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## Supplemental information

# miRNA/mRNA co-profiling identifies the miR-200 family as a central regulator of SMC quiescence

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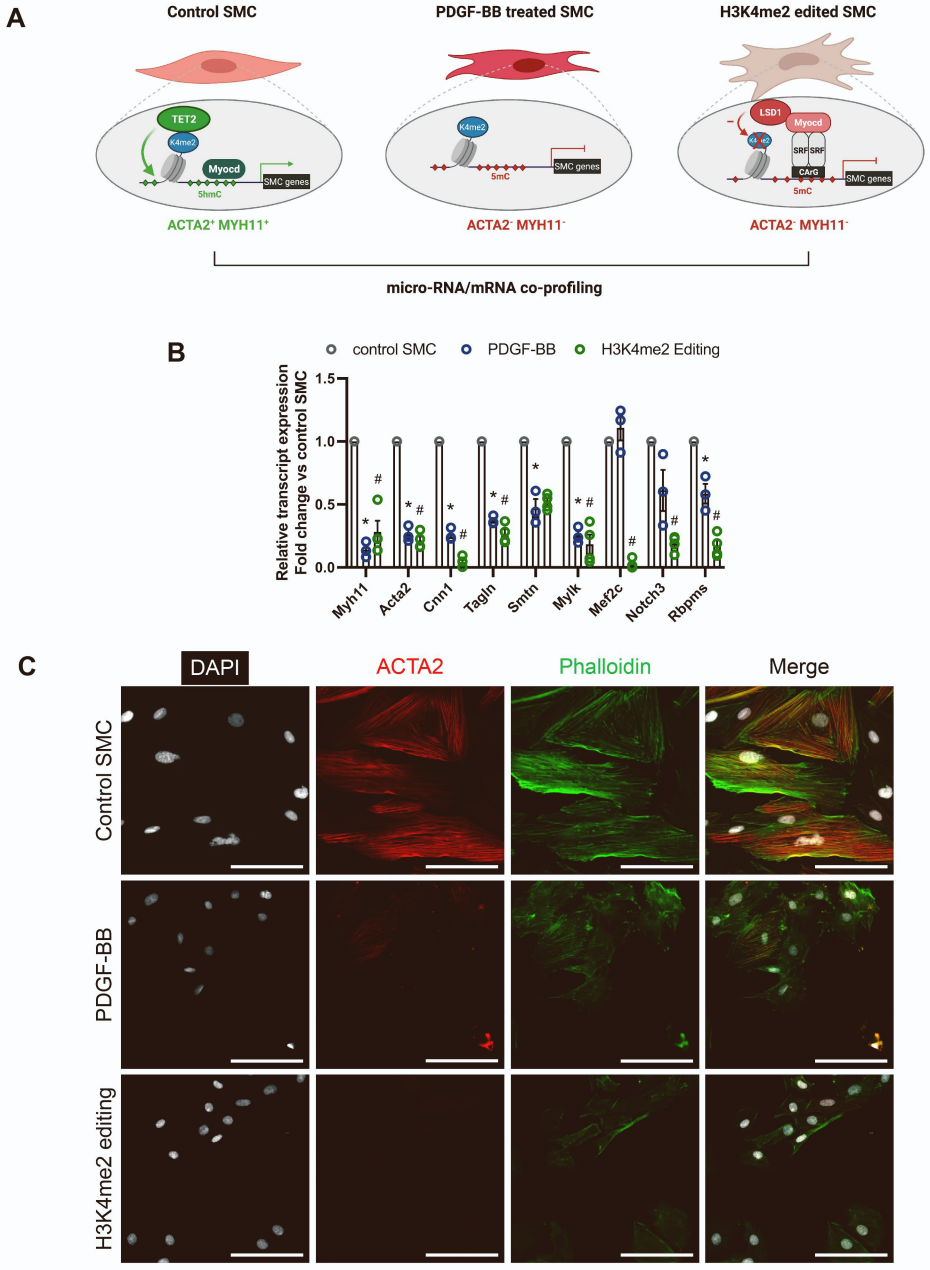
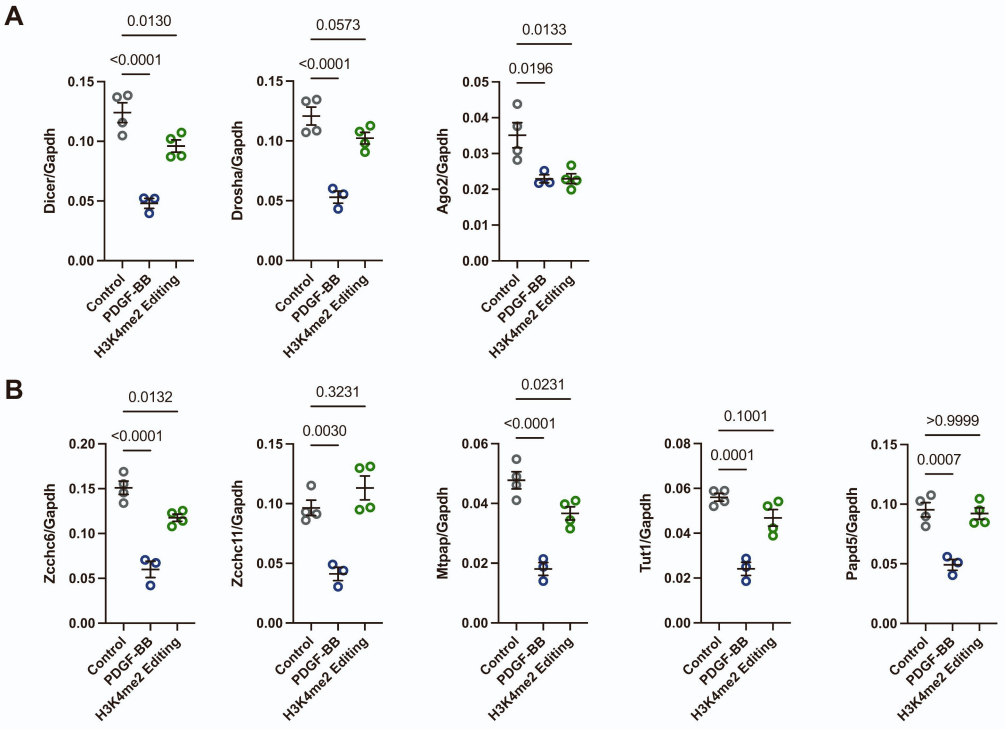
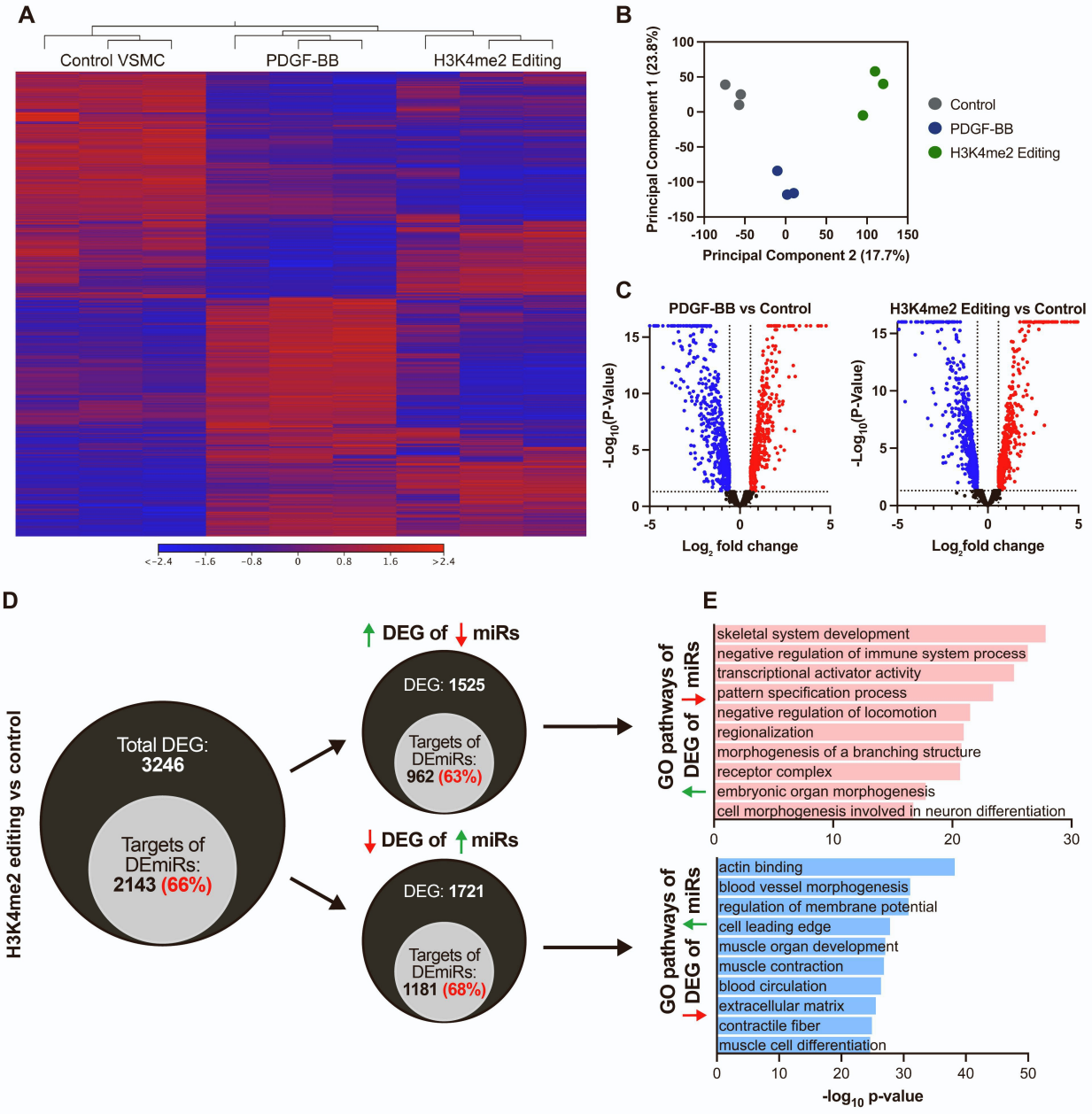


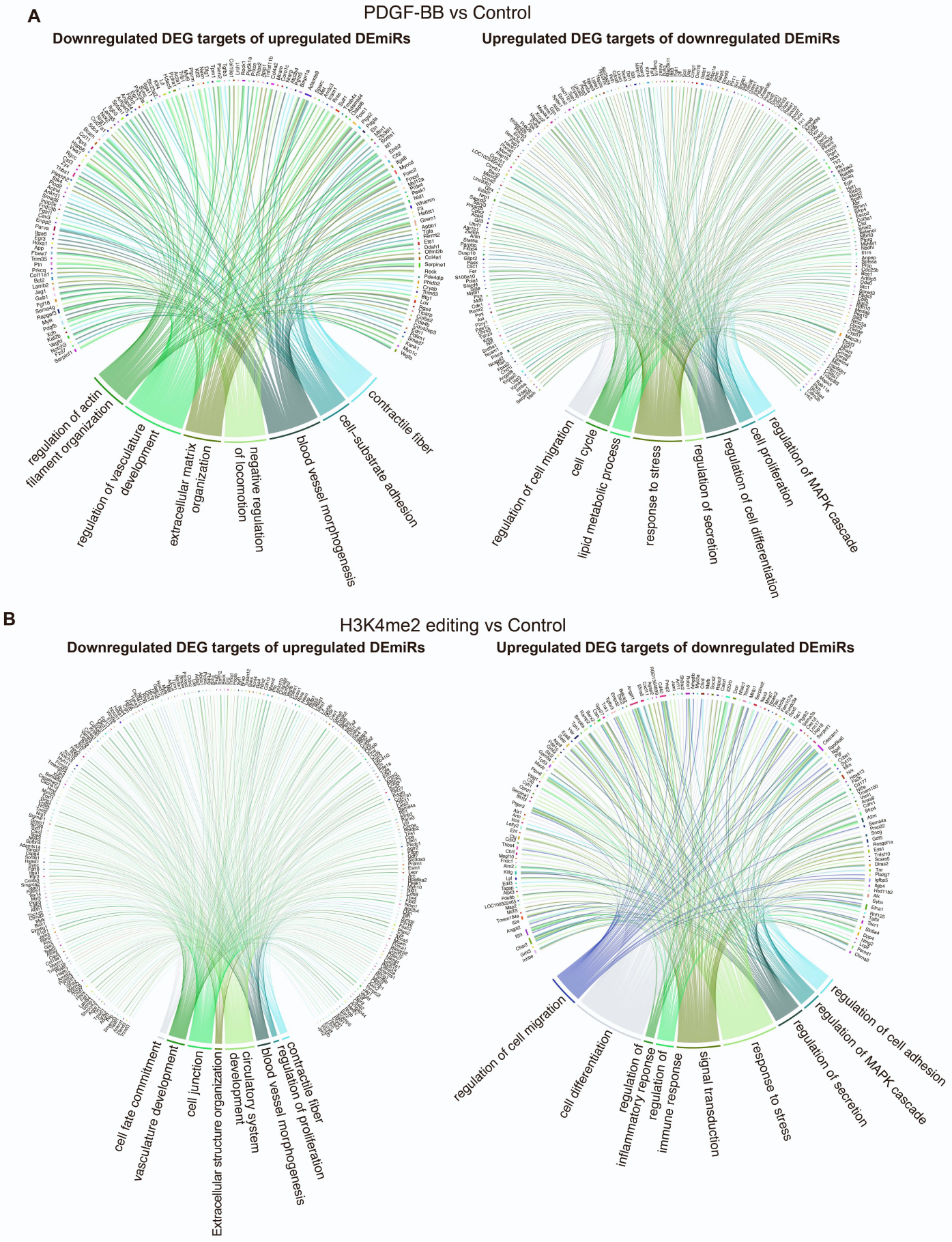

**Supplementary Figure 1, related to Figure 1. Characterization of in vitro SMC dedifferentiation models induced by PDGF-BB treatment or H3K4me2 editing**



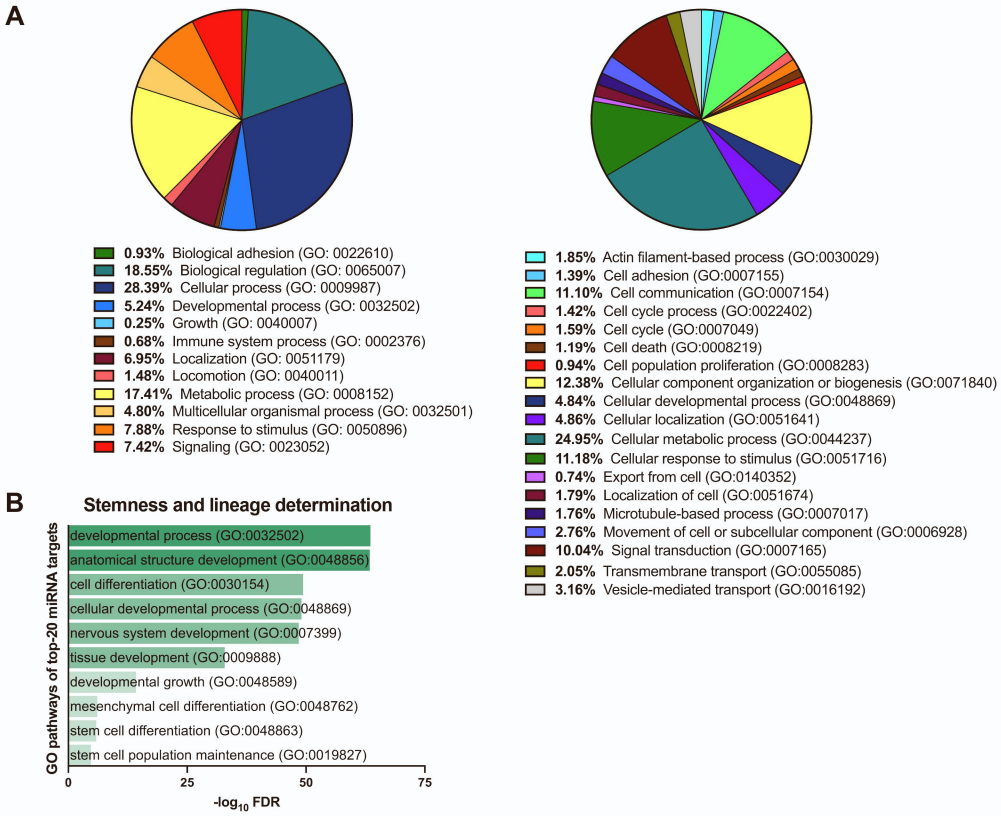

**Supplementary Figure 2, related to Figure 1. Alteration of miRNA processing and editing enzyme expression in dedifferentiated SMC**



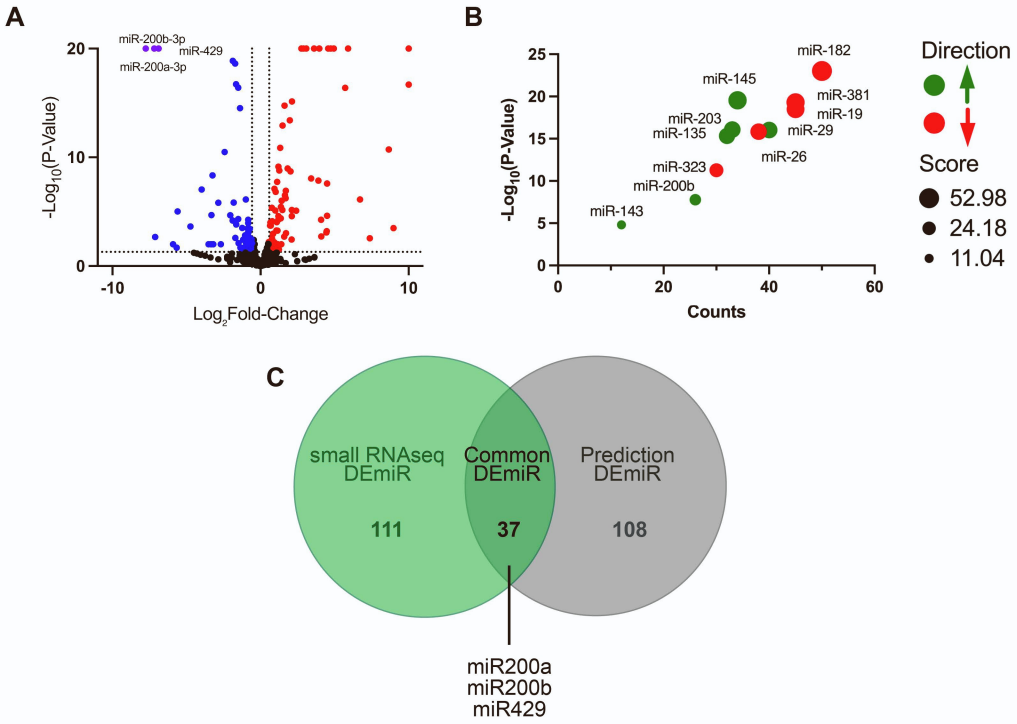

**Supplementary Figure 3, related to Figure 1. Contribution of DEmiRs in the regulation of DEG in H3K4me2 edited SMC**



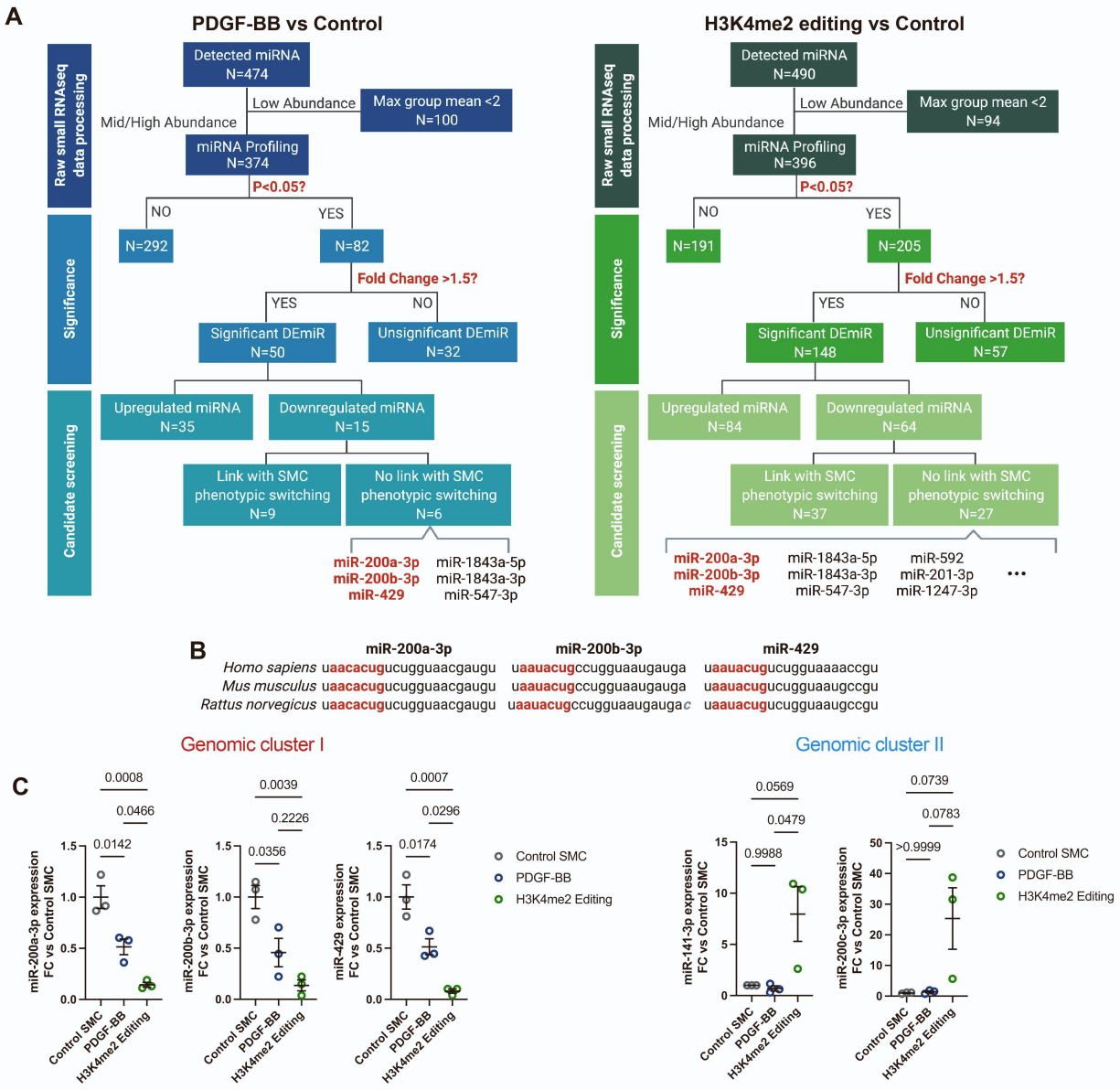

**Supplementary Figure 4, related to Figure 1. Prediction of pathways modulated by DEmiRs during SMC dedifferentiation induced by PDGF-BB**



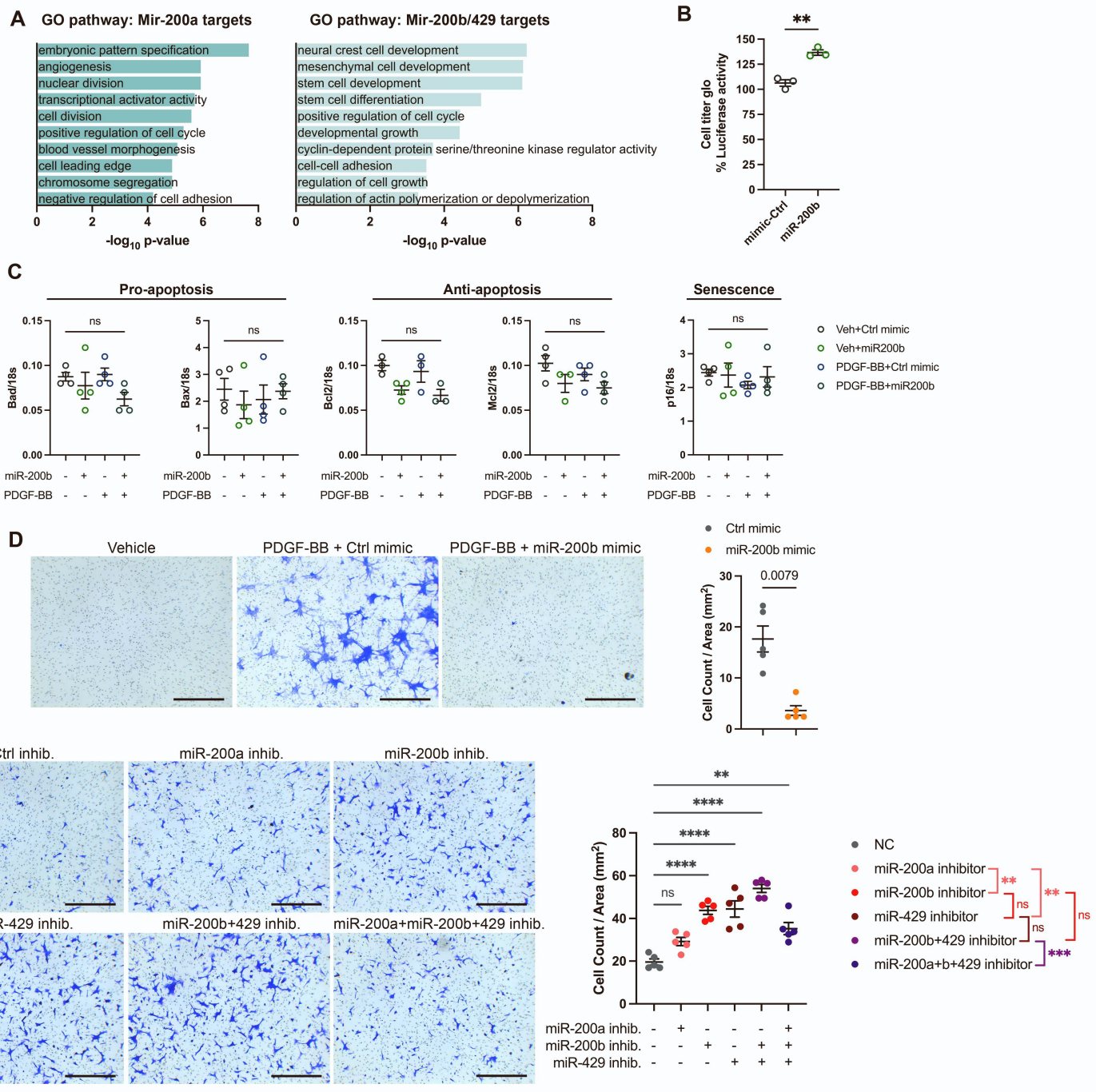

**Supplementary Figure 5, related to Figure 2. Identification of pathways regulated by most abundantly expressed miRNAs in contractile SMC**



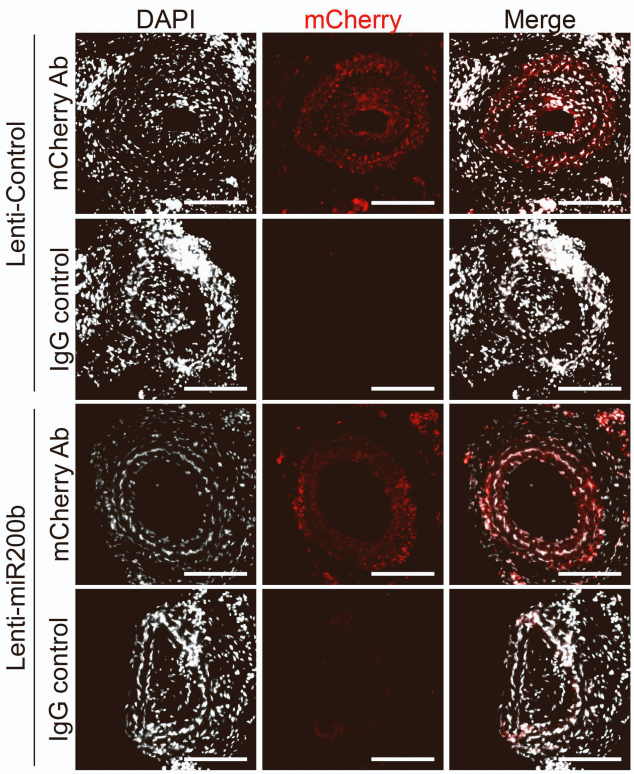

**Supplementary Figure 6, related to Figure 2. Experimental and predictive determination of DEmiRs in H3K4me2 vs. control SMC**



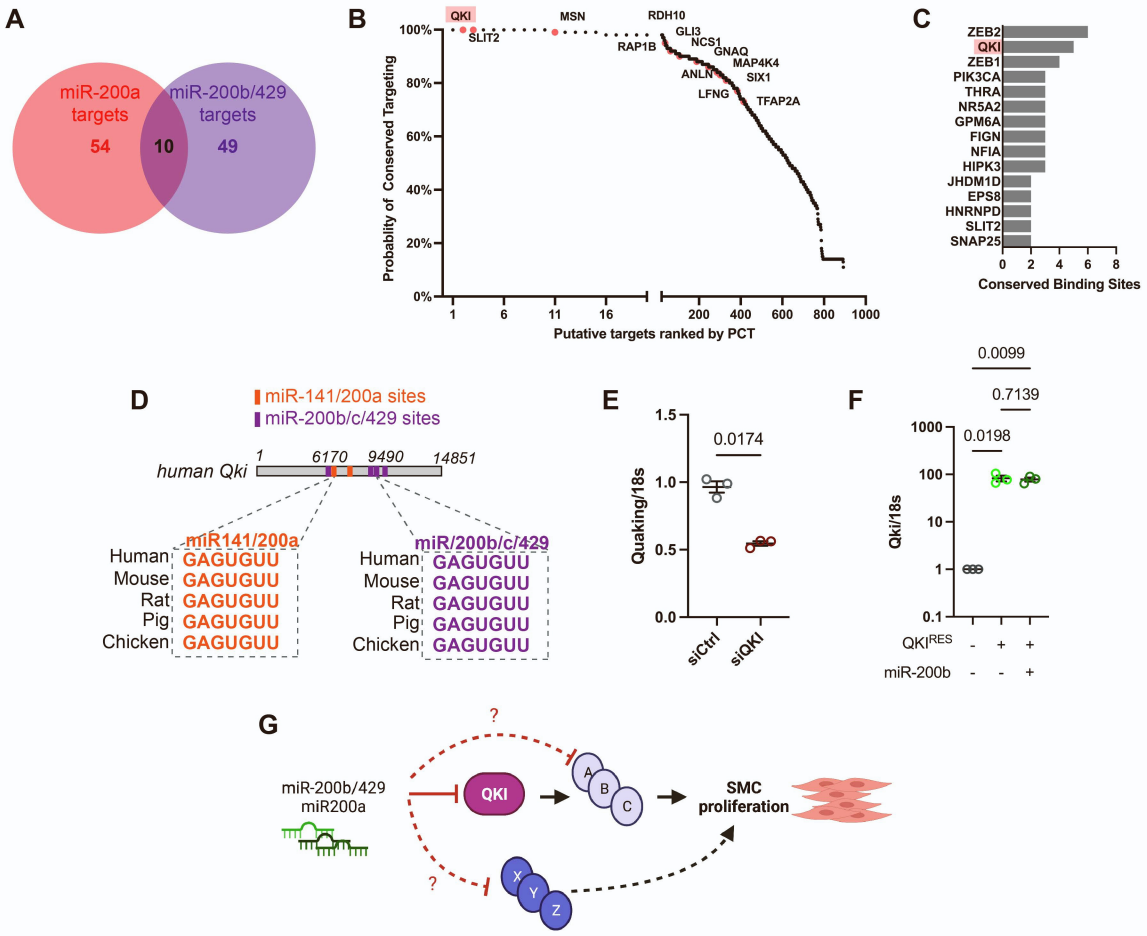

**Supplementary Figure 7, related to Figure 3. miR-200a, miR-200b, and miR-429 downregulation in dedifferentiated SMC**




**Supplementary Figure 8, related to Figure 4. miR-200b/429 inhibits SMC migration**




**Supplementary Figure 9, related to Figure 5. Lentiviral delivery of miR-200b-mCherry in carotid arteries after ligation.**




**Supplementary Figure 10, related to Figure 6. QKI is a top target of miR-200a/200b.**

**Supplementary Figure 1, related to Figure 1. Characterization of in vitro SMC dedifferentiation models induced by PDGF-BB treatment or H3K4me2 editing.** (**A**) Schematic of transcriptional and epigenetic processes controlling SMC contractile gene expression in PDGF-BB treated and H3K4me2 edited SMC. PDGF-BB treatment represses SMC contractile genes through multiple mechanisms, including the downregulation of TET2, which leads to an increase in DNA methylation and gene repression. Selective removal of H3K4me2 on the myocardin-regulated SMC lineage gene repertoire by a Myocardin-LSD1-based epigenome editing system abolishes the recruitment of TET2 and induces a profound loss of contractility and lineage identity. (**B**) Transcript expression of SMC contractile genes and differentiation master regulators in control, PDGF-BB treated, and Myocd-LSD1 expressing SMC. Data are expressed as mean ± SEM. Groups were compared by one-way ANOVA. * and # $p < 0.05$. (**C**) ACTA2, phalloidin, and DAPI immunostaining in control, PDGF-BB treated, and Myocd-LSD1 expressing SMC. Scale bar = 25 μm.

**Supplementary Figure 2, related to Figure 1. Alteration of miRNA processing and editing enzyme expression in dedifferentiated SMC.** Transcript expression of (**A**) miRNA processing enzymes and (B) miRNA editing enzymes in control, PDGF-BB treated, and H3K4me2 edited SMC measured by qPCR. Data are expressed as mean ± SEM. Groups were compared by one-way ANOVA. $p < 0.05$ is considered statistically significant.

**Supplementary Figure 3, related to Figure 1. Contribution of DEmiRs in the regulation of DEG in H3K4me2 edited SMC.** (**A**) Heatmap of differentially expressed genes in control, PDGF-BB and H3K4me2 edited SMC. Data generated with statistic parameters as Euclidean distance, complete linkage cluster, and cutoff value as fold change > 1.7 and P-value < 0.05. (**B**) Principal Component Analysis of control, PDGF-BB treated and H3K4me2 edited SMC. (**C**) Volcano plot showing differentially expressed genes in PDGF-BB treated or H3K4me2 edited versus control SMC. (**D**) Venn diagram representing the percentage of DEmiR targets in total, upregulated and downregulated DEG. (**E**) Gene Ontology analysis performed on DEG targeted by DEmiRs (GO pathways associated with upregulated DEG in red and GO pathways associated with downregulated DEG in blue).

**Supplementary Figure 4, related to Figure 1. Prediction of pathways modulated by DEmiRs during SMC dedifferentiation induced by PDGF-BB.** (**A**) Chord plot representing miRNA-mRNA functional network of DEG targeted by DEmiRs during PDGF-BB induced SMC phenotypic modulation. (**B**) Chord plot representing miRNA-mRNA functional network of DEG targeted by DEmiRs during SMC dedifferentiation induced by H3K4me2 editing.

**Supplementary Figure 5, related to Figure 2. Identification of pathways regulated by most abundantly expressed miRNAs in contractile SMC.** (**A**) Pie charts of the biological processes (upper chart) and cellular processes (bottom chart) regulated by the targets of the top 20 miRNAs expressed in differentiated SMC. (**B**) Gene ontology analysis on transcripts targeted by the top 20 miRNAs expressed in control differentiated SMC revealed the enrichment of pathways associated with other lineage differentiation programs.

**Supplementary Figure 6, related to Figure 2. Experimental and predictive determination of DEmiRs in H3K4me2 vs. control SMC.** (**A**) Volcano plot representing differentially expressed miRNAs in H3K4me2 edited SMC compared to control SMC. The miR200 cluster is among the most downregulated miRNAs (purple dots). (**B**) Prediction of upregulated (green) and downregulated (red) miRNAs in H3K4me2 edited SMC vs. control based on RNAseq data and differentially expressed genes. (**C**) Comparison between H3K4me2 edited SMC DEmiRs identified by small RNAseq and RNAseq-based prediction.

**Supplementary Figure 7, related to Figure 3. miR-200a, miR-200b, and miR-429 downregulation in dedifferentiated SMC.** (**A**) Pipelines for identifying potential miRNA candidates for functional studies, including the miR-200 cluster. (**B**) Sequence homology of miR-200a/200b/429 (genomic cluster I) in human, mouse, and rat. The seed sequence is highlighted in red. (**C**) qPCR validation of miR-200 genomic cluster I (B) and II (C) miRNA expression in control, PDGF-BB, and H3K4me2 edited SMC. Data are expressed as mean ± SEM. Groups were compared by one-way ANOVA. $p < 0.05$ is considered statistically significant.

**Supplementary Figure 8, related to Figure 4. miR-200b/429 inhibits SMC migration.** (**A**) Gene Ontology pathway analysis on genes significantly upregulated in PDGF-BB treated SMC vs. control SMC, predicted targets of miR-200a or miR-200b/miR-429. (**B**) Survival assay (cell titer glo luciferase assay) in SMC transfected with control or miR-200b mimic. Data are expressed as mean ± SEM. Groups were compared by Student's t-test. $p < 0.05$ is considered statistically significant. ** < 0.01. (**C**) mRNA expression of pro-apoptotic, anti-apoptotic, and senescence markers in SMC treated with miR-200b mimic and PDGF-BB. Data are expressed as mean ± SEM. Groups were compared by one-way ANOVA. $p < 0.05$ is considered statistically significant. (**D**) Transwell assay measuring migration of control and PDGF-BB treated SMC transfected with miR-200b mimic or control mimic. Scale bar = 100 μm. Data are expressed as mean ± SEM. Groups were compared by Student's t-test. $p < 0.05$ is considered statistically significant. (**E**) Transwell assay measuring migration of control SMC transfected with miR-200a, miR-200b, miR-429, or control inhibitors. Scale bar = 200 μm. Data are expressed as

mean ± SEM. Groups were compared by one-way ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

**Supplementary Figure 9, related to Figure 5. Lentiviral delivery of miR-200b-mCherry in carotid arteries after ligation.** Immunostaining using mCherry or IgG control antibodies showed a high expression of miR-200b-mCherry or control miRNA-mCherry in injured carotid arteries. Scale bar = 100 μm.

**Supplementary Figure 10, related to Figure 6. QKI is a top target of miR-200a/200b.** (**A**) Venn Diagram representing the overlap between miR-200a and miR-200b targetomes predicted by TargetScan. (**B**) Ranking of miR-200b targets based on miR-200b binding sequence number, conservation, and homology. (**C**) Number of miR-200b conserved binding sequences on the predicted top miR-200b targets. (**D**) Schematics representing the miR-200a and miR-200b binding sequences on human Qki transcript and their conservation across species. (**E**) QKI expression after siQKI or siRNA control transfection in control SMC. Data are expressed as mean ± SEM. Groups were compared by Student's t-test. p < 0.05 is considered statistically significant. (**F**) QKI expression after transfection of pLX317-QKI$^{RES}$ or pLX317 control SMC in the presence or absence of miR-200b mimic. Data are expressed as mean ± SEM. Groups were compared by one-way ANOVA. p < 0.05 is considered statistically significant.

**SUPPLEMENTARY TABLE**

**Supplementary Table 1, related to STAR METHODS and Key Resources Table (Oligonucleotides).** List of primers used for mRNA and miRNA detection.

| Primer (Target/orientation) | Sequence |
|---|---|
| **Approach: mRNA qRT-PCR** | |
| rXrcc2-Fw | TTGCTAAATGGCGTTGCTGG |
| rXrcc2-Rv | GGGCAAGGAGCTCGGTC |
| rRdh10-Fw | TCTCTGGGACATCAACACGC |
| rRdh10-Rv | CCGTTCCCAGCTTGCAGA |
| rTfap2a-Fw | CGGGTGCTCGAGGCGTG |
| rTfap2a-Rv | CCGTCGTGACGGTCCATAGC |
| rZswim4-Fw | GTGCTGCAAGTGGTGGGAATA |
| rZswim4-Rv | AGGTCTCGATTGTCACAGCC |
| rMap4k4-Fw | CTCCATCTCAGCGCCTTGAA |
| rMap4k4-Rv | GCCAACGCAGTCAAGTCAATC |
| rLfng-Fw | TCATCGCCGTCAAGACCACC |
| rLfng-Rv | GAGTACCACATTGCCTGTGTGC |
| rGnaq-Fw | TCCCAGAATATGATGGACCCCA |
| rGnaq-Rv | AAGCGGATGTTCTCCGTGTC |
| rNcs1-Fw | TTGGAGACCCCACCAAGTTC |
| rNcs1-Rv | AAAGCTTGAAGGCCCACCTC |
| rRap1b-Fw | AAGACACTGACGATGTCCCAA |
| rRap1b-Rv | CTCCACTGTCTTGCCAGGTT |
| rMMP16-Fw | ATCTTACTCGCATTCAGCTC |
| rMMP16-Rv | TTCTGTAACCAAACCTCCACA |
| rPenk-Fw | GGCGCAGTTCCTGAGACTTT |
| rPenk-Rv | GAGTGTGCATGCCAGGAAGT |
| rPtprf-Fw | TACAGCACAGTCCACCCCAT |
| rPtprf-Rv | CTGGGTGATAATGCCGTTGC |
| rSlit2-Fw | GTGATGAGGAAGAAGGTCACCA |
| rSlit2-Rv | CTGTGATGGTCTCAGGCAGA |
| rAbi2-Fw | ATTGCCCTATAGACGCCCTC |
| rAbi2-Rv | GTGGTGTATCTGAAACTGGATT |
| rMsn-Fw | TCGGGACCAGAAGAAGACTCA |

| | |
|---|---|
| rMsn-Rv | CCTGGACCATCTGGGCCTTT |
| rFn1-Fw | CCACCATCACTGGTCTGGAG |
| rFn1-Rv | GGGTGTGGAAGGGTAACCAG |
| rAnln-Fw | CACCCGTTTCAAATGCCTCA |
| rAnln-Rv | TAGCAGATGGTCCACTTGCA |
| rKlf14-Fw | GTTTACGCGTTCAGACGAGC |
| rKlf14-Rv | GATGGTAAGTTGGGTGGCGA |
| rQk-Fw | CCCAGCGGTGTGTTAGAGTG |
| rQk-Rv | ATCCAGCAACTCAATGGGCT |
| rZeb2-Fw | GACATAAATACGAACACACAGGAAA |
| rZeb2-Rv | TTGCCACATTTGTCACACTG |
| rZeb1-Fw | GCGGCGCAATAACGTTACAA |
| rZeb1-Rv | CATCATCTGGCACACCACCT |
| rTet2-Fw | GGTTCCTGTTCTGCTACGGT |
| rTet2-Rv | GCCCTTTGAATGAATCCAGCAG |
| rMyocd-Fw | CAAGGGTGTGCACAGATGACTG |
| rMyocd-Rv | GCTGTTTCACTGCATCTTCGT |
| 18s-Fw | CGGCTACCACATCCAAGGAA |
| 18s-Rv | AGCTGGAATTACCGCGGC |
| GAPDH-Fw | ACCACAGTCCATGCCATCAC |
| GAPDH-Rv | TCCACCACCCTGTTGCTGTA |
| **Approach: Taqman miRNA qRT-PCR** | |
| rno-miR-200a-3p | #000502 (Thermo Fisher, miRVana) |
| rno-miR-200b-3p | #001800 (Thermo Fisher, miRVana) |
| rno-miR-429 | #001077 (Thermo Fisher, miRVana) |
| rno-miR-145-5p | #002278 (Thermo Fisher, miRVana) |
| U6 | #001973 (Thermo Fisher, miRVana) |
| **Approach: miRNA In Situ Hybridization** | |
| LNA miR-200b probe | # YD00619853 (Geneglobe) |
| LNA scrambled microRNA probe | # YD00699004 (Geneglobe) |