SUPPLEMENTAL MATERIALS

Supplemental Methods

Establishment of patient-derived iPSCs

Human epicardial coronary arteries and foreskin were procured from the explanted hearts of organ donors or recipients and patients undergoing a circumcision, respectively, within the operating room under protocols approved by the institutional review boards of Yale University School of Medicine and the New England Organ Bank. The vessels were exposed to intra-arterial and/or topical perfusion with ice-cold saline. Adventitia and perivascular fat were carefully removed from the arterial media, the vessels were minced, and smooth muscle cells (SMCs) were obtained by explant outgrowth. Isolated SMCs were serially cultured and expanded in M199 medium supplemented with 20% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA). The cells were used for experiments after 3-4 passages. Foreskin fibroblasts were derived by slicing the dermis followed by 15-20 min collagenase type IA (3mg/ml) (Sigma) treatment at 37^0C. Cells were next spun down and plated in fibroblast media [OPTI-MEM (Invitrogen), 5% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin]. The cells were used for experiments after 3-4 passages. SVAS and WBS patient-derived human iPSC clones were established from the patient's vascular SMCs and foreskin fibroblasts, respectively, by lentiviral delivery of four reprogramming factors (OCT4, KLF4, SOX2 and C-MYC). Briefly, a previously published single lentiviral vector (hSTEMCCA) containing a polycistronic cassette encoding human *OCT4*, *SOX2*, *KLF4* and c-MYC was packaged as follows [1]. The hSTEMCCA plasmid was co-transfected into HEK-293T cells together with two lentiviral

packaging plasmids encoding the VSVG envelope and gag/pol. Virus-containing supernatant medium was collected 48 hours after transfection and used for two rounds of SMCs or foreskin fibroblast infection, each lasting 12 hours. After this, cells were cultured in MEF media for 1 day, followed by human ESC media with 10 ng/ml FGF and 2% ES grade FBS for another 5 days. They were then re-plated at a density of $1–2\times10^5$ cells per well on a mitotically inactivated feeder layer of mouse embryonic fibroblasts (MEF), cultured in human ESC medium with 10 ng/ml FGF for a month. Several human iPSC clones, which morphologically resembled human ESCs were selected for TRA-1-60 staining. The positive clones were expanded for further characterization.

Genomic sequencing

Genomic DNA was isolated from the patient-derived human iPSC colonies using the RED Extract-N-Amp ™ Tissue PCR Kit (Sigma). The relevant DNA fragments of genes of interest were amplified by PCR reaction using a genomic DNA template (primer sequences are detailed in Supplemental Table 1). PCR products were then sequenced by the Keck Core Facility at Yale University.

Immunofluorescence and alkaline phosphatase staining

Colonies of undifferentiated human iPSCs were fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton (Sigma) and blocked with 5% horse serum. Specimens were incubated overnight at 4°C with primary antibodies targeting Tra-1-60, OCT4 (Abcam), NANOG (Abcam), SSEA4 (R&D), nestin (Millipore), α-fetoprotein (Cell Marque), desmin (Thermo), SM α-actin (Abcam), calponin (Sigma) and elastin (Novus). The preparations were incubated with secondary antibodies for 1h at

room temperature. Nuclei were counterstained with Hoechst 33258 (Sigma). Preparations were examined using a fluorescent microscope (Leica) or a Volocity 6.0 on a Nikon eclipse Ti Spinning Disk Microscope (Perkin Elmer). Alkaline phosphatase activity was detected in live cultures using the alkaline phosphatase detection kit (Millipore) according to the manufacturer's instructions.

Chromosome integrity (karyotype) and fluorescence *in situ* hybridization (FISH) analyses

Karyotype analysis was performed using standard G-banding chromosome analysis by the Yale Cytogenetic Services or Cell Line Genetics (Madison, WI) according to standard procedures. The WBS iPSCs grown on Matrigel-coated coverslips were sent to the Cytogenetics Lab at Yale for FISH analysis to detect the deletion of *ELN* gene.

Bisulphite sequencing

Human iPSCs were cultured on matrigel for 3-4 days and collected by accutase (Sigma-Aldrich). Cell pellets were washed with PBS three times and centrifuged at 1000 rpm for 5 min. Then, SVAS patient-derived iPSCs, WBS patient-derived iPSCs, control iPSCs, and human ESC line H7 as positive control were sent to EpigenDx Inc (Worcester, MA) for quantitative methylation analyses of three CpG islands in the *NANOG* promoter via pyrosequencing, spanning positions -565 to -431 relative to the ATG start site.

Gene expression analysis

Undifferentiated human iPSCs and differentiated embryoid bodies were frozen in liquid nitrogen. RNA was isolated using Trizol-RNA isolation assay (Qiagen). Reverse transcription into cDNA was

conducted using the iscript™ cDNA synthesis kit (Bio-rad). Briefly, each RT–PCR included the following PCR program: 5 min at 95°C, 30s at 95°C, 30s at 60°C, and 30s at 72°C. 2.5ng of cDNA was used from each sample. SYBR-Green real-time PCR studies were performed using IQ™ SYBR Super Green mix (Bio-rad) and primers (Supplemental Table 1). All real-time PCR experiments were conducted in triplicate. Samples were cycled 45 times using a CFX96 real-time system (Bio-rad). CFX96 real-time system cycle conditions were as follows: 2min at 50°C, 15min at 95°C followed by 45 cycles of 15s at 95°C, 30s at 60°C and 30s at 72°C. Cycle threshold (CT) was calculated under default settings for real-time sequence detection software (Bio-rad).

Teratoma formation assay

Undifferentiated human iPSCs were cultured on MEF feeder layers for 5 days, isolated by collagenase IV dissociation, and injected subcutaneously into NOD/SCID mice. Palpable tumors were felt by touch 3 weeks after injection and observed another 2-3 weeks later. Tumor samples were collected at 6 weeks after injection, processed by 10-μm paraffin-sectioning, and stained with hematoxylin and eosin.

Western blot analysis

Control, SVAS iPSC-SMCs or WBS iPSC-SMCs were directly lysed in tissue culture wells with RIPA buffer (Boston Bioproducts) supplemented with proteinase and phosphatase inhibitor cocktail mixture (Boston Bioproducts). Samples were resolved by SDS-PAGE. A G Box (SynGene) detection system was used to visualize and quantify protein bands after being incubated with Super Signal West Pico stable peroxide solution. Rabbit polyclonal anti-elastin (1:200) (Novus), mouse monoclonal

anti-tubulin (1:1000) (Sigma), rabbit polyclonal anti-smooth muscle actin (1:1000) (Abcam), mouse monoclonal anti-calponin (1:1000) (Sigma), mouse monoclonal anti-cyclin D1 (1:1000) (Cell Signaling Technology), rabbit anti-phosphorylated ERK (1:1000) (Cell Signaling Technology), rabbit anti-total ERK (1:1000) (Cell Signaling Technology) were used as primary antibodies, and HRP-conjugated goat anti-rabbit or goat anti-mouse antibody was used as secondary antibody (1:10000) (Sigma) for the appropriate primary antibodies.

Fluorescence-activated cell sorting (FACS) analysis

Human iPSC-SMCs were dissociated from culture dishes using 0.05% trypsin (Invitrogen) and fixed with 2% paraformaldehyde in PBS. After three washes using PBST (PBS containing 0.1% (v/v) Triton X-100), cells were pre-incubated with 10% goat serum (Invitrogen) in PBST for 45 min. Cells were next incubated with primary antibody [rabbit SM α-actin (Abcam), mouse calponin (Sigma) or rabbit isotype control antibody for SMA (Santa Cruz), mouse isotype control antibody for calponin (Invitrogen)] in a solution containing 5% goat serum in PBST. After three washes with PBST, cells were incubated with FITC-conjugated secondary antibody (Invitrogen) in PBST containing 5% goat serum for 2 hrs. Samples were analyzed using FACSCalibur and Cell Quest software (BD Pharmingen) following three washes.

Blockage of ERK signalling pathway and analysis of cell proliferation

Control and SVAS iPS-SMCs were plated at the same cell density. 12-14 hours after plating, cells were treated with 10μM U0126 (Cell Signaling Technology) for 72 hours followed by western blot analysis for ERK signalling with cyclin D1 (Cell Signaling Technology), phosphorylated ERK (Cell

Signaling Technology) and total ERK (Cell Signaling Technology) antibodies, direct cell counting with hemocytometer (Fisher), or BrdU immunostaining analysis. Medium with U0126 was replaced every 48 hours. Cells were treated with 10μM BrdU (Sigma) 12 hours before fixation. BrdU was detected with a rat anti-BrdU antibody (Abcam). Nuclei were stained with Hoechst 33258. The images were taking using an inverted fluorescent microscope DM IRB with a Leica DC350FX camera (Leica).

Calcium imaging

In order to examine the functional maturity of iPSC-SMCs, we measured the intracellular calcium influx between control and SVAS iPSC-SMCs (Supplemental Figure 12). Human iPSC-SMCs plated on 25 mm glass coverslips were loaded with 10 μmol/L Fura-2 acetoxymethyl ester (Molecular Probe) and 0.1% pluronic F-127 (Sigma) in Tyrode solution for 20 minutes at room temperature. Non-incorporated dye was washed away with Tyrode solution containing NaCl 148mmol/L, KCl 5mmol/L, $CaCl_2$ 2mmol/L, $MgCl_2$ 1mmol/L glucose 10 mmol/L, Hepes 10 mmol/L, pH 7.4. Ca^{2+} transients were evoked by the treatment of 100 μmol/L carbachol (Sigma) or 50mmol/L of KCl (NaCl 90 mmol/L, KCl 50 mmol/L, $CaCl_2$ 2 mmol/L, $MgCl_2$ 1 mmol/L glucose 10 mmol/L, Hepes 10 mmol/L, pH 7.4) for 2 minutes. Ionomycin at 1 μmol/L was used as an internal control. Fluorescence intensities at 510 nm with 340 nm and 380 nm excitation were collected at a rate of 1 Hz using CoolSNAP HQ2 (Photometrics) and data were analyzed using NIS-Elements (Nikon). Cytosolic Ca^{2+} was measured by ratio of fluorescence intensity at 340 nm and 380 nm (F340/F380) normalized to that of the Ca^{2+} signal elicited by 1 μmol/L Ionomycin as previously reported [2].

Supplemental Table 1: List of primers used in SVAS mutation sequencing and the real-time PCR studies

SVAS mutation sequencing forward	ATATAAGCTTAGATGACTTCCGAAACTCGTGGGA
SVAS mutation sequencing reverse	ATTATAGGCATGAGCCACTGCGTC
SVAS allele-specific forward primer to detect mutant *ELN* mRNA	GCTGCCAGGTGTATGTAT
SVAS reverse primer to detect mutant *ELN* mRNA	TGGGATACCCCAGTGGGAC
Gene silencing OCT4 forward	CACTGCACTGTACTCCTCGGTC
Gene silencing OCT4 reverse	CACCTGCAAGTTTCAGCAAATC
Gene silencing SOX2 forward	GCTTTACATGTGTTTAGTCG
Gene silencing SOX2 reverse	CTTCAGCTCCGTCTCCATC
Gene silencing cMYC forward	CCACTAACTTCTCCCTGTTG
Gene silencing cMYC reverse	GAAATACGGCTGCACCGAGTC
Gene silencing KLF4 forward	GGTGCGCCAGTAAAGCAGACATTAAAC
Gene silencing KLF4 reverse	CAGACGCGAACGTGGAGAAAGA
Endogenous gene activation OCT4 forward	GCAGCTCGGAAGGCAGAT
Endogenous gene activation OCT4 reverse	TGGATTTTAAAAGGCAGAAGACTTG
Endogenous gene activation OCT4 forward (set 2)	GAAGGAGAAGCTGGAGCAAA

Endogenous gene activation OCT4 reverse (set 2)	CTTCTGCTTCAGGAGCTTGG
Endogenous gene activation NANOG forward	GATTTGTGGGCCTGAAGAAA
Endogenous gene activation NANOG reverse	ATGGAGGAGGGAAGAGGAGA
Endogenous gene activation Rex1 forward	ACCAGCACACTAGGCAAACC
Endogenous gene activation Rex1 reverse	TTCTGTTCACACAGGCTCCA
Endogenous gene activation FOXD3 forward	AAGCCCAAGAACAGCCTAGTG
Endogenous gene activation FOXD3 reverse	GGGTCCAGGGTCCAGTAGTTG
Endogenous gene activation SOX2 forward	AACCCCAAGATGCACAACTC
Endogenous gene activation SOX2 reverse	GCTTAGCCTCGTCGATGAAC
GAPDH forward	TGTTGCCATCAA TGACCCCTT
GADPDH reverse	CTCCACGACGTACTCAGCG

Supplemental Figure Legends

Supplemental Figure 1. Establishment and characterization of human iPSCs. **(A)** Mutant *ELN* mRNA undergoes nonsense-mediated decay. Primary SMCs from a control donor or a SVAS patient were plated and treated with 100μg/ml of cycloheximide (CHX) or DMSO (control) for 4 hours. Cells were lysed for RNA isolation, and mutant *ELN* mRNA was detected by RT-PCR using an allele-specific forward primer that contained a 4-nucleotide GTAT insertion at its 3′ end and an *ELN* downstream reverse primer. This results in the detection of a PCR product specifically in the SVAS cells but not in the control cells. GAPDH was used as internal control. The representative image was from three independent experiments. The PCR product from SVAS SMC + CHX was further sequenced, and the GTAT insertion was confirmed (data not shown). **(B)** Immunostaining showing expression of the pluripotency markers TRA-1-60, SSEA-4, NANOG, OCT4, and alkaline phosphatase (AP) activity in control and SVAS iPSCs (clone 2). Also provided are images of the undifferentiated colonies of these human iPSC clones showing human ESC-like morphology (left panels). Scale bars, 100 μm. **(C)** Karyotypic stability (46XY) by G-banding analysis of control iPSCs and SVAS iPSCs (clone 2). **(D)** Pyrosequencing analysis of bisulfite-treated genomic DNA provides quantification of methylation of the human *NANOG* promoter across 3 CpG islands in primary tissue-derived SMCs prior to reprogramming, as well as in the control and SVAS iPSC (clone 2) clone. Abbreviations: H7 ESCs, H7 human embryonic stem cells; iPSCs, induced pluripotent stem cells.

Supplemental Figure 2. Silencing of the four lentiviral transgenes and reactivation of the endogenous levels of the pluripotency markers in human iPSCs. **(A)** Silencing of the four lentiviral transgenes in hiPSCs. Real-time quantitative PCR analysis of the four transgenes used for cell reprogramming.

Analysis for the levels of cMYC, OCT 3/4, KLF4 and SOX2 demonstrated down-regulation of these factors in SVAS iPSC clones as well as in the healthy control iPSCs. Values are normalized to the house-keeping gene GAPDH and expressed as mean ± s.e.m. (n=4). Expression values are relative to 293T cells transiently transfected with the lentiviral plasmid containing all four reprogramming transcription factors used to produce the viruses. **(B)** Real-time quantitative PCR analysis evaluating the endogenous levels of the pluripotency markers OCT3/4 and SOX2 and additional characteristic pluripotency markers (FOXD3, NANOG, and REX1) in the iPSC clones. The levels of these pluripotency markers were evaluated in the control and the primary SVAS SMCs prior to reprogramming and in both control and SVAS iPSC clones. Values were normalized to the house-keeping gene GAPDH and presented as mean ± s.e.m. (n=4). Expression values are relative to levels in the relevant primary SMCs.

Supplemental Figure 3. Differentiation potential shown by *in vitro* embryoid body (EB) assay and *in vivo* teratoma formation in human iPSCs. **(A)** In vitro immunostaining of differentiating embryoid bodies from control iPSCs and SVAS iPSC clone (clone 2) for nestin (ectoderm), α-fetoproptein (AFP, endoderm) and desmin (mesoderm). Scale bars, 50μm. **(B)** Teratoma formation following injection of undifferentiated control iPSCs and SVAS iPSCs (clone2) in NOD/SCID mice. Note the formation of pigmented epithelium (ectoderm), gastrointestinal epithelium (endoderm) and hyaline cartilage (mesoderm), as identified by the arrows. Scale bars, 200μm.

Supplemental Figure 4. Establishment and characterization of WBS iPSCs. **(A)** Fluorescence *in situ* hybridization (FISH) analysis was performed in the metaphase stage control iPSCs and WBS iPSCs

using the probes for the *ELN* gene at 7q11.23 and the *TWIST1* gene (control) at 7p21.1 (Rainbow Inc.). Note that one copy of the *ELN* gene is deleted while the control gene, *TWIST1*, is intact in WBS iPSCs. **(B)** Characterization of the pluripotency markers for WBS iPSCs. Shown from left to right are a typical human iPSC colony, positive immunostaining for pluripotency markers (TRA-1-60, SSEA-4, NANOG, and OCT4), and positive staining for alkaline phosphatase (AP). Scale bars, 100 μm. **(C)** Karyotype analysis of WBS and control iPSCs. A male complement with a deletion at the 7q11.23 was observed (Arrow). Concurrent FISH analysis confirmed this deletion (A)**. (D)** Pyrosequencing analysis of bisulfite-treated genomic DNA provides quantification of methylation of the human *NANOG* promoter across 3 CpG islands in the control and WBS foreskin fibroblasts prior to reprogramming, as well as in the control and WBS iPSCs. **(E)** Immunostaining of differentiated embryoid bodies for α-fetoproptein (AFP, endoderm), desmin (mesoderm) and nestin (ectoderm). Scale bars, 50 μm. **(F)** Teratoma formation following injection of undifferentiated control iPSCs and WBS iPSCs in NOD/SCID mice. Note the formation of neural rosettes (ectoderm), gastrointestinal epithelium (endoderm) and hyaline cartilage (mesoderm), as identified by the arrows. Scale bars, 200 μm.

Supplemental Figure 5. Silencing of the four lentiviral transgenes and reactivation of the endogenous levels of the pluripotency markers in control and WBS iPSCs. **(A)** Silencing of the four lentiviral transgenes in control and WBS iPSCs. Real-time quantitative PCR analysis of the four transgenes used for cell reprogramming. Analysis for the levels of cMYC, OCT 3/4, KLF4 and SOX2 demonstrated down-regulation of these factors in WBS iPSC clones as well as in the healthy control iPSCs. Values are normalized to the house-keeping gene GAPDH and expressed as mean ± s.e.m.

(n=4). Expression values are relative to 293T cells transiently transfected with the lentiviral plasmid containing all four reprogramming transcription factors used to produce the viruses. **(B)** Real-time quantitative PCR analysis evaluating the endogenous levels of the pluripotency markers OCT3/4 and SOX2 and additional characteristic pluripotency markers (FOXD3, NANOG, and REX1) in the iPSC clones. The levels of these pluripotency markers were evaluated in the control and the primary WBS foreskin fibroblasts prior to reprogramming and in both control and WBS iPSC clones. Values were normalized to the house-keeping gene GAPDH and presented as mean ± s.e.m. (n=4). Expression values are relative to levels in the relevant primary foreskin fibroblasts.

Supplemental Figure 6. SM α-actin filament bundle formation in healthy control iPSCs, SVAS iPSCs and H7 human ESCs. **(A)** Control iPSC-SMCs and SVAS iPSC-SMCs (clone 2) were immunostained with antibodies for SM α-actin and calponin. Nuclei were counterstained with Hoechst 33258. Scale bar, 50 μm. **(B)** The SM α-actin filament bundle and calponin-positive cells in each group from (A) were quantified as number of cells stained positively divided by total number of cells. Mean ± s.e.m. (n=4). *$p<0.05$. **(C)** Control iPSC-SMCs and SVAS iPSC-SMCs were dissociated from culture dishes, fixed, immunostained with antibodies for SM α-actin and calponin, and analyzed using FACSCalibur and Cell Quest software. Mean ± s.e.m. (n=5). *$p<0.05$. **(D)** SVAS iPSC-SMCs and control iPSC-SMCs were lysed and immunoblotted with anti-SM α-actin, anti-calponin and anti-tubulin antibody. **(E)** SMCs derived from H7 human embryonic stem cells were immunostained with antibodies for SM α-actin and calponin and were quantified as number of cells stained positively divided by total number of cells from three independent experiments. Nuclei were counterstained with Hoechst 33258. Scale bar, 50 μm.

Supplemental Figure 7. Defective ELN expression level in SVAS iPSC-SMCs. **(A)** Control iPSC-SMCs and SVAS iPSC-SMCs were immunostained with antibodies for ELN. Nuclei were counterstained with Hoechst 33258. Scale bar, 50 μm. **(B)** SVAS iPSC-SMCs and control iPSC-SMCs were lysed and immunoblotted with anti-ELN and anti-tubulin antibody. **(C)** Quantification of the ELN protein expression level in SVAS iPSC-SMCs and control iPSC-SMCs using tubulin as loading control. Mean ± s.e.m. ($n \geq 3$). *$p < 0.05$ vs. control iPSC-SMCs. Note that there is no significant (N.S.) difference of ELN levels between SVAS clone 1 and clone 2.

Supplemental Figure 8. Regulation of SM α-actin filament bundle formation by recombinant ELN and small GTPase RhoA. **(A)** Immunostaining of SM α-actin in control iPSC-SMCs and SVAS iPSC-SMCs (clone 2) with or without 50 μg/ml ELN treatment. **(B)** Quantification of cells with organized SM α-actin filament bundle staining in (A) from at least three independent experiments. *$p < 0.05$. **(C)** Immunostaining of control iPSC-SMCs transfected with cMyc-tagged RhoA dominant negative mutant (Negative RhoA) or cMyc-tagged RhoA constitutive active mutant (Active RhoA) for SM α-actin. Rabbit anti-SM α-actin conjugated with Alexa 565 and mouse anti-cMyc conjugated with Alexa 488, together with nuclear staining of Hoechst 33258, were used to detect SM α-actin and cMyc-tagged active or negative RhoA. Panel a, b & c: The constitutively active mutant RhoA has no effect on the formation of SM α-actin filament bundle in control iPSC-SMCs. Panel d, e & f: RhoA dominant negative mutant knocks down the formation of SM α-actin filament bundle in control iPSC-SMCs. Scale bars, 50 μm. **(D)** The ratio of transfected, control iPSC-SMCs with the expression of SM α-actin filament bundles in total transfected cells. Mean ± s.e.m. ($n=4$, minimum 100 cells/experiment). *$p < 0.05$.

Supplemental Figure 9. Cellular proliferation of control iPSC-SMCs and SVAS iPSC-SMCs. **(A)** Assay measuring cell numbers demonstrates that SVAS iPSC-SMCs (clone 2) proliferate at a much higher rate than control iPSC-SMCs. Mean ± s.e.m. ($n \geq 4$). *$p<0.05$. **(B)** 7-day old control iPSC-SMC and SVAS iPSC-SMC (clone 2) culture were immunostained with antibodies for BrdU. Nuclei were counterstained with Hoechst 33258. Scale bar, 50 μm. **(C)** the BrdU and nuclear positive cells (Blue) in each group from (B) were quantified. Mean ± s.e.m. (n=4). *$p<0.05$. **(D)** A modified Boyden chamber assay was used to determine the total number of migrated cells in five to seven randomly selected fields under the fluorescence microscopy. Data shown are the Mean±s.e.m. from four independent experiments. *$p<0.05$.

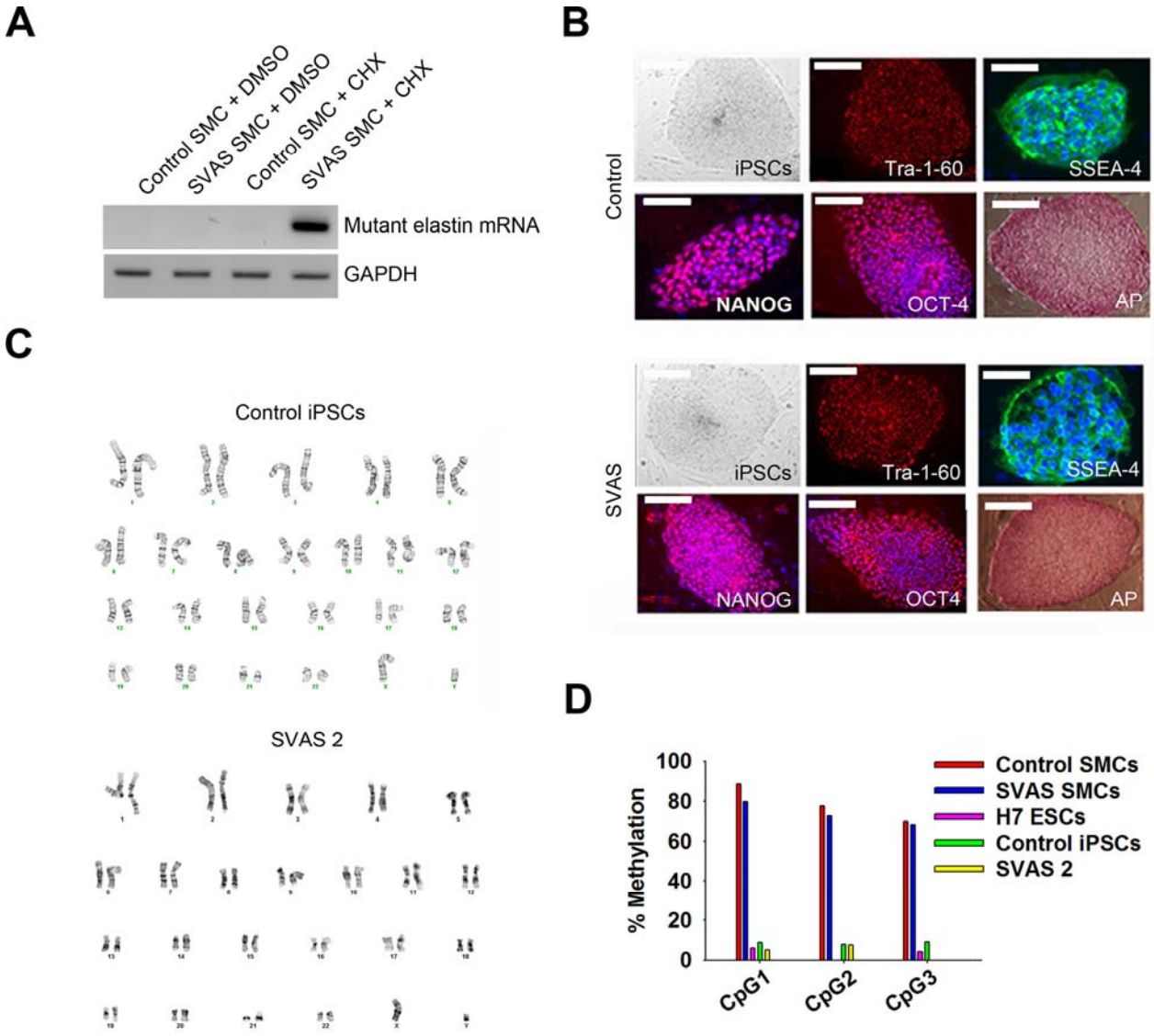
Supplemental Figure 10. Characterization of WBS iPSC-SMCs. **(A)** WBS iPSC-SMCs and control iPSC-SMCs were immunostained with antibodies for SM α-actin and calponin. Nuclei: Hoechst 33258. Scale bar, 50 μm. **(B)** SM α-actin filament bundle and calponin positive cells from each group in (A) were quantified as number of cells stained positively divided by total number of cells (mean ± s.e.m., n=4; *$p<0.05$). **(C)** WBS iPSC-SMCs and control iPSC-SMCs were lysed and immunoblotted with anti-ELN, anti-SM α-actin, anti-calponin and anti-tubulin antibodies. **(D)** Immunostaining of SM α-actin in control and WBS iPSC-SMCs cultured in the presence or absence of 50 μg/ml ELN treatment. Nuclei: Hoechst 33258. **(E)** Quantification of cells with organized SM α-actin filament bundles in (D) (mean ± s.e.m., n=4; *$p<0.05$).

Supplemental Figure 11. WBS iPSC-SMCs proliferate and migrate at higher rates than that of control iPSC-SMCs. **(A)** Cellular proliferation of WBS iPSC-SMCs and control iPSC-SMCs. Mean± s.e.m.

(n=4). *p<0.05. (B-C) 7-day old WBS iPSC-SMC and control iPSC-SMC culture were labeled with BrdU, immunostained with antibodies for BrdU **(B)**, and quantified **(C)**. Nuclei: Hoechst 33258. Scale bar, 50 μm. Mean± s.e.m. (n=4). *p<0.05. **(D)** A modified Boyden chamber assay was used to determine the total number of migrated cells in five to seven randomly selected fields under the fluorescence microscopy. Data shown are the mean±s.e.m. from four independent experiments. *p<0.05.

Supplemental Figure 12. Comparable intracellular calcium influx response triggered by membrane depolarization (KCl) or vasoconstrictor (carbachol) between SVAS iPSC-SMCs and control iPSC-SMCs. **(A)** Representative graphs showing changes in intracellular Ca^{2+} in response to vasoconstrictor (100 μM carbachol, bottom panel) and membrane depolarization (50 mM KCl, top panel) in control-iPSC SMCs and SVAS-iPSC SMCs. Intracellular calcium concentration, $[Ca^{2+}]_i$, was measured using calcium indicator Fura-2 and present as a ratio of fluorescence intensity at 340 and 380 nm (Ratio 340/380). The time points for application of KCl and carbachol are shown by arrows and arrowheads, respectively. **(B)** Bar graphs show the net changes in $[Ca^{2+}]_i$ following treatment with carbachol and KCl. Changes of $[Ca^{2+}]_i$ = peak$[Ca^{2+}]_i$ – resting$[Ca^{2+}]_i$; Mean ± s.e.m. (n=5). Kruskal-Wallis test revealed that there is no significant differences of changes of $[Ca^{2+}]_i$ triggered by KCl (p=0.31) or carbachol (p=0.65) between control iPSC-SMCs and SVAS iPSC-SMCs.

Supplemental Figure 1



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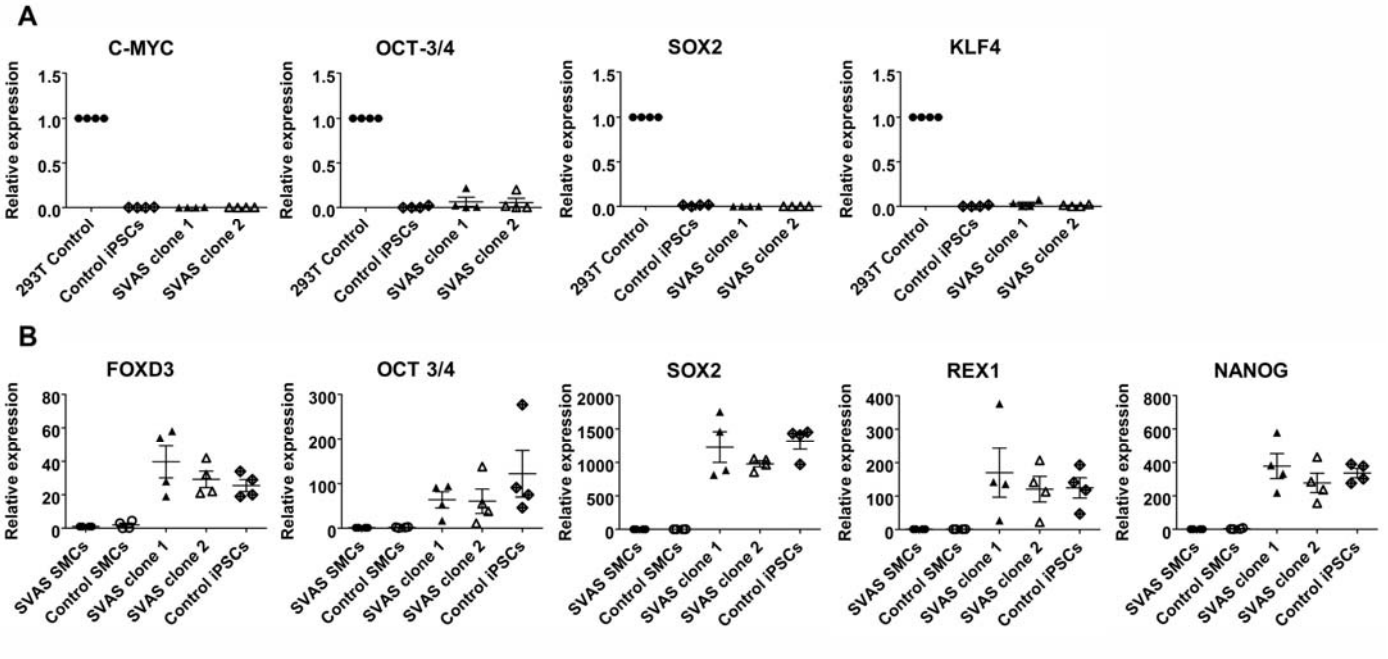

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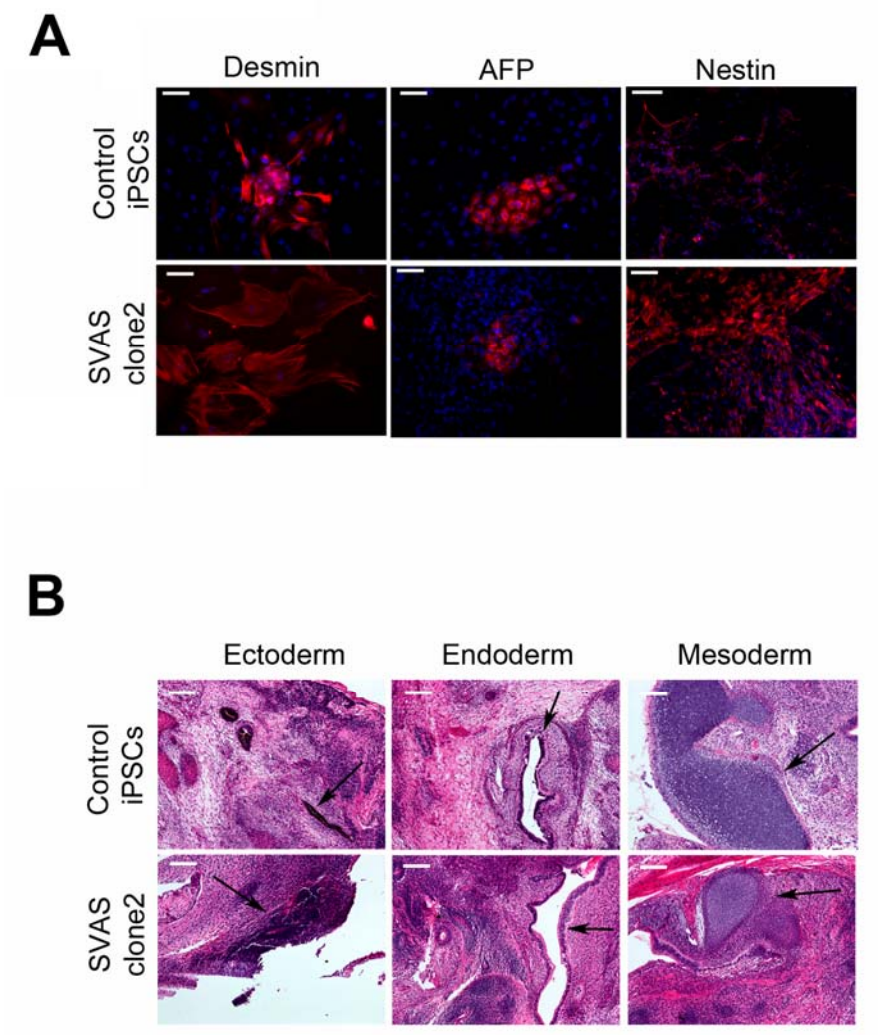

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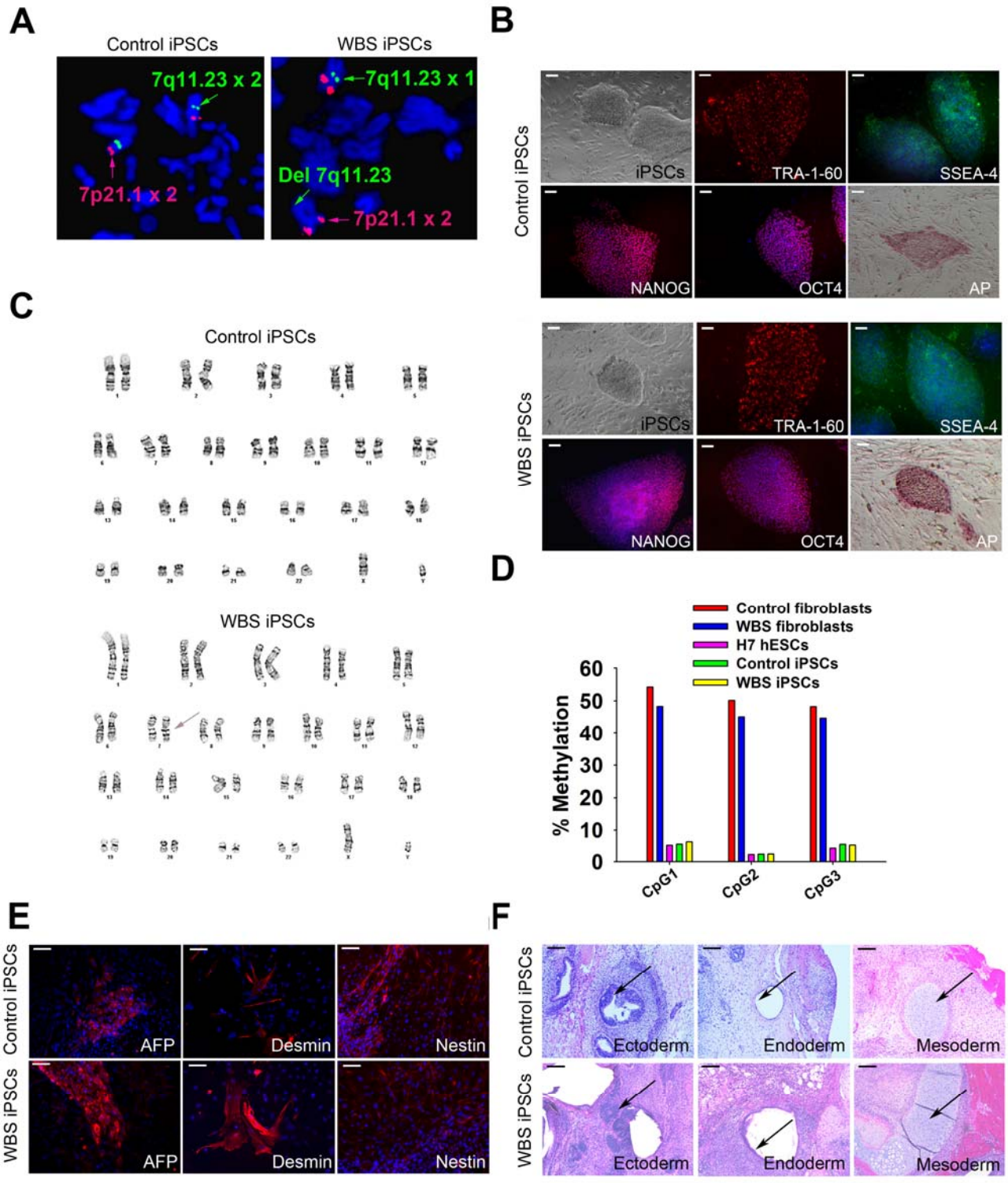

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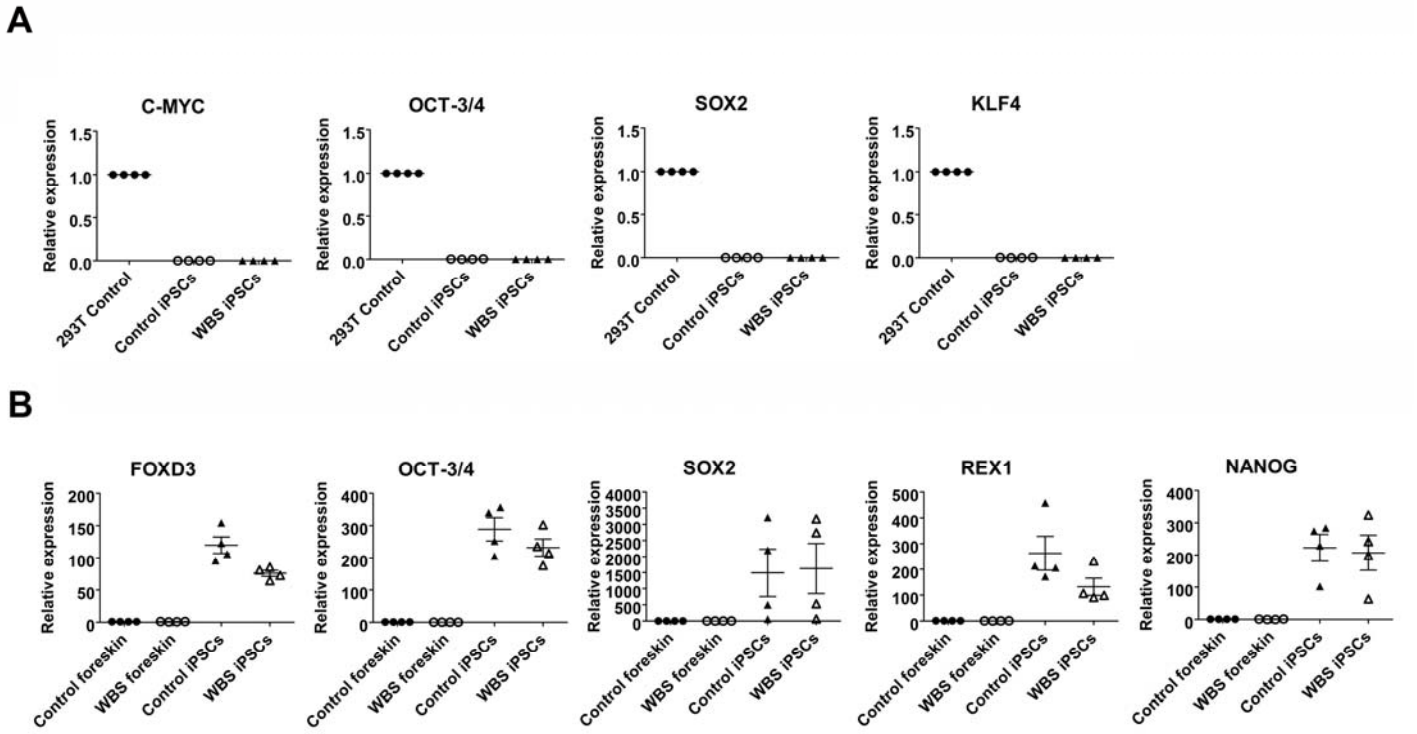

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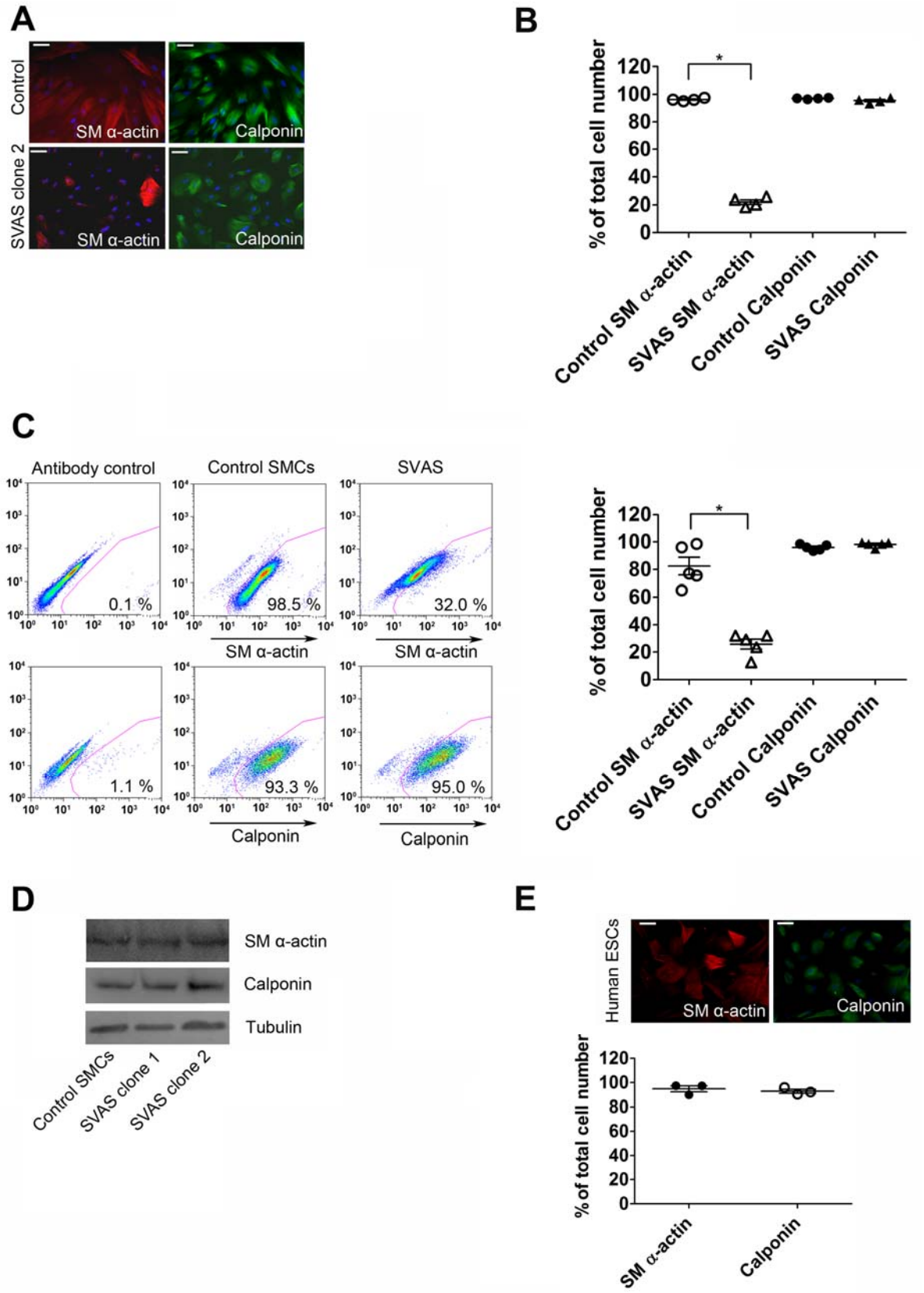

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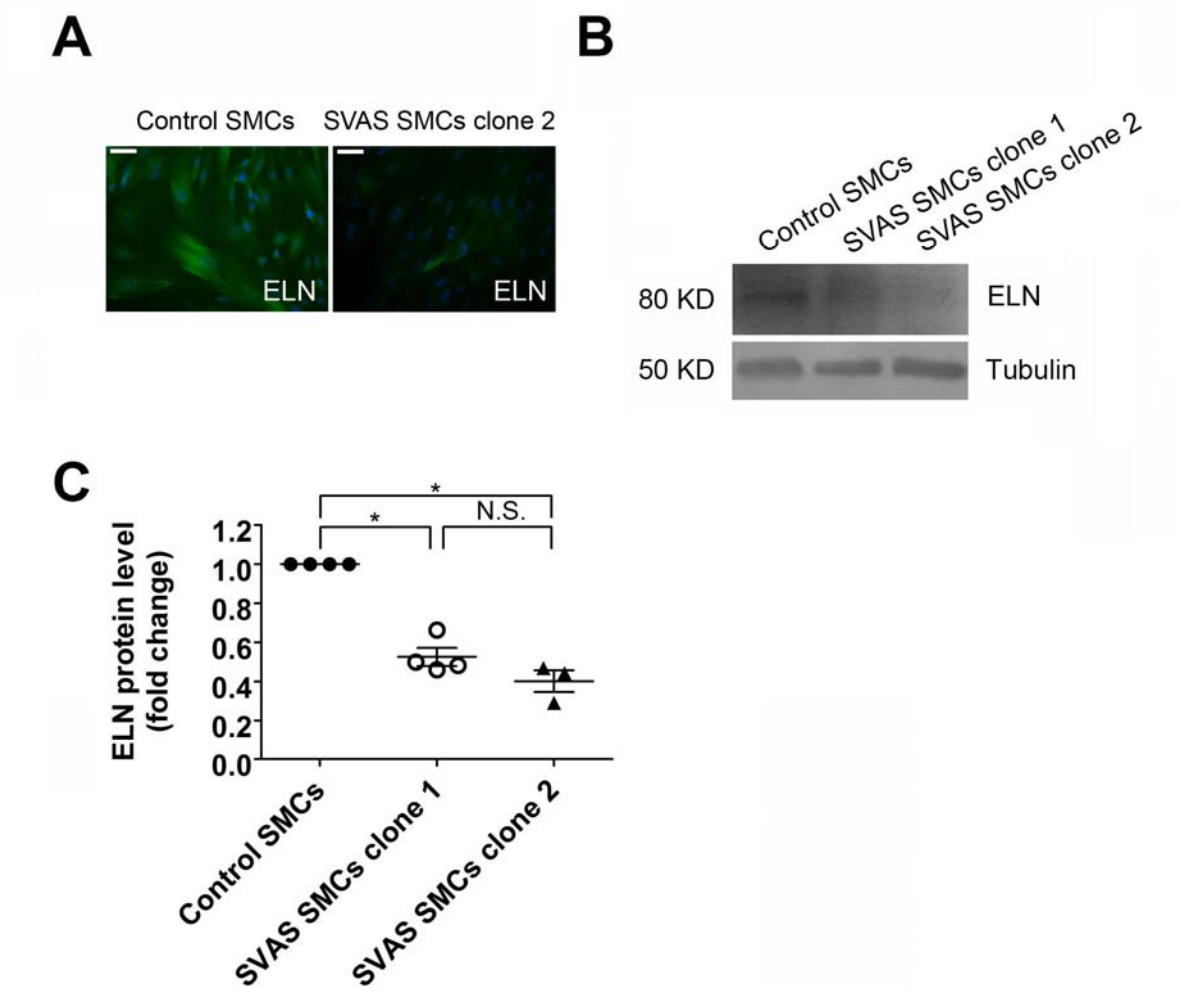

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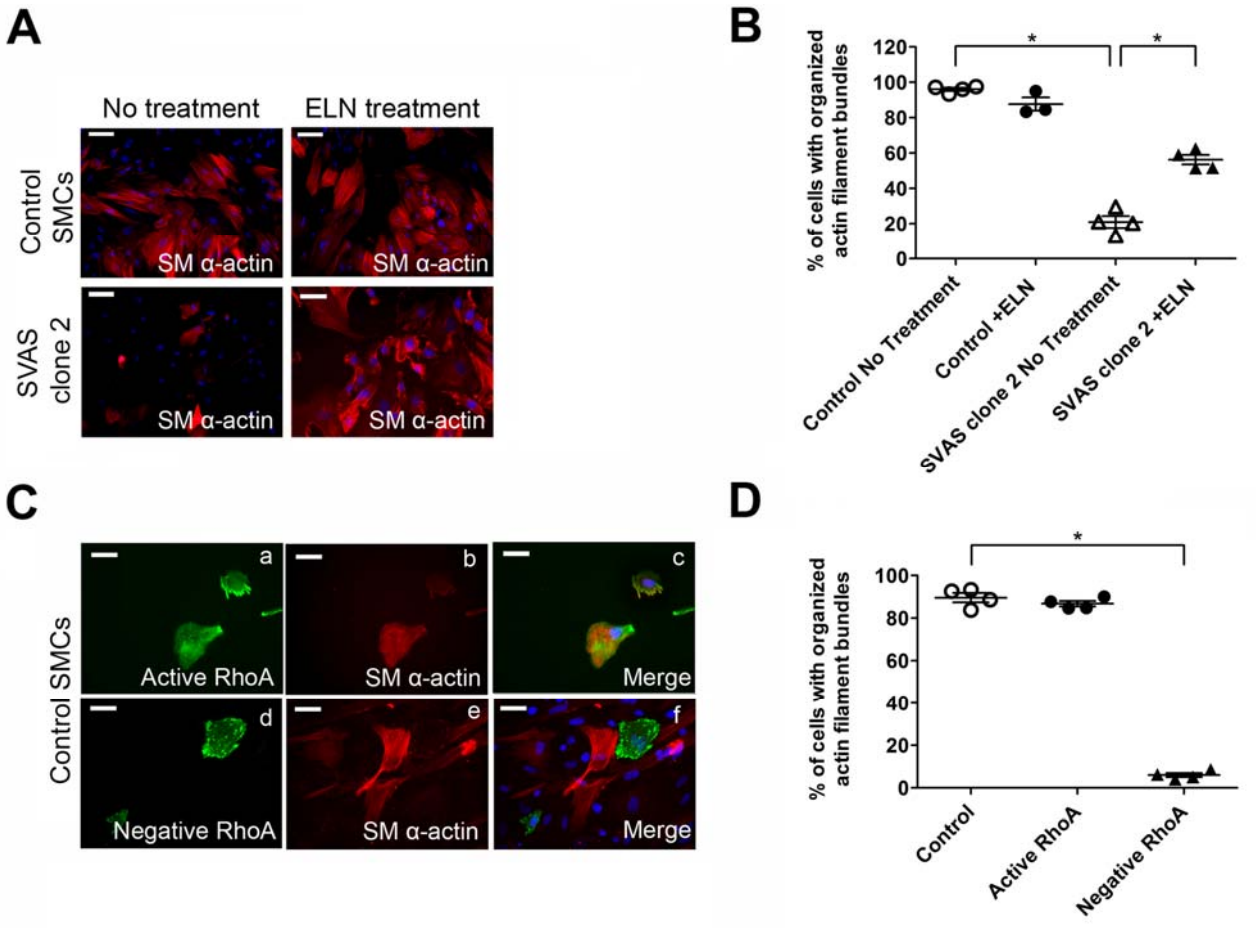

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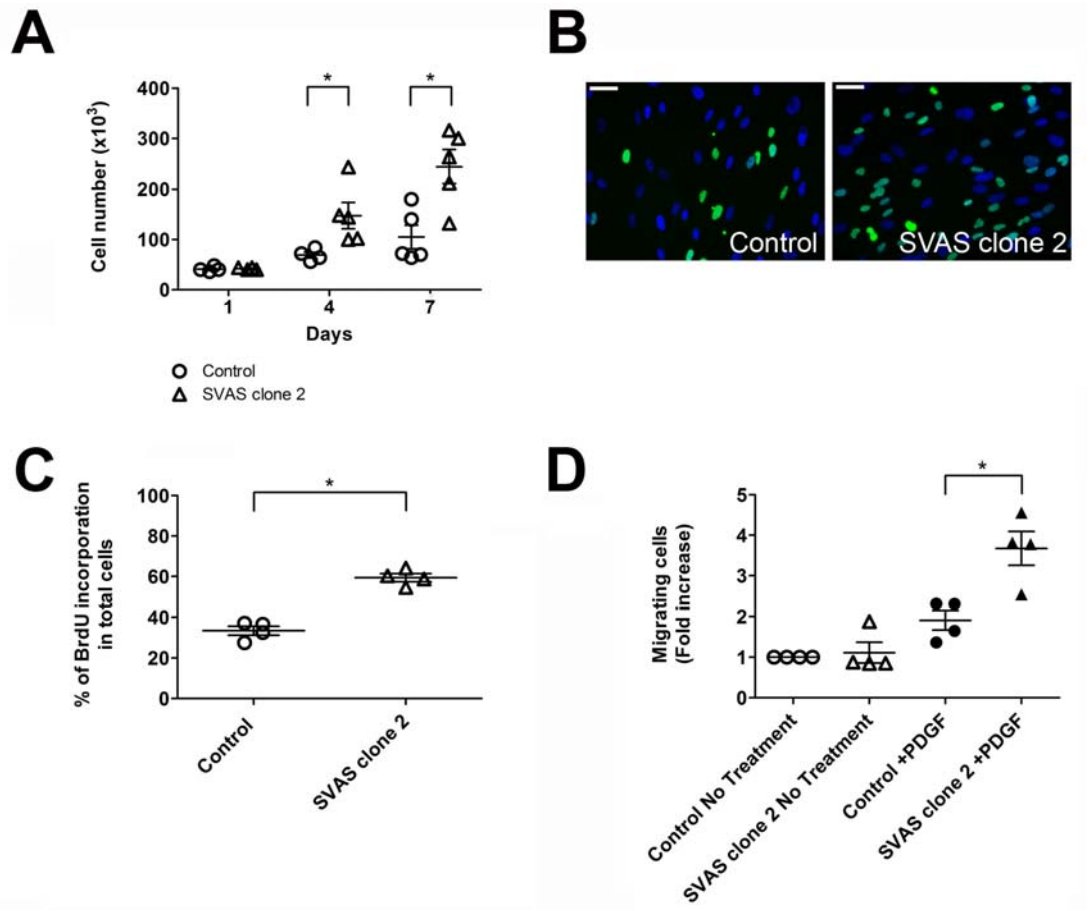

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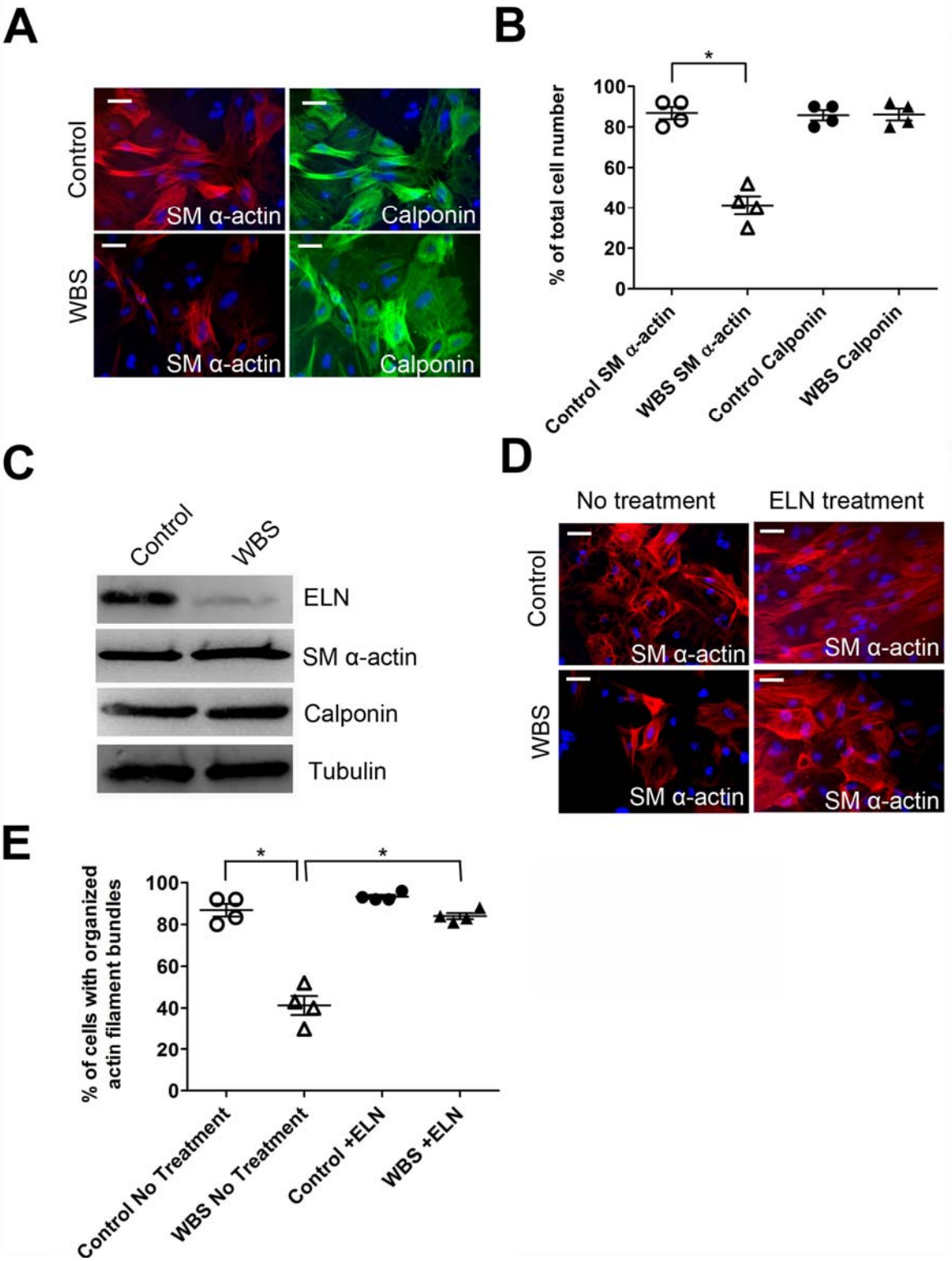

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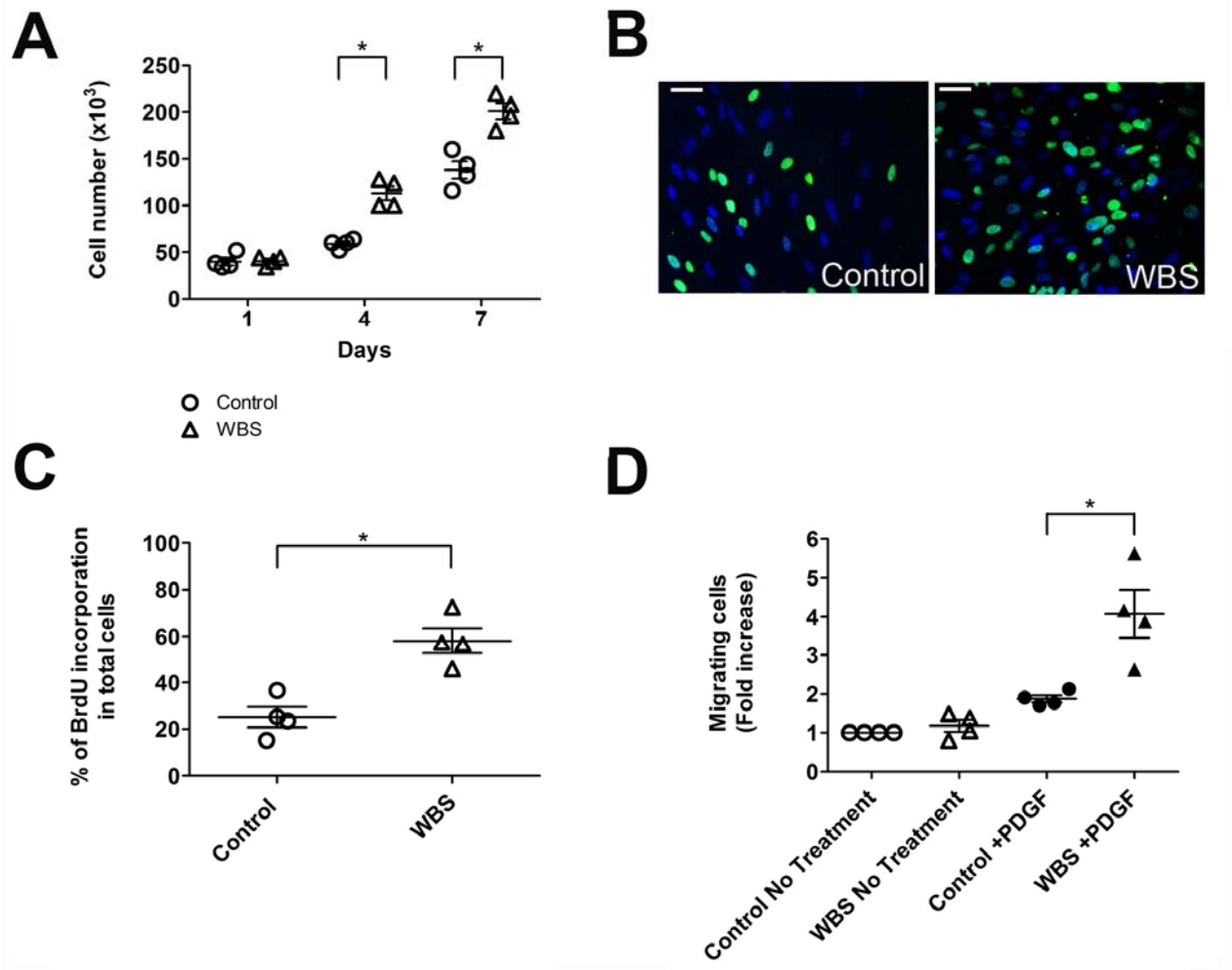
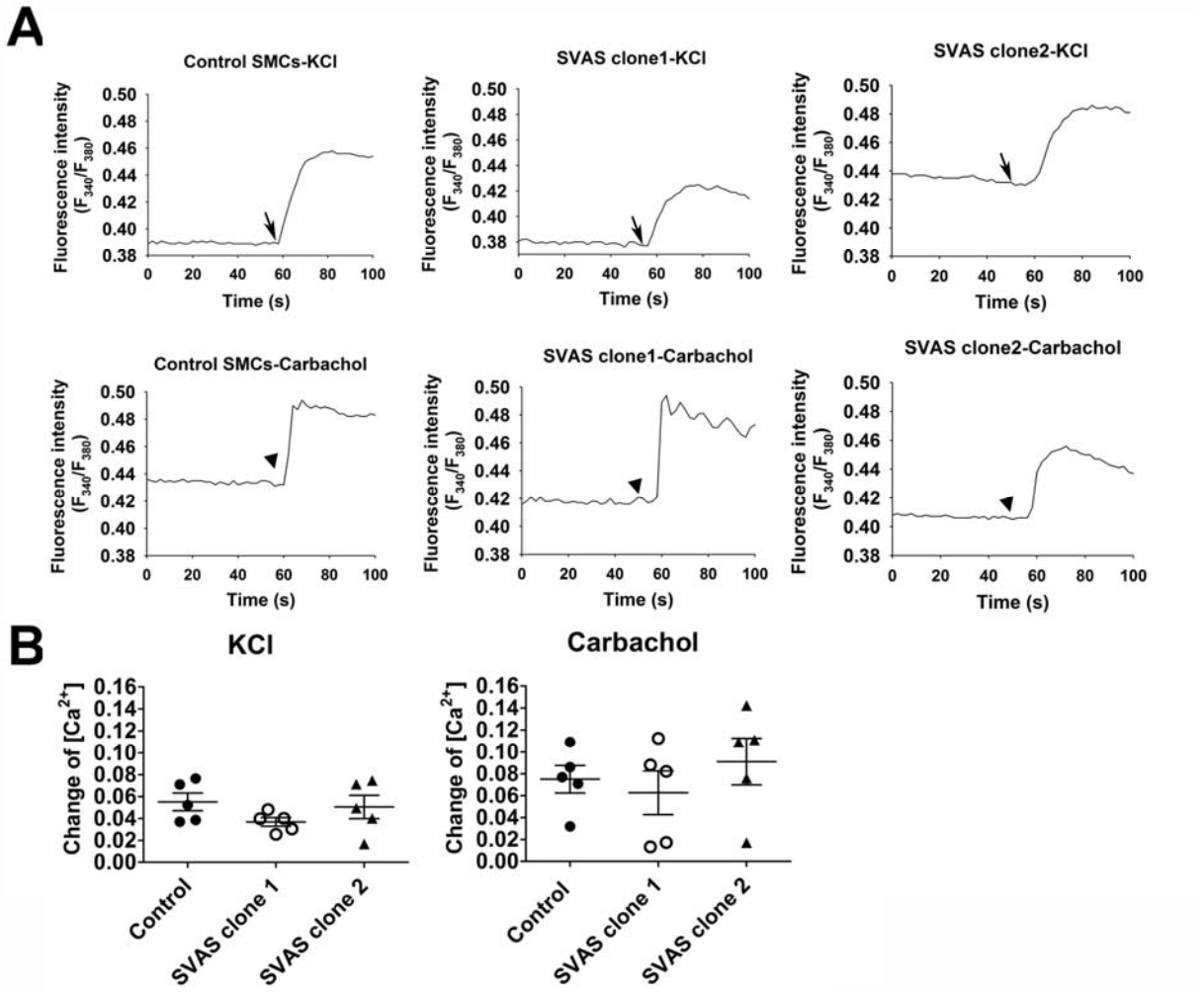


Supplemental Figure 12



Supplemental References

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