SUPPLEMENTARY INFORMATION

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Supplementary Figure 1 Retinoic acid-induced neuronal differentiation of P19 cells. (a) Western blot analysis of protein extracts derived from different stages of RAinduced P19 neuronal differentiation (b) Northern blot analysis of selected microRNA on total RNA from different stages of RA-induced P19 neuronal differentiation.

pri-miR-9-2 and miR-9 in d0 and d4 of P19 cell differentiation



pri-miR-10a and miR-10a in d0 and d4 of P19 cell differentiation



pri-miR-16-1 and miR-16 in d0 and d4 of P19 cell differentiation



Supplementary Figure 2 RNAseq and small RNAseq data from d0 and d4 of retinoic acidinduced neuronal differentiation of P19 cells. Red bars represent normalized reads from undifferentiated P19 cells, whereas yellow bars represent normalized reads from differentiating d4 P19 cells. RNAseq and small RNAseq data shows primary and mature microRNA expression, respectively.



Supplementary Figure 3 *In vitro* pri-miR processing in Hela cell extracts depleted of DGCR8 or Drosha. Internally radiolabeled primary transcripts $(50 \times 10^3 \text{ c.p.m.})$ (counts per minute), ~20 pmol) were incubated in the presence of wild type, DGCR8 or Drosha-depleted cell extracts. The products were analyzed on an 8% denaturing polycrylamide gel. [M] - RNA size marker.



pri-miRNAs Over Expression (OE) in d0 P19

Supplementary Figure 4 Real-time qRT–PCR on miR-9 and let-7a on P19 cells transfected with pri-miR-9-1 (white bar), pri-miR-9-1/miR-16 TL (black bar), pri-let-7a-1 (light grey bar) and pri-let-7a-1/miR-16 TL (dark grey bar). The fold changes in the corresponding microRNA abundance were plotted relative to values derived from mock transfected cells, which were set to 1. The values were normalized to miR-16 levels. Mean values and standard deviations (SD) of three independent experiments are shown. Statistical significance was calculated using a *t*-test (*) -P \leq 0.05, (**) -P \leq 0.01, (***) -P \leq 0.001.



Supplementary Figure 5 Levels of Lin28a-GFP and Lin28a in the P19 FRT/Lin28a cell lines are similar. Western blot analysis of protein extracts derived from d9 of RA-induced P19 neuronal differentiation. Lanes 1, 4 and 7 represent results from P19 Lin28a/GFP cell line, lanes 2, 5 and 8 represent results from P19 FRT/Lin28a 3# and lanes 3, 6 and 9 represent results from P19 FRT/Lin28a 4# cell line. Different concentrations of extracts were loaded on the SDS gel, as indicated.



Supplementary Figure 6 Real-time qRT–PCR on miR-9 primary transcripts at d9 of RA-induced neuronal differentiation of P19 FRT (black bars), P19 FRT/Lin28 3# (grey bars) or P19 FRT/Lin28 4# (white bars) cells. The fold changes in the corresponding pri-miRNA abundance were plotted relative to values derived from d0, which were set to 1. The values were normalized to Cyclophilin A mRNA levels. Mean values and standard deviations (SD) of three independent experiments are shown.



Supplementary Figure 7 *In vitro* processing assays were performed with internally labeled pre-miR transcripts (50×10^3 c.p.m. (counts per minute), ~20 pmol). Pre-microRNAs were incubated in d0 P19 (Lane 2) or d9 (Lane 3). The controls (-; lane 1) were incubated without extract. [M] - RNA size marker. Pre-miR-9-1/miR-16 TL is a substrate, in which wild type terminal loop has been substituted with the terminal loop from miR-16.



Supplementary Figure 8 (a) *In vitro* processing assays were performed with 5'-end labeled pre-miR transcripts (50×10^3 c.p.m. (counts per minute), ~20 pmol). Pre-microRNAs were incubated in d0 P19 RNAi control or d0 Lin28a RNAi cell extracts for 1min (1'), 5min (5'), 10min (10') or 15min (15'). The controls (-; Lane 1) was incubated without extract for 15'. [M] - RNA size marker. (**b**) The percentage of pre-microRNA substrate intensity remaining after corresponding *in vitro* processing reactions was plotted, relative to the control reactions set to 100.



Supplementary Figure 9 Pre-miR-9 is degraded in extracts derived from differentiated cells constitutively expressing untagged but not GFP-tagged Lin28a. (a) *In vitro* processing assays were performed with internally labeled pre-miR transcripts $(50 \times 10^3 \text{ c.p.m.})$ (counts per minute), ~20 pmol). Pre-microRNAs were incubated in d0 P19 (Lanes 2, 4, 6 and 8) or d9 (Lanes 3, 5, 7 and 9) cell extracts derived from P19 FRT, P19 Lin28a/GFP, P19 FRT/Lin28a 3# and P19 FRT/Lin28a 4# cells. The controls (-; Lane 1) was incubated without extract. [M] - RNA size marker. (b) The percentage of pre-microRNA substrate intensity remaining after corresponding *in vitro* processing reactions was plotted, relative to the control reactions set to 100. Mean values and standard deviations (SD) of three independent experiments are shown. Statistical significance was calculated using a *t*-test (*) -P ≤ 0.05



Supplementary Figure 10 Full scans of western blots presented in the manuscript. Boxed regions correspond to cropped fragments used in the figures.

Supplementary Table 1 Specific and common proteins bind to pre-miR-9-1 and prelet-7a-1 in P19 d0 cell extract. Shades of yellow, orange and red represent increasing fold enrichment of proteins binding to the RNA, whereas blue represent proteins non annotated (NaN) in the MS analysis.

	Fold Enrichment	
Protein	(pre-miR/be	ads p19 d0)
	pre-let-7a-1	pre-miR-9-1
Hnrnpa0	67.4	13.4
Hnrnpab	58.8	28.1
Hnrnpa1	57.1	65.3
Hnrnpd	52.7	17.4
Srsf3	50	30.3
Hnrnpa2b1	45.4	33.6
Hnrpdl	42.1	33.5
Rbmxl1	28.9	10
Ncl	26.9	13.3
Hnrnpa3	25.4	23.3
Hnrnpf	25.1	6.3
Hnrnph1	23.2	12.3
Srsf5	20.5	13.8
Rps20	18.1	NaN
Dhx9	15.5	9
Ssb	15	21.4
Dazap1	14.8	6.3
Elavl1	14.6	9.4
Syncrip	14.2	10.2
Fus	14.2	11.3
Lin28a	12.7	9.5
Dhx36	12.1	6.7
UPF0568	10.7	4
Srsf1	9.8	33
lgf2bp1	9.5	7.8
Ubap2l	8.7	4.3
Khsrp	8.4	21.4

Protein (pre-miR-9-1) pre-let-7a-1 Hnrnpa1 65.3 57.1 Cnbp 34.9 NaN Hnrnpa2b1 33.6 45.4 Hnrpdl 33.5 42.1 Srsf1 33 9.8 Srsf7 30.5 NaN Srsf3 30.3 50 Hnrnpab 28.1 58.8 MAST1 25.4 NaN Srsf2 25.4 3 Hnrnpab 28.1 8.8 MAST1 25.4 NaN Srsf2 25.4 3 Hnrnpa3 23.3 25.4 Khsrp 21.4 8.4 Ssb 21.4 15 Rpl22 17.9 NaN Hnrnpd 17.4 6.2 Srsf5 13.8 20.5 Hnrmp1 12.3 23.2 Hnrmp1 12.3 23.2 Hnrmp1 12.3 3.6 Fusp9 10.4	Drotoin	Fold Enrichment	
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Csda 8.7 6.2 Cpsf6 8.6 NaN Tial1 8.6 NaN	Rpl26-ps4	8.8	NaN
Cpsf6 8.6 NaN	Csda	8.7	6.2
Tial1 8.6 NaN	Cpsf6	8.6	NaN
	Tial1	8.6	NaN

Primer name	Sequence
Pri-let-7a-1_F_qRT-PCR	CAGGAAATGAAACCACAGCA
Pri-let-7a-1_R_qRT-PCR	CCTCCTCGGTAATCCTGGTT
Pri-miR-16_F_qRT-PCR	TGGGGTTCGATCTTAACAGG
Pri-miR-16_R_qRT-PCR	TGTCACGATGGTAGGCAAAA
Pri-miR-9-1_F_qRT-PCR	GGAGCCTTTCCACTAGCA
Pri-miR-9-1_R_qRT-PCR	AGGTCGGGAATCTAGGCTGAAACCAAGC
Pri-miR-9-2_F_qRT-PCR	AGCTTGCTGCACCTTAGTCT
Pri-miR-9-2_R_qRT-PCR	TGTGTGCGGCTAGAACATCC
Pri-miR-9-3_F_qRT-PCR	CCATTGTAAGGCTGGGTGGT
Pri-miR-9-3_R_qRT-PCR	CTCCTCTCGCAGGCTAATCG
Pri-miR-302a_F_qRT-PCR	TTCTGGAGGAGAACACGAATC
Pri-miR-302a_R_qRT-PCR	TGAGGAGAAAGAAAACAAAATGG
CypA_F_qRT-PCR	TCGAGCTCTGAGCACTGGAG
CypA_R_qRT-PCR	CATTATGGCGTGTAAAGTCACCA
Pri-miR-9-1_T7_F	<i>TAATACGACTCACTATAGG</i> CCAGGAGGCGGGGTTGGTT
Pri-miR-9-1_T7_R	CCCTCTGCGCAGTGGTATGGGG
Pri-miR-124-1_T7_F	<i>TAATACGACTCACTATAGG</i> CCTCAGGAGAAAGGCCTCTC
Pri-miR-124-1_T7_R	GGCCCTCGCCGACCCACGGT
Pri-let-7a-1_T7_F	<i>TAATACGACTCACTATAGG</i> ATGTTCTCTTCACTGTGGG
Pri-let-7a-1_T7_R	TGCAGACTTTTCTATCACGTTAGG
Pri-miR-101_T7_F	<i>TAATACGACTCACTATA</i> GGGAGCCTTCAGAGAGAGT
Pri-miR-101_T7_R	AGCCACCTGTTTCACATTCC
Pri-miR-302a_T7_F	<i>TAATACGACTCACTATAGG</i> ACTTAAACGTGGATGTACT
Pri-miR-302a_T7_R	TCACCAAAACATGGAAGCA
Pre-miR-9-1_T7_F	<i>TAATACGACTCACTATAGG</i> TCTTTGGTTATCTAGCTGTA
Pre-miR-9-1_T7_R	ACTTTCGGTTATCTAGCTTTA
Pre-let-7a-1_T7_F	<i>TAATACGACTCACTATAGG</i> TGAGGTAGTAGGTTGTATAG
Pre-let-7a-1_T7_R	GAAAGACAGTAGATTGTATA
Pre-miR-101_T7_F	<i>TAATACGACTCACTATAGG</i> CAGTTATCACAGTGCTGATG

Supplementary Table 2 List of custom made primers used in the study.

Pre-miR-101_T7_R	TTCAGTTATCACAGTACTG
miR-9-1/miR-16 TL_F	GAATCTTACTCATACAGCTAGATAACCA
miR-9-1/miR-16 TL_R	TAAAATTATTCATAAAGCTAGATAACCG

Supplementary Table 3 List of custom made RNAs used in the study.

RNA name	Sequence
miR-9-1_CTL	UAUGAGUGGUGUGGAGUCUUCAU
Pre-miR-9-1	UCUUUGGUUAUCUAGCUGUAUGAGUG
	GUGUGGAGUCUUCAUAAAGCUAGAU
Pre-miR-9-2	UCUUUGGUUAUCUAGCUGUAUGAGUG
	UAUUGGUCUUCAUAAAGCUAGAU
Pre-let-7a-1	UGAGGUAGUAGGUUGUAUAGUUUUAGGGUCACA
	CCCACCACUGGGAGAUAACUAUACAAUCUACUGUCUUUC