Annual Summary for CDN

In our previous work, we performed paired single-nucleus RNA-seq and single-nucleus ATAC-seq (sn-multiomics) on three endocardial fibroelastosis (EFE) and three control samples. From these data, we identified two pathological transitional endothelial cell (EC) populations within EFE tissues and discovered a transcription factor (TF), *FLI1*, with significantly elevated chromatin occupancy that may drive the abnormal cell transition/migration (**Fig.1**).

In the current study, we extended our computational framework, TRACED, to identify putative target genes exhibiting time-lagged regulatory responses following *FLI1* expression. Specifically, TRACED models the regulatory interaction between a TF and its target genes using an ordinary differential equation (ODE) framework that captures the temporal dynamics of gene regulation. We benchmarked TRACED's performance on simulated data and demonstrated that it accurately recovers known regulatory targets (**Fig.2**).

Applying TRACED to our sn-multiomics dataset, we identified 32 high-confidence putative targets of *FLI1*, including *HMCN1* (**Fig.3A**). Functional enrichment analysis revealed that these targets are significantly associated with biological processes including cell-cell junction organization and sprouting angiogenesis (**Fig.3B**), suggesting that the *FLI1*-mediated gene regulatory network (GRN) is functionally linked to EFE pathogenesis.

To further characterize the activity of this regulatory network, we developed two novel modules within TRACED: (1) a velocity inference algorithm that leverages TF-target gene relationships to predict regulatory dynamics, and (2) a visualization framework for assessing TF-mediated GRN activity scores across cell populations. Applying these tools, we observed that *FLI1* GRN shows high activity in the EC_2 and EC_3 transitional cell populations within EFE tissues (**Fig.3C**), consistent with our previous findings of increased *FLI1* chromatin occupancy in these cells.

Collectively, our results indicate that *FLI1*, a well-characterized transcription factor, may function as a master regulator driving EFE disease progression through its effects on cell

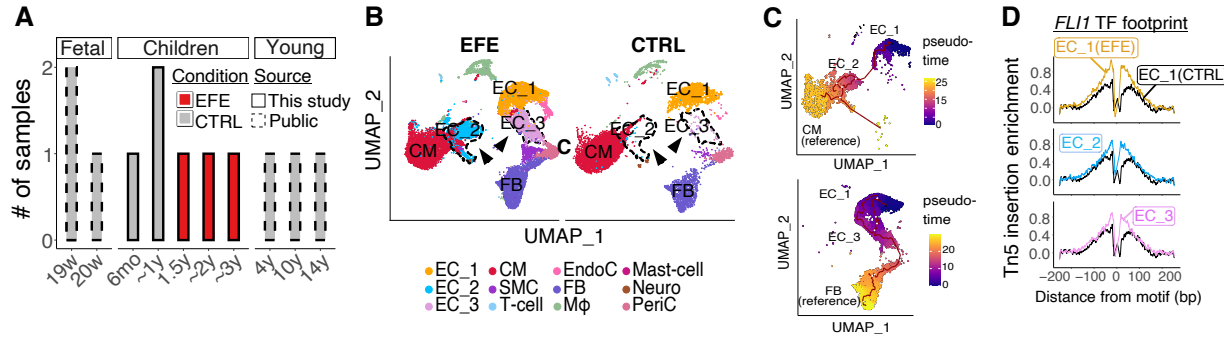

Figure 1. Transitional cells and elevated *FLI1* activity in EFE. (A). Sample information. (B). Annotated cell clusters. (C). Pseudotime trajectory of EC_2 and EC_3 (EC_1 origin) using cardiomyocytes and fibroblasts as reference cell types, respectively. (D). Footprint of *FLI1* in EC populations.

migration and angiogenesis. This study establishes TRACED as a powerful framework for dissecting TF-mediated regulatory dynamics in disease contexts.

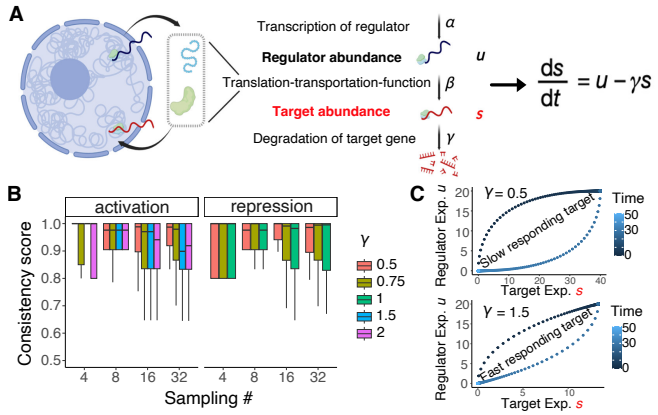

Figure 2. TRACED framework and performance. (A). TRACED framework with ordinary differential equation (ODE) modeling to infer regulator-target gene networks/relationships based on time-lag information. α,β,γ represent transcription activity of the regulator, translation-transportation and degradation speed of the target gene, respectively; *u* and *s* represent regulator and target gene abundance, respectively. (B). Accuracy score of TRACED using simulated data. The x-axis represents the number of real/pseudo-time points simulated. The parameter γ represents the time-lag range being simulated. An accuracy score of 1 indicates 100% accuracy of recapturing the true regulator-target relationships in both activating and repressing relationships. (C). The range of γ and the associated time-lag range between regulators and their target genes.

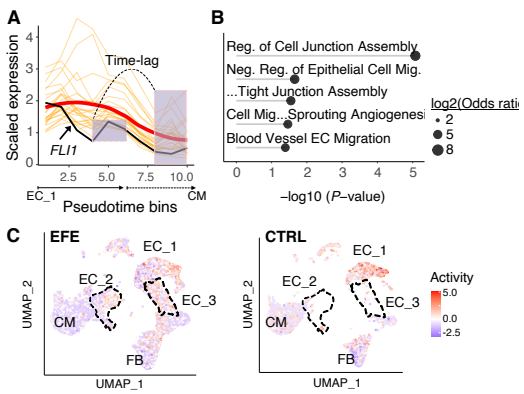

Figure 3. *FLI1* as a potential master regulator of EFE cell transitions. (A). TRACED inferred target genes of *FLI1* in EC_2 trajectory. Black line is the averaged *FLI1* expression across pseudotime bins, orange lines are individual 32 target gene expressions, red line is smoothed expression for 32 target genes. (B). Top functional annotation of *FLI1* target genes. Reg.: regulation; Mig.: migration. (C). *FLI1*-associated gene regulatory network normalized activity scores inferred by TRACED.

Future Directions and Data Availability:

The TRACED software package will be made publicly available upon publication. Additionally, we will extend our analyses to incorporate spatial transcriptomics data to integrate spatial coordinate information, enabling the contextualization of TF regulatory activity within the tissue microenvironment.