Supplementary Figure 1

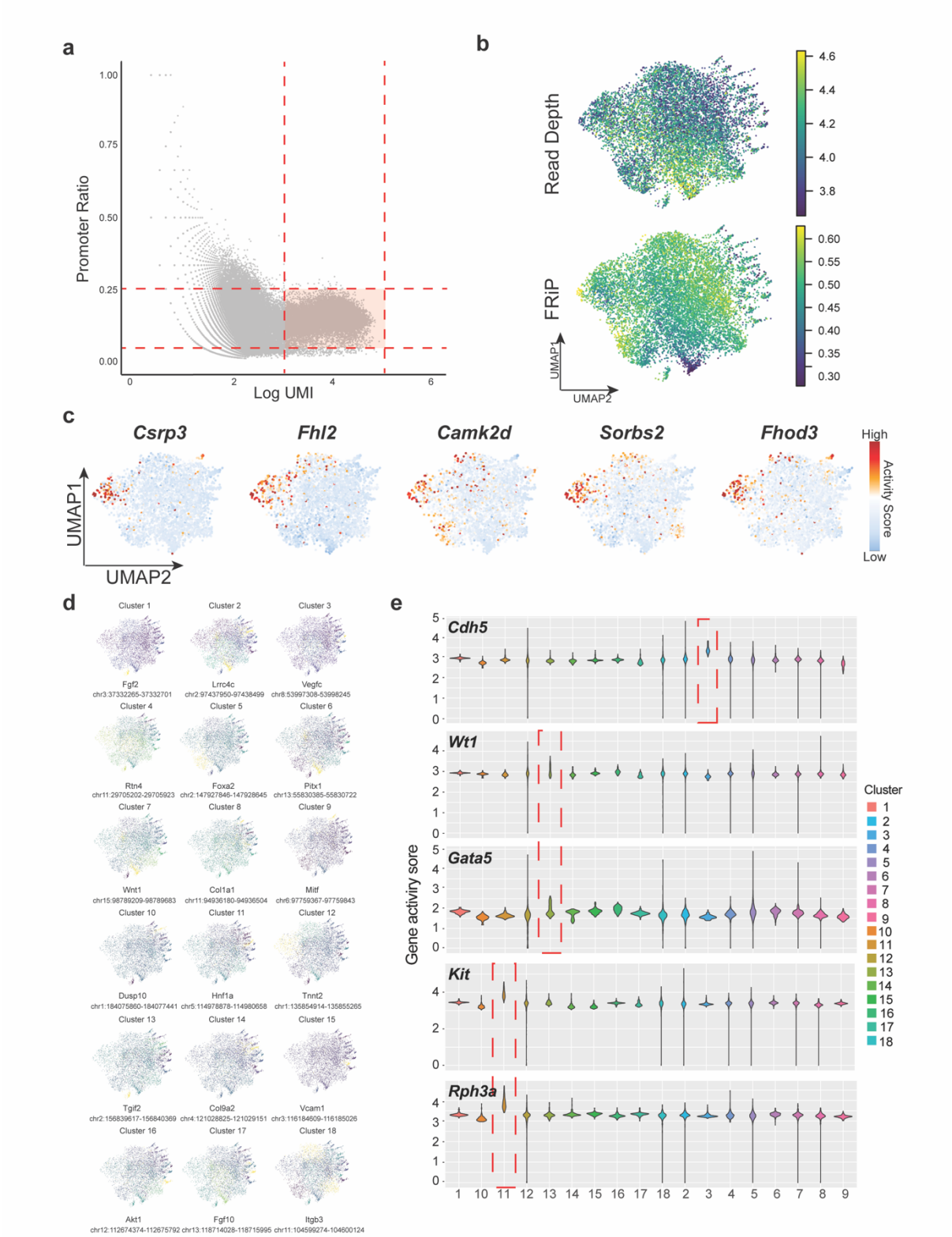

Figure S1. Single-cell ATAC profiling of mouse cardiac reprogramming

a) Scatter plot showing that the low-quality barcodes whose 1) log10(UMI) <= 3.5 or >= 5; or 2) promoter ration <= 0.05 or >= 0.25 were filtered out from the dataset. b) UMAP visualization of Read Depth and the fraction of reads in peaks (FRiP). C) Violin plot showing the gene activity score of genes related to endothelial (*Cdh5*), epicardial (*Wt1*, *Gata5*), and neuron progenitor (*Kit*, *Rph3a*). d) Accessibility score of DARs specific to each cluster superimposed onto UMAP visualization of our scATAC-seq libraries. The nearest gene of each DAR is shown above the DAR's coordinate. e) Gene activity score of cardiac genes of each cell superimposed onto UMAP visualization of our scATAC-seq libraries. Red represents high gene activity score. Blue represents low gene activity score.

Supplementary Figure 2

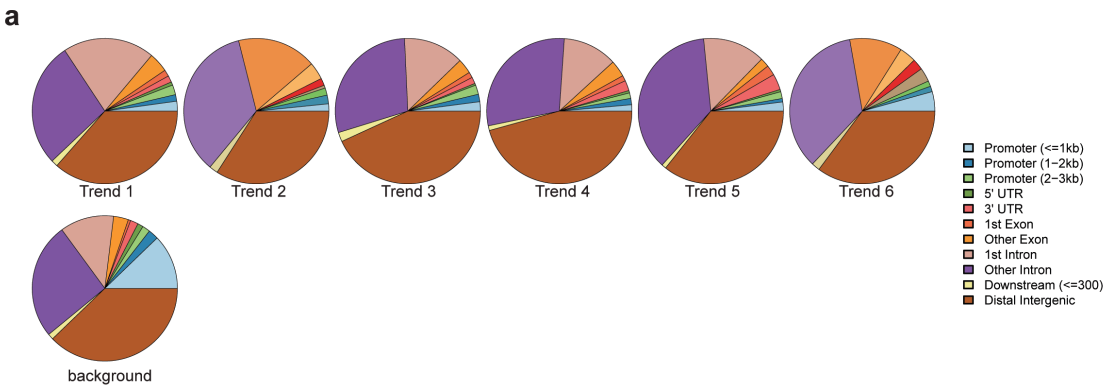

Figure S2. Chromatin dynamics of the cardiac reprogramming along Pseudo-temporal trajectory

a) The pie chart showing the genomic feature composition of DARs in each trend. The genomic feature composition of total ATAC-seq peaks was used as background for comparison. Each color represents one genomic feature.

Supplementary Figure 3

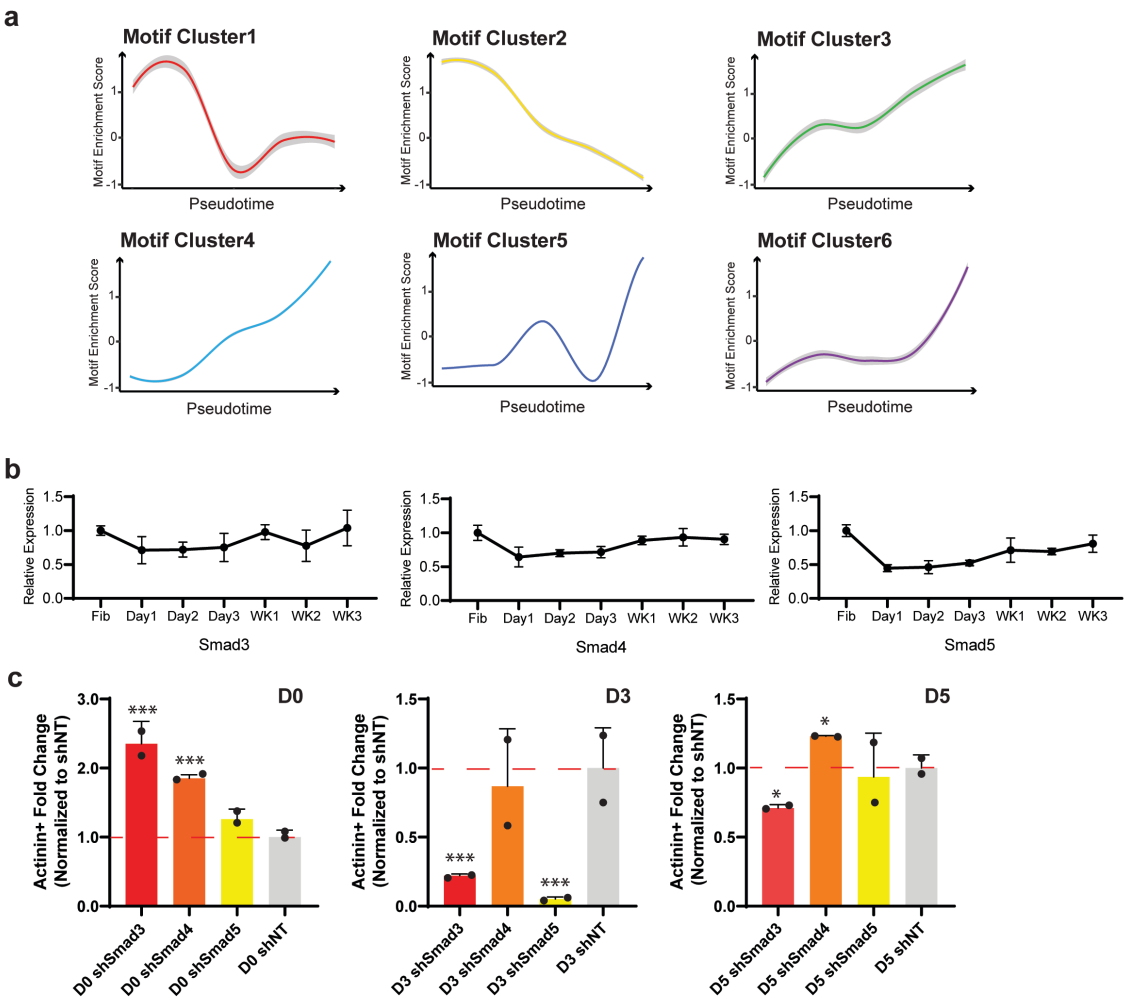

Figure S3. Transcription factor networks of cardiac reprogramming

a) The motif enrichment patterns of six clusters identified in the unsupervised clustering. b) The expression of Smad 3/4/5 along the cardiac reprogramming process. Y-axis represents the relative expression normalized to gene's expression in Fibroblast. Experiments were performed in biological replicates, n=3. Error-bar represents the standard deviation. c) Bar chart showing the fold change of reprogrammed cells (αActinin+) compared to shNT (empty pLKO vector) when knocking down Smad Family TFs, Smad3(red), Smad4(orange), and Smad5(yellow), at D0(left), D3(middle), and D5(right). The red dashed line is added at a fold change of 1 for easy comparison between treatment groups. Error-bar represents the standard deviation. Student's t-test was used for statistical analysis. * represent a p-value less than 0.05. *** represent a p-value less than 0.001.

Supplementary Figure 4

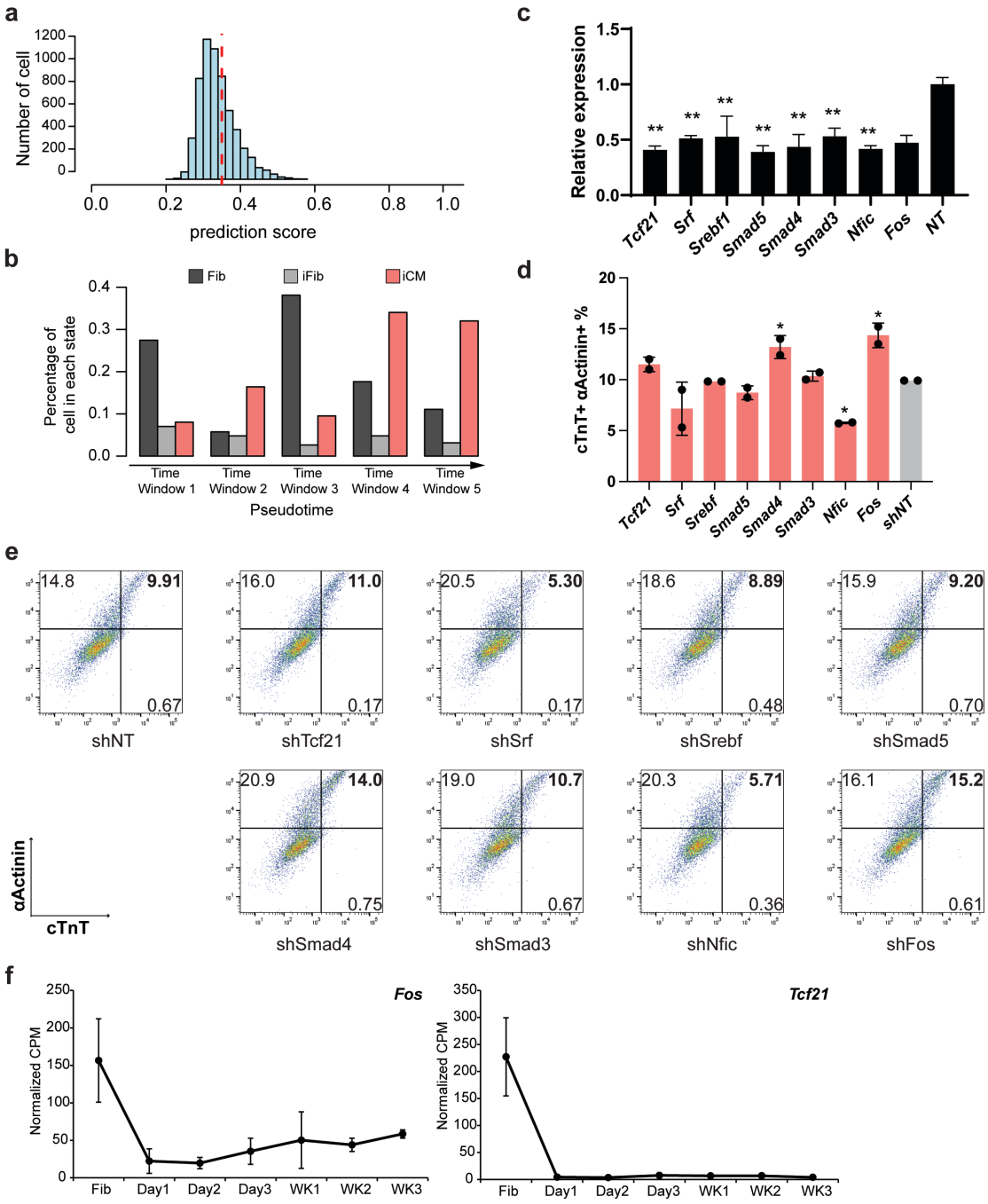

Figure S4. Integrative scATAC-seq and scRNA-seq analysis identified active TFs of cardiac reprogramming

a) The distribution of prediction score of ATAC-seq and reprogramming states for each cell. X-axis represent the prediction score. Y-axis represent the number of cells in each range of prediction score. The red-dashed line represents the 0.35 cut-off line. b) The distribution of three cell states along the Pesudotime trajectory. Y-axis represents the percentage of cells in each cell state. X-axis represents the pseudotime windows. c) Bar chart showing the knockdown efficiency of shRNA designed to target putative active TFs. Y-axis represents the

relative expression normalized to empty shRNA vector (shNT). Experiments were performed in biological replicates, n =3. Error-bar represents the standard deviation. Student's t-test was used for statistical analysis. ** represents p-value <0.01. d) Bar chart showing the percentage of reprogrammed cells (αActinin+/cTnT+) when knocking down these putative active TFs. Error bar represents the standard deviation of replicates within each shRNA KD treatment group. Student's t-test was used for statistical analysis. * represent a p-value less than 0.05. e) Representative flow cytometry plot showing the percentage of αActinin+ cTnT+ cells after knocking down putative active TFs using shRNA. f) The expression of Fos and Tcf21 along the cardiac reprogramming process. Y-axis represents the normalized CPM (counts per million) based on published RNA-seq dataset during cardiac reprogramming. Experiments were performed in biological replicates, n=3. Error-bar represents the standard deviation.