SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. *Dgcr8-/-* ESCs display characteristics of mesenchymal cells. (**A**) Morphology of wild type and *Dgcr8-/-* ESCs at different passages off mouse embryonic fibroblast feeders. (**B**) Examples of spread cells (red) and un-spread cells (green) marked by the Cell-IQ Analyzer program. Blue dots indicate cell debris or unknown particles. (**C**) Heat-map showing mRNA expression changes of epithelial and mesenchymal markers in wild type and *Dgcr8-/-* ESCs. Shown are log2 fold changes relative to the mean of all samples. Heatmap is generated with pheatmap package in R. (**D**) Western analysis of CDH1 and CDH2. GAPDH was used as a loading control. Representative gels are shown. Data were normalized to wild type ESCs. Shown are mean±range. n=2.

Supplementary Figure 2. MiR-294/302 and let-7 antagonistically regulate the EMT in ESCs. (**A**) Morphology of mock, control mimic, miR-293, miR-294, miR-295, and miR-302a transfected *Dgcr8-/-* ESC colonies. (**B**) Number of cells attached in 40 min after plating. (**C**) Western analysis of CDH1 and CDH2. GAPDH was used as a loading control. Representative gels are shown. Data were normalized to wild type ESCs. Shown are mean±range. n=2. (**D**) qRT-PCR analysis of epithelial and mesenchymal markers in mock and let-7c transfected *Dgcr8-/-* ESCs on days 1, 2, and 3. The β-actin gene was used as a control. For each gene, data were normalized to the mRNA level of mock transfected *Dgcr8-/-*ESCs. Shown are mean ± SD, n = 3. (**E**) Western analysis of CDH1 and CDH2 in let-7c transfected *Dgcr8-/-*ESCs. Actin was used as a loading control.

Supplementary Figure 3. Activation of the EMT or apoptotic pathways inhibits the pluripotency program in wild type ESCs. (**A**) Western analysis of CDH1 and CDH2 in Snai1 overexpressing ESCs. Actin was used as a loading control. (**B**) qRT-PCR analysis of pluripotency markers in wild type ESCs without (-Dox) or with (+Dox) Snai1 overexpression. The β-actin gene was used as a control. Data were normalized to wild type ESCs without Snai1 overexpression. Shown are mean ± SD, n = 3. (**C**) qRT-PCR analysis of pluripotency markers in wild type ESCs without or with PAC1 treatment. The β-actin gene was used as a control. Data were normalized to wild type ESCs without PAC1 treatment. Shown are mean ± SD, n = 3.

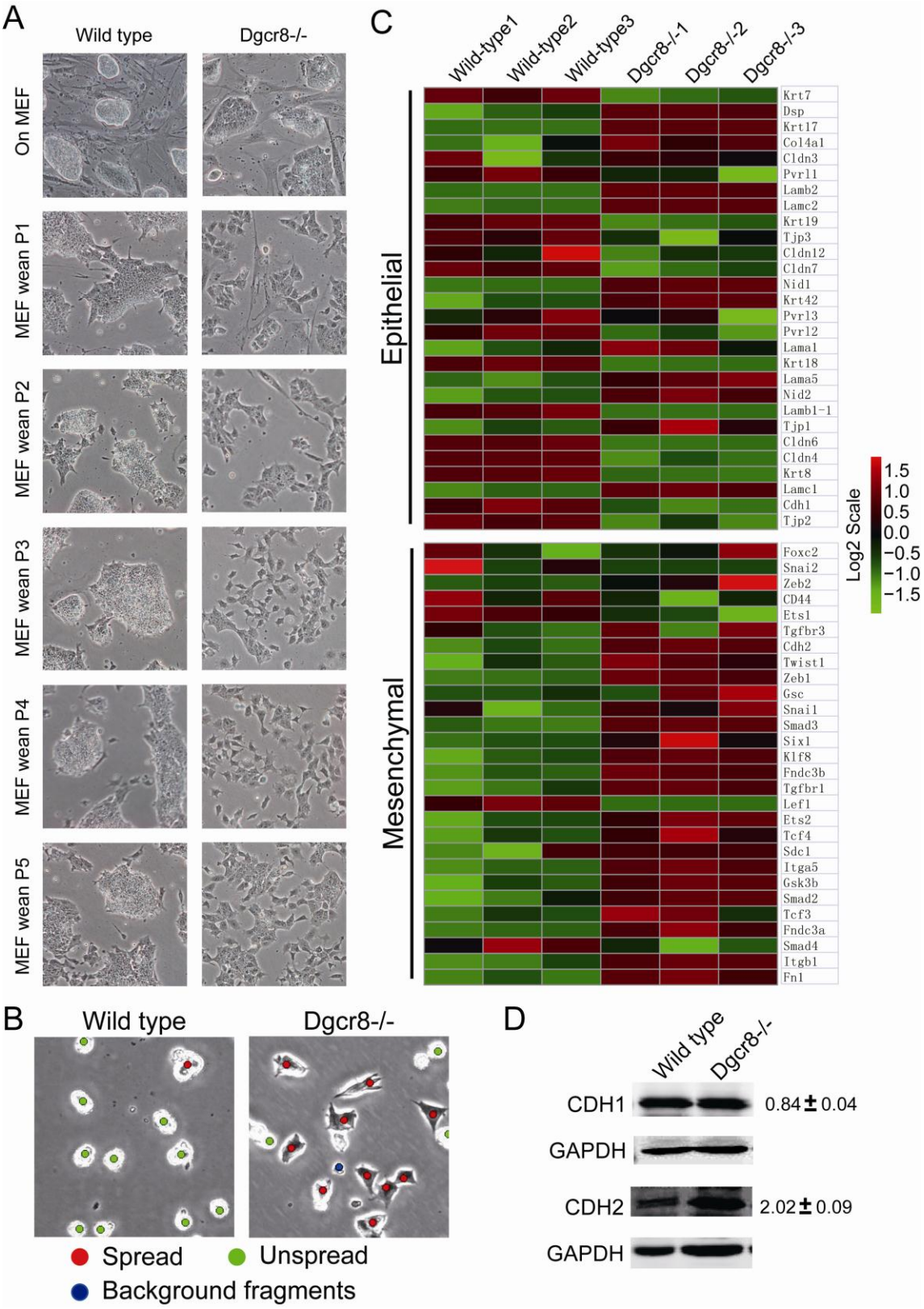
Supplementary Figure 4. Identification of mRNA targets of miR-294 that may regulate EMT process in ESCs. (**A**) Strategy to identify miR-294 targets. (**B**) Luciferase reporter assays. Shown are mean±SD, n=4-8. P value < 0.05 for solid triangle and round dots. (**C**) qRT-PCR analysis of candidate targets in wild type and *Dgcr8-/-* ESCs. (**D**) qRT-PCR analysis of candidate targets in mock and miR-294 transfected *Dgcr8-/-* ES cells. For both **C** and **D**, the β-actin gene was used as a control. Shown are mean±SD, n=3. (**E**) Western analysis of GSK3A, GSK3B and VIM. GAPDH was used as a loading control. Representative gels are shown. Data were normalized to wild type (upper panels) or mock transfected *Dgcr8-/-* ESCs (lower panels).

Supplementary Figure 5. Identification of mRNA targets of miR-294/302. (**A**) qRT-PCR analysis for knockdown efficiency of siRNAs to candidate miR-294 targets. The β-actin gene was used as a control. For each gene, data were normalized to control mimic transfected *Dgcr8-/-* ESCs. n=1-2. For n=2, shown are mean±SD. Two sets of siRNAs were used except for Vim.

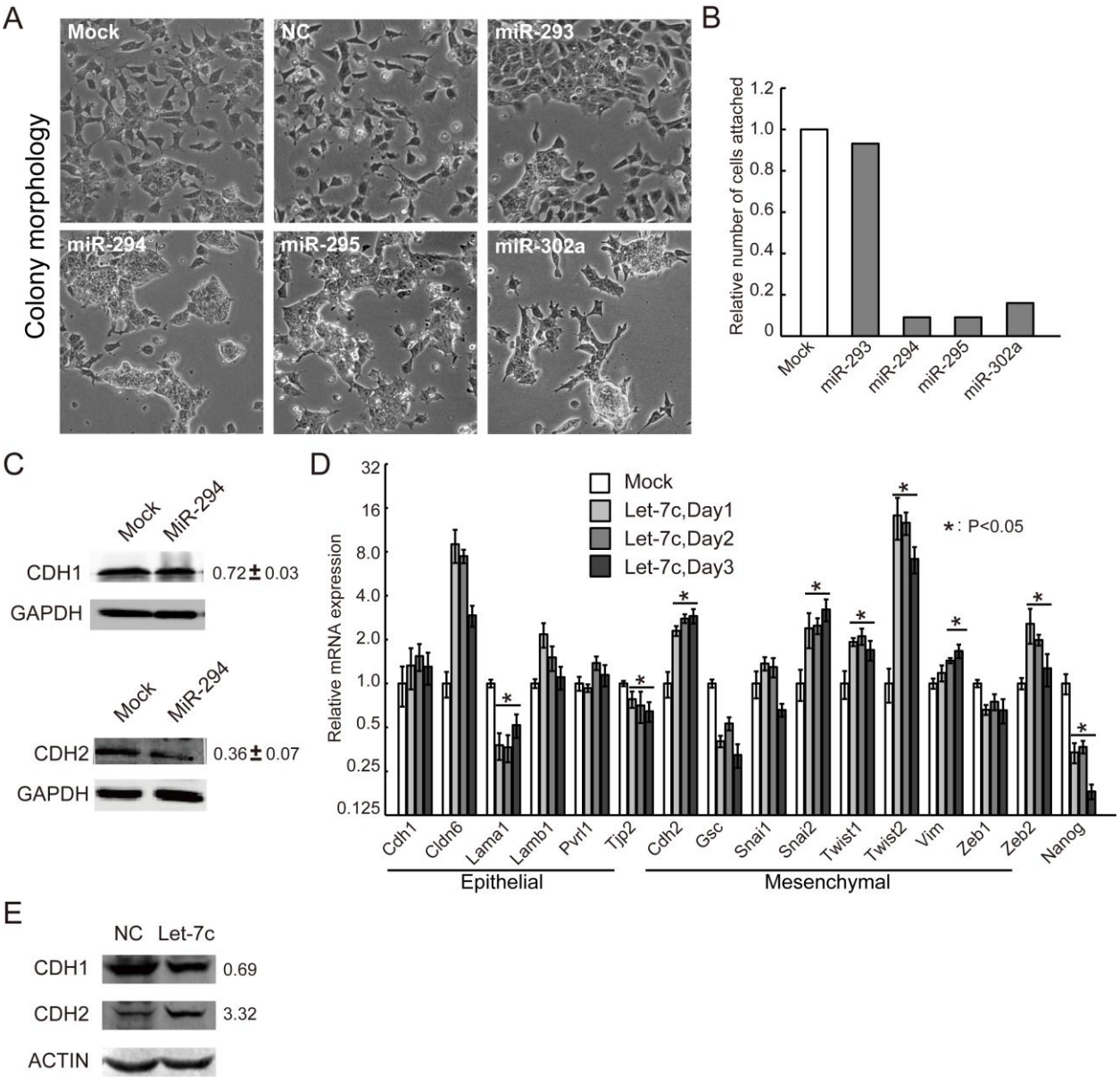
(**B**) Colony morphology of *Dgcr8-/-* ESCs mock transfected or transfected with Mmp23 siRNAs after 48 hours. (**C**) Cell attachment analysis. Shown are mean ±SD, n=6-9.

Supplementary Figure 6. Combined suppression of the EMT and apoptotic pathways antagonizes multiple differentiation-inducing miRNAs. (**A**) Western analysis of CDH2 in R&C treated *Dgcr8-/-* ESCs. Actin was used as a loading control. Representative gels are shown. Data were normalized to DMSO treated ESCs. Shown are mean ±range. n=2. (**B**) qRT-PCR analysis of the pluripotency markers Esrrb, Klf4, and Rex1 in let-7c transfected *Bak-/-*, *Bax-/flox, Dgcr8-/-* ESCs treated with DMSO, RepSox, or R&C. The β-actin gene was used as a control. For each gene, data were normalized to mock transfected *Bak-/-*, *Bax-/flox*, *Dgcr8-/-* ESCs treated with DMSO. Shown are mean ± SD, n = 3. qRT-PCR analysis of the pluripotency markers Oct4, Sox2, and Nanog in miR-26a, miR-99b, miR-193, miR-199a-5p, and miR-218 transfected (**C**) *Dgcr8-/-* ESCs and (**D**) *Bak-/-, Bax-/flox, Dgcr8-/-* ESCs treated with DMSO or R&C. The β-actin gene was used as a control. For each gene, data were normalized to mock transfected, DMSO treated ESCs with the same genotype. Shown are mean ± SD, n = 3-6. (**E**) AP staining for miR-26a, miR-99b, and miR-218 transfected *Bak-/-*, *Bax-/flox, Dgcr8-/-* ESCs treated with DMSO or R&C. (**F**) qRT-PCR analysis of epithelial and mesenchymal markers in *Dgcr8-/-* ESCs transfected with differentiation-inducing miRNAs. The β-actin gene was used as a control. For each gene, data were normalized to the mRNA level of mock transfected *Dgcr8-/-* ESCs. Shown are mean ± SD, n = 2. (**G**) Apoptosis analysis by Annexin V and propidium iodide staining in *Dgcr8-/-* ESCs transfected with differentiation- inducing miRNAs. Shown are mean ± SD, n = 2.

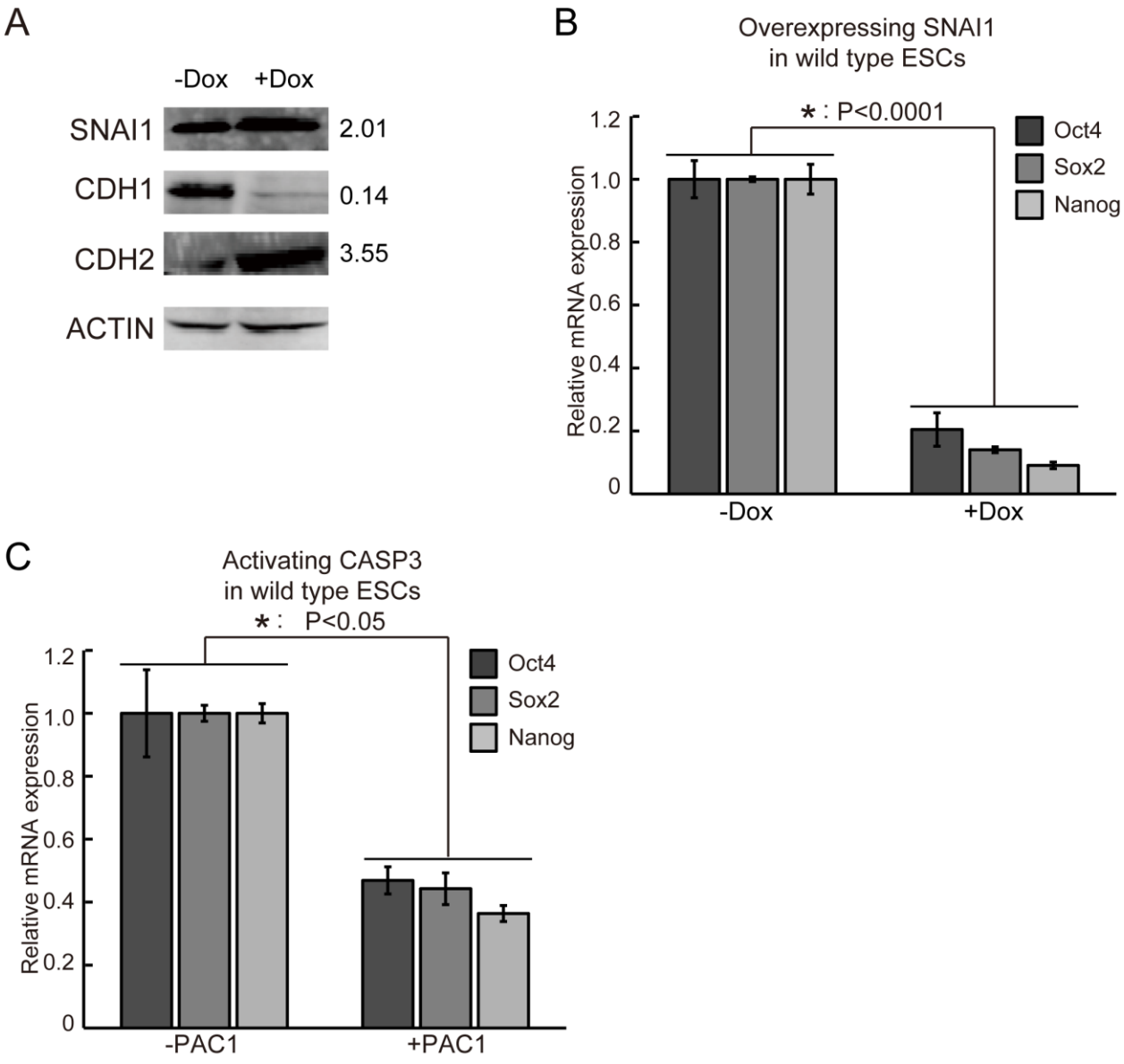
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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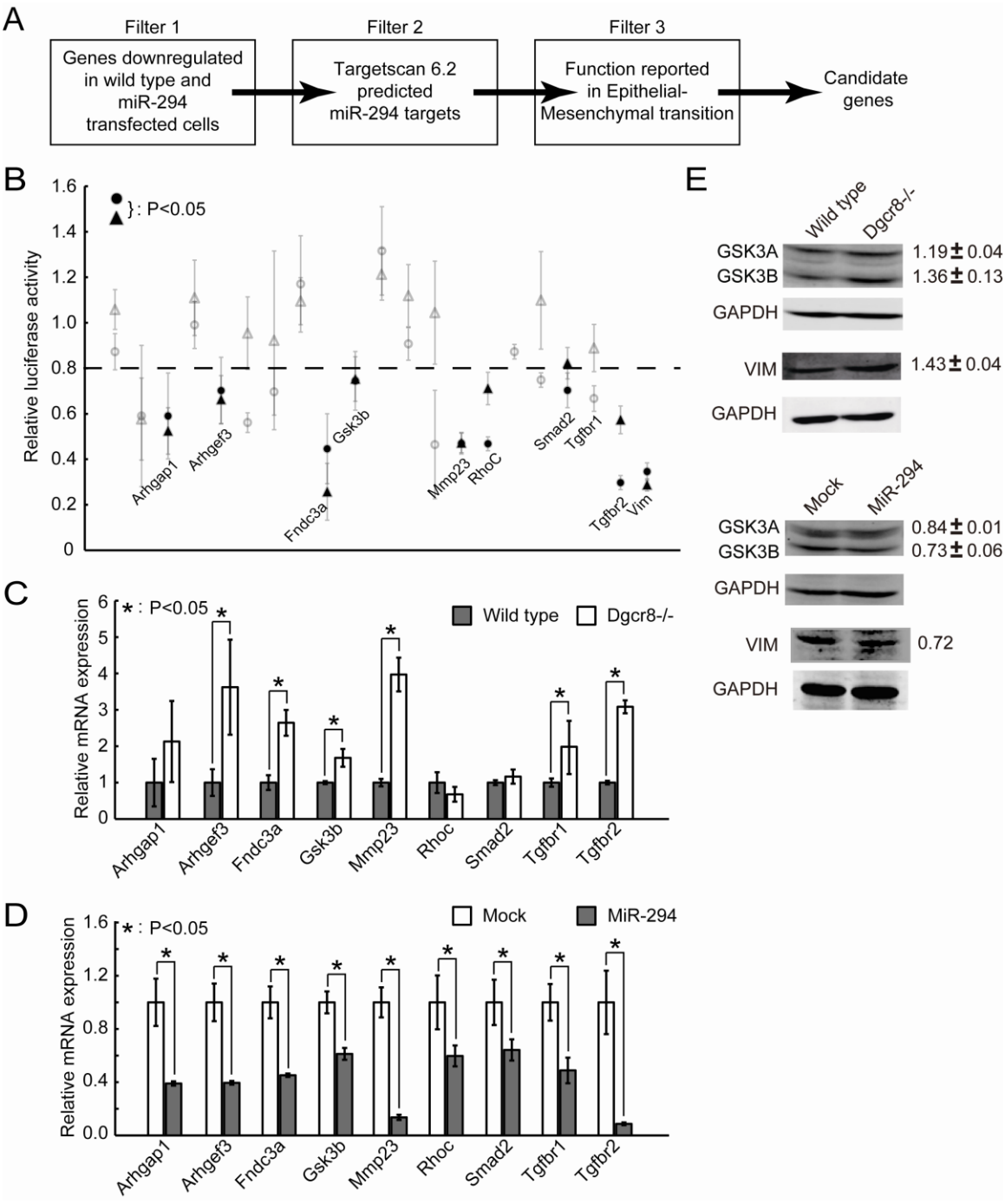
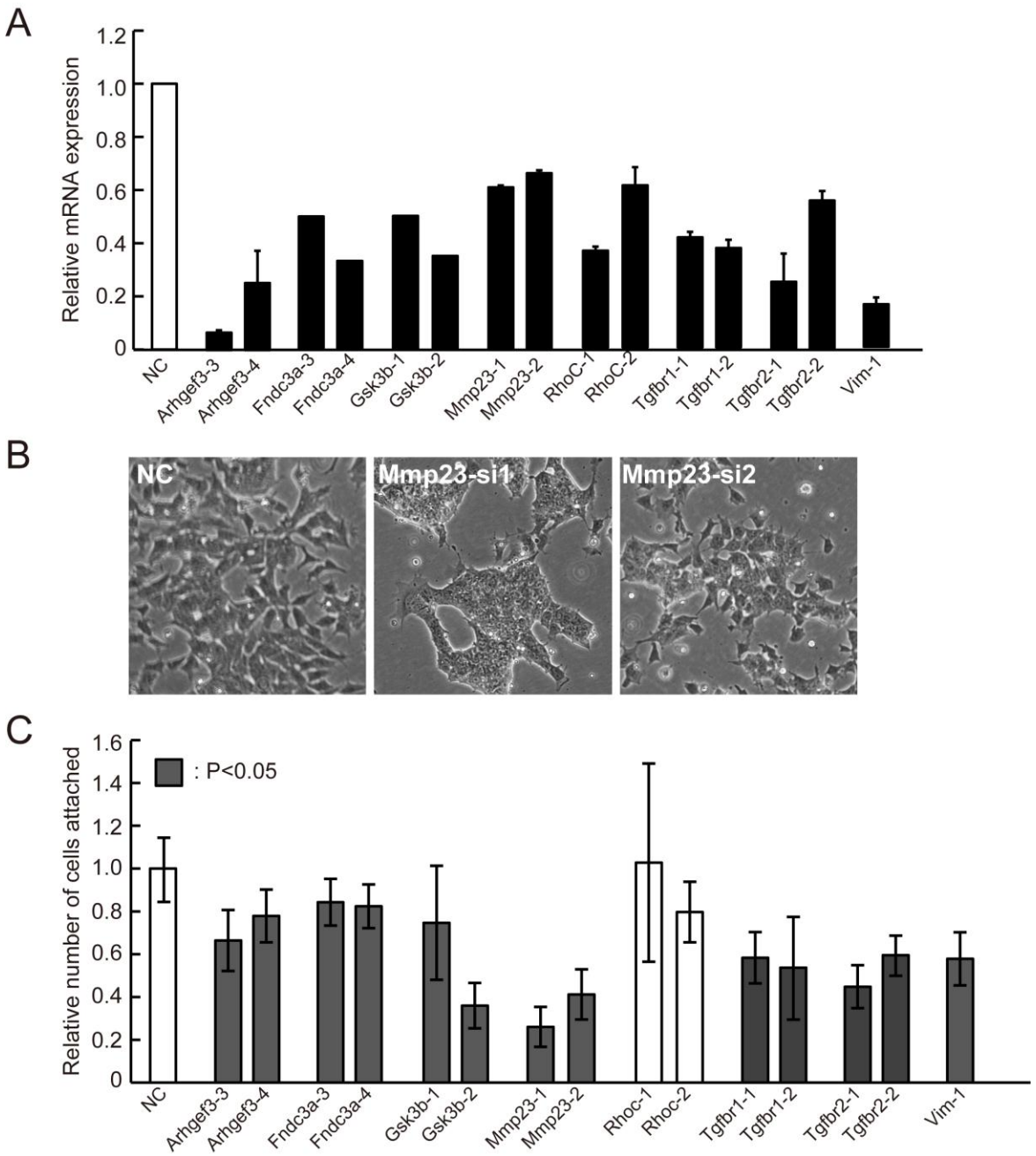
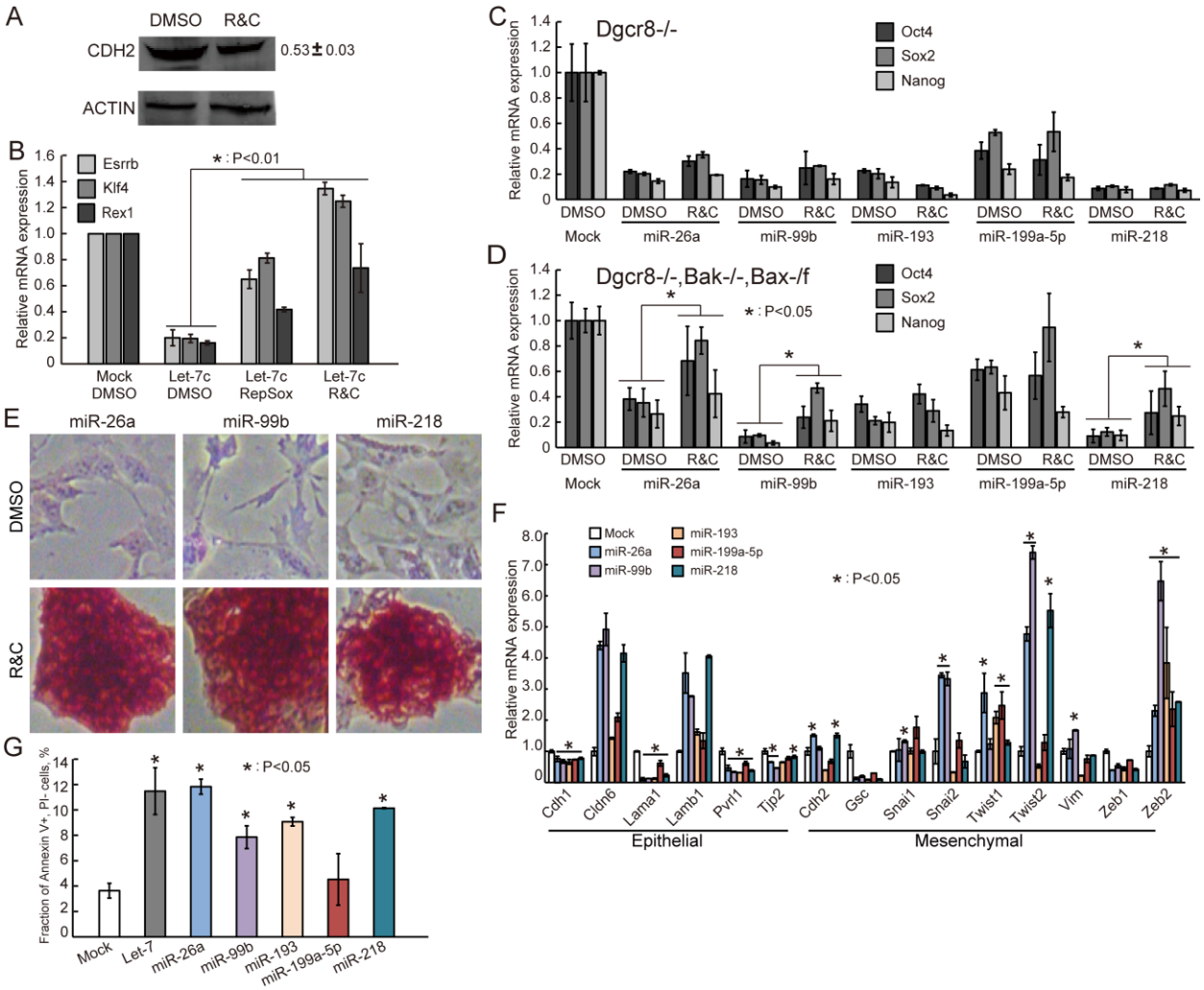


Supplementary Figure 5



Supplementary Figure 6



Supplementary Table 1: Sequences of primers for cloning 3'UTRs of candidate targets

Gene	Primer sequences (5' to 3')
Akt2	3UTR-F: GACACTCGAGACATCTAGAAGTT
Akt2	3UTR-R: GACCGCGGCCGCTTGTACCGTACAAATA
Akt3	3UTR-F: GACACTCGAGGTTCCTTTCAGTCTGTTTC
Akt3	3UTR-R: CTTGCGGCCGCAGATTGACATAGGGCTTTA
Arhgap1	3UTR-F: GACACTCGAGTTATACCTCTCACTG
Arhgap1	3UTR-R: GACCGCGGCCGCTCATTTACATTT
Arhgap26	3UTR-F: GCTACTCGAGACACTCAGGATCTCAGGAT
Arhgap26	3UTR-R: GCTAGCGGCCGCATCTGTCATAGGCCACACAT
Arhgef12	3UTR-F: GGCACCTCGAGGCCTTACTTCTTGCCTGTTCCC
Arhgef12	3UTR-R: GGCACGCGGCCGCGCTGAGTGACCAGGCTTACTG
Arhgef3	3UTR-F: GACACTCGAGCAGAAGACTGTGACCCTGC
Arhgef3	3UTR-R: GACACGCGGCCGCAAGTATTCATTTCACCAA
Arhgef7	3UTR-F: GACACTCGAGGCTGACTTCATTCTCATAGG
Arhgef7	3UTR-R: CTTGCGGCCGCTTAATAATTGCAAGGGTG
Fndc3a	3UTR-F: GGCACCTCGAGGTGGCATTTAGCACTGGCATTGA
Fndc3a	3UTR-R: GACACGCGGCCGCAGATACTTTTGCTACATAGTTTATGTATTC
Gsk3b	3UTR-F: GGCACCTCGAGGCGGGAAAGACCAGCACTTAC
Gsk3b	3UTR-R: GGCACGCGGCCGCGGACAAGCTCACTCACAGTGG
Itga5	3UTR-F: GACACTCGAGTGATCTCAGACTCATGAT
Itga5	3UTR-R: GACAGCGGCCGCCTTGCAACAGAGTTTA
Itgav	3UTR-F: GCTACTCGAGCACCCAGCAGTCTCAGT
Itgav	3UTR-R: GCTAGCGGCCGCCAGGGAGTTGCACACGCTT
Itgb3	3UTR-F: GACACTCGAGTGAGACCATCTTCAGATGA
Itgb3	3UTR-R: CTTGCGGCCGCGGAAAGCACATTTAATTC
Mmp23	3UTR-F: GGCACCTCGAGCACTGCTCAGAGCCTCTGTGCTGGAAGGCAGCAGATAAA
Mmp23	3UTR-R: GGCACGCGGCCGCTCAGAGAGAAAGTGCTTTATCTGCTGCCTTCCAGCACA
Rock2	3UTR-F: GTGTAGATCCTAGTGAGACAT
Rock2	3UTR-R: GCTAGCGGCCGCGATGACATCACCTCATCTACT
Rragd	3UTR-F: GCTACTCGAGAACTGTCAGGAGCCTGAGT
Rragd	3UTR-R: GCTAGCGGCCGCCACTGTCCAGCTACAACAT
Smad2	3UTR-F: GGCACCTCGAGGCTGGTTGCAGTGGTAAACACTA
Smad2	3UTR-R: GGCACGCGGCCGCGACCTTCAACGCCAACTACTC
Tgfbr1	3UTR-F: GGCACCTCGAGCACCGTGGGAACTCTGCTCT
Tgfbr1	3UTR-R: GACACGCGGCCGCAGGATGAAATTTAAGCATCTCCCTAGC
Tgfbr2	3UTR-F: GGCACCTCGAGTCCTCTAGCCAAAGACCAGAGG
Tgfbr2	3UTR-R: GACACGCGGCCGCGAATACATGAATATGGCCGAAGTGTTC
Vim	3UTR-F: GCACACACTTGGTGCAACAGT
Vim	3UTR-R: GAAGCAGTAACAAGTTGGTCAG
Fn1	3UTR-F: GGCACCTCGAGTCTTTCCAGCCCCACCCTACAAGT
Fn1	3UTR-R: GACACGCGGCCGCACTTGTCTTTCCACAGTAGTAAAGCGTT
RhoC	3UTR-F: GACACTCGAGCTTCCCCAAAGCTT
RhoC	3UTR-R: GACCGCGGCCGCAACCTTTGACCTTTATTCAT

Note: Fn1 was chosen as a negative control without miR-294 binding site in its 3'UTR; RhoC was not significantly downregulated in wild type ESCs, but was included as it has been shown to be targeted by miR-294. (Subramanyam, D., Lamouille, S., Judson, R. L., Liu, J. Y., Bucay, N., Derynck, R., and Blelloch, R. (2011). Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. Nat. Biotechnol 29, 443-8.)

Supplementary Table 2: Sequences of siRNAs to candidate miR-294 targets

Gene	siRNA sequences (5' to 3')
Arhgef3-si-3	sense:GCUUCGAGAUGUUCGGAAATT
Arhgef3-si-3	anti-sense:UUUCCGAACAUCUCGAAGCTT
Arhgef3-si-4	sense:GUGCACAGCGGAAACGAAATT
Arhgef3-si-4	anti-sense:UUUCGUUUCCGCUGUGCACTT
Fndc3a-si-3	sense:CAGCAUAGACCUCGGAGAUTT
Fndc3a-si-3	anti-sense:AUCUCCGAGGUCUAUGCUGTT
Fndc3a-si-4	sense:GUGAAGAGGUCCUGUACUATT
Fndc3a-si-4	anti-sense:UAGUACAGGACCUCUUCACTT
Gsk3b-si-1	sense:GAUCAUUUGGUGUGGUAUATT
Gsk3b-si-1	anti-sense:UAUACCACACCAAAUGAUCTT
Gsk3b-si-2	sense:GGAGCAAAUUAGAGAAAUGTT
Gsk3b-si-2	anti-sense:CAUUUCUCUAAUUUGCUCCTT
Mmp23-si-1	sense:GGGAAAUGUAGAUGCGCCATT
Mmp23-si-1	anti-sense:UGGCGCAUCUACAUUUCCCTT
Mmp23-si-2	sense:GGGAAAGUAUACUGGUACATT
Mmp23-si-2	anti-sense:UGUACCAGUAUACUUUCCCTT
RhoC-si-1	sense:CUAUAUAGCCGACAUCGAATT
RhoC-si-1	anti-sense:UUCGAUGUCGGCUAUAUAGTT
RhoC-si-2	sense:GGAUCAGUGCCUUUGGCUATT
RhoC-si-2	anti-sense:UAGCCAAAGGCACUGAUCCTT
Tgfbr1-si-1	sense:GAUUUAUAGCAGCAGACAATT
Tgfbr1-si-1	anti-sense:UUGUCUGCUGCUAUAAAUCTT
Tgfbr1-si-2	sense:GAACAGAAGUUAAGGCCAATT
Tgfbr1-si-2	anti-sense:UUGGCCUUAACUUCUGUUCTT
Tgfbr2-si-1	sense:UGGGAGAAGUGAAGGAUUATT
Tgfbr2-si-1	anti-sense:UAAUCCUUCACUUCUCCCATT
Tgfbr2-si-2	sense:GGAGGAAGAACGACAAGAATT
Tgfbr2-si-2	anti-sense:UUCUUGUCGUUCUUCCUCCTT
Vim-si-1	sense:GUCUUGACCUUGAACGGAATT
Vim-si-1	anti-sense:UUCCGUUCAAGGUCAAGACTT

Supplementary Table 3: Quantitative RT-PCR primers

Gene	Primer sequences (5'to 3')
Actb-F	CCACCATGTACCCAGGCATT
Actb-R	CCGATCCACACAGAGTACTT
Arhgap1-F	CGAAGGAATGTCTTGAGGATGAC
Arhgap1-R	GAGAAAAAAACCGAATGACCTATCA
Arhgef3-F	CCAGTCCCTCAGAGTCAAGCA
Arhgef3-R	TTTCCCCCTGCCACTTTCA
Cdh1-F	GAGCGTGCCCCAGTATCG
Cdh1-R	CTGCCTTCAGGTTTTCATCGA
Cdh2-F	CTCTTTATCCCGCCGTTTCA
Cdh2-R	TTGCACAGACAGTCGATGCTACT
Cldn6-F	GCCCACTCTATCATCCAGGACTT
Cldn6-R	CCCCAGCTCCCGCTTT
Esrrb-F	CCTGAGCGGACACACTGCTT
Esrrb-R	CCCAACTGTCACTGGTCCAT
Fndc3a-F	AGGAATCTCCCAATTCACTTGTG
Fndc3a-R	TCTGTGTGCAGACTGTCAGGTAAG
Gsc-F	GCTAGCTCCTCGTTGCTTTCTC
Gsc-R	ACCCCCCATACCCTTTTGTC
Gsk3b-F	CACGGTCTCCAGCATTAGTATCTG
Gsk3b-R	GCTGCCGGTGGACTTTGA
Klf4-F	CTATGCAGGCTGTGGCAAAA
Klf4-R	TGGTAAGGTTTCTCGCCTGTGT
Lama1-F	CCCCCACACCCATTCCA
Lama1-R	GCAGGATAGCCACCCACATAA
Lamb1-F	AATCTGAAACGGCAGCTTCTG
Lamb1-R	CACGTTCCTCTCAAGCTTGCT
Mmp23-F	CACCAACCTTGTTTTCGTTCTG
Mmp23-R	TGTATCCGTCAGGCAAAAGAAA
Nanog-F	GCTCAGCACCAGTGGAGTATCC
Nanog-R	TCCAGATGCGTTCACCAGATAG
Oct4-F	ATGTTCTTAAGGCTGAGCTGCAA
Oct4-R	CCGTATCAGTGCACGTTCGA
Pvrl1-F	GCCCCCACCCCAAATATG
Pvrl1-R	TCAGCCTCATCCACAGTGAAGT
Rex1-F	GGTGTGGATGCGGATATGG
Rex1-R	GATTGTGGAGCCATACATTGCA
RhoC-F	CGCACCCCTTCCTTAGTCTTG
RhoC-R	GATTTCTGTTATGAATTCCCATTTCC
Smad2-F	TGTGCAGAGCCCCAACTGT
Smad2-R	CAGCCTGGTGGGATCTTACAC
Snai1-F	CACATCCGAGTGGGTTTGG
Snai1-R	CACGCAGTCGCTGAACGA

Snai2-F	GGGAGCATACAGCCCTATTACTGT
Supplementary Table 3 continued	
Snai2-R	GGCCACTGGGTAAAGGAGAGT
Sox2-F	GCAAGTACTGGCAAGACCGTT
Sox2-R	CGATATCAACCTGCATGGACAT
Tgfbr1-F	CCAAGGAACAAAGAAATGATTTACAA
Tgfbr1-R	TGCCAGTGCTAACCCAGTAGCT
Tgfbr2-F	CACTAGTGGACCAGCATTCTGTAAA
Tgfbr2-R	GCCATCATCGCTATCCTTCTG
Tjp2-F	CCCCCGCACATGAGTTCTA
Tjp2-R	TGGTGTCCTGGTAAAGTCTGGAA
Twist1-F	GACCTGGTACAGGAAGTCGATGT
Twist1-R	TCCAGATCGATGTGGACGTTT
Twist2-F	CGCTCCCCTCTGACAAGCT
Twist2-R	GAACCTGGTAGAGGAAGTCTATGTACCT
Vim-F	ATACTGCTGGCGCACATCAC
Vim-R	CCCTTTCCCCAGTTTTTAATAGG
Zeb1-F	GCTGGGCCAACTCTTAACAGA
Zeb1-R	CCTGTGATGCGTATCAGAAAAG
Zeb2-F	TTTCCCCCTGCCACTTTCA
Zeb2-R	CCAGTCCTGGGTATGGTCGTA