International Journal of
*Molecular Sciences*

MDPI

*Supplementary materials*

# CircRNAs as Potential Blood Biomarkers and Key Elements in Regulatory Networks in Gastric Cancer

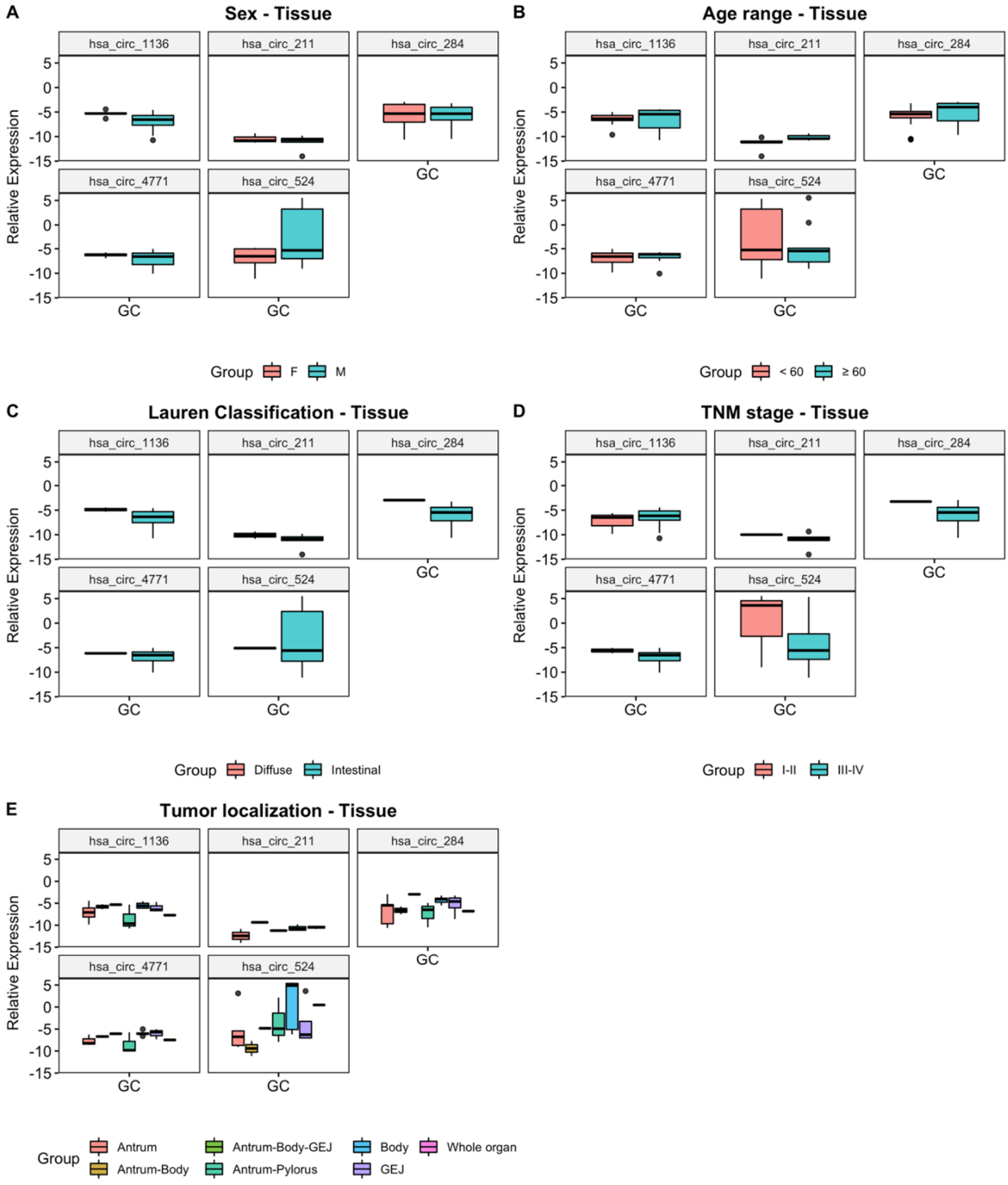


**Figure S1.** Correlation of circRNAs expression in gastric cancer tissue samples with clinicopathological data investigated in patients with gastric adenocarcinoma. GC: gastric cancer.

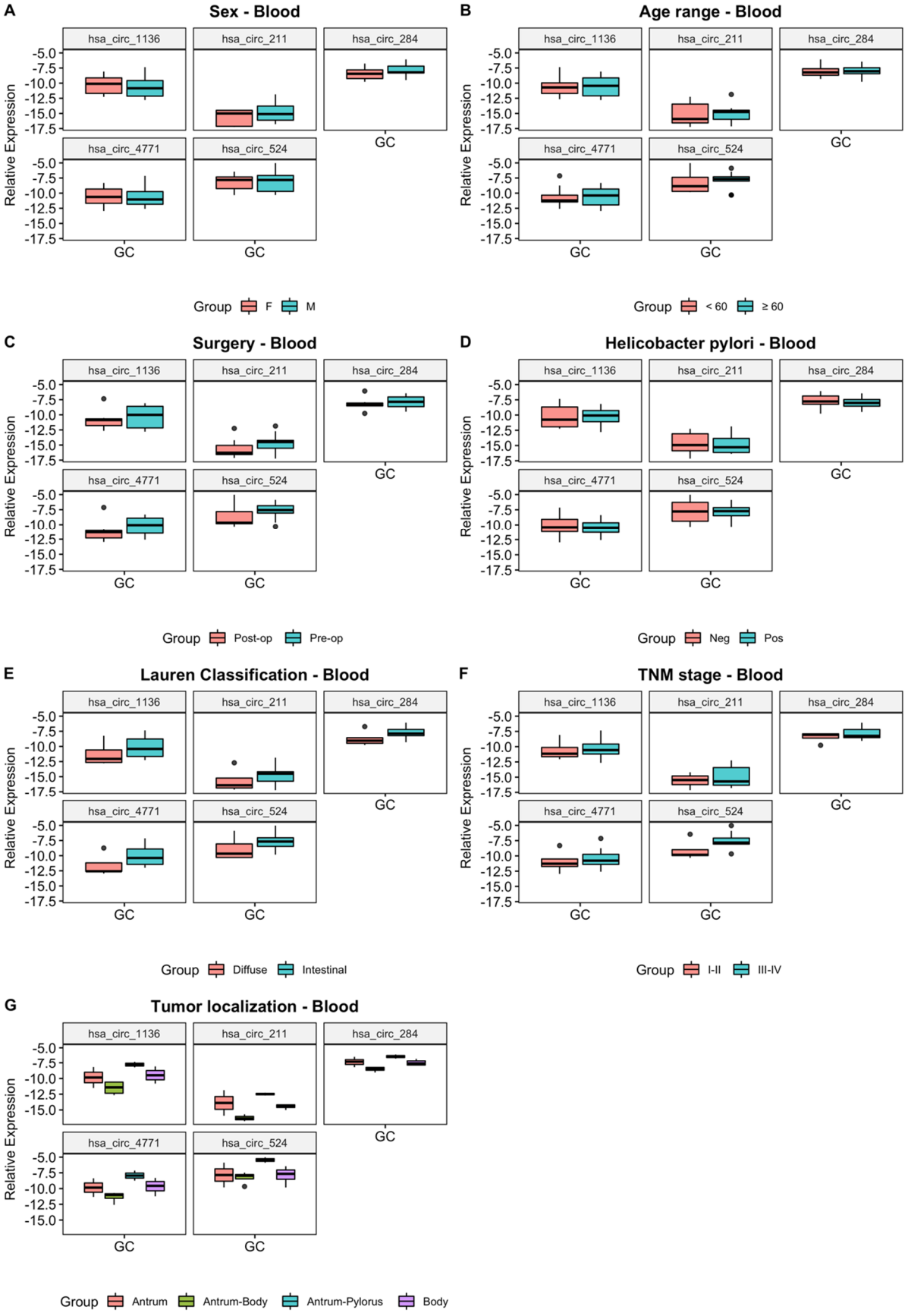


**Figure S2.** Correlation of circRNAs expression in gastric cancer blood samples with clinicopathological data investigated in patients with gastric adenocarcinoma. GC: gastric cancer.

**Table S1.** Expression and accuracy profile (AUC) analysis of circular RNAs in frozen gastric tissue and peripheral blood samples from patients with gastric adenocarcinoma and cancer-free individuals.

| Circular RNAs | Sample Type | Shapiro-Wilk | | | Kruskal-Wallis | *p* Values Wilcoxon (tissue) or T-Test (blood) | | | Fold Change | | | AUC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NC | GC | ADJ | NC/GC/ADJ | GC vs. NC | ADJ vs. NC | GC vs. ADJ | GC vs. NC | ADJ vs. NC | GC vs. ADJ | CG+ADJ vs. NC |
| hsa_circ_0000211 | Tissue | 0.99 | 0.01 | 0.43 | 0.0003 | 0.001 | 0.001 | ns | 5.23 | 6.55 | –1.25 | 0.86 |
| | Blood | 0.85 | 0.28 | - | - | 0.004 | - | - | 2.96 | - | - | 0.77 |
| hsa_circ_0000284 | Tissue | 0.46 | 0.04 | 0.05 | 0.026 | 0.048 | 0.048 | ns | 2.34 | 2.55 | –1.09 | 0.69 |
| | Blood | 0.36 | 0.67 | - | - | $1.3 \times 10^{-5}$ | - | - | 2.84 | - | - | 0.90 |
| hsa_circ_0000524 | Tissue | 0.004 | 0.01 | 0.003 | 0.0002 | 0.0008 | 0.001 | ns | –83.95 | –40.39 | –2.08 | 0.79 |
| | Blood | 0.44 | 0.21 | - | - | 0.0073 | - | - | 2.43 | - | - | 0.73 |
| hsa_circ_0001136 | Tissue | 0.21 | 0.02 | 0.17 | ns | - | - | - | 2.04 | 1.53 | 1.33 | - |
| | Blood | 0.42 | 0.17 | - | - | 0.0005 | - | - | 3.61 | - | - | 0.80 |
| hsa_circ_0004771 | Tissue | 0.79 | 0.04 | 0.96 | 0.002 | 0.0023 | ns | ns | 3.19 | 1.73 | 1.84 | 0.73 |
| | Blood | 0.17 | 0.35 | - | - | 0.0005 | - | - | 3.35 | - | - | 0.82 |

NC: Non cancer, GC: gastric cancer, ADJ: tumor-adjacent.

**Table S2.** Analyses of clinicopathological data investigated in patients with gastric adenocarcinoma.

| Clinical Features | | Tissue Samples (%) | | *p*-Values * | | | | | Blood Samples (%) | | *p*-Values * | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | Female | 6 | (27.3%) | 0.49 Δ | 1.0 Δ | 0.014 Δ | 0.014 Δ | 0.14 Δ | 6 | (33.3%) | 0.27 Δ | 0.31 Δ | 0.74 Δ | 0.7Δ | 0.96Δ |
| | Male | 16 | (72.7%) | | | | | | 12 | (66.7%) | | | | | |
| Age | <60 | 12 | (54.5%) | 0.08 Δ | 0.54 Δ | 0.34 Δ | 0.9 Δ | 0.9 Δ | 8 | (44.4%) | 0.92 Δ | 0.95 Δ | 0.68 Δ | 1.0 Δ | 0.98 Δ |
| | ≥60 | 10 | (45.4%) | | | | | | 10 | (55.6%) | | | | | |
| Surgery | Pre-op | | - | - | - | - | - | - | 11 | (61.1%) | 0.27 Δ | 0.64 Δ | 0.27 Δ | 0.53 Δ | 0.31 Δ |
| | Post-op | | - | | | | | | 7 | (38.9%) | | | | | |
| *H. pylori* | Negative | | - | - | - | - | - | - | 6 | (33.3%) | 0.97 Δ | 0.76 Δ | 0.92 Δ | 0.97 Δ | 0.8 Δ |
| | Positive | | - | | | | | | 4 | (22.2%) | | | | | |
| | NA ** | 22 | (100%) | | | | | | 8 | (44.4%) | | | | | |
| Lauren's classification | Intestinal | 20 | (90.9%) | 0.4 Δ | $1.4\times 10^{-5}$ Δ | 0.21 Δ | 0.044 Δ | 0.61 Δ | 12 | (66.7%) | 0.43 β | 0.2 β | 0.44 β | 0.52 β | 0.28 β |
| | Diffuse | 2 | (9.1%) | | | | | | 5 | (27.8%) | | | | | |
| | Indeterminate | | - | | | | | | 1 | (5.5%) | | | | | |
| Tumor location | Antrum | 6 | (27.3%) | 0.25 β | 0.45 β | 0.29 β | 0.33 β | 0.21 β | 2 | (11.1%) | 0.035 ∂ | 0.018 ∂ | 0.23 β | 0.057 β | 0.075 β |
| | Antrum-Body | 2 | (9.1%) | | | | | | 4 | (22.2%) | | | | | |
| | Antrum-Body-GEJ | 1 | (4.5%) | | | | | | | - | | | | | |
| | Antrum-Pylorus | 3 | (13.6%) | | | | | | 2 | (11.1%) | | | | | |
| | Body | 5 | (22.7%) | | | | | | 4 | (22.2%) | | | | | |
| | GEJ | 4 | (18.2%) | | | | | | | - | | | | | |
| | Whole organ | 1 | (4.5%) | | | | | | | - | | | | | |
| | NA ** | | - | | | | | | 6 | (33.3%) | | | | | |
| TNM stage | I-II | 3 | (13.6%) | 0.042 Δ | $6.2\times 10^{-5}$ Δ | 0.45 Δ | 0.56 Δ | 0.16 Δ | 4 | (22.2%) | 0.49 Δ | 0.34 Δ | 0.17 Δ | 0.79 Δ | 0.65 Δ |
| | III-IV | 19 | (86.4%) | | | | | | 8 | (44.4%) | | | | | |
| | NA ** | | - | | | | | | 6 | (33.3%) | | | | | |

* hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 and hsa_circ_0004771 *p*-values respectively. ** Data disregarded in statistical analysis. NA = Not available; GEJ = Gastroesophageal junction. Δ: T-test; β: ANOVA; ∂: TUKEY HSD when comparing "Antrum-Pylorus" vs. "Antrum-Body".

**Table S3.** Potential circRNAs-miRNAs-mRNAs interactions.

| Circular RNA | Sites | microRNAs | Target Genes with Strong Evidence |
|---|---|---|---|
| hsa_circ_0000211 | 1 | hsa-miR-182-5p | *BCL2* *CDKN1A* *SMAD4* |
| hsa_circ_0000211 | 1 | hsa-miR-188-3p | |
| hsa_circ_0000211 | 1 | hsa-miR-198 | |
| hsa_circ_0000211 | 1 | hsa-miR-224-5p | *BCL2* *KRAS* *SMAD4* |
| hsa_circ_0000211 | 1 | hsa-miR-582-3p | |
| hsa_circ_0000211 | 1 | hsa-miR-587 | |
| hsa_circ_0000211 | 1 | hsa-miR-622 | *KRAS* |
| hsa_circ_0000211 | 1 | hsa-miR-626 | *SLC7A5* |
| hsa_circ_0000211 | 1 | hsa-miR-665 | *CD274* |
| hsa_circ_0000211 | 1 | hsa-miR-7 | *BCL2* *SLC7A5* |
| hsa_circ_0000211 | 1 | hsa-miR-885-3p | *CD274* |
| hsa_circ_0000211 | 1 | hsa-miR-942 | *CDKN1A* |
| hsa_circ_0000211 | 1 | hsa-miR-1278 | |
| hsa_circ_0000211 | 1 | hsa-miR-1290 | |
| hsa_circ_0000211 | 1 | hsa-miR-1296 | |
| hsa_circ_0000211 | 1 | hsa-miR-1305 | |
| hsa_circ_0000284 | 1 | hsa-miR-149 | *FGFR1* *SP1* |
| hsa_circ_0000284 | 1 | hsa-miR-192 | *ACVR2B*, *ALCAM*, *BCL2*, *CAV1*, *CDC7*, *CUL5*, *DICER1*, *DLG5*, *DTL*, *ERCC3*, *ERCC4*, *HRH1*, *HOXA10*, *KIF20B*, *LMNB2*, *MAD2L1*, *MCM10*, *MIS12*, *PIM1*, *PRPF38A*, *RACGAP1*, *RB1*, *SEPT10*, *SMARCB1*, *WNK1* |
| hsa_circ_0000284 | 1 | hsa-miR-215 | *ACVR2B*, *ALCAM*, *CTNNBIP1*, *RB1*, *WNK1*, *ZEB2* |
| hsa_circ_0000284 | 1 | hsa-miR-326 | *NOB1*, *NOTCH1*, *NOTCH2*, *PKM*, *SMO* |
| hsa_circ_0000284 | 1 | hsa-miR-330-5p | |
| hsa_circ_0000284 | 2 | hsa-miR-338-3p | *CCND1* *SMO* *UBE2Q1* *ZEB2* |
| hsa_circ_0000284 | 1 | hsa-miR-346 | *GSK3B* *LIF* |
| hsa_circ_0000284 | 1 | hsa-miR-375 | *BCL2*, *BCL2L11*, *CAB39*, *CASP3*, *ELAVL4*, *ERBB2*, *IGF1R*, *ITGB1*, *LDHB*, *LEPROTL1*, *MTDH*, *MTPN*, *PARP1*, *PHLPP1*, *RAB10*, *RHOA*, *SP1*, *TIMM8A*, *USP1*, *YWHAZ* |
| hsa_circ_0000284 | 1 | | |
| hsa_circ_0000284 | 1 | | |
| hsa_circ_0000284 | 1 | hsa-miR-377 | *PPM1A* *SOD2* |
| hsa_circ_0000284 | 1 | hsa-miR-382 | *PTEN* |
| hsa_circ_0000284 | 1 | hsa-miR-485-3p | *MAT1A* |
| hsa_circ_0000284 | 1 | hsa-miR-490-5p | *FOS* |
| hsa_circ_0000284 | 1 | hsa-miR-495 | *MAT1A* *PTP4A3* *SOX9* |
| hsa_circ_0000284 | 1 | hsa-miR-508-3p | |
| hsa_circ_0000284 | 1 | hsa-miR-513a-3p | |
| hsa_circ_0000284 | 1 | hsa-miR-515-5p | |
| hsa_circ_0000284 | 1 | hsa-miR-558 | |
| hsa_circ_0000284 | 1 | hsa-miR-561 | |
| hsa_circ_0000284 | 1 | hsa-miR-579 | |
| hsa_circ_0000284 | 1 | hsa-miR-580-3p | *TWIST1* |
| hsa_circ_0000284 | 1 | hsa-miR-584 | |
| hsa_circ_0000284 | 1 | hsa-miR-599 | |
| hsa_circ_0000284 | 1 | hsa-miR-606 | |
| hsa_circ_0000284 | 1 | hsa-miR-607 | |
| hsa_circ_0000284 | 1 | hsa-miR-619 | |
| hsa_circ_0000284 | 1 | hsa-miR-637 | |

| | | | | |
|---|---|---|---|---|
| hsa_circ_0000284 | 1 | hsa-miR-640 | | |
| hsa_circ_0000284 | 2 | hsa-miR-653 | | |
| hsa_circ_0000284 | 1 | hsa-miR-668 | | |
| hsa_circ_0000284 | 1 | hsa-miR-766 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1178 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1179 | | |
| hsa_circ_0000284 | 2 | hsa-miR-1231 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1243 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1250 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1278 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1283 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1286 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1290 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1294 | | |
| hsa_circ_0000284 | 2 | hsa-miR-1305 | | |
| hsa_circ_0000284 | 1 | hsa-miR-1825 | | |
| hsa_circ_0000524 | 1 | hsa-miR-338-3p | | |
| hsa_circ_0000524 | 1 | hsa-miR-520g | *VEGFA* | |
| hsa_circ_0000524 | 1 | hsa-miR-520h | *VEGFA* | |
| hsa_circ_0000524 | 1 | hsa-miR-876-3p | | |
| hsa_circ_0000524 | 1 | hsa-miR-1178 | | |
| hsa_circ_0000524 | 1 | hsa-miR-1208 | | |
| hsa_circ_0001136 | 1 | hsa-miR-576-5p | | |
| hsa_circ_0001136 | 1 | hsa-miR-616 | | |
| hsa_circ_0001136 | 1 | hsa-miR-767-3p | *MGMT* | |
| hsa_circ_0001136 | 1 | hsa-miR-1179 | | |
| hsa_circ_0001136 | 1 | hsa-miR-1244 | | |
| hsa_circ_0001136 | 1 | hsa-miR-1248 | | |
| hsa_circ_0001136 | 1 | hsa-miR-1304 | | |
| hsa_circ_0001136 | 1 | hsa-miR-1827 | | |
| hsa_circ_0004771 | 1 | hsa-miR-149 | *BBC3* | *SP1* |
| hsa_circ_0004771 | 1 | hsa-miR-515-5p | | |
| hsa_circ_0004771 | 1 | hsa-miR-532-3p | | |
| hsa_circ_0004771 | 1 | hsa-miR-562 | | |
| hsa_circ_0004771 | 1 | hsa-miR-580-3p | | |
| hsa_circ_0004771 | 1 | hsa-miR-589 | | |
| hsa_circ_0004771 | 1 | hsa-miR-595 | | |
| hsa_circ_0004771 | 1 | hsa-miR-648 | | |
| hsa_circ_0004771 | 1 | hsa-miR-653 | | |
| hsa_circ_0004771 | 1 | hsa-miR-924 | | |
| hsa_circ_0004771 | 1 | hsa-miR-1200 | | |
| hsa_circ_0004771 | 1 | hsa-miR-1203 | | |
| hsa_circ_0004771 | 1 | hsa-miR-1253 | | |

**Table S4.** Potential circRNAs-RBPs interactions.

| RBP | Circular RNA | Site |
|---|---|---|
| ACIN1 | hsa_circ_0000211, hsa_circ_0001136 | 5, 7 |
| ALYREF | hsa_circ_0000284, hsa_circ_0001136 | 2, 1 |
| BUD13 | hsa_circ_0000284, hsa_circ_0001136 | 3, 4 |
| CAPRIN1 | hsa_circ_0000284 | 1 |
| CNBP | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0001136 | 2, 7, 3 |
| CPSF6 | hsa_circ_0000524, hsa_circ_0001136 | 2, 1 |
| CSTF2T | hsa_circ_0000284, hsa_circ_0001136 | 1, 1 |
| DDX3X | hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 3, 4, 2 |
| DDX54 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 2, 20, 3, 6 |
| DGCR8 | hsa_circ_0000524, hsa_circ_0001136 | 1, 1 |
| DICER1 | hsa_circ_0000284 | 1 |
| DKC1 | hsa_circ_0000284, hsa_circ_0001136 | 1, 1 |
| EIF4A3 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 3, 10, 2, 4 |
| EIF4G2 | hsa_circ_0000524, hsa_circ_0001136 | 1, 1 |
| ELAVL1 | hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 3, 2, 2 |
| ELAVL3 | hsa_circ_0000284 | 2 |
| FAM120A | hsa_circ_0000284 | 6 |
| FBL | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 5, 5, 1, 3 |
| FMR1 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 7, 35, 6, 9 |
| FUS | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524 | 1, 6, 1 |
| FXR1 | hsa_circ_0000284, hsa_circ_0000524 | 7, 1 |
| FXR2 | hsa_circ_0000284, hsa_circ_0001136 | 3, 1 |
| GNL3 | hsa_circ_0000284 | 2 |
| GTF2F1 | hsa_circ_0000524 | 1 |
| HNRNPA1 | hsa_circ_0000284, hsa_circ_0000524 | 2, 1 |
| HNRNPA2B1 | hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 2, 4, 1 |
| HNRNPU | hsa_circ_0000524 | 2 |
| IGF2BP1 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 3, 15, 2, 5 |
| IGF2BP2 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 14, 31, 4, 14 |
| IGF2BP3 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 2, 6, 1, 2 |
| KHDRBS2 | hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 1, 1, 1 |
| LIN28A | hsa_circ_0000284, hsa_circ_0001136 | 3, 1 |
| LIN28 | hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 3, 2, 1 |
| LIN28B | hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 11, 1, 6 |
| MBNL2 | hsa_circ_0000284 | 1 |
| METTL3 | hsa_circ_0001136 | 1 |

| | | |
|---|---|---|
| MOV10 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 11, 47, 1, 11 |
| MSI2 | hsa_circ_0000211, hsa_circ_0000284 | 2, 3 |
| NOP56 | hsa_circ_0001136 | 2 |
| NOP58 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 6, 5, 2, 3 |
| NPM1 | hsa_circ_0000284 | 1 |
| NUMA1 | hsa_circ_0000524 | 2 |
| PRPF8 | hsa_circ_0000211, hsa_circ_0001136 | 1, 3 |
| PTBP1 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 1, 3, 4, 2 |
| RBFOX2 | hsa_circ_0000524 | 4 |
| RBM5 | hsa_circ_0000524, hsa_circ_0001136 | 1, 1 |
| RBM10 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 7, 3, 4, 4 |
| RBM47 | hsa_circ_0000284, hsa_circ_0001136 | 4, 1 |
| SF3B4 | hsa_circ_0001136 | 2 |
| SLTM | hsa_circ_0000284 | 1 |
| SMNDC1 | hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 1, 1, 2 |
| SND1 | hsa_circ_0000284, hsa_circ_0000524 | 2, 2 |
| SRSF1 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 4, 9, 7, 9 |
| SRSF3 | hsa_circ_0000211, hsa_circ_0000284 | 1, 4 |
| SRSF7 | hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 2, 1, 2 |
| SRSF9 | hsa_circ_0000284, hsa_circ_0001136 | 2, 2 |
| SRSF10 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 1, 1, 1, 1 |
| TAF15 | hsa_circ_0000211, hsa_circ_0000284 | 1, 2 |
| TARDBP | hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 1, 2, 1 |
| U2AF1 | hsa_circ_0001136 | 3 |
| U2AF2 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 11, 4, 2, 8 |
| UPF1 | hsa_circ_0000211, hsa_circ_0000284, hsa_circ_0000524, hsa_circ_0001136 | 5, 8, 3, 3 |
| YTHDC1 | hsa_circ_0000284 | 2 |
| YTHDF1 | hsa_circ_0000284 | 1 |
| YWHAG | hsa_circ_0001136 | 2 |

**Table S5.** Primers sequences used for qRT-PCR.

| RNA Circular | Type | Forward Primer | Reverse Primer | Product Size (pb) |
|---|---|---|---|---|
| hsa_circ_0000211 | Spanning junction divergent primer | 5′ GGAAGTGTTTAAGCAATCAAAG 3′ | 5′ TTCCTCCAACATTTTCAATC 3′ | 220 |
| hsa_circ_0000284 | Divergent primer | 5′ TATGTTGGTGGATCCTGTTCGGCA 3′ | 5′ TGGTGGGTAGACCAAGACTTGTGA 3′ | 146 |
| hsa_circ_0000524 | Spanning junction divergent primer | 5′ GGGGAGACAAGCAAATCTGAC 3′ | 5′ CCTTTGTTGCTCATCCTCTTCT 3′ | 105 |
| hsa_circ_0001136 | Spanning junction divergent primer | 5′ CCTTTTCACGCTCAAGGTATTAG 3′ | 5′CTGCTCATTTCCTTTAGTCCTTCT 3′ | 102 |
| hsa_circ_0004771 | Divergent primer | 5′ CAGCCTTCTCAATTTTCTTTCTCAG 3′ | 5′ GCTGTGTTTCTCCCAAATGTT 3′ | 130 |
| ACTB | Convergent primer | 5′ GCTCGTCGTCGACAACGGCTC 3′ | 5′ CAAACATGATCTGGGTCATCTTCTC 3′ | 487 |