FIG. 1

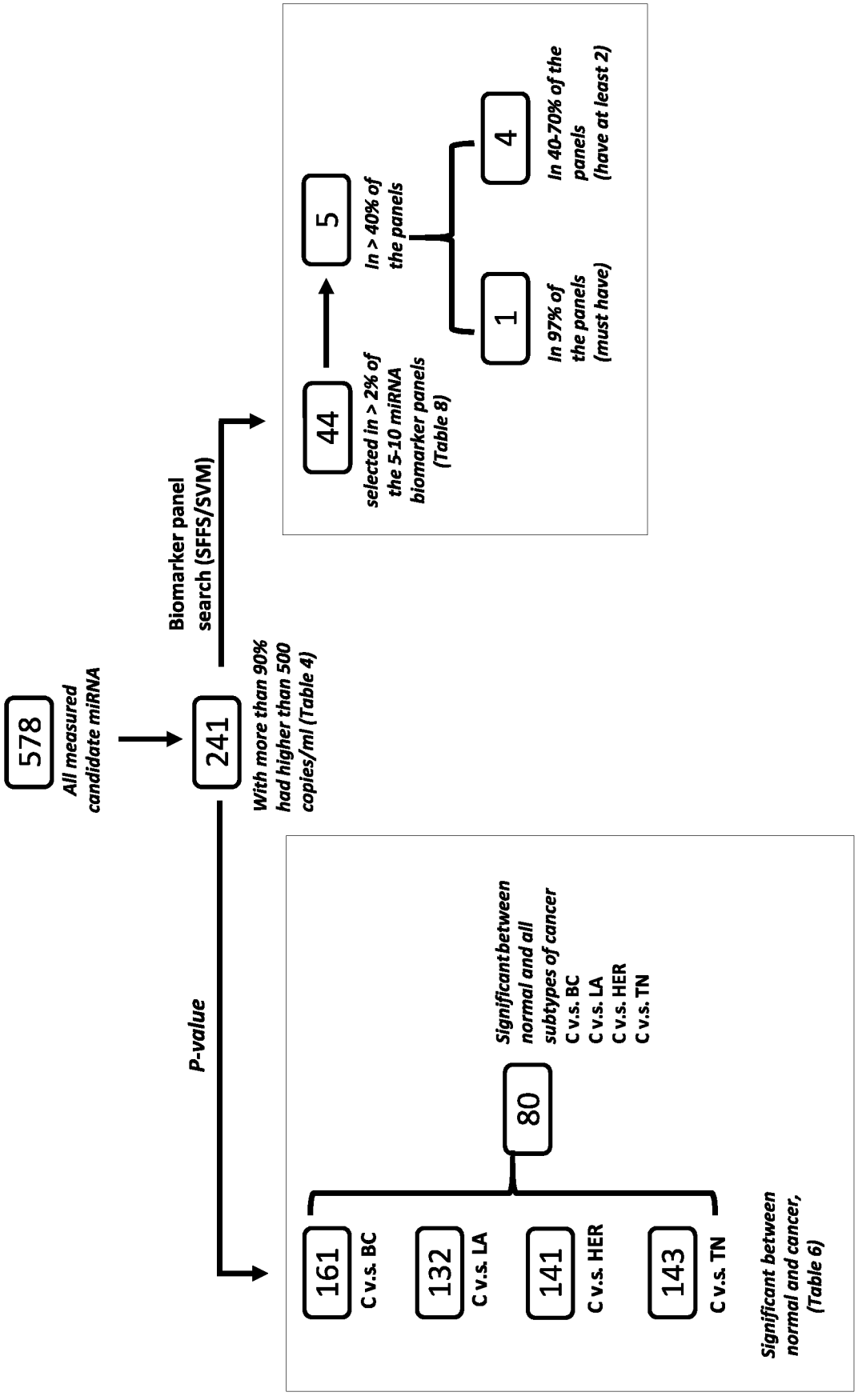

578
All measured candidate miRNA
241
With more than 90% had higher than 500 copies/ml (Table 4)
Biomarker panel search (SFFS/SVM)
44
selected in > 2% of the 5-10 miRNA biomarker panels (Table 8)
5
In > 40% of the panels
1
In 97% of the panels (must have)
4
In 40-70% of the panels (have at least 2)
P-value
161
C v.s. BC
132
C v.s. LA
141
C v.s. HER
143
C v.s. TN
80
Significant between normal and all subtypes of cancer
C v.s. BC
C v.s. LA
C v.s. HER
C v.s. TN
Significant between normal and cancer, (Table 6)

FIG. 2

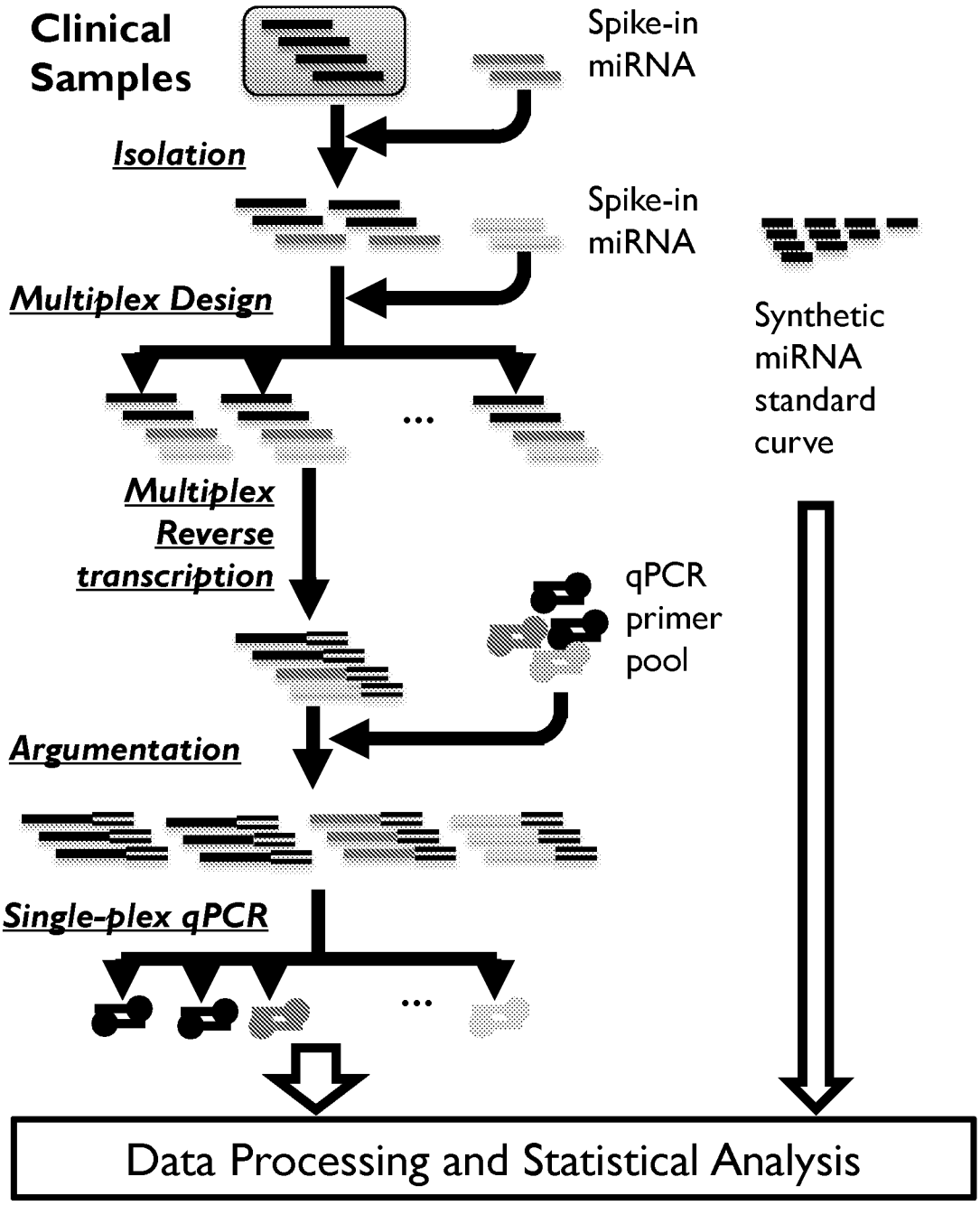
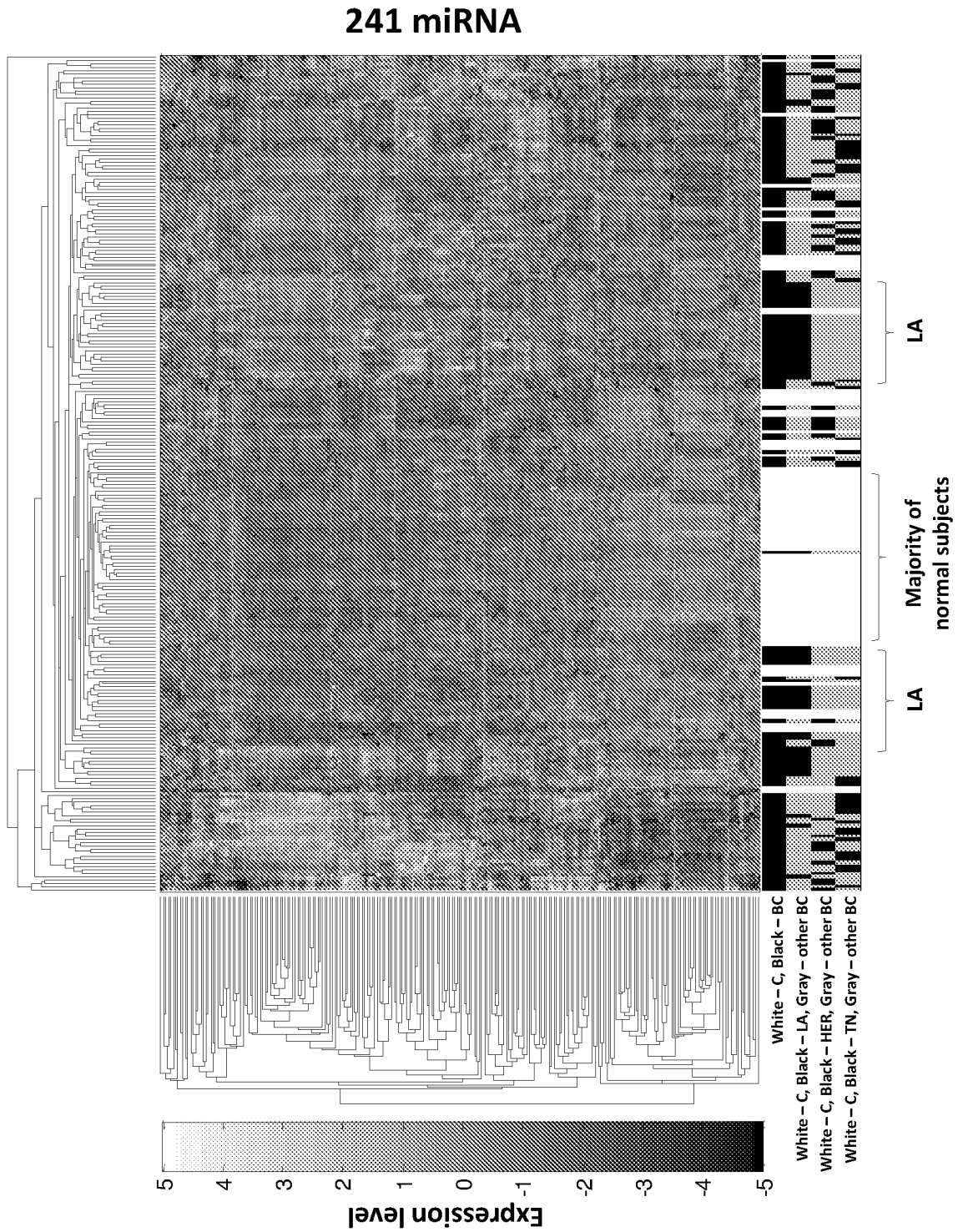

Clinical Samples
Spike-in miRNA
Isolation
Spike-in miRNA
Multiplex Design
Synthetic miRNA standard curve
Multiplex Reverse transcription
qPCR primer pool
Argumentation
Single-plex qPCR
Data Processing and Statistical Analysis

FIG. 3


241 miRNA
Expression level
5
4
3
2
1
0
-1
-2
-3
-4
-5
White – C, Black – BC
White – C, Black – LA, Gray – other BC
White – C, Black – HER, Gray – other BC
White – C, Black – TN, Gray – other BC
LA
Majority of normal subjects
LA

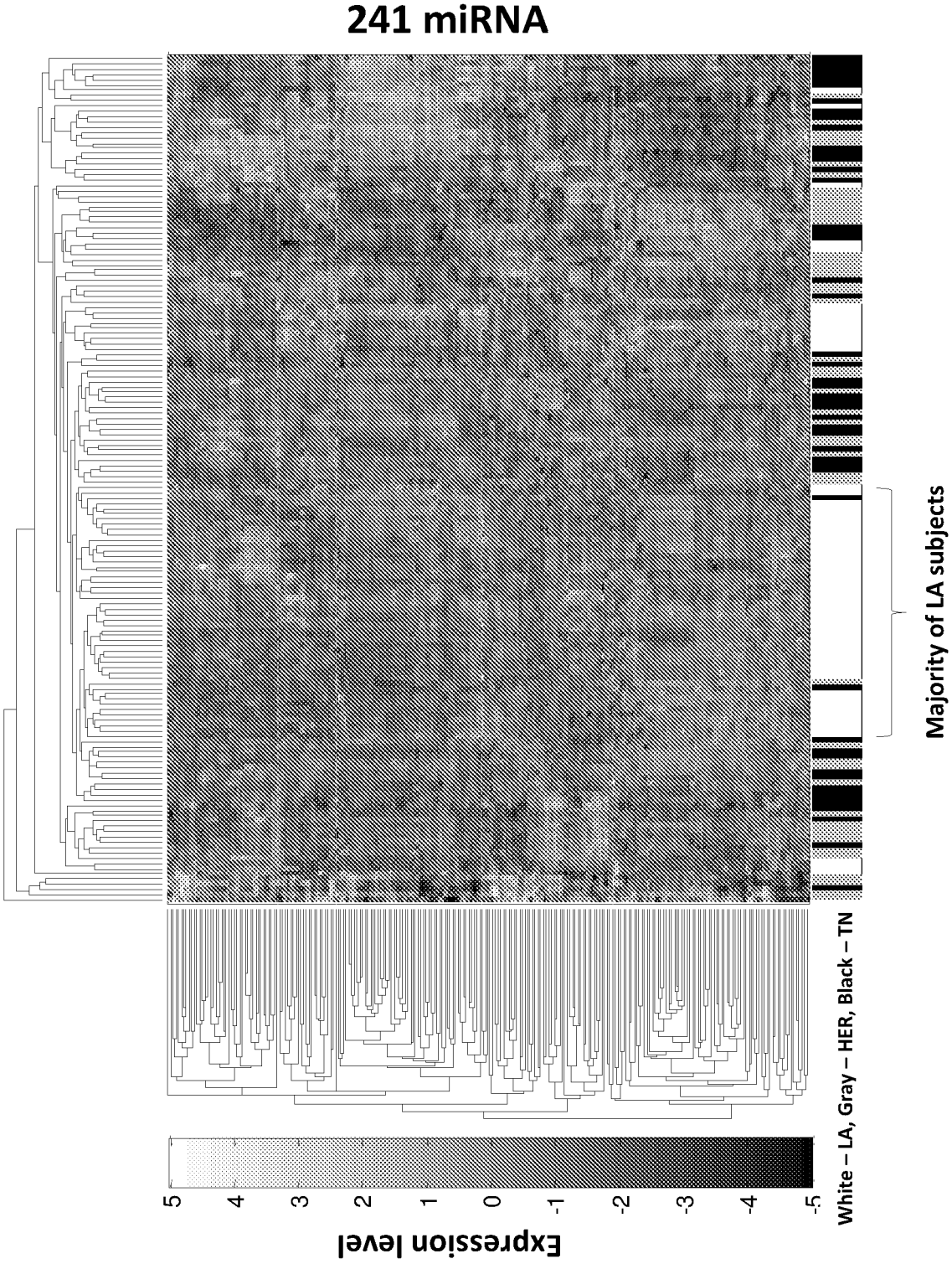

241 miRNA
Expression level
5
4
3
2
1
0
-1
-2
-3
-4
-5
White – LA, Gray – HER, Black – TN
Majority of LA subjects

FIG. 4

FIG. 5

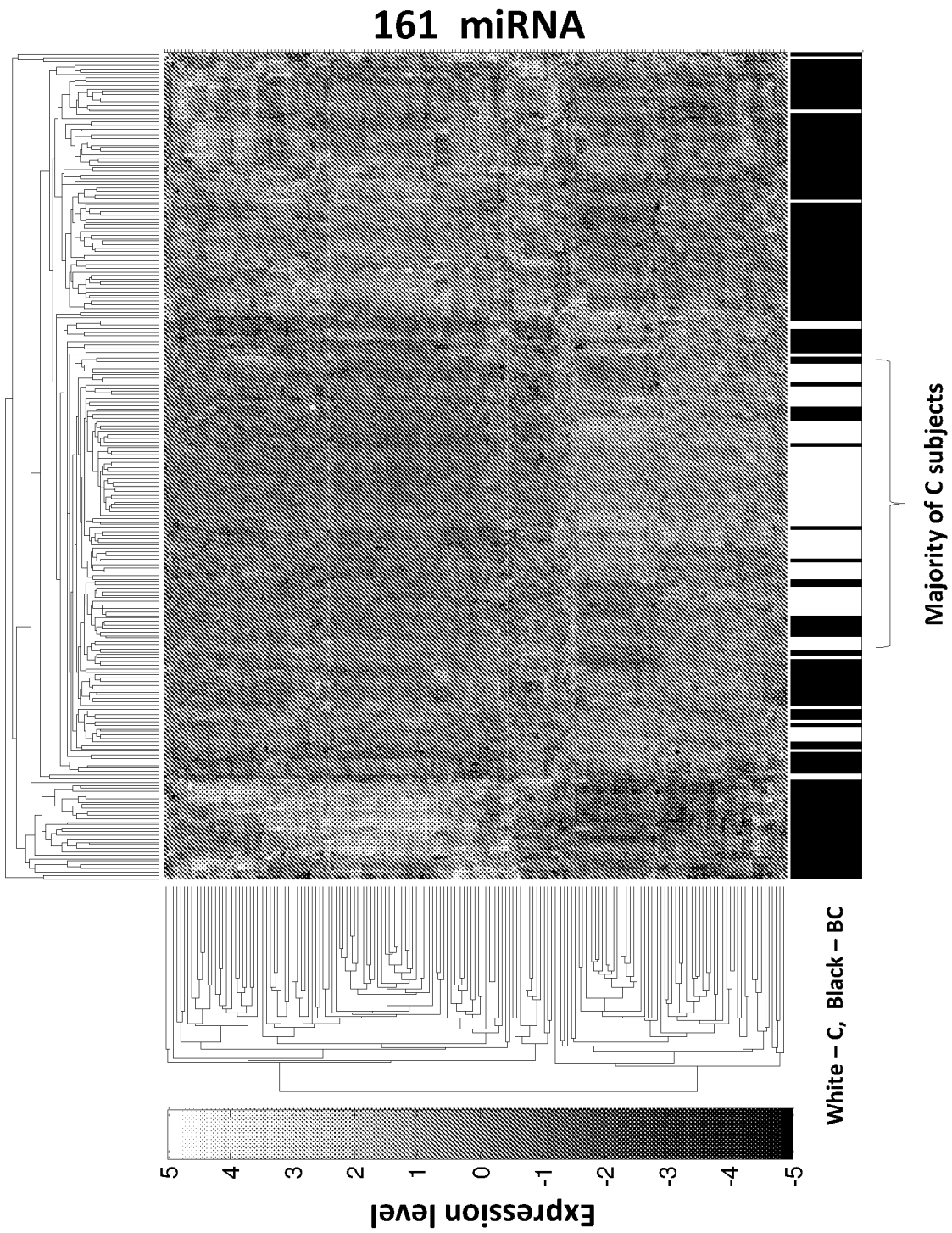

161 miRNA
Majority of C subjects
White – C, Black – BC
Expression level
5
4
3
2
1
0
-1
-2
-3
-4
-5

FIG. 6

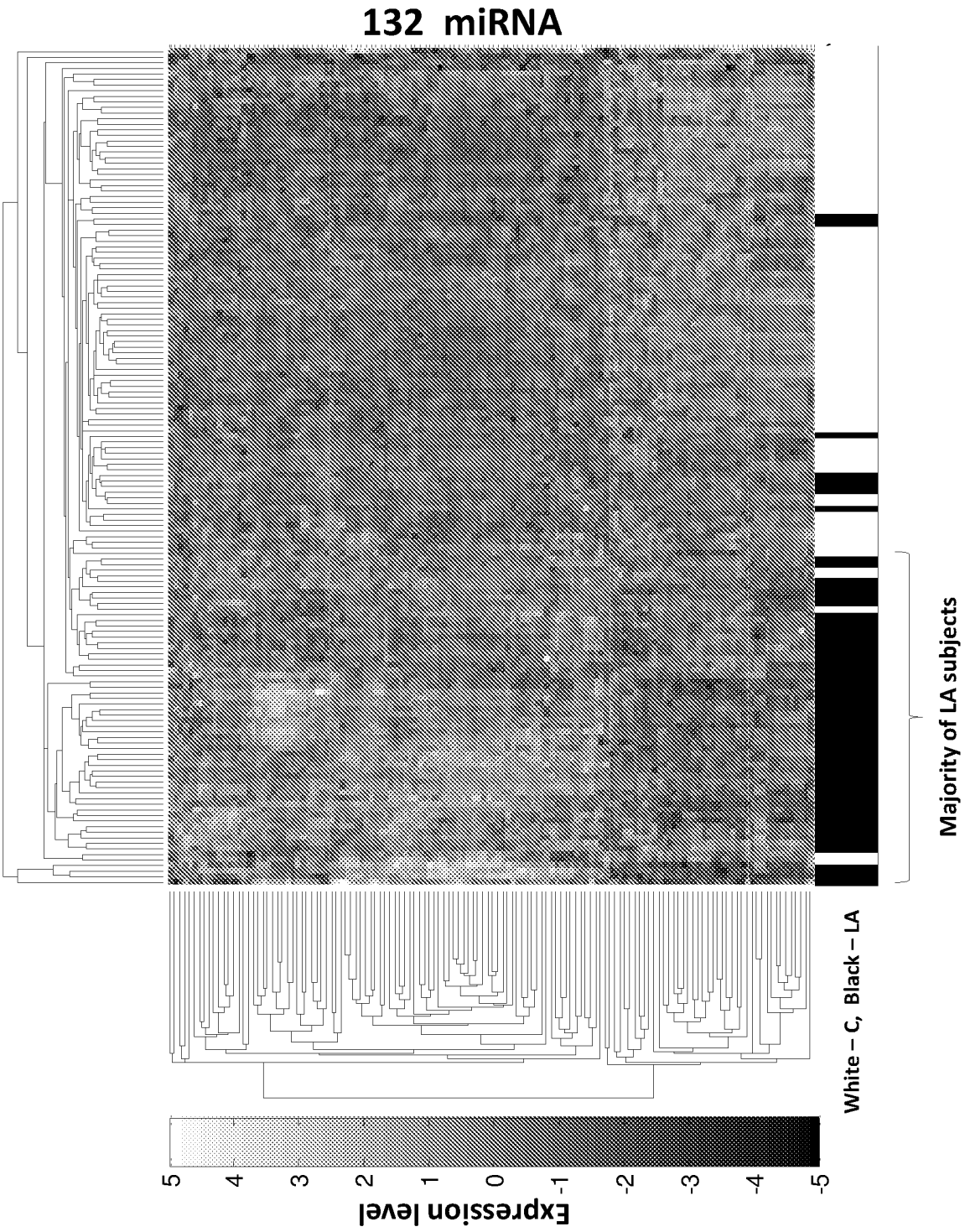

132 miRNA
Expression level
5
4
3
2
1
0
-1
-2
-3
-4
-5
White – C, Black – LA
Majority of LA subjects

FIG. 7

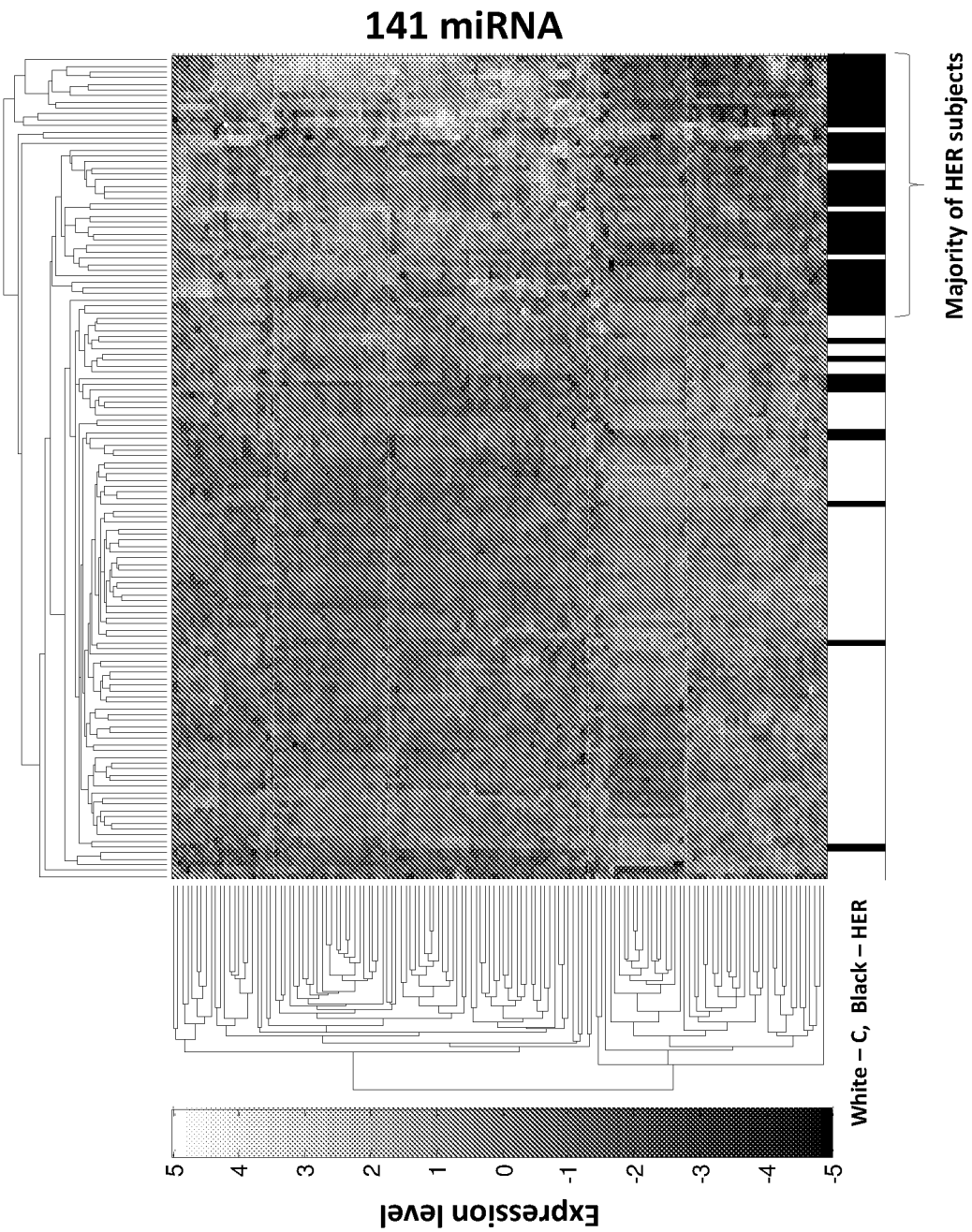

141 miRNA
Majority of HER subjects
White – C, Black – HER
Expression level
5
4
3
2
1
0
-1
-2
-3
-4
-5

FIG. 8

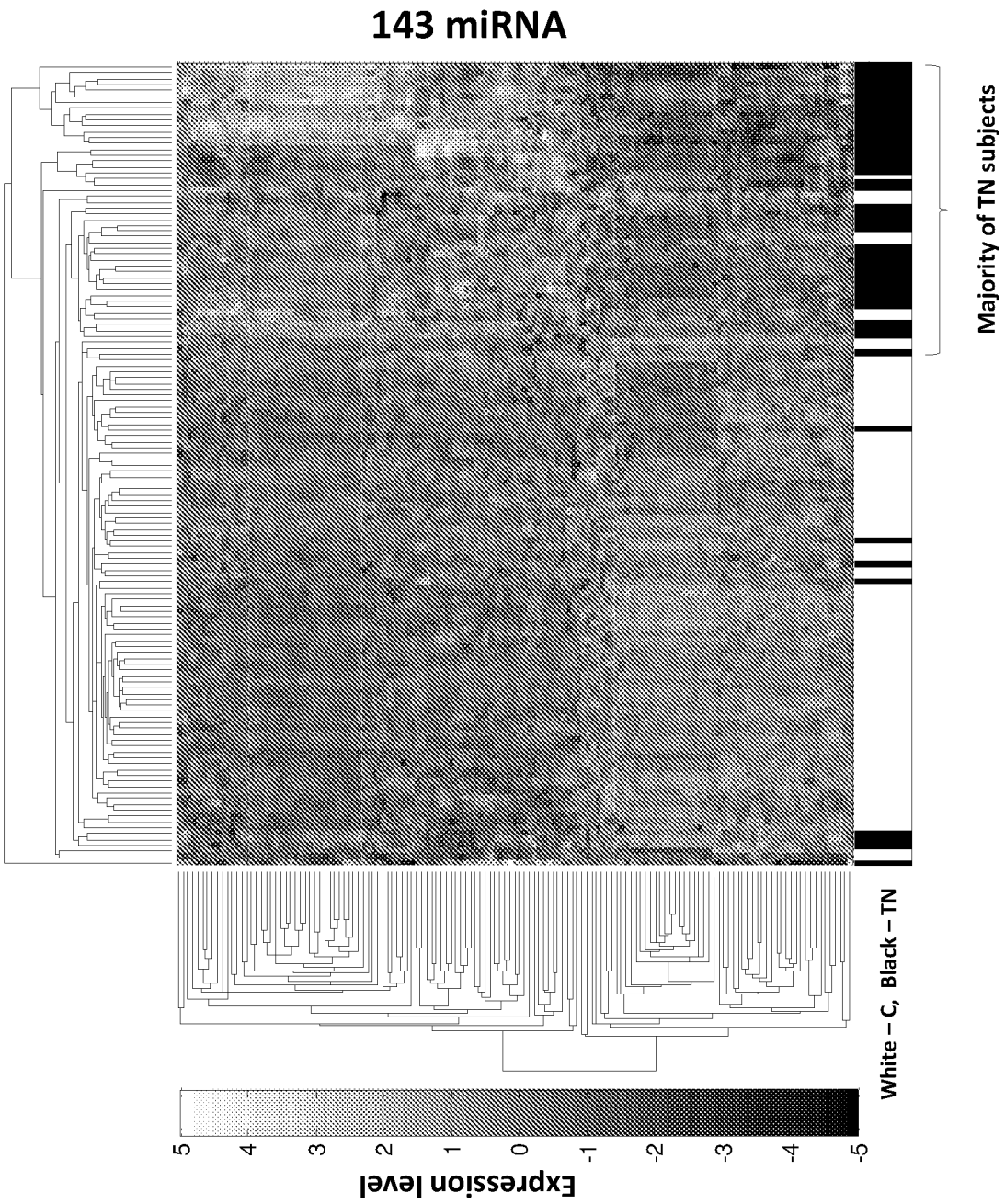

143 miRNA
Majority of TN subjects
White – C, Black – TN
Expression level
5
4
3
2
1
0
-1
-2
-3
-4
-5

FIG. 9

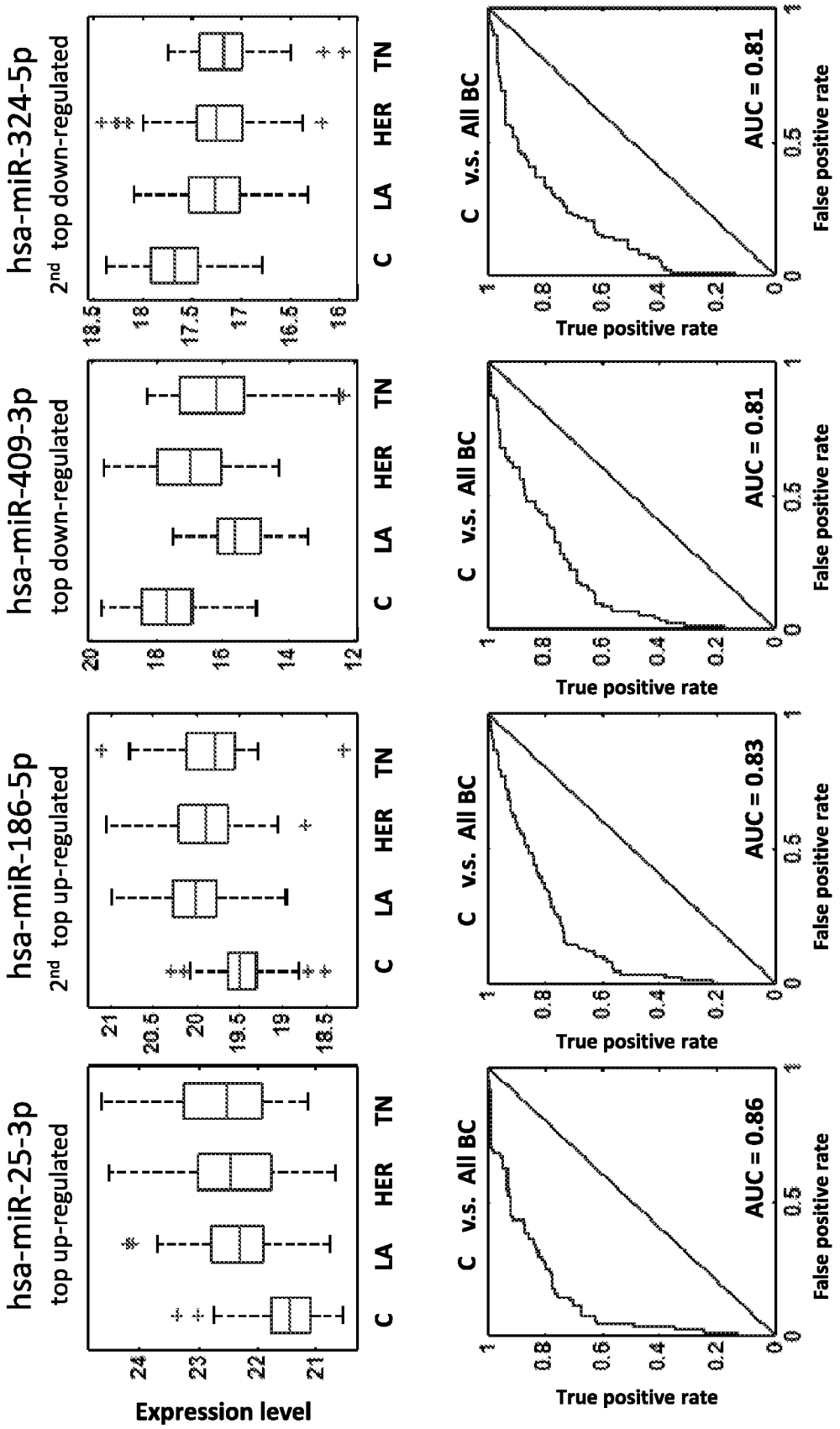

hsa-miR-25-3p
top up-regulated
hsa-miR-186-5p
2nd top up-regulated
hsa-miR-409-3p
top down-regulated
hsa-miR-324-5p
2nd top down-regulated
C LA HER TN
Expression level
C v.s. All BC
AUC = 0.86
AUC = 0.83
AUC = 0.81
AUC = 0.81
True positive rate
False positive rate

FIG. 10

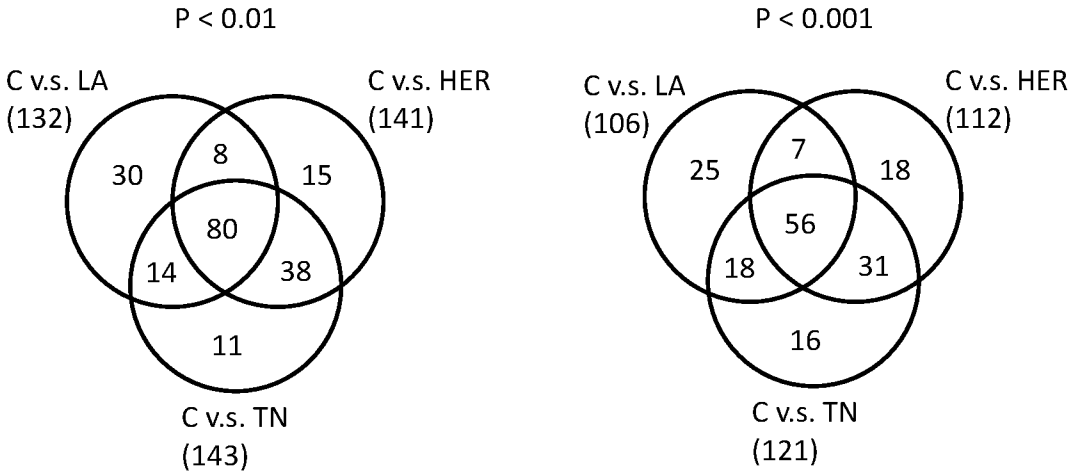

P < 0.01
C v.s. LA
(132)
C v.s. HER
(141)
30
8
15
80
14
38
11
C v.s. TN
(143)
P < 0.001
C v.s. LA
(106)
C v.s. HER
(112)
25
7
18
56
18
31
16
C v.s. TN
(121)

FIG. 11

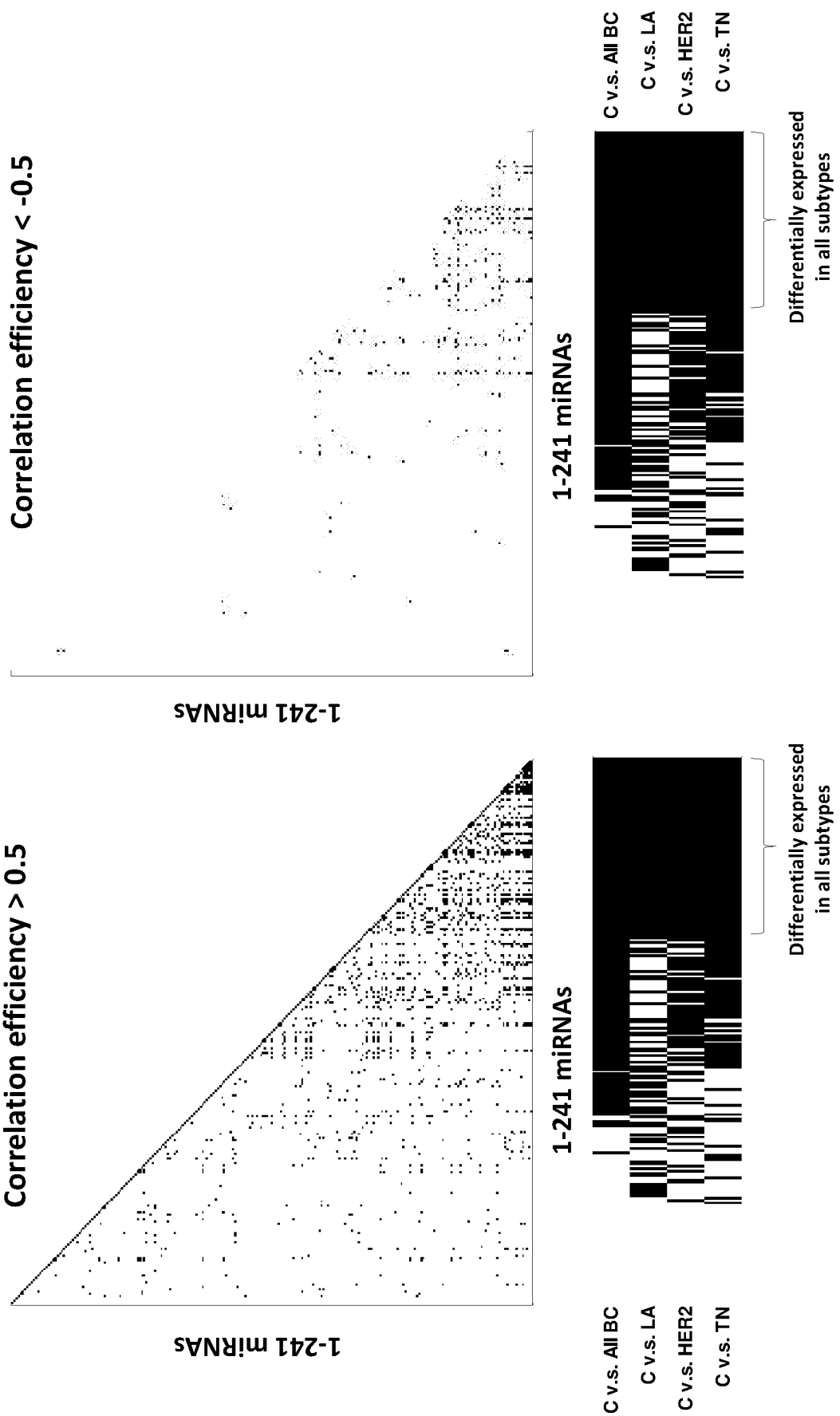

Correlation efficiency > 0.5
1-241 miRNAs
1-241 miRNAs
1-241 miRNAs
C v.s. All BC
C v.s. LA
C v.s. HER2
C v.s. TN
Differentially expressed in all subtypes
Correlation efficiency < -0.5
1-241 miRNAs
1-241 miRNAs
C v.s. All BC
C v.s. LA
C v.s. HER2
C v.s. TN
Differentially expressed in all subtypes

FIG. 12

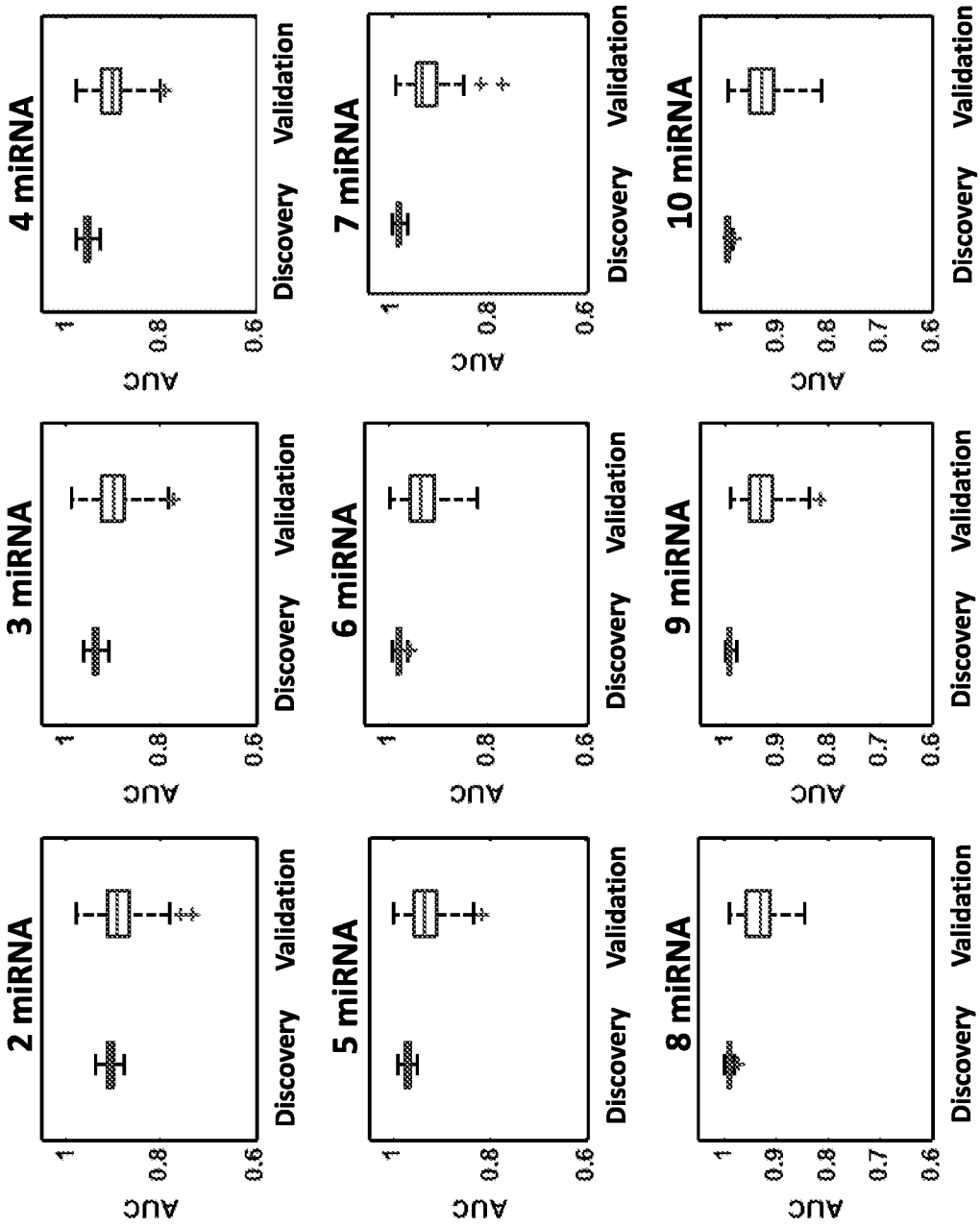

2 miRNA
3 miRNA
4 miRNA
5 miRNA
6 miRNA
7 miRNA
8 miRNA
9 miRNA
10 miRNA
AUC
Discovery
Validation

FIG. 13

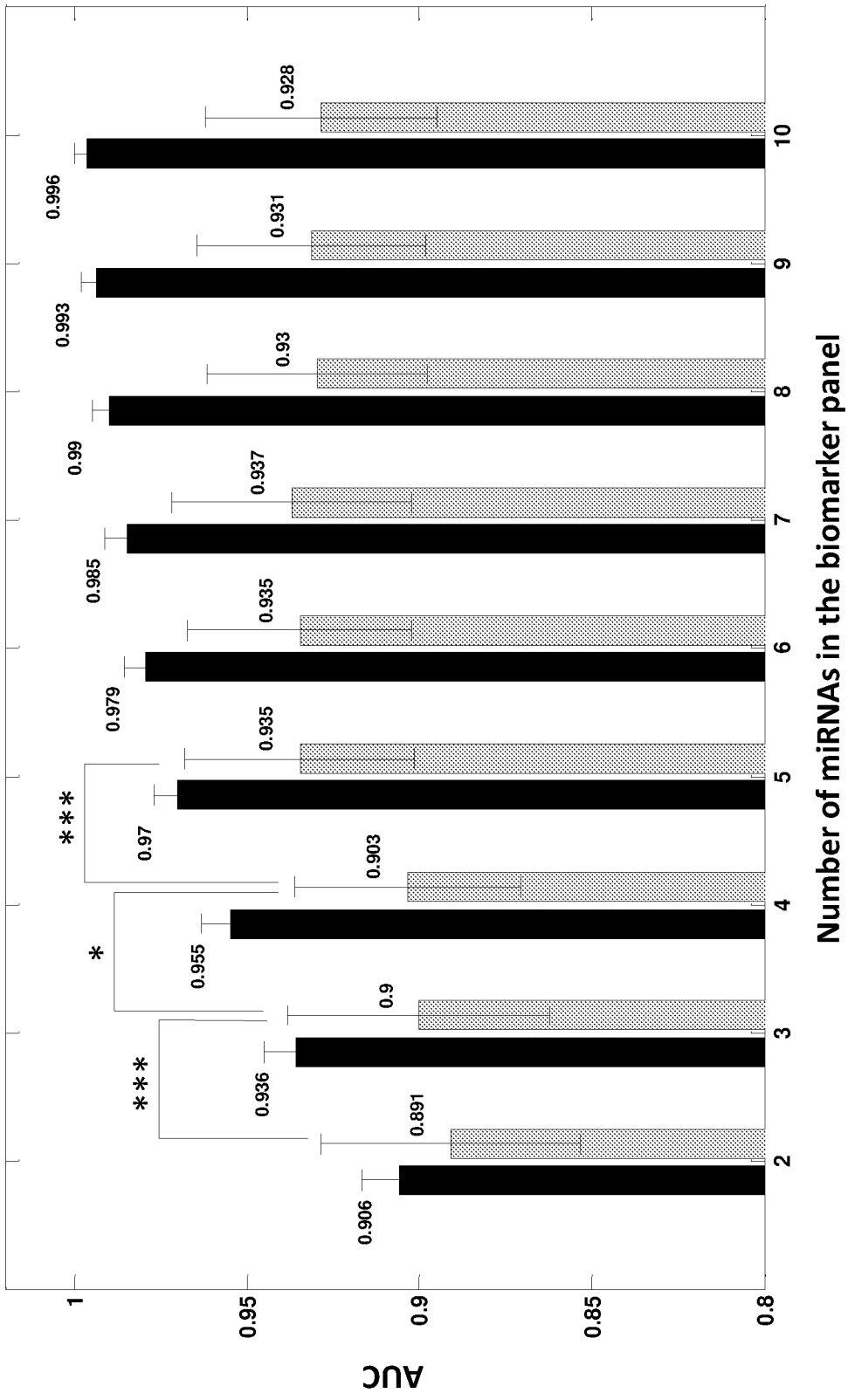

AUC
1
0.95
0.9
0.85
0.8
0.906
0.891
0.936
0.9
0.955
0.903
0.97
0.935
0.979
0.935
0.985
0.937
0.99
0.93
0.993
0.931
0.996
0.928
***
***
*
2
3
4
5
6
7
8
9
10
Number of miRNAs in the biomarker panel

FIG. 14

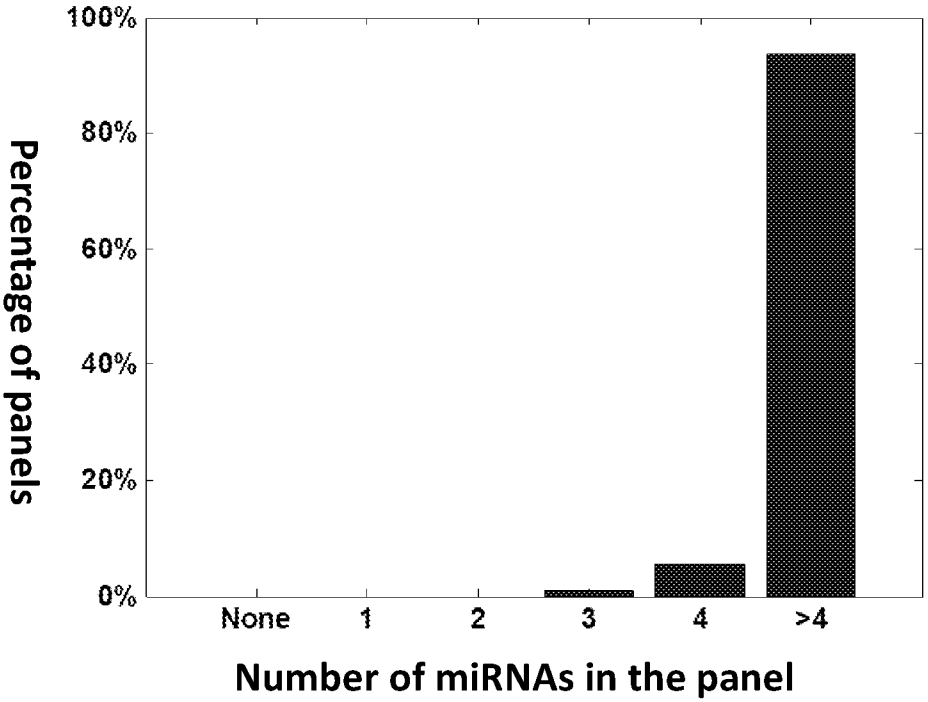

100%
80%
60%
40%
20%
0%
Percentage of panels
None
1
2
3
4
>4
Number of miRNAs in the panel

FIG. 15

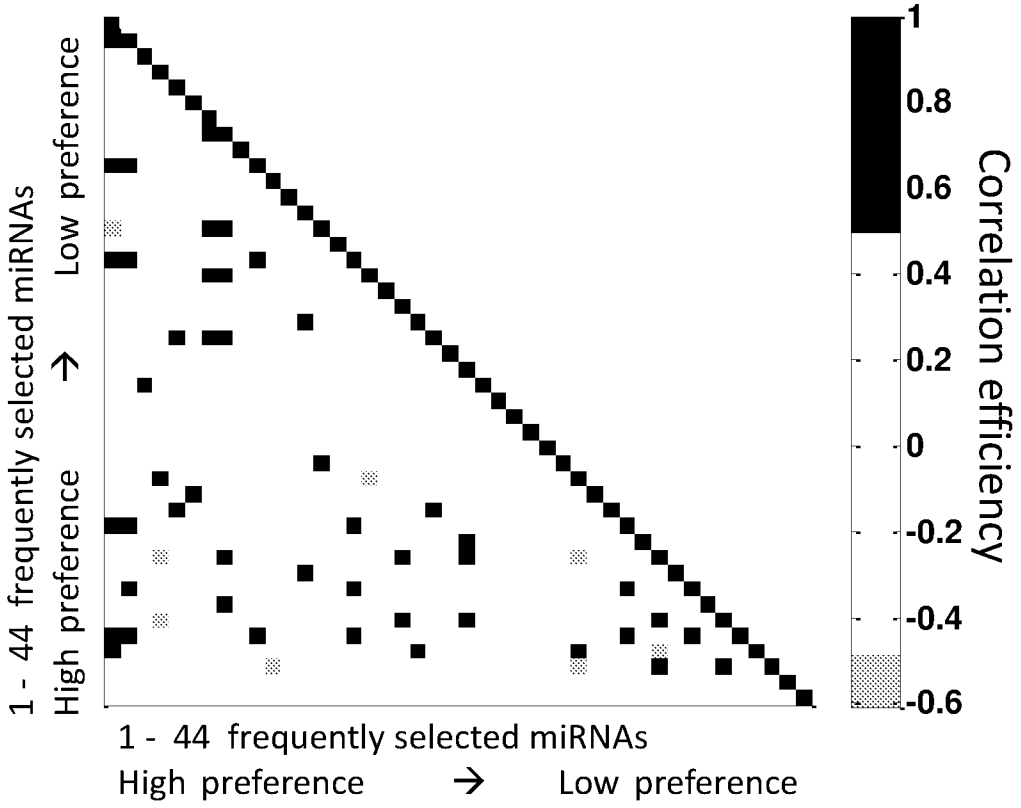
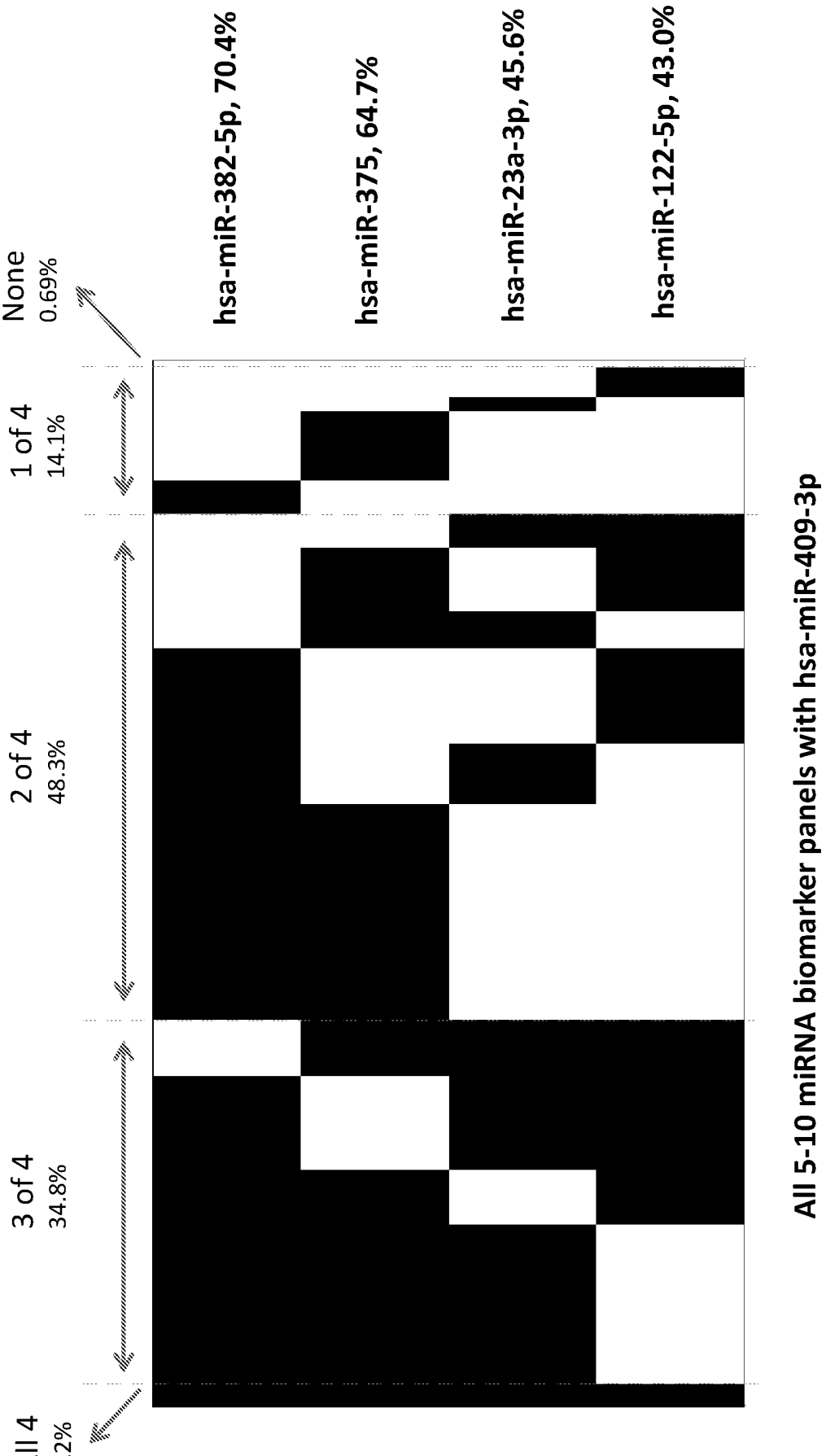

1 - 44 frequently selected miRNAs
High preference → Low preference
1 - 44 frequently selected miRNAs
High preference → Low preference
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
Correlation efficiency

FIG. 16


All 4
2.2%
3 of 4
34.8%
2 of 4
48.3%
1 of 4
14.1%
None
0.69%
hsa-miR-382-5p, 70.4%
hsa-miR-375, 64.7%
hsa-miR-23a-3p, 45.6%
hsa-miR-122-5p, 43.0%
All 5-10 miRNA biomarker panels with hsa-miR-409-3p