Supplementary Data

Supplementary Table S1. Primers Used for Real-Time-Polymerase Chain Reaction

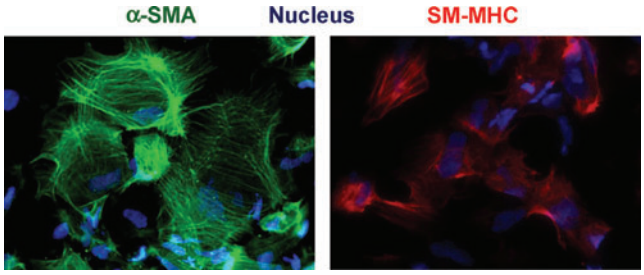
Gene	*Primer sequence*	*Sequence ID*	*Product size (bp)*
18S RNA	Forward: 5′-ggaagggcaccaccaggagt-3′ Reverse: 5′-tgcagccccggacatctaag-3′	NR_003278	317
MyoCD	Forward: 5′-gtgggcccagcattttcaac-3′ Reverse: 5′-tttccggtatcgtgctttcctc-3′	NM_145136	156
α-SMA	Forward: 5′-ggcatccacgaaaccacctat-3′ Reverse: 5′-agccaccgatccagacagagta-3′	NM_007392	214
SMMHC	Forward: 5′-atgctgggaaggtggactacaa-3′ Reverse: 5′-gtgcggaacatgccctttt-3′	NM_013607	216
KLF4	Forward: 5′-agcgctacaatcatggtcaagt-3′ Reverse: 5′-gtaagtccgggcatgttcaagt-3′	NM_004235	221
OCT3/4	Forward:5′-gaaggtattcagccaaacga-3′ Reverse:5′-aaattctccaggttgcctct-3′	NM_013633	216
AFP	Forward:5′-ggcctcttccagaaactagg-3′ Reverse:5′-ccacaggccaatagtttgtc-3′	NM_007423	170
GATA2	Forward:5′-agacgacaaccaccacctta-3′ Reverse:5′-atgcactttgacagctcctc-3′	NM_008090	205
NeuroD1	Forward:5′-ctttcaaacacgaaccatcc-3′ Reverse:5′-aactgacgtgcctctaatcg-3′	NM_010894	237

MyoCD, myocardin; α-SMA, α-smooth muscle actin; SMMHC, smooth muscle myosin heavy chain; KLF4, Kruppel-like factor 4. OCT3/4, octamer-binding protein 3/4; AFP, alpha-fetoprotein; GATA2, GATA binding protein 2.

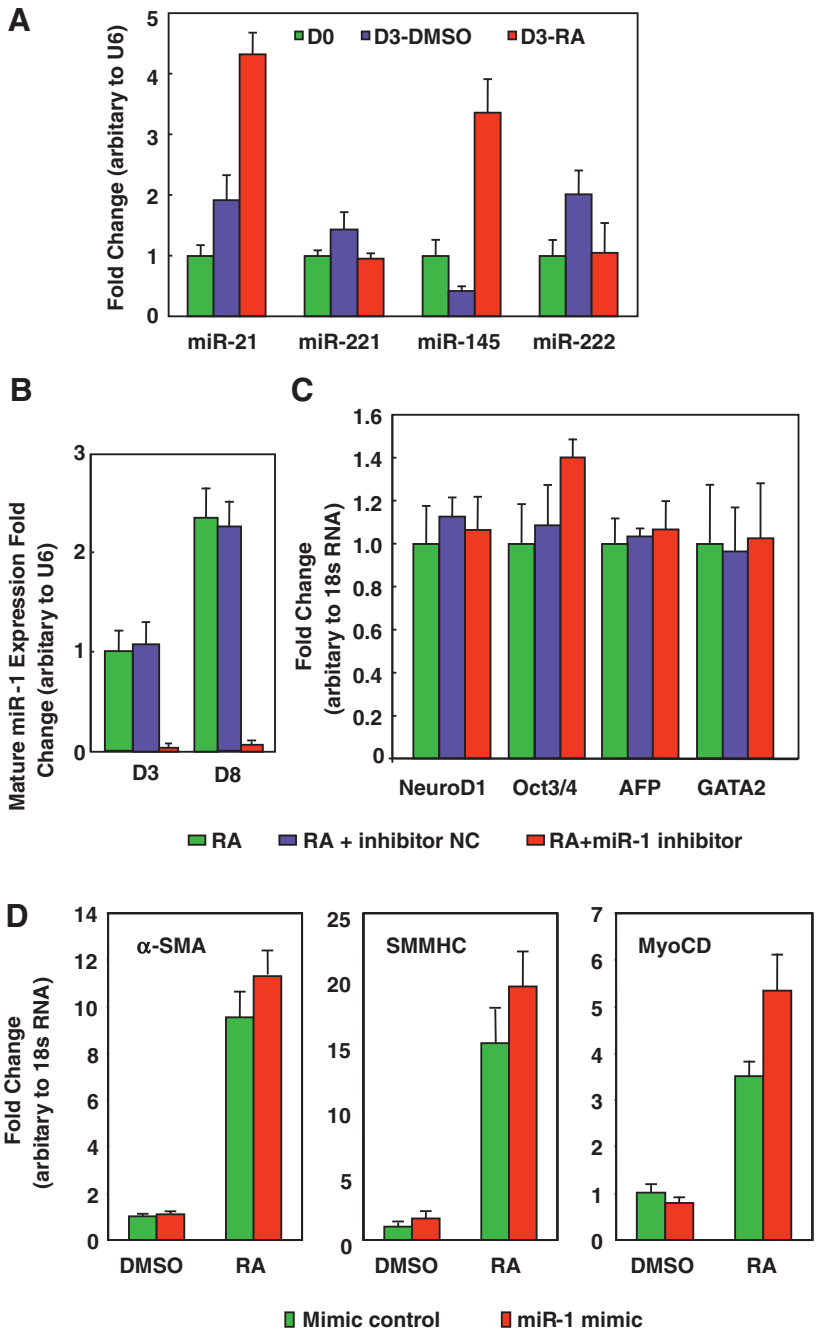
Supplementary Table S2. Primers Used for Kruppel-Like Factor 4 3′ Untranslated Region Cloning

Gene	*Primer sequence*
KLF4 WT 3′UTR	Forward: 5′-ggaggacgactatcccacgtagtggatgtga-3′ Reverse: 5′-ggaggaagcttcttatttctcaccttgagtatgc-3′
KLF4 Mutant 3′UTR	Forward: 5′-ctggatggatcttctatcataggtataccaaatccaacttg-3′ Reverse: 5′-caagttggatttggtatacctatgatagaagatccatccag-3′

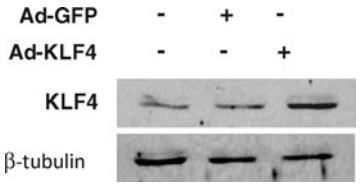
KLF4, kruppel-like factor 4; WT, wild type; 3′UTR, 3′ untranslated region.



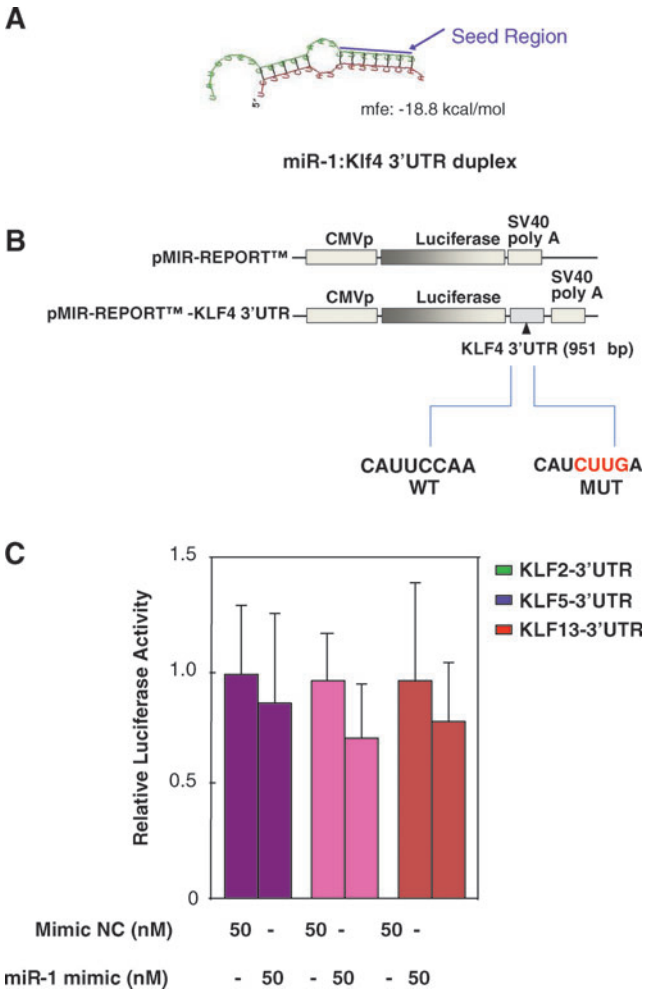
SUPPLEMENTARY FIG. S1. Representative figures with immunofluorescence staining in the derived cell population treated with 10 μM RA for 8 days. (Left panel) α-SMA stained with green and (right panel) SMMHC with red overlapped with blue stained nuclei. α-SMA, α-smooth muscle actin; SMMHC, smooth muscle myosin heavy chain.



SUPPLEMENTARY FIG. S2. **(A)** miR-21, 221, 145, and 222 expression during RA-induced SMC from ESC cultured for 0, 3, 6, or 9 days, at which time miR-1 were measured by quantitative real time PCR (qRT-PCR) and normalized to U6. Fold changes are shown with respect to DMSO-treated cells, where miR-1 levels on day 0 were set to a value of 1. **(B)** miR-1 upregulation could be repressed by miR-1 inhibitor. miR-1 inhibitor (50 nM) or inhibitor NC were transfected into mouse ESCs after RA treatment for 1 day. Cells were continuously cultured in RA-containing differentiating medium for the indicated days. miR-1 expression was measured with TaqMan RT-PCR at the indicated days. Fold changes are shown with respect to DMSO-treated cells, where miR-1 levels on day 0 were set to a value of 1. **(C)** miR-1 inhibitor had no effect on cell-lineage markers, including NeuroD1, AFP, and GATA2, though this inhibition can slightly upregulate OCT3/4 expression on day 6 of ESC/SMC differentiation. Fold changes are shown with respect to inhibitor NC, where the individual gene expression levels were set to a value of 1. **(D)** miR-1 mimic alone cannot enhance SMC differentiation in Dicer$^{-/-}$ ESCs, as evidenced by expression of SMC-specific markers, including α-SMA, SMMHC, and MyoCD with qRT-PCR. $N = 4$. miR-1, microRNA-1; RA, retinoic acid; SMC, smooth muscle cell; ESC, embryonic stem cell; DMSO, dimethyl sulfoxide; NC, negative control; PCR, polymerase chain reaction. OCT3/4, octamer-binding protein 3/4; AFP, alpha-fetoprotein; GATA2, GATA binding protein 2.



SUPPLEMENTARY FIG. S3. Mouse ESCs were infected with adenovirus expressing KLF4 (Ad-KLF4) during differentiate into SMCs with RA. The derived cells were subject to Western blot analysis and expression of KLF4 was detected in extracts from cells differentiated for 6 days. β-tubulin served as internal control. KLF4, Kruppel-like factor 4. Ad-KLF4, adenovirus expressing KLF4; Ad-GFP, adenovirus expressing green fluorescence protein.



SUPPLEMENTARY FIG. S4. **(A)** Predicted folding structure from mFOLD between miR-1 (green) and the Kfl4 (red). The mfe is indicated, and the seed region is shown by the purple line and arrow. **(B)** Schematic for generation of luciferase reporter plasmids containing KLF4 3′UTR downstream of the luciferase reporter gene (WT) and the mutant miR-1 binding site controls. **(C)** KLF2, KLF5, and KLF13 3′UTR-luciferase reporter constructs were co-transfected into HEK 293 cells with miR mimic or mimic NC as indicated. Individual luciferase activity was normalized to the responding thymidine kinase (TK) promoter–*Renilla*-luciferase activity. Relative luciferase activities were expressed as mean ± standard deviation. Data shown are representative samples from at least 3 independent experiments, each done in triplicates. mfe, minimal free energy; WT, wild type; 3′UTR, 3′ untranslated region.