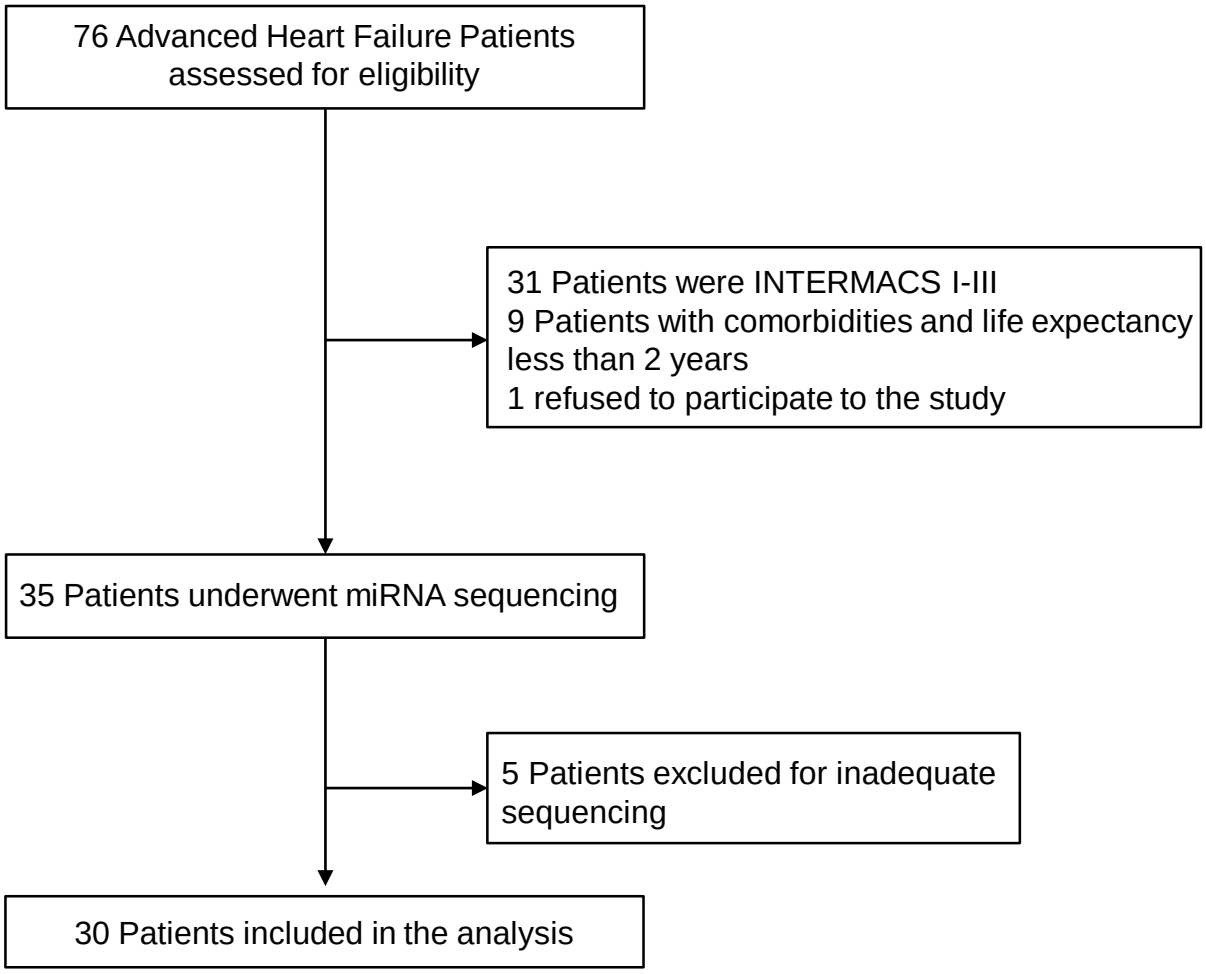
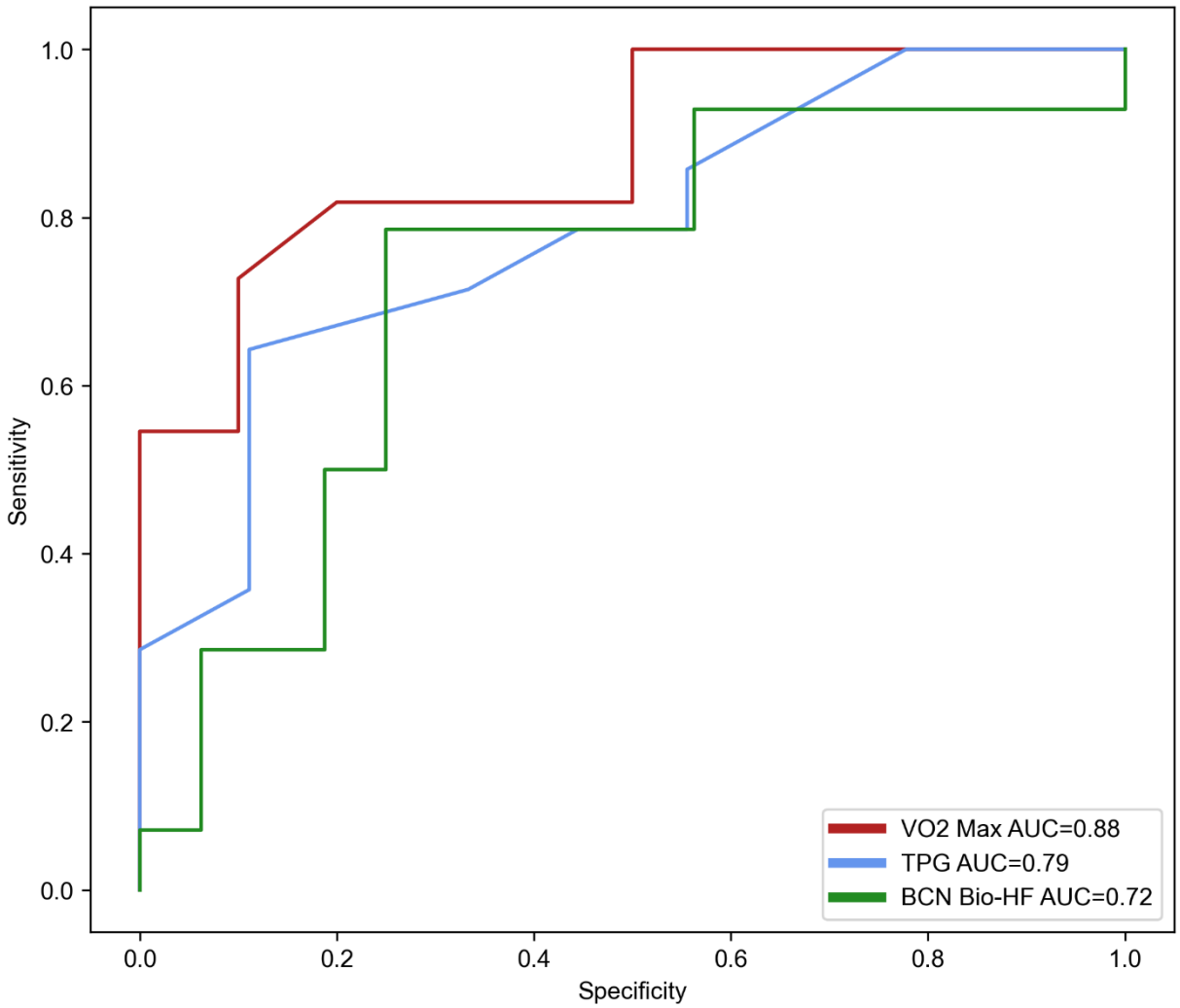
Supplementary Material

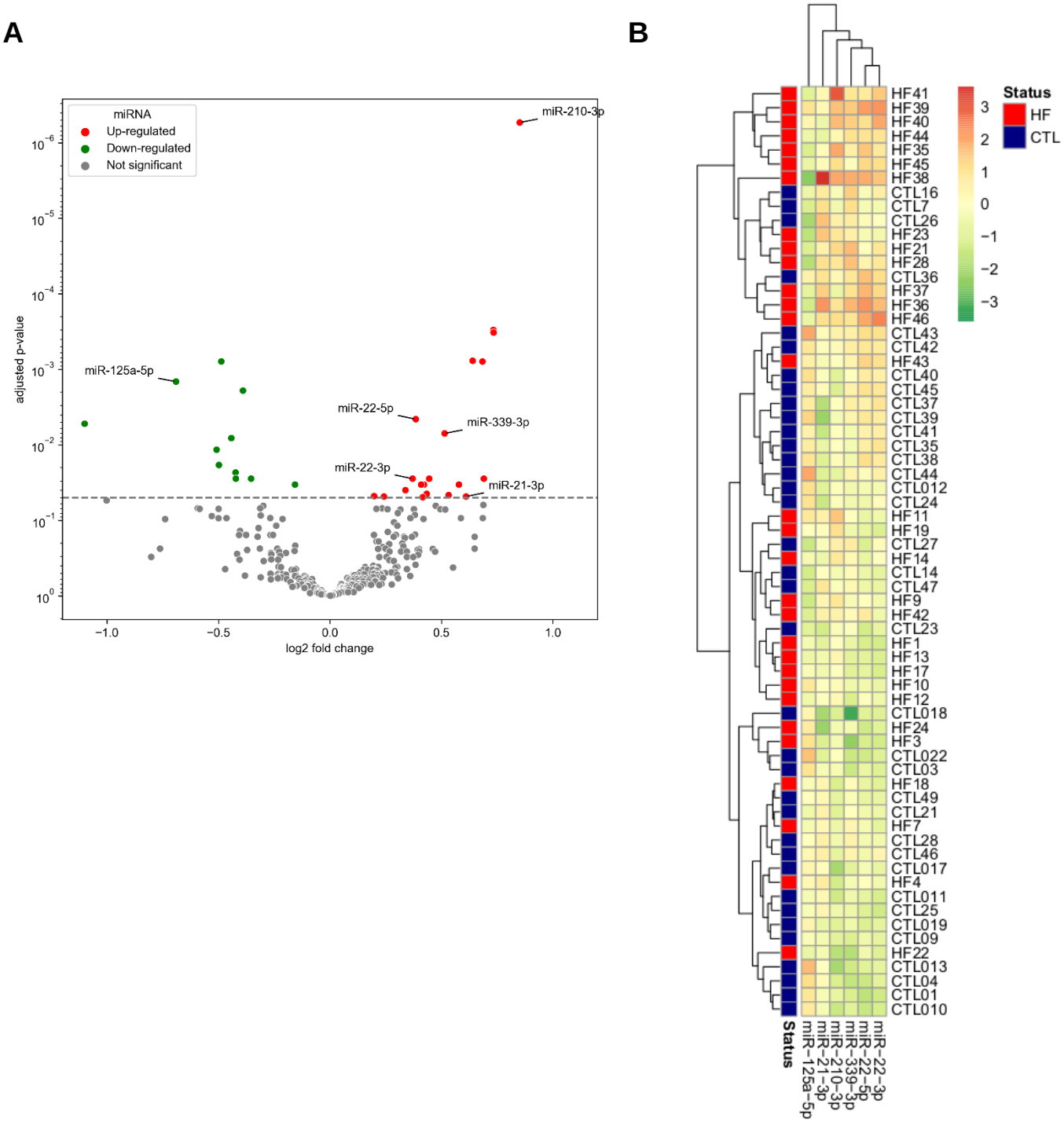
The supplementary material contains:
- Supplementary Figures and Legends (Supplementary Figure S1, S2, S3, S4 and S5)
- Supplementary Methods



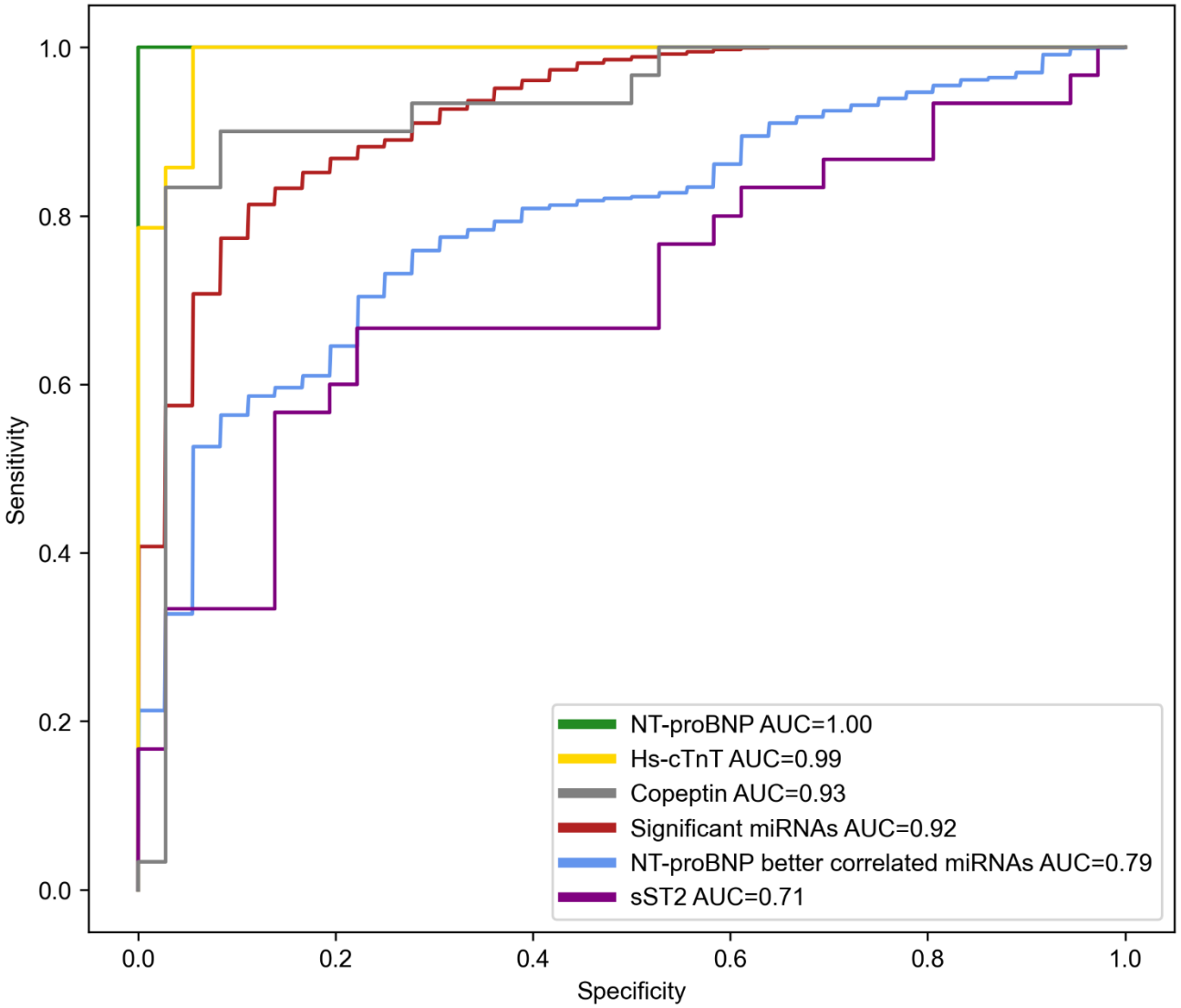
Supplementary Figure S1. Workflow of the study.



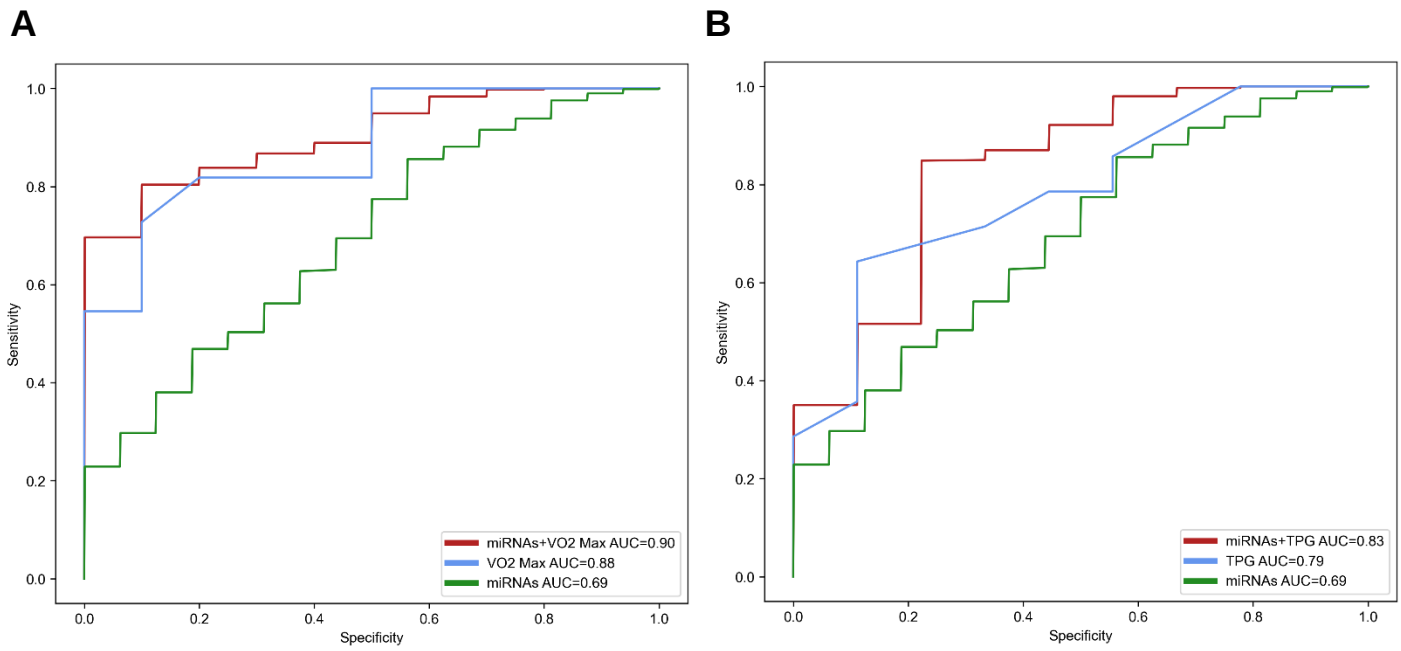
Supplementary Figure S2. Receiver operating characteristic (ROC) curves built for the main clinical and laboratory variables that resulted differentially distributed among patients encountering primary endpoint (HF-PE group) and those without events (HF-noPE group). TPG: trans-pulmonary gradient. The values of area under the curve (AUC) were reported.



Supplementary Figure S3. (A) Volcano Plot reporting all miRNAs differentially expressed between cases and controls (red: up-regulated, green: down-regulated). The six miRNAs better correlated with NT-proBNP are reported. (B) Heatmap reporting the six miRNAs better correlated with NT-proBNP and significantly differentially expressed between cases (HF, red) and controls (CTL, blue).



Supplementary Figure S4. Receiver operating characteristic (ROC) curves built for HF biomarkers (NT-proBNP, Hs-cTNT, Copeptin, and sST2) that resulted differentially distributed among cases and controls, for 32 differentially expressed miRNAs between cases and controls, and 6 miRNAs better correlated with NT-proBNP and significantly differentially expressed between cases and controls. The values of area under the curve (AUC) were reported.



Supplementary Figure S5. Receiver operating characteristic (ROC) curves built for the differentially expressed miRNAs among patients encountering primary endpoint (HF-PE group) and those without events (HF-noPE group), the 3 differentially expressed miRNAs alone or together with (A) VO2 max or (B) TPG. TPG: trans-pulmonary gradient. The values of area under the curve (AUC) were reported.

Supplementary Methods

Clinical and hemodynamic assessment

Echocardiography was performed using Philips i33 (Philips Medical Systems, Andover, MA, USA). Left ventricular (LV) chamber size and systo-diastolic function (with ejection fraction (EF) evaluation and E/E' ratio) were measured. Aortic and mitral regurgitation was assessed using color Doppler. Basal right ventricular (RV) end-diastolic diameter and tricuspid annular plane systolic excursion (TAPSE) were measured. Trans-tricuspid systolic gradient (TR gradient) and systolic pulmonary artery pressure (sPAP) were estimated by tricuspid regurgitation peak velocity (TRV) and right atrial pressure.

Hemodynamic data of right heart catheterization (RHC) were reported when available: cardiac output (CO) according to the thermo-dilution or Fick technique in case of severe tricuspid insufficiency, cardiac index (CI), Pulmonary Capillary Wedge Pressure (PCWP), Pulmonary Arterial Pressure (PAP), right atrial pressure (RAP), and Pulmonary Vascular Resistance (PVR). Pulmonary Artery Pulsatility index was computed as (systolic PAP – diastolic PAP)/RAP.

Next Generation Sequencing miRNAs profile

Library preparation was performed with the NEBNext Multiplex Small RNA Library Prep Set for Illumina (Cat No. E7300, New England BioLabs, Ipswich, Massachusetts, USA). For each library, 6 μL of RNA were used in all the experimental procedures as starting material. Each library was prepared with a unique indexed primer so that the libraries could all be pooled into one sequencing lane. Multiplex adaptor ligations, reverse transcription primer hybridization, reverse transcription reaction and PCR amplification were performed according to the protocol for library preparation (Protocol E7330, New England BioLabs). After PCR amplification, the cDNA constructs were purified with the QIAQuick PCR Purification Kit (Cat No. 28104, Qiagen) following the modifications suggested by the NEBNext Multiplex Small RNA Library Prep Protocol and loaded on

the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, California, USA) using the DNA High Sensitivity Kit (Agilent Technologies) according to the manufacturer's protocol. Libraries were pooled together (24-plex) and further purified with a gel size selection. A concluding Bioanalyzer 2100 run with the High Sensitivity DNA Kit (Agilent Technologies) allowed the evaluation of size, purity and concentration of the pooled DNA libraries.

The obtained sequence libraries were subjected to the Illumina sequencing pipeline, passing through clonal cluster generation on a single-read flow cell (Illumina, San Diego, California, USA) by bridge amplification and 75 cycles sequencing-by-synthesis on the NextSeq550 (Illumina).

Real-Time PCR analysis

Circulating total RNA was extracted from 200 µl of plasma using the Mirneasy serum/plasma advanced kit (Cat No. 17204 Qiagen) following manufacturer's instructions. To improve RNA isolation efficiency, MS2 carrier (Cat. No**.** 10165948001 Roche) was added to each sample before lysis step. The C. Elegans cel-miR-39-3p miR mimic spike-in (Cat No./ID: 219610, Qiagen) was added as exogenous control. RNA elution was performed in 30 µl of nuclease free water. Four µl of total RNA was used for the reverse transcription performed by miRCURY LNA RT kit (Cat No. 339340, Qiagen). Quantitative PCR assay was performed on ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) using the mastermix miRCURY LNA SYBR Green PCR Kit (Cat No. 339345, Qiagen) and the primers miRCURY LNA miRNA PCR Assay (specific *per* each selected miRNAs, Cat No. 339306, Qiagen). PCR reactions for each sample were performed in triplicate and normalized to miR-Cel-39 gene expression.

Computational and statistical analyses

Raw reads adapter clipping was performed with the Cutadapt software (version 1.18) [Martin M et al. 2011]. Reads longer than 14 nucleotides were mapped to a sncRNA (small non-coding RNAs) reference with the bwa alignment software (version 0.7.17-r1188) [Li H et al. *Bioinformatics* 2012;

28: 1838-1844], using the mem algorithm and a seed length of 10. Only alignments without mismatches or indels were considered and those with the highest quality were used to assign each read to a unique sncRNA. Thus, sncRNAs were quantified for each sample and then merged into a single count matrix, setting missing sncRNAs to zero. sncRNAs whose counts were less than 20 in more than half of the samples were discarded. Differential expression analysis was performed with the DESeq2 Bioconductor's package (version 1.22.2) [Love MI et al. *Genome Biology* 2014; 15: 550]. For each model, samples with missing covariates were dropped. sncRNAs were considered significantly associated with a condition or a trend if their p-value, after adjustment for multiple testing by FDR, was below the 0.05 threshold.