptor	Membrane	GSC maintenance and metabolism.	PN GSC	FC	[54]
CD44	Homing cell adhesion molecule	Receptor	Membrane	Adhesion, migration and invasion via extracellular matrix recognition. GSC promotion.	MES GSC	RT-qPCR/FC	[11, 38, 51]
CD74	HLA class II histocompatibility antigen gamma chain	Receptor	Membrane	MIF and DDT receptor. Expressed in GBM. GSC maintenance.	GBM/GSC	RT-qPCR/FC	[45, 55]
CD90	Thymocyte differentiation antigen 1 (Thy-1)	Cell surface immunoglobulin	Membrane	Expressed in GBM. Macrophages interaction. Expressed in GSC but not necessary for stemness.	GSC	FC	[26, 27]
E-Cadherin	*CDH1*	Cell adhesion molecule	Membrane	Higher expression in GBM.	EMT	RT-qPCR	[56]
N-Cadherin	*CDH2*	Cell adhesion molecule	Membrane	Higher expression in GBM. Prognosis factor. Associated with resistance.	EMT	RT-qPCR	[56, 57]
CXCL12	Stromal cell-derived factor 1 (SDF-1)	Cytokine	Cytoplasm-secreted	Associated to CXCR4/7.	GSC	RT-qPCR	[58]
CXCR2	Interleukin 8 receptor, beta	Receptor	Membrane	MIF receptor, IL-8 receptor (interaction with endothelial cells) involved in CSC growth and maintenance.	GBM/GSC	RT-qPCR	[55, 59]
CXCR4	CD184	Receptor	Membrane	MIF & CXCL12 receptor. Migration, invasion and therapeutic resistance. GSC maintenance.	GSC	RT-qPCR/FC	[58, 60, 61]
CXCR7	Atypical chemokine receptor 3 (ACKR3)	Receptor	Membrane	Co receptor for CXCL12/MIF. Apoptosis escape. Associated with differentiated cells.	GSC	RT-qPCR	[62-64]
EZH2	Enhancer of zest homolog 2	Enzyme involved in gene repression	Cytoplasm-Nucleus	Stem cell self-renewal. Growth and survival in stress condition: therapeutic resistance.	PN GSC	RT-qPCR	[49, 50]
Fibronectin 1	*FN1*	ECM (cell adhesion)	Cytoplasm-secreted	Mesenchymal cell marker. Adhesion, invasion, migration.	EMT	RT-qPCR	[65]
GFAP	Glial fibrillary acidic protein	Cytoskeleton	Cytoplasm	Glial (astrocytic) differentiation marker also associated with neural stem cells.	GBM	ICC	[14, 31, 66]
Integrin α5	CD49e	Adhesion molecule	Cytoplasm	Associated with EMT (and resistance) in other cancers. Invasion in GBM.	EMT	RT-qPCR/FC	[67]
Integrin α6	CD49f	Adhesion molecule	Cytoplasm	GSC self-renewal and proliferation. Potential GSC target.	GSC	RT-qPCR/FC	[35]
L1CAM	L1 cell adhesion molecule	Adhesion molecule	Membrane	NSC maintenance. Potential GSC target. Associated with resistance and invasion.	GSC	RT-qPCR	[65, 68]
MIF	Macrophage migration inhibitory	Cytokine	Cytoplasm-secreted	Proliferation, migration, apoptosis escape, GSC maintenance. Recognized by CXCR4/7 CD74 and CD44 heteromers.	GSC and EMT	RT-qPCR	[45, 69]
Nanog	Homeobox protein NANOG (hNanog)	Transcription factor	Cytoplasm-Nucleus	Pluripotency maintenance.	GSC	RT-qPCR	[70]
Nestin	Neuroepithelial stem cell protein	Cytoskeleton	Cytoplasm	Neural progenitor marker. Expressed in GBM and GSC.	GSC	RT-qPCR/ICC	[14, 52, 53]

Oct3/4	POU domain, class 5, transcription factor 1 (*POU5F1*)	Transcription factor	Cytoplasm-Nucleus	Pluripotency maintenance.	GSC	RT-qPCR	[71, 72]
Olig2	Oligodendrocyte transcription factor	Transcription factor	Cytoplasm-Nucleus	Glial progenitor proliferation. Gliomagenesis. GSC maintenance.	PN GSC	RT-qPCR/ICC	[51, 73]
S100A4	Fibroblast-specific protein 1 (FSP1)	Intracellular protein	Cytoplasm-Nucleus	Expressed in GSC, associated with EMT. Potential target. Cell cycle and differentiation regulator.	EMT	RT-qPCR	[74]
Snail2	*SLUG*	Transcription factor	Cytoplasm-Nucleus	Expressed in GSC, associated with EMT. Antiapoptotic activity.	EMT	RT-qPCR	[75]
Sox2	SRY (sex determining region Y)-box 2	Transcription factor	Cytoplasm-Nucleus	Pluripotency maintenance. GSC stemness. Associated to EMT.	GSC	RT-qPCR	[8, 76]
Osteopontin	*SPP1*	ECM (cell adhesion)	Cytoplasm-secreted	Binds CD44. GSC promotion. Proliferation, invasion angiogenesis.	GSC	RT-qPCR	[39, 77]
SSEA-1	Stage-specific embryonic antigen 1 or CD15	Cell surface antigen	Membrane	Associated with stem cells. Involved in cell-cell recognition.	GSC	RT-qPCR/FC	[49, 78]
VEGF-A	Vascular endothelial growth factor A	Growth factor	Cytoplasm-secreted	Angiogenesis. Upregulation of CXCR4 and CXCL12. Upregulated by CXCR pathways.	GBM	RT-qPCR	[63, 79]
VEGF-C	Vascular endothelial growth factor C	Growth factor	Cytoplasm-secreted	Angiogenesis. Upregulation of CXCR4 and CXCL12. Upregulated by CXCR pathways.	GBM	RT-qPCR	[63, 79]
ZEB1	Zinc finger E-box-binding homeobox 1	Transcription factor	Cytoplasm-Nucleus	Increased expression in GBM. Induces N-Cadherin expression, E-Cadherin repression.	EMT	RT-qPCR	[56]

Abbreviation: CSC, Cancer Stem Cells; GBM, Glioblastoma; GSC, Gliobastoma Stem Cell; ECM, Extracellular matrix; EMT, Epithelial-mesenchymal transition; FC, Flow cytometry; ICC, Immunocytochemistry; PN, Proneural; RT-qPCR, Reverse Transcription-quantitative Polymerase Chain Reaction.

Table S2. Antibodies used for flow cytometry analysis

Target	Fluorochrome	Species	Isotype	Reference
CXCR4	APC	Mouse	IgG2ak	130-123-814
–	APC	Mouse	IgG2a	130-113-269
CD44	FITC	Mouse	IgG1k	130-113-334
–	FITC	Mouse	IgG1	130-113-199
CD133	PE	Recombinant human	IgG1	130-110-962
CD49e (ITGA5)	PE	Recombinant human	IgG1	130-110-590
CD49f (ITGA6)	PE	Recombinant human	IgG1	130-119-807
–	PE	Recombinant human	IgG1	130-113-438
CD74	APC-Vio770	Mouse	IgG1k	130-101-533
–	APC-Vio770	Mouse	IgG1k	130-113-759
A2B5	PE	Mouse	IgM	130-123-953
–	PE	Mouse	IgM	130-120-156
CD90	FITC	Recombinant human	IgG1	130-114-859
–	FITC	Recombinant human	IgG1	130-113-449
CD36	Viogreen	Recombinant human	IgG1	130-110-883
–	Viogreen	Recombinant human	IgG1	130-113-456
CD15	Vioblue	Mouse	IgM	130-114-014
–	Vioblue	Mouse	IgM	130-098-589

All antibodies were purchased from Miltenyi.

Table S3. Antibodies used for immunocytochemistry and corresponding dilutions

Target	Species	Isotype	Supplier	Reference	Dilution
GFAP	Rabbit	IgG	Sigma	G4546	1:200
Nestin	Rabbit	IgG	Sigma	N5413	1:200
Olig2	Rabbit	IgG	Diagomics	BSB2562	1:150

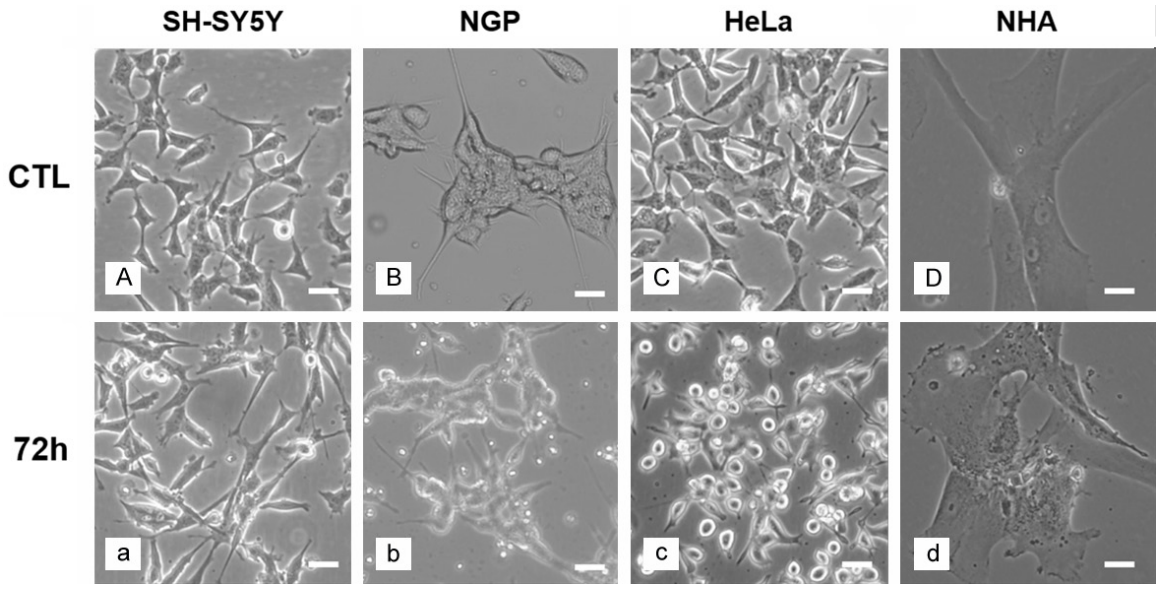


Figure S1. Non-glioblastoma cell lines do not form spheres in dedifferentiation medium. Microscope observations of SH-SY5Y (A, a), NGP (B, b), HeLa (C, c) and NHA (D, d) cells. Capital letters are used for cells in classical medium (CTL) and lowercase letter for cells incubated 72 h in dedifferentiated medium (72 h). Scale is 30 µm.

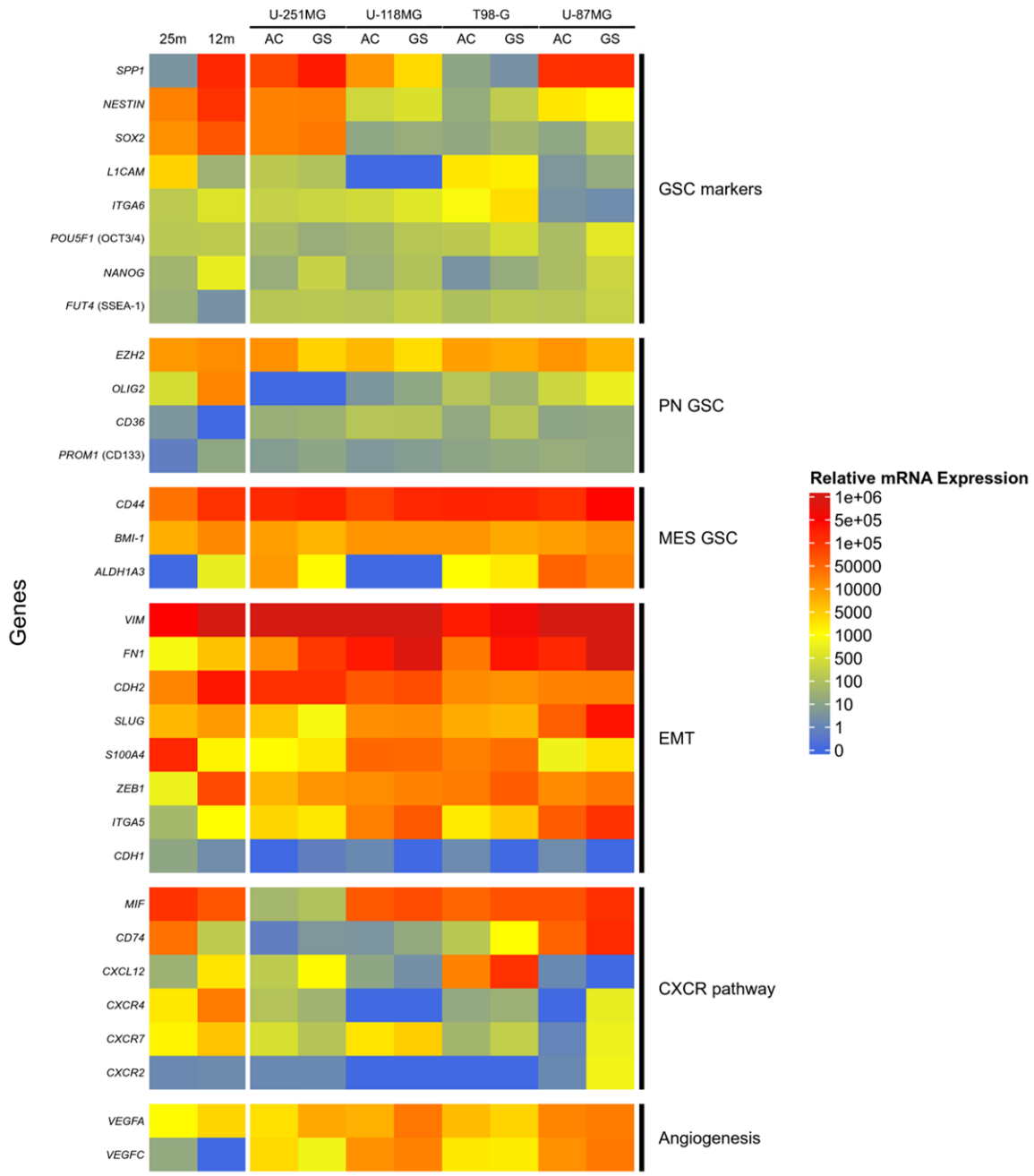
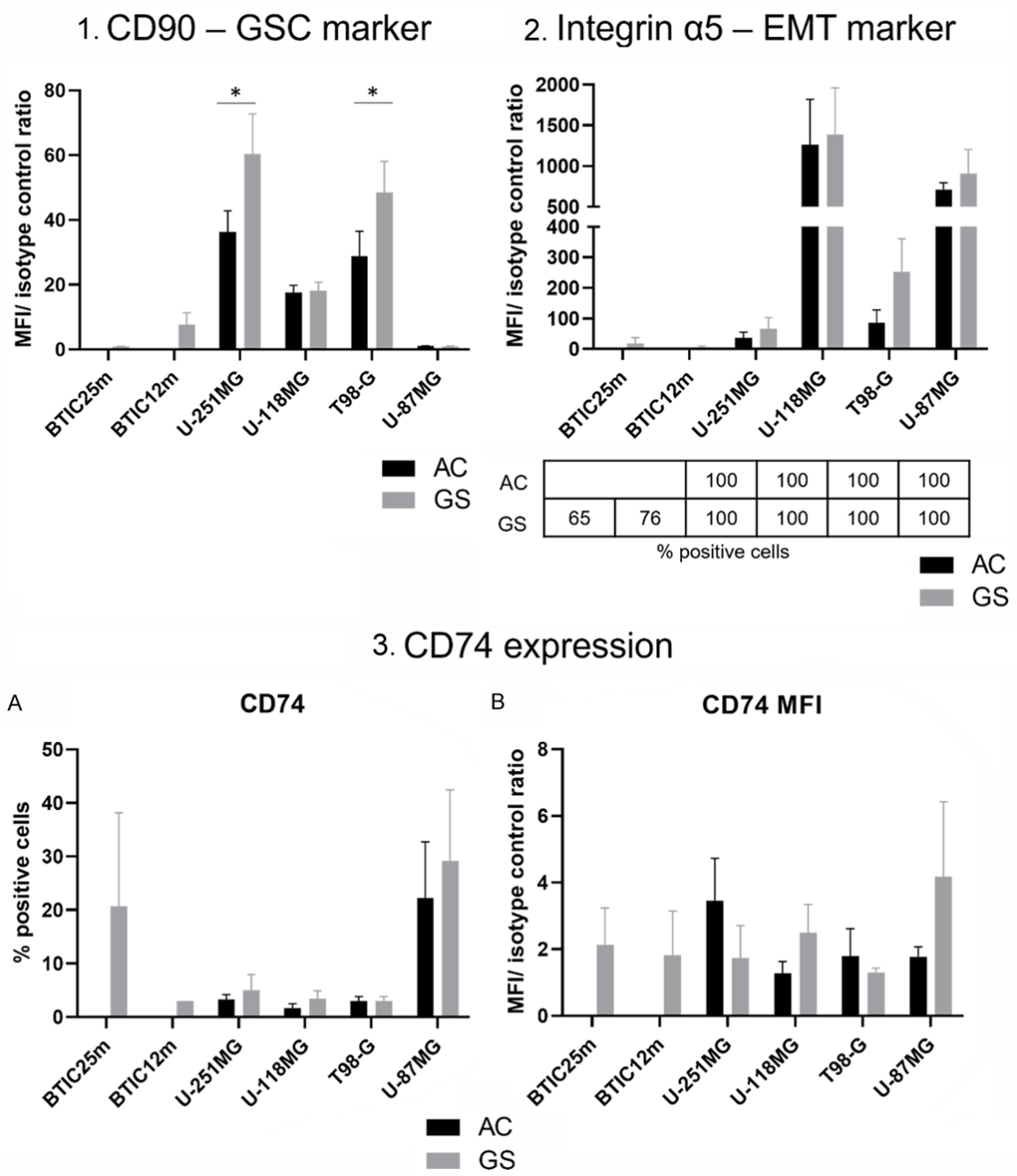


Figure S2. Transcriptional expression supports a mesenchymal GSC profile in gliospheres and dedifferentiation is associated with higher expression of stem-cell, EMT and CXCR associated genes. RT-qPCR analysis of gene expression in U-251MG, U-118MG, T98-G and U-87MG adherent cells (AC) and dedifferentiated gliospheres (GS) and in BTIC 25m and 12m. Relative mRNA expression was calculated and normalized according to Vandesompele method with two control genes (GAPDH and HPRT1) and an internal calibrator. Expression levels are colored from blue to red with increasing values. Genes are sorted by mean expression level in each group. PN: Proneural, MES: Mesenchymal, EMT: Epithelial-Mesenchymal Transition.



AC			100	100	100	100
GS	65	76	100	100	100	100

Figure S3. Change in membrane markers expression supports acquisition of GSC with a mainly mesenchymal phenotype in dedifferentiated gliospheres models. Flow cytometry analysis of CD90 GSC marker, integrin α5 EMT marker and CD74 in U-87MG, U-118MG, U-251MG, T98-G and BTIC 25m and 12m. Adherent cells (AC) were used as control for dedifferentiated gliospheres (GS). CD90 staining results are shown as mean fluorescence ratio (1), similarly to integrin α5 with corresponding positive cells percentage in the table below (2). Percentage of positive cells are represented for CD74 with corresponding mean fluorescence ratios (A and B). Mean fluorescence ratio is calculated as mean fluorescence intensity (MFI) of stained sample on corresponding isotype control MFI. Statistical significance was calculated using Mann-Whitney test on n=4 independent experiments. *p-value <0.05.

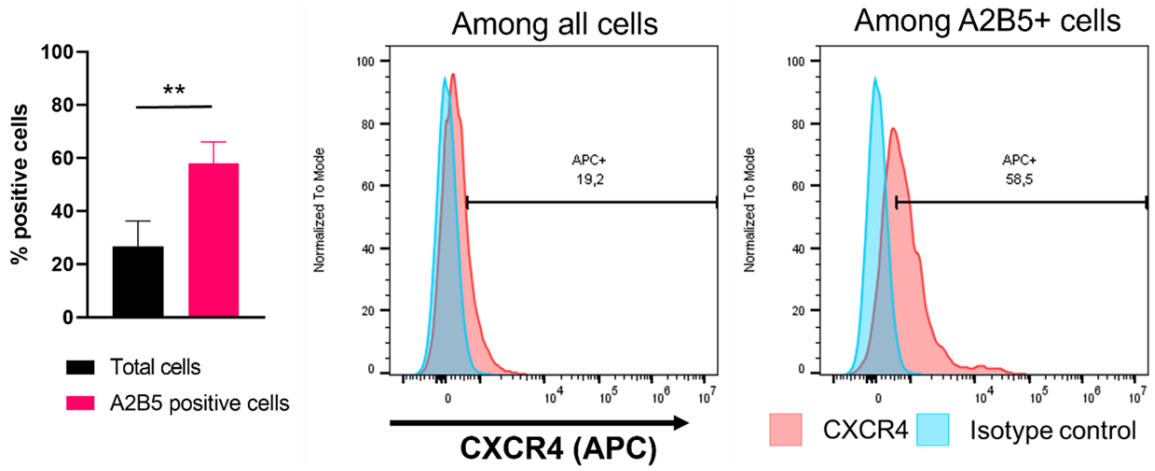


Figure S4. CXCR4 expression is higher in A2B5 positive population in U-118MG dedifferentiated gliospheres. Flow cytometry analysis of CXCR4 staining in U-118MG double stained for A2B5 marker. Percentage of positive cells are represented for n=5 independent experiments (left). Statistical significance was calculated using Mann-Whitney test (P=0.0079). Example of a corresponding flow cytometry histogram (right).