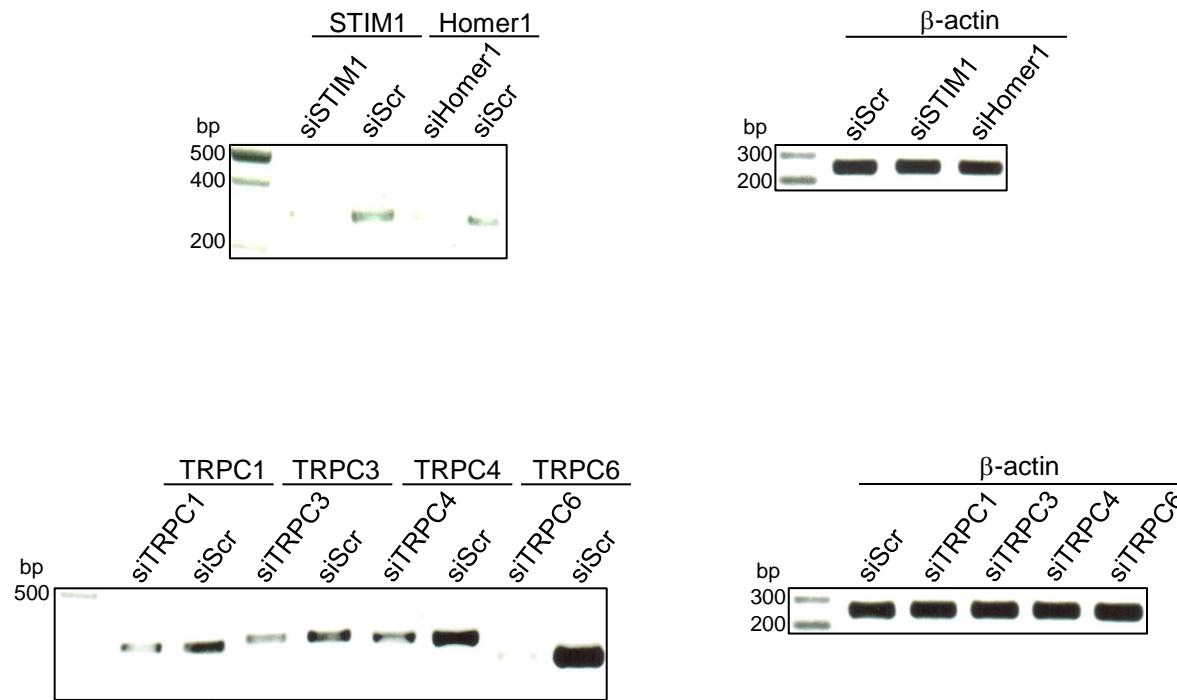


Manuscript Title: Homer binds to Orai1 and TRPC channels in the neointima and regulates vascular smooth muscle cell migration and proliferation

Author List: Shuping Jia, Miguel Rodriguez, Arthur G. Williams, Jr., and Joseph P. Yuan

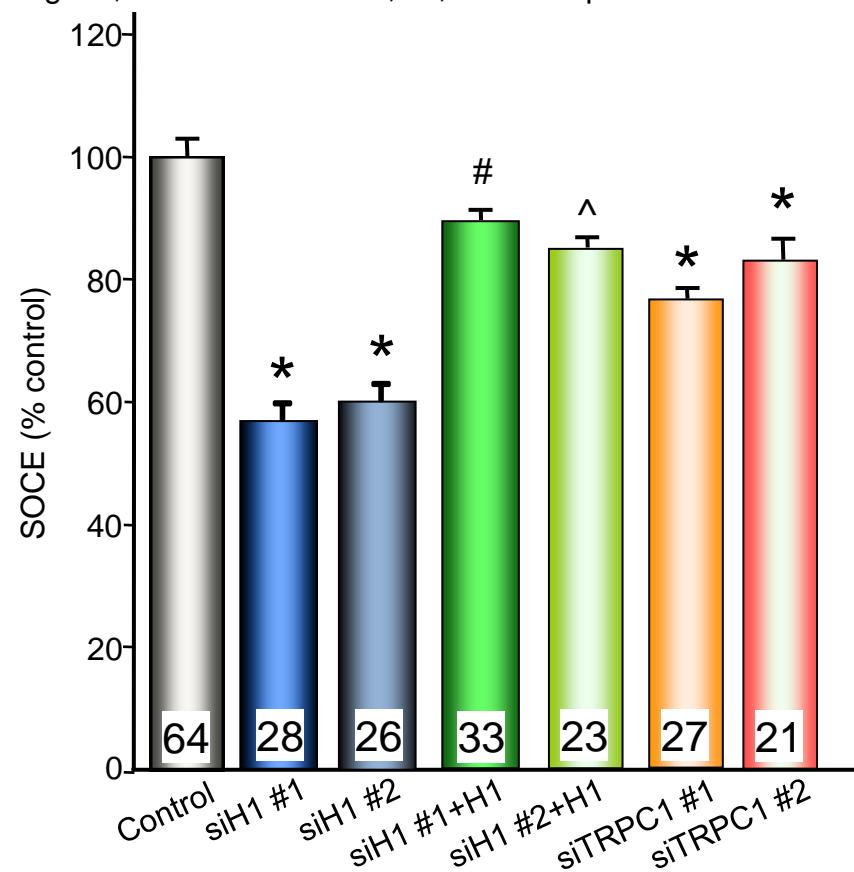


Supplementary Figure 1: RT-PCR from total mRNA from cultured VSMCs treated with siScrambled, siSTIM1, siHomer1, or siTRPC1/3/4/6

Complementary DNA (cDNA) expression levels of STIM1, Homer1, and TRPCs are reduced in cells treated with their respective siRNAs. Total mRNA was harvested from cultured VSMCs, and RT-PCR using gene-specific primers was performed according to standard protocols.

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Supplementary Figure 2: Two independent siRNA's for Homer1 and TRPC1 show similar effects on reducing store-operated Ca²⁺ entry (SOCE) in cultured VSMCs

Cultured aortic VSMCs were transfected with (a) Homer1(H1 #1) and Homer1(H1 #2) siRNAs targeting different regions of Homer1 and (b) TRPC1 #1 and TRPC1 #2 siRNAs targeting different regions of TRPC1. SOCE was measured after depleting ER Ca²⁺ stores with 25 mM cyclopiazonic acid (CPA). SOCE values are normalized to peak Ca²⁺ store depletion (CPA response) and indicated as % control (scrambled siRNA), which is set to 100%. SOCE values: siH1 #1: 56.0 ± 4.0%; siH1 #2: 60.0 ± 5.9%; siH1 #1 + H1(250ng): 90.6 ± 3.1%; siH1 #2 + H1(250ng): 88.6 ± 3.3%; siTRPC1 #1: 78.2 ± 2.3%; siTRPC1 #2: 85.6 ± 4.5%. siH1 #1 and siTRPC1 #1 are used in Figures 3 and 4. *, p < 0.01 compared to control; #, p < 0.01 compared to siH1 #1; ^, p < 0.01 compared to siH1 #2.

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Supplementary Table 1: Sequences for siRNA oligos and list of primers used for RT-PCR

siRNA oligonucleotides (oligos)

siHomer1 #1: 5'-GGAAUUUAGUUAUGAAGAGUUUAAA-3'

siHomer1 #2: 5'-ACCAGAAAGUUUAAGUAGACACTT-3'

siSTIM1: 5'-GCCAUAGUCACAGUGAGAAGAAUAC-3'

siTRPC1 #1: 5'-GGAUAAAGAAUGGAAGUUUGCUCGA-3'

siTRPC1 #2: 5'-GGAAUACUCAACAACAAUGGAUGTT-3'

siTRPC3: 5'-UGGACAGAAAUGCUALUUUUGGUCT-3'

siTRPC4: 5'-GGACUCAAGCAUAGAUUAUGAUUTA-3'

siTRPC6: 5'-AGAGUCGAAACAAGAGGAAAGCCGC-3'

RT-PCR primers used to examine mRNA expression in cultured aortic vascular smooth muscle cells (VSMCs)

Homer1 forward primer: 5'-CAAGACAGAGCTGAGTCAGACAGTCAGGAGC-3'

Homer1 reverse primer: 5'-TCTCTAGGAGTCTTCAGGTTACTGCGGAAAGC-3'

STIM1 forward primer: 5'-CCTGAGGCCCTGCAGAAGTGGCTGC-3'

STIM1 reverse primer: 5'-TATTGACAATCTGGAAACCACAGAGGATCTGA-3'

TRPC1 forward primer: 5'-AAAGGCAAGGTCAAGACGGCAGAACAGCT-3'

TRPC1 reverse primer: 5'-TTGGGATAAAACATAGCATATTAGAAGTCCGAAAGC-3'

TRPC3 forward primer: 5'-AGGTTAACCTCTTCACTCAGTCTAACCGAGAGTTTG-3'

TRPC3 reverse primer: 5'-GGTCAGTTCTCACTGAGTTATGAATCAAGATGGC-3'

TRPC4 forward primer: 5'-ATGAGAAGAGCCACAGCGAAGAAGAAATTACTCG-3'

TRPC4 reverse primer: 5'-CAATCTCGTGGTCACATAATCTTCATGGGCA-3'

TRPC6 forward primer: 5'-AACAAACAATCAAGTACAAGGAGCTCAGAAGATTCC-3'

TRPC6 reverse primer: 5'-TAAAGACAGTCTCTCCCCAAGCTTCTAATGAGTTCT-3'

β -actin forward primer: 5'-ATGAAACTACATTCAATTCCATCATGAAGTGTGACG-3'

β -actin reverse primer: 5'-CATCGTACTCCTGCTTGATCCACATCTG-3'