Fig. 1

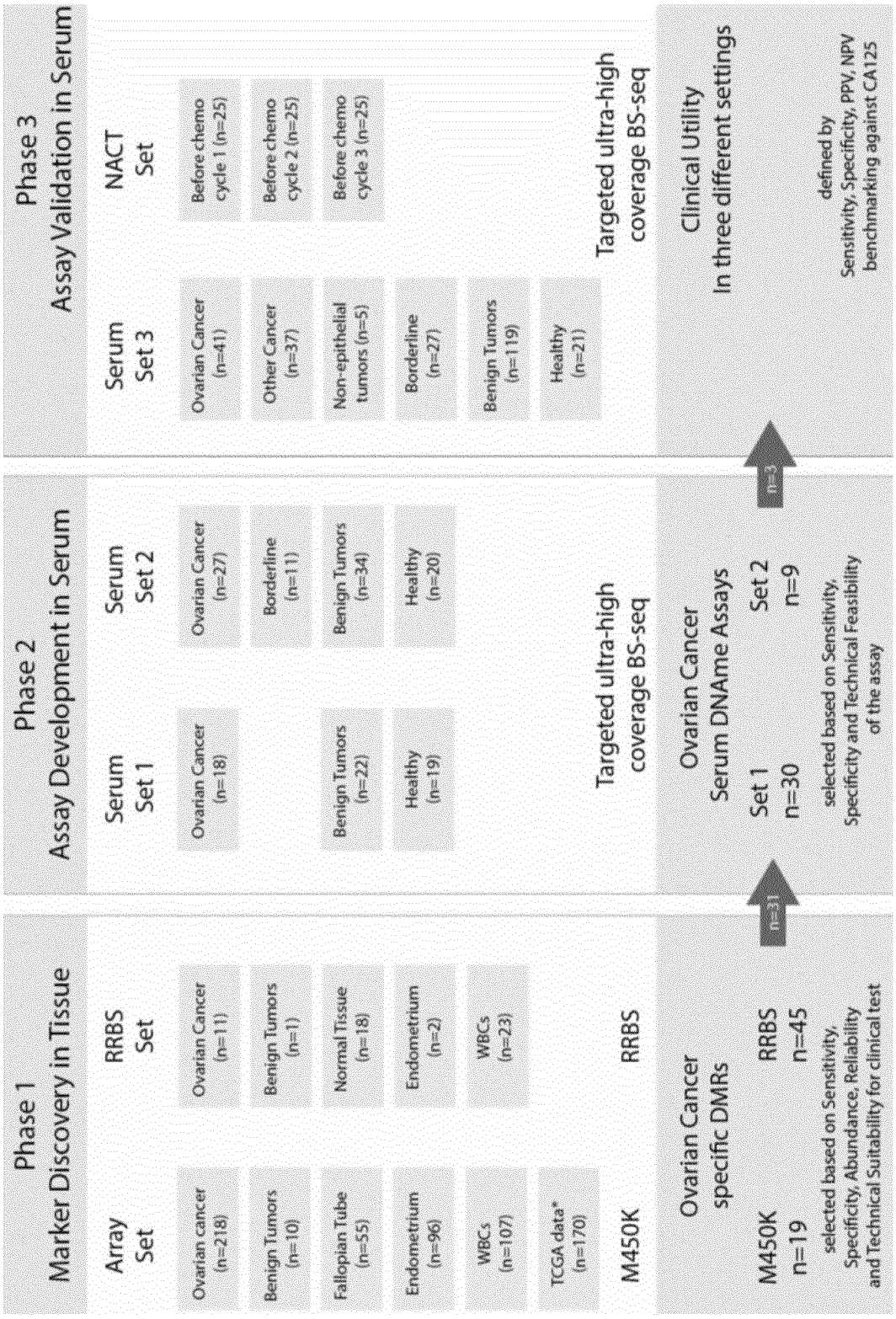

Phase 1
Marker Discovery in Tissue
Array Set
Ovarian cancer (n=218)
Benign Tumors (n=10)
Fallopian Tube (n=55)
Endometrium (n=96)
WBCs (n=107)
TCGA data* (n=170)
M450K
RRBS Set
Ovarian Cancer (n=11)
Benign Tumors (n=1)
Normal Tissue (n=18)
Endometrium (n=2)
WBCs (n=23)
RRBS
Ovarian Cancer specific DMRs
M450K n=19
RRBS n=45
selected based on Sensitivity, Specificity, Abundance, Reliability and Technical Suitability for clinical test
n=31
Phase 2
Assay Development in Serum
Serum Set 1
Ovarian Cancer (n=18)
Benign Tumors (n=22)
Healthy (n=19)
Serum Set 2
Ovarian Cancer (n=27)
Borderline (n=11)
Benign Tumors (n=34)
Healthy (n=20)
Targeted ultra-high coverage BS-seq
Ovarian Cancer Serum DNAme Assays
Set 1 n=30
Set 2 n=9
selected based on Sensitivity, Specificity and Technical Feasibility of the assay
n=3
Phase 3
Assay Validation in Serum
Serum Set 3
Ovarian Cancer (n=41)
Other Cancer (n=37)
Non-epithelial tumors (n=5)
Borderline (n=27)
Benign Tumors (n=119)
Healthy (n=21)
NACT Set
Before chemo cycle 1 (n=25)
Before chemo cycle 2 (n=25)
Before chemo cycle 3 (n=25)
Targeted ultra-high coverage BS-seq
Clinical Utility
In three different settings
defined by Sensitivity, Specificity, PPV, NPV benchmarking against CA125

Fig. 2

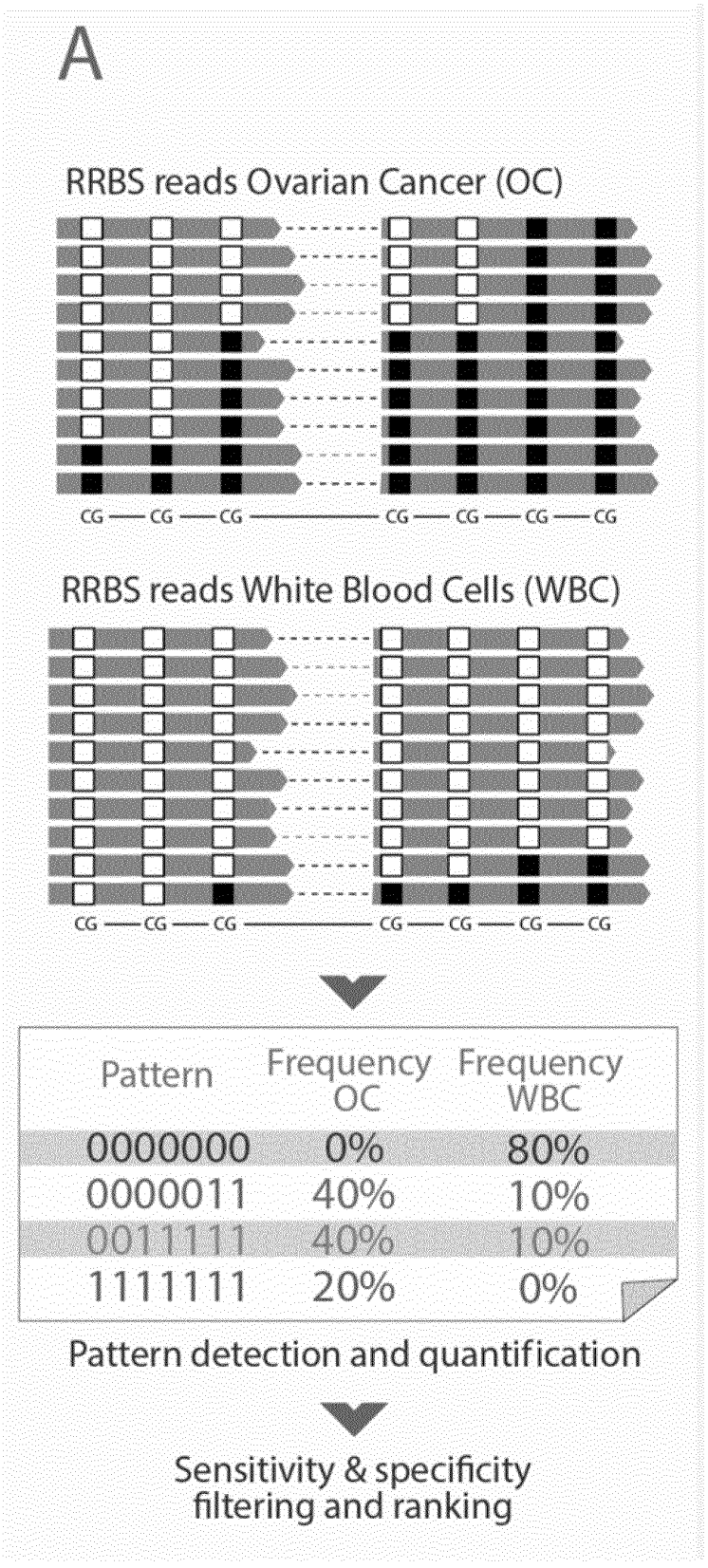

A
RRBS reads Ovarian Cancer (OC)
CG CG CG CG CG CG CG
RRBS reads White Blood Cells (WBC)
CG CG CG CG CG CG CG
Pattern
Frequency OC
Frequency WBC
0000000 0% 80%
0000011 40% 10%
0011111 40% 10%
1111111 20% 0%
Pattern detection and quantification
Sensitivity & specificity filtering and ranking

Fig. 2

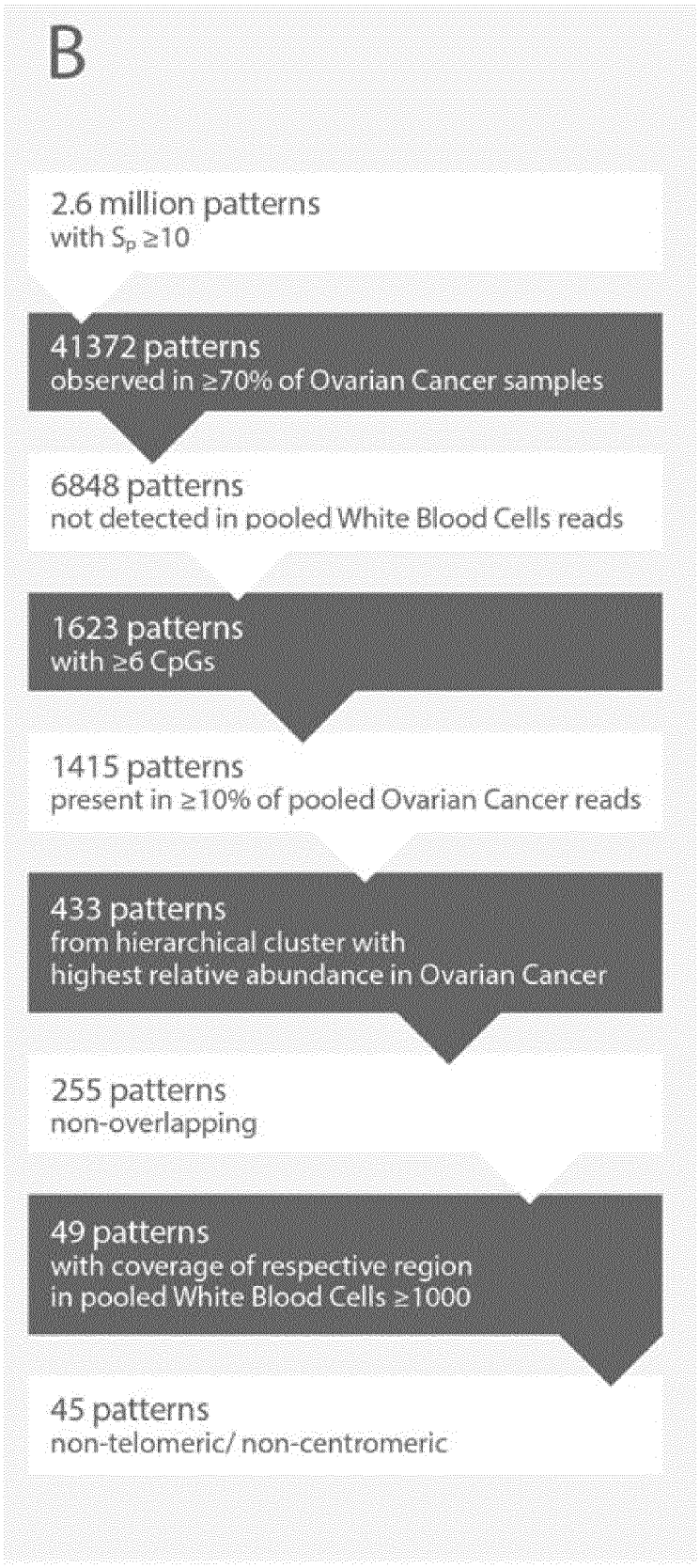

B
2.6 million patterns
with $S_p \geq 10$
41372 patterns
observed in ≥70% of Ovarian Cancer samples
6848 patterns
not detected in pooled White Blood Cells reads
1623 patterns
with ≥6 CpGs
1415 patterns
present in ≥10% of pooled Ovarian Cancer reads
433 patterns
from hierarchical cluster with
highest relative abundance in Ovarian Cancer
255 patterns
non-overlapping
49 patterns
with coverage of respective region
in pooled White Blood Cells ≥1000
45 patterns
non-telomeric/ non-centromeric

Fig. 2

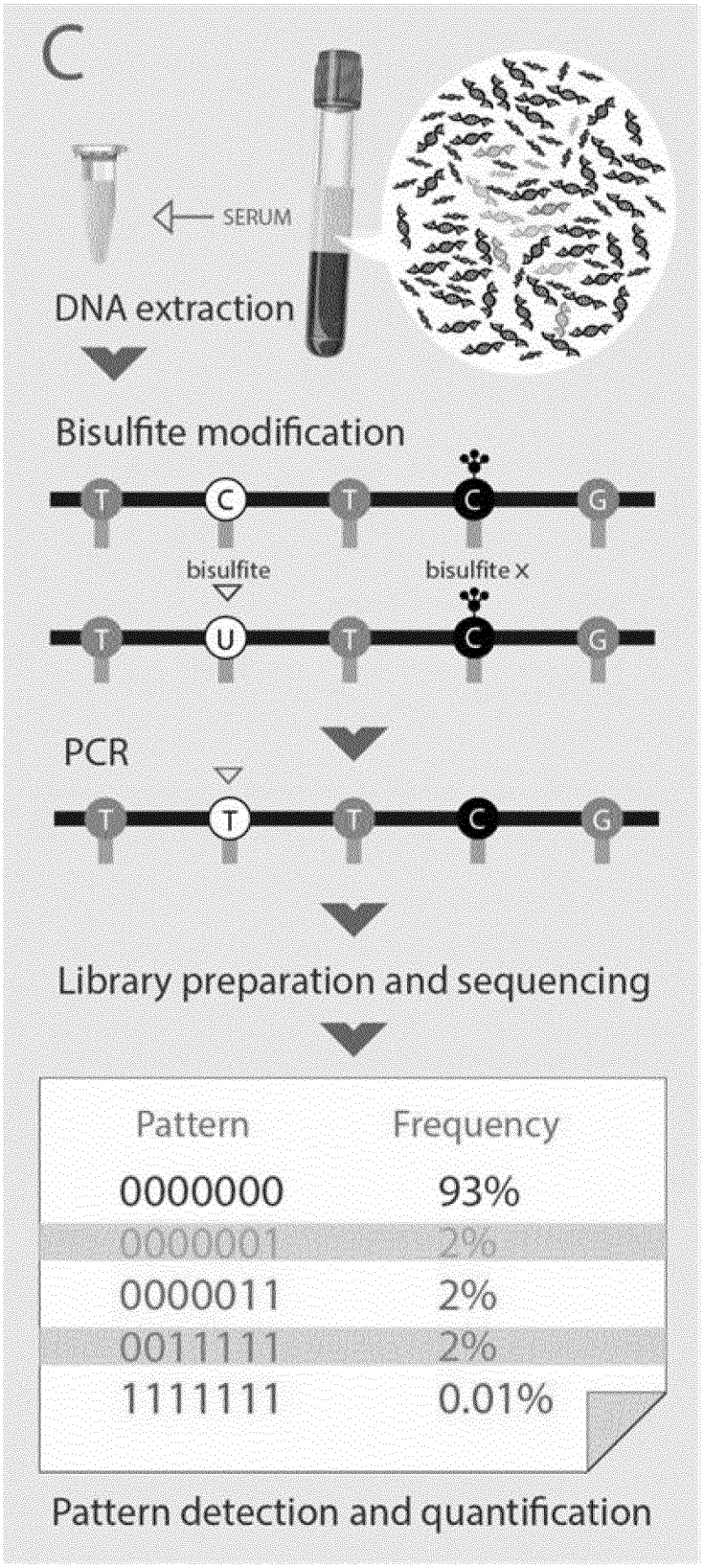
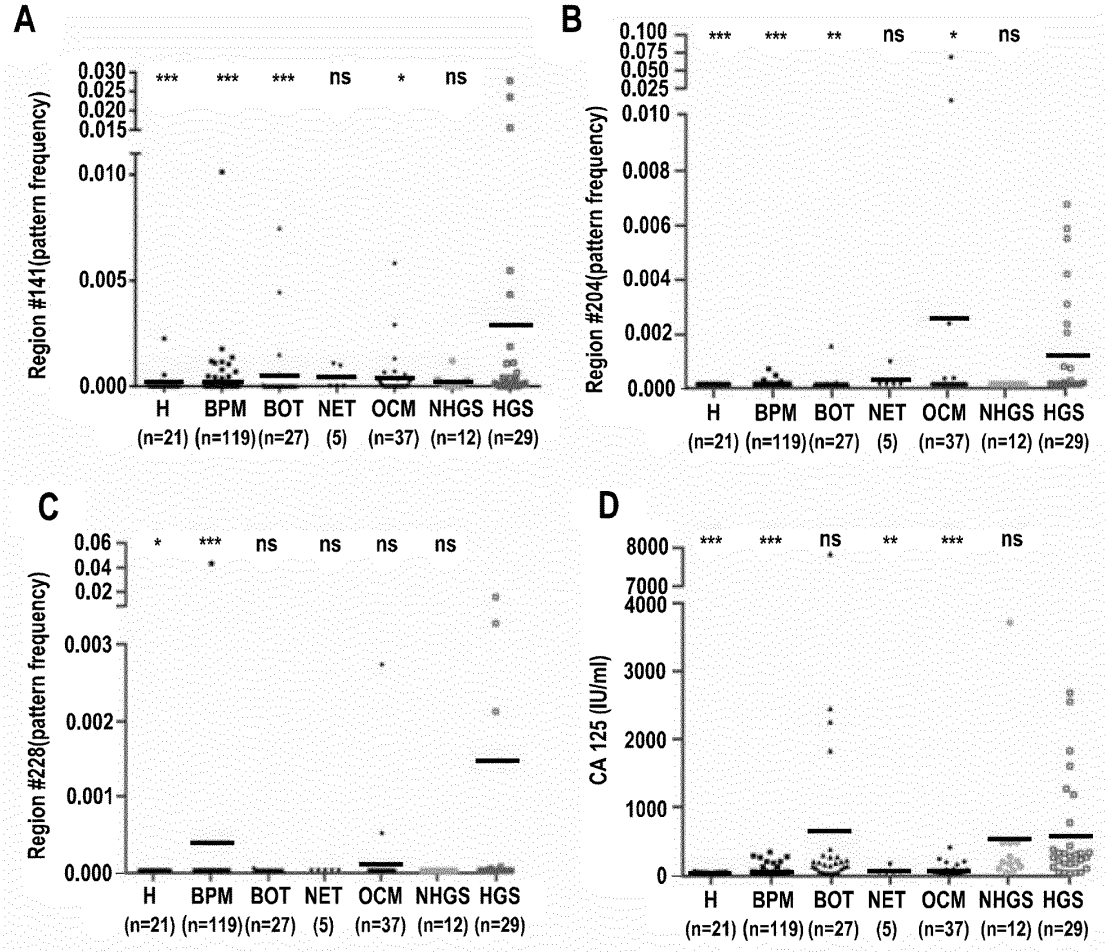

C
SERUM
DNA extraction
Bisulfite modification
T C T C G
bisulfite
bisulfite x
T U T C G
PCR
T T T C G
Library preparation and sequencing
Pattern Frequency
0000000 93%
0000001 2%
0000011 2%
0011111 2%
1111111 0.01%
Pattern detection and quantification

Fig. 3



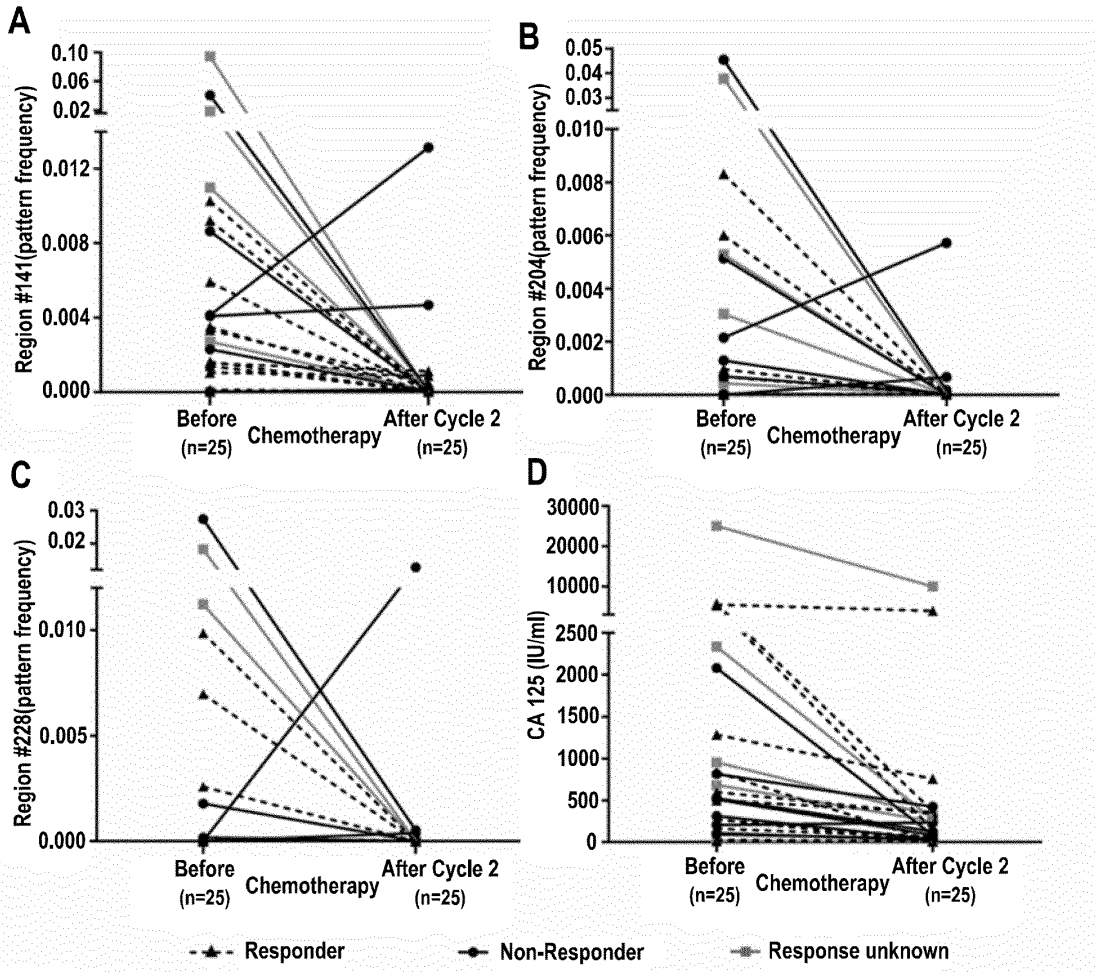
E

		Specificity	Sensitivity
Set 3	CA125 (cut-off 35 IU/mL)	122/140 (87.1%:95%CI 80.1–92.0%)	24/29 (82.8%:95%CI 63.5–93.5%)
	Three DNAme-Marker Panel (thresholds based on Sets 1 & 2)	127/140 (90.7%:95%CI 84.3–94.8%)	12/29 (41.4%:95%CI 24.1–60.9%)
Set 2 & 3	Three DNAme-Marker Panel (thresholds based on Sets 1, 2 & 3)	178/194 (91.8%:95%CI 86.7–95.1%)	28/48 (58.3%:95%CI 43.2–72.1%)

F

		H & BPM		HGS	
		CA125 negative	CA125 positive	CA125 negative	CA125 positive
Three DNAme-Marker Panel (new thresholds)	negative	108	18	4	9
	positive	14	0	1	15

Fig. 4



E

Prediction chemosensitivity	Responder		Non-Responder	
	All	R0/1	All	R0/1
CA125 Positive → Negative	2/10 (20%)	2/8 (25%)		
CA125 Positive → Positive			6/8 (75%)	4/6 (66.7%)
DNAme Positive → Negative	7/9 (77.8%)	7/9 (77.8%)		
DNAme Positive or Negative → Positive			6/7 (85.7%)	6/6 (100%)

Fig. 5

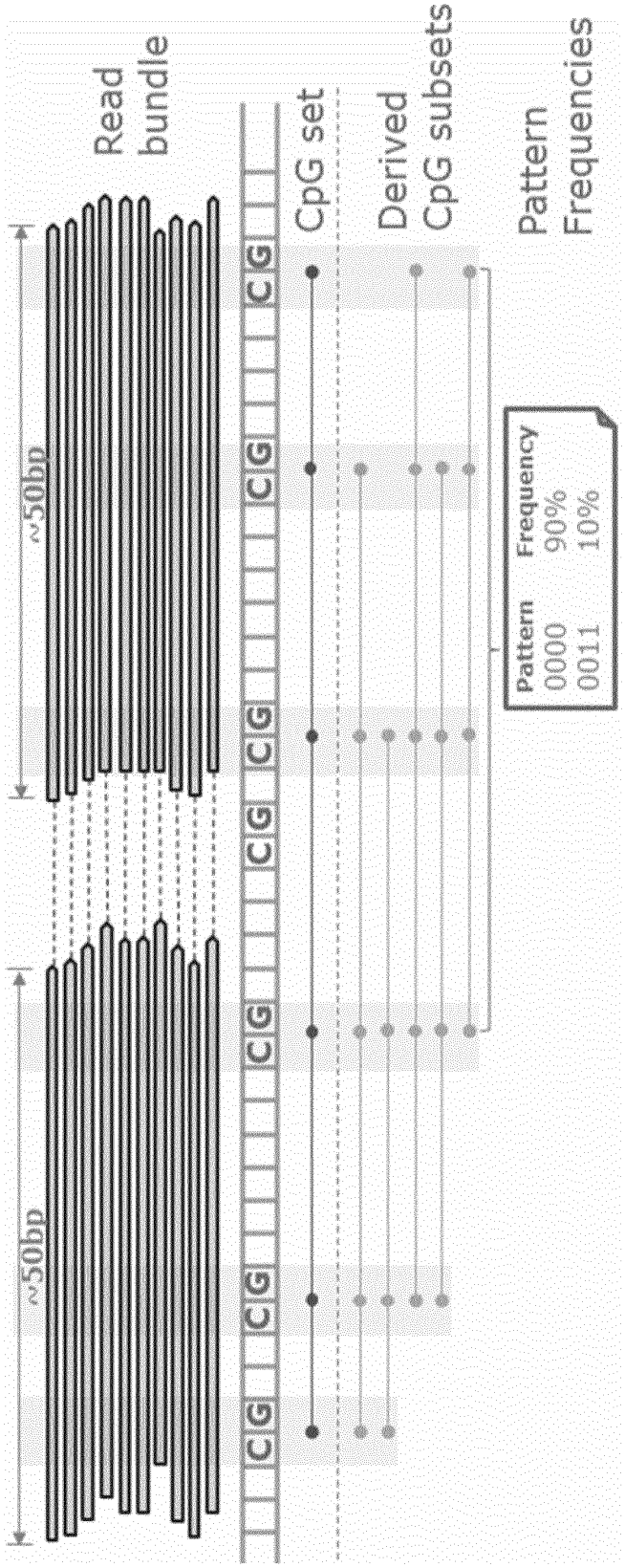

Read bundle
CpG set
Derived CpG subsets
Pattern Frequencies
~50bp
~50bp
CG
Pattern Frequency
0000 90%
0011 10%

Fig. 6

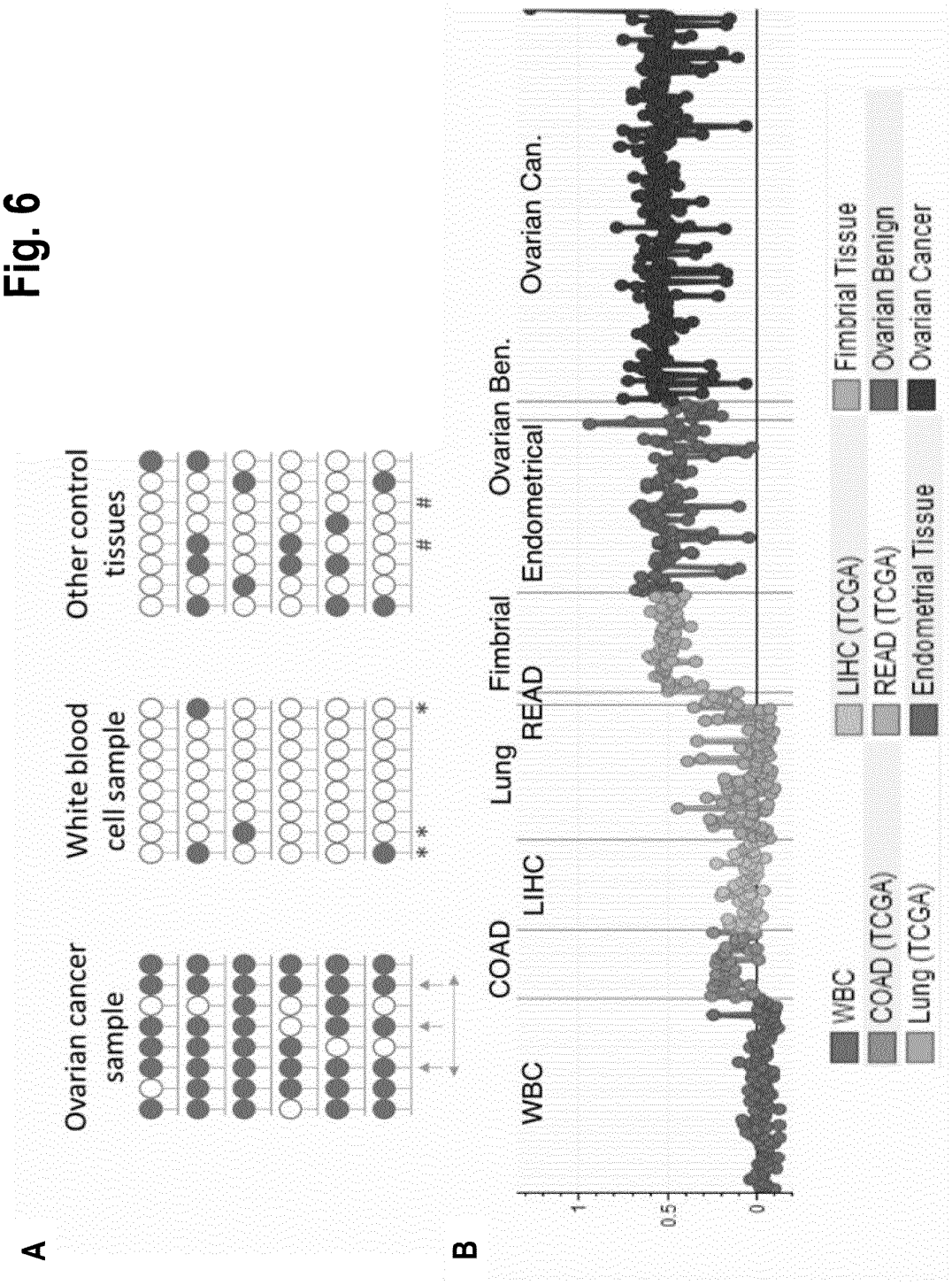

A
Ovarian cancer sample
White blood cell sample
Other control tissues
B
WBC
COAD
LIHC
Lung
READ
Fimbrial
Endometrical
Ovarian Ben.
Ovarian Can.
1
0.5
0
WBC
COAD (TCGA)
Lung (TCGA)
LIHC (TCGA)
READ (TCGA)
Endometrial Tissue
Fimbrial Tissue
Ovarian Benign
Ovarian Cancer

Fig. 7

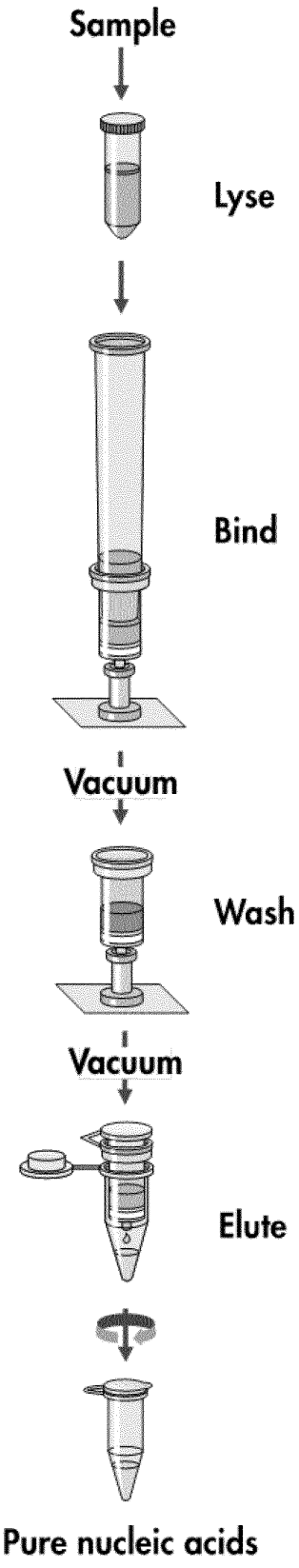

Sample
Lyse
Bind
Vacuum
Wash
Vacuum
Elute
Pure nucleic acids

Fig. 8

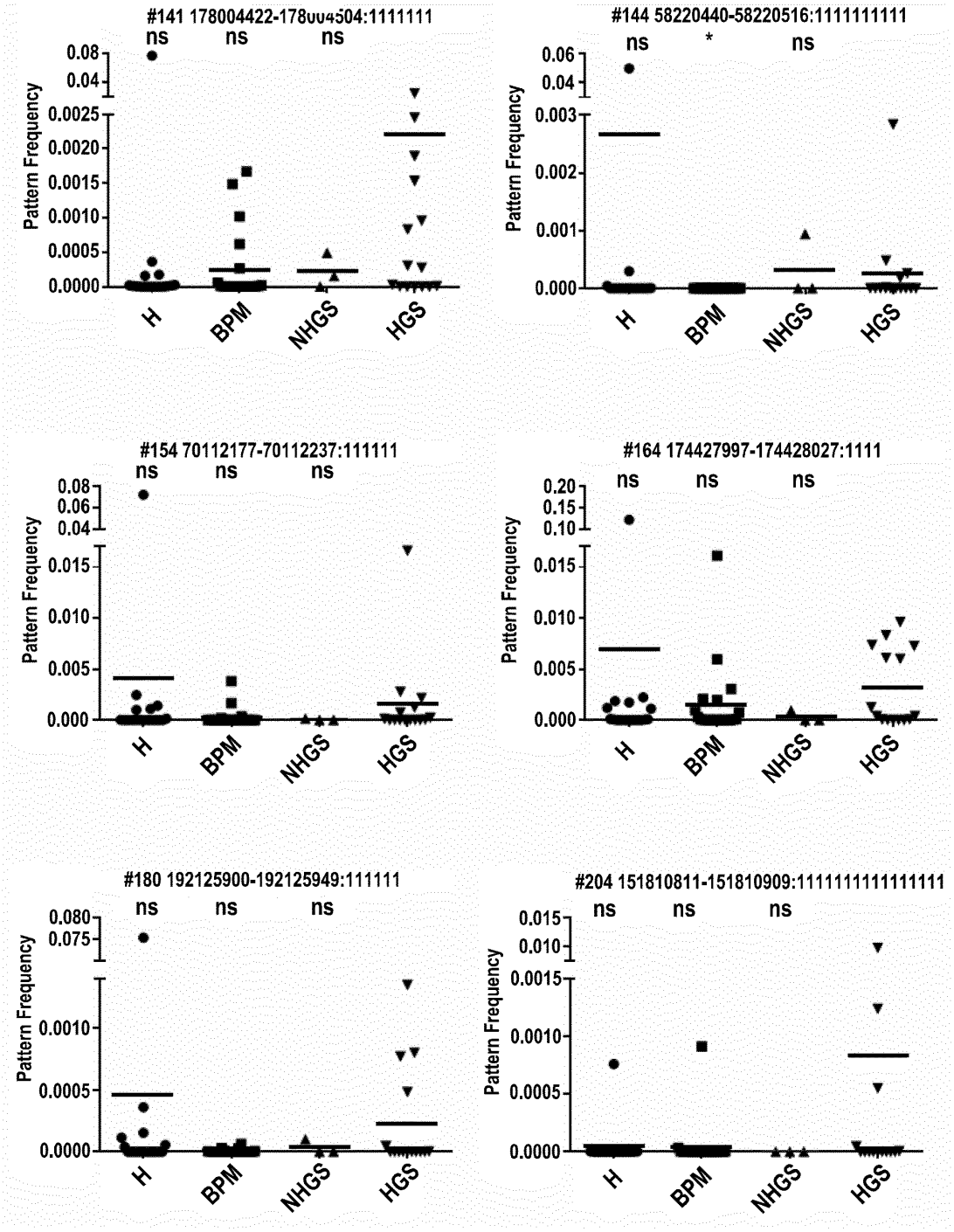

#141 178004422-178004504:1111111
ns ns ns
Pattern Frequency
0.08
0.04
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
H BPM NHGS HGS
#144 58220440-58220516:1111111111
ns * ns
Pattern Frequency
0.06
0.04
0.003
0.002
0.001
0.000
H BPM NHGS HGS
#154 70112177-70112237:111111
ns ns ns
Pattern Frequency
0.08
0.06
0.04
0.015
0.010
0.005
0.000
H BPM NHGS HGS
#164 174427997-174428027:1111
ns ns ns
Pattern Frequency
0.20
0.15
0.10
0.015
0.010
0.005
0.000
H BPM NHGS HGS
#180 192125900-192125949:111111
ns ns ns
Pattern Frequency
0.080
0.075
0.0010
0.0005
0.0000
H BPM NHGS HGS
#204 151810811-151810909:1111111111111111
ns ns ns
Pattern Frequency
0.015
0.010
0.0015
0.0010
0.0005
0.0000
H BPM NHGS HGS

Fig. 9

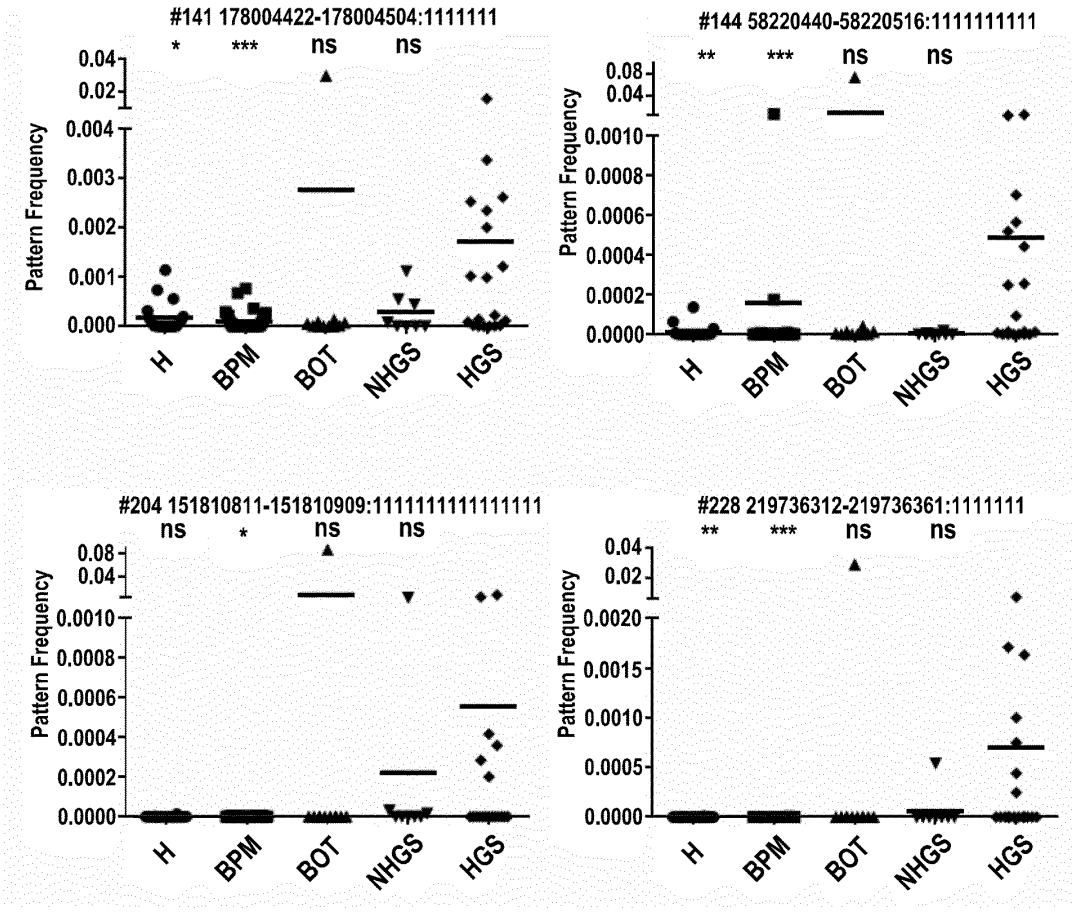

#141 178004422-178004504:1111111
* *** ns ns
Pattern Frequency
0.04
0.02
0.004
0.003
0.002
0.001
0.000
H
BPM
BOT
NHGS
HGS
#144 58220440-58220516:1111111111
** *** ns ns
Pattern Frequency
0.08
0.04
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
H
BPM
BOT
NHGS
HGS
#204 151810811-151810909:1111111111111111
ns * ns ns
Pattern Frequency
0.08
0.04
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
H
BPM
BOT
NHGS
HGS
#228 219736312-219736361:1111111
** *** ns ns
Pattern Frequency
0.04
0.02
0.0020
0.0015
0.0010
0.0005
0.0000
H
BPM
BOT
NHGS
HGS

Fig. 10

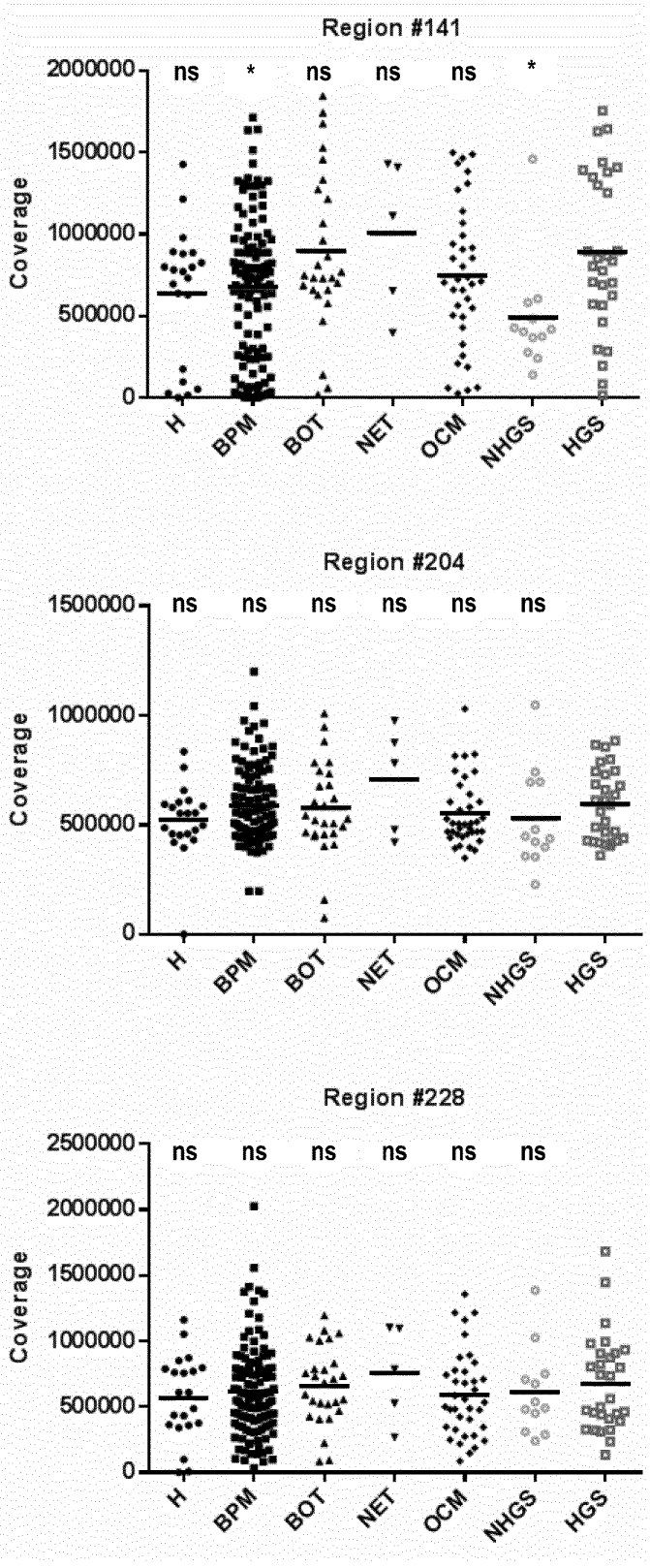

Region #141
Coverage
2000000
1500000
1000000
500000
0
ns
*
ns
ns
ns
*
H
BPM
BOT
NET
OCM
NHGS
HGS
Region #204
Coverage
1500000
1000000
500000
0
ns
ns
ns
ns
ns
ns
H
BPM
BOT
NET
OCM
NHGS
HGS
Region #228
Coverage
2500000
2000000
1500000
1000000
500000
0
ns
ns
ns
ns
ns
ns
H
BPM
BOT
NET
OCM
NHGS
HGS

Fig. 11

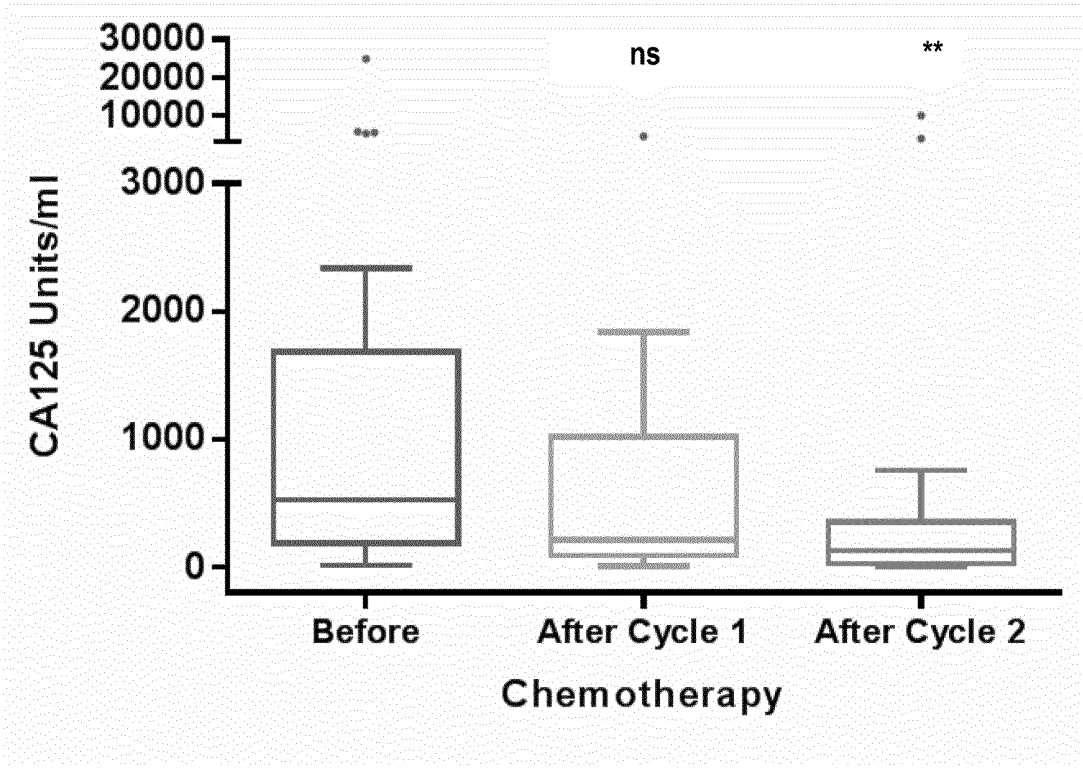

30000
20000
10000
3000
2000
1000
0
CA125 Units/ml
ns
**
Before
After Cycle 1
After Cycle 2
Chemotherapy

Fig. 12

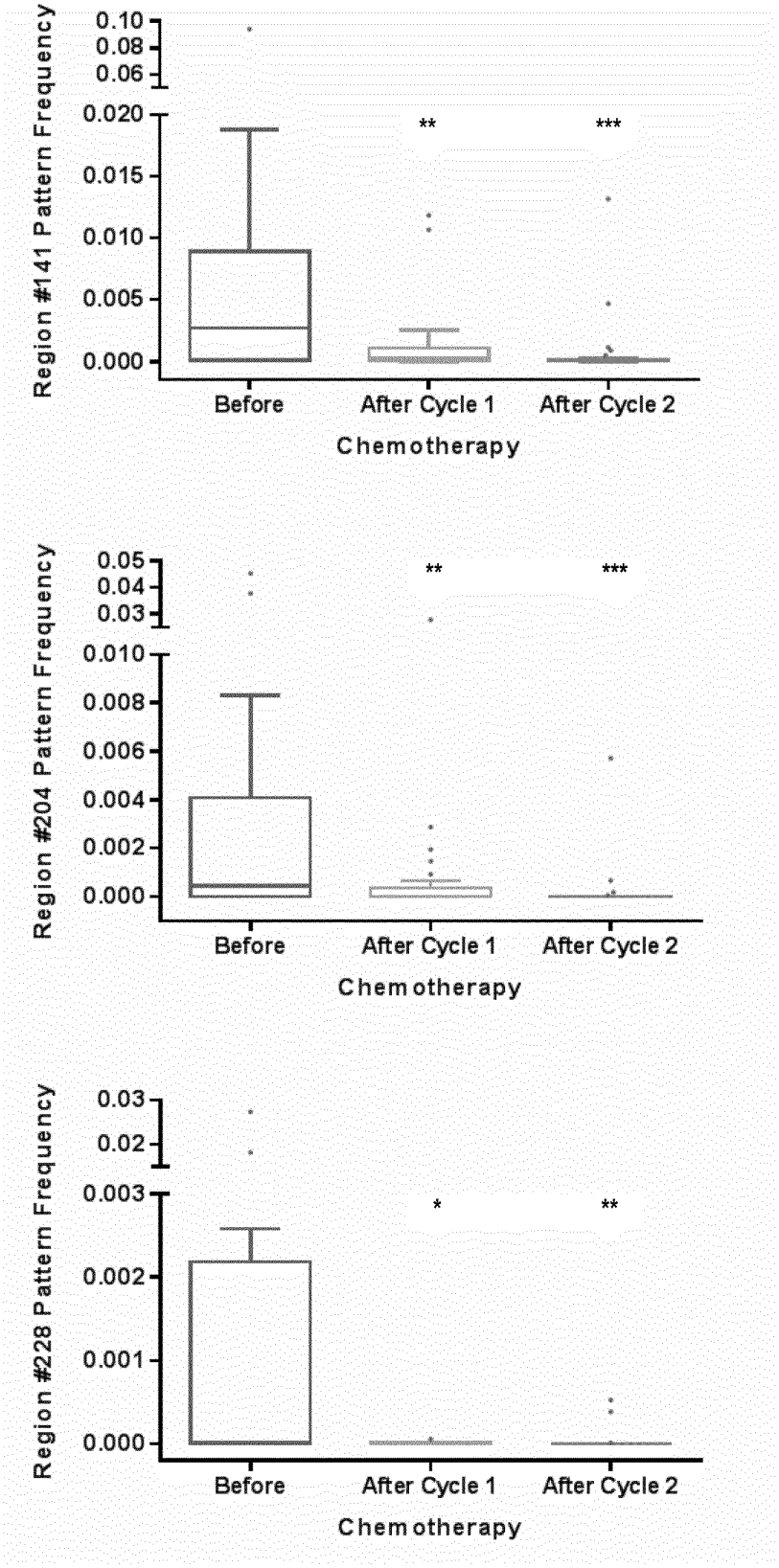

Region #141 Pattern Frequency
0.10
0.08
0.06
0.020
0.015
0.010
0.005
0.000
**
***
Before
After Cycle 1
After Cycle 2
Chemotherapy
Region #204 Pattern Frequency
0.05
0.04
0.03
0.010
0.008
0.006
0.004
0.002
0.000
**
***
Before
After Cycle 1
After Cycle 2
Chemotherapy
Region #228 Pattern Frequency
0.03
0.02
0.003
0.002
0.001
0.000
*
**
Before
After Cycle 1
After Cycle 2
Chemotherapy

Fig. 13

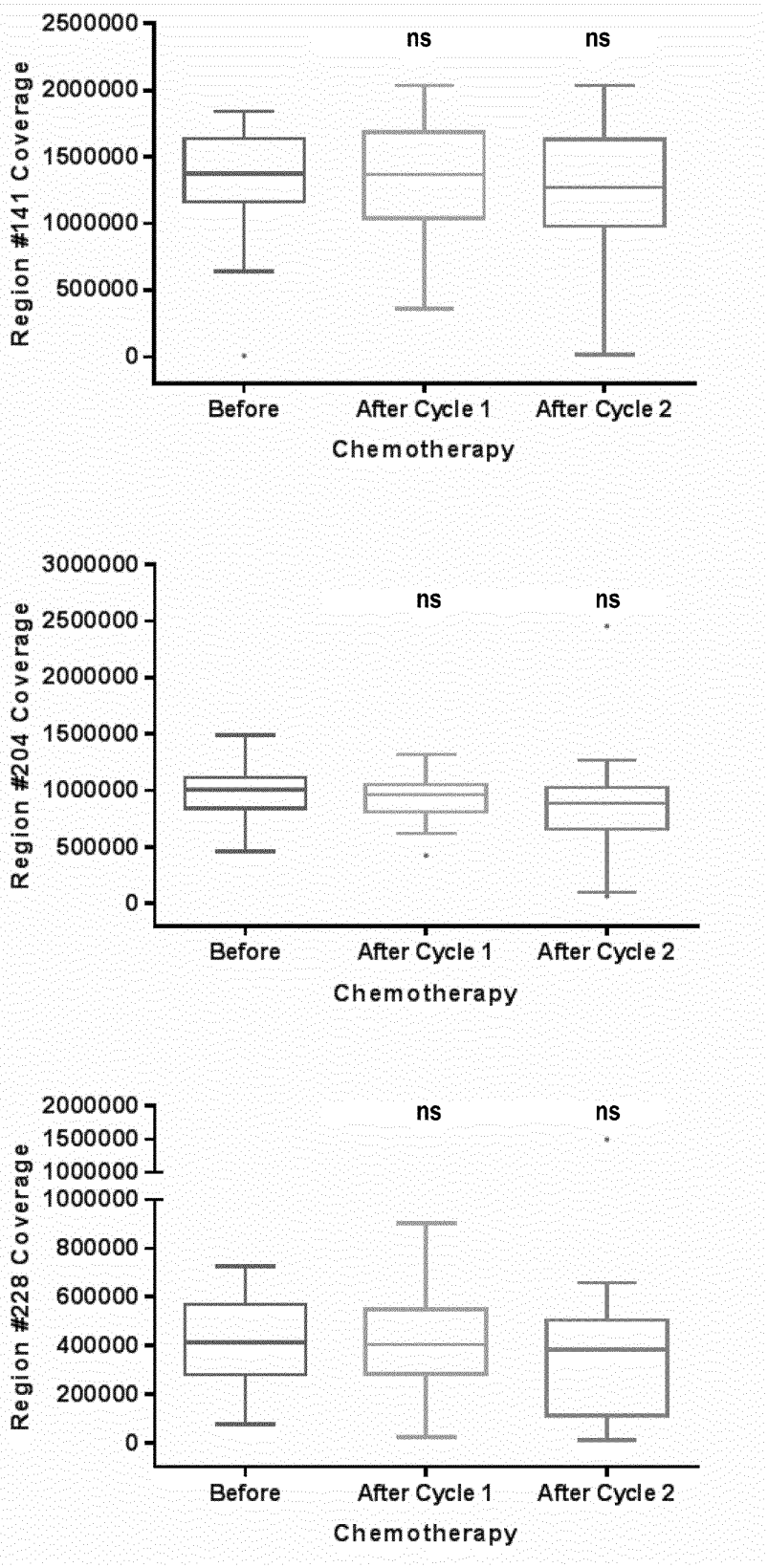

Region #141 Coverage
2500000
2000000
1500000
1000000
500000
0
ns
ns
Before
After Cycle 1
After Cycle 2
Chemotherapy
Region #204 Coverage
3000000
2500000
2000000
1500000
1000000
500000
0
ns
ns
Before
After Cycle 1
After Cycle 2
Chemotherapy
Region #228 Coverage
2000000
1500000
1000000
1000000
800000
600000
400000
200000
0
ns
ns
Before
After Cycle 1
After Cycle 2
Chemotherapy

Fig. 14

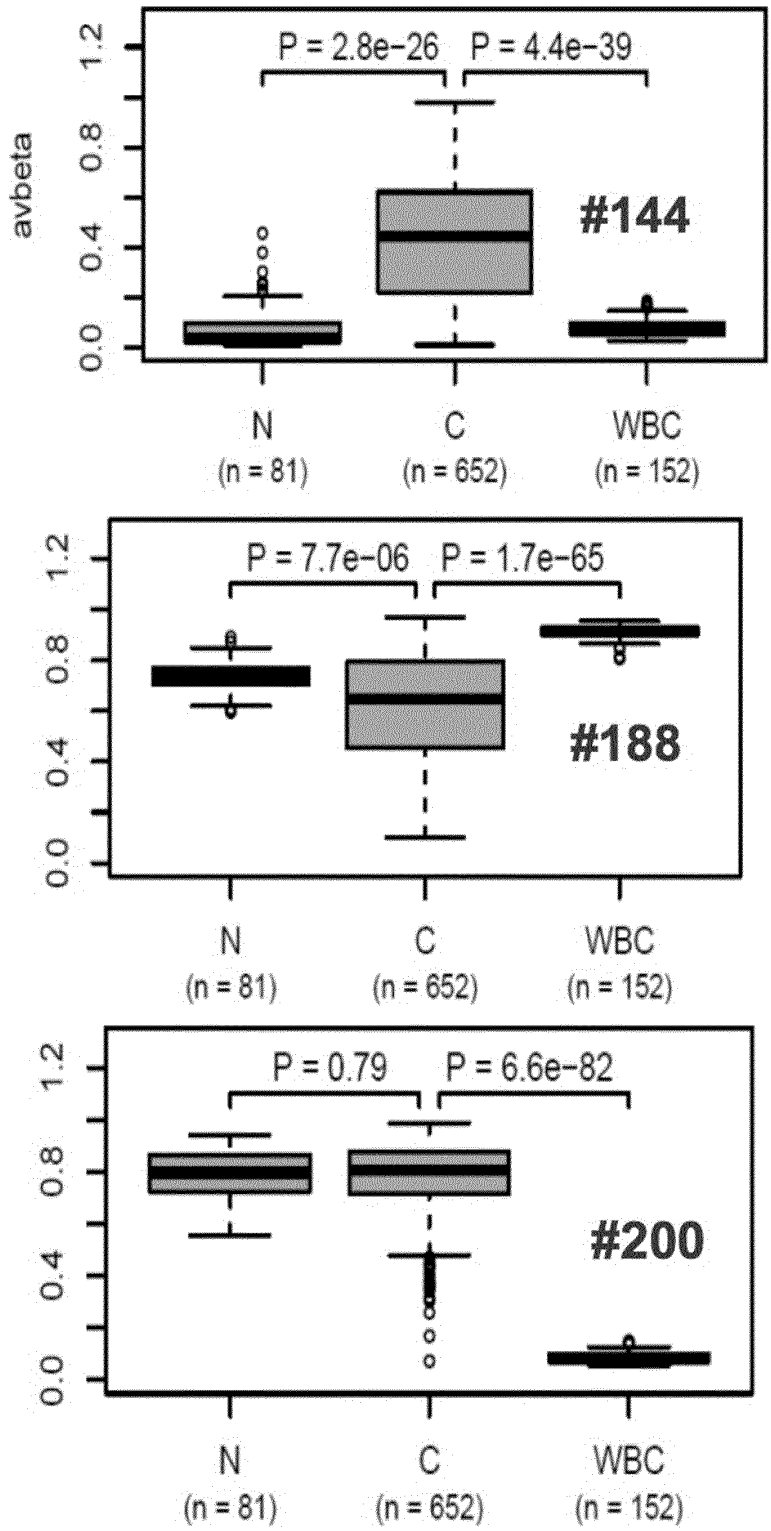

avbeta
0.0
0.4
0.8
1.2
P = 2.8e−26
P = 4.4e−39
#144
N
(n = 81)
C
(n = 652)
WBC
(n = 152)
P = 7.7e−06
P = 1.7e−65
#188
P = 0.79
P = 6.6e−82
#200

Fig. 14 (cont)

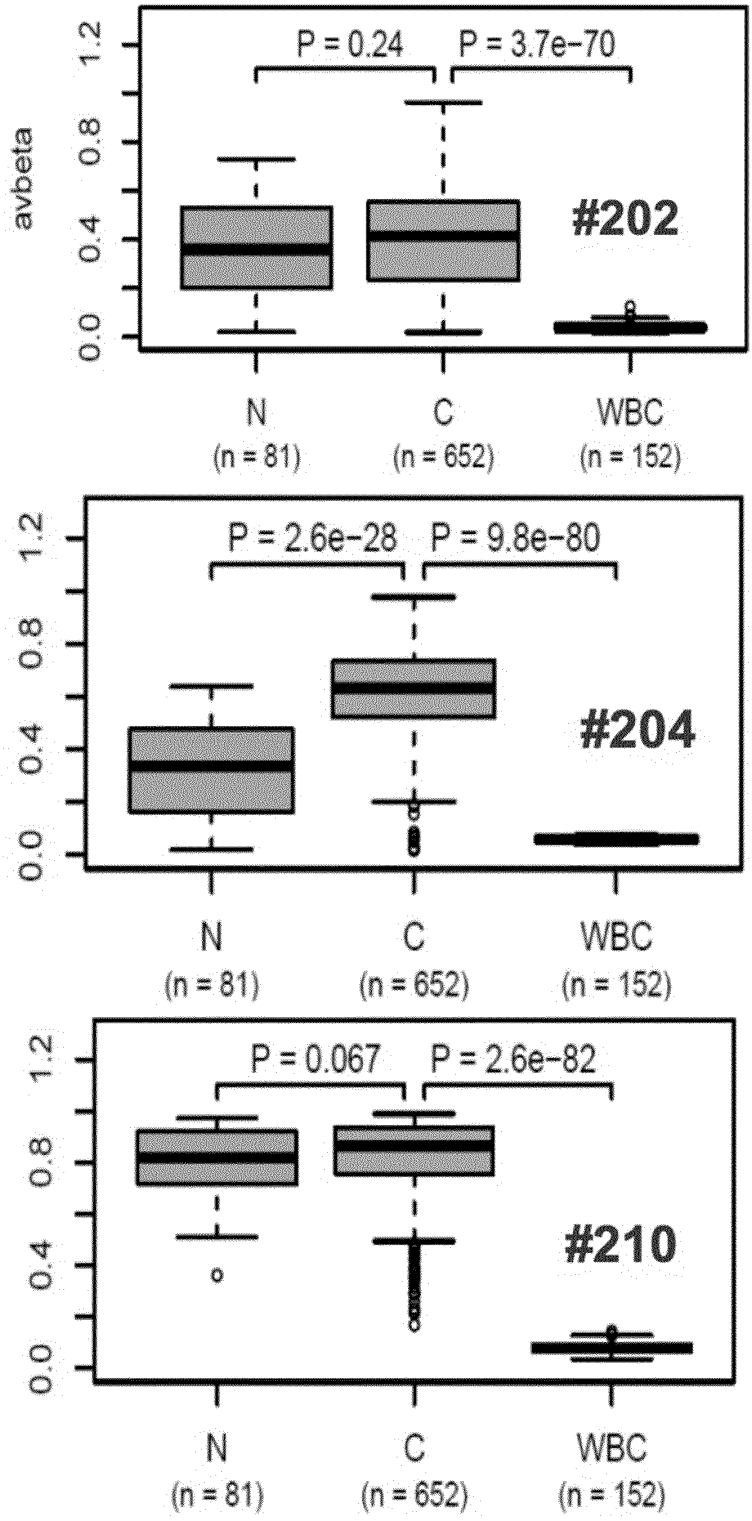

avbeta
0.0
0.4
0.8
1.2
P = 0.24
P = 3.7e−70
#202
N
(n = 81)
C
(n = 652)
WBC
(n = 152)
P = 2.6e−28
P = 9.8e−80
#204
P = 0.067
P = 2.6e−82
#210

Fig. 14 (cont)

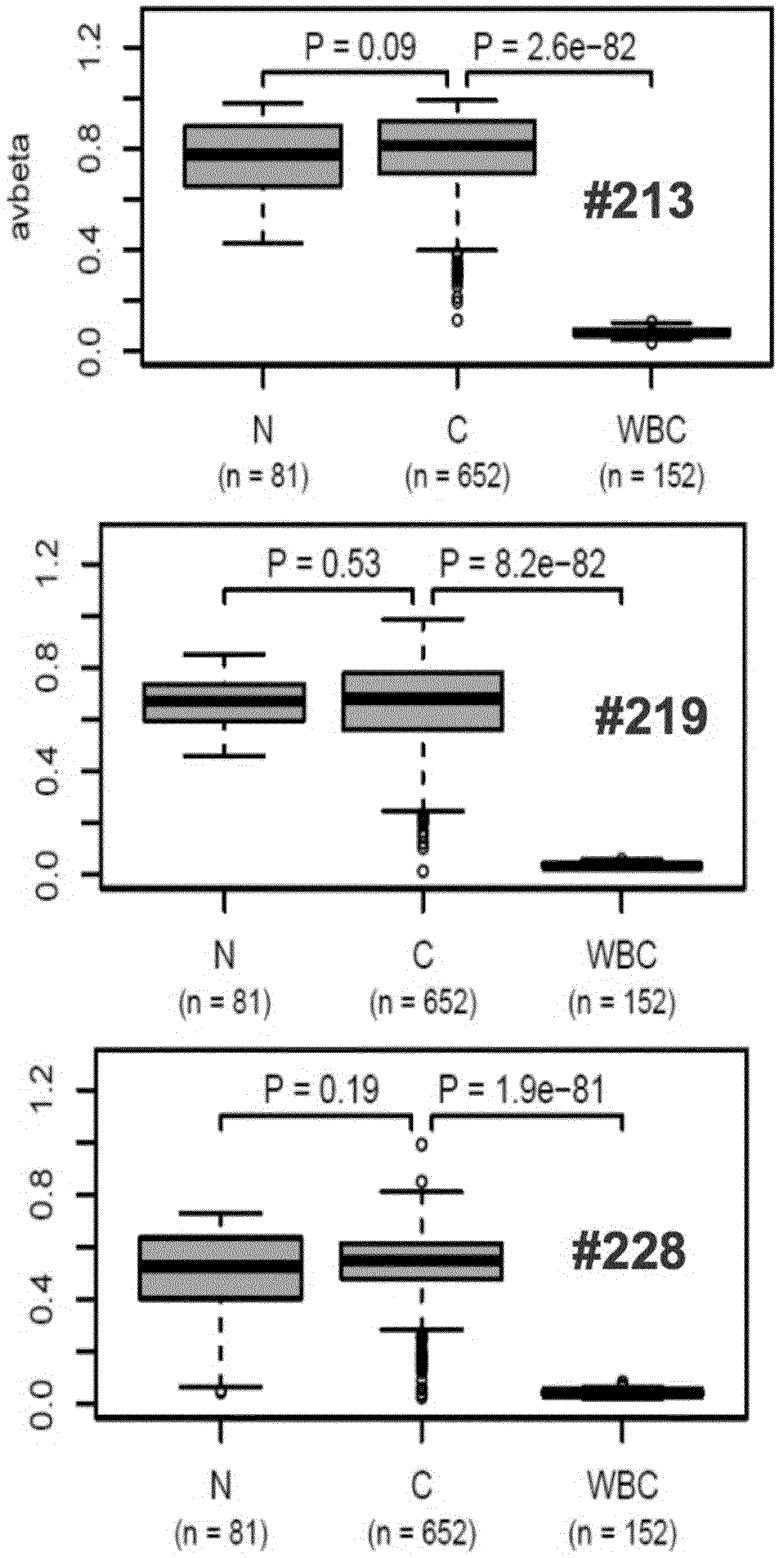

avbeta
0.0
0.4
0.8
1.2
P = 0.09
P = 2.6e−82
#213
N
(n = 81)
C
(n = 652)
WBC
(n = 152)
P = 0.53
P = 8.2e−82
#219
N
(n = 81)
C
(n = 652)
WBC
(n = 152)
P = 0.19
P = 1.9e−81
#228
N
(n = 81)
C
(n = 652)
WBC
(n = 152)

Fig. 15

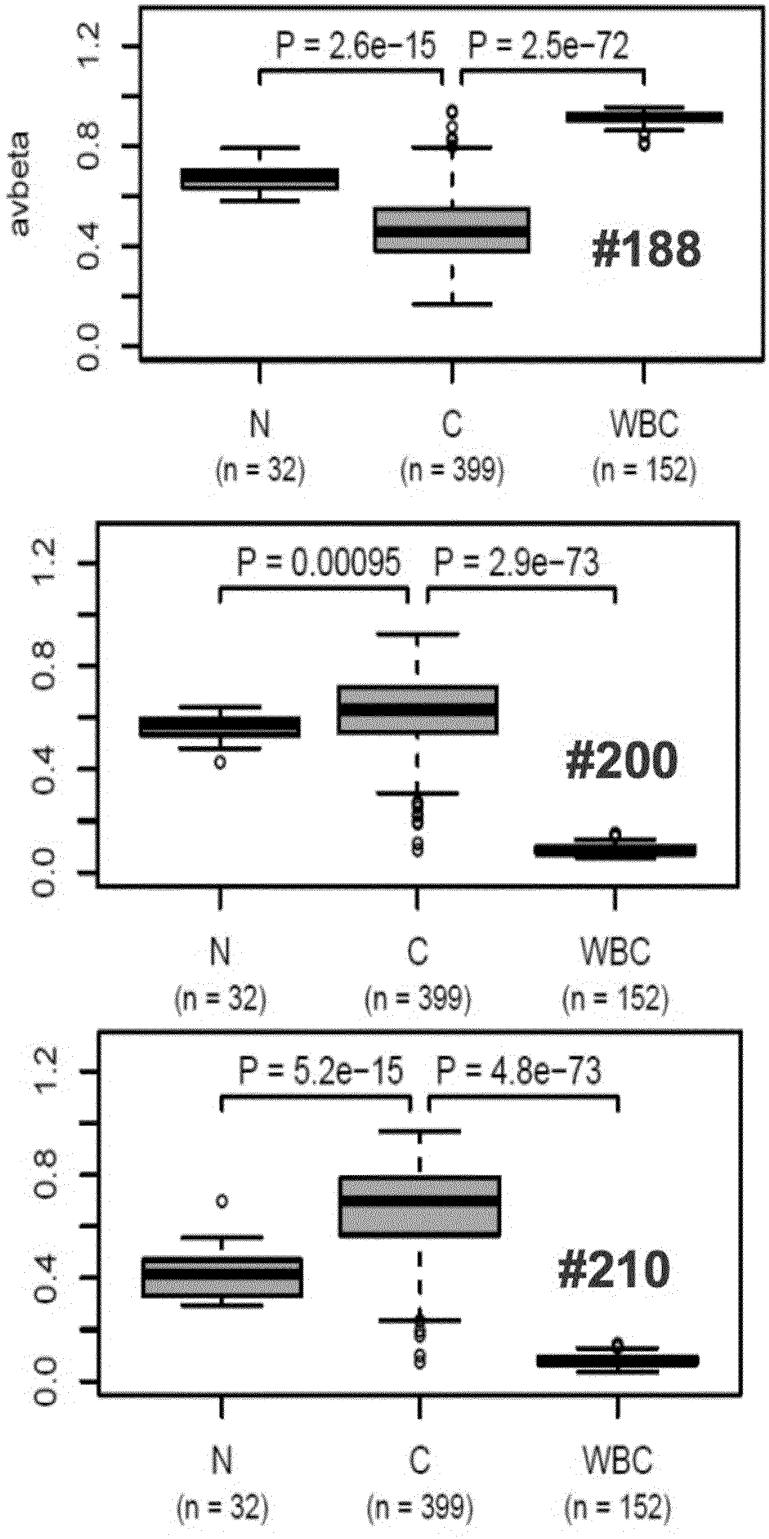

P = 2.6e−15
P = 2.5e−72
avbeta
0.0
0.4
0.8
1.2
#188
N
(n = 32)
C
(n = 399)
WBC
(n = 152)
P = 0.00095
P = 2.9e−73
#200
P = 5.2e−15
P = 4.8e−73
#210

Fig. 15 (cont)

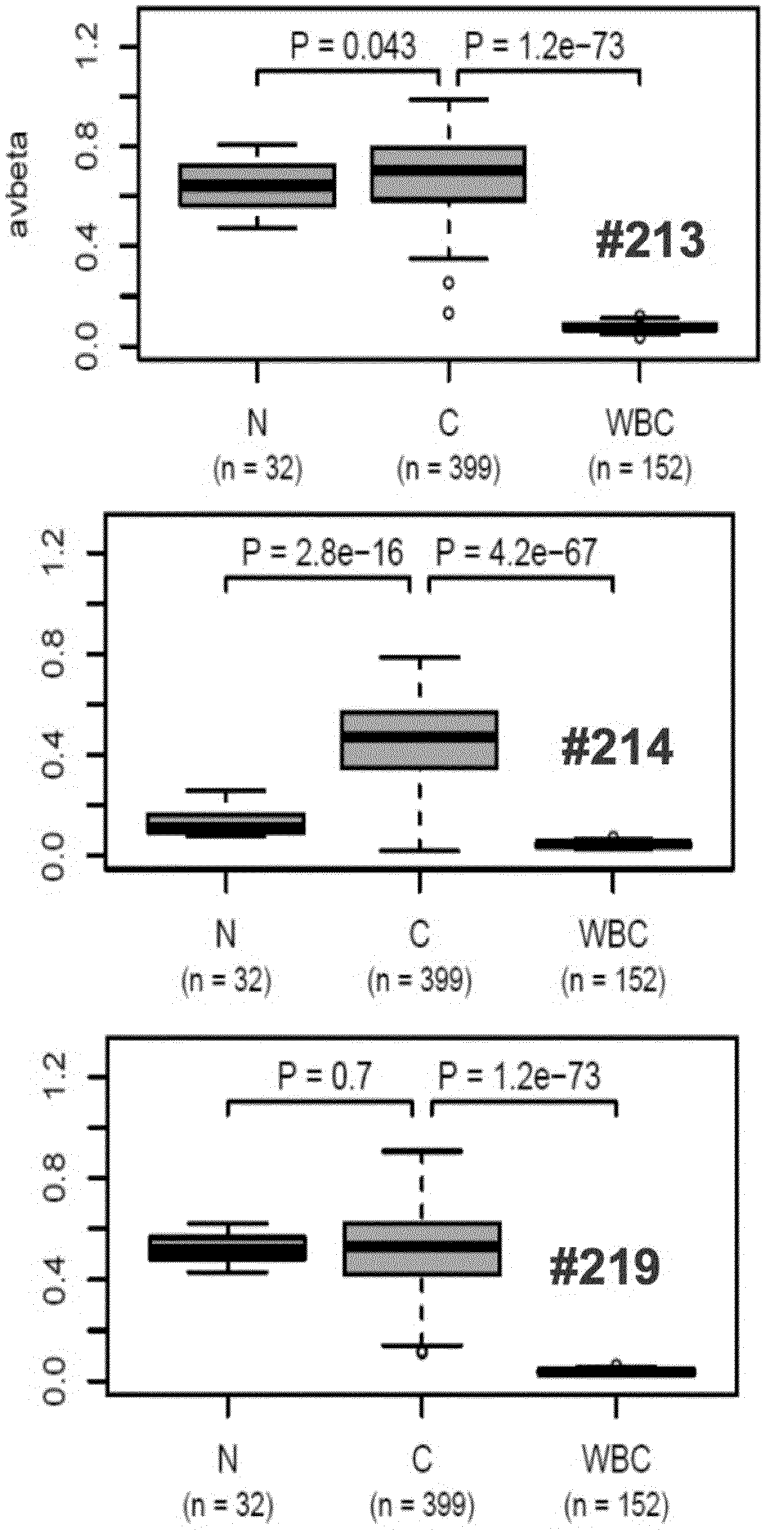

avbeta
0.0
0.4
0.8
1.2
P = 0.043
P = 1.2e−73
#213
N
(n = 32)
C
(n = 399)
WBC
(n = 152)
P = 2.8e−16
P = 4.2e−67
#214
P = 0.7
P = 1.2e−73
#219

Fig. 15 (cont)

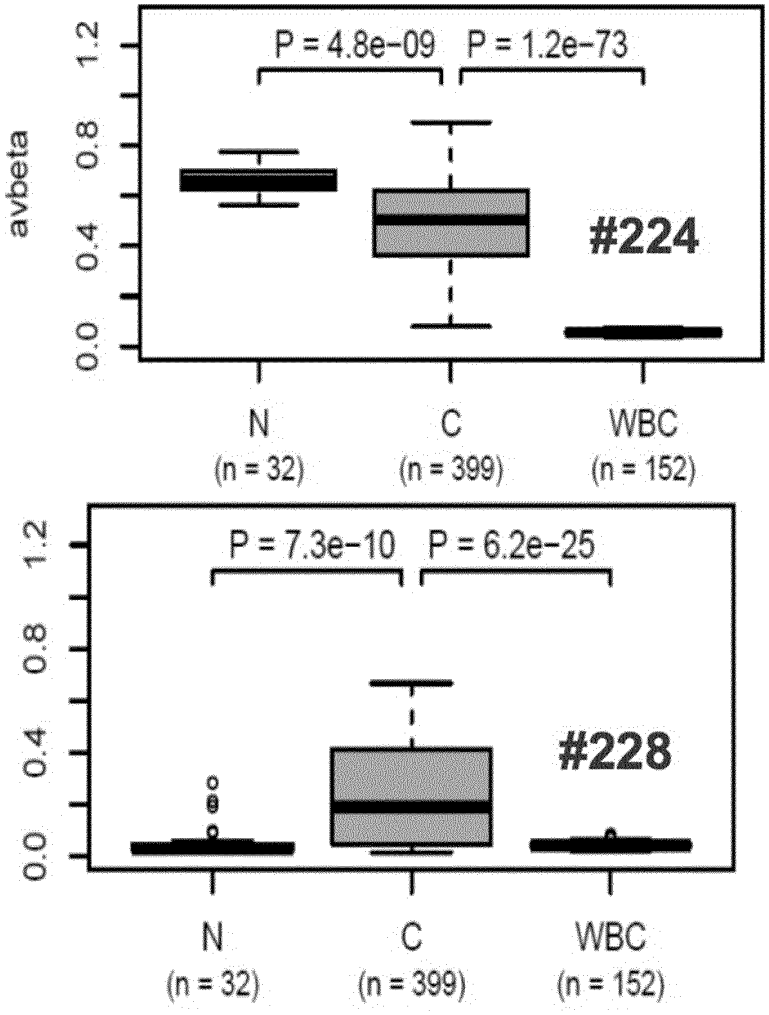

avbeta
0.0
0.4
0.8
1.2
P = 4.8e−09
P = 1.2e−73
#224
N
(n = 32)
C
(n = 399)
WBC
(n = 152)
P = 7.3e−10
P = 6.2e−25
#228
N
(n = 32)
C
(n = 399)
WBC
(n = 152)

Fig. 16

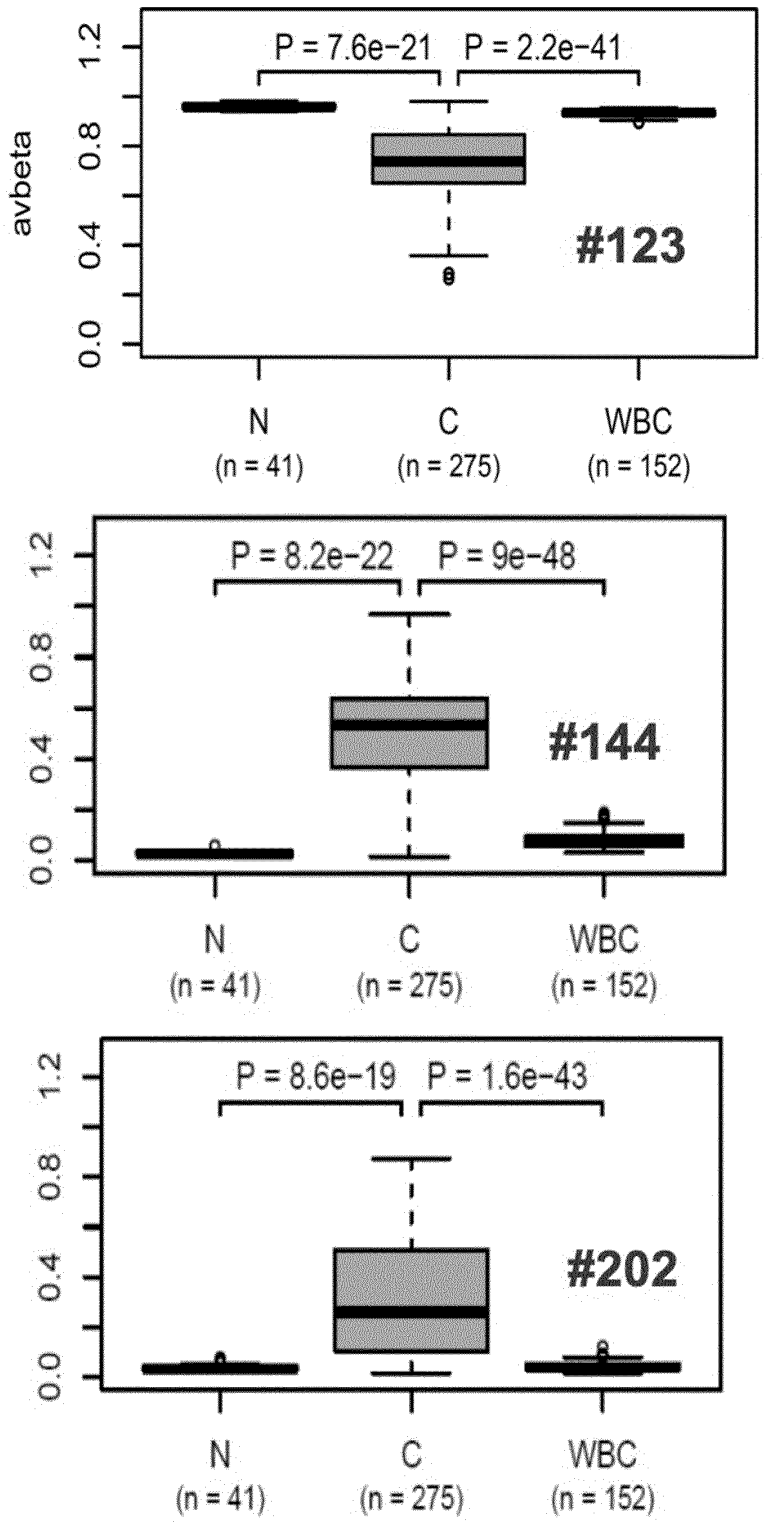

avbeta
0.0
0.4
0.8
1.2
P = 7.6e−21
P = 2.2e−41
#123
N
(n = 41)
C
(n = 275)
WBC
(n = 152)
P = 8.2e−22
P = 9e−48
#144
N
(n = 41)
C
(n = 275)
WBC
(n = 152)
P = 8.6e−19
P = 1.6e−43
#202
N
(n = 41)
C
(n = 275)
WBC
(n = 152)

Fig. 16 (cont)

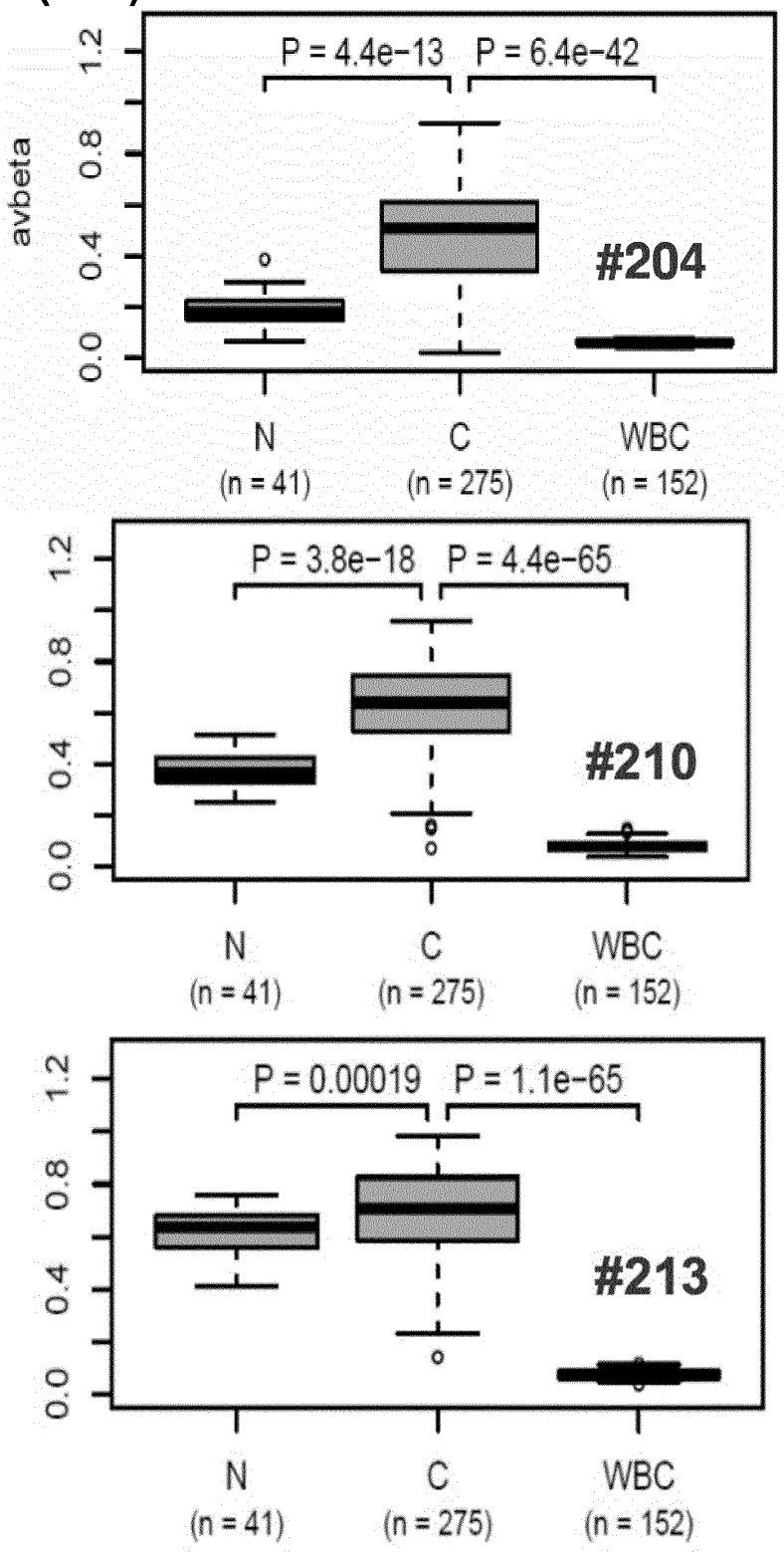

avbeta
0.0
0.4
0.8
1.2
P = 4.4e−13
P = 6.4e−42
#204
N
(n = 41)
C
(n = 275)
WBC
(n = 152)
P = 3.8e−18
P = 4.4e−65
#210
P = 0.00019
P = 1.1e−65
#213

Fig. 16 (cont)

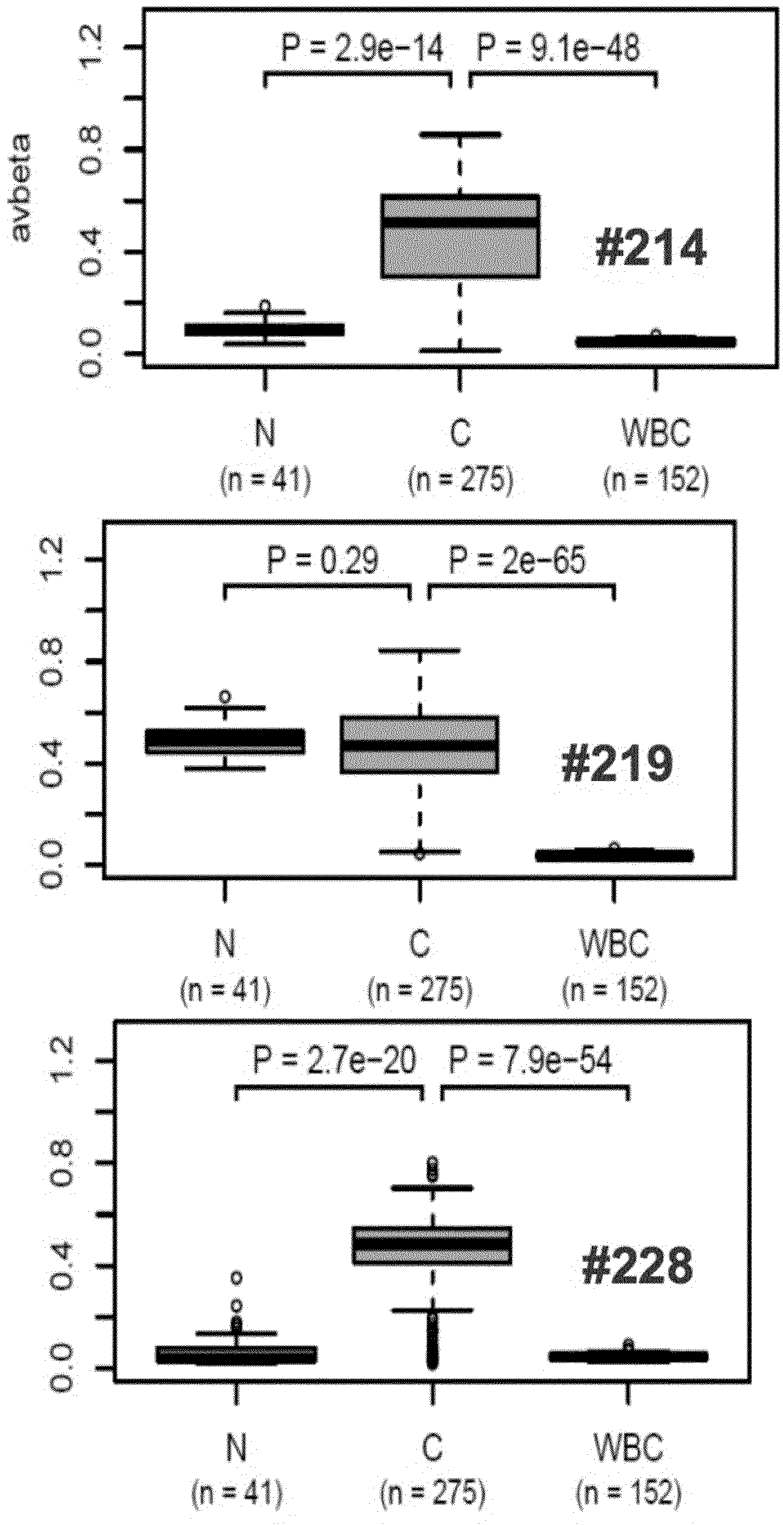

avbeta
0.0
0.4
0.8
1.2
P = 2.9e−14
P = 9.1e−48
#214
N
(n = 41)
C
(n = 275)
WBC
(n = 152)
P = 0.29
P = 2e−65
#219
P = 2.7e−20
P = 7.9e−54
#228

Fig. 17

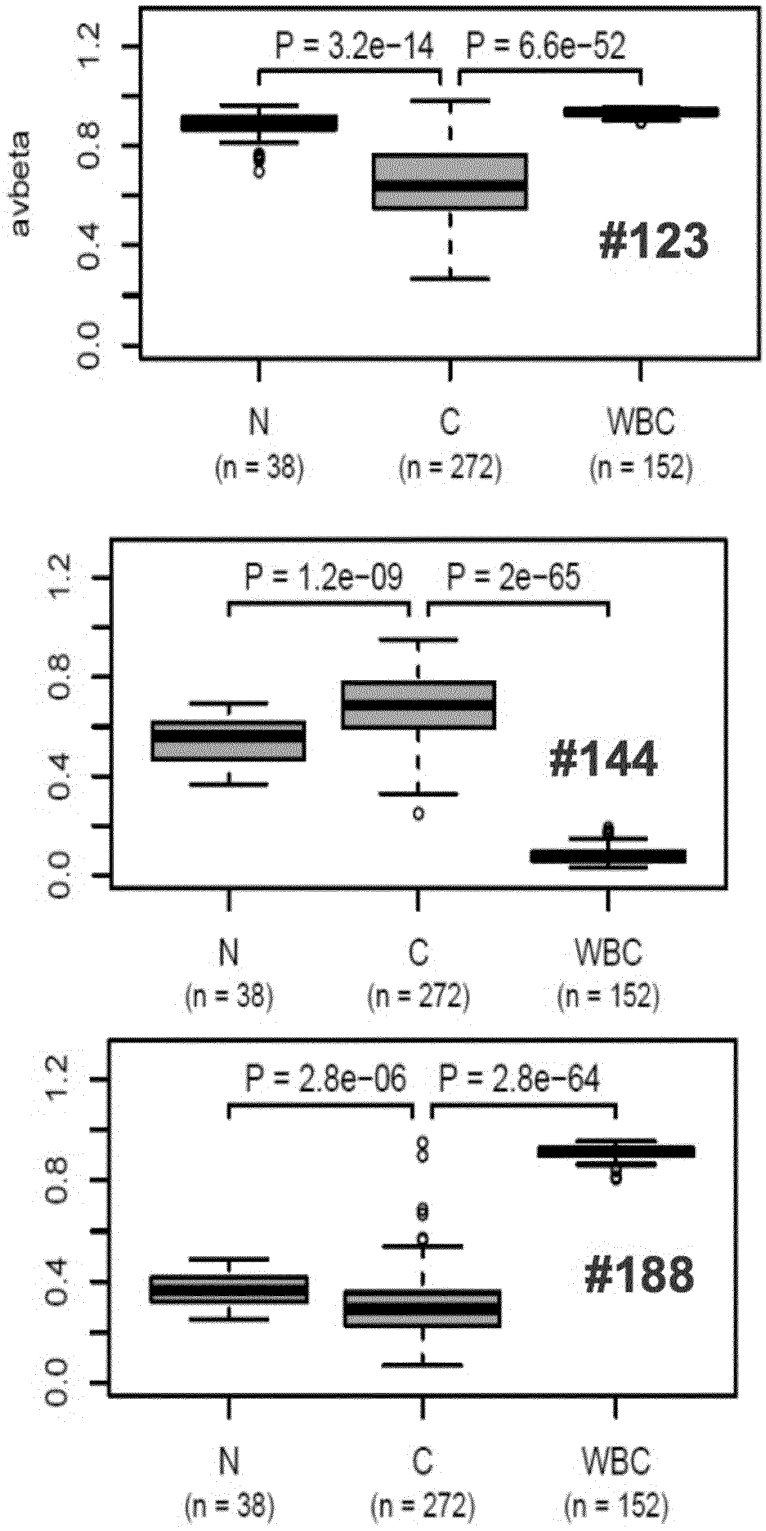

avbeta
0.0
0.4
0.8
1.2
P = 3.2e−14
P = 6.6e−52
#123
N
(n = 38)
C
(n = 272)
WBC
(n = 152)
P = 1.2e−09
P = 2e−65
#144
N
(n = 38)
C
(n = 272)
WBC
(n = 152)
P = 2.8e−06
P = 2.8e−64
#188
N
(n = 38)
C
(n = 272)
WBC
(n = 152)

Fig. 17 (cont)

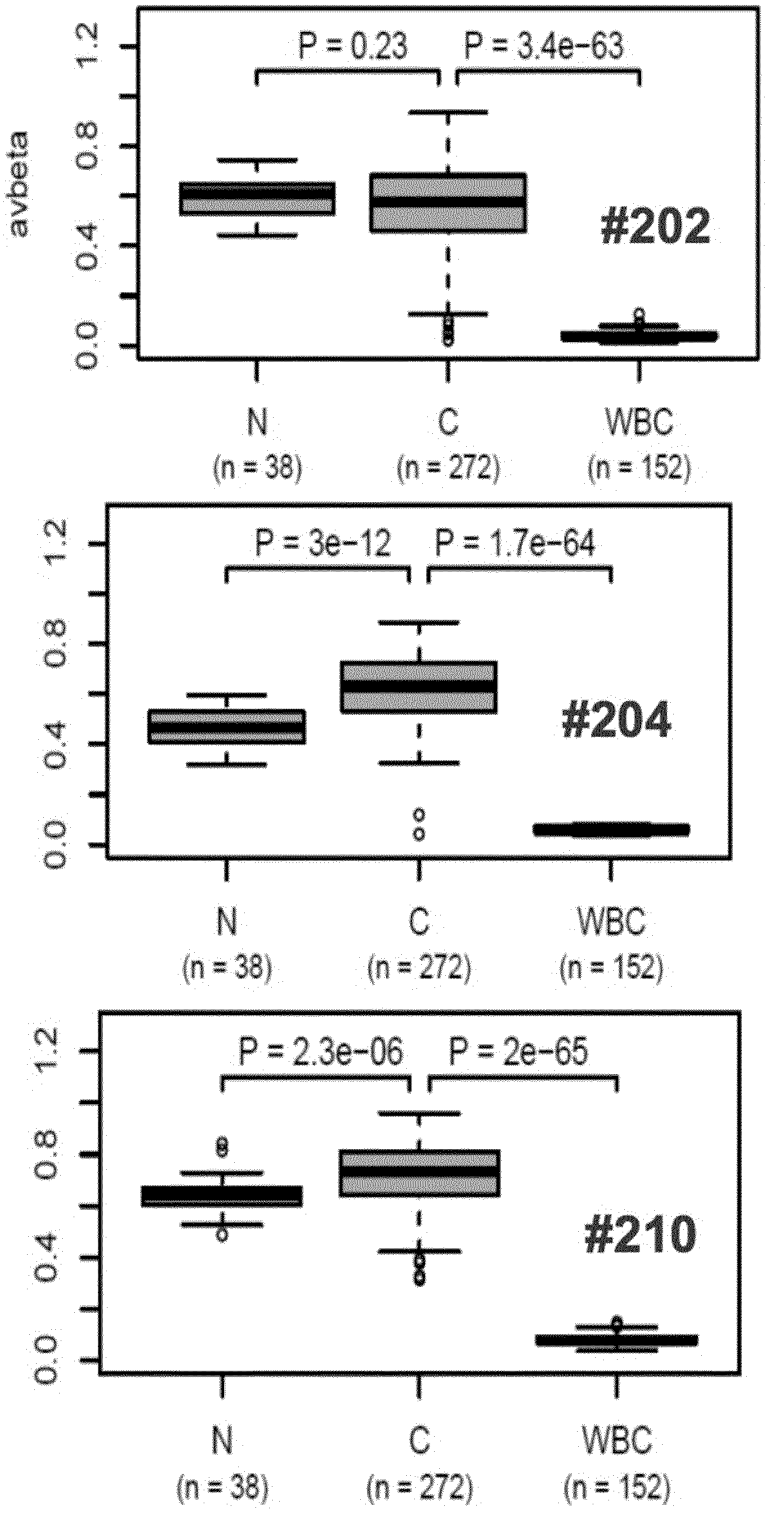

avbeta
0.0
0.4
0.8
1.2
P = 0.23
P = 3.4e−63
#202
N
(n = 38)
C
(n = 272)
WBC
(n = 152)
P = 3e−12
P = 1.7e−64
#204
P = 2.3e−06
P = 2e−65
#210

Fig. 17 (cont)

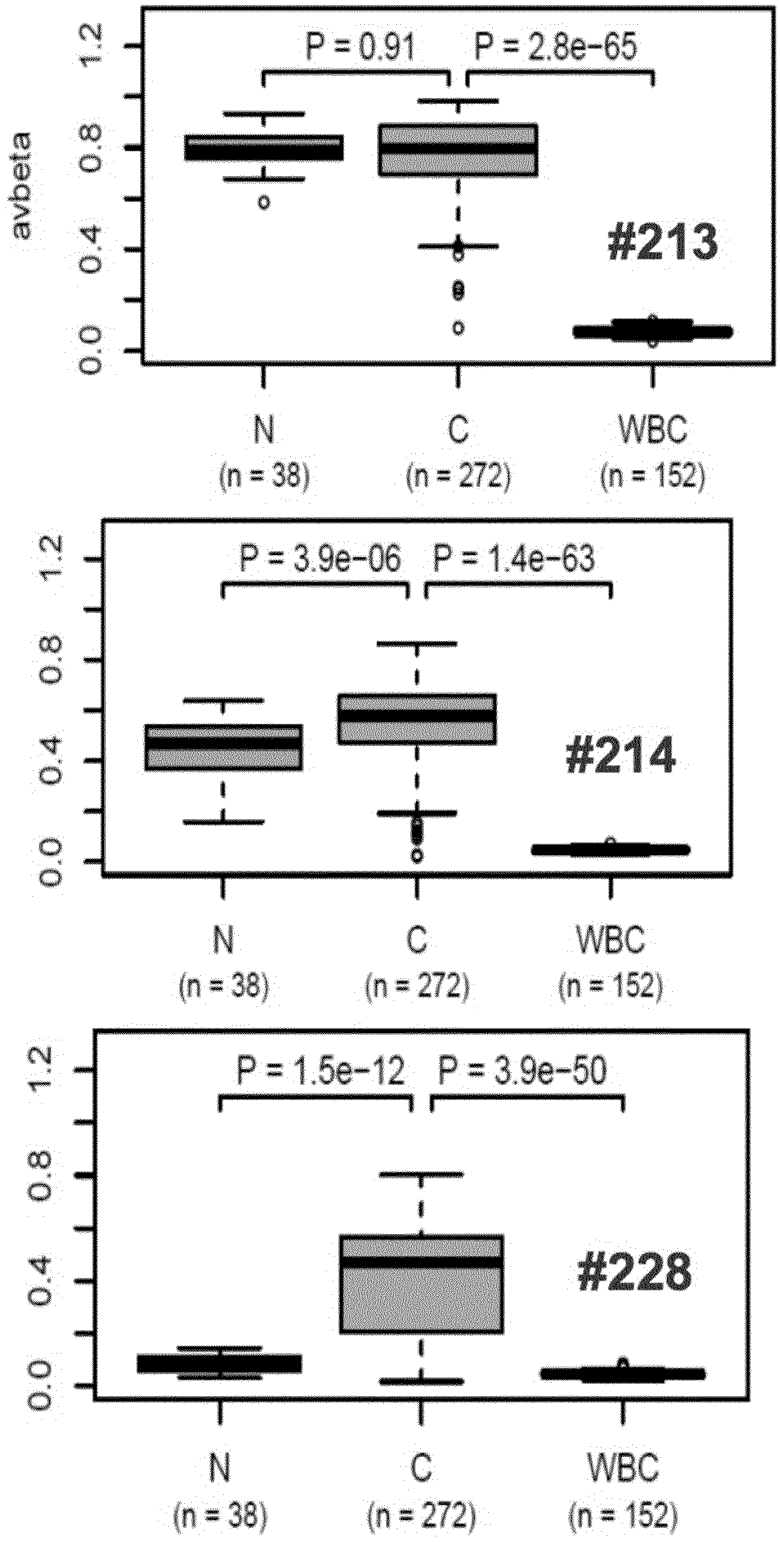

avbeta
0.0
0.4
0.8
1.2
P = 0.91
P = 2.8e−65
#213
N
(n = 38)
C
(n = 272)
WBC
(n = 152)
P = 3.9e−06
P = 1.4e−63
#214
N
(n = 38)
C
(n = 272)
WBC
(n = 152)
P = 1.5e−12
P = 3.9e−50
#228
N
(n = 38)
C
(n = 272)
WBC
(n = 152)

Fig. 18

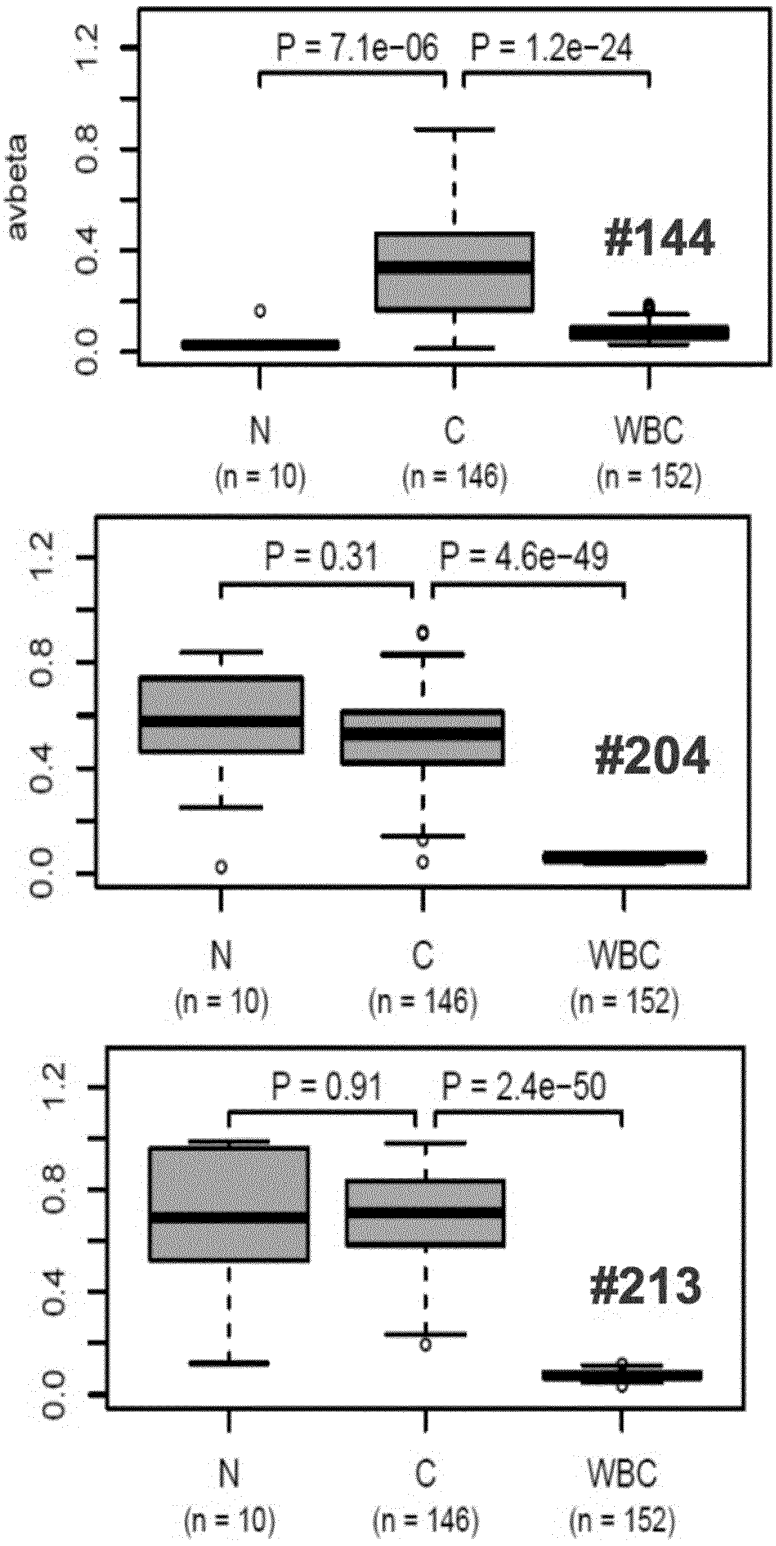

avbeta
0.0
0.4
0.8
1.2
P = 7.1e−06
P = 1.2e−24
#144
N
(n = 10)
C
(n = 146)
WBC
(n = 152)
P = 0.31
P = 4.6e−49
#204
N
(n = 10)
C
(n = 146)
WBC
(n = 152)
P = 0.91
P = 2.4e−50
#213
N
(n = 10)
C
(n = 146)
WBC
(n = 152)

Fig. 18 (cont)

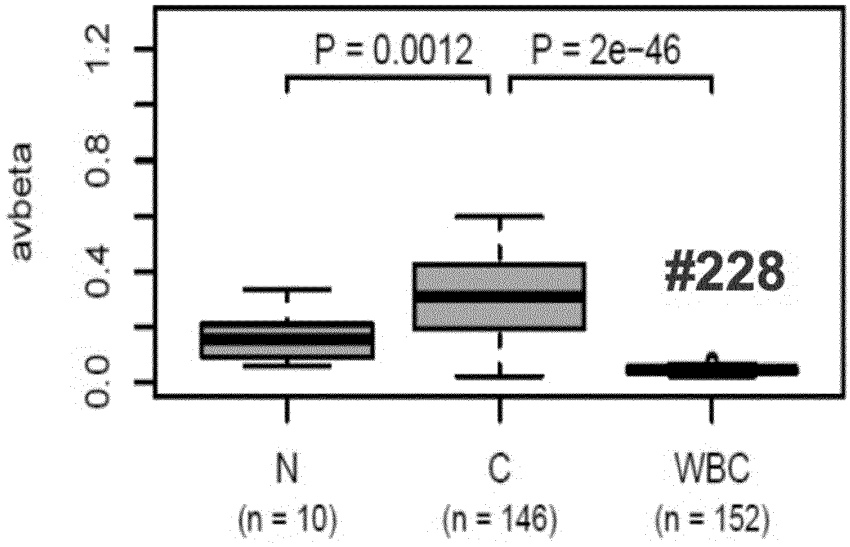


Fig. 19

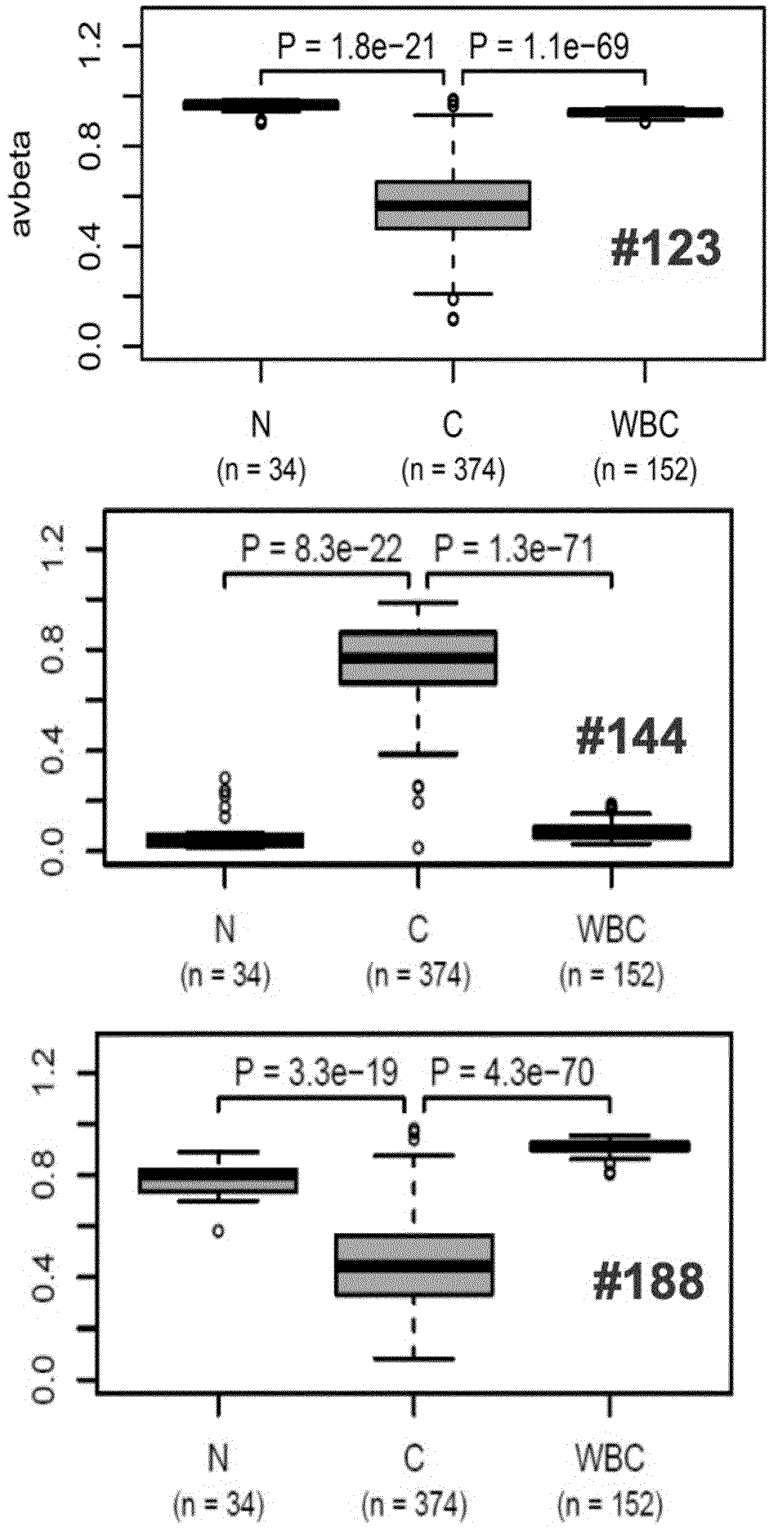

avbeta
0.0
0.4
0.8
1.2
P = 1.8e−21
P = 1.1e−69
#123
N
(n = 34)
C
(n = 374)
WBC
(n = 152)
P = 8.3e−22
P = 1.3e−71
#144
P = 3.3e−19
P = 4.3e−70
#188

Fig. 19 (cont)

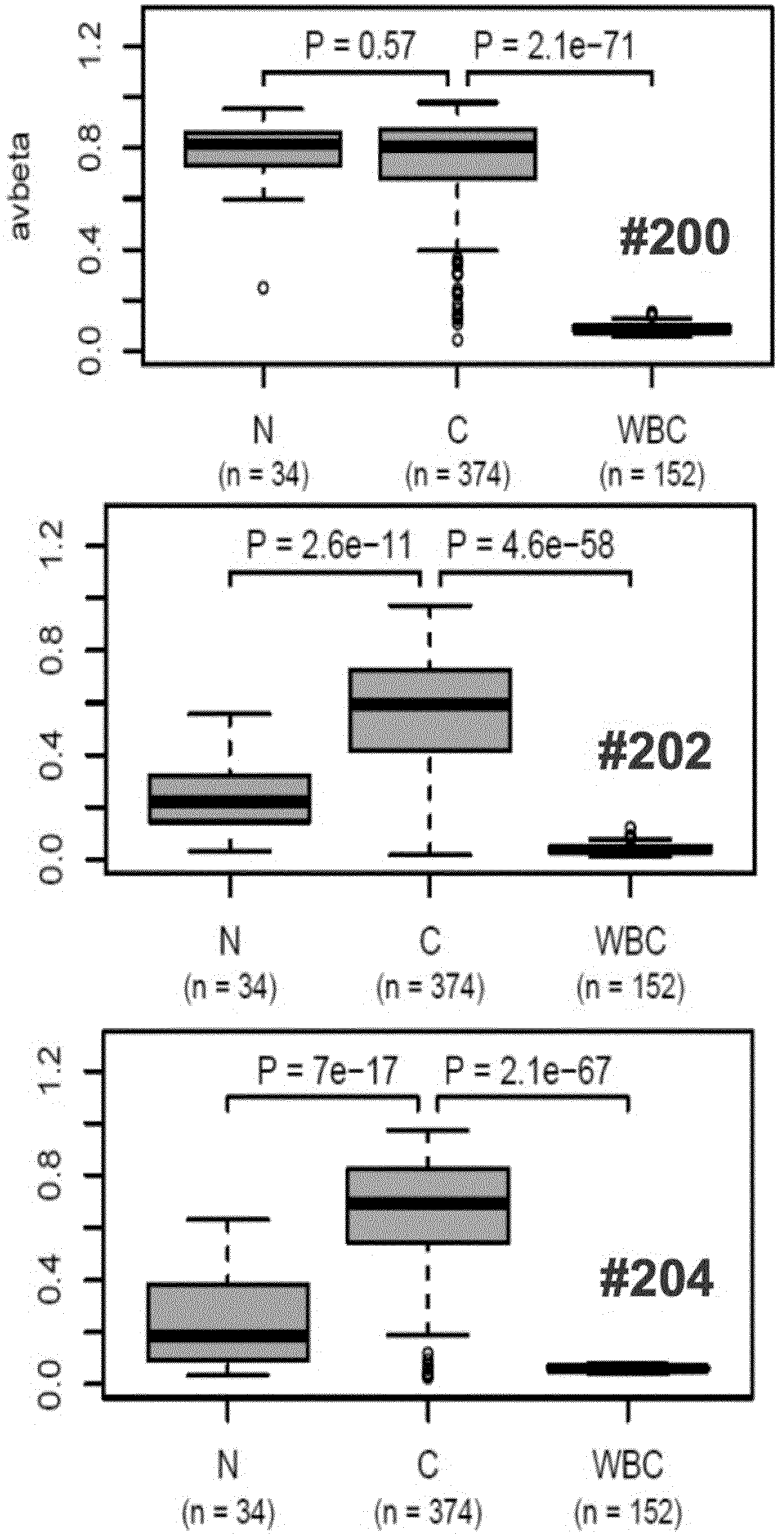

avbeta
0.0
0.4
0.8
1.2
P = 0.57
P = 2.1e−71
#200
N
(n = 34)
C
(n = 374)
WBC
(n = 152)
P = 2.6e−11
P = 4.6e−58
#202
N
(n = 34)
C
(n = 374)
WBC
(n = 152)
P = 7e−17
P = 2.1e−67
#204
N
(n = 34)
C
(n = 374)
WBC
(n = 152)

Fig. 19 (cont)

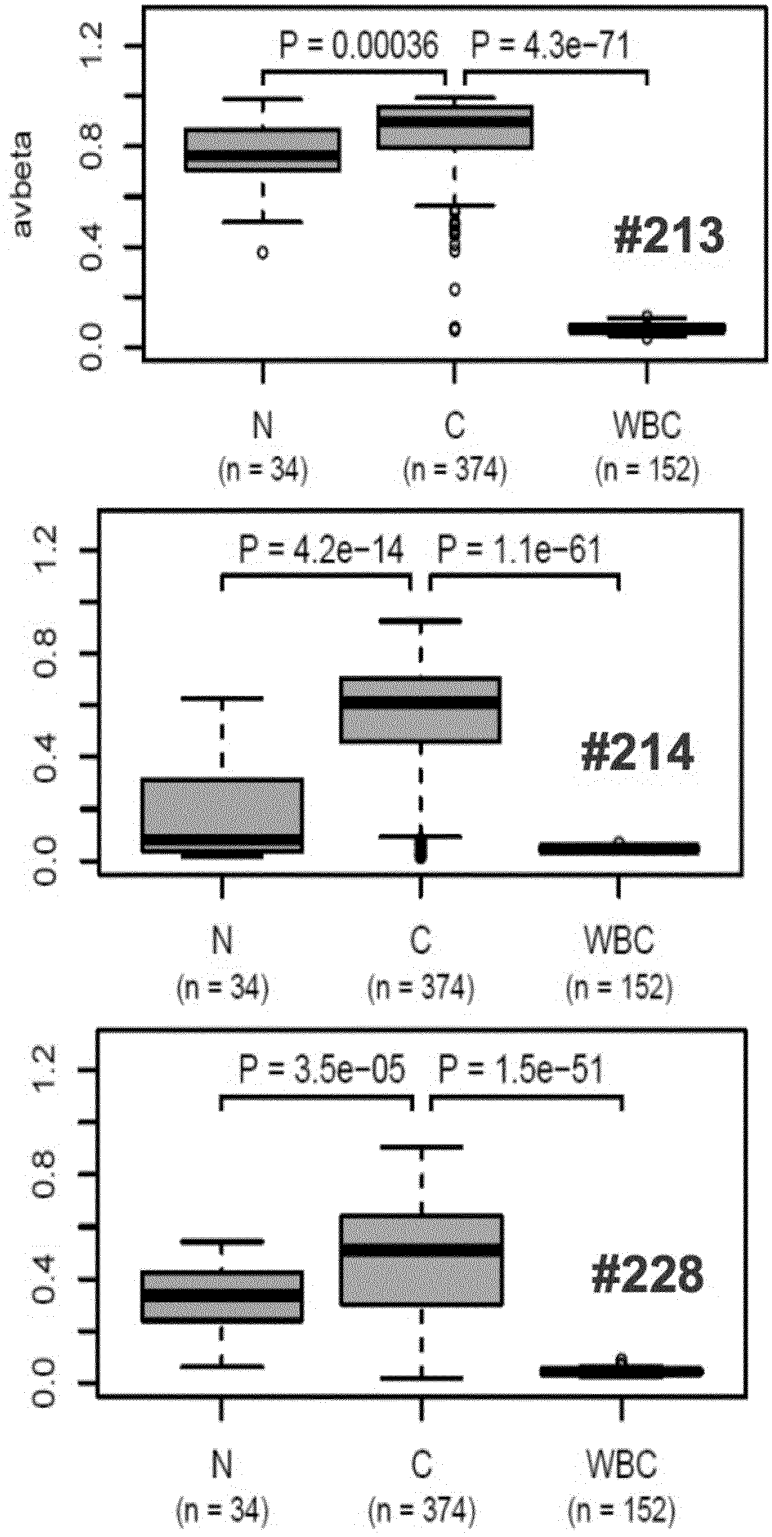

avbeta
0.0
0.4
0.8
1.2
P = 0.00036
P = 4.3e−71
#213
N
(n = 34)
C
(n = 374)
WBC
(n = 152)
P = 4.2e−14
P = 1.1e−61
#214
N
(n = 34)
C
(n = 374)
WBC
(n = 152)
P = 3.5e−05
P = 1.5e−51
#228
N
(n = 34)
C
(n = 374)
WBC
(n = 152)

Fig. 20

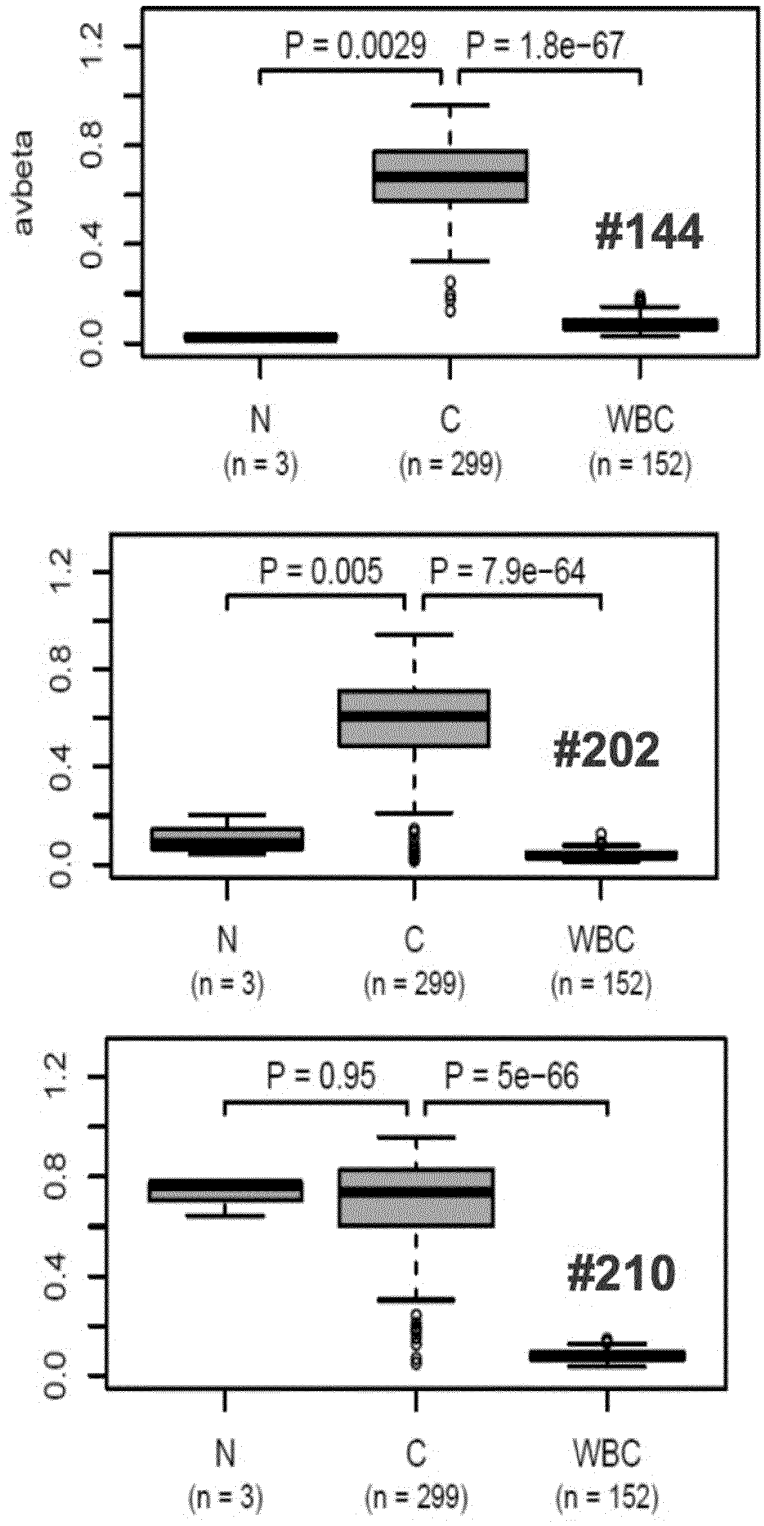

avbeta
0.0
0.4
0.8
1.2
P = 0.0029
P = 1.8e−67
#144
N
(n = 3)
C
(n = 299)
WBC
(n = 152)
P = 0.005
P = 7.9e−64
#202
N
(n = 3)
C
(n = 299)
WBC
(n = 152)
P = 0.95
P = 5e−66
#210
N
(n = 3)
C
(n = 299)
WBC
(n = 152)

Fig. 20 (cont)

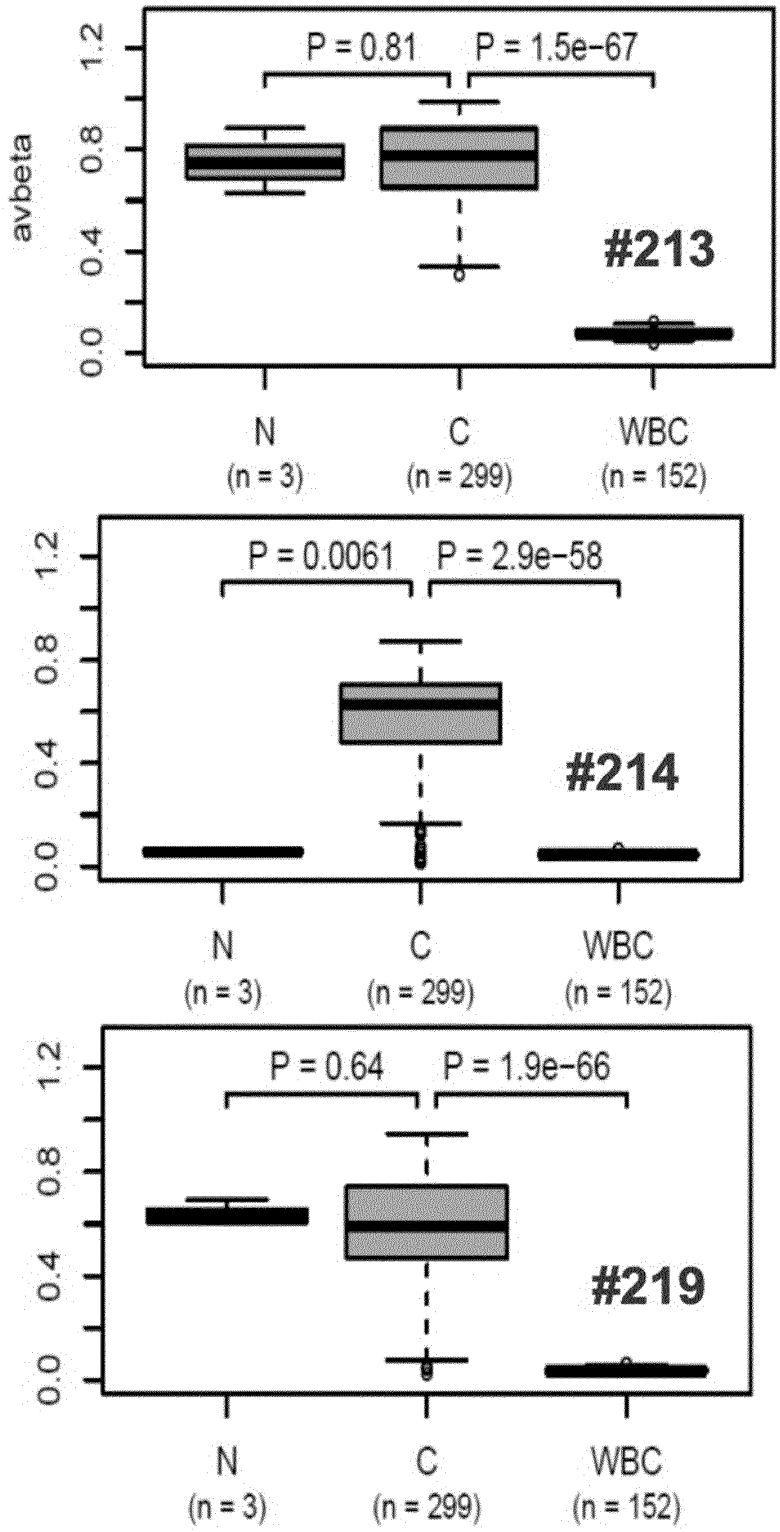

avbeta
0.0
0.4
0.8
1.2
P = 0.81
P = 1.5e−67
#213
N
(n = 3)
C
(n = 299)
WBC
(n = 152)
P = 0.0061
P = 2.9e−58
#214
P = 0.64
P = 1.9e−66
#219

Fig. 20 (cont)

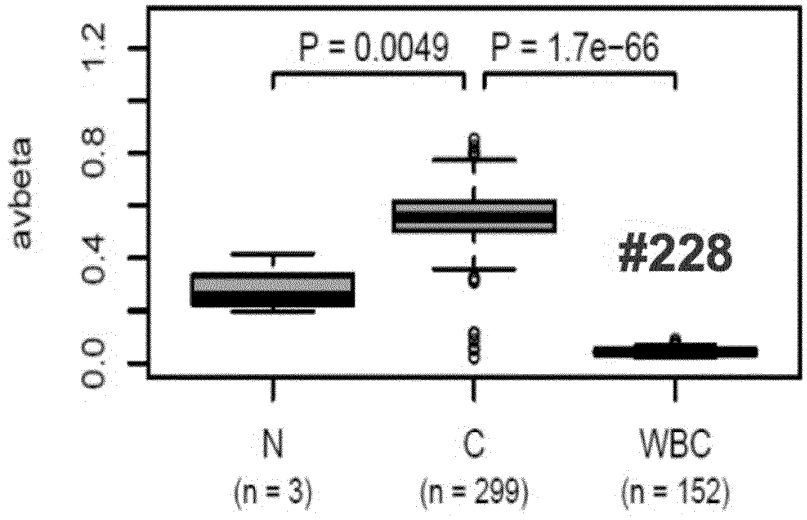


Fig. 21

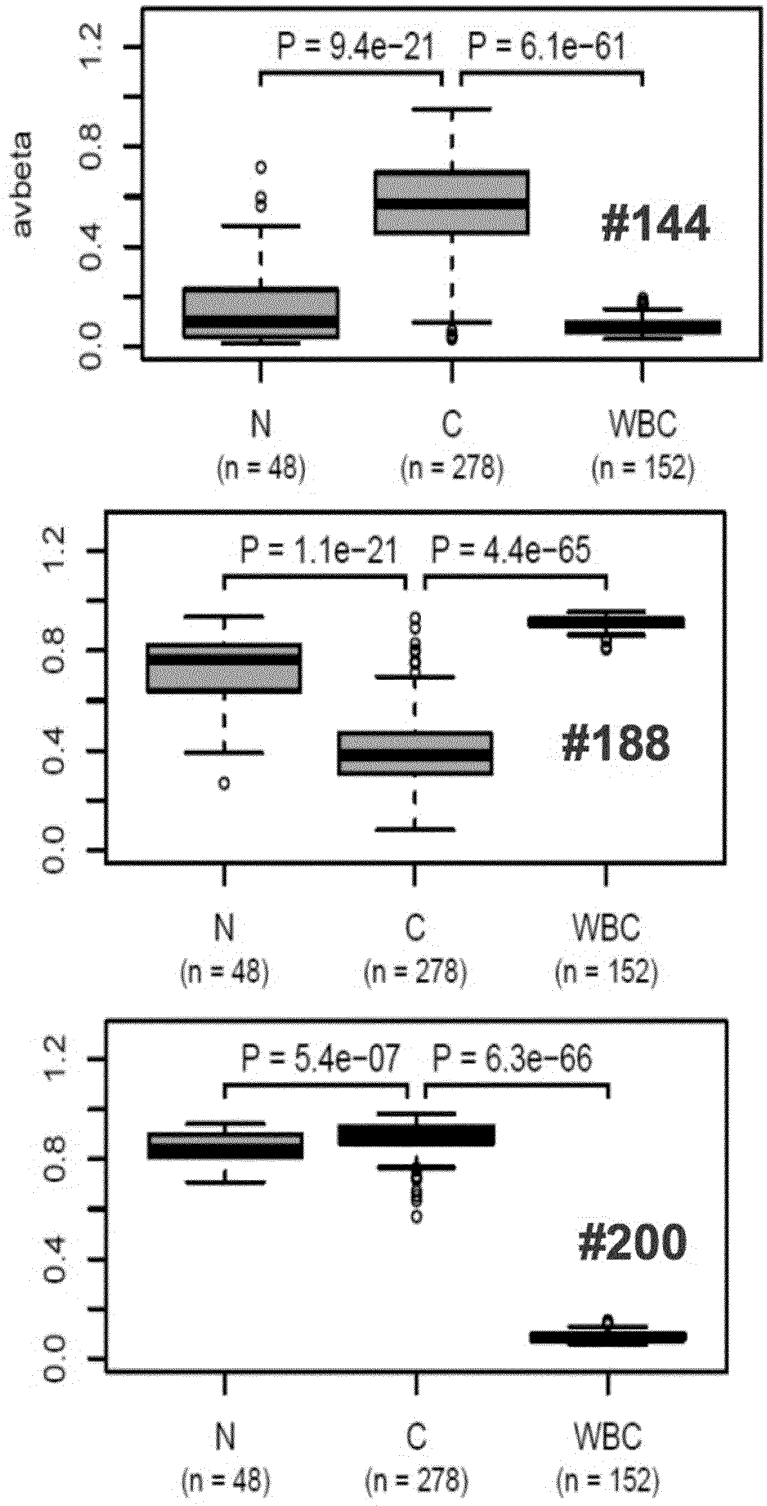

avbeta
0.0
0.4
0.8
1.2
P = 9.4e−21
P = 6.1e−61
#144
N
(n = 48)
C
(n = 278)
WBC
(n = 152)
P = 1.1e−21
P = 4.4e−65
#188
N
(n = 48)
C
(n = 278)
WBC
(n = 152)
P = 5.4e−07
P = 6.3e−66
#200
N
(n = 48)
C
(n = 278)
WBC
(n = 152)

Fig. 21 (cont)

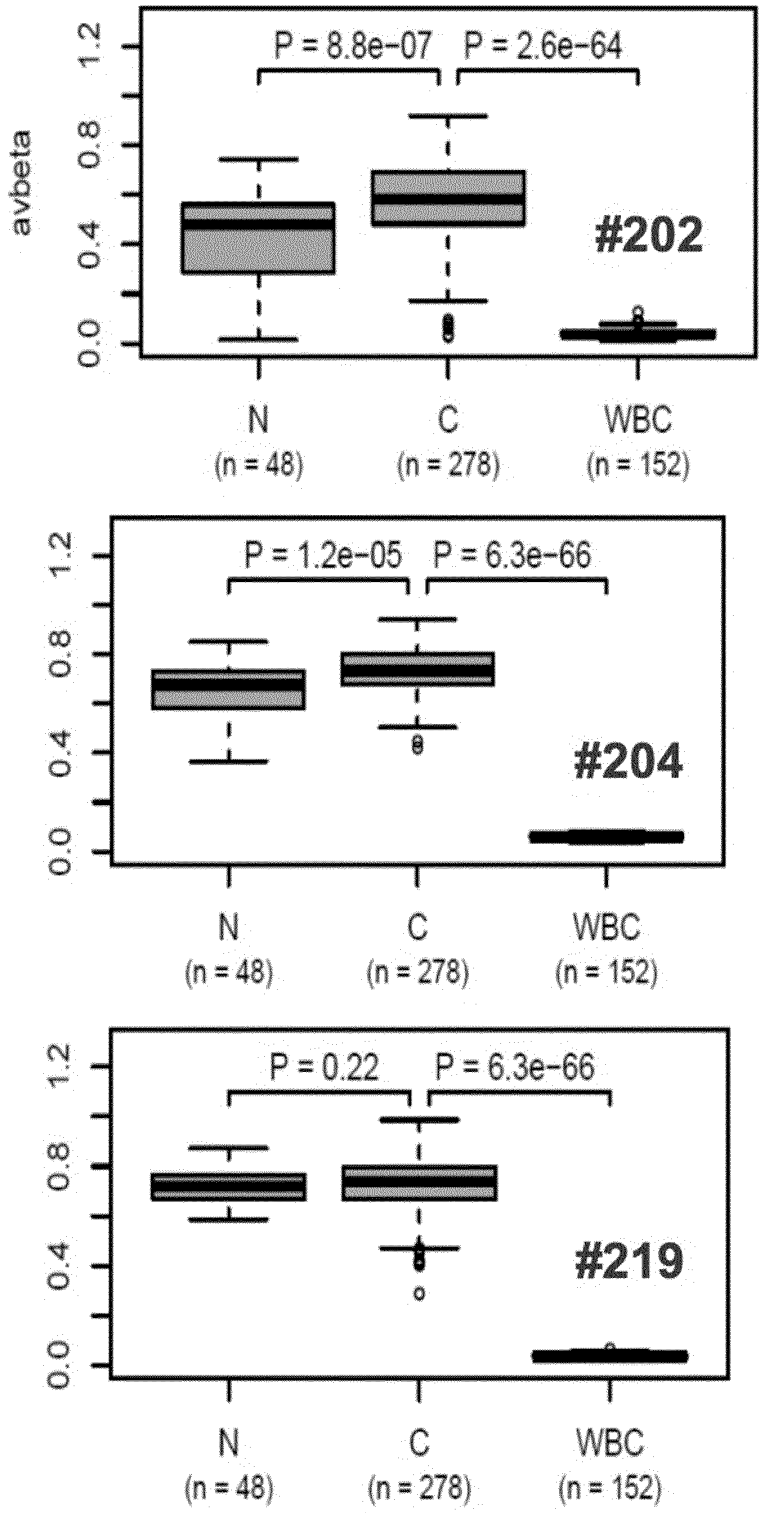

avbeta
0.0
0.4
0.8
1.2
P = 8.8e-07
P = 2.6e-64
#202
N
(n = 48)
C
(n = 278)
WBC
(n = 152)
P = 1.2e-05
P = 6.3e-66
#204
N
(n = 48)
C
(n = 278)
WBC
(n = 152)
P = 0.22
P = 6.3e-66
#219
N
(n = 48)
C
(n = 278)
WBC
(n = 152)

Fig. 21 (cont)

