

## Supplementary Information

**Table S1. A list of primers used in the reactions for clone PCR.**

<b>Primer</b>	
miR-33a-5p-clone-F	CATTTGCTCCAGCGGTTTGC
miR-33a-5p-clone-R	GGGAGTGGCTGAGAATCTGAATC
ALK5-CDS-F	GCCACCATGGAGGCGGCGGTC
ALK5-CDS-R	TTACATTTTGATGCCTTCCTGTTGAC
ALK5-T204D-F	TTGCGAGAGATATTGTGTTACAAGAAAGCATTG GC
ALK5-T204D-R	ACACAATATCTCTCGCAATTGTTCTCTGAACAA GC
ZEB1-3'UTR-F	TTGTACTGTATGTCTTCAAACCTGGC
ZEB1-3'UTR -R	AGGCAGTGAGGAATGTGAGAG
TGFBR1-3'UTR-F	GTAACGTTTCGGTGGATCCTCTG
TGFBR1-3'UTR -R	GGATGGACCAGGGATGTCTATGC

**Table S2. List of primers used for real-time RT-PCR.**

<b>Primer</b>	
ZEB1-up	GGCATAACCTACTCAACTACGG
ZEB1-dn	TGGGCGGTGTAGAATCAGAGTC
SREBF2-up	CTCCATTGACTCTGAGCCAGGA
SREBF2-dn	GAATCCGTGAGCGGTCTACCAT
TGF- $\beta$ R1-up	GACAACGTCAGGTTCTGGCTCA
TGF- $\beta$ R1-dn	CCGCCACTTTCCTCTCCAAACT
IL11-up	TGAAGACTCGGCTGTGACC
IL11-dn	CCTCACGGAAGGACTGTCTC
PTHRP-up	ACTCGCTCTGCCTGGTTAGA
PTHRP-dn	GGAGGTGTCAGACAGGTGGT
CTGF-up	GCTACCACATTTCTACCTAGAAATCA
CTGF-dn	GACAGTCCGTCAAAACAGATTGTT
NEDD9 -up	CTACAGGGTAAGGAGGAGTTT
NEDD9-dn	TGGGTCTCACATTGGTCAT
MMP13-up	AACATCCAAAAACGCCAGAC
MMP13-dn	GGAAGTTCTGGCCAAAATGA
ADAM19-up	TTTCTCAGAACAGCGGGACT
ADAM19-dn	TGTTGATCACCTTTCGCTTG
THBS1-up	TTGTCTTTGGAACCACACCA
THBS1-dn	TTGTCAAGGGTGAGGAGGAC
COL1A1-up	CCTGGATGCCATCAAAGTCT
COL1A1-dn	CGCCATACTCGAACTGGAAT
VEGFA-up	AAGGAGGAGGGCAGAATCAT
VEGFA-dn	CACACAGGATGGCTTGAAGA
GAPDH-up	ATTCCACCCATGGCAAATTC
GAPDH-dn	TGGGATTTCCATTGATGACAAG

**Table S3. Clinicopathological features of 205 prostate cancer patients**

Parameters	Number of cases
Age (years)	
<72	102
$\geq 72$	103
Median	72
Differentiation	
Well/moderate	90
Poor	115
Serum PSA at diagnosis, $\mu\text{g/ml}$	
<69.2	102
>69.2	103
Median	69.2
SD	561.1
Mean	224.9
Gleason grade	
$\leq 7$	105
>7	100
miR-33a-5p expression	
<4.512	103
>4.512	102
Median	4.5119
SD	2.065
Mean	4.236
ZEB1 expression	
<2.4823	102
>2.4823	103
Median	2.4823
SD	1.971
Mean	2.945
BM status	
nBM	165
BM	40

**Abbreviation: PSA, Prostate-specific Antigen; SD, Standard Deviation; BM, Bone Metastasis; n-BM: Non-bone Metastasis.**

**Table S4. List of primers used for ChIP assay**

<b>Primer</b>	
P1-F	AGCACTCTGACTAGGTAAGC
P1-R	TCTGGAGCGAGGTGACTTTG
P2-F	CATGGAAGATGGCCTTCACC
P2-R	GTGGCAGCATTGGAGTCAAC
P3-F	TTTCTTAGGCTCCCAGAGTC
P3-R	TGGAGCAAATGCAGGTGAAC
P4-F	CCTGGGATGGCTGTGACTTG
P4-R	ACTGAAGGCCCTATCAGGTG

**Table S5. Relationship between miR-33a-5p and clinicopathological features in 205 patients with prostate cancer.**

Parameters	Number of cases	miR-33a-5p expression		P-values
		Low	High	
<b>Age (years)</b>				
<72	102	59	47	0.125
≥72	103	44	55	
<b>Differentiation</b>				
Well/moderate	90	23	67	<0.001*
Poor	115	80	35	
<b>Serum PSA</b>				
<69.2	102	34	68	<0.001*
≥69.2	103	69	34	
<b>Gleason grade</b>				
≤7	105	29	76	<0.001*
>7	100	74	26	
<b>BM status</b>				
nBM	165	66	99	<0.001*
BM	40	37	3	

**Table S6. Cox regression univariate and multivariate analyses of prognostic factors for overall survival**

Variable	Univariate analysis			Multivariate analysis		
	P-value	Relative risk	95% CI	P-value	Relative risk	95% CI
Age						
≤71						
>71	0.818	1.10	0.49-2.48		Not included	
Differentiation						
Well/moderate						
Poor	0.016	2.82	1.26-6.30		Not included	
Serum PSA, µg/ml						
≤63.1						
>63.1	0.016	2.70	1.21-6.04	/	/	/
Gleason grade						
≤7						
>7	0.084	2.06	0.91-4.66	/	/	/
miR-33a-5p expression						
<4.8589						
≥4.8589	0.014	0.36	0.16-0.81	0.02	0.33	0.13-0.84

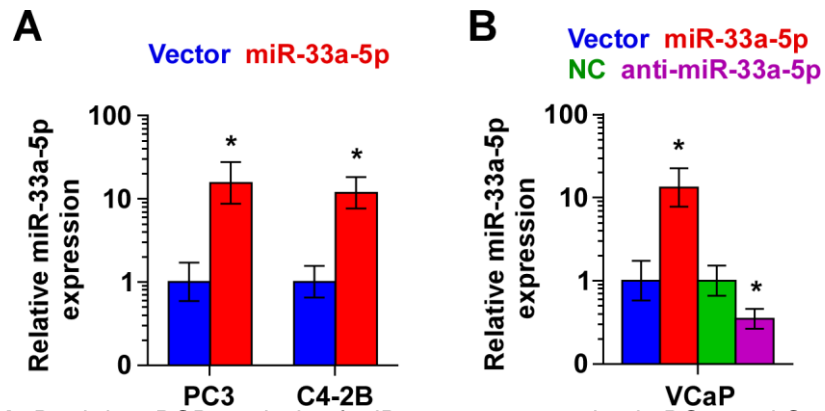
“/” means variables not in the Equation

**Table S7. Cox regression univariate and multivariate analyses of prognostic factors for bone metastasis-free survival**

Variable	Univariate analysis			Multivariate analysis		
	P-value	Relative risk	95% CI	P-value	Relative risk	95% CI
Age						
≤71						
>71	0.046	2.04	1.01-4.09	0.002	3.34	1.58-7.04
Differentiation						
Well/moderate						
Poor	0.001	3.42	1.71-6.86		Not included	
Serum PSA, µg/ml						
≤63.1						
>63.1	0.143	1.68	0.84-3.37		Not included	
Gleason grade						
≤7						
>7	0.002	3.11	1.52-6.34	0.019	2.06	1.18-6.01
miR-33a-5p expression						
<4.8589						
≥4.8589	0.002	0.33	0.16-0.66	0.021	0.35	0.14-0.85

“/” means variables not in the Equation

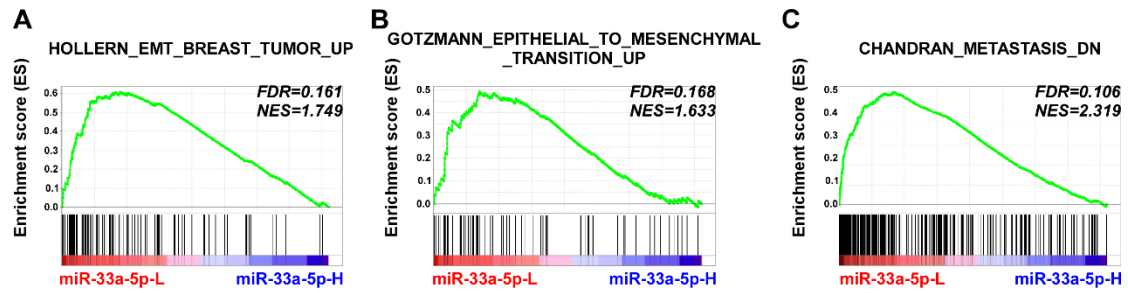
## Supplementary Figures



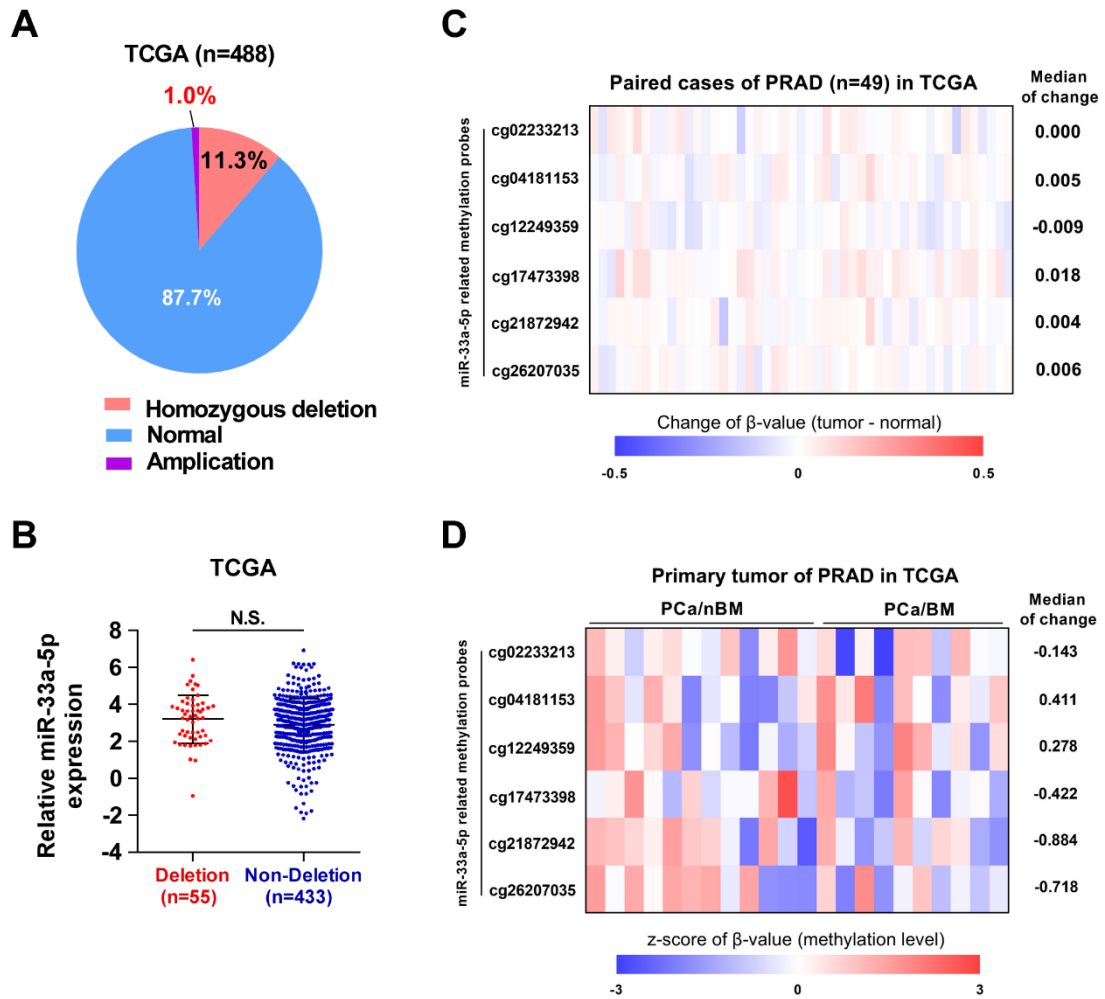
**Figure S1. A**, Real-time PCR analysis of miR-33a-5p expression in PC-3 and C4-2B cells transduced with miR-33a-5p compared to controls. Transcript levels were normalized by *U6* expression. Error bars represent the mean  $\pm$  s.d. of three independent experiments. \* $P < 0.05$ .

**B**, Real-time PCR analysis of miR-33a-5p expression in VCaP cells transduced with miR-33a-5p or transfected with anti-miR-33a-5p compared to controls. Transcript levels were normalized by *U6* expression. Error bars represent the mean  $\pm$  s.d. of three independent experiments. \* $P < 0.05$ .

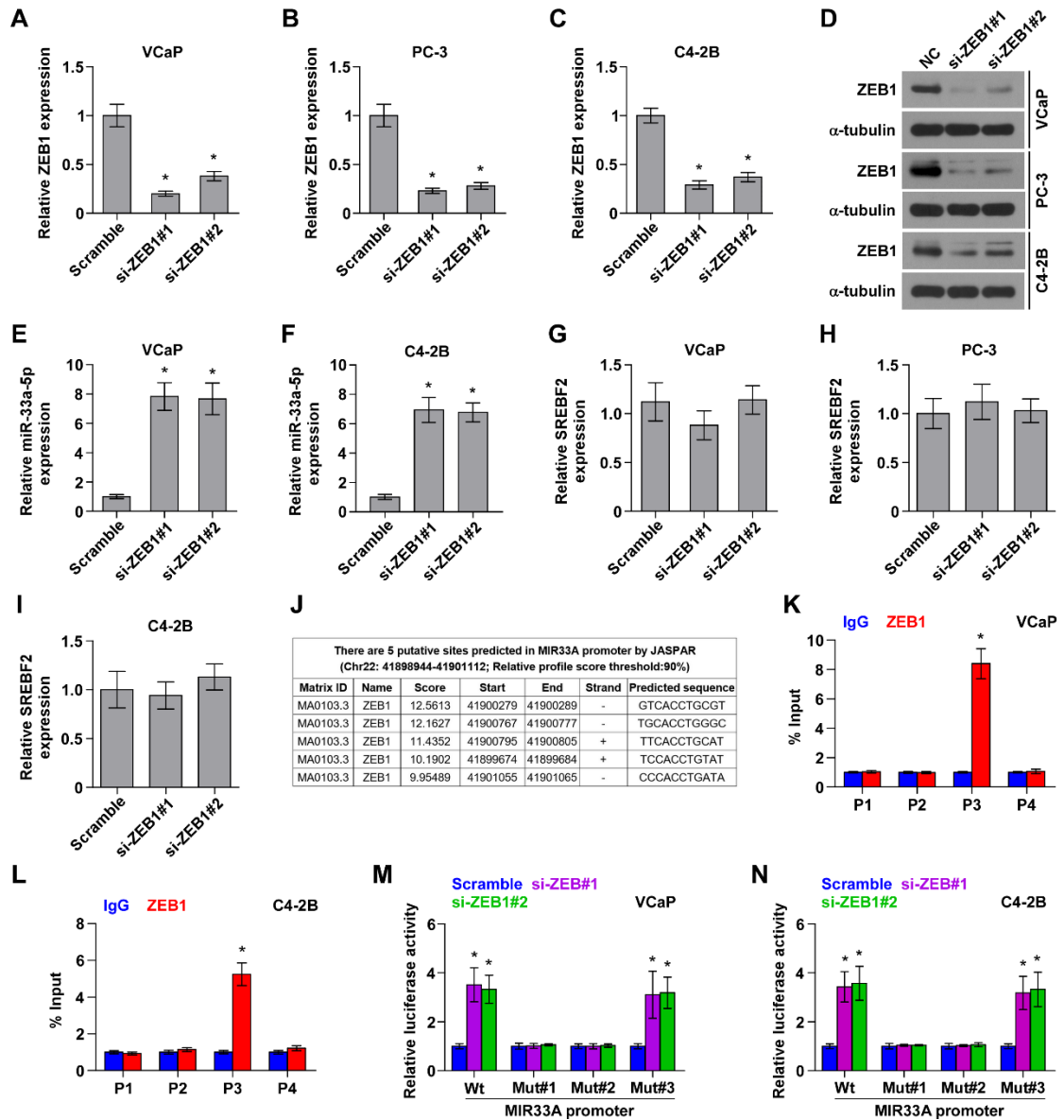




**Figure S2. A and B**, Gene set enrichment analysis (GSEA) revealed that downexpression of miR-33a-5p significantly and positively correlated with the EMT signatures. **C**, GSEA revealed that downexpression of miR-33a-5p significantly and positively correlated with metastatic propensity.

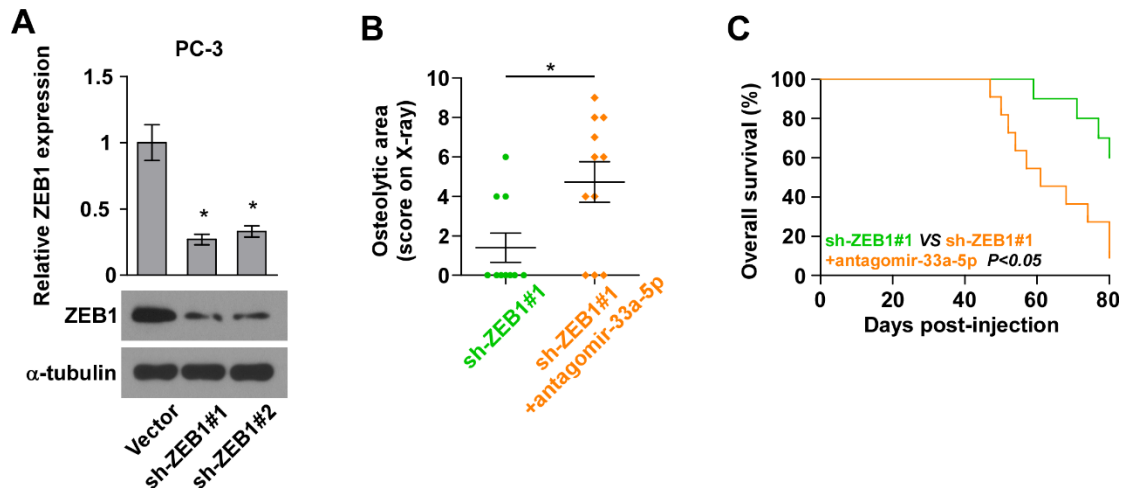


**Figure S3. A**, Deletion and amplification levels of MIR33A in the PCa dataset from TCGA. **B**, miR-33a-5p expression levels in PCa tissues with deletion and in those without deletion in the PCa dataset from TCGA. **C**, Methylation levels of MIR33A in 49 paired PCa tissues from the TCGA dataset. **D**, Methylation levels of MIR33A between PCa/BM and PCa/nBM tissues from the TCGA dataset.

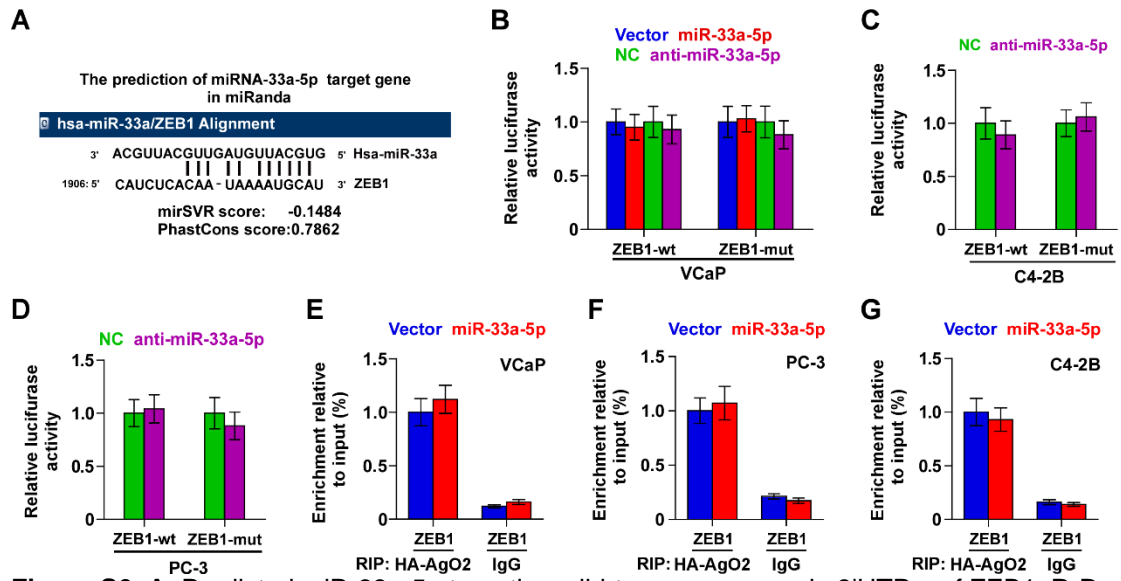


**Figure S4. A-D**, Real-time PCR and Western blot analysis of ZEB1 expression in the indicated PCa cells.  $\alpha$ -Tubulin served as the loading control. Error bars represent the mean  $\pm$  S.D. of three independent experiments.  $*P < 0.05$ . **E and F**, Real-time PCR analysis of miR-33a-5p expression in the indicated VCaP and C4-2B cells. Error bars represent the mean  $\pm$  S.D. of three independent experiments.  $*P < 0.05$ . **G-I**, Real-time PCR analysis of SREBF2 expression in the indicated PCa cells. Error bars represent the mean  $\pm$  S.D. of three independent experiments.  $*P < 0.05$ . **J**, The putative binding site of ZEB1 in the promoter of MIR33A by analyzing JASPAR. **K, and I**, ChIP analysis of ZEB1 binding sites in the promoter of MIR33A using an anti-ZEB1 antibody or a control IgG respectively in VCaP (**K**) and C4-2B (**I**) cells.  $*P < 0.05$ . **M and N**, The luciferase activity was measured by transfecting wild-type and different types of mutant MIR33A promoter in the indicated VCaP (**M**) and C4-2B (**N**) cells.

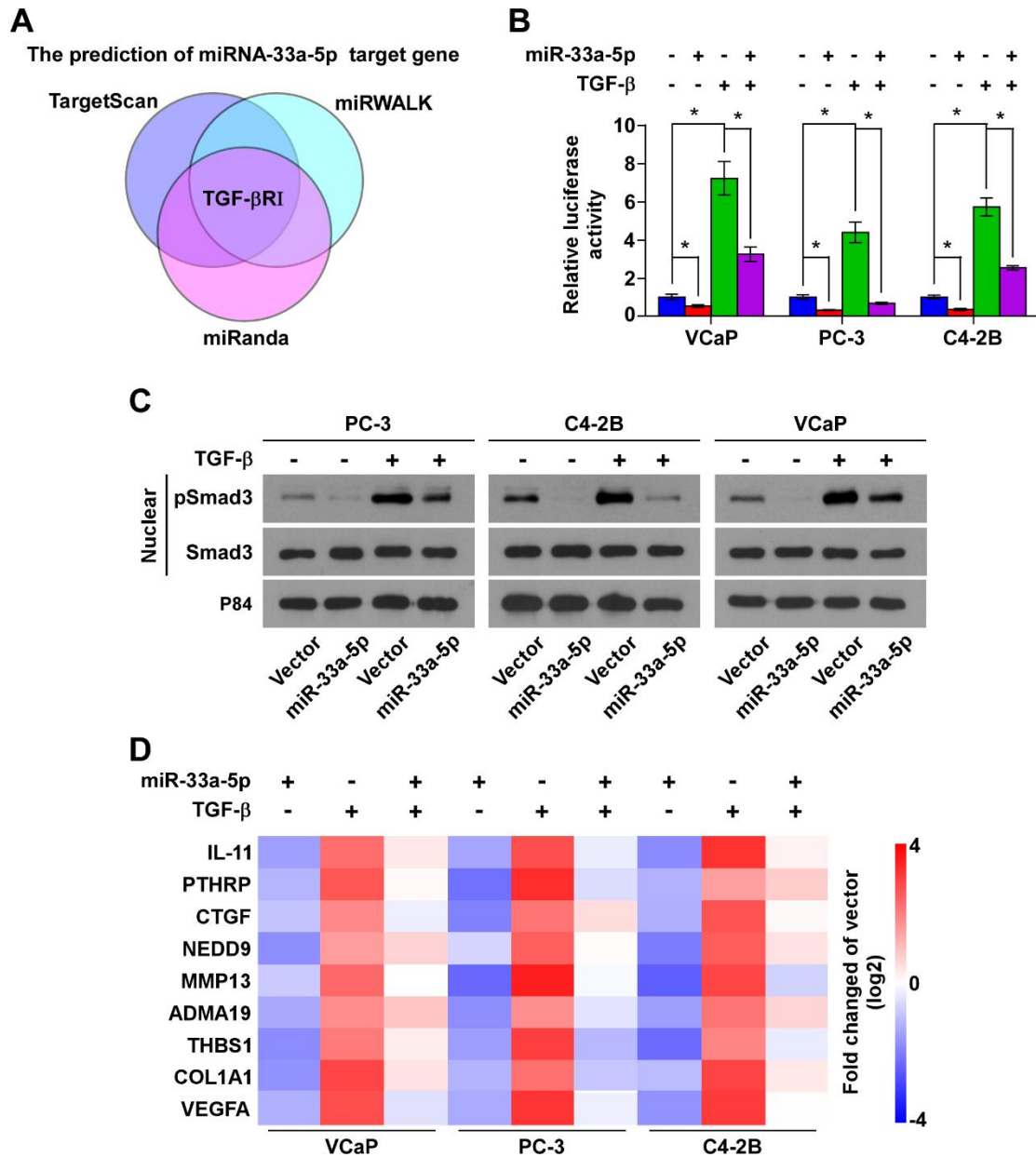
Error bars represent the mean  $\pm$  S.D. of three independent experiments. \* $P < 0.05$ .



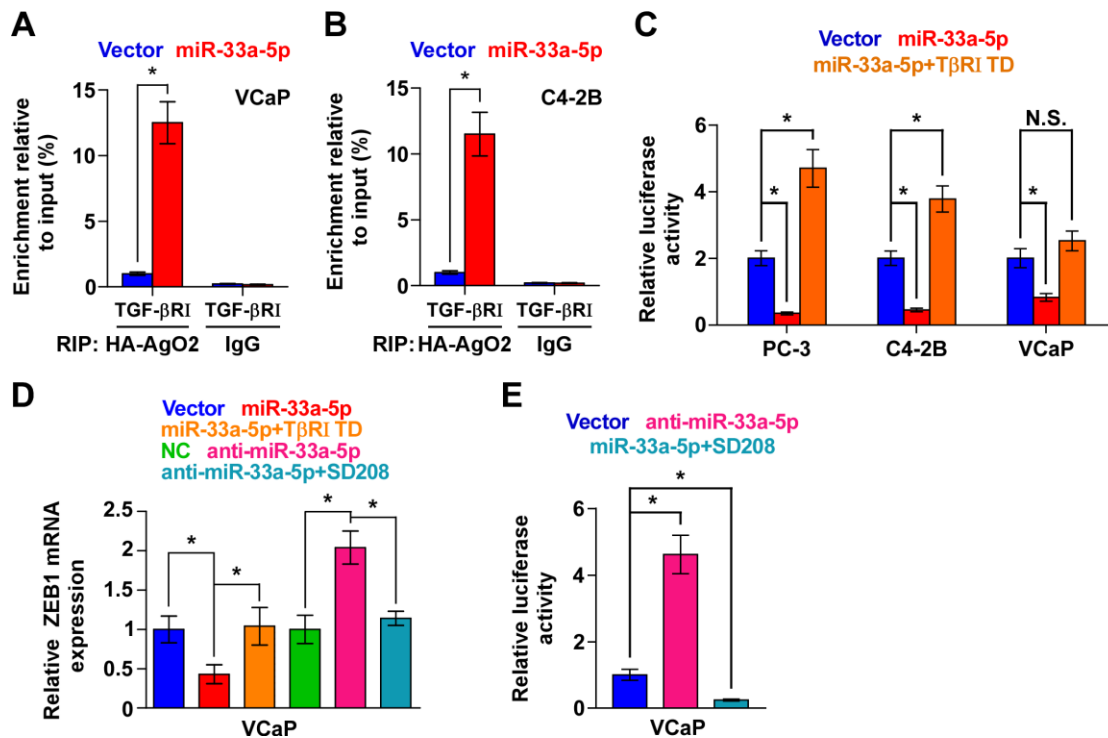
**Figure S5.** **A**, Western blot analysis of ZEB1 expression in ZEB1-stably downexpressing PC-3 cells.  $\alpha$ -Tubulin served as the loading control. **B**, The sum of bone metastasis scores for each mouse in tumor-bearing mice inoculated with ZEB1-silencing cells ( $n = 10$ ) or ZEB1-silencing cells plus antagomir-33a-5p ( $n = 11$ ). **C**, Kaplan-Meier analysis of mouse bone metastasis-free overall survival in the indicated mice groups.



**Figure S6.** **A**, Predicted miR-33a-5p targeting wild-type sequences in 3'UTR s of ZEB1. **B-D**, Luciferase assay of cells transfected with pmirGLO-3'UTR reporter of ZEB1 in the miR-33a-5p overexpressing and silencing PCa cells. \* $P < 0.05$ . **E-G**, MiRNP IP assay showing the association between miR-33a-5p and ZEB1 transcripts in PCa cells cells. Pulldown of IgG antibody served as the negative control.

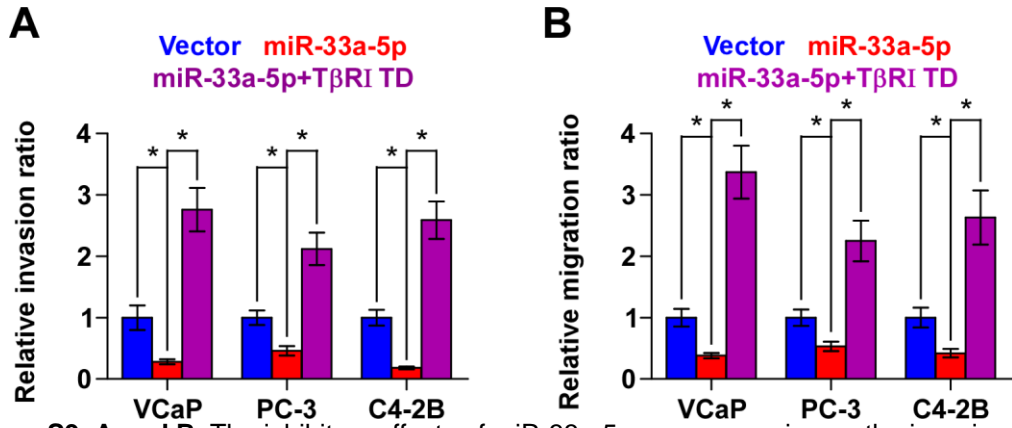


**Figure S7. A**, Predictive target genes of miR-33a-5p from TargetScan, miRanda and miRWALK. **B**, The effects of miR-33a-5p upregulation on transcriptional activity of TGF- $\beta$  signaling based on a TGF- $\beta$ /Smad-responsive luciferase reporter in the absence or presence of TGF- $\beta$  (5 ng/ml). \*P < 0.05. **C**, Western blot analysis showing that upregulation of miR-33a-5p decreased nuclear translocation of pSMAD3 in PCa cells in the absence or presence of TGF- $\beta$ . The nuclear protein p84 was used as a nuclear protein marker. **D**, Real-time PCR analysis of downstream bone metastasis-related genes of the TGF- $\beta$  pathway, including CTGF, PTHRP, IL-11, NEDD9, MMP13, ADAM19, THBS1, COL1A1 and VEGFA, in the indicated cells in the absence or presence of TGF- $\beta$ . Transcript levels were normalized by GAPDH expression.



**Figure S8. A and B**, MiRNP IP assay showing the association between miR-33a-5p and TGFβRI transcripts in VCaP and C4-2B cells. Pulldown of IgG antibody served as the negative control. **C**, The inhibitory effects of miR-33a-5p overexpression on the transcriptional activity of a TGF-β/Smad-responsive luciferase reporter in PCa cells were attenuated by reconstitution of TGFβRI. Error bars represent the mean ± S.D. of three independent experiments. \*P < 0.05. **D**, Real-time PCR analysis ZEB1 expression in the indicated VCaP cells. \*P < 0.05. **E**, The stimulatory effects of miR-33a-5p downregulation on the transcriptional activity of a TGF-β/Smad-responsive luciferase reporter in PCa cells were attenuated by TGF-β signaling inhibitor SD208 (1 μM). Error bars represent the mean ± S.D. of three independent experiments. \*P < 0.05.

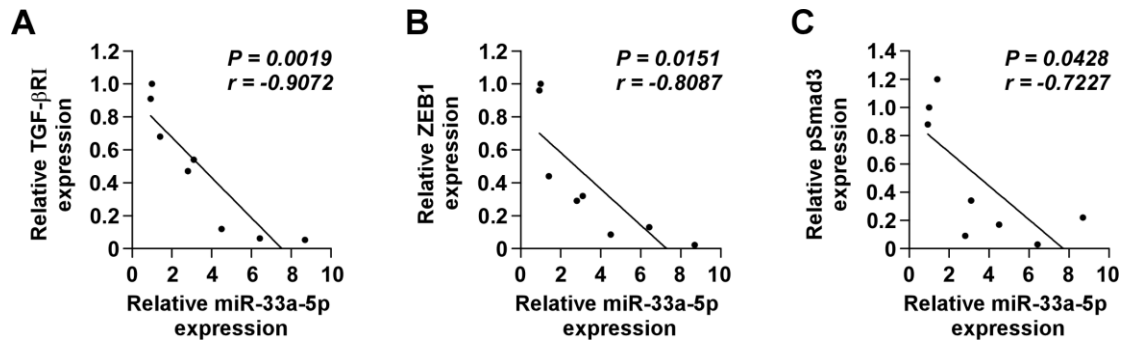




**Figure S9. A and B,** The inhibitory effects of miR-33a-5p overexpression on the invasion (A)

and migration (B) abilities of PCa cells were attenuated by reconstitution of TGFβRI. Error

bars represent the mean ± S.D. of three independent experiments. \*P < 0.05.



**Figure S10. A-C,** Correlation between miR-33a-5p levels and TGFBR1, ZEB1 and pSMAD3

expression in PCa tissues. The protein expression levels of TGFBR1, ZEB1 and pSMAD3 expression in PCa tissues were quantified by densitometry using Image J Software, and normalized to the levels of  $\alpha$ -tubulin and p84, respectively. The sample 1 was used as a standard. The relative expressions of miR-33a-5p and these proteins were used to perform the correlation analysis.