Supplementary Methods

Catalyzed Reporter Deposition (CARD) - Fluorescence In Situ Hybridization (FISH)

Paraffin embedded sections of 6 µm thinness prepared as described above for immunohistochemistry were collected on polylysine coated glass slides (3 per each slide) and subsequently subjected to CARD-FISH.

- For CARD-FISH, tissue sections were incubated 3X5 min in 100% Xylol (The Geyer GmbH, Renningen, Germany) followed by incubation in decreasing ethanol series (3X5 min in ethanol 99% followed by 5 min in 95%, 80% and 50% ethanol respectively). After that, the slides were dipped in wash buffer (WB) for 1 min followed by incubation in 0.2M HCL in WB for 10 min at RT and washing for 1 min again in WB.
- Permeabilization was achieved by dipping the glass slides containing tissue sections sequentially in proteinase K-buffer, lysozyme-buffer and achromopeptidase-buffer (see table 5) each for 5 min at 37^{o}C with 1 minute long washing steps at RT in WB in between enzymatic treatments.
- The slides were air dried and the sections were circled using Pap-pen and were prepared for the hybridization step.
- The sections were hybridized for 3 h at 46°C in a pre-warmed hybridization chamber (made out of a 50 mL Falcon tube, in a humid environment created by soaking a paper tissue with 35% formamide in ultrapure water and place it at the bottom of the tube) using pre-warmed hybridization buffer containing the HRP-labelled probes (s. table 5).
- Following hybridization, glass slides containing sections were incubated in 50 mL of pre-warmed WB2 for 15 min at 48°C.
- After washing, filters were incubated for 15 min at RT in 1× PBS (pH=7.6) to equilibrate the HRP-labeled probe. Subsequently, CARD-FISH was performed by incubating the sections for 20 min at 46°C in the dark in amplification buffer.
- Afterwards, the slides containing sections were rinsed in 1× PBS for 10 min at RT followed by staining with 4´,6-diamidino-2-phenylindole (DAPI) 1µg mL^{-1} for 10 min at RT, washing in ultrapure water, air dried and embedded in mounting medium and stored at -20^{o}C prior to imaging.

For the CARD-FISH we used a mixture of HRP-labelled EUB338 I, II and III (Aman et al., 1990; Daims et al., 1999) probes and the HRP-labelled NON338 (NONEUB) (Wallner et al., 1993) probe, with NONEUB probe being used to detect the potential false positive signals on hybridized tissue sections and to test probe specificity. In addition, tissue sections were used for the complete CARD-FISH procedure but using hybridization probe-free buffer, in order to identify potential false positive signals due to tissue endogenous peroxidase activity. False positive signals were not observed during microscopic investigations, although microbial cells could be observed on the tissue sections by DAPI (Supplementary Figure S2)

All the steps performed with the glass slides containing paraffin sections were performed under sterile conditions. All the glass jars used for the above treatments were autoclaved, MQ water was autoclaved and filter sterilized, using 0.2µm PC Isopore Membrane (MERCK, Darmstadt, Germany) and used to prepare all buffers. The buffers were freshly prepared before usage. Hybridization, washing and amplification buffers were prepared with molecular grade DNase-, RNase-free solutions and autoclaved, filter sterilized MQ water. In addition, we performed controls to verify the sterility of all solutions. An empty polylisine coated slide was run alongside slides containing tissue sections following each experimental step described above. Moreover, a second control was implemented consisting of 500 µL of HB containing probe, which was incubated at 46^{o}C for 3 h and further filtered on the 0.2 µm pore size GTTP type polycarbonate filter and run for the tyramide amplification reaction being further imaged under fluorescence microscope. Sterility controls used i.e. the empty polylisine slide, the paraffin sections containing slide and the filter containing filtrate of the hybridization buffer did not showed any positively hybridized or DAPI stained cell on their surface.

Supplementary Tables

Supplementary Table 1: Statistics for fit of db-RDA per tissue; 1000 permutations

	dbRDA1	dbRDA2	R^2	q_{FDR}
Mesenteric				
LBP	0.24	-0.97	0.07	0.20
TNFA	0.94	0.35	0.03	0.51
IL6	-0.20	-0.98	0.16	0.04
age	-0.94	-0.35	0.02	0.60
BMI	-0.46	-0.89	0.15	0.03
CRP	-0.22	-0.98	0.09	0.09
HOMA	0.81	-0.59	0.88	0.001
HbA1c	0.24	-0.97	0.12	0.06
Bact load	0.98	0.18	0.82	0.001
Omental				
LBP	0.35364	-0.93538	0.28	0.003
TNFA_Serum	0.68956	0.72423	0.17	0.017
IL6_Serum	0.42405	-0.90564	0.07	0.203
age	-0.99998	0.00672	0.06	0.287
BMI	-0.13176	0.99128	0.13	0.053
CRP	-0.72075	-0.69319	0.06	0.235
HOMA	0.99839	-0.05671	0.57	0.001
HbA1c	0.68907	-0.7247	0.1	0.095
Adipocyte_size	0.27558	-0.96128	0.02	0.665
ATM	0.61315	-0.78997	0.01	0.734
bact_load	0.99873	-0.0504	0.22	0.007
Subcutaneous				
LBP	0.94998	-0.31232	0.08	0.177
TNFA_Serum	-0.19994	0.97981	0.28	0.003
IL6_Serum	-0.60836	-0.79366	0.1	0.089
age	0.45129	-0.89238	0.06	0.254
BMI	0.67544	-0.73742	0	0.932
CRP	0.91348	-0.40689	0.47	0.001
HOMA	0.93652	0.35061	0.11	0.08
HbA1c	-0.21669	0.97624	0.14	0.037
Adipocyte_size	-0.99888	-0.04723	0.01	0.779
ATM	-0.78253	0.62261	0.16	0.024
bact_load	0.84125	0.54065	0.29	0.005
Blood				
LBP	-0.98464	-0.17461	0.19	0.008
TNFA_Serum	-0.14505	0.98942	0.44	0.001
IL6_Serum	-0.97247	-0.23301	0.49	0.001
age	-0.93292	0.36009	0.27	0.001
BMI	0.92286	0.38513	0.02	0.564
CRP	-0.63027	0.77638	0.33	0.001
HOMA	0.59456	0.80405	0.01	0.708
HbA1c	-0.1643	0.98641	0.11	0.066
bact_load	0.71806	-0.69599	0.08	0.117

***Supplementary Table 2*: Statistics differential abundance (DESeq2)**

Phylum Level

baseMean	log2FC	lfcSE	stat	pvalue	q_{FDR}	Phylum
omental vs blood						
2.18	-2.49	2.37	-1.05	2.9E-01	8.3E-01	Patescibacteria
0.20	-1.62	2.90	-0.56	5.8E-01	8.3E-01	Armatimonadetes
41.46	-13.11	2.90	-4.53	6.0E-06	3.9E-05	Chloroflexi
10.03	-27.62	2.90	-9.53	1.6E-21	2.1E-20	Planctomycetes
11.72	1.72	1.83	0.94	3.5E-01	8.3E-01	Actinobacteria
0.00	-0.50	1.21	-0.41	6.8E-01	8.9E-01	Gemmatimonadetes
1395.24	1.98	0.68	2.92	3.5E-03	1.5E-02	Firmicutes
0.40	-2.10	2.90	-0.72	4.7E-01	8.3E-01	Tenericutes
0.28	-1.82	2.90	-0.63	5.3E-01	8.3E-01	Bacteroidetes
16481.48	0.37	0.61	0.61	5.4E-01	8.3E-01	Proteobacteria
mesenteric vs blood						
15.05	7.12	2.89	2.46	1.4E-02	2.9E-02	Cyanobacteria
0.22	-2.90	2.90	-1.00	3.2E-01	3.9E-01	Armatimonadetes
42.73	-6.82	2.75	-2.48	1.3E-02	2.9E-02	Chloroflexi
10.68	-27.31	2.90	-9.42	4.4E-21	5.7E-20	Planctomycetes
1322.63	9.72	2.18	4.46	8.3E-06	5.4E-05	Actinobacteria
10.30	7.65	2.57	2.97	3.0E-03	1.3E-02	Elusimicrobia
0.05	-1.20	2.90	-0.42	6.8E-01	7.3E-01	Gemmatimonadetes
1422.09	1.73	0.60	2.88	4.0E-03	1.3E-02	Firmicutes
0.40	-2.83	2.90	-0.98	3.3E-01	3.9E-01	Tenericutes
0.93	-0.58	2.34	-0.25	8.0E-01	8.0E-01	Fusobacteria
0.32	-3.15	2.90	-1.09	2.8E-01	3.9E-01	Bacteroidetes
30.30	3.16	1.31	2.42	1.6E-02	2.9E-02	Patescibacteria
15691.88	1.09	0.56	1.94	5.2E-02	8.4E-02	Proteobacteria
subcutaneous vs blood						
0.20	-2.27	2.53	-0.90	3.7E-01	4.6E-01	Cyanobacteria
0.25	-3.56	2.86	-1.24	2.1E-01	3.2E-01	Armatimonadetes
57.73	-13.79	2.89	-4.76	1.9E-06	2.3E-05	Chloroflexi
18.58	-4.15	2.89	-1.44	1.5E-01	3.1E-01	Planctomycetes
29.27	3.63	1.70	2.14	3.3E-02	1.4E-01	Actinobacteria
0.78	0.47	2.89	0.16	8.7E-01	8.7E-01	Elusimicrobia
2423.65	0.55	0.63	0.87	3.9E-01	4.6E-01	Firmicutes
0.57	-4.13	2.90	-1.43	1.5E-01	3.1E-01	Tenericutes
25.28	4.44	2.57	1.73	8.4E-02	2.5E-01	Fusobacteria
1.13	-3.64	2.90	-1.26	2.1E-01	3.2E-01	Bacteroidetes
41.68	3.48	1.65	2.12	3.4E-02	1.4E-01	Patescibacteria
16250.03	0.47	0.61	0.77	4.4E-01	4.8E-01	Proteobacteria
omental vs mesenteric						
13.82	-7.55	2.90	-2.61	9.1E-03	2.3E-02	Cyanobacteria
3.54	-3.33	2.83	-1.18	2.4E-01	3.0E-01	Chloroflexi
1265.84	-6.63	1.74	-3.81	1.4E-04	6.1E-04	Actinobacteria
9.66	-9.75	2.61	-3.74	1.8E-04	6.1E-04	Elusimicrobia
0.04	1.02	0.86	1.18	2.4E-01	3.0E-01	Gemmatimonadetes
2535.17	0.91	0.61	1.49	1.4E-01	2.3E-01	Firmicutes
0.04	1.15	0.69	1.67	9.5E-02	1.9E-01	Tenericutes
0.34	0.33	1.42	0.23	8.2E-01	8.2E-01	Fusobacteria
24.21	-6.98	1.59	-4.39	1.2E-05	1.2E-04	Patescibacteria
24708.17	0.12	0.49	0.25	8.0E-01	8.2E-01	Proteobacteria

omental vs subcutaneous							
1.15	3.74	2.90	1.29	2.0E-01	4.3E-01	Cyanobacteria	
0.01	2.15	0.94	2.27	2.3E-02	6.3E-02	Chloroflexi	
0.64	-0.82	2.89	-0.28	7.8E-01	8.2E-01	Planctomycetes	
46.03	-0.57	1.61	-0.35	7.2E-01	8.2E-01	Actinobacteria	
0.59	-0.65	2.89	-0.22	8.2E-01	8.2E-01	Elusimicrobia	
0.01	2.22	0.94	2.36	1.8E-02	6.3E-02	Gemmatimonadetes	
4147.83	0.39	0.62	0.63	5.3E-01	7.3E-01	Firmicutes	
14.06	-7.00	2.75	-2.54	1.1E-02	6.0E-02	Fusobacteria	
0.55	2.10	2.90	0.72	4.7E-01	7.3E-01	Bacteroidetes	
36.89	-7.40	1.82	-4.07	4.7E-05	5.2E-04	Patescibacteria	
29047.32	0.53	0.52	1.01	3.1E-01	5.7E-01	Proteobacteria	
subcutaneous vs mesenteric							
1.64	-3.21	2.90	-1.11	2.7E-01	5.3E-01	Cyanobacteria	
2732.19	-6.49	1.72	-3.77	1.6E-04	2.0E-03	Actinobacteria	
21.42	-4.90	2.49	-1.96	5.0E-02	1.6E-01	Elusimicrobia	
5.60	-5.57	2.89	-1.92	5.4E-02	1.6E-01	Chloroflexi	
15.48	5.11	2.17	2.36	1.8E-02	1.1E-01	Fusobacteria	
32540.68	-0.69	0.49	-1.42	1.6E-01	3.7E-01	Proteobacteria	
Genus level							
baseMean	log2FC	lfcSE	stat	pvalue	padj	Phylum	
omental vs blood							
36.55	-29.09	2.90	-10.04	1.0E-23	3.9E-22	Chloroflexi	UTCFX1
6.80	-5.90	2.32	-2.55	1.1E-02	4.6E-02	Firmicutes	Lactococcus
2348.03	3.99	1.05	3.79	1.5E-04	8.2E-04	Firmicutes	Streptococcus
1.96	-26.57	2.90	-9.16	5.1E-20	3.2E-19	Proteobacteria	
12.94	-27.61	2.82	-9.79	1.3E-22	1.6E-21	Proteobacteria	Bdellovibrio
4.09	-8.15	2.90	-2.81	4.9E-03	2.3E-02	Proteobacteria	Tahibacter
67639.97	9.36	1.02	9.22	2.9E-20	2.2E-19	Proteobacteria	Acinetobacter
7.49	-28.39	2.90	-9.79	1.2E-22	1.6E-21	Proteobacteria	Sutterella
5.14	-27.88	2.90	-9.62	6.7E-22	6.4E-21	Proteobacteria	Delftia
mesenteric vs blood							
54.05	-9.39	2.89	-3.25	1.2E-03	4.8E-03	Chloroflexi	UTCFX1
2.41	-25.61	2.90	-8.83	1.0E-18	5.5E-18	Proteobacteria	
48.03	30.00	2.90	10.36	4.0E-25	1.5E-23	Proteobacteria	Pseudochrobactrum
16.91	-28.21	2.90	-9.74	2.1E-22	3.9E-21	Proteobacteria	Methylocystis
5.92	-26.82	2.90	-9.25	2.2E-20	1.7E-19	Proteobacteria	Tahibacter
3.58	26.16	2.90	9.02	1.8E-19	1.1E-18	Proteobacteria	Escherichia-Shigella
5259.91	5.84	0.89	6.53	6.4E-11	3.0E-10	Proteobacteria	Acinetobacter
9.91	27.84	2.90	9.61	7.5E-22	9.2E-21	Proteobacteria	Thauera
6.38	-26.92	2.90	-9.29	1.6E-20	1.5E-19	Proteobacteria	Delftia
442.50	6.70	2.47	2.71	6.7E-03	2.5E-02	Proteobacteria	Rhodoferax
subcutaneous vs blood							
43.25	-13.24	2.90	-4.57	4.9E-06	1.6E-05	Chloroflexi	UTCFX1
13.20	28.12	2.51	11.19	4.6E-29	1.5E-27	Firmicutes	Gemella
719.71	-8.26	2.89	-2.86	4.2E-03	1.1E-02	Firmicutes	Lactobacillus
2098.89	3.03	0.93	3.26	1.1E-03	3.2E-03	Firmicutes	Streptococcus
2.29	-26.24	2.90	-9.05	1.5E-19	5.9E-19	Proteobacteria	
16.13	-28.88	2.90	-9.97	2.1E-23	2.3E-22	Proteobacteria	Methylocystis
15.99	-28.87	2.79	-10.34	4.6E-25	7.3E-24	Proteobacteria	Bdellovibrio
7.85	27.55	2.90	9.51	1.9E-21	1.2E-20	Patescibacteria	uncultured bacterium
4.82	-27.24	2.90	-9.40	5.7E-21	3.1E-20	Proteobacteria	Tahibacter
5.33	26.51	2.90	9.15	5.9E-20	2.7E-19	Proteobacteria	Enterobacter
2664.98	4.90	0.87	5.62	1.9E-08	6.7E-08	Proteobacteria	Acinetobacter

11.56	-28.43	2.90	-9.81	1.0E-22	8.3E-22	Proteobacteria	Sutterella
omental vs mesenteric							
178.70	-30.00	2.90	-10.35	4.0E-25	3.7E-23	Proteobacteria	Pseudochrobactrum
6.08	-28.18	2.90	-9.72	2.5E-22	7.7E-21	Proteobacteria	Thauera
8.92	-28.68	2.90	-9.90	4.3E-23	2.0E-21	Proteobacteria	Deefgea
3.81	-27.53	2.90	-9.49	2.2E-21	5.2E-20	Proteobacteria	uncultured
omental vs subcutaneous							
2.66	-27.03	2.90	-9.32	1.1E-20	2.6E-19	Elusimicrobia	uncultured bacterium
5.07	-27.91	2.90	-9.63	6.0E-22	1.9E-20	Patescibacteria	uncultured bacterium
21.46	-29.90	2.90	-10.32	5.7E-25	2.8E-23	Proteobacteria	Enterobacter
63369.91	2.50	0.83	3.02	2.5E-03	4.6E-02	Proteobacteria	Acinetobacter
25.22	-29.88	2.90	-10.32	6.0E-25	2.8E-23	Proteobacteria	uncultured
subcutaneous vs mesenteric							
2.28	-26.26	2.90	-9.05	1.4E-19	2.5E-18	Firmicutes	Nosocomiicoccus
11.03	25.00	2.49	10.04	9.9E-24	3.6E-22	Firmicutes	Gemella
26.07	-29.58	2.90	-10.21	1.8E-24	1.0E-22	Proteobacteria	Sphingopyxis
284.47	-30.00	2.90	-10.36	3.9E-25	4.3E-23	Proteobacteria	Pseudochrobactrum
6.57	25.97	2.90	8.96	3.2E-19	5.0E-18	Patescibacteria	uncultured bacterium
30.55	28.14	2.90	9.71	2.6E-22	5.7E-21	Proteobacteria	Enterobacter
11.56	-28.46	2.90	-9.82	9.2E-23	2.5E-21	Proteobacteria	Deefgea

Supplementary Table 3: Tested primers and amplification combinations thereof

Name	Sequence (5'-3')	Reference
BAc08F	agagtttgatcctggctcag	(Baker et al., 2003; Salipante et al., 2013)
V3-R	attaccgcggctgctgg	(Claesson et al., 2010)
V3-F	actcctacgggaggcagcag	(Claesson et al., 2010)
V4-F	actgggcgtaaagcg	(Claesson et al., 2010)
V5-R	ccgtcaattcctttgagttt	(Claesson et al., 2010)
V6-R	cgacagccatgcagcacct	(Claesson et al., 2010)
R1492-R	tacggttaccttgttacgactt	(Baker et al., 2003)
V6-F	ttgacgggggcccgcac	(Salipante et al., 2013)
Uni1390-R	gacgggcggtgtgtacaa	(Siala et al., 2008)
806-R	ggactaccagggtatctaat	(Bik et al., 2006)
927-R	cccgtcaattcatttgagtt	(Bik et al., 2006)
63-F	gcaggcctaacacatgcaagtc	(Claesson et al., 2010)
355-R	ctgctgcctcccgtaggagt	(Claesson et al., 2010; Salipante et al., 2013)
8-fw	agagtttgatymtggctcag	(Klindworth et al., 2012)
357-rev	ctgctgcctyccgta	(Klindworth et al., 2012)
341-fw	cctacgggnggcwgcag	(Klindworth et al., 2012)
805-rev	gactachvgggtatctaatcc	(Klindworth et al., 2012)
785-fw	ggmttagatacccbdgta	(Klindworth et al., 2012)
1068-rev	ctgacgrcrgccatgc	(Klindworth et al., 2012)
1053-fw	gcatggcygycgtcag	(Klindworth et al., 2012)
1510-rev	rgytaccttgttacgactt	(Klindworth et al., 2012)
564-fw	aytgggydtaaagng	(Klindworth et al., 2012)
540-rev	ctnygtmttaccgcggctgc	(Klindworth et al., 2012)
8-fw-short	agagtttgatcmtggc	(Klindworth et al., 2012)
357-rev-long	tgctgcctcccgtaggagt	(Klindworth et al., 2012)
V-region	**Results**	
V1-2	Additional but distinguishable human product	
V1-3	One band with human product	
V1-4	Only bacterial product, but low yield	
V1-5	Only bacterial product, but low yield	
V3	One band with human product	
V3-6	Only bacterial product, but low yield	
V4-5	Only bacterial product, good results	
V4-6	Only bacterial product, but low yield	
V4-9	Only bacterial product, but low yield	
V6	Two bands with human products	
V6-8	Only bacterial product, good results	
V6-9	Low yield	

Supplementary Table 4: Tested polymerases and results for low bacterial biomass samples

Polymerase	Results	Supplier
Taq DNA polymerase, recombinant 5 U/µl	No products	ThermoFisher (#EP0402)
Accuprime HS	No products	ThermoFisher (#12339016)
VitaTaq	Only NC were amplified	Procomcure Biotech
FastStart PCR master	Good results, i.e. amplified samples and NC were clean	Sigma-Aldrich
Biozym Long Range PCR	No products	Biozym
Phusion II (HS)	Contaminated	ThermoFisher (F549S)
GoTaq	Good results, i.e. amplified samples and NC were clean	Promega
Q5 high fidelity HS	Good results, i.e. amplified samples and NC were clean	NEB
DreamTaq	Some NC contaminated	ThermoFisher

Supplementary Table 5: CARD-FISH solutions and materials

Buffer type	composition
Wash buffer	20 mM Tris-HCl (pH 7.8) (Invitrogen, Carlsbad, CA, USA)
Proteinase K- buffer	10 µg mL^{-1} proteinase K (Sigma-Aldrich, St. Louise, Missouri, USA) 0.01M Tris-HCL (pH=7.8), 0.05 EDTA (pH=8), 0.5% SDS buffer
Lysozyme-buffer	10 µg mL^{-1} lysozyme (Sigma-Aldrich, St. Louise, Missouri, USA) 0.1M Tris-HCL (pH=7.8), 0.05M EDTA (pH=8)
Achromopeptidase-buffer	60 U mL^{-1} achromopeptidase (Sigma-Aldrich, St. Louise, Missouri, USA) 0.01M Tris-HCL, 0.01M NaCL
HPR-labelled probe Hybridization buffer	0.166 ng mL^{-1} HPR labelled probe (Fluka, Waltham, USA Boehringer, Mannheim Germany) 0.9M NaCl, 20mM Tris–HCl (pH=7.5), 10% (w/v) dextran sulfate,0.02% (w/v) SDS 35% (v/v) 35% formamide, 1% (w/v) blocking reagent
Wash buffer 2	70mM NaCl, 5mM EDTA (pH=8.0), 20mM Tris–HCl (pH=7.5), and 0.01% SDS
Amplification buffer with Alexa 594-labeled tyramides	1 µg mL^{-1} Alexa 594-labeled tyramides (ThermoFisherScientific, Waltham, Massachusetts,USA) 1× PBS, 2M NaCl, 0.1% (w/v) blocking reagent, 10% (w/v) dextran sulfate, 0.0015% (v/v) H_2O_2
Mounting medium	low fluorescence glycerol mountant (Citifluor AF1, Citifluor, London, GB) , mounting fluid Vecta Shield (Vecta Laboratories Burlingame, CA, USA) in 4:1 (v/v) ratio