

**Supplemental information**

**Integrative spatial analysis reveals  
a multi-layered organization of glioblastoma**

**Alissa C. Greenwald, Noam Galili Darnell, Rouven Hoefflin, Dor Simkin, Christopher W. Mount, L. Nicolas Gonzalez Castro, Yotam Harnik, Sydney Dumont, Dana Hirsch, Masashi Nomura, Tom Talpir, Merav Kedmi, Inna Goliand, Gioele Medici, Julie Laffy, Baoguo Li, Vamsi Mangena, Hadas Keren-Shaul, Michael Weller, Yoseph Addadi, Marian C. Neidert, Mario L. Suvà, and Itay Tirosh**

**Table S1: Overview of patient cohort, related to Figure 1**

This cohort

Sample-ID	Patient-ID	Region	Tumor	Histology	Grade	MGMT-status	Location	Sex	Age	Visium	CODEX
MGH258	MGH258	bulk	IDH WT	GBM	4	methyalted	left frontal	F	43	1	1
ZH881_1A	ZH881	bulk	IDH WT	GBM	4	partially methylated	right temporal	M	79	1	
ZH881_1B	ZH881	bulk	IDH WT	GBM	4	partially methylated	right temporal	M	79	1	1
ZH881_2	ZH881	bulk	IDH WT	GBM	4	partially methylated	right temporal	M	79	1	
ZH881_T1	ZH881	T1	IDH WT	GBM	4	partially methylated	right temporal	M	79	1	1
ZH881_inf	ZH881	inf	IDH WT	GBM	4	partially methylated	right temporal	M	79	1	1
ZH916_bulk	ZH916	bulk	IDH WT	GBM	4	methyalted	left temporal	M	62	1	
ZH916_T1	ZH916	T1	IDH WT	GBM	4	methyalted	left temporal	M	62	1	1
ZH916_inf	ZH916	inf	IDH WT	GBM	4	methyalted	left temporal	M	62	1	1
ZH1007_nec	ZH1007	nec	IDH WT	GBM	4	unmethylated	left occipital	F	50	1	1
ZH1007_inf	ZH1007	inf	IDH WT	GBM	4	unmethylated	left occipital	F	50	1	1
ZH1007_bulk	ZH1007	bulk	IDH WT	GBM	4	unmethylated	left occipital	F	50		1
ZH1019_T1	ZH1007	T1	IDH WT	GBM	4	methyalted	left temporal	F	67	1	1
ZH1019_inf	ZH1007	inf	IDH WT	GBM	4	methyalted	left temporal	F	67	1	1
ZH1041_T1	ZH1041	T1	IDH WT	GBM	4	methyalted	left temporal	F	76		1
BWH23	BWH23	bulk	IDH-mut	ODG	4	unmethylated	left frontal	M	32	1	
BWH24	BWH24	bulk	IDH-mut	AA	4	methyalted	right parietal	F	54	1	
BWH25	BWH25	bulk	IDH-mut	AA	4	methyalted	bifrontal	F	51	1	
BWH28	BWH28	bulk	IDH-mut	AA	2/3	partially methylated	right frontotemporal	M	26	1	
MGH259	MGH259	bulk	IDH-mut	ODG	3	partially methylated	left frontal	F	40	1	
BWH35	BWH35	bulk	IDH-mut	ODG	2	methyalted	right parietal	F	36	1	
BWH911-organoid	BWH911	NA	IDH WT	GBM	4	NA	NA	M	58		1

External cohort from Ravi et al.

Sample-ID	Patient-ID	Region	Tumor	Histology	Grade	MGMT-status	Location	Sex	Age	Visium
#255UKF	#255UKF	core	IDH WT	GBM	NA	NA	temporal	f	81	1
#313UKF	#313UKF	core	IDH WT	GBM	NA	NA	temporal	m	57	1
#243UKF	#243UKF	core	IDH WT	GBM	NA	NA	frontal	f	55	1
#260UKF	#260UKF	tumor	IDH WT	GBM	NA	NA	temporal	m	81	1
#334UKF	#334UKF	tumor	IDH WT	GBM	NA	NA	frontal	f	73	1
#248UKF	#248UKF	tumor	IDH WT	GBM	NA	NA	frontal	m	44	1
#259UKF	#259UKF	tumor	IDH WT	GBM	NA	NA	temporal	f	75	1
#275UKF	#275UKF	tumor	IDH WT	GBM	NA	NA	frontal	f	80	1
#296UKF	#296UKF	tumor	IDH WT	GBM	NA	NA	temporal	m	34	1
#251UKF	#251UKF	tumor	IDH WT	GBM	NA	NA	temporal	m	61	1
#266UKF	#266UKF	tumor	IDH WT	GBM	NA	NA	temporal	f	76	1
#269UKF	#269UKF	tumor	IDH WT	GBM	NA	NA	occipital	m	51	1
#304UKF	#304UKF	tumor	IDH WT	GBM	NA	NA	temporal	m	81	1

bulk: tumor bulk, T1: T1-contrast enhancing, inf: infiltrating, NA: not annotated, GBM: Glioblastoma, AA: Astrocytoma, ODG: Oligodendroglioma

**Table S3: Overview of antibodies and experimental design, related to Figure 3**

Cycle #	Target	Reporter	Dilution (1:x)	Exposure time (ms)	Company	Cat #	RRID	Clone	Fluorochrome
Cycle 1	CD45	RX001	200	150	Akoya	4150003	AB_2895052	HI30	AF488
	CD3	RX015	400	150	Akoya	4550103	NA	UCHT1	AF647
	CD69	RX041	200	150	Akoya	4250022	AB_2909513	FN50	Atto550
Cycle 2	CD90	RX022	400	150	Akoya	4150021	AB_2895055	THY1	AF488
	CD11c	RX027	200	150	Akoya	4550107	AB_2936079	118/A5	AF647
	Ki67	RX047	200	150	Akoya	4250019	AB_2895046	B56	Atto550
Cycle 3	CD8	RX004	200	200	Akoya	4150004	AB_2895049	SK1	AF488
	CD4	RX021	200	200	Akoya	4550105	NA	SK3	AF647
	HLA-DR	RX026	265	150	Akoya	4250006	AB_2895054	EPR3692	Atto550
Cycle 4	FN1	RX034	1300	150	Abcam	ab268022	AB_2927764	EPR23110	AF488
	SOX10	RX050	200	170	Abcam	ab220078	NA	EPR4007-104	AF647
	CD31	RX032	200	200	Akoya	4250009	AB_2895051	WM59	Atto550
Cycle 5	VIM	RX025	1000	150	BioLegend	677802	AB_2565911	O91D3	AF488
	PLP1	RX006	600	150	Abcam	ab275751	AB_2915963	EPR23504-106	AF647
	PDPN	RX023	100	150	Akoya	4250004	NA	NC-08	Atto550
Cycle 6	GFAP	RX031	600	150	BioLegend	837404	AB_2650686	SMI24	AF488
	EGFR	RX030	225	150	Abcam	ab272293	AB_2909619	EP38Y	AF647
	CD279	RX014	200	200	Akoya	4250010	AB_2909510	EH12.2H7	Atto550
Cycle 7	SOX4	RX007	100	150	Abcam	ab237903	NA	SOX4/2540	AF488
	CD14	RX024	100	175	BioLegend	325602	AB_830675	HCD14	AF647
	DCX	RX020	400	150	Abcam	ab222921	NA	EPR19997	Atto550
Cycle 8	AQP4	RX010	200	150	Abcam	ab282586	AB_2923003	EPR24281-65	AF488
	OLIG2	RX045	200	150	Abcam	ab220796	AB_2923001	EPR2673	AF647
	NeuN	RX054	150	175	Abcam	ab209898	NA	EPR12763	Atto550
Cycle 9	PDGFRA	RX016	100	150	Abcam	ab234965	NA	EPR22059-720	AF488
	CA9	RX033	300	150	Abcam	ab270401	NA	EPR23055-5	AF647
	GLUT1	RX052	1400	150	Abcam	ab252403	NA	EPR3915	Atto550
Cycle 10	BCAN	RX040	800	150	Abcam	ab285169	NA	EPR25743-59	AF488
	CD44	RX042	400	150	Novus	NBP1-41266	AB_2291286	IM7	AF647
	p53	RX037	400	150	Abcam	ab225531	NA	E26	Atto550
Cycle 11	GAP43	RX019	400	150	Abcam	ab219582	NA	EP890Y	AF488
	CD163	RX043	400	150	BioLegend	333602	AB_1088991	GHI/61	AF647
	NDRG1	RX029	100	175	Abcam	ab226082	NA	EPR5593	Atto550
Cycle 12	APOD	RX028	600	150	Abcam	ab261911	NA	EPR22967-72	AF488
	SOX2	RX036	65	200	Abcam	ab215970	AB_2928047	EPR3131	AF647
Cycle 13	S100B	RX046	300	150	CellSignaling	42397	NA	E7C3A	AF647
	APOE	RX005	65	200	BioLegend	803301	AB_2564662	D6E10	Atto550
Cycle 14									
	CHI3L1	RX017	65	200	Abcam	ab255864	NA	EPR19078-157	Atto550
Cycle 15	CD19	RX003	200	150	Akoya	4550099	AB_2924255	HIB19	AF647
	MAP2	RX035	100	150	Abcam	ab288577	NA	RM1037	Atto550

**Table S4: Criteria for CODEX cluster assignment, related to Figure 3**

cell type/state	Threshold z-score
<b>Vasc</b>	CD31 > 2
<b>Mac</b>	CD45 + CD14 or CD163 or CD11c > 1.5 & MHCII > 0
<b>Inflammatory-Mac</b>	CD45 + CD14 or CD163 or CD11c > 1.5 & MHCII < 0
<b>T_cell</b>	CD3 > 2
<b>B_cell</b>	CD19 > 2
<b>Neuron</b>	NeuN > 2
<b>Oligo</b>	OLIG2 or APOD + SOX10 > 1 & SOX2 < 0
<b>OPC</b>	OLIG2 > 1 & SOX2 > 0
<b>Reactiv-Ast</b>	S100B + VIM or GFAP > 1 & EGFR < 0
<b>AC</b>	S100B + AQP4 or APOE > 1 & EGFR > 0
<b>NPC</b>	DCX + SOX4 > 1 & SOX2 > 0
<b>MES</b>	PDPN + CHI3L1 > 1
<b>MES-Hyp</b>	NDRG1 + PDPN > 1
<b>Chromatin-Reg</b>	NDRG1 + PDPN > 1 & DCX or SOX4 > 1