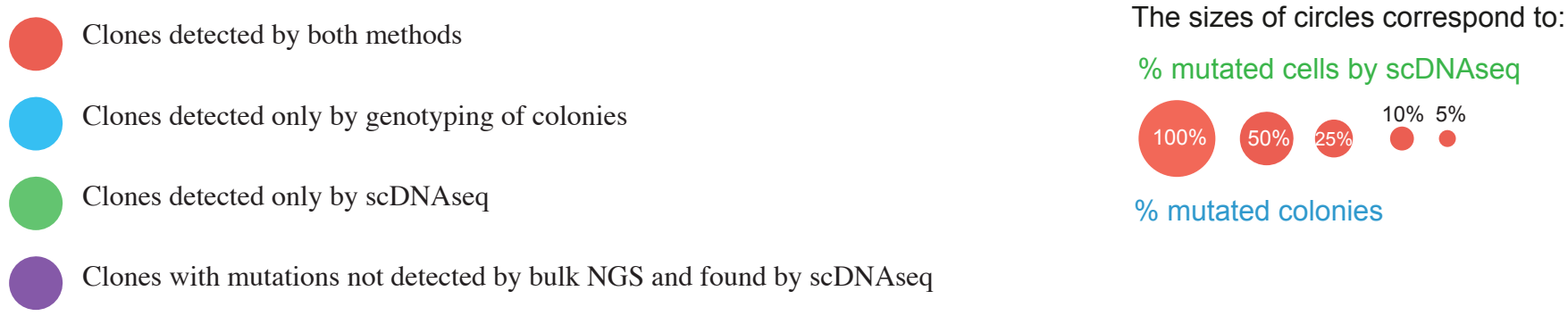
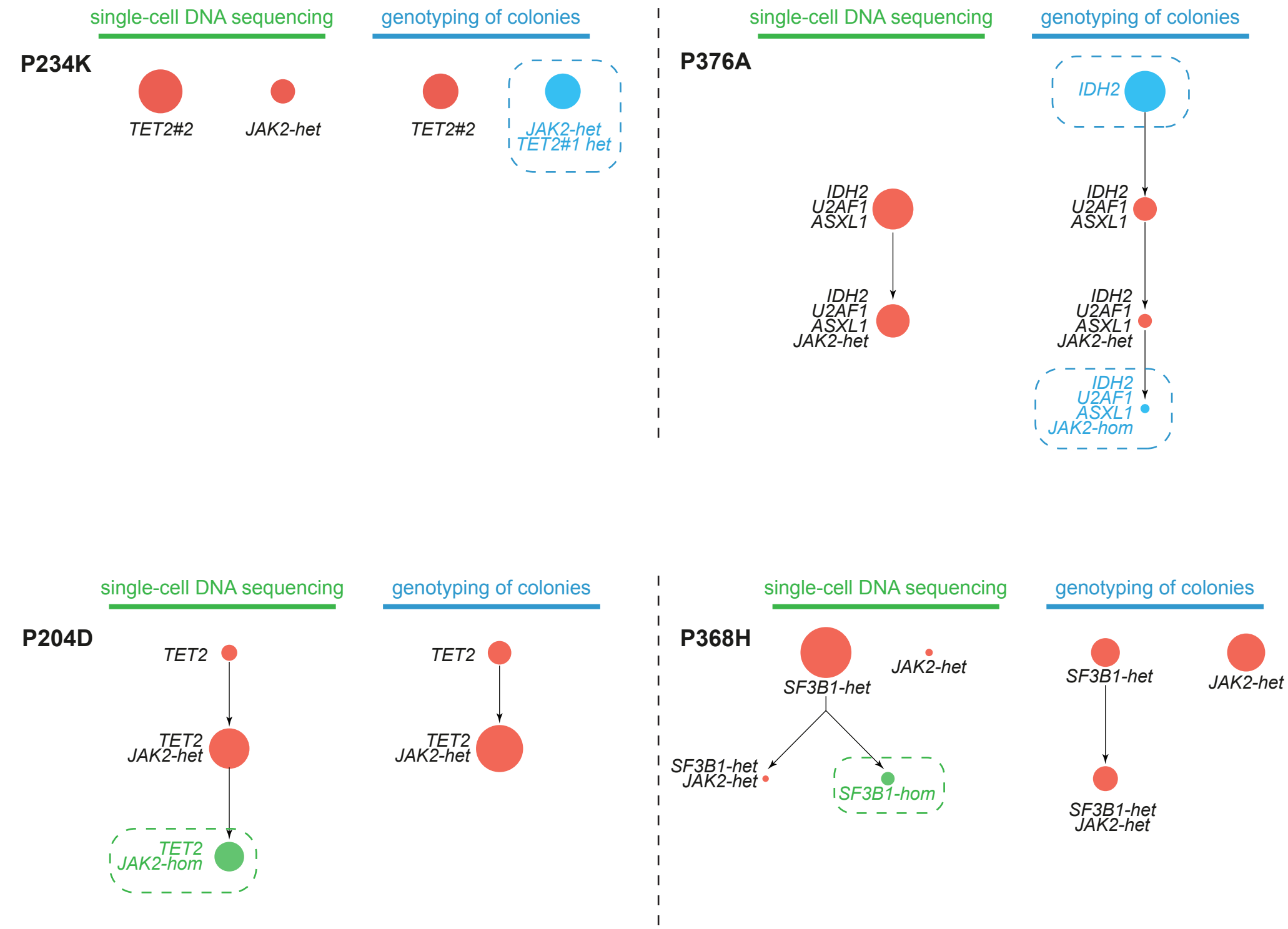
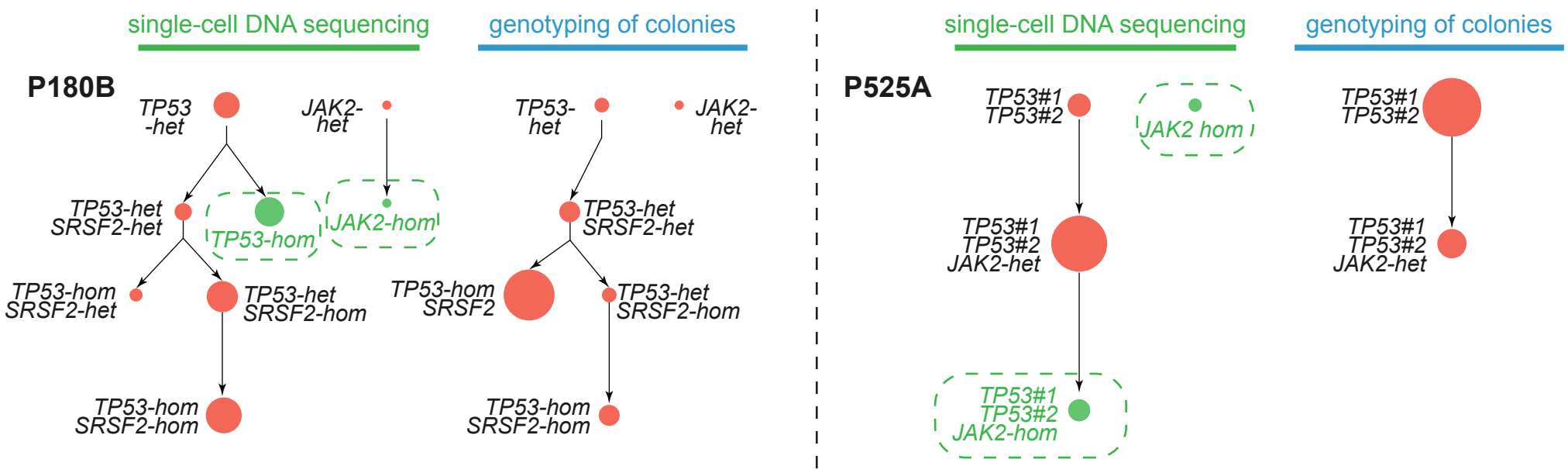
Supplemental Figure S1 (related to Figure 3)



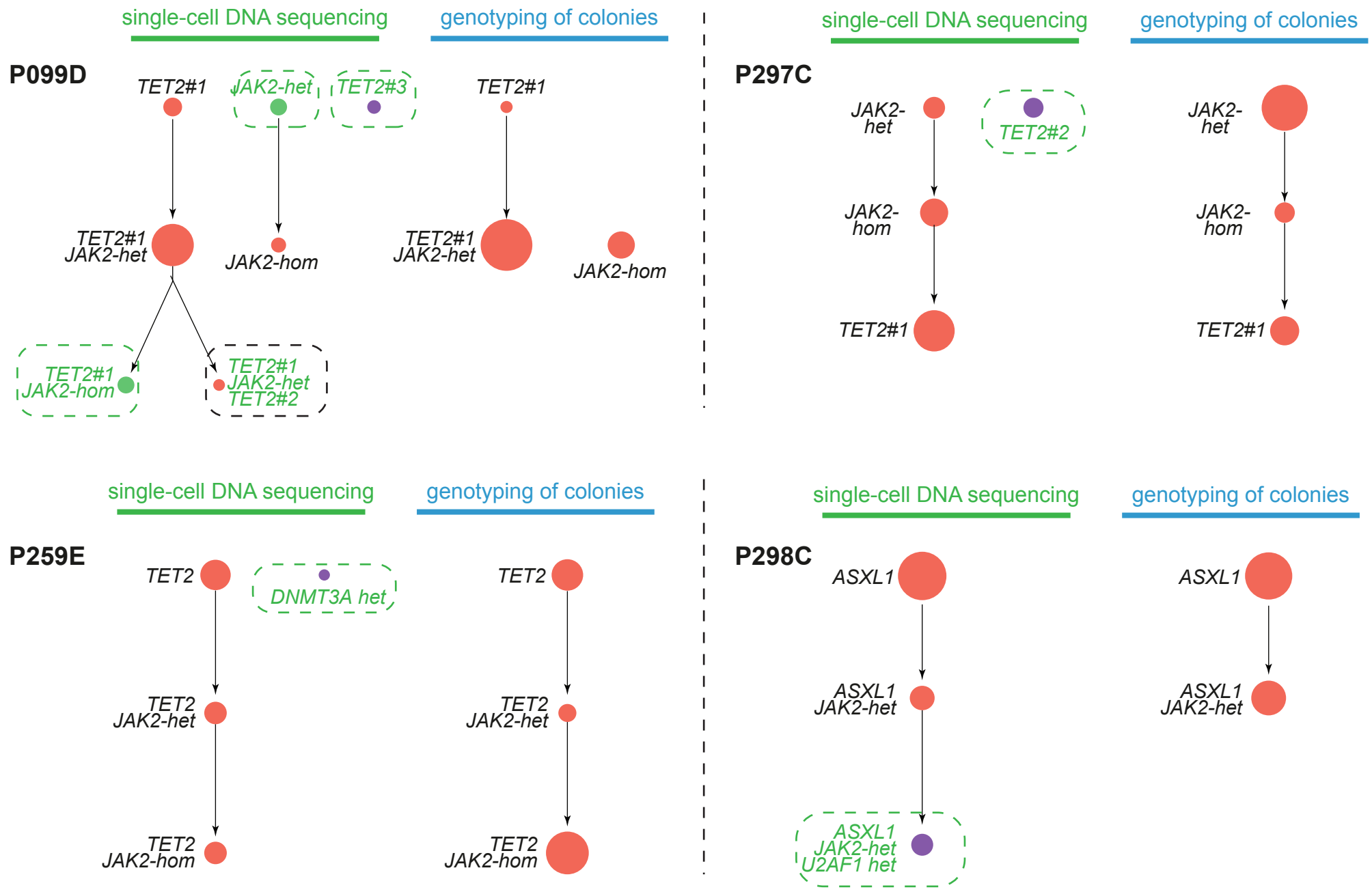
A Patients with subclones detected only by genotyping of colonies



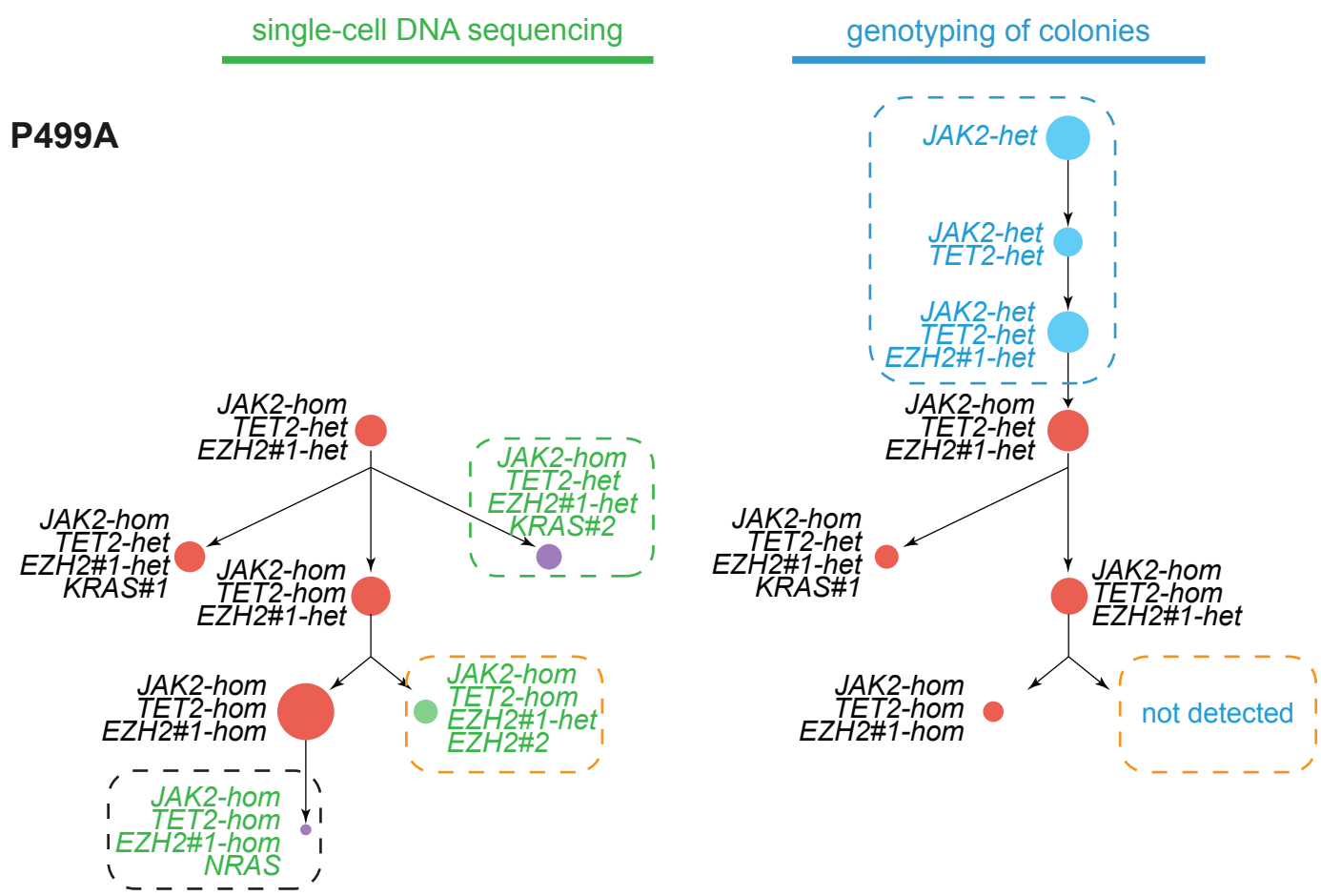
B Patients with subclones detected only by single-cell DNA sequencing of PBMCs



C Patients with subclones detected only by single-cell DNA sequencing of PBMCs and not previously detected by targeted NGS



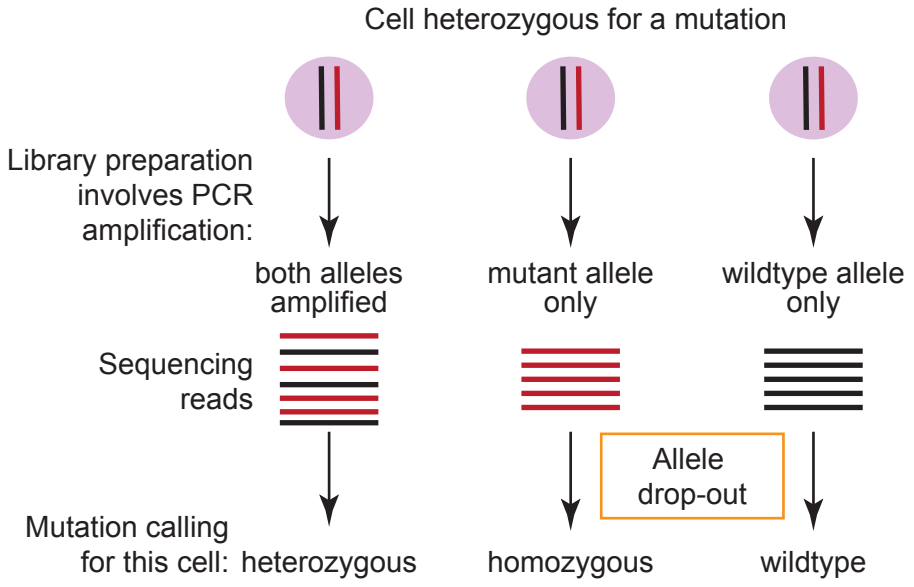
D Complex case with some subclones detected only by single-cell DNA sequencing and others detected only be genotyping of colonies



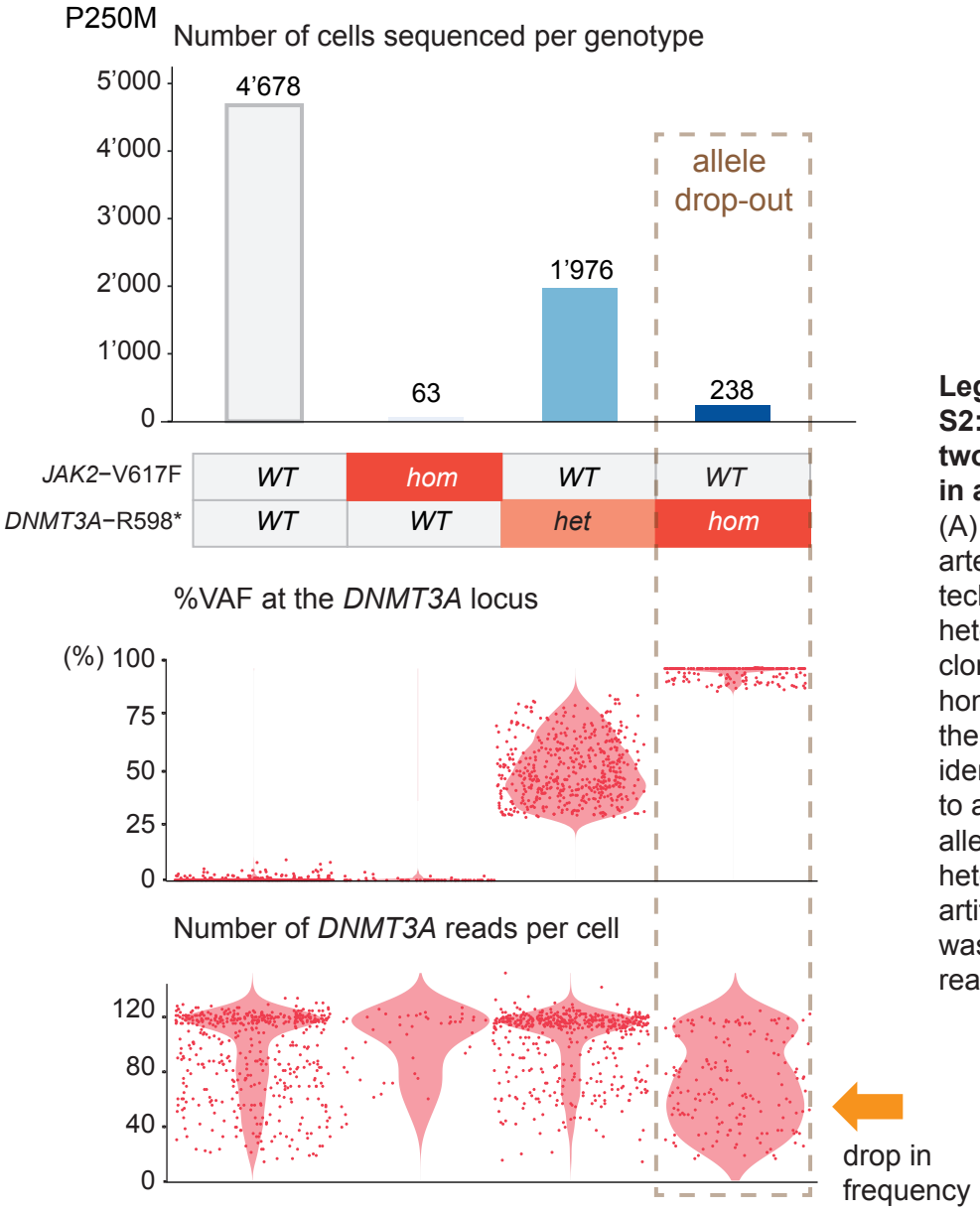
Legend to Supplemental Figure S1: Comparison between clonal architecture derived from from scDNAseq of PBMCs versus genotyping of colonies. The order of acquisition of mutations is drawn from top to bottom. Each subclone is represented by a circle. The size of each circle corresponds to it's percentage when the sum of all mutated subclones is set to 100%.(A) Clones highlighted in blue only by genotyping of colonies , and (B) clones highlighted in green were detected only by scDNA-seq. (C) Note that some subclones with violet circles were found only by scDNAseq and represent new mutations not previously detected by bulk NGS. All gene mutations previouls identified by bulk NGS were also detected by genotyping of colonies, with the exception of (D) one subclone with a *EZH2* gene mutation in patient P499A.

Supplemental Figure S2 (related to Figure 3)

A Amplification of only one of the two alleles results in allele drop-out

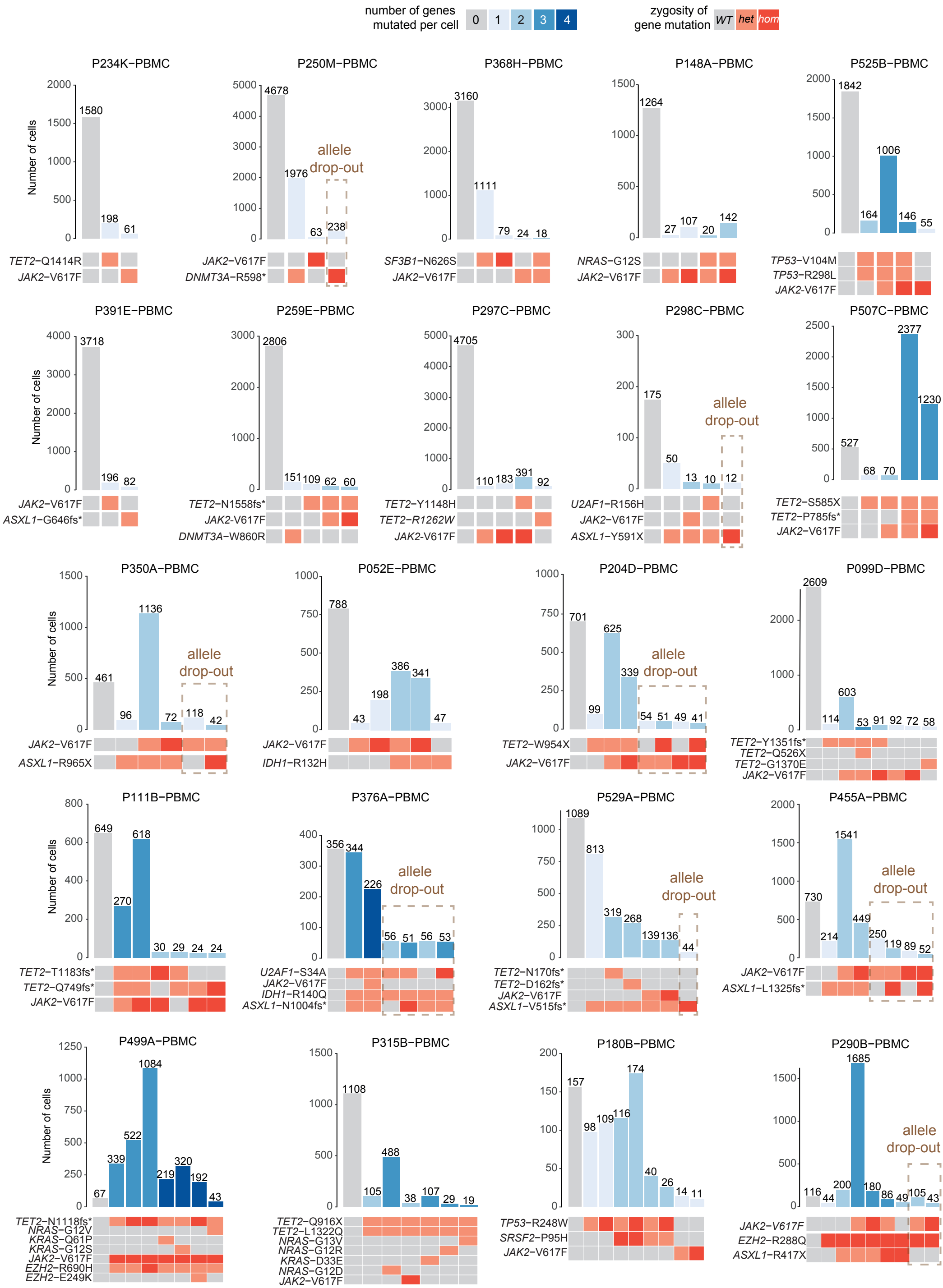


B Example of allele drop-out in a patient with *JAK2* and *DNMT3A*



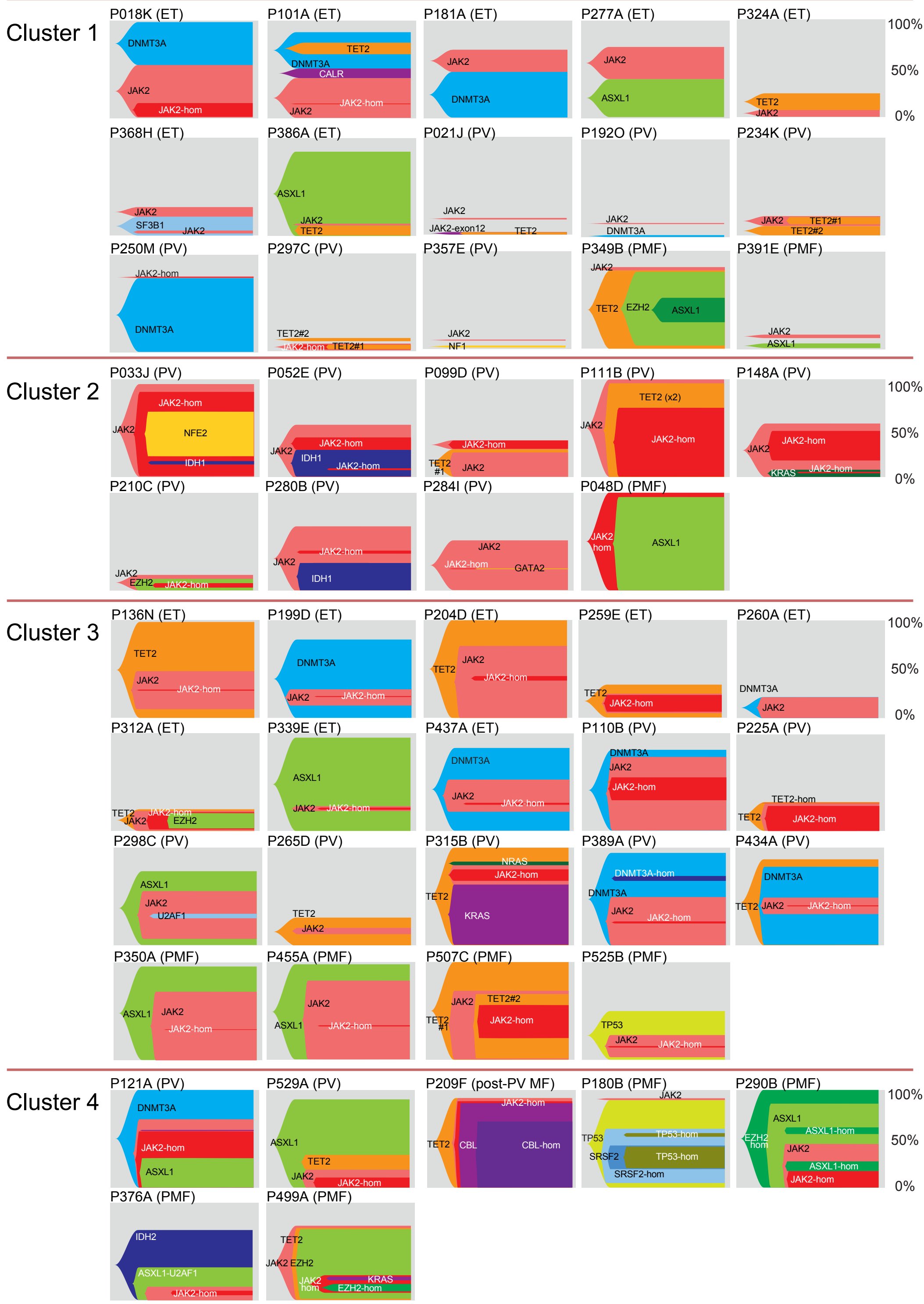
Legend to Supplemental Figure S2: Failure to amplify one of the two alleles by in scDNAseq results in allele drop-out artefacts. (A) Allele drop-out is an inherent artefact due to the scDNAseq technology producing from a heterozygous mutation false positive clone with either wildtype or homozygous mutation. (B) Curating the sequencing data allowed to identify and exclude the clones due to allele drop-out. In patient P250M, allele drop-out of a DNMT3A heterozygous mutation produced an artifactual homozygous clone which was identify because of a drop in read depth.

Supplemental Figure S3 (related to Figure 3)



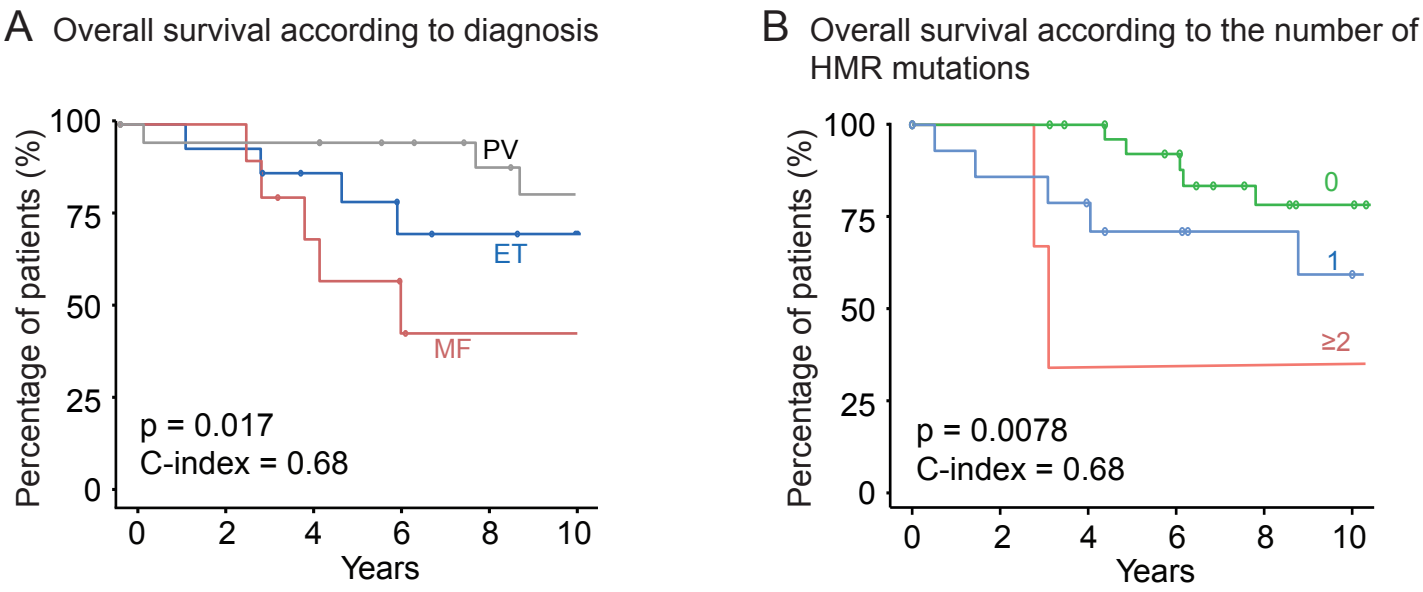
Legend to Supplemental Figure S3 (related to Figure 3): Bar graphs showing the number of cells for each genotype. Gray bars indicate the number of cells with wildtype genotype for all genes studied. Blue color darkness correlates with increased number of somatic mutations per cell, and the gray and red boxes below the bars indicate the zygosity for each of the gene mutations, as indicated by the scale on top. Subclones suspected to represent allele drop-out are marked by dashed boxes.

Supplemental Figure S4 (related to Figure 5)



Legend to Supplemental Figure S4. Fishplots representing the clonal architecture of all 50 MPN patients studied ordered according their assigned clusters. Clonal architecture was derived from genotyping of hematopoietic colonies (in methylcellulose or in liquid culture) and the size of subclones was proportionnal to the percentage of colonies with the corresponding gene mutation. Y-axis is set from 0 to 100% of colonies, as indicated on top right.

Supplemental Figure S5 (related to Figure 6)



Legend to Supplemental Figure S5: Overall survival according to diagnosis or the number of HMR mutations

Kaplan-Meier curves representing the percentage of overall survival according to the subtype of diagnosis (A) or the number of HMR mutations (i.e. ASXL1, EZH2, IDH1, IDH2, SRSF2 and U2AF1) (B).