

Extended Data Figure legends:

Extended Data Fig.1:

(a) Tumor mutational allele frequency analysis by whole-exome sequencing in autologous kryo (hypometabolic tumor lesion) on the x-axis and zesto (hypermetabolic tumor lesion) on the y-axis from two glioblastoma patients with low tumor content in kryo. Zesto and kryo-specific variants are marked respectively with red and blue. Data points represent mutations, and the red dotted line represents perfect uniformity.

Table 1. Shows the demographics of glioblastoma multiforme (GBM) patients involved in the study.

Extended Data Fig.2: MRI, [^{18}F] FDG-PET, [^{11}C] MET-PET, and co-registration images from all glioblastoma patients showing the location of the hypometabolic (blue circle), hypermetabolic (red circle), and akri (green circle) lesions. Blue intensity represents the lowest, and red represents the highest accumulation of radiotracers.

Extended Data Fig.3: Spatial genomic heterogeneity in autologous lesion in glioblastoma

(a) Merged transverse MRI, concordant FDG-PET, and MET-PET images exhibiting different geo locations of lesions and textboxes represent key lesion-specific chromosomal alterations in the patients.

(b) A connectivity map of the significant protein-protein interaction involving genes from zesto lesion-specific CNVs from all six glioblastoma patients. 9 distinct functional clusters (illustrated in a discrete color) were detected, and disconnected nodes were excluded from the map.

Extended Data Fig.4: Single-cell transcriptomic profiling highlights proliferating tumor cell clusters and co-expression of hypermetabolic lesion-specific genes involved in CNVs events.

(a) Uniform Manifold Approximation and Projection (UMAP) plot of the location-averaged transcriptome for all cells (5604 cells) from a glioblastoma patient (male 57 years) obtained from 10x Genomics. Different cell types are colored by cluster (top left), the proliferative tumor cluster (based on MKI67 expression) is marked in the UMAP plot, and the top 10 highly expressed genes in the proliferative tumor cluster.

(b) The expression of genes (*DLGPA3*, *NMU*, *PAICS*, *CEP135*, *PPAT*, among other genes) involved in zesto lesion-specific CNV events shows a cellular colocalization with proliferative tumor cell gene signature.

(c) mRNA expression and copy number alteration of zesto lesion-specific cancer driver genes in the glioblastoma and low-grade glioma samples from the TCGA dataset (Homdel: Homozygous deletion, HetLoss: Loss of heterozygosity).

(d) Overall patient survival analysis of glioblastoma (163 patients) and low-grade glioma (518 patients) based on the high and low expression of zesto lesion-specific signature genes. Signature genes are mentioned in the lower left corner of plots, data analyzed by the GEPIA-2 web tool.

Extended Data Fig.5: MTUS2 as a potential tumor suppressor, regulator of metabolism, and cell proliferation in glioblastoma:

(a) Single-cell mRNA expression pattern of metabolic zesto lesion-specific SNV genes in a glioblastoma patient from 10X genomics dataset. The top left UMAP plots specify the major cellular subtype, and the rest of the UMAP plots indicate the expression or no expression of the zesto lesion-specific SNV genes. Most SNV genes overexpressed in the glioblastoma dataset are expressed in tumor and endothelial cell clusters. MTUS2, DLGAP3, RTN1, CRLF1, and PDIA2, have the lowest expression in the majority of cell types.

(b) mRNA expression of putative tumor suppressor genes MTUS2, DLGAP3, and RTN1 in glioblastoma and low-grade glioma dataset, statistical significance ($P < 0.01$) were analyzed by one-way analysis of variance (ANOVA) and is indicated with an asterisk (analyzed by GEPIA2).

(c) MTUS2 mRNA expression negatively correlates with glucose metabolism-associated genes such as HK2, GALM, GAPDH, ENO1, HK3, PKM, and LDHA mRNA expression (TCGA-glioblastoma dataset: CBioportal).

(d) Protein abundance of MTUS2, SLC12A5, and RTN1 negatively correlates with cell proliferation/cycle-associated proteins KI67 and TK1. The data were analyzed using the cBioPortal web tool.

(e) zesto lesion-specific genes (MTUS2, DLGAP3, RTN1, CRLF1, and PDIA2) mRNA expression shows a positive correlation with each other, text box indicating the Spearman's and Pearson correlation and p-value in the bracket (TCGA-glioblastoma dataset: CBioportal).

Extended Data Fig.6: Low expression of putative tumor suppressor signature genes associated with poor survival of glioma and glioblastoma patients.

(a) Kaplan– Meier overall survival estimates of glioblastoma and low-grade glioma (667 patients) patients according to the high and low expression of genes.

(b) Kaplan Meier plot displaying CGGA dataset overall glioblastoma (WHO grade IV, 220 patients) survival distributions of patients stratified and colored by high and low expression of genes. Data was analyzed by the GlioVis web tool.

(c) and (d) gene expression and overall survival of Polyhomeotic Homolog 3 (*PHC3*) and Collagen Type XVIII Alpha 1 Chain (*COL18A1*) in TCGA (The Cancer Genome Atlas) IDH-wildtype glioblastoma (GBM), IDH-mutated 1p19q co-deleted oligodendroglioma (O) and IDH-mutated 1p19q non-co-deleted astrocytoma. *PHC3* and *COL18A1* were found to be mutated in hypermetabolic lesion.

(e) OncoGrid from WGS analysis performed on TCGA-GBM samples shows genetic alterations of the selected driver genes from the hypermetabolic lesion-specific gene signature.

(f) Volcano plot of drugs from the DSigDB gene set. Each point represents a single term, plotted by the corresponding odds ratio (x-position) and $-\log_{10}(p\text{-value})$ (y-position) from the enrichment results of the metabolic zesto lesion-specific gene set. Top 5 candidate drugs identified in drug-target enrichment using DSigDB gene datasets analyzed by

Enrichr, the drug targets pathways contributed by zesto lesion-specific SNV genes. The larger and darker-colored the point, the more significantly enriched the input gene set is for the drug.

(g) Heatmap and bar chart of top enriched drug and pathway terms from the DSigDB gene set library. The top 10 enriched most significant terms for the metabolic hotspot-specific gene set are displayed based on the $-\log_{10}(\text{p-value})$. The term at the top has the most significant overlap with the metabolic hotspot-specific gene set.

Extended Data Fig.7: The hypermetabolic (zesto) lesion exhibits micro-amplification and deletion of the chromosomal region that could potentially enhance tumor aggressiveness.

(a) Global copy number variation in autologous lesions from glioblastoma patients (2,3,4, 5 and 6) as identified using the whole exome sequencing workflows by the VarSeq analysis tool. The x-axis depicts the genomic location, and the Y-axis indicates the log ratio of copy number. Arrows indicating lesion-specific copy number alterations.

(b) mRNA expression of genes located in the amplicon of patient-2, Chromosome-9p, marked with a red arrow.

(c) mRNA expression of genes located in the deleted region of the zesto lesion (Patient#2, Chromosome#9p, marked with red arrow), genes are arranged in the chromosomal order to show the pattern of CNV and gene expression difference between autologous zesto, kryo, and akri lesions.

(d) mRNA expression of *CDKN2A*, *CDKN2B*, and *MTAP* gene located in the zesto's deleted region (Patient#5, Chromosome-9p, marked with red arrow).

Extended Data Fig.8: Hypermetabolic tumor lesions have higher genomic instabilities: Circos plots of the genome of autologous lesions from different glioblastoma patients showing genomic rearrangements and chromothripsis (marked with a red arrow in zesto lesions from patients 3 and 6). The outer ring depicts the SNVs from the whole exome sequencing analysis. The middle ring indicates the copy number variation from whole exome sequencing, the red color indicates the gain, the green indicates the loss of chromosomes, and the blue shows heterozygosity. The innermost circle shows the structural variation analyzed by whole genome sequencing. The red arrow marks chromothripsis events, and chromoplexy events are marked by a dark grey arrow.

Extended Data Fig.9: Hypermetabolic tumor lesions have higher genomic instabilities:

(a) Circos plots of the genome from the zesto, kryo, and akri from patient 6. The outer ring depicts the SNVs from the whole genome sequencing analysis.

(b) Substitution plots in the respective lesions, indicating the highest substitution events were found in zesto compared to kryo and akri, C to T substitution was the most dominant and followed by T to C substitutions.

(c) Bar plots showing the quantification of deletion, insertions, and global rearrangements in different lesions from whole genome sequencing analysis.

Extended Data Fig.10: Hypermetabolic tumour lesions exhibit a greater degree of genomic instability.

A global overview of B- allele frequency analysis on all 29 samples to highlight the major occurrence of a CNV, such as a deletion, duplication, or loss of heterozygosity (LOH), in the whole genome region.

Extended Data Fig.11: *DPEP1* a novel marker for hypermetabolic lesion of glioblastoma

(a) mRNA expression of *DPEP1* in the CGGA-GBM-LGG dataset shows higher expression in glioblastoma compared to other low-grade gliomas and in IDH mutant vs wild type gliomas.

(b) mRNA expression (log2) of the Dipeptidase 1 gene (*DPEP1*) in the TCGA-GBM database is depicted, contrasting non-tumour brain tissue. Statistical significance ($P < 0.01$) was analyzed by one-way analysis of variance (ANOVA) and is indicated with an asterisk.

(c) A connectivity map illustrating the significant proteins from the glutathione metabolism pathway that interact with the dipeptidase 1 enzyme. The colours of the nodes are depicted in the key provided below.

(d) Kaplan-Meier plot illustrates overall glioblastoma survival distributions of patients, stratified and colored by high and low expression of *DPEP1* from CGGA (n=220), Rembrandt dataset (n=88), TCGA-GBM-LGG (n=495), and Korean SMC (n=57) datasets. Data were analyzed using the GlioVis web tool.

(e) and (f) UMAP of 143,793 nuclei shows the expression pattern of *DPEP1* in normal brain cells, venous cell clusters show the highest expression of *DPEP1* gene. (https://twc-stanford.shinyapps.io/human_bbb).

(g) Representative confocal microscopy images depict the colocalization of dipeptidase 1-positive cells (in blue to orange) and CD34-positive cells (in red) in the glioblastoma tissue section and normal brain.

(h) Kaplan-Meier plot illustrates overall glioblastoma survival distributions of patients, stratified and colored by high and low expression of *CD34* from CGGA (n=220), Rembrandt dataset (n=88) datasets. Data were analyzed using the GlioVis web tool.

Extended Data Fig.12: Representative immunohistochemistry images show dipeptidase 1 expression in human tissues:

(a) Positive control tissues include kidney, testis, and tonsil.

(b) Samples of human glioblastoma, normal brain, and intestine are displayed. Magnified areas are shown in the bottom panel, with a scale bar of 50 μ m, while the top panel has a scale bar of 500 μ m.

(c) Representative CD34 staining illustrates vascular structure in a normal human brain and glioblastoma.

(d) Immunohistochemistry images depicting a longitudinal (left) and horizontal (right) section of a microvessel within a glioblastoma tumor. *DPEP1* expression is exclusively

localized to the endothelial tip and vascular sprouts of hypermetabolic tumor lesions and not in mature vessels.

(e) Quantification of CD34 in zesto, kryo, and akri micro lesions, the violin plot indicate the positive fraction per lesion. The indicated p-value is derived from a paired t-test.

(f) The mRNA expression ($\log_2$) of genes, displaying the spatial distribution of vascular endothelial tip cell gene signature, illustrates the colocalization and enrichment with DPEP1-positive lesions.

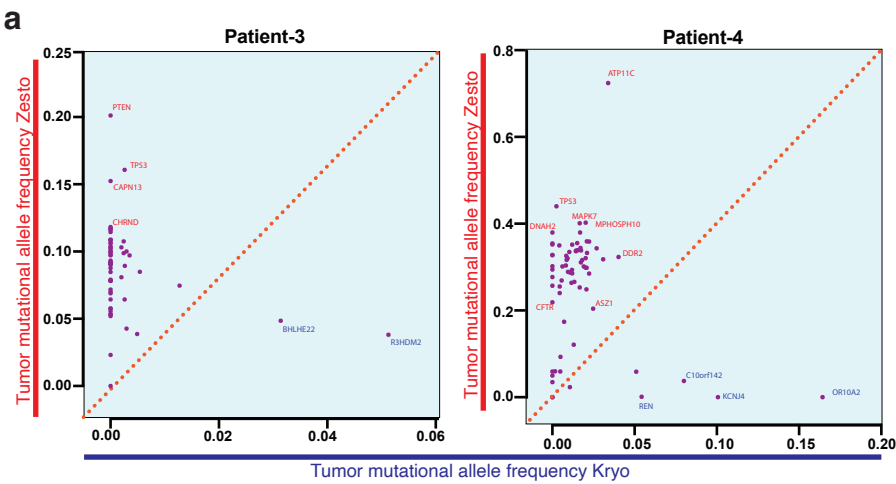
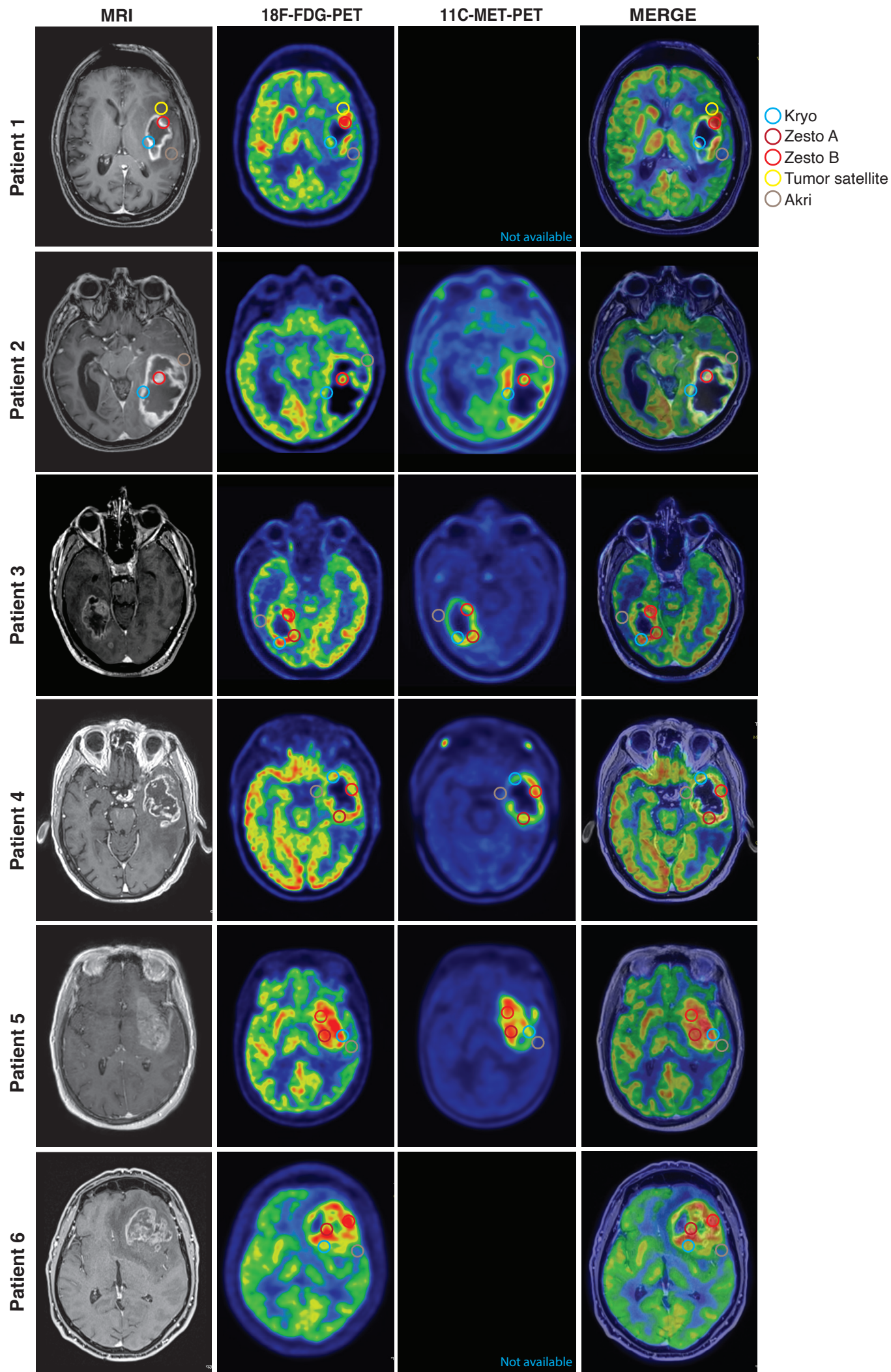
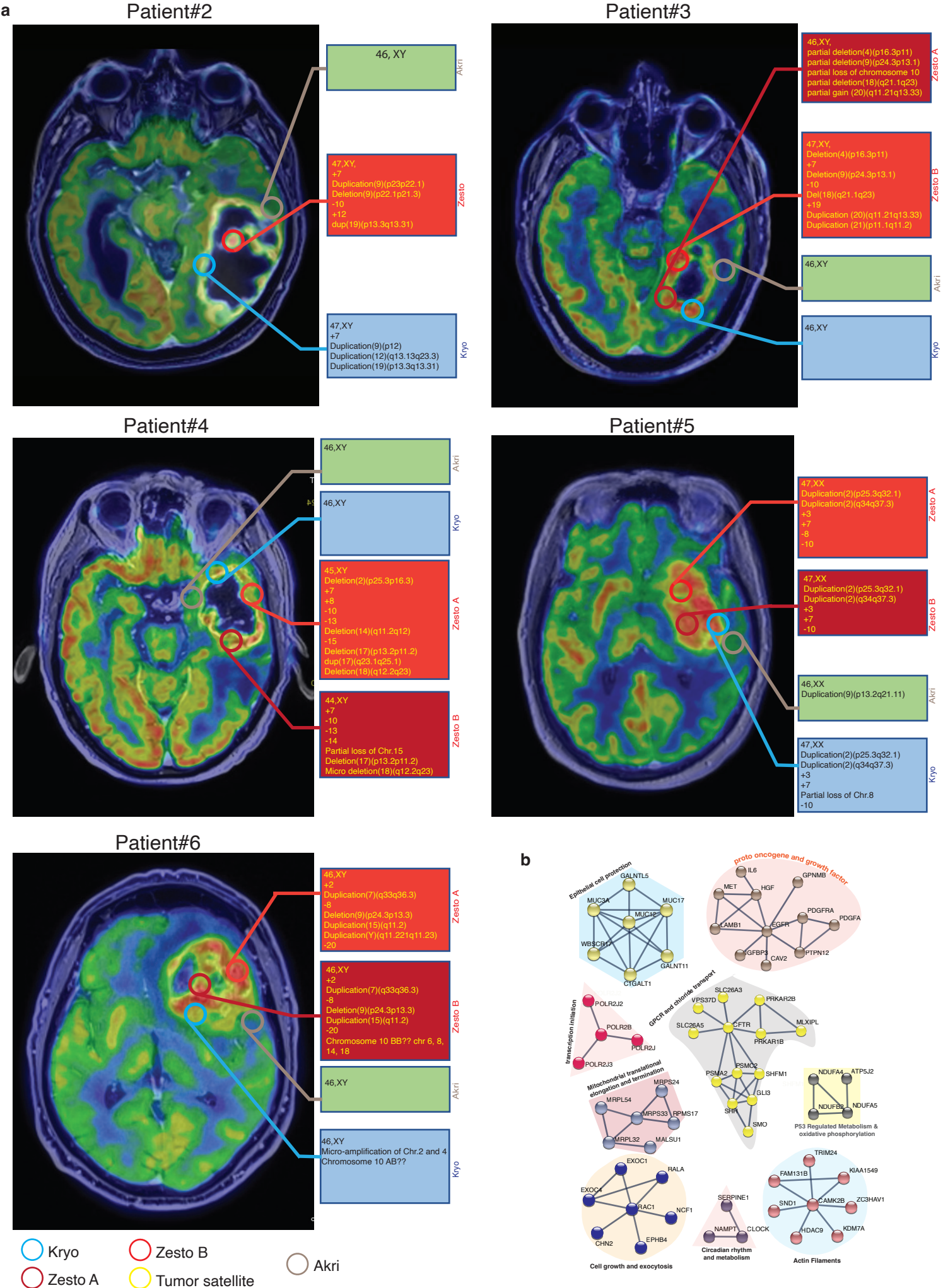


Table 1. Demographics of GBM patients

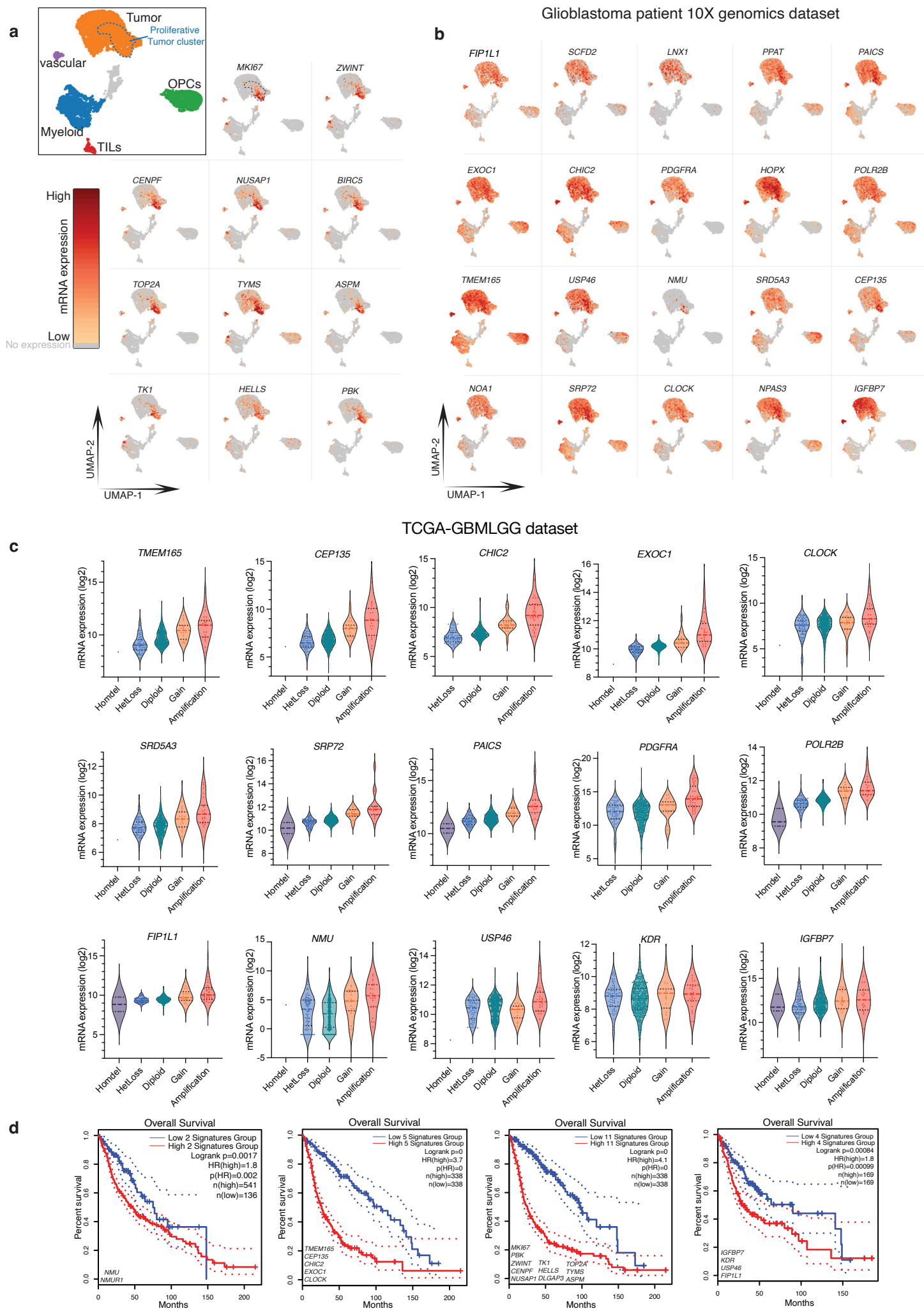
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1	68	male	GBM	unmethylated	wildtype	4	5	4	No
2	74	male	GBM	methylated	wildtype	3	4	3	No
3	48	male	GBM	methylated	wildtype	4	5	4	No
4	76	male	GBM	unmethylated	wildtype	4	5	4	No
5	72	female	GBM	unmethylated	wildtype	4	5	4	No
6	72	male	GBM	methylated	wildtype	4	5	4	No

Extended Data Fig.2

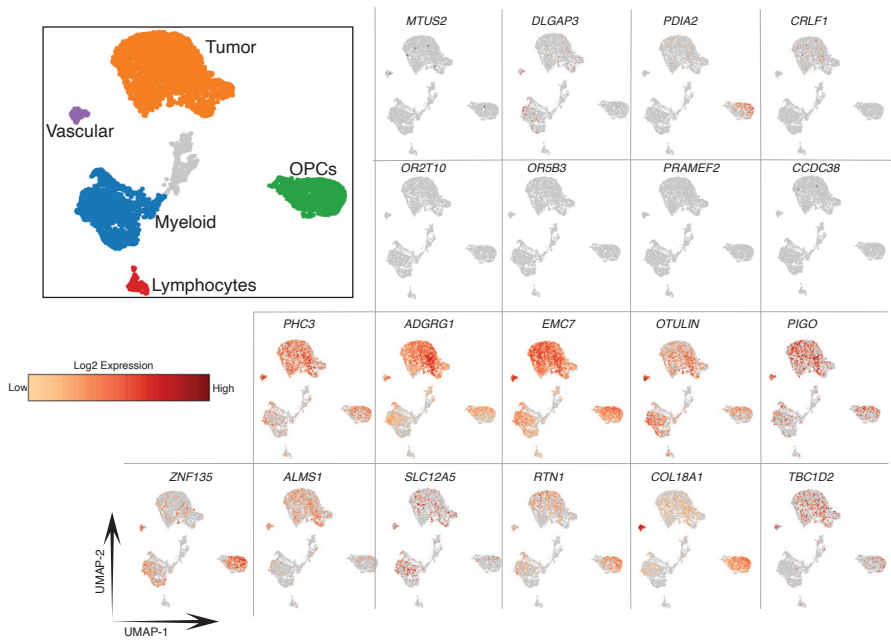




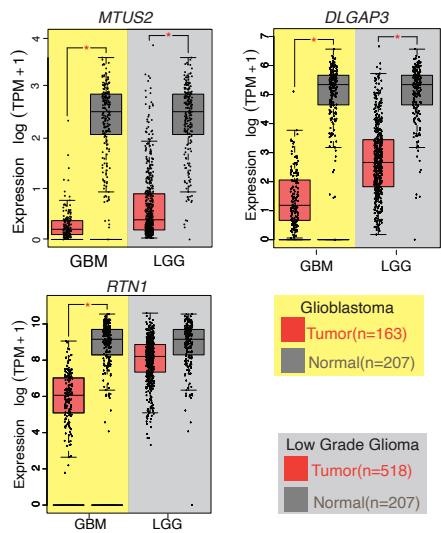
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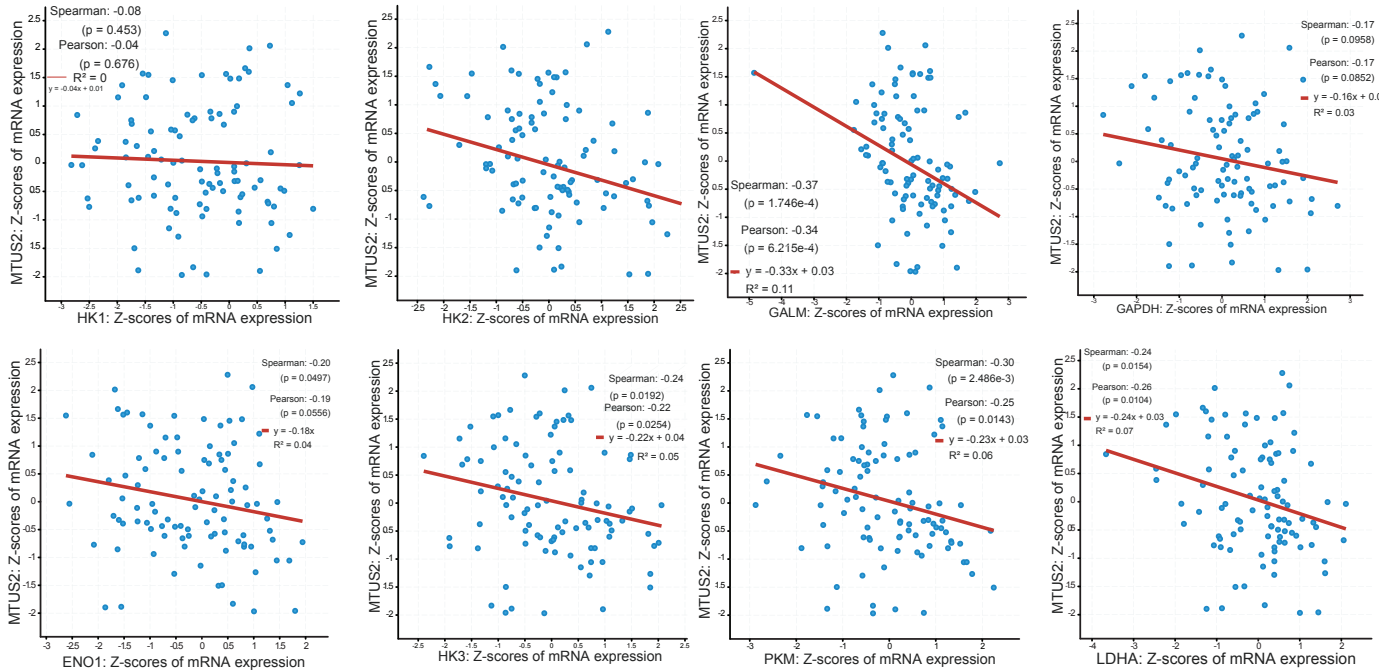
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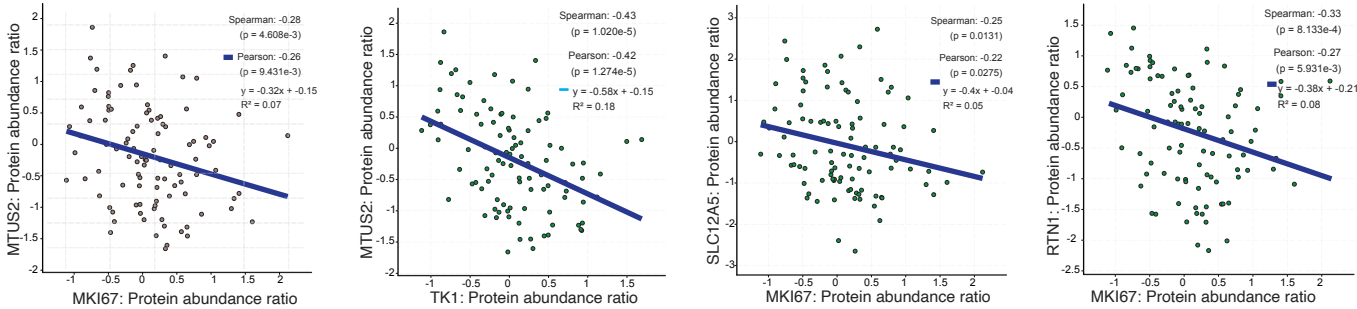
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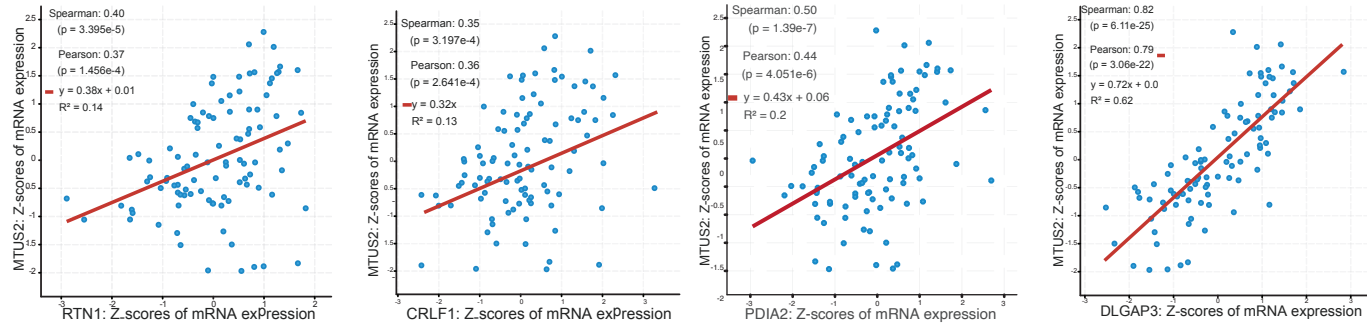
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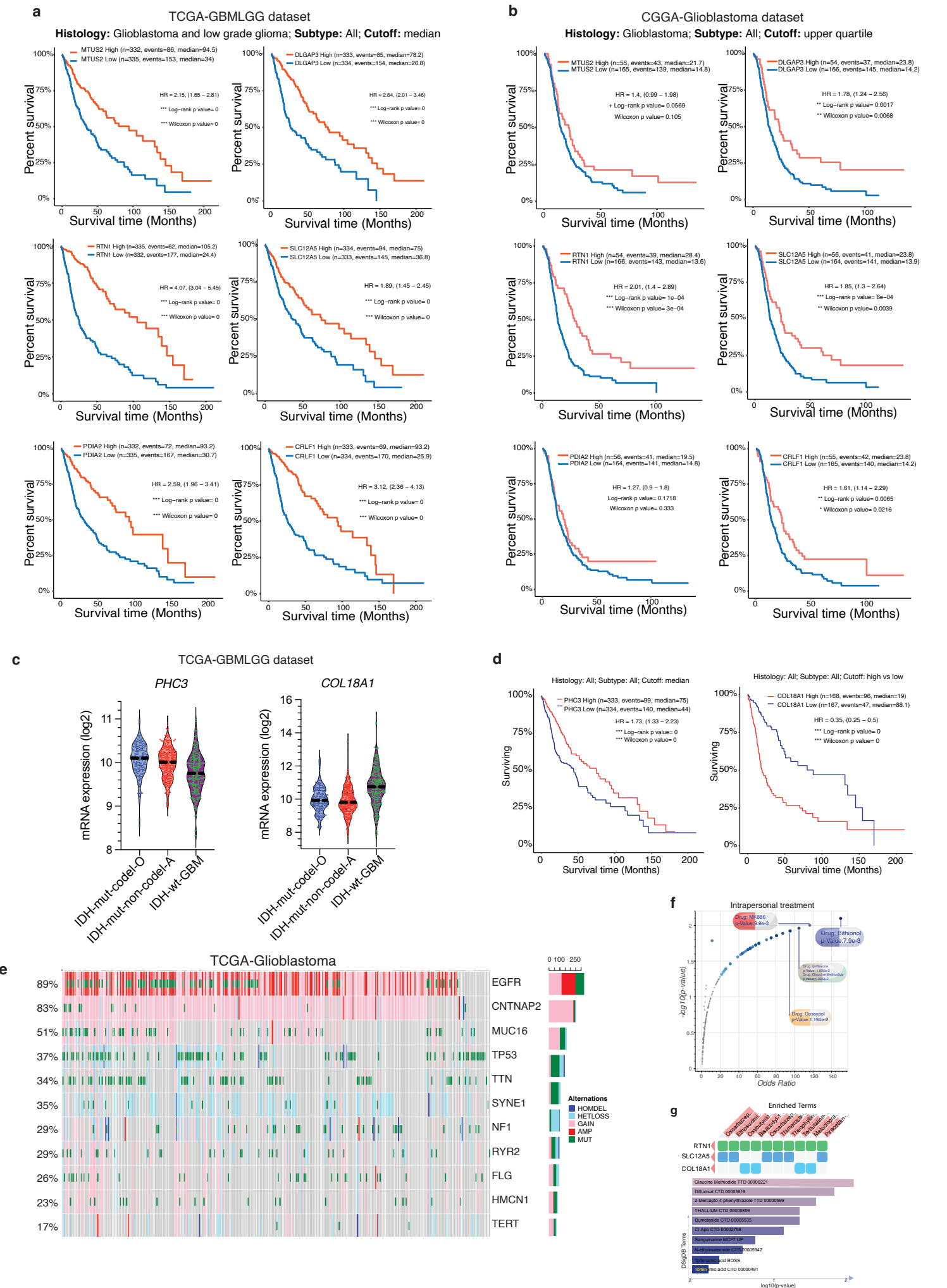


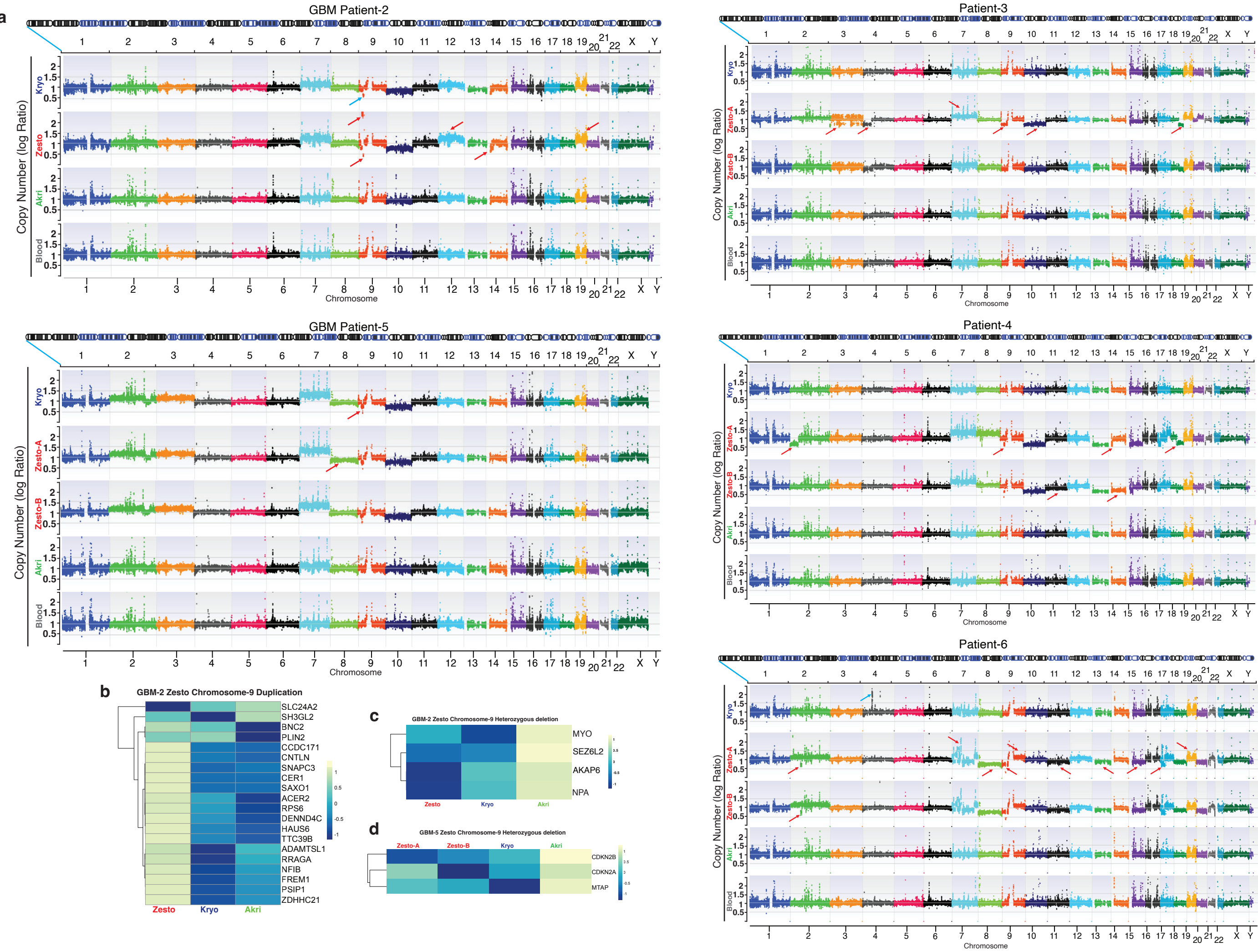
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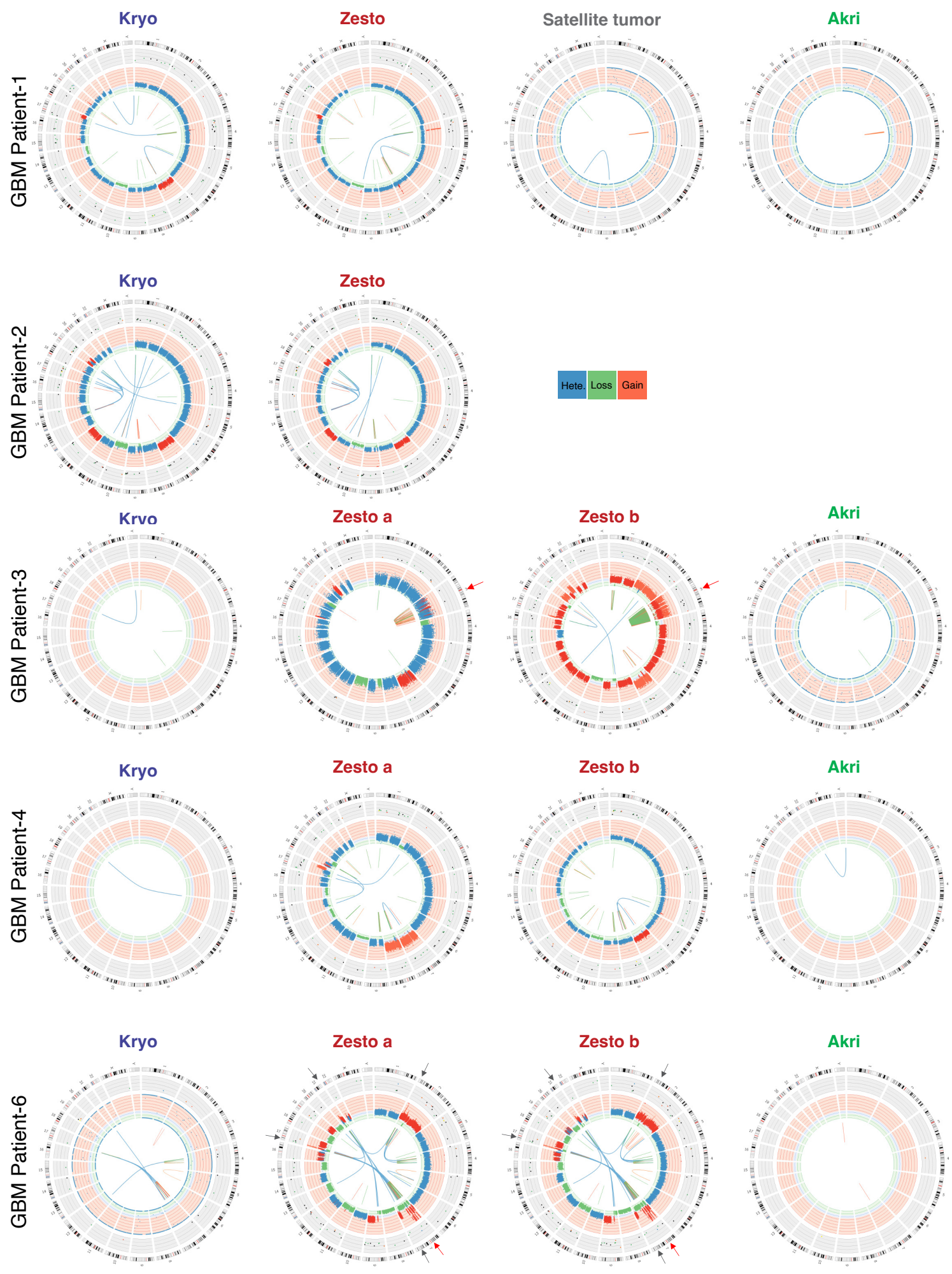
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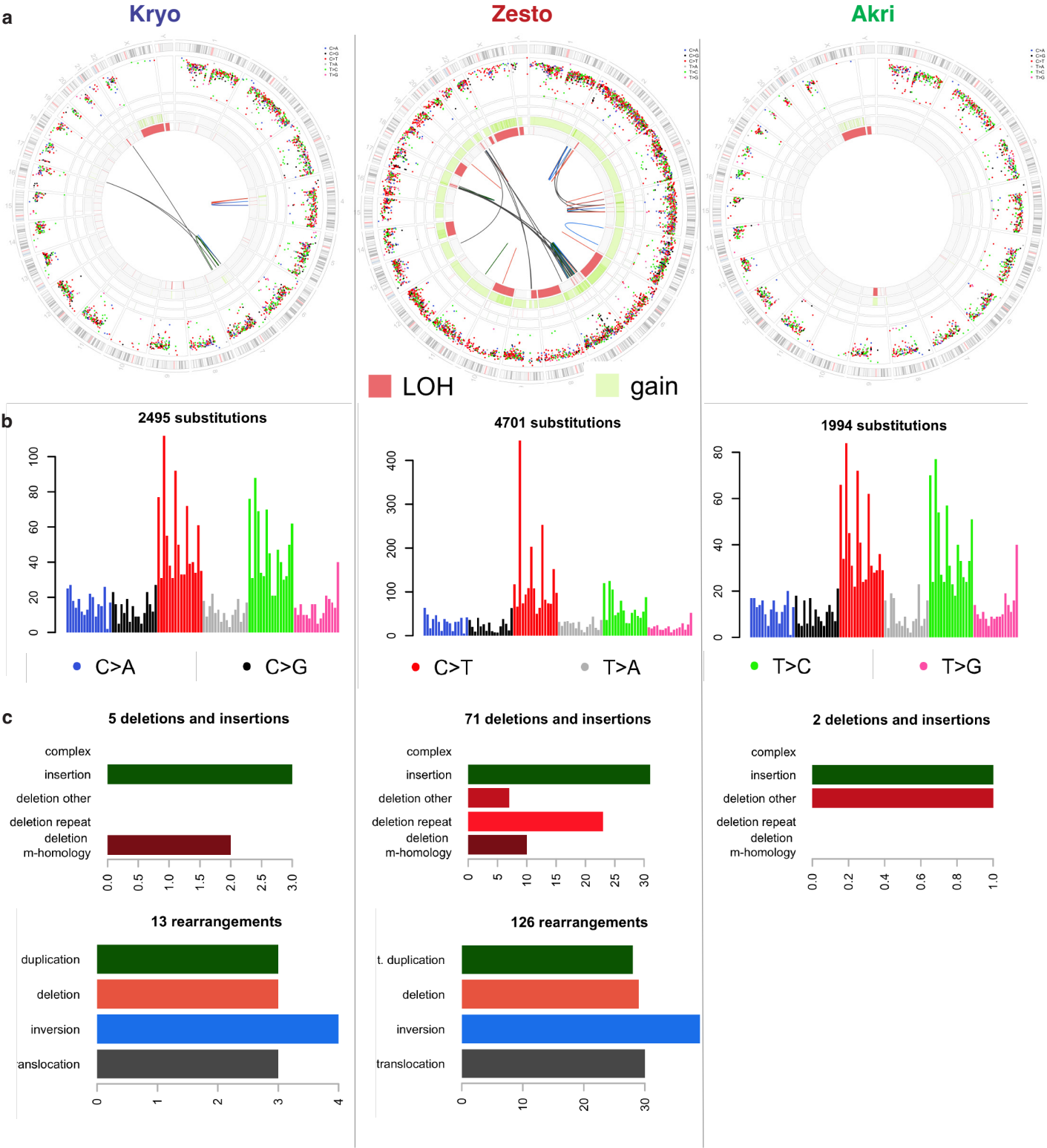


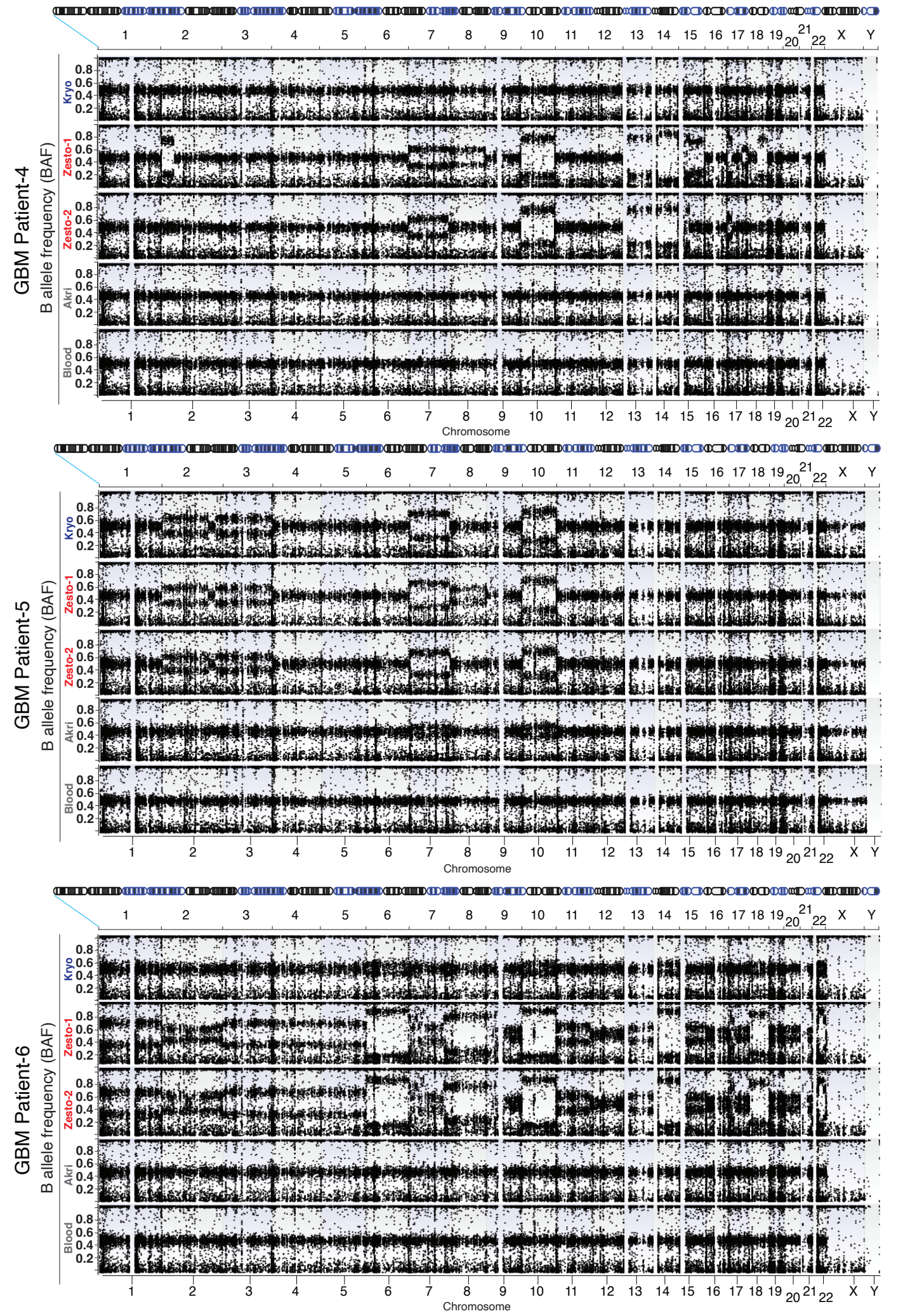
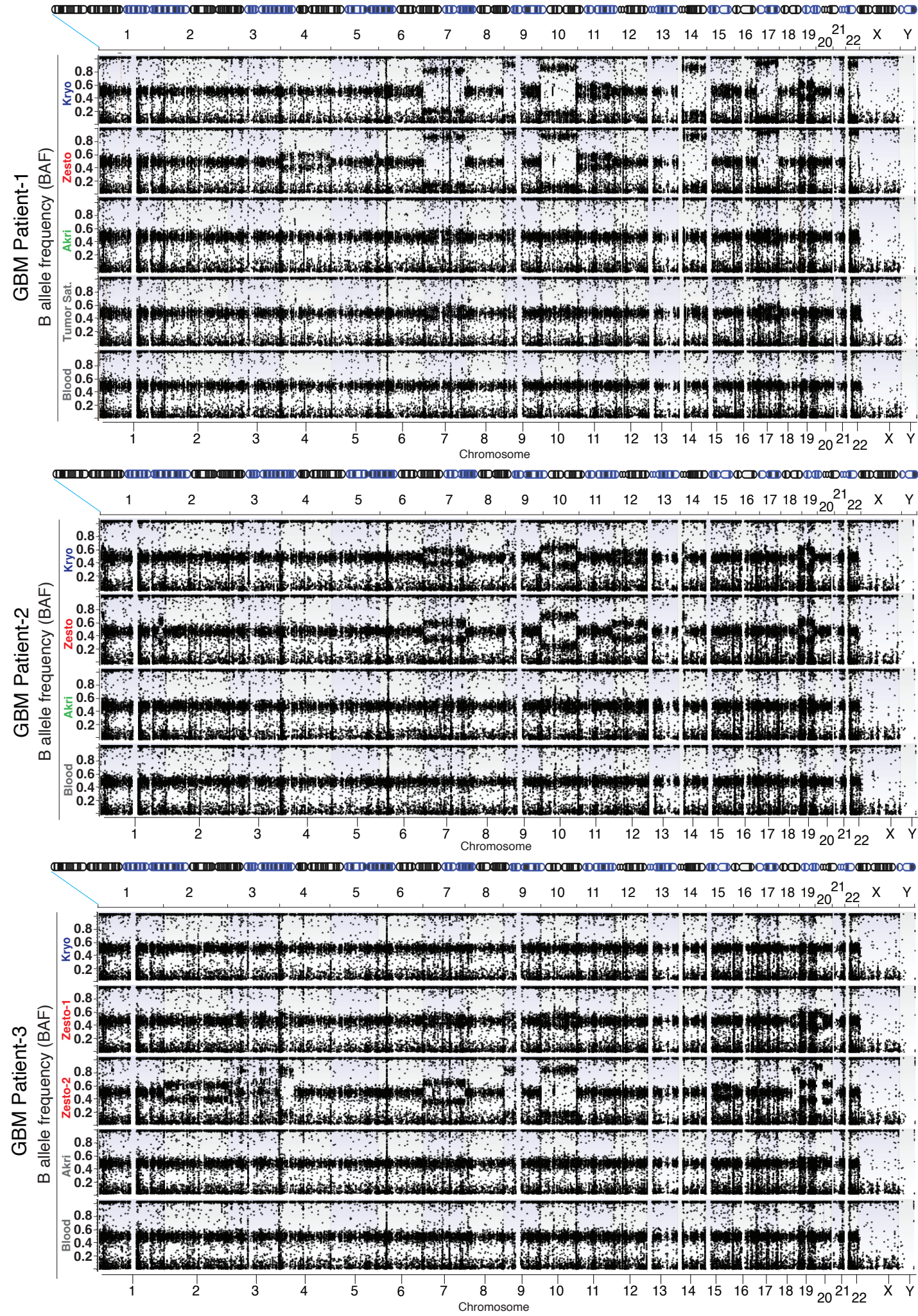


Extended Data Fig.8

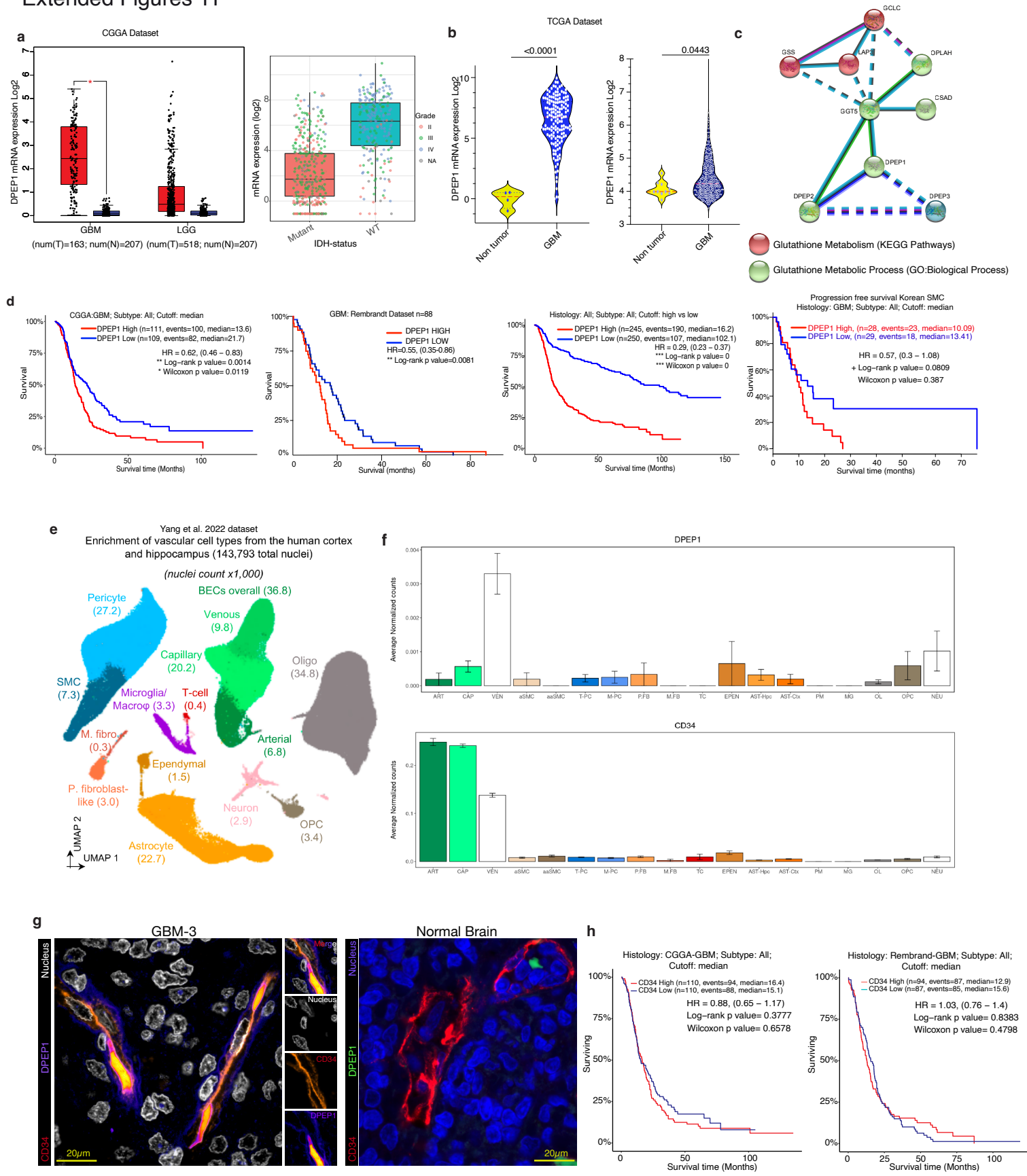


Extended Data Fig.9





Extended Figures 11



Extended Figures 12

