

Supplementary Material

Impact of Preanalytical Procedures on Complement Biomarkers in Cerebrospinal Fluid and Plasma from Controls and Alzheimer's Disease Patients

Supplementary Table 1. Percent recovery of the complement proteins from unsupplemented (No Addition) in plasma

Analyte	Additive	Mean % recovery from no addition	Min % recovery from no addition	Max % recovery from no addition	SD	SE
C4a	EDTA	104	75	140	24	8
C4a	EDTA+FUT	90	56	131	28	9
C4a	PIC	121	22	203	66	22
C4	EDTA	108	95	132	11	4
C4	EDTA+FUT	108	92	156	19	6
C4	PIC	115	100	187	28	9
C3a	EDTA	87	75	99	7	2
C3a	EDTA+FUT	89	77	104	8	3
C3a	PIC	93	78	104	9	3
C3	EDTA	101	93	110	6	2
C3	EDTA+FUT	104	89	159	21	7
C3	PIC	109	64	184	32	11
Bb	EDTA	104	96	129	10	3
Bb	EDTA+FUT	95	75	114	12	4
Bb	PIC	107	89	136	14	5
FB	EDTA	102	88	114	7	2
FB	EDTA+FUT	98	83	141	17	6
FB	PIC	116	96	186	27	9

Min % recovery, Minimum % recovery; Max % recovery, Maximum % recovery; SD, Standard deviation; SE, Standard error

Supplementary Table 2. Percent recovery of the complement proteins from unsupplemented (No Addition) in CSF

Analyte	Additive	Mean % recovery from no addition	Min % recovery from no addition	Max % recovery from no addition	SD	SE
C4a	EDTA	99	76	115	11	4
C4a	EDTA+FUT	107	88	116	10	3
C4a	PIC	113	91	122	10	3
C4	EDTA	98	93	106	4	1
C4	EDTA+FUT	97	91	103	4	1
C4	PIC	100	98	105	3	1
C3a	EDTA	62	36	102	23	8
C3a	EDTA+FUT	57	39	80	14	5
C3a	PIC	243	62	663	182	61
C3	EDTA	98	86	105	6	2
C3	EDTA+FUT	100	83	105	7	2
C3	PIC	114	99	130	12	4
Bb	EDTA	95	87	100	4	1
Bb	EDTA+FUT	78	68	85	5	2
Bb	PIC	102	96	111	5	2
FB	EDTA	103	100	107	3	1
FB	EDTA+FUT	80	72	85	4	1
FB	PIC	107	104	109	2	1

Min % recovery, Minimum % recovery; Max % recovery, Maximum % recovery; SD, Standard deviation; SE, Standard error

Supplementary Table 3. Percent recovery of the complement proteins from the first freeze-thaw cycle in plasma

Analyte	Additive	Mean %	Mean %	Mean %	SD	SD	SD	SE	SE	SE
		recovery	recovery	recovery	FT2	FT3	FT4	FT2	FT3	FT4
C4a	No Addition	102	157	139	38	66	35	13	22	12
C4a	EDTA	151	173	152	87	84	56	29	28	19
C4a	EDTA+FUT	213	196	174	114	102	81	38	34	27
C4a	PIC	192	209	271	167	184	277	56	61	92
C4	No Addition	121	111	130	43	49	52	14	16	17
C4	EDTA	114	111	109	28	51	32	9	17	11
C4	EDTA+FUT	109	117	116	23	20	20	8	7	7
C4	PIC	111	96	109	16	19	17	5	6	6
C3a	No Addition	128	140	150	37	36	28	12	12	9
C3a	EDTA	160	169	192	26	41	41	9	14	14
C3a	EDTA+FUT	155	179	185	35	30	32	12	10	11
C3a	PIC	156	151	165	40	28	35	13	9	12
C3	No Addition	102	93	104	46	48	46	15	16	15
C3	EDTA	100	93	95	35	43	40	12	14	13
C3	EDTA+FUT	92	92	92	23	24	23	8	8	8
C3	PIC	95	84	89	25	27	22	8	9	7
Bb	No Addition	124	122	121	21	15	23	7	5	8
Bb	EDTA	121	113	109	23	24	16	8	8	5
Bb	EDTA+FUT	100	96	88	15	10	18	5	3	6
Bb	PIC	121	121	121	22	20	18	7	7	6
FB	No Addition	98	87	107	15	24	32	5	8	11
FB	EDTA	98	92	98	16	27	26	5	9	9
FB	EDTA+FUT	89	89	89	14	14	12	5	5	4
FB	PIC	102	87	99	14	19	14	5	6	5

FT, Freeze thaw; SD, Standard deviation; SE, Standard error

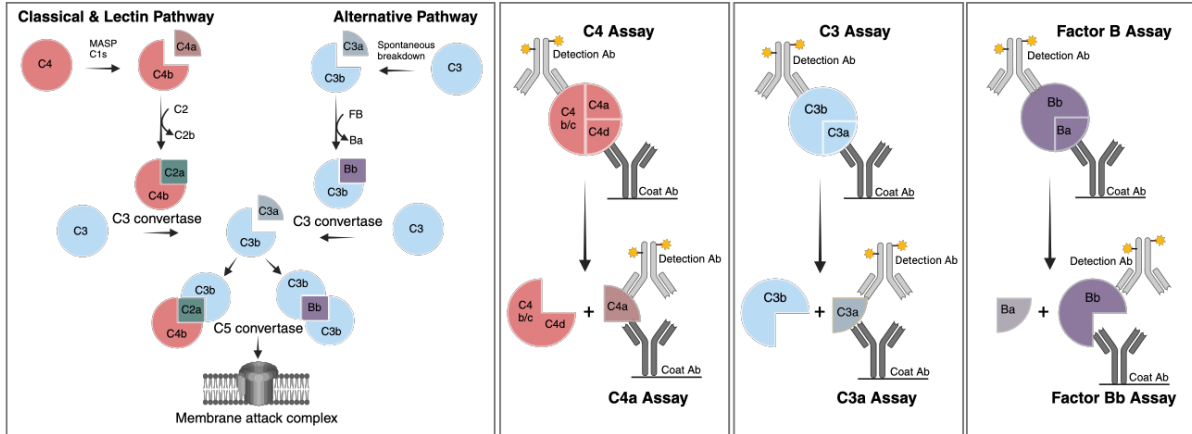
Supplementary Table 4. Percent recovery of the complement proteins from the first freeze-thaw cycle in CSF

Analyte	Additive	Mean %	Mean %	Mean %	SD	SD	SD	SE	SE	SE
		recovery FT2	recovery FT2	recovery FT4	FT2	FT3	FT4	FT2	FT3	FT4
C4a	No Addition	93	95	90	8	14	14	3	5	5
C4a	EDTA	85	85	85	9	9	13	3	3	4
C4a	EDTA+FUT	84	87	88	8	8	5	3	3	2
C4a	PIC	90	94	90	9	14	8	3	5	3
C4	No Addition	88	89	87	6	4	6	2	1	2
C4	EDTA	87	90	89	6	5	5	2	2	2
C4	EDTA+FUT	87	89	89	6	5	6	2	2	2
C4	PIC	92	90	91	9	5	5	3	2	2
C3a	No Addition	181	201	212	72	41	33	24	14	11
C3a	EDTA	129	146	164	17	26	42	6	9	14
C3a	EDTA+FUT	137	182	186	27	44	40	9	15	13
C3a	PIC	259	245	193	60	40	41	20	13	14
C3	No Addition	102	101	97	6	6	7	2	2	2
C3	EDTA	95	93	92	9	7	7	3	2	2
C3	EDTA+FUT	91	89	89	11	11	10	4	4	3
C3	PIC	111	98	92	13	9	9	4	3	3
Bb	No Addition	112	114	115	6	8	6	2	3	2
Bb	EDTA	117	106	118	12	12	7	4	4	2
Bb	EDTA+FUT	105	112	111	8	7	9	3	2	3
Bb	PIC	125	134	131	9	9	9	3	3	3
FB	No Addition	98	99	98	12	9	13	4	3	4
FB	EDTA	95	96	99	7	8	4	2	3	1
FB	EDTA+FUT	83	86	87	8	7	6	3	2	2
FB	PIC	96	98	95	11	9	8	4	3	3

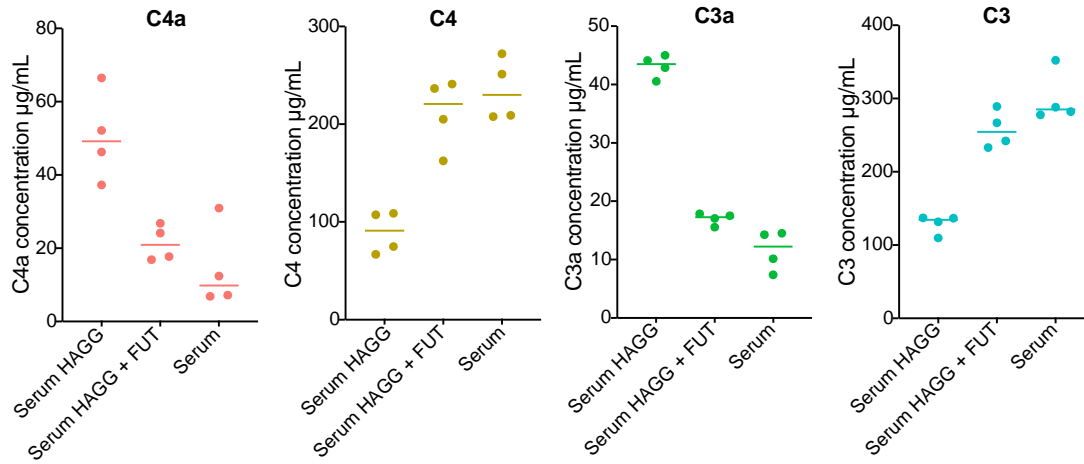
FT, Freeze thaw; SD, Standard deviation; SE, Standard error

Supplementary Figure 1. (a) Schematic of the complement pathway (left) and the C4a, C3a, Bb, C4, C3, and FB assay configuration (right). Note that the C4 assay is cross-reactive with C4 and C4b (see Table 1). Created with BioRender.com. (b) Concentrations of C4a ($\mu\text{g/mL}$), C3a ($\mu\text{g/mL}$), C4 ($\mu\text{g/mL}$) and C3 ($\mu\text{g/mL}$) in neat serum (serum), serum incubated with the complement classical pathway activator HAGG (HAGG) or serum pre-incubated with the C1s inhibitor Futhan-175 followed by HAGG (HAGG+FUT). N = 4.

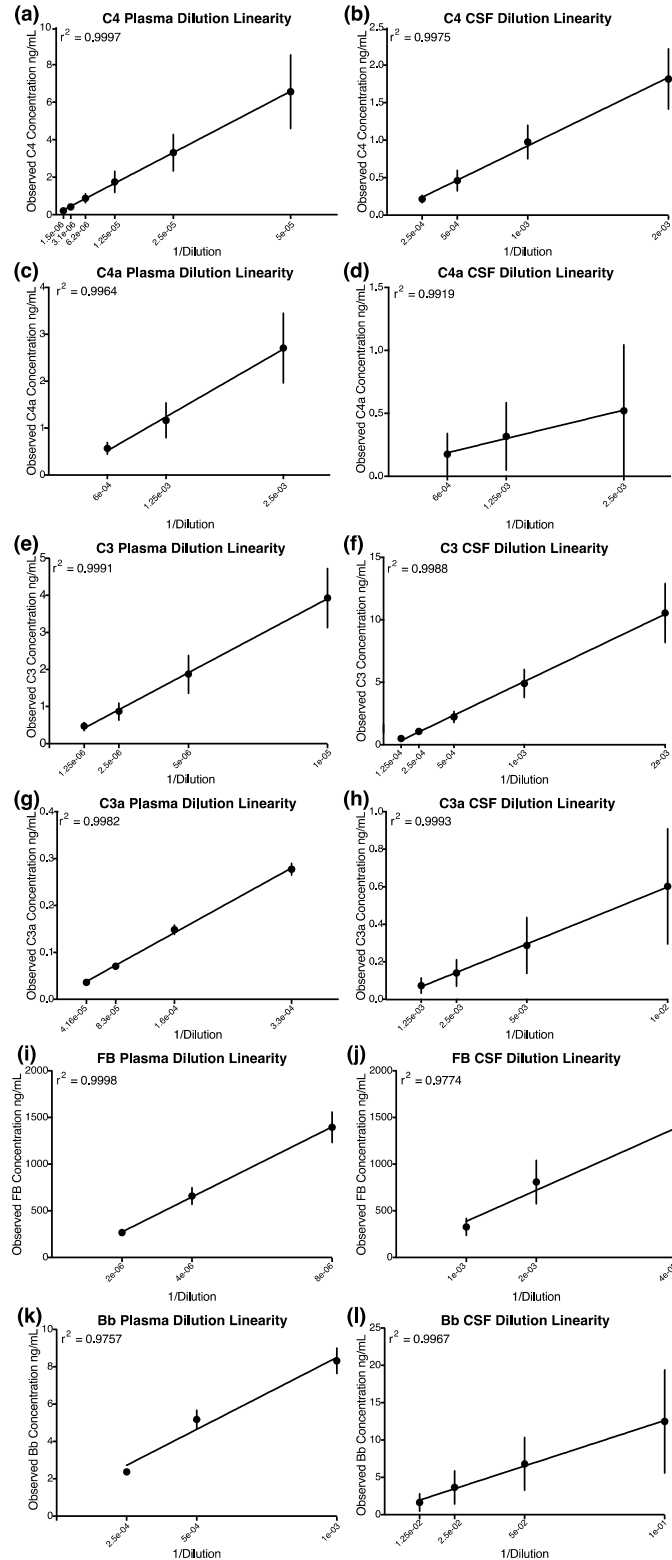
(a)



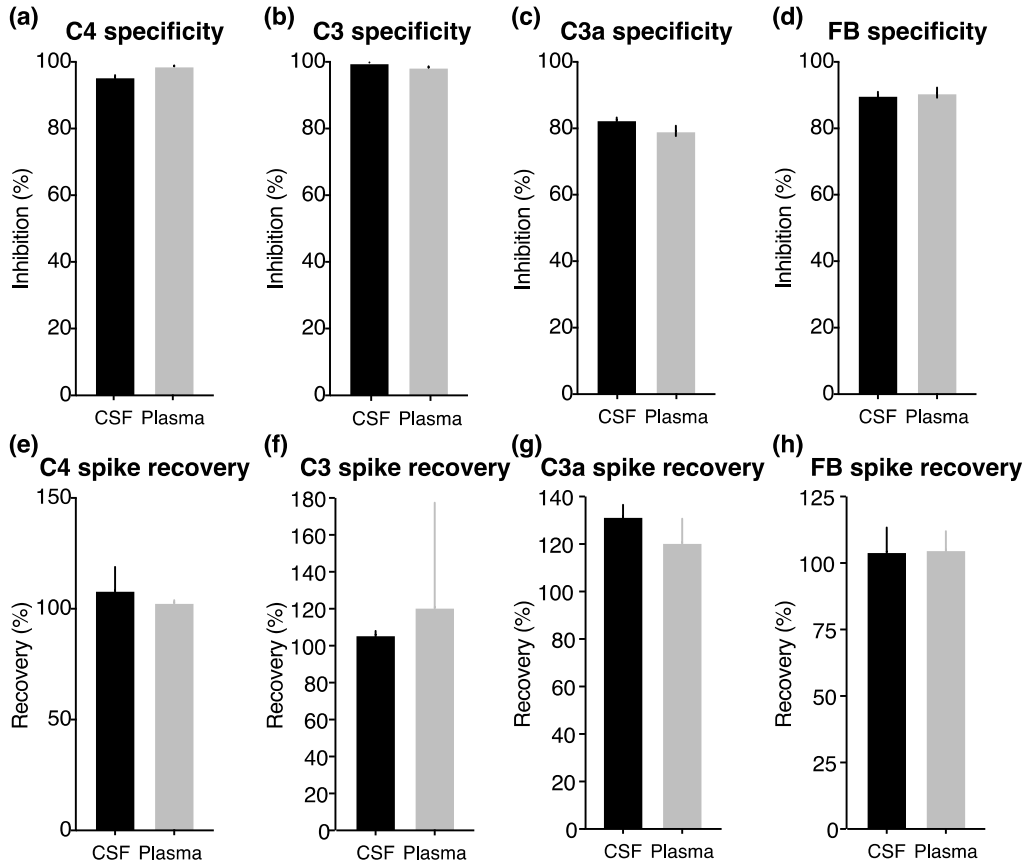
(b) Sensitivity of developed immunoassays to ex vivo CP activation and inhibition



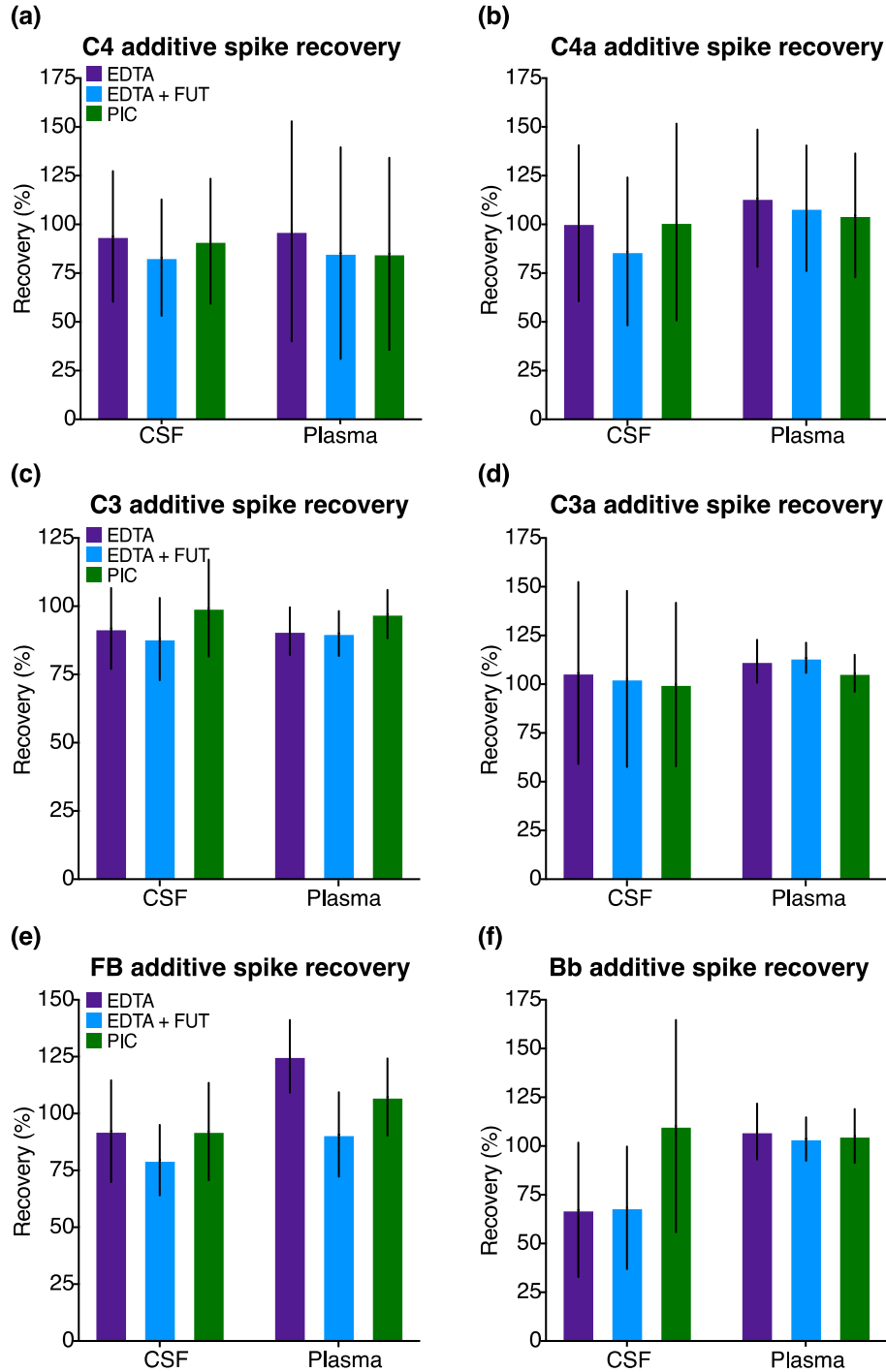
Supplementary Figure 2. (a-l) Dilution linearity shown as observed concentration of C4 (a-b), C4a (c-d), C3 (e-f), C3a (g-h), FB (i-j), Bb (k-l) over 1/Dilution factor in plasma and CSF. The Pearson correlation values are represented in the top left corner ($N=3$ per dilution). Each data point represents the mean of 3 donors \pm SD.



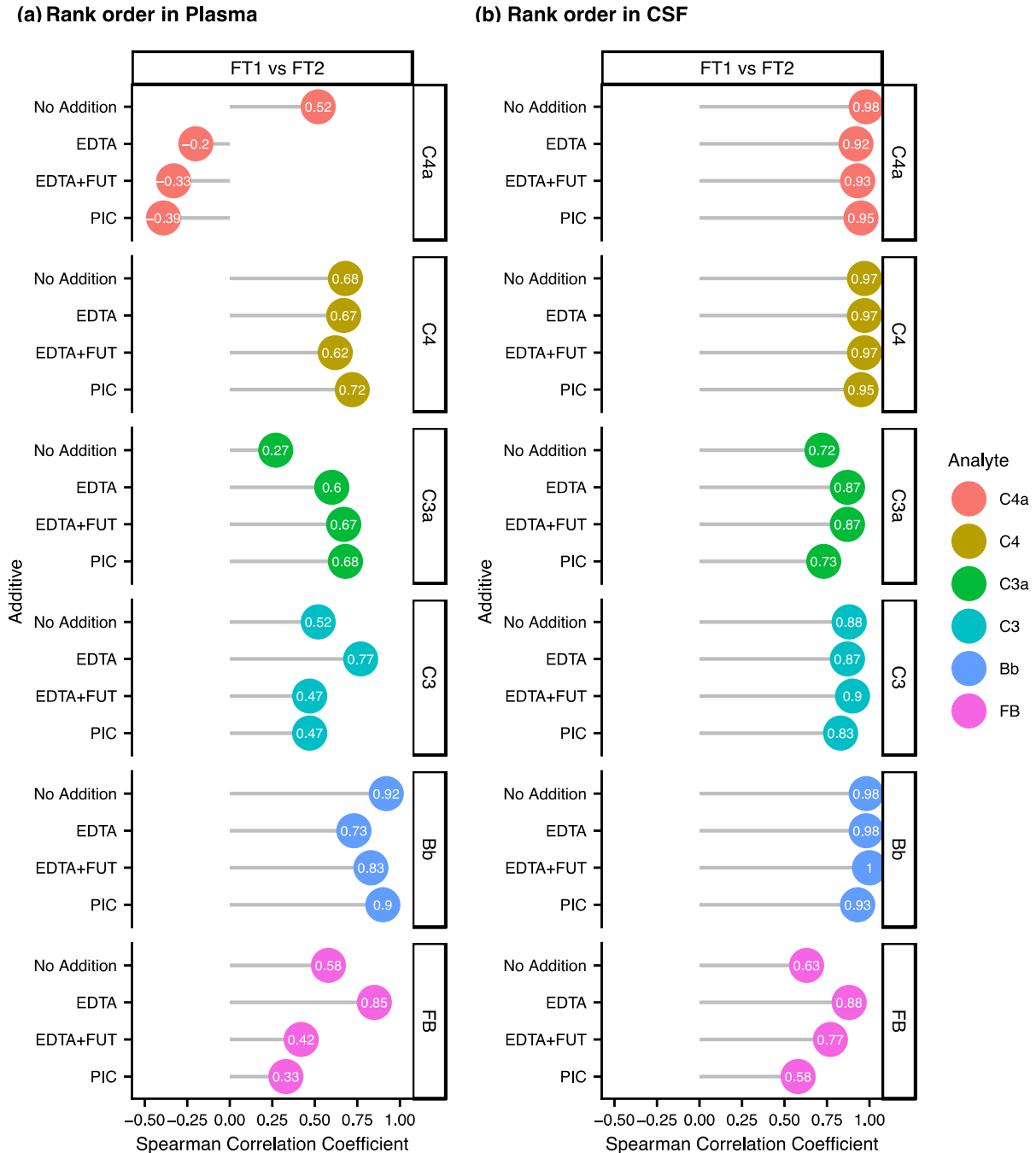
Supplementary Figure 3. (a-d) Specificity of the C4 (a), C3 (b), C3a (c) and FB (d) assays in CSF and plasma (N=3 per condition). All assays showed 80-100% inhibition upon incubation of the samples with the capture antibody. (e-h) Spike recovery of the C4 (e), C3 (f), C3a (g) and FB (h) assays in CSF and plasma. Measured recovered analyte levels were similar to expected levels based on spiked amounts (N=3 per condition). Each data point represents the mean of 3 donors \pm SD.



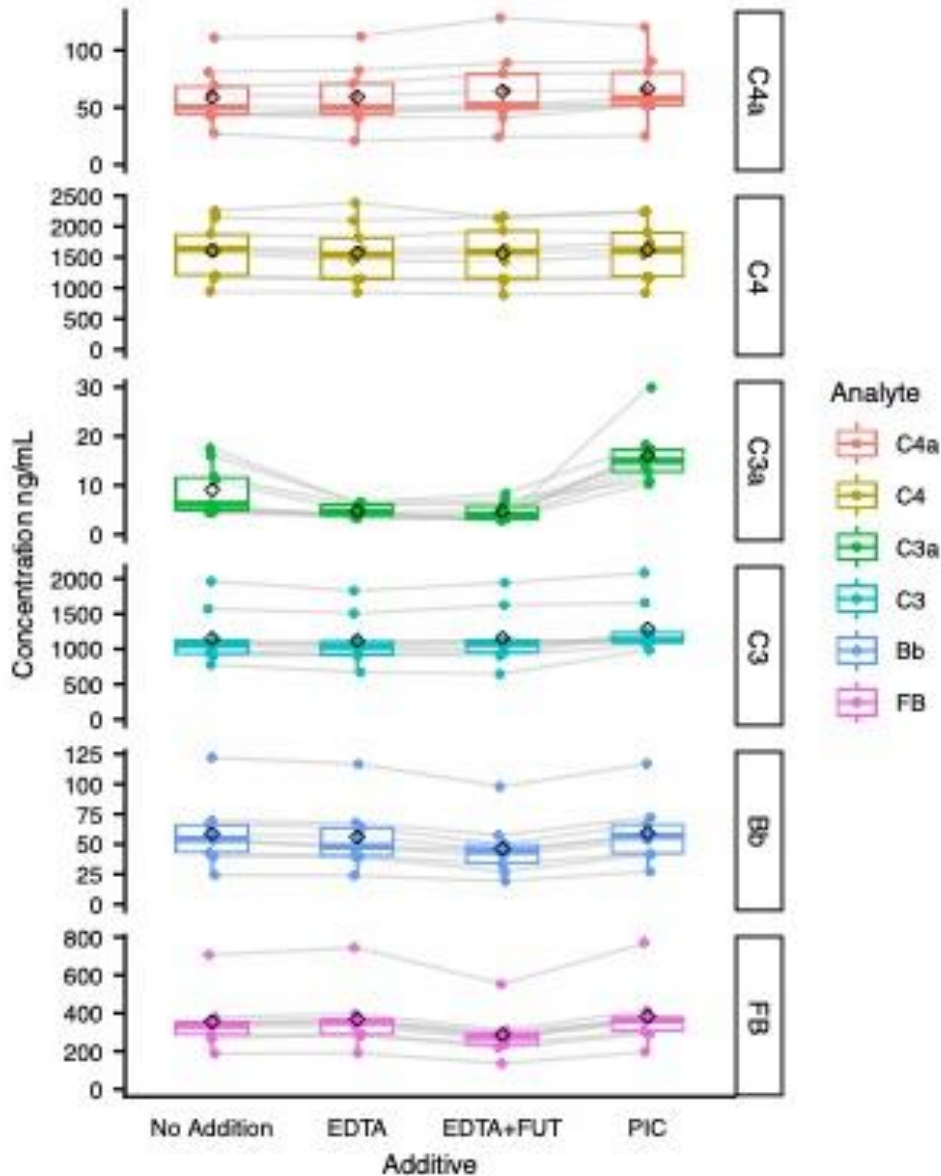
Supplementary Figure 4. (a-f) Interference of EDTA, EDTA+FUT or PIC with the C4 (a), C4a (b), C3 (c), C3a (d), FB (e) and Bb (f) assays in CSF and plasma. For most analytes, close to 100% recovery from the unsupplemented (No Addition) condition was obtained in CSF or plasma samples spiked with EDTA, EDTA+FUT or PIC. Each data point represents the mean of 3 donors \pm SEM.



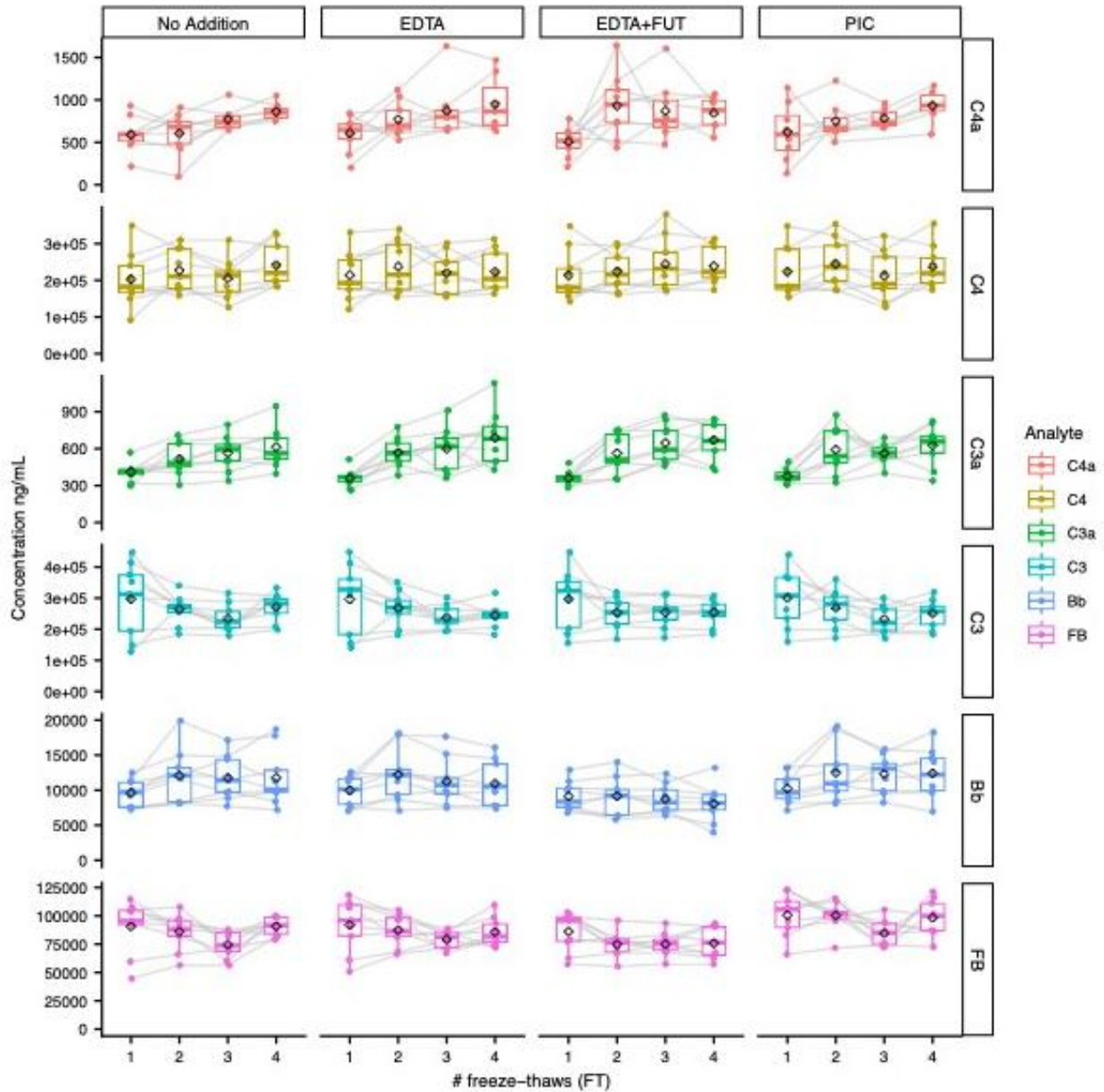
Supplementary Figure 5. Impact of the different additives on absolute complement levels in plasma. Shown are the concentrations of complement proteins C4a, C3a, Bb, C4, C3, and FB (ng/mL) in unsupplemented (No Addition) or supplemented plasma aliquots (EDTA, EDTA+FUT and PIC). Box plots shown comprise data from N=9 individual donors. Boxes represent the median and interquartile range (IQR); The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The upper whisker extends from the hinge to the largest value no further than $1.5 * IQR$ from the hinge. The lower whisker extends from the hinge to the smallest value at most $1.5 * IQR$ of the hinge. The diamond shape represents the mean. Individual data points are plotted over the box plots.



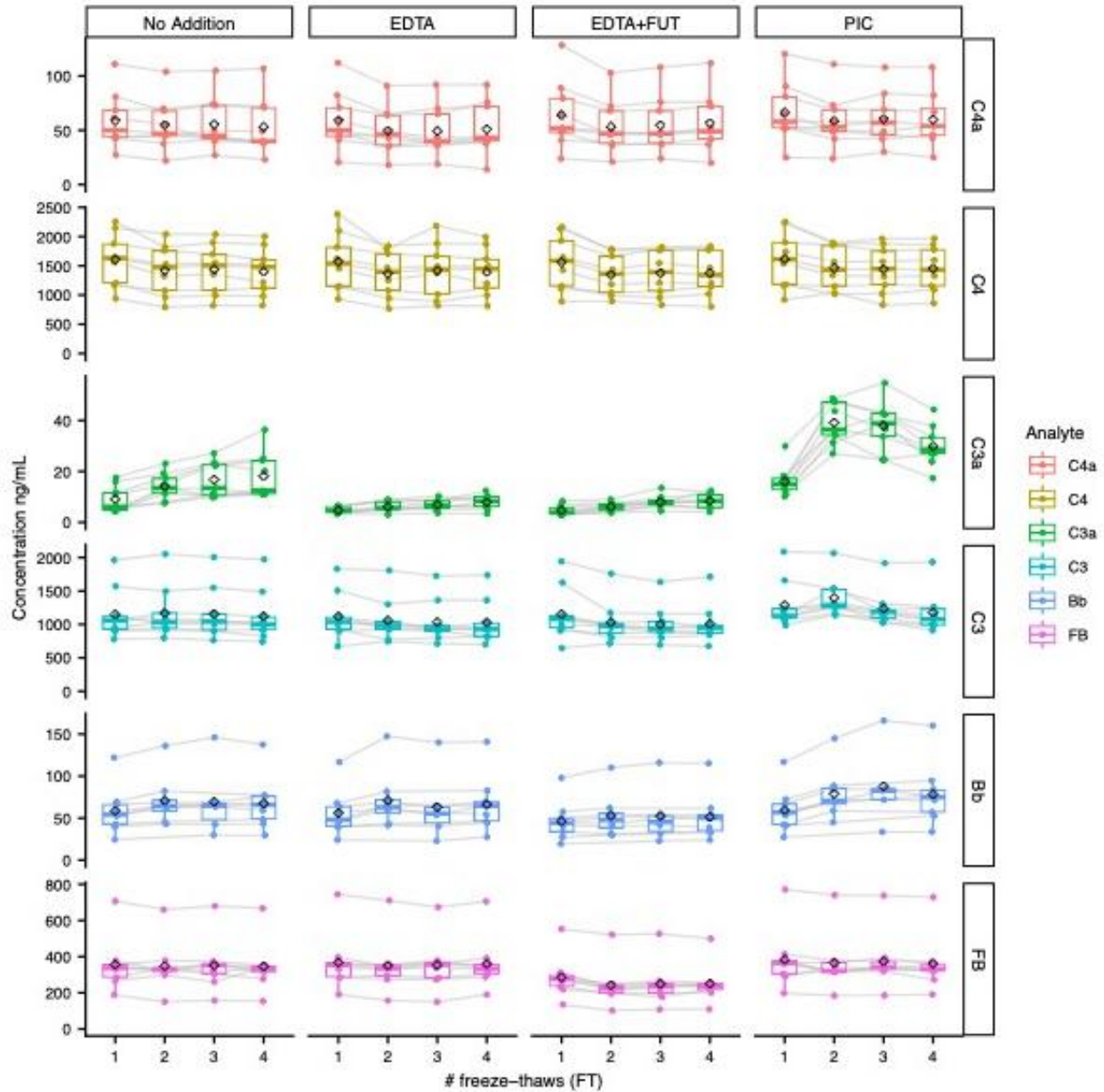
Supplementary Figure 6. Impact of the different additives on absolute complement levels in CSF. Shown are the concentrations of complement proteins C4a, C3a, Bb, C4, C3 and FB (ng/mL) in unsupplemented (No Addition) or supplemented CSF aliquots (EDTA, EDTA+FUT and PIC). Box plots shown comprise data from N=9 individual donors. Boxes represent the median and interquartile range (IQR); The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The upper whisker extends from the hinge to the largest value no further than $1.5 * IQR$ from the hinge. The lower whisker extends from the hinge to the smallest value at most $1.5 * IQR$ of the hinge. The diamond shape represents the mean. Individual data points are plotted over the box plots.



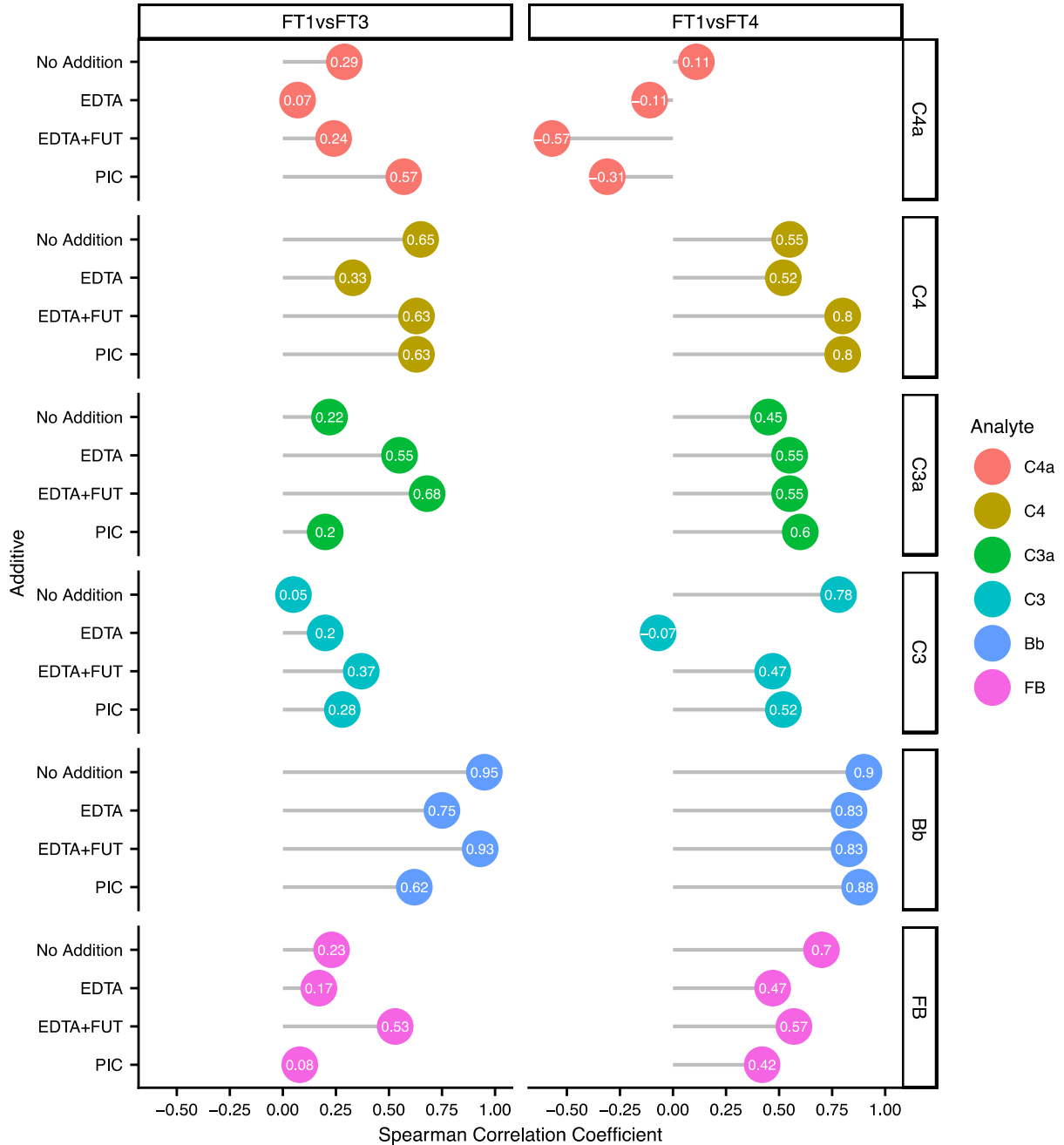
Supplementary Figure 7. Impact of the freeze-thaw cycles on absolute complement levels in plasma. Shown are the concentrations of complement proteins C4a, C3a, Bb, C4, C3, and FB (ng/mL) in unsupplemented (No Addition) or supplemented plasma aliquots (EDTA, EDTA+FUT and PIC) submitted to 1, 2, 3 or 4 FT cycles. Box plots shown comprise data from N=9 individual donors. Boxes represent the median and interquartile range (IQR); The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The upper whisker extends from the hinge to the largest value no further than $1.5 * IQR$ from the hinge. The lower whisker extends from the hinge to the smallest value at most $1.5 * IQR$ of the hinge. The diamond shape represents the mean. Individual data points are plotted over the box plots.



Supplementary Figure 8. Impact of the freeze-thaw cycles on absolute complement levels in CSF. Shown are the concentrations of C4a, C3a, Bb, C4, C3 and FB (ng/mL) in unsupplemented (No Addition) or supplemented CSF aliquots (EDTA, EDTA+FUT and PIC) submitted to 1, 2, 3 or 4 FT cycles. Box plots shown comprise data from N=9 individual donors. Boxes represent the median and interquartile range (IQR); The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The upper whisker extends from the hinge to the largest value no further than $1.5 * IQR$ from the hinge. The lower whisker extends from the hinge to the smallest value at most $1.5 * IQR$ of the hinge. The diamond shape represents the mean. Individual data points are plotted over the box plots.



Supplementary Figure 9. Rank order preservation of complement levels between the first (FT1) and third (FT3) or fourth (FT4) freeze-thaw cycle in plasma. Lollipop plot showing the Spearman Correlation Coefficient between the concentrations at FT1 and the concentrations at FT3 or FT4 in unsupplemented (No Addition) or supplemented (EDTA, EDTA+FUT, PIC) aliquots for C4a, C3a, Bb, C4, C3 and FB.



Supplementary Figure 10. Rank order preservation of complement levels between the first (FT1) and third (FT3) or fourth (FT4) freeze-thaw cycle in CSF. Lollipop plot showing the Spearman Correlation Coefficient between the concentrations at FT1 and the concentrations at FT3 or FT4 in unsupplemented (No Addition) or supplemented (EDTA, EDTA+FUT, PIC) aliquots for C4a, C3a, Bb, C4, C3 and FB.

