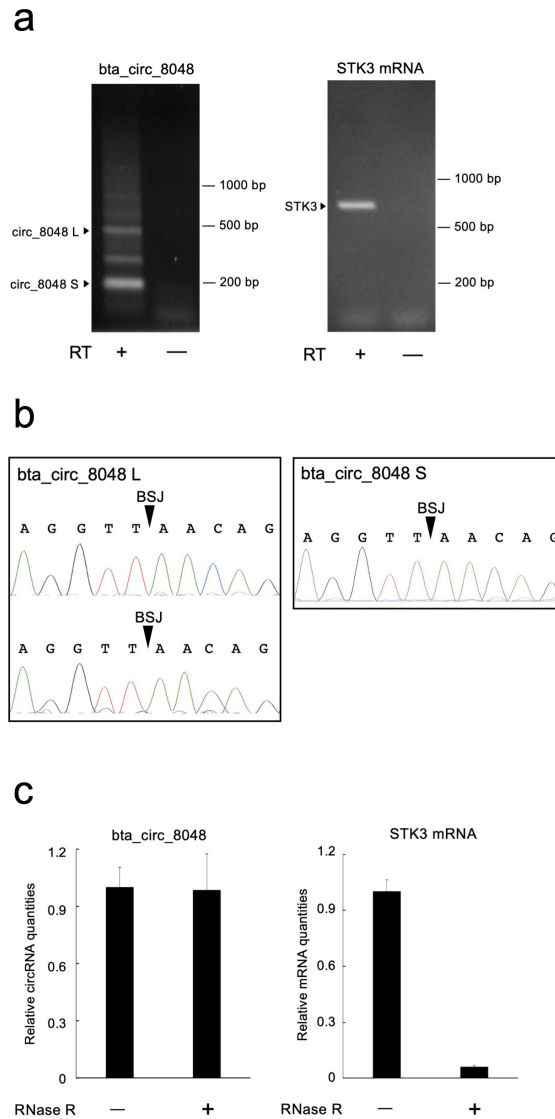
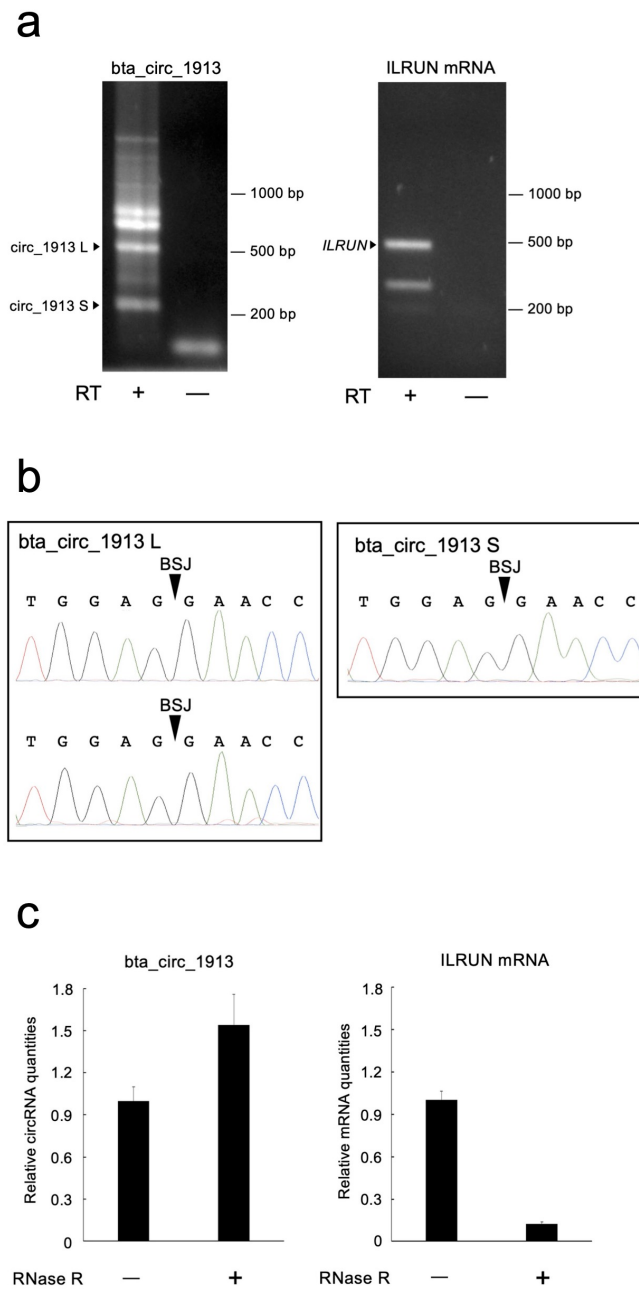


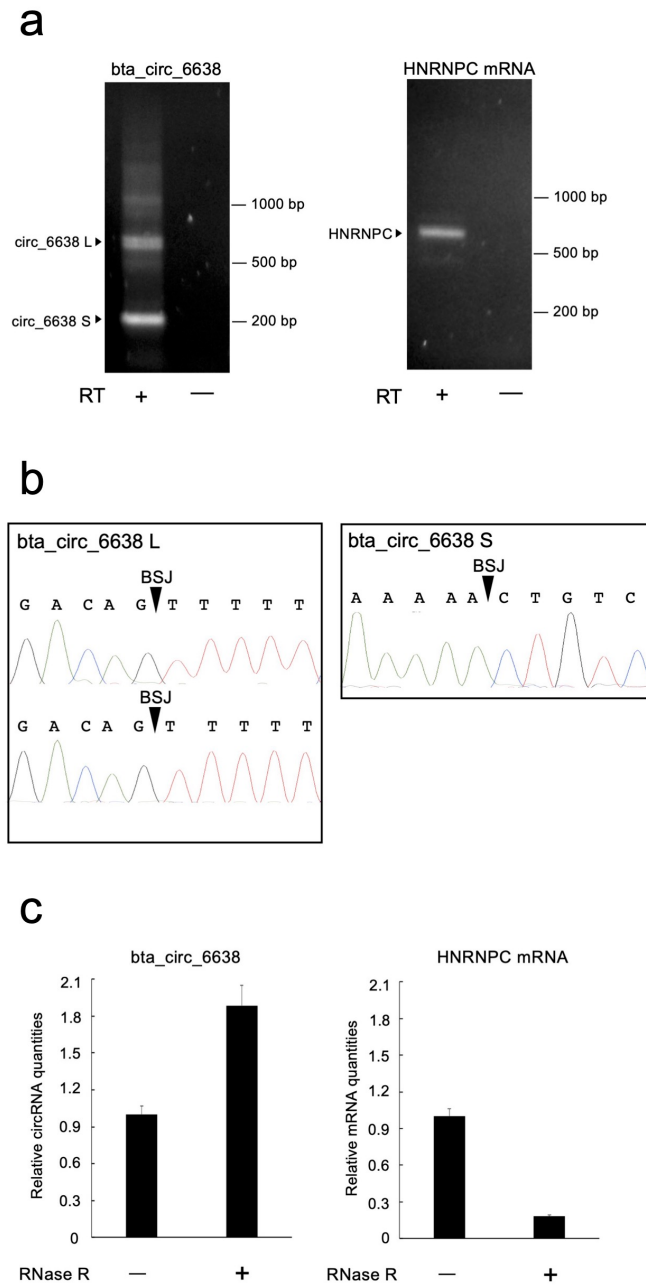
Supplementary Figures



Supplementary Figure S1 Detection of bta_circ_8048 and validation of its circular structure. (a) cDNA was synthesized from bovine placental total RNA with (RT+) or without (RT-) SuperScript IV reverse transcriptase. The expression of circ_8048 (left) and its host gene mRNA, STK3 (right), was measured using conventional PCR. The circ_8048 L and S in panel (left) are indicate long and short form PCR products of circ_8048 RT-PCR, respectively. (b) RT-PCR products for circ_8048 L (left) and S (right) were subcloned into a sequencing vector, and sequencing analysis was performed using Sanger sequencing. BSJ sites of the circRNAs are indicated by black arrowheads on the DNA sequence chromatograms. (c) Total RNA isolated from the bovine placenta was treated with or without RNase R, and cDNA was then synthesized. The quantities of circ_8048 (left) and STK3 (right) in cDNA synthesized from placental RNA treated with or without RNase R (RNase R + or -) were measured using RT-qPCR. The circRNA and mRNA levels are indicated relative to the quantities of the RNase R-untreated control (RNase R -). The values are expressed as the mean $\pm$ SE (n=5).

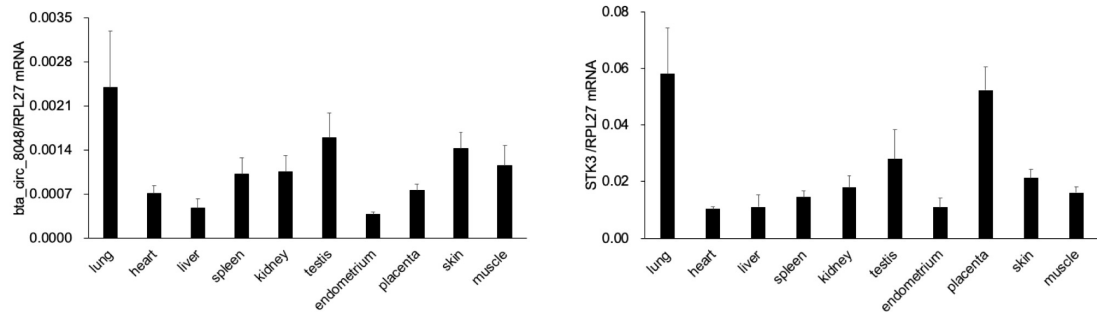


Supplementary Figure S2 Detection of bta_circ_1913 and validation of its circular structure. (a) cDNA was synthesized from bovine placental total RNA with (RT+) or without (RT-) SuperScript IV reverse transcriptase. The expression of circ_1913 (left) and its host gene mRNA, ILRUN (right), was measured using conventional PCR. The circ_1913 L and S in panel (left) are indicate long and short form PCR products of circ_1913 RT-PCR, respectively. (b) RT-PCR products for circ_1913 L (left) and S (right) were subcloned into a sequencing vector, and sequencing analysis was performed using Sanger sequencing. BSJ sites of the circRNAs are indicated by black arrowheads on the DNA sequence chromatograms. (c) Total RNA isolated from the bovine placenta was treated with or without RNase R, and cDNA was then synthesized. The quantities of circ_1913 (left) and ILRUN (right) in cDNA synthesized from placental RNA treated with or without RNase R (RNase R + or -) were measured using RT-qPCR. The circRNA and mRNA levels are indicated relative to the quantities of the RNase R-untreated control (RNase R -). The values are expressed as the mean $\pm$ SE (n=5).

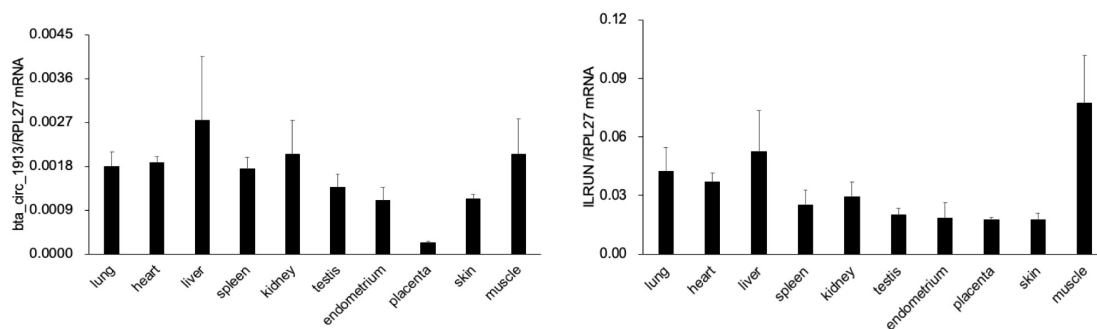


Supplementary Figure S3 Detection of bta_circ_6638 and validation of its circular structure. Detection of bta_circ_6638 and validation of its circular structure. (a) cDNA was synthesized from bovine placental total RNA with (RT+) or without (RT-) SuperScript IV reverse transcriptase. The expression of circ_6638 (left) and its host gene mRNA, HNRNPC (right), was measured using conventional PCR. The circ_6638 L and S in panel (left) are indicate long and short form PCR products of circ_6638 RT-PCR, respectively. (b) RT-PCR products for circ_6638 L (left) and S (right) were subcloned into a sequencing vector, and sequencing analysis was performed using Sanger sequencing. BSJ sites of the circRNAs are indicated by black arrowheads on the DNA sequence chromatograms. (c) Total RNA isolated from the bovine placenta was treated with or without RNase R, and cDNA was then synthesized. The quantities of circ_6638 (left) and HNRNPC (right) in cDNA synthesized from placental RNA treated with or without RNase R (RNase R + or -) were measured using RT-qPCR. The circRNA and mRNA levels are indicated relative to the quantities of the RNase R-untreated control (RNase R -). The values are expressed as the mean $\pm$ SE (n=5).

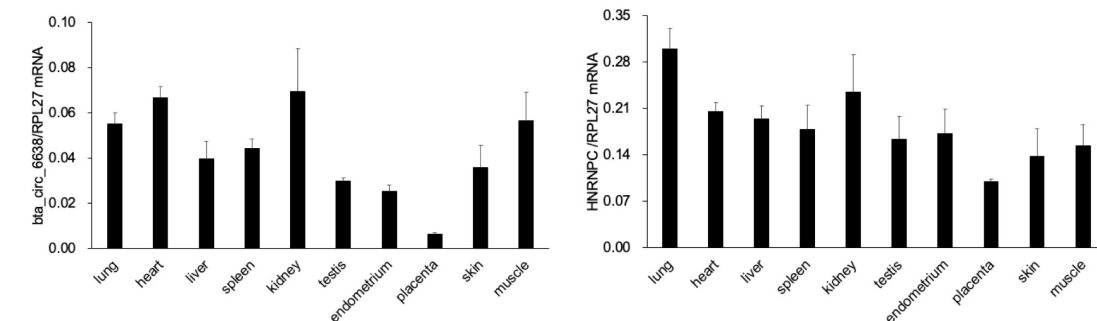
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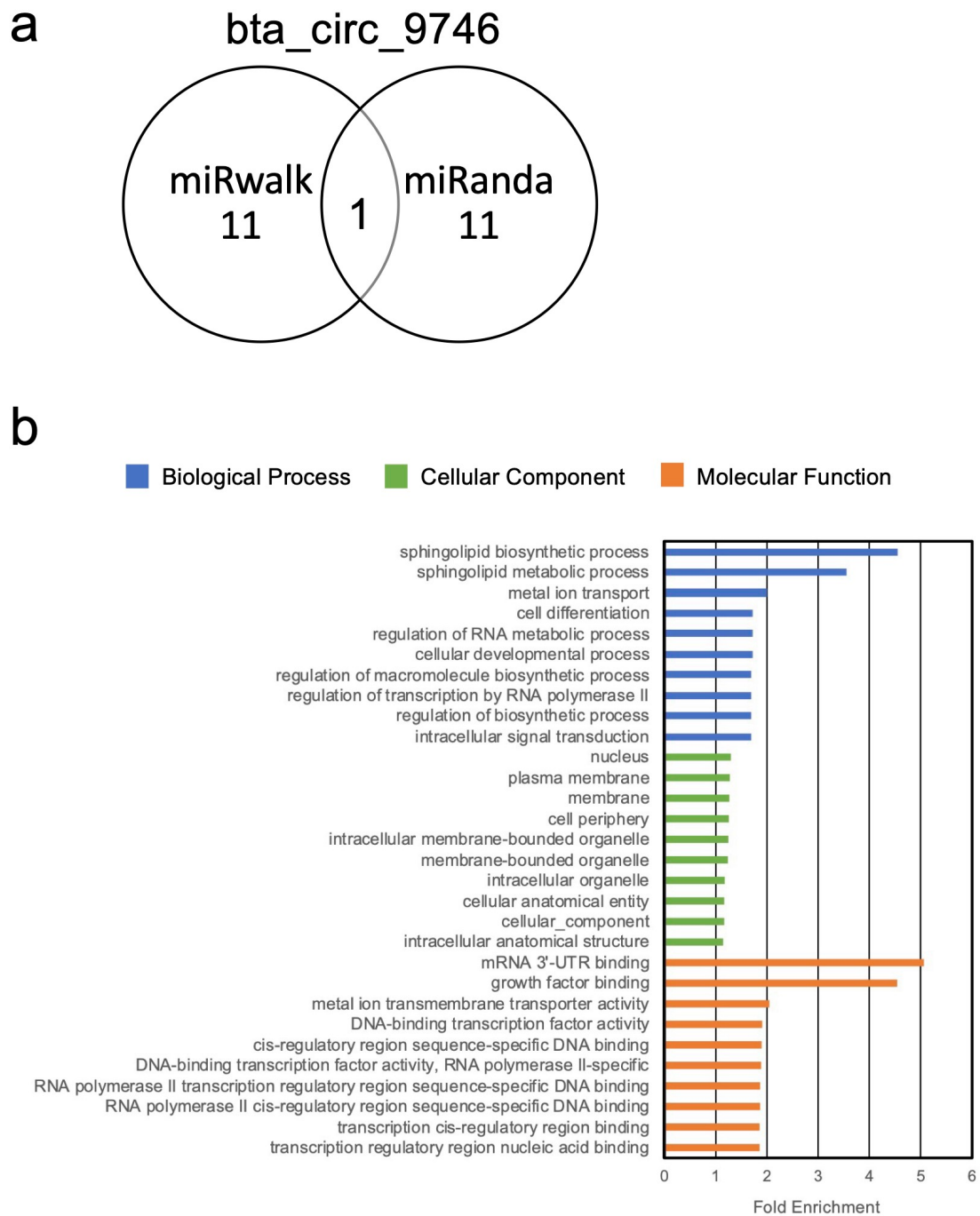
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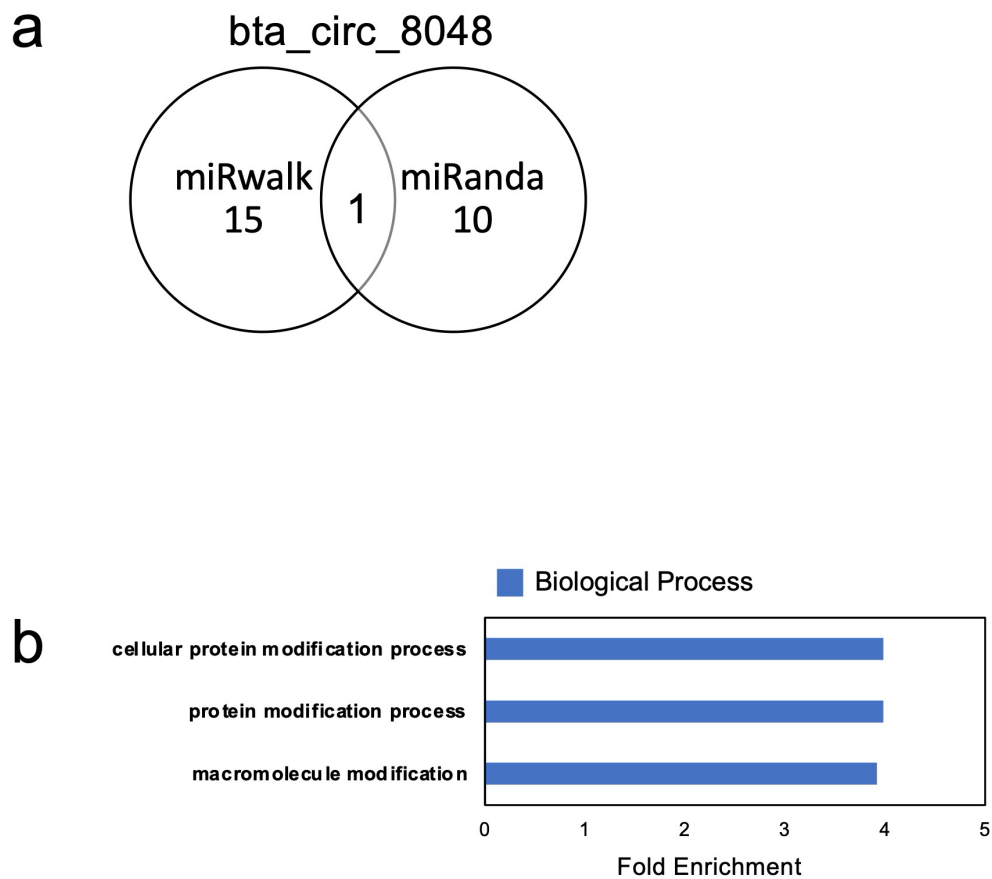
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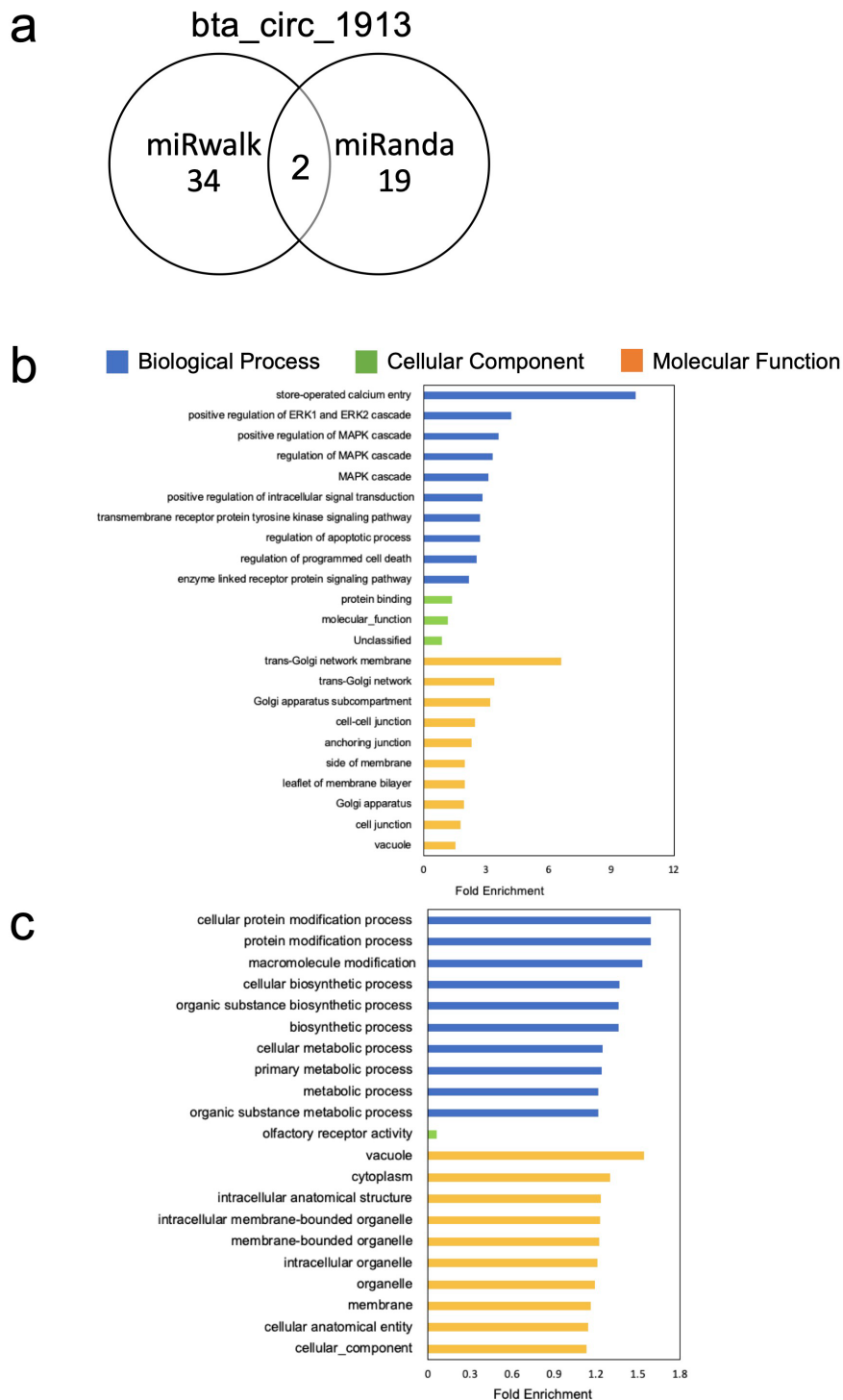
Supplementary Figure S4 Distribution of bta_circ_8048, bta_circ_1913, bta_circ_6638 and these host gene mRNA expression in bovine tissues. The expression circRNA and its host gene mRNA of in various bovine tissues, including lung, heart, liver, spleen, kidney, testis, endometrium, placenta, skin and muscle was analyzed by RT-qPCR. (a) bta_circ_8048 (left) and STK3 mRNA (right), (b) bta_circ_1913 (left) and ILRUN mRNA (right), (c) bta_circ_6638 (left) and HNRNPC (right). Cotyledonary tissue at around Day 60 of gestation was used as bovine placental sample. The values are shown as the mean $\pm$ SE (placenta n=5, other sample n=3).



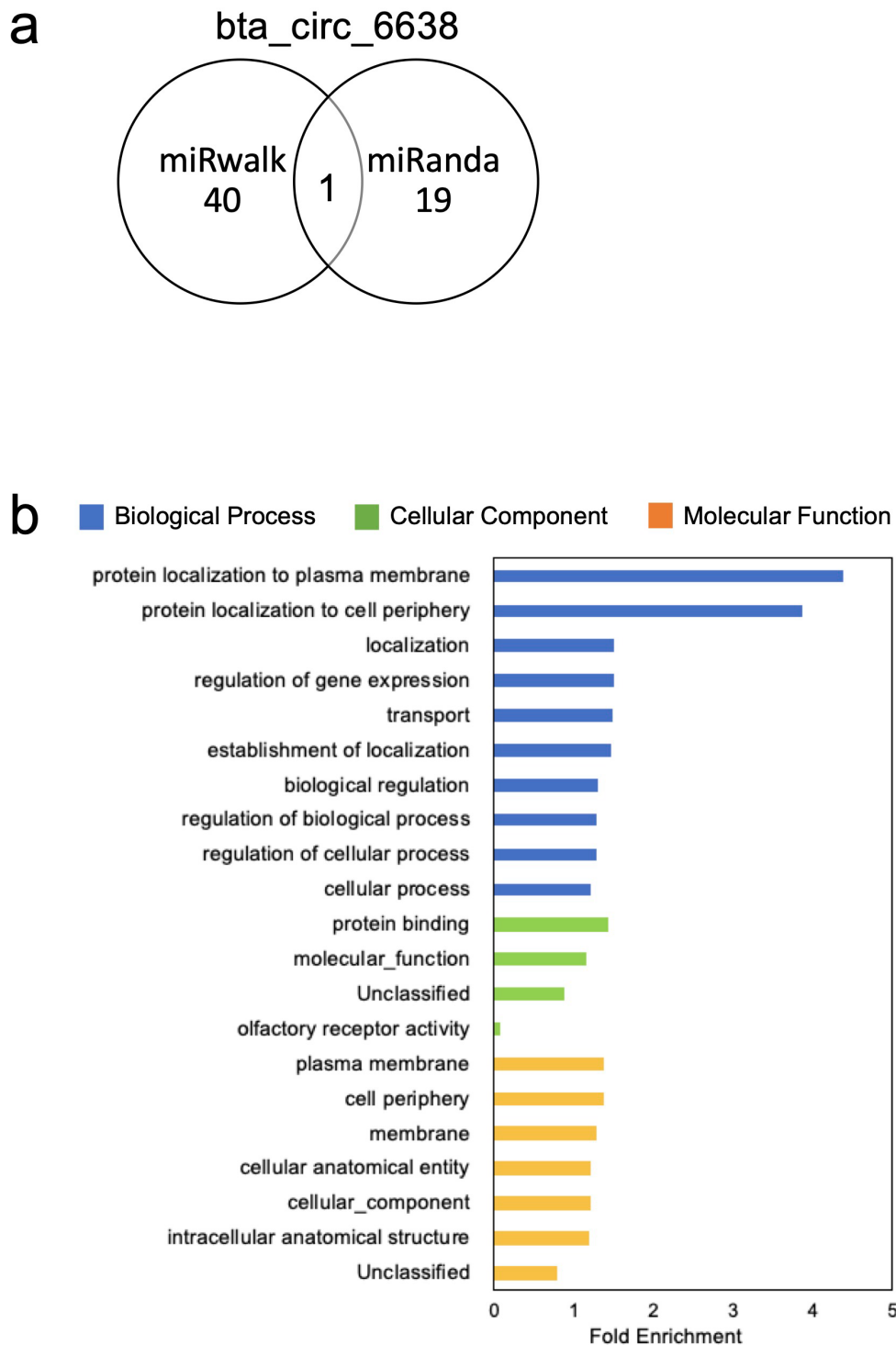
Supplementary Figure S5 Prediction of bta_circ_9746–miRNA–mRNA interaction network. Screening of miRNAs that interact with bta_circ_9746 by using the miRWalk and miRanda database. (a) Venn diagram revealing the overlap of miRNA predicted by the miRWalk and miRanda. As a result, only one miRNA, bta-miR-1306 was extracted as potential binding miRNA to bta_circ_9746. (b) After searching for mRNAs regulated by bta-miR-1306 in miRWalk, and the GO analysis was performed on these mRNAs.



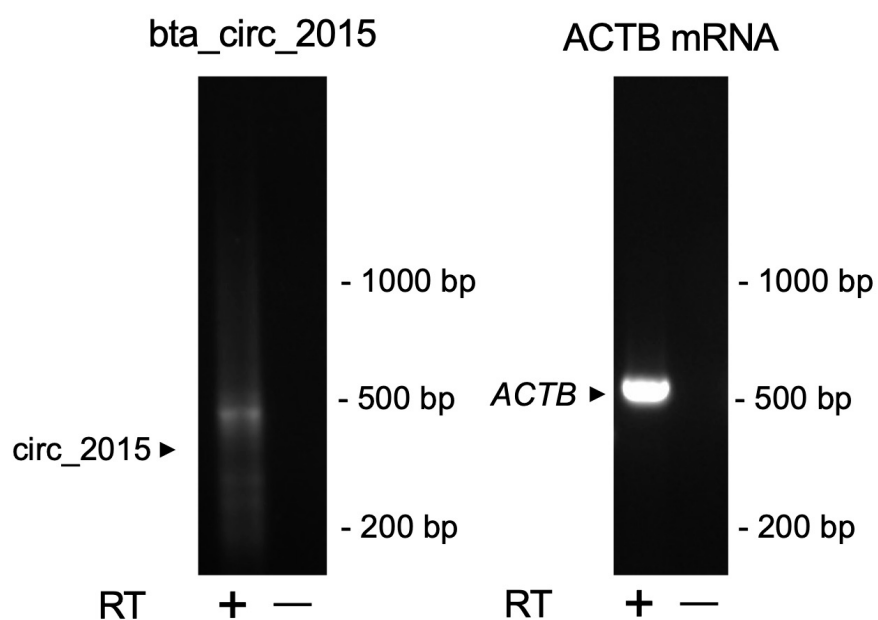
Supplementary Figure S6 Prediction of bta_circ_8048–miRNA–mRNA interaction network. Screening of miRNAs that interact with bta_circ_8048 by using the miRWalk and miRanda database. (a) Venn diagram revealing the overlap of miRNA predicted by the miRWalk and miRanda. As a result, only one miRNA, bta-miR-2303 was extracted as potential binding miRNA to bta_circ_8048. (b) After searching for mRNAs regulated by bta-miR-2303 in miRWalk, and the GO analysis was performed on these mRNAs.



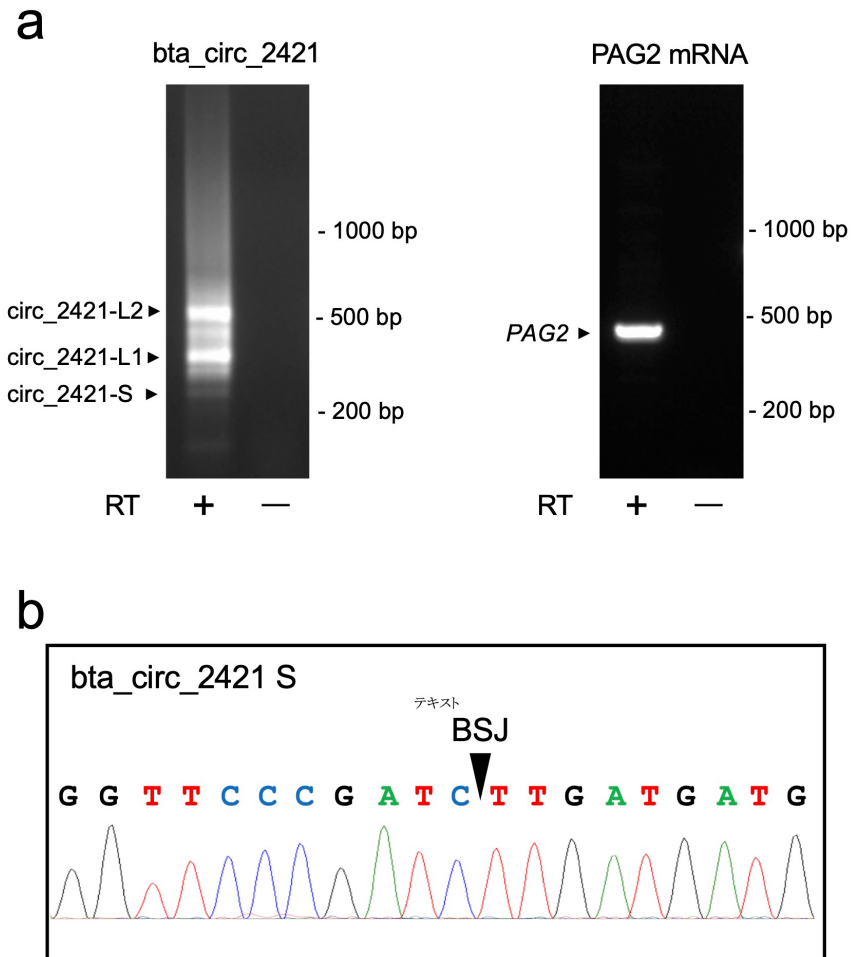
Supplementary Figure S7 Prediction of bta_circ_1913–miRNA–mRNA interaction network. Screening of miRNAs that interact with bta_circ_1913 by using the miRWalk and miRanda database. (a) Venn diagram revealing the overlap of miRNA predicted by the miRWalk and miRanda. As a result, two miRNAs, bta-miR-331-3p and bta-miR-2416 were extracted as potential binding miRNA to bta_circ_1913. After searching for mRNAs regulated by bta-miR-331-3p (b) and bta-miR-2416 (c) in miRWalk, and the GO analysis was performed on these mRNAs.



Supplementary Figure S8 Prediction of bta_circ_6638–miRNA–mRNA interaction network. Screening of miRNAs that interact with bta_circ_6638 by using the miRWalk and miRanda database. (a) Venn diagram revealing the overlap of miRNA predicted by the miRWalk and miRanda. As a result, only one miRNA, bta-miR-331-3p and bta-miR-2416 were extracted as potential binding miRNA to bta_circ_6638. (b) After searching for mRNAs regulated by bta-miR-2882 in miRWalk, and the GO analysis was performed on these mRNAs.



Supplementary Figure S9 Detection of bta_circ_2015 and validation of its circular structure. cDNA was synthesized from bovine placental total RNA with (RT+) or without (RT-) SuperScript IV reverse transcriptase. The expression of circ_2015 (left) and ACTB (right), was measured using conventional PCR.



Supplementary Figure S10 Detection of bta_circ_2421 and validation of its circular structure. (a) cDNA was synthesized from bovine placental total RNA with (RT+) or without (RT-) SuperScript IV reverse transcriptase. The expression of circ_2421 (left) and its host gene mRNA, PAG2 (right), was measured using conventional PCR. The circ_2421-S, -L1 and -L2 in panel (left) are indicate short and long form PCR products of circ_2421 RT-PCR, respectively. (b) RT-PCR products for circ_2421-S were subcloned into a sequencing vector, and sequencing analysis was performed using Sanger sequencing. BSJ sites of the circRNAs are indicated by black arrowheads on the DNA sequence chromatogram.