Supplementary data

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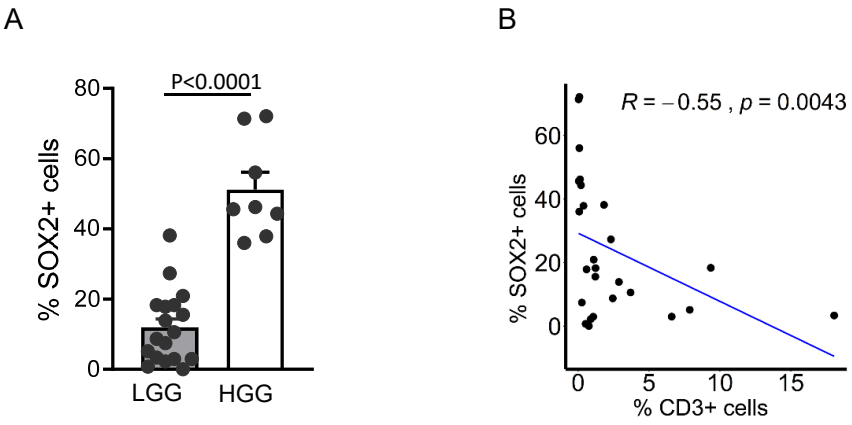
Supplementary Figure 1



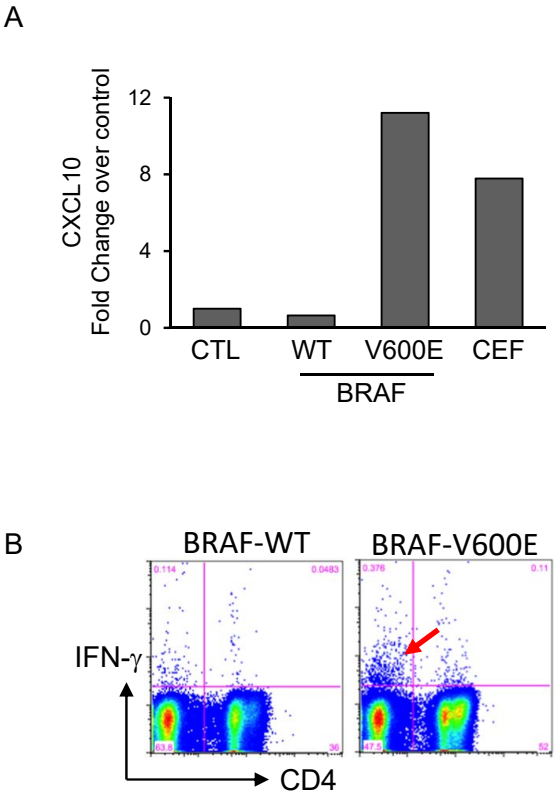
Supplementary Figure 1. Correlation between T cell infiltration and SOX2 expression.

Multiplex IHC slides were analyzed for presence of SOX2 positive tumor cells and CD3+T cells

A. Bar graph shows SOX2 positive T cells in tissue from low grade glioma (n=18, PAX, GG and PA) and high grade glioma (HGG n=8, GBM +AA).

B. Scatter plot showing negative correlation between abundance of SOX2 expressing tumor cells and CD3+ T cells.

Supplementary Figure 2



Supplementary Figure 2. Detection of $BRAF^{V600E}$ mutation specific T cells.

A. Peripheral blood mononuclear cells were cultured alone (CTL), with wild type (WT) or V600E mutant BRAF peptides. Pooled viral peptides from Cytomegalovirus, Epstein-Barr Virus, and Influenza Virus (CEF) were used as positive control. Figure shows $BRAF^{V600E}$ T cells reactivity using a CXCL10 luminex assay.

B. Expansion of IFNγ secreting $BRAF^{V600E}$ specific T cells following stimulation with peptide loaded autologous dendritic cells.

Supplementary Table 1A. Patient characteristics: samples used for multiplex IHC

	Low Grade Glioma			High Grade Glioma
Tumor Type (n)	**PXA (5)**	**GG (7)**	**PA (6)**	**HGG (7)**
Age mean in years (range)	12.4 (7-17)	10.1 (3-18)	7.5 (2-16)	11.9 (0.5-17)
Gender – Male	4 (80%)	3 (43%)	3 (50%)	5 (71%)
Tumor Location				
Parietal	3 (60%)	1 (14%)	1 (17%)	
Temporal	2 (40%)	4 (57%)		5 (71%)
Thalamus		1 (14%)	1 (17%)	2 (29%)
Brain stem			1 (17%)	
Posterior fossa/Cerebellar			3 (50%)	
Not specified		1 (14%)		
BRAF status				
BRAF V600E	4 (80%)	4 (57%)	0%	1(14%)
BRAF-KIAA1549 fusion	ND	ND	2 (33%)	ND
Other genetic alterations				
				IDHwt 6 (86%) H3F3AG34R 3 (43%) H3K27M 1 (14%) TP53 4 (57%) ATRX 3 (43%) Amplification: MYCN, MDM4, PIK3C2B, EGFR (14% each) PTEN loss 1 (14%)

ND – Not determined

Supplementary Table 1B. Patient characteristics: samples used for CyTOF and functional assays

	Low Grade Glioma			High Grade Glioma
Tumor Type (n)	**GG (2)**	**PA (5)**	**Other (2)**	**Anaplastic GG (1)**
Age mean in years (range)	7 (6-8)	8.9 (2-14)	8.5 (2-15)	18
Gender – Male	0	2 (40%)	1(50%)	0
Tumor Location				
Parietal		2 (40%)	1(50%)	
Temporal				1 (100%)
Thalamus				
Brain stem	1 (50%)			
Posterior fossa/Cerebellar	1 (50%)	3 (60%)	1(50%)	
BRAF status				
BRAF V600E	1 (50%)			1 (100%)

Supplementary Table 1C. Patient characteristics: recurrent pilocytic astrocytomas

Pt	Location	Gross Total resection at Dx	Time to recurrence (months)
1	Thalamus	No	75
2	Parietal	Yes	26
3	Brain Stem	No	34

Supplementary Table 2. Antibodies used for single cell mass cytometry

Antibody	Clone	Supplier
CD45	HI30	Fluidigm
CD11b	ICMF44	Fluidigm
CD19	HIB19	Fluidigm
HLADR	L243	Fluidigm
CD69	FN50	Fluidigm
CD4	RPA-T4	Fluidigm
CD8	RPA-T8	Fluidigm
CD14	RM052	Fluidigm
CD127	A019D5	Fluidigm
SOX2	245610	R&D Systems
CD103	Ber-ACT8	Fluidigm
CD11c	Bu15	Fluidigm
TIGIT	MBSA43	Fluidigm
TIM3	F38-2E2	Fluidigm
CD27	L128	Fluidigm
NKG2A	Z199	Beckman Coulter
CD33	WM53	Fluidigm
CCR7	G043H7	Fluidigm
CD163	GHI/61	Fluidigm
CD95	DX2	Fluidigm
CD45R0	UCHL1	Fluidigm
NKG2D	OW72	Fluidigm
CD25	2A3	Fluidigm
CD3	UCHT1	Fluidigm
PD-L2	24F.10C12	Fluidigm
CD57	HCD57	BioLegend
PD-L1	CD274	Fluidigm
PD-1	EH12.2H7	Fluidigm
CD56	HCD56	BioLegend
CD16	3G8	Fluidigm
TCF1	7F11A10	BioLegend
Tbet	4B10	Fluidigm
FOXP3	PCH101	Fluidigm
EOMES	WD1928	Ebioscience
GATA3	TWAJ	Fluidigm
Ki67	Ki-67	Fluidigm
Granzyme	GB11	Fluidigm
OX-40	ACT35	Biolegend
CD11c	Bu15	Fluidigm
CD200	OX104	Fluidigm
CXCR5	J25204	Biolegend
BTLA	MIH26	Fluidigm
CD38	HIT2	Fluidigm
4-1BB	4841	Biolegend

Supplementary Table 3. Antibodies used for multiplex IHC staining

Order	Antibody	Clone	Vendor	Antibody Dilution Factor	Incubation time (minutes)	Opal
1	SOX2	245610	R&D Systems	1:400	40	570
2	CD103	EPR4166(2)	Abcam	1:500	40	480
3	TCF1	C63D9	Cell Signaling	1:100	32	520
4	CD3	SP7	Abcam	1:100	44	620
5	CD31	PECAM-1	Cell Signaling	1:100	40	690
6	DAPI				16	