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Original Article

A set of miRNAs predicts T2DM remission in patients with coronary heart disease: from the CORDIOPREV study

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MicroRNAs (miRNAs) regulate the expression of genes associated with the development of diseases, including type 2 diabetes mellitus (T2DM). However, the use of miRNAs to predict T2DM remission has been poorly studied. Therefore, we aimed to investigate whether circulating miRNAs could be used to predict the probability of T2DM remission in patients with coronary heart disease. We included the newly diagnosed T2DM (n = 190) of the 1,002 patients from the CORDIOPREV study. Seventy-three patients reverted from T2DM after 5 years of dietary intervention with a low-fat or Mediterranean diet. Plasma levels of 56 miRNAs were measured by OpenArray. Generalized linear model, receiver operating characteristic (ROC), Cox regression, and pathway analyses were performed. ROC analysis based on clinical variables showed an area under the curve (AUC) of 0.66. After a linear regression analysis, seven miRNAs were identified as the most important variables in the group's differentiation. The addition of these miRNAs to clinical variables showed an AUC of 0.79. Cox regression analysis using a T2DM remission score including miRNAs showed that high-score patients have a higher probability of T2DM remission (hazard ratio $[HR]_{low\ versus\ high}$, 4.44). Finally, 26 genes involved in 10 pathways were related to the miRNAs. We have identified miRNAs (hsa-let-7b, hsa-miR-101, hsa-miR-130b-3p, hsa-miR-27a, hsa-miR-30a-5p, hsa-miR-375, and hsa-miR-486) that contribute to the prediction of T2DM remission in patients with coronary heart disease.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most severe health problems worldwide.[1] High blood pressure, dyslipidemia, alcohol intake, and a sedentary lifestyle are risk factors of T2DM development. These factors are directly associated with overweight and obesity characterized by low-grade systemic inflammation and oxidative stress, which damages blood vessels and leads to endothelial dysfunction, thus increasing the risk of heart disease or stroke.[2] Patients with acute myocardial infarction (AMI) and T2DM have a higher risk of developing a new cardiovascular event than do those without T2DM.[3]

With this background, the question arises whether disease remission is possible in patients diagnosed with T2DM. The American Diabetes Association (ADA) has defined the term "remission" as "… achieving glycemia below the diabetic range in the absence of active pharmacologic or surgical therapy."[4] T2DM remission has been associated with the weight loss achieved by bariatric surgery, and the Swedish Obese Subjects (SOS) project showed remission rates of 72.3% after 2 years and 30.4% after 5 years of bariatric surgery.[5] Additionally, weight loss has also been achieved through caloric restriction. The Diabetes Remission Clinical Trial (DiRECT), which includes the delivery by a primary care nurse or dietitian of a low-calorie diet replacement (<800 kcal/day), during 12–20 weeks, showed weight loss averaging 15 kg, a decrease in fasting plasma glucose from 148.9 ± 6.8 to 102.2 ± 2.2 mg/dL, and a 1.5-fold decrease in hemoglobin A1c (HbA1c) to 5.9% ± 0.1%.[6,7] Finally, intervention with physical exercise has resulted in an average weight loss of 9 kg and a decrease in HbA1c from 6.8 to 6.2.[8]

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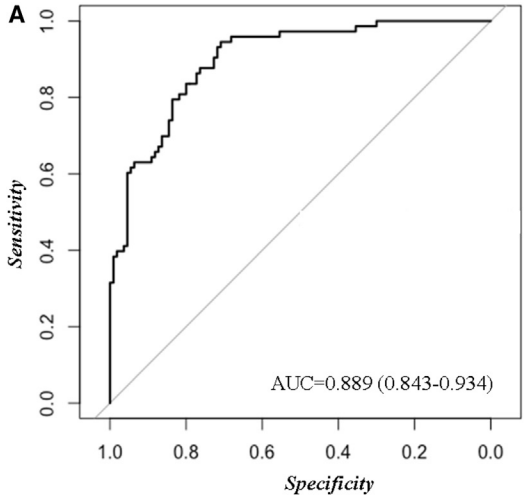

B

miRNA	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*	*Sig.*	*q-value*
hsa-let7b	-1.0399	0.4067	-2.557	0.0106	*	0.0997
hsa-miR-101	1.4592	0.6228	2.343	0.0191	*	0.1098
hsa-miR-130b-3p	2.5509	0.6896	3.699	0.0002	***	0.0092
hsa-miR-27a	-2.0242	0.8066	-2.509	0.0121	*	0.0795
hsa-miR-30a-5p	-1.9770	0.7790	-2.538	0.0112	*	0.1030
hsa-miR-375	1.0920	0.4351	2.510	0.0121	*	0.0928
hsa-miR-486	2.4616	0.7017	3.508	0.0005	***	0.0115

Figure 1. Remission of T2DM assessed by a ROC curve model including 45 miRNAs

(A and B) The ROC analysis was carried out using R software through the glm function (fitting generalized linear models) and pROC libraries, including 45 miRNAs in the model: (A) ROC curve model and (B) summary table with the Pr(>|z|) values of each variable within the model. *p < 0.05, ***p < 0.01. Multiple comparisons in the large-scale analyses were assessed by false discovery rate (FDR) using the Benjamini and Hochberg method (Q value).

T2DM remission involves genetic and epigenetic (DNA methylation and microRNAs [miRNAs]) regulation of several pathways, including those involved in lipid and glucose metabolism.[9] Specifically, previous studies have proposed using miRNAs as biomarkers to evaluate the risk of T2DM development.[10–13] Based on this evidence, we aimed to identify whether circulating miRNAs could also predict T2DM remission after 5 years of dietary intervention with a low-fat or Mediterranean (Med) diet in patients with coronary heart disease (CHD).

RESULTS

Baseline characteristics of the study population

After analyzing the baseline characteristics of the patients included in the study, we observed that body weight, body mass index (BMI), waist circumference, HbA1c, glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and the hepatic insulin resistance index (HIRI) were higher in the non-responders than in responders. In contrast, the insulin sensitivity index (ISI) and disposition index (DI) were lower (Table S1). No differences between diets were observed in the T2DM remission (Table S2). Moreover, the adherence of both diets, that is, low-fat and Mediterranean diets, increased significantly and in a very similar way after a 5-year follow-up period in the two biological groups (responders and non-responders) (Table S3). Despite the baseline differences in body weight between responders and non-responders, these differences were maintained during the dietary intervention and no relevant changes were observed (Table S4).

Receiver operating characteristic curve analysis

Of the 56 miRNAs selected for the study, 9 did not amplify in at least 80% of the samples and 2 (hsa-miR-143 and hsa-miR-144) were used for data normalization, as previously described by our group.[10,11]

With the remaining 45 miRNAs we carried out a receiver operating characteristic (ROC) curve analysis, getting an area under curve (AUC) of 0.89 (95% confidence interval [CI], 0.84–0.93; sensitivity, 0.85; specificity, 0.77; accuracy, 0.80). Additionally, based on the estimate SE z, or Pr(>|z|), values registered in the summary of the fitting generalized linear models (glm) function, hsa-let-7b, hsa-miR-101, hsa-miR-130b-3p, hsa-miR-27a, hsa-miR-30a-5p, hsa-miR-375, and hsa-miR-486 were identified as the most important miRNAs in the group's differentiation (responders versus non-responders) (Figure 1; Table S5).

Next, based on the clinical variables used to evaluate the probability of T2DM remission in diabetic patients, we performed a ROC curve analysis, including body mass index, age, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs). Our results showed an AUC of 0.66 (95% CI, 0.59–0.74; sensitivity, 0.79; specificity, 0.47; accuracy, 0.60) (Figure 2A). To improve this model, we carried out an ROC analysis based on the seven miRNAs identified above (hsa-let-7b, hsa-miR-101, hsa-miR-130b-3p, hsa-miR-27a, hsa-miR-30a-5p, hsa-miR-375, and hsa-miR-486) added to the clinical variables (BMI, age, HDL-C, and triglycerides). Our results showed an AUC of 0.79 (95% CI, 0.70–0.84; sensitivity, 0.78; specificity, 0.64; accuracy, 0.70) (Figure 2B). A DeLong test between the model based on miRNAs added to the clinical variables and the model based only on the clinical variables showed a Z value = −3.0272 and a p value = 0.0025.

Additionally, to improve the models analyzed so far, we carried out three additional ROC curve analyses based on miRNAs, β cell function indexes, and insulin resistance indexes. According to the AUC of the models, no model was observed to have a higher capacity to differentiate between groups (Table 1).

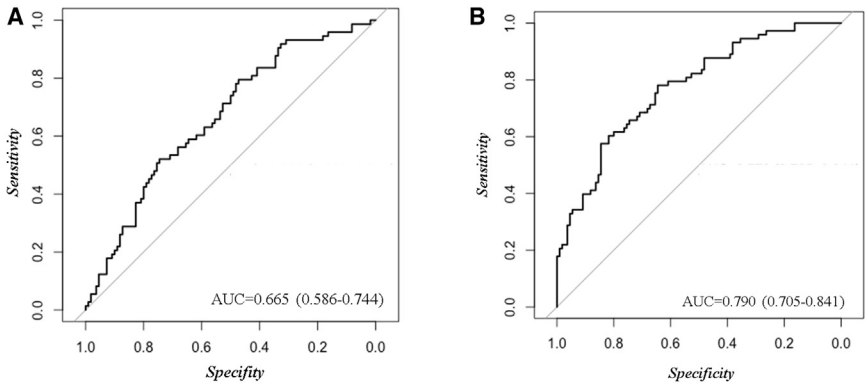

Figure 2. Remission of T2DM assessed by ROC curve models based on clinical variables and miRNAs

The ROC analysis was carried out using R software through the glm function and pROC libraries. (A) Model based on clinical variables, including body mass index (BMI), age, HDL-C, and triglycerides. (B) Model based on the seven miRNAs (hsa-let-7b, hsa-miR-101, hsa-miR-130b-3p, hsa-miR-27a, hsa-miR-30a-5p, hsa-miR375, and hsa-miR-486) added to clinical variables.

T2DM remission score based on miRNAs

In order to evaluate the probability of remission, a T2DM remission score based on miRNAs was calculated. Next, we classified the population according to tertiles of the score and carried out a Cox regression analysis using as a reference the tertile with a low probability of T2DM remission (tertile 1 = low score). Finally, the hazard ratios (HRs) of the analysis were assessed. Our results showed $HR_{T1\ versus\ T2}$ of 2.615 (95% CI, 1.284–5.326) and $HR_{T1\ versus\ T3}$ of 4.440 (95% CI, 2.246–8.776), where T1, T2, and T3 indicate T2DM remission scores (from low to high) (Figure 3).

Pathways regulated by the miRNAs studied and target genes in each pathway

Considering that the miRNAs included in the present study were selected according to their relationship with insulin signaling and β cell function, we analyzed the relationship between the biological processes in which the set of seven miRNAs are involved.

We identified 10 T2DM-related pathways regulated by seven or at least six of the miRNAs studied (hsa-let-7b, hsa-miR-101, hsa-miR-130b-3p, hsa-miR-27a, hsa-miR-30a-5p, hsa-miR-375, and hsa-miR-486) (Table 2).

Additionally, in the analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and TarBase v8 software, a database of experimentally supported miRNA-gene interactions, the results suggested that in the 10 T2DM-related pathways, hsa-let-7b regulated the expression of 16 genes, hsa-miR-101 regulated 87 genes, hsa-miR-130b-3p regulated 44 genes, hsa-miR-27a regulated 131 genes, hsa-miR-30a-5p regulated 80 genes, hsa-miR-375 regulated 50 genes, and hsa-miR-486 regulate 19 genes. In summary, 26 genes were identified as the most significant genes regulated by these seven miRNAs (Table 3; Figures S1–S10). These genes are involved in biological processes related with T2DM, such as apoptosis, cell cycle, ubiquitin-mediated proteolysis, activation of inflammatory cytokines, oxidative stress and DNA repair, inhibition of angiogenesis and metastasis, microtubule organization, lipolysis and lipid biosynthesis, iron metabolism, and glycolysis and gluconeogenesis (Figures S1–S10).

DISCUSSION

Our study showed that the circulating plasma levels of 7 out of a selected set of 45 miRNAs measured in 183 diabetic subjects at the baseline of the CORDIOPREV study were associated with the probability of T2DM remission. These seven miRNAs when added to clinical variables (BMI, age, HDL-C, and triglycerides) were able to differentiate between patients continuing as diabetics (non-responders) and those who reverted from T2DM during the 5 years of follow-up (responders), with an AUC in the ROC model of 0.79. Additionally, through a T2DM remission score based on miRNAs, the Cox regression analysis showed that subjects with a high score (T3) have a higher probability of T2DM remission ($HR_{low\ versus\ high}$, 4.44). Finally, 26 genes regulated by the largest number of miRNAs and involved in biological processes associated with T2DM were identified.

T2DM is one of the leading causes of death globally and is a risk factor for other diseases such as cardiovascular diseases. The etiology of T2DM is complex and is associated with irreversible risk factors such as age, genetics, race, and ethnicity, and reversible factors such as smoking, physical activity, and diet. The common basis of T2DM is overweight and obesity. It has been recognized that the processes underlying T2DM can be reversed and its remission can be achieved.[14] T2DM remission is defined as the state in which hyperglycemia decreases to levels below the thresholds for diabetes, according to the ADA. Previous studies based on bariatric surgery, caloric restriction diets (medium, low, and very low of total energy), and physical exercise demonstrated that remission of T2DM is associated with weight loss.

This evidence suggests that it is important for early identification of diabetic patients with a probability of achieving T2DM remission, in order to take personalized, effective, and efficient therapeutic action focused on disease remission. In terms of predicting T2DM remission, a variety of scores and variables have been used to identify subjects with the probability of remission. In a study by Still et al.,[15] evaluating 259 clinical variables, four parameters were identified (insulin, age, HbA1c, and type of antidiabetic medication) and the DiaRem score was suggested. This score identified 22% of the partially

Table 1. T2DM remission models performed in our study by ROC curve analysis

ROC model	AUC	95% CI	Sensitivity	Specificity	Accuracy	Threshold
45 miRNAs	0.889	0.843–0.934	0.8493	0.7727	0.8033	0.3624
7 miRNAs	0.717	0.644–0.791	0.8767	0.4727	0.6339	0.3313
Clinical variables (age, BMI, HDL-C, triglycerides)	0.665	0.586–0.744	0.7945	0.4727	0.6011	0.3484
7 miRNAs + clinical variables	0.790	0.705–0.841	0.7808	0.6455	0.6995	0.3768
Indexes (ISI, HOMA-IR, HIRI, IGI, DI)	0.716	0.638–0.793	0.6857	0.6606	0.6704	0.3492
7 miRNAs + indexes	0.770	0.712–0.849	0.6571	0.7706	0.7263	0.4082

The models were carried out using R softwar through the glm (fitting generalized linear models) and pROC libraries. The models included clinical variables (BMI, sex, HDL-C, and triglycerides), seven miRNAs (let-7b, miR-101, miR-130b-3p, miR-27a, miR-30a-5p, miR-375, and miR-486), and β cell function and insulin resistance indexes (ISI, HOMA-IR, HIRI, IGI, and DI).

remitted patients and 78% with total remission. Additionally, previous studies by Lee et al.[16] showed, through the application of the ABCD score, which included age, BMI, C-peptide level, and duration of T2DM (years), 25% of patients with complete remission and 23.8% with partial remission were identified. However, all of these variables and scores were designed to predict T2DM remission in the preoperative stages of bariatric surgery and Roux-en-Y gastric bypass to ensure successful surgery and remission. Another recent primary care study in an Egyptian population included 63 patients who gained non-surgical remission from T2DM and 396 patients who served as a control, matched for age, sex, and BMI, all of whom received standards of care according to the updated guidelines. Different variables were analyzed independently by ROC curves, observing that the highest sensitivity was achieved with a short duration of T2DM (85%) and the highest specificity with HDL-C (69%).[17]

In the field of precision medicine, there are no previous studies that have focused on the use of epigenetic markers such as miRNAs to evaluate the probability of T2DM remission, or which are based on dietary intervention in patients with coronary heart disease. However, recent studies have demonstrated significant alterations in the transcriptome after bariatric surgery. Specifically, in the differential expression of miRNAs, Zhu et al.[18] observed in 12 patients with T2DM after 12 months of bariatric surgery the downregulation of *miR-29a-3p*, *miR-122-5p*, *miR-124-3p*, and *miR-320a* from peripheral blood mononuclear cells. The deregulation of circulating miR-7-5p, let-7f-5p, miR-15b-5p, let-7i-5p, miR-320c, miR-205-5p, and miR-335-5p was also observed in 29 patients with T2DM after 21 days of surgery.[19] A meta-analysis identified 13 studies in humans with study periods after surgery of 3, 6, 12, and up to 24 months, in which changes in the expression of miR-93-5p, miR-106b-5p, let-7b-5p, let-7i-5p, miR-16-5p, miR-19b-3p, miR-92a-3p, miR-222-3p, miR-142-3p, miR-140-5p, and miR-155-5p were observed.[20]

In our study, a dietary intervention study in patients with coronary heart disease, the results showed a ROC curve model based on BMI, age, HDL-C, and triglycerides, with an AUC of 0.66. The addition of let-7b, miR-101, miR-130b-3p, miR-27a, miR-30a-5p, miR-375, and miR-486 to the clinical variables improved the model by approximately 20% (19.6%) (AUC of 0.79), supported by the DeLong test where the p value was statistically significant between the two models ($p = 0.0025$). In addition, through a T2DM remission score based on these seven miRNAs, we observed that patients with a high score had a higher probability of T2DM remission than did those with a low score ($HR_{low\ versus\ high}$, 4.44). These results demonstrate that the use of epigenetic markers, such as miRNAs, allows us to assess the probability of T2DM remission during a period of dietary intervention with two healthy diets (low-fat diet and Mediterranean diet) in patients with coronary heart disease.

T2DM remission implies changes at different levels, including decreased liver fat content, the concentration of blood lipid molecules (very-low-density lipoprotein [VLDL], triglycerides, and HDL-C), plasma glucose levels, and increased insulin secretion in the first phase of remission, which suggests recovery of β cell functionality.[7] All of these biological processes involve regulating biochemical and metabolic mechanisms such as fatty acid synthesis and metabolism, glucose metabolism, insulin signaling and synthesis, and cell proliferation, among others. Previous studies have shown that after bariatric surgery, the deregulation of miRNAs was associated with biochemical and metabolic mechanisms such as lipid metabolism, insulin secretion, β cell function, and insulin resistance.[18–20] Our results showed that the seven miRNAs included in the T2DM remission score primarily regulate the expression of 26 genes involved in 10 biochemical and metabolic pathways, including insulin, phosphatidylinositol 3-kinase (PI3K)-Akt, AMP-activated protein kinase (AMPK), p53, fatty acid biosynthesis, tumor necrosis factor (TNF), protein processing in the endoplasmic reticulum, FoxO, mTOR, and HIF-1 signaling pathways. Deregulation by miRNAs of these genes leads to changes in biological processes such as activation of inflammatory cytokines, apoptosis, oxidative stress, lipid metabolism, and glycolysis and gluconeogenesis, which are related to T2DM.

The *NRF2* and *TSP1* genes have been associated with inflammation. The first gene decreases cytokine stress in the β cell, improving insulin synthesis, and the other acts at the level of adipose tissue by regulating pro-inflammatory molecules and insulin resistance.[21,22] The *NRF2* gene is the central point and primary regulator in conditions of oxidative stress. In this sense, the *MnSOD* gene actively participates in the elimination of reactive oxygen species and has been associated with an

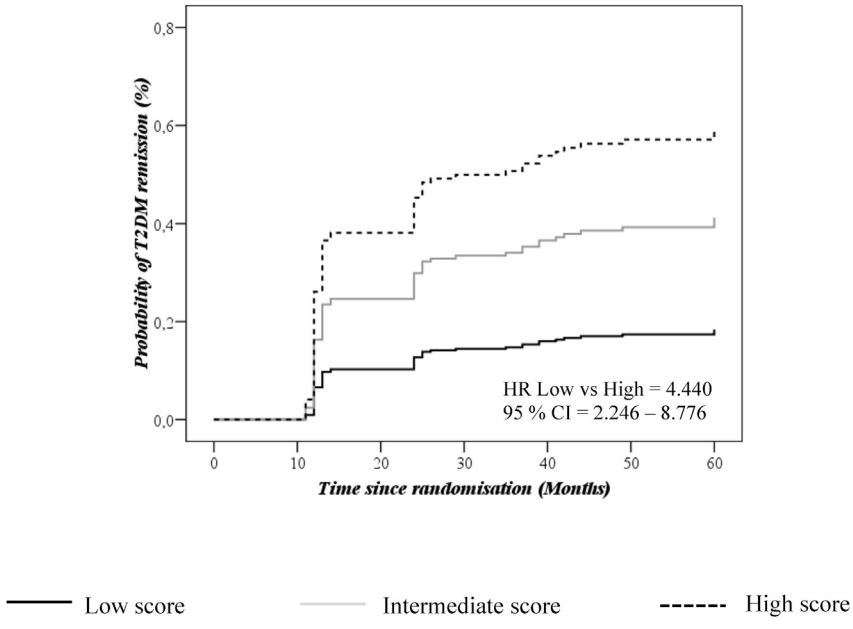

Figure 3. Probability of disease remission analyzed by a T2DM remission score based on seven miRNAs

The analysis was performed using a Cox regression curve by tertiles of the T2DM remission score and adjusted by BMI, sex, diet, age, HDL-C, triglycerides. and statins. Continuous black line indicates low score (T1), continuous gray line indicates medium score (T2), and black dotted line indicates high score (T3). The analysis was carried out using SPSS (now PASW Statistic for Windows, version 21) (IBM, Chicago, IL, USA).

increase in triglyceride levels.[23,24] The genes *Bim*, *p21*, and *CASP3* have shown antiapoptotic activity at the β cell level.[25–27] The *GSK3* gene is involved in glycogen synthesis and is a key target for the development of new therapies for T2DM.[28] The *PTP1B* and *p38* genes regulate the synthesis and subsequent insulin sensitivity, decreasing fasting glucose levels.[29,30] The *CCND1* gene activates β cell proliferation and is highly expressed in pancreatic islets.[31] Finally, an inverse relationship between *FASN* levels and lower insulin resistance has been observed in adipose tissue.[32] In summary, our *in silico* analysis suggests that the miRNAs included in the T2DM remission score regulate the expression of genes directly involved in the biological process associated with the disease.

The results of our study open new avenues for the use of miRNAs to evaluate the probability of T2DM remission after an intervention with two healthy diets in patients with coronary heart disease, without differences in the remission rate between diets. Consequently, this should reduce the probability of a new cardiovascular event. Additionally, we unraveled a T2DM remission score based on miRNAs in diabetic patients without relevant weight loss. Nevertheless, our study has limitations. One of them is that we included a set of miRNAs selected based on previous knowledge. Therefore, we did not include other miRNAs that had not previously demonstrated their relationship with the disease. Finally, our study was conducted in a population with a mean age of 60 years with coronary heart disease, which suggests that our results should not be extrapolated to the general population.

In conclusion, we have identified a set of miRNAs (hsa-let-7b, hsa-miR-101, hsa-miR-130b-3p, hsa-miR-27a, hsa-miR-30a-5p, hsa-miR-375, and hsa-miR-486) that have the potential to evaluate the probability of T2DM remission in patients with coronary heart disease.

MATERIALS AND METHODS

Study subjects

This work was conducted within the framework of the CORDIOPREV study. The rationale, methods, and baseline characteristics have been reported by Delgado-Lista et al.[33] and provided in ClinicalTrials.gov: NTC00924937. The CORDIOPREV study is an ongoing prospective, randomized, single-blind, controlled dietary intervention trial in 1,002 patients with coronary heart disease, at high cardiovascular risk, aged between 20 and 75 years old, who had their last coronary event more than 6 months before enrolment, and had no severe diseases or a life expectancy of fewer than 5 years. The subjects were randomized into two different dietary models (Mediterranean and low-fat diets) during 8 years. Written consent was obtained from all the subjects before recruitment, and the study protocol and all amendments were approved by the Ethics Committee of Hospital Reina Sofia, all of which follow the Declaration of Helsinki and good clinical practices.

The present study (CORDIOPREV-DIRECT) included all newly diagnosed T2DM patients who had not been receiving glucose-lowering treatment at the beginning of the study (190 out of 1,002 patients). Of these, seven patients were excluded due to their inability to perform the diagnostic test used in this work. T2DM remission was evaluated in the remaining 183 patients during the 5-year follow-up period. Moreover, three participants died during the follow-up period without achieving diabetes remission. The 183 newly diagnosed T2DM patients were classified as responders, that is, patients who reverted from T2DM during the 5 years of dietary intervention without the use of diabetes medication (n = 73); or non-responders, that is, patients who did not achieve diabetes remission at the end of the follow-up period (n = 110) (Table S1). T2DM remission was defined as glycosylated hemoglobin <6.5%, fasting plasma glucose <126 mg/dL, and 2-h plasma glucose after an oral glucose tolerance test (OGTT) <200 mg/dL, for at least 2 consecutive years and without the use of diabetes medication to lower blood glucose levels.[34]

Diet, dietary assessment, and follow-up visits

The patients were randomized into two different healthy dietary patterns: a Mediterranean diet rich in fat from olive oil, with 35% of calories from fat (22% monounsaturated, 6% polyunsaturated, <10%

Table 2. Pathways regulated by miRNAs included in the T2DM remission score

KEGG pathways	p value	No. miRNAs
Protein processing in endoplasmic reticulum	1.57E−08	7
FoxO signaling pathway	1.93E−07	7
p53 Signaling pathway	3.28E−05	7
AMPK signaling pathway	8.67E−05	7
mTOR signaling pathway	0.000146	7
HIF-1 signaling pathway	0.003033	7
PI3K-Akt signaling pathway	0.007077	7
Insulin signaling pathway	0.012962	7
TNF signaling pathway	0.047134	7
Fatty acid biosynthesis	3.88E−06	6

The analysis was carried out through the bioinformatics tool DIANA Tools v3. The T2DM-related pathways were selected according to the lower p value and involving all seven miRNAs together or at least six of them.

saturated), and a maximum of 50% carbohydrates; and the low-fat, high-complex carbohydrate diet (LFHCC) recommended by the National Cholesterol Education Program and the American Heart Association, comprising <30% total fat (<10% saturated fat, 12%–14% monounsaturated fatty acid [MUFA] fat, and 6%–8% polyunsaturated fatty acid [PUFA] fat), 15% protein, and a minimum of 55% carbohydrates. Dietitians administered personalized individual interviews at inclusion and every 6 months, and quarterly group education sessions were held with up to 20 participants per session and separate sessions for each group. The general guidelines into the Mediterranean diet group were as follows: abundant use of virgin olive oil for cooking and for dressing salads and other dishes (participants received free extra virgin olive oil and were informed to use the oil as much as they needed in their regular diet), consumption of two or more servings (125 g/serving) per day of vegetables (at least one of them as a salad), three or more servings (125 g/serving) per day of fresh fruit, three or more servings (40 g/serving) per week of legumes, three or more servings (150 g/serving) per week of fish or seafood, three or more servings (25 g/serving) per week of nuts or seeds, white meats instead of red meats or processed meats, and regular preparation of a homemade sauce with tomato, garlic, onion, and spices with olive oil to dress vegetables, pasta, rice, and other dishes. Optionally, for alcohol drinkers, moderate consumption of red wine (seven glasses/week) was also allowed. Recommendations were also given to avoid and limit the consumption of butter, cream, fast foods, sweets, pastries, and sugar-sweetened beverages. The participants randomized to the LFHCC diet received the recommendations according to the American Heart Association and the National Cholesterol Education Program dietary guidelines, which focus on limiting all types of fat (from both animal or vegetable sources) and increasing the intake of complex carbohydrates.

Dietary adherence was assessed using the 14-item Mediterranean Diet Adherence Screener (MEDAS) to measure adherence to the Mediterranean diet. Mediterranean diet adherence was categorized into low (0–5), medium (6–9), and high (10–14) adherence, as previously published.[35] A nine-item dietary screener assessing adherence to the low-fat diet guidelines was also administered. This tool was developed and used in the PREDIMED study, and the total score ranged from 0 to 9 points. Low-fat diet adherence was categorized as low (0–3), medium (4–6), and high (7–9) adherence. The dietary adherence from the CORDIOPREV study was previously reported by Quintana-Navarro et al.[35] Full study diets, dietary assessment, and follow-up visits have been previously reported.[33]

Biochemical measurements of metabolic parameters

Venous blood from the participants was collected in tubes containing EDTA after a 12-h overnight fast. Lipid variables, glucose homeostasis variables, and inflammatory variables (hs-PCR [sensibility-Protein C reactive]) were determined as previously reported.[11]

OGTT, determination of insulin resistance, and secretion by β cell-related indexes

OGTT and the determination of insulin resistance and secretion by β cell-related indexes were previously reported.[11,36] In summary, patients underwent a standard Matsuda test at baseline and year-to-year during the follow-up period. After an overnight fast, blood was sampled from a vein before the oral glucose intake (0 min) and again after a 75 g flavored glucose load (75 g dextrose monohydrate in 250 mL water, NUTER-TEC glucosa 50). Blood samples were taken at 30, 60, 90, and 120 min to determine glucose and insulin concentrations.[37] The Matsuda ISI, HOMA-IR, insulinogenic index (IGI), DI, and HIRI were calculated as previously reported[37–41] and in our group by Jiménez-Lucena et al.,[11] Blanco-Rojo et al.,[42] and Roncero-Ramos et al.[36]

Isolation of circulating miRNAs from plasma samples

Venous blood from the 183 newly diagnosed T2DM patients and who had not been receiving glucose-lowering treatment at the beginning of the study was collected at baseline (day 0 before dietary intervention) in tubes containing EDTA. Since hemolysis can influence the content of miRNAs in plasma samples, and in order to reduce its effect, whole blood was collected in EDTA tubes and subjected to gentle agitation using a rotary tube shaker for six times, immediately after which the samples were kept on ice for no longer than 30 min. Next, the blood samples were centrifuged at 2,000 × *g* for 10 min at 4°C to separate the plasma from the blood cells. RNA isolation was carried out from plasma samples, as previously described by Jiménez-Lucena et al.[11]

cDNA synthesis and circulating miRNA levels by real-time PCR

The cDNA synthesis was carried out using the TaqMan miRNA reverse transcription kit (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA, USA) following the manufacturer's instructions, as previously described in our group by Jiménez-Lucena et al.[11]

The circulating miRNAs study was carried out on 56 miRNAs, of which our group had previously studied 28 in a population of non-diabetic patients.[10,11] The remaining 28 miRNAs were selected based

Table 3. Target genes regulated by the miRNAs included in the T2DM remission score

Pathway	Genes regulated by miRNAs included in the T2DM remission score
Protein processing in endoplasmic reticulum	*Sec62*, *Sec63*, *Sec23*, *Sec24*, *NRF2*
FoxO	*SET9*, *CK1*, *Bim*, *MnSOD*
p53	*p21*, *TSP1*
AMPK	*cyclin A*
mTOR	*GSK3B*, *SESN2*, *CLIP-170*
HIF-1	*EDN1*, *p21*, *p27*,
PI3K-AKT	*JAK*, *CCND1*, *Bim*, *p21*
Insulin signaling	*PTP1B*, *GSK3B*
TNF	*TAK1*, *CASP3*, *Edn1*, *p38*
Fatty acid biosynthesis	*FASN*

The genes were identified after the pathway analysis through the bioinformatics tool TarBase v8, a database of experimentally supported miRNA-gene interactions. Genes were selected when there was experimental support for a miRNA-gene interaction in T2DM-related pathways and when they were also regulated by the highest number of miRNAs.

on a bibliographic search according to their association with insulin sensitivity, insulin secretion, inflammation, and growth and proliferation of β cells (Table S6). We measured the levels of miRNAs at the baseline of the CORDIOPREV study with the OpenArray platform (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA, USA) following the manufacturer's instructions. The relative expression data were analyzed using OpenArray real-time qPCR analysis software (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA, USA), and the normalization method has been previously described.[11]

Pathways regulated by the miRNAs studied and target genes in each pathway

To study the role of miRNAs in the cellular processes related to T2DM, we performed an analysis with DIANA Tools using miRPath v3 software. DIANA-miRPath is a web server that provides accurate statistics and can accommodate advanced pipelines. miRPath can utilize predicted miRNA targets (in coding sequence [CDS] or 3′ UTR regions) provided by the DIANA-microT-CDS algorithm, or even experimentally validated miRNA interactions derived from Database: DIANA-TarBase.[43] The T2DM-related biological processes were selected based on a lower p value and involving all seven miRNAs together or at least six of them.

Additionally, to identify the target genes regulated by the miRNAs studied, we carried out an analysis through the Database: KEGG, listing all of the genes involved in each pathway. Next, through the DIANA Tools bioinformatics tool using TarBase v8 software,[44] a database of experimentally supported miRNA-gene interactions, we identified the genes regulated by the seven miRNAs in each pathway associated with the development of T2DM (Figures S1–S10).

Statistical analysis

The differences between responders and non-responders in the study population's baseline characteristics were assessed by one-way ANOVA analysis using the SPSS software (now PASW Statistic for Windows, version 21) (IBM, Chicago, IL, USA). p values <0.05 were considered statistically significant. Multiple comparisons in the large-scale analyses were assessed by false discovery rate (FDR) using the Benjamini and Hochberg method (Q value). Before analysis, clinical variables (BMI, age, HDL-C, and triglycerides), β cell function and insulin resistance indexes (ISI, HOMA-IR, HIRI, IGI, and DI), and miRNAs data were transformed (centering and scaling) using the preProcess function from the caret package through the free statistical software R version 3.6.1. This function uses the "method = center" to subtract the mean of the predictor's data from the predictor values, while "method = scale" divides data by the standard deviation.

ROC curve analysis

We built six models using ROC analysis based on miRNAs, β cell function indexes, insulin resistance indexes, and clinical variables. The statistical analyses were performed with the free statistical software R version 3.6.1, using RStudio version 1.2.5019. The ROC analysis was carried out in two steps, first through the glm function, which is used to fit generalized linear models, and is specified by giving a symbolic description of the linear predictor and a description of the error distribution.[45] Next, we performed the ROC curve using the pROC package. pROC is a tool for visualizing, smoothing, and comparing ROC curves. The AUC can be compared with a statistical test based on U statistics or bootstrap anaysis. After the analysis, we evaluated the AUC, sensitivity, specificity, accuracy and threshold values for each model built. The most significant variables were identified by $Pr(>|z|)$ values <0.05, registered in the summary of each glm analysis. Finally, the DeLong test was performed to assess the statistical difference between the ROC curves, and for this, we used the roc.test function of the *pROC* package using software R. The roc.test function compares the AUC or partial AUC of two correlated (or paired) or uncorrelated (unpaired) ROC curves through the bootstrap and Venkatraman methods with 2,000 bootstrap replicates or permutations.[46]

T2DM remission score based on miRNAs

In order to evaluate the probability of T2DM remission using seven selected miRNAs, we calculated a T2DM remission score in three steps. First, we performed a glm analysis, including the seven miRNAs and we assessed the z value of each miRNA in the summary analysis (Table S7). Next, we multiplied the z value of each miRNA by the expression value of miRNAs in all patients included in the study. Third, the seven values were then added together to obtain a single value per subject. Finally, the 183 patients included in our study were classified according to the tertiles of the T2DM remission score (T1, low score; T2, intermediate score; T3, high score). Using the SPSS software (now PASW Statistic for Windows, version 21) (IBM, Chicago, IL, USA), we performed a Cox regression analysis adjusting by BMI, sex, diet, age, HDL-C, triglycerides, and statins, and assessing the HR where T1 was the reference.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2020.11.001.

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AUTHOR CONTRIBUTIONS

O.A.R.-Z., A.C., and J.L.-M. conceived and designed the experiments. J.F.A.-D., G.M.Q.-N., A.L.-A., F.G.-D., J.D.-L., P.P.-M., and J.L.-M. participated in the recruitment and carried out the clinical and nutritional control of the volunteers. O.A.R.-Z., C.V.-D., and Y.K. performed the experiments and collected the data. O.A.R.-Z., C.V.-D., J.F.A.-D., Y.K., J.D.-L., A.C., and J.L.-M. analyzed and interpreted the data. O.A.R.-Z., A.C., and J.L.-M. drafted the manuscript. J.L.-M. conceived and designed the study. O.A.R.-Z., C.V.-D., and Y.K. performed the statistical analysis of data. R.M.L., J.M.O., and J.L.-M. provided critical revision of the paper for the important intellectual content. J.D.-L., P.P.-M., A.C., and J.L.-M. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All of the authors were involved in writing the paper and gave their final approval to the submitted and published versions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

A set of miRNAs predicts T2DM remission in patients with coronary heart disease: from the CORDIOPREV study

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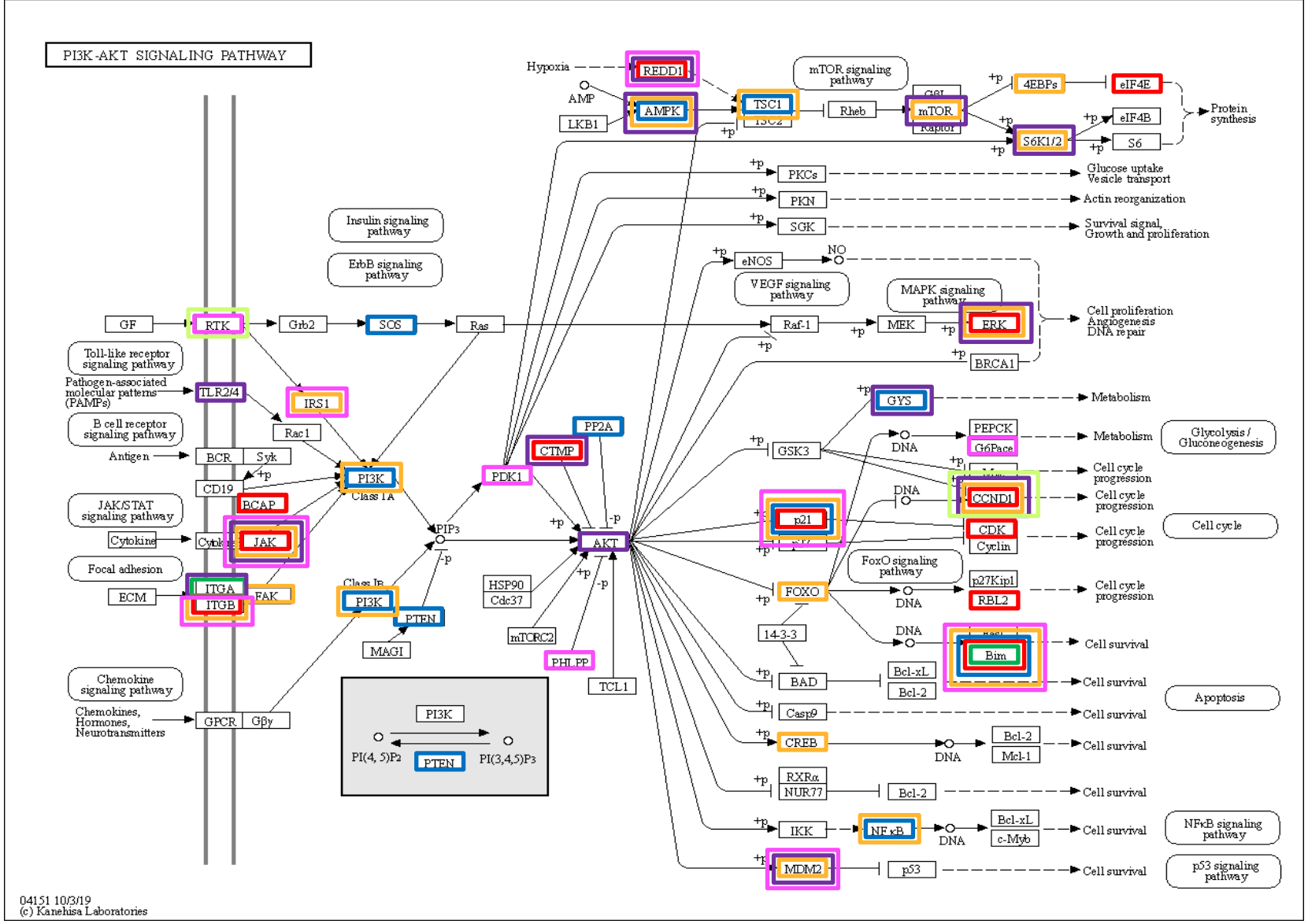
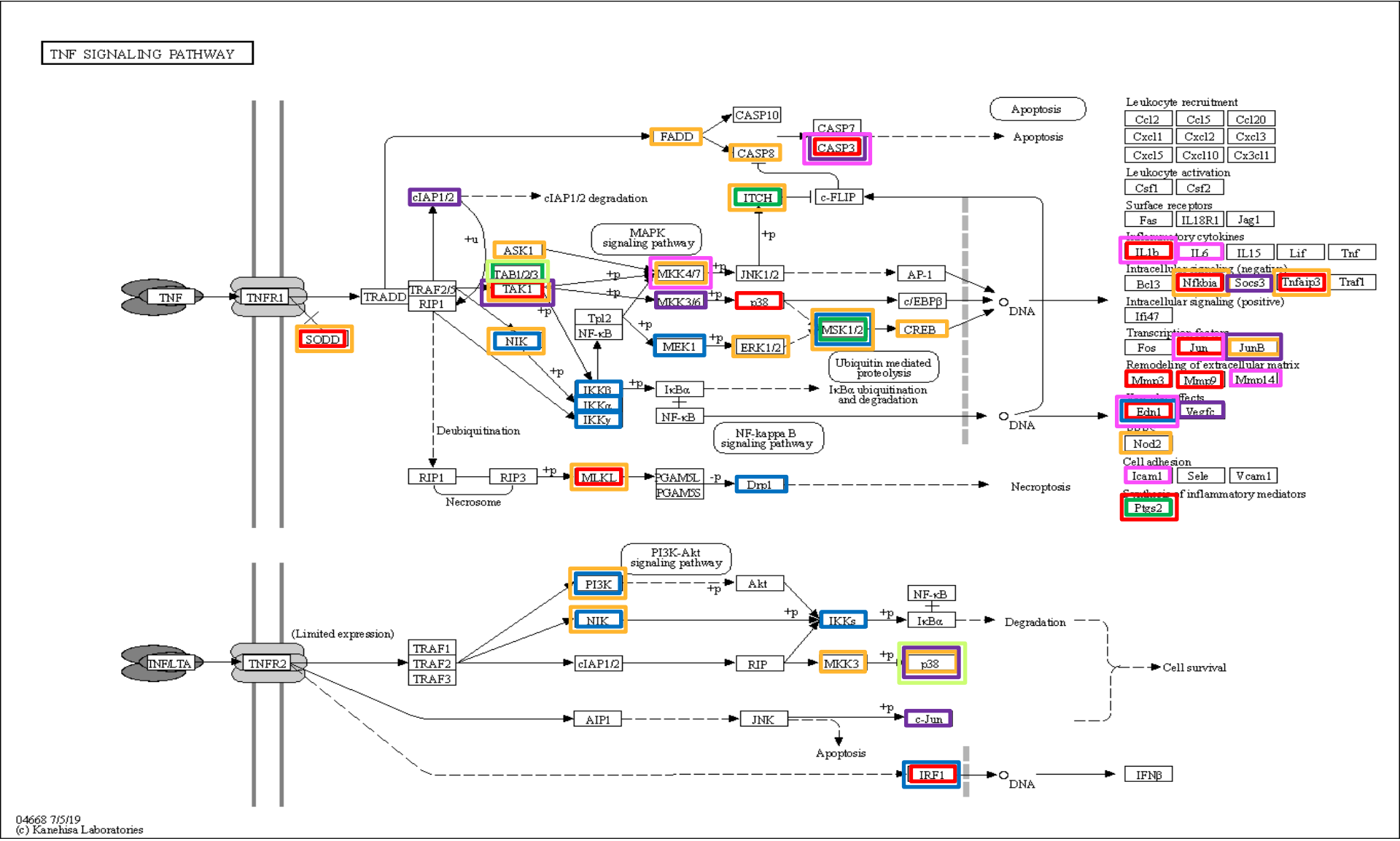


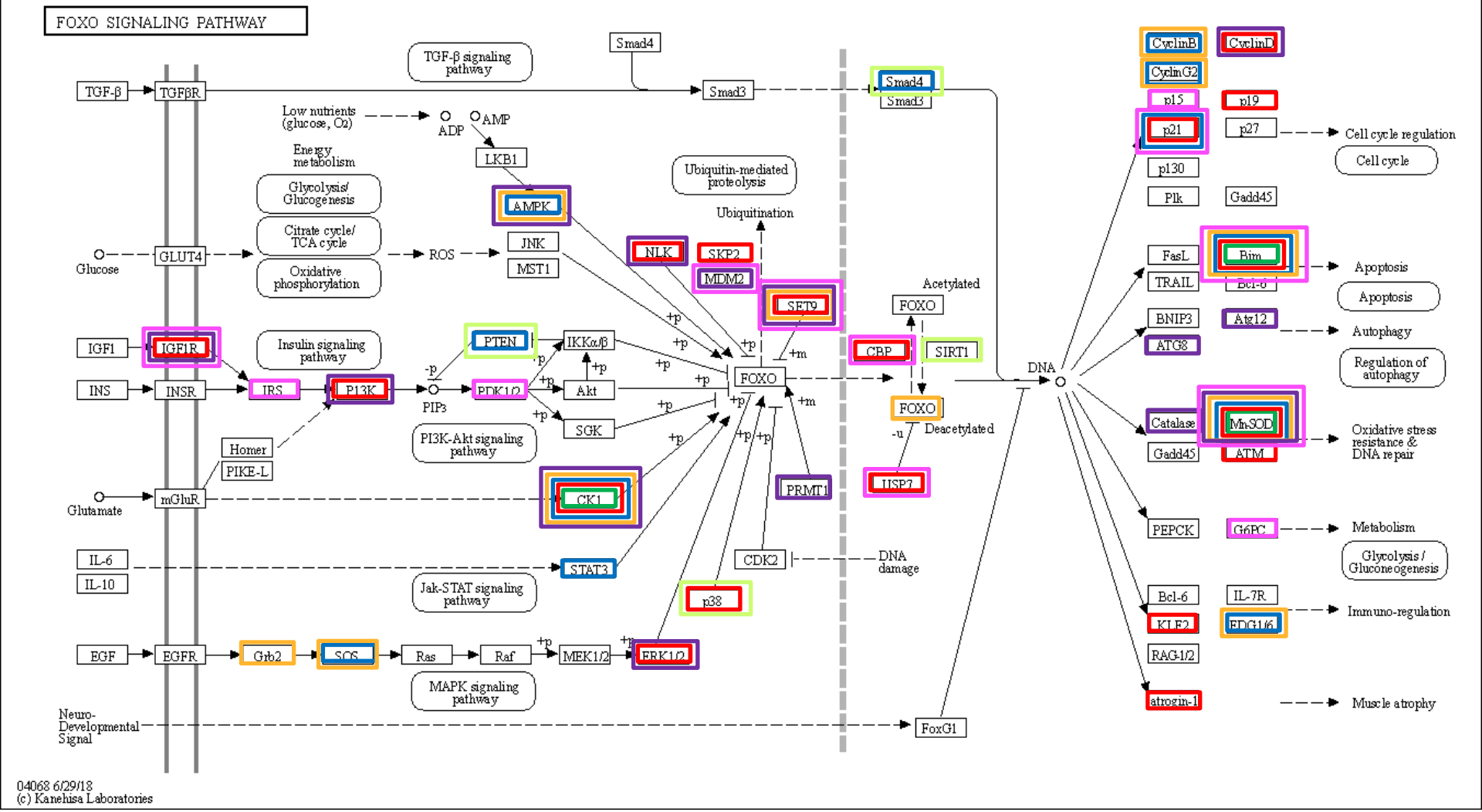
Let7b 2 genes | miR-101 12 genes | miR-130b-3p 10 genes | miR-27a 19 genes | miR-30a-5p 13 genes | miR-375 11 genes | miR-486 2 genes

Figure S1. Genes belonging to the PI3K-AKT pathway and that are regulated by the seven miRNAs included in the T2DM remission score



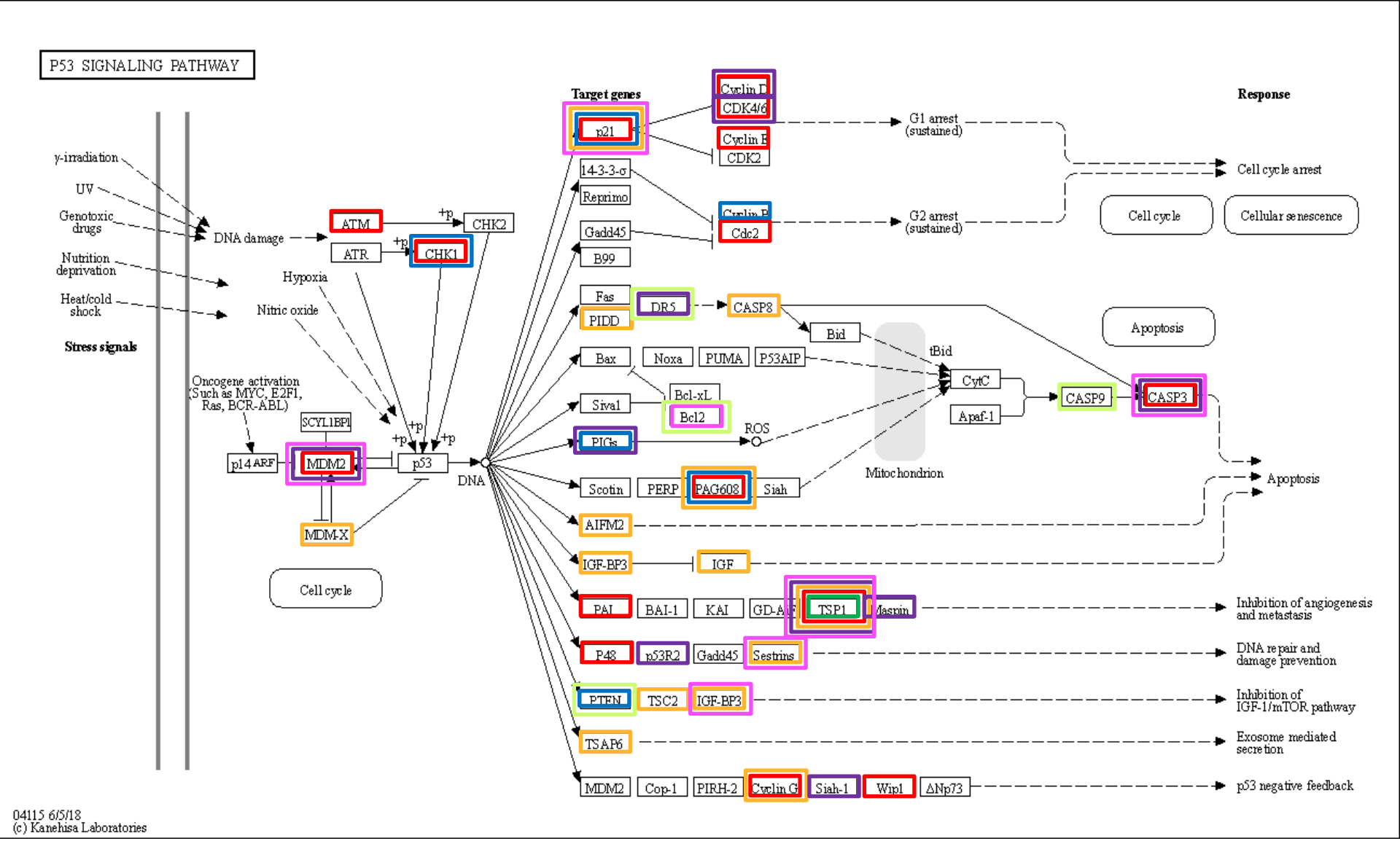
Let7b	miR-101	miR-130b-3p	miR-27a	miR-30a-5p	miR-375	miR-486
4 genes	14 genes	8 genes	19 genes	9 genes	8 genes	2 genes

Figure S2. Genes belonging to the TNF pathway and that are regulated by the seven miRNAs included in the T2DM remission score



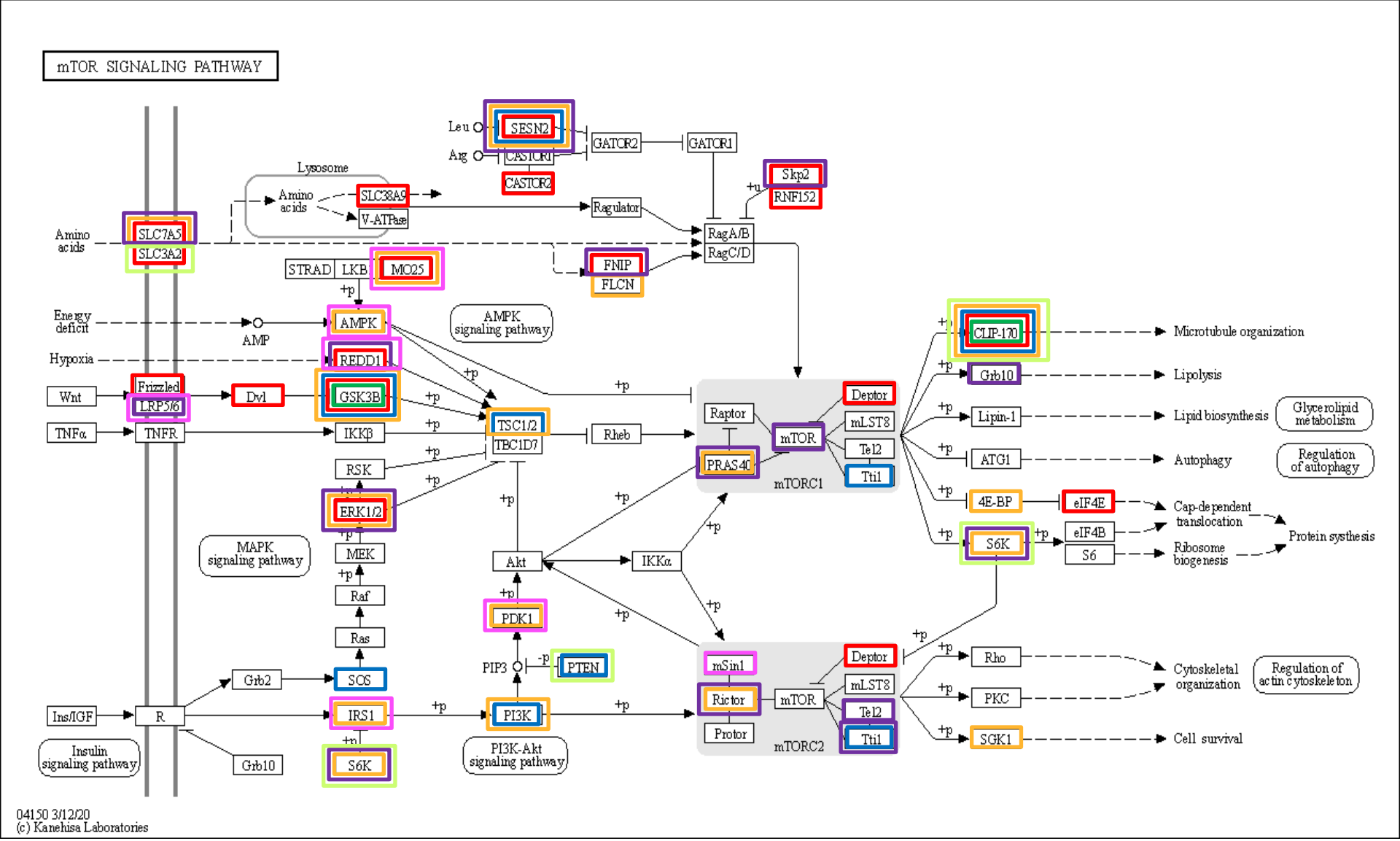
Let7b	miR-101	miR-130b-3p	miR-27a	miR-30a-5p	miR-375	miR-486
3 genes	18 genes	12 genes	20 genes	15 genes	12 genes	4 genes

Figure S3. Genes belonging to the FOXO pathway and that are regulated by the seven miRNAs included in the T2DM remission score



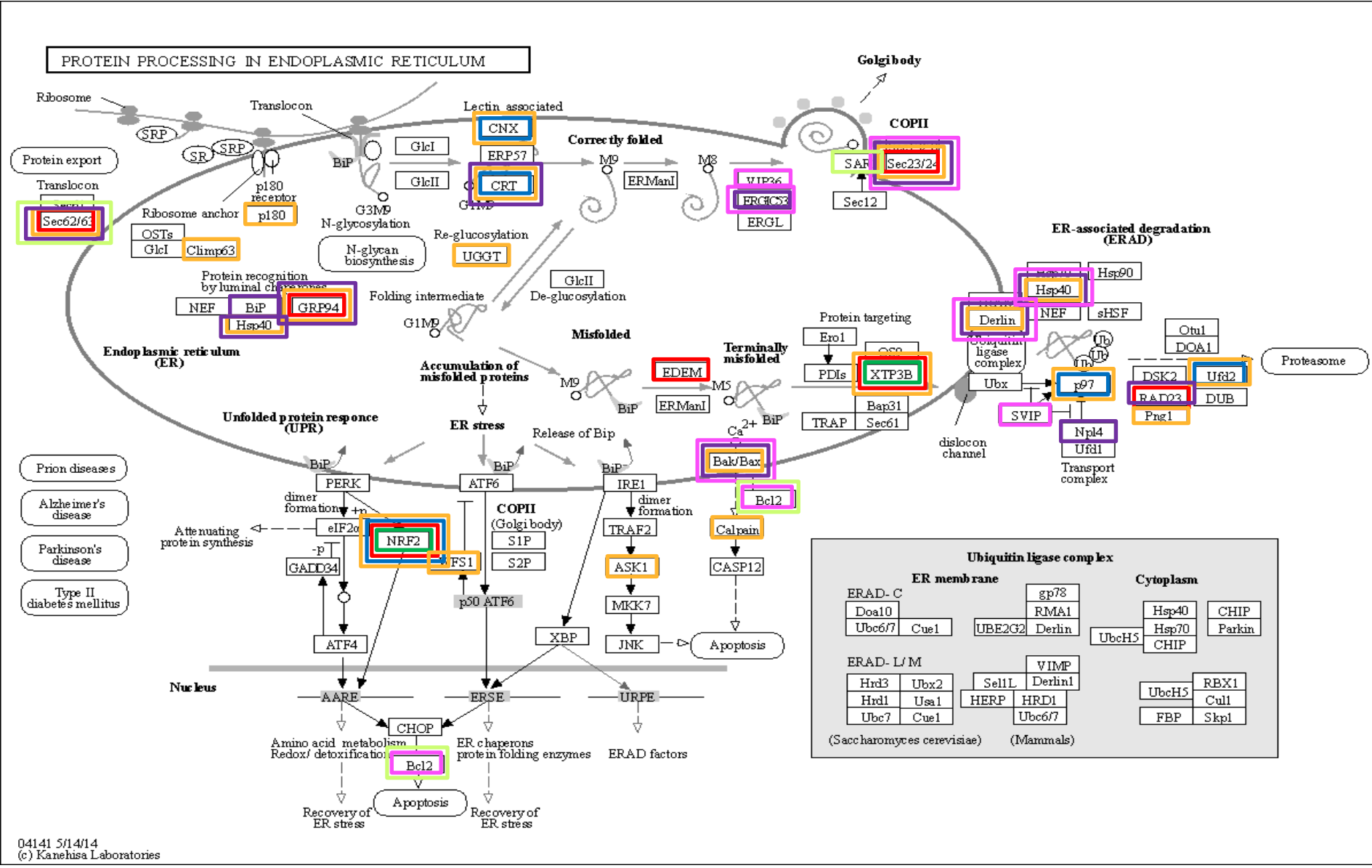
Let7b	miR-101	miR-130b-3p	miR-27a	miR-30a-5p	miR-375	miR-486
1 genes	14 genes	6 genes	16 genes	10 genes	7 genes	4 genes

Figure S4. Genes belonging to the p53 pathway and that are regulated by the seven miRNAs included in the T2DM remission score



Let7b	miR-101	miR-130b-3p	miR-27a	miR-30a-5p	miR-375	miR-486
2 genes	17 genes	8 genes	20 genes	14 genes	7 genes	4 genes

Figure S5. Genes belonging to the mTOR pathway and that are regulated by the seven miRNAs included in the T2DM remission score



Let7b	miR-101	miR-130b-3p	miR-27a	miR-30a-5p	miR-375	miR-486
2 genes	8 genes	5 genes	19 genes	12 genes	8 genes	3 genes

Figure S6. Genes belonging to the protein processing in the endoplasmic reticulum pathway and that are regulated by the seven miRNAs included in the T2DM remission score

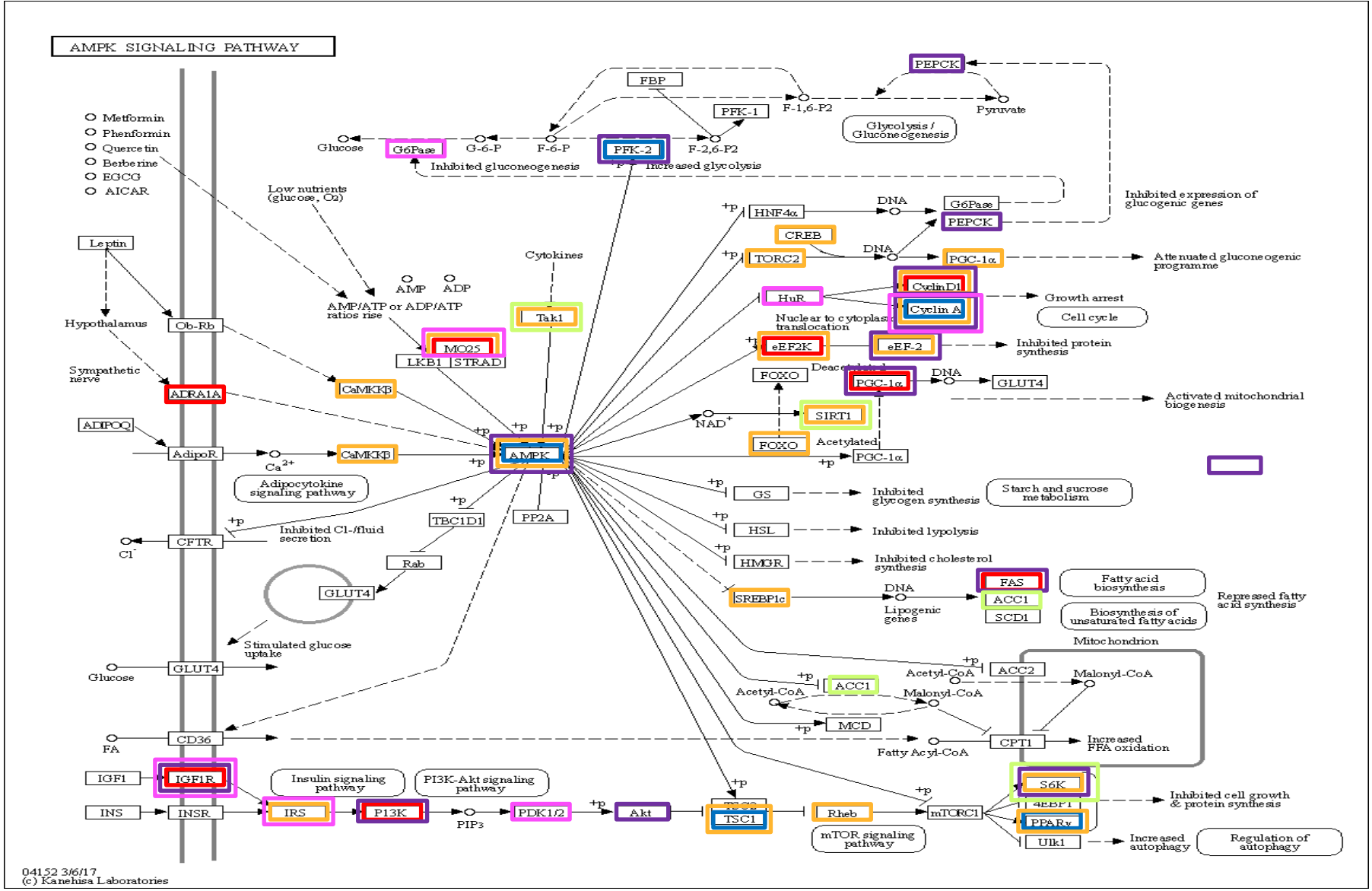
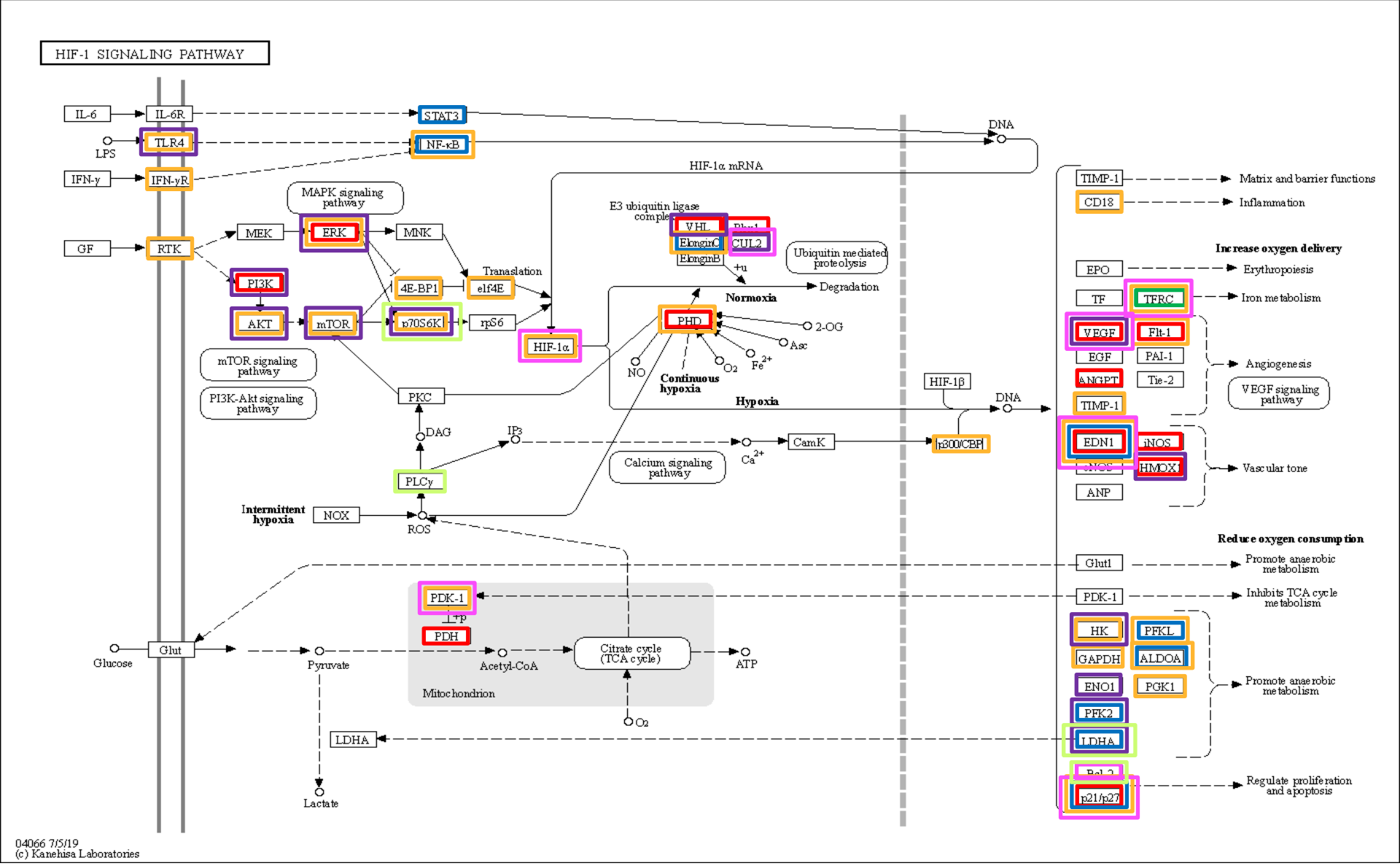
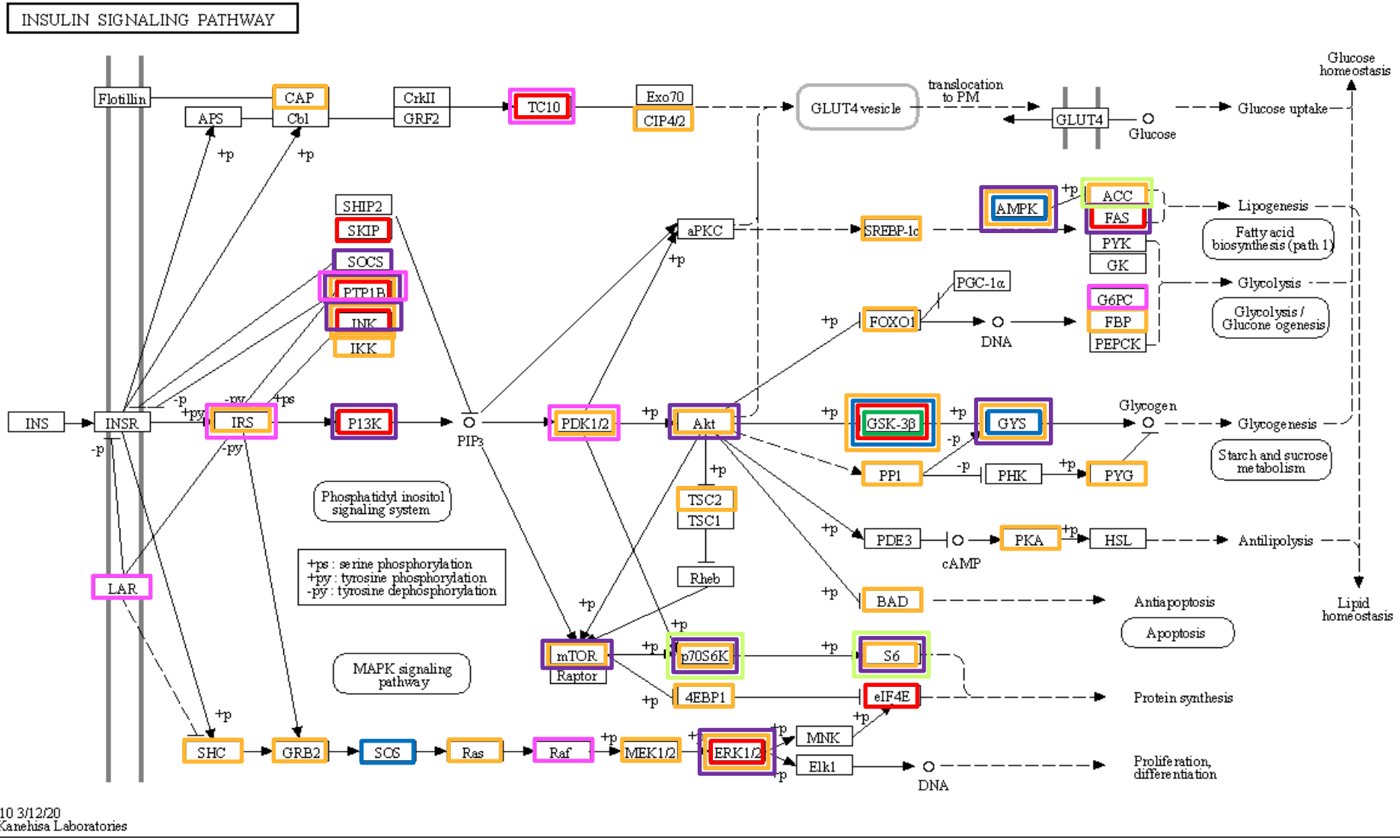


Figure S7. Genes belonging to the AMPK pathway and that are regulated by the seven miRNAs included in the T2DM remission score



Let7b	miR-101	miR-130b-3p	miR-27a	miR-30a-5p	miR-375	miR-486
1 genes	12 genes	9 genes	25 genes	14 genes	8 genes	4 genes

Figure S8. Genes belonging to the HIF-1 pathway and that are regulated by the seven miRNAs included in the T2DM remission score



Let7b	miR-101	miR-130b-3p	miR-27a	miR-30a-5p	miR-375	miR-486
1 genes	9 genes	4 genes	28 genes	11 genes	7 genes	2 genes

Figure S9. Genes belonging to the insulin signaling pathway and that are regulated by the seven miRNAs included in the T2DM remission score

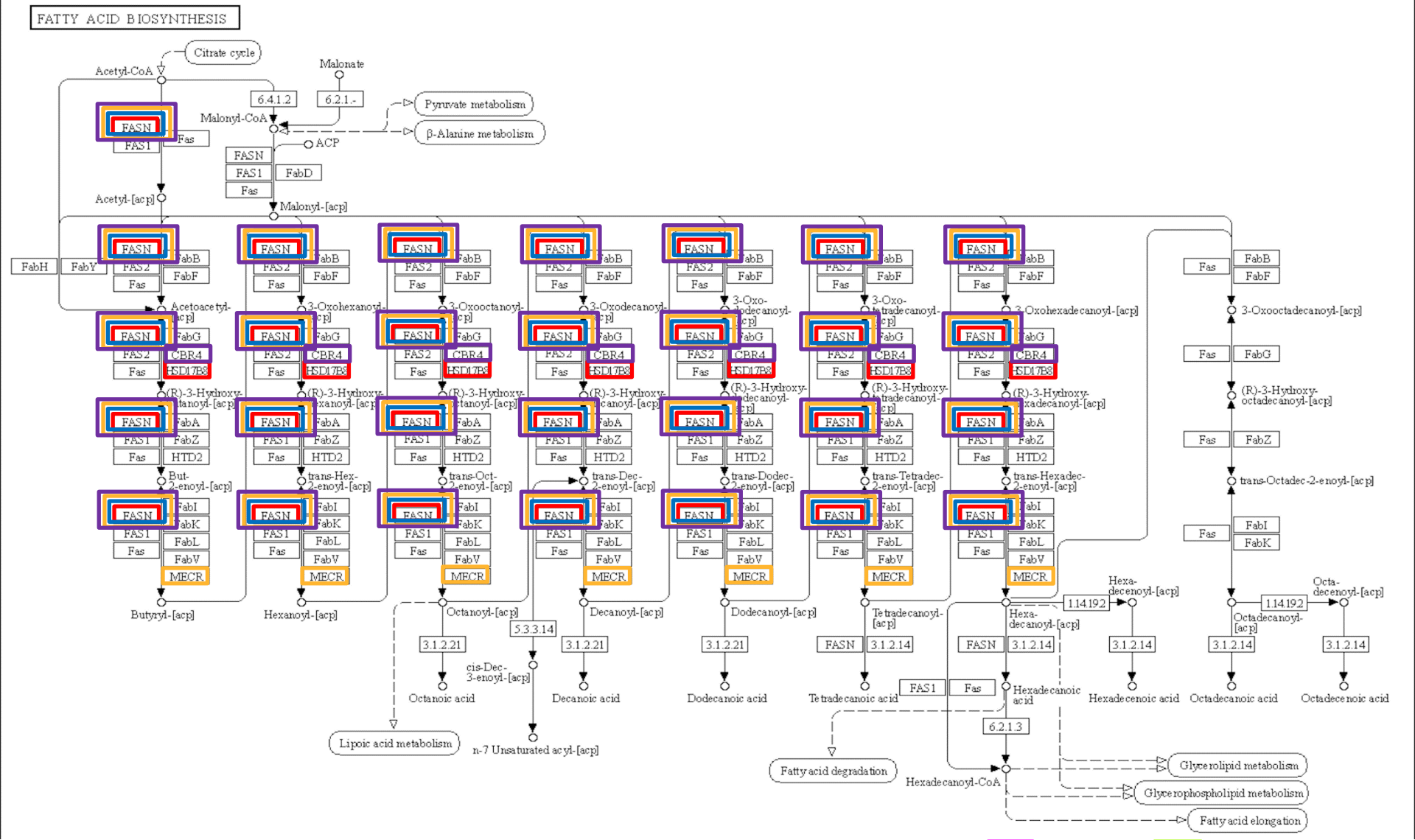


Figure S10. Genes belonging to the fatty acid biosynthesis pathway and that are regulated by the seven miRNAs included in the T2DM remission score

Table S1. Baseline characteristics of the study population. Data are mean±SEM. Responders group: patients who reverted from type 2 diabetes after 60 months of dietary intervention follow-up. Non-Responders group: patients who remained with type 2 diabetes after 60 months of follow-up. * $p < 0.05$. Differences in continuous variables were tested by One-way ANOVA. Differences in gender (men/women) and drugs (%) ingestion were tested by Chi square analysis. Multiple comparisons in the large-scale analyses were assessed by False Discovery Rate (FDR) using the Benjamini and Hochberg method (Q-value).

	Responders (n=73)	**Non-Responders (n=110)**	***p*-value**	***q-value***
Men/Women	60/13	92/18	0.799	0.841
Age (years)	60.8±1.0	59.3±0.9	0.252	0.335
Weight (kg)	80.2±1.3	88.4±1.4	<0.001*	<0.001
Body mass index (kg/m^2)	29.9±0.4	32.1±0.4	0.001*	0.033
Waist circumference (cm)	101±1	108±1	<0.001*	<0.001
Triglycerides (mmol/L)	1.48±0.10	1.69±0.07	0.090	0.150
Total-cholesterol (mmol/L)	4.16±0.08	4.31±0.08	0.203	0.290
HDL-cholesterol (mmol/L)	1.11±0.03	1.06±0.02	0.141	0.216
LDL-cholesterol (mmol/L)	2.31±0.07	2.42±0.07	0.302	0.377
C-reactive protein (nmol/L)	37.1±5.3	33.5±3.6	0.558	0.620
HbA1c (mmol/mol)	47.8±0.9	50.7±0.9	0.032*	0.058
HbA1c (%)	6.53±0.08	6.79±0.08	0.032*	0.064
Glucose (mmol/L)	5.50± 0.09	6.58±0.14	<0.001*	<0.001
Insulin (nmol/L)	64.4± 5.5	93.3±7.8	0.007*	0.020
HOMA-IR	3.49±0.42	4.84±0.32	0.010*	0.220
Insulin sensitivity index	3.16±0.20	2.37±0.12	<0.001*	<0.001
Insulinogenic index	0.70±0.19	0.68±0.14	0.921	0.921
Hepatic insulin resistance index	1421±168	1970±129	0.009*	0.022
Muscle Insulin sensitivity index (x10^2)	1.93±0.22	2.20±0.25	0.452	0.532
Disposition Index	0.68±0.06	0.43±0.02	<0.001*	<0.001
Anti-Aggregates	94.5	96.4	0.339	1.000
Beta-blockers	57.5	65.5	0.279	1.000
Angiotensin-I receptor blockers	13.7	82.7	0.517	1.000
Angiotensin-II receptor antagonist	20.5	23.6	0.624	1.000
Diuretics	39.7	41.8	0.778	1.000
Calcium Antagonists	11.0	19.1	0.140	1.000
Oral anticoagulants	1.4	0.9	0.769	1.000
Other Hypolipidemics	6.8	7.3	0.913	0.946

Table S2. Frequency of subjects Responders and Non-Responders after the dietary intervention period with each of the diets. The analysis was carried out using SPSS (now PASW Statistic for Windows, version 21) (IBM, Chicago, IL, USA) and corresponds to a chi-square test between the two diets for each group independently. $p<0.005$ was considered with a statistically significant difference.

	LFHCC diet	**Med Diet**	**chi-square**
Responders	40	33	0.413
Non-Responders	60	47	0.127

Table S3. Adherence to diets by Responders and non-Responders participants before and after dietary intervention. One-Way ANOVA p-values (p<0.05). ANOVA for repeated measures: b=P<0.05 between baseline and five years in Responders; b=p<0.05 between baseline and five years in non-Responders. *Data at year five correspond to the patients randomized to each diet. LFHCC: low-fat, high–complex carbohydrate diet. Med: Mediterranean diet.

	Baseline			**5-years***		
	Responders	**non-Responders**	***p-value***	**Responders**	**non-Responders**	***p-value***
n	73	110		73	108	
LFHCC diet adherence score	3.88 ± 0.2	3.78 ± 0.1	0.653	7.39 ± 0.3[a]	7.24 ± 0.2[b]	0.646
Med diet adherence score	9.01 ± 0.2	8.62 ± 0.2	0.136	11.53 ± 0.3[a]	11.33 ± 0.2[b]	0.583

Table S4. Weight loss of Responders and non-Responders before and after dietary intervention. The analysis corresponds to a t-test for related samples between the baseline and after the 5 years of follow-up period, independent for each biological group. *= $p<0.05$ between baseline and five years in Responders.

	Responders			Non-Responders		
	Baseline	**5 years of follow-up**	***p-value***	**Baseline**	**5 years of follow-up**	***p-value***
Weight, Kg	80.2 ± 1.3	78.5 ± 1.5*	0.002	88.4 ± 1.4	87.5 ± 1.5	0.100

Table S5. Statistics of the ROC model including 45 miRNAs after a `glm` analysis using the software "R". The variables with the greatest potential for differentiation within the model are marked with *. * p <0.05 and *** p < 0.01. Multiple comparisons in the large-scale analyses were assessed by False Discovery Rate (FDR) using the Benjamini and Hochberg method (Q-value).

	Estimate	Std. Error	z-value	Pr(>|z|)	q-value
(Intercept)	-0.9361	0.3436	-2.724	0.0065	0.0997
hsa-let7b	-1.0399	0.4067	-2.557	0.0106 *	0.1219
hsa-miR-101	1.4592	0.6228	2.343	0.0191 *	0.1098
hsa-miR-103	1.1004	0.5987	1.838	0.0661	0.2534
hsa-miR-107	0.548	0.6944	0.789	0.4300	0.5994
hsa-miR-125b	-0.257	0.5692	-0.452	0.6516	0.8326
hsa-miR-126	-1.5892	0.9714	-1.636	0.1018	0.2602
hsa-miR-130b-3p	2.5509	0.6896	3.699	0.0002 ***	0.0092
hsa-miR-130b-5p	-1.533	0.975	-1.572	0.1159	0.2666
hsa-miR-141	-1.783	1.2463	-1.431	0.1525	0.2923
hsa-miR-142-3p	-0.1409	0.6799	-0.207	0.8359	0.9155
hsa-miR-145	0.7778	0.435	1.788	0.0738	0.2611
hsa-miR-146b-3p	0.0499	0.4012	0.124	0.901	0.9420
hsa-miR-150	-0.0034	0.3828	-0.009	0.9929	0.9929
hsa-miR-15a	-0.2255	0.5361	-0.421	0.6741	0.8381
hsa-miR-17	-0.1425	0.4957	-0.287	0.7738	0.8682
hsa-miR-182	-0.0578	0.3418	-0.169	0.8658	0.9262
hsa-miR-191	0.2797	0.705	0.397	0.6916	0.8157
hsa-miR-192	-0.0181	0.287	-0.063	0.9498	0.9709
hsa-miR-197-5p	-0.7657	0.4552	-1.682	0.0926	0.2662
hsa-miR-199a-3p	-0.1942	0.4637	-0.419	0.6754	0.8176
hsa-miR-200b	-0.4941	0.4471	-1.105	0.2691	0.4421
hsa-miR-21	-0.0966	0.2931	-0.33	0.7418	0.8531
hsa-miR-210	-0.9943	0.7606	-1.307	0.1912	0.3518
hsa-miR-210-5p	0.7942	0.4254	1.867	0.0619	0.3164
hsa-miR-221	-0.7624	0.5236	-1.456	0.1454	0.3185
hsa-miR-222	-1.3886	0.7515	-1.848	0.0646	0.2972
hsa-miR-223	1.2723	0.8848	1.438	0.1504	0.3008
hsa-miR-24	1.7038	1.0042	1.697	0.0898	0.2754
hsa-miR-26	1.6474	0.8934	1.844	0.0652	0.2727

hsa-miR-27a	-2.0242	0.8066	-2.509	0.0121 *	0.0795
hsa-miR-28-3p	-0.8597	1.2655	-0.679	0.4969	0.6723
hsa-miR-29a	0.9765	0.6141	1.59	0.1118	0.2707
hsa-miR-29b	0.7097	0.397	1.788	0.0738	0.2425
hsa-miR-30a-5p	-1.977	0.779	-2.538	0.0112 *	0.1030
hsa-miR-30d	1.0268	0.6151	1.669	0.0950	0.2571
hsa-miR-320	-0.9127	1.0128	-0.901	0.3675	0.5453
hsa-miR-365	-0.5004	0.5057	-0.99	0.3224	0.4943
hsa-miR-374	-1.2479	1.1822	-1.056	0.2911	0.4617
hsa-miR-375	1.092	0.4351	2.51	0.0121 *	0.0928
hsa-miR-423-5p	-0.4462	0.7769	-0.574	0.5658	0.7436
hsa-miR-429	0.4055	0.3129	1.296	0.1951	0.3452
hsa-miR-486	2.4616	0.7017	3.508	0.0005 ***	0.0115
hsa-miR-7	-0.5475	0.4761	-1.15	0.2502	0.4263
hsa-miR-9	0.6318	0.437	1.446	0.1482	0.3099
hsa-miR-140	0.7803	0.8768	0.89	0.3735	0.5369

Table S6. miRNAs included in the OpenArray panel (LifeTechnologies– Thermofisher Scientific, Carlsbad, CA, USA) in the circulating miRNAs study.

Order	Assay Name	Mature microRNA Sequence
1	**hsa-miR-320**	AAAAGCUGGGUUGAGAGGGCGA
2	**hsa-miR-199a-3p**	ACAGUAGUCUGCACAUUGGUUA
3	**hsa-miR-191***	GCUGCGCUUGGAUUUCGUCCCC
4	**hsa-miR-494-5p**	AGGUUGUCCGUGUUGUCUUCUCU
5	**hsa-miR-146b-3p**	UGCCCUGUGGACUCAGUUCUGG
6	**hsa-miR-200a***	CAUCUUACCGGACAGUGCUGGA
7	**hsa-miR-200c***	CGUCUUACCCAGCAGUGUUUGG
8	**hsa-miR-429**	UAAUACUGUCUGGUAAAACCGU
9	**hsa-miR-141***	CAUCUUCCAGUACAGUGUUGGA
10	**hsa-miR-142-3p**	UGUAGUGUUUCCUACUUUAUGGA
11	**hsa-miR-101**	UACAGUACUGUGAUAACUGAA
12	**hsa-miR-125b**	UCCCUGAGACCCUAACUUGUGA
13	**hsa-miR-130b***	ACUCUUUCCCUGUUGCACUAC
14	**hsa-miR-423-5p**	UGAGGGGCAGAGAGCGAGACUUU
15	**hsa-miR-34a***	CAAUCAGCAAGUAUACUGCCCU
16	**hsa-miR-132***	ACCGUGGCUUUCGAUUGUUACU
17	**hsa-miR-374**	UUAUAAUACAACCUGAUAAGUG
18	**hsa-miR-210**	CUGUGCGUGUGACAGCGGCUGA
19	**hsa-miR-24**	UGGCUCAGUUCAGCAGGAACAG
20	**hsa-miR-20b***	ACUGUAGUAUGGGCACUUCCAG
21	**hsa-miR-197-5p**	CGGGUAGAGAGGGCAGUGGGAGG
22	**hsa-miR-29b**	UAGCACCAUUUGAAAUCAGUGUU
23	**hsa-miR-486**	UCCUGUACUGAGCUGCCCCGAG
24	**hsa-miR-221***	ACCUGGCAUACAAUGUAGAUUU
25	**hsa-miR-17***	ACUGCAGUGAAGGCACUUGUAG
26	**hsa-miR-210-5p**	AGCCCCUGCCCACCGCACACUG
27	**hsa-miR-365**	UAAUGCCCCUAAAAAUCCUUAU
28	**mmu-miR-140**	CAGUGGUUUUACCCUAUGGUAG
29	**hsa-miR-130b**	CAGUGCAAUGAUGAAAGGGCAU
30	**hsa-miR-146a***	CCUCUGAAAUUCAGUUCUUCAG
31	**hsa-miR-19a***	AGUUUUGCAUAGUUGCACUACA
32	**hsa-miR-7**	UGGAAGACUAGUGAUUUUGUUG
33	**hsa-miR-15a**	CAGGCCAUAUUGUGCUGCCUCA
34	**hsa-miR-28-3p**	CACUAGAUUGUGAGCUCCUGGA
35	**hsa-miR-29a**	UAGCACCAUCUGAAAUCGGUU
36	**hsa-miR-30d**	CUUUCAGUCAGAUGUUUGCUGC
37	**hsa-miR-103**	AGCAGCAUUGUACAGGGCUAUGA

38	**hsa-miR-107**	AGCAGCAUUGUACAGGGCUAUCA
39	**hsa-miR-126**	UCGUACCGUGAGUAAUAAUGCG
40	**hsa-miR-144**	GGAUAUCAUCAUAUACUGUAAG
41	**hsa-miR-150**	CUGGUACAGGCCUGGGGGACAG
42	**hsa-miR-182**	UUUGGCAAUGGUAGAACUCACA
43	**hsa-miR-192**	CUGCCAAUUCCAUAGGUCACAG
44	**hsa-miR-223**	UGUCAGUUUGUCAAAUACCCCA
45	**hsa-miR-375**	UUUGUUCGUUCGGCUCGCGUGA
46	**hsa-miR-let7b**	CUAUACAACCUACUGCCUUCCC
47	**hsa-miR-143**	UGAGAUGAAGCACUGUAGCUCA
48	**hsa-miR-145**	GGAUUCCUGGAAAUACUGUUCU
49	**hsa-miR-21**	CAACACCAGUCGAUGGGCUGU
50	**hsa-miR-30a-5p**	UGUAAACAUCCUCGACUGGAAG
51	**hsa-miR-9**	UCUUUGGUUAUCUAGCUGUAUGA
52	**hsa-miR-26a**	UUCAAGUAAUCCAGGAUAGGCU
53	**hsa-miR-200b**	CAUCUUACUGGGCAGCAUUGGA
54	**hsa-miR-222**	AGCUACAUCUGGCUACUGGGU
55	**hsa-miR-27a**	UUCACAGUGGCUAAGUUCCGC
56	**RNU6B**	CGCAAGGATGACACGCAAATTCGTGAAGCGTTCCATATTTTT

Table S7. Summary table of the `glm` analysis. The table shows the z-values of the fit generalized linear model based on the 7 miRNAs. The analysis was performed using the `glm` function with the free statistical software "R", version 3.6.1, using RStudio version 1.2.5019.

Coefficients:

	Estimate	**Std. Error**	**z-value**
(Intercept)	-0.5325	0.1898	-2.806
hsa-let7b	-1.0618	0.3274	-3.243
hsa-miR-101	0.1011	0.2208	0.458
hsa-miR-130b-3p	0.7864	0.2397	3.281
hsa-miR-27a	0.2255	0.2289	0.985
hsa-miR-30a-5p	-0.4505	0.3102	-1.453
hsa-miR-375	0.3979	0.1964	2.026
hsa-miR-486	0.4307	0.269	1.602