

Supplemental Table 1. MicroRNAs regulated by ZEB1.

<i>down-regulated</i>			
miRNAs	function[†]	cancer	roles [representative target]
miR-34a	TS	prostate cancer (1)	inhibits clonogenic expansion, tumor regeneration, and metastasis [CD44]
miR-210	TS	head & neck and pancreatic cancer (2)	regulates the hypoxic response of tumor cells and tumor growth
miR-326	TS	medulloblastoma (3)	induces differentiation, cell maturation and growth inhibition
miR-224	TS	ovarian cancer (4)	inhibits cell proliferation
miR-193a	TS	acute myeloid leukemia (5)	induces growth inhibition, apoptosis and differentiation [c-KIT]
miR-370	TS	cholangiocarcinoma (6)	down-regulated by IL-6 [MAP3K8]
miR-331	-		
miR-183	TS OG	pancreatic and colorectal cancer (7) rhabdomyosarcoma, and colon cancer (8)	suppresses expression of stem cell factors in cancer cells promotes tumor cell migration [EGR1]
miR-96	TS OG	pancreatic cancer (9) breast cancer (10)	inhibits Akt signaling and induces apoptosis [KRAS] [FOXO1]
miR-182	OG	breast cancer (10)	[FOXO1]
miR-141	TS	breast cancer (11)	suppresses EMT [ZEB1]
miR-200b	TS	breast cancer (11)	suppresses EMT [ZEB1]
miR-200c	TS	breast cancer (11)	suppresses EMT [ZEB1]
miR-200a	TS	breast cancer (11)	suppresses EMT [ZEB1]
miR-206	TS	rhabdomyosarcoma (12)	inhibits cell proliferation and migration [c-MET]
miR-148a	TS	gastric cancer (13)	suppresses tumor cell invasion and metastasis [ROCK1]
miR-605	-		
miR-126	TS	lung cancer (14)	inhibits proliferation by inducing G1 cell cycle arrest
miR-658	-		
miR-92b	-		
miR-467a	-		
miR-297	-		
miR-466	-		
miR-203	TS	pancreatic and colorectal cancer (7)	suppresses expression of stem cell factors in cancer cells
miR-429	TS	breast cancer (11)	suppresses EMT [ZEB1]
miR-516-5p	-		
miR-555	-		
<i>up-regulated</i>			
miRNAs	function[†]	cancer	roles [representative target]
miR-470	-		
miR-581	-		
miR-542-3p	TS	colon cancer (15)	inhibits cell proliferation by inducing cell cycle arrest [BIRC5]
miR-351	-		
miR-503	TS	hepatocellular carcinoma (16)	inhibits proliferation, migration and invasion
miR-324-5p	TS	medulloblastoma (3)	induces differentiation, cell maturation and growth inhibition
miR-181a*	-		
miR-140*	-		
miR-142-5p	TS	lung cancer (17)	represses lung cancer cell growth
miR-181b	OG	hepatocellular carcinoma (18)	induced by TGFβ, enhances MMP2/9 activity and promoted growth, clonogenic survival, migration and invasion [TIMP3]
	TS	glioma (19)	induces growth inhibition and apoptosis, and inhibits invasion
miR-99a	TS	prostate cancer (20)	induces growth inhibition
miR-181a	TS	glioma (19)	induces growth inhibition and apoptosis, and inhibits invasion
miR-614	-		

miR-181d	OG	hepatocellular carcinoma (18)	induced by TGF β , enhances MMP2/9 activity and promoted growth, clonogenic survival, migration and invasion [TIMP3]
miR-298	-		
miR-10a	OG	pancreatic cancer (21)	promotes metastasis [HOXB1/B3]
miR-344	-		
miR-185	TS	ovarian cancer (22)	inhibits cell migration and tumor growth in vivo [SIX1]
miR-181c	-		

[†]TS: tumor suppressive, OG: oncogenic microRNAs

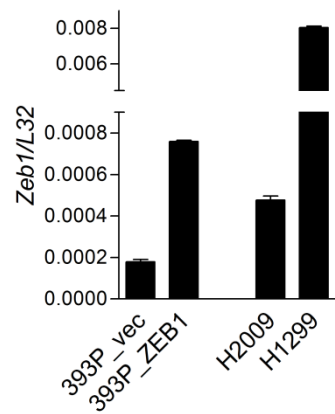
References

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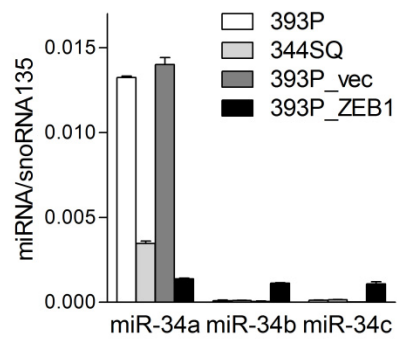
Supplemental table 2. Q-PCR primers

murine gene	forward (5'→3')	reverse (5'→3')
<i>Acbd3</i>	AAACGTCCCAGCGTTGCCCTG	ACAGACAGCAATGCGCCCTCC
<i>Acs14</i>	GTGAAGGCTCTGCTGGGAGGGA	GCGCCGCCAGACAGCATCAT
<i>Adam10</i>	GCGGTAAACCCGTGAGGAGGC	GTCGGCAACACCATCTTCCCCG
<i>Adam17</i>	AGAAGGTTTCCCAGAGAGGTGGTGG	GGAGACGCCGCCTCATGTTCC
<i>Bmp7</i>	CCGTAGTATCCGGTCCACGGGG	GGAGCGATTCTGGCTGCGCT
<i>Cdc42</i>	CGGAGAAGCTGAGGACAAGATCT	CACCATCACCAACAACAACACT
<i>Cdh1</i>	TACGGCGGTGGTGAGGACGA	GCCACACGGGGGAGACTTGC
<i>Chd2</i>	CCTCCAGAGTTTACTGCCATGAC	CCACCACTGATTCTGTATGCCG
<i>Cln3</i>	TTCAGCGGGTCAGGGCTGGT	CTGGCGAGGTGACAGCCGAG
<i>Crb3</i>	CGGACCCTTTCACAAATAGCA	CGTTGGACTCATCACCTGGG
<i>Ctnnb1</i>	GCCATCTTAAGCCCTCGCTCGG	CACAGGACACGAGCTGACGG
<i>DNp63</i>	TTGTACCTGGAAAACAATG	GCATCGTTTCACAACCTCG
<i>E2f5</i>	CGTTGCTGCAGGAGGGCGCA	GCAGCCGCTTTGAGATCCAGGAC
<i>E2f8</i>	GGGGGTCAAGATCCCAAAGCCCT	CTAAGCGCACGACCGGGCAT
<i>Galant7</i>	GGAGCATGGGACTGGAGTATGCTCT	CGAGAGGTCAGAGGCACCCGT
<i>Gmfb</i>	AAGGGGCCCAAGAGATCGCACG	TCCTTCCGGTCGTCAGCGGC
<i>Hif1a</i>	ACAAGCCGGGGGAGGACGAT	AGCACCTTCCACGTTGCTGACT
<i>Hspb1</i>	CACGCAGTCAGCGGAGATCACC	GGCGCGGGCCTCGAAAGTAA
<i>Itgav</i>	CACCTGGGGCATTAGCCGG	GATCACCCACACAGGCACAGGC
<i>Lepr</i>	TCCCAGCAGCTATGGTCTCCCTT	TGCTTTCAGGGTCTGGTGTGGTC
<i>Marcks</i>	GTGGCCTCGTCGCCTTCCAAA	TTACGTGGCCATTCTCCTGCCCA
<i>Plk4</i>	CCTCCTGTGGACCCAAGCTGC	TGCTTGCTGACGCCTGCTCT
<i>Pxn</i>	GGACGAGCTCATGGCGTCACT	TCCCCTGGGCCATGAACTTGAAA
<i>Rpl32 (L32)</i>	GGAGAAGGTTCAAGGGCCAG	TGCTCCATAACCGATGTTTG
<i>Scrib</i>	CCCAGGAAGGCCGTGGCAAG	GCCTCAGCCAGCCGTTCCAA
<i>Smad3</i>	AGAGGGAACCGGCACTGCTCA	GTGTGCCTCCAGAAGCCGCC
<i>Snai1</i>	CCCAAGCCGTAGAGCTGA	GCTTTTGCCACTGTCCTCATC
<i>Snai2</i>	ATCCTCACCTCGGGAGCATA	TGCCGACGATGTCCATACAG
<i>T</i>	ATCCACCCAGACTCGCCCAATT	CTCTCACGATGTGAATCCGAGG
<i>T2</i>	TCCACCTCCCTTGCTAACATGG	CTCTTGGTGGAACATCTGCCTG
<i>TAp63</i>	TGTATCCGCATGCAAGACTC	CCGGGTAATCTGTGTTGGAG
<i>Tgfb1</i>	GCTGCCCCCTGGCTCAGAGAC	AGGCTGGAGGTGGGGTCAGGA
<i>Tshz1</i>	AGGTCACCAATGGCTGCGGC	CCGGCGAGTGGTCCGTGATG
<i>Twist1</i>	TCGACTTCCCTGTACCAGGTCTT	CCATCTTGGAGTCCAGCTCG
<i>Vcl</i>	GCCTCAGATGCTGGTCGCTGG	CGGTTGGCCCGACGAGCAAT
<i>Vim</i>	GCGTGCGGCTGCTTCAAGAC	ATGGCGTCGGCCAGCGAGAA
<i>Wif1</i>	CGGAACCTGCCACGAACCCA	CGTGCCAGCCCTCTCGACAC
<i>Zeb1</i>	GCTCAGCCAGGAACCCGCAG	TGGGCACCCTCTGCCACACA
<i>Zeb2</i>	AGGCGCGAGAGAAAGGGCAC	CCCGTTTCATCAGCAGCTCGG
human gene	forward (5'→3')	reverse (5'→3')
<i>ARHGAP1</i>	CACCACCGAGGGCATCTTCCG	CCGGACCACTTGGGTGTTGGC
<i>CDH1</i>	GACACACCCCCTGTTGGTGT	CAGCCATCCTGTTTCTTTCAA
<i>CDH2</i>	ATCTCGGGTCAGCTGTCCG	GGCTATCTGCTCGCGATCC
<i>RPL32 (L32)</i>	CCTTGTGAAGCCCAAGATCG	TGCCGGATGAACTTCTTGGT
<i>SNAI2</i>	TGTGTGGACTACCGCTGCTC	GAGAGGCCATTGGGTAGCTG
<i>VIM</i>	GGAACAGCATGTCCAAATCGA	GCCGTGAGGTGAGGCTTG
<i>ZEB1</i>	CACTGGTGGTGGCCATTAC	TGCACCATGCCCTGAGG



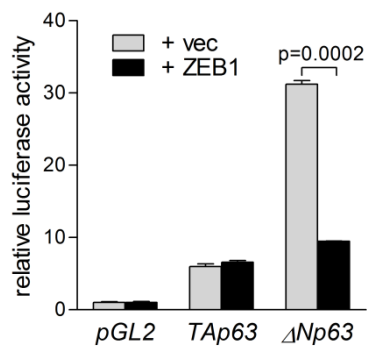
Supplemental Figure 1. Ectopic *Zeb1* levels in 393P_ZEB1 cells relative to that of endogenous *ZEB1* levels in human lung cancer cells.

Q-PCR analysis of *Zeb1* mRNA levels. Results normalized on the basis of snoRNA-135. Mean±SD, n=3.



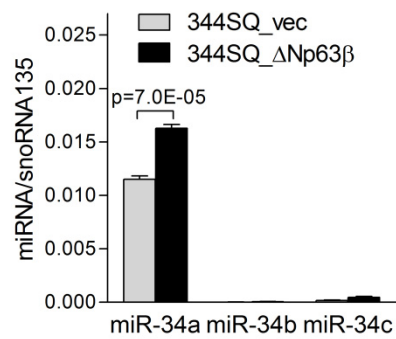
Supplemental Figure 2. ZEB1 down-regulates the expression of miR-34a but not miR-34b or miR-34c.

Q-PCR analysis of miR-34a, -34b, and -34c expression in KP cells with high ZEB1 (344SQ and 393P_ZEB1) or low ZEB1 (393P and 393P_vec). Mean \pm SD, n=3. P-value, two-tailed Student's t-test.



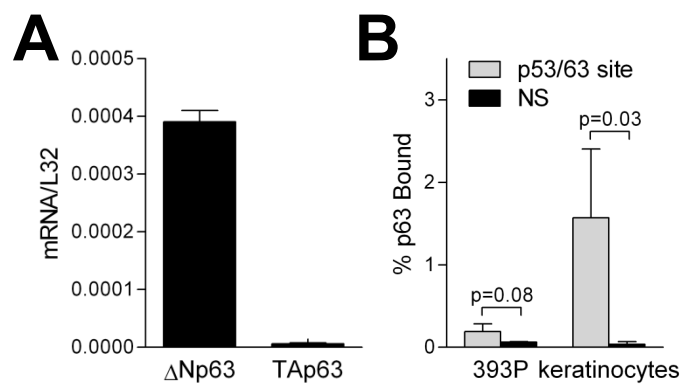
Supplemental Figure 3. ZEB1 down-regulates $\Delta Np63$ but not $TAp63$ promoter activity.

Luciferase activity in 393P cells co-transfected with ZEB1 (+ ZEB1) or empty (+ vec) expression vectors and pGL2 reporter plasmids subcloned with promoters from $TAp63$ or $\Delta Np63$ genes or nothing (pGL2). Results expressed relative to pGL2 transfectants, which was set at 1.0. Mean \pm SD, n=3. P-value, two-tailed Student's t-test.



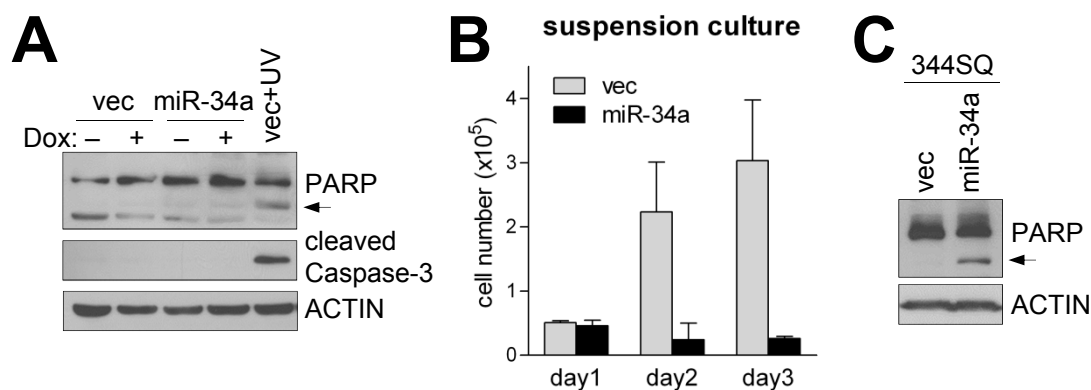
Supplemental Figure 4. Δ Np63 β up-regulates the expression of miR-34a but not miR-34b or miR-34c.

Q-PCR analysis of miR-34a, -34b, and -34c levels. Results normalized on the basis of snoRNA-135. Mean \pm SD, n=3. P-value, two-tailed Student's t-test.



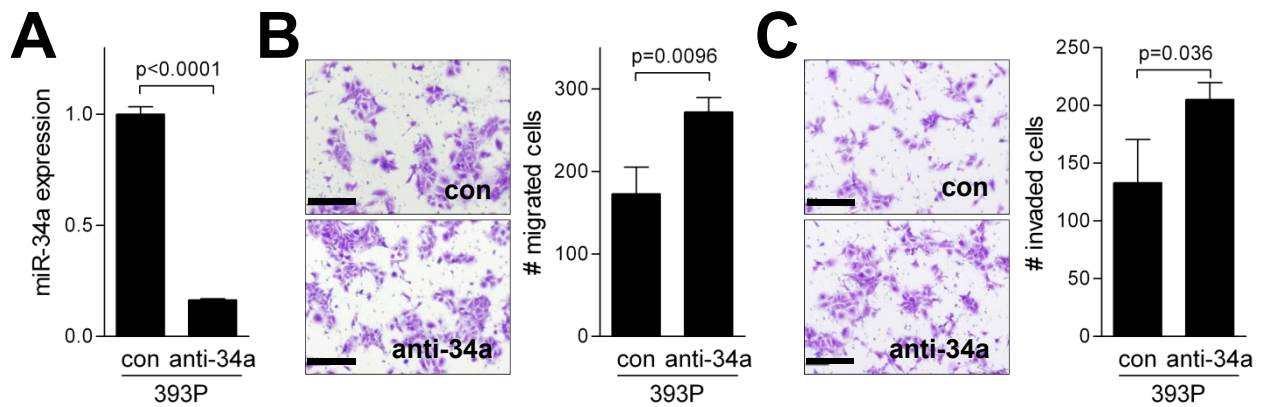
Supplemental Figure 5. 344SQ cells express primarily $\Delta Np63$ isoforms, which bind to the miR-34a promoter.

(A) Q-PCR analysis of $\Delta Np63$ and TAp63 isoforms in 344SQ cells using primers that recognize all alternatively spliced variants (α , β , and γ) of $\Delta Np63$ and TAp63 gene transcripts. Values were normalized on the basis of L32 levels. Mean \pm SD, n=3. (B) Binding of endogenous p63 to the miR-34a promoter in 393P cells (left bar graph) and mouse primary keratinocytes isolated from E18.5 day embryos (right bar graph). Graph denotes DNA amount at specific (p53/p63) and non-specific (NS) sites of the miR-34a promoter precipitated by anti-p63 antibody corrected for non-specific binding activity by IgG precipitation and expressed as % p63 bound. Mean \pm SD, n=3. P-values are indicated (two-tailed Student's t-test).



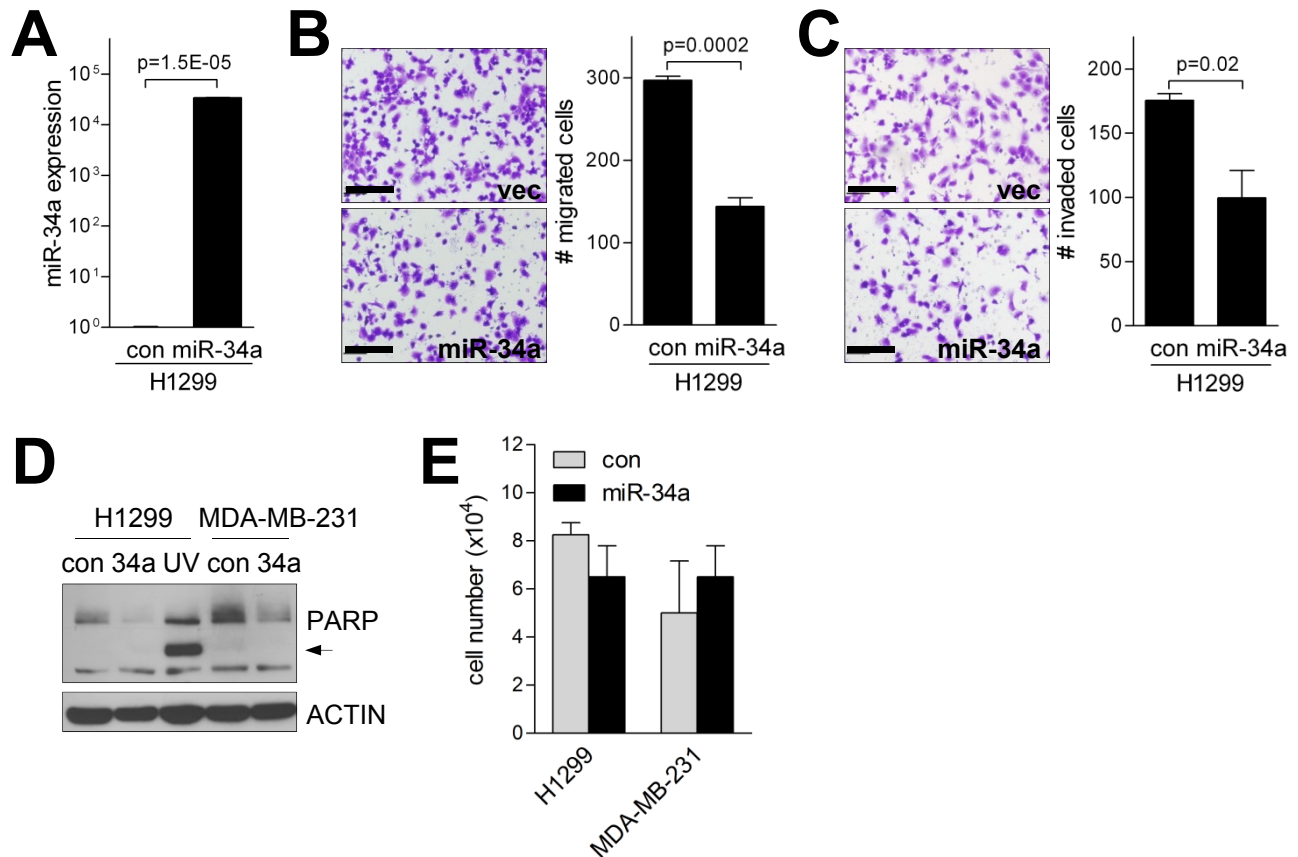
Supplemental Figure 6. Ectopic miR-34a expression induces apoptosis of 344SQ cells in anchorage-independent but not anchorage-dependent conditions.

(**A**) Western blot analysis of PARP and cleaved Caspase-3 in adherent 344SQ_vec and 344SQ_miR-34a cells cultured in the presence or absence of doxycycline (Dox). UV-irradiated (250 J/m², 24 h-incubation) 344SQ_vec cells were used as a positive control. Cleaved PARP band (arrow). ACTIN used as loading control. (**B** and **C**) 344SQ_vec and 344SQ_miR-34a cells were cultured in suspension on low-adhesion plates and counted (**B**) or subjected to western blot analysis of PARP (**C**) at day 2. Mean±SD, n=4. Cleaved PARP band (arrow). ACTIN used as loading control.



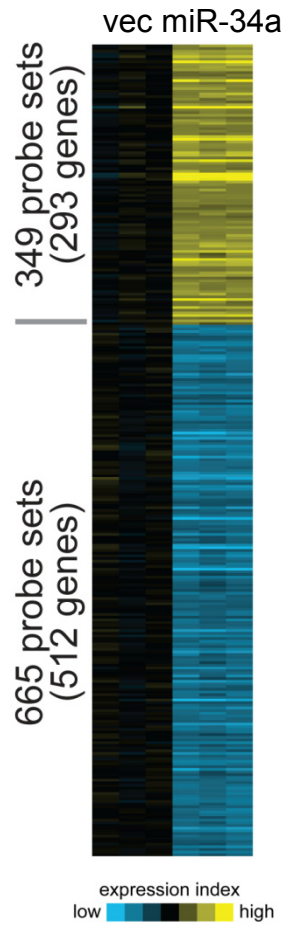
Supplemental Figure 7. miR-34a hairpin inhibitor enhances the migration and invasion of 393P cells.

(A) Q-PCR quantification of miR-34a in 393P cells transiently transfected with 100 nM of miRIDIAN[®] negative control (con) or miR-34a hairpin inhibitor (anti-34a). Migrating (B) and invading (C) cells in Boyden chambers were photographed (images) and counted (bar graphs). Scale bars=100 μ m. Mean \pm SD, n=3. P-values, two-tailed Student's t-test.



Supplemental Figure 8. miR-34a inhibits the migration and invasion of H1299 human lung cancer cells.

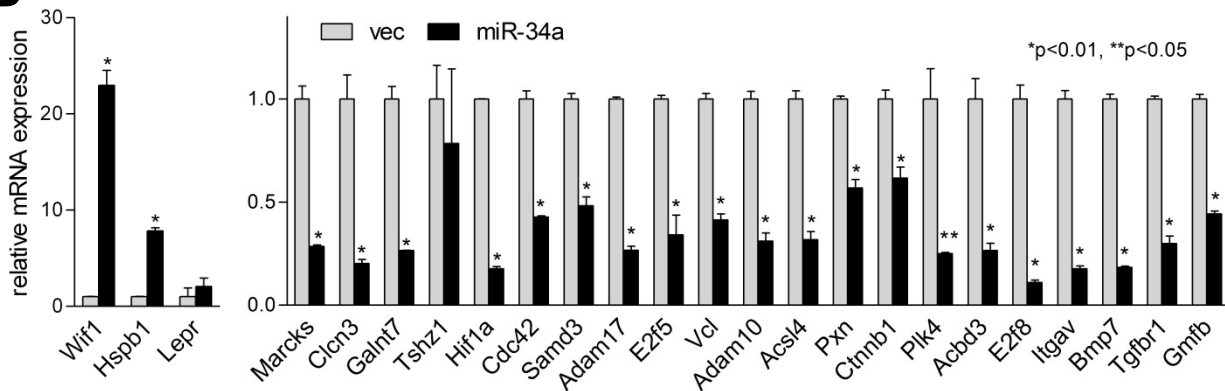
(A) Q-PCR quantification of miR-34a levels in H1299 cells transiently transfected with 40 nM of Pre-miR™ negative control (con) or Pre-miR-34a (miR-34a). Mean±SD, n=3. Migrating (B) and invading (C) cells in Boyden chambers were photographed (images) and counted (bar graphs) (Scale bars=100 μm. Mean±SD, n=3. P-values, two-tailed Student's t-test. (D and E) To exclude the possibility that the reduced migration and invasion was a consequence of apoptosis or reduced proliferation, H1299 cells and MDA-MB-231 cells were transiently transfected with 40 nM of Pre-miR™ negative control (con) or Pre-miR-34a (miR-34a), seeded in equal numbers onto 24-well plates, and subjected to western blot analysis of PARP (D) or counted (E) after 24 hrs. UV-irradiated (250 J/m², 24 h-incubation) H1299 cells were used as a positive control for PARP cleavage (arrow). ACTIN used as loading control. Mean±SD, n=4.



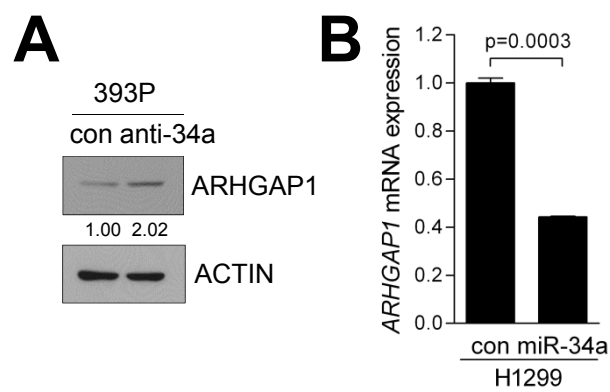
Supplemental Figure 9. Global mRNA profiling of tumors. Heat map depiction of 805 mRNAs up- or down-regulated in 344SQ_miR-34a (miR-34a) tumors, using 344SQ_vector tumors (vec) as the reference. Scale bar at bottom.

A

Symbol	Gene Title	fold [†]	P-value
<i>Wif1</i>	Wnt inhibitory factor 1	15.1446	0.00471
<i>Hspb1</i>	Heat shock protein 1	10.1599	0.03498
<i>Lepr</i>	Leptin receptor	7.2687	0.00152
<i>Marcks</i>	Myristoylated alanine rich protein kinase C substrate*	0.6462	0.00152
<i>Cln3</i>	Chloride channel 3*	0.6321	0.00123
<i>Galnt7</i>	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 7*	0.6273	0.00320
<i>Tshz1</i>	Teashirt zinc finger family member 1*	0.6257	0.00689
<i>Hif1a</i>	Hypoxia inducible factor 1, alpha subunit	0.6018	0.00080
<i>Cdc42</i>	Cell division cycle 42 homolog	0.5862	0.00056
<i>Smad3</i>	MAD homolog 3	0.5855	0.00171
<i>Adam17</i>	A disintegrin and metallopeptidase domain 17	0.5770	0.00131
<i>E2f5</i>	E2F transcription factor 5*	0.5487	0.00086
<i>Vcl</i>	Vinculin*	0.5350	0.00155
<i>Adam10</i>	A disintegrin and metallopeptidase domain 10	0.5348	0.00196
<i>Acs14</i>	Acyl-CoA synthetase long-chain family member 4*	0.5142	0.00097
<i>Pxn</i>	Paxillin	0.4999	0.00364
<i>Ctnnb1</i>	Catenin (cadherin associated protein), beta 1	0.4805	0.00163
<i>Plk4</i>	Polo-like kinase 4	0.4745	0.00063
<i>Acbd3</i>	Acyl-Coenzyme A binding domain containing 3*	0.4724	0.00081
<i>E2f8</i>	E2F transcription factor 8	0.4452	0.00185
<i>Itgav</i>	Integrin alpha V	0.4448	0.00306
<i>Bmp7</i>	Bone morphogenetic protein 7	0.4377	0.00173
<i>Tgfb1</i>	Transforming growth factor, beta receptor I	0.4322	0.00059
<i>Gmfb</i>	Glia maturation factor, beta*	0.3683	0.00288

B

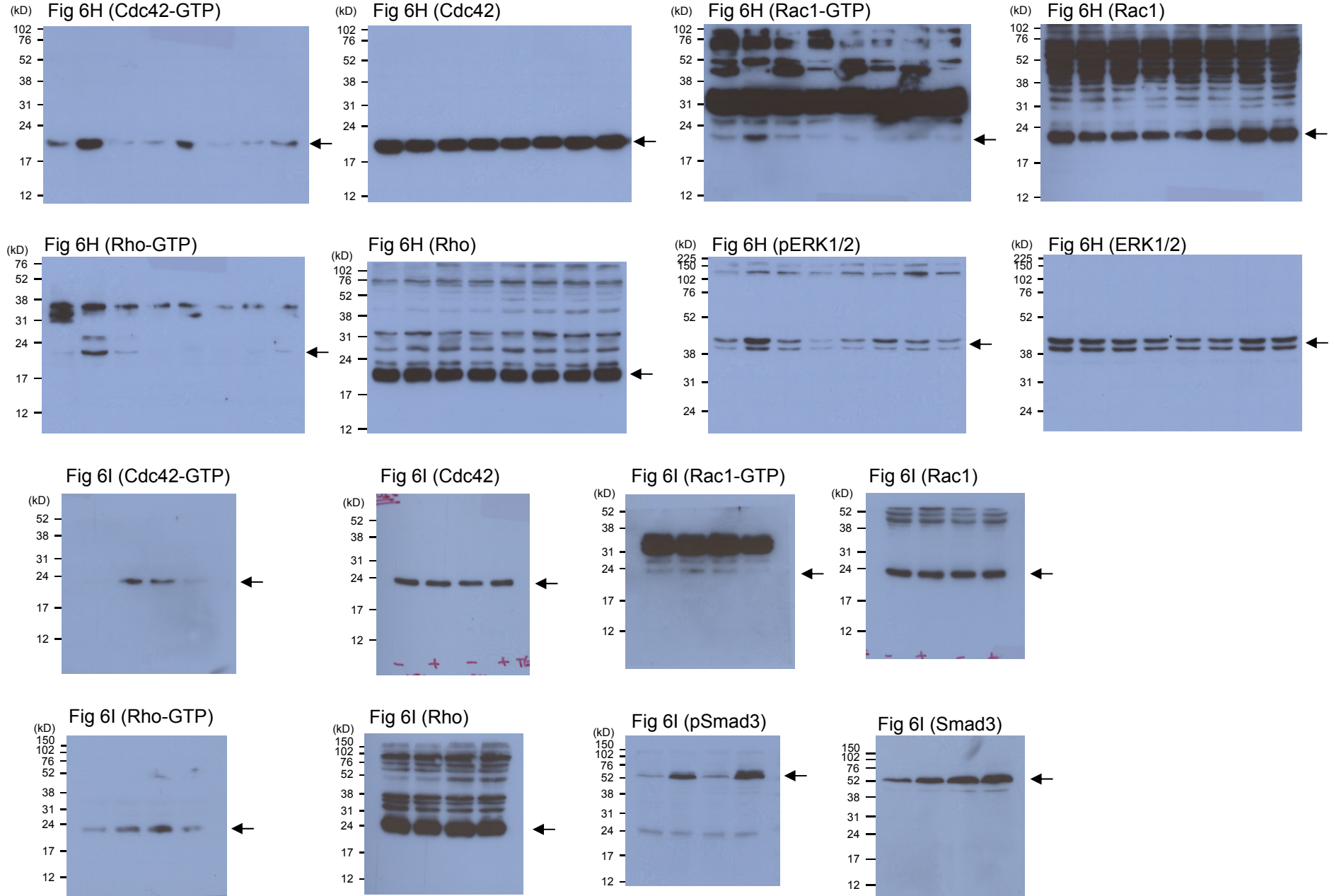
Supplemental Figure 10. Q-PCR confirmation of mRNAs up- or down-regulated in microarrays. (A) List of genes tested by Q-PCR analysis. [†]fold = miR-34a/vector; P-values, two-tailed Student's t-test; *putative miR-34a targets from TargetScan. (B) Q-PCR analysis of mRNAs in 344SQ_vector (vec) and 344SQ_miR-34a (miR-34a) tumor samples. Mean \pm SD, n=3 RNA samples. Results expressed relative to that of 344SQ_vector, which was set at 1.0. *p<0.01, **p<0.05 (two-tailed Student's t-test).



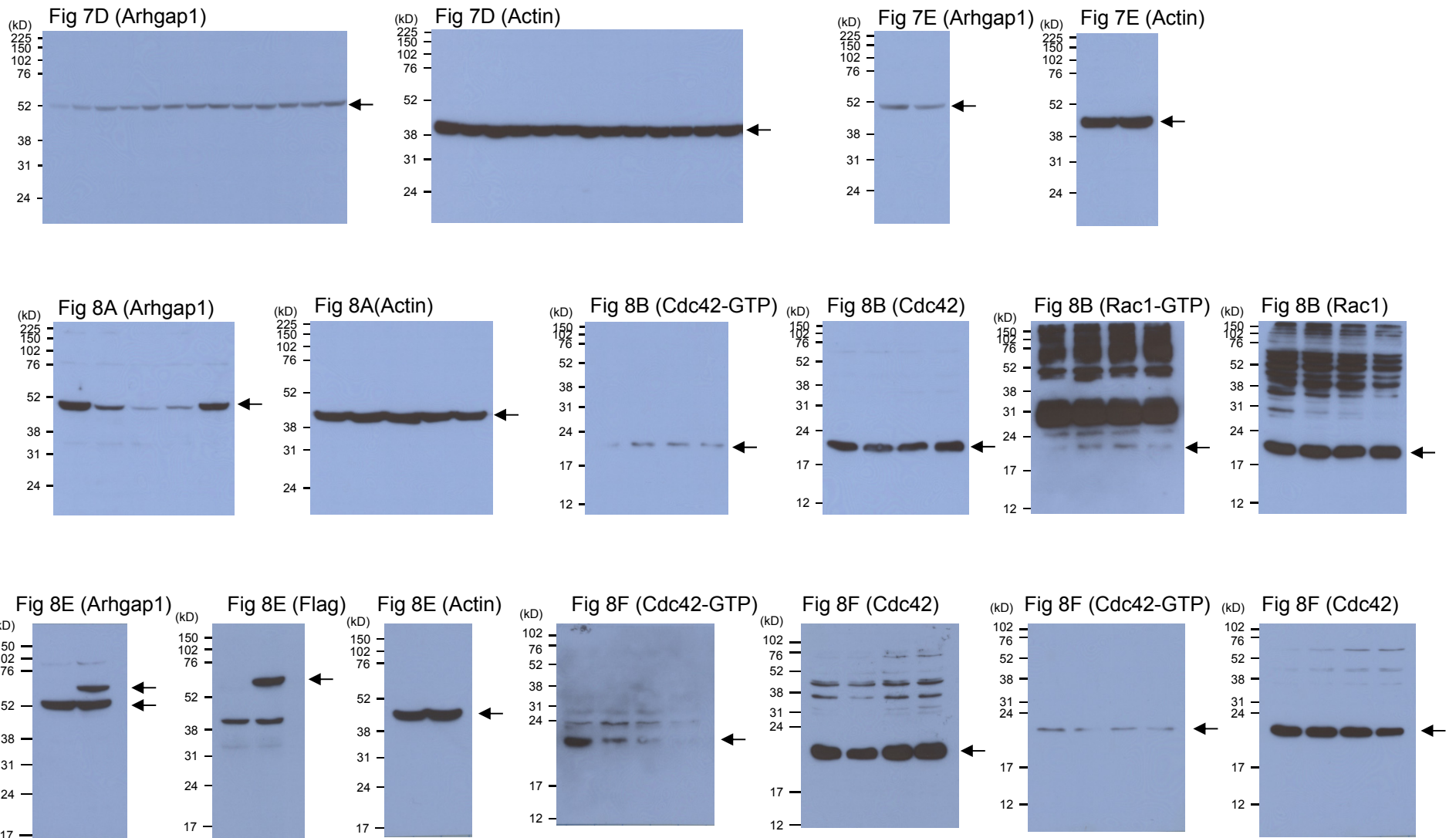
Supplemental Figure 11. miR-34a down-regulates ARHGAP1 expression.

(A) Western blot analysis of ARHGAP1 and ACTIN in 393P cells transiently transfected with 100 nM of miRIDIAN® negative control (con) or miR-34a hairpin inhibitor (anti-34a). Densitometric analysis of ARHGAP1 protein levels normalized to those of ACTIN and expressed relative to control transfectants, which was set at 1.0. (B) Q-PCR analysis of *ARHGAP1* mRNA levels in H1299 human lung cancer cells 72 hrs after transient transfection with Pre-miR™ negative control (con) or Pre-miR-34a (miR-34a). Results normalized on the basis of mRNA for ribosomal protein *L32*. Mean±SD, n=3. P-value, two-tailed Student's t-test.

Full uncut blots-1



Full uncut blots-2



Full uncut blots-3

