Suppl.Fig1.

**Digital quantification of bcl-2 positive cells and comparison to Nanostring normalized counts.** A) Representative patches of PID #27 showing the raw picture (top), overlay of segmentations and compartmentalization (middle) and nuclear membrane compartment in which a threshold of mean DAB intensity of >0.4 was used for classification of the entire cell (bottom). B) Bland-Altman plot comparing Nanostring® normalized counts and immunohistochemical counts in the initial tumors. C) Bland-Altman plot comparing Nanostring® normalized counts and immunohistochemical counts in the recurrent tumors. Red line; Median of residuals, Black lines; 2.5- and 97.5-quantile borders, IHC; immunohistochemistry.

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Suppl.Fig2.

**Visualization of the inter-sample distribution of the raw Nanostring counts as a quality control prior to normalization.** A) mRNA counts across all samples. B) mRNA variance from mean dispersion for initial, normal and recurrent samples, respectively. C) miRNA counts across all samples. D) miRNA variance from mean dispersion for initial, normal and recurrent samples, respectively. E) Quality control of gender distribution of two nominated gene raw counts.​

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Suppl.Fig3.

**Descriptive analysis of matched cohort of GBM cases with late recurrence.** A) cutoff of <1year of relapse interval applied as exclusion criteria of the entire cohort of recurrent GBM (n=97). B) Patient age distribution (at diagnosis) of patients with late recurrence (n=43). C) Spatial tumour location at diagnosis and at recurrence (n=43). D) MIB1 marker expression (assessed by IHC) in initial and recurrent tumor pairs (n=43). E) Distribution of the MGMT methylation status in initial and recurrent tumors assessed by methylation-specific PCR.

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Suppl.Fig4.

**Detailed analysis of mutational signatures and TMB in tumors with late recurrence.** A) Paired boxplots indicating the total number of mutations contributing to the SBS15, SBS16, SBS3, SBS30, SBS40, SBS5 and SBS8 signatures in initial and recurrent samples. B) Mutations in MMR pathway genes (all genes from KEGG MMR pathway gene list hsa03430 with at least one mutation are shown), TMB category, % contribution of COSMIC mutational signature SBS11 (Temozolomide) and SBS15 (defective MMR) and days until relapse. C) Spearman correlation between TMB (mut/Mb) in the initial tumor and age of the patient (years).

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Suppl.Fig5.

**Copy number profiles of tumors with late recurrence**. GISTIC gene level copy number data (panel A&B). A) Frequency of copy number alterations across initial (top), recurrent (middle) and all (bottom) tumors for which copy number data was available. B) Copy number profiles of all tumors for which copy number data was available.

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Suppl.Fig6.

**GISTIC analysis of tumors with late recurrence**. GISTIC amplification (A) and deletion (B) plot.

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Suppl.Fig7.

**Oncoprint of genes in which mutations private to the recurrence sample were found in ≥5 patients.** Each column represents a patient. Vertical bars represent the number of alterations per patient and horizontal bars represent the number of mutations per gene.

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Suppl.Fig8.

**Tumor evolution of GBM patients with late recurrence.** A) Fish plots showing additional examples of tumors with early branching evolution. B,C) Examples of tumors with targetable alterations. B) Patient #052 carrying an activating EGFR p.A289V mutation is presumably not responsive to a therapy with Lapatinib because of illusion of clonality. C) Patient #086 carrying a classical BRAF p.V600E mutation in a common ancestor may be responsive to a therapy with BRAF inhibitors.

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Suppl.Fig9.

**Unsupervised clustering and samples with mRNA and microRNA profile similarities** A) Poisson sample distances after rlog transformation for A) mRNA data and B) miRNA.​

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Suppl.Fig10.

**EGFR expression, a surrogate marker of the classical subtype of GBM, is not consistently downregulated in recurrent GBM.** Pairwise comparison of initial and recurrent GBM from the same patient for EGFR expression using the Nanostring dataset.

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Suppl.Fig11.

**Differentially expressed mRNAs upon long-term recurrence** Circos plot indicating pairwise distribution of gene expression for top differentially expressed genes. Genes and patient IDs are indicated on the outer circle. Range of counts for each gene are gauged on the inner circle and connected with a line to the specific patient that in a pairwise manner. Gender is color labelled. Note that enhanced levels of ACVR1C, LTBP1, RASAL1 and HDAC11, and reduced levels of MDM2 and LEFTY2 are detected in multiple patients. In contrast, enhanced COMP expression level is only detected in patients 73 and 18.

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Suppl.Fig12.

**High LTBP1 expression is significantly associated with worse survival**. Forest plot depicting the genes of interest, included sample sizes, hazard ratios with corresponding confidence intervals and p-values.

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Suppl.Fig13.

**Gene network analysis of genes differentially regulated in recurrent GBM, which serve as hubs for responsive elements resulting in phosphorylation of proteins and proliferation of vascular cells.** Inhibitory interactions are indicated by blue lines and activating interactions are indicated by orange lines (p<0.05).

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Suppl.Fig14.

**Biological function prediction.** Radar graph scored based on pathway analysis using IPA for microRNA data, considering only the oppositely regulated targets.​

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Suppl.Fig15.

**Dichotomous role of ALK7 in GBM cell lines**  A) ALK7 overexpression with a dominant negative mutant (ALK7-DN) confers significant TMZ sensitivity (measured by number of apoptotic cells) at both 50uM and 100uM dose 48hours post treatment. B) ALK7 overexpression with a constitutively active mutant (ALK7-CA) induces significant apoptotic cell counts. ns (P > 0.05), \*\* (P ≤ 0.01), \*\*\* (P ≤ 0.001).

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Figure S16:

**ACVR1C/ALK7 expression correlates with apoptosis resistance in the TCGA dataset of primary GBM.** Significant positive correlation of ALK7 and BCL2 expression and inverse correlation of ALK7 and BAK or BAX in the (A) TCGA dataset of primary GBM and (B) inverse correlation of ALK7 and BAX of our cohort of primary and recurrent GBM. Significance is assessed by Pearson correlation.