Supplementary material for "Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma"

Supplementary Table 1. Quality Control Results for Serum PFAS Measurements in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial [a]

	Below Limit of Detection		Coefficients of Variation			
PFAS	**Controls**	**Cases**	**Intra-batch**	**Inter-batch**	**Overall**	**ICCs**
PFOA	0.0%	0.0%	8.2%	3.2%	8.8%	0.97
PFOS	0.0%	0.0%	7.2%	4.7%	8.6%	0.96
PFHxS	0.0%	0.0%	11.7%	14.1%	18.3%	0.92
PFUnDA	51.2%	49.7%	16.6%	16.2%	23.1%	0.92
PFNA	2.2%	1.5%	10.7%	9.5%	14.3%	0.94
EtFOSAA	0.0%	0.0%	8.5%	11.2%	14.1%	0.97
MeFOSAA	0.0%	0.3%	11.2%	16.5%	20.0%	0.92
PFDA	28.1%	28.4%	10.6%	20.0%	22.6%	0.94

Abbreviations: EtFOSAA, 2-N-ethyl-perfluorooctane sulfonamido acetic acid; ICC, intraclass correlation coefficient PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; MeFOSAA, 2-N-methyl-perfluorooctane sulfonamido acetic acid.

[a] Results were calculated from a total of 59 quality control samples from 22 individuals, with replicate samples from the same individual distributed within and across batches (including within-batch paired samples from all 22 individuals and an additional across-batch sample from 15 individuals).

Supplementary Table 2. Spearman Correlation Coefficients of Serum PFAS Concentrations among Control Participants (N=324) in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

PFAS	PFOA	PFOS	PFHxS	PFUnDA	PFNA	EtFOSAA	MeFOSAA	PFDA
PFOA	1.00							
PFOS	0.62	1.00						
PFHxS	0.42	0.45	1.00					
PFUnDA	0.12	0.30	0.21	1.00				
PFNA	0.42	0.54	0.36	0.57	1.00			
EtFOSAA	0.38	0.50	0.02	-0.02	0.07	1.00		
MeFOSAA	0.27	0.32	0.17	0.04	0.29	0.18	1.00	
PFDA	0.25	0.48	0.27	0.81	0.68	0.07	0.15	1.00

Abbreviations: EtFOSAA, 2-N-ethyl-perfluorooctane sulfonamido acetic acid; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; MeFOSAA, 2-N-methyl-perfluorooctane sulfonamido acetic acid.

Supplementary Table 3. Adjusted Geometric Mean (95% CI)[a] Serum PFAS Concentrations (μg/L) among Controls (N=324) in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

Characteristic	N	PFOA	PFOS	PFHxS	PFUnDA	PFNA	EtFOSAA	MeFOSAA	PFDA
Age[b], years									
55-59	95	4.4 (3.4-5.6)	33.8 (25.9-44.2)	3.2 (2.2-4.7)	0.2 (0.1-0.3)	0.6 (0.5-0.9)	0.9 (0.6-1.5)	1.5 (1.1-2.1)	0.2 (0.2-0.4)
60-64	112	4.3 (3.4-5.4)	34.3 (26.5-44.4)	2.5 (1.7-3.6)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.1 (0.7-1.8)	1.5 (1.1-2.1)	0.3 (0.2-0.4)
65-69	80	4.1 (3.3-5.3)	35.6 (27.2-46.5)	2.7 (1.9-4.0)	0.2 (0.1-0.2)	0.7 (0.5-1.0)	1.1 (0.7-1.8)	1.4 (1.0-1.9)	0.2 (0.2-0.4)
70+	37	4.4 (3.3-5.8)	34.4 (25.3-47.0)	2.3 (1.5-3.6)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.2 (0.7-2.1)	1.3 (0.9-1.9)	0.2 (0.2-0.4)
Center[c]									
Upper Midwest Region	130	3.9 (3.0-5.0)	33.3 (25.3-43.9)	2.4 (1.6-3.6)	0.1 (0.1-0.2)	0.5 (0.4-0.8)	1.0 (0.6-1.6)	1.3 (0.9-1.8)	0.2 (0.1-0.3)
Western and Southern Region	102	4.6 (3.7-5.8)	37.6 (29.4-48.1)	2.7 (1.9-3.8)	0.2 (0.1-0.2)	0.7 (0.5-0.9)	1.3 (0.8-2.0)	1.6 (1.2-2.1)	0.2 (0.1-0.3)
Eastern States Region	92	4.3 (3.4-5.6)	32.9 (25.0-43.2)	2.9 (2.0-4.3)	0.2 (0.2-0.4)	0.9 (0.7-1.3)	1.0 (0.6-1.7)	1.4 (1.0-2.0)	0.3 (0.2-0.5)
Gender									
Female	108	4.0 (3.2-5.1)	31.3 (24.0-40.7)	2.3 (1.6-3.3)	0.2 (0.1-0.2)	0.7 (0.5-0.9)	1.1 (0.7-1.8)	1.4 (1.0-2.0)	0.2 (0.1-0.3)
Male	216	4.5 (3.6-5.7)	38.1 (29.5-49.2)	3.1 (2.2-4.5)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.0 (0.6-1.7)	1.4 (1.1-1.9)	0.3 (0.2-0.4)
Race									
White, non-Hispanic	287	4.8 (3.9-5.8)	30.9 (24.7-38.8)	3.3 (2.4-4.5)	0.1 (0.1-0.1)	0.6 (0.5-0.8)	1.1 (0.7-1.7)	1.3 (1.0-1.8)	0.2 (0.1-0.2)
Black, non-Hispanic	21	4.5 (3.4-6.0)	52.3 (38.2-71.6)	3.3 (2.1-5.1)	0.3 (0.2-0.4)	0.9 (0.6-1.3)	1.1 (0.6-2.1)	1.7 (1.2-2.4)	0.4 (0.3-0.7)
Other	16	3.6 (2.6-5.1)	25.4 (17.7-36.6)	1.8 (1.1-3.0)	0.2 (0.1-0.4)	0.6 (0.4-0.9)	0.9 (0.5-1.9)	1.3 (0.8-2.0)	0.2 (0.1-0.4)
Body Mass Index[b], kg/m^2									
18.5-<25	83	4.0 (3.3-4.9)	35.2 (28.2-43.9)	2.4 (1.8-3.3)	0.2 (0.1-0.3)	0.8 (0.6-1.0)	1.1 (0.7-1.6)	1.3 (1.0-1.6)	0.3 (0.2-0.4)
25-<30	158	4.5 (3.7-5.3)	35.6 (29.1-43.6)	2.6 (2.0-3.5)	0.2 (0.1-0.2)	0.8 (0.6-1.0)	1.1 (0.7-1.6)	1.5 (1.2-1.9)	0.2 (0.2-0.3)
30+	76	4.5 (3.7-5.6)	36.6 (29.3-45.7)	2.5 (1.8-3.5)	0.2 (0.1-0.2)	0.8 (0.6-1.0)	1.2 (0.8-1.8)	1.5 (1.2-2.0)	0.2 (0.1-0.3)
History of Hypertension[b]									
No	216	4.3 (3.4-5.5)	35.7 (27.5-46.2)	2.8 (1.9-4.0)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.0 (0.6-1.7)	1.5 (1.1-2.0)	0.3 (0.2-0.4)
Yes	108	4.3 (3.4-5.4)	33.4 (25.8-43.3)	2.6 (1.8-3.7)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	1.1 (0.7-1.8)	1.4 (1.0-1.9)	0.2 (0.2-0.3)
Smoking Status[b]									
Never	155	4.4 (3.5-5.6)	36.3 (28.0-47.2)	3.0 (2.1-4.3)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	1.0 (0.6-1.6)	1.5 (1.1-2.0)	0.3 (0.2-0.4)
Current	35	4.0 (3.1-5.3)	30.8 (22.8-41.5)	2.3 (1.5-3.5)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	1.1 (0.6-1.9)	1.4 (1.0-2.1)	0.2 (0.1-0.3)
Former	134	4.4 (3.5-5.5)	36.8 (28.5-47.5)	2.8 (1.9-4.0)	0.2 (0.1-0.3)	0.7 (0.6-1.0)	1.1 (0.7-1.8)	1.4 (1.0-1.9)	0.2 (0.2-0.4)
eGFR[d], mL/min/1.73 m^2									
90+	109	4.5 (3.5-5.7)	34.6 (26.7-44.9)	2.7 (1.9-3.9)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.0 (0.6-1.7)	1.4 (1.0-1.9)	0.3 (0.2-0.4)
<90-60	197	4.4 (3.5-5.5)	34.8 (27.1-44.7)	2.8 (2.0-4.0)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	1.1 (0.7-1.8)	1.3 (1.0-1.8)	0.2 (0.2-0.3)
<60	18	4.0 (2.9-5.4)	34.2 (24.3-48.1)	2.5 (1.6-4.1)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.1 (0.6-2.1)	1.5 (1.0-2.3)	0.2 (0.1-0.4)

Calendar Year[d]									
1993-1995	84	4.0 (3.1-5.1)	33.1 (25.2-43.6)	2.7 (1.8-4.0)	0.2 (0.1-0.3)	0.6 (0.5-0.9)	1.2 (0.7-2.0)	1.3 (0.9-1.8)	0.2 (0.1-0.3)
1996-1997	116	4.3 (3.4-5.5)	35.0 (26.8-45.7)	2.5 (1.7-3.6)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.1 (0.7-1.9)	1.4 (1.0-1.9)	0.2 (0.2-0.4)
1998-2002	124	4.6 (3.6-5.8)	35.5 (27.4-46.0)	2.8 (2.0-4.1)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	0.9 (0.6-1.5)	1.6 (1.2-2.1)	0.3 (0.2-0.4)

Abbreviations: eGFR, estimated glomerular filtration rate; EtFOSAA, 2-N-ethyl-perfluorooctane sulfonamido acetic acid; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; MeFOSAA, 2-N-methyl-perfluorooctane sulfonamido acetic acid.

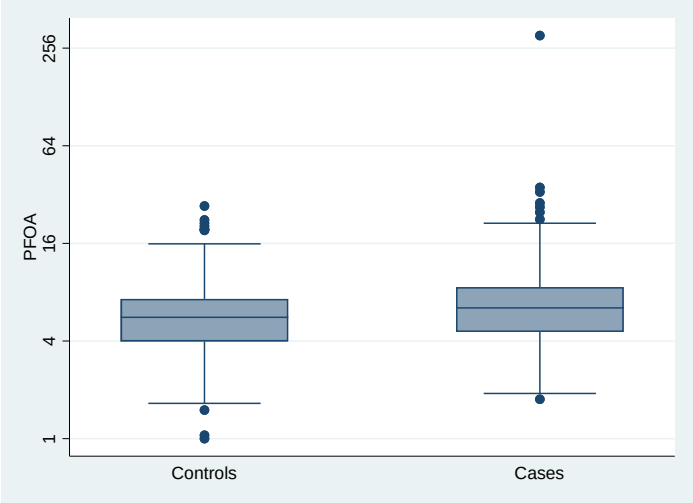
[a] Geometric LS-MEANS among the 324 controls were estimated after adjusting for age category (55-59, 60-64, 65-69, 70+), center, gender (male, female), race (white, black, other), body mass index (underweight, normal, overweight, obese, missing), hypertension (no/missing, yes), smoking status (never, former, current), eGFR (90+, <90-60, <60), previous freeze-thaw cycle, calendar year of blood draw (1993-1995,1996-1997,1998-2002), blood collection (baseline, follow up visit).

[b] Self-reported at study baseline.

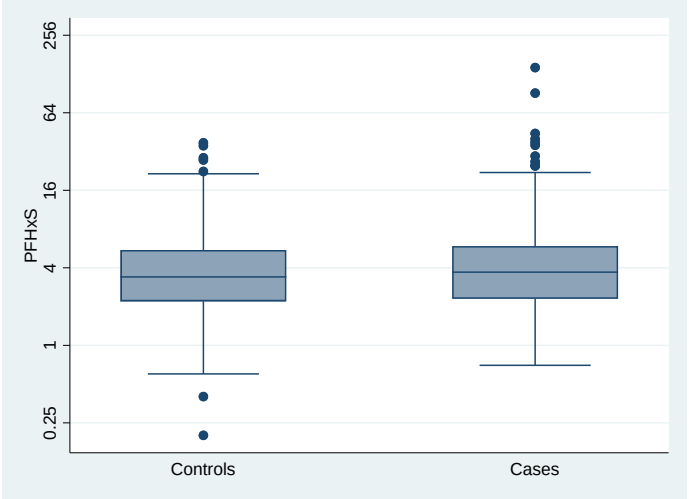
[c] Upper Midwest Region: Minnesota or Marshfield; Western and Southern Region: Colorado, Hawaii, Washington University, University of Utah, or University of Alabama; Southern Region: Georgetown, Henry Ford, or University of Pittsburgh).

[d] At blood draw.

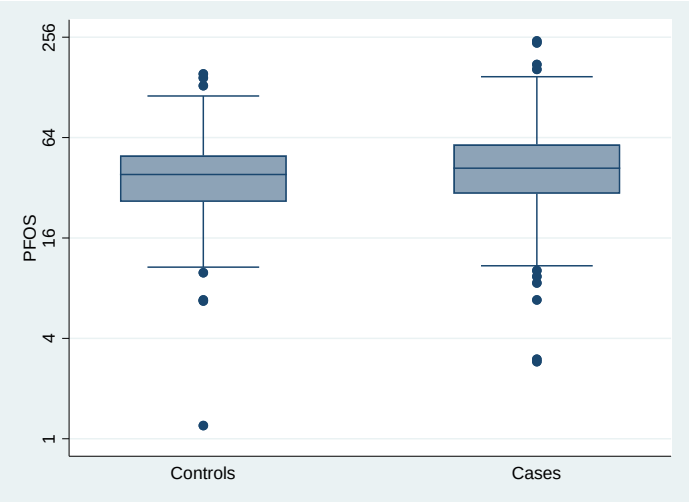
PFOA



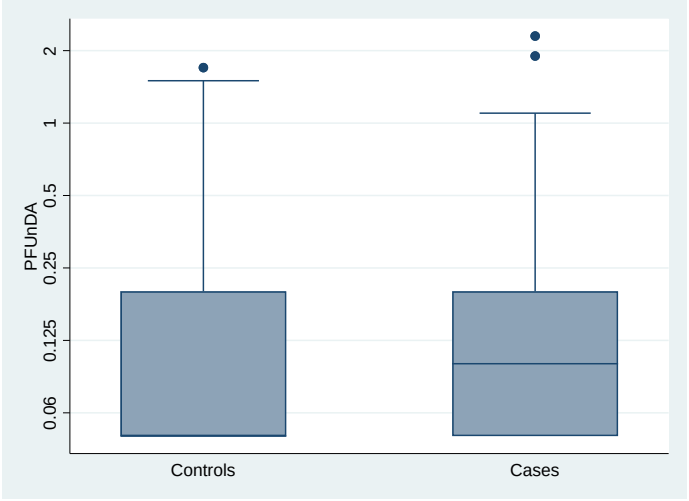
PFHxS

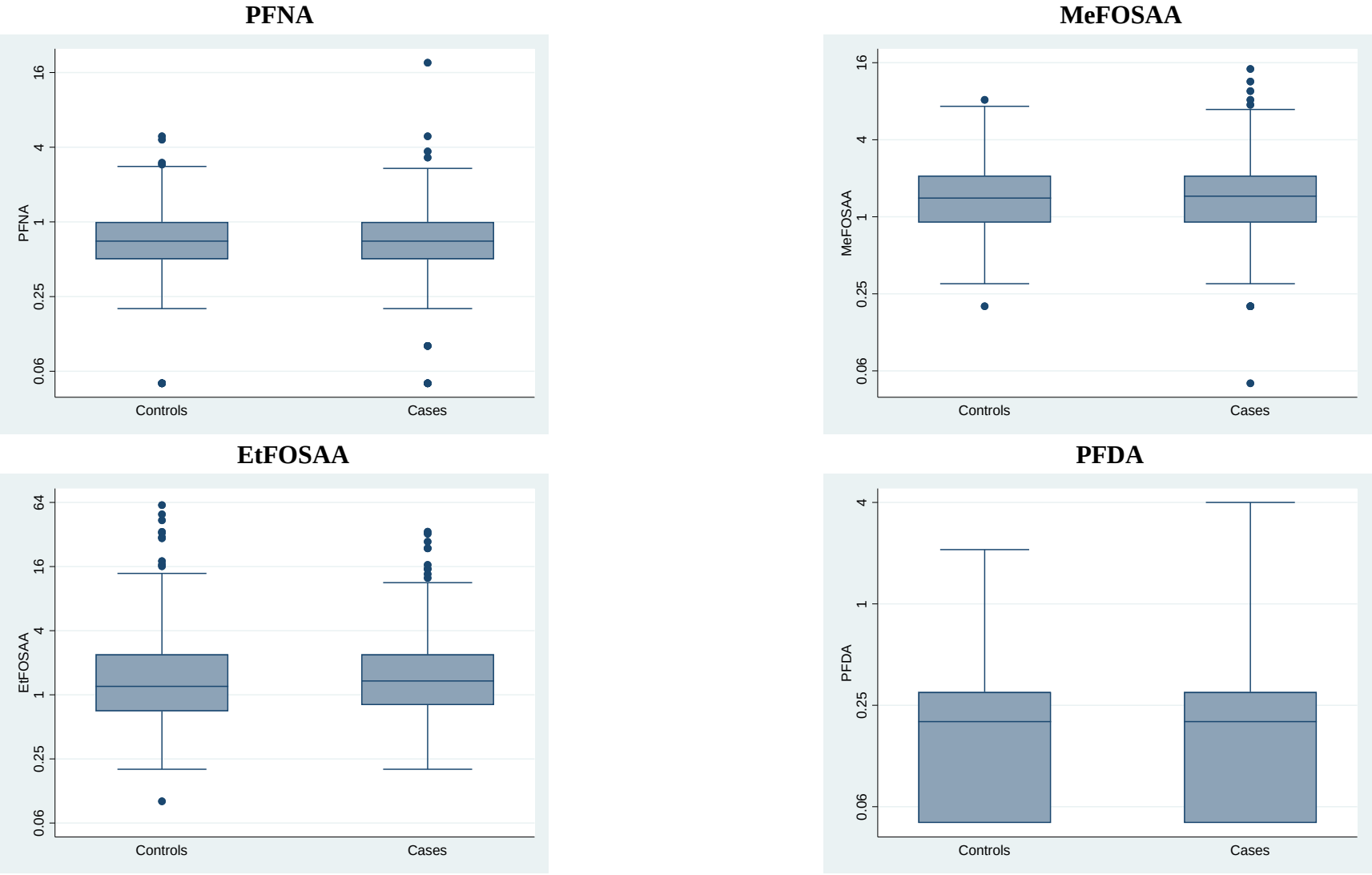


PFOS

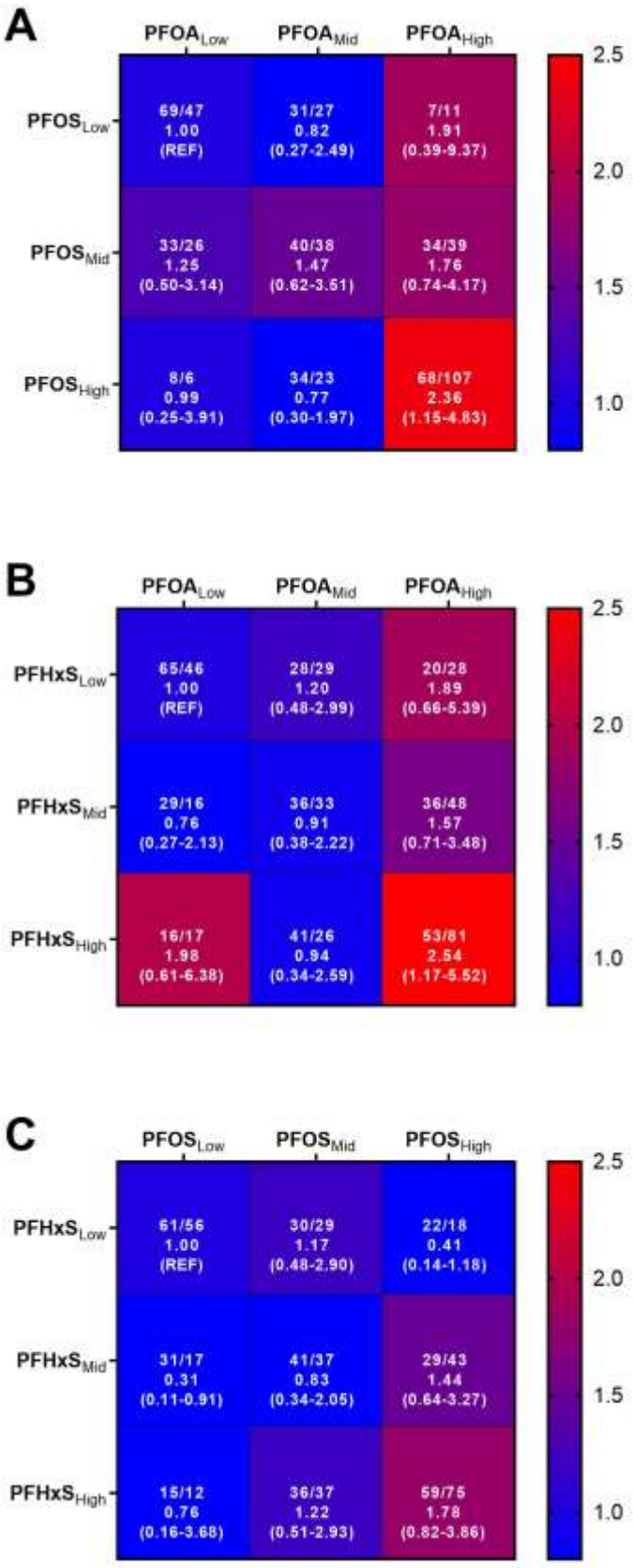


PFUnDA

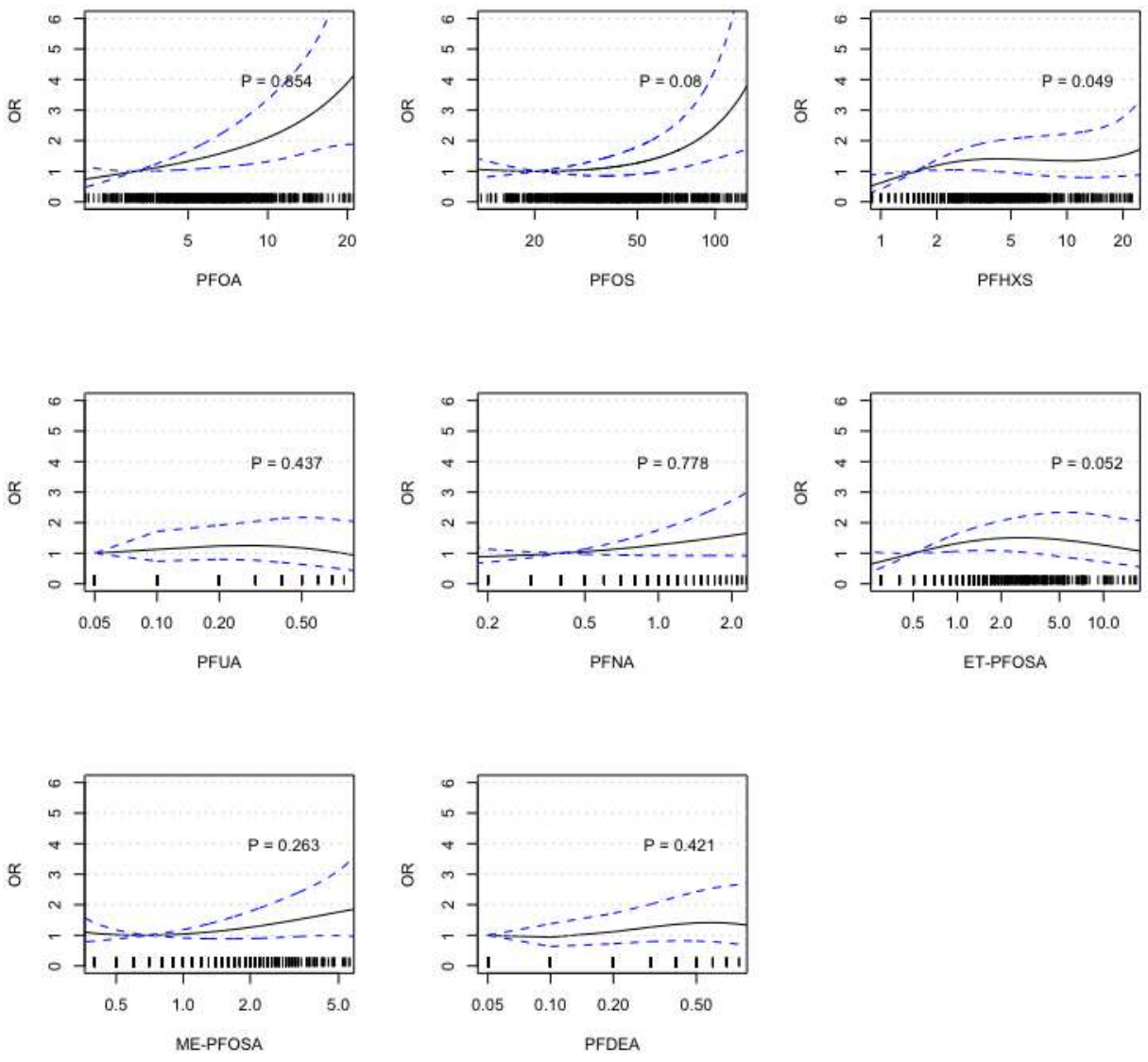




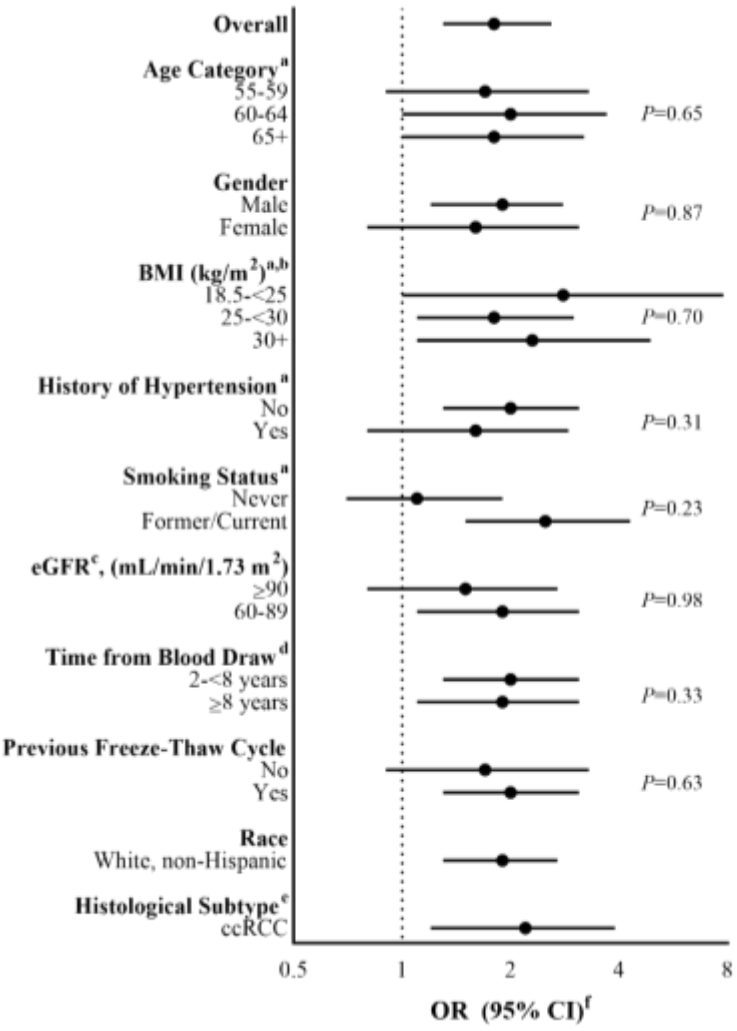
Supplementary Figure 1. Box plots for serum PFAS concentrations (ug/L) among RCC cases and controls



Supplementary Figure 2: Joint Analyses of Select PFAS and RCC Risk in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. (A) PFOA and PFOS, (B) PFOA and PFHxS, and (C) PFOS and PFHxS. Results are shown as the $N_{controls}/N_{cases}$ and ORs (95% CI), adjusted for body mass index (missing, <18.5, 18.5-<25, 25-<30, or ≥30 kg/m^2), smoking status (never, former, current), history of hypertension (no, yes), estimated glomerular filtration rate (continuous), previous freeze-thaw cycle, and calendar year of blood draw (1993-1995, 1996-1997, 1998-2002). Tertile values (based on PFAS distributions of controls) are as follows: $PFOA_{Low}$ ≤4.5 µg/L; 4.5< $PFOA_{Mid}$ ≤6.5 µg/L; $PFOA_{High}$ >6.5 µg/L; $PFOS_{Low}$ ≤30 µg/L; 30< $PFOS_{Mid}$ ≤45.6 µg/L; $PFOS_{High}$ >45.6 µg/L; $PFHxS_{Low}$ ≤2.5 µg/L; 2.5< $PFHxS_{Mid}$ ≤4.4 µg/L; $PFHxS_{High}$ >4.4 µg/L.



Supplementary Figure 3. The relationship between the PFAS levels (X-axis) and OR (Y-axis). The black and blue curves illustrate the estimated effect and associated 95% confidence interval for a range of PFAS levels. The dark black marks along the x-axis indicate observed serum PFAS concentrations (µg/L). Here, we use conditional logistic regression with the log2-transformed PFAS level modeled by a natural spline with three degrees of freedom. We assessed the improvement in the model fit, compared with including only a linear term for the log2-transformed level, using a likelihood ratio test and report the resulting p-value. Note, the figure is trimmed at the 2.5th and 97.5th PFAS percentiles for presentation.



Supplementary Figure 4. Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for Stratified and Sensitivity Analyses Evaluating Serum PFOA Concentrations and RCC Risk in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial with Further Adjustment for Other PFAS. Abbreviations: BMI, body mass index; ccRCC, clear cell renal cell carcinoma; eGFR, estimated glomerular filtration rate; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid. [a] Self-reported at study enrollment. [b] BMI specific analyses exclude individuals with missing or <18.5 kg/m2 BMIs. [c] At blood draw. [d] Time from blood to diagnosis for cases. [e] ICD-O-2 code = 8310. [f] Continuous OR (95% CI), which corresponds to a 1 unit increase on the $\log_2$ scale or an approximate doubling in analyte level, was estimated using unconditional multivariable logistic regression models adjusted for age at enrollment (55-59, 60-64, 65-69, 70+ years), sex (male, female), race/ethnicity (white non-Hispanic, black non-Hispanic, or other), estimated glomerular filtration rate (continuous), body mass index (<18.5 kg/m^2, 18.5 to <25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2, missing), history of hypertension (no/missing, yes), smoking status (never, former, current), previous freeze-thaw cycle, calendar year of blood draw (1993-1995, 1996-1997, 1998-2002), study year of blood draw (enrollment, other), and study center ([1]Minnesota or Marshfield; [2] Colorado, Hawaii, Washington University, University of Utah, or University of Alabama; [3] Georgetown, Henry Ford, or University of Pittsburgh), further adjusted for other PFAS (i.e., $\log_2$ transformed concentrations of PFOS and PFHxS). *P*-values represent Wald tests of heterogeneity across strata.