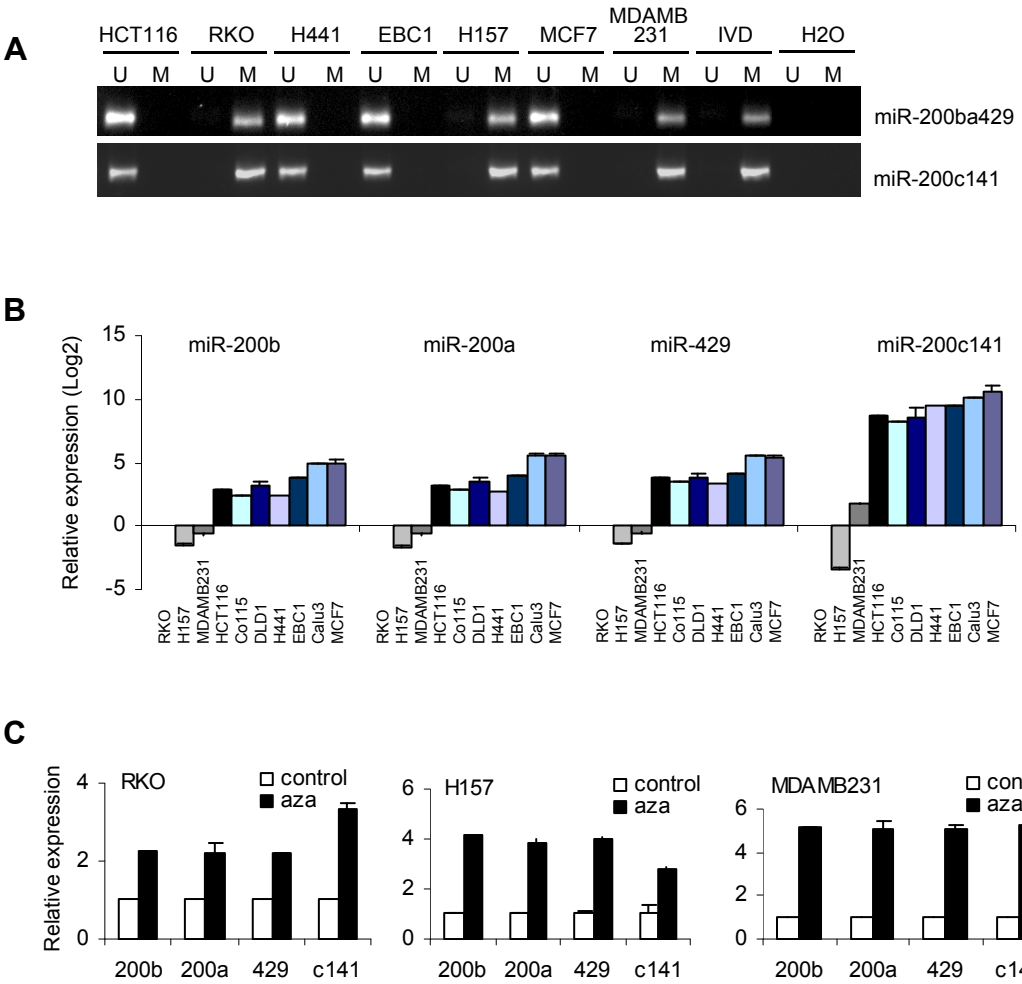
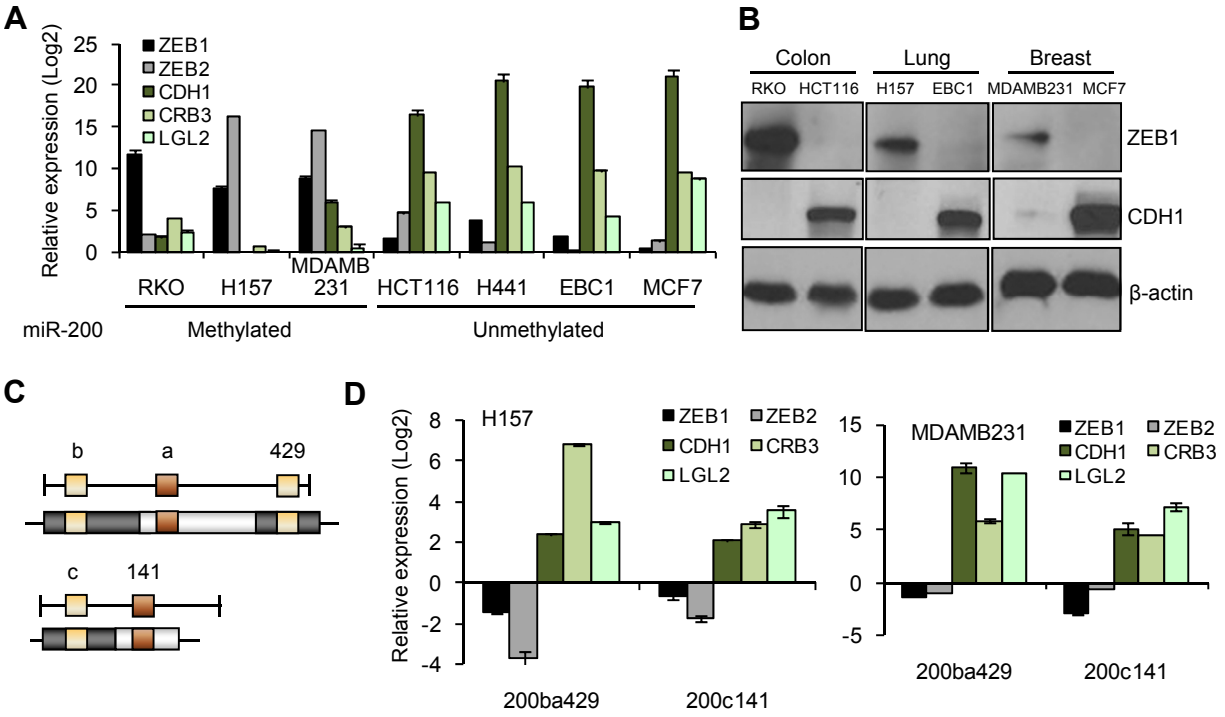
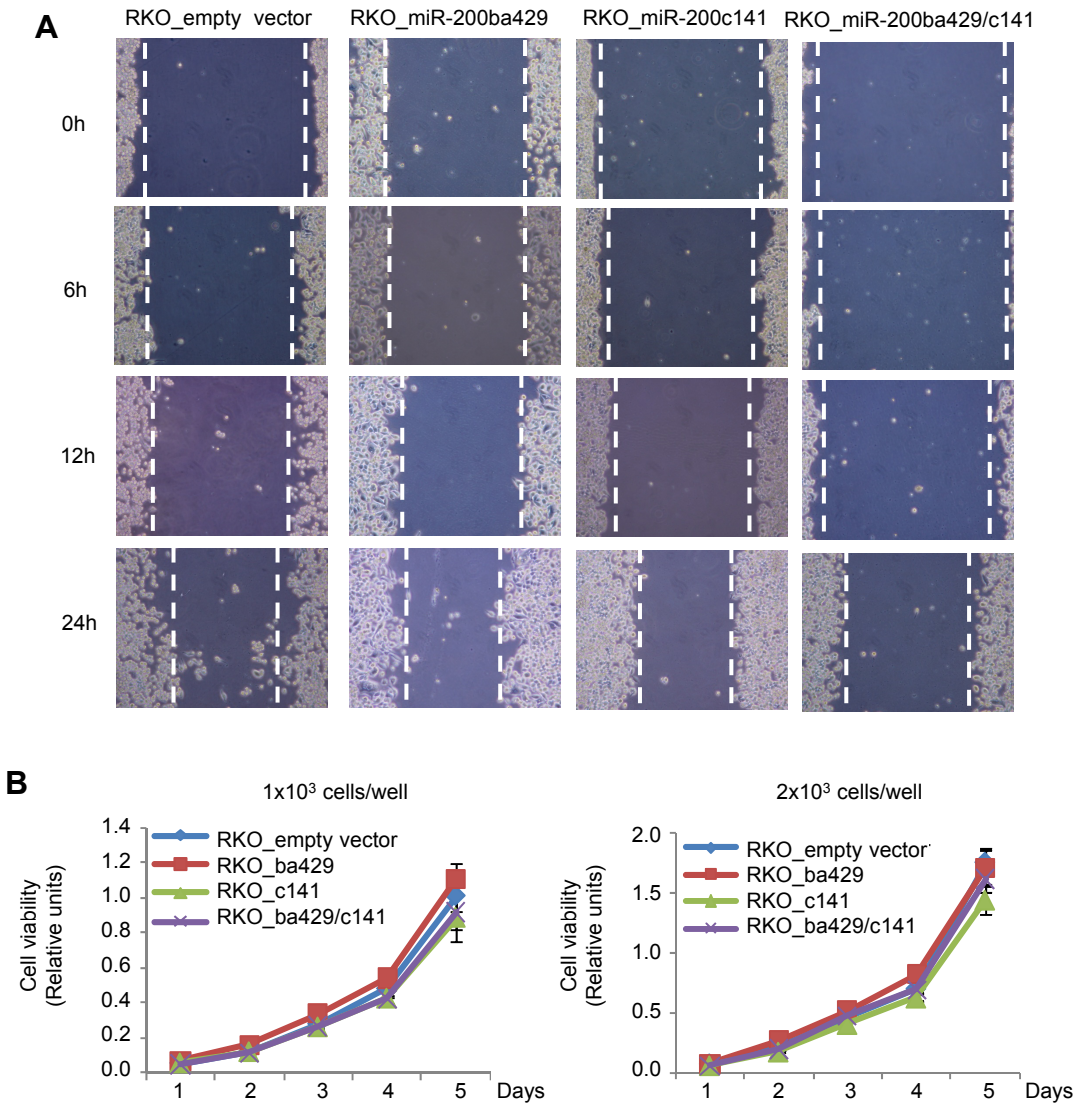


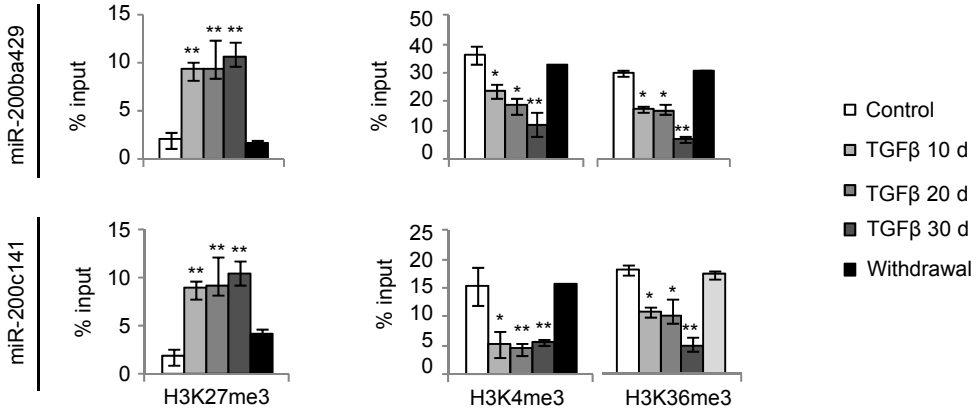
Supplementary Figure 1.
(A) Methylation-specific PCR (MSP) analyses of miR-200 CpG islands in cancer cell lines. The presence of a band under the U lane indicates unmethylated alleles, whilst the presence of a band under the M lane indicates methylated alleles. IVD: *in vitro* methylated DNA. **(B)** Expression of primary miR-200 in human cancer cell lines, evaluated by qRT-PCR. **(C)** Restored primary miR-200 expression upon treatment with DNA demethylating agent 5-aza-2'-deoxycytidine (aza) in miR-200 CpG island methylated cell lines. Data represent the relative quantification by qRT-PCR, using untreated controls as reference.



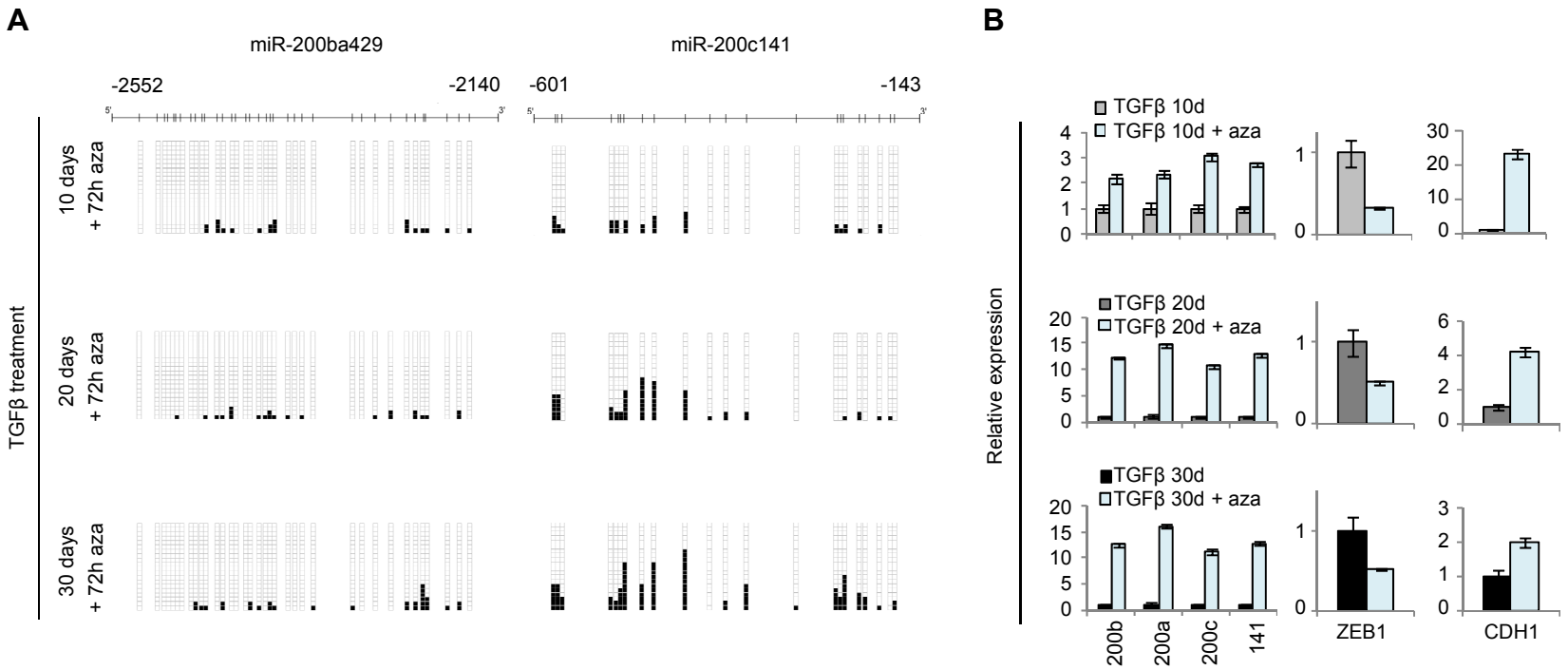
Supplementary Figure 2.
Epigenetic silencing of the miR-200 family upregulates ZEB1/ZEB2 and downregulates their target genes. (**A**) miR-200 methylated cancer cell lines have higher levels of the miR-200 targets ZEB1 and ZEB2, and lower levels of E-cadherin and cell polarity genes CRB3 and LGL2 inhibited by these transcriptional repressors, in comparison with the miR-200 unmethylated cell lines. Data represent relative quantification of mRNA expression evaluated by qRT-PCR (means ± SD). (**B**) High expression of ZEB1 and opposite minimal levels of E-cadherin in miR-200 methylated cancer cell lines were confirmed by western blot. β-actin was used as loading control. (**C**) Schematic depiction of genomic regions surrounding the pri-miRNA sequences of pri-miR-200ba429 and pri-miR-200c141 that were directionally cloned in pSilencer 4.1-CMV puro. (**D**) Stable transfection of pri-miR-200ba429 and pri-miR-200c141 in the CpG island hypermethylated cancer cell lines H157 and MDAMB231 caused a downregulation of their target genes ZEB1 and ZEB2 and an increase in the expression of the downstream cell adherence and polarity genes E-cadherin, CRB3 and LGL2. Data derived from qRT-PCR (means ± SD), using empty vector-transfected cells as reference.



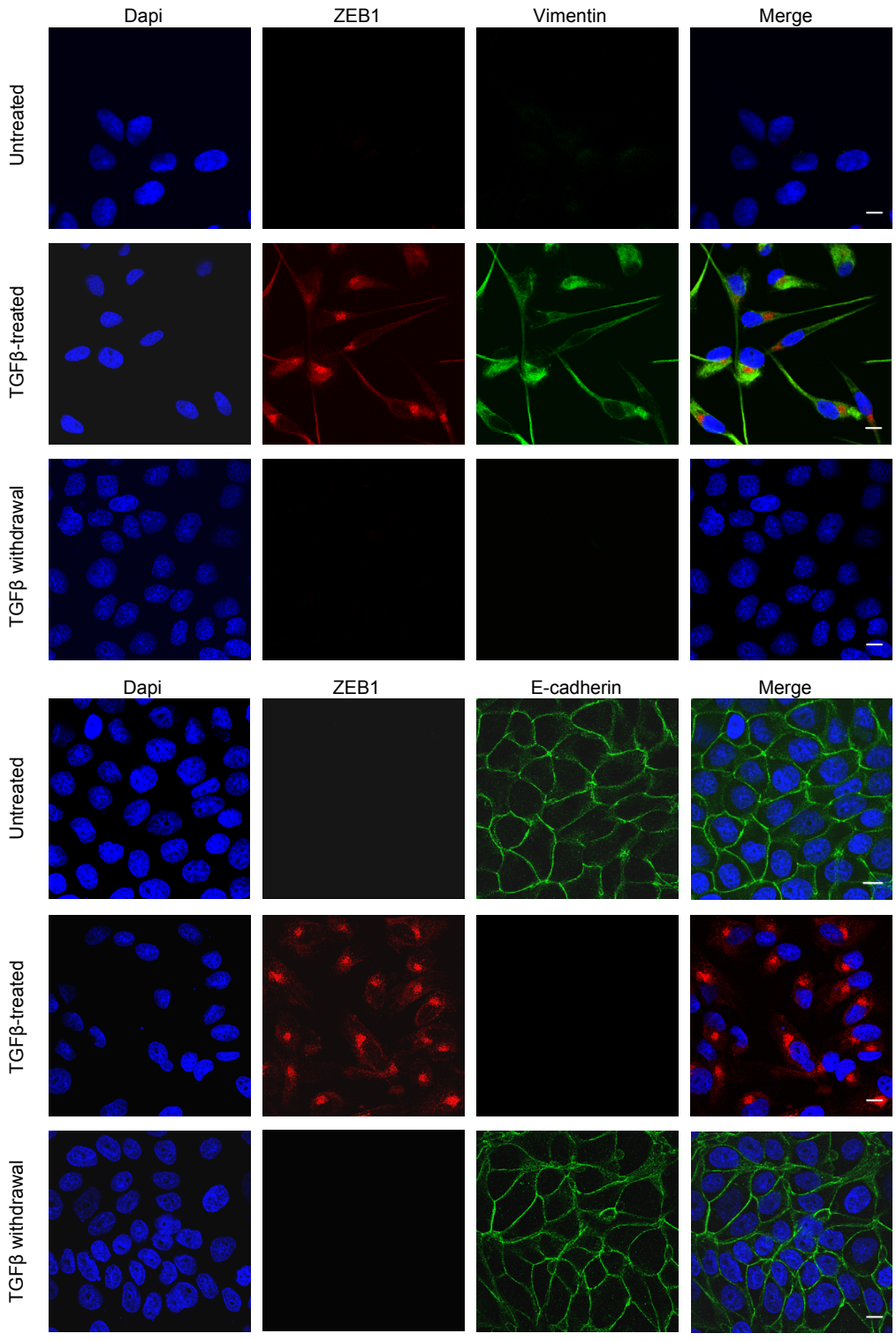
Supplementary Figure 3.
(**A**) Short-time wound healing assay in RKO cells stably transfected with pri-miR-200ba429, pri-miR-200c141 or both clusters (pri-miR-200ba429 and -200c141). Photographs were taken at 0, 6, 12 and 24 hours. (**B**) Cell proliferation and viability were analyzed daily by MTT assay during 5 days.



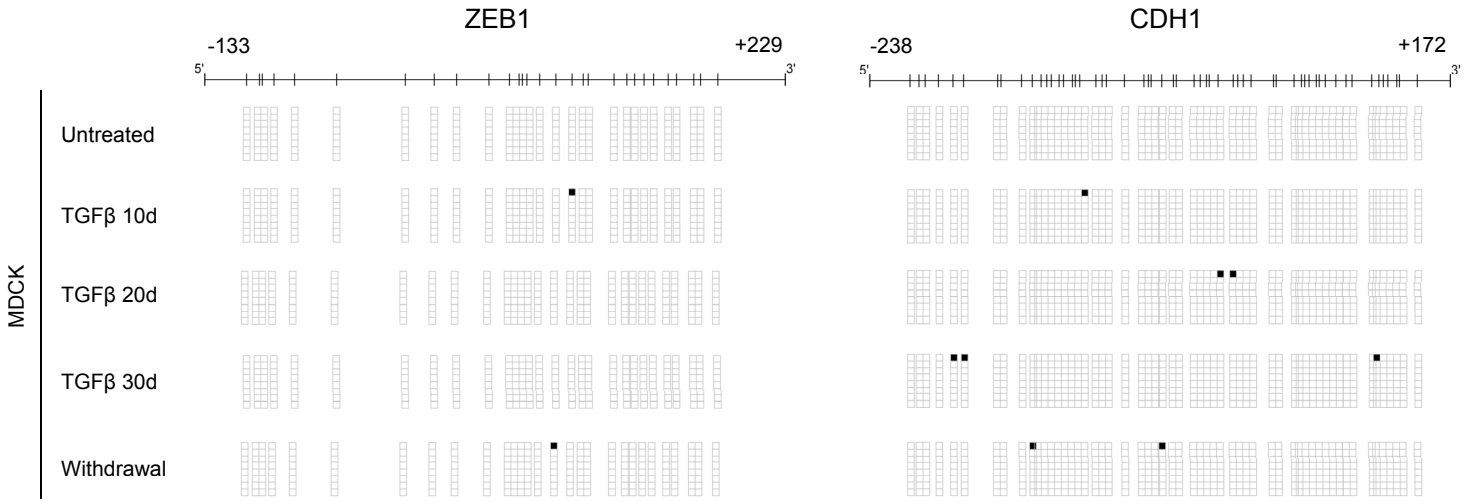
Supplementary Figure 4.
Dynamic epigenetic regulation of miR-200 evidenced by changes in histone modifications in miR-200 promoter regions during TGFβ treatment in MDCK cells. Gain of histone modification associated to transcriptional inactivation (H3K27me3) and decrease in active marks (H3K4me3 and H3K36me3) were detected throughout TGFβ-induced miR-200 silencing using chromatin immunoprecipitation (ChIP) assay. Re-establishment of original status was achieved after TGFβ withdrawal. (* $p<0.05$, ** $p<0.01$ Student`s t test, relative to control untreated cells).



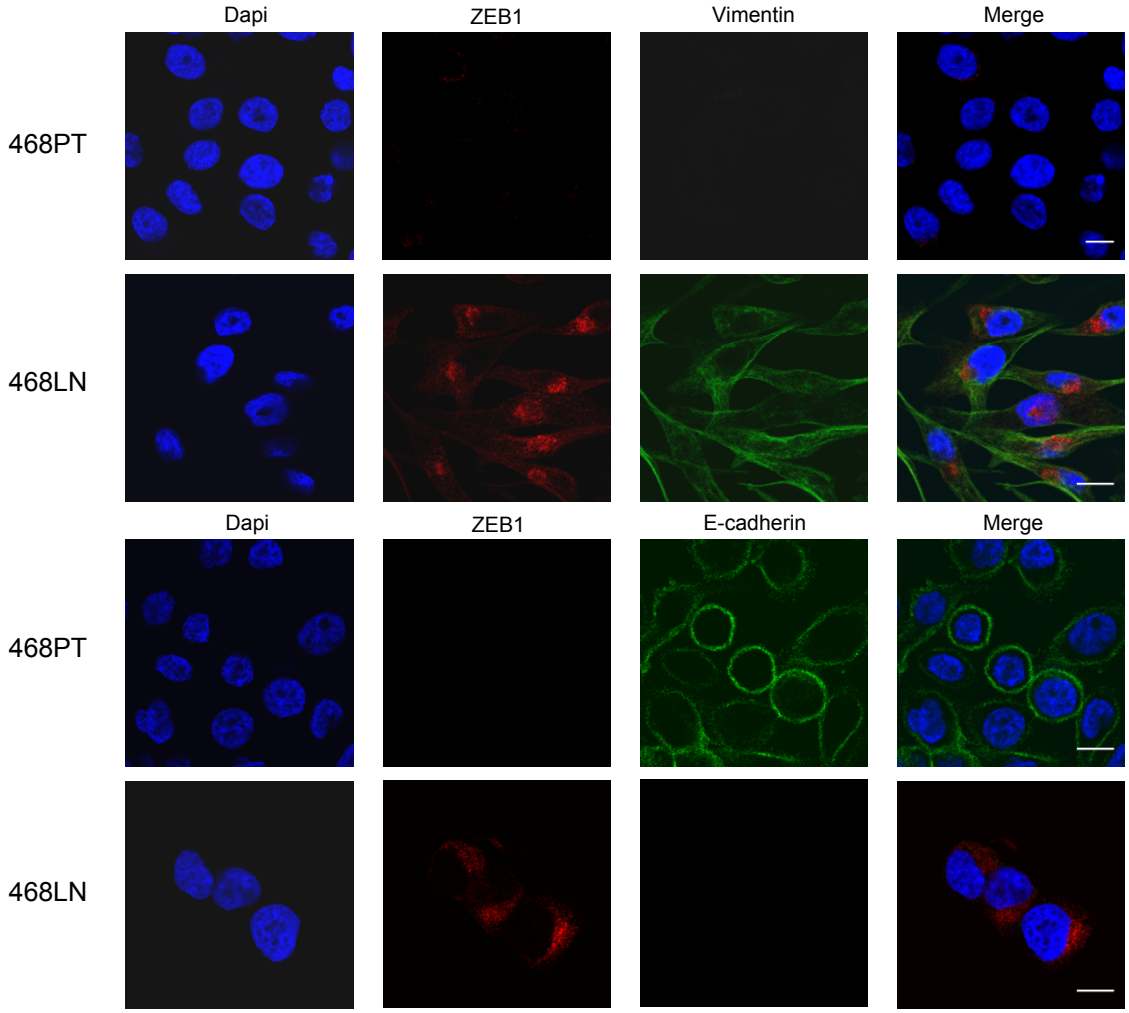
Supplementary Figure 5.
Treatment with DNA demethylating agent 5-aza-2'-deoxycytidine (aza) during TGFβ-induced EMT in MDCK cells. **(A)** Loss of CpG methylation in both clusters of miR-200 family is achieved upon aza treatment in cells undergoing TGFβ-induced EMT, revealed by bisulfite genomic sequencing. Twenty single clones were evaluated in each experimental condition. Presence of unmethylated or methylated CpGs is indicated by white or black squares, respectively. **(B)** Restored expression of miR-200 family members in TGFβ-treated cells upon DNA demethylating treatment, detected by qRT-PCR (Student's t test $p<0.05$). Loss of miR-200 CpG methylation by aza treatment was linked to decreased ZEB1 expression and increased E-cadherin. Data represent relative quantification of miRNAs, using 10, 20 or 30 days TGFβ-treated cells as reference.



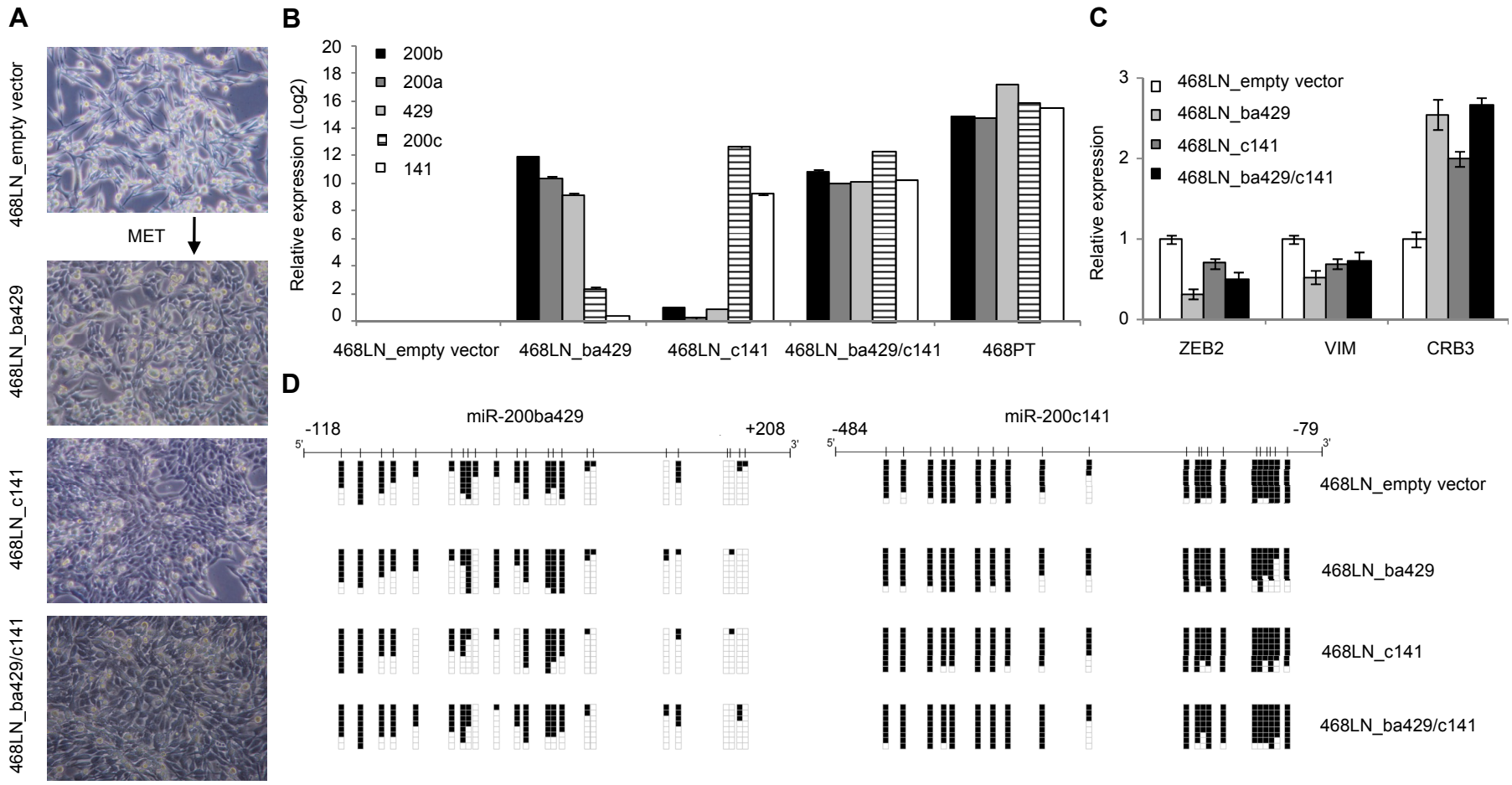
Supplementary Figure 6.
In vitro induced EMT and MET in epithelial MDCK cells. CpG island hypermethylation-associated silencing of the miR-200 family in the TGFβ-treated MDCK cells was linked to upregulation of miR-200 target ZEB1, gain of the mesenchymal marker Vimentin (upper panel) and downregulation of E-cadherin (lower panel). Remarkably, re-establishment of miR-200 CpG island unmethylated status after TGFβ withdrawal was associated with restoration of epithelial features resembling those observed in untreated MDCK cells, such as downregulation of ZEB1 and Vimentin (upper panel) and upregulation of E-cadherin (lower panel). DAPI staining (blue) was used to detect nuclei in indirect immunofluorescence experiments. Scale bars represent 10 μm.



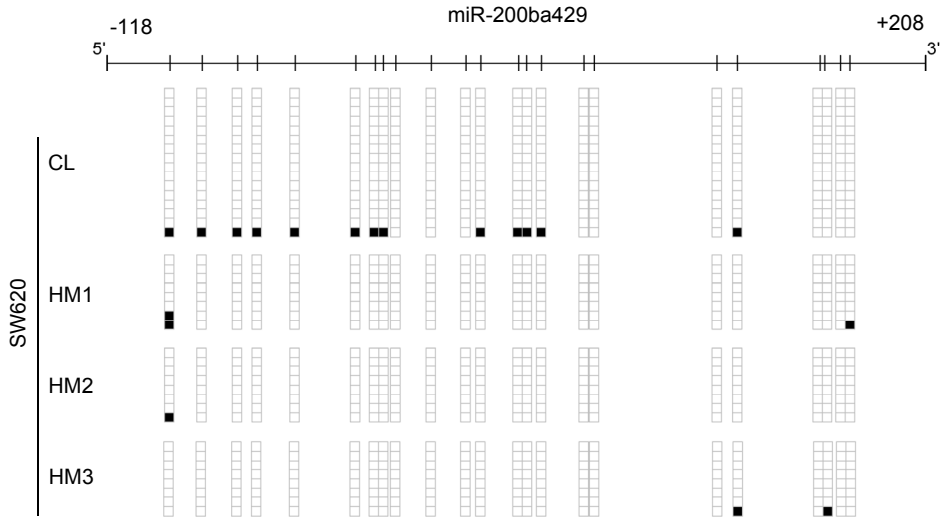
Supplementary Figure 7.
Bisulfite genomic sequencing analysis of ZEB1 and CDH1 CpG islands in TGFβ-induced EMT model in MDCK cells. Eight single clones are represented for sample. Presence of unmethylated or methylated CpGs is indicated by white or black squares, respectively.



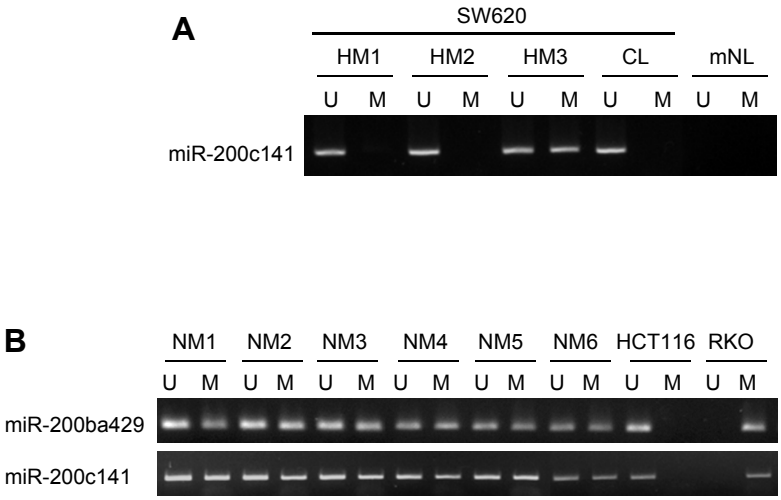
Supplementary Figure 8.
CpG island hypermethylation-associated silencing of the miR-200 family in the metastatic 468LN cell line was linked to re-expression of miR-200 target ZEB1, gain of the mesenchymal marker Vimentin and downregulation of E-cadherin, in contrast to the epithelial features in the parental 468PT cell line. DAPI staining (blue) was used to detect nuclei in indirect immunofluorescence experiments. Scale bars represent 10 µm.



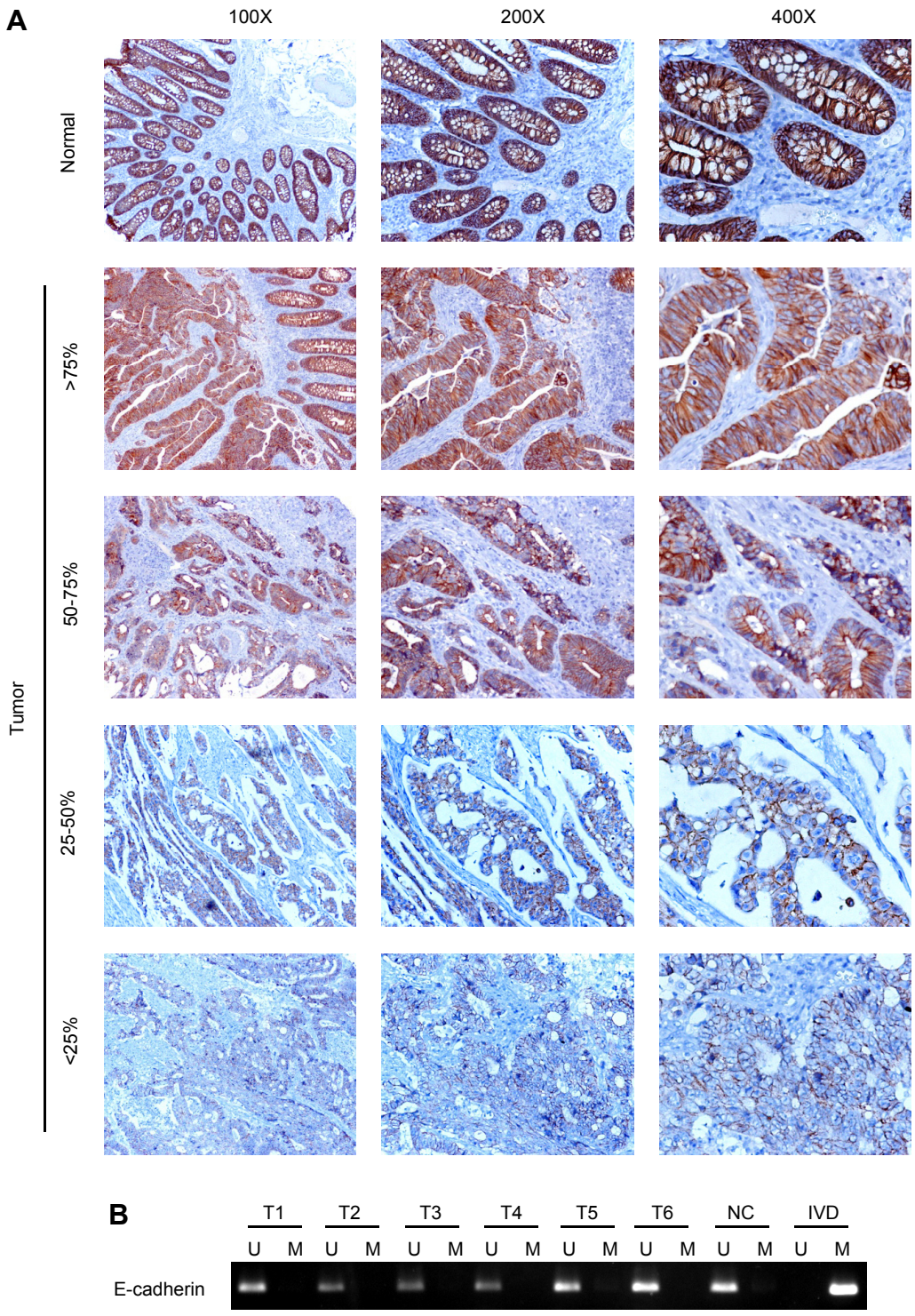
Supplementary Figure 9.
(A) Mesenchymal to epithelial transition (MET) in 468LN cell line upon stable transfection of each or both miR-200 clusters. **(B)** miR-200 stable expression in transfected 468LN cells was validated by qRT-PCR, using mature miRNA TaqMan assays. **(C)** Forced expression of miR-200 in 468LN decreases ZEB2 and Vimentin expression, and increases CRB3 levels. **(D)** Original CpG methylated status of miR-200 promoter regions are conserved in miR-200 stable transfected 468LN cells.



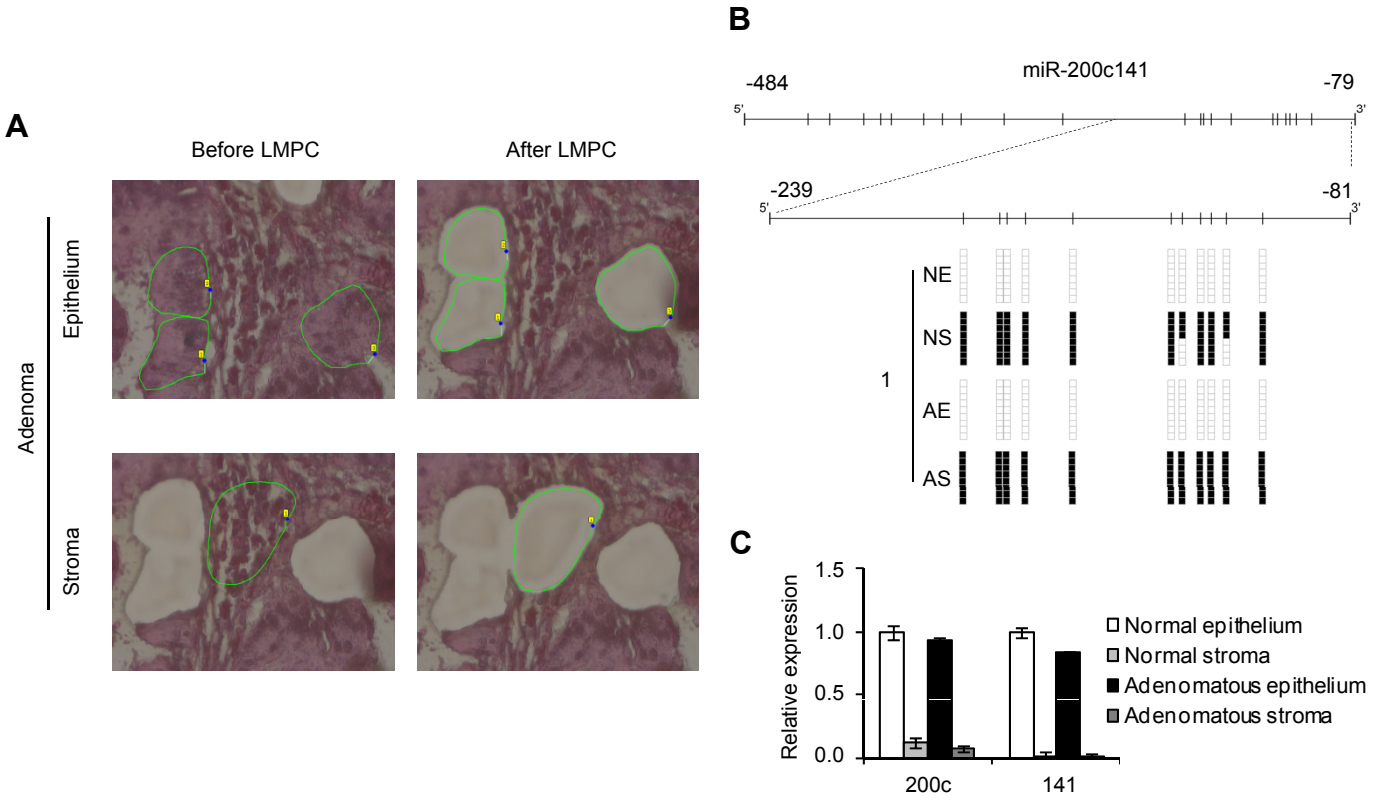
Supplementary Figure 10.
Lack of miR-200ba429 5'-CpG island methylation in hepatic metastases (HM) derived from intrasplenic injection of SW620 cells (CL) in nude mice. Presence of unmethylated or methylated CpGs is indicated by white or black squares, respectively.



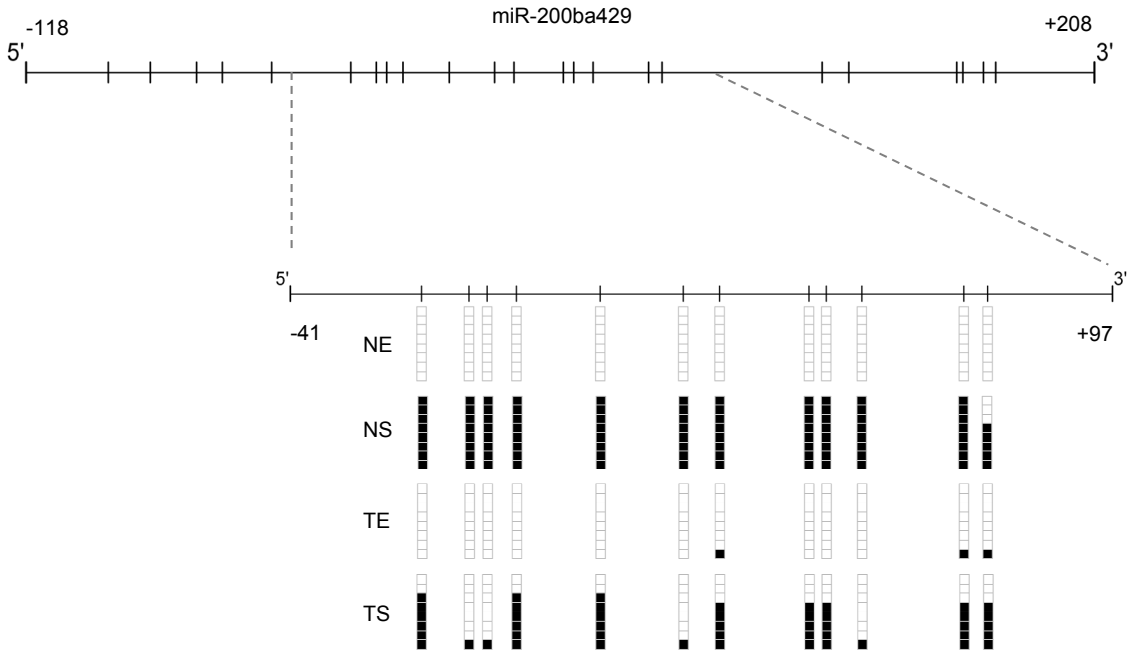
Supplementary Figure 11.
CpG methylation status of miR-200 clusters studied by methylation-specific PCR (MSP). The presence of a band under the U lane indicates unmethylated alleles, whilst the presence of a band under the M lane indicates methylated alleles. **(A)** miR-200c141 CpG island methylation analysis of the hepatic metastases derived from SW620 shows hypermethylation of the cluster in one of them (HM3), in contrast to the unmethylated status of the original SW620 cell line (CL). mNL: mouse normal liver. **(B)** miR-200ba429 and miR-200c141 5'-CpG island methylation analyses by MSP in primary total normal colon mucosa samples demonstrated a mixture of unmethylated and methylated alleles. HCT166 and RKO human colorectal cancer cells were used as unmethylated and methylated controls, respectively.



Supplementary Figure 12.
(A) E-cadherin expression in samples obtained from normal colonic mucosa and colorectal tumors, analyzed by immunohistochemistry (IHC). Expression level in tumor samples was evaluated considering the percentage of tumor cells expressing E-cadherin (<25%, 25-50%, 50-75% or >75%). **(B)** Methylation-specific PCR (MSP) analyses of E-cadherin in human colorectal tumors (T). The presence of a band under the U lane indicates unmethylated alleles, whilst the presence of a band under the M lane indicates methylated alleles. Normal colon (NC) and *in vitro* methylated DNA (IVD) were used as positive control of unmethylation and methylation, respectively.



Supplementary Figure 13.
CpG methylation status of miR-200c141 promoter region in human colorectal adenomas. **(A)** Isolation of epithelial and stromal cells from adenomas using laser microbeam microdissection and pressure catapulting (LMPC). **(B)** Lack of miR-200c141 CpG island hypermethylation in adenomatous epithelium (AE) and corresponding normal epithelial crypts (NE), evaluated by bisulfite genomic sequencing. Both normal stroma (NS) and adenomatous stroma (AS) samples shown dense methylation. **(C)** High expression of mature miR-200c and miR-141 in normal and adenomatous epithelium. Both normal stroma (NS) and adenomatous stroma (AS) samples shown low levels of miR-200c141. Data represent mean ± SD, obtained by qRT-PCR.



Supplementary Figure 14.
Illustrative status of miR-200ba429 5'-CpG island in the four different tissue components observed in the colorectal cancer patients: the normal epithelium (NE, the crypts) and stroma (NS) neighboring the colorectal tumor and, within the malignant pathological mass, the tumor epithelium (TE) and tumor stroma (TS). The samples were obtained by laser microbeam microdissection and pressure catapulting (LMPC). Presence of unmethylated or methylated CpGs is indicated by white or black squares, respectively.