Supplemental Materials

Quantitative proteomics reveals reduction of endocytic machinery components in gliomas

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Supplementary Figures

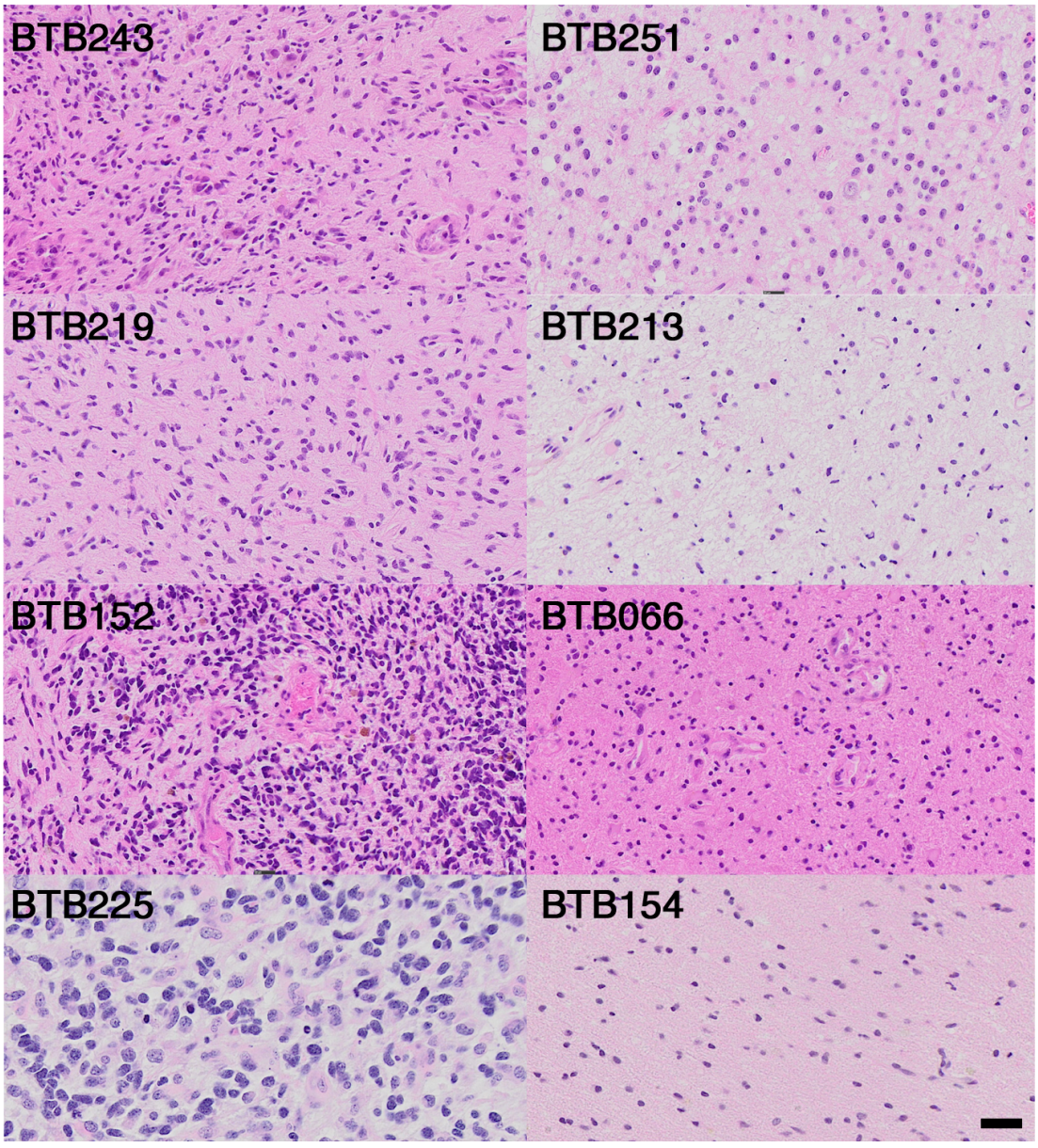

Fig. S1. Histomorphological features of glioma biopsies subjected to proteomic analysis. Hematoxylin- and eosin-stained, formalin-fixed, paraffin-embedded sections of proteomically analysed tumor biopsies. Bar: 20 µm.

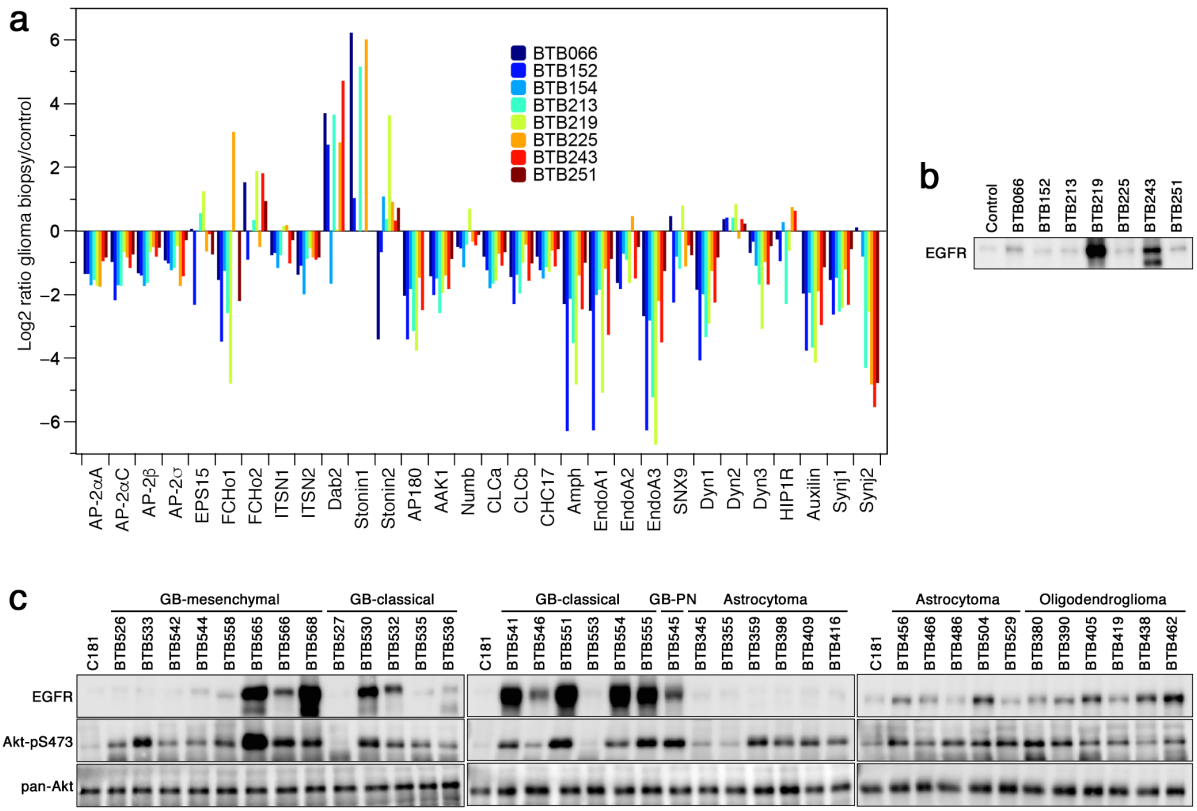

Fig. S2. Proteomic and immunoblot analysis of glioma biopsies. **(a)** Proteomic quantification of endocytic machinery components shown for each individual biopsy. Log2 ratio of the individual endocytic components compared between glioma biopsies and control (see also Fig. 2d). Full names of proteins can be found in Table S4. **(b)** Immunoblot analysis of EGFR protein levels of biopsies subjected to proteomic analysis. Of note, BTB219 and BTB243 carry *EGFR* amplification (see also Table S1). **(c)** Immunoblot analysis of EGFR protein levels of biopsies derived from different glioma subclasses, including GB (classical), GB (mesenchymal), GB (proneural (PN)), astrocytoma and oligodendroglioma (see also Fig. 3c). Disregarding any genetic background, biopsies in general demonstrated an increased steady-state activation of the PI3K/AKT downstream pathway, as judged by pAKT-S473 levels relative to pan-Akt.

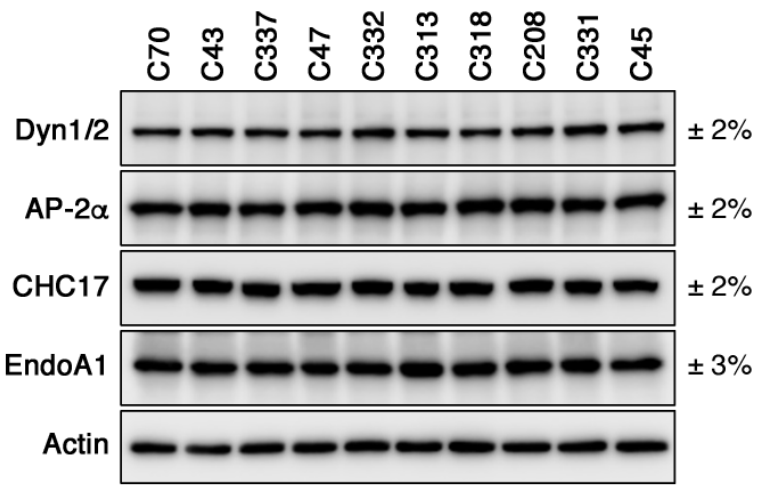

Fig. S3. Immunoblot analysis of additional control white matter samples (C). While endocytic machinery components (Dyn1/2, AP-2α, CHC17, EndoA1) among glioma subclasses were robustly and largely depleted, additional control white matter specimens showed a constant expression level throughout. White matter biopsy controls were free of brain tumor/metastasis. Percentage displays statistical variation in control specimen expression.

Supplementary Tables

Table S1. Grading and summary of the mutational status of the biopsies used in this study. Legend: WT, wild-type.

Biopsy	Grade	*EGFR*	*PDGFRA*	*IDH1/2*	*TERT*	*CKDN2A*
BTB219	III	amplification	amplification	*IDH1/2* WT	*TERT* G250A	deletion
BTB243	IV	amplification	no amplification	*IDH1/2* WT	*TERT* G228A	deletion
BTB225	IV	no amplification	amplification	*IDH1/2* WT	*TERT* G228A	deletion
BTB066	IV	no amplification	amplification	*IDH1/2* WT	*TERT* G228A	no deletion
BTB251	II	no amplification	amplification	*IDH2* R172K	*TERT* G228A	no deletion
BTB154	II	no amplification	amplification	*IDH1* R132S	*TERT* G250A	no deletion
BTB152	IV	no amplification	amplification	*IDH1* R132H	*TERT* WT	no deletion
BTB213	II	no amplification	amplification	*IDH1* R132H	*TERT* WT	no deletion

Table S2. Clinical/surgical characteristics, histopathological diagnosis and survival of the patients for biopsy sample analysis by proteomics. Legend: AA, anaplastic astrocytoma; GBM, glioblastoma; OA, oligoastrocytoma; ODG, oligodendroglioma; FU, follow-up; GTR, gross total resection; PR, partial resection; F, female; M, male; FU, follow-up.

Biopsy	Grade	Age	Sex	Histological diagnosis	Overall survival (days)	Degree of resection
BTB219	III	48	F	AA	405	GTR (awake craniotomy)
BTB243	IV	60	M	GBM	325	GTR
BTB225	IV	68	M	GBM	205	GTR
BTB066	IV	71	M	GBM	138	PR
BTB251	II	35	F	ODG	Alive, FU 6y	GTR
BTB154	II	50	F	ODG	Alive, FU 5y	GTR
BTB152	IV	27	F	GBM	Alive, FU 6y	GTR
BTB213	II	38	M	A	Alive, FU 4y	GTR (awake craniotomy)

Table S3. REVIGO proteomic cluster analysis of down- and upregulated proteins.

Cluster name	Annotated Terms	Sample association
Upregulated proteins		
Cluster I	TAP binding, HLA class-I histocompatibility	BTB 066, 213, 219, 243, 251
	β2-microglobulin binding	BTB219
	Death receptor binding (RIPK1, TLR signaling)	BTB219
	PDGFR-binding	BTB152/154
Cluster II	"protease terms" • cysteine-type endopeptidase activity • serine-type carboxypeptidase activity • caspase 1-9 (except caspase 5) • sentrin-specific protease 2, 3, 6, 7 • otulin	All samples
Cluster III	"RNA/DNA binding"	All samples
Cluster IV	"Upregulation of epigenetic events" • histone acetyltransferase • histone lysine N-methyltransferase • histone kinase	All samples
Downregulated proteins		
Cluster V	"transporter and channel activities" • glucose • hydrogen • phosphate • inorganic ion	All samples
Cluster VI	"binding of …" • bone morphogenetic protein • neuroligin • myosin • microtubule • transcription factor • clathrin	All samples
Cluster VII	"binding of small molecules" • phosphatidyl serine • phospholipids (calcium-dependent) • calcium ions • glycine	All samples

Table S4. Overview of endocytic machinery components. Protein accession number is from UniProtKB.

Gene name	Protein name	Accession number	Description
AAK1	AAK1	Q2M2I8	AP-2-associated protein kinase 1
AMPH	Amph	P49418	Amphiphysin
AP2A1	AP-2 α(A)	O95782	AP-2 complex subunit α(A)
AP2A2	AP-2 α(C)	O94973	AP-2 complex subunit α(C)
AP2B1	AP-2 β	P63010	AP-2 complex subunit β
AP2S1	AP-2 σ	P53680	AP-2 complex subunit σ
CLTA	CLCa	P09496	Clathrin light chain a
CLTB	CLCb	P09497	Clathrin light chain b
CLTC	CHC17	Q00610	Clathrin heavy chain 17
DAB2	Dab2	P98082	Disabled homolog 2
DNAJC6	Auxilin	O75061	Uncoating phosphatase auxilin
DNM1	Dyn 1	Q05193	Dynamin 1
DNM2	Dyn 2	P50570	Dynamin 2
DNM3	Dyn 3	Q9UQ16	Dynamin 3
EPS15	EPS15	P42566	EGFR substrate 15
FCHO1	FCHo1	O14526	F-BAR domain-containing Fer/Cip4 homology domain-only proteins 1
FCHO2	FCHo2	Q0JRZ9	F-BAR domain-containing Fer/Cip4 homology domain-only proteins 2
HIP1R	HIP1R	O75146	Huntingtin-interacting protein 1-related protein
ITSN1	ITSN1	Q15811	Intersectin 1
ITSN2	ITSN2	Q9NZM3	Intersectin 2
NUMB	Numb	P49757	Protein numb homolog
SH3GL1	EndoA2	Q99961	Endophilin A2
SH3GL2	EndoA1	Q99962	Endophilin A1
SH3GL3	EndoA3	Q99963	Endophilin A3
SNAP91	AP180	O60641	Clathrin coat assembly protein AP180
SNX9	SNX9	Q9Y5X1	Sorting nexin 9
STON1	Stonin 1	Q9Y6Q2	Stonin 1
STON2	Stonin 2	Q8WXE9	Stonin 2
SYNJ1	Synj 1	O43426	Synaptojanin 1
SYNJ2	Synj 2	O15056	Synaptojanin 2

Table S5. Clinical/surgical characteristics, histopathological diagnosis and survival of the patients for additional biopsy sample analysis Western blot/validation. Legend: AA, anaplastic astrocytoma; GBM, glioblastoma; s GBM, secondary glioblastoma; s GBM o, secondary glioblastoma with oligodendroglia component; OA, oligoastrocytoma; OG, oligodendroglioma; FU, follow-up; GTR, gross total resection; PR, partial resection; n.a., not applicable. The overall survival information was not available at time of publication.

BTB	Grade	Age	Sex	Histological diagnosis	Degree of resection
BTB181	n.a.	Epilepsy control	M	n.a.	n.a.
BTB345	III	42	M	AA	GTR
BTB355	III	25	M	OG	GTR
BTB359	IV	29	F	s GBM	GTR
BTB380	IV	61	M	s GBM o	GTR
BTB390	III	44	M	AA	GTR
BTB398	III	37	M	OA	GTR
BTB405	IV	47	F	s GBM o	GTR
BTB409	III	20	M	OA	GTR
BTB416	III	49	F	OA	GTR
BTB419	II	58	F	OG	GTR
BTB438	II	34	M	OG	GTR
BTB456	II	51	F	OA	GTR
BTB462	II	51	M	OG	GTR
BTB466	IV	73	M	s GBM	GTR
BTB486	IV	63	M	s GBM	GTR
BTB504	IV	36	M	s GBM	GTR
BTB526	IV	59	M	GBM	GTR
BTB527	IV	60	F	GBM	GTR
BTB529	III	45	F	AA	GTR
BTB530	IV	76	F	GBM	GTR
BTB532	IV	59	M	GBM	GTR
BTB533	IV	81	M	GBM	GTR
BTB535	IV	71	F	GBM	GTR
BTB536	IV	68	M	GBM	Re-resection
BTB541	IV	68	F	GBM	GTR
BTB542	IV	77	M	GBM	Subtotal resection
BTB544	IV	57	M	GBM	GTR
BTB545	IV	65	M	GBM	GTR
BTB546	IV	56	M	GBM	GTR
BTB551	IV	51	M	GBM	GTR
BTB553	IV	57	F	GBM	GTR
BTB554	IV	76	F	GBM	GTR
BTB555	IV	64	M	GBM	GTR
BTB558	IV	28	M	GBM	GTR
BTB565	IV	50	M	GBM	GTR
BTB566	IV	52	F	GBM	GTR
BTB568	IV	59	M	GBM	GTR

Table S6. Grading and summary of the mutational and molecular status of the additional biopsies used in this study. See also Capper et al., 2018 for more detailed information regarding the subclass.[1] Legend: WT, wild-type; Subclass according to methylation profiler: MES, mesenchymal; ND, not determined; RET, retention; MET, methylated; UNM, unmethylated; LOH, loss of heterozygosity; n; number of amplification; n.a, not applicable.

BTB	Grade	Subclass (methylation profiler)	*TERT*	*IDH*	1p19q	*MGMT*	*EGFR*
BTB181	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
BTB345	III	n.a.	WT	R132H	RET	n.a.	WT
BTB355	III	n.a.	WT	R132H	ND	n.a.	n.a.
BTB359	IV	n.a.	WT	R132G	RET	n.a.	n.a.
BTB380	IV	n.a.	G228A	R132H	LOH	n.a.	n.a.
BTB390	III	n.a.	G228A	R132H	LOH	n.a.	n.a.
BTB398	III	n.a.	WT	R132H	RET	n.a.	n.a.
BTB405	IV	n.a.	G228A	R132H	LOH	n.a.	n.a.
BTB409	III	n.a.	WT	R132L	LOH	n.a.	n.a.
BTB416	III	n.a.	WT	R132H	LOH	n.a.	n.a.
BTB419	II	n.a.	G228A	R132H	LOH	n.a.	n.a.
BTB438	II	n.a.	G228A	R132H	LOH	n.a.	n.a.
BTB456	II	n.a.	WT	R132C	RET	n.a.	n.a.
BTB462	II	n.a.	G228A	R132H	LOH	n.a.	n.a.
BTB466	IV	n.a.	WT	R132H	RET	n.a.	n.a.
BTB486	IV	n.a.	WT	R132H	RET	n.a.	n.a.
BTB504	IV	n.a.	n.a.	R132H	LOH 19q	MET	n.a.
BTB526	IV	MES	G250A	WT	no	UNM	2n
BTB527	IV	RTK II CL	G250A	WT	no	MET	>2n
BTB529	III	A	WT	R132H	no	MET	2n
BTB530	IV	RTK II CL	G250A	WT	no	MET	>2n
BTB532	IV	RTK II CL	G228A	WT	no	ND	>2n
BTB533	IV	MES	ND	WT	ND	UNM	>2n
BTB535	IV	RTK II CL	ND	WT	no	UNM	2n
BTB536	IV	RTK II CL	WT	WT	no	UNM	>2n
BTB541	IV	RTK II CL	G250A	WT	no	MET	>2n
BTB542	IV	MES	G228A	WT	no	MET	2n
BTB544	IV	MES	G250A	WT	no	MET	2n
BTB545	IV	RTK I PN	G250A	WT	no	MET	>2n
BTB546	IV	RTK II CL	G250A	WT	no	MET	>2n
BTB551	IV	RTK II CL	ND	WT	no	UNM	>2n
BTB553	IV	RTK II CL	G228A	WT	no	UNM	>2n
BTB554	IV	RTK II CL	n.a.	WT	no	MET	>2n
BTB555	IV	RTK II CL	n.a.	WT	no	UNM	>2n
BTB558	IV	MES	n.a.	WT	no	MET	2n
BTB565	IV	MES	n.a.	WT	no	MET	>2n
BTB566	IV	MES	n.a.	WT	no	MET	2n
BTB568	IV	MES	n.a.	WT	no	UNM	>2n

References

1. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018;555(7697):469-74.