Article

MicroRNAs within Basal-Like Signature of Quadruple Negative Breast Cancer Impact Overall Survival in African Americans

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Abstract

Background: We previously found that QNBC tumors are more frequent in African Americans compared to TNBC tumors. To characterize this subtype further, we sought to determine the miRNA-mRNA profile in QNBC patients based on race. **Methods:** Both miRNA and mRNA expression data were analyzed from TCGA and validated using datasets from the METABRIC, TCGA proteomic, and survival analysis by KMPLOT. **Results:** miRNA-mRNAs which include FOXA1 and MYC (mir-17/20a targets); GATA3 and CCNG2 (mir-135b targets); CDKN2A, CDK6, and B7-H3 (mir-29c targets); and RUNX3, KLF5, IL1-β, and CTNNB1 (mir-375 targets) were correlated with basal-like and immune subtypes in QNBC patients and associated with a worse survival. **Conclusion:** Thus, QNBC tumors have an altered gene signature implicated in racial disparity and poor survival.

Keywords: QNBC (Quadruple negative breast cancer); AR (Androgen receptor); AA (African American); CA (Caucasian); BC (Breast cancer)

Introduction

Breast cancer remains a leading cause of death among women globally due to its lack of response to therapy, poor prognosis, and low survival rate. Although breast tumors are typically categorized by multiple subtypes based on gene expression, women of African Ancestry are frequently diagnosed with TNBC, which is a

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heterogeneous disease that is characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). We recently identified that a subset of TNBC patients that also lack Androgen Receptor (AR) and we named them Quadruple Negative breast cancer (QNBC) and associated them with basal-like (BL1 and BL2) subtype, an immunomodulatory (IM) subtype (1, 2). Furthermore, these QNBC basal-like tumors are commonly found in younger AA women and have worse outcomes compared to other breast cancer subtypes, including TNBC tumors (1). Hence, there is a need for identifying the underlying mechanisms associated with the QNBC subtype.

miRNAs are short, non-coding RNAs (~18-24 nucleotides) that modulate gene expression post-transcriptionally (3) at the mRNA level. Under certain conditions, miRNAs can be either oncogenes or tumor suppressors, regulating a number of rate-limiting events in carcinogenesis, including sustaining cellular proliferation, escaping growth suppressors, apoptotic resistance, angiogenesis, and promoting invasion and metastasis (4). A given miRNA can target multiple mRNAs (5); and these mRNA–miRNA interactions can result in either reduced mRNA stability or inhibition of translation into proteins. In this way, miRNAs regulate gene expression and have clinical relevance as biomarkers (6-8). Novel experimental therapeutics that target miRNAs are now gaining attention in preclinical studies and clinical trials (9, 10).

Here, we report that levels of miRNAs mir-17, mir-20a, mir-135b, mir-584, and mir-532 are overexpressed and levels of miRNAs mir-29c, mir-10a, and mir-375 are decreased that these changes correlate with a lower survival for AA patients regardless of subtype. Furthermore, these miRNAs appear to target mRNAs of FOXA1 and MYC (mir-17/20a targets), GATA3 and CCNG2 (mir-135b targets), CDKN2A, CDK6, and CD276 (mir-29c targets), RUNX3, KLF5, IL1-β, and CTNNB1 (mir-375 targets), all these molecules have been associated with driving both the basal-like and immune gene signatures in QNBC tumors. These findings highlight a regulator role of miRNA associated with race and within the QNBC subtype.

Materials and Methods

RNA sequencing, proteomics data analysis, and determination of AR status

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BRCA miRNA quantification files, metadata, and clinical files for 1206 samples were downloaded from TCGA data portal (https://portal.gdc.cancer.gov/) in 2016. There were 103 solid normal tissue samples, seven metastatic tumor samples, and 1096 primary tumor samples. The miRNA files were merged into a single file. The DESEQ2 package in R was used for normalization and for assessing differential gene expression. On January 13, 2017, BRCA gene expression data in the form of fragments per kilobase of transcript per million mapped reads upper quartile (FPKM-UQ) units and metadata were downloaded from TCGA (11). For primary tumor samples (n= 1102), gene expression was evaluated. As previously described, quantile was used as a threshold to determine AR status (1). AR expression greater than quartile 1 or 25th percentile was considered as AR-positive. AR expression lower than quartile 1 or 25th percentile was considered AR-negative. For 105 primary tumor samples and three solid normal tissue samples, TCGA breast cancer proteomics data and associated clinical information files were downloaded from the Clinical Proteomic Tumor Analysis Consortium (CPTAC). Unshared log-ratio units were used for protein expression of the genes (11).

Microarray data set analysis

Two Gene Expression Omnibus (GEO) datasets, GSE22220 (Illumina humanRef-8 v1.0 expression / human v1 miRNA Bead chip) and GSE19783 (Agilent 014850 whole genome / 019118 miRNA microarray), were used (12, 13). On February 16, 2018, files containing mRNA and miRNA data from primary breast cancer tumor samples were downloaded. GSE22220 mRNA and miRNA data, and GSE19783 mRNA data in log2 form were normalized. GSE19783 miRNA data were normalized using the quantile normalization method and Limma package in R. After normalization, samples were matched by mRNA and miRNA sample IDs. Quantile was used as a threshold to determine AR status. AR expression greater than quartile 1 or 25th percentile was considered as AR-positive. AR expression lower than quartile 1 or 25th percentile was considered as AR-negative. Log2 fold change was calculated for miRNA expression.

Molecular subtype classification

A clinical file was obtained for TCGA breast cancer dataset. Immunohistochemical (IHC) status for ER, PR, and Her2 were considered for subtype classification, and AR mRNA status was used as described above. The

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samples were classified as luminal A (ER- and/or PR-positive and HER2 negative), luminal B (ER- and or PR-positive and HER2-positive), HER2 enriched or HER2 type (ER-negative, PR-negative and HER2-positive), TNBC (ER-negative, PR-negative, and HER2-negative), AR-positive TNBC (ER-negative, PR-negative, HER2-negative, and AR-positive), QNBC (ER-negative, PR-negative, HER2-negative, and AR-negative) (14-17).

Data analysis

Heatmaps were generated with R by use of clinical parameters for clustering. P-adjusted and p-values were calculated for differential gene expression analysis using the DESEQ2 package in R. Venny 2.1 was used for visualization in the form of Venn diagrams (https://bioinfogp.cnb.csic.es/tools/venny/index.html). JMP statistical analysis software was used to prepare box plots and for statistical analysis for the non-parametric Wilcoxon test. In some cases, a two-tailed t-test was used to obtain statistical significance between groups. Pathway Studio MammalPlus was used to assess miRNA interactions with genes or proteins using a network builder tool (https://www.pathwaystudio.com/). A graph pad was also used for data visualization. The KM plotter was used to prepare plots for miRNAs for AAs and CAs in the overall breast cancer TCGA dataset using a pan-cancer tab (18). KM plots for miRNAs for molecular subtype TNBC patients in TCGA or METABRIC datasets were accomplished using the miRNA breast cancer tab (19). KM plots for breast cancer mRNAs were used for the miRNA target genes for survival analysis by the TNBC Pietenpol subtypes(20, 21). The probability of survival was calculated using the KM method, and log-rank tests were used to calculate the p-values.

Results

miRNA profiles in AR-positive versus AR-negative tumors

Using DESEQ2 analysis of TCGA to reveal miRNA signatures of AR-negative vs. AR-positive cancers, we identified 45 miRNAs that were differentially expressed and clustered by AR status and subtype (Figure S2A and Table S4). Among these 45 miRNAs, AR-negative cancers demonstrated upregulation of mir-519a-1,

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mir-519a-2, and mir-516a-1, which are associated with the miRNA-19 cluster, and mir-17, mir-18a, mir-17, and mir-20a, which are associated with the miRNA17-92 cluster. In contrast, mir-449a/b and mir-135a-1/2 were downregulated in AR-negative cancers. The miRNA signatures of AR-negative cancers were similarly expressed in QNBC , and miRNA expression within AR-positive cases was associated with luminal A/B and TNBC AR-positive cases (Figure S2A). When compared to miRNA differential expression for AR negative vs AR positive cases in TCGA and GEO datasets (GSE19783 and GSE22220), 15 miRNAs overlapped among the three independent datasets (Figure S2B).

To determine miRNAs that are associated with race and QNBCs, we performed four DESEQ2 analyses (Figure 1B and S1A). First, we found there are 45 miRNAs differentially expressed in overall AR-negative vs. -positive cases, accounted for 24% and 76% of those being down- and upregulated, respectively (Table S4 and Figure S2A). Second, 63 miRNAs were differentially expressed in overall AA vs. CA cases (Table S6 and Figure S3), with 54% and 46% being down- and upregulated, respectively. Third, 40 miRNAs differentially expressed in QNBCs as compared to AR-positive TNBCs showed 37% and 63% being down- and upregulated, respectively (Table S5 and Figure 1A). Lastly, in QNBCs, 66 miRNAs were differentially expressed in AAs vs. CAs (Table S7 and Figure S4) with proportional up- and downregulation of 85% and 15%, respectively. Moreover, we evaluated the overlap between the differential miRNA expression for results from four DESEQ analysis (Table S4, S5, S6 and S7) by Venn diagram (Figure 1B).

To determine miRNAs differentially expressed in QNBCs, we performed DESEQ2 analysis for QNBC vs. TNBC AR-positive tissues, for which 40 miRNAs were found to be dysregulated in QNBCs (Figure 1A), with mir-17 and 20a demonstrating elevated expression in overall AR-negative cases, AA cases, and QNBC cases. Most of the downregulated miRNAs in AA QNBCs are located on chromosome 14 (Figure S5 and Table S7). Additionally, 13 miRNAs appear to be race specific as they are differentially expressed in both overall AR negative and QNBCs groups irrespective of AR status (Figure 1B).

22 miRNAs, which are dysregulated in QNBC were selected. The miRNA expression levels of these 22 miRNAs were compared by box plot. Eight miRNAs were found to be highly expressed and these miRNAs

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were considered for further analysis (Figure 1C). To evaluate the expression pattern of 8 highly expressed miRNAs by AR status, race in overall samples, and QNBCs, we evaluated their expression by box plots, which showed high levels of hsa-mir-17, 20a, 584, 135b, and 532, and low levels of hsa-mir-29c,10a, and 375 in overall AR-negative samples (Table S1 and Figure 2A). Further, there were high levels of hsa-mir-17, 20a, 584, 135b, and 532 and low levels of hsa-mir-29c,10a, and 375 in QNBCs relative to AR-positive TNBCs (Table S2 and Figure 2B).

The eight selected miRNAs have a differential impact on survival of AA patients

To determine the correlation of miRNAs (hsa-mir-17, hsa-mir-20a, hsa-mir-135b, hsa-mir-584, hsa-mir-532, hsa-mir-10a, hsa-mir-375, and hsa-mir-29c) with overall survival for AA and CA patients with tumors across all breast cancer subtypes, METABRIC and TCGA datasets were used with KM plotter (Figures 3A, B, C, D and 4A, B, C, D). For AA patients having cancers with high levels of mir-17, 20a, 135b, and 584, regardless of subtype, there were lower survival rates compared to CA patients. Further, all patients, irrespective of race, with high levels of mir-532 and low levels of mir-29c and miR-10a had lower survival rates. However, AA cancers, regardless of subtype, and TNBCs with low levels of mir-375 appeared to be associated with lower survival, compared to high levels of mir-375 in CA cancers that appeared to be associated with a lower survival rate. Thus mir-375 may have opposite functions in AA and CA.

Based on the outcomes of selected miRNAs in Figures 3 and 4, higher expression of hsa-mir-17 was evident for 53 (AA, overall) and 85 (TNBC) patients at risk of lower survival; however, based on hsa-mir-135b upregulation, only 102 TNBC patients were at risk of lower survival based on the level of statistical significance. Similarly, high expression of hsa-mir-532 was linked to 49 (AA, overall) and 455 (CA, overall) patients at risk of low survival with no statistical significance among TNBC patients, coupled with a similar pattern based on hsa-mir-10a upregulation with 54 (AA, overall) and 71 (CA, overall) patients at risk of low survival. Furthermore, higher levels of hsa-mir-375 were linked to 46 (AA, overall) and 70 (TNBC) patients at risk of low survival with no statistical significance on the part of CA overall patients. Only cancers of CAs,

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overall, seemed to be affected by hsa-mir-29c upregulation, with 230 patients being at risk of low survival (Figure 4D).

The survival analysis suggests a trend that higher expression of mir 17, mir 20a, mir 135b, mir 584, mir 532 and lower expression of mir 10a, mir 375 and mir 29c in AA patients and TNBC seems to be associated with lower survival (Figure 3 and 4). However, there was discrepancy in survival analysis results for TNBC datasets for TCGA vs Metabric datasets. This could be results of complexity of the patient derived data and the lower number on TNBC patients in TCGA dataset compared to Metabric dataset (Figure S9).

Genes associated with miRNAs are dysregulated in QNBCs

Since miRNAs control gene expression, we next sought to determine the genes associated with expression of the selected miRNAs in QNBCs while making a statistical comparison with AR-positive TNBCs. To assess gene targets, we used the Pathway Studio database to find the genes related to each differentially expressed miRNA through network enrichment analysis. Pathway Studio revealed that the mir-135b targets, CCNG2 and GATA3, were lower in QNBCs as compared to AR-positive TNBCs (Figure 5A). Further, as determined by mRNA and proteomics expression, mir-135b negatively correlated with CCNG2 and GATA3 (Table S3). mir-10a, which targets TNFα, was higher in QNBCs as compared to AR-positive TNBCs (Figure 5B). Confirming these findings, mir-10a negatively correlated with TNFα by mRNA and proteomics expression based on data from CPTAC (Table S3).

MYC, a transcription factor, appears to regulate the expression of MIR17HG in addition to mir-17 and mir-20a. Expressions of MYC and MIR17HG were higher, and FOXA1 expression was lower in QNBCs relative to AR-positive TNBCs. Furthermore, mir-17 and 20a positively correlated with MYC and MIR17HG, and, conversely, negatively correlated with FOXA1 in overall primary tumor samples (Figure 5C and Table S3). miR-375, which targets CTNNB1, IL1B, KLF5, and RUNX3, was higher in QNBCs as compared to AR-positive TNBCs in mRNA level (Figure 5D). Lastly, mir-29c, which targets CDK6, CDKN2A, B7H3, and CDKN2A, was higher in QNBCs as compared to AR-positive TNBCs. Further, mir-29c negatively correlated

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with CD276/B7H3, CDK6, and CDKN2A at both the RNA and proteomic expression levels (Figure 5E and Table S3).

The predicted targets of selected 8 miRNAs were obtained by Pathway Studio. Expressions of gene targets were evaluated in all BC samples by all subtypes (Figure S6A) and in TNBC samples by comparing TNBC AR positive Vs. QNBC subtype (Figure S6B) by heatmap. The miRNA-targets/ gene expression showed a pattern of lower expression of HOXB3, HIF1AN, CCNG2, FOXA1 and GATA3 in QNBC. And higher expression for CD276/B7H3, CTNB1, TGFBR2, IL1B, TNF, KLF5, CDK6, RUNX3, MYC, MIR17HG, CDKN2A and E2F3 was observed in QNBC subtype.

Association of AR, mir-29c, and CD276/B7H3 in QNBCs

Since mir-29c had a significant correlation with survival probability for patients with TNBCs, we next determined if, in QNBCs, loss of AR regulates mir-29c expression as well its gene target CD276/B7H3. CD276/B7H3, located on the cell membrane, is clinically relevant due to its overexpression in tumor tissues and limited expression in normal tissues, coupled with an immunoregulatory role in tumor microenvironment modeling and expansion (22). In TCGA mRNA data for overall primary tumor samples (n=1083), mir-29c positively correlated with AR and negatively correlated with CD276/B7H3. Similarly, in TCGA whole primary tumor samples proteomics data (n=105), mir-29c positively correlated with AR and negatively correlated with CD276/B7H3 (Figure S7), indicating that AR may upregulate mir-29c and subsequently downregulate its target CD276/B7H3.

As compared to apparently healthy tissue, CD276/B7H3 expression was higher in primary tumor tissues in various cancer subtypes. CD276/B7H3 expression was higher in QNBCs as compared to AR-positive TNBCs (Figure S7). In overall samples, CD276/B7H3 mRNA expression was higher in breast cancers of AAs compared to CAs. Also, there was a similar pattern in proteomics data. AAs had higher CD276/B7H3 as compared to CAs ($p=0.08$), but the difference was not statistically significant (Figure S8). Lastly, we performed survival analyses using breast cancer datasets for the CD276/B7H3 (probe ID 224859_at) subtype

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of TNBC. Patients with high CD276/B7H3 in basal-like 1 TNBC had a lower overall survival. Further, high CD276/B7H3 was associated with lower survival for patients with the BL-2, mesenchymal, and mesenchymal stem-like subtypes. Also, there was a similar pattern for patients having cancers with high CD276/B7H3 and was associated with a lower survival for both AA and CA patients, although the difference was not statistically significant (Figure S8).

Discussion

The versatility of miRNAs at physiological and pathological levels makes them indispensable factors for many types of cancer, including mammary cancers. This is corroborated by the fact that they have a function in racial cancer disparities based on diagnosis, prognosis, disease progression, and response to various therapeutic modalities (23). For instance, mir-655 expression positively correlates with COX-2 in genetically diverse breast cancer cells grown as spheroids, thereby linking it with stem-like cells (24). Clinically, miRNAs such as miR-155, miR-222, miR-125b, and miR-21 are associated with tumor resistance to the most common systemic treatments, qualifying them as potential predictors of response to breast cancer therapeutics (25). Our group recently demonstrated (26) that miRNAs like miR-17-5p, miR-432, miR-663a, and miR-1225 are implicated in the TNBC-to-QNBC transition, further underscoring their roles in cancer disparities. miRNA expression levels impact the levels of downstream genes or clinically relevant biomarkers. However, in previous literature it is unknown that how miRNA signatures differ among AA with CA patients relative to AR-positive TNBCs and QNBC subtypes. Consequently, the present communication helps to fill this knowledge gap via establishing relevant baseline information.

Around 70% of TNBCs are QNBCs (1, 27, 28). AR appears to add prognostic benefits to determine which tumors are aggressive and which are non-aggressive. In our previous report, we demonstrated that QNBCs of AA women have distinctive basal and immune gene signatures (1). In a follow-up study using IHC of clinically relevant protein markers, we found that metastatic QNBCs has higher levels of EGFR expression and lower levels of PTEN and the Wnt signaling inactivator, TLE3 (1, 14). These studies comparing miRNA signatures

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and mRNA-mediated genomic expression uncovered differences in QNBCs and AR-positive TNBCs that could explain the aggressive character of this disease.

To understand the regulatory networks associated with QNBCs, we assessed miRNA expression. We found high levels of has-mir-17, mir-20a, mir-584, mir-135b, and mir-532 and low levels of mir-29c, mir-10a and mir-375 in overall AR-negative, overall AA, and QNBC cases. Only a few studies have evaluated miRNA in association with AR status in breast cancers. In 2017, Shi *et al*. compared AR-negative and AR-positive cell lines; the results did not coincide with our findings for patients (29). Breast cancer cell lines treated with dihydrotestosterone showed an increase in the expression of mir-363 and mir-21 (17, 30). Although these studies were limited to cell lines, they provide evidence of AR-regulated miRNAs. In our results, however, mir-21 and mir-363 did not change with AR status. Our findings are consistent with those of Gong *et al.*, who reported that breast cancers of AA patients have lower levels of mir-10a (31). Moreover, Sugita et al. compared miRNA expression patterns in a separate TNBC cohort of AAs (n =27) and non-Hispanic whites (n=30). Consistent with our results, hsa-mir-17-5p and hsa-mir-18a-5p were upregulated in AA TNBCs; however, in contrast, mir-532-3p was low in AA TNBCs (32). Telonis *et al.* found multiple miRNA isoforms and showed that tRNA-derived fragments were associated with TNBCs and disparities (33). Relative to these results, our results seem to be different because we focused on investigating miRNA expression in QNBCs and on disparities.

Based upon our findings and literature reports, miRNAs are altered in QNBCs, resulting in a basal-like phenotype and causing alterations in the immune response. The target genes of miRNAs and transcription factors responsible for miRNA transcription showed an association with the basal-like phenotype and immune function (34). In the present study, we identified an association of the miRNA/gene network that leads to aggressive disease in QNBCs, as highlighted below.

Firstly, mir-135b was elevated in QNBCs, overall AR-negative breast cancers, and AA breast cancers. The target genes of miR-135b, involved in inhibition of the EMT (CCNG2) and inflammatory responses (GATA3) are lower in QNBCs (35, 36). Also, Uva *et al.* categorized mir-135b as upregulated in the basal-like subtype

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compared to the non-basal like subtype (37). Secondly, mir-17 and 20a expressions were higher in overall AR-negative and QNBC samples. MYC positively regulates mir-17 and 20a (38) (39). MYC expression was higher in QNBCs, and FOXA1 was lower in QNBCs. Loss in FOXA1 expression is associated with the basal subtype (40, 41). Also, MYC and FOXA1 are among the PAM50 genes, which we have previously found in QNBCs. This evidence indicates that AR loss in QNBCs could cause an increase in MYC that further increases transcription of mir-20a and -17 and down-regulation FOXA1. In consequence, loss of FOXA1 and an increase in MYC could lead to a basal-like phenotype in QNBCs. Thirdly, mir-375 is downregulated in overall AR-negative breast cancers and QNBCs. A decrease in mir-375 expression is associated with the basal subtype and malignant breast cancer (42). We observed that mir-29c was low in overall AR-negative cases and QNBCs. CD276/B7H3 is a clinically relevant target of mir-29c (43). Patients with high CD276/B7H3 in basal-like cancers have a lower survival rate(44). Finally, our findings showed that mir-10a is downregulated in AR-negative cases, AA cases, and QNBCs. mir-10a is a regulator of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway (45, 46). There is a negative association with mir-10a and TNFα (47, 48). TNFα expression was higher in QNBCs, and TNFα negatively correlated with mir-10a, suggesting that, in QNBCs, a loss in mir-10a could cause an increase in TNFα.

Our study has several strengths, including the large sample size, validation in independent datasets by AR IHC in TNBC samples, a comprehensive analysis of eight highly expressed miRNAs, evaluation of miRNA targets, and assessment of survival probability. Also, our results provide the underlying molecular mechanisms of the basal-like gene signature in QNBCs and in breast cancers of AA women. Therefore, to strengthen our findings, research on copy number variations of the miRNA host genes, AR binding patterns near the transcription start sites of the miRNAs, methylation, and possible single nucleotide polymorphisms of the miRNAs is needed. Further, there should be investigations into the ancestry-related correlation of miRNAs and miRNA host genes, and there is a need for more research to understand the cause of AR loss. Nevertheless, our results provide evidence that miRNAs have a function in breast cancer and highlight the importance of using miRNAs as biomarkers for clinical risk assessment and as potential therapeutic targets.

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A limitation of this study is the complexity of patient-derived data. Survival probability depends on external factors such as time of disease progression and vital status. The patients undergo different treatments, and the procedures may have an impact on vital status or gene expression. So, there is a discrepancy in the TNBC cohort's survival probability in TCGA and METABRIC datasets. For miRNAs mir-17, mir-20a, mir-135b, mir-584, mir-10a, and mir-375, there is a discrepancy between datasets for TNBC cases. For both datasets, however, mir-532 and -29c were consistent. We speculate that this could be due to the inclusion of several patients with AR-like TNBCs, or to different breast cancer stages associated with patients in the TNBC group. Also, the directionality of miRNAs (3p or 5p) is not included in TCGA data. Lastly, we cannot rule out other factors that can influence the vital status and miRNA signatures, such as patient characteristics or breast cancer stages, and exposure to environmental factors.

Conclusion

In our previous study, IHC of metastatic tumors showed that the percentage of tumors positive for EGFR was higher, and those positive for PTEN and TLE3 were lower in QNBCs compared to TNBCs. This evidence showed that QNBC is a consistent subtype, and that AR is a reliable marker to predict metastasis and recurrence. Furthermore, we established that QNBC tumors tend to be basal-like, with elevated EGFR expression and higher rates of Wnt signaling activity. We also evaluated miRNA expression in breast cancers of AA women with the QNBC subtype. By use of TCGA data, we found that expression of miRNAs in QNBCs differs from AR-positive TNBC cases. High levels of mir-17, mir-20a, mir-584, mir-135b, and mir-532 and low levels of mir-29c, mir-10a, and mir-375 were present in overall AR-negative breast cancers and QNBCs. In addition, in QNBCs, miRNA-associated genes such as MYC are upregulated, and FOXA1 is downregulated. We confirmed that miRNAs and their associated gene signatures are altered in QNBCs and breast cancers of AA patients, which could explain the distinctive basal-like and immune gene signatures in QNBCs of AAs. In summary, our study demonstrated that AR negativity is associated with breast cancers of AA women, and that QNBC is an aggressive subtype with an altered gene signature.

Competing Interest Statement

CY is a shareholder in Riptide Biosciences and is a consultant for QED Therapeutics, Riptide Biosciences, and Amgen.

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Author contributions

Concept and design: CY and WDC. Generation of data and performance of analyses: AA. Help with computational analyses: RH. Analysis of the data: CY, RM, MD, and AM. Patient sample collection: SH, MH, AK, TM, EA, WB, and BK. Immunohistochemistry support: SMA. Writing and editing of the manuscript: WDC, CY, MD, AA, TM, and AM.

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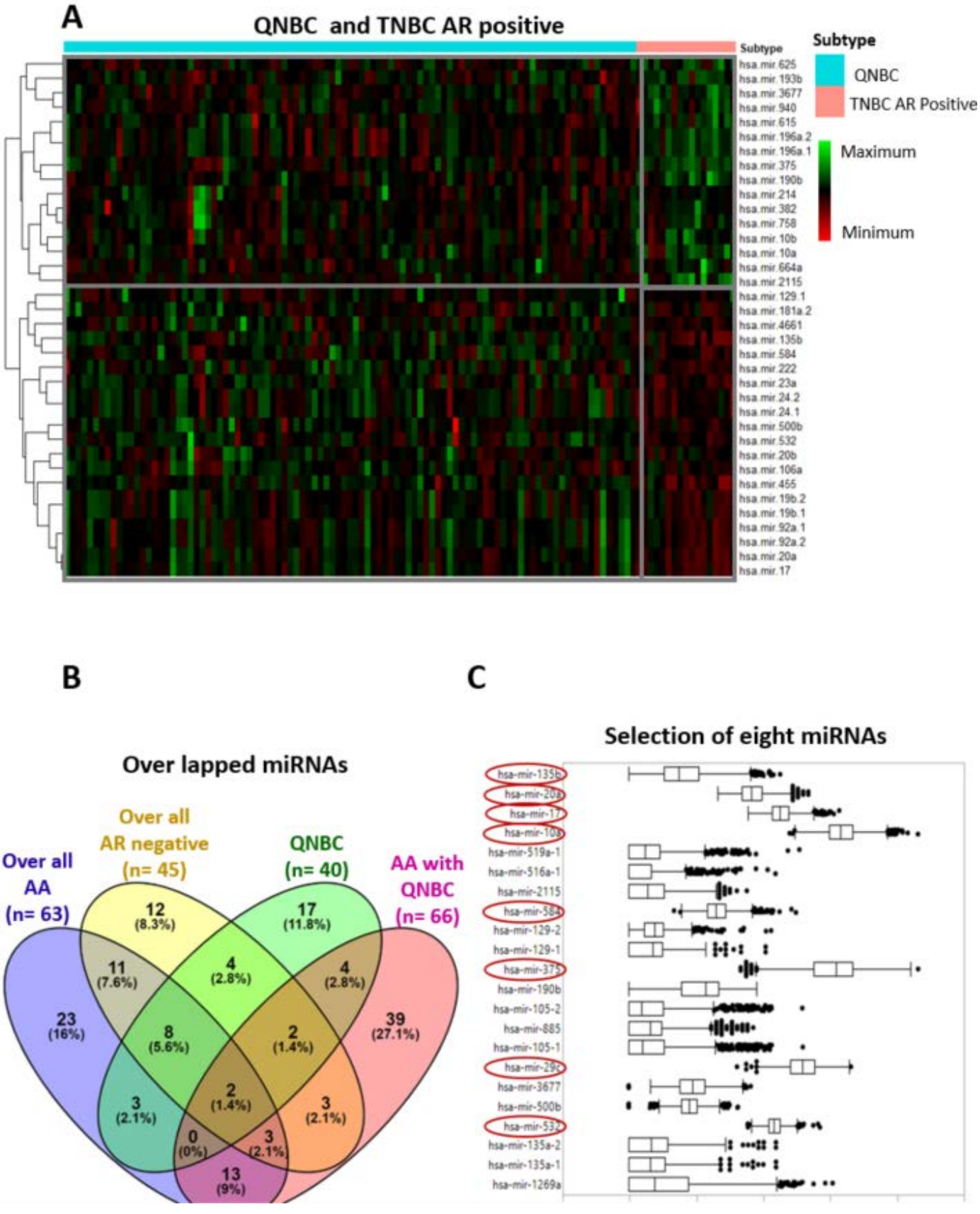

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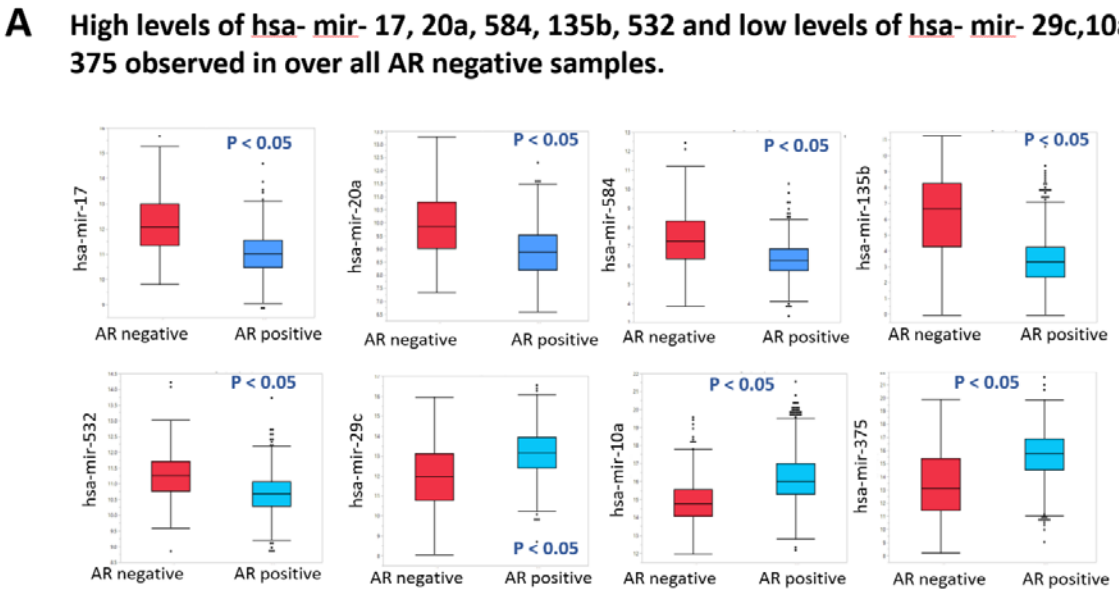
Figure 1. The miRNA signature of QNBCs differs from that of TNBCs that are AR-positive, and QNBC tumors of AAs exhibit a distinctive miRNA signature. A list of 40 miRNAs dysregulated in QNBCs and eight highly expressed miRNAs were selected for further analysis. **(A)** miRNAs differentially regulated in QNBCs (n=97) compared to AR-positive TNBCs (n=17). The heatmap shows the expression of 40 miRNAs that are dysregulated in QNBCs. The fold change of these miRNAs is shown in Table S5. The columns of the heatmap represent the samples, and the rows represent the miRNAs. The expression of miRNA is in the log2 form of the normalized miRNA expression. The heatmap is clustered by subtype. miRNAs in the upper cluster are downregulated in QNBCs, and lower clusters of miRNAs are higher in QNBCs. Presented is a list of 16 miRNAs that are downregulated in QNBCs, and a list of 20 miRNAs that are upregulated in QNBCs. These miRNAs may be associated with a basal-like phenotype. **(B)** A butterfly plot showing the overlapped miRNAs in between four differential miRNA expressions. The list of miRNAs differentially expressed in four DESEQ2 analyses was obtained. Presented are a list of 45 miRNAs dysregulated in AR-negative samples compared to AR-positive overall samples and a list of 40 miRNAs dysregulated in QNBCs compared to AR-positive TNBCs. Presented are a list of 63 miRNAs dysregulated in AA breast cancers compared to breast cancers of CAs, a list of 66 miRNAs dysregulated in AA QNBCs, and a list of 22 selected miRNAs overlapping by race and QNBC. **(C)** A forest plot showing the log2 values of miRNAs as represented in the X-axis. The Y-axis presents the 22 selected miRNAs. Eight miRNAs whose expressions are high were selected for further analysis. Red color shapes highlight those eight miRNAs.



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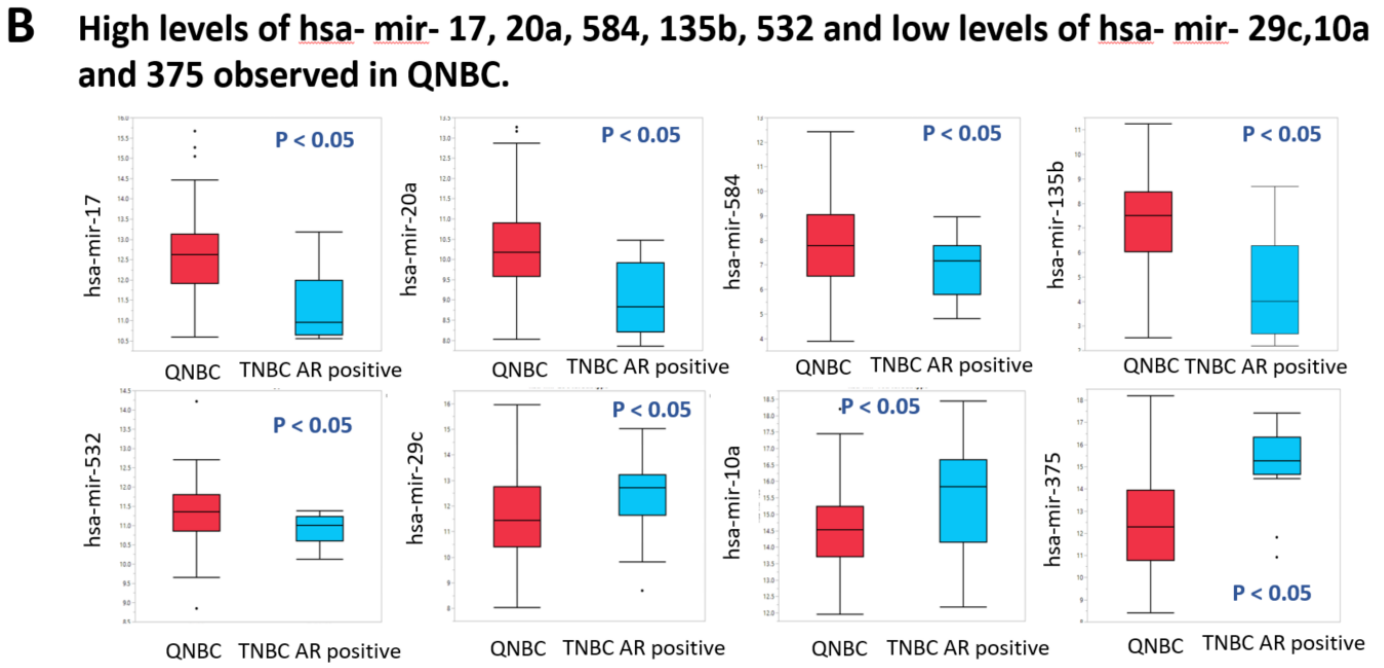
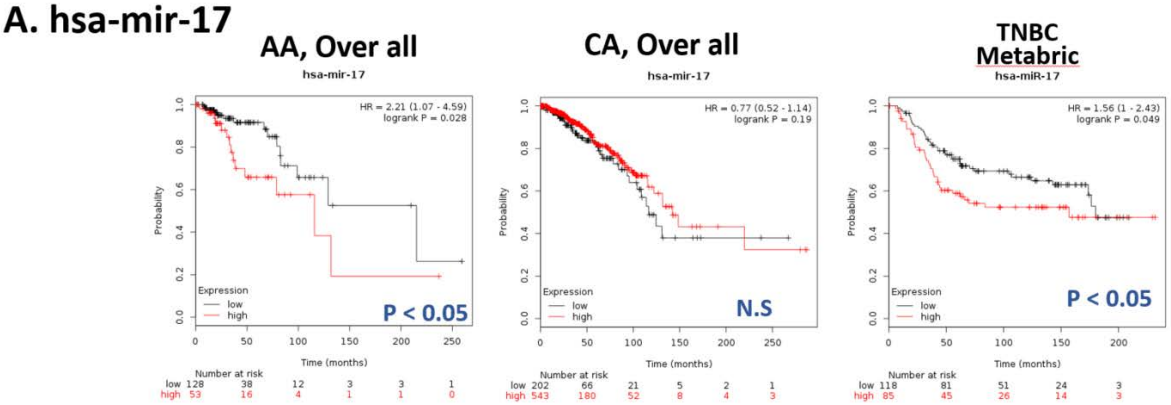


Figure 2. High levels of mir-17, mir-20a, mir-584, mir-135b, and mir-532 and low levels of mir-29c, mir-10a, and mir-375 are present in overall AR-negative breast cancers and QNBCs. (A) AR-negative vs. AR-positive overall. There were high levels of mir-17, mir-20a, mir-584, mir-135b, and mir-532 and low levels of mir-29c, mir-10a, and mir-375 in overall AR-negative samples. Eight highly expressed miRNAs were selected; the box plot shows the expression of these miRNAs in AR-negative (n=267) and AR-positive (n=823) primary breast cancers (nonparametric Wilcoxon test P < 0.05). The X-axis represents the AR status (negative or positive), and the Y-axis represents the miRNA expression normalized and log2 scale for the corresponding miRNA. **(B)** QNBCs vs. AR-positive TNBCs, high levels of mir- 17, mir- 20a, mir- 584, mir-135b, and mir-532 and low levels of mir-29c, mir-10a, and mir-375 in QNBCs. The box plot shows the expression of these miRNAs in QNBC primary breast cancers (n= 97) and AR-positive TNBC (n=17) samples (non-parametric Wilcoxon test P < 0.05). The X-axis represents the subtype (QNBC or TNBC AR-positive), and the Y-axis represents the miRNA expression normalized and the log2 scale for the corresponding miRNA.



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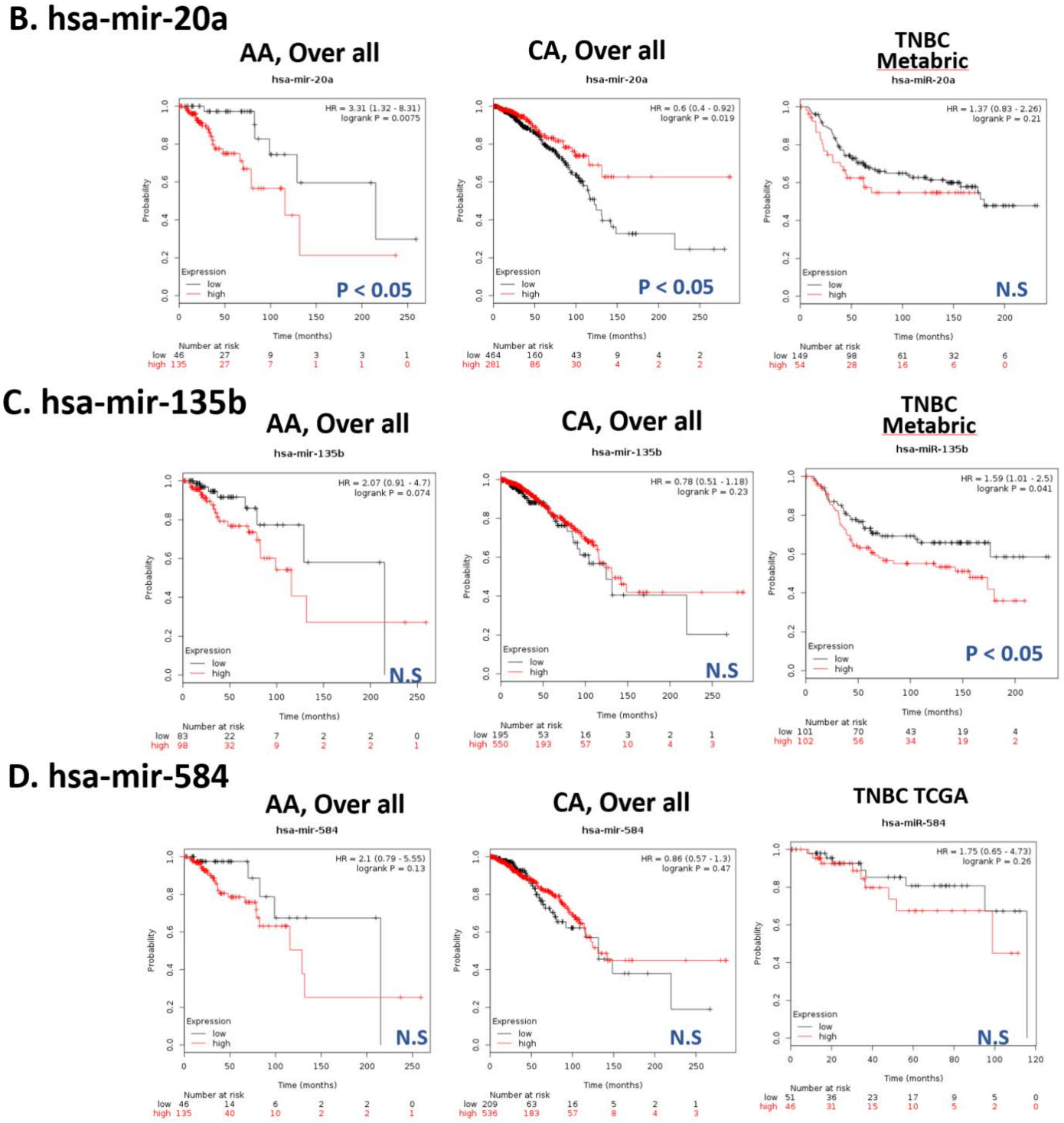

Figure 3. Survival analysis for mir-17, mir-20a, mir-135b, and mir-584 in overall AAs, CAs, and TNBCs. High levels of mir-17, mir-20a, mir-135b, and mir-584 for AAs and TNBCs are associated with a lower survival rate. In contrast, low levels of mir-17, mir-20a, mir-135b, and mir-584 for CA patients are associated with a lower survival rate. Overall AA TCGA (n =181), overall CA TCGA (n=745) and TNBC (METABRIC n = 203, TCGA n= 97). Log-rank p-values are indicated. **(A)** hsa-mir-17: As determined with KM Plotter for overall TCGA samples, AAs with high mir-17 have lower survival. In contrast, there is a trend that CAs with low levels of mir-17 have a lower survival rate (p=0.19); however, the trend is not statistically significant. Using KM plotter for METABRIC cases showed that patients with high mir-17 have a lower survival rate for TNBCs. **(B)** mir-20a: Using KM Plotter for overall TCGA samples, AAs with high mir-20a have lower survival; however, CAs with lower mir-20a have a lower survival. Use of KM plotter for TNBC

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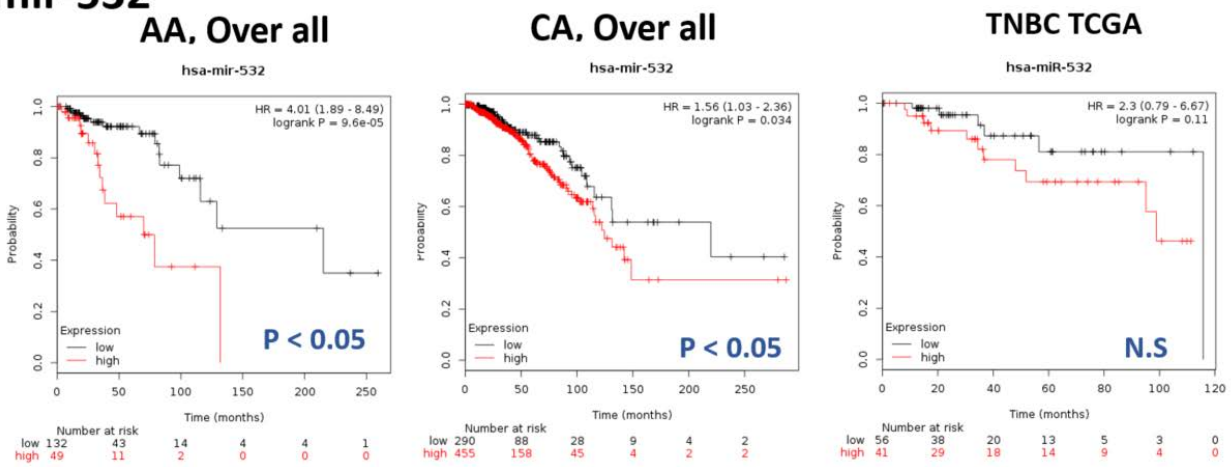
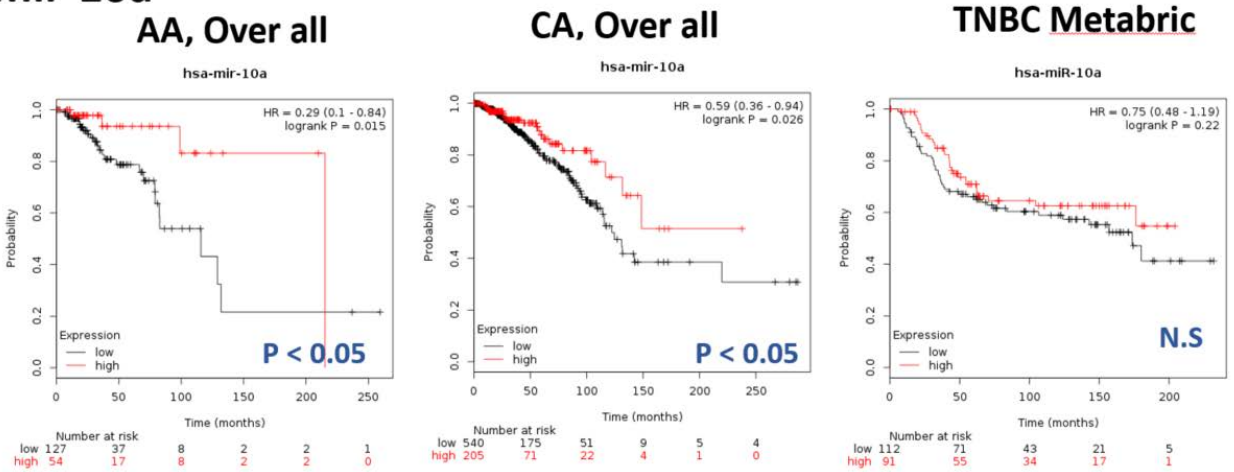
METABRIC cases showed a trend that TNBC patients with high hsa-mir-20a have a lower survival rate (p=0.21); however, the trend is not statistically significant. **(C)** mir-135b: Using KM Plotter for overall TCGA samples, there is a trend that AAs with high mir-135b have lower survival (p=0.07), and CAs with lower mir-135b have a lower survival (0.23); however, the trends are not statistically significant. Using KM plotter for TNBC METABRIC cases showed that patients with high mir-135b in their TNBCs have a lower survival rate. **(D)** mir-584: using KM Plotter for overall TCGA samples, there is a trend that AAs with high mir-584 in their cancers have a lower survival rate (p= 0.13 not significant), and CAs with lower mir-584 have lower survival (p=0.47, not significant). For TCGA TNBCs, there is a trend that patients with high mir-584 in their cancers have lower survival (p=0.26); however, the trend is not statistically significant.

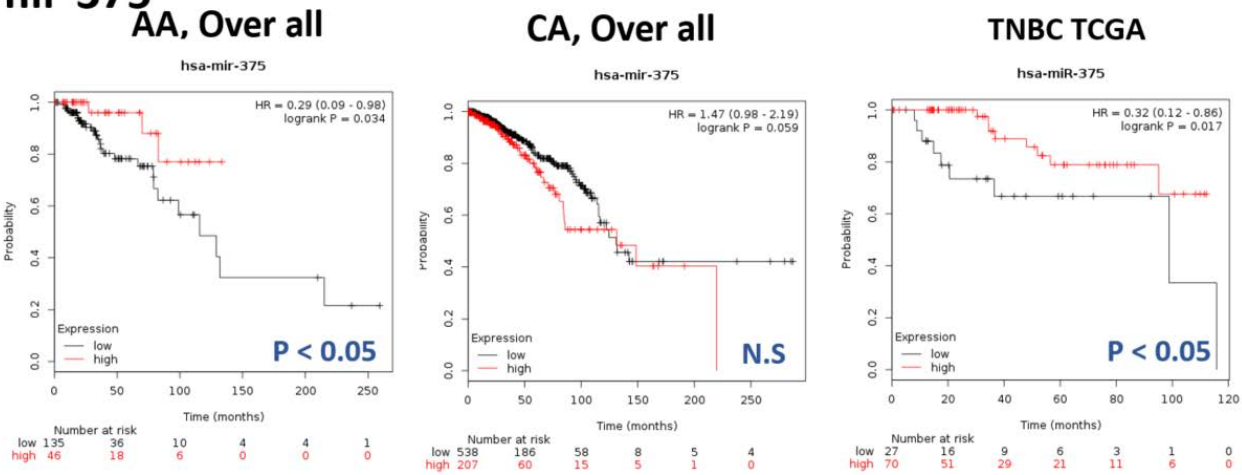
A. hsa-mir-532



B. hsa-mir-10a



C. hsa-mir-375



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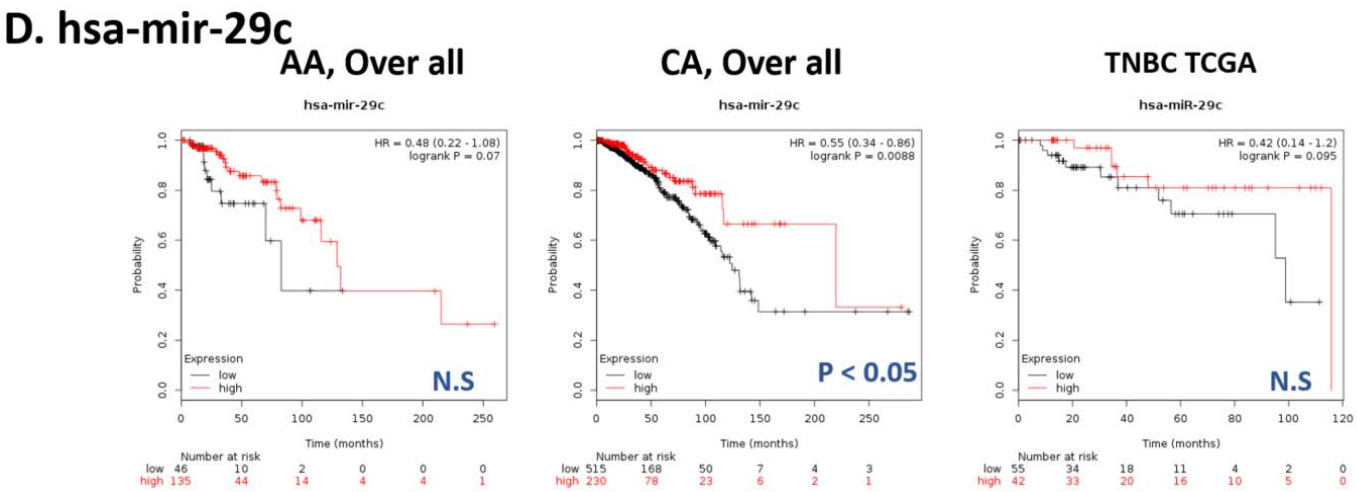

Figure 4. Survival analysis of mir-532, mir-10a, mir-29c, and mir-375 for overall AAs, CAs, and TNBCs. AA, CA, and TNBC patients with high levels of mir-532 in their cancers are associated with a lower survival rate. AA, CA, and TNBC patients with low levels of hsa-mir-29c and hsa-mir-10a have a lower survival rate. Also, AA and TNBC patients with low levels of hsa-mir-375 in their cancers are associated with a lower survival rate. In contrast, CA patients with high levels of mir-375 are associated with a lower survival rate. Overall AA, TCGA (n =181), overall CA TCGA (n= 745), and TNBC (METABRIC n = 203, TCGA n= 97). Log-rank p-values are indicated. **(A)** mir-532: as determined by KM Plotter for overall TCGA samples, AAs with high mir-532 in their cancers have lower survival. Similarly, CAs with higher mir-532 have a lower survival rate. There is a trend in TCGA TNBC cases: patients with high mir-532 in their cancers have lower survival (p=0.11), however, the trend is not statistically significant. **(B)** mir-10a: as shown by use of KM plotter for overall TCGA samples, AAs with low mir-10a in their cancers have lower survival. Similarly, CAs with lower mir-10a have a lower survival rate. Use of KM plotter for TNBC METABRIC cases shows a trend that TNBC cases with low mir-10a have a lower survival rate (p=0.22); however, the trend is not statistically significant. **(C)** mir-375: as determined by use of KM Plotter for overall TCGA samples, AAs with low mir-375 have lower survival. In contrast, there is a trend that CAs with higher mir-375 have a lower survival rate (p=0.05); however, the trend is not statistically significant. Using KM plotter for TNBC TCGA cases showed that TNBC cases with low mir-375 have a lower survival rate. **(D)** mir-29C: Using KM Plotter for overall TCGA samples, there is a trend that AAs with low mir-29C have lower survival (p= 0.07); however, the trend is not statistically significant. Similarly, CAs with low levels of mir-29c in their cancers have a lower survival rate. Using KM plotter for TNBC TCGA cases shows a trend that TNBC cases with low mir-29c have a lower survival rate (P= 0.09); however, the trend is not statistically significant.

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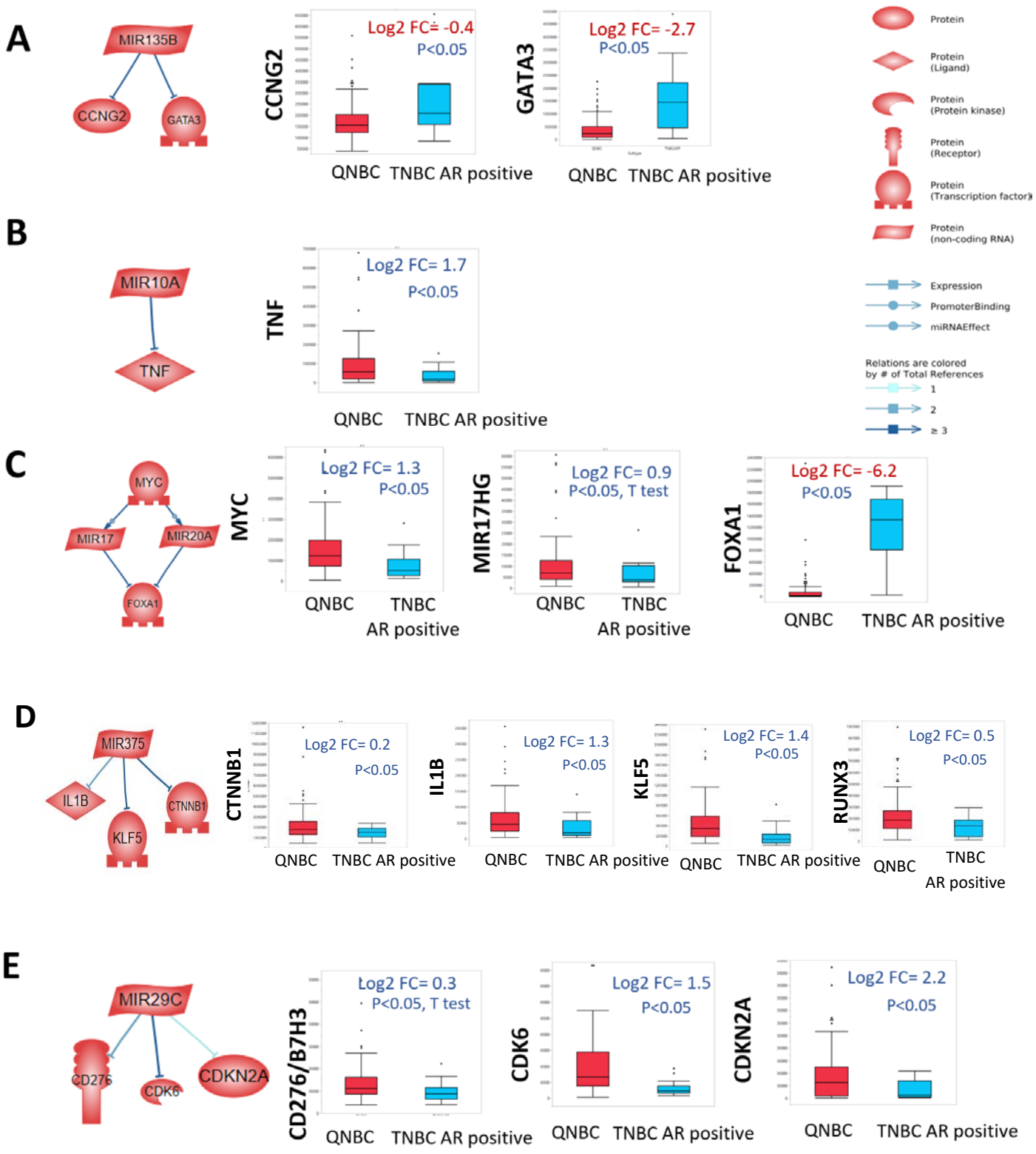

Figure 5. Genes associated with miRNAs are dysregulated in QNBCs. Predicted targets of miRNAs were obtained by use of Pathway Studio. The expression of gene targets was evaluated in QNBCs, and the correlations of miRNAs and genes were obtained for overall TCGA samples**.** Non-parametric Wilcoxon test p-values are presented. **(A)** mir-135b targets CCNG2 and GATA3, the expressions of which were lower in QNBCs. **(B)** mir-10a targets TNFα, the expression of which was higher in QNBCs. **(C)** Pathway Studio

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networks revealed that MYC positively regulates mir-17 and -20a, which target FOXA1. MYC and mir-17HG were higher in QNBCs, and FOXA1 was lower in QNBCs. **(D)** Pathway Studio analysis showed that mir-375 targets IL-1B, KLF5, and CTNNB1. Literature reports show that mir-375 targets RUNX family proteins (49). We observed that IL1B, KLF5, CTNNBA, and RUNX3 are upregulated in QNBCs. **(E)** From network analysis, Pathway Studio revealed that mir-29C targets CD276, CDK6, and CDKN2A. In QNBCs, CD276 ($p < 0.05$ by T-test and $p = 0.05$ by Wilcoxon), CDK6, and CDKN2A are upregulated.

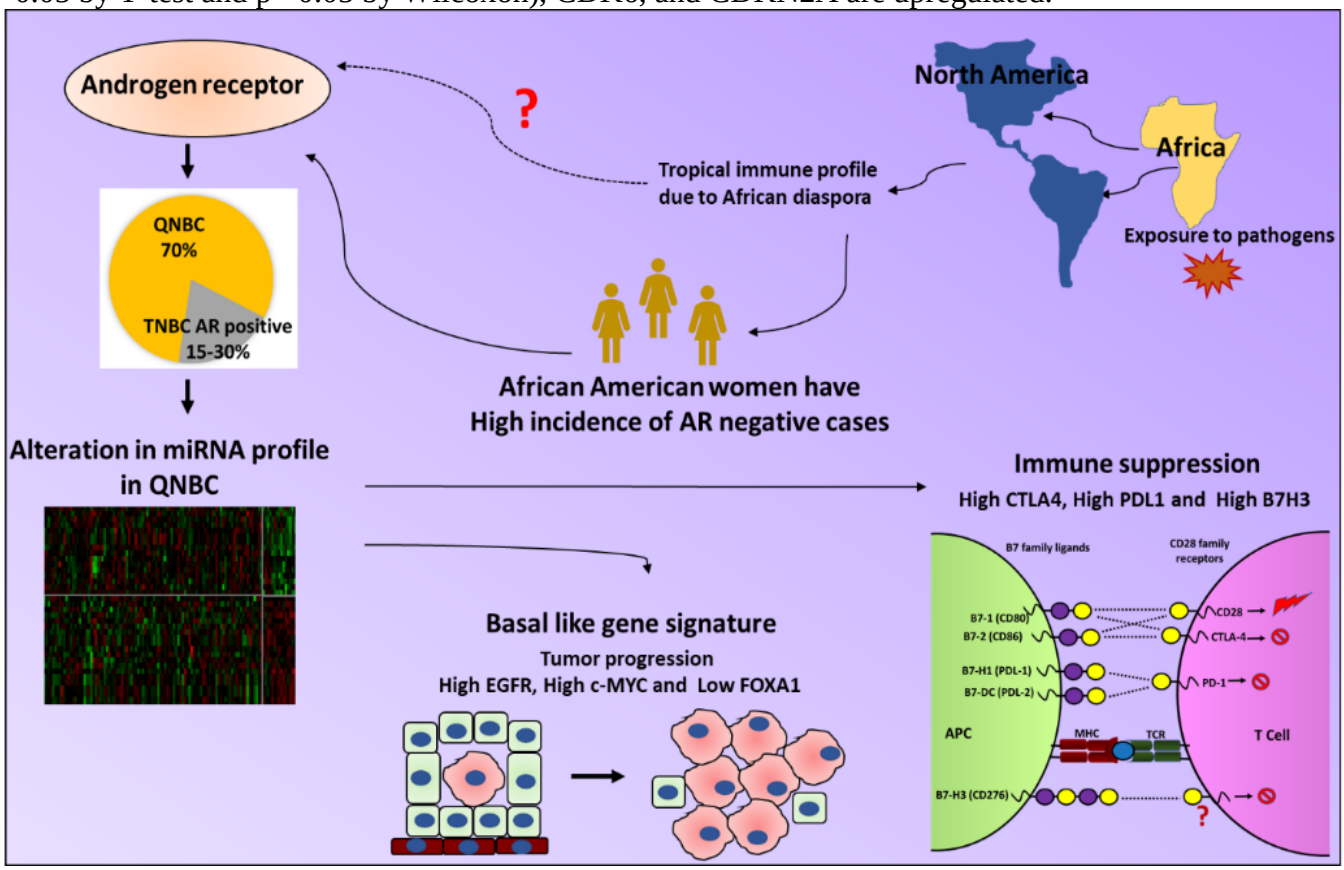

Figure 6. Summary figure/graphical abstract indicating that altered miRNA and gene signatures in QNBCs and cancers of AA patients could be the basis for the immune suppression and tumor progression of QNBCs.

SUPPLEMENTAL FIGURE LEGENDS

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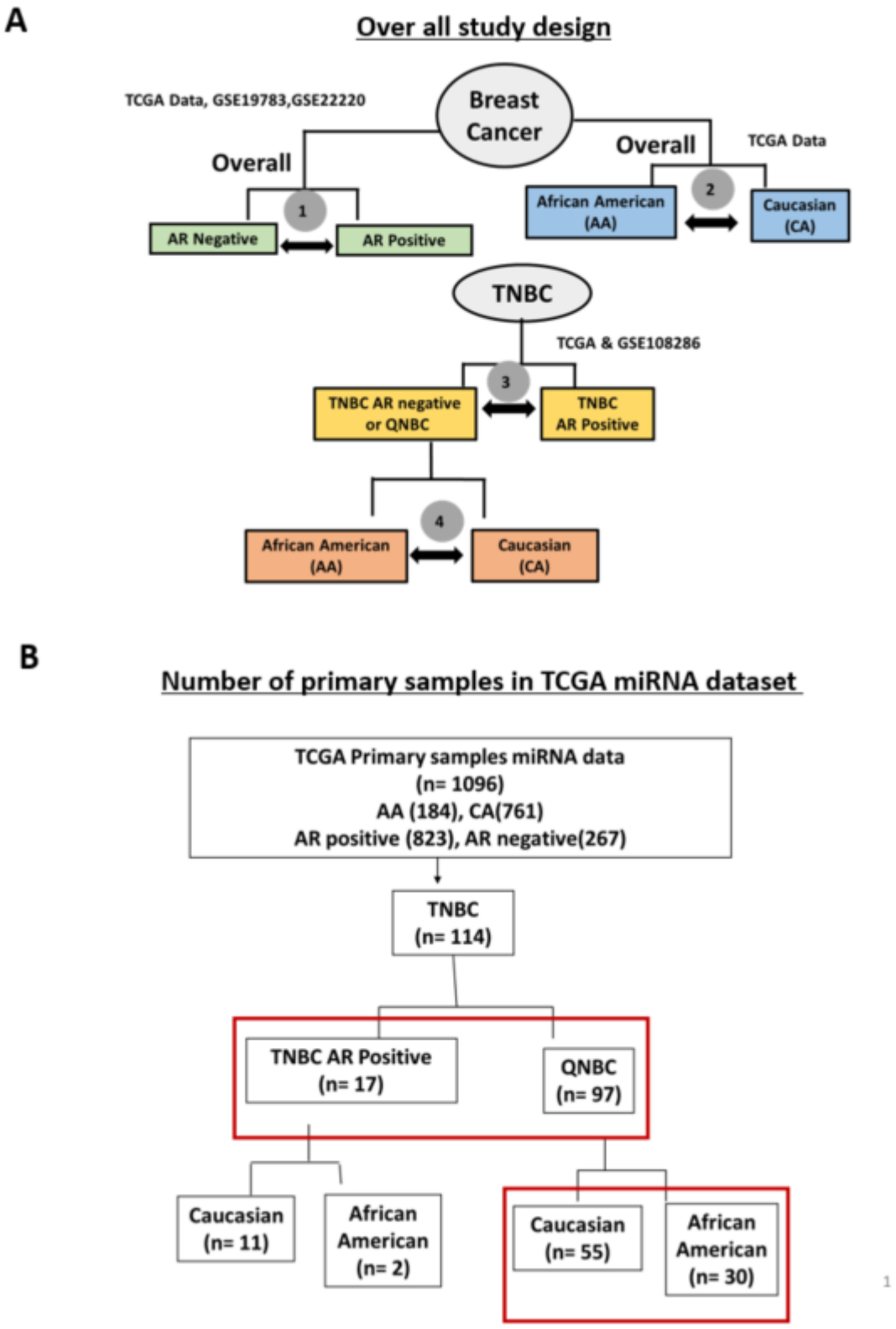

Figure S1. Overall study design. (A) The flow chart represents the overall study design. DESEQ2 was performed for four groups of primary breast cancer samples: 1) Overall AR in contrast to AR-positive (in TCGA and two GEO datasets); 2) Overall AA to CA; 3) QNBC to TNBC AR-positive; and 4) AA with QNBC to CA. **(B)** Numbers of samples in the TCGA miRNA primary sample dataset. The flowchart represents the numbers of samples in each group in TCGA miRNA primary breast cancer samples. There were a total of 1096 samples. The boxes highlighted in red were considered for the present study.

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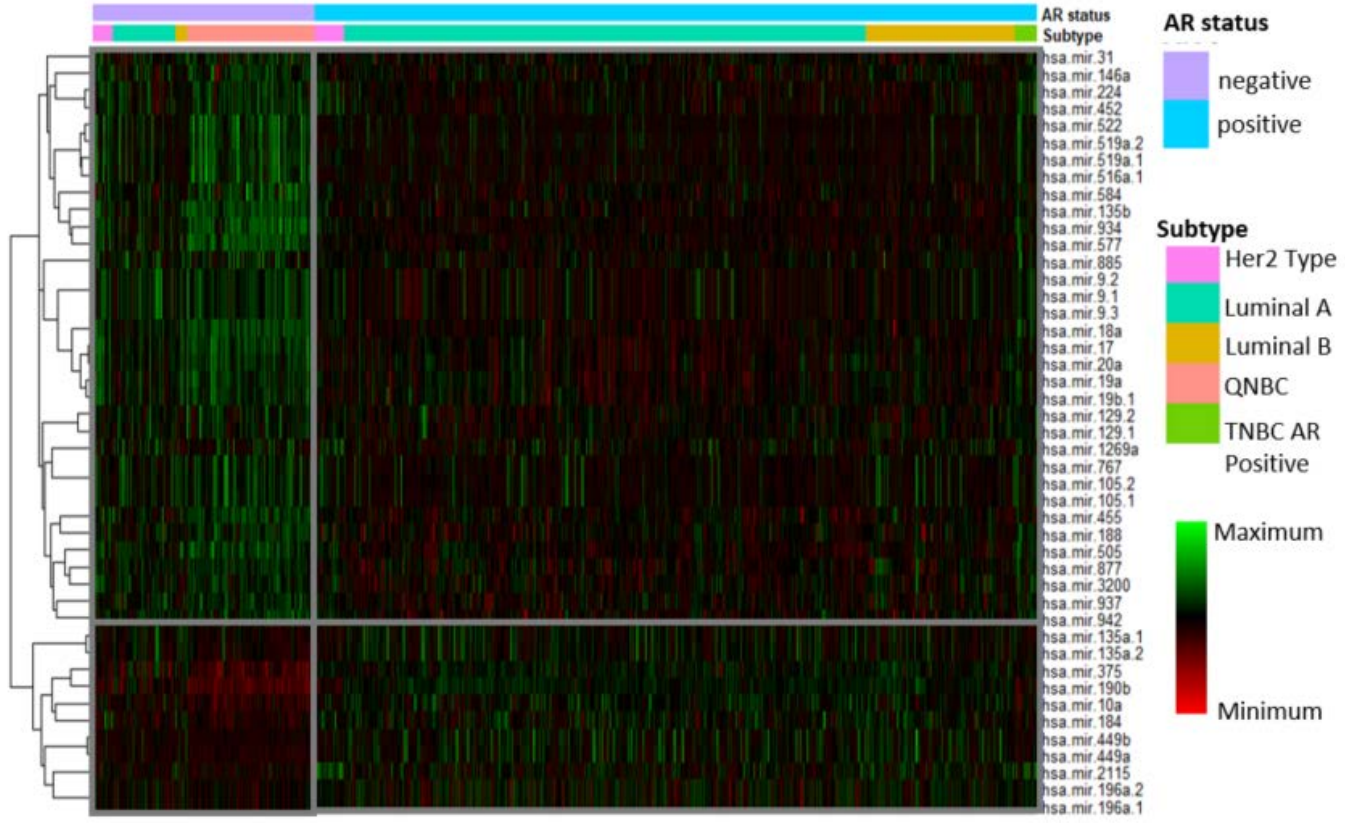

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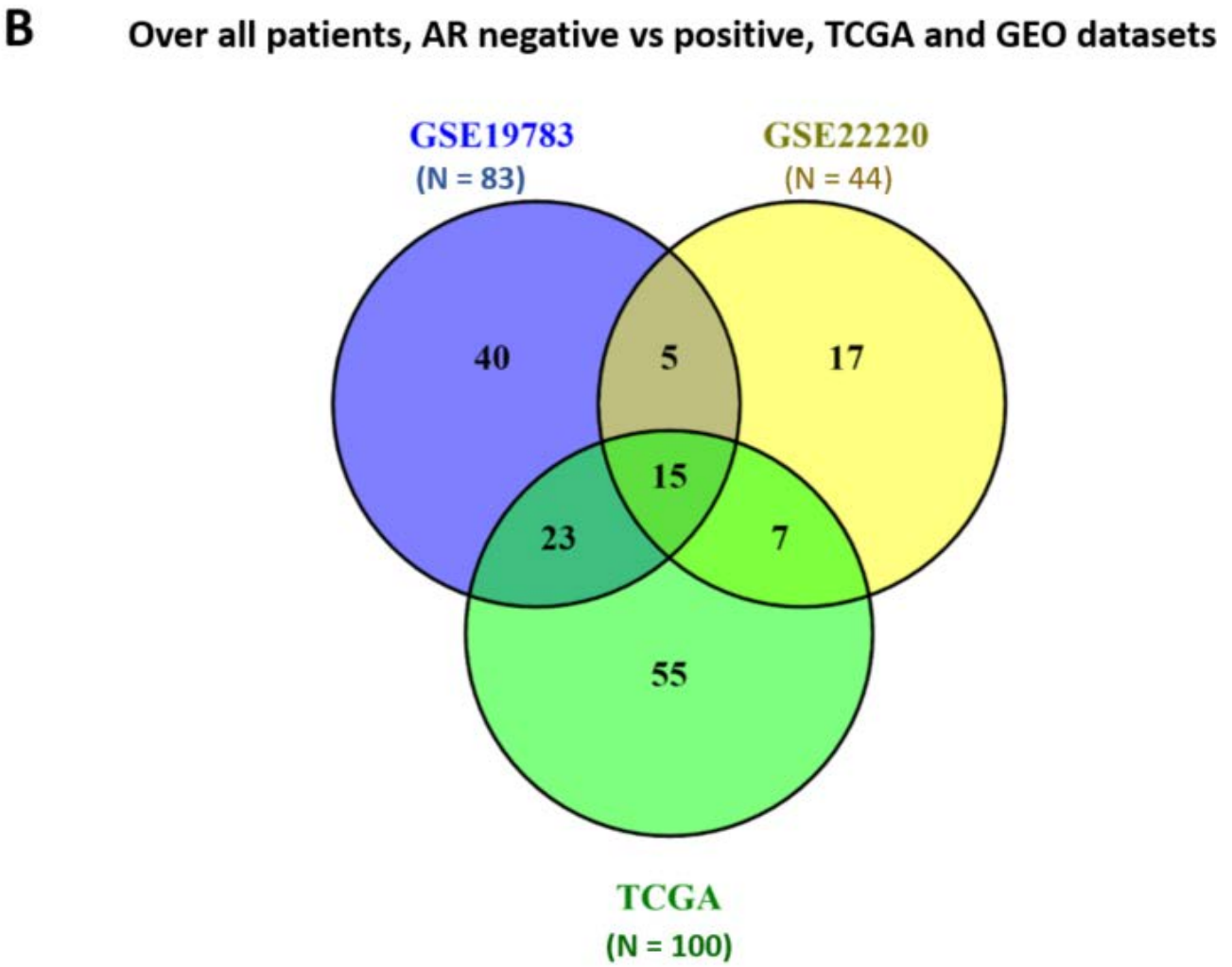

Figure S2. Most AR-negative cases have miRNA expression like QNBCs and the Her2 type; AR-positive cases have miRNA expression like luminal A/B and TNBC AR-positive types.
(A) Heatmap showing a list of 45 miRNAs, differentially expressed in AR-negative cases (n=267) compared to AR-positive cases (n=823) (P-adj < 0.05, p <0.05 and base mean > 5, Log2 fold change < -1 and >1). In this heatmap, columns represent the individual or samples, and rows represent miRNAs. The samples are clustered by AR status and subtype, as shown in the heatmap legend. The fold change and base mean expression are shown in Table S4. Most AR-negative cancers have miRNA expression like QNBCs and the Her2 type. AR-positive cancers have miRNA expression like luminal A/B and AR-positive TNBCs. mir-519a-1, mir-519a-2, and 516a-1 belong to miRNA-19 cluster, and mir-17, mir-18a, mir-17, and mir-20a from miRNA cluster 17-92 are upregulated in AR-negative cases. In contrast, hsa-mir-449a and b, and mir-135a-1/2 are downregulated in AR-negative cases. **(B)** GSE19783 (AR negative n= 26, AR-positive n = 73) and GSE22220 (AR negative n= 49, AR-positive n = 158), two breast cancer GEO datasets, were analyzed to determine differentially expressed miRNAs in AR-negative cases compared to AR-positive cases. The Venn diagram shows the overlap between the GEO datasets and TCGA. A list of 15 miRNAs were overlapped in the three datasets. Also, there were 23 miRNAs overlapped between TCGA and GSE19783, seven miRNAs between TCGA and GSE22220, and five miRNAs between GSE19783 and GSE22220.

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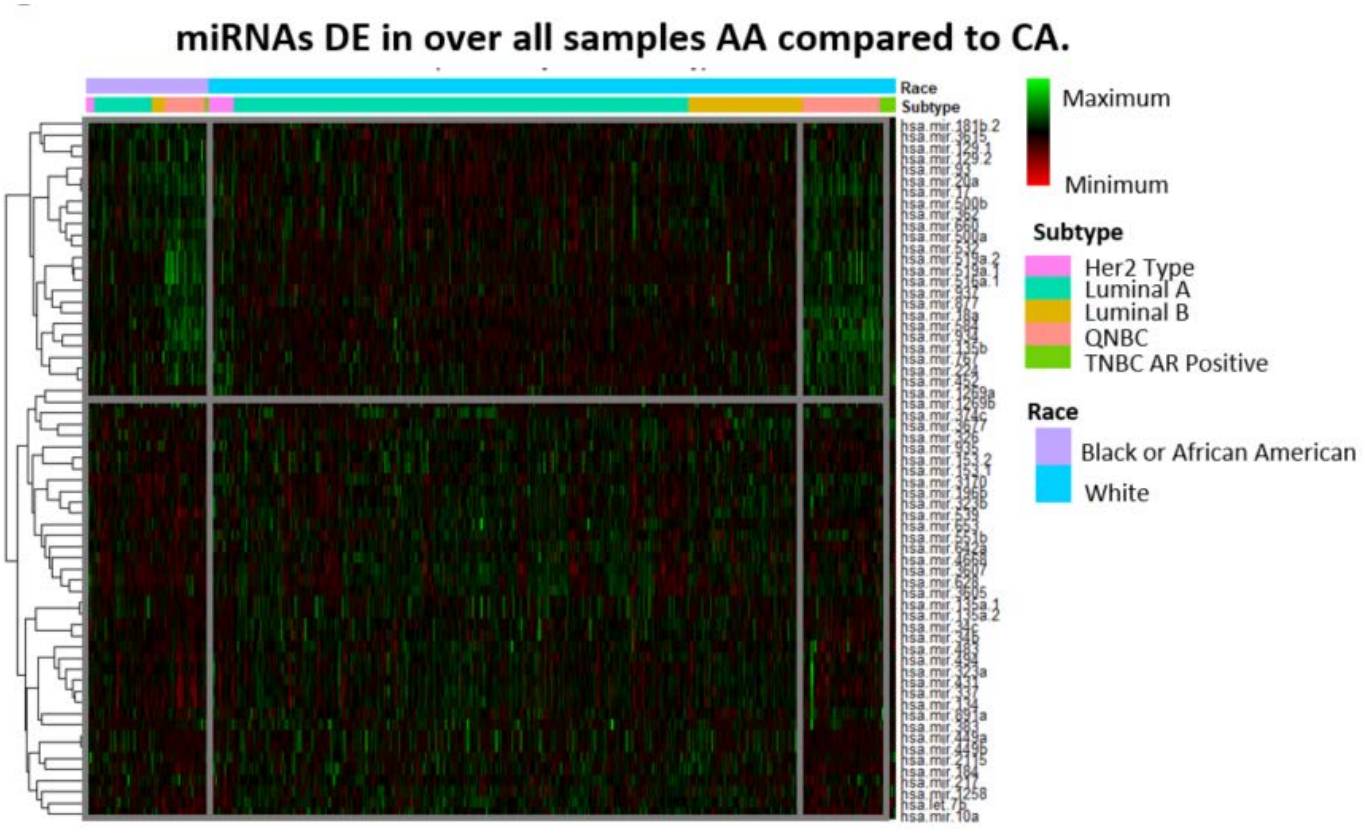

Figure S3. List of 63 miRNAs differentially regulated in AA breast cancers compared to CA breast cancers. The heatmap shows the expression of 63 miRNAs that are dysregulated in AA breast cancers. (P-adj < 0.05, p <0.05 and base Mean > 5, log2 fold change < -0.5 and >0.5). The fold change of these miRNAs is presented in Table S6. The columns of the heatmap represent the individuals or sample, and the rows represent the miRNAs. The expression of miRNA is in the log2 form of the normalized miRNA expression. As shown in the figure legend, the heatmap is clustered by subtype and race. miRNAs in the upper cluster are upregulated in AA breast cancers; the lower two clusters of miRNAs are downregulated in AA breast cancers.

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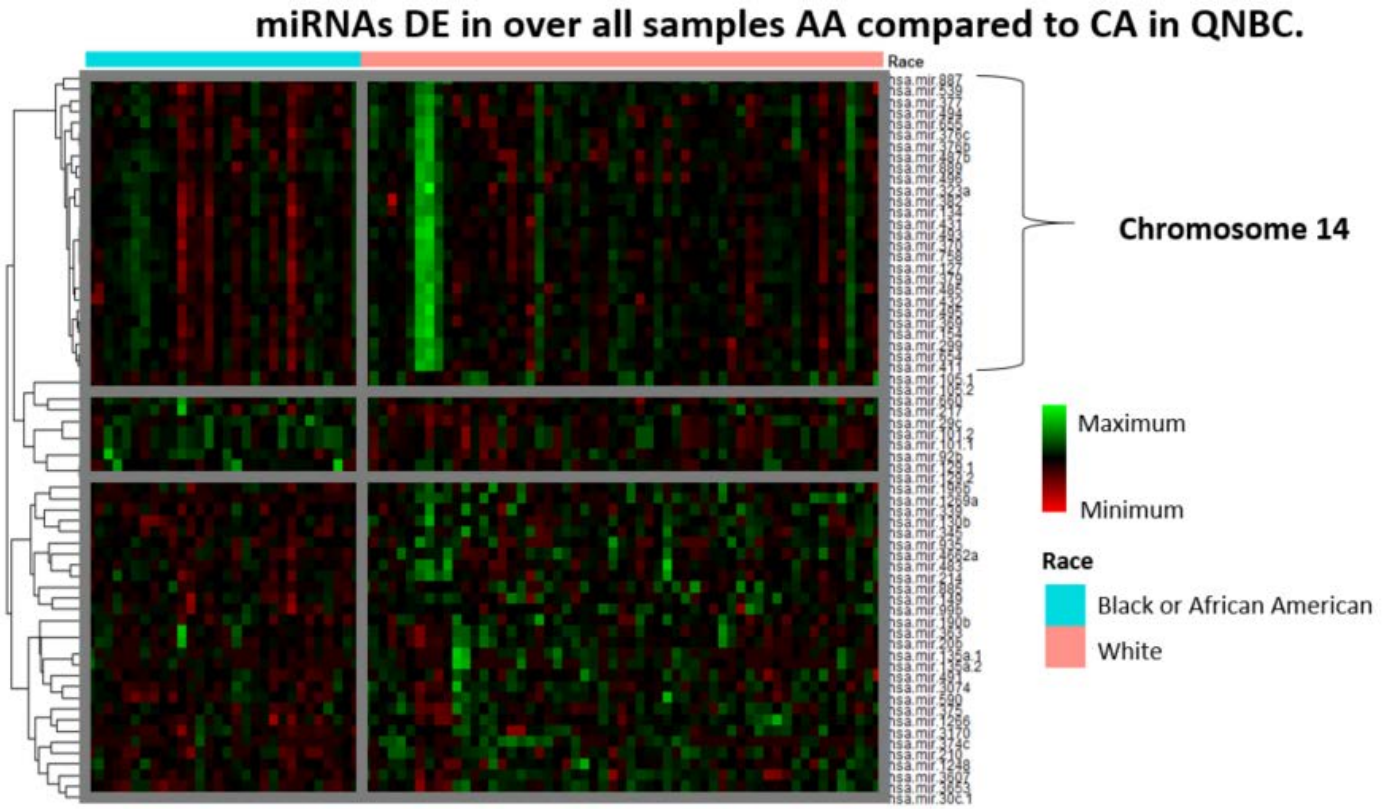

Figure S4. List of 66 miRNAs differentially regulated in QNBCs of AAs relative to QNBCs of CAs. The heatmap shows the expression of 66 miRNAs that are dysregulated in QNBCs of AAs. (P-adj < 0.05, p <0.05 and base Mean > 5, Log2 fold change < -0.5 and >0.5). The fold change of these miRNAs is presented in Table S7. The columns of the heatmap represent the individual or sample, and the rows represent the miRNAs. The expression of miRNAs is in the log2 form of the normalized miRNA expression. As shown in the figure legend, the heatmap is clustered by race. Most miRNAs shown in the heatmap (top and lower two clusters of miRNAs) are downregulated in QNBCs of AAs. In the second miRNA cluster from the top is a list of 7 miRNAs higher in QNBCs of AAs.

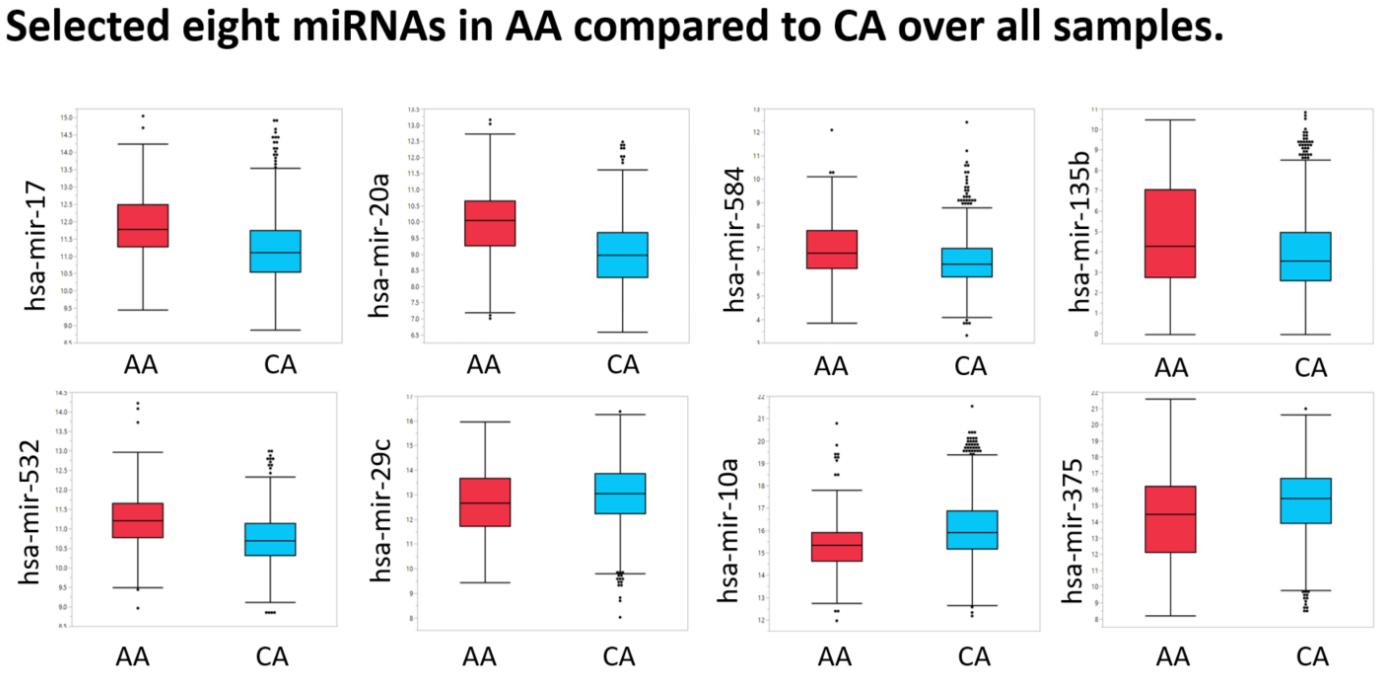

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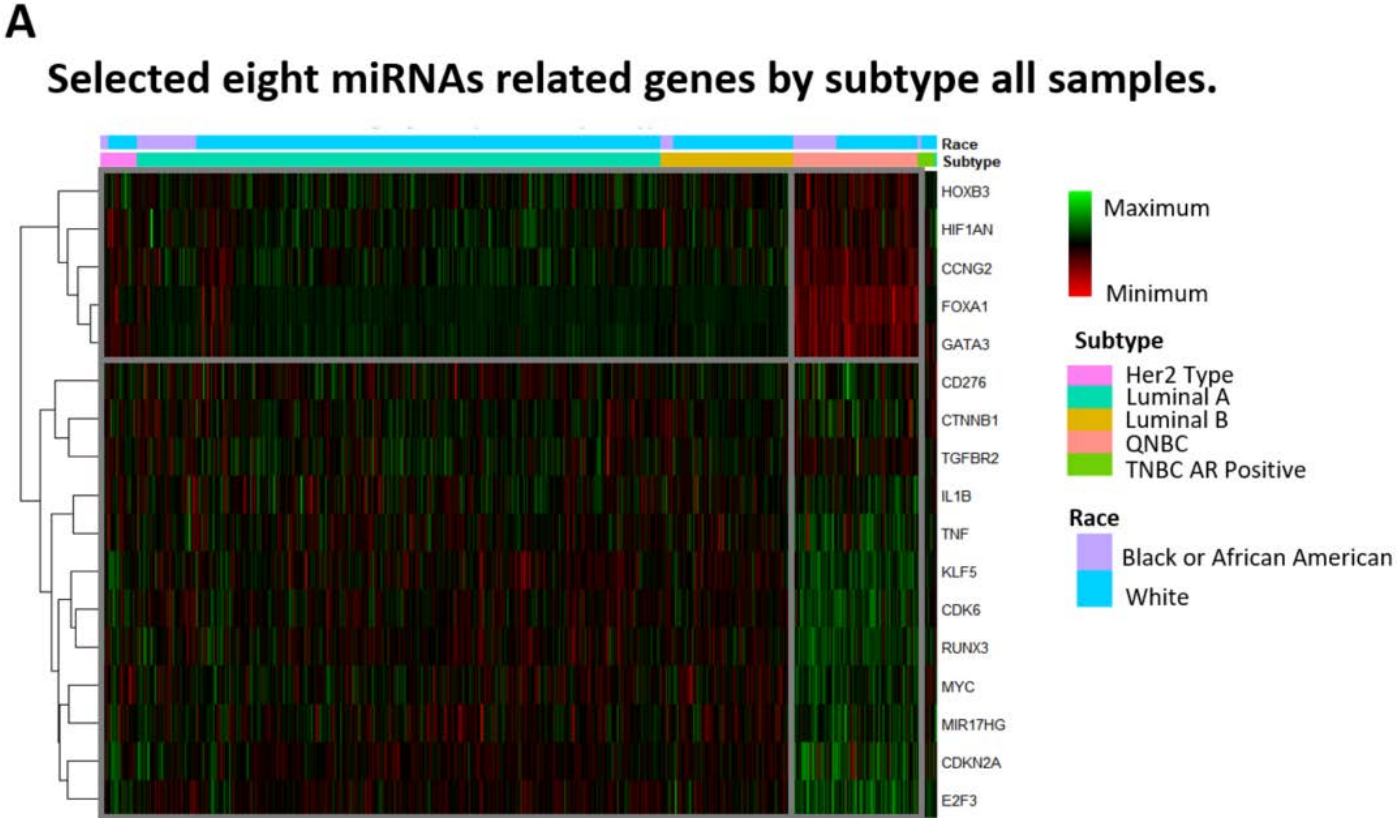
Figure S5. High levels of mir-17, mir-20a, mir-584, mir-135b, and mir-532 and low levels of mir-29c, mir-10a and mir-375 are present in breast cancers of AAs. High levels of mir-17, mir-20a, mir-584, mir-135b, and has-mir-532, and low levels of mir-10a are present in AA samples overall, as shown in Table S8. There is a trend that mir-29c and hsa-mir-375 are lower in AA breast cancers (p adj =0.1 and 0.6), however, the trend is not statistically significant.



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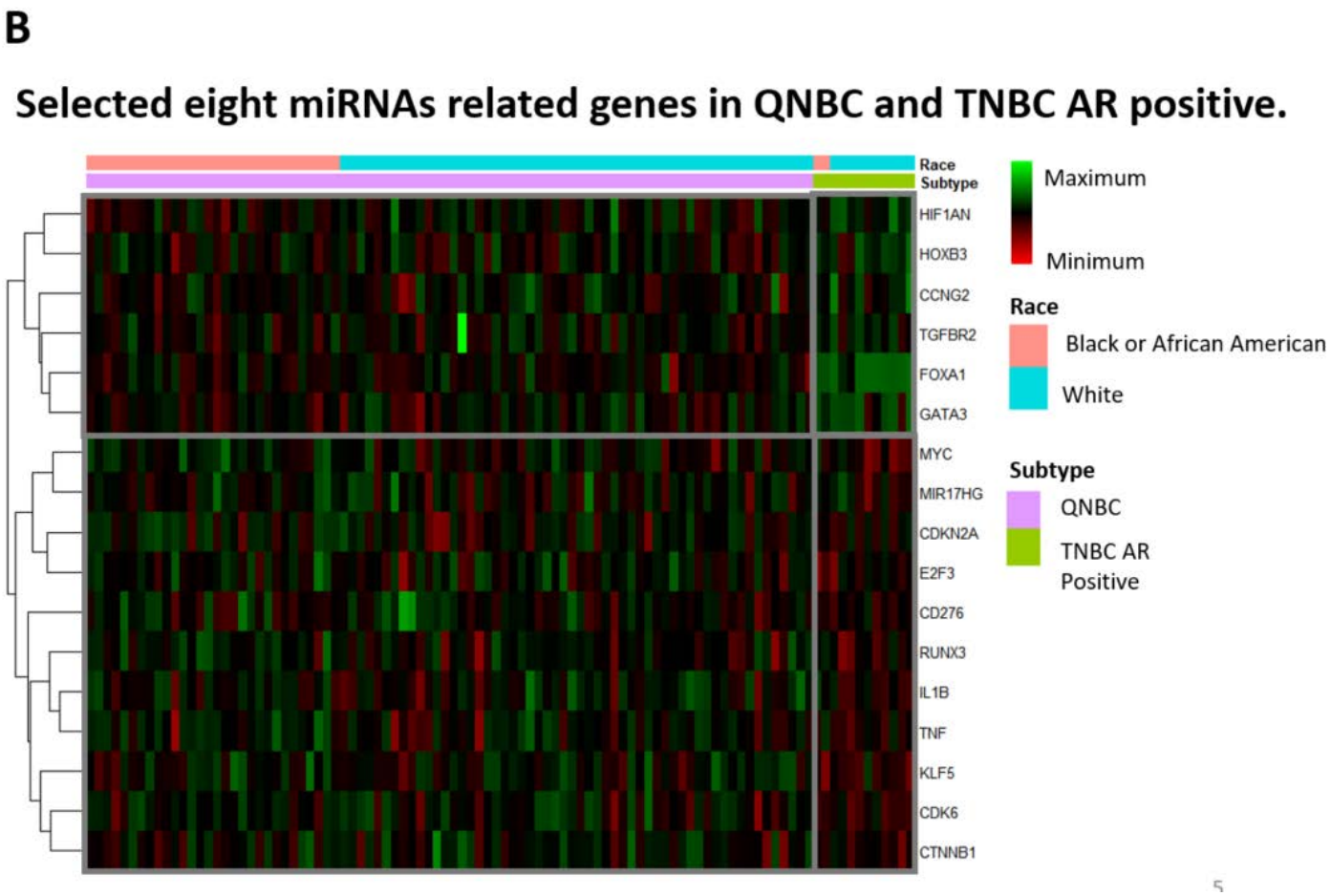

Figure S6. The predicted targets of miRNAs were obtained by Pathway Studio. Expressions of gene targets were evaluated in QNBCs and overall TCGA samples. (A) The heatmap shows the expression of genes dysregulated in QNBCs in overall TCGA samples, clustered by subtype and race. The columns in the heatmap represent the individual or samples, and rows represent expression of the miRNAs. Samples with unknown subtype or receptor status and race were removed. Red color represents downregulated genes in QNBCs, and green represents upregulated genes in QNBCs. **(B)** The heatmap shows the expression of genes dysregulated in QNBCs and AR-positive TNBC samples, clustered by subtype. Samples with unknown subtype or receptor status and race were removed.

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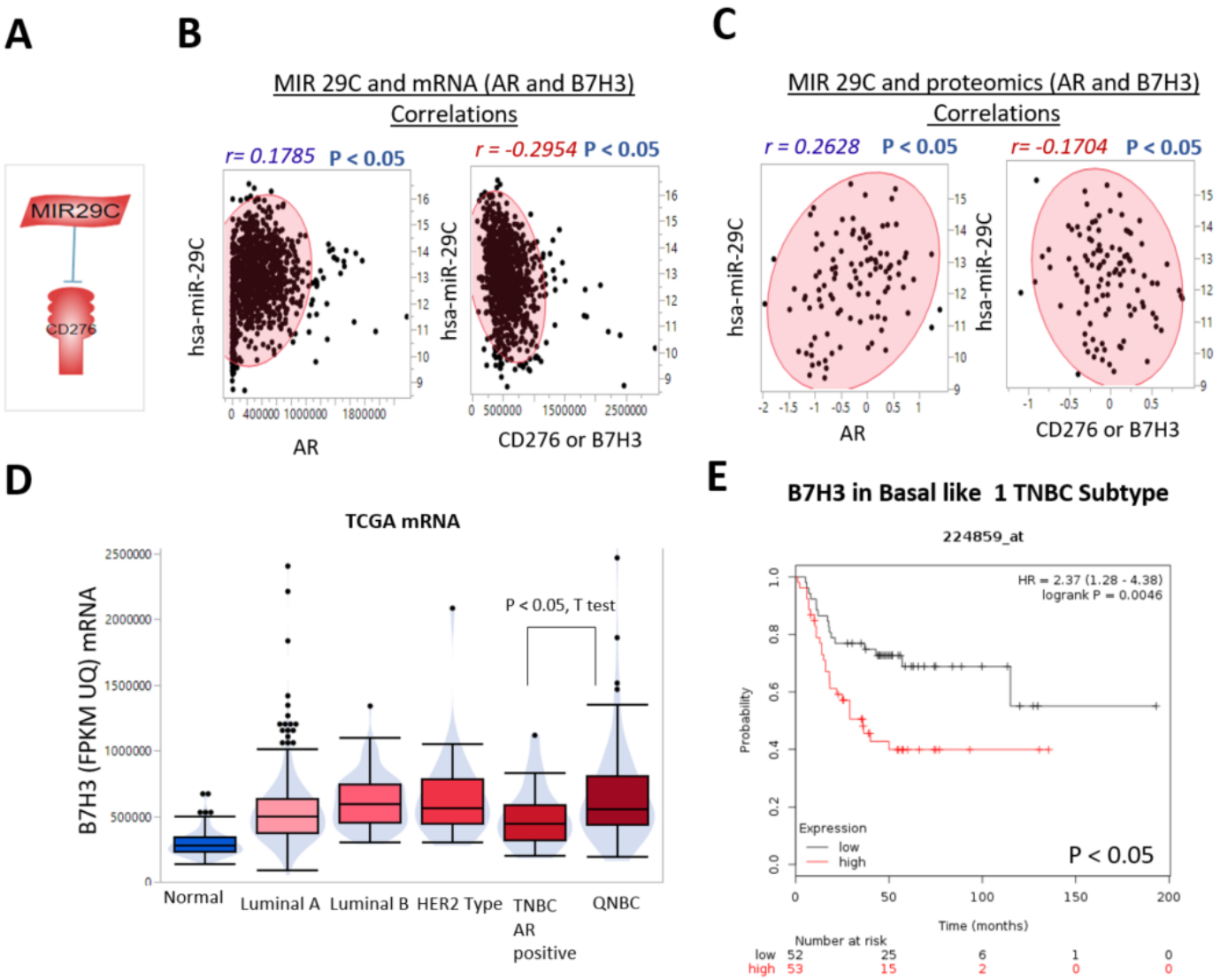

Figure S7. Correlations of AR, miR29C, and B7H3. AR positively correlated with mir-29C, which negatively correlates with B7H3. B7H3 is high in QNBCs compared to AR-positive TNBCs. In TNBCs, high B7H3 is associated with lower survival of patients with the basal-like 1 subtype. Non-parametric t-test p-values are indicated. **(A)** Pathway Studio analysis revealed that mir-29C targets CD276/B7H3, which is located on the cell membrane and known to be clinically relevant. **(B)** Correlation scatter plots show the Pearson correlation coefficients. In mRNA data (n=1083) for TCGA overall primary tumor samples, mir-29C positively correlates with AR and negatively correlates with B7H3. **(C)** In proteomics data for TCGA overall primary tumor samples (n=105), mir-29C positively correlates with AR and negatively correlates with B7H3. **(D)** The box plot shows the mRNA gene expression of B7H3 in FPKM UQ units. As compared to healthy tissues, B7H3 expression was higher in primary breast cancer tissues of various BC subtypes. B7H3 expression was higher in QNBCs as compared to AR-positive TNBCs. **(E)** Survival analysis was accomplished with KM Plotter with breast cancer datasets for B7H3 (probe ID 224859_at) by the Pietenol subtypes of TNBCs. Patients with high B7H3 in basal-like 1 TNBCs have lower survival.

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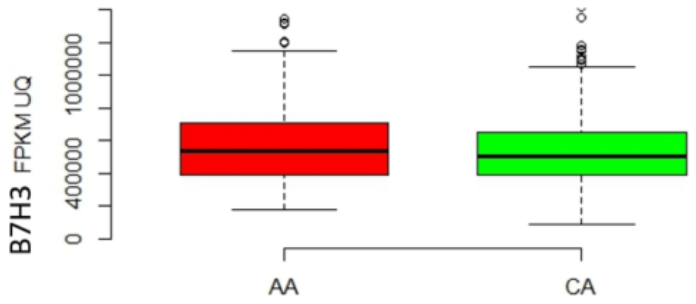
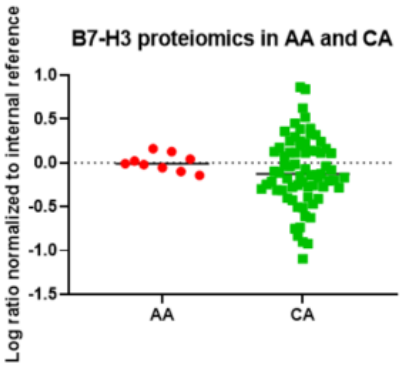
A



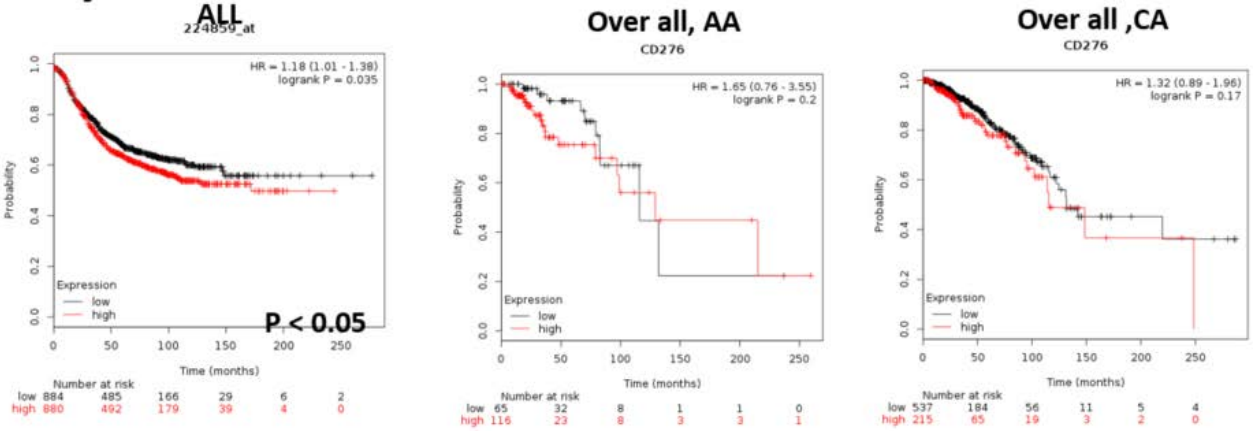
	B7H3_AA	B7H3_CA
Median	538314.7	501981.1
Mean	593893.6	546241.5
N	182	752
	P-value	0.043253

B

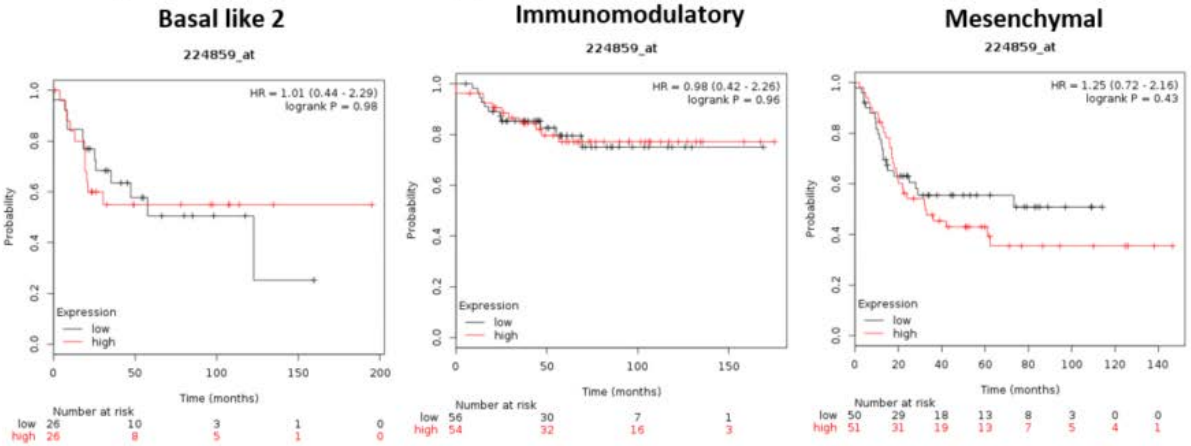


	AA	CA
Median	-0.00945	-0.12574
mean	0.003121	-0.09661
count	9	71
pvalue		0.087323

C. B7H3 by Over all race



D. B7H3 by Pietenol subtype



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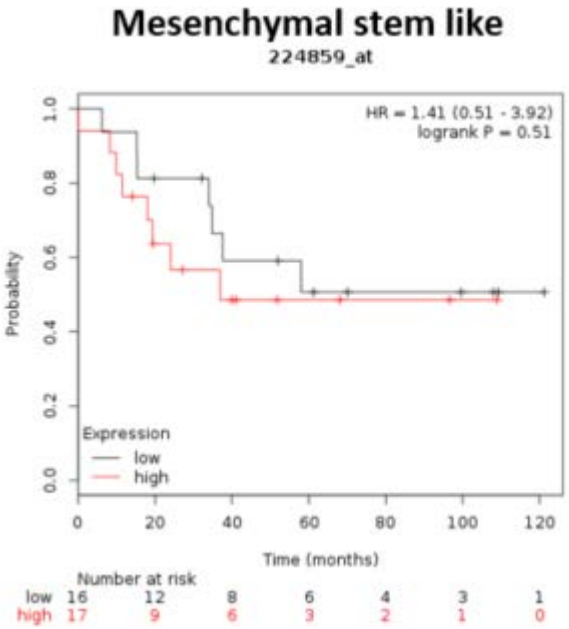

Figure S8. B7H3 expression 1s high in AA breast cancers compared to CA breast cancers. There is a trend that AAs with high B7H3 in their cancers have lower survival, but it is not statistically significant. **(A)** B7H3 expression in FPKM UQ units was obtained from TCGA. B7H3 expression was higher in overall AA breast cancers (n= 182) compared to CA breast cancers (n= 752). P-values were obtained using a non-parametric t-test. **(B)** B7H3 protein expression was obtained from TCGA with the unshared log-ratio normalized to an internal reference. There is a trend that B7H3 expression was higher in overall AA breast cancers (n= 9) compared to CA breast cancers (n=71). P-values were obtained using a non-parametric t-test ($p = 0.08$); however, the trend was not statistically significant. **(C)** Using the KM plotter database using mRNA breast cancer tab, B7H3/Agilent probe ID 224859_at survival analysis was performed for the overall sample. Patients with high B7H3 have a lower survival rate. There was a trend that AAs with high B7H3 in their cancers have lower survival, and CAs with high B7H3 in their cancers also have a lower survival rate, but the trends were not statistically significant. **(D)** KM plots represent the survival curves for B7H3 in various TNBC subtypes (LAR and basal-like 1 are shown in Figure S9). There is a trend that patients with high B7H3 in their cancers have lower survival in the basal-like 2, mesenchymal, and mesenchymal stem-like subtypes of TNBCs; however, the trends are not statistically significant.

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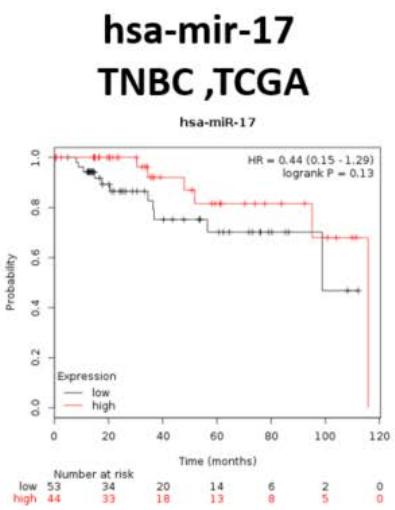

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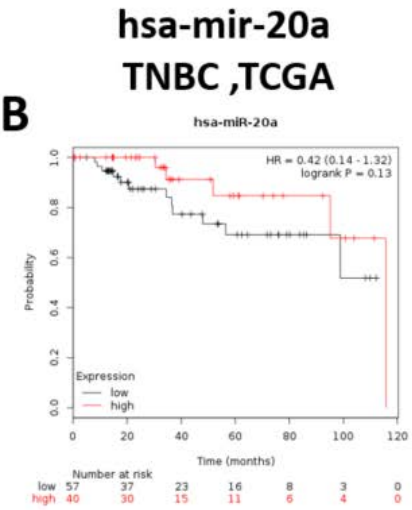

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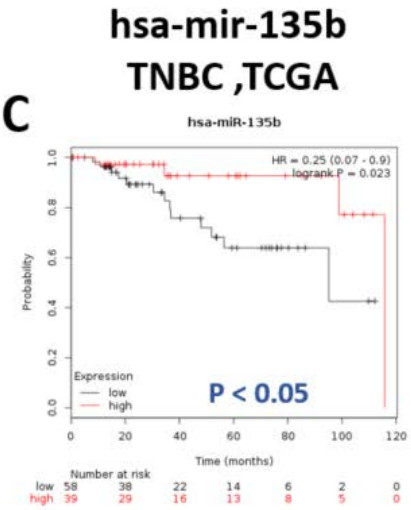

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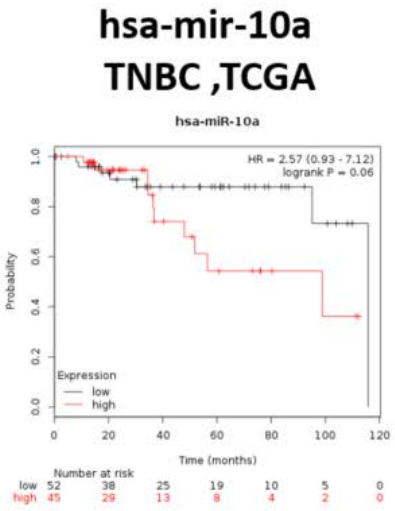

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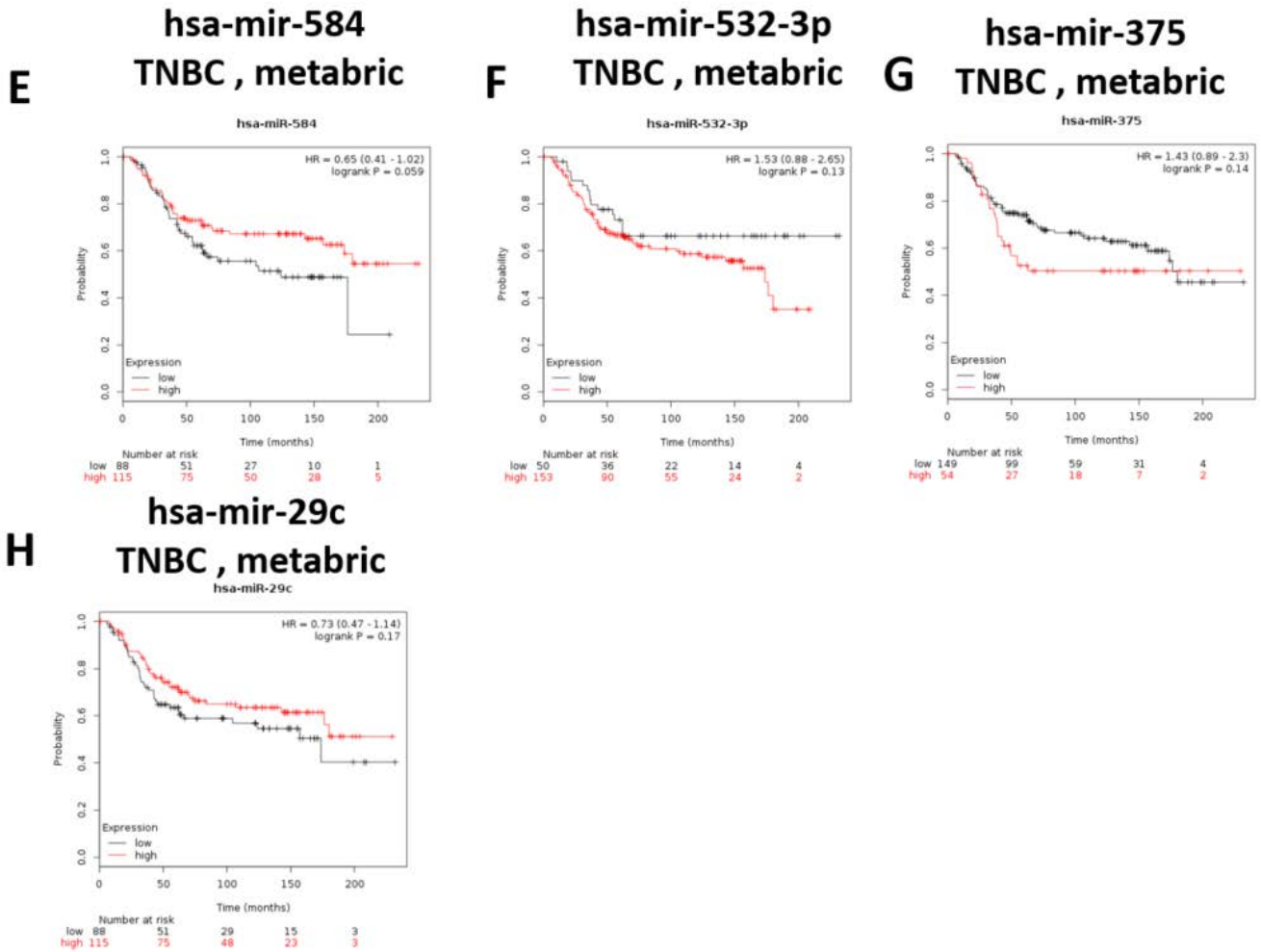

Figure S9. miRNA survival analysis of TNBC TCGA and METABRIC data. (A) In TCGA, there is a trend that TNBC patients with low hsa-mir-17 have a lower survival rate (P =0.1); this trend is not statistically significant. **(B)** In TCGA, there is a trend that TNBC patients with low hsa-mir-20a in their cancers have a lower survival rate (p=0.1); this trend is not statistically significant. **(C)** For TCGA data, KM plots show that TNBC patients with low hsa-mir-135b in their cancers have a lower survival rate. **(D)** In TCGA data, there is a trend that patients having TNBCs with high hsa-mir-10a have a lower survival rate (p= 0.06); this trend is not statistically significant. **(E)** In METABRIC data, there is a trend that patients with TNBCs with low mir-584 have lower survival (p= 0.05); this trend is not statistically significant. **(F)** In METABRIC data, there is a trend that patients having TNBC with high hsa-mir-532-3p have a lower survival rate (p= 0.13), but this trend is not statistically significant. **(G)** In METABRIC data, there is a trend that patients having TNBCs with high hsa-mir-375 have a lower survival rate (p=0.14); this trend is not statistically significant. **(H)** In METABRIC data, there is a trend that patients having TNBCs with low mir-29c have lower survival (p=0.17); this trend is not statistically significant.

SUPPLEMENTAL TABLE LEGENDS

Table S1. High levels of mir-17, mir-20a, mir-584, mir-135b, and mir-532 and low levels of mir-29c, mir-10a, and mir-375 are present in overall AR-negative cases. The expressions of eight selected miRNAs were obtained for total AR-negative (n= 267) and AR-positive samples (n=823). The log2 fold change and p-adj values are less than 0.05.

Table S1. Over all AR negative vs Positive		
miRNA	log2FC	P-adj

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hsa-mir-135b	3.3 (Up)	5.21E-126
hsa-mir-584	1.7 (Up)	1.04E-104
hsa-mir-17	1.4 (Up)	9.36E-116
hsa-mir-20a	1.2 (Up)	2.39E-60
hsa-mir-532	0.6 (Up)	1.58E-44
hsa-mir-29c	-0.9 (Down)	2.72E-26
hsa-mir-375	-1.0 (Down)	2.52E-14
hsa-mir-10a	-1.4 (Down)	2.74E-45

Table S2. High levels of mir-17, mir-20a, mir-584, mir-135b, and mir-532 and low levels of mir-29c, mir-10a, and mir-375 are present in QNBCs. The expressions of eight selected miRNAs were obtained for QNBCs (n= 97) and AR-positive TNBC samples (n=17). The log2 fold change is shown. P-adj is less than 0.05 for all miRNAs, except mir-29c.

Table S2. QNBC compared to TNBC AR positive		
miRNA	**log2FC**	**p-adj**
hsa-mir-135b	2.2 (Up)	0.006415156
hsa-mir-584	1.5 (Up)	0.000490142
hsa-mir-20a	1.4 (Up)	4.27E-05
hsa-mir-17	1.3 (Up)	6.62E-05
hsa-mir-532	0.6 (Up)	0.025283529
hsa-mir-10a	-1.3 (Down)	0.016664581
hsa-mir-375	-1.8 (Down)	0.006761255
hsa-mir-29c	-0.7 (Down)	0.162012123

Table S3. miRNA to related gene correlation coefficient. Correlation of miRNAs to their target genes or regulatory genes as determined by mRNA and protein expression.

Table S3. miRNA to related gene correlation coefficient				
		MYC	**MIR17HG**	**FOXA1**
hsa-mir-17	*mRNA*	0.47	0.5	-0.41
	protein	0.28	N/A	-0.44
hsa-mir-20a	*mRNA*	0.42	0.47	-0.35
	protein	0.25	N/A	-0.38
		CCNG2	**GATA3**	
hsa-mir-135b	*mRNA*	-0.28	-0.46	
	protein	N/A	-0.47	
		TNF		
hsa-mir-10a	*mRNA*	-0.21		

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	protein	N/A			
		CTNNB1	**IL1B**	**KLF5**	**RUNX3**
hsa-mir-375	*mRNA*	-0.12	-0.16	-0.25	-0.37
	protein	-0.16	-0.27	-0.12	-0.17
		CD276	**CDK6**	**CDKN2A**	
hsa-mir-29c	*mRNA*	-0.3	-0.33	-0.29	
	protein	-0.17	-0.31	-0.24	

Table S4. List of 45 miRNAs differentially expressed in AR-negative cases as compared to AR-positive cases in all samples. The table presents 45 miRNAs, differentially expressed in AR-negative cases (n=267) compared to AR-positive cases (n=823) (P-adj < 0.05, p <0.05 and base Mean > 5, Log2 fold change < -1 and >1). In this table, the columns represent base mean expression of miRNAs, log2 fold change, p-adj values from DESEQ2 analysis, and chromosomal location of the miRNA. Blue color represents upregulated miRNAs, and red represents down-regulated miRNAs in AR-negative cases.

Table S4. miRNAs DE in over all AR negative compared to positive.

miRNA	Base Mean	log2FC	P-adj	Chromosome
hsa-mir-934	20.03	4.8	8.50E-142	chrX
hsa-mir-577	36.51	4.7	9.49E-176	chr4
hsa-mir-522	5.68	3.9	1.53E-37	chr19
hsa-mir-519a-1	13.19	3.8	2.27E-69	chr19
hsa-mir-516a-1	5.12	3.8	2.18E-57	chr19
hsa-mir-135b	65.7	3.3	5.21E-126	chr1
hsa-mir-519a-2	7.83	2.8	1.76E-27	chr19
hsa-mir-105-2	45.85	2.3	2.26E-13	chrX
hsa-mir-885	8.06	2.3	2.05E-33	chr3
hsa-mir-9-2	2942.54	2.3	1.48E-63	chr5
hsa-mir-9-1	2944.73	2.3	2.20E-63	chr1
hsa-mir-9-3	2951.94	2.3	1.91E-63	chr15
hsa-mir-767	32.23	2.2	4.00E-12	chrX
hsa-mir-18a	53.2	2.1	2.91E-123	chr13
hsa-mir-105-1	39.49	2	4.57E-10	chrX
hsa-mir-584	167.52	1.7	1.04E-104	chr5
hsa-mir-937	11.95	1.5	1.54E-35	chr8
hsa-mir-129-2	5.85	1.5	8.61E-26	chr11
hsa-mir-224	157.96	1.5	9.21E-27	chrX
hsa-mir-1269a	186	1.4	1.02E-06	chr4
hsa-mir-455	769.34	1.4	1.35E-72	chr9
hsa-mir-17	3323.2	1.4	9.36E-116	chr13
hsa-mir-31	24.7	1.3	4.86E-32	chr9
hsa-mir-129-1	5.37	1.3	6.33E-20	chr7
hsa-mir-19a	90.58	1.3	2.49E-70	chr13
hsa-mir-452	200.43	1.2	2.03E-28	chrX
hsa-mir-505	140.56	1.2	2.23E-88	chrX
hsa-mir-877	6.06	1.2	1.04E-33	chr6
hsa-mir-20a	769.92	1.2	2.39E-60	chr13
hsa-mir-188	8.33	1.1	1.58E-44	chrX
hsa-mir-3200	14.25	1.1	2.05E-23	chr22
hsa-mir-942	16.05	1	2.97E-41	chr1
hsa-mir-146a	417.27	1	5.94E-37	chr5
hsa-mir-19b-1	226.61	1	1.64E-56	chr13
hsa-mir-375	87622.86	-1	2.52E-14	chr2

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hsa-mir-196a-2	1347.35	-1.1	2.30E-18	chr12
hsa-mir-196a-1	1162.97	-1.2	9.32E-19	chr17
hsa-mir-135a-1	8.08	-1.2	7.38E-09	chr3
hsa-mir-2115	5.39	-1.2	2.30E-09	chr3
hsa-mir-135a-2	9.6	-1.3	2.36E-10	chr12
hsa-mir-10a	121503.21	-1.4	2.74E-45	chr17
hsa-mir-190b	65.09	-1.7	2.96E-44	chr1
hsa-mir-184	179.08	-1.8	5.57E-21	chr15
hsa-mir-449b	6.18	-3	5.32E-26	chr5
hsa-mir-449a	34.19	-4	3.75E-59	chr5

FC= Fold change, Blue color = Upregulated miRNA ,Red color = Down regulated miRNA

Table S5. A list of 40 miRNAs dysregulated in QNBCs compared AR-positive TNBCs. Shown are miRNAs differentially regulated in QNBCs (n=97) compared to AR-positive TNBCs (n=17). The table shows the expression of 40 miRNAs that are dysregulated in QNBCs (P-adj < 0.05, p <0.05 and base Mean > 5, log2 fold change < -0.5 and >0.5). In this table, columns represent base mean expression of miRNAs, log2-fold change, p-adj value from DESEQ2 analysis, and chromosomal location of the miRNA. Blue color represents upregulated miRNAs, and red represents down-regulated miRNAs in QNBCs.

Table S5. miRNAs DE in QNBC compared to TNBC AR positive.

miRNA	Base Mean	log2FC	P-adj	Chromo-some
hsa-mir-519a-1	18.65	3.6	0.003044	chr19
hsa-mir-516a-2	6.09	3.6	0.016665	chr19
hsa-mir-516a-1	6.7	3.5	0.016326	chr19
hsa-mir-129-2	5.77	2.4	0.000589	chr11
hsa-mir-129-1	5.3	2.4	0.00131	chr7
hsa-mir-135b	65.7	2.2	0.006415	chr1
hsa-mir-584	167.52	1.5	0.00049	chr5
hsa-mir-455	769.34	1.4	0.000564	chr9
hsa-mir-20a	769.92	1.4	4.27E-05	chr13
hsa-mir-4661	12.93	1.4	0.010915	chr8
hsa-mir-20b	118.21	1.3	0.033979	chrX
hsa-mir-17	3323.2	1.3	6.62E-05	chr13
hsa-mir-106a	145.7	1.1	0.016326	chrX
hsa-mir-92a-1	14131.15	0.9	0.001852	chr13
hsa-mir-92a-2	12805.4	0.9	0.001873	chrX
hsa-mir-500b	24.6	0.9	0.017844	chrX
hsa-mir-222	157.81	0.9	0.025284	chrX
hsa-mir-19b-2	203.04	0.8	0.016665	chrX
hsa-mir-214	114.82	0.8	0.032058	chr1
hsa-mir-19b-1	226.61	0.8	0.01506	chr13
hsa-mir-23a	20190.19	0.7	0.000589	chr19
hsa-mir-181a-2	4819.78	0.6	0.032058	chr9
hsa-mir-532	2049.49	0.6	0.025284	chrX
hsa-mir-24-2	2095.89	0.6	0.024223	chr19
hsa-mir-24-1	2042.37	0.6	0.030062	chr9
hsa-mir-664a	146.92	-0.7	0.032058	chr1
hsa-mir-193b	414.91	-0.7	0.032058	chr16
hsa-mir-625	839.22	-0.8	0.043634	chr14
hsa-mir-382	79.17	-0.8	0.039173	chr14
hsa-mir-758	61.97	-0.9	0.039173	chr14
hsa-mir-10b	285851.01	-1	0.016665	chr2
hsa-mir-615	36.07	-1.2	0.032058	chr12

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hsa-mir-3677	39.36	-1.2	0.033979	chr16
hsa-mir-940	16.21	-1.3	0.019216	chr16
hsa-mir-10a	121503.21	-1.3	0.016665	chr17
hsa-mir-196a-2	1347.35	-1.5	0.032058	chr12
hsa-mir-196a-1	1162.97	-1.6	0.025522	chr17
hsa-mir-375	87622.86	-1.8	0.006761	chr2
hsa-mir-190b	65.09	-2.7	3.04E-07	chr1
hsa-mir-2115	5.75	-4.7	8.22E-08	chr3

FC= Fold change, Blue color = Upregulated miRNA ,Red color = Down regulated miRNA

Table S6. List of 63 miRNAs dysregulated in breast cancers of overall AAs compared to CAs. Shown are miRNAs differentially regulated in AA breast cancers (n=184) compared to those of CAs (n=761). The table shows the expression of 63 miRNAs that are dysregulated in AA breast cancers (p-adj < 0.05, p <0.05 and base Mean > 5, log2 fold change < -0.5 and >0.5). In this table, columns represent base mean expression of miRNAs, log2 fold change, p-adj value from DESEQ2 analysis, and chromosomal location of the miRNA. Blue color represents upregulated miRNAs, and red represents down-regulated miRNAs in AA cancers.

Table S6. miRNAs DE in over all samples AA compared to CA.

miRNA	Base Mean	Log2FC	P-adj	Chromosome
hsa-mir-519a-1	9.87	2	3.26E-12	chr19
hsa-mir-516a-1	5.12	2	4.95E-09	chr19
hsa-mir-1269b	24.92	1.4	0.011747	chr17
hsa-mir-767	26.37	1.3	0.000791	chrX
hsa-mir-519a-2	5.69	1.3	0.00013	chr19
hsa-mir-129-2	5.85	1.2	2.30E-12	chr11
hsa-mir-129-1	5.37	1.2	2.98E-11	chr7
hsa-mir-2115	5.75	1.1	2.18E-05	chr3
hsa-mir-224	157.96	1.1	4.24E-10	chrX
hsa-mir-452	200.43	1.1	8.08E-15	chrX
hsa-mir-934	20.03	1	0.001379	chrX
hsa-mir-217	67.9	1	3.09E-15	chr2
hsa-mir-20a	769.92	0.9	2.52E-24	chr13
hsa-mir-135b	65.7	0.9	2.62E-05	chr1
hsa-mir-660	104.61	0.9	2.26E-37	chrX
hsa-mir-18a	53.2	0.8	2.92E-09	chr13
hsa-mir-383	5.7	0.7	0.024105	chr8
hsa-mir-937	11.95	0.7	2.34E-06	chr8
hsa-mir-500b	24.6	0.7	1.05E-18	chrX
hsa-mir-93	16091.63	0.7	7.04E-26	chr7
hsa-mir-3615	13.24	0.7	4.08E-12	chr17
hsa-mir-584	167.52	0.7	2.55E-10	chr5
hsa-mir-362	35.39	0.7	2.00E-14	chrX
hsa-mir-17	3323.2	0.6	3.83E-12	chr13
hsa-mir-135a-2	14.47	0.6	0.040678	chr12
hsa-mir-500a	1446.64	0.6	1.08E-17	chrX
hsa-mir-181b-2	589.08	0.6	9.41E-13	chr9
hsa-mir-877	6.06	0.6	7.18E-06	chr6
hsa-mir-532	2049.49	0.6	2.74E-23	chrX
hsa-mir-494	5.56	-0.6	5.88E-06	chr14
hsa-mir-3677	39.36	-0.6	5.33E-06	chr16

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hsa-mir-34c	48.96	-0.6	4.88E-06	chr11
hsa-mir-431	41.22	-0.6	8.48E-07	chr14
hsa-let-7b	94864.09	-0.6	2.27E-12	chr22
hsa-mir-326	33.92	-0.6	4.06E-07	chr11
hsa-mir-642a	15.55	-0.6	3.54E-06	chr19
hsa-mir-3605	11.67	-0.6	3.86E-08	chr1
hsa-mir-628	117.77	-0.6	4.56E-09	chr15
hsa-mir-34b	9.24	-0.6	3.16E-06	chr11
hsa-mir-323b	14.4	-0.6	5.45E-06	chr14
hsa-mir-337	137.77	-0.7	3.97E-11	chr14
hsa-mir-134	1321.87	-0.7	2.55E-14	chr14
hsa-mir-3170	6.79	-0.7	4.91E-09	chr13
hsa-mir-935	11.77	-0.7	0.000358	chr19
hsa-mir-323a	13.83	-0.7	6.39E-08	chr14
hsa-mir-10a	121503.21	-0.7	1.18E-08	chr17
hsa-mir-4668	14.52	-0.7	1.25E-11	chr9
hsa-mir-196b	1097.65	-0.8	1.63E-10	chr7
hsa-mir-539	36.09	-0.8	9.74E-14	chr14
hsa-mir-653	66.83	-0.8	1.79E-10	chr7
hsa-mir-551b	6.22	-0.8	1.37E-09	chr3
hsa-mir-374c	7.29	-0.9	0.01274	chrX
hsa-mir-135a-1	10.89	-0.9	0.001425	chr3
hsa-mir-1258	5.22	-0.9	4.92E-09	chr2
hsa-mir-3607	284.27	-0.9	1.18E-16	NA
hsa-mir-891a	7.35	-1	1.23E-07	chrX
hsa-mir-449a	34.71	-1.2	0.000128	chr5
hsa-mir-449b	6.32	-1.3	0.000503	chr5
hsa-mir-483	24.76	-1.4	2.74E-23	chr11
hsa-mir-184	220.46	-1.6	1.59E-11	chr15
hsa-mir-153-2	155.5	-1.6	6.07E-24	chr7
hsa-mir-1269a	240.32	-1.9	2.57E-07	chr4
hsa-mir-153-1	8.52	-1.9	5.71E-23	chr2

Table S7. A list of 66 miRNAs dysregulated in AA QNBCs compared to CA QNBCs. Shown are miRNAs differentially regulated in AA QNBCs (n=30) compared to CAs with QNBCs (n=55). The table shows the expression of 66 miRNAs that are dysregulated in QNBCs of AAs (P-adj < 0.05, p <0.05 and base Mean > 5, log2 fold change < -0.5 and >0.5). In the table, columns represent base mean expression of miRNAs, log2 fold change, p-adj value from DESEQ2 analysis, and chromosomal location of the miRNA. Blue color represents upregulated miRNA, and red represents down-regulated miRNAs in QNBCs of AAs.

Table S7. miRNAs DE in over all samples AA compared to CA in QNBC

miRNA	Base Mean	log2FC	P-value	P-adj	Chromo-some
hsa-mir-129-2	8.07	3.5	2.26E-13	2.82E-11	chr11
hsa-mir-129-1	7.27	3.4	4.52E-12	4.03E-10	chr7
hsa-mir-217	77.86	1.9	2.41E-08	1.16E-06	chr2
hsa-mir-363	57.36	1.2	0.000435	0.005311	chrX
hsa-mir-29c	11003.73	1.2	3.78E-06	8.75E-05	chr1
hsa-mir-20b	127.08	1	0.006116	0.038945	chrX
hsa-mir-92b	393.4	0.9	0.000179	0.002661	chr1
hsa-mir-660	104.61	0.7	4.36E-05	0.000798	chrX

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hsa-mir-101-2	17318.62	0.7	0.000498	0.005649	chr9
hsa-mir-101-1	17210.19	0.7	0.000527	0.00587	chr1
hsa-mir-339	165.74	-0.6	0.001391	0.01204	chr7
hsa-mir-590	57.44	-0.6	0.001905	0.015645	chr7
hsa-mir-99b	124367.1	-0.6	0.000823	0.008147	chr19
hsa-mir-491	13.17	-0.6	0.007189	0.044417	chr9
hsa-mir-130b	73.03	-0.6	0.002804	0.021081	chr22
hsa-mir-127	2846	-0.7	0.001409	0.01204	chr14
hsa-mir-30c-1	2833.08	-0.8	0.000212	0.003002	chr1
hsa-mir-889	42.7	-0.8	0.002475	0.018937	chr14
hsa-mir-3074	44.72	-0.8	0.000798	0.008147	chr9
hsa-mir-495	24.48	-0.9	0.000878	0.008429	chr14
hsa-mir-323a	12.42	-0.9	0.00355	0.02517	chr14
hsa-mir-382	80.14	-0.9	0.000113	0.001802	chr14
hsa-mir-149	516.81	-0.9	0.005524	0.03667	chr2
hsa-mir-485	14.97	-0.9	0.000575	0.006289	chr14
hsa-mir-377	10.77	-0.9	0.000456	0.005371	chr14
hsa-mir-134	1321.87	-0.9	4.48E-05	0.000798	chr14
hsa-mir-431	37.07	-0.9	0.000494	0.005649	chr14
hsa-mir-379	2568.02	-0.9	0.000256	0.003463	chr14
hsa-mir-345	74.64	-1	0.000205	0.002979	chr14
hsa-mir-887	30.51	-1	0.00195	0.015799	chr5
hsa-mir-3607	284.27	-1	0.000709	0.007501	NA
hsa-mir-496	8.12	-1	0.000953	0.008871	chr14
hsa-mir-3170	6.79	-1	0.000841	0.008203	chr13
hsa-mir-299	11.68	-1	0.00011	0.001802	chr14
hsa-mir-655	8.12	-1	0.000156	0.002432	chr14
hsa-mir-1266	50.71	-1.1	3.85E-05	0.000728	chr15
hsa-mir-654	72.56	-1.1	2.69E-05	0.000526	chr14
hsa-mir-539	36.09	-1.1	8.02E-05	0.001387	chr14
hsa-mir-3653	33.19	-1.1	1.18E-05	0.000237	NA
hsa-mir-190b	65.09	-1.1	0.007751	0.047417	chr1
hsa-mir-214	114.82	-1.1	2.83E-07	8.70E-06	chr1
hsa-mir-196b	1097.65	-1.1	0.000178	0.002661	chr7
hsa-mir-487b	16.3	-1.1	9.23E-06	0.000206	chr14
hsa-mir-370	34.06	-1.2	3.32E-06	8.29E-05	chr14
hsa-mir-369	32.21	-1.2	2.12E-07	7.37E-06	chr14
hsa-mir-758	64.31	-1.2	3.12E-06	8.10E-05	chr14
hsa-mir-210	2086.53	-1.2	0.00081	0.008147	chr11
hsa-mir-154	23.11	-1.2	7.20E-08	3.21E-06	chr14
hsa-mir-375	87622.86	-1.3	0.001349	0.011852	chr2
hsa-mir-1248	6.63	-1.3	8.22E-05	0.001387	chr3
hsa-mir-411	22.05	-1.4	1.57E-07	5.78E-06	chr14
hsa-mir-935	11.77	-1.4	0.006044	0.038878	chr19
hsa-mir-4662a	108.99	-1.5	2.25E-06	6.09E-05	chr8
hsa-mir-494	5.56	-1.5	1.89E-06	5.37E-05	chr14
hsa-mir-432	22.95	-1.6	1.05E-07	4.10E-06	chr14
hsa-mir-493	42.03	-1.8	2.67E-12	2.78E-10	chr14
hsa-mir-376c	18.94	-1.8	5.30E-12	4.14E-10	chr14
hsa-mir-376b	6.98	-2.1	2.70E-11	1.68E-09	chr14
hsa-mir-135a-2	11.21	-2.4	0.0009	0.008512	chr12
hsa-mir-483	22.12	-2.4	1.26E-10	7.14E-09	chr11
hsa-mir-374c	7.29	-2.4	0.003216	0.023613	chrX
hsa-mir-135a-1	8.73	-2.5	0.000443	0.005311	chr3

hsa-mir-105-1	46.05	-2.9	0.003655	0.025625	chrX
hsa-mir-105-2	45.85	-2.9	0.003405	0.024708	chrX
hsa-mir-885	8.06	-3.2	2.93E-07	8.70E-06	chr3
hsa-mir-1269a	240.32	-10.3	3.67E-27	2.29E-24	chr4

Table S8: High levels of mir-17, mir-20a, mir-584, mir-135b, miR-532, and low levels of mir-29c, mir-10a, and mir-375 are present in AA breast cancers. The expressions of eight selected miRNAs were measured in AA (n= 184) and CA breast cancer cases (n= 761). The log2 fold change and p-adj values were less than 0.05, except for two miRNAs (mir-29C and mir-375).

Table S8. AA vs CA over all

miRNA	log2FC	P-adj
hsa-mir-20a	0.9 (Up)	2.52E-24
hsa-mir-135b	0.9 (Up)	2.62E-05
hsa-mir-584	0.7 (Up)	2.55E-10
hsa-mir-17	0.6 (Up)	3.83E-12
hsa-mir-532	0.6 (Up)	2.74E-23
hsa-mir-10a	-0.7 (Down)	1.18E-08
hsa-mir-29c	-0.2 (Down)	0.111358
hsa-mir-375	-0.1 (Down)	0.640543