1. Supplementary results of the GBM data.

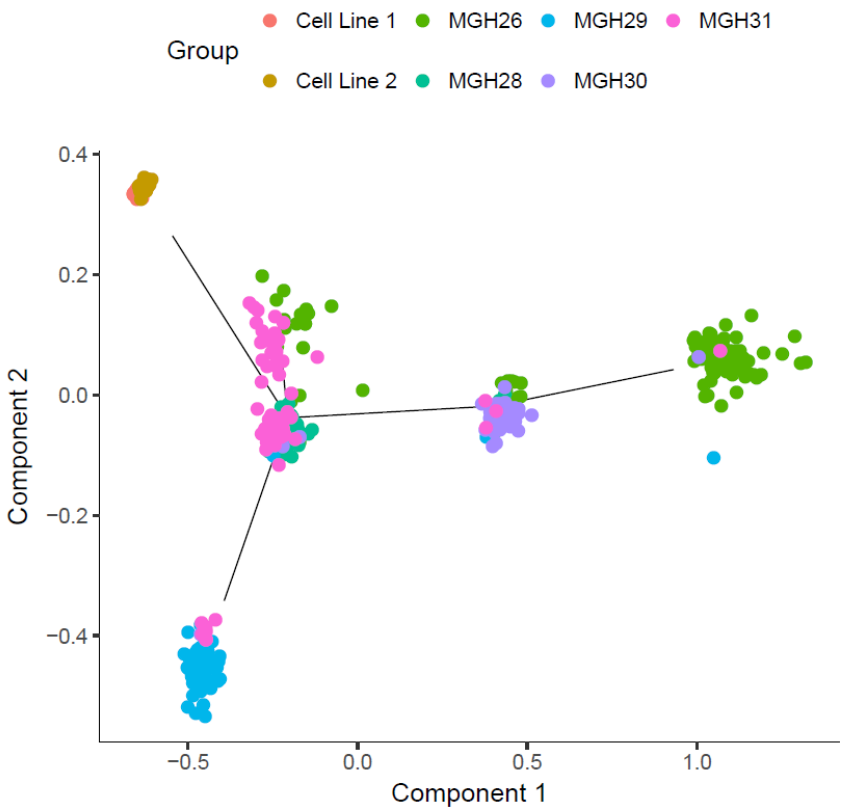


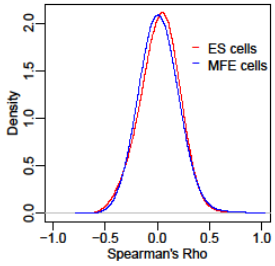
Fig. S1. Cell clustering result based on the expressions of DCGs.

2. A comparison of embryonic stem (ES) cells and embryonic fibroblasts (MEF) cells

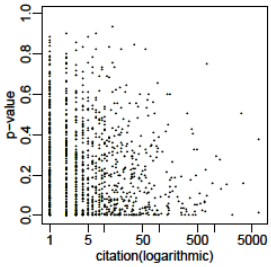
This study includes 48 mouse ES cells and 44 mouse embryonic fibroblasts (MFE) (Islam et al. 2011). The data are read counts in all genes. We first normalized the data by total counts in each cell, and then took a log transformation. The log transformed normalized counts are used for downstream analysis. After sparsity thresholding, 1575 gene candidates were selected for differential connectivity analysis between MFE and ES. As control, we also randomly split the ES and MFE into two groups with equal sample sizes and conducted differential connectivity test.

The overall correlation levels are distinctly different between the two cell types, with ES showing more positive correlations between genes in general (Fig. S2A). For the test between MFE and ES, we discover 121 significantly differentially connected genes at fdr < 0.001, which is 7.7% of all the genes. However, at the same significance level, the test within MFE only found 3 significantly differentially connected gene and the test within ES failed to find any significant gene (Fig. S2C). Furthermore, the consistency check shows a significant association between *P*-value and *Citation* where the slope term is –1.28 with *P* -value < 2e-16 (Fig. S2B), where the keyword here is "embryonic".

A Distribution of Spearman's Rho



B Consistency Analysis



C Number of differentially connected genes

	ES cells	MEF cells
ES cells	0	121
MEF cells	-	3

Fig. S2. Differential Single Cell Gene Connectivity Analysis of ES/MFE Transcriptional study. A: Histogram of the Spearman' Rho. **B:** Consistency analysis between the testing significance with the number of citation from PubMed. **C:** The number of significant differentially connected genes based on a fdr < 0.001.

GO biological process enrichment analysis shows that the predominant biological function of the DCGs is translation and protein localization (Figures and Tables can be found at the supplementary website at http://web1.sph.emory.edu/users/tyu8/SCDGN/MFE.html). It has been documented that in ES cells, the protein synthesis process is stringently controlled, partly through histone modification in sync with the ES cell cycle (Zaidi et al. 2016). Given the scRNA-seq data measures multiple cells that may be at different stages of the cell cycle, the RNA abundance of the ribosomal proteins would be highly correlated with each other. The differential correlation results indicate that when ES cells differentiate into MFE cells, the

tight control over protein synthesis is loosened to give priority to other, possibly developmental processes. The full results of connectivity pattern and related biological functions can be found at http://web1.sph.emory.edu/users/tyu8/SCDGN/MFE.html.

We further conduct clustering analysis using monocle (Trapnell et al. 2014) based on the selected DCGs (Fig. S3). Although the DCGs are selected based on differential co-expression, we find their expression levels separate the groups reasonably well. The MFE samples are closer together with each other in the dimension-reduced space, indicating a higher consistency within the MFE group. This is expected as the MFE cells are more specialized. Interestingly, the ES cells are separated, with a small group clustering with the MFE cells, indicating potential existence of sub-classes of cells with regard to the expression of the 121 DCGs.

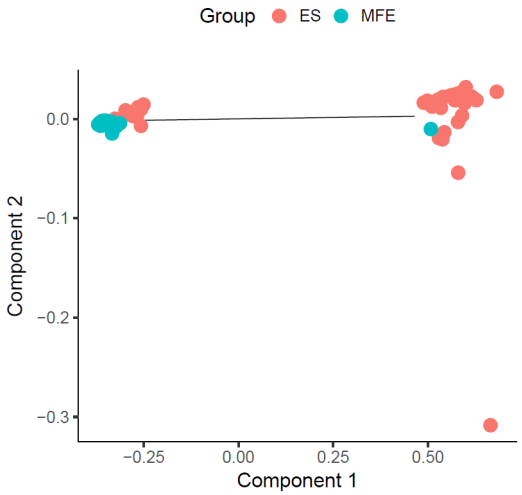


Fig. S3. Cell clustering result using the selected DCGs.

References:

Islam, S., Kjallquist, U., Moliner, A., Zajac, P., Fan, J. B., Lonnerberg, P.Linnarsson, S., 2011. Characterization of the single-cell transcriptional landscape by highly multiplex RNA-seq. Genome Res 21, 1160-1167.

Trapnell, C., Cacchiarelli, D., Grimsby, J., Pokharel, P., Li, S., Morse, M., Lennon, N. J., Livak, K. J., Mikkelsen, T. S.Rinn, J. L., 2014. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. Nat Biotechnol 32, 381-386.

Zaidi, S. K., Boyd, J. R., Grandy, R., Medina, R., Lian, J. B., Stein, G. S.Stein, J. L., 2016. Expression of Ribosomal RNA and Protein Genes in Human Embryonic Stem Cells Is Associated with the Activating H3K4me3 Histone Mark. J Cell Physiol.