Evidence for STIM1- and Orai1 (CRACM1)-dependent Store-Operated Calcium Influx through I_{CRAC} in Vascular Smooth Muscle cells: Role in Proliferation and Migration

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Online Data Supplement

LEGENDS TO SUPPLEMENTARY FIGURES

Figure S1. RT-PCR from synthetic cultured VSMC showing mRNA expression of STIM and Orai isoforms.

Figure S2. Western Blots analysis showing protein levels of STIM1 (**A**), Orai1 (**B**), Orai3 (**C**), TRPC1 (**D**), TRPC4 (**E**) and TRPC6 (**F**) with or without gene specific siRNA transfection. A scrambled siRNA and anti-Actin Western Blots are used as controls. Arrows point to specific protein bands. The same blots have been stripped and reprobed several times and asterisks point to bands presumably representing actin.

Figure S3. Vectors carrying shRNA hairpins against STIM1 (**A**; control n=36; shSTIM1 n=20) or Orai1 (**B**; control n=20; shOrai1 n=14) were transfected into synthetic cultured VSMC (the empty vector was used as a control) and SOCE was evoked by 2μM thapsigargin and measured using Fura2 imaging. Data are representative of 3 independent experiments.

Figure S4. Western Blots analysis of STIM1 (**A**) and Orai1 (**B**) protein levels in cultured synthetic rat aortic VSMC (Synth. VSMC) and their quiescent freshly isolated counterparts (Quiesc. VSMC).

Figure S5. A; photographs of scratch wound migration assay in primary cultured VSMC in the presence of 10% serum at 24 hours post-wound in control siRNA- , Orai2 siRNA-, Orai3 siRNA- and STIM2 siRNA-transfected cells. Data (represented as % of control) are representative of 2 different siRNA sequences with 3 independent experiments per siRNA (4 wells per transfection) and statistical analysis is shown in **B**. **C**; Proliferation in VSMC transfected with scrambled control siRNA, Orai2 siRNA, Orai3 siRNA and STIM2 siRNA was assayed at different times post-transfection. Data are represented as % of control and are representative of 4 independent experiments (12 wells per condition).

PCR Primers

	Forward (5'-3')	Reverse (3'-5')	Size Amplified (bp)
rSTIM1	GGCCAGAGTCTCAGCCATAG	CATAGGTCCTCCACGCTGAT	305
rSTIM2	TAAGCTGTCTCGCTGCTTCA	CAGTAGCGCTCTCGGGTTAC	241
rOrai1	ACGTCCACAACCTCAACTCC	ACTGTCGGTCCGTCTTATGG	362
rOrai2	CACCTATTTGCCCTGCTCAT	AGCTTGTGCAGTTCCTCGAT	386
rOrai3	CTGTCCACCAGTCACCACAC	CCACCAAGGATCGGTAGAAA	422
rTRPC1	TATGGGGAAGAACTGCAGTCC	CAGATCTTGGCGCAGTTCATT	467
rTRPC4	GCCTACACCTTTCAATGTCATCCC	CTTAGGTTATGTCTCTCGGAGGC	492
rTRPC6	GTGCCAAGTCCAAAGTCCCTGC	CTGGGCCTGCAGTACGTATC	315

rGAPDH	TCACCATCTTCCAGGAGCGA	CACAATGCCGAAGTGGTCGT	293

siRNA Sequences

Scrambled control	UGGUUUACAUGUCGACUAA
TRPC1-1	CTGCTCATCGTAACAACTA
TRPC1-2	GAGAAATGCTGTTACCATA
TRPC4-1	GGCTCAGTTCTATTACAAA
TRPC4-2	CCACGAGGTCCGCTGTAAC
TRPC6-1	GGACCAGCATACATGTTTA
TRPC6-2	GTGTGGATTACATGGGCCA
STIM1-1	UAAGGGAAGACCUCAAUUA
STIM1-2	CAUCAGAAGUGUAUAACUG
STIM2-1	GGACUUACAAGCUUUAAUG
STIM2-2	GGGACUGUUUUCACUUUUA
Orai1-1	CAACAGCAAYCCGGAGCUU
Orai1-2	GCCAUAAGACGGACCGACA
Orai2-1	GCCACAACCGUGAGAUCGA
Orai2-2	GCAUGCACCCGUACAUCGA
Orai3-1	GGGUCAAGUUUGUGCCCAU
Orai3-2	CCACGUACCGGGAGUUCGU

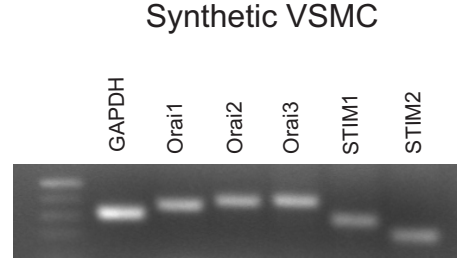
shRNA Sequences

Orai1-1	TGGATCGGCCAGAGTTACTCCGAGGTGAT
Orai1-2	GACCGACAGTTCCAGGAGCTCAACGAGCT
STIM1-1	GATGATGCCAATGGTGATGTGGATGTGGA
STIM1-2	CTGCTGGTTTGCCTATATCCAGAACCGTT

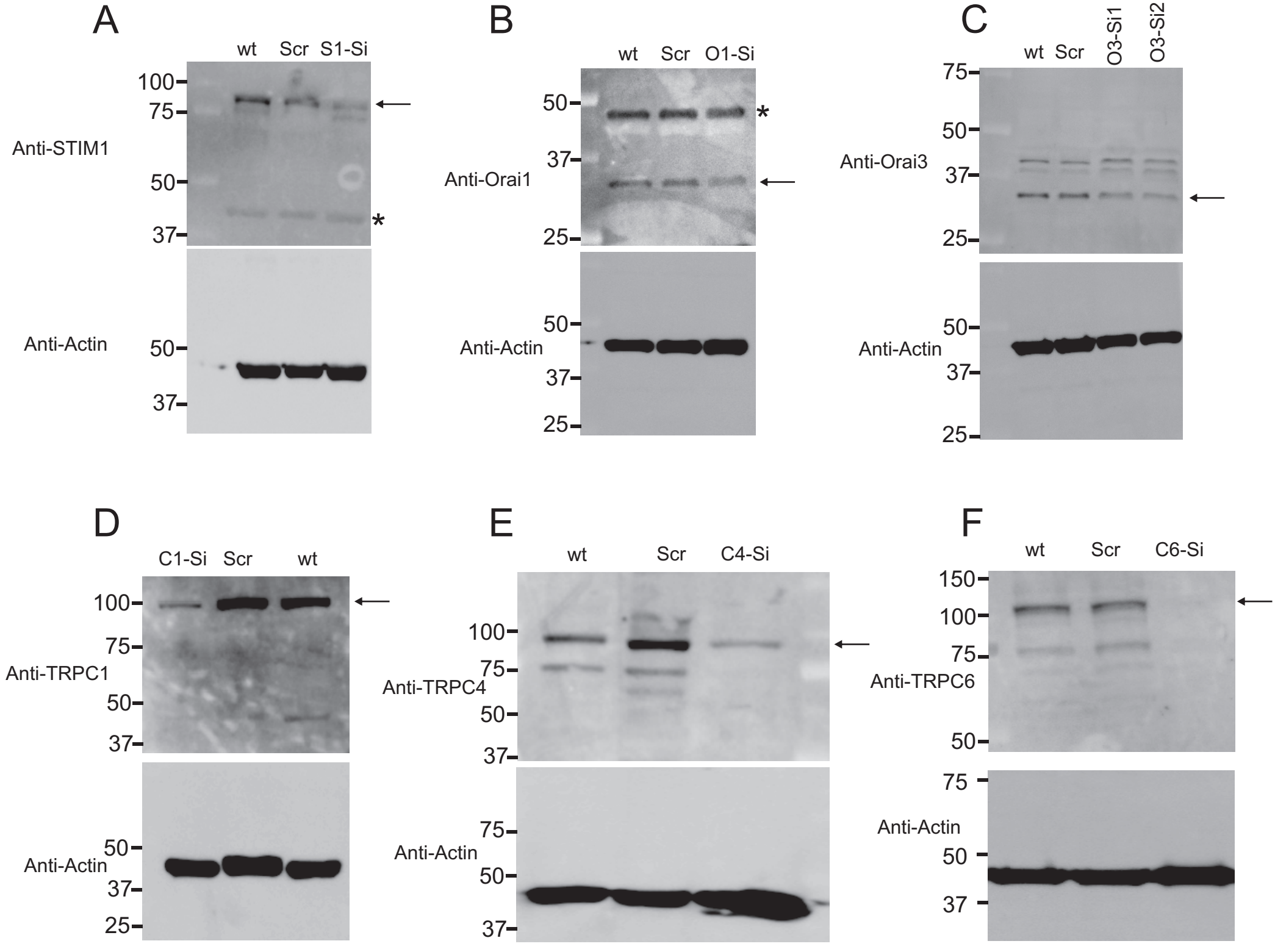
Supplementary Table: Sequences of primers used in PCR and qPCR. STIM1, STIM2, Orai1, Orai2, Orai3 primers were designed using Primer3 Program from MIT. The TRPC1 primers were from Ohba et al(1), while all other TRPC primers were from Bergdhal et al(2). SiRNA and shRNA sequences are also listed; siRNA sequences were designed using the siDESIGN CENTER on Dharmacon website. All siRNA were purchased from Dharmacon while shRNA were purchased from Origene.

1. Ohba, T., Watanabe, H., Murakami, M., Takahashi, Y., Iino, K., Kuromitsu, S., Mori, Y., Ono, K., Iijima, T., and Ito, H. (2007) Upregulation of TRPC1 in the development of cardiac hypertrophy. *Journal of molecular and cellular cardiology* **42**, 498-507
2. Bergdahl, A., Gomez, M. F., Wihlborg, A. K., Erlinge, D., Eyjolfson, A., Xu, S. Z., Beech, D. J., Dreja, K., and Hellstrand, P. (2005) Plasticity of TRPC expression in arterial smooth muscle: correlation with store-operated Ca2+ entry. *American journal of physiology* **288**, C872-880

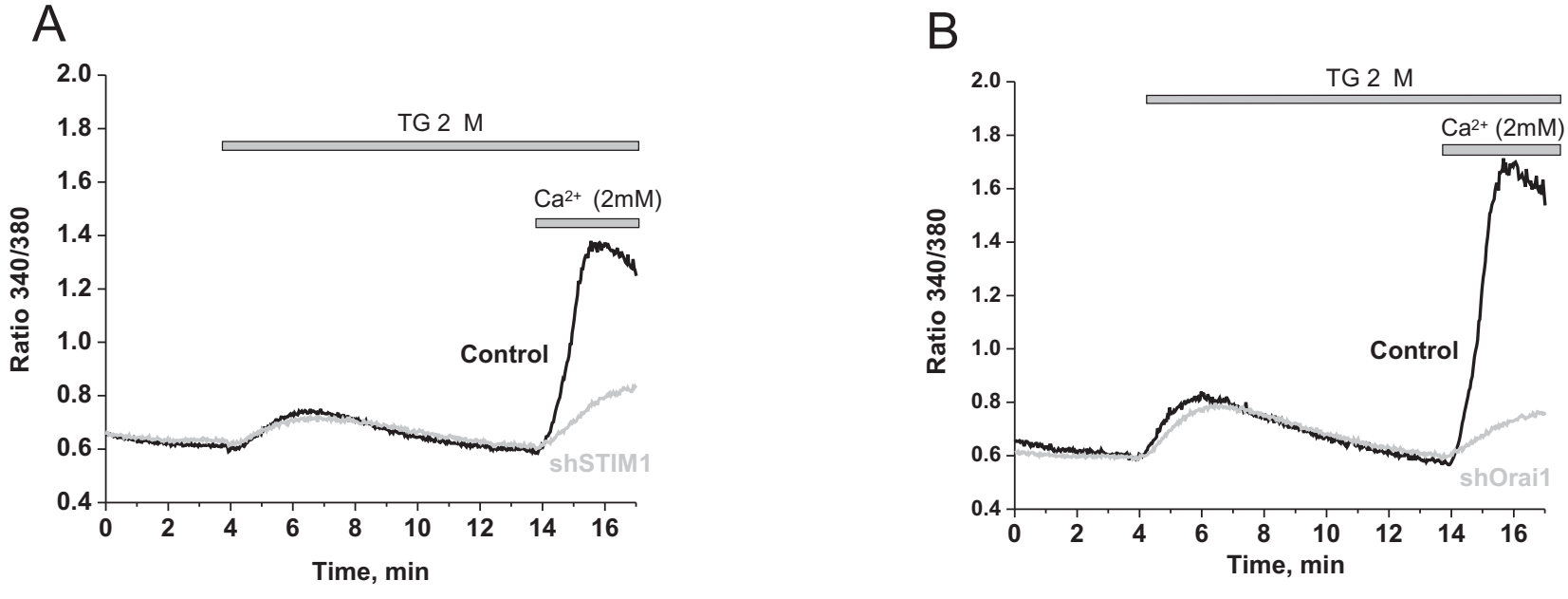
Supplementary Figure 1



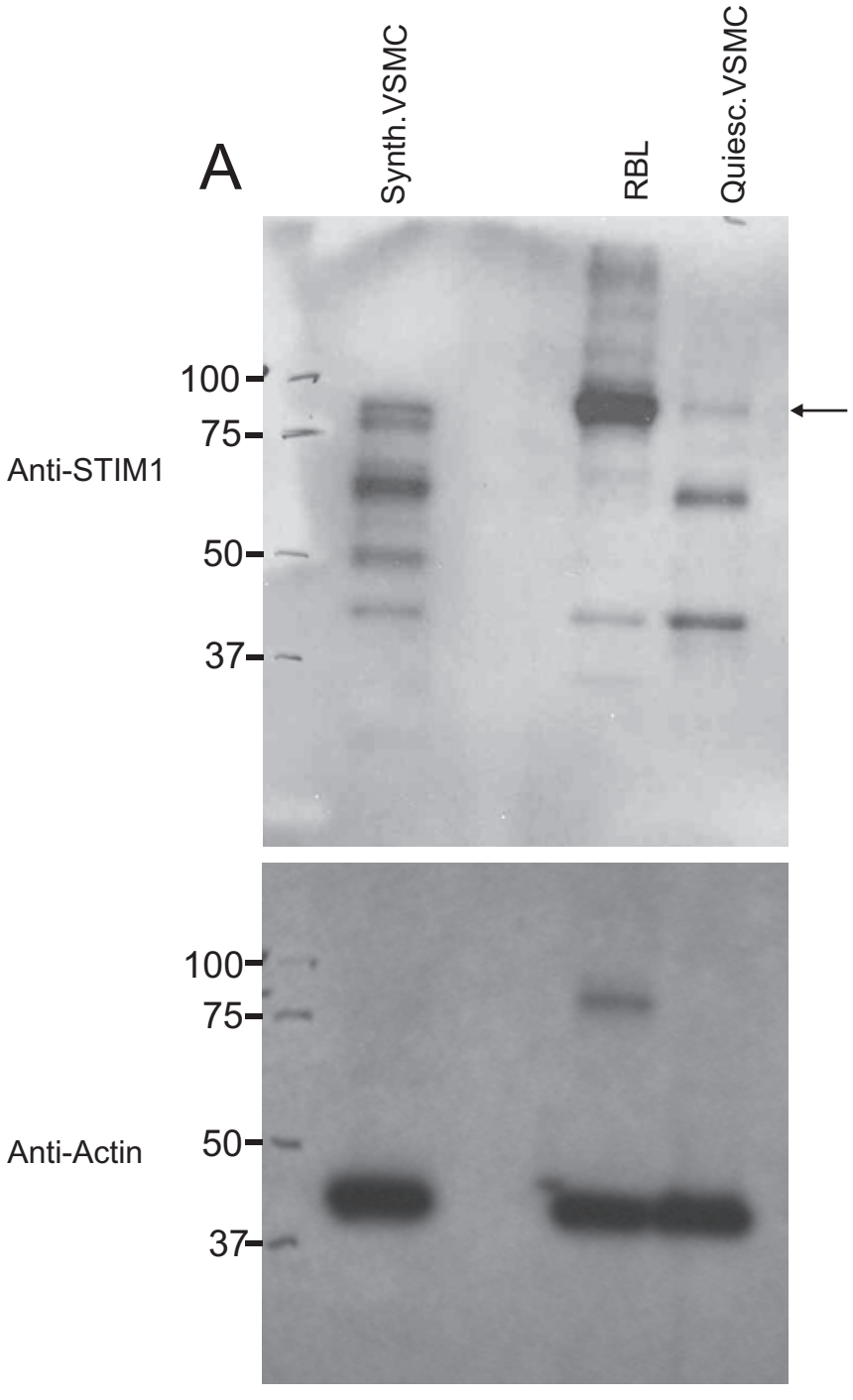
Supplementary Figure 2

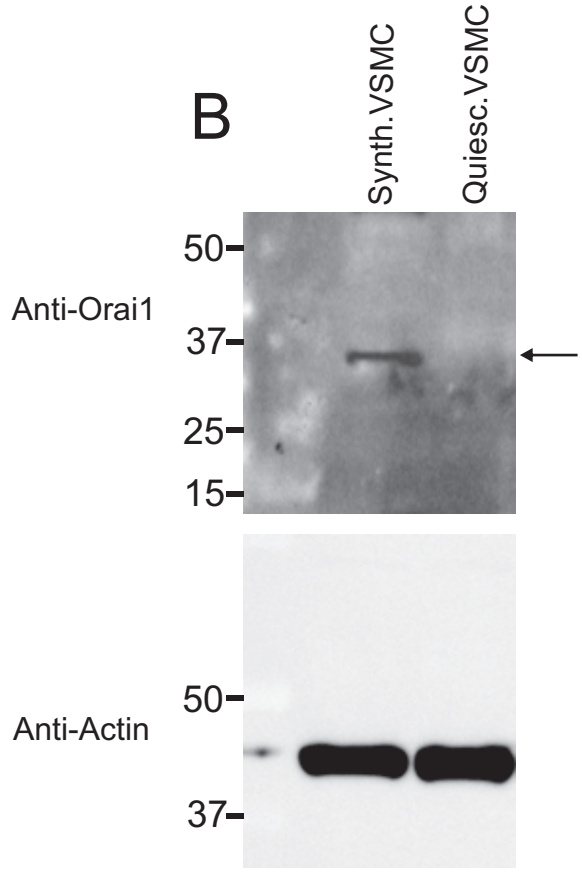


Supplementary Figure 3



Supplementary Figure 4





Supplementary Figure 5