Supplementary figure 1. Analysis of 564 EV-TRACK knowledgebase[1] records on blood-derived EV. a. The most frequently used blood collection tubes for EV research. b. Study aims in blood-derived EV research.

Supplementary figure 2. The number of BCT and performance metrics evaluated by other BCT performance studies and this study.

Supplementary figure 3. Illustrative overview of EV separation and analysis. After blood sample collection, serum or PDP is prepared by serial centrifugation. Hematology analysis is performed on whole blood. Particle analysis (EV subtypes, lipoprotein particles (presumably chylomicrons), residual platelets) is performed by flow cytometry on processed blood (serum or PDP) without additional EV separation. For rEV recovery analysis, a crude plasma EV preparation is obtained by size-exclusion chromatography (SEC). For integrative omics analysis (LC-MS/MS and small RNAseq), EV are separated from serum or PDP by the orthogonal implementation of SEC and ODG. * rEV spike-in in whole blood samples is only performed for rEV recovery analysis.

Supplementary figure 4. Characterization of EV preparations separated from plasma using SEC (left) and SEC + ODG (right). a. NTA of EV-enriched fractions. NTA calculated size distributions are depicted as mean (black line) with standard error (red area) and total particle number, mean particle size and mode are shown for each EV-enriched fraction. b. Western blot analysis for EV- (Flotillin-1) and non-EV (Apolipoprotein A1) associated proteins. c. Transmission electron microscopy of EV-enriched fractions (scale bar: 200 nm). White arrows indicate EV and black arrows indicate lipoprotein particles. d. Technical evaluation of methodological repeatability of SEC+ODG separation of citrate plasma EV (PEV) by mass spectrometry-based proteomic analysis (LC-MS/MS) of 5 technical replicates (each replicate indicated by number). LC-MS/MS data from EV-enriched fractions are compared by correlation analysis. Data are provided as Pearson's *r* coefficients.

Supplementary figure 5. Impact of BCT type (n=10) on hematology testing of full blood samples prior to sample preparation (n=10). Data are depicted as individual values with means. The asterisk indicates a statistically significant difference compared to the reference BCT (EDTA). a. Leukocyte (10^3/µL), erythrocyte (10^6/µL) and platelet (10^3/µL) count. b. Mean cellular volume (fL).

Supplementary figure 6. Impact of BCT type (n=10) on the ratio of activated platelet EV (CD61+Lac+; RI<1.42) to total EV (RI<1.42), measured by flow cytometry (n=10). Data are depicted as individual values with means.

Supplementary figure 7. Impact of BCT type (n=10) on EV (RI<1.42) size distribution, measured by flow cytometry (n=10). Data are provided as the decay constant (Mu) of the size distribution slope. Data are depicted as individual values with means.

Supplementary figure 8. Principal component analysis of EV proteome (LC-MS/MS) data impacted by BCT type. Data points are colored according to biological replicate. Serum (red) and Roche cfDNA (pink) clusters are indicated by colored ellipses.

Supplementary figure 9. GSEA of EV proteome (LC-MS/MS) data impacted by BCT type. a. The serum EV proteome is enriched in a phenotype corresponding to platelet activation, signaling and aggregation b. The Roche cfDNA EV proteome is enriched in a phenotype corresponding to the erythrocyte membrane and cytosol. Significance threshold was set at FDR < 0.05. The heat map corresponds to the enrichment of phenotypic proteins in individual samples.

Supplementary figure 10. Principal component analysis of EV miRNA (small RNA seq) data impacted by BCT type. Data points are colored according to biological replicate. Serum (red) and Roche cfDNA (pink) clusters are indicated by colored ellipses.

Supplementary figure 11. Impact of BCT type (n=5) on hematology testing of full blood samples prior to sample preparation (n=5) after 60 min (T1), 8h (T2) and 72h (T3) (BPI evaluation). Data are depicted as individual values with means. The asterisk indicates a statistically significant difference compared to the reference BPI (T1). a. Leukocyte (10^3/µL), erythrocyte (10^6/µL) and platelet (10^3/µL) count. b. Mean cellular volume (fL). c. Residual platelet (10^3/µL) count on hematology testing after plasma preparation.

Supplementary figure 12. GSEA of EV proteome (LC-MS/MS) data (BPI evaluation). a. The EV proteome at T3 is enriched in a phenotype corresponding to platelet activation, signaling and aggregation compared to T1. b. The EV proteome at T3 is enriched in a phenotype corresponding to the erythrocyte membrane and cytosol compared to T1. Significance threshold was set at FDR < 0.05. The heat map corresponds to the enrichment of phenotypic proteins in individual samples.

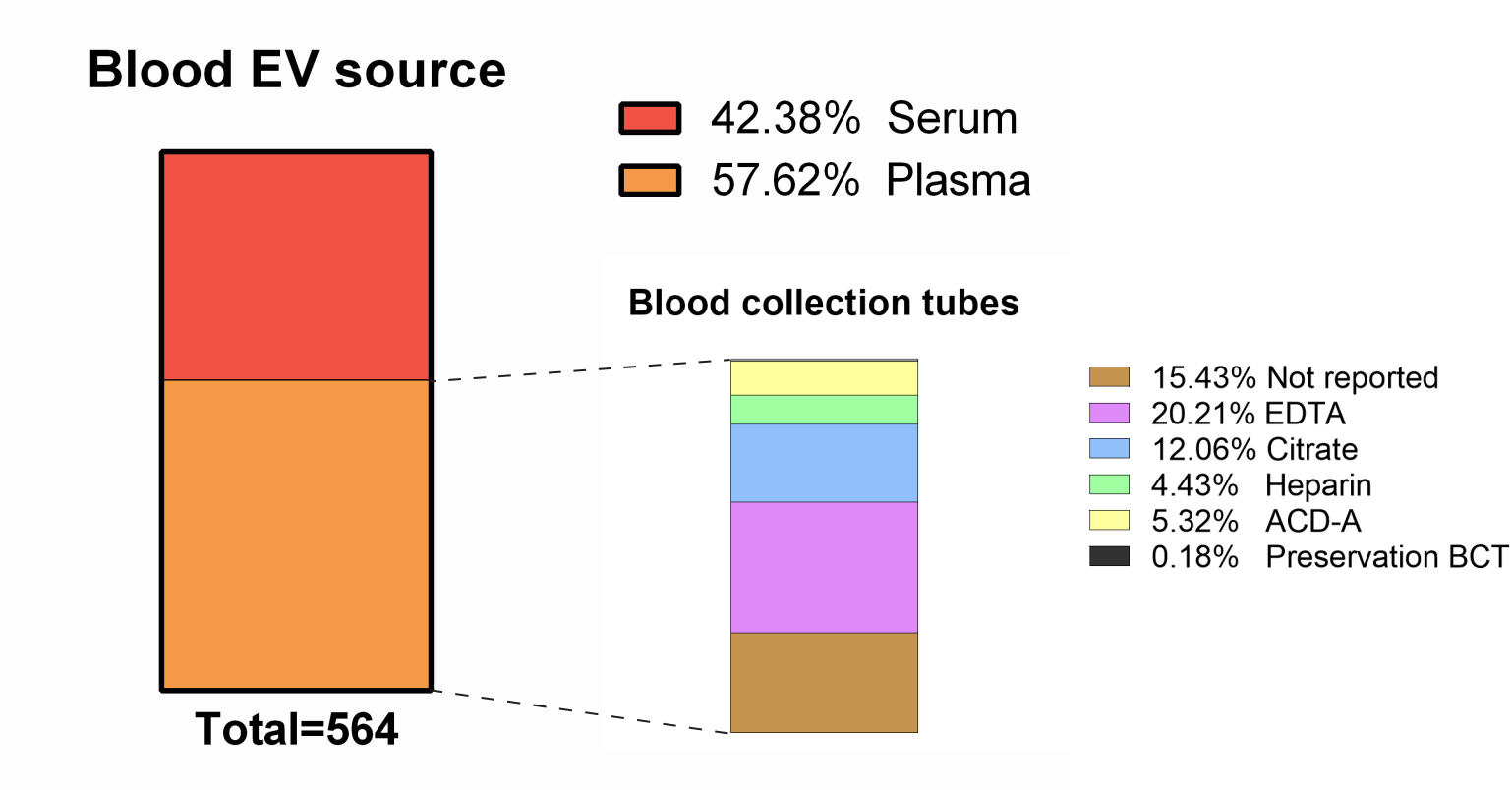
Supplementary figure 13. Impact of BCT type (n=5) on the ratio of a. Activated platelet EV (CD61+Lac+; RI<1.42) to total EV (RI<1.42), b. Non-platelet EV (CD61-Lac+; RI<1.42) to total EV (RI<1.42) and c. Erythrocyte EV (CD235+; RI<1.42) to total EV (RI<1.42), measured by flow cytometry (n=5) in plasma 60 min (T1), 8h (T2) and 72h (T3) following sample collection (BPI evaluation). Data are depicted as individual values with means.

Supplementary figure 14. Impact of BCT type (n=5) on the EV proteome, analyzed by LC-MS/MS (n=3) in EV-enriched fractions prepared from plasma 60 min (T1), 8h (T2) and 72h (T3) following sample collection (BPI evaluation). The EV proteome at T2 and T3 is compared to the EV proteome at T1 by correlation analysis. Data are provided as Pearson's *r* coefficients and are depicted as individual values with means.

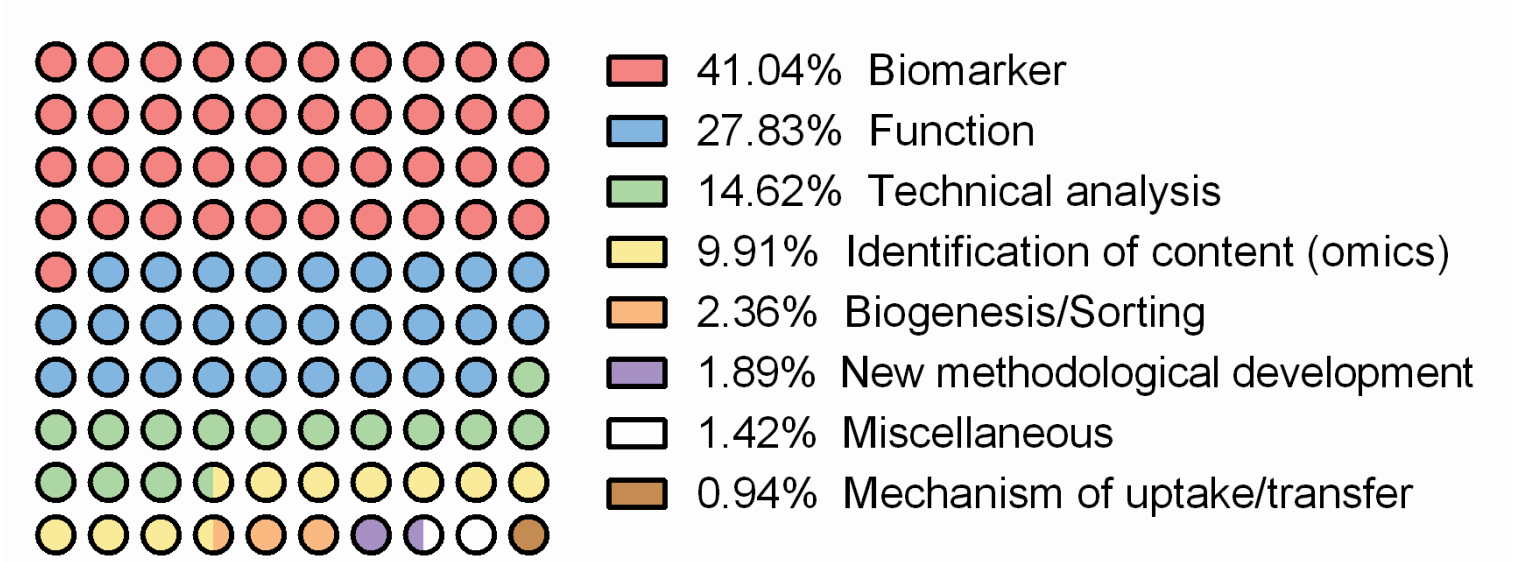
Supplementary figure 15. Impact of BCT type (n=5) on the EV miRNA profile, analyzed by small RNAseq (n=3) in EV-enriched fractions prepared from plasma 60 min (T1), 8h (T2) and 72h (T3) following sample collection (BPI evaluation). The EV miRNA profile at T2 and T3 is compared to the EV miRNA profile at T1 by correlation analysis. Data are provided as Pearson's *r* coefficients and are depicted as individual values with means.

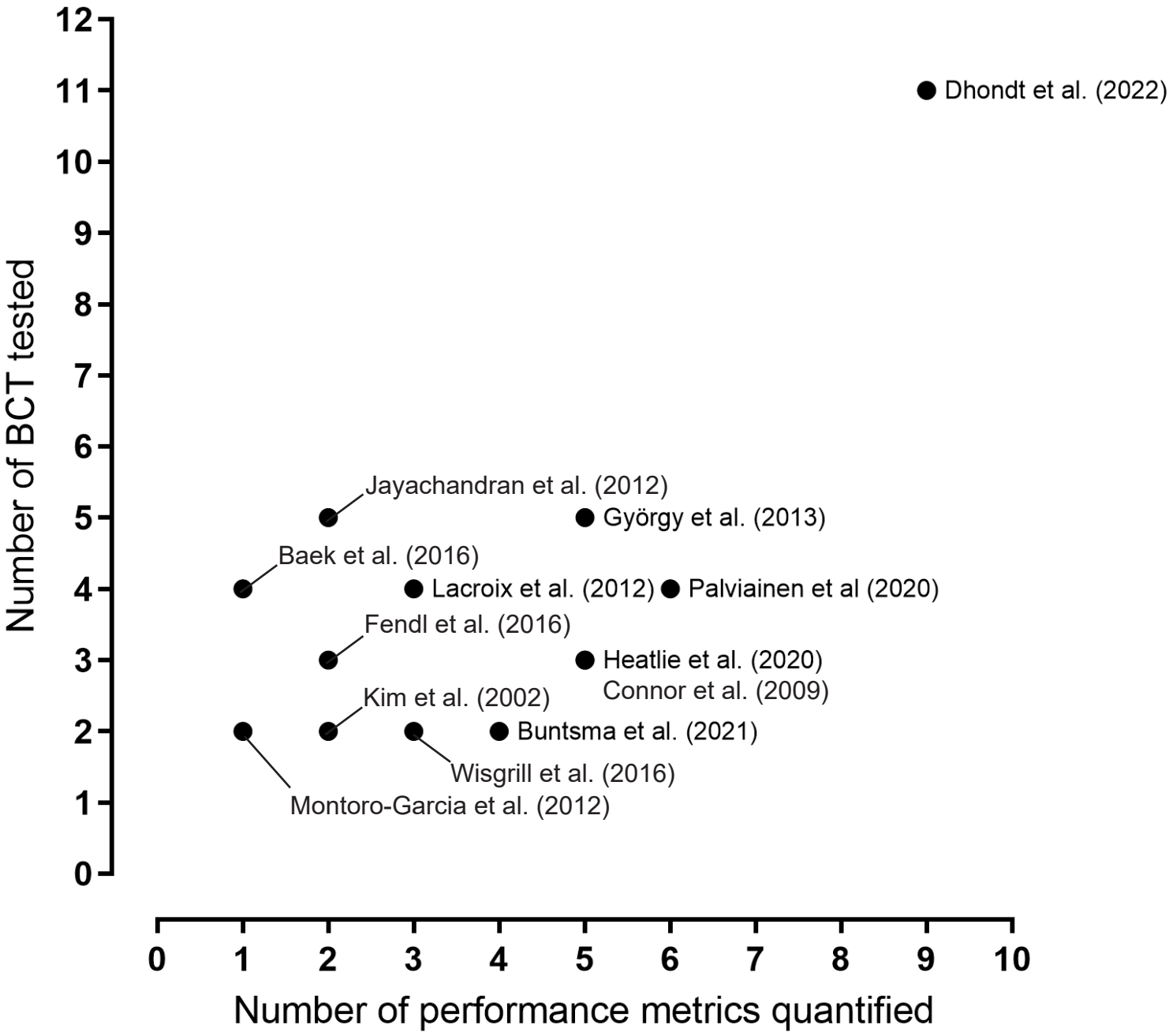
Supplementary figure 16. Impact of BCT type (n=5) on the volume of plasma 60 min (T1), 8h (T2) and 72h (T3) following sample collection (BPI evaluation).

a. Blood EV research: sample types

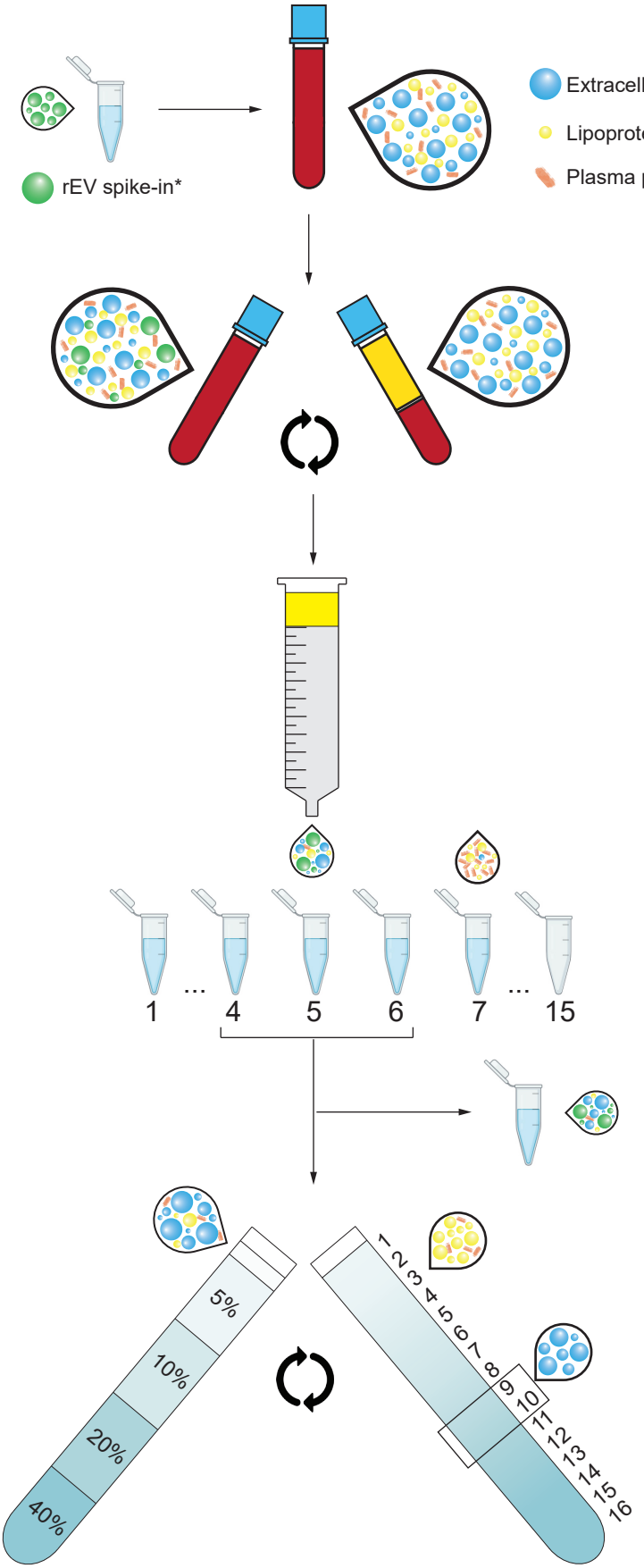


b. Blood EV research: study aim




Number of BCT tested
0
1
2
3
4
5
6
7
8
9
10
11
12
Number of performance metrics quantified
0
1
2
3
4
5
6
7
8
9
10
Dhondt et al. (2022)
Jayachandran et al. (2012)
György et al. (2013)
Baek et al. (2016)
Lacroix et al. (2012)
Palviainen et al (2020)
Fendl et al. (2016)
Heatlie et al. (2020)
Connor et al. (2009)
Kim et al. (2002)
Buntsma et al. (2021)
Wisgrill et al. (2016)
Montoro-Garcia et al. (2012)

EV separation



Blood analysis

Whole blood

Hematology analysis
Leukocytes
Erythrocytes
Platelets
Mean cellular volume

Processed blood

ELISA
Hemolysis (Hb)
Platelet activation (BTG and PF-4)

Flow cytometry
EV subtypes
Residual platelets
Lipoprotein particles

EV-enriched SEC fractions

Fluorescent NTA
rEV recovery

EV-enriched ODG fractions

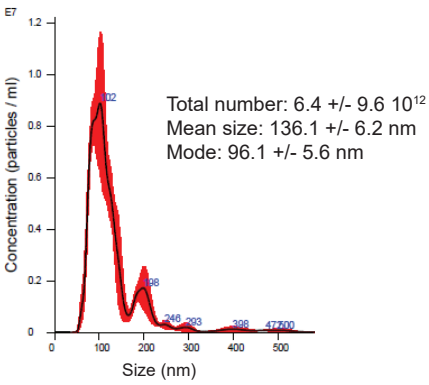
Transmission electron microscopy
EV ultrastructure

LC-MS/MS and small RNAseq
Molecular signatures

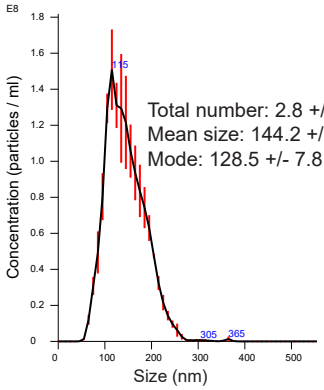
SEC

SEC + ODG

a. Nanoparticle tracking analysis

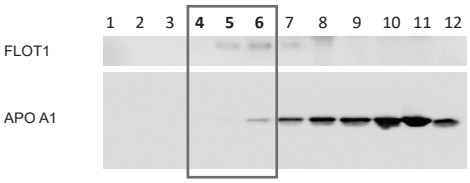


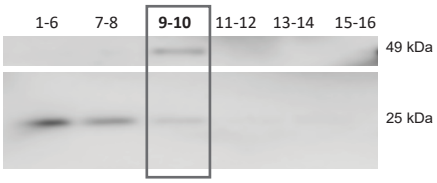
Total number: 6.4 +/- 9.6 10^{12}
Mean size: 136.1 +/- 6.2 nm
Mode: 96.1 +/- 5.6 nm



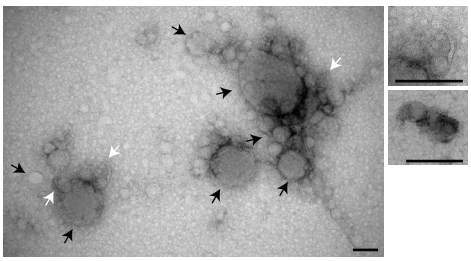
Total number: 2.8 +/- 0.7 10^{10}
Mean size: 144.2 +/- 2.0 nm
Mode: 128.5 +/- 7.8 nm

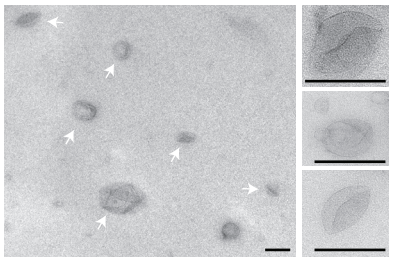
b. Western blot analysis





c. Electron microscopy

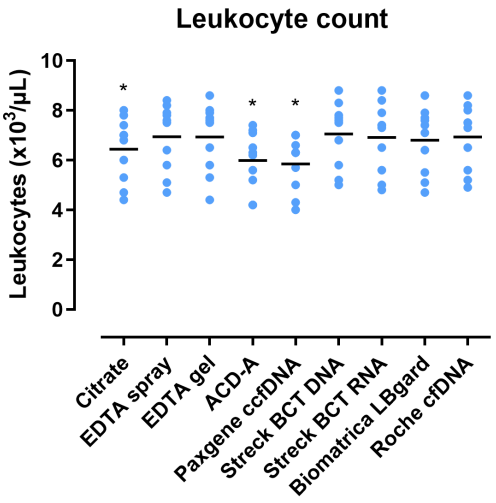


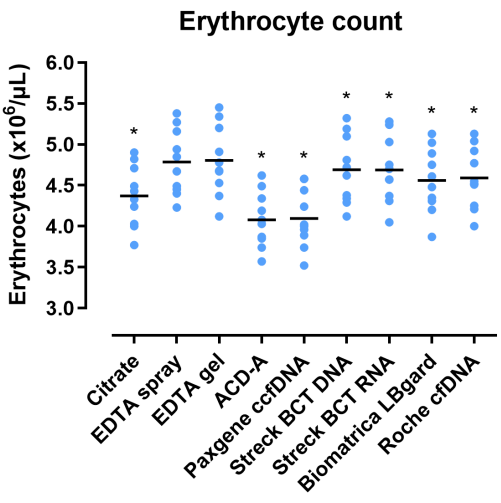


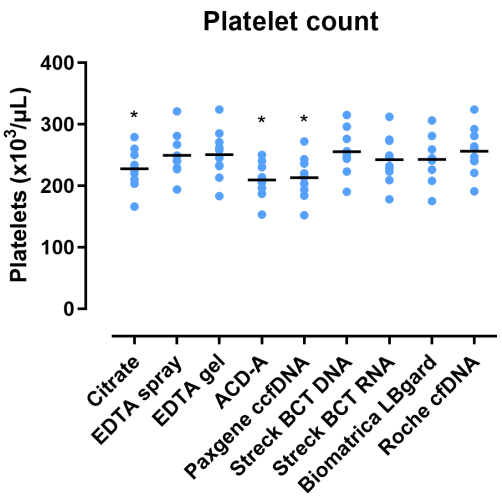
d. Repeatability

	PEV 1	PEV 2	PEV 3	PEV 4	PEV 5
PEV 1	1.00	0.99	0.97	0.98	0.96
PEV 2	0.99	1.00	0.96	0.98	0.97
PEV 3	0.97	0.96	1.00	0.96	0.95
PEV 4	0.98	0.98	0.96	1.00	0.95
PEV 5	0.96	0.97	0.95	0.95	1.00

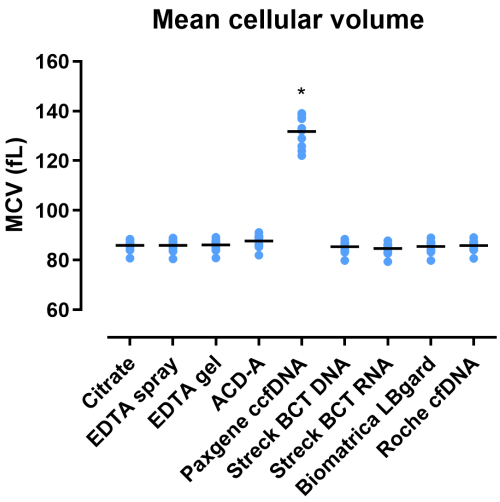
a

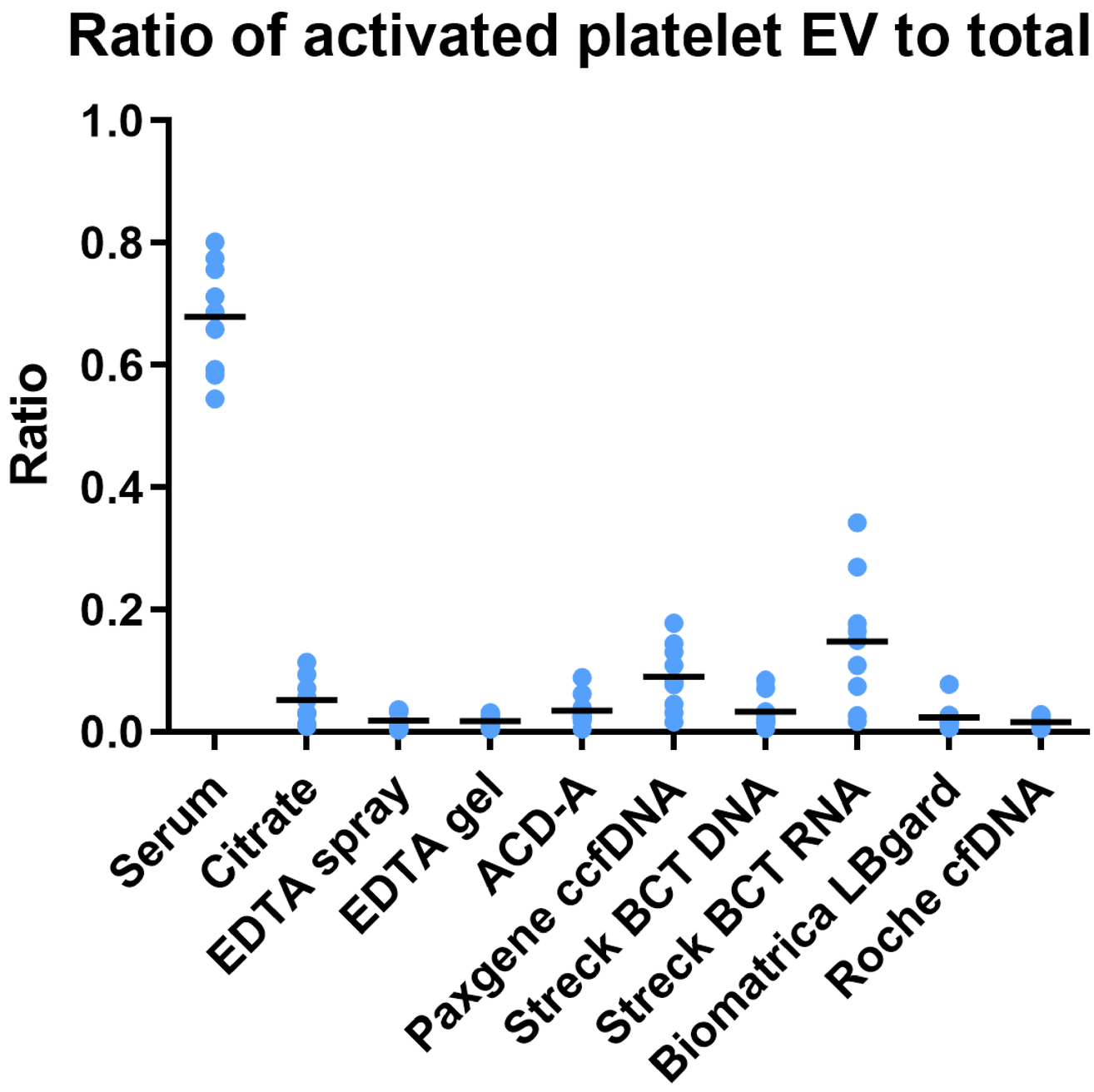

Leukocyte count
Leukocytes (x10^3/µL)
10
8
6
4
2
0
Citrate
EDTA spray
EDTA gel
ACD-A
Paxgene ccfDNA
Streck BCT DNA
Streck BCT RNA
Biomatrica LBgard
Roche cfDNA

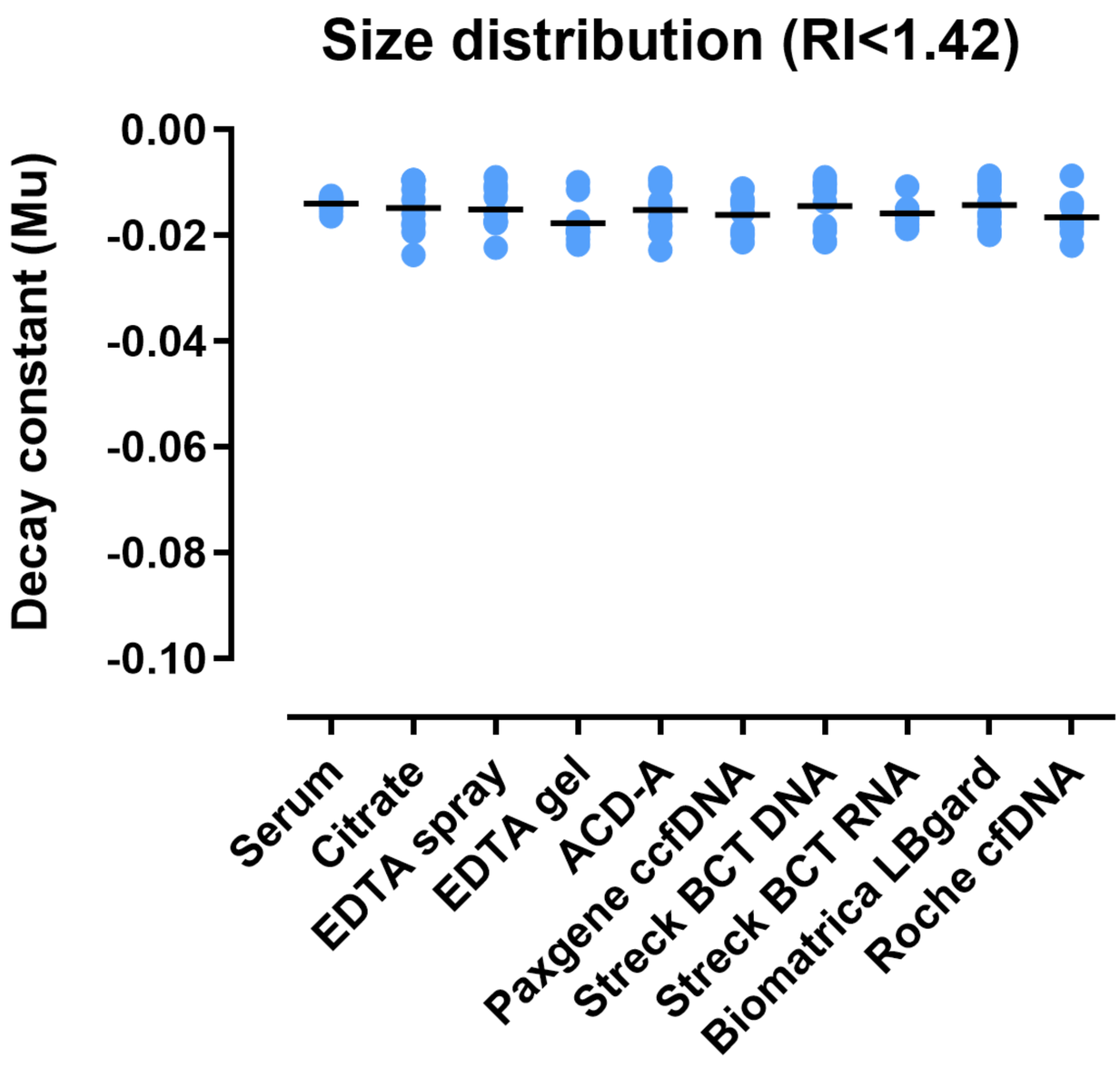

Erythrocyte count
Erythrocytes (x10^6/µL)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Citrate
EDTA spray
EDTA gel
ACD-A
Paxgene ccfDNA
Streck BCT DNA
Streck BCT RNA
Biomatrica LBgard
Roche cfDNA

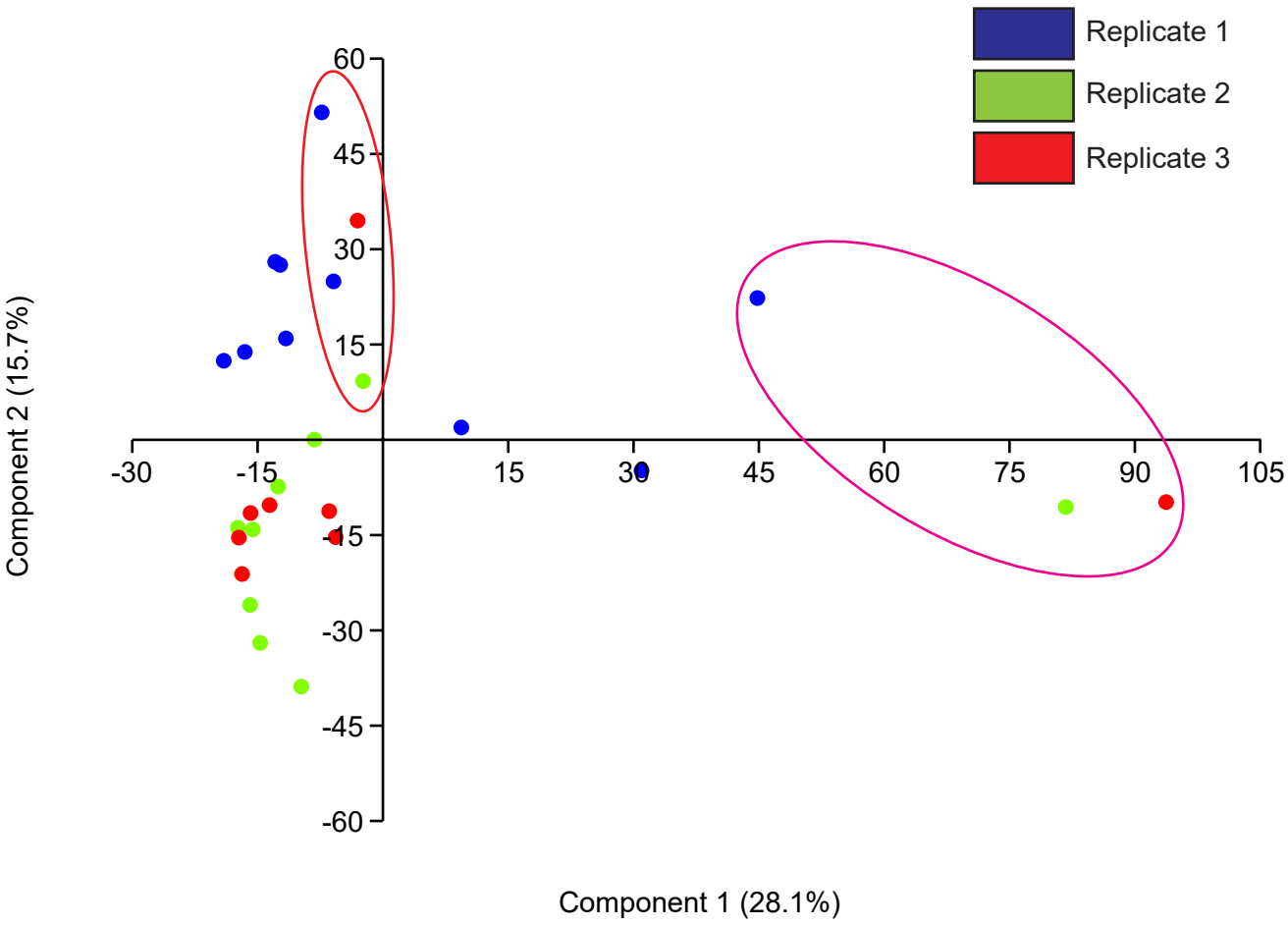

Platelet count
Platelets (x10^3/µL)
400
300
200
100
0
Citrate
EDTA spray
EDTA gel
ACD-A
Paxgene ccfDNA
Streck BCT DNA
Streck BCT RNA
Biomatrica LBgard
Roche cfDNA

b


Mean cellular volume
MCV (fL)
160
140
120
100
80
60
Citrate
EDTA spray
EDTA gel
ACD-A
Paxgene ccfDNA
Streck BCT DNA
Streck BCT RNA
Biomatrica LBgard
Roche cfDNA

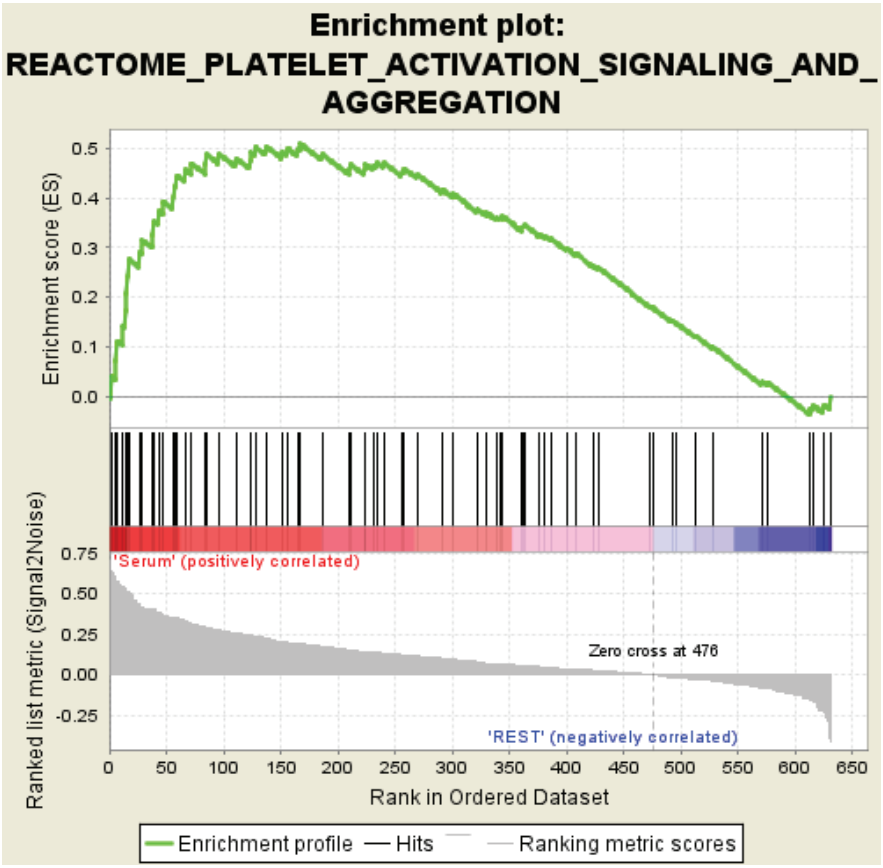

Ratio of activated platelet EV to total EV
Ratio
1.0
0.8
0.6
0.4
0.2
0.0
Serum
Citrate
EDTA spray
EDTA gel
ACD-A
Paxgene ccfDNA
Streck BCT DNA
Streck BCT RNA
Biomatrica LBgard
Roche cfDNA

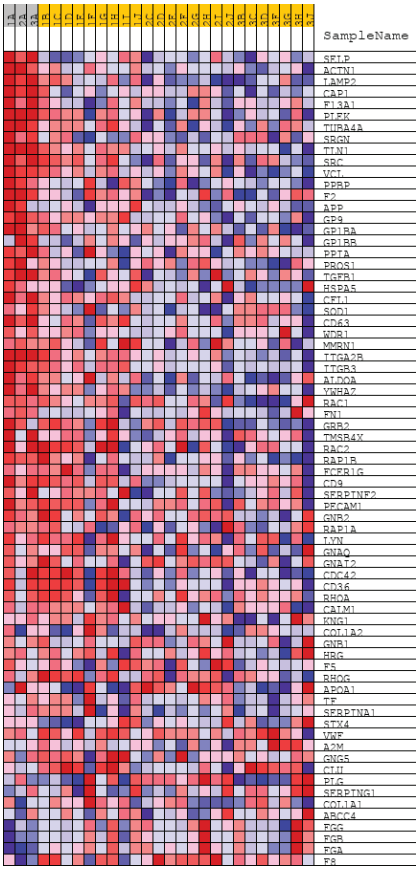

Size distribution (RI<1.42)
Decay constant (Mu)
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
Serum
Citrate
EDTA spray
EDTA gel
ACD-A
Paxgene ccfDNA
Streck BCT DNA
Streck BCT RNA
Biomatrica LBgard
Roche cfDNA


Replicate 1
Replicate 2
Replicate 3
60
45
30
15
-15
-30
-45
-60
-30
-15
15
30
45
60
75
90
105
Component 2 (15.7%)
Component 1 (28.1%)

a

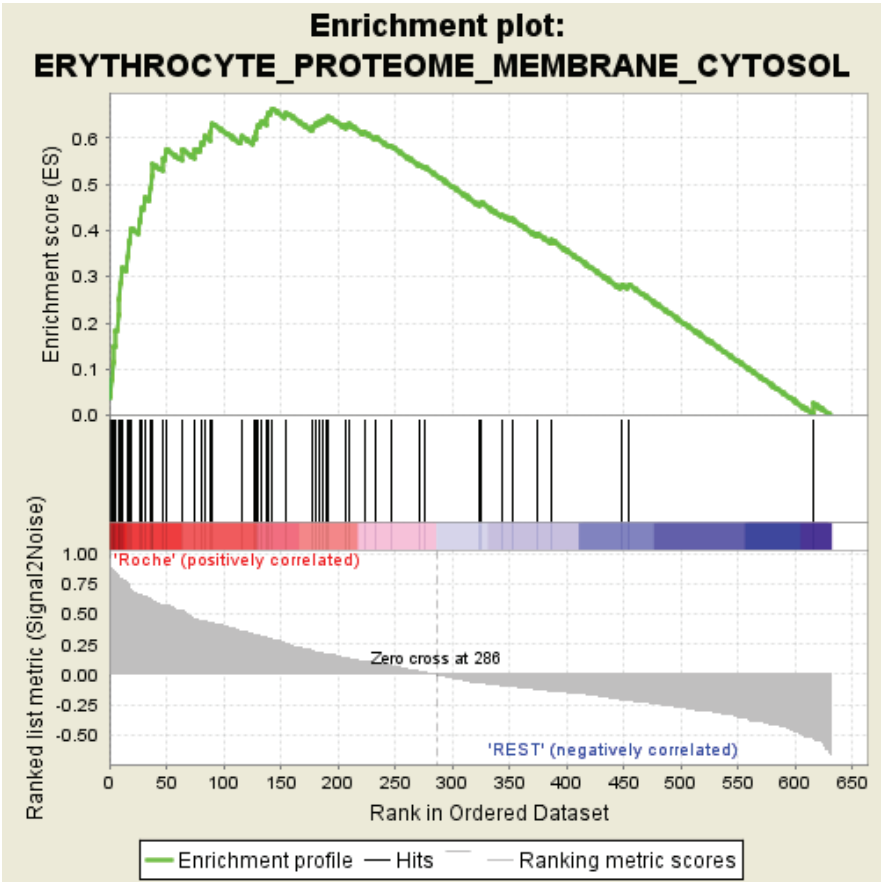
GSEA Serum

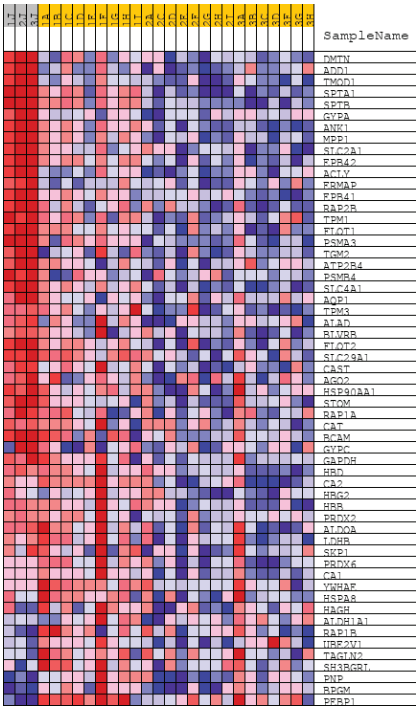


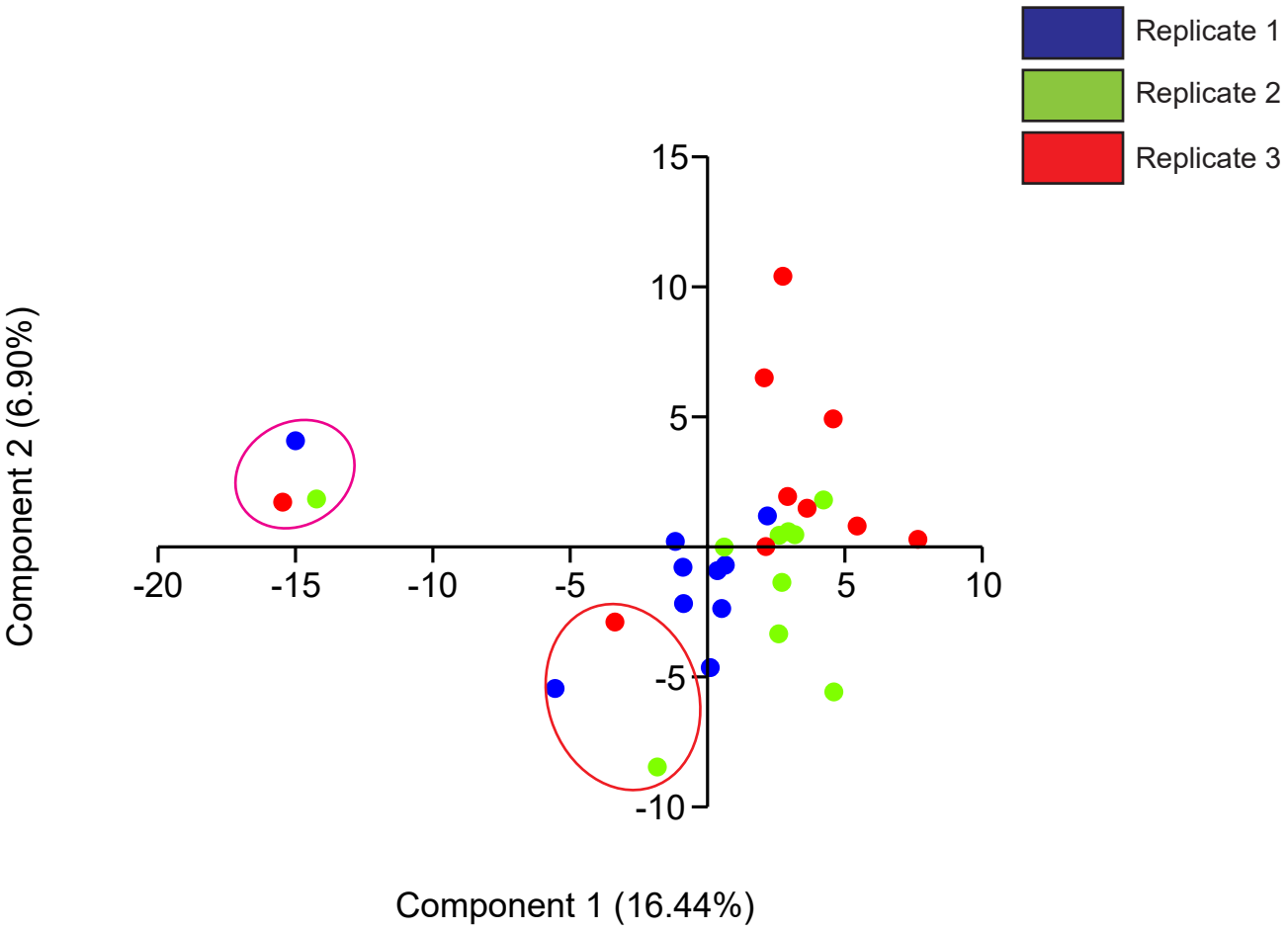


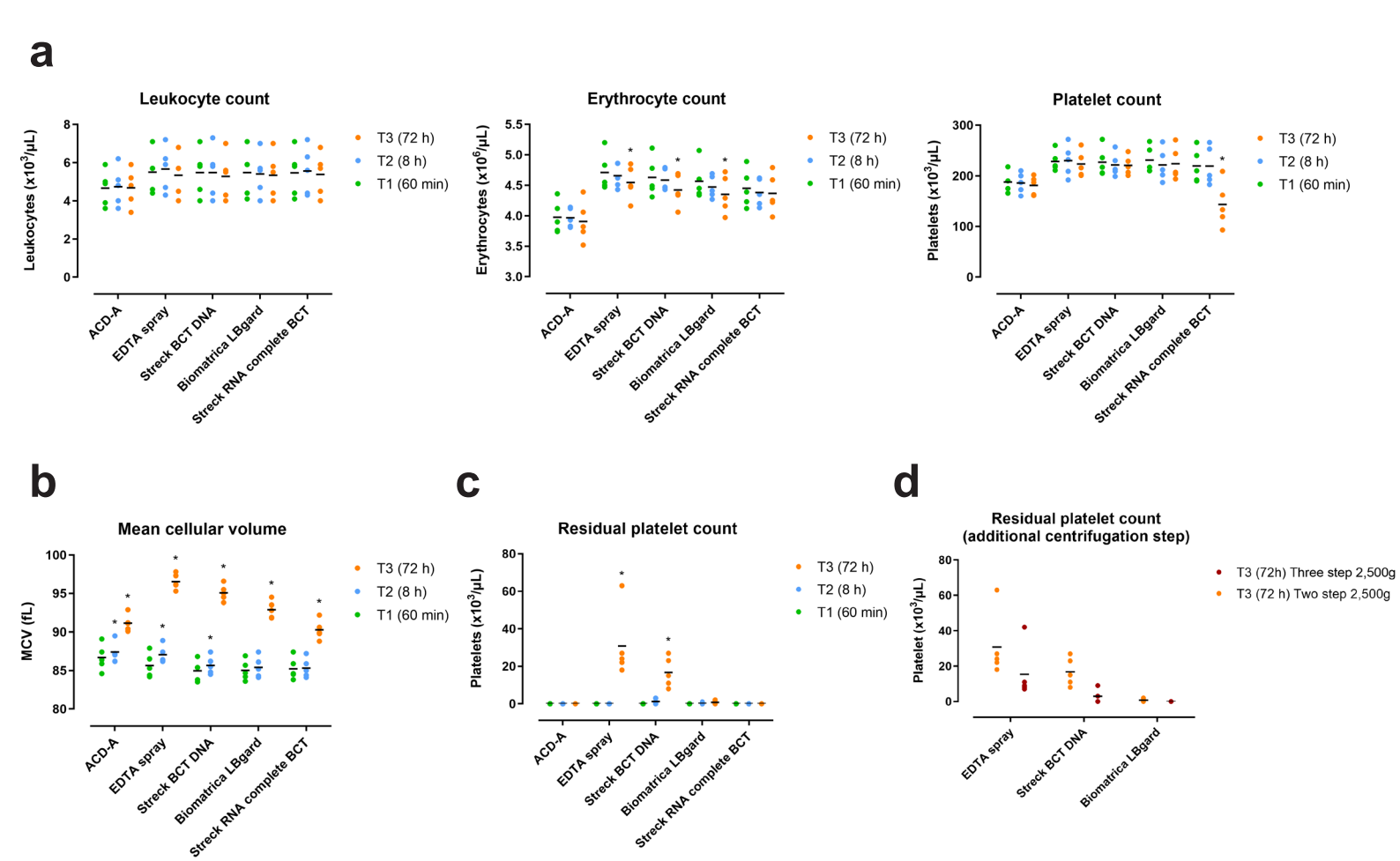
b

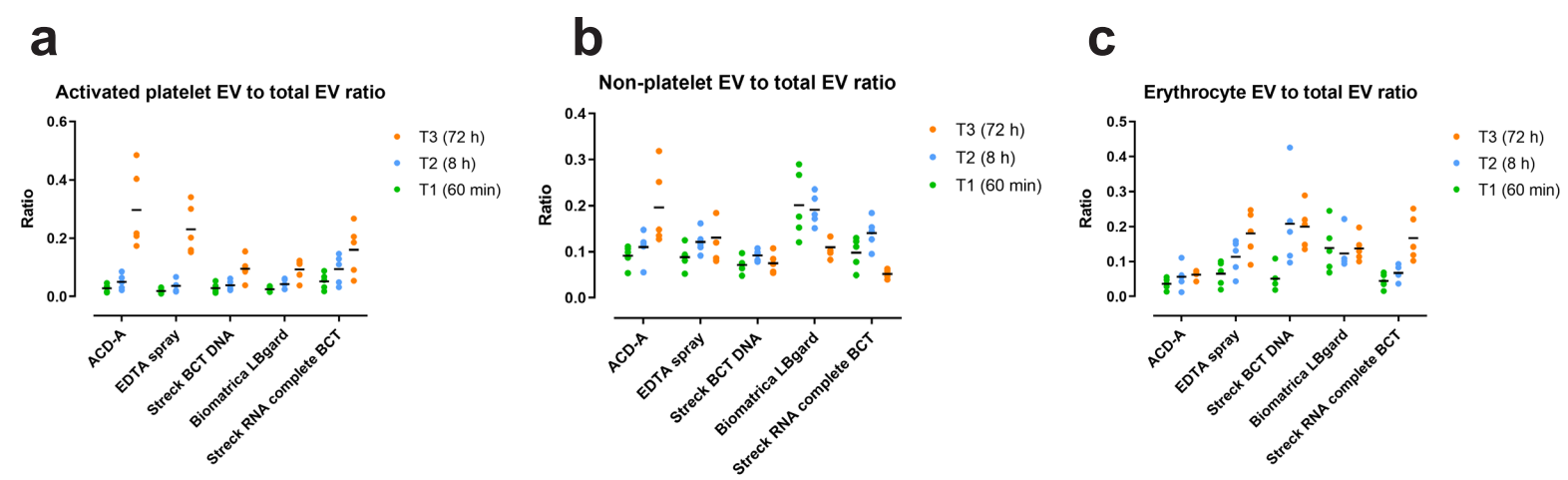
GSEA Roche cfDNA





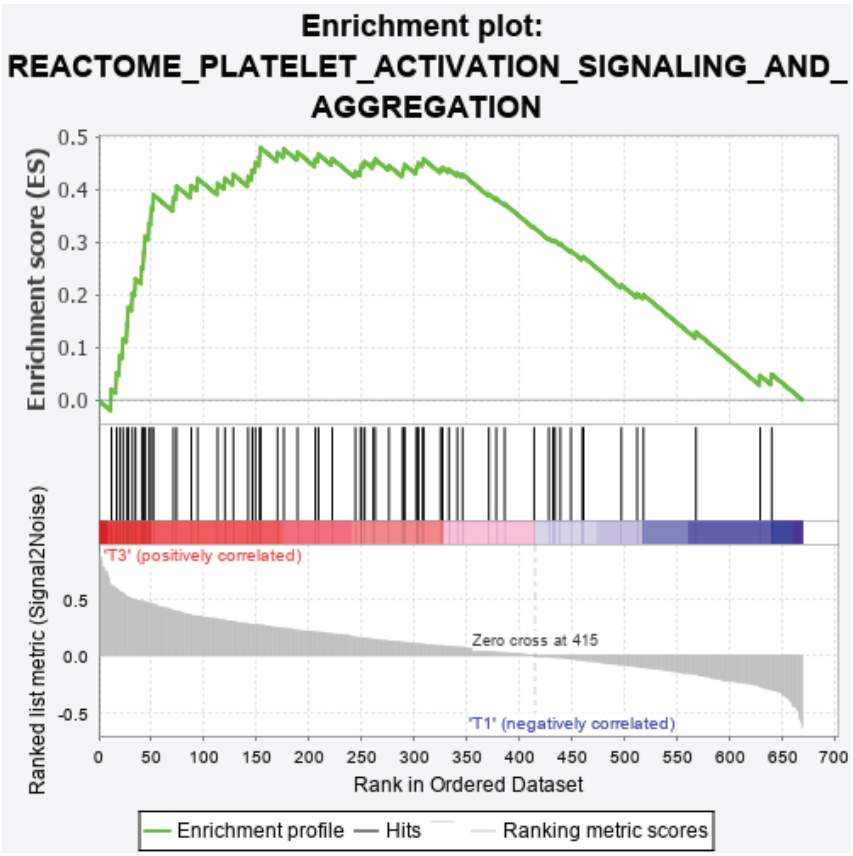

Replicate 1
Replicate 2
Replicate 3
15
10
5
-5
-10
-20
-15
-10
-5
5
10
Component 2 (6.90%)
Component 1 (16.44%)

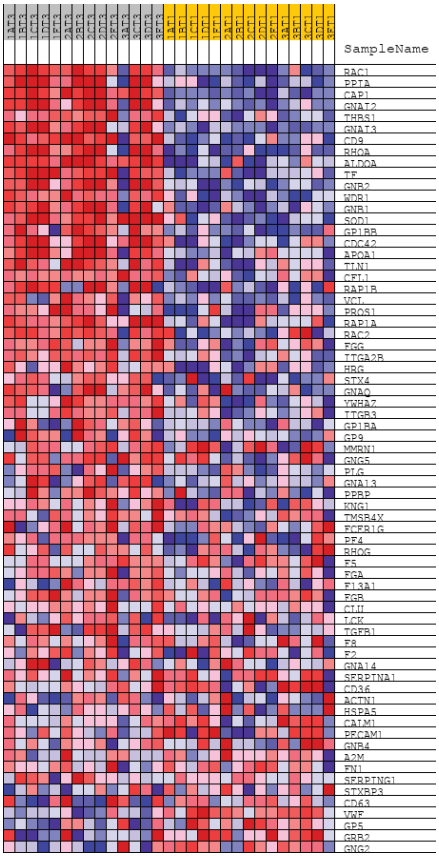

a
Leukocyte count
Leukocytes (x10³/µL)
Erythrocyte count
Erythrocytes (x10⁶/µL)
Platelet count
Platelets (x10³/µL)
T3 (72 h)
T2 (8 h)
T1 (60 min)
ACD-A
EDTA spray
Streck BCT DNA
Biomatrica LBgard
Streck RNA complete BCT
b
Mean cellular volume
MCV (fL)
c
Residual platelet count
Platelets (x10³/µL)
d
Residual platelet count
(additional centrifugation step)
Platelet (x10³/µL)
T3 (72h) Three step 2,500g
T3 (72 h) Two step 2,500g


a
Activated platelet EV to total EV ratio
Ratio
0.6
0.4
0.2
0.0
T3 (72 h)
T2 (8 h)
T1 (60 min)
ACD-A
EDTA spray
Streck BCT DNA
Biomatrica LBgard
Streck RNA complete BCT
b
Non-platelet EV to total EV ratio
Ratio
0.4
0.3
0.2
0.1
0.0
T3 (72 h)
T2 (8 h)
T1 (60 min)
ACD-A
EDTA spray
Streck BCT DNA
Biomatrica LBgard
Streck RNA complete BCT
c
Erythrocyte EV to total EV ratio
Ratio
0.5
0.4
0.3
0.2
0.1
0.0
T3 (72 h)
T2 (8 h)
T1 (60 min)
ACD-A
EDTA spray
Streck BCT DNA
Biomatrica LBgard
Streck RNA complete BCT

a

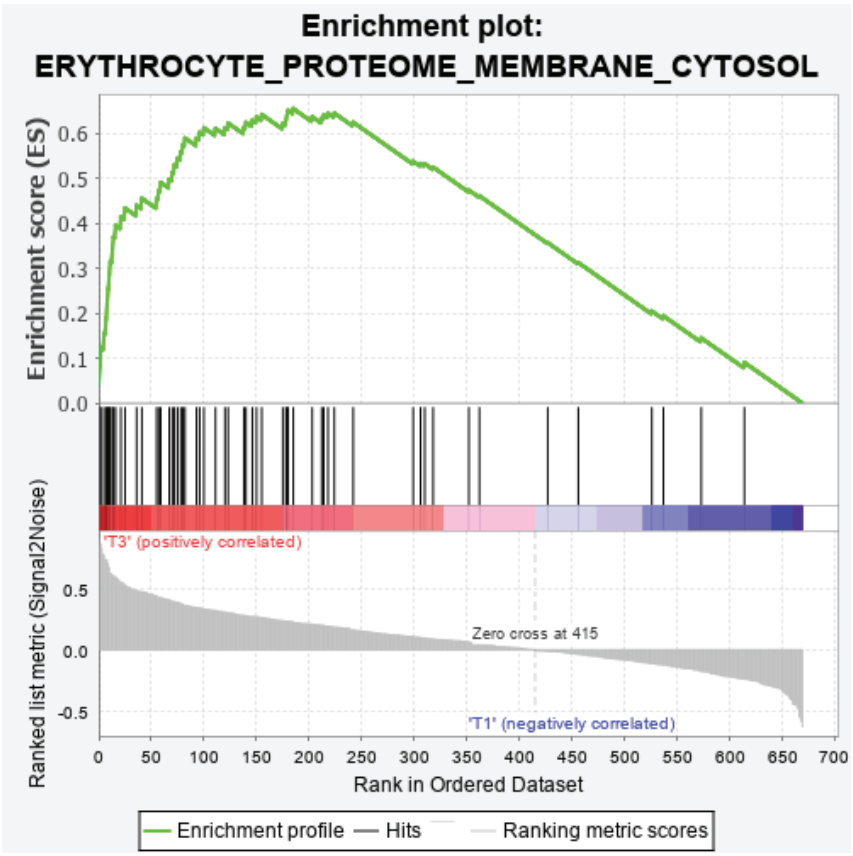
GSEA T3-T1

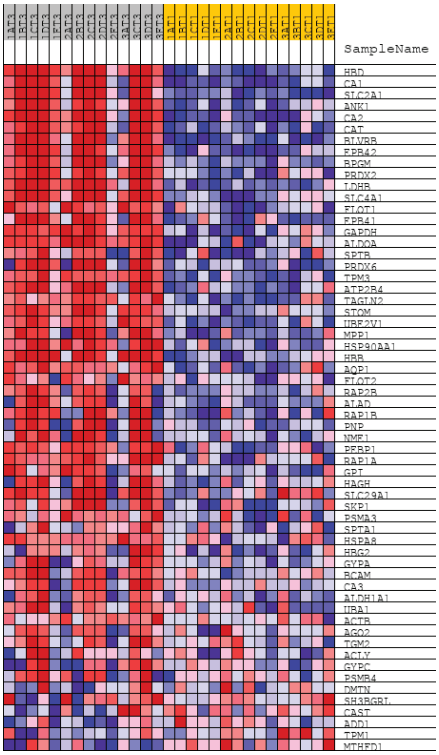


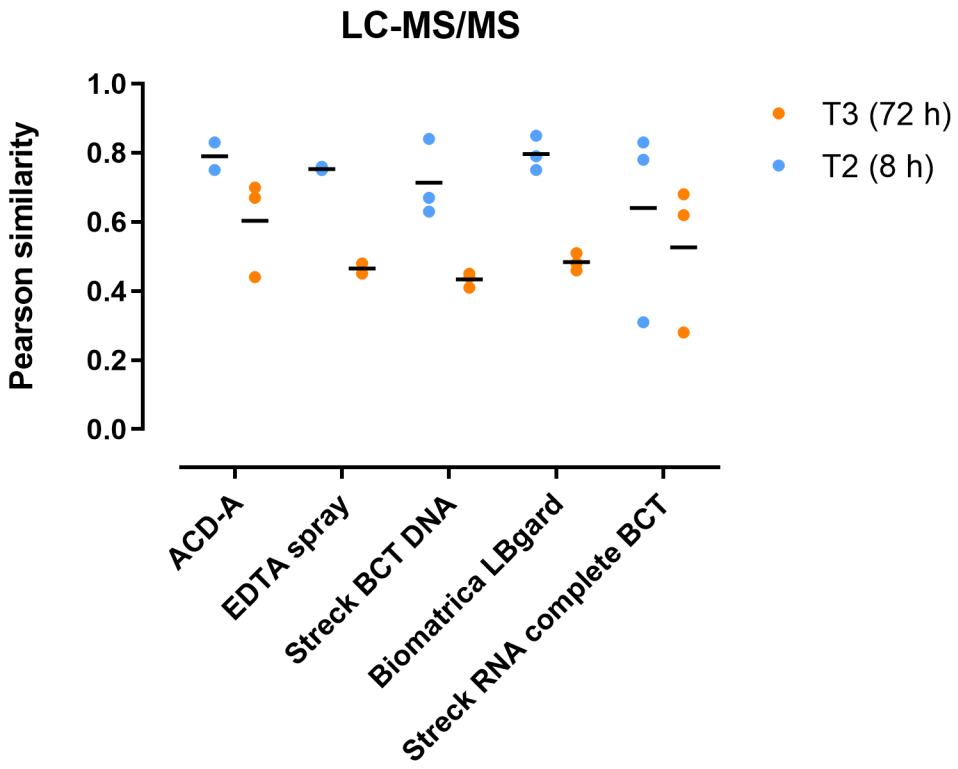


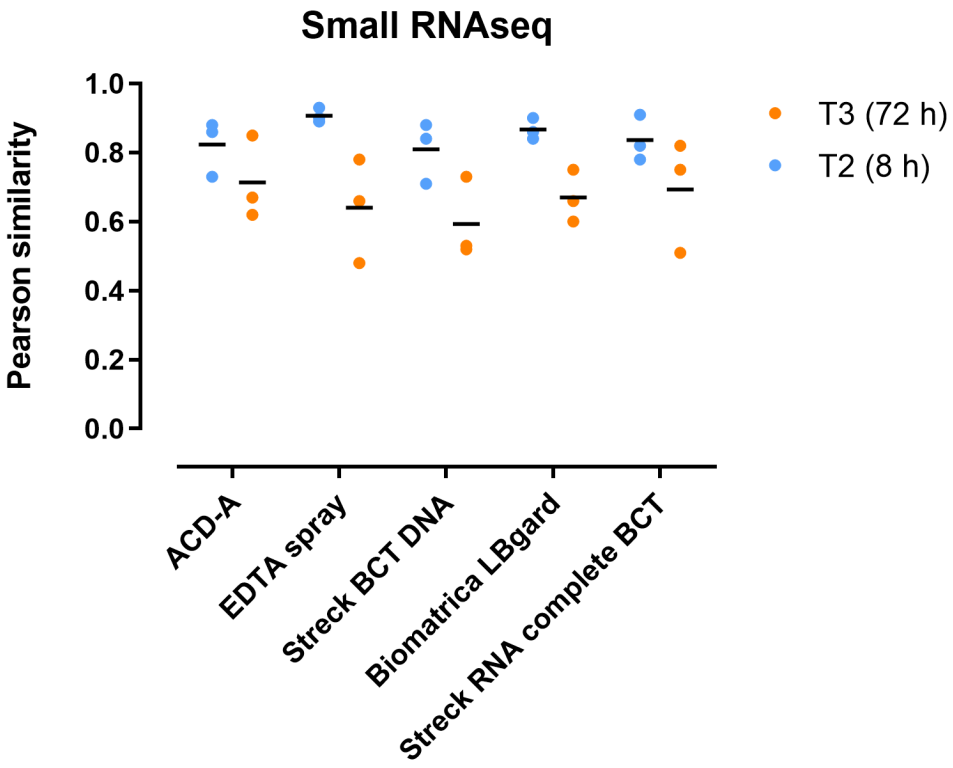
b

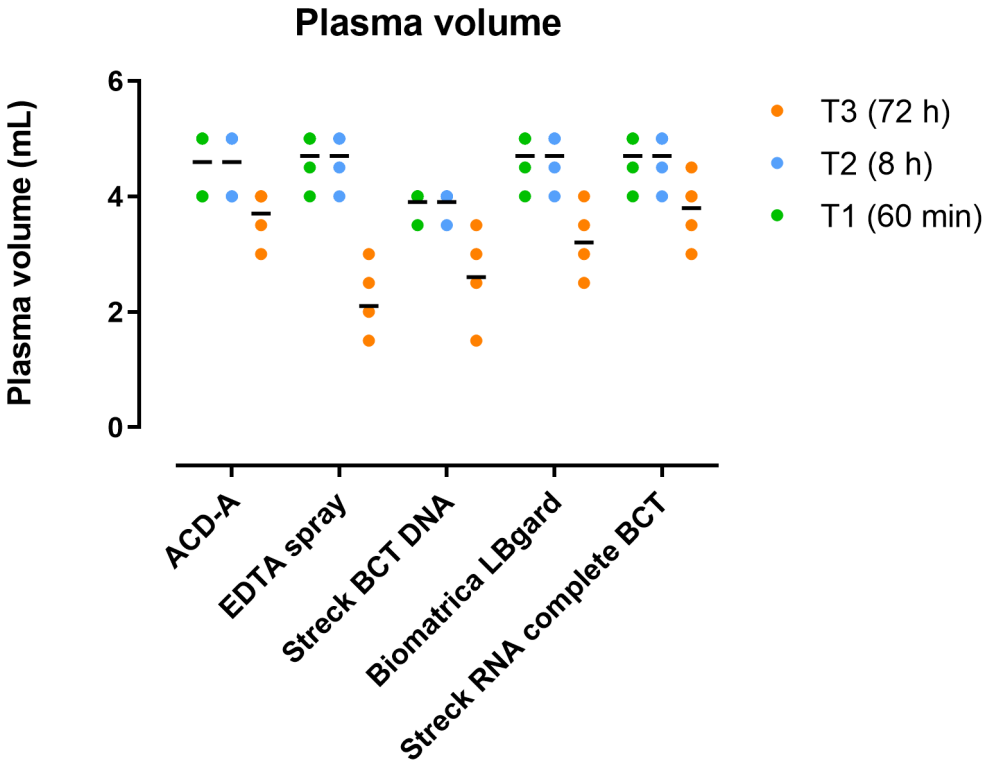
GSEA T3-T1






LC-MS/MS
Pearson similarity
1.0
0.8
0.6
0.4
0.2
0.0
ACD-A
EDTA spray
Streck BCT DNA
Biomatrica LBgard
Streck RNA complete BCT
T3 (72 h)
T2 (8 h)


Small RNAseq
Pearson similarity
1.0
0.8
0.6
0.4
0.2
0.0
ACD-A
EDTA spray
Streck BCT DNA
Biomatrica LBgard
Streck RNA complete BCT
T3 (72 h)
T2 (8 h)


Plasma volume
Plasma volume (mL)
6
4
2
0
ACD-A
EDTA spray
Streck BCT DNA
Biomatrica LBgard
Streck RNA complete BCT
T3 (72 h)
T2 (8 h)
T1 (60 min)