Supplementary Table 1. Association between ROCK1 staining and prostate cancer phenotype in ERG negative cancers.

| Parameter                        | N   | ROCK1 (%) |     |     |     |     |
|----------------------------------|-----|-----------|-----|-----|-----|-----|
|                                 |     | Negative  | Weak| Moderate | Strong | P    |
| Tumor stage                     |     |           |     |         |       | <0.0001 |
| pT2                             | 3508| 12.4      | 26.4| 49.5    | 11.7  |      |
| pT3a                            | 1031| 10.3      | 26.6| 49.5    | 13.7  |      |
| pT3b–pT4                        | 644 | 7.0       | 23.6| 48.1    | 21.3  |      |
| Gleason grade                   |     |           |     |         |       | <0.0001 |
| ≤3+3                            | 1042| 18.3      | 34.5| 41.1    | 6.0   |      |
| 3+4                             | 2798| 10.6      | 25.3| 51.4    | 12.6  |      |
| 3+4 Tert.5                     | 227 | 8.4       | 22.0| 54.6    | 15.0  |      |
| 4+3                            | 574 | 7.5       | 22.6| 50.0    | 19.9  |      |
| 4+3 Tert.5                     | 330 | 5.5       | 20.0| 51.5    | 23.0  |      |
| ≥4+4                           | 302 | 5.3       | 21.9| 50.3    | 22.5  |      |
| Quantitative Gleason grade      |     |           |     |         |       | <0.0001 |
| ≤3+3                           | 1042| 18.3      | 34.5| 41.1    | 6.0   |      |
| 3+4 ≤5%                        | 766 | 11.1      | 26.6| 51.8    | 10.4  |      |
| 3+4 6–10%                      | 721 | 11.4      | 25.2| 50.9    | 12.5  |      |
| 3+4 11–20%                     | 624 | 11.5      | 23.7| 51.8    | 13.0  |      |
| 3+4 21–30%                     | 315 | 9.2       | 23.5| 53.0    | 14.3  |      |
| 3+4 31–49%                     | 273 | 8.8       | 27.8| 46.5    | 16.8  |      |
| 3+4 Tert.5                     | 227 | 8.4       | 22.0| 54.6    | 15.0  |      |
| 4+3 50–60%                     | 236 | 9.3       | 22.9| 51.3    | 16.5  |      |
| 4+3 61–100%                    | 330 | 5.5       | 20.0| 51.5    | 23.0  |      |
| 4+3 Tert.5                     | 281 | 6.8       | 21.0| 48.4    | 23.8  |      |
| ≥4+4                           | 302 | 5.3       | 21.9| 50.3    | 22.5  |      |
| Lymph node metastasis           |     |           |     |         |       | <0.0001 |
| N0                              | 3003| 10.5      | 23.6| 51.1    | 14.8  |      |
| N+                              | 288 | 6.6       | 20.1| 46.2    | 27.1  |      |
| Preoperative PSA level (ng/ml)   |     |           |     |         |       | 0.3238 |
| ≤4                             | 552 | 9.4       | 26.1| 49.8    | 14.7  |      |
| 4–10                           | 3068| 10.9      | 26.0| 49.9    | 13.2  |      |
| 10–20                          | 1134| 13.1      | 26.2| 48.7    | 12.0  |      |
| >20                            | 402 | 11.7      | 25.6| 47.0    | 15.7  |      |
| Surgical margin                 |     |           |     |         |       | 0.0071 |
| Negative                       | 4154| 11.3      | 26.1| 50.1    | 12.5  |      |
| Positive                       | 960 | 10.9      | 26.7| 45.8    | 16.6  |      |
**Supplementary Table 2. Association between ROCK1 staining and prostate cancer phenotype in ERG positive cancers.**

| Parameter               | N     | ROCK1 (%) |        |        |        |        |        |        |        |        |        |        | P       |
|-------------------------|-------|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|
|                         |       | Negative  | Weak   | Moderate | Strong |        |        |        |        |        |        |        |         |
| Tumor stage             |       |           |        |        |        |        |        |        |        |        |        |        | <0.0001 |
| pT2                     | 2421  | 2.7       | 16.0   | 60.1   | 21.2   |        |        |        |        |        |        |        |         |
| pT3a                    | 1068  | 2.4       | 13.1   | 55.5   | 28.9   |        |        |        |        |        |        |        |         |
| pT3b-pT4                | 550   | 2.9       | 13.6   | 46.5   | 36.9   |        |        |        |        |        |        |        |         |
| Gleason grade           |       |           |        |        |        |        |        |        |        |        |        |        | <0.0001 |
| ≤3+3                    | 827   | 3.7       | 23.8   | 61.7   | 10.8   |        |        |        |        |        |        |        |         |
| 3+4                     | 2396  | 2.4       | 13.2   | 59.0   | 25.3   |        |        |        |        |        |        |        |         |
| 3+4 Tert. 5             | 124   | 0.8       | 14.5   | 54.0   | 30.6   |        |        |        |        |        |        |        |         |
| 4+3                     | 409   | 1.7       | 11.5   | 47.4   | 39.4   |        |        |        |        |        |        |        |         |
| 4+3 Tert. 5             | 225   | 1.8       | 9.3    | 48.9   | 40.0   |        |        |        |        |        |        |        |         |
| ≥4+4                    | 163   | 3.1       | 9.8    | 47.2   | 39.9   |        |        |        |        |        |        |        |         |
| Quantitative Gleason grade |     |           |        |        |        |        |        |        |        |        |        |        |         |
| ≤3+3                    | 827   | 3.7       | 23.8   | 61.7   | 10.8   |        |        |        |        |        |        |        | <0.0001 |
| 3+4 ≤5%                 | 591   | 2.9       | 17.9   | 58.9   | 20.3   |        |        |        |        |        |        |        |         |
| 3+4 6-10%               | 642   | 2.5       | 11.2   | 63.2   | 23.1   |        |        |        |        |        |        |        |         |
| 3+4 11-20%              | 513   | 1.9       | 11.3   | 56.9   | 29.8   |        |        |        |        |        |        |        |         |
| 3+4 21-30%              | 300   | 1.7       | 10.3   | 57.0   | 31.0   |        |        |        |        |        |        |        |         |
| 3+4 31-49%              | 215   | 2.3       | 13.0   | 53.0   | 31.6   |        |        |        |        |        |        |        |         |
| 3+4 Tert.5              | 124   | 0.8       | 14.5   | 54.0   | 30.6   |        |        |        |        |        |        |        |         |
| 4+3 50-60%              | 181   | 0.6       | 16.6   | 49.2   | 33.7   |        |        |        |        |        |        |        |         |
| 4+3 61-100%             | 225   | 1.8       | 9.3    | 48.9   | 40.0   |        |        |        |        |        |        |        |         |
| 4+3 Tert.5              | 186   | 2.2       | 7.0    | 44.6   | 46.2   |        |        |        |        |        |        |        |         |
| ≥4+4                    | 163   | 3.1       | 9.8    | 47.2   | 39.9   |        |        |        |        |        |        |        |         |
| Lymph node metastasis   |       |           |        |        |        |        |        |        |        |        |        |        | 0.1389  |
| N0                      | 2315  | 1.9       | 13.3   | 55.5   | 29.3   |        |        |        |        |        |        |        |         |
| N+                       | 256   | 3.1       | 10.9   | 51.2   | 34.8   |        |        |        |        |        |        |        |         |
| Preoperative PSA level (ng/ml) |     |           |        |        |        |        |        |        |        |        |        |        | 0.4117  |
| <4                       | 562   | 1.6       | 14.9   | 58.4   | 25.1   |        |        |        |        |        |        |        |         |
| 4–10                     | 2470  | 2.8       | 14.3   | 57.9   | 25.0   |        |        |        |        |        |        |        |         |
| 10–20                    | 729   | 3.0       | 16.3   | 54.5   | 26.2   |        |        |        |        |        |        |        |         |
| >20                      | 248   | 2.4       | 17.3   | 52.0   | 28.2   |        |        |        |        |        |        |        |         |
| Surgical margin          |       |           |        |        |        |        |        |        |        |        |        |        | 0.0091  |
| Negative                 | 3190  | 2.5       | 15.2   | 58.2   | 24.1   |        |        |        |        |        |        |        |         |
| Positive                 | 791   | 3.0       | 14.3   | 53.0   | 29.7   |        |        |        |        |        |        |        |         |
Supplementary Table 3. Association between ROCK1 staining and patient age in all cancers, ERG negative cancers and in ERG positive cancers.

| Age   | n   | ROCK1 IHC result (%) | p    |
|-------|-----|----------------------|------|
|       |     | negative | weak   | moderate | strong | <0.0001 |
| all cancers |     |           |       |          |        |         |
| <50   | 292 | 6.2       | 18.8  | 61.6     | 13.4   |         |
| 50–70 | 2670| 6.7       | 20.1  | 55.4     | 17.8   |         |
| >70   | 1548| 7.6       | 19.6  | 52.3     | 20.5   |         |
| <50   | 80  | 10.0      | 23.8  | 53.8     | 12.5   |         |
| ERG negative |     |           |       |          |        | 0.4249  |
| 50–70 | 1007| 10.6      | 25.4  | 50.6     | 13.3   |         |
| >70   | 722 | 11.1      | 27.4  | 49.4     | 12.0   |         |
| <50   | 134 | 2.4       | 16.6  | 60.0     | 21.1   | <0.0001 |
| ERG positive |     |           |       |          |        |         |
| 50–70 | 1045| 3.3       | 17.5  | 54.2     | 25.0   |         |
| >70   | 444 | 2.3       | 9.9   | 53.8     | 34.0   |         |

Supplementary Table 4. Multivariate analysis including ROCK1 expression in all cancers, ERG negative and ERG positive cancers.

| Subset    | Scenario | N     | Preoperative PSA-Level | pT-stage | cT-stage | Gleason grade prostatectomy | Gleason grade biopsy | pN-stage | R-stage | ROCK1-expression |
|-----------|----------|-------|------------------------|----------|----------|----------------------------|----------------------|----------|---------|-----------------|
| All       | 1        | 5789  | <0.0001                | <0.0001  | -        | <0.0001                   | -                    | <0.0001  | 0.0075  | 0.0029          |
|           | 2        | 9373  | <0.0001                | <0.0001  | -        | <0.0001                   | -                    | <0.0001  | 0.0004  |                 |
|           | 3        | 9224  | <0.0001                | -        | <0.0001  | <0.0001                   | -                    | -        | 0.0008  |                 |
|           | 4        | 9103  | <0.0001                | -        | <0.0001  | <0.0001                   | -                    | <0.0001  | -       | <0.0001         |
| ERG-negative | 1         | 2912  | <0.0001                | <0.0001  | -        | <0.0001                   | -                    | 0.0013   | 0.4537  | 0.0165          |
|           | 2        | 4600  | <0.0001                | <0.0001  | -        | <0.0001                   | -                    | 0.0068   | 0.0601  |                 |
| ERG-positive | 3         | 4550  | <0.0001                | -        | <0.0001  | <0.0001                   | -                    | -        | 0.0024  |                 |
|           | 4        | 4495  | <0.0001                | -        | <0.0001  | <0.0001                   | -                    | -        | <0.0001        |
|           | 1        | 2264  | 0.0009                 | <0.0001  | -        | <0.0001                   | -                    | 0.0236   | 0.0335  | 0.017           |
|           | 2        | 3582  | 0.0009                 | <0.0001  | -        | <0.0001                   | -                    | 0.0002   | 0.0018  |                 |
|           | 3        | 3504  | <0.0001                | -        | <0.0001  | <0.0001                   | -                    | -        | 0.0067  |                 |
|           | 4        | 3455  | <0.0001                | -        | <0.0001  | <0.0001                   | -                    | -        | <0.0001        |

Scenario 1 includes all postoperatively available parameters (pathological tumor (pT) stage, lymph node (pN), surgical margin (R) status, preoperative 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.
Supplementary Table 5. Cox proportional hazards for PSA recurrence-free survival after prostatectomy of established preoperative prognostic parameter and ROCK1 expression.

| Variable                          | Univariate analysis | Multivariate analysis |
|-----------------------------------|---------------------|-----------------------|
| **Gleason grade biopsy**          |                     |                       |
| 3+4 vs. ≤3+3                      | 2.20 (2.00-2.41) ***| 1.81 (1.63-2.01) ***  |
| 4+3 vs. 3+4                       | 1.85 (1.66-2.05) ***| 1.65 (1.47-1.86) ***  |
| ≥4+4 vs. 4+3                      | 1.48 (1.32-1.66) ***| 1.33 (1.17-1.52) ***  |
| **cT-stage**                      |                     |                       |
| ≥T2c vs. T1c                       | 3.95 (3.24-4.76) ***| 2.11 (1.70-2.59) ***  |
| **Preoperative PSA-level (ng/µl)**|                     |                       |
| 4-10 vs. <4                       | 1.26 (1.12-1.42) ** | 1.34 (1.15-1.57) **   |
| 10-20 vs. 4-10                     | 2.08 (1.93-2.25) ***| 1.58 (1.43-1.74) ***  |
| >20 vs. 10-20                      | 1.95 (1.76-2.16) ***| 1.65 (1.44-1.88) ***  |
| **ROCK1 expression**              |                     |                       |
| Strong vs. negative                | 2.04 (1.72-2.44) ***| 1.55 (1.30-1.86) ***  |
| ERG negative subset                | 2.41 (1.91-3.07) ***| -                     |
| ERG positive subset                | 1.61 (1.11-2.42) *  | -                     |

Confidence interval (95%) in brackets; asterisk indicate significance level: * p≤0.05, ** p≤0.001, *** p≤0.0001.
Supplementary Table 6. Pathological and clinical data of the arrayed prostate cancers.

|                        | Study cohort on TMA* | Biochemical relapse among categories |
|------------------------|----------------------|--------------------------------------|
| **Follow-up**          |                      |                                      |
| n                      | 11 665               | 2769 (23.7%)                         |
| Mean / Median (month)  | 62 / 49              | -                                    |
| **Age (y)**            |                      |                                      |
| ≤50                    | 334                  | 81 (24.3%)                           |
| 51-59                  | 3061                 | 705 (23%)                            |
| 60-69                  | 7188                 | 1610 (22.4%)                         |
| ≥70                    | 1761                 | 370 (21%)                            |
| **Pretreatment PSA (ng/ml)** |                |                                      |
| <4                     | 1585                 | 242 (15.3%)                          |
| 4-10                   | 7480                 | 1355 (18.1%)                         |
| 10-20                  | 2412                 | 737 (30.6%)                          |
| >20                    | 812                  | 397 (48.9%)                          |
| **pT stage (AJCC 2002)** |                    |                                      |
| pT2                    | 8187                 | 1095 (13.4%)                         |
| pT3a                   | 2660                 | 817 (30.7%)                          |
| pT3b                   | 1465                 | 796 (54.3%)                          |
| pT4                    | 63                   | 51 (81%)                             |
| **Gleason grade**      |                      |                                      |
| ≤3+3                   | 2848                 | 234 (8.2%)                           |
| 3+4                    | 6679                 | 1240 (18.6%)                         |
| 3+4 Tert.5             | 433                  | 115 (26.6%)                          |
| 4+3                    | 1210                 | 576 (47.6%)                          |
| 4+3 Tert.5             | 646                  | 317 (49.1%)                          |
| ≥4+4                   | 596                  | 348 (58.4%)                          |
| **pN stage**           |                      |                                      |
| pN0                    | 6970                 | 1636 (23.5%)                         |
| pN+                    | 693                  | 393 (56.7%)                          |
| **Surgical margin**    |                      |                                      |
| Negative               | 9990                 | 1848 (18.5%)                         |
| Positive               | 2211                 | 853 (38.6%)                          |

* Numbers do not add up to 12 427 in all categories due to missing values.
AJCC American Joint Committee on Cancer