Supplementary Table 1 – Detailed composition of the experimental diets used in the study

Diet formulation provided as gram of ingredients per 100g for each of the 10 diets used in the study. The diets are isocaloric at 14.5 MJ/kg with the following net metabolizable energy (NME) assigned: casein (13.3 kJ/g; 3.2 kCal/g), L-methionine (18 kJ/g; 4.3 kCal/g), Canola oil (36.6 kJ/g; 8.7 kCal/g), Wheat starch (14.13 kJ/g; 3.4 kCal/g), Dextrinized Starch (14.6 kJ/g; 3.49 kCal/g), Sucrose 14.92 kCal/g and cellulose was given a NME of 0 kCal/g.

		Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**	**Diet 7**	**Diet 8**	**Diet 9**	**Diet 10**
	%P	60	5	5	33	33	5	14	14	42	24
	%C	20	75	20	47	20	47	29	57	29	38
	%F	20	20	75	20	47	48	57	29	29	38
INGREDIENTS											
Protein	**Casein**	64.87	5.04	5.04	35.50	35.50	5.04	14.83	14.83	45.29	25.71
	L-Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Fat	**Canola Oil**	7.91	7.91	29.65	7.91	18.58	18.98	22.53	11.46	11.46	15.03
Carbohydrate	**Wheat Starch**	12.59	47.87	12.60	29.91	12.60	29.91	18.37	36.34	18.37	24.13
	Dextrinized Starch	4.10	15.57	4.10	9.73	4.10	9.73	5.98	11.82	5.98	7.85
	Sucrose	3.11	11.81	3.11	7.38	3.11	7.38	4.54	8.97	4.54	5.96
Minerals	**$CaCO_3$**	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31
	NaCl	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
	AIN93 Trace Minerals	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
	KH_2PO_4	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
	K_2SO_4	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
	KCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	$C_5H_{14}ClNO$	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamins	**AIN93 vitamins**	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cellulose	**Cellulose**	3.06	7.44	41.15	5.21	21.75	24.60	29.39	12.23	10.00	16.97

Supplementary Table 2 – Statistical output for mixture models relating to sIgA

Model (Scheffé Polynomials)	Akaike Information Criterion	Degrees of freedom
1	1168.363	4
2	1168.552	7
3	1156.861	11
4	1170.262	8
Null	1181.324	2

Model 1 Coefficients			
Components	**Estimate (Std. Error)**	**t value**	**P(>|t|)**
Protein	2094.2 (264.7)	7.913	2.05e−11
Fat	517.6 (214.5)	2.413	0.018334
Carbohydrate	868.8 (220.6)	3.939	0.000185
Adjusted R-squared	0.8076		
p-value	< 2.2e−16		

Supplementary Table 3 – Statistical output for mixture models relating to plasma IgA

Model (Scheffé Polynomials)	Akaike Information Criterion	Degrees of freedom
1	1160.604	4
2	1142.835	7
3	1149.336	11
4	1144.762	8
Null	1171.433	2

Model 2 Coefficients			
Components	**Estimate (Std. Error)**	**t value**	**P(>|t|)**
Protein	1244.4 (600.3)	2.073	0.041706
Fat	2042.4 (371.3)	5.501	5.31e−07
Carbohydrate	2110.2 (375.5)	5.619	3.30e−07
Protein:Fat	−3432.3 (1666.8)	−2.059	0.043043
Protein:Carbohydrate	−4738.6 (1667.2)	−2.842	0.005804
Fat:Carbohydrate	−6169.1 (1657.3)	−3.722	0.000385
Adjusted R-squared	0.7243		
p-value	< 2.2e−16		

Supplementary Table 4 – Statistical output for mixture models relating to plasma IgM

Model (Scheffé Polynomials)	Akaike Information Criterion	Degrees of freedom
1	696.561	4
2	700.119	7
3	701.771	11
4	700.027	8
Null	693.555	2

Supplementary Table 5 – 16S rRNA sequencing quality control

	Total reads	Reads post filtering	Reads post merging and chimera filtering	Reads retained
HP 1	135805	119366	97727	71.96%
HP 2	49009	43630	43301	88.35%
HP 3	120743	107426	91056	75.41%
HP 4	59370	51662	50927	85.78%
HP 5	71171	62355	61855	86.91%
HP 6	55324	49431	49288	89.09%
HP 7	24290	21876	21826	89.86%
HP 8	111569	96616	95494	85.59%
HC 1	89660	79158	78435	87.48%
HC 2	133887	119303	102883	76.84%
HC 3	52702	47334	46964	89.11%
HC 4	29954	26904	26437	88.26%
HC 5	121070	100002	94779	78.28%
HC 6	86078	71337	71144	82.65%
HC 7	69380	59366	59010	85.05%
HC 8	136477	117920	101334	74.25%
HF 1	44447	41330	40566	91.27%
HF 2	135418	126495	117867	87.04%
HF 3	13618	12835	12692	93.20%
HF 4	19284	18178	17971	93.19%
HF 5	23368	22069	21948	93.92%
HF 6	19495	18154	18134	93.02%
HF 7	120323	113333	103573	86.08%
HF 8	102020	95906	84963	83.28%

Supplementary Table 6 – Statistical output for the comparison of different dietary groups of different beta diversity distance metric, based on PERMANOVA test (permutation = 9999)

PERMANOVA	HP vs HC	HP vs HF	HC vs HF
Unweighted UniFrac	R^2= 0.18861 P= 0.005	R^2= 0.34924 P= 2e-4	R^2= 0.35927 P= 3e-4
Weighted UniFrac	R^2= 0.45964 P= 2e-4	R^2= 0.67843 P= 2e-4	R^2= 0.81691 P= 2e-4
Atchison's distance	R^2= 0.35152 P= 3e-04	R^2= 0.53433 P= 1e-04	R^2= 0.52121 P= 3e-04

Supplementary Table 7 –Statistical output of ALDEx2 analysis comparing HP and HC at the genera level. CLR, centered log-ratio transformed; FDR, Benjamini–Hochberg corrected false discovery rate. Two-sided tests used.

Genus	Median CLR (HP)	Median CLR (HC)	Effect size	FDR (Welch's t test)	FDR (Wilcoxon test)
Akkermansia	5.230	-1.047	-1.365	0.032	0.013
Lactococcus	7.187	3.426	-1.481	0.024	0.009
Pseudomonas	-4.515	-6.937	-0.439	0.404	0.427
Turicibacter	-5.442	-1.88	0.555	0.317	0.315
Barnesiella	7.097	7.503	0.257	0.628	0.773
Lactobacillus	9.757	7.297	-0.683	0.185	0.278
Romboutsia	-3.655	0.020	0.071	0.815	0.880
Allobaculum	7.151	8.922	1.063	0.061	0.050
Bifidobacterium	9.147	7.640	-0.506	0.275	0.382
Olsenella	8.893	8.177	-0.419	0.362	0.442
Clostridium_XlVa	-2.954	-5.438	-0.439	0.416	0.437
Acetatifactor	1.263	2.133	0.582	0.165	0.194
Desulfovibrio	3.196	5.494	0.794	0.118	0.074
Parabacteroides	-5.583	-5.925	-0.041	0.698	0.771
Coprobacter	-0.637	2.605	0.732	0.145	0.175
Dorea	2.102	3.489	0.531	0.194	0.190
Sporobacter	-6.180	-4.408	0.346	0.440	0.541
Ruminococcus2	-1.070	-3.723	-0.300	0.528	0.567
Clostridium_sensu_st ricto	2.372	-6.863	-1.355	0.047	0.049
Enterorhabdus	1.809	2.222	0.128	0.660	0.833
Clostridium_IV	-0.607	-0.186	0.084	0.801	0.913
Mesorhizobium	-0.669	-0.841	-0.057	0.828	0.905
Intestinimonas	-4.610	-5.968	-0.253	0.609	0.679
Corynebacterium	-6.419	0.715	0.818	0.166	0.228
Murimonas	-2.197	-6.755	-0.743	0.235	0.214
Staphylococcus	-3.963	2.500	0.642	0.211	0.168
Escherichia/Shigella	-5.458	-5.203	0.026	0.697	0.770
Ralstonia	-4.315	-3.492	0.156	0.635	0.743
Burkholderia	-3.611	-3.850	0.031	0.773	0.860
Enterococcus	-6.467	-2.824	0.633	0.276	0.286
Bacillus	-6.061	-2.534	0.627	0.265	0.297
Senegalimassilia	-3.162	-3.831	-0.118	0.694	0.787

Supplementary Table 8 –Statistical output of ALDEx2 analysis comparing HP and HF at the genera level. CLR, centered log-ratio transformed; FDR, Benjamini–Hochberg corrected false discovery rate. Two-sided tests used.

Genus	Median CLR (HP)	Median CLR (HF)	Effect size	FDR (Welch's t test)	FDR (Wilcoxon test)
Akkermansia	5.201	8.460	1.823	0.002	0.003
Lactococcus	7.158	6.614	-0.458	0.244	0.447
Pseudomonas	-4.552	2.817	0.604	0.233	0.434
Turicibacter	-5.315	9.107	4.267	0.000	0.001
Barnesiella	7.137	4.406	-0.925	0.040	0.071
Lactobacillus	9.804	9.049	-0.428	0.276	0.367
Romboutsia	-3.374	7.604	1.943	0.006	0.001
Allobaculum	7.201	-6.456	-3.674	0.000	0.001
Bifidobacterium	9.221	-5.819	-4.804	0.000	0.001
Olsenella	8.948	-6.443	-4.254	0.000	0.001
Clostridium_XlVa	-2.878	1.792	0.587	0.224	0.194
Acetatifactor	1.231	0.101	-0.157	0.616	0.828
Desulfovibrio	3.181	3.842	0.119	0.672	0.671
Parabacteroides	-5.452	-3.252	0.407	0.370	0.395
Rhodococcus	-6.354	0.442	0.699	0.213	0.272
Coprobacter	-0.594	1.230	0.245	0.575	0.612
Dorea	2.128	1.273	-0.194	0.752	0.603
Ruminococcus2	-1.074	-5.474	-0.784	0.136	0.135
Clostridium_sensu_stricto	2.420	-6.603	-1.184	0.041	0.048
Enterorhabdus	1.855	3.425	0.536	0.254	0.347
Clostridium_IV	-0.654	-2.718	-0.290	0.552	0.515
Mesorhizobium	-0.700	0.052	0.298	0.546	0.477
Intestinimonas	-4.387	-5.404	-0.235	0.545	0.557
Murimonas	-2.214	-2.454	0.098	0.657	0.781
Staphylococcus	-3.825	-2.447	0.326	0.500	0.538
Escherichia/Shigella	-5.473	-3.606	0.494	0.249	0.338
Ralstonia	-4.262	0.189	0.518	0.314	0.250
Burkholderia	-3.616	-2.998	0.075	0.701	0.754
Enterococcus	-6.275	-2.906	0.500	0.329	0.310
Senegalimassilia	-3.159	-5.558	-0.427	0.327	0.332

Supplementary Table 9 –Statistical output of ALDEx2 analysis comparing HC and HF at the genera level. CLR, centered log-ratio transformed; FDR, Benjamini–Hochberg corrected false discovery rate. Two-sided tests used.

Genus	Median CLR (HC)	Median CLR (HF)	Effect size	FDR (Welch's t test)	FDR (Wilcoxon test)
Akkermansia	5.201	8.460	1.823	0.002	0.003
Lactococcus	7.158	6.614	-0.458	0.244	0.447
Pseudomonas	-4.552	2.817	0.604	0.233	0.434
Turicibacter	-5.315	9.107	4.267	0.000	0.001
Barnesiella	7.137	4.406	-0.925	0.040	0.071
Lactobacillus	9.804	9.049	-0.428	0.276	0.367
Romboutsia	-3.374	7.604	1.943	0.006	0.001
Allobaculum	7.201	-6.456	-3.674	0.000	0.001
Bifidobacterium	9.221	-5.819	-4.804	0.000	0.001
Olsenella	8.948	-6.443	-4.254	0.000	0.001
Clostridium_XlVa	-2.878	1.792	0.587	0.224	0.194
Acetatifactor	1.231	0.101	-0.157	0.616	0.828
Desulfovibrio	3.181	3.842	0.119	0.672	0.671
Parabacteroides	-5.452	-3.252	0.407	0.370	0.395
Rhodococcus	-6.354	0.442	0.699	0.213	0.272
Coprobacter	-0.594	1.230	0.245	0.575	0.612
Dorea	2.128	1.273	-0.194	0.752	0.603
Ruminococcus2	-1.074	-5.474	-0.784	0.136	0.135
Clostridium_sensu_stricto	2.420	-6.603	-1.184	0.041	0.048
Enterorhabdus	1.855	3.425	0.536	0.254	0.347
Clostridium_IV	-0.654	-2.718	-0.290	0.552	0.515
Mesorhizobium	-0.700	0.052	0.298	0.546	0.477
Intestinimonas	-4.387	-5.404	-0.235	0.545	0.557
Murimonas	-2.214	-2.454	0.098	0.657	0.781
Staphylococcus	-3.825	-2.447	0.326	0.500	0.538
Escherichia/Shigella	-5.473	-3.606	0.494	0.249	0.338
Ralstonia	-4.262	0.189	0.518	0.314	0.250
Burkholderia	-3.616	-2.998	0.075	0.701	0.754
Enterococcus	-6.275	-2.906	0.500	0.329	0.310
Senegalimassilia	-3.159	-5.558	-0.427	0.327	0.332

Supplementary Table 10 – Concentration of small intestine luminal metabolites detected by nuclear magnetic resonance (NMR). Concentrations are expressed as mM per mg of SI content (SD).

	HP	HC	HF
2-Aminobutyrate	1.076 (2.789)	0.753 (0.893)	0.624 (1.205)
Acetate	0.792 (0.199)	0.633 (0.127)	0.330 (0.159)
Alanine	3.981 (1.553)	2.561 (1.291)	0.000 (1.365)
Choline	1.208 (0.357)	1.434 (0.272)	1.068 (0.148)
Fumarate	0.094 (0.009)	0.066 (0.038)	0.000 (0.009)
Glutamate	5.568 (1.792)	3.293 (0.462)	2.850 (1.271)
Glycine	1.335 (0.309)	1.054 (0.553)	1.252 (0.361)
Homoserine	0.000 (1.212)	2.054 (0.877)	1.703 (0.466)
Isoleucine	3.090 (0.571)	2.070 (0.734)	1.408 (0.503)
Lactose	2.468 (0.628)	1.450 (0.295)	0.946 (0.152)
Leucine	9.563 (1.047)	5.229 (1.082)	4.309 (0.857)
Lysine	3.674 (1.803)	3.284 (0.877)	2.852 (0.463)
Malonate	0.366 (0.366)	0.310 (0.310)	0.291 (0.291)
Methionine	2.791 (0.425)	1.393 (0.711)	1.219 (0.468)
Pyruvate	0.133 (0.242)	0.264 (0.129)	0.125 (0.119)
Serine	1.945 (0.942)	1.227 (0.832)	0.000 (0.431)
Succinate	0.039 (0.005)	0.017 (0.011)	0.013 (0.008)
Valine	5.303 (1.046)	3.182 (0.688)	2.429 (0.800)

Supplementary Table 11 – Concentration of cecal content metabolites detected by nuclear magnetic resonance (NMR). Concentrations are expressed as mM per mg of cecal content (SD).

	HP	HC	HF
Acetate	23.21 (4.367)	35.50 (15.36)	14.87 (2.479)
Alanine	0.4942 (0.6841)	0.5355 (0.6802)	0.2778 (0.2390)
Butyrate	3.558 (0.8711)	8.759 (3.395)	2.027 (1.026)
Glutamate	4.083 (1.991)	2.808 (2.193)	1.026 (1.109)
Isoleucine	0.6867 (0.3561)	0.1078 (0.2042)	0 (0)
Lactate	1.457 (0.8441)	1.119 (1.143)	1.909 (1.849)
Leucine	1.597 (0.9185)	0.5091 (0.4334)	0.1735 (0.2790)
Lysine	0.8053 (0.9037)	0.6552 (0.6331)	0 (0)
Malonate	0.3504 (0.1826)	0.3163 (0.1561)	0.1881 (0.1885)
Methionine	0.2958 (0.2621)	0.1782 (0.1760)	0.03506 (0.07632)
Propionate	5.771 (1.606)	2.777 (0.8856)	3.297 (0.6736)
Pyruvate	1.445 (0.6071)	0.6418 (0.2915)	0.4338 (0.1698)
Succinate	0.7279 (0.2722)	0.2428 (0.08354)	0.1241 (0.07370)
Valine	0.8662 (0.4700)	0.4675 (0.3449)	0.03995 (0.1130)

Supplementary Table 12 – Antibodies used for flow cytometry

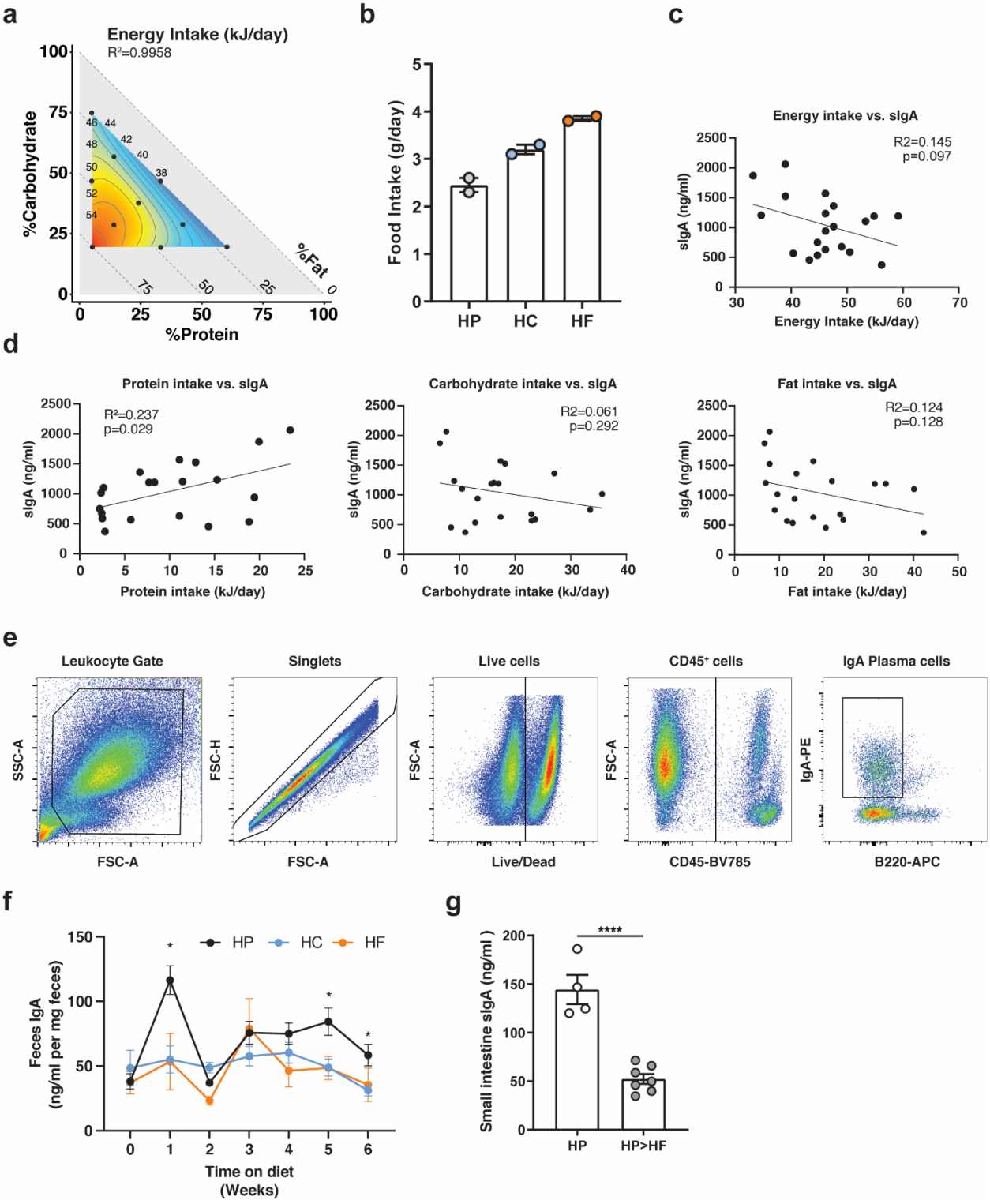
Target	Conjugate	Clone	Dilution	Manufacturer
CD45	BV785	30-F11	1:400	BioLegend
CD95	APC	SA367H8	1:200	BioLegend
GL-7	FITC	GL7	1:200	BioLegend
B220	VioGreen	REA755	1:50	Miltenyi Biotec
B220	APC	RA3-6B2	1:200	BioLegend
IgA	PE	11-44-2	1:200	eBioscience
CD4	PerCP	RM4-5	1:200	BioLegend

Supplementary Table 13 – Primers used in this study and their sequence

Pre-designed and validated primers used in this study. All primers were purchased from Sigma-Aldrich (KiCqStart SYBR® Green primers).

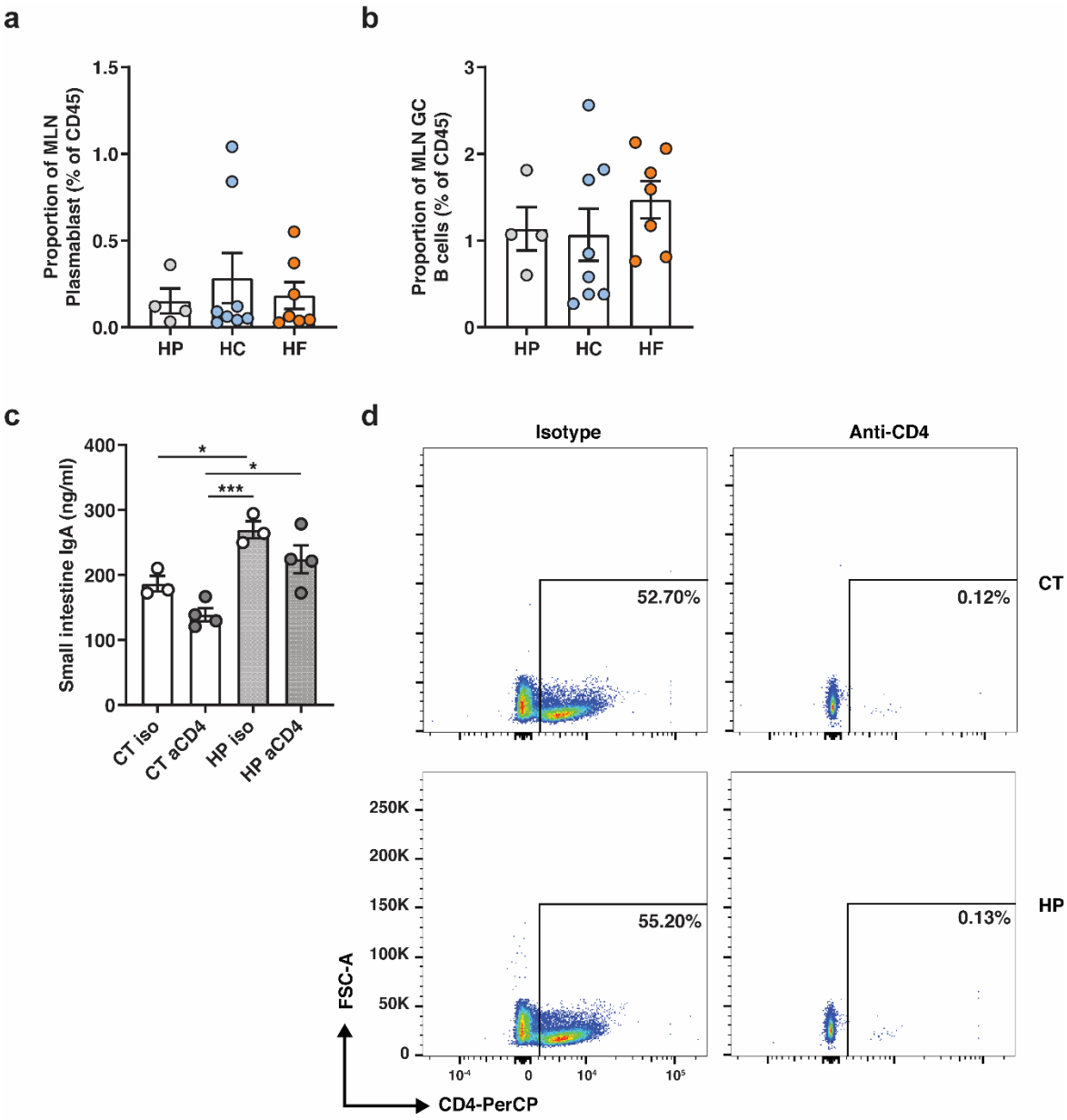
Gene target	Specie	Forward (5'-3')	Reverse (5'-3')
Pigr	Mouse	AAGAACTCCAGAGATTTGGG	GTGGTAGTCACGATTTCATC
Ccl28	Mouse	GAGGTGTCTCATCATGTTTC	ATACGTTTTCTCTGCCATTC
Tnfsf13	Mouse	TCTATAGTCAGGTCCTGTTTC	GGCATACTTCTGATACATCG
Baff	Mouse	ATCTACAGCCAGGTTCTATAC	AGCTGAATCTCATCTCCTTC
Tgfb	Mouse	GGATACCAACTATTGCTTCAG	TGTCCAGGCTCCAAATATAG
Tslp	Mouse	CCTGAAACTGAGAGAAATGAC	ACACCCTTAGTATTCTGTCC
Il10	Mouse	AAGGGTTACTTGGGTTGCCA	AAATCGATGACAGCGCCTCAG
Il4	Mouse	CTGGATTCATCGATAAGCTG	TTTGCATGATGCTCTTTAGG
Muc2	Mouse	ATTCTGAAGCCTGGGGAGAT	GAAGTCGGGACAGGTGATGT
Tjp1	Mouse	GTCTGCCATTACACGGTCCT	GGCTTAAGTCCAGGGGAGTC
Ocln	Mouse	CGGTACAGCAGCAATGGTAA	CTCCCCACCTGTCGTGTAGT
Tlr4	Mouse	GATCAGAAACTCAGCAAAGTC	TGTTTCAATTTCACACCTGG
Rpl13a	Mouse	ATCCCTCCACCCTATGACAA	GCCCCAGGTAAGCAAACTT
GAPDH	Human	GAAGGTGAAGGTCGGAGTCA	CAGAGTTAAAAGCAGCCCTGG
CCL28	Human	GAGGTGTCTCATCATGTTTC	ATACGTTTTCTCTGCCATTC
APRIL	Human	CAGGTGTCTTCCATTTACAC	TGGAGAGAGGTTAAGTTTCG
PIGR	Human	GACCGAGTTTCAATCAGAAG	TTGTCATTGGCTCCAAATTC

Supplementary Figure 1



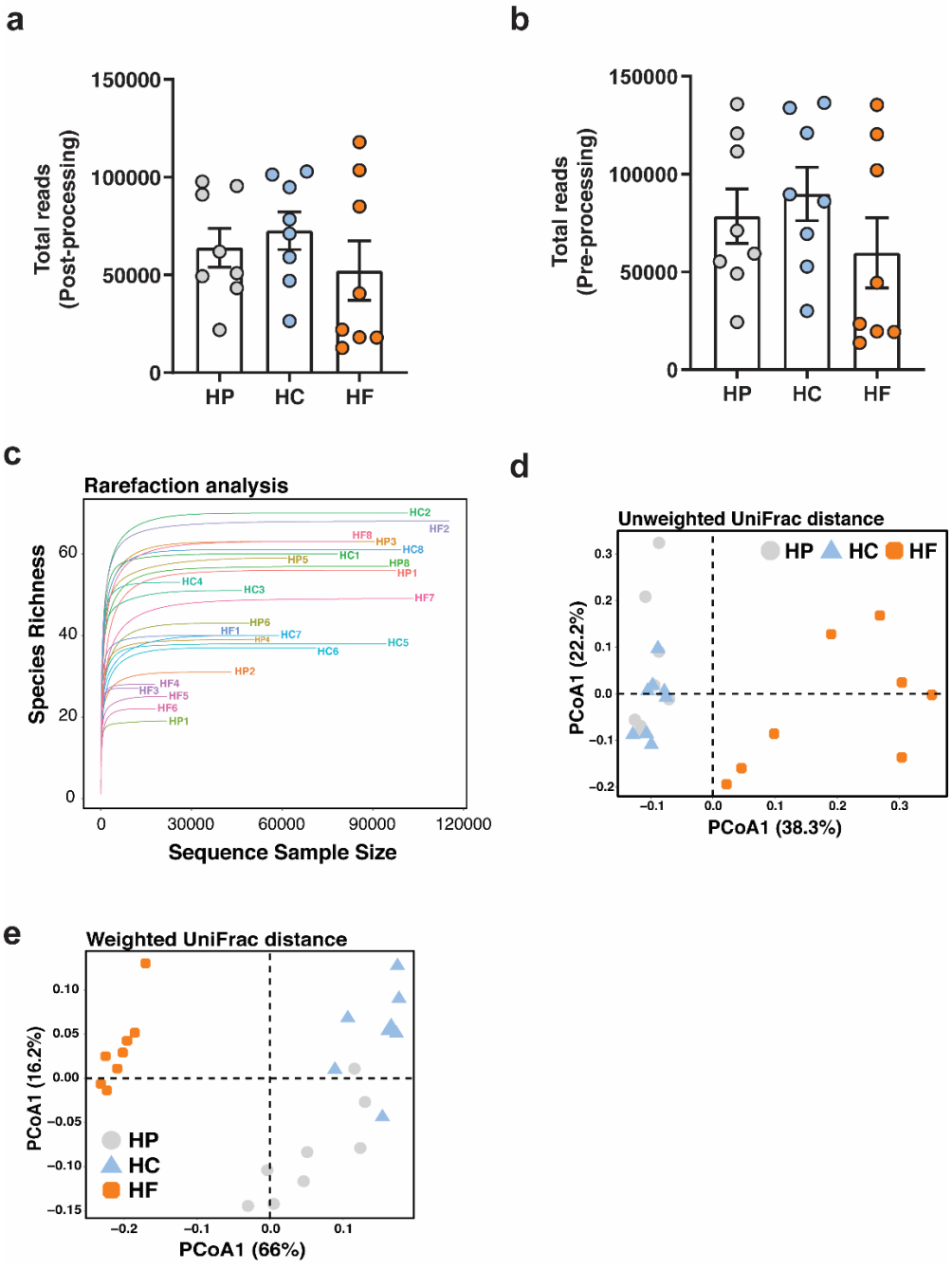
Supplementary Figure 1: Mice were fed on one of 10 diets encompassing a macronutrient range of 5-60% protein, 20-75% carbohydrate, and 20-75% fat for 6 weeks. (a) Contribution of macronutrient composition to average total daily energy intake (kJ/day) (averaged cage data) was modelled by Scheffé mixture model and represented on a right-angled mixture triangle. 3 component mixture model represented on a right-angled mixture triangle comprising of carbohydrate (y-axis), protein (x-axis) and fat (hypotenuse) with average total daily energy intake (kJ/day) as the response variable. Red represents higher average total daily energy intake while blue represents lower average total daily energy intake (kJ/day) in the nutrient mixture space. Each dot represents one of the 10 diets used for modelling response surface. (b) Average food intake (each point represents averaged cage data of n=4 mice per cage) of mice fed on a high-protein (HP), high-carbohydrate (HC) or high-fat (HF) diet. Linear regression of (c) total energy intake vs. sIgA levels as well as (d) protein, carbohydrate and fat eaten vs. sIgA levels were performed by simple linear regression analysis. (e) Gating strategy for the identification of $B220^{-}IgA^{+}$ IgA plasma cells by flow cytometry. (f) Mice were fed on a HP, HC or HF diet for 6 weeks and feces were collected weekly for determination of feces IgA by ELISA (n=6 mice per group for Week 0-2 and n=10 mice per group for Week 3-6). (g) Mice were fed on a HP diet for 6 weeks or fed on a HP diet for 6 weeks then switched to a HF diet for an additional 6 weeks and sIgA determined by ELISA (n=4 mice per group for HP and n=7 mice per group for HP>HF; $p = <0.0001$ by two-tailed unpaired t-test). Data are represented as mean ± SEM. Results represent n=3 independent (a-b), n=2 (c-e) and n=1 experiments (f-g).

Supplementary Figure 2



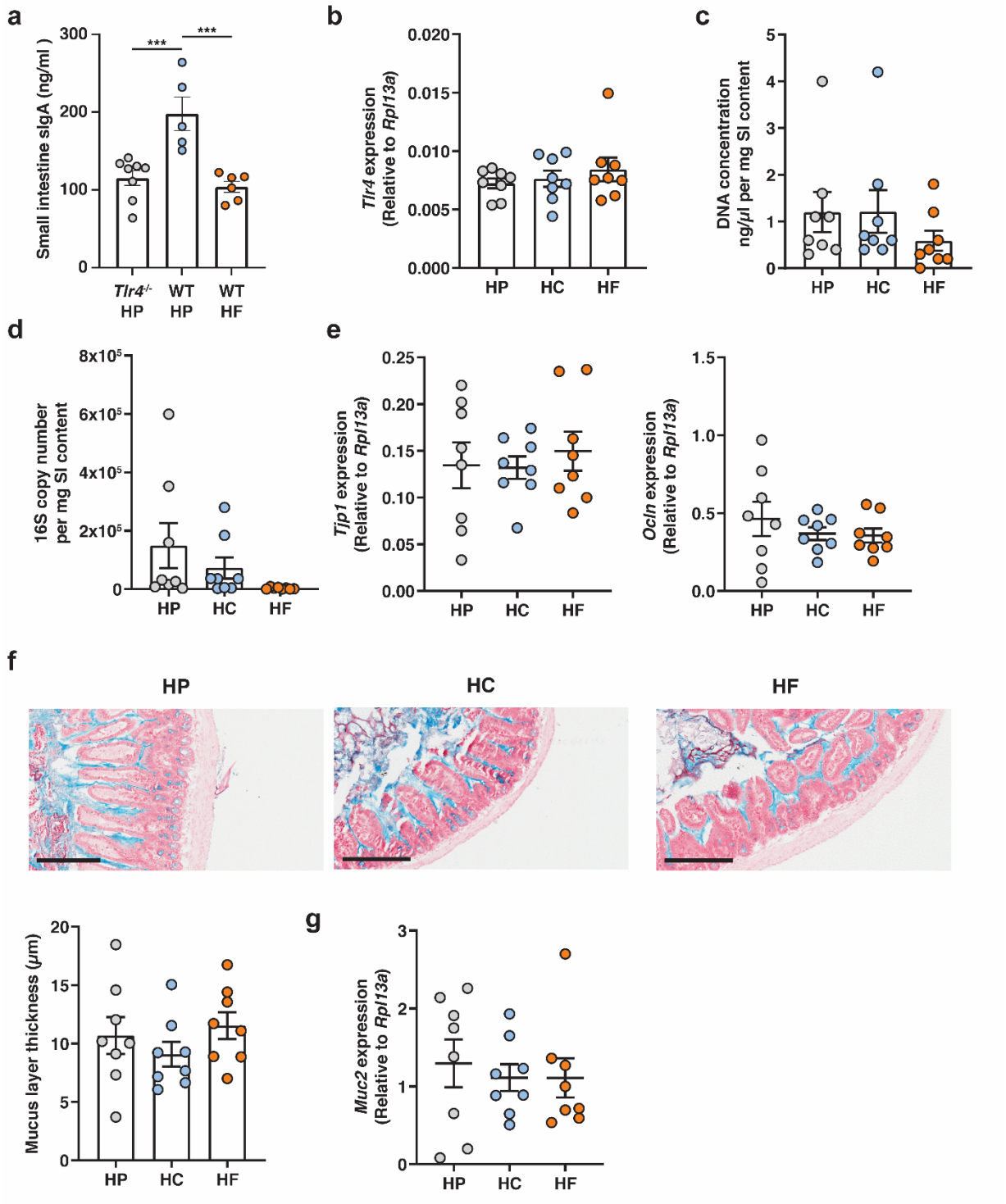
Supplementary Figure 2: (a) Proportion of $B220^{+}IgA^{+}$ plasmablast and (b) $CD95^{+}GL7^{+}$ germinal centre B cells in the mesenteric lymph nodes (n=4, n=7 and n=8 mice per diet for HP, HC and HF diet respectively) were determined by flow cytometry from mice fed on either a high-protein (HP), high-carbohydrate (HC) or high-fat (HF) diet for 6 weeks. (c) Mice were administered 200µg of anti-CD4 (GK1.5) or isotype control (LTF-2) antibody one day prior to either HP or control (AIN93G) diet feeding. Antibody treatment was administered twice weekly and small intestine sIgA determined 6 weeks later (n=3 mice per group for isotype control and n=4 mice per group for anti-CD4 treated groups; CT iso vs. HP iso p=0.0276, CT aCD4 vs HP iso p=0.0008, CT aCD4 vs. HP aCD4 p=0.01 by ordinary one-way ANOVA followed by Tukey's multiple comparisons test). Data are represented as mean ± SEM. (d) Representative flow cytometry plot demonstrating the successful systemic depletion of $CD4^{+}$ lymphocytes by flow cytometry. Results represent n=2 independent (a-b) and n=1 independent experiment (c-d).

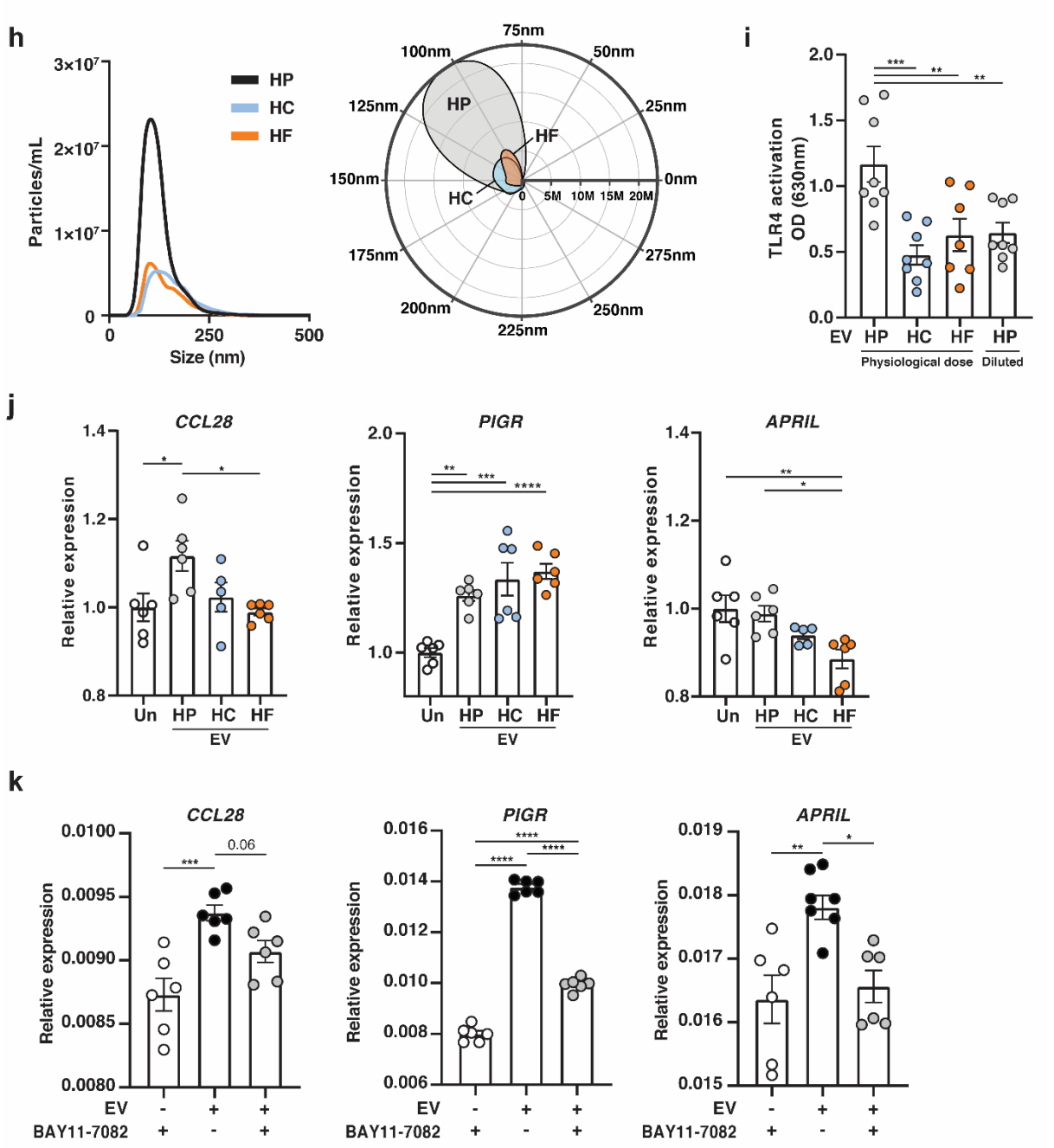
Supplementary Figure 3



Supplementary Figure 3: DNA from luminal content was extracted for 16S rRNA DNA sequencing from mice fed on a high-protein (HP), high-carbohydrate (HC) or high-fat (HF) diet for 6 weeks (n=8 per group). The total number of reads per samples (a) post and (b) pre-processing were quantified and no statistical significance between groups were found (Kruskal-Wallis test). Data are represented as mean ± SEM. (c) Rarefaction analysis was performed for each sample demonstrating that sequencing depth was sufficient to uncover all unique taxa (ASV) present in each sample. (d-e) Differences in the structure of the small intestinal microbiota communities were determined by principal coordinate analysis (PCoA) of both (d) Unweighted and (e) Weighted UniFrac distances. Results represent n=1 independent experiment.

Supplementary Figure 4

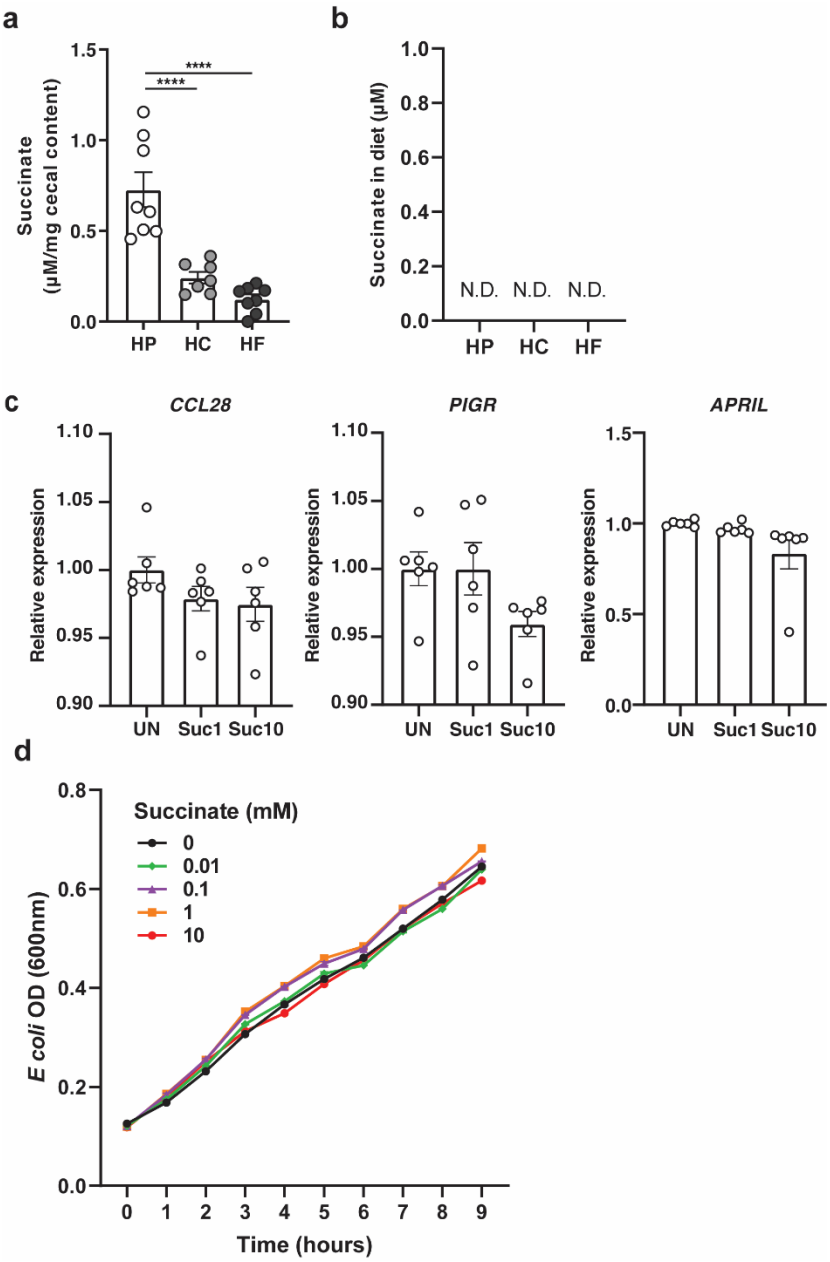




Supplementary Figure 4: (a) *Tlr4*$^{-/-}$ or wild type (WT) mice were fed on a high-protein (HP) diet, or WT mice fed on a high-fat (HF) diet for 6 weeks and sIgA levels were determined by ELISA (n=8, n=5 and n=6 mice per group for *Tlr4*$^{-/-}$ HP, WT HP and WT HF respectively; *Tlr4*$^{-/-}$ HP vs. WT HP p=0.008, WT HP vs. WT HF p=0.0004). Wild type mice were fed on either a high-protein (HP), high-carbohydrate (HC) or high-fat (HF) diet for 6 weeks and small intestine gene expression of (b) *Tlr4*, (e) *Tjp1* and *Ocln* and (g) *Muc2* was determined by qPCR (n=8 mice per group). Their small intestine contents were collected for DNA extraction and (c) DNA concentration was quantified by the Qubit™ dsDNA HS Assay Kit and (d) total 16S copy number by qPCR (n=8 mice per group). Wild type mice were fed on either a high-protein (HP), high-carbohydrate (HC) or high-fat (HF) diet for 6 weeks and (f) average mucus layer thickness was determined by histological analysis of Alcian blue-stained small intestine sections with the scale bar representing

100µm. (n=8 mice per group) (h) Cecum microbiota-derived extracellular vesicles (EV) were characterised by Nanoparticle Tracking Analysis (n=2 per diet pooled from n=4 mice each) and represented as an XY plot (left): 0-500nm vs. particle number/mL, or polar plot (right): angular axis represents particle size between 0-300nm and radial axis represents particle concentration in number/mL). (i) Cecum microbiota-derived EV were incubated at physiological ratio (approximately 3:1:1 of HP:HC:HF; n=8,n=8 and n=7 mice per group respectively), or at 1:1:1 ratio (HP diluted, n=8 mice per group) overnight with HEK-Blue TLR4 cell line and TLR activation measured at 630nm (HP vs. HC p=0.0004, HP vs. HF p=0.0072, HP vs. HP diluted p=0.007). (j) HT-29 were stimulated with cecum microbiota-derived EV isolated from HP ($3x10^9$ EV per well) or HC/HF ($1x10^9$ EV per well) fed animals for 16 hours and expression of CCL28 (Un vs. HP p=0.0372, HP vs. HF p=0.0213), PIGR (Un vs. HP p=0.0027, Un vs. HC p=0.0002, Un vs. HF p=<0.0001) and APRIL (Un vs. HF p=0.0067, HP vs. HF p=0.0156) were quantified by qPCR (n=6 wells per condition) and as well as (k) in the presence or absence of 10µM of NF-κB inhibitor BAY 11-7082, or with BAY 11-7082 alone and expression of CCL28 (-EV+Bay11-7082 vs. +EV-BAY11-7082 p=0.008), PIGR (-EV+Bay11-7082 vs. +EV-BAY11-7082 p=<0.0001, -EV+Bay11-7082 vs. +EV+Bay11-7082 p=<0.0001, +EV-BAY11-7082 vs. +EV+Bay11-7082 p=<0.0001) and APRIL (-EV+Bay11-7082 vs. +EV-BAY11-7082 p=0.0069, +EV-BAY11-7082 vs. +EV+BAY11-7082 p=0.0188) were quantified by qPCR (n=6 wells per condition). Data are represented as mean ± SEM. Results represent n=2 (b-e, g-k) and n=1 independent experiment(s) (a, f). *p<0.05, **<0.01, ***<0.001, ****<0.0001 by ordinary one-way ANOVA followed by Tukey's multiple comparisons test.

Supplementary Figure 5



Supplementary Figure 5: (a) Levels of succinate in the cecum were quantified by NMR spectroscopy from mice fed on a high-protein (HP), high-carbohydrate (HC) or high-fat (HF) diet for 6 weeks (n=7 and n=8 mice per diet for HC and HP/HF diet respectively; HP vs. HC p=<0.0001, HP vs. HF p= p=<0.0001). (b) Levels of succinate in food pellet of the HP, HC or HF diet was quantified by NMR spectroscopy (n=2 pellet from each diet were analyzed to confirm undetectable levels of succinate). (c) HT29 were stimulated overnight with vehicle control (PBS), or 1mM or 10mM of succinate and gene expression levels of CCL28, PIGR and APRIL quantified by qPCR (n=6 wells per condition). (d) Growth of *E coli,* cultured with the indicated concentrations of succinate, was quantified by spectrophotometry (OD 600nm). Data are represented as mean ± SEM. Results represent n=2 (d) and n=1 independent experiments (a-c). ****p<0.0001 by ordinary one-way ANOVA followed by Tukey's multiple comparisons test.