Supporting Information for

Habitual Coffee Intake Shapes the Gut Microbiome and Modifies Host Physiology and Cognition

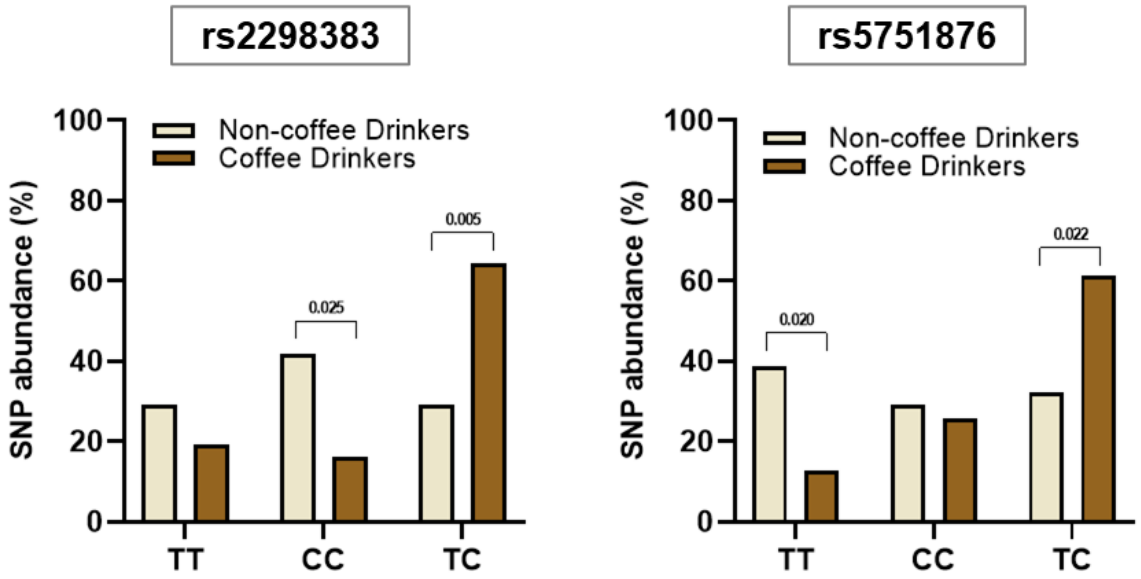

Fig. S1 Study Participant ADORA2A SNP Genotyping

Genotyping analysis of the rs2298383 and rs5751876 single nucleotide polymorphisms (SNP) of ADORA2A gene (N= 31 per group). Coffee drinkers are represented in dark brown, non-coffee drinkers are presented in light brown. Data is presented as percentage SNP abundance; significant differences are indicated by p-values using the Pearson's chi-squared test.

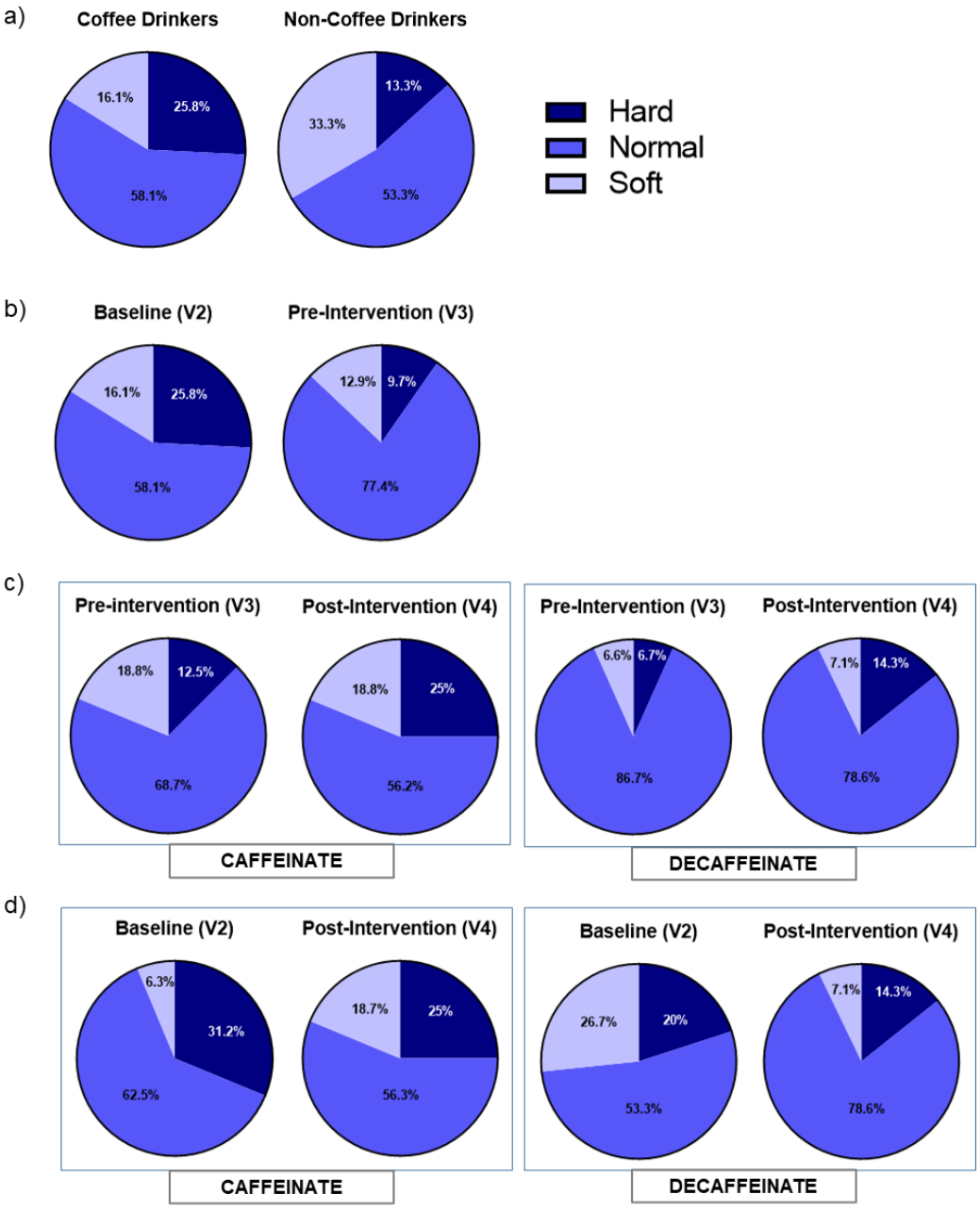

Figure S2: Bristol Stool Score Comparisons during study timepoints.

a) coffee drinker's vs non-coffee drinkers at baseline; b) before and after coffee washout in coffee drinkers (Visit 2 vs Visit 3); c) before and after intervention in caffeinate and decaffeinate groups (Visit 3 vs Visit 4); d) baseline compared to post-intervention in caffeinate and decaffeinate groups. Data are presented as percentages of patients per group with hard (navy), normal (blue) and soft stools (light blue). See methods for further details.

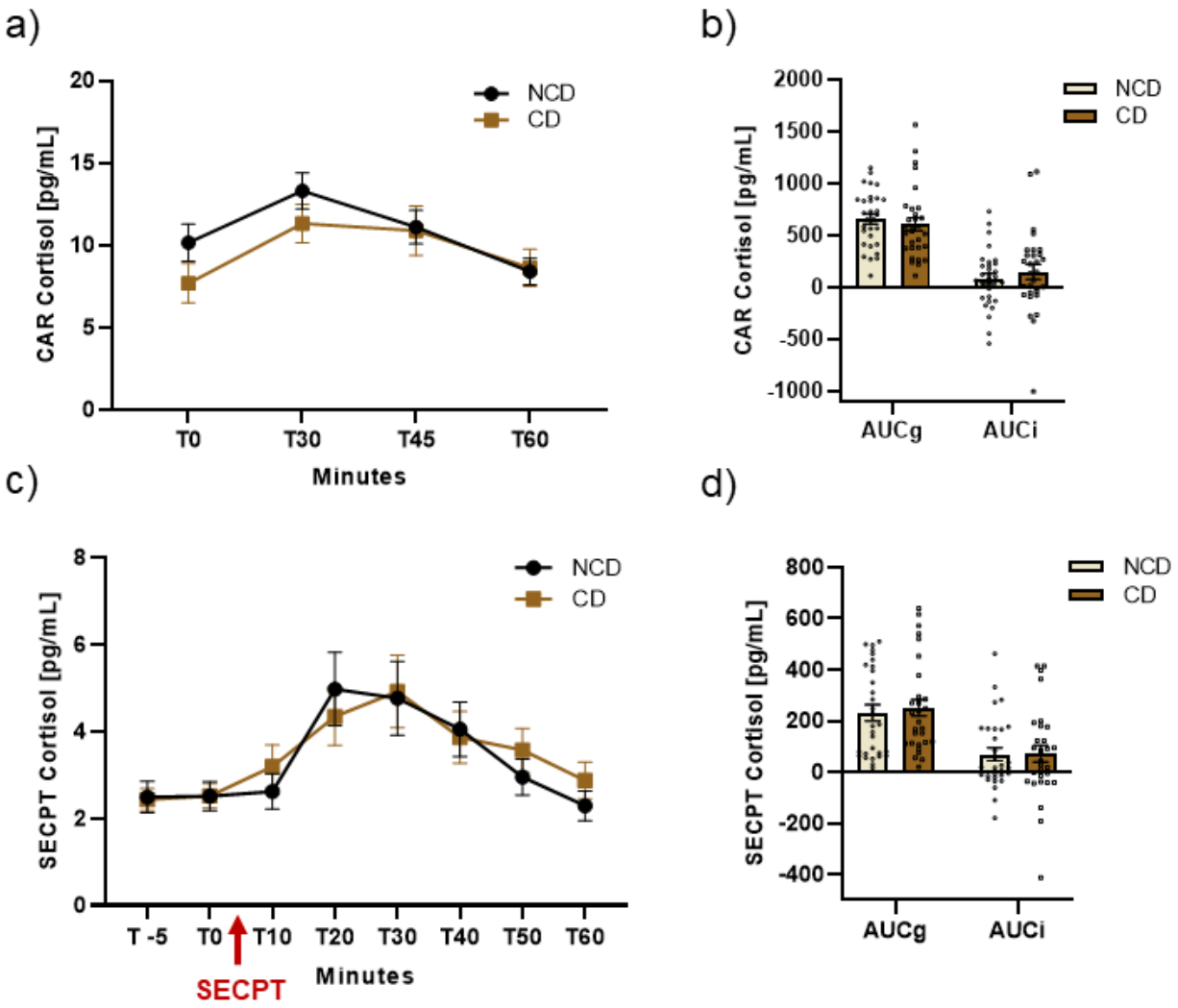

Figure S3: Similar baseline cortisol response between coffee drinkers and non-coffee drinkers.

Salivary cortisol awakening response (CAR) and cortisol levels during SECPT (socially evaluated cold pressor test) procedure at the baseline (Visit 2) of non-coffee drinker (NCD, light brown) and coffee drinker (CD. Dark brown) participants. a) Cortisol concentration trajectory during the hour after awakening. b) Area under the curve with respect to ground (AUCg) and with respect to increase (AUCi) of the CAR. c) Cortisol concentration trajectory during the SECPT, performed at the time indicated by the red arrow. d) AUCg and AUCi of the cortisol during SECPT. N=31 per group.

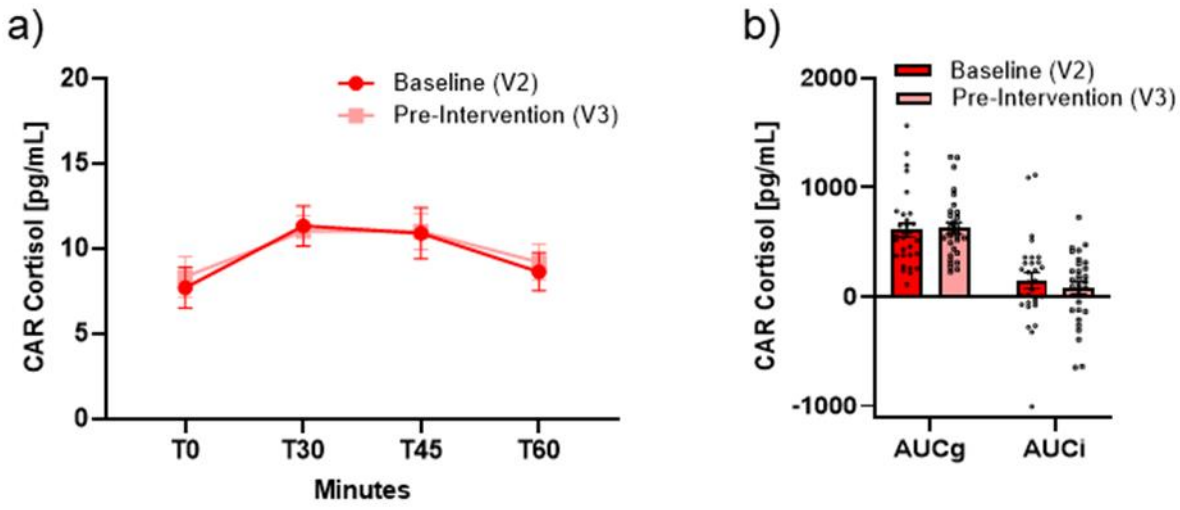

Figure S4: Caffeine abstinence does not influence salivary cortisol production following coffee abstinence.

Salivary cortisol awakening response (CAR) before and after coffee washout i.e., at baseline (Visit 2, red) compared to pre-intervention visit (Visit 3, pink) of coffee drinkers. a) Cortisol concentration trajectory during the hour after awakening. b) Area under the curve with respect to ground (AUCg) and with respect to increase (AUCi) of the CAR. N= 31 per group.

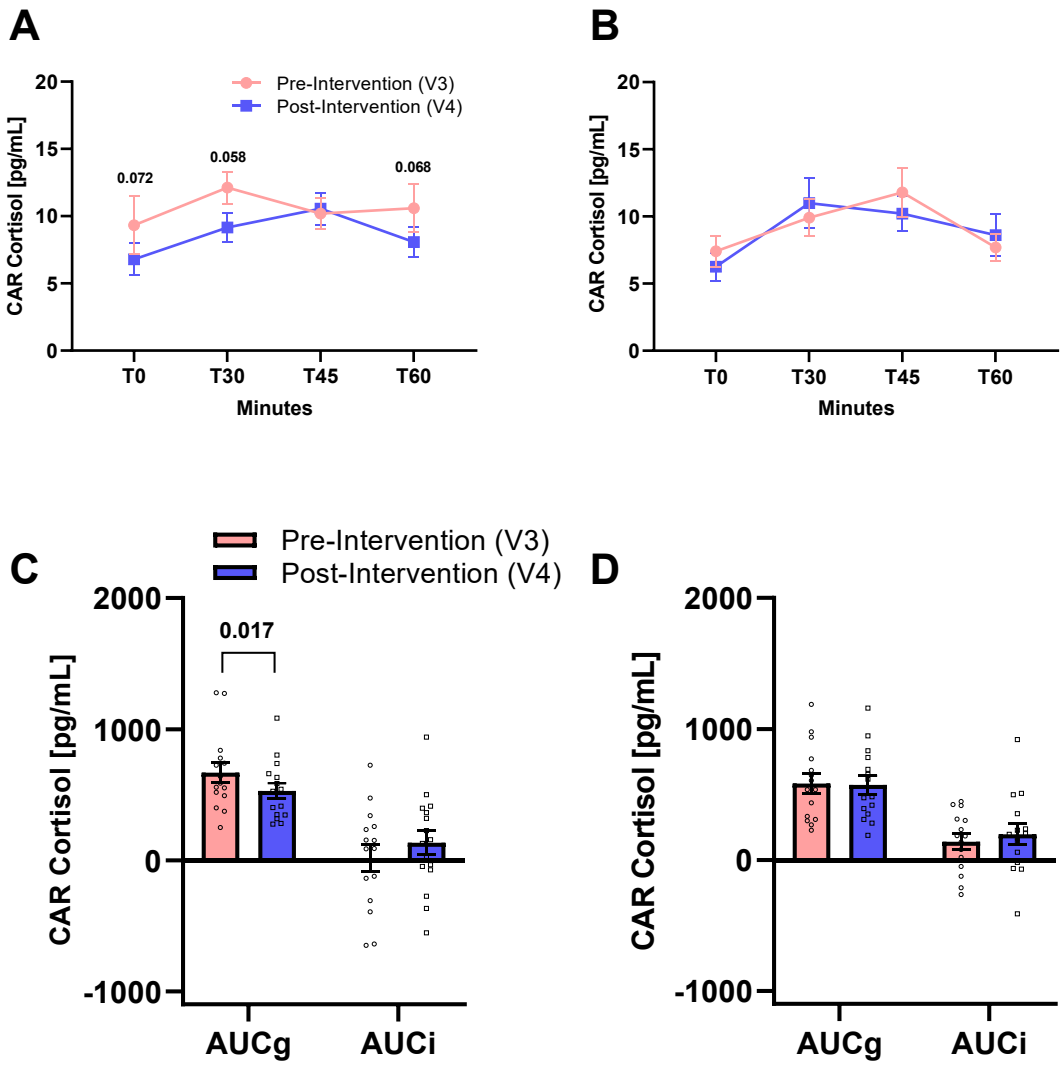

Figure S5: Caffeinated and decaffeinated coffee does not influence the cortisol awakening response.

Salivary cortisol awakening response (CAR) before and after caffeinated and decaffeinated coffee intervention i.e., pre-intervention (Visit 3, pink) compared to post-intervention (Visit 4, blue) in habitual coffee drinkers. a) Cortisol concentration trajectory during the hour after awakening in caffeinate group. b) Cortisol concentration trajectory during the hour after awakening in decaffeinate group. c) Area under the curve with respect to ground (AUCg) and with respect to increase (AUCi) in the CAR in caffeinate group. d) Area under the curve with respect to ground (AUCg) and with respect to increase (AUCi) in the CAR of decaffeinate group.

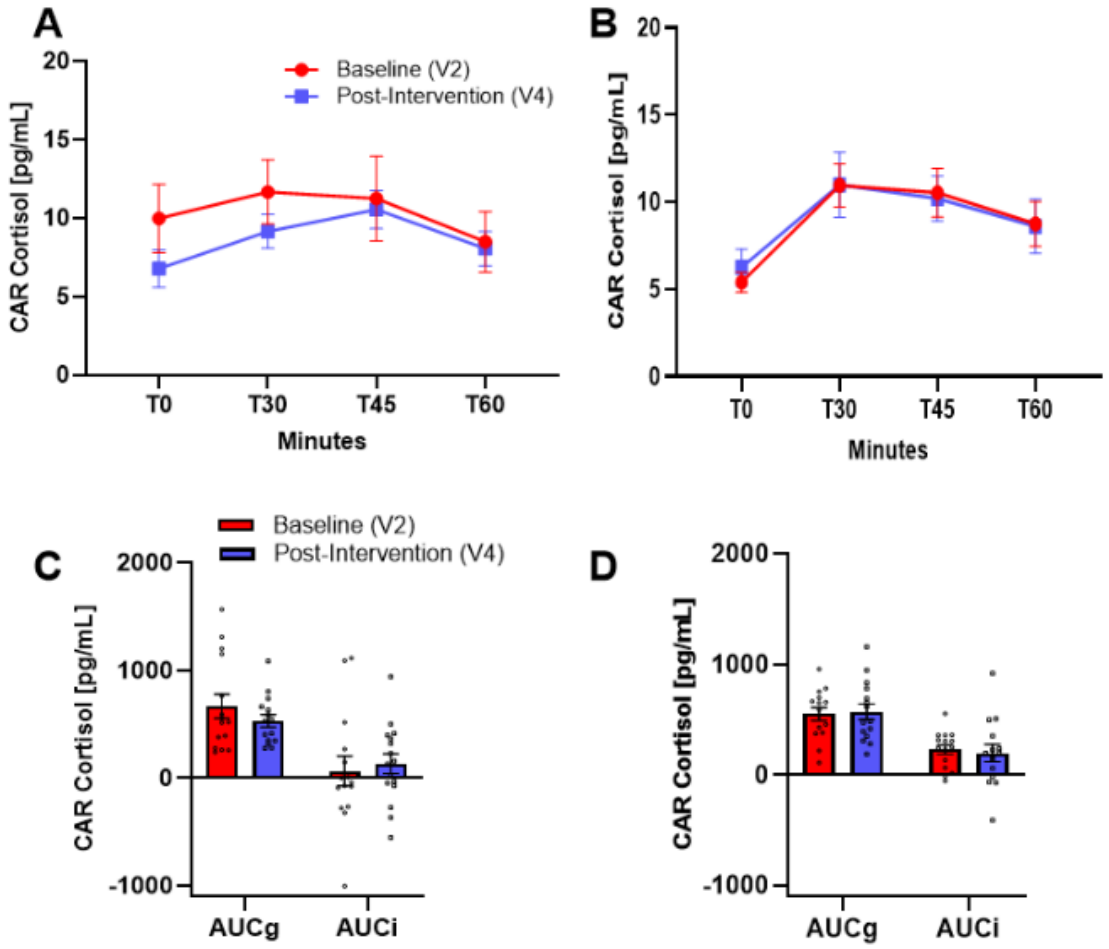

Figure S6 No observed differences in salivary cortisol awakening response (CAR) at the baseline (Visit 2) compared to post-intervention (Visit 4) in habitual coffee drinkers.

Salivary cortisol levels during CAR at the baseline (Visit 2, red) compared to post-intervention (Visit 4, blue) in habitual coffee drinkers. a) Cortisol concentration trajectory during the hour after awakening in caffeinate group. b) Cortisol concentration trajectory during the hour after awakening in decaffeinate group. c) Area under the curve with respect to ground (AUCg) and with respect to increase (AUCi) in the CAR in caffeinate group. d) Area under the curve with respect to ground (AUCg) and with respect to increase (AUCi) in the CAR of decaffeinate group.

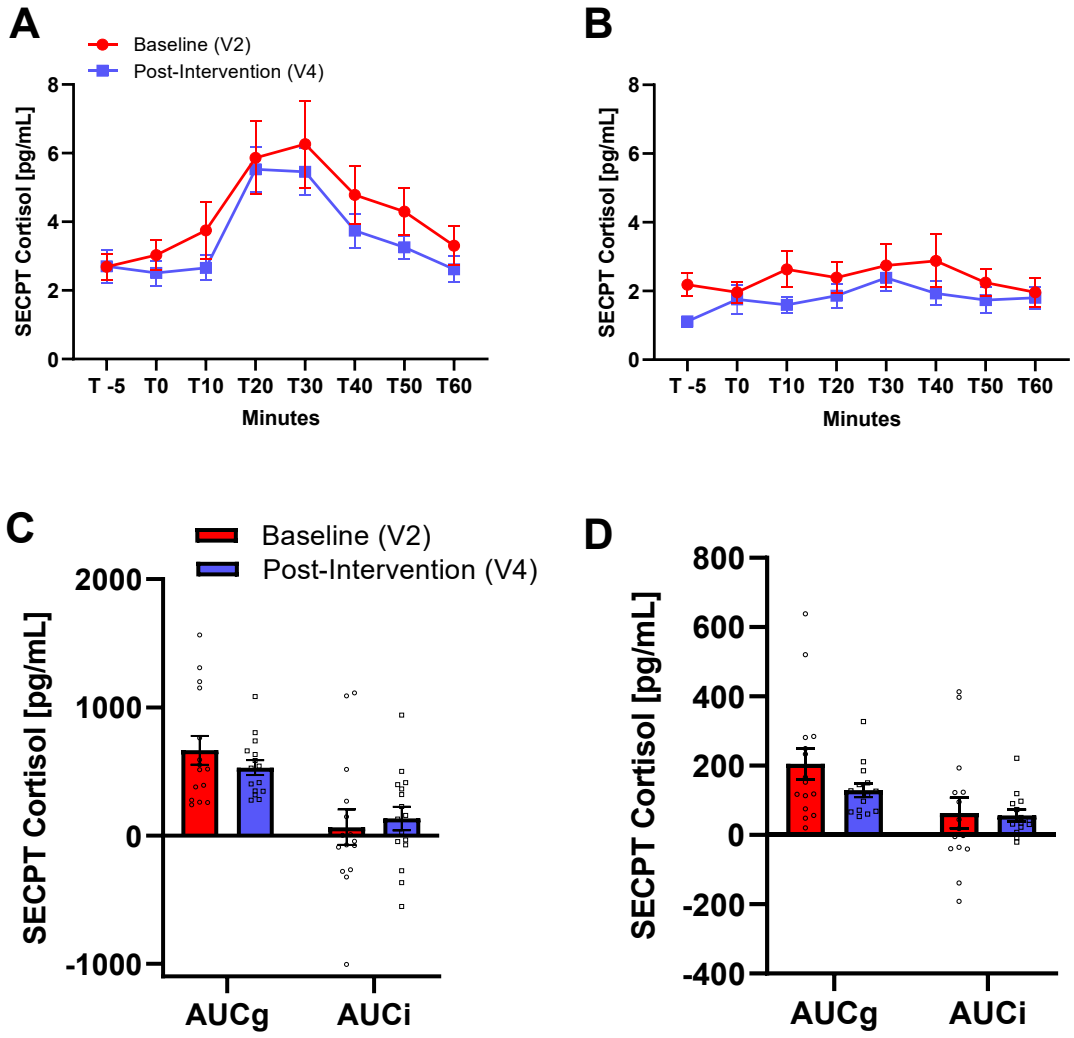

Figure S7: No observed differences in salivary cortisol levels during SECPT (socially evaluated cold pressor test) procedure at the baseline (Visit 2) compared to post-intervention (Visit 4) in habitual coffee drinkers.

Salivary cortisol levels during SECPT (socially evaluated cold pressor test) procedure at the baseline (Visit 2, red) compared to post-intervention (Visit 4, blue) in habitual coffee drinkers. a) Cortisol concentration trajectory during the SECPT in the caffeinated group. b) Cortisol concentration trajectory during the SECPT in the decaffeinated group. c) Area under the curve with respect to ground (AUCg) and with respect to increase (AUCi) in the SECPT in caffeinated group. d) Area under the curve with respect to ground (AUCg) and with respect to increase (AUCi) in the SECPT of decaffeinated group.

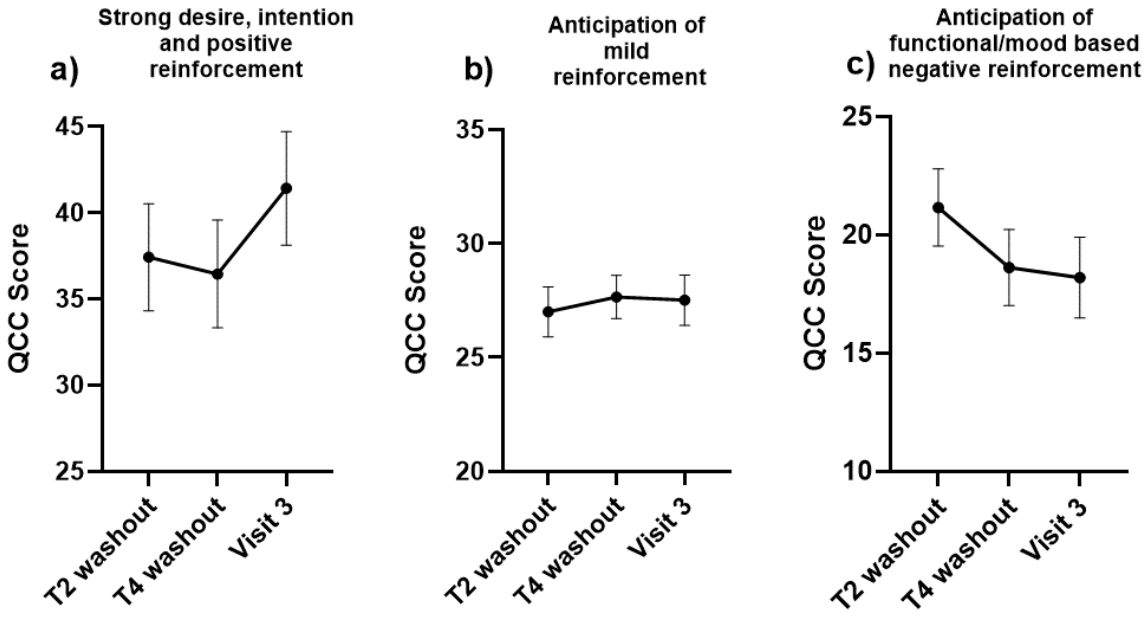

Figure S8: No observed differences in Sub-scores of Questionnaires of Caffeine cravings (QCC) during coffee washout in habitual coffee drinkers.

Sub-scores of Questionnaires of Caffeine cravings (QCC) during coffee washout (T2 washout, T4 washout, visit 3)

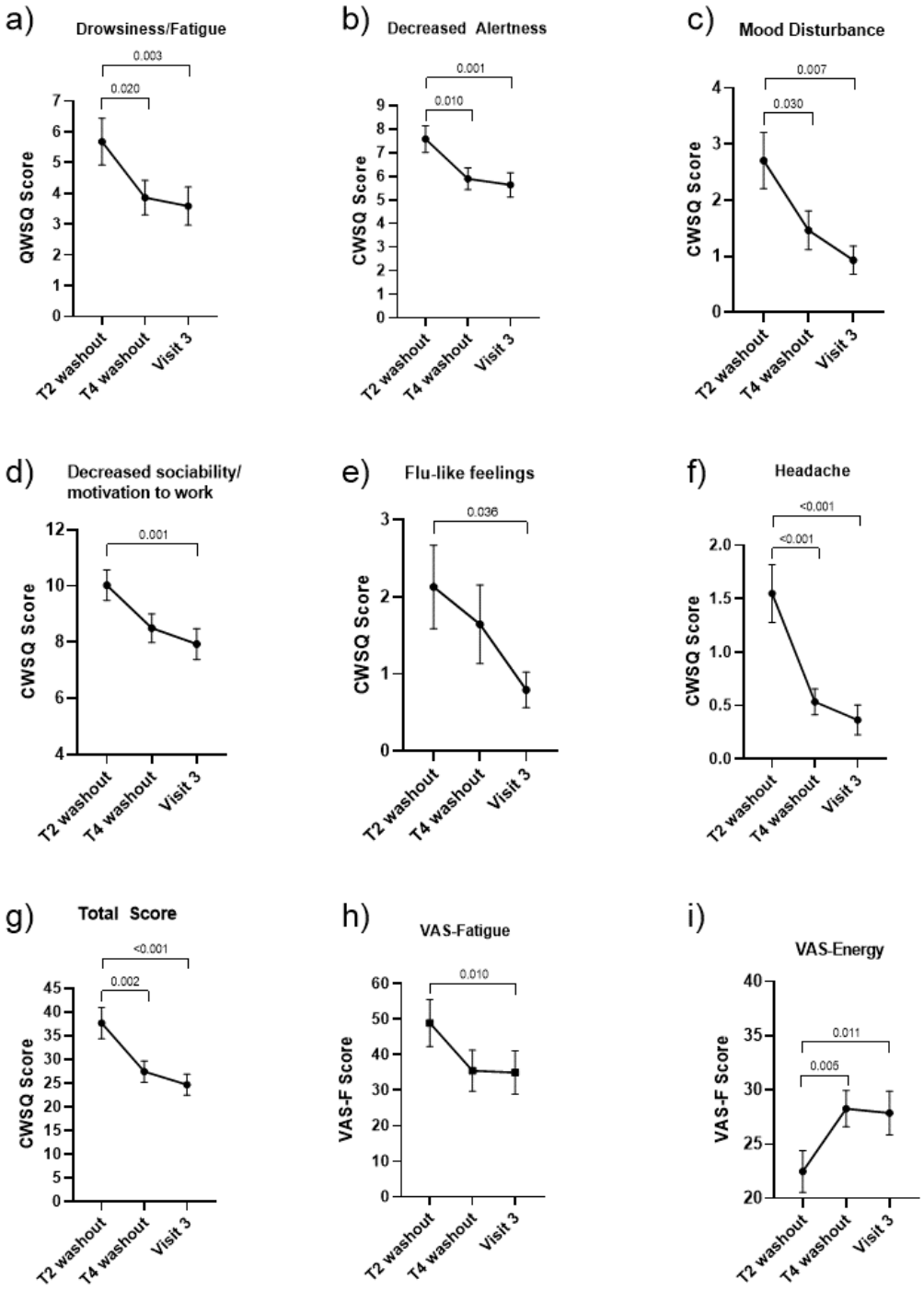

Figure S9: Differences in caffeine withdrawal symptoms, fatigue, and energy during a two-week period of coffee abstinence.

During a two-week period of caffeine abstinence, participants completed CWSQ and VAS questionnaires. Participants were scored for drowsiness and fatigue a), decreased alertness b), mood disturbance c), decreased sociability or motivation to work d), flu-like feelings e), headache f), and total CWSQ score g). Patients also completed a visual analogue scale for fatigue h) and energy i). Groups showing a p value <0.05 (time points comparison): * = <0.05, ** = <0.01, *** = < 0.001) are significant. Data is presented as mean ± S.E.M

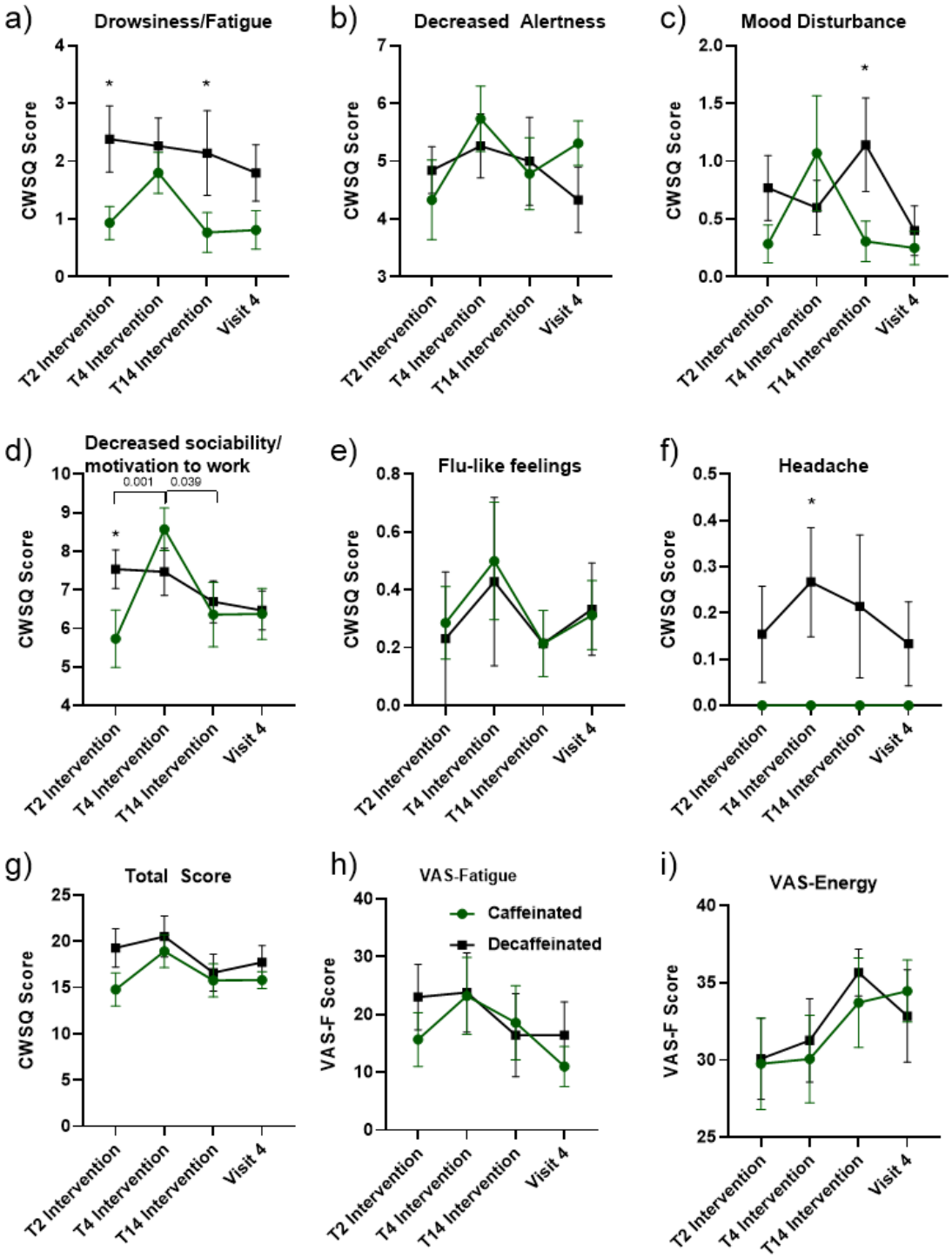

Figure S10: Divergent effects of caffeinated and decaffeinated coffee on CWSQ, fatigue and energy levels over a 3-week intervention.

Sub-scores of visual analogue scales-fatigues (VAS-F) during coffee washout (T2 washout, T4 washout, visit 3) and during coffee intervention (T2 intervention, T4 intervention, T14 intervention, Visit 4) in habitual coffee drinkers. Groups showing a p value <0.05 (time points comparison) or *

(Caffeinate vs Decaffeinate comparison: * = <0.05, ** = <0.01, *** = < 0.001) are significant. Data are presented as mean ± S.E.M. Caffeinated participants data represented by green lines and circles reflect different timepoints during the intervention. Participants receiving decaffeinated coffee are represented by black lines and timepoints are represented by black squares.

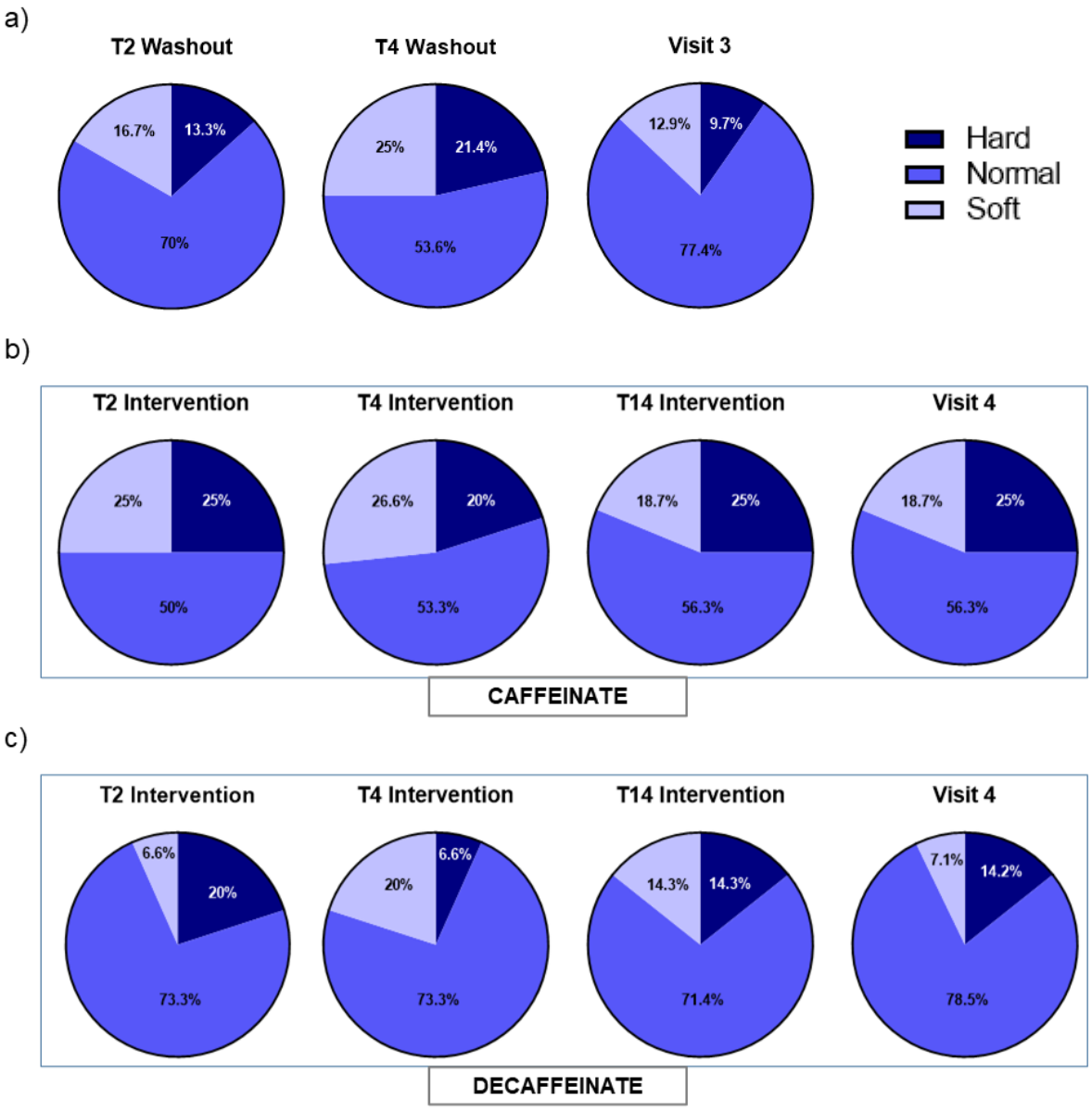

Figure S12: Stool consistency score increases following caffeine withdrawal, though non-significantly.
Bristol Stool Charts. a) time points 2 days and 4 days after the start of coffee washout and Visit 3; b) time points 2, 4 and 14 days after the start of the intervention and Visit 4 in the caffeinate group; c) time points 2, 4 and 14 days after the start of the intervention and Visit 4 in the decaffeinate group. Hard (navy), normal (blue) and soft stools (light blue).

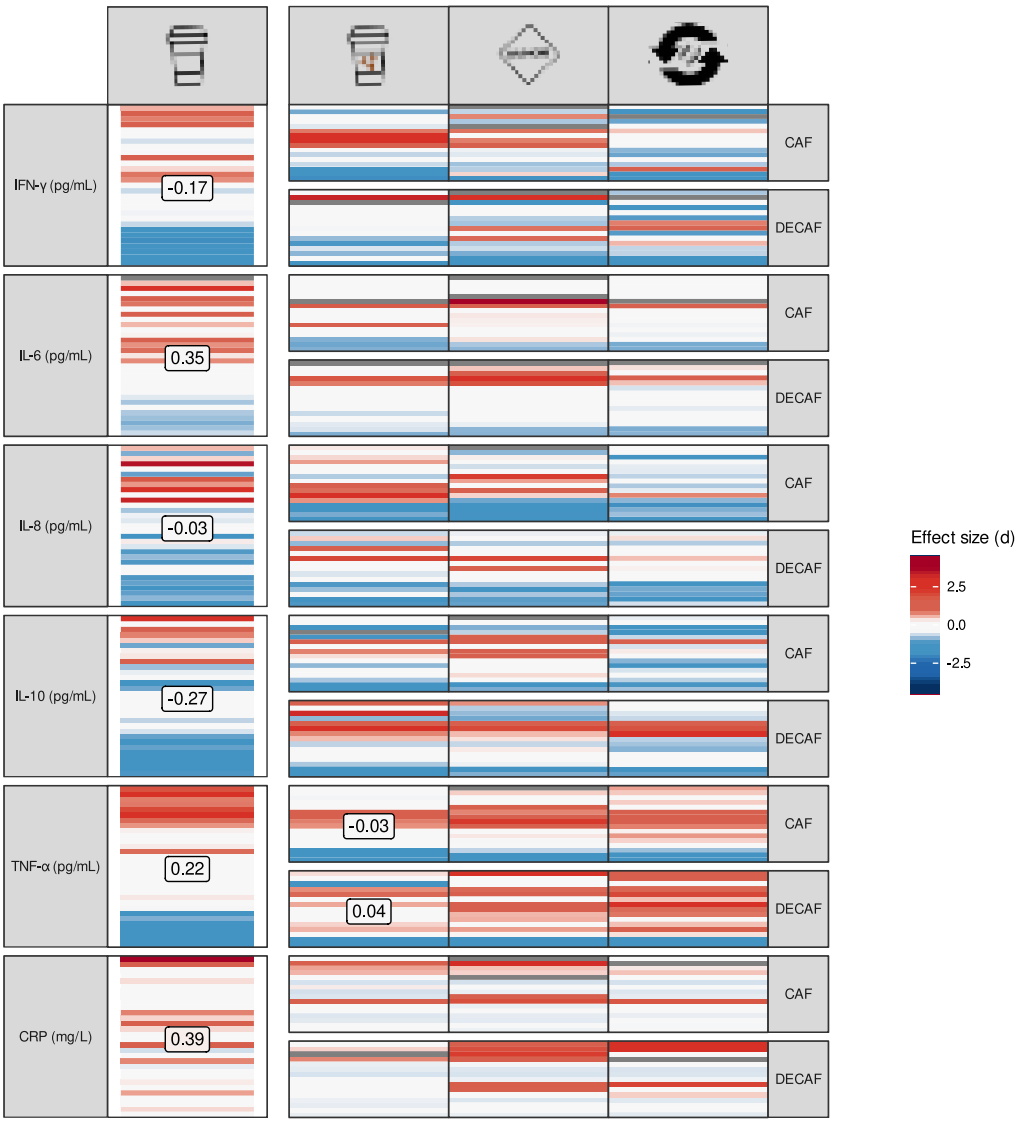

Fig S13: Coffee and its components influence basal cytokine concentration.

Heatmap of unstimulated whole blood cytokine concentrations relative to baseline coffee drinkers (CD). For the coffee drinker subset, which features repeated measurements, measurements from the same participant are aligned on the y-axis. Red colour indicates positive effect size while blue reflects negative effect size, with intensity of red or blue corresponding to increased or decreased effect sizes respectively and white reflecting zero effect. Red colour indicates positive effect size while blue reflects negative effect size, with intensity of red or blue corresponding to increased or decreased effect sizes respectively and white reflecting zero effect. Panels containing a text box with numbers reflects those comparisons with a Cohen's d effect size of > 0 5 reflecting medium sized effects. Column one =cytokine, column two = NCD, column three = CD, column four washout period for CD and column five reintroduction of caffeinated or decaffeinated coffee. N=31 at baseline for CD and NCD, N= 31 during washout period, N=15 during reintroduction of decaffeinated coffee and N=16 during reintroduction of caffeinated coffee. IFN = Interferon, IL= interleukin, TNF = tumour necrosis factor, CRP= C-reactive protein. Analysis performed on data expressed as picogram per millilitre (IFNγ, IL-6, IL-8. IL-10, TNFα) or milligram per litre, CRP).

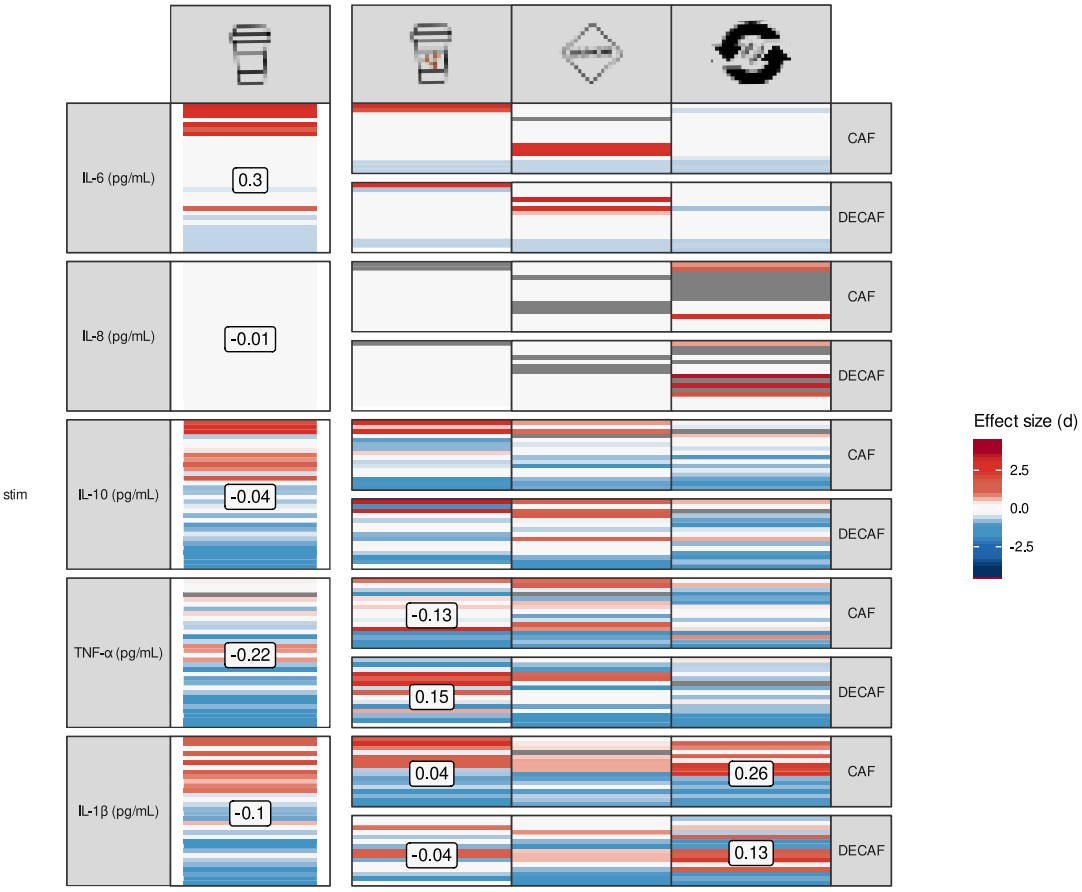

Fig S14: Coffee and its components influence cytokine secretion following LPS stimulation.

Heatmap of LPS stimulated whole blood cytokine concentrations relative to baseline coffee drinkers (CD). For the coffee drinker subset, which features repeated measurements, measurements from the same participant are aligned on the y-axis. Red colour indicates positive effect size while blue reflects negative effect size, with intensity of red or blue corresponding to increased or decreased effect sizes respectively and white reflecting zero effect. Red colour indicates positive effect size while blue reflects negative effect size, with intensity of red or blue corresponding to increased or decreased effect sizes respectively and white reflecting zero effect. Panels containing a text box with numbers reflects those comparisons with a Cohen's d effect size of > 0 5 reflecting medium sized effects. Column one =cytokine, column two = NCD, column three = CD, column four washout period for CD and column five reintroduction of caffeinated or decaffeinated coffee. N=31 at baseline for CD and NCD, N= 31 during washout period, N=15 during reintroduction of decaffeinated coffee and N=16 during reintroduction of caffeinated coffee. IFN = Interferon, IL= interleukin, TNF = tumour necrosis factor, LPS = lipopolysaccharide. Analysis performed on data expressed as picogram per millilitre.

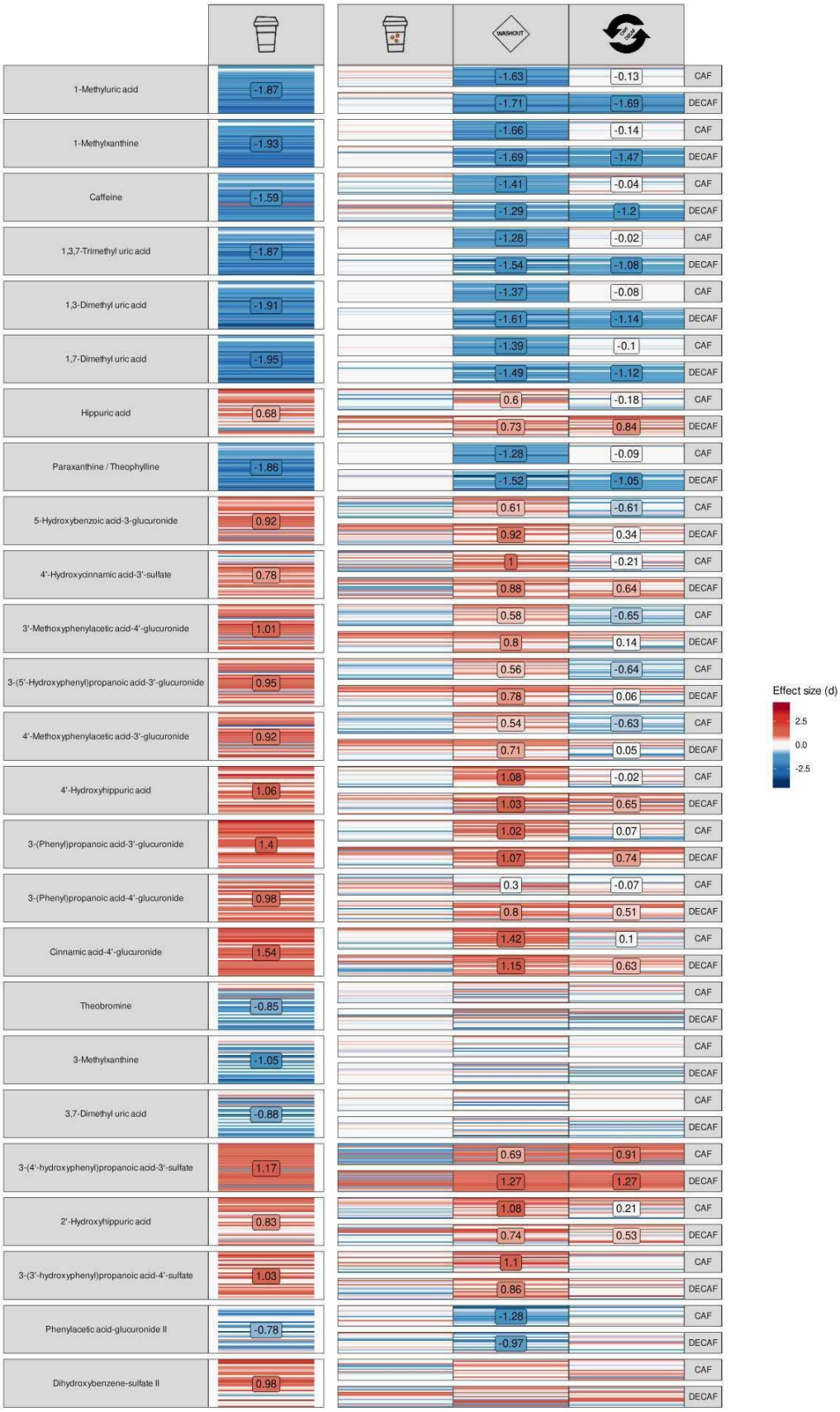
WASHOUT
1-Methyluric acid
-1.87
-1.63
-0.13
CAF
-1.71
-1.69
DECAF
1-Methylxanthine
-1.93
-1.66
-0.14
CAF
-1.69
-1.47
DECAF
Caffeine
-1.59
-1.41
-0.04
CAF
-1.29
-1.2
DECAF
1,3,7-Trimethyl uric acid
-1.87
-1.28
-0.02
CAF
-1.54
-1.08
DECAF
1,3-Dimethyl uric acid
-1.91
-1.37
-0.08
CAF
-1.61
-1.14
DECAF
1,7-Dimethyl uric acid
-1.95
-1.39
-0.1
CAF
-1.49
-1.12
DECAF
Hippuric acid
0.68
0.6
-0.18
CAF
0.73
0.84
DECAF
Paraxanthine / Theophylline
-1.86
-1.28
-0.09
CAF
-1.52
-1.05
DECAF
5-Hydroxybenzoic acid-3-glucuronide
0.92
0.61
-0.61
CAF
0.92
0.34
DECAF
4'-Hydroxycinnamic acid-3'-sulfate
0.78
1
-0.21
CAF
0.88
0.64
DECAF
3'-Methoxyphenylacetic acid-4'-glucuronide
1.01
0.58
-0.65
CAF
0.8
0.14
DECAF
3-(5'-Hydroxyphenyl)propanoic acid-3'-glucuronide
0.95
0.56
-0.64
CAF
0.78
0.06
DECAF
4'-Methoxyphenylacetic acid-3'-glucuronide
0.92
0.54
-0.63
CAF
0.71
0.05
DECAF
4'-Hydroxyhippuric acid
1.06
1.08
-0.02
CAF
1.03
0.65
DECAF
3-(Phenyl)propanoic acid-3'-glucuronide
1.4
1.02
0.07
CAF
1.07
0.74
DECAF
3-(Phenyl)propanoic acid-4'-glucuronide
0.98
0.3
-0.07
CAF
0.8
0.51
DECAF
Cinnamic acid-4'-glucuronide
1.54
1.42
0.1
CAF
1.15
0.63
DECAF
Theobromine
-0.85
CAF
DECAF
3-Methylxanthine
-1.05
CAF
DECAF
3,7-Dimethyl uric acid
-0.88
CAF
DECAF
3-(4'-hydroxyphenyl)propanoic acid-3'-sulfate
1.17
0.69
0.91
CAF
1.27
1.27
DECAF
2'-Hydroxyhippuric acid
0.83
1.08
0.21
CAF
0.74
0.53
DECAF
3-(3'-hydroxyphenyl)propanoic acid-4'-sulfate
1.03
1.1
CAF
0.86
DECAF
Phenylacetic acid-glucuronide II
-0.78
-1.28
CAF
-0.97
DECAF
Dihydroxybenzene-sulfate II
0.98
CAF
DECAF
Effect size (d)
2.5
0.0
-2.5

Fig S15: Coffee and its components influence urinary metabolite secretion.

Heatmap of metabolite concentrations relative to baseline coffee drinkers (CD). For the coffee drinker subset, which features repeated measurements, measurements from the same participant are aligned on the y-axis. Red colour indicates positive effect size while blue reflects negative effect size, with intensity of red or blue corresponding to increased or decreased effect sizes respectively and white reflecting zero effect. Red colour indicates positive effect size while blue reflects negative effect size, with intensity of red or blue corresponding to increased or decreased effect sizes respectively and white reflecting zero effect. Panels containing a text box with numbers reflects those comparisons with a Cohen's d effect size of > 0 5 reflecting medium sized effects. Column one =cytokine, column two = NCD, column three = CD, column four washout period for CD and column five reintroduction of caffeinated or decaffeinated coffee. N=31 at baseline for CD and NCD, N= 31 during washout period, N=15 during reintroduction of decaffeinated coffee and N=16 during reintroduction of caffeinated coffee.

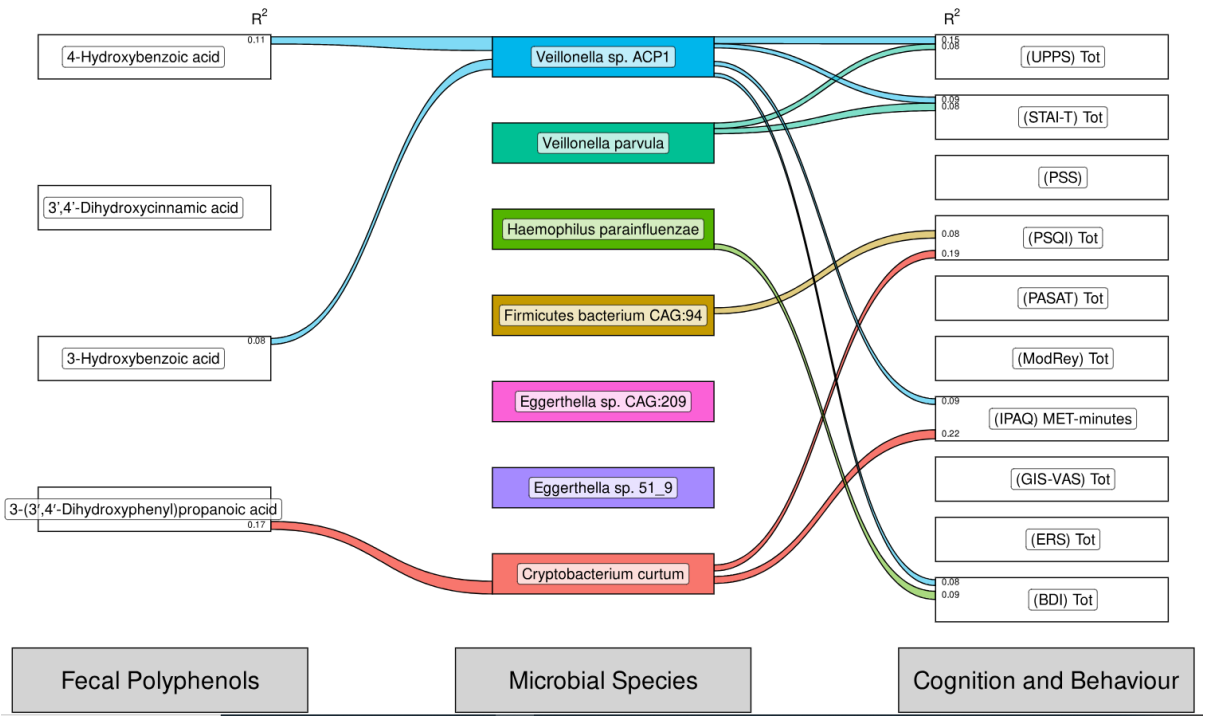

Figure S16 Limited Influence of coffee on faecal poly(phenol) metabolites on cognition and behaviour.

Sankey diagram summarising pairwise statistical associations between metabolites (left), microbial species (centre) and cognition and behaviour (right) based on the integration of data from Fig1, 2 and 5. Each node represents a species, metabolite, or behavioural or cognitive output. Each line connecting a node represents a statistical association between that microbial species, metabolite, and cognitive output, as measured with a generalised linear mixed-effects model. The left (microbial species) and right (cognition and behaviour) nodes contain R^2 values that represent the determination coefficient between that column and a centre node (metabolites). The thickness of the connecting line represents the marginal, not conditional, R^2 value of that particular model, scaled by the total R^2 of associations with that feature. Lines are opaque if $R^2 > 0.3$ and translucent if $0.3 > R^2 > 0.1$. Only marginal associations with Benjamini-Hochberg adjusted p-values of < 0.001 are shown.

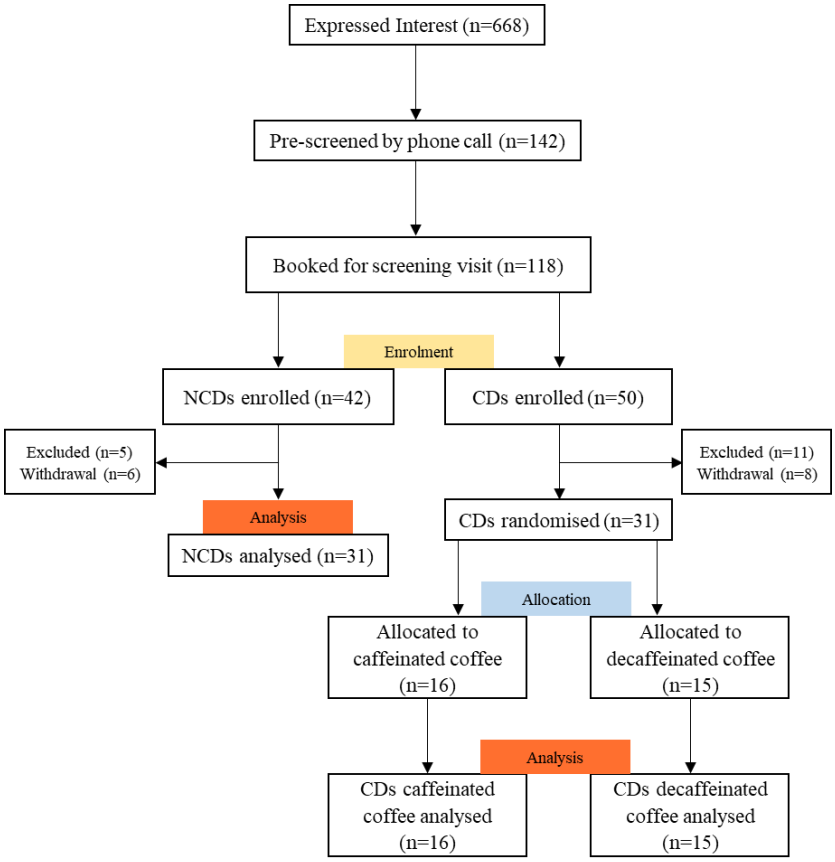

Figure S17: CONSORT flow diagram of the study.

NCD = non-Coffee drinker, CD = Coffee drinker.

Supplementary References

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