Supplemental Note regarding Supplemental Figures S2

The HGG-IDHmut-A / B subgrouping is not biased by patient age or tumor content

Upon identification of the proteome-based classification, we aimed to link this classification to patient's demographic data or histological correlates. While 1p/19q-codel tumor patients were younger than IDHmut patients without 1p/19q-codeletion, the HGG-IDHmut-B and HGG-IDHmut-A entities did not differ significantly regarding patient age (Fig. S2A). Nevertheless, stratifying samples into an 'old' and a 'young' group at a 50%/50% balance of HGG-IDHmut-A to HGG-IDHmut-B did not result in significant proteome differences between these groups (Fig. S2A). To check for histological correlates to this sub-classification, a neuropathologist re-evaluated the IDHmut tissue sections. No discerning histmorphological feature could be identified. The fractions of the tissue section occupied by the solid tumor, the tumor infiltration zone, and the reactive CNS tissue were estimated to investigate a potential tumor content bias more carefully. The HGG-IDHmut-B sample group had a moderately higher total tumor area, wherein total area refers to the sum of solid and infiltration zone area (Fig. S2A). However, stratification of samples into two groups of either 'high total tumor area' and a 'low total tumor area' as above for patient age did not show significant proteome differences (Fig. S2A). Similarly, the HGG-IDHmut-B sample group had a higher solid tumor content, but again, stratification according to solid tumor content did not result in significant proteome differences (data not shown).

Tumor content is linked to independent cancer drivers

To systematically deconvolute tumor content-associated effects, we employed a linear regression model to predict protein intensities of IDHmut tumors using the codeletion status, HGG-IDHmut-B/HGG-IDHmut-A status, solid tumor area, and infiltration zone area as variables. The regression model largely confirmed the differences of the proteome-based classification including the top outlier proteins and the mitochondrial respiratory chain complex signature (Fig. S2B). After correction for the other covariates, the significance of proteome differences between the 1p/19q-codel and non-codel entity was larger but the previous top outliers were conserved (Fig. S2B). Outlier proteins associated with solid or infiltration tumor area were distinct to the proteins linked to either subgrouping (HGG-IDHmut-A/B or 1p/19q status) but protein association with the two tumor area parameters was highly correlated (Fig. S2B). Amongst the outliers associated with high tumor content, several have previously been implicated as oncogenes or have genetic variants associated with cancer, predominantly in other cancer types. These include NID1, SYNE2, DEK, DDX6, DES, GATAD2A, SNRPE, ALOX5, HAPLN1, LAMA5, NCOR1, and RBM10[145-157]. Conversely, outlier proteins associated with low tumor content included functional CNS proteins such as myelin sheath proteins MBP and MAG and neuronal proteins NFASC and STMN2. Moreover, these outliers also contained proteins implicated as tumor suppressors such as NRGN, CARNS1, and GPD1[158-160]

The HGG-IDHmut-A/B stratification does not reflect differential neuronal or glial content

To check the possibility that differential neuronal or astrocytic content causes the proteomic differences between HGG-IDHmut-B and HGG-IDHmut-A, we stratified samples according to GFAP (astrocyte marker) or NEFL (neuronal marker) abundance into 'high expression' and 'low expression' groups with a balanced ratio of the HGG-IDHmut-A and HGG-IDHmut-B subgroups (Fig. S2C). None of these stratifications resulted in any significant proteome alterations or in the differences of mitochondrial respiratory chain proteins as shown by the HGG-IDHmut-A/HGG-IDHmut-B stratification (Fig. S2C). Microglial marker proteins were not regulated between HGG-IDHmut-A / B (Fig. S2D).
Furthermore, mitochondrial protein abundance differences associated with the HGG-IDHmut-A/B stratification do not at all correlate with the abundance differences between isolated brain cell types (astrocytes vs neurons, astrocytes vs oligodendrocytes, astrocytes vs microglia) accessible for mouse (Fig. S2E)[144].
Lastly, none of the control comparisons above (i.e. the stratifications for age, tumor content, cell type abundance, comparison to the isolated cell types) reproduce the apparent differences in HGG-IDHmut-A / B outlier proteins such as CCAR1, YBX1, and ERH.
Comparison of our data with IDHwt glioma proteome data revealed that HGG-IDHmut-A and B correspond to nmf1/proneural-likethat inherently higher expression of neuronal proteins in the glioma cells of the proneural subtype and by equivalence in glioma cells of the HGG-IDHmut-A subtype account for the mild differences of neuronal protein expression observed in our dataset. and nmf3/classical-like subtypes of IDHwt glioma, respectively, (Fig. 5). It is conceivable that inherently higher expression of neuronal proteins in the glioma cells of the proneural subtype and by equivalence in glioma cells of the HGG-IDHmut-A subtype account for the mild differences of neuronal protein expression observed in our dataset.