SUPPLEMENTAL DATA

A synthetic peptide designed to neutralize lipopolysaccharides attenuates metaflammation and diet-induced metabolic derangements in mice

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Supplemental Figures

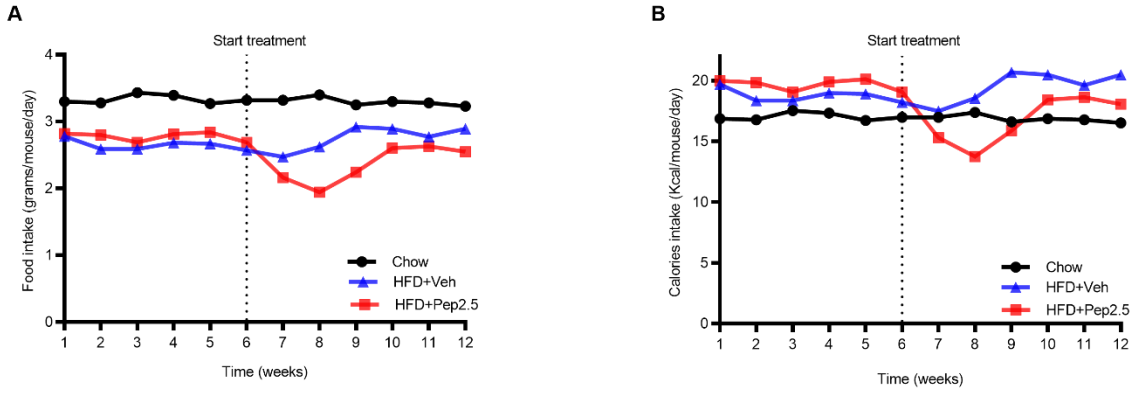

Figure S1: Effect of peptide 19-2.5 administration on food and caloric intake in HFD-fed mice. Weekly data was collected to report **(A)** calories intake (Kcal/mouse/day) and **(B)** food intake (grams/mouse/day). Data was analyzed by one-way ANOVA, with Bonferroni *post-hoc* test. Data are expressed as mean ± SEM; n = 20 per group.

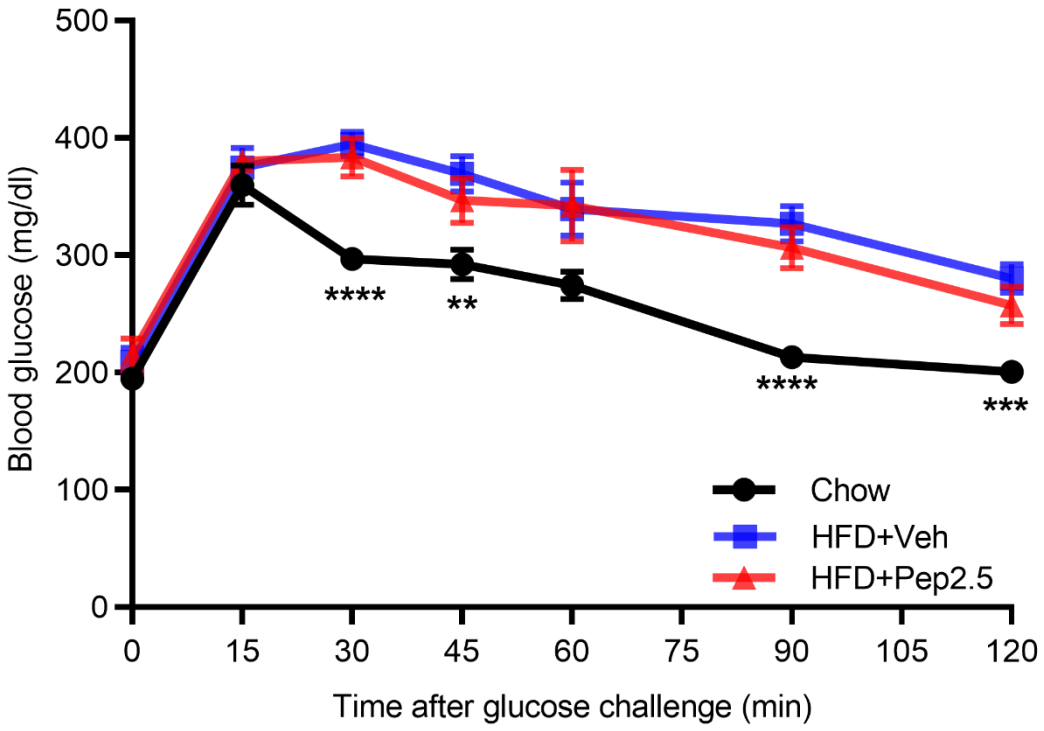

Figure S2: Effect of peptide 19-2.5 administration on OGTT at week 6 in HFD-fed mice. Oral glucose tolerance (OGTT) was assessed over 120 min, after receiving the oral dose of glucose at week 6 (mg/dl). Data are expressed as mean ± SEM; n = 20 per group. **P < 0.01 and ****P < 0.0001 vs. HFD + Veh.

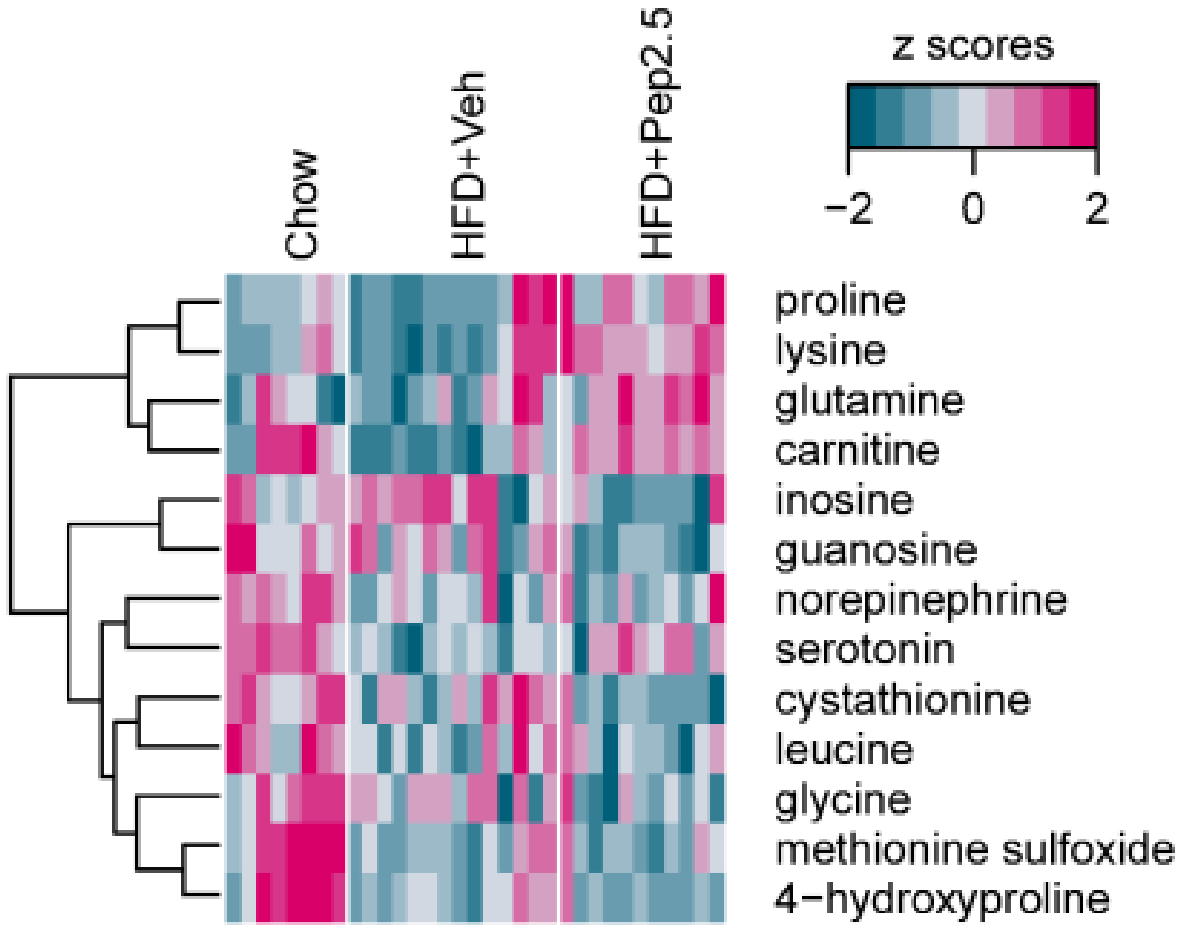

Figure S3: Z score heatmap of primary metabolites. Heatmap of all primary metabolites that are significantly different for any of the tested groupwise comparisons. Z scores are shown for all samples. Each column represents an individual sample and each row defines an analyte. Samples from Chow (n=8), HFD+Veh (n=13) and HFD+Pep2.5 (n=11) were arranged from left to right. Analytes were hierarchically clustered using Ward's minimum variance method[1] and an euclidian distance between z scores. Dendrograms provide information about distances between clusters.

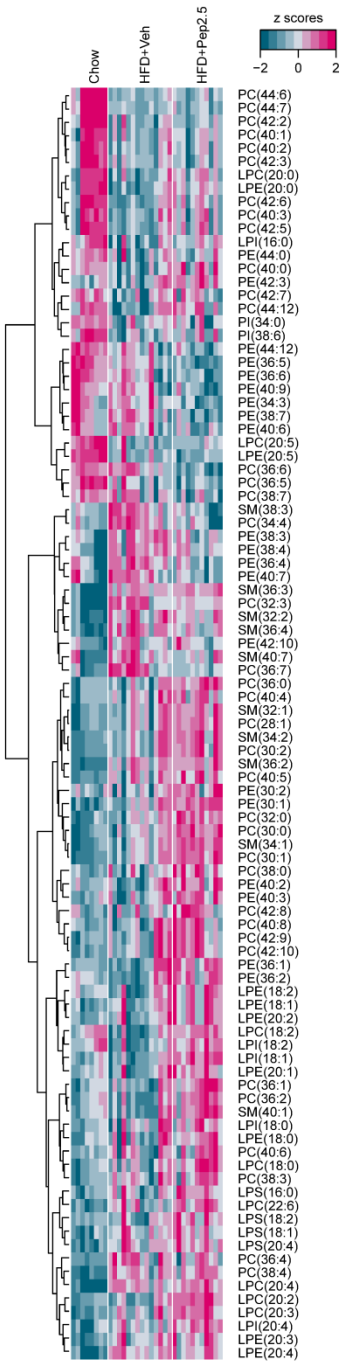

Figure S4. Z score heatmap of phospholipids. Heatmap of all phospholipids that are significantly different for any of the tested groupwise comparisons. Z scores are shown for all samples. Each column represents an individual sample and each row defines an analyte. Samples from Chow (n=8), HFD+Veh (n=13) and HFD+Pep2.5 (n=11) were arranged from left to right. Analytes were hierarchically clustered using Ward's minimum variance method [1] and an euclidian distance between z scores . Dendrograms provide information about distances between clusters. LPC: Lysophosphatidylcholine, PC: phosphatidylcholine, LPE: lysophosphatidylethanolamin, PE: phosphatidylethanolamine, LPI: lysophosphatidylinositol, PI: phosphatidylinositol, LPS: lysophosphatidylserine, SM: sphingomyeline.

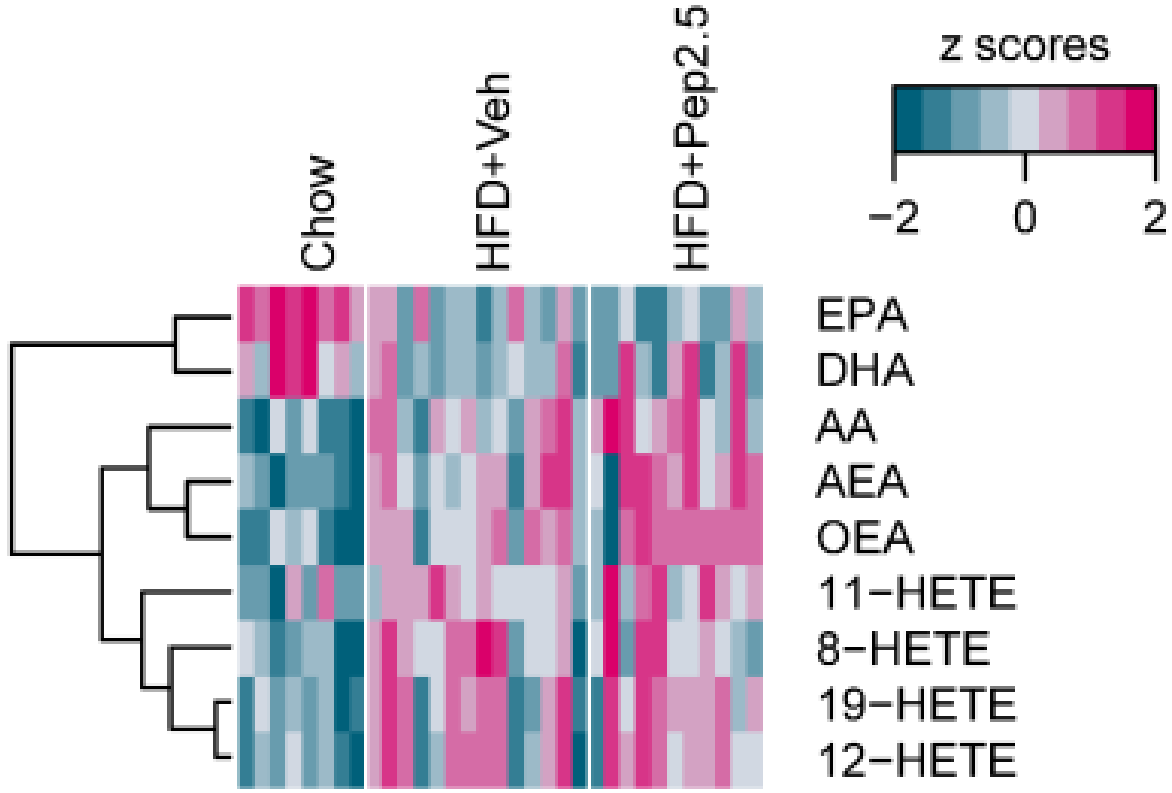

Figure S5: Z score heatmap of lipid mediators. Heatmap of all lipid mediators that are significantly different for any of the tested groupwise comparisons. Z scores are shown for all samples. Each column represents an individual sample and each row defines an analyte. Samples from Chow (n=8), HFD+Veh (n=13) and HFD+Pep2.5 (n=11) were arranged from left to right. Analytes were hierarchically clustered using Ward's minimum variance method [1] and an euclidian distance between z scores. Dendrograms provide information about distances between clusters. EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, AA: arachidonic acid, AEA: arachidonoylethanolamide, OEA: oleoylethanolamine, HETE: hydroxyeicosatetraenoic acid.

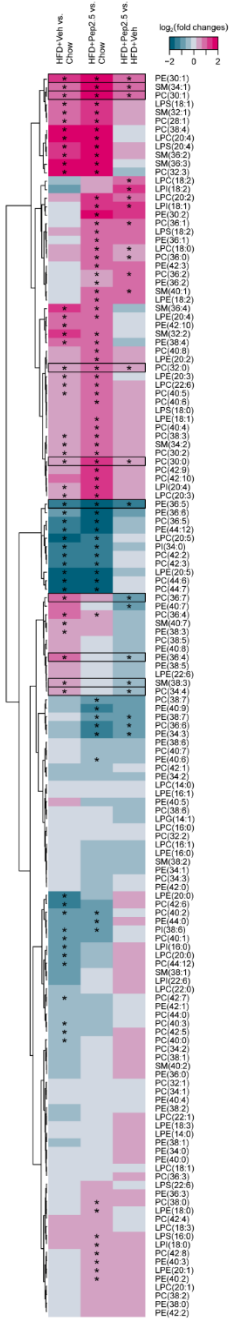

Figure S6: Log2 fold change heatmap of phospholipids. Heatmap of all detected phospholipids. Log2 fold changes and * P< 0.05 are shown for all tested groupwise comparisons: HFD+Veh (n = 13) vs. Chow (n = 8), HFD+Pep2.5 (n = 11) vs. Chow and HFD+Pep2.5 vs. HFD+Veh. Each column represents a groupwise comparison and each row defines an analyte. Analytes were hierarchically clustered using Ward's minimum variance method [1] and an euclidian distance between log2 fold changes. Dendrograms provide information about distances between clusters. Black frames show analytes with significant differences between the Chow and HFD+Veh group as well as between the HFD+Veh and HFD+Pep2.5 group. LPC: lysophosphatidylcholine, PC: phosphatidylcholine, LPE: lysophosphatidylethanolamine, PE: phosphatidylethanolamine, LPI: lysophosphatidylinositol, PI: phosphatidylinositol, LPS: lysophosphatidylserine, SM: sphingomyeline.

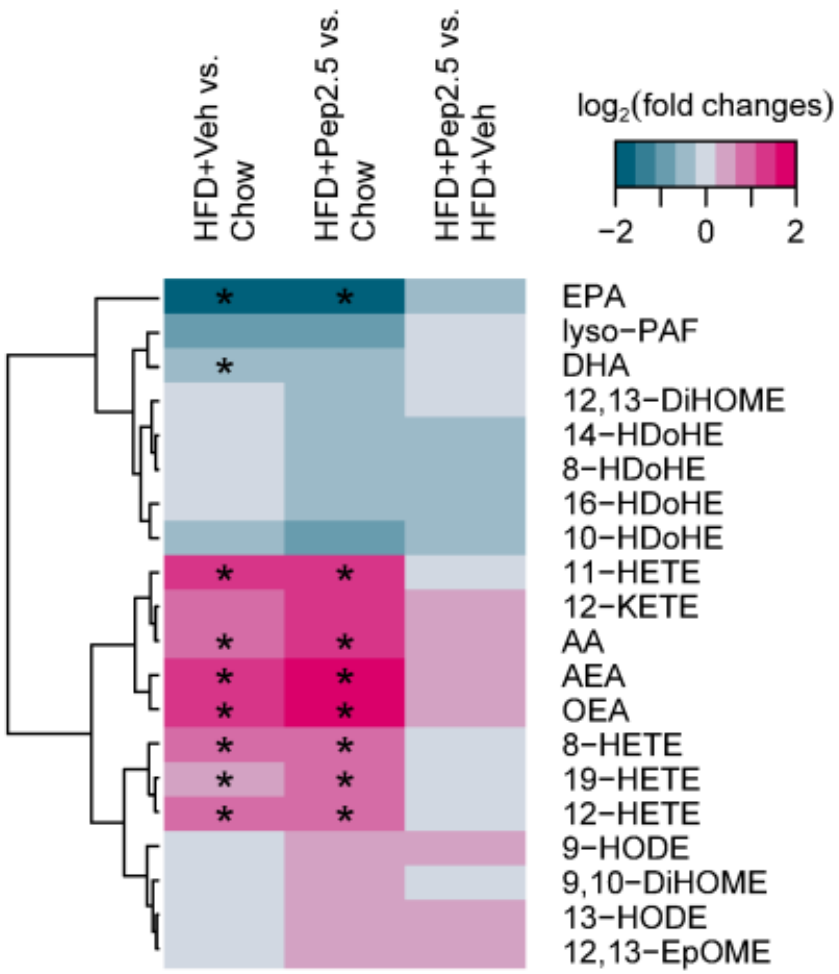

Figure S7: Log2 fold change heatmap of lipid mediators. Heatmap of all detected lipid mediators. Log2 fold changes and * P< 0.05 are shown for all tested groupwise comparisons: HFD+Veh (n = 13) vs. Chow (n = 8), HFD+Pep-2.5 (n = 11) vs. Chow and HFD+Pep2.5 vs. HFD+Veh. Each column represents a groupwise comparison and each row defines an analyte. Analytes were hierarchically clustered using Ward's minimum variance method [1] and an euclidian distance between log2 fold changes. Dendrograms provide information about distances between clusters. Black frames show analytes with significant differences between the Chow and HF+Veh group as well as between the HFD+Veh and HFD+Pep2.5 group. EPA: eicosapentaenoic acid, Lyso-PAF: lyso-platelet activating factor, DHA: docosahexaenoic acid, DiHOME: dihydroxy-octadecenoic acid, HDoHE: hydroxy-docosahexaenoic acid, HETE: hydroxyeicosatetraenoic acid, AA: arachidonic acid, AEA: arachidonoyl ethanolamide, OEA: oleoylethanolamine, HODE: Hydroxyoctadecadienoic acid, EpOME: epoxyoctamonoenoic acid.

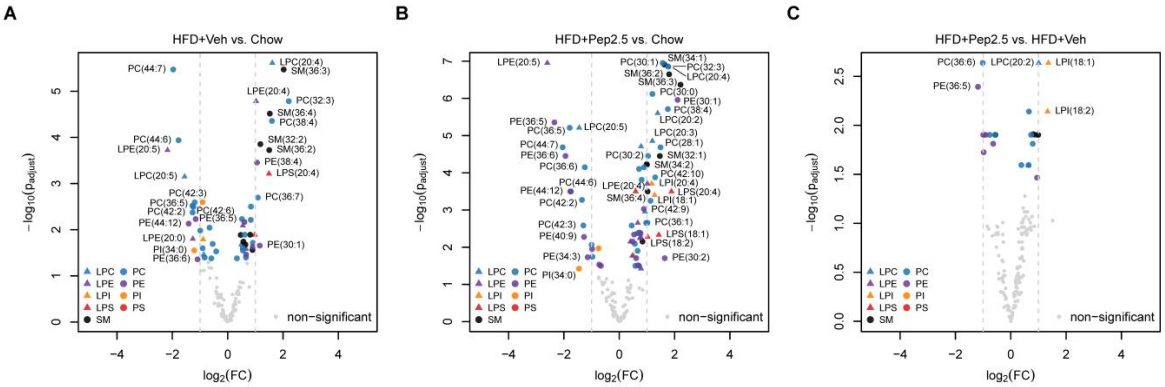

Figure S8: Volcano plots of phospholipids. Volcano plots of all tested groupwise comparisons of phospholipids showing log2 fold changes and adjusted P-values. (A) HFD+Veh (n = 13) vs. Chow (n = 8), (B) HFD+Pep2.5 (n = 11) vs. Chow and (C) HFD+Pep2.5 vs. HFD+Veh. Significantly different analytes are colored according to their lipid classes. Analytes with absolute log2 fold changes above 1 (grey dashed lines) are labeled. Grey dots represent analytes that were not significantly different for the respective groupwise comparison. LPC: lysophosphatidylcholine, PC: phosphatidylcholine, LPE: lysophosphatidylethanolamine, PE: phosphatidylethanolamine, LPI: lysophosphatidylinositol, PI: phosphatidylinositol, LPS: lysophosphatidylserine, SM: sphingomyeline.

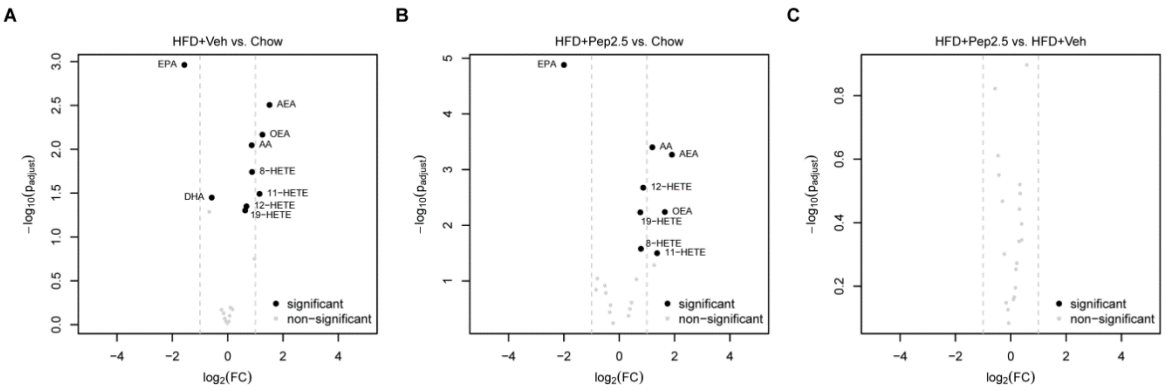

Figure S9: Volcano plots of lipid mediators. Volcano plots of all tested groupwise comparisons of lipid mediators showing log2 fold changes and adjusted P-values. (A) HFD+Veh (n=13) vs. Chow (n=8), (B) HFD+Pep2.5 (n=11) vs. Chow and (C) HFD+Pep2.5 vs. HFD+Veh. Significantly different analytes are depicted as black circles. Grey dots represent analytes that were not significantly different for the respective comparison. Grey dashed lines indicate absolute log 2fold change of one. EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, AA: arachidonic acid, AEA: arachidonoylethanolamide,OEA: oleoylethanolamine, HETE: hydroxyeicosatetraenoic acid.

Supplemental Tables

Table S1. Patient sample from healthy and T2DM patients.

	Healthy (n=10)		**T2DM patients (n=12)**
Age (years)	63 (61-68)		65 (54-74) (*)
Gender (n, men, %)	5 (50)		9 (75) (*)
Current smoking habit (n,%)	5 (50)		7 (58)
Current use of alcoholic drings (n (%))	4 (40)		7(58)
BMI (Kg/m2)	26.48 (3.78)		30.11 (7.50) (*)
waist circumference (cm)	90.67 (8.28)		101.77 (16.32) (*)
systolic blood pressure (mmHg)	138.60 (12.50)		131.50 (17.00)
diastolic blood pressure (mmHg)	83.60 (4.75)		81.50 (13.75)
anti-hypertensives (n,%)	6 (60)		10 (83) (*)
glucose (mg/dl)	99.0 (9.6)		153.3 (76.5) (*)
oral glucose lowering drugs (n,%)	0 (0)		9 (75) (*)
insulin analogues (n,%)	0 (0)		4 (33) (*)
total cholesterol (mg/dl)	244.60 (31.0)		196.16 (40.40) (*)
HDL-C (mg/dl)	54.70 (5.76)		44.66 (11.85) (*)
Triglycerides (mg/dl)	110.00 (70.00-130.00)		200.00 (95.00-250.75) (*)
LDL-C (mg/dl)	167.80 (24.60)		114.58 (38.40) (*)
Lipid lowering therapies (n,%)	3 (30)		7 (58)
creatinine (mg/dl)	0.81 (0.78-0.96)		1.66 (0.70-2.12) (*)
Glomerular Filtration Rate (Cockroft-Gault formula, ml/min/1.73 m2)	79.56 (18.15)		47.57 (27.43-80.52) (*)
Alanine Aminotransferase (ALT) (UI/ml)	25 (22-28)		20 (15-35)
Aspartate Aminotransferase (AST) (UI/ml)	24 (19-27)		20 (16-23)
Gamma-glutamyl Transpeptidase (GGT) (UI/ml)	21 (18-37)		21 (16-33)
C-Reactive Protein (mg/L)	0.18 (0.06-0.32)		0.21 (0.07-0.43) (*)

*P<0.05 data are presented either as mean (standard error) or median (inter-quartile range) based on their distribution (Shapiro-Wilk test for normality)

Table S2. HPLC programs. Solvent A: of 0.1% formic acid in water, solvent B: 0.1% formic acid in acetonitrile, solvent C: methanol, solvent D: 2-propanol. The column oven temperature was set to 50 °C.

time **[min]**	flow **[mL/min]**	solvent A concentration **[%]**	solvent B concentration **[%]**	solvent C concentration **[%]**	solvent D concentration **[%]**	solvent B and C curve*
primary metabolites						
0.01	0.25	100				
2.00	0.25	100				
5.00	0.25	75				
11.00	0.25	65				
15.00	0.25	5				
20.00	0.25	5				
20.01	0.25	100				
25.00	stop					
lipid mediators						
0	0.2	90	10			
10.0	0.2	75	25			
20.0	0.2	65	35			
40.0	0.2	25	75			
40.2	0.2	5	95			
50.0	0.2	5	90			
50.2	0.2	90	10			
59.0	Stop					
phospholipids						

0.0	0.15	80	10	10	
2.0	0.15	80	10	10	
4.0	0.15	60	20	20	-3
50.0	0.15	7.6	46.2	46.2	
52.0	0.15	0	50	50	
70.0	0.15	0	50	50	
70.2	0.15	80	10	10	
80.0	stop				

sphingosine-1-phosphate and sphingosine

0	0.4	90	10	
0.01	0.4	0	100	
3.00	0.4	0	100	
5.00	0.8	0	100	
7.00	0.8	0	100	
7.01	0.8	90	10	
7.80	0.8	90	10	
8.30	0.3	90	10	
9.50	0.3	90	10	
9.51	Stop			

*sets the gradient curve of the solvent (-10 to 10)

Table S3: Mass spectrometer (LCMS 8050) settings

source conditions	parameters
nebulizing gas flow rate	3.0 L/min
heating gas flow rate	10.0 L/min
drying gas flow rate	10.0 L/min
collision-induced dissociation gas pressure	230 kPa
interface temperature	300 °C
desolvation line temperature	250 °C
block heater temperature	400 °C
ionization mode	electrospray ionisation (ESI)

Table S4: Mass transitions for identified significantly changed primary metabolites. The target ion shows the multiple reaction monitoring (MRM) transitions, the ionization polarity (IP) shows the ionization mode of the electrospray ionization (ESI) source and the internal standard (IS) column assigns the number of the internal standard to the compounds with which they were evaluated. The internal standard is marked in bold letters. Injection volume of 20 µl sample.

no.	compound	target ion	IP	IS
1	4-hydroxyproline	132.10>86.05	+	1
2	acetylcarnitine	204.10>85.05	+	1
3	adenosine monophosphate	348.00>136.05	+	1
4	alanine	89.90>89,90	+	1
5	arginine	175.10>70.10	+	1
6	asparagine	133.10>87.15	+	1
7	aspartic acid	134.00>74.05	+	1
8	bilirubin	583.30>285.25	-	1
9	carnitine	162.10>103.05	+	1
10	choline	104.10>60.05	+	1
11	citric acid	191.20>111.10	-	1
12	citrulline	176.10>70.05	+	1
13	creatine	132.10>44.05	+	1
14	creatinine	114.10>44.05	+	1
15	cystathionine	223.00>88.05	+	1
16	cystine	241.00>151.95	+	1
17	cytidine	244.10>112.05	+	1
18	cytidine monophosphate	324.00>112.05	+	1
19	dimethylarginine (symmetric/asymmetric)	203.10>70.15	+	1
20	dimethylglycine	104.10>58.05	+	1
21	glutamic acid	147.90>84.10	+	1

22	glutamine	147.10>84.15	+	1
23	glycine	75.90>30.15	+	1
24	guanosine	284.00>152.00	+	1
25	histidine	155.90>110.10	+	1
26	hypoxanthine	137.00>55.05	+	1
27	inosine	269.10>137.05	+	1
28	isocitric acid	191.20>111.10	-	1
29	isoleucine	132.10>69.15	+	1
30	kynurenine	209.10>192.05	+	1
31	leucine	132.10>30.05	+	1
32	lysine	147.10>84.10	+	1
33	methionine	149.90>56.10	+	1
34	methionine sulfoxide	166.00>74.10	+	1
35	niacinamide	123.10>80.05	+	1
36	norepinephrine	170.10>152.15	+	1
37	ornithine	133.10>70.10	+	1
38	phenylalanine	166.10>120.10	+	1
39	proline	116,10>70,15	+	1
40	serine	105.90>60.10	+	1
41	serotonin	177.10>160.10	+	1
42	threonine	120.10>74.15	+	1
43	tryptophan	205.10>188.15	+	1
44	tyrosine	182.10>136.10	+	1
45	uracil	113.00>70.00	+	1
46	uric acid	167.10>123.95	-	1
47	uridine	245.00>113.05	+	1
48	valine	118.10>72.15	+	1
49	**2-morpholinoethanesulfonic acid (IS)**	**194.00>80.15**	**-**	**1**

Table S5: Mass transitions for identified significantly changed phospholipids. The target ion shows the multiple reaction monitoring (MRM) transitions, the ionization polarity (IP) shows the ionization mode of the ESI source and the internal standard (IS) column assigns the number of the internal standard to the lipid species with which they were evaluated. The internal standard is marked in bold letters. (LPC: lysophosphatidylcholine,PC:phosphatidylcholine,LPE:lysophosphatidylethanolamine, PE:phosphatidylethanolamine,LPG:lysophosphatidylglycerol,PG:phosphatidylglycerol, LPI: lysophosphatidylinositol, PI:phosphatidylinsitol, LPS:lysophosphatidylserine, PS:phosphatidylserine, SM:sphingomyelin)

no.	lipid species	target ion	IP	IS
1	LPC(14:0)	468.3>184.10	+	1
2	LPC(16:0)*	496.4>184.10	+	1
3	LPC(16:1)	494.3>184.10	+	1
4	LPC(18:0)*	524.4>184.10	+	1
5	LPC(18:1)*	522.4>184.10	+	1
6	LPC(18:2)*	520.4>184.10	+	1
7	LPC(18:3)	518.3>184.10	+	1
8	LPC(20:0)	552.4>184.10	+	1
9	LPC(20:1)	550.4>184.10	+	1
10	LPC(20:2)	548.4>184.10	+	1
11	LPC(20:3)	546.4>184.10	+	1
12	LPC(20:4)	544.4>184.10	+	1
13	LPC(20:5)	542.3>184.10	+	1
14	LPC(22:0)	580.5>184.10	+	1
15	LPC(22:1)	578.4>184.10	+	1
16	LPC(22:6)	568.4>184.10	+	1
17	PC(28:1)	676.5>184.10	+	2
18	PC(30:0)	706.6>184.10	+	2
19	PC(30:1)	704.5>184.10	+	2
20	PC(30:2)	702.5>184.10	+	2
21	PC(32:0)	734.6>184.10	+	2
22	PC(32:1)	732.6>184.10	+	2
23	PC(32:2)	730.6>184.10	+	2
24	PC(32:3)	728.5>184.10	+	2
25	PC(34:1)*	760.6>184.10	+	2
26	PC(34:2)*	758.6>184.10	+	2
27	PC(34:3)	756.6>184.10	+	2
28	PC(34:4)	754.6>184.10	+	2
29	PC(36:0)	790.7>184.10	+	2
30	PC(36:1)*	788.6>184.10	+	2
31	PC(36:2)*	786.6>184.10	+	2

32	PC(36:3)*	784.6>184.10	+	2
33	PC(36:4)*	782.6>184.10	+	2
34	PC(36:5)*	780.6>184.10	+	2
35	PC(36:6)	778.6>184.10	+	2
36	PC(36:7)	776.5>184.10	+	2
37	PC(38:0)	818.7>184.10	+	2
38	PC(38:1)	816.7>184.10	+	2
39	PC(38:2)	814.7>184.10	+	2
40	PC(38:3)*	812.6>184.10	+	2
41	PC(38:4)*	810.6>184.10	+	2
42	PC(38:5)*	808.6>184.10	+	2
43	PC(38:6)*	806.6>184.10	+	2
44	PC(38:7)*	804.6>184.10	+	2
45	PC(40:0)	846.7>184.10	+	2
46	PC(40:1)	844.7>184.10	+	2
47	PC(40:2)	842.7>184.10	+	2
48	PC(40:3)	840.7>184.10	+	2
49	PC(40:4)	838.7>184.10	+	2
50	PC(40:5)	836.6>184.10	+	2
51	PC(40:6)*	834.6>184.10	+	2
52	PC(40:7)*	832.6>184.10	+	2
53	PC(40:8)	830.6>184.10	+	2
54	PC(42:1)	872.7>184.10	+	2
55	PC(42:2)	870.7>184.10	+	2
56	PC(42:3)	868.7>184.10	+	2
57	PC(42:4)	866.7>184.10	+	2
58	PC(42:5)	864.7>184.10	+	2
59	PC(42:6)	862.7>184.10	+	2
60	PC(42:7)	860.6>184.10	+	2
61	PC(42:8)	858.6>184.10	+	2
62	PC(42:9)	856.6>184.10	+	2
63	PC(42:10)	854.6>184.10	+	2

64	PC(44:0)	902.8>184.10	+	2
65	PC(44:6)	890,7>184,10	+	2
66	PC(44:7)	888.7>184.10	+	2
67	PC(44:12)	878.6>184.10	+	2
68	LPE(14:0)	426.3>285.25	+	2
69	LPE(16:0)	454.3>313.25	+	2
70	LPE(16:1)	452.3>311.25	+	2
71	LPE(18:0)	482.3>341.30	+	2
72	LPE(18:1)	480.3>339.30	+	2
73	LPE(18:2)	478.3>337.25	+	2
74	LPE(18:3)	476.3>335.25	+	2
75	LPE(20:0)	510.4>369.35	+	2
76	LPE(20:1)	508.4>367.30	+	2
77	LPE(20:2)	506.3>365.30	+	2
78	LPE(20:3)	504.3>363.30	+	2
79	LPE(20:4)	502.3>361.25	+	2
80	LPE(20:5)	500.3>359.25	+	2
81	LPE(22:6)	526.3>385.25	+	2
82	PE(30:1)	662.5>521.45	+	2
83	PE(30:2)	660.5>519.45	+	2
84	PE(34:0)	720.6>579.55	+	2
85	PE(34:1)	718.6>577.50	+	2
86	PE(34:2)	716.5>575.50	+	2
87	PE(34:3)	714.5>573.50	+	2
88	PE(36:0)	748.6>607.55	+	2
89	PE(36:1)	746.6>605.55	+	2
90	PE(36:2)	744.6>603.55	+	2
91	PE(36:3)	742.6>601.50	+	2
92	PE(36:4)	740.5>599.50	+	2
93	PE(36:5)	738.5>597.50	+	2
94	PE(36:6)	736.5>595.45	+	2
95	PE(38:0)	776.6>635.60	+	2

96	PE(38:1)	774.6>633.60	+	2
97	PE(38:2)	772.6>631.55	+	2
98	PE(38:3)	770.6>629.55	+	2
99	PE(38:4)	768.6>627.55	+	2
100	PE(38:5)	766.6>625.50	+	2
101	PE(38:6)	764.5>623.50	+	2
102	PE(38:7)	762.5>621.50	+	2
103	PE(40:0)	804.7>663.65	+	2
104	PE(40:2)	800.6>659.60	+	2
105	PE(40:3)	798.6>657.60	+	2
106	PE(40:4)	796.6>655.55	+	2
107	PE(40:5)	794.6>653.55	+	2
108	PE(40:6)	792.6>651.55	+	2
109	PE(40:7)	790.6>649.50	+	2
110	PE(40:8)	788.5>647.50	+	2
111	PE(40:9)	786.5>645.50	+	2
112	PE(42:0)	832.7>691.65	+	2
113	PE(42:1)	830.7>689.65	+	2
114	PE(42:2)	828.7>687.65	+	2
115	PE(42:3)	826.7>685.60	+	2
116	PE(42:10)	812.5>671.50	+	2
117	PE(44:0)	860.7>719.70	+	2
118	PE(44:12)	836.5>695.50	+	2
119	LPG(14:1)	455.3>283.15	+	2
120	LPI(16:0)	571.3>241.00	-	2
121	LPI(18:0)	599.3>241.00	-	2
122	LPI(18:1)	597.3>241.00	-	2
123	LPI(18:2)	595.3>241.00	-	2
124	LPI(20:4)	619.3>241.00	-	2
125	LPI(22:6)	643.3>241.00	-	2
126	PI(34:0)	837.6>241.00	-	2
127	PI(38:6)	881.5>241.00	-	2

128	LPS(16:0)	498.3>313.25	+	2
129	LPS(18:0)	526.3>341.30	+	2
130	LPS(18:1)	524.3>339.30	+	2
131	LPS(18:2)	522.3>337.25	+	2
132	LPS(20:4)	546.3>361.25	+	2
133	LPS(22:6)	570.3>385.25	+	2
134	SM(32:1)	675.6>184.10	+	2
135	SM(32:2)	673.6>184.10	+	2
136	SM(34:1)	703.6>184.10	+	2
137	SM(34:2)	701.6>184.10	+	2
138	SM(36:2)	729.6>184.10	+	2
139	SM(36:3)	727.6>184.10	+	2
140	SM(36:4)	725.6>184.10	+	2
141	SM(38:1)	759.7>184.10	+	2
142	SM(38:2)	757.6>184.10	+	2
143	SM(38:3)	755.6>184.10	+	2
144	SM(40:1)	787.7>184.10	+	2
145	SM(40:2)	785.7>184.10	+	2
146	SM(40:7)	775.6>184.10	+	2
147	**sphingosine (17:0) IS**	**286.3>268.40**	**+**	**1**
148	**LPC(17:0) IS**	**510.4>184.30**	**+**	**2**

*1 µl sample injection volume

Table S6: Mass transitions for identified significantly changed lipid mediators. The target ion shows the multiple reaction monitoring (MRM) transitions, the ionization polarity (IP) shows the ionization mode of the ESI source and the internal standard (IS) column assigns the number of the internal standard to the lipid mediators with which they were evaluated. The internal standard is marked in bold letters.

no.	lipid mediator		target ion	IP	IS
1	12,13-DiHOME	12,13-dihydroxy-octadecenoic acid	313,2>183,1	-	1
2	9,10-DiHOME	9,10-dihydroxy-octadecenoic acid	313,2>201,2	-	1

3	19-HETE	19-hydroxyeicosatetraenoic acid	319,2>275,2	-	1
5	Lyso-PAF	lyso-platelet activating factor	482,3>184,1	-	1
6	13-HODE	13-hydroxyoctadecadienoic acid	295,2>195,1	-	1
7	9-HODE	9-hydroxyoctadecadienoic acid	295,2>171,1	-	1
8	16-HDoHE	16-hydroxy-docosahexaenoic acid	343,2>233,2	-	1
9	11-HETE	11-hydroxyeicosatetraenoic acid	319,2>167,1	-	1
10	10-HDoHE	10-hydroxy-docosahexaenoic acid	343,2>153,1	-	1
11	8-HETE	8-hydroxyeicosatetraenoic acid	319,2>155,1	-	1
12	14-HDoHE	14-hydroxy-docosahexaenoic acid	343,2>205,2	-	1
13	12-HETE	12-hydroxyeicosatetraenoic acid	319,2>179,1	-	1
14	8-HDoHE	8-hydroxy-docosahexaenoic acid	343,2>109,1	-	1
15	12-KETE	12-oxo-eicosatetraenoic acid	317,2>153,1	-	1
16	12,13-EpOME	12,13-epoxyoctamonoenoic acid	295,2>195,2	-	1
17	AEA	arachidonoyl ethanolamide	348,2>62,1	+	1
18	OEA	oleoylethanolamine	326,2>62,1	+	1
19	EPA	eicosapentaenoic acid	301,2>257,2	-	1
20	DHA	docosahexaenoic acid	327,2>283,2	-	1
21	AA	arachidonic acid	303,2>303,2	-	1
22	**Sph(17:0) IS**	**sphingosine (17:0) IS**	**286.3>268.4**	**+**	**1**

Table S7: Mass transitions for sphingosine-1-phosphate and sphingosine. The target ion shows the multiple reaction monitoring (MRM) transitions, the ionization polarity (IP) shows the ionization mode of the ESI source and the internal standard (IS) column assigns the number of the internal standard to the compounds with which they were evaluated. The internal standard is marked in bold letters.

no.	compound	target ion	IP	IS
1	sphingosine-1-phosphate	366.3>250.4	+	1

2	sphingosine	300.4>282.4	+	1
3	**sphingosine(17:0) IS**	**286.3>268.4**	**+**	**1**

Supplemental results

Z score heatmaps

Z score heatmaps illustrate 13 significantly altered primary metabolites, including 8amino acids, 2amino acid derivates, 2nucleosides and 1catecholamine (Figure S4).

Among the detected 146 phospholipids significant alterations were observed for 8lysophosphatidylcholines (LPC), 37phosphatidylcholines (PC), 9lysophosphatidylethanolamines (LPE), 20phosphatidylethanolamines (PE), 5lysophosphatidylinositols (LPI), 2phosphatidylinositols (PI), 4lysophosphatidylserines (LPS) and 10sphingomyelines (SM) (Supplementary figure5). Nine lipid mediators changed significantly, e.g. eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), oleoyl ethanolamide (OEA), arachidonoyl ethanolamide (AEA) and arachidonic acid (AA) and 4 of its monohydroxides: 11-hydroxyeicosatetraenoic acid (11-HETE), 8-HETE, 19-HETE und 12-HETE (FigureS6).

Log2 fold change heatmaps

Phospholipid analysis revealed 71 phospholipids without significant differences between the groups, Chow and HFD+Pep2.5 (Figure S74). Within these phospholipids PC(34:4), PC(36:7), PE(36:4) and SM(38:3) were significantly elevated in the Chow group compared with the HFD+Veh group as well as significantly elevated in the HFD+Veh group compared with the HFD+Pep2.5 group. Additionally, the group comparison of Chow and HFD+Pep2.5 identified 75 phospholipids with significant differences. Among these phospholipids PC(30:1), PC(30:1), PC(32:0), PE(30:41), SM(34:1) were significantly increased and PE(36:5) were significantly decreased in the groupwise comparisons of HFD+Veh vs. Chow as well as of HFD+Pep2.5 vs. HFD+Veh. Twelve lipid mediators were detected without significant differences and 8 were determined with significant differences when comparing the Chow and HFD+Pep2.5 group. The highlighted (black framed) metabolites in all log2 fold change heatmaps were significantly altered different between Chow and HFD+Veh group as well as between HFD+Veh and HFD+Pep2.5 group. None of the lipid mediators

showed significant differences in groupwise comparisons of HFD+Veh vs. Chow as well as of HFD+Pep2.5 vs. HFD+Veh (Figure S8).

Volcano plots

Volcano plot analysis highlights significantly different analytes with at least twofold changed phospholipids and lipid mediators. The comparison between the Chow group and HFD+Veh group showed a significant decrease or increase of 11phospholipids in HFD Control group (Supplementary figure 9A), subdivided in 4 reduced classes: PC (6lipidspecies), LPE(3), PE(2), PI(1) and 5elevated classes: LPC(1), PC(3), LPE(1), PE(2) and SM(4). Comparing the Chow and HFD+Pep2.5 group identified 13significantly decreased and 26 significantly increased phospholipids in HFD+Pep2.5 group (Figure S9B), subdivided in 5reduced classes: LPC(1), PC(6), LPE(1), PE(4) and PI(1) and 7elevated classes: LPC(3), PC(9), LPE(1), PE(2), LPS(3), LPS(2) and SM(6). Only 1PC and 1PE were significantly decreased and 1LPC and 2LPI were significantly increased in the HFD+Pep2.5 group compared with the HFD+Veh group (Figure S9C). The analysis of lipid mediators identified significantly decreased EPA and significantly increased AEA, OEA and 11-HETE in the HFD+Veh group compared with Chow group (Figure S10A) as well as in the HFD+Pep2.5 group compared with Chow group (Figure S10B). The latter group comparison revealed an additional significant elevated lipid mediator: AA. Comparing the HFD+Veh group and HFD+Pep2.5 group, no significant changes for lipid mediators were observed (Figure S10B).

References

[1] Doudney CO, Rinaldi CN. Modification of UV-induced mutation frequency and cell survival of Escherichia coli B/r WP2 trp E65 by treatment before irradiation," *J. Bacteriol.* 1984;160(1):233-238.