

Inference of cell type-specific gene regulatory networks on cell lineages from single cell omic datasets

Shilu Zhang¹, Saptarshi Pyne¹, Stefan Pietrzak¹, Alireza Fotuhi Siahpirani¹, Rupa Sridharan^{1,3},
and Sushmita Roy^{1,2*}

¹Wisconsin Institute for Discovery, University of Wisconsin-Madison, Madison, WI, USA

²Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison,
Madison, WI, USA

³Department of Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI,
USA

*To whom correspondence should be addressed. Corresponding author email:
sroy@biostat.wisc.edu

1 **Abstract**

2 Cell type-specific gene expression patterns are outputs of transcriptional gene regulatory networks
3 (GRNs) that connect transcription factors and signaling proteins to target genes. These networks recon-
4 figure during dynamic processes such as cell fate specification to drive diverse cellular states. Single-cell
5 transcriptomic technologies, such as single cell RNA-sequencing (scRNA-seq) and single cell Assay
6 for Transposase-Accessible Chromatin using sequencing (scATAC-seq), can examine the transcriptional
7 state of individual cells, allowing the study of cell-type specific gene regulation at unprecedented detail.
8 However, current approaches to infer cell type-specific gene regulatory networks from these datasets are
9 limited in their ability to integrate scRNA-seq and scATAC-seq measurements and to model network
10 dynamics on a cell lineage. To address this challenge, we have developed single-cell Multi-Task Net-
11 work Inference (scMTNI), a multi-task learning framework to infer the gene regulatory network for each
12 cell type on a lineage from scRNA-seq and scATAC-seq data. Using simulated, published and newly
13 collected single cell omic datasets, we show that scMTNI is able to accurately infer gene regulatory net-
14 works and captures meaningful network dynamics that identify GRN components associated with cell
15 type transitions. Application of our method to mouse cellular reprogramming identified key regulators
16 associated with cell populations that reprogram versus those that are stalled. Taken together, scMTNI
17 is a powerful framework to infer cell type-specific gene regulatory networks and their dynamics from
18 scRNA-seq and scATAC-seq datasets.

19

Introduction

20

Transcriptional gene regulatory networks (GRNs) specify connections between regulatory proteins and target genes and determine the spatial and temporal expression patterns of genes^{1,2}. These networks reconfigure during dynamic processes such as development or disease progression, to specify cell type specific expression levels. Recent advances in single cell omic techniques such as single cell RNA-sequencing (scRNA-seq) and single cell Assay for Transposase-Accessible Chromatin using sequencing (scATAC-seq)³ enable collecting high resolution molecular phenotypes of a developing system and offer unprecedented opportunities for the discovery of cell type-specific regulatory networks and their dynamics. However, computational methods to systematically leverage these datasets to identify regulatory networks driving cell type-specific expression patterns, are limited.

29

Existing methods of network inference from single cell omic data^{4–16} have primarily used transcriptomic measurements and have low recovery of experimentally verified interactions^{17,18}. Recently a small number of methods have attempted to integrate scRNA-seq and scATAC-seq datasets^{19,20} to examine gene regulation, however, the primary focus of these methods is to define cell clusters and the network is defined entirely based on accessible sequence-specific motif matches. This restricts the class of regulators that can be incorporated into the regulatory network to those with known motifs. Furthermore, existing methods infer a single GRN for the entire dataset or do not model the cell population structure which is important to discern dynamics and transitions in the inferred networks for cell type-specificity.

37

To overcome the limitations of existing methods, we have developed single-cell Multi-Task Network Inference (scMTNI), a multi-task learning framework that integrates the cell lineage structure, scRNA-seq and scATAC-seq measurements to enable joint inference of cell type-specific GRNs. scMTNI takes as input a cell lineage tree, scRNA-seq data and scATAC-seq based prior networks for each cell type. scMTNI uses a novel probabilistic prior to incorporate the lineage structure during network inference and outputs GRNs for each cell type on a cell lineage. We performed a comprehensive benchmarking study of multi-task learning approaches including scMTNI on simulated data and show that incorporation of multi-task learning and tree structure is beneficial for GRN inference.

45

We applied scMTNI to a novel scRNA-seq and scATAC-seq time course dataset for cellular reprogramming in mouse and a published scRNA-seq and scATAC-seq cell-type specific dataset for human hematopoietic differentiation. We demonstrate the advantage of integration of scATAC-seq and scRNA-seq datasets for inferring cell type specific GRNs and their dynamics. We examined how the inferred networks change along the trajectory and identified regulators and network components specific to dif-

50 ferent parts of the lineage tree. Our predictions include known as well as novel regulators of cell popu-
51 lations transitioning to different lineage paths, providing insight into regulatory mechanisms associated
52 with hematopoietic specification and reprogramming efficiency.

53 **Results**

54 **Single-cell Multi-Task learning Network Inference (scMTNI) for defining regula-
55 tory networks on cell lineages**

56 We developed scMTNI, a multi-task graph learning framework for inferring cell type-specific gene reg-
57 ulatory networks from scRNA-seq and scATAC-seq datasets (**Figure 1A**), where a cell type is defined
58 by a cluster of cells with a distinct transcriptional and accessibility profile. scMTNI models a GRN
59 as a Dependency network²¹, a probabilistic graphical model with random variables representing genes
60 and regulators, such as transcription factors (TFs) and signaling proteins. scMTNI takes as input cell
61 clusters with gene expression and accessibility profiles and a lineage structure linking the cell clusters
62 (**Figure 1**). Such inputs can be obtained from existing methods for integrative clustering²² and lineage
63 construction²³. scMTNI uses the scATAC-seq data for each cell cluster to define cell type-specific se-
64 quence motif-based TF-target interactions (e.g., a motif for a particular TF, which is accessible only in
65 specific cell types will result in a TF-target interaction only in those cell types) which are used as a prior
66 to guide network inference (**Methods**). The output of scMTNI is a set of cell type-specific GRNs one
67 for each cell cluster in the lineage tree. scMTNI's multi-task learning framework incorporates a novel
68 lineage tree prior, which uses the lineage tree structure to influence the similarity of gene regulatory
69 networks on the lineage. This prior models the change of a GRN from a start state (e.g., progenitor cell
70 state) to an end state (e.g. more differentiated state) as a series of individual edge-level probabilistic
71 transitions. While scMTNI was developed to incorporate both scRNA-seq and scATAC-seq data, it can
72 be applied to situations where scATAC-seq, and therefore a cell type-specific prior network, is not avail-
73 able. We refer to the versions of our approach as scMTNI+prior and scMTNI depending upon whether it
74 uses prior knowledge or not. The output networks of scMTNI are analyzed using two dynamic network
75 analysis methods: edge-based k-means clustering and topic models (**Figure 1B**). These approaches iden-
76 tify key regulators and subnetworks associated with a particular cell cluster or a set of cell clusters on a
77 branch.

78 **Multi-task learning algorithms outperform single-task algorithms for single cell
79 network inference**

80 To evaluate scMTNI and other existing algorithms with known ground truth networks on single-cell tran-
81 scriptomic data, we set up a simulation framework, which entailed creation of a cell lineage, generating

82 synthetic networks and corresponding single-cell expression datasets for each cell type on the lineage
83 (**Figure 2A**). We used a probabilistic process of network structure evolution to simulate the network
84 structure for three cell types, each containing 15 regulators and 65 genes and between 202-239 edges
85 (**Methods**). Next, we applied BoolODE¹⁷ to simulate the *in silico* single-cell expression data using each
86 cell type's simulated network. To mimic the sparsity in single-cell expression data, we set 80% of the
87 values to 0. We created three datasets with different numbers of cells: 2000, 1000, 200, referred here as
88 dataset 1, dataset 2, dataset 3.

89 We asked whether multi-task learning is beneficial compared to single-task learning for network in-
90 ference from scRNA-seq data. To this end we compared scMTNI and four other multi-task learning
91 algorithms, MRTLE²⁴, GNAT²⁵, Ontogenet²⁶, and AMuSR²⁷ to three single-task algorithms, LASSO
92 regression²⁸, INDEP, and SCENIC²⁹ (**Methods**). Of these methods only SCENIC uses a non-linear
93 regression model while the others are based on linear models. INDEP is similar to scMTNI but does
94 not incorporate the lineage prior. Each algorithm was applied within a stability selection framework and
95 evaluated with Area under the Precision recall curve (AUPR) and F-score of top k edges, where k is
96 the number of edges in the true network (**Figure 2B, C**). On dataset 1, based on AUPR, scMTNI, MR-
97 TLE and AMuSR are able to recover the network structure (**Figure 2B**) better than the other multi-task
98 learning and single-task learning algorithms. Ontogenet performs better than the single-task learning
99 algorithms in at least two cell types. Finally, GNAT performs comparably to the single-task learning
100 algorithms. When comparing algorithms based on F-score of top k edges, we have similar observations
101 that scMTNI and MRTLE have a better performance than other algorithms (**Figure 2C**). Ontogenet per-
102 forms better than LASSO and INDEP in at least two cell types, and comparable to SCENIC, except that
103 Ontogenet in cell type 3 is worse than SCENIC. GNAT is comparable to the single-task learning algo-
104 rithms for at least 2 of the cell types. The low F-score of AMuSR is because the inferred networks are
105 too sparse, with fewer than 100 edges, while the other algorithms inferred similar number of edges with
106 the true networks. These results remain consistent for datasets 2 and 3 which have fewer cells (1000 and
107 200, respectively), scMTNI and MRTLE remain superior in performance than other algorithms mea-
108 sured by both AUPR and F-score (**Figure 2B, C**). We expect scMTNI to be better since the network
109 simulation procedure is similar, but the data generated is different and independent. Finally, we aggre-
110 gated the results across all three cell types and datasets to obtain an overall comparison of the algorithms.
111 Here we considered algorithms across all parameter settings tested as well as the best parameter setting
112 determined by the best F-score or AUPR. Based on the AUPR of “all parameter setting”, we found
113 that multi-task learning methods, especially scMTNI and MRTLE are generally better than single-task

learning methods with higher AUPRs (**Supplementary Figure 1A,C**). AMuSR also outperformed the single-task algorithms based on AUPRs, although this was not as significant as MRTLE and scMTNI. When considering the “best parameter setting” the methods were not significantly different when using AUPR, though MRTLE and scMTNI had the highest AUPR (**Supplementary Figure 1B,D**). When using the F-score, scMTNI and MRTLE remained top performing algorithms for the “all parameter setting” (**Supplementary Figure 2A,C**) and the “best parameter setting” (**Supplementary Figure 2B,D**). Further, GNAT and Ontogenet had a higher F-score than the single-task learning method LASSO for the “all parameter” and “best parameter” settings. AMuSR suffered for the F-score metric due to the high sparsity in the inferred networks. Across different single-task algorithms, LASSO had the worst performance. Overall, the results on the simulated networks suggest that multi-task learning algorithms have a better performance than single-task algorithms for network inference on sparse datasets, similar to single-cell transcriptomic data. Furthermore, scMTNI and MRTLE are able to more accurately infer networks than other multi-task learning algorithms.

Inference of gene regulatory networks of somatic cell reprogramming to induced pluripotent stem cells

Cellular reprogramming is the process of converting cells in a differentiated state to a pluripotent state and is important in regenerative medicine as well as for generating patient-specific disease models. However, this process is inefficient as a small fraction of cells get reprogrammed to the pluripotent state³⁰. To gain insight into the gene regulatory networks that govern the dynamics of this process, we profiled single cell accessibility (scATAC-seq) during the reprogramming process from mouse embryonic fibroblasts (MEFs) to the induced pluripotent state and four intermediate timepoints, day3, day6, day9 and day12, to constitute a dataset of 6 timepoints. We used LIGER to integrate the scRNA-seq and scATAC-seq datasets (**Figure 3A, B**) and identified 8 clusters (**Methods**). Of these clusters, C4 is MEF-specific while C5 is ESC-specific (**Figure 3C, D**) and showed good integration of the scRNA-seq and scATAC-seq profiles. We removed C6 as it did not have scRNA-seq cells and applied a minimum spanning tree (MST²³) approach to construct the cell lineage tree from the 7 cell clusters with both scRNA-seq and scATAC-seq (**Methods, Figure 3E**). The MEF-specific cluster (C4) is at one end of the tree, while the ESC-specific cluster (C5) is at the other end. This is consistent with the starting and end state of the reprogramming process and we considered C4 to represent the root of the tree.

We applied scMTNI, scMTNI+prior (scMTNI with prior network), INDEP, INDEP+prior (INDEP with prior network) and SCENIC to this dataset (**Figure 3F**). We used the matched scATAC-seq clus-

ters to obtain transcription factor (TF)-target prior interactions for each scRNA-seq cluster needed for INDEP+prior and scMTNI+prior (**Methods**). We assessed the quality of the inferred networks by comparing to three gold standard datasets in mouse embryonic stem cells (mESCs, **Table 2**), one derived from ChIP-seq experiments (referred to as “ChIP”) from ESCAPE or ENCODE databases^{31,32}, one from regulator perturbation experiments (referred to as “Perturb”)^{31,33}, and the third from the intersection of edges in ChIP and Perturb (referred to as “ChIP+Perturb”). We compared the performance of the methods using F-score on the top 500, 1k and 2k edges across methods (**Figure 3F, Supplementary Figure 3, 4**). On Perturb and Perturb+ChIP, scMTNI+Prior had a higher average performance, outperforming other methods significantly in Perturb. On ChIP, SCENIC was generally better than other methods. To examine the poorer performance of scMTNI+Prior for the ChIP gold standard, we compared the regulators and targets in the inferred networks from each method. Between SCENIC and scMTNI, the number of regulators are similar, but SCENIC’s networks have more target genes, which recovered more targets from the gold standard datasets, resulting in a higher F-score. scMTNI+prior outperformed scMTNI in all but the ChIP dataset, and INDEP+prior outperformed INDEP, indicating that addition of priors based on scATAC-seq data was beneficial.

To gain an initial assessment of the network dynamics on the cell lineage, we computed F-score between each pair of inferred networks defined by the top 4k edges (**Figure 3G**). Both scMTNI and scMTNI+prior networks diverged in a manner consistent with the lineage structure. scMTNI networks formed three groups of cell types, (C4, C8, C1, C7), (C2, C3) and (C5 (ESC)). scMTNI+prior found similar groupings but placed C5 (ESC) closer to (C1, C7, C8, C4) branch. Both methods showed that C5 is closest to C1, which could be an important transitioning state of cells during reprogramming. SCENIC showed similarity among C1, C4, C7, however had lower similarity scores for most pairwise comparisons which made it difficult to discern a clear lineage structure. The networks inferred by the other methods were very divergent which is not biologically realistic because the reprogramming system is heterogeneous with a number of transitioning populations. Overall, these results suggest that scMTNI+prior recovered regulatory networks are of high quality and the networks exhibit a gradual rewiring of structure from the MEF to the pluripotent state.

172 **scMTNI predicts key regulatory nodes and GRN components that are rewired 173 during reprogramming**

174 To gain insight into which cell populations successfully reprogram versus those that do not and to fur-
175 ther characterize these different cell clusters, we examined the specific rewired network components

176 in each cell type-specific network inferred by scMTNI+prior using two complementary approaches: k-
177 means edge clustering and Latent Dirichlet Allocation (LDA, **Methods**). In the k-means edge clustering
178 approach, we represented each edge in the top 4k confidence set of any cell cluster, by a vector of confi-
179 dence scores in each cell cluster-specific network (if an edge is not inferred in the network it is assigned
180 a weight of 0). Next, we clustered edges based on their edge confidence pattern into 20 clusters de-
181 termined by the Silhouette Coefficient optimization (**Figure 4A**). The largest “edge clusters” exhibited
182 interactions specific to one cell cluster (e.g., E4, E6, E7, E11, E13, E15 and E16), while smaller clusters
183 exhibited conserved edges for more than one cell cluster (e.g., E2, E5, E12). To interpret these edge clus-
184 ters, we identified the top regulators associated with each of the edge clusters (**Figure 4B**). E16, which
185 was MEF-specific (C4) had Npm1, Nme2, Thy1, Ddx5 and Loxl2 as the top regulators which are known
186 MEF-specific genes. In contrast, E11, which was ESC-specific (C5) had Klf4, Lhx2, Elf4 which have
187 known roles in stem cell maintenance (Klf4) and differentiation into neural (Lhx2³⁴) and hematopoietic
188 lineage (Elf4³⁵). Edge clusters that shared edges across multiple cell clusters, e.g. E5 (C4, C8 and C1),
189 shared some of the top-ranking regulators such as Npm1 and Thyb1 with the MEF-specific cluster and
190 also identified other fibroblast-specific genes such as Col5a2 and Ybx1. Finally, E2 which comprised
191 shared edges between cell clusters C1 and C5, contained Esrrb, as its top regulator (**Figure 4B**). Esrrb
192 plays an important role for establishing naive pluripotency. This further supports the lineage structure
193 that C1 likely represents a population of cells that are committed to becoming pluripotent.

194 While the k-means analysis identified regulatory hubs specific to individual cell clusters, it was chal-
195 lenging to identify sub-network components that rewired at specific branch points likely because it treats
196 each edge independently. We developed an approach by adopting Latent Dirichlet Allocation (LDA) that
197 was recently used to study regulatory network rewiring from transcription factor ChIP-seq datasets³⁶
198 (**Methods**). In this approach, each TF is treated as a “document” and target genes are treated as “words”
199 in the document. Each document (TF) is assumed to have words (genes) from a mixture of topics, each
200 topic in turn interpreted as a pathway. TFs across cell clusters are treated as separate documents. We
201 applied LDA with $k = 10$ topics (**Figure 4C, D, Supplementary Figure 5,6, 7**), and examined each
202 of the topics based on their Gene Ontology process enrichment (**Supplementary Figure 8**), and the
203 tendency and identity of specific regulators to rewire across the cell clusters (**Methods**). Topic 3 net-
204 works were among the most divergent networks across the cell populations and identified several known
205 regulators for the pluripotency fate (**Figure 4C**). In particular, Esrrb was a hub in C5 (ESC) and C1
206 (closest to ESC) but absent in the other cell clusters. Topic 3 is enriched for cell cycle and developmen-
207 tal terms (**Supplementary Figure 8**). Comparison of the regulators in the (C1,C5) branch and (C7, C3,

208 C2) branch showed that the latter branch had regulators such as Wt1. Wt1 was a major regulator in the
209 starting MEF cluster as well suggesting the incomplete suppression of the MEF-specific program in the
210 C7-C3-C2 branch. Wt1 is an important regulator of cellular developmental processes and can act both as
211 a tumor suppressor and an oncogene³⁷. Topic 9 was also interesting in that it identified the persistence of
212 the regulators Ccng1 and Nme2 from the MEF-specific cell cluster (C4) in the C7-C3-C2 branch. Ccng1
213 is a cyclin that is part of the p53 pathway, which has been previously identified to be associated with
214 the inefficiency of cellular reprogramming^{38,39}. Nme2 is known to regulate Myc, which is an oncogene
215 and also one of the four reprogramming factors⁴⁰. The cellular reprogramming process has been consid-
216 ered to be similar to tumorigenesis which is supported by the identification of regulators associated with
217 cancer signaling pathways for populations that do not reprogram. Inhibition of these regulators could
218 potentially improve the reprogramming process. In total, using scMTNI and network rewiring analysis
219 we identified known cell population-specific regulators and also predicted new regulators that can be
220 perturbed to examine the impact on cellular reprogramming efficiency.

221 **Inferring gene regulatory networks in human hematopoietic differentiation**

222 To examine the utility of scMTNI in a different cell fate specification system, we applied scMTNI to a
223 published scATAC-seq and scRNA-seq dataset for human hematopoietic differentiation⁴¹. This dataset
224 profiled accessibility and transcriptomic state of immunophenotypic populations that were sorted based
225 on cell surface markers in hematopoietic differentiation and enabled studies of how multipotent pro-
226 genitors transit into lineage-restricted cell states. We considered the cell populations measured with
227 both scATAC-seq and scRNA-seq datasets: hematopoietic stem cell (HSC), common myeloid progeni-
228 tor (CMP), granulocyte-macrophage progenitors (GMP) and monocyte (Mono). These populations are
229 known to be heterogeneous comprising multiple sub-populations⁴¹. To identify these sub-populations
230 we again applied LIGER²² and identified 10 integrated clusters of RNA and accessibility (**Figure 5A-D**). Most clusters exhibited a mixed composition: C8 is mainly composed of HSCs but also included
231 CMP0 cells; C6 and C9 are composed of GMP and CMP0 cells. C1 (73 cells) and C4 (37 cells) were
232 mainly composed of Mono cells and were combined into C1. C5 had too few RNA cells (22 cells) and
233 was excluded from further analysis. We next inferred a cell lineage tree from these 8 cell clusters using
234 a minimal spanning tree approach²³ as described in the reprogramming study (**Figure 5E, Methods**).
235 As C8 is largely made up of HSC cells and HSC is the starting cell type, we treat C8 as the root of the
236 lineage.
237

238 We applied the same set of network inference algorithms to this dataset as the reprogramming dataset:

239 scMTNI, scMTNI+prior, INDEP, INDEP+prior and SCENIC. We assessed the quality of the inferred net-
240 works from each method by comparing them to gold-standard edges from published ChIP-seq and regu-
241 lator perturbation assays from several human hematopoietic cell types. This included ChIP-seq datasets
242 from the UniBind database (Unibind⁴²), ChIP-seq (Cus_ChIP) and regulator perturbation (Cus_KO) ex-
243 periments in the GM12878 lymphoblastoid cell line from Cusanovich et al⁴³ and the intersection of ChIP
244 and perturbation studies (Cus_KO+Cus_ChIP, Cus_KO+Unibind). In total we had five gold standard net-
245 works. We used F-score of the top 500, 1k, 2k edges in the inferred network (**Methods, Figure 5F,**
246 **Supplementary Figure 9**). The relative performance of the algorithms depended upon the gold stan-
247 dard. Algorithms that did not use priors (INDEP, SCENIC and scMTNI) performed comparably (with no
248 significant difference) on three of the five gold standards. On Unibind and Cus_KO+Unibind, SCENIC
249 is significantly better than INDEP and scMTNI (**Supplementary Figure 10**). Methods that used pri-
250 ors, INDEP+prior, scMTNI+prior, were generally better than methods without priors. INDEP+prior and
251 scMTNI+prior are comparable across the gold standard datasets with no significant difference in per-
252 formance. For the Unibind dataset, we had ChIP-seq based gold standard edges for different blood cell
253 types, with 1 to 48 transcription factors (**Table 3**). When comparing to these cell type-specific gold stan-
254 dards, prior based methods have a better performance especially for datasets with more TFs among top
255 500 and 1k edges (**Supplementary Figure 11,Supplementary Figure 12**). Furthermore, INDEP+prior
256 had the best overall performance indicating that incorporation of accessibility priors is more important
257 rather than the lineage information. However, these gold standards were much smaller and therefore can
258 assess smaller portion of the inferred networks.

259 We next examined the inferred networks for the extent of change on the lineage structure (**Figure 5G**).
260 The single-task learning methods INDEP and INDEP+prior exhibited a low overlap across each pair of
261 cell lines and did not as such obey the lineage structure. SCENIC recovers part of the lineage structure,
262 but placed C7 (common myeloid) close to C6 (granulocyte-macrophage progenitors (GMP)) rather than
263 C10, which has similar sample composition as C7. In contrast, scMTNI and scMTNI+prior were able to
264 find two groups of cell types, one corresponding to HSC and CMP2 branch consisting of C8, C3 and C2,
265 and the second corresponding to the CMP0, CMP1 and GMP branch (C6, C9, C10 and C7). The exces-
266 sive divergence identified by the single-task learning methods makes it difficult to identify and prioritize
267 specific network level changes driving cell fate decisions.

268 **Inferring shared and lineage-specific regulators for hematopoietic differentiation**

269 Similar to our cellular reprogramming study, we examined the scMTNI+prior networks to identify cell
270 type-specific regulators and network components (**Figure 6**). We applied k-means edge clustering to top
271 5k edges in any of the cell clusters and identified 19 edge clusters (**Methods**). Compared to the repro-
272 gramming study, a larger portion (94% vs 86%) of the edges are specific to one cell cluster (**Figure 6A**).
273 We used these edge clusters to identify differences among cell clusters which had similar compositions
274 of the initial cell types identified based on cell surface markers, e.g., C7 and C10 had similar compo-
275 sition of CMP0, CMP1, CMP2 cells and C6 and C9 had similar composition of GMP and CMP cells.
276 Edge cluster E2 had edges specific to cell cluster C7 and was associated with PLEX, YBX1, EEF1A1,
277 TSC22D3. PLEK and YBX1⁴⁴ are known to be involved in directing fate of HSCs, while both EEF1A1
278 and TSC22D3 have immune-related functions. In contrast, E8 which had edges specific to C10 had
279 different regulators, namely KLF7, ETV5, MBD2, ZNF202, EPM2A, ULK4. Of these, KLF7, ETV5
280 and MBD2 have known regulatory roles in hematopoiesis, with ETV5 regulating a population of Th9
281 cells⁴⁵ and KLF7 suppressing the formation of myeloid cells⁴⁶. Edge cluster E11, which was specific
282 to C6 ranked SP4, TYR03, ZNF417, MNDA highly. MNDA is associated with granulocyte-monocyte
283 lineage⁴⁷. In contrast, E6, which was specific to C9 had a different set of top regulators including
284 L3MBTL4, GABPA, ELF4, and RGS14. Both GABPA and ELF4 have important roles in hematopoei-
285 sis^{48,49}. A few edge clusters represented shared network components, e.g. E19 had edges from C6, C9,
286 C10, C7 that represented the GMP and CMP populations and E12 representing edges from C10 and C7.
287 Both E19 and E12 had YBX1 and TSC22D3 as top regulators (**Figure 6B**). YBX1, is known to have high
288 expression in myeloid progenitor cells⁴⁴, and regulates CCL5 expression during monocyte/macrophage
289 differentiation⁵⁰. TSC22D3, which is a glucocorticoid leucine zipper⁵¹, is involved in differentiation of
290 hematopoietic stem cells⁵². Taken together, the k-means edge clustering approach helped identify the
291 key regulators with known or plausible roles in hematopoiesis that could explain the differences among
292 the cell clusters.

293 To identify cell type-specific network rewiring that are associated with lineage decisions, we again
294 examined the regulatory networks of each cell cluster using LDA (**Methods**, **Figure 6C, D**). The top-
295 ics were enriched for diverse biological processes such as cell cycle (Topic 1 and 8, **Supplementary**
296 **Figure 16**), blood related processes (Topic 9) and represented subnetworks with different extents of con-
297 servation across the lineage. For example, topic 2 showed a gradual rewiring of an ID2-specific network
298 from the HSC populations (C8, C3, C2), to KLF1 and MYC centered networks for C7 and C10 which
299 represented the CMP0 population. ID2 is known to negatively regulate differentiation, which is con-

300 sistent with its presence in the C8, C3, C2 branches. KLF1 is an essential regulator for the erythroid
301 lineage^{53,54}, which is derived from the myeloid progenitor cells and therefore the association of KLF1
302 with these cells is consistent with the literature. Topics 1, 6 and 10 exhibited a conserved core around
303 HMGB2, TSC22D3, and YBX1 respectively, across all cells clusters (**Supplementary Figure 13, 14,**
304 **15**). HMGB2 is an important regulator for HSCs⁵⁵. Both YBX1 and TSC22D3, which were also identi-
305 fied in our k-means analysis, have known role in hematopoiesis⁴⁴. Topic 8 was associated with various
306 cell cycle and chromatin remodeling regulators such as TOP2A, CDC20 and CCNB1 (**Supplementary**
307 **Figure 15, 16**). Taken together, the LDA analysis identified differential subnetworks centered to candi-
308 date cell fate drivers in hematopoiesis that could be followed up with functional studies.

309

Discussion

310

Single-cell technologies have transformed our ability to study cellular heterogeneity and cell-type specific gene regulation of known and novel cell populations. Defining gene regulatory networks from scRNA-seq data of developmental systems has remained challenging as most existing methods have assumed a static view of the GRN and do not leverage accessibility to inform the GRN structure. To address this need, we develop single-cell Multi-Task Network Inference (scMTNI), a probabilistic graphical model-based approach that uses multi-task learning to infer cell type-specific GRNs on a cell lineage tree by integrating scRNA-seq and scATAC-seq data and model the dynamics of these regulatory interactions on a lineage.

318

Multi-task learning is well-suited for the inference of cell type-specific GRNs. However, a key question is how to implement multi-task learning for GRN inference. A number of multi-task learning algorithms were developed for inferring GRNs and functional networks from bulk transcriptomic data but have not been systematically compared for their effectiveness on single-cell transcriptomic data. Some approaches, such as AMuSR²⁷ have used a flat hierarchy where all the tasks are considered equally related. For heterogeneously related datasets, a hierarchy or a tree is well-suited to model the dependence across datasets. Such hierarchies can be implemented as a phylogenetic tree with observed data at the tips of the tree as in GNAT²⁵ and MRTLE²⁴, or as a cell-lineage tree with observations at all nodes in the tree. scMTNI and MRTLE both use a tree-based structure prior, whereas AMuSR, GNAT and Ontogenet used a regularized regression parameter to implement multi-task learning. scMTNI and MRTLE have better performance in predicting the gene regulatory relationships than single-task learning algorithms. The performance of Ontogenet is better than the single-task learning algorithms LASSO and INDEP in at least two cell types, and comparable to SCENIC. A prominent factor contributing to the difference in the performance of the algorithms was whether the models inferred a directed graph versus an undirected graph, with GNAT generally suffering likely due to this reason. Performance of GNAT is worst among multi-task learning algorithms and comparable to the single-task learning algorithms. We speculate that the undirected relationship in the graphical model of GNAT might be a reason that the performance is not as good as other multi-task learning algorithms. We also examined the performance of algorithms across different parameter settings that control for sparsity as well as for sharing information. We found that the algorithms were generally robust to the setting of sharing and more sensitive to the extent of sparsity. However, multi-task learning algorithms generally outperformed single-task learning algorithms indicating that this is a useful direction for methodological development for GRN inference from single cell

340 omic datasets. Importantly, single-task learning infers very different networks that makes it challenging
341 to study transitions across the networks.

342 Once GRNs are inferred across multiple cell types, the next challenge is to examine which compo-
343 nents of the GRNs change along the lineage. We developed two complimentary techniques to study dy-
344 namics. Our k-means edge clustering method was able to find regulatory connections that were unique
345 to each cell cluster, while our topic model-based dynamic network analysis highlighted subnetworks
346 that were activated or deactivated along the lineage. We applied our tools to study GRN dynamics in
347 hematopoietic cell differentiation and reprogramming from mouse embryonic fibroblasts to embryonic
348 stem cells. We found that both these systems exhibited different dynamics, with the reprogramming
349 system exhibiting more edges shared across populations compared to the hematopoietic system which
350 identified most edges as cell cluster-specific. In both systems, our analysis identified known and novel
351 regulators. For example, in the reprogramming system, we found that cells that were closer to the end
352 point pluripotent state already had an Esrrb-centered GRN component active. In contrast, for cells that
353 were on an alternate trajectory had several oncogenes such as Wt1 as key regulators. In the hematopoietic
354 system, our analysis examined immuno-phenotypically similar populations by identifying different
355 set of hematopoietic regulators associated with such populations.

356 scMTNI currently assumes that the input lineage structure is accurate. However, lineage construc-
357 tion, especially from integrated scRNA-seq and scATAC-seq datasets is a challenging problem. One
358 direction of future work is to assume the initial lineage structure is inaccurate and incorporate the re-
359 finement of the lineage structure as part of the GRN inference procedure. A second direction of work
360 is to model more fine-grained transitions within each cell population, for example using RNA veloc-
361 ity or pseudotime, which will complement the coarse-grained dynamics that scMTNI currently handles.
362 Studies from bulk RNA-seq data have shown that estimating hidden transcription factor activity (TFA)⁵⁶
363 can further improve the performance of network inference. Thus, another direction of future work is to
364 estimate hidden TFA and incorporate these to improve the accuracy of the inferred networks. Finally,
365 SCENIC performs very well among the single-task learning algorithms, which is likely because of its
366 regression-tree based model that captures non-linear dependencies and is less prone to the sparsity of
367 the dataset. While scMTNI's stability selection framework can capture some non-linearities, another
368 direction of future work is to extend scMTNI to model more non-lineage dependencies.

369 In summary, scMTNI is a tool to infer cell type-specific regulatory networks and their dynamics
370 on a cell lineage which combines scRNA-seq and scATAC-seq data. As single cell multi-omic datasets
371 become increasingly available, we expect scMTNI to be broadly applicable to predict GRNs and identify

372 important regulators associated with regulatory network dynamics across cell types in diverse cell-fate
373 specification processes.

374

Methods

375

Single-cell Multi-Task Network Inference (scMTNI)

376

Single-cell Multi-Task Network Inference (scMTNI) is a probabilistic graphical model-based approach that uses multi-task learning to infer gene regulatory networks for cell types related on a cell lineage tree (**Figure 1**). We define a cell type to be a group of cells with similar transcriptome and accessibility levels as defined by existing cell clustering methods. Each task learns the gene regulatory network (GRN), $\mathbf{G}^{(d)}$ for each cell type or cell cluster d . Given cell type-specific datasets for M cell types, $\mathcal{D} = \{D^{(1)}, \dots, D^{(M)}\}$, our task is to find the set of graphs $\mathcal{G} = \{\mathbf{G}^{(1)}, \dots, \mathbf{G}^{(M)}\}$ and parameters $\Theta = \{\theta^{(1)}, \dots, \theta^{(M)}\}$ for each of the cell types. $\mathbf{G}^{(d)}$ is modeled as a dependency network²¹, a class of probabilistic graphical models for inferring directed, predictive relationships among random variables (regulators and genes). Each gene is modeled as a random variable $X_i^{(d)}$ which encodes the expression level of gene i in each cell. A conditional probability distribution $P(X_i^{(d)} | \mathbf{R}_i^{(d)})$ models the relationship between gene i and its set of regulators, $\mathbf{R}_i^{(d)}$ in cell type d . In a dependency network, GRN inference entails estimating the regulators $\mathbf{R}_i^{(d)}$ for each gene i in each cell type d . To enable joint learning of these cell type-specific networks our goal is to find the set $\mathcal{G} = \{\mathbf{G}^{(1)}, \dots, \mathbf{G}^{(M)}\}$ and parameters $\Theta = \{\theta^{(1)}, \dots, \theta^{(M)}\}$ by estimating the posterior distribution of these two sets and finding their maximum a posteriori values:

$$P(\mathcal{G}, \Theta | \mathcal{D}) \propto P(\mathcal{D} | \mathcal{G}, \Theta) P(\Theta | \mathcal{G}) P(\mathcal{G}) \quad (1)$$

391

$P(\mathcal{D} | \mathcal{G}, \Theta)$ is the data likelihood, expanded as $\prod_d P(\mathcal{D}^{(d)} | \mathbf{G}^{(d)}, \theta^{(d)})$. In a dependency network, pseudo likelihood²¹ is used to approximate the data likelihood for each cell type, defined as the products of the conditional distribution of each random variable $X_i^{(d)}$ given its neighbor set $\mathbf{R}_i^{(d)}$ in cell type d , $P(X_i^{(d)} | \mathbf{R}_i^{(d)}, \theta_i^{(d)})$. Thus, the likelihood can be written as:

$$P(\mathcal{D} | \mathcal{G}, \Theta) \propto \prod_{d \in \{1, \dots, M\}} \prod_{i \in \{1, \dots, N\}} P(X_i^{(d)} | \mathbf{R}_i^{(d)}, \theta_i^{(d)}) \quad (2)$$

395

Given the neighbor set $\mathbf{R}_i^{(d)}$, the above quantity can be computed efficiently. We assume that each variable $X_i^{(d)}$ and its neighbor set $\mathbf{R}_i^{(d)}$ in cell type d are from a multi-variate Gaussian distribution. Thus, $P(X_i^{(d)} | \mathbf{R}_i^{(d)}, \theta_i^{(d)})$ can be modeled using a conditional Gaussian distribution with mean $\mu_{X_i^{(d)} | \mathbf{R}_i^{(d)}}$ and variance $\sigma_{X_i^{(d)} | \mathbf{R}_i^{(d)}}^2$ which can be estimated in closed form. $\mathbf{R}_i^{(d)}$ is selected from the input list of

399 regulators using a greedy search algorithm, executed in parallel across all cell types (See **Supplementary Methods**). The second term $P(\Theta|\mathcal{G})$ in **Equation** (1) is estimated using the maximum likelihood
 400 settings of the parameters. The third term $P(\mathcal{G}) = P(\mathbf{G}^{(1)}, \dots, \mathbf{G}^{(M)})$ in the objective function is the
 401 structure prior and is defined in a way to capture the state of an edge across all cell types modeled, where
 402 $\mathcal{G} = \{\mathbf{G}^{(1)}, \dots, \mathbf{G}^{(M)}\}$. We assume that $P(\mathcal{G})$ is composed of two priors, one is the cell-type specific
 403 prior $P(\mathbf{T})$, where $\mathbf{T} = \{T^{(1)}, \dots, T^{(M)}\}$, and the other one is a cell lineage structure prior $P(\mathbf{S})$ which
 404 captures the similarity between related cell types along the cell lineage tree, where $\mathbf{S} = \{S^{(1)}, \dots, S^{(M)}\}$.
 405

$P(\mathbf{T})$ is the cell-type specific prior, which decomposes over a product of cell-type specific graphs:
 $P(T^{(1)}, \dots, T^{(M)}) = \prod_{d=1}^M P(T^{(d)})$. The $P(T^{(d)})$ decomposes over a product of individual edge configurations, $P(I_{u,v}^{(d)})$, where $I_{u,v}^{(d)}$ is an indicator function that represents whether there exists an edge
 between regulator u to target gene v in cell type d , $X_u \rightarrow X_v$ as follows:

$$I_{u,v}^{(d)} = \begin{cases} 1, & \text{if there is an edge from } u \text{ to } v \text{ in cell type } d, \\ 0, & \text{otherwise.} \end{cases}$$

406 As in Roy et al⁵⁷, we model the prior probability using a logistic function:

$$P(I_{u,v}^{(d)} = 1) = \frac{1}{1 + e^{-(\beta_0 + \beta_1 * m_{uv}^{(d)})}} \quad (3)$$

407 The β_0 parameter is a sparsity prior that controls the penalty of adding of a new edge to the network,
 408 which takes a negative value ($\beta_0 < 0$). A smaller value of β_0 will result in a higher penalty on adding
 409 new edges and will therefore infer sparser networks. The β_1 parameter controls how strongly motifs are
 410 incorporated as prior ($\beta_1 \geq 0$). A higher value of β_1 will result in motif presence being valued more
 411 strongly to select an edge. β_1 is set to 0 when there is no cell type-specific motif information available.
 412 $m_{uv}^{(d)}$ is the weight of the edge from regulator u to target v in the prior network and is computed based
 413 on the motif instance score if gene v has a motif of regulator u in its promoter region that overlaps an
 414 ATAC-seq peak. Thus, we have

$$P(\mathbf{T}) = \prod_{d=1}^M P(T^{(d)}) = \prod_{d=1}^M \prod_{u,v; u \neq v} P(I_{u,v}^{(d)}) \quad (4)$$

415 The cell lineage structure prior $P(\mathbf{S})$ is constructed to make use of multi-task learning. We define
 416 that $P(S^{(1)}, \dots, S^{(M)})$ can be rewritten as a product over a set of edges between regulators and target
 417 genes: $\prod_{u,v; u \neq v} P(I_{u,v}^{(1)}, \dots, I_{u,v}^{(M)})$. Under the assumption that the prior probability of the edge state in

418

one cell type is only dependent upon its state in the predecessor cell type, we have:

$$P(\mathbf{S}) = \prod_{u,v; u \neq v} P(I_{u,v}^{(1)}, \dots, I_{u,v}^{(M)}) = \prod_{u,v; u \neq v} \prod_{d \in \{1, \dots, M\}} P(I_{u,v}^{(d)} | I_{u,v}^{pa(d)}) P(I_{u,v}^{(r)}), \quad (5)$$

419

where $pa(d)$ denotes the predecessor cell of cell type d on the cell lineage tree and r denotes the starting root cell. $P(I_{u,v}^{(d)} | I_{u,v}^{pa(d)})$ is a measure of overall regulatory gain and loss of regulatory connections between related cell types, and is assumed to be the same across the set of edges. Thus, it can be specified by three parameters: the probability of gaining a regulatory edge in the starting cell, $p_r = P(I_{u,v}^{(r)})$, the probability of gaining a regulatory edge in cell type d given that the edge does not exist in its predecessor cell $p_g^{(d)} = P(I_{u,v}^{(d)} = 1 | I_{u,v}^{pa(d)} = 0)$, and the probability of maintaining a regulatory edge in cell type d , given its presence in its predecessor cell $p_m^{(d)} = P(I_{u,v}^{(d)} = 1 | I_{u,v}^{pa(d)} = 1)$. These parameters of the priors can be set by the user or estimated empirically by analyzing different configurations and selecting those values with the best agreement with existing biological knowledge of the system. scMTNI uses a greedy score-based structure learning algorithm. Please refer to **Supplementary Methods** for details.

420

421

422

423

424

425

426

427

428

429

430

431

432

433

434

435

436

437

438

439

440

441

442

443

444

Input Datasets

Simulated Datasets

To benchmark the performance of different multi-task and single-task learning algorithms, we simulated single cell expression data from a lineage resembling a linear differentiation process for three cell types (**Figure 2A**). We simulated network dynamics on a lineage tree and controlled the extent of similarity with the three prior parameters: p_r , the probability of having an edge in the starting/root cell type; $p_g^{(d)}$, the probability of gaining an edge in cell type d that is not in the predecessor cell type; $p_m^{(d)}$, the probability of maintaining an edge in cell type d from the predecessor cell type. We set $p_r = 0.5$, $p_g^{(d)} = 0.4$ and $p_m^{(d)} = 0.7$ or 0.8 and simulated three networks from a linear lineage tree for each of the three cell types, each with 15 regulators and 65 genes. Next, we applied BoolODE on the simulated gene regulatory networks and generated single cell expression data for 2000 cells for each cell type. To mimic the dropouts in the scRNA-seq data, we added 80% sparsity uniformly to all genes on the simulation data. We refer to this simulated dataset as data 1, consisting of 65 genes and 2,000 cells for three cell types. We generated smaller sample sizes of these datasets, data 2 and data 3 by downsampling data 1 to 1,000 cells (data 2) and 200 cells (data 3). We applied each of the algorithms on these three datasets within a stability selection framework and evaluated their performance based on AUPR and F-score as

445 described in the **Evaluation** section.

446 **Human hematopoietic differentiation data**

447 Buenrostro et al.⁴¹ measured single-cell accessibility (scATAC-seq) and single-cell RNA sequencing
448 (scRNA-seq) data to study the regulatory dynamics during human hematopoietic differentiation for mul-
449 tiple immuno-phenotypic cell types: hematopoietic stem cells (HSCs), common myeloid progenitors
450 (CMPs) and granulocyte-macrophage progenitors (GMPs) and Monocytes (Monos). We downloaded
451 fragment files for the scATAC-seq data and processed scRNA-seq data for each cell type. For the
452 scATAC-seq data we mapped the fragments into 23,347,540 bins with length of 1000bp. Next, we
453 mapped 1kb bins to the nearest gene and extracted cells with cell barcodes labeled as HSC, CMP, GMP
454 and Mono cells. Next, we filtered out genes with sum of counts in all samples less than 100 producing
455 a processed scATAC-seq dataset with 54,344 genes and 1,315 cells across the four cell types. We ex-
456 tracted the count matrix of scRNA-seq from these four cell types. After filtering out genes with non-zero
457 expression in less than 5 cells, the scRNA-seq data had 12,558 genes and 4,165 cells. We normalized
458 the count matrix for depth and variance stabilization based on the pagoda pipeline⁵⁸. We kept 12,393
459 common genes between scATAC-seq and scRNA-seq data and applied LIGER²² to define integrated cell
460 populations. We applied LIGER with $k \in 8, 10, 12, 15, 20$ and found 10 cell subpopulations to be most
461 appropriate. C8 was mainly composed of HSCs, C6 was mainly composed of GMP cells, C7 was mainly
462 CMP0 cells, C1 was composed of Mono cells, and the rest clusters were a combination of several cell
463 types. C5 had too few RNA cells (22 cells) so we excluded it from further analysis. Since the composi-
464 tion of C1 (73 cells) and C4 (37 cells) are very similar, mainly GMP and Mono cells, we combined these
465 two clusters as C1. We inferred a cell lineage tree from the 8 cell clusters using a minimal spanning tree
466 approach (python package `scipy.sparse.csgraph`).

467 To derive the prior network for each cell cluster we created cluster-specific bam files from the
468 scATAC-seq data using the LIGER clusters. We pooled these bam files to generate pseudo bulk accessi-
469 bility coverage and applied MACS2 to identify scATAC-seq peaks for each cell cluster⁵⁹. We obtained
470 sequence-specific motifs from the Cis-BP database⁶⁰ and used the script `pwmmatch.exact.r` avail-
471 able from the PIQ toolkit⁶¹ to identify significant motif instances genome-wide using the human genome
472 assembly of hg19. We mapped motifs to each scATAC-seq peak and mapped the peak to a gene if it was
473 within ± 5000 bp of the transcription start site (TSS) of a gene. In this case, we connect motifs to TSS that
474 are mapped to the same scATAC-seq peak. We used the max motif score from `pwmmatch.exact.r`
475 for each motif-TSS pair and took the maximum value among all TSSs of a gene as the value for each

476 motif-gene pair. The motif instance score is the log ratio of the PWM to a uniform background. Finally,
477 to generate the edge weight for each TF-gene pair, we used the max score among all motifs mapped
478 to the same TF. To normalize the edge weights across TFs, we converted these weights into percentile
479 scores and selected the top 20% of edges as prior edges.

480 **Mouse reprogramming data**

481 We generated a novel scATAC-seq time course dataset for cellular reprogramming from mouse embry-
482 onic fibroblast (MEF) reprogramming to induced pluripotent cells (iPSC). The dataset contains had a
483 total of 6 time points corresponding to the starting MEF, the end pluripotent state (mESC), and four
484 intermediate timepoints of day3, day6, day9 and day12. We downloaded scRNA-seq datasets (GEO:
485 GSE108222) for the same time points from Tran et al⁶². The scATAC-seq data was first processed
486 through CellRanger ATAC pipeline to provide the `frags.txt` file. We binned the genome at non-
487 overlapping 1kb bin and computed the number of fragments mapped to each 1kb bin. Next, we mapped
488 1kb bins to the nearest gene for all of the samples. For scRNA-seq data, we concatenated the expres-
489 sion data from two replicates at each time point and normalized the concatenated matrix for depth and
490 variance stabilization based on the pagoda pipeline⁵⁸. Next, for each time point, we removed genes with
491 expression in less than 5 cells. We took the union of genes among all time points and concatenated the
492 expression data across all time points as our final scRNA-seq data matrix. The processed scATAC-seq
493 data contains 25,824 genes and 30,344 cells. The processed scRNA-seq dataset contains 14,953 genes
494 and 3,460 cells. We had a total of 11,926 genes in common between the two datasets, which were used
495 for downstream analysis. We applied LIGER with $k \in 8, 10, 12, 15, 20$ and found $k = 8$ to provide
496 the optimal clustering of the scRNA-seq and scATAC-seq data determined based on the clustering of the
497 accessibility and transcriptome of the MEF and ESC time points. We used the mean expression profiles
498 across samples of these cell clusters and computed the Euclidean distance between every cell clusters.
499 Then, we inferred a minimal spanning tree using the distance matrix and used it as the cell lineage tree
500 using `scipy.sparse.csgraph` in python. The prior motif was generated in the same way as for
501 the hematopoiesis differentiation dataset using motifs for mouse from the CisBP database⁶⁰. We used
502 mouse genome mm10 for this analysis.

503 Application of network inference algorithms on simulated datasets

504 We used the simulated datasets to perform extensive benchmarking of the different network inference
505 algorithms. We also used this dataset to study the sensitivity of the algorithms to the different parameter
506 settings. Below we describe each of the algorithms as well as the parameters used for each of the
507 algorithms for the simulated datasets. For all three simulation datasets, we applied all algorithms other
508 than SCENIC within a stability selection framework to estimate the confidence score for each edge in
509 the predicted networks. For stability selection we subsampled each dataset 20 times randomly using
510 half of the cells and all genes. SCENIC has its own internal sub-sampling and directly outputs the edge
511 confidence.

512 **scMTNI.** scMTNI has five hyper-parameters: p_r , probability of having an edge in the starting cell
513 type; $p_g^{(d)}$, probability of gaining an edge in a child cell type d ; $p_m^{(d)}$ the probability of maintaining
514 an edge in d from its immediate predecessor cell type; a sparsity penalty β_0 , that controls penalty for
515 adding edges; β_1 , that controls the strength of incorporating prior network. We tried different configu-
516 rations of the hyper-parameters: $p_r \in \{0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5\}$, and $p_g^{(d)} \in \{0.05,$
517 $0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45\}$, and $p_m^{(d)} \in \{0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9\}$, $\beta_0 \in$
518 $\{-0.005, -0.01, -0.05, -0.1, -0.5\}$. β_1 was set to 0 as there is no prior network in the simulations. If
519 the size of the predicted network for a parameter setting was smaller than the size of the simulated net-
520 work, we disregarded this parameter setting for comparison. We used the area under the precision-recall
521 curve (AUPR) to compare the scMTNI inferred networks to simulated networks. We also computed
522 F-score on top K edges ranked by the confidence score (where K is the number of edges in the simulated
523 network, see **Table 1**). Overall performance of scMTNI was stable across different parameter configu-
524 rations (**Supplementary Figure 17, Supplementary Methods**). To compare against methods, we used
525 values from the best parameter settings for each dataset and cell type as well as all parameter settings
526 (**Supplementary Figure 1,2**).

527 **MRTLE.** Multi-species regulatory network learning (MRTLE)²⁴ is a probabilistic graphical model-
528 based algorithm that uses phylogenetic structure, transcriptomic data for multiple species, and sequence-
529 specific motifs to infer the genome-scale regulatory networks across these species simultaneously. It was
530 developed for bulk transcriptomic data. It uses a dependency network model to specify the directed rela-
531 tionship among regulators to target genes. Sequence-specific motif instances can be incorporated as prior
532 knowledge to favor edge supported with presence of motifs. The multi-task learning framework is em-

533 bedded in the phylogenetic prior, which captures the evolutionary dynamics of regulatory edge gain and
534 loss guided by the phylogenetic structure. The MRTLE algorithm has four parameters: p_g , the probability
535 of gaining an edge in a child species s that is not in the ancestor species; p_m , the probability of main-
536 taining an edge in a species s given that is also in s 's immediate ancestor of s ; β_0 , a sparsity penalty that
537 controls penalty for adding edges, and a penalty β_1 that controls the strength of motif prior. In the sim-
538 ulation case, we examined different parameter configurations: $p_g \in \{0.05, 0.1, 0.15, 0.2, 0.3, 0.4\}$, $p_m \in$
539 $\{0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85\}$, $\beta_0 \in \{-0.005, -0.01, -0.05, -0.1, -0.5, -1\}$. β_1 was set to 0. The
540 overall performance of MRTLE was stable across different parameter configurations (**Supplementary**
541 **Figure 18**). Similar to scMTNI, we used the AUPR and F-score of top K edges to select the best param-
542 eter setting. The best parameter setting and all parameter settings were used to compare against other
543 algorithms.

544 **GNAT.** The GNAT²⁵ algorithm uses a hierarchy of tissues to share information between related tissue
545 and infers tissue-specific gene co-expression networks. It was developed for bulk transcriptomic data.
546 GNAT models each network using a Gaussian Markov Random Field (GMRF). It has two parameters:
547 the L_1 penalty λ_s that controls the sparsity of the network, and the L_2 penalty λ_p that encourage the
548 precision matrix of children to be similar to its parent precision matrix. It initially learns a co-expression
549 network for each leaf tissue. Then it infers the networks in internal nodes using the networks in the leaf
550 nodes and updates the networks in leaf nodes for several iterations until convergence. Since GNAT learns
551 undirected networks, we transformed them to directed networks by adding edges from a regulator to a
552 target. If the nodes of an edge are both candidate regulators, we output the edge in both directions. We
553 tried different parameter configurations of λ_s and λ_p . For data 1 (n=2000), λ_s were set to {30, 31, 32,...,
554 37}, and λ_p were set to {30, 31, 32,..., 40}. For data 2 (n=1000), λ_s were set to {18, 19,..., 22}, and λ_p
555 were set to {18, 19,..., 25}. For data 3 (n=200), λ_s were set to {5, 6, 7, 8}, and λ_p were set to {5, 6, 7,
556 8}. We found that λ_s dominates the performance and under the same λ_s , changing λ_p does not change
557 the performance a lot (**Supplementary Figure 19**). If the size of the predicted network for a parameter
558 setting is smaller than the size of the simulated network, we removed this parameter setting. In this case,
559 the ranges of λ_s and λ_p are slightly different and varying across different datasets. We used AUPR and
560 F-score of top K edges to select the best parameter settings. We compared the algorithms using these
561 and all parameter settings.

562 **Ontogenet.** The Ontogenet²⁶ algorithm was developed to reconstruct lineage-specific regulatory net-
563 works using cell type-specific gene expression data across cell lineages. It was developed for bulk
564 transcriptomic data. To infer the regulatory networks for each cell type, it uses a fused LASSO frame-
565 work combined with an additional L_2 penalty. The L_1 penalty is introduced to control the sparsity of
566 regulators, while the L_2 penalty is used to select correlated predictors. The multi-task learning comes
567 in the fused LASSO framework with additional L_1 penalty on the difference of the regression weight of
568 related cell types, which encourage the consistency of regulatory programs between related cell types.
569 Ontogenet was applied on the same subsample of the three simulation datasets within a stability selec-
570 tion framework to estimate the confidence score for each edge in the networks. The Ontogenet algorithm
571 has three parameters: the L_1 penalty λ that controls the sparsity of the network, the L_2 penalty κ that
572 handles correlated predictors, and γ that encourage the similarity of regulatory programs between related
573 cell types. We tried different parameter configurations of λ , γ and κ . For data 1 (n=2000), λ were set to
574 {1000,1250,1500,1750,2000,2250,2500}, and γ were set to {1000,1250,1500,1750,2000,2250,2500}.
575 For data 2 (n=1000), λ were set to {500,1000,2000,3000}, and γ were set to {500,1000,2000,3000}.
576 For data 3 (n=200), λ were set to {475,500,525}, and γ were set to {475,500,525}. κ was set to {1, 5,
577 10} for each of the datasets. We found that λ and γ dominate the performance and while changing κ
578 does not change the performance significantly (**Supplementary Figure 20**). If the size of the predicted
579 network for a parameter setting is smaller than the size of the simulated network, we removed this pa-
580 rameter setting. The ranges of λ and γ are slightly different and varying across different datasets in order
581 to infer similarly sized networks for different datasets. We used AUPR and F-score of top K edges to
582 select the best parameter settings. We compared the algorithms using these and all parameter settings.

583 **AMuSR.** The Inferelator-AMuSR²⁷ algorithm uses sparse block-sparse regression to estimates the
584 activities of transcription factors and infer gene regulatory networks from expression datasets. The multi-
585 task learning approach decomposes the model coefficients matrix into a dataset-specific component us-
586 ing a sparse penalty and a conserved component using a block-sparse penalty to capture both conserved
587 interactions and dataset-unique interactions. It is able to incorporate prior knowledge from multiple
588 resources and robust to false interactions in the prior network. For our simulation setting, we applied
589 AMuSR without TFA estimation by setting `worker.set_tfa(tfa_driver=False)` in the `SingleCellWorkflow`
590 from Inferelator 3.0 package. To be comparable across different algorithms, AMuSR was applied on
591 the same subsample of the three simulation datasets within a stability selection framework to estimate
592 the confidence score for each edge in the AMuSR networks. The AMuSR algorithm has two sparsity

parameters: λ_s that controls the sparsity of the network for each dataset, the block-sparse penalty λ_b that controls the sparsity of the conserved network across all datasets. AMuSR has its own parameter selection framework (see²⁷ for details) and uses extended Bayesian information criterion (EBIC) to select the optimal (λ_s , λ_b). We additionally externally tuned the parameters by setting c to {0.01, 0.02154435, 0.04641589, 0.1, 0.21544347, 0.46415888, 1., 2.15443469, 4.64158883, 10 } and set $\lambda_b = c * \sqrt{\frac{d * \log(p)}{n}}$ as suggested in the paper, where d is the number of cell types and n is the number of samples and p is the number of genes. However, by setting λ_b to 0 and λ_s to 0, we found that the inferred networks are too sparse with 7-100 edges for data 1, and 71-129 edges for data 2. We kept two settings for AMuSR, one using our criteria to select the best setting based on AUPR and F-scores among different c settings (AMuSR_tuned) and another version using AMuSR's default optimal parameter selection (AMuSR_default). We computed AUPR and F-score of top K edges (where K is the number of edges in the simulated network) for AMuSR inferred networks with optimal parameter settings for comparison with other algorithms. We compared the algorithms using the optimal and all parameter settings.

INDEP. The INDEP algorithm is the single-task framework of scMTNI which does not have the prior for sharing information across cell types and infers a regulatory network for each cell type independently. It also models each network using a dependency network as scMTNI. INDEP learns the graphs for each cell type using a greedy graph learning algorithm with a score-based search, where the score contains only the data likelihood. At each iteration, the algorithm computes the change in data likelihood score²¹ for all candidate regulators for each target gene, selects the best regulator for the target gene and adds this (regulator, target) edge to the current graph. INDEP has two parameters in the model: a sparsity penalty β_0 that controls penalty for adding edges, and a penalty β_1 that controls the strength of motif prior. In the simulation case, β_0 were set to {-0.005, -0.01, -0.05, -0.1, -0.5, -1}, and β_1 were set to 0. AUPR and F-score of top K edges were used to select the best parameter settings (**Supplementary Figure 21**). If the size of the predicted network for a parameter setting is smaller than the size of the simulated network, we removed this parameter setting. As above, we compared INDEP to other algorithms using best and all parameter settings for a dataset.

LASSO. The LASSO regression is linear regression with L_1 regularization. For each gene, we use the expression profiles of candidate regulators to predict the expression profiles of this gene. The regulators with non-zero coefficients are inferred as the regulators for this gene and these edges are added to the gene regulatory network. We used matlab implementation of the LASSO regression. Similarly to

623 scMTNI and MRTLE, LASSO was run on the same subsample of the three simulation datasets within
624 a stability selection framework to estimate the confidence score for each edge in the networks. LASSO
625 has only the L_1 penalty λ that controls the sparsity of the network. In the simulation case, λ were set
626 to $\{0.01, 0.02, 0.03, 0.04, 0.05, 0.06\}$. AUPR and F-score of top K edges were used to select the best
627 parameter settings (**Supplementary Figure 22**). If the size of the predicted network for a parameter set-
628 ting is smaller than the size of the simulated network, we removed this parameter setting. We compared
629 LASSO to other algorithms using the best and all parameter settings.

630 **SCENIC.** The SCENIC²⁹ algorithm uses GENIE3 or GRNBoost2 to infer TF-target relationships
631 available as part of the Arboreto framework⁶³. We used the GRNBoost2 algorithm with default parame-
632 ters for network inference. SCENIC is based on ensemble models with its own bootstrapping and hence
633 was directly applied to each cell type-specific dataset in the simulation. SCENIC uses the feature impor-
634 tance score of each edge to rank the edges in the inferred network. We computed AUPR and F-score of
635 top K edges (where K is the number of edges in the simulated network) for SCENIC inferred networks
636 for comparison with other algorithms.

637 Application of network inference algorithms to cellular reprogramming data

638 We applied scMTNI, scMTNI+prior, INDEP, INDEP+prior and SCENIC to this dataset. scMTNI and
639 INDEP algorithms were applied within a stability selection framework to estimate edge confidence.
640 SCENIC has its own subsampling framework which can estimate an edge importance. In the stability
641 selection framework, we subsampled the data 50 times, each with 12,216 genes and $\frac{2}{3}$ of the cells, ap-
642 plied the algorithms to each subsample and used the inferred networks to estimate the confidence score
643 for each TF-target edge in the predicted networks. In both scMTNI and scMTNI+prior, we used the fol-
644 lowing hyper-parameter settings for the lineage structure prior $p_r = 0.2$, $p_g^{(d)} = 0.2$ and $p_m^{(d)} = 0.8$. For
645 the sparsity prior we set $\beta_0 = -0.9$ for scMTNI, and $\beta_0 \in \{-0.9, -2, -3, -4\}$ for scMTNI+prior. To
646 generate prior network, we used the matched scATAC-seq clusters to obtain TF-target prior interactions
647 for each scRNA-seq cluster. For scMTNI+prior which uses the scATAC-seq prior, we set $\beta_1 \in \{2, 4\}$.
648 INDEP and INDEP+prior were applied on the same subsampled data followed by edge confidence esti-
649 mation. We used the same settings for β_0 and β_1 for INDEP as scMTNI. Final results of scMTNI+prior
650 are using $\beta_0 = -4$ and $\beta_1 = 4$, which was determined by the distribution of edges at different confi-
651 dences. Final results for INDEP+prior are using $\beta_0 = -4$ and $\beta_1 = -4$. SCENIC was applied to the
652 entire dataset with default parameter settings.

653 **Application of network inference algorithms to human hematopoietic differentiation data**
654

655 We used a similar workflow for the human hematopoietic differentiation dataset as the reprogramming
656 system. We subsampled the scRNA-seq data for each cell cluster 50 times, each with 11,994 genes and $\frac{2}{3}$
657 of the cells, and applied scMTNI, scMTNI+prior, INDEP, INDEP+prior on each subsample to estimate
658 the edge confidence of the GRNs. For scMTNI and scMTNI+prior, the lineage structure prior parameters
659 were set as follows: $p_r = 0.2$, $p_g^{(d)} = 0.2$, $p_m^{(d)} = 0.8$. The sparsity prior β_0 was set to -0.9 for scMTNI.
660 For scMTNI+prior, the sparsity prior was set $\beta_0 \in \{-0.9, -2, -3, -4\}$ and $\beta_1 \in \{2, 4\}$. For INDEP and
661 INDEP+prior, we used the same settings for β_0 and β_1 for as scMTNI and scMTNI+prior respectively.
662 Final results of scMTNI+prior are with $\beta_0 = -4$ and $\beta_1 = 4$ and final results for INDEP+prior are using
663 $\beta_0 = -4$ and $\beta_1 = -4$. SCENIC was applied to the entire dataset with default parameter settings.

664 **Evaluation**

665 **Gold standard datasets**

666 To evaluate the predicted networks of different inference algorithms on real data, we downloaded and
667 processed several gold standard datasets (**Tables 2, 3**). For human hematopoietic cell types, we have five
668 gold standard datasets. Two gold standard datasets were a ChIP-based (Cus_ChIP) and a regulator knock
669 down-based (Cus_KO) gold standard dataset in GM12878 lymphoblastoid cell line downloaded from
670 Cusanovich et al⁴³. For the knockout dataset, we had TF-target relationships at two p-value thresholds,
671 0.01 and 0.05. We used the one at 0.01 to have a more stringent gold standard. The third gold standard
672 was from human hematopoietic cell types from the UniBind database ([https://unibind.uio.
673 no/](https://unibind.uio.no/))⁴², which has high confidence TF binding site predictions from ChIP-seq experiments. To obtain
674 the TF-gene network, we mapped TF binding sites to the nearest gene if there is overlap between the
675 TF binding sites and the promoter of the gene define by ± 5000 bp. If multiple ChIP-seq datasets were
676 available for the same TF in a given cell type, we took the union of TF-gene edges for the same cell
677 type. We took the union of these individual cell type-specific gold standards to create our Unibind
678 gold standard (UniBind). Finally, we took the intersection of the ChIP-based gold standards with the
679 knockdown based gold standards, Unibind+Cus_KO and CusChIP+Cus_KO to produce the fourth and
680 fifth gold standards. The statistics of the gold standard datasets are provided in **Table 3**.

681 For mouse reprogramming study we curated multiple experimentally derived networks of regulatory
682 interactions from the literature and existing databases. The statistics of the gold standard datasets are

683 provided in **Table 2**. One of these experiments is ChIP based gold standard (referred to as “ChIP”) from
684 ESCAPE or ENCODE databases^{31,32}, which contains ChIP-chip or ChIP-seq experiments in mouse
685 ESCs. Another is knock-down based gold standard (referred to as “Perturb”), which is derived from
686 regulator perturbation followed by global transcriptome profiling^{31,33}. We took a union of the networks
687 from LOGOF (loss or gain of function) based gold standard networks from ESCAPE database³¹ and
688 the networks from Nishiyama et al³³ as the perturbation interactions. Finally, we took the intersection
689 of the interactions between ChIP and knock-down based gold standard to create the third gold standard
690 network referred to as “ChIP+Perturb”.

691 Area Under the Precision Recall Curve

692 To evaluate the performance of scMTNI and other algorithms, we compared the inferred networks to the
693 simulated networks or interactions from the gold standard datasets based on Area under the precision
694 recall curve (AUPR). Edge weights for all but the SCENIC algorithm were obtained using stability
695 selection. In our stability selection framework, we generated N random subsamples of the data, inferred
696 a network for each subsample, and calculated a confidence score for each edge as the fraction of how
697 many times this edge was present in the inferred networks across all subsamples. Next, we ranked
698 the edges by the confidence score and estimated precision and recall at different confidence thresholds
699 ranging from 0 to 1. Precision P is defined as the fraction of the number of edges that are true positives
700 among the total number of predicted edges. Recall R is defined as the fraction of the number of edges
701 that are true positives among the total number of true edges. Then, we plotted the precision recall curve
702 and estimated the area under this curve using the AUCCalculator package developed by Davis et al.⁶⁴.
703 The area under the precision recall curve is computed as an overall assessment of the inferred networks
704 compared to “true” networks. The higher AUPR, the better the performance is. For the real scRNA-seq
705 datasets, we filtered the inferred networks to include TFs and targets that were in the gold standard.

706 F-score

While AUPR uses a ranking of the edges, F-score is a metric to compare a set of predicted edges to a set
of “true” edges. F-score is defined as the harmonic mean of the precision (P) and recall (R),

$$\text{F-score} = \frac{2*P*R}{P + R}$$

707 F-score enables us to control for the number of edges across network inference algorithms as these
708 can vary significantly across algorithms. To control for number of edges in the predicted networks, we
709 ranked the predicted network by the confidence score or edge weight, selected top K edges and com-
710 puted F-score compared to simulated networks or gold standard networks. K in the simulated datasets
711 corresponded to the size of the simulated networks. For the real datasets, we considered top 500, 1000,
712 2000 edges. We obtained the top K edges after filtering the inferred networks based on the TFs and
713 targets in the gold standard networks.

714 **Examining network dynamics on cell lineages**

715 We used several global and subnetwork-level methods to examine how regulatory networks change on a
716 cell lineage. These include F-score based comparison of all pairs of networks on the lineage, k-means
717 based edge clustering and Latent Dirichlet Allocation (LDA).

718 **F-score based analysis of inferred network change along cell lineage tree**

719 To examine the overall conservation and divergence between the inferred cell type-specific networks
720 along the cell lineage tree, we computed F-score on the predicted networks between each pair of cell
721 types and applied hierarchical clustering on the inferred networks based on the F-score. To compute
722 F-score, we selected top X edges ranked by confidence score to obtain a reliable network for each cell
723 type, where X was close to the median of the number of 80% confident edges across all cell types. This
724 was 4k in the mouse reprogramming dataset and 5k in the hematopoietic differentiation dataset. We
725 visualized the dendrogram obtained from the hierarchical clustering and compared this to the original
726 cell lineage tree.

727 **k-means based edge clustering**

728 For each cell cluster, we selected top K edges, where K was close to the median number of edges with at
729 least 80% confidence across all cell types. This corresponded to 4k edges for the mouse reprogramming
730 dataset and 5k edges for the hematopoietic differentiation dataset. We merged the confidence score of
731 each edge across all cell types as an edge by cell type matrix, each entry corresponding to the edge
732 confidence and with as many edges as in the union of top K edges from any cell type. We applied
733 k-means clustering on this matrix to find subnetworks with different patterns of conservation. We tried a
734 range of number of clusters and selected the one that has the highest silhouette coefficient.

735 **Latent Dirichlet Allocation (LDA) model for regulatory network rewiring**

736 We adopted Latent Dirichlet Allocation (LDA) to examine subnetwork level rewiring as shown in Top-
737 icNet³⁶. LDA was originally developed to cluster documents based on their word distributions. Each
738 document, i is assumed to have a certain composition of topics, as captured by a θ_i parameter and each
739 topic, k , is assumed to have a specific distribution of words as captured by a φ_k parameter. In the appli-
740 cation of LDA to a regulatory network, we first concatenated the TF by target network across cell types
741 to have as many rows as there are TFs times the number of cell types. Each TF in a cell type is treated as
742 a document and its targets are treated as words in the document. The topic distribution for all documents
743 constitutes a $M \times K$ matrix for document-topic distribution, where M is the total number of TFs in any
744 of the networks and K is the total number of topics. The distribution of words (genes) in each topic is
745 captured by $K \times V$ matrix for V genes. Each gene can be assigned to a topic based on its maximum
746 probability across topics. We applied LDA model to the 80% confidence networks of all cell clusters
747 inferred from scMTNI with 10 or 15 topics and found 10 topics to be suitable for both datasets. We ex-
748 tracted the subnetworks in each cell type associated with each topic by obtaining the induced graph for
749 the genes and regulators associated with each topic and visualized the giant components of each network
750 to identify change across cell clusters within the same topic.

751 For the mouse reprogramming dataset, we used the results of LDA application with 10 topics on
752 the 80% confidence networks of all cell clusters (**Supplementary Figure 5, 6, 7**). To interpret the
753 topics in each cell type, we tested the genes in the cell type-specific subnetwork for each topic for en-
754 richment of gene ontology (GO)⁶⁵ processes using a hypergeometric test with FDR correction. We used
755 an FDR <0.01 to determine significant enrichment (**Supplementary Figure 8**). For the hematopoiesis
756 dataset, we also used LDA results with 10 topics on the 80% confidence networks of all cell clusters
757 (**Supplementary Figure 13, 14, 15**) and used FDR <0.01 to determine significantly enriched terms
758 (**Supplementary Figure 16**).

759 **Data and code availability**

760 Pre-processed datasets are available at scMTNI Supplementary website at <https://github.com/Roy-lab/scMTNI>. The reprogramming scATAC-seq dataset has been deposited to Gene Expression
761 Omnibus (GEO). The scMTNI code and associated MATLAB, python and R scripts to compute various
762 validation metrics are available at <https://github.com/Roy-lab/scMTNI>.

763

Cell type	Number of edges
C1	202
C2	217
C3	239

Table 1. Statistics of the edges in each cell type for simulated networks.

Gold standards	Number of TFs	Number of targets
ChIP	54	31367
Perturb	179	21019
Perturb+ChIP	47	6109

Table 2. Statistics of the gold standard datasets in mouse ESC from ESCAPE³¹ and ENCODE³² databases and Nishiyama et al³³.

Gold standard	Number of tfs	Number of targets
Hematopoietic stem cells (HSC)	6	9173
CD14_monocytes	1	6523
megakaryocytes	4	8733
erythroid_progenitors	1	7955
R3R4_erythroid_cells	1	8494
megakaryocytes	4	8733
CD34_hematopoietic_stem_cells-derived_proerythroblasts	3	5847
T-cells	3	6189
B-cells	1	7036
GM_B-cells	48	10597
UniBind	56	10621
Cus_ChIP	149	6179
Cus_KO	50	6108
Cus_KO+Cus_ChIP	26	2124
Cus_KO+UniBind	12	2020

Table 3. The statistics of the gold standard datasets in human hematopoietic cell types from UniBind database⁴² and Cusanovich et al⁴³.

764

References

765

- [1] Constantinos Chronis, Petko Fiziev, Bernadett Papp, Stefan Butz, Giancarlo Bonora, Shan Sabri, Jason Ernst, and Kathrin Plath. Cooperative Binding of Transcription Factors Orchestrates Reprogramming. *Cell*, 168(3):442–459.e20, January 2017.
- [2] Zachary D Smith, Camille Sindhu, and Alexander Meissner. Molecular features of cellular reprogramming and development. *Nature Reviews Molecular Cell Biology*, 17(3):139–154, 2016.
- [3] Amos Tanay and Aviv Regev. Scaling single-cell genomics from phenomenology to mechanism. *Nature*, 541(7637):331–338, 2017.
- [4] Andrew McDavid, Raphael Gottardo, Noah Simon, and Mathias Drton. Graphical Models for Zero-Inflated Single Cell Gene Expression. *The Annals of Applied Statistics*, 13(2):848–873, June 2019. arXiv: 1610.05857.
- [5] Thalia E. Chan, Michael P. H. Stumpf, and Ann C. Babtie. Gene Regulatory Network Inference from Single-Cell Data Using Multivariate Information Measures. *Cell Systems*, 5(3):251–267.e3, September 2017.
- [6] Hirotaka Matsumoto, Hisanori Kiryu, Chikara Furusawa, Minoru S. H. Ko, Shigeru B. H. Ko, Norio Gouda, Tetsutaro Hayashi, and Itoshi Nikaido. SCODE: an efficient regulatory network inference algorithm from single-cell RNA-Seq during differentiation. *Bioinformatics*, 33(15):2314–2321, August 2017.
- [7] Chee Yee Lim, Huange Wang, Steven Woodhouse, Nir Piterman, Lorenz Wernisch, Jasmin Fisher, and Berthold Göttgens. BTR: training asynchronous Boolean models using single-cell expression data. *BMC Bioinformatics*, 17(1):355, September 2016.
- [8] Xiaojie Qiu, Arman Rahimzamani, Li Wang, Qi Mao, Timothy Durham, José L. McFaleine-Figueroa, Lauren Saunders, Cole Trapnell, and Sreeram Kannan. Towards inferring causal gene regulatory networks from single cell expression Measurements. *bioRxiv*, page 426981, September 2018.
- [9] Jukka Intosalmi, Henrik Mannerström, Saara Hiltunen, and Harri Lähdesmäki. SCHiRM: Single Cell Hierarchical Regression Model to detect dependencies in read count data. *bioRxiv*, page 335695, May 2018.

766

767

768

769

770

771

772

773

774

775

776

777

778

779

780

781

782

783

784

785

786

787

788

789

790

791

- 792 [10] Alicia T. Specht and Jun Li. LEAP: constructing gene co-expression networks for single-cell RNA-
793 sequencing data using pseudotime ordering. *Bioinformatics*, 33(5):764–766, March 2017.
- 794 [11] Sara Aibar, Carmen Bravo González-Blas, Thomas Moerman, Ván Anh Huynh-Thu, Hana Imri-
795 chova, Gert Hulselmans, Florian Rambow, Jean-Christophe Marine, Pierre Geurts, Jan Aerts, Joost
796 van den Oord, Zeynep Kalender Atak, Jasper Wouters, and Stein Aerts. SCENIC: single-cell reg-
797ulatory network inference and clustering. *Nature Methods*, 14(11):1083–1086, November 2017.
- 798 [12] Rong Zhang, Zhao Ren, and Wei Chen. SILGGM: An extensive R package for efficient statistical
799 inference in large-scale gene networks. *PLOS Computational Biology*, 14(8):e1006369, August
800 2018.
- 801 [13] Andrea Ocne, Laleh Haghverdi, Nikola S. Mueller, and Fabian J. Theis. Reconstructing gene
802 regulatory dynamics from high-dimensional single-cell snapshot data. *Bioinformatics*, 31(12):i89–
803 i96, June 2015.
- 804 [14] Chee Yee Lim, Huang Wang, Steven Woodhouse, Nir Piterman, Lorenz Wernisch, Jasmin Fisher,
805 and Berthold Göttgens. Btr: training asynchronous boolean models using single-cell expression
806 data. *BMC bioinformatics*, 17(1):1–18, 2016.
- 807 [15] Thalia E Chan, Michael PH Stumpf, and Ann C Babtie. Gene regulatory network inference from
808 single-cell data using multivariate information measures. *Cell systems*, 5(3):251–267, 2017.
- 809 [16] Hirotaka Matsumoto, Hisanori Kiryu, Chikara Furusawa, Minoru SH Ko, Shigeru BH Ko, Norio
810 Gouda, Tetsutaro Hayashi, and Itoshi Nikaido. Scode: an efficient regulatory network inference
811 algorithm from single-cell rna-seq during differentiation. *Bioinformatics*, 33(15):2314–2321, 2017.
- 812 [17] Aditya Pratapa, Amogh P Jalihal, Jeffrey N Law, Aditya Bharadwaj, and TM Murali. Benchmark-
813 ing algorithms for gene regulatory network inference from single-cell transcriptomic data. *Nature
814 methods*, 17(2):147–154, 2020.
- 815 [18] Matthew Stone, Sunnie Grace McCalla, Alireza Fotuhi Siahpirani, Viswesh Periyasamy, Junha
816 Shin, and Sushmita Roy. Identifying strengths and weaknesses of methods for computational net-
817 work inference from single cell RNA-seq data. preprint, Bioinformatics, June 2021.
- 818 [19] Camden Jansen, Ricardo N Ramirez, Nicole C El-Ali, David Gomez-Cabrero, Jesper Tegner,
819 Matthias Merkenschlager, Ana Conesa, and Ali Mortazavi. Building gene regulatory networks

- 820 from scatac-seq and scrna-seq using linked self organizing maps. *PLoS computational biology*,
821 15(11):e1006555, 2019.
- 822 [20] Wanwen Zeng, Xi Chen, Zhana Duren, Yong Wang, Rui Jiang, and Wing Hung Wong. Dc3 is a
823 method for deconvolution and coupled clustering from bulk and single-cell genomics data. *Nature
communications*, 10(1):1–11, 2019.
- 824
- 825 [21] David Heckerman, David Maxwell Chickering, Christopher Meek, Robert Rounthwaite, and Carl
826 Kadie. Dependency networks for inference, collaborative filtering, and data visualization. *Journal
of Machine Learning Research*, 1(Oct):49–75, 2000.
- 827
- 828 [22] Joshua D Welch, Velina Kozareva, Ashley Ferreira, Charles Vanderburg, Carly Martin, and Evan Z
829 Macosko. Single-cell multi-omic integration compares and contrasts features of brain cell identity.
830 *Cell*, 177(7):1873–1887, 2019.
- 831
- 832 [23] Lennart Kester and Alexander van Oudenaarden. Single-cell transcriptomics meets lineage tracing.
Cell stem cell, 23(2):166–179, 2018.
- 833
- 834 [24] Christopher Koch, Jay Konieczka, Toni Delorey, Ana Lyons, Amanda Socha, Kathleen Davis,
835 Sara A Knaack, Dawn Thompson, Erin K O’Shea, Aviv Regev, et al. Inference and evolutionary
836 analysis of genome-scale regulatory networks in large phylogenies. *Cell systems*, 4(5):543–558,
2017.
- 837
- 838 [25] Emma Pierson, Daphne Koller, Alexis Battle, Sara Mostafavi, GTEx Consortium, et al. Shar-
839 ing and specificity of co-expression networks across 35 human tissues. *PLoS Comput Biol*,
11(5):e1004220, 2015.
- 840
- 841 [26] Vladimir Jojic, Tal Shay, Katelyn Sylvia, Or Zuk, Xin Sun, Joonsoo Kang, Aviv Regev, and Daphne
842 Koller. Identification of transcriptional regulators in the mouse immune system. *Nature immunol-
ogy*, 14(6):633–643, 2013.
- 843
- 844 [27] Dayanne M Castro, Nicholas R De Veaux, Emily R Miraldi, and Richard Bonneau. Multi-study
845 inference of regulatory networks for more accurate models of gene regulation. *PLoS computational
biology*, 15(1):e1006591, 2019.
- 846
- 847 [28] Robert Tibshirani. Regression shrinkage and selection via the lasso. *Journal of the Royal Statistical
Society: Series B (Methodological)*, 58(1):267–288, 1996.

- 848 [29] Sara Aibar, Carmen Bravo González-Blas, Thomas Moerman, Hana Imrichova, Gert Hulselmans,
849 Florian Rambow, Jean-Christophe Marine, Pierre Geurts, Jan Aerts, Joost van den Oord, et al.
850 Scenic: single-cell regulatory network inference and clustering. *Nature methods*, 14(11):1083–
851 1086, 2017.
- 852 [30] Rupa Sridharan and Kathrin Plath. Illuminating the black box of reprogramming. *Cell Stem Cell*,
853 2(4):295–297, April 2008.
- 854 [31] Huilei Xu, Caroline Baroukh, Ruth Dannenfelser, Edward Y Chen, Christopher M Tan, Yan Kou,
855 Yujin E Kim, Ihor R Lemischka, and Avi Ma’ayan. Escape: database for integrating high-
856 content published data collected from human and mouse embryonic stem cells. *Database (Oxford)*,
857 2013:bat045, 2013.
- 858 [32] ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human
859 genome. *Nature*, 489(7414):57–74, 2012.
- 860 [33] Akira Nishiyama, Li Xin, Alexei A Sharov, Marshall Thomas, Gregory Mowrer, Emily Mey-
861 ers, Yulan Piao, Samir Mehta, Sarah Yee, Yuhki Nakatake, et al. Uncovering early response of
862 gene regulatory networks in escs by systematic induction of transcription factors. *Cell stem cell*,
863 5(4):420–433, 2009.
- 864 [34] Pei-Shan Hou, Ching-Yu Chuang, Cheng-Fu Kao, Shen-Ju Chou, Lee Stone, Hong-Nerng Ho,
865 Chung-Liang Chien, and Hung-Chih Kuo. LHX2 regulates the neural differentiation of human
866 embryonic stem cells via transcriptional modulation of PAX6 and CER1. *Nucleic Acids Research*,
867 41(16):7753–7770, September 2013.
- 868 [35] Aileen M. Smith, Fernando J. Calero-Nieto, Judith Schütte, Sarah Kinston, Richard T. Timms,
869 Nicola K. Wilson, Rebecca L. Hannah, Josette-Renee Landry, and Berthold Göttgens. Integra-
870 tion of Elf-4 into stem/progenitor and erythroid regulatory networks through locus-wide chromatin
871 studies coupled with in vivo functional validation. *Molecular and Cellular Biology*, 32(4):763–773,
872 February 2012.
- 873 [36] Shaoke Lou, Tianxiao Li, Xiangmeng Kong, Jing Zhang, Jason Liu, Donghoon Lee, and Mark
874 Gerstein. Topicnet: a framework for measuring transcriptional regulatory network change. *Bioin-
875 formatics*, 36(Supplement_1):i474–i481, 2020.

- 876 [37] L1 Yang, Y Han, F Saurez Saiz, and MD Minden. A tumor suppressor and oncogene: the wt1
877 story. *Leukemia*, 21(5):868–876, 2007.
- 878 [38] Yan Liu, Ruben Hoya-Arias, and Stephen D Nimer. The role of p53 in limiting somatic cell
879 reprogramming. *Cell Research*, 19(11):1227–1228, 2009.
- 880 [39] Yang Zhao, Xiaolei Yin, Han Qin, Fangfang Zhu, Haisong Liu, Weifeng Yang, Qiang Zhang,
881 Chengang Xiang, Pingping Hou, Zhihua Song, et al. Two supporting factors greatly improve the
882 efficiency of human ipsc generation. *Cell stem cell*, 3(5):475–479, 2008.
- 883 [40] Kazutoshi Takahashi and Shinya Yamanaka. Induction of pluripotent stem cells from mouse em-
884 bryonic and adult fibroblast cultures by defined factors. *cell*, 126(4):663–676, 2006.
- 885 [41] Jason D Buenrostro, M Ryan Corces, Caleb A Lareau, Beijing Wu, Alicia N Schep, Martin J Aryee,
886 Ravindra Majeti, Howard Y Chang, and William J Greenleaf. Integrated single-cell analysis maps
887 the continuous regulatory landscape of human hematopoietic differentiation. *Cell*, 173(6):1535–
888 1548, 2018.
- 889 [42] Rafael Riudavets Puig, Paul Boddie, Aziz Khan, Jaime Abraham Castro-Mondragon, and Anthony
890 Mathelier. Unibind: maps of high-confidence direct tf-dna interactions across nine species. *bioRxiv*,
891 pages 2020–11, 2021.
- 892 [43] Darren A. Cusanovich, Bryan Pavlovic, Jonathan K. Pritchard, and Yoav Gilad. The functional
893 consequences of variation in transcription factor binding. *PLoS Genet*, 10(3):e1004226+, March
894 2014.
- 895 [44] Jasjeet Bhullar and Vincent E Sollars. Ybx1 expression and function in early hematopoiesis and
896 leukemic cells. *Immunogenetics*, 63(6):337–350, 2011.
- 897 [45] Byunghee Koh, Matthew M. Hufford, Duy Pham, Matthew R. Olson, Tong Wu, Rukhsana Jabeen,
898 Xin Sun, and Mark H. Kaplan. The ETS Family Transcription Factors Etv5 and PU.1 Function
899 in Parallel To Promote Th9 Cell Development. *Journal of Immunology (Baltimore, Md.: 1950)*,
900 197(6):2465–2472, September 2016.
- 901 [46] Laura G. Schuettpelz, Priya K. Gopalan, Felipe O. Giuste, Molly P. Romine, Ronald van Os, and
902 Daniel C. Link. Kruppel-like factor 7 overexpression suppresses hematopoietic stem and progenitor
903 cell function. *Blood*, 120(15):2981–2989, October 2012.

- 904 [47] R. C. Briggs, W. Y. Kao, L. L. Dworkin, J. A. Briggs, E. N. Dessimis, and J. Clark. Regulation
905 and specificity of MNDA expression in monocytes, macrophages, and leukemia/B lymphoma cell
906 lines. *Journal of Cellular Biochemistry*, 56(4):559–567, December 1994.
- 907 [48] Shuyang Yu, Kairong Cui, Raja Jothi, Dong-Mei Zhao, Xuefang Jing, Keji Zhao, and Hai-Hui
908 Xue. Gabp controls a critical transcription regulatory module that is essential for maintenance and
909 differentiation of hematopoietic stem/progenitor cells. *Blood, The Journal of the American Society*
910 of Hematology
- 911 [49] H Daniel Lacorazza, Takeshi Yamada, Yan Liu, Yasuhiko Miyata, Mariela Sivina, Juliana Nunes,
912 and Stephen D Nimer. The transcription factor mef/elf4 regulates the quiescence of primitive
913 hematopoietic cells. *Cancer cell*, 9(3):175–187, 2006.
- 914 [50] Christina Alidousty, Thomas Rauen, Lydia Hanssen, Qiang Wang, Setareh Alampour-Rajabi,
915 Peter R Mertens, Jürgen Bernhagen, Jürgen Floege, Tammo Ostendorf, and Ute Raffetseder.
916 Calcineurin-mediated yb-1 dephosphorylation regulates ccl5 expression during monocyte differ-
917 entiation. *Journal of Biological Chemistry*, 289(31):21401–21412, 2014.
- 918 [51] Zenobio Viana de Barros, Stefano Bruscoli, Sara Flaminii, Tiziana Frammartino, Andrea Gagliardi,
919 Graziella Migliorati, Carlo Riccardi, and Oxana Bereshchenko. 3142–glucocorticoid-induced
920 leucine zipper (gilz) intrinsically regulates hematopoietic stem cell function. *Experimental Hema-*
921 *tology*, 88:S82, 2020.
- 922 [52] M Dolores Delgado and Javier León. Myc roles in hematopoiesis and leukemia. *Genes & cancer*,
923 1(6):605–616, 2010.
- 924 [53] Louis C Doré and John D Crispino. Transcription factor networks in erythroid cell and megakary-
925 ocyte development. *Blood, The Journal of the American Society of Hematology*, 118(2):231–239,
926 2011.
- 927 [54] Miroslawa Siatecka and James J Bieker. The multifunctional role of eklf/klf1 during erythropoiesis.
928 *Blood, The Journal of the American Society of Hematology*, 118(8):2044–2054, 2011.
- 929 [55] Cuiping Zhang, Yvonne N. Fondufe-Mittendorf, Chi Wang, Jin Chen, Qiang Cheng, Daohong
930 Zhou, Yi Zheng, Hartmut Geiger, and Ying Liang. Latexin regulation by HMGB2 is required for
931 hematopoietic stem cell maintenance. *Haematologica*, 105(3):573–584, March 2020.

- 932 [56] Emily R. Miraldi, Maria Pokrovskii, Aaron Waters, Dayanne M. Castro, Nick De Veaux, Jason
933 Hall, June-Yong Lee, Maria Ciofani, Aviv Madar, Nick Carriero, and et al. Leveraging chromatin
934 accessibility for transcriptional regulatory network inference in t helper 17 cells. *Genome Research*,
935 page gr.238253.118, Jan 2019.
- 936 [57] Sushmita Roy, Stephen Lagree, Zhonggang Hou, James A. Thomson, Ron Stewart, and Audrey P.
937 Gasch. Integrated module and Gene-Specific regulatory inference implicates upstream signaling
938 networks. *PLoS Comput. Biol.*, 9(10):e1003252+, October 2013.
- 939 [58] Jean Fan, Neeraj Salathia, Rui Liu, Gwendolyn E Kaeser, Yun C Yung, Joseph L Herman, Fiona
940 Kaper, Jian-Bing Fan, Kun Zhang, Jerold Chun, et al. Characterizing transcriptional heterogeneity
941 through pathway and gene set overdispersion analysis. *Nature methods*, 13(3):241–244, 2016.
- 942 [59] Yong Zhang, Tao Liu, Clifford A Meyer, Jérôme Eeckhoute, David S Johnson, Bradley E Bernstein,
943 Chad Nusbaum, Richard M Myers, Myles Brown, Wei Li, et al. Model-based analysis of chip-seq
944 (macs). *Genome biology*, 9(9):1–9, 2008.
- 945 [60] Matthew T. Weirauch, Ally Yang, Mihai Albu, Atina G. Cote, Alejandro Montenegro-Montero,
946 Philipp Drewe, Hamed S. Najafabadi, Samuel A. Lambert, Ishminder Mann, Kate Cook, Hong
947 Zheng, Alejandra Goity, Harm van Bakel, Jean-Claude Lozano, Mary Galli, Mathew G. Lewsey,
948 Eryong Huang, Tuhin Mukherjee, Xiaoting Chen, John S. Reece-Hoyes, Sridhar Govindarajan,
949 Gad Shaulsky, Albertha J M. Walhout, François-Yves Bouget, Gunnar Ratsch, Luis F. Larrondo,
950 Joseph R. Ecker, and Timothy R. Hughes. Determination and inference of eukaryotic transcription
951 factor sequence specificity. *Cell*, 158(6):1431–1443, Sep 2014.
- 952 [61] Richard I Sherwood, Tatsunori Hashimoto, Charles W O'Donnell, Sophia Lewis, Amira A Barkal,
953 John Peter van Hoff, Vivek Karun, Tommi Jaakkola, and David K Gifford. Discovery of directional
954 and nondirectional pioneer transcription factors by modeling dnase profile magnitude and shape.
955 *Nature biotechnology*, 32:171–178, February 2014.
- 956 [62] Khoa A Tran, Stefan J Pietrzak, Nur Zafirah Zaidan, Alireza Fotuhi Siahpirani, Sunnie Grace
957 McCalla, Amber S Zhou, Gopal Iyer, Sushmita Roy, and Rupa Sridharan. Defining reprogramming
958 checkpoints from single-cell analyses of induced pluripotency. *Cell reports*, 27(6):1726–1741,
959 2019.
- 960 [63] Thomas Moerman, Sara Aibar Santos, Carmen Bravo González-Blas, Jaak Simm, Yves Moreau,

- 961 Jan Aerts, and Stein Aerts. Grnboost2 and arboreto: efficient and scalable inference of gene regu-
962 latory networks. *Bioinformatics*, 35(12):2159–2161, 2019.
- 963 [64] Jesse Davis and Mark Goadrich. The relationship between precision-recall and roc curves. In
964 *Proceedings of the 23rd international conference on Machine learning*, pages 233–240, 2006.
- 965 [65] Michael Ashburner, Catherine A Ball, Judith A Blake, David Botstein, Heather Butler, J Michael
966 Cherry, Allan P Davis, Kara Dolinski, Selina S Dwight, Janan T Eppig, et al. Gene ontology: tool
967 for the unification of biology. *Nature genetics*, 25(1):25–29, 2000.
- 968 [66] The ENCODE Project Consortium. Identification and analysis of functional elements in 1% of the
969 human genome by the ENCODE pilot project. *Nature*, 447(7146):799–816, 2007.

970 **Acknowledgements**

971 We thank the Center for High Throughput Computing at University of Wisconsin-Madison for computa-
972 tional resources. This work is supported by the National Institutes of Health NIGMS grant 1R01GM117339.

973 **Author contributions**

974 S.Z. and S.R. designed the scMTNI algorithm and experiments. S.Z. implemented the code and per-
975 formed most of the experiments. S.P. contributed towards creation of the gold standards and evaluating
976 selected algorithms. S.P. and R.S. generated the scATAC-seq data for the reprogramming experiments.
977 All authors contributed towards writing the manuscript.

978 **Competing Interests**

979 The authors declare no competing interests.

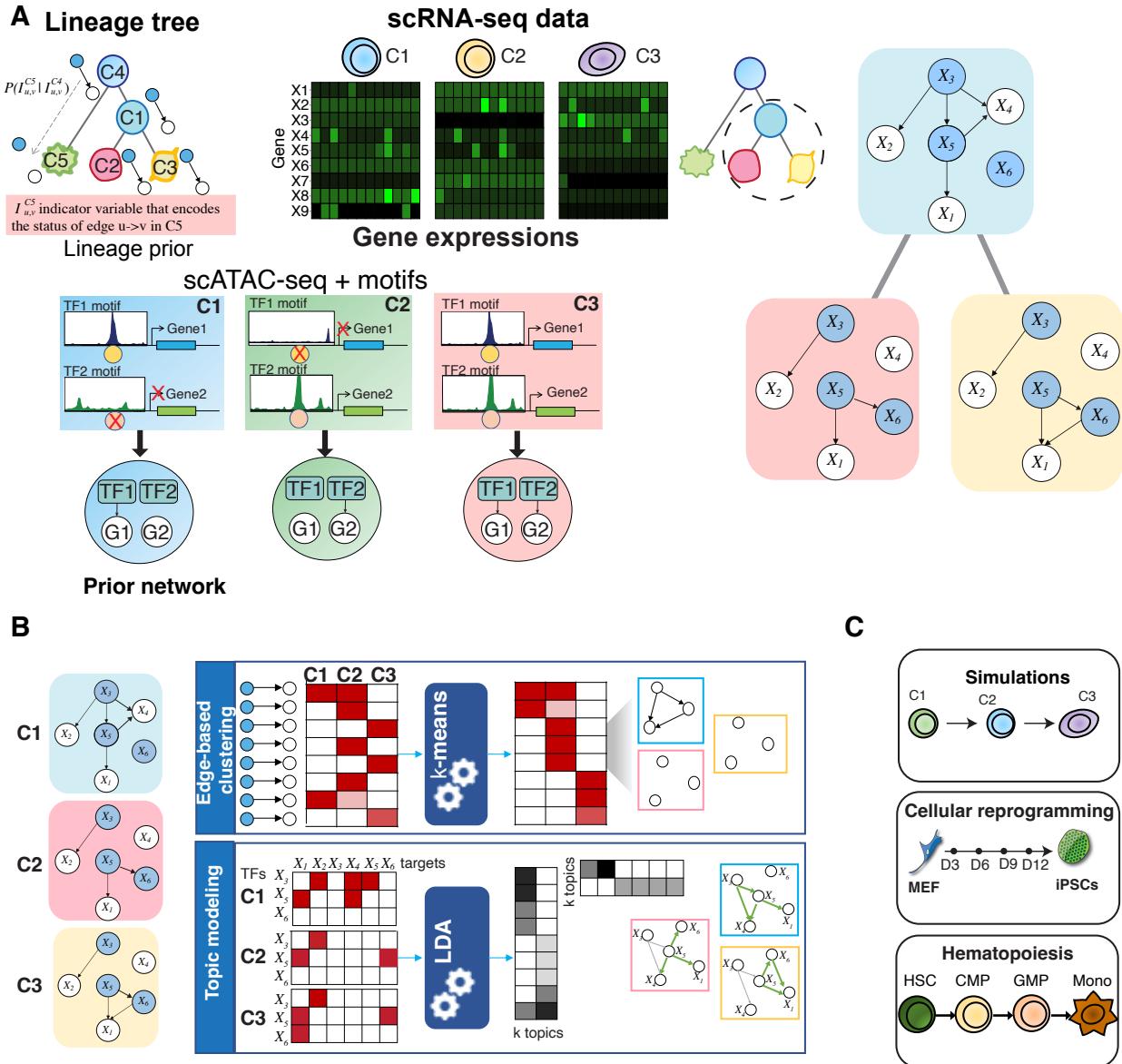


Figure 1. An overview of the scMTNI framework. **A.** scMTNI takes as input a cell lineage tree and cell type-specific scRNA-seq data and cell type-specific prior networks derived from single cell ATAC-seq datasets. If scATAC-seq data is not available, the same prior network can be used for all cell types. The output of scMTNI is a set of cell type-specific gene regulatory networks for each cell type on the cell lineage tree. **B.** The output networks of scMTNI are analyzed using two dynamic network analysis methods: edge-based k-means clustering and Latent Dirichlet Allocation (LDA) based topic models to identify key regulators and subnetworks associated with a particular cell cluster or a set of clusters on a branch. **C.** Datasets used with scMTNI. The simulation data comprised a linear trajectory of three cell types, while the two real datasets came from a reprogramming time-series process and immunophenotypic cell types identified during human hematopoietic differentiation.

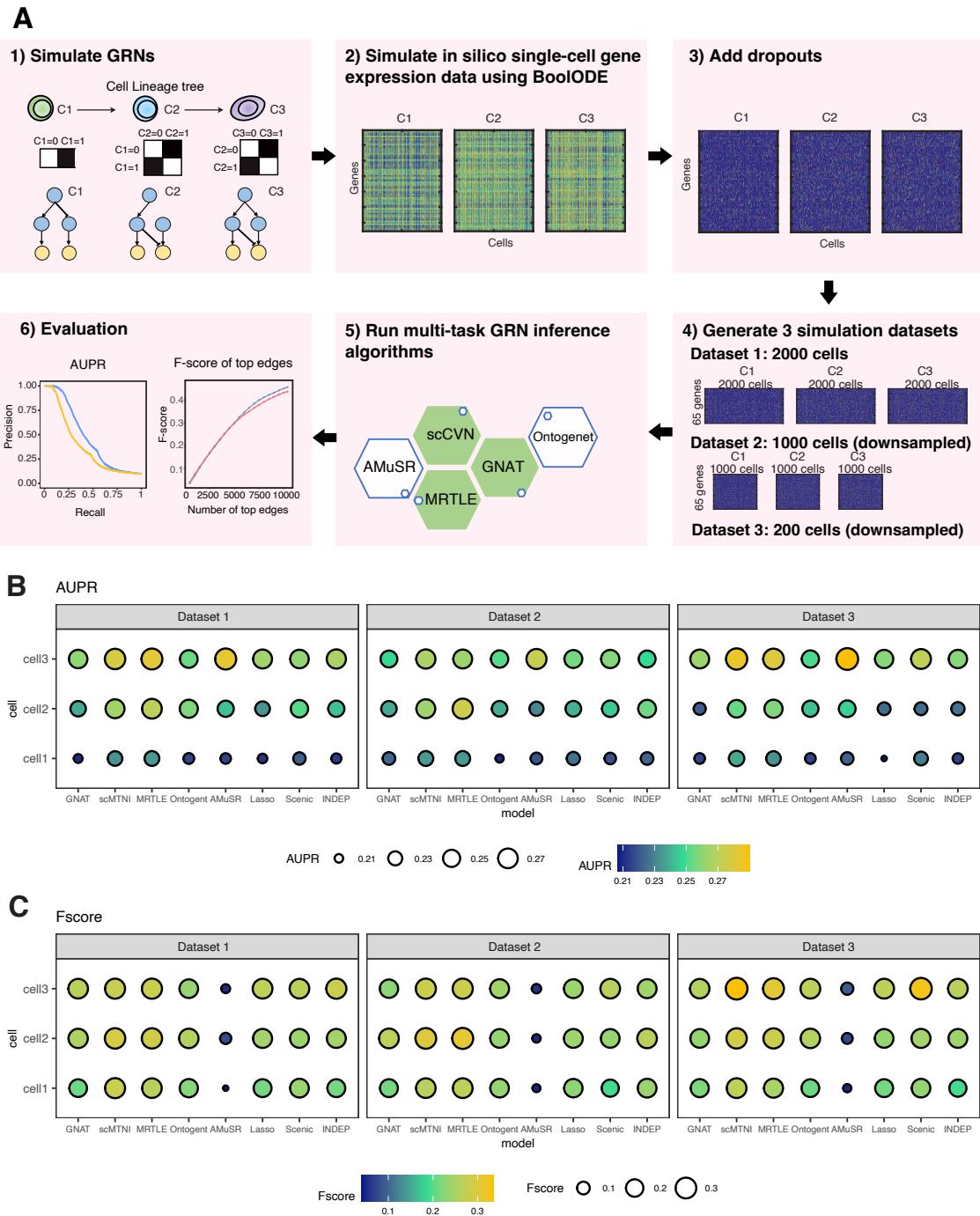


Figure 2. Benchmarking algorithms on simulated data. **A.** Simulation framework for scMTNI. We first simulate GRNs for cell types across a cell lineage tree. Next, we generate *in silico* single-cell gene expression data for each cell type using BoolODE using the simulated GRNs and add 80% zeros in the simulation data. Then, we apply five multi-task learning algorithms for GRN inference to the simulated datasets and predict networks in stability selection framework. We compare the performance of these algorithms based on area under precision and recall curve (AUPR) and F-score of top edges. **B.** AUPR comparing inferred networks to ground truth networks of simulated datasets 1, 2, 3. **C.** F-score comparing top K edges in the inferred networks to those in the ground truth networks of simulated datasets 1, 2, 3. The brighter and larger the circle the better the performance of the algorithm.

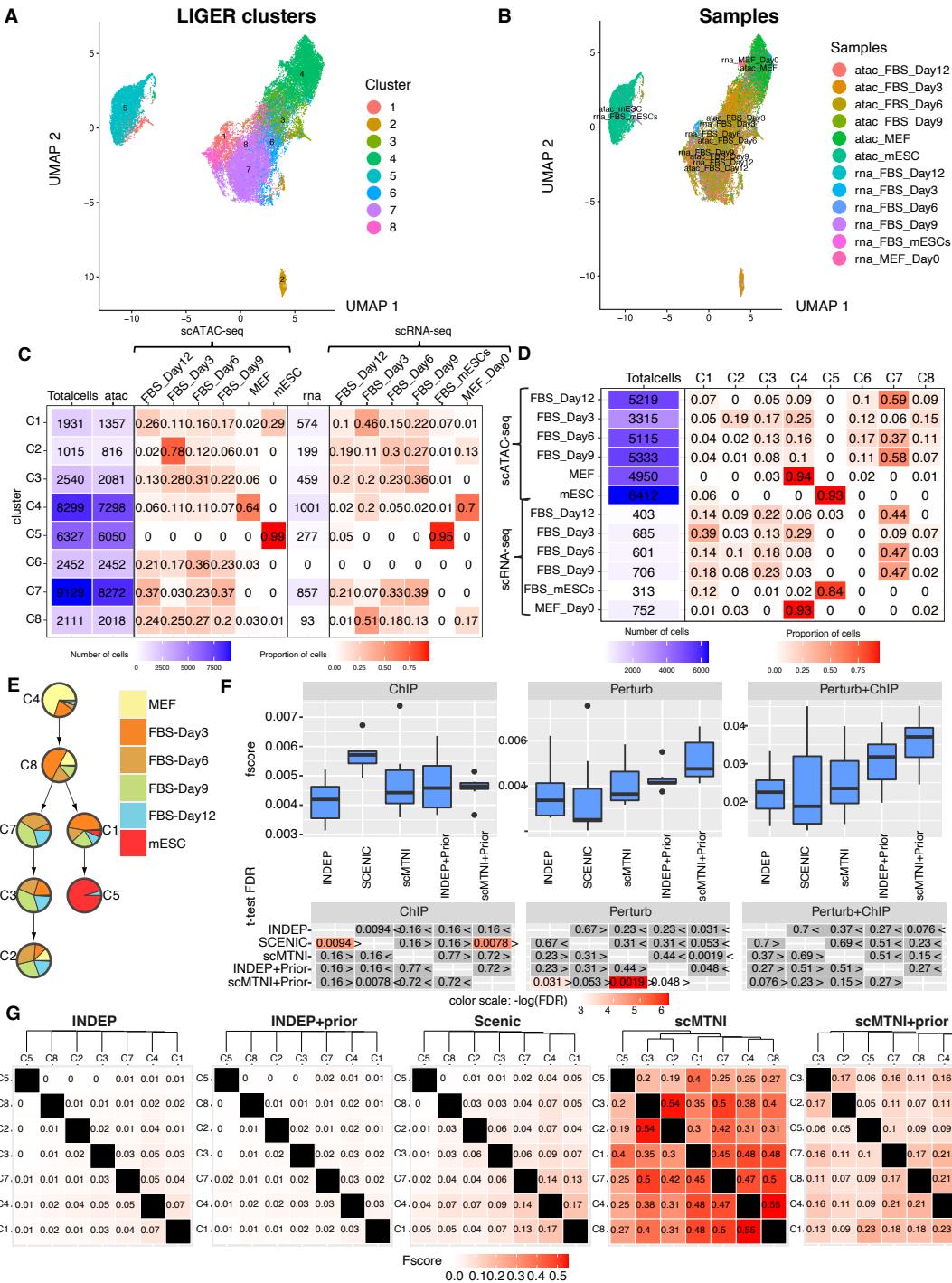


Figure 3. Inference of cell-type specific networks of mouse reprogramming data. **A.** UMAP of LIGER cell clusters on the scATAC-seq data and scRNA-seq data. **B.** UMAP depicting the sample labels of the scATAC-seq and scRNA-seq data. **C.** The distribution of LIGER clusters in each sample. **D.** The distribution of samples for each LIGER cluster. **E.** Inferred lineage structure for scMTNI linking the 7 cell clusters with scRNA-seq measurements. **F.** F-score of top 1k edges in predicted networks of scMTNI, scMTNI+prior, INDEP, INDEP+prior, and SCENIC compared to three gold standard datasets: ChIP, Perturb and Perturb+ChIP. The top boxplots show the F-scores, while the bottom heatmaps show FDR corrected T-test comparing the F-scores of the row algorithm to that of the column algorithm. A FDR<0.05 was considered significantly better. The sign < or > specifies whether the row algorithm's F-scores were worse or better than the column algorithm's F-scores. The color scale is specified for $-\log(FDR)$, with the red color proportional to significance. **G.** Pairwise similarity of networks from each cell cluster using F-score on the top 4k edges. Rows and columns are ordered based on the dendrogram created using the F-score similarity.

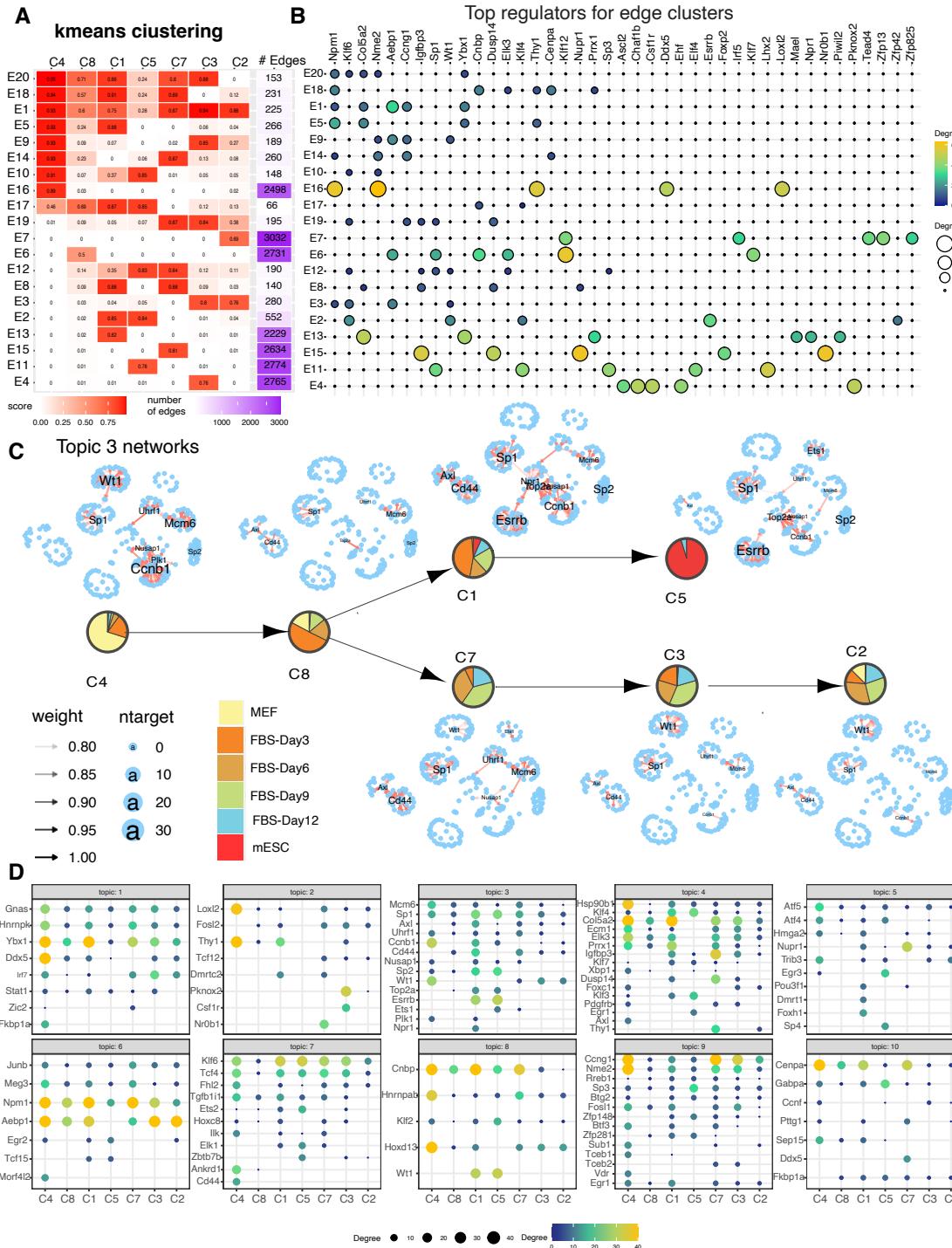


Figure 4. Network dynamics analysis of GRNs from cellular reprogramming. **A.** Kmeans clustering analysis of inferred networks. Shown are the mean profiles of edge confidence of 20 edge clusters. Each row corresponds to an edge cluster and each column corresponds to a cell cluster. The red intensity corresponds to the average confidence of edges in that cluster. Shown also are the number of edges in the edge cluster. **B.** Top regulators for each edge cluster. Shown are only regulators that have at least 10 targets in any edge cluster. The size and brightness of the circle is proportional to the number of targets. **C.** LDA topic 3 networks along the cell lineage. The layout of each network is the same, edges present in a particular cell cluster are shown in red. Labeled nodes correspond to regulators with the largest number of connections **D.** Cell cluster-specific regulators for each topic. The brighter and larger the circle, the greater are the number of targets for the regulator.

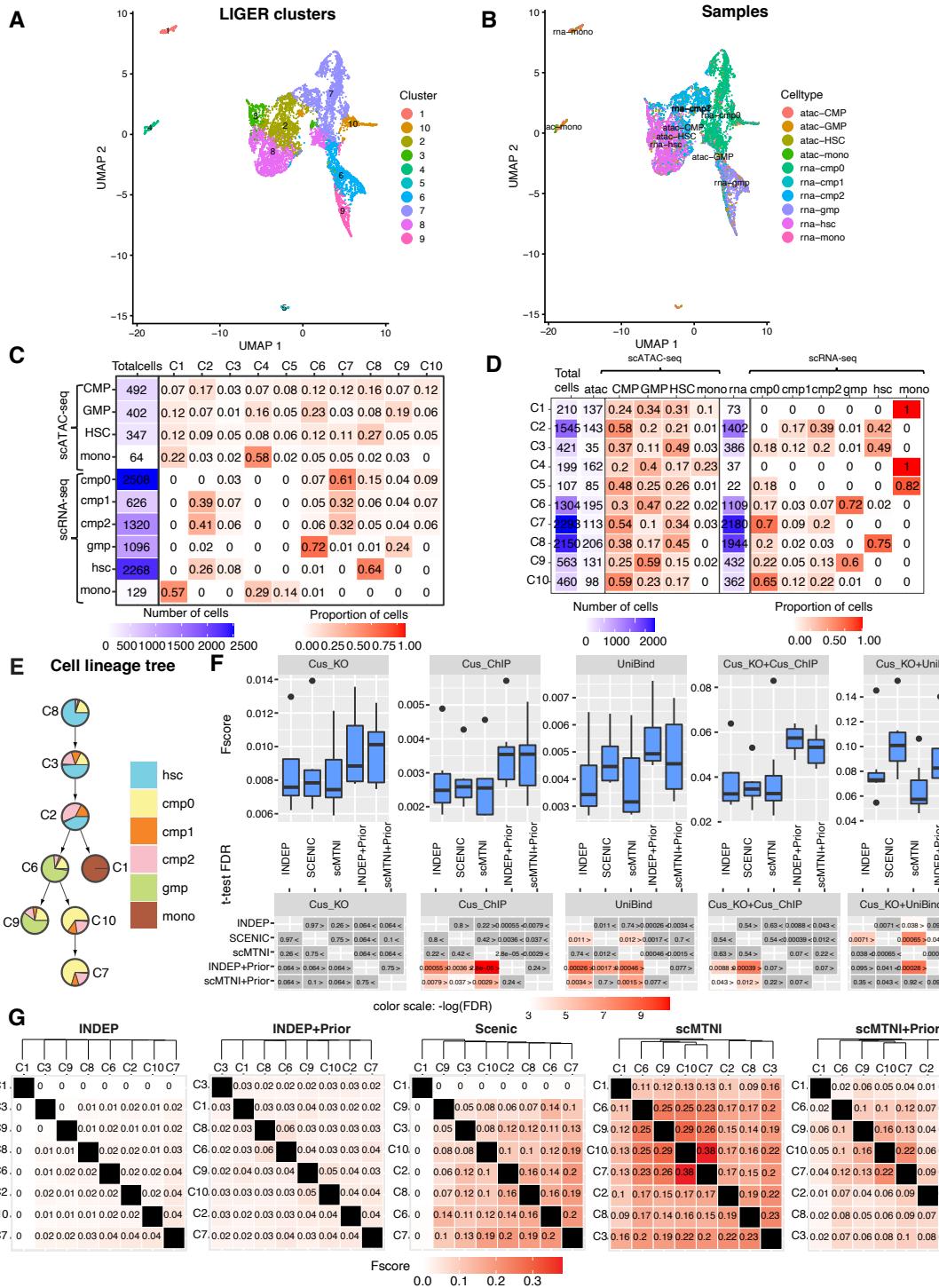


Figure 5. scMTNI networks on human hematopoietic differentiation data. **A.** UMAP of LIGER cell clusters of the scATAC-seq and scRNA-seq data. **B.** UMAP depicting the original cell types (samples) with scATAC-seq and scRNA-seq data. **C.** The distribution of samples for each LIGER cluster. **D.** The distribution of cell clusters for each sample. **E.** Inferred lineage structure linking the eight cell clusters with scRNA-seq and scATAC-seq data. **F.** Boxplots showing F-score of top 1k edges in predicted networks from scMTNI, scMTNI+prior, INDEP, INDEP+prior, and SCENIC compared to gold standard datasets (top). FDR-corrected T-test to compare the F-score of the row algorithm to the F-score of the column algorithm (bottom). A FDR<0.05 was considered significantly better. The sign < or > specifies whether the row algorithm's F-scores were worse or better than the column algorithm's F-scores. The color scale is specified for $-\log(FDR)$, with the red color proportional to significance. **G.** Pairwise similarity of networks from each cell cluster using F-score on the top 5k edges which corresponds to a confidence of ~0.8. Rows and columns ordered by hierarchical clustering using F-score as the similarity measure.

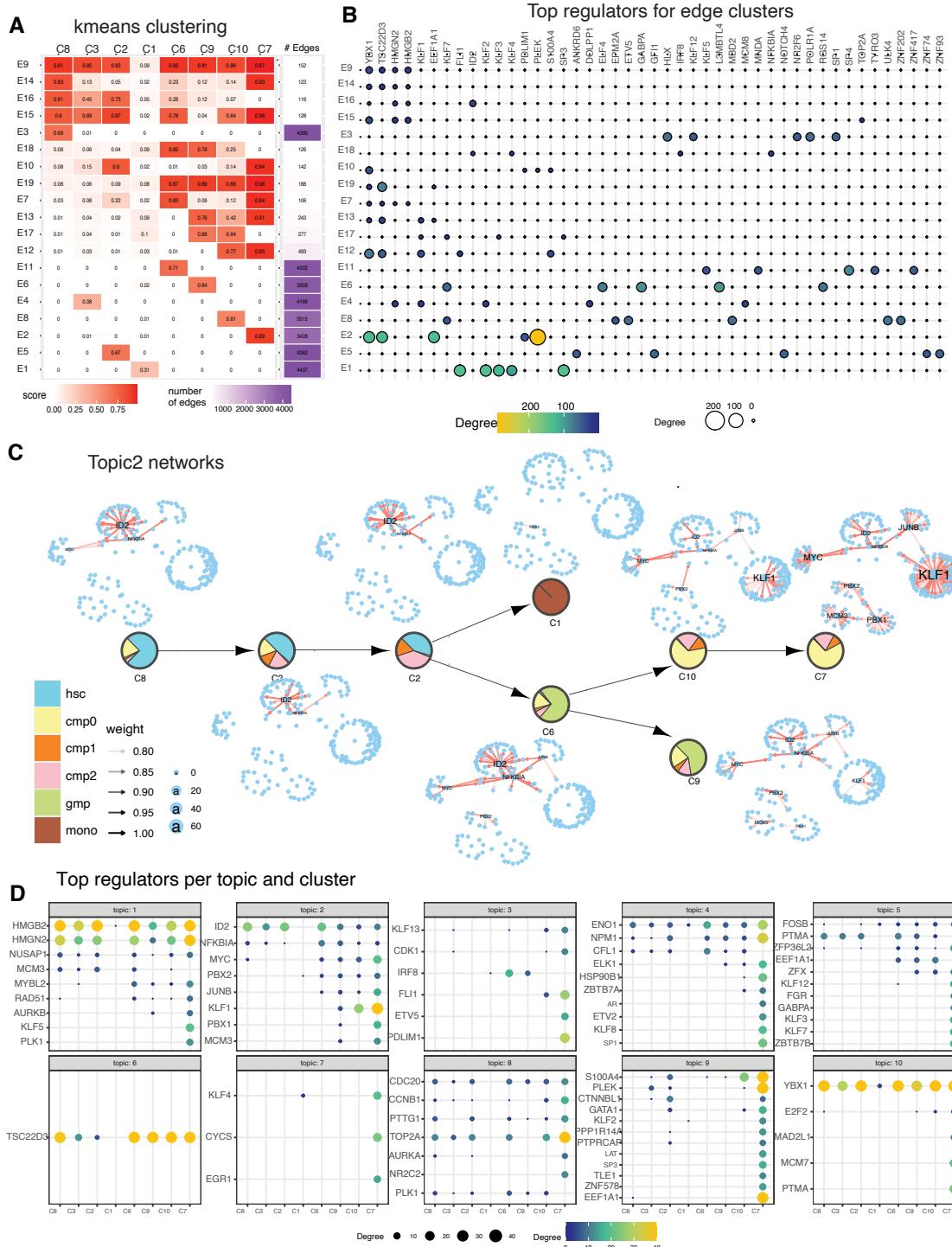


Figure 6. Network rewiring during hematopoietic differentiation. **A.** Kmeans-based edge clusters of the top 5k edges (rows) across 8 cell clusters (columns). The edge confidence matrix was clustered into 19 clusters to identify common and divergent networks. The red intensity corresponds to the average confidence of edges in that cluster. Shown also are the number of edges in the edge cluster. **B.** Top regulators of each edge cluster. Shown are only regulators with at least 10 targets in a given edge cluster. The size and brightness of the circle is proportional to the number of targets. **C.** Topic-specific networks across each cell cluster for topic 4. The layout of each network is the same, edges present in a particular cell cluster are shown in red. Labeled nodes correspond to regulators with the largest number of connections. **D.** Regulators associated with each cell cluster's network in each topic. The brighter and larger the circle, the greater are the number of targets for the regulator.