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Supplementary Material

Prostate Cancer-Induced Changes in Urinary Odors at Biomarker Concentrations of PPQ with Validation by Sniffer Mouse Behavioural Assays

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ABSTRACT

Although prostate-specific antigen (PSA) is a significant tumor marker for prostate cancer at present, the low specificity (approximately 33%) and so on likely lead to an overdiagnosis and patient suffering from highly invasive prostate biopsy. Complementary measures with cancer-characteristic biomarkers could improve the specificity and accuracy of diagnosis before the biopsy. Previously, "sniffer mice" were shown to be super-sensitive to differences in odors and to discriminate between odors of urine mixtures from patients with bladder cancer before and after tumor resection as well as urine odors of mice with or without experimental tumors. Here, we showed that the sniffer mice discriminate efficiently urinary odors of patients with prostate cancer using an odor plume-guided Y-maze behavioural assay. Through discrimination training in forced-odor choice, statistically significant increases in correct odor choice rates showed the super-sensitivity of sniffer mice to the olfactory cue of ppq-level urinary biomarkers for prostate cancer in 10^6-fold diluted urine samples, where donor-unique odors were below the threshold. Moreover, we validated eight volatile urinary biomarkers nearly at their original relative concentrations as the prostate cancer cue even when adding a similar biomarker profile to the post-radical prostatectomy urine samples by the same behavioural score of the sniffer mice. These biomarkers and profiles could be useful for non-invasive tests for prostate and bladder cancers.

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Results

I Variation of Negative Control Urine Samples Induces a Moderate Perturbation in %Correct of Sniffer Mice for the Identical, Learned and Rewarded Odor

Notably, the %Correct fall of −5.1% at the prostate cancer U_m vs. bladder cancer U_m pair was judged as a mismatch to the learned prostate cancer odor, leading to a 100% (1/1) specificity of this behavioural assay for prostate cancer against bladder cancer (Supplementary Table 5, see Materials and Methods). The discrimination pair of prostate cancer U_m vs. bladder cancer U_m was not considered as the positive control pair of the prostate cancer U_m vs. post-RP U_m samples, because sniffer mice were somewhat confused to discriminate this modified odor pair, in which a possible partial similarity between the bladder cancer and prostate cancer odors would made the partner prostate cancer odor slightly and relatively different from the learned odor. This interpretation is further supported by an extended analysis described below.

Based on the criterion, we judged %Correct falls of our previous data for bladder cancer U_m vs. individual patient post-transurethral resection (post-TUR) U_i samples. Considering an expected increase in inter-individual variations in relative concentrations of individual patient-dependent dietary and genetically-controlled compounds among individual patient post-TUR U_i samples compared to the urine mixture

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U_m of 5 patients × 5 samples, a decrease and remained constant in sensitivity of the behavioural assay for pairs of bladder cancer U_m vs. individual patient post-TUR U_i samples, respectively, could be attributed to a negative control-dependent positive odor choice and a complete ignorance of the negative control. As the inter-individual negative control variations increased, a sensitivity of the sniffer mouse behavioural assay (percentage of matched test urine samples) decreased from 78% (7/9) to 25% (1/4) (Supplementary Table 4, and other data were not shown). Notably, all %Correct of the four trials were significantly higher than the chance level. The decreased sensitivity indicates that odor pair of a positive control and a modified negative control differs from the positive control pair, as we treated.

Upon switching between different olfactory cues, %Correct falls often indicated mismatches to the learned odor (data now shown). Five tests of individual urine samples including the bladder cancer odor K:U_m with a much reduced #165 peak (see Materials and Methods, Table 3) for the learned bladder cancer odor resulted in 60% (3/5) specificity of the sniffer mouse behavioural assay for bladder cancer (Supplementary Table 4, and other data were not shown). Similarly, four tests of bladder cancer urines for the learned salient occult blood odor resulted in 75% (3/4) specificity of this assay (Supplementary Table 4, and other data were not shown). Totally, the criterion of −1.29 × SD resulted in a 63-83% sensitivity and a 60-100% specificity of the sniffer mouse behavioural assay for prostate or bladder cancer or occult blood regardless of neoadjuvant endocrine therapy for prostate cancer (Supplementary Table 4). Statistical parameters of pairwise differences by Student's t-test or absolute values of %Correct did not demonstrate any systematic differences in %Correct and their falls or confused statuses of the sniffer mice (Supplementary Tables 3 & 4).

II Calibration of Biomarker Concentrations in Urine Samples of Individual Patients with Prostate Cancer

Concentrations of the nine biomarkers in individual patients were determined with calibration curves of SPMS-GC-MS peak area vs. biomarker concentration (data now shown). High correlation coefficients of >0.9997 were observed for all calibration curves of the nine biomarkers in urine mixture samples, Ums, except for 2-phenyl-2-propanol (r = 0.9990) (data not shown). Similarly, to urine mixture samples, concentrations of the nine biomarkers significantly differed between 83 of 99 pairs of pre- and post-RP individual urine samples (Supplementary Figure 3 and other data were not shown). Inter-patient variability was higher than inter-sample variability, as shown relatively-low box height of 25^{th}-75^{th} percentile compared to differences in mean values for most patients (Supplementary Figure 3), as reported previously [1-3]. For this reason, we did not exclude outliers from the averaging. Provisional cut-off values of biomarkers in concentrations and ratios were determined, based on two pairs of pre- and post-RP (prostate cancer) and pre- and post-TUR (bladder cancer) urine mixture concentrations and rates as pairs of positive and negative controls, respectively, as well as those of healthy volunteers as negative control (Table 3 and Supplementary Table 7).

Although the sniffer mouse behavioural assay and combinatorial method of provisional cut-off values of the ten biomarkers exhibited similar sensitivity for detection of prostate cancer, a half of diagnoses were identical (*P2*, *P5*, *P13*, *Pe1*, *Pe3*, and *Pe7*) but the half was not (*P6*, *P8*, *P10*, *Pe11*, *Pe14*, and *Pe4*). Relative concentrations of dimethyl succinate were lower in *P8* and *Pe4* and higher in *P6* than the others, whereas those of dimethyl glutarate, 2,6-di(propan-2-yl)phenol, and acetophenone were lower in *P8*, higher in *Pe4*, and lower in *P6*, respectively, than the others (data not shown). The concentrations of the others (*P10*, *Pe11*, and *Pe14*) were too low to analyse.

Discussion

In mice, individual-unique body odors are determined genetically by major histocompatibility complex [4-6]. In humans, variations among individual-unique genetically-determined body odors will modify target disease-induced odors in an individual-dependent manner. Our proposed criterion also revealed that the dietary or inter-individual variations among individual patient U_i samples (mixture of 5 samples) as negative control odors are greater than that of the urine mixture U_m of 5 patients × 5 samples and reduced the sensitivity of the sniffer mouse assay from 78-25% for bladder cancer (Supplementary Table 4, and other data were not shown). In the present study, the sensitivity of the sniffer mouse assay was approximately two-times higher than the 41% sensitivity of sniffer dog assays for bladder cancer odor in urine samples [7]. The higher sensitivity of the sniffer mouse behavioural assay than that of sniffer dogs is attributable to the training of sniffer mice for discriminating a positive control odor in an odor plume-like flow in the Y-maze in a wide concentration range initially from the original or 10-fold dilution to finally 10^6- fold dilution for being below the detection level of dietary variations, as well as the stable negative control of 5-patient U_m sample.

Another limitation of this simple assay is that sniffer mice can discriminate a pair of urine samples without the learned olfactory cue of prostate or bladder cancer, possibly reflecting the discrimination of some other different elements. However, repeated assays of the positive control and individual patient urine samples can be used to identify spurious discrimination of non-target olfactory cues based on the proposed criterion. This criterion will be confirmed through testing of this assay for various cancers and diseases as well as inter-individual body odor variations.

Our finding of eight-compound biomarker contrasts with the previously-reported single or few-compound biomarkers [8-10]. The present study first indicates the importance of relative concentrations of biomarkers to each other and other constant compounds. As a single-compound biomarker, heneicosane was proposed as a biomarker for lung cancer by SPME-GC-MS analysis in cell lines of lung cancer, breast cancer, and healthy lung fibroblasts [10]. It also remained to determine specificity and accuracy across various diseases and healthy tissues. Interestingly, an electronic nose (e-nose) sensor array of the Cyranose 320 discriminates the volatile organic compounds of lung cancer cultured medium from those of controlled ones by the linear discriminant analysis [10]. Notably, however, it remains unclear whether and how the e-nose arrays are specifically sensitive to disease-related signals, i.e., their multiple compound profile [11].

Supplementary Table 1: %Correct of sniffer mice for serial 10- or 100-fold diluted equi-occult blood urine samples in a Y-maze.

Odor pairs	%Correct of individual sniffer mice (%)								avg. of 4-8 mice	SE	%Correct for P = 0.05
	wt1	*wt2*	*wt4*	*wt5*	*wt6*	*wt7*	*wt13*	*wt3*			
Pre**- vs. post*-Resect urine mixture (U_m), equi-occult blood											
10^{-6} pre[§]- vs. post[¶]-*N*:U_m	77.8	77.8	72.2	72.2	72.2	77.8	66.7	73.6	73.6	1.4	58.3
10^{-1} pre**- vs. post*-*P*:U_m	72.2	72.2	72.2	66.7	72.2	75.0	72.2	71.2	71.2	1.0	58.3
10^{-2} pre**- vs. post*-*P*:U_m	72.2	77.8	83.3	77.8	72.2	66.7	77.8	75.0	75.0	1.8	58.3
10^{-3} pre**- vs. post*-*P*:U_m	77.8	94.4	91.7	77.8	77.8	58.3	88.9	80.6	80.6	4.0	58.3
10^{-4} pre**- vs. post*-*P*:U_m	72.2	66.7	91.7	72.2	77.8	66.7	83.3	76.0	76.0	3.0	58.3
10^{-6} pre**- vs. post*-*P*:U_m	72.2	72.2	66.7	72.2	72.2	66.7	88.9	73.6	73.6	2.5	58.3
10^{-8} pre**- vs. post*-*P*:U_m	66.7	77.8	75.0	77.8	77.8	66.7	66.7	73.3	73.3	2.0	58.3
10^{-10} pre**- vs. post*-*P*:U_m	66.7	88.9	58.3	66.7	61.1	58.3	72.2	67.4	67.4	3.5	58.3
10^{-12} pre**- vs. post*-*P*:U_m	50.0	66.7	33.3	55.6	61.1	58.3	61.1	55.2	55.2	3.6	58.3
Post assays											
10^{-4} pre**- vs. post*-*P*:U_m	77.8	83.3	66.7	77.8	72.2	66.7	77.8	73.6	73.6	2.3	58.3
10^{-6} pre[§]- vs. post[¶]-*N*:U_m	77.8	77.8	75.0	66.7	72.2	58.3	66.7	72.2	72.2	2.8	58.3
10^{-4} pre**- vs. pre**-*P*:U_m	44.4	55.6	83.3	55.6	44.4	58.3	55.6	55.9	55.9	4.3	58.3
Pre**- vs. post*-Resect urine mixture (U_m), equi-occult blood, from patients after endocrine therapy (preliminary result)											
10^{-6} pre**- vs. post*-*P*:U_m		83.3	72.2	77.8	72.2				76.4	2.7	61.5
10^{-1} pre[‡]- vs. post[†]-*Pe*:U_m		83.3	77.8	72.2	72.2				76.4	2.7	61.5
10^{-2} pre[‡]- vs. post[†]-*Pe*:U_m		94.4	83.3	83.3	83.3				86.1	2.8	61.5
10^{-3} pre[‡]- vs. post[†]-*Pe*:U_m		83.3	88.9	72.2	72.2				79.2	4.2	61.5
10^{-4} pre[‡]- vs. post[†]-*Pe*:U_m		66.7	72.2	66.7	61.1				66.7	2.3	61.5
10^{-6} pre[‡]- vs. post[†]-*Pe*:U_m		77.8	72.2	66.7	66.7				70.8	2.7	61.5
10^{-8} pre[‡]- vs. post[†]-*Pe*:U_m		66.7	72.2	77.8	77.8				73.6	2.7	61.5
10^{-10} pre[‡]- vs. post[†]-*Pe*:U_m		61.1	61.1	61.1	50.0				58.3	2.8	61.5
Post assays											
10^{-4} pre[‡]- vs. post[†]-*Pe*:U_m		83.3	83.3	66.7	72.2				76.4	4.2	61.5
10^{-4} pre[‡]- vs. pre[‡]-*Pe*:U_m		61.1	61.1	61.1	38.9				55.6	5.6	61.5
10^{-6} pre[‡]- vs. post[†]-*Pe*:U_m		72.2	72.2	66.7	72.2				70.8	1.4	61.5
Individual pre-Resect urine mixture (U_i) vs. post-Resect U_m or pre-Resect *P*:U_m vs. pre-transurethral resection *N*:U_m											
10^{-6} pre**- vs. post*-*P*:U_m	88.9	88.9	88.9	77.8	72.2	83.3	72.2		81.7	2.9	58.7
10^{-6} pre-*Pe4*:U_i vs. post*-*P*:U_m	88.9	88.9	88.9	55.6	72.2	83.3	61.1		77.0	5.3	58.7
10^{-6} pre-*P6*:U_i vs. post*-*P*:U_m	77.8	83.3	55.6	77.8	55.6	66.7	72.2		69.8	4.2	58.7
10^{-6} pre**- vs. post*-*P*:U_m	66.7	72.2	72.2	72.2	72.2	72.2	66.7		70.6	1.0	58.7
10^{-6} pre-*P8*:U_i vs. post*-*P*:U_m	77.8	72.2	11.1	77.8	66.7	94.4	66.7		66.7	9.9	58.7
10^{-6} pre-*P9*:U_i vs. post*-*P*:U_m	72.2	94.4	72.2	72.2	83.3	77.8	77.8		78.6	3.1	58.7
10^{-6} pre**- vs. post*-*P*:U_m	72.2	66.7	66.7	72.2	72.2	88.9	72.2		73.0	2.8	58.7
10^{-6} pre-*P13*:U_i vs. post*-*P*:U_m	61.1	61.1	61.1	88.9	61.1	61.1	77.8		67.5	4.3	58.7
10^{-6} pre[&]-*P2*:U_i vs. post*-*P*:U_m	72.2	66.7	77.8	72.2	61.1	72.2	77.8		71.4	2.2	58.7
10^{-6} pre[※]-*P5*:U_i vs. post*-*P*:U_m	61.1	77.8	72.2	88.9	72.2	66.7	72.2		73.0	3.3	58.7
10^{-6} pre**- vs. post*-*P*:U_m	77.8	77.8	72.2	77.8	66.7	83.3	66.7		74.6	2.4	58.7
10^{-6} pre**- vs. post*-*P*:U_m	72.2	72.2	72.2		66.7	61.1			68.9	2.5	58.7
10^{-6} pre-*P10*:U_i vs. post*-*P*:U_m	72.2	94.4	77.8	72.2	72.2	83.3	66.7		77.0	3.5	58.7
10^{-6} pre-*P12*:U_i vs. post*-*P*:U_m	88.9	100.0	77.8	83.3	72.2	83.3	88.9		84.9	3.4	58.7
10^{-6} pre**- vs. post*-*P*:U_m		88.9	66.7	72.2	88.9	72.2	72.2		76.9	3.9	59.4
10^{-6} pre**- vs. post*-*P*:U_m		77.8	72.2		77.8	66.7			73.6	2.7	61.5
10^{-4} pre[‡]-*Pe*:U_m vs. post*-*P*:U_m		66.7	66.7	66.7	61.1	66.7	66.7		65.7	0.9	59.4
10^{-6} pre[‡]-*Pe3*:U_i vs. post*-*P*:U_m		77.8	83.3	72.2	88.9	88.9	72.2		80.6	3.1	59.4
10^{-6} pre[†]-*Pe7*:U_i vs. post*-*P*:U_m		66.7	72.2	77.8	77.8	88.9	72.2		75.9	3.1	59.4
10^{-6} pre**- vs. post*-*P*:U_m		83.3	72.2	72.2	77.8	66.7	66.7		73.1	2.7	59.4
10^{-6} pre-*Pe14*:U_i vs. post*-*P*:U_m		94.4	77.8	72.2	66.7	83.3	83.3		79.6	4.0	59.4
10^{-6} pre-*Pe11*:U_i vs. post*-*P*:U_m		72.2	77.8	77.8	66.7	83.3	72.2		75.0	2.4	59.4
10^{-6} pre[ʃ]-*Pe1*:U_i vs. post*-*P*:U_m		88.9	83.3		77.8	77.8	66.7		81.5	4.4	60.3
10^{-6} pre**- vs. post*-*P*:U_m		83.3	72.2		77.8	72.2			76.4	2.7	61.5
10^{-6} pre**-*P*:U_m vs. pre[§]-*N*:U_m		66.7	83.3	77.8	66.7	66.7	66.7		71.3	3.0	59.4
10^{-6} pre**- vs. post*-*P*:U_m		77.8	66.7	66.7	72.2	77.8	72.2		72.2	2.0	59.4

SE: Standard Error.

Extra-dilution rates for equal-occult blood urine samples were 1/50** v/v, 1/2* v/v, 1/10[‡] v/v, 1/3[†] v/v, 1/13[§] v/v, 1/6[¶] v/v, 1/100[&] v/v, 1/140[※], and 1/90[ʃ] v/v.

Supplementary Table 2: Blood, proteins, glucose testing in *Pe*-series patient urine samples using urine test strips.

Parameters and diluted urine samples	Pre-RP urine samples							Post-RP urine samples						
	Pe1:U_i	*Pe3*:U_i	*Pe7*:U_i	*Pe11*:U_i	*Pe14*:U_i	*Pe*:U_m	*Pe4*:U_i	*Pe1*:U_i	*Pe3*:U_i	*Pe7*:U_i	*Pe11*:U_i	*Pe14*:U_i	*Pe*:U_m	*Pe4*:U_i
Cancer	pros.	pros.	pros.	pros.	pros.	pros.	pros.							
Stage	Ⅲ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	4Ⅱ+1Ⅲ	Ⅱ							
Gleason score	6	6	6	6	6	6.0	7							
Age	58	68	80	69	70	58–80	62							
Gender	♂	♂	♂	♂	♂	♂	♂							
Sampling (5 days)	(-30)–(-1)	(-41)–(-1)	(-34)–(-1)	(-30)–(-1)	(-37)–(-1)	(-41)–(-1)	(-23)–(-1)	1–26	1–22	1–29	1–26	1–26	1–29	1–26
Hemoglobin (Hb, blood) (mg/dℓ)														
stock U_i/U_m	0.90	0.00	0.03	0.00	0.00	0.10	0.00	0.06	0.00	0.00	0.04	0.20	0.03	0.00
10^{-1}-diluted U_i/U_m	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00
10^{-2}-diluted U_i/U_m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10^{-3}-diluted U_i/U_m	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dilution for equi-occult blood of 0.01 mg/dℓ or less Hb														
Extra-dilution rate	90	1	3	1	1	10	1	6	1	1	4	20	3	1
Protein (mg/dℓ)														
stock U_i/U_m	100.0	0.0	0.0	0.0	0.0	30.0	15.0	30.0	30.0	0.0	0.0	0.0	15.0	0.0
10^{-1}-diluted U_i/U_m	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10^{-2}-diluted U_i/U_m	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10^{-3}-diluted U_i/U_m	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucose (mg/dℓ)														
stock U_i/U_m	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-1}-diluted U_i/U_m	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-2}-diluted U_i/U_m	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10^{-3}-diluted U_i/U_m	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Pros: Prostate. Individual patient urine mixture (U_i) of equal volumes of five urine samples from each patient. *Pe* -series urine mixture (*Pe* :U_m) of equal volumes of 25 urine samples from five patient *Pe1, Pe3, Pe7, Pe11,* and *Pe14* on five different days for each pre-radical prostatectomy (pre-RP) after neoadjuvant endocrine therapy with LH-RH and post-RP. The patient *Pe4* was treated with Avolve. Ranges of patient 1st–5th sampling days are shown, when day 0 is the ablative operation day.

Supplementary Table 3: Statistical analyses of %Correct of sniffer mice for prostate cancer odor discrimination in a Y-maze.

Odor pairs for prostate cancer	%Correct		fall from CNT		%Rank (vs. 10 cnt)	Judge: fall > −4.6	*P* value of pairwise difference											%*P*>0.05 (vs. 10 *P*:U_m)	%*P*<0.05 (vs. *Pe*:U_m)
	mean	SE	pre	post			*1st*	*2nd*	*3rd*	*4th*	*5th*	*6th*	*7th*	*8th*	*9th*	*10th*	*Pe*:U_m		
Odor discrimination for the prostate cancer-induced odor control																			
1st control (10^{-6} pre**- vs. post*-RP *P*:U_m)	81.7	2.9		11.1	100	○		0.010	0.091	0.022	0.005	0.516	0.162	0.082	0.239	0.060	0.003	60	100
2nd control (10^{-6} pre**- vs. post*-RP *P*:U_m)	70.6	1.0	-11.1	-2.4	20	≠	0.010		0.448	0.140	0.477	0.203	0.638	0.465	0.215	0.695	0.012	90	100
3rd control (10^{-6} pre**- vs. post*-RP *P*:U_m)	73.0	2.8	2.4	-1.6	40	○	0.091	0.448		0.569	0.512	0.543	1.000	1.000	0.718	0.771	0.082	100	0
4th control (10^{-6} pre**- vs. post*-RP *P*:U_m)	74.6	2.4	1.6	5.7	70	○	0.022	0.140	0.569		0.178	0.611	0.824	0.822	0.789	0.530	0.017	90	100
5th control (10^{-6} pre**- vs. post*-RP *P*:U_m)	68.9	2.5	-5.7	-8.0	10	≠	0.005	0.477	0.512	0.178		0.161	0.092	0.080	0.058	0.308	0.391	90	0
6th control (10^{-6} pre**- vs. post*-RP *P*:U_m)	76.9	3.9	8.0	3.2	90	○	0.516	0.203	0.543	0.611	0.161		0.252	0.175	0.495	0.224	0.058	100	0
7th control (10^{-6} pre**- vs. post*-RP *P*:U_m)	73.6	2.7	-3.2	0.5	60	○	0.162	0.638	1.000	0.824	0.092	0.252		0.391	0.182	1.000	0.103	100	0
8th control (10^{-6} pre**- vs. post*-RP *P*:U_m)	73.1	2.7	-0.5	-3.2	50	○	0.082	0.465	1.000	0.822	0.080	0.175	0.391		0.391	0.771	0.062	100	0
9th control (10^{-6} pre**- vs. post*-RP *P*:U_m)	76.4	2.7	3.2	4.2	80	○	0.239	0.215	0.718	0.789	0.058	0.495	0.182	0.391		0.391	0.041	100	100
10th control (10^{-6} pre**- vs. post*-RP *P*:U_m)	72.2	2.0	-4.2		30	○	0.060	0.695	0.771	0.530	0.308	0.224	1.000	0.771	0.391		0.034	100	100
mean, SE, SD × 1.29	74.1	1.1	4.6																
Odor discrimination for individual patient pre-RP U_i (prostate cancer) after learning the cue of prostate cancer																			
P2 (10^{-6} pre[&]-RP *P2*:U_i vs. post*-RP *P*:U_m)	71.4	2.2	-1.6	-3.2	20	○	0.021	0.788	0.673	0.356	0.749	0.426	0.519	0.721	0.312	0.822	0.041	90	100
P5 (10^{-6} pre[※]-RP *P5*:U_i vs. post*-RP *P*:U_m)	73.0	3.3	0.0	-1.6	40	○	0.130	0.448	1.000	0.715	0.749	0.721	0.391	0.611	0.058	0.562	0.031	100	100
P6 (10^{-6} pre-RP *P6*:U_i vs. post*-RP *P*:U_m)	69.8	4.2	-11.9	-0.8	10	≠	0.037	0.869	0.569	0.248	0.847	0.191	0.297	0.383	0.116	0.444	0.542	90	0
P8 (10^{-6} pre-RP *P8*:U_i vs. post*-RP *P*:U_m)	66.7	9.9	-4.0	-6.3	0	≠	0.218	0.709	0.480	0.415	0.788	0.319	0.544	0.521	0.439	0.509	0.939	100	0
P9 (10^{-6} pre-RP *P9*:U_i vs. post*-RP *P*:U_m)	78.6	3.1	7.9	5.6	90	○	0.476	0.035	0.267	0.356	0.075	0.203	0.103	0.034	0.092	0.025	0.014	70	100
P10 (10^{-6} pre[¶]-RP *P10*:U_i vs. post*-RP *P*:U_m)	77.0	3.5	8.1	0.1	90	○	0.143	0.084	0.411	0.448	0.075	0.842	0.215	0.224	0.252	0.144	0.027	100	100
P12 (10^{-6} pre-RP *P12*:U_i vs. post*-RP *P*:U_m)	84.9	3.4	16.0	8.1	100	○	0.386	0.009	0.047	0.021	0.025	0.191	0.213	0.041	0.239	0.015	0.003	50	100
P13 (10^{-6} pre-RP *P13*:U_i vs. post*-RP *P*:U_m)	67.5	4.3	-5.6	-7.1	0	≠	0.063	0.508	0.334	0.211	0.025	0.304	0.018	0.497	0.010	0.576	0.580	70	0
Odor discrimination for prostate cancer vs. bladder cancer after learning the cue of prostate cancer																			
P (10^{-6} prostate** *P*:U_m vs. bladder[§] N:U_m)	71.3	3.0	-5.1	-0.9	20	≠	0.042	1.000	0.741	0.518	0.495	0.415	0.638	0.679	0.423	0.856	0.111	90	0
Odor discrimination for individual patient post-endocrine therapy pre-RP U_i after learning the cue of prostate cancer																			
Pe1 (10^{-6} pre[ǀ]-RP *Pe1*:U_i vs. post*-RP *P*:U_m)	81.5	4.4	8.3	5.1	90	○	0.809	0.028	0.215	0.082	0.003	0.419	0.058	0.060	0.092	0.117	0.010	70	100
Pe3 (10^{-6} pre[‡]-RP *Pe3*:U_i vs. post*-RP *P*:U_m)	80.6	3.1	6.9	7.4	90	○	1.000	0.020	0.082	0.158	0.046	0.444	0.092	0.121	0.182	0.045	0.010	80	100
Pe7 (10^{-6} pre[†]-RP *Pe7*:U_i vs. post*-RP *P*:U_m)	75.9	3.1	2.3	2.8	70	○	0.383	0.185	0.076	0.576	0.339	0.876	0.718	0.611	1.000	0.328	0.028	100	100
Pe11 (10^{-6} pre-RP *Pe11*:U_i vs. post*-RP *P*:U_m)	75.0	2.4	1.9	-1.4	70	○	0.111	0.175	0.530	0.611	0.278	0.765	0.836	0.695	0.824	0.415	0.004	100	100
Pe14 (10^{-6} pre-RP *Pe14*:U_i vs. post*-RP *P*:U_m)	79.6	4.0	6.5	3.2	90	○	0.793	0.107	0.272	0.203	0.117	0.624	0.368	0.201	0.486	0.062	0.010	100	100
Pe4 (10^{-6} pre-RP *Pe4*:U_i vs. post*-RP *P*:U_m)	77.0	5.3	-4.8	6.3	90	≠	0.200	0.280	0.542	0.649	0.005	0.788	0.162	0.750	0.239	0.624	0.175	90	0
Odor discrimination for post-endocrine therapy-prostate cancer after learning the cue of prostate cancer																			
Pe (10^{-6} pre[‡]-RP *Pe*:U_m vs. post*-RP *P*:U_m)	65.7	0.9	-7.9	-7.4	0	≠	0.003	0.012	0.082	0.017	0.391	0.058	0.103	0.062	0.041	0.034		50	

RP: Radical Prostatectomy; CNT: Control; SE: Standard.

Extra-dilution rates for equal-occult blood urine samples were 1/50** v/v, 1/2* v/v, 1/10 v/v, 1/3 v/v, 1/13[§] v/v, 1/6 v/v, 1/100 v/v, 1/140 , and 1/90 v/v.

Supplementary Table 4: Sensitivity and specificity of sniffer mouse behavioural assays for prostate or bladder cancer odor.

Odor pairs for prostate cancer	%Correct		fall from CNT		%Rank (vs. 10 cnt)	Judge: fall > −4.6	Sensitivity	Specificity
	mean	SE	pre	post				
Odor discrimination for the prostate cancer control								
1st control (10^{-6} pre**- vs. post*-RP *P*: U_m)	81.7	2.9		11.1	100	○		
2nd control (10^{-6} pre**- vs. post*-RP *P*: U_m)	70.6	1.0	-11.1	-2.4	20	≠		
3rd control (10^{-6} pre**- vs. post*-RP *P*: U_m)	73.0	2.8	2.4	-1.6	40	○		
4th control (10^{-6} pre**- vs. post*-RP *P*: U_m)	74.6	2.4	1.6	5.7	70	○		
5th control (10^{-6} pre**- vs. post*-RP *P*: U_m)	68.9	2.5	-5.7	-8.0	10	≠	80%	
6th control (10^{-6} pre**- vs. post*-RP *P*: U_m)	76.9	3.9	8.0	3.2	90	○	(8/10)	
7th control (10^{-6} pre**- vs. post*-RP *P*: U_m)	73.6	2.7	-3.2	0.5	60	○		
8th control (10^{-6} pre**- vs. post*-RP *P*: U_m)	73.1	2.7	-0.5	-3.2	50	○		
9th control (10^{-6} pre**- vs. post*-RP *P*: U_m)	76.4	2.7	3.2	4.2	80	○		
10th control (10^{-6} pre**- vs. post*-RP *P*: U_m)	72.2	2.0	-4.2		30	○		
mean, SE, SD × 1.29	74.1	1.1	4.6					
Odor discrimination for individual patient prostate cancer to the prostate cancer								
P2 (10^{-6} pre[&]-RP *P2*:U_i vs. post*-RP *P*: U_m)	71.4	2.2	-1.6	-3.2	20	○		
P5 (10^{-6} pre[※]-RP *P5*: U_i vs. post*-RP *P*: U_m)	73.0	3.3	0.0	-1.6	40	○		
P6 (10^{-6} pre-RP *P6*:U_i vs. post*-RP *P*: U_m)	69.8	4.2	-11.9	-0.8	10	≠		
P8 (10^{-6} pre-RP *P8*: U_i vs. post*-RP *P*: U_m)	66.7	9.9	-4.0	-6.3	0	≠	63%	
P9 (10^{-6} pre-RP *P9*:U_i vs. post*-RP *P*: U_m)	78.6	3.1	7.9	5.6	90	○	(5/8)	
P10 (10^{-6} pre[¶]-RP *P10*: U_i vs. post*-RP *P*: U_m)	77.0	3.5	8.1	0.1	90	○		
P12 (10^{-6} pre-RP *P12*:U_i vs. post*-RP *P*: U_m)	84.9	3.4	16.0	8.1	100	○		
P13 (10^{-6} pre-RP *P13*: U_i vs. post*-RP *P*: U_m)	67.5	4.3	-5.6	-7.1	0	≠		
Odor discrimination for prostate cancer vs. bladder cancer to the prostate cancer								100%
P (10^{-6} prostate** *P*:U_m vs. bladder[§] *N*: U_m)	71.3	3.0	-5.1	-0.9	20	≠		(1/1)
Odor discrimination for individual patient post-endocrine therapy-prostate cancer to the prostate cancer								
Pe1 (10^{-6} pre[J]-RP *Pe1*:U_i vs. post*-RP *P*: U_m)	81.5	4.4	8.3	5.1	90	○		
Pe3 (10^{-6} pre[‡]-RP *Pe3*: U_i vs. post*-RP *P*: U_m)	80.6	3.1	6.9	7.4	90	○		
Pe7 (10^{-6} pre[†]-RP *Pe7*:U_i vs. post*-RP *P*: U_m)	75.9	3.1	2.3	2.8	70	○	83%	
Pe11 (10^{-6} pre-RP *Pe11*: U_i vs. post*-RP *P*: U_m)	75.0	2.4	1.9	-1.4	70	○	(5/6)	
Pe14 (10^{-6} pre-RP *Pe14*:U_i vs. post*-RP *P*: U_m)	79.6	4.0	6.5	3.2	90	○		
Pe4 (10^{-6} pre-RP *Pe4*:U_i vs. post*-RP *P*: U_m)	77.0	5.3	-4.8	6.3	90	≠		
Odor discrimination for post-endocrine therapy-prostate cancer to the prostate cancer								100%
Pe (10^{-6} pre[‡]-RP *Pe*: U_m vs. post*-RP *P*: U_m)	65.7	0.9	-7.9	-7.4	0	≠		(1/1)

RP: Radical Prostatectomy; CNT: Control; SE: Standard Error; SD: Standard Deviation.

Extra-dilution rates for equal-occult blood urine samples were 1/50** v/v, 1/2* v/v, 1/10[‡] v/v, 1/3[†] v/v, 1/13[§] v/v, 1/6[¶] v/v, 1/100[&] v/v, 1/140[※], and 1/90[J] v/v.

Supplementary Table 4: Sensitivity and specificity of sniffer mouse behavioural assays for prostate or bladder cancer odor. (continued)

Odor pairs for bladder cancer	%Correct		fall from CNT		%Rank (vs. 12 cnt)	Judge: fall > −6.2	Sensitivity	Specificity
	mean	SE	pre	post				
Odor discrimination for the bladder cancer control								
1st control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	69.0	2.0		0.8	25	○	75% (9/12)	
2nd control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	68.3	2.9	-0.8	-11.4	17	≠		
3rd control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	79.6	3.7	11.4	13.0	67	○		
4th control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	66.7	4.8	-13.0	-15.7	8	≠		
5th control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	82.4	2.2	15.7	-0.9	92	○		
6th control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	83.3	1.4	0.9	8.3	100	○		
7th control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	75.0	3.4	-8.3	-3.7	33	≠		
8th control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	78.7	2.7	3.7	0.9	50	○		
9th control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	77.8	1.4	-0.9	-2.8	42	○		
10th control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	80.6	3.7	2.8	0.9	75	○		
11th control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	79.6	2.3	-0.9	-1.9	67	○		
12th control (10^{-6} pre**- vs. post*-TUR *N:* U_m)	81.5	4.2	1.9		83	○		
mean, SE, SD × 1.29	78.5	1.5	6.2					
Odor discrimination for individual patient bladder cancer to the bladder cancer								
N6 (10^{-6} pre*-TUR *N6*:U_i vs. post*-TUR *N:* U_m)	70.6	5.2	1.6	2.4	25	○	78% (7/9)	
N7 (10^{-6} pre-TUR *N7:* U_i vs. post*-TUR *N:* U_m)	72.2	4.8	-7.4	-9.3	25	≠		
N8 (10^{-6} pre†-TUR *N8*:U_i vs. post*-TUR *N:* U_m)	69.0	4.0	0.0	0.8	17	○		
N9 (10^{-6} pre§-TUR *N9:* U_i vs. post*-TUR *N:* U_m)	68.5	4.0	-11.1	-13.0	8	≠		
N10 (10^{-6} pre-TUR *N10*:U_i vs. post*-TUR *N:* U_m)	68.3	4.1	-0.8	0.0	8	○		
N5 (10^{-6} pre‡-TUR *N5:* U_i vs. post*-TUR *N:* U_m)	73.8	3.4	4.8	5.6	25	○		
N8 (10^{-6} pre†-TUR *N8*:U_i vs. post*-TUR *N:* U_m)	75.9	4.0	-3.7	9.3	33	○		
A2 (10^{-6} pre¶-TUR *A2:* U_i vs. post*-TUR *N:* U_m)	78.7	3.9	-0.9	-2.8	50	○		
A3 (10^{-6} pre-TUR *A3*:U_i vs. post*-TUR *N:* U_m)	82.4	5.3	2.8	0.9	92	○		
Odor discrimination for individual patient (antibiotic drug metabolites > bladder cancer) to the bladder cancer								
K3 (10^{-6} pre†-TUR *K3:* U_i vs. post*-TUR *N:* U_m)	87.0	3.4	4.6	3.7	100	○	60% (3/5)	
K4 (10^{-6} pre-TUR *K4*:U_i vs. post*-TUR *N:* U_m)	72.2	1.4	-11.1	-2.8	25	≠		
K5 (10^{-6} pre-TUR *K5:* U_i vs. post*-TUR *N:* U_m)	68.5	3.4	-10.2	-9.3	8	≠		
K4 (10^{-6} pre-TUR *K4*:U_i vs. post*-TUR *N:* U_m)	78.7	1.7	-0.9	-2.8	50	○		
K5 (10^{-6} pre-TUR *K5:* U_i vs. post*-TUR *N:* U_m)	66.7	6.4	-13.0	-14.8	0	≠		
Odor discrimination for individual or 5-patient bladder cancer to the occult blood (in coufusing)								
N6 (10^{-6} pre*-TUR *N6*:U_i vs. post*-TUR *N:* U_m)	64.8	1.9	-14.8	3.7	0	≠	75% (3/4)	
N8 (10^{-6} pre†-TUR *N8*:U_i vs. post*-TUR *N:* U_m)	75.0	3.7	-9.3	13.0	33	≠		
N10 (10^{-6} pre-TUR *N10*:U_i vs. post*-TUR *N:* U_m)	74.1	1.9	11.1	9.3	25	?		
cN: U_m (10^{-6} pre**- vs. post*-TUR *N:* U_m)	63.9	3.4	-0.9	-15.7	0	≠		

CNT: Control; SE: Standard Error; SD: Standard Deviation; TUR: Transurethral Resection of bladder tumor.

Extra-dilution rates for equal-occult blood urine samples were 1/13** v/v, 1/6* v/v, $1/75^{‡}$ v/v, $1/15^{†}$ v/v, $1/10^{§}$ v/v, and 1/9 v/v. *cN:* U_m, *N:* U_m trial in confusing odor choices; "?" means the impossibility of judgement in an unstable condition.

Supplementary Table 5: %Correct of sniffer mice for diluted equal-occult blood urine samples of bladder or prostate cancer and diluted prostate cancer-characteristic odor-mimic urine sample in a Y-maze.

Odor pairs	%Correct of individual sniffer mice (%)						avg. of 5-6 mice	SE	suc-cessive drop	Judge: drop > −7.9 or −5.9	%Correct for P = 0.05
	wt14	*wt15*	*wt16*	*wt17*	*wt18*	*wt19*					
Initial training with enantiomeric odors											
10^{-5} (*R*)-(-)- vs. (*S*)-(+)-carvone	85.0	95.0	85.0	80.0	65.0	75.0	80.8	4.2			58.9
Pre**- vs. post*-Resect urine mixture (U_m), equi-occult blood											
10^{-1} pre§- vs. post¶-TUR *N*:U_m	75.0	95.0	65.0	55.0	75.0	75.0	73.3	5.4	-7.5	≠	58.9
10^{-1} pre§- vs. post¶-TUR *N*:U_m	95.0	85.0	70.0	60.0	65.0	70.0	74.2	5.4	0.8	○	58.9
10^{-3} pre§- vs. post¶-TUR *N*:U_m	85.0	90.0	70.0		75.0	85.0	81.0	3.7	6.8	○	59.8
10^{-1} pre**- vs. post*-RP *P*:U_m	66.7	72.2	61.1	55.6	61.1	83.3	66.7	4.1	-14.3	≠	59.4
10^{-2} pre**- vs. post*-RP *P*:U_m	94.4	77.8	66.7	66.7	72.2	77.8	75.9	4.2	9.3	○	59.4
10^{-3} pre**- vs. post*-RP *P*:U_m	94.4	77.8	66.7	61.1	77.8	72.2	75.0	4.7	-0.9	○	59.4
10^{-3} pre**- vs. post*-RP *P*:U_m	88.9	88.9	72.2	66.7	77.8	77.8	78.7	3.6	3.7	○	59.4
10^{-6} pre**- vs. post*-RP *P*:U_m	100.0	83.3	66.7	77.8	77.8	77.8	80.6	4.5	1.9	○	59.4
10^{-6} pre**- vs. post*-RP *P*:U_m	44.4	94.4	72.2	72.2	77.8	88.9	75.0	7.1	-5.6	○	59.4
10^{-6} post**-RP *P*:U_m+BM-PC vs. post*-RP *P*:U_m	77.8	94.4	61.1	61.1	77.8	77.8	75.0	5.1	0.0	○	59.4
Post assays											
10^{-6} pre**- vs. pre**-RP *P*:U_m	55.6	50.0	38.9	61.1	50.0	44.4	50.0	3.2	-25.0	≠	59.4

SE: Standard Error. Extra-dilution rates for equal-occult blood urine samples were 1/50** v/v, 1/2* v/v, 1/13§ v/v, and 1/6 v/v. *N*, patients with bladder cancer; *P*, patients with prostate cancer; BM- PC, eight-coupound biomarkers (phenol, dimethyl succinate, acetophenone, 2-phenyl-2-propanol, 3,5,5-trimethyl-2-cyclohexenone, dimethyl glutarate, 2,6-xylidine, and piperitone) for prostate cancer; A %Correct fall greater than 5.9% and 7.9% for prostate and bladder cancers, respectively, was judged to be a mismatch to the learned olfactory cue.

Supplementary Table 6: Estimated numbers of biomarker molecules in the urine samples at the lowest discriminative concentrations for odor pairs of healthy volunteers, patients with bladder cancer, and patients with prostate cancer.

Peak #	Compound	Molecular weight (g/mol)	Healthy volunteers		Bladder cancer (post-antibiotic pro.)		Bladder cancer		Prostate cancer (endocrine therapy)		Prostate cancer	
			H1–3	H4–6	post-TUR *K*:U_m	pre-TUR *K*:U_m	post-TUR *N*:U_m	pre-TUR *N*:U_m	post-RP *Pe*:U_m	pre-RP *Pe*:U_m	post-RP *P*:U_m	pre-RP *P*:U_m
Number of molecules in 0.3-mL urine mixture sample at the lowest odor-pair discriminative concentrations												
81	Phenol	94.11	9.3E+08	5.7E+08	0.19	3.1	6.4.E+04	1.2.E+05	4.9.E+05	1.8.E+06	4,292	5,738
101	Dimetyl succinate	146.14	7.2E+06	6.6E+06	0.001	0.04	2,484	939	7,231	3.2.E+04	103	165
104	Acetophenone	120.15	1.3E+07	1.0E+07	0.002	0.02	1,695	730	1.6.E+04	4.5.E+04	175	144
109	2-Phenyl-2-propanol	136.19	2.6E+07	3.3E+07	0.005	0.07	2,821	1,140	3.6.E+04	1.1.E+05	482	423
119	3,5,5-Trimethyl-2-cyclohexenone	138.21	1.8E+06	1.1E+06	0.09	0.00	356	65	6.6.E+04	1.8.E+05	1,156	755
123	Dimethyl glutarate	160.17	4.3E+07	3.6E+07	0.001	0.06	4,815	1,875	2.2.E+04	1.1.E+05	442	607
129	2,6-Xylidine	121.20	not determined due to a significant desorption-resistant amount									
152	Piperitone	152.23	9.8E+06	4.0E+07	0.0000	0.03	342	129	7,284	1.5.E+04	16	109
155	2-Hydroxy-2-methylpropiophenone	164.2	2.2E+07	1.4E+07	0.0003	0.005	503	106	3.1.E+04	8.7.E+04	847	438
165	2,6-Di(propan-2-yl)phenol	178.28	1.6E+05	1.4E+05	0.0000	0.0003	79	465	56	2,014	1.0	6.7

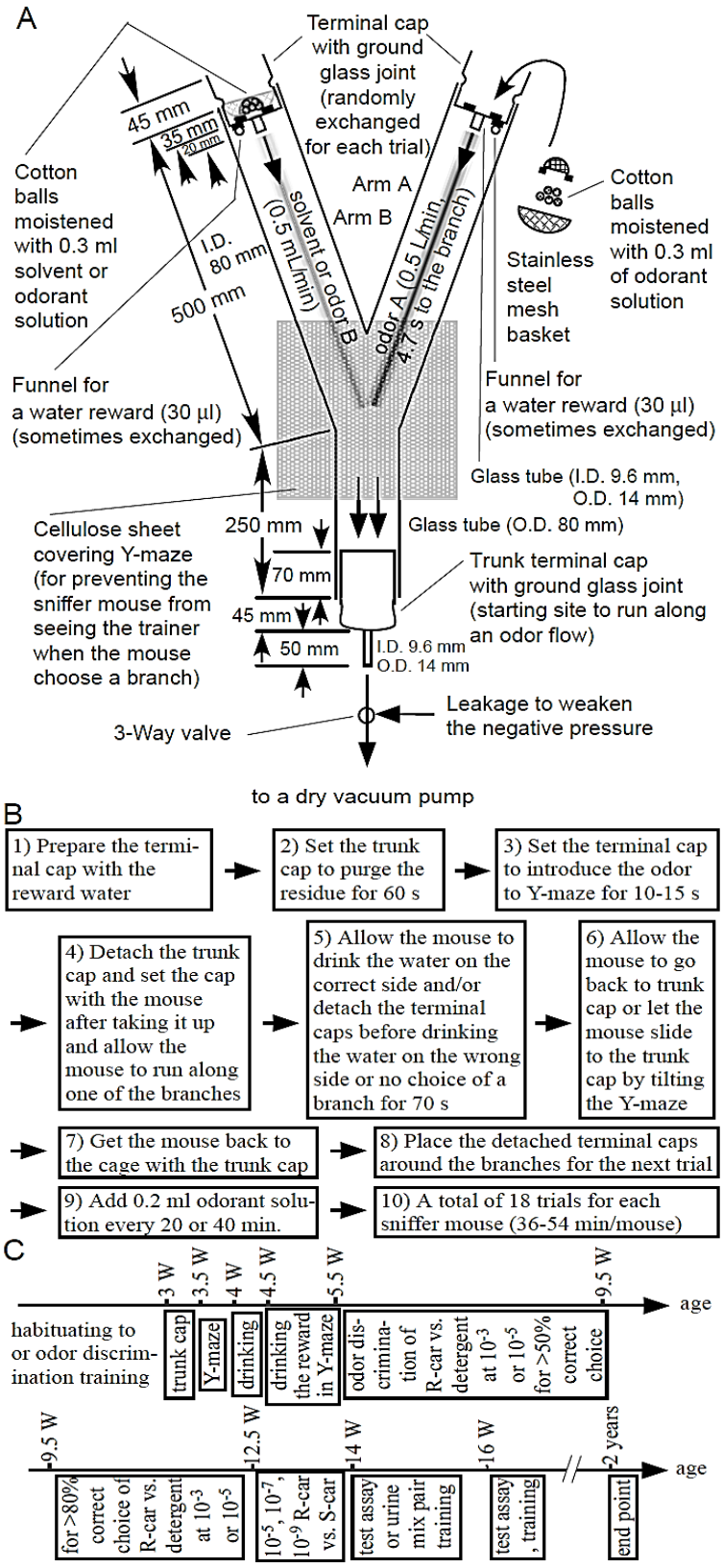
TUR: Transurethral Resection; RP: Radical Prostatectomy.

Post-antibiotic pro., post-TUR *K:* U_m with antibiotic prophylaxis; Numbers of molecules in 0.3-mL urine samples at their lowest discriminative concentrations (10^{-5}, 10^{-5}, 2×10^{-16}, 1.7×10^{-14}, 1.7×10^{-9}, 7.7×10^{-10}, 3.3×10^{-9}, 10^{-9}, 5×10^{-11}, 2×10^{-12}, respectively) for sniffer mice were shown. >10-fold (red) and 2–10-fold (blue) increases from the peak height of the healthy volunteer in were highlighted, as in (Supplementary Table 7).

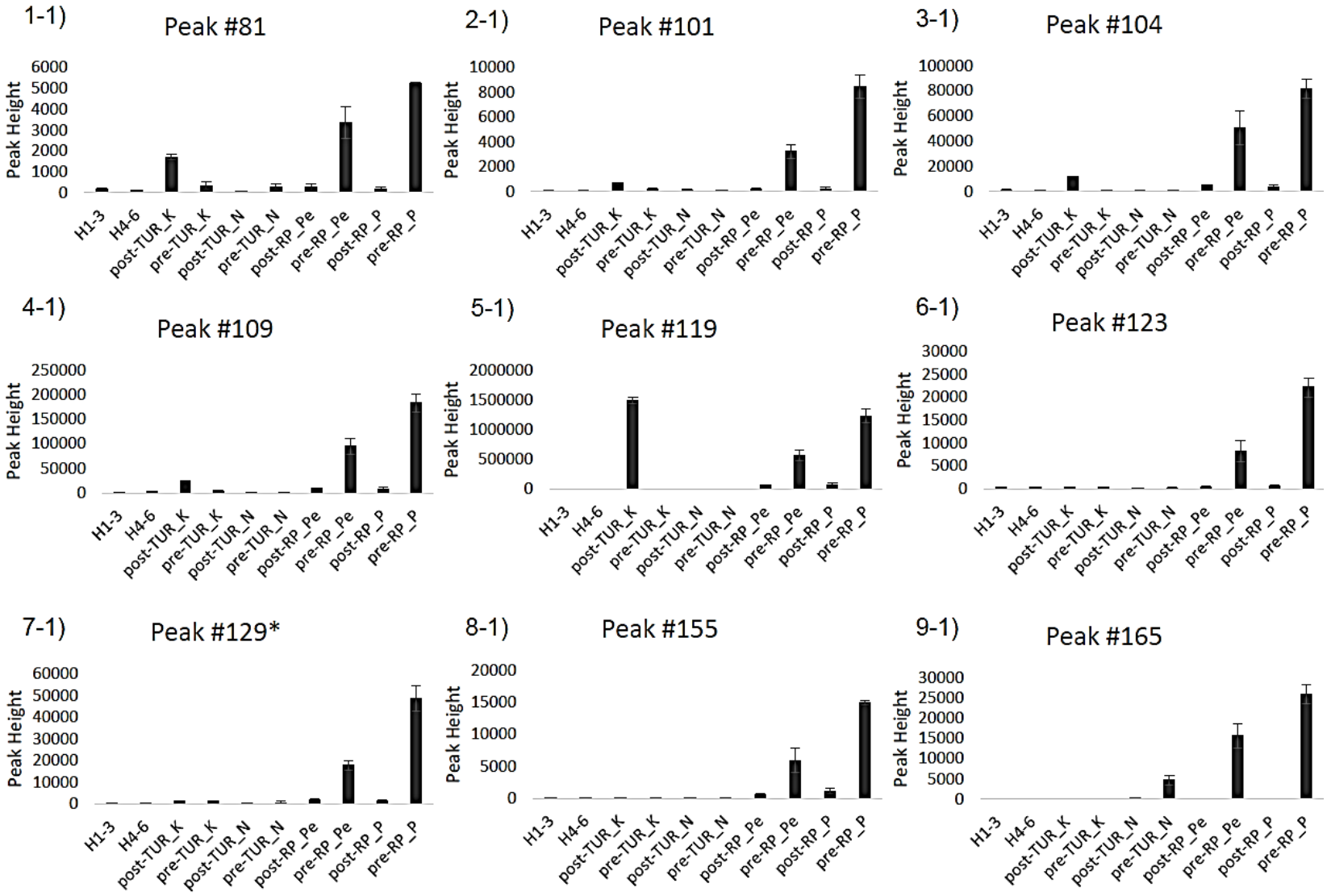
Supplementary Table 7: Provisional cut-off values of concentrations of biomarkers, their sensitivities, and specificities for prostate cancer. **A)** Single biomarkers, **B)** Combinatorial method for nine biomarkers, **C)** Peak height/area (concentration) ratio of biomarkers to a relatively constant compound #70 in urine mixture samples, **D)** Combinatorial method for #70-based ratios of nine biomarkers.

Peak #	Compound	Provisional cut-off value for prostate cancer in urine (ppb)		Healthy volunteer	Prostate cancer		Bladder cancer		Total	
		Conc.	ratio	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
a) Single biomarkers										
81	Phenol	236	6	6/6 (100%)	8/12 (67%)	12/12 (100%)	–	1/1 (100%)	8/12 (67%)	19/19 (100%)*
101	Dimetyl succinate	7.0	10	6/6 (100%)	6/12 (50%)	12/12 (100%)	–	1/1 (100%)	6/12 (50%)	19/19 (100%)*
104	Acetophenone	5.0	13	6/6 (100%)	8/12 (67%)	11/12 (92%)	–	1/1 (100%)	8/12 (67%)	18/19 (95%)*
109	2-Phenyl-2-propanol	33.5	15	6/6 (100%)	8/12 (67%)	12/12 (100%)	–	1/1 (100%)	8/12 (67%)	19/19 (100%)*
119	3,5,5-Trimethyl-2-cyclohexenone	80.0	1,000	6/6 (100%)	7/12 (58%)	12/12 (100%)	–	1/1 (100%)	7/12 (58%)	19/19 (100%)*
123	Dimethyl glutarate	35.3	10	6/6 (100%)	7/12 (58%)	11/12 (92%)	–	1/1 (100%)	7/12 (58%)	18/19 (95%)*
129	2,6-Xylidine	n.d.	20	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
152	Piperitone	8.0	4	5/6 (83%)	5/12 (42%)	11/12 (92%)	–	1/1 (100%)	5/12 (42%)	17/19 (89%)*
155	2-Hydroxy-2-methylpropiophenone	120	40	4/6 (67%)	6/12 (50%)	11/12 (92%)	–	1/1 (100%)	6/12 (50%)	16/19 (84%)*
165	2,6-Di(propan-2-yl)phenol	0.24	24	6/6 (100%)	6/12 (50%)	12/12 (100%)	1/1 (100%)	1/1 (100%)	7/13 (54%)	19/19 (100%)*
b) Combinatorial method for nine biomarkers										
pros.	I) Two or more biomarkers of #81, #101, #104, #109, #119, #123, and #129 > cut-off value + II) one of #152, #155, or #165 biomarker > cut-off value			6/6 (100%)	8/12 (67%)	12/12 (100%)	–	2/2 (100%) (pre- & post-TUR)	8/12 (67%)	20/20 (100%)*
bladd.	I) #165 biomarker > cut-off value + II) all of #104, #109, #119, and #155 biomarkers < cut-off value			6/6 (100%)	–	24/24 (100%) (pre- & post-RP)	1/1 (100%)	1/1 (100%)	1/1 (100%)	31/31 (100%)**
c) Peak height/area (concentration) ratio of biomarkers to a relatively-constant compound #70 in urine mixture samples										
81	Phenol	–	0.12	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
101	Dimetyl succinate	–	0.10	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
104	Acetophenone	–	1.2	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
109	2-Phenyl-2-propanol	–	2.5	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
119	3,5,5-Trimethyl-2-cyclohexenone	–	20	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
123	Dimethyl glutarate	–	0.20	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
129	2,6-Xylidine	–	0.40	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
152	Piperitone	–	0.60	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
155	2-Hydroxy-2-methylpropiophenone	–	0.10	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
165	2,6-Di(propan-2-yl)phenol	–	0.15	2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	3/3 (100%)	6/6 (100%)*
d) Combinatorial method for #70-based ratios of nine biomarkers										
pros.	I) Two or more biomarker ratios of #81, #101, #104, #109, #119, #123, and #129 > cut-off value + II) one of #152, #155, or #165 biomarker ratio > cut-off value			2/2 (100%)†	2/2 (100%)†	2/2 (100%)†	–	1/1 (100%)†	2/2 (100%)	6/6 (100%)*
bladd.	I) #165 biomarker ratio > cut-off value + II) all of #104, #109, #119, and #155 biomarker ratios < cut-off value			2/2 (100%)†	–	4/4 (100%)† (pre- & post-RP)	1/1 (100%)†	1/1 (100%)†	1/1 (100%)	7/7 (100%)**

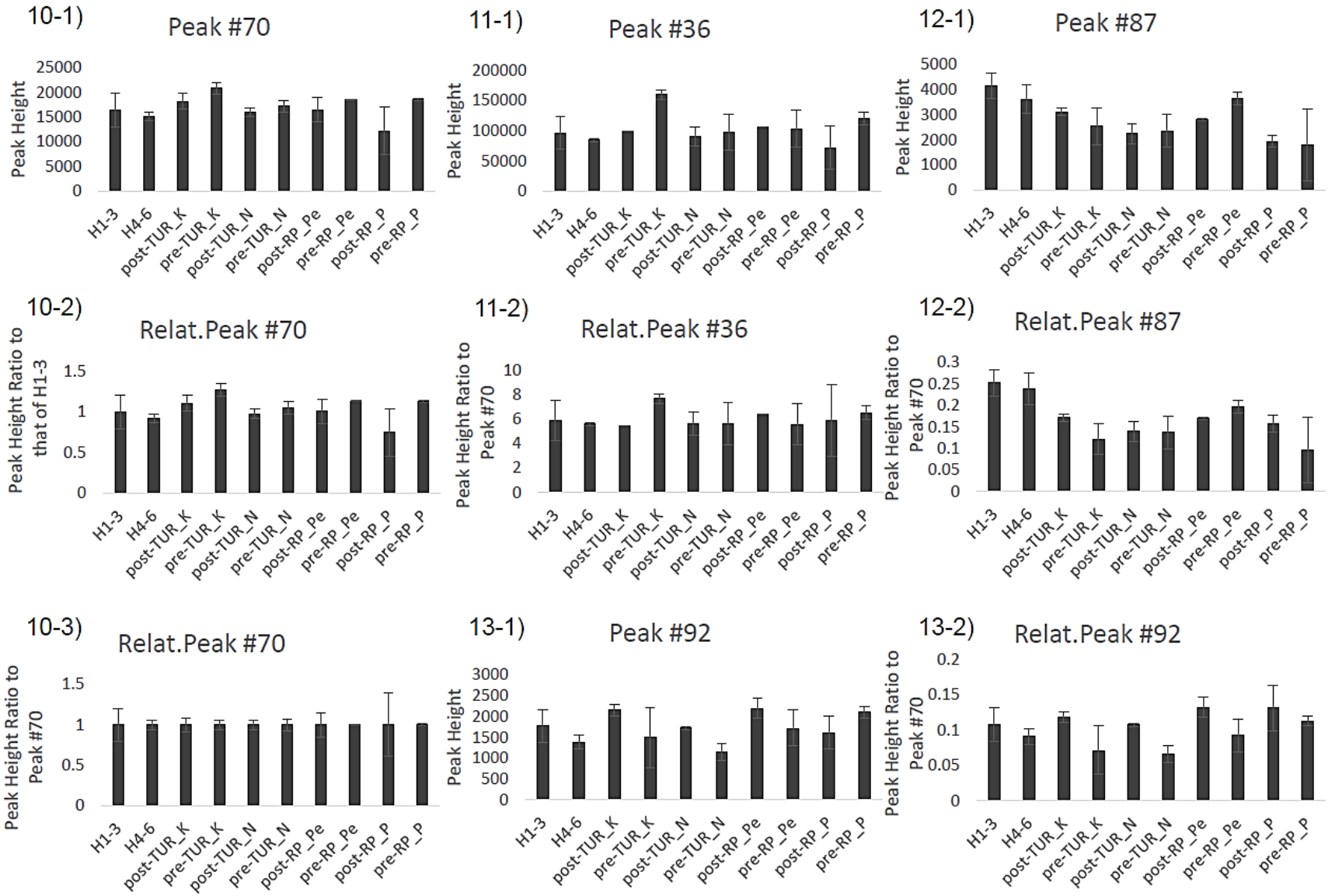
Urine mixture samples of individual patients with prostate cancer or five-patient mixture sample of patients with bladder cancer (N :U_m) and healthy volunteers were judged for prostate cancer by each biomarker or combinatorial method. Sensitivity and specificity were calculated in six (no pre-therapy) + six (neoadjuvant endocrine therapy) pre-PR, six (no pre-therapy) + six (neoadjuvant endocrine therapy) post-PR, one pre-TUR, one post-TUR, and two healthy volunteer urine samples. Conc., concentration; ratio, ratio of patient to healthy volunteer at biomarker concentrations; n.d., not determined. Value* for detection of prostate cancer in samples including one pre-TUR sample and value** for detection of bladder cancer in samples including 12 pre-RP U_i or two pre-RP U_m samples. †Urine mixture samples (not individual samples).



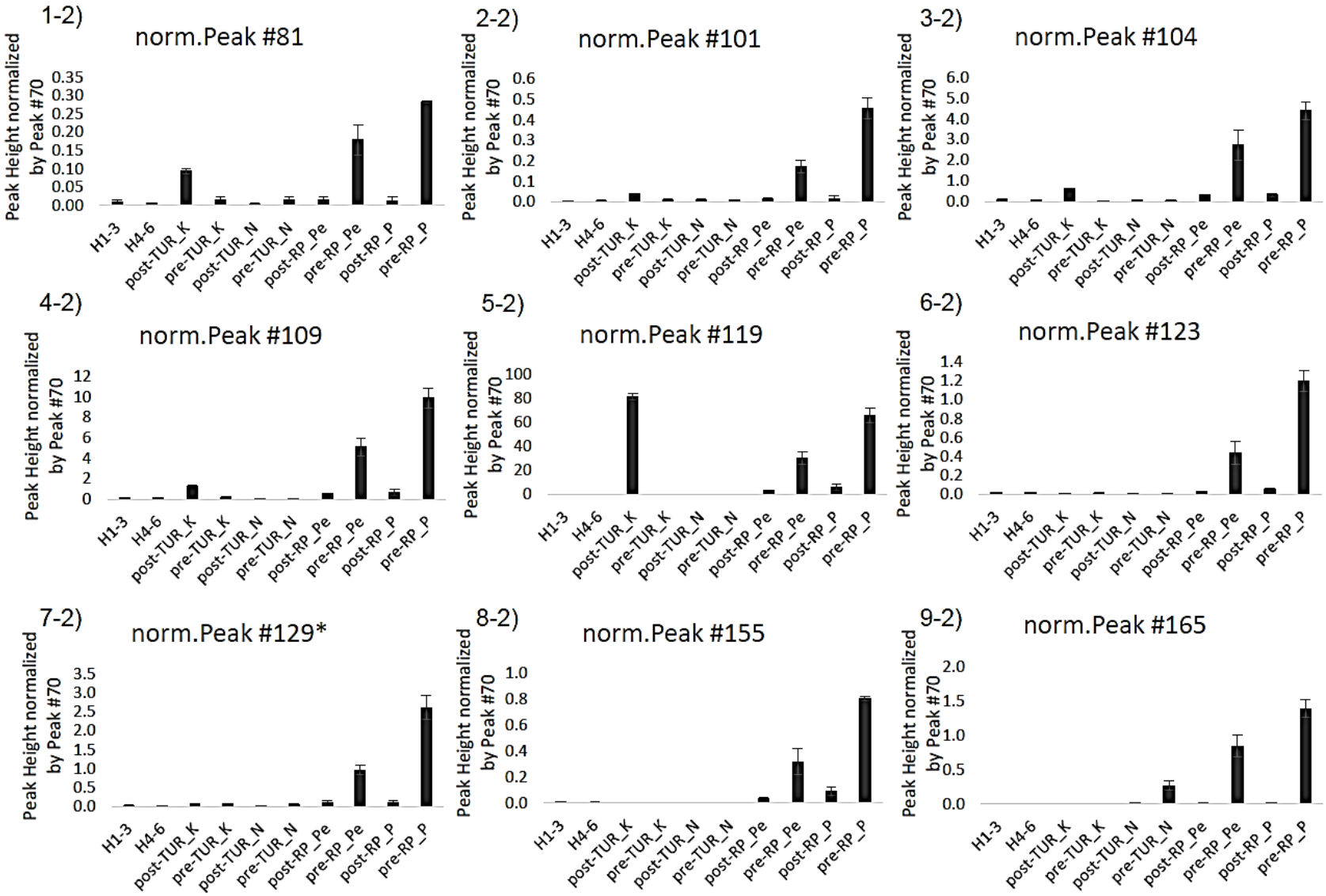
Supplementary Figure 1: A schematic diagram of the odor plume-guided Y-maze and training flow of sniffer mice. **A)** A schematic diagram of the odor plume-guided Y-maze (modified from the reference [17]). **B)** Procedure for each trial of Y-maze behavioural assay. **C)** Training flow of sniffer mice.



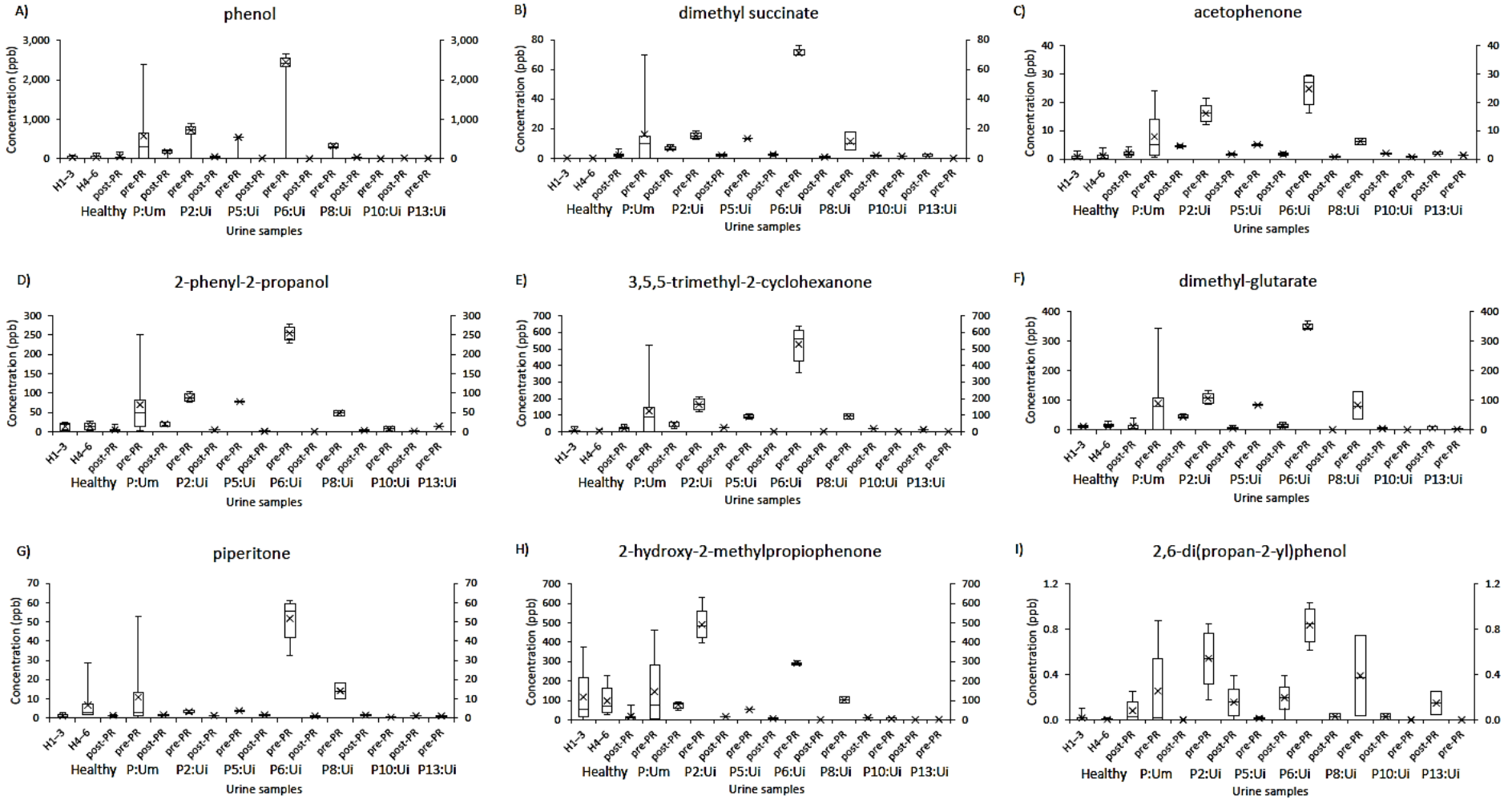
Supplementary Figure 2: Tumor-associated increases in concentrations of volatile compounds between five pairs of urine mixture samples. **A)** Peak height. *Not corrected for a significant desorption-resistant amount., and



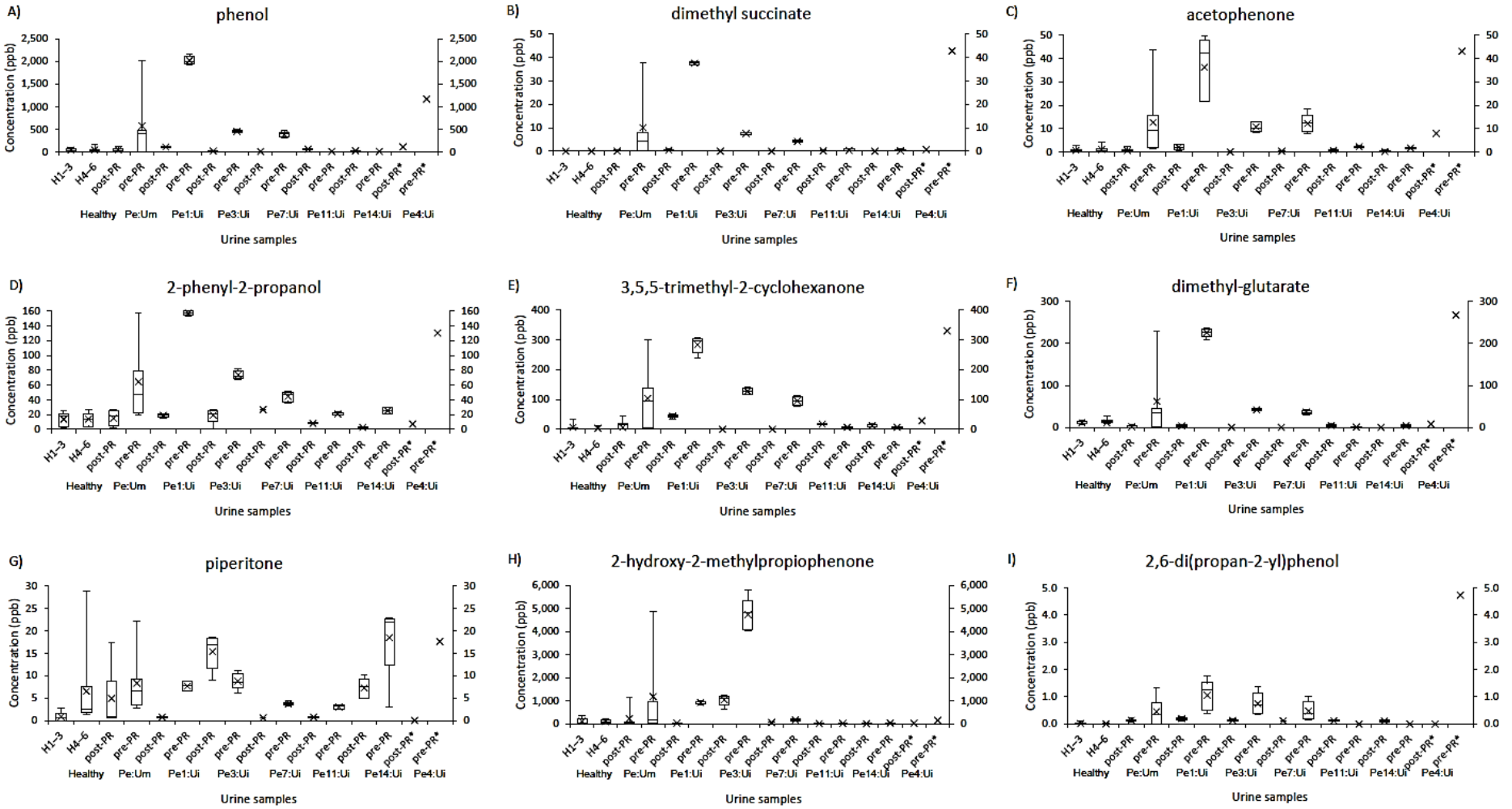
Supplementary Figure 2: (Continued) Tumor-associated increases in concentrations of volatile compounds between five pairs of urine mixture samples. **B)** Relatively constant peaks and their peak height ratios to those of respective Peak #70 or H1-3's one.



Supplementary Figure 2: (Continued) Tumor-associated increases in concentrations of volatile compounds between five pairs of urine mixture samples. **C)** Peak heights normalized by those of respective Peak #70. *Not corrected for a significant desorption-resistant amount of #129 compound.



Supplementary Figure 3: Comparison of nine biomarkers in concentrations between pairs of individual pre- and post-RP urine samples of patients with prostate cancer. A box, the 25th-50th-75th percentiles in concentrations; error bars, 10th-25th and 75th-90th percentiles; ×, mean of individual urine samples. Without endocrine therapy.



Supplementary Figure 3: Comparison of nine biomarkers in concentrations between pairs of individual pre- and post-RP urine samples of patients with prostate cancer. A box, the 25th-50th-75th percentiles in concentrations; error bars, 10th-25th and 75th-90th percentiles; ×, mean of individual urine samples. Treated with endocrine therapy (Continued).

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