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Supplementary Information for

Modifying the maternal microbiota alters the gut-brain metabolome and prevents emotional dysfunction in the adult offspring of obese dams

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Supplementary Results

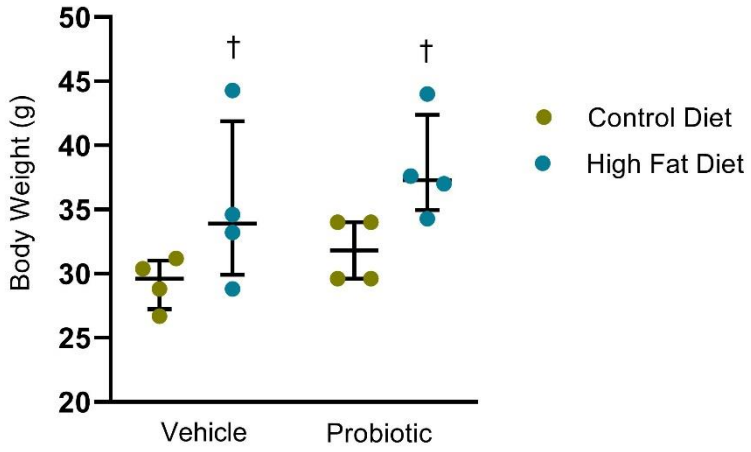

Figure S1 At the time of mating (E0.5), maternal diet constituted the only weight difference between dams ($F_{(1, 12)}$ = 8.75, p = 0.012). †p < 0.05, indicating main effect of diet. Data presented as median ± interquartile range showing all points. N = 4 per group.

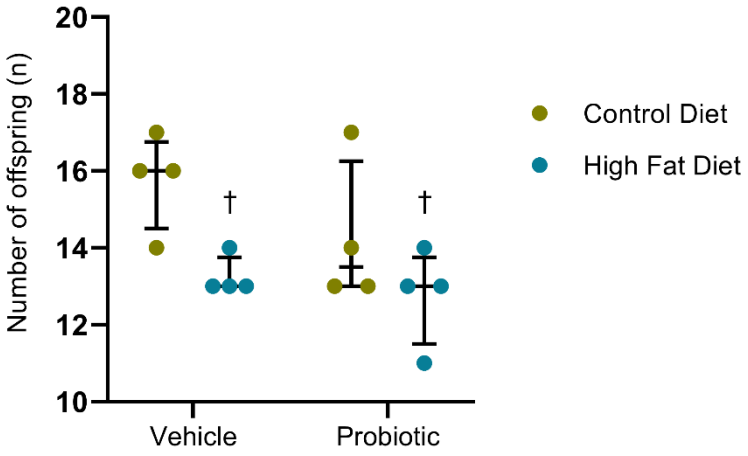

Figure S2 Maternal HFD-induced obesity reduces litter size ($F_{(1, 12)} = 9.14$, $p = 0.011$). †$p < 0.05$, indicating main effect of diet. Data presented as median ± interquartile range showing all points. N = 4 per group.

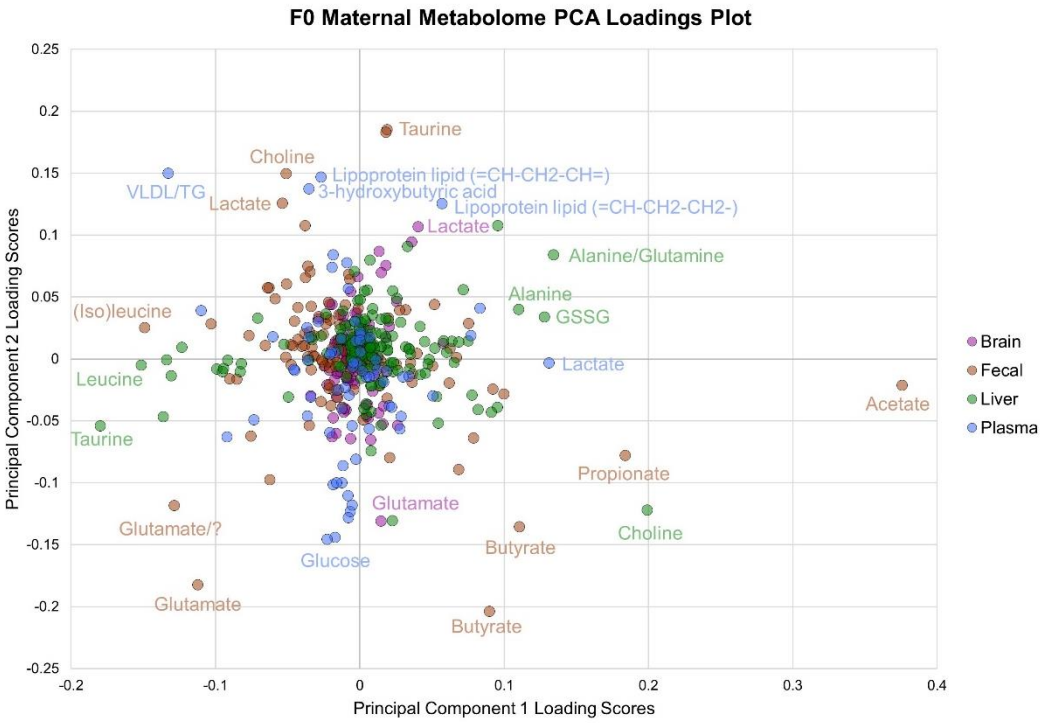

Figure S3 Loadings plot for maternal integrated PCA model. Loadings correspond to the PCA scores plot shown in Figure 1E.

Figure S3 Notes: Fecal acetate, elevated by probiotic supplementation, contributes largely to the clustering of the CD/Probiotic dams. Maternal fecal, liver, and plasma metabolites contribute relatively equally to the clustering observed between the three other groups (CD/Vehicle, HFD/Vehicle, HFD/Probiotic).

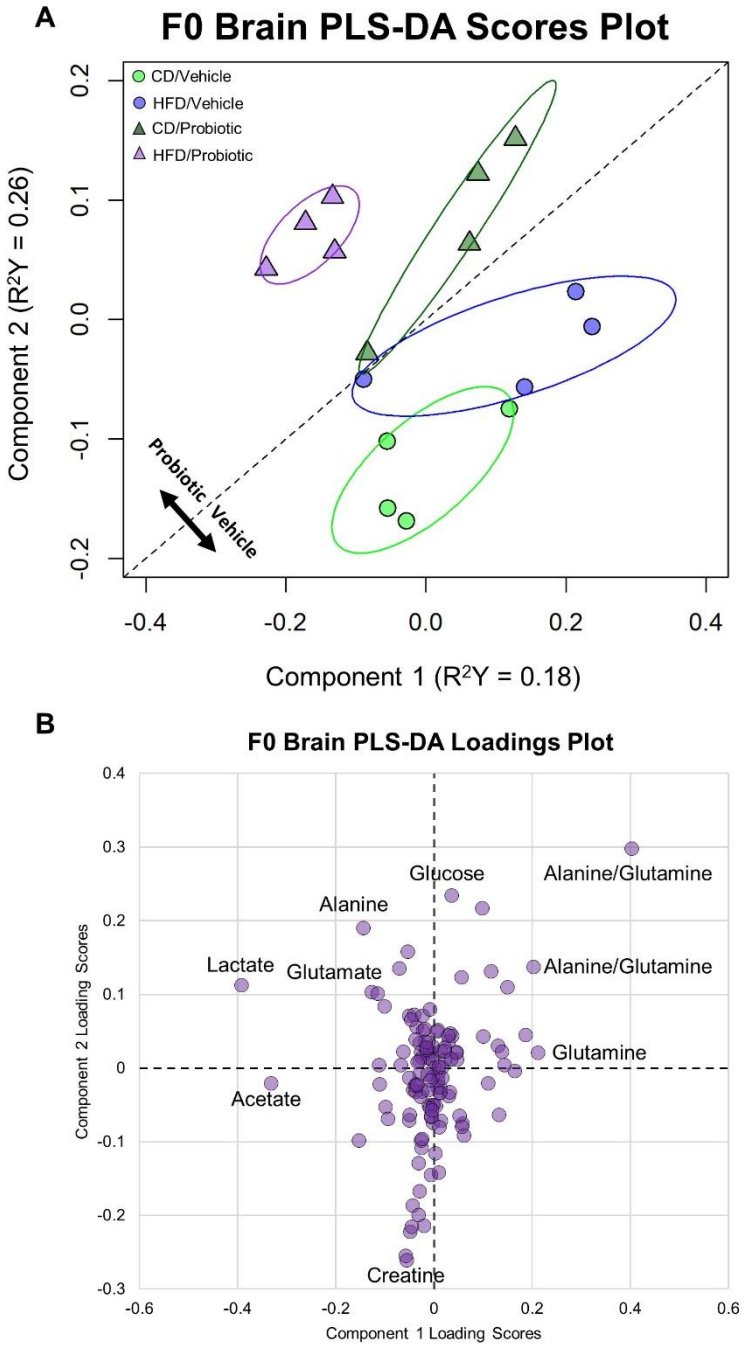

Figure S4 PLS-DA scores and loadings plot of the F0 maternal brain metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 4 per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative $R^2X = 0.49$. Cumulative $R^2Y = 0.44$. Cumulative $Q^2 = 0.19$. 2 component model.

Figure S4 Notes: Greatest group separation can be observed between maternal probiotic-supplemented dams and vehicle, independent of maternal diet.

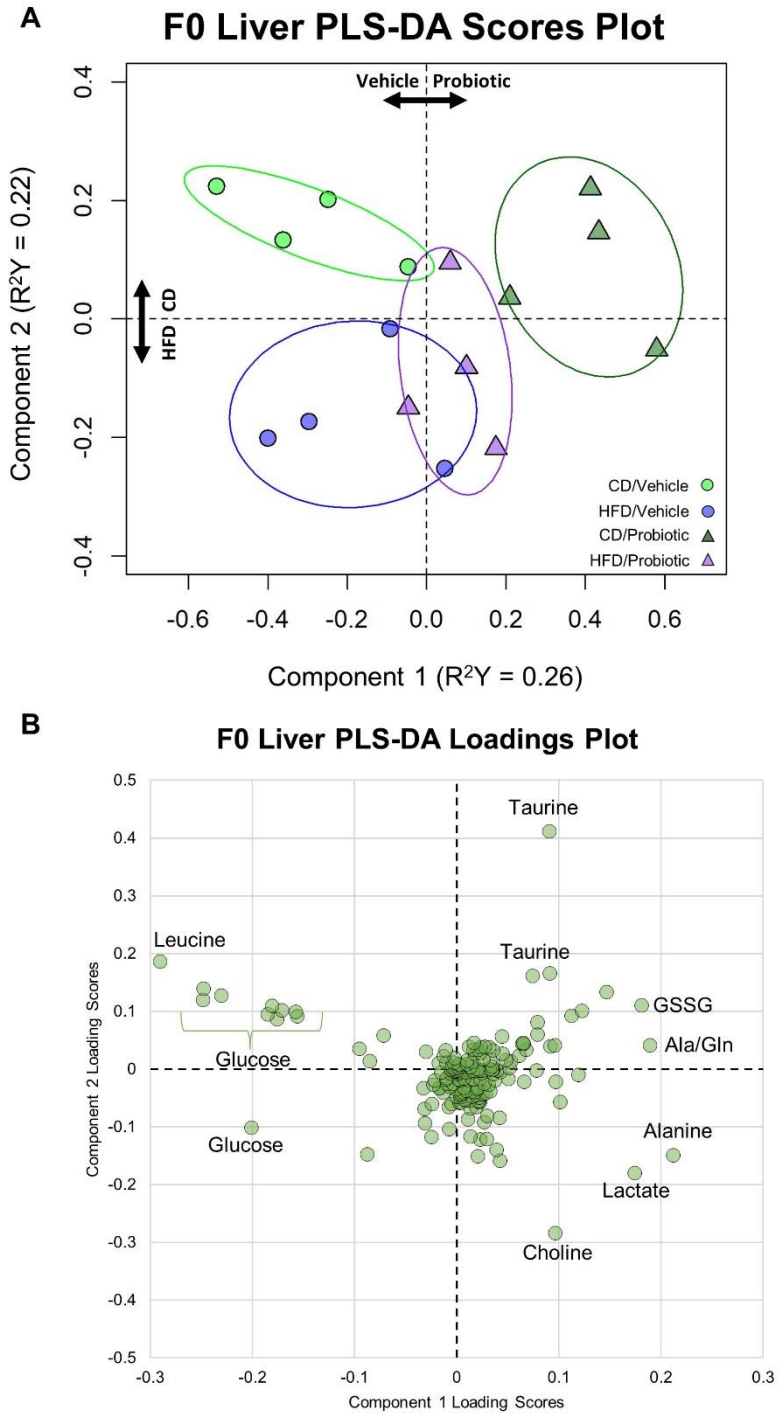

Figure S5 PLS-DA scores and loadings plot of the F0 maternal liver metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 4 per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative R^2X = 0.57. Cumulative R^2Y = 0.48. Cumulative Q^2 = 0.26. 2 component model.

Figure S5 Notes: Group separation between vehicle and probiotic supplementation in the liver metabolome of the dams can be observed in the first component, however; obese dams (in the HFD/probiotic group) cluster between vehicle dams and CD/probiotic dams. Otherwise, the effect of control diet and high-fat diet supplementation is largely independent, with separation occurring orthogonally in the second component.

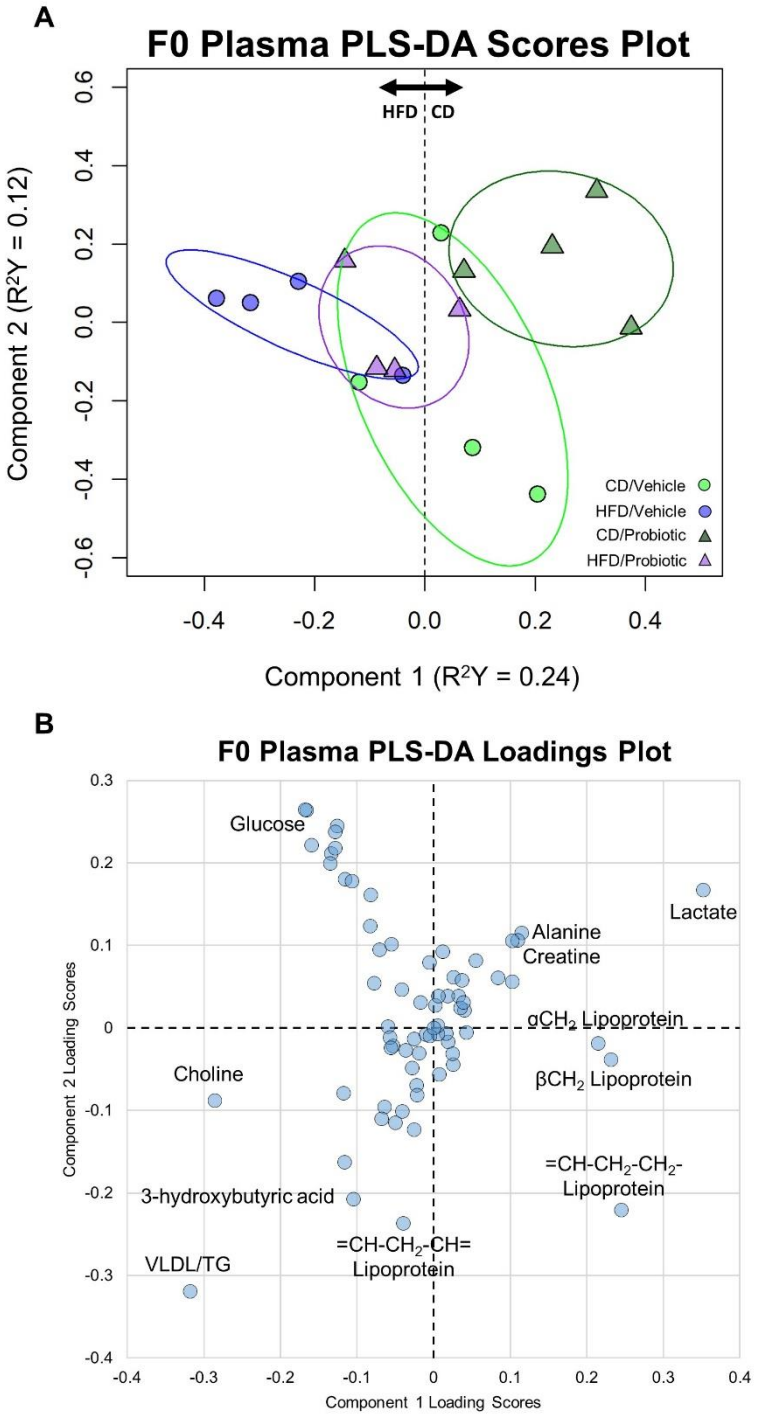

Figure S6 PLS-DA scores and loadings plot of the F0 maternal plasma metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 4 per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative $R^2X = 0.64$. Cumulative $R^2Y = 0.36$. Cumulative $Q^2 = 0.08$. 2 component model.

Figure S6 Notes: Greatest group separation can be observed between dams fed control and high fat diet. A right-shift (towards control dams) in the same component is also observed in probiotic-supplemented dams, leading HFD/probiotic dams to cluster between CD/vehicle and HFD/vehicle dams across the first component. No clustering by group was observed in the second component.

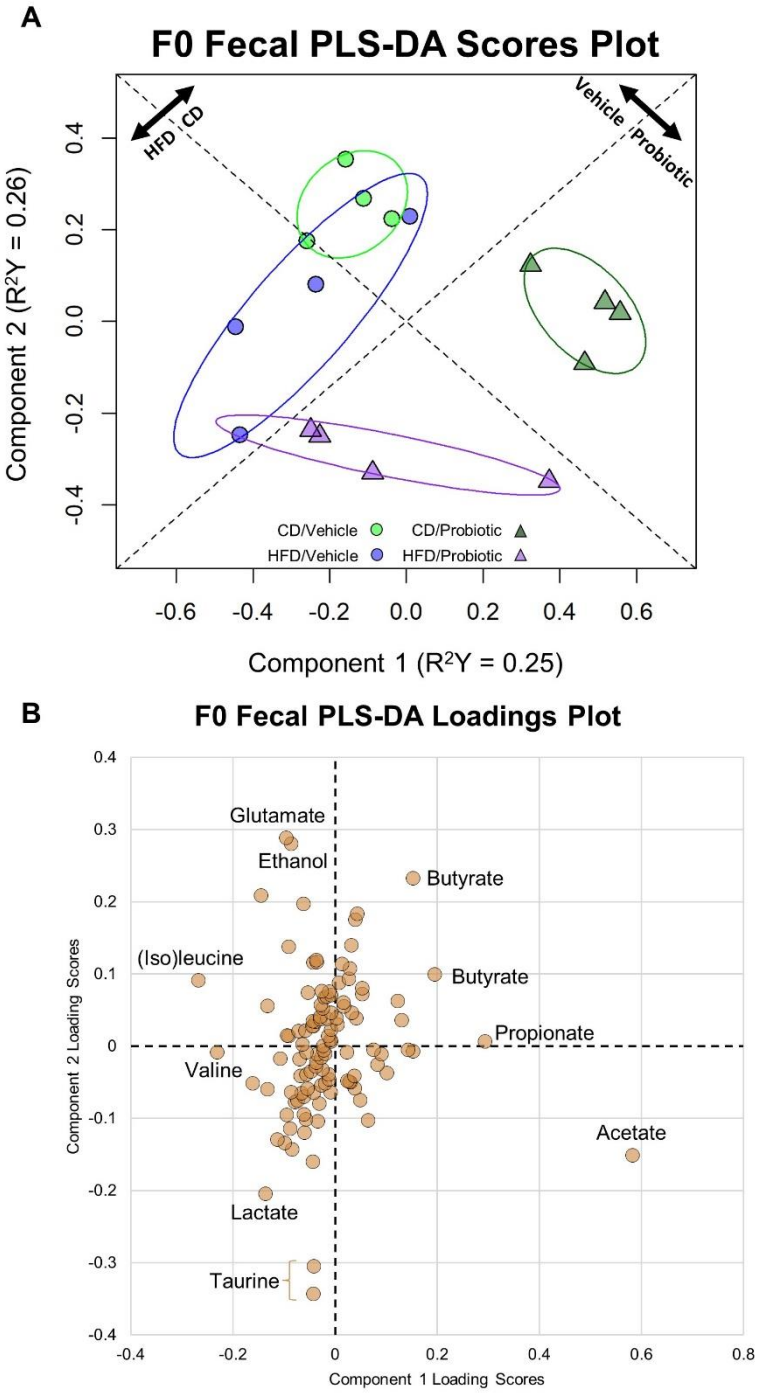

Figure S7 PLS-DA scores and loadings plot of the F0 maternal fecal metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 4 per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative R^2X = 0.64. Cumulative R^2Y = 0.71. Cumulative Q^2 = 0.33. 3 component model.

Figure S7 Notes: Group separation between CD/HFD and vehicle/probiotic dams occur orthogonally (independently) between components 1 and 2. The fecal metabolome of CD/probiotic dams is most different from any other group, due to the relative increase in concentration of short-chain fatty, in particular acetate (Table S1).

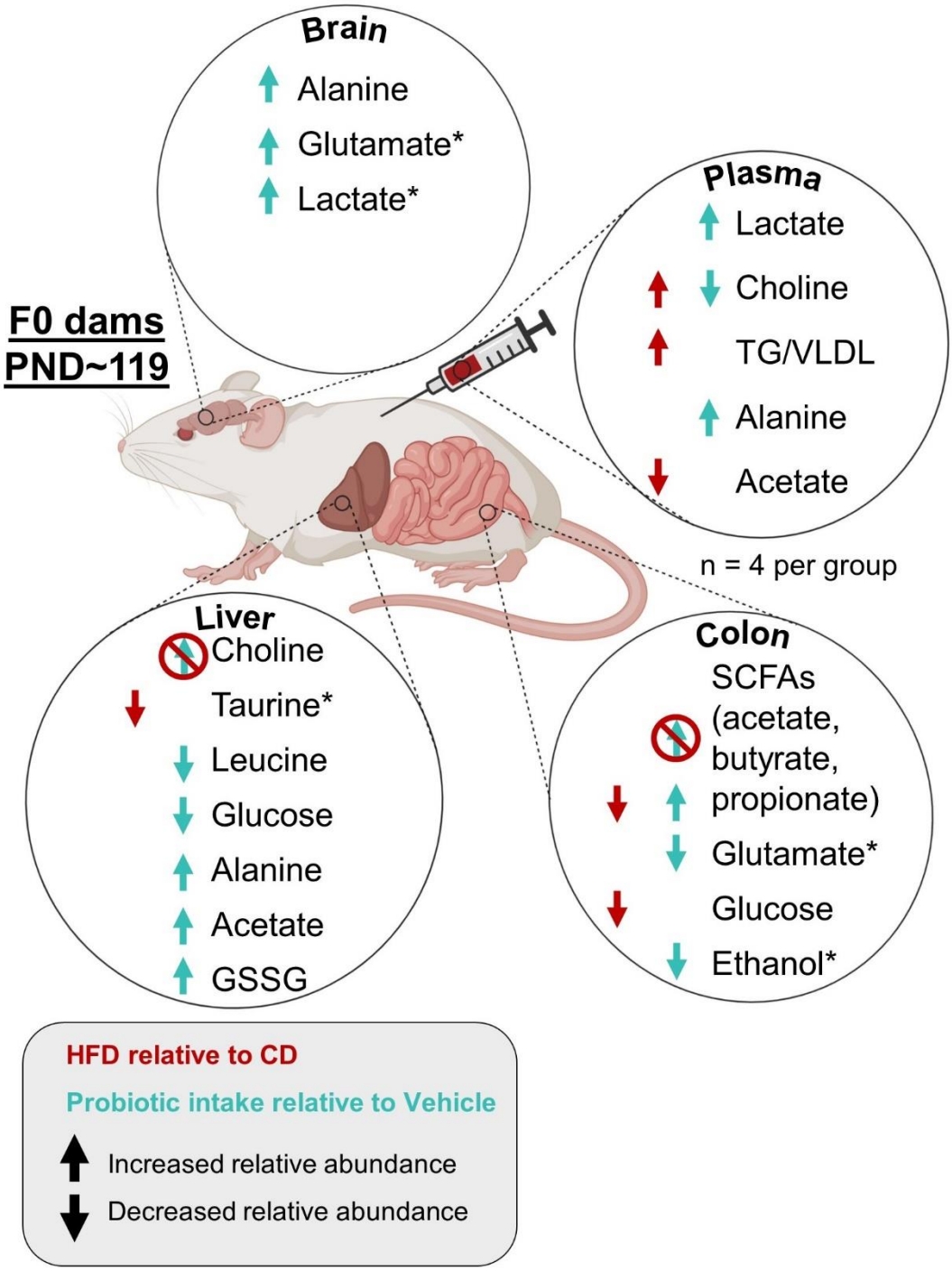

Figure S8 Maternal multi-tissue metabolic profiling indicates composition resembling the juvenile offspring. Summary of top metabolites from partial least squares discriminant analyses (PLS-DA), altered by HFD-induced obesity and/or perinatal probiotic intake, as shown in Figures S4-S7. Brain, liver, plasma, and colonic fecal samples were collected from dams at the same time as the juvenile offspring. Metabolite data is listed in Table S1. *Significant after PLS-DA but not significantly altered after adjusting for multiple testing using the Benjamini & Hochberg method of correction.

Supplementary Table 1 F0 maternal metabolome

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Brain Alanine [1.47-1.49]	0.0074± $5.6x10^{-4}$	0.0076± $8.5x10^{-4}$	**$^{\#\#}$0.0083± $5.4x10^{-4}$**	**$^{\#\#}$0.0093± $5.4x10^{-4}$**	(0.0056)	(0.85)
Brain Glutamate [2.34-2.39]	0.054± 0.0021	0.053± 0.0013	0.056± 0.0018	0.055± 0.0032	(0.079)	(0.85)
Brain Creatine [3.93]	0.019± 0.0045	0.022± 0.0084	0.021± 0.0051	0.014± 0.011	(0.42)	(0.85)
Brain Lactate [1.32-1.34]	0.071± 0.0074	0.064± 0.0068	0.071± 0.0025	0.077± 0.0064	(0.079)	(0.90)
Liver Leucine [3.70-3.74]	0.065± 0.010	0.056± 0.0059	**$^{\#}$0.044± 0.0079**	**$^{\#}$0.051± 0.0040**	(0.027)	(0.95)
Liver Alanine [1.47-1.49]	0.019± 0.0031	0.021± 0.0060	**$^{\#\#}$0.031± 0.0040**	**$^{\#\#}$0.030± 0.0056**	(0.0049)	(0.95)
Liver Taurine [3.41-3.44]	0.13± 0.0060	0.11± 0.0076	0.13± 0.014	0.12± 0.0086	(0.47)	(0.29)
Liver Glucose [4.64-4.66]	0.041± 0.0072	0.036± 0.0043	**$^{\#\#}$0.027± 0.0055**	**$^{\#\#}$0.032± 0.0028**	(0.0049)	(0.95)
Liver Acetate [1.91-1.92]	0.0024± 0.00089	0.0025± 0.00044	**$^{\#}$0.0049± 0.0016**	**$^{\#}$0.0040± 0.00040**	(0.015)	(0.50)
Liver Choline [3.22-3.23]	0.0081± 0.0014	0.012± 0.0021	******0.040± 0.010**	0.012± 0.0046	(<0.0001)	(0.001)
Liver GSSG [2.16-2.19]	0.013± 0.0038	0.013± 0.0037	**$^{\#\#}$0.021± 0.0021**	**$^{\#\#}$0.017± 0.0032**	(0.0049)	(0.95)
Plasma Lactate [1.32-1.34]	0.069± 0.0023	0.066± 0.0090	**$^{\#}$0.086± 0.0059**	**$^{\#}$0.078± 0.014**	(0.030)	(0.23)
Plasma VLDL/Triglyceride [0.84-88]	0.080± 0.013	**†0.087± 0.002**	0.068± 0.011	**†0.084± 0.0047**	(0.42)	(0.040)
Plasma Alanine [1.47-1.49]	0.0064± 0.0021	0.0054± $2.9x10^{-4}$	**$^{\#}$0.0087± $7.7x10^{-4}$**	**$^{\#}$0.0077± 0.0022**	(0.030)	(0.23)
Plasma Choline [3.22-3.23]	0.042± $2.8x10^{-3}$	**††††0.05± $1.8x10^{-3}$**	**$^{\#}$0.037± $3.5x10^{-3}$**	**$^{\#\dagger\dagger\dagger\dagger}$0.044± $1.6x10^{-3}$**	(0.05)	(<0.0001)
Fecal Acetate [1.91-1.92]	0.061± 0.0082	0.062± 0.026	*****0.14± 0.021**	0.089± 0.065	(0.014)	(0.0040)
Fecal Propionate [1.06-1.07]	0.017± 0.0052	**†0.015± 0.0058**	**$^{\#}$0.038± 0.0028**	**$^{\#\dagger}$0.019± 0.0090**	(0.011)	(0.012)
Fecal Butyrate [1.54-1.58]	0.0185± $3.1x10^{-4}$	0.0184± 0.0071	*****0.0341± 0.0030**	0.0163± 0.0030	(0.037)	(0.011)
Fecal Glutamate [2.34-2.39]	0.046± 0.0070	0.052± 0.016	0.033± 0.0077	0.040± 0.0066	(0.056)	(0.94)
Fecal Ethanol [1.17-1.20]	0.035± 0.016	0.019± 0.0056	0.017± 0.0030	0.018± 0.0043	(0.10)	(0.23)
Fecal Glucose [4.64-4.66]	0.0080± 0.0025	**††††0.0017 ±0.00091**	0.0054± 0.0012	**††††0.0017± 0.00062**	(0.35)	(<0.0001)

Table S1 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where a statistically significant diet x probiotic interaction occurred after correcting for the false discovery rate, post-hoc comparisons have been applied and have been explained here.

A significant interaction was identified for liver choline ($F_{(1,12)}$ = 30.64, q = 0.0007). Post-hoc Tukey tests revealed that CD/probiotic dams had significantly higher levels of liver choline relative to all other groups ($p < 0.0001$ compared to all other groups).

A significant interaction was identified for F0 fecal acetate ($F_{(1,11)}$ = 19.35, q = 0.0066). Post-hoc Tukey tests revealed that CD/probiotic dams had significantly higher levels of fecal acetate relative to all other groups (p = 0.0004 compared to CD/vehicle and HFD/vehicle, p = 0.0005 compared to HFD/probiotic).

A significant interaction was identified for F0 fecal butyrate ($F_{(1,12)}$ = 18.84, q = 0.0060). Post-hoc Tukey tests revealed that CD/probiotic dams had significantly higher levels of fecal butyrate relative to all other groups (p = 0.0008 compared to CD/vehicle and p = 0.0009 compared to HFD/vehicle, p = 0.0003 compared to HFD/probiotic).

No other significant interactions occurred.

N=4 per group. Data presented as mean ± standard deviation. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

Supplementary Table 2 F0 maternal brain PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Lactate 1.32....1.34.	3.42
Alanine/Glutamine 3.75....3.79.	3.30
Creatine 3.92....3.94.	2.14
Glutamine/Glutamate 2.11....2.18.	2.08
Alanine 1.47....1.49.	2.04
Taurine 3.39....3.45.	1.94
Glutamate 2.34....2.37.	1.81
Glucose 3.67....3.70.	1.79
Acetate 1.91....1.92.	1.77
GSSG 3.75....3.75.	1.63

Supplementary Table 3 F0 maternal liver PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Choline 3.22....3.23.	3.80
Taurine 3.41....3.44.	3.80
Leucine 3.70....3.74.	2.91
Taurine 3.26....3.28.	2.71
Glucose 4.64....4.66.	2.40
Taurine 3.48....3.51.	2.39
Glucose 3.83....3.85.	2.30
Alanine 1.47....1.50.	2.17
Lactate 1.32....1.34.	1.80
Alanine/Glutamine 3.76....3.79.	1.75
GSSG 2.16....2.19.	1.73

Supplementary Table 4 F0 maternal plasma PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
VLDL/TG 0.84....0.88.	2.78
Lactate 1.32....1.34.	2.62
Choline 3.21....3.23.	2.43
3-hydroxybutyric acid 1.19....1.21.	1.52
Taurine 3.48....3.51.	1.43
βCH_2 Lipoprotein 1.56....1.62.	1.39
αCH_2 Lipoprotein 2.24....2.28.	1.37
Glucose 3.82....3.86.	1.35
$=CH\text{-}CH_2\text{-}CH_2\text{-}$ Lipoprotein 1.96....2.11.	1.31
Glucose 3.45....3.48.	1.19
Glucose 3.39....3.41.	1.15
Glucose 3.73....3.74.	1.15
$=CH\text{-}CH_2\text{-}CH=$ Lipoprotein 2.69....2.82.	1.14
Glucose 3.71....3.72.	1.14
Glucose 3.89....3.91.	1.10
Creatine 3.01....3.04.	1.04
Lactate 4.09....4.13.	1.01
Alanine 1.47....1.49.	1.01

Supplementary Table 5 F0 maternal fecal PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Acetate 1.91....1.92.	3.34
Ethanol 1.17....1.20.	2.53
Glutamate 2.34....2.39.	2.33
Taurine 3.41....3.43.	2.09
Taurine 3.25....3.28.	2.07
Propionate 1.06....1.07.	1.99
Butyrate 0.89....0.91.	1.95
Ethanol 3.65....3.68.	1.93
Glucose 4.64....4.66.	1.68
Butyrate 1.54....1.58.	1.56
(Iso)leucine 0.94....0.98.	1.44
Valine 0.98....1.00.	1.22

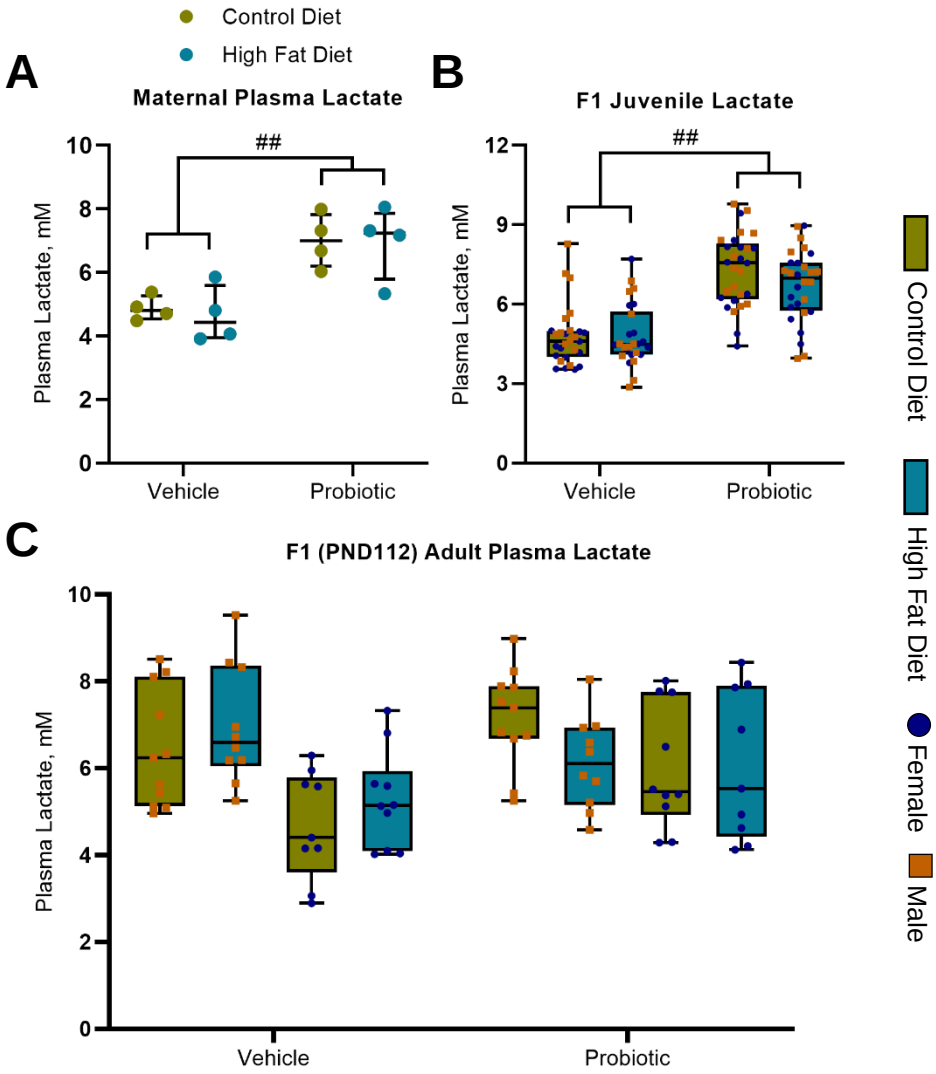

Figure S9 Maternal perinatal probiotic intake increases absolute lactate concentrations in maternal and offspring plasma. (A) Maternal plasma lactate is increased by probiotic supplementation (main effect, $F_{(1, 12)} = 26.55$, q = 0.0011) independent of diet with no interaction. N = 4 per group. **(B)** Juvenile offspring from probiotic fed dams have increased plasma lactate at weaning age (main effect, $F_{(1, 107)} = 84.97$, q = 0.0011) with no interaction and no sex differences. N = 26-29 per group. **(C)** Plasma lactate was not found to be elevated in adult (postnatal day 112) offspring from probiotic fed dams (Male: $F_{(1, 38)} = 0.029$, q = 0.76. Female: $F_{(1, 34)} = 5.43$, q = 0.091). There was also no main effect of maternal diet and no interaction. N = 9-11 per sex per group. All statistics were corrected for multiple testing using the Benjamini & Hochberg method.

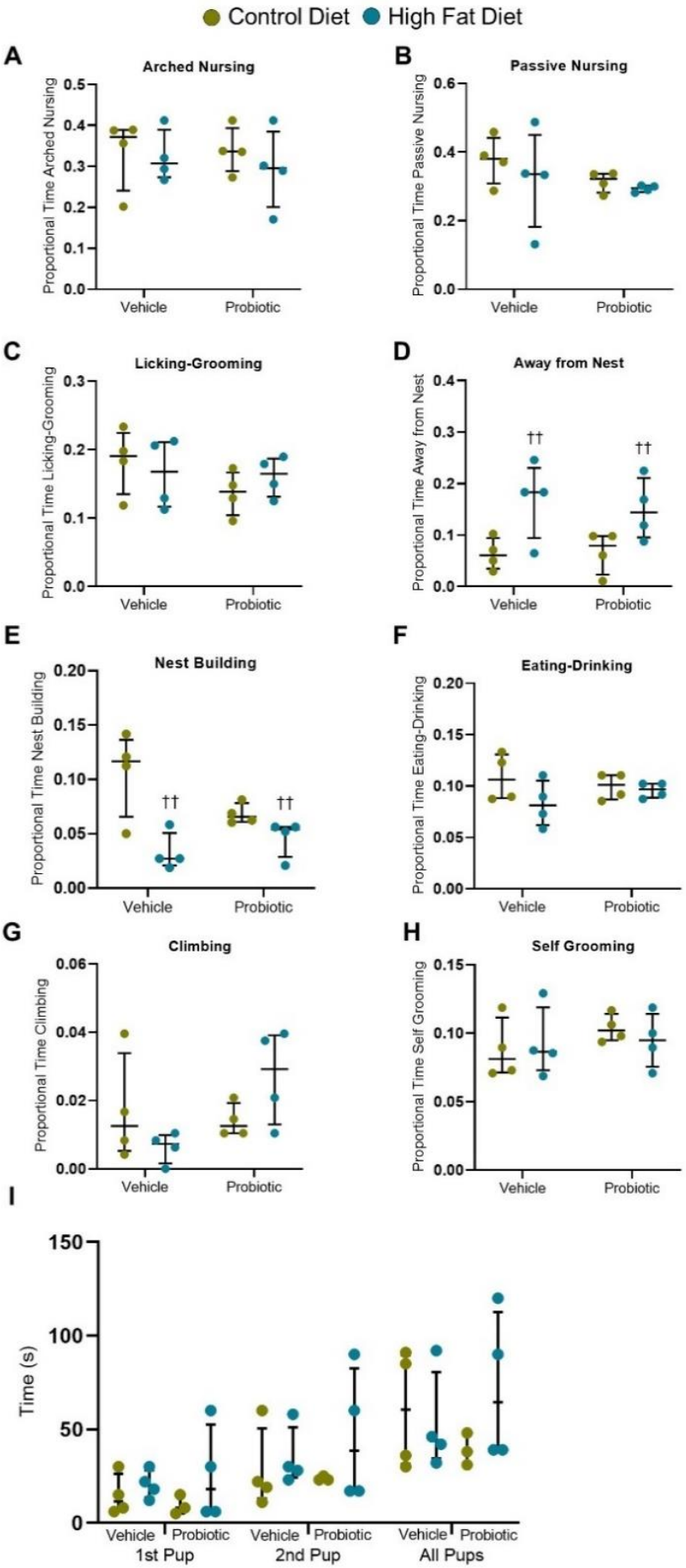
Control Diet
High Fat Diet
A
Arched Nursing
Proportional Time Arched Nursing
0.0
0.1
0.2
0.3
0.4
0.5
Vehicle
Probiotic
B
Passive Nursing
Proportional Time Passive Nursing
0.0
0.2
0.4
0.6
Vehicle
Probiotic
C
Licking-Grooming
Proportional Time Licking-Grooming
0.0
0.1
0.2
0.3
Vehicle
Probiotic
D
Away from Nest
Proportional Time Away from Nest
0.0
0.1
0.2
0.3
0.4
††
††
Vehicle
Probiotic
E
Nest Building
Proportional Time Nest Building
0.00
0.05
0.10
0.15
0.20
††
††
Vehicle
Probiotic
F
Eating-Drinking
Proportional Time Eating-Drinking
0.00
0.05
0.10
0.15
0.20
Vehicle
Probiotic
G
Climbing
Proportional Time Climbing
0.00
0.02
0.04
0.06
Vehicle
Probiotic
H
Self Grooming
Proportional Time Self Grooming
0.00
0.05
0.10
0.15
Vehicle
Probiotic
I
Time (s)
0
50
100
150
Vehicle
Probiotic
Vehicle
Probiotic
Vehicle
Probiotic
1st Pup
2nd Pup
All Pups

Figure S10 Maternal care behavior. There was no main effect of gestational probiotic supplementation on maternal care behavior (MCB) during the first week of nursing. **(A)** arched nursing, **(B)** passive nursing, and **(C)** licking and grooming were unchanged. **(D)** Obese (HFD) dams spent more time passively away from the nest whilst not performing any other stereotypic behavior compared to lean (CD) dams (main effect, $F_{(1, 12)} = 11.94$, $p = 0.0048$), independent of probiotic treatment, which seemed to be at the expense of **(E)** time building the nest (main effect, $F_{(1, 12)} = 16.20$, $p = 0.0017$), which was lower in obese dams independent of probiotic treatment. **(F)** eating/drinking, **(G)** climbing, and **(H)** self-grooming were unaffected by diet or probiotic intake. **(I)** Time to retrieve pups after the period of maternal care observation was not different between groups. Data presented as median ± interquartile range showing all points. No significant interactions. $^{\dagger\dagger}p < 0.01$, indicating main effect of diet. N = 4 per group. No significant diet x probiotic interactions were identified, and therefore no post hoc tests were performed.

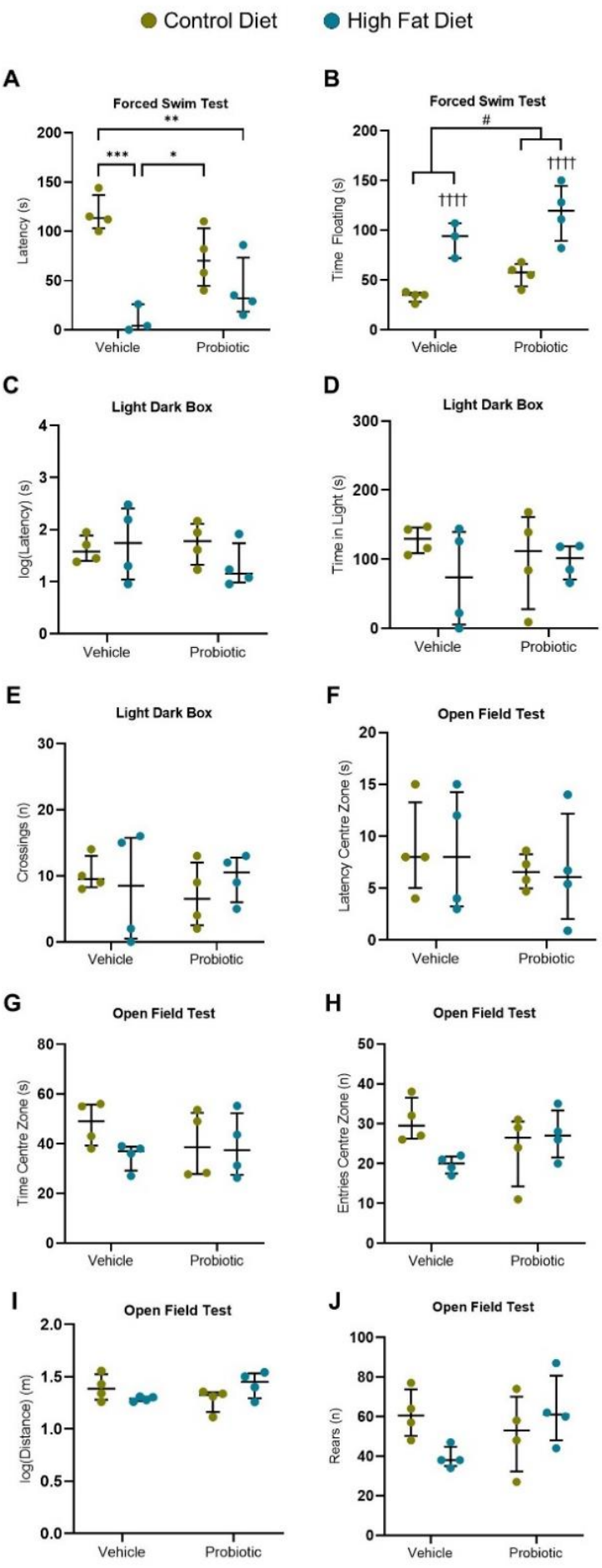
Control Diet
High Fat Diet
A
Forced Swim Test
Latency (s)
Vehicle
Probiotic
B
Forced Swim Test
Time Floating (s)
C
Light Dark Box
log(Latency) (s)
D
Light Dark Box
Time in Light (s)
E
Light Dark Box
Crossings (n)
F
Open Field Test
Latency Centre Zone (s)
G
Open Field Test
Time Centre Zone (s)
H
Open Field Test
Entries Centre Zone (n)
I
Open Field Test
log(Distance) (m)
J
Open Field Test
Rears (n)

Figure S11 Chronic high fat diet exposure increased passive stress coping in dams. A significant interaction ($F_{(1, 11)} = 8.39$, $p = 0.015$) was identified for **(A)** latency to float, and post hoc Tukey tests revealed that CD/vehicle dams had increased latency to float compared to HFD/vehicle ($p = 0.0008$) and HFD/probiotic ($p = 0.0062$) dams, while CD/probiotic dams had an increased latency to float only relative to HFD/vehicle dams ($p = 0.035$). **(B)** Time spent floating was increased by HFD ($F_{(1, 11)} = 40.36$, $p < 0.0001$) and by perinatal probiotic intake ($F_{(1, 11)} = 6.786$, $p = 0.025$). There were no changes to maternal anxiety-like behavior through **(C)** latency, **(D)** time in light, or **(E)** crossings in the light-dark box. **(F)** Latency, **(G)** time, and **(H)** entries to the center zone of the open field test were unaffected, as was **(I)** distance travelled and **(J)** rearing. Data presented as median ± interquartile range showing all points. $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$, indicating main effect of maternal diet. $^{\#}p < 0.05$, indicating main effect of maternal probiotic intake. $^{**}p < 0.01$, $^{***}p < 0.001$ indicating significant Kolmogorov-Smirnov with Bonferroni correction post-hoc comparisons. N = 4 per group.

Supplementary Table 6 F0 maternal prefrontal cortex pro-inflammatory gene expression

	Vehicle		Probiotic	
	Control Diet	High Fat Diet	Control Diet	High Fat Diet
TNF	1.00± 0.09	1.02± 0.30	**1.70± 0.49**$^{\#}$	**1.20± 0.43**$^{\#}$
IL-1B	1.00± 0.31	0.77± 0.17	0.67± 0.25	0.86± 0.07
IL-6	1.00± 0.06	0.92± 0.32	**2.71± 1.18**$^{\#}$	**1.33± 0.55**$^{\#}$
TLR4	1.00± 0.01	**1.48± 0.14**†	0.93± 0.17	**1.76± 0.82**†

Table S6 Notes: Data presented as mean±SD, relative expression compared to vehicle/Control Diet. 2-way ANOVA performed, or non-parametric equivalent. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, , $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey or Kolmogorov-Smirnov post-hoc comparison compared to control group. N = 4 per group.

Brain *TLR4* ($Q = 9.69$, $p = 0.024$) was elevated in obese dams, with no significant diet x probiotic interaction. *TNF* and *IL-6* were increased (*TNF*: $Q = 5.92$, $p = 0.035$, *IL-6*: $F_{(1, 11)} = 10.70$, $p = 0.0075$) in the brain of probiotic-supplemented dams, with no significant diet x probiotic interactions. No significant interaction or main effects occurred for brain IL-1B.

Supplementary Table 7 F0 maternal liver pro-inflammatory gene expression

	Vehicle		Probiotic	
	Control Diet	High Fat Diet	Control Diet	High Fat Diet
TNF	1.00± 0.15	0.90± 0.42	**0.57± 0.33**$^{\#}$	**0.53± 0.11**$^{\#}$
IL-1B	1.00± 0.23	**2.15± 0.67**†	0.98± 0.53	**1.44± 0.69**†
IL-6	1.00± 0.32	0.93± 0.48	**0.36± 0.27**$^{\#\#}$	**0.22± 0.09**$^{\#\#}$
TLR4	1.00± 0.24	**2.03± 0.07**†	1.07± 0.21	**1.26± 0.40**†
SAA-2	1.00± 0.49	**3.93± 1.68****	1.66± 0.53	**2.62± 0.74***

Table S7 Notes: Data presented as mean±SD, relative expression compared to vehicle/Control Diet. 2-way ANOVA performed, or non-parametric equivalent. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, , $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey or Kolmogorov-Smirnov post-hoc comparison compared to control group. N = 4 per group.

Liver *TLR4* ($Q = 7.96$, $p = 0.022$) and *IL-1B* ($F_{(1, 12)} = 8.269$, $p = 0.014$) were elevated in obese dams. *TNF* ($F_{(1, 12)} = 8.142$, $p = 0.015$) and *IL-6* ($F_{(1, 12)} = 17.55$, $p = 0.0013$) cytokines were reduced in the liver after probiotic intake. No diet x probiotic interactions occurred for the expression of these genes in the liver.

For the expression of liver *SAA-2,* a significant diet x probiotic interaction was identified ($F_{(1, 12)} = 4.76$, $p = 0.0497$). Tukey post-hoc testing revealed that HFD/vehicle dams had significantly greater expression of SAA-2 compared to CD/vehicle dams ($p = 0.0026$) and CD/probiotic dams ($p = 0.032$), while HFD/probiotic dams had significantly greater expression compared to CD/vehicle dams only ($p = 0.046$).

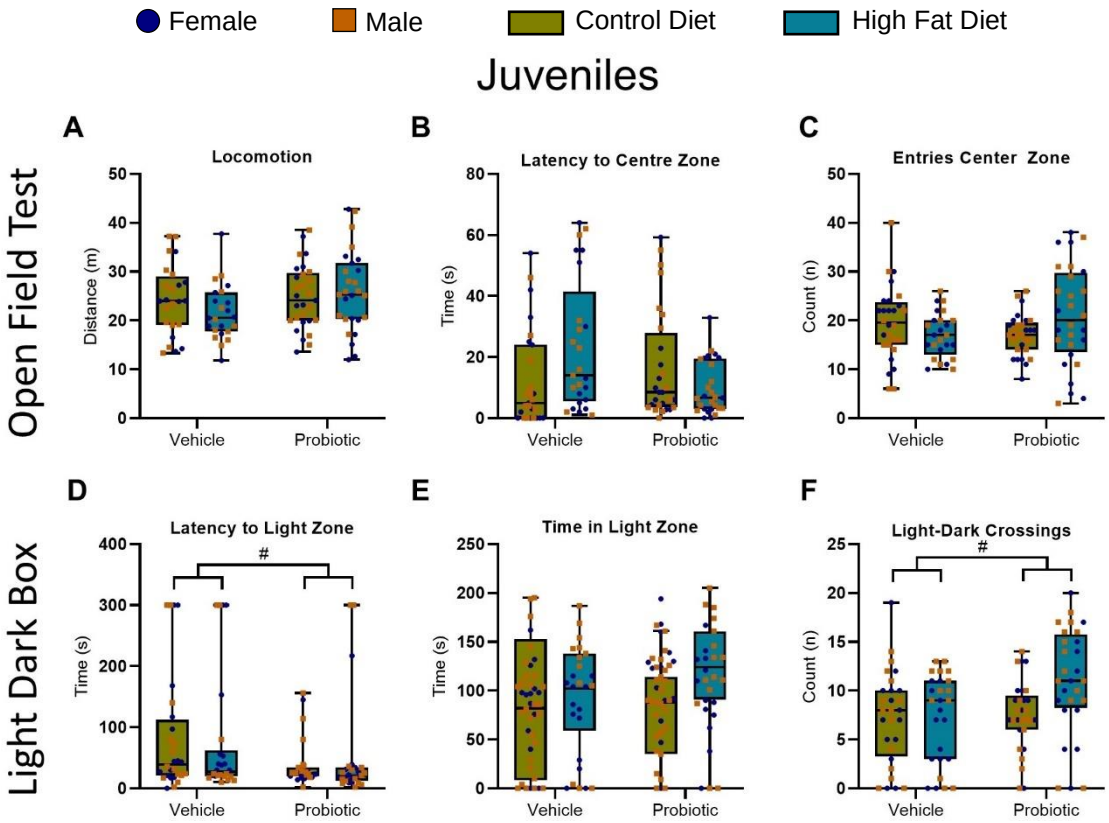

Figure S12 Maternal obesity and probiotic reduces anxiety-like behavior in juvenile offspring. (A) Total distance travelled and **(B)** latency to enter the center zone in the open field test (OFT) were unchanged in juvenile offspring. **(C)** There was a significant interaction between maternal diet and probiotic intake on center zone entries ($Q_{(1, 107)} = 4.87$, $p = 0.033$), though post hoc Tukey tests did not reveal any significant group differences. **(D)** Maternal probiotic intake reduced latency to enter the light zone of the light-dark box (LDB; $Q_{(1, 107)} = 5.12$, $p = 0.031$) without a significant interaction between maternal diet and probiotic treatment. **(E)** No significant differences were found for total time spent in the light zone. **(F)** Maternal probiotic intake increased LDB crossings in offspring (main effect, $F_{(1, 107)} = 4.99$, $p = 0.028$) in a manner that was independent of maternal diet and without interaction. $^{\#}p < 0.05$, indicating main effect of maternal probiotic intake. N = 13-15 per sex, per group.

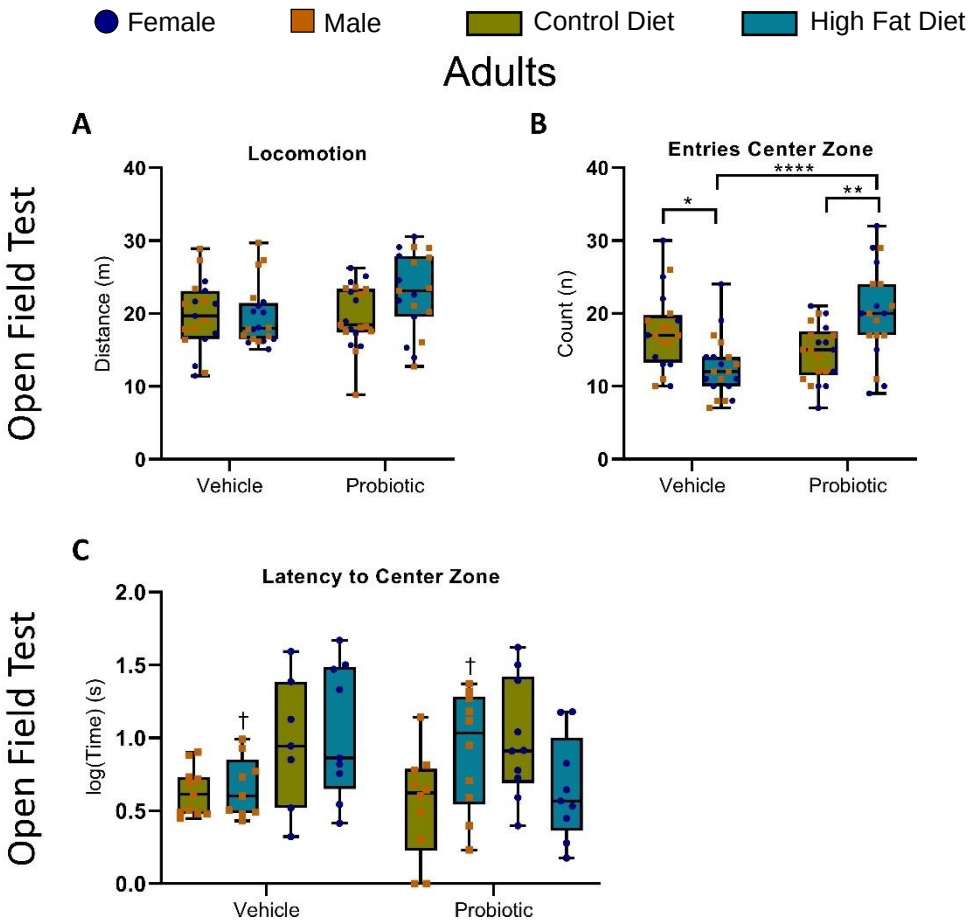

Figure S13 Maternal probiotic intake reverses anxiety-like behavior in the adult offspring of obese dams. (A) Overall activity in the OFT in adult offspring was unaltered in adult offspring. Maternal **(B)** Center zone entries were affected by a significant interaction ($F_{(1, 76)}$ = 21.20, $p < 0.0001$). Post-hoc Tukey tests revealed that HFD/Veh adult offspring entered the center zone significantly fewer times than both CD/Veh offspring ($p = 0.014$) and HFD/Pro offspring ($p < 0.0001$). CD/Pro offspring had significantly fewer entries than HFD/Pro offspring ($p = 0.0057$). **(C)** Maternal diet increased latency to center zone in the OFT in males (main effect, $F_{(1, 36)} = 4.33$, $p = 0.045$) independent of maternal probiotic treatment with no interaction. Data presented as boxplots indicating the median, interquartile range, minimum and maximum points. $^{\dagger}p < 0.05$, indicating main effect of maternal diet. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, indicating significant Tukey post-hoc comparisons computed only in the case of a significant interaction. N = 9-11 per sex, per group.

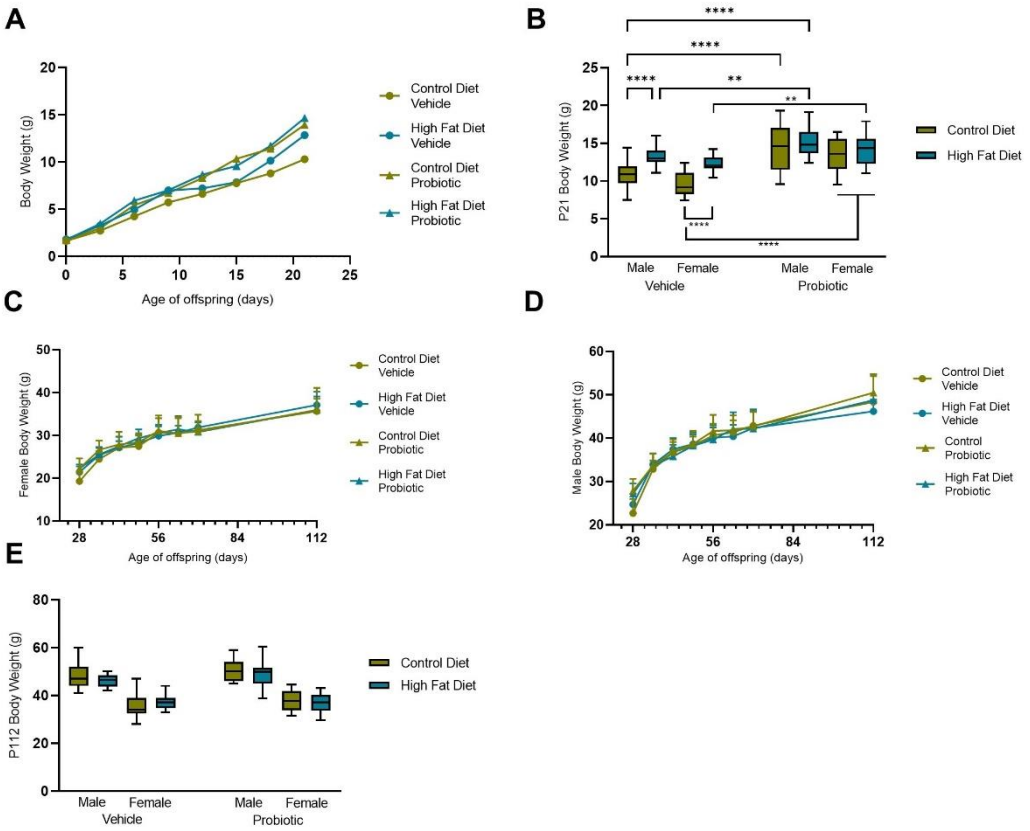

Figure S14 Maternal HFD and probiotic intake increase offspring body weight in early life but not in adulthood. (A) Mixed effects analysis revealed that both maternal probiotic intake ($F_{(1, 217)}$ = 298.7, p < 0.0001) and high-fat diet (HFD; $F_{(1, 217)}$ = 58.56, p < 0.0001) independently increased juvenile body weight compared to controls. There was also a significant main effect of time on increasing body weight ($F_{(3.652, 698.6)}$ = 2928, p < 0.0001) and significant interactions: Time*Probiotic ($F_{(7, 1339)}$ = 55.73, p < 0.0001); Time*Maternal diet ($F_{(7, 1339)}$ = 19.03, p < 0.0001), Probiotic*Maternal diet ($F_{(1, 217)}$ = 19.71, p < 0.0001) and Time*Probiotic*Maternal diet ($F_{(7, 1339)}$ = 5.72, p < 0.0001). **(B)** At postnatal day (PND)21, significant diet x probiotic interactions were present for males ($F_{(1, 107)}$ = 5.32, p = 0.023) and females ($F_{(1, 88)}$ = 9.44, p = 0.0028). Post hoc Tukey testing revealed significant pairwise differences between offspring of HFD/vehicle and control/vehicle dams (p < 0.0001 for males and females) In each post hoc comparison, CD/probiotic and HFD/probiotic offspring were significantly heavier than CD/vehicle and HFD/vehicle offspring. There was no significant difference in body weight between CD/probiotic and HFD/probiotic offspring for males and females. Apart from a main effect of time, body weight was not different between groups in adult **(C)** female or **(D)** male offspring with no significant interactions, also shown at **(E)** PND112. Data presented as mean ± SD **(A,C,D)** or as boxplots indicating the median, interquartile range, minimum and maximum data points **(B,E)**. **p < 0.01, ****p < 0.0001, indicating significant Tukey post-hoc comparisons. N = 55-62 per group **(A)**; 22-33 per group **(B)**; 9-16 per group **(C-E)**.

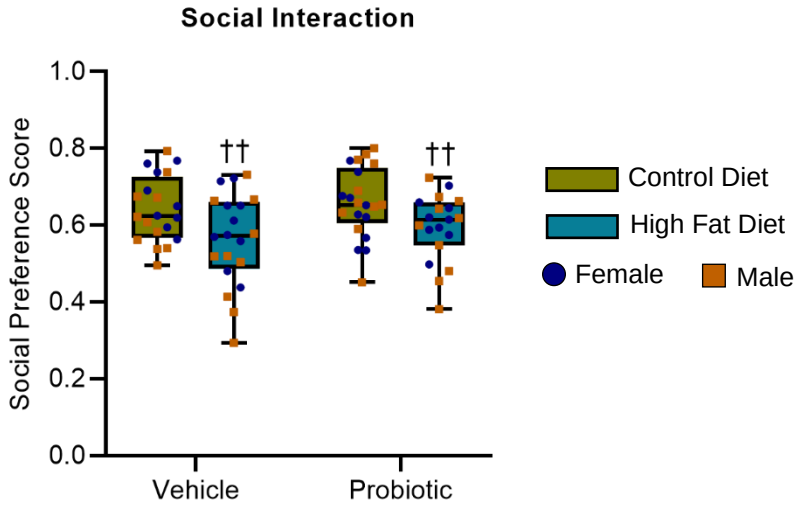

Figure S15 Maternal HFD-induced obesity reduces social interaction in F1 (PND112) adult offspring (main effect, $F_{(1, 76)} = 10.98$, $p = 0.0014$) independent of maternal probiotic treatment with no interaction. ††$p < 0.01$, indicating main effect of maternal diet. N = 9-11 adult offspring per sex per group.

Supplementary Table 8 F0 maternal milk PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
TG/VLDL 1.23....1.32.	4.19
Lactate 1.32....1.34.	3.59
Lactose 3.64....3.69.	2.41
Butyrate/Valerate 0.89....0.90.	2.16
Butyrate/Valerate 0.90....0.91.	1.93
Lactose 3.72....3.75.	1.58
Butyrate 2.15....2.19.	1.50
Formate 8.46....8.46.	1.35
Alanine 1.48....1.49.	0.85

Supplementary Table 9 F0 Milk Metabolome (postnatal day 4)

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Lactate [1.32-1.34]	0.015± 0.005	0.011± 0.004	**$^{\#\#}$0.034± 0.022**	**$^{\#\#}$0.036± 0.016**	(0.021)	(0.95)
Lactose [3.72-3.75]	0.041± $2.4x10^{-3}$	**†0.045± $3.8x10^{-3}$**	**$^{\#}$0.035± $4.3x10^{-3}$**	**$^{\dagger\#}$0.041± $3.5x10^{-3}$**	(0.017)	(0.031)
Butyrate/Valerate [0.89-91]	0.056± $5.5x10^{-3}$	**†0.052± $6.6x10^{-3}$**	**$^{\#}$0.070± $3.3x10^{-3}$**	**$^{\dagger\#}$0.057± $8.3x10^{-3}$**	(0.019)	(0.047)
Alanine [1.47-1.49]	0.0053± $6.4\ x10^{-4}$	0.0037± $8.3\ x10^{-4}$	**$^{\#}$0.0068± 0.0019**	**$^{\#}$0.0058± $7.4\ x10^{-4}$**	(0.021)	(0.084)
Formate [8.46]	$8.1x10^{-5}$± $2.8\ x10^{-5}$	$7.7x10^{-5}$± $1.1\ x10^{-5}$	**$^{\#\#}1.4x10^{-4}$± $2.9\ x10^{-5}$**	**$^{\#\#}2.0x10^{-4}$± $6.5\ x10^{-5}$**	(0.0037)	(0.25)
Creatine [3.04]	0.0029± $3.6\ x10^{-4}$	0.0022± $8.5\ x10^{-4}$	0.0036± 0.0012	0.0031± $6.8\ x10^{-4}$	(0.06)	(0.18)
Triglyceride/VLDL [1.23-1.32]	0.052± 0.011	**†0.062± 0.014**	0.045± 0.009	**†0.069± 0.016**	(0.99)	(0.039)

Table S9 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where a statistically significant diet x probiotic interaction occurred after correcting for the false discovery rate, post-hoc comparisons have been applied and have been explained here.

No significant diet x probiotic interactions occurred in any milk metabolites; thus, post hoc comparisons have not been described.

N=4 per group. Data presented as mean ± standard deviation. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

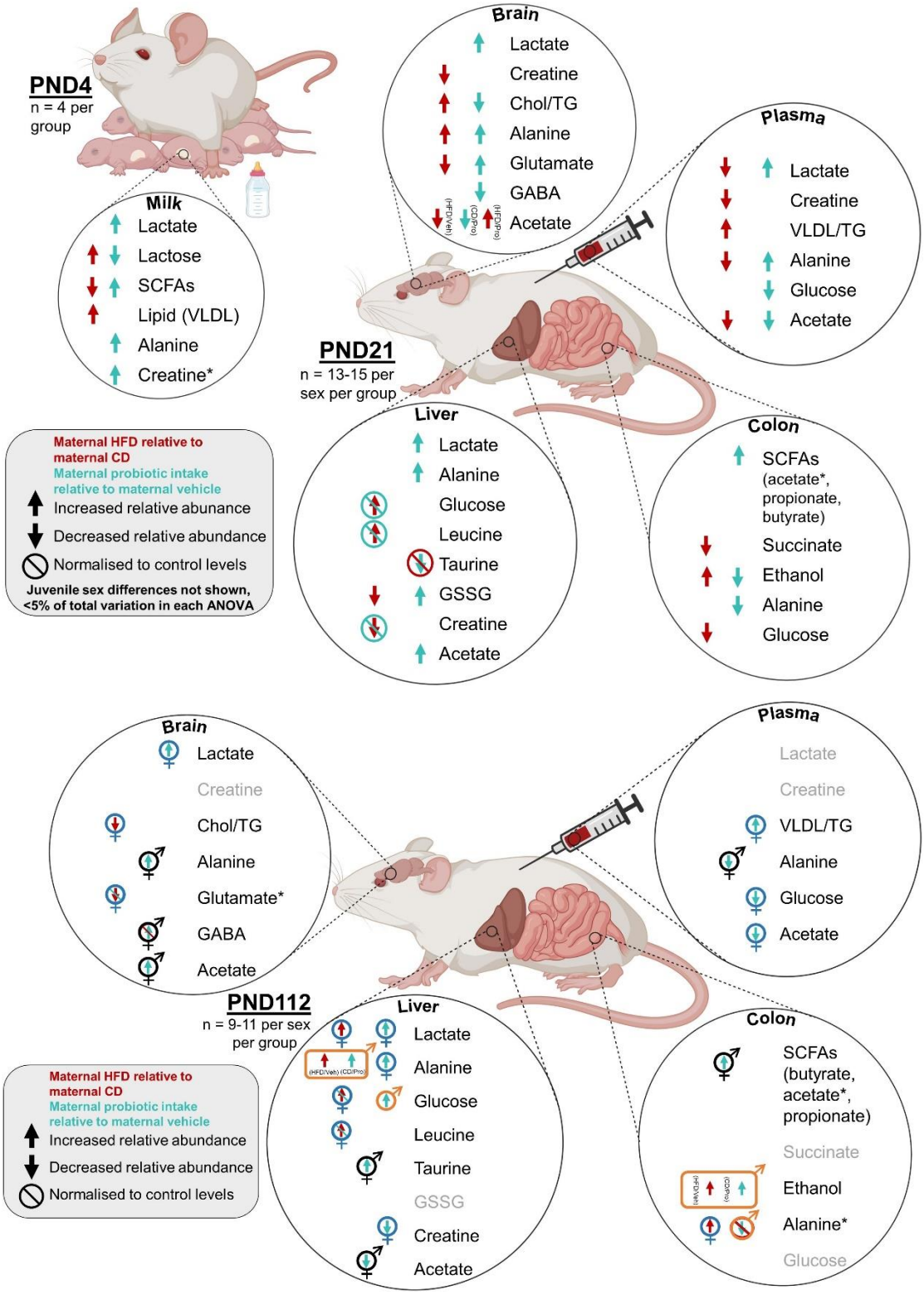

Figure S16 Comprehensive atlas of offspring gut-liver-brain after perinatal exposure to maternal obesity and maternal probiotic intake. Summary of top metabolites from partial least squares discriminant analyses (PLS-DA) shown in Figures S17,S21-S28 in juvenile and adult offspring. Metabolite data is described in Tables S9,S18-S25. *Significant after PLS-DA but not significantly altered after adjusting for multiple testing using the Benjamini & Hochberg method of correction.

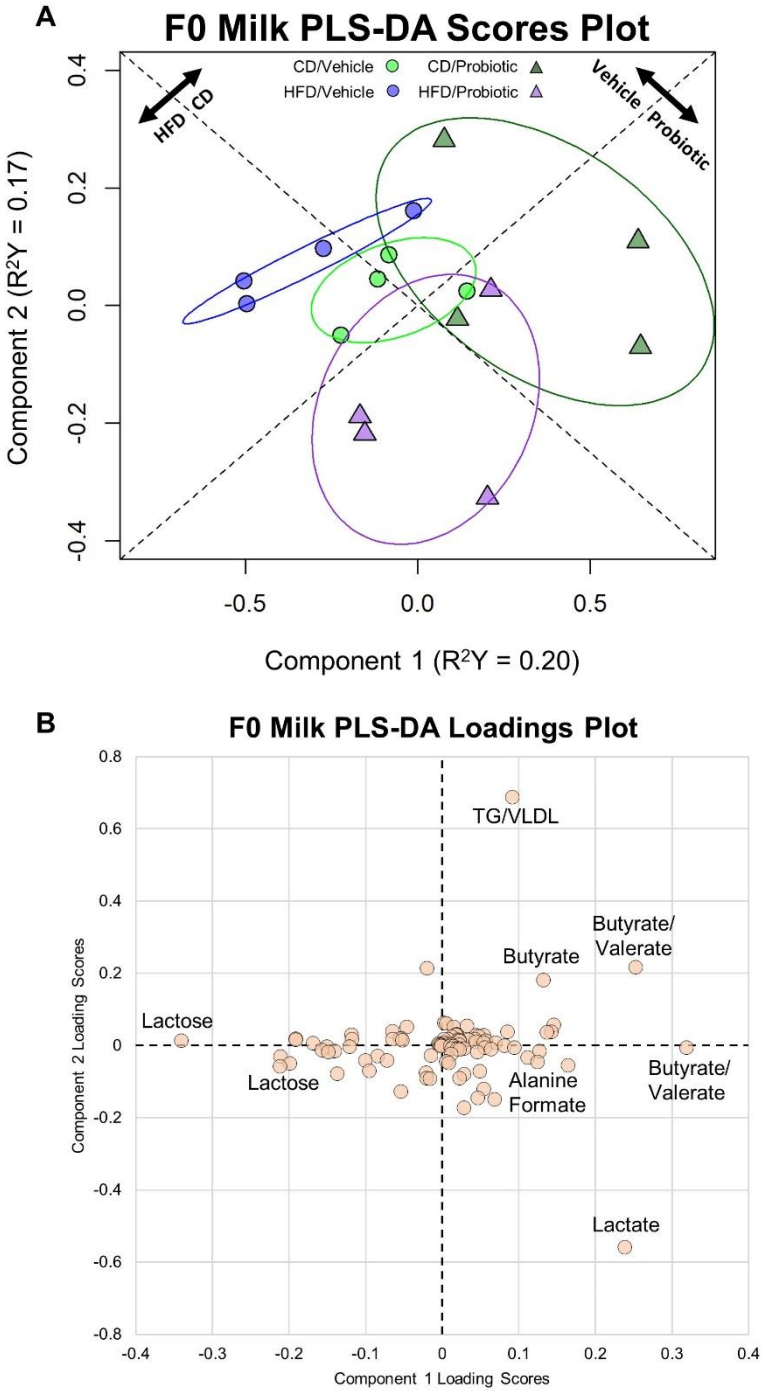

Figure S17 PLS-DA scores and loadings plot of the F0 maternal milk metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 4 per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative $R^2X = 0.79$. Cumulative $R^2Y = 0.37$. Cumulative $Q^2 = 0.12$. 2 component model.

Figure S17 Notes: Similar to the maternal fecal metabolome, group separation between vehicle/probiotic dams occurs between components 1 and 2. The separation between CD and HFD is orthogonal in probiotic-supplemented dams, whereas in dams given vehicle, group separation occurs on the same axis as vehicle/probiotic separation. Also note that the F0 milk PCA plot in Figure 4b was so congruent to the corresponding PLS-DA plot in Figure S17a, that it is unnecessary to provide an additional PCA loadings plot where the PLS-DA loadings plot (Figure S17b) is shown.

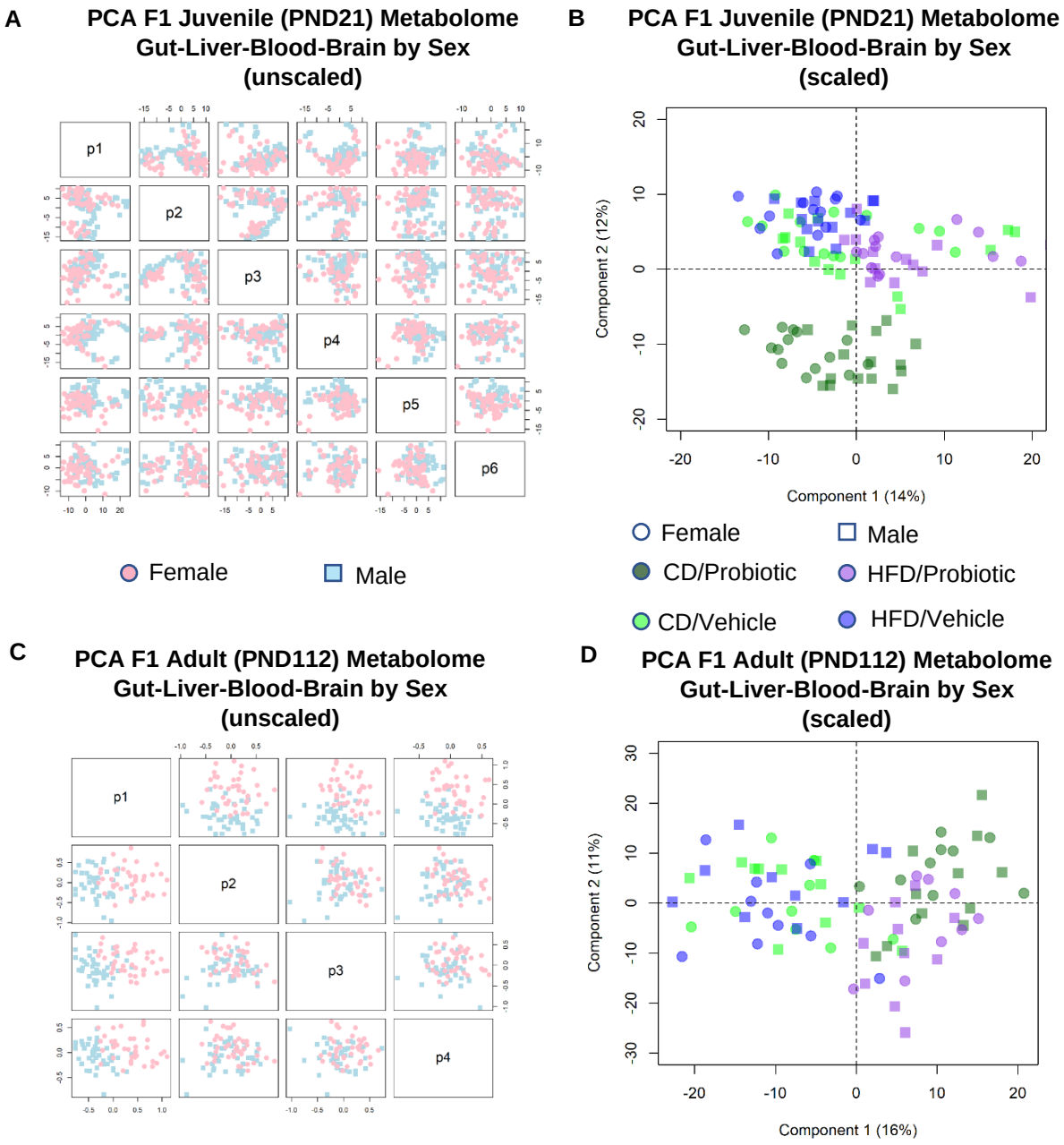

Figure S18 Integrated principal component analysis (PCA) of the offspring gut, liver, blood, and brain metabolome. A) PCA at PND21 (n=111) where male and female offspring are not independently z-scaled. Some clustering is observed in PC5, explaining <5% of total variance. Overall, very minimal clustering by sex is evident. Cumulative $R^2X = 0.51$. **B)** In the same mice where male and female offspring are independently z-scaled, the effect of each treatment (maternal obesity, maternal probiotic intake) affects males and females in the same way compared to the controls. $R^2X = 0.51$ with 6 predictive components. **C)** PCA at PND112 (n=80) where male and female offspring are not independently z-scaled. Strong sex-effects are observed in PC1, explaining 24% of total variance. Cumulative $R^2X = 0.57$. **D)** In the same mice where male and female adult offspring are independently z-scaled, the effect of each treatment (maternal obesity, maternal probiotic intake) affects males and females in the same way compared to the controls. $R^2X = 0.55$ with 6 predictive components.

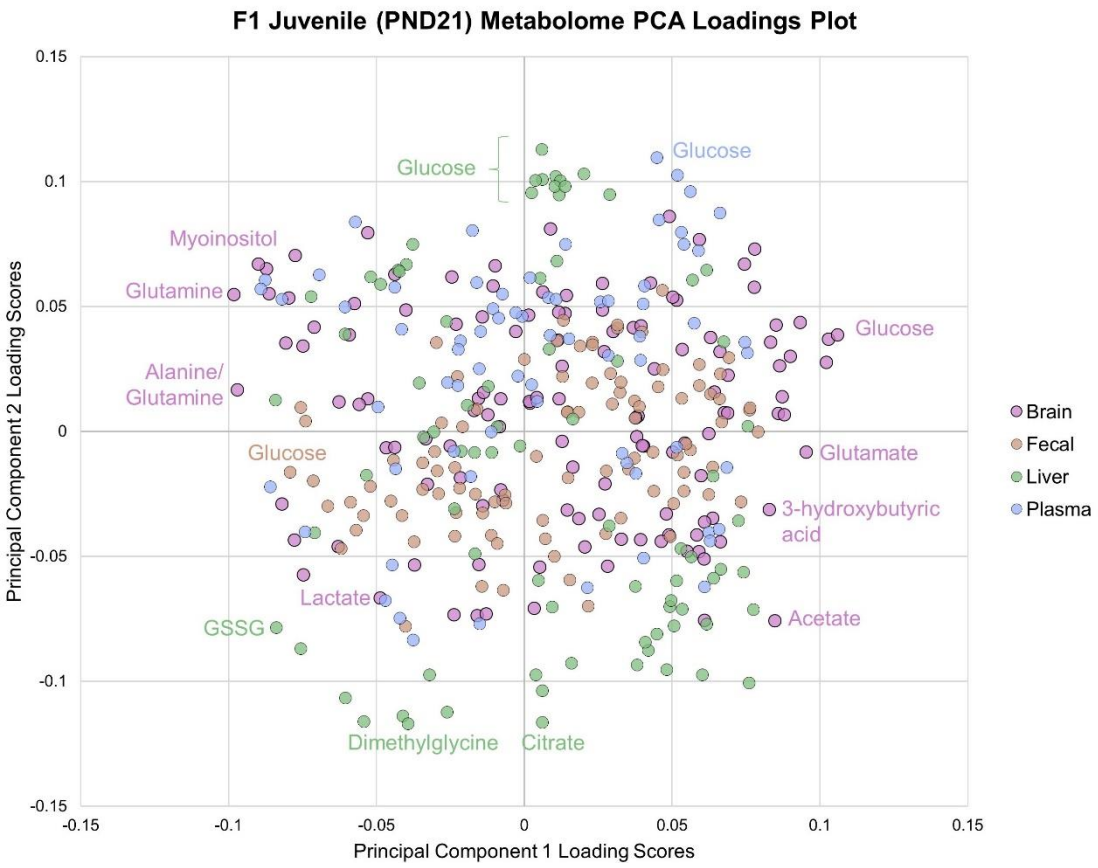

Figure S19 Loadings plot for F1 juvenile integrated PCA model. Loadings correspond to the PCA scores plot shown in Figure 5A.

Figure S19 Notes: Unlike in the dams, changes to the brain metabolome in the juvenile offspring are equal to or greater than the variation explained by fecal, liver, or plasma metabolites. Rather than a select few metabolites being altered by maternal probiotic supplementation or high-fat diet intake, the variation between the juvenile offspring is contributed by many metabolites from each tissue.

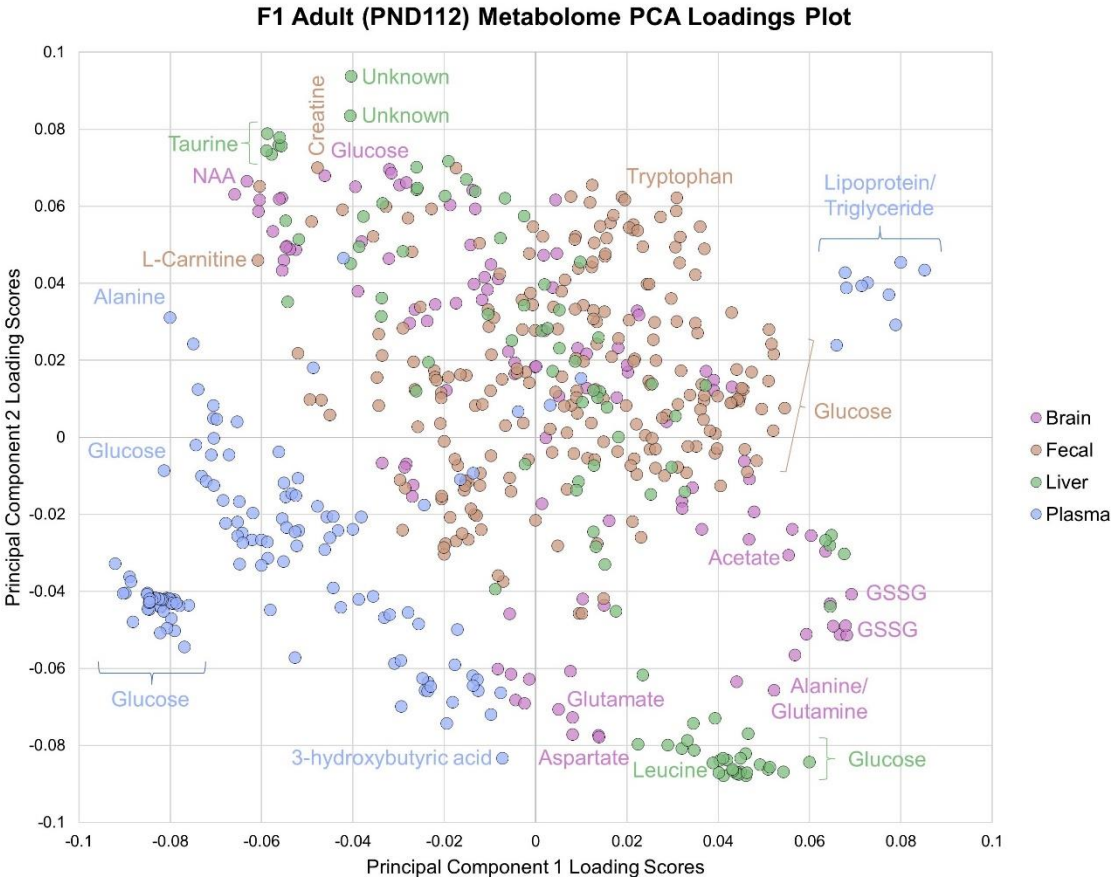

Figure S20 Loadings plot for F1 adult integrated PCA model. Loadings correspond to the PCA scores plot shown in Figure 5C.

Figure S20 Notes: In the adult offspring, plasma metabolites are important in the variation exhibited across the first principal component. Metabolite peaks corresponding to glucose were lower in adult offspring of probiotic-fed dams, whilst plasma lipid and lipoprotein resonances were increased in the adult offspring of probiotic-fed dams and reduced in the adult offspring of obese dams.

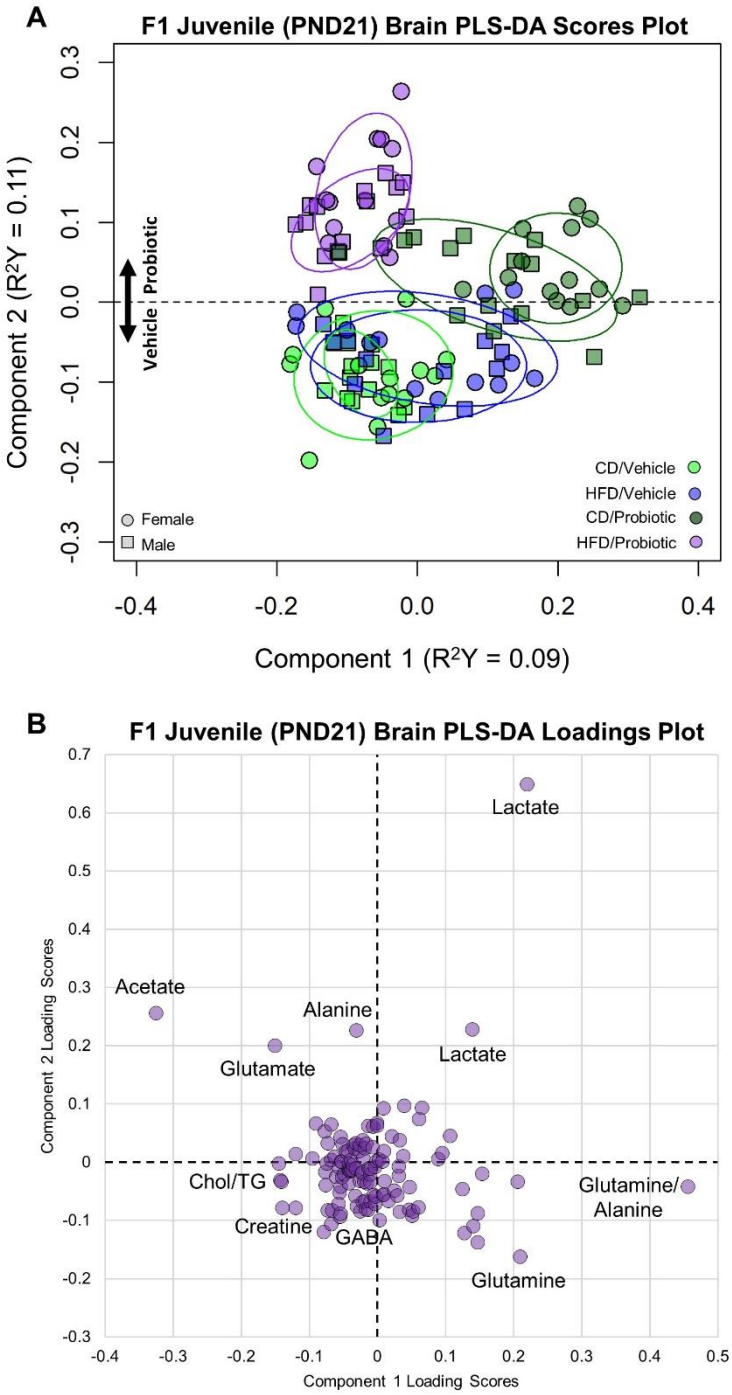

Figure S21 PLS-DA scores and loadings plot of the F1 juvenile (PND21) brain metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 13-15 per sex per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative R^2X = 0.67. Cumulative R^2Y = 0.32. Cumulative Q^2 = 0.19. 5 component model.

Figure S21 Notes: Separation between the juvenile offspring of maternal probiotic and maternal vehicle dams occurs in component 2, largely due to the main effect increase in brain lactate (Table S18). Separation between CD/probiotic and HFD/probiotic offspring occurs in the first component. No clustering by sex is observed in the first 2 components.

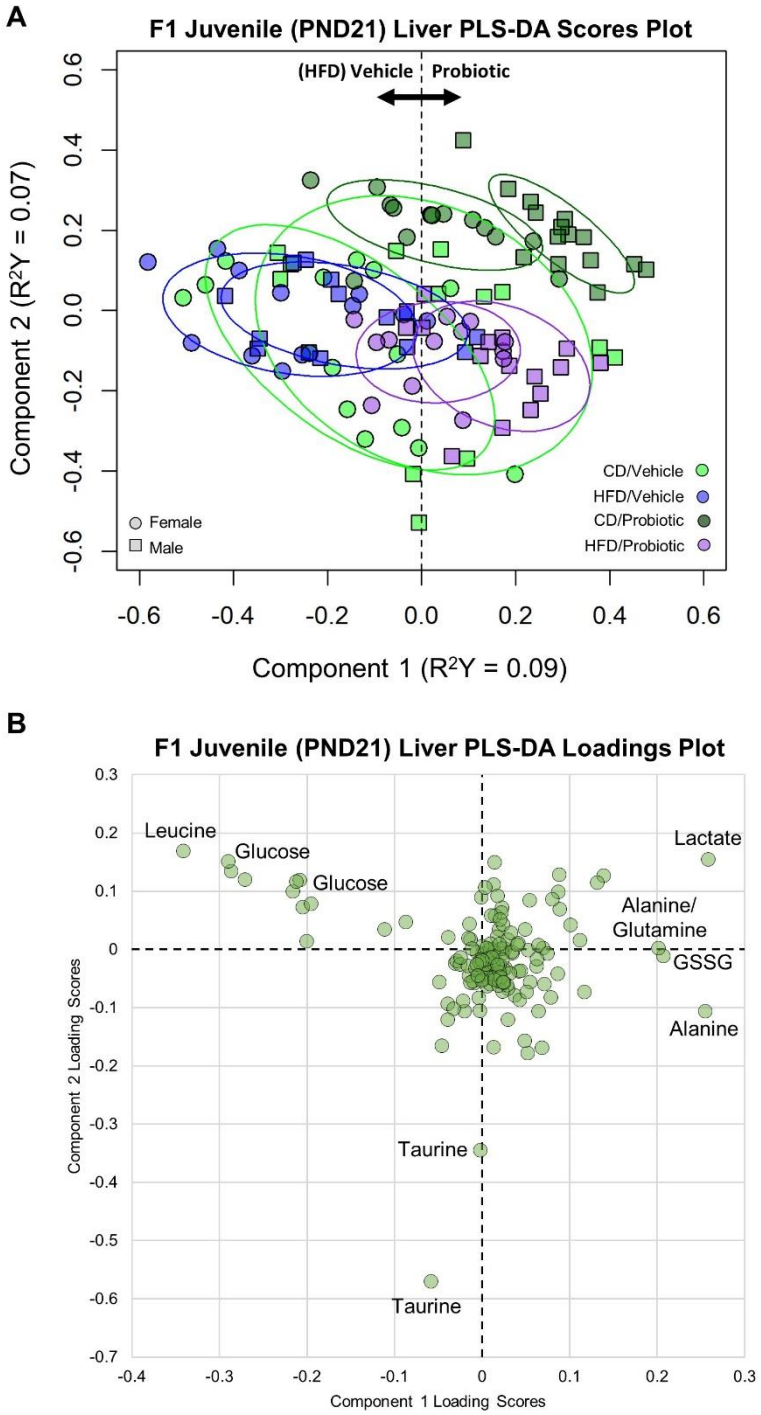

Figure S22 PLS-DA scores and loadings plot of the F1 juvenile (PND21) liver metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 13-15 per sex per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative R^2X = 0.88. Cumulative R^2Y = 0.44. Cumulative Q^2 = 0.25. 8 component model.

Figure S22 Notes: Separation between offspring of maternal vehicle/HFD and maternal probiotic dams occurs in component 1. Separation between CD/probiotic offspring and offspring of obese dams occurs in the second component. Male/female separation is observed in the CD/probiotic group only, in the first component.

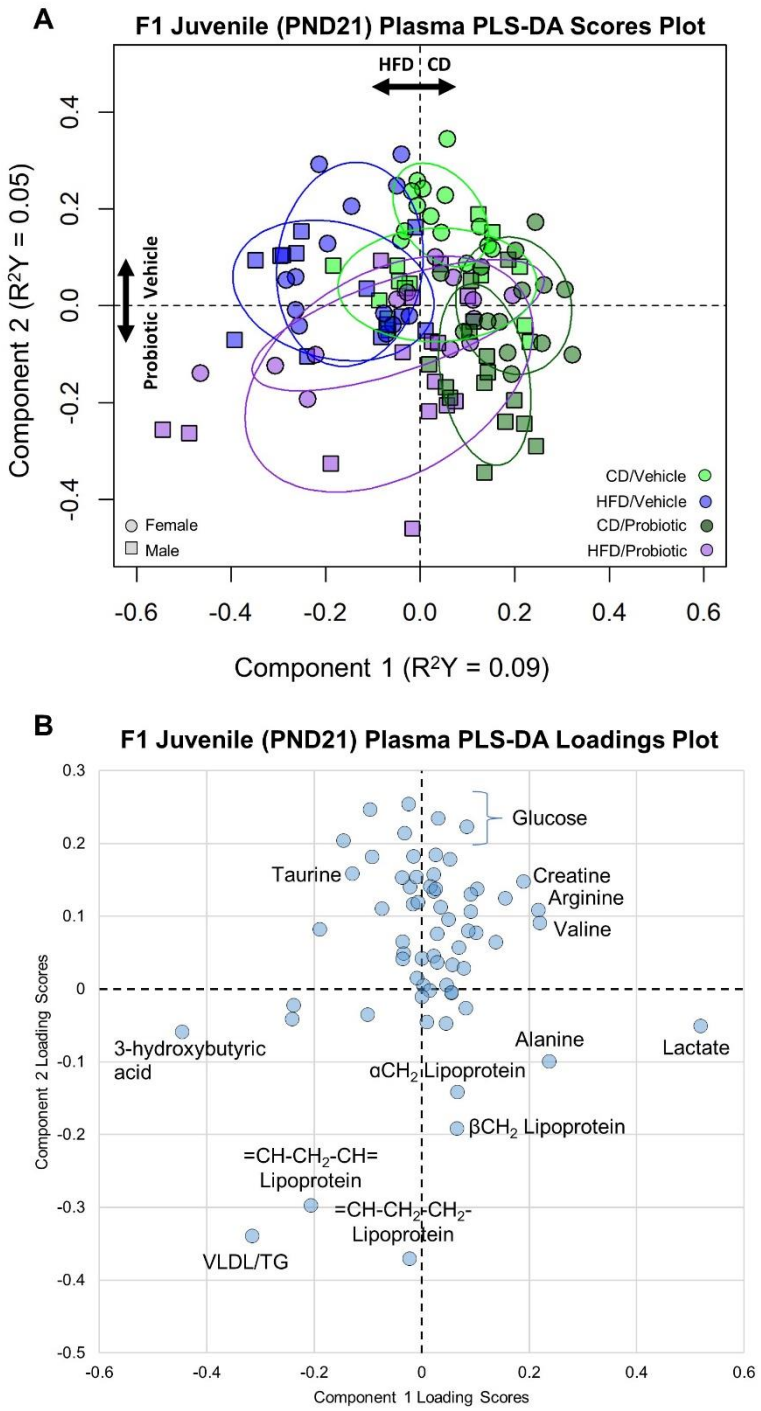

Figure S23 PLS-DA scores and loadings plot of the F1 juvenile (PND21) plasma metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 13-15 per sex per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative R^2X = 0.81. Cumulative R^2Y = 0.30. Cumulative Q^2 = 0.17. 5 component model.

Figure S23 Notes: Little clustering is observed between groups, including by sex. Some separation between offspring of obese and lean dams may occur in component 1. Orthogonally, in component 2, there is some separation between probiotic and vehicle offspring.

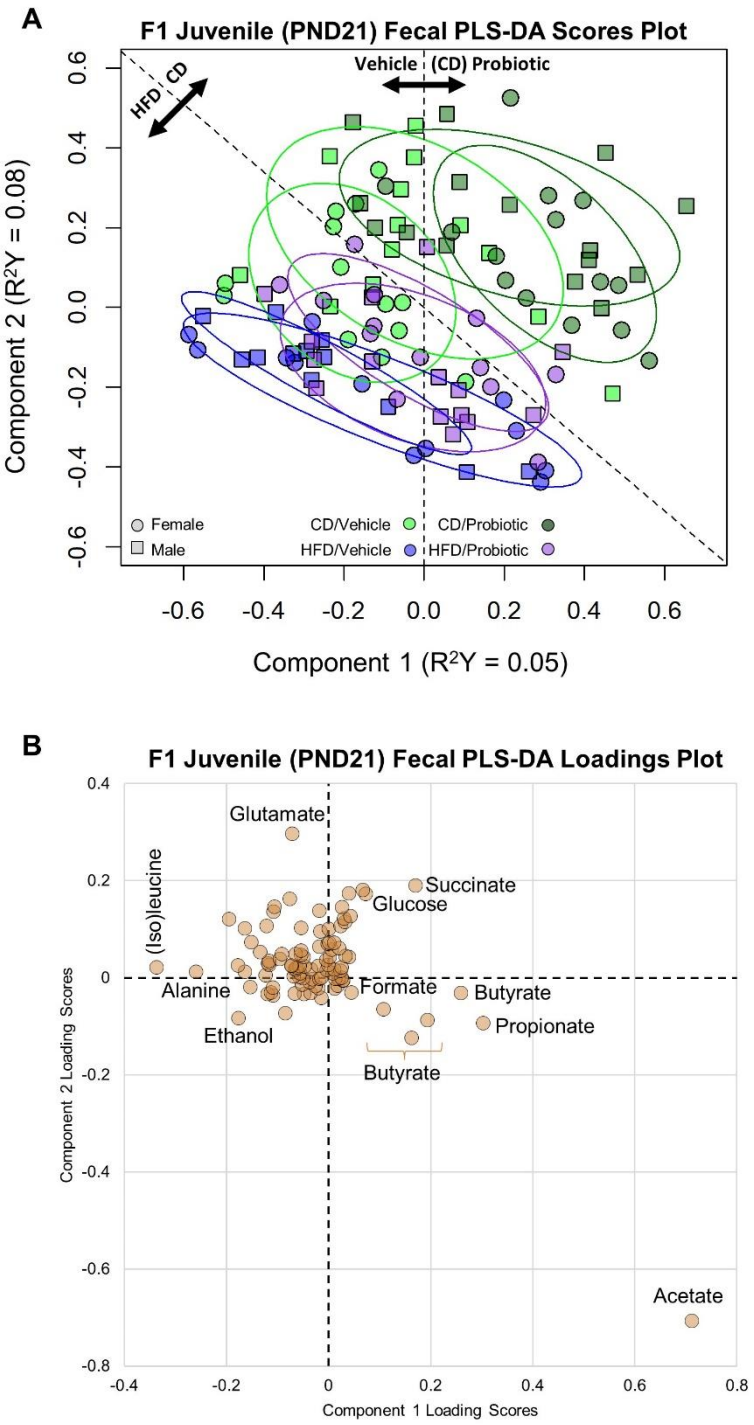

Figure S24 PLS-DA scores and loadings plot of the F1 juvenile (PND21) fecal metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 13-15 per sex per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative R^2X = 0.49. Cumulative R^2Y = 0.22. Cumulative Q^2 = 0.13. 3 component model.

Figure S24 Notes: Offspring of lean and obese dams separate between components 1 and 2, with the fecal metabolome of offspring from CD/probiotic and HFD/vehicle dams being most distinguishable from each other.

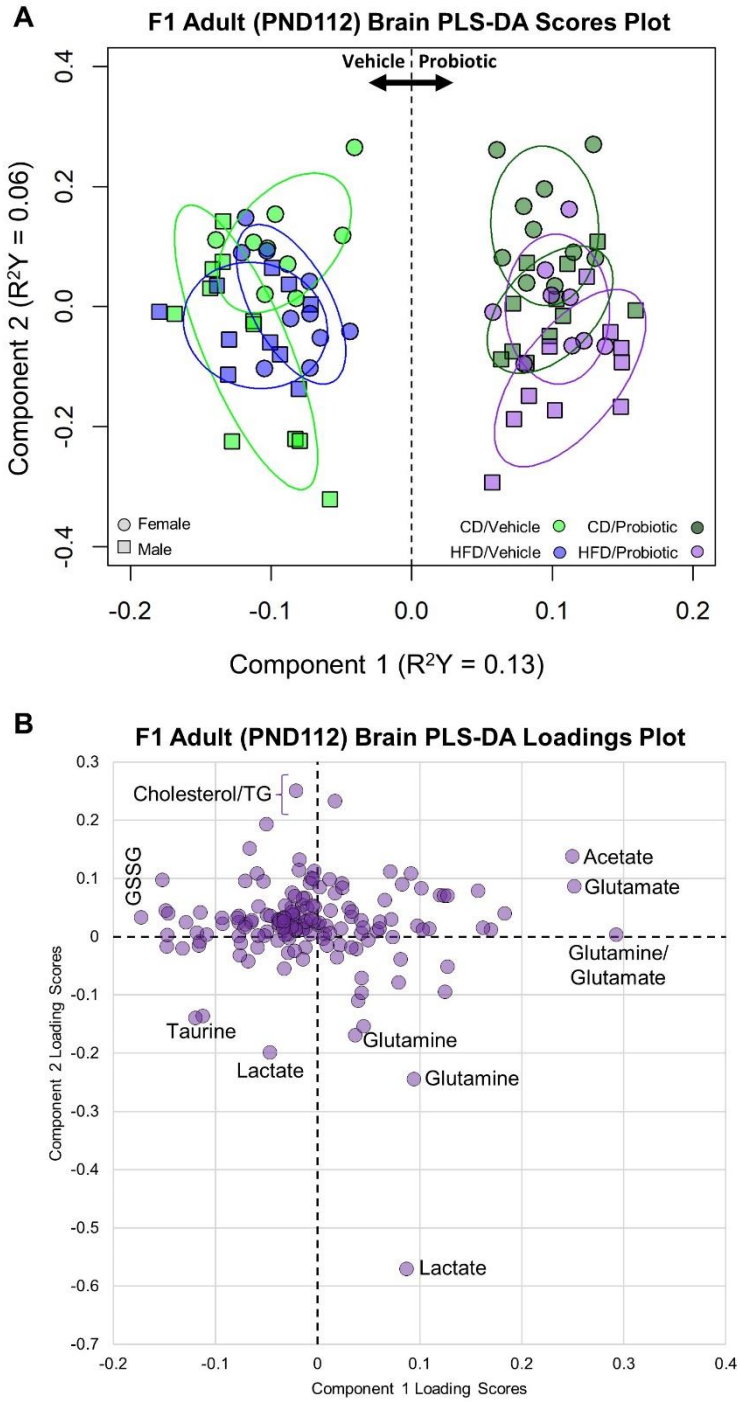

Figure S25 PLS-DA scores and loadings plot of the F1 adult (PND112) brain metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 9-11 per sex per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative $R^2X = 0.44$. Cumulative $R^2Y = 0.19$. Cumulative $Q^2 = 0.15$. 2 component model.

Figure S25 Notes: Marked differences in the brain metabolome of adult offspring are observed between vehicle and probiotic groups in component 1, independent of sex and maternal diet. In the offspring of probiotic-supplemented dams, some sex differences may be observed orthogonally in component 2.

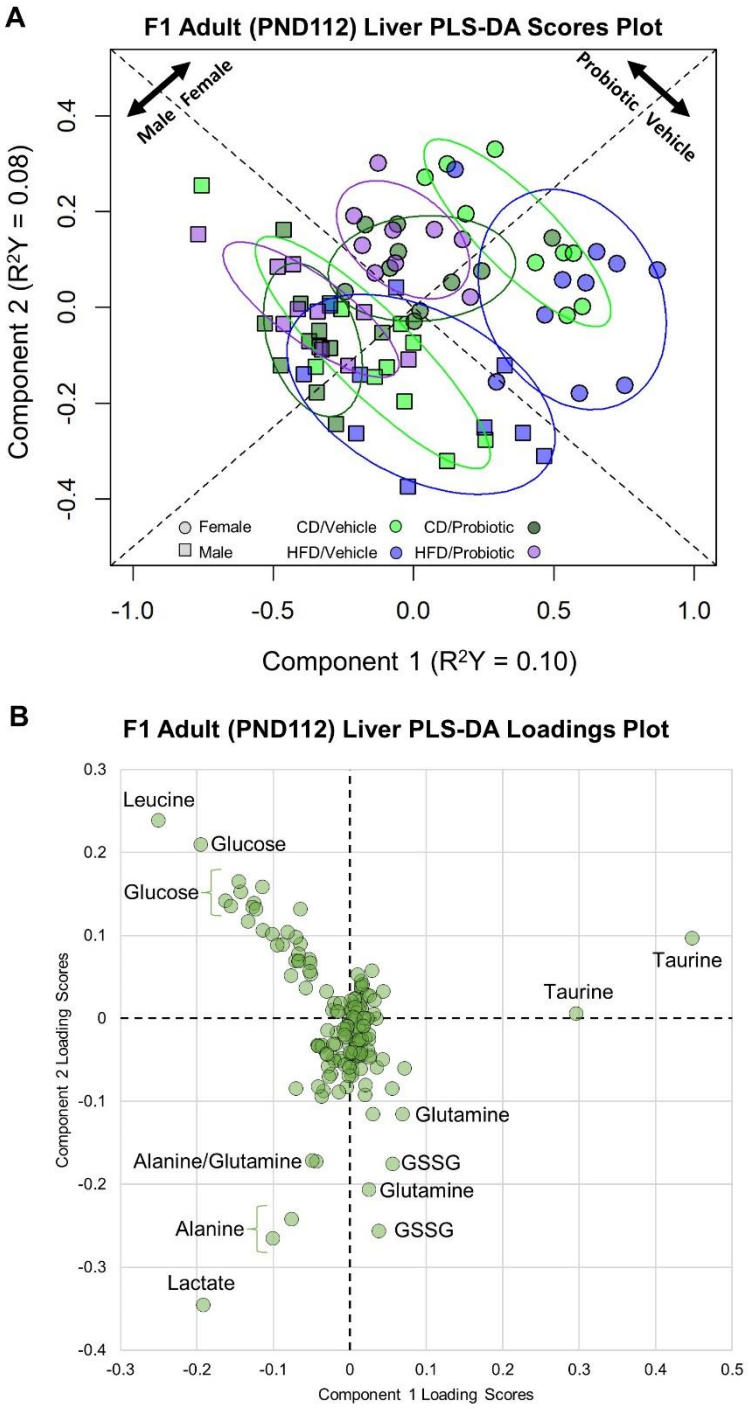

Figure S26 PLS-DA scores and loadings plot of the F1 adult (PND112) liver metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 9-11 per sex per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative $R^2X = 0.65$. Cumulative $R^2Y = 0.18$. Cumulative $Q^2 = 0.14$. 2 component model.

Figure S26 Notes: Some separation in the liver metabolome is observed between adult offspring of probiotic and vehicle dams, independent of maternal diet. Orthogonal to this separation, a sex effect in the adult offspring is clear, independent of maternal factors.

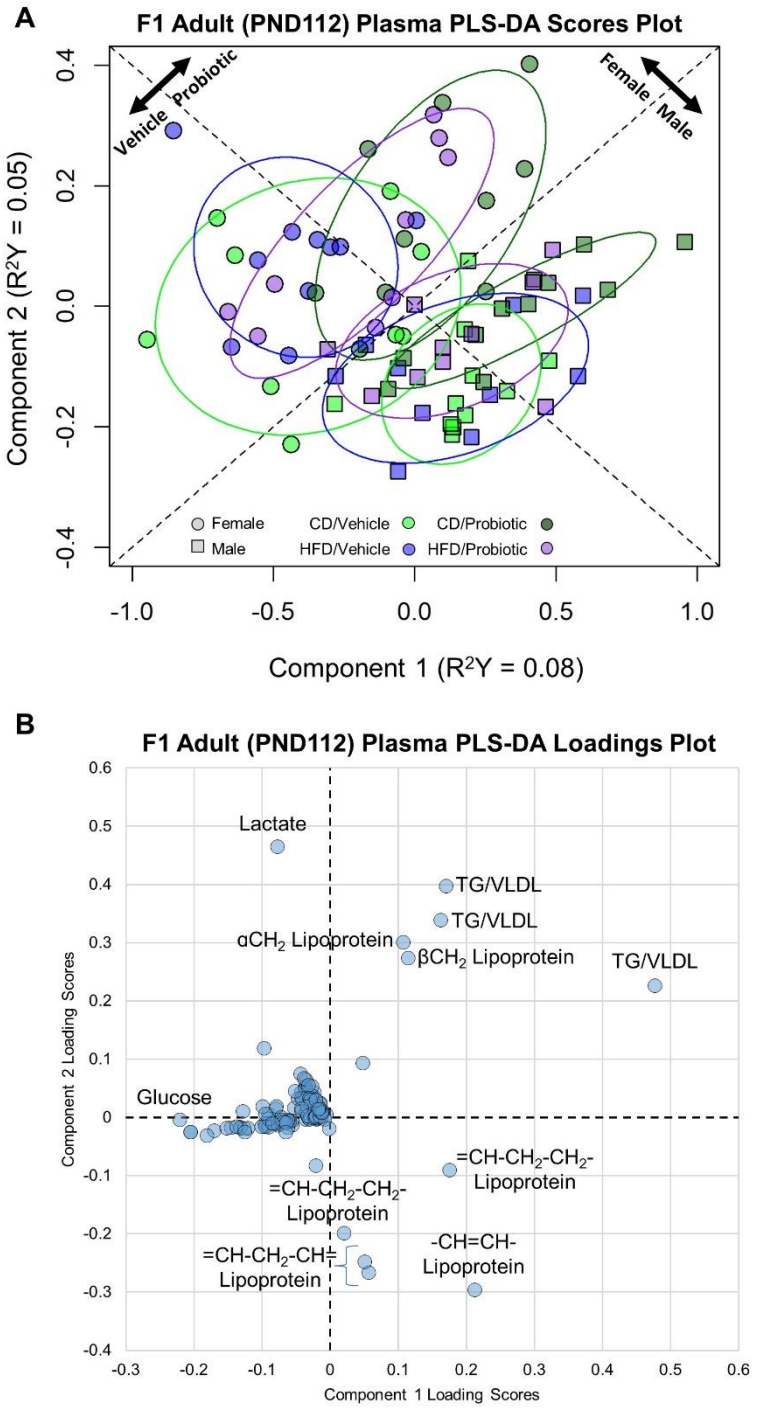

Figure S27 PLS-DA scores and loadings plot of the F1 adult (PND112) plasma metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 9-11 per sex per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative $R^2X = 0.84$. Cumulative $R^2Y = 0.13$. Cumulative $Q^2 = 0.09$. 2 component model.

Figure S27 Notes: Like the liver metabolome of adult offspring, a sex effect is clear in the plasma metabolome between components 1 and 2, largely independent of maternal factors. Orthogonal to this separation, minimal clustering is observed between adult offspring of probiotic and vehicle dams, independent of maternal diet and sex.

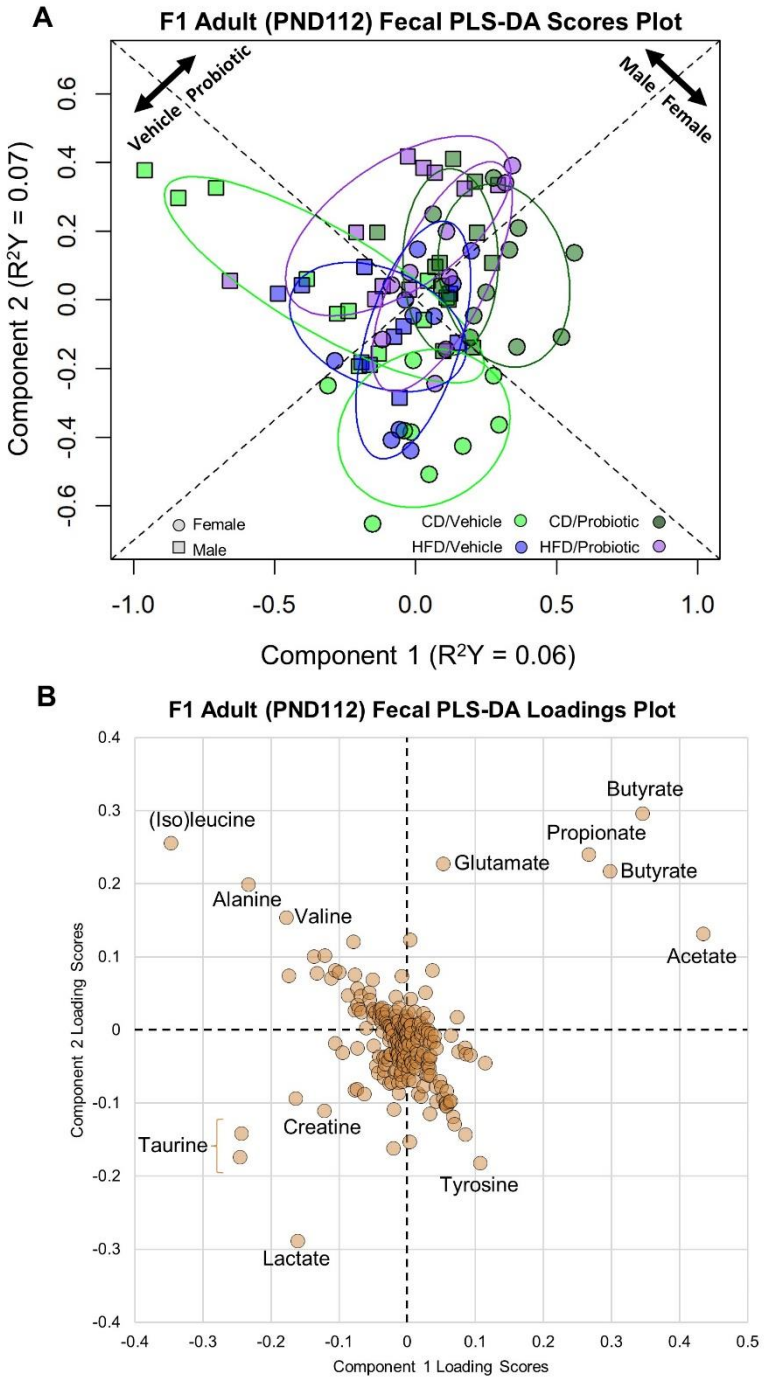

Figure S28 PLS-DA scores and loadings plot of the F1 adult (PND112) fecal metabolome. A) PLS-DA scores plot and accompanying **B)** loadings plot. N = 9-11 per sex per group. Pareto scaling of predictors and standard scaling of response(s). Cumulative $R^2X = 0.46$. Cumulative $R^2Y = 0.13$. Cumulative $Q^2 = 0.07$. 2 component model.

Figure S28 Notes: Very little separation is clear between the fecal metabolomes of adult offspring across the 8 different groups. However, between the vehicle and probiotic adult offspring, some separation is driven by a relative main effect increase in gut butyrate concentration in the latter group (Table S25). Orthogonally, male/female separation is visually apparent.

Supplementary Table 10 F1 juvenile brain PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Lactate 1.32....1.34.	4.23
Acetate 1.91....1.92.	2.79
Alanine/Glutamine 3.75....3.79.	2.70
Creatine 3.04....3.04.	2.10
Creatine 3.92....3.94.	2.06
Lactate 4.09....4.13.	1.91
Alanine 1.47....1.49.	1.74
Glutamine 2.49....2.50.	1.64
Glutamate 2.34....2.37.	1.59
Cholesterol/TG 1.38....1.44.	1.55
3-hydroxybutyric acid 1.19....1.21.	1.50
Glutamine/Glutamate 2.11....2.18.	1.46
Cholesterol/TG 0.86....0.90.	1.39
Glutamine 2.43....2.48.	1.27
GABA 1.88....1.91.	1.22

Supplementary Table 11 F1 juvenile liver PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Lactate 1.32....1.34.	3.62
Taurine 3.26....3.28.	3.20
Alanine 1.47....1.50.	2.92
Glucose 3.41....3.44.	2.25
Glucose 3.56....3.56.	2.18
GSSG/Glucose 3.76....3.79.	2.00
Leucine 3.70....3.74.	1.97
Glucose 3.67....3.69.	1.70
Glucose 4.64....4.66.	1.67
Lactate 4.09....4.13.	1.67
Glutamine 2.45....2.48.	1.59
Glucose 3.83....3.85.	1.55
GSSG 2.16....2.19.	1.53

Supplementary Table 12 F1 juvenile plasma PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Lactate 1.32....1.34.	2.33
Glucose 3.67....3.69.	1.88
VLDL/TG 0.84....0.88.	1.80
3-hydroxybutyric acid 1.19....1.21.	1.79
Valine 0.98....1.05.	1.76
Arginine 4.31....4.35.	1.46
(Iso)leucine 0.93....0.98.	1.44
=CH-CH_2-CH_2- Lipoprotein 1.96....2.11.	1.39
=CH-CH_2-CH= Lipoprotein 2.69....2.82.	1.34
Creatine 3.01....3.04.	1.32
Alanine 1.47....1.49.	1.31
Arginine 1.71....1.77.	1.30
Glucose 3.89....3.91.	1.23
Taurine 3.48....3.51.	1.23
Glucose 3.94....3.98.	1.17
Glucose 3.82....3.86.	1.15
βCH_2 Lipoprotein 1.56....1.62.	1.09
αCH_2 Lipoprotein 2.29....2.32.	1.06

Supplementary Table 13 F1 juvenile fecal PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Acetate 1.91....1.93.	4.09
Ethanol 1.18....1.20.	1.99
Glucose 4.64....4.66.	1.91
Succinate 2.40....2.42.	1.89
Ethanol 3.64....3.68.	1.88
Glutamate 2.34....2.37.	1.82
Taurine 3.48....3.50.	1.48
(Iso)leucine 0.95....0.98.	1.47
Glucose 3.41....3.44.	1.44
Propionate 1.06....1.07.	1.33
Butyrate/Propionate 2.17....2.20.	1.25
Taurine 3.25....3.28.	1.24
Alanine 1.47....1.49.	1.14
Butyrate 1.54....1.58.	1.09

Supplementary Table 14 F1 adult brain PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Lactate 1.32....1.34.	3.68
Glutamine 3.78....3.78.	3.21
Glutamine/Glutamate 2.11....2.17.	2.76
Acetate 1.91....1.92.	2.47
GSSG 2.96....2.97.	2.04
GSSG 4.58....4.61.	1.90
GSSG 2.56....2.57.	1.90
Cholesterol/TG 0.93....0.98.	1.87
GSSG 2.98....2.99.	1.83
GSSG 2.53....2.56.	1.81
Taurine 3.40....3.44.	1.80
Cholesterol/TG 0.86....0.90.	1.73
Taurine 3.25....3.28.	1.62
Glutamate 3.75....3.76.	1.58
Lactate 4.09....4.13.	1.57
Glutamine 2.43....2.47.	1.35
Glutamate 2.34....2.37.	1.22

Supplementary Table 15 F1 adult liver PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Taurine 3.26..28.	4.51
Lactate 1.32....1.34.	3.81
Taurine 3.42....3.44.	3.23
Alanine 1.48....1.49.	2.44
Leucine 3.70….74.	2.41
Glucose 4.64....4.66.	1.94
Glucose 3.41....3.41.	1.85
GSSG 2.15....2.19.	1.70
Glucose 3.89....3.89.	1.63
Glutamine 3.79....3.79.	1.57

Supplementary Table 16 F1 adult plasma PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
Triglyceride/VLDL 1.22....1.32.	4.01
Lactate 1.32....1.34.	3.39
VLDL/Triglyceride 0.88....0.92.	3.38
-CH=CH- Lipoprotein 5.25....5.38.	2.92
Triglyceride/VLDL 1.34....1.39.	2.68
αCH_2 Lipoprotein 2.21....2.27.	2.22
βCH_2 Lipoprotein 1.56....1.62.	1.99
$=CH\text{-}CH_2\text{-}CH_2\text{-}$ Lipoprotein 2.74....2.78.	1.91
$=CH\text{-}CH_2\text{-}CH_=$ Lipoprotein 2.78....2.82.	1.90
Glucose 3.40....3.42.	1.79
$=CH\text{-}CH_2\text{-}CH_2\text{-}$ Lipoprotein 1.97....2.04.	1.71
Glucose 3.83....3.86.	1.70
Glucose 3.89....3.91.	1.69
$=CH\text{-}CH_2\text{-}CH_=$ Lipoprotein 2.69....2.74.	1.68

Supplementary Table 17 F1 adult fecal PLS-DA model VIP scores

Metabolite Bin (ppm)	PLS-DA VIP Score
(Iso)leucine 0.93....0.98.	4.22
Lactate 1.32....1.34.	3.25
Alanine 1.47....1.49.	3.18
Taurine 3.25....3.28.	3.00
Creatine 3.04....3.04.	2.72
Butyrate 0.89....0.91.	2.63
Valine 0.99....1.00.	2.57
Taurine 3.41....3.44.	2.54
Acetate 1.92....1.92.	2.41
Butyrate 1.54....1.58.	2.32
Glutamate 2.34....2.37.	2.30
Propionate 2.15....2.17.	2.04

Supplementary Table 18 F1 (PND21) Juvenile Brain Metabolome

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Brain Lactate [1.32-1.34; 4.09-4.13]	0.0705± 0.0049	0.0739± 0.0044	$^{\#\#\#\#}$**0.0810± 0.0036**	$^{\#\#\#\#}$**0.0817± 0.0053**	(<0.0001)	(0.073)
Brain Creatine [3.04]	0.0507± 0.0018	*****0.0480 ± 0.0016**	0.0497± 0.0009	*****0.0488± 0.0019**	(0.18)	(<0.0001)
Brain Cholesterol/Triglyceride [1.38-1.44]	0.0143± $8.3x10^{-4}$	††††**0.0153± 0.0011**	$^{\#\#}$**0.0136± 0.0012**	$^{\dagger\dagger\dagger\dagger\#\#}$**0.0148± $8.4x10^{-4}$**	(0.0041)	(<0.0001)
Brain Alanine [1.47-1.49]	0.0085± $8.7x10^{-4}$	††††**0.0091± $5.2x10^{-4}$**	$^{\#\#\#\#}$**0.0094± $3.9x10^{-4}$**	$^{\dagger\dagger\dagger\dagger\#\#\#\#}$**0.010± $5.5x10^{-4}$**	(<0.0001)	(<0.0001)
Brain Glutamate [2.34-2.37]	0.0514± 0.0020	†††**0.0509± 0.0017**	$^{\#\#\#\#}$**0.0534± 0.0013**	$^{\dagger\dagger\dagger\#\#\#\#}$**0.0515± 0.0016**	(<0.0001)	(0.0006)
Brain GABA [1.88-1.91]	0.0116± $6.5x10^{-4}$	0.0116± $5.8x10^{-4}$	$^{\#\#\#\#}$**0.0110 ± $4.2x10^{-4}$**	$^{\#\#\#\#}$**0.0110 ± $4.5x10^{-4}$**	(<0.0001)	(0.79)
Brain Acetate [1.92]	0.00534± $7.6x10^{-4}$	*****0.00419± 0.0013**	******0.00400 ± $8.0x10^{-4}$**	******0.00699 ± $9.8x10^{-4}$**	(0.0002)	(<0.0001)
Brain Glutamine [2.43-2.48]	0.0268± $2.4x10^{-3}$	††**0.0259 ± $1.4x10^{-3}$**	0.0266± $1.4x10^{-3}$	††**0.0258± $1.3x10^{-3}$**	(0.75)	(0.010)

Table S18 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where a statistically significant diet x probiotic interaction occurred after correcting for the false discovery rate, post-hoc comparisons have been applied and have been explained here.

There was a significant diet x probiotic interaction for the juvenile offspring for brain creatine ($F_{(1, 106)}$ = 9.24, q = 0.021). Tukey post hoc testing revealed a significant reduction in brain creatine in HFD/vehicle offspring compared to CD/vehicle (p < 0.0001) and CD/probiotic offspring (p = 0.0007). HFD/probiotic offspring also had reduced brain creatine compared to CD/vehicle offspring (p < 0.0001).

There was a significant diet x probiotic interaction for the juvenile offspring for brain acetate ($F_{(1, 105)}$ = 121.1, q < 0.0001). Tukey post hoc testing revealed a significant reduction in brain acetate in HFD/vehicle and CD/probiotic offspring compared to CD/vehicle (p = 0.0002; p < 0.0001, respectively). HFD/probiotic offspring had increased brain acetate compared to all other groups (p < 0.0001), driving the significant main effect of increased brain acetate in the juvenile offspring of probiotic dams relative to the offspring of dams given vehicle, independent of diet.

No other significant diet x probiotic interactions were observed.

N=26-29 per group. Data presented as mean ± standard deviation with combined sexes. †p < 0.05, ††p < 0.01, †††p < 0.001, ††††p < 0.0001 indicating main effect of maternal diet. $^{\#}$p < 0.05, $^{\#\#}$p < 0.01, $^{\#\#\#}$p < 0.001, $^{\#\#\#\#}$p < 0.0001 indicating main effect of maternal probiotic intake. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

Supplementary Table 19 F1 (PND21) Juvenile Liver Metabolome

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Liver Lactate [1.32-1.34]	0.060± 0.012	0.062± 0.014	###**0.071± 0.016**	###**0.073± 0.017**	(0.0003)	(0.45)
Liver Alanine [1.47-1.50]	0.030± 0.0080	0.027± 0.0066	****0.033± 0.0071**	******0.038± 0.0079**	(<0.0001)	(0.45)
Liver Glucose [4.64-4.66]	0.027± 0.0066	****0.032± 0.0052**	0.025± 0.0043	0.024± 0.0040	(<0.0001)	(0.21)
Liver Leucine [3.70-3.74]	0.045± 0.0092	****0.052± 0.0073**	0.043± 0.0068	0.041± 0.0056	(<0.0001)	(0.15)
Liver Taurine [3.26-3.28]	0.095± 0.018	0.090± 0.014	****0.078± 0.0075**	0.095± 0.0091	(0.013)	(0.031)
Liver GSSG [2.16-2.19]	0.018± 0.0036	†**0.016± 0.0028**	####**0.021± 0.0032**	†####**0.020± 0.0031**	(<0.0001)	(0.043)
Liver Creatine [3.04]	0.0018± $5.2x10^{-4}$	****0.0014 ± 1.8x10^-4**	0.0017± $3.3x10^{-4}$	0.0019± $6.8x10^{-4}$	(0.024)	(0.23)
Liver Acetate [1.92]	0.0033± $7.9x10^{-4}$	0.0033± $4.7x10^{-4}$	***0.0038± 5.3x10^-4**	******0.0047 ± 5.6x10^-4**	(<0.0001)	(0.0096)

Table S19 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where a statistically significant diet x probiotic interaction occurred after correcting for the false discovery rate, post-hoc comparisons have been applied and have been explained here.

A significant diet x probiotic interaction was identified for liver alanine ($F_{(1, 105)} = 7.42$, $q = 0.023$). Post hoc Tukey testing revealed that HFD/probiotic offspring had significantly increased liver alanine levels relative to CD/vehicle and HFD/vehicle offspring (both $p < 0.0001$). CD/probiotic offspring also had significantly increased alanine levels compared to HFD/vehicle offspring ($p = 0.0033$), and less levels compared to HFD/probiotic offspring ($p = 0.050$).

A significant diet x probiotic interaction was identified for liver glucose ($F_{(1, 106)} = 9.75$, $q = 0.011$). HFD/vehicle offspring had increase glucose levels compared to CD/vehicle offspring ($p = 0.0042$) and compared to CD/probiotic and HFD/probiotic offspring (both $p < 0.0001$).

Similarly, an interaction was identified for liver leucine ($F_{(1, 107)} = 9.00$, $q = 0.014$) due to an increase in HFD/vehicle offspring compared to CD/vehicle ($p = 0.0060$), CD/probiotic ($p = 0.0002$) and HFD/probiotic ($p < 0.0001$).

A significant interaction was identified for liver taurine ($F_{(1, 107)} = 20.36$, $q < 0.0001$), whereby levels in CD/probiotic juvenile offspring were relatively lower than CD/vehicle ($p < 0.0001$), HFD/vehicle ($p = 0.0018$) and HFD/probiotic ($p < 0.0001$).

A significant interaction was identified for liver creatine ($F_{(1, 105)} = 10.43$, $q = 0.010$), whereby levels were lower in HFD/vehicle offspring only compared to CD/vehicle offspring ($p = 0.0098$) and HFD/probiotic offspring ($p = 0.0011$).

A significant interaction was identified for liver acetate ($F_{(1, 107)} = 15.63$, $q = 0.0008$). Post hoc Tukey tests revealed that this interaction was driven by increased acetate in the HFD/probiotic

offspring relative to all other groups ($p < 0.0001$). However, CD/probiotic offspring also had greater levels of liver acetate than HFD/vehicle offspring ($p = 0.0041$), and CD/vehicle offspring ($p = 0.014$).

No other significant diet x probiotic interactions were observed.

N=26-29 per group. Data presented as mean ± standard deviation with combined sexes. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, , $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

Supplementary Table 20 F1 (PND21) Juvenile Plasma Metabolome

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Plasma Lactate [1.32-1.34]	0.084± 0.012	**††0.076 ± 0.013**	**##0.091± 0.010**	**††##0.084± 0.015**	(0.0035)	(0.0032)
Plasma Creatine [3.03-3.04]	0.0048± 0.0011	**††††0.0038± 7.6x10^{-4}**	0.0044± 0.0010	**††††0.036± 0.0011**	(0.078)	(<0.0001)
Plasma VLDL/TG [0.84-0.88]	0.065± 0.0065	**††††0.073± 0.0069**	0.066± 0.0071	**††††0.074± 0.0083**	(0.25)	(<0.0001)
Plasma Alanine [1.47-1.49]	0.0091± 0.0030	**††††0.0071± 0.0020**	**####0.0132± 0.0022**	**††††####0.0104± 0.0032**	(<0.0001)	(<0.0001)
Plasma Glucose [3.56-59]	0.0137± 0.0011	******0.0125± 6.4x10^{-4}**	******0.0106 ± 0.0011**	******0.0112± 0.0013**	(<0.0001)	(0.12)
Plasma Acetate [1.92]	0.0033± 5.2x10^{-4}	**†0.0031 ± 4.8x10^{-4}**	**###0.0030± 3.8x10^{-4}**	**†###0.0027± 3.2x10^{-4}**	(0.0002)	(0.011)

Table S20 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where a statistically significant diet x probiotic interaction occurred after correcting for the false discovery rate, post-hoc comparisons have been applied and have been explained here.

A significant diet x probiotic interaction was identified for plasma glucose ($F_{(1, 107)}$ = 19.01, p < 0.0001). HFD/vehicle, CD/probiotic, HFD/probiotic juvenile offspring had significantly less glucose levels relative to CD/vehicle offspring (p = 0.0004, p < 0.0001, p < 0.0001, respectively), and both CD/probiotic and HFD/probiotic offspring had less levels compared to HFD/vehicle offspring (p < 0.0001, p = 0.0002, respectively).

No other significant diet x probiotic interactions were observed.

N=26-29 per group. Data presented as mean ± standard deviation with combined sexes. †p < 0.05, ††p < 0.01, †††p < 0.001, ††††p < 0.0001 indicating main effect of maternal diet. #p < 0.05, ##p < 0.01, ###p < 0.001, ####p < 0.0001 indicating main effect of maternal probiotic intake. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

Supplementary Table 21 F1 (PND21) Juvenile Gut Metabolome

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Fecal Acetate [1.91-1.93]	0.14± 0.052	0.18± 0.087	0.19± 0.064	0.19± 0.061	(0.096)	(0.22)
Fecal Propionate [1.06-1.07]	0.027± 0.010	0.025± 0.0088	####**0.037± 0.011**	####**0.031± 0.0085**	(<0.0001)	(0.16)
Fecal Butyrate [1.54-1.58]	0.016± 0.0051	0.017± 0.0050	###**0.019± 0.0043**	###**0.021± 0.0047**	(0.0015)	(0.16)
Fecal Succinate [2.40-2.42]	0.015± 0.0085	††††**0.010± 0.0053**	0.022± 0.014	††††**0.0092± 0.0025**	(0.16)	(<0.0001)
Fecal Ethanol [3.64-3.68]	0.032± 0.0089	††††**0.042± 0.011**	####**0.026± 0.0036**	††††####**0.031± 0.0094**	(<0.0001)	(<0.0001)
Fecal Glucose [4.64-4.66]	0.0062± 0.0022	††††**0.0016± 5.1x10^{-4}**	0.0068± 0.0014	††††**0.0014± 0.0013**	(0.50)	(<0.0001)
Fecal Alanine [1.47-1.49]	0.0332± 0.0067	0.0329± 0.0066	#**0.0255± 0.084**	#**0.0321± 0.0066**	(0.011)	(0.090)

Table S21 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where a statistically significant diet x probiotic interaction occurred after correcting for the false discovery rate, post-hoc comparisons have been applied and have been explained here.

No significant diet x probiotic interactions were observed.

N=26-29 per group. Data presented as mean ± standard deviation with combined sexes. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

Supplementary Table 22 F1 (PND112) Adult Brain Metabolome

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Brain Lactate [1.32-1.34]	M: 0.0917± 0.013 F: 0.0803± 0.0045	M: 0.0847± 0.0075 F: 0.0778± 0.0051	M: 0.0863± 0.0062 **$^{\#}$F: 0.0812± 0.0054**	M: 0.0914± 0.0041 **$^{\#}$F: 0.0854± 0.0048**	M (0.80) F (0.030)	M (0.72) F (0.62)
Brain Cholesterol/ Triglyceride [0.86-0.90]	M: 0.0084± 0.0011 F: 0.0097± 0.0009	M: 0.0083± 0.0006 **††F: 0.0087± 0.0007**	M: 0.0088± 0.0006 F: 0.0099± 0.0012	M: 0.0079± 0.0010 **††F: 0.0086± 0.0007**	M (0.95) F (0.90)	M (0.07) F (0.003)
Brain Alanine [1.47-1.49]	0.0085± 0.0009	0.0084± 0.0007	**$^{\#}$0.0087± 0.0004**	**$^{\#}$0.0089± 0.0006**	(0.016)	(0.74)
Brain Glutamate [2.34-2.37]	M: 0.0561± 0.0037 F: 0.0596± 0.0030	M: 0.0570± 0.0027 F: 0.0578± 0.0022	M: 0.0567± 0.0018 F: 0.0579± 0.0015	M: 0.0598± 0.0015 F: 0.0603± 0.0013	M (0.12) F (0.60)	M (0.053) F (0.70)
Brain GABA [1.88-1.91]	0.0126± 0.0006	0.0124± 0.0007	****0.0133± 0.0007**	0.0124± 0.0005	(0.035)	(0.0018)
Brain Acetate [1.92]	0.0083± 0.0014	0.0086± 0.0007	**$^{\#\#\#\#}$0.011± 0.0010**	**$^{\#\#\#\#}$0.010± 0.0018**	(<0.0001)	(0.67)
Brain Glutamine [2.43-2.47]	0.0215± 0.002	0.0213± 0.002	**$^{\#\#}$0.0234± 0.001**	**$^{\#\#}$0.0220± 0.002**	(0.0046)	(0.070)

Table S22 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where sex differences occur, data is shown individually for male (M) and female (F) adult offspring. Where a statistically significant diet x probiotic interaction occurred after correcting for the false discovery rate, post-hoc comparisons have been applied and have been explained here.

There was a significant diet x probiotic interaction in brain lactate levels in adult male offspring ($F_{(1, 38)}$ = 5.324, q = 0.049), but post hoc Tukey tests did not reveal any significant group differences.

There was a significant diet x probiotic interaction in brain glutamate in adult female offspring ($F_{(1, 34)}$ = 8.87 q = 0.01), but post hoc Tukey tests did not reveal any significant group differences.

There was a significant diet x probiotic interaction in brain GABA in the adult offspring ($F_{(1, 76)}$ = 7.58, q = 0.018). Post hoc Tukey tests revealed that the adult offspring of CD/probiotic dams had increased levels relative to CD/vehicle (p = 0.0017), HFD/vehicle (p = 0.0002), and HFD/probiotic (p = 0.0002).

No other significant diet x probiotic interactions occurred.

N=9-11 per sex per group. Data presented as mean ± standard deviation with combined sexes. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

Supplementary Table 23 F1 (PND112) Adult Liver Metabolome

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Liver Lactate [1.32-1.34]	M: 0.0582± 0.0090 F: 0.0294± 0.0105	M: 0.0594± 0.0161 **††F: 0.0474± 0.0146**	M: 0.0612± 0.0099 **$^{\#}$F: 0.0421± 0.0099**	M: 0.0556± 0.0108 **$^{\dagger\dagger\#}$F: 0.0503± 0.0094**	M (0.90) F (0.04)	M (0.96) F (0.0066)
Liver Alanine [1.48]	M: 0.0091± 0.0037 F: 0.0037± 0.0024	***M: 0.0129± 0.0036** F: 0.0071± 0.0028	*****M: 0.0144± 0.0016** **$^{\#\#\#}$F: 0.0106± 0.0033**	M: 0.0120± 0.0017 **$^{\#\#\#}$F: 0.0092± 0.0043**	M (0.053) F (0.0010)	M (0.94) F (0.75)
Liver Glucose [4.64-4.66]	M: 0.0320± 0.0074 F: 0.0288± 0.0063	M: 0.0297± 0.0067 ***F: 0.0222± 0.0057**	**$^{\#}$M: 0.0351± 0.0041** F: 0.0300± 0.0036	**$^{\#}$M: 0.0368± 0.0056** F: 0.0329± 0.0038	M (0.049) F (0.0027)	M (0.98) F (0.70)
Liver Leucine [3.70-3.74]	M: 0.0551± 0.0136 F: 0.0482± 0.0091	M: 0.0500± 0.0094 ***F: 0.0381± 0.0081**	M: 0.0595± 0.0057 F: 0.0515± 0.0054	M: 0.0611± 0.0080 F: 0.0543± 0.0054	M (0.053) F (0.0010)	M (0.96) F (0.50)
Liver Taurine [3.26-3.28]	M: 0.1060± 0.0188 F: 0.1517± 0.0140	M: 0.1285± 0.0278 F: 0.1658± 0.0157	**$^{\#\#}$M: 0.0942± 0.0078** **$^{\#\#\#}$F: 0.1233± 0.0248**	**$^{\#\#}$M: 0.0942± 0.0153** **$^{\#\#\#}$F: 0.1189± 0.0145**	M (0.0018) F (0.0006)	M (0.30) F (0.75)
Liver Creatine [3.04]	M: 0.0012± 0.0002 F: 0.0015± 0.0003	M: 0.0013± 0.0004 F: 0.0017± 0.0003	M: 0.0012± 0.0002 **$^{\#}$F: 0.0014± 0.0002**	M: 0.0011± 0.0001 **$^{\#}$F: 0.0013± 0.0003**	M (0.17) F (0.017)	M (0.98) F (0.75)
Liver Acetate [1.92]	0.0018± 0.0005	0.0020± 0.0005	**$^{\#\#\#\#}$0.0015± 0.0004**	**$^{\#\#\#\#}$0.0015± 0.0004**	(<0.0001)	(0.69)

Table S23 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where sex differences occur, data is shown individually for male (M) and female (F) adult offspring. Where a statistically significant diet x probiotic interaction occurred after correcting for the false discovery rate, post-hoc comparisons have been applied and have been explained here.

There was a significant diet x probiotic interaction in liver alanine levels in the male adult offspring ($F_{(1, 38)} = 12.69$, q = 0.0060). Tukey post hoc tests revealed that both HFD/vehicle (p = 0.019) and CD/probiotic (p = 0.0004) offspring had significantly greater levels than CD/vehicle control offspring.

In the adult female offspring, significant diet x probiotic interactions occurred for liver glucose ($F_{(1, 34)} = 8.55$, q = 0.036) and leucine ($F_{(1, 34)} = 7.66$, q = 0.045). Post hoc tests showed that, in both cases, levels were significantly lower in the female offspring of HFD/vehicle dams, than other groups (Glucose: p = 0.033 relative to CD/vehicle offspring, p = 0.0075 relative to CD/probiotic offspring, p = 0.0003 relative to HFD/probiotic offspring; Leucine: p = 0.021 relative to CD/vehicle offspring, p = 0.0010 relative to CD/probiotic offspring, p = 0.0001 relative to HFD/probiotic offspring).

No other significant diet x probiotic interactions occurred.

N=9-11 per sex per group. Data presented as mean ± standard deviation with combined sexes. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

Supplementary Table 24 F1 (PND112) Adult Plasma Metabolome

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Plasma Triglyceride/VLDL [1.22-1.32]	M: 0.1862± 0.0211 F: 0.1413± 0.0290	M: 0.1837± 0.0311 F: 0.1396± 0.0208	M: 0.2100± 0.0322 **$^{\#\#}$F: 0.1830± 0.0318**	M: 0.1821± 0.0228 **$^{\#\#}$F: 0.1581± 0.0286**	M (0.20) F (0.0028)	M (0.22) F (0.39)
Plasma Alanine [1.47-1.49]	M: 0.0065± 0.0015 F: 0.0089± 0.0015	M: 0.0074± 0.0020 F: 0.0100± 0.0019	**$^{\#\#\#}$M: 0.0051± 0.0009 $^{\#\#\#\#}$F: 0.0063± 0.0006**	**$^{\#\#\#}$M: 0.0055± 0.0006 $^{\#\#\#\#}$F: 0.0065± 0.0016**	M (0.0009) F (<0.0001)	M (0.23) F (0.39)
Plasma Glucose [3.83-86]	M: 0.0301± 0.0034 F: 0.0383± 0.0067	M: 0.0301± 0.0044 F: 0.0387± 0.0042	M: 0.0262± 0.0048 **$^{\#\#}$F: 0.0308± 0.0044**	M: 0.0295± 0.0047 **$^{\#\#}$F: 0.0344± 0.0056**	M (0.19) F (0.0028)	M (0.23) F (0.39)
Plasma Acetate [1.92]	0.0023± 0.0006	0.0020± 0.0004	**$^{\#\#\#\#}$0.0015± 0.0005**	**$^{\#\#\#\#}$0.0016± 0.0005**	(<0.0001)	(0.29)

Table S24 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where sex differences occur, data is shown individually for male (M) and female (F) adult offspring.

No significant diet x probiotic interactions occurred.

N=9-11 per sex per group. Data presented as mean ± standard deviation with combined sexes. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

Supplementary Table 25 F1 (PND112) Adult Gut Metabolome

Metabolite [ppm]	Vehicle		Probiotic		Vehicle vs Probiotic main effect (q-value)	Control Diet vs High Fat Diet main effect (q-value)
	Control Diet	High Fat Diet	Control Diet	High Fat Diet		
Fecal Acetate [1.91-1.93]	0.079± 0.038	0.092± 0.026	0.093± 0.027	0.104± 0.038	(0.082)	(0.49)
Fecal Propionate [2.15-2.17]	0.032± 0.012	0.037± 0.013	**$^{\#}$0.042± 0.013**	**$^{\#}$0.042± 0.015**	(0.024)	(0.49)
Fecal Butyrate [1.54-58]	0.029± 0.012	0.035± 0.012	**$^{\#}$0.041± 0.013**	**$^{\#}$0.041± 0.015**	(0.013)	(0.49)
Fecal Ethanol [1.17-1.20]	M: 0.0050± 0.0016 F: 0.0062± 0.0010	***M: 0.0064± 0.0011** F: 0.0067± 0.0009	****M: 0.0068± 0.0012** F: 0.0065± 0.0016	M: 0.0050± 0.0008 F: 0.0064± 0.0014	M (0.53) F (0.97)	M (0.86) F (0.58)
Fecal Alanine [1.47-1.49]	M: 0.0271± 0.0097 F: 0.0139± 0.0049	M: 0.0202± 0.0065 **†F: 0.0164± 0.0038**	***M: 0.0187± 0.0036** F: 0.0146± 0.0041	M: 0.0246± 0.0028 **†F: 0.0184± 0.0032**	M (0.52) F (0.55)	M (0.86) F (0.044)

Table S25 Notes: Q-values for main effects (maternal diet; maternal probiotic) are presented in the table, determined from 2-way ANOVA or non-parametric equivalent for each given metabolite with Benjamini & Hochberg correction. Where sex differences occur, data is shown individually for male (M) and female (F) adult offspring. Where a statistically significant diet x probiotic interaction occurred after correcting for the false discovery rate, post-hoc comparisons have been applied and have been explained here.

There were significant diet x probiotic interactions in the adult male offspring for fecal ethanol ($F_{(1, 38)}$ = 18.83, q = 0.0002) and alanine ($F_{(1, 38)}$ = 10.73, q = 0.0023) levels. Tukey testing revealed that fecal ethanol levels were relatively higher in HFD/vehicle offspring than CD/vehicle offspring (p = 0.046) and relative higher in CD/probiotic offspring than both CD/vehicle (p = 0.0048) and HFD/probiotic (p = 0.0081) offspring. Fecal alanine levels were lower in CD/probiotic offspring compared to CD/vehicle offspring only (p = 0.018).

No other significant diet x probiotic interactions occurred.

N=9-11 per sex per group. Data presented as mean ± standard deviation with combined sexes. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, $^{\dagger\dagger\dagger\dagger}p < 0.0001$ indicating main effect of maternal diet. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ indicating main effect of maternal probiotic intake. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, indicating significant Tukey post-hoc comparison, performed only if a significant diet x probiotic interaction was identified.

Supplementary Table 26 Prefrontal cortex gene expression analysis in F1 (PND21) juvenile offspring

	FEMALE JUVENILE OFFSPRING				MALE JUVENILE OFFSPRING			
	Vehicle		Probiotic		Vehicle		Probiotic	
	Control Diet	High Fat Diet	Control Diet	High Fat Diet	Control Diet	High Fat Diet	Control Diet	High Fat Diet
GLUN1	1.00± 0.65	1.42± 0.47	0.85± 0.09	0.95± 0.33	1.00± 0.09	1.00± 0.30	0.55± 0.29	1.00± 0.55
GLUN2A	1.00± 0.31	0.88± 0.22	**0.67± 0.13**$^{\#}$	**0.66± 0.17**$^{\#}$	1.00± 0.23	0.85± 0.10	**0.60± 0.28**$^{\#}$	**0.90± 0.19**$^{\#}$
GLUN2B	1.00± 0.21	0.86± 0.13	**0.43± 0.06**$^{\#}$	**0.60± 0.23**$^{\#}$	1.00± 0.25	0.97± 0.15	0.76± 0.13	0.91± 0.13
GLUN2C	1.00± 0.15	**2.09± 0.30****	1.28± 0.42	1.24± 0.41	1.00± 0.36	0.99± 0.35	0.62± 0.30	0.92± 0.23
PSD-95	1.00± 0.08	1.14± 0.07	0.98± 0.12	0.95± 0.07	1.00± 0.11	1.03± 0.09	0.90± 0.11	0.96± 0.09
5-HT1A	1.00± 0.36	1.60± 0.51	0.88± 0.25	1.30± 0.61	1.00± 0.41	0.93± 0.53	0.75± 0.21	0.86± 0.48
5-HT2A	1.00± 0.25	1.15± 0.32	0.85± 0.21	0.92± 0.21	1.00± 0.44	0.64± 0.24	0.55± 0.15	0.66± 0.33
5-HT6	1.00± 0.27	1.27± 0.26	0.68± 0.17	0.84± 0.07	1.00± 0.48	0.80± 0.26	0.87± 0.43	1.02± 0.46
BDNF	1.00± 0.19	1.23± 0.40	1.19± 0.54	1.16± 0.28	1.00± 0.40	0.99± 0.62	0.95± 0.14	1.16± 0.50
CREB1	1.00± 0.15	1.03± 0.26	1.56± 0.37	1.05± 0.06	1.00± 0.20	0.98± 0.08	**1.33± 0.10**$^{\#}$	**1.10± 0.06**$^{\#}$
SYP	1.00± 0.10	1.06± 0.07	0.99± 0.11	0.99± 0.08	1.00± 0.04	1.02± 0.07	**0.85± 0.11**$^{\#}$	**0.92± 0.07**$^{\#}$
GSK3B	1.00± 0.15	0.86± 0.27	1.28± 0.27	0.84± 0.14	1.00± 0.18	0.86± 0.08	1.28± 0.12	1.02± 0.20
ZIF-268	1.00± 0.16	**1.17± 0.35**†	0.60± 0.10	**1.26± 0.08**†	1.00± 0.12	**1.31± 0.23********	0.67± 0.14	**1.61± 0.15********
cFOS	1.00± 0.49	0.88± 0.31	0.67± 0.11	1.05± 0.49	1.00± 0.47	1.47± 0.56	1.03± 0.25	1.50± 0.44
ΔFOSB	1.00± 0.08	0.82± 0.22	1.38± 0.63	1.36± 0.29	1.00± 0.32	1.09± 0.54	1.94± 0.42	1.65± 0.69
ATP1A2	1.00± 0.20	1.06± 0.12	0.97± 0.19	0.82± 0.09	1.00± 0.12	1.08± 0.08	1.02± 0.13	0.86± 0.10
PFKFB3	1.00± 0.09	1.03± 0.12	**1.21± 0.20**$^{\#}$	**1.11± 0.18**$^{\#}$	1.00± 0.05	1.04± 0.08	**1.32± 0.12**$^{\#}$	**1.28± 0.25**$^{\#}$

Table S26 Notes: Data presented as mean±SD, relative expression compared to respective female or male vehicle/Control Diet offspring. N = 6 per group for 2-way ANOVA for each sex, or N = 12 per group if sex consolidated into a single 2-way ANOVA where no sex differences. $^{\dagger}p < 0.001$, indicating main effect of maternal diet. $^{\#}p < 0.001$ indicating main effect of maternal probiotic intake. *p < 0.001 indicating significant post-hoc comparison if a significant diet x probiotic interaction was identified. Comparisons were adjusted for multiple testing using the Bonferroni method.

GLUN2A: Maternal probiotic intake had a main effect on reducing PFC expression of *GLUN2A* ($F_{(1, 44)} = 13.16$, $p = 0.0007$). No significant sex differences or interaction was observed.
GLUN2B: Expression was reduced in females ($F_{(1, 19)} = 35.26$, $p < 0.0001$) of probiotic dams, but not males ($F_{1, 20)} = 4.216$, $p = 0.053$). No significant interactions were identified after FDR correction.
GLUN2C: 2-way ANOVA revealed a significant maternal diet x probiotic interaction in females only ($F_{(1, 18)} = 15.28$, $p = 0.0010$). Tukey post hoc tests showed that *GLUN2C* expression was increased in the female offspring of HFD/vehicle dams only (post hoc $p = 0.0003$ compared to offspring of CD/vehicle dams, $p = 0.0057$ compared to CD/probiotic, $p = 0.0025$ compared to HFD/probiotic).
CREB1: Main effect of maternal probiotic intake on increasing expression, only in male offspring ($F_{(1, 20)} = 19.80$, $p = 0.0002$). No significant interaction. No significant interactions or main effects in the female juvenile offspring.
SYP: Expression was reduced in male probiotic offspring ($F_{(1, 20)} = 16.77$, $p = 0.0006$) but not females ($F_{(1, 20)} = 1.00$, $p = 0.33$), with no significant interactions.
ZIF-268: There was a main effect of maternal HFD, relative to maternal CD, on increasing expression in female juvenile offspring ($F_{(1, 20)} = 24.99$, $p < 0.0001$) with no significant interaction. In males, there was a significant diet x probiotic interaction ($F_{(1,20)} = 21.41$, $p = 0.0002$). Post hoc Tukey tests revealed significant differences, where male HFD/probiotic offspring had increased expression compared to both CD/probiotic and CD/vehicle male offspring ($p < 0.0001$) and HFD/vehicle male offspring had significantly greater expression compared to CD/probiotic male offspring ($p < 0.0001$).
PFKFB3 expression was increased in all juvenile probiotic offspring ($F_{(1, 44)} = 23.96$, $p < 0.0001$) relative to offspring from dams fed the vehicle.

GLUN1, PSD-95, 5-HT1A/2A/6, BDNF, GSK3B, cFOS, ΔFOSB, ATP1A2: No significant interactions or main effects.

Supplementary Table 27 Prefrontal cortex gene expression analysis in F1 (PND112) adult offspring

	FEMALE ADULT OFFSPRING				MALE ADULT OFFSPRING			
	Vehicle		Probiotic		Vehicle		Probiotic	
	Control Diet	High Fat Diet	Control Diet	High Fat Diet	Control Diet	High Fat Diet	Control Diet	High Fat Diet
GLUN1	1.00± 0.10	0.91± 0.10	0.95± 0.06	0.95± 0.10	1.00± 0.11	1.05± 0.15	1.24± 0.10	1.06± 0.10
GLUN2A	1.00± 0.05	0.80± 0.06	0.80± 0.20	0.86± 0.14	1.00± 0.15	0.93± 0.16	1.13± 0.13	0.97± 0.15
GLUN2B	1.00± 0.19	0.88± 0.10	1.01± 0.09	0.90± 0.14	1.00± 0.20	1.07± 0.22	1.22± 0.16	1.06± 0.15
GLUN2C	1.00± 0.15	1.06± 0.06	0.98± 0.07	0.89± 0.08	1.00± 0.10	1.07± 0.08	1.16± 0.19	1.10± 0.07
PSD-95	1.00± 0.20	1.01± 0.34	1.06± 0.55	0.83± 0.35	1.00± 0.25	1.06± 0.10	1.15± 0.56	0.78± 0.23
5-HT1A	1.00± 0.23	0.78± 0.09	0.76± 0.11	0.63± 0.11	1.00± 0.22	0.93± 0.20	0.85± 0.36	0.78± 0.18
5-HT2A	1.00± 0.24	0.96± 0.12	0.93± 0.21	0.91± 0.11	1.00± 0.15	0.98± 0.13	1.18± 0.15	1.16± 0.10
5-HT6	1.00± 0.25	0.82± 0.09	1.00± 0.16	0.93± 0.17	1.00± 0.20	1.14± 0.16	1.40± 0.18	1.33± 0.27
BDNF	1.00± 0.32	0.75± 0.31	**1.67± 0.34**$^{\#}$	**1.54± 0.47**$^{\#}$	1.00± 0.15	0.68± 0.19	**1.49± 0.30**$^{\#}$	**1.83± 0.38**$^{\#}$
CREB1	1.00± 0.11	0.77± 0.09	0.89± 0.13	1.03± 0.20	1.00± 0.20	0.53± 0.12	0.82± 0.21	0.83± 0.22
SYP	1.00± 0.15	**0.80± 0.23**†	0.87± 0.14	**0.78± 0.16**†	1.00± 0.20	**0.73± 0.21**†	0.82± 0.09	**0.71± 0.08**†
GSK3B	1.00± 0.13	0.88± 0.09	0.80± 0.12	0.84± 0.13	1.00± 0.24	0.71± 0.13	0.76± 0.27	0.69± 0.10
ZIF-268	1.00± 0.50	0.73± 0.36	1.17± 0.18	0.87± 0.29	1.00± 0.45	1.62± 0.58	**3.58± 0.99**$^{\#}$	**3.07± 1.13**$^{\#}$
cFOS	1.00± 0.24	0.77± 0.16	1.05± 0.23	1.61± 0.67	1.00± 0.86	1.41± 1.01	**2.94± 1.22**$^{\#}$	**3.72± 1.03**$^{\#}$
ΔFOSB	1.00± 0.28	0.70± 0.22	**1.55± 0.13**$^{\#}$	**1.42± 0.37**$^{\#}$	1.00± 0.25	0.91± 0.24	**1.75± 0.78**$^{\#}$	**2.01± 0.71**$^{\#}$
ATP1A2	1.00± 0.26	0.89± 0.25	0.91± 0.12	0.94± 0.21	1.00± 0.20	0.78± 0.18	0.91± 0.27	0.70± 0.18
PFKFB3	1.00± 0.15	0.65± 0.17	**1.27± 0.48**$^{\#}$	**1.28± 0.37**$^{\#}$	1.00± 0.24	0.77± 0.16	**1.75± 0.37**$^{\#}$	**1.44± 0.42**$^{\#}$

Table S27 Notes: Data presented as mean±SD, relative expression compared to respective female or male vehicle/Control Diet offspring. N = 6 per group for 2-way ANOVA for each sex, or N = 12 per group if sex consolidated into a single 2-way ANOVA where no sex differences. $^{\dagger}p < 0.001$, indicating main effect of maternal diet. $^{\#}p < 0.001$ indicating main effect of maternal probiotic intake. *p < 0.001 indicating significant post-hoc comparison. Comparisons were adjusted for multiple testing using the Bonferroni method.

BDNF: Significant main effect of maternal probiotic intake, whereby expression was increased relative to adult offspring of vehicle dams ($F_{(1, 44)} = 70.53$, $p < 0.0001$). No significant interactions occurred, with no sex differences.
SYP: There was a main effect of maternal obesity, whereby expression was reduced in the offspring of HFD dams compared to adult offspring of CD dams ($F_{(1, 44)} = 12.86$, $p = 0.0008$), independent of maternal probiotic intake. No sex differences and no significant diet x probiotic interaction occurred.
ZIF-268: In the male adult offspring only, there was a main effect of maternal probiotic intake on increasing expression ($F_{(1, 20)} = 34.99$, $p < 0.0001$), independently of maternal diet, with no significant interaction. In females, no main effects or interactions were observed.
cFOS: In the male adult offspring only, there was a main effect of maternal probiotic intake on increasing expression ($F_{(1, 20)} = 25.17$, $p < 0.0001$), independently of maternal diet, with no significant interaction. In females, no main effects or interactions were observed.
ΔFOSB: There was a main effect of maternal probiotic intake on increasing expression ($F_{(1, 42)} = 33.60$, $p < 0.0001$) independent of maternal diet, with no sex differences. No significant interaction occurred.
PFKFB3: There was a main effect of maternal probiotic intake on increasing *PFKFB3* expression the adult offspring ($Q = 29.96$, $p = 0.001$) relative to the adult offspring from dams given vehicle, independent of maternal diet. No sex differences and no significant interactions occurred.

GLUN1/2A/2B/2C, PSD-95, 5-HT1A/2A/6, CREB1, GSK3B, ATP1A2: No significant interactions or main effects.

Supplementary Table 28 F1 (PND21) juvenile liver and brain pro-inflammatory gene expression

	FEMALE JUVENILE OFFSPRING				MALE JUVENILE OFFSPRING			
	Vehicle		Probiotic		Vehicle		Probiotic	
	Control Diet	High Fat Diet	Control Diet	High Fat Diet	Control Diet	High Fat Diet	Control Diet	High Fat Diet
Liver *TNF*	1.00± 0.50	1.30± 0.32	2.03± 0.96	1.67± 0.96	1.00± 0.44	1.70± 0.29	1.43± 0.36	1.28± 0.46
Liver *IL-1B*	1.00± 0.42	1.31± 0.37	3.03± 1.89	1.22± 0.40	1.00± 0.33	1.28± 0.37	1.26± 0.26	1.10± 0.34
Liver *IL-6*	1.00± 0.53	**2.65± 0.41*****	1.02± 0.63	0.79± 0.34	1.00± 0.43	1.23± 0.48	0.72± 0.31	1.09± 0.61
Liver *TLR4*	1.00± 0.19	1.09± 0.28	**0.60± 0.19**$^{\#}$	**1.04± 0.31**$^{\#}$	1.00± 0.30	1.26± 0.39	**0.77± 0.29**$^{\#}$	**0.81± 0.30**$^{\#}$
PFC *TNF*	1.00 ±0.40	0.90 ±0.13	1.29 ±0.41	1.18 ±0.41	1.00 ±0.12	0.82 ±0.10	1.23 ±0.22	1.07 ±0.28
PFC *IL-1B*	1.00± 0.19	1.04± 0.44	1.04± 0.43	1.02± 0.28	1.00± 0.18	1.01± 0.33	0.69± 0.45	1.16± 0.08
PFC *IL-6*	1.00± 0.24	0.74± 0.20	**1.27± 0.45**$^{\#}$	**1.23± 0.39**$^{\#}$	1.00± 0.36	1.09± 0.29	**1.37± 0.55**$^{\#}$	**1.78± 0.74**$^{\#}$
PFC *TLR4*	1.00 ±0.28	0.80± 0.17	0.86± 0.18	0.87± 0.22	1.00± 0.19	1.12± 0.11	1.42± 0.52	1.34± 0.32

Table S28 Notes: Data presented as mean±SD, relative expression compared to respective female or male vehicle/Control Diet offspring. N = 6 per group for 2-way ANOVA for each sex, or N = 12 per group if sex consolidated into a single 2-way ANOVA where no sex differences. $^{\dagger}p < 0.001$, indicating main effect of maternal diet. $^{\#}p < 0.001$ indicating main effect of maternal probiotic intake. *p < 0.001 indicating significant post-hoc comparison, performed only if a significant diet x probiotic interaction was identified. Comparisons were adjusted for multiple testing using the Bonferroni method.

Liver *IL-6:* In females, a significant interaction was identified ($F_{(1, 18)} = 19.06$, $p = 0.0004$). Post hoc Tukey tests revealed that the female juvenile offspring from HFD/vehicle dams had increased expression relative to the CD/vehicle ($p = 0.0002$), CD/probiotic ($p = 0.0002$) and HFD/probiotic ($p < 0.0001$) conditions. No significant interactions or main effects were observed in the male juvenile offspring.

Liver *TLR4*: There was a main effect of maternal probiotic intake on decreasing expression in the juvenile offspring, independent of maternal diet, with no sex differences and no significant interaction ($F_{(1, 44)} = 12.46$, $p = 0.0010$).

Liver *TNF, IL-1B:* No significant main effects or significant interactions occurred.

Brain *IL-6*: There was a significant main effect of maternal probiotic intake, whereby expression was increased relative to the juvenile offspring of dams given vehicle ($F_{(1, 44)} = 12.49$, $p = 0.001$), independent of maternal diet. No sex differences were observed, and there was no significant diet x probiotic interaction.

Brain *TNF, IL-1B, TLR4:* No significant main effects or significant interactions occurred.

Supplementary Table 29 F1 (PND112) adult liver and brain pro-inflammatory gene expression

	FEMALE ADULT OFFSPRING				MALE ADULT OFFSPRING			
	Vehicle		Probiotic		Vehicle		Probiotic	
	Control Diet	High Fat Diet	Control Diet	High Fat Diet	Control Diet	High Fat Diet	Control Diet	High Fat Diet
Liver *TNF*	1.00± 0.41	0.77± 0.25	1.55± 0.77	1.05± 0.49	1.00± 0.40	1.12± 0.21	1.14± 0.26	1.39± 0.77
Liver *IL-1B*	1.00± 0.48	1.15± 0.33	2.05± 1.21	2.67± 0.49	1.00± 0.33	**1.85± 0.90**†	1.22± 0.48	**3.59± 1.19**†
Liver *IL-6*	1.00± 0.16	0.78± 0.15	**1.81± 0.74**$^{\#}$	**2.51± 1.37**$^{\#}$	1.00± 0.16	**1.55± 0.23**†	1.22± 0.45	**3.38± 1.10**†
Liver *TLR4*	1.00± 0.21	1.03± 0.28	1.00± 0.44	1.09± 0.25	1.00± 0.21	1.13± 0.23	1.07± 0.43	1.45± 0.32
PFC *TNF*	1.00± 0.39	1.47± 0.74	1.67± 0.82	1.10± 0.57	1.00± 0.48	1.23± 0.29	1.08± 0.28	0.96± 0.22
PFC *IL-1B*	1.00± 0.39	0.94± 0.10	0.84± 0.36	0.94± 0.27	1.00± 0.18	1.01± 0.33	1.12± 0.08	0.61± 0.28
PFC *IL-6*	1.00± 0.21	1.63± 0.68	1.26± 0.50	1.42± 0.34	1.00± 0.31	1.06± 0.28	1.51± 0.68	1.09± 0.35
PFC *TLR4*	1.00± 0.22	1.08± 0.37	1.07± 0.23	1.08± 0.24	1.00± 0.19	1.12± 0.11	1.30± 0.36	1.27± 0.51

Table S29 Notes: Data presented as mean±SD, relative expression compared to respective female or male vehicle/Control Diet offspring. N = 6 per group for 2-way ANOVA for each sex, or N = 12 per group if sex consolidated into a single 2-way ANOVA where no sex differences. $^{\dagger}p < 0.001$, indicating main effect of maternal diet. Comparisons were adjusted for multiple testing using the Bonferroni method.

Liver *IL-1B:* In male adult offspring, there was a significant main effect of maternal diet ($F_{(1, 19)} = 26.48$, $p < 0.0001$), whereby *IL-1B* expression was increased in the offspring of obese dams relative to the offspring of lean dams, independent of probiotic and with no significant diet x probiotic interaction. In the female offspring, no significant main effects or interactions were observed ($p > 0.001$).

Liver *IL-6:* In male adult offspring, there was a significant effect of maternal diet ($F_{(1, 19)} = 33.83$, $p < 0.0001$), whereby *IL-6* expression was increased in the offspring of obese dams relative to the offspring of lean dams, independent of probiotic and with no significant diet x probiotic interaction. In the adult female offspring, there was a main effect of maternal probiotic intake on increasing liver *IL-6* expression ($F_{(1, 19)} = 19.64$, $p = 0.0003$) relative to the offspring of dams given vehicle, independent of maternal diet and with no significant diet x probiotic interaction.

Liver *TNF, TLR4:* No significant main effects or significant interactions occurred.

Supplementary Table 30 F1 (PND21) juvenile correlation analysis between prefrontal cortex gene expression and brain metabolites altered by maternal probiotic intake or obesity

	Brain Metabolite			
Brain (PFC) Gene	Lactate	Glutamate	Alanine	Creatine
GLUN2A	**r = -0.47** **q = 0.0080**	r = -0.27 q = 0.16	r = -0.26 q = 0.16	r = -0.20 q = 0.26
GLUN2B	**r = -0.50** **q = 0.0080**	r = -0.26 q = 0.16	r = -0.26 q = 0.16	r = -0.21 q = 0.26
GLUN2C	r = -0.023 q = 0.73	r = -0.11 q = 0.47	r = -0.042 q = 0.68	r = -0.13 q = 0.43
PFKFB3	**r = 0.40** **q = 0.021**	r = 0.13 q = 0.42	r = -0.26 q = 0.16	r = 0.068 q = 0.59
SYP	**r = -0.40** **q = 0.021**	r = -0.10 q = 0.48	r = -0.22 q = 0.24	r = -0.16 q = 0.36
IL-6	r = 0.14 q = 0.42	r = 0.20 q = 0.26	**r = 0.44** **q = 0.013**	r = -0.13 q = 0.42

N = 48 per correlation analysis (24 female, 24 male).

Supplementary Table 31 F1 (PND21) juvenile correlation analysis between prefrontal cortex gene expression and gut metabolites altered by maternal probiotic intake

	Gut Metabolite		
Brain (PFC) Gene	Acetate	Propionate	Butyrate
GLUN2A	r = -0.25 q = 0.10	**r = -0.35** **q = 0.029**	r = -0.30 q = 0.056
GLUN2B	r = -0.22 q = 0.13	**r = -0.43** **q = 0.0088**	r = -0.24 q = 0.11
GLUN2C	r = -0.19 q = 0.18	r = -0.17 q = 0.20	**r = -0.34** **q = 0.038**
PFKFB3	**r = 0.38** **q = 0.025**	**r = 0.53** **q = 0.0013**	**r = 0.44** **q = 0.0088**
SYP	r = -0.19 q = 0.18	**r = -0.31** **q = 0.047**	**r = -0.37** **q = 0.025**
IL-6	r = 0.10 q = 0.36	r = 0.056 q = 0.49	r = 0.25 q = 0.10

N = 48 per correlation analysis (24 female, 24 male).

Supplementary Table 32 F1 (PND112) adult correlation analysis between prefrontal cortex gene expression and gut metabolites

	Gut Metabolite		
Brain (PFC) Gene	Acetate	Propionate	Butyrate
PFKFB3	r = 0.089 q = 0.50	r = 0.11 q = 0.49	**r = 0.40** **q = 0.027**
SYP	r = -0.34 q = 0.053	r = -0.14 q = 0.43	r = -0.24 q = 0.18

N = 48 per correlation analysis (24 female, 24 male).

Supplementary Table 33 F1 (PND112) adult correlation analysis between prefrontal cortex gene expression and brain metabolites

	Brain Metabolite		
Brain (PFC) Gene	Lactate	Glutamate	Alanine
PFKFB3	r = 0.36 q = 0.057	r = 0.16 q = 0.57	r = 0.34 q = 0.57
SYP	r = -0.01 q = 1.00	r = -0.09 q = 0.88	r = -0.04 q = 0.97

N = 48 per correlation analysis (24 female, 24 male).

Supplementary Methods

Probiotic preparation

The dose and route of administration were chosen in keeping with previous research conducted on probiotics (1, 2) that have demonstrated effects on gut microbiota composition. Oral administration through drinking water is the most ethically sound and minimally stressful method. To prepare the probiotic, 800mg ($8x10^9$ colony forming units) Bio-Kult Advance® were added to 8mL sterile water and the solution thoroughly mixed. The solution was left for five minutes to allow separation, and the supernatant (~6mL) containing live bacteria was aspirated and dispensed into a sterile water bottle. Each water bottle was topped up to 200mL sterile water and mixed again. Water bottles were changed Monday, Wednesday, Friday, with fresh probiotic. The assignment of the female mice to probiotic intake or vehicle was random.

Supplementary Table 34 List of bacterial strains in the Bio-Kult Advanced probiotic.

Bacillus subtilis PXN® 21®
Bifidobacterium bifidum PXN® 23™
Bifidobacterium breve PXN® 25™
Bifidobacterium infantis PXN® 27™
Bifidobacterium longum PXN® 30™
Lactobacillus acidophilus PXN® 35™
Lactobacillus delbrueckii ssp. *bulgaricus* PXN® 39™
Lactobacillus casei PXN® 37™
Lactobacillus plantarum PXN® 47™
Lactobacillus rhamnosus PXN® 54™
Lactobacillus helveticus PXN® 45™
Lactobacillus salivarius PXN® 57™
Lactococcus lactis ssp. *lactis* PXN® 63™
Streptococcus thermophilus PXN® 66™

Behavior

Mice were habituated to the room for 30 minutes prior to behavioral testing. Male mice were always run before females. Otherwise, the order in which animals were run was randomised. Behavior was videotaped using a 1080p webcam (Huafu HI-Tech, China) and recorded on Apple QuickTime (Cupertino, CA).

Open field test (OFT)

The OFT is one of the most widely used tools in the assessment of animal behavior. Given that rodents show innate aversions to novel, open environments, shorter assessments of OFT activity (2-10 minutes) can sensitively detect differences in emotional 'anxiety-like' behavior (3). Longer or consecutive tests are more suited to assessing general activity after habituation to the novel environment. This apparatus (50x50x50cm^3, *lxwxh*) contained four identical open fields, allowing for the simultaneous assessment of multiple animals. Lighting was fixed at 10 lux.

Introduction of mice to this environment was used to assess anxiety-like traits, primarily through quantifying the aversion of a pre-defined central zone (25x25cm^2) – the most open area of the field. Aversion of open area and preference for the solid walls is known as thigmotaxis and is used as a proxy for anxiety-like behavior. It is a commonly used parameter to screen for novel anxiolytic compounds or demonstrate anxiety-like behavior in genetic models (4-6). Distance travelled was used to interpret activity levels displayed in a novel environment. Rearing behavior was quantified as a measure of exploratory behavior, which was expected to be reduced in more anxious animals. A rear was scored when the mouse lifted its forepaws and stood on its hind legs, either leaning onto the walls of the open field, or in the middle of the arena.

Here, mice were placed individually into the corner of the open field and were allowed to explore uninterrupted for five minutes. ANY-maze software (Stoelting Co., USA) was used to measure distance travelled (m), time spent in the central zone (s), and the number of entries into the central zone. The number of rears was scored manually. Each open field was cleaned with 70% ethanol between each animal tested.

Light-dark box (LDB)

The innate preference of rodents for darker areas over lighter areas can be exploited as a measure of anxious behavior in the light-dark box. The dissonance between a natural tendency to explore novel environments and neophobia, avoidance of the unfamiliar (particularly to more bright areas) is a mild stressor and can be used to quantify anxiety-like behavior in a scenario that somewhat mimics a stressful environment frequently encountered by wild mice. Increased tendency to explore the larger light compartment, which include total time spent, reduced latency to enter, and increased total number of entries, are interpreted as reduced anxiety-like behavior.

Here, a small black covered compartment (21x16x16cm^3; *lxwxh*) was separated from the white, lighter (15 lux) open compartment (46.5x21x21cm^3, *lxwxh*) by a small opening (3x2.7cm^2, *lxw*). One face of this lighter compartment was transparent and faced the camera. Animals were placed gently in the dark enclosure and the lid was immediately put on. The test was run for five minutes. Time in the light compartment (s), latency to enter the light compartment (s), and the number of crossings between compartments (with all 4 paws crossing over the threshold), were scored manually. The apparatus was cleaned with 70% ethanol between each animal.

Forced swim test (FST)

The FST remains a 'gold-standard' for assessing depressive-like behavior in rodents (7). Antidepressants reduce immobility or 'floating' behavior in the FST, which is the key parameter assessed (8). In the FST, mice are placed gently into a tub of tepid water from which escape is not possible. Initially, the mice exhibit frantic escape behavior typified by swimming around the container and climbing behavior (peddling of the immersed paws against the side of the container). After a variable period, activity is interspersed with periods of (or continuous) floating behavior. This immobility is described as a passive coping behavior, whereby helplessness to the environment is learned (9). Because of this, behaviors of the forced swim test are also referred to as behavioral despair or passive stress coping.

A rectangular box (32x17x12 cm3, *lxwxh*) was filled nine centimetres deep with water (30°C). Light was set at 5 lux. To start the test, a mouse was placed facing the center of the apparatus. The first two minutes were not scored to avoid the effect of stress-induced hyper-locomotion

(10). Time floating (s) and the latency to float (s) were scored for the last three minutes. Floating behavior was defined as the animal doing the minimal amount of movement required to remain above water. Water was changed after every 2-3 mice. Mice were placed in a separate cage with paper towel for five minutes to allow for drying prior to being returned to their home cage.

Three-chamber social interaction test (SIT)

Mice are social creatures by nature. They communicate to forage food, defend territory, nurse pups, and reproduce. While wild mice undoubtedly possess and require a more diverse and adaptable array of social behaviors, the natural tendency for laboratory mice to explore unfamiliar conspecifics over inanimate novel objects is rooted in their social instincts. Maternal peripheral immune activation, as well as various genetic defects, have been shown to reduce interaction time. Social dysfunction in humans characterises aspects of both internalising and externalising disorders, such as autism and schizophrenia. The three-chamber SIT has been used to screen mouse models of these human diseases using this paradigm (11, 12). In humans, anxiety and social deficits are related and may co-occur in autism. Social anxiety is an internalising behavior associated with mood disorders (13).

The SIT used in this study was adopted from an established protocol (14). The apparatus consisted of a red transparent plexiglass box measuring 65x30x20 cm^3 (*lxwxh*). This was separated into three chambers of equal size by manually operated sliding doors. Room lighting was 0 lux. The SIT is divided into an initial habituation period followed by the testing phase. In the morning of testing (~0800-1100 hours), mice were individually placed in the central chamber with no access to the peripheral chambers. The doors were slid open after five minutes to then allow each mouse to explore the entire apparatus for a further five minutes. In the afternoon (~1300-1600 hours), each mouse was assessed individually during a ten-minute testing phase. Here, a novel C57Bl/6 mouse of the same sex was enclosed in a small cage at the end of the right chamber. An empty cage was used as a novel object, placed in the corresponding position in the left chamber. The mouse to be tested was placed in the center of the apparatus and the doors were immediately opened. The time spent in either the novel mouse (NM) or novel object (NO) chamber was recorded, and the social preference score calculated as time spent in the chamber with the novel mouse as a percentage of the total time exploring either of the

peripheral chambers (NM or NO chambers). The apparatus, including each small cage, was cleaned with disinfectant and water (to remove odour) between each mouse tested.

Maternal care behavior (MCB)

Mice exhibit natural variations in postpartum maternal care (15). Evaluating the amount and quality of maternal care behavior is critical when trying to comprehensively understand the influence of a maternal treatment or challenge, particularly if instigated during gestation or nursing, on offspring. This is particularly relevant if a chronic treatment, for example high fat diet, is expected to result in depressive-like behaviors during this period. Variations in MCB are known to affect the behavior and gene expression in offspring (16). Home cage observation is the primary method of assessing MCB as it is highly ethologically relevant. If performed appropriately discreet, it allows for the continuous non-disruptive assessment of maternal care. Cages can be videotaped, though live scoring is more accurate as the nest is often constructed away from the front of the cage. Similar maternal behaviors can also be difficult to discern.

Conversely, the pup retrieval test is used as a simple method of assessing maternal motivation toward her pups. This is a commonly used paradigm as it is quick and objective, quantifying the latency to retrieve pups dispersed from the nest (16, 17). Because this test requires disruption to the nest, it should not overlap with home cage observation.

Home cage observation:

Methods of maternal care behavior observation were adapted from a published protocol (18). All home cage observations were carried out by the same researcher, which negated inter-researcher variability. Cages were scored live, for 4 hours daily between P1 and P6. Scoring was conducted in 3-minute intervals between 1000 and 1200 hours, and 1500 and 1700 hours. This resulted in 80 observations per day, and a total of 480 time-point observations per litter. These included arched nursing, passive nursing, licking/grooming, self-grooming, nest building, eating or drinking (dam), climbing, or time spent passively away from the nest. The frequency of each behavior during observation was calculated for each day and overall, from P1 to P6. Not all behaviors were mutually exclusive. Some could be conducted in parallel, for example, nursing and licking/grooming.

Pup Retrieval Test

After the home cage observation of maternal care behavior was complete (1700 hours P6), the cage was placed in an animal transfer station (biological safety cabinet) and the lid removed. The damn was briefly moved to a separate cage while three pups were displaced equidistant from the nest. Some disruption to the nest may occur to collect the pups if the nest was initially covered rather than open. The dam was then placed back on the nest, and the latency to retrieve each pup was recorded live.

RNA extraction, cDNA conversion, and quantitative (q)PCR

Frozen whole brain was cut into two hemispheres on dry ice. The left hemisphere was thawed on ice and the prefrontal cortex quickly dissected for RNA extraction. The right hemisphere was stored at -80°C for metabolomic analysis. RNA extraction was carried out using the Qiagen RNEasy Mini Kit (Qiagen Ltd, Manchester, UK) as per the manufacturer's instructions. Total RNA and RNA purity was measured using a NanoDrop 1000 (Thermo Fisher, Loughborough, UK) and was deemed suitable for cDNA conversion if the 260/280nm ratio (indicating the extent of genomic DNA contamination) was >2, and 260/230 ratio (indicating phenol contamination) was between 1.8 and 2.2. 1000ng of RNA was then converted to cDNA using the high-capacity cDNA kit (Life Technologies, Paisley, UK), as per the manufacturer's instructions.

qPCR was performed using a LightCycler® 480 instrument (Roche Diagnostics, West Sussex, UK) with SYBR green master mix (Primerdesign, Hampshire, UK). 25ng cDNA was used per reaction. Primers were purchased from either Primerdesign, Merck Life Science Ltd (Dorset, UK), or Bio-Rad Laboratories Ltd (Hertfordshire, UK), and used at a working concentration of 300nM. Gapdh was used as the reference gene. Primer sequences can be found in Table S35. The generation of a melt curve was included after each run to confirm only a single PCR product was generated for a given reaction. Samples were run in duplicate, and $2^{-\Delta\Delta Ct}$ was calculated for each sample, where $\Delta Ct = (Ct_{\text{target gene}} - Ct_{\text{reference gene}})$. Data were analysed as fold change in gene expression relative to the control group.

Supplementary Table 35 List of Primers

Gene	Forward sequence (5' to 3')	Reverse sequence (5' to 3')	Reference
GAPDH	AACGACCCCTTCATTGAC	TCCACGACATACTCAGCAC	PrimerDesign
TNF	GCCTCCCTCTCATCAGTTCTAT	TTTGCTACGACGTGGGCTA	PrimerDesign
IL-1B	CAACCAACAAGTGATATTCTCCAT	GGGTGTGCCGTCTTTCATTA	PrimerDesign
IL-6	TCCATCCAGTTGCCTTCTTG	GGTCTGTTGGGAGTGGTATC	PrimerDesign
TLR4	CTGGCTAGGACTCTGATCATG	GCATTGGTAGGTAATATTAGGAACTA	(19)
SAA-2	TGGCTGGAAAGATGGAGACAA	AAAGCTCTCTCTTGCATCACTG	PrimerDesign
BDNF	GGATATTGCGAAGGGTTATTAGATT	GGAAGGTAATGTGTCTTGTTTGAA	PrimerDesign
5-HT$_{1A}$	AACCAGTTTTGTGTCCTCTCA	AGCACCTAAATAATTTTCTTCTCTGA	PrimerDesign
5-HT$_{2A}$	CAGGCAAGTCACAGGATAGC	TTAAGCAGAAAGAAAATCCCACAG	(20)
5-HT$_{6}$	CCTGACCCTCGGCATCCTG	GGTTCATGGTGCTATTACAGTATCC	PrimerDesign
GLUN1	CCAGACTAAAGATAGTGACAA	ACCATTGACTGTGAACTC	PrimerDesign
GLUN2A	GCTTTCCTTGAACCCTTCAG	GGGGAGCTTTCCCTTTGGCTAAGTT	(20)
GLUN2B	TTGGTGAGGTGGTCATGAAG	GGCTCTAAGAAGGCAGAAGGT	
GLUN2C	GGTTGCCATCACTGTCTTCA	CCACACGGACTTGCCAAT	PrimerDesign
CREB1	AGTGACTGAGGAGCTTGTACCA	TGTGGCTGGGCTGAAC	(21)
GSK3B	GACAAGCATTTAAGAACCGAGA	ACCAGGTAAGGTAGACCTACATC	
PSD-95	TCTGTGCGAGAGGTAGCAGA	AAGCACTCCGTGAACTCCTG	(22)
SYP	BioRad unique assay ID: qMmuCID0023269		BioRad
ATP1A2	BioRad unique assay ID: qMmuCID0024259		BioRad
PFKFB3	BioRad unique assay ID: qMmuCID0014746		BioRad
cFOS	AACCGCATGGAGTGTGTTGTTCC	TCAGACCACCTCGACAATGCATGA	(21)
ΔFOSB	AGGCAGAGCTGGAGTCGGAGAT	GCCGAGGACTTGAACTTCACTCG	
ZIF-268	TCGGCTCCTTTCCTCACTCA	CTCATAGGGTTGTTCGCTCGG	
16S	CGGCAACGAGCGCAACCC	CCATTGTAGCACGTGTGTAGCC	(23)
Lactobacillus	GGAAACAGRTGCTAATACCG	CACCGCTACACATGGAG	(24)
Bifidobacterium	TCGCGTCYGGTGTGAAAG	CCACATCCAGCRTCCAC	(25)

Metabolomic profiling

^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{1}H NMR spectra were acquired by using a 700 MHz Bruker AVII spectrometer operating at 16.4 Tesla equipped with a ^{1}H (^{13}C/^{15}N) TCI cryoprobe, as described (20, 26). Sample temperature was stable at 310K. ^{1}H NMR spectra were acquired by using a one-dimensional nuclear Overhauser effect spectroscopy presaturation scheme for attenuation of the water resonance with a 2 s presaturation.

For brain samples, 32 data collections were performed. For liver, plasma, and fecal samples, an addition pulse sequence, the Wasted-II (formerly Painless-II) sequence (27), was applied with 32 repetitions of the pulse sequence (or 128 for juvenile fecal extracts), an acquisition time of 1.5s, a relaxation delay of 2s, and an inter-pulse delay of 287μs. For milk and plasma, a spin-echo Carr-Purcell-Meiboom-Gill (CPMG) sequence was used under the same conditions as Wasted-II, but with a longer pulse interval of 400μs. Milk was run with 64 repetitions. These sequences were used for all future analyses of their respective samples. A fixed receiver gain was used for samples.

Sample preparation for ^{1}H NMR spectroscopy

Samples were prepared by using an optimized approach based on published methods (28). Samples of one type from multiple groups were extracted altogether to minimize batch variation. Tissue and biofluid-specific extraction methods are described below.

Brain and Liver

Between 100 and 125mg of fresh snap-frozen liver or brain (right cerebrum, excluding cerebellum and brainstem) was homogenised with a pestle and mortar on dry ice. Grinding dry tissue is more reproducible than homogenising wet tissue (29). Equipment was wiped using a dry paper towel to remove particulate matter between samples. Each ground sample was diluted 8-fold (μL/mg) in 50% acetonitrile (Sigma; v/v) in distilled water and further homogenised by vortex. Samples were centrifuged at 5,060xg for 5 minutes at 4°C. 750μL of supernatant was collected into a fresh tube, lyophilised, and stored at -80°C until the day of NMR analysis. Lyophilized brain and

liver tissue samples were resuspended by vortex in 600µL of 75mM 'NMR buffer' (5:1 disodium phosphate [Na2HPO4] and monosodium phosphate [NaH2PO4] in 100% D2O, pH = 7.4). Samples were centrifuged at 2,500xg for 5 minutes at 4°C to remove any particulate matter, before being transferred to a 5mm borosilicate NMR tube (Norell) using a glass pipette dropper.

Plasma

100µL plasma was added to 500µL 75mM NMR buffer and mixed by vortex. This was transferred to a 5mm borosilicate NMR tube (Sigma) using a glass pipette dropper.

Fecal

Fecal metabolites were extracted as described previously (20). ~30mg of starting material was used from juvenile mice, and ~75mg from F0 and F1 adult mice. Material was taken directly from the colon and multiple fecal pellets were collected and used for each mouse. 30mg fecal material was homogenised in 500µL NMR buffer, and 75mg in 750µL buffer, using a mechanical pestle homogeniser (DWK Life Sciences) and centrifuged at 16,100xg for 15 minutes at 4°C. The supernatant was collected into a clean Eppendorf and centrifuged again. 550µL was aspirated and added to 50µL 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid (TSP) to be used as an external reference. The final concentration of TSP in each sample was 5µg/mL. Samples were kept at -80°C until the day of NMR analysis, where the sample was added to a 5mm borosilicate NMR tube (Norell) using a glass pipette dropper.

Milk

At postnatal day (PND)4, pups from each litter were observed until the cessation of a nursing period. One pup from each litter was killed by cervical dislocation and the stomach rapidly dissected via visualisation of the 'milk spot', indicating a stomach full of recently ingested milk. The milk was transferred to a small tube, weighed, and diluted 8-fold (µL/mg) in 50% acetonitrile (Sigma; v/v) in distilled water. This mixture was homogenised using a mechanical pestle homogeniser, and centrifuged for 10 minutes at 13,000xg at 4°C. 600µL of supernatant was collected, and the remainder of

supernatant discarded. The pellet was resuspended in the same volume of 50% acetonitrile, centrifuged, and another 600µL of supernatant was collected. 1.2mL supernatant was lyophilised and stored at -80°C until the day of NMR analysis. Lyophilized milk samples were resuspended by vortex in 600µL of 75mM NMR buffer (5:1 disodium phosphate [Na2HPO4] and monosodium phosphate [NaH2PO4] in 100% D2O, pH = 7.4). Samples were centrifuged at 2,500xg for 5 minutes at 4°C prior to being transferred to a 5mm borosilicate NMR tube (Norell) using a glass pipette dropper.

NMR data pre-processing

Resulting free induction decays (FIDs) were zero-filled by a factor of 2 and multiplied by an exponential function corresponding to 0.30 Hz line broadening prior to Fourier transformation. All spectra were manually phased, baseline corrected (using a 3rd degree polynomial), and chemical shifts referenced to the lactate-CH_3 doublet resonance at δ = 1.33 ppm in Topspin 2.1 (Bruker, Germany), or in the case of fecal extracts to TSP at δ = 0 ppm. Spectra were visually examined for errors in baseline correction, referencing, spectral distortion, or contamination and then exported to ACD/Labs Spectrus Processor Academic Edition 12.01 (Advanced Chemistry Development, Inc.). Baseline regions outside the spectrum region of interest were discounted from further analyses. All spectra of the same tissue were overlayed, and the region of each spectra between 0.86–8.00 ppm, excluding the water region, was then binned by manually adding bins around each resonance signal, and the integral of each bin normalised to the sum of all integrals in the spectrum of each sample. Bins labelled with their corresponding treatment group and animal ID were exported to R (R Foundation for Statistical Computing; v3.3.1). Principal component analysis (PCA) and other supervised multivariate analyses were performed.

Statistical analysis

Univariate (non-parametric data)

Spearman's rank correlation test for heteroscedasticity was used to assess whether larger values of the outcome variable tended to have larger residuals, thus invalidating ANOVA.

Significance was set at $p < 0.05$, the null hypothesis being homoscedasticity. The Shapiro-Wilk test for normally distributed data was also used, where $p < 0.01$. Tukey's correction for multiple comparisons was performed on normally distributed, homoscedastic data, to investigate group differences. Data that rejected the null hypothesis of a Gaussian distribution or heteroscedasticity were either log- or square root-transformed. This was most often the case for behavioral data. If these transformations still failed to satisfy the assumptions required to perform ANOVA, robust analogous ANOVAs using 20% trimmed means were run in R using the WRS2 package (30). These generated a Q statistic, analogous to the F statistic in 2-way ANOVA. This allowed the computation of main effects and interactions. For post-hoc pairwise comparisons of nonparametric data, the Kolmogorov-Smirnov test was performed in Prism, using the Bonferroni correction method for multiple comparisons. These tests are robust to outliers and do not require normally distributed data or equal variances. Three-way effects models were used to assess the main effects of maternal diet and probiotic treatment on offspring weight over time.

Multivariate

Metabolomic analysis was performed as described previously (20, 26) using in-house scripts and the ropls package (31). Principal component analysis (PCA) incorporated all metabolomic data from brain, liver, feces, and plasma, and was used to initially visualise group differences in an unbiased manner. PCA was performed for individual tissues to inspect the data and identify outliers. Partial least squares discriminant analysis (PLS-DA) was then used to investigate group differences in the metabolome of each tissue/biofluid, and importantly, which variables (metabolites) contributed most to the variability between groups (e.g., maternal obesity vs healthy, or maternal probiotic intake vs vehicle control). Orthogonal (O)PLS-DA was then used to confirm main effect differences using ten-fold external cross validation with 100 iterations using a random sample of matched class sizes (the training set) to build an ensemble of 1000 models. For the maternal generation, including milk, eight-fold cross validation with 125 iterations was used due to the small class sizes. As part of this cross validation, an independent test set corresponding to 10% of the dataset was used to predict which class a sample belonged to, and thus the mean accuracy, sensitivity, and specificity over 1000 models was reported, ±

standard error of the mean. Model performance metrics were also retrieved to assess goodness of fit, including R^2X, R^2Y, and Q^2.

OPLS-DA models were further validated against models built with randomly permuted data using the same external cross validation method, representing a null distribution. All models performed significantly better than random chance, and the most important metabolites found to be driving these group differences were identified using average variable importance in projection (VIP) scores. OPLS-DA data are shown in Figures S29-S31 and Tables S36-S46.

F0 and F1 juvenile metabolomic heatmaps were generated by integrating the metabolites with the highest 3-4 VIP scores from each PLS-DA comparison for each tissue. Sum-normalised spectral bins corresponding to each metabolite were z-scaled. Male and female metabolites were z-scaled separately in both juvenile and adult offspring, to remove sex as a confounder in adult offspring. To allow for a direct comparison between juvenile and adult offspring, the F1 adult metabolomic heatmap was generated using the same list of metabolites as the F1 juvenile heatmap.

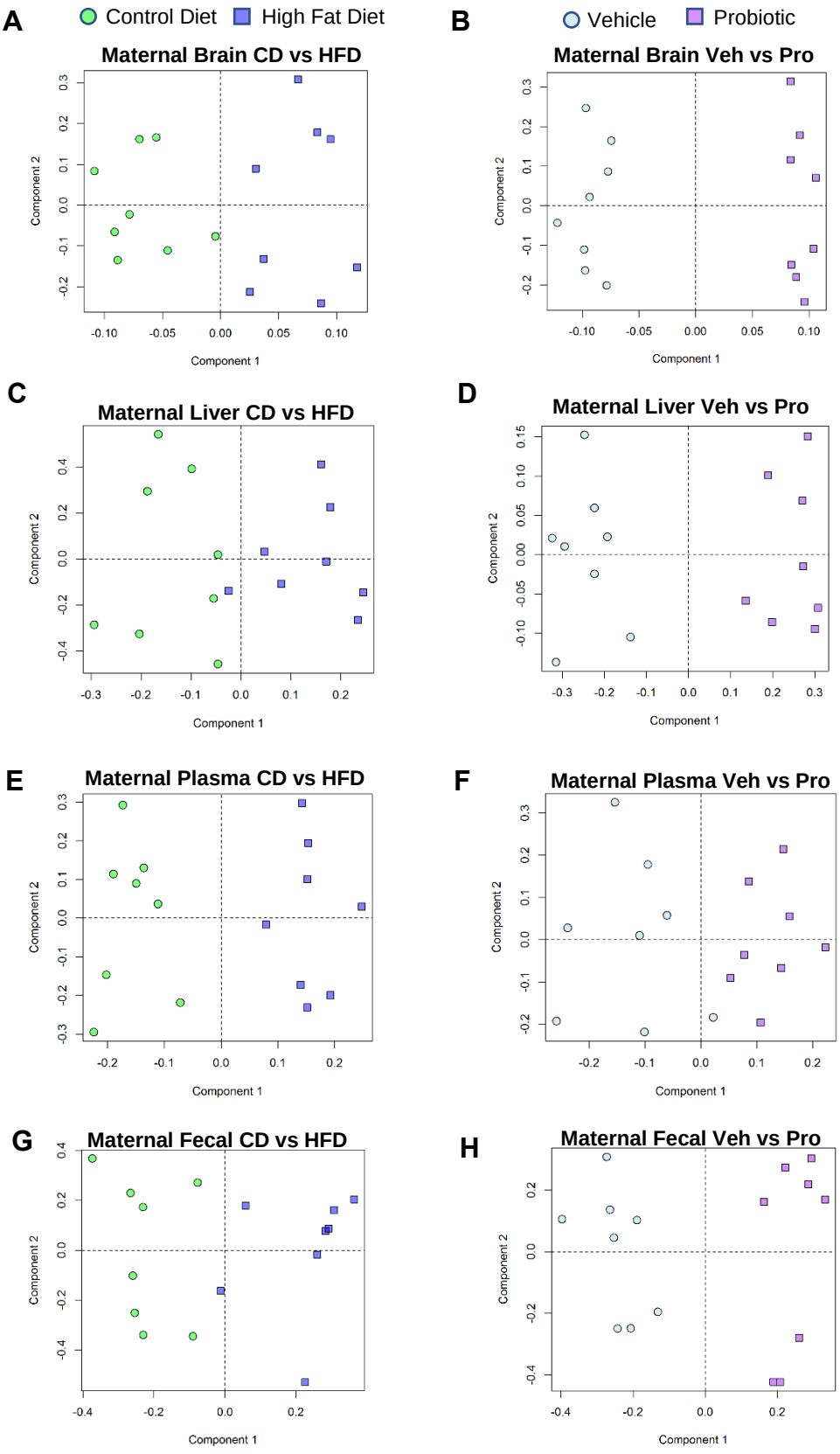
A
Control Diet
High Fat Diet
Maternal Brain CD vs HFD
B
Vehicle
Probiotic
Maternal Brain Veh vs Pro
C
Maternal Liver CD vs HFD
D
Maternal Liver Veh vs Pro
E
Maternal Plasma CD vs HFD
F
Maternal Plasma Veh vs Pro
G
Maternal Fecal CD vs HFD
H
Maternal Fecal Veh vs Pro
Component 1
Component 2

Figure S29 Orthogonal partial least squares discriminate analysis (OPLS-DA) scores plots comparing the entire metabolome of brain, liver, plasma, and fecal material of maternal mice. Binary comparisons were made between maternal mice fed control diet (CD) and high fat diet (HFD) and all maternal mice treated with Veh (vehicle) and Pro (probiotic). **(A)** Brain CDvsHFD, $R^2X = 0.70\pm0.01$, $R^2Y = 0.90\pm0.01$, $Q^2 = 0.15\pm0.11$. **(B)** Brain VehvsPro, $R^2X = 0.70\pm0.02$, $R^2Y = 0.98\pm0.01$, $Q^2 = 0.82\pm0.02$. **(C)** Liver CDvsHFD, $R^2X = 0.66\pm0.04$, $R^2Y = 0.84\pm0.04$, $Q^2 = 0.24\pm0.14$. **(D)** Liver VehvsPro, $R^2X = 0.71\pm0.01$, $R^2Y = 0.97\pm0.01$, $Q^2 = 0.22\pm0.07$. **(E)** Plasma CDvsHFD, $R^2X = 0.78\pm0.03$, $R^2Y = 0.90\pm0.02$, $Q^2 = 0.66\pm0.05$. **(F)** Plasma VehvsPro, $R^2X = 0.74\pm0.04$, $R^2Y = 0.85\pm0.03$, $Q^2 = 0.13\pm0.10$. **(G)** Fecal CDvsHFD, $R^2X = 0.66\pm0.04$, $R^2Y = 0.92\pm0.02$, $Q^2 = 0.52\pm0.07$. **(H)** Fecal VehvsPro, $R^2X = 0.70\pm0.04$, $R^2Y = 0.97\pm0.02$, $Q^2 = 0.82\pm0.03$. N = 8 per group comparison. Variable importance in projection (VIP) scores for key metabolites are detailed in Table S37.

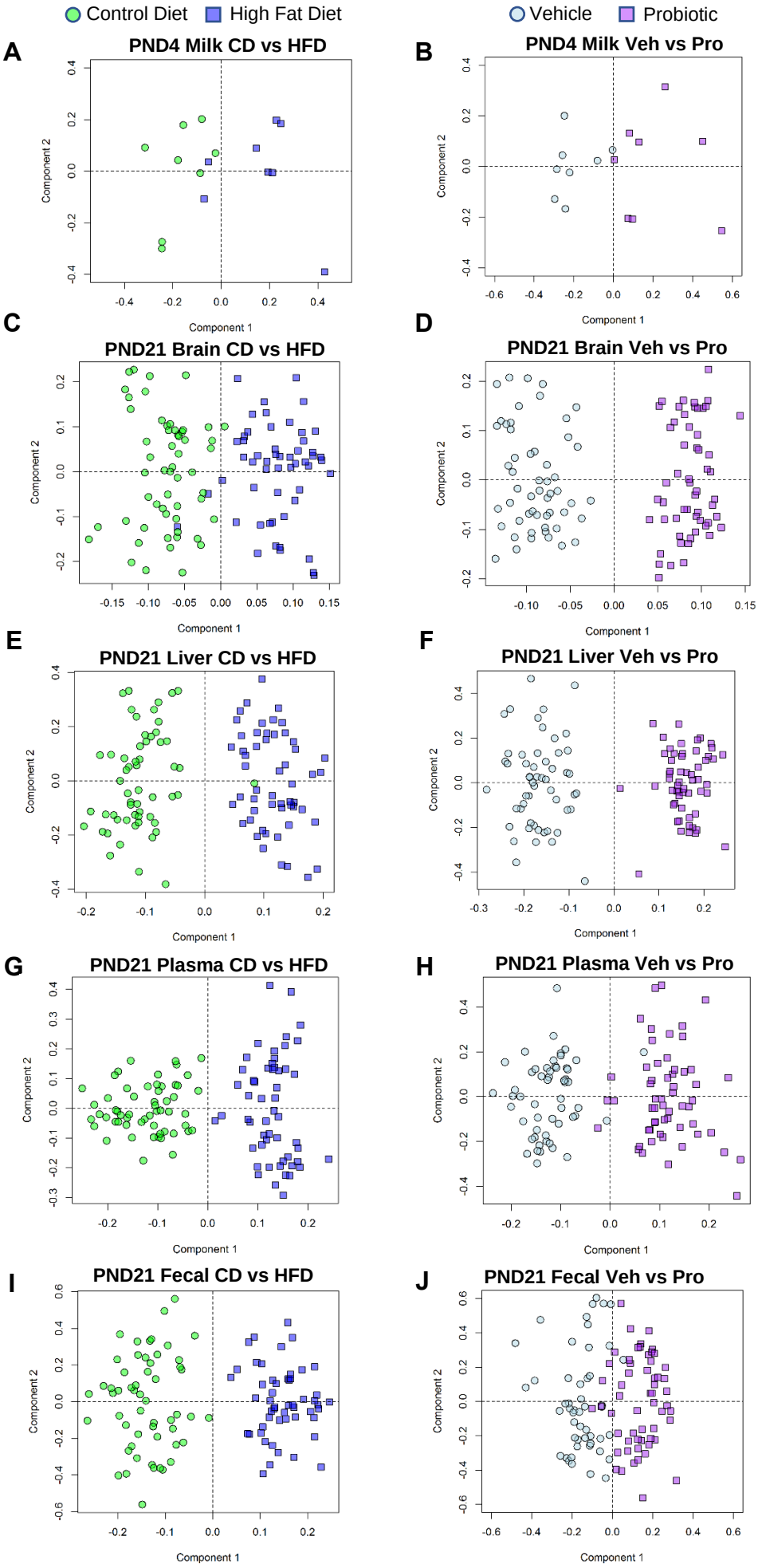
Control Diet
High Fat Diet
Vehicle
Probiotic
A
PND4 Milk CD vs HFD
B
PND4 Milk Veh vs Pro
C
PND21 Brain CD vs HFD
D
PND21 Brain Veh vs Pro
E
PND21 Liver CD vs HFD
F
PND21 Liver Veh vs Pro
G
PND21 Plasma CD vs HFD
H
PND21 Plasma Veh vs Pro
I
PND21 Fecal CD vs HFD
J
PND21 Fecal Veh vs Pro
Component 1
Component 2

Figure S30 Individual OPLS-DA scores plots comparing the entire metabolome of milk, brain, liver, plasma, and fecal material for each pairwise comparison in juvenile offspring. Corresponding model performance indices, including model accuracy, for each scores plot can be found in Table S36. **(A)** Maternal control diet (CD) vs high fat diet (HFD) offspring milk samples. **(B)** Maternal vehicle vs probiotic offspring milk samples. **(C)** Maternal CD vs HFD offspring brain samples. **(D)** Maternal vehicle vs probiotic offspring brain samples. **(E)** Maternal CD vs HFD offspring liver samples. **(F)** Maternal vehicle vs probiotic offspring liver samples. **(G)** Maternal CD vs HFD offspring plasma samples. **(H)** Maternal vehicle vs probiotic offspring plasma samples. **(I)** Maternal CD vs HFD offspring fecal samples. **(J)** Maternal vehicle vs probiotic offspring fecal samples. N = 54-57 per group for each OPLS-DA model. Variable importance in projection (VIP) scores for key metabolites are detailed in Tables S38-S42.

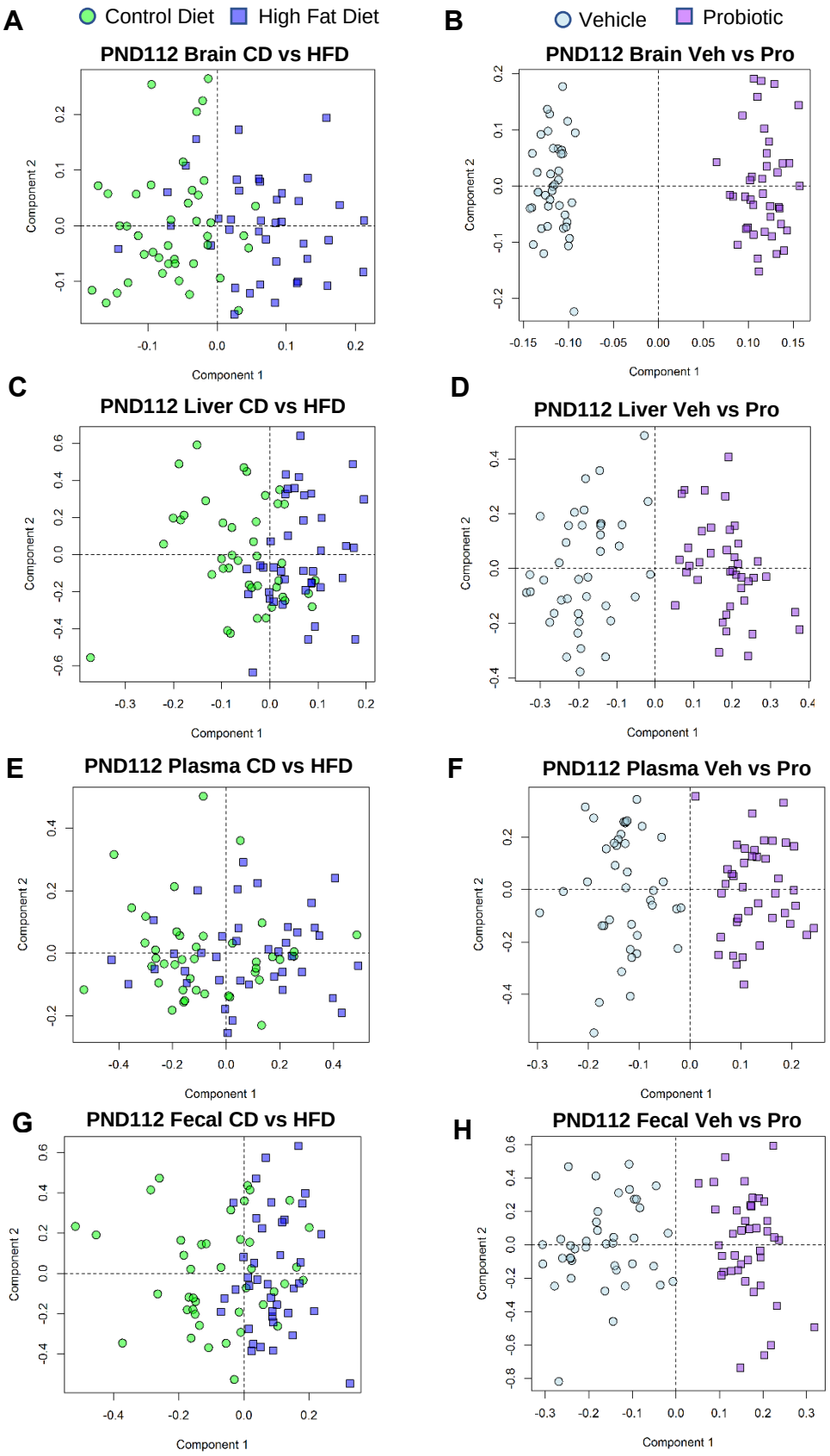
A
Control Diet
High Fat Diet
PND112 Brain CD vs HFD
B
Vehicle
Probiotic
PND112 Brain Veh vs Pro
C
PND112 Liver CD vs HFD
D
PND112 Liver Veh vs Pro
E
PND112 Plasma CD vs HFD
F
PND112 Plasma Veh vs Pro
G
PND112 Fecal CD vs HFD
H
PND112 Fecal Veh vs Pro
Component 1
Component 2

Figure S31 Individual OPLS-DA scores plots comparing the entire metabolome of brain, liver, plasma, and fecal material for each pairwise comparison in adult offspring. Corresponding model performance indices, including model accuracy, for each scores plot can be found in Table S36. **(A)** Maternal CD vs HFD offspring brain samples. **(B)** Maternal vehicle vs probiotic offspring brain samples. **(C)** Maternal CD vs HFD offspring liver samples. **(D)** Maternal vehicle vs probiotic offspring liver samples. **(E)** Maternal CD vs HFD offspring plasma samples. **(F)** Maternal vehicle vs probiotic offspring plasma samples. **(G)** Maternal CD vs HFD offspring fecal samples. **(H)** Maternal vehicle vs probiotic offspring fecal samples. N = 38-42 per group for each OPLS-DA model. Variable importance in projection (VIP) scores for key metabolites are detailed in Tables S43-S46.

Supplementary Table 36 OPLS-DA cross-validated model performance indices for metabolomics

	OPLS-DA Model	Accuracy	Specificity	Sensitivity	R^2X	R^2Y	Q^2
F0 (F1 PND4) Milk	CD vs HFD	69.4%	74.7%	65.8%	0.81	0.70	0.22
	Vehicle vs Probiotic	69.5%	63.7%	77.4%	0.90	0.80	-0.13
F1 Brain	CD vs HFD	88.1%	88.8%	87.5%	0.68	0.77	0.60
	Vehicle vs Probiotic	99.2%	99.7%	98.9%	0.58	0.89	0.84
F1 Liver	CD vs HFD	97.0%	97.5%	96.1%	0.84	0.88	0.77
	Vehicle vs Probiotic	98.9%	98.2%	99.6%	0.82	0.94	0.88
F1 Plasma	CD vs HFD	98.9%	98.8%	98.9%	0.85	0.90	0.85
	Vehicle vs Probiotic	97.2%	97.2%	97.4%	0.86	0.88	0.80
F1 Fecal	CD vs HFD	96.8%	99.2%	94.4%	0.57	0.89	0.80
	Vehicle vs Probiotic	86.0%	86.8%	85.7%	0.50	0.70	0.56
F1 Adult Brain	CD vs HFD	74.8%	77.6%	73.9%	0.46	0.50	0.16
	Vehicle vs Probiotic	99.8%	99.6%	100%	0.64	0.96	0.92
F1 Adult Liver	CD vs HFD	59.4%	62.5%	57.5%	0.70	0.43	-0.06
	Vehicle vs Probiotic	95.4%	99.9%	90.8%	0.69	0.87	0.79
F1 Adult Plasma	CD vs HFD	58.0%	60.6%	56.7%	0.91	0.27	-0.20
	Vehicle vs Probiotic	91.6%	93.3%	90.3%	0.95	0.78	0.64
F1 Adult Fecal	CD vs HFD	65.4%	75.1%	57.3%	0.56	0.41	0.12
	Vehicle vs Probiotic	93.3%	93.0%	94.2%	0.66	0.84	0.69

Supplementary Table 37 OPLS-DA VIP scores for key F0 metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP score	Control Diet vs High Fat Diet OPLS-DA VIP score
Brain Alanine [1.47-1.49]	2.6	1.6
Brain Glutamate [2.34-2.39]	3.0	0.7
Brain Creatine [3.93]	1.7	0.7
Brain Lactate [1.32-1.34]	4.6	1.7
Liver Leucine [3.70-3.74]	2.1	1.3
Liver Alanine [1.47-1.49]	3.6	1.0
Liver Taurine [3.41-3.44]	0.7	4.8
Liver Glucose [4.64-4.66]	4.4	0.7
Liver Acetate [1.91-1.92]	1.3	0.9
Liver Choline [3.22-3.23]	2.9	2.2
Liver GSSG [2.16-2.19]	2.0	1.0
Plasma Lactate [1.32-1.34]	4.2	1.4
Plasma VLDL/Triglyceride [0.84-88]	0.9	2.8
Plasma Alanine [1.47-1.49]	1.7	0.6
Plasma Choline [3.22-3.23]	1.9	2.6
Fecal Acetate [1.91-1.92]	5.2	2.3
Fecal Propionate [1.06-1.07]	2.5	2.1
Fecal Butyrate [1.54-1.58]	1.2	1.8
Fecal Glutamate [2.34-2.39]	2.6	0.6
Fecal Ethanol [1.17-1.20]	1.9	2.1
Fecal Glucose [4.64-4.66]	0.6	2.1

Supplementary Table 38 OPLS-DA VIP scores for key F0 milk (F1 PND4) metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP Score	Control Diet vs High Fat Diet OPLS-DA VIP Score
Lactate [1.32-1.34]	4.5	0.8
Lactose [3.72-3.75]	1.7	2.1
Butyrate/Valerate [0.89-91]	0.7	1.8
Alanine [1.47-1.49]	1.1	0.9
Formate [8.46]	1.3	0.1
Creatine [3.04]	0.7	0.6
Triglyceride/VLDL [1.23-1.32]	1.1	2.1

Supplementary Table 39 OPLS-DA VIP scores for key F1 (PND21) brain metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP Score	Control Diet vs High Fat Diet OPLS-DA VIP Score
Brain Lactate [1.32-1.34; 4.09-4.13]	6.9	2.0
Brain Creatine [3.04]	0.7	2.7
Brain Cholesterol/Triglyceride [1.38-1.44]	1.2	2.4
Brain Alanine [1.47-1.49]	2.3	1.9
Brain Glutamate [2.34-2.37]	1.8	1.1
Brain GABA [1.88-1.91]	1.8	0.2
Brain Acetate [1.92]	1.3	2.3
Brain Glutamine [2.43-2.48]	0.7	1.8

Supplementary Table 40 OPLS-DA VIP scores for key F1 (PND21) liver metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP Score	Control Diet vs High Fat Diet OPLS-DA VIP Score
Liver Lactate [1.32-1.34]	3.5	1.1
Liver Alanine [1.47-1.50]	2.9	0.8
Liver Glucose [4.64-4.66]	2.3	1.0
Liver Leucine [3.70-3.74]	2.6	1.4
Liver Taurine [3.26-3.28]	2.1	3.2
Liver GSSG [2.16-2.19]	2.5	1.4
Liver Creatine [3.04]	0.1	0.2
Liver Acetate [1.92]	1.3	0.7

Supplementary Table 41 OPLS-DA VIP scores for key F1 (PND21) plasma metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP Score	Control Diet vs High Fat Diet OPLS-DA VIP Score
Plasma Lactate [1.32-1.34]	2.2	2.2
Plasma Creatine [3.03-3.04]	0.4	2.2
Plasma VLDL/TG [0.84-0.88]	0.7	2.9
Plasma Alanine [1.47-1.49]	2.2	1.3
Plasma Glucose [3.56-59]	1.9	0.2
Plasma Acetate [1.92]	0.5	0.8

Supplementary Table 42 OPLS-DA VIP scores for key F1 (PND21) gut metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP Score	Control Diet vs High Fat Diet OPLS-DA VIP Score
Fecal Acetate [1.91-1.93]	3.8	1.8
Fecal Propionate [1.06-1.07]	2.4	1.0
Fecal Butyrate [1.54-1.58]	1.2	0.6
Fecal Succinate [2.40-2.42]	0.9	3.4
Fecal Ethanol [3.64-3.68]	3.2	2.0
Fecal Glucose [4.64-4.66]	0.2	3.1
Fecal Alanine [1.47-1.49]	1.5	1.3

Supplementary Table 43 OPLS-DA VIP scores for key F1 (PND112) brain metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP Score	Control Diet vs High Fat Diet OPLS-DA VIP Score
Brain Lactate [1.32-1.34]	3.0	1.3
Brain Cholesterol/ Triglyceride [0.86-0.90]	0.1	2.1
Brain Alanine [1.47-1.49]	0.9	0.3
Brain Glutamate [2.34-2.37]	1.1	3.1
Brain GABA [1.88-1.91]	0.8	2.2
Brain Acetate [1.92]	2.7	0.6
Brain Glutamine [2.43-2.47]	1.8	2.0

Supplementary Table 44 OPLS-DA VIP scores for key F1 (PND112) liver metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP Score	Control Diet vs High Fat Diet OPLS-DA VIP Score
Liver Lactate [1.32-1.34]	0.8	4.6
Liver Alanine [1.48]	2.1	1.2
Liver Glucose [4.64-4.66]	2.3	1.9
Liver Leucine [3.70-3.74]	1.8	1.4
Liver Taurine [3.26-3.28]	4.8	4.2
Liver Creatine [3.04]	0.3	0.4
Liver Acetate [1.92]	0.6	0.2

Supplementary Table 45 OPLS-DA VIP scores for key F1 (PND112) plasma metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP Score	Control Diet vs High Fat Diet OPLS-DA VIP Score
Plasma Triglyceride/VLDL [1.22-1.32]	5.0	6.2
Plasma Alanine [1.47-1.49]	2.4	1.2
Plasma Glucose [3.83-86]	2.3	1.9
Plasma Acetate [1.92]	1.0	0.53

Supplementary Table 46 OPLS-DA VIP scores for key F1 (PND112) gut metabolites

Metabolite [ppm]	Vehicle vs Probiotic OPLS-DA VIP Score	Control Diet vs High Fat Diet OPLS-DA VIP Score
Fecal Acetate [1.91-1.93]	3.0	5.0
Fecal Propionate [2.15-2.17]	3.2	1.6
Fecal Butyrate [1.54-58]	3.5	1.8
Fecal Ethanol [1.17-1.20]	0.6	0.5
Fecal Alanine [1.47-1.49]	0.5	1.6

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