Supplementary Data

TUSC3 Loss Alters the ER Stress Response and Accelerates Prostate Cancer Growth *in vivo*

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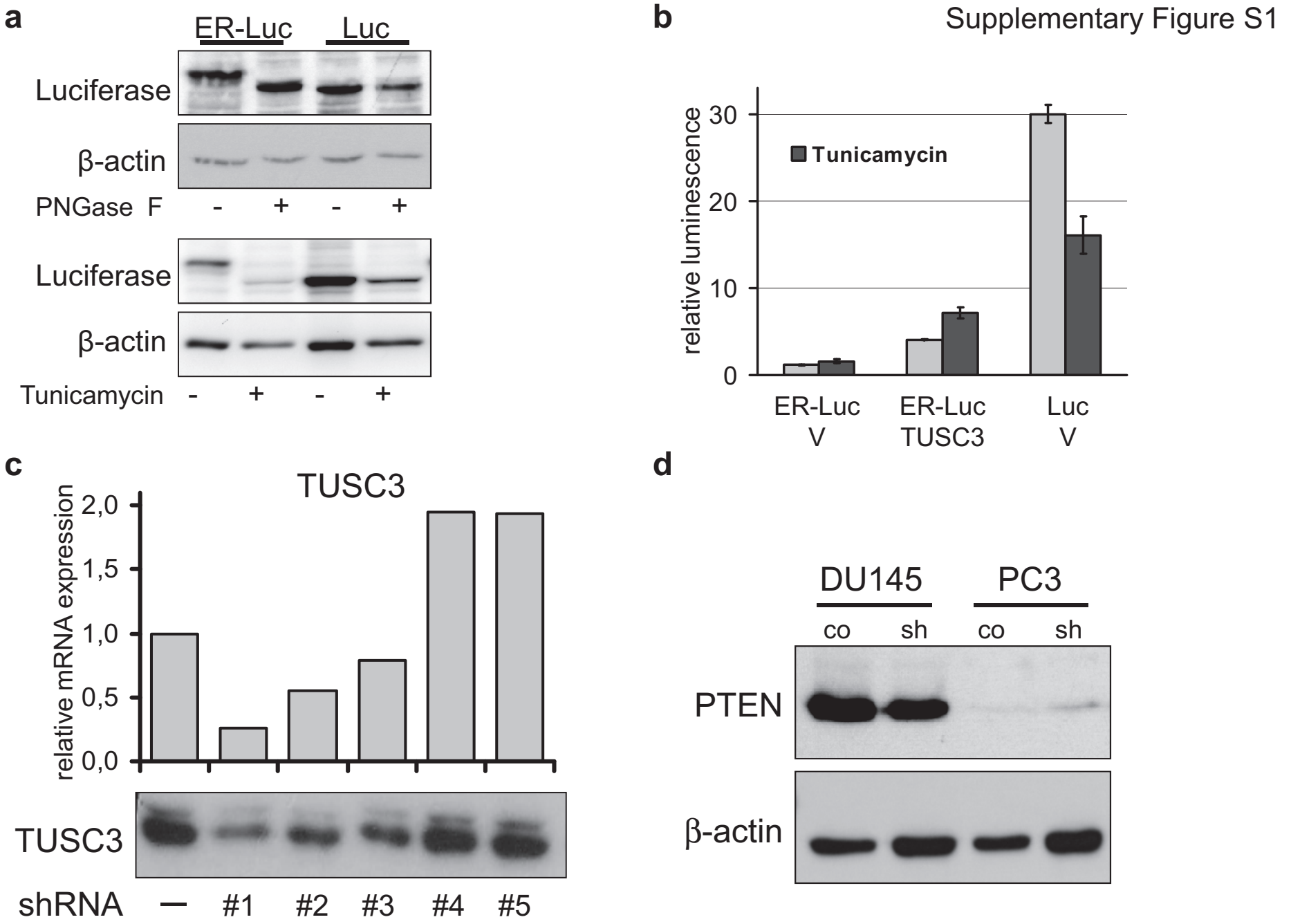
Supplementary Figure Legends

Supplementary Figure S1. (**a**) Modified, ER targeted luciferase (ER-Luc) causes an electrophoretic gel shift corresponding to increased glycosylation in HEK293T cells. Treatment of cell lysates with N-Glycosidase F (PNGase F), an amidase that cleaves between the GlcNAc and asparagine residues, or treatment of cells with 5μM tunicamycin leads to deglycosylation of the ER-Luc and molecular weight shift. (**b**) HEK293T cells were co-transfected with wild type luciferase (Luc) or ER-Luc and pcDNA 3.1 vector with (TUSC3) or without (V) full length TUSC3 and seeded in 96-well plates. Transfected cells were treated with 0.5μM tunicamycin for 24h before measurement. Luminesence was measured 48 hours after transfection and normalized to the cell count. The increase in luciferase activity in ER-Luc was less than twofold, though significant. Tunicamycin driven induction of UPR might have an inhibitory effect on protein translation, lessening the effects observed in this experiment. (**c**) Knockdown efficiency of 5 different short hairpin RNAs (cloned in pLKO.1 puro vector) against TUSC3 was measured in HEK293T cells. shRNA #1 was used in further silencing experiments by lentiviral delivery in prostate cancer cell lines. mRNA and protein expression are shown. (**d**) Knockdown of TUSC3 (sh) in prostate cancer cell lines DU145 and PC3 does not influence PTEN expression levels.

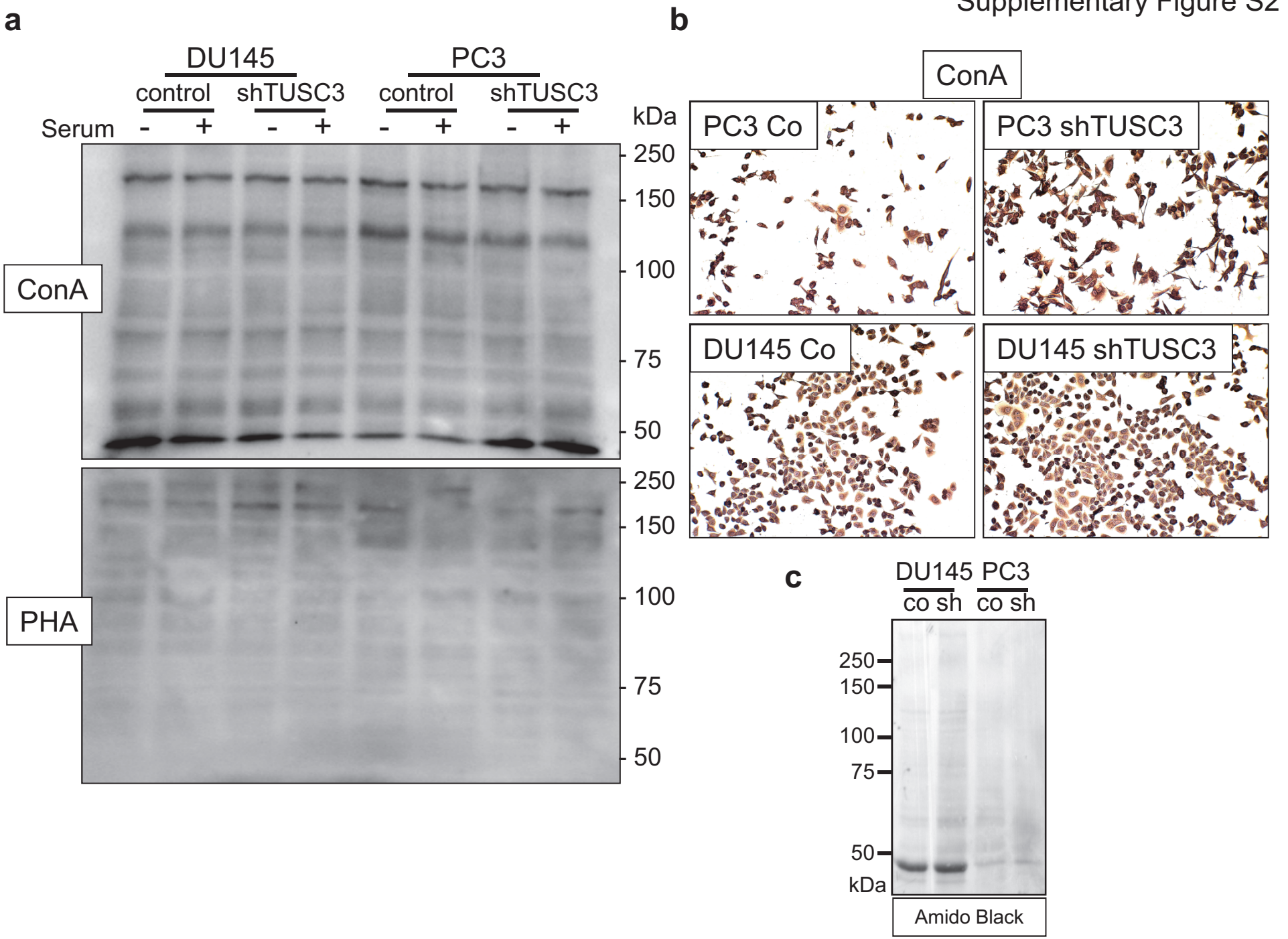
Supplementary Figure S2. Lectin binding in prostate cancer cell lines. (**a**) Lectin blotting using Concanavalin A and Phytohaemagglutinin-L HRP conjugates after SDS-PAGE shows no differences in lectin binding to whole cell protein lysates between control and shTUSC3 prostate cancer cell lines. Binding did not differ in full serum conditions (+) and was not affected by serum starvation for 72 hours (-). (**b**) Lectin cytochemistry with Concanavalin A HRP conjugate (ConA) in prostate cancer cell lines did not detect significant differences in staining intensity or distribution. Magnification 200x. (**c**) A loading control for the lectin blots of cell surface protein lysates of prostate cancer cells after serum starvation. Amido black was used for visualizing the proteins on the membrane.

Supplementary Figure S3. Prostate cancer TMA and *in silico* analysis of TCGA data. (**a**) Representative immunohistochemical TUSC3 stainings of tissue microarray samples from prostate cancer patients. Absent (0), weak (1) moderate (2) and strong (4) stainings were observed. 40x magnification. (**b**) Pie chart of immunohistochemical TUSC3 expression in prostate cancer tissue (TMA) and copy number analysis of TCGA data, showing homozygous (del), heterozygous (het) deletions as well as wild-type copy numbers for *TUSC3*. (**c**) Significant negative correlation between *TUSC3* mRNA expression and extent of *TUSC3* promoter methylation was calculated using Pearson's correlation coefficient (left graph). More advanced prostate cancers (pathological stage T3/T4 vs. T2) display significantly lower mRNA expression levels of *TUSC3* (right graph).

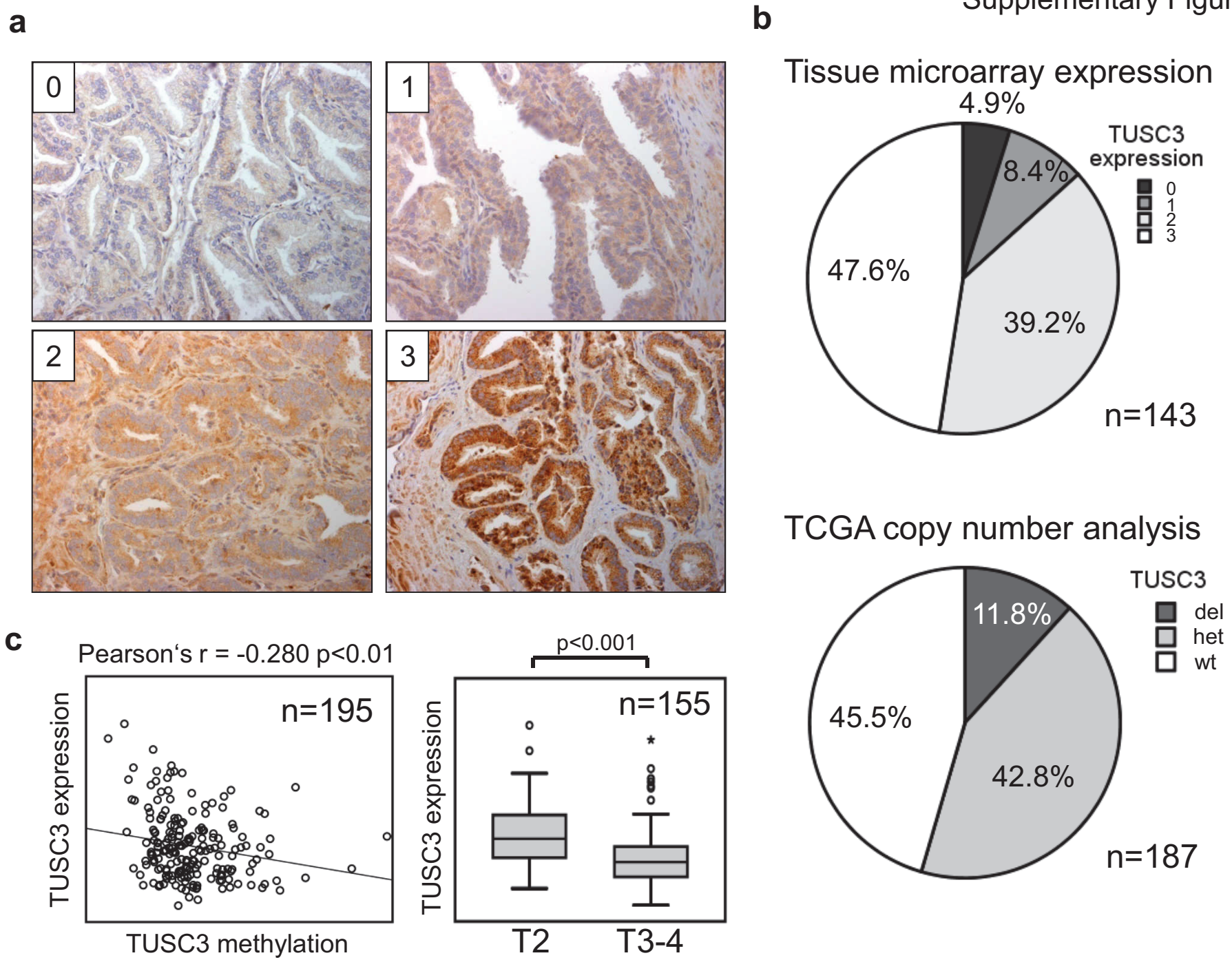
Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3



Supplementary Table 1. Clinical and pathological characteristics of TMA patients

Prostate Cancer Samples	n	%	mean TUSC3 staining
TUSC3 IHC Staning	143		
0	7	4.9	
1	12	8.4	
2	56	39.2	
3	68	47.6	
Age			
mean	56.1	years	
range	39 – 71	years	
<60 years	33		1.37
≥60 years	16		1.25
missing	94		
PSA			
mean	6.4	ng/ml	
range	1.2 – 15.9	ng/ml	
<6.0	24		1.56
≥6.0	25		1.08
missing	94		
T Stage			
2a	5	3.5	1.07
2b	38	26.6	1.35
3a	4	2.8	1.33
3b	2	1.4	1.67
missing	94		
Gleason			
5	3	2.1	0.67
6	22	15.4	1.60
7	24	16.8	1.16
missing	94		
Follow-up			
median	3.9	years	
range	0.3 – 7.0	years	