SUPPLEMENTAL METHODS

Cell culture

Normal primary prostate epithelial cells (PPEC) were maintained in prostate epithelial cell basal media (ATCC) supplemented with prostate epithelial cell growth kit (ATCC) under recommended conditions. LNCaP and PC3 cells were cultured in RPMI 1640 media while Du145 cells were maintained in MEM media, each supplemented with 10% fetal bovine serum (FBS) (Atlanta biologicals) and 1% penicillin/streptomycin (UCSF cell culture facility). PC3M-luc-C6 cells (UCSF Core facility) were cultured in MEM/EBSS media supplemented with 10% FBS and 200 ug/ml zeocin (Invitrogen) under recommended conditions. All cell lines were maintained in an incubator with a humidified atmosphere of 95% air and 5% CO2 at 37°C.

Laser capture microdissection (LCM)

Microdissections were performed using the AutoPix System (Arcturus) as described previously [1-3]. Briefly, 8 μm sections were placed on glass slides, deparaffinized, stained with hematoxylin, dehydrated, and microdissected with the AutoPix instrument using manufacturer's instructions. Areas of interest were captured with infrared laser pulses onto CapSure Macro LCM Caps.

Quantitative real-time PCR

Mature miRNAs and mRNAs were assayed using the TaqMan MicroRNA Assays and Gene Expression Assays respectively, in accordance with the manufacturer's instructions (Applied Biosystems). miRNA and mRNA expressions were normalized to RNU48 or GAPDH controls respectively (Applied Biosystems). Taqman assays employed were hsa-miR-383 (assay ID 000573), RNU48 (assay ID 001006), CD44 (Hs00174139_m1), GAPDH (Hs99999905_m1).

The comparative Ct method was used to calculate the relative changes in gene expression with the 7500 Fast Real Time PCR System.

TCGA data

The results described in this manuscript are partly based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/ [4]" miRNA seq data and corresponding clinical data for prostate adenocarcinomas were downloaded from the Cancer Genome Atlas (TCGA) data portal (https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm) while copy number data for prostate adenocarcinomas in the TCGA dataset were retrieved from cBioportal [5,6]. At the time of download, miRNA seq data from 187 tumors and copy number data from 246 tumors was available. Z- scores were calculated as per the following formula: z = (expression in tumor sample) - (mean expression in normal samples) / (standard deviation of expression in normal samples).

RNA and miRNA extraction

Total RNA was extracted from microdissected FFPE tissues using a miRNeasy FFPE Kit (Qiagen) and a miRNeasy mini kit (Qiagen) was used for miRNA extraction from cultured cells following the manufacturer's instructions.

Transient transfections

Cells were plated in growth medium without antibiotics ~24hrs before transfections. Transient transfection of miRNA mimics (Ambion)/siRNAs (Origene) were performed using Lipofectamine 2000 (Invitrogen) transfection reagent according to the manufacturer's protocol. Mimics employed were: miR-383 miRVANA mimic (4464066) or negative control (miR-CON) (4464058) (Ambion). For siRNA-mediated knockdown, Trilencer-27 predesigned siRNA (Origene) against *CD44* (SR300683) were used. All miRNA/siRNA transfections were for 72h

followed by functional assays. For rescue experiments, *CD44* ORF expression clone (cat no. EX-Z0381-M02-B) or control ORF clone were obtained from Genecopoeia.

Generation of miR-383 overexpressing stable cell line

PC3M-luc-C6 cells were plated 2 days prior to transfection in 10 cm dishes. Cells were transfected with the following constructs (Applied Biological Materials): miR-383 expression construct (Cat no. mh40579) or control construct (m003). 72 h post-transfection, the stably transfected cells were selected by culturing in the presence of puromycin (1µg/ml).

Xenograft tumors

Subcutaneous injections of PC-3M-luc-C6 cells (5 $X10^6$) stably overexpressing miR-CON/miR-383 cells were performed in nude mice (4- 5 week-old, Simonsen Laboratories, n=8 per group) Mice were randomized and miR-CON/miR-383 overexpressing cells were injected in the right flanks of mice in a volume of 100 µl. Once palpable tumors developed, caliper measurements were taken once a week and tumor volumes were calculated on the basis of width (x) and length (y): $x^2y/2$, where $x< y$. Investigator was blinded to the groups during tumor measurements.

For primary prostate tumors, we considered a 50% inhibition in tumor volume/burden in miRNA treated animals compared to controls as a reasonable inhibition. For a 50% difference with type I error of 0.05 and type II error of 0.1 (or testing power of 90%), the estimated sample size was about 8.

Experimental metastasis model

6-weeks old male nude mice (strain nu/nu; Simonsen Laboratories) (n=16) were randomized into two groups (n=8 per group). Bioluminescent PC-3M-luc-C6 cells stably overexpressing miR-CON/miR-383 (1 x 10^6 cells in 100µl PBS) were injected into left ventricles of these groups in a volume of 100 µl. Successful intra-cardiac injections were confirmed by bioluminescent imaging

(BLI). To monitor development of metastases, bioluminescence imaging at periodic intervals was performed with an IVIS100 Imaging System (Xenogen) with the use of the Living Image acquisition and analysis software (Caliper Life Sciences). Before imaging, the mice were anesthetized with 2.5% isofluorane and 150 mg/kg D-luciferin firefly potassium salt (Perkin Elmer) was administered intraperitoneally. At 15 min post-injection, serial imaging of the tumor burden was done to capture peak luciferase intensities. Investigator was blinded to the groups during tumor imaging.

Xenograft tumor processing

Tumors were processed as described previously [7-9] [1]. Briefly, DU145, PC3 and LAPC9 xenograft tumors were harvested from mice, minced into ~1mm^3 pieces in DMEM media supplemented with 10% FBS. Tumors were rinsed (2X) in the same medium and incubated with 1X Accumax (Innovative Cell Technologies, Inc) at a concentration of 10 ml/1 g tissue in D-PBS for ~30 min at RT. Following accumax digestion, supernatants were filtered through a 40-µm cell strainer and single-cell suspensions were then loaded onto a layer of Histopaque-1077 gradient to get rid of red blood cells, dead cells, and debris. Cells were centrifuged at 400 g for 30 min and used to deplete murine cells using the MACS Lineage Cell Depletion Kit (Miltenyi Biotech) as described in [7,9]. The resultant cell suspensions were then stained for CD44 using human specific FITC-conjugated anti-CD44 antibody (Miltenyi Biotech, 130-095-195) as per manufacturer's protocol. Fluorescence-activated cell sorting (FACS) was performed to isolate CD44+ and CD44- subpopulations.

Purification of CD44+ subpopulations of xenograft tumors

Xenograft human prostate tumors LAPC9 were courtesy of R. Reiter [10,11]. DU145, PC3 and LAPC9 xenograft tumors were established using early passage cells and maintained in

NOD/SCID mice (Charles River Laboratories) under standard conditions. CD44+ subpopulations of xenograft tumors were isolated as described previously [1]. Single cell suspensions were obtained from xenograft tumors (detailed in supplemental methods), stained for CD44 using human specific FITC-conjugated anti-CD44 antibody (Miltenyi Biotech, 130-095-195) as per manufacturer's protocol and purified using fluorescence-activated cell sorting (FACS). Post-sort analysis confirmed purities of CD44+ and CD44- subpopulations (>95%).

Cell cycle and Apoptosis Assays

Fluorescence-activated cell-sorting (FACS) for analyzing cell cycle and apoptosis was done 72 hours post-transfection. Cells were harvested, washed with cold PBS, and resuspended in the nuclear stain DAPI for cell cycle analysis. Cells were stained with 7-AAD and Annexin-V-FITC using ANNEXIN V-FITC /7-AAD KIT (Beckman Coulter) for apoptosis analysis according to the manufacturer's protocol. Stained cells were immediately analyzed by FACS (Cell Lab Quanta SC; Beckman Coulter, Inc).

Luciferase assays

The wild type CD44 3'UTR target sequence containing miR-383 binding site was cloned downstream of the luciferase gene in the pmiRGLO luciferase vector (Promega). Mutant CD44 3'UTR sequence (represented in Fig. 5C) was cloned in the same vector. The primers used for cloning were synthesized from Invitrogen and are listed in Table S5. Control/wt CD44/mutant CD44 3'-UTR constructs (0.2 ug) were transfected into DU145/PC3 cells cultured in 24-well plates along with 50nM miR-383 or miR-CON mimics (Ambion) using Lipofectamine 2000 (Invitrogen). After 48 hrs, cells were harvested and firefly and renilla luciferase activities were measured by using the dual luciferase reporter assay system (Promega) according to the manufacturer's protocol. Firefly luciferase was normalized to renilla luciferase activity.

miR-383 inhibition

miR-383 inhibition in primary prostate epithelial cells was performed by transient transfection with anti-miR-383 oligonucleotides (Ambion) according to the manufacturer's protocol. As a control, anti-miR-CON oligonucleotides (Ambion) were transfected. All transfections were for 72h.

Cell viability assays

Cell viability was determined at 24, 48, 72 hours by using the CellTiter 96 AQueousOne Solution Cell Proliferation Assay Kit (Promega), according to the manufacturer's protocol. Briefly, 20ul of MTS reagent was added to cells in 100ul media in 96-well plates, followed by incubation at 37°C. Absorbances were read at 490nm using a plate reader.

Sphere formation assays

Single cells were counted and plated at 1,000 cells/well in 6-well ultralow attachment dishes (Fisher Scientific Co.). Cells were grown in serum-free prostate epithelial basal medium (PrEBM) supplemented with 4μg/mL insulin (Sigma), B27, 20ng/mL EGF, and 20 ng/mL basic FGF (Invitrogen). Floating spheres that arose in 1–2 weeks were counted under microscope.

Clonogenicity assays

Cells were counted, seeded at low density (1000 cells/plate) and allowed to grow until visible colonies appeared. Then, cells were stained with Giemsa, and colonies were counted.

Migration and invasion assays

Migration and invasion assay kit (Cell Biolabs, Inc.) was used for assaying cellular migration and invasiveness according to the manufacturer's protocol. Briefly, 48 hrs post-transfection, cells were counted and placed on control inserts (for migration) or Matrigel inserts (for invasion) at 1 x10^5 cells/ml in serum-free medium and were allowed to migrate for 20 h at 37°C. Cells were

removed from the top of the inserts and cells that migrated/invaded though the polycarbonate/basement membrane were fixed, stained and quantified at OD 560nm after extraction.

Western blotting

Whole cell extracts were prepared in RIPA buffer [50 mmol/L Tris (pH 8.0), 150 mmol/L NaCl, 0.5% deoxycholate, 0.1% SDS, and 1.0% NP-40] containing protease inhibitor cocktail (Roche). Total protein was electrophoresed by SDS-PAGE and Western blotting was carried out according to standard protocols. The following antibodies were used for Western blotting: CD44 (Epitomics, 3381-1) and GAPDH (Santa Cruz Biotechnology, sc-32233).

Copy number assays

Genomic DNA extracted from clinical tissues was used for copy number analyses using Taqman copy number assays (Applied Biosystems) as per manufacturer's instructions. Briefly, genomic DNA was combined with the test assay (Hs05044921_cn) and the reference assay hTERT (4403316) alongwith the Taqman genotyping master mix. The comparative Ct method was used to calculate the relative changes in copy number on the 7500 Fast Real Time PCR System [12].

Statistics

All experiments were performed in triplicate and repeated at least three times with similar results each time. All quantified data for functional assays represent an average of triplicate samples and were used to generate P-values.

The Wilcoxon Signed Rank test was used to assess the difference between miR-383 expression in clinical tissues (tumor/normal adjacent). For Kaplan-Meier survival analysis, patients were stratified into low miR-383 and high miR-383/no change groups based on miR-338 levels and survival analyses were performed with MedCalc version 10.3.2. Receiver operating curves

(ROC) were calculated to determine the potential of miR-383 and PSA to discriminate between tumor and normal samples. miR-383 is modeled as a categorical variable (no change, low expression, high expression). Areas under the ROC curve (AUC) were estimated and reported with 95% DeLong Confidence Intervals (CI). AUC of miRNA and PSA were compared using bootstrap. The discriminatory ability of miR-383 was further characterized based on a dichotomous variable (no change, high expression vs. low expression); using apparent prevalence, true prevalence, sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio. Logistic regression model was employed to compare the ability of miR-383 and PSA to identify a tumor sample by comparing probability of tumor conditional on miR-383 values vs. the probability conditional on PSA values. PSA values for normal samples were randomly generated from the Uniform distribution on the age-defined ranges.

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1 miR-383 overexpression in prostate cancer cell lines

Relative miR-383 expression in DU145/PC3/LNCaP cell lines transfected with miR-CON /miR-383 mimics or mock transfected cells as assessed by real time PCR. Data was normalized to RNU48 control.

Fig. S2 miR-383 silencing induces tumorigenicity in normal prostate epithelial cells

Inhibition of miR-383/miR-CON was performed in primary prostate epithelial cells (PPEC) by transient transfection of anti-miR-inhibitors followed by functional assays, 72 hrs post-transfection (* $P < .05$).

A. Assessment of miR-383 expression levels by RT-PCR. Data were normalized to RNU48 control and are represented as mean ± SEM.

B. MTS cell viability assay, C. Transwell invasion assay and D. Transwell migration assay in anti-miR-CON/ anti-miR-383 treated PPEC.

Fig. S3 miR-383 and stemness related gene targets

Immunoblots of endogenous STAT3, LIN28A, CD44 and LEF1 in Du145/PC3 cells transfected with mock/miR-CON/miR-383. GAPDH was used a loading control.

Fig. S4 miR-383 directly regulates prostate cancer stem cell marker CD44 in prostate cancer

Immunoblots of endogenous CD44 in LNCaP cells transfected with mock/miR-CON/miR-383. GAPDH was used a loading control.

Fig. S5 CD44+ cells in mice xenograft tumors derived from prostate cancer cell lines

Xenograft tumors were generated in mice from prostate cancer cell lines Du145, PC3 and LAPC9. Tumors were processed and stained for CD44 using human specific FITC-conjugated anti-CD44 antibody followed by FACS to isolate CD44+ and CD44- subpopulations. The average percentage of CD44+ subpopulation observed with Du145 (upper panel), PC3 (middle panel) and LAPC9 (lower panel) cells are represented.

Fig. S6 CD44 knockdown induces G0-G1 cell cycle arrest in Du145 cell line

Du145 cells were transfected with two sets of siRNAs specific to CD44 (si1 and si2) or a nonspecific (NS) control siRNA followed by cell cycle analyses (performed 72 hrs post-transfection). Cell cycle analyses in Du145 cells after NS siRNA (left panel) or CD44 siRNA-1 and siRNA-2 transfections.

SUPPLEMENTAL TABLE LEGENDS

Table S1 Clinicopathologic characteristics of prostate cancer patients

Clinicopathologic characteristics of prostate cancer tissues used for analyses of miR-383 expression.

Table S2 Multivariate regression analyses for miR-383 expression and known prognostic factors

Multivariate regression analyses were performed for overall survival for SFVAMC cohort. Independent variables included miR-383 expression, serum PSA at diagnosis, Gleason grade and clinical stage. Cases with complete clinical information were included in these analyses using A. entry, B. forward, C. backward, and D, stepwise methods. These analyses showed that miR-383 expression is an independent predictor of overall survival ($P < 0.0001$).

Table S3 Correlation of miR-383 expression with serum PSA levels

Correlation of miR-383 expression with age-adjusted serum PSA levels in PCa patients. (* P< .05).

Table S4 miR-383 and stemness related gene targets

Schematic representation of 3'-UTRs of potential stemness related genes showing putative miR-383 target sites with mirSVR scores generated by the miRANDA algorithm.

Table S5 List of primers used for CD44 3' UTR cloning

Primers used for cloning of wild-type and mutant 3'UTR region of CD44 represented in Fig. 4A. 3'UTR region containing wt/mutant miR-383 target sequence were cloned downstream of the luciferase gene in the pmiRGLO luciferase vector (Promega) using the indicated primers.

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Fig. S1 miR-383 overexpression in prostate cancer cell lines

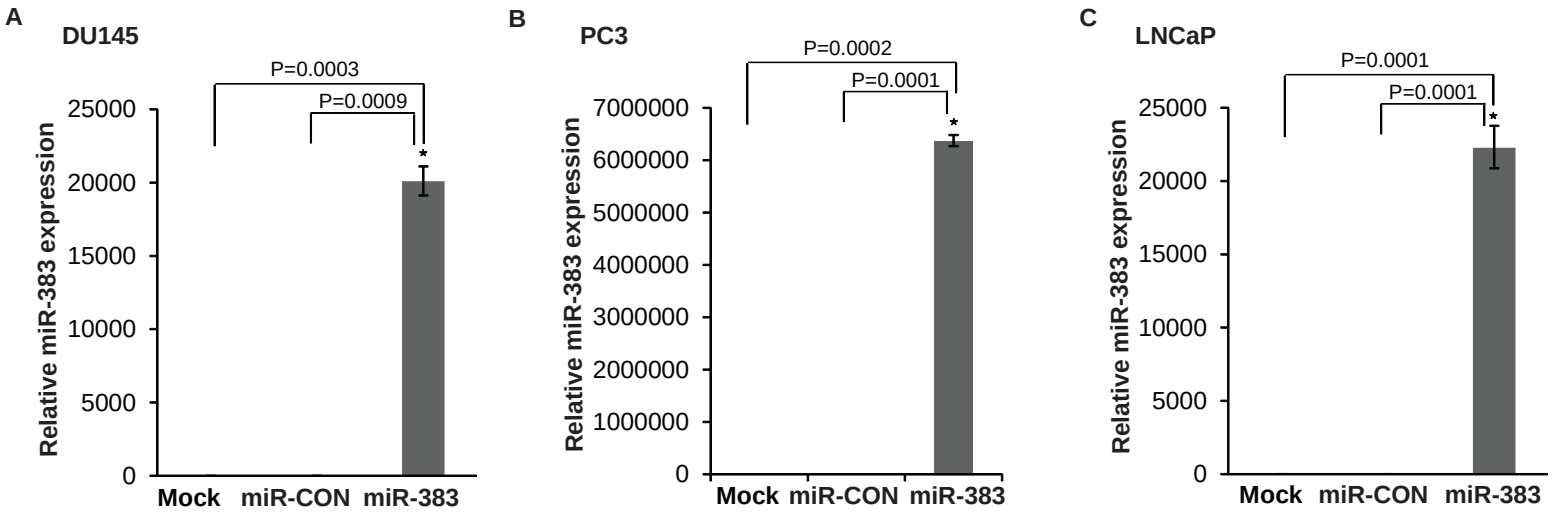

Fig. S2 miR-383 silencing induces tumorigenicity in normal prostate epithelial cells

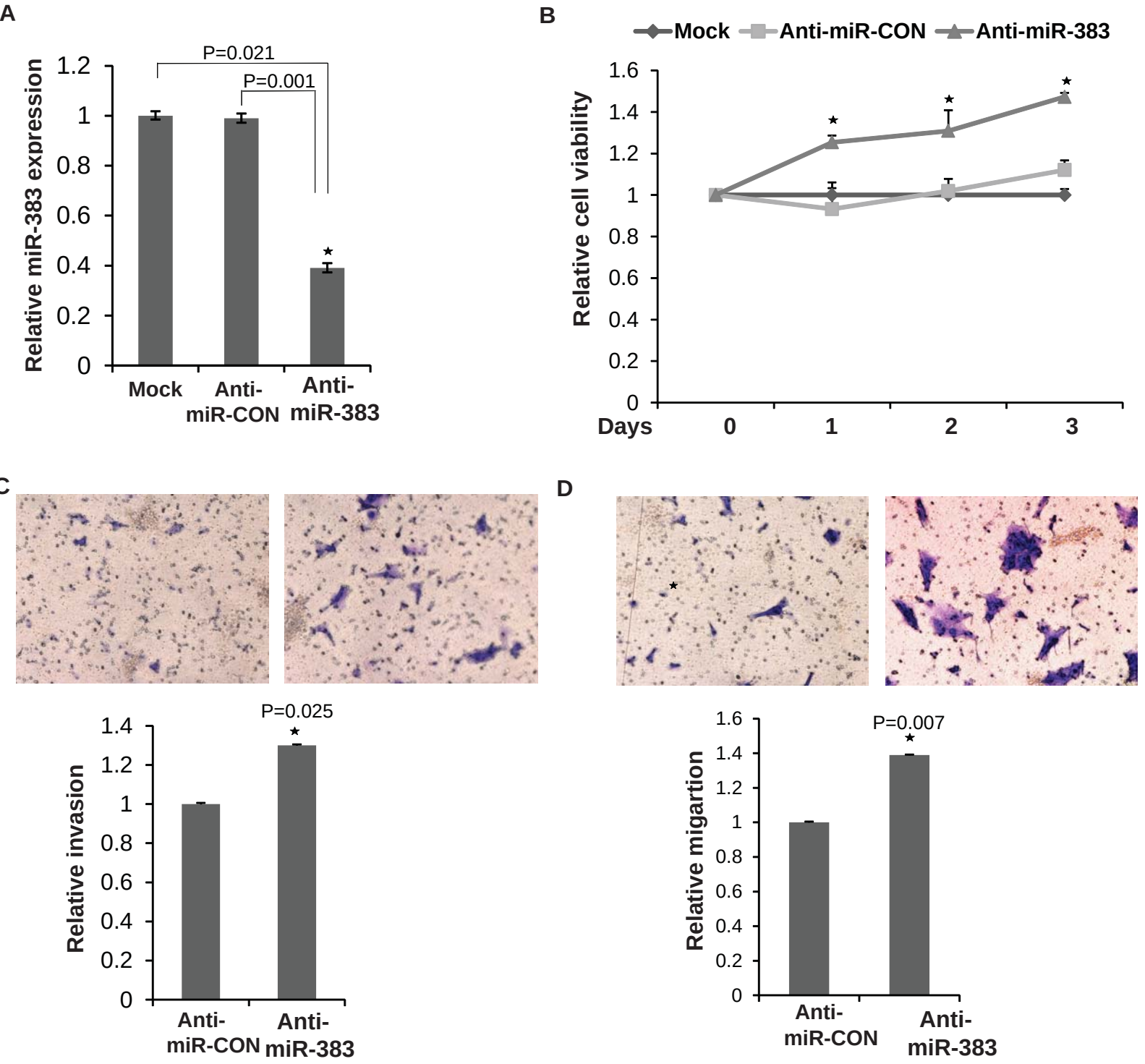

Fig. S3 miR-383 and stemness related gene targets

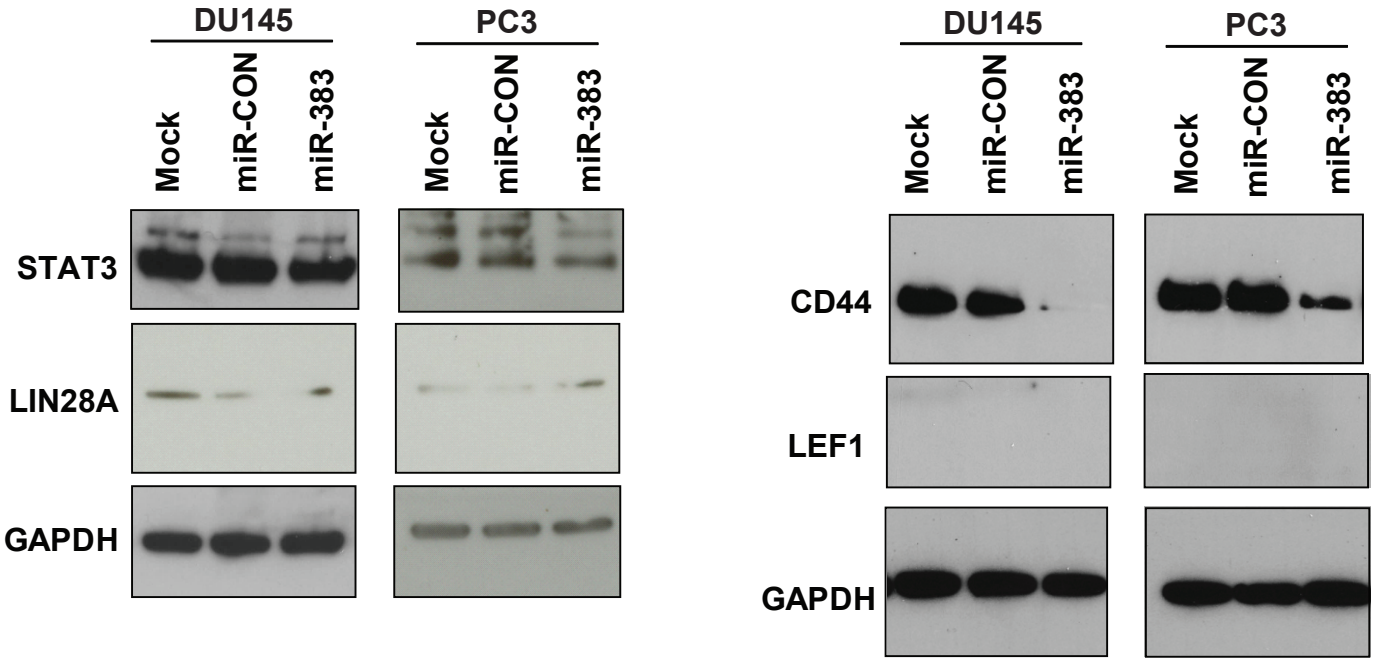

Fig. S4 miR-383 directly regulates prostate cancer stem cell marker CD44 in LNCaP cells

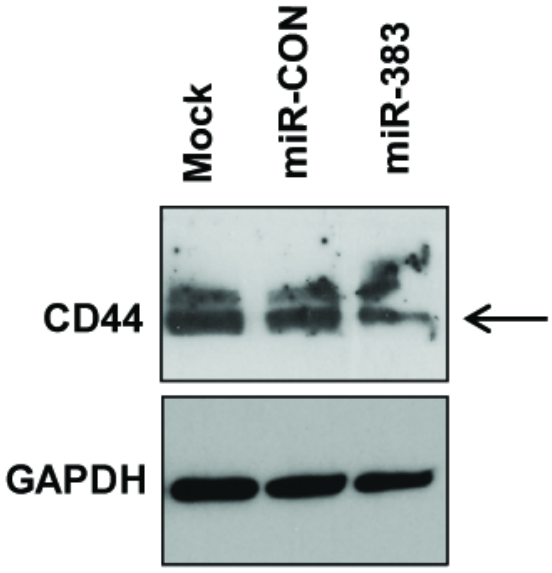

Fig. S5 CD44+ cells in mice xenograft tumors derived from prostate cancer cell lines

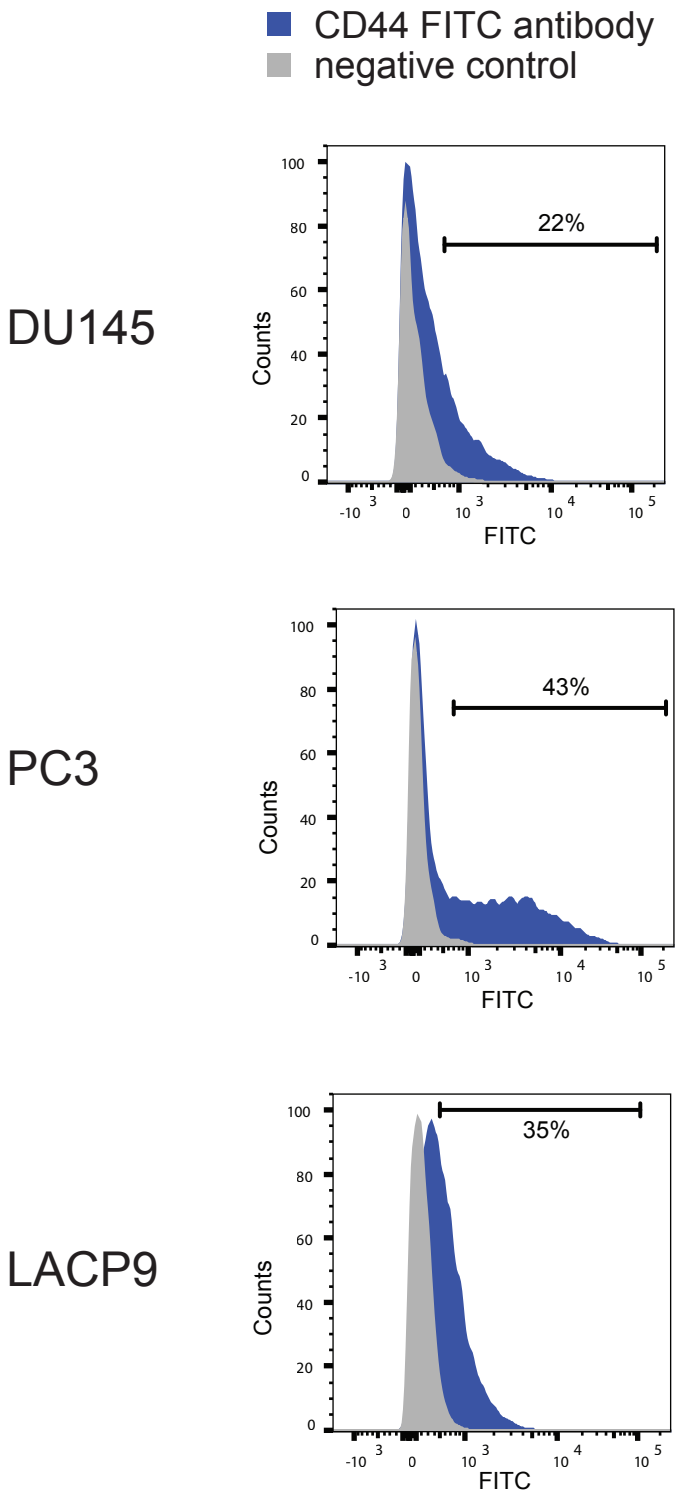

Table S1 Clinicopathologic characteristics of prostate cancer patients.

	SFVAMC cohort (N=112*)	TCGA cohort (N=187**)
Characteristic	**N (%)**	**N (%)**
Age, Years		
Mean	63	65
Median	61.5	66
Range	49-83	44-73
T-stage		
pT2	75 (67)	-
pT3	23 (21)	-
pT4	1 (1)	2 (6)
Gleason Score		
4-6	56 (50)	1 (3)
7 (3+4)	34 (30)	13 (33)
7 (4+3)	11 (10)	13 (33)
8-10	9 (8)	22 (55)
PSA		
Median	4.0	17.15
<median	18 (16)	15 (38)
>median	85 (76)	15 (38)
Biochemical recurrence (PSA failure)	39 (35)	No information
N-stage		
N0/NX	111 (99)	40 (100)
N1	1 (1)	
M-stage		

M0/MX	112 (100)	35 (88)
M1	-	5 (12)
Pathological diagnosis		
Adenocarcinoma	112 (100)	40 (100)

* Information not available for SFVAMC cohort: PSA values for 9, Gleason score for 2, tumor stage for 11, recurrence for 24 cases.

** Information not available for TCGA cohort: Age for 61, PSA for 69, Gleason score for 61, tumor stage for 33, recurrence for 30 cases.

Supplemental Table S2 Multivariate regression analyses for miR-383 expression and known prognostic factors

A. Multiple Regression Enter Method

Method	Enter
Sample size	86
Coefficient of determination R^2	0.2295
R^2-adjusted	0.1915
Multiple correlation coefficient	0.4791
Residual standard deviation	8.1262

Regression Equation

Independent variables	Coefficient	Std. Error	$r_{partial}$	t	P	VIF
miR_383_expression	-1.2082	0.2511	-0.4715	-4.812	<0.0001	1.031
Gleason	-1.9721	1.5216	-0.1425	-1.296	0.1986	1.311
Stage	0.03042	2.3248	0.001454	0.0131	0.9896	1.299
PSA_Dx	-0.07864	0.1613	-0.05409	-0.488	0.6272	1.177

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	4	1593.6273	398.4068
Residual	81	5348.8311	66.0350

F-ratio	6.0333
Significance level	P=0.0003

Zero order and simple correlation coefficients

Variable	Time	miR_383_expression	Gleason	Stage
miR_383_expression	-0.4496			
Gleason	-0.09616	-0.1351		
Stage	-0.04677	-0.06739	0.4420	
PSA_Dx	-0.02773	-0.1459	0.3186	0.3174

B. Multiple Regression Forward Method

Method	Forward
Enter variable if P<	0.05
Remove variable if P>	0.1

Sample size	86
Coefficient of determination R^2	0.2021
R^2-adjusted	0.1926
Multiple correlation coefficient	0.4496
Residual standard deviation	8.1205

Regression Equation

Independent variables	Coefficient	Std. Error	$r_{partial}$	t	P	VIF
miR_383_expression	-1.1398	0.2471	-0.4496	-4.613	<0.0001	1.000

Variables not included in the model
Gleason
PSA_Dx
Stage

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	1	1403.3389	1403.3389
Residual	84	5539.1195	65.9419

F-ratio	21.2814
Significance level	P<0.0001

Zero order and simple correlation coefficients

Variable	Time	Gleason	PSA_Dx	Stage
Gleason	-0.09616			
PSA_Dx	-0.02773	0.3186		
Stage	-0.04677	0.4420	0.3174	
miR_383_expression	-0.4496	-0.1351	-0.1459	-0.06739

C. Multiple Regression Backward Method

Method	Backward
Enter variable if P<	0.05
Remove variable if P>	0.1

Sample size	86
Coefficient of determination R^2	0.2021
R^2-adjusted	0.1926
Multiple correlation coefficient	0.4496
Residual standard deviation	8.1205

Regression Equation

Independent variables	Coefficient	Std. Error	$r_{partial}$	t	P	VIF
miR_383_expression	-1.1398	0.2471	-0.4496	-4.613	<0.0001	1.000

Variables not included in the model
PSA_Dx
Stage
Gleason

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	1	1403.3389	1403.3389
Residual	84	5539.1195	65.9419

F-ratio	21.2814
Significance level	P<0.0001

Zero order and simple correlation coefficients

Variable	Time	miR_383_expression	PSA_Dx	Stage
miR_383_expression	-0.4496			
PSA_Dx	-0.02773	-0.1459		
Stage	-0.04677	-0.06739	0.3174	
Gleason	-0.09616	-0.1351	0.3186	0.4420

D. Multiple Regression Stepwise Method

Method	Stepwise
Enter variable if P<	0.05
Remove variable if P>	0.1

Sample size	86
Coefficient of determination R^2	0.2021
R^2-adjusted	0.1926
Multiple correlation coefficient	0.4496
Residual standard deviation	8.1205

Regression Equation

Independent variables	Coefficient	Std. Error	$r_{partial}$	t	P	VIF
miR_383_expression	-1.1398	0.2471	-0.4496	-4.613	<0.0001	1.000

Variables not included in the model
PSA_Dx
Stage
Gleason

Analysis of Variance

Source	DF	Sum of Squares	Mean Square
Regression	1	1403.3389	1403.3389
Residual	84	5539.1195	65.9419

F-ratio	21.2814
Significance level	P<0.0001

Zero order and simple correlation coefficients

Variable	Time	miR_383_expression	PSA_Dx	Stage
miR_383_expression	-0.4496			
PSA_Dx	-0.02773	-0.1459		
Stage	-0.04677	-0.06739	0.3174	
Gleason	-0.09616	-0.1351	0.3186	0.4420

Age	PSA limit	Serum PSA*							
		<upper limit				>upper limit			
			miR-383 expression N(%)				miR-383 expression N(%)		
		Total (N=24)	Low	No change	High	Total (N=79)	Low	No change	High
40-49	≤2.5	1	1/1 (100)	-	-	3	3/3 (100)	-	-
50-59	≤3.5	5	3/5 (60)	1/5 (20)	1/5 (20)	30	22/30 (73)	2/30 (7)	6/30 (20)
60-69	≤4.5	12	5/12 (42)	-	7/12 (58)	34	28/34 (82)	5/34 (15)	1/34 (3)
70-79	≤6.5	6	4/6 (67)	-	2/6 (33)	11	10/11 (91)	1/11 (9)	-
80-89	≤7.2	-	-	-	-	1	1/1 (100)	-	-
Totals		24/112 (21)	13/24 (54)	1/24 (4)	10/24 (42)	79/112 (71)	64/79 (81)	8/79 (10)	7/79(9)

Table S3 Correlation of miR-383 expression with serum PSA levels

* Serum PSA data was available for a total of 103 samples out of 112 analysed.

miR-383 expression is correlated with age-adjusted PSA levels in SFVAMC cohort of tissues (P= 0.0012).

Table S4 miR-383 and stemness related gene targets

```
hsa-miR-383          3′  UCGGUGU-UAGUGGAAGACUAGA 5′ miRSVR score -0.0075
                                    ||||||
CD44              301:5′..CUACACAUAUGUAUUCCUGAUCG.. 3′

hsa-miR-383           3′  UCGGUGUUAGUGGAAGACUAGA 5′ miRSVR score -0.0008
                                   ||||||
STAT3              1264:5′..CCGACCCCAGUCCCCCUGAUCC.. 3′

hsa-miR-383           3′  UCGGUGUUAGUGGAAGACUAGA 5′  miRSVR score -0.0133
                                   ||||||
STAT3              826:5′..ACCACCUUGCCUCAGCUGAUCA.. 3′

hsa-miR-383           3′ UCGGUGUUAGUGGAAGACUAGA 5′  miRSVR score -0.0963
                                   |||||||
LIN28             1538:..AUUGGGUGGUGUGUGUCUGAUCC.. 3′

hsa-miR-383           3′ UCGGUGUUAGUGGAAGACUAGA 5′  miRSVR score -0.0296
                                   ||||||
LIN28             3030:  ..CUGUCUGUUUUUUUCCUGAUCC.. 3′

hsa-miR-383           3′ UCGGUGUUAGUGGAAGACUAGA 5′  miRSVR score -0.0530
                                   ||||||
LEF1              156:5' ..UCACUGCUAGAGACGCUGAUCC 3′
```

Primer	Sequence (5’-3’)
CD44 sense	AAACTAGCGGCCGCTAGTCTACACATATGTATTCCTGATCGCCAACCTTTCCCCCT
CD44 antisense	CTAGAGGGGGAAAGGTTGGCGATCAGGAATACATATGTGTAGACTAGCGGCCGCTAGTTT
CD44 mutant-sense	AAACTAGCGGCCGCTAGT CTACACATATGTATTCGTCATGG CCAACCTTTCCCCCT
CD44 mutant-antisense	CTAGAGGGGGAAAGGTTGGCCATGACGAATACATATGTGTAGACTAGCGGCCGCTAGTTT

Table S5 List of primers used for CD44 3’ UTR cloning

Fig. S6 CD44 knockdown induces G0-G1 cell cycle arrest in Du145 cell line