

Section S1: performance evaluation metrics

To evaluate the performance of different methods in inferring gene regulatory networks (GRNs), we choose area under the receiver operating characteristic curve (AUROC) and area under the precision-recall curve (AUPRC) as metrics. AUROC represents the area under the curve of true positive rate against false positive rate, while AUPRC denotes the area under the curve of precision against recall. Higher AUROC and AUPRC scores indicate better performance of the corresponding methods and more accurate and reliable inferred GRNs.

$$\text{FPR} = \frac{\text{FP}}{\text{FP} + \text{TN}}, \quad (\text{S1})$$

$$\text{TPR} = \frac{\text{TP}}{\text{TP} + \text{FN}}, \quad (\text{S2})$$

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}}, \quad (\text{S3})$$

$$\text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}}, \quad (\text{S4})$$

$$\text{AUROC} = \int_0^1 \text{TPR}(\text{FPR}) \cdot d\text{FPR}, \quad (\text{S5})$$

$$\text{AUPRC} = \int_0^1 \text{Precision}(\text{Recall}) \cdot d\text{Recall}, \quad (\text{S6})$$

where TP denotes the number of true regulatory edges predicted to be positive, FN denotes the number of true regulatory edges predicted to be negative, FP denotes the number of false regulatory edges predicted to be positive, and TN denotes the number of false regulatory edges predicted to be negative.

Section S2: summary of supplementary tables

Table S1 Performance comparison between scGRAIL and its six variants under the AUROC metric on a TF500+ scale Specific class real network dataset

Variant/ dataset	TF500+						
	hESC	hHEP	mDC	mESC	mHSC-E	mHSC-GM	mHSC-L
w/o N	0.866	0.889	0.712	0.908	0.906	0.909	0.820
w/o S	0.864	0.868	0.707	0.902	0.906	0.899	0.857
w/ M	0.855	0.895	0.717	0.892	0.918	0.924	0.864
w/ G	0.867	0.897	0.705	0.902	0.916	0.921	0.863
w/ A	0.870	0.898	0.713	0.902	0.917	0.926	0.869
w/ V	0.859	0.891	0.704	0.917	0.915	0.924	0.864
scGRAIL	0.876	0.902	0.722	0.919	0.925	0.929	0.872

hESC: human embryonic stem cell; hHEP: human hepatocyte; mDC: mouse dendritic cell; mESC: mouse embryonic stem cell; mHSC-E: mouse hematopoietic stem cell-erythroid; mHSC-GM: mouse hematopoietic stem cell-granulocyte/monocyte; mHSC-L: mouse hematopoietic stem cell-lymphoid.

Table S2 AUROC of scGRAIL on Non-Specific and STRING real networks using TF500+ and TF1000+ gene scale datasets, under varying batch_size and hidden_dim configurations

Dataset/AUROC	batch_size				hidden_dim			
	512	256	128	64	[512,256]	[256,128]	[128,64]	[64,32]
Non-Specific 500+	0.738	0.770	0.742	0.746	0.727	0.736	0.770	0.737
Non-Specific 1000+	0.730	0.758	0.730	0.758	0.732	0.741	0.758	0.721
STRING 500+	0.862	0.880	0.717	0.872	0.866	0.873	0.880	0.871
STRING 1000+	0.885	0.893	0.838	0.881	0.868	0.885	0.893	0.885

STRING: search tool for the retrieval of interacting genes/proteins; AUROC: area under the receiver operating characteristic curve.

Table S3 Statistical results of the average running time of our scGRAIL versus six existing methods on four classes of real networks and different gene-size datasets

Item	Average running time (s)			
	Lofgof	Non-Specific	Specific	STRING
scGRAIL	7.79	3.88	28.67	5.84
scMGATGRN	75.76	76.31	367.57	100.04
GENELink	44.70	27.42	204.62	34.12
GCLink	29.50	19.61	140.28	24.52
DeepRIG	104.12	89.24	107.30	122.92
DeepSEM	107.27	120.53	116.12	118.75
DGRNS	332.07	257.63	1515.18	381.21

STRING: search tool for the retrieval of interacting genes/proteins.

Table S4 Performance comparison (AUROC and AUPRC) of scGRAIL on the Specific network datasets of TF500+ and TF1000+ gene scales under unimputed and DeepImpute imputed statuses

Item	TF500+		TF1000+	
	Unimputed	Imputed	Unimputed	Imputed
Dataset/AUROC				
hESC	0.732	0.877	0.877	0.880
hHEP	0.731	0.902	0.903	0.904
mDC	0.816	0.816	0.755	0.770
mESC	0.806	0.920	0.927	0.927
mHSC-E	0.779	0.928	0.937	0.938
mHSC-GM	0.779	0.928	0.936	0.939
mHSC-L	0.748	0.869	0.891	0.895
Dataset/AUPRC				
hESC	0.877	0.880	0.615	0.620
hHEP	0.903	0.904	0.847	0.848
mDC	0.755	0.770	0.144	0.158
mESC	0.927	0.927	0.855	0.860
mHSC-E	0.937	0.938	0.952	0.952
mHSC-GM	0.936	0.939	0.946	0.950
mHSC-L	0.891	0.895	0.884	0.891

hESC: human embryonic stem cell; hHEP: human hepatocyte; mDC: mouse dendritic cell; mESC: mouse embryonic stem cell; mHSC-E: mouse hematopoietic stem cell-erythroid; mHSC-GM: mouse hematopoietic stem cell-granulocyte/monocyte; mHSC-L: mouse hematopoietic stem cell-lymphoid; AUROC: area under the receiver operating characteristic curve; AUPRC: area under the precision-recall curve.

Table S5 Performance landscape of scGRAIL versus the other four models under different proportions of noise interference on three mouse datasets (mHSC-E, mHSC-GM, and mHSC-L) at the TF500+ gene scale in the Specific-like real network

Dataset	Model	AUROC				
		0%	10%	20%	30%	40%
mHSC-E	scGRAIL	0.925	0.919	0.912	0.909	0.903
	GENELink	0.884	0.804	0.734	0.502	0.754
	GCLink	0.895	0.886	0.883	0.886	0.885
	GNNLink	0.880	0.796	0.794	0.829	0.823
	DeepRIG	0.742	0.729	0.699	0.667	0.665
mHSC-GM	scGRAIL	0.928	0.923	0.915	0.905	0.898
	GENELink	0.900	0.818	0.841	0.791	0.742
	GCLink	0.905	0.908	0.903	0.896	0.894
	GNNLink	0.890	0.876	0.872	0.869	0.876
	DeepRIG	0.636	0.700	0.643	0.672	0.666
mHSC-L	scGRAIL	0.872	0.859	0.856	0.855	0.850
	GENELink	0.841	0.817	0.775	0.784	0.773
	GCLink	0.828	0.842	0.838	0.838	0.837
	GNNLink	0.850	0.833	0.831	0.839	0.839
	DeepRIG	0.656	0.694	0.567	0.518	0.504

mHSC-E: mouse hematopoietic stem cell-erythroid; mHSC-GM: mouse hematopoietic stem cell-granulocyte/monocyte; mHSC-L: mouse hematopoietic stem cell-lymphoid.

Section S3: supplementary data for case studies

As shown in Fig. S1, among the top 200 high-confidence prediction edges for the TF1000+ gene scale, 183 are known regulatory relationships in the standard network, 1 exists in the STRING network, and 16 are prediction edges that have not yet been covered by existing databases. The regulatory role of these key TFs has been confirmed by several literatures and databases, which further supports the biological plausibility of the model predictions.

In addition, we analyzed the top 20 TF–target gene regulatory pairs with the highest prediction scores in the hESC dataset, as shown in Table S6, and all of these prediction edges have been validated in standard networks.

In summary, scGRAIL is not only capable of accurately restoring known gene regulatory relationships, but also has the ability to mine potential regulatory mechanisms, which provides a basis for researchers to screen subsequent experimental targets and lays the foundation for improving biological regulatory data.

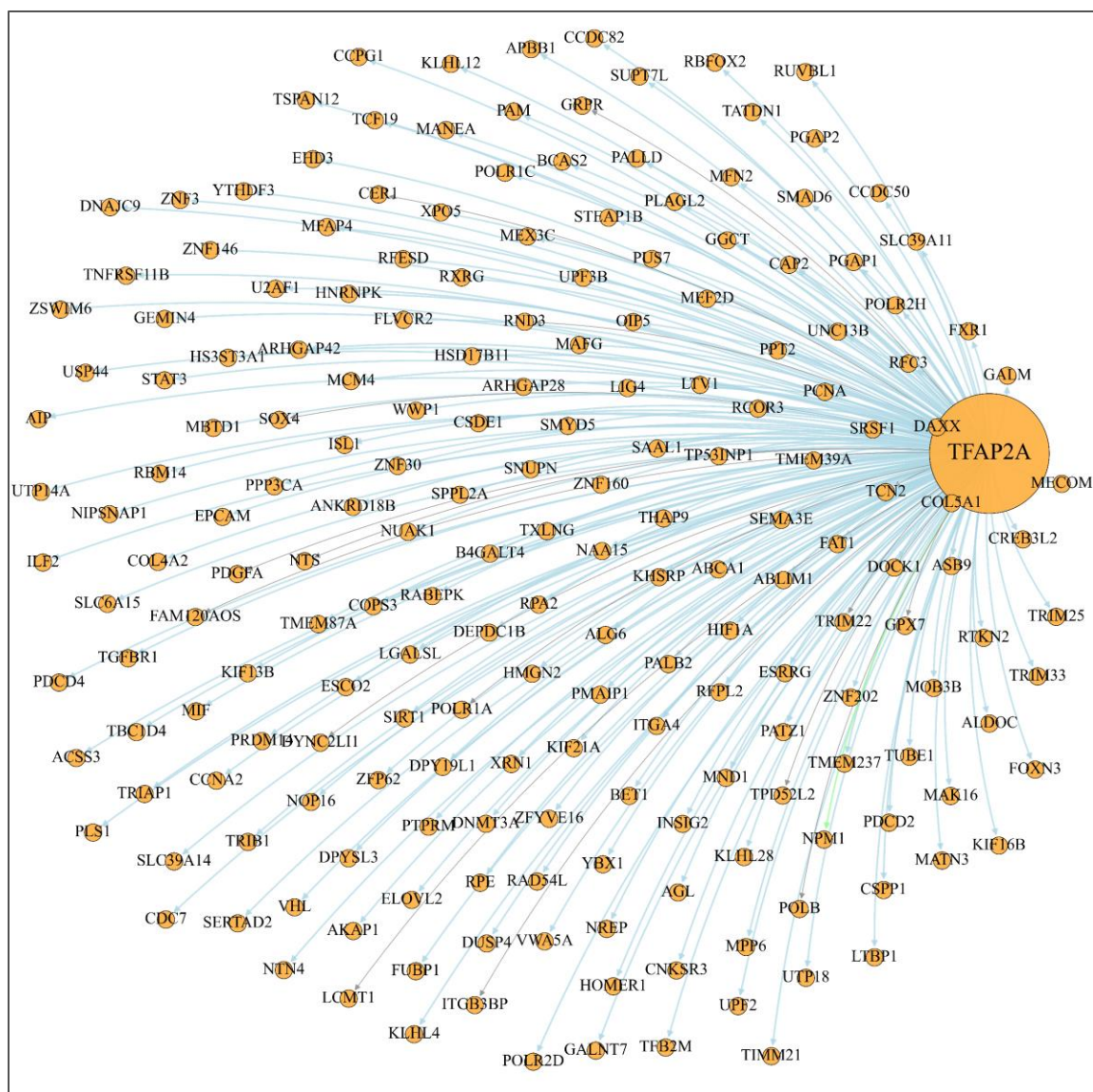


Table S6 Top 20 high-confidence TF–target gene pairs inferred by scGRAIL on the hESC datasets (TF500+ and TF1000+) of the Specific-like network (Diacou et al., 2022; Wang et al., 2024)

No.	Specific network			
	hESC-TF500+		hESC-TF1000+	
	TF	Target	TF	Target
1	TFAP2A	NUP54	TFAP2A	SLC39A11
2	TFAP2A	SALL2	TFAP2A	CDC7
3	TFAP2A	PHB2	TFAP2A	DNAJC9
4	TFAP2A	ZNF202	TFAP2A	RUVBL1
5	TFAP2A	EEF1A1	TFAP2A	TCF19
6	TFAP2A	ANKRD28	TFAP2A	NAA15
7	TFAP2A	DNMT3A	TFAP2A	SLC39A14
8	TFAP2A	RGS5	TFAP2A	TGFBR1
9	TFAP2A	FUBP1	TFAP2A	DNMT3A
10	TFAP2A	CDCA2	TFAP2A	INSIG2
11	TFAP2A	HNRNPD	TFAP2A	ZFP62
12	TFAP2A	ETV1	TFAP2A	KIF21A
13	TFAP2A	CSDE1	TFAP2A	POLR1C
14	TFAP2A	TCF4	TFAP2A	ILF2
15	TFAP2A	SNRNPB	TFAP2A	TFB2M
16	TFAP2A	SIRT1	TFAP2A	XPO5
17	TFAP2A	BCAR3	TFAP2A	RFC3
18	TFAP2A	FAM117B	TFAP2A	FUBP1
19	TFAP2A	SFPQ	TFAP2A	MAK16
20	TFAP2A	BRWD1	TFAP2A	PDCD4

hESC: human embryonic stem cell; TF: transcription factor; TFAP2A: transcription factor AP-2 alpha; NUP54: nucleoporin 54; SALL2: spalt like transcription factor 2; PHB2: prohibitin 2; ZNF202: zinc finger protein 202; EEF1A1: eukaryotic translation elongation factor 1 alpha 1; ANKRD28: ankyrin repeat domain 28; DNMT3A: DNA methyltransferase 3 alpha; RGS5: regulator of G protein signaling 5; FUBP1: far upstream element binding protein 1; CDCA2: cell division cycle associated 2; HNRNPD: heterogeneous nuclear ribonucleoprotein D; ETV1: ETS variant transcription factor 1; CSDE1: cold shock domain containing E1; TCF4: transcription factor 4; SNRNPB: small nuclear ribonucleoprotein polypeptides B and B1; SIRT1: sirtuin 1; BCAR3: breast cancer anti-estrogen resistance 3; FAM117B: family with sequence similarity 117 member B; SFPQ: splicing factor proline and glutamine rich; BRWD1: bromodomain and WD repeat domain containing 1; SLC39A11: solute carrier family 39 member 11; CDC7: cell division cycle 7; DNAJC9: DnaJ heat shock protein family (Hsp40) member C9; RUVBL1: RuvB like AAA ATPase 1; TCF19: transcription factor 19; NAA15: N-alpha-acetyltransferase 15; SLC39A14: solute carrier family 39 member 14; TGFBR1: transforming growth factor beta receptor 1; INSIG2: insulin induced gene 2; ZFP62: zinc finger protein 62; KIF21A: kinesin family member 21A; POLR1C: RNA polymerase I subunit C; ILF2: interleukin enhancer binding factor 2; TFB2M: transcription factor B2, mitochondrial; XPO5: exportin 5; RFC3: replication factor C subunit 3; MAK16: MAK16 homolog; PDCD4: programmed cell death 4.

References

- Diacou R, Nandigrami P, Fiser A, et al., 2022. Cell fate decisions, transcription factors and signaling during early retinal development. *Prog Retin Eye Res*, 91:101093.
<https://doi.org/10.1016/j.preteyeres.2022.101093>
- Wang DG, Gao J, Wang J, et al., 2024. TFAP2A drives non-small cell lung cancer (NSCLC) progression and resistance to targeted therapy by facilitating the ESR2-mediated MAPK pathway. *Cell Death Discov*, 10(1):491.
<https://doi.org/10.1038/s41420-024-02251-5>