Supplementary information:

Supplementary Figure 1 (Related to Figure 1): Lin28A physically interacts with Zcchc11. (A) Co-immunoprecipitation (co-IP): Hela cells were co-transfected with Human myc-Lin28A, or myc-Lin28B with a titration of Flag-Zcchc11 plasmid (1X), Flag-Zcchc11 (5X), or control (Mock). Flag-IP and Flag- and Myc-western blots were performed to detect expression and interaction respectively. (B) Flag-Zcchc11 interacts with endogenous Lin28A. Igrov1 cells were transfected with Flag-Zcchc11 (or Mock) and the IP was analyzed by Western blot using antibodies to detect Lin28A.

Supplementary Figure 2 (Related to Figure 4): Stoichiometry shifts of Lin28A and Lin28B. The data are plotted as the fraction of bound labeled RNA versus molar equivalents of recombinant protein. The 7:1 curve most closely approximates the data for both proteins.

Supplementary Figure 3 (Related to Figure 5): Lin28B-mediated transformation and tumorigenicity is Zcchc11-independent. (A) Lin28B mRNA expression levels are increased during transformation of MCF10A ER-Src cells. Lin28B mRNA expression was evaluated 1, 4, 12, 24, 36h post tamoxifen (TAM) treatment of MCF10A ER-Src cells by real-time PCR analysis. Error bars ±S.E.M. (n=3) (B) Inhibition of Lin28B expression does not block the transformation ability of MCF10A ER-Src cells. Phase-contract images of MCF10A ER-Src treated with TAM for 36h in the presence or absence of a siRNA against Lin28B (siLin28B), a monoclonal antibody against IL6 (Ab-IL6) or a siRNA against Zcchc11 (siZcchc11#1). (C) Inhibition of Lin28B but not Zcchc11 blocks the tumorigenicity of MCF10A ER-Src cells. MCF10A ER-Src transformed cells untreated or treated with siRNA negative control (siRNA NC), two different siRNAs against Zcchc11 (siZcchc11#1, siZcchc11#2), Ab-IL6 and siLin28B were plated in soft agar and their ability to form colonies was evaluated 20 days later. The experiment was repeated thrice and the statistical significance was calculated using Student's t test. (D) Effectiveness of siRNA inhibition of Zcchc11 expression in MCF10A ER-Src cells. MCF10A ER-Src cells were treated with siRNA NC or siZcchc11#1 or siZcchc11#2 and Zcchc11 mRNA expression was tested by real-time PCR 24h post transfection.

Error bars ±S.E.M. (n=3) (E) Inhibition of Lin28B but not of Zcchc11 allows up-regulation of let-7a microRNA expression in MCF10A ER-Src transformed cells. Let-7a expression level was tested by real-time PCR analysis 24h post transfection. Error bars ±S.E.M. (n=3) (F) Inhibition of Lin28B but not of Zcchc11 results in increased IL6 production levels expression in MCF10A ER-Src transformed cells. IL6 production was examined by ELISA assay in MCF10A ER-Src transformed cells 48h post transfection. mean ± SD n=3 (G) Inhibition of Lin28B but not of Zcchc11 suppresses MCF10A ER-Src tumor growth in xenografts. The treatments with siRNA NC, siZcchc11#1 and siLin28B were performed intraperitoneally (i.p.) for 5 cycles starting on day 15. Error bars ±S.E.M. (n=3). (H) Efficiency of siRNA inhibition of Zcchc11 in xenograft tumors (day 39). Zcchc11 mRNA expression levels were tested by real-time PCR on tumors untreated or treated with siRNA NC or siZcchc11#1. Error bars ±S.E.M. (n=3) (I) Inhibition of Lin28B but not of Zcchc11 allows up-regulation of let-7a expression levels in xenograft tumors (day 30). Let-7a expression levels were tested by real-time PCR on tumors untreated or treated with siRNA NC or siZcchc11#1 or siLin28B. Error bars ±S.E.M. (n=3)

Supplementary Figure 4 (Related to Figure 7): Lin28A, Lin28B and let-7a expression in colon adenocarcinomas. (A) A subset of colon adenocarcinomas expresses significantly higher levels of Lin28A compared to the uninvolved normal tissues. Sections were subjected to immunohistochemistry for Lin28A (red) and counterstained with haematoxylin. (B) A different subset of adenocarcinomas expresses significantly higher levels of Lin28B compared to the uninvolved tissues. Sections were subjected to immunohistochemistry for Lin28B (red) and counterstained with haematoxylin. (C) Let-7a levels are decreased in colon adenocarcinomas in comparison to normal tissue. Sections were subjected to in situ hybridization for let-7a and counterstained with nuclear fast red. Bar, 50 μm.

Supplementary Table 1 (Related to Experimental Procedures): List of primers used.

	Cloning to pBK-EF1
Myc-Lin28A AgeI For	TGCAGACCGGTGAGCAGAAACTCATAAGCGAAGAGGACCTGGGCTCCGTGT CCAACCAG
Myc-Lin28A HindIII Rev	GACATGAAGCTTTCAATTCTGTGCCTCCGGGAG
Myc-Lin28B AgeI For	TGCAGACCGGTGAGCAGAAACTCATAAGCGAAGAGGACCTGGCCGAAGGCG GGGCTAG
Myc-Lin28B XbaI Rev	TACGATTCTAGATTATGTCTTTTTCCTTTTTTGAACTGAAGGC
Myc-Lin28BΔNLS#1 Rev	AGTCTATCTAGATTAGCTTTGCTCTTCTGGTGC
	Site-directed mutagenesis Lin28B NoLS
Lin28B Mut1 For	GACACTACAGGGAAGAGGACCAGGGGGAGATAG
Lin28B Mut1 Rev	CTATCTCCCCCTGGTCCTCTTCCCTGTAGTGTC
Lin28B Mut2 For	GGAAGTGAAAGAGGACCCGGAGGGAAGACACTA
Lin28B Mut2 Rev	TAGTGTCTTCCCTCCGGGTCCTCTTTCACTTCC
	Cloning to pFlag-CMV2
Lin28A XbaI For	TATCGATCTAGAGGCTCCGTGTCCAACCAGCAG
Lin28A BamHI Rev	TATCGAGGATCCTTAATTCTGTGCCTCCGGGAGCAGGG
Lin28B XbaI For	TATCGATCTAGAGCCGAAGGCGGGGCTAGCAAAG
Lin28B BamHI Rev	CTCGCAGGATCCTTATGTCTTTTTCCTTTTTTGAACTG
Lin28BΔNLS#1 BamHI Rev	CGTCAGGGATCCTTAGCTTTGCTCTTCTGGTGC
	Cloning Primers for CT-GFP-Topo
Lin28A For	GCCGCCATGGGCTCCGTGTCCAACCAGC
Lin28A Rev	CATTCTGTGCCTCCGGGAGCAG
Lin28B For	GCCGCCATGGCCGAAGGCGGGGCTAGC
Lin28B Rev	CTGTCTTTTTCCTTTTTTGAACTG
Lin28BΔNLS#1 Rev	AAACTGAAGGCCCCTTTTTGC
NLS#1 For	GCCGCCATGAAAAAGGGGCCTTCAGTTCAAAAAAGGAAAAAGACAGA
NLS#1 Rev	TCTGTCTTTTTCCTTTTTTGAACTGAAGGCCCCTTTTTCATGGCGGCA
NLS#2 For	GCCGCCATGGGAAGAAGACCCAAAGGGAAGACACTACAGAAAAGAAAACCAAAGGA
NLS#2 Rev	CCTTTGGTTTTCTTTTCTGTAGTGTCTTCCCTTTGGGTCTTCTTCCCATGGCGGCA
	Cloning to Pet21a(+)
His-Lin28A- BamHI For	GGATCCcatcatcaccatcaccacGGCTCCGTGTCCAACCAGCAG
Lin28A-NotI Rev	GCGGCCGCTTACAGTTTGCGTACCAATAAG
His-Lin28B-BamHI For	GGATCCcatcatcaccatcaccacGCCGAAGGCGGGGCTAGCAAAG
Lin28B-NotI Rev	TTATGTCTTTTTCCTTTTTTGAAC
	q.RT-PCR Primers
hsa-pri-let-7g For	AGCGCTCCGTTTCCTTTT
hsa-pri-let-7g Rev	CCCCACTTGGCAGCTG
hsa-pri-let-7a-1 For	CCTGGATGTTCTCTTCACTG
hsa-pri-let-7a-1 For	GCCTGGATGCAGACTTTTCT
hsa-pri-mir-21 For	GCTTATCAGACTGATGTTGACTG
hsa-pri-mir-21 Rev	CAGCCCATCGACTGGTG
U6 For	CTCGCTTCGGCAGCACA
U6 Rev	AACGCTTCACGAATTTGCGT
Lin28A For	AAGCGCAGATCAAAAGGAGA
Lin28A Rev	CTGATGCTCTGGCAGAAGTG
Lin28B For	TGATAAACCGAGAGGGAAGC
Lin28B Rev	TGTGAATTCCACTGGTTCTCC
GAPDH For	ATGTTCGTCATGGGTGTGAA
GAPDH Rev	GGTGCTAAGCAGTTGGTGGT

Supplementary Table 2 (Related to Experimental Procedures): List of shRNA sequences used.

Control shRNA#1	ACCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTTGAATTC
Control shRNA#2	ACCGGGCCCGCAAGCTGACCCTGAAGTTCATTCAAGAGATGAACTTCAGGgTCAGCTTGCTTTTTGAATTC
Control shRNA#3	ACCGGGTCGGCTTACGGCGGTGATTTCTCGAGAAATCACCGCCGTAAGCCGACTTTTTGAATTC
Zcchc11 shRNA #1	ACCGGGTCAGTTACATTCAGCAGAAACTCGAGTTTCTGCTGAATGTAACTGACTTTTTTGAATTC
Zcchc11 shRNA#2	ACCGGCGTGATAGTGATCTGGATATTCTCGAGAATATCCAGATCACTATCACGTTTTTTGAATTC
Zcchc11 shRNA#3	ACCGGGCTTCTGACCTTAATGATGATCTCGAGATCATCATTAAGGTCAGAAGCTTTTTTGAATTC
Zcchc11 shRNA#4	ACCGGGCAACAGACATGTACAGATAACTCGAGTTATCTGTACATGTCTGTTGCTTTTTTGAATTC
Lin28A shRNA	ACCGGGAACCCTTCCATGTGCAGCTTTTCGAAGCTGCACATGGAAGGGTTCCTTTTTTGAATTC
Lin28B shRNA	CCGGGCCTTGAGTCAATACGGGTAACTCGAGTTACCCGTATTGACTCAAGGCTTTTTTG

Supplementary Experimental Procedures

Cell Culture

HEK293, Hela, H1299, Igrov1, HepG2, T47D, MDA-MB-231, CaCO2, and SK_Mel_28 cells were maintained in DMEM (Gibco, Invitrogen), supplemented with 10% FBS, Pen/Strep, L-Glutamine and Non-essential Amino Acids (Gibco, Invitrogen). K562 cell line was grown in IMDM (Gibco, Invitrogen) with the same supplements. MCF10A cells containing the ER-Src fusion protein were grown in DMEM/F12 medium supplemented with 5% donor HS, 20 ng/ml epidermal growth factor (EGF), 10 mg/ml insulin, 100 mg/ml hydrocortisone, 1 ng/ml cholera toxin, and 50 units/ml pen/strep, with the addition of puromycin(Iliopoulos et al., 2009). To induce transformation, the Src oncogene was activated by the addition of 1 mM tamoxifen (Sigma, St. Louis) to confluent cell cultures.

RNA extraction and qPCR

RNA was harvested from cells and from xenograft tumors on day 30 using Trizol (Invitrogen) per manufacturer's instructions. TaqMan miRNA assays (Applied Biosystems) were used to quantify mature miRNA expression as described previously (Hagan et al., 2009). Pri-miRNA levels were

analyzed by qPCR. First, gene specific cDNA was made with the reverse primer for each pri-miRNA, using SuperScriptIII cDNA Synthesis Kit (Invitrogen). cDNA was used for qPCR with iQ SYBRGreen Supermix (BioRad). U6 was used as a normalizer.

Immunoprecipitation and Western blotting

Whole cell lysates were prepared using lysis buffer (20 mM Tris/pH8.0, 137 mM NaCl, 1 mM EDTA, 1% Triton X100, 10% Glycerol, 1.5 mM $MgCl_2$, 1 mM DTT, with protease inhibitors (Roche)). Flag-immunoprecipitations were done using Flag-agarose beads (Sigma) for 90 min at 4°C. Beads were washed with Buffer containing 300mM KCl (BC300). Elutions were done with Flag peptide (Sigma). Anti-Flag-HRP Antibody (Sigma, A8592) was used at 1:1000 dilution in 5% milk for an hour. For myc-immunoprecipitation, myc-antibody (Covance, PRB-150C) was added to Protein G-agarose beads (Sigma). Myc-IP was performed overnight at 4°C. Anti-myc antibody was used at 1:1000 in 5% milk. Secondary anti-mouse IgG-HRP (Sigma, A9044) was used in 2.5% milk at 1:10000 dilution for an hour. For endogenous protein western blots, concentration of cell lysates was measured using Bradford reagent (Biorad). The following antibodies were used: Lin28A (Cell Signaling, 3978), Lin28B (Cell Signaling, 4196), Zcchc11 (Protein Tech Group, 18980-1-AP), DGCR8 (Protein Tech Group, 10996-1-AP) at 1:1000 and β-Tubulin (Abcam AB6046) at and 1:5000. Secondary anti-rabbit IgG-HRP (Sigma #A9169) secondary antibodies were used at 1:10,000 dilutions in 2.5% milk. Antibody against Zcchc11 (Imgenex, IMX-3587) was used at 1:1,000 and a donkey anti-goat IgG-HRP (Santa Cruz, sc-2033) secondary antibody was used at 1:5,000. Antibody for Fibrillarin (Abcam, ab18380) was used at 1:1,000 in 5% milk, and anti-mouse IgG-HRP (Sigma) was used at 1:10,000 in 2.5% milk.

Subcellular fractionation

Cellular fractionation was done with the NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce). Cells were grown on 3.5 mm dishes and harvested per manufacturer's instructions. Large-scale fractionation of Nucleoli was performed as as follows: Cells pellets were resuspended in an equal volume of buffer A [10mM Tris, (pH 8.0), 1.5mM $MgCl_2$, 10mM KCl, 0.5mM DTT, 0.2mM PMSF]. Cells

were incubated for 10 min on the rotator, pelleted and resuspended in 2 volumes of buffer A. Cells were manually homogenized with a pestle, spun down to pellet the nuclei. Nuclei were resuspended in buffer C [20mM Tris, (pH 7.9), 25% Glycerol, 0.42M NaCl, 1.5 mM $MgCl_2$, 0.2mM EDTA, 0.5mM DTT, 0.5mM PMSF] and homogenized with a pestle. Nuclei were incubated on the rotator for 30 min at 4°C, and spun down at 12,000 rpm for 30 min to pellet nucleoli. Nucleoli were resuspended in buffer E [50mM Tris, (pH 7.9), 25% Glycerol, 0.5mM EDTA, 5mM $MgCl_2$], homogenized with a pestle and spun down to separate the soluble and insoluble fractions. The nucleolar pellet fraction was resuspended in Lysis buffer (20 mM Tris (pH 8.0), 137 mM NaCl, 1 mM EDTA, 1% Triton X100, 10% Glycerol, 1.5 mM $MgCl_2$, 1 mM DTT, with protease inhibitors (Roche)].

Recombinant Lin28A and Lin28B protein purification and EMSA

Transformed BL21-CodonPlus® Competent bacteria (Stratagene) were grown to an OD600nm of 0.4-0.6. Expression was induced 100μM IPTG for 2-3 hours. Cell pellets were resuspended in cold lysis buffer [20mM imidazole pH 8.0 in PBS, 0.1% Phenylmethyl sulfonyl fluoride (PMSF)] and sonicated. Cleared lysates were incubated with Ni-NTA beads and after 90 minutes incubation at 4°C the beads were washed with 80 column volumes wash buffer [10mM Tris (pH 7.8), 50mM imidazole pH 8.0, 500mM NaCl, 0.1% PMSF). Bound His-tagged proteins were eluted from the column with 1 volume elution buffer [10mM Tris (pH 7.8), 500mM imidazole pH 8.0, 500mM NaCl, 0.1% fresh PMSF] and dialyzed overnight against BC100 [20 mM Tris-HCl (pH 7.8), 100 mM KCl, 0.2 mM EDTA, 10% glycerol]. EMSA with end-labeled synthetic pre-let-7 RNA was performed as described but without competitor yeast tRNA (Piskounova et al., 2008). Briefly, reactions were set up in binding buffer [50mM Tris, (pH7.5), 100mM NaCl, 10mM βMe, 20U RNaseIN (Promega)] with 0.5nM end-labeled pre-let-7g and incubated for 45 min at room temperature. Bound complexes were resolved on native 5% polyacrylamide gels and visualized by radiography. Band intensities of scanned gels were quantified using ImageJ software and used to calculate percentage of probe bound. Graph-Pad Prism was used to plot the data. For both recombinant Lin28A and Lin288B, the percent active protein was

determined using stoichiometric binding reactions as described in (Ryder et al., 2008) with 10nM pre-let-7g probe. Hills equation for specific binding with one site was, Y=Bmax*X^h/(Kd^h + X^h), was used to calculate Kd. Error is expressed as SEM, n=3. EMSA with pri-miRNA was performed using in vitro transcribed radiolabeled RNA in the same conditions and resolved by 3.5% PAGE.

RNA-immunoprecipitation (RIP)

Hela cells were transfected with Flag-constructs of Lin28A and Lin28B, or empty Flag-plasmid for 48 hours. Flag-immunoprecipitations were done using Flag-agarose beads (Sigma) for 90 min at 4C. Beads were washed with BC500. RNA was eluted from the beads with Trizol. RNA was extracted following manufacturer's protocol. Pri-microRNA levels were analyzed by q.RT-PCR. Error was shown as SEM, n=3.

Immunofluorescence

Cells were grown on coverslips for 24-48 hours, before fixing with 4% paraformaldehyde for 20 min at room temperature. Cells were then blocked and permeabilized with 5% serum with 0.2% Triton for 20 min at room temperature. Cells were incubated at 4°C overnight with primary antibodies at 1:400 dilutions. The next day, cells were washed with PBS and incubated with the secondary antibodies at 1:400 dilution for 1 hour (Invitrogen, Anti-mouse A21202, Anti-Rabbit A21207), in the dark at room temperature. The cover slips were mounted with Vectashield mounting solution with DAPI (VectorLabs). For GFP-fusions, the Topo CT-GFP cloning kit (Invitrogen) was used. Cells were transfected with GFP-fusion constructs, and after 48 hours fixed as described above. Cells were washed with PBS three times, the last wash contained 0.2% Triton. Coverslips were mounted as described above.

Phase-contrast Images

In order to study the morphological changes and phenotypic transformation of MCF10A ER-Src TAM-induced (36h) cells, phase-contrast pictures were taken in a microscope (10x objective). Furthermore,

phase-contrast pictures were taken MCF10A ER-Src cells induced by TAM for 36h and simultaneously treated with an siRNA against Lin28B (siLin28B) (100nM), a monoclonal antibody against IL6 (Ab-IL6) (2ug/ml) (Mab206, R&D Systems) or a siRNA against Zcchc11 (siZcchc11#1) (100nM). Cell morphology was assessed by phase contrast microscopy (10X objective), and percentage of transformed ER-Src cells was calculated by evaluation of cell morphology by Metamorph v5.0 software.

Colony Formation Assay

MCF10A ER-Src transformed cells, MDA-MB-231 cells and T47D cells were transfected with different siRNAs for 48h. The siRNAs used in this experiment were the following: i) siRNA negative control (siRNA NC) (100nM), (cat no. AM4611, Ambion Inc); ii) siRNA against Zcchc11 (siZcchc11#1) (100nM) (cat no. s23551, Ambion Inc); iii) siRNA against Zcchc11 (siZcchc11#2) (100nM) (cat no. s23553, Ambion Inc); iv) siRNA against Lin28B (siLin28B) (100nM) (cat no. s52477, Ambion Inc); v) siRNA against Lin28A (siLin28A) (100nM) (cat no. s36195, Ambion Inc). Then, triplicate samples of 10^5 cells from each cell line were mixed 4:1 (v/v) with 2.0% agarose in growth medium for a final concentration of 0.4% agarose. The cell mixture was plated on top of a solidified layer of 0.5% agarose in growth medium. Cells were fed every 6 to 7 days with growth medium containing 0.4% agarose. The number of colonies was counted after 20 days. The experiment was repeated thrice and the statistical significance was calculated using Student's t test.

Interleukin 6 ELISA Assay

The concentration of interleukin 6 released to the supernatant of MCF10A ER-Src TAM-treated (36h) cells treated together with 100nM siRNA NC, or siZcchc11#1 or siZcchc11#2 or siLin28B was measured via IL6 ELISA assays (cat no. D6050), according to manufacturer instructions (R&D Systems).

Mouse experiments

In this study, the following mouse experiments were performed:

a) $5x10^6$ MCF10A ER-Src TAM-treated (36h) cells were injected subcutaneously in the right flank of athymic nude mice (Charles River Laboratories). Tumor growth was monitored every five days and tumor volumes were calculated by the equation $V(mm^3)=axb^2/2$, where a is the largest diameter and b is the perpendicular diameter. When the tumors reached a size of ~$100mm^3$ (day 15) were randomly distributed in 4 groups (5 mice/group). The first group was used as control (non-treated), the second group was i.p treated with 5mg/kg siRNA negative control, the third group was i.p treated with 5mg/kg siZcchc11#1 and the fourth group was i.p. treated with 5mg/kg siLin28B. Tumor volumes were monitored for 45 days.

b) $2x10^6$ MDA-MB-231 cells were injected subcutaneously in the right flank of athymic nude mice (Charles River Laboratories). Tumor growth was monitored every five days. When the tumors reached a size of ~$100mm^3$ (day 15) were randomly distributed in 5 groups (5 mice/group). The first group was used as control (non-treated), the second group was i.p treated with 5mg/kg siRNA negative control, the third group was i.p treated with 5mg/kg siZcchc11#1 and the fourth group was i.p. treated with 5mg/kg siLin28B and the fifth group was i.p. treated with 5mg/kg let-7a microRNA. Tumor volumes were monitored for 45 days.

c) $2x10^6$ T47D cells were injected subcutaneously in the right flank of athymic nude mice (Charles River Laboratories). Tumor growth was monitored every five days. When the tumors reached a size of ~$100mm^3$ (day 15) were randomly distributed in 5 groups (5 mice/group). The first group was used as control (non-treated), the second group was i.p treated with 5mg/kg siRNA negative control, the third group was i.p treated with 5mg/kg siZcchc11#1 and the fourth group was i.p. treated with 5mg/kg siLin28A and the fifth group was i.p. treated with 5mg/kg let-7a microRNA. Tumor volumes were monitored for 45 days.

d) 10^6 HepG2 cells were injected subcutaneously in the right flank of athymic nude mice (Charles River Laboratories). Tumor growth was monitored every five days. When the tumors reached a size of ~$200mm^3$ (day 15) were randomly distributed in 4 groups (5 mice/group). The first group was used as control (non-treated), the second group was i.p treated with 5mg/kg siRNA negative

control, the third group was i.p treated with 5mg/kg siZcchc11#1 and the fourth group was i.p. treated with 5mg/kg siLin28B. Tumor volumes were monitored for 45 days.

e) $5x10^6$ H1299 cells were injected subcutaneously in the right flank of athymic nude mice (Charles River Laboratories). Tumor growth was monitored every five days. When the tumors reached a size of ~200mm^3 (day 15) were randomly distributed in 4 groups (5 mice/group). The first group was used as control (non-treated), the second group was i.p treated with 5mg/kg siRNA negative control, the third group was i.p treated with 5mg/kg siZcchc11#1 and the fourth group was i.p. treated with 5mg/kg siLin28B. Tumor volumes were monitored for 45 days.

f) $2x10^6$ Igrov1 cells were injected subcutaneously in the right flank of athymic nude mice (Charles River Laboratories). Tumor growth was monitored every five days. When the tumors reached a size of ~180mm^3 (day 15) were randomly distributed in 4 groups (5 mice/group). The first group was used as control (non-treated), the second group was i.p treated with 5mg/kg siRNA negative control, the third group was i.p treated with 5mg/kg siZcchc11#1 and the fourth group was i.p. treated with 5mg/kg siLin28A. Tumor volumes were monitored for 45 days.

g) $5x10^6$ SK_MEL_28 melanoma cells were injected subcutaneously in the right flank of athymic nude mice (Charles River Laboratories). Tumor growth was monitored every five days. When the tumors reached a size of ~200mm^3 (day 15) were randomly distributed in 4 groups (5 mice/group). The first group was used as control (non-treated), the second group was i.p treated with 5mg/kg siRNA negative control, the third group was i.p treated with 5mg/kg siZcchc11#1 and the fourth group was i.p. treated with 5mg/kg siLin28B. Tumor volumes were monitored for 45 days.

h) $5x10^6$ CaCO2 colon cancer cells were injected subcutaneously in the right flank of athymic nude mice (Charles River Laboratories). Tumor growth was monitored every five days. When the tumors reached a size of ~180mm^3 (day 15) were randomly distributed in 4 groups (5 mice/group). The first group was used as control (non-treated), the second group was i.p treated with 5mg/kg siRNA negative control, the third group was i.p treated with 5mg/kg siZcchc11#1 and the fourth group was i.p. treated with 5mg/kg siLin28B. Tumor volumes were monitored for 45 days.

For all the experiments described above, we used In Vivo Ready siRNAs from Ambion Inc which are high-quality siRNAs that are purified especially for introduction into animals. Each siRNA strand is individually purified by HPLC, desalted, and annealed with its complementary strand. In Vivo Ready siRNAs are then further purified using a process that removes excess salt via a semi-permeable membrane. The result is a highly pure siRNA with minimal salt content, suitable for in vivo applications. These siRNAs are then filtered through a 0.2-μm pre-sterilized filter and tested for the presence of endotoxin. Next, we mixed the siRNAs with Invivofectamine 2.0 liposomes (Ambion Inc) and injected them in mice in a volume of 100ul using a 20G needle. The experiments described above were performed in accordance with Dana-Farber Institutional Animal Care and Use Committee procedures and guidelines.

Real-time RT-PCR Analysis for Human Cancer Tissues

Real-time RT-PCR was performed to determine the expression levels of let-7a in human colon and breast normal tissues and carcinomas. RNA was isolated, using Trizol (15596-026, Invitrogen). Reverse Transcription was carried out using the Universal cDNA synthesis kit (203300). Real-time PCR was carried out in triplicate using the SYBR Green master mix (203450) and primer for let-7a (204775, Exiqon) in a CFX384 Real Time PCR detection system (Bio-Rad). Let-7a expression levels were normalized to the levels of U6 snRNA (203907, Exiqon). Furthermore, RNAs were purchased from Origene from the following cancer tissues (8 renal cell carcinomas, 8 hepatocellular carcinomas, 8 squamous cell lung carcinomas, 8 ovarial adenocarcinomas, 8 prostate adenocarcinomas, 8 papillary thyroid carcinomas) and were used to test Lin28A and Lin28B mRNA expression levels.

Real time RT-PCR was employed to determine the expression levels of Lin28A and Lin28B. Reverse Transcription was carried out using the Retroscript Kit (AM1710, Applied Biosystems). Real time PCR for was carried out using IQ SYBR Green supermix (170-8882, Bio-Rad). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control.

NF-kB/p65 ActivELISA Assay

The NF-kB/p65 ActivELISA Kit measured nuclear p65 levels in nuclear protein lysates derived from 12 breast cancer tissues purchased from AMS Biotechnology Inc and Biochain Inc. The anti-p65 antibody coated plate captures free p65 and the amount of bound p65 is detected by adding a second anti-p65 antibody followed by alkaline phosphatase (AKP) -conjugated secondary antibody using colorimetric detection in an ELISA plate reader at absorbance 405nm. Each sample was loaded on triplicate and data are presented as mean ± SD.

In situ microRNA hybridization

Double-DIG labeled Mircury LNA Detection probe for the detection of hsa-let-7 (1800-15, Exiqon) by *in situ* hybridization, was used as previously described (Iliopoulos et al., 2009) with modifications. Sections of colon adenocarcinomas and adjacent uninvolved tissues were deparaffinized with xylene (3x5 min), followed by treatment with serial dilutions of ethanol (3x100%, 2x96% and 3x70%) and by two changes of DEPC-PBS. Tissues were then digested with proteinase K (15 μg/ml) for 20 min at 37^{o}C, rinsed with 3xDEPC-PBS. Sections were dehydrated with 2x70%, 2x96% and 2x100% ethanol, air-dried and hybridized for 1 hour with the hsa-let-7 probe (40 nM) or the double-DIG labeled U6 Control Probe (99002-15) diluted in microRNA ISH buffer (90000, Exiqon), at 54^{o}C. Following hybridization, sections were rinsed twice with 5XSSC, 2x1XSSC and 3x0.2XSSC, 5 min each, at 54^{o}C, and PBS. The slides were incubated with blocking solution (11585762001, Roche) for 15 min and then with anti-DIG antibody (1:800) in 2% sheep serum (013-000-121, Jackson Immunoresearch) blocking solution for 1 hour, at RT. Following three washes with PBS-T (PBS, 0.1% Tween-20), slides were incubated with the AP substrate buffer (NBT-BCIP tablet [11697471001, Roche] in 10 ml 0.2 mM Levamisole [31742, Fluka]) for 2 hours at 30^{o}C in the dark. The reaction was stopped with 2 washes of AP stop solution (50 mM Tris-HCl, 150 mM NaCl, 10 mM KCl) and 2 washes with water. Tissues were counter stained with Nuclear Fast Red for 1 min and rinsed with water. At the end, sections were dehydrated with 2x70%, 2x96% and 2x100% ethanol and mounted with coverslips in Eukitt mounting medium (361894G, VWR). Images were captured with a Nikon 80i Upright Microscope equipped with

a Nikon Digital Sight DS-Fi1 color camera, using the NIS-Elements image acquisition software. All images were captured and processed using identical settings.

Immunohistochemistry

Sections of the colon adenocarcinomas and adjacent uninvolved tissues were deparaffinised with xylene (3x5 min) followed by treatment with serial dilutions of ethanol (100%, 100%, 95% and 95%, 10 min each) and by two changes of ddH_2O. Antigen unmasking was achieved by boiling the slides (95-99^oC) for 10 min, in 10 mM sodium citrate, pH 6.0. Sections were then rinsed three times with ddH_2O, immersed in 3% H_2O_2 for 10 minutes, washed twice with ddH_2O and once with TBS-T (TBS, 0.1% Tween-20) and blocked for 1 hour with blocking solution (5% normal goat serum [5425, Cell Signaling Technology] in TBS-T). Lin28A (3978, Cell Signaling Technology) and Lin28B (LS-B3423, LSBio) antibodies were diluted 1:50 in signal stain antibody diluent (8112, Cell Signaling Technology) and 1:100 in blocking solution respectively, and incubated with the sections overnight at 4^oC. Following incubation with the antibodies, sections were washed three times, 5 min each, with TBS-T and incubated for 1 hour at room temperature with anti-rabbit biotin antibody (LS-D1, LSBio) diluted in blocking solution (1:300). Sections were washed three times, 5 min each, with TBS-T, incubated with the Vectastain ABC-AP reagent (AK-5000) for 30 min, washed and stained with the Vector Red Alkaline Phosphatase Substrate Kit (SK-5100), and with the hematoxylin QS counterstain (H-3404, Vector Laboratories). Finally tissues were dehydrated and mounted in Eukitt medium.