**SUPPLEMENTARY TABLE S1:** Precision and accuracy for 1,3-butadiene metabolites in urine,
Inter-day (N=10)

| Analyte                        | Added amount (ng/mL) | Expected amount a (ng/mL) | Measured mean (ng/mL) | Accuracy (percent of expected) | Precision CV (%) |
|--------------------------------|----------------------|----------------------------|-----------------------|-------------------------------|-----------------|
| Sum of MHBMA-1 and MHBMA-2 (MHBMA1+2) | 0                    | 0.11                       | 0.11                  | 78.9                          | 11.2            |
|                                | 0.5                  | 0.610                      | 0.481                 | 80.0                          | 11.3            |
|                                | 1.0                  | 1.11                       | 0.887                 | 80.0                          | 11.3            |
|                                | 5.0                  | 5.11                       | 4.07                  | 79.6                          | 7.73            |
|                                | 10                   | 10.11                      | 8.17                  | 80.8                          | 7.93            |
| LOQ                            | 0.1                  |                            |                       |                               |                 |
| MHBMA-3                        | 0                    | 0.035                      |                       |                               |                 |
|                                | 1.0                  | 1.04                       | 1.04                  | 100                           | 8.53            |
|                                | 2.5                  | 2.54                       | 2.42                  | 95.5                          | 4.82            |
|                                | 10                   | 10.0                       | 9.55                  | 95.2                          | 4.84            |
|                                | 20                   | 20.0                       | 17.7                  | 88.3                          | 5.16            |
| LOQ                            | 0.1                  |                            |                       |                               |                 |

a Added amount + mean amount measured in non-smoker’s urine

MHBMA-1+2 = sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid; MHBMA-3 = 4-hydroxy-2-buten-1-yl-mercapturic acid

LOQ = limit of quantitation
SUPPLEMENTARY TABLE S2: Concentrations of urinary biomarkers for Black and White smokers. Biomarkers are presented as raw concentrations, covariate-adjusted standardization of urinary biomarker concentrations, and creatinine-normalized concentrations. The study included a subset of Black and White smokers over the age of 40 who participated in a randomized clinical trial of reduced nicotine content cigarettes between June 2013 and July 2014 at 10 sites across the U.S.

| Biomarker                        | Blacks (GM, 95% CI) | Whites (GM, 95% CI) | p value |
|----------------------------------|---------------------|---------------------|---------|
| TNE (nmol/mL)                    | 40.8 (36.2 - 46.0)  | 43.2 (38.2 - 49.0)  | 0.301   |
| TNE (nmol/mL) (covariate-adjusted) | 40.6 (36.7 - 45.0)  | 43.3 (38.7 - 48.5)  | 0.152   |
| TNE (nmol/mg) (creatinine-normalized) | 40.6 (36.6 - 45.0)  | 56.9 (50.7 - 63.8)  | <0.001  |
| NNA (pmol/mL)                    | 1.10 (0.96 - 1.27)  | 1.45 (1.28 - 1.65)  | 0.005   |
| NNA (pmol/mL) (covariate-adjusted) | 1.09 (0.97 - 1.23)  | 1.45 (1.30 - 1.61)  | <0.001  |
| NNA (pmol/mg) (creatinine-normalized) | 1.09 (0.97 - 1.24)  | 1.91 (1.72 - 2.13)  | <0.001  |
| 2-HPMA (ng/mL)                   | 62.1 (54.5 - 70.7)  | 62.6 (53.5 - 73.4)  | 0.756   |
| 2-HPMA (ng/mL) (covariate-adjusted) | 62.1 (56.0 - 68.9)  | 62.6 (55.8 - 70.3)  | 0.832   |
| 2-HPMA (ng/mg) (creatinine-normalized) | 62.0 (55.9 - 68.8)  | 82.3 (73.3 - 92.5)  | <0.001  |
| 3-HPMA (ng/mL)                   | 1007 (869 - 1166)   | 965 (834 - 1116)    | 0.882   |
| 3-HPMA (ng/mL) (covariate-adjusted) | 1007 (907 - 1117)   | 965 (864 - 1077)    | 0.709   |
| 3-HPMA (ng/mg) (creatinine-normalized) | 1005 (904 - 1119)   | 1268 (1136 - 1416)  | 0.001   |
| AAMA (ng/mL)                     | 205 (181 - 232)     | 200 (175 - 229)     | 0.834   |
| AAMA (ng/mL) (covariate-adjusted) | 205 (190 - 220)     | 200 (185 - 217)     | 0.856   |
| AAMA (ng/mg) (creatinine-normalized) | 204 (190 - 220)     | 263 (243 - 285)     | <0.001  |
| CNEMA (ng/mL)                    | 182 (158 - 210)     | 184 (159 - 212)     | 0.377   |
| CNEMA (ng/mL) (covariate-adjusted) | 182 (164 - 202)     | 184 (163 - 206)     | 0.281   |
| CNEMA (ng/mg) (creatinine-normalized) | 182 (164 - 202)     | 241 (214 - 272)     | <0.001  |
| HEMA (ng/mL)                     | 3.67 (3.09 - 4.36)  | 3.46 (2.94 - 4.06)  | 0.739   |
| HEMA (ng/mL) (covariate-adjusted) | 3.67 (3.18 - 4.24)  | 3.46 (3.02 - 3.95)  | 0.732   |
| HEMA (ng/mg) (creatinine-normalized) | 3.67 (3.17 - 4.24)  | 4.54 (3.97 - 5.20)  | 0.015   |
| HPMMA (ng/mL)                    | 209 (160 - 274)     | 201 (156 - 260)     | 0.556   |
| HPMMA (ng/mL) (covariate-adjusted) | 209 (162 - 270)     | 201 (159 - 256)     | 0.402   |
| HPMMA (ng/mg) (creatinine-normalized) | 209 (162 - 269)     | 265 (209 - 336)     | 0.013   |
| MHBMA-1+2 (ng/mL)                | 1.23 (1.03 - 1.46)  | 1.20 (0.99 - 1.46)  | 0.603   |
| MHBMA-1+2 (ng/mL) (covariate-adjusted) | 1.23 (1.05 - 1.44)  | 1.20 (1.01 - 1.44)  | 0.619   |
| MHBMA-1+2 (ng/mg) (creatinine-normalized) | 1.23 (1.05 - 1.43)  | 1.58 (1.32 - 1.89)  | 0.004   |
| MHBMA-3 (ng/mL)                  | 0.12 (0.11 - 0.14)  | 0.14 (0.13 - 0.15)  | 0.056   |
| MHBMA-3 (ng/mL) (covariate-adjusted) | 0.12 (0.11 - 0.14)  | 0.14 (0.12 - 0.16)  | 0.082   |
| MHBMA-3 (ng/mg) (creatinine-normalized) | 0.12 (0.11 - 0.14)  | 0.18 (0.16 - 0.21)  | <0.001  |
| MMA (ng/mL)                      | 19.8 (16.6 - 23.6)  | 14.9 (12.9 - 17.3)  | 0.023   |
| MMA (ng/mL) (covariate-adjusted) | 19.8 (17.0 - 23.1)  | 14.9 (13.3 - 16.7)  | 0.003   |
| MMA (ng/mg) (creatinine-normalized) | 19.8 (16.9 - 23.2)  | 19.6 (17.5 - 22.0)  | 0.954   |
| PMA (ng/mL)                      | 1.16 (1.00 - 1.35)  | 1.25 (1.06 - 1.49)  | 0.242   |
| PMA (ng/mL) (covariate-adjusted) | 1.16 (1.02 - 1.32)  | 1.25 (1.08 - 1.46)  | 0.257   |
| PMA (ng/mg) (creatinine-normalized) | 1.16 (1.02 - 1.32)  | 1.65 (1.41 - 1.92)  | <0.001  |

TNE = total nicotine equivalents (nmol/mL); NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (pmol/mL); units of VOC metabolites are ng/mL; 2-HPMA = 2-hydroxypropylmercapturic acid (propylene oxide); 3-HPMA = 3-hydroxypropylmercapturic acid (acrolein); AAMA = 2-
carbamoylethylmercapturic acid (acrylamide); CNEMA = 2-cyanoethylmercapturic acid (acrylonitrile); HEMA = 2-hydroxyethylmercapturic acid (acrylonitrile, vinyl chloride, ethylene oxide); HPMMA = 3-hydroxy-1-methyl-propylmercapturic acid (crotonaldehyde); MHBMA-1+2 = sum of isomers 1-hydroxy-3-buten-2-yl-mercapturic acid and 2-hydroxy-3-buten-1-yl-mercapturic acid (1,3-butadiene); MHBMA-3 = 4-hydroxy-2-buten-1-yl-mercapturic acid (1,3-butadiene); MMA = methylmercapturic acid (methylating agents); and, PMA = phenylmercapturic acid (benzene)

† The covariate-adjusted concentrations were determined using a method from a previously published paper (“Method 3”).(1)

REFERENCES

1. O’Brien KM, Upson K, Cook NR, Weinberg CR. Environmental chemicals in urine and blood: improving methods for creatinine and lipid adjustment. Environ Health Perspect. 2016;124:220-7.