

Supplementary Information

Bioinformatic prediction of Crohn's disease-associated microbial metabolites

To predict the microbial metabolites associated with Crohn's disease, a previously reported perspective IBD PRISM cohort was used in this study. The following steps were used to generate an initial list of screening metabolites. Step 1: The public available data were downloaded from NCBI (BioProject ID numbers 82111). The microbial compositions were generated from 16S rRNA gene amplicon sequencing data for the healthy and Crohn's disease subjects in the PRISM cohort. We generated a table of the microbial taxa in each sample after the standard pipeline for sequence cleaning and phylotype assignment using MacQiime. Step 2: We imputed the metagenomics function by using a public available software, PICRUST (<http://picrust.github.io/picrust/>). The normalized OTU table were used as an input to predict metagenome by functional KEGG ortholog predictions. Step 3: MelonnPan (an R software, <https://huttenhower.sph.harvard.edu/melonnpan>) was used to predict metabolite compositions. The data matrix of relative abundances of metabolites for each sample were generated. Step 4: Disease-associated metabolites were determined using a multivariate model comparing predicted compound levels in health versus Crohn's disease subjects. We performed a generalized linear mixed model (glmmPQL, implemented in R), correcting for age and medications (antibiotics, immunosuppressants and anti-inflammatory agents). Disease-associated metabolites were ranked by FDR-corrected q value. From this raw list, we selected the top ranked commercially available molecules, tabulated in Supplementary Table 1.

Supplementary Table 1. List of screened metabolites

Metabolite Name	HMDB_ID	KEGG_ID	Cat.# (Sigma-Aldrich)
L-Phenylalanine	HMDB00159	C00079	P5482-25G
gamma-L-Glutamyl-L-cysteine	HMDB01049	C00669	G0903-25MG
Sulfite	HMDB00240	C00094	S0505-250G
D-Fructose 1,6-bisphosphate	HMDB01058	C00354	F6803-1G
Thiamin monophosphate	HMDB02666	C01081	T8637-5G
(R)-5-Phosphomevalonate	HMDB01343	C01107	07841-10MG
O-Acetyl-L-serine	HMDB03011	C00979	A6262-10MG
L-Lysine	HMDB00182	C00047	L5501-10MG
2-Oxoglutarate	HMDB00208	C00026	K1875-1G
dTMP	HMDB01227	C00364	T7004-100MG
(S)-3-Hydroxy-3-methylglutaryl-CoA	HMDB01375	C00356	H6132-5MG
beta-Alanine	HMDB00056	C00099	A9920-100G
alpha-D-Glucose 6-phosphate	HMDB01401	C00668	G7250-10MG
Phosphoenolpyruvate	HMDB00263	C00074	P7127-100MG
Glutathione	HMDB00125	C00051	PHR1359-500MG
UTP	HMDB00285	C00075	U6875-100MG
dTTP	HMDB01342	C00459	GE27-1880-04
Hexanoyl-CoA	HMDB02845	C05270	H2012-5MG
D-Fructose 6-phosphate	HMDB00124	C00085	F3627-10MG
2-Dehydro-3-deoxy-D-gluconate	HMDB01353	C00204	12271-1MG
O-Phospho-L-homoserine	HMDB03484	C01102	04668-50MG
Succinate	HMDB00254	C00042	W327700-1KG-K
Glyoxylate	HMDB00119	C00048	G10601-25G
L-Ornithine	HMDB00214	C00077	O2375-10MG
GDP-mannose	HMDB01163	C00096	G5131-10MG
D-Glyceraldehyde 3-phosphate	HMDB01112	C00118	39705-1ML
IMP	HMDB00175	C00130	I4625-5G
L-Histidine	HMDB00177	C00135	H6034-10MG
Citrate	HMDB00094	C00158	W302600-1KG-K
Adenosine	HMDB00050	C00212	A9251-1G
D-Mannose 1-phosphate	HMDB06330	C00636	M1755-10MG
LL-2,6-Diaminoheptanedioate	HMDB01370	C00666	89469-10MG
Urocanate/4-Imidazoleacrylic acid	HMDB00301	C00785	859796-5G
Isopentenyl diphosphate	HMDB04196	C00129	00297-10MG

AMP	HMDB00045	C00020	01930-5G
CoA	HMDB01423	C00010	C4282-10MG
UDP	HMDB00295	C00015	94330-100MG
L-Glutamate	HMDB00148	C00025	G1501-100G
Glycine	HMDB00123	C00037	G7126-100G
L-Arginine	HMDB00517	C00062	A5006-100G
CTP	HMDB00082	C00063	C1506-25MG
Thiamin diphosphate	HMDB01372	C00068	C8754-1G
L-Ascorbate	HMDB00044	C00072	A7631-25G/A4034-100G
L-Methionine	HMDB00696	C00073	M9625-5G
Fumarate	HMDB00134	C00122	F1506-25G
L-Leucine	HMDB00687	C00123	L8000-25G
dATP	HMDB01532	C00131	D6500-10MG
myo-Inositol	HMDB00211	C00137	I5125-50G
L-Homocysteine	HMDB00742	C00155	69453-50MG
Acetoacetate	HMDB00060	C00164	A8509-10MG
Agmatine	HMDB01432	C00179	A7127-1G
D-Glucuronate	HMDB00127	C00191	G8645-5G
Acetyl phosphate	HMDB01494	C00227	A0262-500MG
L-Homoserine	HMDB00719	C00263	H6515-10MG
D-Mannose 6-phosphate	HMDB01078	C00275	M3655-100MG
Creatine	HMDB00064	C00300	C0780-10MG
Isocitrate	HMDB00193	C00311	58790-250MG
L-Citrulline	HMDB00904	C00327	C7629-1G
6-Phospho-D-gluconate	HMDB01316	C00345	55962-10MG
Phosphatidylethanolamine	HMDB60501	C00350	1535744-100MG
cis-Aconitate	HMDB00072	C00417	A3412-1G
(R)-Mevalonate	HMDB59629	C00418	50838-10MG
5,6-Dihydrouracil	HMDB00076	C00429	D7628-5G
dCTP	HMDB00998	C00458	D4635-25MG
Glutaryl-CoA	HMDB01339	C00527	G9510-5MG
Pyridoxine phosphate	HMDB01319	C00627	82890-10MG
(S)-Methylmalonyl-CoA	HMDB02310	C00683	M1762-1MG
Deamino-NAD (NAADP)	HMDB01179	C00857	N6506-25MG
L-Histidinol	HMDB03431	C00860	H6647-10MG
Crotonoyl-CoA	HMDB02009	C00877	28007-5MG
Dephospho-CoA	HMDB01373	C00882	D3385-5MG
L-Histidinol phosphate		C01100	41486-10MG

L-Galactono-1,4-lactone	HMDB02541	C01115	05313-100MG
D-Ribulose 1,5-bisphosphate	HMDB11688	C01182	83895-10MG
3-Methylbutanoyl-CoA	HMDB01113	C02939	I9381-10MG
cis-Homoaconitate		C04002	40487-10MG
1-(5'-Phosphoribosyl)-5-amino-4-imidazolecarboxamide (AICAR)	HMDB01517	C04677	A9978-25MG
Succinyl-CoA	HMDB01022	C00091	S1129-5MG
Glycerone phosphate	HMDB01473	C00111	37442-100MG-F
Proline betaine	HMDB04827	C10172	sc-296420(Santa Cruz)
Suberic acid	HMDB00893	C08278	S5200-5G
Oleic acid	HMDB00207	C00712	O1008-1G
Tyramine	HMDB00306	C00483	T90344-5G
Niacinamide	HMDB01406	C00153	N5535-100G
Arachidonic acid	HMDB01043	C00219	A3555-10MG
Caprylic acid	HMDB00482	C06423	PHR1202-1G
Linoleic acid	HMDB00673	C01595	L1376-1G
L-Tyrosine	HMDB00158	C00082	T8566-25G
Butyric acid	HMDB00039	C00246	B103500-100ML
cis-4,7,10,13,16,19-Docosahexaenoic acid	HMDB02183	C06429	D2534-25MG
L-Isoleucine	HMDB00172	C00407	I2752-5G
Sebacic acid	HMDB00792	C08277	283258-5G
4-Vinylphenol	HMDB04072	C05627	sc-267756; (Santa Cruz), 10% solution in propylene glycol
Oleamide	HMDB02117	C19670	O2136-100MG
Propionic acid	HMDB00237	C00163	P1386-500ML
Stigmasterol	HMDB00937	C05442	S2424-1G
Shikimic acid	HMDB03070	C00493	S5375-1G
N-Acetylputrescine	HMDB02064	C02714	A8784-25MG
Hexanoic acid	HMDB00535	C01585	153745-2.5G
Pyridoxamine dihydrochloride	HMDB01431	C00534	P9380-1G
N-a-Acetyl-L-arginine	HMDB04620		S451029-250MG
L-Carnitine	HMDB00062	C00318	C0158-1G
Azelaic acid (98%)	HMDB00784	C08261	246379-25G
Dodecanedioic acid	HMDB00623	C02678	D1009-100G
Nicotinic acid	HMDB01488	C00253	N4126-5G
1,2,4-Trimethylbenzene	HMDB13733	C14533	T73601-25ML
Isovaleric acid	HMDB00718	C08262	129542-100ML

Taurine	HMDB00251	C00245	T0625-10G
Phenylacetic acid	HMDB00209	C07086	P16621-5G
Putrescine	HMDB01414	C00134	51799-100MG
L-(+)-Lactic acid	HMDB00190	C00186	L1750-10G
Piperidine	HMDB34301	C01746	76046- 100MG/411027
gamma-butyrobetaine/(3-Carboxypropyl)trimethylammonium chloride	HMDB01161	C01181	403245-1G
D-Glyceric acid	HMDB00139	C00258	61786-10MG
Cyclohexylamine	HMDB31404	C00571	G6503-1G
4-Guanidinobutyric acid	HMDB03464	C01035	G6503-1G
1-Oleoyl-sn-glycero-3-phosphocholine(synthetic, ≥99%)	HMDB02815	C04230	L1881-5MG
Cadaverine	HMDB02322	C01672	33211-10ML-F
L(-)-Fucose	HMDB00174	C01019	F2252-10MG
2-Palmitoylglycerol	HMDB11533		75614-25MG
Cholesteryl oleate	HMDB00918	C14641	C9253-100MG
Histamine	HMDB00870	C00388	H7125-1G
D-Pantothenic acid	HMDB00210	C00864	P5155-100G
dGDP	HMDB00960	C00361	D9250-25MG
Tetradecanoyl-CoA	HMDB01521	C02593	M4414-5MG
NAD	HMDB00902	C00003	N7004-250MG
ADP	HMDB01341	C00008	A2754-100MG
FAD	HMDB01248	C00016	F6625-10MG
S-Adenosyl-L-methionine	HMDB01185	C00019	A7007-25MG
Acetyl-CoA	HMDB01206	C00024	A2056-10MG
GDP	HMDB01201	C00035	G7127-25MG
FMN	HMDB01520	C00061	F2253-25MG
GMP	HMDB01397	C00144	G8377-500MG
D-Erythrose 4-phosphate	HMDB01321	C00279	E0377-10MG
dGTP	HMDB01440	C00286	D4010-10MG
Acetoacetyl-CoA	HMDB01484	C00332	A1625-10MG
dUMP	HMDB01409	C00365	D3876-100MG
5-Aminolevulinate	HMDB01149	C00430	A3785-500MG
Homogentisate	HMDB00130	C00544	H0751-100MG

Supplementary Table 2. Gut OTUs containing enzymes of the D-sorbitol pathway

OTU_IDs	Consensus Lineage
156580	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Burkholderiaceae; g__Burkholderia; s__
4322414	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Burkholderiaceae; g__Burkholderia; s__
49998	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Burkholderiaceae; g__Burkholderia; s__
4311006	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Burkholderiaceae; g__Burkholderia; s__
135993	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Burkholderiaceae; g__Burkholderia; s__

Supplementary Table 3. Gut OTUs containing enzymes of the 2,5-diketo-D-gluconic acid pathway

OTU_IDs	Consensus Lineage
4368451	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Phyllobacteriaceae; g__ ; s__
221365	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
4369186	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
4343884	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
648004	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
772368	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
88754	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
2228274	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
1044767	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
639311	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
163322	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__ ; s__
929312	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodospirillales; f__Acetobacteraceae; g__Roseomonas; s__mucosa
236142	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
1119540	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
510870	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
819636	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
4456892	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
213522	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
540402	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
219248	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__

3944484	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
819387	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
1110763	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
238820	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
4432891	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ ; s__
578058	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
239362	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
9939	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
138826	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
806143	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
813564	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
4430681	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
4318990	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
656889	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
4067355	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
835346	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
9826	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
9912	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
1022184	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
1134377	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
4417158	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
688934	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__
167120	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__ Erwinia; s__

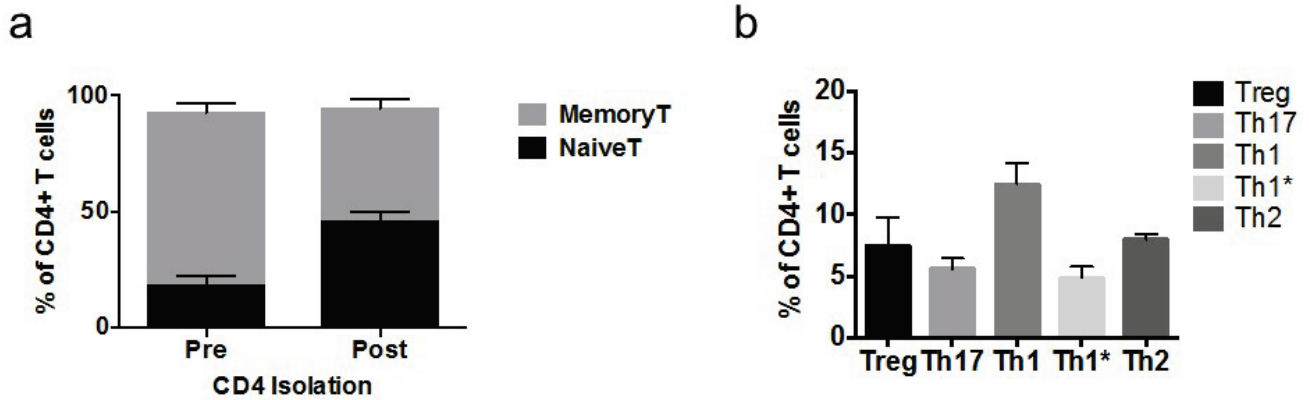
4250206	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia; s__
585701	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia; s__
9918	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia; s__
817254	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia; s__ dispersa
539107	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia; s__ dispersa
808540	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia; s__ dispersa
169538	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia; s__ dispersa
825033	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia; s__ dispersa
372473	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia; s__ soli
3159130	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Serratia; s__
1726426	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Serratia; s__
4454068	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Serratia; s__
540793	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Serratia; s__
4360511	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Serratia; s__
759061	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Yersinia; s__
244248	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__ ; s__
4382169	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Azorhizophilus; s__
358042	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
133961	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
139321	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
813216	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
279948	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
171527	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__

350105	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
242070	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
104313	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
3290367	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
141206	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
218267	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
271238	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
553648	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
617271	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
4435983	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
144452	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
219457	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
99682	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
4475523	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
4320653	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
828623	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
217410	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
513808	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
28841	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
269901	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
1119175	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
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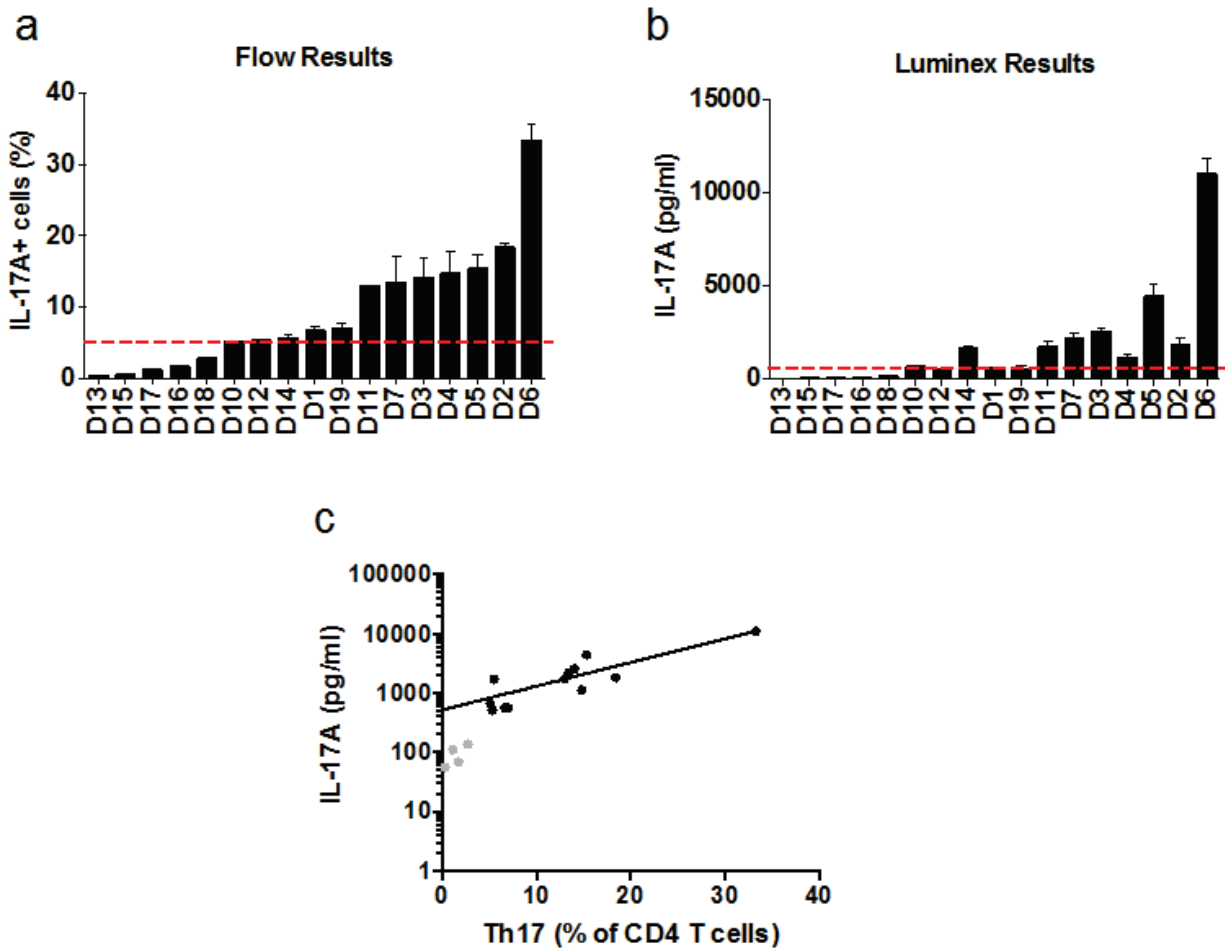
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4451011	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
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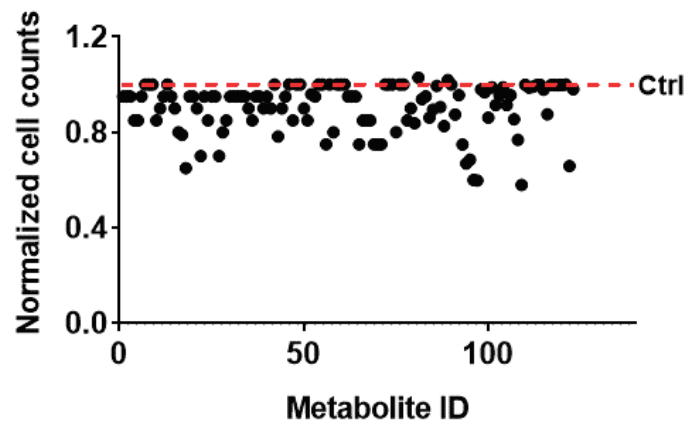
Supplementary Figure 1. T cell subsets in the CD4+ T population before culture. (a) Pre- and post-isolated CD4+ T cells were stained with cell surface markers, and assessed for % naïve/memory population by flow cytometry. (b) Isolated CD4+ T cells stained with cell surface surrogate markers for T cell subsets, and assessed for % T cell subsets by flow cytometry. (Treg: CD4+CD25+CD127-; Th17: CD4+CD45RO+CXCR3-CCR4+CCR6+; Th1:CD4+CD45RO+CXCR3+CCR4-CCR6-; Th1*:CD4+CD45RO+CXCR3+CCR4-CCR6+; Th2: CD4+CD45RO+CXCR3-CCR4+CCR6-)



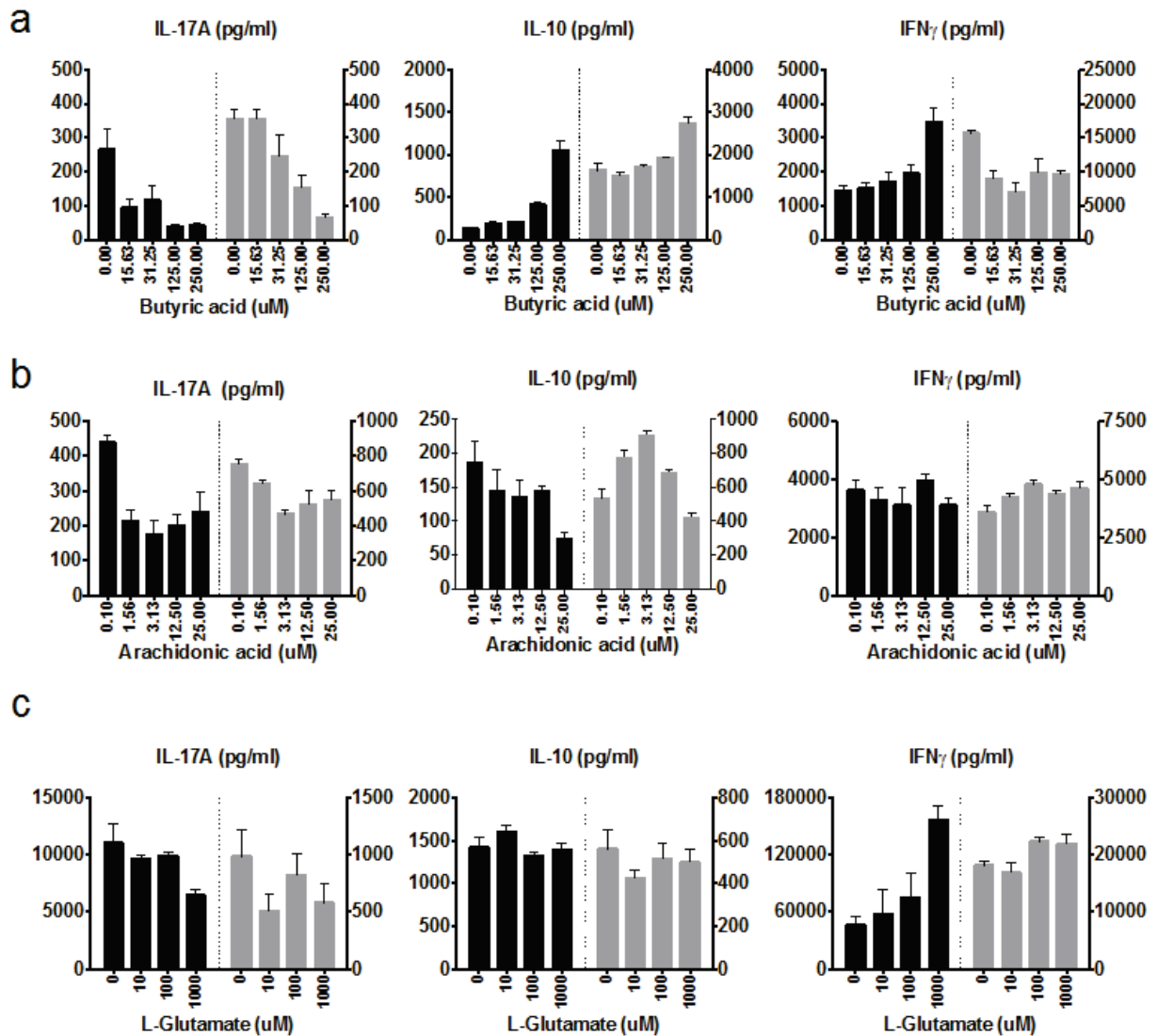
Supplementary Figure 2. Levels of human Th17 induction among study donors. (a, b) We cultured isolated CD4+ T cells for 14 days with Th17 polarizing cytokines, and thereafter assessed cells for intracellular IL-17A expression by flow cytometry and supernatant IL-17A levels by multiplex Luminex assay. Each bar represents data from one donor. Mean \pm SEM calculated from 3 technical replicates. (c) XY-plot shows the correlation between intracellular and secreted IL-17A. Each dot represents one donor. Gray dots indicate donor samples that did not meet assay quality control cut-off.



Supplementary Figure 3. Normalized cell numbers after 14-days culture with each metabolite. Absolute counts were determined by using CountBright™ Absolute Counting Beads (Molecular Probes Cat C36950) and assayed via flow cytometry. The highest metabolite concentration is presented after excluding all toxic concentrations. The cell counts were normalized by the polarized, metabolite untreated condition performed in the same batch of experiment (shown as Ctrl, set as 1).

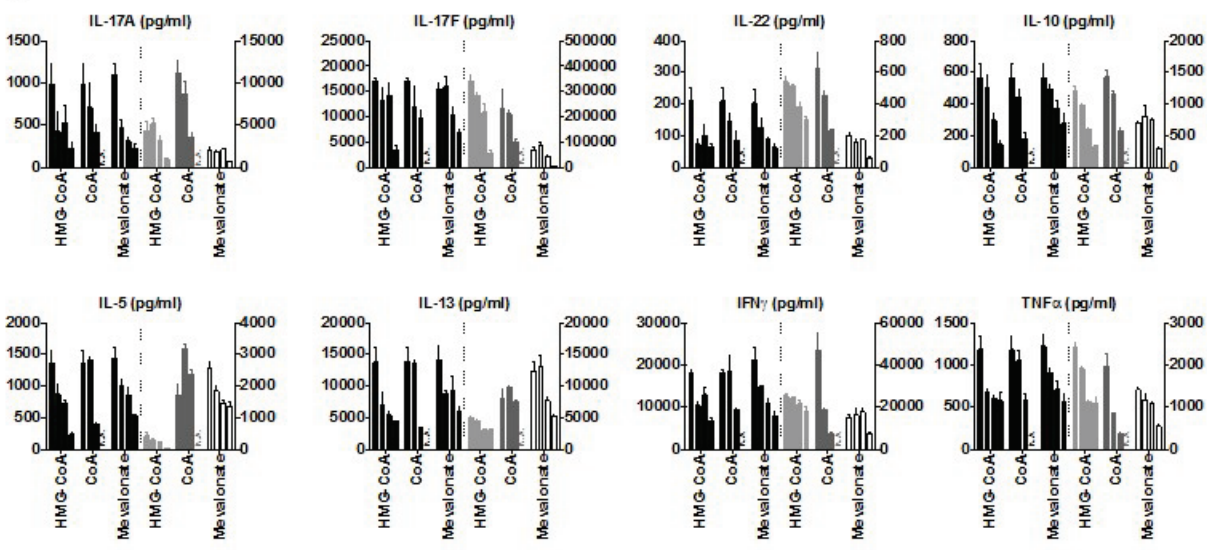


Supplementary Figure 4. Bioactivity of metabolites with previously reported effects on CD4+ T cells. Secreted cytokines levels of CD4+ T cells cultured under polarizing Th17 conditions with the indicated metabolites. (a) Butyric acid, (b) arachidonic acid, and (c) L-glutamate. We show data from two biological replicates (black and gray). Mean \pm SEM calculated from three technical replicates.

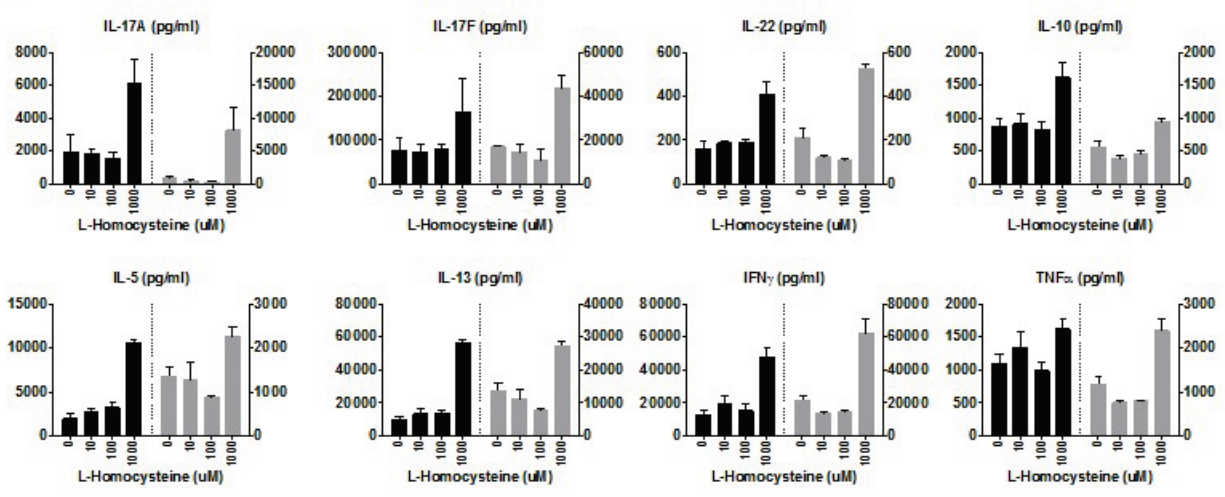


Supplementary Figure 5. Metabolites with pan-effects on cytokine production. Secreted cytokines from CD4+ T cells cultured under polarizing Th17 conditions with the indicated metabolites. We show data from independent biological replicates for each metabolite (black, dark-gray, light-gray, and white). CoA: Coenzyme A; HMG-CoA: 3-hydroxy-3-methyl-glutaryl-coenzyme A. (a) Metabolites with apparent pan-inhibition. (b) Metabolites with apparent pan-enhancement. N.A., conditions causing cell death and hence excluded. Mean \pm SEM calculated from 3 technical replicates.

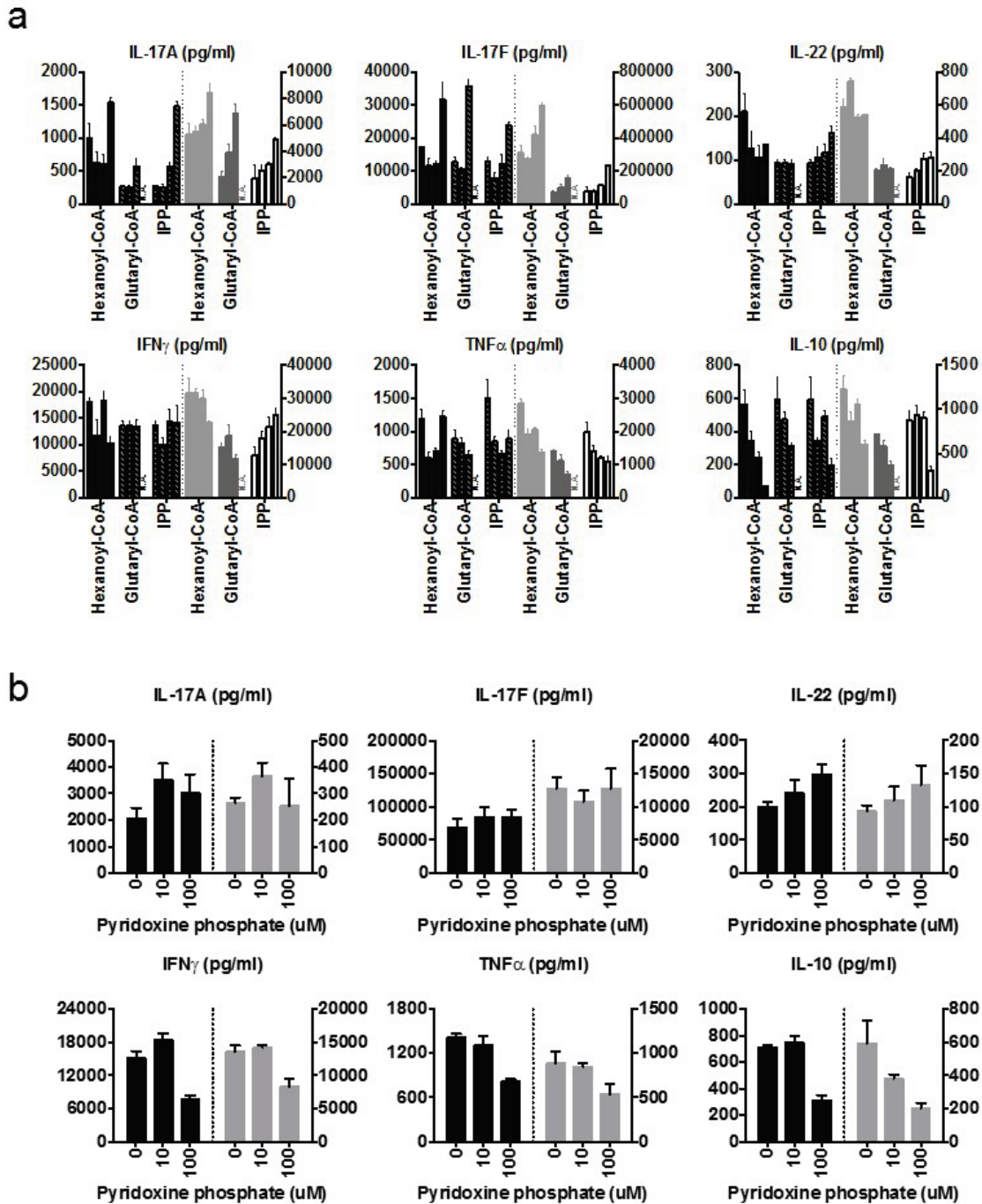
a



b



Supplementary Figure 6. Metabolites with selective effects on cytokine production. Secreted cytokines from CD4+ T cells cultured under polarizing Th17 conditions with the indicated metabolites. (a) IPP: isopentenyl diphosphate. (b) Pyridoxine phosphate. N.A.: conditions that caused cell death and excluded. Mean \pm SEM calculated from 3 technical replicates.



Supplementary Figure 7. GLUT1 knockdown efficiency. The knockdown efficiencies of GLUT1 siRNA were determined by immunoblotting using anti-GLUT1 antibody (Abcam ab115730). Isolated CD4⁺ T cells were transiently transfected with GLUT1 or scramble siRNA clones purchased from Dharmacon (ON-TARGETplus). After 4 hours of transfection, cells were activated with anti-CD3 and anti-CD28 with IL-2 supplement for additional 48 hours and lysed for western blot. Three independent experiments were performed and the representative plot is shown.

