Figure S1. Hierarchical clustering of the tumors is stable with regard to the number of underlying top hub genes. To select genes for the clustering, genes in each module of the 'Gravendeel' dataset were ranked in the descending order of Pearson correlation between their expression profile and the module eigengene (ME). Thus, for a given module, genes at the top of the list best conformed to the module's expression profile and therefore were referred to as modules' hub genes. Tumor samples in the 'Gravendeel' dataset (276 samples) were hierarchically clustered based on expression profiles of the modules' hub genes: top 10 hub genes per module (A), top 25 hub genes per module (B), top 100 hub genes per module (C). In the heatmaps, columns represent patients, rows – genes. Red denotes higher expression relative to green. Biological characteristics of the samples are shown below the heatmaps (patients' diagnosis and tumor grade).

Figure S2. Structure of the transcriptome is similar across the patients' cohorts. In the consensus modules, genes that were present on both microarray platforms (Affymetrix U133A and U133A Plus 2.0) were selected. Within each module, these genes were ranked in the descending order of kME (the kME values were averaged across the five datasets). The top 25 genes from each module were selected as hubs for the subsequent clustering (note that in the M10 module only 16 genes were found, whereas all the other modules provided 25 genes each). Tumors in each of the 5 datasets were hierarchically clustered based on expression profiles of the selected genes. Note that identical gene sets were used to cluster tumors in all the datasets; therefore, the heatmap confirms that these genes are reproducibly coexpressed. Below the heatmaps, we color-code glioma subtypes from the present and the respective original studies. Modules that were originally shown to form higher-order groups in the 'Gravendeel' dataset (groups A, B, C, D and E on the right of the heatmap) tend to be coexpressed in all the patient cohorts. Similarly, the major tumor clusters marked by up-regulation of the respective module groups tend to be conserved across the datasets. The consistency between the heatmaps demonstrates that the global structure of the glioma transcriptome is conserved across independent patients' cohorts.

Figure S3. Definition of the samples' classification scheme. In the 'Gravendeel' dataset, samples were clustered based on expression levels of the top 25 hub genes from the consensus modules M8, M11, M15 and M20 (K-means clustering, 5 clusters, Euclidean distance). Panel A shows the resulting clusters as a heatmap: 'Mesenchymal', 'Proliferative', 'Proastrocytic', 'Proneural' and 'Other' (rows correspond to the 100 genes, columns – to the 276 patients). Based on the K-means clustering result, a centroid was defined for each tumor subtype by averaging expression values of the 100 genes within each cluster. Panel B shows the centroids of the 5 glioma subtypes.

Figure S4. Expression patterns that underlie centroid-based classification of samples. For each dataset, a heatmap of the 100 marker genes that comprised the centroids is shown. Rows represent genes, columns represent samples. Samples in each dataset are grouped by the predicted tumor subtype. Below the heatmaps, the tumor subtypes are color-coded (red – 'Mesenchymal', orange – 'Proliferative', blue – 'Proastrocytic', green – 'Proneural', grey – 'Other'). The heatmap sizes differ due to the fact that each dataset contains a unique number of patients.

Figure S5. Expression levels of the proastrocytic and proneural modules are negatively correlated across differentiated tumors. From each dataset we selected differentiated tumors, i.e. those where the proastrocytic (M15) and/or neurogenesis (M20) modules were up-regulated (Z-score normalized expression values of the proastrocytic ME and/or proneural ME higher than 1.0). Heatmaps were created based on the top 25 hub genes from the proastrocytic and neurogenesis modules. In each heatmap, samples were ranked in the

increasing order of the proastrocytic ME. Pearson correlations were calculated between the proastrocytic ME and the neurogenesis ME across the selected tumors.

Table S1. Patient clinical data. This table combines patient characteristics (survival, diagnosis, WHO grade, age) obtained from the supplementary materials of the respective original studies. The table does not include other clinical characteristics that were not used in our analysis.

Table S2. Module gene composition is highly preserved across independent patient cohorts. Module projections were compared based on their gene composition across all possible pairs of the 5 datasets. To prevent overestimation of the similarity, hubs that were used for generation of the module projections were excluded from the comparison. Projections of the same module were always more similar to each other than to those of the other modules (data not shown). Fisher's exact test P-values denote statistical significance of overlap in gene composition between projections of a given module across a pair of datasets. Additionally, the corresponding percentages of overlapping genes are provided.

Table S3. Consensus module gene lists. For each module, consensus gene members are listed (see Supplementary Methods). The following information is provided for each gene: gene symbol, gene name, cytoband (chromosomal location), Entrez gene ID, number of datasets in which the gene was a member of the respective module, kME (averaged across the five datasets), an indicator whether expression level of the gene is measured by both microarray platforms (U133A and U133A Plus 2.0) or only the U133A Plus 2.0 chip.

Table S4. Functional annotation of the modules. An overall functional annotation of the modules is supported by the corresponding over-represented terms. For each over-represented term the table specifies: data source from which the term's gene list was extracted, number of genes overlapping between this gene list and the module, statistical significance of the overlap (Fisher's exact test P-value). For functional categories that were assigned to modules based on a literature search, the supporting genes and references are provided in the additional table.

Table S5. Centroids for tumor classification. For each tumor class ('Mesenchymal', 'Proliferative', 'Proastrocytic', 'Proneural', and 'Other'), a representative expression profile, i.e. a centroid, is provided.

Table S6. Samples classification in multiple datasets. Each spreadsheet corresponds to an individual dataset ('Gravendeel', 'Li Train', 'Li Test', 'Phillips', 'Freije'). Three mappings of samples to classes are provided: (1) obtained from the supplementary materials of the dataset-specific original studies; (2) obtained by classifying samples based on the 3 centroids that we reproduced according to the study of Phillips and colleagues (*Mes*, *Prolif* and *PN*); (3) obtained by classifying samples based on the 5 centroids that we defined in our study ('Mesenchymal', 'Proliferative', 'Proastrocytic', 'Proneural', and 'Other'). In the 'Phillips' cohort, minor differences were observed between the stratification of samples from the 'Phillips' supplementary materials and the one that we inferred based on the reproduced 'Phillips' centroids: apparently due to minor differences in the microarray data normalization procedures.

Table S7. Cross-study comparison of the glioma subtypes. For each subtype from the present study the matching subtypes from the previous studies are shown ($P < 0.05$, Fisher's exact test, based on the number of samples shared by the subtypes). When none of the previously described subtypes reached a statistical significance for a given subtype from the present study, the published subtype with the lowest P-value was shown in the table (grey

cells). *(A) Subtypes from the original studies.* Our subtypes were compared with the subtypes described in the dataset-specific original studies (the mapping of samples to subtypes was extracted from supplementary materials of the articles). *(B) Inferred Phillips subtypes.* Our subtypes were compared with the inferred 'Phillips' subtypes that we obtained based on the *Mes*, *Prolif* and *PN* centroids reproduced from the original study.

Table S8. Association of the modules with amplification and up-regulation of the EGFR gene. *(A) Expression change upon EGFR amplification*. P-values denote statistical significance of the modules' differential expression between tumors with amplified and wild-type EGFR (Wilcoxon test applied to module eigengenes). The 'FoldChange' column contains expression fold-change values averaged across all genes in each module. *(B) Correlation with EGFR expression level.* The columns provide Pearson correlation coefficients between the module MEs and the EGFR gene expression profile (with the respective P-values). Module M3 ('Protein kinase regulation') is marked green to denote that it is the module most strongly associated with the EGFR gene amplification and expression up-regulation.