The cancer-associated cell migration protein TSPAN1 is under direct control of androgens and its upregulation increases prostate cancer cell migration

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Supplementary Information:

Supplementary Data

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Supplementary Figure 3: (A) Overall survival and relapse free survival for patients with either low or high TSPAN1. (B) TSPAN1 histoscores correlation with prostate cancer patient clinical parameters: i) PSA levels at patient relapse, ii) hormone sensitive or castrate resistant disease.

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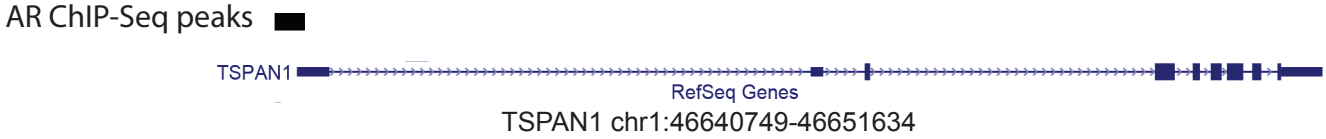
Full length western blots are also included for supplementary information

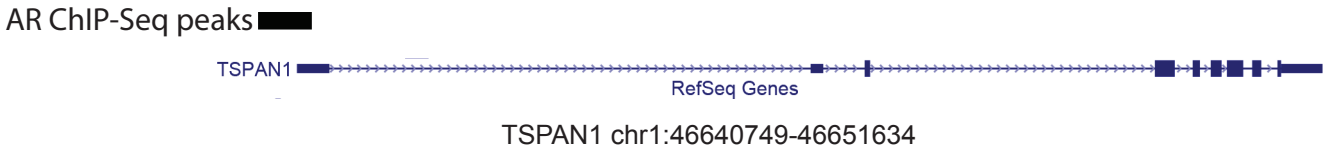
Supplementary Figure 1

Androgen Receptor ChIP-Seq peaks in LNCaP and VCaP cells identified by Massie et al. 2011

LNCaP cells



VCaP cells



Predicted AR binding sites:

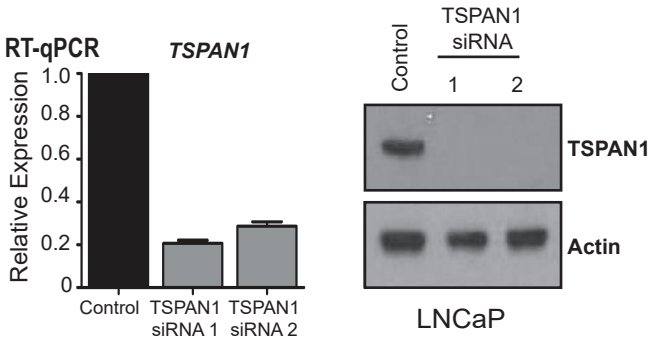
LNCaP cells: chr1: 46640495-46640802

VCaP cells: chr1:46640361-46640945

Supplementary Figure 2

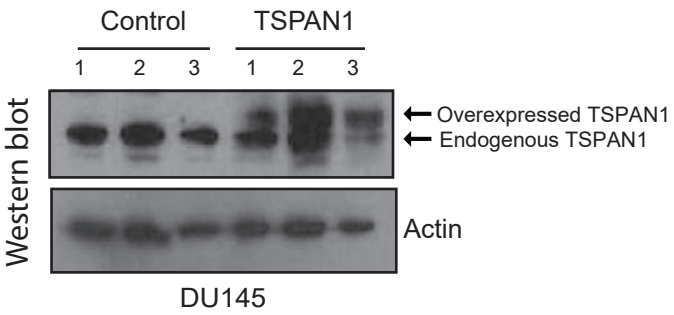
TSPAN1 antibody validation

A

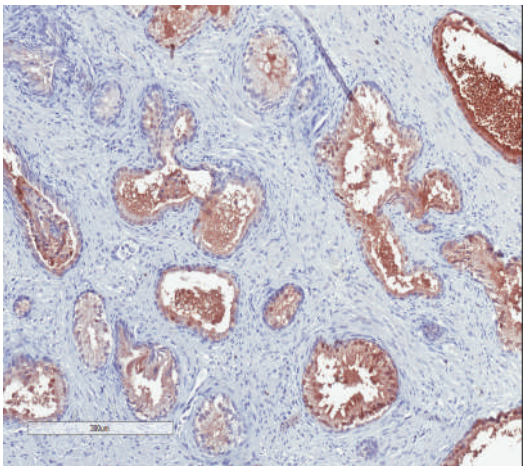


(duplicated from Figure 3A)

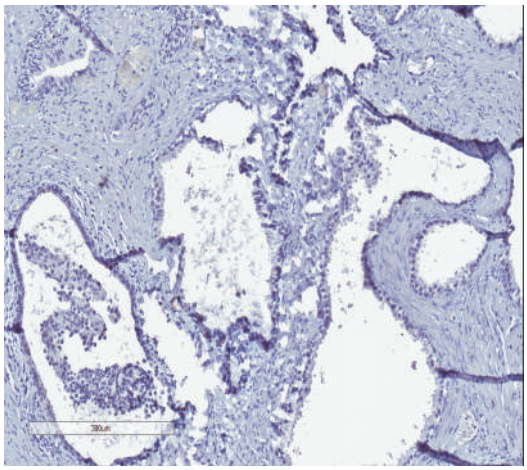
B



C



Prostate cancer tissue stained with TSPAN1 antibody at 1:1500

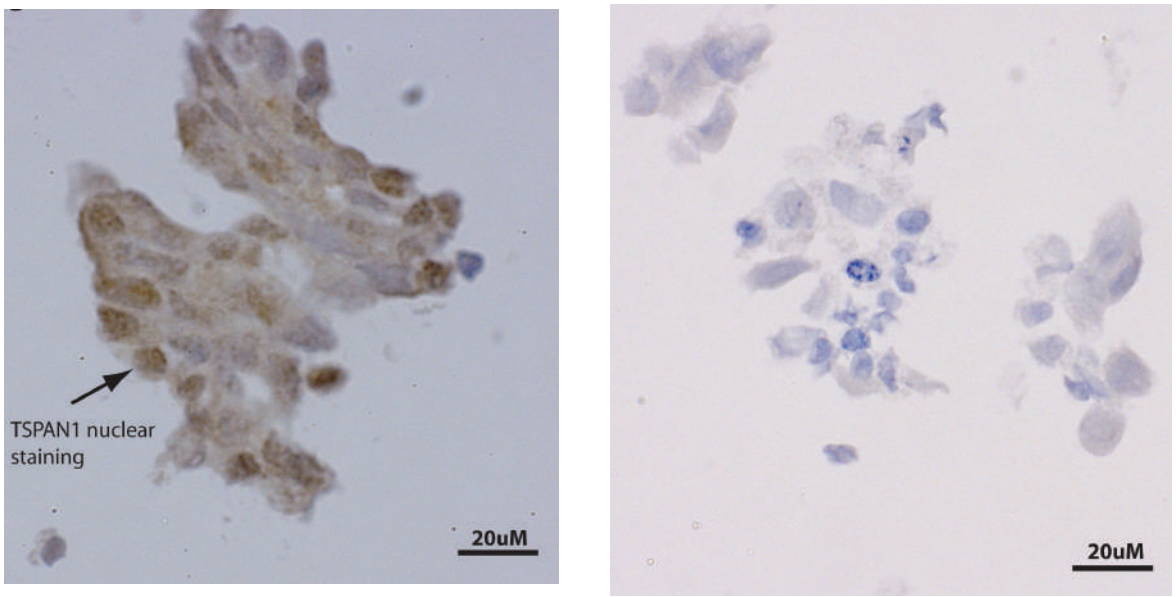


Prostate cancer tissue stained with TSPAN1 antibody at 1:1500 following pre-incubation with TSPAN1 immunising peptide

D

Control siRNA

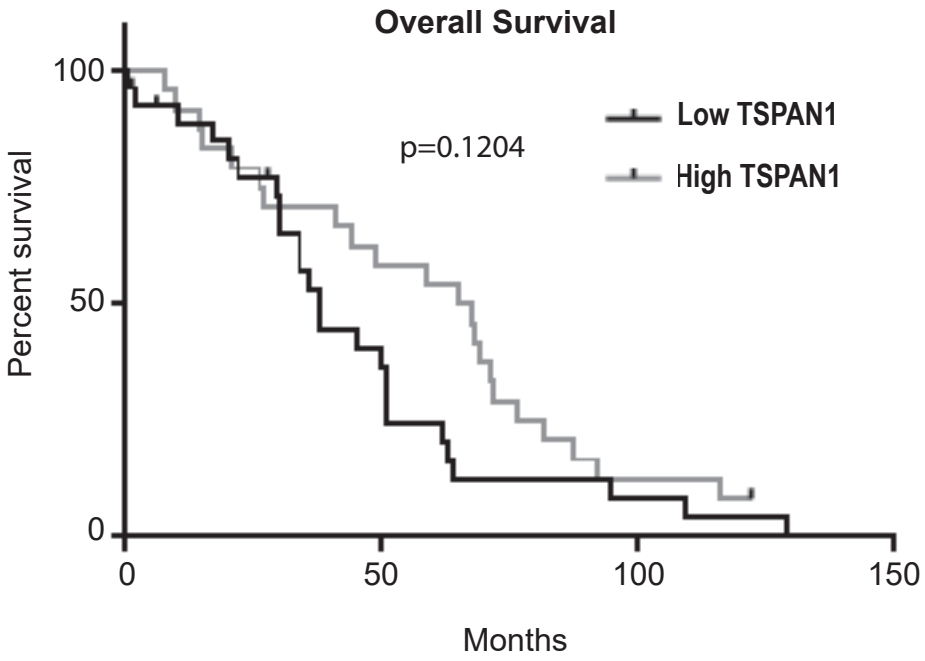
TSPAN1 siRNA

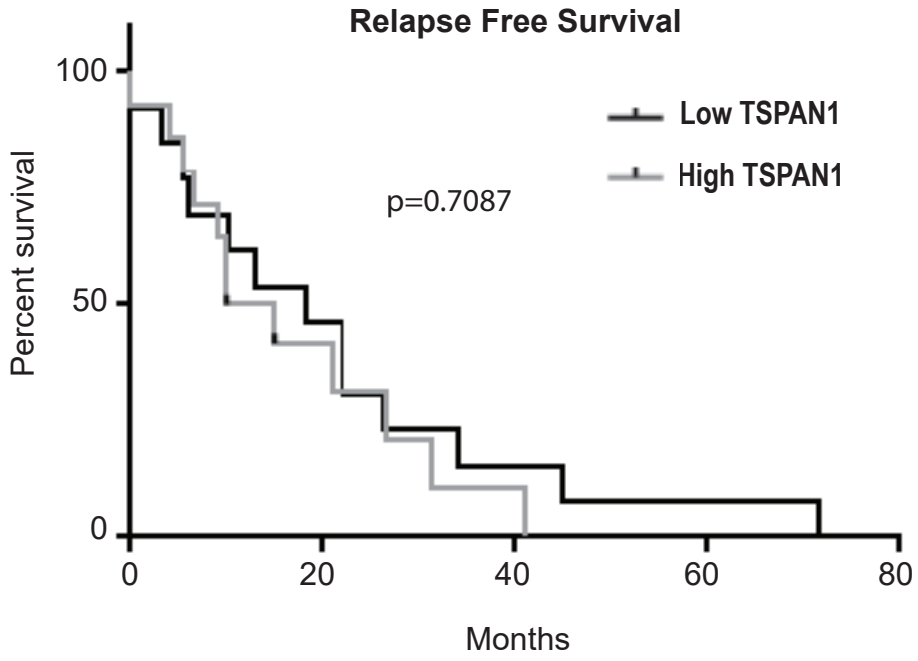


Detection of siRNA mediated depletion of TSPAN1 in FFEP LNCaP cell pellets stained with TSPAN1 antibody

Supplementary Figure 3A

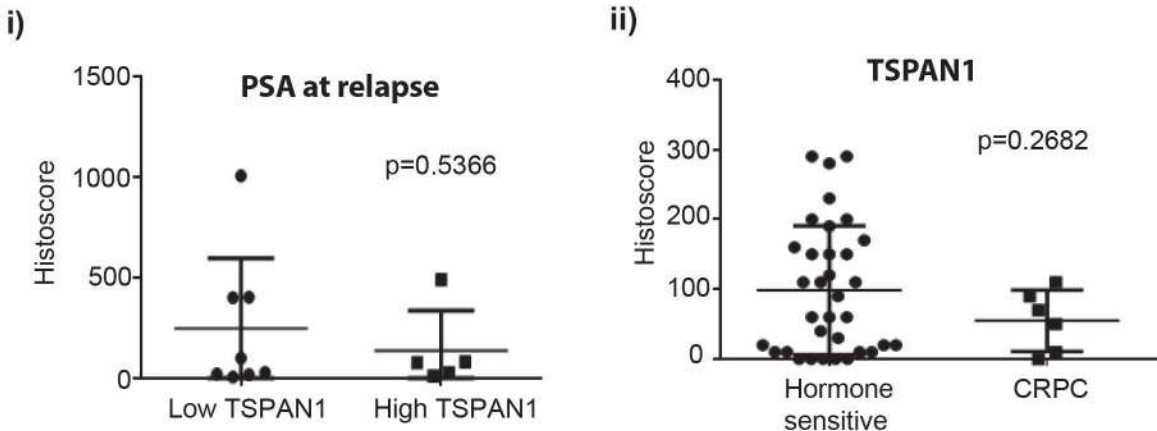
Overall survival and relapse free survival for patients with either low or high TSPAN1





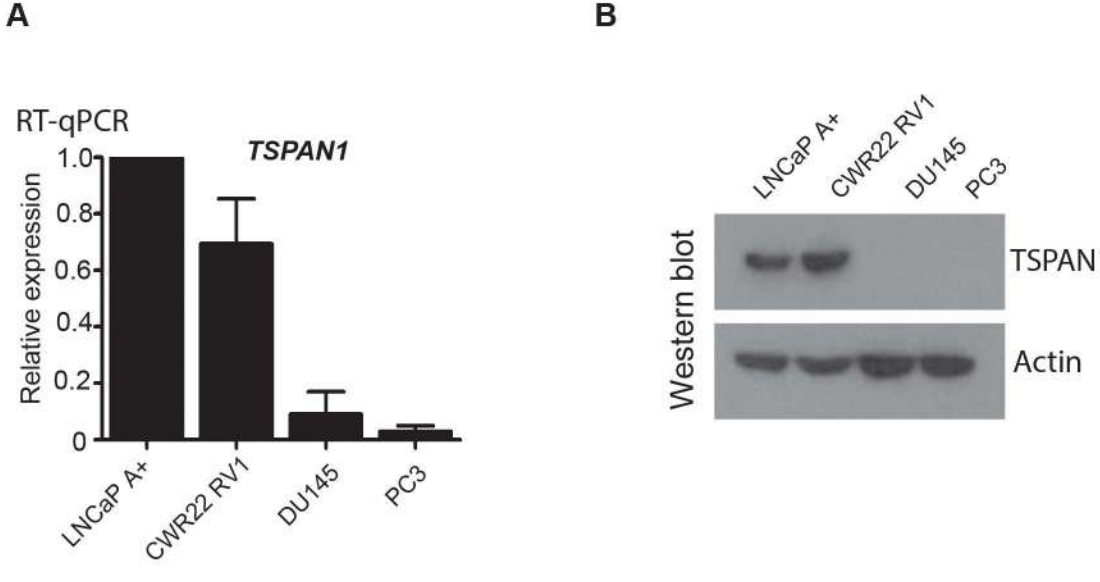
Supplementary Figure 3B

TSPAN1 histoscores correlation with prostate cancer patient clinical parameters



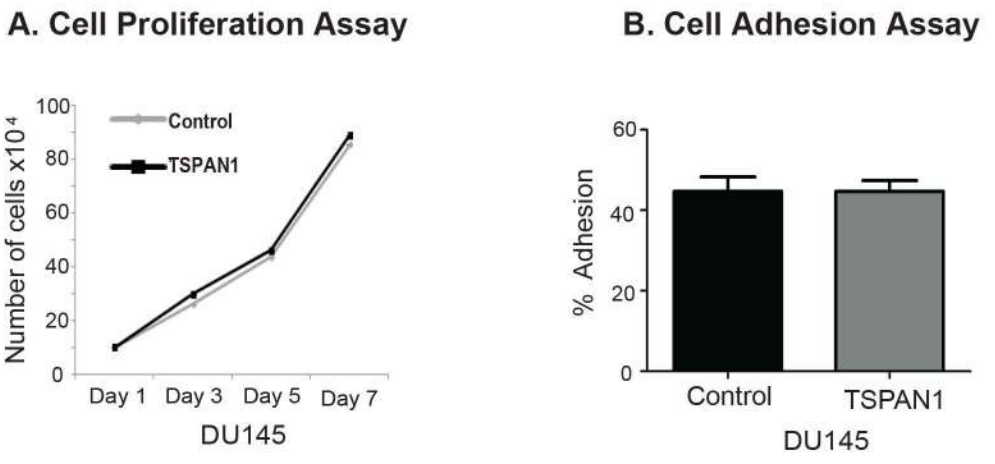
Supplementary Figure 4

Relative TSPAN1 expression levels in different prostate cancer cell lines



Supplementary Figure 5

Analysis of cell proliferation and cell adhesion in DU145 cells stably transfected with TSPAN1



Munkley et al. Full western blot images

The cancer-associated cell migration protein TSPAN1 is under direct control of androgens and its upregulation increases prostate cancer cell migration

Figure 1D

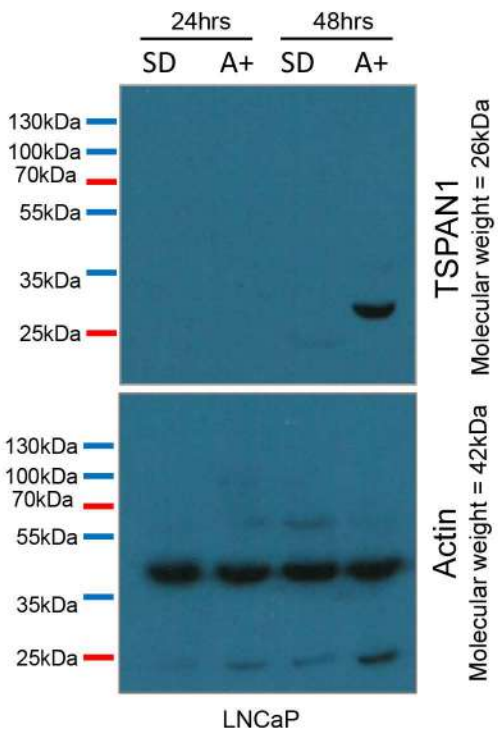


Figure 1E

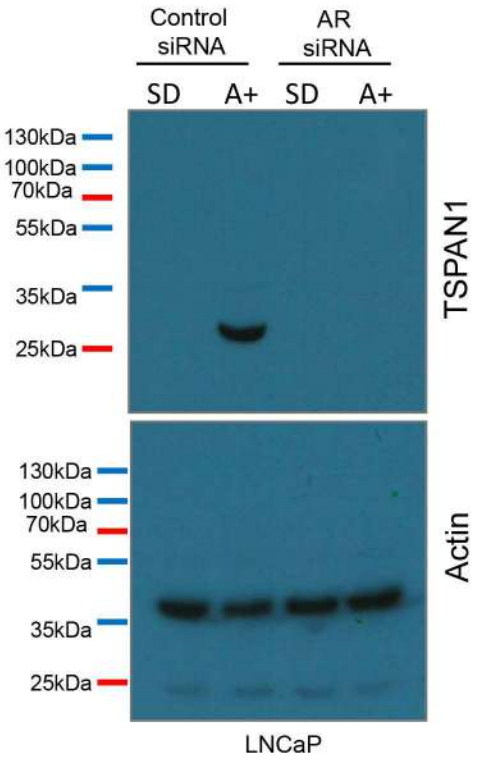


Figure 1F

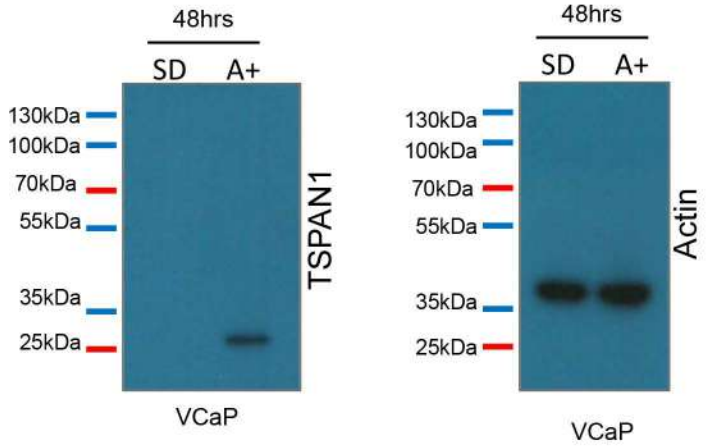


Figure 3C

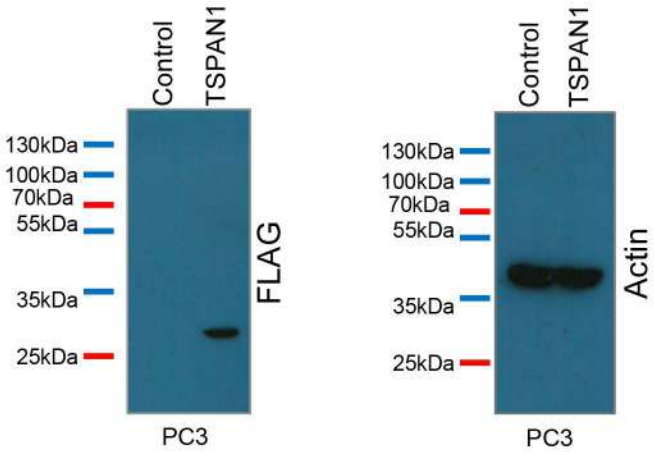


Figure 3E

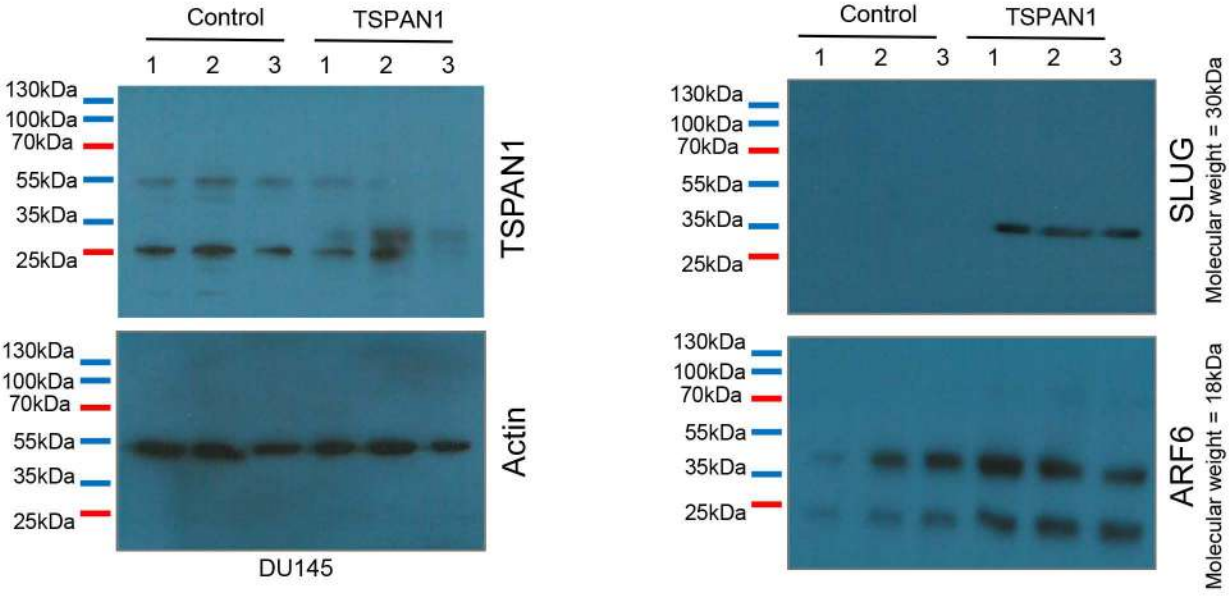


Figure 4A

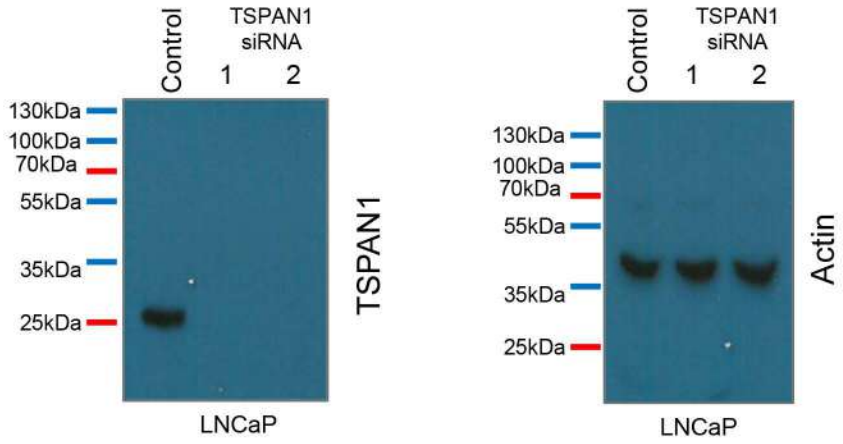
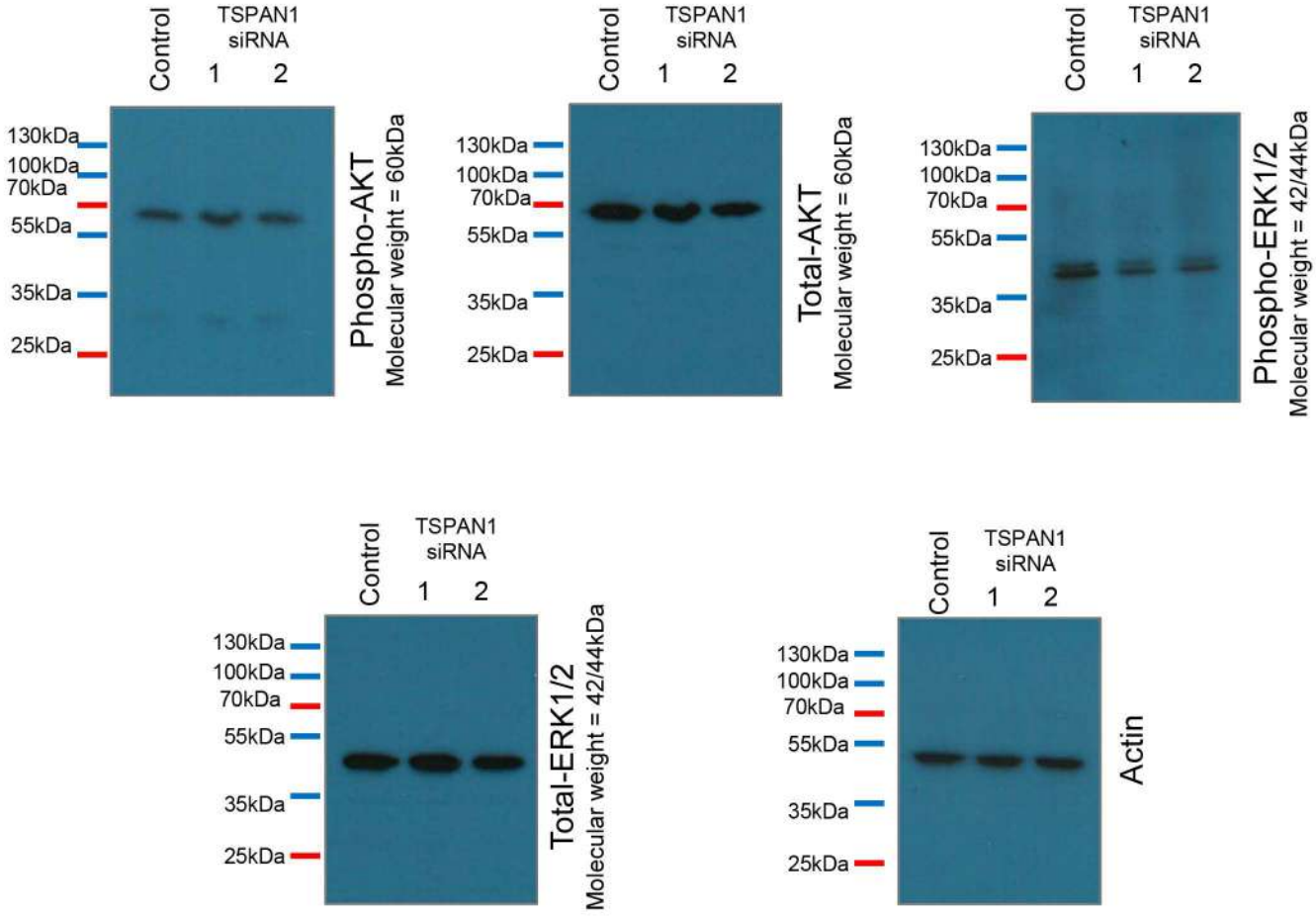


Figure 4C



Supplementary Table 1: Meta-analysis of *TSPAN1* expression in prostate cancer

1012 samples

	Grasso et al. 2012	**Taylor et al. 2010**	**Singh et al. 2002**
	Prostate carcinoma v normal	Prostate carcinoma v normal	Prostate carcinoma v normal
	122 samples	185 samples	102 samples
Fold Change	2.214	1.844	8.438
p value	1.31E-06	1.72E-07	3.98E-08

	Yu et al. 2004	**Vanaja et al. 2003**	**LaTulippe et al. 2002**
	112 samples	40 samples	35 samples
	Prostate carcinoma v normal	Prostate carcinoma v normal	Prostate carcinoma v normal
Fold Change	2.322	2.213	7.751
p value	2.55E-05	4.64E-05	0.123

	Wallace et al. 2008	**Arredouani et al. 2009**	**LaPointe et al. 2004**
	Prostate carcinoma v normal	Prostate carcinoma v normal	Prostate carcinoma v normal
	89 samples	21 samples	112 samples
Fold Change	2.276	1.612	1.59
p value	2.00E-03	0.049	4.19E-04

	Luo et al. 2002	**Holzbeierlein et al. 2004**	**Welsh et al. 2001**
	Prostate carcinoma v normal	Prostate carcinoma v normal	Prostate carcinoma v normal
	30 samples	54 samples	34 samples
Fold Change	1.41	1.366	3.971
p value	1.15E-01	2E-01	4.33E-05

	Varambally et al. 2005	**Liu et al. 2006**
	19 samples	Prostate carcinoma v normal
	Prostate carcinoma v normal	57 samples
Fold Change	2.303	1.574
p value	0.007	8.87E-04

TSPAN1 significantly upregulated in 11/14 datasets

Supplementary Table 2: Basic clinical data for patient tissue samples used in TMA.

Samples used in this study were collected between January 1989 and June 2003 from the Freeman Hospital patients. All samples were first analysed by a trained pathologist before selection of cores for TMA construction was performed.

Gleason	4 n=1	5 n=1	6 n=5	7 n=11	8 n=17	9 n=24	10 n=7	uk n=11
Age at diagnosis	40-49 n=1	50-59 n=5	60-69 n=12	70-79 n=28	80-89 n=4			uk n=26
Stage	T1 n=16	T1B n=7	T2 n=11	T3 n=24	T3C n=3	T4 n=11	T4B n=1	uk n=4
PSA	<10 n=8	10-19 n=11	20-29 n=4	30-49 n=10	50-99 n=6	100-200 n=4	<200 n=4	Uk n=30

Supplementary Table 3: Real-time PCR screen for 72 cell motility genes in DU145 TSPAN1 stable cell line

10 genes with significant changes:

TSPAN1	RQ	SE
Control	1.00	0.00
TSPAN1	6.62	2.16

rac2	RQ	SE
Control	1	
TSPAN1	2.01	0.19

actn1	RQ	SE
control	1.00	
TSPAN1	1.95	0.20

ARF6	RQ	SE
control	1.00	
TSPAN1	2.04	0.39

DIAPH1	RQ	SE
control	1.00	
TSPAN1	2.28	0.27

ICAM1	RQ	SE
control	1.00	
TSPAN1	2.00	0.28

SNAI2/Slug	RQ	SE
control	1.00	
TSPAN1	3.27	0.40

actn3	RQ	SE
control	1.00	
TSPAN1	0.65	0.11

cav1	RQ	SE
Control	1.000	
TSPAN1	0.500	0.05

ptk2b	RQ	SE
Control	1.000	
TSPAN1	0.510	0.03

pxn	RQ	SE
Control	1	
TSPAN1	0.36	0.030

67 genes with no change:

wasf2	capn1	stat3
fgf2	msn	baiap2
vegfa	myl9	plcg1
pld1	rock1	svil
myh9	ptk2	rnd3
itgb1	tln1	rdx
wipf1	ezr	crk
mapk1	vasp	rac1
igfr1	pak4	limk1
egfr	pfn1	pak1
met	tmp2	twist2
plaur	arhgdia	snai1
itgb3	ptpn1	vim
itgb2	akt1	cdh1
rac2	actn4	cdh2
prkca	actr2	
capn2	wasf1	
mmp14	fap	
ptk2	cfl1	
pten	pik3ca	
csf1	actr3	
enah	sh3pxd2a	
ilk	cttn	
vcl	mylk	
rasa1	cdc42	
bcar1	egf	

Supplementary Table 4: Differential analysis of somatic copy number and gene expression in neuroendocrine prostate cancer

Beltran et al. 2016

	% CRPC-NE deletion	% CRPC-Adeno deletion
TSPAN1	0.023255814	0.037735849
ARF6	0.093023256	0
SLUG	0.069767442	0

	% CRPC-NE amplification	% CRPC-Adeno amplification
TSPAN1	0.279069767	0.283018868
ARF6	0.325581395	0.377358491
SLUG	0.372093023	0.58490566

Supplementary Table 5: Real-time PCR primer sequences

	Forward	Reverse
TSPAN1	GAAAGGGCTCAAGTGCTGTG	TCATTGGCTGTGTTGGTGAC
rac2	GTTCCCCTTCCGTTTCCTC	ATCTCCCACCACCACACACT
actn1	GCTCCTGTGGTGTCAGAGAA	GCTTCCCGTAGTCAATCAGC
ARF6	CGCCATAATCCTCATCTTCG	TCATAGAGTCCGTCCCCTGA
DIAPH1	AGTCCCTTCGTGTGTCTCTCA	GGCTATCGTAACTCCCAGCA
ICAM1	CACTCTGGCTCCTTCACCAT	*AACCACTGACTCCCCTTCCT*
SNAI2/Slug	TGTCATACCACAACCAGAGA	CTTGGAGGAGGTGTCAGAT
actn3	CAGAGGAAGACAGCACCGTA	CTTTCGCAGTTTGGCGTAGT
cav1	ACTGGTTTTACCGCTTGCTG	GGAATAGACACGGCTGATGC
ptk2b	ATCATCACCTCCATCCTGCT	TCATACCTCCACTCGGCTTC
pxn	GGCTCTCTCATCATCCATCC	TTCAGGACCACATCTTCACG
Actin	CATCGAGCACGGCATCGTCA	TAGCACAGCCTGGATAGCAAC
GAPDH	AAC AGC GAC ACC CAT CCT C	CAT ACC AGG AAA TGA GCT TGA CAA
Btubulin	CTTCGGCCAGATCTTCAGAC	AGAGAGTGGGTCAGCTGGAA

Supplementary Table 6: Concentration of antibodies used

Antibody	Supplier	Product number	Concentration for WB	Concentration for IHC
TSPAN1	Sigma-Aldrich	HPA011909	1 to 2000	1 to 1500
AR	BD Bioscience	554226	0.5 µg/ml	
p21 Waf1/Cip1	Cell Signaling	2947	1 to 1000	
Slug	Cell Signaling	9585	1 to 1000	
p44/42 MAPK	Cell Signaling	9106	1 to 250	
Total ERK2	Santa Cruz	sc-1647	0.2 µg/ml	
pAKT	Sigma-Aldrich	SAB4300042	1 to 1000	
Total AKT	Santa Cruz	sc-8312	0.2 µg/ml	
ARF6	Cell Signaling	5740	1 to 1000	
Flag	Sigma-Aldrich	F3165	1 to 2000	
Actin	Sigma-Aldrich	A2668	1 to 2000	
normal rabbit IgG	Jackson labs	711-035-152	1 to 2000	
normal mouse IgG	Jackson labs	715-036-150	1 to 2000	