A DGCR8-Independent Stable MicroRNA Expression Strategy Reveals Important Functions of miR-290 and miR-183–182 Families in Mouse Embryonic Stem Cells

Xi-Wen Wang,1 Jing Hao,1 Wen-Ting Guo,1 Le-Qi Liao,1 Si-Yue Huang,1 Xiangpeng Guo,2 Xichen Bao,2 Miguel A. Esteban,2 and Yangming Wang1,*

1Beijing Key Laboratory of Cardiometabolic Molecular Medicine, Institute of Molecular Medicine, Peking University, Beijing 100871, China
2Laboratory of RNA, Chromatin, and Human Disease, Key Laboratory of Regenerative Biology and Guangdong Provincial Key Laboratory of Stem Cells and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China

*Correspondence: yangming.wang@pku.edu.cn

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SUMMARY

DGCR8 knockout cells provide a great means to understand the function of microRNAs (miRNAs) in vitro and in vivo. Current strategies to study miRNA function in DGCR8 knockout cells depend on transient transfection of chemically synthesized miRNA mimics, which is costly and not suitable for long-term study and genetic selection of miRNA function. Here, we developed a cost-effective DGCR8-independent stable miRNA expression (DISME) strategy based on a short hairpin RNA vector that can be precisely processed by DICER. Using DISME, we found that miR-294 promoted the formation of meso-endoderm lineages during embryonic stem cell differentiation. Furthermore, DISME allowed for a pooled screen of miRNA function and identified an miR-183–182 cluster of miRNAs promoting self-renewal and pluripotency in mouse embryonic stem cells. Altogether, our study demonstrates that DISME is a robust and cost-effective strategy that allows for long-term study and genetic selection of miRNA function in a DGCR8 knockout background.

INTRODUCTION

MicroRNAs (miRNAs) are a class of small noncoding RNAs ~22 nucleotides in length. As one of the most important classes of post-transcriptional regulators, they are predicted to fine-tune the expression of hundreds of mRNAs in mammals (Bartel, 2009; Jonas and Izaurralde, 2015). Numerous reports have demonstrated an essential function of miRNAs in a variety of biologic processes, including development (Amin et al., 2015; Hasuwa et al., 2013; Parchem et al., 2015), regeneration (Tian et al., 2015), and cancer (He et al., 2005; Lujambio and Lowe, 2012). Typically, miRNAs are generated by two sequential cleavages of pri-miRNAs, first by a microprocessor consisting of DROSHA and DGCR8 in nucleus (Denli et al., 2004; Ha and Kim, 2014; Han et al., 2006), then by DICER1 in cytoplasm (Hammond, 2005; Hutvagner et al., 2001). Once loaded into the RNA-induced silencing complex, miRNAs bind their targets through partial complementarity with the 3′ UTR of their target mRNAs (Chendrimada et al., 2005). The second to the eighth nucleotide at the 5′ end of miRNA is essential for the recognition of its mRNA targets, which is termed the “seed sequence” (Lewis et al., 2003).

Tackling the function of miRNAs is often impeded by the extraordinary redundancy among miRNAs. Mammalian genomes encode thousands of miRNAs, and many of them share identical or similar seed sequences, therefore acting redundantly to regulate gene expression (Fischer et al., 2015; Olive et al., 2015). For example, the let-7 family has 13 different members encoded by 10 loci in the mouse genome. In addition, miRNAs with different seed sequences could also repress the same mRNAs or even different mRNAs to exert similar functions, which makes up additional layers of redundancy (Fischer et al., 2015; Olive et al., 2015). To solve the redundancy issue, we and others have constructed in vitro and in vivo miRNA deficient models that do not express any canonical mature miRNAs by knocking out DGCR8 or Dicer1 (Kanellopoulou et al., 2005; Wang et al., 2007). Recently, using DGCR8 knockout cells, Liu et al. (2015) found that canonical miRNAs as a whole facilitate but are not required for the reprogramming of induced pluripotent stem cells. Typically, for specific phenotypic defects found in DGCR8 or Dicer1 knockout cells, screens with a library of chemically synthetic miRNA mimics are then designed to identify miRNAs that can rescue corresponding defects (Wang et al., 2008). Using this strategy, we and others have identified many miRNAs that play important functions in controlling the cell cycle, metabolism, pluripotency, and differentiation of embryonic stem cells (ESCs) (Cao et al., 2015; Gu et al., 2016; Ma et al., 2015; Sinkkonen et al., 2008; Wang et al., 2008, 2013). However, due to the transient nature of transfection of miRNA mimics, studies on the long-term function of miRNAs are limited. In addition, the transfection efficiency of miRNA mimics varies a lot in different cell types, therefore limiting this strategy to only a subset of cells. More importantly, genetic selection cannot be designed based on miRNA mimics, which makes the identification of functional miRNAs a laborious and expensive procedure.
Therefore, a stable miRNA expression system in Dgcr8 knockout cells will accelerate the functional study of miRNAs and generally benefit the miRNA field.

In this study, we developed a cost-effective DGCR8-independent stable miRNA expression (DISME) strategy. We showed that DISME is generally applicable for the expression of miRNAs with different sequences. The miR-290 family of miRNAs are important for the rapid proliferation and maintenance of pluripotency in ESCs (Guo et al., 2015; Melton et al., 2010; Wang et al., 2013). We achieved high expression of miR-294 in Dgcr8 knockout ESCs with DISME and showed that the DISME of miR-294 can efficiently rescue various defects of Dgcr8 knockout ESCs in proliferation and differentiation. In addition, we studied the long-term function of miR-294 in the embryoid body by DISME and found that miR-294 is sufficient to promote the formation of mesoderm and endoderm lineages. More importantly, we demonstrated that DISME can be adapted for genetic selection of miRNA function and identified miR-183–182 cluster of miRNAs acting redundantly with miR-290 cluster to promote pluripotency in mouse ESCs. Therefore, DISME is a robust and cost-effective strategy that allows for long-term study and genetic selection of miRNA function in Dgcr8 knockout cells.

RESULTS

Design of DISME

We designed a DISME strategy based on a Pol III-driven short hairpin RNA (shRNA) strategy that implements a “loop-counting” rule for the precise processing of DICER, as described previously by Gu et al. (2012) (Figure 1A). The construct begins with a guanine for Pol III recognition and ends with six uridines as a termination signal for transcription. The first to the 18th nucleotide sequence of a given miRNA is placed at the 3’ arm of shRNA to allow precise processing. We did not use the full sequence of miRNAs since the 3’ end sequence of miRNAs is not important for their function.

DISME Expresses miRNAs with Precise 5’ Ends in Dgcr8−/− ESCs

To evaluate the universality of DISME, we chose 21 of the most abundant miRNAs expressed in mouse ESCs as candidates (Yan et al., 2017) (Table S1). A pool of plasmids containing a mixture of DISME cassettes was transfected into Dgcr8 knockout ESCs. After brief selection with blasticidin, RNAs were extracted from the cells and small RNAs were analyzed by deep sequencing. Remarkably, we found that DISME of all miRNAs generated reads mapped to shRNA constructs (Figures 1B and 2), indicating that DGCR8 is dispensable for the processing of shRNA. More importantly, for all miRNAs except miR-7a, at least 90% of small RNA reads have 5’ ends mapped to the canonical cleavage site (Figures 1B, 2, and S1), indicating the generality of DISME in expressing miRNAs in Dgcr8 knockout cells.

DISME of miR-294 Is Functional in Dgcr8−/− ESCs

Next, we tested whether miRNAs expressed by DISME were functional. The miR-290 cluster encodes three miRNAs, including miR-291a-3p, miR-294, miR-295 with a seed sequence AAGUGCU, that are highly expressed and play essential functions in promoting proliferation and pluripotency in mouse ESCs (Melton et al., 2010; Wang et al., 2008). We chose miR-294 for further tests since its functions are well characterized in ESCs. To increase the expression level of miR-294, we inserted four tandem units of a U6 promoter-driven expression cassette in the same vector. We gained a cell line that expressed miR-294 at similar level as wild-type ESCs (Figure 3A). We first analyzed the expression of known direct and indirect targets of miR-294 (Cao et al., 2015; Melton et al., 2010; Wang et al., 2008, 2013) and found that all of them were essentially rescued by DISME of miR-294 in Dgcr8 knockout ESCs to the level seen in wild-type ESCs (Figure 3B). Excitingly, miR-294 DISME cells also proliferated at a rate similar to wild-type ESCs (Figure 3C). We then tested whether DISME of miR-294 can promote the self-renewal and pluripotency of Dgcr8−/− ESCs in the presence of differentiation-inducing miRNA let-7 (Melton et al., 2010; Wang et al., 2013). Based on colony morphology, alkaline phosphatase staining, and the expression of key pluripotency genes, the DISME of miR-294 successfully blocked let-7c-induced differentiation in Dgcr8−/− ESCs (Figures 3D and 3E). Moreover, the DISME of miR-294 also blocked the function of other previously reported pluripotency silencing inducers, including miR-26a, miR-218, miR-199b, miR-99b, and miR-193 (Wang et al., 2013) (Figure 3F). Taken together, these data demonstrate that DISME can produce high enough levels of miRNAs that recapitulate previously identified functions of synthetic miRNA mimics.

miR-294 Promotes the Formation of Mesoderm and Endoderm Lineages in Embryoid Bodies

We previously showed that Dgcr8−/− ESCs cannot differentiate properly during embryoid body differentiation even when more cells were added to compensate for proliferation defects (Wang et al., 2007). Interestingly, the miRNAs from the miR-290 family are continuously highly expressed in embryoid bodies for at least 8 days (Hadjimichael et al., 2016) (Figure S2A), suggesting that this family may have functions during embryoid body differentiation. Transfection of miRNA mimics into embryoid body is apparently impractical, so we investigated the function of miR-294 in embryoid body differentiation using DISME.
Interestingly, large cysts were readily observed in both wild-type and miR-294 DISME but not Dgcr8−/− ESCs (Figure 4A). The yolk-sac-like cyst structure indicated that miR-294 may promote ESC differentiation into mesoderm and endoderm. Indeed, qPCR analysis of endoderm (Hnf4, Sox17, Foxa2, and Afp) and mesoderm (T, Mix1, Gata4, and Cdx2) markers showed that miR-294 partially rescued the defects of Dgcr8−/− ESCs in differentiation into these lineages (Figures 4B and 4C). However, qPCR analysis of ectoderm (Sox3, Pax6, Tubb3, and Nestin) markers indicate that miR-294 has little impact (comparing Dg8KO and Dg8KO-miR-294) on ectoderm differentiation (Figure 4D). These data indicate that miR-294 initiated meso- and endoderm differentiation by facilitating the exit of the
pluripotency state at early stages of differentiation. Consistent with this, miR-294 DISME promoted the silencing of naive pluripotency genes and the upregulation of primed markers during transition from naive ESC state to primed epiblast-like cell (EpiLC) state in differentiation media containing basic fibroblast growth factor (Figure S2B), although not as efficiently as observed in wild-type ESCs. Taken together, these data validated that DISME can be

**Figure 2. Observed Cleavage Efficiency at the 5' End of miRNAs from Small RNA-Seq**

Arrows and associated numbers indicate the percentage of sequences starting at the 5’ end of each miRNA relative to the total number of miRNA strand reads.
Figure 3. Stable Overexpression of miR-294 by DISME Rescues the Proliferation and Self-Renewal Defects of Dgcr8⁻/- ESCs
(A) The relative expression level of DISME miR-294 compared with miR-294 in wild-type cells by miRNA qPCR. miR-183 and miR-293 are two negative controls showing that other miRNAs are not expressed in miR-294 DISME Dgcr8⁻/- ESCs. Data were normalized to 5S rRNA and then to wild-type ESCs. Shown are means ± SD, n = 3 independent experiments.
(B) qRT-PCR analysis of direct targets of miR-294 and other genes indirectly upregulated by miR-294. The β-actin gene was used as a control. For each gene, data were normalized to the mRNA level of wild-type ESCs. Shown are means ± SD, n = 3 independent experiments.
(C) Population doubling time of wild-type, Dgcr8⁻/- and DISME miR-294 rescued Dgcr8⁻/- ESCs. Shown are means ± SD, n = 4 independent experiments.
(D) Representative images of alkaline phosphatase staining of Dgcr8⁻/- and DISME miR-294 rescued Dgcr8⁻/- cells after mock or let-7c transfection.
(E) qRT-PCR for Oct4, Sox2, and Nanog for Dgcr8⁻/- and DISME miR-294 rescued Dgcr8⁻/- cells after mock or let-7c transfection. The β-actin gene was used as a control. For each gene, data were normalized to the mRNA level of mock-transfected ESCs. Shown are means ± SD, n = 3 independent experiments.
(F) qRT-PCR for Oct4, Sox2, and Nanog for Dgcr8⁻/- and DISME miR-294-rescued Dgcr8⁻/- cells after transfection with various differentiation-inducing miRNAs. The β-actin gene was used as a control. For each gene, data were normalized to the mRNA level of mock-transfected ESCs. Shown are means ± SD, n = 3 independent experiments.
used for the long-term study of miRNA functions and identified the meso-endoderm-promoting function of miR-294 during embryoid body differentiation.

**miR-183/-182 Act Redundantly with the miR-290 Family to Promote the Self-Renewal and Pluripotency of ESCs**

The let-7 family of miRNAs are highly enriched in somatic cells and rapidly upregulated during ESC differentiation (Landgraf et al., 2007). Interestingly, let-7c silences the self-renewal of Dgcr8−/− but not wild-type ESCs. The miR-290 family of miRNAs are reported to be responsible for blocking the differentiation-inducing function of let-7c (Melton et al., 2010). However, whether the miR-290 family of miRNAs are the only miRNAs required to block the function of let-7c is not clear. We investigated this question with miR-290−/− ESCs. Surprisingly, let-7c was not able to silence the self-renewal of ESCs lacking the miR-290 family (Figure S3), indicating that there are other miRNAs capable of blocking let-7c function in ESCs.
To identify miRNAs other than the miR-290 family that can block the function of let-7c, we designed a selection strategy based on DISME. The selection criterion is that Dgcr8<sup>−/−</sup> ESCs expressing miRNAs blocking let-7c will be enriched after introduction of let-7c. We first transfected Dgcr8<sup>−/−</sup> ESCs with a mixture of DISME vectors encoding miRNAs that are abundant in ESCs. After 5 days of blastocidin treatment to get rid of non-transfected cells, let-7c was transfected to enrich DISME of miRNAs that can promote the self-renewal and pluripotency of ESCs. After three rounds of selection, cells were collected for miRNA qPCR (Figure 5A). Interestingly, miR-182 was identified as the most significant hit. Moreover, its paralog miR-183 was also enriched in the selection as the third most significant hit (Figure 5B). miR-182 and miR-183 were produced from the miR-183–182 cluster, shared similar seed sequences, were highly enriched in mouse ESCs, and downregulated during embryoid differentiation (Hadjimichael et al., 2016) (Figure S4A). We found that the expression of miR-182 and miR-183 was also higher in naive ESCs than primed EpilCs (Gu et al., 2016) (Figure S4B), suggesting their function is associated with naive pluripotency. Furthermore, we verified the function of miR-182 and miR-183 in blocking let-7c with an miRNA mimics approach (Figures 5C, 5D, S4C, and S4D). Excitingly, miR-182 also suppressed two other differentiation-inducing miRNAs, including miR-26 and miR-218, in silencing the self-renewal of Dgcr8<sup>−/−</sup> ESCs, suggesting its general function against differentiation-inducing miRNAs (Figures 5C and 5D). To further test whether miR-182 and miR-183 act together with the miR-290 family of miRNAs to promote the self-renewal and pluripotency of ESCs, we constructed miR-290<sup>−/−</sup> and miR-183–182<sup>−/−</sup> double knockout mouse ESCs (DKO) using CRISPR/CAS9 (Figures S5A–S5F). Excitingly, while deletion of either miR-290 or the miR-183–182 cluster retained the ability to self-renew after the introduction of let-7c, the self-renewal of DKO ESCs was largely silenced by let-7c as demonstrated by qPCR analysis and alkaline phosphatase staining (Figures 5E and 5F). Therefore, miR-290 and miR-183–182 clusters functioned redundantly in promoting self-renewal and pluripotency in mouse ESCs. However, the precise pattern for the rescue of Oct4, Sox2, and Nanog expression by miR-182 was not exactly the same as miR-294 (Figure S5F), suggesting the two classes of miRNAs function through different mechanisms. In addition, DKO ESCs grew much slower than wild-type or single knockout ESCs (Figure 5G), suggesting possible redundancy of two miRNA families in regulating the proliferation of ESCs. Finally, consistent with its function in promoting ESC self-renewal and pluripotency, overexpressing miR-183–182 cluster increased the number of Oct4-GFP reporter-positive colonies ~1.7-fold in the reprogramming of mouse embryonic fibroblasts by Yamanaka factors (OCT4, SOX2, KLF4, and MYC) (Figure 5H). Taken together, DISME selection successfully identified miR-183–182 as another miRNA cluster that promotes self-renewal and pluripotency in ESCs.

**DISCUSSION**

In summary, we established that DISME can stably express miRNAs in Dgcr8<sup>−/−</sup> cells. Previously, functional studies of miRNAs in Dgcr8<sup>−/−</sup> cells could only be performed with chemically synthesized miRNA mimics (Cao et al., 2015; Gu et al., 2016; Guo et al., 2015; Ma et al., 2015; Melton et al., 2010; Sinkkonen et al., 2008; Wang et al., 2008, 2013). Here, we showed that DISME can produce high enough levels of miRNAs that can almost fully rescue...
defects of $Dgcr8^{−/−}$ cells in proliferation, gene expression, and differentiation. Therefore, DISME provides an alternative and cost-efficient approach to study miRNA functions in $Dgcr8^{−/−}$ cells. Two significant advantages of DISME versus miRNA mimics include its application in the study of long-term function and genetic selection of miRNAs (Figure 6) in $Dgcr8^{−/−}$ cells. Taking advantage of DISME, we found that miR-294 played a role in driving the formation of mesoderm and endoderm lineages. In addition, we identified an miR-182 cluster of miRNAs acting redundantly with an miR-290 cluster in promoting the self-renewal and pluripotency of ESCs. Together, our study demonstrates the applicability of DISME for long-term functional studies and genetic selection of miRNAs in $Dgcr8^{−/−}$ cells.

How the miR-182 family of miRNAs promotes self-renewal is not clear. The seed sequence of miR-182 is significantly different from that of miR-294. In addition, miR-294 but not miR-182 increased the proliferation rate of $Dgcr8^{−/−}$ ESCs (Wang et al., 2008). In future, knockout and DISME strategies should be used to elucidate the functions of miR-182 and miR-294 families in the differentiation of ESCs and during early embryonic development. Moreover, identification of functional mRNA targets of miR-182 and miR-294 will likely contribute to the understanding of redundant and non-redundant functions of these two miRNA families. Likewise, how miR-294 promotes the formation of meso- and endoderm is also not clear. Future work should focus on dissecting the function of miR-294 families at different stages of meso- and endoderm differentiation. This may be achieved through a conditional knockout strategy using tissue-specific Cre recombinase. Intercrossing the $Dgcr8^{flox/flox}$ mouse line with mouse lines expressing tissue-specific promoter-driven Cre recombinase provides the means to study the function of miRNAs in specific tissue development or disease. Using this strategy, miRNAs were reported to be essential for the development or disease progression in a variety of tissues, including skin (Yu et al., 2009), the immune system (Bezman et al., 2010; Steiner et al., 2011), vascular smooth muscle (Chen et al., 2012), adipocytes (Kim et al., 2014), heart (Rao et al., 2009), kidney (Bartram et al., 2015), and brain (Hsu et al., 2012; Lin et al., 2015). However, a lack of approaches to stably express miRNAs in $Dgcr8^{−/−}$ cells has prevented identification of the exact miRNAs responsible for the defects uncovered by conditional $Dgcr8$ knockout mouse models. By adopting DISME into appropriate virus-based vectors, we expect that DISME will help accelerate the discovery of important miRNAs in normal function and disease progression of different tissues.
**EXPERIMENTAL PROCEDURES**

**Plasmids and Vector Construction**

miRNA sequences were cloned downstream of a U6 promoter in a piggyBac vector that contains a blasticidin resistance gene driven by human GAPDH promoter. We used an 18 nt sequence from the 5′ end of miRNAs to design oligonucleotides (“N1_N18” is 18 bp of the miRNA sequence, while “R1_R18” is the reverse complementary sequence of “N1_N18”). Four oligos were synthesized, then annealed at 95°C for 5 min and at room temperature for 1 hr. Annealed oligos were ligated with T4 PNK (NEB, M0201), then annealed at 95°C for 5 min and at room temperature for 1 hr. Annealed oligos were finally ligated into HpaI- and XhoI-digested pCMV-Tag3B (Stratagene). Sequences for oligos to generate miRNA expression plasmids can be found in Table S1.

**Cell Culture, Plasmid Transfection, and Alkaline Phosphatase Staining**

Wild-type and Dicer−/− ESCs were cultured on gelatin or irradiated mouse fibroblasts as reported previously (Wang et al., 2008). Approximately 1 day after plating, miRNA expression plasmids were transfected into cells, along with PBase expression plasmids using Lipofectamine 2000 (Life Technologies). After transfection, cells were treated with 10 μg/mL blasticidin S (Gibco) for 4 days. After that, cells were treated with TRIzol for RNA extraction or plated at single-cell density for colony picking. For alkaline phosphatase staining, cells were fixed with 4% paraformaldehyde for 10 min at single-cell density for colony picking. For alkaline phosphatase staining, cells were treated with TRIzol for RNA extraction or plated at single-cell density for colony picking. For alkaline phosphatase staining, cells were fixed with 4% paraformaldehyde for 10 min at room temperature, then stained for alkaline phosphatase staining, cells were fixed with 4% paraformaldehyde for 10 min at room temperature, then stained for alkaline phosphatase staining.

**Small RNA Deep Sequencing and Data Analysis**

Small RNAs libraries were generated using NEBNext small RNA library Prep Set for Illumina (E7330) and NEBNext multiplex oligos for Illumina (E7335) according to the manufacturer’s instructions. Libraries were sequenced using the Illumina HiSeq2500. After trimming the adapters, the remaining sequence reads were matched to the miRBase r19 miRNA stem-loop sequences by Bowtie. Then the unmatched sequence reads were matched to rRNA, tRNA, small nuclear RNA (snRNA), repeats, intron, and mRNA. In each step, reads with zero or one mismatch were retained and the unmatched sequences were rematched in the next step. The “-best-strata” options were specified to allow mismatches and favor high-quality alignments. Mouse ribosomal, tRNA, and snoRNA sequences were extracted from Ensembl (Mus_musculus.GRCm38.69.ncbi). Repeats, introns, and miRNAs were extracted from UCSC Tables (mm9). The miRNA expression profile was obtained according to the criterion described previously (Gu et al., 2016).

**qRT-PCR and miRNA RT-PCR**

RNA was extracted using TRIzol (Roche) and quantified by Biodropers BD2000. Around 500 ng of RNA was reverse transcribed with a first-strand cDNA synthesis kit (Vazyme). qPCR was performed on ABI Step One Plus (Applied Biosystems). For miRNA qPCR, RNA samples were first polyadenylated with poly(A)polymerase and then reverse transcribed using a modified oligo-IT primer as described previously (Gu et al., 2016). Sequences for primers are listed in Table S2.

**Embryoid Body Formation**

Undifferentiated mESCs were first cultured in standard medium. Embryoid bodies were generated in low-attached dishes containing leukemia inhibitory factor (LIF)-free medium. The medium was changed every other day. RNA was extracted at day 0, 8, and 16 of differentiation and analyzed by RT-qPCR.

**Cell Proliferation Assay and Cell-Cycle Analysis**

Cells were plated at 4,000 cells per well in 96-well plates, incubated for 0, 24, and 48 hr before MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Stock solution was added to a final concentration of 1 ng/μL. After incubation at 37°C for 3 hr, the MTT solution was removed, and 100 μL of DMSO was added to dissolve the precipitate. Absorbance was recorded at 540 nm using a MultiskanFC (Thermo Scientific). Cell-cycle analysis was performed as described previously (Wang et al., 2008).

**miRNA Cluster Knockout Strategy**

To knock out miR-290 and the miR-183/182 cluster, a pair of guide RNA sequences for each locus were designed by http://crispr.mit.edu/. Knockout of miR-290 and miR-183–182 clusters was verified by genomic PCR and miRNA qRT-PCR. Guide RNA sequences and the primers used in the knockout experiments are listed in Table S3.

**Somatic Cell Reprogramming**

OG2 mouse embryonic fibroblasts (MEFs) bearing a transgenic Oct4-GFP reporter were isolated, cultured, and reprogrammed as previously described (Li et al., 2010). For reprogramming, ~15,000–20,000 MEFs per well in 12-well plates were infected twice with retroviral supernatants expressing Yamanaka factors and the miR-183–182 cluster of miRNAs. After infection, MEFs were cultured in mouse ESC medium (DMEM supplemented with 15% fetal bovine serum [GIBCO], non-essential amino acids, sodium pyruvate, β-mercaptoethanol, penicillin/streptomycin and LIF). GFP-positive colonies were counted at day 15 after the first infection.

**Statistical Analysis**

The data are presented as means ± SD except where indicated otherwise. The two-tailed unpaired Student’s t-test was used to determine statistical significance. A p value <0.05 was considered statistically significant.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes five figures and three tables and can be found with this article online at https://doi.org/10.1016/j.stemcr.2017.08.027.
AUTHOR CONTRIBUTIONS

X.W.W. performed all the experiments except Figure 5H with help from the other authors. J.H. performed the bioinformatics analysis. W.T.G. assisted in performing experiments in Figures 5C and 5D. L.Q.L. and S.Y.H. assisted in making the DISME constructs. X.B., X.G., and M.A.E. performed the reprogramming experiment in Figure 5H. All authors were involved in the interpretation of data. Y.W. conceived and supervised the project. Y.W. and X.W.W. wrote the manuscript.

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