Combined promoter-capture Hi-C and Hi-C analysis reveals a fine-tuned regulation of 3D chromatin architecture in colorectal cancer

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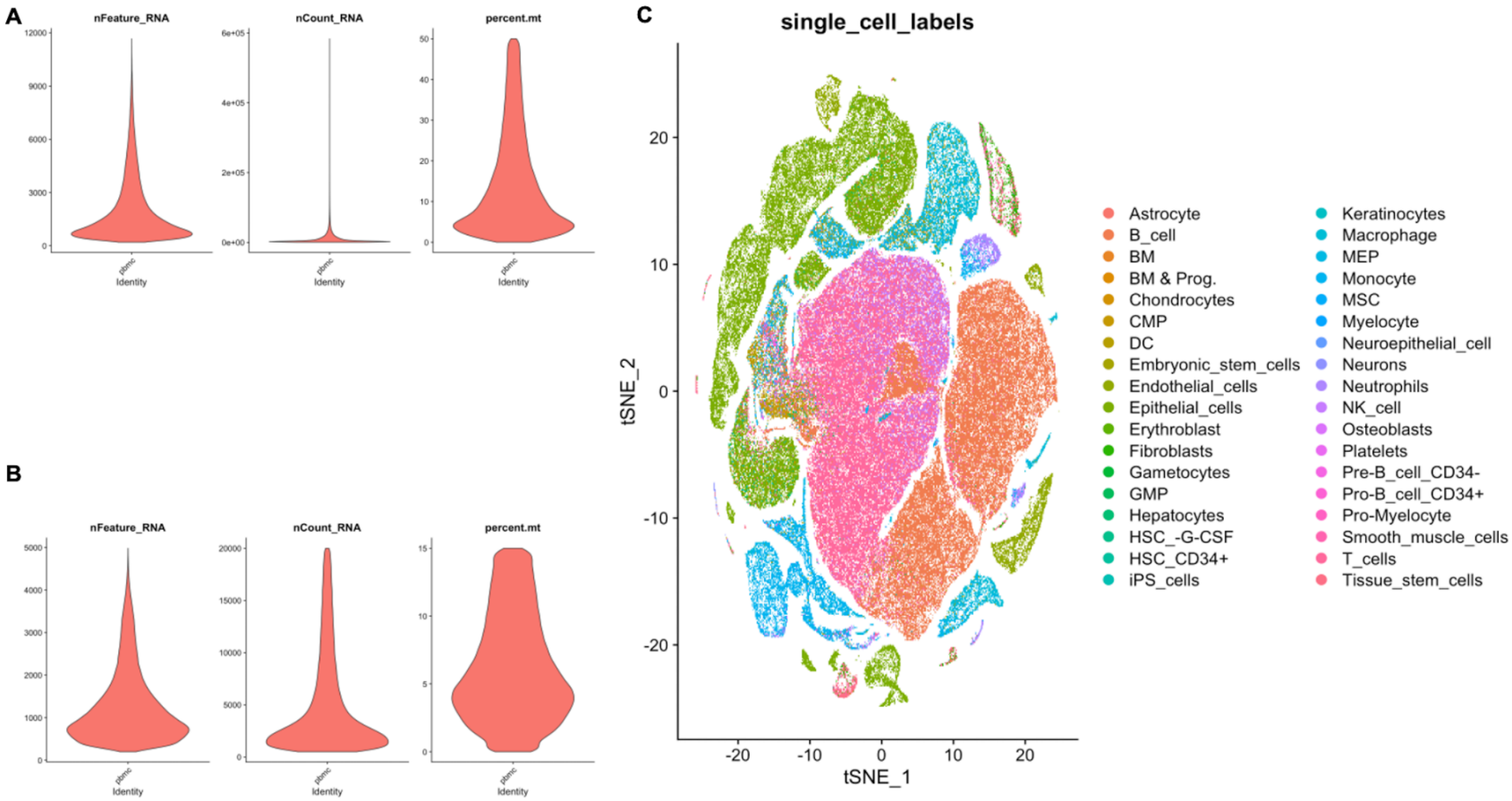
Supplementary Material M1

Expression pattern of statistically significant genes for therapeutic target in single cell human colon cancer atlas

In the analysis step, first we preprocessed data for preventing outlier cells which could influence downstream analysis. We generated a violin plot before and after performing the quality control (Figure M1(A-B)). In the quality control step, we manually selected the threshold level of number of features (transcripts), number of counts against each feature and mitochondrial percentage of cells. Using the processed data, we generated a t-SNE plot (Figure M1C). The t-SNE plot separated the diverse cell population into 36 clusters enabling high-resolution depiction of the cellular diversity and heterogeneity. Here, we were interested in monitoring the expression patterns derived from the integrative bulk-seq study at single cell level across different cell populations. This helped us to gauge the distribution of the expression patterns of targeted genes across diverse cell populations (Figure M1C). In this study we selected a few statistically significant genes (MALAT1, NEAT1, FTX, PVT1, SNORA26, SNORA71A, TMPRSS11D, TSPEAR and DSG4) as potential therapeutic targets in early colorectal cancer detection or prevention. Out of these 9 genes, 3 genes (SNORA26, SNORA71A, TMPRSS11D) were missing in the single cell gene annotation files. Therefore, we monitored the expression pattern of the remaining six genes using the single cell human colon cancer atlas database[1]. FTX and PVT1 exhibit lower expression levels compared to MALAT1 and NEAT1 (Figure M1D). FTX gene showed significant expression only in B cell, T cell, endothelial cell, epithelial cell, macrophage, and monocyte populations. PVT1 gene showed significant expression only in B cell, T cell, and epithelial cell cells population. However, TSPEAR and DSG4 did not exhibit any significant expression level differences among distinct cell populations. Expression profiling at single cell level helped us to monitor the distribution of gene expression across distinct cell types which could be considered as a key factor in the assessment of their potential as a biomarker.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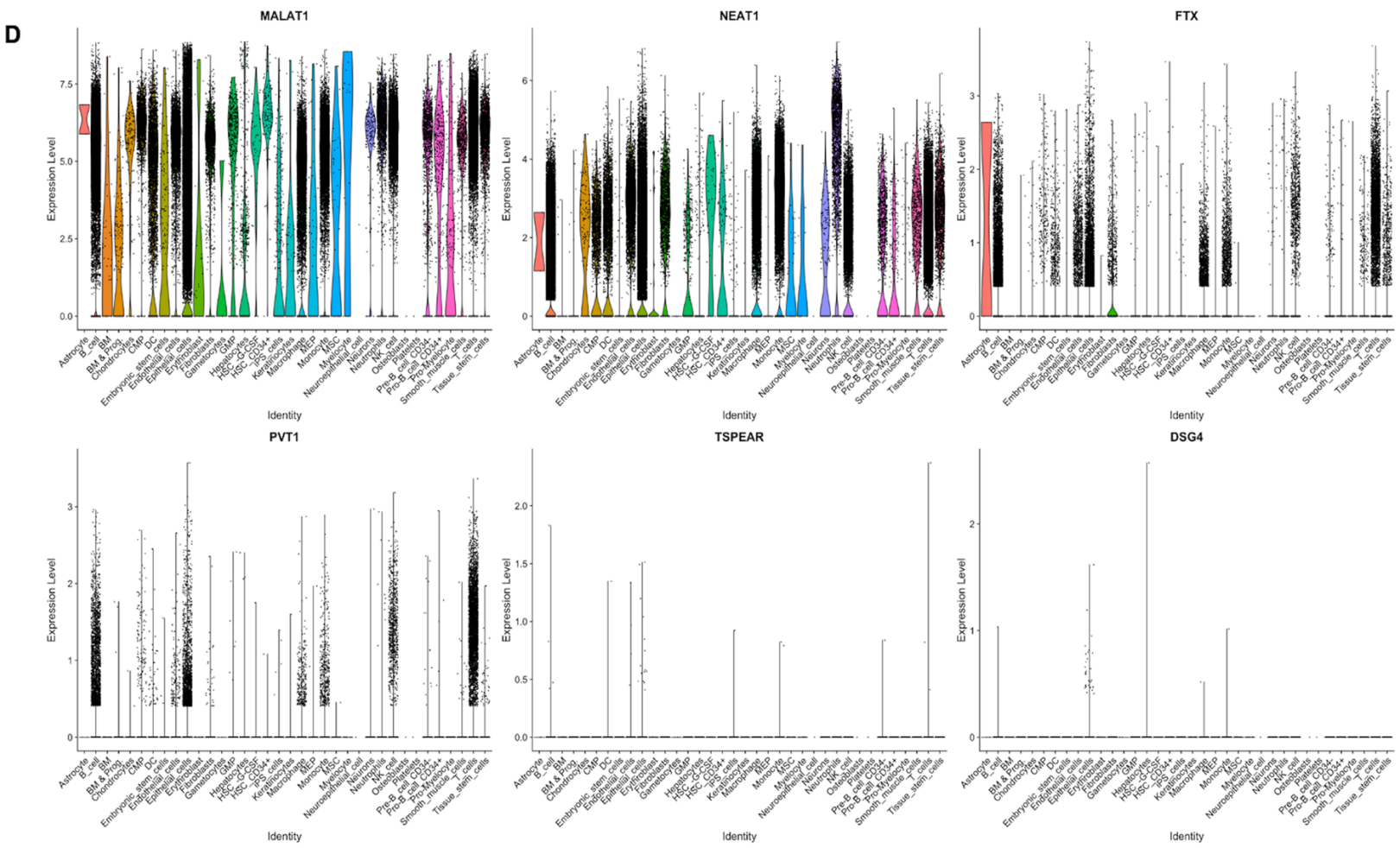

Figure M1. Single-cell RNA-Seq analysis of 371,223 tumor and adjacent normal cells collected from single cell human colon cancer atlas database reveals contributions of target genes across diverse cell populations. (A and B) Violin plot of number of features, number of counts and mitochondrial percentage before and after quality control. (C) t-SNE plot of 371,223 tumor and adjacent normal cells separated the cell population into 36 clusters enabling high-resolution depiction of the cellular diversity and heterogeneity. (D) Violin plots of targeted gene enrichment scores across 36 diverse cell type populations.

Reference

1. Pelka, K. *et al.* Spatially organized multicellular immune hubs in human colorectal cancer. *Cell* **184**, 4734-4752.e20 (2021).