

Supplementary

Table S1. Assay ID for each miRNA used in the study.

Name	Assay ID
hsa-miR-34a-5p	000426
hsa-miR-146a-5p	000468
hsa-miR-155-5p	002623
hsa-miR-200b-3p	002251
hsa-miR-200c-3p	002300
hsa-miR-223-3p	002295
U6 snRNA	001973
Cel-miR-39	000200

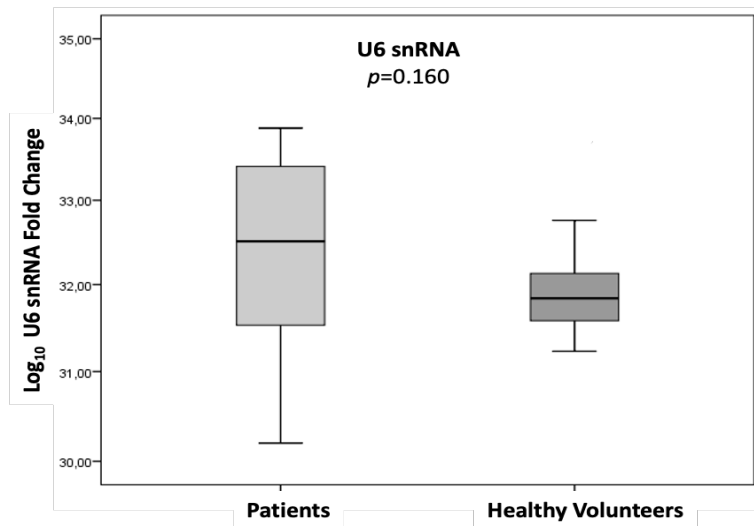


Figure S1. U6 snRNA expression levels between patients with NSCLC and healthy donors. Mann-Whitney test was used to determine statistically significant differences and the results were displayed on box plots. Horizontal line depicts the median Ct value, whereas the length of the boxes is the interquartile range that represents values between the 75th and 25th percentiles of individual Ct values. p values are shown.

Table S2. Median Ct values, SD and median miRNA expression values in the plasma of patients with NSCLC (N=72) and healthy volunteers (N=33).

	NSCLC patients (N=69)			Healthy Volunteers (N=33)		<i>p</i> value
	Ct	SD	Median	Ct	SD	
miR-34a	32.76	1.56	1.42	34.55	1.15	<i>p</i> <0.001
miR-146a	26.07	1.36	110.97	28.50	0.99	<i>p</i> <0.001
miR-155	31.55	1.16	3.09	32.02	0.77	<i>p</i> =0.646
miR-200b	34.06	1.96	0.48	34.76	1.47	<i>p</i> <0.001
miR-200c	32.90	2.22	1.36	34.26	1.12	<i>p</i> <0.001
miR-223	21.94	1.35	1798.33	24.61	1.23	<i>p</i> <0.001

Ct, cycle threshold; SD, standard deviation; miRNA expression values were calculated by the 2- Δ Ct method, Mann-Whitney test.

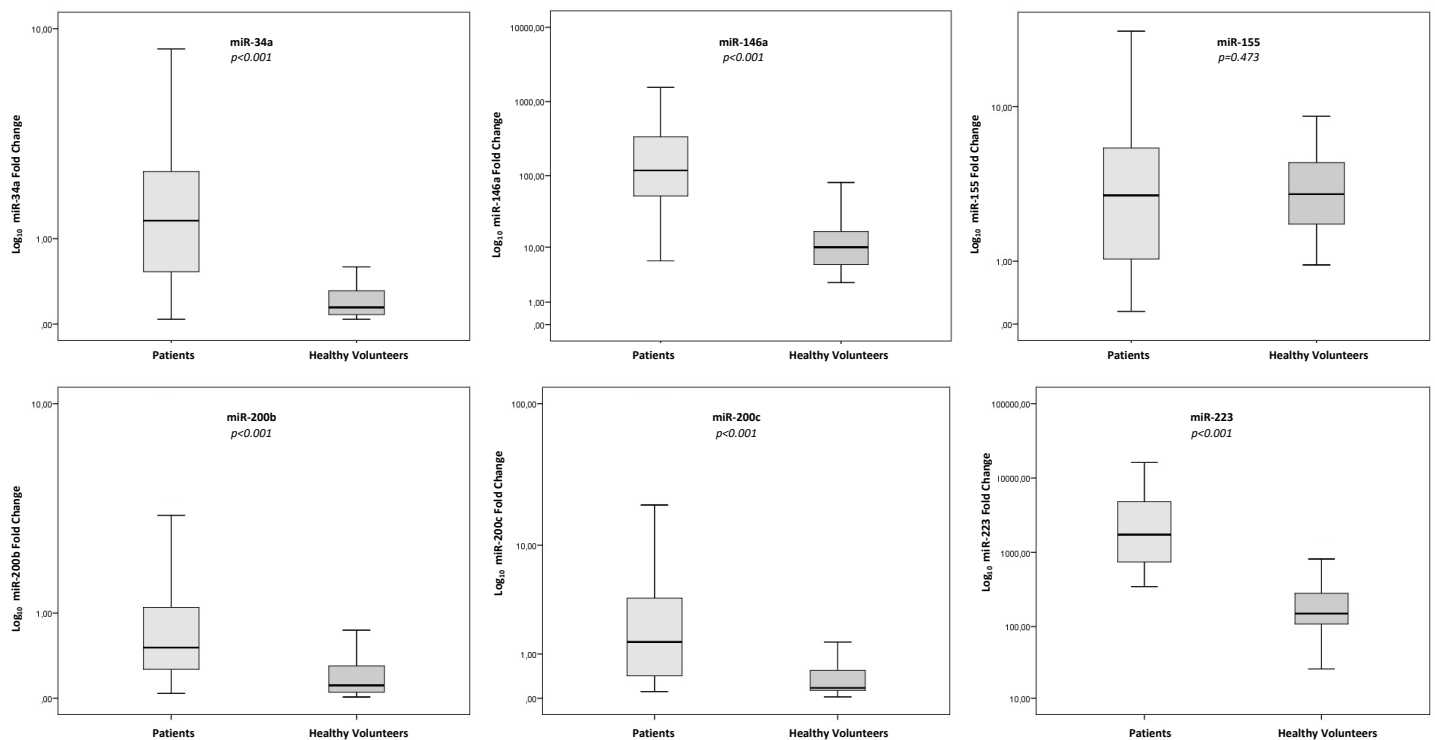
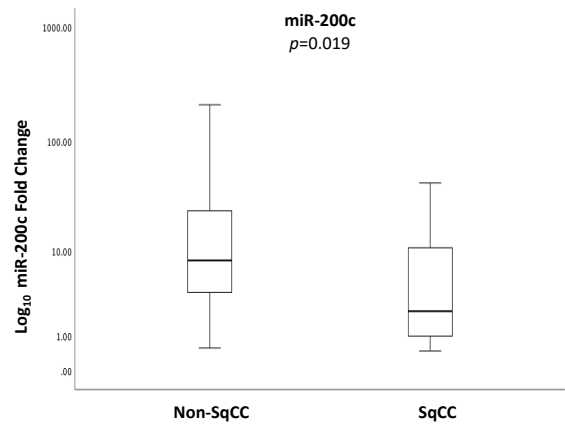
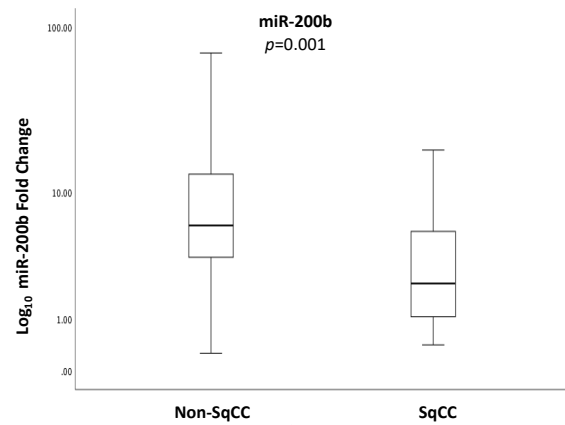
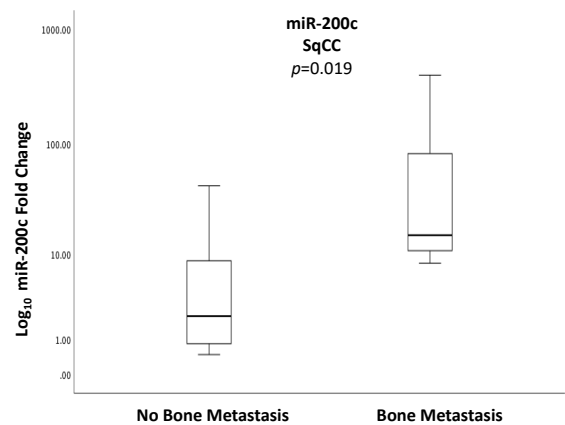
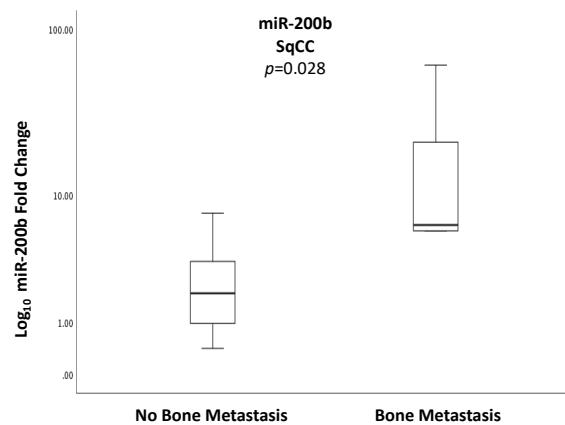
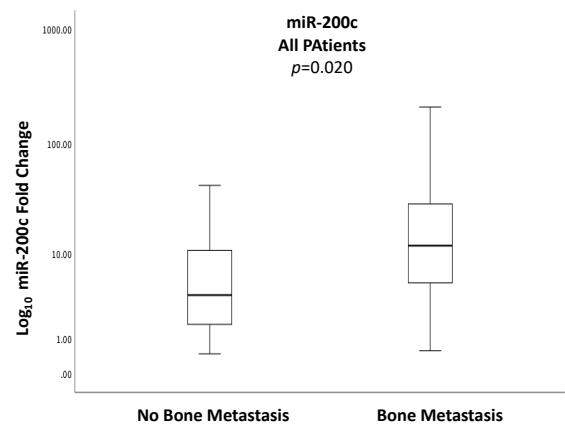
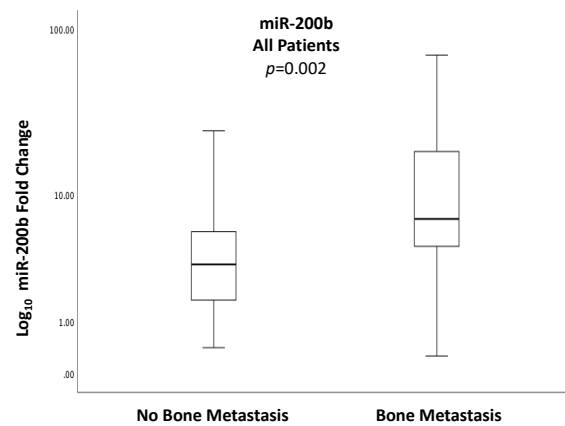


Figure S2. Differential expression of miR-34a, miR-146a, miR-155, miR-200b, miR-200c and miR-223 in the plasma of NSCLC patients (N=69) treated with Nivolumab compared to healthy donors (N=33).

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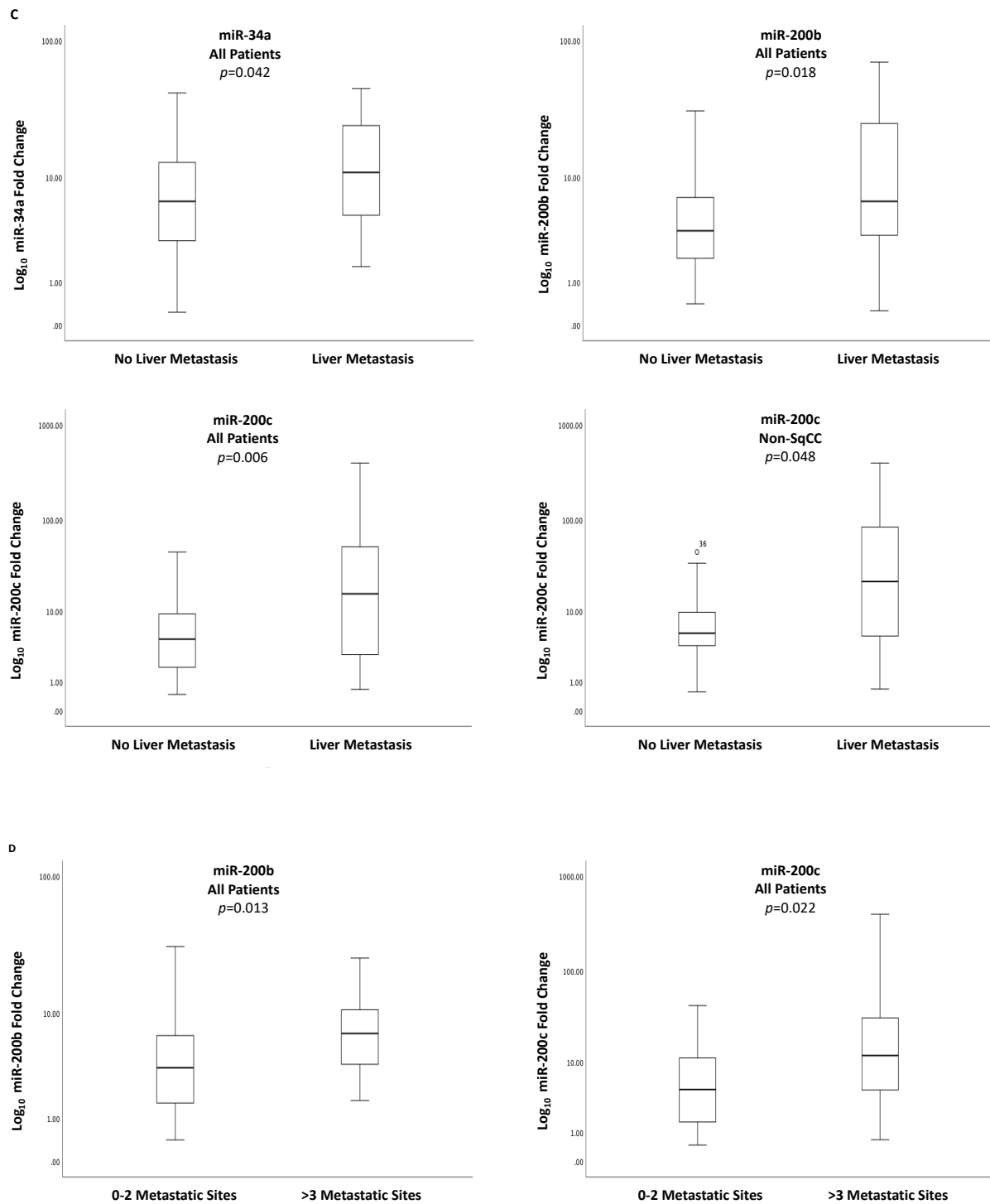


Figure S3. Correlation of miRNAs expression levels and patients' clinicopathological characteristics. Correlation with (A) the histologic subtype, (B) bone metastasis, (C) liver metastasis and (D) the presence of multiple metastatic sites. Expression levels of miRNAs were assessed by the $2^{-\Delta\Delta C_t}$ method and U6 snRNA was used as a reference gene. Statistically significant differences were determined by the Mann-Whitney test and the results are displayed on box plots. Horizontal line depicts median, whereas the length of the boxes is the interquartile range that represents values between the 75th and 25th percentiles of individual fold change expression values. Relative expression values on the y -axis are plotted on a \log_{10} scale. p values are shown.

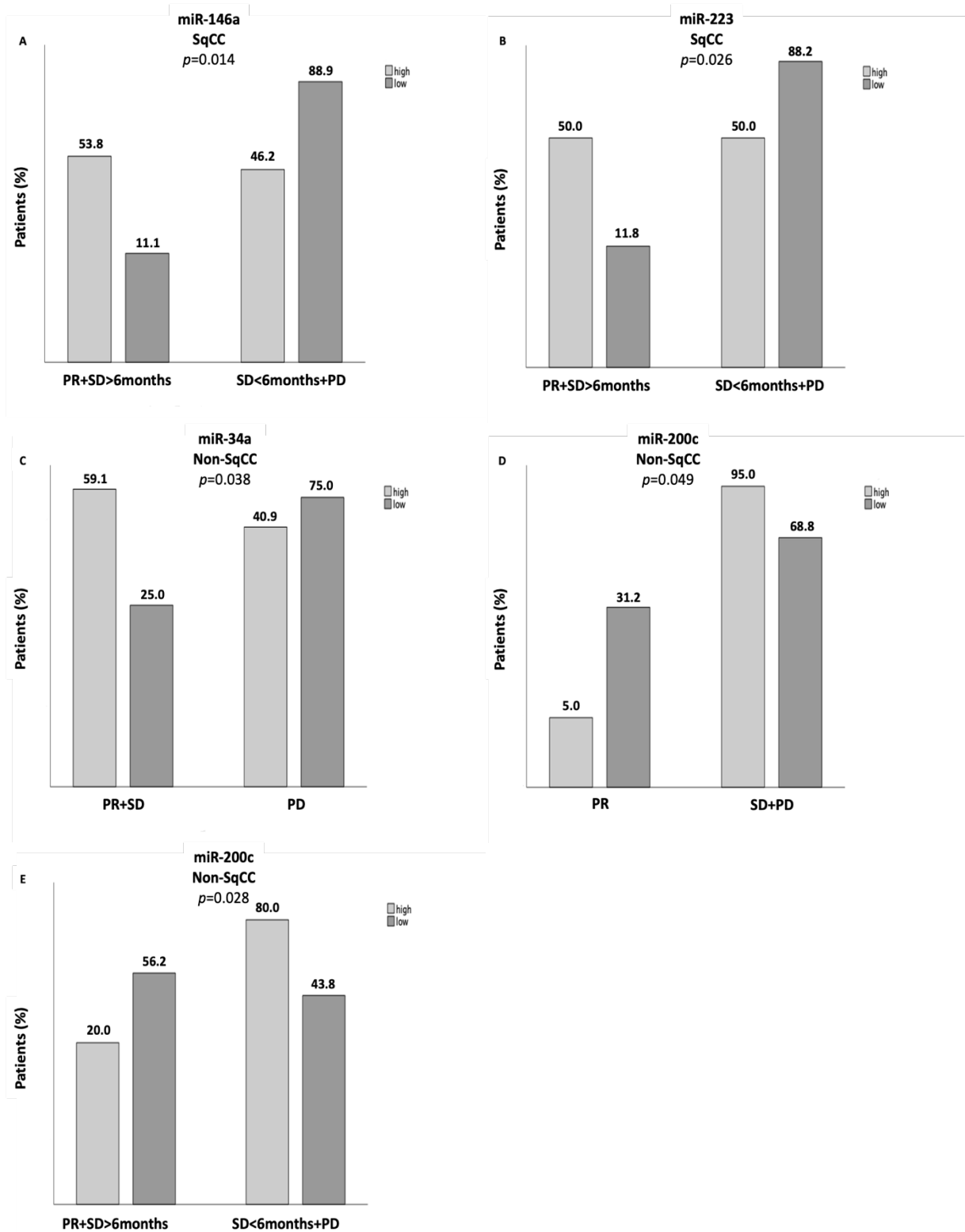
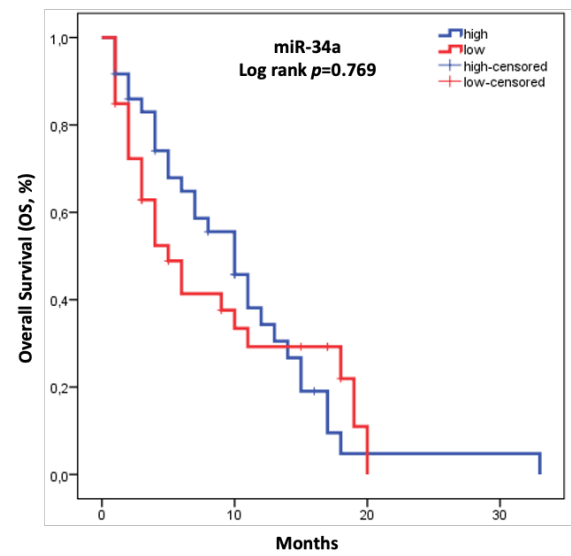
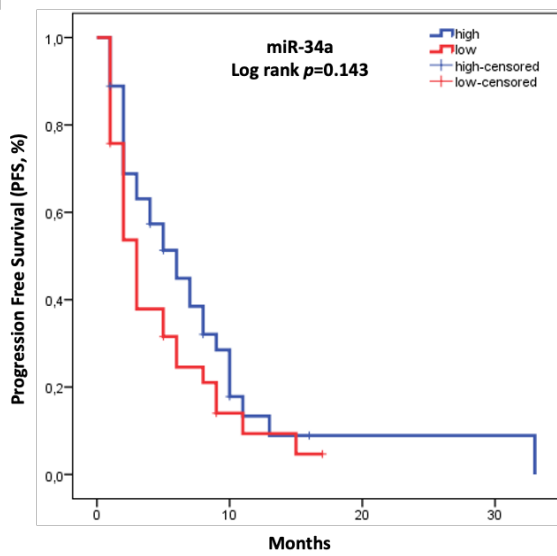
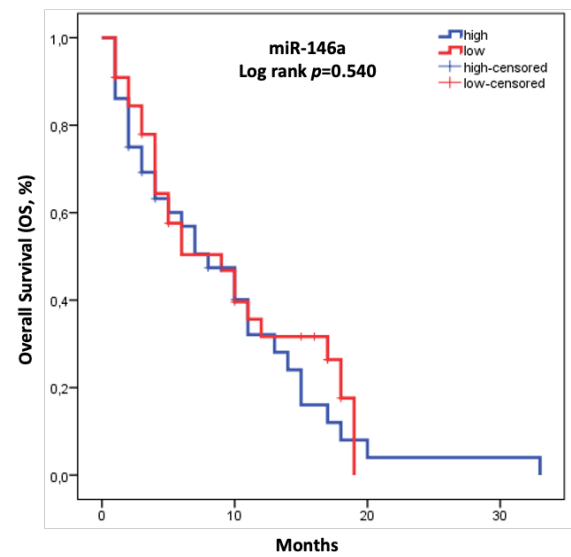
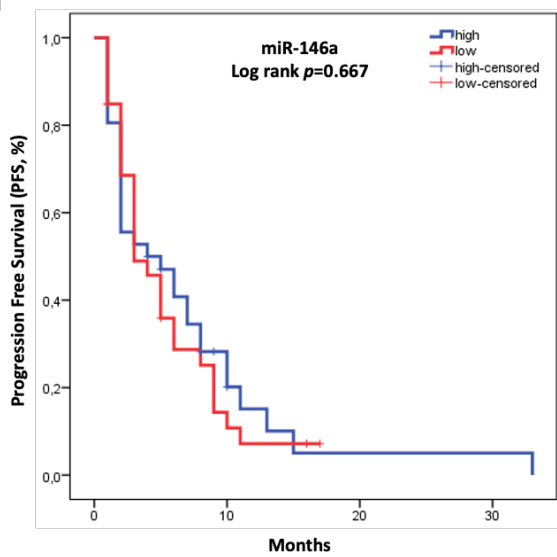
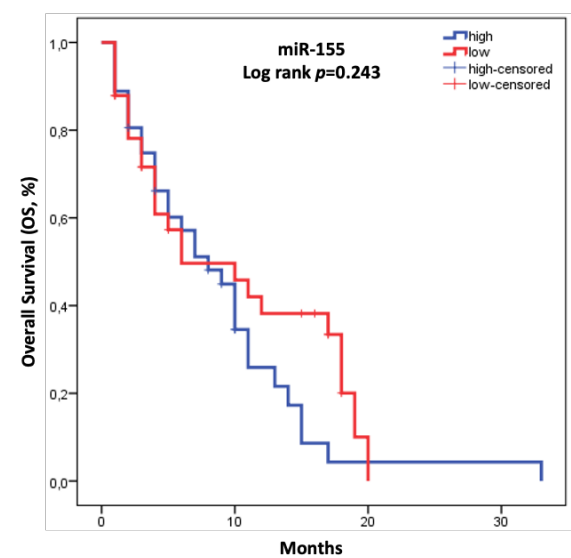
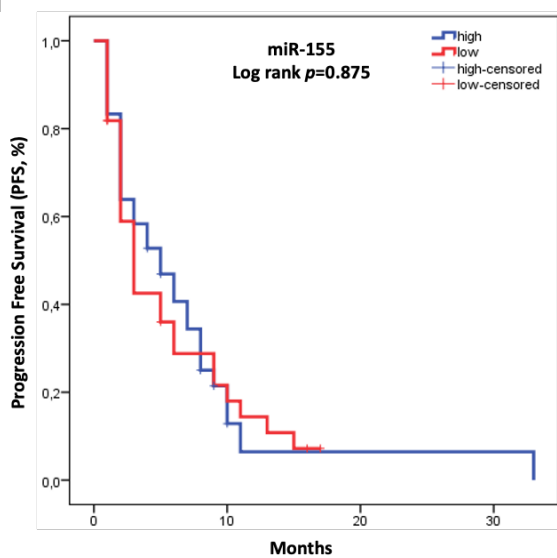


Figure S4. Correlation of miRNAs expression levels and response to immunotherapy, (A) Correlation of miR-146a and PDDC in the SqCC subgroup (B) Correlation of miR-223 and PDDC in the SqCC subgroup, (C) Correlation of miR-34a and DCR in the Non-SqCC subgroup, (D) Correlation of miR-200c and ORR in the Non-SqCC subgroup and (E) Correlation of miR-200c and PDDC in the Non-SqCC subgroup. p values are shown.

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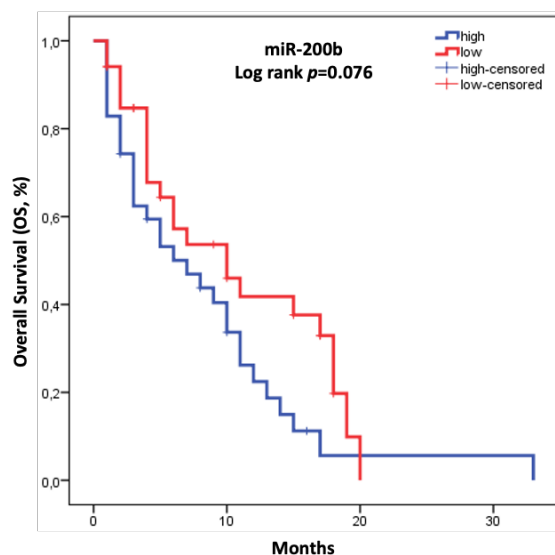
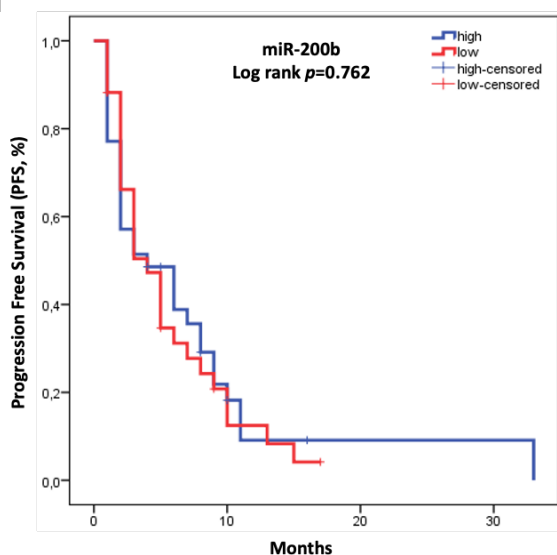
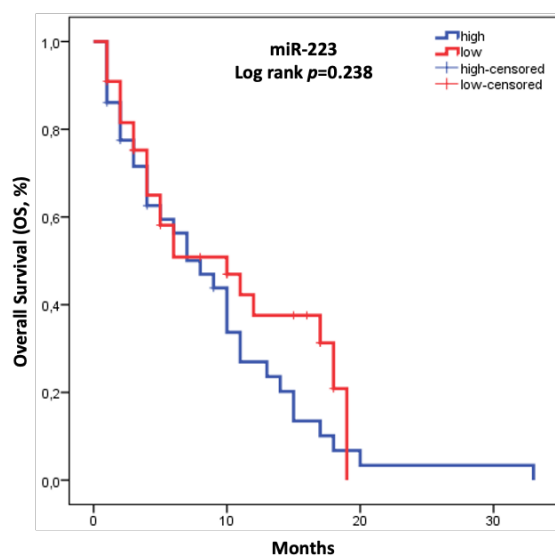
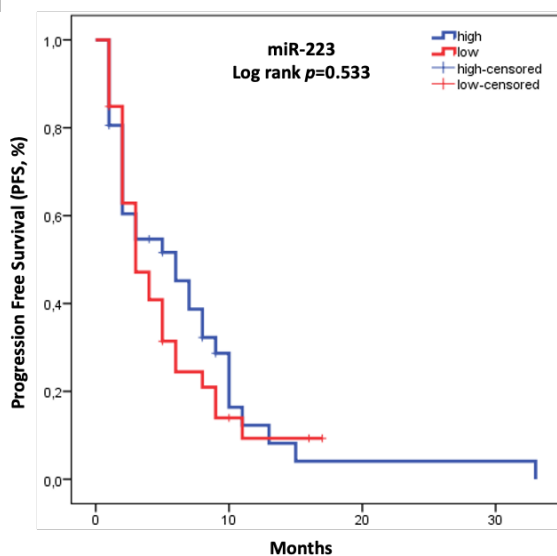
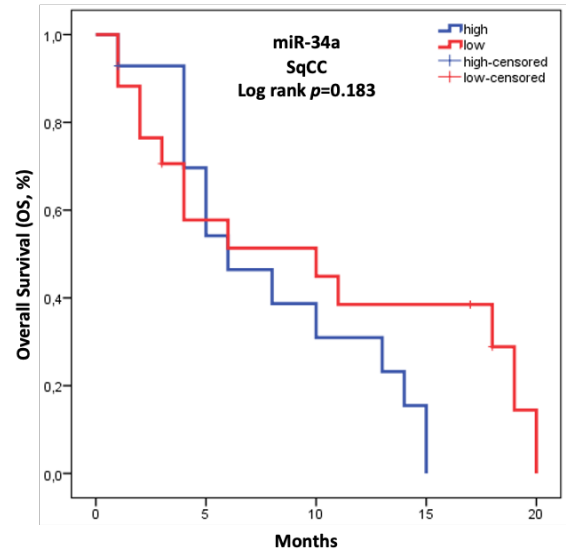
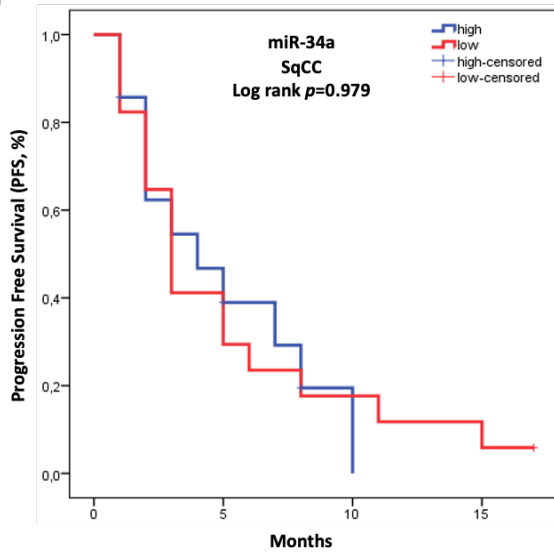
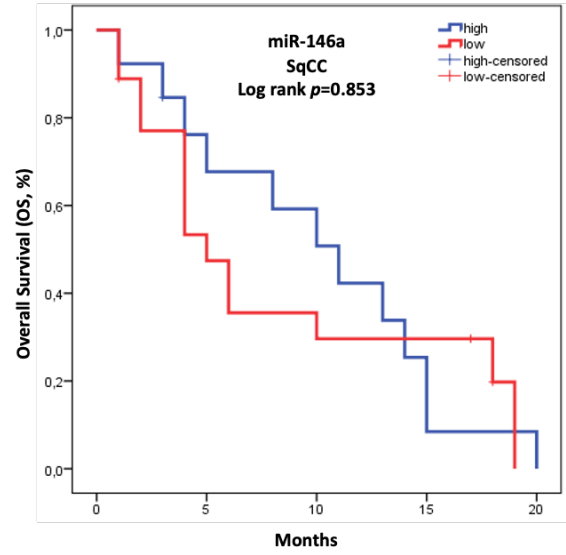
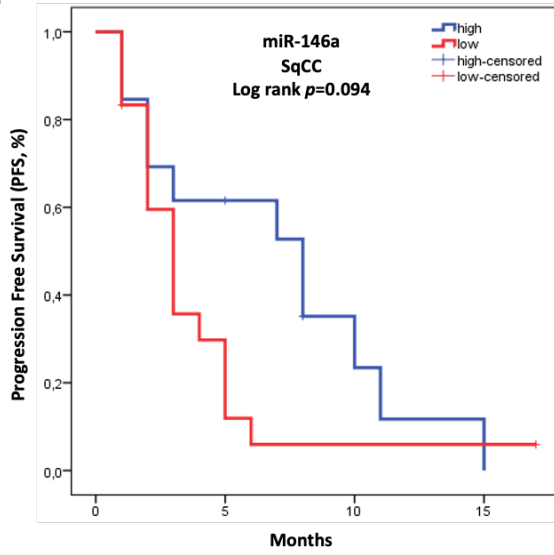
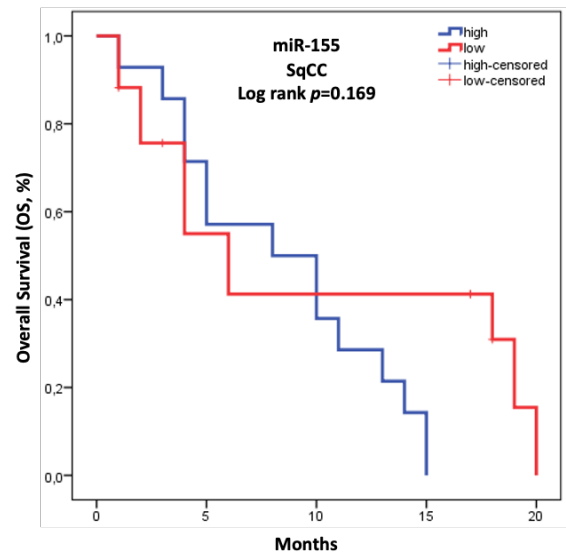
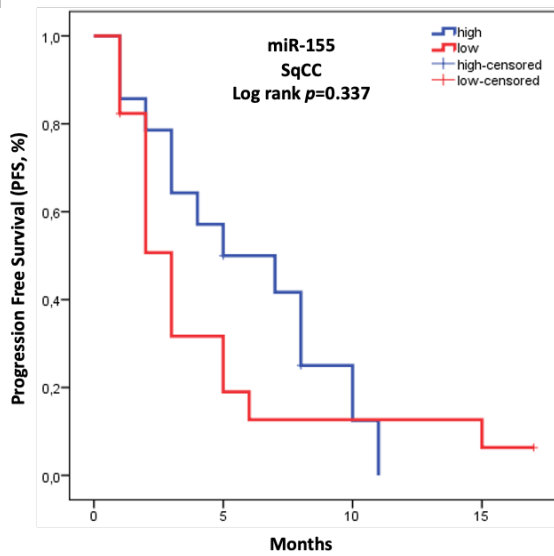
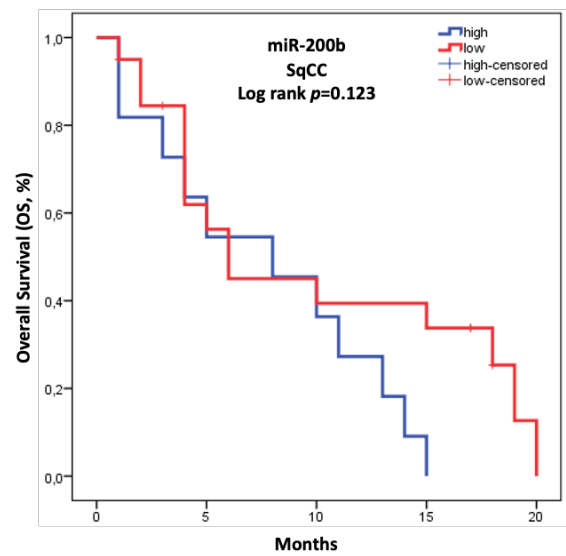
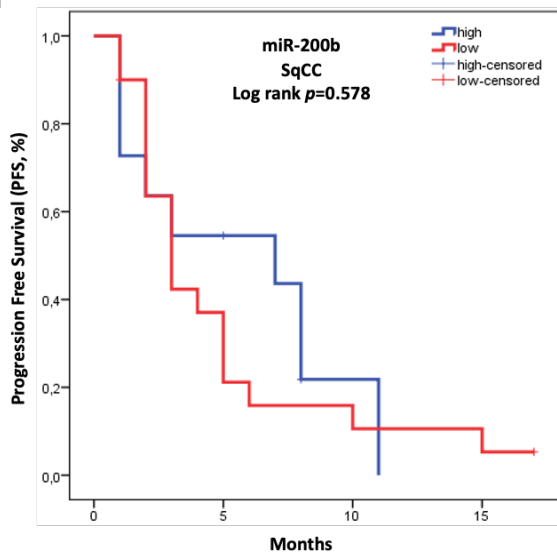
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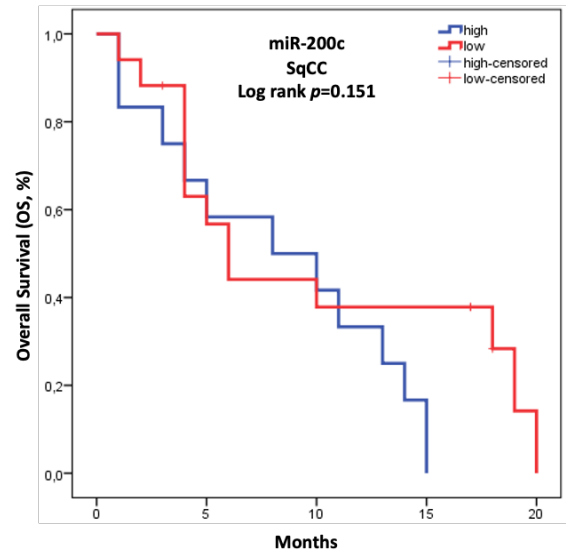
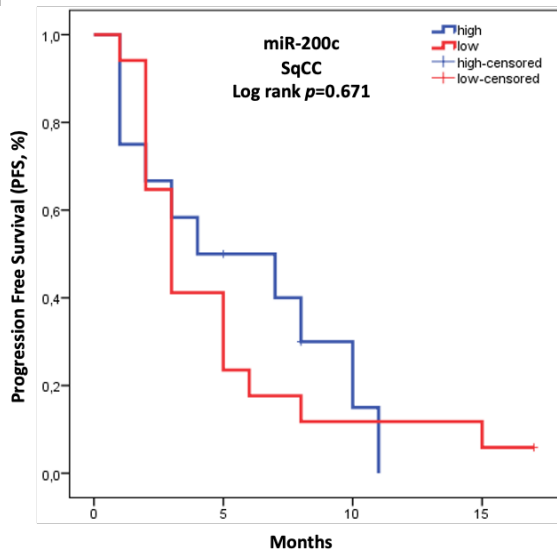
Figure S5. Kaplan-Meier analysis for PFS (left column) and OS (right column) (A) miR-34a, (B) miR-146a, (C) miR-155, (D) miR-200b and (E) miR-223, based on the microRNA's expression levels in the plasma of patients with NSCLC (N=69). Median expression values for each microRNA subcategorized patients into high and low expression groups. Curves were compared using the log rank test. p values are shown.

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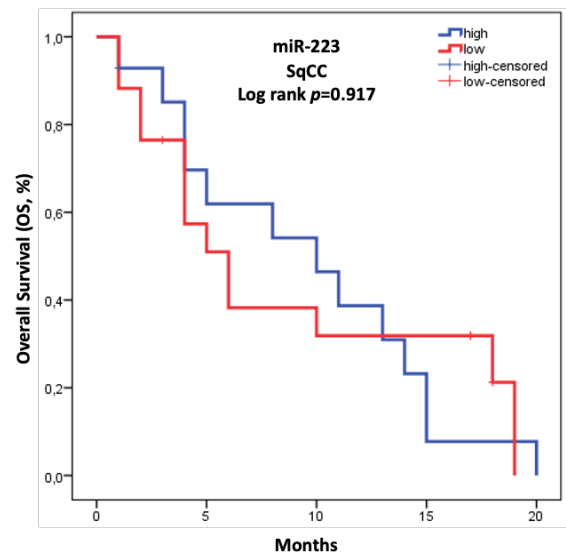
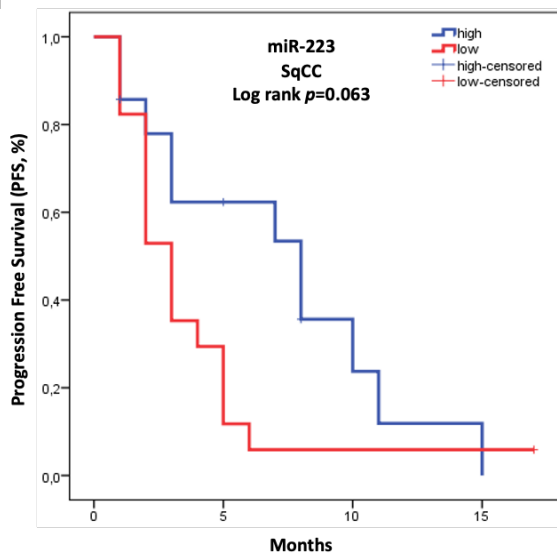


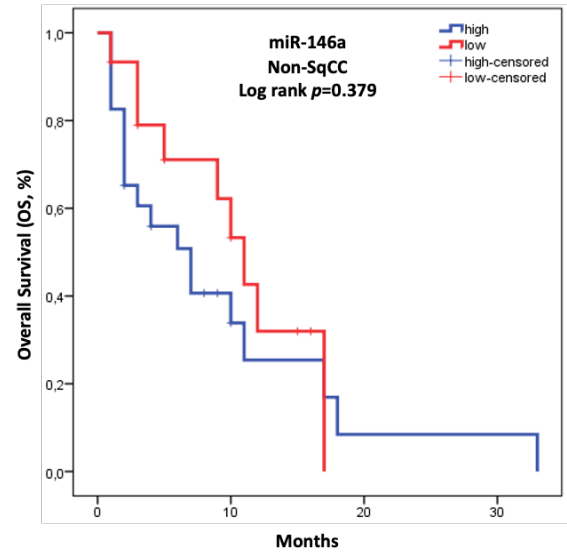
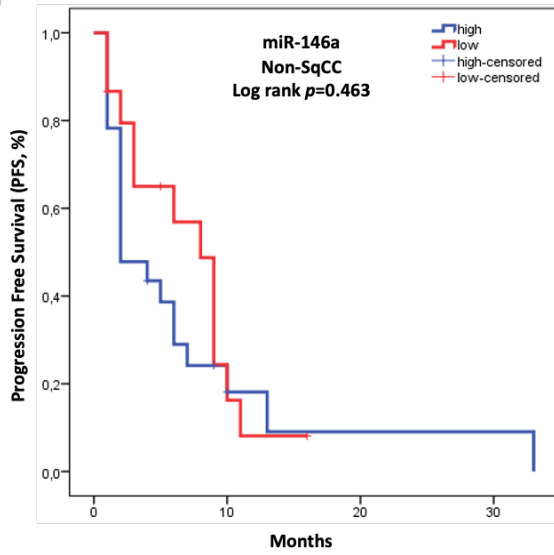
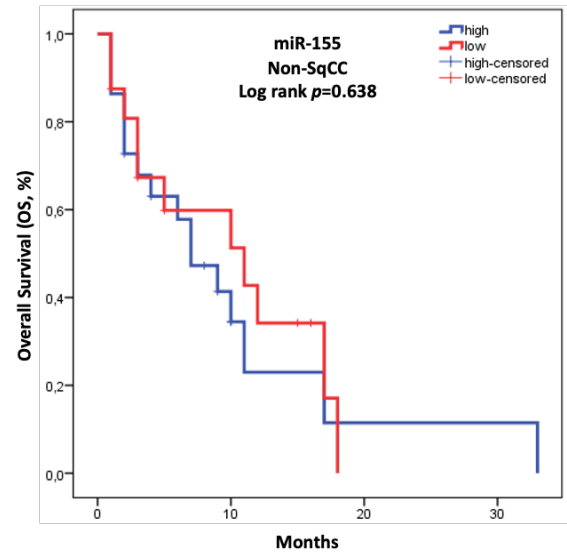
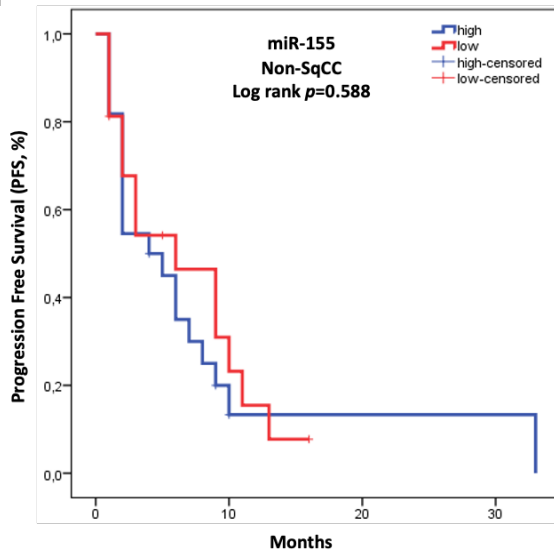
Figure S6. Kaplan-Meier analysis for PFS (left column) and OS (right column) based on the miRNAs' expression levels in the plasma of patients with SqCC subtype(N=31): (A) miR-34a, (B) miR-146a, (C) miR-155, (D) miR-200b,

(E) miR-200c and (F) miR-223. Median expression values for each microRNA subcategorized patients into high and low expression groups. Curves were compared using the log rank test. *p* values are shown.

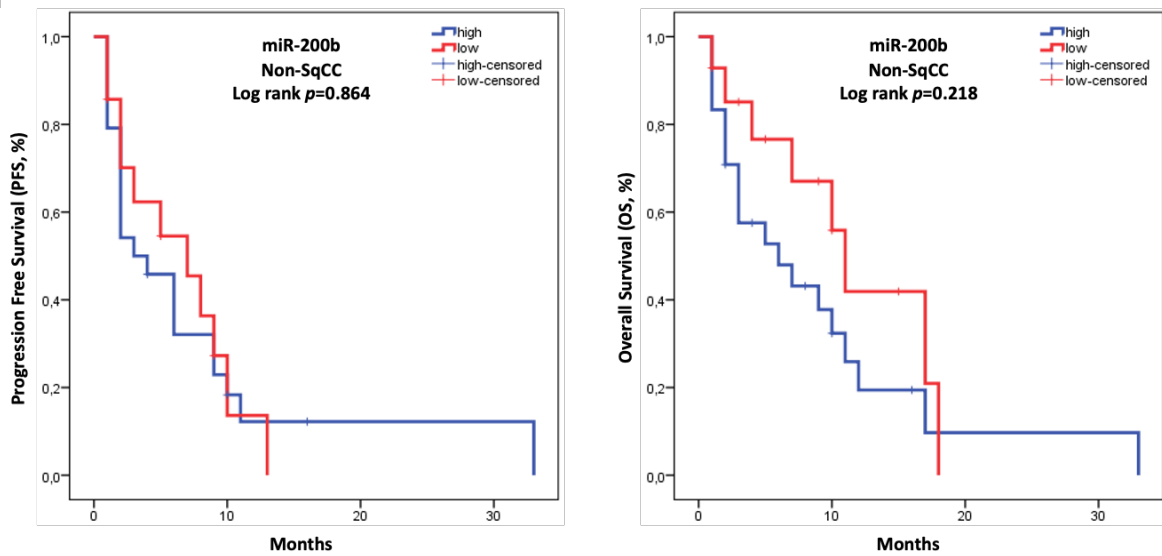
Table S3. Univariate and Multivariate Cox regression analysis for Progression Free Survival (PFS) and Overall Survival (OS) in patients with SqCC subtype (N=31) treated with Nivolumab.

Variable	Progression Free Survival (PFS)				Overall Survival (OS)			
	Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	<i>p</i> Value	HR (95% CI)	<i>p</i> Value	HR (95% CI)	<i>p</i> Value	HR (95% CI)	<i>p</i> Value
Age (<60 vs. ≥60)	4.157 (0.892-19.381)	0.070	-	-	1.880 (0.249-14.182)	0.540	-	-
Gender (male vs. female)	1.540 (0.203-11.688)	0.676	-	-	5.497 (0.641-47.109)	0.120	-	-
Smoker (Yes vs. No)	1.540 (0.203-11.688)	0.676	-	-	5.497 (0.641-47.109)	0.120	-	-
ECOG PS (≥2 vs. 0-1)	1.894 (0.747-4.804)	0.178	-	-	4.267 (1.584-11.494)	0.004*	-	-
Immunotherapy Line (2 nd vs. 3 rd)	1.256 (0.294-5.377)	0.758	-	-	2.494 (0.335-18.584)	0.372	-	-
No. of Metastatic Sites (≥3 vs. 0-2)	1.232 (0.418-3.631)	0.706	-	-	2.227 (0.734-6.753)	0.157	-	-
miR-34a (low vs. high)	1.010 (0.457-2.231)	0.980	-	-	1.713 (0.743-3.952)	0.207	-	-
miR-146a (high vs. low)	1.883 (0.834-4.248)	0.128	-	-	1.075 (0.485-2.384)	0.859	-	-
miR-155 (high vs. low)	1.425 (0.648-3.131)	0.378	-	-	1.761 (0.751-4.127)	0.193	-	-
miR-200b (high vs. low)	1.240 (0.545-2.824)	0.608	-	-	1.859 (0.808-4.274)	0.145	-	-
miR-200c (high vs. low)	1.176 (0.523-2.642)	0.695	-	-	1.797 (0.771-4.189)	0.175	-	-
miR-223 (high vs. low)	2.006 (0.893-4.506)	0.092	-	-	1.041 (0.472-2.296)	0.921	-	-

HR, Hazard Ratio; CI, Confidence Intervals; ECOG PS, Eastern Cooperative Oncology Group Performance Status; patients classified into high and low expression groups according to the median value of each miRNA; Cox regression, * *p* < 0.05. |

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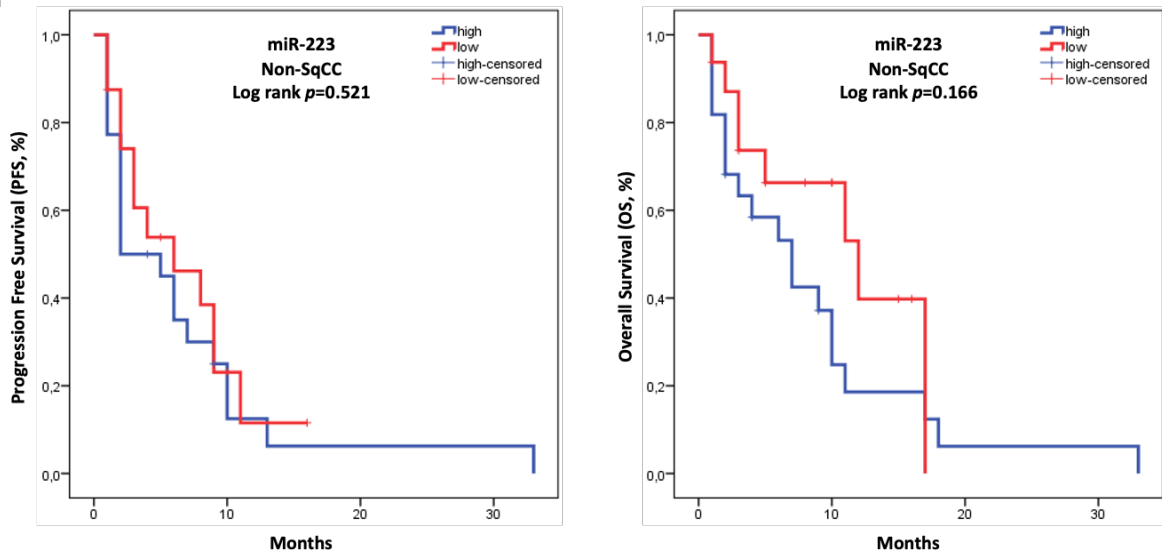


Figure S7. Kaplan-Meier analysis for PFS (left column) and OS (right column) based on the miRNAs' expression levels in the plasma of patients with non-SqCC subtype (N=38): (A) miR-146a, (B) miR-155, (C) miR-200b and (D) miR-223. Median expression values for each microRNA subcategorized patients into high and low expression groups. Curves were compared using the log rank test. p values are shown.