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Supplemental Material to:

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Activation of miR200 by c-Myb depends on ZEB1 expression and miR200 promoter methylation

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Supplementary Table 1. Correlation between c-MYB and mir200 in GSE22220 dataset (ER- breast cancer patients)

GENE (probe ID)	miRNA (probe ID)	Pearson correlation	p value
c-MYB (7000594)	HSA-miR-200c (ILMN_3167002)	0.40	0.01
c-MYB (7000594)	HSA-miR-200b (ILMN_3168294)	0.30	0.005
c-MYB (7000594)	HSA-miR-200a (ILMN_3167801)	0.09	0.1
c-MYB (7000594)	HSA-miR-141 (ILMN_3168064)	0.02	0.1
c-MYB (7000594)	HSA-miR-429 (ILMN_3167806)	-0.09	0.80

Supplementary Table 2. Primers used in the study.

Cloning	Forward 5'-3'	Reverse 5'-3'
human miR200ba429 promoter	ACCTGCTAGCGTGACCTTTCTTCT CACGGAG	CAGGAGATCTCCTGGCACAGGAAG TCAGTT
human miR200c141 promoter	ACGAACGCGTGCACCGAGATGAC GGCTG	ACGACTCGAGAGATCCCTGGCTCC CATC
human ZEB1-A	TAAGGATCCGCCACCATGAAAGT TACAAATTA TAATACTGTGGTAGAAACA	TGAGAGCTCTTCTGCACTTGGTTGT GCATTCAAAG C
human ZEB1-B	GAAGAGCTCTCAAAAATTGCTGA TTCAGTAAACCTACCAC	GGCTTCATTTGTCTTTTCTTCAGAC ACTTGCTCACTACTC

Deletion	Forward 5'-3'	Reverse 5'-3'
Mut1 miR200ba429 promoter	ACCTGCTAGCGTCAGAGGTCACC GGTGC	CAGGAGATCTCCTGGCACAGGAAG TCAGTT
Mut2 miR200ba429 promoter	ACCTGCTAGCTTCCAGCGAGAAG AGAGCCAG	CAGGAGATCTCCTGGCACAGGAAG TCAGTT
Mut11 miR200c141 promoter	ACGAACGCGTGTCCCCTCTGGCG GGAACA	ACGACTCGAGAGATCCCTGGCTCC CATC
Mut2 miR200c141 promoter	ACGAACGCGTGACTTGGACTCCA CTGAGGGC	ACGACTCGAGAGATCCCTGGCTCC CATC
Mut3 miR200c141 promoter	ACGAACGCGTTAAAGCCCCTTCG TCTCC	ACGACTCGAGAGATCCCTGGCTCC CATC

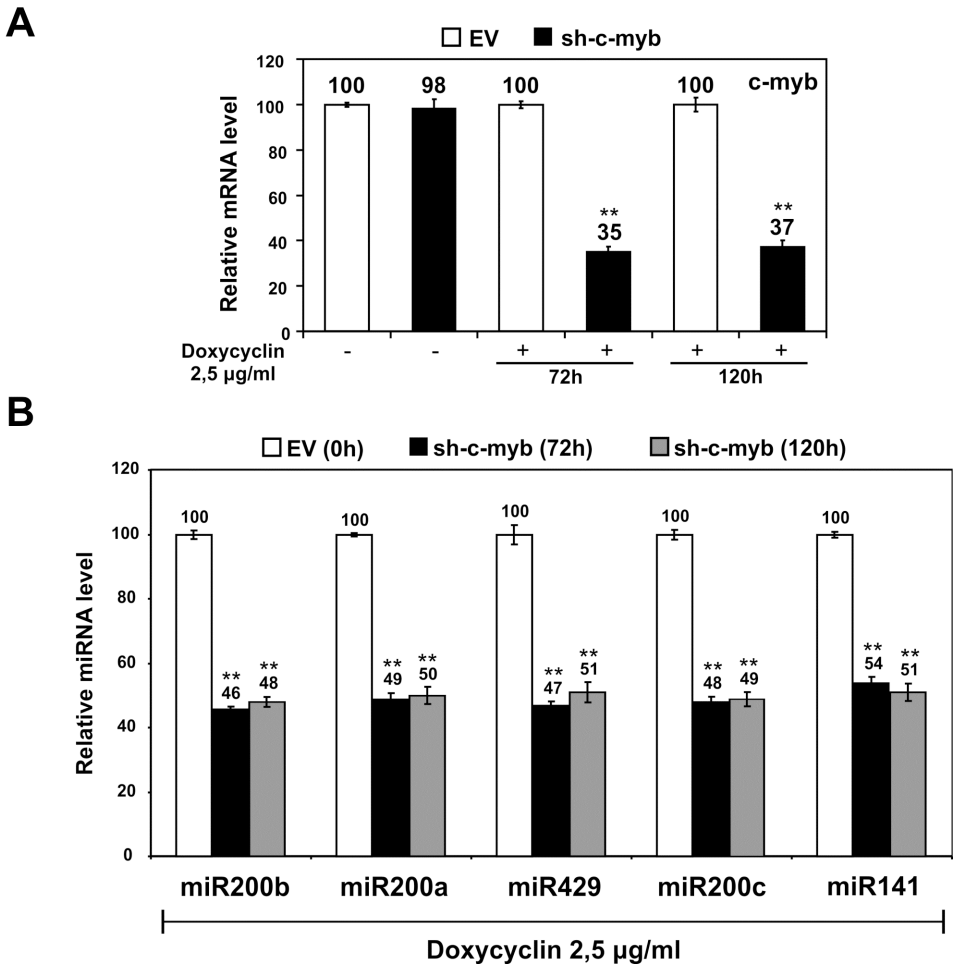
Site directed mutagenesis	Forward 5'-3'	Reverse 5'-3'
miR200ba429 promoter MUT1	GACTGTCCGGGGGAGGGAgTtTTc GGGCAAGGGCAGTGG	CCACTGCCCTTGCCCgAAaAcTCCCT CCCCGGGACAGTC
miR200ba429 promoter MUT2	CACCGGTGCCCCCAGGACgAtTTc CCAGCGAGAAGAGA	TCTCTTCTCGCTGGgAAaTcGTCCTG GGGGCACCGGTG
miR200c141 promoter MUT1	AACTAAGAAGAGTAGTTtAaAaCC GGAAGCGCAGACCTG	CAGGTCTGCGCTTCCGGtTtTaAACT ACTCTTCTTAGTT
miR200c141 promoter MUT2	CAAGTCCCACCTCCTCTtAaaaCCC TTGGGCTACCTGC	GCAGGTAGCCCAAGGGtttTaAGAGG AGGTGGGACTTG

Supplementary Table 2. Primers used in the study.

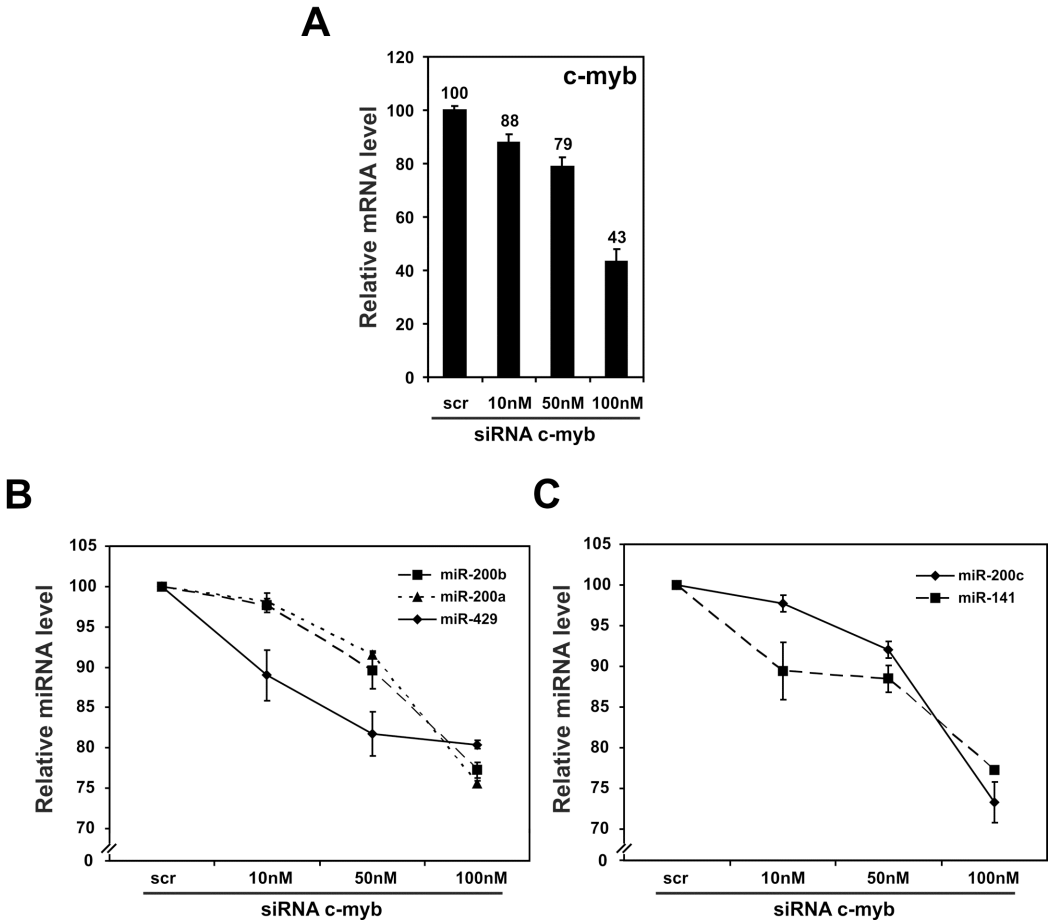
ChIP	***Forward 5'-3'***	***Reverse 5'-3'***
hsa miR200ba429 promoter Distal 1 and 2	CAGACACCTGAGTGGGCATCAG	CCCAGAGGAAGAGCCCATAAT
hsa miR200ba429 promoter Proximal	AGATTGGCCAGCGGGA	CCTGGCACAGGAAGTCAG
hsa miR200c141 promoter Distal 1	TCACAGGGCTATGGAACAGTGA	CACGTCAGCCAGCTCTTCAA
hsa miR200c141 promoter l Distal 2	GAGGGATTGAGCAACCCAATAG	GCCCTCAGTGGAGTCCAAGTC
hsa miR200c141 promoter Proximal	CCAAGCCTTAGAGGAGGTGC	GAGACGAAGGGGCTTTAAGG
ADA	CTCCTCCTTTTTGTCTTCCT	GAAACTCAGTCTCCTTTGTTCCCC

qPCR	**Forward 5'-3'**	**Reverse 5'-3'**
c-myb	ACCATGACTATGATGGGCTGC	TCCCCAAGTGACGCTTTCC
Desmoplakin	GCTAAACGCCGCCAGGAT	CCGCATGACTGTGTTGGAAT
Occludin	GCCGGTTCCTGAAGTGGTT	CGAGGCTGCCTGAAGTCATC
Vimentin	CCAGCCGCAGCCTCTACG	GCGAGAAGTCCACCGAGTCC
MRPL19	TCCTCGGGTCCAGGAGATT	CAAGCTATCATCCAGCCGTTTC
Zeb1	GAACAGGACTCAAGACATCTCAG TGT	GGTTTATTCTCTATCTTTTGCCGTA TCT
E-Cadherin	CCGCTGGCGTCTGTAGGAAGG	GGCTCTTTGACCACCGCTCTCC
N-Cadherin	CTGTGGGAATCCGACGAATGG	GTCATTGTCAGCCGCTTTAAGG

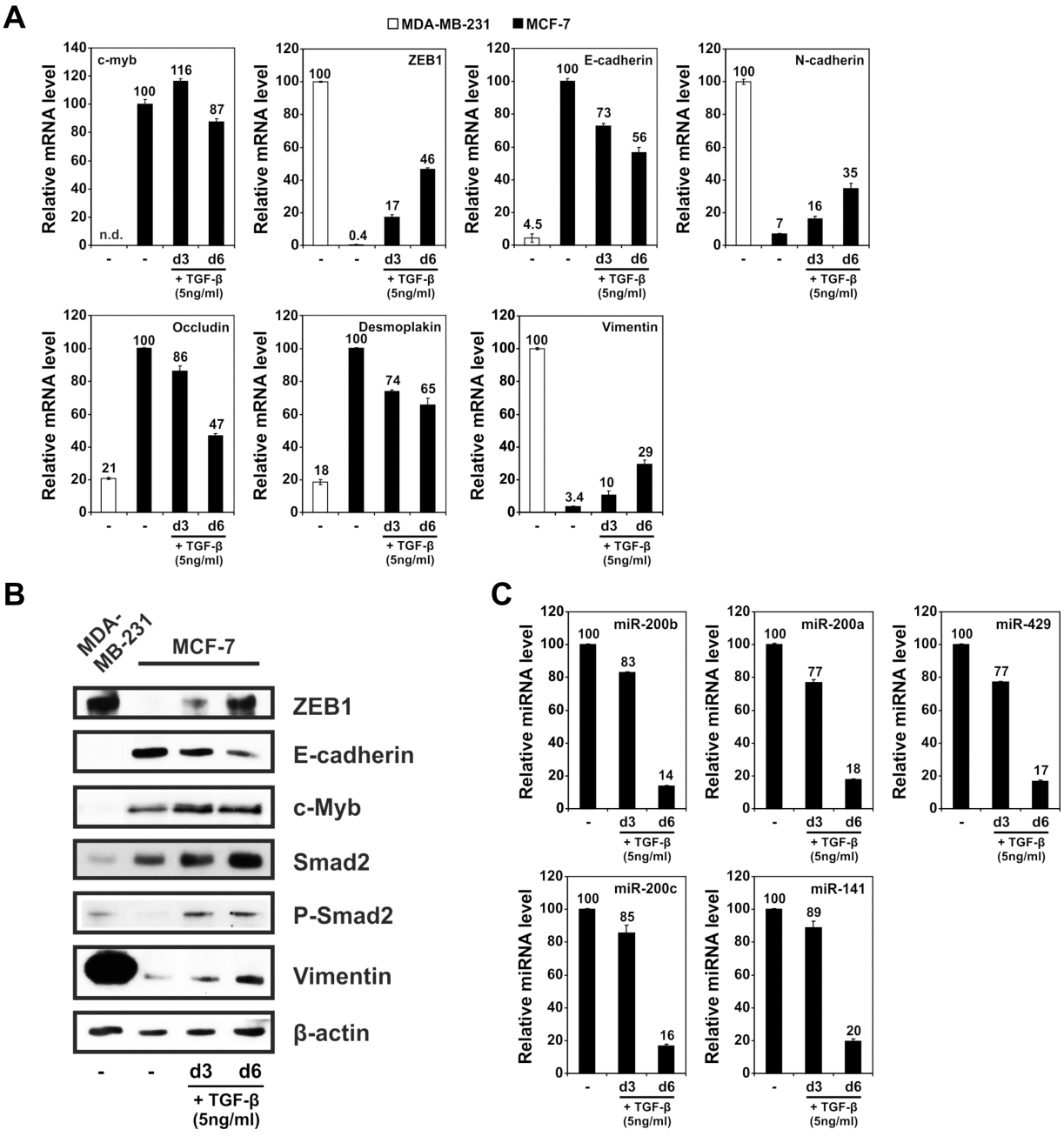
Lower case, red letters indicate mutated nucleotides. * indicate biotinylated PCR primer.



Supplementary Fig. 1. A: HEK293 cells were infected with lentiviral vector pLV-sh-c-myb (sh-c-myb) or with the empty vector pLV-EV (EV) and c-Myb expression was analyzed in sh-c-myb and EV infected cells after 72 and 120 hours of treatment with 2.5 mg/ml of doxycyclin by qRT-PCR. The relative mRNA level in untreated Ctrl cells was arbitrarily set to 100. B: qRT-PCR to analyze the expression level of each miRNA of the miR200 family was carried out in sh-c-myb-infected cells treated with doxycyclin for 72 and 120 h. The expression level of each miRNA in untreated sh-c-myb-infected cells was arbitrarily set to 100. Statistical significance was calculated by two-tailed Student's t test. ** $p \leq 0.01$. qRT-PCR analyses were carried out in triplicate in two independent experiments.



Supplementary Fig.2. A: c-myb mRNA levels were analyzed by qRT-PCR in MCF-7 cells transfected with siRNA directed against c-myb at the indicated concentrations. MCF-7 transfected with scrambled siRNA at 100 nM were used as control. B and C: qRT-PCR analysis to detect the levels of miR200b, miR200a, miR42 (B) , miR200c and miR141 (C) in MCF-7 cells transfected at the indicated concentrations of siRNA against c-myb. Scrambled: as in a. Analyses were carried out 72 hours after transfections. qRT-PCR analyses were carried out twice in triplicate. Data are reported ± standard deviation. Statistical significance was calculated by Student's t test.



Supplementary Fig. 3. A: analysis of mRNA level for the indicated genes was carried out by qRT-PCR in MCF-7 cells treated with TGF-β (5 ng/ml) for 3 and 6 days. B: western blot analysis was carried out to detect c-Myb, ZEB1, E-cadherin, and Vimentin in MCF-7 cells treated with TGF-β as in A. C: miRNA levels for miR200b, miR200a, miR429, miR200c and miR141 were analyzed by qRT-PCR in MCF-7 cells treated with TGF-β as in a. qRT-PCR analyses were carried out twice in triplicate. Data are reported ± standard deviation. Statistical significance was calculated by Student's t test.