**Supplementary Methods**

**Identification of PBMC-Expressed miRNAs for Rheumatoid Arthritis**

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**Supplementary Methods**

**Study subjects and sample preparation**

For the transcriptome-wide miRNA expression microarray analysis, a total of 25 subjects with active RA and 18 healthy controls were recruited. For the miRNA and mRNA expression validation, another sample of 35 subjects with active RA and 35 healthy controls were recruited. The subjects were recruited in the department of rheumatology of the First Affiliated Hospital of Soochow University, during December 2014 to July 2015. All the patients with RA met the standard of American College of Rheumatology and the European Union League Against Rheumatism set in 2010. All patients had an examination of tender and swollen joints and disease activity score of 28 joints (DAS28) recorded. Laboratory investigations included C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Active RA was defined as having a 28-joint disease activity score (DAS28) of 2.6 or higher. For health controls, subjects with severe cardiovascular diseases, liver and kidney dysfunction, malignant tumor and other immune diseases including systemic lupus erythematosus, ankylosing spondylitis were excluded. No significant differences in age and BMI were detected between the cases and controls (**Table S6**). The study was approved by the ethical committee of Soochow University. All subjects signed informed consents.

Peripheral blood samples were collected from each subject and stored in vacuum blood collection tubes containing sodium citrate. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation from blood samples (Greiner Bio-one, Frickenhausen, Germany). Total RNA was then extracted from PBMCs using TRIzol reagent (Invitrogen, USA) according to manufacturer’s instructions. The total RNA was purified using mirVana™ miRNA Isolation Kit (Ambion, Woodward, Austin, TX, USA) or NucleoSpin® RNA clean-up kit (MACHEREY- NAGEL, Germany), quantified using NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, USA) and controlled using Agilent Bioanalyzer 2100 (Agilent Technologies, USA). All samples showed good quality of RNA (260/280 nm absorbance ratios >1.8 and RNA Integrity Number (RIN)>7).

**Transcriptome-wide miRNA and mRNA expression profiling**

The transcriptome-wide miRNA expression profiling was analyzed using Affymetrix miRNA 4.0 (CapitalBio, Beijing, China). The microarray contains mature miRNAs across 203 species in Sanger miRBasev20.0, including 2,578 human mature miRNAs. About 1.0 ug of total RNA per sample was converted in microarray to profile miRNA expression. Briefly, the total RNA was poly-A tailed using the poly A polymerase and connected with the biotin-labeled 3DNA dendrimer for the miRNA labeling. The labeling process was completed using FlashTagTM Biotin RNA Labeling Kit (Genisphere, Hatfield, PA, USA). The hybridization cocktail was then added into biotin-labeled RNA using Eukaryotic Hybridization Control Kit (Affymetrix, Santa Clara, CA, USA). After incubating for 5 minutes at 99°C, 5 minutes at 45°C and centrifugation for 5minutes at 13200rpm to remove insoluble impurities, the mixture was hybridized on miRNA arrays at 48°C for 16 hours. The microarrays were then washed and stained using Affymetrix Fluidics Station 450, and scanned using Affymetrix GeneChip Scanner 3000. The scanning image was converted into digital signal using Affymetrix GeneChip Command Console (AGCC).The data normalization and quality control was processed by Robust Multi-array Average (RMA) plug in of Affymetrix Expression Console. Fold-change and P value were calculated to index the expression difference of miRNAs between RA patients and healthy controls.

The transcriptome-wide mRNA expression profiling was analyzed using lncRNA & mRNA Human Gene Expression Microarray V4.0 (CaptialBio, Beijing, China) according to the manufacturer’s instructions. Total RNA was reverse-transcribed into double stranded cDNA with T7 Oligo (dT) Primer and T7 random hexamers followed by the cRNA generation. The cRNA was then purified and reverse transcribed into cDNA using CbcScript reverse transcriptase. The purified cDNA was labeled by Cy5-dCTP and hybridized to the assay at 45°C overnight. After washing, the microarray was scanned using Agilent G2565CA Microarray Scanner and analyzed using Agilent Feature Extraction v10.7. The data was finally normalized and controlled using Agilent GeneSpring GX program v12.0. Probes with incomplete annotation information and detection rate less than 80% were excluded. Only the most significant probe of mRNA associated with RA was retained when there were multiple probes for the same mRNA. Two-sided Student’s *t*-test was used to identify differentially expressed genes between RA patients and healthy controls.

**Expression validation of differentially expressed miRNAs and corresponding targets in population**

The differential expression of significant miRNAs and mRNAs were validated in another sample including 35 RA patients and 35 healthy controls using real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from PBMC using TRIzol reagent.

For the miRNAs, 9 out of 18 miRNAs were selected for validation. The selection was mainly based on the following criteria: (1) the miRNA has not been validated in RA patients by RT-qPCR in the previous studies; (2) the miRNA had the relatively higher fold change value in the present study; (3) the miRNA had relatively higher expression values in microarray analysis. 100 ng of total RNA was reverse-transcribed into cDNA using a miRNA Reverse Transcription Kit (CT Bioscience, Changzhou, China) with stem-loop RT primer according to the manufacture’s protocol. Briefly, the reactions were incubated for 30min at 16℃, 30min at 42℃, 5min at 85℃ and then stored at 4℃. RT-qPCR was performed by Roche light cycler 480II with SYBR Green MasterMix (CT Bioscience, Changzhou, China). The reactions were incubated at 95℃ for 2min, followed by 45 cycles of 95℃ for 10s, 60℃ for 20s and 40℃ for 30s.

For mRNAs, we used mRNA Reverse Transcription Kit (CT Bioscience, Changzhou, China) to reverse-transcribe total RNA into cDNA. The reactions were incubated for 60min at 42℃, 5min at 85℃ and stored at 4℃ thereafter. In the qPCR analyses, the reactions were incubated at 95℃ for 10min, followed by 45 cycles of 95℃ for 10s, 60℃ for 20s and 40℃ for 30s.

The differential expression of miR-99b-5p in primary T cells was validated in additional samples including 7 RA patients and 7 healthy controls by using RT-qPCR. 10 ml peripheral blood was obtained and PBMCs were purified by density gradient centrifugation over Ficoll-Paque (Lymphoprep, density: 1.077g/ml, STEMCELL Technologies, Vancouver, Canada). T cells were then isolated using the EasySep Human Whole Blood CD3 Positive Selection Kit (STEMCELL Technologies, Vancouver, Canada). An aliquot of CD3+ T lymphocytes were harvested for RNA extraction. The expression level of miR-99b-5p was detected using qRT-PCR as shown above.

Each sample was analyzed in triplicate. All the specific primers were designed and purchased from CT Bioscience (CT Bioscience, Changzhou, China). The gene expression levels were relatively quantified by using the comparative cycle threshold (Ct) method. The miRNA and mRNA expression levels were normalized against RNU48/U6 and beta-2-microglobulin (B2M), respectively.

**Functional assays of miR-99b-5p in Jurkat T cells**

**Cell culture**

Jurkat T cells and 293 T cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and were cultured in a humidified incubator at 37℃ and 5% CO2. Jurkat T cells were maintained in RPMI 1640 cell culture medium (Hyclone, Thermo scientific, USA) supplemented with 20% fetal bovine serum (FBS; Biological Industries, Israel) and 1% penicillin-streptomycin (TransGen, Beijing, China). 293T cells were cultured in DMEM (Hyclone, Thermo scientific, USA) supplemented with 10% FBS and 1% penicillin-streptomycin.

**Lentiviral construction, production and transfection**

Lentivirus vectors were constructed using the pCDH-CMV-MCS-EF1-copGFP system (System Biosciences, USA; https://www.systembio.com/products/gene-expression-systems/lentiviral-vectors/pcdh-cmv-mcs-ef1-alpha-copgfp/). Briefly, we first exacted DNA from PBMCs of human blood samples and performed PCR amplification for the miRNA fragment with the primer designed by Primer5 (**Table S7**).The primer was based on the whole and 1000bp up- and downstream sequences of miR-99b-5p obtained from Ensembl (http://www.ensembl.org). The fragment was then cloned into the pCDH-CMV-MCS-EF1-copGFP miRNA expression vector. With the process of enzyme digestion, purification and enzyme connection, the target plasmid (pCDH-99b-GFP) was generated. Gel electrophoresis and DNA sequencing were used to validate the plasmid. The empty vector of pCDH was used as a negative control in this study.

The sequencing-verified recombinant plasmid and the lentivirus Packaging plasmid ps-PAX2, envelope plasmid pMD2.G and pCDH- CMV-MCS-EF1-copGFP plasmids (SBI, Mountain View, CA) were then co-transfected into 293T cells with Lipofectamine 3000 (Invitrogen, USA) to generate the lentivirus. The viral titer of lentivirus concentrated from the culture supernatants was detected according to the manufacture’s protocols (Applied Biological Materials Inc, Canada).

The recombinant lentivirus was then harvested and transfected into Jurkat T cells to construct the miR-99b-5p over-expression cell lines. The lentivirus transfection efficiency was measured by flow cytometry (Beckman Instruments Inc., USA) and the miRNA expression was assessed using RT-qPCR (primer information shown in **Table S7)**, which were performed to guarantee the quality of target cell line. Briefly, 2ug of total RNA was reverse-transcribed with TransScript miRNA First-Strand cDNA Synthesis kit (TranGen Biotech, Beijing, China). The real-time qPCR were then performed using Promega GoTaq qPCR Master Mix (Promega Corp, USA) by Applied Biosystems Quanstudio6 Flex (Thermo Fisher Scientific, USA). The reactions were incubated at 94℃ for 30s, followed by 45 cycles of 94℃ for 5s, 55℃ for 15s and 72℃ for 10s.

The miR-99b-5p inhibitor was synthesized by GenePharma (Shanghai, China). 160nM of the inhibitor or the negative control was added into the Jurkat T cells to inhibit the expression of the miR-99b-5p in Jurkat cell lines. The miRNA expression of miR-99b-5p was validated by RT-qPCR using the same protocol shown above. The primer information of the miR-99b-5p inhibitor was shown in Table S7.

**Cell proliferation assay**

The miR-99b-5p stably transfected cells or negative control cells were seeded into 96-well plates at a density of 5×104 cells per well. After 12, 24 and 48 hours of culture, the cells were treated with 10uL cell counting kit-8 (CCK-8) solution and incubated for 1h. The optical density (OD) at the wavelength of 450nm was detected by enzyme-linked immunometric meter (BioTek Instruments Inc., USA). Each group was made in quintuplicate.

The Jurkat T cells with 200nM of the inhibitor and the negative control was seeded into 96-well plates at a density of 2×104 cells per well and cultured for 48h. The cell proliferation was conducted at 0/24/48/72h after the incubation according to the standard protocol as previously described.

**Cell apoptosis assay**

The cell apoptosis was assessed using PE Annexin V Apoptosis Detection Kit (BD Biosciences, Becton, Dickinson and Company, USA). The stably transfected cells were harvested by centrifugation, washed twice by PBS and stained with Annexin V and PI according to the manufacturer’s instructions. After incubation in the dark for 15min at room temperature, cells were resuspended with 1×Annexin V binding buffer and examined by flow cytometry (Beckman Instruments Inc., USA).

**Cell cycle assay**

For the assay of cell cycle, the stably transfected cells were collected, washed, and fixed with ice-cold 70% ethanol according to the protocol of Beyotime Biotech (Shanghai, China). After storage at 4℃ overnight, the cells were washed, resuspended with PBS, and treated with propidium iodide(PI) staining solution mix (Beyotime) for 30min in the dark. Flow cytometry (Beckman Instruments Inc., USA) was used for detection of the cells in different phases.

**Cell activation assay**

The stably transfected cells were treated with or without 5ug/ml phytohemagglutinin (PHA; Beyotime Biotechnology, Shanghai, China) for 48h. Afterwards, the cells were collected, washed and incubated with 3uL allophycocyanin (APC)-conjugated human CD25 or human CD69 antibody (BioLegend, USA) for 30min in the dark. The positive rate of CD25 and CD69 in Jurkat T cells were assessed by flow cytometry.

RT-qPCR was performed to measure the mRNA expression levels of 7 cytokines in Jurkat cells overexpressing miR-99b-5p according to the standard protocol. Briefly, total RNA was extracted from stably transfected Jurkat T cells and negative controls. The RNA quality was controlled and quantified using electrophoresis and NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, USA). A total of 2ug RNA was reverse-transcribed using GoScript Reverse Transcriptase Kit (Promega, USA). The qPCR analysis was performed using GoTaq qPCR Master Mix (Promega, USA) with Applied Biosystems Quanstudio6 Flex (ABI, Thermo Fisher Scientific, USA). The expression of 7 miRNA target genes in Jurkat T cells was also assessed according to the standard protocol. The primer information was shown in **Table S7**.

**Luciferase reporter assay**

The luciferase reporter plasmid was constructed using the pmirGLO vector (Promega, USA; https://www.promega.com.cn/products/reporter-assays-and-transfection/reporter-vectors-and-cell-lines/pmirglo-dual-luciferase-mirna-target-expression-vector/?catNum=E1330). The fragment of wild-type and mutant-type *RASSF4* 3’UTR were amplified by PCR and cloned into the pmirGLO vector (primer information shown in **Table S7**). The plasmids were validated by DNA sequencing. Jurkat T cells were seeded in 6-well plates the day before transfection. 2.0µg of pmirGLO vector with wild-type or mutant-type *RASSF4* 3’UTR was co-transfected with miR-99b-5p overexpression lentivirus into Jurkat T cells using DharmaFECT Duo Transfection Reagent (Active Motif, USA). The relative luciferase activity was assessed using Dual-Luciferase Reporter Assay system (Promega, USA) and normalized for transfection efficiency by Renilla-luciferase.

**Fig. S1 Overview of the study strategy**

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**Fig. S2 Volcano-Plot of the identified DEMIRs in RA cases vs. healthy controls**

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**Fig. S3 Hierarchical clustering analysis of the 18 identified DEMIRs**

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**Fig. S4 Expression validations by RT-qPCR of miR-99b-5p in T lymphocytes in vivo**

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**Fig. S5 Functional roles of miR-99b-5p in Jurkat T cells analyzed by flow cytometry**



**Fig. S6 Expression validations by RT-qPCR of the identified miRNAs with insignificant differential expression or inconsistent direction with the discovery sample**



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| --- | --- | --- | --- | --- | --- |
| **Table S1. Significant and negative correlated pairs between 9 DEMIRs and 141 target mRNAs (genes)** | | | | | |
| **Gene** | **Fold change** | **P value** | **Corresponding miRNA** | **Correlation coefficient** | **P value for correlation** |
| SOCS5 | 1.82 | 1.62E-04 | miR-101-3p | -0.33 | 3.08E-02 |
| ZFP36L2 | 1.98 | 8.82E-07 | miR-101-3p | -0.33 | 2.87E-02 |
| CDYL | 1.55 | 6.96E-05 | miR-101-3p | -0.57 | 6.83E-05 |
| RANBP9 | 1.42 | 2.13E-04 | miR-101-3p | -0.45 | 2.24E-03 |
| NLK | 1.26 | 4.27E-02 | miR-101-3p | -0.41 | 6.91E-03 |
| ATXN1L | 1.39 | 8.50E-05 | miR-101-3p | -0.38 | 1.08E-02 |
| STAG2 | 1.81 | 7.11E-06 | miR-101-3p | -0.41 | 6.64E-03 |
| RAP2C | 1.44 | 7.91E-05 | miR-101-3p | -0.42 | 4.89E-03 |
| ZNF746 | 2.00 | 2.25E-06 | miR-101-3p | -0.41 | 7.00E-03 |
| UBE2A | 1.46 | 9.42E-05 | miR-101-3p | -0.30 | 4.85E-02 |
| ARID1A | 1.79 | 6.78E-08 | miR-101-3p | -0.66 | 1.41E-06 |
| SMARCD1 | 1.79 | 1.34E-06 | miR-101-3p | -0.44 | 2.83E-03 |
| SH2B3 | 1.61 | 1.58E-06 | miR-101-3p | -0.45 | 2.72E-03 |
| ICK | 1.49 | 8.07E-04 | miR-101-3p | -0.32 | 3.90E-02 |
| CBFA2T2 | 1.28 | 2.67E-04 | miR-101-3p | -0.33 | 2.85E-02 |
| ZBTB34 | 1.96 | 6.14E-06 | miR-101-3p | -0.33 | 3.26E-02 |
| ATXN1 | 1.75 | 4.62E-08 | miR-101-3p | -0.48 | 1.12E-03 |
| SUB1 | 1.41 | 1.45E-05 | miR-101-3p | -0.49 | 7.75E-04 |
| GLCCI1 | 1.34 | 5.23E-03 | miR-101-3p | -0.32 | 3.57E-02 |
| UBE2D2 | 1.87 | 9.16E-06 | miR-101-3p | -0.34 | 2.69E-02 |
| ZSWIM6 | 1.34 | 4.61E-04 | miR-101-3p | -0.36 | 1.64E-02 |
| ERBB2IP | 1.84 | 2.65E-06 | miR-101-3p | -0.40 | 8.35E-03 |
| ANKRD11 | 1.36 | 3.94E-05 | miR-101-3p | -0.43 | 3.90E-03 |
| ZNF207 | 1.62 | 1.09E-05 | miR-101-3p | -0.40 | 8.55E-03 |
| GAB1 | 1.80 | 5.77E-04 | miR-101-3p | -0.32 | 3.58E-02 |
| ARAP2 | 1.50 | 2.07E-04 | miR-101-3p | -0.46 | 2.08E-03 |
| RAB1A | 2.04 | 1.02E-05 | miR-101-3p | -0.32 | 3.43E-02 |
| SYNCRIP | 1.47 | 1.96E-03 | miR-101-3p | -0.31 | 4.01E-02 |
| DDIT4 | 1.64 | 4.60E-04 | miR-101-3p | -0.53 | 2.67E-04 |
| IMPA1 | 1.28 | 3.59E-04 | miR-101-3p | -0.33 | 3.17E-02 |
| RAP1B | 1.46 | 7.75E-04 | miR-101-3p | -0.42 | 5.22E-03 |
| DOT1L | 1.35 | 4.52E-07 | miR-101-3p | -0.53 | 2.62E-04 |
| EDEM3 | 1.65 | 2.52E-06 | miR-101-3p | -0.44 | 3.53E-03 |
| ASAP1 | 1.59 | 1.16E-07 | miR-101-3p | -0.57 | 7.58E-05 |
| RAB4A | 1.36 | 6.54E-04 | miR-101-3p | -0.34 | 2.45E-02 |
| METAP1 | 1.36 | 1.93E-03 | miR-101-3p | -0.30 | 4.97E-02 |
| KDM3B | 2.79 | 3.20E-07 | miR-101-3p | -0.40 | 7.40E-03 |
| MAML3 | 1.11 | 3.19E-02 | miR-101-3p | -0.35 | 2.31E-02 |
| ZBED4 | 1.77 | 2.33E-05 | miR-101-3p | -0.36 | 1.61E-02 |
| HNRNPF | 1.56 | 1.50E-05 | miR-101-3p | -0.32 | 3.50E-02 |
| BBX | 1.58 | 5.00E-05 | miR-101-3p | -0.33 | 3.17E-02 |
| FAM60A | 1.74 | 4.01E-06 | miR-101-3p | -0.34 | 2.80E-02 |
| SACM1L | 1.32 | 7.94E-04 | miR-101-3p | -0.36 | 1.93E-02 |
| ZCCHC2 | 1.68 | 1.60E-05 | miR-101-3p | -0.36 | 1.93E-02 |
| NSD1 | 1.33 | 1.33E-02 | miR-101-3p | -0.34 | 2.76E-02 |
| KDM6B | 1.47 | 1.15E-03 | miR-101-3p | -0.34 | 2.45E-02 |
| RNF38 | 1.56 | 4.91E-08 | miR-101-3p | -0.54 | 2.17E-04 |
| MLEC | 1.28 | 4.25E-03 | miR-101-3p | -0.40 | 7.94E-03 |
| GNB1 | 1.24 | 4.45E-03 | miR-101-3p | -0.46 | 2.00E-03 |
| UBR7 | 1.38 | 1.56E-04 | miR-101-3p | -0.42 | 4.67E-03 |
| PHTF2 | 1.81 | 2.97E-07 | miR-101-3p | -0.40 | 7.34E-03 |
| TMF1 | 1.61 | 5.01E-06 | miR-101-3p | -0.40 | 8.06E-03 |
| C1orf52 | 1.33 | 1.32E-04 | miR-101-3p | -0.44 | 3.37E-03 |
| SMARCA4 | 1.39 | 6.77E-05 | miR-101-3p | -0.41 | 5.80E-03 |
| GDE1 | 2.47 | 1.91E-06 | miR-101-3p | -0.44 | 3.44E-03 |
| COTL1 | 1.61 | 1.22E-06 | miR-101-3p | -0.51 | 5.18E-04 |
| USP38 | 1.22 | 1.05E-02 | miR-101-3p | -0.41 | 6.05E-03 |
| C10orf12 | 1.53 | 8.99E-05 | miR-101-3p | -0.48 | 1.13E-03 |
| RAB11FIP2 | 1.97 | 5.91E-08 | miR-1184 | -0.51 | 4.83E-04 |
| NPPA | 1.50 | 2.67E-05 | miR-1184 | -0.45 | 2.78E-03 |
| ENDOD1 | 1.69 | 9.13E-04 | miR-1184 | -0.35 | 2.31E-02 |
| DNM3 | 1.55 | 4.30E-03 | miR-1184 | -0.33 | 2.94E-02 |
| TPP1 | 1.92 | 3.24E-07 | miR-1184 | -0.47 | 1.58E-03 |
| PHF5A | 1.54 | 2.33E-05 | miR-1184 | -0.38 | 1.23E-02 |
| LRRC59 | 1.26 | 3.06E-02 | miR-1184 | -0.30 | 4.91E-02 |
| VDAC2 | 1.52 | 9.24E-06 | miR-1184 | -0.35 | 2.30E-02 |
| RILPL2 | 1.72 | 3.50E-07 | miR-1184 | -0.38 | 1.31E-02 |
| ZNF223 | 1.22 | 2.20E-02 | miR-1184 | -0.31 | 4.19E-02 |
| UBR7 | 1.38 | 1.56E-04 | miR-1184 | -0.33 | 3.00E-02 |
| ZSWIM6 | 1.34 | 4.61E-04 | miR-1184 | -0.46 | 1.87E-03 |
| BCL2L11 | 1.53 | 1.42E-05 | miR-1184 | -0.34 | 2.70E-02 |
| PRNP | 2.29 | 2.12E-06 | miR-1184 | -0.36 | 1.78E-02 |
| ARID1A | 1.79 | 6.78E-08 | miR-1184 | -0.41 | 5.90E-03 |
| SLK | 1.25 | 2.16E-04 | miR-1184 | -0.45 | 2.19E-03 |
| TAL1 | 1.99 | 8.03E-04 | miR-1184 | -0.40 | 7.94E-03 |
| CD97 | 1.61 | 8.91E-05 | miR-1184 | -0.32 | 3.81E-02 |
| DPH3 | 1.69 | 4.97E-05 | miR-1184 | -0.35 | 1.97E-02 |
| SPRED2 | 1.51 | 2.99E-06 | miR-1184 | -0.49 | 8.17E-04 |
| SLC30A7 | 2.26 | 1.97E-07 | miR-1184 | -0.32 | 3.78E-02 |
| C10orf32 | 2.21 | 9.42E-08 | miR-1184 | -0.40 | 8.20E-03 |
| CDKN2D | 2.01 | 4.76E-06 | miR-1184 | -0.50 | 5.69E-04 |
| VAPB | 2.06 | 1.45E-06 | miR-1184 | -0.31 | 4.12E-02 |
| NR1D2 | 1.82 | 2.46E-04 | miR-1184 | -0.37 | 1.42E-02 |
| RNF11 | 1.37 | 8.52E-03 | miR-1184 | -0.43 | 4.38E-03 |
| RAB27A | 1.48 | 7.14E-04 | miR-1184 | -0.30 | 4.73E-02 |
| GRIA4 | 1.13 | 4.63E-02 | miR-1184 | -0.38 | 1.29E-02 |
| ZNF217 | 1.43 | 6.32E-03 | miR-1184 | -0.41 | 6.71E-03 |
| CREBL2 | 1.88 | 5.05E-07 | miR-1246 | -0.35 | 2.34E-02 |
| SEPHS1 | 1.51 | 2.39E-05 | miR-1246 | -0.34 | 2.44E-02 |
| MIER1 | 1.55 | 2.58E-06 | miR-1246 | -0.33 | 2.85E-02 |
| FERMT2 | 1.52 | 3.54E-05 | miR-1246 | -0.59 | 3.45E-05 |
| QTRTD1 | 1.65 | 3.19E-05 | miR-1246 | -0.31 | 4.60E-02 |
| IFIT5 | 1.86 | 6.26E-05 | miR-1246 | -0.38 | 1.31E-02 |
| MBNL2 | 1.40 | 2.20E-03 | miR-1246 | -0.34 | 2.80E-02 |
| NUP153 | 2.01 | 4.04E-07 | miR-1246 | -0.35 | 2.20E-02 |
| FRMD3 | 1.95 | 5.39E-05 | miR-1246 | -0.34 | 2.50E-02 |
| AKAP2 | 3.66 | 3.98E-07 | miR-1246 | -0.37 | 1.55E-02 |
| CDC73 | 1.30 | 4.65E-03 | miR-1246 | -0.32 | 3.42E-02 |
| SEC22C | 1.25 | 1.70E-02 | miR-1246 | -0.31 | 4.35E-02 |
| SDHC | 1.60 | 5.71E-08 | miR-1246 | -0.37 | 1.53E-02 |
| ISCA1 | 1.56 | 7.30E-05 | miR-1246 | -0.37 | 1.59E-02 |
| TAP2 | 1.25 | 2.43E-02 | miR-1246 | -0.39 | 9.45E-03 |
| SH2B3 | 1.61 | 1.58E-06 | miR-1246 | -0.36 | 1.69E-02 |
| BACH1 | 1.42 | 7.94E-04 | miR-142-3p | -0.39 | 1.05E-02 |
| TFG | 1.36 | 4.96E-08 | miR-142-3p | -0.46 | 1.77E-03 |
| FNDC3A | 1.27 | 2.02E-03 | miR-142-3p | -0.41 | 6.41E-03 |
| AHR | 2.05 | 8.72E-05 | miR-142-5p | -0.31 | 4.56E-02 |
| RAP1A | 1.60 | 5.96E-07 | miR-142-5p | -0.40 | 7.89E-03 |
| ACTN4 | 1.52 | 3.19E-06 | miR-142-5p | -0.31 | 3.99E-02 |
| PTP4A1 | 1.68 | 2.61E-04 | miR-142-5p | -0.50 | 7.19E-04 |
| KLF10 | 1.93 | 8.07E-04 | miR-142-5p | -0.32 | 3.38E-02 |
| FAM199X | 1.26 | 1.90E-02 | miR-142-5p | -0.38 | 1.18E-02 |
| HIPK1 | 2.05 | 1.27E-06 | miR-142-5p | -0.33 | 3.34E-02 |
| RHOQ | 1.48 | 4.07E-06 | miR-142-5p | -0.38 | 1.14E-02 |
| ICK | 1.49 | 8.07E-04 | miR-142-5p | -0.36 | 1.86E-02 |
| NFE2L2 | 2.34 | 7.34E-09 | miR-142-5p | -0.44 | 2.97E-03 |
| TMF1 | 1.61 | 5.01E-06 | miR-142-5p | -0.37 | 1.48E-02 |
| CUL4A | 1.22 | 1.87E-04 | miR-142-5p | -0.39 | 8.83E-03 |
| CUL2 | 1.32 | 6.17E-04 | miR-142-5p | -0.45 | 2.63E-03 |
| SYNJ1 | 1.23 | 2.72E-03 | miR-142-5p | -0.41 | 6.56E-03 |
| BTBD1 | 1.41 | 1.67E-03 | miR-142-5p | -0.32 | 3.94E-02 |
| SGMS1 | 1.32 | 8.57E-03 | miR-142-5p | -0.48 | 1.05E-03 |
| BNIP2 | 2.54 | 1.48E-07 | miR-142-5p | -0.31 | 4.44E-02 |
| ARHGEF6 | 1.33 | 4.87E-03 | miR-142-5p | -0.33 | 3.08E-02 |
| RPS6KA5 | 1.46 | 9.56E-03 | miR-142-5p | -0.42 | 5.50E-03 |
| LEPROT | 1.44 | 1.36E-03 | miR-142-5p | -0.40 | 7.29E-03 |
| MGAT4B | 1.58 | 3.28E-05 | miR-142-5p | -0.47 | 1.29E-03 |
| DUSP2 | 1.37 | 1.63E-03 | miR-142-5p | -0.45 | 2.73E-03 |
| DNAJC7 | 1.19 | 4.86E-03 | miR-142-5p | -0.47 | 1.57E-03 |
| EIF4E3 | 1.27 | 2.08E-02 | miR-142-5p | -0.44 | 3.10E-03 |
| SPCS2 | 1.16 | 2.43E-02 | miR-142-5p | -0.41 | 5.83E-03 |
| TRIP11 | 1.34 | 2.98E-03 | miR-142-5p | -0.31 | 4.11E-02 |
| PCBP2 | 1.29 | 3.56E-05 | miR-142-5p | -0.54 | 2.17E-04 |
| DDHD1 | 1.21 | 1.53E-02 | miR-142-5p | -0.38 | 1.26E-02 |
| FAM107B | 1.88 | 9.34E-07 | miR-142-5p | -0.39 | 9.34E-03 |
| TIAM1 | 1.66 | 1.76E-04 | miR-142-5p | -0.33 | 3.18E-02 |
| SLC30A7 | 2.26 | 1.97E-07 | miR-142-5p | -0.33 | 3.30E-02 |
| WDR26 | 1.87 | 3.29E-06 | miR-142-5p | -0.31 | 4.20E-02 |
| CD69 | 2.17 | 1.33E-04 | miR-142-5p | -0.51 | 4.97E-04 |
| IPO7 | 1.25 | 1.83E-02 | miR-195-5p | -0.31 | 4.10E-02 |
| RAB10 | 1.45 | 1.54E-04 | miR-195-5p | -0.31 | 4.07E-02 |
| EDEM3 | 1.65 | 2.52E-06 | miR-26b-5p | -0.33 | 3.27E-02 |
| TAF11 | 1.11 | 3.75E-02 | miR-29b-3p | -0.43 | 3.86E-03 |
| ERC1 | 1.88 | 5.07E-08 | miR-29b-3p | -0.34 | 2.53E-02 |
| GNA13 | 1.40 | 1.51E-05 | miR-29b-3p | -0.37 | 1.46E-02 |
| RFX7 | 1.99 | 9.39E-07 | miR-29b-3p | -0.40 | 8.27E-03 |
| TRMT6 | 1.74 | 7.01E-07 | miR-3201 | -0.37 | 1.46E-02 |
| SLC7A1 | 1.53 | 5.75E-04 | miR-3201 | -0.39 | 1.07E-02 |
| PNRC1 | 1.54 | 1.74E-04 | miR-3201 | -0.30 | 4.97E-02 |
| *Notes*: The fold change was calculated by the division of expression levels between RA patients and controls. P value was generated from the Student’s *t*-test. The association between miRNA and mRNA was evaluated by Pearson's correlation analysis. | | | | | |
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**Table S2. Analysis for the genes negatively correlated with DEMIRs using DAVID (A) and literatures searching (B)**

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| **Table S2-A Enrichment analysis for the 141 genes negatively correlated with DEMIRs using DAVID** | | | |
| **KEGG Pathway** | **Genes** | **P Value** | **Fold Enrichment** |
| Neurotrophin signaling pathway | RPS6KA5, GAB1, SH2B3, RAP1A, RAP1B | 0.013 | 5.31 |
| Renal cell carcinoma | CUL2, GAB1, RAP1A, RAP1B | 0.014 | 7.72 |
| Endocytosis | DNM3, RAB11FIP2, RAB4A, ASAP1, RAB10, ARAP2 | 0.037 | 3.17 |

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| **Table S2-B Literatures searching for the 57 genes that have strong negative correlations (r<-0.4) with DEMIRs** | | | |
| **Target gene** | **DEMIRs** | **RA susceptibility** | **Immune/Bone-related** |
| ARID1A | miR-101-3p、miR-1184 |  | PMID:25625625、18852284 |
| SH2B3 | miR-101-3p | PMID:27744395 | PMID:21723852 |
| DDIT4 | miR-101-3p |  | PMID:21917559 |
| RAP1B | miR-101-3p |  | PMID:25092872 |
| DOT1L | miR-101-3p |  | PMID:22566624 |
| ASAP1 | miR-101-3p |  | PMID:24865276 |
| SMARCA4 | miR-101-3p |  | PMID:23321680 |
| COTL1 | miR-101-3p | PMID:19307756 |  |
| TPP1 | miR-1184 | PMID:21833529 |  |
| SPRED2 | miR-1184 | PMID:23460240 | PMID:28993690 |
| NFE2L2 | miR-142-5p |  | PMID:24311378 |
| DUSP2 | miR-142-5p |  | PMID:30458195 |

*Notes:* PMID: Pubmed ID, search website: [www.ncbi.nlm.nih.gov/pubmed/](http://www.ncbi.nlm.nih.gov/pubmed/). We search the 57 genes but only 12 genes have known immune inflammation functions.

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| **Table S3. Clinical evaluation for the 18 identified DEMIRs** | | | | | | | |
| **miRNA** | **ROC analyses** | | **Correlation with clinical variables (R(P-value))** | | | | |
| **AUC (95%CI)** | **P value** | **CRP** | **ESR** | **DAS28** | **TJC** | **SJC** |
| hsa-miR-4448 | 0.876(0.755-0.996) | <0.001 | -0.115(0.583) | 0.3(0.145) | 0.373(0.066) | 0.208(0.318) | **0.398(0.049)** |
| hsa-miR-1246 | 0.858(0.736-0.979) | <0.001 | -0.212(0.31) | -0.012(0.954) | 0.162(0.44) | 0.147(0.482) | 0.289(0.162) |
| hsa-miR-142-5p | 0.82(0.685-0.955) | <0.001 | -0.047(0.824) | -0.135(0.518) | 0.179(0.392) | 0.145(0.489) | 0.245(0.237) |
| hsa-miR-3607-5p | 0.813(0.677-0.95) | 1.00E-03 | -0.136(0.518) | -0.06(0.776) | 0.177(0.397) | 0.06(0.775) | 0.264(0.203) |
| hsa-miR-29c-3p | 0.804(0.661-0.948) | 1.00E-03 | -0.194(0.352) | -0.111(0.598) | 0.214(0.304) | 0.239(0.249) | 0.29(0.16) |
| hsa-miR-101-3p | 0.798(0.649-0.947) | 1.00E-03 | 0.157(0.455) | 0.138(0.512) | 0.354(0.083) | 0.185(0.377) | 0.372(0.067) |
| hsa-miR-1184 | 0.789(0.653-0.925) | 1.00E-03 | -0.142(0.499) | -0.124(0.555) | 0.35(0.086) | **0.459(0.021)** | 0.126(0.549) |
| hsa-miR-26b-5p | 0.767(0.609-0.924) | 3.00E-03 | **-0.425(0.034)** | 0.101(0.631) | 0.07(0.739) | 0.138(0.509) | 0.396(0.05) |
| hsa-miR-29b-3p | 0.747(0.59-0.904) | 6.00E-03 | -0.114(0.587) | 0.104(0.62) | 0.177(0.396) | 0.187(0.37) | 0.301(0.144) |
| hsa-miR-7641 | 0.747(0.599-0.894) | 6.00E-03 | -0.387(0.056) | -0.157(0.453) | 0.205(0.326) | 0.374(0.065) | 0.189(0.365) |
| hsa-miR-142-3p | 0.74(0.579-0.901) | 8.00E-03 | -0.058(0.784) | 0.097(0.645) | 0.215(0.303) | 0.296(0.151) | 0.14(0.504) |
| hsa-miR-3201 | 0.739(0.59-0.888) | 8.00E-03 | -0.305(0.138) | **-0.409(0.043)** | -0.094(0.654) | 0.073(0.727) | -0.11(0.601) |
| hsa-miR-99b-5p | 0.738(0.585-0.891) | 8.00E-03 | -0.112(0.595) | -0.039(0.854) | -0.167(0.424) | -0.196(0.348) | 0.107(0.612) |
| hsa-miR-3651 | 0.738(0.587-0.889) | 8.00E-03 | -0.203(0.331) | -0.393(0.052) | 0.016(0.939) | -0.031(0.882) | 0.115(0.583) |
| hsa-miR-4668-5p | 0.727(0.578-0.876) | 1.20E-02 | -0.18(0.388) | -0.069(0.742) | 0.371(0.068) | **0.496(0.012)** | 0.111(0.597) |
| hsa-miR-195-5p | 0.722(0.565-0.88) | 1.40E-02 | -0.126(0.55) | -0.005(0.981) | 0.05(0.813) | 0.018(0.931) | 0.172(0.412) |
| hsa-miR-8084 | 0.711(0.558-0.864) | 1.90E-02 | -0.323(0.115) | **-0.407(0.043)** | -0.16(0.444) | 0.052(0.804) | -0.136(0.518) |
| hsa-miR-3613-3p | 0.698(0.542-0.854) | 2.80E-02 | -0.007(0.975) | 0.029(0.889) | **0.423(0.035)** | **0.498(0.011)** | 0.132(0.531) |
| *Notes*: Receiver-operating characteristic (ROC) curve analysis was used to evaluate the discriminative capacity of the differentially expressed miRNAs. AUC, area under the curve; 95%CI, 95% confidence interval. Pearson’s correlation analysis was performed to evaluate the associations between miRNAs and clinical variables. R, correlation coefficient; CRP, C-reaction protein; ESR, erythrocyte sedimentation rate; DAS28, 28-joint disease activity score; TJC, Tender joint count; SJC, Swollen joint count. | | | | | | | |
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**Table S4. Analysis for miR-99b-5p target genes by expression detection in vivo and vitro (A) and enrichment analysis using DAVID (B)**

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| **Table S4-A miR-99b-5p target gene expression in PBMCs in vivo and in Jurkat T cells in vitro** | | | | | | | | | | | | |
| **Gene** | **Corresponding miRNA** | **Discovery Phase (in PBMC)** | | | | | **Validation Phase (in Jurkat cell)** | | | | **Validation Phase (in PBMC)** | |
| **Correlation coefficient** | **P value for correlation** | | **Fold change** | **P value** | **Fold change** | | **P value** | | **Fold change** | **P value** |
| mTOR | miR-99b-5p | -0.62 | 8.41E-06 | | -1.17 | 3.89E-02 | -2.26 | | 1.09E-03 | | -2.22 | 4.87E-02 |
| NLRC5 | miR-99b-5p | -0.52 | 4.08E-04 | | -1.24 | 2.57E-02 | -4.38 | | 3.41E-04 | | NA | NA |
| C2orf56 | miR-99b-5p | -0.48 | 1.10E-03 | | -1.25 | 3.79E-03 | -4.69 | | 1.02E-04 | | NA | NA |
| TRIM52 | miR-99b-5p | -0.42 | 5.51E-03 | | -1.12 | 4.41E-02 | -4.83 | | 9.24E-06 | | NA | NA |
| OGFOD2 | miR-99b-5p | -0.40 | 7.57E-03 | | -1.18 | 1.45E-02 | -1.48 | | 7.52E-02 | | NA | NA |
| PRKCZ | miR-99b-5p | -0.39 | 1.07E-02 | | -1.22 | 2.10E-04 | -3.49 | | 1.39E-03 | | NA | NA |
| RASSF4 | miR-99b-5p | -0.36 | 1.75E-02 | | -1.45 | 1.14E-02 | -1.59 | | 1.66E-02 | | -1.82 | 2.18E-03 |
| ZNF226 | miR-99b-5p | -0.44 | 3.32E-03 | | -1.18 | 6.86E-04 | NA | | NA | | NA | NA |
| LRCH4 | miR-99b-5p | -0.36 | 1.93E-02 | | -1.12 | 3.64E-02 | NA | | NA | | NA | NA |
| CTBP2 | miR-99b-5p | -0.32 | 3.45E-02 | | -1.52 | 4.85E-05 | NA | | NA | | NA | NA |
| *Notes*: Two targets (mTOR, RASSF4) were subjected to validations in independent sample by qRT-PCR. Among all the miR-99b-5p target genes, mTOR was the strongest correlated one, and RASSF4 presented the largest fold change of differential expression in discovery phase. Primers for the last three target genes failed to work. NA: not available. | | | | | | | | | | | | |
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| **Table S4-B Enrichment analysis for the target genes of miR-99b-5p using DAVID** | | | | | | | | | | | | |
| **KEGG Pathway** | | | | **Genes** | | | | **P-Value** | | **Fold Enrichment** | | |
| Type II diabetes mellitus | | | | PRKCZ, mTOR | | | | 0.014 | | 95.54 | | |
| Insulin resistance | | | | PRKCZ, mTOR | | | | 0.031 | | 42.46 | | |
| Insulin signaling pathway | | | | PRKCZ, mTOR | | | | 0.040 | | 33.23 | | |

**Table S5. Correlations between inflammatory cytokines and targets of miR-99b-5p**

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| --- | --- | --- | --- | --- | --- |
|  | | **In Jurkat** | | **In PBMC** | |
| **R** | **P value** | **R** | **P value** |
| mTOR  RASSF4 | IL-2 | **-0.878** | **0.021** | NA | NA |
| IL-6 | -0.711 | 0.113 | -0.251 | 0.104 |
| TNFα | -0.024 | 0.964 | 0.104 | 0.507 |
| IFNΓ  IL-2 | -0.806  **-0.852** | 0.053  **0.031** | **0.328**  NA | **0.032**  NA |
| IL-6 | **-0.914** | **0.011** | **-0.471** | **0.001** |
| TNFα | -0.042 | 0.937 | -0.136 | 0.385 |
| IFNΓ | -0.628 | 0.182 | 0.235 | 0.129 |

*Notes*: R, correlation coefficient; NA, the gene expression of IL-2 was not been detected in microarray due to the lack of probes.

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| **Table S6. Basic characteristics of the female study subjects** | | | | | | |
|  | **Discovery Phase** | | | **Validation Phase** | | |
| **Cases** | **Controls** | **P value** | **Cases** | **Controls** | **P value** |
| Sample size (n) | 25 | 18 | - | 35 | 35 | - |
| Age (year) | 45.60±9.84 | 47.11±14.09 | 0.68 | 46.44±10.99 | 47.57±13.99 | 0.75 |
| BMI (kg/m2) | 22.07±3.31 | 22.32±2.79 | 0.81 | 22.24±3.67 | 22.32±2.79 | 0.94 |
| DAS28 | 4.46±0.99 | NA | - | 5.08±1.32 | NA | - |
| CRP (mg/L) | 13.51±16.74 | NA | - | 18.37±31.17 | NA | - |
| ESR (mm/h） | 42.61±27.50 | NA | - | 48.29±29.46 | NA | - |
| TJC | 8.64±6.49 | NA | - | 10±7.70 | NA | - |
| SJC | 5.48±3.83 | NA | - | 6.79±5.43 | NA | - |
| *Notes*: Variables were expressed as the mean±standard deviation. P-value was from the result of two-sided Student’s *t*-test between active RA cases and healthy controls. BMI, body mass index; DAS28, 28-joint Disease Activity Score; CRP, C reactive protein; ESR, equivalent series resistance; TJC, tender joint count; SJC, swollen joint count; NA, not applicable. | | | | | | |
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| **Table S7. Primer information** | | |
|  | **Sense primers** | **Antisense primers** |
| miR-99b-5p (lenti) | forward: TCTAGAGAAGCCAGGTTTTCTATCAG | reverse: GAATTCTCCTCCTCAACTATACAACC |
| miR-99b-5p inhibitor | forward: CGCAAGGUCGGUUCUACGGGUG |  |
| miR-99b-5p (qPCR) | forward: CACCCGTAGAACCGACCTT |  |
| U6 (qPCR) | forward: CTCGCTTCGGCAGCACA | reverse: AACGCTTCACGAATTTGCGT |
| IL-1β | forward:GCTTATTACAGTGGCAATGAGG | reverse:AGATTCGTAGCTGGATGCC |
| IL-2 | forward:CATTGCACTAAGTCTTGCACTTGTCA | reverse:CGTTGATATTGCTGATTAAGTCCCTG |
| IL-4 | forward:CGGCAACTTTGACCACGGACACAAGTGCGATA | reverse:ACGTACTCTGGTTGGCTTCCTTCACAGGACAG |
| IL-6 | forward:GATTCAATGAGGAGACTTGCC | reverse:TGTTCTGGAGGTACTCTAGGT |
| IL-8 | forward:ATGACTTCCAAGCTGGCCGTGGCT | reverse:TCTCAGCCCTCTTCAAAAACTTCTC |
| TNF-α | forward:GGCTCCAGGCGGTGCTTGTTC | reverse:AGACGGCGATGCGGCTGATG |
| IFN-γ | forward:GCATCGTTTTGGGTTCTCTTGGCTGTTACTGC | reverse:CTCCTTTTTCGCTTCCCTGTTTTAGCTGCTGG |
| mTOR | forward:TTCATTCTTTCATTGGAGACGG | reverse:CTCGAACCCTGTTAATAATCTGG |
| RASSF4 | forward:CACTTCTACAATCATAAGACCTCC | reverse:TTGTCATGGTGCTGTTGAC |
| PRKCZ | forward:CTTTAACAGGAGAGCGTACTG | reverse:CAGTTGATGCACCTGTAGC |
| TRIM52 | forward:GACAAAGAGGCCATCTGTG | reverse:CAGTTATGCCTGGTACTCCT |
| C2orf56 | forward:AGAAGCCTTCATACAACATGAC | reverse:GAAAGTTCCTCGATGATAACACC |
| NLRC5 | forward:CTGTACCTGCTGGAGACAC | reverse:ACTCACTTAGCCTGAGTGTC |
| OGFOD2 | forward:ACAGTATCGGAGGAGAAGC | reverse:AGTTGTTCATGGTGTTGGG |
| GAPDH | forward:GAAGGTGAAGGTCGGAGT | reverse:CTTCTACCACTACCCTAAAG |
| RASSF4 WT-3'UTR | forward:GGGTTTAAACAGGAATGCTGCTGGGAAAAG | reverse:GCTCTAGAGCACACCTTTATTGAGCACC |
| RASSF4 MT-3'UTR | forward:GAAGCTCCAGTAATCCTGCCCATCAAGAGCATAGCTTGGAAGCCACCA | reverse:CTTGATGGGCAGGATTACTGGAGCTTCTACATGCATTAGAGATGG |
| *Notes*: miR-99b-5p (lenti) was used in the PCR amplification for the miRNA fragment. miR-99b-5p (qPCR) and U6 (qPCR) were used for the validation of miRNA expression. RASSF4 WT-3'UTR and RASSF4 MT-3'UTR were used in the PCR amplification for the fragment of the wide-type (WT) and mutant-type (MT) 3' untranslated region (3'UTR) of RASSF4. | | |
|