Exploring the Changing Landscape of Cell-to-Cell Variation After CTCF Knockdown via Single Cell RNA-seq

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Abstract

Background: CCCTC-Binding Factor (CTCF), also known as 11-zinc finger protein, participates in many cellular processes, including insulator activity, transcriptional regulation and organization of chromatin architecture. Based on single cell flow cytometry and single cell RNA-FISH analyses, our previous study showed that deletion of CTCF binding site led to a significantly increase of cellular variation of its target gene. However, the effect of CTCF on genome-wide landscape of cell-to-cell variation is unclear. Results: We knocked down CTCF in EL4 cells using shRNA, and conducted single cell RNA-seq on both wild type (WT) cells and CTCF-Knockdown (CTCF-KD) cells using Fluidigm C1 system. Principal component analysis of single cell RNA-seq data showed that WT and CTCF-KD cells concentrated in two different clusters on PC1, indicating gene expression profiles of WT and CTCF-KD cells were systematically different. Interestingly, GO terms including regulation of transcription, DNA binding, Zinc finger and transcription factor binding were significantly enriched in CTCF-KD-specific highly variable genes, indicating tissue-specific genes such as transcription factors were highly sensitive to CTCF level. The dysregulation of transcription factors potentially explain why knockdown of CTCF lead to systematic change of gene expression. In contrast, housekeeping genes such as rRNA processing, DNA repair and tRNA processing were significantly enriched in WT-specific highly variable genes, potentially due to a higher cellular variation of cell activity in WT cells compared to CTCF-KD cells. We further found cellular variation-increased genes were significantly enriched in down-regulated genes, indicating CTCF knockdown simultaneously reduced the expression levels and increased the expression noise of its regulated genes. Conclusions: To our knowledge, this is the first attempt to explore genome-wide landscape of cellular variation after CTCF knockdown. Our study not only advances our understanding of CTCF function in maintaining gene expression and reducing expression noise, but also provides a framework for examining gene function.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

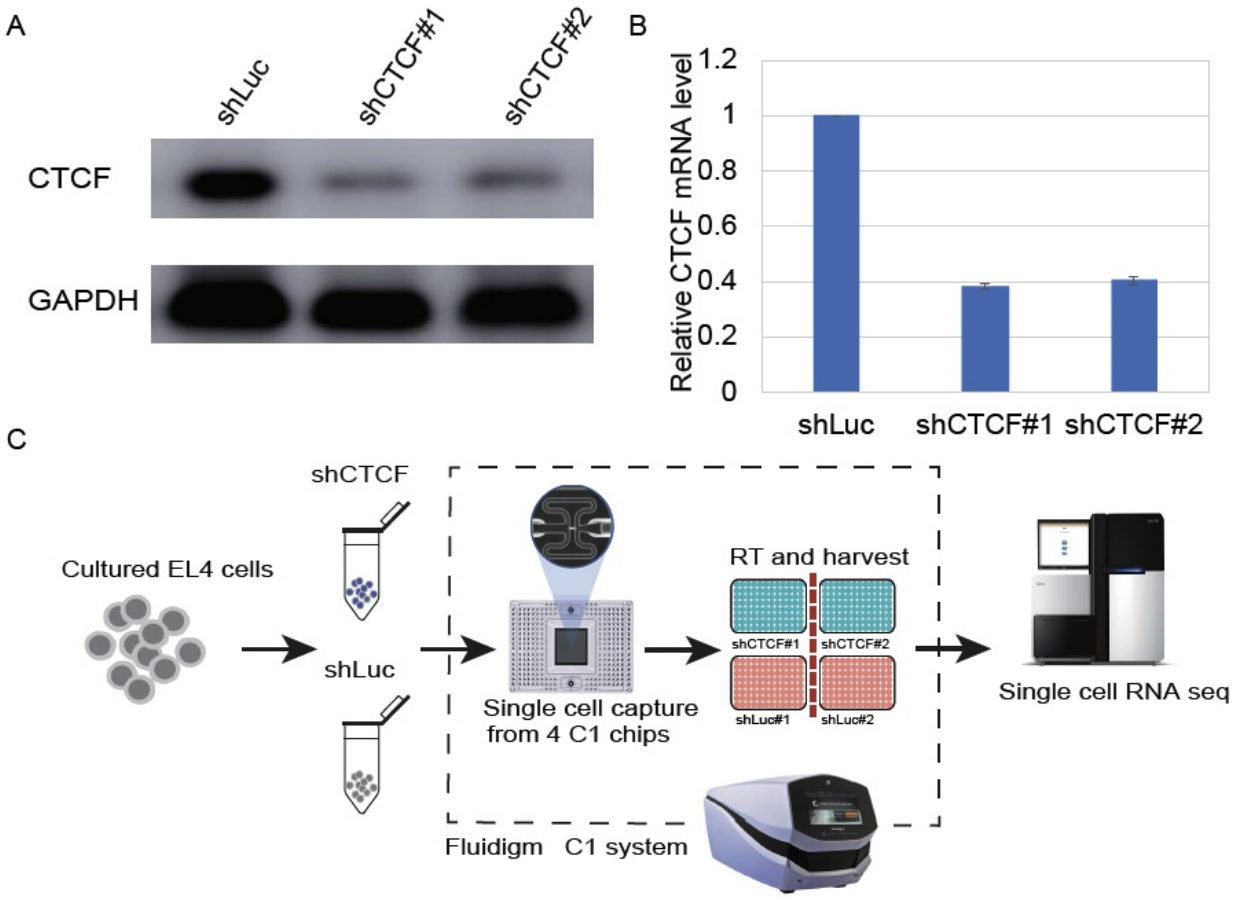


Figure 1

Knockdown of CTCF and schema of single cell sequencing. A. Western blot analysis of CTCF in luciferase control (shLuc) and CTCF-KD cells (shCTCF#1 and shCTCF#2). EL4 cells were infected with retroviral particles encoding GFP and an shRNA targeting CTCF or a control sequence for 5 days. B. Real-time quantitative PCR (RT-qPCR) analysis of CTCF expression in luciferase control (shLuc) and knockdown (shCTCF#1 and shCTCF#2) cells. The expression level of CTCF was normalized to GAPDH. C. Schema of single cell RNA sequencing using Fluidigm C1 system.

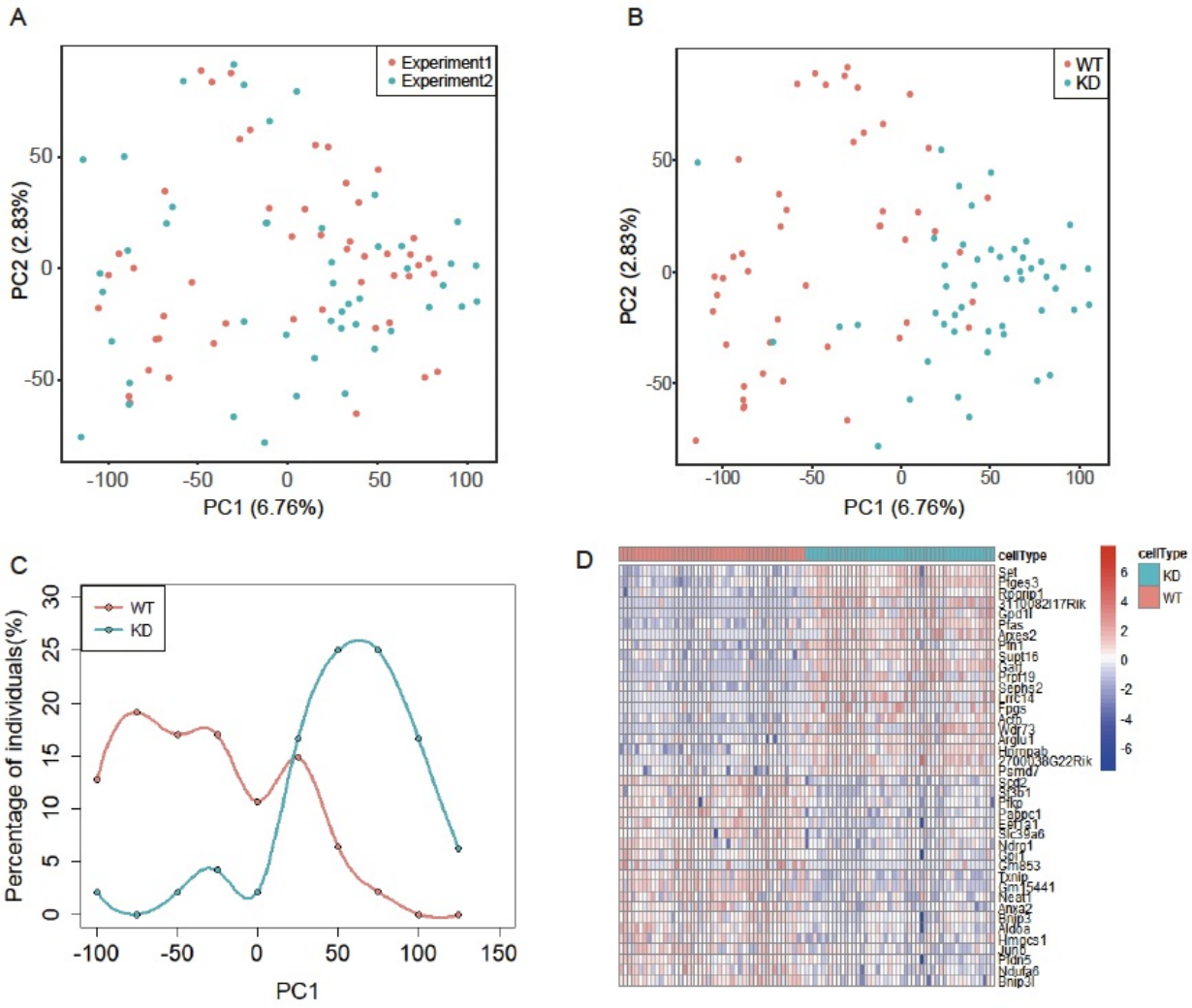
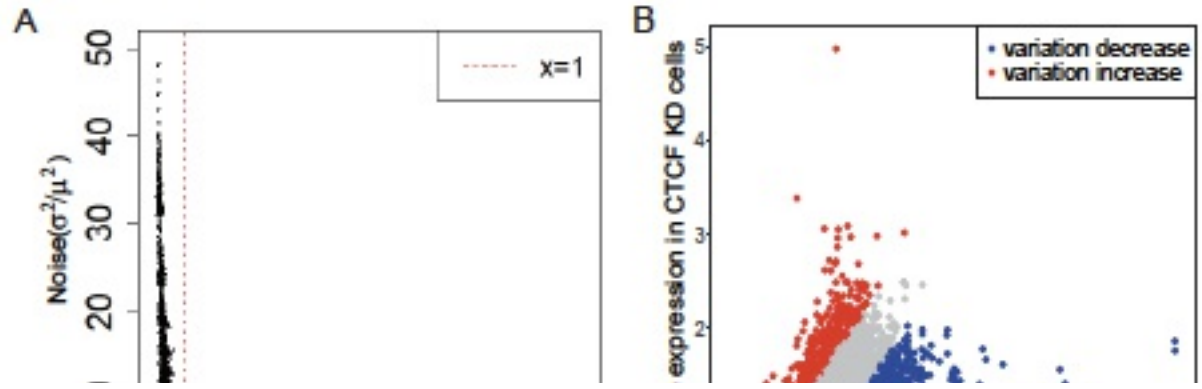


Figure 2

There is systematic difference between CTCF-KD and WT cells. A. No significant batch effect among the experimental repeats based on PCA analysis. B. CTCF-KD cells were largely distinguishable from WT cells on PCA projection. C. Distribution of individual WT cells and individual CTCF-KD cells on PC1. D. Heatmap of differentially expressed genes (TOP 20) between WT cells and CTCF-KD cells.



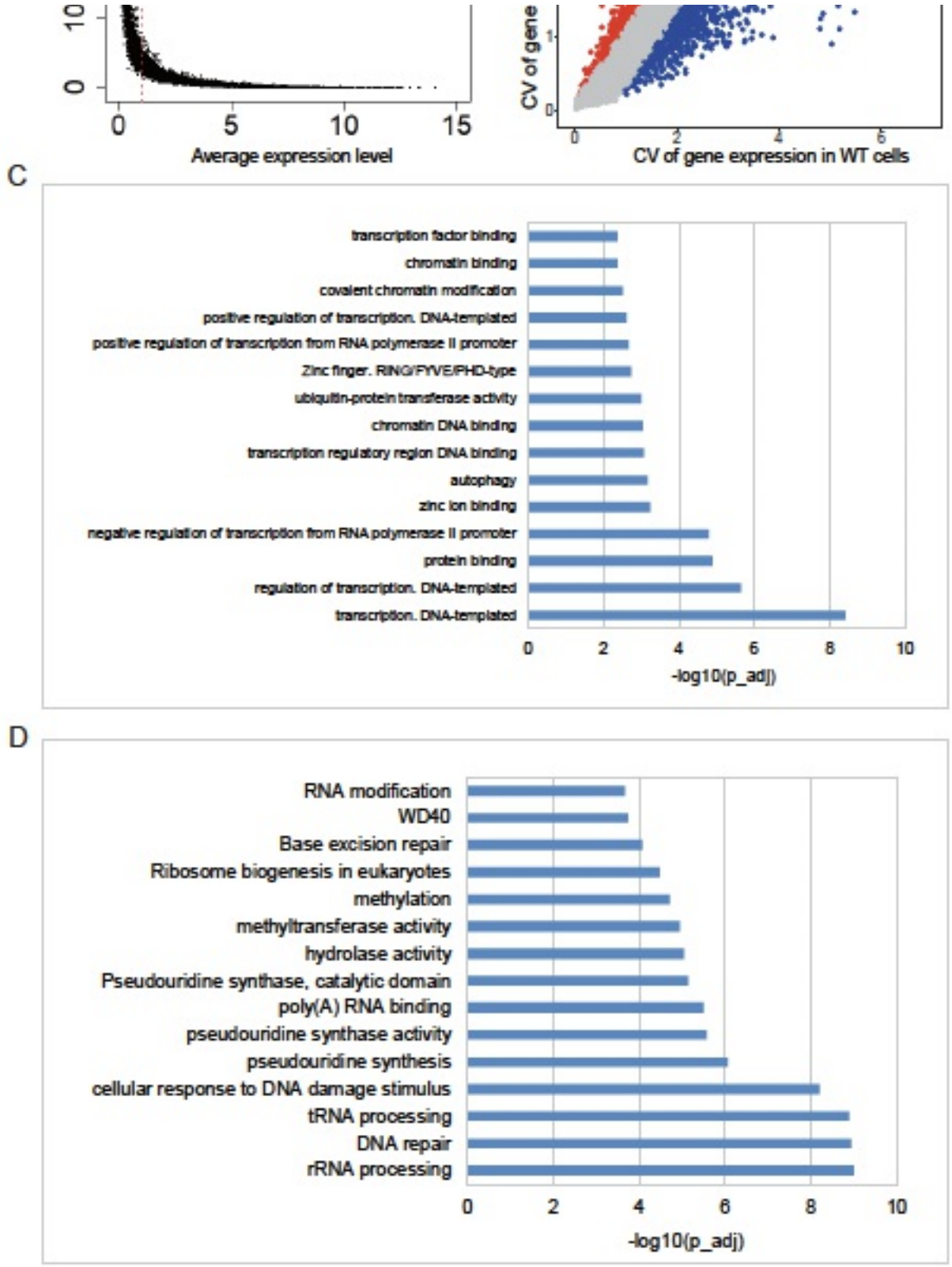


Figure 3

Identification and analyses of genes showing cellular variation-changed after CTCF KD. A. The relationship between expression level and noise level of reference genes. Genes with

low cellular variation was used for further analyses. B. Scatter plot of the cellular variation-changed genes after CTCF KD. Blue and red indicate the variation decrease and variation increase, respectively. C. The top 15 gene categories enriched in variation increased gene. D. The top 15 gene categories enriched in variation decreased gene.

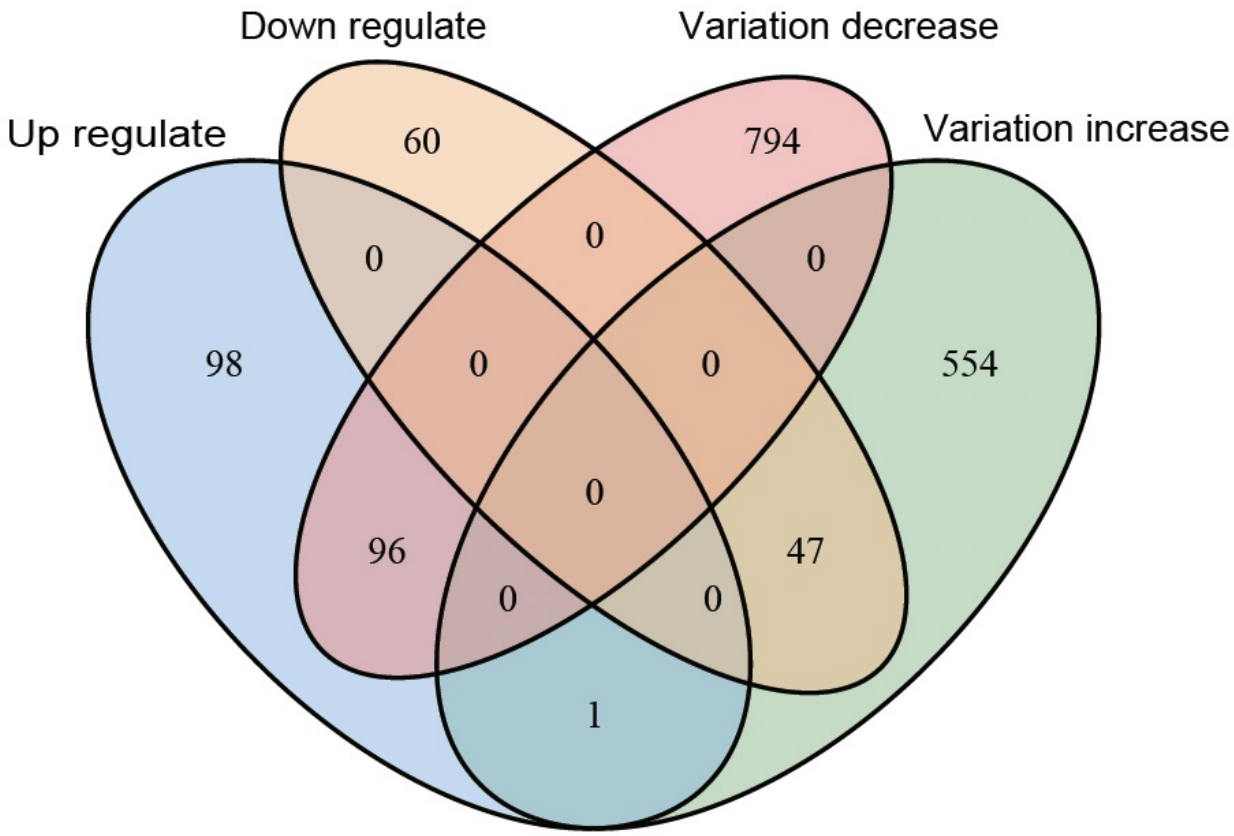


Figure 4

Genes showing cellular variation change are more likely to be differentially expressed genes. Genes with expression decreased and cellular variation increased were significantly over-represented (P=0.29×10-23, χ2 test). Genes with decreased cellular variation and increased expression level were significantly over-represented (P=0.48×10-23, χ2 test).

Supplementary Files

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supple_2.pdf
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