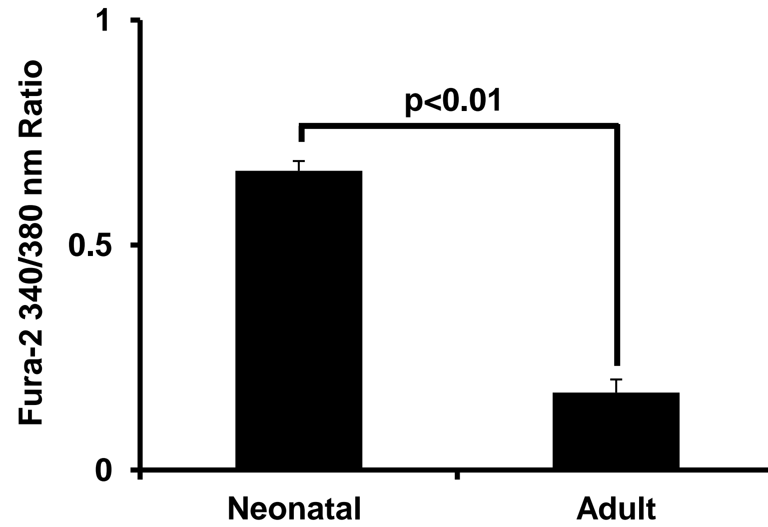

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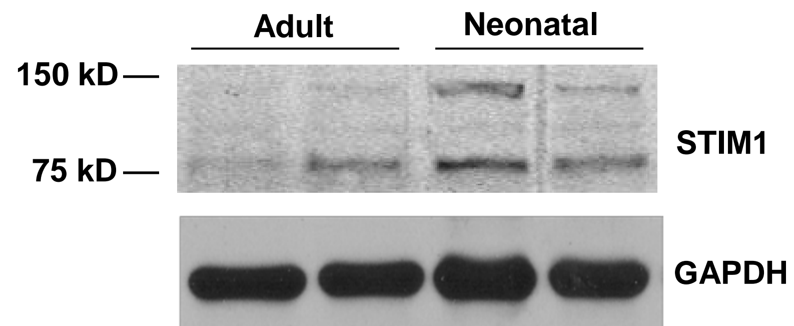

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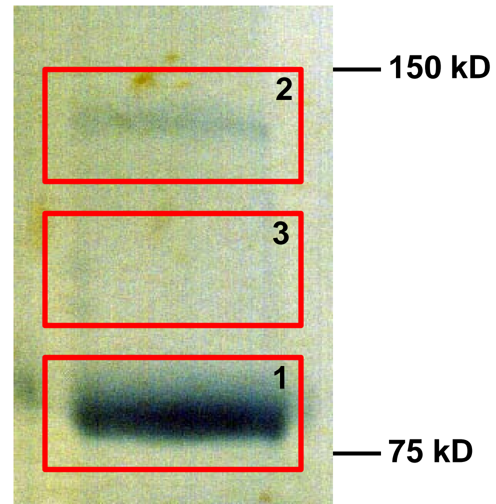

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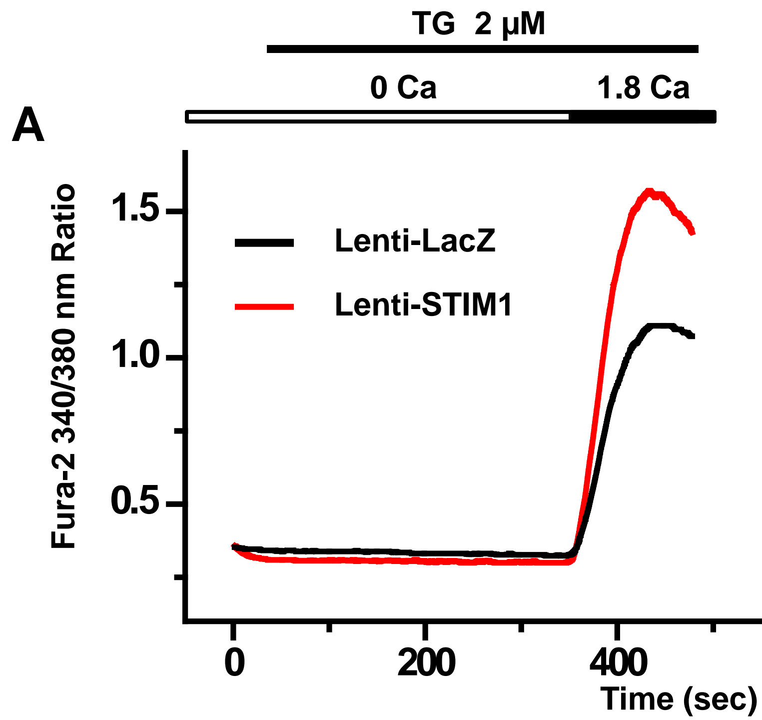

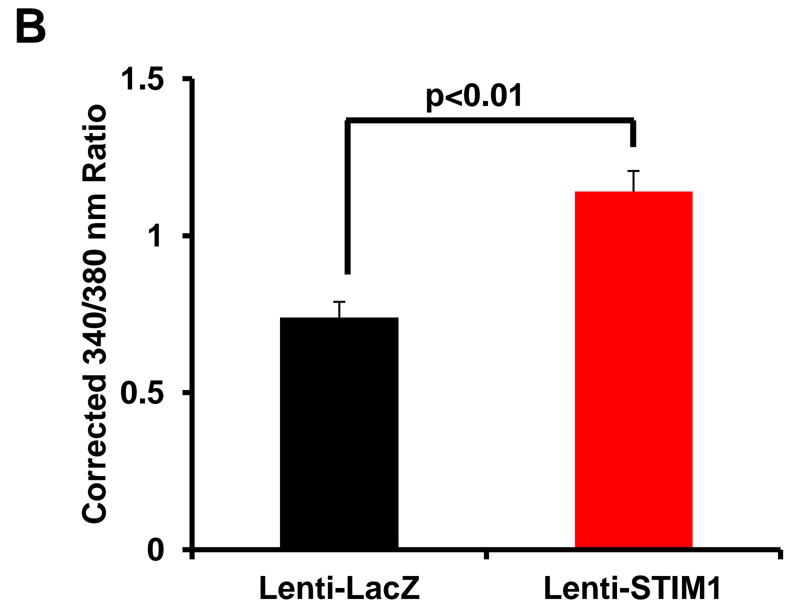

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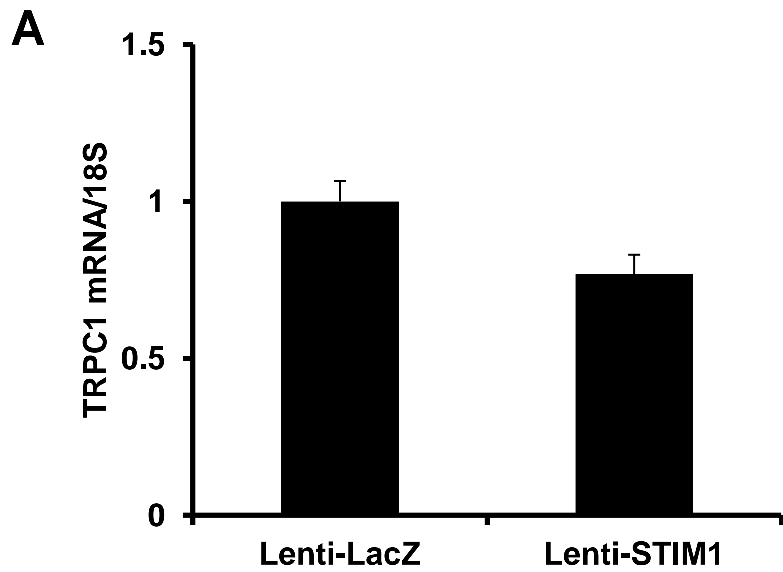

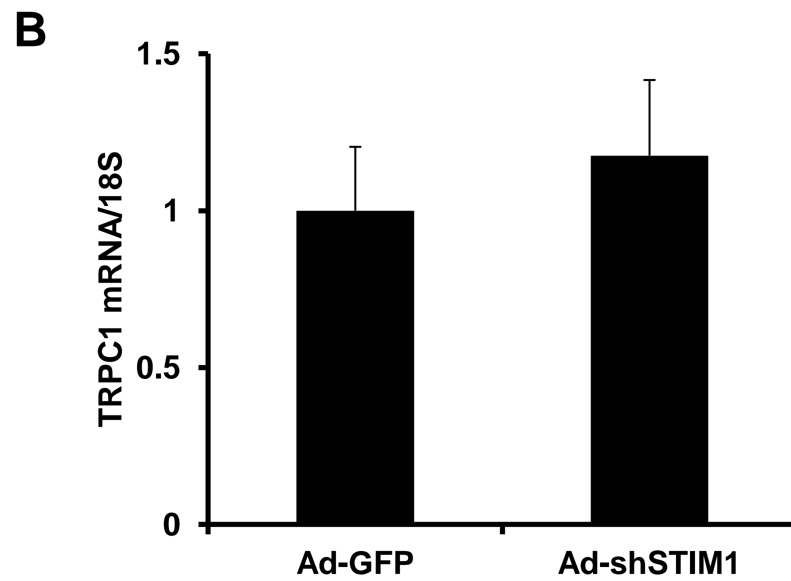

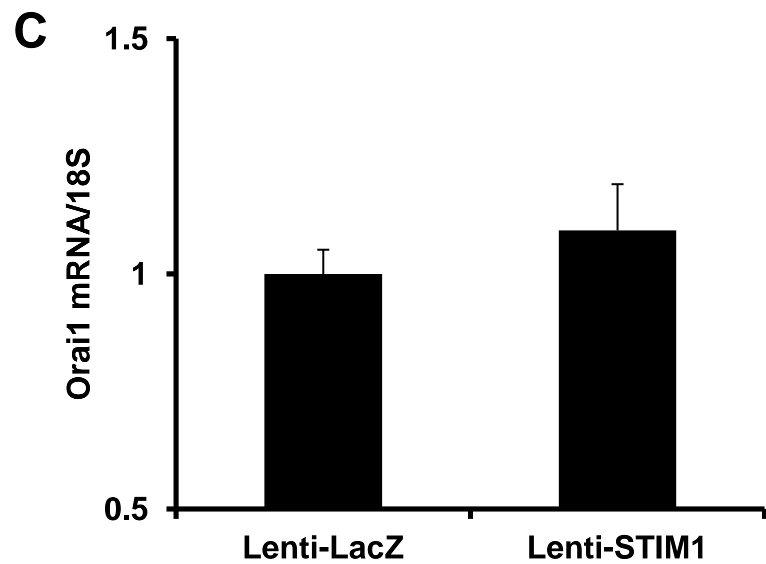

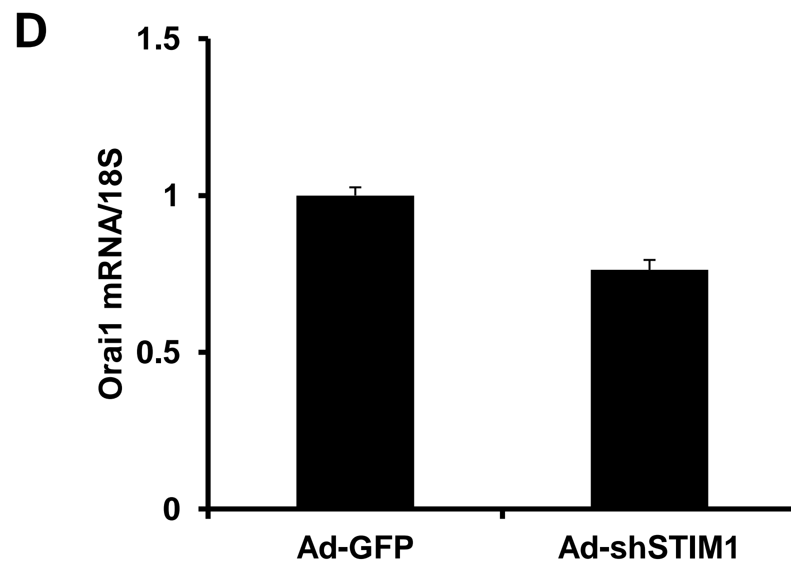

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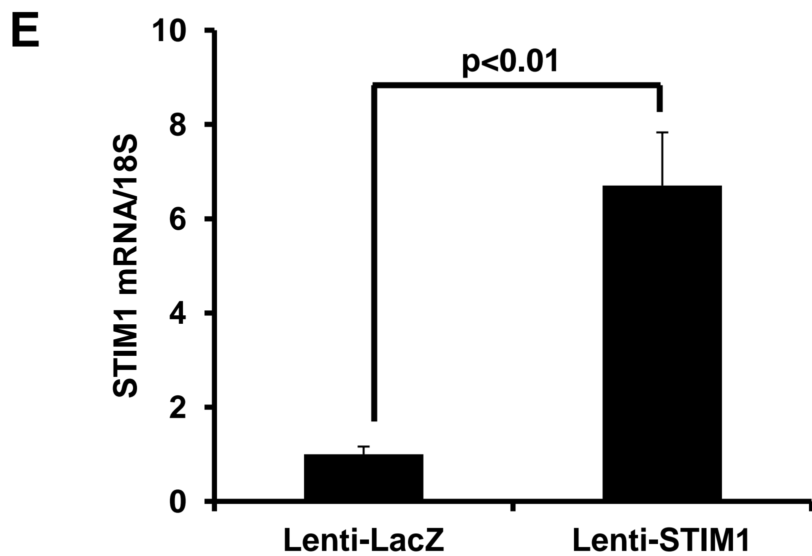

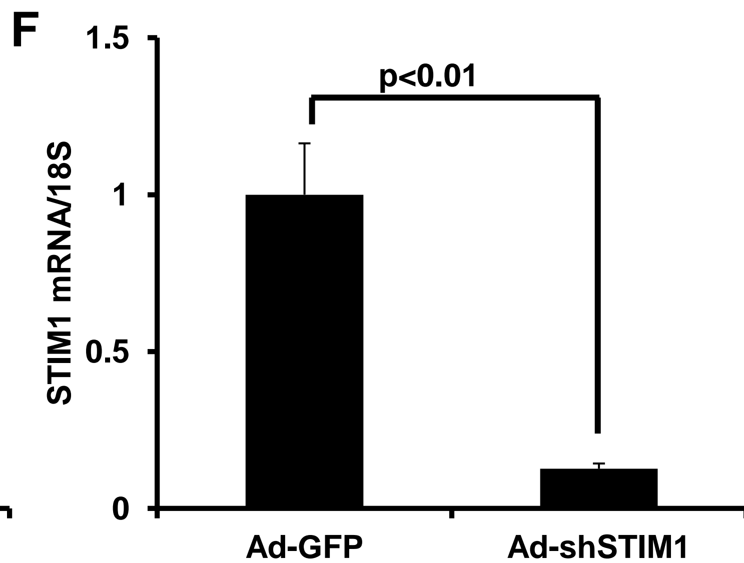

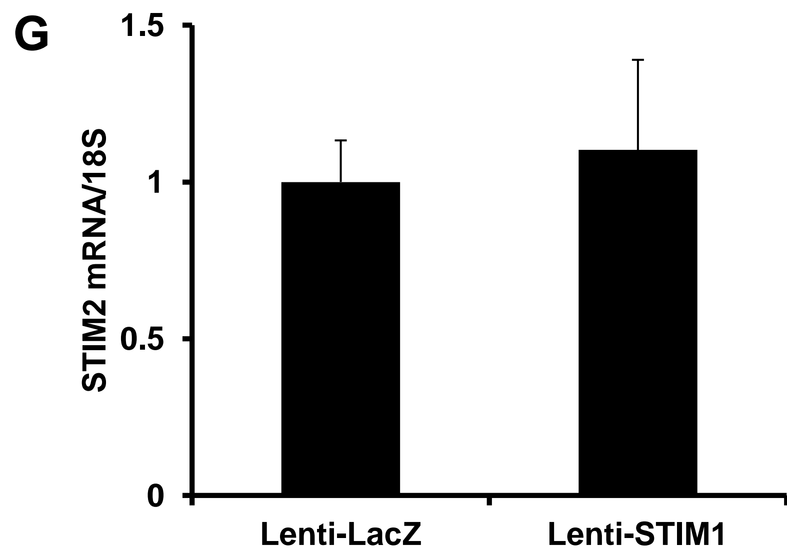

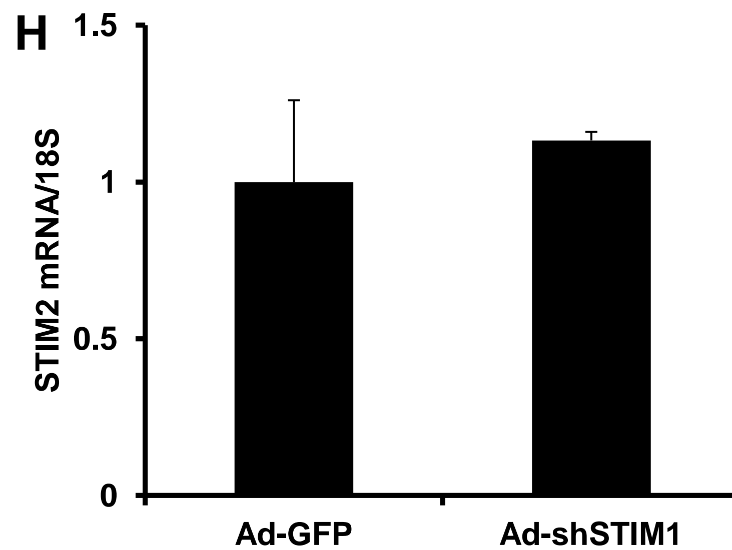

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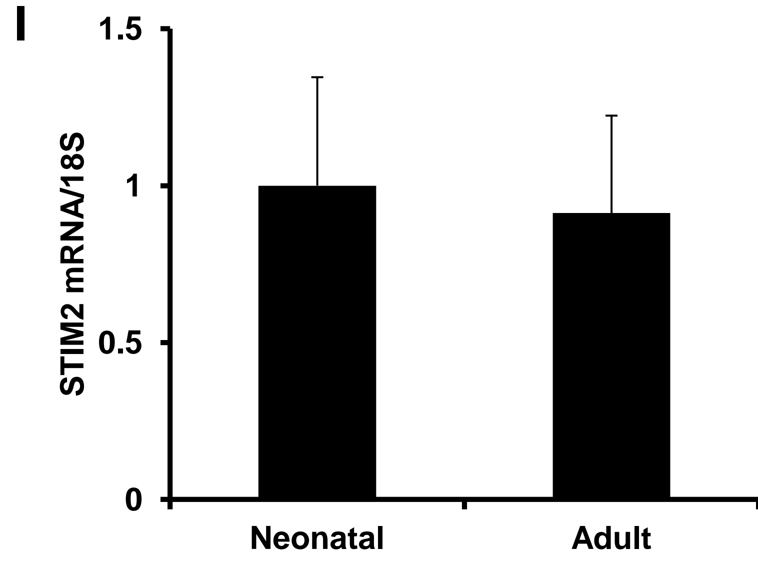

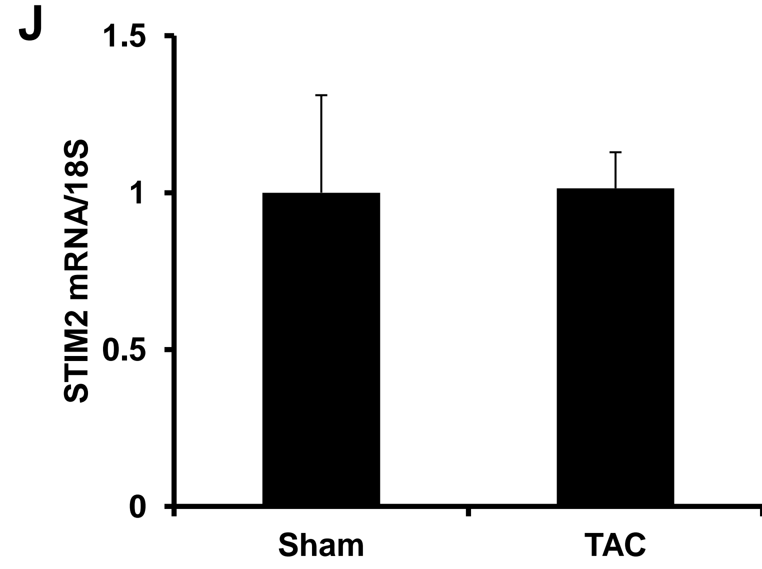

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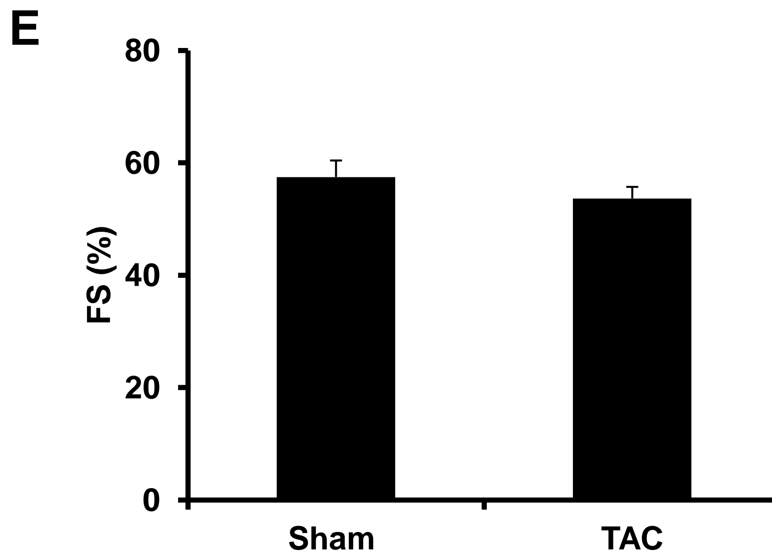
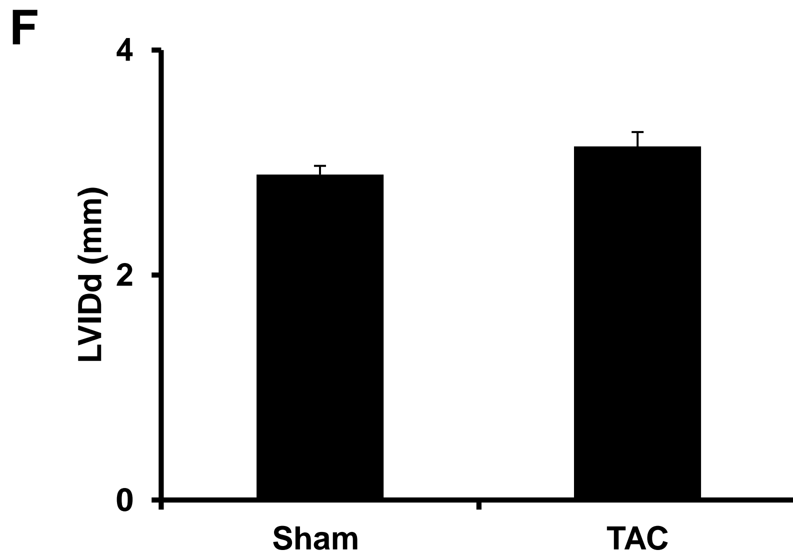
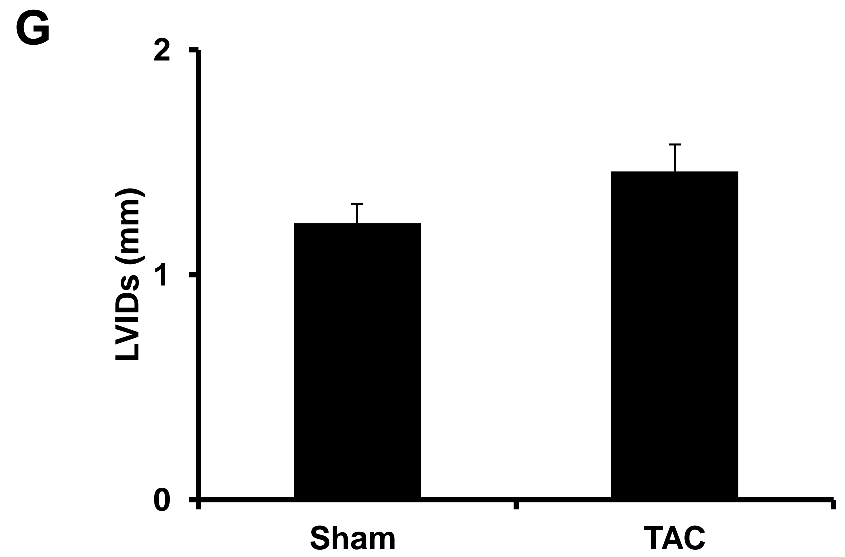
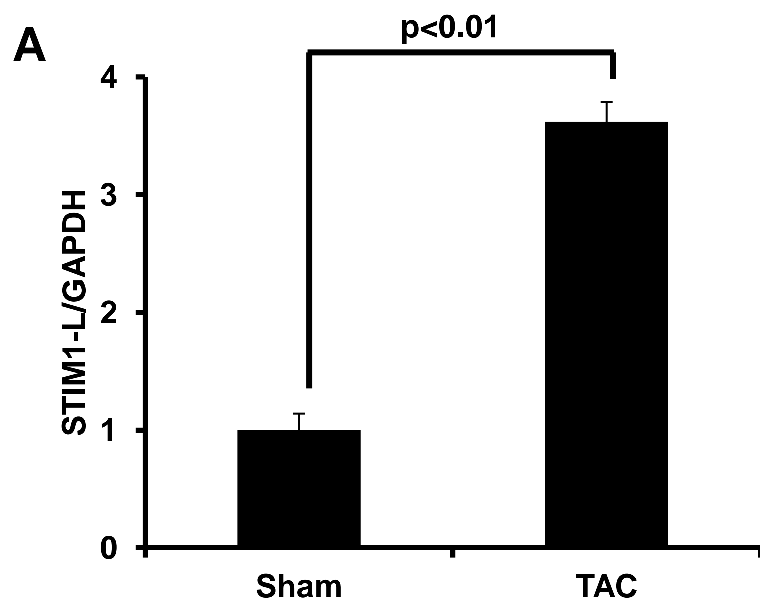
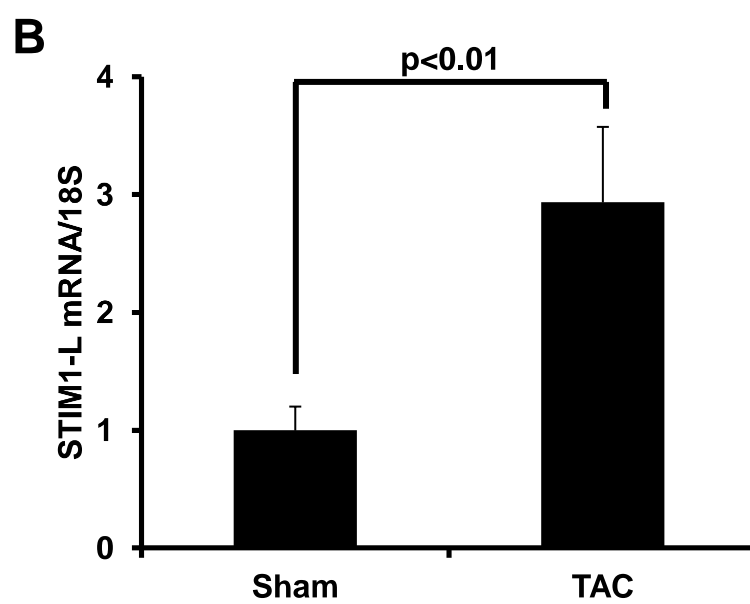


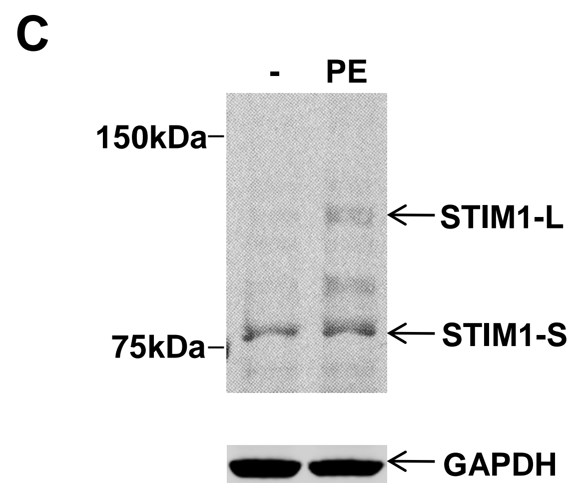




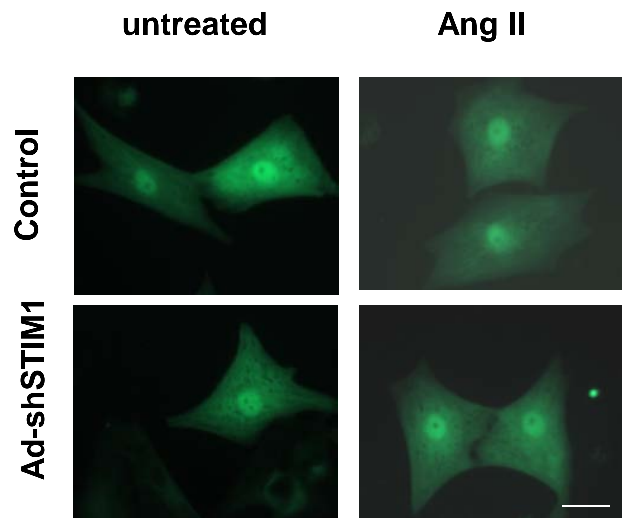
Supplemental Figure 6

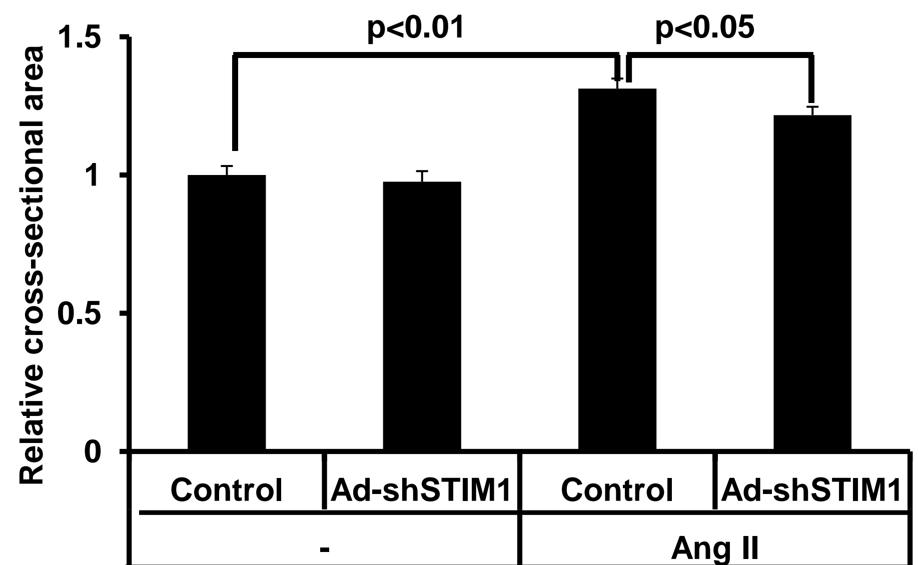




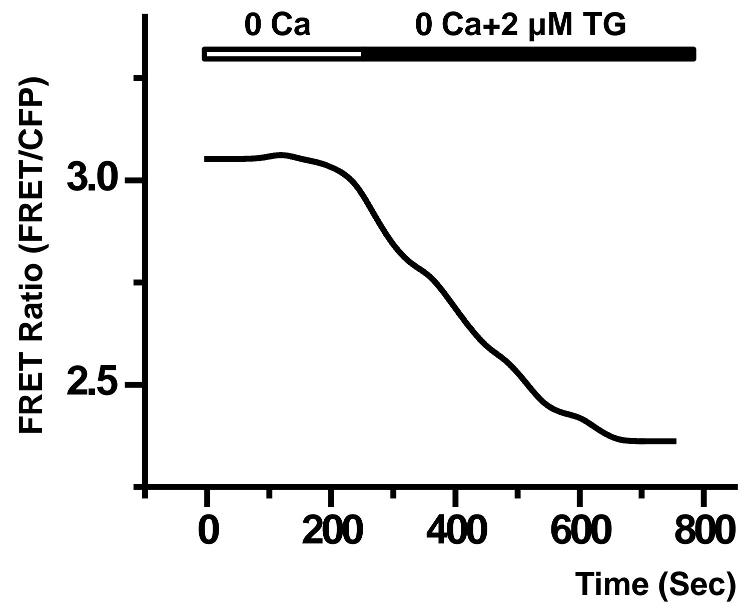


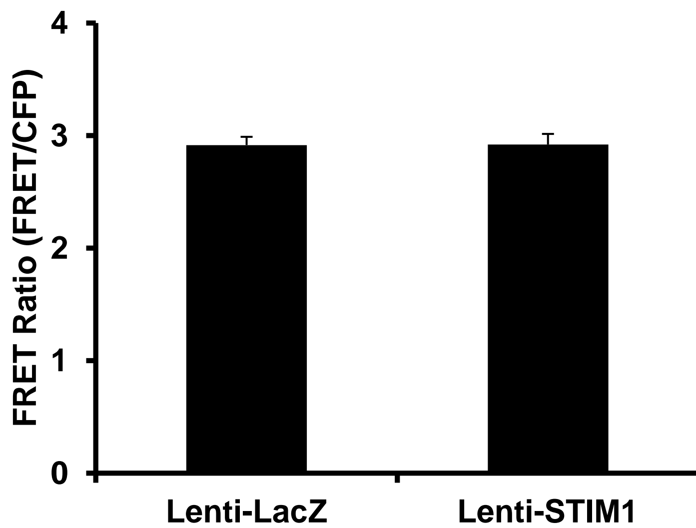
Supplemental Figure 7

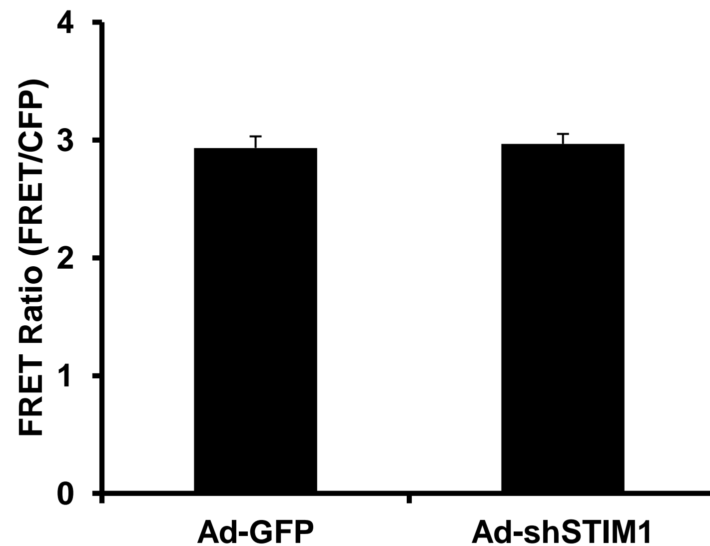




Supplemental Figure 8







Supplemental 9

Supplemental Data

Figure Legends

Supplemental Figure 1. Quantitative analyses of detectable SOCE depicted as the corrected average peak Ca^{2+} levels in NRVMs (n=78) and adult cardiomyocytes (n=89). N=3-5 independent experiments.

Supplemental Figure 2. Specificity of the STIM1 antibody (#54680, AnaSpec, Inc.) used in this study was supported by additional Western blot analysis using an independent polyclonal STIM1 antibody (ProSci Inc., #4119). Similar results were obtained.

Supplemental Figure 3. Ventricular lysates from TAC mice were immunoprecipitated with STIM1 antibody and subjected to SDS-PAGE. Coumassie blue staining revealed two protein bands which were subjected to mass spectrometric analysis: Sample 1 (80 kD band), Sample 2 (130 kD band). As a control, a region between these two bands was analyzed (Sample 3).

Supplemental Figure 4. SOCE activity was increased in NRVMs with STIM1 over-expression. (**A**) Representative Ca^{2+} traces demonstrating SOCE in cultured NRVMs infected with lentivirus expressing LacZ and STIM1. (**B**) Quantitative analysis of SOCE depicted as the corrected average peak Ca^{2+} levels in lenti-LacZ or lenti-STIM1-infected NRVMs (n=20-22) from three independent experiments.

Supplemental Figure 5. STIM1 over-expression (**A**, **C**) or knockdown (**B**, **D**) provoked no difference by quantitative real-time RT-PCR in TRPC1 (**A**, **C**) or Orai1 (**B**, **D)** expression levels. Alteration in STIM1 levels (**E, F**) had no effect on STIM2 (**G, H**) in NRVMs with STIM1 over-expression (**E, G**) or knockdown (**F, H**). N=3 independent experiments. Additionally, no difference in STIM2 expression levels was detected in hearts between neonatal and adult (**I**), Sham and TAC mice (**J**).

Supplemental Figure 6. Cardiac function measured in Sham and TAC mice 3 weeks post-surgery (n=5-8). There were no significant difference in FS (**A**), left ventricular internal diameter

in diastole, LVIDd (**B**), and left ventricular internal diameter in systole, LVIDs (**C**) between two groups. FS, fractional shortening at the diastole; LVIDd, LV diastolic diameter; LVIDs, LV systolic diameter.

Supplemental Figure 7. Quantitative analyses of STIM1-L expression depicted in **Figure 4A** (**A**). Quantitative real-time RT-PCR analysis of STIM-L mRNA levels in Sham and TAC hearts (**B**). Western blot analysis showing STIM1-L is up-regulated in cultured ARVMs exposed to PE (50 μM) for 36 hr (**C**).

Supplemental Figure 8. (A) Representative images of Ad-GFP- or Ad-shSTIM1-infected neonatal cardiomyocytes exposed to Ang II or vehicle; scale bar, 20 μm. **(B)** Cell cross-sectional areas from experiments shown in panel C. Sixty to eighty randomly selected cells from each group were measured.

Supplemental Figure 9. D1ER signals from NRVMs shown as FRET ratio for ER Ca^{2+} content measurement, application of 2 μM TG caused a rapid drop in FRET ratio, indicating a release of Ca^{2+} from the ER store (**A**). Basal ER Ca^{2+} content was not affected in NRVMs (n=11-14) with STIM1 over-expression (**B**) or knockdown (**C**) (3 independent experiments).

Supplemental Materials and Methods

Immunoprecipitation and Coumassie blue staining. Left ventricular tissue lysates from Sham- and TAC-operated mice were extracted at 4°C in buffer containing Tris-buffered saline, 0.1% Triton X-100, 4% glycerol, 1mM DTT, 1mM EDTA, protease inhibitors (Roche), and phosphatase inhibitors (Sigma). Homogenates were passed over glass wool to remove DNA, and protein concentrations were determined using the Bio-Rad Bradford assay. Tissue extracts were incubated with STIM1 antibody (AnaSpec Inc.) at 4°C overnight on a shaking platform, followed by incubation with Protein A sepharose beads (Roche) overnight and washing 5 times with lysis buffer. Bound proteins were eluted by boiling 5 min in 20 μL 2x SDS-PAGE loading buffer. Protein gels were stained with 0.2% Brilliant Blue R250 for 1 hour, followed by washing with 30% methanol and kept in 0.5% acetic acid for mass spectrometry.

Mass spectrometry. Gel bands were excised and subjected to in-gel reduction, alkylation, and tryptic digestion using 10 ng/μL trypsin. The resulting tryptic peptides were extracted, dried, and cleaned in a C18 Zip-tip (Millipore). Peptides were separated in an LTQ-2D ion trap mass spectrometer (Thermo Scientific) equipped with a nano-electrospray ionization source, coupled with an Agilent 1100 nano-flow HPLC system and subjected to data-dependent tandem mass spectrometry analysis. The tandem mass spectra were searched against the *Mus musculus* entries of the NCBI-nr database using the search engine MASCOT (Version 2.2, Matrix Science).

Proteomics analyses. Data are reported as "protein score", a measure of the statistical significance of a PMF (Peptide Mass Fingerprint) match. This commonly used measure of scoring mass spectrometry-derived protein identification (Version 2.2, Matrix Science) is calculated as -10*Log(P), where P is the probability that the observed match is a random event. Protein scores greater than 67 are significant ($p<0.05$). Here, we employed a standard that only proteins with Mascot score > 150 are considered reliable.

ER Ca^{2+} content measurement. A fluorescence resonance energy transfer (FRET)-based chameleon Ca^{2+} indicator D1ER was employed to measure ER Ca^{2+} content in NRVMs. D1ER expression plasmid was kindly provided by Dr. Roger Tsien (University of California, San Diego) [1]. D1ER is composed of enhanced cyan fluorescent protein (ECFP) and citrin

fluorescent protein separated by a linker encoding calmodulin (CaM) and CaM-binding M13 peptide sequence. Calreticulin (CRT) targeting sequence (MLLPVLLLGLLGAAAD) was added to the N terminus, and an ER retention sequence (KDEL) was added to the C terminus of D1ER protein to facilitate ER targeting and retention [2].

Cultured NRVMs were transfected with D1ER plasmid and lenti-STIM1, Ad-shSTIM1 or control plasmids using FuGENE HD transfection reagent (Promega). Imaging experiments were performed 48h after transfection using a Deltavision RT wide-field epifluorescence deconvolution microscope (Applied Precision) equipped with a Photometrics CoolSNAPHQ monochromatic digital camera (Roper Scientific) controlled by the SoftWorx image acquisition software package (Applied Precision). The filter sets used for FRET imaging experiments were CFPx 436 nm/10 nm and yellow fluorescent protein (YFPx) 492 nm/18 nm for excitation (x) and CFPm 465 nm/30 nm and YFPm 535 nm/30 nm for emission (m). A series of three images CFPx/CFPm (CFP), YFPx/YFPm (YFP), and CFPx/YFPm (FRET) was acquired every 25 s. Analysis of the imaging data was performed using MetaMorph imaging software. ER Ca^{2+} content is expressed as FRET/CFP ratio.

Primary culture of adult rat cardiomyocytes and adeno- or lenti-virus infection. Male Sprague-Dawley rats (250 g) were anesthetized with Euthasol (Virbac Corporation). Adult rat cardiomyocytes (ARVMs) were isolated by a retrograde aortic perfusion method. Briefly, the heart was removed, washed with Gerard medium (in mM, NaCl 128, KCl 4.0, $MgSO_4 \cdot 7H_2O$ 1.39, NaH_2PO_4 0.19, Na_2HPO_4 1.01, HEPES 10.0, glucose 5.5 and pyruvic acid 2.0 at 37 °C, pH 7.4 containing 10 mM 2,3-butanedione monoxime) and retroperfused with 0.12% (wt/vol) collegenase A (Roche) digestion buffer for 30 min. Digested heart was mechanically shattered in digestion buffer, and the supernatant was collected, the remaining tissues were further digested in a sterile flask for 10 min and the supernatants collected, then centrifuged at 500 rpm for 30 seconds. Pellets containing cardiac myocytes were resuspended in Gerard medium and followed by Ca^{2+} reintroduction, then plated on laminin-precoated dishes or coverslips. Following overnight incubation, cells were transferred to serum-free medium and used for adeno- or lenti-virus infection (multiplicity of infection 10).

References

[1] Palmer AE, Jin C, Reed JC, Tsien RY. Bcl-2-mediated alterations in endoplasmic reticulum Ca2+ analyzed with an improved genetically encoded fluorescent sensor. Proc Natl Acad Sci U S A. 2004;101:17404-9.

[2] Palmer AE, Tsien RY. Measuring calcium signaling using genetically targetable fluorescent indicators. Nat Protoc. 2006;1:1057-65.