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Supplementary Materials for

Accurate detection of acute sleep deprivation using a metabolomic biomarker—A machine learning approach

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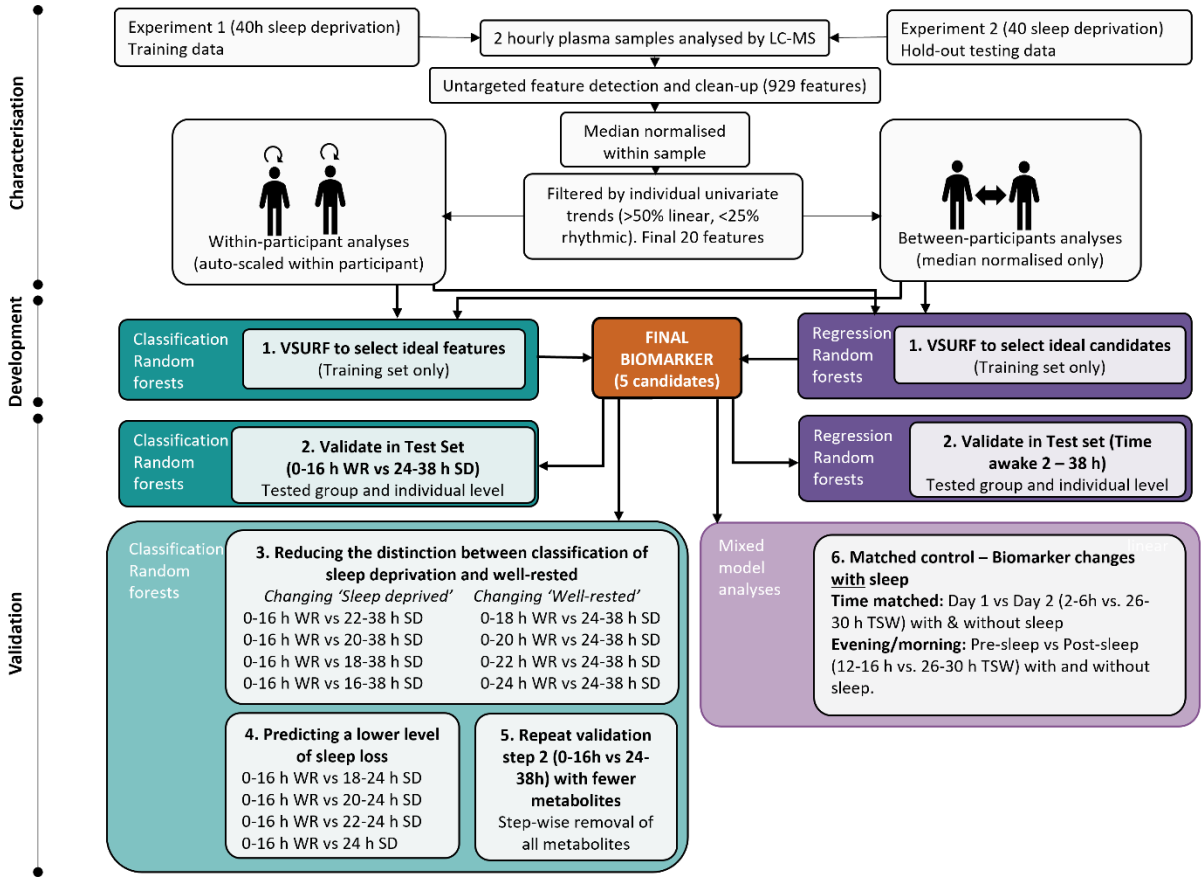

Figure S1: Graphical Representation of our Approach and Analysis Plan. Biomarkers were characterised by linear/circadian trends with time awake to reduce the final number of metabolites for development and modelling (only those with strong linear changes without circadian confounds). **[1]** For biomarker development, machine learning techniques identified the best candidates (using VSURF) to predict a level of sleep loss (classification) or time awake (regression). The 'best' five metabolites formed our final biomarker. **[2]** We validated our biomarker in an independent test set for both classification and regression, within and between subjects. **[3]** We assessed biomarker accuracy when classifying lower cut-offs of sleep deprivation (e.g., 22h, 20h etc) and less conservative cut-offs for well-rested (e.g., 18h, 20h). **[4]** We then examined accuracy with a lower, more real-world level of sleep loss (<16h versus 18-24h, i.e., day versus night only). **[5]** As some metabolites may not be useful in future, we re-tested the primary analyses [1] but with all possible metabolite combinations. **[6]** Finally, we checked the 'recovery' of the biomarker with a period of sleep using both circadian time matched AND pre/post sleep.

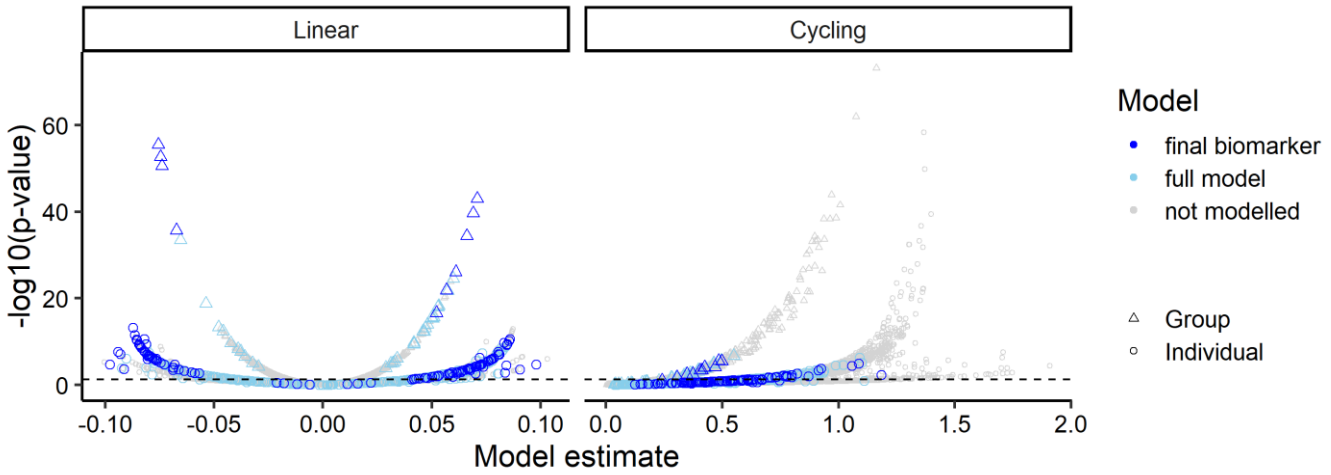

Figure S2. Volcano plots showing group and individual level significance (-log$_{10}$(p-value)) against model estimate (slope for linear models and amplitude for cosinor models) of 929 features detected with hydrophilic interaction liquid chromatography-mass spectrometry (HILIC LC-MS). Symbol colours indicate if features were included in the final biomarker (dark blue), passed the data reduction filter (full model; light blue) or were not modelled (grey). Group models are displayed as triangles and individual models are displayed as circles. Significance at the p = 0.05 is indicated with a dashed line.

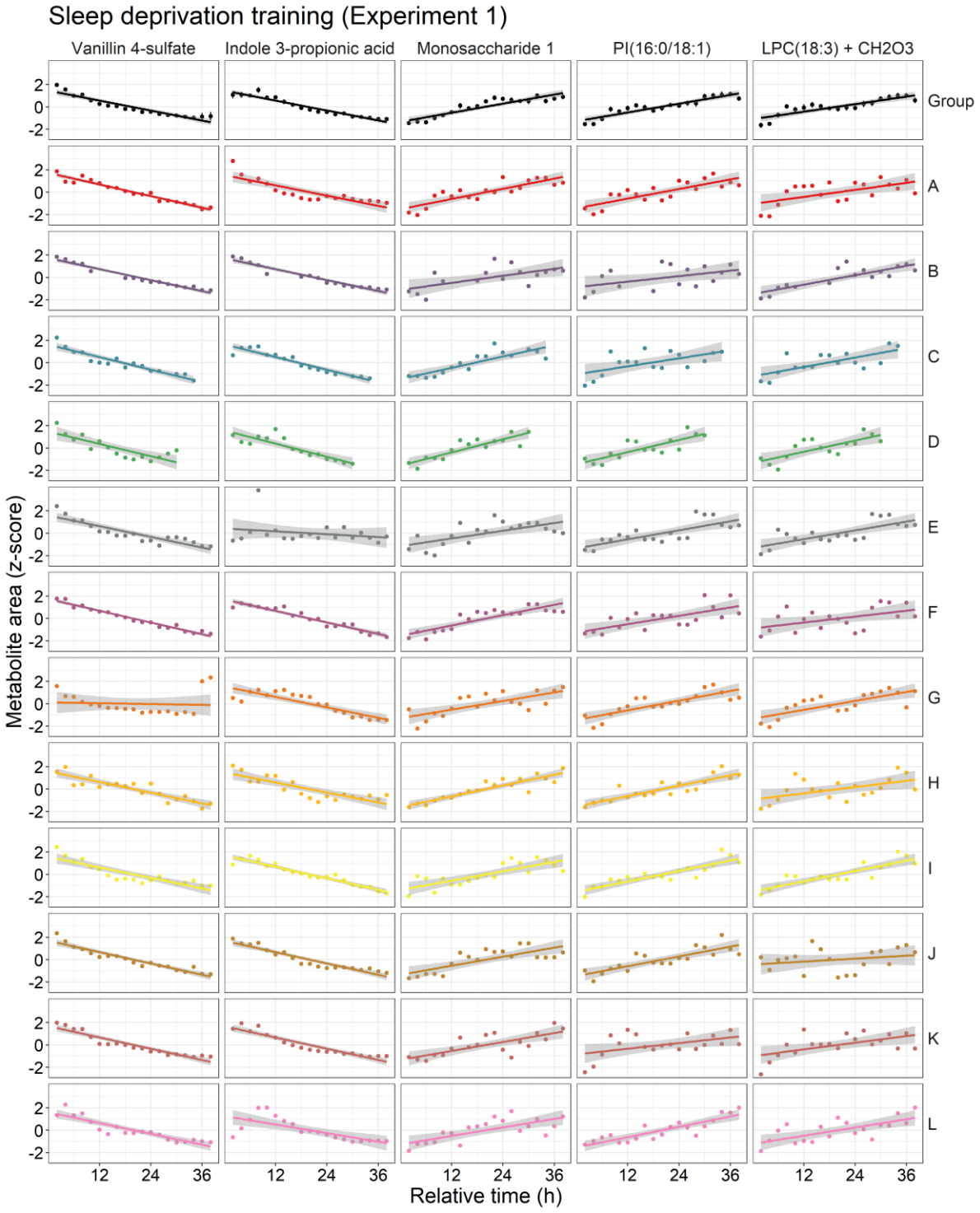

Figure S3. Within-participant group and individual level trends of five consistently important metabolites across time since wake in Experiment 1 (sleep deprivation training). Displaying metabolite peak area (median normalised z-scored within participant) and linear fit with shaded 95% CI for each participant (A-L) and **standard error bars for group points**.

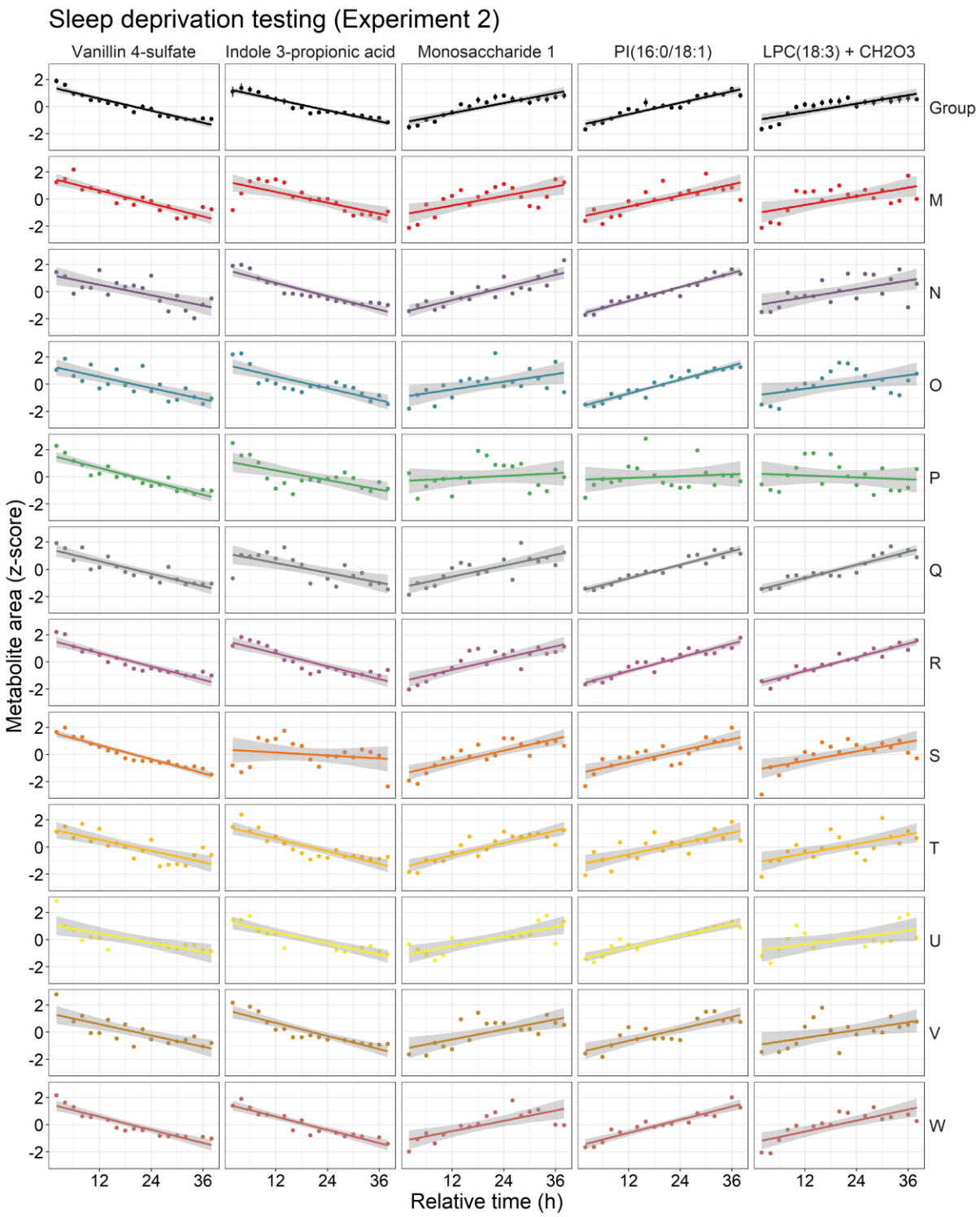

Figure S4. Within-participant group and individual level trends of five consistently important metabolites across time since wake in Experiment 2 (sleep deprivation testing). Displaying metabolite peak area (median normalised z-scored within participant) and linear fit with shaded 95% CI for each participant (M-W) and standard error bars for group points.

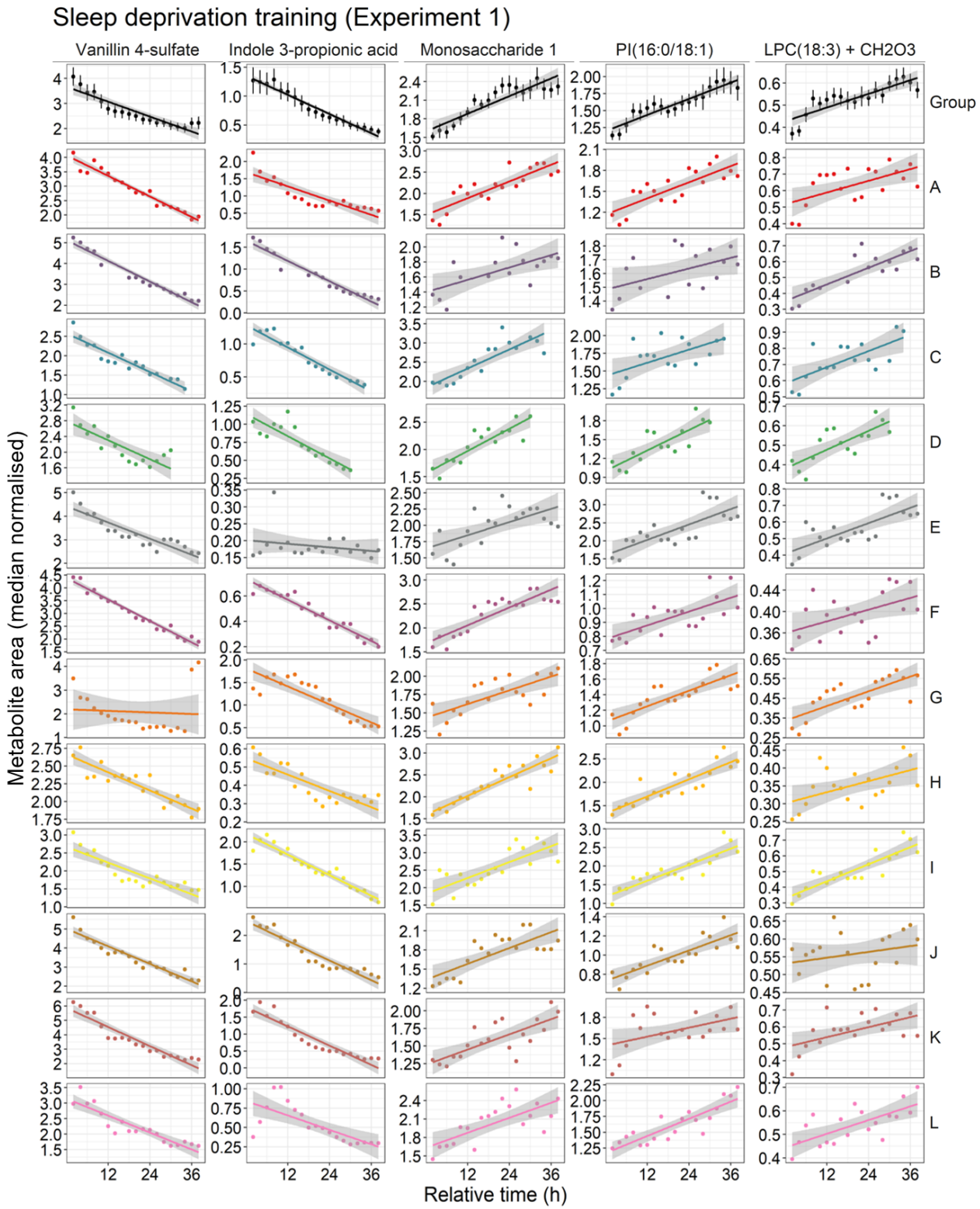

Figure S5. Between-participants group and individual level trends of five consistently important metabolites across time since wake in Experiment 1 (sleep deprivation training). Displaying metabolite peak area (median normalised) and linear fit with shaded 95% CI for each participant (A-L) and standard error bars for group points.

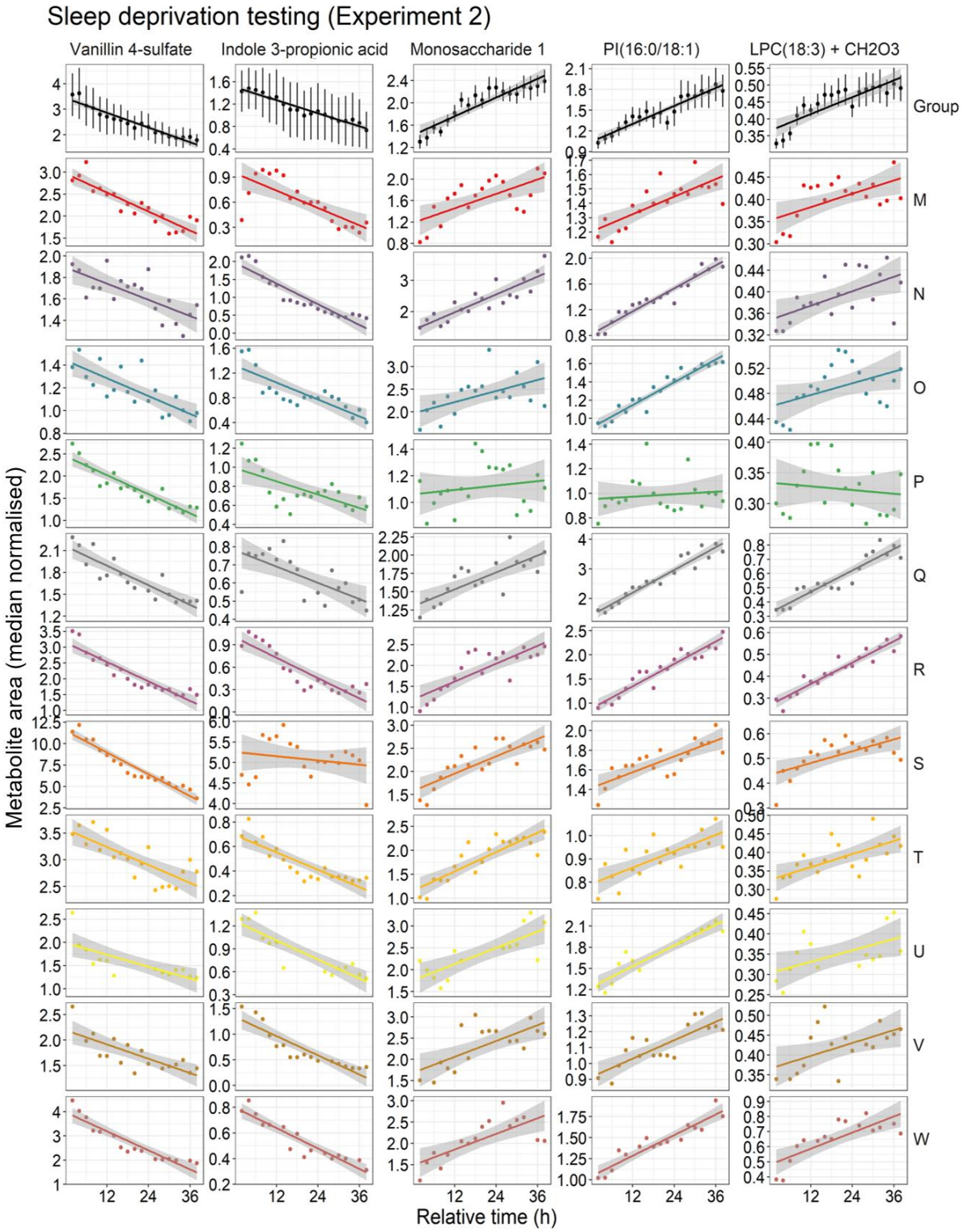

Figure S6. Between-participants group and individual level trends of five consistently important metabolites across time since wake in Experiment 2 (sleep deprivation testing). Displaying metabolite peak area (median normalised) and linear fit with shaded 95% CI for each participant (M-W) and standard error bars for group points.

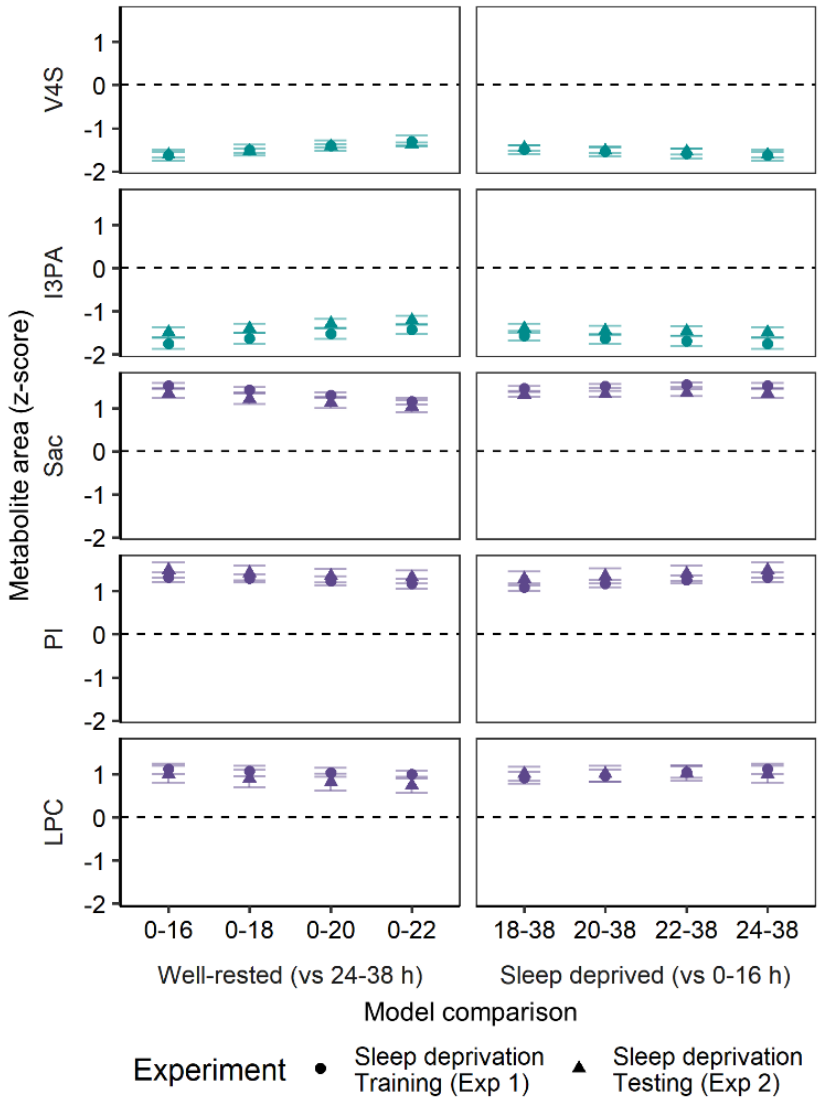

Figure S7. Magnitude of change (expressed as a z-score) in metabolite features as a function of sleep deprivation. *Left Panels:* Relative to sleep deprivation (24-38h), group level changes for varying levels of well-rested ranging from typical well-rested state (0-16h) to less conservative well-rested classifications. *Right Panels*: Relative to well-rested (0-16h), group level changes for varying levels of sleep deprivation thresholds ranging from 18hours and beyond to 24hours and beyond.

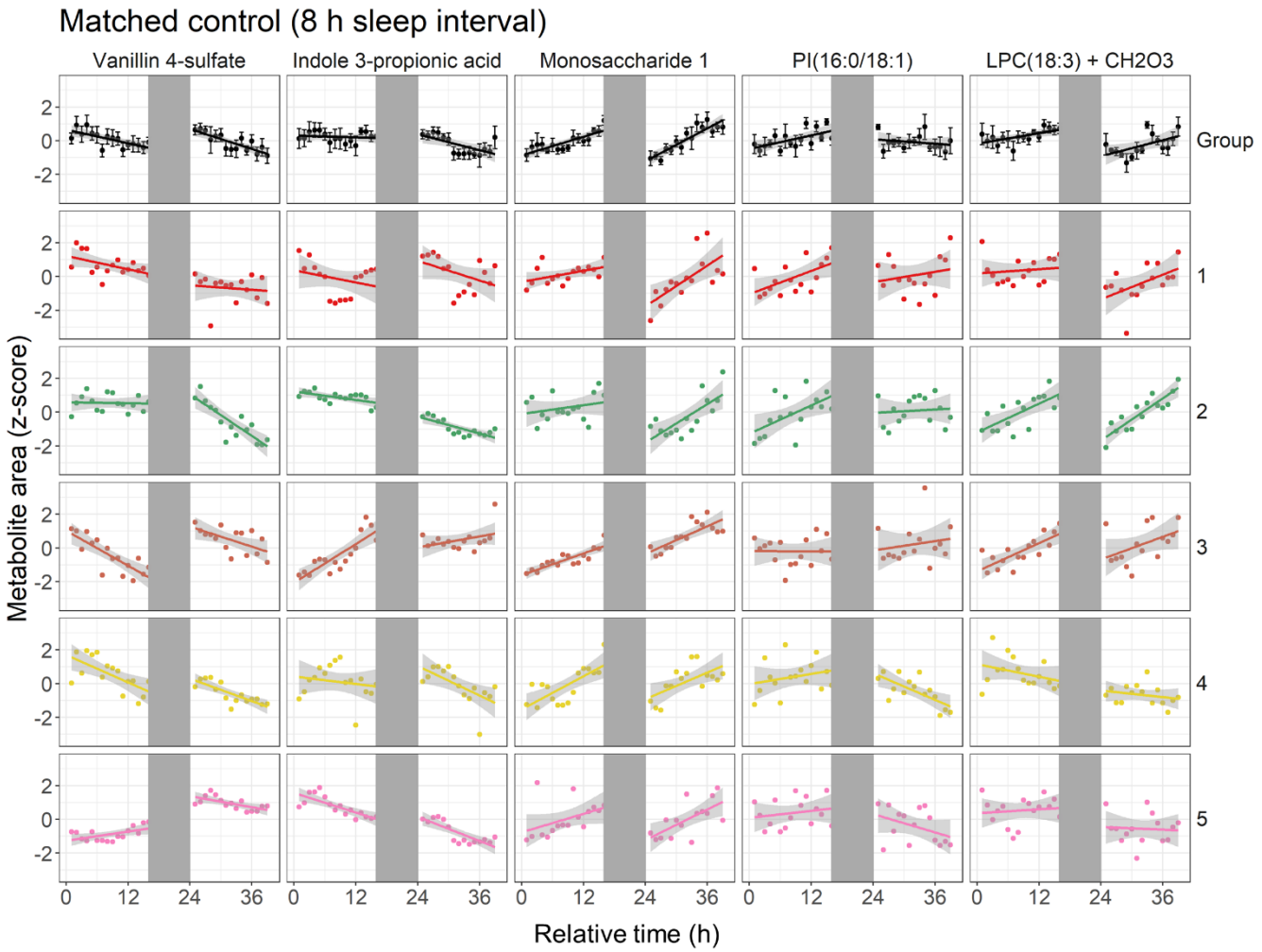

Figure S8. Within-participant group and individual level trends of five consistently important metabolites across time since wake in Matched Control experiment. Displaying metabolite peak area (median normalised z-scored within participant) and linear fit with shaded 95% CI for each participant (1-5) and standard error bars for group points. Grey band from 16 - 24 relative Time (h) indicates an 8 h sleep opportunity.

Table S1.

Filtered HILIC LC-MS features included in predictive modelling. Including mass to charge ratio (m/z) and retention time (RT), calculated formulas, ppm difference between formula and candidate m/z, putative identified name, and level of identification reached as described by the metabolite standards initiative, features in bold were confirmed to level 1 identification using chemical standards.

Putative identified name	Feature name	m/z	Accurate mass	RT (min)	Formula	ppm	Level
Vanillin 4-sulfate	**Unidentified 262**	**230.9955**	**232.0028**	**4.0**	**C8H8SO6**	**-5.9**	**1**
Indole 3-propionate	**Unidentified_181**	**188.0722**	**189.0795**	**7.3**	**C11H11NO2**	**2.5**	**1**
Monosaccharide 1	Unidentified 158	179.0561	180.0633	13.3	C6H12O6	-0.3	3
PI(16:0/18:1)	**Unidentified_906**	**835.5352**	**836.5425**	**3.3**	**C43H81O13P**	**1.2**	**1**
(7S,8S)-DiHODE	Unidentified 394	311.2221	312.2293	4.0	C18H32O4	-2.3	3
4-Pyridoxate	**Unidentified_165**	**182.0458**	**183.0531**	**4.5**	**C8H9NO4**	**-0.2**	**1**
pABA-Glc	Unidentified 368	298.0966	299.1039	13.7	C13H17NO7	11.4	3
LPC(18:3) + CH2O3	**Unidentified_753**	**578.3101**	**579.3174**	**4.1**	**C27H50NO10P**	**0.3**	**1**
Indan-1-ol	Unidentified_86	133.0657	134.0729	3.9	C9H10O	-1.4	2
Mercaptopropanoic acid +FA	Unidentified 112	151.0072	152.0144	12.2	C4H8O4S	-7.9	2
Monosaccharide analogue 1	Unidentified_135	165.0769	166.0841	12.7	C6H14O5	0.2	3
PS(O-20:0/22:6)	Unidentified_919	848.5975	849.6048	3.5	C48H84NO9P	19.3	3
2-Keto-glutamarate	Unidentified_99	144.0303	145.0375	12.8	C5H7NO4	0.1	2
PI(36:1)	Unidentified_934	863.5627	864.5699	3.3	C45H85O13P	3.6	3
DGCC(20:5/20:5)	Unidentified_935	864.5655	865.5728	3.3	C50H77NO8	3.8	2
L-Proline	**X1.Proline**	**114.0562**	**115.0635**	**14.6**	**C5H9NO2**	**0**	**1**
L-Proline C13	Unidentified_58	115.0591	116.0664	14.6	C5H9NO2(13C)	0	2
PE(40:5)	Unidentified_879	792.5442	793.5514	3.5	C45H80NO8P	2.6	3
PE(40:5) C13	Unidentified 880	793.5487	794.5560	3.5	C45H80NO8P(13C)	1.9	3
Monosaccharide 2	Unidentified_222	209.0664	210.0736	13.9	C7H14O7	-1.4	3

Table S2.

Results from regression (predicting time since wake) and classification (predicting clock time matched >24 h awake (0-16 h to 24-38 h awake)) random forest models for within-participant and between-participant analyses. Showing models trained on Experiment 1 (tested Experiment 2) and sanity checks (trained on Experiment 2 and tested on Experiment 1). Displaying the number of features used to build the models (all filtered features (20 or 13[a]), variables selected with the VSURF variable selection tool (various[v]), and the five final biomarker candidates (final[f])). For regression models, variance explained (as R^2) and root mean square error (RMSE) are displayed. For classification models, model accuracy (Lower – Upper 95% CI), Area Under the receiver operating Curve (AUC, Lower – Upper 95% CI), negative and positive prediction accuracy (NPV and PPV%), specificity (SP%) and sensitivity (SN%) are displayed.

Training Experiment	Comparison	Model	Variables (Classification)	Tree depth (Regression)	R^2 (%)	RMSE	Variables (Classification)	Tree depth (Classification)	Accuracy (%) (Lower - Upper)	AUC (%) (Lower - Upper)	SP (%)	SN (%)	NPV (%)	PPV (%)
Experiment 1	Within-participant	Training[a]	20	171	98.6	1.27	20	35	98.4 (95.3 - 99.7)	99.9 (99.7 - 100)	100	96.7	96.8	100
		Testing[a]	20		86.1	4.14	20		95.9 (91.7 - 98.3)	99.4 (98.7 - 100)	96.4	95.3	95.3	96.4
		Training[v]	9	173	98.4	1.35	6	35	97.8 (94.5 - 99.4)	99.8 (99.4 - 100)	98.9	96.7	96.8	98.9
		Testing[v]	9		87.2	3.98	6		96.4 (92.4 - 98.7)	99.4 (98.7 - 100)	97.6	95.3	95.3	97.6
		Training[f]	5	159	98.0	1.52	5	35	97.8 (94.5 - 99.4)	99.7 (99.3 - 100)	98.9	96.7	96.8	98.9
		Testing[f]	5		86.7	4.06	5		94.7 (90.1 - 97.5)	99.2 (98.3 - 100)	96.3	93.1	92.9	96.4
	Between-participants	Training[a]	20	167	96.5	2.03	20	53	95.1 (90.8 - 97.7)	98.6 (97.1 - 100)	96.7	93.5	93.5	96.6
		Testing[a]	20		32.6	9.12	20		72.8 (65.4 - 79.3)	88.4 (83.5 - 93.4)	67.3	83.9	89.4	56.0
		Training[v]	13	165	96.6	2.01	14	53	95.1 (90.8 - 97.7)	98.9 (97.6 - 100)	95.7	94.4	94.6	95.5
		Testing[v]	13		39.3	8.66	14		72.2 (64.8 - 78.8)	90.1 (85.6 - 94.6)	67.0	82.5	88.2	56.0
		Training[f]	5	171	94.0	2.66	5	53	92.9 (88.1 - 96.1)	97.7 (95.8 - 99.6)	94.4	91.3	91.4	94.4
		Testing[f]	5		48.2	8.00	5		79.3 (72.4 - 85.1)	89.1 (84.4 - 93.8)	76.0	83.6	85.9	72.6
Experiment 2	Within-participant	Training[a]	20	157	97.9	1.63	20	33	95.9 (91.7 - 98.3)	99.5 (99 - 100)	96.4	95.3	95.3	96.4
		Testing[a]	20		86.4	4.00	20		96.7 (93 - 98.8)	99.7 (99.3 - 100)	96.8	96.6	96.8	96.6
		Training[v]	14	155	97.8	1.63	6	31	96.4 (92.4 - 98.7)	99 (97.8 - 100)	97.6	95.3	95.3	97.6
		Testing[v]	14		86.8	3.95	6		95.1 (90.8 - 97.7)	99.6 (99.1 - 100)	96.7	93.5	93.5	96.6
		Training[f]	5	145	97.2	1.86	5	31	96.4 (92.4 - 98.7)	99.5 (98.8 - 100)	97.6	95.3	95.3	97.6
		Testing[f]	5		89.6	3.49	5		97.8 (94.5 - 99.4)	99.7 (99.3 - 100)	100	95.7	95.7	100
	Between-participants	Training[a]	13	147	95.8	2.28	13	55	91.7 (86.5 - 95.4)	97.8 (96 - 99.6)	91.8	91.7	91.8	91.7
		Testing[a]	13		46.3	7.95	13		84.6 (78.5 - 89.5)	89.5 (84.4 - 94.7)	83.5	85.9	87.1	82.0
		Training[v]	7	147	95.8	2.29	8	55	91.7 (86.5 - 95.4)	97.9 (96.2 - 99.6)	92.8	90.7	90.6	92.9
		Testing[v]	7		42.5	8.23	8		79.1 (72.5 - 84.8)	88.2 (82.9 - 93.5)	75.7	84.0	87.1	70.8
		Training[f]	5	145	94.4	2.62	5	51	91.1 (85.8 - 94.9)	96.9 (94.6 - 99.2)	93.8	88.8	88.2	94.0
		Testing[f]	5		52.7	7.46	5		80.8 (74.3 - 86.2)	90.7 (86.6 - 94.9)	81.5	80.0	80.6	80.9

Table S3.

Results from sanity check analysis (trained on Experiment 2 and tested on Experiment 1) for group and individual level regression (predicting time since wake) and classification (predicting clock matched >24 h awake (0-16 h to 24-38 h TSW)) random forest models for within-participant and between-participant analyses built with the five final biomarker candidates. For regression models, variance explained (as R^2) and root mean square error (RMSE) are displayed (Tree depth was 145, for both within-participant and between participants regression training models). For classification models, model accuracy (Lower – Upper 95% CI), Area Under the receiver operating Curve (AUC, Lower – Upper 95% CI), negative and positive prediction accuracy (NPV and PPV%), specificity (SP%) and sensitivity (SN%) are displayed (Tree depth was 31 and 51, for within-participant and between participants classification training models, respectively). Note if PPV or NPV are 0 SN and SP cannot be calculated.

Comparison	Model	Participant	Accuracy (%) (Lower - Upper)	AUC (%) (Lower - Upper)	SP (%)	SN (%)	NPV (%)	PPV (%)	R^2 (%)	RMSE
Within-participant	Training	Group	96.4 (92.4 - 98.7)	99.5 (98.8 - 100)	97.6	95.3	95.3	97.6	97.2	1.86
	Testing	Group	97.8 (94.5 - 99.4)	99.7 (99.3 - 100)	100	95.7	95.7	100	89.6	3.49
		A	100 (79.4 - 100)	100 (100 - 100)	100	100	100	100	93.7	2.74
		B	100 (75.3 - 100)	100 (100 - 100)	100	100	100	100	86.1	4.31
		C	100 (75.3 - 100)	100 (100 - 100)	100	100	100	100	86.1	3.65
		D	91.7 (61.5 - 99.8)	100 (100 - 100)	100	80.0	87.5	100	66.6	4.99
		E	100 (79.4 - 100)	100 (100 - 100)	100	100	100	100	86.8	3.98
		F	100 (79.4 - 100)	100 (100 - 100)	100	100	100	100	94.3	2.61
		G	87.5 (61.7 - 98.4)	96.9 (89.6 - 100)	100	80.0	75.0	100	78.4	5.09
		H	100 (79.4 - 100)	100 (100 - 100)	100	100	100	100	95.5	2.33
		I	100 (79.4 - 100)	100 (100 - 100)	100	100	100	100	96.3	2.11
		J	100 (79.4 - 100)	100 (100 - 100)	100	100	100	100	92.3	3.05
		K	93.8 (69.8 - 99.8)	98.4 (94.1 - 100)	100	88.9	87.5	100	89.1	3.62
		L	100 (79.4 - 100)	100 (100 - 100)	100	100	100	100	96.3	2.11
Between-participants	Training	Group	91.1 (85.8 - 94.9)	96.9 (94.6 - 99.2)	93.8	88.8	88.2	94.0	94.4	2.62
	Testing	Group	80.8 (74.3 - 86.2)	90.7 (86.6 - 94.9)	81.5	80.0	80.6	80.9	52.7	7.46
		A	100 (79.4 - 100)	100 (100 - 100)	100	100	100	100	68.5	6.15
		B	69.2 (38.6 - 90.9)	100 (100 - 100)	55.6	100	100	50.0	51.6	8.05
		C	76.9 (46.2 - 95)	100 (100 - 100)	100	62.5	62.5	100	31.6	8.08
		D	91.7 (61.5 - 99.8)	100 (100 - 100)	100	80.0	87.5	100	81.3	3.74
		E	50 (24.7 - 75.3)	92.2 (76.2 - 100)			0.0	100	4.4	10.71
		F	100 (79.4 - 100)	100 (100 - 100)	100	100	100	100	83.0	4.51
		G	68.8 (41.3 - 89)	98.4 (94.1 - 100)	61.5	100	100	37.5	52.5	7.55
		H	87.5 (61.7 - 98.4)	100 (100 - 100)	100	80.0	75.0	100	57.6	7.13
		I	93.8 (69.8 - 99.8)	100 (100 - 100)	100	88.9	87.5	100	70.9	5.91
		J	50 (24.7 - 75.3)	43.8 (12.3 - 75.2)	50.0		100	0.0	-14.5	11.72
		K	87.5 (61.7 - 98.4)	100 (100 - 100)	80.0	100	100	75.0	69.3	6.07
		L	93.8 (69.8 - 99.8)	100 (100 - 100)	100	88.9	87.5	100	69.4	6.06

Table S4.

Testing model results from classification random forests for within and between-participant analyses. Showing models trained on Experiment 1 and tested Experiment 2 and sanity checks (trained on Experiment 2 and tested on Experiment 1). Classification models predicting clock matched >24 h awake were built and tested closing the distinction between well-rested and sleep deprived classification by altering the Time grouping (h). Models were built with the five consistent features across VSURF selected models. Model accuracy (Lower – Upper 95% CI), Area Under the receiver operating Curve (AUC, Lower – Upper 95% CI), negative and positive prediction accuracy (NPV and PPV%), specificity (SP%) and sensitivity (SN%) are displayed. For the training models tested here, tree depth ranged from 27-73 nodes).

Training Experiment	Comparison	Time grouping (h)	Accuracy (%) (Lower - Upper)	AUC (%) (Lower - Upper)	SP (%)	SN (%)	NPV (%)	PPV (%)
Experiment 1	Within-participant	0-16 vs 18-38	92.4 (87.8 - 95.7)	98 (96.6 - 99.4)	91.7	93.0	90.6	93.8
		0-16 vs 20-38	93.6 (89.1 - 96.7)	98.9 (98.1 - 99.8)	92.9	94.2	92.9	94.2
		0-16 vs 22-38	93.8 (89.2 - 96.9)	98.8 (97.9 - 99.8)	93.0	94.6	94.1	93.5
		0-18 vs 24-38	96.6 (92.8 - 98.8)	99.1 (98.1 - 100)	98.9	94.3	94.7	98.8
		0-20 vs 24-38	92.1 (87.2 - 95.5)	97.9 (96.3 - 99.5)	95.0	88.8	90.5	94.0
		0-23 vs 24-38	90.4 (85.4 - 94.1)	96.5 (94.2 - 98.7)	90.6	90.1	93.0	86.9
	Between-participants	0-16 vs 18-38	70.2 (63.3 - 76.5)	84.7 (79.4 - 90)	64.8	74.5	67.1	72.6
		0-16 vs 20-38	72.3 (65.4 - 78.6)	85.8 (80.6 - 91)	67.7	76.8	74.1	70.9
		0-16 vs 22-38	75.3 (68.3 - 81.4)	87.6 (82.7 - 92.5)	71.1	80.2	81.2	69.9
		0-18 vs 24-38	78.2 (71.4 - 84)	88.4 (83.7 - 93.1)	76.9	80.0	84.2	71.4
		0-20 vs 24-38	77.2 (70.6 - 83)	88.1 (83.5 - 92.7)	78.7	75.3	81.0	72.6
		0-23 vs 24-38	76.8 (70.3 - 82.5)	87.4 (82.8 - 92.1)	79.3	73.2	80.7	71.4
Experiment 2	Within-participant	0-16 vs 18-38	93.6 (89.5 - 96.4)	98.7 (97.6 - 99.8)	92.5	94.4	92.5	94.4
		0-16 vs 20-38	96.1 (92.5 - 98.3)	99.5 (98.8 - 100)	96.7	95.7	94.6	97.3
		0-16 vs 22-38	96.9 (93.4 - 98.9)	99.7 (99.2 - 100)	98.9	95.2	94.6	99.0
		0-18 vs 24-38	95.9 (92 - 98.2)	99.3 (98.6 - 100)	98.0	93.5	94.3	97.8
		0-20 vs 24-38	93.7 (89.5 - 96.6)	98.9 (98 - 99.8)	95.6	91.3	93.2	94.4
		0-23 vs 24-38	90.4 (85.7 - 93.9)	96.6 (94.7 - 98.6)	92.9	87.0	90.7	89.9
	Between-participants	0-16 vs 18-38	76.1 (69.9 - 81.6)	86.7 (82.2 - 91.3)	72.0	79.2	72.0	79.2
		0-16 vs 20-38	77.2 (70.8 - 82.7)	88.3 (84 - 92.7)	75.0	78.9	74.2	79.6
		0-16 vs 22-38	77.8 (71.3 - 83.5)	89.6 (85.4 - 93.8)	77.2	78.4	76.3	79.2
		0-18 vs 24-38	78.4 (71.9 - 83.9)	89 (84.4 - 93.6)	79.4	77.0	81.0	75.3
		0-20 vs 24-38	77.7 (71.4 - 83.2)	86 (81 - 91.1)	78.9	75.9	82.9	70.8
		0-23 vs 24-38	77.5 (71.4 - 82.9)	84.6 (79.3 - 89.9)	80.3	73.3	82.2	70.8

Table S5.

Testing model results from classification random forest models for within- and between-participants analyses. Showing models trained on Experiment 1 and tested Experiment 2 and sanity checks (trained on Experiment 2 and tested on Experiment 1). Classification models predicting well-rested (0-16 h TSW) vs stepwise biological night classifications (time grouping) for the sleep deprived group. Models were built with the five consistent variables across VSURF selected models). Model accuracy (Lower – Upper 95% CI), Area Under the receiver operating Curve (AUC, Lower – Upper 95% CI), negative and positive prediction accuracy (NPV and PPV%), specificity (SP%) and sensitivity (SN%) are displayed. For the training models tested here, tree depth ranged from 19 to 57 nodes).

Training Experiment	Comparison	Time grouping (h)	Accuracy (%) (Lower - Upper)	AUC (%) (Lower - Upper)	SP (%)	SN (%)	NPV (%)	PPV (%)
Experiment 1	Within-participant	0-16 vs 18-24	84.6 (76.9 - 90.4)	95.1 (91.8 - 98.4)	89.3	74.4	88.2	76.3
		0-16 vs 20-24	91.2 (84.3 - 95.7)	97.5 (95.3 - 99.8)	94.1	82.1	94.1	82.1
		0-16 vs 22-24	93.2 (86.5 - 97.2)	97.6 (95.2 - 100)	95.3	82.4	96.5	77.8
		0-16 vs 24	96.8 (91 - 99.3)	98.7 (96.8 - 100)	97.7	87.5	98.8	77.8
	Between-participants	0-16 vs 18-24	69.1 (60.1 - 77.1)	76.9 (68.2 - 85.5)	81.3	50.0	71.8	63.2
		0-16 vs 20-24	73.5 (64.3 - 81.3)	79.1 (70.1 - 88.1)	84.8	47.1	78.8	57.1
		0-16 vs 22-24	78.6 (69.5 - 86.1)	80.2 (69.6 - 90.7)	89.9	41.7	83.5	55.6
		0-16 vs 24	80.9 (71.4 - 88.2)	83.5 (72.2 - 94.7)	94.7	26.3	83.5	55.6
Experiment 2	Within-participant	0-16 vs 18-24	89.4 (83.1 - 93.9)	96.9 (94.4 - 99.4)	89.0	90.2	95.7	77.1
		0-16 vs 20-24	93 (87.2 - 96.8)	98.5 (96.9 - 100)	92.9	93.5	97.8	80.6
		0-16 vs 22-24	93.2 (87 - 97)	98.5 (96.9 - 100)	93.8	90.0	97.8	75.0
		0-16 vs 24	94.3 (88 - 97.9)	99.3 (98.1 - 100)	94.8	87.5	98.9	58.3
	Between-participants	0-16 vs 18-24	74.5 (66.4 - 81.4)	78.4 (70.2 - 86.7)	80.6	62.5	80.6	62.5
		0-16 vs 20-24	79.8 (71.9 - 86.4)	80.7 (71.8 - 89.7)	84.5	65.6	88.2	58.3
		0-16 vs 22-24	86.3 (78.7 - 92)	82.2 (72.3 - 92.1)	90.5	68.2	92.5	62.5
		0-16 vs 24	93.3 (86.7 - 97.3)	81.7 (65.2 - 98.3)	94.8	77.8	97.8	58.3

Table S6.

Results from regression (predicting time since wake) and classification (predicting clock matched >24 h awake (0-16 h to 24-38 h awake)) random forest models for within-participant and between-participant analyses using subset variables from the final biomarker. Showing models trained on Experiment 1 (tested Experiment 2). Displaying the number of features used to build the models. For classification models, model accuracy (Lower – Upper 95% CI), Area Under the receiver operating Curve (AUC, Lower – Upper 95% CI), negative and positive prediction accuracy (NPV and PPV%), specificity (SP%) sensitivity (SN%) and Bonferroni corrected p-values are displayed. For regression models, variance explained (as R^2) and root mean squared error (RMSE) are displayed. Metabolites are abbreviated as: V4S: vanillin 4-sulfate, I3PA: indole 3-propionate, Sac: monosaccharide 1, PI: PI(16:0/18:1) and LPC: LPC(18:3). For the training models tested here, tree depth ranged from 25 to 101 nodes).

Comparison	Reduced candidates	Variables	Accuracy (%) (Lower - Upper)	AUC (%) (Lower - Upper)	SP (%)	SN (%)	NPV (%)	PPV (%)	adjusted p-value	R^2 (%)	RMSE
Within-participant	Final biomarker	5	94.7% (90.1 - 97.5)	99.2 (98.3 - 100)	96.3	93.1	92.9	96.4	<0.001	86.3	4.1
	No LPC	4	96.4% (92.4 - 98.7)	99.1 (98.2 - 100)	96.5	96.4	96.5	96.4	<0.001	84.6	4.4
	No PI	4	96.4% (92.4 - 98.7)	99.2 (98.4 - 100)	97.6	95.3	95.3	97.6	<0.001	88.1	3.8
	No Sac	4	92.3% (87.2 - 95.8)	98.3 (97 - 99.6)	91.9	92.8	92.9	91.7	<0.001	83.1	4.6
	No I3PA	4	97.6% (94.1 - 99.4)	99.8 (99.4 - 100)	97.6	97.6	97.6	97.6	<0.001	84.8	4.3
	No V4S	4	89.3% (83.7 - 93.6)	97.7 (96.2 - 99.3)	92.4	86.7	85.9	92.9	<0.001	80.2	4.9
	No LPC, No PI	3	96.4% (92.4 - 98.7)	99.3 (98.3 - 100)	96.5	96.4	96.5	96.4	<0.001	84.9	4.3
	No Sac, No LPC	3	92.9% (87.9 - 96.3)	98.4 (97.1 - 99.7)	92.0	93.9	94.1	91.7	<0.001	83.3	4.5
	No Sac, No PI	3	92.9% (87.9 - 96.3)	97.6 (95.7 - 99.4)	92.9	92.9	92.9	92.9	<0.001	80.0	5.0
	No Sac, No I3PA	3	95.3% (90.9 - 97.9)	98.7 (97.4 - 100)	93.3	97.5	97.6	92.9	<0.001	80.7	4.9
	No Sac, No V4S	3	87.6% (81.6 - 92.1)	95.5 (92.7 - 98.3)	86.4	88.9	89.4	85.7	<0.001	75.6	5.5
	No I3PA, No LPC	3	97.6% (94.1 - 99.4)	99.8 (99.6 - 100)	96.6	98.8	98.8	96.4	<0.001	86.4	4.1
	No I3PA, No PI	3	95.9% (91.7 - 98.3)	99.4 (98.8 - 100)	95.3	96.4	96.5	95.2	<0.001	82.0	4.7
	No V4S, No LPC	3	89.9% (84.4 - 94)	97.8 (96.2 - 99.4)	92.5	87.6	87.1	92.9	<0.001	81.8	4.7
	No V4S, No PI	3	91.1% (85.8 - 94.9)	97.2 (95.3 - 99.1)	93.8	88.8	88.2	94.0	<0.001	75.7	5.5
	No I3PA, No VS4	3	85.8% (79.6 - 90.7)	92.5 (88.6 - 96.4)	88.6	83.3	82.4	89.3	<0.001	68.2	6.3
	Only I3PA and V4S	2	89.9% (84.4 - 94)	96.6 (94.2 - 99)	92.5	87.6	87.1	92.9	<0.001	75.0	5.6
	Only V4S and PI	2	93.5% (88.7 - 96.7)	98.8 (97.8 - 99.9)	92.0	95.1	95.3	91.7	<0.001	80.6	4.9
	Only IPA and PI	2	88.8% (83 - 93.1)	94.8 (91.4 - 98.2)	88.4	89.2	89.4	88.1	<0.001	75.9	5.5
	Only Sac and PI	2	82.2% (75.6 - 87.7)	92.2 (88.4 - 96)	84.0	80.7	80.0	84.5	<0.001	68.8	6.2
	Only LPC and PI	2	78.7% (71.7 - 84.6)	87.9 (83 - 92.8)	81.0	76.7	75.3	82.1	<0.001	56.5	7.3
	Only Sac and V4S	2	96.4% (92.4 - 98.7)	99.4 (98.6 - 100)	95.4	97.6	97.6	95.2	<0.001	81.9	4.7
	Only LPC and VS4	2	92.3% (87.2 - 95.8)	97.3 (95.2 - 99.5)	89.1	96.1	96.5	88.1	<0.001	76.0	5.4
	Only Sac and I3PA	2	92.9% (87.9 - 96.3)	97.3 (95.4 - 99.3)	95.1	90.9	90.6	95.2	<0.001	74.9	5.6
	Only I3PA and LPC	2	83.4% (77 - 88.7)	92.8 (89 - 96.6)	82.0	85.0	85.9	81.0	<0.001	65.0	6.6
	Only Sac and LPC	2	83.4% (77 - 88.7)	90.3 (85.5 - 95)	87.0	80.4	78.8	88.1	<0.001	53.3	7.6
Between-participants	Final biomarker	5	79.3% (72.4 - 85.1)	89.1 (84.4 - 93.8)	76.0	83.6	85.9	72.6	<0.001	45.8	8.2
	No LPC	4	78.1% (71.1 - 84.1)	89.4 (84.8 - 94)	75.5	81.3	83.5	72.6	<0.001	47.0	8.1
	No PI	4	76.3% (69.2 - 82.5)	89.9 (85.5 - 94.4)	79.2	73.9	71.8	81.0	<0.001	47.3	8.1
	No Sac	4	75.7% (68.6 - 82)	84.3 (78.4 - 90.3)	75.6	75.9	76.5	75.0	<0.001	39.6	8.6
	No I3PA	4	72.8% (65.4 - 79.3)	84.7 (79.1 - 90.4)	68.9	78.8	83.5	61.9	0.044	38.1	8.7
	No V4S	4	79.9% (73 - 85.6)	86.6 (81 - 92.3)	75.2	86.8	89.4	70.2	<0.001	33.6	9.1
	No LPC, No PI	3	81.1% (74.3 - 86.7)	90.3 (85.8 - 94.7)	86.3	77.1	74.1	88.1	<0.001	46.4	8.1
	No Sac, No LPC	3	73.4% (66 - 79.9)	85.2 (79 - 91.5)	81.2	68.6	61.2	85.7	0.071	30.6	9.3
	No Sac, No PI	3	82.2% (75.6 - 87.7)	85.9 (80.1 - 91.8)	84.0	80.7	80.0	84.5	<0.001	35.5	8.9
	No Sac, No I3PA	3	66.9% (59.2 - 73.9)	73.5 (66 - 80.9)	69.3	64.9	61.2	72.6	0.098	19.7	10.0
	No Sac, No V4S	3	69.8% (62.3 - 76.6)	83.8 (77.8 - 89.9)	64.9	80.0	87.1	52.4	1	24.3	9.7
	No I3PA, No LPC	3	74% (66.7 - 80.4)	81.2 (74.8 - 87.6)	75.3	72.7	71.8	76.2	<0.001	30.0	9.3

Comparison	Reduced candidates	Variables	Accuracy (%) (Lower - Upper)	AUC (%) (Lower - Upper)	SP (%)	SN (%)	NPV (%)	PPV (%)	adjusted p-value	R^2 (%)	RMSE
	No I3PA, No PI	3	72.8% (65.4 - 79.3)	83.6 (77.7 - 89.5)	69.3	77.9	82.4	63.1	0.015	34.1	9.0
	No V4S, No LPC	3	79.3% (72.4 - 85.1)	88.1 (83.1 - 93)	78.4	80.2	81.2	77.4	<0.001	39.2	8.7
	No V4S, No PI	3	77.5% (70.5 - 83.6)	87.8 (82.5 - 93.2)	72.8	84.8	88.2	66.7	<0.001	26.8	9.5
	No I3PA, No VS4	3	75.1% (67.9 - 81.5)	83.8 (77.6 - 89.9)	69.0	87.5	91.8	58.3	0.638	22.3	9.8
	Only I3PA and V4S	2	74% (66.7 - 80.4)	84.1 (77.5 - 90.8)	84.7	68.2	58.8	89.3	0.445	19.9	10.0
	Only V4S and PI	2	62.7% (55 - 70)	71.5 (63.8 - 79.1)	66.2	60.4	52.9	72.6	1	-11.8	11.8
	Only IPA and PI	2	69.8% (62.3 - 76.6)	78.9 (72.2 - 85.7)	71.8	68.1	65.9	73.8	0.001	22.6	9.8
	Only Sac and PI	2	71.6% (64.2 - 78.3)	80.6 (74.1 - 87.2)	69.1	75.0	78.8	64.3	0.005	21.3	9.9
	Only LPC and PI	2	65.1% (57.4 - 72.2)	73.2 (65.7 - 80.7)	60.7	76.6	87.1	42.9	1	3.8	10.9
	Only Sac and V4S	2	71.6% (64.2 - 78.3)	78.4 (71.7 - 85.2)	77.6	67.6	61.2	82.1	0.079	27.0	9.5
	Only LPC and VS4	2	64.5% (56.8 - 71.7)	70.6 (62.8 - 78.5)	67.6	62.2	56.5	72.6	1	-0.4	11.1
	Only Sac and I3PA	2	77.5% (70.5 - 83.6)	86.3 (81 - 91.7)	75.3	80.3	82.4	72.6	<0.001	35.1	9.0
	Only I3PA and LPC	2	74% (66.7 - 80.4)	78.2 (71 - 85.4)	68.5	84.5	89.4	58.3	0.677	5.7	10.8
	Only Sac and LPC	2	70.4% (62.9 - 77.2)	79.9 (73.1 - 86.8)	65.8	79.3	85.9	54.8	1	9.3	10.6

Table S7.

Statistical results from individual t-tests comparing a 4 h block pre- and post- habitual sleep interval for clock time matched (2-6 h day 2 vs 2-6 h day 3) and evening/morning (12-16 h day 2 vs 2-6 h day 3) in the Matched Control experiment (5 participants n = 3 per treatment).

		Clock time Matched					Evening/Morning				
Metabolite	Participant	t-value	df	mean difference	p-value (fdr adjusted)	Trend	t-value	df	mean difference	p-value (fdr adjusted)	Trend
Vanillin 4-sulfate	1	2.7	2.9	-2.6	0.186	Not significant	1.7	2.1	-1.5	0.376	Not significant
	2	0.4	3.3	-0.3	0.809	Not significant	0.1	3.0	-0.1	0.969	Not significant
	3	-1.0	4.0	0.2	0.510	Not significant	-8.6	4.0	2.0	**0.021**	Increasing
	4	19.4	2.3	-1.7	**0.021**	Decreasing	-1.2	2.1	0.5	0.508	Not significant
	5	-8.9	3.7	2.4	**0.021**	Increasing	-6.2	4.0	1.8	**0.044**	Increasing
Indole 3-propionate	1	-2.5	3.8	0.7	0.183	Not significant	-3.1	3.4	0.8	0.163	Not significant
	2	5.0	3.8	-1.7	0.071	Not significant	3.7	3.9	-1.3	0.092	Not significant
	3	-5.1	3.0	1.3	0.071	Not significant	0.7	2.2	-0.4	0.661	Not significant
	4	-1.7	3.3	0.7	0.335	Not significant	-2.3	2.3	1.8	0.249	Not significant
	5	5.6	4.0	-1.4	0.051	Not significant	1.2	2.4	-0.2	0.505	Not significant
Monosaccharide 1	1	-1.0	2.3	0.6	0.510	Not significant	1.0	3.7	-0.9	0.510	Not significant
	2	-1.0	3.9	0.5	0.510	Not significant	1.8	2.1	-0.6	0.352	Not significant
	3	4.4	3.9	-0.7	0.071	Not significant	-0.6	2.6	0.2	0.703	Not significant
	4	-0.6	3.9	0.3	0.703	Not significant	0.5	4.0	-0.3	0.758	Not significant
	5	0.8	2.9	-0.5	0.615	Not significant	1.0	2.5	-0.6	0.510	Not significant
PI(16:0/18:1)	1	1.8	2.4	-0.9	0.335	Not significant	4.4	3.1	-1.3	0.092	Not significant
	2	3.1	3.4	-1.6	0.163	Not significant	2.7	3.1	-1.5	0.183	Not significant
	3	-3.2	2.8	1.1	0.183	Not significant	0.1	4.0	-0.1	0.969	Not significant
	4	-0.1	2.8	0.0	0.969	Not significant	1.9	3.9	-1.6	0.249	Not significant
	5	-1.1	2.3	0.4	0.510	Not significant	1.4	4.0	-0.8	0.376	Not significant
LPC(18:3)+CH2O3	1	2.6	3.2	-0.7	0.183	Not significant	2.4	2.2	-1.4	0.249	Not significant
	2	0.1	3.6	0.0	0.969	Not significant	4.8	3.4	-1.8	0.071	Not significant
	3	0.0	3.8	0.0	0.969	Not significant	2.1	3.4	-1.4	0.249	Not significant
	4	2.9	2.9	-1.7	0.183	Not significant	2.6	3.4	-0.8	0.183	Not significant
	5	2.1	3.0	-1.0	0.249	Not significant	4.4	3.9	-1.3	0.071	Not significant

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