

Genomic Catastrophe Defines the Evolutionary Trajectory of Adrenocortical Carcinoma

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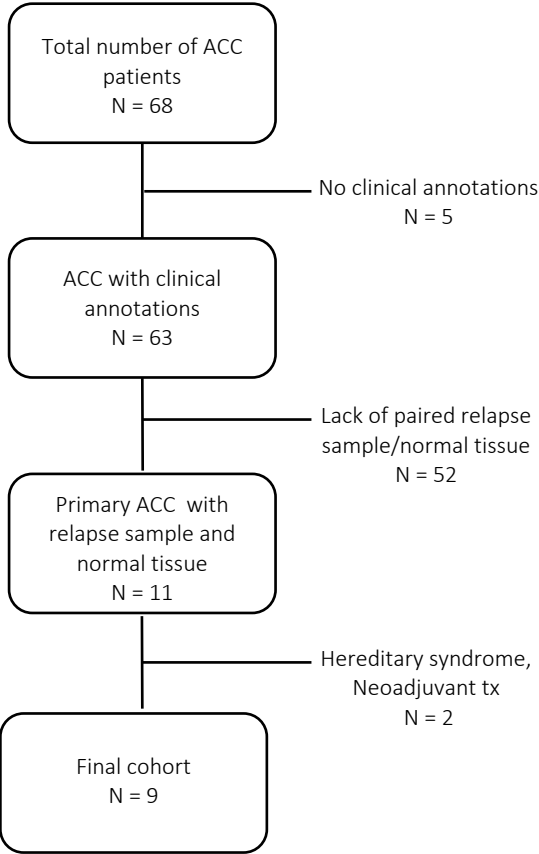
Tobias Åkerström

Department of Surgical Sciences, Uppsala University hospital.

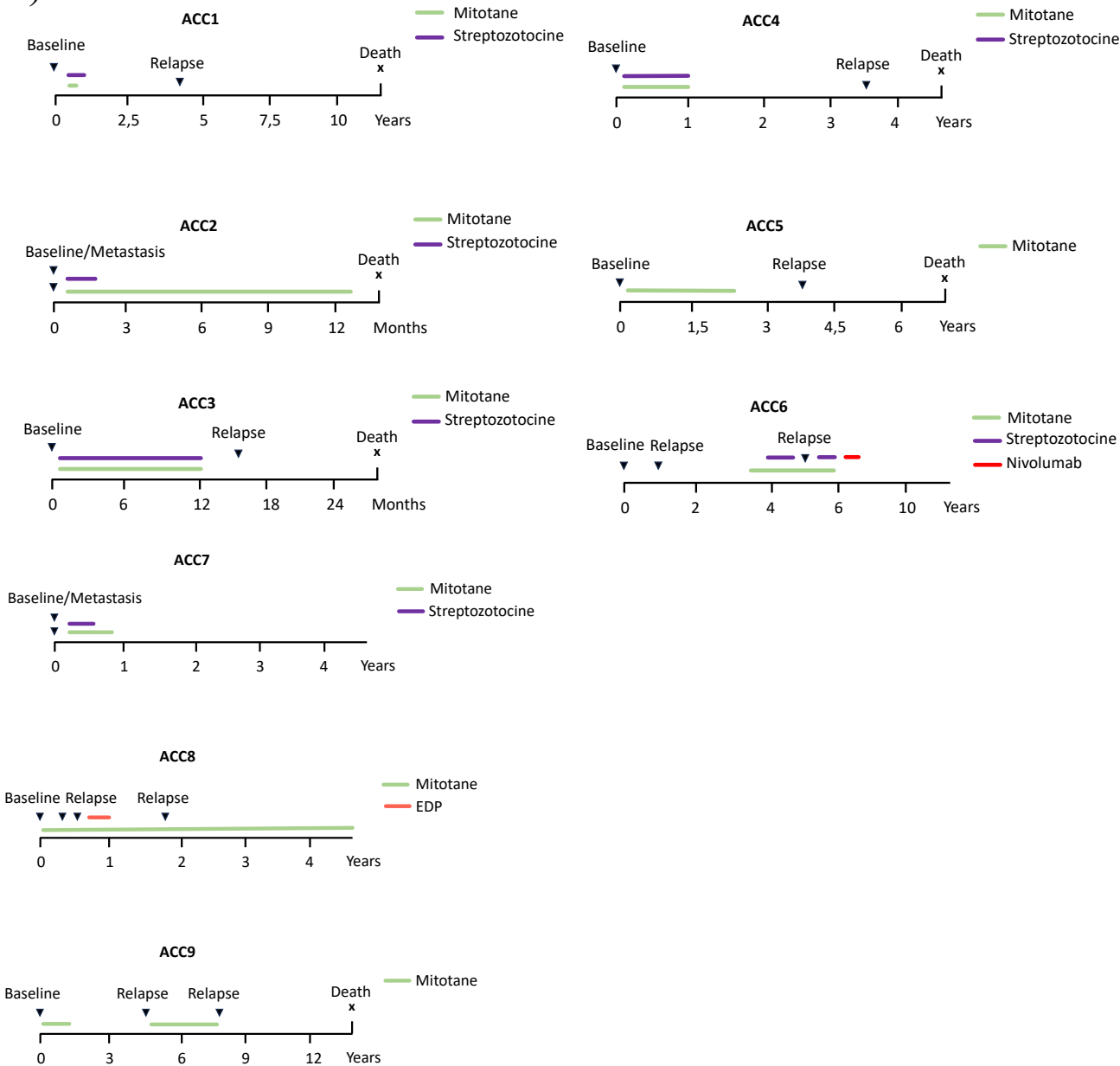
75185, Uppsala, Sweden.

Supplementary Figure 1. A) Flowchart illustrating patient and sample inclusion for the cohort. From the total number of patients initially identified, cases were excluded based on defined criteria specified in the figure. **B)** Clinical course and treatment timeline of patients included in the cohort. Black arrowheads denote baseline sampling and relapse events, while black crosses mark time of death. Time is shown in years or months as specified on each axis.

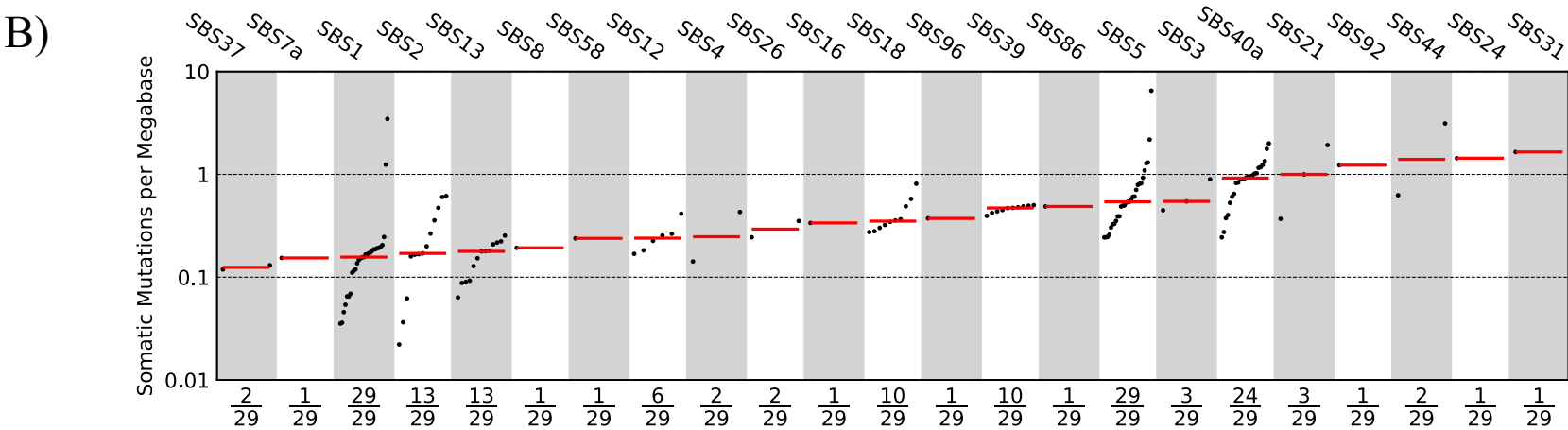
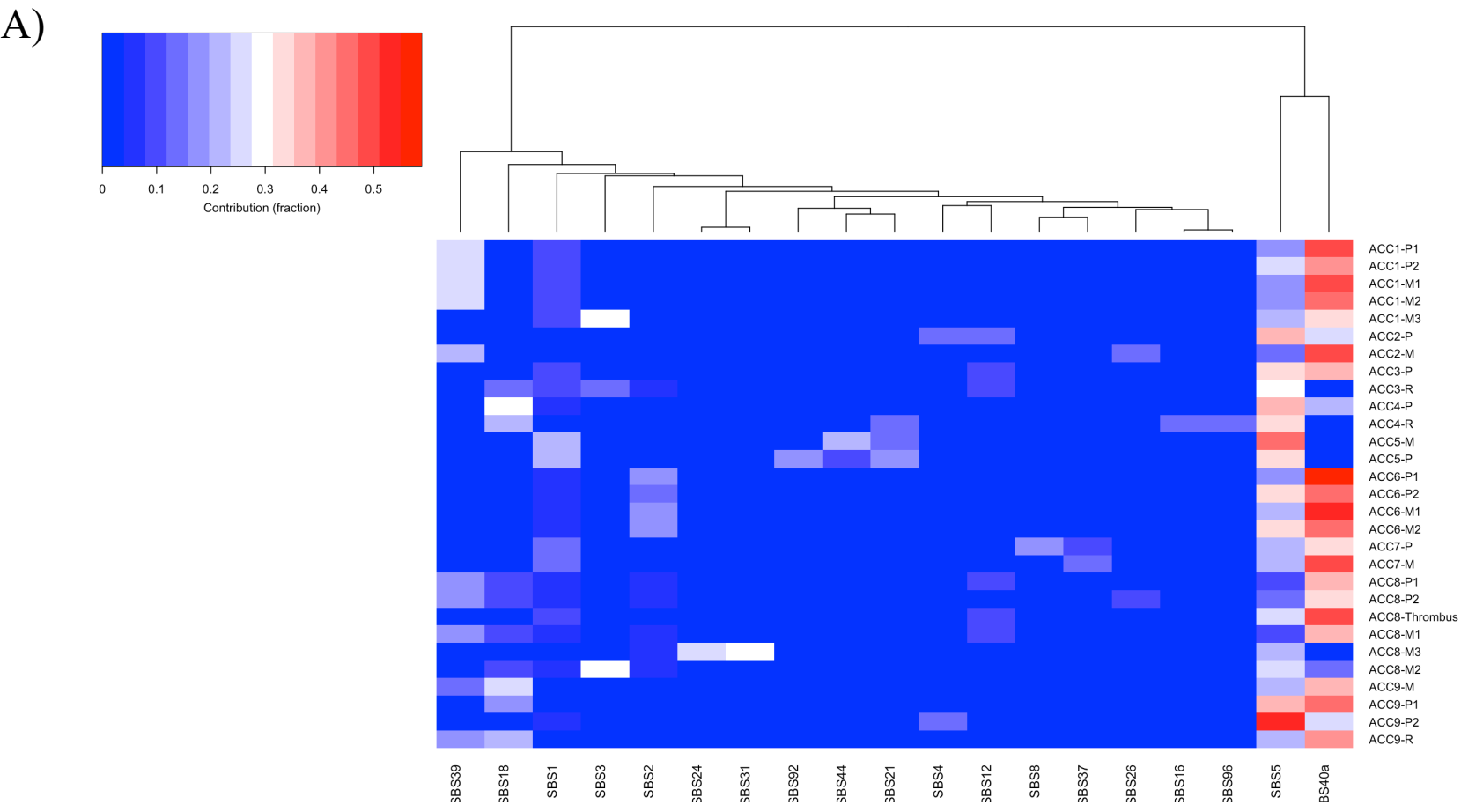
A)



B)

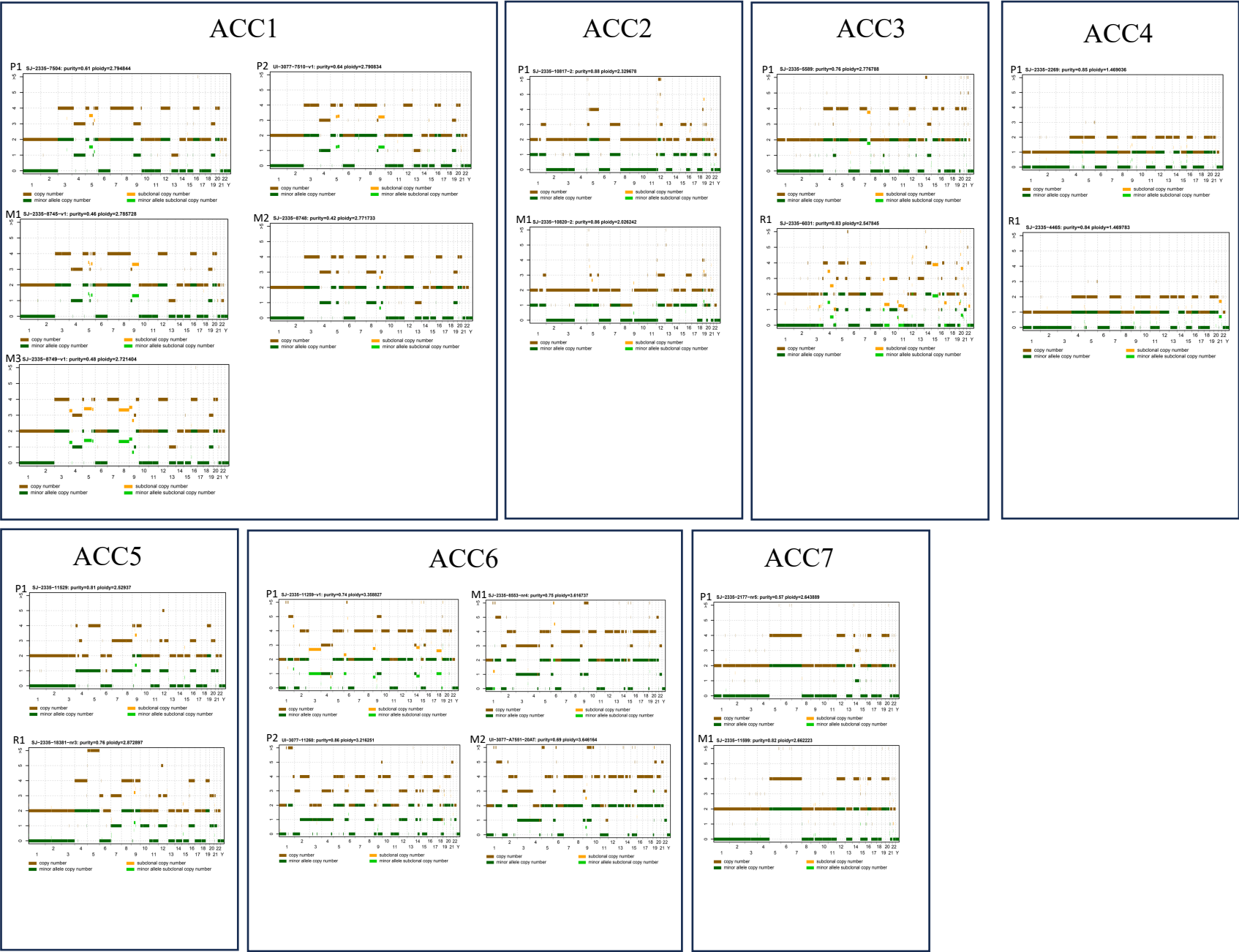


Supplementary Figure 2. Mutational signature analysis. A) Heatmap displaying different mutational signatures. B) Number of samples displaying the different mutational signatures.

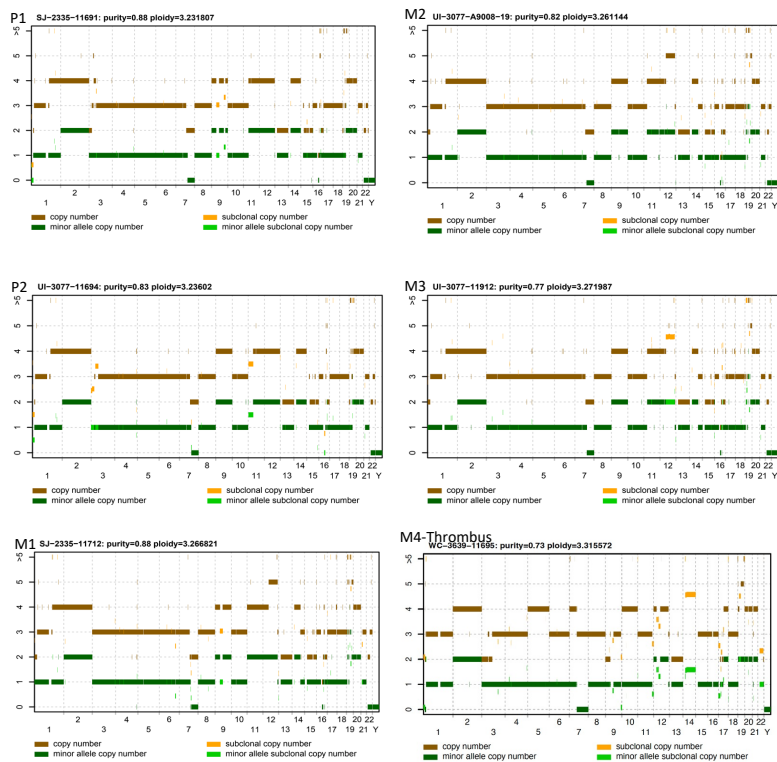


*Showing samples with counts more than 0

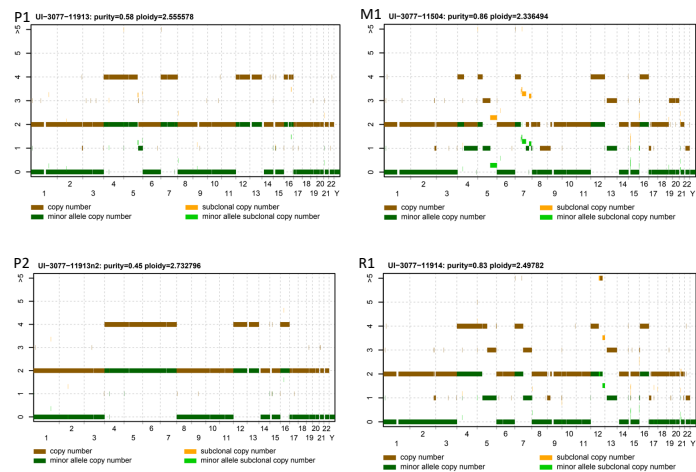
Supplementary Figure 3. Genome-wide allele-specific copy number profiles in adrenocortical carcinoma (ACC). Allele-specific copy number plots are shown for five representative ACC samples. Each panel displays the total copy number (brown) and the minor allele copy number (green) across all autosomes (x-axis). The y-axis indicates the absolute copy number.



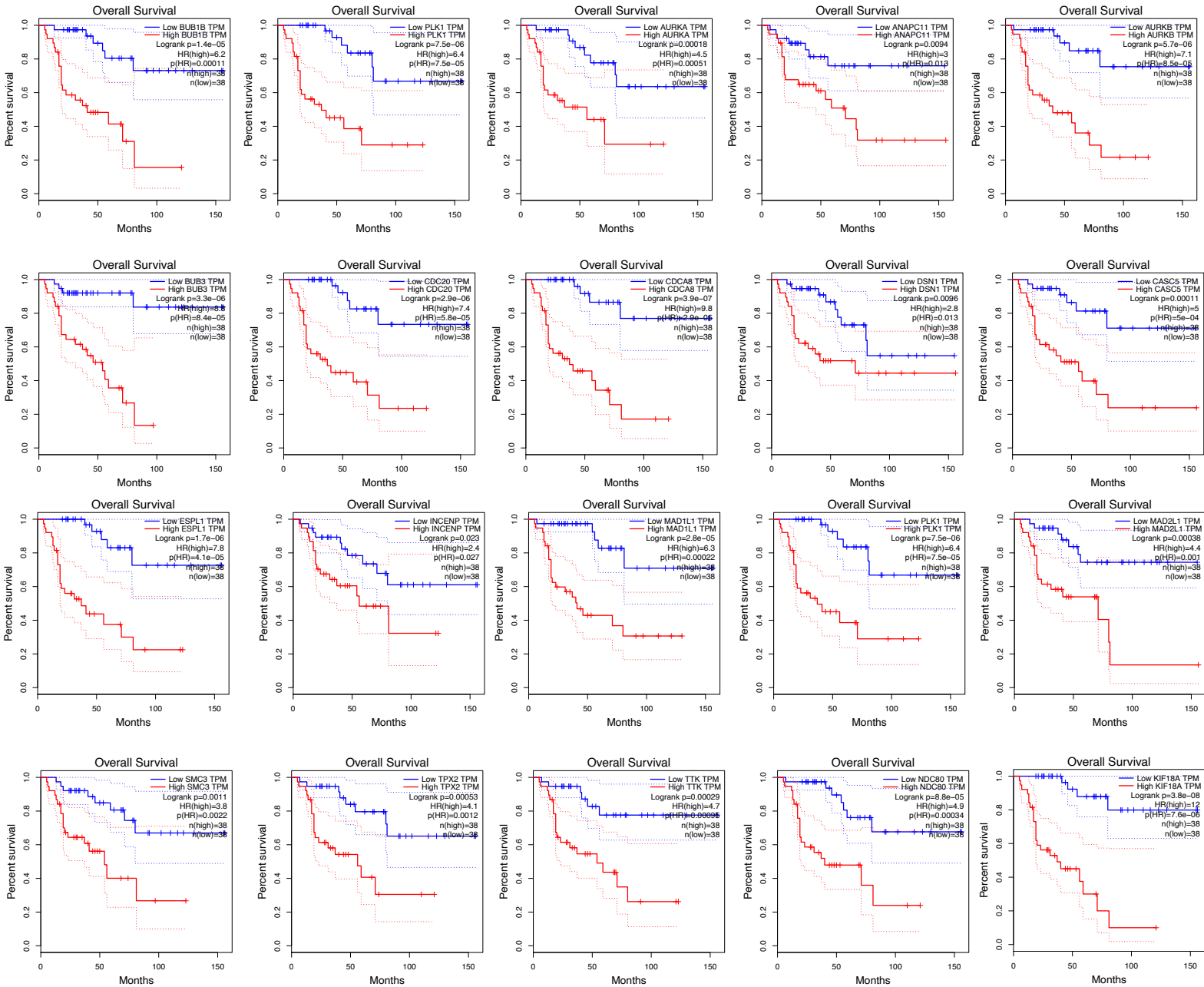
ACC8



ACC9

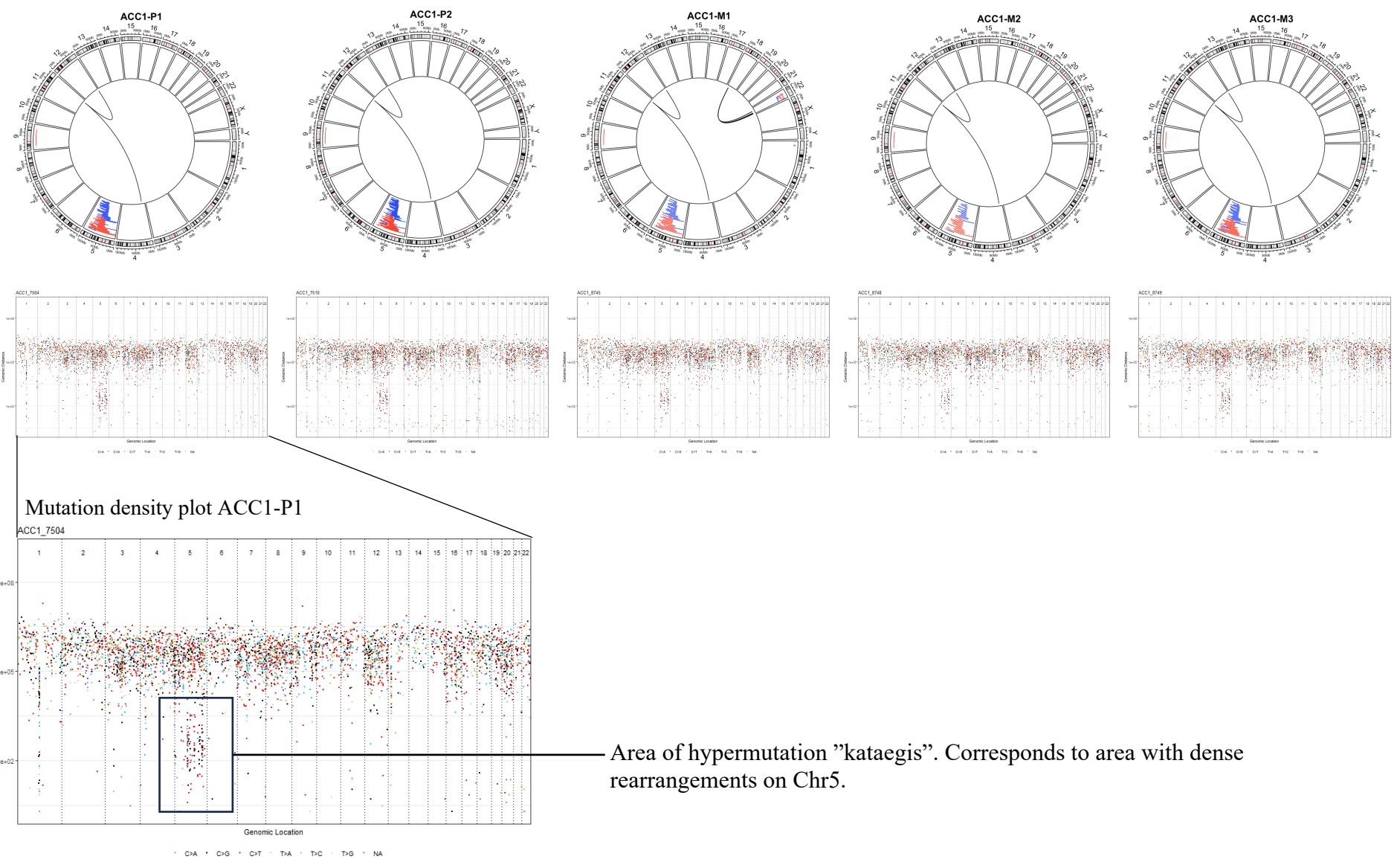


Supplementary Figure 4. Association between Spindle assembly checkpoint proteins and overall survival in adrenocortical carcinoma (ACC) using the TCGA cohort. Kaplan–Meier survival analysis comparing overall survival between patients with high (red) and low (blue). Figures adapted using GEPIA.

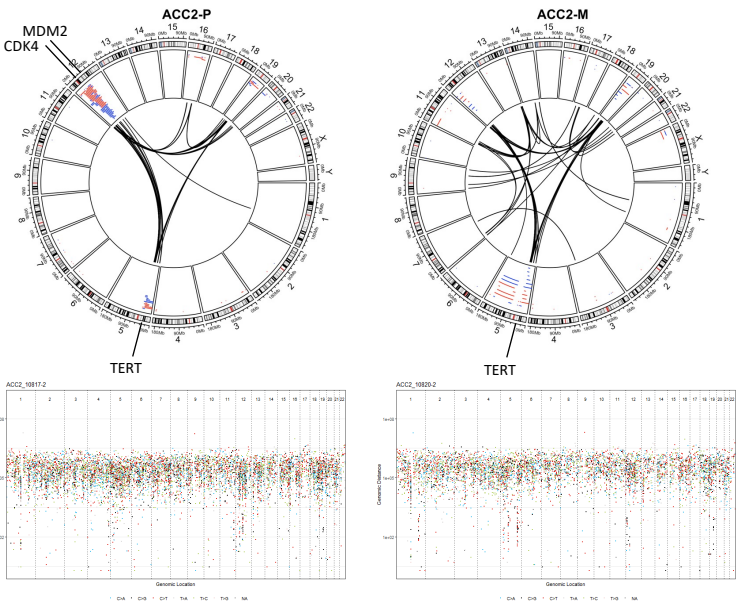


Supplementary Figure 5. Top row shows circos plots with structural rearrangements. Inner area depicts translocations; intrachromosomal deletions (blue) and duplications (red) are shown in the middle area. Middle row: Rainfall plots depicting mutation density. Bottom row: Rainfall plot of one tumour showing areas of hypermutation (Black square). P1: Primary tumour 1, R1: Relapse sample 1.

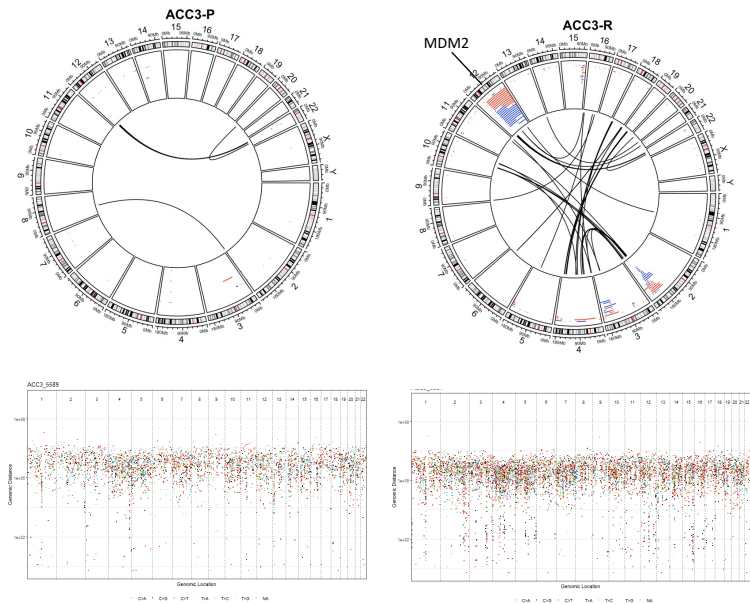
ACC1



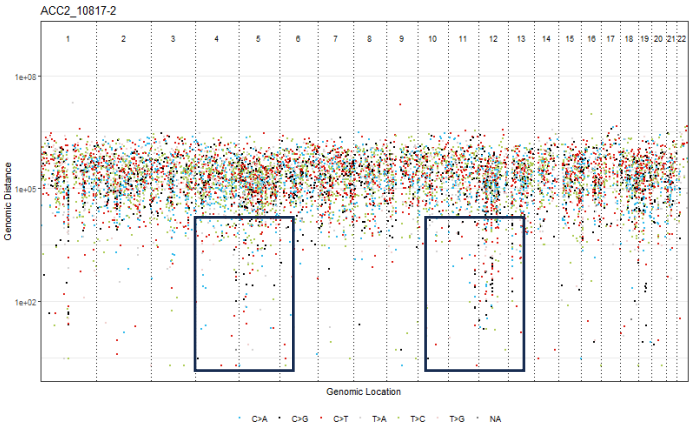
ACC2



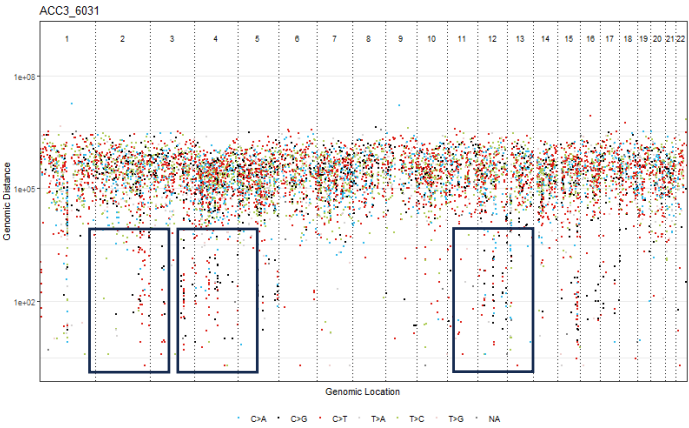
ACC3



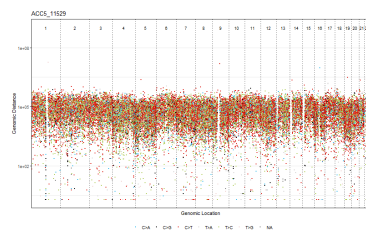
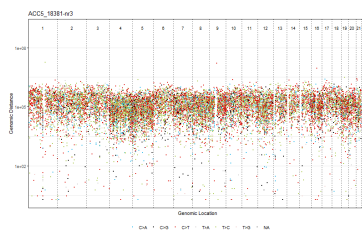
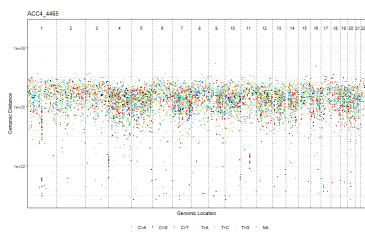
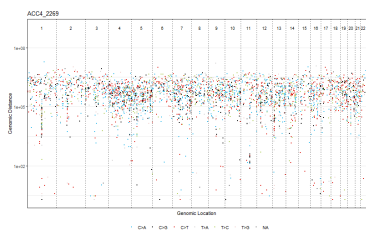
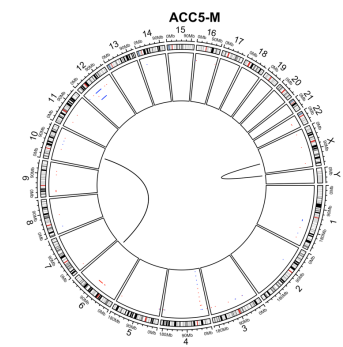
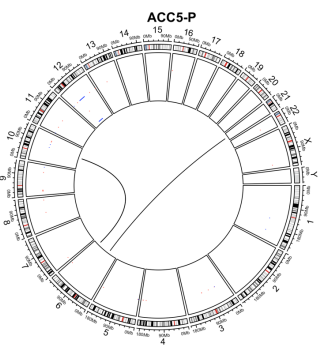
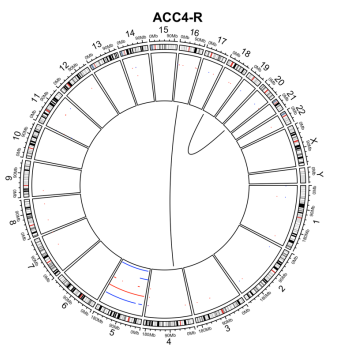
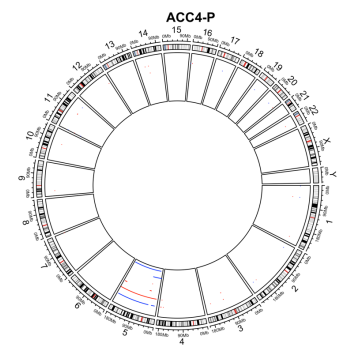
Mutation density plot ACC2_P1



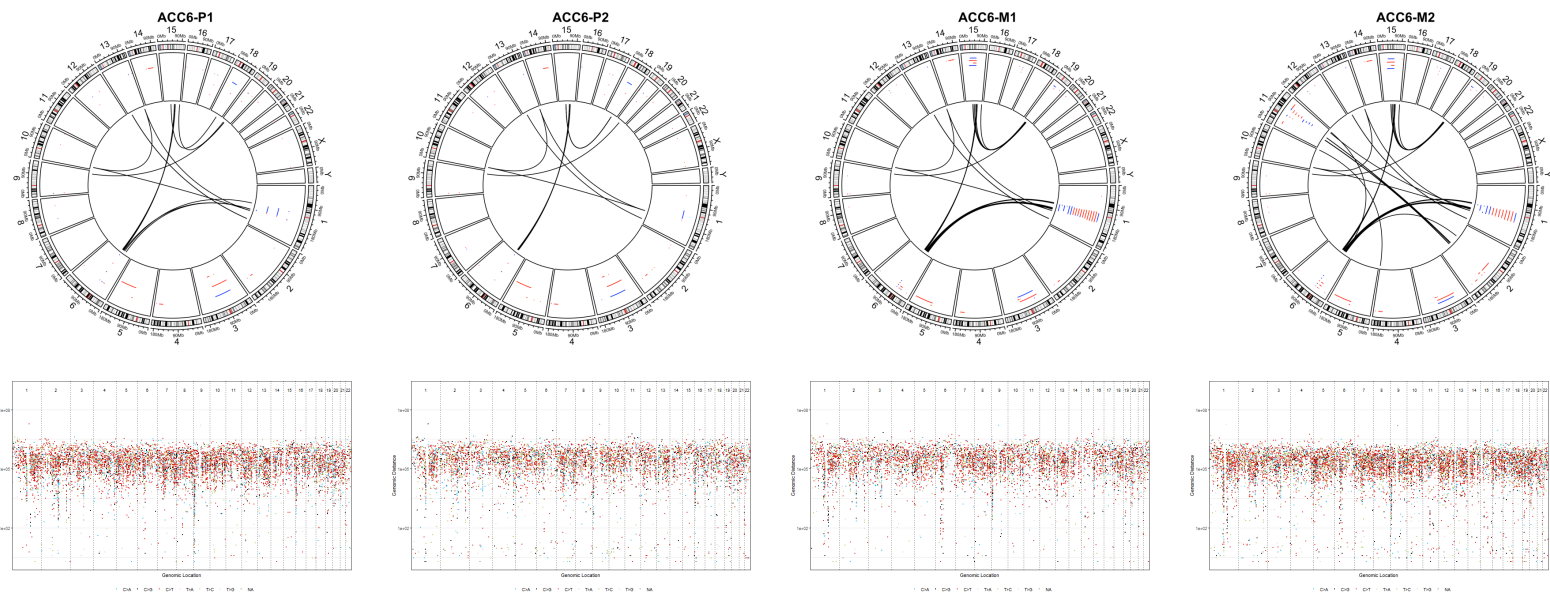
Mutation density plot ACC3_R1



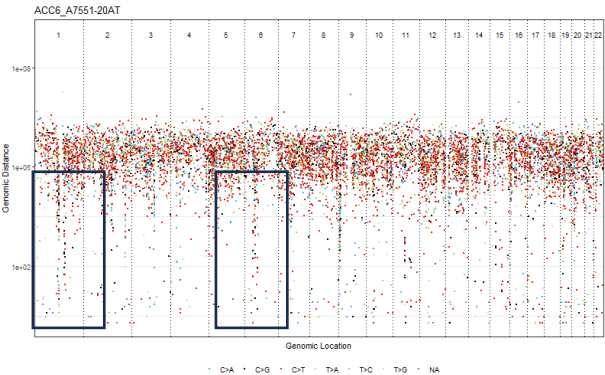
ACC4



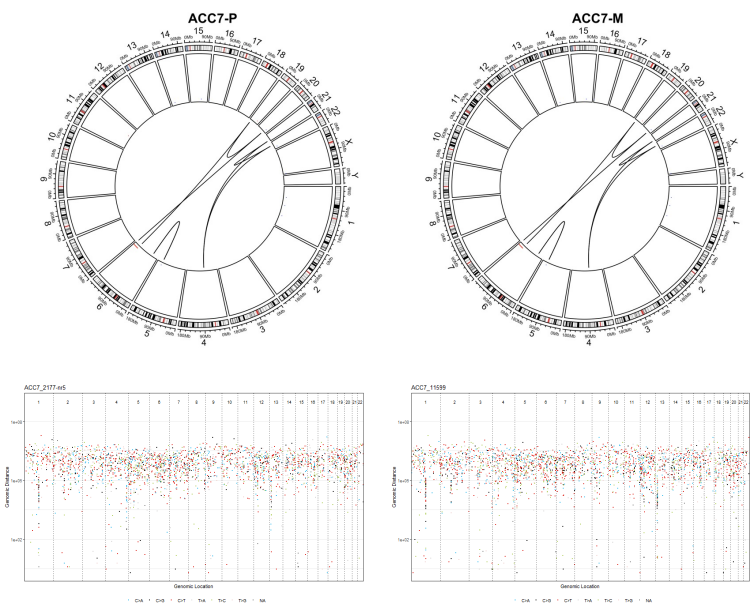
ACC6



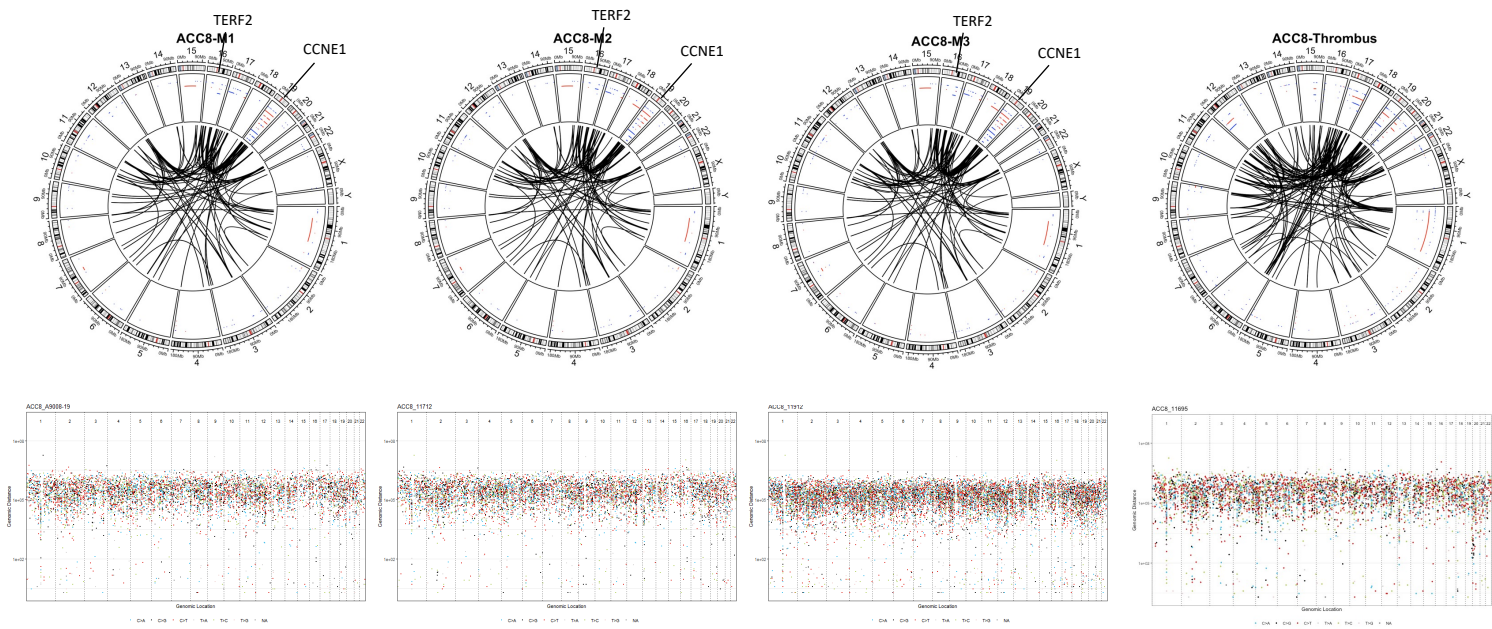
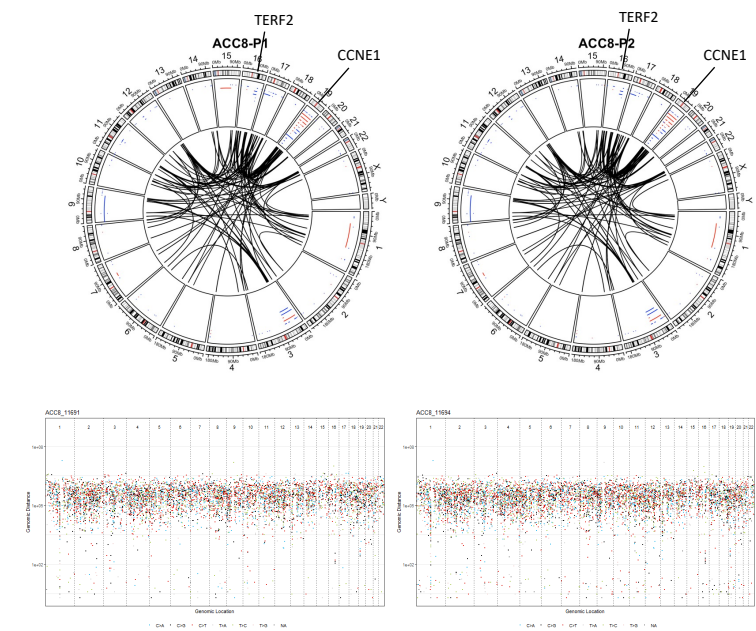
Mutation density plot ACC6_R2



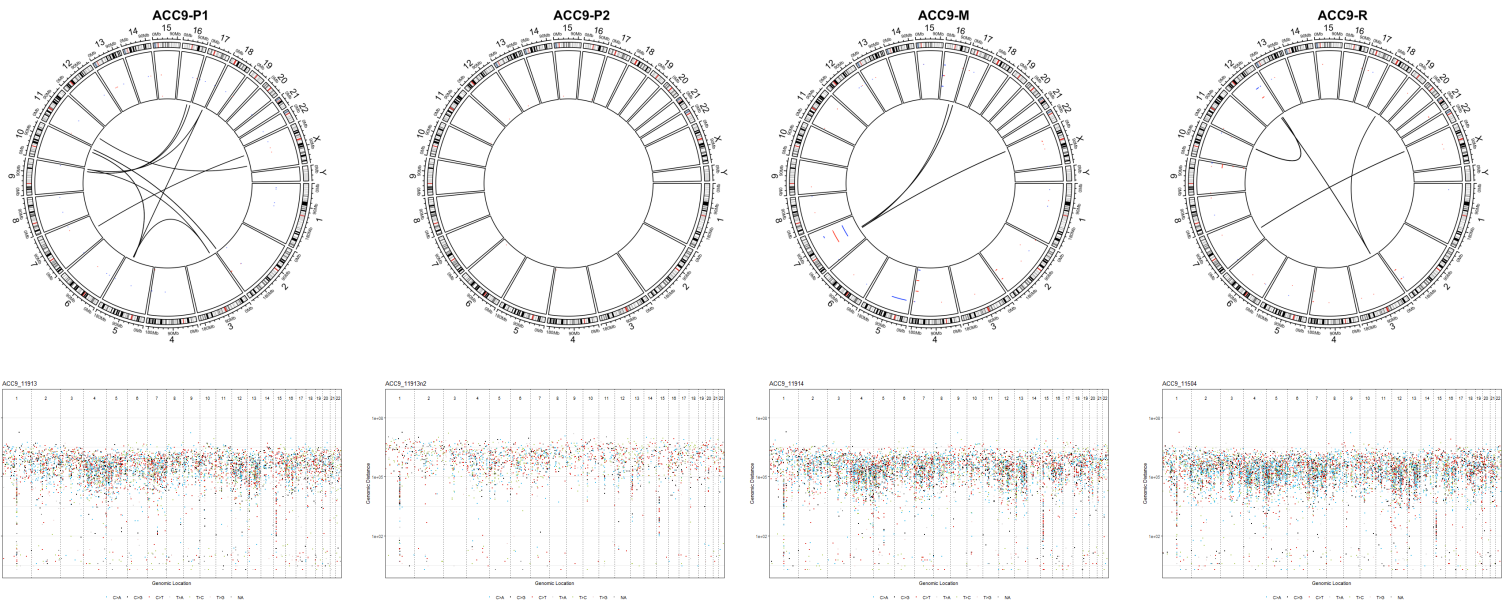
ACC7



ACC8

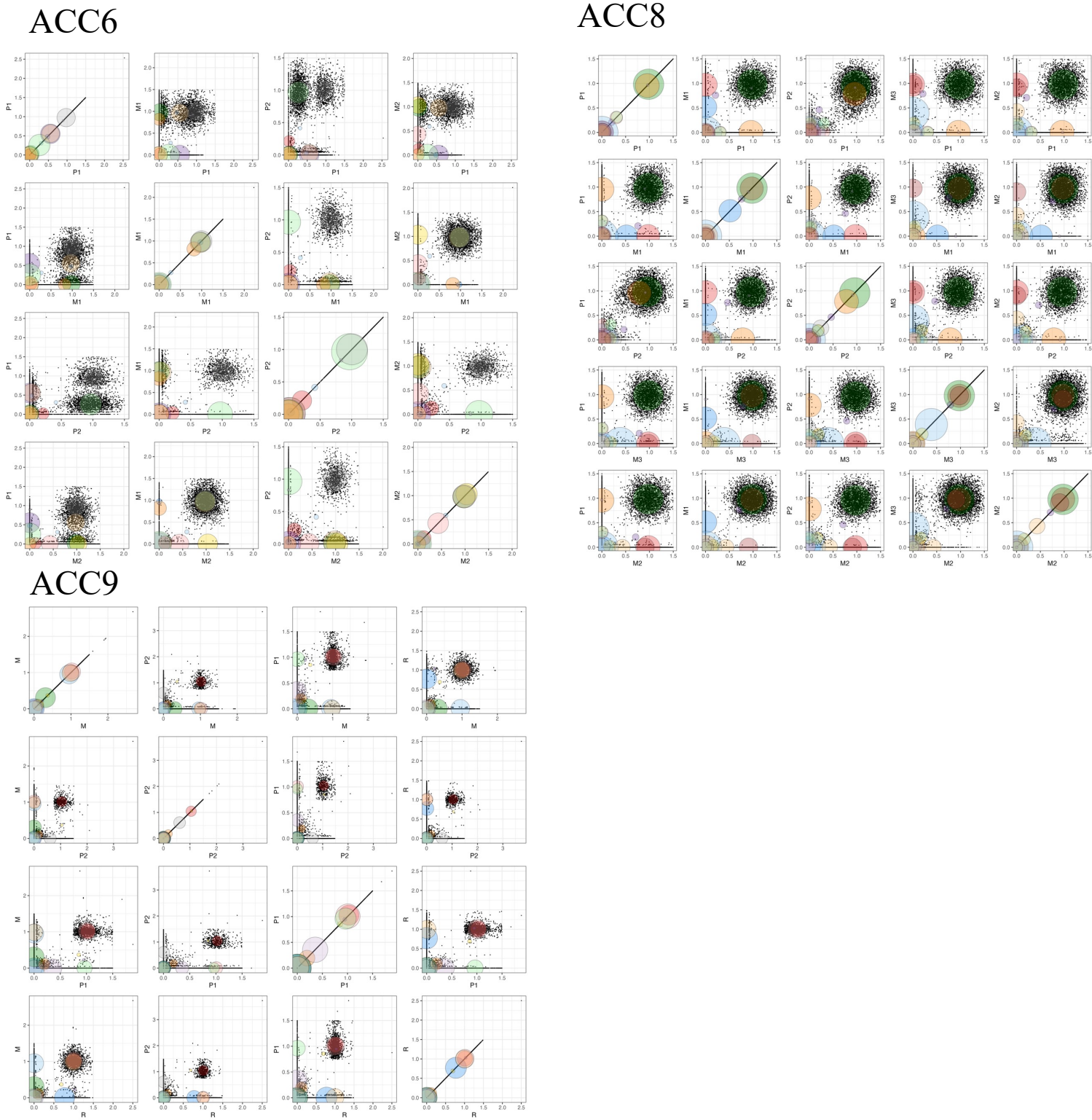


ACC9

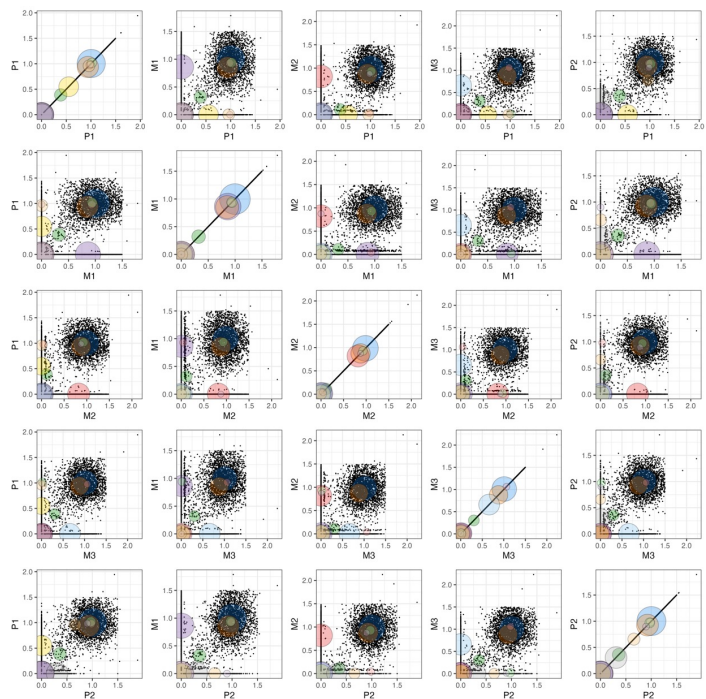


Supplementary Figure 6. Clonal architecture inferred by PyClone analysis.

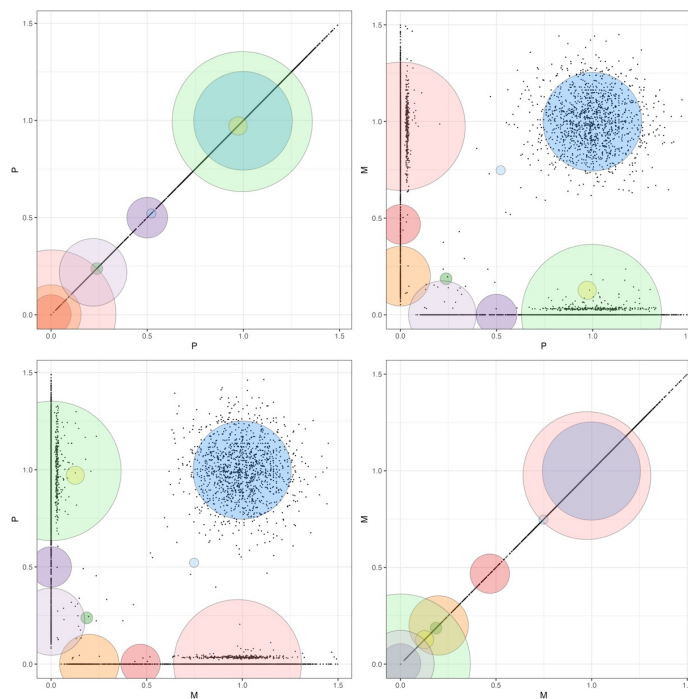
Scatter plot showing inferred cellular prevalence of somatic mutations based on PyClone clustering analysis. Each dot represents a somatic variant, plotted according to its cancer cell fraction (CCF) across samples. Colored circles indicate cellular populations inferred by Bayesian clustering, corresponding to distinct subclones within the tumour.



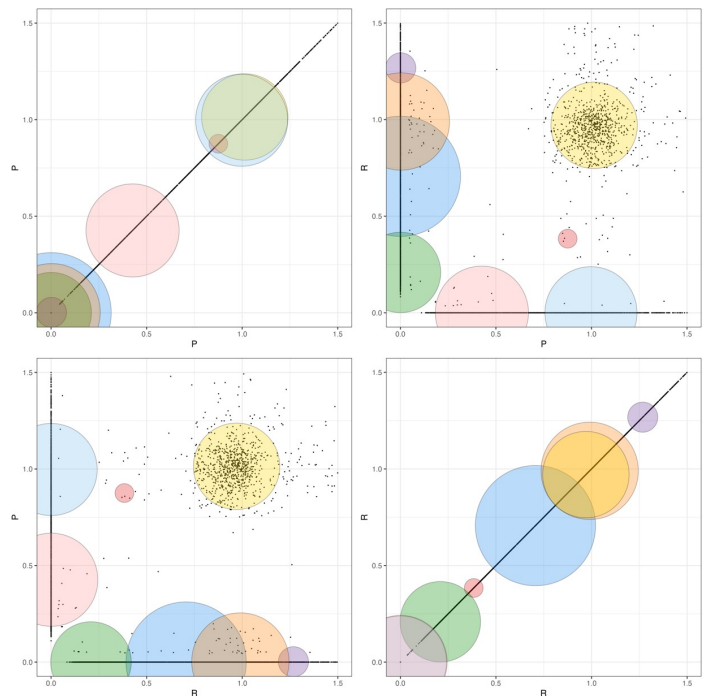
ACC1



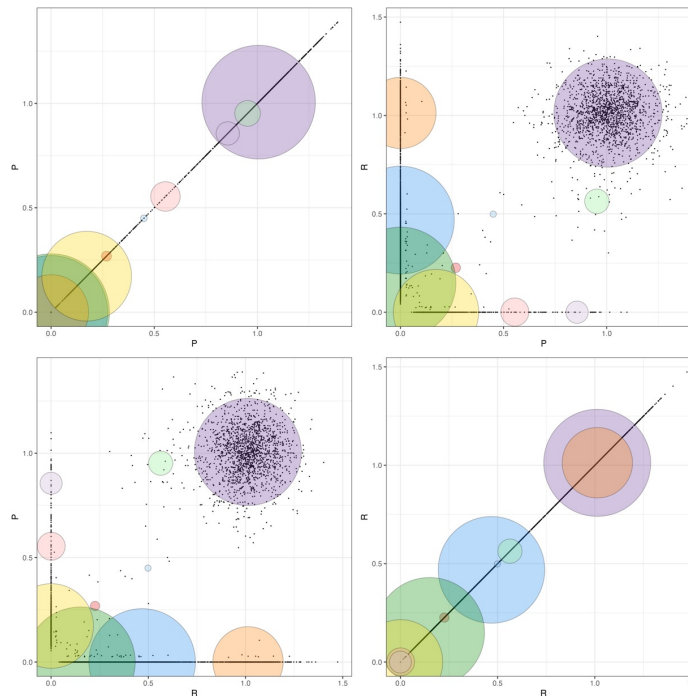
ACC2



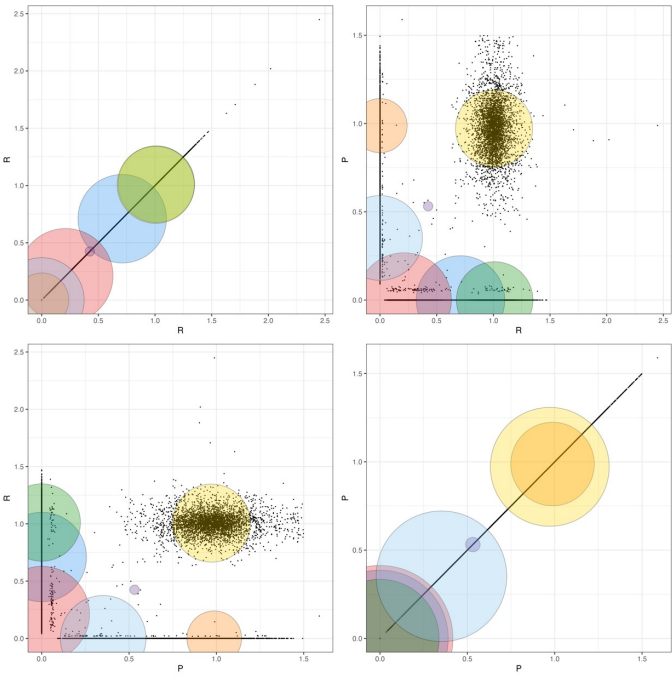
ACC3



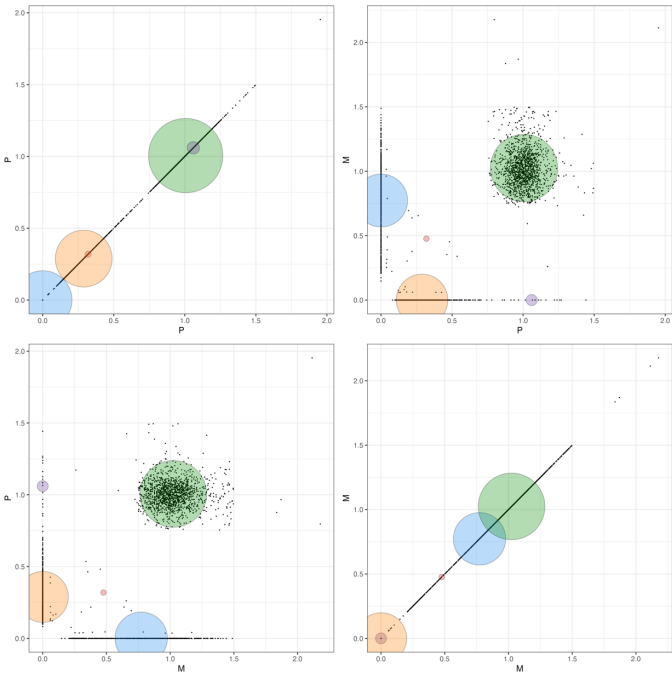
ACC4



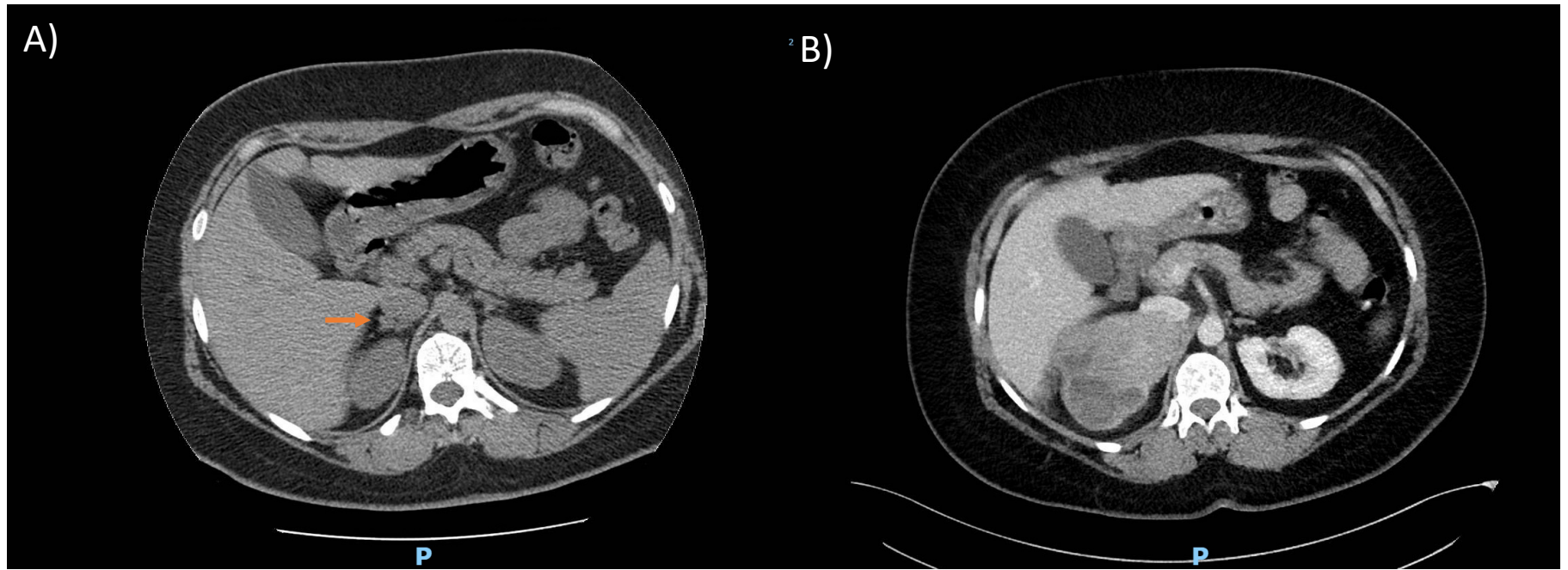
ACC5



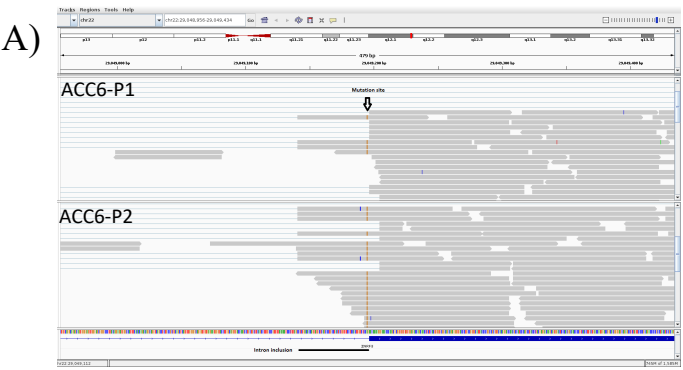
ACC7



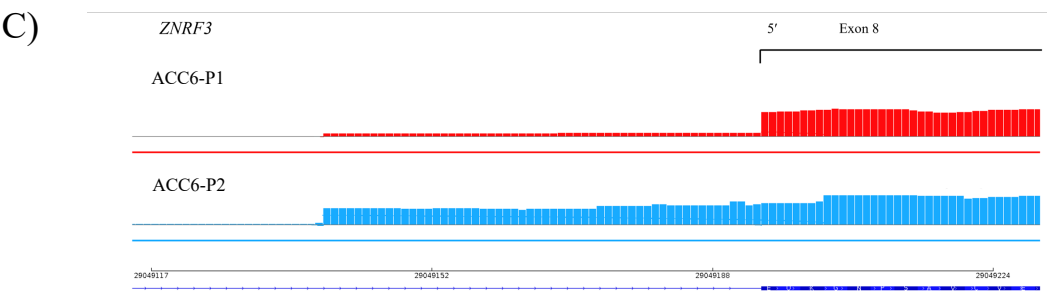
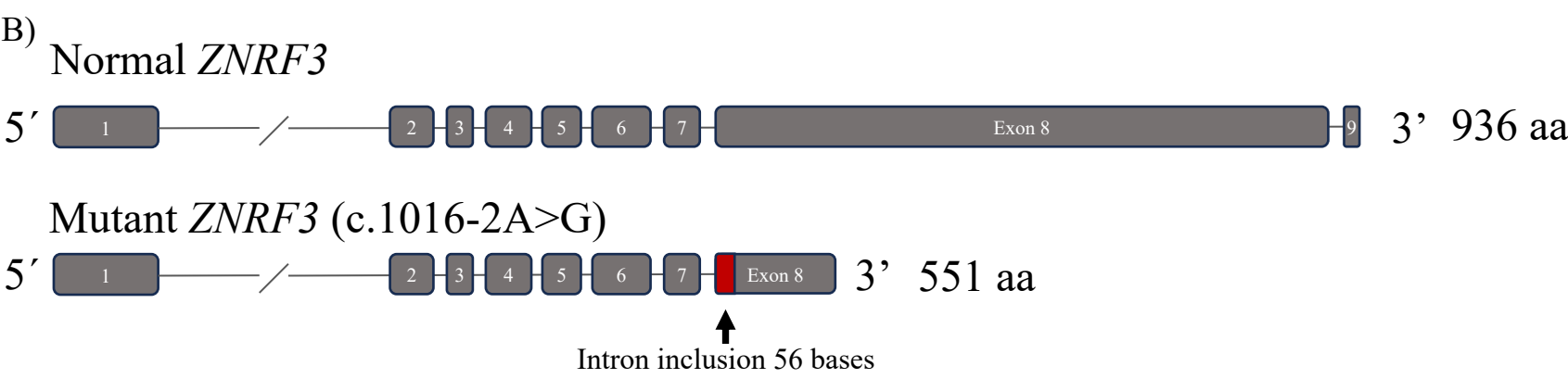
Supplementary Figure 7. Computer tomography of ACC6. A) CT scan of adrenals T=0 showing a 10x7 mm nodule (arrow) on the right adrenal measuring 33 Hounsfield units. B) CT scan at T+4 years with a 10 cm ACC.



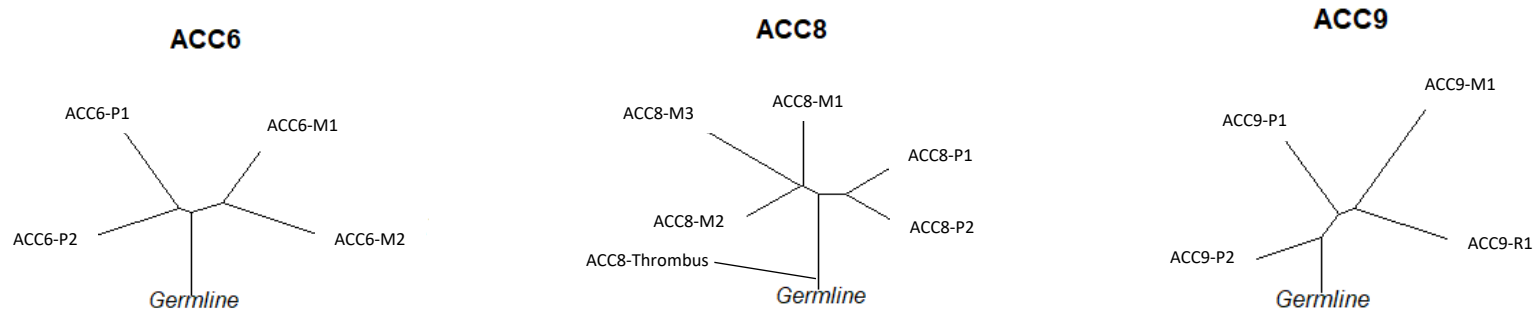
Supplementary Figure 8. A) Read frames for the c.1016-2A>G mutation in *ZNRF3*. Top row – ACC6_P1 with low allele frequency of *ZNRF3* mutation. Bottom row ACC6_P2. **B)** Visualization of the consequence on the mRNA molecule of the c.1016-2A>G mutation (Ensembl ENSG00000183579). C) Plot displaying genomic expression consequences of the c.1016-2A>G mutation in wild type ACC6_P1 and mutated ACC6_P2.



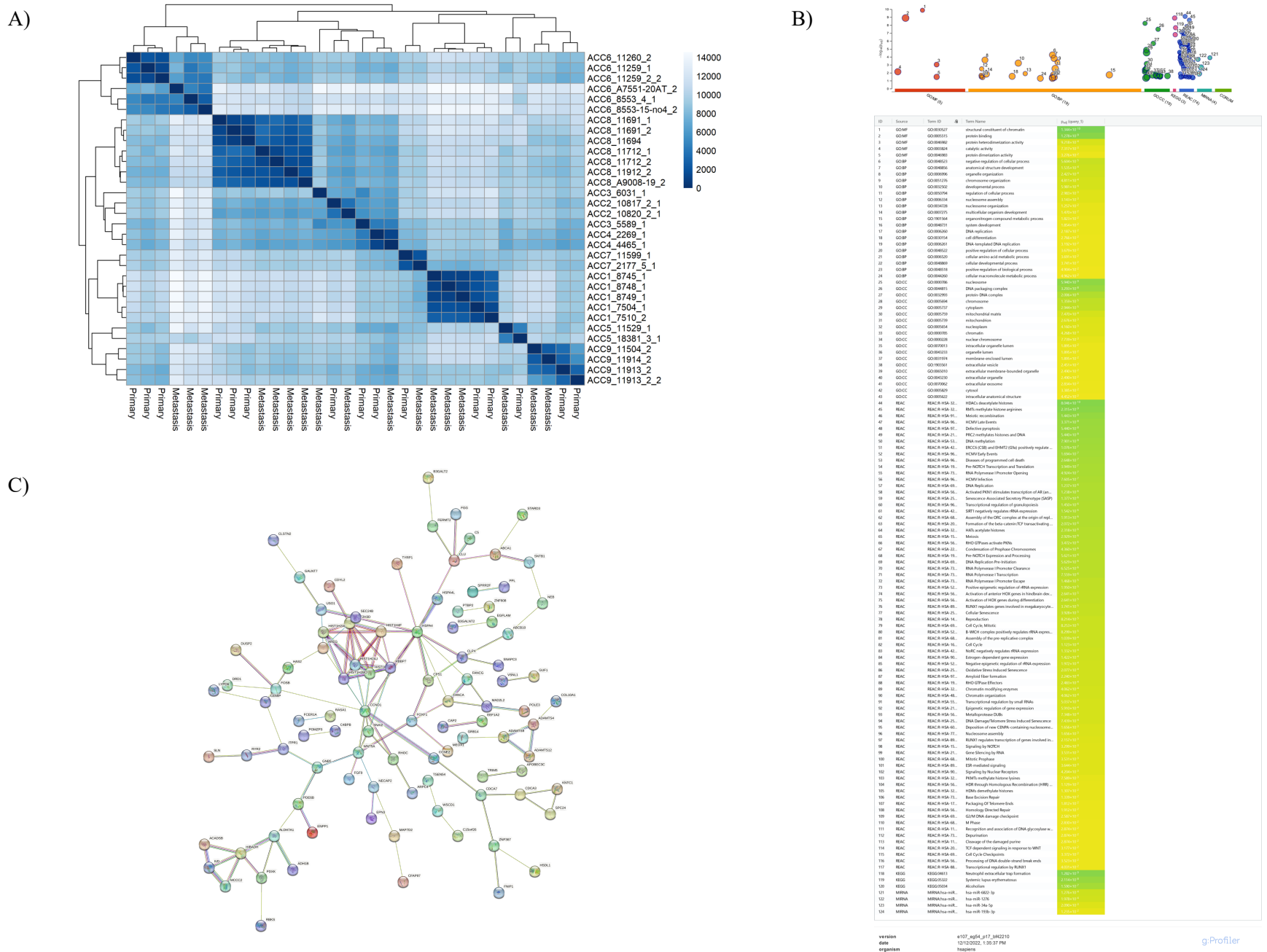
Inclusion of bases chr22:29,049,141-29,049,197



Supplementary Figure 8. Raw phylogenetic tree based on somatic single nucleotide substitutions identified across multiple tumours and matched germline DNA from patient ACC6, ACC8 and ACC9. Branch lengths represent the number of private or shared mutations between samples. .



Supplementary Figure 9. A) Heatmap displaying pairwise correlation of RNA expression profiles among all tumour samples in the cohort. The x-axis denotes tumour type, and the y-axis lists individual samples. ACC6_11259_2_2; ACC8_11691_2 were used as controls. **B)** Gene ontology (GO) enrichment analysis. Functional enrichment analysis was performed using g:Profiler. The upper panel shows the distribution of enriched GO terms grouped by functional category. The lower panel lists the top enriched pathways ranked by adjusted *p*-value (Benjamini–Hochberg correction). **C)** Protein–protein interaction and pathway enrichment network of genes differentially expressed between primary and relapse samples. Network visualization of significantly enriched pathways derived from differentially expressed genes between primary and relapse ACC tumours. The analysis was performed using STRING. Interconnected clusters centered around genes involved in cell cycle regulation, mitotic spindle assembly, chromatin organization, and DNA repair.



Supplementary Figure 10. Heatmap showing DNA methylation clustering. Each column represents an individual CpG site, and each row corresponds to a tumour sample. Color intensity reflects methylation level, ranging from low (blue) to high (yellow). Colored side bars indicate patient identity, white: primary; black: metastatic; grey: recurrent sample.

