Figure S1. Predicted secondary structure of Oct4-R2 where Oct4-95 (enclosed in the rectangle) was derived from.
**An introduction to Figure S2:**

RNA-binding proteins, in general, exert their functions by binding to target RNAs in the form of ribonucleoprotein particles (RNPs). In our previous studies we isolated Lin28-containing RNPs from human ES cells using antibodies specific for Lin28. The identities of associated mRNAs were determined by high throughput deep sequencing. We found that approximately 5% of mRNAs were enriched >2.5-fold in Lin28 IP versus control IP samples (38). Gene Ontology analysis revealed that the top mRNA class selected by Lin28 represent genes encoding RNP proteins, followed by genes involved in translation (including ribosomal proteins and key translation initiation and elongation factors) and genes participating in cellular metabolism. The association of Lin28 with mRNAs for ribosomal proteins and metabolic enzymes was of particular interest as their expression levels are known to be coupled to cell growth and survival. Subsequent validation studies using polysome profiling and reporter gene analysis suggested that Lin28 stimulates the expression of many or most of these targets at the translational level. These findings thus offered a mechanistic insight into why down-regulation of Lin28 expression negatively impacts the growth and survival of both human embryonic stem (ES) cells and embryonal carcinoma (EC) cells (38).

A careful inspection of our genome-wide data (Supplemental tables S1 and S2 in (38)) revealed that the RPS19 and HER2 mRNAs were enriched by 7.7 and 2.8-fold in Lin28 IP from human ES cell extracts, respectively. Figure S2A shows screenshots of normalized UCSF Genome Browser alignments of Lin28 IP and preimmune IP sequences. To confirm the interactions between Lin28 and RPS19 and HER2 mRNAs, here we performed IP using extracts from PA-1 cells (a human EC line), followed by reverse transcription and quantitative real-time PCR (RT-qPCR). PA-1 cells have been used as a surrogate for human ES cells in studies of post-transcriptional regulation of gene expression mediated by Lin28, owing to many shared properties with human ES cells (38-40). Figure S2B recapitulates our previous
observations (38) that both the Oct4 and HMGA1 mRNAs were enriched over 2.5-fold in Lin28 IP vs. preimmune IP, while the non-target beta-actin mRNA was not. Significantly, the RPS19 and HER2 mRNAs were also enriched (~15 and 7-fold, respectively) under the same conditions (Fig. S2B), demonstrating that the interactions of Lin28 with RPS19 and HER2 mRNAs can be reproduced in PA-1 cells.

Lin28 promotes the translation of target mRNAs, including oct4 and hmgal, as determined by various analyses (38-43). We speculated that Lin28 might regulate RPS19 and HER2 mRNAs in a similar fashion. It is widely believed that increased polysome association of an mRNA indicates increased translation of that particular mRNA. Using polysome profiling combined with heterologous expression of a nonfunctional Lin28 mutant (Lin28ΔC) in PA-1 cells we demonstrated that the increase in both Oct4 and HMGA1 expression was at least in part due to translational stimulation by Lin28 (38-40). Lin28ΔC contains a 35-amino acid deletion in the carboxy terminal region of Lin28 (Fig. S2C). This mutant is capable of RNA binding but is incapable of interacting with RNA helicase A (RHA), thus functioning as a dominant-negative inhibitor of Lin28-mediated stimulation of translation (38, 40). As expected, when an epitope-tagged FL-Lin28ΔC was expressed in PA-1 cells by transfection, we observed selective inhibition of translation of oct4 and hmgal, as determined by polysome profiling analysis (38, 40). Here, in the presence of FL-Lin28ΔC, the association of RPS19, HER2, Oct4, and HMGA1 mRNAs with polysomes decreased by 51%, 45%, 62% and 45%, respectively, when compared to vector only control cells (Fig. S2D, compare light grey bars to black bars), while the polysome association of beta-actin mRNA did not change under these same conditions (Fig. S2D, first column from right). These results support the notion that selective binding of the Lin28 mutant protein to target mRNAs prevents endogenous wild-type Lin28 from binding to the same mRNAs, hence interfering with their translation. The fact that the association of RPS19 and HER2 mRNAs with polysomes also
decreased in the presence of FL-Lin28ΔC supports that these mRNAs are *in vivo* targets of Lin28 regulation at the translational level.

**Figure S2.** RPS19 and HER2 mRNAs are targets of Lin28 post-transcriptional regulation. (A) RPS19 and HER2 mRNAs were enriched in Lin28-containing RNPs in human ES cells as revealed by IP and deep sequencing. RNA-Seq libraries were generated using RNAs captured by IP using anti-Lin28 or preimmune IgG. The libraries derived from Lin28 IP and preimmune IP samples were individually used for deep sequencing. About 10 million 75-nt reads were obtained from each IP sample, and these sequences were uniquely aligned to a combined database of the human genome and splice junctions. The read counts were further analyzed using normalized values to identify transcripts that were significantly different between the Lin28 IP and preimmune IP samples. The heights of the peaks indicate frequencies of the 75-nt sequence reads that match the particular exon regions of the genome marked as blue boxes at the bottom of the histograms. (B) RPS19 and HER2 mRNAs are
enriched in Lin28-containing RNPs in PA-1 cells. RNPs were isolated from PA-1 cells using anti-Lin28 antibody or preimmune IgG, followed by RNA extraction and RT-qPCR. Relative abundance of the indicated mRNAs associated with anti-Lin28 vs. preimmune IP was plotted as relative fold enrichment.

(C) A diagram of wild-type and mutant Lin28 proteins with the two RNA-binding domains CSD (cold shock domain) and CCHC (cys-cys-his-cys) zinc finger motif marked. Numbers are in amino acids. (D) Polysome profile analysis. PA-1 cells were transfected with FL-Lin28ΔC or empty vector. Polysome fractionations were carried out 48 h later. RNAs were extracted from each fraction (RNP, 40S, 60S, 80S, and polysomes) and subjected to RT-qPCR using primers specific for the indicated genes. The efficiency of translation was calculated, after normalization of beta-tubulin mRNA, by comparing the RNA level in polysomes with total fractions (combining the polysomal and non-polysomal fractions). Polysome association of mRNAs in vector-transfected cells was arbitrarily set as 1. Numbers are mean ± SD (n=3). (E) Representative polysome profiles of experiments in D.
Figure S3. A predicted secondary structure of the 456-nt long LRE from the coding region of human RPS13 mRNA. The numbers at the 5’ and 3’ ends of the RNA are in nucleotides relative to the transcriptional start sites of RPS13. The putative “A” bulges are highlighted in red.
Figure S4. A predicted secondary structure of the 330-nt long LRE from the coding region of human EEF1G mRNA. The numbers at the 5’ and 3’ ends of the RNA are in nucleotides relative to the transcriptional start sites of EEF1G. The putative “A” bulges are highlighted in red.
Supplementary References

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