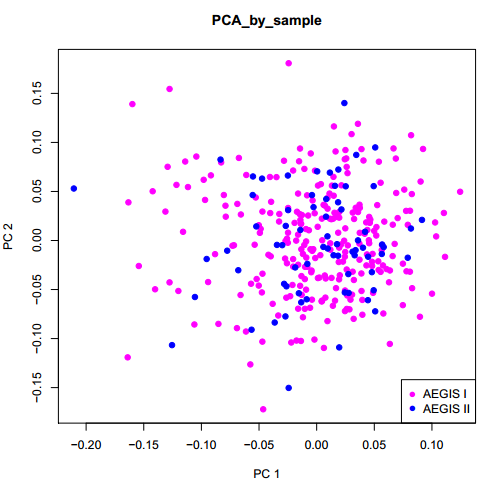
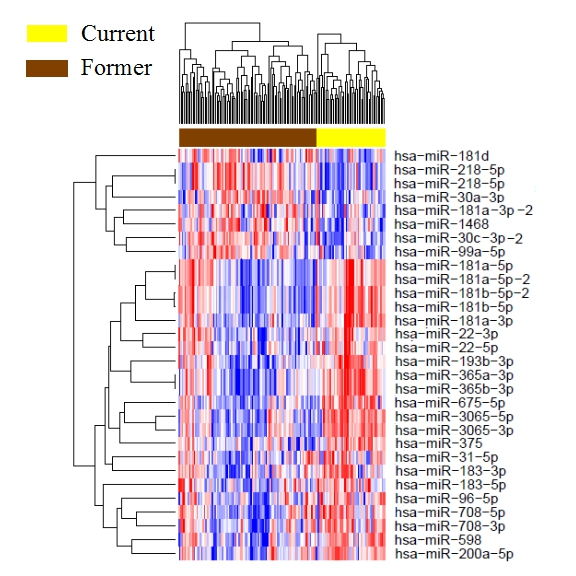
**Supplementary Figures and Tables**



**Supplementary Figure 1. Principal Components Analysis (PCA) of global microRNA expression.** PCA was performed using the expression of all 463 detected microRNA, and the distribution of samples along the first two components is plotted with the samples colored based on the cohort in which each patient was enrolled. The plot shows that there are no major differences in microRNA expression in samples obtained from either cohort.



**Supplementary Figure 2. Significantly differentially expressed microRNAs between current and former smokers** **(q<0.01)**. Some of these microRNAs have been previously associated with smoking status, such as miR-218, miR-365, miR-30 and miR-99a.

**Supplementary Table 1. Patient demographics stratified by cancer status.** The association of cancer with gender, smoking status and nodule size were computing using a Fisher’s exact test; the association of cancer with age and cumulative smoke exposure were computed using a Student’s t-test; n indicates number of patients with clinical data available; SD indicates standard deviation.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | Discovery set  n=138 | | | Test set  n=203 | | |
| Lung Cancer  n=88 | Benign  n=50 | p | Lung Cancer n=103 | Benign  n=100 | p |
| Gender | Females | 25 | 37 | 0.84 | 38 | 46 | 0.2 |
| Males | 63 | 13 | 65 | 54 |  |
| Age (SD; n) | | 61  (10; 88) | 56  (13; 50) | 0.01 | 60  (9; 103) | 58  (12; 100) | 0.29 |
| Smoking | Current | 32 | 14 | 0.35 | 47 | 41 | 0.57 |
| Former | 56 | 36 | 56 | 59 |
| Cumulative Smoke Exposure - pack-yr. (SD; n) | | 38  (22; 88) | 33  (27; 49) | 0.2 | 40  (28; 102) | 32  (30; 97) | 0.05 |
| Race | White | 69 | 40 | 0.98 | 74 | 75 | 0.8 |
| Black | 15 | 9 | 26 | 20 |
| Unknown | 4 | 1 | 3 | 5 |
| Lesion Size | <3cm | 30 | 22 | 4∙10-4 | 20 | 51 | 8∙10-14 |
| >=3cm | 47 | 11 | 73 | 18 |
| Infiltrate | 4 | 11 | 6 | 25 |
| Unknown | 7 | 6 | 4 | 6 |

**Supplementary Table 2. Smoking associated microRNAs (p<0.05)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| miRBase ID | microRNA name | p-value | t-statistic | Direction in current smokers |
| MI0000767\_MIMAT0000710 | hsa-miR-365a-3p | 6.99E-14 | 8.384407 | UP |
| MI0000769\_MIMAT0022834 | hsa-miR-365b-3p | 7.00E-14 | 8.384203 | UP |
| MI0005416\_MIMAT0004284 | hsa-miR-675-5p | 7.43E-12 | 7.527167 | UP |
| MI0000289\_MIMAT0000270 | hsa-miR-181a-3p | 1.97E-11 | 7.344121 | UP |
| MI0014228\_MIMAT0015066 | hsa-miR-3065-5p | 2.92E-11 | 7.269376 | UP |
| MI0000294\_MIMAT0000275 | hsa-miR-218-5p | 5.99E-11 | -7.13266 | DOWN |
| MI0000295\_MIMAT0000275 | hsa-miR-218-5p | 6.17E-11 | -7.12678 | DOWN |
| MI0014228\_MIMAT0015378 | hsa-miR-3065-3p | 8.02E-11 | 7.076626 | UP |
| MI0000683\_MIMAT0000257 | hsa-miR-181b-5p | 9.07E-11 | 7.053075 | UP |
| MI0000270\_MIMAT0000257 | hsa-miR-181b-5p | 9.43E-11 | 7.04563 | UP |
| MI0003137\_MIMAT0002819 | hsa-miR-193b-3p | 3.94E-10 | 6.768818 | UP |
| MI0000783\_MIMAT0000728 | hsa-miR-375 | 2.92E-07 | 5.408163 | UP |
| MI0000269\_MIMAT0000256 | hsa-miR-181a-5p | 1.63E-06 | 5.022647 | UP |
| MI0000289\_MIMAT0000256 | hsa-miR-181a-5p | 1.63E-06 | 5.022619 | UP |
| MI0003782\_MIMAT0006789 | hsa-miR-1468 | 1.09E-05 | -4.57523 | DOWN |
| MI0000254\_MIMAT0004550 | hsa-miR-30c-2-3p | 1.17E-05 | -4.55862 | DOWN |
| MI0003610\_MIMAT0003266 | hsa-miR-598 | 2.26E-05 | 4.396201 | UP |
| MI0005543\_MIMAT0004927 | hsa-miR-708-3p | 2.89E-05 | 4.334139 | UP |
| MI0005543\_MIMAT0004926 | hsa-miR-708-5p | 6.18E-05 | 4.140067 | UP |
| MI0000089\_MIMAT0000089 | hsa-miR-31-5p | 9.02E-05 | 4.040998 | UP |
| MI0000078\_MIMAT0004495 | hsa-miR-22-5p | 9.43E-05 | 4.02934 | UP |
| MI0000088\_MIMAT0000088 | hsa-miR-30a-3p | 0.000115 | -3.97679 | DOWN |
| MI0000269\_MIMAT0004558 | hsa-miR-181a-2-3p | 0.000137 | -3.93045 | DOWN |
| MI0000737\_MIMAT0001620 | hsa-miR-200a-5p | 0.000146 | 3.912748 | UP |
| MI0000273\_MIMAT0004560 | hsa-miR-183-3p | 0.000157 | 3.893655 | UP |
| MI0000098\_MIMAT0000095 | hsa-miR-96-5p | 0.000289 | 3.724799 | UP |
| MI0000101\_MIMAT0000097 | hsa-miR-99a-5p | 0.000291 | -3.72306 | DOWN |
| MI0000273\_MIMAT0000261 | hsa-miR-183-5p | 0.000306 | 3.7089 | UP |
| MI0003139\_MIMAT0002821 | hsa-miR-181d | 0.000411 | -3.62574 | DOWN |
| MI0000078\_MIMAT0000077 | hsa-miR-22-3p | 0.000626 | 3.505042 | UP |
| MI0000737\_MIMAT0000682 | hsa-miR-200a-3p | 0.000845 | 3.416487 | UP |
| MI0000746\_MIMAT0004678 | hsa-miR-99b-3p | 0.000904 | -3.39641 | DOWN |
| MI0003820\_MIMAT0003884 | hsa-miR-454-5p | 0.000906 | -3.39591 | DOWN |
| MI0003205\_MIMAT0002888 | hsa-miR-532-5p | 0.000947 | -3.38262 | DOWN |
| MI0005545\_MIMAT0004929 | hsa-miR-190b | 0.000975 | -3.37397 | DOWN |
| MI0016436\_MIMAT0018204 | hsa-miR-676-3p | 0.001084 | -3.34211 | DOWN |
| MI0000081\_MIMAT0000080 | hsa-miR-24-3p | 0.001205 | 3.310141 | UP |
| MI0000064\_MIMAT0000064 | hsa-let-7c | 0.001216 | -3.30728 | DOWN |
| MI0000080\_MIMAT0000080 | hsa-miR-24-3p | 0.001217 | 3.307088 | UP |
| MI0000470\_MIMAT0004603 | hsa-miR-125b-2-3p | 0.001225 | -3.30498 | DOWN |
| MI0014186\_MIMAT0015032 | hsa-miR-3158-3p | 0.001507 | -3.24164 | DOWN |
| MI0014187\_MIMAT0015032 | hsa-miR-3158-3p | 0.001534 | -3.23617 | DOWN |
| MI0000460\_MIMAT0004600 | hsa-miR-144-5p | 0.002046 | -3.14643 | DOWN |
| MI0005562\_MIMAT0004951 | hsa-miR-887 | 0.002052 | 3.145495 | UP |
| MI0016010\_MIMAT0018000 | hsa-miR-23c | 0.002269 | 3.113702 | UP |
| MI0001733\_MIMAT0001636 | hsa-miR-452-3p | 0.002611 | 3.068962 | UP |
| MI0000079\_MIMAT0000078 | hsa-miR-23a-3p | 0.003471 | 2.976788 | UP |
| MI0000301\_MIMAT0000281 | hsa-miR-224-5p | 0.003558 | 2.968621 | UP |
| MI0000439\_MIMAT0000418 | hsa-miR-23b-3p | 0.003769 | 2.949658 | UP |
| MI0002470\_MIMAT0002177 | hsa-miR-486-5p | 0.003934 | -2.93552 | DOWN |
| MI0000805\_MIMAT0004694 | hsa-miR-342-5p | 0.003953 | -2.93395 | DOWN |
| MI0000077\_MIMAT0004494 | hsa-miR-21-3p | 0.004101 | 2.921693 | UP |
| MI0003591\_MIMAT0003249 | hsa-miR-584-5p | 0.00437 | -2.90054 | DOWN |
| MI0000750\_MIMAT0000082 | hsa-miR-26a-5p | 0.004811 | -2.86833 | DOWN |
| MI0000083\_MIMAT0000082 | hsa-miR-26a-5p | 0.004813 | -2.86818 | DOWN |
| MI0006415\_MIMAT0005929 | hsa-miR-1275 | 0.004879 | -2.86359 | DOWN |
| MI0003581\_MIMAT0003239 | hsa-miR-574-3p | 0.005154 | 2.845097 | UP |
| MI0000441\_MIMAT0004589 | hsa-miR-30b-3p | 0.005343 | -2.83292 | DOWN |
| MI0000234\_MIMAT0000222 | hsa-miR-192-5p | 0.005851 | -2.80193 | DOWN |
| MI0000448\_MIMAT0000425 | hsa-miR-130a-3p | 0.005886 | -2.79989 | DOWN |
| MI0000082\_MIMAT0000081 | hsa-miR-25-3p | 0.005893 | -2.7995 | DOWN |
| MI0000077\_MIMAT0000076 | hsa-miR-21-5p | 0.006117 | 2.786672 | UP |
| MI0001733\_MIMAT0001635 | hsa-miR-452-5p | 0.006251 | 2.779215 | UP |
| MI0000272\_MIMAT0000259 | hsa-miR-182-5p | 0.006668 | 2.756908 | UP |
| MI0001729\_MIMAT0001631 | hsa-miR-451a | 0.00711 | -2.73462 | DOWN |
| MI0000089\_MIMAT0004504 | hsa-miR-31-3p | 0.007343 | 2.72339 | UP |
| MI0003589\_MIMAT0003247 | hsa-miR-582-5p | 0.007882 | 2.69853 | UP |
| MI0000748\_MIMAT0000691 | hsa-miR-130b-3p | 0.008389 | 2.676552 | UP |
| MI0000088\_MIMAT0000087 | hsa-miR-30a-5p | 0.008469 | -2.67318 | DOWN |
| MI0000736\_MIMAT0004674 | hsa-miR-30c-1-3p | 0.008581 | -2.66855 | DOWN |
| MI0000272\_MIMAT0000260 | hsa-miR-182-3p | 0.008731 | 2.662408 | UP |
| MI0000470\_MIMAT0000423 | hsa-miR-125b-5p | 0.009243 | -2.64211 | DOWN |
| MI0000446\_MIMAT0000423 | hsa-miR-125b-5p | 0.009518 | -2.63162 | DOWN |
| MI0000471\_MIMAT0000445 | hsa-miR-126-3p | 0.009523 | -2.63144 | DOWN |
| MI0003589\_MIMAT0004797 | hsa-miR-582-3p | 0.010005 | 2.613703 | UP |
| MI0000743\_MIMAT0004677 | hsa-miR-34c-3p | 0.010512 | -2.59587 | DOWN |
| MI0000080\_MIMAT0000079 | hsa-miR-24-1-5p | 0.010513 | 2.595842 | UP |
| MI0003834\_MIMAT0003887 | hsa-miR-769-3p | 0.011142 | 2.574744 | UP |
| MI0000271\_MIMAT0000258 | hsa-miR-181c-5p | 0.011176 | -2.57363 | DOWN |
| MI0000271\_MIMAT0004559 | hsa-miR-181c-3p | 0.01136 | -2.5677 | DOWN |
| MI0000469\_MIMAT0004602 | hsa-miR-125a-3p | 0.011383 | -2.56696 | DOWN |
| MI0017290\_MIMAT0019731 | hsa-miR-4662a-5p | 0.011406 | -2.56622 | DOWN |
| MI0003575\_MIMAT0004794 | hsa-miR-551b-5p | 0.012532 | -2.53171 | DOWN |
| MI0006406\_MIMAT0005923 | hsa-miR-1269a | 0.013649 | -2.50014 | DOWN |
| MI0000434\_MIMAT0004585 | hsa-let-7i-3p | 0.014389 | 2.480456 | UP |
| MI0000456\_MIMAT0004597 | hsa-miR-140-3p | 0.014819 | -2.46945 | DOWN |
| MI0016789\_MIMAT0018965 | hsa-miR-4446-3p | 0.016347 | -2.43247 | DOWN |
| MI0000764\_MIMAT0000707 | hsa-miR-363-3p | 0.016384 | -2.43161 | DOWN |
| MI0015995\_MIMAT0017982 | hsa-miR-3605-3p | 0.017059 | -2.41627 | DOWN |
| MI0003186\_MIMAT0004775 | hsa-miR-502-3p | 0.017377 | -2.40923 | DOWN |
| MI0001519\_MIMAT0001413 | hsa-miR-20b-5p | 0.018296 | -2.38951 | DOWN |
| MI0003657\_MIMAT0020924 | hsa-miR-642a-3p | 0.018614 | -2.38289 | DOWN |
| MI0000812\_MIMAT0004700 | hsa-miR-331-5p | 0.018661 | 2.381911 | UP |
| MI0002470\_MIMAT0004762 | hsa-miR-486-3p | 0.019508 | -2.3648 | DOWN |
| MI0003190\_MIMAT0004776 | hsa-miR-505-5p | 0.021653 | -2.32421 | DOWN |
| MI0000101\_MIMAT0004511 | hsa-miR-99a-3p | 0.021731 | -2.3228 | DOWN |
| MI0000288\_MIMAT0000269 | hsa-miR-212-3p | 0.02358 | 2.290676 | UP |
| MI0000253\_MIMAT0000243 | hsa-miR-148a-3p | 0.023882 | 2.285637 | UP |
| MI0000107\_MIMAT0000100 | hsa-miR-29b-3p | 0.02423 | 2.279899 | UP |
| MI0003184\_MIMAT0002871 | hsa-miR-500a-3p | 0.024263 | -2.27936 | DOWN |
| MI0000105\_MIMAT0000100 | hsa-miR-29b-3p | 0.024364 | 2.277713 | UP |
| MI0000732\_MIMAT0000460 | hsa-miR-194-5p | 0.025292 | -2.26283 | DOWN |
| MI0000267\_MIMAT0000254 | hsa-miR-10b-5p | 0.025327 | -2.26227 | DOWN |
| MI0000086\_MIMAT0000085 | hsa-miR-28-5p | 0.026716 | 2.240894 | UP |
| MI0000791\_MIMAT0000738 | hsa-miR-383 | 0.027841 | -2.22429 | DOWN |
| MI0000434\_MIMAT0000415 | hsa-let-7i-5p | 0.029044 | -2.20717 | DOWN |
| MI0000488\_MIMAT0000460 | hsa-miR-194-5p | 0.029417 | -2.20198 | DOWN |
| MI0005561\_MIMAT0004949 | hsa-miR-877-5p | 0.030033 | 2.193535 | UP |
| MI0003639\_MIMAT0004808 | hsa-miR-625-3p | 0.030715 | 2.184364 | UP |
| MI0000460\_MIMAT0000436 | hsa-miR-144-3p | 0.032348 | -2.16311 | DOWN |
| MI0000812\_MIMAT0000760 | hsa-miR-331-3p | 0.033042 | 2.154355 | UP |
| MI0016413\_MIMAT0018183 | hsa-miR-3909 | 0.033468 | 2.149058 | UP |
| MI0000113\_MIMAT0000103 | hsa-miR-106a-5p | 0.034881 | -2.13191 | DOWN |
| MI0000288\_MIMAT0022695 | hsa-miR-212-5p | 0.035107 | 2.129219 | UP |
| MI0003685\_MIMAT0003339 | hsa-miR-421 | 0.036102 | 2.11756 | UP |
| MI0000439\_MIMAT0004587 | hsa-miR-23b-5p | 0.03665 | 2.111253 | UP |
| MI0006358\_MIMAT0005886 | hsa-miR-1297 | 0.036658 | -2.11116 | DOWN |
| MI0000462\_MIMAT0000438 | hsa-miR-152 | 0.036967 | 2.107647 | UP |
| MI0006357\_MIMAT0005885 | hsa-miR-1295a | 0.037339 | -2.10344 | DOWN |
| MI0006394\_MIMAT0005911 | hsa-miR-1260a | 0.038609 | -2.08937 | DOWN |
| MI0005765\_MIMAT0004984 | hsa-miR-941 | 0.03994 | 2.075042 | UP |
| MI0005763\_MIMAT0004984 | hsa-miR-941 | 0.03994 | 2.075042 | UP |
| MI0003132\_MIMAT0003161 | hsa-miR-493-3p | 0.040122 | -2.07311 | DOWN |
| MI0000342\_MIMAT0000318 | hsa-miR-200b-3p | 0.041066 | 2.063223 | UP |
| MI0005530\_MIMAT0002881 | hsa-miR-509-3p | 0.041551 | -2.05823 | DOWN |
| MI0005717\_MIMAT0002881 | hsa-miR-509-3p | 0.041551 | -2.05823 | DOWN |
| MI0003196\_MIMAT0002881 | hsa-miR-509-3p | 0.041551 | -2.05823 | DOWN |
| MI0016053\_MIMAT0018073 | hsa-miR-3653 | 0.042775 | -2.04582 | DOWN |
| MI0000742\_MIMAT0004676 | hsa-miR-34b-3p | 0.043272 | -2.04088 | DOWN |
| MI0000290\_MIMAT0000271 | hsa-miR-214-3p | 0.044466 | -2.02918 | DOWN |
| MI0015825\_MIMAT0016847 | hsa-miR-378c | 0.045614 | 2.018188 | UP |
| MI0000481\_MIMAT0000454 | hsa-miR-184 | 0.047471 | 2.000892 | UP |
| MI0000479\_MIMAT0000451 | hsa-miR-150-5p | 0.048959 | -1.98744 | DOWN |
| MI0000094\_MIMAT0000092 | hsa-miR-92a-3p | 0.049239 | -1.98495 | DOWN |
| MI0003195\_MIMAT0002880 | hsa-miR-508-3p | 0.049502 | -1.98262 | DOWN |

**Supplementary Table 3. Gender associated microRNAs (p<0.05)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| miRBase ID | microRNA name | p-value | t-statistic | Direction in males |
| MI0003591\_MIMAT0003249 | hsa-miR-584-5p | 8.71E-05 | 4.050113 | UP |
| MI0006406\_MIMAT0005923 | hsa-miR-1269a | 0.00038 | -3.64802 | DOWN |
| MI0000261\_MIMAT0000250 | hsa-miR-139-5p | 0.000421 | 3.619431 | UP |
| MI0002470\_MIMAT0002177 | hsa-miR-486-5p | 0.00051 | 3.564016 | UP |
| MI0002470\_MIMAT0004762 | hsa-miR-486-3p | 0.000597 | 3.518597 | UP |
| MI0000764\_MIMAT0000707 | hsa-miR-363-3p | 0.001246 | 3.299905 | UP |
| MI0000482\_MIMAT0000455 | hsa-miR-185-5p | 0.001287 | 3.290067 | UP |
| MI0005767\_MIMAT0004985 | hsa-miR-942 | 0.00245 | 3.089317 | UP |
| MI0001729\_MIMAT0001631 | hsa-miR-451a | 0.00249 | 3.084152 | UP |
| MI0000267\_MIMAT0000254 | hsa-miR-10b-5p | 0.003311 | 2.992199 | UP |
| MI0017878\_MIMAT0021044 | hsa-miR-5010-3p | 0.003669 | 2.958545 | UP |
| MI0005524\_MIMAT0004902 | hsa-miR-891a | 0.003731 | 2.95302 | UP |
| MI0000487\_MIMAT0004614 | hsa-miR-193a-5p | 0.004155 | 2.917373 | UP |
| MI0000737\_MIMAT0001620 | hsa-miR-200a-5p | 0.004183 | -2.91512 | DOWN |
| MI0003205\_MIMAT0002888 | hsa-miR-532-5p | 0.00421 | 2.912991 | UP |
| MI0016005\_MIMAT0017994 | hsa-miR-3615 | 0.004315 | 2.904792 | UP |
| MI0000460\_MIMAT0004600 | hsa-miR-144-5p | 0.0044 | 2.898319 | UP |
| MI0000762\_MIMAT0000705 | hsa-miR-362-5p | 0.004953 | 2.858559 | UP |
| MI0003132\_MIMAT0003161 | hsa-miR-493-3p | 0.005383 | 2.830367 | UP |
| MI0000471\_MIMAT0000445 | hsa-miR-126-3p | 0.00618 | 2.783127 | UP |
| MI0000113\_MIMAT0000103 | hsa-miR-106a-5p | 0.00626 | 2.778729 | UP |
| MI0000743\_MIMAT0000686 | hsa-miR-34c-5p | 0.008081 | -2.68977 | DOWN |
| MI0003184\_MIMAT0002871 | hsa-miR-500a-3p | 0.008227 | 2.683471 | UP |
| MI0006359\_MIMAT0005887 | hsa-miR-1299 | 0.008432 | 2.674752 | UP |
| MI0000482\_MIMAT0004611 | hsa-miR-185-3p | 0.008837 | 2.658095 | UP |
| MI0000742\_MIMAT0000685 | hsa-miR-34b-5p | 0.008861 | -2.65714 | DOWN |
| MI0003129\_MIMAT0002809 | hsa-miR-146b-5p | 0.008881 | 2.656329 | UP |
| MI0000737\_MIMAT0000682 | hsa-miR-200a-3p | 0.009483 | -2.63292 | DOWN |
| MI0000082\_MIMAT0000081 | hsa-miR-25-3p | 0.009953 | 2.615567 | UP |
| MI0003583\_MIMAT0003241 | hsa-miR-576-5p | 0.011609 | 2.559776 | UP |
| MI0000734\_MIMAT0004672 | hsa-miR-106b-3p | 0.01165 | 2.55849 | UP |
| MI0003132\_MIMAT0002813 | hsa-miR-493-5p | 0.011967 | 2.548663 | UP |
| MI0000461\_MIMAT0000437 | hsa-miR-145-5p | 0.012154 | 2.542988 | UP |
| MI0014234\_MIMAT0015072 | hsa-miR-320e | 0.012943 | 2.519831 | UP |
| MI0000748\_MIMAT0000691 | hsa-miR-130b-3p | 0.013366 | 2.507912 | UP |
| MI0000471\_MIMAT0000444 | hsa-miR-126-5p | 0.013573 | 2.502228 | UP |
| MI0000459\_MIMAT0000435 | hsa-miR-143-3p | 0.014702 | 2.472407 | UP |
| MI0000448\_MIMAT0000425 | hsa-miR-130a-3p | 0.015185 | 2.460286 | UP |
| MI0000438\_MIMAT0004586 | hsa-miR-15b-3p | 0.01567 | 2.448448 | UP |
| MI0000814\_MIMAT0004701 | hsa-miR-338-5p | 0.015785 | 2.445691 | UP |
| MI0000342\_MIMAT0000318 | hsa-miR-200b-3p | 0.01587 | -2.44367 | DOWN |
| MI0001519\_MIMAT0001413 | hsa-miR-20b-5p | 0.016517 | 2.428529 | UP |
| MI0000300\_MIMAT0004570 | hsa-miR-223-5p | 0.017687 | 2.402462 | UP |
| MI0000094\_MIMAT0000092 | hsa-miR-92a-3p | 0.017863 | 2.398675 | UP |
| MI0000461\_MIMAT0004601 | hsa-miR-145-3p | 0.018111 | 2.393409 | UP |
| MI0000434\_MIMAT0000415 | hsa-let-7i-5p | 0.01864 | 2.382346 | UP |
| MI0000093\_MIMAT0000092 | hsa-miR-92a-3p | 0.019155 | 2.37185 | UP |
| MI0000816\_MIMAT0004703 | hsa-miR-335-3p | 0.019155 | 2.371841 | UP |
| MI0000438\_MIMAT0000417 | hsa-miR-15b-5p | 0.020899 | 2.338061 | UP |
| MI0003129\_MIMAT0004766 | hsa-miR-146b-3p | 0.021041 | 2.335417 | UP |
| MI0000281\_MIMAT0000232 | hsa-miR-199a-3p | 0.021208 | 2.332323 | UP |
| MI0000282\_MIMAT0004563 | hsa-miR-199b-3p | 0.02133 | 2.330082 | UP |
| MI0000242\_MIMAT0000232 | hsa-miR-199a-3p | 0.021332 | 2.330055 | UP |
| MI0000115\_MIMAT0000069 | hsa-miR-16-5p | 0.021953 | 2.318821 | UP |
| MI0000115\_MIMAT0004518 | hsa-miR-16-2-3p | 0.022134 | 2.315603 | UP |
| MI0000070\_MIMAT0000069 | hsa-miR-16-5p | 0.022163 | 2.315092 | UP |
| MI0005565\_MIMAT0004954 | hsa-miR-543 | 0.023505 | 2.291934 | UP |
| MI0003772\_MIMAT0010214 | hsa-miR-151b | 0.023652 | 2.289467 | UP |
| MI0000290\_MIMAT0000271 | hsa-miR-214-3p | 0.025275 | 2.263094 | UP |
| MI0000778\_MIMAT0000722 | hsa-miR-370 | 0.025438 | 2.260537 | UP |
| MI0003185\_MIMAT0002872 | hsa-miR-501-5p | 0.026337 | 2.246644 | UP |
| MI0003667\_MIMAT0003322 | hsa-miR-652-3p | 0.028277 | 2.218005 | UP |
| MI0000472\_MIMAT0000446 | hsa-miR-127-3p | 0.031579 | 2.172998 | UP |
| MI0000460\_MIMAT0000436 | hsa-miR-144-3p | 0.031737 | 2.170951 | UP |
| MI0000265\_MIMAT0000252 | hsa-miR-7-5p | 0.031786 | 2.170322 | UP |
| MI0000264\_MIMAT0000252 | hsa-miR-7-5p | 0.031836 | 2.169671 | UP |
| MI0000263\_MIMAT0000252 | hsa-miR-7-5p | 0.032048 | 2.166942 | UP |
| MI0000255\_MIMAT0000245 | hsa-miR-30d-5p | 0.033298 | -2.15116 | DOWN |
| MI0000749\_MIMAT0000692 | hsa-miR-30e-5p | 0.033377 | -2.15018 | DOWN |
| MI0000790\_MIMAT0000737 | hsa-miR-382-5p | 0.033437 | 2.14944 | UP |
| MI0000085\_MIMAT0000084 | hsa-miR-27a-3p | 0.033468 | -2.14906 | DOWN |
| MI0000747\_MIMAT0004679 | hsa-miR-296-3p | 0.034018 | 2.142316 | UP |
| MI0003186\_MIMAT0004775 | hsa-miR-502-3p | 0.034607 | 2.135181 | UP |
| MI0003632\_MIMAT0003287 | hsa-miR-618 | 0.035611 | 2.123284 | UP |
| MI0000802\_MIMAT0004692 | hsa-miR-340-5p | 0.036036 | -2.11833 | DOWN |
| MI0000090\_MIMAT0004505 | hsa-miR-32-3p | 0.037585 | -2.10069 | DOWN |
| MI0000456\_MIMAT0004597 | hsa-miR-140-3p | 0.038434 | 2.091286 | UP |
| MI0000457\_MIMAT0004598 | hsa-miR-141-5p | 0.03844 | -2.09122 | DOWN |
| MI0000813\_MIMAT0000762 | hsa-miR-324-3p | 0.038692 | 2.08846 | UP |
| MI0000786\_MIMAT0000731 | hsa-miR-378a-5p | 0.041886 | 2.054805 | UP |
| MI0015995\_MIMAT0017982 | hsa-miR-3605-3p | 0.0445 | 2.028849 | UP |
| MI0000650\_MIMAT0000617 | hsa-miR-200c-3p | 0.045047 | -2.02359 | DOWN |
| MI0020364\_MIMAT0023712 | hsa-miR-6087 | 0.047024 | 2.005 | UP |
| MI0003127\_MIMAT0002808 | hsa-miR-511 | 0.049781 | 1.980159 | UP |
| MI0000458\_MIMAT0000433 | hsa-miR-142-5p | 0.049834 | 1.979692 | UP |

**Supplementary Table 4. Age associated microRNAs (p<0.05)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| miRBase ID | microRNA name | p-value | t-statistic | Direction with age increase |
| MI0000102\_MIMAT0000098 | hsa-miR-100-5p | 0.004609 | 2.882752 | UP |
| MI0000681\_MIMAT0000646 | hsa-miR-155-5p | 0.008066 | -2.69041 | DOWN |
| MI0000289\_MIMAT0000256 | hsa-miR-181a-5p | 0.009424 | -2.63516 | DOWN |
| MI0000269\_MIMAT0000256 | hsa-miR-181a-5p | 0.009427 | -2.63504 | DOWN |
| MI0000289\_MIMAT0000270 | hsa-miR-181a-3p | 0.009663 | -2.62621 | DOWN |
| MI0005416\_MIMAT0004284 | hsa-miR-675-5p | 0.012408 | -2.53539 | DOWN |
| MI0003195\_MIMAT0002880 | hsa-miR-508-3p | 0.014651 | 2.473715 | UP |
| MI0000086\_MIMAT0000085 | hsa-miR-28-5p | 0.016109 | -2.43802 | DOWN |
| MI0000683\_MIMAT0000257 | hsa-miR-181b-5p | 0.016941 | -2.41891 | DOWN |
| MI0000270\_MIMAT0000257 | hsa-miR-181b-5p | 0.01724 | -2.41225 | DOWN |
| MI0003198\_MIMAT0002883 | hsa-miR-514a-3p | 0.024898 | 2.269085 | UP |
| MI0003200\_MIMAT0002883 | hsa-miR-514a-3p | 0.024898 | 2.269085 | UP |
| MI0003199\_MIMAT0002883 | hsa-miR-514a-3p | 0.024898 | 2.269085 | UP |
| MI0005767\_MIMAT0004985 | hsa-miR-942 | 0.030666 | -2.18502 | DOWN |
| MI0003760\_MIMAT0004819 | hsa-miR-671-3p | 0.032443 | -2.1619 | DOWN |
| MI0000542\_MIMAT0000510 | hsa-miR-320a | 0.033491 | -2.14878 | DOWN |
| MI0000441\_MIMAT0000420 | hsa-miR-30b-5p | 0.034698 | 2.134098 | UP |
| MI0003601\_MIMAT0004800 | hsa-miR-550a-5p | 0.038568 | -2.08981 | DOWN |
| MI0003600\_MIMAT0004800 | hsa-miR-550a-5p | 0.038664 | -2.08876 | DOWN |
| MI0000476\_MIMAT0000430 | hsa-miR-138-5p | 0.041213 | -2.06171 | DOWN |
| MI0000458\_MIMAT0000433 | hsa-miR-142-5p | 0.043524 | -2.03838 | DOWN |
| MI0000269\_MIMAT0004558 | hsa-miR-181a-2-3p | 0.04365 | -2.03714 | DOWN |
| MI0000764\_MIMAT0000707 | hsa-miR-363-3p | 0.04401 | -2.03361 | DOWN |
| MI0000481\_MIMAT0000454 | hsa-miR-184 | 0.045025 | 2.023794 | UP |
| MI0000115\_MIMAT0004518 | hsa-miR-16-2-3p | 0.047756 | -1.99829 | DOWN |

**Supplementary Table 5. Pack-years (PY) associated microRNAs (p<0.05)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| miRBase ID | microRNA name | p-value | t-statistic | Direction with PY increase |
| MI0000071\_MIMAT0000071 | hsa-miR-17-3p | 0.011764 | -2.55466 | DOWN |
| MI0003183\_MIMAT0002870 | hsa-miR-499a-5p | 0.016027 | -2.4397 | DOWN |
| MI0015997\_MIMAT0017985 | hsa-miR-3607-3p | 0.019021 | 2.374323 | UP |
| MI0000300\_MIMAT0004570 | hsa-miR-223-5p | 0.019299 | 2.368731 | UP |
| MI0000434\_MIMAT0004585 | hsa-let-7i-3p | 0.020197 | -2.35112 | DOWN |
| MI0005544\_MIMAT0004928 | hsa-miR-147b | 0.030047 | 2.193163 | UP |
| MI0000088\_MIMAT0000088 | hsa-miR-30a-3p | 0.030984 | 2.180624 | UP |
| MI0019308\_MIMAT0022494 | hsa-miR-5701 | 0.036403 | 2.113925 | UP |
| MI0019593\_MIMAT0022494 | hsa-miR-5701 | 0.036403 | 2.113925 | UP |
| MI0000068\_MIMAT0000067 | hsa-let-7f-5p | 0.037702 | 2.099219 | UP |
| MI0000067\_MIMAT0000067 | hsa-let-7f-5p | 0.039373 | 2.080928 | UP |
| MI0003780\_MIMAT0005794 | hsa-miR-1296 | 0.039909 | -2.0752 | DOWN |
| MI0000101\_MIMAT0004511 | hsa-miR-99a-3p | 0.040476 | -2.06922 | DOWN |
| MI0000300\_MIMAT0000280 | hsa-miR-223-3p | 0.04588 | 2.015523 | UP |

**Supplementary Table 6. Cancer associated microRNAs (p<0.05)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| miRBase ID | microRNA name | p-value | t-statistic | Direction in cancer |
| MI0000813\_MIMAT0000761 | hsa-miR-324-5p | 0.000649 | -3.49412 | DOWN |
| MI0000300\_MIMAT0000280 | hsa-miR-223-3p | 0.000714 | -3.46634 | DOWN |
| MI0000477\_MIMAT0000449 | hsa-miR-146a-5p | 0.000808 | -3.4298 | DOWN |
| MI0000300\_MIMAT0004570 | hsa-miR-223-5p | 0.001586 | -3.22597 | DOWN |
| MI0003834\_MIMAT0003887 | hsa-miR-769-3p | 0.002842 | -3.04179 | DOWN |
| MI0003632\_MIMAT0003287 | hsa-miR-618 | 0.003085 | -3.01518 | DOWN |
| MI0000814\_MIMAT0004701 | hsa-miR-338-5p | 0.005204 | -2.8418 | DOWN |
| MI0000286\_MIMAT0000267 | hsa-miR-210 | 0.007292 | -2.72581 | DOWN |
| MI0001652\_MIMAT0001545 | hsa-miR-450a-5p | 0.008086 | -2.68953 | DOWN |
| MI0000441\_MIMAT0000420 | hsa-miR-30b-5p | 0.008275 | -2.68142 | DOWN |
| MI0003187\_MIMAT0001545 | hsa-miR-450a-5p | 0.008425 | -2.67505 | DOWN |
| MI0000268\_MIMAT0000255 | hsa-miR-34a-5p | 0.009728 | -2.62381 | DOWN |
| MI0003667\_MIMAT0003322 | hsa-miR-652-3p | 0.013213 | -2.51217 | DOWN |
| MI0000114\_MIMAT0000104 | hsa-miR-107 | 0.016073 | -2.43885 | DOWN |
| MI0000783\_MIMAT0000728 | hsa-miR-375 | 0.018023 | 2.395261 | UP |
| MI0000080\_MIMAT0000079 | hsa-miR-24-1-5p | 0.018845 | 2.378128 | UP |
| MI0005562\_MIMAT0004951 | hsa-miR-887 | 0.020166 | -2.35195 | DOWN |
| MI0005544\_MIMAT0004928 | hsa-miR-147b | 0.022438 | -2.31024 | DOWN |
| MI0000089\_MIMAT0000089 | hsa-miR-31-5p | 0.022548 | -2.30832 | DOWN |
| MI0000735\_MIMAT0004673 | hsa-miR-29c-5p | 0.022925 | -2.30179 | DOWN |
| MI0000813\_MIMAT0000762 | hsa-miR-324-3p | 0.023499 | -2.29203 | DOWN |
| MI0000825\_MIMAT0000772 | hsa-miR-345-5p | 0.024099 | -2.28205 | DOWN |
| MI0005531\_MIMAT0004909 | hsa-miR-450b-5p | 0.026429 | -2.24524 | DOWN |
| MI0003834\_MIMAT0003886 | hsa-miR-769-5p | 0.027019 | -2.23636 | DOWN |
| MI0000808\_MIMAT0000756 | hsa-miR-326 | 0.027116 | -2.23493 | DOWN |
| MI0005762\_MIMAT0004983 | hsa-miR-940 | 0.028261 | -2.21824 | DOWN |
| MI0000433\_MIMAT0000414 | hsa-let-7g-5p | 0.029329 | -2.20319 | DOWN |
| MI0003589\_MIMAT0003247 | hsa-miR-582-5p | 0.030653 | -2.1852 | DOWN |
| MI0000748\_MIMAT0004680 | hsa-miR-130b-5p | 0.030991 | 2.180711 | UP |
| MI0000089\_MIMAT0004504 | hsa-miR-31-3p | 0.03322 | -2.15213 | DOWN |
| MI0016902\_MIMAT0019074 | hsa-miR-378i | 0.033939 | -2.14328 | DOWN |
| MI0003190\_MIMAT0002876 | hsa-miR-505-3p | 0.039021 | -2.08488 | DOWN |
| MI0006384\_MIMAT0005901 | hsa-miR-1249 | 0.039448 | -2.08028 | DOWN |
| MI0000298\_MIMAT0000278 | hsa-miR-221-3p | 0.039583 | -2.07884 | DOWN |
| MI0014197\_MIMAT0015041 | hsa-miR-1260b | 0.039908 | -2.07538 | DOWN |
| MI0014249\_MIMAT0015085 | hsa-miR-3200-3p | 0.042866 | -2.04492 | DOWN |
| MI0001448\_MIMAT0001343 | hsa-miR-425-3p | 0.044056 | -2.03317 | DOWN |
| MI0017438\_MIMAT0019963 | hsa-miR-4791 | 0.044608 | -2.02781 | DOWN |
| MI0003780\_MIMAT0005794 | hsa-miR-1296 | 0.045033 | -2.02372 | DOWN |
| MI0017308\_MIMAT0019761 | hsa-miR-4677-3p | 0.045417 | -2.02006 | DOWN |
| MI0000273\_MIMAT0000261 | hsa-miR-183-5p | 0.045741 | 2.016984 | UP |
| MI0000283\_MIMAT0000264 | hsa-miR-203a | 0.049459 | 1.983001 | UP |

**Supplementary Table 7. Biomarker performance in the test set (\* significant increase in AUC compared to mRNA biomarker p<0.05;** † **ROC-curve Operating Point set to 90% sensitivity in the training set)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | AUC (95% CI) | Sens† | Spec† | NPV† | PPV† |
| mRNA biomarker | 0.66 (0.58-0.73) | 0.94 | 0.27 | 0.78 | 0.60 |
| mRNA biomarker + miR-146a-5p | 0.71\* (0.64-0.78) | 0.93 | 0.33 | 0.83 | 0.59 |
| mRNA biomarker + miR-324-5p | 0.65 (0.57-0.72) | 0.94 | 0.23 | 0.79 | 0.55 |
| mRNA biomarker + miR-223-3p | 0.67 (0.60-0.75) | 0.96 | 0.27 | 0.87 | 0.58 |
| mRNA biomarker + miR-223-5p | 0.67 (0.60-0.75) | 0.95 | 0.26 | 0.84 | 0.57 |