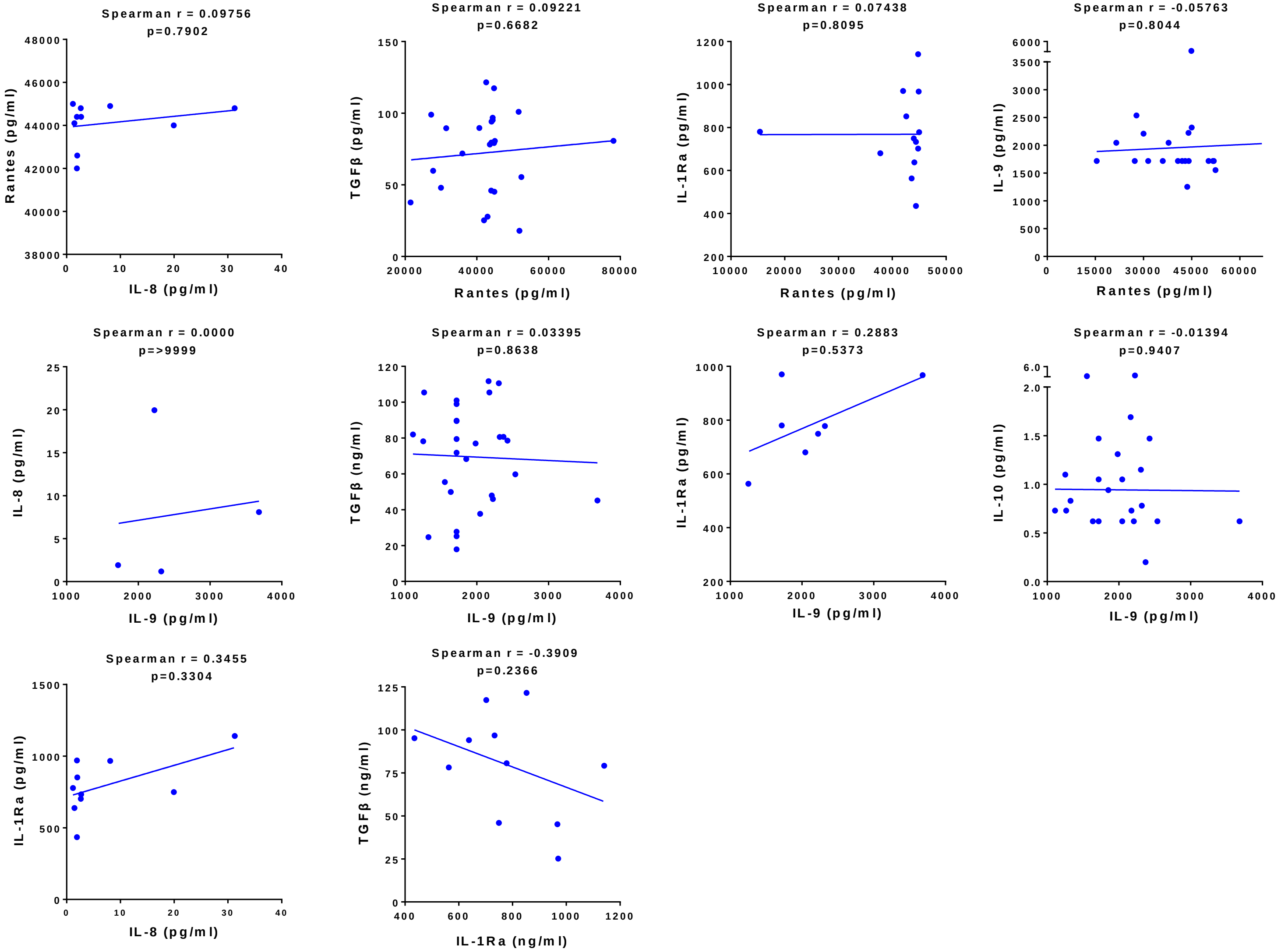
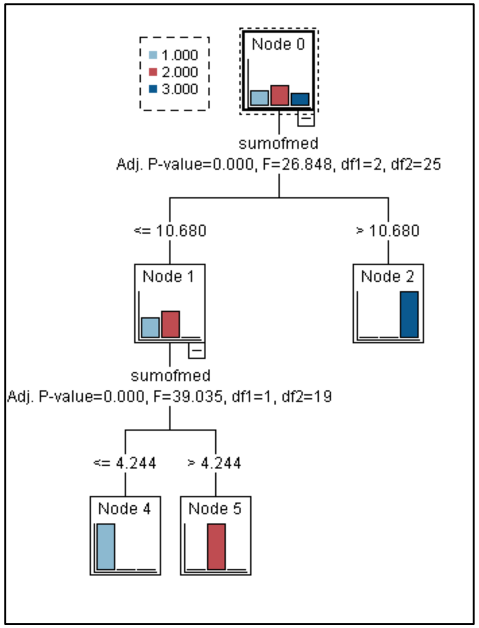
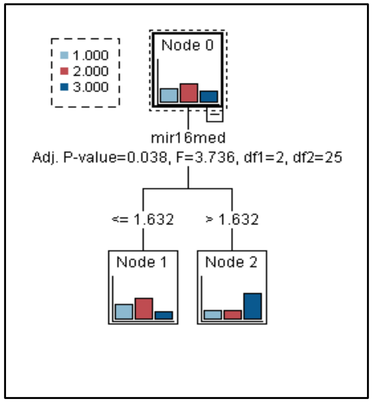
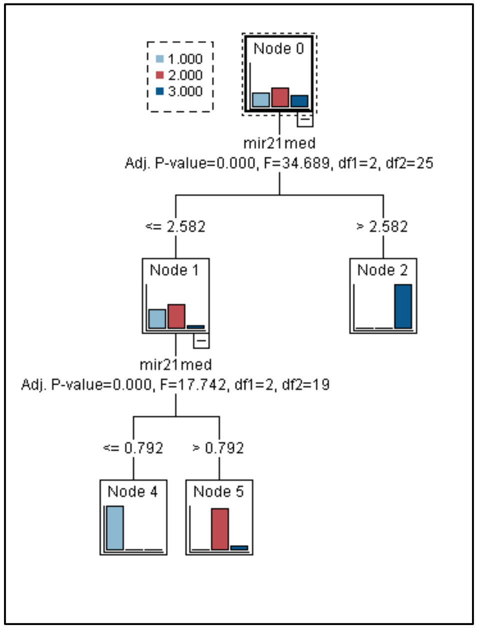


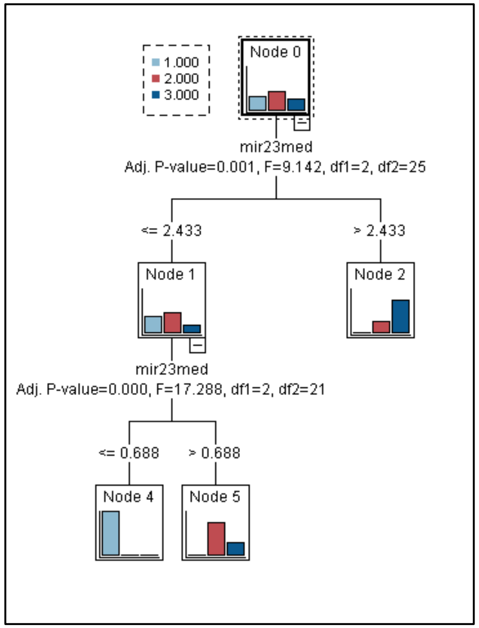
Supplementary Figure 1 XY plots depicting the correlations of cytokines/chemokines in healthy donors, that had a correlation in BCa patients. The significance (p) and Spearman correlation coefficients (r) are presented above their respective graphs. The lines represent the correlation generated for each set of cytokines/chemokines.

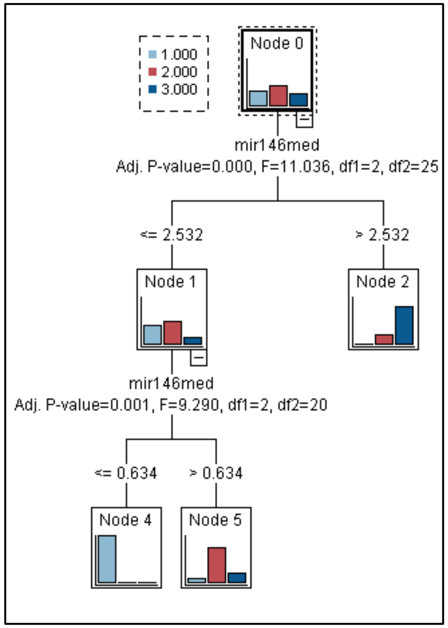
Individual distinctive capability of each parameter by QUEST classification

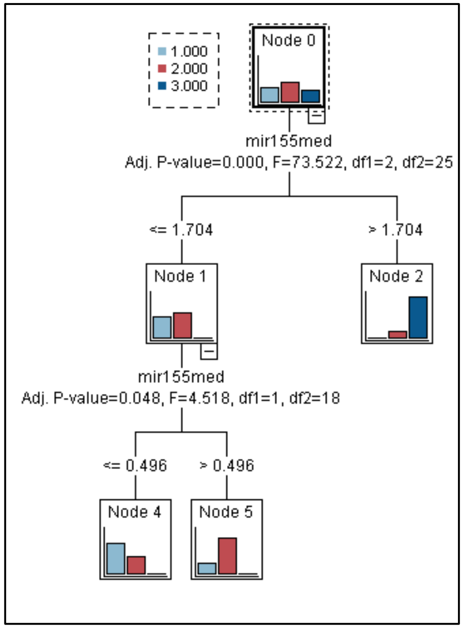


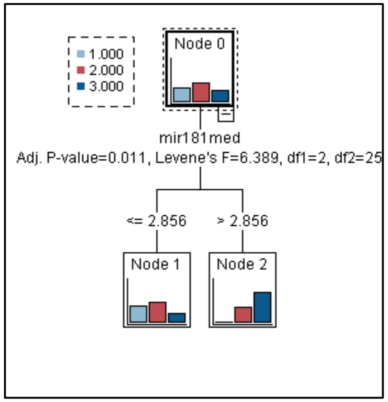








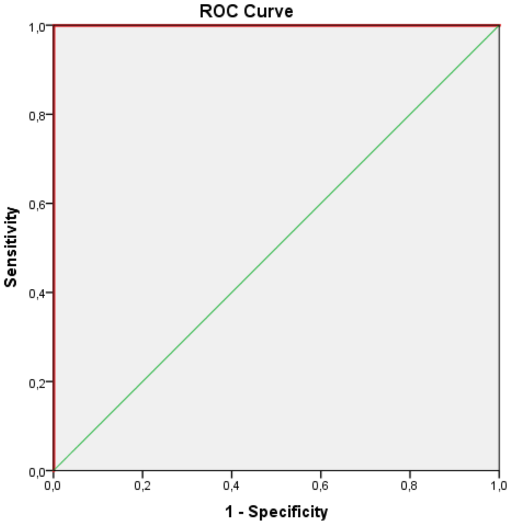




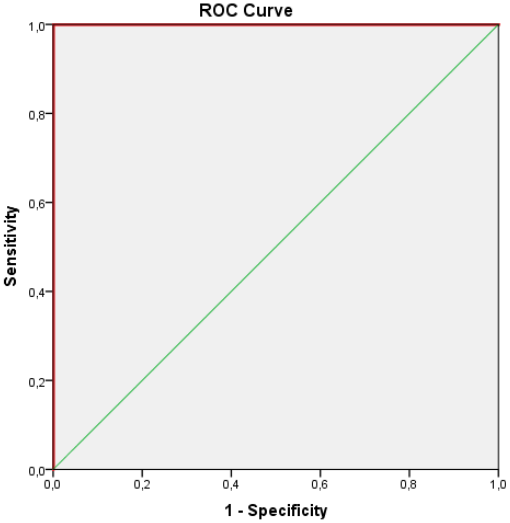
miRNAs	# of Nodes	C1 disc.	C2 disc.	C3 disc.
Sum	3	100%	100%	100%
miR-16	2	no	no	no
miR-21	3	100%	100%	86%
miR-23α	3	100%	92%	43%
miR-146α	3	89%	92%	56%
miR-155	3	78%	58%	100%
miR-181α	2	no	no	no

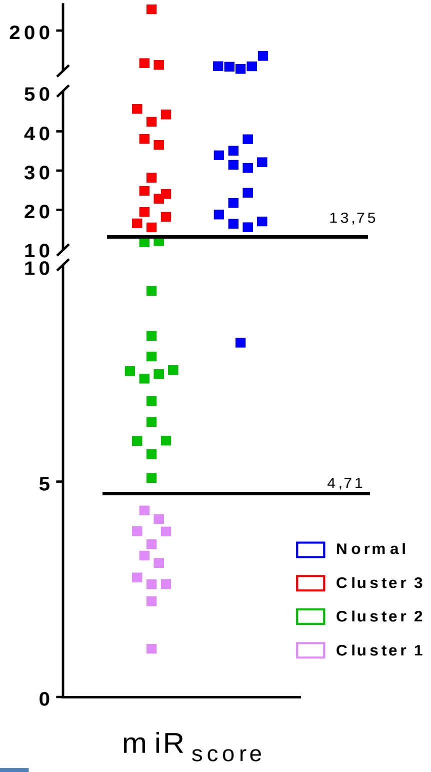
Supplementary Figure 2 Bar charts generated using the QUEST classification trees, when we try to evaluate each variable's distinctive capability separately (sum of ratios and 6 miRNAs). C1 is presented in light blue, C2 in red and C3 in dark blue. The table presents the discrimination percentage for each variable. Discrimination percentage is set as the final value, after we substract from a perfect score (100%) the percentage of wrongly classified patients for each cluster.

C1 vs non c1



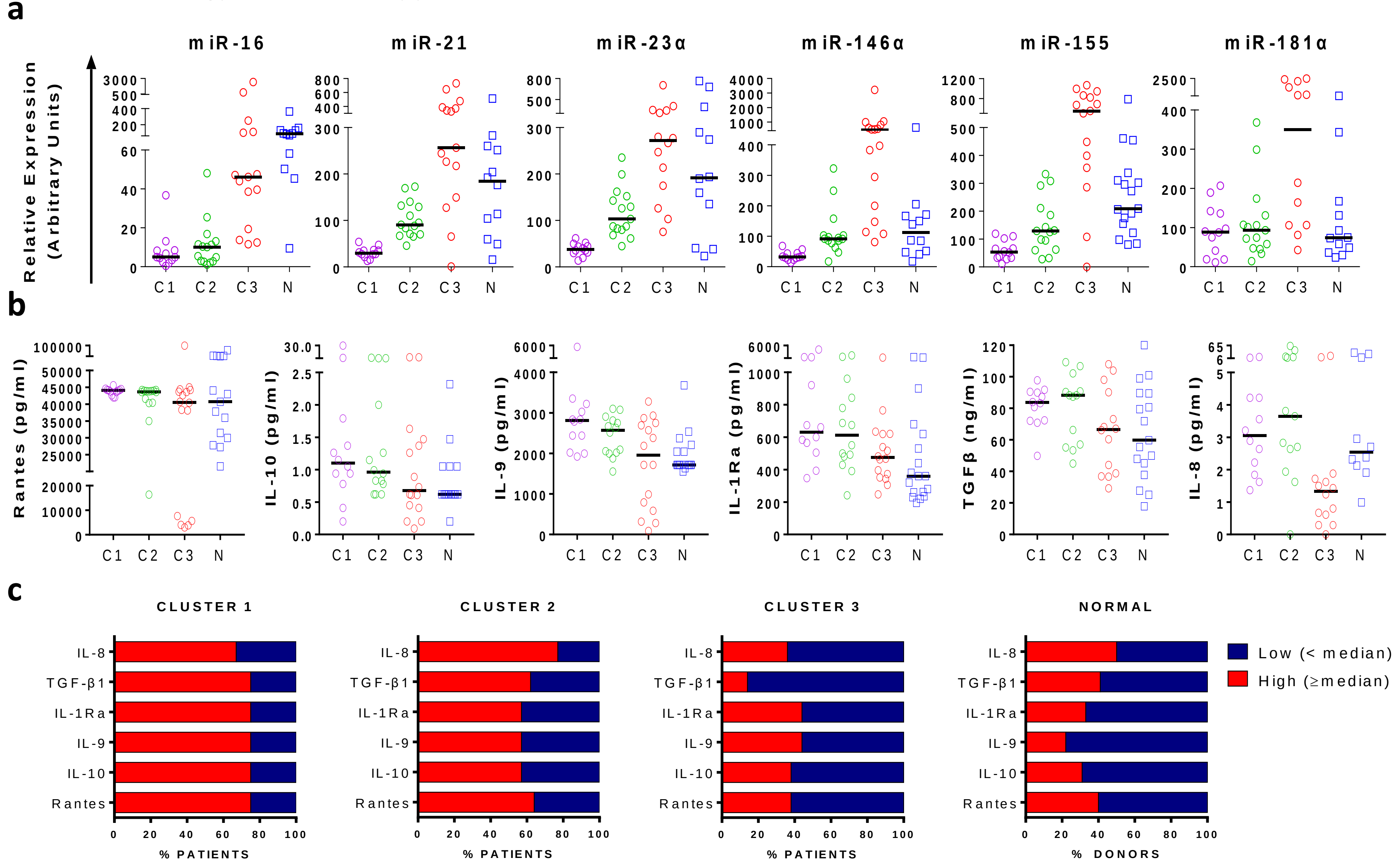
C1 and c2 vs non c1c2





	AUC	AUC 95%CI	miRSCORE value for max sensitivity - min 1-specificity	Youden Statistic (J)
C1 vs non C1	1.000	1.000-1.000	4.71	1.000
C1 and C2 vs non C1, C2	1.000	1.000-1.000	13.75	1.000

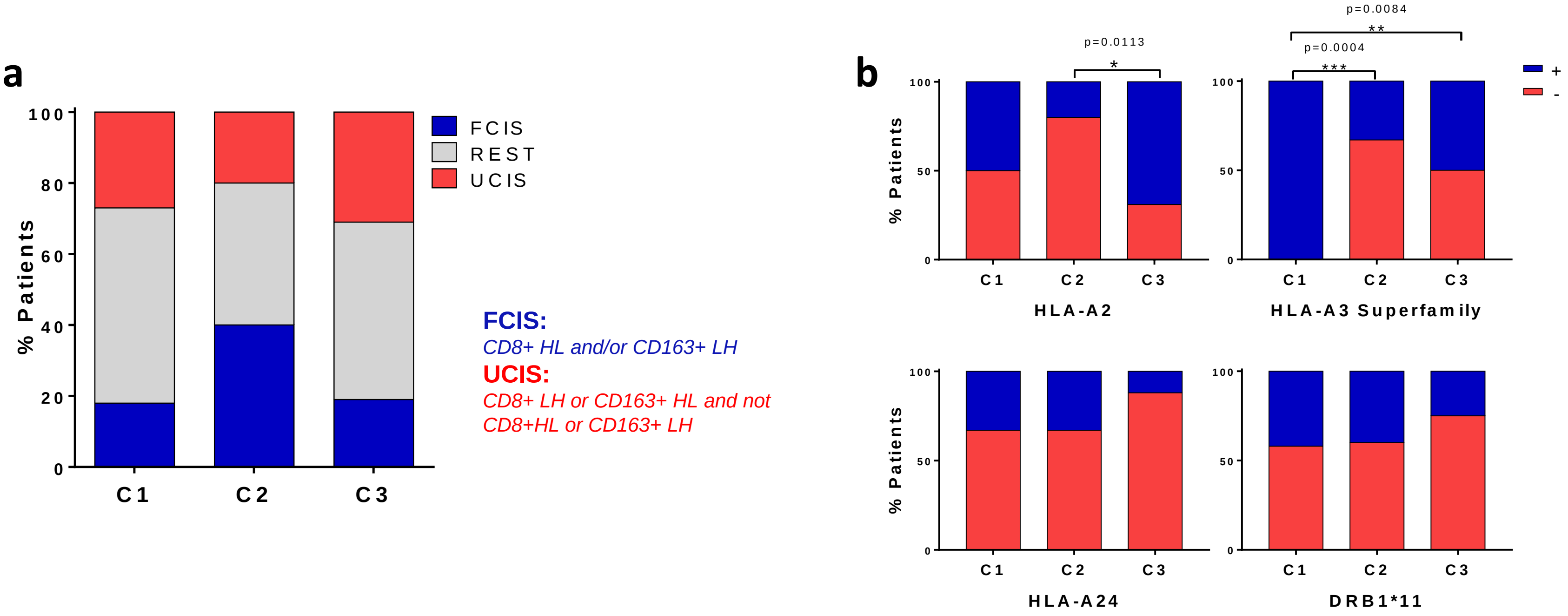
Supplementary Figure 3 ROC curves presenting the discriminatory capabilities of our algorithm miR$_{SCORE}$. The AUC, Youden's J and the algorithm value for the best combination of sensitivity-specificity is presented on the table. These algorithm values are used as the Cluster thresholds as presented in the scatter diagram on the right.



Supplementary Figure 4 a) Circulating miRNA values between the 3 clusters (C1 pink, C2 green, C3 red) and Healthy donors, including those stratified by our algorithm, in arbitrary units. The horizontal lines represent the median values of their respective groups.

b) Circulating cytokine/chemokine values between the 3 clusters (C1 pink, C2 green, C3 red) and Healthy donors, including those stratified by our algorithm. The horizontal lines represent the median values of their respective groups.

c) Bar charts presenting the percentages of patients within each group that have high (>= of the median on total patients, red) or low (<= of the median on total patients, blue) amounts of each circulating cytokine/chemokine we investigated.



FCIS:

CD8+ HL and/or CD163+ LH

UCIS:

CD8+ LH or CD163+ HL and not CD8+HL or CD163+ LH

Supplementary Figure 5 a) Bar chart depicting the distribution of our established combined immune signatures between the 3 clusters in percentages. Patient tumors with the FCIS are presented in blue, with the UCIS in red and the Rest group is presented in grey.
b) Bar chart depicting the distribution of 4 HLA alleles (or groups of alleles), A2, A3 superfamily (A3, A11, A31, A33, A68), A24 and DRB1*11 between our 3 clusters in percentages. The percentage of positive patients for each allele is presented in blue, while the negative in red. The p values presented above the charts are calculated using the number of patients and not the percentages.

Supplementary Table 1. Clinicopathological characteristics of patients

Total Number of Patients	
n=48	
Median age (years)	Range
56	27-78
Tumor size	n (%)
T1	20 (42)
T2	25 (52)
T3	3 (6)
LN status	n (%)
N0	19 (40)
N1	16 (33)
N2	11 (23)
N3	2 (4)
AJCC stage (TNM)	n (%)
I	14 (29)
IIA	10 (21)
IIB	11 (23)
IIIA	12 (25)
IIIB*	0
IIIC	1 (2)
Grade	n (%)
1	0
2	25 (52)
3	23 (48)
Hormone receptor	n (%)
positive	40 (83)
negative	8 (17)
HER-2/neu	n (%)
positive	15 (31)
negative	33 (69)
Ki67	n (%)
<20%	24 (50)
≥20%	24 (50)
Immune Signature	n (%)
FCIS	13 (27)
UCIS	11 (23)
Rest	24 (50)

* Stage IIIB patients were not eligible

Serum	*miR-16*	*miR-21*	*miR-23α*	*miR-146α*	*miR-155*	*miR-181α*
miR-16	-	Spearman r= 0.4462 P=0.0027 **	Spearman r= 0.6663 P<0.0001 ***	Spearman r= 0.7012 P<0.0001 ***	Spearman r= 0.4444 P=0.0032 **	Spearman r= 0.3726 P=0.0195 *
miR-21	Spearman r= 0.4462 P=0.0027 **	-	Spearman r= 0.7826 P<0.0001 ***	Spearman r= 0.7793 P<0.0001 ***	Spearman r= 0.4894 P=0.0010 **	Spearman r= 0.3841 P=0.0144 *
miR-23α	Spearman r= 0.6663 P<0.0001 ***	Spearman r= 0.7826 P<0.0001 ***	-	Spearman r= 0.8570 P<0.0001 ***	Spearman r= 0.6420 P<0.0001 ***	Spearman r= 0.3835 P=0.0160 *
miR-146α	Spearman r= 0.7012 P<0.0001 ***	Spearman r= 0.7793 P<0.0001 ***	Spearman r= 0.8570 P<0.0001 ***	-	Spearman r= 0.4383 P=0.0029 **	Spearman r= 0.4102 P=0.0086 **
miR-155	Spearman r= 0.4444 P=0.0032 **	Spearman r= 0.4894 P=0.0010 **	Spearman r= 0.6420 P<0.0001 ***	Spearman r= 0.4383 P=0.0029 **	-	Spearman r= 0.1659 P=0.3129 ns
miR-181α	Spearman r= 0.3726 P=0.0195 *	Spearman r= 0.3841 P=0.0144 *	Spearman r= 0.3835 P=0.0160 *	Spearman r= 0.4102 P=0.0086 **	Spearman r= 0.1659 P=0.3129 ns	-

Supplementary Table 2 Table presenting the correlations between the recorded values of the 6 miRNAs investigated, as measured in the patient sera. The significance (p) and Spearman correlation coefficient (r) are presented for each set of miRNAs

PBMCs	***miR-16***	***miR-21***	***miR-23α***	***miR-146α***	***miR-155***	***miR-181α***
miR-16	-	**Spearman r= 0.7219 P<0.0001 *****	**Spearman r= 0.7112 P<0.0001 *****	**Spearman r= 0.8087 P<0.0001 *****	**Spearman r= 0.5824 P=0.0002 *****	**Spearman r= 0.7639 P<0.0001 *****
miR-21	**Spearman r= 0.7219 P<0.0001 *****	-	**Spearman r= 0.8615 P<0.0001 *****	**Spearman r= 0.8451 P<0.0001 *****	**Spearman r= 0.6655 P<0.0001 *****	**Spearman r= 0.6088 P=0.0001 *****
miR-23α	**Spearman r= 0.7112 P<0.0001 *****	**Spearman r= 0.8615 P<0.0001 *****	-	**Spearman r= 0.8603 P<0.0001 *****	**Spearman r= 0.8212 P<0.0001 *****	**Spearman r= 0.6101 P=0.0001 *****
miR-146α	**Spearman r= 0.8087 P<0.0001 *****	**Spearman r= 0.8451 P<0.0001 *****	**Spearman r= 0.8603 P<0.0001 *****	-	**Spearman r= 0.8599 P<0.0001 *****	**Spearman r= 0.7692 P<0.0001 *****
miR-155	**Spearman r= 0.5824 P=0.0002 *****	**Spearman r= 0.6655 P<0.0001 *****	**Spearman r= 0.8212 P<0.0001 *****	**Spearman r= 0.8599 P<0.0001 *****	-	**Spearman r= 0.6339 P<0.0001 *****
miR-181α	**Spearman r= 0.7639 P<0.0001 *****	**Spearman r= 0.6088 P=0.0001 *****	**Spearman r= 0.6101 P=0.0001 *****	**Spearman r= 0.7692 P<0.0001 *****	**Spearman r= 0.6339 P<0.0001 *****	-

Supplementary Table 3 Table presenting the correlations between the recorded values of the 6 miRNAs investigated, as measured in the patient PBMCs. The significance (p) and Spearman correlation coefficient (r) are presented for each set of miRNAs