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Supplementary Table 1. Expression of PSA from recombinant engineered PSA constructs quantified by DELFIA PROSTATUS immunoassay

Protein name	Free PSA (µg/L)	Total PSA (µg/L)	Free/total (f/t) ratio (%)
Wt PSA	37.9	43.3	83
Thr^{163} PSA	40.4	45.4	88
Ala^{195} PSA	5	5.6	89

Supplementary Table 2. Participant characteristics

Strata	PCa[a]		Family History Controls		Family History Cases		Risk - case only			Advanced[b]	
	controls	**cases**	**No**	**Yes**	**No**	**Yes**	**High**	**Intermediate**	**Low**	**No**	**Yes**
PRACTICAL Consortium											
Australia	1557	3996	798	114	768	618	1162	2102	118	169	132
Belgium	103	166	0	0	28	12	112	44	4	3	8
Bulgaria	89	192	89	0	192	0	108	77	6	0	0
Canada	455	668	338	77	486	155	96	314	143	0	0
Croatia	149	146	149	0	118	0	75	57	9	2	10
Denmark	1031	2057	0	0	766	87	802	1017	95	91	75
Finland	1183	2421	0	62	0	70	675	1021	543	79	148
France	691	923	606	85	629	294	417	501	3	8	13
Germany	493	781	484	9	578	203	308	424	7	64	33
Multi_Center	693	877	0	0	0	0	161	331	94	21	28
Nederland	65	71	0	0	0	0	0	0	0	0	0
Norway	0	1443	0	0	0	0	51	484	0	182	761
Poland	317	484	0	0	432	5	241	175	56	0	0
Portugal	180	374	0	0	181	190	182	161	29	0	1
Spain	819	1322	434	34	659	122	359	689	223	60	34
Sweden	2834	5976	750	79	1436	294	1599	2473	1380	539	624
UK	10854	17565	4881	1156	9129	3543	6396	5302	1566	1073	2307
USA	10488	10479	7410	1060	5977	1599	1303	3936	1766	1313	579
Total (PRACTICAL)	**32001**	**49941**	**15939**	**2676**	**21379**	**7192**	**14047**	**19108**	**6042**	**3604**	**4753**

[a]Prostate Cancer; [b]Identification of advanced disease cases was based on Gleason score 8+, metastatic disease, PSA>100 or death from PCa.

Supplementary Table 3. Risk of prostate cancer for rs17632542 SNP

Analysis	OR (95% CI)[a]	P-value
All prostate cancers	0.70 (0.67-0.73)	9.6E-69
Positive family history status	0.75 (0.71-0.79)	2.7E-26
Age of disease onset	0.75 (0.71-0.79)	5.2E-29
High risk[b] vs Low[c]	1.58 (1.42-1.76)	1.23E-17
High risk vs Low/Intermediate[d]	1.42 (1.33-1.51)	1.41E-26
Risk lethal vs controls	1.33 (1.16-1.51)	2.29E-05

[a]Odds-ratio and (95% confidence interval); we defined [b]High risk as tumor stage T3/T4 or N1 or M1 or GleasonScore ≥8 or PSA >20 ng/mL; [c]low risk as tumor stage ≤T1 and GleasonScore ≤6 and PSA <10 ng/mL; and [d]intermediate risk as tumor stage T2 or GleasonScore=7 or PSA=10-20 ng/mL.

Supplementary Table 4: Frequency distribution for the rs17632542 SNP

Category	Coding	TT	CT	CC	Total	Genotype Frequency		
						p(TT)	p(CT)	p(CC)
T-Stage	T0	19	4	0	23	0.826087	0.173913	0
	T1	13433	1408	38	14879	0.902816	0.09463	0.002554
	T2	13496	1543	45	15084	0.894723	0.102294	0.002983
	T3	6317	926	41	7284	0.867243	**0.127128**	0.005629
	T4	686	103	8	797	0.860728	**0.129235**	0.010038
Total		33951	3984	132	38067			
GLEASON Score_Range	1	306	38	1	345	0.886957	0.110145	0.002899
	2	7697	890	32	8619	0.893027	0.10326	0.003713
	3	1182	203	8	1393	0.848528	**0.145729**	0.005743
	4	3	0	0	3	1	0	0
Total		9188	1131	41	10360			
N-Stage	N0	11883	1402	43	13328	0.891582	0.105192	0.003226
	N1	1001	182	12	1195	0.837657	**0.152301**	0.010042
Total		12884	1584	55	14523			
M-Stage	M0	13839	1709	55	15603	0.886945	0.10953	0.003525
	M1	1413	215	8	1636	0.863692	**0.131418**	0.00489
Total		15252	1924	63	17239			
SEER Staging	Local	27928	3056	85	31069	0.898902	0.098362	0.002736
	Regional	6454	948	43	7445	0.866891	**0.127334**	0.005776
	Distant	1421	216	8	1645	0.86383	**0.131307**	0.004863
Total		35803	4220	136	40159			

Significant frequencies for the genotype highlighted in bold.

Detailed description for each “Category”

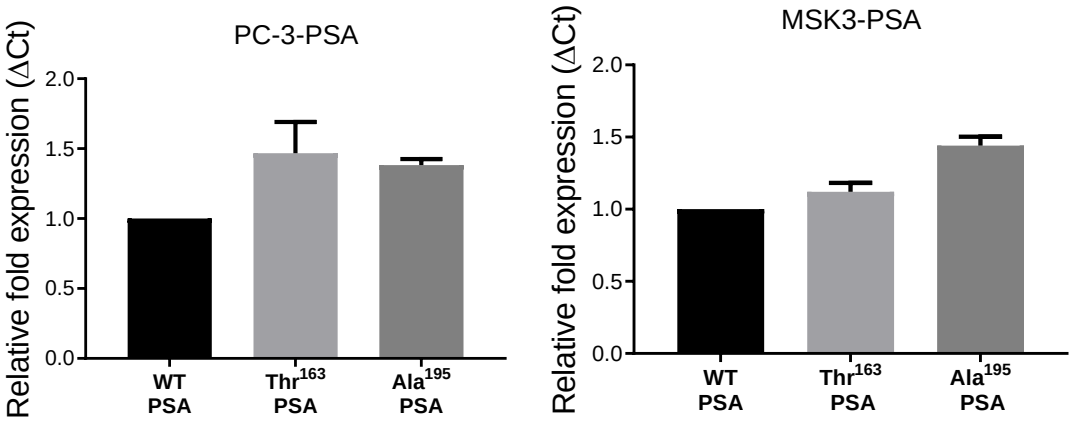
Category	Variable name	Description	Coding
Cancer Stage	T-Stage	T-Stage: Size or direct extent of the primary tumor ('T')	T0=no evidence of tumor, T1=tumor present, but not detectable clinically or with imaging, T2=the tumor can be felt (palpated) on examination, but has not spread outside the prostate, T3=the tumor has spread through the prostatic capsule, T4=the tumor has invaded other nearby structures

Gleason	Gleason Score_Range	Range based on Gleason Score	1=Gleason Score<5. 2=Gleason Score 5,6,7. 3=Gleason Score 8, 9, 10. 4=undifferentiated.
	N-Stage	Degree of spread to regional lymph nodes ('N')	N0=there has been no spread to the regional lymph nodes, N1=there has been spread to the regional lymph nodes
	M-Stage	Presence of metastasis ('M')	M0=there is no distant metastasis, M1=there is distant metastasis
	SEER Staging	Prostate cancer SEER staging	Local=Confined to the prostate, Regional=Direct extension involving adjacent local structures and local lymph node, Distant=Direct extension or metastasis

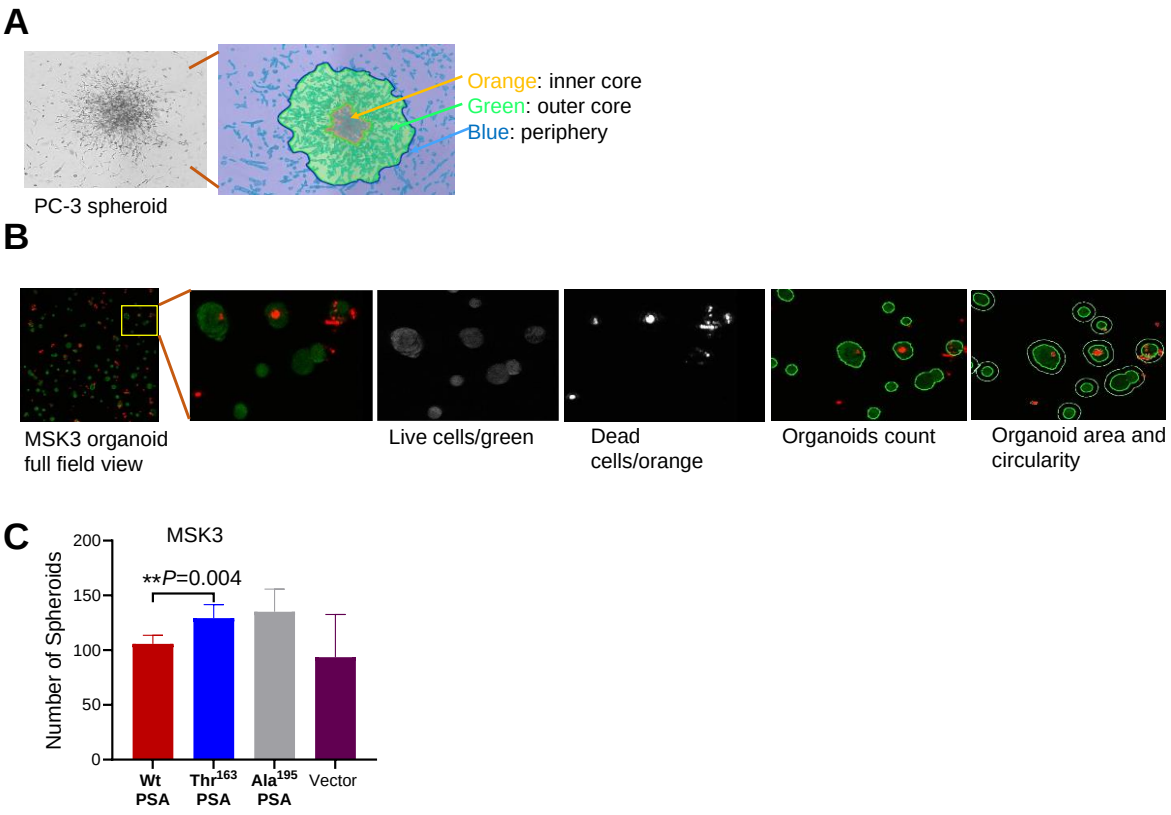
Supplementary Table 5. Primers used for the study

Gene	Primer sequences (5' to 3')	Reference/Source
KLK3 (Exon 2-3)	F1-AGTGCGAGAAGCATTCCCAAC R1-AACAAAAGCGTGATCTTGCTGG	[1]
GAPDH	F-GCAAATTCCATGGCACCGT R- TCGCCCCACTTGATTTTGG	[2]
pcDNA3.1 vector sequencing primers	F-TAATACGACTCACTATAGGG R-TAGAAGGCACAGTCGAGG	(Invitrogen™)
SD2 (minigene assay)	TCTGAGTCACCTGGACAACC	(Life Technologies)
SA2 (minigene assay)	ATCTCAGTGGTATTTGTGAGC	(Life Technologies)
7SL	F-ATCGGGTGTCCGCACTAAGTT R-CAGCACGGGAGTTTTGACCT	(Life Technologies)
Alpha-signal	GGACGGATCCAAACGATGAGATTTCCTTCA	Sigma Aldrich, Australia
PSA mature	F-CTCTCGAGAAAAGAATTGTGGGAGGCTGGGAGT R-CAGCCTCCCACAATTCTTTTCTCGAGAGATACC	Sigma Aldrich, Australia
Site-directed mutagenesis primers for PSA-inactive mutant A^{195}	F-GCACCTGCTCGGGTGATGCTGGGGGCCCACTTGTC R-GACAAGTGGGCCCCCAGCATCACCCGAGCAGGTGC	(Primer X)
Site-directed mutagenesis primers for rs17632542	F-GTGGACCTCCATGTTACTTCCAATGACGTGTG R-CACACGTCATTGGAAGTAACATGGAGGTCCAC	(Primer X)

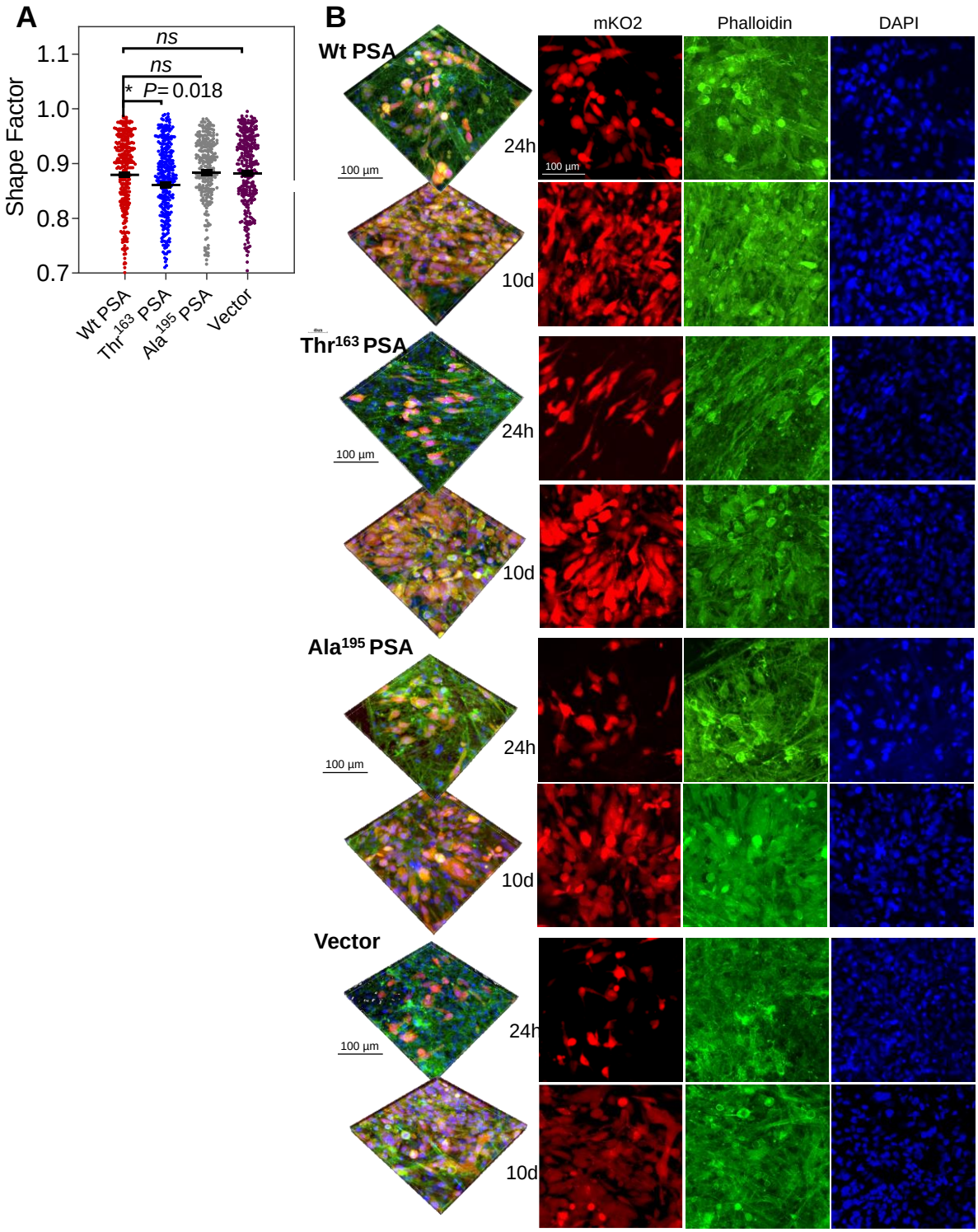
Supplementary Figure 1. *KLK3* expression in overexpression models



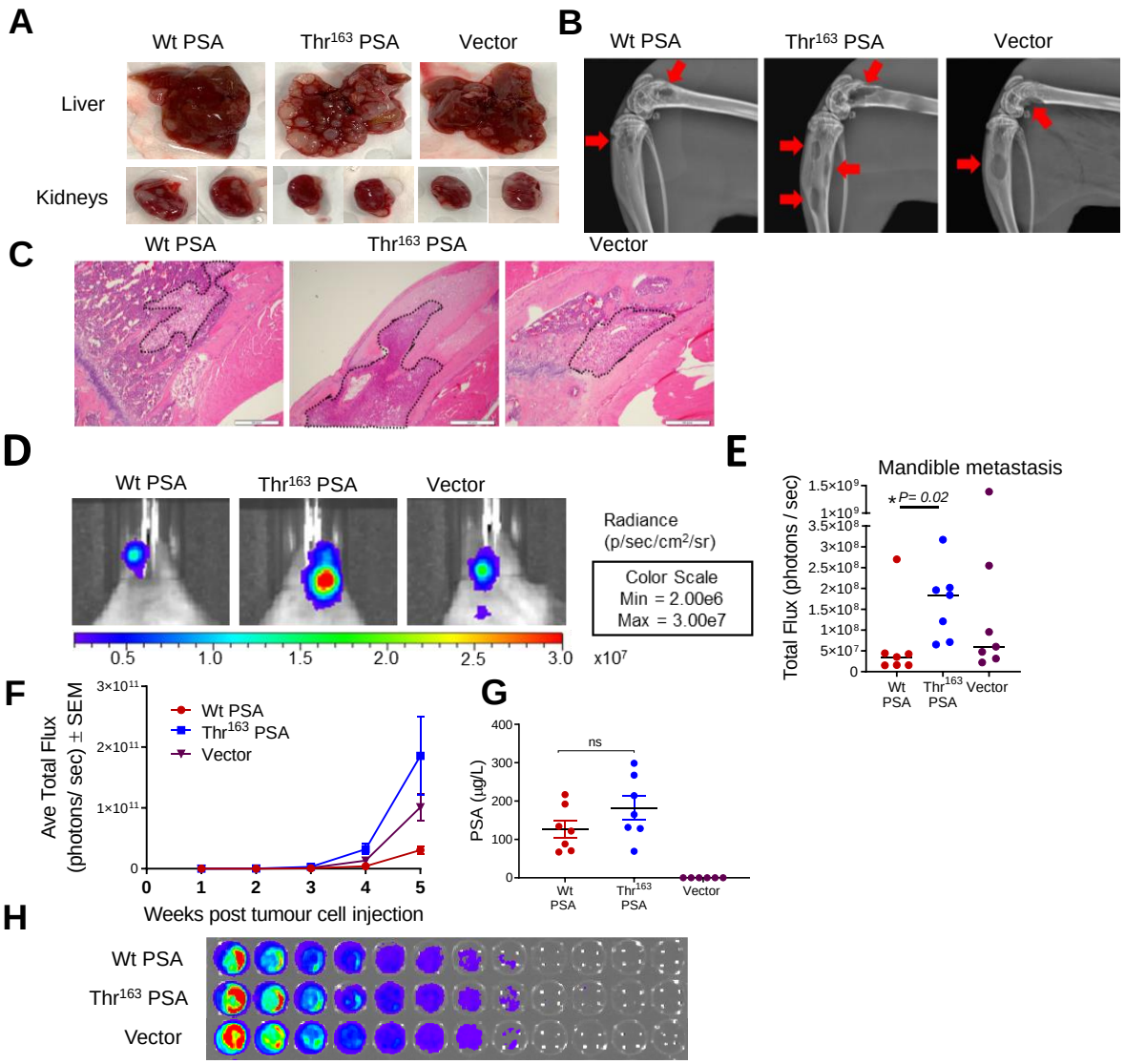
Supplementary Figure 2. Digital spheroid analysis of PC3 and MSK3cells



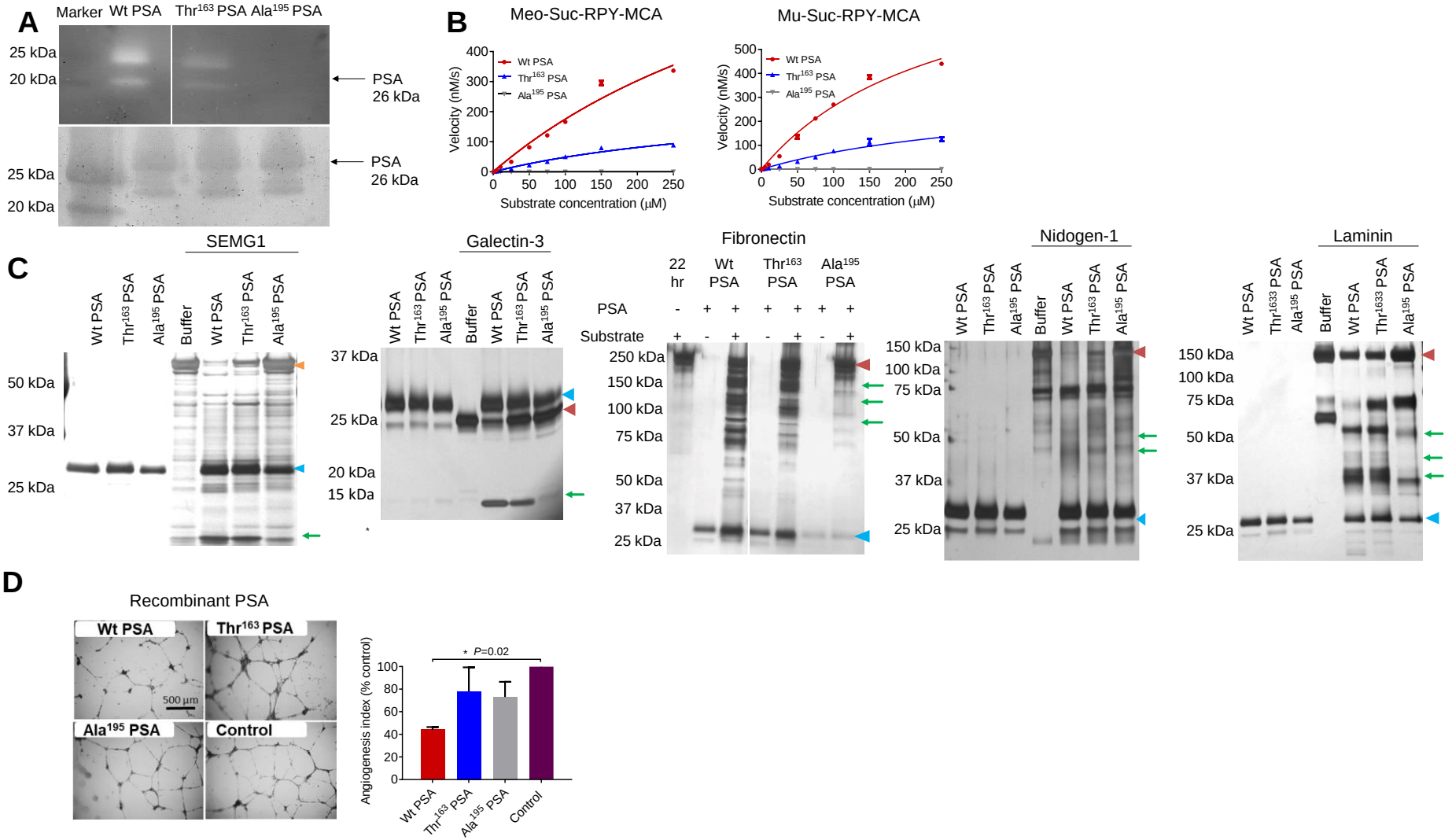
Supplementary Figure 3. Confocal Microscopy for PSA and vector transfected PC3 cells on OBM constructs



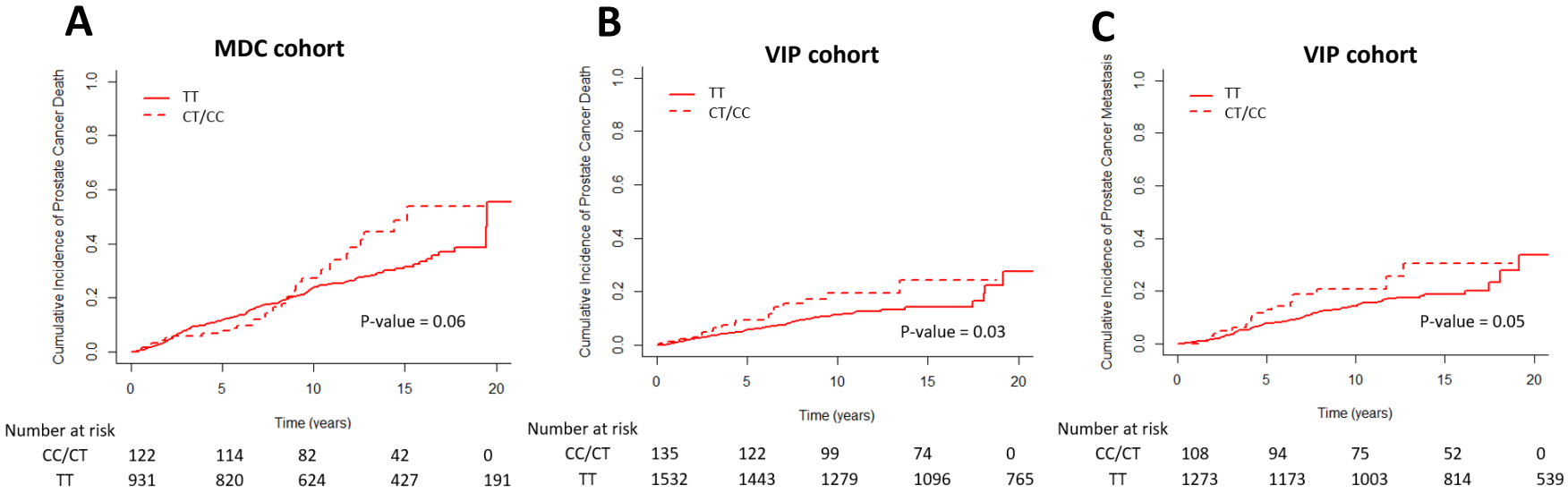
Supplementary Figure 4. Effect of rs17632542 SNP on PC3 cell metastasis in an experimental metastasis mouse model



Supplementary Figure 5. Proteolysis of peptide and full-length protein substrates by mature PSA protein variants



Supplementary Figure 6. Overall- and metastasis-free survival of MDC and VIP cohorts for the rs17632542 SNP



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