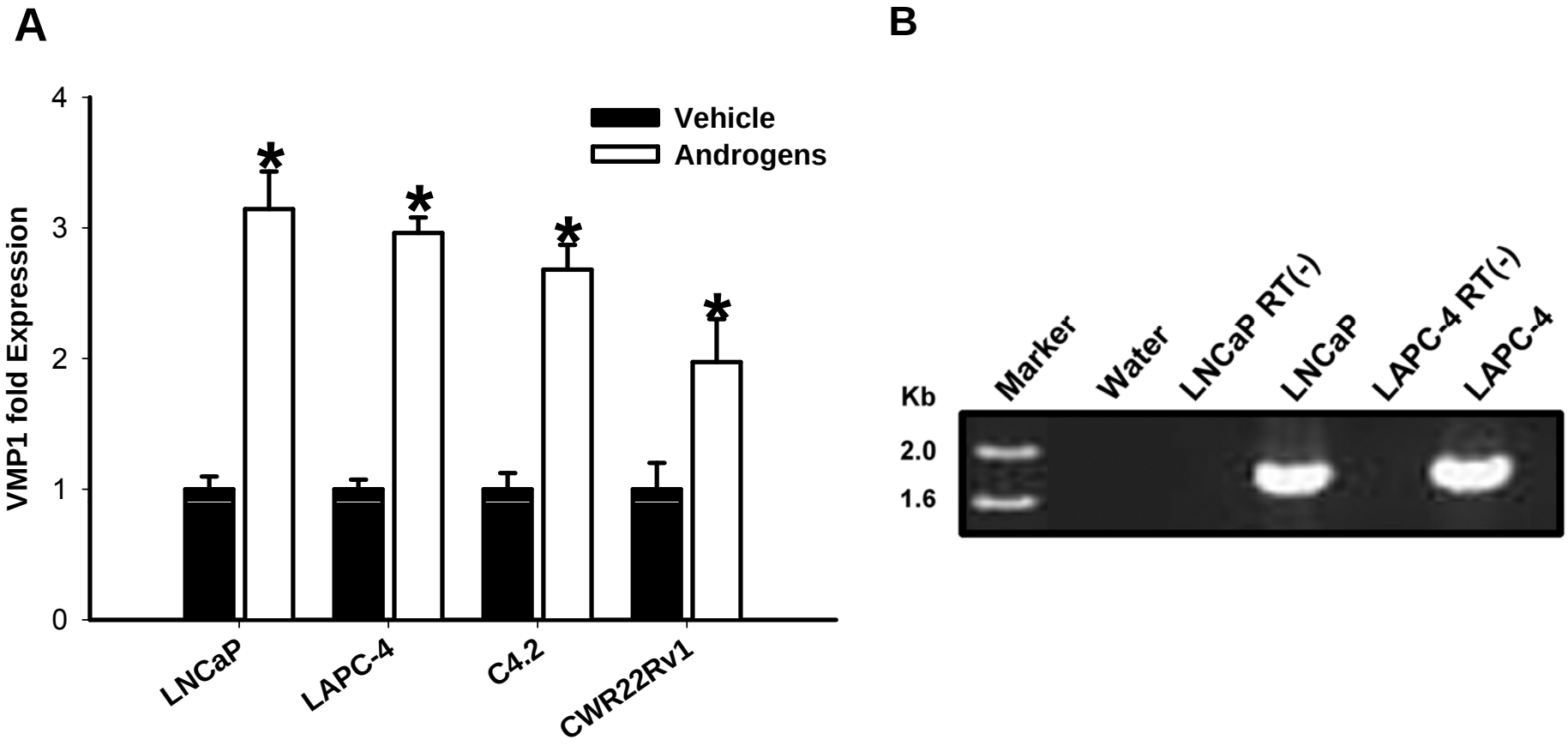
SUPPLEMENTARY MATERIALS AND METHODS

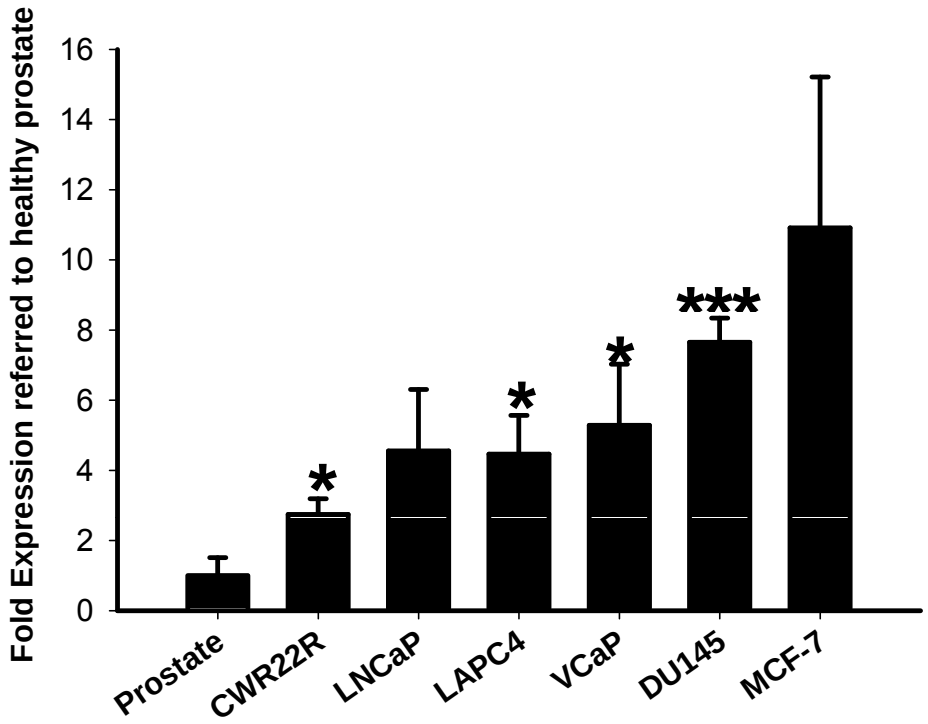
Cell lines and chemicals. Prostate cell lines LNCaP and LAPC-4 were kindly provided by Johns Isaacs (Johns Hopkins University, Baltimore, Maryland). LNCaP, C4-2 and CWR22Rv1 were grown in RPMI 1640 (Cellgro). LAPC-4 cell line was cultivated in Iscove´s medium (Invitrogen) plus 1 nmol/L of the synthetic AR agonist R1881 (Perkin-Elmer). All media contained ciprofloxacin hydrochloride (U.S biological) and 10% fetal bovine serum. For androgen stimulation, all cells were maintained in the appropriate media depleted from phenol red and supplemented with 10% charcoal-stripped serum (Hyclone) for 24 h prior to androgen (R1881) treatment. Ethanol was the vehicle utilized to dissolve R1881 and therefore was the control in our treatments. Healthy prostate RNA was kindly provided by Charles Ewing and William Isaacs (Johns Hopkins University, Baltimore, Maryland) and consisted of pooled prostate RNA from 30 healthy young men purchased from Sigma-Aldrich.

VMP1 Stability Studies. MCF-7 and LAPC4 cells were seeded in 6-well plates at 60% confluency. 24 hours later, cells were transfected with either 50 nM Neg siRNA or Drosha siRNA with HiPerFect Transfection Reagent (Qiagen) following the manufacturer's recommended protocol. Cells were then treated with PBS or Actynomicin D at a concentration of 100ng/ml. After 48 hours of treatment, RNA was harvested for analysis via qRT-PCR as described in the methods section of the manuscript.

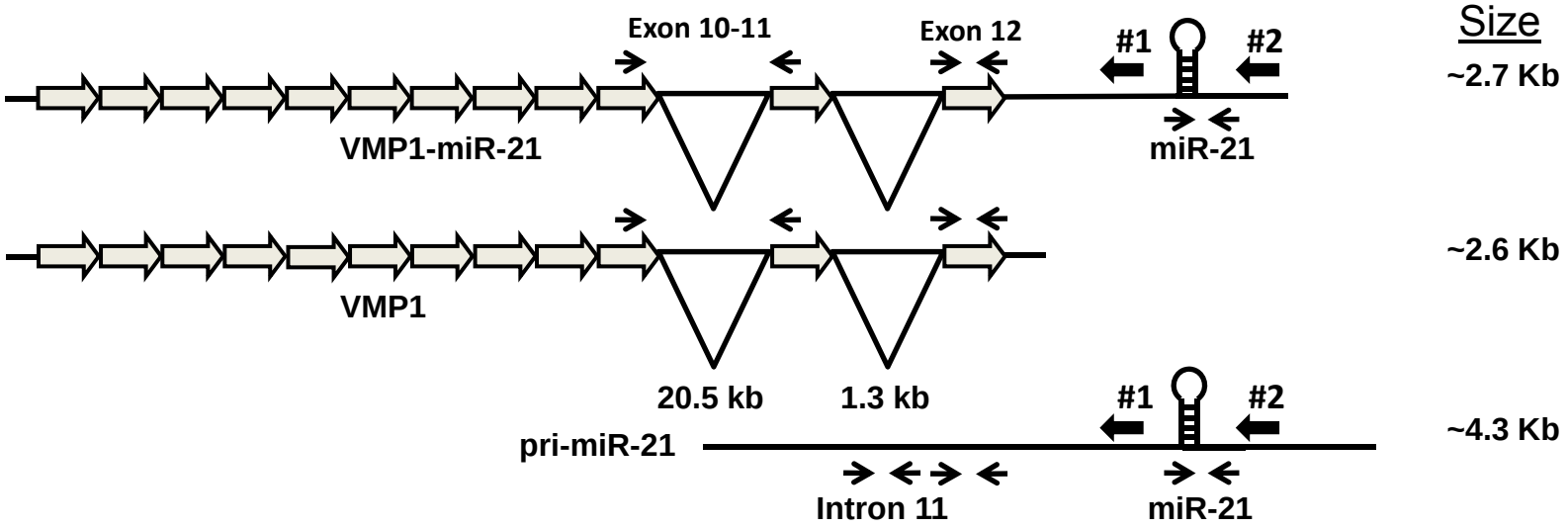
Bisulfite Sequencing. 2 µgs of HEK293 and HCT116 were bisulfited converted using the EZ DNA Methylation Gold Kit (Zymo) as per the manufacturer's instructions with elution in 40 uL at final spin elution step. Two uL of converted DNA were amplified by PCR in a reaction containing 3U Platinum Taq (Invitrogen), 1.5 mM $MgCl_2$, 1 mM dNTPs, 10 µg BSA, 400 nM each forward and reverse primer, and 5% DMSO v/v. Cycling conditions were as follows: 95ºC for 3 min; 35 cycles of 95ºC for 30 seconds, 53ºC (for mir21) or 50ºC (for VMP Downstream) for 30 seconds, 72ºC for 1 minute; followed by an extension step of 72ºC for 5 minutes; then a 10ºC hold. Products were purified using the DNA Clean & Concentrator-5 kit (Zymo) and cloned with TOPO TA cloning kit (Invitrogen). Colonies were picked into a 96 well plate for glycerol stocks, then shipped to Beckman Coulter Genomics for Sanger sequencing.



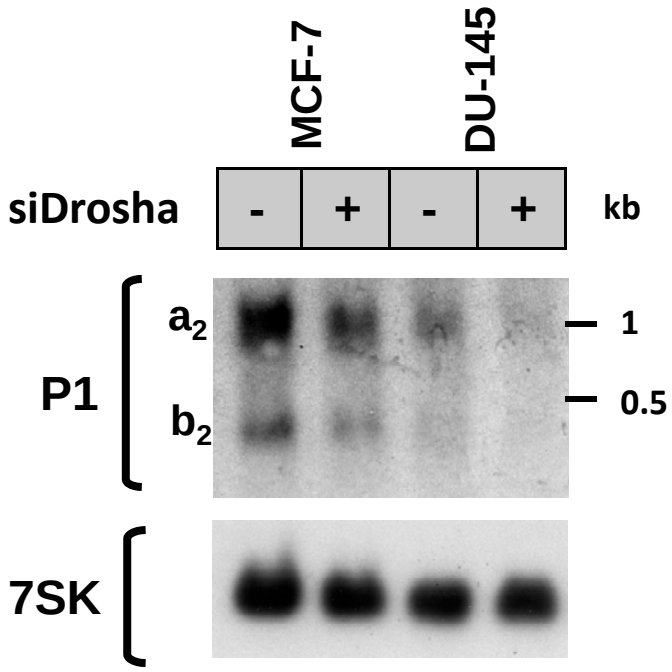
Supplementary Figure S1. VMP1 and VMP1-miR-21 expression in androgen-sensitive PCa cell lines .
A, Quantitative RT-PCR of VMP1 mRNA after 72h of 10nM R1881(androgens) treatment in four androgen receptor positive prostate cancer cell lines. “VMP1 fold Expression” indicates the induction relative to cells treated with the vehicle after referring the values to actin. Data are mean (columns) ± SE (bars) of tripiclates from two independent experiments. * $P < 0.05$ (Student´s test). **B**, Detection of a VMP1-miR-21 fusion transcript by RT-PCR spanning exon 9 of VMP1 through the miR-21 hairpin (see Supplementary Table SI - PRIMERS FOR NESTED PCR OF VMP1 EXON 9/MIR-21). RNA from Drosha depleted cells was reverse transcribed followed by a nested PCR. Lane 1 is a DNA marker. Lane 2 is the blank for the PCR reaction. Lanes 3 and 5 are identical to lanes 4 and 6 but without addition of reverse transcriptase.



Supplementary Figure S2. VMP1 expression is elevated in prostate cancer cell lines and MCF-7 compared to normal prostate. "Fold Expression referred to healthy prostate" indicates the expression of VMP1 mRNA as quantified by real-time RT-PCR and normalized to actin and referred to healthy prostate. Data are mean (columns) ± SE (bars) from three independent wells. * $P < 0.05$; *** $P < 0.001$ (Student´s test).

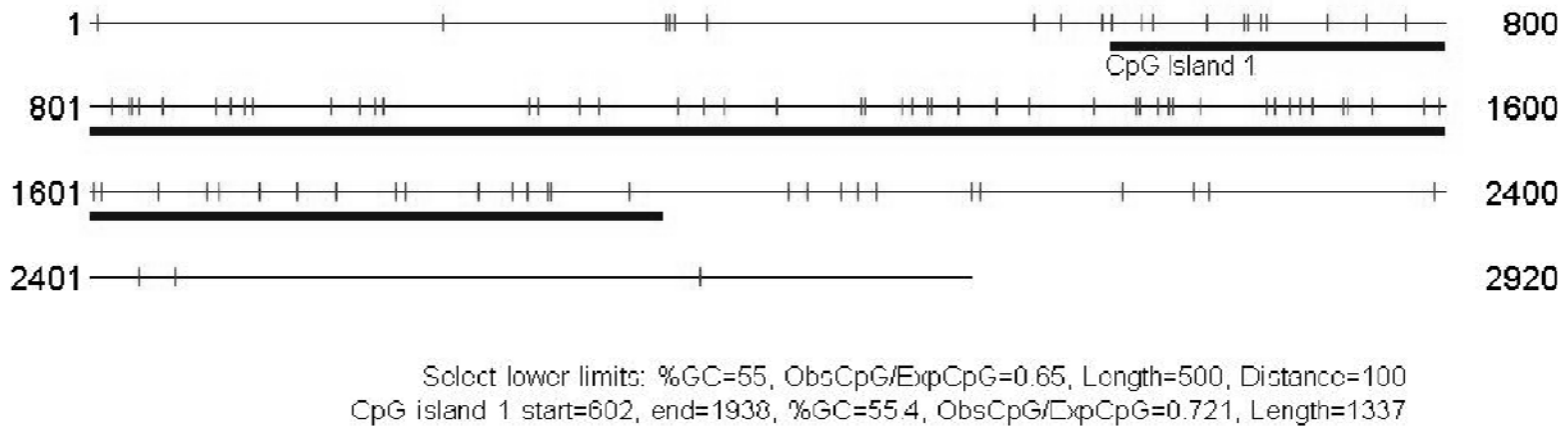


Supplementary Figure S3. Schematic of VMP1 and miR-21 transcripts with mapped amplicons and primer binding sites. VMP1, VMP1-miR-21 and pri-miR-21 with their observed size are schematized. Coding exons are depicted as grey arrows whereas introns and non coding sequences are represented as black lines. Spliced introns are inverted triangles and their size is provided below. RT-PCR primers (facing arrows), reverse transcription primer (single arrow, #2) and the biotinylated oligonucleotide for RNA Pull-down of transcripts containing miR-21 (single arrow, #1) are annotated.

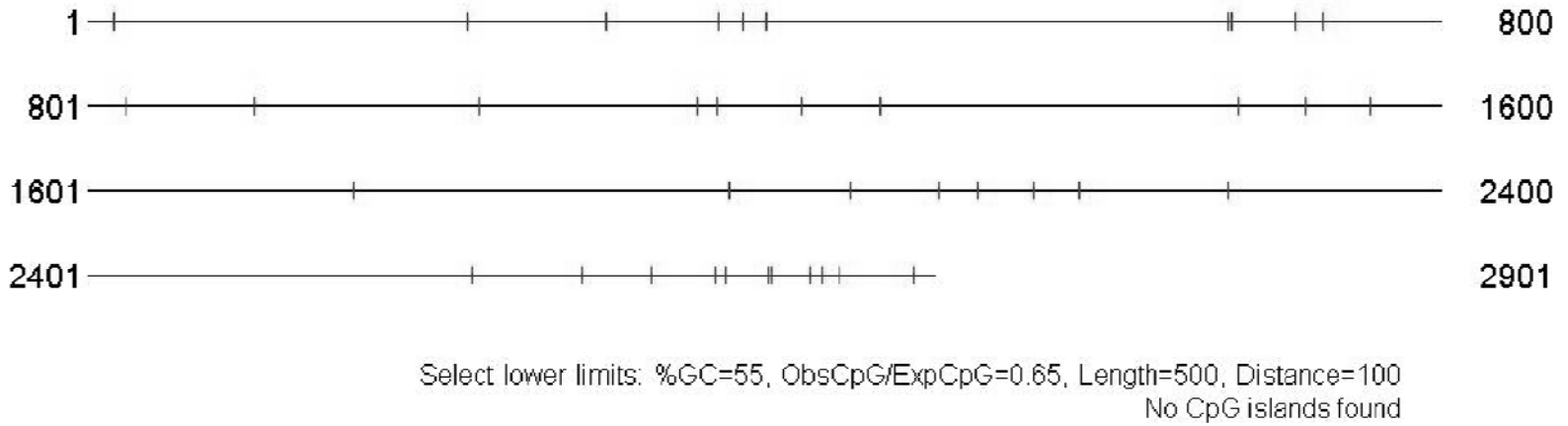


Supplementary Figure S4. Drosha proscessing. MCF-7 and DU-145 cells were transfected for 48 h with Drosha siRNA (siDrosha, +) or a control siRNA (siDrosha, -) and evaluated by Northern blot for Microprocessor cleavage products with Probe 1 (P1). Inhibition of Drosha reduced levels of 1kb and 0.3 kb bands (Band a_2 and b_2, respectively, as mapped in Figure 2A).

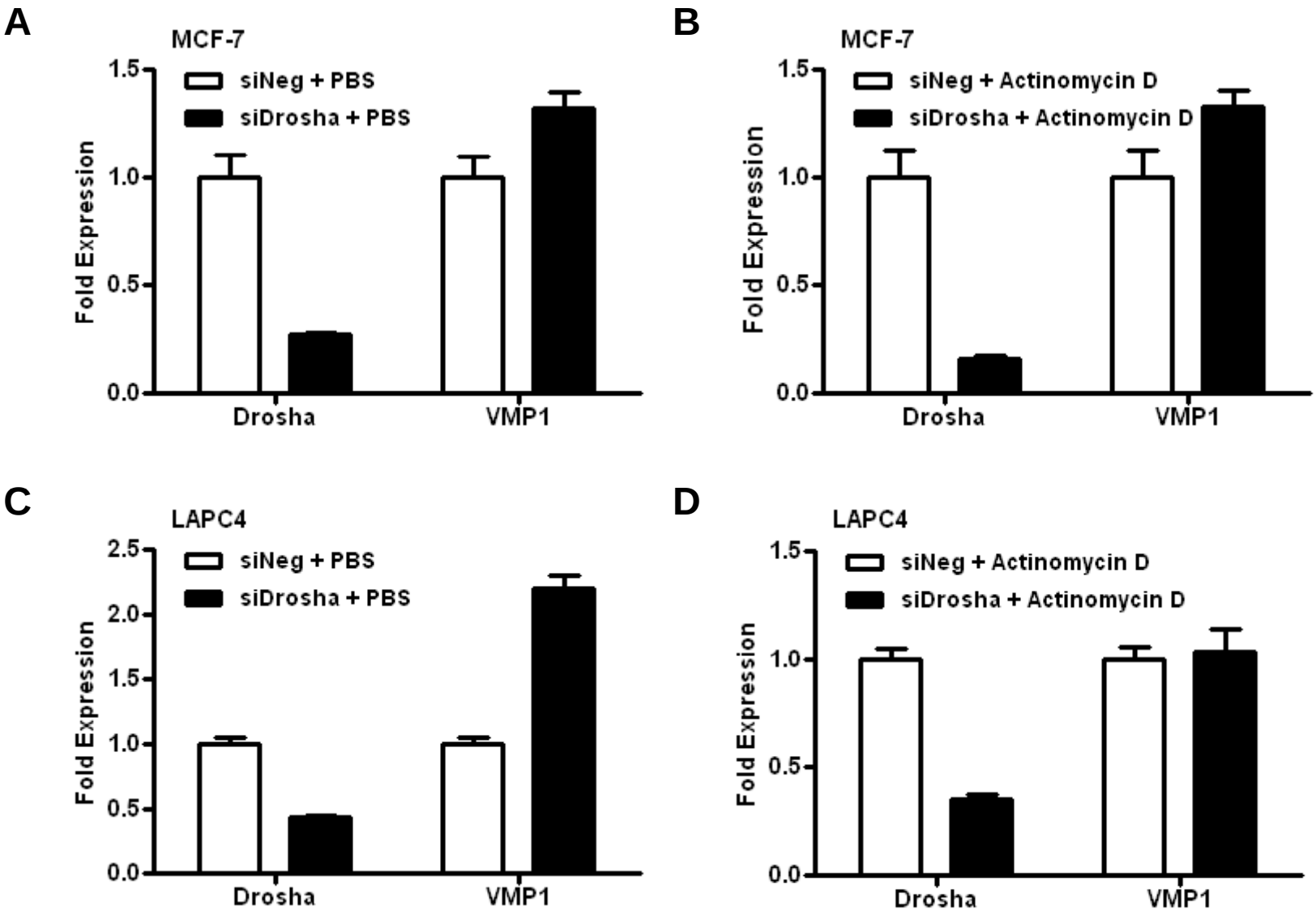
A VMP1



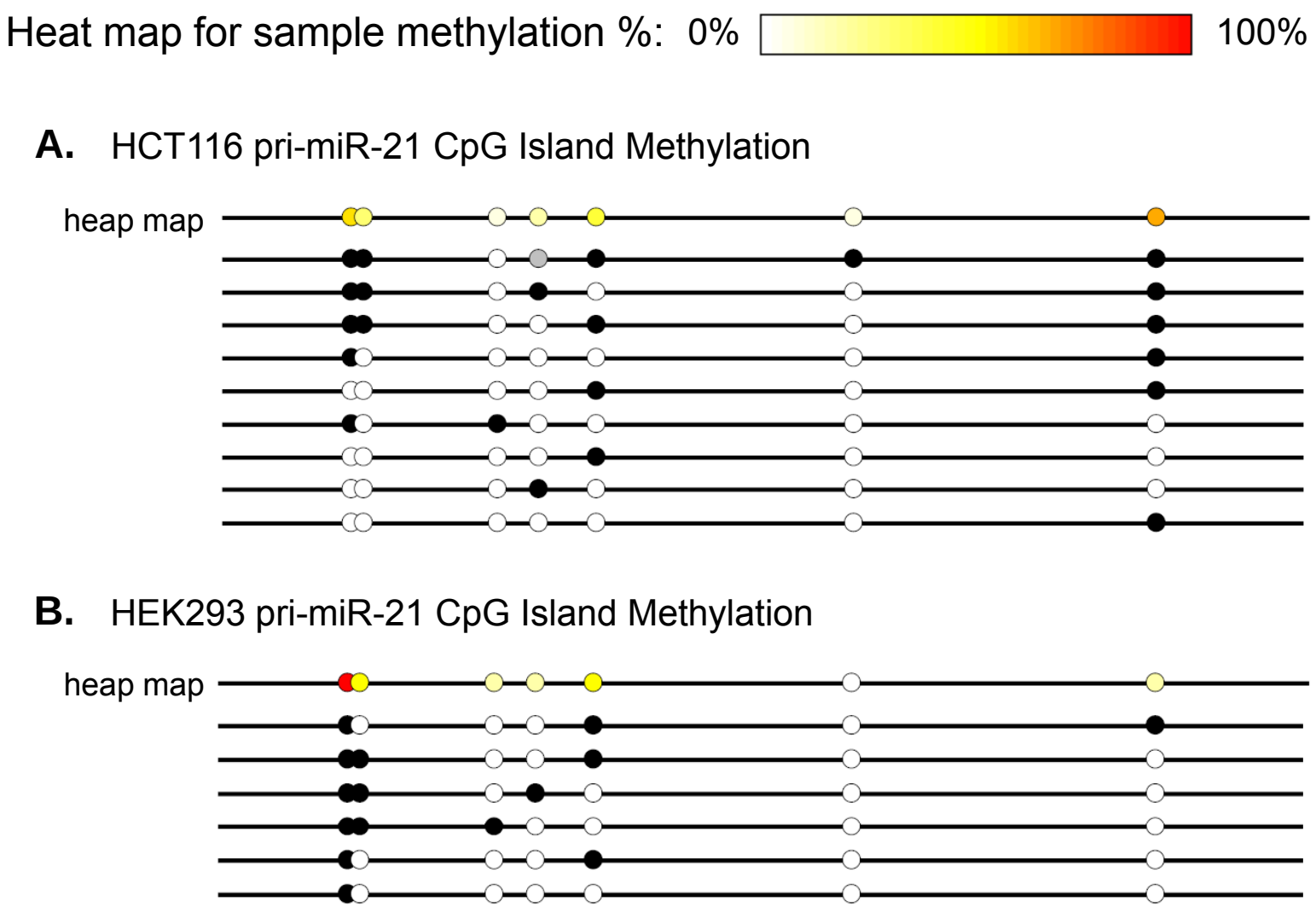
B miPRR-21



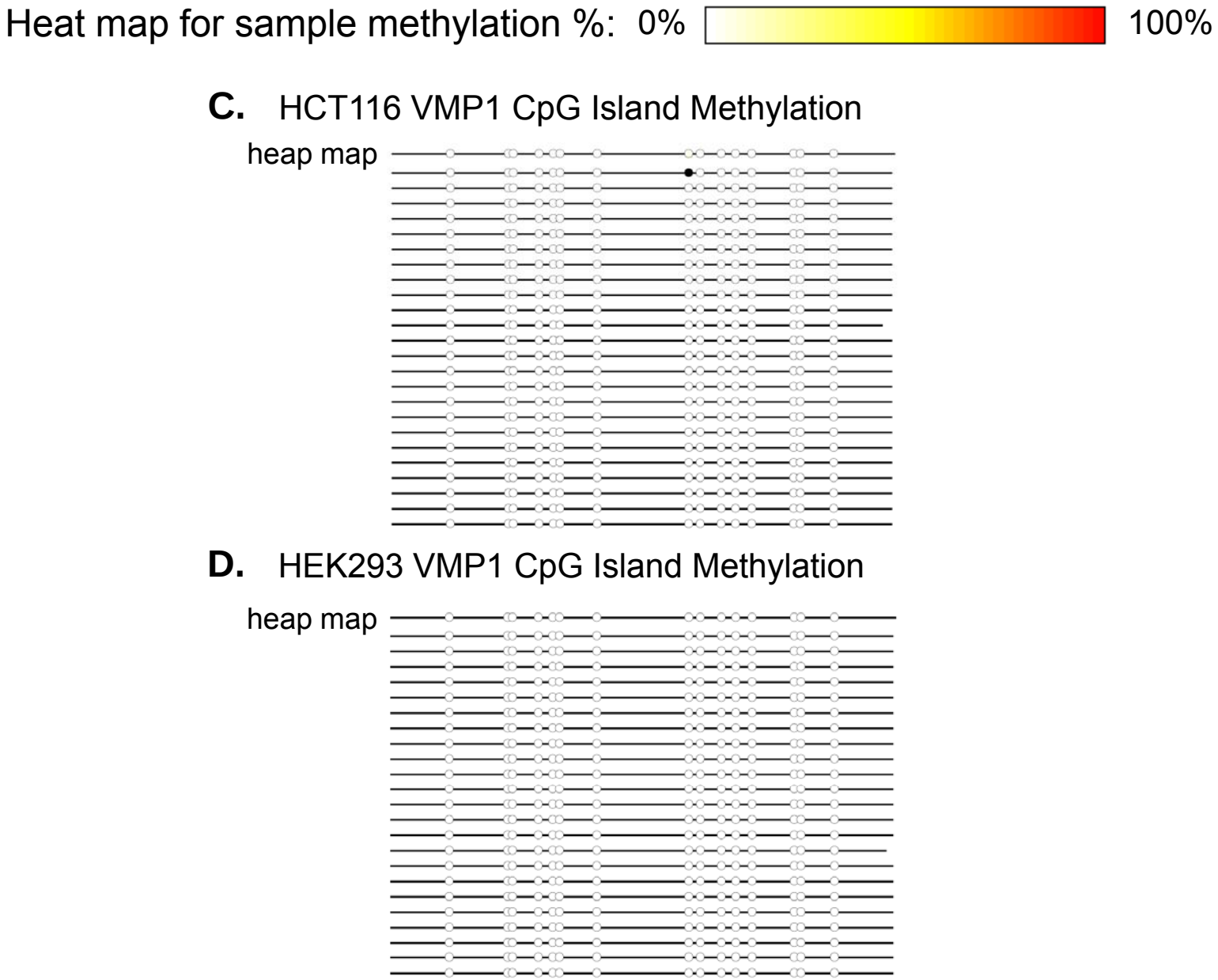
Supplementary Figure S5. CpG island search.
A, Schematic of the 2.9 kb area surrounding the VMP1 Transcription Start Site (TSS) predicted by Marson and workers and **B,** an area of 2.9 kb including the pri-miR-21 promoter (miPPR-21) as analyzed by the CpG island Searcher Program (http://cpgisland.usc.edu/). Transcripts are represented under the form of a horizontal line. Vertical lines indicate a C followed by a G. A thick line below the transcript marks the presence of a CpG island of at least 500 bp of length defined by the presence of >50% GC.



Supplementary Figure S6. Effect of Drosha on VMP1 transcript stability following transcriptional inhibition. Total RNA was prepared from MCF-7 and LAPC-4 cells which had been transfected with a negative control siRNA (siNeg) or Drosha siRNA (siDrosha) and treated with vehicle (A and C) or the transcriptional inhibitor Actinomycin D (B and D). Expression of Drosha and VMP1, relative to GAPDH, was measured by quantitative RT-PCR. These results further support that Drosha processing effects on VMP1 mRNA levels and reveal that these changes can be due to alteration of transcript stability (MCF-7) as well as by changes in transcription (LAPC4).



Supplementary Figure S7. Bisulfite sequencing of putative pri-miR-21 and VMP1 CpG Islands.
Schematic representation of bisulfite sequencing results with circles denoting the methylation status of each selected clone. Black and white circles are methylated and unmethylated CG dinucleotides, respectively. Grey circles indicate that the CpG was not identifiable in the sequencing data. Colorimetric heat maps are used to quantify relative mehtylation for each site. **A,** methylation of pri-miR-21 CpG island from HCT116 cells. **B,** methylation of pri-miR-21 CpG island from HEK293 cells.



Supplementary Figure S7. Bisulfite sequencing of putative pri-miR-21 and VMP1 CpG Islands (continued). **C,** methylation of VMP1 CpG island from HCT116 cells. **B,** methylation of VMP1 CpG island from HEK293 cells.

PRIMERS FOR NORTHERN PROBES		
Name	**Sense**	**Antisense**
P1	CCTTTAGGAGCATTATGAGCATT	GGTCATGAAGACTATCCCCATT
P2	CATGGCGACTTCAGAGTTGA	TGCAGAACAATGCAATGAAA
P3	TGGCAGTTCAAAAACTAGTACAGAA	ATGAAAGCCACCATTTGCTC
P4	CAAAGTTATGCCAAACGAATCCAGCAGCG	CAATTTCCCCAGAGGGTGTGCAATG
P5	CAGCCTCTAGTCGTGGTTCA	CATGCCACAGCTGAACACTT
PRIMERS FOR NESTED PCR OF VMP1 EXON 9/MIR-21		
First round	CCTGTGCTTCAATTCCAAATCC	CTGCTGCATTATGGCACAAA
Second round	CCTTTATTTGATCTGGCTGG	GTGTTGCCATGAGATTCAAC
PRIMERS FOR RT-PCR		
Amplicon	**Sense**	**Antisense**
Exon 10-11	GCAAGCACATAGTGGAGCAAAT	CCGTTGAGCCTCCAGGTACTC
Intron 11	CATGGCGACTTCAGAGTTGA	TGGGATGAGTGTGAGGTTAGC
Exon 12	TGGAGTGGATGGGTTCTGC	TCCTTCCCGGCTTGGAGT
miR-21	ATGGGCTGTCTGACATTTTGGTA	CATTGGATATGGATGGTCAGATGA
Neg. Control	TGGCACACCACCTACAGGG	GACATCCACTGCCTTCTTTGG
Drosha	GGAGAAAGAATAGGCTGTGG	TGCTCTCTTTCCCACCTCAT
β-Actin	AGGCACCAGGGCGTGAT	GCCCACATAGGAATCCTTCTGAC

Supplementary Table SI.

PRIMERS AND PROBES	
5'-3' biotinylated probe	GTAGGCAAAACAAGCAGACAGTCAGGCAGG
GSP for reverse transcription	CCCATTTCTCCATAAAATCCTCCCTC

Supplementary Table SII.