Supplementary information of:

METHANOGENIC ARCHAEA QUANTIFICATION IN THE HUMAN GUT MICROBIOME WITH F_{420} AUTOFLUORESCENCE BASED FLOW CYTOMETRY

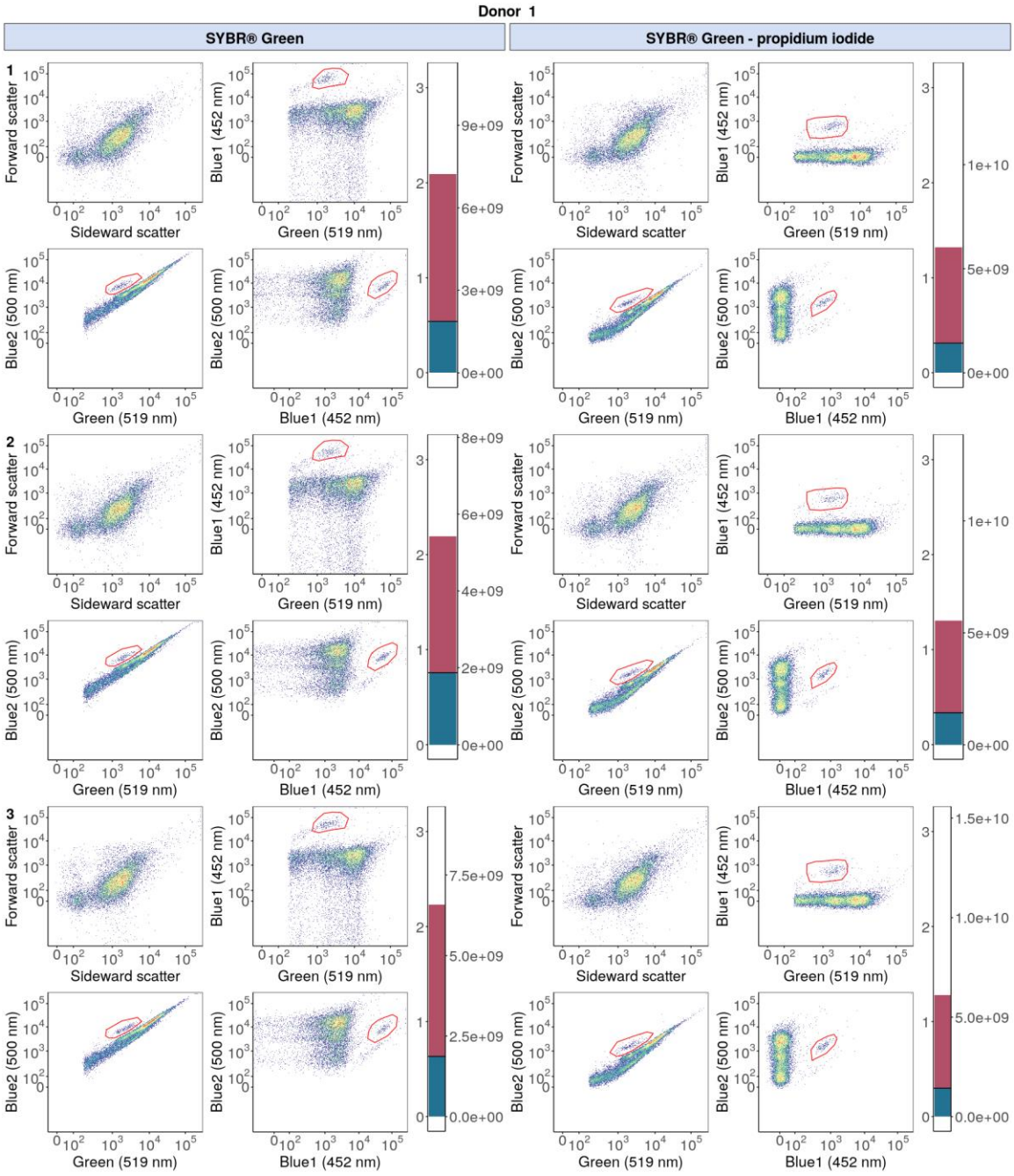

Figure S1: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 1. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the detected methanogen population in the filtered sample depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

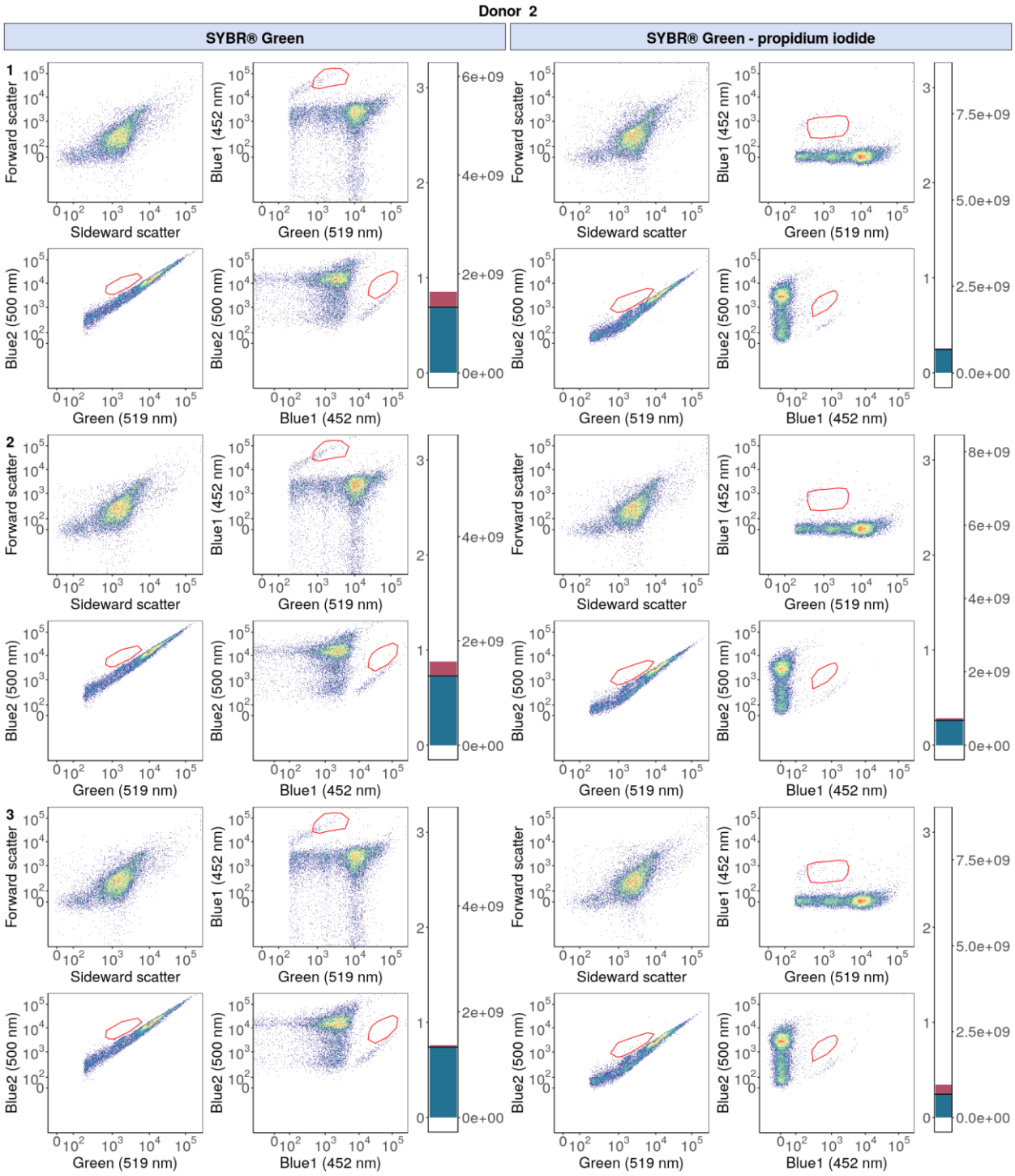

Figure S1 - continued: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 2. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the LOD depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

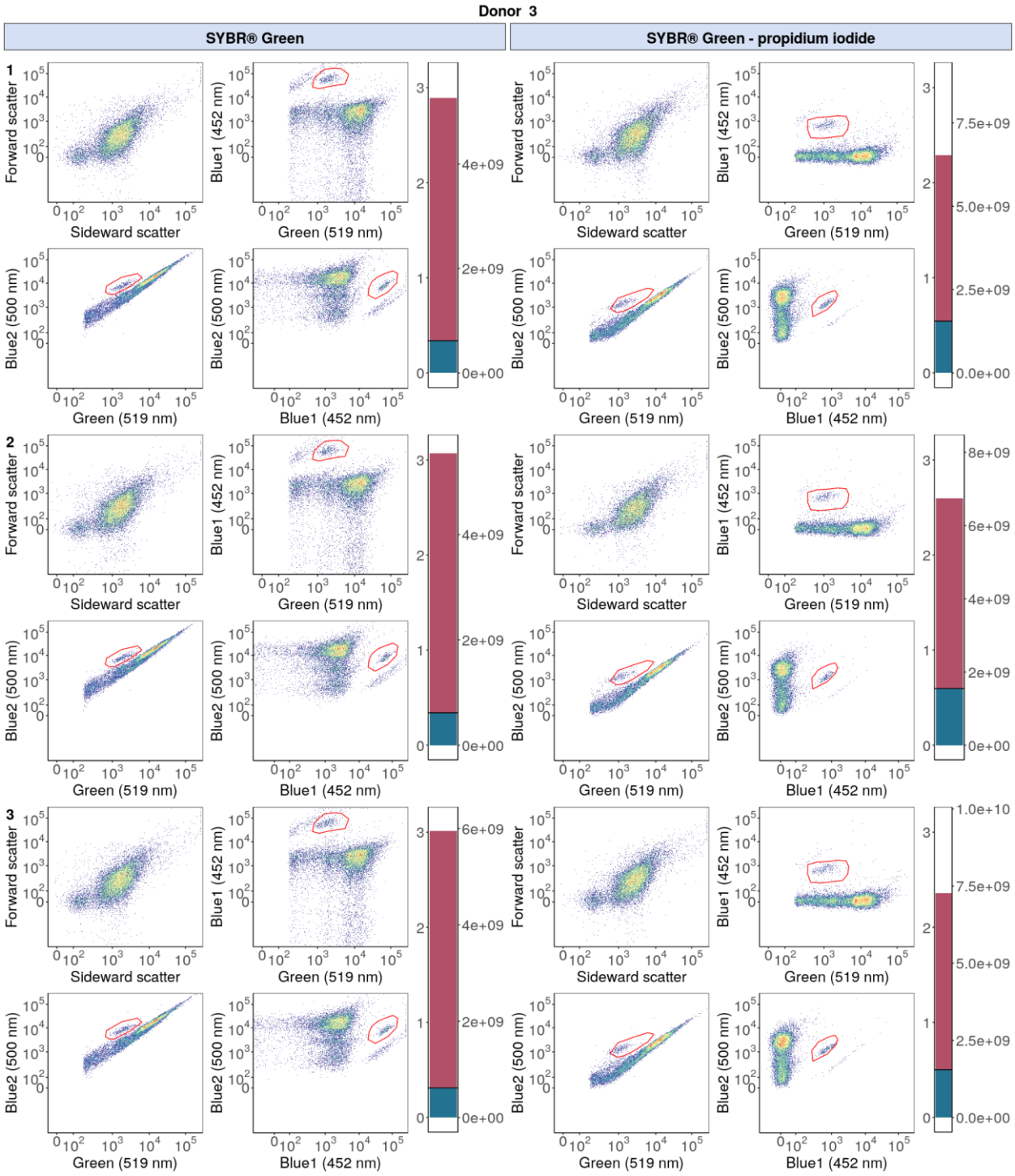

Figure S1 - continued: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 3. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the detected methanogen population in the filtered sample depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

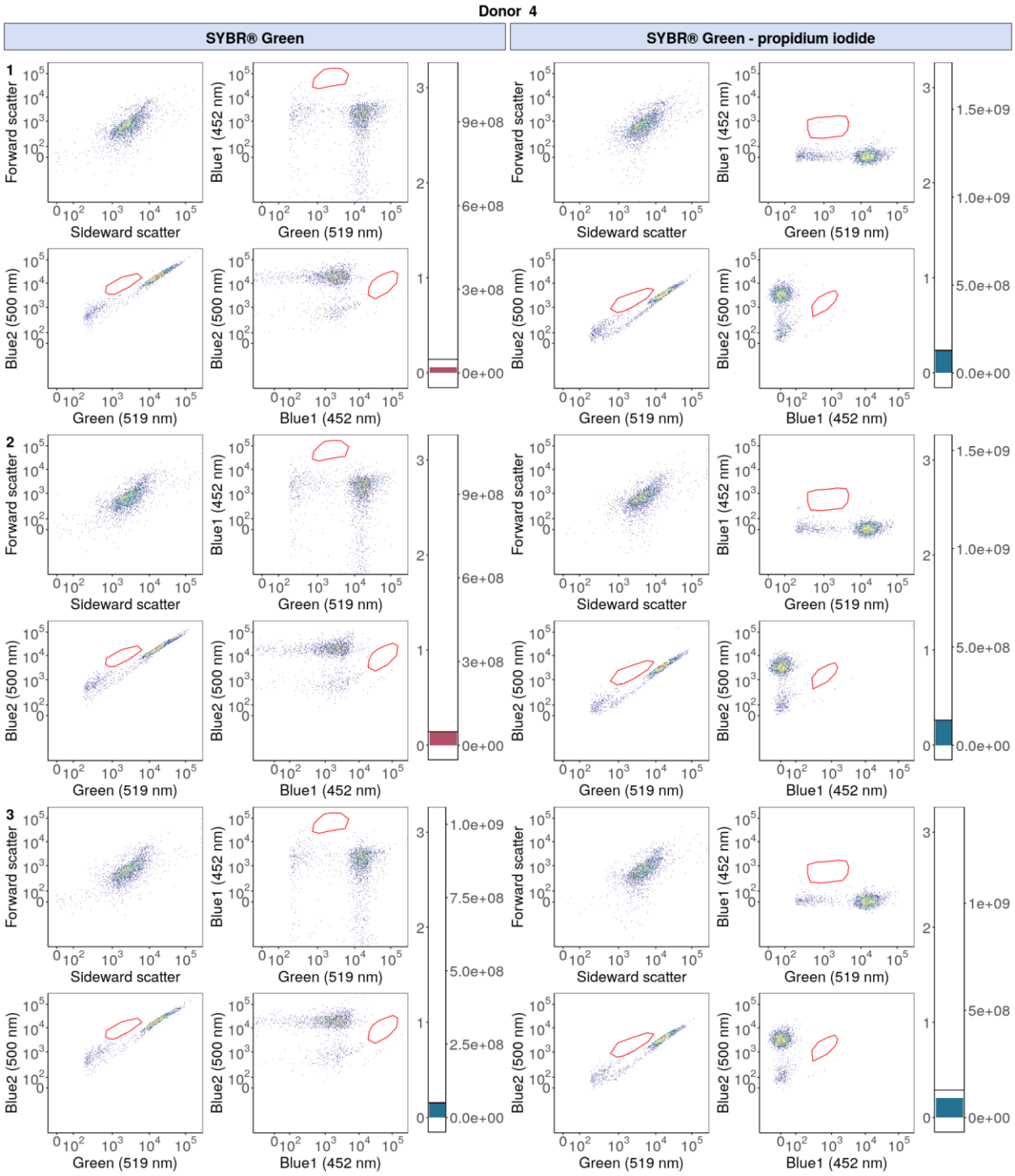

Figure S1 - continued: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 4. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the detected methanogen population in the filtered sample depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

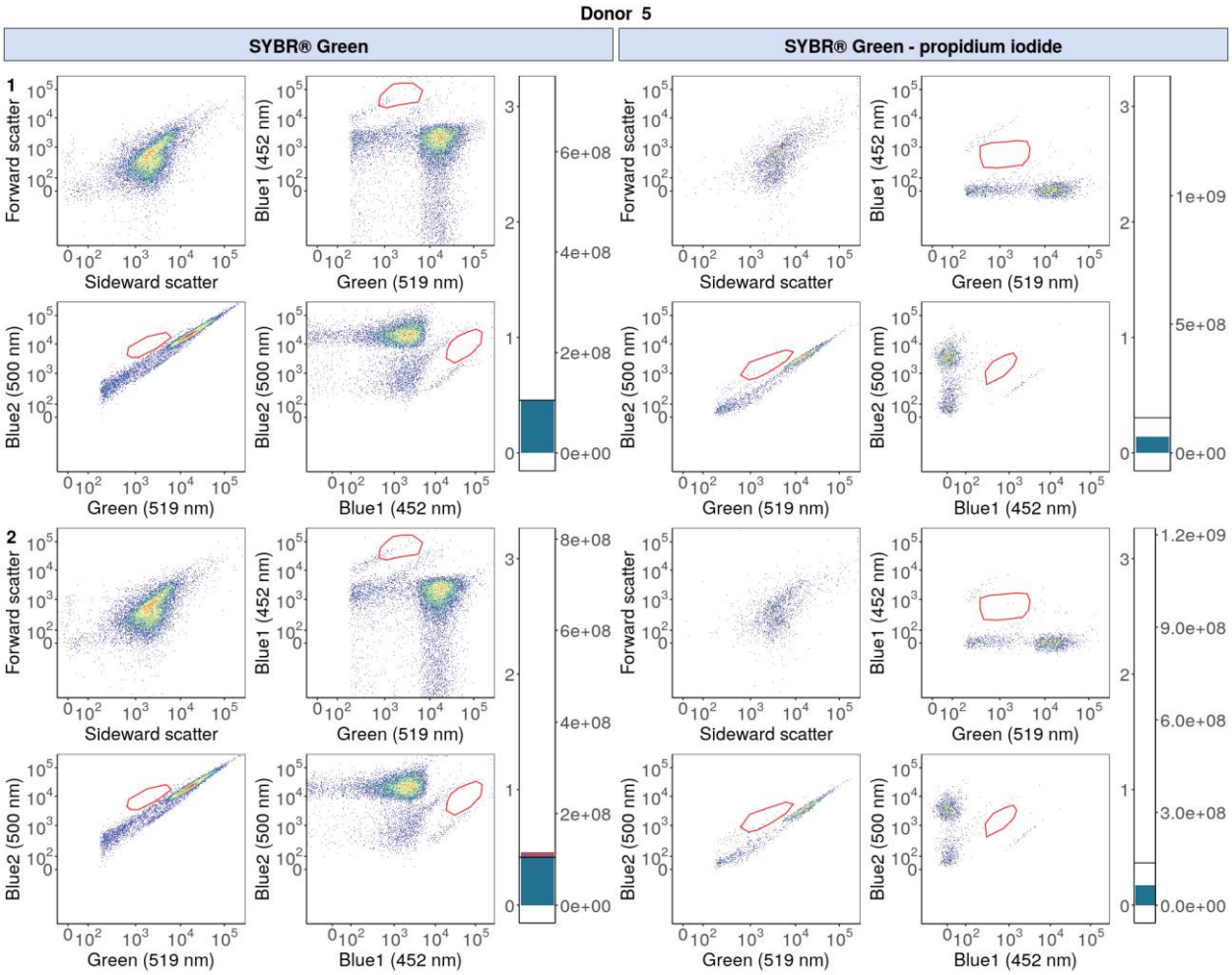

Figure S1 - continued: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 5. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the detected methanogen population in the filtered sample depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

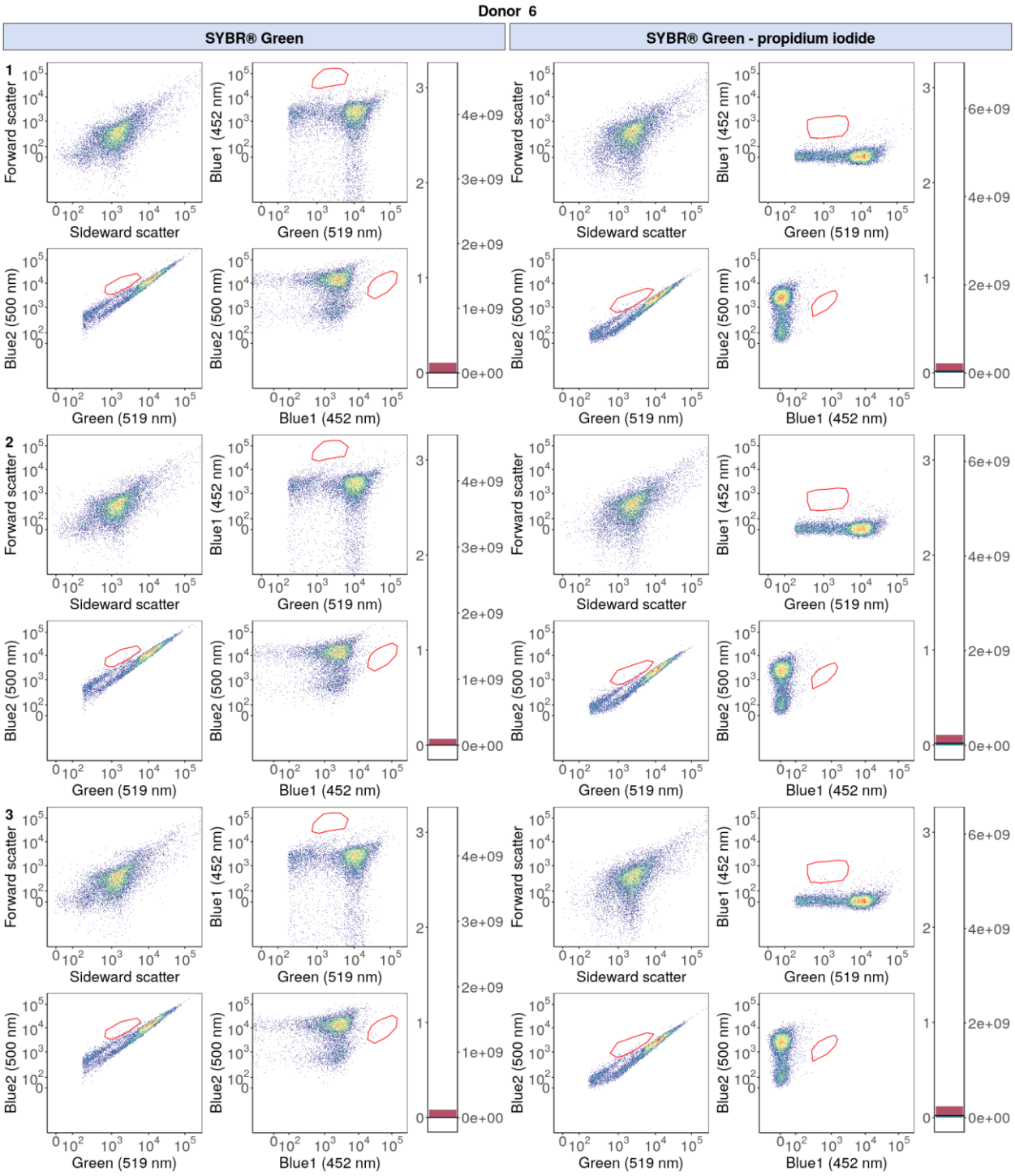

Figure S1 - continued: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 6. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the detected methanogen population in the filtered sample depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

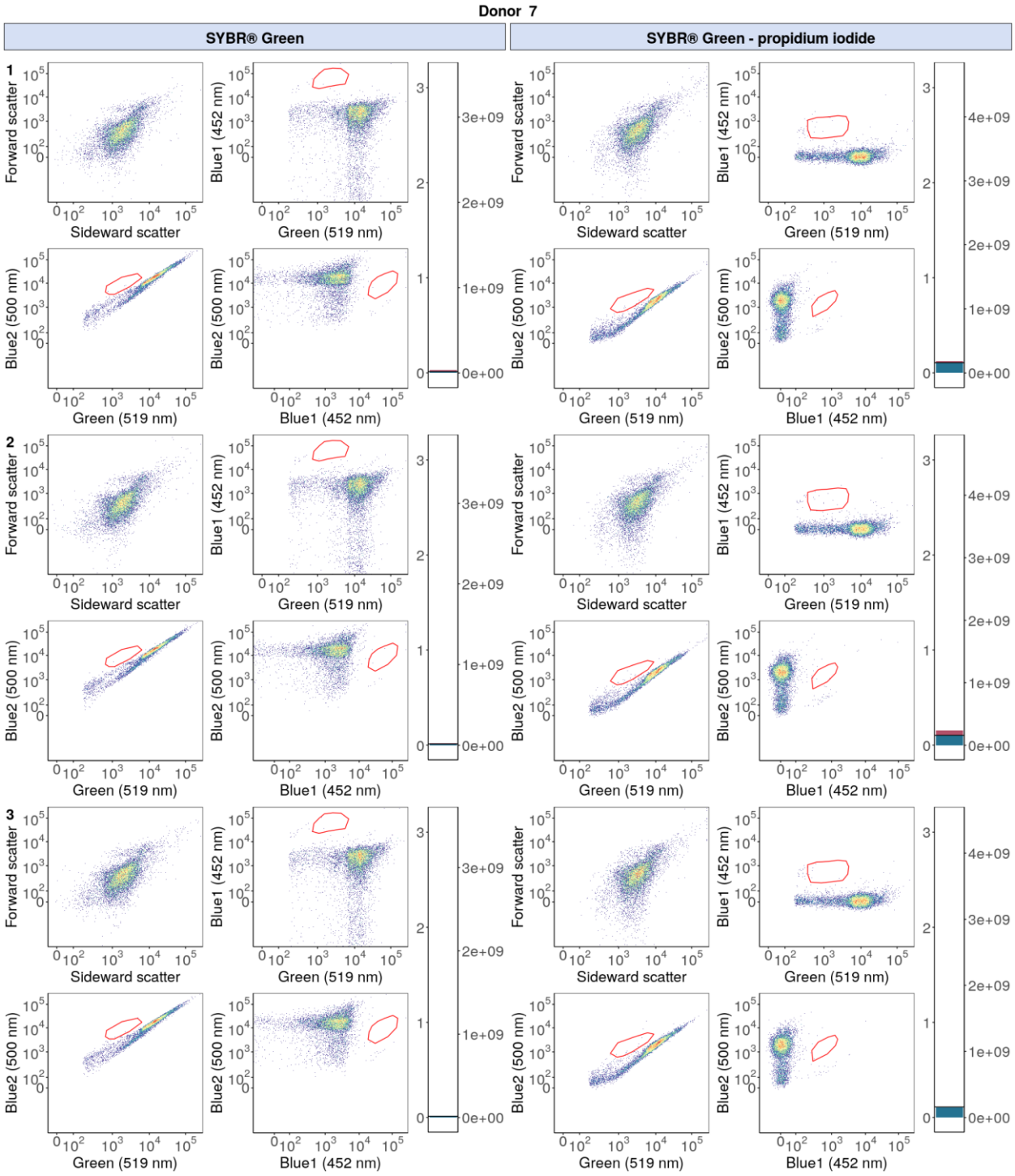

Figure S1 - continued: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 7. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the detected methanogen population in the filtered sample depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

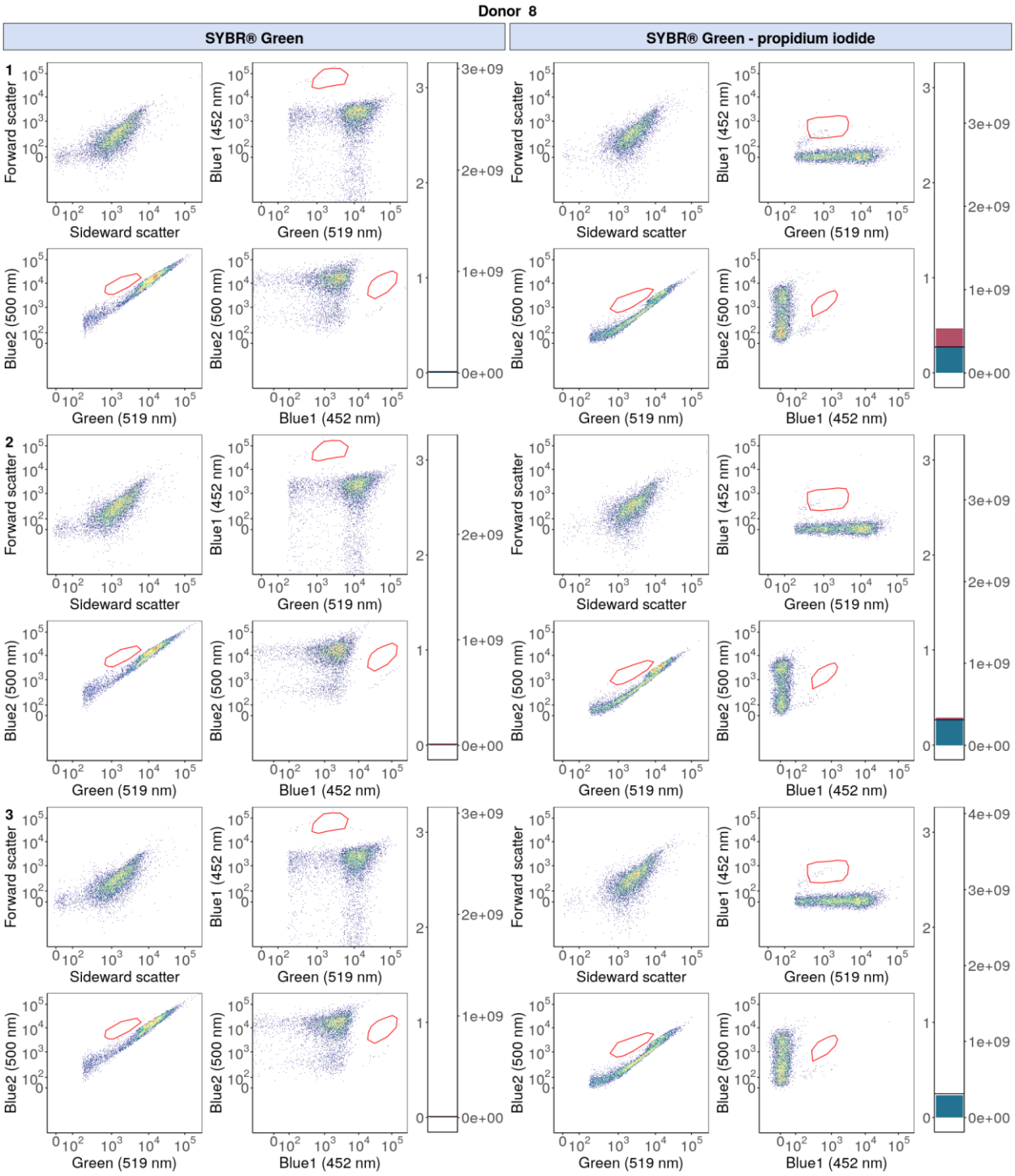

Figure S1 - continued: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 8. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the detected methanogen population in the filtered sample depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

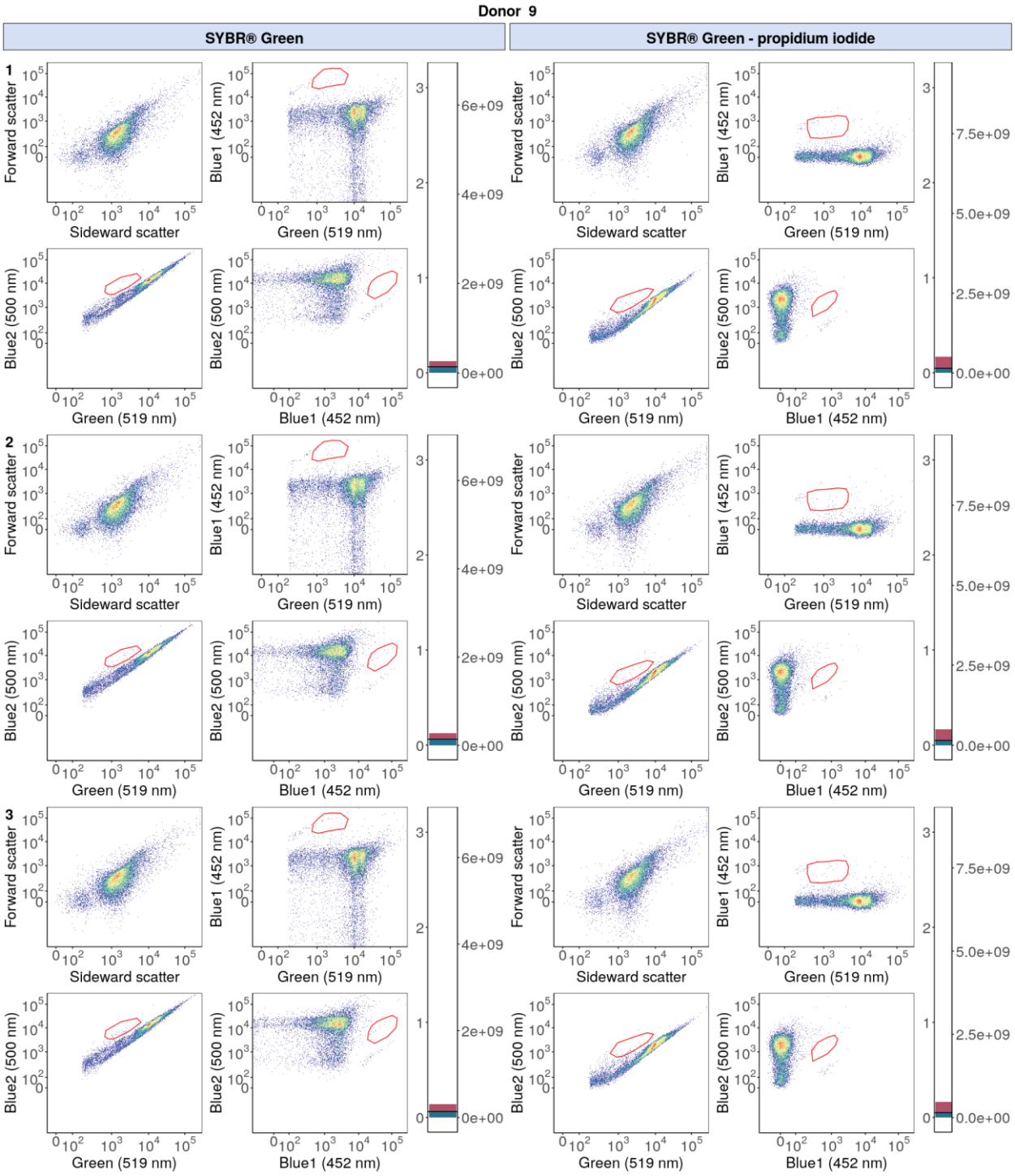

Figure S1 - continued: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 9. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the detected methanogen population in the filtered sample depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

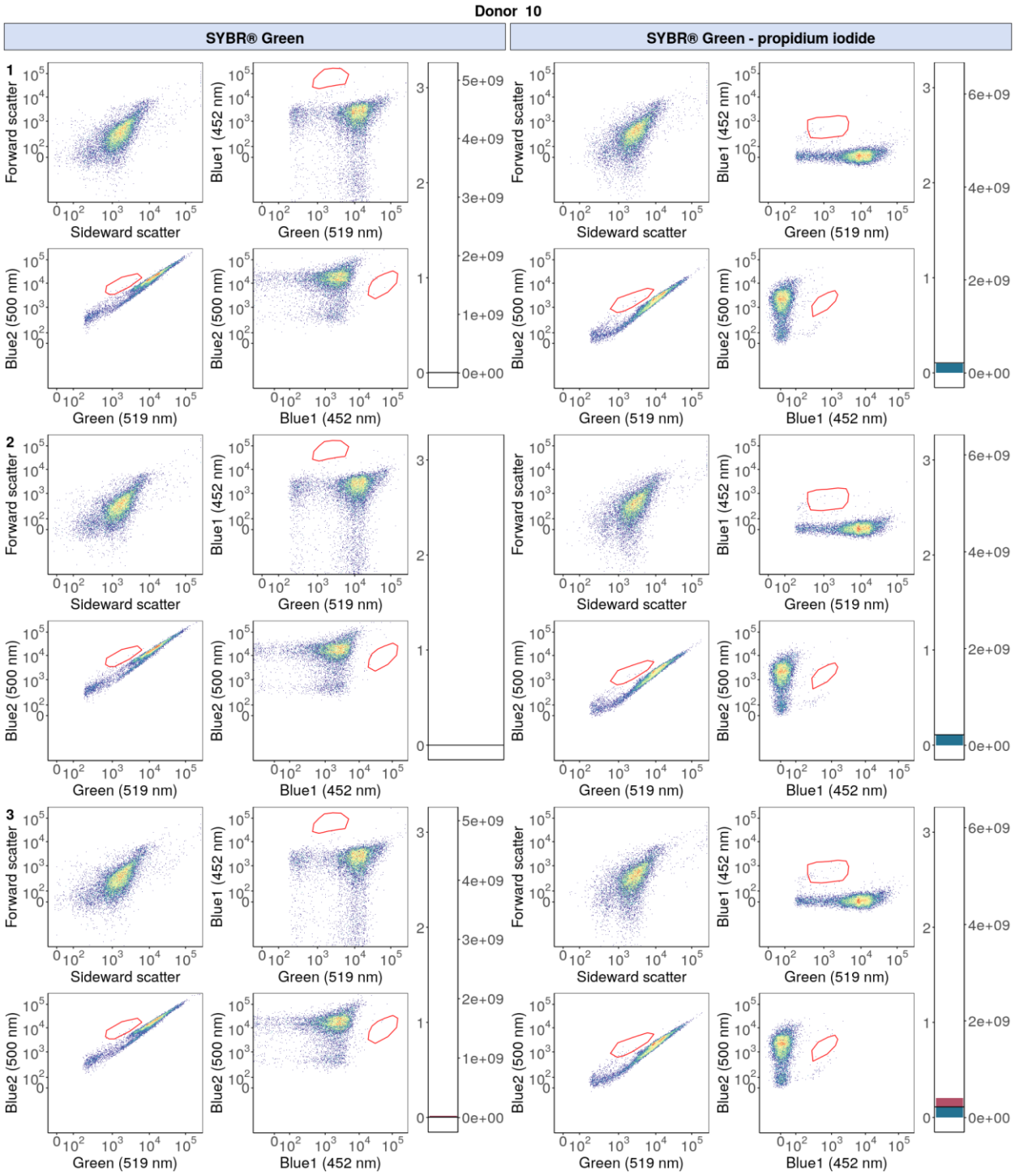

Figure S1 - continued: Visualisation of cofactor F_{420} auto fluorescence (gated in red) in Blue1 (452 nm) and Blue2 (500 nm) fluorescence channels as a function of the Green (519 nm) fluorescence channels, Blue2 fluorescence as a function of the Blue1 fluorescence channels and the microbial community visualisation in the forward scatter as a function of the sidewards scatter channels of technical replicates of a faecal sample derived from donor 10. The replicates were stained with either SYBR® Green (left) or a SYBR® Green – propidium iodide staining mix (right). The proportional (left y-axis) and absolute (right y-axis) cell counts are depicted as a bar chart on the right, with the detected methanogen population in the filtered sample depicted as a horizontal line. The absolute methanogen population below and above the filtered sample population is depicted in blue and red, respectively.

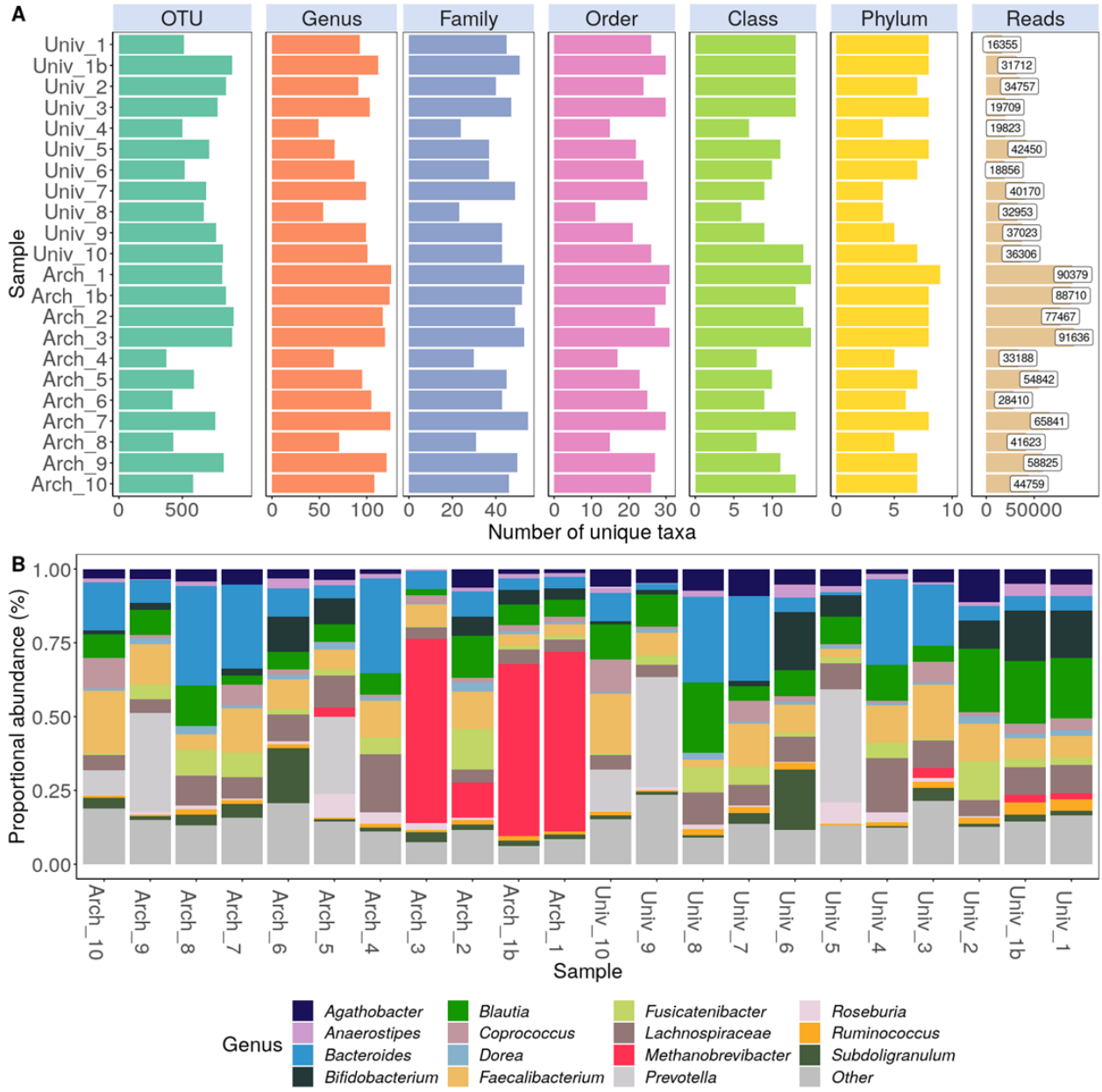

Figure S2: (**A**) Number of unique taxa in the 16S rRNA gene amplicon sequencing samples. (**B**) Proportional abundance (%) of the 15 most abundant genera in all samples. Less abundant genera are pooled into "Other". The V3-V4 region of 16S rRNA gene was amplified with either universal (Univ, U341F – U806) or Archaeal (Arch, 340F – 1000R, nested with U341F – U806R) primers. Higher level taxa are to be interpreted as unclassified genus belonging to the respective taxon.

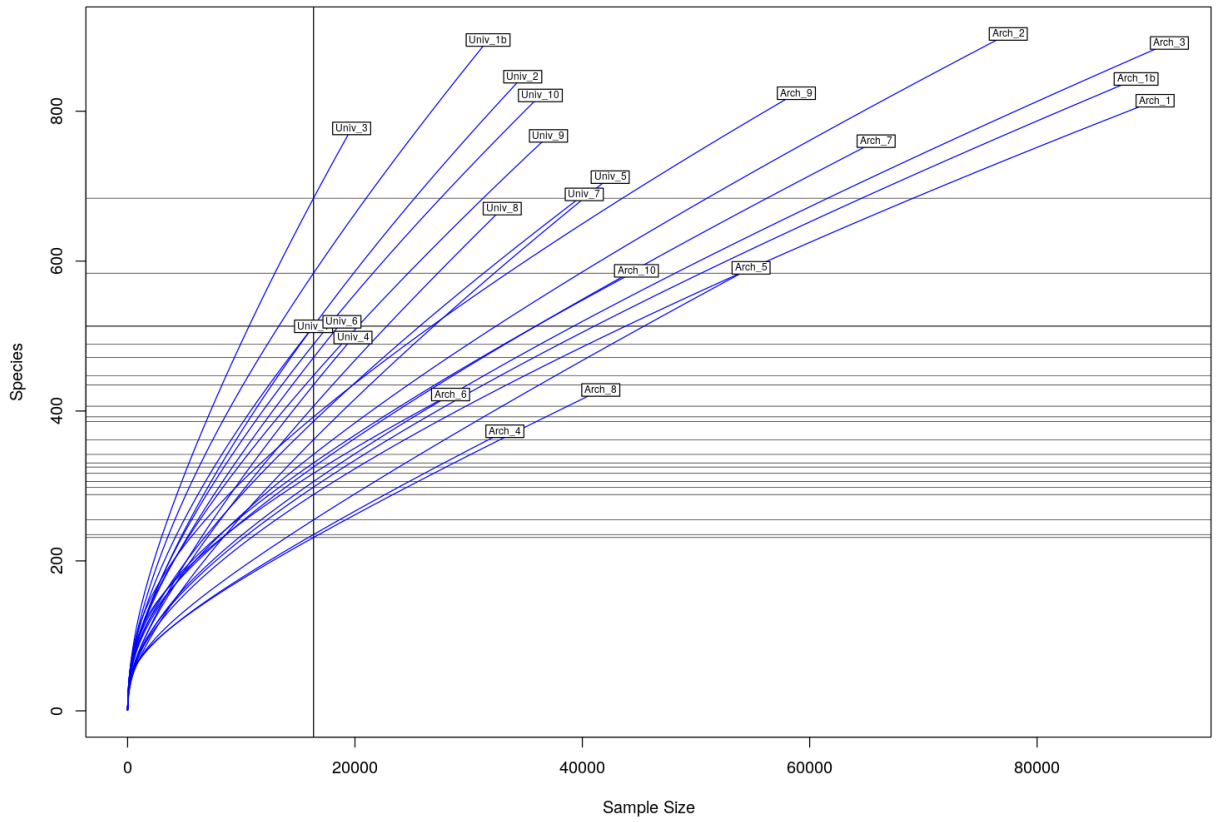

Figure S3: Rarefaction curves of 16S rRNA gene sequence count data. The V3-V4 region of 16S rRNA gene was amplified with either universal (Univ, U341F – U806) or Archaeal (Arch, 340F – 1000R, nested with U341F – U806R) primers. The raw data corresponding to the sample identifiers can be found in the EBI ENA submission (ERP138722).

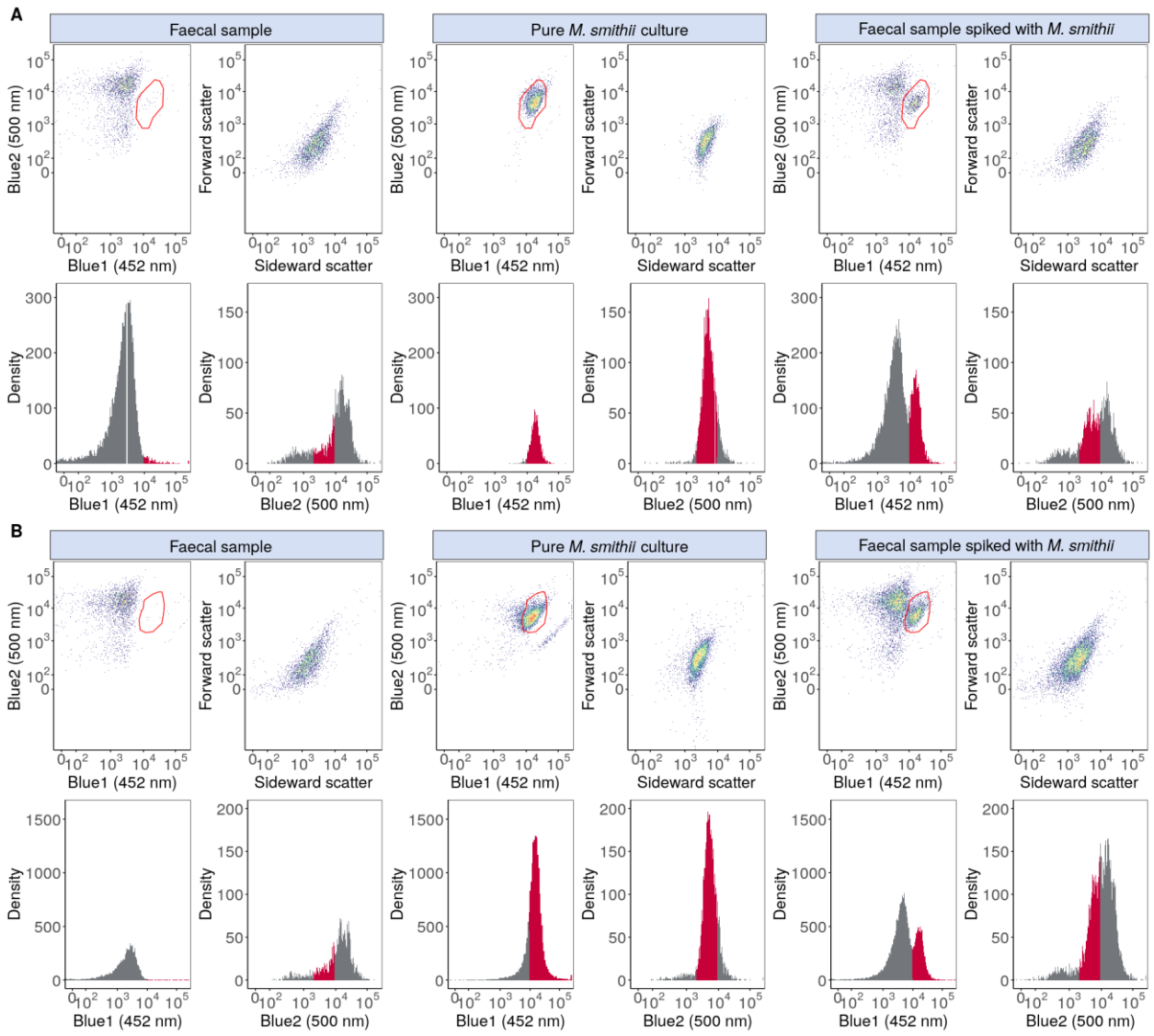

Figure S4: Flow cytometric visualisation and gating of the cofactor F_{420} auto fluorescence (gated in red) in the Blue2 (500 nm) fluorescence channel as a function of the Blue1 (452 nm) fluorescence channel and community visualisation in the forward scatter channel in function of the sideward scatter channel. The one-dimensional histograms of the 452 and 500 nm emissions are depicted below the density plots with the F_{420} auto fluorescence peaks in red. The samples were stained with either SYBR® Green – propidium iodide (**A**) or SYBR® Green (**B**), of a faecal sample, pure *M. smithii* culture and faecal sample spiked with *M. smithii*.

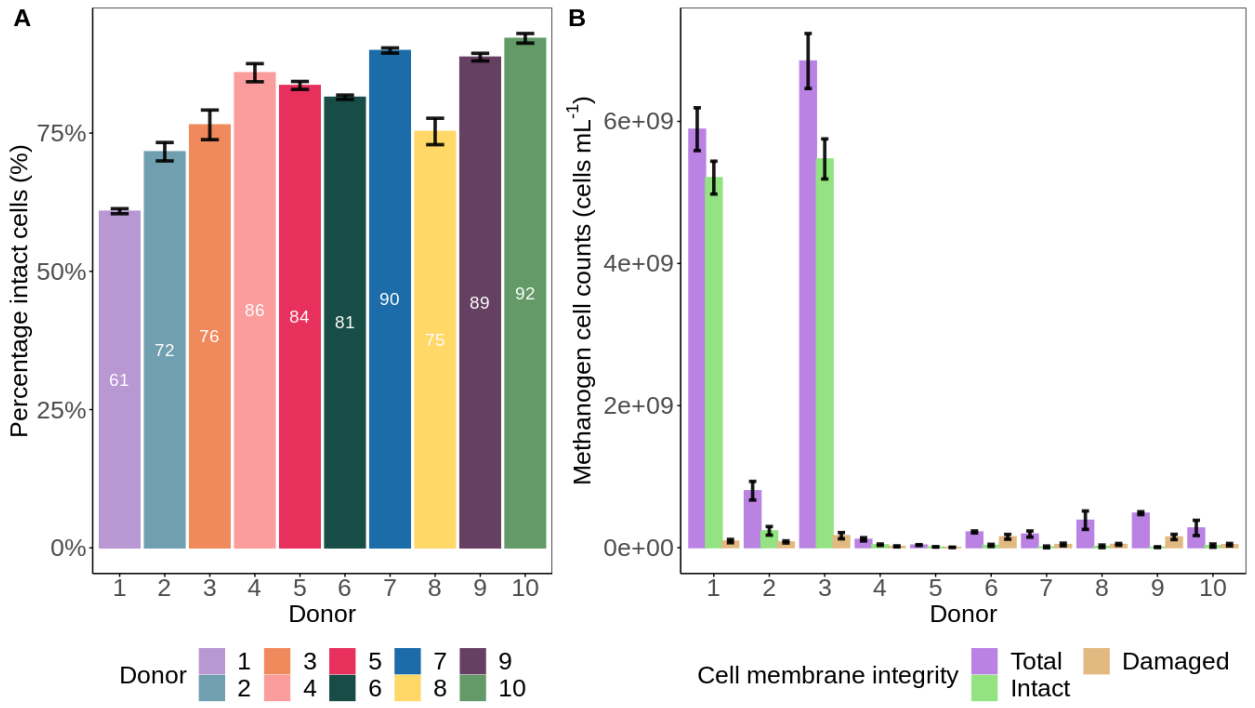

Figure S5: (**A**) The total percentage of intact cells. Methanogen cell counts (**B**) of all samples stained with Sybr Green – Propidium iodide (purple), and its subsequent intact fraction (green) and damaged fraction (orange).

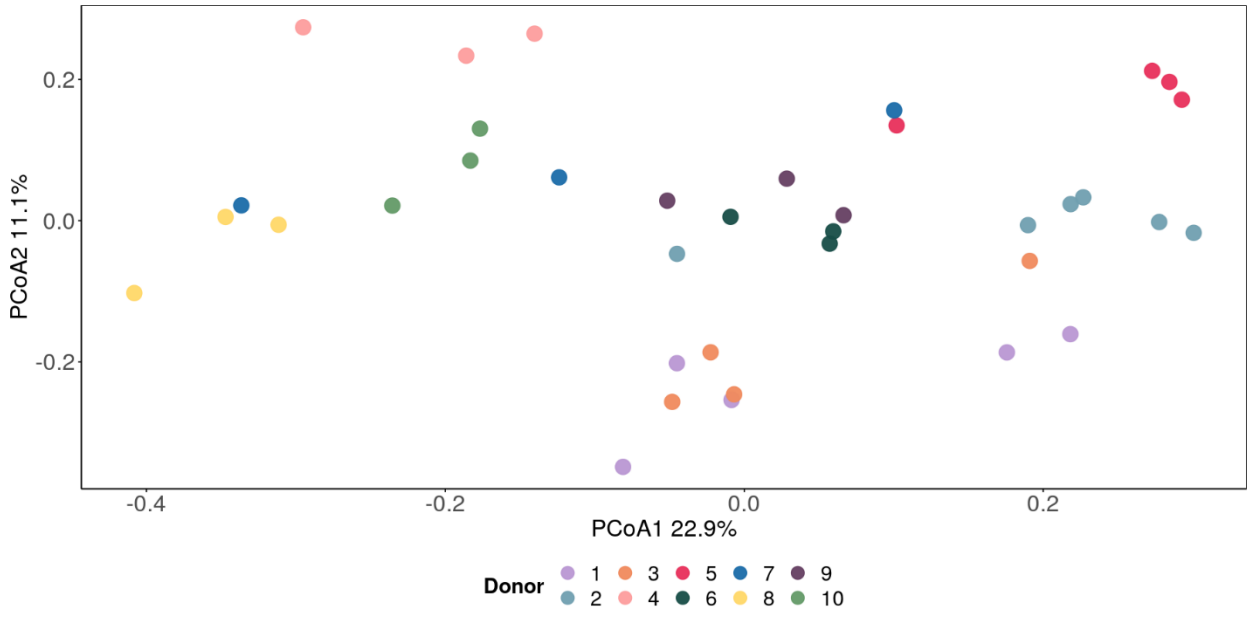

Figure S6: Community β diversity assessment of the flow cytometric fingerprint data of all cellular information based on six phenotypic parameters (4 fluorescence and 2 scatter signals), stained with SYBR® Green. Ordination was performed with principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity.

Table S1: Components of DMSZ medium 1523. Modified *Methanobacterium* medium (DSMZ, 2015).

Component	Weight	Unit	Conc (gL^{-1})	Conc (mM)
KH_2PO_4	0.50	g	0.50	3.67
$MgSO_4$ x 7 H_2O	0.40	g	0.40	1.62
NaCl	0.40	g	0.40	6.85
NH_4Cl	0.40	g	0.40	7.48
$CaCl_2$ x 2 H_2O	0.05	g	0.05	0.34
Trace element solution SL-10 (Table S2)	1.00	mL	-	-
Brain Heart Infusion	6.00	g	6.00	-
Proteose peptone	6.00	g	6.00	-
Yeast extract	2.00	g	2.00	-
Na-acetate	1.00	g	1.00	12.19
Na-formate	2.00	g	2.00	29.41
Na-resazurin solution (0.1% w/v)	0.50	mL	5.00E-4	-
$NaHCO_3$	4.00	g	4.00	47.62
Vitamins solution (Table S3)	1.00	mL	-	-
L-Cysteine-HCl x H_2O	0.50	g	0.50	2.85
Na_2S x 9 H_2O	0.50	g	0.50	2.08
Distilled water	1000.00	mL	-	-

Table S2: Components of trace element solution SL-10, derived from DSMZ medium 320 [2].

Component	Weight	Unit	Conc (gL^{-1})	Conc (mM)
HCl (25%; 7.7 M)	10.00	mL	2.50	68.57
$FeCl_2$ x 4 H_2O	1.50	g	1.50	7.55
$ZnCl_2$	70.00	mg	0.07	0.51
$MnCl_2$ x 4 H_2O	100.00	mg	0.10	0.51
H_3BO_3	6.00	mg	0.01	0.10
$CoCl_2$ x 6 H_2O	190.00	mg	0.19	1.46
$CuCl_2$ x 2 H_2O	2.00	mg	0.002	0.01
$NiCl_2$ x 6 H_2O	24.00	mg	0.02	0.19
Na_2MoO_4 x 2 H_2O	36.00	mg	0.04	0.15
Distilled water	990.00	mL	-	-

Table S3: Components in the seven vitamins solution, derived from DSMZ medium 503 [3]

Component	Weight	Unit	Conc (gL^{-1})	Conc (mM)
Vitamin B12	100.00	mg	0.10	0.07
p-Aminobenzoic acid	80.00	mg	0.08	0.58
D(+)-Biotin	20.00	mg	0.02	0.08
Nicotinic acid	200.00	mg	0.20	1.63
Calcium pantothenate	100.00	mg	0.10	0.21
Pyridoxine hydrochloride	300.00	mg	0.30	1.46
Thiamine-HCl x 2 H_2O	200.00	mg	0.20	0.54
Distilled water	1000.00	mL	-	-

Table S4: Reducing anaerobic phosphate buffer (pH 6.8) was sparged and flushed with N_2-gas prior to autoclaving.

Component	Composition ($g\ L^{-1}$)	Molar conc. (mM)	Manufacturer
L-Cystein- HCl	1.00	6.34e-3	J&K Scientific, Lommel, Belgium
$C_2H_3O_2SNa$	1.00	8.75e-3	Sigma Aldrich, St. Louis, MO, USA
$NaHCO_3$	1.40	2.40e-2	Chem-lab, Zedelgem, Belgium
NaCl	0.90	1.07e-2	Carl Roth, Karlsruhe, Germany
KH_2PO_4	6.80	5.00e-2	Carl Roth, Karlsruhe, Germany
K_2HPO_4	8.72	5.00e-2	Carl Roth, Karlsruhe, Germany

Table S5: Classification measures (accuracy, P-value, sensitivity, specificity, precision, F1-score and positive and negative predictive values) of the methanogen classification method on ten faecal samples, stained with either SGPI or SG, as verified with 16S rRNA gene amplicon (16S) Archaea abundances, sequenced with either universal or Archaeal primers. Methanogen classifications were marked as positive when methanogen cell counts were higher than both the sum of the average, sample wide, filtered background noise and standard deviation, and the sample specific filtered background noise.

16S primer	Stain	Accuracy	P-value	Sensitivity	Specificity	Precision	F1	Pos. Pred. Value	Neg. Pred. Value
Universal	SGPI	1.00	0.11	1.00	1.00	1.00	1.00	1.00	1.00
Universal	SG	0.90	0.38	1.00	0.88	0.67	0.80	1.00	0.67
Archaeal	SGPI	0.80	0.17	0.50	1.00	1.00	0.67	1.00	0.75
Archaeal	SG	0.90	0.05	0.75	1.00	1.00	0.86	1.00	0.86

Table S6: Confusion matrices of the predicted methanogen classification method (FCM) on ten faecal samples, stained with either SGPI or SG, as verified with 16S rRNA gene amplicon (16S) Archaea abundances, sequenced with either universal or Archaeal primers. Methanogen classifications were marked as positive when methanogen cell counts were higher than both the sum of the average, sample wide, filtered background noise and standard deviation, and the sample specific filtered background noise.

		16S - Universal Reference	
		Methanogen	Non Methanogen
FCM - SGPI Prediction	Methanogen	2	0
	Non methanogen	0	8

		16S - Universal Reference	
		Methanogen	Non Methanogen
FCM - SG Prediction	Methanogen	2	0
	Non methanogen	1	7

		16S - Archaeal Reference	
		Methanogen	Non Methanogen
FCM - SGPI Prediction	Methanogen	2	0
	Non methanogen	2	6

		16S - Archaeal Reference	
		Methanogen	Non Methanogen
FCM - SG Prediction	Methanogen	3	0
	Non methanogen	1	6

Table S7: Classification measures (**A**) (accuracy, P-value, sensitivity, specificity, precision, F1-score and positive and negative predictive values) and confusion matrix (**B**) of the predicted methanogen classification method (FCM) on 241 SHIME samples, stained with SGPI and verified with the presence of methane (CH_4) in SHIME headspace and the presence of methanogenic Archaea. Methanogen classifications were marked as positive when methanogen cell counts were higher than the average, sample wide, filtered background noise and standard deviation.

A

Reference	Accuracy	P-value	Sensitivity	Specificity	Precision	F1-score	Pos Pred Value	Neg Pred Value
CH_4 production	0.71	0.98	0.65	0.72	0.42	0.51	0.42	0.87
Archaea	0.75	7.57E-07	0.65	0.82	0.70	0.67	0.70	0.78

B

FCM - SGPI Prediction	CH_4 detected reference: Methanogen	CH_4 detected reference: Non Methanogen
Methanogen	37	51
Non methanogen	20	133

FCM - SGPI Prediction	Archaea detected reference: Methanogen	Archaea detected reference: Non Methanogen
Methanogen	62	26
Non methanogen	34	119