Supplementary materials

The miR-125 family is an important regulator of the expression and maintenance of maternal effect genes during preimplantational embryo development

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Running head: miR-125 family regulates MEG expression

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**Supplementary Figure 1.** Endogenous *Sebox* and *Lin28a* transcript levels were differentially regulated by different mimics in different cell types, including oocytes and mESC. The endogenous *Sebox* transcript level was measured by quantitative real-time RT-PCR after oocytes (a) and mESCs (b) were treated with mimics of each miR-125 family member. The endogenous *Lin28a* transcript level was measured after oocytes (c) and mESCs (d) were treated with mimics of each miR-125 family member. NC, treatment with negative control mimic. *p*<0.05 compared with the negative control mimic-transfected or negative control mimic-microinjected group.
Supplementary Figure 2. Downregulation of c-Myc by Lin28a siRNA. (a) The expression pattern of Lin28a during early embryogenesis. The relative gene expression of Lin28a in a single oocyte or embryo throughout development was determined by quantitative real-time RT-PCR. Synthetic GFP RNA was used as an internal control for normalization. The results are expressed as the mean±SEM of at least three experiments. GV, germinal vesicle; MII, metaphase II; PN, pronuclear stage; 2C, 2-cell stage; 4C, 4-cell stage; MO, morula stage; BL, blastocyst stage. (b) Expression of Lin28a transcript in mouse oocytes. Lin28a mRNA levels during oocyte maturation were determined by quantitative real-time RT-PCR. *p<0.05 compared with GV oocytes. (c) Expression of Lin28a protein in mouse oocytes, as determined by Western blot analysis. The experiment was conducted three times, with 100 oocytes per lane. The blots were reprobed with an Actin antibody as a loading control. (d) Lin28a RNAi suppressed Lin28a and c-Myc mRNA levels but did not affect the expression of other reprogramming factors (Oct4, Sox2, and Klf4) in Lin28a-silenced oocytes. H1foo was used as an internal control. (e) Lin28a RNAi disrupts Lin28a and c-Myc protein expression, as confirmed by Western blot analysis. The lysates of 200 MII oocytes injected with control siRNA or Lin28a siRNA were separated by SDS-PAGE. Proteins were visualized using specific antibodies against Lin28a, c-Myc and Actin (loading control).
**Supplementary Table 1.** miR-125 family prediction score for Sebox and Lin28a using each computational algorithm.

| Gene | miRNAs   | Algorithm       |          |          |          |
|------|----------|-----------------|----------|----------|----------|
|      |          | TargetScan      | miRanda  | miRmap   |          |
|      |          | Context+ score<sup>a</sup> | mirSVRscore<sup>b</sup> | miRmapscore<sup>c</sup> |          |
| Sebox| miR-125a-5p | -0.37            | -0.1229  | 84.41    |          |
|      | miR-125b-5p | -0.35            | -0.1205  | 88.10    |          |
|      | miR-351-5p  | -0.33            | -0.1340  | 84.85    |          |
| Lin28a| miR-125a-5p | -0.28            | -0.6884  | 89.78    |          |
|      | miR-125b-5p | -0.28            | -0.6847  | 89.30    |          |
|      | miR-351-5p  | -0.29            | -0.6847  | 91.25    |          |

The more negative the value of the context+ score<sup>a</sup> from TargetScan and the mirSVRscore<sup>b</sup> from miRanda, the more favorable and higher the probability of mRNA binding.

<sup>c</sup>The miRmap score ranges from 0 to 100, where 0 represents no complementarity and 100 represents complete complementarity.
Supplementary Table 2. List of the MEGs identified as predicted targets of the miR-125 family via dry lab methods (i.e., using three computational algorithms: miRanda, TargetScan, and miRmap). Among the list of MEGs whose depletion has been reported to lead to embryonic arrest at the 2C stage, only *Filia* and *Nlrp5* appear to be unlikely targets of miR-125 family members; the rest of the MEGs were predicted as tentative targets of at least one of the miR-125 family members.

| Gene     | Computational algorithm | miRanda | TargetScan | miRmap |
|----------|-------------------------|---------|------------|--------|
| Filia    |                         | -       | -          | -      |
| Nlrp5    |                         | -       | -          | -      |
| Bnc1     |                         | miR-125a-5p | miR-125b-3p | miR-125b-3p |
| Lin28a   | miR-125a-5p | miR-125b-5p | miR-125a-5p | miR-125b-3p |
|          | miR-125b-5p | miR-125b-5p | miR-125b-3p | miR-351-5p |
|          | miR-351    | miR-125b-3p | miR-351-5p |
| Ooep     |                         | -       | miR-125a-3p | miR-125a-3p |
| Sebox    | miR-125a-5p | miR-125a-5p | miR-125a-5p |
|          | miR-125b-5p | miR-125b-5p | miR-125b-3p |
|          | miR-351    | miR-351-5p | miR-351-5p |
| Smarca4  |                         | -       | miR-125a-3p | miR-125b-3p |
|          |               |         | miR-125b-3p |
| Trim24   |                         | -       | miR-125a-5p | -      |
|          |               |         | miR-125a-3p |
|          |               |         | miR-125b-3p |
|          |               |         | miR-351-5p |
| Ube2a    | miR-125a-5p | miR-125a-3p | -      |
|          | miR-125b-5p | miR-351-3p |
|          | miR-351    | miR-351-3p |
| Zfp36l2  | miR-125a-3p | miR-125a-3p | miR-125a-3p |