

Supporting Information

Bidirectional effect of long-term Δ^9 -tetrahydrocannabinol-treatment on mTOR activity and metabolome

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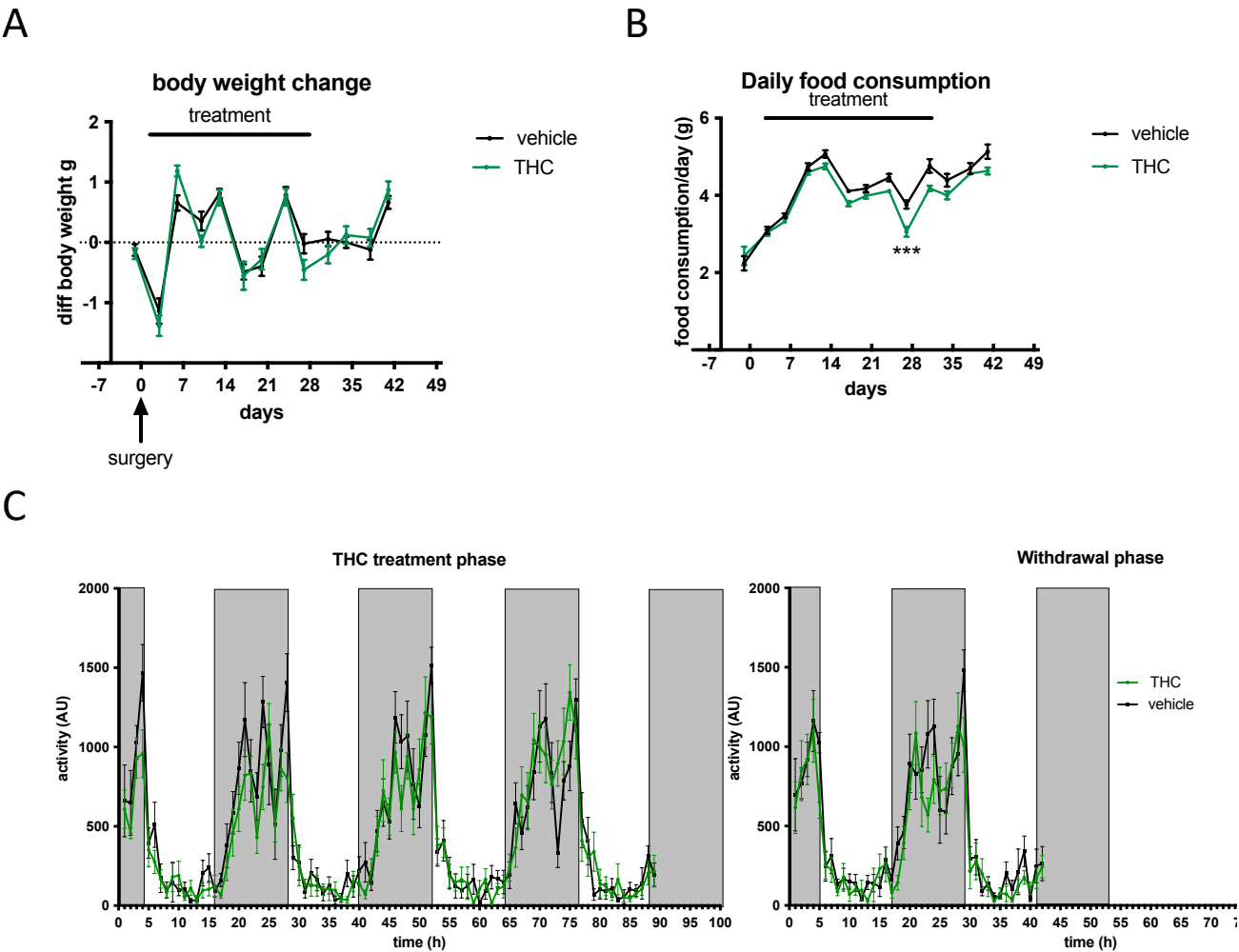
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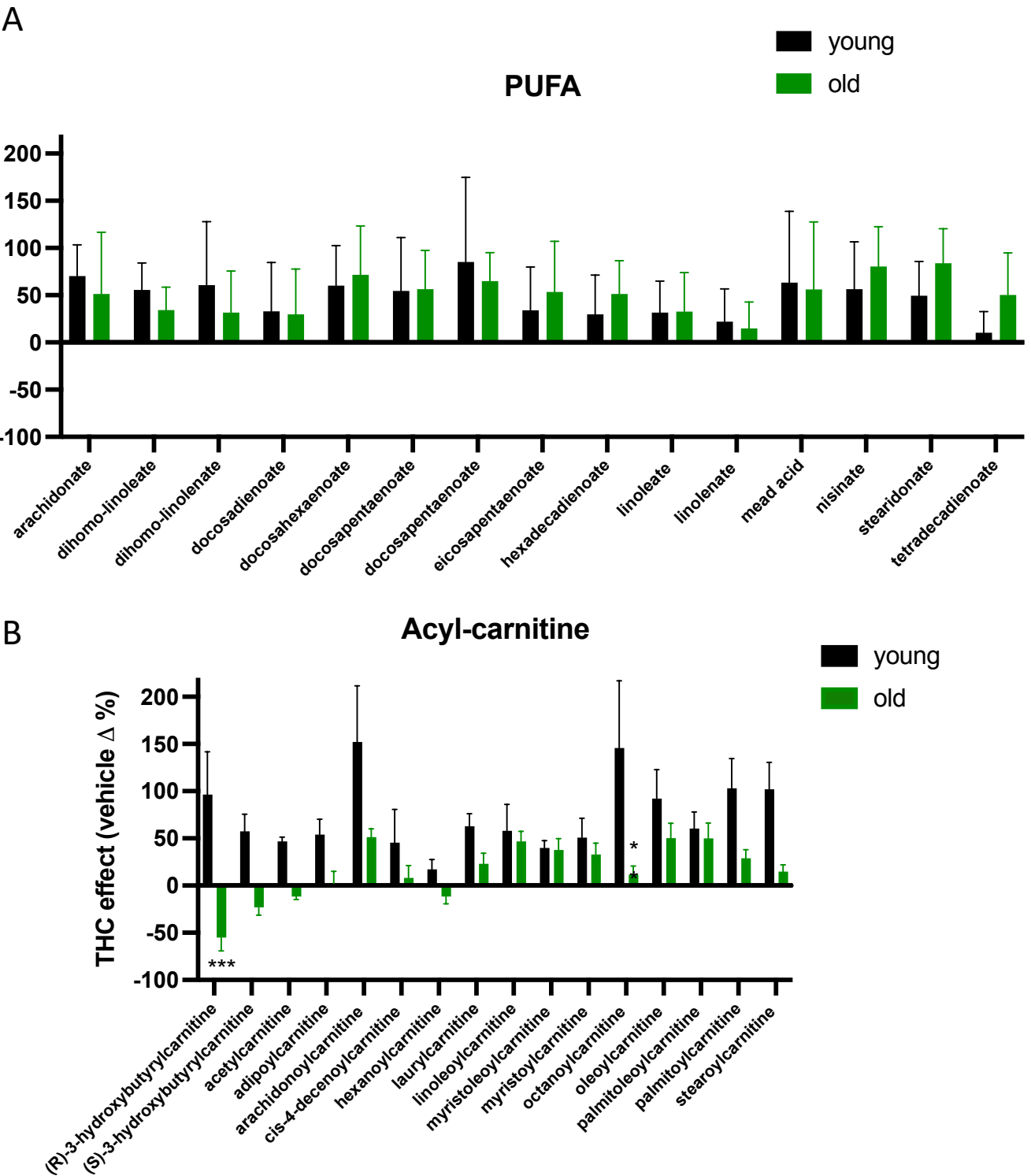
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Figure S1



Long-term, low dose THC-treatment did not influence **(A)** body weight change and **(B)** only slightly reduced food intake – significantly at day 28. **(C)** No change in the home cage activity or in the diurnal cycle at the end of the THC-treatment or at the end of the withdrawal phase. Dots represent mean values, whiskers standard error of mean for panels A-C. *** $p < 0.001$ according to two-way ANOVA followed by Bonferroni t-test for panel B.

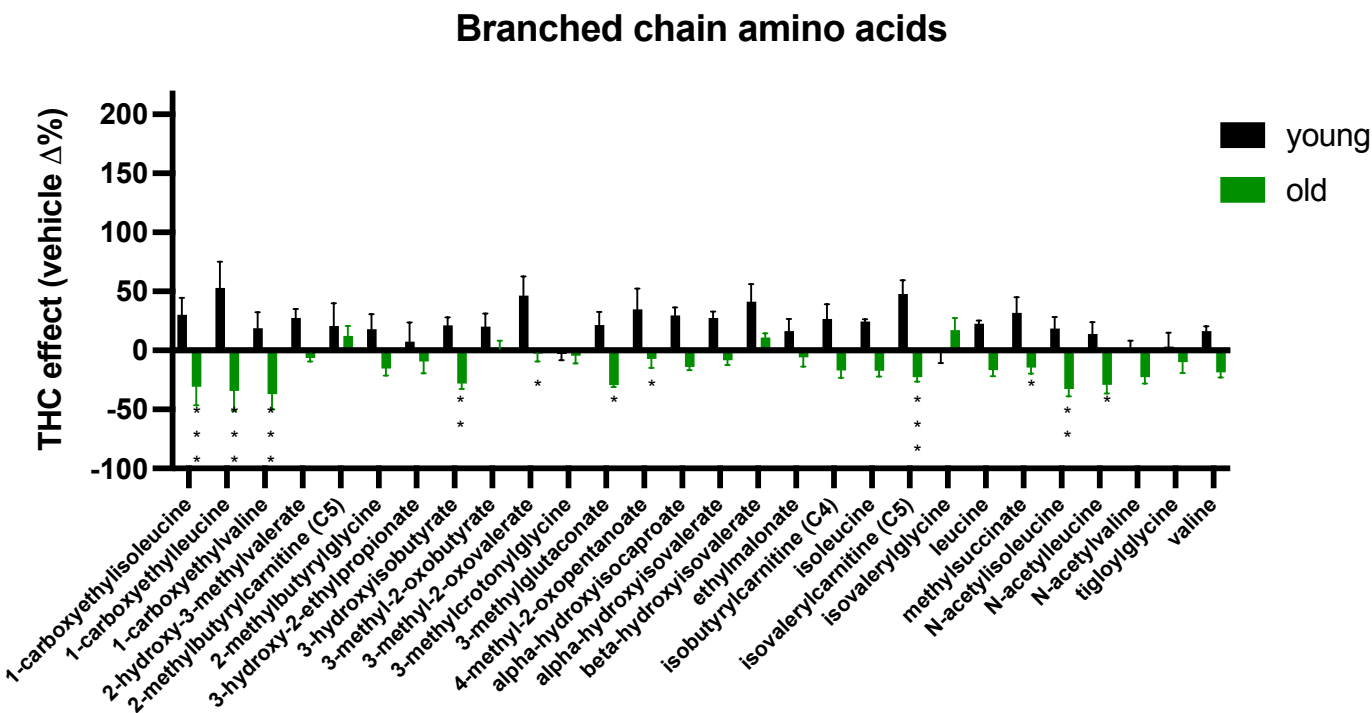
Figure S2



Similar effect of THC-treatment on **(A)** polyunsaturated fatty acid (PUFA) metabolite levels in the blood plasma of young (4-month-old) and old (18-month-old) animals at day 28. **(B)** Generally higher blood plasma levels of acyl-carnitine metabolites in THC-treated young than in old mice with the same treatment at day 28. Columns represent mean values, whiskers standard error. ** $p < 0.01$; *** $p < 0.001$ significant difference between the age-groups according two-way ANOVA followed by Bonferroni t-test.

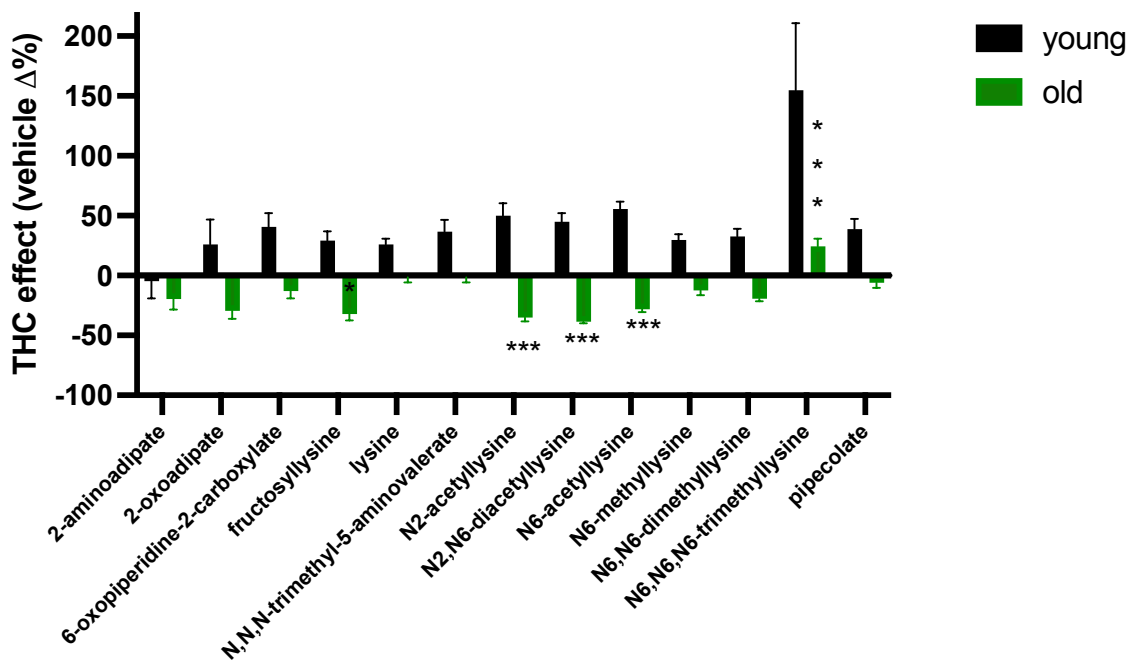
Figure S3

A



B

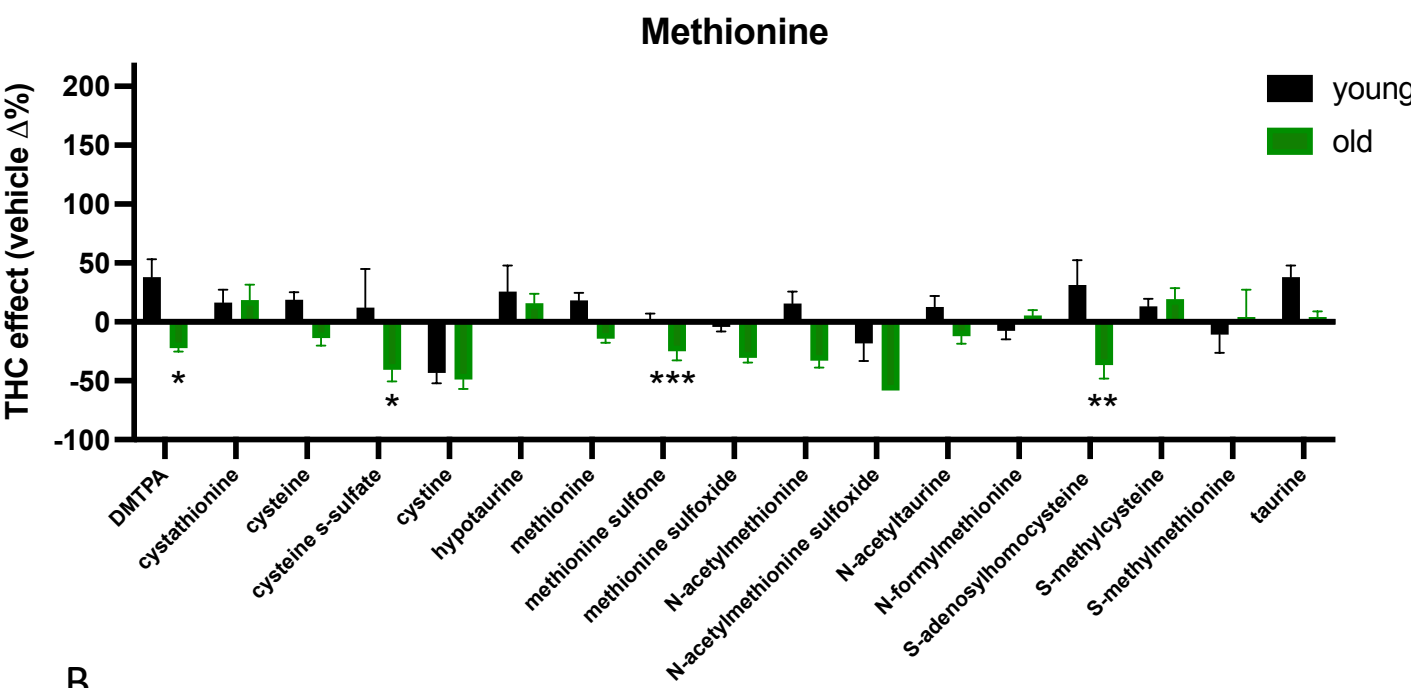
Lysine



Dominant opposite change in **(A)** branched chain amino acid and **(B)** lysine metabolite levels in the blood plasma of THC-treated young (4-month-old) and old (18-month-old) animals at day 28. Columns represent mean values, whiskers standard error. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ significant difference in the effect of THC on the metabolite levels between young (4-month-old) and old (18-month-old) animals according two-way ANOVA followed by Bonferroni t-test.

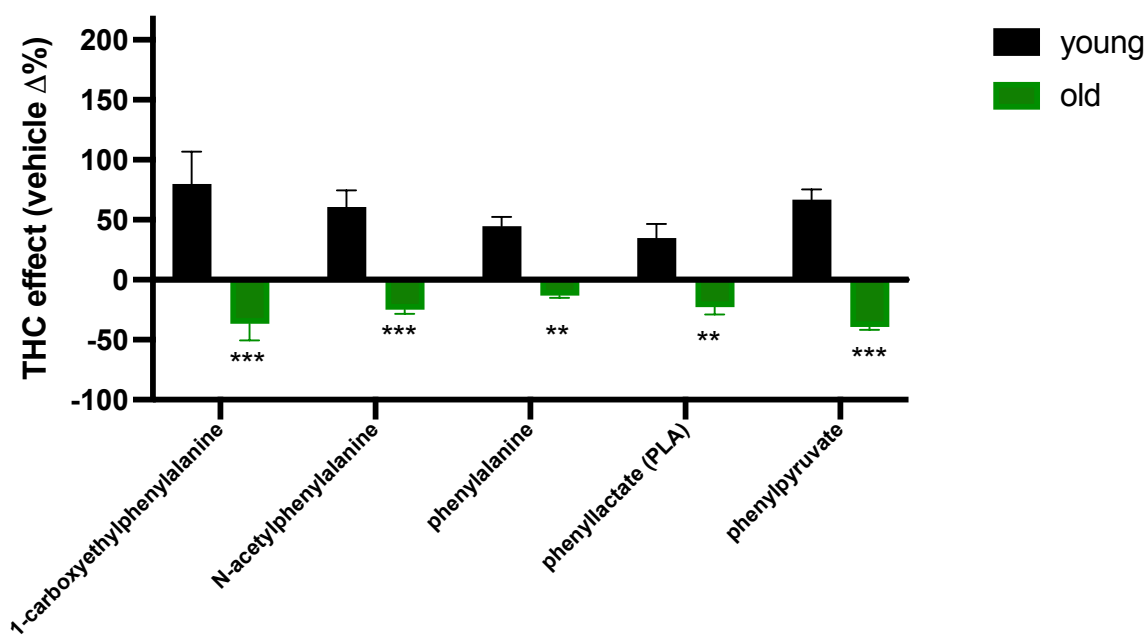
Figure S4

A



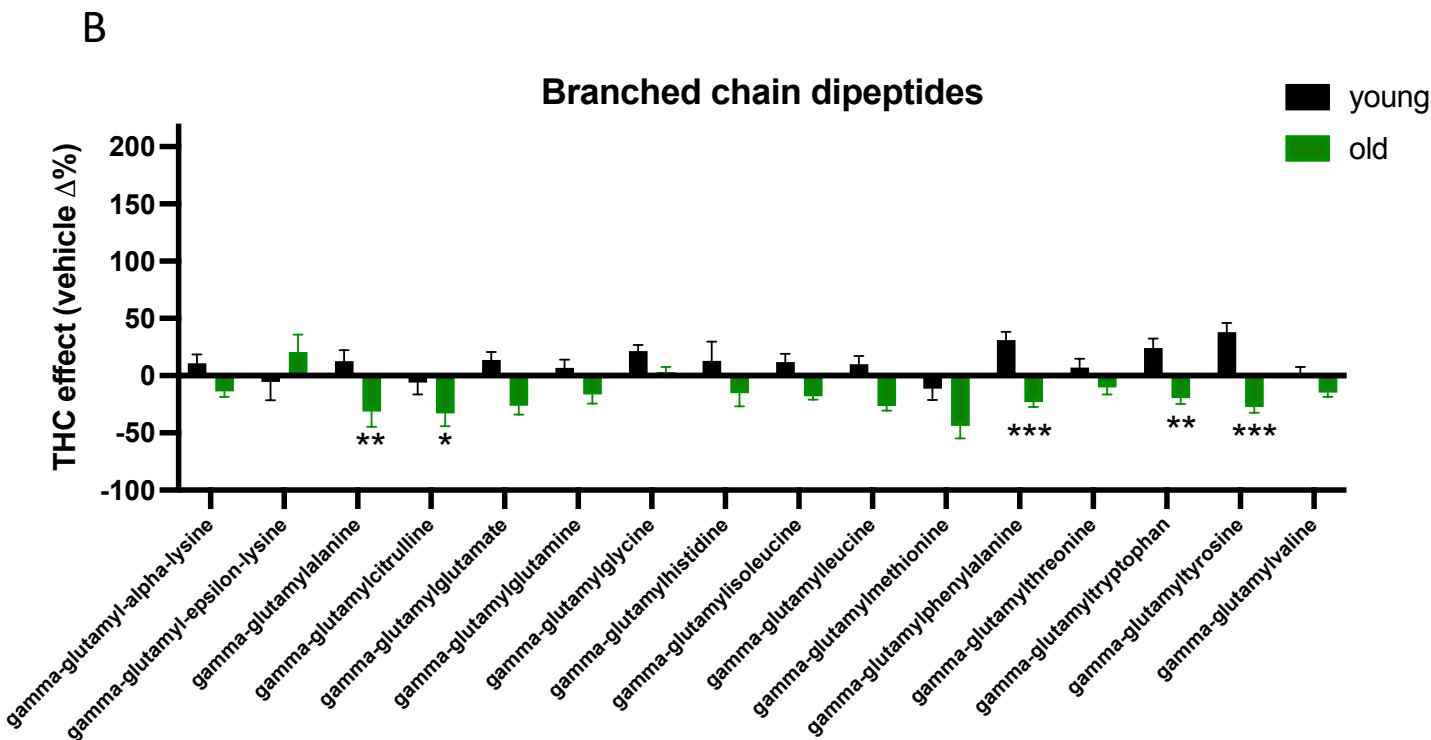
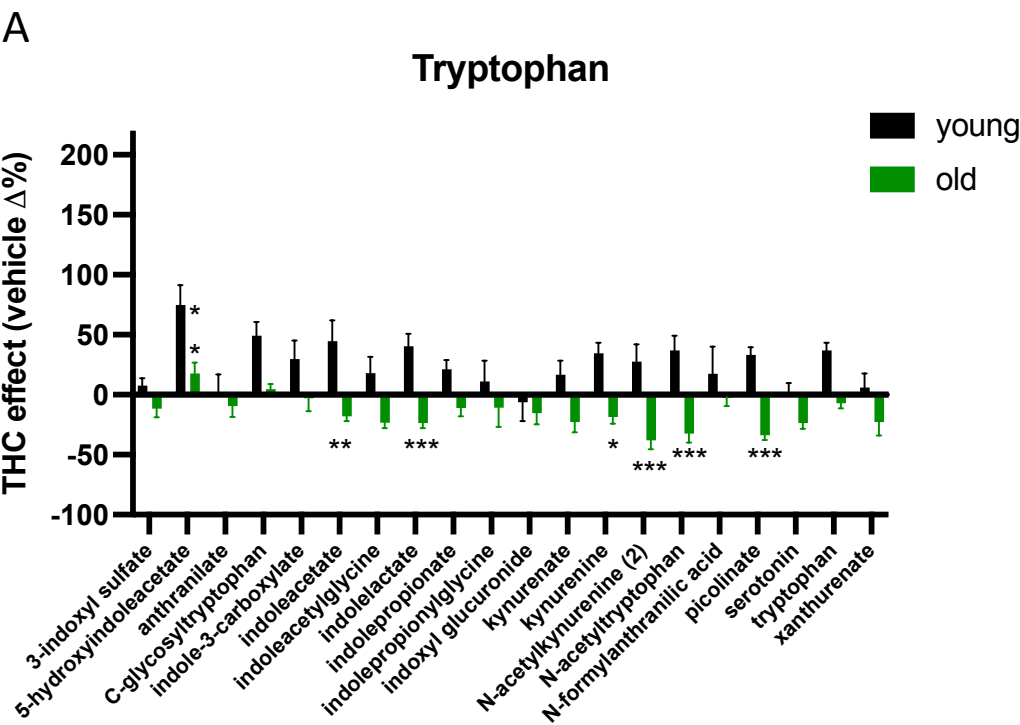
B

Phenylalanine



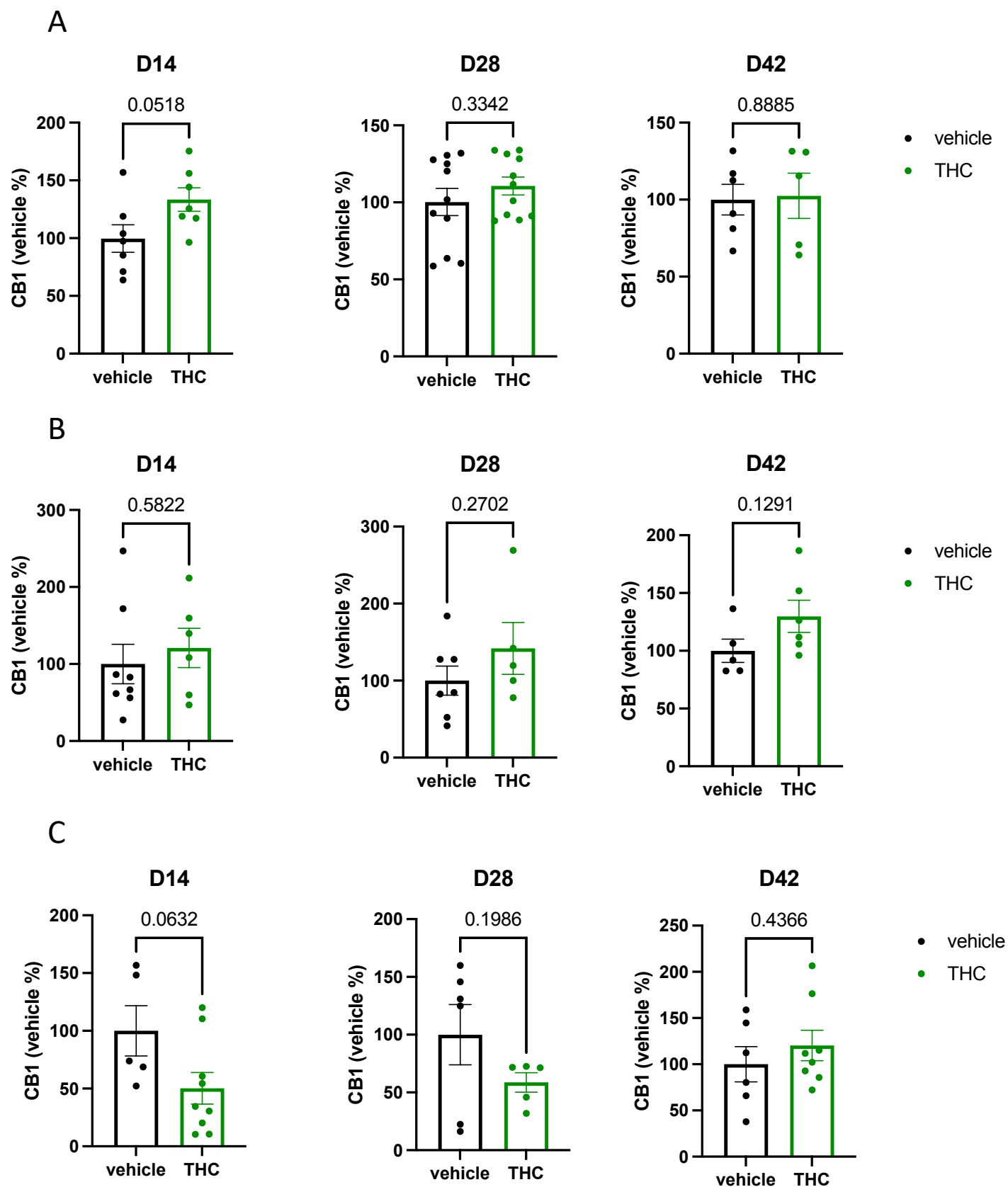
Compound-specific opposite effect of THC-treatment between young (4-month-old) and old (18-month-old) animals on the **(A)** methionine and **(B)** phenylalanine metabolite levels in the blood plasma at day 28. Columns represent mean values, whiskers standard error. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ significant difference in the effect of THC on the metabolite levels between young (4-month-old) and old (18-month-old) animals according two-way ANOVA followed by Bonferroni t-test.

Figure S5



Compound-specific opposite effect of THC-treatment between young (4-month-old) and old (18-month-old) animals on the **(A)** tryptophan and **(B)** branched chain dipeptide metabolite levels in the blood plasma at day 28. Columns represent mean values, whiskers standard error. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ significant difference in the effect of THC on the metabolite levels between young (4-month-old) and old (18-month-old) animals according two-way ANOVA followed by Bonferroni t-test.

Figure S6



Long-term, low dose THC-treatment or withdrawal did not alter CB1 receptor levels in the **(A)** cortex, **(B)** hippocampus or **(C)** adipose tissue

Table S1

Pentose

compound	THC (vehicle %)		q value	individual p value
	mean	sd		
arabinose	116.683	30.824	0.218	0.242
arabitol/xylitol	105.424	37.818	0.711	0.902
arabonate/xylonate	138.008	59.858	0.042	0.020
ribitol	119.370	38.373	0.218	0.212
ribonate	129.878	35.904	0.074	0.047
ribose	125.313	29.665	0.076	0.060
ribulonate/xylulonate	140.477	50.904	0.022	0.007
sedoheptulose	142.963	24.924	0.015	0.002

Fructose

compound	THC (vehicle %)		q value	individual p value
	mean	sd		
fructose	151.870	57.296	0.070	0.045
galactonate	124.575	18.688	0.070	0.038
galactose 1-phosphate	159.873	110.498	0.247	0.235
mannitol/sorbitol	110.505	8.731	0.070	0.033
mannose	164.415	75.537	0.070	0.037
mannose-6-phosphate	160.771	76.315	0.078	0.062

Monoacylglycerol

compound	THC (vehicle %)		q value	individual p value
	mean	sd		
1-arachidonylglycerol (20:4)	191.168	109.082	0.102	0.030
1-dihomo-linolenylglycerol (20:3)	132.854	51.828	0.220	0.175
1-docosahexaenoylglycerol (22:6)	170.008	90.594	0.102	0.048
1-oleoylglycerol (18:1)	138.502	39.132	0.126	0.080
2-arachidonoylglycerol (20:4)	185.673	98.728	0.102	0.042
2-oleoylglycerol (18:1)	124.137	60.576	0.357	0.340

Metabolite groups with significantly increased hippocampal levels after 14 days THC-treatment according to two-way ANOVA. Individual metabolites with significantly altered concentration according to Welch’s t-tests are highlighted with red.

Table S1

Lysophospholipids

compound	THC (vehicle %)		q value	individual p value
	mean	sd		
1-arachidonoyl-GPC (20:4n6)	112.373	40.171	0.682	0.498
1-arachidonoyl-GPE (20:4n6)	108.926	36.044	0.699	0.608
1-arachidonoyl-GPI (20:4)	98.266	20.107	>0.9999	0.953
1-linoleoyl-GPC (18:2)	161.960	93.538	0.444	0.257
1-linoleoyl-GPG (18:2)	126.183	45.487	0.336	0.120
1-oleoyl-GPC (18:1)	114.888	35.603	0.455	0.283
1-oleoyl-GPE (18:1)	104.426	32.093	0.891	0.811
1-oleoyl-GPG (18:1)	140.955	51.021	0.336	0.071
1-oleoyl-GPI (18:1)	120.655	27.855	0.336	0.157
1-oleoyl-GPS (18:1)	172.261	67.246	0.238	0.020
1-palmitoleoyl-GPC (16:1)	119.229	36.165	0.336	0.167
1-palmitoyl-GPC (16:0)	120.262	42.730	0.360	0.194
1-palmitoyl-GPE (16:0)	108.285	29.105	0.682	0.529
1-palmitoyl-GPG (16:0)	129.372	25.625	0.336	0.061
1-palmitoyl-GPI (16:0)	118.560	23.237	0.336	0.160
1-palmitoyl-GPS (16:0)	212.613	96.386	0.054	0.002
1-stearoyl-GPC (18:0)	119.841	34.514	0.336	0.150
1-stearoyl-GPE (18:0)	108.333	31.502	0.682	0.565
1-stearoyl-GPG (18:0)	131.145	45.939	0.336	0.134
1-stearoyl-GPI (18:0)	114.472	27.661	0.455	0.301
1-stearoyl-GPS (18:0)	139.216	56.516	0.336	0.102
2-palmitoyl-GPC (16:0)	128.292	37.227	0.336	0.072
2-stearoyl-GPE (18:0)	105.789	19.224	0.682	0.544

Table S1

Glutamate

compound	THC (vehicle %)		q value	individual p value
	mean	sd		
alpha-ketoglutaramate	150.857	50.626	0.230	0.159
beta-citrylglutamate	124.072	21.719	0.182	0.026
carboxyethyl-GABA	104.692	9.996	0.453	0.393
gamma-aminobutyrate (GABA)	112.805	22.819	0.230	0.160
glutamate	117.758	27.787	0.230	0.115
glutamate, gamma-methyl ester	120.239	26.277	0.230	0.085
glutamine	120.764	33.288	0.230	0.093
N-acetyl-aspartyl-glutamate (NAAG)	117.704	32.496	0.230	0.150
N-acetylglutamate	106.493	21.172	0.519	0.494
N-acetylglutamine	111.741	27.426	0.417	0.325
pyroglutamine	115.243	15.321	0.182	0.031

Table S1

Branched chain amino acids

compound	THC (vehicle %)		q value	individual p value
	mean	sd		
1-carboxyethylisoleucine	105.433	25.788	0.642	0.541
1-carboxyethylleucine	117.439	27.699	0.314	0.219
1-carboxyethylvaline	109.722	27.236	0.584	0.458
2-methylbutyrylcarnitine (C5)	102.133	32.576	0.732	0.697
3-hydroxyisobutyrate	111.884	17.574	0.314	0.229
3-methylglutaconate	121.242	30.923	0.199	0.075
alpha-hydroxyisovalerate	120.870	41.688	0.314	0.211
beta-hydroxyisovalerate	114.794	12.801	0.199	0.056
ethylmalonate	139.888	58.530	0.199	0.078
isobutyrylcarnitine (C4)	84.301	55.769	0.642	0.576
isoleucine	122.970	41.119	0.314	0.184
isovalerylcarnitine (C5)	117.320	25.608	0.314	0.219
leucine	147.922	52.134	0.176	0.027
methylsuccinate	133.129	43.425	0.199	0.056
N-acetylleucine	125.404	27.293	0.176	0.023
N-acetylvaline	120.953	32.630	0.302	0.136
valine	135.975	45.583	0.176	0.030

Fatty acid amids

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
heptadecenamide (17:1)*	425.692	301.076	0.006	0.004
linoleamide (18:2n6)	309.091	140.223	0.006	0.003
oleamide	390.895	262.994	0.006	0.003
palmitoleamide (16:1)*	416.666	269.383	0.006	0.006

Phosphatidylinositols

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
1-oleoyl-2-arachidonoyl-GPI (18:1/20:4)	90.985	19.965	0.520	0.744
1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)	106.980	13.944	0.413	0.394
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	158.194	25.050	0.020	0.009
1-palmitoyl-2-oleoyl-GPI (16:0/18:1)	212.666	58.888	0.020	0.007
1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	92.179	4.888	0.520	0.658
1-stearoyl-2-linoleoyl-GPI (18:0/18:2)	137.349	18.335	0.045	0.032

Metabolite groups with significantly changed levels in the blood plasma after 14 days THC-treatment according to two-way ANOVA. Individual metabolites with significantly altered concentration according to Welch’s t-tests are highlighted with red.

Phosphatidylethanolamines

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
1-linoleoyl-2-arachidonoyl-GPE (18:2/20:4)	163.084	34.506	0.040	0.015
1-oleoyl-2-arachidonoyl-GPE (18:1/20:4)	125.492	27.424	0.065	0.055
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)	140.796	28.068	0.040	0.020
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)	128.384	28.041	0.040	0.030
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)	96.796	24.182	0.807	1.000
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	139.667	32.107	0.040	0.028
1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	206.656	44.344	0.007	0.001
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	116.156	15.994	0.129	0.123
1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6)	106.927	26.172	0.394	0.450
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	145.136	27.663	0.040	0.024
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	181.112	35.349	0.027	0.008
1,2-dilinoleoyl-GPE (18:2/18:2)	134.848	28.064	0.129	0.135
1,2-dipalmitoyl-GPE (16:0/16:0)	283.564	68.915	0.000	<0.0001

Table S2

Diacylglycerols

compound	THC (vehicle %)		q value	individual p value
	mean	sd		
diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])	220.712	77.910	0.013	0.003
linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1]	121.404	56.750	0.202	0.289
linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]	139.978	40.006	0.067	0.076
linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [2]	118.277	27.474	0.152	0.188
linoleoyl-linolenoyl-glycerol (18:2/18:3) [2]	217.203	93.657	0.038	0.028
linoleoyl-linoleoyl-glycerol (18:2/18:2) [1]	159.567	41.247	0.044	0.046
oleoyl-arachidonoyl-glycerol (18:1/20:4) [1]	132.359	78.789	0.163	0.217
oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]	178.315	38.556	0.020	0.010
oleoyl-linoleoyl-glycerol (18:1/18:2) [1]	170.779	53.632	0.044	0.044
oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	177.846	48.492	0.038	0.029
oleoyl-oleoyl-glycerol (18:1/18:1) [2]	262.902	97.325	0.016	0.005
palmitoleoyl-linoleoyl-glycerol (16:1/18:2) [1]	165.908	63.823	0.038	0.025
palmitoyl-linoleoyl-glycerol (16:0/18:2) [1]	153.984	76.579	0.044	0.044
palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]	257.869	69.869	0.017	0.007
palmitoyl-oleoyl-glycerol (16:0/18:1) [2]	320.685	148.301	0.013	0.002

Table S2

Ceramides

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
ceramide (d16:1/24:1, d18:1/22:1)	134.647	39.169	0.182	0.087
ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)	121.834	63.886	0.346	0.274
ceramide (d18:2/24:1, d18:1/24:2)	121.595	32.485	0.205	0.130
N-behenoyl-sphingadienine (d18:2/22:0)	164.418	62.575	0.143	0.045
N-palmitoyl-sphingosine (d18:1/16:0)	131.498	25.256	0.143	0.026
N-stearoyl-sphingosine (d18:1/18:0)	94.742	15.591	0.936	0.891

(Hypo)xanthine/inosine containing purine metabolites

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
allantoin	88.558	17.191	0.127	0.202
hypoxanthine	11.368	17.814	0.021	0.019
inosine	7.534	13.008	0.021	0.014
inosine 5'-monophosphate	107.916	61.165	0.424	0.941
urate	81.596	16.020	0.021	0.027
xanthine	13.800	24.156	0.021	0.021
xanthosine	94.855	54.786	0.309	0.588

Branched chain amino acids

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
1-carboxyethylisoleucine	69.147	44.388	0.096	0.055
1-carboxyethylleucine	65.724	47.441	0.098	0.061
1-carboxyethylvaline	62.972	36.513	0.054	0.028
2-hydroxy-3-methylvalerate	93.487	8.166	0.310	0.281
2-methylbutyrylcarnitine (C5)	112.304	23.558	0.268	0.204
2-methylbutyrylglycine	84.547	16.805	0.146	0.105
3-hydroxy-2-ethylpropionate	90.562	27.906	0.546	0.676
3-hydroxyisobutyrate	71.992	13.287	0.020	0.003
3-methyl-2-oxobutyrate	101.074	20.292	0.636	0.849
3-methyl-2-oxovalerate	97.701	19.688	0.582	0.748
3-methylcrotonylglycine	95.486	18.208	0.533	0.609
3-methylglutaconate	70.656	4.842	0.008	0.000
4-methyl-2-oxopentanoate	92.860	21.860	0.525	0.575
alpha-hydroxyisocaproate	86.078	7.552	0.054	0.026
alpha-hydroxyisovalerate	91.684	11.295	0.127	0.085
beta-hydroxyisovalerate	110.979	10.031	0.546	0.652
ethylmalonate	94.257	22.472	0.388	0.388
isobutyrylcarnitine (C4)	83.040	17.824	0.315	0.300
isoleucine	82.813	14.054	0.027	0.010
isovalerylcarnitine (C5)	77.460	10.862	0.020	0.004
isovalerylglycine	117.294	29.082	0.301	0.244
leucine	83.388	14.764	0.041	0.018
methylsuccinate	85.508	14.681	0.310	0.269
N-acetylisoleucine	67.345	17.458	0.020	0.006
N-acetylleucine	70.791	20.810	0.025	0.008
N-acetylvaline	77.323	15.092	0.020	0.005
tigloylglycine	90.048	25.707	0.400	0.419
valine	81.456	12.463	0.020	0.006

Metabolite groups with significantly changed levels in the blood plasma after 28 days THC-treatment according to two-way ANOVA. Individual metabolites with significantly altered concentration according to Welch’s t-tests are highlighted with red.

Table S3

Lysine

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
2-aminoadipate	80.359	24.433	0.107	0.131
2-oxoadipate	70.432	18.772	0.050	0.055
6-oxopiperidine-2-carboxylate	87.163	17.972	0.266	0.398
fructosyllysine	67.984	15.768	0.014	0.009
lysine	98.589	12.091	0.315	0.515
N,N,N-trimethyl-5-aminovalerate	98.464	12.231	0.343	0.606
N2-acetyllysine	64.880	8.638	0.002	0.000
N2,N6-diacetyllysine	61.376	3.705	0.011	0.004
N6-acetyllysine	71.758	6.130	0.014	0.009
N6-methyllysine	87.469	10.711	0.021	0.017
N6,N6-dimethyllysine	80.642	5.707	0.002	0.001
N6,N6,N6-trimethyllysine	124.387	18.058	0.030	0.028
pipecolate	94.014	12.469	0.266	0.375

Methionine

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)	80.256	8.122	0.021	0.005
cystathionine	126.663	39.417	0.332	0.343
cysteine	85.242	18.248	0.139	0.077
cysteine s-sulfate	61.885	28.940	0.060	0.024
cystine	51.333	23.114	0.031	0.010
hypotauroine	127.419	25.625	0.275	0.240
methionine	87.946	9.901	0.116	0.055
methionine sulfone	78.784	22.322	0.206	0.147
methionine sulfoxide	69.369	11.047	0.016	0.001
N-acetylmethionine	68.006	17.367	0.021	0.005
N-acetyltaurine	89.270	17.738	0.332	0.318
N-formylmethionine	108.578	12.624	0.366	0.407
S-adenosylhomocysteine (SAH)	63.627	32.600	0.177	0.112
S-methylcysteine	119.115	26.673	0.244	0.194
S-methylmethionine	112.023	71.738	0.660	0.838
taurine	106.637	13.824	0.464	0.553

Table S3

Phenylalanine

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
1-carboxyethylphenylalanine	63.625	39.736	0.042	0.040
N-acetylphenylalanine	74.955	9.099	0.007	0.004
phenylalanine	86.176	5.587	0.003	0.001
phenyllactate (PLA)	77.607	17.033	0.039	0.030
phenylpyruvate	61.054	6.548	0.000	<0.0001

Tryptophan

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
3-indoxyl sulfate	88.368	20.050	0.227	0.216
5-hydroxyindoleacetate	117.774	25.454	0.208	0.145
anthranilate	90.430	25.023	0.414	0.435
C-glycosyltryptophan	104.752	12.043	0.414	0.470
indole-3-carboxylate	97.312	30.927	0.660	0.828
indoleacetate	82.054	11.110	0.050	0.019
indoleacetyl glycine	76.677	12.639	0.208	0.143
indolelactate	76.514	12.555	0.024	0.006
indolepropionate	88.939	19.940	0.226	0.181
indolepropionyl glycine	89.218	45.604	0.414	0.473
indoxyl glucuronide	84.668	26.576	0.226	0.201
kynurenate	77.178	23.869	0.208	0.142
kynurenine	81.364	15.291	0.084	0.037
N-acetylkynurenine (2)	62.042	20.973	0.024	0.002
N-acetyltryptophan	67.732	21.159	0.024	0.005
N-formylanthranilic acid	98.049	21.204	0.660	0.838
picolinate	66.163	11.155	0.024	0.008
serotonin	76.309	13.147	0.024	0.006
tryptophan	92.896	12.039	0.226	0.189
xanthurenate	77.119	31.187	0.131	0.066

Gamma-glutamyl containing branched dipeptides

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
gamma-glutamyl-alpha-lysine	93.046	14.615	0.223	0.230
gamma-glutamyl-epsilon-lysine	135.492	48.453	0.329	0.366
gamma-glutamylalanine	66.203	36.463	0.160	0.140
gamma-glutamylcitrulline	65.318	31.331	0.132	0.095
gamma-glutamylglutamate	74.733	22.233	0.043	0.023
gamma-glutamylglutamine	82.580	21.779	0.144	0.115
gamma-glutamylglycine	104.753	13.238	0.530	0.673
gamma-glutamylhistidine	92.362	35.001	0.468	0.557
gamma-glutamylisoleucine	84.200	8.661	0.023	0.006
gamma-glutamylleucine	75.211	11.382	0.006	0.001
gamma-glutamylmethionine	54.758	29.725	0.043	0.017
gamma-glutamylphenylalanine	78.638	11.726	0.007	0.001
gamma-glutamylthreonine	91.063	17.750	0.203	0.193
gamma-glutamyltryptophan	84.194	15.583	0.043	0.024
gamma-glutamyltyrosine	78.699	15.569	0.094	0.059
gamma-glutamylvaline	85.463	10.847	0.023	0.007

Table S4

Alanine

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
alanine	78.663	9.494	0.065	0.010
asparagine	82.269	9.986	0.135	0.107
aspartate	50.586	17.656	0.132	0.084
N-acetylalanine	86.747	11.724	0.084	0.027
N-acetylasparagine	73.376	12.407	0.114	0.054
N-acetylaspartate (NAA)	102.972	16.192	0.583	0.555

Phospholipids

Compound	THC (vehicle %)		q value	individual p value
	mean	sd		
choline	70.221	13.964	0.428	0.408
choline phosphate	52.396	19.289	0.140	0.066
glycerophosphoethanolamine	65.685	20.290	0.140	0.025
glycerophosphorylcholine (GPC)	73.187	18.752	0.254	0.176
phosphoethanolamine	71.472	15.483	0.140	0.061
trimethylamine N-oxide	80.321	23.394	0.254	0.202

Metabolite groups with significantly changed levels in blood plasma on day 42, after 14 days of THC withdrawal, according to two-way ANOVA.