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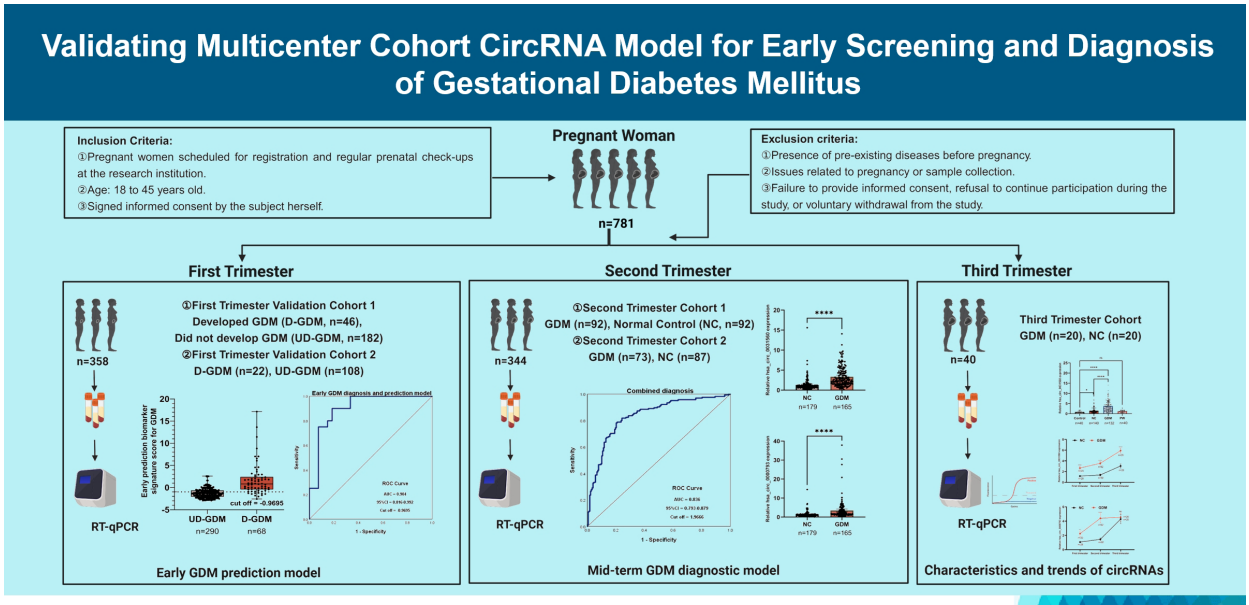
Diabetes Metab J 2025;49:462-474 | *https://doi.org/10.4093/dmj.2024.0205*



Conclusion

Hsa_circ_0031560, hsa_circ_0000793, and the developed model serve as biomarkers for early prediction or mid-term diagnosis of GDM, offering clinical tools for early GDM screening.

Highlights

- **This study identified sensitive biomarkers for early GDM screening using circRNAs.**
- **We validated hsa_circ_0031560 and hsa_circ_0000793 as reliable biomarkers for GDM.**
- **We developed an early GDM prediction model (E-GDMM) with high diagnostic accuracy.**
- **The model was validated in multiple cohorts, showing strong sensitivity and specificity.**

How to cite this article:
Ma S, Chen Y, Gu Z, Wang J, Zhao F, Yao Y, et al. Validating Multicenter Cohort Circular RNA Model for Early Screening and Diagnosis of Gestational Diabetes Mellitus. Diabetes Metab J 2025;49:462-474. https://doi.org/10.4093/dmj.2024.0205

Original Article
Metabolic Risk/Epidemiology

Diabetes Metab J 2025;49:462-474
https://doi.org/10.4093/dmj.2024.0205
pISSN 2233-6079 · eISSN 2233-6087

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Validating Multicenter Cohort Circular RNA Model for Early Screening and Diagnosis of Gestational Diabetes Mellitus

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Background: Gestational diabetes mellitus (GDM) is a metabolic disorder posing significant risks to maternal and infant health, with a lack of effective early screening markers. Therefore, identifying early screening biomarkers for GDM with higher sensitivity and specificity is urgently needed.
Methods: High-throughput sequencing was employed to screen for key circular RNAs (circRNAs), which were then evaluated using reverse transcription quantitative polymerase chain reaction. Logistic regression analysis was conducted to examine the relationship between clinical characteristics, circRNA expression, and adverse pregnancy outcomes. The diagnostic accuracy of circRNAs for early and mid-pregnancy GDM was assessed using receiver operating characteristic curves. Pearson correlation analysis was utilized to explore the relationship between circRNA levels and oral glucose tolerance test results. A predictive model for early GDM was established using logistic regression.
Results: Significant alterations in circRNA expression profiles were detected in GDM patients, with hsa_circ_0031560 and hsa_circ_0000793 notably upregulated during the first and second trimesters. These circRNAs were associated with adverse pregnancy outcomes and effectively differentiated GDM patients, with second trimester cohorts achieving an area under the curve (AUC) of 0.836. In first trimester cohorts, these circRNAs identified potential GDM patients with AUCs of 0.832 and 0.765, respectively. The early GDM prediction model achieved an AUC of 0.904, validated in two independent cohorts.
Conclusion: Hsa_circ_0031560, hsa_circ_0000793, and the developed model serve as biomarkers for early prediction or mid-term diagnosis of GDM, offering clinical tools for early GDM screening.

Keywords: Biomarkers; Cohort studies; Diabetes, gestational; Predictive learning models; ROC curve

INTRODUCTION

Gestational diabetes mellitus (GDM) is a pregnancy complication involving abnormal glucose tolerance, with increasing incidence due to dietary changes [1,2]. It poses serious risks to mothers, such as postpartum hemorrhage, metabolic syndrome, gestational hypertension, and type 2 diabetes mellitus, and to fetuses, increasing the chances of macrosomia, hypogly-

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Received: Apr. 20, 2024; Accepted: Nov. 15, 2024

cemia, and distress [3,4]. The oral glucose tolerance test (OGTT), conducted between the 24th and 28th weeks of pregnancy, is the standard for diagnosing GDM [5]. Early identification of predictive and diagnostic biomarkers is essential to reduce perinatal complications and improve long-term health outcomes for mothers and infants.

Circular RNAs (circRNAs), a distinct class of non-coding RNAs, possess a unique covalently closed loop structure, providing resistance to RNase R enzyme degradation and greater biological stability compared to linear RNAs [6-9]. They act as potent biomarkers and have significant effects in diseases like cancer and GDM [10-12]. Research indicates their diagnostic potential, such as hsa_circRNA_0054633's correlation with glycated hemoglobin A1 (GHBA1) and GHBA1c levels during pregnancy, and hsa-circRNA_0039480's association with OGTT results in GDM patients [13,14]. Despite these promising findings regarding the diagnostic potential of circRNAs for GDM, most research has predominantly concentrated on identifying diagnostic markers during the second trimester, serving as an auxiliary tool. These studies have not yet provided a comprehensive framework for the early detection and diagnosis of GDM.

In this study, we aimed to identify more sensitive and specific early diagnostic markers for GDM by comparing the expression profiles of circRNAs in serum samples from GDM patients and matched controls. We identified two key circRNAs, hsa_circ_0031560 and hsa_circ_0000793, which are significantly upregulated in the serum of GDM patients. Our findings indicate that hsa_circ_0031560 and hsa_circ_0000793 can effectively distinguish between GDM patients and controls during the second trimester, and this distinction is also applicable during the early stages of GDM. By constructing an early GDM prediction model (E-GDMM) utilizing these circRNAs, we were able to significantly differentiate potential patients who may develop GDM. Thus, our study presents a novel opportunity for the early diagnosis of GDM.

METHODS

Study design

This study, approved by the Ethics Committee of Nanjing Maternity and Child Health Care Hospital (Ethical Review Report Number: NFKSL-077) and the Clinical Research Ethics Committee of Zhongda Hospital, Southeast University (Ethical Review Report Number: 2024ZDSYLL299-P01), is both a retrospective case-control study and a nested case-control study. All participants provided informed consent. Pregnant women underwent a 75-g OGTT between the 24th and 28th weeks of gestation. According to the criteria set forth by the International Diabetes and Pregnancy Study Groups, GDM was diagnosed if any of the following glucose levels were observed: fasting plasma glucose ≥5.1 mmol/L, 1-hour post-load glucose ≥10.0 mmol/L, and 2-hour post-load glucose ≥8.5 mmol/L. Exclusion criteria encompassed individuals with other pregnancy-related pathologies, chronic hypertension, multiple gestations, gynecological disorders, hepatic or renal diseases, malignancies, pre-existing type 1 or type 2 diabetes mellitus, or obesity. Subjects who were smokers were also excluded. Serum samples were obtained from all pregnant women, and a comprehensive set of clinical parameters was recorded at the time of sample collection. These parameters included maternal age, gestational age, systolic and diastolic blood pressure, body mass index (BMI), levels of alanine aminotransferase, aspartate aminotransferase, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, total cholesterol, serum creatinine, and blood urea nitrogen.

This study initially included 781 pregnant women at different stages of pregnancy. After excluding six cases with pre-pregnancy type 2 diabetes mellitus, eight cases of multiple pregnancies, and 25 cases lost to follow-up during the second trimester, a total of 358 first trimester, 344 second trimester, and 40 third trimester samples were collected and divided into five cohorts: (1) second trimester cohort 1: 184 second trimester women from Nanjing Maternal and Child Health Hospital, including 92 cases of GDM and 92 controls; (2) second trimester cohort 2: 160 second trimester women from Zhongda Hospital, Southeast University, including 73 GDM cases and 87 controls; (3) third trimester cohort: 80 third trimester women from Zhongda Hospital, Southeast University, including 40 GDM cases and 40 controls; (4) first trimester validation cohort 1: 228 first trimester women from Nanjing Maternal and Child Health Hospital, with 46 cases developing GDM in the second trimester and 182 cases not developing GDM in the second trimester (48 of these were used for model construction, and the remaining were assigned to the early validation cohort); and (5) first trimester validation cohort 2: 130 first trimester women from Zhongda Hospital, Southeast University, with 22 cases developing GDM in the second trimester and 108 cases not developing GDM in the second trimester. In this study, serum samples were collected from all women during

the first trimester (10–14 weeks), second trimester (24–28 weeks) and third trimester (28–35 weeks). First trimester women underwent an OGTT during the second trimester, and based on the results, they were categorized into the group that develop GDM (D-GDM group) and the group that did not develop GDM (UD-GDM group). In addition, we collected serum samples from an additional 40 age-matched healthy non-pregnant women (control group) undergoing routine health check-ups, as well as 40 postpartum women (PW) within 1 week after delivery (PW group) to serve as controls.

Construction and validation of the E-GDMM

The development and validation of the cohort involved the analysis of serum samples from early pregnancy in 20 patients who later D-GDM and 28 who did not. This study focused on evaluating the expression levels of hsa_circ_0031560 and hsa_circ_0000793. Incorporating the expression levels of these circRNAs as independent variables and GDM outcomes as the dependent variable, the E-GDMM was constructed using logistic regression methods. The diagnostic efficacy of the model was validated using receiver operating characteristic (ROC) curve analysis, obtaining key indicators such as sensitivity and specificity to determine the optimal cut-off value.

To verify the stability of the model, early pregnancy cohorts from Nanjing Maternal and Child Health Hospital and Zhongda Hospital, Southeast University, were collected. The expression levels of circRNAs in these samples were similarly measured and input into the model. The sensitivity and specificity in different cohorts were assessed based on the identified cut-offs from the model, thereby validating its robustness and predictive accuracy across multiple populations.

This refined text improves clarity and cohesiveness, making it suitable for academic publication.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 9.5 (GraphPad Software Inc., San Diego, CA, USA) and SPSS version 26.0 (IBM Co., Armonk, NY, USA). Variables were expressed as mean±standard deviation, median (interquartile range), or percentages, depending on their distribution. The Kolmogorov-Smirnov test was first applied to determine the normality of data distributions. For normally distributed variables, the homogeneity of variance test was subsequently performed. When both normal distribution and homogeneity of variance were confirmed, an independent samples *t*-test was utilized. Otherwise, the Mann-Whitney *U* test was employed. Pearson's chi-square test was applied to compare frequencies of categorical variables. A *P* value of less than 0.05 was considered statistically significant. Pearson correlation analysis was used to assess the relationship between two variables. To evaluate the accuracy of circRNAs in distinguishing different groups, ROC curve analysis was performed, and the area under the curve (AUC) was calculated.

RESULTS

Identification of circRNA expression profiles in GDM

To study the expression profile of circRNAs in GDM patients, we reanalyzed next-generation sequencing data from the placental villi tissues of three GDM patients and three normal pregnancies from the study by Yan et al. [15] (Supplementary Methods). A total of 33,574 circRNAs were identified, including 19,359 previously known circRNAs from the circBase database (http://circrna.org/) and 14,215 newly identified circRNAs (Supplementary Fig. 1A). Compared to the 48,270 circRNAs reported by Yan et al. [15], our results differ, possibly due to improved data filtering strategies and updated databases. In addition to features not mentioned in Yan et al.'s study [15], we further discovered that these circRNAs are distributed across all chromosomes, mainly composed of 2–4 exons, with most lengths between 201 and 600 base pairs. Additionally, most genes produce only 1 or 2 circRNAs (Supplementary Fig. 1B-E). This distribution and structural characteristics of circRNAs provide new insights into their functions in GDM. After screening for differentially expressed circRNAs between the two groups, 474 circRNAs exhibited significant changes in expression, with 268 upregulated and 206 downregulated ($|\log_2$ fold change [FC]$| \geq 1$, $P<0.05$) (Fig. 1A and B). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that these differentially expressed circRNAs are mainly involved in pathways such as homologous recombination, lysine degradation, and cortisol synthesis and secretion (Fig. 1C). Furthermore, Gene Ontology (GO) analysis revealed that these circRNAs may influence the development of GDM by regulating biological processes like protein degradation, metabolism, and epithelial cell migration (Fig. 1D). Although our pathway analysis results are similar to those of Yan et al. [15], indicating a close relationship between these circRNAs and glucose and lipid metabolism, we further refined the functional speculations of the differentially expressed circRNAs. These circRNAs may play

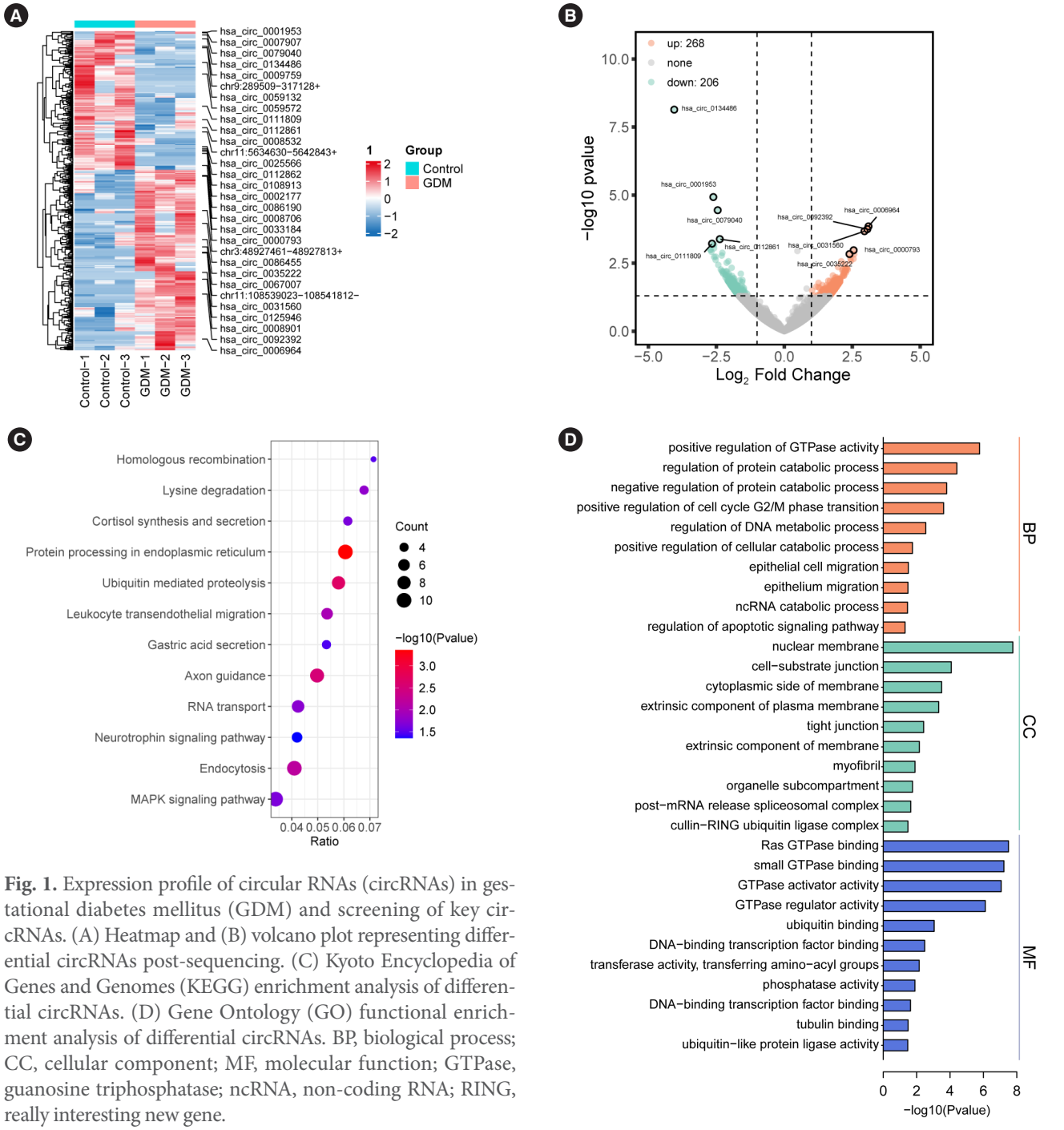

Fig. 1. Expression profile of circular RNAs (circRNAs) in gestational diabetes mellitus (GDM) and screening of key circRNAs. (A) Heatmap and (B) volcano plot representing differential circRNAs post-sequencing. (C) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of differential circRNAs. (D) Gene Ontology (GO) functional enrichment analysis of differential circRNAs. BP, biological process; CC, cellular component; MF, molecular function; GTPase, guanosine triphosphatase; ncRNA, non-coding RNA; RING, really interesting new gene.

more complex roles in the occurrence and development of GDM, providing new clues for future mechanistic studies.

Screening and identification of hsa_circ_0031560 and hsa_circ_0000793

To identify circRNAs with biomarker potential, we employed a progressively stringent filtering approach. First, we conducted an initial screening of the raw data using $|\log_2 FC| > 1$ and $P < 0.05$ as criteria, identifying 474 differentially expressed circRNAs, with 268 upregulated and 206 downregulated (Supplementary Table 1). Next, we tightened the filtering criteria to $|\log_2 FC| > 2$ and $P < 0.05$, selecting 87 differentially expressed cir-

cRNAs, which included 43 upregulated and 44 downregulated circRNAs. Given that highly expressed circRNAs exhibit higher sensitivity and better linear relationships, we focused on the 43 upregulated circRNAs (Supplementary Table 2). Further analysis of the expression stability of these circRNAs in samples led us to select those detected in over 90% of the sequencing samples. Ultimately, five circRNAs met this standard and were identified as potential biomarkers (Supplementary Table 3). Subsequently, we assessed the expression levels of these circRNAs in serum samples from 20 GDM patients in the second trimester and normal control (NC) pregnant women. The results indicated that only hsa_circ_0031560 and hsa_circ_0000793 were significantly upregulated in the serum of GDM patients, consistent with the sequencing data (Fig. 2A). The sequences of relevant primers are listed in Supplementary Table 4. Consequently, these two circRNAs were selected for further investigation. Using the circPrimer software, we identified that hsa_circ_0031560 and hsa_circ_0000793 are derived from the HEAT repeat containing 5A (HEATR5A) and ubiquitin specific peptidase 32 (USP32) genes, respectively, comprising 8 and 4 exons with lengths of 1,160 and 419 bp (Supplementary Fig. 2A). Additionally, agarose gel electrophoresis and Sanger sequencing confirmed the primer specificity for these circRNAs (Fig. 2B, Supplementary Fig. 2B). Furthermore, following RNase R and actinomycin D treatment, both circRNAs demonstrated greater stability compared to their corresponding linear parental genes (Fig. 2C and D). To explore the secretion dynamics of these circRNAs, we cultured the human trophoblast cell line human trophoblast 8 (HTR-8)/SV40 large T-antigen-transfected cells (SVNEO) for 48 hours and measured the expression levels of hsa_circ_0031560 and hsa_circ_0000793 in the culture supernatants at various time points. We observed that the expression of both circRNAs progressively increased over time (Fig. 2E). Collectively, these findings suggest that hsa_circ_0031560 and hsa_circ_0000793 are upregulated in the serum of GDM patients and hold potential as novel biomarkers.

Expression characteristics and clinical significance of hsa_circ_0031560 and hsa_circ_0000793 in GDM

To investigate the expression patterns and clinical significance of hsa_circ_0031560 and hsa_circ_0000793 in GDM, we performed reverse transcription quantitative polymerase chain reaction analysis on serum samples from four groups of women: 140 women who remained free from GDM throughout pregnancy (NC group), 132 women with GDM, 40 PW group, and age-matched non-pregnant women (control group). The analysis revealed that the expression levels of both circRNAs were significantly upregulated during pregnancy compared to the non-pregnant group, with an even more pronounced upregulation observed in the GDM patients. Notably, after delivery and placental removal, the expression levels of both circRNAs rapidly declined, returning to levels comparable to those seen in non-pregnant women (Fig. 3A and B). This suggests a potential link between circRNA expression and the presence of the placenta. Further examination of circRNA expression throughout different pregnancy stages showed that hsa_circ_0031560 expression progressively increased with gestation, with a more marked increase in the GDM group (Fig. 3C). In contrast, hsa_circ_0000793 reached peak expression in the second trimester in GDM patients' serum but exhibited similar levels between the GDM and control groups during the third trimester (Fig. 3D). Clinical data from the second and third trimester groups showed significant differences in OGTT results (Supplementary Table 5). Correlation analysis revealed a strong association between OGTT results and hsa_circ_0031560 expression (Fig. 3E), while a moderate correlation between hsa_circ_0031560 and hsa_circ_0000793 expression was also observed (Fig. 3F). In conclusion, these circRNAs may play critical roles in the development and progression of GDM, particularly through their involvement in placental function and glucose metabolism regulation.

Association of hsa_circ_0031560 and hsa_circ_0000793 with adverse pregnancy outcomes

We systematically monitored and documented the incidence of adverse pregnancy outcomes among women in both the GDM and NC groups across different stages of pregnancy. Our findings revealed a notably higher incidence of adverse pregnancy outcomes in the GDM group compared to the NC group at all stages (Supplementary Table 6). To investigate the potential association between key clinical characteristics, the expression levels of the two circRNAs, and adverse pregnancy outcomes, we performed univariate and multivariate logistic regression analyses on several critical factors. Both analyses identified maternal age, BMI, GDM status, and elevated expression levels of hsa_circ_0031560 and hsa_circ_0000793 as independent risk factors for adverse pregnancy outcomes ($P<0.05$) (Fig. 4A and B). These findings suggest that increased expression levels of hsa_circ_0031560 and hsa_circ_0000793 are significantly associated with a heightened risk of adverse preg-

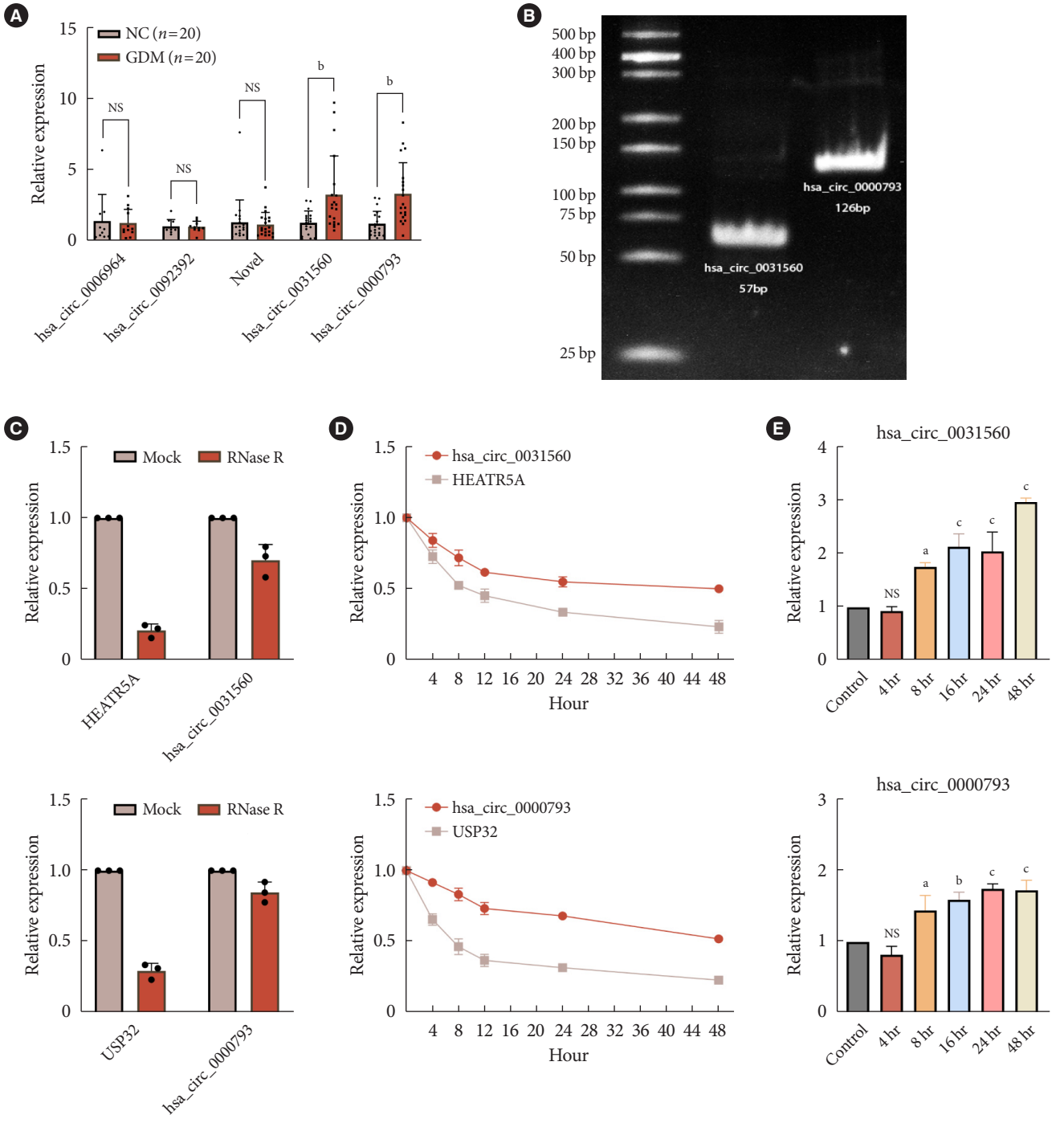

Fig. 2. Screening and characterization of hsa_circ_0031560 and hsa_circ_0000793. (A) The expression levels of the five selected circular RNAs (circRNAs) in the serum of gestational diabetes mellitus (GDM) patients ($n=20$) compared to the matched normal control (NC) group ($n=20$). (B) Agarose gel electrophoresis results of hsa_circ_0031560 and hsa_circ_0000793. Expression of circRNAs and their host genes after treatment with (C) RNase R enzyme or (D) actinomycin D. (E) Expression of circRNAs in cell culture supernatant after continuous cultivation for 48 hours. NS, not significant; HEATR5A, HEAT repeat containing 5A; USP32, ubiquitin specific peptidase 32. [a]$P<0.01$, [b]$P<0.001$, [c]$P<0.0001$.

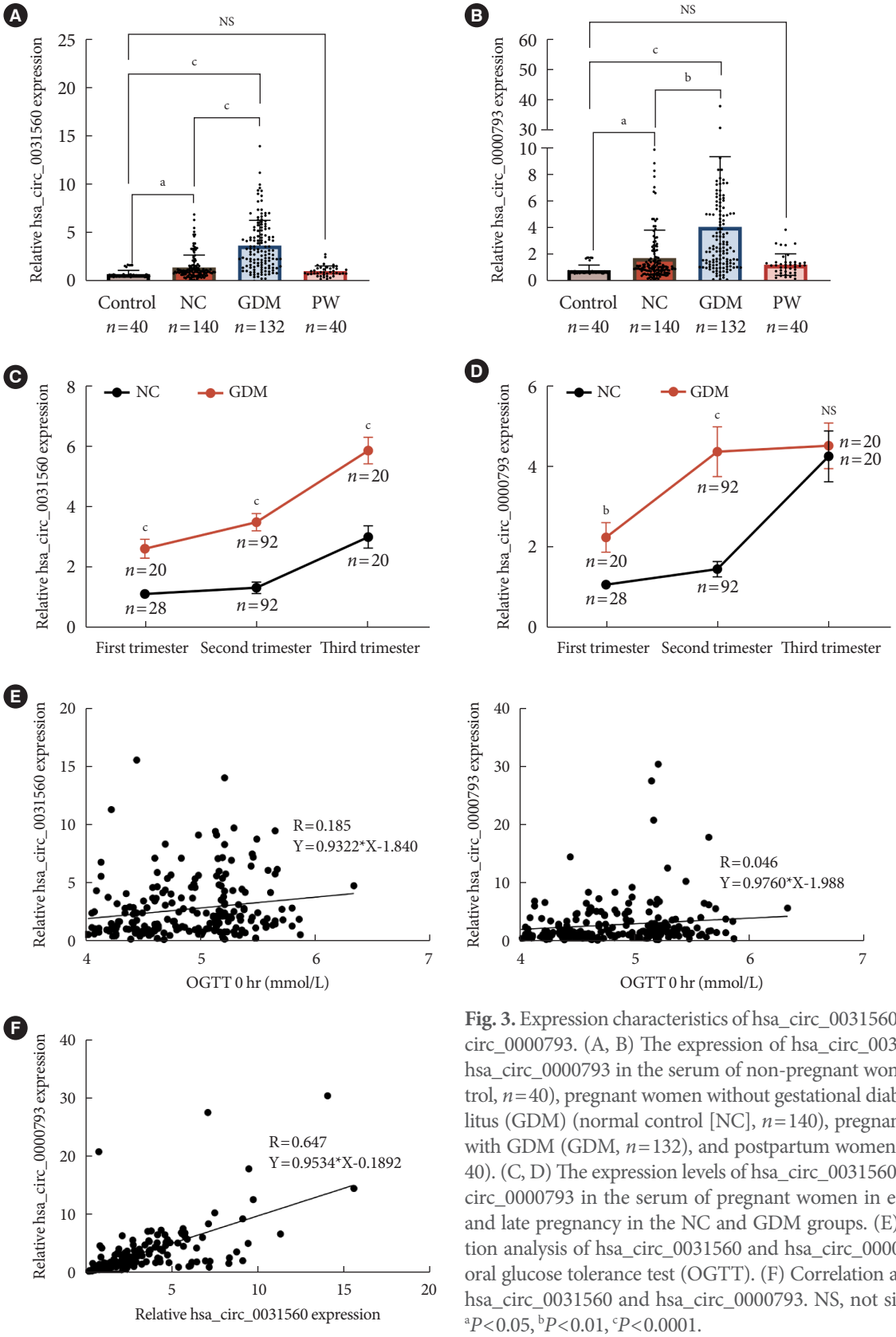

Fig. 3. Expression characteristics of hsa_circ_0031560 and hsa_circ_0000793. (A, B) The expression of hsa_circ_0031560 and hsa_circ_0000793 in the serum of non-pregnant women (control, $n=40$), pregnant women without gestational diabetes mellitus (GDM) (normal control [NC], $n=140$), pregnant women with GDM (GDM, $n=132$), and postpartum women (PW, $n=40$). (C, D) The expression levels of hsa_circ_0031560 and hsa_circ_0000793 in the serum of pregnant women in early, mid, and late pregnancy in the NC and GDM groups. (E) Correlation analysis of hsa_circ_0031560 and hsa_circ_0000793 with oral glucose tolerance test (OGTT). (F) Correlation analysis of hsa_circ_0031560 and hsa_circ_0000793. NS, not significant. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.0001$.

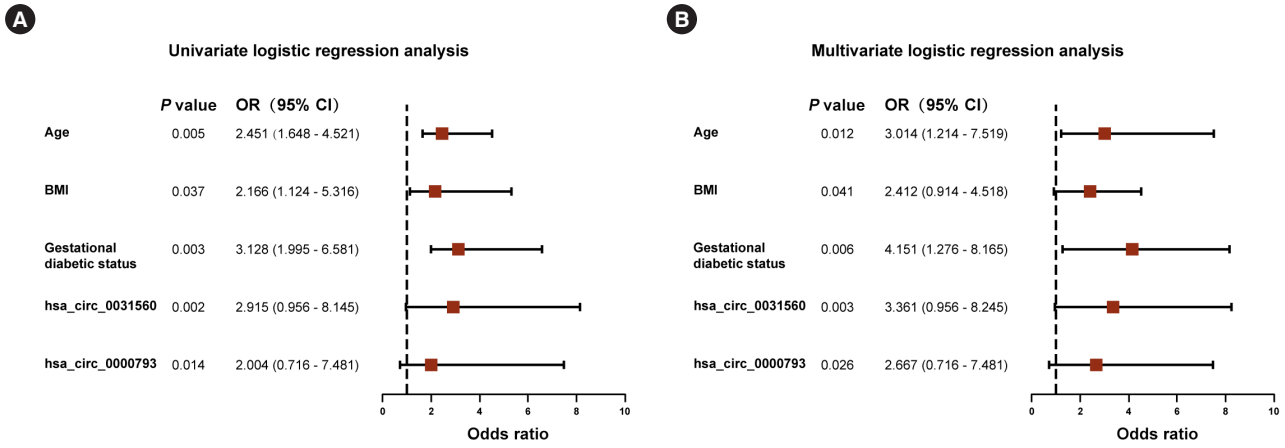

Fig. 4. (A) Univariate and (B) multivariate logistic regression analysis of hsa_circ_0031560 and hsa_circ_0000793 with key clinical characteristics and adverse pregnancy outcomes. OR, odds ratio; CI, confidence interval; BMI, body mass index.

nancy outcomes. Furthermore, advanced maternal age, higher BMI, and GDM status contribute to this elevated risk. This underscores the critical role these circRNAs may play in the occurrence of adverse pregnancy outcomes.

Diagnostic potential of hsa_circ_0031560 and hsa_circ_0000793 for GDM

To assess the viability of hsa_circ_0031560 and hsa_circ_0000793 as diagnostic biomarkers for GDM, we evaluated their expression levels in two independent cohorts and examined their diagnostic performance using ROC curves. In the first cohort during the second trimester, both hsa_circ_0031560 and hsa_circ_0000793 showed significant upregulation in the serum of GDM patients (Supplementary Fig. 3A). The AUC values for diagnosing GDM were 0.802 for hsa_circ_0031560 and 0.789 for hsa_circ_0000793, with a combined AUC of 0.809 (Supplementary Fig. 3B and C). In the second cohort of the second trimester, the upregulated hsa_circ_0031560 and hsa_circ_0000793 demonstrated AUC values of 0.850 and 0.788, respectively, for the diagnosis of GDM. When combined, the AUC increased to 0.865 (Supplementary Fig. 3D-F). Additionally, pooled data from both cohorts facilitated further ROC curve analysis across the entire sample set, resulting in consistent findings. The AUC values for diagnosing GDM using hsa_circ_0031560 and hsa_circ_0000793 were 0.829 and 0.789, respectively, with the combined AUC reaching 0.836 (Fig. 5). The combined diagnostic model exhibited a sensitivity of 78.79% and a specificity of 79.21% (Supplementary Table 7). These results suggest that the combined use of hsa_circ_0031560 and hsa_circ_0000793 holds substantial promise as diagnostic biomarkers for GDM, demonstrating strong sensitivity and specificity across independent cohorts.

Development of an early prediction model for GDM (E-GDMM) using circRNAs and validation in independent cohorts

Recognizing the critical importance of early detection and intervention in GDM to reduce adverse maternal and neonatal outcomes [16], we investigated the roles of hsa_circ_0031560 and hsa_circ_0000793 in the early diagnosis of GDM. Our findings revealed that the expression levels of these two circRNAs were significantly elevated in patients who D-GDM compared to those who did not (UD-GDM) (Supplementary Fig. 4A). ROC curve analysis demonstrated that hsa_circ_0031560 and hsa_circ_0000793 could effectively distinguish D-GDM, with AUC values of 0.894 and 0.835 (Supplementary Fig. 4B). To enhance early screening accuracy, we developed an early prediction model (E-GDMM) using logistic regression, with the formula: E-GDMM = −3.663+1.063×Exp (hsa_circ_0031560)+0.973×Exp (hsa_circ_0000793). This model yielded an AUC of 0.904 and a sensitivity of 85% (Fig. 6A), with an optimal cut-off value of −0.9695 (Fig. 6B). To validate the model's accuracy, we collected early pregnancy serum samples from two independent centers. The clinical characteristics of these validation cohorts showed significant differences in hypertension, OGTT at 0 and 1 hour, and triglyceride levels (Supplementary Table 8). Consistent with our earlier findings, expression levels of hsa_circ_0031560 and hsa_circ_0000793

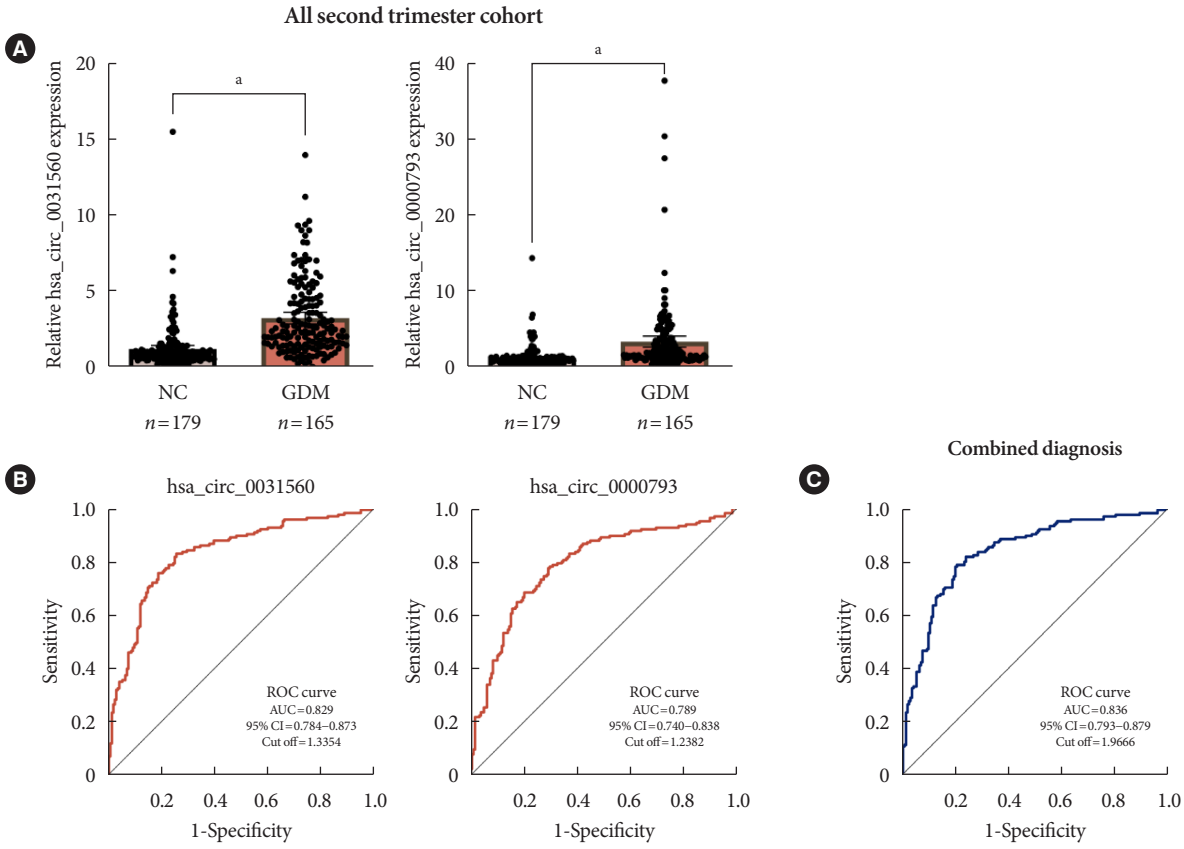

Fig. 5. hsa_circ_0031560 and hsa_circ_0000793 can distinguish gestational diabetes mellitus (GDM) patients in the mid-trimester of pregnancy. (A) Expression levels of hsa_circ_0031560 and hsa_circ_0000793 in the GDM and normal control (NC) groups in all second trimester cohort. (B) Receiver operating characteristic (ROC) curve analysis of hsa_circ_0031560 and hsa_circ_0000793 for GDM patients in all second trimester cohort. (C) Combined ROC curve analysis of hsa_circ_0031560 and hsa_circ_0000793 for GDM patients in all second trimester cohort. AUC, area under the curve; CI, confidence interval. [a]$P<0.0001$.

were elevated in the D-GDM groups across both validation cohorts (Supplementary Fig. 5A, D, and G). The AUC values for hsa_circ_0031560 were 0.860 and 0.774 in the two first trimester validation cohorts, and 0.832 in the combined first trimester validation cohort, while the AUC values for hsa_circ_0000793 were 0.807 and 0.763 in the two first trimester validation cohorts, and 0.765 in the combined first trimester validation cohort (Supplementary Fig. 5A, D, and G). The prediction model showed AUC values of 0.895 in first trimester validation cohort 1, with a sensitivity of 88.46% and specificity of 72.08%, and 0.798 in first trimester validation cohort 2, with a sensitivity of 81.82% and specificity of 66.67% (Supplementary Fig. 5B, C, E, and F). The AUC for the overall cohort was 0.852, with a sensitivity of 89.13% and specificity of 73.63% (Fig. 6C and D, Supplementary Table 9). The model's cut-off value of –0.9695 demonstrated robust performance in distinguishing D-GDM patients, suggesting that it could serve as a valuable tool for the early prediction of GDM.

DISCUSSION

GDM is one of the common metabolic disorders during pregnancy, characterized by elevated blood glucose levels that typically resolve after delivery [17]. GDM poses significant impacts on pregnant women and fetuses worldwide [17,18]. Thus, early prediction of GDM is crucial for preventing pregnancy complications and long-term metabolic diseases. Currently, besides the widely accepted OGTT screening test conducted between 24 and 28 weeks of pregnancy, numerous studies have reported various biochemical markers as independent risk factors for GDM [19]. These include glycosylated hemoglobin [20], peripheral blood leukocyte count [21], C-reactive protein [22],

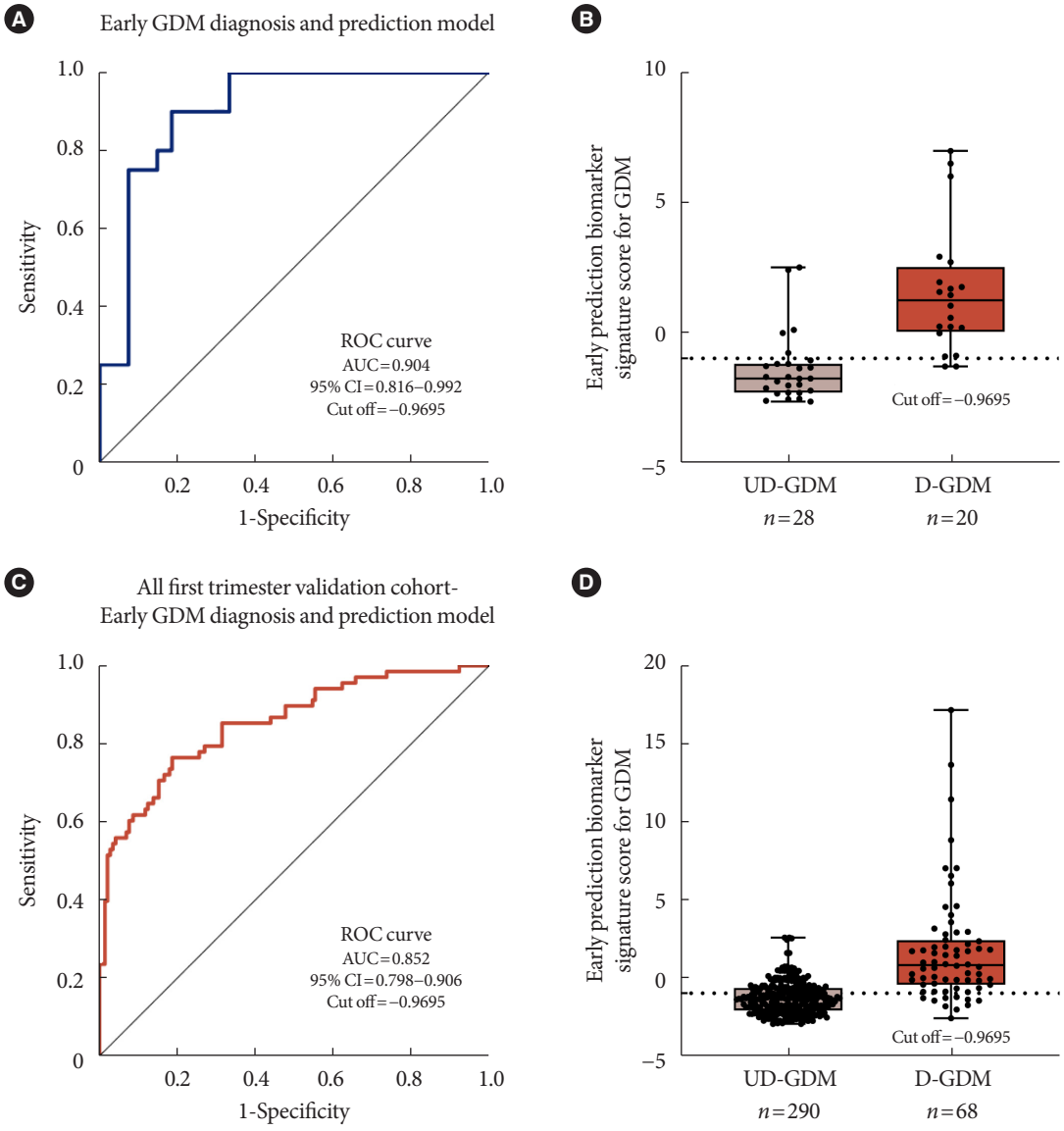

Fig. 6. Construction and validation of the early warning model for gestational diabetes mellitus (GDM). (A) The receiver operating characteristic (ROC) curve for distinguishing GDM patients in the modeling cohort using the model. (B) The optimal cut-off value set by the model. (C, D) The ROC curve and cut-off value for distinguishing GDM patients in all first trimester validation cohort using the model. AUC, area under the curve; CI, confidence interval; UD-GDM, did not develop GDM; D-GDM, develop GDM.

interleukin-6 [23], tumor necrosis factor-alpha [24], and adipocytokines [25]. However, current clinical markers are somewhat limited in sensitivity and finding accurate cut-off points. Therefore, there is a need to identify more effective and accurate early biomarkers to address these challenges.

CircRNAs, a newly identified class of non-coding RNAs characterized by their unique circular structure, have increasingly been recognized due to advancements in high-throughput sequencing technologies [26]. Notably, several circRNAs have shown potential as biomarkers for GDM. For example, hsa_circRNA_0039480, which is highly expressed in GDM patients, has been reported to distinguish GDM with an AUC of 0.898 [14]. Similarly, hsa_circRNA_0054633, also upregulated in GDM patients, exhibits a diagnostic AUC of 0.793 for second trimester GDM [13]. Consistent with these previous findings, our study identified key circRNAs, hsa_circ_0031560 and

hsa_circ_0000793, in the serum of GDM patients and validated their expression in two independent cohorts. Both circRNAs were upregulated in the serum of GDM patients across different cohorts, effectively distinguishing second trimester GDM. The optimal AUC values for diagnosing mid-pregnancy GDM were 0.850 for hsa_circ_0031560 and 0.789 for hsa_circ_0000793. Among these, hsa_circ_0031560 consistently demonstrated higher AUC values, sensitivity, and specificity across second trimester cohort 1, second trimester cohort 2, and all second trimester cohort compared to hsa_circ_0000793, suggesting a stronger potential for differentiating GDM. To enhance diagnostic accuracy, we performed a combined ROC analysis of both circRNAs, which yielded a maximum AUC of 0.865 in different cohorts, with improved sensitivity and specificity compared to the individual markers. This combined analysis highlights the potential of these circRNAs as robust biomarkers for GDM diagnosis.

While combined diagnostics can effectively differentiate second trimester GDM, diagnosing GDM at this stage may not be optimal, as earlier prediction could substantially reduce the risk of severe outcomes. Indeed, recent studies have increasingly focused on early GDM prediction. For example, circLRP6 was found to be upregulated in GDM patients, distinguishing potential GDM cases with an AUC of 0.8809 [27]. Another study identified hsa_circ_0003218, which was significantly downregulated in GDM patients, achieving an AUC of 0.743, with a sensitivity of 54.35% and specificity of 87.10% [28]. Additionally, hsa_circRNA_0039480, highly expressed in the plasma exosomes of GDM patients, demonstrated diagnostic value for early pregnancy GDM with an AUC of 0.704. Moreover, combining hsa_circRNA_0039480 and hsa_circRNA_0026497 significantly improved the diagnostic capability for early pregnancy GDM, reaching an AUC of 0.754 [14]. However, previous early diagnostic biomarkers have faced challenges, such as low sensitivity, making it difficult to screen potential GDM populations effectively, and many studies were based on single-center cohorts, limiting representativeness. To address these limitations, we investigated the potential of hsa_circ_0031560 and hsa_circ_0000793 in identifying patients at risk for GDM. Our study demonstrated that both circRNAs were upregulated in the serum of early pregnancy patients in the D-GDM group, serving as independent diagnostic markers across different cohorts. To further enhance screening accuracy, we developed an early prediction model for GDM using logistic regression, which demonstrated high sensitivity and specificity in distinguishing potential GDM patients. This model was validated across multiple cohorts, showing a robust ability to differentiate D-GDM patients. Unlike previous studies, our approach improved diagnostic accuracy through combined analysis and model construction, thereby increasing screening efficiency and addressing the limitations of single-molecule detection. Although specificity slightly decreased with the combined diagnosis and model, the sensitivity was significantly enhanced. Therefore, our model offers a more effective tool for identifying patients at high risk for GDM, facilitating timely intervention and improving outcomes.

This study found that the expression of hsa_circ_0031560 and hsa_circ_0000793 was significantly upregulated during pregnancy, particularly in GDM patients, and rapidly declined to non-pregnant levels after delivery. Therefore, this finding suggests that the expression of these two circRNAs may be closely related to the presence of the placenta and plays an important role in the pathogenesis of GDM. Further analysis showed that high expression levels of these two circRNAs, along with maternal age, BMI, and GDM status, were independent risk factors for adverse pregnancy outcomes. This finding suggests that, in addition to traditional risk factors, abnormal circRNA expression may be a key mechanism affecting pregnancy outcomes, thus supporting their potential as biomarkers for GDM. Moreover, our study observed significant differences in various metabolic parameters between the GDM and control groups at different stages of pregnancy, with the most notable differences in glucose metabolism and BMI occurring in late pregnancy. This phenomenon further indicates that GDM begins to affect the maternal metabolic environment early in pregnancy and progressively worsens as the pregnancy advances. Previous studies have also supported the regulatory role of circRNAs in GDM [29,30]. For example, circular RNA produced by mitogen activated protein kinase saucer 4 (circMAP3K4) has been positively correlated with pregnancy weight gain and the OGTT AUC, regulating insulin resistance via the miR-6795-5p/protein tyrosine phosphatase, non-receptor type 1 (PTPN1) axis [31]. Additionally, hsa_circ_0005243 was significantly downregulated in the placenta and plasma of GDM patients, potentially contributing to the pathogenesis of GDM by regulating the β-catenin and nuclear factor-κB signaling pathways [32]. These studies suggest that circRNAs play multiple regulatory roles in the pathological processes of GDM. Our research further revealed the specific expression patterns and potential mechanisms of hsa_circ_0031560 and

hsa_circ_0000793 in GDM. In line with previous research on the critical regulatory role of circRNAs in GDM, our study further confirmed the specific expression patterns and potential mechanisms of hsa_circ_0031560 and hsa_circ_0000793 in GDM. Notably, the rapid decline in the expression of these two circRNAs postpartum strongly suggests that their high expression may primarily originate from placental tissue. Therefore, future research should focus on further validating their specific functions and regulatory mechanisms in the placenta, with the goal of providing new insights and potential targets for the diagnosis and treatment of GDM.

In summary, hsa_circ_0031560 and hsa_circ_0000793 are significantly upregulated during the different stages of GDM, demonstrating potential as independent biomarkers for early prediction and mid-term diagnosis of the condition. Notably, we have developed a robust early prediction model for GDM, which may serve as a valuable clinical tool to assist healthcare professionals in screening and identifying GDM at an earlier stage.

SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found online at https://doi.org/10.4093/dmj.2024.0205.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: S.M., Y.C., G.W.
Acquisition, analysis, or interpretation of data: all authors.
Drafting the work or revising: S.M., Y.C., Y.Y., M.M.
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FUNDING

This work was Supported by the National Natural Science Foundation of China (82373781 and 82302609), Natural Science Foundation of Jiangsu Province (BK20230840), Jiangsu Provincial Medical Key Discipline (Laboratory) Cultivation Unit (JSDW202240), the Jiangsu Provincial Key Laboratory of Critical Care Medicine (JSKLCCM202202015), Southeast University Doctoral Students Innovation Ability Enhancement Program (CXJH_SEU_24219), Zhongda Hospital Affiliated to Southeast University, Jiangsu Province High-Level Hospital Pairing Assistance Construction Funds (zdyyxy10) and Zhongda Hospital Affiliated to Southeast University, Jiangsu Province High-Level Hospital Pairing Assistance Construction Funds (zdlyg09).

ACKNOWLEDGMENTS

We appreciate all patients implicated in this study and thank all individuals contributed to this study.

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SUPPLEMENTARY METHODS

Sample collection and processing

Pregnant women's serum samples were collected from September 2022 to July 2024 at Nanjing Maternity and Child Health Care Hospital and Zhongda Hospital, Southeast University. All blood collections were performed in the morning on an empty stomach. Peripheral venous blood (3 mL) was collected, centrifuged at 4,000 rpm for 10 minutes at room temperature, and the serum was collected and stored at –80°C until further use.

Reanalysis of circRNAs sequencing

To investigate the expression profile of circular RNAs (circRNAs) in gestational diabetes mellitus, we reanalyzed the data from the National Center for Biotechnology Information Sequence Read Archive (Accession number: SRP127316, Project ID: PRJNA423147). The dataset can be downloaded from https://www.ncbi.nlm.nih.gov/bioproject/PRJNA423147.

First, adapter contamination was removed from the raw FASTQ files. The cleaned sequences were then aligned to the reference genome hg38 using STAR v2.7.1a (https://github.com/alexdobin/STAR). CircRNAs were identified using the CIRCexplorer program (v2.3.8), which detects both circularization junctions and the spliced sequences of circRNAs. All candidate circRNAs with fewer than two junction reads were discarded, and the remaining circRNAs were considered bona fide circRNAs. For differential expression analysis between the two conditions/groups, we employed the limma R package version 3.46.0 (R Foundation for Statistical Computing, Vienna, Austria). By default, a threshold of $P<0.05$ and $|\log_2$ fold change [FC]$| > 1$ was used to determine significant differential expression.

Total RNA extraction and reverse transcription quantitative polymerase chain reaction

Total RNA was extracted from all serum samples using TRIzol LS Reagent (Invitrogen, Karlsruhe, Germany). The concentration and purity of total RNA were measured using a ultraviolet (UV) spectrophotometer. After quantification, RNA was reverse transcribed into cDNA using HiScript III RT SuperMix for qPCR (Vazyme Biotech Co. Ltd., Nanjing, China) under the conditions of 50°C for 15 minutes and 85°C for 5 seconds. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was then performed using the Taq Pro Universal SYBR qPCR Master Mix (Vazyme Biotech Co. Ltd.). Amplification was carried out on the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) with the program set at 95°C for 10 seconds for denaturation, followed by 60°C for 30 seconds for annealing, over 40 cycles (total volume of 20 μL). The internal reference used was 18s rRNA. Supplementary Table 4 lists the specific primers for all circRNAs (Sangon Biotech, Shanghai, China). Analyses were performed using the $2^{-\Delta\Delta CT}$ method.

Agarose gel electrophoresis

A 3% gel was prepared by repeatedly heating Tris-acetate-EDTA (TAE) buffer and agarose powder in a microwave. Once the agarose had melted, 10,000× GelRed nucleic acid dye (SolarBio Life Science, Beijing, China) was added. After thorough mixing, the solution was poured into a gel casting tray to cool and solidify at room temperature. The gel was then placed in an electrophoresis tank filled with TAE buffer, and PCR products were loaded into the wells. Electrophoresis was conducted at 100 V for 30 to 60 minutes. After electrophoresis, the gel was observed and photographed under UV light.

RNase R and actinomycin D experiments

Total RNA (5 μL) was treated with RNase R enzyme (Geneseed, Guangzhou, China). In a 20 μL reaction mixture, RNase R was incubated at 37°C for 1 minute, followed by deactivation of RNase R at 85°C for 10 minutes. The treated RNA was then reverse transcribed into cDNA for subsequent PCR validation. Actinomycin D was diluted to 2.5 μg/mL with RPMI-1640 medium (Corning, New York, NY, USA) from a stock concentration of 1 mg/mL. Cells were evenly seeded in 6-well plates and, once they reached 80% confluency, the culture medium was replaced with medium containing actinomycin D. Total RNA from the cells was extracted at 0, 4, 8, 12, 24, and 48 hours for PCR analysis.

Cell culture and transfection

The human trophoblast cell line HTR-8/SVneo, purchased from Shanghai Zhong Qiao Xin Zhou Biotechnology Co. Ltd. (Shanghai, China), was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco, Waltham, MA, USA). All cells were authenticated using short tandem repeat (analysis and were free of mycoplasma contamination throughout the experiments). Cells were cultured at 37°C in a 5% CO_2 incubator.

Supplementary Table 1. Differentially expressed circRNAs identified with $|\log_2 FC| > 1$ and $P < 0.05$

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0006964	TOP2A	1	0	1	4	17	6	3.099736006	0.00014028
hsa_circ_0092392	CWF19L1	1	2	0	7	8	7	3.056641667	0.000178133
Novel	EXPH5	1	2	1	10	13	5	2.950583014	0.000211661
hsa_circ_0031560	HEATR5A	1	3	1	6	9	5	2.571415173	0.001489214
hsa_circ_0000793	USP32	4	2	1	5	13	11	2.551783943	0.001058451
hsa_circ_0125946	SORBS2	0	0	0	1	9	9	2.547952063	0.002191674
hsa_circ_0008901	PTPN12	1	0	0	2	5	17	2.446616668	0.002637654
hsa_circ_0086455	PLIN2	0	3	0	14	2	13	2.433069339	0.00210411
hsa_circ_0035222	USP50	0	0	5	15	10	9	2.398799071	0.001460012
hsa_circ_0092998	ATE1	0	0	0	3	7	3	2.333333333	0.005388766
hsa_circ_0082958	KMT2C	1	0	0	7	4	5	2.302296865	0.00490459
hsa_circ_0125480	NR3C2	0	1	0	5	4	7	2.302296865	0.00490459
hsa_circ_0117611	RIF1	0	0	0	3	6	3	2.269118307	0.006934337
hsa_circ_0113557	NRDC	1	0	0	2	7	8	2.251629167	0.006036939
hsa_circ_0126836	KIAA0232	0	2	0	7	7	4	2.245655198	0.005405924
hsa_circ_0131944	HMGCLL1	0	1	0	4	5	6	2.238081839	0.006376199
hsa_circ_0005855	TPM4	3	0	0	5	13	4	2.238081839	0.005039587
hsa_circ_0103783	TRPM7	1	0	0	2	13	4	2.238081839	0.006376199
hsa_circ_0067007	HSPBAP1	3	0	0	10	13	7	2.22727468	0.00339134
Novel	CRYBG3	0	0	0	6	1	6	2.204903281	0.008855832
hsa_circ_0083172	DNAJB6	1	0	0	3	5	7	2.1949875	0.007569235
Novel	SSH2	0	0	0	1	5	7	2.1949875	0.009190574
hsa_circ_0084941	INTS8	0	0	2	6	4	7	2.181440172	0.007038683
hsa_circ_0097961	ERC1	1	0	0	4	5	5	2.163951032	0.008545251
hsa_circ_0047972	ZNF236	0	3	0	8	9	7	2.163951032	0.005349764
Novel	MIR100HG	0	0	1	16	4	1	2.136463645	0.009499478
hsa_circ_0077141	PHIP	1	0	4	5	13	9	2.130772474	0.005672506
hsa_circ_0033184	WARS	8	0	3	8	24	24	2.095904127	0.002681542
hsa_circ_0000096	AC118553.2	0	1	0	3	5	5	2.056641667	0.012813474
Novel	ADAM12	0	0	1	3	5	5	2.056641667	0.012813474
hsa_circ_0109717	MARK4	0	2	0	3	8	5	2.056641667	0.011478365
hsa_circ_0013298	SLC35A3	1	0	0	5	5	3	2.056641667	0.012813474
Novel	TAB3	1	0	0	2	3	11	2.056641667	0.012813474
hsa_circ_0005537	CDC25C	0	0	0	2	5	3	2.056641667	0.015144179
hsa_circ_0036624	PDE8A	0	0	0	3	2	5	2.056641667	0.015144179
hsa_circ_0002393	FAM106A	0	0	0	1	6	4	2.043094339	0.015874663
hsa_circ_0007339	GPRASP2	0	0	0	4	6	1	2.043094339	0.015874663
hsa_circ_0087750	ERP44	1	0	0	5	4	8	2.025605199	0.011896089
hsa_circ_0128057	FAM13B	0	0	1	8	2	4	2.025605199	0.014348223
hsa_circ_0003309	KCTD3	0	0	1	2	8	4	2.025605199	0.014348223
hsa_circ_0118029	UBR3	3	0	0	5	8	4	2.025605199	0.011896089
hsa_circ_0127602	EPB41L4A	0	1	0	3	2	10	2.01479804	0.014918459
hsa_circ_0128672	NSD1	0	0	0	4	12	0	2.007455938	0.017942015
hsa_circ_0034349	SLC12A6	1	0	0	6	2	5	1.992426641	0.016160861

(*Continued to the next page*)

Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
Novel	RNF213	0	1	0	2	6	5	1.992426641	0.016160861
hsa_circ_0093546	CCDC7	0	0	1	1	8	6	1.992426641	0.016160861
hsa_circ_0002824	JAK2	0	0	0	6	2	2	1.992426641	0.018880367
hsa_circ_0068379	VPS8	0	0	0	2	6	2	1.992426641	0.018880367
Novel	STK3	0	0	0	6	2	2	1.992426641	0.018880367
Novel	ARIH2	0	10	7	29	19	8	1.979770691	0.004394791
hsa_circ_0122384	NR2C2	0	0	3	4	5	7	1.968963532	0.014725838
hsa_circ_0008078	DOP1B	1	0	0	3	2	9	1.968963532	0.017557904
hsa_circ_0026238	LARP4	1	0	0	5	3	4	1.968963532	0.017557904
hsa_circ_0018952	POLR3A	0	0	0	4	5	1	1.968963532	0.020428722
hsa_circ_0131343	SMOC2	0	0	0	5	1	4	1.968963532	0.020428722
hsa_circ_0069399	ARAP2	1	0	4	6	13	5	1.959248083	0.011639433
hsa_circ_0036103	UACA	0	0	4	2	13	6	1.959248083	0.014354089
Novel	RHOBTB3	1	0	3	10	6	5	1.950583014	0.012921827
hsa_circ_0043138	TAF15	4	0	1	7	8	7	1.949332302	0.012108816
hsa_circ_0056552	RAB3GAP1	0	0	0	10	0	4	1.927119905	0.023456726
hsa_circ_0129884	RASA1	0	0	2	2	5	8	1.918295834	0.019048247
Novel	ST3GAL1	0	1	0	5	5	2	1.918295834	0.020929174
Novel	TTI2	0	0	0	5	2	2	1.918295834	0.024140769
hsa_circ_0008624	LNPK	0	0	0	2	5	2	1.918295834	0.024140769
hsa_circ_0002463	BAZ2B	0	0	0	8	5	0	1.918295834	0.024140769
hsa_circ_0005729	RNF138	0	0	0	2	2	5	1.918295834	0.024140769
hsa_circ_0006467	SIRT3	1	3	2	10	12	8	1.914944612	0.010339502
hsa_circ_0054345	LRPPRC	0	2	0	7	3	4	1.912321865	0.0194535
hsa_circ_0118239	CWC22	0	0	2	4	3	7	1.912321865	0.0194535
hsa_circ_0064789	CLASP2	0	0	3	4	6	5	1.904748506	0.018613424
hsa_circ_0059061	SEPT2	0	1	0	6	4	2	1.904748506	0.021918353
Novel	FHDC1	0	0	0	0	3	12	1.900146573	0.025599285
hsa_circ_0010064	PLEKHM2	1	2	1	8	3	16	1.890808447	0.014401931
hsa_circ_0080835	PTPN12	0	0	17	4	14	11	1.881285397	0.013134101
hsa_circ_0108786	KDSR	0	0	3	9	3	4	1.881285397	0.020236525
Novel	CYP19A1	1	0	17	6	11	20	1.871569948	0.010714856
hsa_circ_0010144	FBXO42	0	0	2	6	2	6	1.871569948	0.022417881
hsa_circ_0087428	SPIN1	0	0	0	0	6	6	1.871569948	0.028040202
hsa_circ_0097828	NCOR2	0	0	0	6	0	6	1.871569948	0.028040202
hsa_circ_0067594	TFDP2	1	0	2	3	8	7	1.861654167	0.019585964
hsa_circ_0022324	CYB561A3	0	0	2	2	5	7	1.861654167	0.023194236
hsa_circ_0100186	FLT1	0	0	2	1	5	11	1.861654167	0.023194236
hsa_circ_0083823	HMBOX1	1	0	0	3	2	7	1.861654167	0.025331685
hsa_circ_0109427	CEP89	1	0	0	5	3	3	1.861654167	0.025331685
hsa_circ_0073836	RAPGEF6	0	0	0	3	3	2	1.861654167	0.028929514
hsa_circ_0105188	UQCRC2	0	0	0	2	3	3	1.861654167	0.028929514
Novel	CCAR2	0	0	0	3	2	3	1.861654167	0.028929514
Novel	SVEP1	0	0	0	7	0	5	1.861654167	0.028929514
hsa_circ_0017992	ARHGAP21	0	0	0	3	2	3	1.861654167	0.028929514

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Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
Novel	FERMT2	0	0	0	3	3	2	1.861654167	0.028929514
Novel	CAMK2G	0	0	0	3	3	2	1.861654167	0.028929514
Novel	STRN	0	0	0	3	2	3	1.861654167	0.028929514
hsa_circ_0027364	MON2	0	0	0	3	2	3	1.861654167	0.028929514
hsa_circ_0115722	JAM2	0	0	0	2	3	3	1.861654167	0.028929514
hsa_circ_0110829	ILF2	0	0	0	2	3	3	1.861654167	0.028929514
hsa_circ_0120795	WDR92	2	2	0	5	9	6	1.848106839	0.018448969
hsa_circ_0006212	UBAP1	0	2	0	3	6	4	1.848106839	0.024291456
hsa_circ_0008753	AC112229.3	1	1	5	10	9	9	1.839441769	0.014139706
hsa_circ_0000942	VASP	0	1	5	4	4	21	1.839441769	0.017557505
hsa_circ_0138381	DENND4C	1	0	2	12	2	6	1.835931547	0.021484511
hsa_circ_0006565	CUL1	2	4	1	14	5	14	1.830617699	0.013592171
hsa_circ_0002946	MTDH	7	0	0	8	4	7	1.830617699	0.020320263
hsa_circ_0120031	MAP4K3	3	0	0	3	2	14	1.830617699	0.024153844
hsa_circ_0063410	TNRC6B	0	2	0	4	2	8	1.830617699	0.025772208
Novel	WDR48	0	0	1	5	4	2	1.830617699	0.028057772
hsa_circ_0003115	IPO9	1	0	0	2	9	2	1.830617699	0.028057772
hsa_circ_0102147	FERMT2	1	0	0	5	2	4	1.830617699	0.028057772
Novel	OTUD4	0	0	1	2	4	5	1.830617699	0.028057772
hsa_circ_0003277	ST7L	0	0	0	4	0	8	1.830617699	0.031858148
hsa_circ_0111362	CEP350	0	0	0	2	4	2	1.830617699	0.031858148
Novel	MRPL33	1	0	12	12	3	21	1.81981054	0.014907729
hsa_circ_0104352	MAP2K5	2	2	1	9	10	6	1.806263211	0.017672304
hsa_circ_0079943	SUGCT	2	1	0	3	7	7	1.8050125	0.023970091
Novel	TMC4	3	0	1	7	6	5	1.797439141	0.022909752
hsa_circ_0086648	NFX1	0	0	1	5	1	6	1.797439141	0.031239226
Novel	AMPD3	0	1	0	6	1	5	1.797439141	0.031239226
Novel	SEMA3A	0	0	0	5	0	6	1.797439141	0.035238902
hsa_circ_0007370	NUP153	4	0	1	7	3	12	1.792837208	0.021972523
hsa_circ_0049835	NOTCH3	2	2	4	12	10	12	1.789485986	0.013999993
hsa_circ_0060927	CYP24A1	19	0	6	16	19	16	1.789190253	0.008974397
hsa_circ_0008096	YEATS2	1	1	3	4	10	11	1.788774071	0.019569501
hsa_circ_0101677	NPAS3	1	3	1	5	11	8	1.779950001	0.02023331
hsa_circ_0095918	CELF1	0	0	1	2	8	2	1.779950001	0.033033661
hsa_circ_0093883	SIRT1	1	0	6	9	6	7	1.773976032	0.021487199
hsa_circ_0124757	EPHA3	1	0	2	4	15	2	1.773976032	0.02670393
hsa_circ_0103678	SLC30A4	1	0	0	4	3	3	1.773976032	0.033665883
Novel	HUWE1	0	0	0	1	4	3	1.773976032	0.037790246
hsa_circ_0130055	MCTP1	0	0	0	7	0	4	1.773976032	0.037790246
Novel	PHLDB2	0	0	0	4	7	0	1.773976032	0.037790246
hsa_circ_0003722	FAM45A	0	0	0	4	0	7	1.773976032	0.037790246
hsa_circ_0104218	TRIP4	0	0	0	4	3	1	1.773976032	0.037790246
Novel	FAM172A	0	0	0	4	1	3	1.773976032	0.037790246
Novel	PDPR	0	0	0	1	3	4	1.773976032	0.037790246
hsa_circ_0032931	TTC7B	5	0	1	3	16	6	1.769951754	0.022747167

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Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0042860	CRLF3	0	2	5	10	7	7	1.763168872	0.020836977
hsa_circ_0074828	RNF145	1	1	6	6	13	10	1.755595514	0.018795945
hsa_circ_0133959	FAM126A	3	0	0	5	4	4	1.742939563	0.032435829
hsa_circ_0111933	RPS6KC1	1	0	0	4	2	4	1.742939563	0.037114078
Novel	TCTN3	1	0	0	2	4	4	1.742939563	0.037114078
hsa_circ_0030131	DGKH	2	0	5	7	20	3	1.740797474	0.022660377
hsa_circ_0001960	MYO19	3	3	1	13	5	13	1.733224115	0.01968493
hsa_circ_0102474	TMEM229B	0	2	1	3	4	10	1.732132404	0.030798472
hsa_circ_0070268	COPS4	0	1	2	3	4	10	1.732132404	0.030798472
Novel	CSGALNACT1	9	2	0	6	12	11	1.728622182	0.020442298
hsa_circ_0005004	OGDH	1	8	0	9	4	12	1.724790302	0.024045934
hsa_circ_0006656	POLD3	3	1	1	7	5	11	1.723308334	0.024963265
hsa_circ_0008529	ACTR2	1	1	0	3	5	5	1.723308334	0.034581872
hsa_circ_0004568	USP54	1	1	2	7	8	5	1.723308334	0.026910813
hsa_circ_0006704	SNX5	0	2	1	8	5	3	1.723308334	0.031725435
Novel	MTMR10	5	0	0	5	8	3	1.723308334	0.031725435
hsa_circ_0008513	HSPA4	0	0	4	9	5	2	1.723308334	0.033008565
Novel	MED13	0	0	2	8	3	2	1.723308334	0.036608012
hsa_circ_0017701	UPF2	2	0	0	8	3	2	1.723308334	0.036608012
Novel	AC092042.3	0	0	1	1	5	5	1.723308334	0.039441973
hsa_circ_0041195	YWHAE	0	0	1	5	1	5	1.723308334	0.039441973
hsa_circ_0007095	SETBP1	0	1	0	1	8	3	1.723308334	0.039441973
Novel	GLDN	0	0	0	2	2	3	1.723308334	0.043767409
hsa_circ_0016353	RCOR3	0	0	0	5	0	5	1.723308334	0.043767409
Novel	EFTUD2	0	0	0	5	2	1	1.723308334	0.043767409
hsa_circ_0008861	WDR41	0	0	0	2	3	2	1.723308334	0.043767409
hsa_circ_0105948	GLG1	0	0	0	2	2	3	1.723308334	0.043767409
hsa_circ_0020767	TOLLIP	0	0	0	2	3	2	1.723308334	0.043767409
Novel	SNX8	0	0	0	0	5	5	1.723308334	0.043767409
hsa_circ_0009358	GNB1	0	0	0	1	8	1	1.723308334	0.043767409
hsa_circ_0079259	TNRC18	0	0	0	2	1	5	1.723308334	0.043767409
hsa_circ_0133691	WDR86	1	0	10	3	12	14	1.715966232	0.023495195
hsa_circ_0006178	UGGT2	1	0	4	3	7	10	1.712501175	0.029245143
Novel	NEK7	0	1	1	4	6	3	1.709761006	0.036130287
hsa_circ_0101973	LINC01588	0	0	2	6	4	2	1.709761006	0.038210814
hsa_circ_0006824	TNRC18	0	0	1	1	4	6	1.709761006	0.041117464
hsa_circ_0100898	MYCBP2	0	1	0	9	6	0	1.709761006	0.041117464
hsa_circ_0122723	PLD1	0	0	1	4	6	1	1.709761006	0.041117464
hsa_circ_0113574	TUT4	0	0	0	0	4	6	1.709761006	0.04547612
Novel	MAN1A2	0	0	0	4	0	6	1.709761006	0.04547612
hsa_circ_0009017	MRPS35	0	0	0	0	6	4	1.709761006	0.04547612
hsa_circ_0008631	UIMC1	5	1	3	16	13	6	1.705736728	0.01930741
hsa_circ_0110791	GABPB2	4	0	2	7	7	7	1.697703135	0.027848392
hsa_circ_0069236	PROM1	8	3	0	16	8	7	1.695820947	0.021964878
hsa_circ_0102404	SYNE2	0	4	0	7	2	6	1.690129776	0.036794042

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Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0026810	ERBB3	3	2	2	9	14	7	1.686297896	0.022787409
hsa_circ_0005312	SEC23IP	2	0	0	3	4	4	1.686297896	0.041123253
hsa_circ_0063328	DDX17	1	6	10	16	14	19	1.683164997	0.014045392
hsa_circ_0094081	SGPL1	1	3	0	3	10	5	1.681464706	0.034242168
hsa_circ_0017099	NID1	0	2	1	5	10	2	1.681464706	0.036441913
hsa_circ_0000179	DTL	1	0	0	2	10	1	1.681464706	0.044805752
Novel	ATRNL1	0	0	0	2	10	0	1.681464706	0.049198276
hsa_circ_0068957	NOP14	1	2	1	7	6	6	1.676582448	0.031702098
hsa_circ_0002154	RIC8B	1	1	4	5	8	11	1.672640636	0.02827605
hsa_circ_0087265	GNAQ	0	4	1	7	4	7	1.666666667	0.034225422
Novel	GUCY2F	2	2	0	7	8	3	1.666666667	0.035050342
Novel	SUZ12P1	2	0	1	3	5	7	1.666666667	0.038242392
hsa_circ_0106109	ZC3H18	2	0	0	2	3	7	1.666666667	0.043697717
hsa_circ_0008393	DNMBP	0	2	0	7	5	1	1.666666667	0.043697717
Novel	MTMR10	0	0	2	3	3	5	1.666666667	0.043697717
hsa_circ_0004978	HERC2P2	0	0	1	3	1	7	1.666666667	0.046839673
Novel	ANK3	0	0	1	1	3	7	1.666666667	0.046839673
hsa_circ_0009034	MAP3K5	0	0	10	4	9	6	1.663926498	0.033795941
hsa_circ_0071410	PALLD	0	0	3	2	5	6	1.659093308	0.042436807
Novel	XPO6	1	0	0	6	2	2	1.659093308	0.047909334
hsa_circ_0031256	HAUS4	0	1	0	2	2	6	1.659093308	0.047909334
hsa_circ_0003222	LARP4	4	4	1	7	13	13	1.656951218	0.023118473
Novel	SMOC2	1	2	2	6	7	9	1.653119339	0.031151668
hsa_circ_0127161	WDFY3	1	1	2	10	2	10	1.639621079	0.035975311
hsa_circ_0079557	RAPGEF5	3	0	1	11	4	3	1.635630199	0.039845531
hsa_circ_0068851	SLBP	0	1	2	8	3	4	1.635630199	0.042256542
hsa_circ_0137217	WWP1	0	1	1	3	5	4	1.635630199	0.045648166
hsa_circ_0001440	SCLT1	0	0	3	7	4	2	1.635630199	0.045648166
hsa_circ_0018206	CCNY	1	1	0	4	5	3	1.635630199	0.045648166
hsa_circ_0115205	PANK2	0	0	2	9	0	8	1.635630199	0.048035369
Novel	PTPN3	0	2	0	4	5	2	1.635630199	0.048035369
hsa_circ_0004114	ADAMTS6	1	3	7	15	6	16	1.631605921	0.023664385
hsa_circ_0005116	SP100	10	1	1	11	11	8	1.626806128	0.026961574
hsa_circ_0104568	UBE2Q2	8	0	9	11	9	21	1.624823039	0.021812894
hsa_circ_0029549	ANKLE2	0	2	1	6	4	4	1.62208287	0.044113558
hsa_circ_0069789	TMEM165	10	2	9	16	19	27	1.616807881	0.014021416
hsa_circ_0005663	HAUS4	3	1	4	5	15	11	1.615998969	0.028845465
hsa_circ_0059893	RALY	0	2	2	5	6	5	1.602451641	0.043302312
hsa_circ_0115580	PRPF6	3	1	0	6	7	3	1.602451641	0.04435382
hsa_circ_0060664	ARFGEF2	0	1	2	6	3	5	1.602451641	0.046922115
Novel	FNDC3A	0	1	2	3	5	6	1.602451641	0.046922115
hsa_circ_0106238	TTC19	0	0	4	13	1	4	1.602451641	0.048545927
hsa_circ_0007317	CNKSR3	2	0	5	8	5	8	1.584962501	0.039445356
hsa_circ_0077304	UBE2J1	0	6	0	6	2	8	1.584962501	0.048124558
hsa_circ_0078539	EZR	1	1	3	6	5	9	1.571415173	0.04239527

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Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0062525	BCR	2	2	1	12	3	8	1.566813239	0.041935582
hsa_circ_0017105	NID1	6	1	16	5	78	12	1.564788401	0.020084471
hsa_circ_0062563	SMARCB1	1	2	1	3	6	10	1.560608013	0.046709822
hsa_circ_0008403	SPRY4-AS1	0	8	7	11	7	18	1.554321671	0.030802261
hsa_circ_0072575	DEPDC1B	5	2	0	4	6	12	1.553265911	0.043876849
hsa_circ_0110148	AC118553.2	4	1	3	5	11	13	1.551783943	0.036461631
hsa_circ_0088681	FAM129B	2	2	1	6	7	7	1.545809974	0.044976083
hsa_circ_0008246	TBC1D14	5	3	0	6	11	6	1.538236615	0.04325451
hsa_circ_0007808	PPP4R1	0	1	9	11	7	4	1.528320834	0.046570941
Novel	BIRC6	1	2	2	5	8	7	1.528320834	0.047642516
hsa_circ_0073816	ADAMTS19	3	4	4	16	9	13	1.524296556	0.031412563
Novel	TFPI	2	0	6	9	6	6	1.514773505	0.048186332
hsa_circ_0030720	UBAC2	3	4	11	14	17	19	1.497284365	0.02665705
hsa_circ_0110388	SORT1	1	5	2	9	8	8	1.497284365	0.045267901
hsa_circ_0002419	NAV3	2	0	8	4	10	10	1.495301277	0.04864788
hsa_circ_0000479	EPSTI1	2	3	18	16	14	19	1.494463839	0.027426088
Novel	UBE2Q2P1	6	0	6	9	8	11	1.487368584	0.043523331
hsa_circ_0056826	WDSUB1	8	2	2	10	8	17	1.486477206	0.038403993
hsa_circ_0001185	IFNGR2	3	1	13	12	16	10	1.479993085	0.036001762
hsa_circ_0001486	GPBP1	2	3	15	26	9	14	1.466247897	0.032438049
hsa_circ_0007168	HECTD2	1	3	9	11	13	9	1.464105808	0.041799327
hsa_circ_0102727	CEP128	16	0	21	9	28	25	1.444484783	0.028684084
hsa_circ_0118751	WDR12	6	0	11	5	13	19	1.440642698	0.044912158
hsa_circ_0070040	NUP54	4	2	4	9	14	9	1.440642698	0.046201211
hsa_circ_0006970	RAD1	6	1	9	6	17	21	1.435809508	0.039958927
Novel	LINC02109	3	5	6	9	24	12	1.424635527	0.039624968
hsa_circ_0022623	NAA40	5	3	5	18	11	11	1.415975838	0.042698878
hsa_circ_0100875	CLN5	4	4	4	13	11	13	1.41129602	0.045095135
hsa_circ_0006689	SLC15A4	8	0	15	9	14	17	1.40960623	0.043717561
hsa_circ_0083220	ESYT2	8	2	10	14	15	22	1.40537781	0.036194205
hsa_circ_0129299	KIF2A	10	3	12	20	21	22	1.405146554	0.029388108
hsa_circ_0046534	TBCD	8	6	25	31	40	22	1.40113144	0.020261535
hsa_circ_0107593	ABCA5	13	1	16	24	25	12	1.383305954	0.034208717
hsa_circ_0075157	NSD1	4	4	18	18	22	18	1.37587776	0.035292904
hsa_circ_0002024	NAB1	21	2	4	14	12	26	1.331965201	0.046734823
hsa_circ_0005350	MYO1E	16	3	8	16	13	40	1.331660642	0.039092587
hsa_circ_0005720	TMEM56	10	8	29	26	34	46	1.300837385	0.0259526
hsa_circ_0008470	WSB1	5	5	15	20	13	27	1.279034088	0.049094224
hsa_circ_0002457	ATXN2	14	31	27	65	55	49	1.260453238	0.016740721
hsa_circ_0007201	IQGAP1	18	9	34	31	73	37	1.252747418	0.023855699
hsa_circ_0005571	AC007192.1	18	4	15	23	31	24	1.219654361	0.046985962
hsa_circ_0132015	F13A1	65	14	18	107	53	36	1.173338714	0.0233586
hsa_circ_0001246	ATXN10	42	8	21	36	48	44	1.08679831	0.048783056
hsa_circ_0006965	RANBP2	79	16	47	67	82	96	1.022687226	0.031883032
hsa_circ_0000754	SSH2	15	12	23	7	2	11	−1.371825739	0.041658826

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Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0065149	SETD2	9	11	26	0	9	15	−1.446616668	0.036946572
hsa_circ_0008197	FAF1	16	7	11	1	3	9	−1.450165749	0.043947464
hsa_circ_0003856	PDE8A	13	3	16	0	4	8	−1.467654889	0.047553183
hsa_circ_0066290	FLNB	3	10	16	4	0	6	−1.472537148	0.049544675
hsa_circ_0008572	TTC3	9	6	10	3	0	8	−1.472929878	0.049166395
Novel	ANO2	9	10	6	1	5	2	−1.472929878	0.049166395
Novel	GTF3C2	25	5	9	7	2	2	−1.479135104	0.040684674
hsa_circ_0007846	CLEC16A	12	11	26	5	3	7	−1.485109073	0.030090093
hsa_circ_0137606	NIPSNAP3A	29	0	29	0	0	40	−1.485409729	0.045731842
hsa_circ_0006727	SLC25A43	19	21	14	5	4	9	−1.486477206	0.025885628
hsa_circ_0043082	LIG3	13	12	13	6	3	3	−1.502598213	0.033034581
hsa_circ_0079440	PHF14	8	5	14	6	0	4	−1.510831694	0.04350014
Novel	DDX3Y	0	42	47	0	0	86	−1.522761253	0.032890817
hsa_circ_0070635	SEC24B	8	4	7	4	0	2	−1.528320834	0.049502703
hsa_circ_0070473	PDLIM5	5	9	5	4	2	0	−1.528320834	0.049502703
hsa_circ_0008191	KIAA0355	11	13	6	3	2	3	−1.538236615	0.036518788
hsa_circ_0007750	RABGGTA	37	16	33	10	8	8	−1.541190525	0.013207808
hsa_circ_0007486	TBC1D1	24	13	37	2	10	15	−1.551581502	0.015694712
Novel	RERE	20	9	25	5	5	5	−1.553265911	0.021841754
hsa_circ_0015210	PRRC2C	6	2	9	3	0	1	−1.571415173	0.048939162
hsa_circ_0005548	AMBRA1	7	4	7	0	3	2	−1.578988531	0.044005557
hsa_circ_0104256	IGDCC4	14	5	23	3	9	1	−1.584962501	0.026535778
hsa_circ_0044969	PPM1D	9	14	10	3	0	14	−1.593786571	0.027940657
hsa_circ_0138482	SMARCA2	5	4	10	2	0	3	−1.593786571	0.041922265
hsa_circ_0003943	CCZ1B	6	6	8	0	3	3	−1.594878282	0.039187642
hsa_circ_0137288	SLC26A7	28	44	30	7	12	13	−1.598745254	0.007717007
hsa_circ_0008778	PTK2	6	7	11	5	0	3	−1.602451641	0.034660195
hsa_circ_0055160	DYSF	8	3	11	2	0	4	−1.615998969	0.037025758
hsa_circ_0019589	FBXW4	5	2	7	0	0	4	−1.615998969	0.046553154
hsa_circ_0005090	SMYD3	4	4	6	2	0	1	−1.62208287	0.044113558
hsa_circ_0111490	SWT1	10	3	3	1	0	2	−1.624823039	0.043732672
hsa_circ_0040707	USP10	13	1	15	2	0	4	−1.633488109	0.034876413
Novel	GRHL1	8	7	9	1	1	5	−1.635630199	0.030804173
hsa_circ_0000925	CEP89	5	4	5	0	2	1	−1.635630199	0.042256542
Novel	ALG8	5	3	4	0	3	0	−1.635630199	0.045648166
hsa_circ_0109350	ZNF77	5	4	3	0	0	3	−1.635630199	0.045648166
Novel	FBRSL1	4	5	2	2	0	0	−1.635630199	0.048035369
hsa_circ_0003715	UBQLN1	20	12	29	0	9	26	−1.640944046	0.013607372
hsa_circ_0102486	RAD51B	10	5	7	0	0	16	−1.652310426	0.031690231
hsa_circ_0066726	CBLB	6	9	3	0	2	2	−1.653119339	0.036673593
Novel	PECAM1	4	7	6	2	0	2	−1.653119339	0.036673593
hsa_circ_0014982	PPOX	6	2	5	0	1	1	−1.659093308	0.042436807
Novel	LINC01524	7	7	3	3	1	0	−1.666666667	0.035975277
hsa_circ_0119531	FAM228B	7	3	2	0	0	2	−1.666666667	0.043697717
hsa_circ_0067209	EEFSEC	3	1	7	0	1	0	−1.666666667	0.046839673

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Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0076373	UBR2	6	5	13	2	0	5	−1.676582448	0.028655043
hsa_circ_0000017	EXOSC10	10	5	4	4	0	1	−1.681464706	0.032546115
hsa_circ_0138631	DNAJA1	8	4	8	2	1	1	−1.692271866	0.03002005
hsa_circ_0005115	SEC63	5	4	8	3	1	0	−1.692271866	0.03302055
Novel	MORC4	8	4	5	0	3	1	−1.692271866	0.03302055
hsa_circ_0134619	RFC2	1	0	16	0	0	0	−1.695820947	0.047283818
Novel	CREB3L2	10	6	15	2	1	5	−1.698953846	0.021699808
hsa_circ_0008693	TRPM7	10	4	9	3	0	3	−1.701095936	0.027055612
hsa_circ_0099007	AC078927.1	14	1	6	0	2	1	−1.709761006	0.03319356
Novel	DTNB	6	3	4	1	1	0	−1.709761006	0.036130287
hsa_circ_0040559	KARS	4	2	6	0	0	2	−1.709761006	0.038210814
hsa_circ_0003691	ASAP1	4	6	1	1	0	0	−1.709761006	0.041117464
Novel	UTY	0	4	6	0	0	0	−1.709761006	0.04547612
hsa_circ_0079039	PRKAR1B	0	4	6	0	0	0	−1.709761006	0.04547612
hsa_circ_0008172	PARD3B	9	3	7	2	2	0	−1.717334364	0.029496039
Novel	RRN3P1	1	8	5	0	0	2	−1.723308334	0.036608012
Novel	TGM2	1	5	2	0	0	0	−1.723308334	0.043767409
Novel	THSD7A	2	2	3	0	0	0	−1.723308334	0.043767409
hsa_circ_0007789	ZNF510	5	5	0	0	0	0	−1.723308334	0.043767409
hsa_circ_0061307	NCAM2	5	0	5	0	0	0	−1.723308334	0.043767409
hsa_circ_0092745	ABLIM1	5	1	2	0	0	0	−1.723308334	0.043767409
Novel	EPAS1	8	1	7	1	0	1	−1.723308334	0.034581872
hsa_circ_0069865	ADGRL3	13	2	25	1	0	14	−1.728622182	0.020442298
hsa_circ_0007836	ZSWIM4	6	12	3	0	4	1	−1.728622182	0.027643667
Novel	SVEP1	7	9	10	1	0	11	−1.732132404	0.02155824
hsa_circ_0001786	LEPROTL1	8	6	6	0	2	3	−1.733224115	0.025976067
Novel	ADAM12	13	7	7	0	2	7	−1.740797474	0.020856938
hsa_circ_0015068	PBX1	6	3	3	0	2	0	−1.740797474	0.034621296
hsa_circ_0123064	SENP2	6	3	3	0	0	2	−1.740797474	0.034621296
hsa_circ_0000618	DPP8	4	4	2	0	1	0	−1.742939563	0.037114078
Novel	L3MBTL1	7	7	2	0	0	4	−1.754344802	0.029765387
hsa_circ_0008327	NOP58	7	3	14	0	3	2	−1.773976032	0.022414864
hsa_circ_0022007	CELF1	9	3	5	1	2	0	−1.773976032	0.02670393
hsa_circ_0071123	ARHGAP10	7	5	4	0	1	2	−1.773976032	0.02670393
hsa_circ_0132765	FBXL4	3	3	4	0	0	1	−1.773976032	0.033665883
hsa_circ_0133298	KLHDC10	9	1	3	0	0	1	−1.773976032	0.033665883
Novel	HIP1	4	1	3	0	0	0	−1.773976032	0.037790246
Novel	ARHGAP27P1-BPTFP1-KP-NA2P3	4	1	3	0	0	0	−1.773976032	0.037790246
hsa_circ_0032363	SMOC1	3	4	1	0	0	0	−1.773976032	0.037790246
Novel	LPIN1	3	4	1	0	0	0	−1.773976032	0.037790246
hsa_circ_0100623	LRCH1	1	3	4	0	0	0	−1.773976032	0.037790246
Novel	ARIH2	3	1	4	0	0	0	−1.773976032	0.037790246
hsa_circ_0016121	SOX13	8	2	8	0	1	2	−1.779950001	0.026158335

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Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0006509	MYO9A	2	2	8	0	1	0	−1.779950001	0.033033661
hsa_circ_0080368	TMEM248	12	6	8	3	0	4	−1.785263849	0.018565169
Novel	ADAM12	5	7	5	0	0	6	−1.78752336	0.02454236
hsa_circ_0030694	FARP1	4	4	4	0	0	2	−1.793607261	0.029156585
hsa_circ_0061232	PCMTD2	6	4	5	0	0	4	−1.797439141	0.025706042
hsa_circ_0059071	FARP2	9	2	6	4	0	0	−1.797439141	0.025706042
hsa_circ_0079884	ELMO1	3	2	6	1	0	0	−1.797439141	0.031239226
Novel	PAPPA	6	2	3	0	0	1	−1.797439141	0.031239226
Novel	SHANK2	6	5	1	0	0	1	−1.797439141	0.031239226
hsa_circ_0027244	AC137834.1	2	3	6	1	0	0	−1.797439141	0.031239226
hsa_circ_0004237	ERMARD	6	2	1	0	0	0	−1.797439141	0.035238902
hsa_circ_0089922	PIR	0	5	6	0	0	0	−1.797439141	0.035238902
hsa_circ_0029590	CHFR	2	6	1	0	0	0	−1.797439141	0.035238902
Novel	DHX15	6	1	2	0	0	0	−1.797439141	0.035238902
Novel	PRKAR1B	0	5	6	0	0	0	−1.797439141	0.035238902
hsa_circ_0026728	COPZ1	6	1	2	0	0	0	−1.797439141	0.035238902
hsa_circ_0008949	ANKRD13A	3	7	3	0	0	2	−1.8050125	0.028076219
hsa_circ_0006799	QRICH2	10	8	3	2	0	2	−1.81981054	0.020481503
hsa_circ_0098837	ATP5F1B	8	7	4	0	3	1	−1.830617699	0.020320263
hsa_circ_0006461	KDM4B	4	5	8	2	1	0	−1.830617699	0.021895503
hsa_circ_0064459	NR2C2	4	3	8	0	0	3	−1.830617699	0.024153844
hsa_circ_0054853	XPO1	8	1	4	0	1	0	−1.830617699	0.028057772
hsa_circ_0006030	TMEM120B	4	2	5	0	0	1	−1.830617699	0.028057772
hsa_circ_0105169	LYRM1	4	2	2	0	0	0	−1.830617699	0.031858148
hsa_circ_0094044	DDX21	2	4	2	0	0	0	−1.830617699	0.031858148
hsa_circ_0131402	KIF13A	4	2	2	0	0	0	−1.830617699	0.031858148
hsa_circ_0118684	BMPR2	2	2	4	0	0	0	−1.830617699	0.031858148
hsa_circ_0044966	APPBP2	2	2	4	0	0	0	−1.830617699	0.031858148
Novel	GMEB1	4	2	2	0	0	0	−1.830617699	0.031858148
hsa_circ_0009164	AC012213.5	2	4	2	0	0	0	−1.830617699	0.031858148
hsa_circ_0075011	NPM1	2	2	4	0	0	0	−1.830617699	0.031858148
hsa_circ_0137355	VIRMA	4	2	2	0	0	0	−1.830617699	0.031858148
hsa_circ_0003504	HBS1L	18	4	11	0	4	4	−1.836987306	0.014087844
Novel	RGPD6	10	6	5	0	1	4	−1.843273649	0.018236968
hsa_circ_0003777	UBR4	9	9	6	4	0	2	−1.848106839	0.015882101
hsa_circ_0002958	FARS2	13	4	7	3	2	0	−1.848106839	0.016987258
hsa_circ_0050834	RYR1	9	1	6	2	0	0	−1.848106839	0.024291456
Novel	MUC20-OT1	10	9	16	1	4	3	−1.84896482	0.011353414
hsa_circ_0086462	DENND4C	14	5	7	2	0	4	−1.861654167	0.015061932
hsa_circ_0121951	ACAD11	8	3	3	0	0	2	−1.861654167	0.023194236
hsa_circ_0090830	FGD1	5	3	3	1	0	0	−1.861654167	0.025331685
hsa_circ_0064557	TBC1D5	2	3	3	0	0	0	−1.861654167	0.028929514
hsa_circ_0044895	TUBD1	3	2	3	0	0	0	−1.861654167	0.028929514
Novel	BANP	8	5	8	9	0	0	−1.867628136	0.016635673
hsa_circ_0003060	SUCLG2	5	2	10	1	0	1	−1.876452207	0.020585354

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Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0002754	KAT6A	17	3	17	0	1	12	−1.879803428	0.011648474
hsa_circ_0008830	EXT2	3	4	4	0	1	0	−1.881285397	0.023725075
Novel	RAP1GAP2	4	2	9	0	2	0	−1.881285397	0.0216786
hsa_circ_0002849	POMT2	4	3	4	0	0	1	−1.881285397	0.023725075
hsa_circ_0096833	AMOTL1	17	1	19	1	0	6	−1.894832725	0.013502286
Novel	SND1	12	0	3	0	0	0	−1.900146573	0.025599285
hsa_circ_0014878	DCAF8	2	4	6	0	0	1	−1.904748506	0.021918353
hsa_circ_0007294	ANKS1B	8	8	19	4	0	5	−1.918295834	0.009379418
hsa_circ_0011115	EYA3	8	5	5	1	0	2	−1.918295834	0.015901355
hsa_circ_0064656	TGFBR2	8	2	3	1	0	0	−1.918295834	0.020929174
Novel	FAM13A	2	5	2	0	0	0	−1.918295834	0.024140769
hsa_circ_0044270	NPEPPS	2	2	5	0	0	0	−1.918295834	0.024140769
hsa_circ_0001192	BRWD1	5	2	2	0	0	0	−1.918295834	0.024140769
Novel	AF117829.1	10	0	4	0	0	0	−1.927119905	0.023456726
hsa_circ_0067494	IL20RB	3	1	6	0	0	0	−1.935784974	0.022800697
hsa_circ_0002503	TRPA1	16	21	17	2	4	7	−1.936642955	0.004680557
hsa_circ_0065390	PFKFB4	5	6	10	0	1	3	−1.950583014	0.012921827
hsa_circ_0074332	HDAC3	7	4	5	3	0	0	−1.968963532	0.014725838
hsa_circ_0011385	EIF3I	8	4	3	0	0	2	−1.968963532	0.01589049
hsa_circ_0075267	RUFY1	2	3	4	0	0	0	−1.968963532	0.020428722
Novel	GABRR2	2	3	4	0	0	0	−1.968963532	0.020428722
hsa_circ_0110195	UBE4B	2	3	4	0	0	0	−1.968963532	0.020428722
hsa_circ_0072548	GPBP1	9	1	2	0	0	0	−1.968963532	0.020428722
Novel	TBC1D5	5	1	4	0	0	0	−1.968963532	0.020428722
hsa_circ_0003605	ZMIZ1	2	3	4	0	0	0	−1.968963532	0.020428722
hsa_circ_0105938	GLG1	11	0	4	0	0	0	−1.968963532	0.020428722
hsa_circ_0035554	ANXA2	10	4	8	0	1	3	−1.983761572	0.011340108
Novel	GLUD1	6	2	2	0	0	0	−1.992426641	0.018880367
Novel	NFIB	7	5	7	1	2	0	−2	0.011637498
Novel	TFRC	3	1	7	0	0	0	−2	0.018402429
hsa_circ_0013215	BCAR3	9	4	12	0	1	4	−2.007455938	0.009574398
Novel	NR1D2	10	1	2	0	0	0	−2.01479804	0.017498269
hsa_circ_0088036	SUSD1	4	6	3	1	0	0	−2.043094339	0.013465057
hsa_circ_0004776	PMS1	10	6	10	2	1	1	−2.04708522	0.007602408
hsa_circ_0008706	PALM2-AKAP2	14	11	41	2	4	6	−2.056641667	0.002682346
hsa_circ_0076710	PTCHD4	17	3	3	0	1	1	−2.056641667	0.010554544
Novel	SYNJ2	2	7	2	0	0	0	−2.056641667	0.015144179
hsa_circ_0062997	SYN3	3	5	2	0	0	0	−2.056641667	0.015144179
hsa_circ_0129814	RASGRF2	5	1	5	0	0	0	−2.056641667	0.015144179
hsa_circ_0100626	LRCH1	7	2	2	0	0	0	−2.056641667	0.015144179
Novel	SRGAP2B	5	2	3	0	0	0	−2.056641667	0.015144179
hsa_circ_0044962	APPBP2	4	4	2	0	0	0	−2.076272897	0.014136922
Novel	TFPI	74	0	0	0	0	0	−2.076272897	0.014136922
hsa_circ_0069864	ADGRL3	7	1	18	0	3	0	−2.082642504	0.009533516
hsa_circ_0062888	LIMK2	3	9	1	0	0	0	−2.107309365	0.01266206

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Supplementary Table 1. Continued

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0098656	SENP1	12	6	7	0	2	2	−2.112623213	0.006390308
hsa_circ_0086190	DOCK8	15	10	20	4	2	2	−2.119965315	0.003036541
Novel	BTBD3	7	4	14	6	0	0	−2.140487923	0.006226111
hsa_circ_0004500	BCAR1	2	0	59	0	0	1	−2.163951032	0.008545251
hsa_circ_0002537	UBE2V2	8	3	4	0	0	1	−2.163951032	0.008545251
hsa_circ_0017728	DHTKD1	4	1	8	0	0	0	−2.163951032	0.010310554
Novel	RARS2	4	6	5	1	0	0	−2.238081839	0.006376199
hsa_circ_0055855	AFF3	6	2	4	0	0	0	−2.238081839	0.007811826
hsa_circ_0047104	MIB1	6	1	7	0	0	0	−2.269118307	0.006934337
hsa_circ_0008532	CBFA2T2	15	14	20	0	3	10	−2.279925467	0.001316616
hsa_circ_0112861	SCCPDH	35	9	7	0	2	7	−2.302296865	0.001655549
hsa_circ_0103482	RTF1	7	2	4	0	0	0	−2.302296865	0.006093058
hsa_circ_0109754	DHX34	3	3	7	0	0	0	−2.333333333	0.005388766
hsa_circ_0007907	ZFY	1	63	114	0	0	105	−2.372523199	0.000413796
Novel	AC104389.4	0	2	51	0	0	0	−2.428467406	0.003656458
hsa_circ_0079040	PRKAR1B	6	99	167	3	14	11	−2.450558479	3.57934E-05
hsa_circ_0025566	PDE3A	7	3	11	1	0	0	−2.528320834	0.00182107
hsa_circ_0059132	D2HGDH	5	7	3	0	0	0	−2.528320834	0.002388549
hsa_circ_0112862	SCCPDH	11	7	19	2	2	0	−2.578988531	0.000661384
Novel	DOCK8	14	11	5	0	4	0	−2.584962501	0.000893061
hsa_circ_0009759	EXOSC10	3	8	5	0	0	0	−2.584962501	0.001859674
hsa_circ_0001953	ZFY	0	233	549	0	0	563	−2.611367059	1.1772E-05
hsa_circ_0111809	HHAT	6	11	14	0	0	4	−2.659093308	0.000604967
hsa_circ_0059572	RALGAPA2	10	2	7	0	0	0	−2.681464706	0.001196069
hsa_circ_0002177	ARHGAP10	6	4	7	0	0	0	−2.709761006	0.001047065
hsa_circ_0108913	ATP9B	7	3	8	0	0	0	−2.723308334	0.000981867
hsa_circ_0134486	PRKAR1B	4	80	100	0	0	8	−4.050021526	7.22909E-09

log_2FC, log_2 fold change; circRNAs, circular RNAs; GDM, gestational diabetes mellitus.

Supplementary Table 2. Screen the specific information of 43 circRNAs that meet the criteria of $log_2FC > 2$ and $P < 0.05$

circBase_id	Gene	Control-1_ Count	Control-2_ Count	Control-3_ Count	GDM-1_ Count	GDM-2_ Count	GDM-3_ Count	Log_2FC	*P* value
hsa_circ_0000096	AC118553.2	0	1	0	3	5	5	2.056641667	0.012813474
Novel	ADAM12	0	0	1	3	5	5	2.056641667	0.012813474
hsa_circ_0092392	CWF19L1	1	2	0	7	8	7	3.056641667	0.000178133
hsa_circ_0083172	DNAJB6	1	0	0	3	5	7	2.1949875	0.007569235
hsa_circ_0127602	EPB41L4A	0	1	0	3	2	10	2.01479804	0.014918459
hsa_circ_0097961	ERC1	1	0	0	4	5	5	2.163951032	0.008545251
hsa_circ_0087750	ERP44	1	0	0	5	4	8	2.025605199	0.011896089
Novel	EXPH5	1	2	1	10	13	5	2.950583014	0.000211661
hsa_circ_0128057	FAM13B	0	0	1	8	2	4	2.025605199	0.014348223
hsa_circ_0031560	HEATR5A	1	3	1	6	9	5	2.571415173	0.001489214
hsa_circ_0131944	HMGCLL1	0	1	0	4	5	6	2.238081839	0.006376199
hsa_circ_0067007	HSPBAP1	3	0	0	10	13	7	2.22727468	0.00339134
hsa_circ_0084941	INTS8	0	0	2	6	4	7	2.181440172	0.007038683
hsa_circ_0003309	KCTD3	0	0	1	2	8	4	2.025605199	0.014348223
hsa_circ_0126836	KIAA0232	0	2	0	7	7	4	2.245655198	0.005405924
hsa_circ_0082958	KMT2C	1	0	0	7	4	5	2.302296865	0.00490459
hsa_circ_0109717	MARK4	0	2	0	3	8	5	2.056641667	0.011478365
Novel	MIR100HG	0	0	1	16	4	1	2.136463645	0.009499478
hsa_circ_0125480	NR3C2	0	1	0	5	4	7	2.302296865	0.00490459
hsa_circ_0113557	NRDC	1	0	0	2	7	8	2.251629167	0.006036939
hsa_circ_0077141	PHIP	1	0	4	5	13	9	2.130772474	0.005672506
hsa_circ_0086455	PLIN2	0	3	0	14	2	13	2.433069339	0.00210411
hsa_circ_0008901	PTPN12	1	0	0	2	5	17	2.446616668	0.002637654
hsa_circ_0013298	SLC35A3	1	0	0	5	5	3	2.056641667	0.012813474
Novel	TAB3	1	0	0	2	3	11	2.056641667	0.012813474
hsa_circ_0006964	TOP2A	1	0	1	4	17	6	3.099736006	0.00014028
hsa_circ_0005855	TPM4	3	0	0	5	13	4	2.238081839	0.005039587
hsa_circ_0103783	TRPM7	1	0	0	2	13	4	2.238081839	0.006376199
hsa_circ_0118029	UBR3	3	0	0	5	8	4	2.025605199	0.011896089
hsa_circ_0000793	USP32	4	2	1	5	13	11	2.551783943	0.001058451
hsa_circ_0035222	USP50	0	0	5	15	10	9	2.398799071	0.001460012
hsa_circ_0033184	WARS	8	0	3	8	24	24	2.095904127	0.002681542
hsa_circ_0047972	ZNF236	0	3	0	8	9	7	2.163951032	0.005349764

circRNAs, circular RNAs; log_2FC, log_2 fold change; GDM, gestational diabetes mellitus.

Supplementary Table 3. Detailed information on the five selected circRNAs

circBase_id	Gene	Control-1_Count	Control-2_Count	Control-3_Count	GDM-1_Count	GDM-2_Count	GDM-3_Count	Log_2FC	*P* value	Positon	Type
hsa_circ_0006964	TOP2A	1	0	1	4	17	6	3.099736	0.00014	chr17:40395448-40399131-	Exon
hsa_circ_0092392	CWF19L1	1	2	0	7	8	7	3.056642	0.000178	chr10:100243697-100250332-	Exon
Novel	EXPH5	1	2	1	10	13	5	2.950583	0.000212	chr11:108539023-108541812-	Exon
hsa_circ_0031560	HEATR5A	1	3	1	6	9	5	2.571415	0.001489	chr14:31347747-31374968-	Exon
hsa_circ_0000793	USP32	4	2	1	5	13	11	2.551784	0.001058	chr17:60265411-60271481-	Exon

circRNAs, circular RNAs; GDM, gestational diabetes mellitus; log_2FC, log_2 fold change.

Supplementary Table 4. Primer sequences used in this study

circRNAs	Primer sequences (5′-3′)	
	Forward	Reverse
hsa_circ_0006964	GAGAACCAGAAAATAAGCCT	GAACCTTTTGCTGGGCTTCT
hsa_circ_0092392	CAACATCGGCACACATAAACC	AGGGTTTTCAGTGACATCCG
Novel (EXPH5)	TCCCGGATGAGCTTCAGATC	CCATTCACCAGTCACTCCCT
hsa_circ_0031560	TGATGTGCAGGTCCTGCAGT	CCACAACAGCAGAACTCGAG
hsa_circ_0000793	CACAAAGTGGAGGAATCAGACAT	CACAAAGTGGAGGAATCAGACAT
18s rRNA	CGGCTACCACATCCAAGGAA	CGGCTACCACATCCAAGGAA

circRNAs, circular RNAs.

Supplementary Table 5. Clinical characteristics of independent cohorts in the first, second, and third trimesters

Variable	First trimester cohort			Second trimester cohort 1			Third trimester cohort		
	D-GDM (*n*=20)	UD-GDM (*n*=28)	*P* value	GDM (*n*=92)	NC (*n*=92)	*P* value	GDM (*n*=40)	NC (*n*=40)	*P* value
Age, yr	29.65±2.15	30.11±3.14	0.527	30.25±3.72	29.75±3.10	0.405	30.15±2.84	31.20±3.55	0.541
Gestational, day	70.25±12.36	70.92±14.58	0.715	172.85±11.79	171.25±8.08	0.358	212.00±16.52	217.59±6.85	0.215
Systolic pressure, mm Hg	120.25±13.65	116.67±14.25	0.135	114.82±9.44	111.44±10.69	0.067	120.58±9.61	121.78±12.64	0.446
Diastolic pressure, mm Hg	71.36±8.26	69.38±7.14	0.328	75.06±6.91	70.45±6.90	<0.0001	71.52±9.12	77.51±7.52	0.581
BMI, kg/m^2	22.58±3.98	22.12±4.87	0.334	23.99±3.18	21.76±2.81	<0.0001	27.16±4.12	24.25±3.55	<0.0001
OGTT 0 hr, mmol/L	4.68±0.28	4.28±0.61	0.089	5.06±0.44	4.51±0.41	<0.0001	5.51±0.52	4.25±0.63	<0.0001
OGTT 1 hr, mmol/L	9.02±1.55	8.31±1.69	0.061	9.68±1.69	7.65±1.47	<0.0001	10.12±2.18	7.25±1.85	<0.0001
OGTT 2 hr, mmol/L	7.92±1.05	6.58±1.18	0.147	8.52±1.61	6.65±1.21	<0.0001	8.96±1.55	6.58±1.24	<0.0001
Total cholesterol, mmol/L	4.85±5.36	4.66±3.15	0.841	6.26±0.94	6.21±1.03	0.801	5.29±1.08	5.38±0.76	0.724
Triglycerides, mmol/L	1.95±0.36	1.25±0.61	0.061	2.12±1.12	2.56±1.25	0.022	2.45±1.09	2.04±0.63	0.015
LDL-C, mmol/L	1.71±0.61	1.91±0.81	0.041	2.90±0.70	2.26±0.42	<0.0001	2.68±0.54	2.73±0.38	0.071
HDL-C, mmol/L	2.81±0.82	3.02±1.04	0.514	2.59±0.71	3.22±0.87	<0.0001	2.69±0.81	2.61±0.92	0.152
ALT, U/L	24.81±19.62	22.14±16.84	0.718	21.64±11.75	21.68±12.43	0.985	23.56±4.89	22.18±3.17	0.261
AST, U/L	20.51±6.81	22.51±7.94	0.338	21.52±5.84	19.94±8.02	0.219	22.98±3.81	23.58±4.12	0.312
Serum creatinine, μmol/L	33.18±7.18	34.82±7.51	0.229	36.04±6.29	34.71±6.23	0.239	33.88±6.94	32.05±7.12	0.116
BUN, mmol/L	2.85±0.64	3.12±0.21	0.591	2.94±0.88	2.92±0.71	0.910	3.05±0.32	2.91±0.47	0.165

Values are presented as mean±standard deviation.
D-GDM, develop GDM; UD-GDM, did not develop GDM; GDM, gestational diabetes mellitus; NC, normal control; BMI, body mass index; OGTT, oral glucose tolerance test; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

Supplementary Table 6. Specific details of adverse pregnancy outcomes across different groups in cohorts at various stages of pregnancy

Pregnancy period grouping	Group	Maternal						Fetal					
		Polyhydramnios, *n*	Oligohydramnios, *n*	Dystocia, *n*	Postpartum hemorrhage, *n*	Preeclampsia, *n*	Placenta previa, *n*	Placenta previa, *n*	Macrosomia, *n*	Neonatal asphyxia, *n*	Neonatal hypoglycemia, *n*	Incidence of adverse outcomes, *n* (%)	*P* value
First trimester	GDM (*n*=20)	1	0	0	0	1	4	1	2	0	0	9 (45.00)	0.0021
	NC (*n*=28)	0	0	0	0	0	2	0	0	0	0	2 (7.14)	
Second trimester	GDM (*n*=92)	10	8	1	2	5	13	3	5	1	7	55 (59.78)	<0.0001
	NC (*n*=92)	2	0	1	1	2	4	1	1	0	0	12 (13.04)	
Third trimester	GDM (*n*=40)	6	5	0	1	0	6	1	1	0	0	20 (50.00)	0.0134
	NC (*n*=40)	4	1	0	0	0	2	0	1	0	0	8 (20.00)	

GDM, gestational diabetes mellitus; NC, normal control.

Supplementary Table 7. The diagnostic value for gestational diabetes mellitus in different cohorts

circRNAs	Cohort	Sensitivity, %	Specificity, %	Accuracy, %
hsa_circ_0031560	Second trimester cohort 1	78.26	79.12	78.69
	Second trimester cohort 2	90.41	70.11	79.38
	All second trimester cohort	83.03	74.16	78.43
hsa_circ_0000793	Second trimester cohort 1	75.00	74.73	74.86
	Second trimester cohort 2	83.56	65.52	73.75
	All second trimester cohort	78.18	70.22	74.05
Combined diagnosis	Second trimester cohort 1	68.48	87.91	78.14
	Second trimester cohort 2	93.15	67.82	79.38
	All second trimester cohort	78.79	79.21	79.01

circRNAs, circular RNAs.

Supplementary Table 8. Clinical characteristics of an independent cohort in early pregnancy

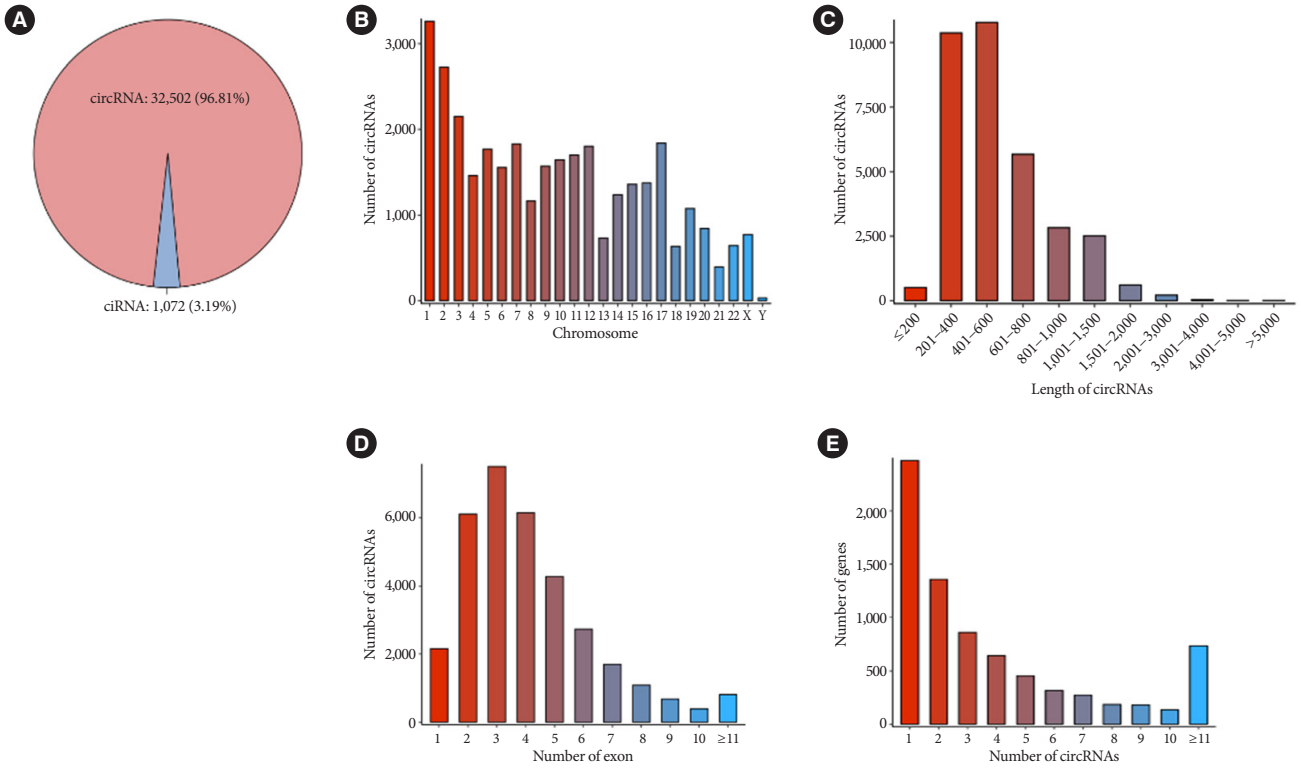
Variable	First trimester validation cohort 1			First trimester validation cohort 2		
	D-GDM (n=26)	UD-GDM (n=154)	P value	D-GDM (n=22)	UD-GDM (n=108)	P value
Age, yr	29.84±4.76	30.41±4.37	0.609	31.17±4.57	30.75±4.67	0.720
Gestational, day	71.36±18.52	71.02±18.93	0.128	67.76±17.37	61.28±15.28	0.128
Systolic pressure, mm Hg	119.09±12.82	112.26±11.54	0.033	115.13±15.02	114.05±10.33	0.760
Diastolic pressure, mm Hg	74.23±9.71	70.98±7.38	0.137	75.87±7.51	72.68±7.21	0.154
BMI, kg/m^2	23.88±4.93	22.86±3.00	0.209	23.63±4.09	22.68±3.17	0.398
OGTT 0 hr, mmol/L	4.90±0.57	4.56±0.62	0.034	4.71±0.54	4.54±0.37	0.141
OGTT 1 hr, mmol/L	8.40±1.42	7.37±2.03	0.146	10.63±0.09	7.78±1.42	0.009
OGTT 2 hr, mmol/L	7.07±1.78	6.48±1.02	0.211	8.36±1.75	6.71±1.21	0.078
Total cholesterol, mmol/L	4.83±0.82	4.96±1.14	0.725	6.40±7.14	4.36±1.04	0.216
Triglycerides, mmol/L	1.20±0.57	1.46±0.92	0.364	2.02±1.52	1.17±0.46	0.023
LDL-C, mmol/L	1.87±0.55	1.95±0.44	0.652	1.98±0.37	1.59±0.53	0.030
HDL-C, mmol/L	2.56±0.50	2.59±0.81	0.908	2.20±0.55	2.30±0.64	0.636
ALT, U/L	25.52±20.78	27.97±62.87	0.851	32.36±33.34	25.37±31.95	0.393
AST, U/L	20.50±7.52	24.90±35.71	0.546	23.28±6.83	22.31±12.47	0.724
Serum creatinine, μmol/L	36.47±5.11	38.93±5.87	0.081	37.95±5.35	39.76±4.90	0.158
BUN, mmol/L	4.10±5.36	8.51±35.93	0.545	3.15±0.76	3.11±0.87	0.867

Values are presented as mean±standard deviation.
UD-GDM, did not develop GDM; D-GDM, develop GDM; BMI, body mass index; OGTT, oral glucose tolerance test; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

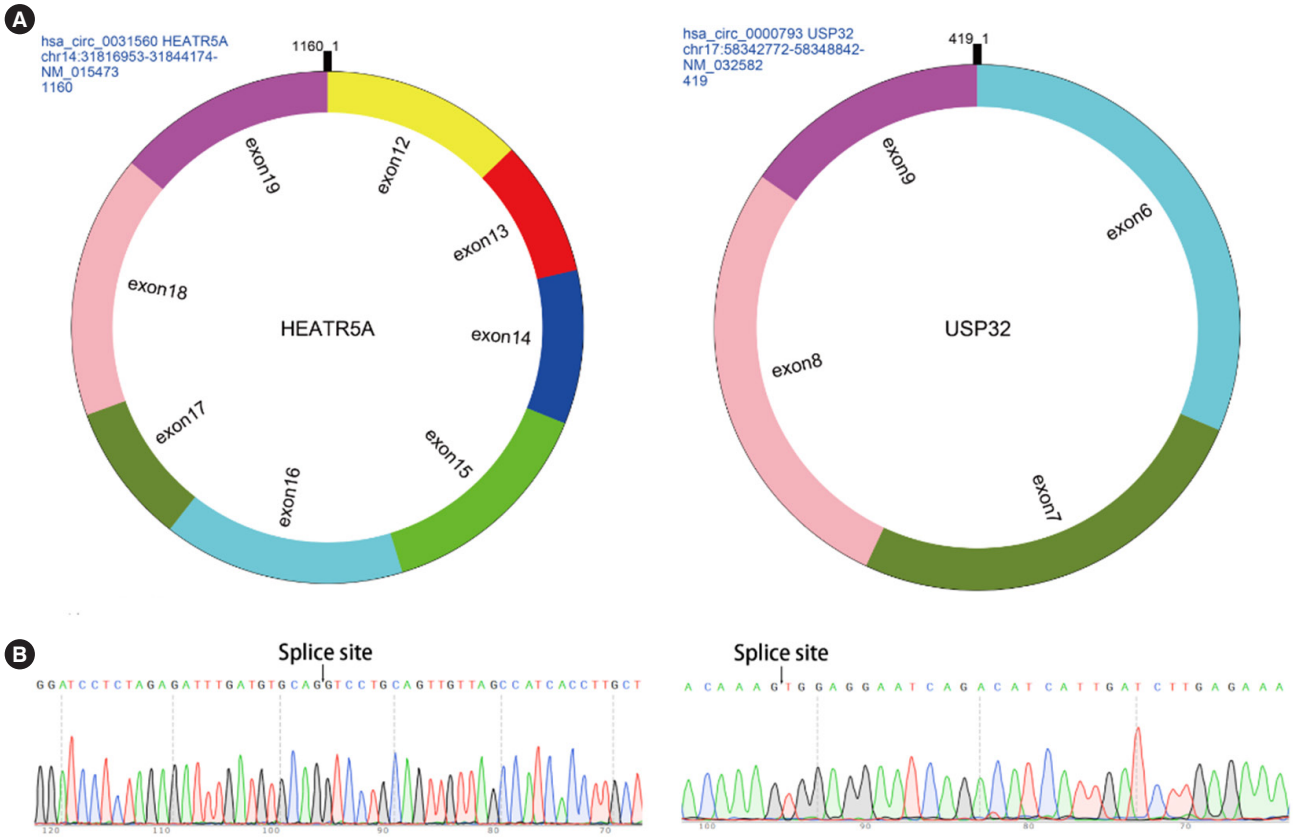
Supplementary Table 9. The diagnostic value of hsa_circ_0031560 and hsa_circ_0000793 for D-GDM in different cohorts

circRNAs	Cohort	Sensitivity, %	Specificity, %	Accuracy, %
hsa_circ_0031560	First trimester validation cohort 1	84.62	68.18	70.56
	First trimester validation cohort 2	72.73	72.22	72.31
	All first trimester validation cohort	84.78	71.43	74.12
hsa_circ_0000793	First trimester validation cohort 1	69.23	79.87	78.33
	First trimester validation cohort 2	68.18	72.22	71.54
	All first trimester validation cohort	73.91	80.77	79.39
E-GDMM	First trimester validation cohort 1	88.46	72.08	74.44
	First trimester validation cohort 2	81.82	66.67	69.23
	All first trimester validation cohort	89.13	73.63	76.75

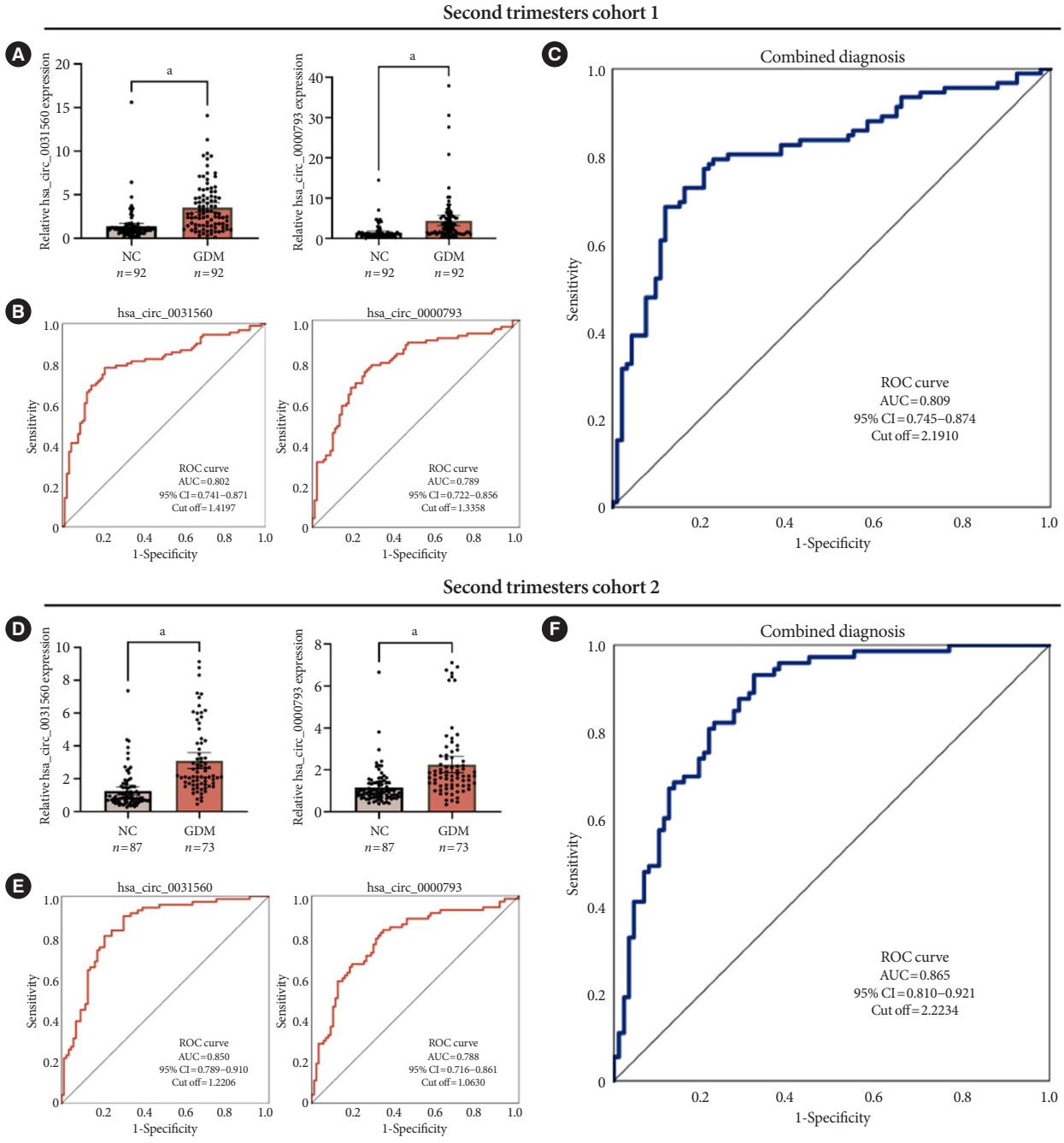
D-GDM, develop gestational diabetes mellitus (GDM); circRNAs, circular RNAs; E-GDMM, early GDM prediction model.



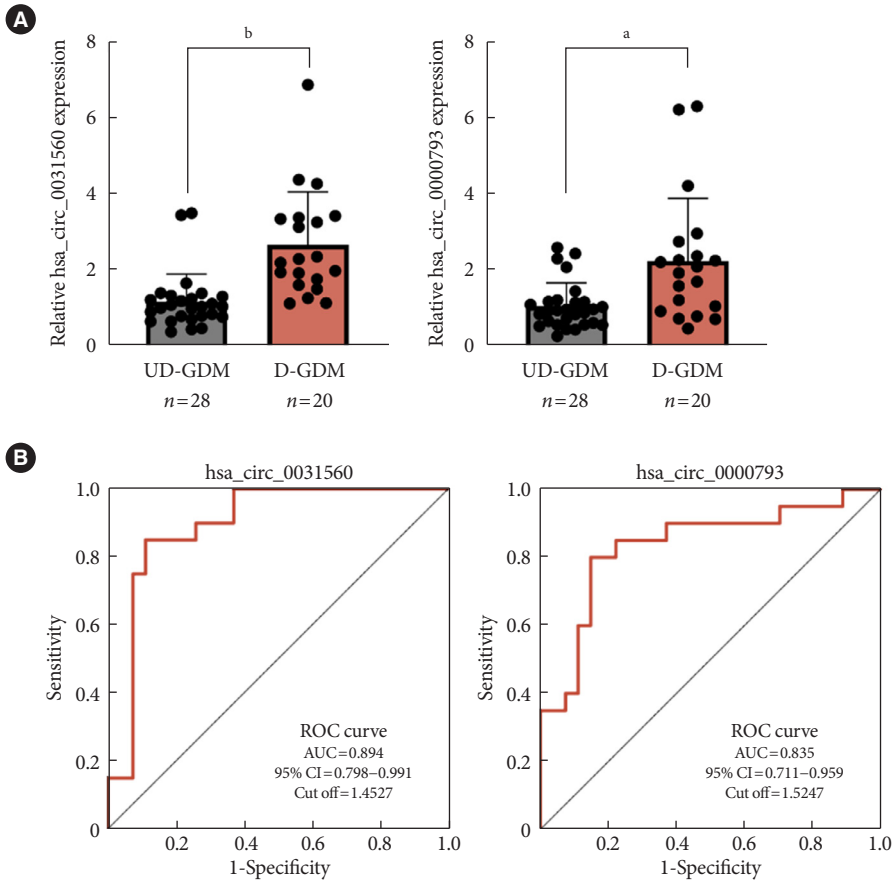
Supplementary Fig. 1. Sequencing information of circular RNAs (circRNAs). (A) Types, (B) chromosomal locations, (C) lengths, (D) exon compositions, and (E) number of circRNAs generated from individual genes after sequencing.



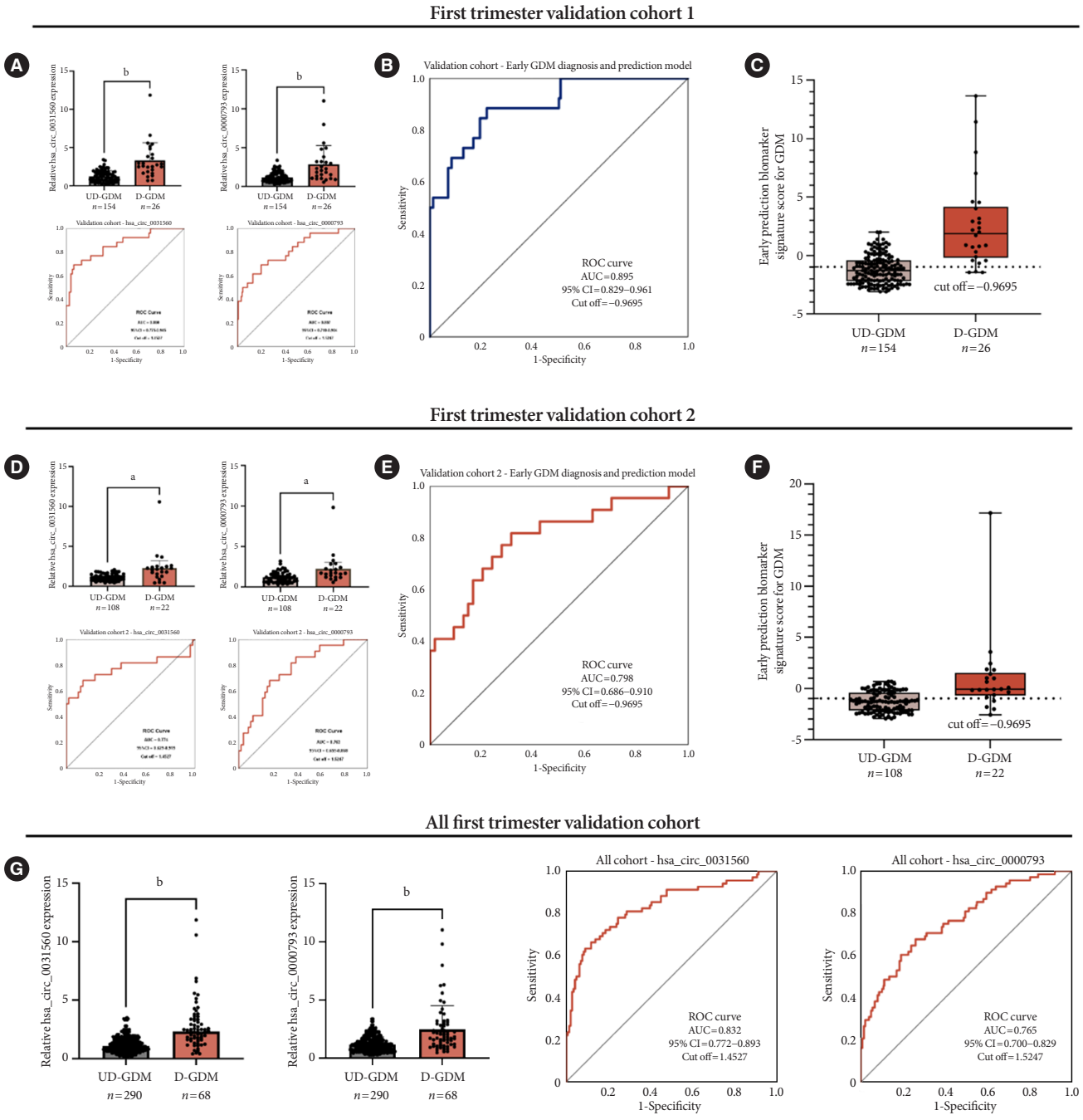
Supplementary Fig. 2. Basic information of hsa_circ_0031560 and hsa_circ_0000793. (A) Basic information of hsa_circ_0031560 and hsa_circ_0000793. (B) Sanger sequencing results of hsa_circ_0031560 and hsa_circ_0000793. HEATR5A, HEAT repeat containing 5A; USP32, ubiquitin specific peptidase 32.



Supplementary Fig. 3. Expression and receiver operating characteristic (ROC) curve analysis of hsa_circ_0031560 and hsa_circ_0000793 in different mid-pregnancy cohorts. (A) Expression levels of hsa_circ_0031560 and hsa_circ_0000793 in the serum of gestational diabetes mellitus (GDM) and normal control (NC) groups of pregnant women in second trimesters cohort 1. (B) ROC curve analysis using hsa_circ_0031560 and hsa_circ_0000793 for GDM patients in second trimesters cohort 1. (C) Combined ROC curve analysis of hsa_circ_0031560 and hsa_circ_0000793 for GDM patients in second trimesters cohort 1. (D) Expression levels of hsa_circ_0031560 and hsa_circ_0000793 in the serum of GDM and NC groups of pregnant women in second trimesters cohort 2. (E) ROC curve analysis using hsa_circ_0031560 and hsa_circ_0000793 for GDM patients in second trimesters cohort 2. (F) Combined ROC curve analysis of hsa_circ_0031560 and hsa_circ_0000793 for GDM patients in second trimesters cohort 2. AUC, area under the curve; CI, confidence interval. [a]$P<0.0001$.



Supplementary Fig. 4. The expression levels of hsa_circ_0031560 and hsa_circ_0000793 in the serum of pregnant women with gestational diabetes mellitus (GDM) and normal control (NC) groups in the modeling cohort (A) and receiver operating characteristic (ROC) curve analysis (B). UD-GDM, did not develop GDM; D-GDM, develop GDM; AUC, area under the curve; CI, confidence interval. [a]$P<0.01$, [b]$P<0.0001$.



Supplementary Fig. 5. Expression and diagnosis of hsa_circ_0031560, hsa_circ_0000793, and early gestational diabetes mellitus (GDM) prediction model (E-GDMM) in the independent validation cohort. (A) Expression levels and receiver operating characteristic (ROC) curve analysis of hsa_circ_0031560 and hsa_circ_0000793 in the serum of pregnant women from the GDM and normal control (NC) groups in the first trimester validation cohort 1. (B, C) ROC curve analysis and cut-off values of E-GDMM in GDM patients from the first trimester validation cohort 1. (D) Expression levels and ROC curve analysis of hsa_circ_0031560 and hsa_circ_0000793 in the serum of pregnant women from the GDM and NC groups in the first trimester validation cohort 2. (E, F) ROC curve analysis and cut-off values of E-GDMM in GDM patients from the first trimester validation cohort 2. (G) Expression levels and ROC curve analysis of hsa_circ_0031560 and hsa_circ_0000793 in the serum of pregnant women from the GDM and NC groups in the all first trimester validation cohorts. UD-GDM, did not develop GDM; D-GDM, develop GDM; AUC, area under the curve; CI, confidence interval. [a]$P<0.001$, [b]$P<0.0001$.