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Supplemental Information

scTenifoldNet: A Machine Learning Workflow for Constructing and Comparing Transcriptome-wide Gene Regulatory Networks from Single-Cell Data

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Supplementary Data

Supplementary Tables

Table S1. Running time comparison between the gene regulatory network (GRN) construction algorithms.

We compared principal component regression (PCR), Spearman's correlation coefficient (SCC), mutual information (MI), and GENIE3 using 10-times microbenchmark evaluations. PCR implementation is 246 times faster than GENIE3. The test is done on the same workstation, PCR can construct an all-by-all GRN for 15,000 genes in less than 50 minutes, whereas GENIE3 requires more than 24 hours. Abbreviations: EXPR – defines the instruction used in R to perform the network construction; MIN – the minimum; LQ – the 25th percentile; MEAN – the average; MEDIAN – the 50th percentile; UQ – the 75th percentile; MAX – the maximum, time (in seconds) taken to compute the same network across 10 independent evaluations. Note: values are also available at https://github.com/cailab-tamu/scTenifoldNet/blob/master/inst/benchmarking/results/

EXPR	**MIN**	**LQ**	**MEAN**	**MEDIAN**	**UQ**	**MAX**
PCR(countMatrix)	0.820	0.847	0.929	0.887	0.985	1.233
SCC(countMatrix)	0.074	0.075	0.082	0.082	0.087	0.097
MI(countMatrix)	0.016	0.017	0.020	0.019	0.024	0.026
GENIE3(countMatrix)	220.670	225.088	226.736	226.714	229.138	232.545

Table S2. 56 differentially regulated genes in scTenifoldNet analysis of transcriptional responses of mouse neurons to acute morphine treatment

Identified 56 differentially regulated genes sorted according to the significance level based on the scTenifoldNet differential regulation test. Target genes of the RARB transcription factor (21 out of 56 genes) are highlighted in bold font. The full list (reporting 8,138 genes) is available for downloading at https://raw.githubusercontent.com/cailab-tamu/scTenifoldNet/master/inst/manuscript/results/sym10X500morphineNeuron_Itensor_Dalignment.csv

GENE	**DISTANCE**	**Z**	**FC**	**P-VALUE**	**FDR**
Ppp3ca	0.000505417	1.261436871	437.1982883	4.41E-97	3.59E-93
Hpca	0.000451657	1.252762791	349.1376011	6.53E-78	2.66E-74
Pcp4l1	0.000438705	1.250518947	329.4005652	1.30E-73	3.52E-70
Rgs7bp	0.00037183	1.237766693	236.6292622	2.14E-53	4.35E-50
Akap5	0.000369005	1.23717862	233.0464964	1.29E-52	2.10E-49
Slc24a2	0.000333128	1.229294319	189.9326813	3.29E-43	4.46E-40
Atp2a2	0.000326077	1.227645545	181.9778237	1.79E-41	2.08E-38
Atp2b1	0.000262899	1.211050974	118.2926978	1.50E-27	1.52E-24
Ppp1r1b	0.000237531	1.203234585	96.56518433	8.64E-23	7.81E-20
Foxp1	0.000235088	1.20243811	94.58854285	2.34E-22	1.91E-19
Spock3	0.000234686	1.20230651	94.26586162	2.76E-22	2.04E-19
Arpp19	0.000184148	1.183632617	58.03775404	2.57E-14	1.74E-11
Gpr88	0.000179385	1.181615521	55.07460401	1.16E-13	7.26E-11
Rgs9	0.000176012	1.180154126	53.02265609	3.30E-13	1.92E-10
Adcy5	0.00016515	1.175251138	46.68025395	8.36E-12	4.53E-09
Gnal	0.00014669	1.166129206	36.82790684	1.29E-09	6.56E-07
Ubb	0.000144813	1.165138306	35.89150024	2.09E-09	9.99E-07
Scn4b	0.000141973	1.163614607	34.4977928	4.27E-09	1.93E-06
Actb	0.000141033	1.163103413	34.04242857	5.39E-09	2.31E-06
Calm2	0.000140195	1.162644572	33.63881652	6.64E-09	2.70E-06
Penk	0.000137206	1.160986891	32.22008587	1.38E-08	5.33E-06
mt-Rnr1	0.000132469	1.158283381	30.03338755	4.25E-08	1.57E-05
Arpp21	0.000130444	1.157098489	29.12234279	6.79E-08	2.40E-05
Phactr1	0.000120406	1.150938919	24.81276515	6.32E-07	0.000214225
Cck	0.000119607	1.150426534	24.48437556	7.49E-07	0.000243863
Eif1	0.000117922	1.149335785	23.7996985	1.07E-06	0.000334595
mt-Nd1	0.000110779	1.144529614	21.00352911	4.58E-06	0.001381767
mt-Cytb	0.000110139	1.144083836	20.76143989	5.20E-06	0.001511921
Spred1	0.00010886	1.143185982	20.28227019	6.68E-06	0.00187502
mt-Nd2	0.000107265	1.142051021	19.69233806	9.10E-06	0.002467587
mt-Co1	0.00010359	1.139369985	18.36596353	1.82E-05	0.004785283
Hspa4l	0.000101727	1.137974627	17.71134934	2.57E-05	0.006385139
Nrn1	0.000101688	1.137945289	17.69783898	2.59E-05	0.006385139
Scn8a	0.000101237	1.137603657	17.54127063	2.81E-05	0.006729133
Chn1	0.000100641	1.13714947	17.33525951	3.13E-05	0.007285116
Diras2	9.86E-05	1.135567947	16.6365887	4.53E-05	0.010233366
Cpe	9.71E-05	1.134395301	16.13677372	5.89E-05	0.012961116
Ramp1	9.68E-05	1.134188236	16.05008839	6.17E-05	0.013211132
B3galt2	9.58E-05	1.133376704	15.71481014	7.36E-05	0.015042375
Chst15	9.58E-05	1.133358441	15.7073458	7.39E-05	0.015042375

Grin2b	9.47E-05	1.132461883	15.34524062	8.95E-05	0.017773833
Lamp1	9.38E-05	1.131764442	15.06933254	0.000103633	0.020080194
Rasd2	9.36E-05	1.131594715	15.00294167	0.000107344	0.020315423
Gabrg1	9.28E-05	1.130922747	14.74294867	0.000123207	0.022787777
Chst1	9.26E-05	1.130763107	14.68184644	0.000127266	0.023015378
3110035E14Rik	9.16E-05	1.129872801	14.34569311	0.000152127	0.026913304
Nfia	9.13E-05	1.129690493	14.27781314	0.000157713	0.027307825
Samd4	8.98E-05	1.128415938	13.81212882	0.000202028	0.034252132
Sacs	8.94E-05	1.128012573	13.66793622	0.000218148	0.036230303
Nrxn1	8.83E-05	1.127134063	13.35907405	0.000257175	0.041857844
D3Bwg0562e	8.78E-05	1.126626656	13.18386957	0.000282369	0.044568192
Akap9	8.77E-05	1.126580167	13.16793243	0.000284781	0.044568192
Rtn1	8.73E-05	1.126226571	13.04734237	0.000303715	0.04663459
Ahsa1	8.70E-05	1.125961129	12.95754223	0.000318635	0.04717659
D430041D05Rik	8.70E-05	1.12591797	12.94299968	0.00032112	0.04717659
Syn2	8.69E-05	1.125857388	12.92261423	0.000324636	0.04717659

Table S3. Summary of enriched functional annotations for 56 differentially regulated genes in scTenifoldNet analysis of transcriptional responses of mouse neurons to acute morphine treatment

Abbreviations: DB = Database reporting the gene set, TERM = Name of the gene set, P-VALUE: P-value result of the hypergeometric test, FDR = Adjusted P-value, ODDS RATIO = Odds ratio computed by the hypergeometric test. GENES = Differentially regulated genes included in the gene set. Full GSEA enrichment is available at https://raw.githubusercontent.com/cailab-tamu/scTenifoldNet/master/inst/manuscript/results/annotations/A_morphineNeurons.csv

DB	**TERM**	**PVALUE**	**FDR**	**ODDS RATIO**	**GENES**
KEGG	Parkinson disease	3.03E-06	0.0003	14.88	*Gnal, Ubb, Nd1, Cytb, Nd2, Adcy5*
KEGG	Dopaminergic synapse	2.08E-06	0.0003	15.87	*Ppp3ca, Gnal, Ppp1r1b, Grin2b, Calm2, Adcy5*
KEGG	Amphetamine addiction	1.31E-06	0.0004	26.26	*Ppp3ca, Ppp1r1b, Grin2b, Calm2, Adcy5*
BioPlanet	G-protein signaling pathways	2.62E-07	0.0004	22.56	*Ppp3ca, Gnal, Akap9, Akap5, Calm2, Adcy5*
KEGG	Cocaine addiction	9.78E-06	0.0007	29.76	*Ppp1r1b, Rgs9, Grin2b, Adcy5*
KEGG	cAMP signaling pathway	2.69E-05	0.0016	10.16	*Ppp1r1b, Atp2a2, Atp2b1, Grin2b, Calm2, Adcy5*
KEGG	Adrenergic signaling in cardiomyocytes	5.85E-05	0.0030	12.07	*Atp2a2, Atp2b1, Scn4b, Calm2, Adcy5*
BioPlanet	Myometrial relaxation and contraction pathways	4.64E-06	0.0035	13.82	*Atp2a2, Rgs9, Calm2, Ramp1, Actb, Adcy5*
KEGG	Alzheimer disease	1.29E-04	0.0049	10.20	*Ppp3ca, Atp2a2, Cytb, Grin2b, Calm2*
KEGG	cGMP-PKG signaling pathway	1.19E-04	0.0051	10.38	*Ppp3ca, Atp2a2, Atp2b1, Calm2, Adcy5*
KEGG	Calcium signaling pathway	1.85E-04	0.0062	9.45	*Ppp3ca, Gnal, Atp2a2, Atp2b1, Calm2*
KEGG	Pancreatic secretion	2.14E-04	0.0065	13.61	*Atp2a2, Cck, Atp2b1, Adcy5*
BioPlanet	Transmission across chemical synapses	1.49E-05	0.0075	11.28	*Gnal, Akap9, Akap5, Syn2, Grin2b, Adcy5*
BioPlanet	Nitric oxide signaling pathway	3.08E-05	0.0116	48.70	*Ppp3ca, Grin2b, Calm2*
KEGG	Thermogenesis	4.65E-04	0.0128	7.73	*Nd1, Cytb, Nd2, Actb, Adcy5*
BioPlanet	Calcium regulation in the cardiac cell	6.04E-05	0.0182	11.98	*Atp2a2, Atp2b1, Rgs9, Calm2, Adcy5*
BioPlanet	Opioid signaling	7.44E-05	0.0187	17.86	*Ppp3ca, Gnal, Ppp1r1b, Adcy5*
KEGG	Oxytocin signaling pathway	9.10E-04	0.0197	9.28	*Ppp3ca, Calm2, Actb, Adcy5*
KEGG	Long-term potentiation	8.77E-04	0.0204	15.99	*Ppp3ca, Grin2b, Calm2*
KEGG	Retrograde endocannabinoid signaling	8.25E-04	0.0208	9.52	*Nd1, Nd2, Gabrg1, Adcy5*
BioPlanet	Calcium signaling pathway	1.40E-04	0.0234	10.03	*Ppp3ca, Gnal, Atp2a2, Atp2b1, Calm2*
BioPlanet	Alzheimer’s disease	1.09E-04	0.0236	10.57	*Ppp3ca, Atp2a2, Cytb, Grin2b, Calm2*
KEGG	Gastric acid secretion	1.17E-03	0.0237	14.48	*Calm2, Actb, Adcy5*
KEGG	Renin secretion	1.26E-03	0.0240	14.10	*Ppp3ca, Calm2, Adcy5*
KEGG	Salivary secretion	1.36E-03	0.0243	13.74	*Atp2b1, Calm2, Adcy5*

Reactome	Neurotransmitter Receptor Binding And Downstream Transmission In The Postsynaptic Cell Homo sapiens R-HSA-112314	4.80E-05	0.0245	12.58	*Gnal, Akap9, Akap5, Grin2b, Adcy5*
BioPlanet	Neuronal system	1.36E-04	0.0258	7.57	*Gnal, Akap9, Akap5, Syn2, Grin2b, Adcy5*
Reactome	Cardiac conduction Homo sapiens R-HSA-5576891	3.77E-05	0.0288	13.23	*Scn8a, Akap9, Atp2a2, Atp2b1, Scn4b*
Reactome	Opioid Signalling Homo sapiens R-HSA-111885	7.81E-05	0.0299	17.64	*Ppp3ca, Gnal, Ppp1r1b, Adcy5*
KEGG	Dilated cardiomyopathy (DCM)	2.06E-03	0.0346	11.90	*Atp2a2, Actb, Adcy5*
KEGG	Alcoholism	2.33E-03	0.0372	7.18	*Ppp1r1b, Grin2b, Calm2, Adcy5*
KEGG	Rap1 signaling pathway	2.78E-03	0.0383	6.84	*Grin2b, Calm2, Actb, Adcy5*
KEGG	Aldosterone synthesis and secretion	2.93E-03	0.0387	10.50	*Atp2b1, Calm2, Adcy5*
KEGG	Circadian entrainment	2.70E-03	0.0389	10.82	*Grin2b, Calm2, Adcy5*
KEGG	Phototransduction	2.58E-03	0.0391	26.46	*Rgs9, Calm2*
Reactome	Transmission across Chemical Synapses Homo sapiens R-HSA-112315	2.69E-05	0.0411	10.16	*Gnal, Akap9, Akap5, Syn2, Grin2b, Adcy5*
Reactome	Muscle contraction Homo sapiens R-HSA-397014	2.19E-04	0.0478	9.11	*Scn8a, Akap9, Atp2a2, Atp2b1, Scn4b*

Table S4. 125 differentially regulated genes in scTenifoldNet analysis of transcriptional responses of a carcinoma (HNSCC) cell line to cetuximab.

Identified 125 differentially regulated genes sorted according to the significance level based on the scTenifoldNet differential regulation test. Target genes of the *SMAD2* and *SMAD3* transcription factors (39 out of 125 genes) are highlighted in bold font. The full list (reporting 11,140 genes) is available for downloading at https://raw.githubusercontent.com/cailab-tamu/scTenifoldNet/master/inst/manuscript/results/sym10x500SCC6_Itensor_Dalignment.csv

GENE	DISTANCE	Z	FC	P-VALUE	FDR
EEF1A1	0.000218237	1.299249543	149.2144722	2.57E-34	2.87E-30
HMGA1	0.000211927	1.293259249	140.7103801	1.86E-32	1.04E-28
EEF1D	0.000151671	1.225774559	72.07036265	2.08E-17	7.22E-14
ACTG1	0.00015121	1.225168134	71.63351925	2.59E-17	7.22E-14
PHLDA1	0.000146704	1.219138613	67.42708529	2.19E-16	4.87E-13
HBEGF	0.000128775	1.19330152	51.95353611	5.68E-13	1.06E-09
EEF1B2	0.000124669	1.186913746	48.69368397	2.99E-12	4.76E-09
COTL1	0.000123388	1.184879672	47.69781626	4.97E-12	6.92E-09
TNFRSF12A	0.000121793	1.182320275	46.4727078	9.29E-12	1.15E-08
DDIT3	0.000116961	1.174369662	42.85796136	5.89E-11	6.56E-08
MYC	0.000115498	1.171903518	41.79307541	1.01E-10	1.03E-07
FOSL1	0.000114923	1.170925666	41.37796006	1.25E-10	1.16E-07
PAWR	0.000111194	1.16447104	38.73619062	4.85E-10	4.16E-07
YBX1	0.00010905	1.160667676	37.25670524	1.04E-09	8.24E-07
CYTOR	0.000108661	1.159969197	36.99100606	1.19E-09	8.81E-07
EIF4A2	0.000104968	1.15322954	34.51963405	4.22E-09	2.94E-06
PTP4A1	0.000104387	1.152148564	34.1384092	5.13E-09	3.36E-06
IER2	0.000103444	1.150383391	33.5246257	7.04E-09	4.35E-06
MIR4435-2HG	0.000102664	1.148911568	33.02101632	9.12E-09	5.35E-06
AKAP12	0.000101109	1.145944397	32.02789264	1.52E-08	8.46E-06
SSFA2	9.99E-05	1.143584916	31.25884091	2.26E-08	1.20E-05
ERRFI1	9.77E-05	1.139256559	29.89422132	4.56E-08	2.31E-05
EEF2	9.64E-05	1.136618233	29.09087687	6.91E-08	3.34E-05
ITGA3	9.28E-05	1.129407286	27.00026396	2.03E-07	9.44E-05
SLC38A2	9.15E-05	1.126611132	26.229334	3.03E-07	0.000135098
LUC7L3	9.08E-05	1.125066298	25.81258025	3.76E-07	0.000161199
AHCY	9.01E-05	1.123678212	25.44357981	4.56E-07	0.00018794
GAPDH	8.91E-05	1.121519831	24.87992038	6.10E-07	0.000242751
PROSER2	8.83E-05	1.119773807	24.43280055	7.69E-07	0.000288103
RICTOR	8.83E-05	1.119711043	24.41687352	7.76E-07	0.000288103
MARCKSL1	8.62E-05	1.115121377	23.27901576	1.40E-06	0.00050353
DUSP4	8.61E-05	1.114831586	23.20891909	1.45E-06	0.000505362
MRPL51	8.60E-05	1.114594811	23.15179798	1.50E-06	0.000505362
HDGF	8.58E-05	1.114158241	23.04683302	1.58E-06	0.000518019
EREG	8.56E-05	1.113669821	22.92994702	1.68E-06	0.000534764
PIM1	8.49E-05	1.112242993	22.59176067	2.00E-06	0.000619947
RND3	8.47E-05	1.11180148	22.48809409	2.11E-06	0.000636636
PIM3	8.42E-05	1.110680764	22.22701292	2.42E-06	0.00071015
GPRC5A	8.40E-05	1.110121069	22.09772663	2.59E-06	0.000740143
UQCRB	8.26E-05	1.10703097	21.39691779	3.73E-06	0.001039838
MAFF	8.25E-05	1.106664534	21.31525266	3.90E-06	0.001046422
FERMT2	8.24E-05	1.10655672	21.29128235	3.95E-06	0.001046422
SRSF5	8.23E-05	1.106311856	21.23693839	4.06E-06	0.001051478
SH2D3A	8.20E-05	1.105426312	21.04152218	4.49E-06	0.001137346
NDUFS5	8.19E-05	1.105234327	20.99938619	4.59E-06	0.001137346

SLC25A5	8.14E-05	1.104178013	20.76900625	5.18E-06	0.001254819
COX6C	8.09E-05	1.103024964	20.52031865	5.90E-06	0.001398464
AREG	8.08E-05	1.102725145	20.45612726	6.10E-06	0.001416031
ASAP1	8.07E-05	1.102411242	20.38912877	6.32E-06	0.001436554
MIF	8.00E-05	1.100887201	20.06684625	7.48E-06	0.00166614
C19orf33	7.95E-05	1.099550799	19.78830117	8.65E-06	0.001889662
CDV3	7.85E-05	1.097107477	19.28867762	1.12E-05	0.002407327
DDX21	7.78E-05	1.095418446	18.95044968	1.34E-05	0.002819842
RBX1	7.68E-05	1.093058232	18.48740833	1.71E-05	0.003528296
AVPI1	7.62E-05	1.091471589	18.18230087	2.01E-05	0.00406581
POLR2F	7.59E-05	1.090688683	18.0335496	2.17E-05	0.004317665
KLF3	7.45E-05	1.087307437	17.40452242	3.02E-05	0.005904323
TMSB4X	7.42E-05	1.08636165	17.23240552	3.31E-05	0.006271549
FTL	7.41E-05	1.086317035	17.22432717	3.32E-05	0.006271549
TPM4	7.39E-05	1.085795708	17.13020221	3.49E-05	0.006380564
NME4	7.39E-05	1.085784952	17.12826542	3.49E-05	0.006380564
SNRPE	7.37E-05	1.085240573	17.03052012	3.68E-05	0.006609217
ARHGAP29	7.36E-05	1.085027905	16.99248158	3.75E-05	0.006635926
PPP1R15A	7.35E-05	1.084669256	16.92851808	3.88E-05	0.006756073
AP1S1	7.33E-05	1.084198833	16.84497333	4.06E-05	0.006951465
ATP5PF	7.28E-05	1.082818778	16.60217603	4.61E-05	0.0077702
HSP90AB1	7.27E-05	1.082670218	16.57624212	4.67E-05	0.0077702
POLR1D	7.25E-05	1.081922579	16.44632114	5.00E-05	0.00819891
DYNLL1	7.18E-05	1.080171626	16.14589097	5.86E-05	0.009468299
CNPY2	7.14E-05	1.079140502	15.97145976	6.43E-05	0.010233625
BACH1	7.09E-05	1.077843839	15.75469363	7.21E-05	0.011313974
TXNIP	7.07E-05	1.077382676	15.67828803	7.51E-05	0.011469432
ELL2	7.07E-05	1.077370851	15.67633351	7.52E-05	0.011469432
VMP1	7.03E-05	1.076098356	15.46738497	8.39E-05	0.012501241
VDAC1	7.03E-05	1.076067574	15.46236391	8.42E-05	0.012501241
H3F3A	7.01E-05	1.07554502	15.37736753	8.80E-05	0.012904293
IMP3	6.99E-05	1.075101762	15.30562332	9.14E-05	0.013229675
VAMP8	6.98E-05	1.074795697	15.25627379	9.39E-05	0.013405768
SLC25A6	6.96E-05	1.074382762	15.18993641	9.72E-05	0.013709273
SNRNP35	6.95E-05	1.073973106	15.12440144	0.000100654	0.014016046
METRN	6.94E-05	1.073701024	15.0810262	0.000102993	0.014036374
DYNLT1	6.94E-05	1.07366349	15.07505198	0.00010332	0.014036374
HSPA5	6.92E-05	1.073242266	15.00816331	0.000107047	0.014228519
HNRNPA1	6.92E-05	1.07321542	15.00390992	0.000107289	0.014228519
NDUFB3	6.90E-05	1.072522623	14.89455015	0.000113691	0.014678557
S100A11	6.89E-05	1.072461874	14.88499765	0.000114268	0.014678557
ATP5MF	6.89E-05	1.072423397	14.87895036	0.000114635	0.014678557
NDUFC2	6.82E-05	1.070515482	14.58205147	0.000134187	0.01683991
STK17A	6.82E-05	1.070483505	14.57712466	0.000134538	0.01683991
TXN	6.77E-05	1.068946672	14.34222181	0.000152408	0.018864729
COASY	6.75E-05	1.06841975	14.26252827	0.000158999	0.01946426
TAF1D	6.73E-05	1.068024076	14.202967	0.000164112	0.019871767
NDUFA13	6.72E-05	1.067748235	14.16158653	0.000167761	0.020095213
P4HB	6.67E-05	1.066104193	13.91736244	0.000191026	0.022373668
PPM1G	6.66E-05	1.066063663	13.91139336	0.000191633	0.022373668
HSPA1A	6.66E-05	1.065985677	13.89991494	0.000192807	0.022373668
HERPUD1	6.64E-05	1.065425113	13.81767733	0.000201432	0.023133541
ATP5MD	6.64E-05	1.065253644	13.79261609	0.000204137	0.023204956
PFDN4	6.63E-05	1.065057694	13.76403062	0.000207267	0.023322761
SOX4	6.61E-05	1.064677602	13.70874541	0.000213458	0.023728819
TRAPPC1	6.61E-05	1.064576284	13.69404473	0.000215136	0.023728819

TRAF4	6.60E-05	1.064114903	13.62729364	0.000222921	0.024346468
F3	6.55E-05	1.062923603	13.45639198	0.000244173	0.0264086
WDR83OS	6.52E-05	1.061825605	13.3007118	0.000265305	0.028418288
HSPE1	6.51E-05	1.061584641	13.2667804	0.000270151	0.02866171
CCNB1IP1	6.49E-05	1.061205707	13.21359004	0.000277926	0.029208502
MINOS1	6.46E-05	1.060283344	13.08497981	0.000297673	0.030834041
OTUD6B-AS1	6.46E-05	1.060226468	13.07708893	0.00029893	0.030834041
ATP5IF1	6.44E-05	1.059674018	13.00068279	0.000311377	0.031823343
CTNNAL1	6.43E-05	1.059412837	12.96471087	0.000317417	0.032089877
CLDN1	6.43E-05	1.059313278	12.9510241	0.000319747	0.032089877
PDCD6	6.42E-05	1.058921981	12.8973664	0.000329045	0.032728207
FOSL2	6.38E-05	1.057943703	12.76415615	0.000353325	0.034826429
SEC61B	6.38E-05	1.057813456	12.74652136	0.000356672	0.034826429
ABL2	6.37E-05	1.057703501	12.73165224	0.000359519	0.034826429
CITED4	6.36E-05	1.057119428	12.65294985	0.000374974	0.036010392
SEM1	6.34E-05	1.056609211	12.58458433	0.000388942	0.036789535
ATP5MPL	6.34E-05	1.056582262	12.58098326	0.000389692	0.036789535
NEAT1	6.33E-05	1.056317467	12.54565341	0.000397129	0.037176612
CFL1	6.32E-05	1.055997784	12.50312796	0.000406271	0.037715513
NDUFS6	6.29E-05	1.055165051	12.39300793	0.000430944	0.039413981
CLDN4	6.29E-05	1.055142074	12.38998276	0.000431643	0.039413981
SMIM13	6.26E-05	1.054415102	12.29463831	0.000454261	0.041142048
LIF	6.26E-05	1.054160083	12.2613602	0.000462434	0.041544502
DKK1	6.23E-05	1.053406692	12.16355616	0.000487324	0.043430292

Table S5. Summary of enriched functional annotations for 125 differentially regulated genes in scTenifoldNet analysis of transcriptional responses of a carcinoma (HNSCC) cell line to cetuximab.

Abbreviations: DB = Database reporting the gene set, TERM = Name of the gene set, P-VALUE: P-value result of the hypergeometric test, FDR = Adjusted P-value, ODDS RATIO = Odds ratio computed by the hypergeometric test. GENES = Differentially regulated genes included in the gene set. Full GSEA enrichment is available at: https://raw.githubusercontent.com/cailab-tamu/scTenifoldNet/master/inst/manuscript/results/annotations/A_SCC.csv

DB	TERM	P-VALUE	FDR	ODDS RATIO	GENES
BioPlanet	EGFR1 pathway	1.24E-11	1.87E-08	13.684	*DUSP4, ITGA3, LIF, DDX21, AREG, EREG, CLDN4, MYC, COTL1, TXNIP, LUC7L3, PHLDA1, HBEGF*
BioPlanet	Prolactin regulation of apoptosis	1.98E-10	1.49E-07	14.915	*EEF1A1, DUSP4, HSP90AB1, HSPA5, MAFF, CITED4, HNRNPA1, CLDN1, PHLDA1, AREG, RBX1*
BioPlanet	T cell receptor regulation of apoptosis	6.64E-09	3.34E-06	5.041	*HSP90AB1, HSPA5, MIF, YBX1, EEF2, DYNLL1, COX6C, HSPE1, MYC, DDIT3, PIM1, VDAC1, SEC61B, SLC25A5, PFDN4, HNRNPA1, GAPDH, IER2, HSPA1A*
BioPlanet	BDNF signaling pathway	9.87E-09	3.73E-06	7.969	*DUSP4, HSPA5, DKK1, RND3, HERPUD1, MYC, DDIT3, MAFF, TXNIP, SRSF5, IER2, S100A11, HSPA1A*
BioPlanet	Parkinson’s disease	1.36E-07	4.09E-05	10.992	*UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, VDAC1, SLC25A5, COX6C, SLC25A6*
KEGG	Huntington disease	3.59E-07	5.43E-05	8.333	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, POLR2F, VDAC1, SLC25A5, COX6C*
BioPlanet	Huntington’s disease	2.42E-07	6.08E-05	8.696	*UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, POLR2F, VDAC1, SLC25A5, COX6C, SLC25A6*
BioPlanet	Electron transport chain	3.12E-07	6.73E-05	12.190	*UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, SLC25A5, COX6C, SLC25A6*
KEGG	Protein processing in endoplasmic reticulum	8.67E-07	8.76E-05	8.834	*PPP1R15A, HSP90AB1, HSPA5, DDIT3, SEC61B, P4HB, RBX1, HERPUD1, HSPA1A*
KEGG	Parkinson disease	3.04E-07	9.23E-05	10.000	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, VDAC1, SLC25A5, COX6C*
BioPlanet	Respiratory electron transport, ATP biosynthesis by chemiosmotic coupling, and heat production by uncoupling proteins	7.45E-07	0.000141	13.827	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C*
BioPlanet	Protein processing in the endoplasmic reticulum	1.01E-06	0.000169	8.675	*PPP1R15A, HSP90AB1, HSPA5, DDIT3, SEC61B, P4HB, RBX1, HERPUD1, HSPA1A*
KEGG	Non-alcoholic fatty liver disease (NAFLD)	4.89E-06	0.000370	8.477	*NDUFA13, UQCRB, NDUFS6, DDIT3, NDUFS5, NDUFB3, NDUFC2, COX6C*
KEGG	Alzheimer disease	1.44E-05	0.000874	7.314	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C, GAPDH*
KEGG	Oxidative phosphorylation	2.14E-05	0.001081	8.358	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C*
BioPlanet	Tricarboxylic acid (TCA) cycle and respiratory electron transport	8.83E-06	0.001333	9.573	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C*
Reactome	Respiratory electron transport Homo sapiens R-HSA-611105	1.31E-06	0.002008	12.727	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C*
BioPlanet	Translation factors	1.49E-05	0.002043	16.000	*EIF4A2, EEF1B2, EEF1A1, EEF1D, EEF2*
Reactome	Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins. Homo sapiens R-HSA-163200	5.53E-06	0.002820	10.275	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C*

Reactome	Uptake and function of diphtheria toxin Homo sapiens R-HSA-5336415	4.70E-06	0.003597	80.000	*HSP90AB1, EEF2, HBEGF*
KEGG	Thermogenesis	0.000103	0.004479	5.541	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C, ACTG1*
Reactome	Complex I biogenesis Homo sapiens R-HSA-6799198	1.35E-05	0.005150	16.327	*NDUFA13, NDUFS6, NDUFS5, NDUFB3, NDUFC2*
BioPlanet	FSH regulation of apoptosis	4.16E-05	0.005230	5.475	*AKAP12, PTP4A1, GPRC5A, ITGA3, STK17A, PAWR, DKK1, AREG, EREG*
KEGG	ErbB signaling pathway	0.000183	0.006935	9.524	*MYC, ABL2, AREG, EREG, HBEGF*
Reactome	B-WICH complex positively regulates rRNA expression Homo sapiens R-HSA-5250924	3.09E-05	0.009465	13.793	*TAF1D, H3F3A, POLR1D, DDX21, POLR2F*
BioPlanet	Disease	8.29E-05	0.009629	3.323	*HSPA5, H3F3A, HMGA1, RBX1, HERPUD1, MYC, DDIT3, AP1S1, POLR2F, RICTOR, HDGF, SLC25A5, SLC25A6, HSPA1A*
BioPlanet	Alzheimer’s disease	9.38E-05	0.010122	6.627	*UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C, GAPDH*
Reactome	The NLRP3 inflammasome Homo sapiens R-HSA-844456	5.03E-05	0.010998	40.000	*HSP90AB1, TXNIP, TXN*
Reactome	The citric acid (TCA) cycle and respiratory electron transport Homo sapiens R-HSA-1428517	5.01E-05	0.012780	7.320	*NDUFA13, UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C*
Reactome	Infectious disease Homo sapiens R-HSA-5663205	6.75E-05	0.012909	4.598	*HSP90AB1, HMGA1, AP1S1, POLR2F, EEF2, SLC25A5, RBX1, HBEGF, SLC25A6, HSPA1A*
BioPlanet	Inflammasomes	0.000152	0.015302	28.235	*HSP90AB1, TXNIP, TXN*
BioPlanet	Interactions of HIV Vpr protein with host cellular proteins	0.000163	0.015352	14.545	*HMGA1, SLC25A5, HSPA1A, SLC25A6*
Reactome	Positive epigenetic regulation of rRNA expression Homo sapiens R-HSA-5250913	9.41E-05	0.015996	10.959	*TAF1D, H3F3A, POLR1D, DDX21, POLR2F*
Reactome	Cellular responses to stress Homo sapiens R-HSA-2262752	0.000105	0.016019	4.360	*EEF1A1, HSP90AB1, HSPA5, H3F3A, HMGA1, TXN, P4HB, DYNLL1, RBX1, HSPA1A*
Reactome	Metabolism of proteins Homo sapiens R-HSA-392499	0.000123	0.017128	2.682	*EIF4A2, TRAPPC1, HSPA5, H3F3A, TXN, EEF2, DYNLL1, AREG, HERPUD1, EEF1B2, EEF1A1, EEF1D, DDIT3, VDAC1, HDGF, SEC61B, PFDN4, SLC25A6*
BioPlanet	Myc active pathway	0.000194	0.017194	9.412	*FOSL1, MYC, PIM1, HMGA1, GAPDH*
BioPlanet	TGF-beta regulation of extracellular matrix	0.000223	0.017726	3.398	*FOSL1, TNFRSF12A, MYC, PIM1, PAWR, CLDN1, DKK1, AREG, F3, CTNNAL1, S100A11, HERPUD1*
BioPlanet	Oxidative phosphorylation	0.000214	0.017958	7.059	*UQCRB, NDUFS6, NDUFS5, NDUFB3, NDUFC2, COX6C*
Reactome	Inflammasomes Homo sapiens R-HSA-622312	0.000152	0.019381	28.235	*HSP90AB1, TXNIP, TXN*
Reactome	Disease Homo sapiens R-HSA-1643685	0.000178	0.019424	3.090	*HSP90AB1, HMGA1, EEF2, DKK1, EREG, RBX1, MYC, AP1S1, POLR2F, RICTOR, SLC25A5, HBEGF, SLC25A6, HSPA1A*
Reactome	Unfolded Protein Response (UPR) Homo sapiens R-HSA-381119	0.000205	0.020860	9.302	*HSPA5, DDIT3, HDGF, SEC61B, HERPUD1*
Reactome	Gene Expression Homo sapiens R-HSA-74160	0.000178	0.020898	2.256	*EIF4A2, IMP3, H3F3A, DDX21, TXN, YBX1, EEF2, COX6C, ELL2, EEF1B2, EEF1A1, TAF1D, EEF1D, MYC, POLR1D, POLR2F, SNRPE, RICTOR, SRSF5, SEC61B, SNRNP35, HNRNPA1, HSPA1A*
BioPlanet	Interleukin-5 regulation of apoptosis	0.000291	0.022008	6.667	*DUSP4, TPM4, PIM1, IER2, FOSL2, HBEGF*
BioPlanet	ERBB signaling pathway	0.000310	0.022274	8.511	*MYC, ABL2, AREG, EREG, HBEGF*

BioPlanet	Translation	0.000376	0.025801	6.358	*EIF4A2, EEF1B2, EEF1A1, EEF1D, SEC61B, EEF2*
Reactome	mRNA Splicing Homo sapiens R-HSA-72172	0.000291	0.027874	6.667	*POLR2F, SNRPE, SRSF5, YBX1, SNRNP35, HNRNPA1*
Reactome	mRNA Splicing - Minor Pathway Homo sapiens R-HSA-72165	0.000312	0.028093	12.308	*POLR2F, SNRPE, YBX1, SNRNP35*
BioPlanet	Tumor suppressor Arf inhibits ribosomal biogenesis	0.000438	0.028770	20.000	*MYC, POLR1D, HSPA1A*
Reactome	Translation Homo sapiens R-HSA-72766	0.000376	0.031952	6.358	*EIF4A2, EEF1B2, EEF1A1, EEF1D, SEC61B, EEF2*
Reactome	RNA Polymerase I Chain Elongation Homo sapiens R-HSA-73777	0.000445	0.035803	11.228	*TAF1D, H3F3A, POLR1D, POLR2F*
BioPlanet	PERK-regulated gene expression	0.000696	0.043821	17.143	*HSPA5, DDIT3, HERPUD1*
Reactome	Uptake and actions of bacterial toxins Homo sapiens R-HSA-5339562	0.000625	0.047790	17.778	*HSP90AB1, EEF2, HBEGF*

Table S6. 29 differentially regulated genes in scTenifoldNet analysis of transcriptional responses of human dermal fibroblasts to the double-stranded RNA stimulus.

Identified 29 differentially regulated genes sorted according to the significance level based on the scTenifoldNet differential regulation test. Target genes of the *RELA* transcription factor (14 out of 29 genes) are highlighted in bold font. The full list (containing 7,904 genes) is available for downloading at https://raw.githubusercontent.com/cailab-tamu/scTenifoldNet/master/inst/manuscript/results/sym10X500DF_Itensor_Dalignment.csv

GENE	DISTANCE	Z	FC	P-VALUE	FDR
SOD2	0.011713	2.19348	543.7713	2.85E-120	2.25E-116
GBP1	0.011486	2.187157	522.8897	9.95E-116	3.93E-112
WARS	0.011135	2.177163	491.4463	6.90E-109	1.82E-105
ZC3HAV1	0.011076	2.175438	486.2045	9.54E-108	1.89E-104
EGR1	0.010945	2.171631	474.8289	2.85E-105	4.51E-102
BBC3	0.010762	2.166204	459.0516	7.73E-102	1.02E-98
ISG15	0.01039	2.154919	427.8377	4.80E-95	5.42E-92
HLA-B	0.010152	2.147514	408.474	7.88E-91	7.78E-88
ZFP36	0.009915	2.139969	389.6078	1.01E-86	8.85E-84
PPP1R15A	0.009793	2.136045	380.1296	1.17E-84	9.22E-82
JUN	0.00973	2.133988	375.2511	1.35E-83	9.67E-81
IFI6	0.009546	2.127923	361.209	1.54E-80	1.01E-77
JUNB	0.009401	2.123037	350.2658	3.71E-78	2.26E-75
B2M	0.009257	2.118148	339.6356	7.66E-76	4.32E-73
APOL2	0.009111	2.113113	329.0091	1.58E-73	8.32E-71
HLA-A	0.008515	2.091738	287.3475	1.88E-64	9.29E-62
IER3	0.007784	2.06358	240.1286	3.69E-54	1.71E-51
SAT1	0.007619	2.056891	230.0592	5.79E-52	2.54E-49
NFKBIA	0.00635	2.0005	159.836	1.23E-36	5.11E-34
NNMT	0.005542	1.958886	121.7369	2.64E-28	1.04E-25
FN1	0.004237	1.87818	71.16211	3.29E-17	1.24E-14
IFITM3	0.003611	1.830964	51.69072	6.50E-13	2.31E-10
MEG3	0.003609	1.830771	51.62276	6.73E-13	2.31E-10
NEAT1	0.003331	1.807323	43.97505	3.33E-11	1.10E-08
COL1A1	0.002785	1.755558	30.74931	2.94E-08	9.28E-06
PLEKHA4	0.002488	1.723228	24.52613	7.33E-07	0.000223
EEF1A1	0.002222	1.69118	19.56002	9.75E-06	0.002854
SOCS1	0.002039	1.667151	16.48527	4.90E-05	0.01384
SERF2	0.001944	1.653789	14.98184	0.000109	0.029586

Table S7. Summary of enriched functional annotations for 29 differentially regulated genes in scTenifoldNet analysis of transcriptional responses of human dermal fibroblasts to the double-stranded RNA stimulus.

Abbreviations: DB = Database reporting the gene set, TERM = Name of the gene set, P-VALUE: P-value result of the hypergeometric test, FDR = Adjusted P-value, ODDS RATIO = Odds ratio computed by the hypergeometric test. GENES = Differentially regulated genes included in the gene set. Full GSEA enrichment is available for downloading at https://raw.githubusercontent.com/cailab-tamu/scTenifoldNet/master/inst/manuscript/results/annotations/A_DF.csv

DB	**TERM**	**P-VALUE**	**FDR**	**ODDS RATIO**	**GENES**
BioPlanet	Interferon signaling	1.45E-12	2.20E-09	36.946	*IFITM3, EGR1, SOCS1, IFI6, HLA-B, ISG15, HLA-A, GBP1, B2M*
BioPlanet	Interferon alpha/beta signaling	3.62E-12	2.73E-09	75.431	*IFITM3, EGR1, SOCS1, IFI6, HLA-B, ISG15, HLA-A*
Reactome	Interferon Signaling Homo sapiens R-HSA-913531	5.86E-12	4.48E-09	31.668	*IFITM3, EGR1, SOCS1, IFI6, HLA-B, ISG15, HLA-A, GBP1, B2M*
Reactome	Interferon alpha/beta signaling Homo sapiens R-HSA-909733	5.62E-12	8.60E-09	70.994	*IFITM3, EGR1, SOCS1, IFI6, HLA-B, ISG15, HLA-A*
BioPlanet	Immune system signaling by interferons, interleukins, prolactin, and growth hormones	1.42E-10	7.17E-08	22.167	*IFITM3, EGR1, SOCS1, IFI6, HLA-B, ISG15, HLA-A, GBP1, B2M*
BioPlanet	Oncostatin M	1.01E-08	2.54E-06	17.740	*COL1A1, JUN, SOCS1, FN1, SOD2, JUNB, B2M, IER3*
BioPlanet	T cell receptor regulation of apoptosis	6.88E-09	2.60E-06	11.437	*IFITM3, NFKBIA, EGR1, JUN, HLA-B, SOD2, GBP1, JUNB, BBC3, IER3*
BioPlanet	Type II interferon signaling (interferon-gamma)	9.02E-09	2.72E-06	68.966	*SOCS1, IFI6, HLA-B, ISG15, GBP1*
Reactome	Cytokine Signaling in Immune system Homo sapiens R-HSA-1280215	8.97E-09	4.57E-06	11.123	*IFITM3, EGR1, SOCS1, IFI6, HLA-B, FN1, ISG15, HLA-A, GBP1, B2M*
BioPlanet	CD8/T cell receptor downstream pathway	4.36E-08	9.41E-06	50.710	*EGR1, JUN, HLA-A, JUNB, B2M*
BioPlanet	Immune system	6.83E-08	1.29E-05	7.601	*IFITM3, NFKBIA, EGR1, JUN, SOCS1, IFI6, HLA-B, ISG15, HLA-A, GBP1, B2M*
Reactome	Immune System Homo sapiens R-HSA-168256	7.06E-08	2.70E-05	5.795	*IFITM3, EGR1, JUN, IFI6, HLA-B, FN1, ISG15, HLA-A, NFKBIA, SOCS1, GBP1, B2M, IER3*
BioPlanet	Endosomal/vacuolar pathway	2.29E-07	3.84E-05	229.885	*HLA-B, HLA-A, B2M*
BioPlanet	Interferon-gamma signaling pathway	2.62E-07	3.95E-05	35.549	*SOCS1, HLA-B, HLA-A, GBP1, B2M*
Reactome	Interferon gamma signaling Homo sapiens R-HSA-877300	2.12E-07	6.49E-05	37.078	*SOCS1, HLA-B, HLA-A, GBP1, B2M*
BioPlanet	Interleukin-1 regulation of extracellular matrix	7.57E-07	0.000104	28.736	*NFKBIA, ZFP36, JUN, HLA-B, SOD2*
Reactome	Endosomal/Vacuolar pathway Homo sapiens R-HSA-1236977	5.98E-07	0.000152	172.414	*HLA-B, HLA-A, B2M*
BioPlanet	AP-1 transcription factor network	3.06E-06	0.000385	39.409	*EGR1, JUN, HLA-A, JUNB*
Reactome	Antigen Presentation: Folding, assembly and peptide loading of class I MHC Homo sapiens R-HSA-983170	6.17E-06	0.001349	82.759	*HLA-B, HLA-A, B2M*
BioPlanet	Interleukin-2 signaling pathway	1.94E-05	0.002257	6.514	*IFITM3, ZFP36, JUN, SOCS1, WARS, GBP1, BBC3, IER3*
Reactome	Innate Immune System Homo sapiens R-HSA-168249	1.37E-05	0.002619	6.837	*NFKBIA, JUN, SOCS1, HLA-B, FN1, ISG15, B2M, IER3*
BioPlanet	Prolactin regulation of apoptosis	2.44E-05	0.002631	23.378	*EEF1A1, EGR1, GBP1, IER3*
KEGG	Osteoclast differentiation	3.36E-05	0.003392	21.552	*NFKBIA, JUN, SOCS1, JUNB*

KEGG	Human T-cell leukemia virus 1 infection	2.47E-05	0.003746	14.075	*NFKBIA, EGR1, ZFP36, JUN, B2M*
KEGG	AGE-RAGE signaling pathway in diabetic complications	1.32E-05	0.004001	27.313	*COL1A1, EGR1, JUN, FN1*
BioPlanet	Regulation of NFAT transcription factors	4.26E-05	0.004286	44.021	*EGR1, JUN, JUNB*
BioPlanet	Interleukin-5 regulation of apoptosis	5.32E-05	0.005022	19.157	*EGR1, SOCS1, SOD2, IER3*
BioPlanet	Nef-mediated downregulation of MHC class I complex cell surface expression	9.07E-05	0.008056	137.931	*HLA-A, B2M*
BioPlanet	Interleukin-12-mediated signaling events	0.000113	0.008956	31.830	*SOCS1, HLA-A, B2M*
BioPlanet	Erythropoietin-mediated neuroprotection through NF-kB	0.000111	0.009291	125.392	*NFKBIA, SOD2*
BioPlanet	CTL mediated immune response against target cells	0.000157	0.011837	106.101	*HLA-A, B2M*
BioPlanet	Prolactin activation of MAPK signaling	0.000173	0.012406	27.586	*NFKBIA, JUN, SOCS1*
BioPlanet	Antigen processing and presentation	0.000217	0.013636	25.543	*HLA-B, HLA-A, B2M*
BioPlanet	Apoptosis modulation and signaling	0.000209	0.013715	25.862	*NFKBIA, JUN, BBC3*
BioPlanet	Antigen processing: cross presentation	0.000201	0.013814	26.189	*HLA-B, HLA-A, B2M*
Reactome	Nef mediated downregulation of MHC class I complex cell surface expression Homo sapiens R-HSA-164940	0.000091	0.015418	137.931	*HLA-A, B2M*
BioPlanet	Cadmium-induced DNA biosynthesis and proliferation in macrophages	0.000272	0.016452	81.136	*NFKBIA, JUN*
Reactome	ER-Phagosome pathway Homo sapiens R-HSA-1236974	0.000113	0.017243	31.830	*HLA-B, HLA-A, B2M*
BioPlanet	Senescence and autophagy	0.000392	0.021113	20.899	*COL1A1, JUN, FN1*
BioPlanet	4-1BB-dependent immune response	0.000380	0.021224	68.966	*NFKBIA, JUN*
BioPlanet	TSH regulation of gene expression	0.000369	0.021415	21.330	*PPP1R15A, EGR1, JUN*
BioPlanet	ERBB1 downstream pathway	0.000478	0.023296	19.519	*EGR1, ZFP36, JUN*
BioPlanet	Interleukin-4 signaling pathway	0.000452	0.023553	19.894	*NFKBIA, COL1A1, SOCS1*
BioPlanet	Antigen presentation: folding, assembly, and peptide loading of class I MHC proteins	0.000477	0.024008	10.818	*SOCS1, HLA-B, HLA-A, B2M*
KEGG	Epstein-Barr virus infection	0.000318	0.024054	12.046	*NFKBIA, JUN, ISG15, B2M*
BioPlanet	Ras-independent pathway in NK cell-mediated cytotoxicity	0.000549	0.024396	57.471	*HLA-A, B2M*
BioPlanet	Interleukin-4 regulation of apoptosis	0.000567	0.024465	10.332	*JUN, NNMT, IFI6, FN1*
BioPlanet	FSH regulation of apoptosis	0.000536	0.024517	10.489	*IFITM3, ISG15, GBP1, IER3*
BioPlanet	BDNF signaling pathway	0.000521	0.024568	10.569	*EGR1, ZFP36, JUN, JUNB*
BioPlanet	Alpha-9 beta-1 integrin pathway	0.000597	0.025022	55.172	*FN1, SAT1*
BioPlanet	Oxidative stress	0.000697	0.026975	51.086	*SOD2, JUNB*
BioPlanet	Immunoregulatory interactions between a lymphoid and a non-lymphoid cell	0.000670	0.027356	17.386	*HLA-B, HLA-A, B2M*
BioPlanet	TNF/stress-related signaling	0.000697	0.027685	51.086	*NFKBIA, JUN*
BioPlanet	Nef in HIV-1 replication and disease pathogenesis	0.000750	0.028298	49.261	*HLA-A, B2M*
BioPlanet	Interleukin-1 signaling pathway	0.000773	0.028481	16.552	*NFKBIA, ZFP36, JUN*
BioPlanet	T cell receptor calcium pathway	0.000804	0.028921	47.562	*JUN, JUNB*
BioPlanet	Tumor necrosis factor (TNF) pathway	0.000861	0.029552	45.977	*NFKBIA, JUN*

BioPlanet	CD40/CD40L signaling	0.000920	0.030189	44.494	*NFKBIA, JUN*
BioPlanet	Inflammatory response pathway	0.000861	0.030239	45.977	*COL1A1, FN1*
BioPlanet	Alpha-V beta-3 integrin/OPN pathway	0.000920	0.030860	44.494	*NFKBIA, JUN*
Reactome	Antigen processing-Cross presentation Homo sapiens R-HSA-1236975	0.000225	0.031259	25.231	*HLA-B, HLA-A, B2M*
Reactome	Toll Like Receptor 2 (TLR2) Cascade Homo sapiens R-HSA-181438	0.000316	0.032191	22.489	*NFKBIA, JUN, SOCS1*
KEGG	TNF signaling pathway	0.000533	0.032298	18.809	*NFKBIA, JUN, JUNB*
BioPlanet	Toll-like receptor signaling pathway regulation	0.001118	0.034464	14.570	*NFKBIA, JUN, SOCS1*
Reactome	Toll Like Receptor TLR6:TLR2 Cascade Homo sapiens R-HSA-168188	0.000316	0.034490	22.489	*NFKBIA, JUN, SOCS1*
BioPlanet	EPO receptor signaling	0.001107	0.034810	40.568	*JUN, SOCS1*
BioPlanet	EGF receptor transactivation by GPCRs in cardiac hypertrophy	0.001107	0.035551	40.568	*NFKBIA, JUN*
Reactome	Toll Like Receptor TLR1:TLR2 Cascade Homo sapiens R-HSA-168179	0.000316	0.037143	22.489	*NFKBIA, JUN, SOCS1*
BioPlanet	FRA pathway	0.001310	0.038790	37.279	*JUN, JUNB*
BioPlanet	Allograft rejection	0.001310	0.039566	37.279	*HLA-B, HLA-A*
Reactome	Nef-mediates down modulation of cell surface receptors by recruiting them to clathrin adapters Homo sapiens R-HSA-164938	0.000419	0.040075	65.681	*HLA-A, B2M*
BioPlanet	Signal transduction through IL-1R	0.001382	0.040121	36.298	*NFKBIA, JUN*
Reactome	MyD88:Mal cascade initiated on plasma membrane Homo sapiens R-HSA-166058	0.000316	0.040239	22.489	*NFKBIA, JUN, SOCS1*
KEGG	Measles	0.001164	0.044100	14.368	*NFKBIA, JUN, BBC3*
KEGG	Relaxin signaling pathway	0.000886	0.044739	15.794	*NFKBIA, COL1A1, JUN*
BioPlanet	Adaptive immune system	0.001632	0.045634	5.690	*NFKBIA, SOCS1, HLA-B, HLA-A, B2M*
BioPlanet	Graft-versus-host disease	0.001607	0.045792	33.642	*HLA-B, HLA-A*
BioPlanet	TWEAK signaling pathway	0.001686	0.046292	32.841	*NFKBIA, JUN*
BioPlanet	Type 1 diabetes mellitus	0.001767	0.046805	32.077	*HLA-B, HLA-A*
KEGG	Apoptosis	0.001096	0.047432	14.674	*NFKBIA, JUN, BBC3*
BioPlanet	Beta-3 integrin cell surface interactions	0.001767	0.047640	32.077	*COL1A1, FN1*
BioPlanet	Gastrin pathway	0.001849	0.048145	31.348	*JUN, IER3*

Supplementary Figures

Figure S1. Benchmarking of scTenifoldNet/PC-regression and 11 other existing methods for single-cell gene regulatory network construction using BEELINE.

Performance of 12 methods for single-cell gene regulatory network construction evaluated using BEELINE and the same curated synthetic dataset. Methods are ranked by average performance among three different metrics: the precision-recall curve (AUPRC), the receiver operating characteristic curve (AUROC), and the time of computing. Briefly, we used the GSD dataset included in the BEELINE package and added our scTenifoldNet/PC regression to the list of other available methods (PIDC, PPCOR, LEAP, GRNBOOST2, GENIE3, SCINGE, SINCERITIES, GRISLI, SCODE, GRNVBEM, and SCNS) included in their pipeline to compare the performance for the network construction. We compared the results obtained (see the figure below) and ranked the methods based on their average performance. scTenifoldNet performed better than almost all other existing methods on this test data set. Note that GSD is the largest curated reference data set (in terms of the number of genes and number of cells included) in the BEELINE package.

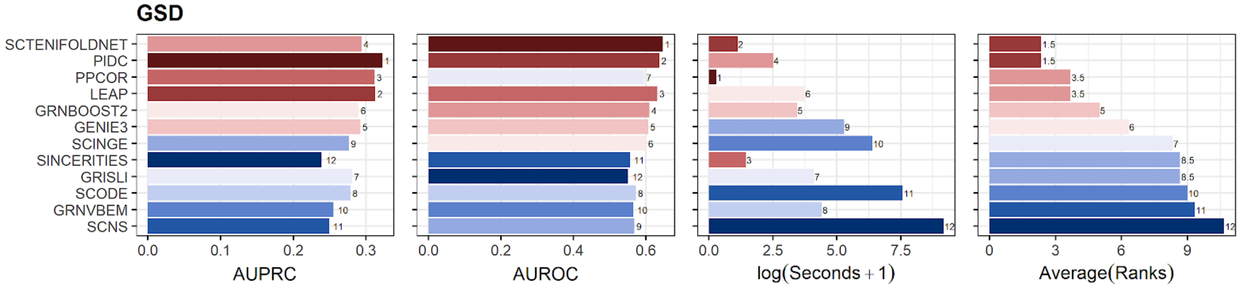


Figure S2. Influence of input data normalization on network construction.

Snapshots (showing 500 x 500 adjacency matrices) of the constructed networks using the same input (real data set 1: mock-treated mouse neurons) subjected to normalizations using the library size-based and DESeq methods.

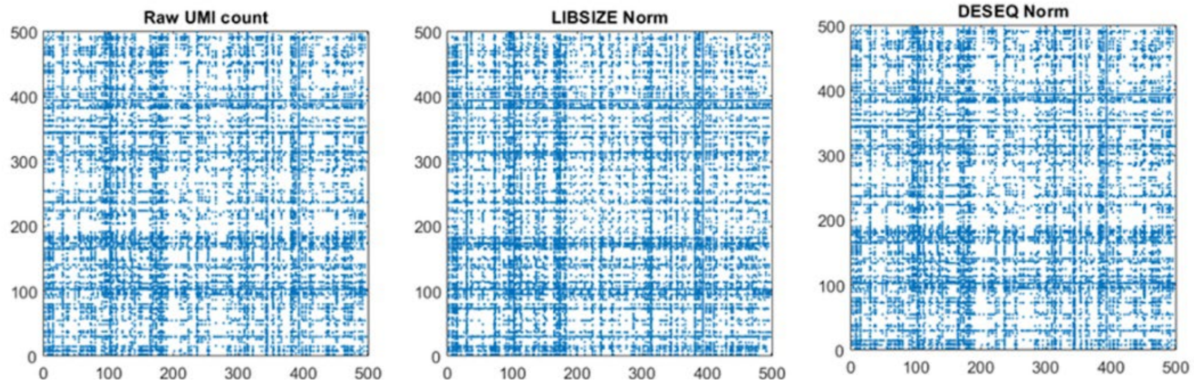
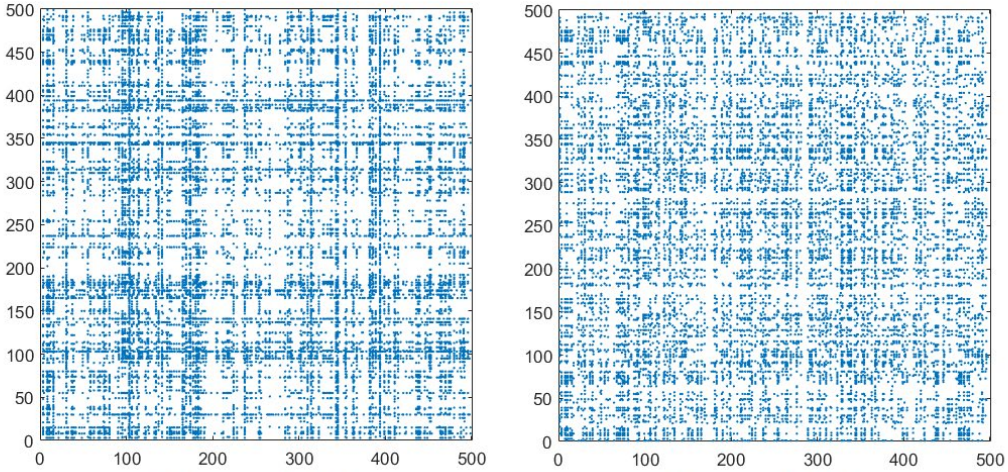


Figure S3. Influence of input data imputation on network construction.

Snapshot (showing 500 x 500 adjacency matrices) of the constructed networks using the same input (real data set 1: mock-treated mouse neurons) before and after the expression matrix imputation using MAGIC.



Before using MAGIC

After using MAGIC

Figure S4. Sensibility of scTenifoldNet to different sample sizes used as input.

Comparison of the ranked list (Z-score of the computed distance used as the metric) returned by scTenifoldNet using different combinations of balanced and unbalanced sample sizes as input. Cells were subsampled from the mouse neurons dataset to generate different input sizes for scTenifoldNet under the parameters by default. In the left panel, results are presented as a heatmap of the ranked lists and labels of the genes associated with morphine addiction in the KEGG database. At the top of the right panel, the principal component analysis of the ranked lists. In the middle of the left panel, the Spearman coefficient correlation (SCC) between ranked lists, and at the bottom of the right panel is the number of tested genes in the function of the sample size used as input in the balanced scenarios.

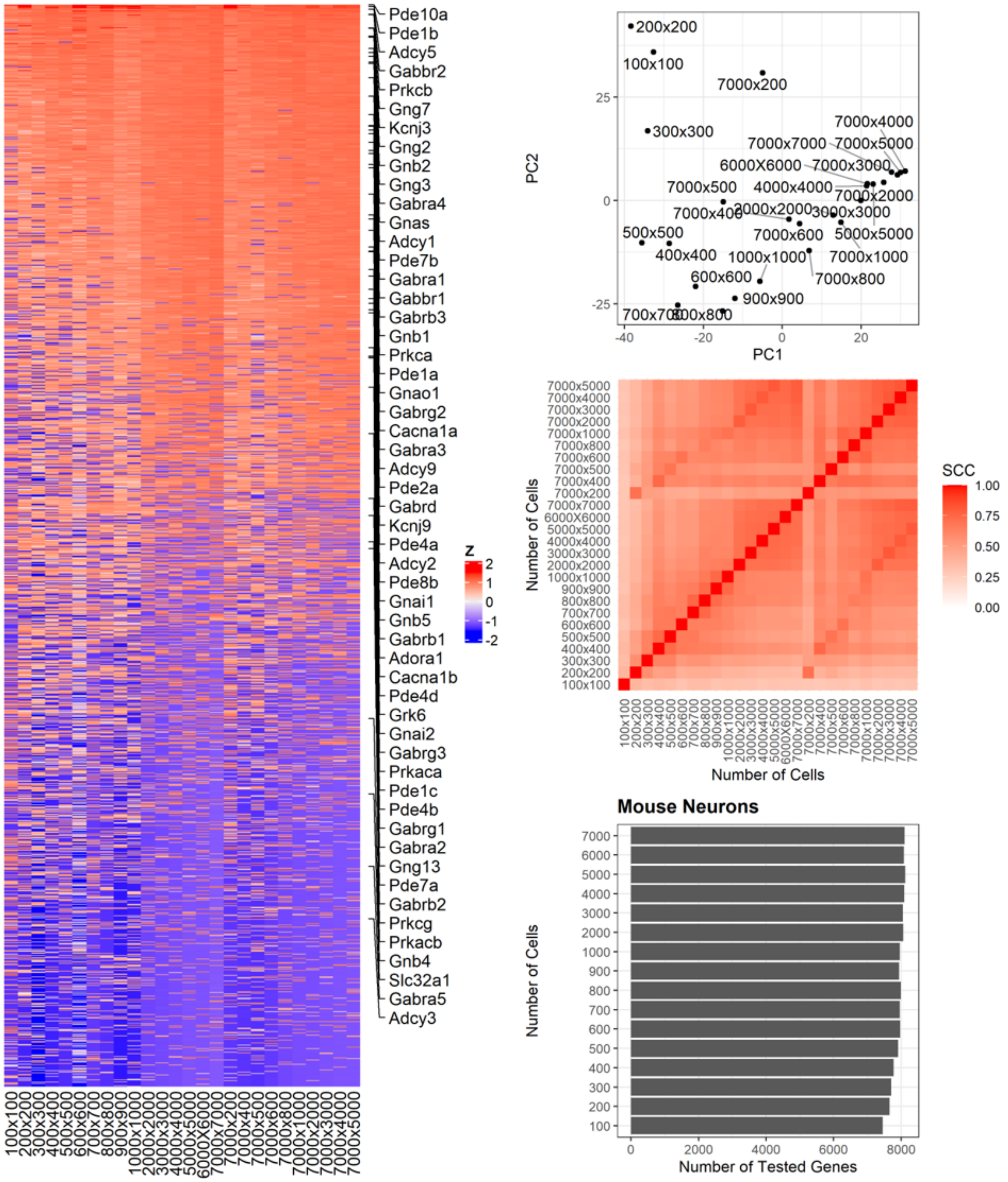


Figure S5. Relationship between node degree and average gene expression level across the output of scTenifoldNet/PC-regression and 11 other existing methods for single-cell gene regulatory network construction.

Comparison of the relationship between the node degree and gene expression values across the output of 11 methods included in the BEELINE pipeline and scTenifoldNet/PC-regression using the same dataset as input. For each package, using the GSD data set included in the BEELINE package, we generated a single-cell gene regulatory network and computed the degree for each gene. The calculated degree was then correlated (using Spearman's correlation coefficient) with the average gene expression level calculated from the input expression matrix. Left panel shows that, among all the tested methods, scTenifoldNet displays the smallest absolute correlation. Right panel shows the mean correlation values and their associated standard deviations after ten tests as a function of the number of cells in resampled matrices.