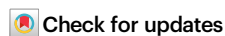


Decomposing multi-scale dynamic regulation from single-cell multiomics with scMagnify

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Deciphering the highly coupled regulatory circuits that drive cellular dynamics remains a fundamental goal in biology. However, capturing the multi-scale time-lagged dynamics and combinatorial regulatory logic of gene regulation remains challenging. Here we present scMagnify, a deep-learning-based framework that leverages chromatin accessibility and gene expression via Granger causality to reconstruct and decompose multi-scale gene regulatory networks (GRNs). Diverse benchmarks demonstrate scMagnify's favorable performance. scMagnify employs tensor decomposition to identify combinatorial TF modules and their activation profiles across multiple time-lags. We applied scMagnify to human hematopoiesis and mouse pancreas development, where it recovered known lineage-driving regulators and provided further insights into the combinatorial logic underlying cell fate decisions. Furthermore, in the context of kidney injury, scMagnify's intercellular communication module mapped key signaling-to-transcription cascades linking microenvironment cues to pathological epithelial cell changes. In summary, scMagnify provides a robust computational framework for dissecting the multi-scale regulatory logic that governs complex biological processes in development and disease.

Cell fates and complex organismal structures are established through a sophisticated series of molecular cues that orchestrate intricate and highly specific programs of gene expression. These programs are governed by interconnected molecular networks, which are shaped by a repertoire of cell-type-specific transcription factors (TFs)^{1–3}. Importantly, TFs typically bind to cis-regulatory regions containing clusters of diverse TF binding sites. When associated TFs are co-expressed in overlapping spatial domains, their combinatorial binding generates precise spatiotemporal transcriptional activity patterns^{4,5}. This context-dependent regulation allows TFs to integrate multiple signals, activating or repressing target genes (TGs) with high specificity, thereby driving the intricate gene expression programs required for cellular specialization and organismal development⁶.

Over the past decades, a variety of computational methods have been proposed to unravel the intricate regulatory mechanisms controlling gene expression^{7,8}. Transcriptome-based methods, such as WGCNA⁹, GENIE3¹⁰, GRNBoost2¹¹ and PIDC¹², infer TF–TG relationships by capturing covariation in gene expression. Although tree-based methods, such as GENIE3 and GRNBoost2, can infer directed regulator–target associations when candidate regulators are specified, expression-only approaches generally remain limited in distinguishing direct from indirect effects and lack explicit causal interpretability^{13,14}.

To overcome these limitations, recent advances have taken two major directions. First, transcriptional regulation unfolds dynamically during cell-state transitions, where TF activity and TG expression may be temporally offset. Incorporating temporal or pseudotemporal information is therefore important for distinguishing regulatory

drivers from downstream responses. Although single-cell profiles are typically measured as static snapshots rather than true time-series observations, pseudotime-ordering^{15,16} and RNA-velocity^{17,18} methods provide temporal ordering and transition directionality, offering a basis for modeling dynamic regulatory relationships along cellular transitions (Supplementary Note 1). These approaches, including widely used frameworks such as Monocle¹⁹, Slingshot²⁰, Palantir²¹, and scVelo²², have been systematically reviewed and benchmarked in previous studies. More recently, CellRank^{23,24} has enabled unified fate mapping in multiview single-cell data by integrating diverse transition information to infer fate probabilities and lineage relationships in complex cellular landscapes. Building on these representations, pseudotime-informed GRN inference methods model regulatory relationships along cellular transitions, often using time-lagged or autoregressive strategies^{25–28}. Among these, classical Granger causality and its nonlinear or deep-learning-based extensions infer directed regulatory relationships by evaluating whether earlier states of candidate regulators improve the prediction of their putative targets^{29,30}. However, these models often assume a reliable ordering and approximately uniform spacing of observations along pseudotime. In real single-cell systems, these assumptions can be challenged by sparse sampling, uneven cell-state densities, branching or convergent trajectories, and feedback-driven or cyclic regulatory interactions^{26,28,31,32}. Recent frameworks, such as Velorama³³ and RegVelo³⁴, have begun to address parts of this challenge by integrating dynamic modeling with regulatory information, highlighting the need to bridge cell-state dynamics and gene-regulatory mechanisms.

Second, complementary molecular modalities, such as chromatin accessibility (e.g., DNase-seq³⁵, ATAC-seq³⁶) or TF binding profiles (e.g., ChIP-seq³⁷), have been integrated to provide mechanistic evidence for regulatory interactions. These allow the identification of regulatory elements (REs), which can be linked to TFs via motif matching and associated with nearby TGs, forming TF–RE–TG connections³⁸. While these strategies offer mechanistic insights, they often rely on separate measurements from different samples or cell populations, limiting their resolution and ability to resolve dynamic regulatory relationships.

The development of single-cell sequencing has begun to overcome these challenges by profiling the transcriptome and epigenome at cellular resolution, thereby enabling the investigation of causal and temporal relationships among critical regulatory events^{39–41}. Pioneering methods like SCENIC⁴² reconstructed cell-type-specific GRNs by integrating scRNA-seq data with population-level chromatin information. Subsequent approaches, including Pando⁴³, FigR⁴⁴, and SCENIC+⁴⁵, introduced various optimizations by leveraging single-cell chromatin accessibility data in more intricate ways to better define regulatory connections. Furthermore, LINGER⁴⁶ employs a lifelong learning framework to build highly accurate GRNs by integrating knowledge from atlas-scale external bulk data across diverse cellular contexts. Beyond improving GRN inference accuracy, a new generation of methods has introduced powerful downstream applications to provide more biologically meaningful insights. For example, CellOracle⁴⁷ utilizes the inferred GRNs to simulate the effects of TF perturbations and predict their function in driving cell state transitions, while Dictys⁴⁸ moves beyond a static view by modeling how the GRNs dynamically rewire across a continuous cellular process.

Despite these significant advances, several key limitations remain⁴¹. First, for inference accuracy, most methods do not simultaneously leverage multimodal and pseudotemporal information, which are critical for identifying causal regulatory interactions. Second, for downstream application, current approaches tend to focus on individual regulator activity in discrete cell states, overlooking the continuous dynamics of cellular transitions and the combinatorial logic³ of regulatory modules central to complex biological processes.

Here, we present scMagnify (**M**ulti **s**cA**l**e **G**ene regulatory **N**etwork **I**n**F**erence and **a**naly**S**is), an integrated computational framework designed to address these challenges. To improve inference accuracy, scMagnify synergistically integrates single-cell multi-omic data and pseudotemporal information through a Granger causality model^{49,50} to infer multi-scale GRNs. For downstream analysis, it enables a multi-level investigation of gene regulation, moving from individual regulators to the identification of combinatorial regulatory modules via tensor decomposition⁵¹, and further linking these intracellular dynamics to extracellular cues by modeling signaling-to-transcription cascades. We demonstrate the power and versatility of scMagnify in both developmental and disease contexts, where it rediscovers known master regulators and characterizes multi-scale regulatory programs driving cell state transitions.

Results

Overview of scMagnify

scMagnify is a versatile Python toolkit for inferring and analyzing gene regulatory networks (GRNs) from single-cell multi-omic datasets. To ensure a targeted and robust GRN inference, scMagnify first identifies a set of high-confidence genes intrinsically linked to the specified cellular dynamics. It achieves this by establishing cellular trajectories and using generalized additive models (GAMs)⁵² to select genes whose expression significantly correlates with the pseudotime axis. To further constrain the model with plausible chromatin interactions, scMagnify then constructs a basal TF binding network for each cell state. This structural scaffold is built by linking genes to potential cis-regulatory elements (cCREs) via expression-accessibility correlation and scanning these regions for known TF binding motifs⁵³. Together, these steps yield a focused, biologically relevant search space for the final GRN inference (Fig. 1a and “Methods”).

Within this focused search space from the previous step, scMagnify performs GRN inference using a partial ordering-based^{33,54} Granger causality framework. This framework is implemented via a self-explaining neural network featuring a multi-scale architecture. More specifically, we consider these lagged TF expression values as predictors for the regulation coefficient matrices in a feedforward neural network. To capture regulatory effects across different time scales, the model uses parallel branches, each examining cellular dynamics over a different retrospective time lag. Finally, an attention mechanism automatically learns to weight the information from each branch, integrating these multi-scale signals to construct the final GRN (Fig. 1b and Supplementary Fig. 1, “Methods”).

scMagnify includes a suite of functions to analyse context-specific GRNs. To isolate the most robust interactions, the network is filtered based on user-defined statistical thresholds (“Methods”). The resulting GRN then serves as a basis for functional and structural analysis. A primary application is the inference of regulatory activity, where the activity of each TF is quantified from the collective expression of its TGs (its regulon) using a multivariate linear model⁵⁵ (Fig. 1c and “Methods”). Beyond this functional insight, the network structure can be investigated through standard graph-theoretic metrics to identify pivotal hub regulators within the cellular circuitry (Fig. 1e).

It is well-established that TFs often act in a combinatorial manner to regulate their target genes^{56,57}. To systematically explore these regulatory synergies within dynamic processes, scMagnify employs a modified Tucker decomposition approach. This regfactor decomposition method is applied to a tensor comprising the regulatory coefficients (Reg. scores) derived from the multi-scale Granger causality model. This approach enables the simultaneous identification of co-regulating TF modules and their shared sets of TGs across different time lags (Fig. 1d, e, and Supplementary Fig. 1b, “Methods”).

Furthermore, scMagnify links intercellular communication to intracellular regulation by dissecting signaling-to-transcription cascades along the pseudotime axis. To achieve this, it first identifies key

pathways consisting of ligand-receptor pairs and their downstream TFs from prior knowledge^{58–60}. It then correlates each receptor's expression with its target TF's expression across pseudotime and validates these interactions with a permutation test⁶¹. This analysis reveals robust signaling axes that are active during specific cellular transitions (Fig. 1e and “Methods”).

Collectively, scMagnify dissects the multi-level architecture of gene regulation—from the activity of individual regulators, to the combinatorial logic of regulatory modules, and finally to the systemic coordination via intercellular communication—enabling mechanistic insights into the regulation of cellular dynamics.

Comprehensive benchmarks support the accuracy and robustness of scMagnify

To provide a comprehensive evaluation, we benchmarked scMagnify against a set of leading GRN inference methods (Supplementary Data 1). The selected methods represent a variety of modeling strategies and differ in their core capabilities. For example, a major class of methods, including SCENIC+, LINGER, Dictys, CellOracle, and FigR, excel at integrating multi-omic data to infer GRNs, but they do so in a static manner without explicitly modeling temporal dynamics. Conversely, another class of methods, such as Velorama³³ and SINCERITIES⁶², can model cellular dynamics using pseudotime information, but they are limited to single-cell transcriptomic data. This benchmark design allows us to specifically highlight the advantages of scMagnify's integrated framework, which combines multi-modal data with pseudotemporal information to reconstruct GRNs. We also reported the results from scMagnify without the TF binding network (scMagnify-nobasal) to isolate the contribution of the chromatin prior and to enable a fair comparison with methods that rely solely on transcriptomic data (Supplementary Fig. 3a).

Our evaluation was performed on both synthetic datasets with known ground-truth network structures and well-characterized real-world multi-omic datasets. We systematically compared the methods from multiple perspectives, including their accuracy in predicting TF–TG interactions, their ability to recover key driver TFs, and the computational stability and scalability (Fig. 2a).

First, we applied scMultiSim⁶³ to generate synthetic single-cell multiomics datasets with different parameters (Supplementary Fig. 2a). We used the area under the precision-recall curve (AUPR) and the F1-score to assess the accuracy of ground-truth network reconstruction, as both metrics are well-suited for the sparse nature of GRNs. scMagnify achieved significantly higher AUPR and F1 score than CellOracle in the synthetic benchmark, based on one-sided paired Wilcoxon signed-rank tests across simulation replicates (adjusted $P = 0.000122$ for AUPR and 0.003357 for F1; Fig. 2b and “Methods”). We further expanded the synthetic benchmark by varying cell number, GRN size, GRN density, transcript dropout, and ATAC-derived chromatin-prior noise; scMagnify maintained higher AUPR across most perturbation settings, whereas F1 scores were broadly comparable between the two methods under some conditions (Supplementary Fig. 3c, d).

Next, to evaluate scMagnify's real-world performance, we applied our cell state transition module to uniformly processed single-cell multi-omic datasets spanning hematopoietic and pancreas endocrine differentiation trajectories (Supplementary Fig. 2b–g and “Methods”). Each of these lineages represents a distinct dynamic process and serves as an independent test set for GRN inference. We then curated a corresponding gold-standard set of TF–TG interactions from a comprehensive ChIP-seq database to serve as ground truth. Specifically, we collected ChIP-seq experiments from the Cistrome database⁶⁴ to assess whether each method could successfully recover these experimentally supported TF–TG interactions, with performance quantified by the AUPR and F1-score (“Methods”). Across the three real-data benchmarks, scMagnify generally achieved among the highest AUPR

values, although performance differences varied across datasets, metrics, and competing methods. Compared with the best-performing competitor, scMagnify achieved significantly higher AUPR (adjusted $P < 0.01$), whereas the difference in F1 score was not significant and remained dataset-dependent and broadly comparable among the top-performing methods (Fig. 2c and Supplementary Fig. 4a, b). These results support the competitive edge-recovery performance of scMagnify across diverse real-data benchmarks. In conclusion, our systematic set of benchmarks on real-world data demonstrates the comparable or superior performance of scMagnify, highlighting its reliability and high accuracy across a range of dynamic cellular processes.

While edge-level accuracy is a fundamental metric for GRN inference, a more stringent test of any inferred GRN is whether its network topology can be used to identify biologically important regulators^{24,65}. We therefore performed a driver TF recovery benchmark to assess if scMagnify could prioritize known lineage-driving TFs (Supplementary Data 2) more effectively than competing methods. For each inferred network, we ranked TFs by their degree centrality and measured how well this ranking recovered a curated gold-standard list of drivers for each lineage. scMagnify achieved the highest aggregate driver-recovery score among the tested methods (adjusted $P < 0.01$), with individual lineage-level recovery curves provided in the Supplementary Figs. (Fig. 2d and Supplementary Fig. 5a, b). We further compared the rank correlation between inferred and reference degree centrality values. scMagnify showed consistently positive Spearman correlations across most lineages and ranked among the top-performing methods, indicating that its inferred networks better preserve regulator-level topological structure (Fig. 2e). This suggests that the topology of the GRN from scMagnify is not only accurate but also structurally meaningful, successfully identifying key regulators based on their network connectivity.

A critical test for any GRN inference method is its ability to generate networks that are both reproducible and context-specific⁴⁸. To assess scMagnify against this standard, we compared GRNs generated from 10 independent runs with different random seeds across representative lineages in the T-cell-depleted bone marrow dataset. The resulting Jaccard similarity heatmap revealed a clear block-diagonal structure, a pattern arising from high intra-lineage similarity (reproducibility) and low inter-lineage similarity (specificity) (Fig. 2f). Using the CD34+ bone marrow dataset, scMagnify maintained stable AUPR and F1 scores under moderate cell subsampling down to 40% retained cells and under increasing synthetic transcript dropout, with lineage-specific trends shown alongside the median response (Fig. 2g, h). These results validate that scMagnify can robustly infer GRNs that are both consistent within a given biological context and unique to it.

Finally, we evaluated the contribution and robustness of scMagnify's model design. A forward K-scan showed that performance improved as additional pseudotime-lag branches were introduced and plateaued around $K = 5$, supporting its use as the default setting (Supplementary Fig. 6a). Conversely, reverse ablation of lag-specific graph-diffused TF inputs or coefficient-generating branches reduced AUPR and F1 score, indicating that multiple lag branches contribute to GRN reconstruction (Supplementary Fig. 6b–d). Sensitivity analyses using alternative trajectory inputs and transition-matrix perturbations further showed broadly stable GRN performance and network similarity under reasonable trajectory variations (Supplementary Figs. 7 and 8). Scalability profiling showed that runtime increased approximately linearly with cell number, gene number, and lag depth, with manageable CPU and GPU memory requirements across the tested settings (Supplementary Fig. 9a–c).

Together, these benchmarks show that scMagnify achieves competitive GRN reconstruction performance, prioritizes lineage-associated regulators, and produces reproducible, context-specific networks across synthetic and real single-cell multiome datasets.

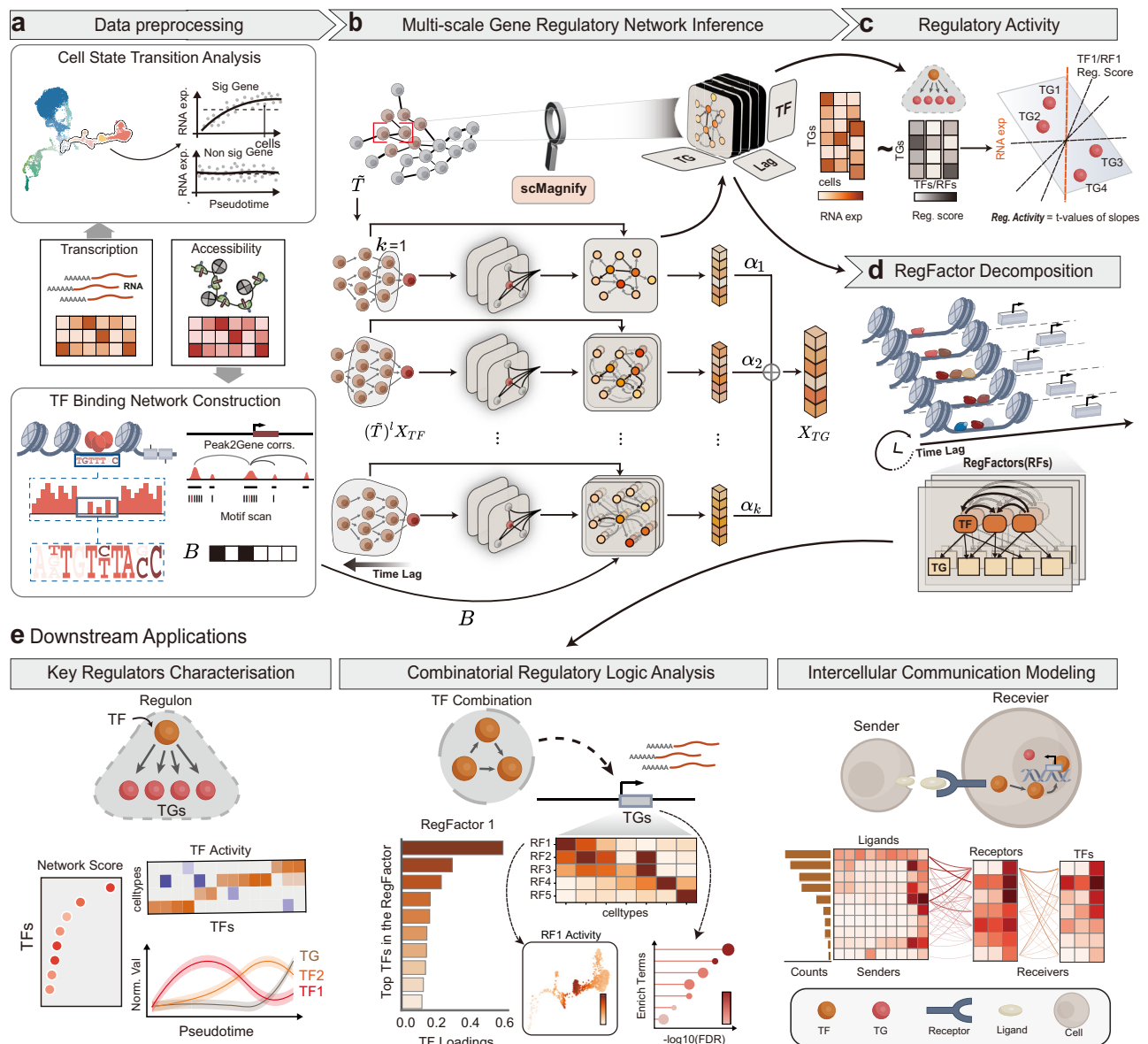


Fig. 1 | Overview of the scMagnify framework. **a** Data preprocessing. scMagnify integrates single-cell transcriptomic and chromatin accessibility data. It first identifies genes with dynamic expression along a pseudotime trajectory. Concurrently, it constructs a basal GRN by linking transcription factors (TFs) to target genes (TGs) based on peak-to-gene correlations and TF motif scanning. **b** Multi-scale GRN inference. A self-explaining neural network implementing Granger causality infers the GRN. The model uses a multi-scale architecture with parallel branches to capture regulatory effects across different time lags, constrained by the basal GRN. An attention mechanism integrates these signals to predict TG expression. **c** Regulatory activity inference. The activity of a TF or RF is quantified using a multivariate linear model based on the expression of its target regulon. The

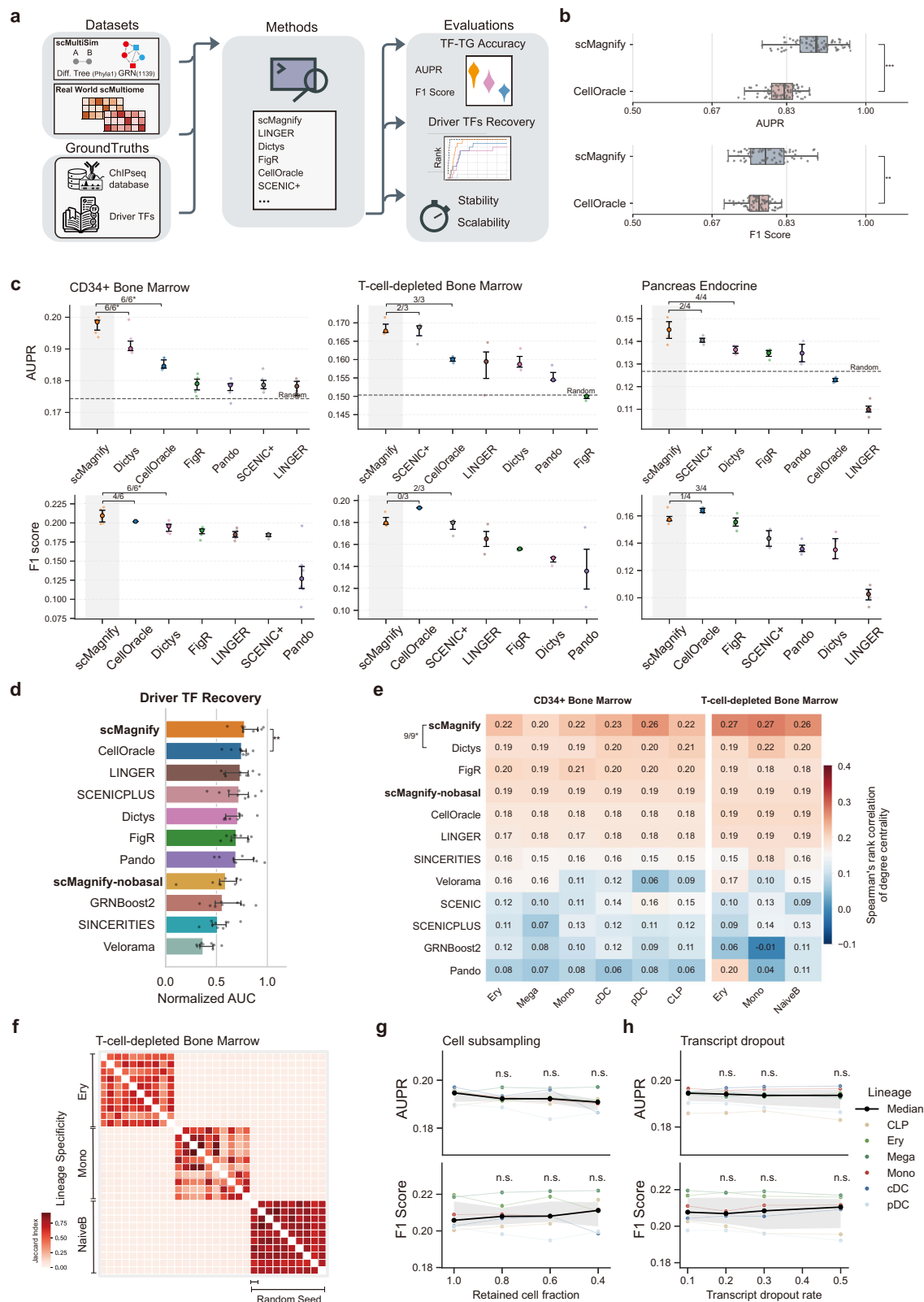
resulting t -value from the model fit serves as the final activity score. **d** RegFactor decomposition. The inferred multi-scale regulatory scores are organized into a third-order tensor (TFs, TGs, time-lags). Tucker decomposition is applied to this tensor to deconvolve co-regulating TF modules, termed RegFactors (RFs). **e** Downstream applications. The inferred GRNs and RegFactors enable multiple analyses: (left) characterization of key regulators by network centrality and activity dynamics over pseudotime; (middle) analysis of regulatory synergy through TF loadings, activity patterns, and functional enrichment of RegFactors; and (right) modeling of intercellular communication by linking ligand-receptor pairs to downstream TF activation in receiver cells. All schematic elements were created by the authors using Adobe Illustrator.

scMagnify dissects lineage-specific key regulators in human hematopoiesis

To demonstrate the utility of scMagnify in developmental contexts, we obtained a human blood dataset containing single-cell multi-omic data of CD34+ T-cell-depleted bone marrow cells from healthy donors⁶⁶, a paradigmatic system where tightly regulated transcriptional programs drive the differentiation of hematopoietic stem cells into a complex hierarchy of distinct lineages⁶⁷. We first classified the data into three lineages and selected 3281 genes that were significantly associated with pseudotime ($\text{FDR} < 0.0001$, $A > 0.3$) (Supplementary Fig. 2b–d and “Methods”). As shown in Fig. 3a, HSCs follow three main

developmental trajectories—the erythroid, monocyte, and Naïve B cell lineages—which are defined by a progression through distinct intermediate cell states. Then we applied scMagnify to investigate the lineages separately to construct the lineage-specific GRN and identify the key regulators driving cell fate decisions.

By comparing GRNs across different lineages (“Methods”), scMagnify identified major lineage-defining TFs with highly specific target sets, including EBF1, PAX5, IRF4 and SPIB for the B cell lineage^{68–71}; GATA2 and KLF1 for the erythroid lineage^{72,73}; and IRF8, EGR1 and KLF4 for the monocyte lineage⁷⁴ (Fig. 3b, c, and Supplementary Fig. 10a, b). In contrast, the analysis also identified TFs with



broader, non-lineage-specific regulatory roles. For instance, *ERG*⁷⁵ and *BACH2*⁷⁶ regulate a large number of targets that are distributed across two or more lineages, indicating their function as more general regulators in hematopoiesis (Fig. 3c).

To better understand how transcriptional programs are orchestrated in different lineages, we next analysed the transcriptional and regulatory dynamics along trajectories. This analysis revealed distinct

waves of TF expression that define each lineage, with key regulatory modules being activated in a highly specific temporal order (Fig. 3d). Specifically, the erythroid trajectory is characterized by the strong early expression of a module including *GATA2* and *KLF1*, the monocytic (Mono) trajectory is driven by the subsequent induction of pioneer factors, such as *CEBPB* and *CEBPB*, while the Naive B-cell lineage

Fig. 2 | scMagnify demonstrates competitive performance in comprehensive benchmarks. **a** Schematic of the benchmarking strategy. **b** AUPR and F1 scores for scMagnify and CellOracle on synthetic datasets. **c** AUPR and F1 scores for each method across the CD34+ bone marrow, T-cell-depleted bone marrow, and pancreas endocrine datasets. **d** Normalized AUC for recovery of curated lineage-associated driver TFs. **e** Spearman rank correlations between reference and inferred TF degree-centrality rankings across lineages and datasets. **f** Jaccard similarities between GRNs inferred from 10 runs with different random seeds in three lineages. Robustness to cell subsampling (**g**) and synthetic transcript dropout (**h**) in the CD34+ bone marrow dataset. Colored lines are individual lineages and black lines are medians. All schematic elements in **a** were created by the authors using Adobe Illustrator. For **b**, $n = 15$ matched simulation replicates; boxes show the median and interquartile range (25th–75th percentiles), whiskers extend to $1.5 \times$ the interquartile range, and points indicate individual simulation replicates. One-sided paired Wilcoxon signed-rank tests (alternative: scMagnify greater than CellOracle) gave raw/adjusted $P = 0.000061/0.000122$ (AUPR) and $0.003357/0.003357$ (F1).

For **c**, each small point is one matched lineage-level task ($n = 6, 3$, and 4 for the CD34+ bone marrow, T-cell-depleted bone marrow, and pancreas endocrine datasets, respectively; pooled $n = 13$); within each method, large markers show the median, and error bars span the interquartile range across lineage-level tasks. Dashed lines indicate random-predictor performance. Pre-specified one-sided paired Wilcoxon signed-rank tests were Benjamini–Hochberg-adjusted within each dataset $\times$ metric family. For **d**, bars show mean $\pm$ s.e.m. across $n = 9$ matched tasks; $P = 0.001953$ versus CellOracle (one-sided paired Wilcoxon signed-rank test). For **e**, $n = 9$ matched lineage-level tasks; the bracket compares scMagnify with Dictys using a one-sided paired Wilcoxon signed-rank test (raw and adjusted $P = 0.001953$). For **g**, **h**, colored lines represent $n = 6$ matched lineage-level tasks and black lines indicate medians; baseline and perturbed results were compared using two-sided paired Wilcoxon signed-rank tests with Benjamini–Hochberg adjustment within each metric and perturbation family; n.s., adjusted $P \geq 0.05$. Exact P values and source data are provided in the Source Data file.

is established through the robust co-activation of key B-cell determinants like EBF1, PAX5, and BCL11A⁷⁷ (Supplementary Data 2).

Our analysis also highlights the temporal decoupling between the expression of a transcription factor and its regulatory activity^{45,78} (Fig. 3d and “Methods”). To avoid relying on visual comparison of expression and activity curves, we quantified lagged regulation using three complementary summaries: the center-of-mass shift between TF RNA expression and inferred TF activity, the model-inferred regulatory lag, and the dominant lag usage of TF–TG edges (Fig. 3e, Supplementary Fig. 11, and Supplementary Note 2). By inferring GRNs that operate across multiple time-lag scales, scMagnify provides a framework to quantify the regulatory lag of individual transcription factors. We interpret this regulatory lag as a relative, model-derived local pseudotime-lag index, rather than an absolute molecular response time (“Methods”).

Consistent with previous studies, we next examined whether the model-derived lag index was associated with network topology and functional annotation^{33,79}. The model-derived lag index showed positive associations with degree centrality across lineages, although these associations were attenuated after controlling for regulon size and target-gene number. (Supplementary Fig. 10c–e). After controlling for target number, regulon-size downsampling, and lag-shuffling controls, the association was attenuated but remained positive in most lineages (Supplementary Fig. 10f). Functionally, these long-lag TFs, which include the B-cell determinants EBF1, PAX5, and TCF3, are highly enriched for foundational processes like “pioneer-like properties”⁸⁰, “B cell differentiation,” and “Cell fate commitment.” In contrast, TFs with shorter lags are associated with more immediate cellular responses, such as “B cell receptor signaling” and “BCR signaling events” (Fig. 3f).

In summary, scMagnify systematically dissects the key regulators and their dynamic regulation across different hematopoietic lineages. These analyses suggest that scMagnify can nominate lineage-associated regulators and summarize their relative pseudotime-lag patterns, while the inferred lag should be interpreted as a model-derived regulatory feature rather than direct evidence of absolute biological timing. Building on this principle of temporal regulation, we next investigated the combinatorial regulatory logic governing B cell commitment.

scMagnify recovers regulatory logic associated with B cell fate commitment

Deciphering the regulatory logic that drives B cell commitment is challenging, as the process relies on temporally orchestrated combinatorial inputs rather than a simple linear TF cascade^{81–83}. To systematically explore these dynamic regulatory patterns, we therefore applied scMagnify’s regfactor decomposition, hypothesizing that distinct co-regulating TF modules controlling distinct cellular programs can be deconvolved from our multi-scale GRNs.

By decomposing the multi-scale GRNs, we identified several RegFactors. Analysis revealed that each of these RegFactors is regulated by a different combination of TFs, activated at a different developmental stage, and is associated with distinct biological functions.

Specifically, our analysis identified 5 RegFactors that are associated with sequential stages of B-cell lineage progression (Fig. 4a and Supplementary Fig. 12a–c). RegFactor 1, predominantly active in the earliest progenitor states (HSC/HMP), includes key TFs, such as MECOM, RORA, and PBX1, suggesting its role in stem cell maintenance, proliferation, and alternative lineage priming^{84–86}. From this stage, progenitors in HMP/CLP initiate lineage priming through the activation of RegFactor 2, suggesting a transitional regulatory program. This is followed by RegFactor 3, which is highly enriched in cell-cycle regulators^{87,88} like E2F3 and TFDP1 and drives the robust proliferation of these primed progenitors, preparing them for subsequent lineage commitment. In contrast, cells that commit to the B-lineage down-regulate these programs and instead activate RegFactor 4 during the pro-B and pre-B stages. Governed by B-cell regulators IRF4, PAX5, TCF3, and EBF1, these factors are central to establishing B-cell identity⁸⁹. Finally, RegFactor 5, driven by ERG and IRF4, governs the functional readiness of B cells by orchestrating the molecular machinery required for both antigen recognition and presentation⁹⁰ (Fig. 4b, c, and Supplementary Data 3).

To understand the molecular programs underlying B cell commitment, we focused our investigation on RegFactor 4. Consistent with the overall activity of RegFactor 4, its key TFs showed tightly correlated expression and activity patterns peaking in the pro-B and pre-B cell stages (Fig. 4d and Supplementary Fig. 12d). Several high-loading TF pairs exhibited AND-like/synergy-like activity patterns, with the double-high state showing the strongest RegFactor activity (Supplementary Fig. 15a, b). Together, these core TFs orchestrate a broad downstream program of TGs critical for B-cell function, including components of immunoglobulin chains (*IGHM*, *IGKC*), B-cell receptor signaling (*BLK*, *LYN*)⁹¹, and chromatin modification (*CHD7*, *HDAC9*)⁹² (Fig. 4e).

To examine chromatin-level support for this RegFactor-associated program, we focused on the PAX5 locus, a canonical B-cell identity gene. Highly correlated peaks linked to PAX5 showed increasing accessibility along B-cell differentiation and were associated with PAX5 expression. These linked regions contained motif occurrences for RegFactor 4 TFs, including IRF4, EBF1, TCF3, and SOX4, providing chromatin-level support consistent with a coordinated regulatory program around PAX5 (Fig. 4f).

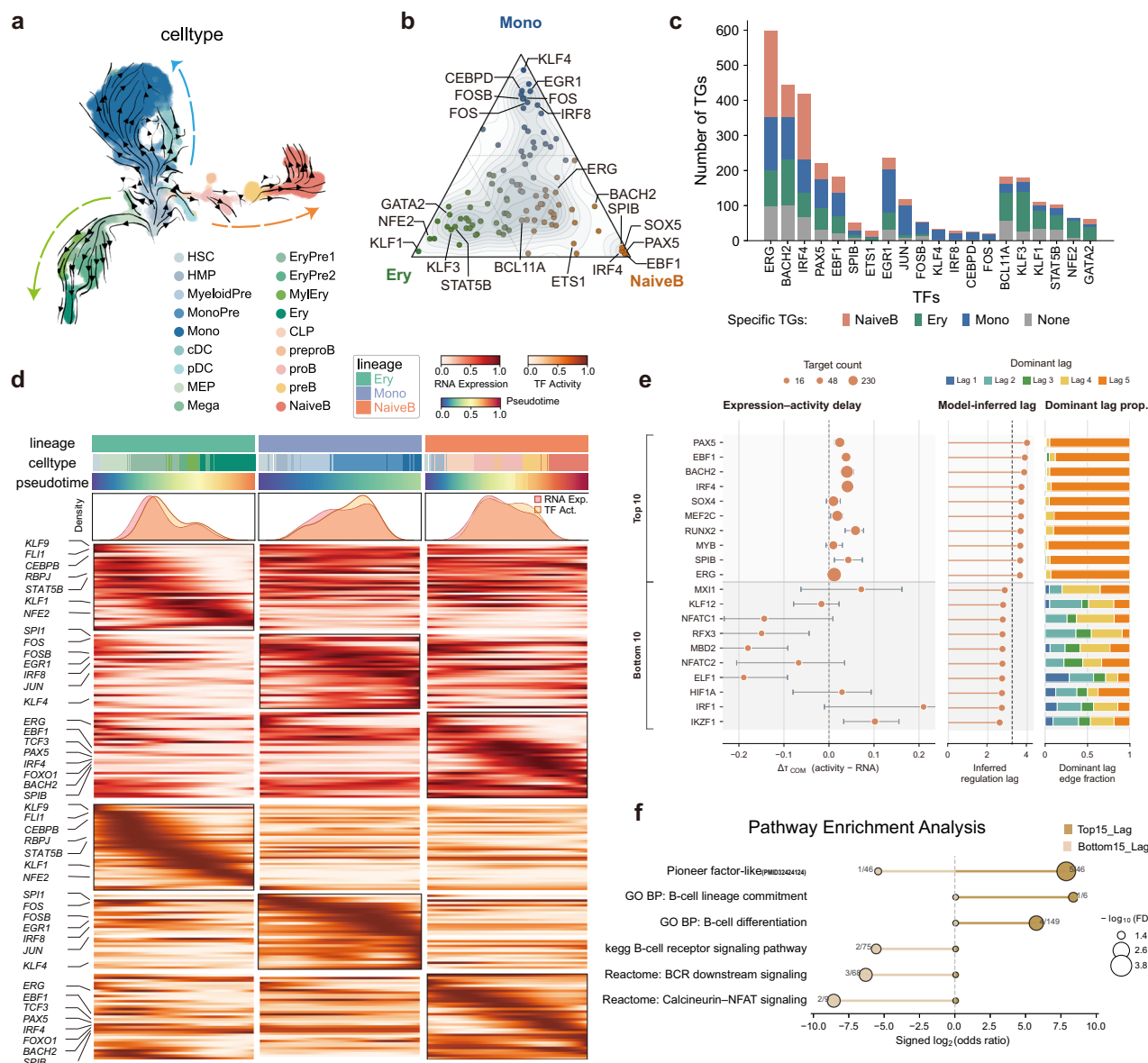


Fig. 3 | scMagnify dissects lineage-specific regulatory programs in human hematopoiesis. a UMAP embedding of human T-cell-depleted bone marrow dataset, colored by cell type. Arrows indicate the three major differentiation trajectories: erythroid, monocyte, and Naive B cell. **b** Ternary plot of key TFs associated with the erythroid, monocyte, and Naive B cell lineages. TFs are positioned according to their lineage specificity. **c** Bar plot quantifying the number and lineage specificity of target genes (TGs) for key TFs. Bar colors represent targets specific to Naive B, erythroid, or monocyte lineages, or those shared across lineages. **d** Heatmap displaying the dynamics of TF gene expression (top panel) and inferred regulatory activity (bottom panel) along the pseudotime axis for the three hematopoietic lineages. **e** Quantitative analysis of TF regulatory lag in the Naive B-cell lineage. The 10 TFs with the highest and lowest model-inferred regulatory lag are shown. In the left panel, points indicate the expression-activity delay ($\Delta\tau_{COM}$),

calculated as the center-of-mass shift between GAM-smoothed TF RNA expression and inferred regulatory activity across lineage cells. Point size represents the number of target genes, and error bars show 95% confidence intervals obtained from 200 bootstrap resamples of cells with replacement for each TF. The middle panel shows the model-inferred local regulatory lag index, and the right panel shows the fraction of retained TF-TG edges assigned to each dominant lag branch. **f** Pathway enrichment analysis comparing the 15 TFs with the highest (Top15_Lag) and lowest (Bottom15_Lag) inferred regulatory lag. The x-axis shows the signed log2 odds ratio, and point size represents $-\log_{10}(\text{FDR})$. Enrichment was assessed using one-sided over-representation analysis against MSigDB gene sets, with a background of 20,000 protein-coding genes and Benjamini-Hochberg correction. Exact adjusted *P* values, overlap counts, and source data are provided in the Source Data file.

scMagnify deciphers regulatory programs contributing to pancreatic lineage maturation

To test the versatility of scMagnify, we applied it to a single-cell multi-omic dataset of mouse embryonic pancreas development. This dataset encompasses the diversification of endocrine progenitors into the primary alpha (alpha) and beta (beta) cell fates, alongside delta (δ) and epsilon (ϵ) lineages (Fig. 5a and Supplementary Fig. 12e, f). We leveraged this developmental map to identify candidate TFs and time-

varying regulatory programs associated with alpha and beta lineage progression.

Based on this dataset, scMagnify inferred dynamic, multi-scale GRNs that captured regulatory changes along endocrine differentiation trajectories. These networks prioritized TFs with lineage-biased inferred activities, including regulators with known roles in endocrine differentiation and maturation.

To systematically identify the regulatory modules, we employed the RegFactor decomposition. The GRNs were decomposed into 5

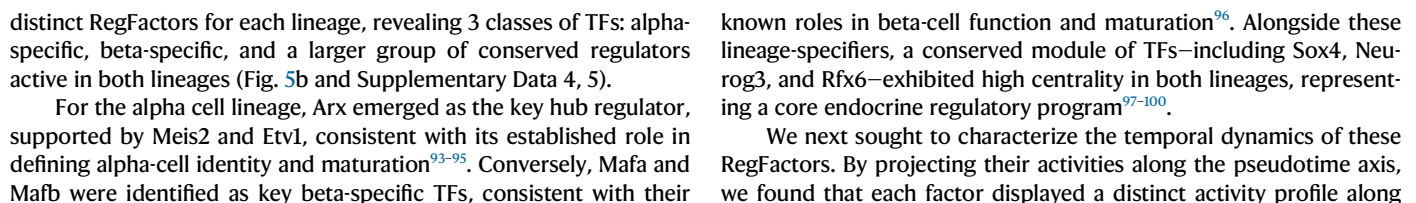


Fig. 4 | RegFactor decomposition reveals combinatorial regulatory logic underlying B cell commitment. **a** A circular heatmap summarizing the 5 RegFactors (RFs) identified in the Naive B cell lineage. From outer to inner rings, the plot shows contributing TFs, TF loadings for each RF, and the average regulatory lag of each RF. **b** Characterization of the RegFactors. For each RF, the plots show the top TFs by TF loading (left bar plot), the activity projected on the UMAP embedding (middle), and the activity distribution across cell types (right violin plot). **c** Dot plot showing functional enrichment of the top 500 target genes for each RegFactor. Rows represent biological pathways, dot color indicates the scaled enrichment score, and dot size represents $-\log_{10}(\text{FDR})$. **d** Comparison of inferred TF activity and RNA expression for major RegFactor 4 TFs. UMAP plots show inferred TF activity (top row) and RNA expression (bottom row) for IRF4, PAX5, TCF3 and EBF1 along the B-cell differentiation trajectory. **e** A subnetwork view of RegFactor 4, organized in three layers to illustrate the regulatory cascade. Inner hexagonal

nodes represent the core TFs. The middle ring of diamond nodes represents highly correlated peaks through the peak–gene association analysis. The outer ring of circular nodes represents target genes (TGs). For TFs, node size corresponds to degree centrality, and for TGs, node color indicates scaled RNA expression. Red edges highlight the specific regulatory interactions targeting the PAX5 gene, corresponding to the genomic analysis in **f**. **f** Genomic view of the PAX5 locus. Chromatin accessibility tracks are shown for different stages of B cell development. The bottom tracks indicate the positions of cCREs (peaks) and predicted binding motifs for key RegFactor 4 TFs. Violin plots on the right show PAX5 RNA expression across the corresponding cell types. For **c**, enrichment was assessed using one-sided over-representation analysis of the top 500 target genes per RegFactor against MSigDB Gene Ontology Biological Process terms, using a background of 20,000 protein-coding genes, with Benjamini–Hochberg correction. Source data for the loading and enrichment panels are provided in the Source Data file.

both the alpha and beta cell lineages (Fig. 5c and Supplementary Fig. 13a, b). This revealed that the lineage-specific RegFactor 5 for each fate exhibited the most striking stage-specific pattern. In both lineages, the activity of their respective RegFactor 5 surged dramatically only at the final maturation stage. To identify the specific TFs driving these terminal programs, we examined the TF loadings for each lineage's RegFactor 5 (Fig. 5d). The analysis confirmed that they are composed of largely distinct TF cohorts: the alpha-specific module is overwhelmingly dominated by *Arx*¹⁰¹, while the beta-specific module is principally led by *Mafk*¹⁰². Consistently, TF-pair quadrant analysis identified AND-like/synergy-like activity patterns for *Isl1–Mafk* and *Arx–Mafk* within the alpha-lineage RegFactor 5 (Supplementary Fig. 15c, d, and Supplementary Data 8). These results nominate lineage-associated TFs that may contribute to late maturation programs.

To gain a mechanistic view of the alpha-specific RegFactor 5 program, we visualized the Alpha-RegFactor 5 regulatory network (Fig. 5e). This revealed a sophisticated architecture organized around a core of co-regulating TFs, with *Arx* acting as a central hub in the inferred network. Its dense connections to other module members, such as *Pax4* and *Sox4*, highlight the coordinated nature of this regulatory program.

Most critically, the network further linked Alpha-RegFactor 5 to alpha-cell functional genes. We identified a regulatory connection from *Arx* to *Gcg* (Glucagon), the definitive hormone produced by alpha cells (Fig. 5g). Underscoring this critical link, an analysis of TG loadings showed that *Gcg* is the highest-weighted gene in this module (Fig. 5f). Beyond direct hormone synthesis, the module coordinates the broader functional machinery required for its activity. For example, it targets the prohormone convertase *Pcsk2*, essential for processing proglucagon, and other genes involved in secretion modulation like *Ffar1*¹⁰³. Epigenetic analysis confirms this link, showing *Gcg* expression correlates with accessibility at enhancer regions containing motif occurrences for key module TFs, including *Arx*, *Pax4*, and *Sox4* (Fig. 5h).

scMagnify maps signaling-to-transcription cascades associated with epithelial cell adaptation during kidney injury

Intercellular communication via ligand-receptor interactions is fundamental to coordinating cellular responses in development and disease. However, how these extracellular signals are dynamically translated into specific intracellular transcriptional programs to orchestrate cell state transition remains largely unresolved^{61,104}. To address this, we developed a dynamic communication module within scMagnify that links intercellular signaling to intracellular TF activity along a pseudotemporal trajectory. Briefly, after establishing ligand-receptor-TF links from a knowledge base, we aggregated cells into metacells ordered along a pseudotime trajectory. We then correlated receptor and TF expression across these metacells and employed a permutation test to identify robust, time-varying signaling-to-transcription axes (Fig. 6a and “Methods”).

To validate scMagnify's capability to infer signaling pairs driving pathological cell state transitions in a complex disease setting, we analyzed a single-cell multi-omic dataset from the cortex of patients with acute and/or chronic kidney disease (AKI/CKD)¹⁰⁵ (Fig. 6b). While this dataset comprises diverse kidney cell types, our analysis focuses on the epithelial populations to investigate their adaptive responses to injury. The dataset captures the transition of thick ascending limb (TAL) epithelial cells from a healthy, cortical state (C-TAL) to a dysregulated adaptive state (aTAL). Trajectory inference using a diffusion map resolved a continuous progression from healthy (C-TAL/M-TAL) to adaptive TAL cell states (aTAL), establishing a pseudotemporal axis to dissect the dynamics of the injury response (Fig. 6c and Supplementary Fig. 14b).

Building on the inferred trajectory, we constructed the GRNs for the TAL, allowing us to identify key regulators (Supplementary Fig. 14a), and then decomposed the network into 5 RegFactors (Fig. 6d, Supplementary Fig. 14d–f, and Supplementary Data 6). Notably, RegFactor 3, which governs TGs functionally enriched for pathological processes like cell adhesion and signaling (Supplementary Fig. 14c), exhibited activity that was highly and specifically enriched in the aTAL state (adjusted $P < 0.0001$; Fig. 6d, e). It is principally driven by the profibrotic TFs *ZEB1*, *SMAD3*, and *KLF6*^{106–109}, which are upregulated in the aTAL state (Fig. 6f and Supplementary Data 8). This prompted us to focus our analysis on this module to dissect the regulatory program driving the adaptive injury response.

To identify the key upstream signaling axes, we prioritized ligand-receptor pairs based on the correlation strength between the receptor's expression and the expression of the core RegFactor 3 TFs (Fig. 6f, g). Among the top-ranked couplings, we identified a candidate *SPP1–CD44/ITGAV* axis, in which broadly expressed *SPP1* from stromal (STR) and immune (IMM) sender populations was linked to *CD44/ITGAV* expression and RegFactor 3 TF activity in aTAL cells. Its receptor, *CD44*, was significantly upregulated on aTAL cells, nominating a candidate injury-associated signaling axis¹¹⁰. In contrast, our analysis identified a putative *NCAM1–FGFR1* signaling axis. Unlike the broadly sourced *SPP1* signal, *NCAM1* originated predominantly from immune cells, indicating a more cell-type-restricted mode of communication. The inferred connection to *EGRI* is consistent with the established coupling of *FGFR* signaling to *MAPK–ERK*-dependent immediate-early transcriptional responses, whereas the predicted link to *RUNX1* may reflect a more context-dependent regulatory cascade (Fig. 6g). Independent renal injury datasets provided orthogonal support for selected RegFactor 3-associated TF and target programs, including injury–recovery dynamics in GSE139107 and altered responses following *Klf6* or *Smad3* deletion, although these non-TAL-specific analyses do not directly validate the complete TAL network (Supplementary Fig. 16). These findings illustrate how scMagnify can deconvolve the complex signaling landscape into both broad-acting and cell-type-specific pathological pathways.

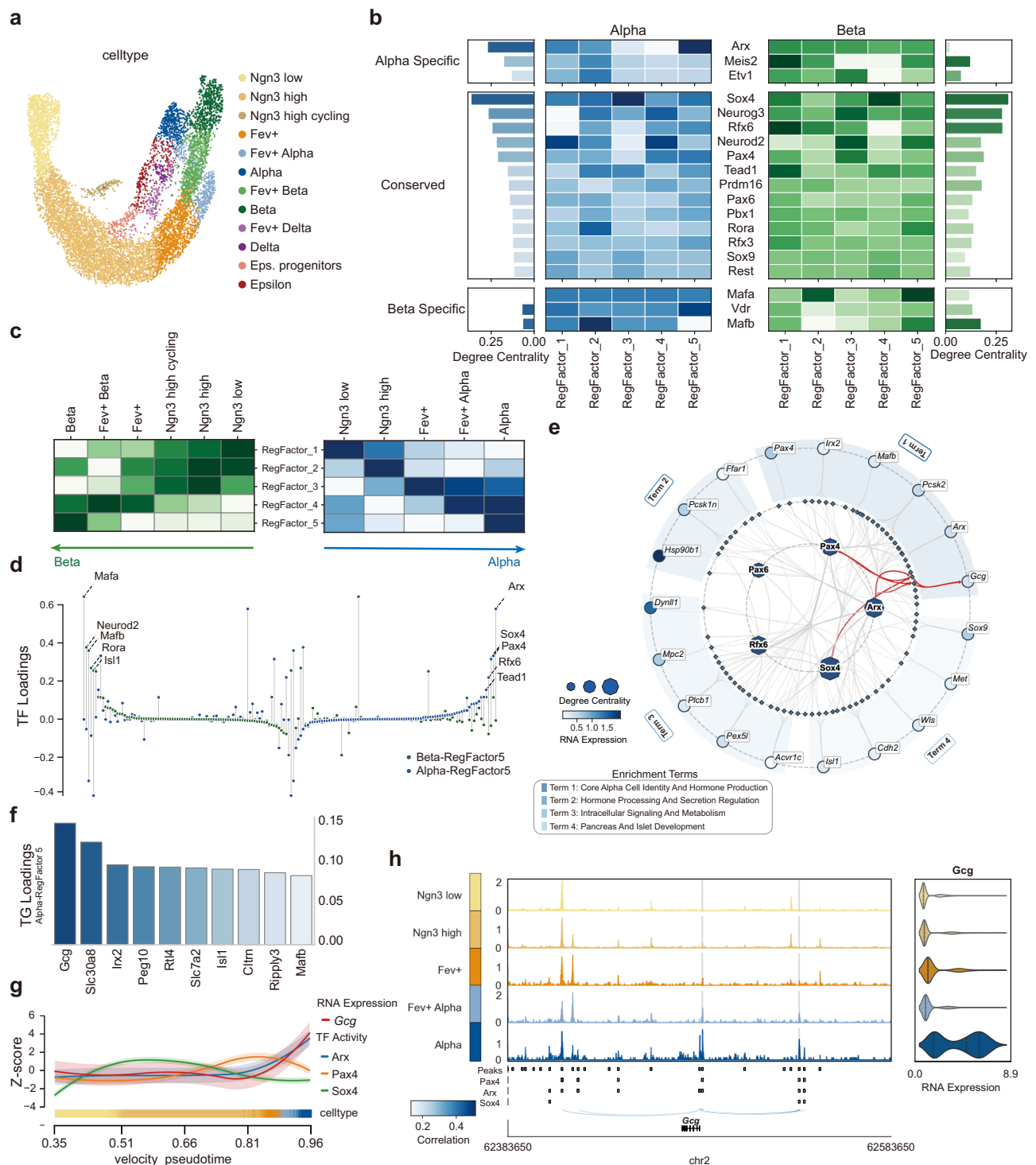


Fig. 5 | scMagnify dissects the regulatory programs associated with pancreatic alpha and beta cell differentiation. a UMAP embedding of mouse embryonic pancreas cells, colored by cell type, showing the endocrine differentiation landscape. **b** Heatmaps showing TF contributions to the five RegFactors for the alpha (left) and beta (right) lineages. TFs are grouped as lineage-specific or conserved. Adjacent bar plots show the degree centrality for each TF. **c** Heatmaps of the inferred activity for each of the five RegFactors across different cell states in the beta (left) and alpha (right) differentiation trajectories. **d** Scatter plot of TF loadings for the Beta-RegFactor 5 and Alpha-RegFactor 5. **e** Subnetwork view of the Alpha-RegFactor 5 module. Node size represents degree centrality, and color indicates RNA expression. Functionally related target genes are grouped by enrichment

terms. **f** Bar plot of the top target gene (TG) loadings for the Alpha-RegFactor 5 module. **g** Line plot showing the dynamics of Glucagon (Gcg) gene expression and the inferred regulatory activity of its key TFs (Arx, Pax4, and Sox4) along the alpha-cell pseudotime trajectory. **h** Genomic view of the Gcg locus. Tracks show chromatin accessibility across different cell states of the alpha lineage. Bottom tracks indicate predicted binding motifs for key TFs. Violin plots on the right show Gcg gene expression across the corresponding cell types. For **g**, lines show GAM-smoothed, min-max-normalized trends along alpha-cell pseudotime ($n_{\text{splines}} = 5$); shaded bands indicate ± 1 s.d. of the GAM fit across cells. Source data for the loading panels are provided in the Source Data file.

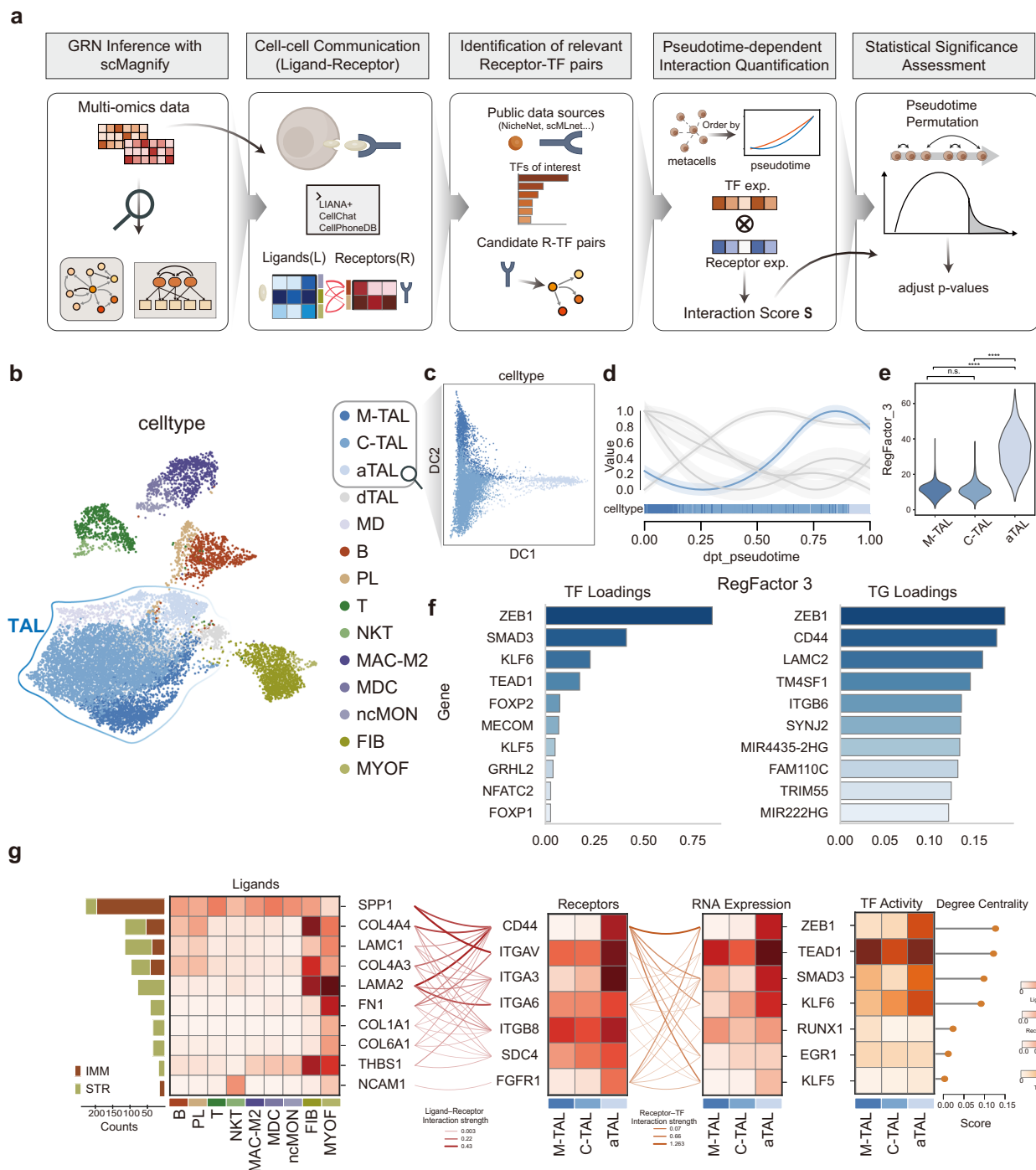


Fig. 6 | scMagnify maps signaling-to-transcription cascades associated with epithelial cell adaptation during kidney injury. a A schematic overview of the scMagnify intercellular communication module. The workflow integrates GRN inference, ligand-receptor analysis, and pseudotime ordering to quantify and statistically assess signaling-to-transcription cascades. **b** UMAP embedding of single cells from the human kidney cortex, colored by cell type. **c** A diffusion map of the thick ascending limb (TAL) epithelial cell populations, showing the inferred injury trajectory from healthy (M-TAL, C-TAL) to adaptive (aTAL) states. **d** A line plot showing the inferred activity of RegFactor 3 along the TAL pseudotime trajectory. **e** A violin plot comparing the activity of RegFactor 3 across the M-TAL, C-TAL, and aTAL cell states. **f** Bar plots showing the TF loadings (left) and TG loadings (right) for the aTAL-specific RegFactor 3. **g** A summary plot of the key signaling-to-transcription cascades targeting RegFactor 3 TFs. Heatmaps show the expression of ligands in various sender cell populations (left), the expression of corresponding

receptors in TAL cells (middle), and the RNA expression and activity of downstream TFs in TAL cells (right). Lines connect ligands to their receptors and receptors to downstream TFs. All schematic elements in a were created by the authors using Adobe Illustrator. For **d**, the line shows a GAM-smoothed, min-max-normalized RegFactor 3 activity trend along DPT pseudotime (n splines = 5); the shaded band indicates ± 1 s.d. of the GAM fit across TAL cells. For **e**, pairwise differences in cell-level RegFactor 3 activity were assessed using two-sided Mann-Whitney U tests with Benjamini-Hochberg correction across the three comparisons (C-TAL versus M-TAL: $P = P_{\text{adj}} = 0.061$, n.s.; C-TAL versus aTAL and M-TAL versus aTAL: $P_{\text{adj}} < 1 \times 10^{-4}$). Violin plots summarize $n = 5513$, 1714, and 853 cells in C-TAL, M-TAL, and aTAL, respectively. Cells were treated as observational units and not as independent biological replicates. Source data for the loading and interaction-summary panels are provided in the Source Data file.

Discussion

In this manuscript, we present scMagnify, an integrated computational framework designed to reconstruct, decompose, and analyze dynamic, multi-scale gene regulatory networks (GRNs) from single-cell multi-omic datasets. To achieve this, scMagnify synergistically integrates single-cell multi-omic data with pseudotemporal information through a Granger causality model implemented within an interpretable multi-scale neural network. The inference is further constrained by chromatin accessibility-derived priors to enhance accuracy. A key innovation of our framework is its ability to reconstruct the GRN as a multi-scale tensor, explicitly capturing time-lagged regulatory interactions along a dynamic process. This unique structure is pivotal, as it enables subsequent analyses, such as tensor decomposition to identify dynamic regulatory modules and the linking of these modules to upstream signaling-to-transcription cascades.

Our comprehensive benchmarks on both synthetic and real-world datasets demonstrate that scMagnify achieves competitive performance, achieved competitive or favorable performance across network reconstruction and driver-TF recovery benchmarks and the critical task of identifying driver TFs. Beyond benchmarks, the application of scMagnify to complex biological systems nominated biologically interpretable regulatory programs and testable hypotheses. In developmental contexts, our analyses of human hematopoiesis and mouse pancreas development revealed relative offsets between TF RNA expression and inferred regulatory activity, and nominated lineage- and maturation-associated RegFactor programs linked to cell-state progression. In a disease context, scMagnify successfully mapped the signaling cascades from the microenvironment that were associated with pathological cell-state transitions in kidney injury. These findings, which are often missed by conventional analyses, underscore the power of a multi-scale approach.

While scMagnify provides a flexible framework for dynamic GRN inference and downstream regulatory analysis, several limitations should be noted. First, scMagnify depends on the quality of the input multi-omic data and on the accuracy of the upstream pseudotime or transition-matrix estimation. Although our sensitivity analyses showed that the inferred networks were broadly stable across reasonable trajectory choices and perturbations, substantial loss of cell-state coverage or inaccurate trajectory ordering may affect GRN reconstruction. Second, the chromatin-derived basal GRN currently relies on peak-to-gene correlations and motif matching, which provide useful regulatory priors but do not directly establish functional enhancer–gene links or TF binding events. Future versions could incorporate improved peak-to-gene linking strategies^{111,112}, TF footprinting⁷⁹, perturbation-based priors¹¹³, or context-specific chromatin interaction data¹¹⁴ to refine these priors.

Another important limitation is that scMagnify uses a predictive, Granger-style regulatory model rather than a fully mechanistic biochemical model. Therefore, the inferred regulatory relationships should be interpreted as computationally prioritized TF–TG associations, not as direct evidence of causal regulation. Similarly, the inferred regulatory lag represents a relative, model-derived local pseudotime-lag index along the cell-state transition graph, rather than an absolute chronological delay or molecular response time. Although intermediate regulatory coefficients can take positive or negative values, the final reported GRNs are treated as unsigned TF–TG association networks, and scMagnify does not currently assign activating or inhibitory labels to final regulatory edges. Finally, because the current formulation relies on a directed transition structure along pseudotime, it may not fully capture complex feedback loops or cyclic regulatory dynamics²⁸ (Supplementary Fig. 17). Addressing these limitations, together with extending scMagnify to additional modalities, such as single-cell proteomics¹¹⁵, spatial transcriptomics¹¹⁶, or perturbation screens¹¹⁷, represents an important direction for future development.

The advent of single-cell multi-omics is providing an unprecedented opportunity to understand complex biological processes with high resolution. To translate this rich data into a mechanistic understanding, it is essential to move beyond static, single-scale views of gene regulation. Frameworks like scMagnify, which embrace the dynamic, multi-scale, and combinatorial nature of regulatory networks, are critical for this endeavor. By providing a tool to dissect these complex systems, scMagnify can help generate novel, testable hypotheses and will be a valuable resource for advancing our understanding of development and disease. Ultimately, integrating such dynamic regulatory maps will be an essential step toward realizing the goals of large-scale initiatives aiming to build “virtual cells,” which seek to create predictive, multi-scale computational models of cellular function and behavior^{118,119}.

Methods

Overview of scMagnify

scMagnify is a computational framework to infer gene regulatory networks (GRNs) and explore dynamic regulation synergy from single-cell multiomics data. The construction of GRNs is predominantly achieved through multivariable Granger causal inference, using an interpretable multi-scale neural network. Moreover, scMagnify integrates prior knowledge of chromatin conformation by incorporating a domain-specific constraint into the model structure.

The scMagnify workflow consists of several steps: (1) multi-omic data preprocessing; (2) multi-scale gene regulatory network inference; and (3) downstream regulation analysis.

We implemented and tested scMagnify in Python and designed it for use in the Jupyter notebook environment. scMagnify code is open source and available on GitHub (<https://github.com/LiHongCSBLab/scMagnify>) along with detailed descriptions of functions and tutorials.

Multi-omic data preprocessing

Cell state transition analysis. Cell state transition can be modeled as a multi-scale process. Existing approaches, such as CellRank, combine similarity-based Markov models with multi-view single-cell data for cellular fate mapping.

Transition matrix construction. We begin by applying CellRank to calculate cell–cell transition probabilities, represented by a transition matrix $T \in \mathbb{R}^{N \times N}$, which reflects the likelihood of one cell transitioning to another along the pseudotime trajectory, which we index by t .

Cell state selection. Cells are then assigned to specific fates or lineages through clustering based on their fate probabilities. This step groups cells that are likely to follow similar developmental paths, allowing for the identification of distinct lineages.

Feature association test. Feature association is tested using GAMs, which model gene expression as a function of pseudotime t with cubic spline regression. The GAM model is compared to an unconstrained model using an F-test, and p -values are adjusted for multiple testing using the Bonferroni correction. This identifies key genes associated with cell state transitions along pseudotime.

TF binding network construction. For each cell state, scMagnify constructs a TF binding network that contains unweighted, directional edges between a TF and its TG. scMagnify uses the peak-gene correlations and TF-binding motifs for this task.

Identification of cCREs via peak-gene correlations. To identify candidate cis-regulatory elements (CREs), scMagnify first aggregates the single-cell multiomics data into metacells using SEACells⁶⁶. For each gene, the method then computes the Pearson correlation between its normalized metacell expression and the normalized

accessibility of each ATAC peak located within 100 kb upstream and 100 kb downstream. The significance of each peak-gene correlation is assessed against an empirical null distribution, which is generated by sampling 100 background peaks matched for GC content and accessibility¹²⁰. This analysis identifies the subset of regions significantly correlated with gene expression, retaining pairs that meet the criteria of a correlation coefficient >0.1 and an empirical $P < 0.1$.

Motif scanning. Following the identification of cCREs, we scanned their DNA sequences to identify TF binding motifs, thereby linking TFs to potential TGs. This motif scanning was performed for all candidate regions using MOODS¹²¹. By default, scMagnify uses the HOCOMOCO v11 core collection¹²² for human and mouse as its motif database; however, users may also provide alternative motif collections, such as CIS-BP- or HOMER-derived motifs, or custom position weight matrices through the motif-scanning interface. The results were aggregated into a binary adjacency matrix, hereafter referred to as the basal TF binding network $B \in \{0,1\}^{P \times Q}$. An entry $B_{p,q} = 1$ indicates that a motif for TF_p was found within a cCRE linked to TG_q , establishing a potential regulatory edge. In addition to this network, scMagnify also reports the specific genomic coordinates of each identified TF-binding site.

Multi-scale gene regulatory network inference

To address the inherent limitations of classical Granger causal inference in handling dynamical systems with branching points, scMagnify employs a directed acyclic graph (DAG)-structured partial ordering³³ to establish robust temporal relationships among variables. This approach ensures a principled representation of causal dependencies in complex systems, particularly in single-cell genomics.

Input data and preprocessing. The training of scMagnify relies on three primary inputs:

- (1) A gene expression matrix X , where $X \in \mathbb{R}^{N \times M}$, with N cells and M genes.
- (2) A cell transition matrix T , where $T \in \mathbb{R}^{N \times N}$, encoding pairwise relationships between cells.
- (3) A TF binding network $B \in \{0,1\}^{P \times Q}$, which incorporates prior biological knowledge to guide the inference process.

Preprocessing of X . To ensure data comparability, raw expression counts were first normalized by scaling each cell's library size to the median library size of the dataset. The resulting normalized counts were then log-transformed to stabilize variance and reduce skewness.

Preprocessing of T . To account for variability in the in-degrees of the DAG, we precomputed a modified matrix $\tilde{T}$, derived from the normalized transpose of T . Specifically, $\tilde{T}$ is defined such that each row sums to 1, ensuring a consistent scaling of influence across cells. For rows of T^T (the transpose of T) that consist entirely of zeros, no normalization is applied. Formally, $\tilde{T}$ is computed as:

$$\tilde{T} = \text{normalize}(T^T) \quad (1)$$

where the normalization ensures $\sum_j (\tilde{T})_{ij} = 1$ for all rows i with non-zero entries. This matrix $\tilde{T}$ serves as a graph diffusion operator, enabling the aggregation of information over each observation's ancestors in the DAG.

Model structure. Our model infers dynamic regulatory relationships by conceptualizing gene regulation as a sophisticated, non-linear extension of the classical Vector Autoregressive (VAR) framework, which is foundational to Granger causality¹²³ (Supplementary Note 1). The model's architecture is built up progressively to address the complexities of biological systems. The foundational linear VAR model posits that TG expression can be predicted from a linear combination

of past TF expression:

$$\hat{X}_{TG} = \sum_{k=1}^K C_k \cdot X_{TF,t-k} + \epsilon \quad (2)$$

Here, C_k represents a constant coefficient matrix for a given time lag k . To capture the non-linear dynamics inherent in gene regulation, we first extend this model by replacing the constant matrix C_k with a neural network $\Psi_k: \mathbb{R}^P \rightarrow \mathbb{R}^{P \times Q}$, which learns a multi-scale regulatory coefficient matrix based on historical TF expression⁴⁹. To ensure biological plausibility, we impose a structural constraint on these learned coefficients by applying an element-wise multiplication with a prior TF binding network, B . This constrained model is formulated as:

$$\hat{X}_{TG} = \sum_{k=1}^K (\Psi_k(X_{TF,t-k}) \odot B) \cdot X_{TF,t-k} + \epsilon \quad (3)$$

The final model further advances this framework to simultaneously account for regulatory effects across multiple time scales and to integrate the cell-state transition topology (Supplementary Fig. 1a). This is achieved through a multi-branch architecture with an attention mechanism, which operates on features propagated along a cell transition graph. The complete model can be formulated as:

$$\hat{X}_{TG} = \sum_{k=1}^K [\alpha_k \sum_{1 \leq l \leq k} (\Psi_k^l((\tilde{T})^l X_{TF}) \odot B \cdot (\tilde{T})^l X_{TF})] + \epsilon \quad (4)$$

In this architecture, the model is composed of K independent and parallel branches, where each branch k acts as a “receptive field” considering historical information up to k steps away (Supplementary Fig. 1b). Instead of simple time lags, we use a graph diffusion operator $(\tilde{T})^k X_{TF}$ to aggregate TF expression information from k -hop ancestral cells in the trajectory graph, thereby embedding cell-state relationships into the temporal model.

$$(\tilde{T})^k X = \tilde{T} \left((\tilde{T})^{k-1} X \right) \quad (5)$$

Within each branch k , an additional index l (where $1 \leq l < k$) is used to sum over all intermediate time lags, allowing the model to capture both immediate and delayed causal effects. Finally, an additional neural network produces learnable attention weights α_k , which determine the emphasis placed on each branch, effectively merging all extracted causal information based on its relevance. Here, $\Psi_k^l: \mathbb{R}^P \rightarrow \mathbb{R}^{P \times Q}$ is a neural network parameterized by θ_k that includes an input layer, fully connected hidden layers with ReLU activation, and an output layer of size $P \times Q$. (Supplementary Fig. 1c). The model's direct output is the multi-scale regulatory coefficient tensor $\mathcal{T}$, which quantifies the dynamic, context-specific regulatory strengths as determined by the attention-weighted aggregation of the neural network outputs:

$$\mathcal{T} = \sum_{k=1}^K \alpha_k \left[\sum_{1 \leq l \leq k} \Psi_k^l((\tilde{T})^l X_{TF}) \odot B \right] \quad (6)$$

Training procedure. To mitigate spurious inference in multivariate time series, scMagnify is trained by minimizing the following penalized loss function using mini-batch gradient descent:

$$\mathcal{L} = \mathcal{L}_{MSE} + \lambda \mathcal{L}_{\text{sparsity}} + \gamma \mathcal{L}_{\text{smooth}} \quad (7)$$

where λ and γ are hyperparameters that control the strength of the regularization terms. The components of the loss function are defined as:

Mean squared error (MSE) $\mathcal{L}_{MSE}$: This term quantifies the difference between the model's predicted target gene expression $\hat{X}_{TG}$, and the true expression values X_{TG} . It is calculated as the standard mean squared error:

$$\mathcal{L}_{MSE} = \frac{1}{N_c} \sum_{i=1}^{N_c} \|X_{TG,i} - \hat{X}_{TG,i}\|_2^2 \quad (8)$$

where N_c is the number of cells in the batch.

Sparsity-inducing regularization $\mathcal{L}_{\text{sparsity}}$: To promote a sparse GRN and select only the most salient regulatory interactions, we employ a Group Elastic Net penalty¹²⁴ on the output regulatory coefficient tensor $\mathcal{T}$. This penalty encourages the model to either drive all coefficients for a given TF–TG pair across all time lags to zero (group sparsity via L2 norm) or to selectively zero out individual time-lag-specific coefficients (element-wise sparsity via L1 norm). Let $\mathbf{r}_{p,q} = (\mathcal{T}_{1,p,q}, \dots, \mathcal{T}_{K,p,q})$ be the vector of regulatory coefficients for TF p and TG q across all K time lags. The regularization term is defined as:

$$\mathcal{L}_{\text{sparsity}}(\mathcal{T}) = \frac{1}{N_c} \sum_{i=1}^{N_c} R(\mathcal{T}) \quad (9)$$

$$R(\mathcal{T}) = \frac{1}{PQ} \sum_{p=1}^P \sum_{q=1}^Q \left[(1-\beta) \|\mathbf{r}_{p,q}\|_2 + \beta \|\mathbf{r}_{p,q}\|_1 \right] \quad (10)$$

where P and Q are the number of TFs and TGs, respectively, and β is a hyperparameter that mixes the L1 and L2 penalties.

Temporal smoothness regularization $\mathcal{L}_{\text{smooth}}$: To ensure that the inferred regulatory relationships evolve smoothly over the pseudo-time trajectory, we introduce a penalty on the difference between the coefficient tensors of temporally adjacent cells. This term penalizes large, abrupt changes in the GRN structure between consecutive time points. The smoothness loss is calculated as the squared L2 norm of the difference between the coefficient tensor at pseudotime t_i and the tensor at the next time point t_{i+1} :

$$\mathcal{L}_{\text{smooth}}(\mathcal{T}) = \frac{1}{N_c - 1} \sum_{i=1}^{N_c-1} \|\mathcal{T}_{t_{i+1}} - \mathcal{T}_{t_i}\|_2^2 \quad (11)$$

Model inference and postprocessing. After training, the model is applied to the entire dataset to infer cell-specific regulatory dynamics and subsequently post-processed to generate formatted results for downstream analysis (Supplementary Fig. 1d). For each of the N cells in the cell state, the model outputs a regulatory coefficient tensor. These individual tensors are first collected into a comprehensive tensor, which we denote as $\mathcal{T}_{\text{total}} \in \mathbb{R}^{N \times K \times P \times Q}$. This tensor represents the complete set of inferred regulatory coefficients across all cells, time lags, TFs, and TGs.

To derive a robust, context-level GRN, we perform a series of post-processing and aggregation steps. First, to ensure the reliability of inferred interactions, we filter out regulatory coefficients that exhibit high variability across the cell population. This is achieved by calculating the coefficient of variation (CV) for each interaction across the cell dimension of $\mathcal{T}_{\text{total}}$. Interactions with a CV below 0.2 are retained, resulting in a filtered tensor $\mathcal{T}_{\text{final}}$.

From this stabilized tensor, we construct the final GRNs. To obtain a representative multi-scale network that preserves time-lag-specific information, we compute the median of $\mathcal{T}_{\text{final}}$ across the cell

dimension. This yields the multi-scale network $\mathcal{G}_{\text{multi-scale}} \in \mathbb{R}^{K \times P \times Q}$

$$\mathcal{G}_{\text{multi-scale}} = \text{median}_{i=1 \dots N}(\mathcal{T}_{\text{final}}) \quad (12)$$

To create a single, consolidated GRN that summarizes the most dominant interactions across all time lags, we derive two ensemble networks. The ensemble network strength G_{strength} quantifies the maximum unsigned regulatory influence for each TF–TG pair and is constructed by taking the maximum value across the time-lag dimension (k) of the absolute median coefficients:

$$G_{\text{strength}} = \max_{k=1 \dots K} (|\mathcal{G}_{\text{multi-scale}}|) \quad (13)$$

Similarly, the ensemble network activation retains the sign of the dominant aggregated coefficient for downstream activity estimation; these signs are not interpreted as validated activating or inhibitory regulatory effects. By taking the maximum value of the signed median coefficients across the time-lag dimension:

$$G_{\text{activation}} = \max_{k=1 \dots K} (\mathcal{G}_{\text{multi-scale}}) \quad (14)$$

Downstream regulation analysis

Network score calculation. The GRN can be investigated using general network topology metrics to identify key regulators. To facilitate this, we first perform network binarization. This step converts the continuous, weighted ensemble network strength matrix G_{strength} into a binary adjacency matrix by applying a user-defined sparsity threshold. On this resulting binary GRN, we then employ graph-theoretic approaches, such as calculating degree centrality, betweenness centrality, and PageRank, using the Python library `networkx`¹²⁵ to characterize the network's structure and pinpoint influential TFs.

Regulatory activity inference. Following this filtering step, we leverage the filtered GRN to infer transcription factor (TF) activities. Specifically, the activity of a TF is represented by the expression levels of its TGs, collectively referred to as a *regulon*.

To quantify the activity of each regulon, we used a multivariate linear model (MLM) using the `decoupler`⁵⁵ package, which estimates regulator activities by jointly modeling all regulators in the network and assigning activity scores based on the t -values of the fitted model.

For each cell, the MLM estimates a vector of TF activities $\mathbf{a}_{TF}$, that best explains the TG expression vector $\mathbf{x}_{TG}$, given the ensemble network activation matrix $G_{\text{activation}}$, which serves as the score matrix $\mathbf{W}$:

$$\mathbf{x}_{TG} = \mathbf{a}_{TF} \mathbf{W} + \epsilon \quad (15)$$

The final activity score for each TF is the t -value from the fitted model, which provides a normalized measure of activity.

RegFactor decomposition. To identify combinatorial regulatory modules, we apply Tucker decomposition to a third-order multi-scale signed network $\mathcal{G}_{\text{multi-scale}} \in \mathbb{R}^{K \times P \times Q}$. This tensor is constructed by aggregating the regulatory coefficients, derived from the multi-scale Granger causality model, across the time-lags. The decomposition is formulated as:

$$\mathcal{G}_{\text{multi-scale}} \approx \mathbf{C} \times_1 \mathbf{U}^{(\text{lag})} \times_2 \mathbf{U}^{(\text{TF})} \times_3 \mathbf{U}^{(\text{TG})} \quad (16)$$

This formulation approximates the original three-way tensor $\mathcal{G}_{\text{multi-scale}}$, as the n-mode product of a smaller core tensor ($\mathbf{C}$) and three factor matrices. The core tensor $\mathbf{C} \in \mathbb{R}^{R_{\text{lag}} \times R_{\text{TF}} \times R_{\text{TG}}}$ captures the interaction strengths between the decomposed modules. By default, the rank of the decomposition for each mode ($R_{\text{lag}} \times R_{\text{TF}} \times R_{\text{TG}}$) is set to be equal to the number of time lags K and the final rank was selected through a data-driven rank-sweep and elbow-detection procedure centered around (K), as detailed in Supplementary Note 3. The three

factor matrices define the modules themselves: the lag factor matrix $\mathbf{U}^{(\text{lag})} \in \mathbb{R}^{K \times R_{\text{lag}}}$ defines temporal activation profiles across the time lags; the TF factor matrix $\mathbf{U}^{(\text{TF})} \in \mathbb{R}^{P \times R_{\text{TF}}}$ defines co-regulating TF modules; and the TG factor matrix $\mathbf{U}^{(\text{TG})} \in \mathbb{R}^{Q \times R_{\text{TG}}}$ defines shared target gene modules. Together, a specific combination of a TF module, a TG module, and a temporal profile, linked by the core tensor, constitutes a “RegFactor” and its activity can also be estimated using the MLM method, where the TG loadings from the corresponding column of the $\mathbf{U}^{(\text{TG})}$ matrix serve as the weight matrix $\mathbf{W}$.

Further details on RegFactor significance assessment and downstream analyses, including the TF-pair quadrant analysis, are provided in Supplementary Note 3.

Intercellular communication. To link intercellular communication with intracellular transcriptional regulation, scMagnify models signaling-to-transcription cascades along a pseudotime axis. The process begins by identifying ligand-receptor pairs and TFs of interest. Putative signaling pathways are then constructed by linking receptors to their downstream TFs using a prior knowledge base from scMLnetDB³⁹.

For each candidate receptor-TF pair, an interaction score is quantified across the inferred trajectory. Cells are first aggregated into M metacells ordered by pseudotime to reduce noise. Let $\mathbf{x}_R = (x_{R_1}, \dots, x_{R_M})$ and $\mathbf{x}_{TF} = (x_{TF_1}, \dots, x_{TF_M})$ be the vectors of the receptor's and TF's average expression across the metacells, respectively. The interaction score is calculated as the sample covariance between these vectors:

$$S = \text{Cov}(\mathbf{x}_R, \mathbf{x}_{TF}) = \frac{\sum_{i=1}^M (x_{R_i} - \bar{x}_R)(x_{TF_i} - \bar{x}_{TF})}{M - 1} \quad (17)$$

where $\bar{x}_R$ and $\bar{x}_{TF}$ are the mean expression values.

The statistical significance of the observed score S_{obs} is assessed via a permutation test. The pseudotime ordering of the metacells is shuffled N times (by default, $N=1000$), and a score $S_{\text{perm}}^{(j)}$ is calculated for each permutation j . This generates an empirical null distribution. The empirical p -value for a two-tailed test is then calculated as:

$$p\text{-value} = \frac{|\{j | |S_{\text{perm}}^{(j)}| \geq |S_{\text{obs}}|\}| + 1}{N + 1} \quad (18)$$

The resulting p -values are adjusted for multiple testing to identify significant signaling axes.

Datasets

Simulated data. To benchmark the performance of existing GRN inference methods, we used scMultiSim to generate simulated single-cell multiomics datasets with the following configurations: 1139-gene GRN, 1000 cells, 50 CIFs, $\text{rd} = 0.2$, $\text{oi} = 1$, and other default parameters. We generated a total of eight datasets with random seeds from 1 to 5.

To evaluate robustness to dataset scale, regulatory-network complexity, and technical noise, we additionally generated matched simulation series in which we independently varied the number of cells, GRN size, GRN density, transcript dropout rate, and chromatin-prior noise while holding the other parameters at their baseline values. scMagnify and CellOracle were evaluated on the same simulated datasets and under matched preprocessing and evaluation settings.

Technical noise and batch effects were introduced using the default scMultiSim procedures. Transcriptomic noise was evaluated by applying increasing levels of synthetic transcript dropout. Specifically, the technical noise was modeled by randomly adding and removing connections in the simulated ATAC peak-gene matrices, where the probability of these modifications is controlled by a noise-level

parameter p . “Scale” is set to $50 \times p_{\text{pos}}$, where p_{pos} is the proportion of positive edges within each matrix.

Single-cell multi-omic data. Single-cell Multiome ATAC + Gene Expression data were obtained from previously published studies, including: (1) human hematopoiesis datasets from Persad et al., comprising CD34⁺-enriched and T cell-depleted bone marrow cells; (2) a mouse pancreas development dataset from Klein et al., consisting of embryonic pancreatic cells; and (3) human kidney disease datasets from Gisch et al., derived from percutaneous kidney biopsy samples.

The gene expression matrix (GEM) was normalized by total UMI counts and log-transformed using Scanpy with default parameters. The chromatin accessibility data had been deduplicated in the original studies. Cell annotations such as cell type and low-dimensional embeddings were also obtained from the original studies.

Human T-cell-depleted bone marrow data. The single-cell multi-omic data from Persad et al.⁶⁶ were originally described and made available on Zenodo (<https://doi.org/10.5281/zenodo.6383269>). For this study, the preprocessed AnnData objects for gene expression (RNA) and chromatin accessibility (ATAC) were downloaded directly from Mellon¹²⁶ tutorial. The cell type annotations (celltype) and Palantir-derived pseudotime values (palantir_pseudotime) were used directly from the metadata provided in these original files.

Human CD34+ bone marrow data. This dataset also originates from the study by Persad et al.⁶⁶ (Zenodo: <https://doi.org/10.5281/zenodo.6383269>). For this study, the preprocessed AnnData objects for gene expression (RNA) and chromatin accessibility (ATAC) were downloaded directly from SEACells tutorial. Palantir-derived pseudotime values (palantir_pseudotime) were retained from the original data files. For this study, the cell type annotations (celltype) were re-annotated by marker genes.

Mouse pancreatic endocrine data. This dataset was originally published by Klein et al.¹²⁷, with raw data available from the Gene Expression Omnibus (GEO) under accession number GSE275562. The preprocessed MuData object (.h5mu), which contains the necessary AnnData objects for this study, was obtained as described in the mubind¹²⁸ tutorial. RNA velocity-related data and the original cell type annotations were used directly from the metadata provided in this file.

Human kidney injury data. This dataset was originally published by Gisch et al.¹⁰⁵ and made available on Zenodo (<https://doi.org/10.5281/zenodo.8029990>). The preprocessed Seurat objects were downloaded from this repository. The cell type annotations for this study were derived by merging and re-annotating the original subclass.l2 metadata. Pseudotime trajectories were computed using the Diffusion Pseudotime (DPT) algorithm¹²⁹ from the Scanpy package¹³⁰ (scanpy.tl.dpt).

Ethics statement

This study exclusively analyzed publicly available datasets from previously published studies. No new human participants were recruited, and no new human biospecimens or animal samples were collected. Therefore, no additional ethical approval or informed consent was required for the present study. Ethical approval and informed consent for the original data collection were obtained by the respective studies, as described in the original publications.

Evaluations

To evaluate whether scMagnify can correctly identify cell-state-specific GRNs, we benchmarked our method against diverse GRN inference algorithms: SCENIC+, LINGER, Dictys, CellOracle, FigR, Pando, SCENIC, Velorama, and SINCERITIES.

Preparation of input data for GRN inference. For each of the three real-data datasets, we first performed a feature-association test to identify significant genes (Bonferroni-adjusted $P < 0.001$). Next, we subsetted the cells into 13 lineages (Ery/Mono/NaiveB for the T-cell-depleted Bone Marrow dataset; pDC/cDC/Mono/Ery/CLP/Mega for the CD34+ Bone Marrow dataset; Alpha/Beta/Delta/Epsilon for the Pancreas Endocrine dataset).

GRN inference methods. After preprocessing, the same lineage-restricted datasets were used as input for each GRN inference method, following the official tutorials and default hyperparameters unless otherwise specified. For the real-data benchmarks, SCENIC+ was run using lineage-specific consensus open-chromatin peaks as candidate regulatory regions, because its standard pycisTopic topic- and DAR-based feature selection is primarily designed for comparisons across predefined cell groups and was not directly applicable to our continuous lineage setting. Except for CellOracle, SCENIC+ and the other multiome-based GRN inference methods were not included in the scMultiSim benchmark because the simulated regulatory regions lack genomic coordinates and DNA sequences, precluding the motif enrichment, transcription-factor binding-site annotation, and genome-based enhancer-to-gene mapping required by these methods.

Ground-truth data preparation. Cell-type-specific ground-truth GRNs were generated in the same manner as in a previous benchmarking study³³. We obtained tissue-specific ChIP-seq peak data from the Cistrome database⁶⁴. For each experiment (by GSMID), the top 100 peaks (by peak score) were regarded as positive hits and annotated to the nearest gene. The TF binding gold standard was defined as TF–target pairs that were positive hits in at least one of the experiments.

TF–TG interactions evaluation. To assess the accuracy of inferred TF–TG interactions, we benchmarked performance on both synthetic and real-world datasets. For both data types, each method's ranked predictions were evaluated using the area under the precision-recall curve (AUPR) to assess overall ranking quality and the F1-score to evaluate the balance of precision and recall for a set of top-ranked predictions. The F1-score is calculated as:

$$F1 - \text{score} = 2 \cdot \frac{\text{Precision} \cdot \text{Recall}}{\text{Precision} + \text{Recall}} \quad (19)$$

Driver TF recovery evaluation. To evaluate how well each method identifies key regulators, we performed a driver TF recovery benchmark^{24,45}. First, for each inferred network, we ranked all TFs by their degree centrality C_D , calculated as the sum of connections for each TF node i in the adjacency matrix A :

$$C_D(i) = \sum_j A_{ij} \quad (20)$$

We then compared this ranking against a curated list of known driver TFs. To quantify performance, we generated a recovery curve, $\phi(N)$, which measures the number of known drivers found within the top N ranked TFs. The y-axis of this curve is the “Normalized Rank,” which represents the fraction of total known drivers recovered at rank N . The overall performance for each method m was summarized by calculating the area under this curve (AUC):

$$\text{AUC}(m) = \sum_{N=1}^{|T|} \phi^{(m)}(N) \quad (21)$$

where $|T|$ is the total number of TFs. A higher AUC score indicates better performance in prioritizing known driver TFs.

Reproducibility and cell-type specificity evaluations. We evaluated network reproducibility and specificity across three distinct cell lineages. For each lineage, we generated 10 independent GRNs by running the inference algorithm with different random seeds.

The GRN similarity between any two inferred networks (A and B), defined by their sets of regulatory edges, was quantified using the Jaccard Index:

$$J(A, B) = \frac{|A \cap B|}{|A \cup B|} \quad (22)$$

Analysis

Functional enrichment analysis. Functional enrichment was conducted using over-representation analysis (ORA) with gene sets from the Molecular Signatures Database (MSigDB). Statistical significance was assessed using a one-sided Fisher's exact test, which computes the probability of enrichment based on the hypergeometric distribution.

The p -value for observing an overlap of size o (given a query list of size r , a gene set of size g , and a background universe of D) was calculated as:

$$p\text{-value} = \sum_{i=o}^{\min(r,g)} \frac{\binom{g}{i} \binom{D-g}{r-i}}{\binom{D}{r}} \quad (23)$$

Enriched terms were ranked by a “Combined score,” integrating the p -value and the odds ratio (OR):

$$\text{Combined score} = -\log(p\text{-value}) \times \text{Odds Ratio} \quad (24)$$

All resulting p -values were adjusted for multiple testing using the Benjamini–Hochberg FDR correction.

Regulation specificity analysis. To quantify regulation specificity, two matrices were compiled for each gene across the Ery, NaiveB, and Mono lineages: (1) the absolute mean TF activity and (2) the mean expression. Each matrix was independently normalized on a per-gene basis by dividing the gene's value in each lineage by its total sum across all three lineages. A final composite specificity score was computed by averaging these two resulting proportional (normalized) matrices.

Lineage-specific TGs were determined by first calculating the $\log_2(\text{FoldChange})$ for each gene's expression in one lineage against the average expression of the other two lineages. A gene was then assigned to a specific lineage if its maximum $\log_2(\text{FC}) \geq 0.4$ and this maximum value was also at least 0.2 greater than its median $\log_2(\text{FC})$ across all three lineages.

Regulation lag calculation. scMagnify quantifies the characteristic regulatory lag for each transcription factor (TF) based on the inferred multi-scale network tensor, $\mathcal{G}_{\text{multi-scale}} \in \mathbb{R}^{K \times P \times Q}$.

First, for each TF–TG interaction, the vector of regulatory coefficients across all K lags is L1-normalized. This converts the coefficients into a distribution of relative regulatory influence across the different time lags. An estimated lag for the specific interaction is then calculated as the absolute value of the weighted average of the lag indices, using the normalized coefficients as weights⁵. To derive a single, representative “regulation lag” score for each TF, the method then computes the mean of all its associated interaction-specific lag scores across all of its TGs. Further details on the derivation, interpretation, and validation of this metric are provided in Supplementary Note 2.

Statistics and reproducibility

No statistical method was used to predetermine sample size. Sample sizes were determined by the available datasets, matched lineage-level tasks or independent computational replicates, as specified in the figure legends. No data were excluded from the analyses beyond the predefined data-quality and matching criteria described in the

"Methods." Randomization and blinding were not applicable because this study involved retrospective computational analyses of publicly available datasets. Statistical tests, multiple-testing corrections, exact sample sizes, and units of analysis are specified in the corresponding Methods, figure legends, and Source Data. The code and processed data required to reproduce the analyses are publicly available as described in the Code Availability and Data Availability sections.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The public single-cell multi-omic datasets reused in this study are available as follows: the human T-cell-depleted and CD34+ bone marrow datasets are available from Zenodo (<https://doi.org/10.5281/zenodo.6383269>); the mouse pancreatic endocrine dataset is available from the Gene Expression Omnibus under accession [GSE275562](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE275562); and the human kidney injury dataset is available from Zenodo (<https://doi.org/10.5281/zenodo.8029990>). The corresponding raw bone-marrow multiome data are also available from GEO under accession GSE200046. The independent mouse kidney injury datasets used for external validation are available from GEO under accessions GSE139107 (IRI time course), GSE150656 (Klf6 perturbation), and GSE55808 (Smad3-knockout UUO). ChIP-seq datasets used to construct the TF-binding gold standards were obtained from Cistrome DB, and gene sets used for pathway enrichment were obtained from MSigDB.

The processed analysis-ready datasets used in this study are available from Figshare at https://figshare.com/articles/dataset/scMagnify_reproducibility_datasets/30600797. Source data underlying the main and Supplementary Figs. are provided with this paper. No new primary human-participant data were generated in this study. Source data are provided with this paper.

Code availability

scMagnify is released under the BSD-3-Clause license, with code available at <https://github.com/LiHongCSBLab/scMagnify>. Code to reproduce the results in the paper can be found at https://github.com/LiHongCSBLab/scmagnify_reproducibility. The analyses reported in this study were performed using scMagnify version 0.1.1, which is available as the v0.1.1 release tag in the GitHub repository.

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Author contributions

X.C. and H.L. contributed to the initial conceptualization and design of the project. H.L. supervised and conceived the project. X.C. designed and developed the scMagnify algorithms, performed the benchmark tasks and analyzed the datasets, and X.Y., B.S., H.W., and H.L. helped with the data analysis and discussed the results. X.Y., Z.T., and Y.Z. contributed to the software test. H.L., X.Y., P.L., H.Z., and Y.L. contributed to the writing and review. X.C. and H.L. wrote the manuscript with help from other authors. All authors have reviewed and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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