

Supplementary Methods

Bacterial DNA contamination assessment

To assess the potential bacterial DNA contamination from environment and reagents, several negative controls were included and analysed throughout the 16S rRNA gene sequencing pipeline. Ten negative controls consisting of ultrapure water (NC-EXT-WA) were added at the DNA extraction step to account for reagent and environmental contaminants. NC-EXT-WA samples were processed concomitantly with the DNA extraction from whole blood samples. This study comprised 296 whole blood samples. 16 rounds of DNA extraction were performed adding 2 NC-EXT-WA samples to the extraction round 1 (EXT1), 5 (EXT5), 10 (EXT10), 15 (EXT15) and 16 (EXT16).

To overcome the unbalance between the whole blood sample and the control groups (296 vs 10), we performed 10 randomized statistical analyses by comparing the 10 NC-EXT-WA samples to 10 randomly selected whole blood samples. The beta-diversity was measured through Bray-Curtis index. PERMANOVA and PERMDISP were used to test for homogeneity of multivariate dispersions on Bray-Curtis dissimilarity table for the contamination control.

We used diversity analyses and comparisons of the taxonomic profiles in order to confirm that the differences between DNA extraction negative controls and whole blood samples were large enough.

Beta-diversity indices were used to investigate whether blood bacterial DNA differs from that of extraction negative controls. Based on all beta-diversity indices, especially on the Bray-Curtis distance matrix (Supplementary Figure S4), blood bacterial DNA profiles were separated from the negative controls in all the randomized analyses. Moreover, blood samples tended to form a tighter cluster than the negative controls, suggesting that the blood samples shared a specific and more similar DNA bacterial profile. The negative control group was more dispersed, showing more variability in-between samples and suggesting a random profile more evident. PERMANOVA showed statistically significant differences between whole blood samples and negative controls for Bray-Curtis beta-diversity indices (Supplementary Table S2), suggesting that the bacterial communities differed both in composition and relative abundance between biological and negative control samples. PERMDISP confirmed the results.

We could, therefore, consider that the background noise did not impact the results of the study.

Table S1. Distributions of Observed, Chao1, Shannon and Simpson alpha-diversity indices of control, intestinal adenoma (IA), colon and rectal cancer subjects for bacterial genera and OTUs.

		Median (I-III quartiles)				p* Controls vs IA	p* Colon cancer vs IA	p* Colon cancer vs controls	p* Rectal cancer vs IA	p* Rectal cancer vs controls	p* Colon cancer vs rectal cancer
Alpha-diversity		Controls	IA	Colon cancer	Rectal cancer						
Genera	<i>Observed</i>	28.5 (25–32)	28 (25–32)	29.5 (25–36)	30 (26–32)	0.917	0.246	0.251	0.364	0.332	0.691
	<i>Chao1</i>	44.9 (33–57.5)	44.5 (34–55.5)	44.5 (36.3–55.3)	41.3 (35–53)	0.686	0.874	0.774	0.617	0.879	0.493
	<i>Shannon</i>	2.34 (2.02–2.53)	2.33 (2.15–2.51)	2.33 (2.00–2.58)	2.32 (2.12–2.51)	0.575	0.824	0.706	0.958	0.540	0.723
	<i>Simpson</i>	0.87 (0.80–0.89)	0.86 (0.83–0.89)	0.86 (0.81–0.90)	0.86 (0.82–0.89)	0.843	0.808	0.884	0.855	0.792	0.981
OTUs	<i>Observed</i>	34 (31–39)	35 (31–42)	37 (32–47)	37 (33–40)	0.542	0.208	0.105	0.440	0.171	0.617

<i>Chao1</i>	62.1 (48–85)	66 (49.2–90.5)	67.1 (51.0–87.5)	59.9 (49.6–82)	0.297	0.631	0.594	0.299	0.968	0.702
<i>Shannon</i>	2.58 (2.28–2.77)	2.58 (2.40–2.80)	2.61 (2.27–2.87)	2.51 (2.41–2.74)	0.372	0.874	0.496	0.625	0.667	0.581
<i>Simpson</i>	0.90 (0.86–0.92)	0.90 (0.87–0.92)	0.90 (0.86–0.92)	0.89 (0.88–0.91)	0.671	0.879	0.749	0.241	0.482	0.378

* P for heterogeneity estimated from the Wilcoxon rank-sum. SD: standard deviation; IQ: interquartile.

Table S2. PERMANOVA and PERMDISP on beta-diversity Bray-Curtis index for the 10 randomized comparisons between negative controls (CTRL) and whole blood samples (WB). For all randomized comparisons, the sample size is equal to 20 (10 Neg CTRL vs. 10 WB) at the exception of RandExt16 (10 Neg CTRL vs. 9 WB). The statistical testing used were pseudo-F and F-value for PERMANOVA and PERMDISP, respectively. For both tests, 2000 permutations were performed. P-values < 0.05 in yellow, < 0.005 in orange and < 0.0005 in red.

			RandExt01	RandExt05	RandExt10	RandExt15	RandExt16	RandAllExt	Rand1	Rand2	Rand3	Rand4
Bray-Curtis	PERMANOVA	Test statistic	3,49	4,51	3,51	4,01	4,37	3,03	4,08	3,22	2,94	2,82
		P-value	0,0005	0,0005	0,0005	0,0005	0,0005	0,0005	0,0005	0,0005	0,0005	0,0005
	PERMDISP	Test statistic	10,67	29,79	15,52	13,87	28,65	9,96	15,13	18,59	8,28	3,60
		P-value	0,0050	0,0005	0,0025	0,0005	0,0005	0,0130	0,0005	0,0015	0,0190	0,0900

Figure S1. Distribution of 16S rRNA gene copies per µl of whole blood among controls, intestinal adenomas and colorectal cancers.

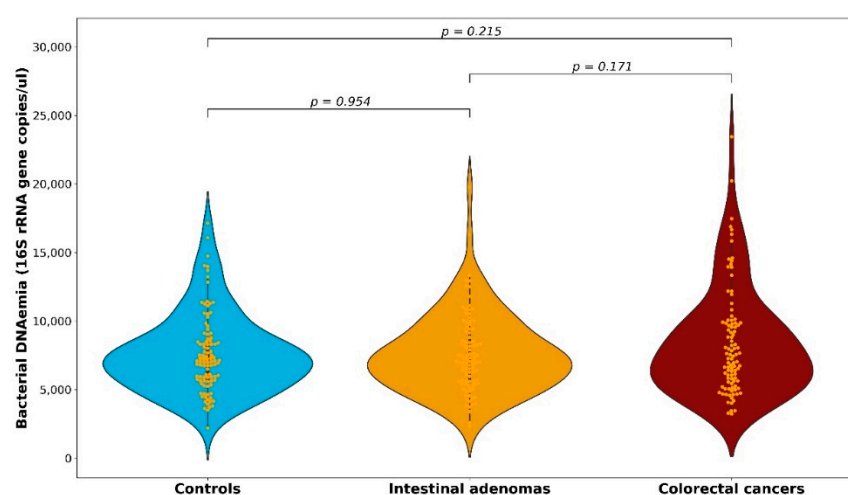


Figure S2. Mean of the taxonomy composition in terms of relative abundance per group of samples at family taxonomic level. The panel on the left shows the taxonomic composition of controls, intestinal adenoma subjects (IA) and both controls/IA, respectively (as per legend to the right side). The panel on the right shows the taxonomic composition of colorectal cancer (CRC), and rectal and colon cancer cases separately (as per legend to the right side).

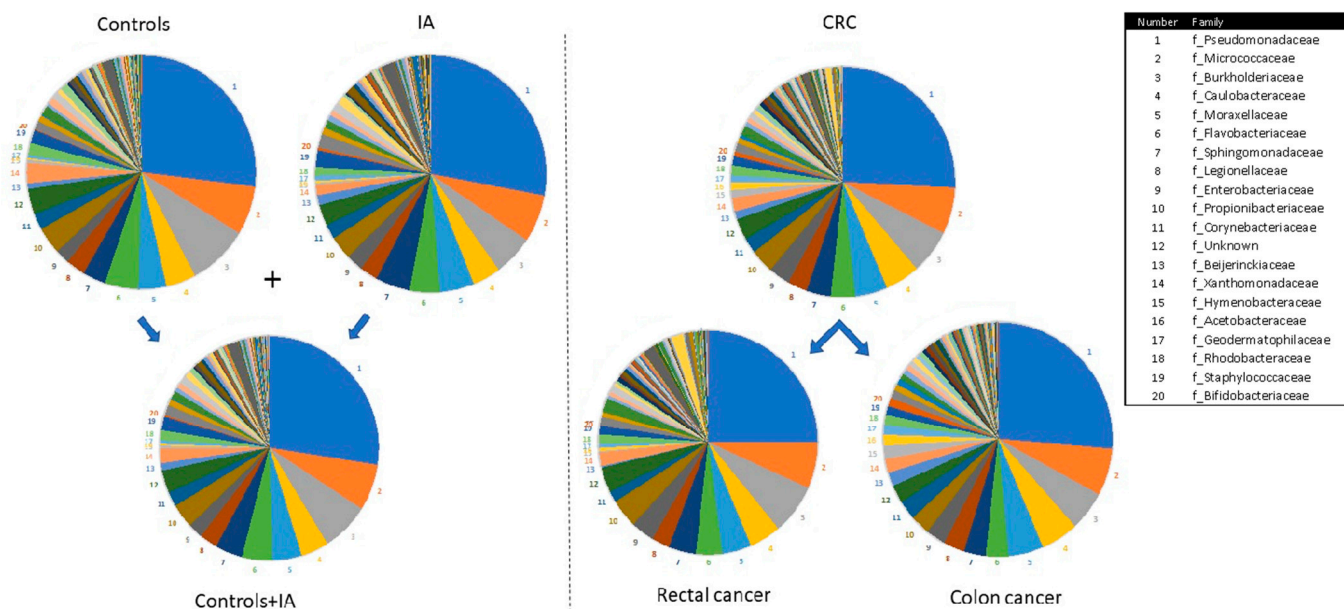


Figure S3. Different taxa between: (A) colorectal cancers (CRC) and controls; (B) CRC and intestinal adenoma (IA); (C) IA and controls by DESeq2 analyses. The taxonomic lineage of each taxon is shown: p, phylum; c, class; o, order; f, family; g, genus; OTU#, Operational Taxonomic Unit. The first two columns show the logarithmic transformation of normalized base mean value for each group. The “padj” column shows the p-value for heterogeneity between groups adjusted for multi-testing analyses. Positive fold changes (shown on a green background) designate taxon overrepresentation in the CRC group. Negative fold changes (shown on a yellow background) designate taxon underrepresentation in the CRC group.

	CRC	Controls	OTU#	Taxonomy	padj	log2FoldChange
A				p_Actinobacteria_c_Acidimicrobia	3E-02	4.92
				p_Actinobacteria_c_Actinobacteria_o_Bifidobacteriales_f_Bifidobacteriaceae	6E-09	10.21
				p_Actinobacteria_c_Actinobacteria_o_Bifidobacteriales_f_Bifidobacteriaceae_g_Bifidobacterium	6E-09	10.21
				p_Actinobacteria_c_Actinobacteria_o_Corynebacteriales_f_Corynebacteriaceae_g_Corynebacterium	9E-05	-7.55
			OTU_54	p_Actinobacteria_c_Actinobacteria_o_Corynebacteriales_f_Corynebacteriaceae_g_Corynebacterium	2E-04	-7.14
				p_Actinobacteria_c_Actinobacteria_o_Frankiales_f_Gemmatimonadetes	2E-02	4.32
				p_Actinobacteria_c_Actinobacteria_o_Micrococcales_f_Micrococaceae_g_Rothia	8E-03	4.45
				p_Actinobacteria_c_Actinobacteria_o_Bifidobacteriales	6E-09	10.21
				p_Actinobacteria_c_Actinobacteria_o_Propionibacteriales_f_Nocardiaceae	2E-02	-4.17
				p_Actinobacteria_c_Thermoplasma	5E-06	7.01
				p_Bacteroidetes_c_Bacteroidia_o_Bacteroidales_f_Bacteroidaceae	2E-09	-6.77
				p_Bacteroidetes_c_Bacteroidia_o_Bacteroidales_f_Bacteroidaceae_g_Bacteroides	2E-09	-6.77
				p_Bacteroidetes_c_Bacteroidia_o_Sphingobacteriales	9E-06	-6.42
				p_Bacteroidetes_c_Bacteroidia_o_Sphingobacteriales_f_Sphingobacteriaceae	1E-04	-5.39
			OTU_62	p_Firmicutes_c_Clostridia_o_Clostridiales_f_Clostridiaceae1_g_Clostridium	9E-05	-5.04
				p_Firmicutes_c_Clostridia_o_Clostridiales_f_Peptostreptococcaceae	3E-02	4.25
			OTU_49	p_Firmicutes_c_Clostridia_o_Clostridiales_f_Peptostreptococcaceae_g_Romboutsia_s_metagenome	1E-06	9.2
				p_Proteobacteria_c_Alphaproteobacteria_o_Acetobacteriales	5E-06	7.38
				p_Proteobacteria_c_Alphaproteobacteria_o_Acetobacteriales_f_Acetobacteraceae	5E-06	7.38
			OTU_44	p_Proteobacteria_c_Alphaproteobacteria_o_Caulobacteriales_f_Caulobacteraceae_g_Caulobacter_s_Unknown	1E-04	-5.53
				p_Proteobacteria_c_Alphaproteobacteria_o_Sphingomonadales_f_Sphingomonadaceae_g_Novosphingobium	2E-02	4.43
			OTU_42	p_Proteobacteria_c_Alphaproteobacteria_o_Sphingomonadales_f_Sphingomonadaceae_g_Sphingomonas_s_Multi-affiliation	9E-05	-5.34
				p_Proteobacteria_c_Deltaproteobacteria_o_Mycococcales	2E-03	4.46
				p_Proteobacteria_c_Deltaproteobacteria_o_Oligoflexales_f_Oligoflexaceae	9E-05	7.86
			OTU_37	p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Burkholderiaceae_g_Cupriavidus_s_Multi-affiliation	7E-07	-7.02
				p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Burkholderiaceae_g_Ralstonia	4E-02	-4.71
				p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Neisseriaceae	5E-06	6.58
				p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Neisseriaceae_g_Neisseria	2E-02	4.12
				p_Proteobacteria_c_Gammaproteobacteria_o_Enterobacteriales_f_Enterobacteriaceae_g_Pantoea	2E-03	7.73
			OTU_48	p_Proteobacteria_c_Gammaproteobacteria_o_Legionellales_f_Legionellaceae_g_Legionella_s_Unknown	3E-03	4.86
			OTU_83	p_Proteobacteria_c_Gammaproteobacteria_o_Legionellales_f_Legionellaceae_g_Legionella_s_Unknown	3E-03	5.35
			OTU_46	p_Proteobacteria_c_Gammaproteobacteria_o_Pseudomonadales_f_Moraxellaceae_g_Acinetobacter_s_Multi-affiliation	2E-13	10.64
			OTU_31	p_Proteobacteria_c_Gammaproteobacteria_o_Pseudomonadales_f_Moraxellaceae_g_Acinetobacter_s_Multi-affiliation	2E-07	-7.15
			OTU_30	p_Proteobacteria_c_Gammaproteobacteria_o_Pseudomonadales_f_Moraxellaceae_g_Acinetobacter_s_Multi-affiliation	2E-02	4.13
B	IA	CRC	OTU#	Taxonomy	padj	log2FoldChange
				p_Actinobacteria_c_Actinobacteria_o_Corynebacteriales_f_CorynebacteriumE1_s_Unknown	9E-07	7.82
			OTU_94	p_Actinobacteria_c_Actinobacteria_o_Micrococcales_f_Micrococaceae_g_Microcococcus_s_Microcococcus	3E-03	5.23
			OTU_6	p_Actinobacteria_c_Actinobacteria_o_Propionibacteriales_f_Propionibacteriaceae_g_Cutibacterium_s_Multi-affiliation	7E-03	1.16
				p_Bacteroidetes_c_Bacteroidia_o_Bacteroidales_f_Bacteroidaceae	1E-05	-4.62
				p_Bacteroidetes_c_Bacteroidia_o_Bacteroidales_f_Bacteroidaceae_g_Bacteroides	1E-05	-4.62
			OTU_52	p_Bacteroidetes_c_Bacteroidia_o_Bacteroidales_f_Bacteroidaceae_g_Bacteroides_s_Multi-affiliation	6E-05	6.65
				p_Bacteroidetes_c_Bacteroidia_o_Chitinophagales	1E-06	7.47
				p_Bacteroidetes_c_Bacteroidia_o_Chitinophagales_f_Chitinophagaceae	3E-04	5.57
				p_Bacteroidetes_c_Bacteroidia_o_Cytophagales_f_Hymenobacteraceae	2E-05	5.85
				p_Bacteroidetes_c_Bacteroidia_o_Cytophagales_f_Hymenobacteraceae_g_Hymenobacter	5E-04	4.86
				p_Bacteroidetes_c_Bacteroidia_o_Flavobacteriales_f_Weekseellaceae_g_Cloacibacterium	5E-04	-5.64
			OTU_26	p_Bacteroidetes_c_Bacteroidia_o_Flavobacteriales_f_Weekseellaceae_g_Cloacibacterium_s_Multi-affiliation	7E-05	5.24
				p_Bacteroidetes_c_Bacteroidia_o_Sphingobacteriales	4E-02	-3.75
				p_Bacteroidetes_c_Bacteroidia_o_Sphingobacteriales_f_Sphingobacteriaceae	4E-02	-3.75
				p_Dependentiae_x_Babellae	3E-02	-4.12
				p_Dependentiae_x_Babellae_g_Babellales	3E-02	-4.12
				p_Firmicutes_x_Bacillo_o_Bacillales_f_FamilyXI	9E-07	6.9
				p_Firmicutes_x_Bacillo_o_Lactobacillales_f_Lactobacillaceae	2E-07	7.56
				p_Firmicutes_x_Bacillo_o_Lactobacillales_f_Lactobacillaceae_g_Lactobacillus	7E-07	7.7
				p_Firmicutes_x_Clostridia_o_Clostridiales_f_Clostridiaceae	3E-09	7.93
				p_Firmicutes_x_Clostridia_o_Clostridiales_f_Lachnospiraceae	4E-02	-4.01
				p_Firmicutes_x_Clostridia_o_Clostridiales_f_Peptostreptococcaceae	5E-12	9.59
				p_Firmicutes_x_Clostridia_o_Clostridiales_f_Peptostreptococcaceae_g_Romboutsia	5E-04	5.89
			OTU_49	p_Firmicutes_x_Clostridia_o_Clostridiales_f_Peptostreptococcaceae_g_Romboutsia_s_metagenome	7E-07	8.36
				p_Firmicutes_x_Clostridia_o_Clostridiales_f_Ruminococcaceae	2E-03	-5.41
				p_Patesobacteria	2E-03	4.76
				p_Patesobacteria_x_Saccharimonadia	8E-07	8.13
				p_Patesobacteria_x_Saccharimonadia_o_Saccharimonadales	8E-07	8.13
				p_Proteobacteria_c_Alphaproteobacteria_o_Acetobacteriales	4E-03	4.7
				p_Proteobacteria_c_Alphaproteobacteria_o_Acetobacteriales_f_Acetobacteraceae	4E-03	4.7
			OTU_5	p_Proteobacteria_c_Alphaproteobacteria_o_Caulobacteriales_f_Caulobacteraceae_g_Unknown_s_Unknown	2E-02	1.27
			OTU_14	p_Proteobacteria_c_Alphaproteobacteria_o_Rhodobiales_f_Rhodobacteriaceae_g_Phylobacterium_s_Multi-affiliation	3E-02	-1.71
			OTU_33	p_Proteobacteria_c_Alphaproteobacteria_o_Rhodobacteriales_f_Rhodobacteriaceae_g_Paracoccus_s_Multi-affiliation	1E-06	6.28
			OTU_42	p_Proteobacteria_c_Alphaproteobacteria_o_Sphingomonadales_f_Sphingomonadaceae_g_Sphingomonas_s_Multi-affiliation	9E-04	-5.01
			OTU_37	p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Burkholderiaceae_g_Cupriavidus_s_Multi-affiliation	4E-05	5.77
				p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Polyphosphatibacter	2E-02	-6
			OTU_51	p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Burkholderiaceae_g_Ralstonia_s_Multi-affiliation	8E-03	4.77
				p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Neisseriaceae_g_Neisseria	1E-02	4.42
				p_Proteobacteria_c_Gammaproteobacteria_o_Enterobacteriales_f_Pantoea	4E-05	8.05
			OTU_32	p_Proteobacteria_c_Gammaproteobacteria_o_Legionellales_f_Legionellaceae_g_Legionella_s_Multi-affiliation	1E-04	5.74
			OTU_46	p_Proteobacteria_c_Gammaproteobacteria_o_Pseudomonadales_f_Moraxellaceae_g_Acinetobacter_s_Multi-affiliation	2E-10	8.85
			OTU_31	p_Proteobacteria_c_Gammaproteobacteria_o_Pseudomonadales_f_Moraxellaceae_g_Acinetobacter_s_Multi-affiliation	3E-05	-6.4
			OTU_30	p_Proteobacteria_c_Gammaproteobacteria_o_Pseudomonadales_f_Moraxellaceae_g_Acinetobacter_s_Multi-affiliation	5E-02	3.29
			OTU_18	p_Proteobacteria_c_Gammaproteobacteria_o_Xanthomonadales_f_Xanthomonadaceae_g_Stenotrophomonas_s_Multi-affiliation	5E-10	4.63
C	IA	Controls	OTU#	Taxonomy	padj	log2FoldChange
				p_Actinobacteria_c_Actinobacteria_o_Bifidobacteriales	1E-06	9.15
				p_Actinobacteria_c_Actinobacteria_o_Bifidobacteriales_f_Bifidobacteriaceae	1E-06	9.25
			OTU_54	p_Actinobacteria_c_Actinobacteria_o_Corynebacteriales_f_Corynebacteriaceae_g_Corynebacterium	3E-05	-8.05
			OTU_43	p_Actinobacteria_c_Actinobacteria_o_Corynebacteriales_f_Corynebacteriaceae_g_Corynebacterium	3E-04	-7.51
				p_Actinobacteria_c_Actinobacteria_o_Corynebacteriales_f_Corynebacteriaceae_g_CorynebacteriumE1_s_Unknown	6E-05	-6.48
				p_Actinobacteria_c_Thermoplasma	2E-04	7.78
				p_Bacteroidetes_c_Bacteroidia_o_Chitinophagales	3E-09	-8.68
				p_Bacteroidetes_c_Bacteroidia_o_Chitinophagales_f_Chitinophagaceae	6E-09	-8.83
				p_Bacteroidetes_c_Bacteroidia_o_Cytophagales_f_Hymenobacteraceae	4E-03	-4.84
				p_Bacteroidetes_c_Bacteroidia_o_Cytophagales_f_Hymenobacteraceae_g_Hymenobacter	4E-03	-4.85
				p_Bacteroidetes_c_Bacteroidia_o_Cytophagales_f_Spirosomaceae	4E-03	-5.27
				p_Firmicutes_x_Bacillo_o_Lactobacillales_f_Lactobacillaceae	1E-05	-6.85
				p_Firmicutes_x_Bacillo_o_Lactobacillales_f_Lactobacillaceae_g_Lactobacillus	8E-06	-6.99
				p_Firmicutes_x_Clostridia_o_Clostridiales_f_Clostridiaceae_g_Clostridium	5E-13	-9.39
			OTU_62	p_Firmicutes_x_Clostridia_o_Clostridiales_f_Clostridiaceae1_g_Clostridium	2E-02	-3.74
				p_Firmicutes_x_Clostridia_o_Clostridiales_f_Clostridiaceae1_g_ClostridiumE1_s_Multi-affiliation	6E-05	-5.54
				p_Firmicutes_x_Clostridia_o_Clostridiales_f_FamilyXI	8E-10	-9.12
				p_Patesobacteria	3E-02	-3.9
				p_Patesobacteria_x_Saccharimonadia	2E-05	-7.75
				p_Patesobacteria_x_Saccharimonadia_o_Saccharimonadales	2E-05	-7.75
				p_Proteobacteria_c_Alphaproteobacteria_o_Rhodobiales_f_Rhodobacteriaceae_g_Bosea	3E-02	-5.05
			OTU_33	p_Proteobacteria_c_Alphaproteobacteria_o_Rhodobacteriales_f_Rhodobacteriaceae_g_Paracoccus_s_Multi-affiliation	8E-06	-5.41
			OTU_37	p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Burkholderiaceae_g_Cupriavidus_s_Multi-affiliation	3E-10	-8.31
			OTU_71	p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Burkholderiaceae_g_Massilia_s_Massilia	1E-06	8.81
				p_Proteobacteria_c_Gammaproteobacteria_o_Betaproteobacteriales_f_Neisseriaceae	5E-07	7.14
			OTU_45	p_Proteobacteria_c_Gammaproteobacteria_o_Diplomicrobiales_f_Diplomicrobiaceae_g_Unknown_s_Unknown	3E-02	4.29
			OTU_53	p_Proteobacteria_c_Gammaproteobacteria_o_Legionellales_f_Legionellaceae_g_Legionella_s_Unknown	2E-02	4.26
			OTU_65	p_Proteobacteria_c_Gammaproteobacteria_o_Pseudomonadales_f_Moraxellaceae_g_Acinetobacter_s_Unknown	3E-08	10
			OTU_1	p_Proteobacteria_c_Gammaproteobacteria_o_Pseudomonadales_f_Pseudomonadaceae_g_Pseudomonas_s_Multi-affiliation	6E-03	0.44
			OTU_18	p_Proteobacteria_c_Gammaproteobacteria_o_Xanthomonadales_f_Xanthomonadaceae_g_Stenotrophomonas_s_Multi-affiliation	3E-10	-4.65

Figure S4. Multidimensional scaling (MDS) differentiates bacterial patterns of study blood samples and DNA extraction negative controls on Bray-Curtis distance matrices for all 10 randomized comparisons. Study blood samples (in Red) separated from negative controls (in Blue) in all of the randomized comparisons.

