Supplemental Material

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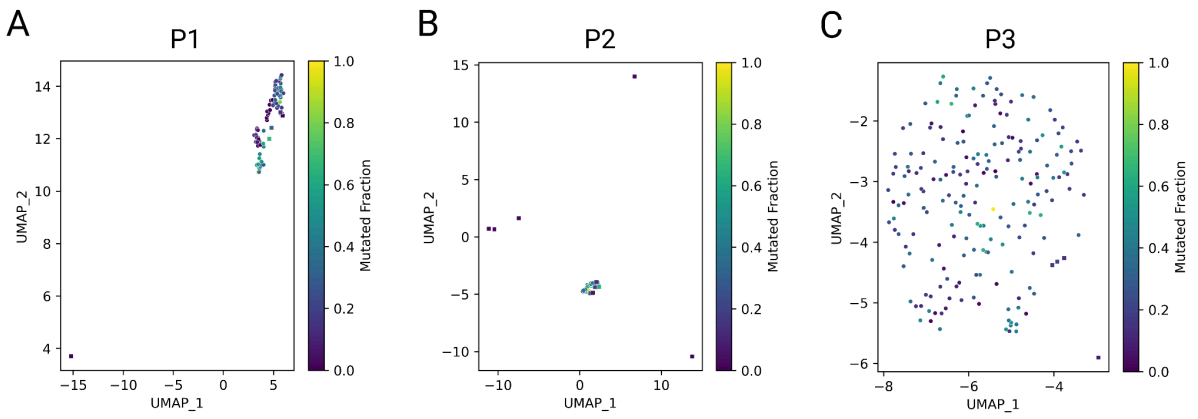
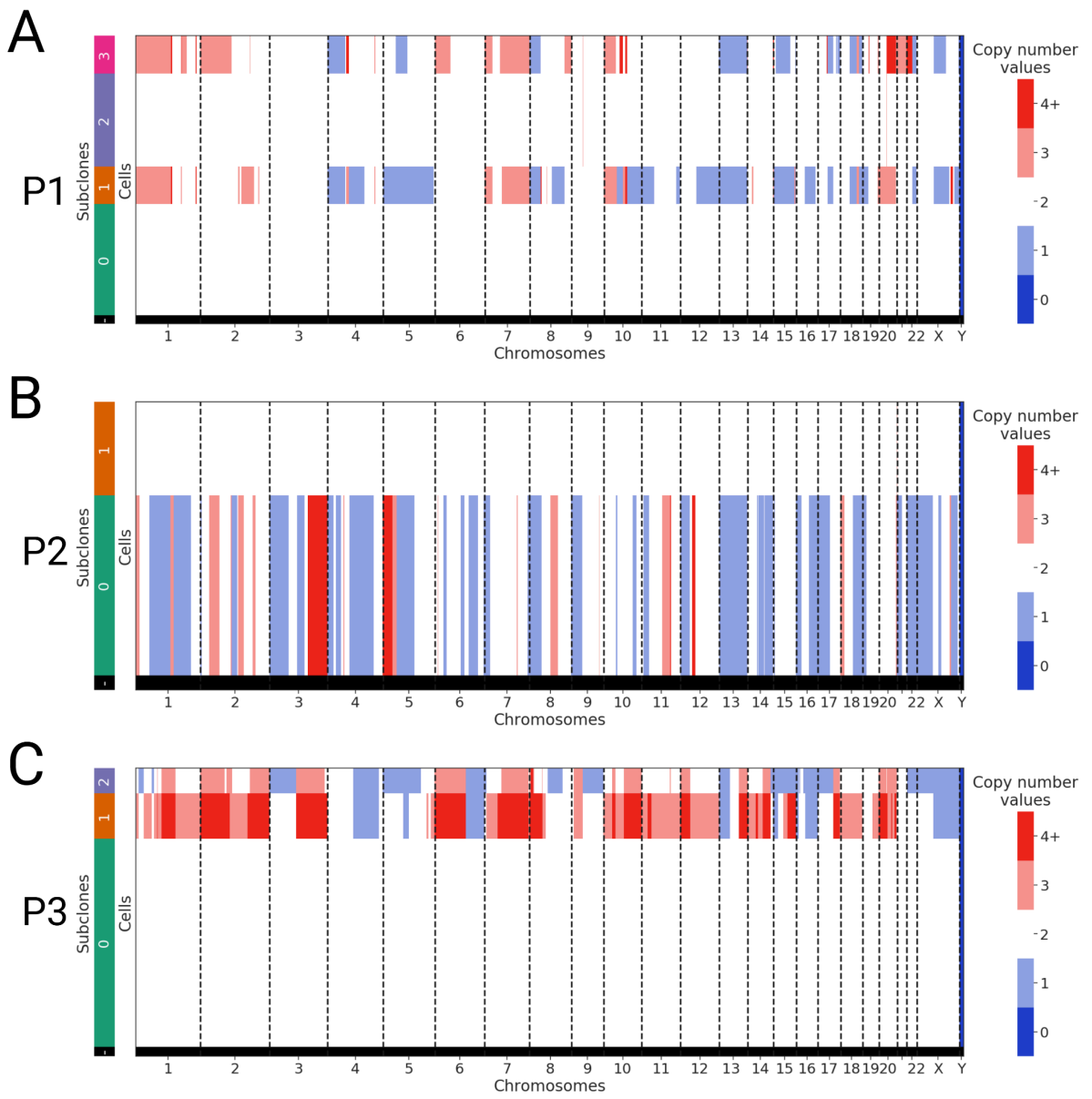


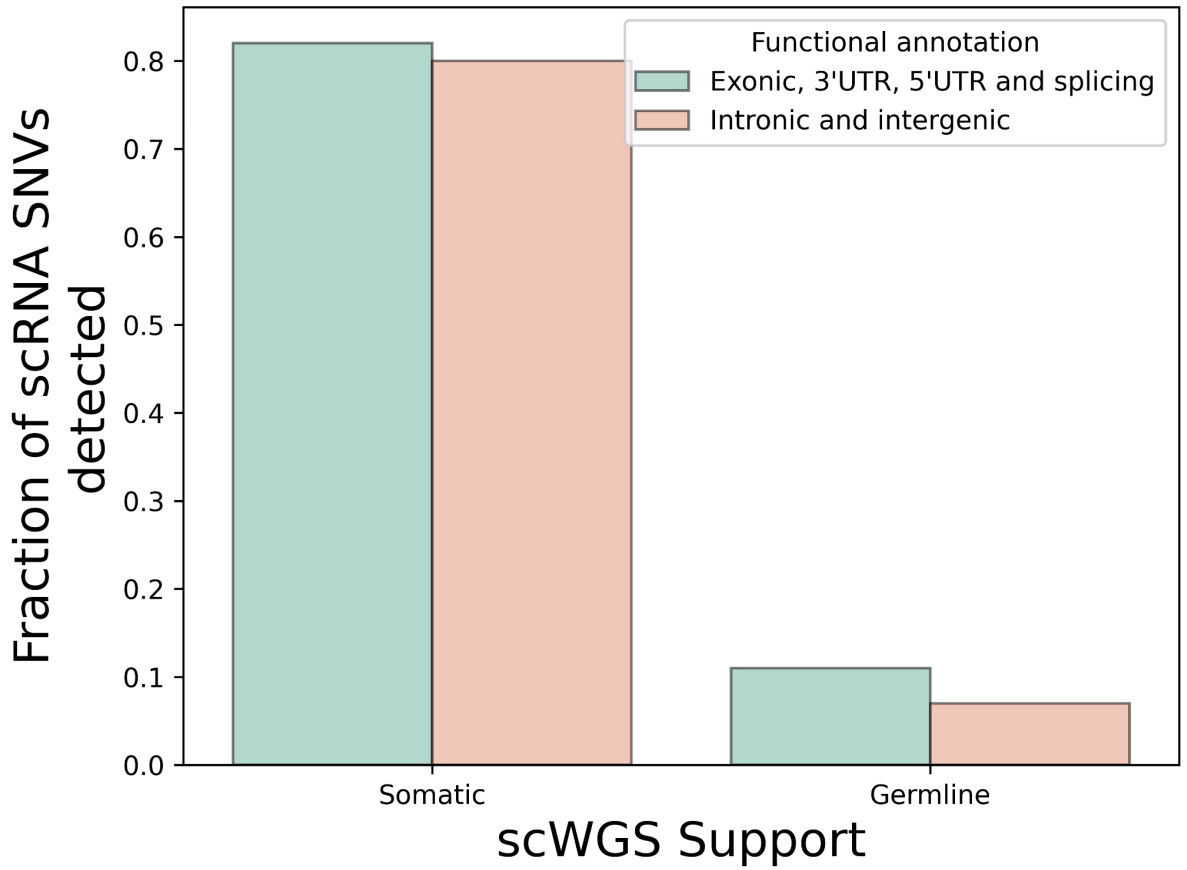
Supplementary Figure S1: Mutational burden of re-annotated cells

UMAP embeddings of LR scRNA-seq expression of cancer cells in patient **(A)** P1, **(B)** P2, **(C)** P3. Cells are colored by the fraction of covered SNV loci that are mutated in the cell, and shaped by their re-annotation status, either previously annotated as cancer and re-annotated as cancer (circle) or previously annotated as noncancer and annotated as cancer (square).



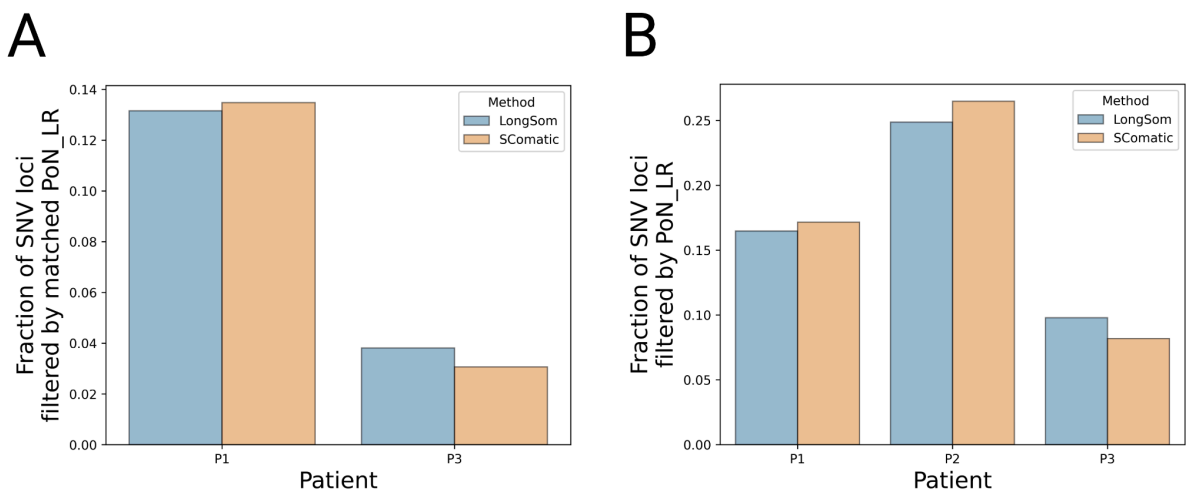
Supplementary Figure S2: scWGS CNA subclones.

scDNA-seq copy number values per subclone in patient **(A)** P1, **(B)** P2, **(C)** P3 data. Subclones with multiple copy number alterations are aneuploid (likely cancer), while copy number neutral subclones are diploid (likely noncancer).



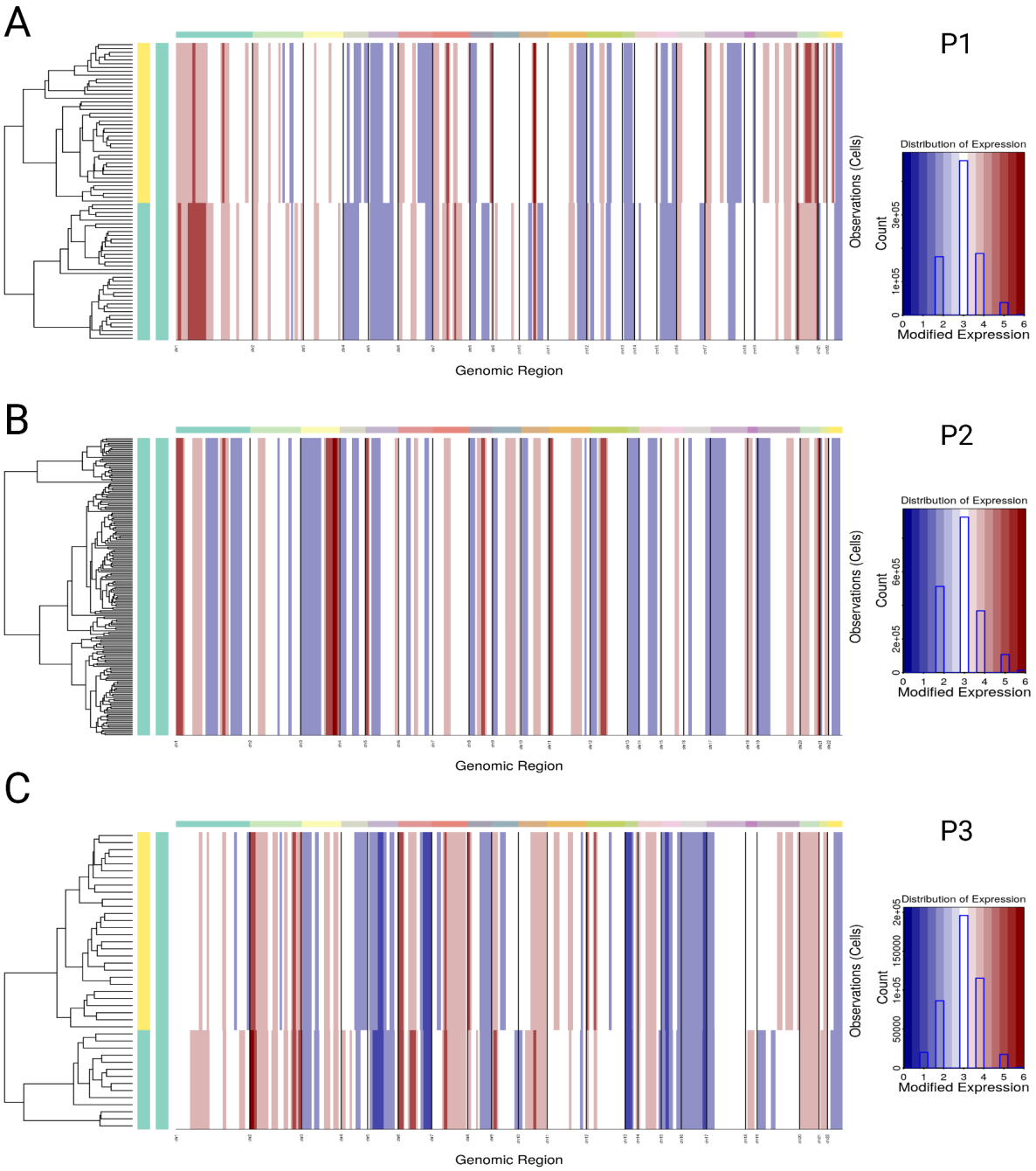
Supplementary Figure S3: scWGS support depending on functional annotation.

Bar plot of the fraction of all loci being supported by scWGS data as either somatic or germline, colored by the functional annotation status of the loci.



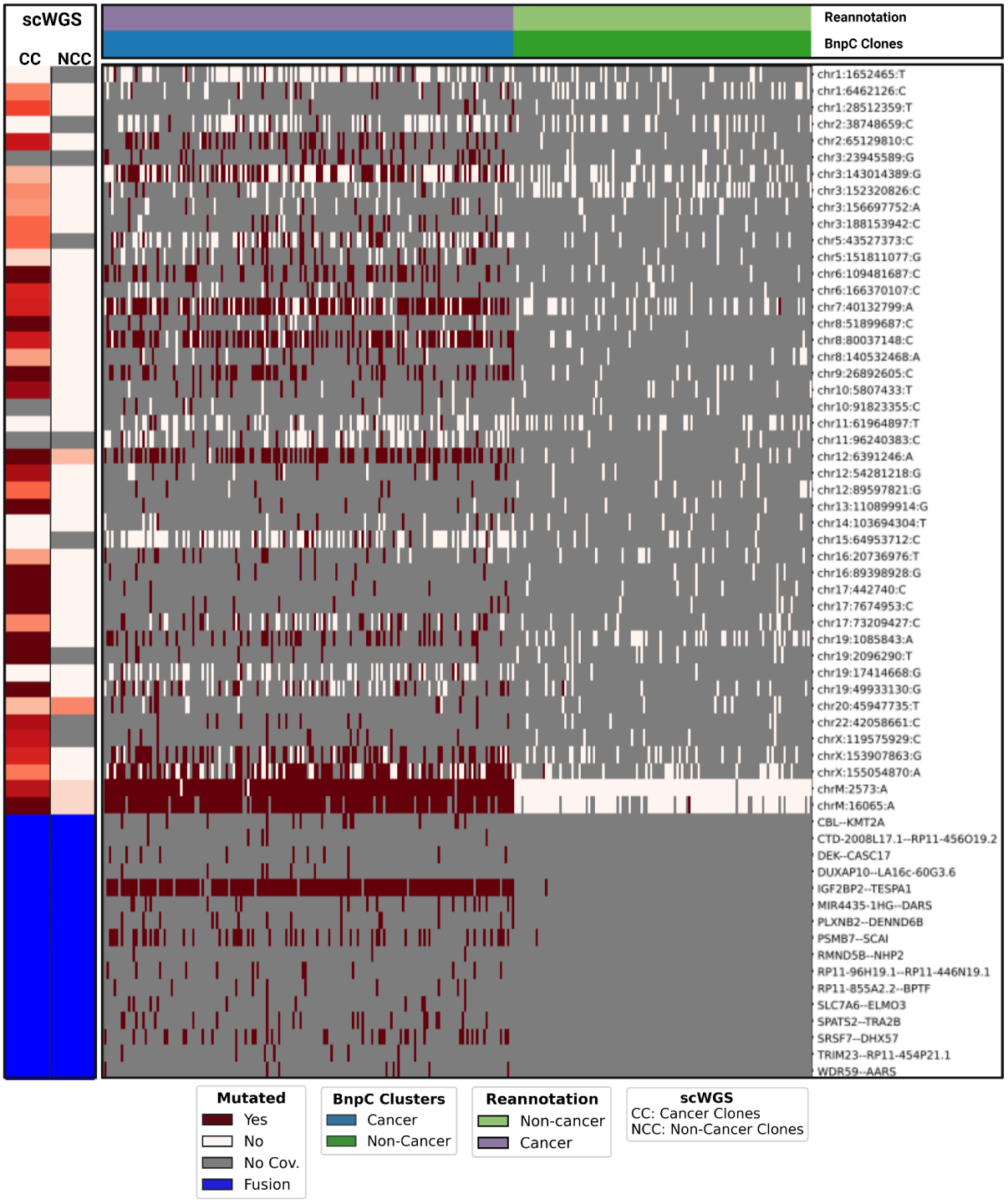
Supplementary Figure S4: Validation using matching normals.

(A) Fraction of LongSom calls in somatic biopsies from patients P1 and P3 that were also called in matched normal samples. **(B)** Calls that were filtered with the LR PoN as the only filtering reason, as a fraction of the final LongSom call set.



Supplementary Figure S5: inferCNV CNA subclones.

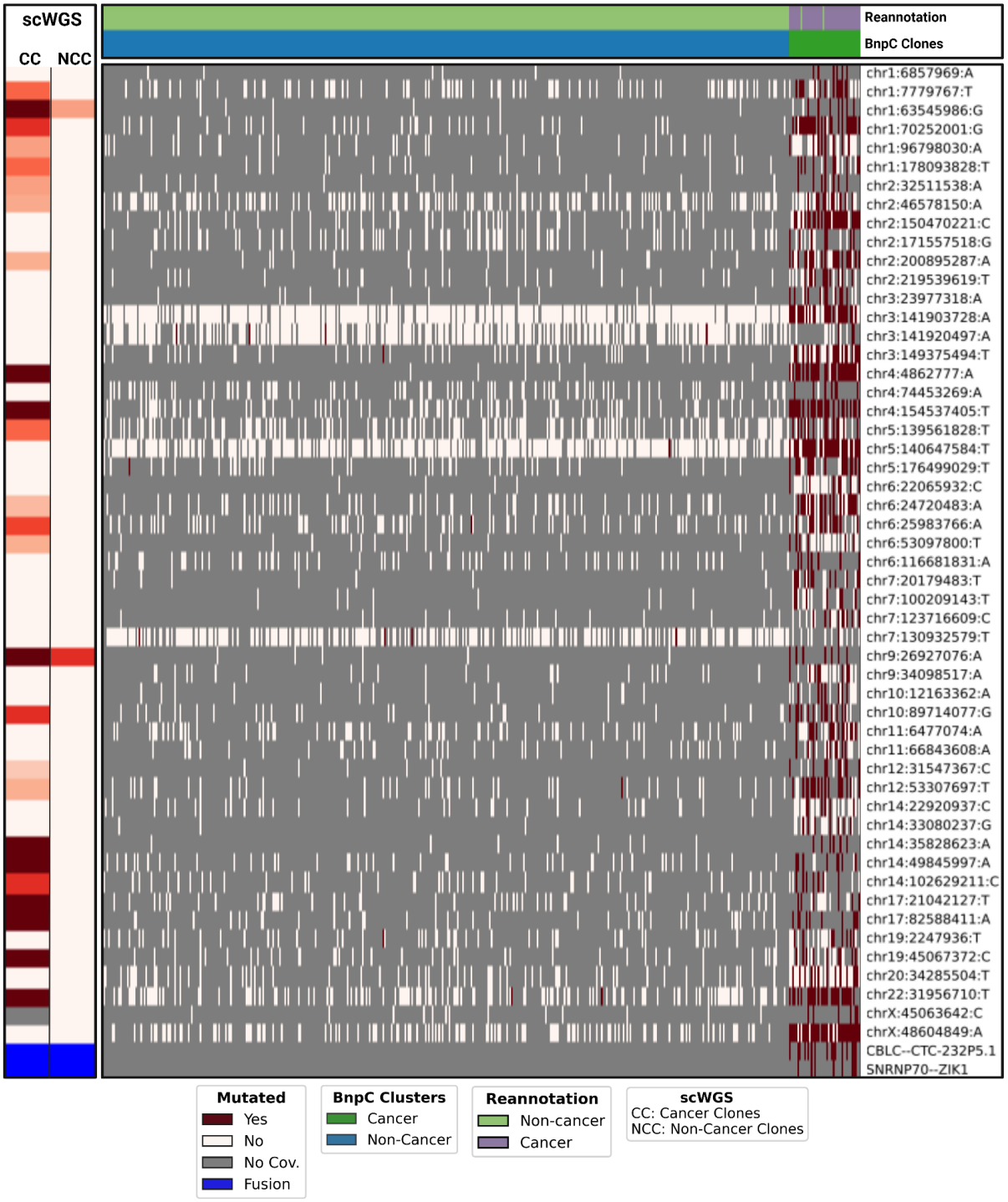
(A-C) CNA profiles of patients **(A)** P1, **(B)** P2, and **(C)** P3 cells computed by LongSom using inferCNV in LR scRNA-seq. Rows are individual cells, and colors (left) indicate subclonal attribution, columns are chromosomes. Regions in red have high copy number values, and in blue low.



Supplementary Figure S6: BnpC clonal reconstruction in Patient P2.

BnpC clustering of single cells from the tumor biopsy of patient P2 (columns) by somatic SNVs and fusions called by LongSom in LR scRNA-seq data (rows). Red indicates that a loci is mutated in a cell (beta-binomial P value < 0.05), white that it is not, and grey indicates no coverage in the cell at a given locus. Rows are colored according to the mutation status of aggregated scWGS diploid (Noncancer Clones) or aneuploid (Cancer Clones) cells. Fusions appear in blue. Columns are colored

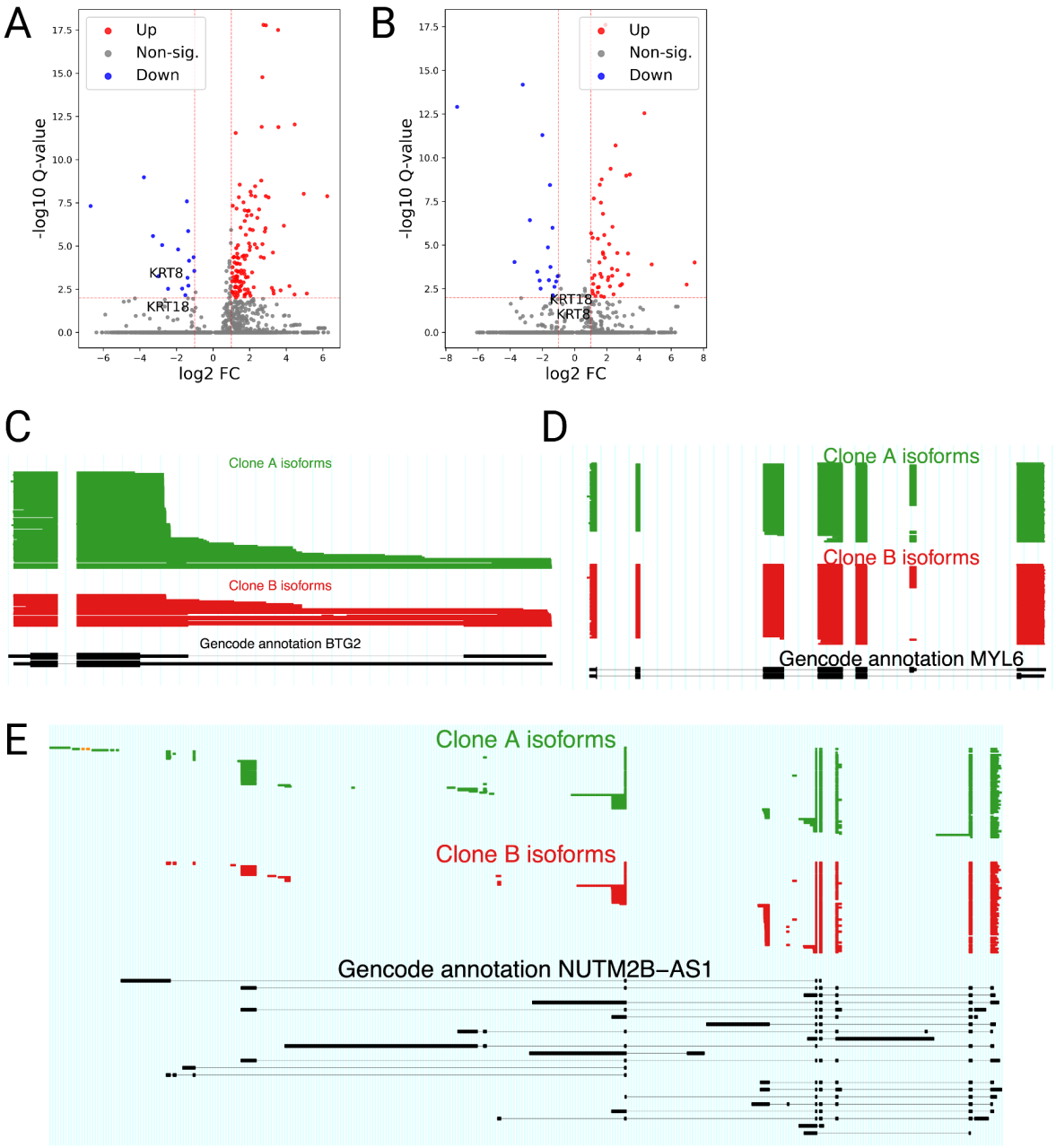
from top to bottom by cell types reannotated by LongSom and BnpC subclones inferred from somatic SNVs and fusions.



Supplementary Figure S7: BnpC clonal reconstruction in Patient P3.

BnpC clustering of single cells from the tumor biopsy of patient P3 (columns) by somatic SNVs and fusions called by LongSom in LR scRNA-seq data (rows). Red indicates that a loci is mutated in a cell (beta-binomial P value < 0.05), white that it is not, and grey indicates no coverage in the cell at a given locus. Rows are colored according to the mutation status of aggregated scWGS diploid (Noncancer Clones) or aneuploid (Cancer Clones) cells. Fusions appear in blue. Columns are colored

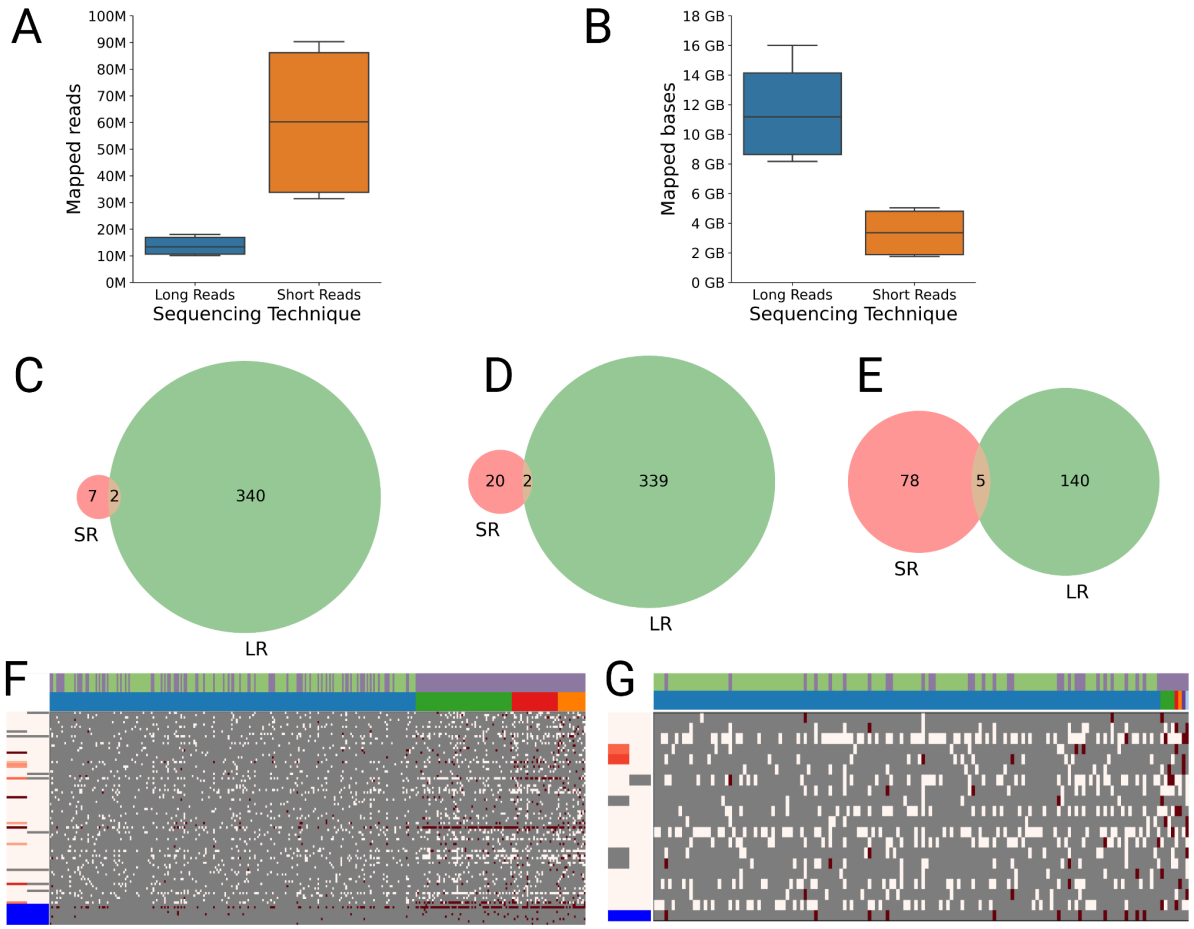
from top to bottom by cell types reannotated by LongSom and BnpC subclones inferred from somatic SNVs and fusions.



Supplementary Figure S8: Differential gene and isoform expression in patient P1 subclones.

(A) Volcano plot of differentially expressed genes identified between Patient P1's subclone B and all other cancer subclones from all patients. **(B)** Volcano plot of differentially expressed genes identified between Patient P1's subclone A and all other cancer subclones from all patients. **(C-E)** ScisorWiz representation of **(C)** *BTG2*, **(D)** *MYL6* and **(E)** *NUTM2B-AS1*

isoforms. Colored areas are exons, whitespace areas are intronic space, not drawn to scale, and each horizontal line represents a single read colored according to subclones. Gencode notable reference isoforms appear in black.



Supplementary Figure S9: Sequencing statistics in LR and SR.

(A) Number of reads mapped across tumor and normal samples (n=5), per sequencing technique. **(B)** Billions of bases mapped across tumor and normal samples (n=5), per sequencing technique. **(C-E)** Venn diagram of the SNVs detected in scRNA-seq SR data by SComatic (red) and LR data by LongSom (green) in **(C)** patient P1, **(D)** patient P2 and **(E)** patient P3. **(F-G)** BnpC clustering of single cells (columns) from the tumor biopsy of patient **(F)** P1 and **(G)** P3 by somatic SNVs and fusions (rows) called in SR scRNA-seq data. Red indicates that a loci is mutated in a cell (bet-binomial P value < 0.05), white that it is not, and grey indicates no coverage in the cell at a given locus. Rows are colored according to the mutation status of aggregated scWGS diploid (Noncancer Clones) or aneuploid (Cancer Clones) cells. Fusions appear in blue. Columns are colored from top to bottom by marker-based annotation and BnpC subclones inferred from somatic SNVs and fusions.

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