

Supplementary data to:

The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2

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Supplementary figure legends:

Figure S1. Expression of 207 different miRNAs in the NCI60 cells ranked according to expression in either epithelial or mesenchymal cell lines. P-values are the result of a two-sample t-test analysis. High expression is indicated in red and low expression in blue. Fold change (\log_2) is indicated.

Figure S2. Comparison of the ratio of E-cadherin/Vimentin protein expression with expression of either miR-141, miR-200a, miR-200b or miR-200c in the NCI60 cells. Pearson correlation coefficients (r) and p-values (p) are reported.

Figure S3. (A) Normalized Δ CTs of the expression of the functional cluster miR-200a/miR-141 miRNAs were added and plotted against the sum of the Δ CTs of miR-200b and miR-200c. (B) Normalized Δ CTs of the expression of the genetic cluster miR-200a and miR-200b miRNAs were added and plotted against the sum of the Δ CTs of miR-200c and miR-141. Residual variances (R^2) are reported.

Figure S4. Effects of only 5 nM of miR-200 on MDA-MB-231 cells. Cells were transfected with either 5 nM scrambled oligo (S) or a mixture of 2.5 nM miR-200a and 2.5 nM miR-200c and after 3 (D3) or 6 days (D6) expression of E-cadherin, ZEB1 and ZEB2 mRNA was determined using real-time PCR.

Figure S5. (A) Analysis of the expression of let-7d and miR-200c in 59 of the NCI60 cells. In the left half, the expression of let-7d in the 11 Type I and 11 Type II cells previously identified among the NCI60 cells (Algeciras-Schimnich et al. 2003) and the SC1 and SC2 cell lines among the 59 NCI60 cells are shown. In the right half the expression of miR-200c in the same cells is shown. (B) Comparison of the ratio of E-cadherin/Vimentin protein expression with expression of let-7d in the NCI60 cells. Cells with either high E-cadherin and no Vimentin (I), E-cadherin/Vimentin ratio of around one (II) and with high Vimentin and low E-cadherin expression (III) are labeled with a stippled oval. (C) Comparison of the ratio of E-cadherin/Vimentin protein expression with expression of miR-200c in the NCI60 cells. Group I, group II and group III cells are labeled. SC1 and SC2 cells within the epithelial group I are labeled in blue and red, respectively. Residual variances (R^2) are given.

References

Algeciras-Schimnich, A., E.M. Pietras, B.C. Barnhart, P. Legembre, S. Vijayan, S.L. Holbeck, and M.E. Peter. 2003. Two CD95 tumor classes with different sensitivities to antitumor drugs. *Proc Natl Acad Sci U S A* **100**: 11445-50.

Figure S2

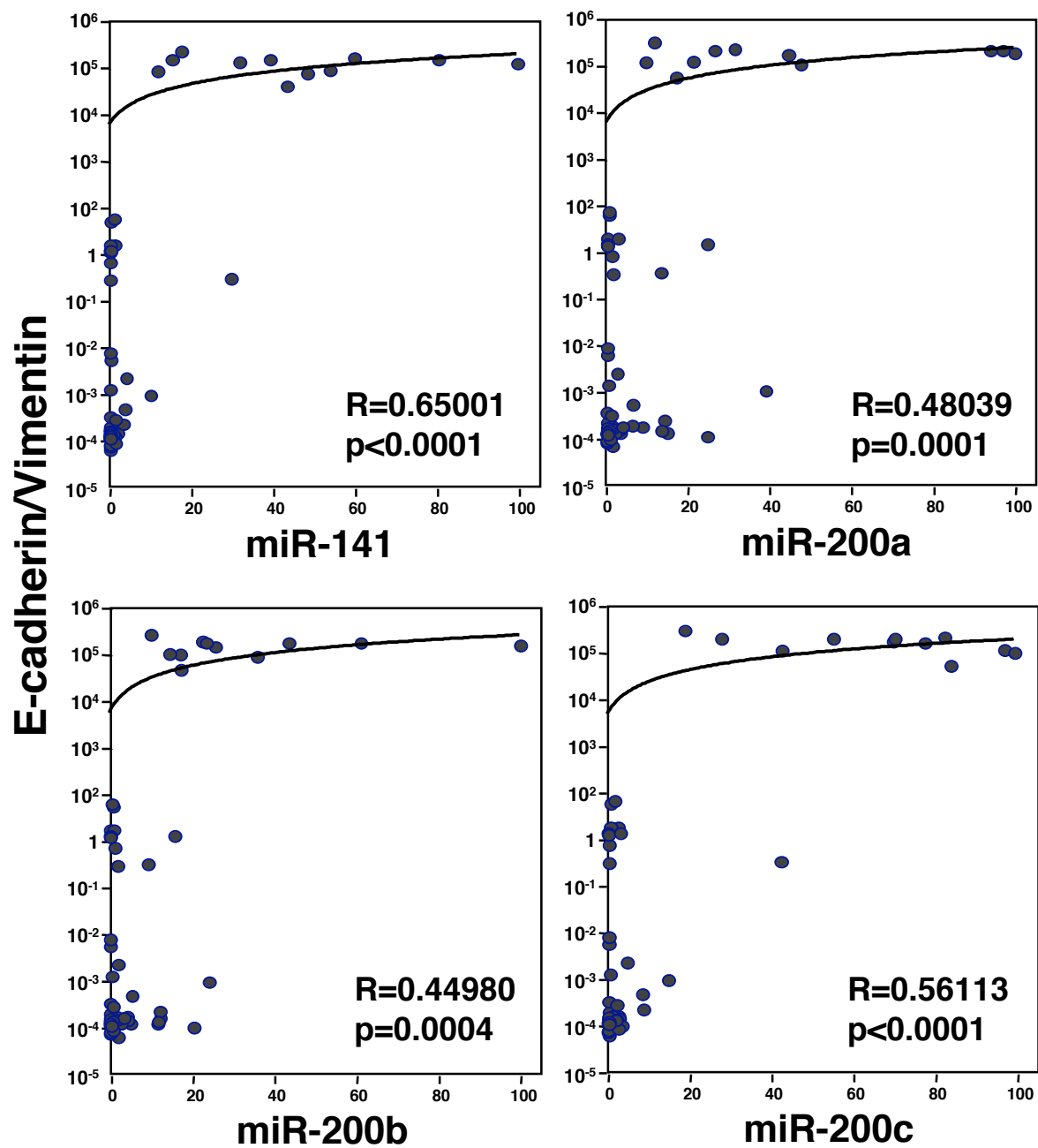


Figure S3

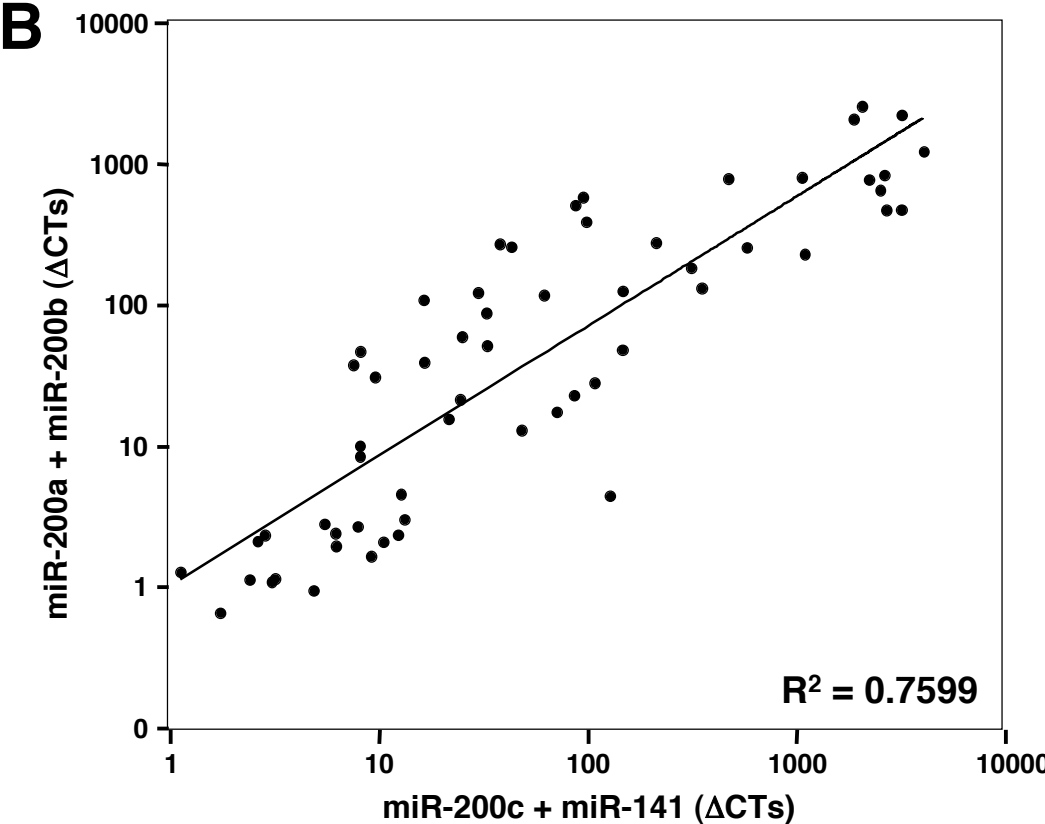
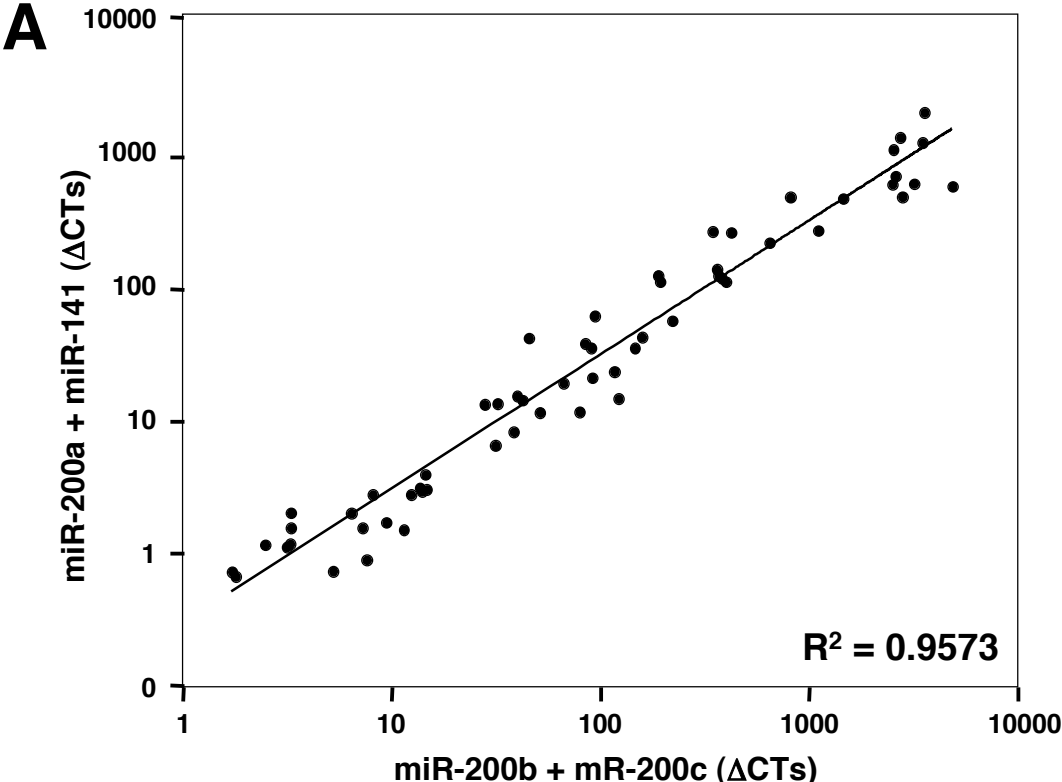


Figure S4

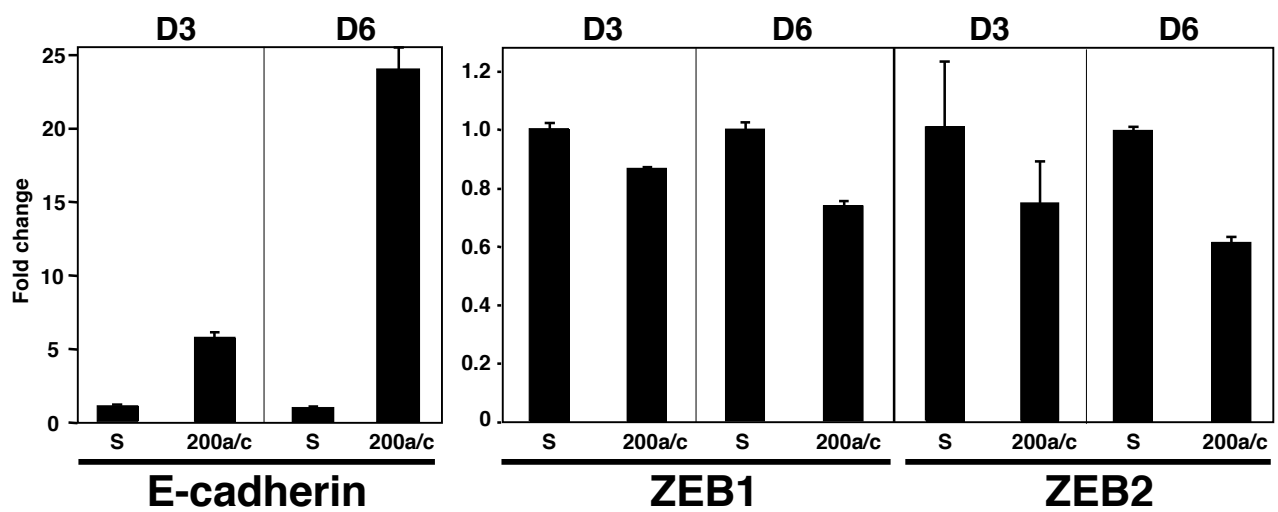


Figure S5

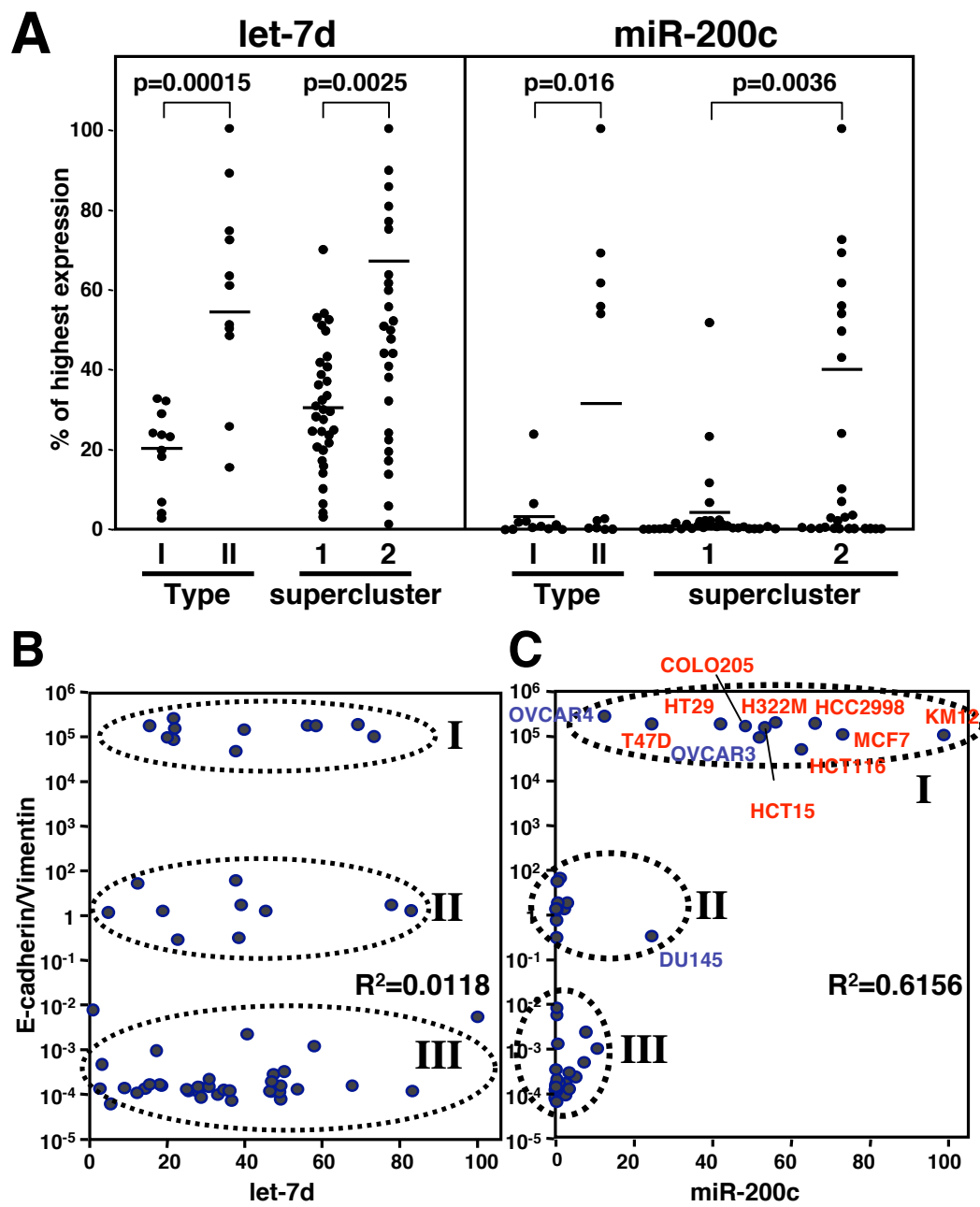


Table S1: Genes downregulated >0.5 in MBA-MD-231 cells transfected with either miR-200a or miR-200c

Gene	miR-200a vs scr log2 change	miR-200c vs scr log2 change	TargetScan		TargetRank		Descriptions
			miR-141/200a	miR-200b,c/429	miR-200a	miR-200c	
ICEBERG	-3.2	-2.6					gb:NM_021571.1 /DB_XREF=gi:10954342 /GEN=ICEBERG /
QKI	-0.7	-1.3	X	X	X	X	gb:AL031781 /DB_XREF=gi:4038570 /FEA=FLmRNA /CNT=
LOX	-0.3	-1.3			X	X	gb:L16895 /DB_XREF=gi:292923 /FEA=DNA /CNT=3 /TID=H
WIPF1	-0.4	-0.9	X	X	X	X	gb:AW058622 /DB_XREF=gi:5934261 /DB_XREF=wy59c01.:
LHFP	-0.4	-0.9		X	X	X	gb:NM_005780.1 /DB_XREF=gi:5031864 /GEN=LHFP /FEA=
Unknown	-0.3	-0.9					gb:AF134802.1 /DB_XREF=gi:4868361 /FEA=FLmRNA /CNT
ZEB1	-0.5	-0.8	X	X	X	X	gb:AI373166 /DB_XREF=gi:4153032 /DB_XREF=qz13b01.x1
Unknown	-0.3	-0.8					Cluster Incl. U79256:Human clone 23719 mRNA sequence /c
Unknown	-0.6	-0.8					gb:BE644809 /DB_XREF=gi:9969120 /DB_XREF=7e57e09.x
Unknown	-0.4	-0.8					gb:AL133706 /DB_XREF=gi:6601894 /DB_XREF=DKFZp761
AP1S2	-0.2	-0.7		X		X	gb:AF251295.1 /DB_XREF=gi:12005731 /FEA=FLmRNA /CN
ZEB1 (1)	-0.4	-0.7	X	X	X	X	gb:AI806174 /DB_XREF=gi:5392740 /DB_XREF=wf06h03.x1
RPS6	-0.4	-0.7					gb:AA142942 /DB_XREF=gi:1712320 /DB_XREF=zl43c04.s1
TAX1BP1	-0.2	-0.7					gb:AL047908 /DB_XREF=gi:4728096 /DB_XREF=DKFZp586
Unknown	-0.8	-0.7					gb:AV703394 /DB_XREF=gi:10720721 /DB_XREF=AV70339
Unknown	-0.7	-0.7					gb:AW119113 /DB_XREF=gi:6087697 /DB_XREF=xd89b09.>
ZEB2	-0.3	-0.7	X	X	X	X	gb:NM_014795.1 /DB_XREF=gi:7662183 /GEN=ZFHX1B /FE
PPAP2B	-0.3	-0.7		X		X	gb:AA628586 /DB_XREF=gi:2540973 /DB_XREF=af39f12.s1
Unknown	-0.4	-0.6					gb:NM_024551.1 /DB_XREF=gi:13375714 /GEN=FLJ21432 /
Unknown	-0.3	-0.6					gb:BF572868 /DB_XREF=gi:11646580 /DB_XREF=6020794<
Unknown	-0.4	-0.6					gb:AL049176 /DB_XREF=gi:4808226 /FEA=FLmRNA /CNT=i
ATP2C1	-0.3	-0.6					gb:AF225981.1 /DB_XREF=gi:7021496 /GEN=ATP2C1 /FEA
Unknown	-0.4	-0.6					gb:AA917899 /DB_XREF=gi:3057789 /DB_XREF=ol76e05.s1
Unknown	-0.5	-0.6					gb:H95263 /DB_XREF=gi:1102896 /DB_XREF=yu20e04.s1 /
Unknown	-0.4	-0.6					gb:AL050199.1 /DB_XREF=gi:4884438 /FEA=mRNA /CNT=2
Unknown	-0.3	-0.6					gb:NM_022753.1 /DB_XREF=gi:12232418 /GEN=FLJ12903 /
Unknown	-0.2	-0.6					gb:NM_021183.1 /DB_XREF=gi:10880976 /GEN=LOC57826
PCDH9	-0.5	-0.6	X		X		gb:AI524125 /DB_XREF=gi:4438260 /DB_XREF=th09d11.x1
ALS2CR2	-0.4	-0.6					gb:AB038950.1 /DB_XREF=gi:12862479 /GEN=ALS2CR2 /FI
TRIP8	-0.3	-0.6					gb:BF002296 /DB_XREF=gi:10702571 /DB_XREF=7h02f04.>
Unknown	-0.4	-0.6					gb:BF593817 /DB_XREF=gi:11686141 /DB_XREF=nac05a10
Unknown	-0.5	-0.6					gb:AI378647 /DB_XREF=gi:4188500 /DB_XREF=tc57a04.x1
Unknown	-0.6	-0.6					gb:AA577678 /DB_XREF=gi:2355862 /DB_XREF=nn22f08.s'
MTND6	-0.9	-0.6					gb:NM_173714.1 /DB_XREF=gi:27754187 /GEN=MTND6 /TI
Unknown	-0.3	-0.6					gb:AK000680.1 /DB_XREF=gi:7020924 /FEA=FLmRNA /CNT
PAPPA	-0.3	-0.5	X		X	X	gb:AA148534 /DB_XREF=gi:1721559 /DB_XREF=zl06d06.s1
COP9	-0.2	-0.5					gb:BC003090.1 /DB_XREF=gi:13111846 /FEA=FLmRNA /CN
LSM5	-0.3	-0.5					gb:AU153477 /DB_XREF=gi:11014998 /DB_XREF=AU15347
Unknown	-0.3	-0.5					gb:AL050164.1 /DB_XREF=gi:4884378 /GEN=DKFZp586C1f

THBD	-0.7	-0.5						gb:NM_000361.1 /DB_XREF=gi:4507482 /GEN=THBD /FEA=
IGFBP1	-0.4	-0.5						gb:NM_000596.1 /DB_XREF=gi:4504614 /GEN=IGFBP1 /FE
ID1H	-0.3	-0.5						gb:D13889.1 /DB_XREF=gi:464181 /GEN=id-1H /FEA=FLmF
ZEB1 (1)	-0.2	-0.5	X	X	X	X		gb:U12170.1 /DB_XREF=gi:529172 /FEA=FLmRNA /CNT=2 ,
Unknown	-0.3	-0.5						gb:AB020681.1 /DB_XREF=gi:4240236 /GEN=KIAA0874 /FE
DNAJC8	-0.3	-0.5						gb:AA843895 /DB_XREF=gi:2930346 /DB_XREF=ak09g10.s
ATM	-0.5	-0.5						gb:U82828 /DB_XREF=gi:2304970 /FEA=mRNA /CNT=113 /
Unknown	-0.4	-0.5						gb:AK000002.1 /DB_XREF=gi:7209304 /GEN=FLJ00002 /FE
RPS21	-0.4	-0.5						gb:AW024383 /DB_XREF=gi:5877913 /DB_XREF=ww03e06.:
MTAP44	-0.4	-0.5						gb:NM_006417.1 /DB_XREF=gi:5453743 /GEN=MTAP44 /FE
Unknown	-0.5	-0.5						gb:AI535683 /DB_XREF=gi:4449818 /DB_XREF=cong1.P11.
LOC55828	-0.3	-0.5						gb:NM_018443.1 /DB_XREF=gi:8923850 /GEN=LOC55828 /I
Unknown	-0.3	-0.5						gb:AL133049.1 /DB_XREF=gi:6453464 /GEN=DKFZp434E13
Unknown	-0.4	-0.5						gb:NM_014650.1 /DB_XREF=gi:7662313 /GEN=KIAA0798 /F
Unknown	-0.4	-0.5						gb:AI742210 /DB_XREF=gi:5110498 /DB_XREF=wg39c02.x'
Unknown	-0.3	-0.5						gb:AA775681 /DB_XREF=gi:2835015 /DB_XREF=zf31a02.s1
ADAMTS1	-0.6	-0.5						gb:AK023795.1 /DB_XREF=gi:10435838 /FEA=mRNA /CNT=1
MAIL	-0.5	-0.5						gb:BE646573 /DB_XREF=gi:9970884 /DB_XREF=7e89c10.x
TRIM6	-0.4	-0.5						gb:AF220030.1 /DB_XREF=gi:12407390 /GEN=TRIM6 /FEA=
CS-1	-0.5	-0.5						gb:AF001540 /DB_XREF=gi:2529712 /DB_XREF=AF001540
Unknown	-0.3	-0.5						gb:AW152589 /DB_XREF=gi:6200489 /DB_XREF=xf76g02.x
Unknown	-0.4	-0.5						gb:BG484552 /DB_XREF=gi:13416831 /DB_XREF=6025057
Unknown	-0.3	-0.5						gb:AA779684 /DB_XREF=gi:2839015 /DB_XREF=af43e03.s'
CALM2	-0.5	-0.5						gb:AL046017 /DB_XREF=gi:5434111 /DB_XREF=DKFZp434
PTMA	-0.6	-0.5						gb:AI110886 /DB_XREF=gi:6359749 /DB_XREF=HA0306 /FE
Unknown	-0.3	-0.5						gb:AW134977 /DB_XREF=gi:6138523 /DB_XREF=UI-H-B11-;
Unknown	-0.4	-0.5						gb:AI692523 /DB_XREF=gi:4969863 /DB_XREF=wd73d06.x'
Unknown	-0.4	-0.5						gb:AI374739 /DB_XREF=gi:4174729 /DB_XREF=tc32d12.x1
Unknown	-0.5	-0.5						gb:AI760013 /DB_XREF=gi:5175680 /DB_XREF=wh83a07.x'
Unknown	-0.4	-0.5						gb:AI937080 /DB_XREF=gi:5675950 /DB_XREF=wp72g12.x'
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Unknown	-0.3	-0.5						gb:AL534095 /DB_XREF=gi:12797588 /DB_XREF=AL534095
SFRS3	-0.4	-0.5						gb:BE927772 /DB_XREF=gi:10453848 /DB_XREF=MR3-CTC
H105E3	-0.3	-0.5						gb:U82671 /DB_XREF=gi:7280331 /FEA=DNA_9 /CNT=6 /TI
Unknown	-0.5	-0.5						gb:N71074 /DB_XREF=gi:1227654 /DB_XREF=za86d11.s1 /I
THBD	-0.5	-0.4						gb:NM_000361.1 /DB_XREF=gi:4507482 /GEN=THBD /FEA=
TM7SF1	-0.7	-0.4						gb:NM_003272.1 /DB_XREF=gi:4507544 /GEN=TM7SF1 /FE
APAF1	-0.5	-0.4				X	X	gb:NM_013229.1 /DB_XREF=gi:7108332 /GEN=APAF1 /FEA
HAS2	-0.5	-0.4						gb:NM_005328.1 /DB_XREF=gi:4885390 /GEN=HAS2 /FEA=
H4FE	-0.7	-0.4						gb:NM_021968.1 /DB_XREF=gi:11415029 /GEN=H4FE /FEA
Unknown	-0.6	-0.4						gb:AB020684.1 /DB_XREF=gi:4240242 /GEN=KIAA0877 /FE
MKPX	-0.5	-0.4						gb:NM_020185.1 /DB_XREF=gi:9910431 /GEN=MKPX /FEA:
Unknown	-0.5	-0.4						Cluster Incl. AA150165:zl06h03.r1 Homo sapiens cDNA, 5 er

PRO1073	-0.5	-0.4
TASR1	-0.5	-0.4
PRO1073	-0.7	-0.4
Unknown	-0.6	-0.4
Unknown	-0.5	-0.4
NNMT	-0.5	-0.4
Unknown	-0.6	-0.4
PTTG3	-0.5	-0.4
GJA1	-0.5	-0.3
Unknown	-0.5	-0.3
Unknown	-0.5	-0.3
Unknown	-0.5	-0.3
Unknown	-0.5	-0.3
Unknown	-0.5	-0.2
KBRAS1	-0.5	-0.2

gb:AI446756 /DB_XREF=gi:4297435 /DB_XREF=tj15d04.x1 /
 gb:AI954700 /DB_XREF=gi:5747010 /DB_XREF=wq32b06.x'
 gb:AI475544 /DB_XREF=gi:4328589 /DB_XREF=tc92a05.y1
 gb:N76327 /DB_XREF=gi:1238905 /DB_XREF=yv39c02.r1 /C
 gb:N32832 /DB_XREF=gi:1153231 /DB_XREF=yw85g06.s1 /
 gb:NM_006169.1 /DB_XREF=gi:5453789 /GEN=NNMT /FEA=
 gb:AB023198.1 /DB_XREF=gi:4589605 /GEN=KIAA0981 /FE
 gb:NM_021000.1 /DB_XREF=gi:10337606 /GEN=PTTG3 /FE
 gb:NM_000165.2 /DB_XREF=gi:4755136 /GEN=GJA1 /FEA=
 gb:NM_014705.1 /DB_XREF=gi:7662263 /GEN=KIAA0716 /F
 gb:AU153138 /DB_XREF=gi:11014659 /DB_XREF=AU15313
 gb:AK023825.1 /DB_XREF=gi:10435876 /FEA=mRNA /CNT=
 gb:NM_017945.1 /DB_XREF=gi:8923656 /GEN=FLJ20730 /F
 gb:AI972416 /DB_XREF=gi:5769332 /DB_XREF=wr39d10.x1
 gb:AI970120 /DB_XREF=gi:5766946 /DB_XREF=wq89d09.x'

(1) The online versions of both TargetScan and TargetRank are based on an incomplete 3'UTR sequence for ZEB of 542 bp. The complete ZEB1 3'UTR contains 1954 bp (gb:U12170) and is predicted to be a target of both miR-200a and miR-200c by TargetScan and TargetRank.

Table S2: The top 20 genes most downregulated in MDA-MB-231 cells transfected with only miR-200a

Gene	miR-200a Fold down	miR-200c Fold down	TargetScan		TargetRank		GenBank Accession #
			miR-141/200a	miR-200b,c/429	miR-200a	miR-200c	
Unknown	12.13	NC					NM_032923.1
DLG2	10.56	NC					NM_001364.1
Unknown	9.85	NC					BC033319.1
SDR-O	8.57	NC					NM_148897.1
Unknown	7.46	NC					AF339829.1
MKP	6.06	NC					AB033338.1
Unknown	6.06	NC					AL832555.1
KCNJ13	3.25	NC				X	AB013889.1
PRO0214	2.14	NC					NM_014120.1
Unknown	2.00	NC					AA662761
Unknown	2.00	NC					AL833020.1
NAP1L3	1.87	NC			X		NM_004538.1
H4F2	1.87	NC					NM_003548.1
TIMELESS	1.74	NC					AK000721.1
Unknown	1.74	NC					AA876372
Unknown	1.74	NC					AI423201
Unknown	1.74	NC					BC043608.1
Unknown	1.74	NC					AK096609.1
IL11	1.62	NC					NM_000641.1
Unknown	1.62	NC					AI382123

Table S3: The top 20 genes most downregulated in MDA-MB-231 cells transfected with only miR-200c

Gene	miR-200a	miR-200c	TargetScan		TargetRank		GenBank
	Fold down	Fold down	miR-141/200a	miR-200b,c/429	miR-200a	miR-200c	Accession #
Unknown	NC	13.93					AI216576
Unknown	NC	13.00					AW452357
H3FA	NC	12.13					NM_003529.1
Unknown	NC	12.13					AF086073.1
ADRB1	NC	9.85	X				AI625747
Unknown	NC	9.19					BF222940
Unknown	NC	8.57					AI655633
Unknown	NC	7.46					BF476913
Unknown	NC	7.46					AI123346
RASGRP2	NC	6.50					AI688812
C1QR	NC	6.06					W72082
Unknown	NC	4.92					AL832158.1
Unknown	NC	4.92					AA156795
Unknown	NC	4.59					NM_014729.1
TCRA	NC	4.29					M15565.1
Unknown	NC	4.29					BC020583.1
COMP	NC	3.03					NM_000095.1
Unknown	NC	2.64					AI961235
Unknown	NC	2.30					NM_014934.1
Unknown	NC	2.30					BC002715.1