Supplementary materials

Table S1 Composition of the prostate prognosis tissue microarray

	No. of patients (%)	
	Study cohort on TMA*	Biochemical relapse among categories
Follow-up		
n	14,464	3,612 (25%)
Mean/median (months)	56/48	–
Age (years)		
≤ 50	433	66 (15.2%)
51–59	4,341	839 (19.3%)
60–69	9,977	2,073 (20.8%)
≥ 70	2,936	634 (21.6%)
Pretreatment PSA (ng/mL)		
< 4	2,225	313 (14.1%)
4–10	10,520	1,696 (16.1%)
10–20	3,662	1,043 (28.5%)
> 20	123	545 (44.3%)
pT stage (AJCC 2002)		
pT2	11,518	1,212 (10.5%)
pT3a	3,842	1,121 (29.2%)
pT3b	2,233	1,213 (54.3%)
pT4	85	63 (74.1%)
Gleason grade		
≤ 3+3	3,570	264 (7.4%)
3+4	9,336	1,436 (15.4%)
3+4 Tert.5	1,697	165 (9.7%)
4+3	2,903	683 (23.5%)
4+3 Tert.5	1,187	487 (41%)
≥ 4+4	999	531 (53.2%)
pN stage		
pN0	10,636	2,243 (21.1%)
pN+	1,255	700 (55.8%)
Surgical margin		
Negative	14,297	2,307 (16.1%)
Positive	3,388	1,304 (38.5%)

AJCC, American Joint Committee on Cancer; cT, clinical tumor; pT, pathological tumor; pN, pathological lymph node; PSA, prostate-specific antigen; TMA, tissue microarray. *Numbers do not always add up to 17,747 in the different categories because of cases with missing data.

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Marx et al. ANO7 expression in prostate cancer

Table S2 ANO7 expression and prostate cancer phenotype in the ERG-negative subset

Parameter	n	Fraction of ANO7 expression (%)				P
		Negative	Weak	Moderate	Strong	
All cancers	4915	7.0	5.3	44.5	43.2	
Tumor stage						< 0.0001
pT2	8889	5.5	7.0	47.4	40.2	
pT3a	2984	9.8	11.0	52.1	27.1	
pT3b-pT4	1668	14.5	18.5	49.6	17.3	
Gleason grade						< 0.0001
≤ 3+3	1002	3.7	3.6	40.4	52.3	
3+4	2670	5.1	4.5	44.0	46.3	
3+4 Tert.5	216	5.6	7.4	41.7	45.4	
4+3	509	15.1	6.3	48.7	29.9	
4+3 Tert.5	282	11.3	8.2	54.3	26.2	
≥ 4+4	233	20.6	13.7	50.2	15.5	
Quantitative Gleason grade						< 0.0001
≤ 3+3	1002	3.7	3.6	40.4	52.3	
3+4 ≤ 5%	730	4.5	3.8	41.1	50.5	
3+4 (6%–10%)	697	3.4	3.6	43.3	49.6	
3+4 (11%–20%)	595	4.5	4.7	45.5	45.2	
3+4 (21%–30%)	291	7.2	7.6	46.7	38.5	
3+4 (31%–49%)	261	8.8	5.4	46.7	39.1	
3+4 Tert.5	216	5.6	7.4	41.7	45.4	
4+3 (50%–60%)	226	11.9	4.9	44.7	38.5	
4+3 Tert.5	282	11.3	8.2	54.3	26.2	
4+3 (61%–100%)	235	16.6	7.2	53.2	23.0	
≥ 4+4	209	20.6	12.0	50.7	16.7	
Lymph node metastasis						< 0.0001
N0	2876	7.0	5.5	45.8	41.7	
N+	248	19.8	13.3	46.4	20.6	
Preoperative PSA level (ng/mL)						< 0.0001
< 4	523	8.0	6.1	44.9	40.9	
4–10	2936	5.8	4.5	43.4	46.4	
10–20	1082	8.0	5.3	47.4	39.3	
> 20	349	12.0	10.9	45.6	31.5	
Surgical margin						< 0.0001
Negative	3951	6.1	4.9	44.5	44.5	
Positive	952	10.6	6.7	44.9	37.8	

ANO7, anoctamin 7; ERG, erythroblast transformation-specific-related gene; PSA, prostate-specific antigen.

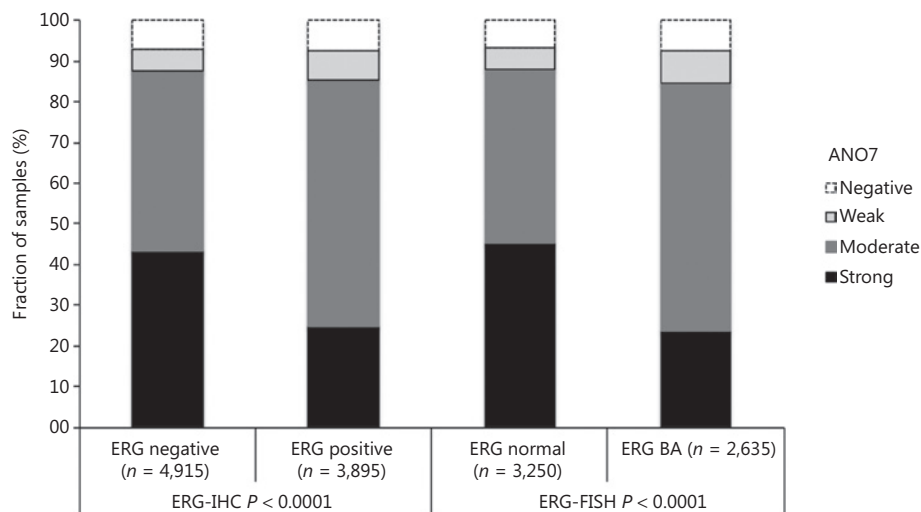**Table S3** ANO7 expression and prostate cancer phenotype in the ERG-positive subset

Parameter	n	Fraction of ANO7 expression (%)				P
		Negative	Weak	Moderate	Strong	
All cancers	3895	7.4	7.4	60.3	24.9	
Tumor stage						< 0.0001
pT2	2367	5.1	5.1	58.9	31.0	
pT3a	1027	10.3	9.2	63.9	16.7	
pT3b-pT4	485	12.8	14.2	59.8	13.2	
Gleason grade						< 0.0001
≤ 3+3	815	4.3	2.8	58.4	34.5	
3+4	2261	6.0	6.6	62.2	25.2	
3+4 Tert.5	116	10.3	10.3	59.5	19.8	
4+3	377	14.6	12.7	57.3	15.4	
4+3 Tert.5	205	10.7	16.1	60.0	13.2	
≥ 4+4	118	24.6	16.9	47.5	11.0	
Quantitative Gleason grade						< 0.0001
≤ 3+3	815	4.3	2.8	58.4	34.5	
3+4 ≤ 5%	569	2.8	4.2	57.8	35.1	
3+4 (6%–10%)	600	5.3	7.2	62.8	24.7	
3+4 (11%–20%)	492	5.7	7.1	66.1	21.1	
3+4 (21%–30%)	283	7.4	8.1	63.3	21.2	
3+4 (31%–49%)	197	11.2	6.1	67.5	15.2	
3+4 Tert.5	116	10.3	10.3	59.5	19.8	
4+3 (50%–60%)	173	6.4	12.7	59.0	22.0	
4+3 Tert.5	205	10.7	16.1	60.0	13.2	
4+3 (61%–100%)	163	20.2	14.1	54.6	11.0	
≥ 4+4	98	21.4	16.3	52.0	10.2	
Lymph node metastasis						< 0.0001
N0	2248	9.2	7.9	58.9	24.1	
N+	220	12.7	15.9	59.5	11.8	
Preoperative PSA level (ng/mL)						< 0.0001
< 4	530	8.3	5.5	62.3	24.0	
4–10	2409	6.0	6.9	59.5	27.6	
10–20	694	9.8	8.1	62.0	20.2	
> 20	233	12.9	12.9	58.4	15.9	
Surgical margin						0.0001
Negative	3072	7.2	6.8	59.5	26.5	
Positive	806	8.2	9.2	63.3	19.4	

ANO7, anoctamin 7; ERG, erythroblast transformation-specific-related gene; PSA, prostate-specific antigen.

Table S4 Multivariate Cox regression analysis with established prognostic parameters and the ANO7 expression in all prostate cancers, the ERG-negative, and ERG-positive subsets

Tumor subset	Scenario	n analyzable	P-value Preoperative PSA level	pT stage	cT stage	Gleason grade prostatectomy	Gleason grade biopsy	pN stage	R stage	ANO7 expression
All cancers	1	7173	< 0.0001	< 0.0001	–	< 0.0001	–	< 0.0001	< 0.0001	< 0.0001
	2	11,141	< 0.0001	< 0.0001	–	< 0.0001	–	-	< 0.0001	< 0.0001
	3	10,965	< 0.0001	–	< 0.0001	< 0.0001	–	–	–	< 0.0001
	4	9527	< 0.0001	–	< 0.0001	–	< 0.0001	–	–	< 0.0001
ERG-negative cancers	1	2877	0.0054	< 0.0001	–	< 0.0001	–	0.0004	0.00537	0.0032
	2	4518	< 0.0001	< 0.0001	–	< 0.0001	–	–	0.0004	0.0028
	3	4474	< 0.0001	–	< 0.0001	< 0.0001	–	–	–	0.0002
	4	4404	< 0.0001	–	< 0.0001	–	< 0.0001	–	–	< 0.0001
ERG-positive cancers	1	2274	0.0016	< 0.0001	–	< 0.0001	–	0.0166	< 0.0001	0.0002
	2	3567	< 0.0001	< 0.0001	–	< 0.0001	–	–	< 0.0001	0.0001
	3	3511	< 0.0001	–	< 0.0001	< 0.0001	–	–	–	< 0.0001
	4	3455	< 0.0001	–	< 0.0001	–	< 0.0001	–	–	< 0.0001

ANO7, anoctamin 7; ERG, erythroblast transformation-specific-related gene. Scenario 1 includes all postoperatively available parameters: pathological tumor (pT) stage, lymph node (pN), surgical margin (R) status, preoperative prostate-specific antigen (PSA) value, and Gleason grade obtained after the morphological evaluation of the entire resected prostate. Scenario 2 excluded the nodal status from analysis. Scenario 3 included preoperative PSA, clinical tumor (cT) stage, and Gleason grade obtained on the prostatectomy specimen. In scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA and cT stage.

Figure S1 Anoctamin 7 (ANO7) expression and erythroblast transformation-specific-related gene (ERG) status [immunohistochemistry (IHC)/fluorescence *in situ* hybridization (FISH)].
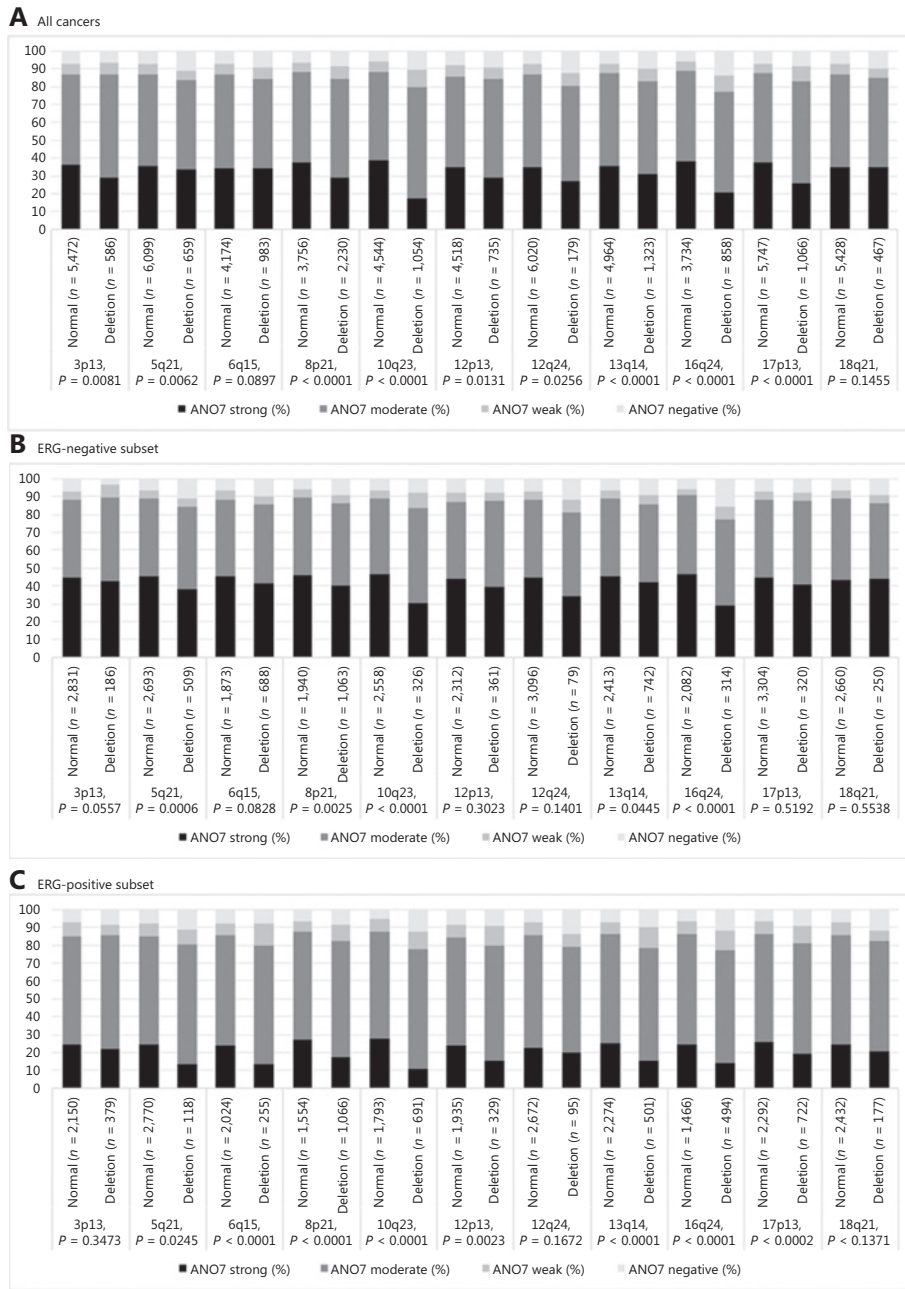
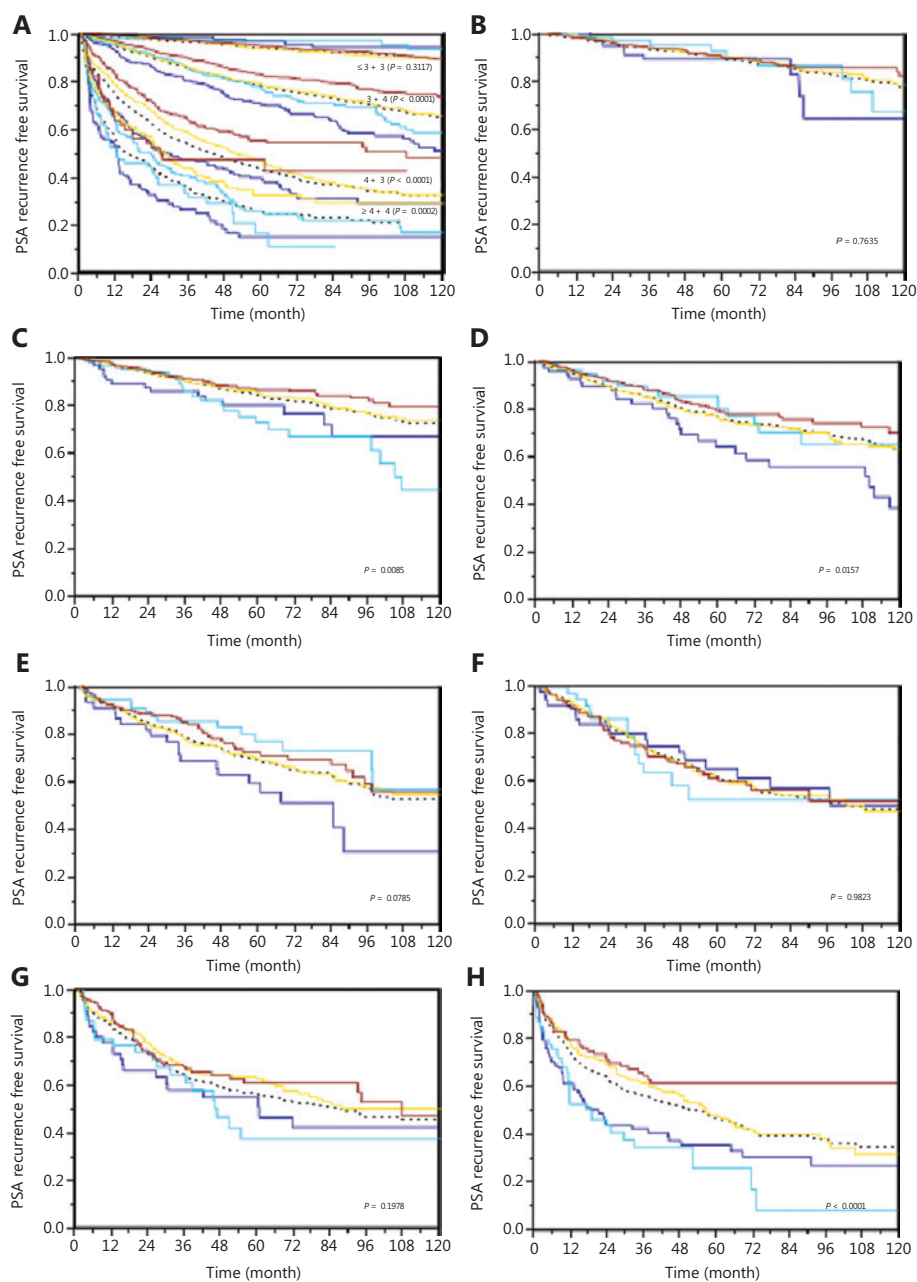**Figure S2**   Anoctamin 7 (ANO7) staining and genomic deletions.

Figure S3 Prognostic impact of anoctamin 7 (ANO7) expression defined by the Gleason score. (A) Impact of ANO7 expression as compared to the classical Gleason score categories; (B–H) as compared to the quantitative Gleason score categories defined by (B) ≤ 5%, (C) 6%–10%, (D) 11%–20%, (E) 21%–30%, (F) 31%–49 %, (G) 50%–60%, and (H) ≥ 61% Gleason 4 patterns. Dotted line shows the reference for the respective Gleason category.