SUPPLEMENTARY MATERIALS

Single cell characterization of CRISPR-modified transcript isoforms with nanopore sequencing

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Fig S1. Guide-RNA designs targeting splicing acceptor of *RACK1*. Red and blue bases indicate protospacer adjacent motif (PAM) and splicing acceptor sites respectively.

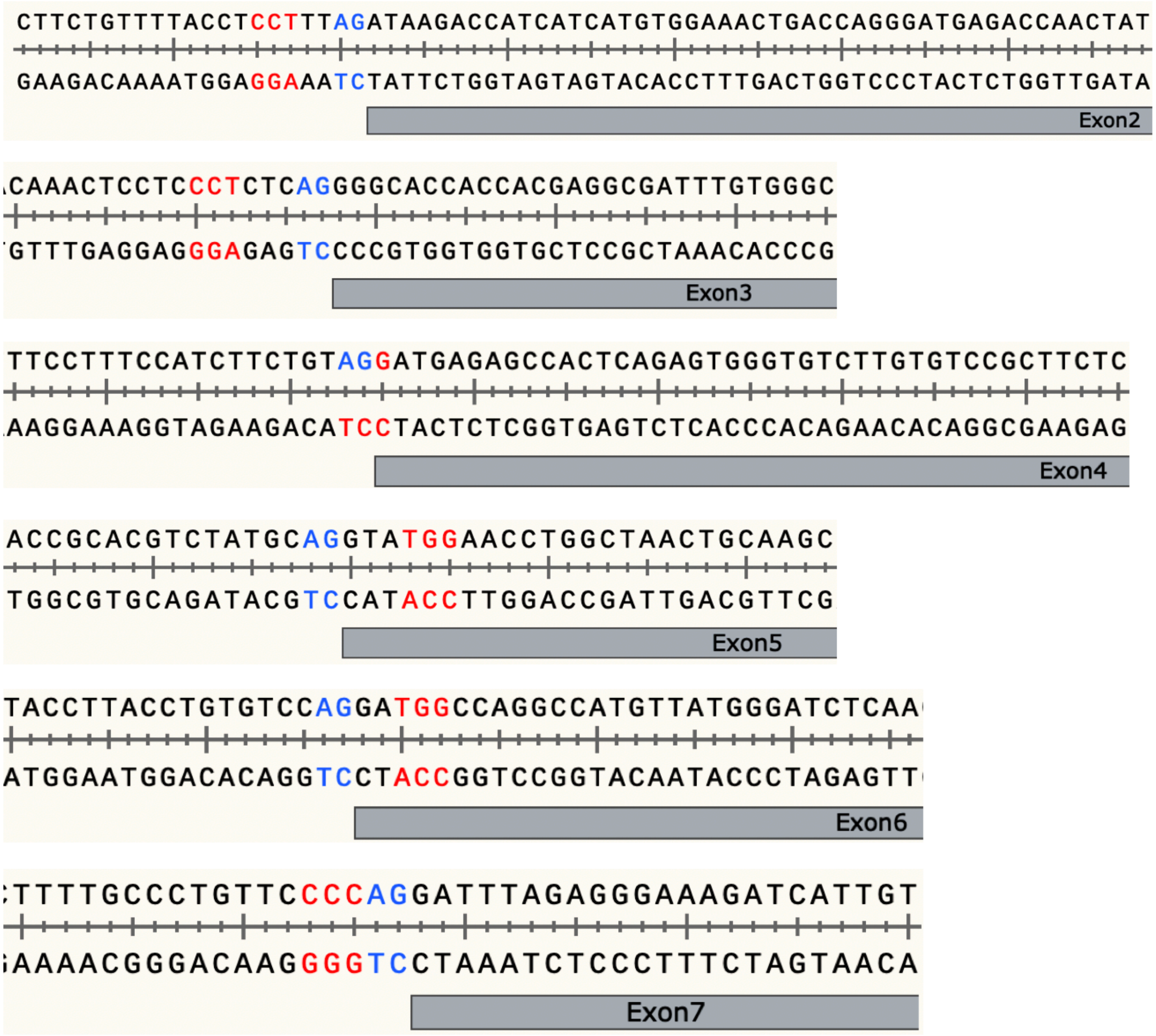
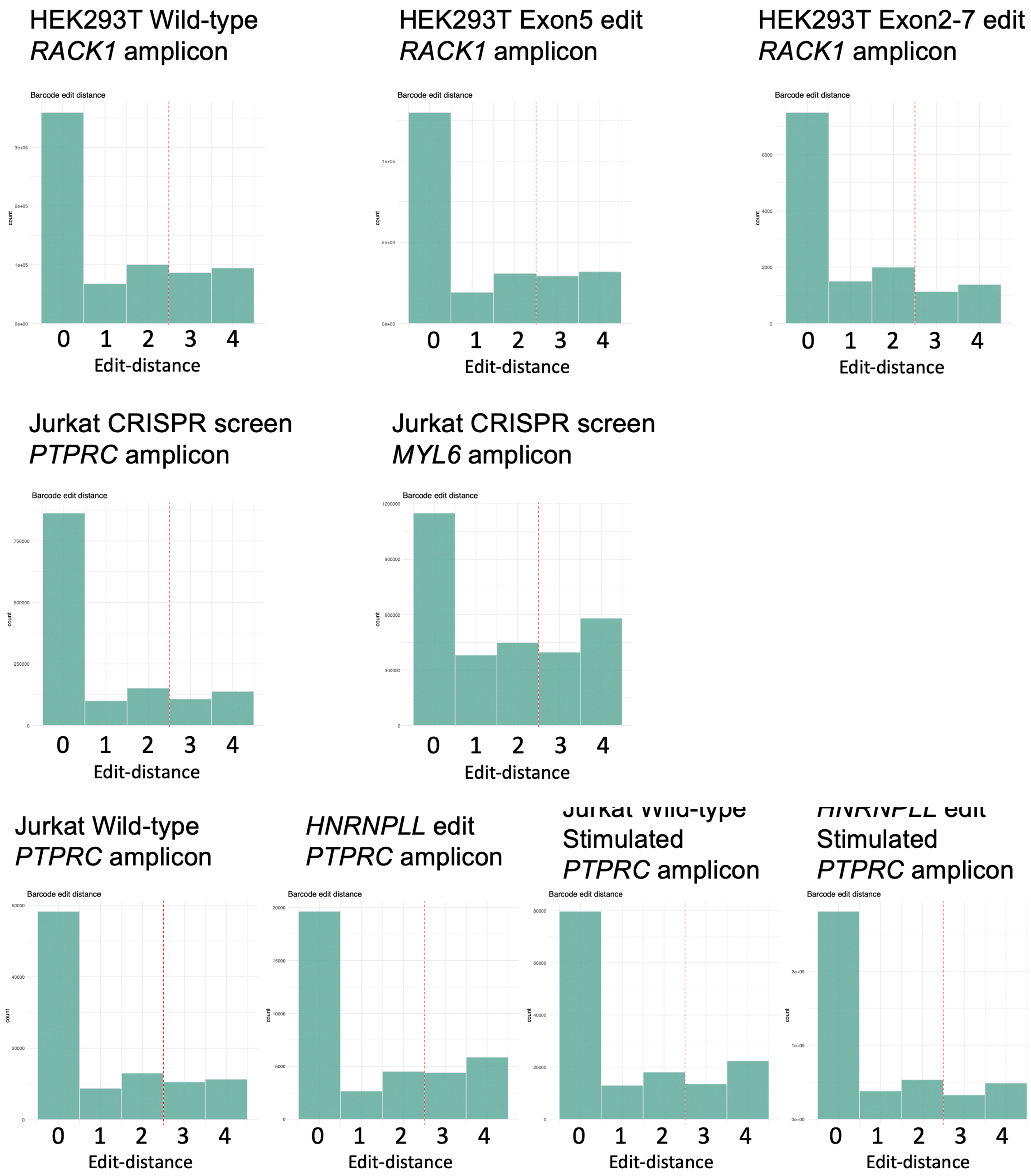


Fig S2. Distribution of edit distance between the cell barcode from each long-read sequence and the matched barcode from the cellranger whitelist. Reads with edit distance >3 (red line) are excluded from the analysis.



HEK293T Wild-type *RACK1* amplicon

HEK293T Exon5 edit *RACK1* amplicon

HEK293T Exon2-7 edit *RACK1* amplicon

Jurkat CRISPR screen *PTPRC* amplicon

Jurkat CRISPR screen *MYL6* amplicon

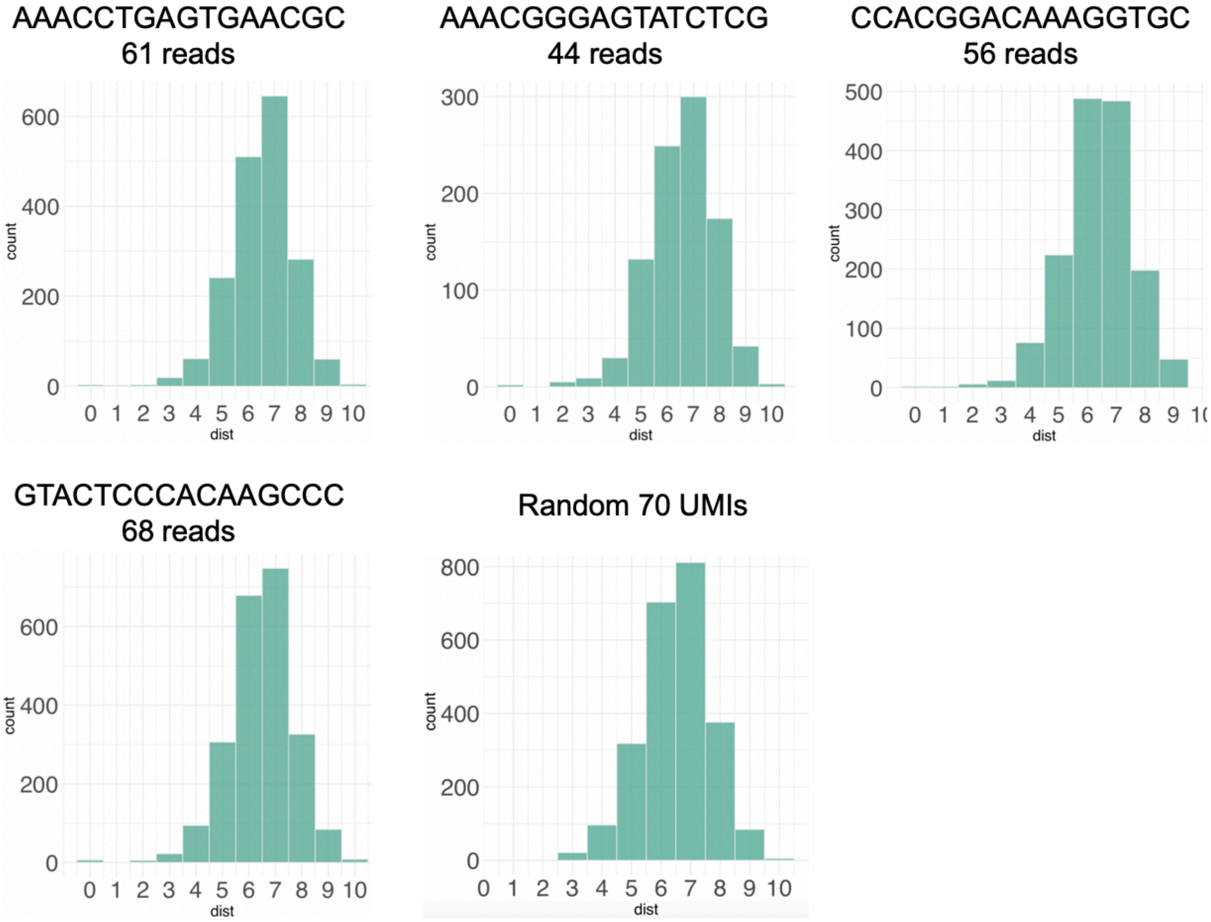
Jurkat Wild-type *PTPRC* amplicon

HNRNPLL edit *PTPRC* amplicon

Jurkat Wild-type Stimulated *PTPRC* amplicon

HNRNPLL edit Stimulated *PTPRC* amplicon

Fig S3. UMI edit distance distribution for a representative sample of cells in the wild-type HEK293T sample. Edit distance between all UMIs in each cell are shown. Cell barcode and read counts per cell are noted.



AAACCTGAGTGAACGC
61 reads

AAACGGGAGTATCTCG
44 reads

CCACGGACAAAGGTGC
56 reads

GTACTCCCACAAGCCC
68 reads

Random 70 UMIs

Fig S4. *RACK1* transcript isoform composition of wild-type HEK293T cells. Heatmap showing proportion of each transcript isoform (x-axis) with each cell (y-axis) and clustering based on a given mRNA transcript isoform proportion.

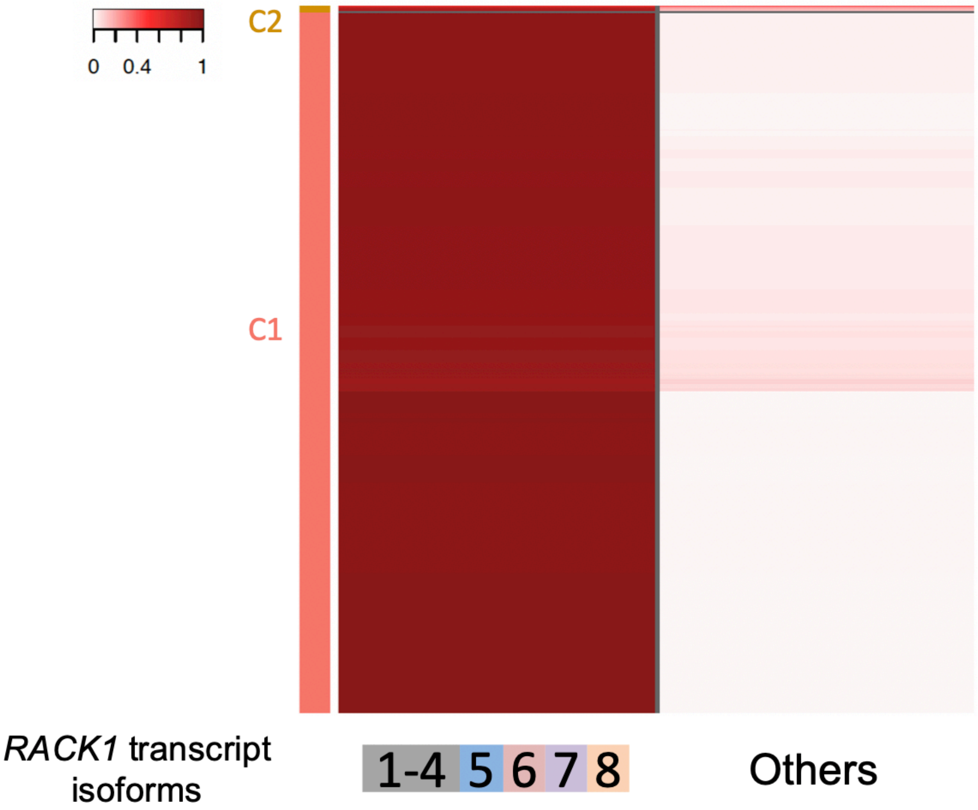


Fig S5. Quantification of *RACK1* expression level for each cluster. Violin plot showing the expression level of *RACK1* in each cluster. P values are calculated in comparison with cluster2. (two-sided t-test)

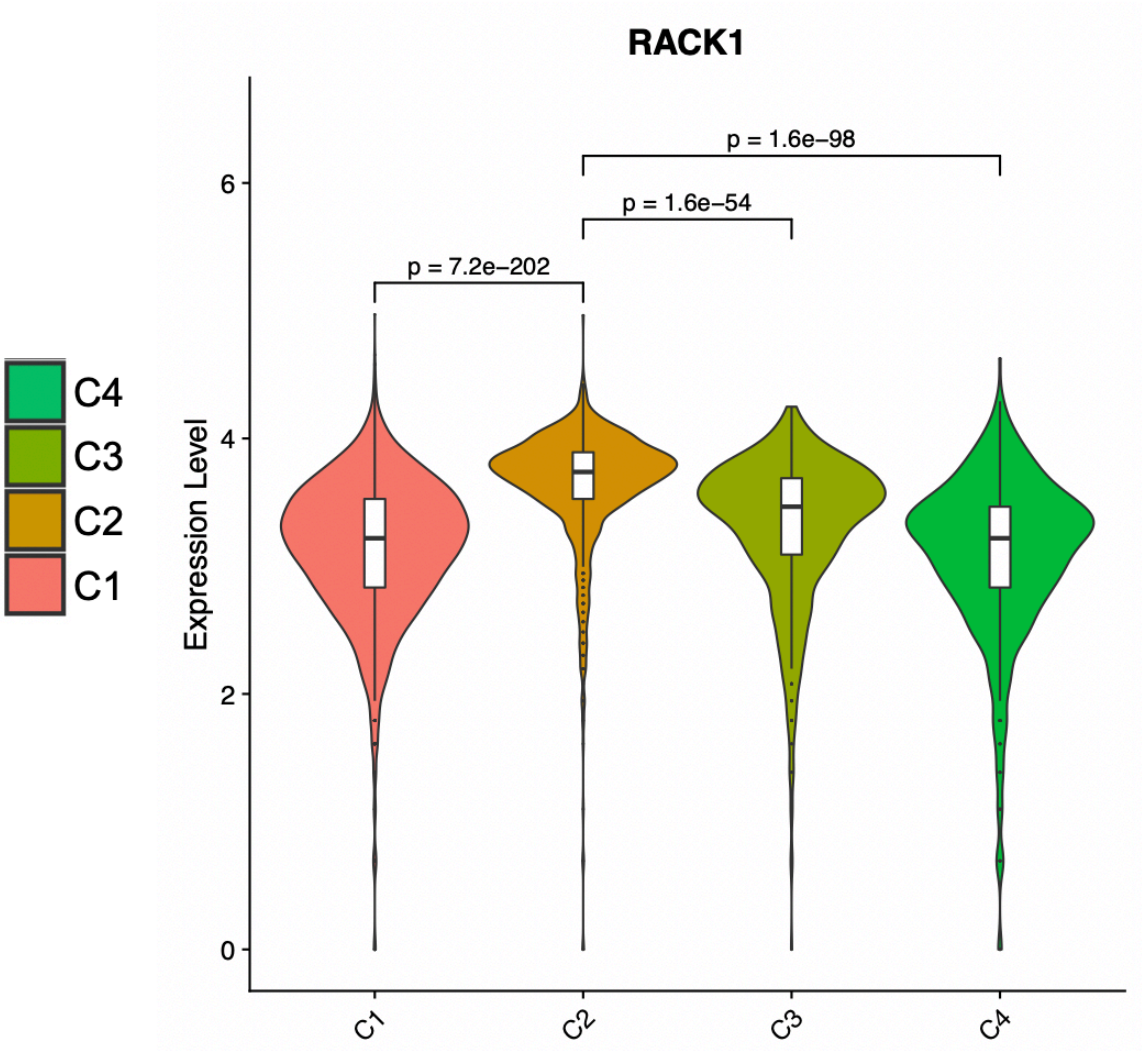


Fig S6. Volcano plot differentially expressed gene between cells with full-length or exon5 skipped RACK1 transcript.

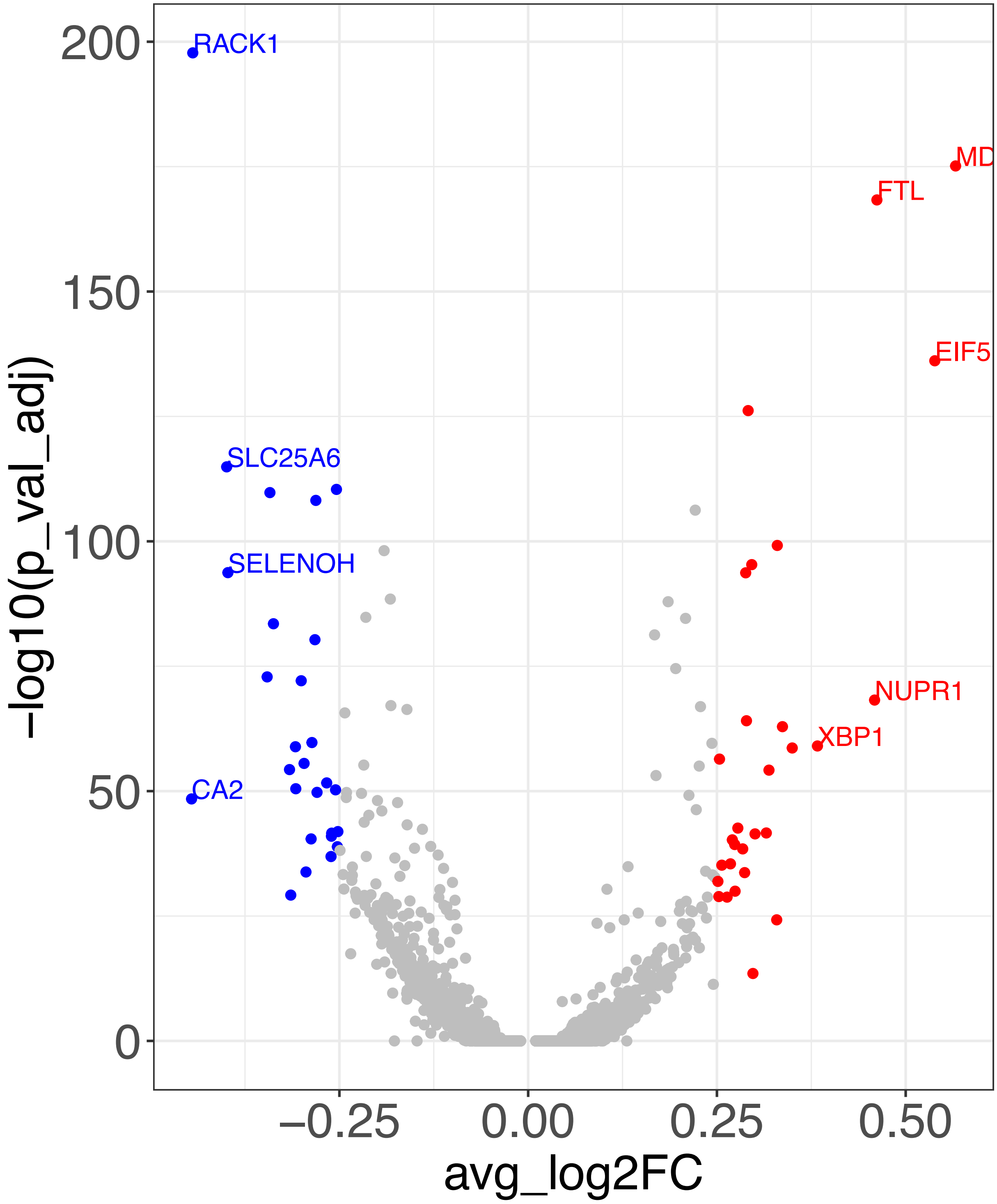
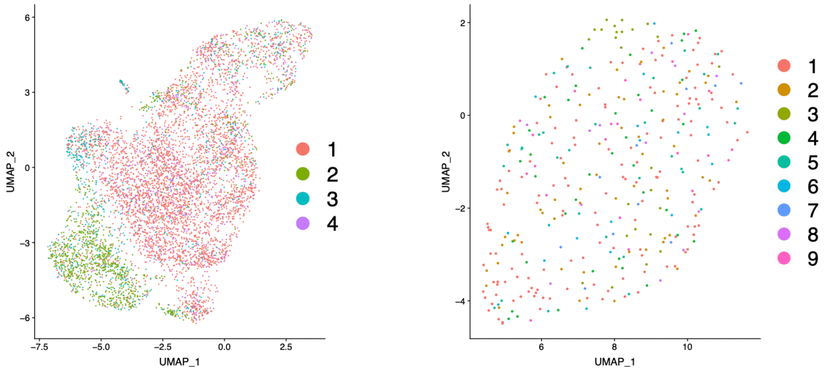
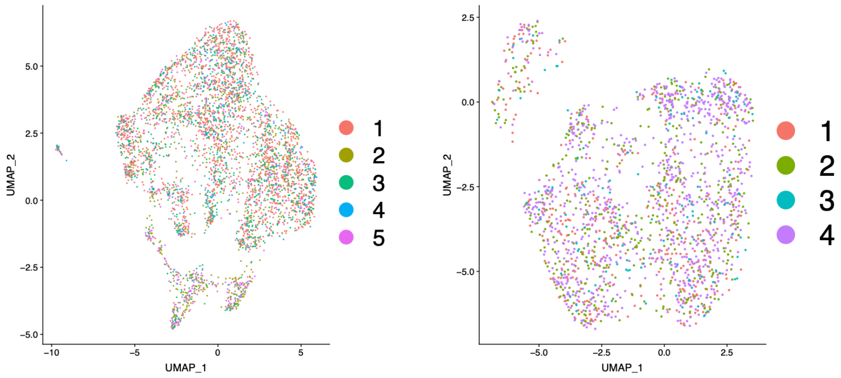


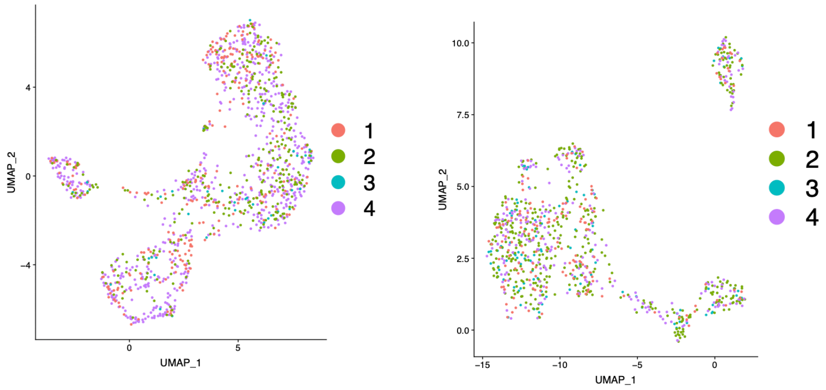
Fig S7. UMAP plots colored by transcript isoform based cell clusters.


RACK1 exon5 targeted

RACK1 exon2-7 targeted


Wild-type Jurkat

HNRNPLL targeted Jurkat


Wild-type Jurkat Stimulated

HNRNPLL targeted Jurkat Stimulated

Fig S8. Structure of major transcript isoforms of *PTPRC*. Structures of major 5 transcripts isoform of *PTPRC.* Exon3 to 7 are highlighted.

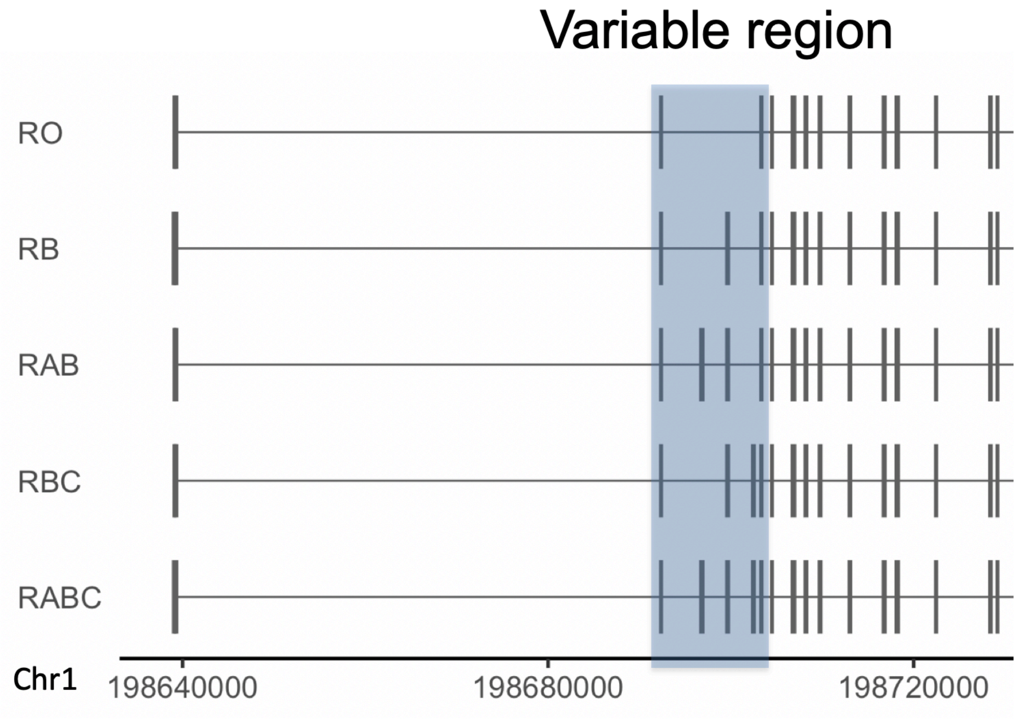


Fig S9. Validation of gRNA identified by short-read sequencing and its consequential CRISPR editing by long-read sequencing per individual cell. (a) Scheme of the experiment. Ad/CB: Sequencing/10x adaptors and cell barcode sequences.

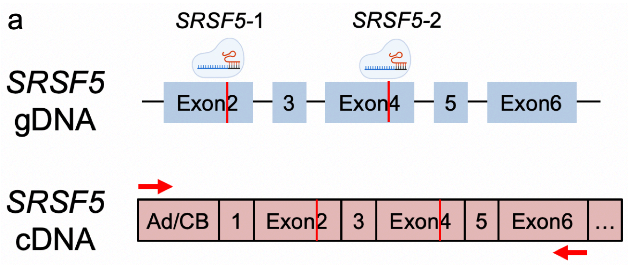


Fig S10. Average short isoform proportion per targeted genes. Heatmap showing the fold change of short *PTPRC* transcripts (RO and RB) proportion per cells having indicated gene targeting gRNA compared to nontarget cells.

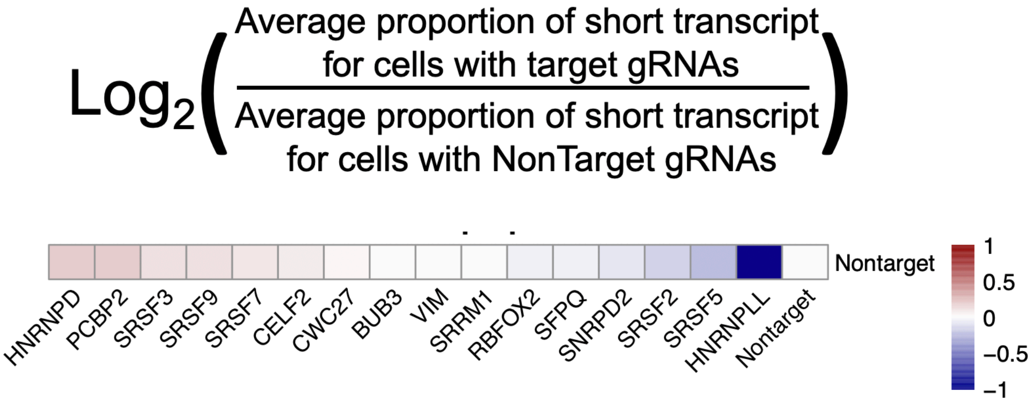


Fig S11. Transcript isoform proportion for cells having nontarget gRNA sequence. Heatmap showing proportion of each transcript isoform (x-axis) with each cell (y-axis).

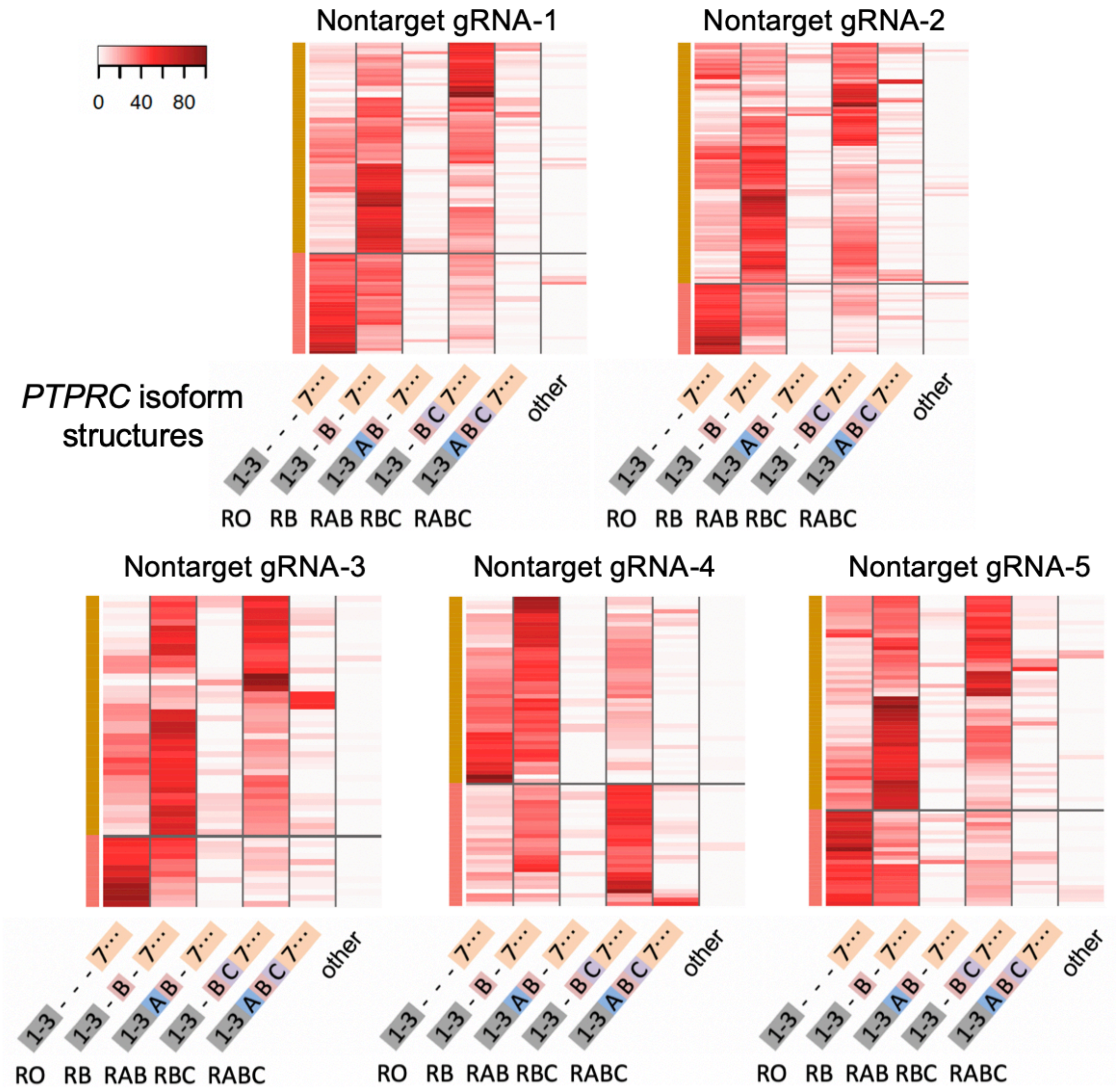


Fig S12. Transcript isoform proportion for cells having *HNRNPLL* and *SRSF5* targeting gRNA sequence. (a) Heatmap showing proportion of each transcript isoform (x-axis) with each cell (y-axis). (b, c) Boxplot showing the ratio of each of long *PTPRC* transcript isoform (RBC and RABC) for cells with the CRISPR target genes.

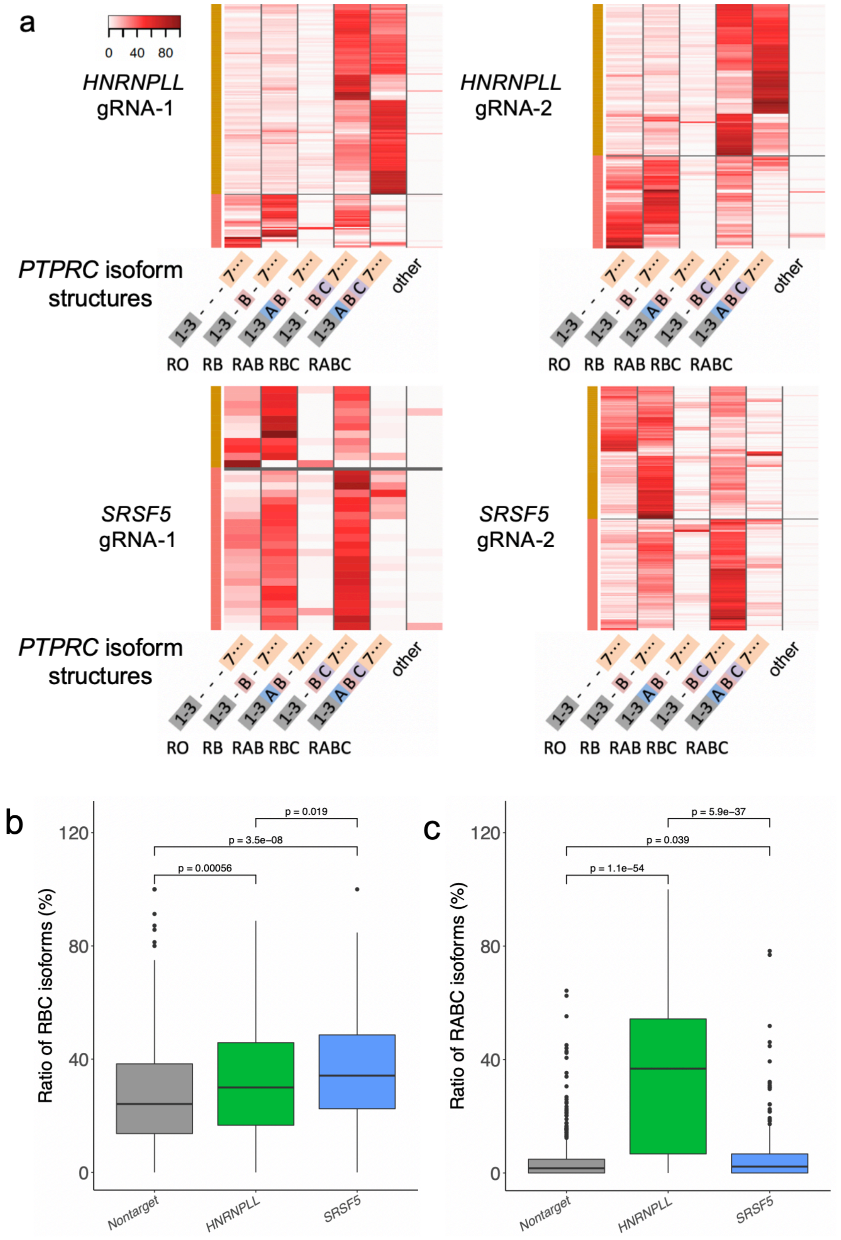


Fig S13. Transcript isoform proportion for cells having *HNRNPD* and *PCBP2* gRNA sequence. (a) Heatmap showing proportion of each transcript isoform (x-axis) with each cell (y-axis). (b, c) Boxplot showing the ratio of each of short *PTPRC* transcript isoform (RO and RB) for cells with the CRISPR targeted genes.

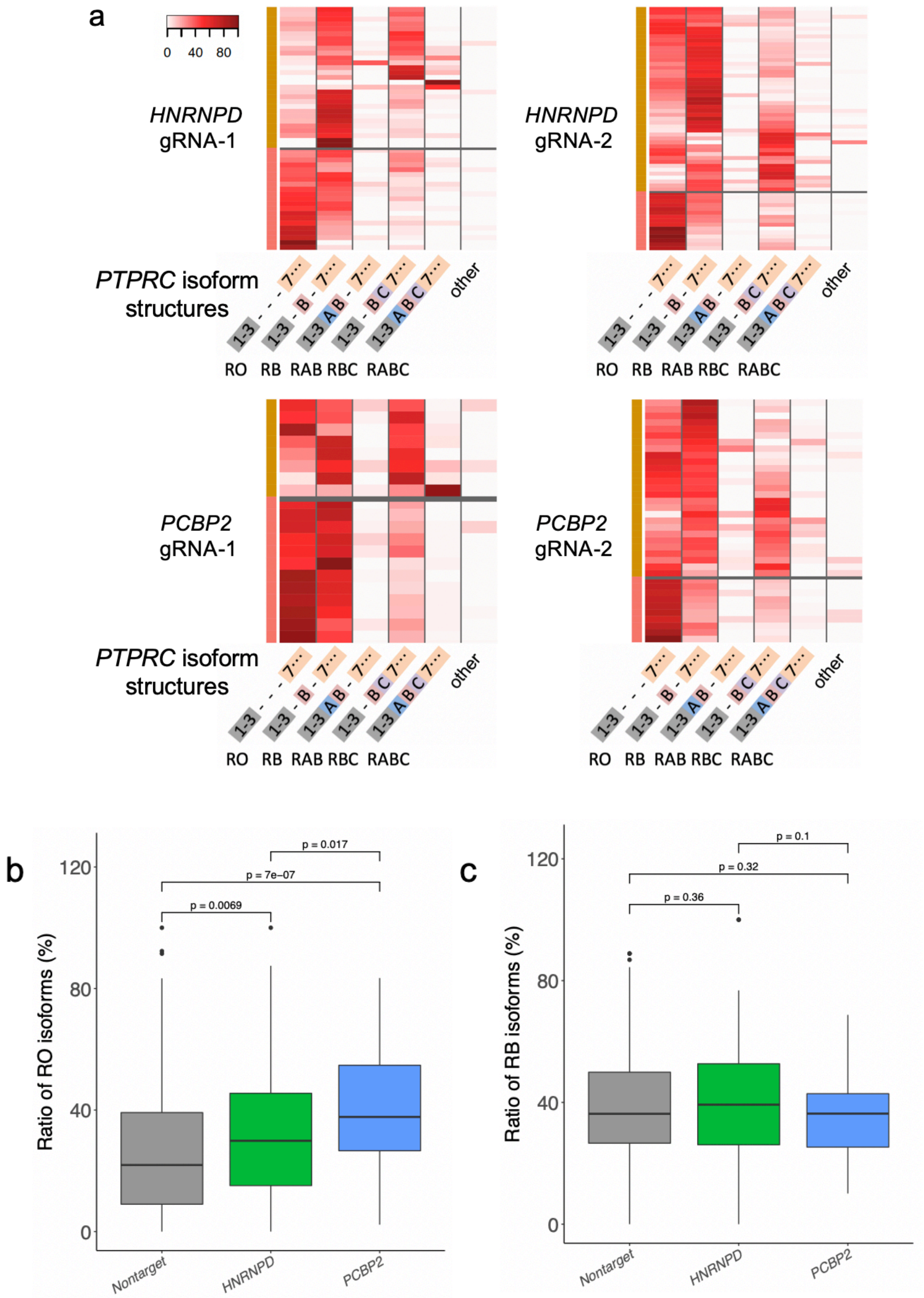


Fig S14. Effect of various splicing factor KO on *MYL6* alternative splicing. (a) Structure of major transcript isoforms of *MYL6.* (b) Boxplot showing the ratio of *MYL6* transcripts with exon6 skipping for cells with gRNAs targeting indicated genes.