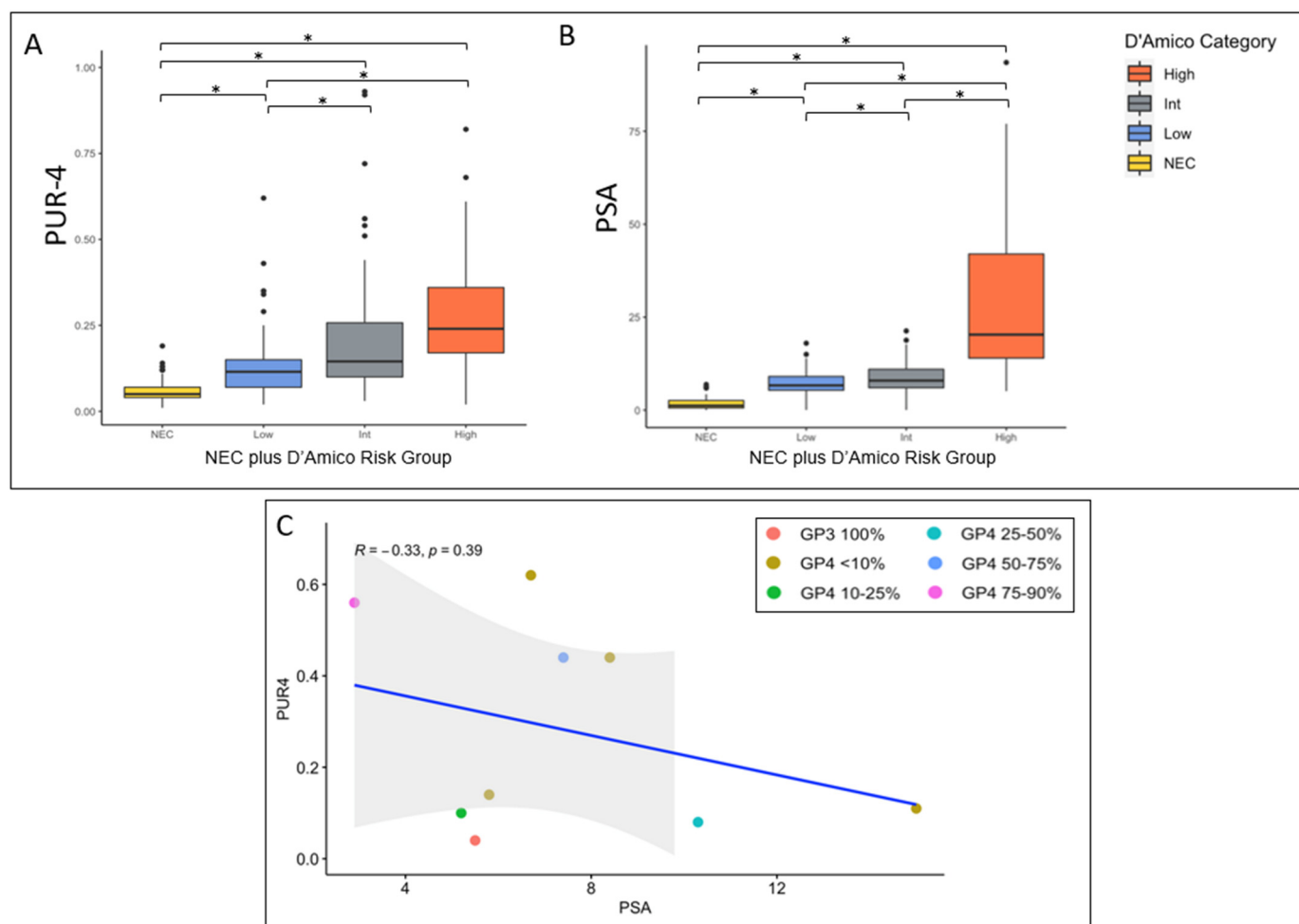


Supplementary materials

## The Urine Biomarker PUR-4 is Positively Associated with the Amount of Gleason 4 in Human Prostate Cancers.

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**Figure S1.** PSA and PUR-4 data in biopsy patients categorized on D'Amico and PSA in the nine RPx men. For 'a' and 'b' Data were analyzed using a non-parametric Kruskal-Wallis analysis of variance followed by Games-Howell post-hoc analysis. A) PUR-4 in men categorized as No Evidence of prostate Cancer (NEC) and D'Amico risk groups low, intermediate and high-risk. PUR-4 correlated with increasing D'Amico risk group (Pearson's correlation  $r=0.62, p=3.2e^{-30}$ ). '\*\*' indicates a significant difference ( $p<0.05$ ). B) PSA correlated with increasing D'Amico risk group (Pearson's correlation  $r=0.73, p=9.8e^{-47}$ ). C) PUR-4 and PSA for the nine prostatectomy cases, no correlation was found ( $\rho = -0.33, p=0.39$ ).

**Table S1.** Clinical characteristics and PUR scores for biopsy cases categorized by Gleason Grade and subcategorized by % biopsy cores positive. All data presented as: 'Median (IQR)', Abbreviations: IQR, interquartile range; GG, Gleason Grade; NEC, No Evidence of Cancer; US, Ultrasound; Age and PSA at time of urine collection; PSA, prostate specific antigen; PSAD, PSA density (PSA/US prostate volume). PUR-1-4, Prostate urine risk signatures 1-4, NA, Not applicable.

	NEC	GG1	GG2	GG3	GG4	GG5	GG1(S)	GG1(L)	GG2(S)	GG2(L)	GG3(S)	GG3(L)
Total (%)	62 (22.4%)	93 (33.6%)	61 (22.0%)	38 (13.7%)	12 (4.3%)	11 (4.0%)	46 (16.6%)	47 (17%)	30 (10.8%)	31 (11.2%)	19 (6.9%)	19 (6.9%)
Age in years,	66 (17)	67 (6.5)	68 (9)	73 (8.25)	71 (12.25)	69 (8.5)	67 (8)	69.5 (8.25)	67.5 (9.5)	69 (7)	75 (7)	70 (10)
PSA ng/ml	1.2 (2)	7.3 (5.5)	7.8 (4.5)	11.35 (11.03)	13.45 (12.68)	18.7 (45.95)	6.75 (5.05)	7.5 (6.2)	6.2 (2.15)	9.5 (5.25)	11.2 (6.75)	11.6 (15.9)
Prostate volume US	NA	23 (7.5)	42.43 (24.73)	49.03 (28.42)	50.39 (22.89)	55.93 (24.97)	25.08 (32.45)	22 (5.25)	55.29 (33.62)	37.95 (20.96)	59 (43.28)	35.94 (18.03)
PSAD	NA	0.292 (0.315)	0.179 (0.122)	0.256 (0.227)	0.287 (0.385)	0.31 (0.565)	0.248 (0.272)	0.417 (0.392)	0.123 (0.055)	0.24 (0.309)	0.19 (0.098)	0.323 (0.454)
biopsy Cores taken	NA	11 (4)	9 (2)	8 (6)	5.5 (5.25)	4 (6.5)	12 (2)	9 (4)	10 (2.75)	8 (2)	10 (3)	6 (4)
Biopsy Cores positive	NA	1 (1)	3 (3)	3.5 (2.75)	3.5 (3.5)	4 (5)	1 (0)	2 (1.5)	2 (2)	5 (2.5)	2 (2)	4 (2.5)
% biopsy cores positive	NA	12.5 (16.67)	37.5 (42.5)	50 (58.57)	50 (36.39)	100 (0)	8.333 (4.17)	18.182 (16.67)	20 (14.21)	62.5 (25)	28.571 (31.79)	80 (35.42)
% biopsy cores positive/US	NA	0.003 (0.004)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.001 (0.002)	0.003 (0.004)	0.003 (0.004)	0.001 (0)	0.001 (0.001)	0.001 (0)	0.001 (0.002)
PUR-1	0.358 (0.206)	0.119 (0.165)	0.078 (0.145)	0.03 (0.119)	0.059 (0.04)	0.07 (0.059)	0.112 (0.104)	0.129 (0.202)	0.152 (0.135)	0.041 (0.061)	0.042 (0.126)	0.019 (0.094)
PUR-2	0.321 (0.036)	0.302 (0.078)	0.275 (0.151)	0.187 (0.217)	0.248 (0.07)	0.201 (0.124)	0.302 (0.06)	0.306 (0.099)	0.315 (0.059)	0.214 (0.143)	0.217 (0.178)	0.152 (0.197)
PUR-3	0.256 (0.129)	0.442 (0.15)	0.452 (0.116)	0.475 (0.119)	0.506 (0.032)	0.489 (0.073)	0.45 (0.102)	0.417 (0.166)	0.411 (0.127)	0.491 (0.098)	0.473 (0.123)	0.483 (0.091)
PUR-4	0.049 (0.036)	0.126 (0.09)	0.162 (0.18)	0.255 (0.269)	0.188 (0.075)	0.171 (0.146)	0.131 (0.068)	0.119 (0.126)	0.107 (0.076)	0.223 (0.174)	0.219 (0.195)	0.302 (0.251)

**Table S2.** Combined RPx and Biopsy clinical characteristics for the 9 radical prostatectomy (RPx) cases examined. GG = Gleason Grade group; Majority, data for most common GG found in each prostate; Highest GG, the highest GG found in each prostate; GP3, GP4, GP5, Gleason Pattern 3, 4, 5 respectively; Age, age at recruitment; US vol, Ultrasound volume of prostate; PCa, prostate cancer; All areas measured from scans of H&E slides (see methods); for GP3 and GP4 amount calculation see results; GG2<sup>+5</sup> indicates GG2 cancer with a tertiary GP5 (<5% of all PCa); PUR-1-4, Prostate urine risk signatures 1-4.

RPx data										Biopsy data				PUR signatures				
GG	Majority GG, %GP4	Highest GG, %GP4	prostate area (mm <sup>2</sup> )	Tumor area (mm <sup>2</sup> )	GP3 area	GP4 area	Age	PSA	GG	US vol	Total Biopsy Cores taken	PCa- Positive Cores	% cores PCa positive	PUR-1	PUR-2	PUR-3	PUR-4	
RPx 1	GG1	GG1, (+GP4<2%)	GG1, (+GP4<2%)	5269.9	486.4	481.6	4.8	54	5.5	GG1	54.5	10	3	30.0	0.45	0.31	0.20	0.04
RPx 2	GG2	GG2, <10% GP4	GG2, <10% GP4	3339.0	341.2	324.1	17.1	64	15	GG1	45.8	8	4	50.0	0.15	0.33	0.42	0.11
RPx 3	GG2	GG2, <10% GP4	GG2, <10% GP4	4347.6	531.8	505.2	26.6	52	5.8	GG1	68.0	12	2	16.7	0.10	0.30	0.46	0.14
RPx 4	GG2 <sup>+5</sup>	GG2, <10% GP4	GG5, GP4+5 ≥50% GP4	4606.0	254.5	233.9	20.5	70	8.4	GG3	59.0	10	3	30.0	0.01	0.08	0.48	0.44
RPx 5	GG2	GG2, 10-25% GP4	GG2, 10-25% GP4	3643.2	172.1	143.3	28.8	66	5.2	GG2	35.2	9	2	22.2	0.17	0.34	0.39	0.10
RPx 6	GG2	GG2, <10% GP4	GG2, 25-50% GP4	4529.7	364.0	302.0	62.0	68	6.7	GG2	36.1	28	18	66.7	0.00	0.03	0.35	0.62
RPx 7	GG3	GG2, 25-50% GP4	GG3, 50-75% GP4	11991.1	92.9	54.8	38.0	67	10.3	GG2	85.6	9	1	11.1	0.23	0.35	0.35	0.08
RPx 8	GG3	GG3, 50-75% GP4	GG3, 50-75% GP4	3729.6	158.7	60.3	98.4	65	7.4	GG2	46.0	23	3	13.0	0.01	0.08	0.47	0.44
RPx 9	GG3	GG3, 75-90% GP4	GG3, 75-90% GP4	3851.4	664.4	122.2	542.2	75	2.9	GG3 <sup>+5</sup>	38.0	10	8	80.0	0.00	0.04	0.40	0.56