legends and data plotted as $-\Delta\Delta$Ct. Left and right panels depict the results for Lin28 and Zcchc11, respectively. For each panel, the upper right quadrant contains those miRNAs that are reproducibly upregulated by both siRNA knockdown sets. Depicted errors bars are ±s.e.m. (b) Luciferase reporter assays to evaluate the in vivo relevance of elevated mature let-7 levels. Renilla luciferase lacked (termed “Absent”), contained an imperfect, or had a perfect let-7 binding site in its 3’UTR. Depicted errors bars are ±s.d.

**Online Methods**

**Mouse Zcchc11 cloning.** We cloned the mouse Zcchc11 open reading frame (derived from BC150791) inframe into an N-terminal 2X-HA tagged expression vector under the control of the mouse EF1α promoter. We performed site-directed mutagenesis to generate a construct in which two of the three aspartates of the catalytic aspartate triad (aa.1008 and aa.1010 relative to AAI50792) were replaced with alanine codons to generate an inactive enzyme. The sense mutagenic primer sequence is 5’-aatggatttggattccgagatagtgcactggccatttgtatgactctggaaggccat-3’. We sequence verified wild-type and mutant clones.

**Affinity purification of HA-Zcchc11 and Flag-Lin28.**

We transfected an expression plasmid for HA-Zcchc11 into HEK293 cells using Lipofectamine 2000 (Invitrogen). We harvested transfected cells in lysis buffer (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 1 mM EDTA, 1% (v/v) Triton x100, 10% (v/v) Glycerol, 1.5 mM MgCl₂, 5 mM DTT, 0.2 mM PMSF), and performed Protein purifications using anti-HA-agarose (Sigma). We used HA peptide (Sigma) to elute purified proteins and confirmed the presence of purified protein by Western Blot analysis with a mouse anti-HA antibody (Roche). We purified Flag-Lin28 from a doxycycline-inducible mouse KH2 embryonic stem cell stable cell line. The cell line was created using the inducible gene targeting kit (Open Biosystems). Using anti-Flag M2 affinity gel (Sigma) we affinity-purified Flag-Lin28, and eluted the purified proteins using Flag peptide. We analyzed
the affinity eluate by Western blot with a mouse anti-Flag-Antibody (Sigma), and analyzed an aliquot of the elution by silver staining. We purified recombinant His<sub>6</sub>-Lin28 as previously described<sup>6,10</sup>.

**Electrophoretic mobility shift analysis**

Electrophoretic mobility shift assay (EMSA) was performed as previously described<sup>10</sup>.

**In vitro uridylation assay**

We incubated purified proteins with 4 pmol of unlabelled synthetic RNA for 1 hour at 37°C in a 30 µl reaction mixture containing 100 mM KCl, 20 mM Tris-HCl pH 7.6, 10% (v/v) Glycerol, 125 nM [α-<sup>32</sup>P]UTP, 3.2 mM MgCl<sub>2</sub>, 40 U µl<sup>-1</sup> RNasein ribonuclease inhibitor (Promega). As a control we used 8 U of recombinant Poly(A) Polymerase. For competition experiments, we added 10x and 100x excess of unlabelled rNTP as indicated. We ran the reaction products on 15% (w/v) denaturing polyacrylamide gels and detected bands by autoradiography.

**In vivo Knockdowns and Real-time RT-PCR.**

For knockdown experiments, we reverse transfected siRNAs (see Supplemental Table 3) in either P19 or feeder-free J1 mouse embryonic stem cells using Lipofectamine2000 in 6 well plates, according to the manufacturer’s protocol (Invitrogen). We isolated total RNA 60 hours post-transfection using TriZol reagent (Invitrogen). To analyze relative mRNA levels, we used 2 µg of total RNA and performed reverse transcription reactions using random hexamers and SuperScriptIII (Invitrogen). To analyze miRNA expression, we used gene-specific stem-loop RT primers (Applied Biosystems) for reverse transcription from 10ng total RNA. Relative levels of mRNAs and miRNAs were determined by TaqMan based real-time PCR, using Gapdh and snoRNA-142 for normalization, respectively. The TaqMan PCR assays that were used are listed
in Supplemental Table 4. For global microRNA profiling, we used the TaqMan Rodent MicroRNA A Array v2.0 with 350ng total RNA as starting material for the multiplex RT with pre-amplification, according to manufacturer’s directions (Applied Biosystems). We normalized the resulting data to the average of the four endogenous controls present on the TLDA card (U6 snRNA, U87 scaRNA, snoRNA-135, and snoRNA-202).