

Figure S1. DNA methylation levels of selected genes in the Prostate Cancer Dataset (PRAD) [9]. Level 3 DNA methylation data, obtained using HM450 platform, were used to generate the plots. The depicted β -values are average values of the gene promoter regions. The boxplot depicts Q1-Q3 quartile, when the line in the box indicates the median, the plus sign indicates the mean, the whiskers represent 10-90% of the range and the outliers are marked as dots. Significant P-values are marked in bold.

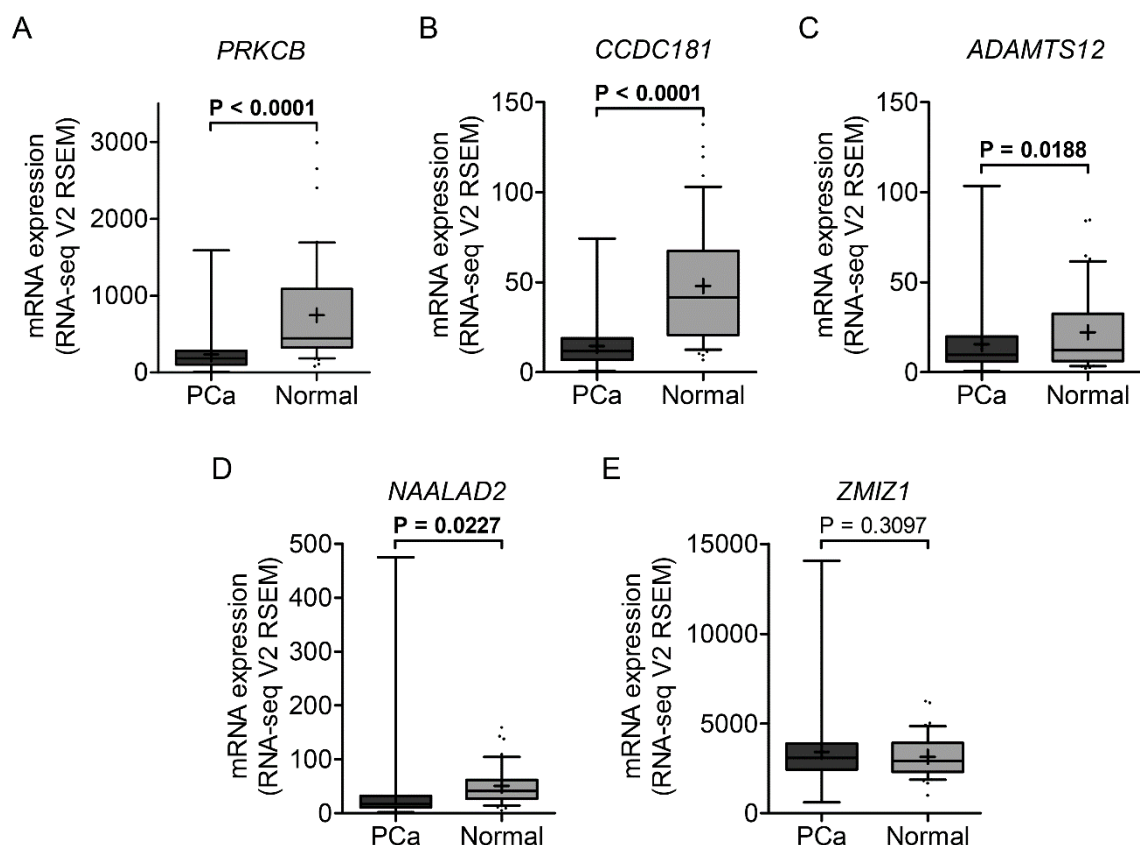


Figure S2. Relative expression levels of selected genes in the Prostate Cancer Dataset (PRAD) [9]. Level 3 PRAD RNA-seq RSEM data were used to generate the plots. The boxplot depicts Q1-Q3 quartile, when the line in the box indicates the median, the plus sign indicates the mean, the whiskers represent 10-90% of the range and the outliers are marked as dots. Significant P-values are marked in bold.

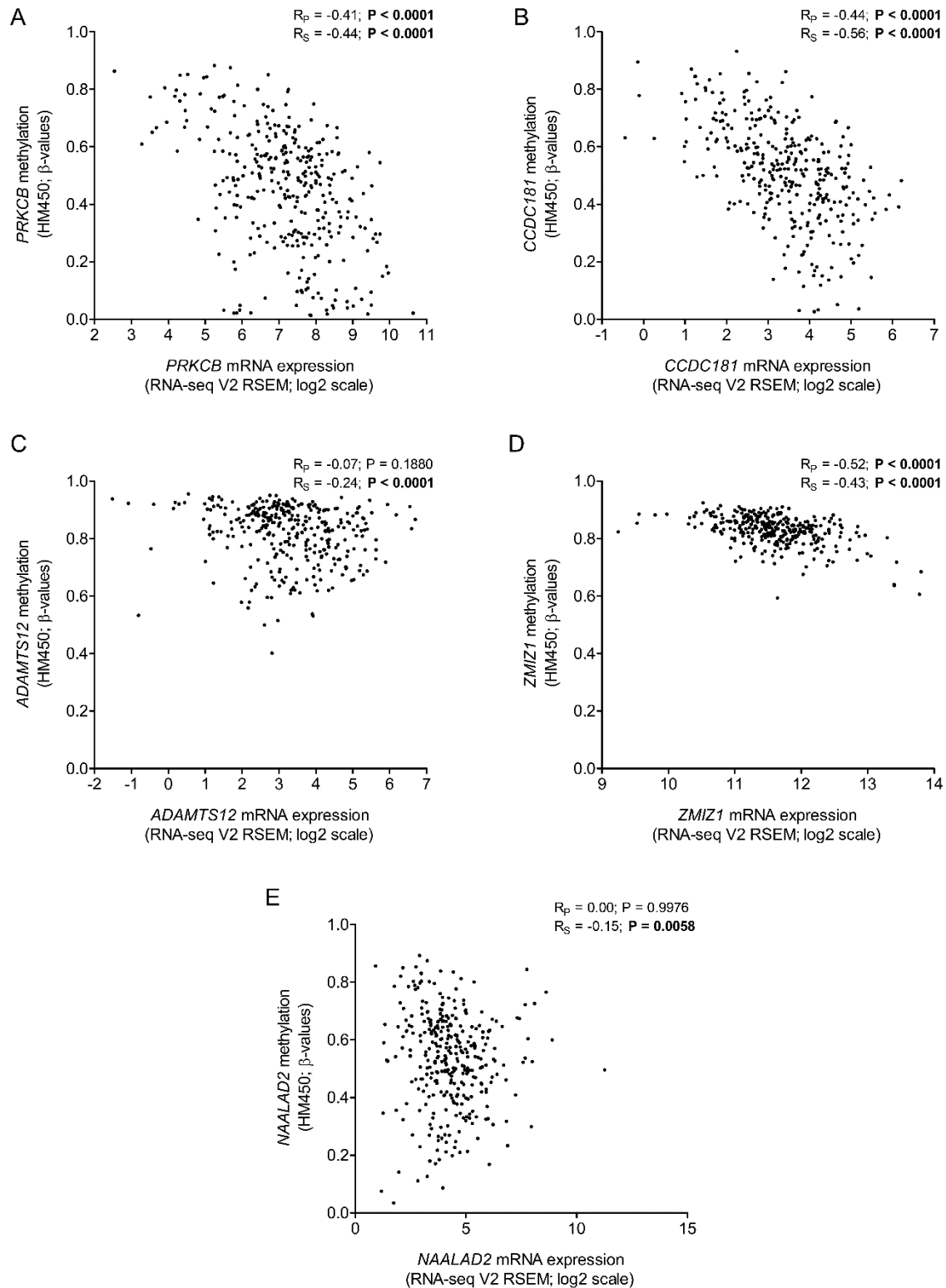


Figure S3. Correlations between promoter methylation status and gene expression levels of selected genes in the Prostate Cancer Dataset (PRAD) [9]. Level 3 DNA methylation data, obtained using HM450 platform, and level 3 PRAD RNA-seq RSEM data were used to generate scatter diagrams. RNA-seq data is plotted on log2 scale. Pearson's R (RP) and Spearman's R (RS) correlation coefficients are provided in the graphs. Significant P-values are marked in bold.

Table S1. Clinico-pathological and molecular characteristics of the analysis groups.

| Parameter | Methylation Analysis Group | | | Gene Expression Analysis Group ¹ | |
|-------------------------------------|----------------------------|------------------------|------------------------|---|-------------------------|
| Group composition | PCa (N = 151) | NPT (N = 51) | BPH (N = 17) | PCa (N = 81) | NPT (N = 25) |
| Tumor stage, N | | | | | |
| ≤pT2 | 96 | - | - | 51 | - |
| ≥pT3 | 55 | - | - | 30 | - |
| ISUP grade group (Gleason score), N | | | | | |
| I (3 + 3) | 34 | - | - | 13 | - |
| II (3 + 4) | 84 | - | - | 48 | - |
| III (4 + 3) | 23 | - | - | 14 | - |
| IV (8) | 3 | - | - | 2 | - |
| V (9) | 5 | - | - | 3 | - |
| Unknown | 2 | - | - | 1 | - |
| Tumor cellularity, N | | | | | |
| 90-100% | 73 | - | - | 49 | - |
| 70-89% | 38 | - | - | 24 | - |
| 50-69% | 40 | - | - | 8 | - |
| 1-3% | - | 3 | - | - | 0 |
| 0% | - | 48 | - | - | 25 |
| BCR status, N | | | | | |
| Yes (mean time to BCR, mo) | 37 (18) | 16 (16) | - | 30 (19) | 7 (20) |
| No (mean follow-up, mo) | 99 (38) | 32 (47) | - | 49 (42) | 17 (58) |
| Unknown | 15 | 3 | - | 2 | 1 |
| PSA, ng/mL | | | | | |
| Mean±SD (range) | 10.6±11.1 [2.5; 84.2] | 9.4±8.6 [2.5; 44.0] | 7.3±6.6 [0.8; 28.1] | 10.8±9.4 [2.5; 44.0] | 10.0±9.5 [2.5; 44.0] |
| Unknown | 2 | 1 | 0 | 2 | 1 |
| Prostate mass, g | | | | | |
| Mean±SD (range) | 48±17 [16; 123] | 50±20 [16; 104] | - | 48±17 [16; 123] | 48±20 [16; 104] |
| TMPRSS2-ERG fusion transcript, N | | | | | |
| Yes | 77 | - | - | 46 | - |
| No | 44 | - | - | 26 | - |
| Unknown | 8 | - | - | 9 | - |
| Age, years | | | | | |
| Mean±SD (range) | 61±8 [41; 82] | 62±7 [46; 74] | 70±8 [59; 80] | 61±8 [41; 82] | 61±6 [48; 74] |

BCR—biochemical disease recurrence, PCa—prostate cancer, NPT—noncancerous prostate tissue, BPH—benign prostatic hyperplasia, PSA—prostate-specific antigen, SD—standard deviation. ¹ The same subset of BPH samples as in “Methylation analysis in group” was also included in the gene expression analysis.

Table S2. Primers used for methylation-specific PCR, location of amplicons, and amplification conditions.

| Gene symbol | Primer pair ID | Forward primer sequence (5'-3') | Reverse primer sequence (5'-3') | Product size, nt | Amplicon location from TSS | Primer annealing T, °C | Number of PCR cycles |
|---------------------------------------|----------------|-----------------------------------|---------------------------------|------------------|----------------------------|------------------------|----------------------|
| <i>PRKCB</i> | M | TAAGCGTAGTTGGACGAGC | AAAACGACGACCGCTACTAC | 124 | -7/+117 | 56 | 36 |
| | U | TGTTAAGTGTAGTTGGATGAGT | AAAACAACAACCACTACTACACC | 127 | -10/+117 | | |
| <i>CCDC181</i> (<i>C1orf114</i>) | M | CGGTATTTTCGCGAGTTTTTATAAC | CGAAAACGACAAAAATCTACG | 164 | -166/-3 | 57 | 35 |
| | U | TAGTGGTATTTTGTGAG- TTTTTATAAT | ACAAAAACAACAAAAATCTACACA | 168 | -169/-2 | | |
| <i>ADAMTS12</i> | M | GAGTTCGGGAGGAAGATGTATC | ACAACGACTACAAAACCTACCCG | 241 | -195/+46 | 62 | 35 |
| | U | GAGTTTGGGAGGAAGATGTATT | AAACAACAACCTACAAAACCTACCA | 243 | -195/+48 | | |
| <i>NAALAD2</i> | M | TATTTATTATGTTTCGGGTTATTGC | CCTACTACATTCGCGAACTTC | 244 | -116/+128 | 58 | 35 |
| | U | GTTATTTATTATGTTTGGGTTATTGT | CCTACTACATTCACAACTTCAA | 246 | -118/+128 | | |
| <i>ZMIZ1</i> | M | TCGTTTCGAAAATTTTTTAAATC | AACTCCCGAAACGCTATC | 246 | -188/+58 | 55 | 38 |
| | U | TGTAGTTTGTGTTT- GAAAATTTTTTAAA | AACTCCCAAAACACTATCACC | 252 | -194/+58 | | |
| <i>FILIP1L</i> | M | TACGGTTCGTTTATACGGTC | CGACCTATAAACGTTACGTCA | 160 | -151/+9 | 57 | 36 |
| | U | GGAATTATGTTTTGTTTATATGGTT | CCCAACCTATAAACATTACATCAC | 167 | -156/+11 | | |
| <i>KCTD8</i> | M | TTTTTATTGTCGTCGTCGTATC | CTCCGCGTACTCCTAACG | 169 | +45/+213 | 58 | 37 |
| | U | GTTTTTTTATTGTTGTTGTTGTATT | ACCCTCCACATACTCCTAACA | 175 | +42/+216 | | |
| <i>CD44</i> | M | TCGTTGAGTTTGGCGTAGATC | ACTACCGCCGAATCCGCG | 89 | +480/+568 | 58 | 35 |
| | U | GTGTTGTTGAGTTTGGTGTAGATT | CAAAAAAACTACCAC- CAAATCCACA | 99 | +477/+575 | | |
| <i>EPAS1</i> | M | ATATATTCGCGTCGGTGTTTC | CGCTCGCGAATATAAACTC | 118 | -100/+18 | 56-57 | 37-38 |
| | U | TGTTTATATATTTGTGTTGGTGTGTTG | CCACTCACAAATATAAACTCCC | 124 | -105/+19 | | |
| <i>NEK9</i> | M | TTAATAGATTTCGAGAGGTCGTATC | CGCGAACTCGATAATAACTC | 253 | -210/+43 | 57 | 38 |
| | U | GGGTAAATAGATTTGAGAGGTT- GTATT | CCACAAAACCTCAATAATAACTCCT | 257 | -213/+44 | | |

All primers were designed with Methyl Primer Express® Software v1.0 (Applied Biosystems™, Thermo Fisher Scientific, Carlsbad, CA, USA). TSS – transcription start site, M/ U – primers specific for methylated/ unmethylated sequence.

Table S3. Primers used for quantitative methylation-specific PCR and location of amplicons.

| Gene symbol | Primer ID | Primer sequence (5'-3') | Amplicon size, bp | Amplicon location from TSS | Reference |
|-----------------------------|-----------|---|-------------------|----------------------------|------------|
| PRKCB | Fw | CGTAGTTGGGGTTAGCGGTG | 145 | -28/+117 | This study |
| | Rv | AAAACGACGACCGCTACTACA | | | |
| | Probe | JOE-TTAGAGTCGGCGTAGGGGAAGCG-BHQ-1 | | | |
| CCDC181 (<i>C1orf114</i>) | Fw | GCGGTATTTTCGCGAGTTTTAT | 131 | -167/-37 | This study |
| | Rv | TATCCTCAAACCACCGACC | | | |
| | Probe | FAM-AGTATCGGGATGGGTGTCGCGA-BHQ-1 | | | |
| ADAMTS12 | Fw | CGGGAGGAAGATGTATCGAGC | 138 | -190/-53 | This study |
| | Rv | TCAACTAACAATATCCGCTTTCG | | | |
| | Probe | Cy5-TTTCGTTTTGGTTATTTTATATTTTCG-BHQ-3 | | | |
| NAALAD2 | Fw | TGCGAAGGTAGCGGAGGT | 139 | +36/+174 | This study |
| | Rv | GACCCCTAAATTCCGCCATAA | | | |
| | Probe | FAM-GAAGTTCGCGAATGTAGTAGGCG-BHQ-1 | | | |
| ACTB | Fw | TGGTGATGGAGGAGGTTTAGTAAGT | 133 | -1629/-1497 | [29] |
| | Rv | AACCAATAAAACCTACTCCTCCCTAA | | | |
| | Probe | FAM-ACCACCACCCAACACACAATAACAAACAC A-BHQ-1 | | | |

TSS – transcription start site, Fw/ Rv – forward/ reverse primer.

Ref [29]. Lehmann, U.; Langer, F.; Feist, H.; Glöckner, S.; Hasemeier, B.; Kreipe, H. Quantitative assessment of promoter hypermethylation during breast cancer development. *Am. J. Pathol.* **2002**, *160*, 605–612.

Table S4. Genes showing the most significant differences of methylation levels according to tissue histology and biochemical disease recurrence status.

| PCa vs. NPT | | | | | | BCR-positive vs. BCR-negative | | | | | |
|-------------|------------------|----------------|--------------|-----|---------|-------------------------------|-----------------|----------------|--------------|-----|---------|
| No. | Gene/ locus | Probe location | Methy-lation | FC | P-value | No. | Gene/ locus | Probe location | Methy-lation | FC | P-value |
| 1 | <i>HOXC4</i> | Intragenic | up | 1.3 | <0.0001 | 1 | <i>COL23A1</i> | Intragenic | up | 1.3 | <0.0001 |
| 2 | <i>CCDC181</i> | Promoter | up | 1.4 | <0.0001 | 2 | <i>HOXC9</i> | Promoter | up | 1.2 | <0.0001 |
| 3 | <i>MAGI2</i> | Intragenic | up | 1.5 | <0.0001 | 3 | <i>MATN4</i> | Promoter | up | 1.2 | <0.0001 |
| 4 | <i>CHGB</i> | Promoter | up | 1.2 | <0.0001 | 4 | <i>POU4F3</i> | Intragenic | up | 1.3 | <0.0001 |
| 5 | <i>C8orf33</i> | Promoter | down | 1.4 | <0.0001 | 5 | <i>MECOM</i> | Promoter | down | 1.2 | <0.0001 |
| 6 | <i>SOX2OT</i> | Intragenic | up | 1.4 | <0.0001 | 6 | <i>CAMTA1</i> | Intragenic | up | 1.4 | <0.0001 |
| 7 | <i>PNMA6A</i> | Intragenic | down | 1.6 | <0.0001 | 7 | <i>C7orf51</i> | Intragenic | up | 1.3 | 0.0001 |
| 8 | <i>CHAT</i> | Promoter | up | 1.4 | <0.0001 | 8 | <i>BMP8A</i> | Intragenic | up | 1.2 | 0.0001 |
| 9 | <i>ARMC9</i> | Intragenic | down | 1.2 | <0.0001 | 9 | <i>RAPGEFL1</i> | Promoter | up | 1.3 | 0.0001 |
| 10 | <i>DGKG</i> | Intragenic | up | 1.3 | <0.0001 | 10 | <i>BARHL1</i> | Promoter | up | 1.2 | 0.0001 |
| 11 | <i>FER1L4</i> | Intragenic | up | 1.3 | <0.0001 | 11 | <i>C1orf170</i> | Promoter | up | 1.5 | 0.0001 |
| 12 | <i>SLC32A1</i> | Intragenic | up | 1.4 | <0.0001 | 12 | <i>TMEM233</i> | Intragenic | up | 1.2 | 0.0001 |
| 13 | <i>PCDHGC4</i> | Intragenic | up | 1.3 | <0.0001 | 13 | <i>C1D</i> | Intragenic | down | 1.2 | 0.0002 |
| 14 | <i>DPYSL3</i> | Promoter | up | 1.3 | <0.0001 | 14 | <i>NFATC1</i> | Promoter | down | 1.3 | 0.0002 |
| 15 | <i>GRIA3</i> | Promoter | up | 1.3 | <0.0001 | 15 | <i>CLDN5</i> | Intragenic | up | 1.3 | 0.0002 |
| 16 | <i>GATA4</i> | Intragenic | up | 1.2 | <0.0001 | 16 | <i>PLK1</i> | Intragenic | up | 1.3 | 0.0002 |
| 17 | <i>SGMS1</i> | Intragenic | up | 1.4 | <0.0001 | 17 | <i>CACNG7</i> | Intragenic | up | 1.3 | 0.0002 |
| 18 | <i>ZIC1</i> | Intragenic | up | 1.2 | <0.0001 | 18 | <i>GSX2</i> | Intragenic | up | 1.2 | 0.0002 |
| 19 | <i>C1orf229</i> | Promoter | down | 1.3 | <0.0001 | 19 | <i>C19orf20</i> | Intragenic | up | 1.3 | 0.0002 |
| 20 | <i>CXCL6</i> | Intragenic | up | 1.4 | <0.0001 | 20 | <i>EPHA10</i> | Intragenic | up | 1.3 | 0.0002 |
| 21 | <i>DRGX</i> | Promoter | up | 1.4 | <0.0001 | 21 | <i>SPRY4</i> | Intragenic | down | 1.2 | 0.0002 |
| 22 | <i>LPPR4</i> | Intragenic | up | 1.3 | <0.0001 | 22 | <i>WNT10B</i> | Intragenic | up | 1.3 | 0.0002 |
| 23 | <i>MKNK1</i> | Promoter | up | 1.2 | <0.0001 | 23 | <i>TRIM54</i> | Intragenic | up | 1.2 | 0.0002 |
| 24 | <i>NR2E1</i> | Intragenic | up | 1.4 | <0.0001 | 24 | <i>SV2A</i> | Intragenic | up | 1.4 | 0.0003 |
| 25 | <i>MCF2L2</i> | Intragenic | up | 1.4 | <0.0001 | 25 | <i>HOPX</i> | Intragenic | up | 1.2 | 0.0003 |
| 26 | <i>DPP10</i> | Intragenic | up | 1.4 | <0.0001 | 26 | <i>RSPH9</i> | Intragenic | up | 1.3 | 0.0003 |
| 27 | <i>SLC24A2</i> | Intragenic | up | 1.3 | <0.0001 | 27 | <i>HOXB4</i> | Intragenic | up | 1.3 | 0.0003 |
| 28 | <i>LOC648809</i> | Promoter | up | 1.2 | <0.0001 | 28 | <i>SYN</i> | Intragenic | up | 1.2 | 0.0003 |
| 29 | <i>ALDH1L2</i> | Intragenic | up | 1.3 | <0.0001 | 29 | <i>C2orf88</i> | Promoter | up | 1.2 | 0.0003 |
| 30 | <i>HOXC11</i> | Intragenic | up | 1.3 | <0.0001 | 30 | <i>CALY</i> | Intragenic | up | 1.3 | 0.0003 |
| 31 | <i>NAALAD2</i> | Promoter | up | 1.5 | <0.0001 | 31 | <i>FAM43B</i> | Intragenic | up | 1.2 | 0.0003 |
| 32 | <i>EZH2</i> | Promoter | down | 1.4 | <0.0001 | 32 | <i>IGFBP7</i> | Intragenic | up | 1.4 | 0.0003 |
| 33 | <i>TTC23L</i> | Intragenic | up | 1.2 | <0.0001 | 33 | <i>PLCXD1</i> | Promoter | down | 1.3 | 0.0004 |

| | | | | | | | | | | | |
|----|----------------|------------|----|-----|---------|----|---------------------|------------|------|-----|--------|
| 34 | <i>SPAG17</i> | Promoter | up | 1.4 | <0.0001 | 34 | <i>WBP2</i> | Promoter | down | 1.2 | 0.0004 |
| 35 | <i>FGD5</i> | Promoter | up | 1.6 | <0.0001 | 35 | <i>SCNN1B</i> | Promoter | up | 1.2 | 0.0004 |
| 36 | <i>GALNT13</i> | Intragenic | up | 1.2 | <0.0001 | 36 | <i>LOC400043</i> | Intragenic | up | 1.2 | 0.0004 |
| 37 | <i>ZSCAN12</i> | Intragenic | up | 1.3 | <0.0001 | 37 | <i>ZNF778</i> | Intragenic | up | 1.2 | 0.0004 |
| 38 | <i>TRIM7</i> | Intragenic | up | 1.3 | <0.0001 | 38 | <i>MMP25</i> | Intragenic | up | 1.3 | 0.0004 |
| 39 | <i>CNPY1</i> | Intragenic | up | 1.3 | <0.0001 | 39 | <i>LOC100287042</i> | Intragenic | down | 1.3 | 0.0004 |
| 40 | <i>HOXC10</i> | Intragenic | up | 1.4 | <0.0001 | 40 | <i>NRXN2</i> | Intragenic | up | 1.4 | 0.0005 |
| 41 | <i>GPR149</i> | Intragenic | up | 1.4 | <0.0001 | 41 | <i>EXOC3L2</i> | Intragenic | up | 1.3 | 0.0005 |
| 42 | <i>PAX7</i> | Intragenic | up | 1.3 | <0.0001 | 42 | <i>INSM1</i> | Promoter | up | 1.3 | 0.0005 |
| 43 | <i>DLL4</i> | Promoter | up | 1.3 | <0.0001 | 43 | <i>SEPT9</i> | Promoter | up | 1.3 | 0.0005 |
| 44 | <i>BHMT</i> | Intragenic | up | 1.3 | <0.0001 | 44 | <i>C1orf190</i> | Promoter | up | 1.2 | 0.0005 |
| 45 | <i>C6orf97</i> | Promoter | up | 1.2 | <0.0001 | 45 | <i>SPOCK2</i> | Intragenic | up | 1.2 | 0.0005 |
| 46 | <i>CA3</i> | Promoter | up | 1.4 | <0.0001 | 46 | <i>ALLC</i> | Promoter | up | 1.6 | 0.0005 |
| 47 | <i>TFPI2</i> | Promoter | up | 1.2 | <0.0001 | 47 | <i>MIR152</i> | Promoter | up | 1.4 | 0.0005 |
| 48 | <i>VSX1</i> | Promoter | up | 1.2 | <0.0001 | 48 | <i>FAM100A</i> | Promoter | down | 1.2 | 0.0005 |
| 49 | <i>C4orf22</i> | Intragenic | up | 1.2 | <0.0001 | 49 | <i>SPTBN4</i> | Promoter | up | 1.3 | 0.0005 |
| 50 | <i>HELT</i> | Intragenic | up | 1.2 | <0.0001 | 50 | <i>ZNF471</i> | Promoter | down | 1.3 | 0.0005 |

Values are given for the most significant microarray probe per gene/ annotated locus. PCa – prostate tumors, NPT – noncancerous prostate tissues, BCR – biochemical disease recurrence, FC – absolute fold change value.

Table S5. Associations of promoter methylation and gene expression with clinico-pathological variables and *TMPRSS2-ERG* fusion status in prostate tumors.

| Promoter methylation | pT3-4 vs. pT2 | | pISUP grade group | | <i>TMPRSS2-ERG</i> + vs. - | | PSA | | Prostate volume | | Age | |
|----------------------|---------------|---------------|-------------------|---------|----------------------------|---------------|-----------------|---------|-----------------|---------|-----------------|---------|
| | % | P-value | H | P-value | % | P-value | Z _{ad} | P-value | Z _{ad} | P-value | Z _{ad} | P-value |
| <i>PRKCB</i> | 81.8 vs. 66.7 | 0.0589 | 7.19 | 0.0659 | 83.1 vs. 61.4 | 0.0151 | 1.91 | 0.0555 | -0.17 | 0.4433 | 0.11 | 0.9123 |
| <i>CCDC181</i> | 94.5 vs. 88.5 | 0.2594 | 2.85 | 0.4150 | 92.2 vs. 88.6 | 0.5260 | 0.22 | 0.8275 | 1.22 | 0.2237 | 1.30 | 0.1924 |
| <i>ADAMTS12</i> | 92.7 vs. 82.3 | 0.0898 | 5.58 | 0.1338 | 89.6 vs. 75.0 | 0.0406 | 0.43 | 0.6665 | -0.70 | 0.4859 | 1.28 | 0.2021 |
| <i>ZMIZ1</i> | 94.5 vs. 81.3 | 0.0273 | 6.46 | 0.0914 | 85.7 vs. 86.4 | >0.9999 | 1.94 | 0.0528 | 0.58 | 0.5593 | 1.41 | 0.1577 |
| <i>FILIP1L</i> | 89.1 vs. 78.1 | 0.1223 | 1.93 | 0.5875 | 88.3 vs. 75.0 | 0.0755 | 0.70 | 0.4810 | 0.70 | 0.4810 | -0.12 | 0.9071 |
| <i>NAALAD2</i> | 74.5 vs. 65.6 | 0.2785 | 7.57 | 0.0557 | 71.4 vs. 70.5 | >0.9999 | 1.60 | 0.1105 | -0.06 | 0.9487 | 0.76 | 0.4483 |
| <i>CD44</i> | 36.4 vs. 33.3 | 0.7248 | 0.60 | 0.8957 | 40.3 vs. 20.5 | 0.0286 | -0.43 | 0.6686 | 0.91 | 0.3612 | 1.12 | 0.2639 |
| <i>KCTD8</i> | 36.4 vs. 12.5 | 0.0008 | 6.49 | 0.0902 | 31.2 vs. 6.8 | 0.0015 | 0.98 | 0.3248 | -0.49 | 0.6275 | 0.44 | 0.6568 |

| Gene expression | pT | | pISUP grade group | | <i>TMPRSS2-ERG</i> + vs. - | | PSA | | Prostate volume | | Age | |
|-----------------|-----------------|---------|-------------------|---------------|----------------------------|---------------|----------------|---------|-----------------|---------|----------------|---------------|
| | Z _{ad} | P-value | R _s | P-value | Z _{ad} | P-value | R _s | P-value | R _s | P-value | R _s | P-value |
| <i>PRKCB</i> | -0.45 | 0.6528 | -0.16 | 0.1439 | 0.17 | 0.8674 | -0.17 | 0.1362 | 0.00 | 0.9815 | 0.13 | 0.2456 |
| <i>CCDC181</i> | -0.65 | 0.5154 | -0.35 | 0.0016 | 2.47 | 0.0136 | -0.20 | 0.0755 | -0.01 | 0.9482 | 0.15 | 0.1698 |
| <i>ADAMTS12</i> | -0.35 | 0.7248 | 0.13 | 0.2570 | 0.97 | 0.3323 | -0.12 | 0.2958 | 0.05 | 0.6676 | 0.01 | 0.9334 |
| <i>ZMIZ1</i> | -0.29 | 0.7692 | -0.07 | 0.5211 | -0.09 | 0.9313 | 0.11 | 0.3433 | 0.03 | 0.8052 | 0.02 | 0.8385 |
| <i>NAALAD2</i> | -1.07 | 0.2864 | -0.35 | 0.0015 | -0.03 | 0.9742 | -0.19 | 0.0972 | -0.03 | 0.8003 | 0.27 | 0.0153 |

pT – pathological tumor stage, pISUP – pathological ISUP grading, ISUP – International Society of Urological Pathology, *TMPRSS2-ERG*+/- – *TMPRSS2-ERG* fusion positive/ negative status, PSA – prostate-specific antigen, H – Kruskal-Wallis's H parameter, Z_{ad} – Mann-Whitney's Z adjusted parameter, R_s – Spearman's correlation coefficient. Significant P-values are in bold.

Table S6. Univariate Cox proportional hazard analysis of molecular and/or clinico-pathological variables in Lithuanian and The Cancer Genome Atlas (TCGA) prostate cancer cohorts. For simplicity, only models' P-values are provided for TCGA data.

| No. | Covariates | Lithuanian cohort | | | TCGA cohort | |
|--------------------------------|---|---------------------|---------------------|-----------------|-----------------------|-----------------|
| | | HR (95% CI) | Covariate's P-value | Model's P-value | HR (95% CI) | Model's P-value |
| Clinico-pathological variables | | | | | | |
| 1 | pT (3 <i>vs.</i> 2) | 4.68 (2.30; 9.51) | < 0.0001 | < 0.0001 | 0.11 (0.03; 0.49) | 0.0001 |
| 2 | pISUP grade group (5 groups) | 2.93 (2.06; 4.16) | < 0.0001 | < 0.0001 | 2.00 (1.44; 2.80) | < 0.0001 |
| 3 | Prostate volume (cont.) | 1.01 (0.99; 1.03) | 0.2001 | 0.2226 | n. a. | n. a. |
| 4 | PSA (cont.) | 1.02 (1.00; 1.04) | 0.0342 | 0.0682 | 1.02 (0.96; 1.08) | 0.4881 |
| 5 | Age (cont.) | 1.00 (0.95; 1.04) | 0.9241 | 0.9239 | 1.02 (0.96; 1.08) | 0.4655 |
| Promoter methylation | | | | | | |
| 6 | <i>PRKCB</i> (M <i>vs.</i> U/ cont.) | 6.31 (1.52; 26.10) | 0.0115 | 0.0008 | 4.90 (0.81; 29.59) | 0.0795 |
| 7 | <i>CCDC181</i> (M <i>vs.</i> U/ cont.) | 4.18 (0.58; 30.29) | 0.1585 | 0.0725 | 7.22 (0.85; 61.18) | 0.0702 |
| 8 | <i>ADAMTS12</i> (M <i>vs.</i> U/ cont.) | >1000 | 0.9496 | 0.0003 | 49.36 (0.60; 4093.06) | 0.0624 |
| 9 | <i>NAALAD2</i> (M <i>vs.</i> U/ cont.) | 6.08 (1.86; 19.84) | 0.0029 | 0.0002 | 7.38 (0.69; 79.12) | 0.0911 |
| 10 | <i>ZMIZ1</i> (M <i>vs.</i> U/ cont.) | 3.51 (0.85; 14.57) | 0.0845 | 0.0368 | 0.28 (0.00; 107.98) | 0.6455 |
| Gene expression | | | | | | |
| 11 | <i>PRKCB</i> (cont.) | 0.60 (0.27; 1.34) | 0.2118 | 0.1836 | 1.02 (1.00; 1.04) | 0.0378 |
| 12 | <i>CCDC181</i> (cont.) | 0.00 (0.00; 28.14) | 0.1329 | 0.1111 | 0.98 (0.94; 1.01) | 0.1856 |
| 13 | <i>ADAMTS12</i> (cont.) | 2.26 (0.02; 262.82) | 0.7375 | 0.7387 | 1.02 (1.00; 1.04) | 0.0321 |
| 14 | <i>ZMIZ1</i> (cont.) | 0.94 (0.82; 1.09) | 0.4090 | 0.3960 | 1.00 (1.00; 1.00) | 0.0548 |
| 15 | <i>NAALAD2</i> (cont.) | 0.00 (0.00; 0.17) | 0.0205 | 0.0059 | 1.00 (0.99; 1.00) | 0.7077 |
| 16 | <i>TMPRSS2-ERG</i> (yes <i>vs.</i> no) | 0.70 (0.34; 1.44) | 0.3328 | 0.3375 | n. a. | n. a. |

M/U – methylated/ unmethylated promoter status, cont. – continuous variable, *TMPRSS2-ERG* – fusion transcript status, pT – pathological tumor stage, pISUP – pathological ISUP grading, ISUP – International Society of Urological Pathology, PSA – prostate-specific antigen, HR – hazard ratio, CI – confidence intervals. Significant P-values are in bold, n.a. – not available/ not analyzed.

Table S7. Multivariate Cox proportional hazard analysis of protein-coding gene methylation and other molecular and/or clinico-pathological variables in Lithuanian and The Cancer Genome Atlas (TCGA) prostate cancer cohorts.

| No. | Covariates | Lithuanian cohort | | TCGA cohort | |
|--|------------------------------------|--------------------|---------------------|-----------------|-----------------|
| | | HR (95% CI) | Covariate's P-value | Model's P-value | Model's P-value |
| Combinations with clinico-pathological variables | | | | | |
| 1 | pT (3 vs. 2) | 4.14 (2.04; 8.42) | 0.0001 | <0.0001* | 0.0002 |
| | <i>PRKCB</i> (M vs. U/ cont.) | 5.18 (1.24; 21.55) | 0.0245 | | |
| 2 | pT (3 vs. 2) | 4.34 (2.14; 8.82) | 0.0001 | <0.0001* | 0.0001 |
| | <i>NAALAD2</i> (M vs. U/ cont.) | 5.55 (1.70; 18.15) | 0.0048 | | |
| 3 | pISUP grade group (5 groups) | 2.65 (1.86; 3.79) | < 0.0001 | <0.0001* | <0.0001 |
| | <i>PRKCB</i> (M vs. U/ cont.) | 4.51 (1.07; 18.93) | 0.0408 | | |
| 4 | pISUP grade group (5 groups) | 2.61 (1.83; 3.72) | < 0.0001 | <0.0001* | <0.0001 |
| | <i>NAALAD2</i> (M vs. U/ cont.) | 4.50 (1.36; 14.94) | 0.0145 | | |
| 5 | PSA (cont.) | 1.02 (1.00; 1.04) | 0.0403 | 0.0008* | 0.5903 |
| | <i>PRKCB</i> (M vs. U/ cont.) | 6.28 (1.52; 26.02) | 0.0117 | | |
| 6 | PSA (cont.) | 1.02 (1.00; 1.04) | 0.0862 | 0.0001 | 0.7838 |
| | <i>PRKCB</i> (M vs. U/ cont.) | 3.76 (0.87; 16.25) | 0.0778 | | |
| | <i>NAALAD2</i> (M vs. U/ cont.) | 3.70 (1.09; 12.60) | 0.0371 | | |
| Combinations of gene promoter methylation | | | | | |
| 7 | <i>PRKCB</i> (M vs. U/ cont.) | 3.84 (0.90; 16.35) | 0.0704 | 0.0001 | 0.1314 |
| | <i>NAALAD2</i> (M vs. U/ cont.) | 4.30 (1.28; 14.46) | 0.0191 | | |
| 8 | <i>ADAMTS12</i> (M vs. U/ cont.) | >1000 | 0.9522 | < 0.0001 | 0.1132 |
| | <i>NAALAD2</i> (M vs. U/ cont.) | 3.81 (1.16; 12.46) | 0.0279 | | |
| 9 | <i>PRKCB</i> (M vs. U/ cont.) | 3.28 (0.79; 13.63) | 0.1033 | < 0.0001 | 0.2001 |
| | <i>ADAMTS12</i> (M vs. U/ cont.) | >1000 | 0.9524 | | |
| | <i>NAALAD2</i> (M vs. U/ cont.) | 3.46 (1.04; 11.45) | 0.0433 | | |
| 10 | <i>NAALAD2</i> (M vs. U/ cont.) | 5.10 (1.18; 22.08) | 0.0303 | 0.0007 | 0.2267 |
| | <i>NAALAD2</i> (expression, cont.) | 0.00 (0.00; 2.70) | 0.0828 | | |
| Combinations with fusion transcript status | | | | | |
| 11 | <i>TMPRSS2-ERG</i> (yes vs. no) | 1.61 (0.80; 3.27) | 0.1872 | 0.0113 | n.a. |
| | <i>PRKCB</i> (M vs. U/ cont.) | 5.30 (1.26; 22.25) | 0.0233 | | |
| 12 | <i>TMPRSS2-ERG</i> (yes vs. no) | 0.48 (0.23; 1.02) | 0.0582 | 0.0002 | n.a. |
| | <i>ADAMTS12</i> (M vs. U/ cont.) | >1000 | 0.9479 | | |
| 13 | <i>TMPRSS2-ERG</i> (yes vs. no) | 1.48 (0.73; 3.00) | 0.2748 | 0.0003 | n.a. |
| | <i>NAALAD2</i> (M vs. U/ cont.) | 8.57 (2.04; 35.92) | 0.0035 | | |

Selected combinations are shown. For simplicity, only models' P-values are provided for TCGA data. M/U – methylated/unmethylated promoter status, *TMPRSS2-ERG* – fusion transcript status, pT – pathological tumor stage, pISUP – pathological ISUP grading, ISUP – International Society of Urological Pathology, PSA – prostate-specific antigen, HR – hazard ratio, CI – confidence intervals. Significant P-values are in bold, n.a. – not analyzed. *Forward entering of covariates.