Supplemental Data

Table 1 Sequences of PCR primers and siRNAs

Supplemental figure legend

Supplemental Figure I. *MYOSLID* and its neighboring genes. **A,** Genome browser track of *MYOSLID* gene locus and its neighboring protein coding genes. *MYOSLID* is located within a lncRNA-rich genomic region. Several distinct lncRNAs are adjacent to *MYOSLID* gene locus and transcribed in an opposite direction. The closest protein coding genes, KLF7 and CREB1, are 70 kb and 200 kb away from its 5' end and 3' end, respectively. **B,** HCASMCs were transduced with adenovirus carrying MYOCD (Ad-MYOCD) or empty control adenovirus (Ad-Control) for 3 days before qRT-PCR analysis of mRNA levels of AC007879.5 and LOC101927865. **C,** HCASMCs were serum starved for 24 hrs, cells were then treated with TGFβ1 (4 ng/ml) or vehicle control for another 24 hrs before RNA extraction for qRT-PCR analysis of mRNA levels of AC007879.5 and LOC101927865. **D,** qRT-PCR analysis of mRNA levels of KLF7 from RNA samples similar to **B.** **E** and **F,** qRT-PCR evaluation of *MYOSLID* knockdown effect on mRNA levels of the indicated genes in HCASMCs 3 days after Dicer substrate siRNA to *MYOSLID* (D-si*MYOSLID*) or same amount of the D-siRNA negative control (D-siControl) transfection. Values are the mean ± SD and all data are representative of 3 separate experiments.

Supplemental Figure II. Characterization of *MYOSLID* transcripts. **A,** Growing HCASMCs were transduced with same amount of Ad-MYOCD or Ad-Vector control for 3 days before RNA isolation for conventional RT-PCR analysis of *MYOSLID_V1* and *MYOSLID_V2*. Multiple primer pairs were used to verify the results. Representative data from > 2 experiments are shown. **B,** Sequence information of full length transcripts of *MYOSLID_V1* and *MYOSLID_V2*.

Supplemental Figure III. Validation of the coding potential of *MYOSLID*. **A,** No predicted proteins/peptides were revealed within the *MYOSLID* gene locus by PhyloCSF. **B,** Quick coupled in vitro transcription/translation combined with Transcend Chemiluminescent Translation Detection was used to experimentally validate the coding potential of *MYOSLID*. *MYOSLID_V1*, *MYOSLID_V2*, vector control pcDNA, and the positive control luciferase plasmid were processed per manufacturer's instruction. 8 µl of translation product was resolved in 15% SDS-PAGE gel and detected by Transcend Chemiluminescent Translation Detection. Note: An expected band (63 KD) was seen in the positive control sample processed from luciferase

plasmid. Compared with the control pcDNA, no specific proteins/peptides were revealed in the samples processed from *MYOSLID_V1* and *MYOSLID_V2* plasmids.

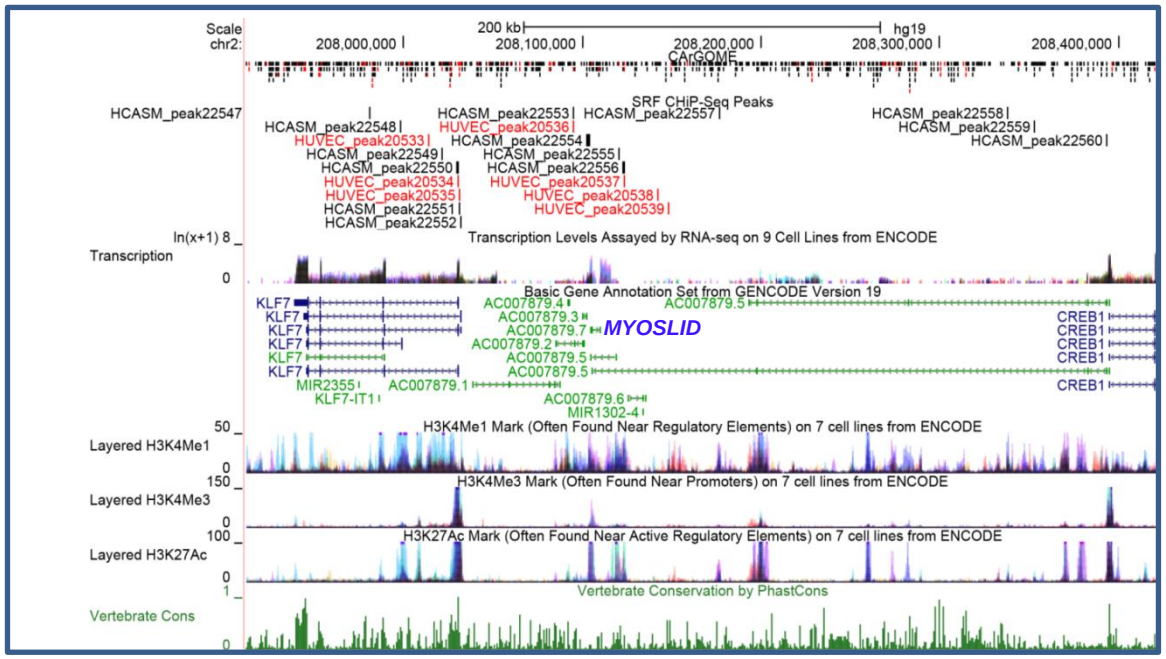
Supplemental Figure IV. mRNA levels of MYOCD and SRF in AVF human samples. qRT-PCR analysis of mRNA levels of *MYOCD* and *SRF* in normal vein and failed AVF samples (diseased vein), as described in Figure 4D (n=6).

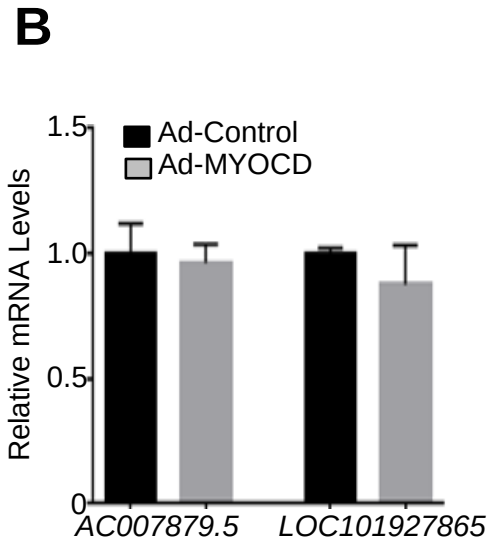
Supplemental Figure V. Effects of *MYOSLID* on VSMC proliferation and migration. **A,** Growing HCASMCs were transduced with lenti-*MYOSLID_V1* or equal amount of lenti-vector control for 48 hrs. Scratch wound was created and cell migration was assessed by time-lapsed microscopy. The representative image at 4 hrs after the creation of scratch wound (upper panel) and the quantitative analysis of the percentage of the area covered by migrating cells to the original wound area (bottom panel) are shown. **B,** Growing HCASMCs were transfected with the indicated siRNAs for 48 hrs and cell migration was assessed, as described in **A**. The representative image at 6 hrs after the creation of the scratch wound (upper panel) and the quantitative analysis (bottom panel) are shown.

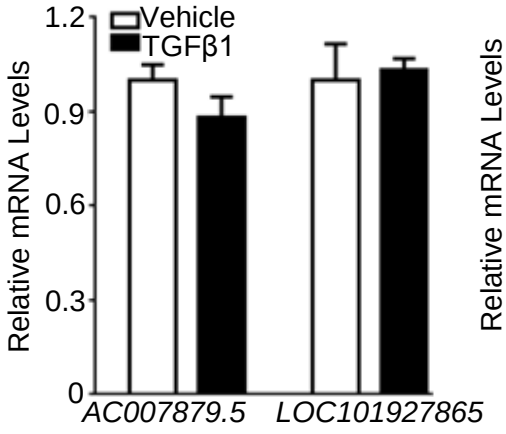
Supplemental Figure VI. Forced expression of *MYOSLID* on F-actin formation. Growing HCASMCs were transfected with siSRF for two days before the lentiviral transduction of *MYOSLID_ V1*. 48 hrs after viral transduction, cells were then fixed with 4% formaldehyde solution before phalloidin staining for F-actin formation. Representative images are shown for each indicated condition. Note: lenti-*MYOSLID_V1* rescues F-actin formation in HCASMCs when SRF is depleted.

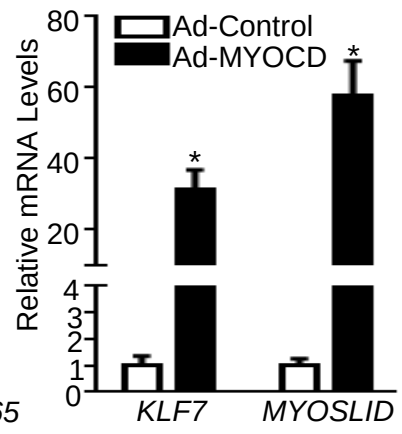
Supplemental Figure VII. Effect of *MYOSLID* knockdown on SRF and MKL1 protein levels and mRNA levels of its neighboring lncRNAs. **A,** Growing HCASMCs were transfected with two different D-siRNAs to *MYOSLID*. Cells were starved overnight before the 24 hrs treatment of ±TGFβ1 (4 ng/ml). Protein was then extracted for Western blotting analysis of SRF and MKL1. Represented data are from 3 separate experiments. **B,** Growing HCASMCs were transfected with two different D-siRNAs to *MYOSLID* for 3 days. qRT-PCR was done to examine the mRNA levels of the indicated genes. Represented data are from 2 independent experiments.

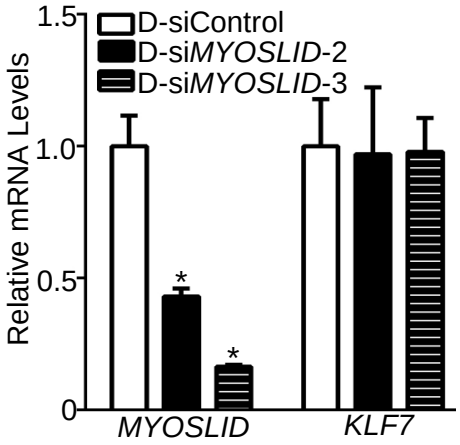
Supplemental Figure I

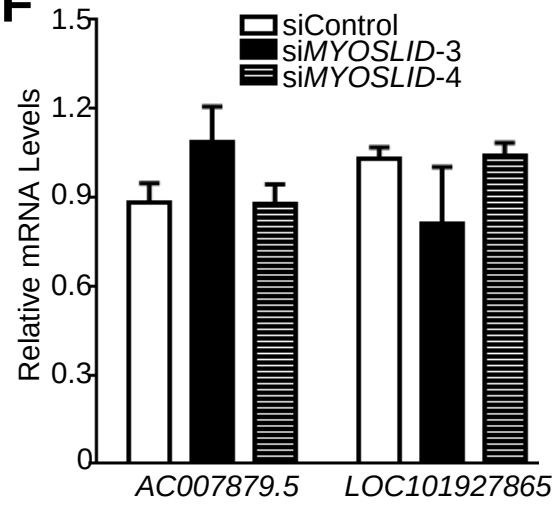






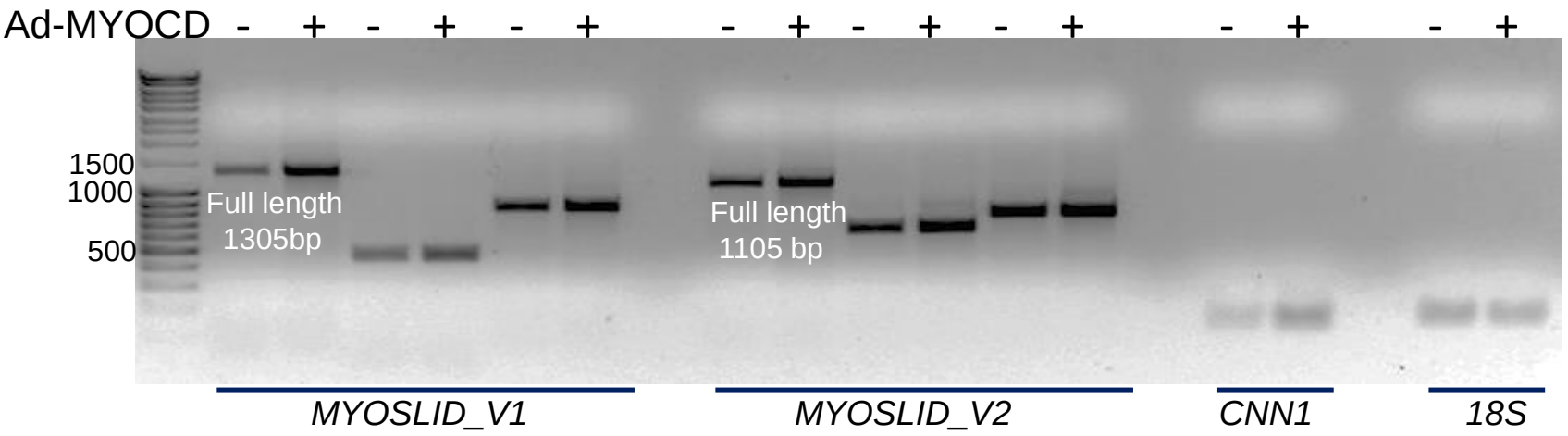






Supplemental Figure II

A



B

MYOSLID_V1 sequence:

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AACTGCA

MYOSLID_V2 sequence:

GGTTTTCCCAGGGCATGGAAAGGACAGGGCTCCCAGTGGAGATATCATGTAGCTATAGGGCCTAGAAGTATTTTT
TCAGTAGATGAAATTTAGAAATAATTTGAATATTTTATGCTGGGGAGGTAGCTTGTGGAGTGAAATGGTCGGGTA
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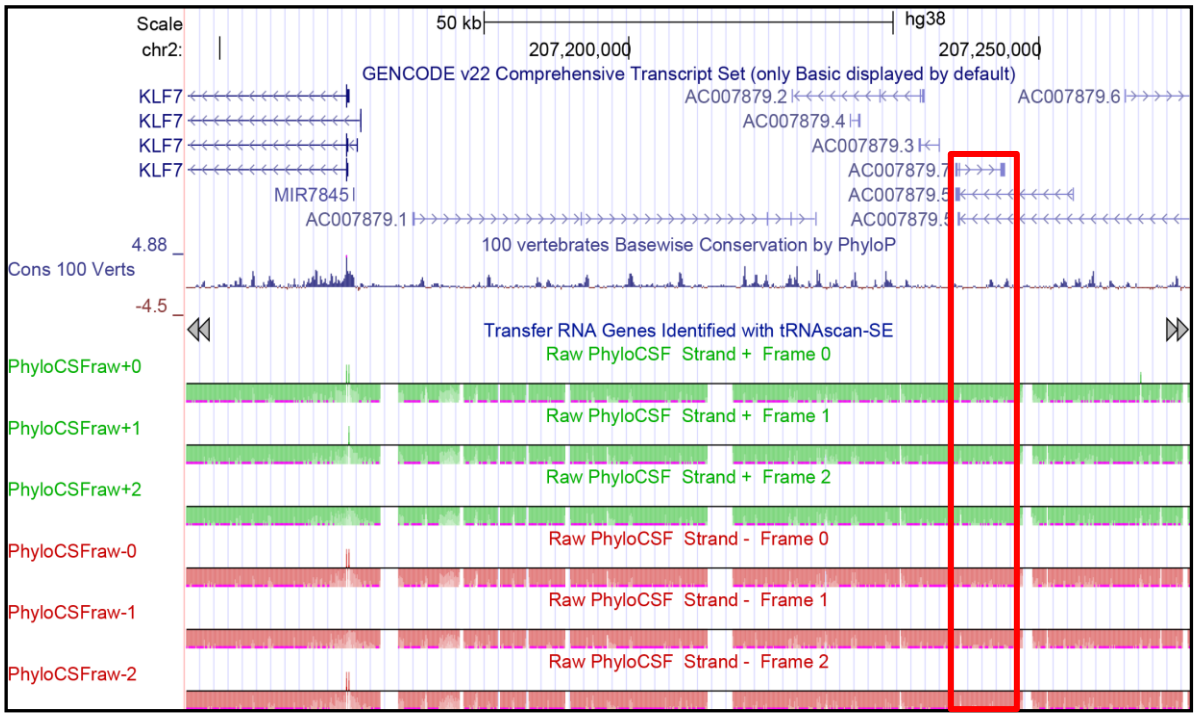
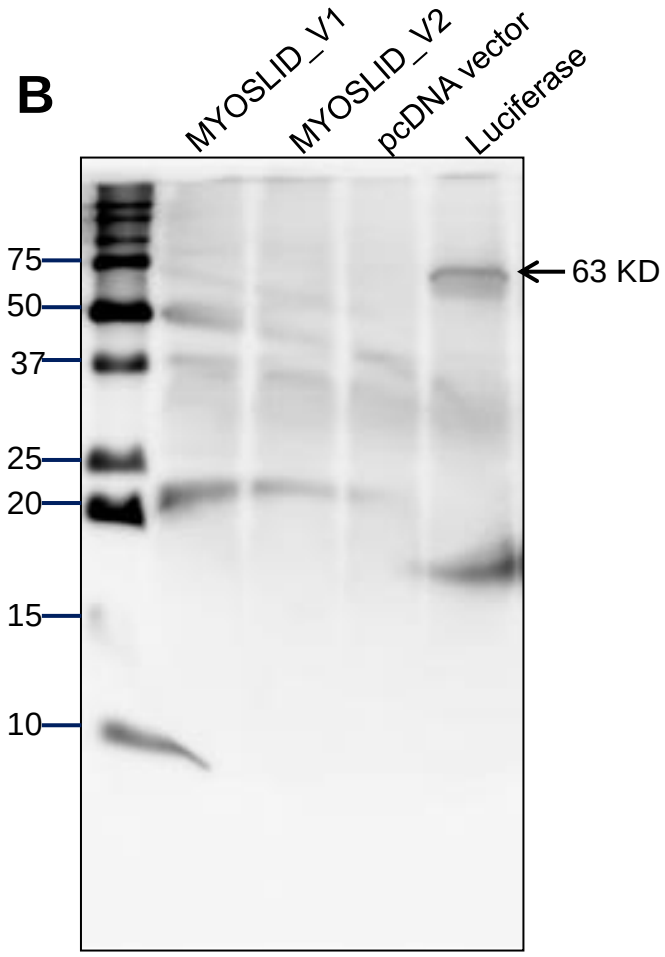
Supplemental Figure III





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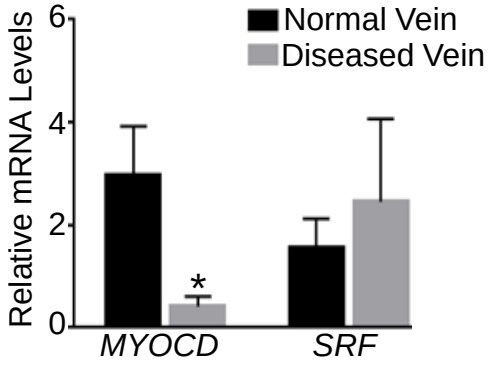

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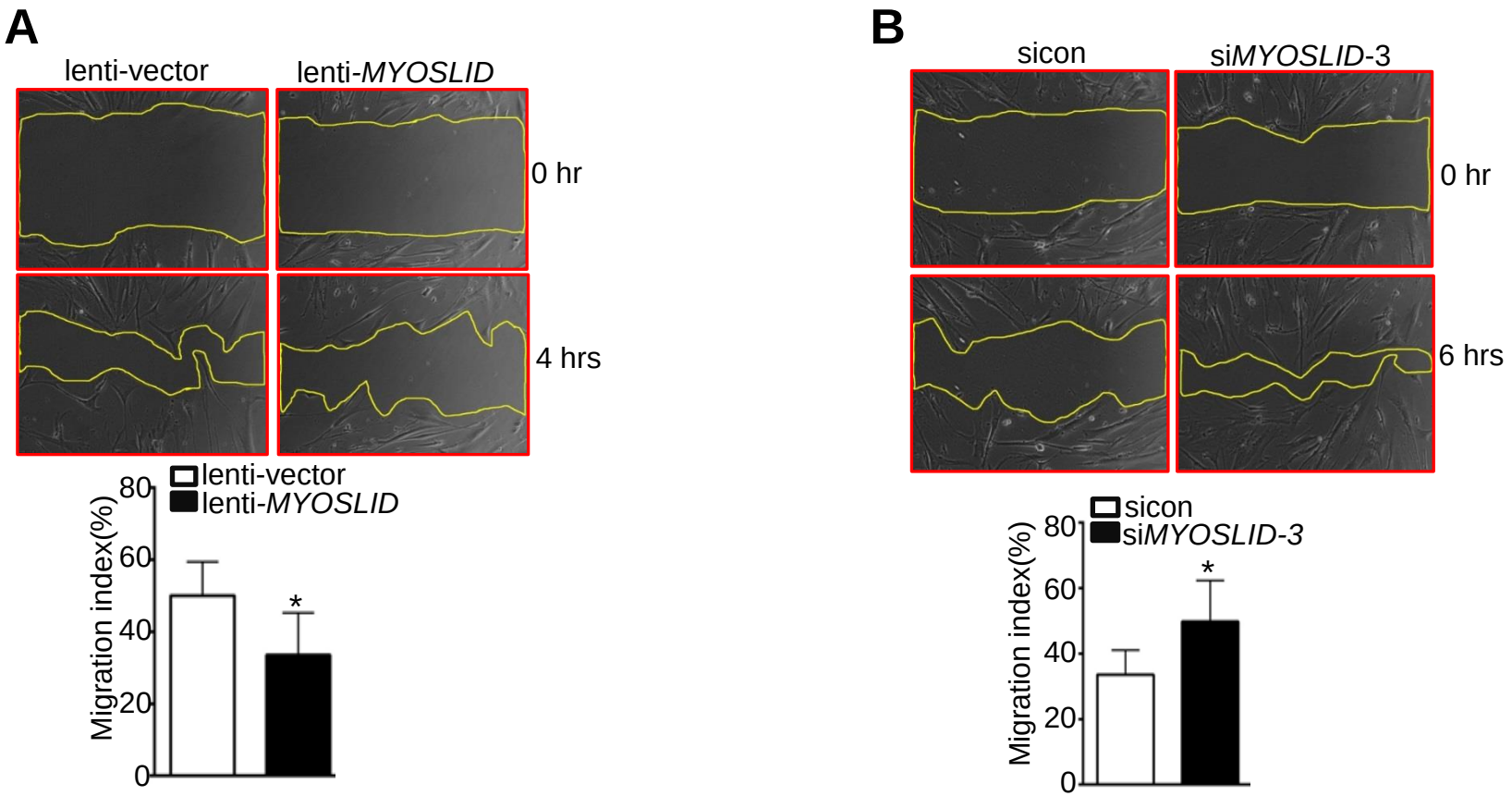
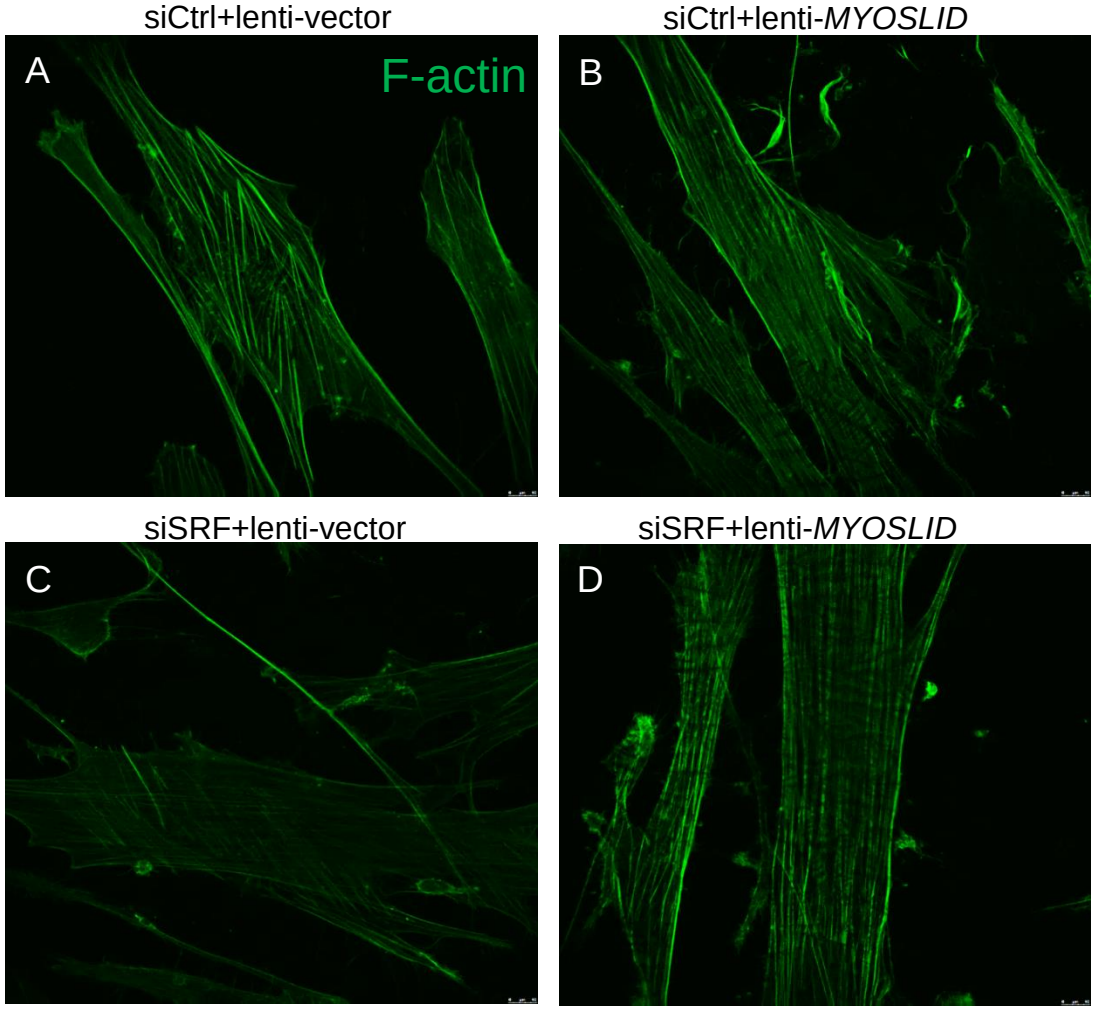
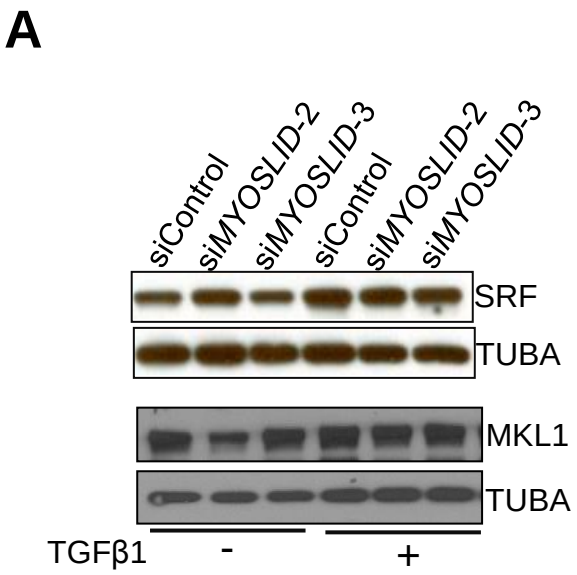
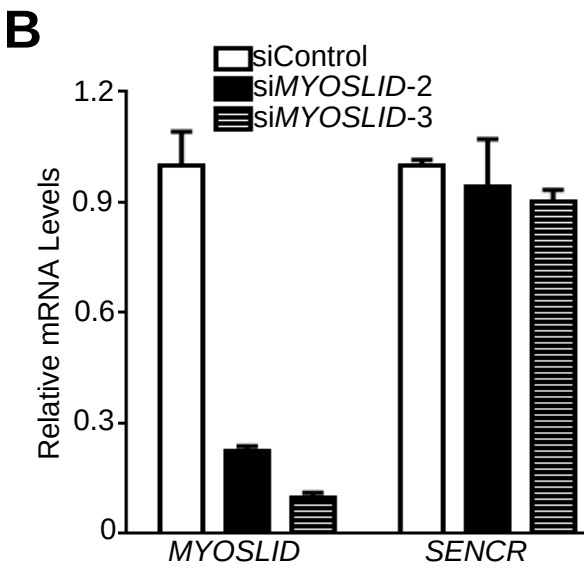


Supplemental Figure VI



Supplemental Figure VII

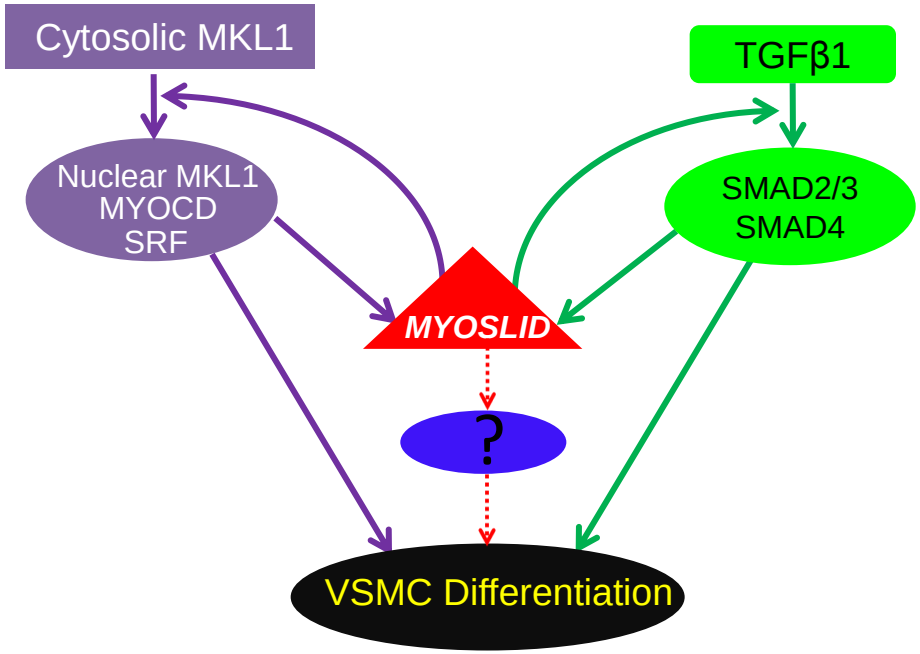




Supplemental Table 1: Sequences of PCR primers and siRNAs

Name of Sequence	Strand	Sequence	Application(s)
MYOCD-lncRNA1	Forward Reverse	CGTTGAGCGAGAGGTTGTGG GAAGAGGCCGAAGCGAAGAG	qRT-PCR
MYOCD-lncRNA2	Forward Reverse	CGCCATGTGCTACGATCTTG CGCCCACCTTACCGCTAG	qRT-PCR
MYOCD-lncRNA3	Forward Reverse	TCGGAGCAGCATCTGAGAGG TTCCAGGCTAGAGCGGAGTAG	qRT-PCR
MYOCD-lncRNA4	Forward Reverse	GTGGATTGGAAGTGATGTTAAGG GAAGGAAGGAAGGAAGGAAGG	qRT-PCR
MYOCD-lncRNA5	Forward Reverse	ACCAAGAATGAAAGGCAACCTATG ATGATGGCTCTGGGTTTCCTTC	qRT-PCR
MYOSLID	Forward Reverse	ACAGGGAGCCAGGACACC GGAACCAGCACCAGGAACC	qRT-PCR
MYOCD-lncRNA7	Forward Reverse	CTGAATGCCTGGGTCCTTA GACATTCTCCCCTGCTCATAC	qRT-PCR
MYOCD-lncRNA8	Forward Reverse	ACATTGAAAACCACATCCTGC GAAAATCGAGGACCAGGGAG	qRT-PCR
MYOCD-lncRNA9	Forward Reverse	ACTCTGACCTCCAGAACTAGG CTAGAAAACCCTTCAGCTCCC	qRT-PCR
MYOCD-lncRNA10	Forward Reverse	TGCACTGGAAATGGCTGG ACCCTGTTTGTGAGGCTTC	qRT-PCR
MYOCD-lncRNA11	Forward Reverse	TTGATGGCATGGACCTGG TGACGCATCTTAAATAGCCTGG	qRT-PCR
MYOCD-lncRNA12	Forward Reverse	AGGTGGACACAATTGATGGG CTTCTGGTGAGGGCTGC	qRT-PCR
MYOCD-lncRNA13	Forward Reverse	CCTAGCACCCTTTTAAGTCCG TGTCAGCCTTACACATTCCG	qRT-PCR
LMOD1	Forward Reverse	GCGGCAGAGAAACCAGAC CCACTTGCTTGCTTTCATCC	qRT-PCR
18S	Forward Reverse	ATGGGCGGCGGAAAATAGC TCTTGGTGAGGTCAATGTCTGC	qRT-PCR
AC007879.5	Forward Reverse	GGAGATTTGAGTCTTAGGAATTTGG AACACCCAGGACAGCTTATG	qRT-PCR
LOC101927865	Forward Reverse	ATTGGTATGGAGACGTGGATG ATGCTGGTGTTGGTCCTTAG	qRT-PCR
KLF7	Forward Reverse	ACATCCCAACTCCAGAAAACG AGGTAGCGTTCCAATTCAAGG	qRT-PCR
MYOSLID_ V1 full length	Forward Reverse	GGTTTTCCCAGGGCATGGAAAG CTATAAGTCAGACATGGTTC	RT-PCR Clone
MYOSLID_ V2 full length	Forward Reverse	GGTTTTCCCAGGGCATGGAAAG CCTTTATGTACCCAGCAGGAACA	RT-PCR Clone
SENCR	Forward Reverse	TTACCTTGTCCACGCTCTCC CCGTCTCTCCGCATTCTCC	qRT-PCR
MYOCD	Forward Reverse	TGCTGCTGTAAAGTCCAAATCC GCGTAGGCTGAGTCCATAGG	qRT-PCR
ACTA2	Forward Reverse	AGCCAAGCACTGTCAGGAATC GAGCCCAGAGCCATTGTCAC	qRT-PCR
SRF	Forward Reverse	CTACACGACCTTCAGCAAGAG TGGAGAGTCTGGCGAGTTG	qRT-PCR
MYOSLID -2kb promoter	Forward Reverse	CTCAAGGTGGCTCAAGATAA CATGATATCTCCACTGGGA	PCR clone
MYOSLID -890 bp promoter	Forward Reverse	CTGGTAAAGCTTGAGGATCA CATGATATCTCCACTGGGA	PCR clone
MYOSLID -157 bp promoter	Forward Reverse	TTGGAGTGCAAGAGAGCCCCTAA CATGATATCTCCACTGGGA	PCR clone
MYOSLID ChIP CArG1	Forward Reverse	ATCTGACCTTATCTTGGCACC AGTCAGAATACCAGCCATGTG	qPCR
MYOSLID ChIP CArG2	Forward Reverse	TACAATGGGACCTTGCTCAC AGAAAGAGCCCTTCTCTGTAATC	qPCR
MYOSLID ChIP CArG3	Forward Reverse	AGGAATGTGTCTGTGGGAAG AGGTCTCTGTCTCTGCCTG	qPCR
MYOSLID ChIP Negative Control	Forward Reverse	TAATCTCTCAGAGCCTCAATTTCC AGGCTTTGTTACTAGGGTTTCTT	qPCR
CNN1 ChIP Intronic CArG1	Forward Reverse	TCGGGCGAGAGACAGAT AAGCAAACCGGCCCTTAT	qPCR
MYH11	Forward Reverse	CGCCAAGCCAGAGACGAGAG GAGGAAGGTGTAGTTGTTGAAGCC	qRT-PCR
CNN1	Forward Reverse	ATGTCCTCTGCTCACTTCAAC GCTGGTGGTCATACTTCTGG	qRT-PCR
TAGLN	Forward Reverse	CATCCTGTCTGTCCGAACC CACTATGATCCACTCCACCAG	qRT-PCR
IL6	Forward Reverse	GTGTTGCCTGCTGCCTTC AGTGCCTCTTTGCTGCTTTC	qRT-PCR
MKL1	Forward Reverse	CAGCCTGAAGGAAGCCATC GCCCATCGGAAGTTGAGAC	qRT-PCR
MYOSLID - 2 kb promoter SBE1/2 mutagenesis	Forward Reverse	ATATGCACCtAcAtAAGATGTCTCTGAtAcAtCAGGAAGG CCTTCCTGaTgTaTCAGAGACATCTTaTgTaGGTGCATAT	mutagenesis
	Forward	TCAAGCTGGTGAAtAcAtCCTGAAAAGCTGA	

MYOSLID -2 kb promoter SBE3 mutagenesis	Reverse	TCAGCTTTTCAGGaTgTaTTCACCAGCTTGA	mutagenesis
SMAD4	Forward Reverse	ACCATCATAACAGCACTAC GGAACACCAATACTCAGG	qRT-PCR
GAPD	Forward Reverse	CGCTCTCTGCTCCTCCTGTTC TTGACTCCGACCTTCACCTTCC	qPCR
MYOSLID_V1	Forward Reverse	GCTGCATCGCAGTTTACTTTC AGGCTTTGTTACTAGGGTTTCTT	RT-PCR
MYOSLID_V2	Forward Reverse	ACAGGGAGCCAGGACACC GGGAAGCTCTACCTCTTGATTAG	RT-PCR
GUSB	Forward Reverse	GCTATGGGATTGTGGTCATCG AGGATTTGGTGTGAGCGATC	qRT-PCR
DsiRNA-MYOSLID-2	AntiSense Sense	rUrUrCrArGrCrUrUrUrArArGrArArGrUrUrCrArUrUrCrUrCrCrArC rGrGrArGrArArUrGrArArCrUrUrCrUrUrArArArGrCrUrGAA	gene knockdown
DsiRNA-MYOSLID-3	AntiSense Sense	rGrGrCrArCrArUrCrCrUrArGrArGrCrArArGrGrUrGrGrCrUrCrUrU rGrArGrCrCrArCrCrUrUrGrCrUrCrUrArGrGrArUrGrUrGCC	gene knockdown
DsiRNA-MYOSLID-4	AntiSense Sense	rCrUrArCrArGrArGrArUrUrCrArArCrArGrCrUrUrCrUrUrCrCrArG rGrGrArArGrArArGrCrUrGrUrUrGrArArUrCrUrCrUrGrUAG	gene knockdown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TGFβ1
Nuclear MKL1
MYOCD
SRF
SMAD2/3
SMAD4
MYOSLID
?
VSMC Differentiation