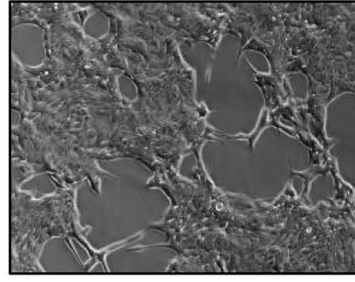
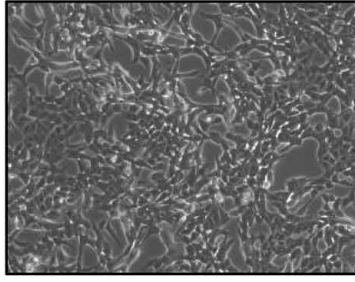
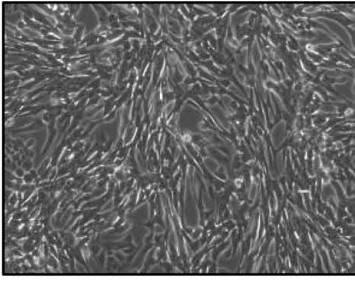
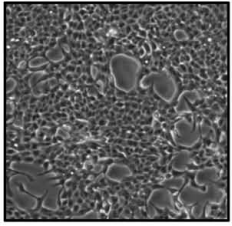


A**MDCK-TGF****+ SB-431542**

Day 0

Day 2

Day 7

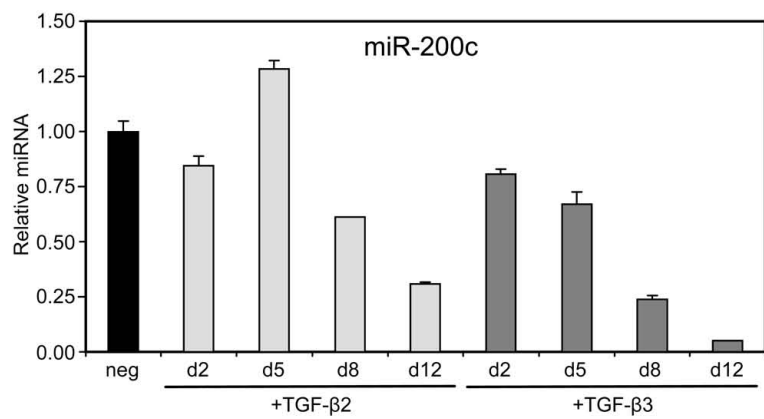
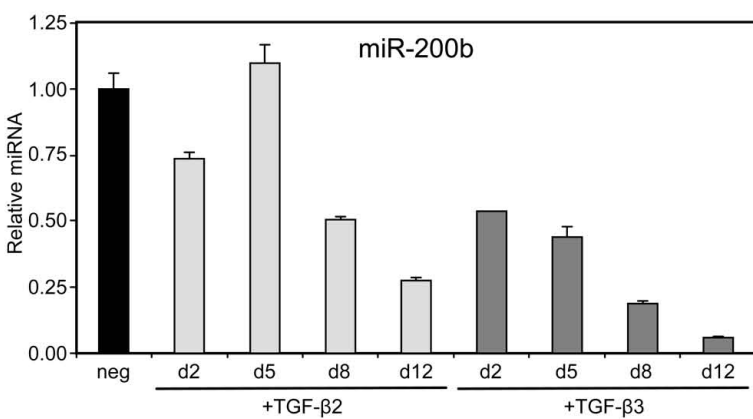
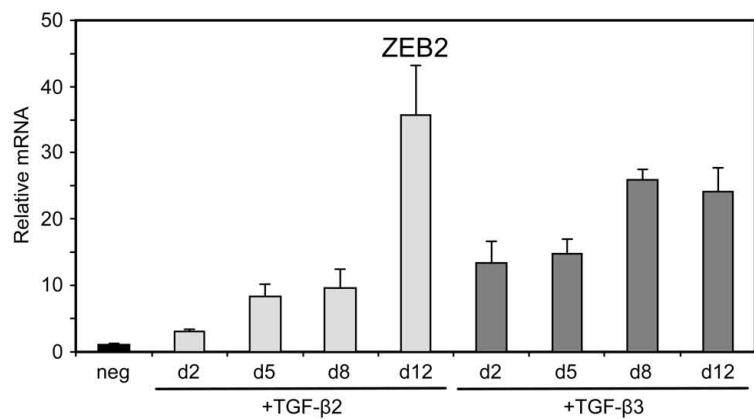
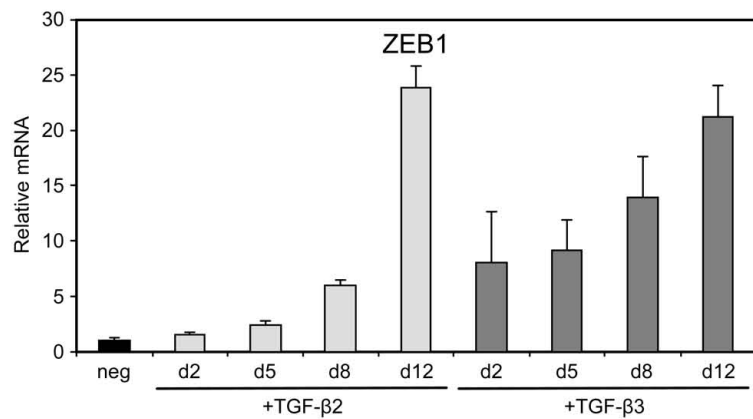
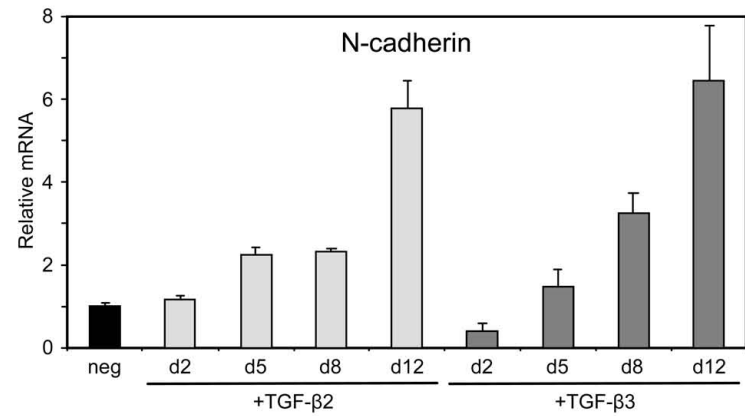
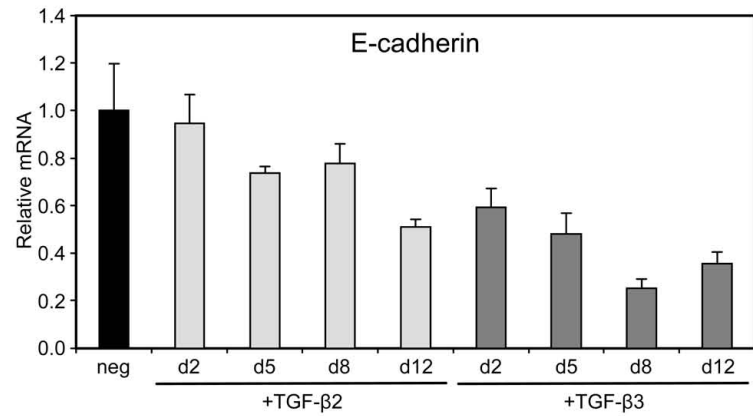
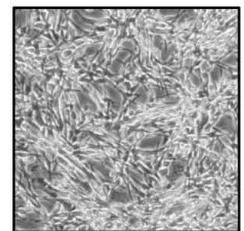
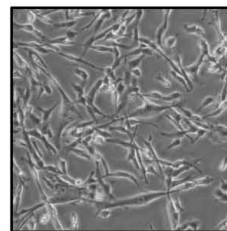
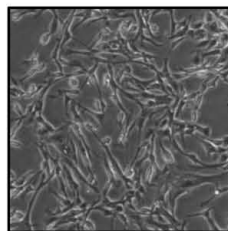
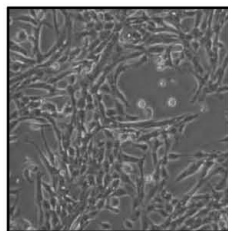
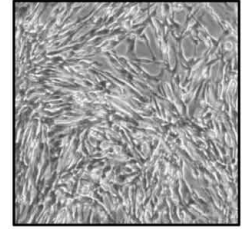
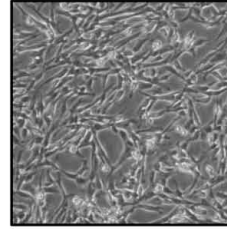
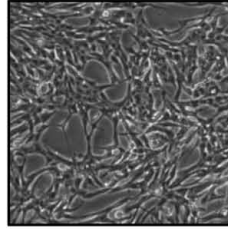
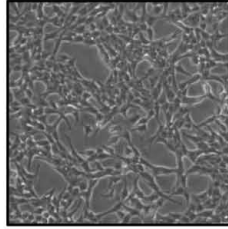
**B****MDCK**+ TGF- β 2- TGF- β + TGF- β 3

Day 2

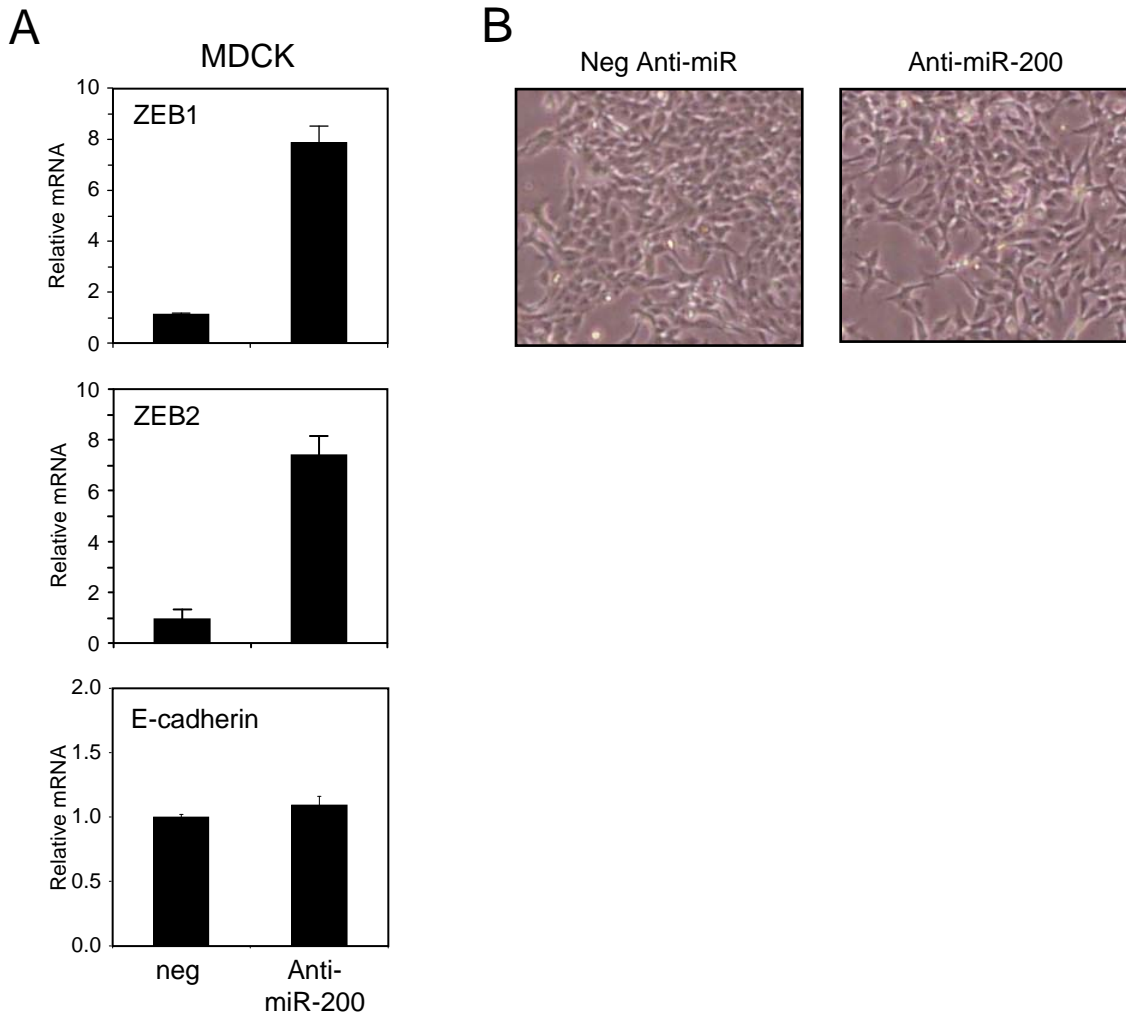
Day 5

Day 8

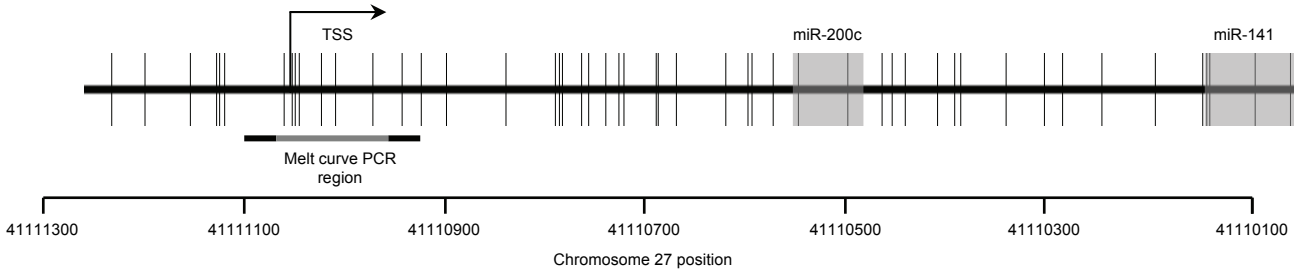
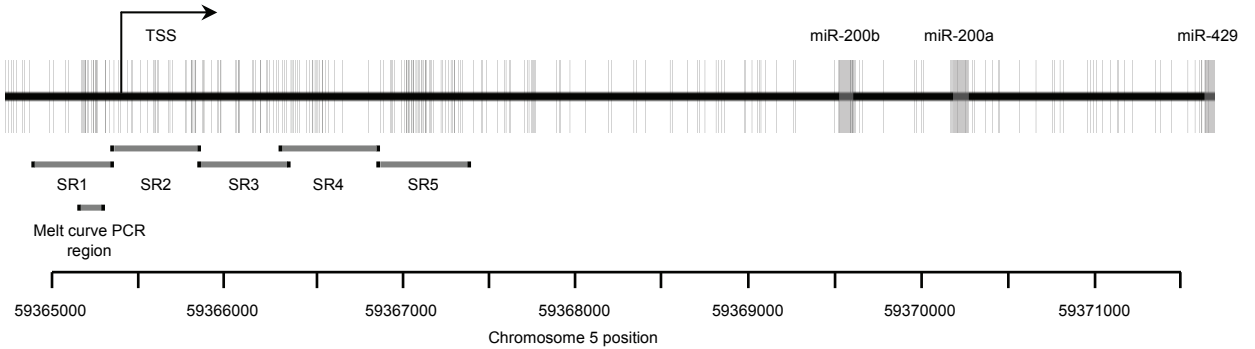
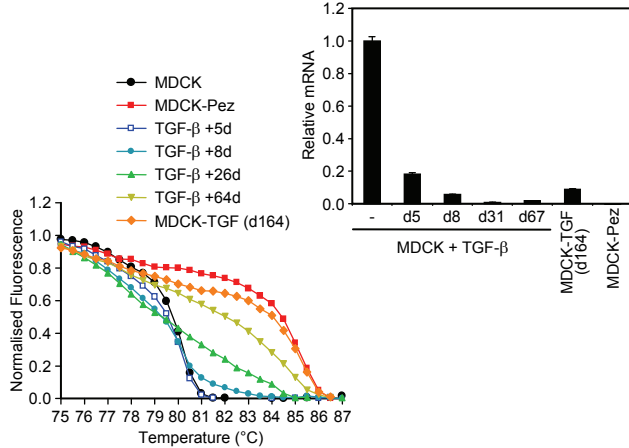
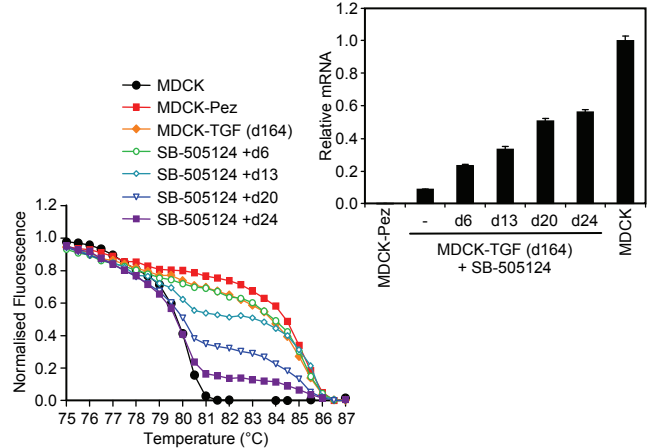
Day 12



Supplementary Figure 1. (A) Cell morphology of MDCK-TGF cells treated with the TGF- β R1 inhibitor SB-431542 (1 μ M) showing they revert to an epithelial phenotype. (B) TGF- β 2 and - β 3 (1ng/ml) induce EMT in MDCK cells with progressive changes in E-cadherin, ZEB and miR-200 expression over a 12 day timecourse. Data is representative of duplicate experiments. Each value shown is the mean \pm SD of 3 replicate measurements.



Supplementary Figure 2. (A) Measurement of EMT markers after transfection of MDCK cells with a control or LNA miR-200 family Anti-miR (10 d). Data are representative of duplicate experiments. Each value shown is the mean \pm SD of 3 replicate measurements. (B) Phase pictures of MDCK cells after miR-200 Anti-miR transfection showing changes in ZEB and TGF- β expression (see Figure 4) precede morphological changes.

A**miR-200c~141 locus (canine)****miR-200b~200a~429 locus (canine)****B****miR-200b~200a~429****C****miR-200b~200a~429**

Supplementary Figure 3. (A) Structure of the canine miR-200b~200a~429 and miR-200c~141 loci. Vertical bars show CpG dinucleotides, the locations of the putative transcription start sites (TSS) are marked with arrows, and the Pre-miR sequences are shaded. The regions analysed in bisulfite sequencing (SR1-5) and melt curve PCR are indicated. (B) PCR melt curve analysis around the miR-200b~200a~429 TSS. MDCK cells were treated with TGF- β 1 for the indicated time periods followed by melt curve analysis with MDCK and MDCK-Pez samples representing unmethylated and methylated profiles. (C) TGF- β signaling was inhibited in MDCK-TGF cells (d164) using the 1 μ M SB-505124 over a 24 d timecourse and methylation of the miR-200b~200a~429 promoter assessed by melt curve analysis.

Supplementary Table 1. mRNA & miRNA correlations in Primary Human IDC

		correlation	Significance of correlation (P value)		correlation	Significance of correlation (P value)	
ZEB1	TGF- β 1	+	<0.0001	miR-200a	TGF- β 1	0.2185	
	TGF- β 2	+	<0.0001		TGF- β 2	0.1041	
	TGF- β 3	+	<0.0001		TGF- β 3	0.6937	
	ZEB2	+	<0.0001		ZEB1	0.0911	
	miR-200a		0.0911		ZEB2	0.1225	
	miR-200b		0.2734		miR-200b	+	<0.0001
	miR-200c	-	0.0014		miR-200c	+	0.0427
	miR-141	-	0.0188		miR-141	+	0.0005
ZEB2	TGF- β 1	+	<0.0001	miR-200b	TGF- β 1	0.3046	
	TGF- β 2	+	0.0017		TGF- β 2	-	0.0158
	TGF- β 3	+	<0.0001		TGF- β 3		0.2447
	ZEB1	+	<0.0001		ZEB1		0.2734
	miR-200a		0.1225		ZEB2		0.3307
	miR-200b		0.3307		miR-200a	+	<0.0001
	miR-200c	-	0.0010		miR-200c	+	0.0050
	miR-141	-	0.0308		miR-141	+	0.0002
TGF- β 1	TGF- β 2	+	0.0024	miR-200c	TGF- β 1	-	0.0090
	TGF- β 3	+	0.0002		TGF- β 2	-	0.0047
	ZEB1	+	<0.0001		TGF- β 3		0.4537
	ZEB2	+	<0.0001		ZEB1	-	0.0014
	miR-200a		0.2185		ZEB2	-	0.0010
	miR-200b		0.3046		miR-200a	+	0.0427
	miR-200c	-	0.0090		miR-200b	+	0.0050
	miR-141		0.1150		miR-141	+	<0.0001
TGF- β 2	TGF- β 1	+	0.0024	miR-141	TGF- β 1		0.1150
	TGF- β 3		0.1477		TGF- β 2	-	0.0040
	ZEB1	+	<0.0001		TGF- β 3		0.9890
	ZEB2	+	0.0017		ZEB1	-	0.0188
	miR-200a		0.1041		ZEB2	-	0.0308
	miR-200b	-	0.0158		miR-200a	+	0.0005
	miR-200c	-	0.0047		miR-200b	+	0.0002
	miR-141	-	0.0040		miR-200c	+	<0.0001
TGF- β 3	TGF- β 1	+	0.0002				
	TGF- β 2		0.1477				
	ZEB1	+	<0.0001				
	ZEB2	+	<0.0001				
	miR-200a		0.6937				
	miR-200b		0.2447				
	miR-200c		0.4537				
miR-141		0.9890					

Correlations between miR-200, ZEB and TGF- β in 27 invasive ductal breast cancer samples. P-values are calculated using Pearson correlation coefficients (significant correlations are highlighted).

Supplemental Table 2 - Primers used for miR-200 loci methylation analysis

Promoter	Region	Primer Sequence	Primer Position ^a	Number of CpGs Analysed ^b
Canine miR-200c~141	MCR	F 5'-GGTAGTTTATGGTAGGAGGATA-3'	chr27:41111055-41111076	7
		R 5'-GAAAAAAAAAAAAAAAAAATCRAAACCCAAAAT-3'	chr27:41110910-41110940	
Canine miR-200b~200a~429	MCR	F 5'-AGTTTTTTTTTTTGGTTTTTGTTTAAGTTG-3'	chr5:59365176-59365205	15
		R 5'-TATATCCCCTAAACTCCATAAACCAA-3'	chr5:59365317-59365343	
	SR1	F 5'-GGTATATAGGGGAGGTAGATT-3'	chr5:59364919-59364939	22
		R 5'-CATACAAATACCTACTCACTAC-3'	chr5:59365375-59365396	
	SR2	F 5'-TYGGTAGTGAGTAGGTATTTG-3'	chr5:59365372-59365392	24
		R 5'-TACCTACCCCAACAAATCTTAC-3'	chr5:59365886-59365907	
	SR3	F 5'-GATTTGTTGGGGTAGGTAAGT-3'	chr5:59365890-59365910	27
		R 5'-AAAAACCTAAATCCAAATCAAACC-3'	chr5:59366398-59366421	
	SR4	F 5'-GGAGGGAAGGGTTTGGTTT-3'	chr5:59366352-59366370	24
		R 5'-ATCCCTATCTTACAACCTATTTTACA-3'	chr5:59366915-59366939	
SR5	F 5'-TGTAATAAGTTGTAAGATAGGGAT-3'	chr5:59366915-59366939	42	
	R 5'-CAAAAATAAATCAAAAACCCAAAAC-3'	chr5:59367444-59367470		
Human miR-200c~141	MCR	F 5'-GGTAGTTTATGGTAGGAGGATA-3'	chr12:7072308-7072329	8
		R 5'-AAACRAAAAACTTTAAAACCCCAA-3'	chr12:7072445-7072470	
Human miR-200b~200a~429	MCR	F 5'-TGGGGYGGGGAGTATTGT-3'	chr1:1098207-1098224	12
		R 5'-TAACACAAAAAATCAATTCAAACCTA-3'	chr1:1098348-1098373	

Y – C/T. R – A/G. ^aPrimer position, as determined by UCSC Genome Browser Database (GBD, <http://genome.ucsc.edu>) Human February 2009 (hg19) or Canine May 2005 (canFam2) assembly. ^bNumber of CpGs in the PCR product between the primer binding sites. SR1-5 designates Sequencing Regions. MCR designates regions analysed by melt curve PCR.