# Cell-specific Network Constructed by Single-cell RNA Sequencing Data

## Supplementary Data

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**Supplementary Note 1: Statistic of cell-specific network**

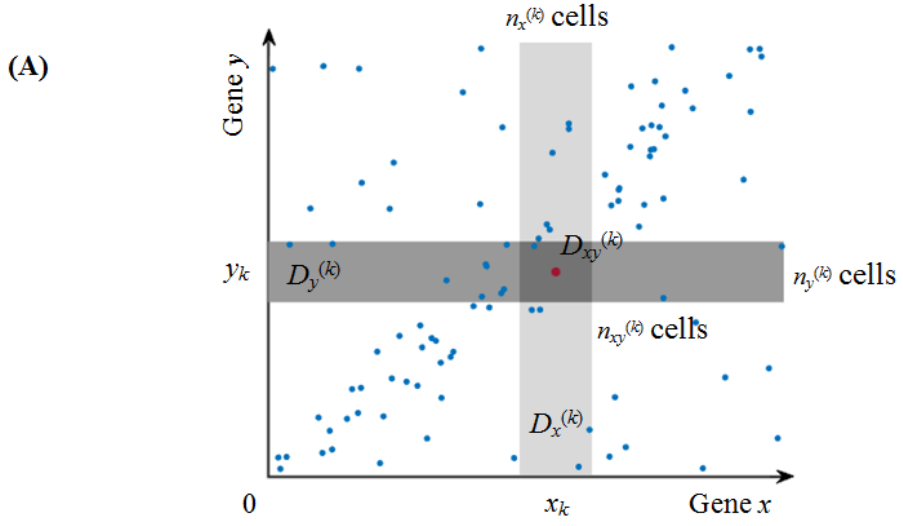
**(1) Statistic**

Assume that there are $n$ cells with $m$ genes in scRNA-seq data. Based on the statistical independency in probability theory (eqn. (1) in the main text), we design a statistic for each gene pair and each cell as

$$\rho_{xy}{}^{(k)} = \frac{n_{xy}{}^{(k)}}{n} - \frac{n_x{}^{(k)}}{n} \cdot \frac{n_y{}^{(k)}}{n} \tag{S-1}$$

where $\rho_{xy}{}^{(k)}$ is the statistic for genes $x$, $y$ of cell $k$ (the red plot in Figure S1(A)), $n_x{}^{(k)}$ and $n_y{}^{(k)}$ are the number of plots (or cells) in the neighborhood of $x_k$ and $y_k$ respectively (light and medium grey boxes in Figure S1(A)), $n_{xy}{}^{(k)}$ is the number of plots in the neighborhood of ($x_k$, $y_k$) (intersection of two boxes and represented as dark grey box in Figure S1(A)), and $n$ is the total number of plots.

In eqn. (S-1), $n_x{}^{(k)}$ and $n_y{}^{(k)}$ are determined in advance ($< n$), and thus this statistic is only changed with $n_{xy}{}^{(k)}$. Hence, as shown in Figure S1 (A), we first draw the two boxes $D_x{}^{(k)}$ and $D_y{}^{(k)}$ to represent the neighborhood of $x_k$ and $y_k$ respectively based on the predetermined $n_x{}^{(k)}$ and $n_y{}^{(k)}$, and then we can straightforwardly have the third box $D_{xy}{}^{(k)}$, which is simply the intersection of the previous two boxes. By counting the plots in $D_{xy}{}^{(k)}$, we can obtain the value of $n_{xy}{}^{(k)}$ and then get the criterion of eqn. (S-1), as shown in Figure S1(B).




**(B)**

$$\rho_{xy}{}^{(k)} = \frac{n_{xy}{}^{(k)}}{n} - \frac{n_x{}^{(k)}}{n} \cdot \frac{n_y{}^{(k)}}{n} \leq 0 \quad \text{for} \quad \text{X} \quad \text{Y}$$

$$\rho_{xy}{}^{(k)} = \frac{n_{xy}{}^{(k)}}{n} - \frac{n_x{}^{(k)}}{n} \cdot \frac{n_y{}^{(k)}}{n} > 0 \quad \text{for} \quad \text{X} \text{—} \text{Y}$$

**Figure S1.** Scatter diagram of the expression values of gene $x$, $y$ for cell $k$, and illustration of the three boxes for our statistic. (A) Near the red plot $k$, make the light and medium grey box $D_x{}^{(k)}$ and $D_y{}^{(k)}$ to represent the neighborhood of $x_k$ and $y_k$ respectively. The number of plots in the two boxes are $n_x{}^{(k)}$ and $n_y{}^{(k)}$, which are predetermined ($< n$). The intersection of the two boxes is the dark grey box $D_{xy}{}^{(k)}$ that represents the neighborhood of ($x_k$ , $y_k$), in which the number of plots is $n_{xy}{}^{(k)}$. (B) Criterion based on the

statistic $\rho_{xy}^{(k)}$. We can identify the association of two genes by statistical test based on the statistic $\rho_{xy}^{(k)}$.

Note that the distribution of genes has no influence on the statistic. The box size of $D_x^{(k)}$ and $D_y^{(k)}$ will change with the density of plots, smaller in the dense area and larger in the sparse area. Then, no matter which distribution the genes follow, the boxes in Figure S1 cover the same plots and the statistic model will not change.

**(2) Mean value and variance of the statistic**

As $n_x^{(k)}$ and $n_y^{(k)}$ are predetermined and $n_{xy}^{(k)}$ follows binomial distribution, based on Bayes formula, we can get

$$P\left(n_{xy}{}^{(k)} = t \middle| n_x{}^{(k)} = i, n_y{}^{(k)} = j\right)$$

$$= \frac{P\left(n_{xy}{}^{(k)} = t \middle| n_x{}^{(k)} = i\right) P\left(n_{xy}{}^{(k)} = t \middle| n_y{}^{(k)} = j\right)}{P\left(n_{xy}{}^{(k)} = t\right)}$$

$$= \frac{\frac{i!}{t!\,(i-t)!} \cdot \left(\frac{p_{xy}}{p_x}\right)^t \left(1 - \frac{p_{xy}}{p_x}\right)^{i-t} \cdot \frac{j!}{t!\,(j-t)!} \cdot \left(\frac{p_{xy}}{p_y}\right)^t \left(1 - \frac{p_{xy}}{p_y}\right)^{j-t}}{\frac{n!}{t!\,(n-t)!} \cdot {p_{xy}}^t \left(1 - p_{xy}\right)^{n-t}}$$

where $p_x$, $p_y$ and $p_{xy}$ is the probability that a plot is located in the $D_x^{(k)}$, $D_y^{(k)}$ and $D_{xy}^{(k)}$ respectively. Then

$$\sum_{t=0}^{n} P\left(n_{xy}{}^{(k)} = t \middle| n_x{}^{(k)} = i, n_y{}^{(k)} = j\right) = 1$$

$$\sum_{t=0}^{n} t \cdot P\left(n_{xy}{}^{(k)} = t \middle| n_x{}^{(k)} = i, n_y{}^{(k)} = j\right)$$

$$= \sum_{t=0}^{n} t \frac{\left[\frac{ip_{xy}}{tp_x} \cdot \frac{(i-1)!}{(t-1)!\,(i-t)!} \left(\frac{p_{xy}}{p_x}\right)^{t-1} \left(1 - \frac{p_{xy}}{p_x}\right)^{i-t}\right] \left[\frac{jp_{xy}}{tp_y} \cdot \frac{(j-1)!}{(t-1)!\,(j-t)!} \left(\frac{p_{xy}}{p_y}\right)^{t-1} \left(1 - \frac{p_{xy}}{p_y}\right)^{j-t}\right]}{\frac{np_{xy}}{t} \cdot \frac{(n-1)!}{(t-1)!\,(n-t)!} \cdot {p_{xy}}^{t-1} \left(1 - p_{xy}\right)^{n-t}}$$

$$= \frac{ij}{n} \cdot \frac{p_{xy}}{p_x p_y} \cdot \sum_{t=1}^{n} P\left(n_{xy}{}^{(k)} = t - 1 \middle| n_x{}^{(k)} = i - 1, n_y{}^{(k)} = j - 1\right) = \frac{ij}{n} \cdot \frac{p_{xy}}{p_x p_y}$$

By the similar calculation, we can get

$$\sum_{t=0}^{n} t^2 \cdot P\left(n_{xy}{}^{(k)} = t \middle| n_x{}^{(k)} = i, n_y{}^{(k)} = j\right) = \frac{ij(i-1)(j-1)}{n(n-1)} \cdot \frac{{p_{xy}}^2}{{p_x}^2 {p_y}^2} + \frac{ij}{n} \cdot \frac{p_{xy}}{p_x p_y}$$

Then the mean value of the statistic is

$$\mu_{xy}{}^{(k)} = E\left(\frac{n_{xy}{}^{(k)}}{n} - \frac{n_x{}^{(k)} n_y{}^{(k)}}{n^2}\right) = \sum_{t=0}^{n} \left(\frac{t}{n} - \frac{ij}{n^2}\right) \cdot P\left(n_{xy}{}^{(k)} = t \middle| n_x{}^{(k)} = i, n_y{}^{(k)} = j\right)$$

$$= \frac{ij}{n^2}\left(\frac{p_{xy}}{p_x p_y} - 1\right)$$

The variance of the statistic is

$$\sigma_{xy}{}^{(k)2} = E\left[\left(\frac{n_{xy}{}^{(k)}}{n} - \frac{n_x{}^{(k)}n_y{}^{(k)}}{n^2}\right)^2\right] - \left[E\left(\frac{n_{xy}{}^{(k)}}{n} - \frac{n_x{}^{(k)}n_y{}^{(k)}}{n^2}\right)\right]^2$$

$$= \sum_{t=0}^{n}\left(\frac{t}{n} - \frac{ij}{n^2}\right)^2 \cdot P\left(n_{xy}{}^{(k)} = t \middle| n_x{}^{(k)} = i, n_y{}^{(k)} = j\right) - \left[\frac{ij}{n^2}\left(\frac{p_{xy}}{p_x p_y} - 1\right)\right]^2$$

$$= \frac{ij}{n^4} \cdot \frac{p_{xy}}{p_x p_y}\left[\frac{(n-i)(n-j)}{n-1} \cdot \frac{p_{xy}}{p_x p_y} + n\left(1 - \frac{p_{xy}}{p_x p_y}\right)\right]$$

If genes *x* and *y* are independent of each other, $p_{xy} = p_x p_y$, and thus

$$\mu_{xy}{}^{(k)} = 0$$

$$\sigma_{xy}{}^{(k)2} = \frac{n_x{}^{(k)}n_y{}^{(k)}\left(n - n_x{}^{(k)}\right)\left(n - n_y{}^{(k)}\right)}{n^4(n-1)}$$

We normalize the statistic $\rho_{xy}{}^{(k)}$ and define

$$\hat{\rho}_{xy}{}^{(k)} = \frac{\left(\frac{n_{xy}{}^{(k)}}{n} - \frac{n_x{}^{(k)}n_y{}^{(k)}}{n^2}\right) - 0}{\sqrt{\frac{n_x{}^{(k)}n_y{}^{(k)}\left(n - n_x{}^{(k)}\right)\left(n - n_y{}^{(k)}\right)}{n^4(n-1)}}} = \frac{\sqrt{n-1} \cdot \left(n \cdot n_{xy}{}^{(k)} - n_x{}^{(k)}n_y{}^{(k)}\right)}{\sqrt{n_x{}^{(k)}n_y{}^{(k)}\left(n - n_x{}^{(k)}\right)\left(n - n_y{}^{(k)}\right)}} \qquad \text{(S-2)}$$

Eqn. (S-2) is the statistic model in this paper, or called the normalized statistic. If genes *x* and *y* are independent of each other, the mean value and variance of $\hat{\rho}_{xy}{}^{(k)}$ for the *n* cells are 0 and 1 respectively.

**(3) Distribution of the statistic**

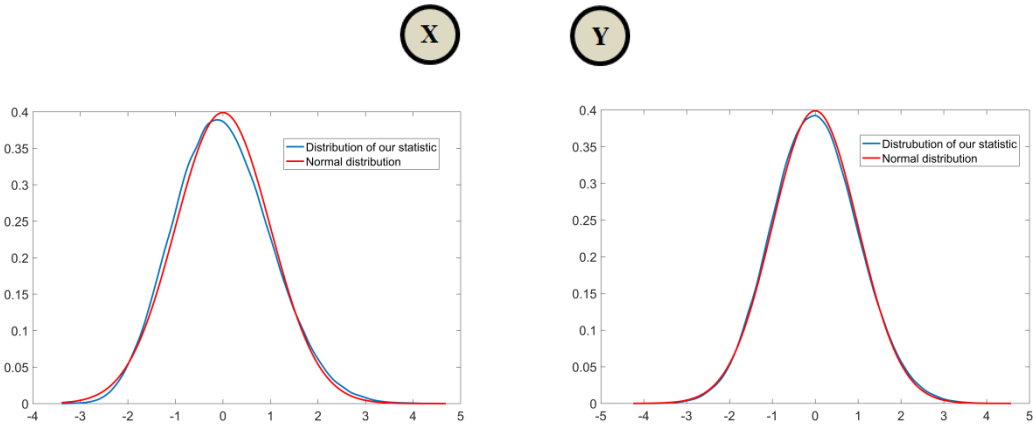
By the similar derivation, we can get the third-order and fourth-order moments of the statistic. If genes *x* and *y* are independent of each other, $n_x{}^{(k)}$ and $n_y{}^{(k)}$ increase in proportion to *n*, and the third-order and fourth-order moments of the normalized statistic will tend to those of the standard normal distribution (0 and 3 respectively) with the increase of *n*.

$$\lim_{n\to\infty} E\left[\left(\hat{\rho}_{xy}{}^{(k)}\right)^3\right] = \lim_{n\to\infty} \frac{\sqrt{n-1}}{n-2} \cdot \frac{\left(n - 2n_x{}^{(k)}\right)\left(n - 2n_y{}^{(k)}\right)}{\sqrt{n_x{}^{(k)}n_y{}^{(k)}\left(n - n_x{}^{(k)}\right)\left(n - n_y{}^{(k)}\right)}} = 0$$

$$\lim_{n\to\infty} E\left[\left(\hat{\rho}_{xy}{}^{(k)}\right)^4\right] = \lim_{n\to\infty} \frac{n-1}{(n-2)(n-3)}\left[3(n+6) - \frac{6n^2}{n_x{}^{(k)}\left(n - n_x{}^{(k)}\right)} - \frac{6n^2}{n_y{}^{(k)}\left(n - n_y{}^{(k)}\right)} + \frac{n^3(n+1)}{n_x{}^{(k)}n_y{}^{(k)}\left(n - n_x{}^{(k)}\right)\left(n - n_y{}^{(k)}\right)}\right] = 3$$

Based on the numerical simulation, we also find that with the increase of *n*, the distribution of the normalized statistic $\hat{\rho}_{xy}{}^{(k)}$ of eqn. (S-2) will tend to standard normal distribution (Figure S2), and actually if $n > 100$, the statistic well follows normal distribution in general. Hence, we assume that if genes *x* and *y* are independent of each other, our normalized statistic follows standard normal distribution. We can use the normal distribution to test the independency or association of

any two genes in a cell $k$ by $\hat{\rho}_{xy}^{(k)}$ of eqn. (S-2).




**(A)** $n$ =100 **(B)** $n$ = 500

**Figure S2.** The comparision of standard normal distribution and the distribution of $\hat{\rho}_{xy}^{(k)}$. The density function is calculated by kernel density estimation based on 200,000 plots, and $n_x$ and $n_y$ are equal to 0.2$n$. The two genes $x$ and $y$ are independent of each other.

**(4) Performance of the statistic in different situations or associations**

We have discussed the mean value, variance and distribution of the statistic $\hat{\rho}_{xy}^{(k)}$ when genes $x$ and $y$ are independent of each other. Figure S3 illustrates the performance of the normalized statistic when genes $x$ and $y$ follow different distributions. We can see no matter which distribution genes $x$ and $y$ follow, if only genes $x$ and $y$ are independent, the distribution of $\hat{\rho}_{xy}^{(k)}$ always approaches normal distribution and few plots are larger than the significant level.

Figure S4 illustrates the performance if genes $x$ and $y$ are correlated in partial cells and uncorrelated in the other cells. We can see no matter if the correlation is positive, negative or nonlinear, the distribution of our statistic always shows the double crest, which indicates that $\hat{\rho}_{xy}^{(k)}$ is able to distinguish the cells with the correlated genes and uncorrelated genes wonderfully.

Figure S5 illustrates the performance if genes $x$ and $y$ are correlated in all cells. We can see no matter if the dependency is linear, nonlinear or complex, the distribution of our statistic is always far from 0 and few plots are smaller than the significant level.

As a summary, our statistic model is able to distinguish the cells with the correlated genes and uncorrelated genes in a reliable manner.

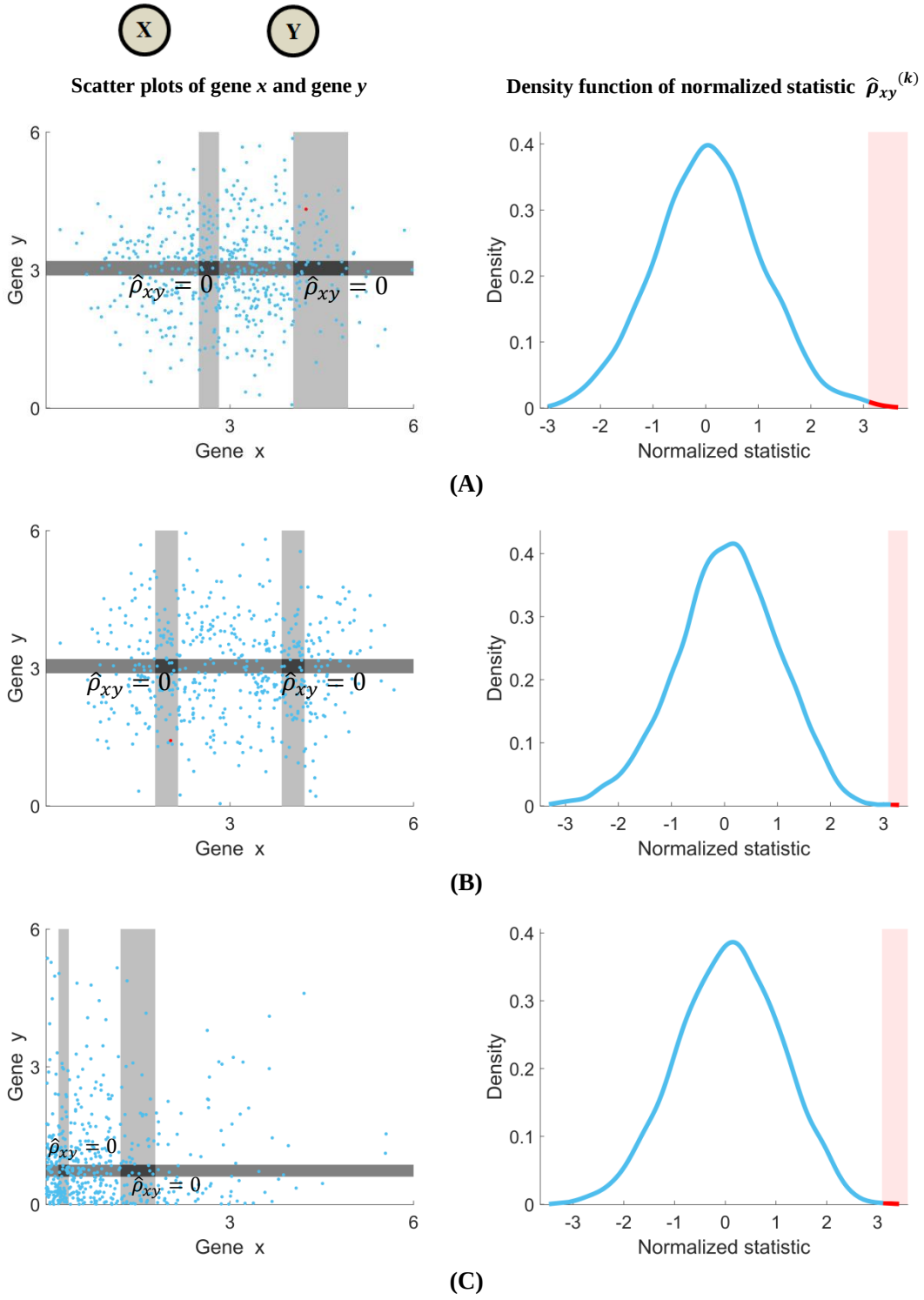



**Figure S3.** Performance of the statistic if genes *x* and *y* are independent of each other. $n_x^{(k)}$ and $n_y^{(k)}$ are set as 60 and $n$ = 500. The box size will be changed with the density of plots, smaller in the dense area and larger in the sparse area. Thus, no matter which distribution the genes follow, the boxes cover the same number of plots, and the statistic model will not change. Red plots in the left figures represent $\hat{\rho}_{xy}^{(k)}$ in these plots are larger than the significant level of 0.001, which is corresponding with the red area in the right figures. We can see if genes *x* and *y* are independent, the distribution of our statistic is always similar to normal distribution and few plots are larger than the significant level.

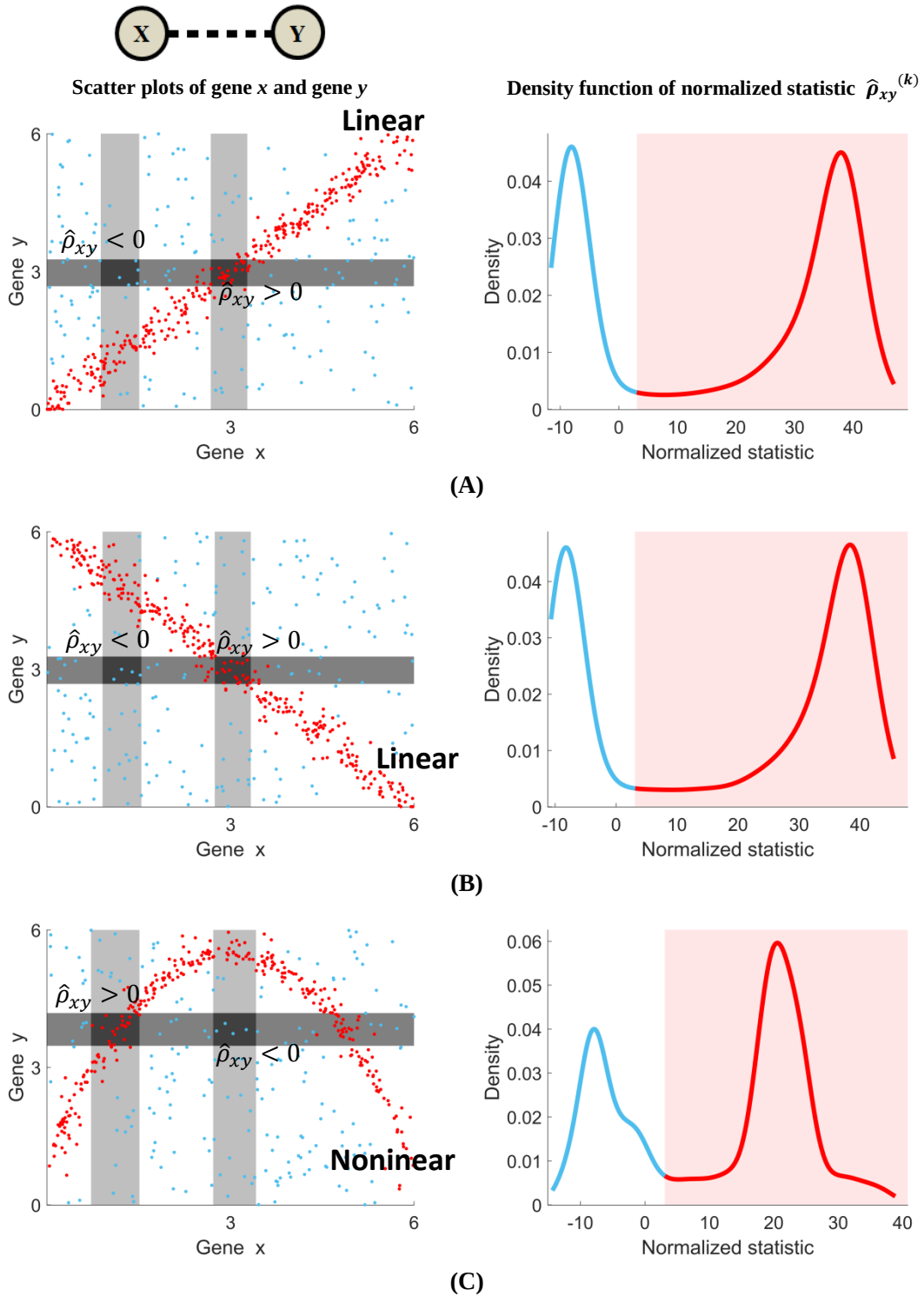



**Figure S4.** Performance of the statistic if genes *x* and *y* are correlated in partial cells and uncorrelated in the other cells. $n_x^{(k)}$ and $n_y^{(k)}$ are set as 60 and $n$ = 500. Red plots in the left figures represent $\hat{\rho}_{xy}^{(k)}$ in these plots are larger than the significant level of 0.001, which is corresponding with the red area in the right figures. We can see the distribution of $\hat{\rho}_{xy}^{(k)}$ always shows the double crest and is able to distinguish the cells with the correlated genes and uncorrelated genes wonderfully, no matter if the correlation is positive, negative or nonlinear.

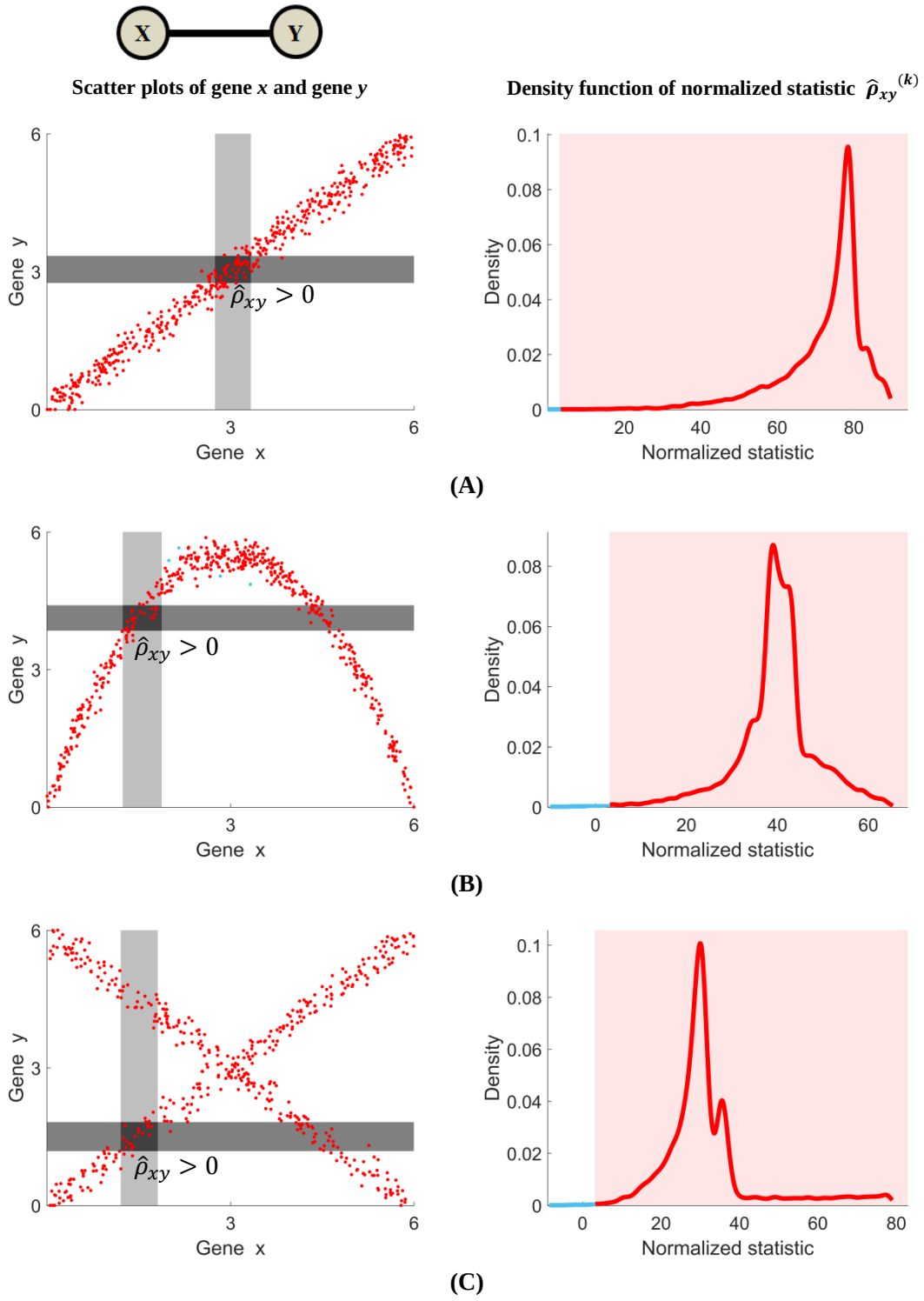



**Figure S5.** Performance of the statistic if genes *x* and *y* are correlated in all cells. $n_x^{(k)}$ and $n_y^{(k)}$ are set as 60 and $n$ = 500. Red plots in the left figures represent $\hat{\rho}_{xy}^{(k)}$ in these plots are larger than the significant level of 0.001, which is corresponding with the red area in the right figures. We can see the correlation can be identified in most cells, no matter if the correlation is positive, negative or complex dependency.

**(5) Performance of the statistic in the case of high dropout rate**

Some experimental platforms such as Drop-seq and 10×genomics will produce the scRNA-seq data with high dropout rate, and then the gene expression matrix may be quite sparse. Figure S6 illustrates the performance of our statistic in the case of high dropout rate. If genes *x* and *y* are expressed in only 10% and 20% of total cells respectively and the expression level is 1, our statistic $\hat{\rho}_{xy}^{(k)}$ can only get four values: $\hat{\rho}_{11}$, $\hat{\rho}_{01}$, $\hat{\rho}_{10}$, $\hat{\rho}_{00}$. Then, as shown in Figure S6 (C, D), if genes *x* and *y* are independent, $\hat{\rho}_{xy}$ still follows normal distribution. If genes *x* and *y* are dependent, for example, Matthew Correlation Coefficient (MCC) = 0.33, $\hat{\rho}_{11}$ and $\hat{\rho}_{00}$ will be much larger than 0, and $\hat{\rho}_{01}$ and $\hat{\rho}_{10}$ will be much smaller than 0, which produce the double crest in the histogram. As a result, no matter in the case of high dropout rate or low dropout rate, our statistic gets similar performance, and thus we do not need to change our statistical model for the sparse gene expression matrix especially.

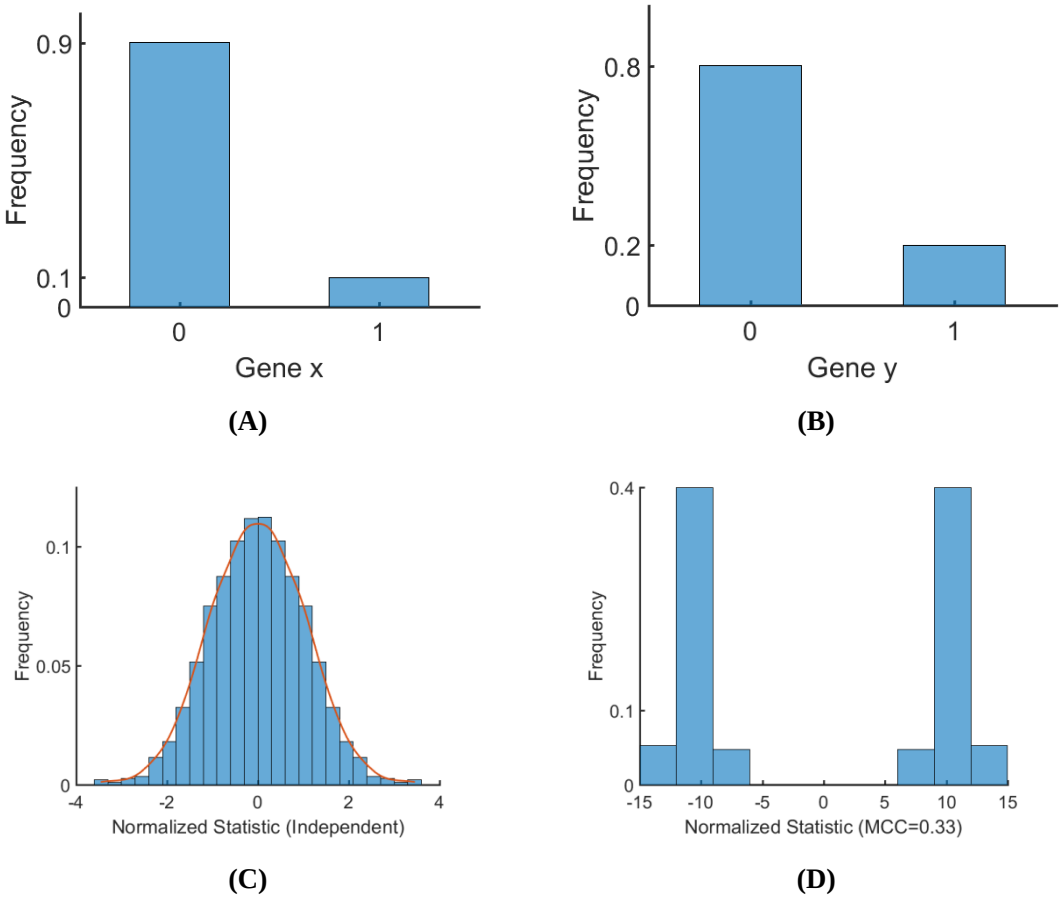



**Figure S6.** Performance of the statistic in the case of high dropout rate. **(A, B)** Histograms of the detected expression of genes *x* and *y*. **(C)** Histogram of the normalized statistic if genes *x* and *y* are independent. **(D)** Histogram of the normalized statistic if genes *x* and *y* are dependent (MCC = 0.33). Note that in (C) and (D), we made 1000 repetitions, and each figure was drawn from 4000 points (four points $\hat{\rho}_{11}$, $\hat{\rho}_{01}$, $\hat{\rho}_{10}$, $\hat{\rho}_{00}$ for each repetition).

**Supplementary Note 2: The robustness of CSNs and NDM on different cell populations**

If the dataset contains only one cell, it is impossible to construct the CSN; but if we have the expression profiles of more than one hundred cells, we can construct the network for each single cell, by exploring the distribution information from the population of cells. From the large number of cells in the dataset, we can estimate the joint distributions of every two genes, and these distributions can be used as the reference for CSN construction. However, if the composition of cells in the dataset is changed, for example, some new cells are added into the dataset, the reference or distribution might change with the new cells and then different CSNs would be constructed. In this section, we will discuss how CSNs change with the new cells or the composition of cells.

(1) First, if the proportion of each cell type in the new cells is the same as that in the original cells (i.e. the number of cells of each cell type increases with the same proportion), the distribution of each gene and the joint distribution $f(x, y)$ of each gene pair will not change, and then the statistical independency measurement $f(x, y) - f_X(x)\, f_Y(y)$ is fixed. As the statistic ${\rho_{xy}}^{(k)}$ in eqn. (S-1) is an approximation of $f(x_k, y_k) - f_X(x_k)\, f_Y(y_k)$, the expectation of the statistic is stable. Note that if we set ${n_x}^{(k)}$ and ${n_y}^{(k)}$ increase in proportion to $n$ (${n_x}^{(k)} = t_1 n$, ${n_y}^{(k)} = t_2 n$), the light grey, medium grey and dark grey boxes in Figure S1 (${D_x}^{(k)}$, ${D_y}^{(k)}$ and ${D_{xy}}^{(k)}$ respectively) will barely change, and then

$$E\left({\rho_{xy}}^{(k)}\right) = \frac{{n_x}^{(k)}{n_y}^{(k)}}{n^2}\left(\frac{p_{xy}}{p_x p_y} - 1\right) = t_1 t_2\left(\frac{p_{xy}}{p_x p_y} - 1\right)$$

is a constant, where $p_x = \int_{{D_x}^{(k)}} f_X(x)dx$, $p_y = \int_{{D_y}^{(k)}} f_Y(y)dy$ and $p_{xy} = \iint_{{D_{xy}}^{(k)}} f(x,y)dxdy$ are the probability that a plot is located in the ${D_x}^{(k)}$, ${D_y}^{(k)}$ and ${D_{xy}}^{(k)}$ respectively. $t_1$ and $t_2$ are constants.

However, with the increase of $n$, the variance will be smaller, and then

$$E\left(\hat{\rho}_{xy}{}^{(k)}\right) = \frac{E\left(\frac{{n_{xy}}^{(k)}}{n} - \frac{{n_x}^{(k)}{n_y}^{(k)}}{n^2}\right)}{\sqrt{\frac{{n_x}^{(k)}{n_y}^{(k)}\left(n - {n_x}^{(k)}\right)\left(n - {n_y}^{(k)}\right)}{n^4(n-1)}}} = \sqrt{n-1}\cdot\frac{t_1 t_2\left(\frac{p_{xy}}{p_x p_y} - 1\right)}{\sqrt{t_1 t_2(1-t_1)(1-t_2)}}$$

will be positive and become larger if $p_{xy} > p_x p_y$, negative and become smaller if $p_{xy} < p_x p_y$, and equal to 0 if $p_{xy} = p_x p_y$.

As a result, with the increase of $n$, the discrimination between the cells with the correlated genes and uncorrelated genes will become larger, the type II error will decrease, and some edges will be rediscovered in the new dataset.

(2) Next, if the new cells do not belong to any cell type in the original dataset (i.e. a new cell type is added into the dataset), the distribution of each gene and the joint distribution $f(x, y)$ of each gene pair will be changed, and then the CSNs constructed by the original dataset may be also changed. Here, to simplify the analysis, we suppose that there is the same distribution of each gene in the new cells as the original cells, but all of the genes do not have any correlations. Then, the new cells are obviously different from any cell type in the original dataset, and will interfere with the edge recognition in the CSN construction, which represents an extreme case.

We set $n_x^{(k)}$ and $n_y^{(k)}$ increase in proportion to sample size, number of the original cells is $n_0$, and number of the new cells is $n$. If CSNs are constructed by the original cells, then

$$E\left(\hat{\rho}_{xy}{}^{(k)}\right) = \sqrt{n_0 - 1} \cdot \frac{t_1 t_2 \left(\frac{p_{xy}}{p_x p_y} - 1\right)}{\sqrt{t_1 t_2 (1 - t_1)(1 - t_2)}}$$

On the other hand, if CSNs are constructed by the original cells and the new cells, then

$$E\left(\hat{\rho}_{xy}{}^{(k)\prime}\right) = \sqrt{n_0 + n - 1} \cdot \frac{t_1 t_2 \left(\frac{\frac{n_0 p_{xy} + n p_x p_y}{n_0 + n}}{p_x p_y} - 1\right)}{\sqrt{t_1 t_2 (1 - t_1)(1 - t_2)}}$$

Thus

$$\frac{E\left(\hat{\rho}_{xy}{}^{(k)\prime}\right)}{E\left(\hat{\rho}_{xy}{}^{(k)}\right)} = \frac{\sqrt{n_0 + n - 1} \cdot \frac{t_1 t_2 \left(\frac{n_0 p_{xy} + n p_x p_y}{p_x p_y (n_0 + n)} - 1\right)}{\sqrt{t_1 t_2 (1 - t_1)(1 - t_2)}}}{\sqrt{n_0 - 1} \cdot \frac{t_1 t_2 \left(\frac{p_{xy}}{p_x p_y} - 1\right)}{\sqrt{t_1 t_2 (1 - t_1)(1 - t_2)}}} = \frac{\sqrt{n_0 + n - 1} \cdot \frac{n_0}{n_0 + n}}{\sqrt{n_0 - 1}} \approx \frac{1}{\sqrt{1 + \frac{n}{n_0}}}$$

We can see the normalized statistic will become smaller with the increase of $n$. Compared with the CSNs constructed from the original dataset, the CSNs from the new dataset will lose some edges. However, if the significance of an edge (i.e. the normalized statistic) is sufficiently large, this edge can still be retained.

As a result, if a new cell type is added into the dataset, CSNs will lose some edges, but the significant edges are retained.

(3) Finally, if the new cells belong to partial cell types in the original dataset, the proportion of each cell type will change. It is obvious that to the cell types with more samples, more edges will be identified, while to the other cell types, the noise will increase and some edges will be lost. Hence, this situation is a combination of the two situations mentioned above.

As a conclusion, the robustness of an edge is determined by its normalized statistic; the larger normalized statistic, the more robust this edge is. In addition, if there are many or more cells in a cell type, the edges of the CSNs in this cell type can be identified more easily. Furthermore, the topological structure of CSN is stable and robust, which means the hub genes that have higher degrees in the CSN are always hub genes no matter how many edges can be identified to some extent.

On the other hand, we further transfer CSNs to a network degree matrix (NDM) to embody the network features and reduce the dimensions simultaneously. The value in this matrix is the number of edges connected to each gene in each CSN, which means that for gene $x$ in the network of cell $k$

$$NDM_{xk} = \sum_{y=1, y \neq x}^{m} edge_{xy}{}^{(k)} \tag{S-3}$$

Then we can get a matrix ***NDM*** with $m \times n$ elements. Moreover, in this work we normalize the ***NDM*** by

$$\widehat{NDM}_{xk} = \frac{NDM_{xk}}{\sum_{i=1}^{m} NDM_{ik}} \cdot \frac{a^2}{c} \tag{S-4}$$

where *a* is the average genes per cell in the scRNA-seq data and is defined as

$$a = \frac{1}{n}\sum_{j=1}^{n}\sum_{i=1}^{m} \mathrm{sgn}\left(GEM_{ij}\right)$$

*c* is a constant, and is chosen as 2000 in this work based on the computational simulation.

***GEM*** is the original gene expression matrix and sgn() is sign function. Thus each cell has the same number of network degrees, which is determined by the average genes per cell in the scRNA-seq data. The normalization is able to improve the robustness and helps to the comparison of the cells from different cell populations. Hence, for NDM, we can easily conclude that if there are more cells in a cell type, there are more edges in the CSNs of this cell type, but if we normalize the NDM by eqn. (S-4), NDM will be robust.

**Supplementary Note 3: The input, output and application fields of our CSN method**

***Input:***

Gene expression matrix (RPKM/FPKM/TPM/count)

Significant level (e.g. 0.001, 0.01, 0.05 …). If the significant level is larger, it is easier to reject the null hypothesis and get more edges. In this paper, we set significant level as 0.01.

Box size. $n_x^{(k)}$ and $n_y^{(k)}$ should be determined in advance. In this paper, we set $n_x^{(k)}$ and $n_y^{(k)}$ approximately equal to $0.1n$. Users can change this parameter as well.

***Output:***

Cell-specific network for each cell (rows = genes, column = genes)

Network degree matrix (rows = genes, column = cells)

Normalized statistic of edge *x*-*y* for all cells ($1 \times n$ vector)

***Application fields:***

The number of cells should be 100 at least.

**Supplementary Note 4: Algorithms and their parameters used in clustering, visualization and pseudo-trajectory analysis**

*Preprocessing*: GEM is preprocessed from initial gene expression matrix by normalization, gene selection (listed in Supplementary Note 5) and logarithm, i.e. $\hat{x} = \log(1 + x)$. NDM is straight transformed from GEM and also preprocessed by logarithm.

*Hierarchical clustering*

Distance between two objects: *Euclidean distance*

Algorithm for computing distance between clusters: *Ward's linkage* (Inner squared distance, minimum variance algorithm)

Number of clusters: *Same as the number of categories in the original dataset*

*k-means clustering*

Centroid initialization: *k-means++ algorithm* (1)

Distance between two objects: *Squared Euclidean distance*

Maximum number of iterations: *1000*

Number of times to repeat clustering using new initial cluster centroid positions: *100*

Number of clusters: *Same as the number of categories in the original dataset*

*k-medoids clustering*

Algorithm to find medoids: *Partitioning Around Medoids (PAM)* (2)

Centroid initialization: *k-means++ algorithm* (1)

Distance between two objects: *Squared Euclidean distance*

Maximum number of iterations: *1000*

Number of times to repeat clustering using new initial cluster centroid positions: *1*

Number of clusters: *Same as the number of categories in the original dataset*

*SNN-Cliq* (3)

Number of neighbors: *5*

Other parameters: *Default*

*SIMLR* (4)

Number of clusters: *Same as the number of categories in the original dataset*

Other parameters: *Default*

*t-SNE* (5)

*In clustering analysis:*

Preprocessed by PCA to reduce the dimensions to: *20*

t-SNE reduce the dimensions to: *5*

Perplexity: *30*

Clustering method: *Hierarchical clustering / k-means*

*In visualization:*

Preprocessed by PCA to reduce the dimensions to: *20*

t-SNE reduce the dimensions to: *2*
Perplexity: *30*

*Wanderlust* (6)

Preprocessed by PCA to reduce the dimensions to: *250 (Chu-time) / 200 (Trapnell)*
Branch: *no branch*
Other parameters: *Default*

**Supplementary Note 5: Datasets used for validation of CSN**

Ten single-cell RNA-seq datasets (7-15) and one bulk RNA-seq dataset are used in this paper. The normalization, gene selection rules and data sources of all datasets are listed below.

| Dataset | Number of cells | Number of cell types | Normalization method | Number of genes used in GEM and NDM | Gene selection rules | Data Sources |
|---|---|---|---|---|---|---|
| *Buettner* | 182 | 3 | FPKM | 8988 | FPKM per cell on average > 5 | *Supplementary Data 2* |
| *Kolodziejczyk* | 704 | 3 | count | 10685 | Counts per cell on average > 10 | ArrayExpress (http://www.ebi.ac.uk/arrayexpress) *E-MTAB-2600* |
| *Pollen* | 249 | 11 | TPM | 14805 | Expressed in at least 10 cells | NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/Traces/sra/) *SRP041736* |
| *Zeisel* | 3005 | 9 | TPM | 15964 | | Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) *GSE60361* |
| *Darmanis* | 420 | 8 | TPM | 15651 | | *GSE67835* |
| *Chu-type* | 1018 | 7 | FPKM | 16619 | | *GSE75748* |
| *Chu-time* | 758 | 6 | FPKM | 15691 | | *GSE75748* |
| *Trapnell* | 372 | 4 | FPKM | 17867 | | *GSE52529* |
| *Kim* | 118 | 3 | TPM | 13392 | | *GSE73121* |
| *Xin* | 1600 | 4 | RPKM | 25040 | | *GSE81608* |
| *TCGA* | 1135 | 4 | FPKM | 33409 | Expressed in at least 500 samples | The Cancer Genome Atlas (TCGA) *Project: TCGA-LUAD and TCGA-LUSC* (https://cancergenome.nih.gov) |

**Supplementary Note 6: The relevance between correlation coefficient and normalized statistic**

We selected 500 genes with the largest variance on *Chu-type* dataset, and then calculated the squared correlation coefficient $R^2$ and average normalized statistic for every two genes in H1 cells. Figure S7 indicates the high relevance between $R^2$ and the normalized statistic of eqn. (S-2). As shown in the figure, a few points get the large $R^2$ but normalized statistic is almost zero, which is because the two genes in most cells are not expressed except one or two cells, and thus $R^2$ approaches 1 by only those cells.

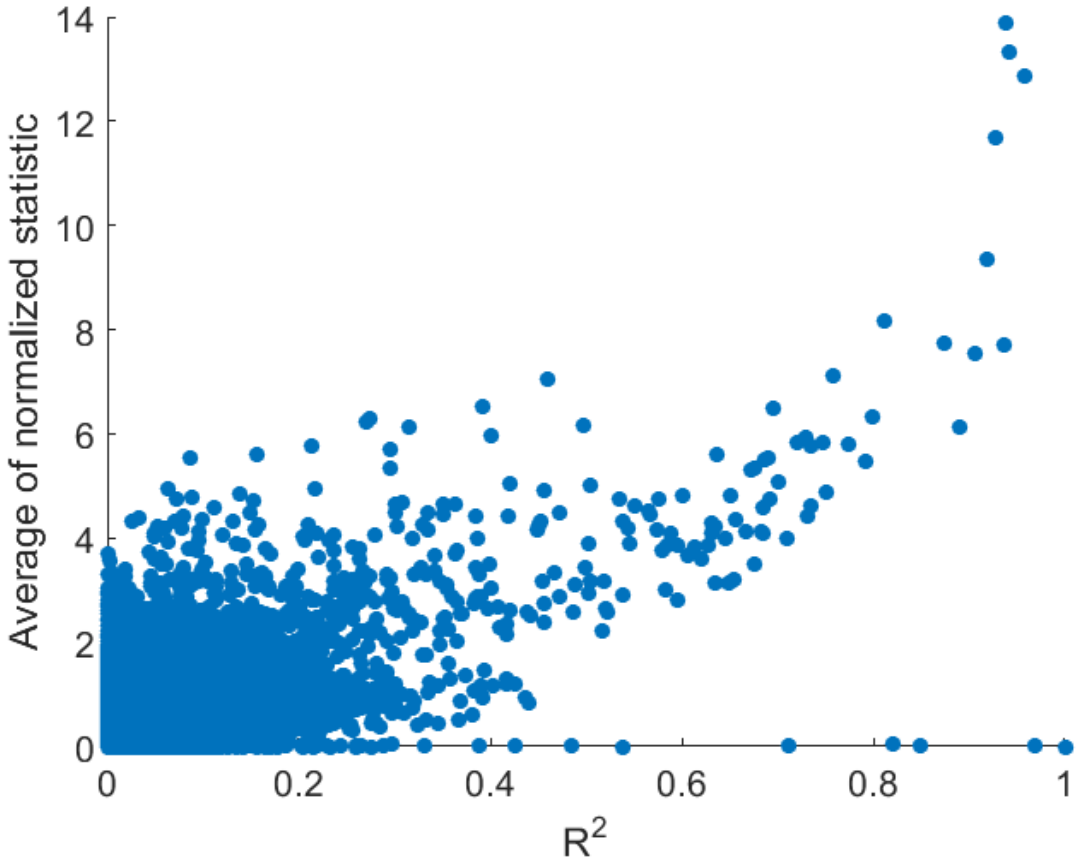



**Figure S7.** The relevance between squared correlation coefficient and average normalized statistic in H1 cells.

**Supplementary Note 7: Illustration of some gene associations**

Figure S8 illustrates some gene associations with POU5F1 based on *Chu-type* dataset. Some literatures have validated the gene interactions between POU5F1 and CDH1 (16), POU5F1 and EPAS1 (17), POU5F1 and NANOG (18), which indicates that it is possible to find the information of gene associations by scRNA-seq data.

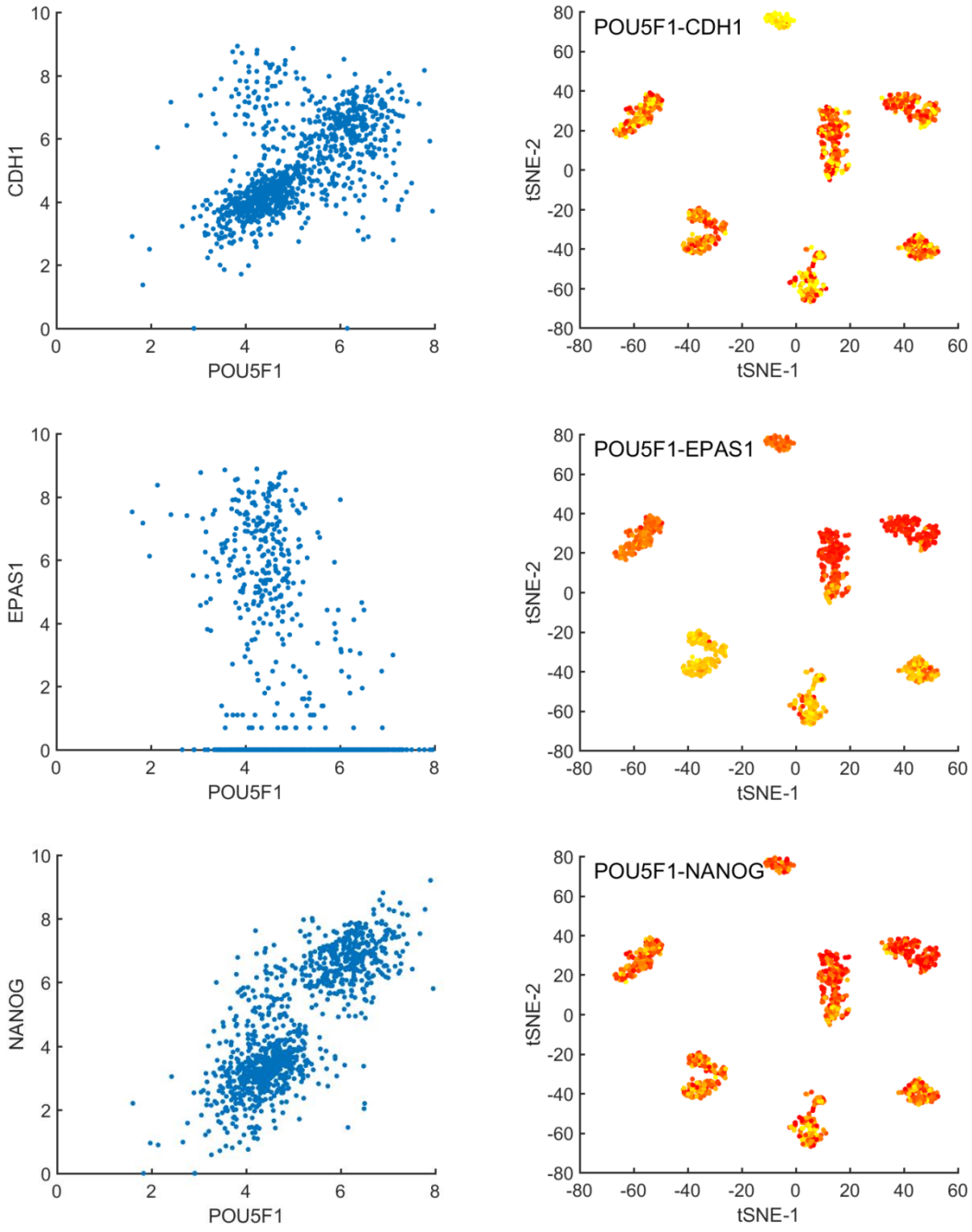



**Figure S8.** Scatter diagrams and performance of the normalized statistic of edge POU5F1 - CDH1, POU5F1 - EPAS1 and POU5F1 – NANOG.

**Supplementary Note 8: The top genes with the highest degrees on Chu-type dataset**

We listed the top 10 genes with the highest degrees of each cell type on *Chu-type* dataset.

| H1 | H9 | DEC | EC | HFF | NPC | TB |
|---|---|---|---|---|---|---|
| PHC1 | DPPA4 | CER1 | PECAM1 | LIN28A | CDH6 | ITGB6 |
| L1TD1 | L1TD1 | SERPINB9 | CXorf36 | B2M | LIX1 | VGLL1 |
| TERF1 | PHC1 | POU2AF1 | MMRN2 | AXL | LRP2 | PLSCR5 |
| DPPA4 | TERF1 | ERBB4 | TFPI | LGALS1 | PAX6 | GABRP |
| TDGF1 | POU5F1 | GATA4 | F2RL2 | ANXA2 | ILDR2 | PIK3C2G |
| POLR3G | ZFP42 | RHOBTB3 | TNFSF10 | CD24 | DLK1 | PAPPA2 |
| USP44 | HSPD1 | STC1 | SPTBN1 | CD59 | DPYSL5 | GUCY1A3 |
| DNMT3B | USP44 | CST1 | SCARF1 | THBS1 | OTX1 | HAND1 |
| POU5F1 | CD24 | FGF17 | CARD8 | COL1A1 | TMSB4X | TPM1 |
| S100A11 | SNURF | MYCT1 | SOX7 | TWSG1 | EFNA5 | P2RY6 |

**Supplementary Note 9: The "darker" genes of each cell type on Chu-type dataset**

We listed the "darker" genes of each cell type on *Chu-type* dataset (p-value in this table is the maximum p-value among the top-expressed cell type and other cell type, and Wilcoxon rank-sum test is used).

| Gene | Cell type | p-value of network degree | p-value of expression | Gene | Cell type | p-value of network degree | p-value of expression |
|---|---|---|---|---|---|---|---|
| TDGF1 | H1 | 3.59E-06 | 0.956 | MSL1 | EC | 3.32E-06 | 0.010 |
| VSNL1 | H1 | 3.62E-08 | 0.094 | PHF8 | EC | 1.07E-06 | 0.086 |
| DNAH11 | H9 | 2.00E-06 | 0.030 | GORAB | EC | 1.01E-06 | 0.573 |
| NPPB | HFF | 1.66E-07 | 0.015 | KANSL3 | EC | 1.51E-07 | 0.026 |
| TMEM97 | NPC | 3.33E-06 | 0.053 | EFNA2 | EC | 1.01E-07 | 0.017 |
| SGPL1 | NPC | 7.88E-06 | 0.818 | UBQLN4 | EC | 2.14E-06 | 0.061 |
| ICAM1 | NPC | 2.18E-07 | 0.038 | SIK2 | EC | 3.12E-06 | 0.016 |
| ARSA | NPC | 6.86E-06 | 0.019 | FBXO33 | EC | 6.57E-06 | 0.794 |
| MX2 | DEC | 9.35E-06 | 0.015 | KANSL1L | EC | 1.01E-06 | 0.016 |
| GATA2 | DEC | 4.92E-06 | 0.504 | STAT5B | EC | 3.74E-07 | 0.031 |
| ACTA2 | DEC | 3.39E-12 | 0.022 | ASB3 | EC | 6.23E-07 | 0.024 |
| LGALS3 | DEC | 1.73E-16 | 0.337 | THAP3 | EC | 1.60E-06 | 0.017 |
| TNFRSF19 | DEC | 5.34E-06 | 0.016 | TTC32 | EC | 8.76E-06 | 0.195 |
| FAM131B | EC | 6.30E-06 | 0.012 | ZHX2 | EC | 2.03E-06 | 0.053 |
| PHF13 | EC | 2.96E-06 | 0.026 | TAF1C | EC | 5.64E-06 | 0.151 |
| ZNF226 | EC | 1.39E-06 | 0.904 | ZNF618 | EC | 2.45E-07 | 0.095 |
| IFT122 | EC | 3.08E-06 | 0.014 | C1orf21 | EC | 2.49E-06 | 0.024 |
| CDH8 | EC | 4.98E-06 | 0.012 | AGBL5 | EC | 1.75E-07 | 0.042 |
| EZH1 | EC | 2.60E-08 | 0.028 | EXOC6 | EC | 6.33E-06 | 0.014 |
| KHDRBS3 | EC | 2.09E-07 | 0.033 | SCMH1 | EC | 1.67E-07 | 0.188 |
| IDH2 | EC | 2.26E-07 | 0.051 | DOCK3 | EC | 1.43E-06 | 0.044 |
| EFCAB7 | EC | 4.92E-08 | 0.024 | FXR2 | TB | 9.83E-06 | 0.017 |

**Supplementary Note 10: Results of clustering analysis**

We used different measurements to evaluate the performances of clustering analysis, which are shown below.

**(1)** ***Adjusted Random Index (ARI)***

| | | Buettner | Kolod ziejczyk | Pollen | Zeisel | Darmanis | Chu-type | Chu-time | Kim | Trapnell |
|---|---|---|---|---|---|---|---|---|---|---|
| Hierarchical | GEM | 0.48 | 0.49 | 0.95 | 0.55 | 0.63 | 0.75 | 0.67 | 0.66 | 0.08 |
| | **NDM** | 0.82 | 0.99 | 0.96 | 0.53 | 0.91 | 0.77 | 0.72 | 0.73 | 0.24 |
| k-means | GEM | 0.31 | 0.53 | 0.90 | 0.39 | 0.58 | 0.73 | 0.59 | 0.60 | 0.14 |
| | **NDM** | 0.74 | 0.80 | 0.87 | 0.43 | 0.77 | 0.77 | 0.70 | 0.83 | 0.44 |
| Hierarchical (tSNE) | GEM | 0.32 | 0.99 | 0.94 | 0.60 | 0.67 | 0.98 | 0.68 | 0.66 | 0.16 |
| | **NDM** | 0.97 | 1.00 | 0.85 | 0.62 | 0.86 | 0.99 | 0.68 | 1.00 | 0.43 |
| k-means (tSNE) | GEM | 0.30 | 0.99 | 0.94 | 0.62 | 0.65 | 0.98 | 0.69 | 0.72 | 0.16 |
| | **NDM** | 0.94 | 1.00 | 0.85 | 0.65 | 0.85 | 0.99 | 0.69 | 1.00 | 0.47 |
| k-medoids | GEM | 0.14 | 0.03 | 0.91 | 0.43 | 0.36 | 0.60 | 0.43 | 0.57 | 0.00 |
| | **NDM** | 0.31 | 0.73 | 0.89 | 0.11 | 0.23 | 0.76 | 0.41 | 0.61 | 0.23 |
| SIMLR | GEM | 0.92 | 0.99 | 0.90 | 0.56 | 0.75 | 0.74 | 0.66 | 0.97 | 0.21 |
| | **NDM** | 1.00 | 1.00 | 0.92 | 0.67 | 0.90 | 0.75 | 0.67 | 0.95 | 0.31 |
| SNN-Cliq | GEM | 0.00 | 0.00 | 0.90 | 0.50 | 0.20 | 0.64 | 0.30 | 0.58 | 0.00 |
| | **NDM** | 0.50 | 0.65 | 0.90 | 0.60 | 0.01 | 0.61 | 0.36 | 0.58 | 0.24 |

**(2)** ***F1-measure***

| | | Buettner | Kolod ziejczyk | Pollen | Zeisel | Darmanis | Chu-type | Chu-time | Kim | Trapnell |
|---|---|---|---|---|---|---|---|---|---|---|
| Hierarchical | GEM | 0.66 | 0.67 | 0.95 | 0.63 | 0.69 | 0.79 | 0.73 | 0.78 | 0.35 |
| | **NDM** | 0.88 | 0.99 | 0.96 | 0.61 | 0.93 | 0.81 | 0.77 | 0.82 | 0.49 |
| k-means | GEM | 0.54 | 0.70 | 0.91 | 0.48 | 0.65 | 0.78 | 0.67 | 0.73 | 0.36 |
| | **NDM** | 0.83 | 0.87 | 0.88 | 0.54 | 0.82 | 0.81 | 0.76 | 0.89 | 0.60 |
| Hierarchical (tSNE) | GEM | 0.55 | 0.99 | 0.95 | 0.67 | 0.73 | 0.98 | 0.74 | 0.78 | 0.38 |
| | **NDM** | 0.98 | 1.00 | 0.86 | 0.69 | 0.88 | 0.99 | 0.74 | 1.00 | 0.60 |
| k-means (tSNE) | GEM | 0.54 | 0.99 | 0.95 | 0.68 | 0.71 | 0.98 | 0.75 | 0.82 | 0.38 |
| | **NDM** | 0.96 | 1.00 | 0.86 | 0.71 | 0.88 | 0.99 | 0.75 | 1.00 | 0.62 |
| k-medoids | GEM | 0.46 | 0.50 | 0.92 | 0.52 | 0.48 | 0.67 | 0.54 | 0.72 | 0.37 |
| | **NDM** | 0.55 | 0.82 | 0.91 | 0.34 | 0.40 | 0.79 | 0.52 | 0.75 | 0.49 |
| SIMLR | GEM | 0.95 | 0.99 | 0.91 | 0.63 | 0.80 | 0.78 | 0.73 | 0.98 | 0.41 |
| | **NDM** | 1.00 | 1.00 | 0.92 | 0.73 | 0.92 | 0.79 | 0.74 | 0.97 | 0.51 |
| SNN-Cliq | GEM | 0.50 | 0.52 | 0.91 | 0.58 | 0.39 | 0.70 | 0.48 | 0.72 | 0.37 |
| | **NDM** | 0.69 | 0.75 | 0.91 | 0.67 | 0.34 | 0.67 | 0.47 | 0.74 | 0.48 |

**(3) *Purity***

| | | Buettner | Kolod ziejczyk | Pollen | Zeisel | Darmanis | Chu-type | Chu-time | Kim | Trapnell |
|---|---|---|---|---|---|---|---|---|---|---|
| Hierarchical | GEM | 0.77 | 0.75 | 0.97 | 0.80 | 0.85 | 0.83 | 0.80 | 0.84 | 0.42 |
| | **NDM** | 0.93 | 1.00 | 0.97 | 0.77 | 0.94 | 0.86 | 0.87 | 0.89 | 0.48 |
| k-means | GEM | 0.70 | 0.76 | 0.92 | 0.78 | 0.84 | 0.82 | 0.75 | 0.81 | 0.45 |
| | **NDM** | 0.91 | 0.92 | 0.89 | 0.74 | 0.83 | 0.87 | 0.87 | 0.93 | 0.65 |
| Hierarchical (tSNE) | GEM | 0.67 | 1.00 | 0.96 | 0.89 | 0.89 | 0.99 | 0.83 | 0.84 | 0.46 |
| | **NDM** | 0.99 | 1.00 | 0.91 | 0.88 | 0.94 | 1.00 | 0.82 | 1.00 | 0.67 |
| k-means (tSNE) | GEM | 0.68 | 1.00 | 0.96 | 0.89 | 0.89 | 0.99 | 0.83 | 0.88 | 0.46 |
| | **NDM** | 0.98 | 1.00 | 0.91 | 0.88 | 0.94 | 1.00 | 0.83 | 1.00 | 0.67 |
| k-medoids | GEM | 0.56 | 0.50 | 0.93 | 0.73 | 0.71 | 0.75 | 0.67 | 0.82 | 0.30 |
| | **NDM** | 0.65 | 0.89 | 0.92 | 0.46 | 0.50 | 0.87 | 0.68 | 0.77 | 0.50 |
| SIMLR | GEM | 0.97 | 1.00 | 0.94 | 0.86 | 0.90 | 0.84 | 0.82 | 0.99 | 0.48 |
| | **NDM** | 1.00 | 1.00 | 0.95 | 0.91 | 0.93 | 0.84 | 0.79 | 0.98 | 0.56 |
| SNN-Cliq | GEM | 0.36 | 0.42 | 0.94 | 0.83 | 0.68 | 0.77 | 0.54 | 0.81 | 0.29 |
| | **NDM** | 0.73 | 0.94 | 0.91 | 0.87 | 0.34 | 0.89 | 0.70 | 0.76 | 0.55 |

**(4) *Entropy***

| | | Buettner | Kolod ziejczyk | Pollen | Zeisel | Darmanis | Chu-type | Chu-time | Kim | Trapnell |
|---|---|---|---|---|---|---|---|---|---|---|
| Hierarchical | GEM | 0.77 | 0.72 | 0.12 | 0.78 | 0.53 | 0.43 | 0.62 | 0.54 | 1.76 |
| | **NDM** | 0.31 | 0.02 | 0.10 | 0.86 | 0.29 | 0.32 | 0.52 | 0.45 | 1.30 |
| k-means | GEM | 1.02 | 0.69 | 0.28 | 0.93 | 0.64 | 0.46 | 0.77 | 0.66 | 1.66 |
| | **NDM** | 0.49 | 0.31 | 0.34 | 1.06 | 0.66 | 0.32 | 0.54 | 0.32 | 1.01 |
| Hierarchical (tSNE) | GEM | 1.02 | 0.01 | 0.14 | 0.52 | 0.30 | 0.07 | 0.55 | 0.52 | 1.62 |
| | **NDM** | 0.07 | 0.00 | 0.28 | 0.59 | 0.21 | 0.03 | 0.55 | 0.00 | 1.02 |
| k-means (tSNE) | GEM | 1.02 | 0.01 | 0.14 | 0.51 | 0.31 | 0.07 | 0.53 | 0.46 | 1.62 |
| | **NDM** | 0.12 | 0.00 | 0.28 | 0.57 | 0.23 | 0.03 | 0.52 | 0.00 | 0.98 |
| k-medoids | GEM | 1.19 | 1.42 | 0.29 | 1.10 | 1.13 | 0.71 | 1.10 | 0.69 | 1.95 |
| | **NDM** | 1.03 | 0.45 | 0.27 | 2.12 | 1.61 | 0.37 | 1.13 | 0.63 | 1.30 |
| SIMLR | GEM | 0.14 | 0.01 | 0.24 | 0.64 | 0.36 | 0.43 | 0.57 | 0.06 | 1.50 |
| | **NDM** | 0.00 | 0.00 | 0.16 | 0.50 | 0.28 | 0.36 | 0.62 | 0.11 | 1.20 |
| SNN-Cliq | GEM | 1.58 | 1.54 | 0.27 | 0.69 | 1.39 | 0.66 | 1.72 | 0.68 | 1.98 |
| | **NDM** | 0.80 | 0.26 | 0.21 | 0.65 | 2.49 | 0.28 | 1.04 | 0.62 | 1.14 |

## Supplementary Note 11: Results of visualization

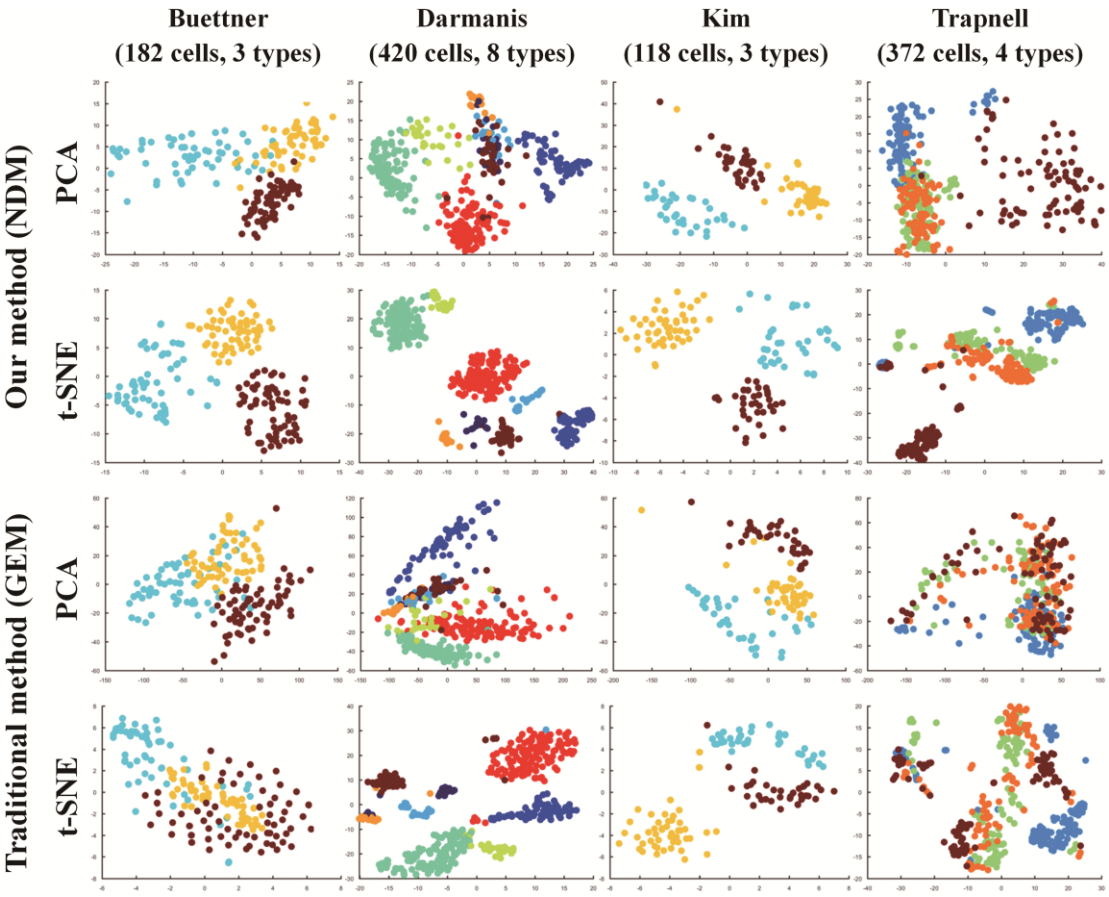



**Figure S9.** The clustering performances of NDM and GEM on four datasets. PCA and t-SNE that represent linear and nonlinear dimension-reduction method respectively are used for visualization. Different colors represent different cell types. x-axis and y-axis in each graph represent PC1 and PC2 (or tSNE1 and tSNE2), respectively.

## Supplementary Note 12: Results of pseudo trajectory analysis

In this paper, we used two datasets with the gold standard from literatures, which include 758 cells with 6 stages (0h, 12h, 24h, 36h, 72h, 96h) in Chu-time dataset (14) and 372 cells with 4 stages (0h, 24h, 48h, 72h) in Trapnell dataset (10). Wanderlust (6) is a method to construct no-branch pseudo trajectory, which gives each cell a value to represent the cell order, and the cells in the later stages will get larger values. GEM and NDM are used for comparison. Figure S10 illustrates that the Wanderlust values increase in accordance with the time sequence in Chu-time dataset, and the results of GEM and NDM are quite similar. But in Trapnell dataset, NDM is able to identify the change at 72h, but GEM fails.

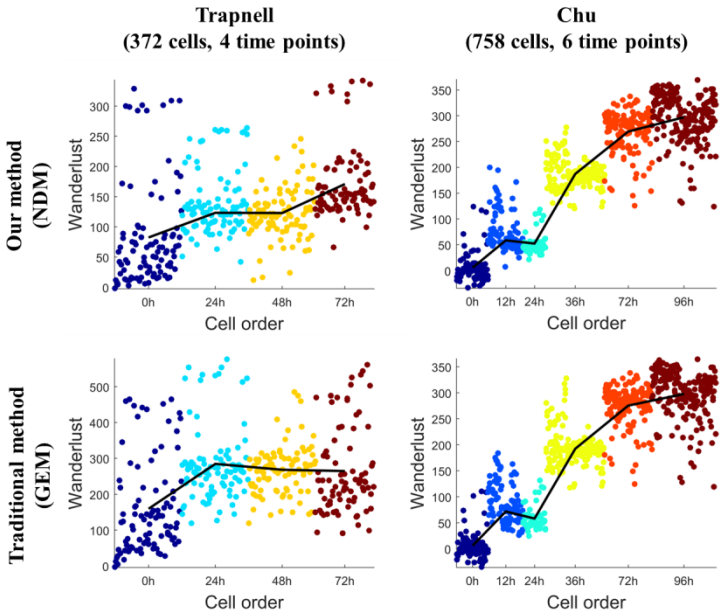



**Figure S10.** The comparison of GEM and NDM in pseudo trajectory analysis. The cells in the later stages will get larger Wanderlust values, and the average at every time points is shown as black line.

**Supplementary Note 13: Comparison of different parameters of CSN**

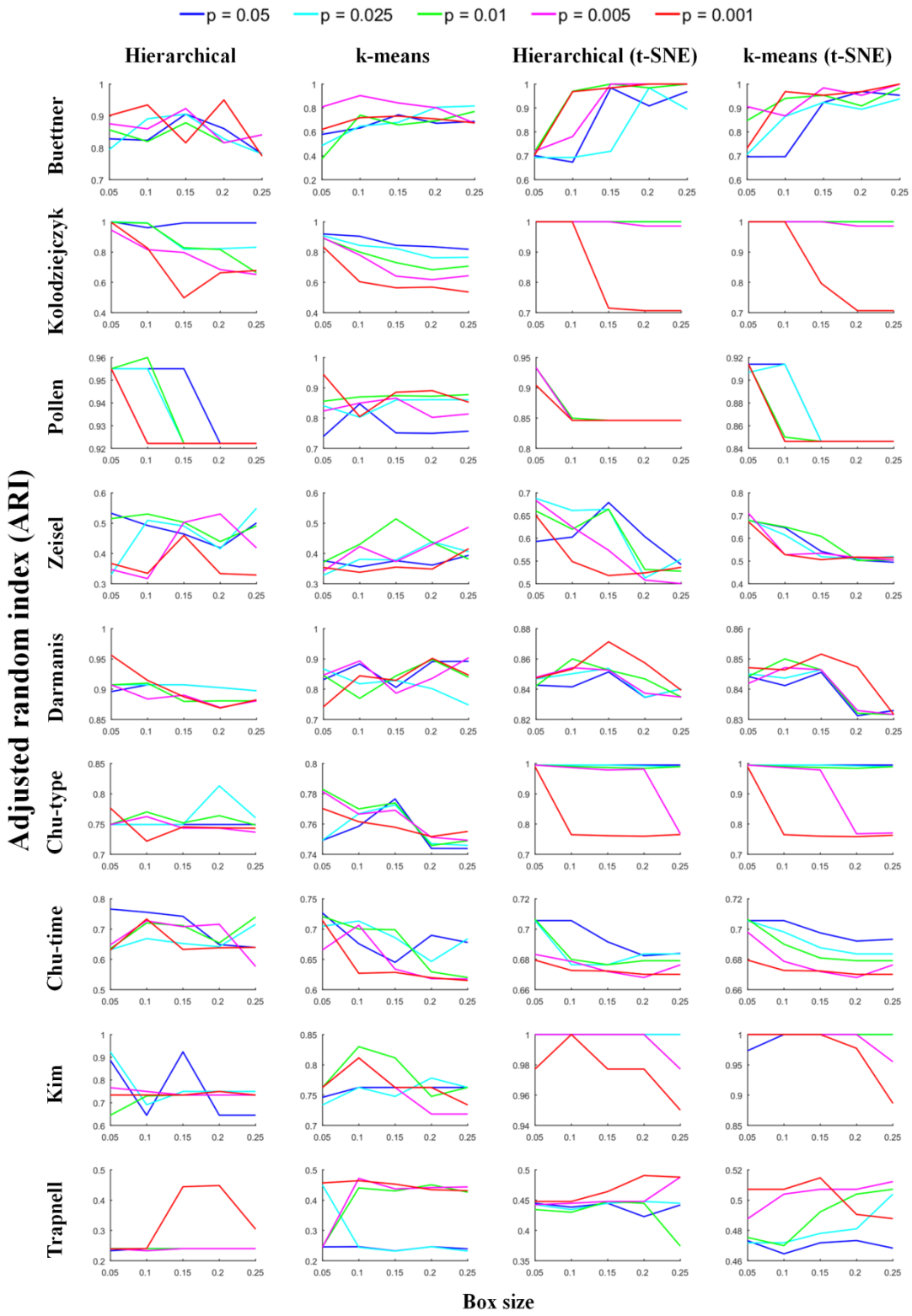



**Figure S11**. Comparison of different parameters of CSN. X-axis is box size and different colors represent different *p*-value. Y-axis is the ARI in clustering analysis. The results show that the optimum box size is about 0.1, and the optimum p-value is about 0.01, on average.

**Supplementary Note 14: Comparison of different normalization methods**

ARI of GEM and NDM in clustering analysis between different normalization methods is listed below. We can see NDM from the GEM normalized by TPM/FPKM/count gets the similar performances on the same dataset, though the result by TPM seems to be better. Thus, our NDM method is not sensitive to the normalization method, and is suitable to various types of gene expression matrix.

| | Kolodziejczyk (NDM) | | Kolodziejczyk (GEM) | | Pollen (NDM) | | Pollen (GEM) | |
|---|---|---|---|---|---|---|---|---|
| | TPM | count | TPM | count | TPM | count | TPM | Count |
| Hierarchical | 1 | 0.99 | 0.73 | 0.49 | 0.96 | 0.92 | 0.95 | 0.95 |
| k-means | 0.93 | 0.80 | 0.70 | 0.53 | 0.87 | 0.82 | 0.90 | 0.87 |
| Hierarchical (tSNE) | 1 | 1 | 0.73 | 0.99 | 0.85 | 0.85 | 0.94 | 0.88 |
| k-means (tSNE) | 1 | 1 | 0.73 | 0.99 | 0.85 | 0.85 | 0.94 | 0.92 |

| | Zeisel (NDM) | | Zeisel (GEM) | | Darmanis (NDM) | | Darmanis (GEM) | |
|---|---|---|---|---|---|---|---|---|
| | TPM | count | TPM | count | TPM | count | TPM | Count |
| Hierarchical | 0.53 | 0.56 | 0.55 | 0.39 | 0.91 | 0.89 | 0.63 | 0.46 |
| k-means | 0.43 | 0.41 | 0.39 | 0.36 | 0.77 | 0.80 | 0.58 | 0.42 |
| Hierarchical (tSNE) | 0.62 | 0.59 | 0.60 | 0.58 | 0.86 | 0.85 | 0.67 | 0.62 |
| k-means (tSNE) | 0.65 | 0.55 | 0.62 | 0.57 | 0.85 | 0.85 | 0.65 | 0.61 |

| | Chu-type (NDM) | | Chu-type (GEM) | | Chu-time (NDM) | | Chu-time (GEM) | |
|---|---|---|---|---|---|---|---|---|
| | TPM | FPKM | TPM | FPKM | TPM | FPKM | TPM | FPKM |
| Hierarchical | 0.75 | 0.77 | 0.75 | 0.75 | 0.67 | 0.72 | 0.79 | 0.67 |
| k-means | 0.75 | 0.77 | 0.75 | 0.73 | 0.72 | 0.70 | 0.72 | 0.59 |
| Hierarchical (tSNE) | 0.99 | 0.99 | 0.99 | 0.98 | 0.72 | 0.68 | 0.72 | 0.68 |
| k-means (tSNE) | 0.99 | 0.99 | 0.99 | 0.98 | 0.72 | 0.69 | 0.72 | 0.69 |

| | Kim (NDM) | | Kim (GEM) | | Trapnell (NDM) | | Trapnell (GEM) | |
|---|---|---|---|---|---|---|---|---|
| | TPM | FPKM | TPM | FPKM | TPM | FPKM | TPM | FPKM |
| Hierarchical | 0.73 | 0.73 | 0.66 | 0.66 | 0.24 | 0.24 | 0.08 | 0.08 |
| k-means | 0.83 | 0.75 | 0.60 | 0.56 | 0.48 | 0.44 | 0.13 | 0.14 |
| Hierarchical (tSNE) | 1 | 1 | 0.66 | 0.95 | 0.44 | 0.43 | 0.20 | 0.16 |
| k-means (tSNE) | 1 | 1 | 0.72 | 0.76 | 0.5 | 0.47 | 0.20 | 0.16 |

**Supplementary Note 15: Comparison of different gene selection rules**

ARI of GEM and NDM in clustering analysis between different gene selection rules is listed below. We can see that the different gene selection rules have just a little influence on the performance of GEM and NDM, and NDM is still superior to GEM clearly.

| | Buettner (NDM) | | | | Buettner (GEM) | | | |
|---|---|---|---|---|---|---|---|---|
| | FPKM per cell on average | | | | FPKM per cell on average | | | |
| | > 1 | > 5 | > 10 | > 50 | > 1 | > 5 | > 10 | > 50 |
| Hierarchical | 0.91 | 0.82 | 0.85 | 0.85 | 0.26 | 0.48 | 0.34 | 0.38 |
| k-means | 0.90 | 0.74 | 0.85 | 0.75 | 0.30 | 0.31 | 0.25 | 0.31 |
| Hierarchical (tSNE) | 0.78 | 0.97 | 0.70 | 0.95 | 0.37 | 0.32 | 0.29 | 0.38 |
| k-means (tSNE) | 0.84 | 0.94 | 0.71 | 0.95 | 0.30 | 0.30 | 0.31 | 0.42 |

| | Darmanis (NDM) | | | | Darmanis (GEM) | | | |
|---|---|---|---|---|---|---|---|---|
| | Detected expression in at least | | | | Detected expression in at least | | | |
| | 1 cell | 5 cells | 10 cells | 50 cells | 1 cell | 5 cells | 10 cells | 50 cells |
| Hierarchical | 0.88 | 0.91 | 0.91 | 0.90 | 0.63 | 0.64 | 0.63 | 0.59 |
| k-means | 0.76 | 0.90 | 0.77 | 0.90 | 0.58 | 0.57 | 0.58 | 0.57 |
| Hierarchical (tSNE) | 0.85 | 0.85 | 0.86 | 0.64 | 0.64 | 0.67 | 0.67 | 0.63 |
| k-means (tSNE) | 0.85 | 0.85 | 0.85 | 0.64 | 0.65 | 0.65 | 0.65 | 0.63 |

| | Chu-type (NDM) | | | | Chu-type (GEM) | | | |
|---|---|---|---|---|---|---|---|---|
| | Detected expression in at least | | | | Detected expression in at least | | | |
| | 1 cell | 5 cells | 10 cells | 50 cells | 1 cell | 5 cells | 10 cells | 50 cells |
| Hierarchical | 0.75 | 0.75 | 0.77 | 0.77 | 0.75 | 0.75 | 0.75 | 0.75 |
| k-means | 0.77 | 0.77 | 0.77 | 0.77 | 0.74 | 0.73 | 0.73 | 0.74 |
| Hierarchical (tSNE) | 0.99 | 0.99 | 0.99 | 0.99 | 0.98 | 0.98 | 0.98 | 0.98 |
| k-means (tSNE) | 0.99 | 0.99 | 0.99 | 0.99 | 0.98 | 0.98 | 0.98 | 0.98 |

| | Chu-time (NDM) | | | | Chu-time (GEM) | | | |
|---|---|---|---|---|---|---|---|---|
| | Detected expression in at least | | | | Detected expression in at least | | | |
| | 1 cell | 5 cells | 10 cells | 50 cells | 1 cell | 5 cells | 10 cells | 50 cells |
| Hierarchical | 0.64 | 0.65 | 0.72 | 0.75 | 0.67 | 0.68 | 0.67 | 0.67 |
| k-means | 0.70 | 0.70 | 0.70 | 0.71 | 0.60 | 0.59 | 0.59 | 0.59 |
| Hierarchical (tSNE) | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 |
| k-means (tSNE) | 0.69 | 0.68 | 0.69 | 0.68 | 0.69 | 0.68 | 0.69 | 0.69 |

| | Trapnell (NDM) | | | | Trapnell (GEM) | | | |
|---|---|---|---|---|---|---|---|---|
| | Detected expression in at least | | | | Detected expression in at least | | | |
| | 1 cell | 5 cells | 10 cells | 50 cells | 1 cell | 5 cells | 10 cells | 50 cells |
| Hierarchical | 0.23 | 0.24 | 0.24 | 0.23 | 0.06 | 0.06 | 0.08 | 0.01 |
| k-means | 0.25 | 0.25 | 0.44 | 0.23 | 0.13 | 0.15 | 0.16 | 0.13 |
| Hierarchical (tSNE) | 0.43 | 0.45 | 0.43 | 0.44 | 0.17 | 0.16 | 0.16 | 0.16 |
| k-means (tSNE) | 0.47 | 0.50 | 0.47 | 0.47 | 0.16 | 0.16 | 0.16 | 0.16 |

| | Kim (NDM) | | | | Kim (GEM) | | | |
|---|---|---|---|---|---|---|---|---|
| | Detected expression in at least | | | | Detected expression in at least | | | |
| | 1 cell | 5 cells | 10 cells | 50 cells | 1 cell | 5 cells | 10 cells | 50 cells |
| Hierarchical | 0.77 | 0.73 | 0.73 | 0.72 | 0.66 | 0.66 | 0.66 | 0.73 |
| k-means | 0.78 | 0.76 | 0.83 | 0.73 | 0.61 | 0.61 | 0.60 | 0.56 |
| Hierarchical (tSNE) | 1.00 | 1.00 | 1.00 | 1.00 | 0.66 | 0.66 | 0.66 | 0.76 |
| k-means (tSNE) | 1.00 | 1.00 | 1.00 | 0.98 | 0.72 | 0.72 | 0.72 | 0.75 |

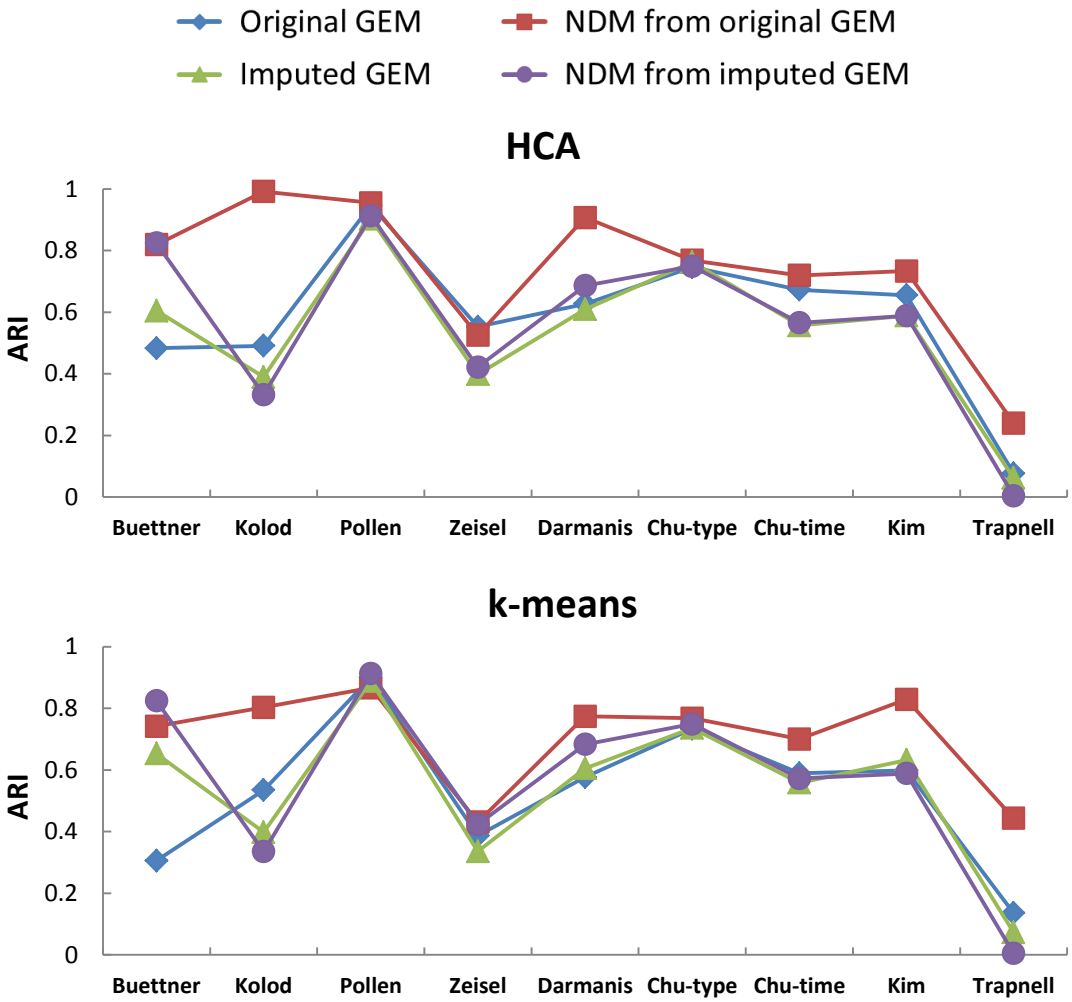
| | Kolodziejczyk (NDM) | | | Kolodziejczyk (GEM) | | |
|---|---|---|---|---|---|---|
| | Counts per cell on average | | | Counts per cell on average | | |
| | > 10 | > 20 | > 50 | > 10 | > 20 | > 50 |
| Hierarchical | 0.99 | 0.81 | 0.83 | 0.49 | 0.51 | 0.53 |
| k-means | 0.80 | 0.76 | 0.61 | 0.53 | 0.53 | 0.52 |
| Hierarchical (tSNE) | 1.00 | 1.00 | 1.00 | 0.99 | 0.99 | 0.99 |
| k-means (tSNE) | 1.00 | 1.00 | 1.00 | 0.99 | 0.99 | 0.99 |

| | Pollen (NDM) | | | Pollen (GEM) | | |
|---|---|---|---|---|---|---|
| | Detected expression in at least | | | Detected expression in at least | | |
| | 10 cells | 20 cells | 50 cells | 10 cell | 20 cells | 50 cells |
| Hierarchical | 0.96 | 0.96 | 0.92 | 0.95 | 0.94 | 0.95 |
| k-means | 0.87 | 0.84 | 0.90 | 0.90 | 0.91 | 0.92 |
| Hierarchical (tSNE) | 0.85 | 0.85 | 0.88 | 0.94 | 0.94 | 0.95 |
| k-means (tSNE) | 0.85 | 0.86 | 0.88 | 0.94 | 0.94 | 0.94 |

**Supplementary Note 16: Clustering analysis for the imputed data**

In this work, we used scImpute (18) to impute the dataset, and then constructed the NDM from the imputed data. Clustering methods including hierarchical clustering algorithm (HCA) and k-means were used for comparison. We also performed the clustering to the data that are preprocessed by t-SNE.

The result is shown in Figure S12. From Figure S12, we can see that the imputed GEM gets better results than original GEM in some datasets, but is usually inferior to the performance of NDM from original GEM. The result of NDM from imputed GEM is a little better than imputed GEM, but is obviously worse than NDM from original GEM. As a conclusion, the imputation based on the current methods is not recommended before CSN construction. In other words, existing imputation method is based on the expression level of scRNA-seq data but does not take into account of the gene-gene interactions, and thus, the identification of edges in CSNs from imputed data may be interfered, which leads to the worse result for the NDM from imputed data.



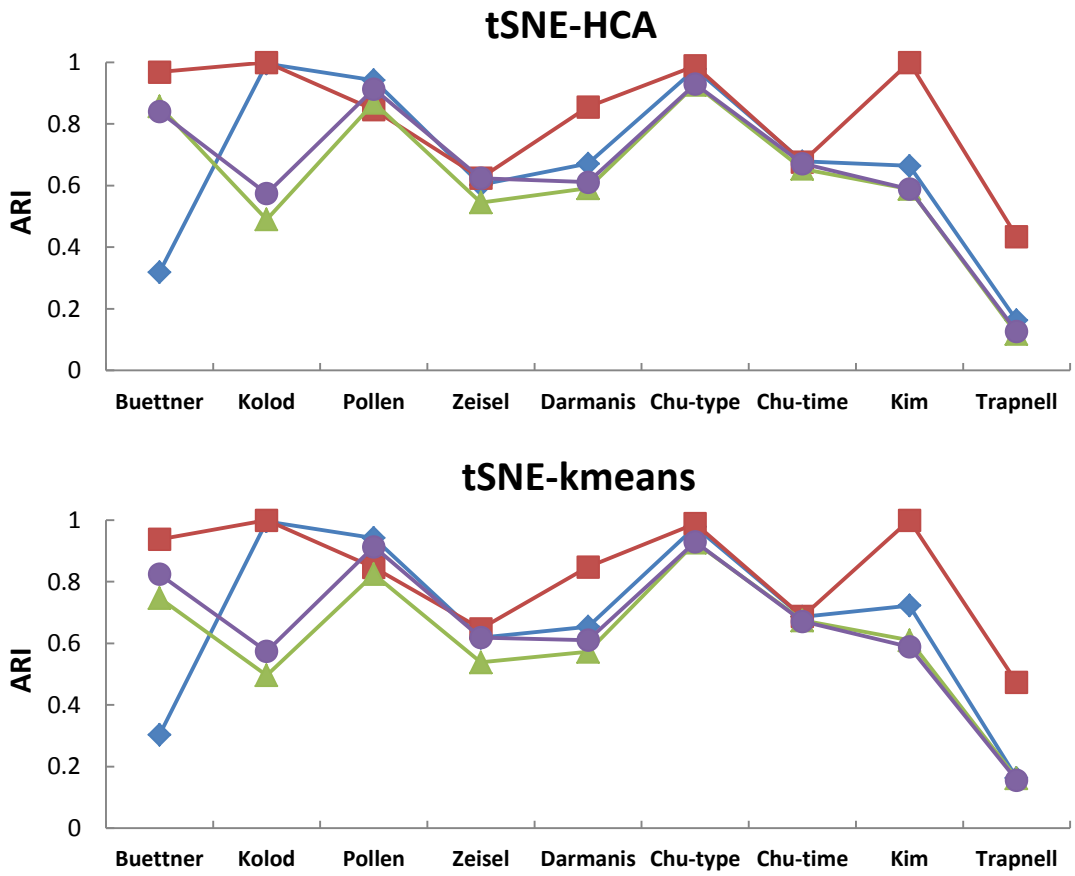



**Figure S12.** The clustering performance of original GEM, imputed GEM, NDM from original GEM and NDM from imputed GEM on the nine datasets. Hierarchical clustering algorithm (HCA) and k-means were used for comparison. We also performed the clustering to the data that is preprocessed by t-SNE.

**Supplementary Note 17: Differential genes in gene expression or network degree between adenocarcinoma and squamous cell carcinoma adjacent normal tissues**

We listed the differential genes in gene expression or network degree between adenocarcinoma and squamous cell carcinoma adjacent normal tissues (Wilcoxon rank sum test, FDR < 0.05 and fold change > 2).

| Ensembl ID | FDR of gene expression | FDR of network degree | Ensembl ID | FDR of gene expression | FDR of network degree |
|---|---|---|---|---|---|
| **ENSG00000090512** | **0.003197** | **0.007644** | ENSG00000213875 | **0.015745** | 0.708816 |
| ENSG00000107807 | 0.055148 | **0.035364** | ENSG00000214097 | 0.215530 | **0.019641** |
| ENSG00000108849 | 0.368530 | **0.045265** | ENSG00000214417 | **0.034325** | 0.806984 |
| ENSG00000118990 | **0.043816** | 0.097390 | ENSG00000216331 | 0.075561 | **0.043294** |
| ENSG00000122043 | 0.303478 | **0.046362** | ENSG00000217159 | 0.180773 | **0.047935** |
| **ENSG00000125998** | **0.028002** | **0.028380** | ENSG00000217653 | 0.365533 | **0.025425** |
| ENSG00000132677 | 0.703875 | **0.019787** | ENSG00000219559 | 0.118403 | **0.026393** |
| **ENSG00000134757** | **0.000013** | **0.018907** | ENSG00000224299 | 0.564278 | **0.016476** |
| ENSG00000143556 | **0.002407** | 0.290305 | ENSG00000225513 | 0.523751 | **0.026656** |
| ENSG00000144785 | 0.117591 | **0.028505** | **ENSG00000226084** | **0.000015** | **0.009083** |
| **ENSG00000147381** | **0.003110** | **0.005170** | **ENSG00000228697** | **0.032383** | **0.027148** |
| ENSG00000158874 | **0.044069** | 0.547680 | **ENSG00000229119** | **0.000003** | **0.000919** |
| ENSG00000159527 | 0.203220 | **0.044516** | ENSG00000231725 | 0.055468 | **0.030999** |
| ENSG00000163209 | **0.000326** | 0.086008 | ENSG00000232385 | **0.001449** | 0.551598 |
| ENSG00000163216 | **0.004361** | 0.843903 | ENSG00000233214 | **0.046598** | 0.468909 |
| ENSG00000169469 | **0.000020** | 0.092241 | ENSG00000233246 | 0.227560 | **0.028999** |
| ENSG00000169474 | **0.000225** | 0.258632 | ENSG00000233260 | 0.187237 | **0.032592** |
| **ENSG00000170454** | **0.019020** | **0.024161** | ENSG00000235175 | **0.000013** | 0.787825 |
| **ENSG00000170465** | **0.004923** | **0.020187** | ENSG00000237409 | 0.483375 | **0.043804** |
| ENSG00000178363 | **0.000016** | 0.103138 | ENSG00000237490 | **0.009014** | 0.084218 |
| ENSG00000183242 | 0.832016 | **0.033203** | **ENSG00000237955** | **0.028421** | **0.000968** |
| **ENSG00000184330** | **0.016564** | **0.015338** | ENSG00000241794 | **0.027922** | 0.850091 |
| ENSG00000185479 | **0.000194** | 0.901974 | **ENSG00000242113** | **0.005058** | **0.001137** |
| ENSG00000186832 | **0.000255** | 0.455530 | ENSG00000244712 | 0.383523 | **0.015151** |
| ENSG00000186847 | **0.001649** | 0.223682 | ENSG00000251377 | 0.170296 | **0.002587** |
| **ENSG00000187054** | **0.000189** | **0.019431** | ENSG00000255723 | 0.170214 | **0.028800** |
| **ENSG00000188373** | **0.039188** | **0.009669** | ENSG00000257759 | 0.136402 | **0.019879** |
| **ENSG00000197641** | **0.015678** | **0.026463** | ENSG00000259303 | 0.088267 | **0.046652** |
| ENSG00000199676 | **0.041426** | 0.878395 | ENSG00000261587 | 0.799625 | **0.041349** |
| **ENSG00000203785** | **0.000052** | **0.001433** | ENSG00000263829 | 0.789961 | **0.012973** |
| ENSG00000205420 | **0.000051** | 0.270627 | ENSG00000265394 | 0.149375 | **0.022218** |
| ENSG00000205628 | 0.313880 | **0.042657** | ENSG00000273962 | **0.018738** | 0.085123 |
| ENSG00000213033 | 0.956406 | **0.022251** | **ENSG00000281550** | **0.039444** | **0.040817** |
| ENSG00000213455 | **0.010947** | 0.746091 | **ENSG00000281591** | **0.032370** | **0.032139** |

**Supplementary Note 18: The two subtypes divided from squamous cell carcinoma samples**

We can divide squamous cell carcinoma samples into two subtypes S1 and S2 based on NDM (Figure 6C in main text), which are listed below.

*Subtype S1* (117 samples):

| | | | | | |
|---|---|---|---|---|---|
| TCGA-18-3409 | TCGA-37-3792 | TCGA-52-7622 | TCGA-60-2716 | TCGA-85-6560 | TCGA-77-A5GH |
| TCGA-18-3410 | TCGA-37-4129 | TCGA-52-7809 | TCGA-60-2725 | TCGA-85-7950 | TCGA-85-A4PA |
| TCGA-21-1078 | TCGA-37-4130 | TCGA-52-7812 | TCGA-63-6202 | TCGA-85-8048 | TCGA-85-A50M |
| TCGA-21-1083 | TCGA-37-4132 | TCGA-56-6546 | TCGA-66-2744 | TCGA-85-8288 | TCGA-85-A510 |
| TCGA-21-5787 | TCGA-37-4135 | TCGA-56-7223 | TCGA-66-2754 | TCGA-85-8352 | TCGA-85-A513 |
| TCGA-22-1000 | TCGA-37-4141 | TCGA-56-7731 | TCGA-66-2755 | TCGA-85-8584 | TCGA-90-A4ED |
| TCGA-22-1002 | TCGA-37-5819 | TCGA-56-8083 | TCGA-66-2756 | TCGA-90-7767 | TCGA-90-A4EE |
| TCGA-22-1005 | TCGA-39-5011 | TCGA-56-8201 | TCGA-66-2757 | TCGA-90-7964 | TCGA-90-A59Q |
| TCGA-22-1016 | TCGA-39-5034 | TCGA-56-8307 | TCGA-66-2769 | TCGA-94-7943 | TCGA-96-A4JL |
| TCGA-22-1017 | TCGA-39-5039 | TCGA-56-8309 | TCGA-66-2785 | TCGA-94-8491 | TCGA-98-A53B |
| TCGA-22-4594 | TCGA-39-5040 | TCGA-56-8624 | TCGA-66-2786 | TCGA-96-7545 | TCGA-98-A53C |
| TCGA-22-4596 | TCGA-43-2576 | TCGA-56-8626 | TCGA-66-2789 | TCGA-98-7454 | TCGA-98-A53D |
| TCGA-22-5472 | TCGA-43-2578 | TCGA-56-8628 | TCGA-68-8250 | TCGA-98-8022 | TCGA-98-A53H |
| TCGA-22-5480 | TCGA-43-2581 | TCGA-58-8386 | TCGA-68-8251 | TCGA-98-8023 | TCGA-L3-A4E7 |
| TCGA-22-5481 | TCGA-43-5668 | TCGA-58-8388 | TCGA-77-6842 | TCGA-33-AASB | TCGA-NK-A5D1 |
| TCGA-22-5483 | TCGA-43-7656 | TCGA-60-2695 | TCGA-77-7335 | TCGA-56-A49D | TCGA-O2-A52Q |
| TCGA-33-4587 | TCGA-43-7658 | TCGA-60-2697 | TCGA-77-7465 | TCGA-56-A4ZJ | TCGA-O2-A5IB |
| TCGA-33-6737 | TCGA-46-3766 | TCGA-60-2706 | TCGA-77-8007 | TCGA-63-A5MM | |
| TCGA-34-2608 | TCGA-46-3767 | TCGA-60-2714 | TCGA-77-8131 | TCGA-63-A5MN | |
| TCGA-34-5234 | TCGA-46-3769 | TCGA-60-2715 | TCGA-85-6175 | TCGA-77-A5FZ | |

*Subtype S2* (384 samples):

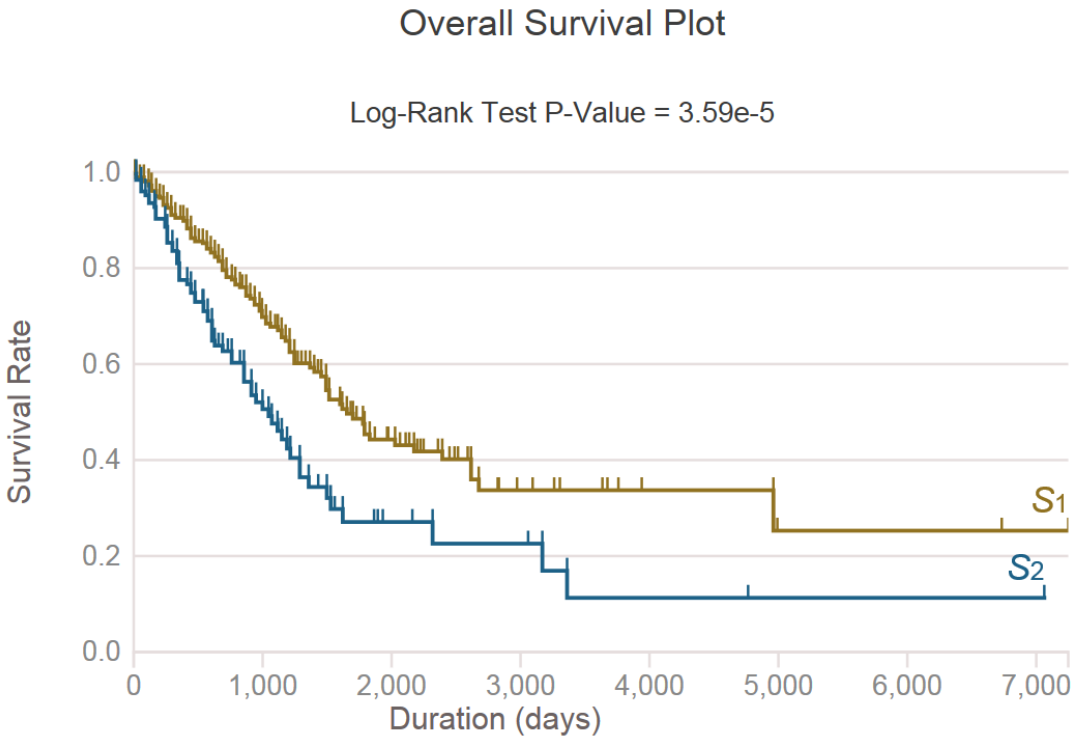
| | | | | | |
|---|---|---|---|---|---|
| TCGA-18-3406 | TCGA-33-6738 | TCGA-56-7822 | TCGA-66-2790 | TCGA-85-8479 | TCGA-63-A5MV |
| TCGA-18-3407 | TCGA-34-2596 | TCGA-56-7823 | TCGA-66-2791 | TCGA-85-8481 | TCGA-63-A5MW |
| TCGA-18-3408 | TCGA-34-2600 | TCGA-56-8082 | TCGA-66-2792 | TCGA-85-8580 | TCGA-63-A5MY |
| TCGA-18-3411 | TCGA-34-5231 | TCGA-56-8304 | TCGA-66-2793 | TCGA-85-8582 | TCGA-68-A59I |
| TCGA-18-3412 | TCGA-34-5232 | TCGA-56-8305 | TCGA-66-2794 | TCGA-85-8664 | TCGA-68-A59J |
| TCGA-18-3414 | TCGA-34-5236 | TCGA-56-8308 | TCGA-66-2795 | TCGA-85-8666 | TCGA-6A-AB49 |
| TCGA-18-3415 | TCGA-34-5239 | TCGA-56-8503 | TCGA-66-2800 | TCGA-90-6837 | TCGA-77-A5G1 |
| TCGA-18-3416 | TCGA-34-5240 | TCGA-56-8504 | TCGA-68-7755 | TCGA-90-7766 | TCGA-77-A5G3 |
| TCGA-18-3417 | TCGA-34-5241 | TCGA-56-8622 | TCGA-68-7756 | TCGA-90-7769 | TCGA-77-A5G6 |
| TCGA-18-3419 | TCGA-34-5927 | TCGA-56-8623 | TCGA-68-7757 | TCGA-92-7340 | TCGA-77-A5G7 |
| TCGA-18-3421 | TCGA-34-5928 | TCGA-56-8625 | TCGA-70-6722 | TCGA-92-7341 | TCGA-77-A5G8 |
| TCGA-18-4083 | TCGA-34-5929 | TCGA-56-8629 | TCGA-70-6723 | TCGA-92-8063 | TCGA-77-A5GA |
| TCGA-18-4086 | TCGA-34-7107 | TCGA-58-8387 | TCGA-77-6843 | TCGA-92-8064 | TCGA-77-A5GB |
| TCGA-18-4721 | TCGA-34-8454 | TCGA-58-8390 | TCGA-77-6844 | TCGA-92-8065 | TCGA-77-A5GF |
| TCGA-18-5592 | TCGA-34-8455 | TCGA-58-8391 | TCGA-77-6845 | TCGA-94-7033 | TCGA-85-A4CL |

| | | | | | |
|---|---|---|---|---|---|
| TCGA-18-5595 | TCGA-34-8456 | TCGA-58-8392 | TCGA-77-7138 | TCGA-94-7557 | TCGA-85-A4CN |
| TCGA-21-1070 | TCGA-37-3783 | TCGA-58-8393 | TCGA-77-7139 | TCGA-94-8035 | TCGA-85-A4JB |
| TCGA-21-1071 | TCGA-37-3789 | TCGA-60-2696 | TCGA-77-7140 | TCGA-94-8490 | TCGA-85-A4JC |
| TCGA-21-1072 | TCGA-37-4133 | TCGA-60-2698 | TCGA-77-7141 | TCGA-96-7544 | TCGA-85-A4QQ |
| TCGA-21-1075 | TCGA-39-5016 | TCGA-60-2703 | TCGA-77-7142 | TCGA-96-8169 | TCGA-85-A4QR |
| TCGA-21-1076 | TCGA-39-5019 | TCGA-60-2704 | TCGA-77-7337 | TCGA-96-8170 | TCGA-85-A50Z |
| TCGA-21-1077 | TCGA-39-5021 | TCGA-60-2707 | TCGA-77-7338 | TCGA-98-8020 | TCGA-85-A511 |
| TCGA-21-1079 | TCGA-39-5022 | TCGA-60-2708 | TCGA-77-7463 | TCGA-98-8021 | TCGA-85-A512 |
| TCGA-21-1080 | TCGA-39-5024 | TCGA-60-2709 | TCGA-77-8008 | TCGA-21-A5DI | TCGA-85-A53L |
| TCGA-21-1081 | TCGA-39-5027 | TCGA-60-2710 | TCGA-77-8009 | TCGA-22-A5C4 | TCGA-85-A5B5 |
| TCGA-21-1082 | TCGA-39-5028 | TCGA-60-2711 | TCGA-77-8128 | TCGA-33-A4WN | TCGA-94-A4VJ |
| TCGA-21-5782 | TCGA-39-5029 | TCGA-60-2712 | TCGA-77-8130 | TCGA-33-A5GW | TCGA-94-A5I4 |
| TCGA-21-5783 | TCGA-39-5030 | TCGA-60-2713 | TCGA-77-8133 | TCGA-33-AAS8 | TCGA-94-A5I6 |
| TCGA-21-5784 | TCGA-39-5031 | TCGA-60-2719 | TCGA-77-8136 | TCGA-33-AASD | TCGA-96-A4JK |
| TCGA-21-5786 | TCGA-39-5035 | TCGA-60-2720 | TCGA-77-8138 | TCGA-33-AASI | TCGA-98-A538 |
| TCGA-22-0940 | TCGA-39-5036 | TCGA-60-2721 | TCGA-77-8139 | TCGA-33-AASJ | TCGA-98-A539 |
| TCGA-22-0944 | TCGA-39-5037 | TCGA-60-2722 | TCGA-77-8140 | TCGA-33-AASL | TCGA-98-A53A |
| TCGA-22-1011 | TCGA-43-3394 | TCGA-60-2723 | TCGA-77-8143 | TCGA-34-A5IX | TCGA-98-A53I |
| TCGA-22-1012 | TCGA-43-3920 | TCGA-60-2724 | TCGA-77-8144 | TCGA-37-A5EL | TCGA-98-A53J |
| TCGA-22-4591 | TCGA-43-5670 | TCGA-60-2726 | TCGA-77-8145 | TCGA-37-A5EM | TCGA-J1-A4AH |
| TCGA-22-4593 | TCGA-43-6143 | TCGA-63-5128 | TCGA-77-8146 | TCGA-37-A5EN | TCGA-L3-A524 |
| TCGA-22-4595 | TCGA-43-6647 | TCGA-63-5131 | TCGA-77-8148 | TCGA-43-A474 | TCGA-LA-A446 |
| TCGA-22-4599 | TCGA-43-6770 | TCGA-63-7020 | TCGA-77-8150 | TCGA-43-A475 | TCGA-LA-A7SW |
| TCGA-22-4601 | TCGA-43-6771 | TCGA-63-7021 | TCGA-77-8153 | TCGA-43-A56U | TCGA-MF-A522 |
| TCGA-22-4604 | TCGA-43-6773 | TCGA-63-7022 | TCGA-77-8154 | TCGA-43-A56V | TCGA-NC-A5HD |
| TCGA-22-4605 | TCGA-43-7657 | TCGA-63-7023 | TCGA-77-8156 | TCGA-56-A4BW | TCGA-NC-A5HE |
| TCGA-22-4607 | TCGA-43-8115 | TCGA-66-2727 | TCGA-79-5596 | TCGA-56-A4BX | TCGA-NC-A5HF |
| TCGA-22-4609 | TCGA-43-8116 | TCGA-66-2734 | TCGA-85-6561 | TCGA-56-A4BY | TCGA-NC-A5HG |
| TCGA-22-4613 | TCGA-43-8118 | TCGA-66-2737 | TCGA-85-6798 | TCGA-56-A4ZK | TCGA-NC-A5HH |
| TCGA-22-5471 | TCGA-46-3765 | TCGA-66-2742 | TCGA-85-7696 | TCGA-56-A5DR | TCGA-NC-A5HI |
| TCGA-22-5473 | TCGA-46-3768 | TCGA-66-2753 | TCGA-85-7697 | TCGA-56-A5DS | TCGA-NC-A5HJ |
| TCGA-22-5474 | TCGA-46-6025 | TCGA-66-2758 | TCGA-85-7698 | TCGA-56-A62T | TCGA-NC-A5HK |
| TCGA-22-5477 | TCGA-46-6026 | TCGA-66-2759 | TCGA-85-7699 | TCGA-58-A46J | TCGA-NC-A5HL |
| TCGA-22-5478 | TCGA-51-4079 | TCGA-66-2763 | TCGA-85-7710 | TCGA-58-A46K | TCGA-NC-A5HM |
| TCGA-22-5479 | TCGA-51-4080 | TCGA-66-2765 | TCGA-85-7843 | TCGA-58-A46L | TCGA-NC-A5HN |
| TCGA-22-5482 | TCGA-51-4081 | TCGA-66-2766 | TCGA-85-7844 | TCGA-58-A46M | TCGA-NC-A5HO |
| TCGA-22-5485 | TCGA-51-6867 | TCGA-66-2767 | TCGA-85-8049 | TCGA-58-A46N | TCGA-NC-A5HP |
| TCGA-22-5489 | TCGA-52-7810 | TCGA-66-2768 | TCGA-85-8052 | TCGA-63-A5M9 | TCGA-NC-A5HQ |
| TCGA-22-5491 | TCGA-52-7811 | TCGA-66-2770 | TCGA-85-8070 | TCGA-63-A5MB | TCGA-NC-A5HR |
| TCGA-22-5492 | TCGA-56-1622 | TCGA-66-2771 | TCGA-85-8071 | TCGA-63-A5MG | TCGA-NC-A5HT |
| TCGA-33-4532 | TCGA-56-5897 | TCGA-66-2773 | TCGA-85-8072 | TCGA-63-A5MH | TCGA-NK-A5CR |
| TCGA-33-4533 | TCGA-56-5898 | TCGA-66-2777 | TCGA-85-8276 | TCGA-63-A5MI | TCGA-NK-A5CT |
| TCGA-33-4538 | TCGA-56-6545 | TCGA-66-2778 | TCGA-85-8277 | TCGA-63-A5MJ | TCGA-NK-A5CX |
| TCGA-33-4547 | TCGA-56-7221 | TCGA-66-2780 | TCGA-85-8287 | TCGA-63-A5ML | TCGA-NK-A7XE |

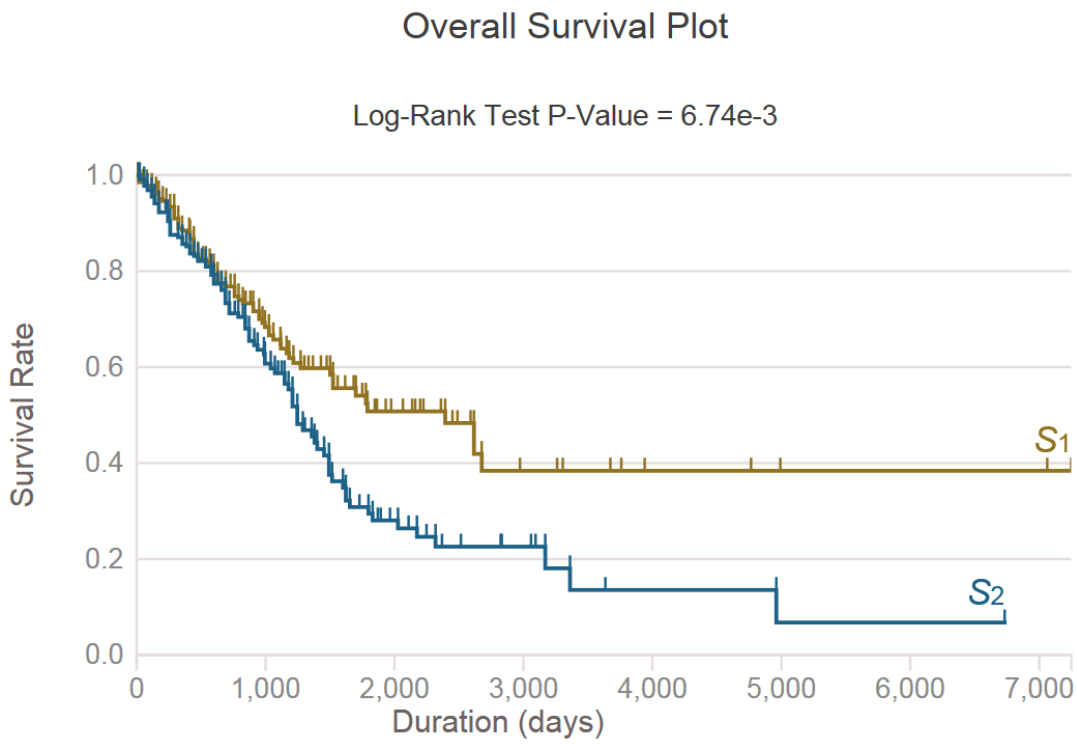
| | | | | | |
|---|---|---|---|---|---|
| TCGA-33-4566 | TCGA-56-7222 | TCGA-66-2781 | TCGA-85-8350 | TCGA-63-A5MP | TCGA-O2-A52N |
| TCGA-33-4582 | TCGA-56-7579 | TCGA-66-2782 | TCGA-85-8351 | TCGA-63-A5MR | TCGA-O2-A52S |
| TCGA-33-4583 | TCGA-56-7580 | TCGA-66-2783 | TCGA-85-8353 | TCGA-63-A5MS | TCGA-O2-A52V |
| TCGA-33-4586 | TCGA-56-7582 | TCGA-66-2787 | TCGA-85-8354 | TCGA-63-A5MT | TCGA-O2-A52W |
| TCGA-33-4589 | TCGA-56-7730 | TCGA-66-2788 | TCGA-85-8355 | TCGA-63-A5MU | TCGA-XC-AA0X |

**Supplementary Note 19: Survival analyses based on "dark" genes**

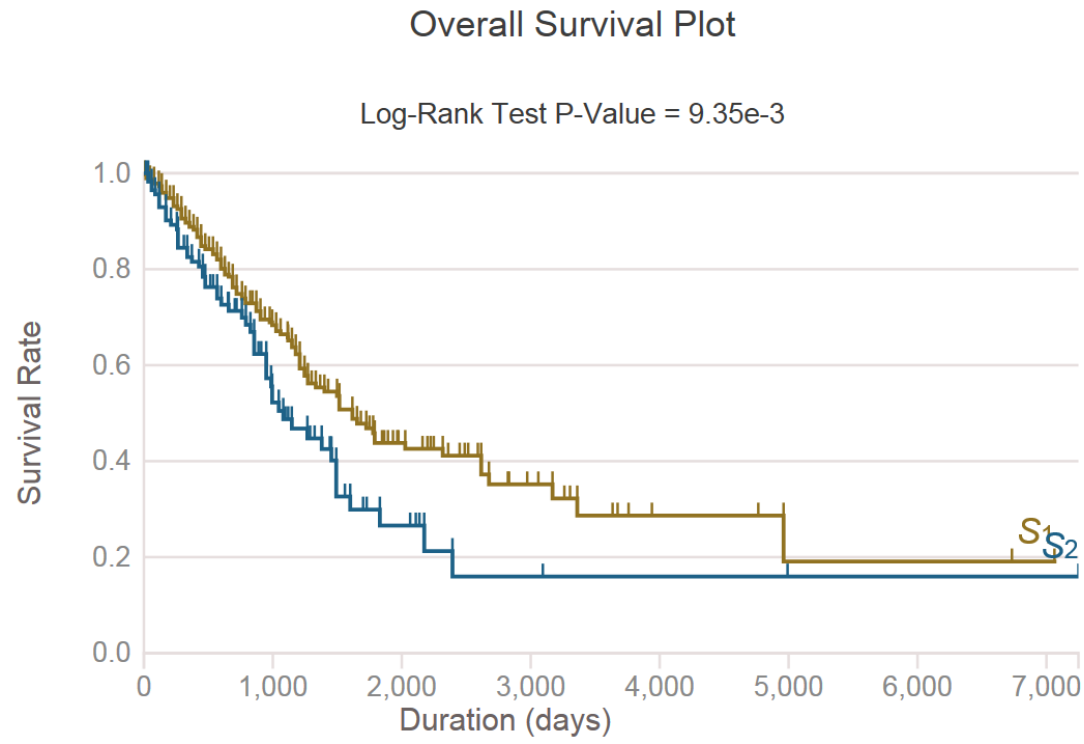
We can find some "dark" genes from TCGA lung cancer bulk RNA-seq data. These "dark" genes have no differential expressions between lung cancer samples and normal samples (FDR of Wilcoxon rank-sum test > 0.05), which are ignored by the traditional methods. But by our CSN method, they have significantly differential network degrees, which are considered important at a network level. As shown in Figure S13, although those "dark" genes have no differential expressions, they can divide the adenocarcinoma samples into two parts based on the normalized network degree. The survival analyses of those "dark" genes indicate the significant difference between the two parts, which implies their prognosis ability, and have potential applications in precision medicine or personalized treatment.




**(A) AC007638.2 (novel transcript, antisense to HLF)**. $S_1$ (391 samples): normalized degree > 8, $S_2$ (133 samples): normalized degree ≤ 8




**(B) AC092574.2 (novel transcript, antisense to ZNF721)**. $S_1$ (288 samples): normalized degree > 14, $S_2$ (236 samples): normalized degree ≤ 14




**(C) AC002563.1 (novel transcript, antisense to CIT)**. $S_1$ (402 samples): normalized degree > 7, $S_2$ (122 samples): normalized degree ≤ 7

**Figure S13**. Survival analyses of three "dark" genes.

**Supplementary Note 20: Source Code (MATLAB)**

**(1) Output is CSNs**

Construction of cell-specific networks from gene expression matrix

```
function csn = csnet(data,c,alpha,boxsize,weighted)
%Construction of cell-specific networks
%The function performs the transformation from gene expression matrix to
%cell-specific network (csn).
%data: Gene expression matrix, rows = genes, columns = cells
%c: Construct the CSNs for all cells, set c = [] (Default);
%   Construct the CSN for cell k, set  c = k
%alpha: Significant level (eg. 0.001, 0.01, 0.05 ...)
%      larger alpha leads to more edges, Default = 0.01
%boxsize: Size of neighborhood, Default = 0.1
%weighted: 1  edge is weighted
%         0  edge is not weighted (Default)
%csn: Cell-specific network, the kth CSN is in csn{k}
%     rows = genes, columns = genes
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Too many cells or genes may lead to out of memory. %
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

if nargin < 5 || isempty(weighted)
    weighted = 0;
end
if nargin < 4 || isempty(boxsize)
    boxsize = 0.1;
end
if nargin <3 || isempty(alpha)
    alpha = 0.01;
end

[n1,n2] = size(data);
if nargin <2 || isempty(c)
    c = 1 : n2;
end

%Define the neighborhood of each plot
upper = zeros(n1,n2);
lower = zeros(n1,n2);
for i = 1 : n1
    [s1,s2] = sort(data(i,:));
    n3 = n2-sum(sign(s1));
```

```
    h = round(boxsize/2*sum(sign(s1)));
    k = 1;
    while k <= n2
        s = 0;
        while k+s+1 <= n2 && s1(k+s+1) == s1(k)
            s = s+1;
        end
        if s >= h
            upper(i,s2(k:k+s)) = data(i,s2(k));
            lower(i,s2(k:k+s)) = data(i,s2(k));
        else
            upper(i,s2(k:k+s)) = data(i,s2(min(n2,k+s+h)));
            lower(i,s2(k:k+s)) = data(i,s2(max(n3*(n3>h)+1,k-h)));
        end
        k = k+s+1;
    end
end

%Construction of cell-specific network
csn = cell(1,n2);
B = zeros(n1,n2);
p = -icdf('norm',alpha,0,1);
for k = c
    for j = 1 : n2
        B(:,j) = data(:,j) <= upper(:,k) & data(:,j) >= lower(:,k);
    end
    a = sum(B,2);
    d = (B*B'*n2-a*a')./sqrt((a*a').*((n2-a)*(n2-a)')/(n2-1)+eps);
    d(1 : n1+1 : end) = 0;
    if weighted
        csn{k} = d.*(d > 0);
    else
        csn{k} = sparse(d > p);
    end
    disp(['Cell ' num2str(k) ' is completed']);
end
```

**(2) Output is NDM**

Construction of network degree matrix from gene expression matrix

```
function ndm = csndm(data,alpha,boxsize,normalize)
%Construction of network degree matrix
%The function performs the transformation from gene expression matrix to
```

```
%network degree matrix (ndm).
%data: Gene expression matrix (TPM/FPKM/count), rows = genes, columns =
cells
%alpha: Significant level (eg. 0.001, 0.01, 0.05 ...), Default = 0.01
%boxsize: Size of neighborhood, Default = 0.1 (nx(k) = ny(k) = 0.1*n)
%normalize: 1  result is normalized (Default); 0  result is not normalized

if nargin < 4 || isempty(normalize)
    normalize = 1;
end
if nargin < 3 || isempty(boxsize)
    boxsize = 0.1;
end
if nargin <2 || isempty(alpha)
    alpha = 0.01;
end

%Define the neighborhood of each plot
[n1,n2] = size(data);
upper = zeros(n1,n2);
lower = zeros(n1,n2);
for i = 1 : n1
    [s1,s2] = sort(data(i,:));
    n0 = n2-sum(sign(s1));
    h = round(boxsize/2*sum(sign(s1)));
    k = 1;
    while k <= n2
        s = 0;
        while k+s+1 <= n2 && s1(k+s+1) == s1(k)
            s = s+1;
        end
        if s >= h
            upper(i,s2(k:k+s)) = data(i,s2(k));
            lower(i,s2(k:k+s)) = data(i,s2(k));
        else
            upper(i,s2(k:k+s)) = data(i,s2(min(n2,k+s+h)));
            lower(i,s2(k:k+s)) = data(i,s2(max(n0*(n0>h)+1,k-h)));
        end
        k = k+s+1;
    end
end

%If gene expression matrix is sparse, use the sparse matrix will accelerate
%the calculation and reduce memory footprint
```

```
%data = sparse(data); upper = sparse(upper); lower = sparse(lower);

%Construction of network degree matrix
ndm = zeros(n1,n2);
B = zeros(n1,n2);
p = -icdf('norm',alpha,0,1);
for k = 1 : n2
    for j = 1 : n2
       B(:,j) = data(:,j) <= upper(:,k) & data(:,j) >= lower(:,k) &
data(:,k);
    end
    %B = sparse(B);
    a = sum(B,2);
    csn = (B*B'*n2-a*a')./sqrt((a*a').*((n2-a)*(n2-a)')/(n2-1)+eps);
    csn = (csn > p);
    ndm(:,k) = sum(csn,2) - diag(csn);
    disp(['Cell ' num2str(k) ' is completed']);
end

%Normalization of network degree matrix
if normalize
    ndm = bsxfun(@rdivide,ndm,sum(ndm))*mean(sum(sign(ndm)))^2/2000;
end
```

**(3) Output is Edge**

Normalized statistic of edge *x*-*y* from the expression values of genes *x* and *y*

```
function edge = csnedge(gx,gy,boxsize)
%The normalized statistic of edge x-y
%gx gy: Gene expression values of gene x and gene y
%      If there are n cells, gx and gy are 1-by-n vectors
%boxsize: Size of neighborhood, Default = 0.1
%edge: 1-by-n vector, the normalized statistic of edge x-y in all cells

if nargin < 3
    boxsize = 0.1;
end

%Define the neighborhood of each plot
n = length(gx);
upper = zeros(1,n);
lower = zeros(1,n);
a = zeros(2,n);
```

```
B = cell(1,2);
for i = 1 : 2
    g = gx*(i==1)+gy*(i==2);
    [s1,s2] = sort(g);
    n0 = n-sum(sign(s1));
    h = round(boxsize/2*sum(sign(s1)));
    k = 1;
    while k <= n
        s = 0;
        while k+s+1 <= n && s1(k+s+1) == s1(k)
            s = s+1;
        end
        if s >= h
            upper(s2(k:k+s)) = g(s2(k));
            lower(s2(k:k+s)) = g(s2(k));
        else
            upper(s2(k:k+s)) = g(s2(min(n,k+s+h)));
            lower(s2(k:k+s)) = g(s2(max(n0*(n0>h)+1,k-h)));
        end
        k = k+s+1;
    end

    B{i} = bsxfun(@le,g',upper) & bsxfun(@ge,g',lower);
    a(i,:) = sum(B{i});
end

%Calculate the normalized statistic of edge x-y in all cells
edge = (sum(B{1} & B{2})*n-a(1,:).*a(2,:))./sqrt(a(1,:).*a(2,:) ...
    .*(n-a(1,:)).*(n-a(2,:))/(n-1));
```

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