Online Supplement

Expanded Materials and Methods

Human mammary artery VSMC and rat VSMC culture and treatment

Waste fragments of internal mammary arteries were obtained from patients undergoing coronary artery bypass surgery at the Cardiology Institute of Hôpital Pitié-Salpêtrière, Paris, France, in accordance with French legislation (L.1211-3-9) and with the principles outlined in the Declaration of Helsinki. On the same day, the mammary artery segments, kept in physiological solution at 4°C, were dissected and VSMC were isolated from the media of mammary arteries from 55-60 years old patients, using a protocol previously described for rat aortic VSMC [1]. Cells were resuspended in human smooth muscle cell basal medium containing 20% of supplement mix (Basic Fibroblast Growth Factor, Epidermal Growth Factor, insulin and fetal calf serum) (Promocell GMBH, Heidelberg, Germany) and 1% GIBCO Antibiotic-antimycotic (Invitrogen, Cergy Pontoise, France). After 2 days the medium was replaced with basal medium containing 5% supplement mix. Cells at passages 2 to 4 were used for the experiments. Alternatively rat VSMC were prepared using aortas from 6 week-old rats. Adult male Wistar rats (Janvier, France) were treated in accordance with our institutional guidelines (Ministère de l'Agriculture, France; authorization 75-1090) and conformed to the Directive 2010/63/EU of the European Parliament. At the time of sacrifice, rats were administered with a sodium pentobarbital (Ceva, Santé Animale, France) intra-peritoneal overdose (200 mg/kg). When the animals were completely non-responsive to toe pinching, a thoracotomy was performed, the heart was removed and aortas were retrieved. Cells were used at passages 2-6 and cultured in DMEM (Life Technologies, Villebon sur Yvette, France) supplemented with 10% fetal bovine serum (Biowest, Nuaillé, France) and 1% penicillin/streptomycin (Life Technologies).

To keep VSMC in a quiescent state, cells were rinsed twice with PBS and maintained at least 2 days in serum-free (rat cells) or 0.1%-serum medium (human cells) and changed every day. Then half of the cells were cultured with serum (10% for rat cells-5% for human cells) for 24 to 72 hours. RNA and proteins were then prepared.

Global miRNA expression profile between quiescent and serum-induced proliferative VSMC

Quiescent human VSMC at passage 2 were stimulated or not with 5% serum for 24 hours. RNA from quiescent and proliferative cells was prepared. MiRNA microarrays (version 9.2 of Ambion® mirVana™ miRNA Bioarrays including 471 human probes and 238 rat probes) were used to compare miRNAexpression profile between proliferative and quiescent VSMC according to the manufacturer's protocol. The microarray analysis using GenePixPro 6.0 indicated that expression of 26 miRNAs common to rat and human was deregulated during proliferation. MiRNAs with fold change >1.3 were verified by qRT-PCR (**supplementary table 1**).

Proliferation of VSMC

Rat aortic VSMC were retro-transfected either with 30 nM Pre-miR™ miRNA Precursor for miR-322 (PM11080) (Pre-322) or Pre-miR™ Negative control (PreNeg) or 100 nM Ambion® miRNA inhibitor of miR-322 (MH11080) (anti-322) or Negative control (anti-Neg) (Life Technologies) with Lipofectamine 2000 transfection reagent (Life Technologies) in 96-well plates according to the manufacturer's recommendations . Twenty four hours later, cells were washed twice and then cultured for 2 days in serum free-DMEM to allow growth arrest. Then 10% FBS or 0%FBS-medium was added for 24 hours and Bromodeoxy-Uridine (BrdU) for the last 16h. The plates were then washed and a colorimetric BrdU cell proliferation assay was performed, as recommended by the manufacturer (Roche Diagnostics, Meylan, France).

Wound migration assay

Rat VSMC were retro-transfected or not with Pre-Neg or Pre-miR-322 in 24-well tissue culture plates and cultured for 2 days until confluence. Then cells were serum starved for 2 days. Confluent cell monolayer was wounded by scrapping with a 200 µl pipette tip and cell migration was stimulated with 10% serum medium while proliferation was blocked by incubation with 40 µmol/L mitomycin C (Sigma-Aldrich). The distance of wound closure was photographed and measured over a 24h period, using Metamorph software (Roper Scientific, Evry, France).

Apoptosis measurement

Rat VSMC were retro-transfected with or without Pre-Neg or Pre-miR-322 in 96-well plates. Twenty four hours later, medium was changed. The next day cells were washed twice and then cultured for 24hours in 10% FBS or 0%FBS-medium. Apoptosis was determined using NucView 488 Caspase-3 Assay kit for live cells (VWR Inernational, Fontenay-sous-bois, France) according to the manufacturer's instructions. After 30 minutes incubation with 5µM NucView 488 Caspase-3 substrate, cells were washed with PBS and green fluorescence was observed with a 538 nm band-pass emission filter under 485 nm laser illumination. Positive apoptotic nuclei were counted on different area and the density of cells was calculated for each area. For positive control, cells were treated 2 hours with 0.5µM staurosporine.

RNA isolation and quality measurement.

Total RNA including miRNA from cultured VSMC was isolated with the mirVana miRNA isolation kit (Life technologies) following the manufacturer's instructions. RNA concentrations were determined with a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Courtaboeuf, France). RNA quality was determined using the RNA nano LabChip® and a 2100 Bioanalyzer (Agilent Technologies, Massy, France). Only samples with a RNA integrity number higher than 7 (as

determined by the Bioanalyzer) were accepted for the following studies. Total RNA from rat carotid arteries were prepared using RNeasy Mini kit from Qiagen (Courtaboeuf, France) according to the manufacturer's instructions.

RT-PCR

Total RNA Reverse transcriptase-PCR analysis was performed using the Absolute QPCR SYBR green mix (ABgene, Courtaboeuf, France) on an MX3005P QPCR system (Stratagene, Agilent Technologies, Massy, France). Each primer pair (list available in a **supplementary table 2)** was designed with Primer Express software (Applied Biosystems) or Primer3 input (http://frodo.wi.mit.edu/primer3/) or previously published and were checked by using a standard curve in order to ensure that the reaction efficiency was between 90% and 110%. Melt curves were analysed to check the specificity of each RT-PCR amplification. Transcript levels were normalized to the RPL32 mRNA. miRNA specific RT-PCR were performed using specific Taqman miRNA assays (Life technologies) and Taqman Universal PCR Master Mix, no AmpErase UNG according to the manufacturer's instructions and normalized either to small RNA RNU6B (U6) probe for human samples or rat specific U87. The relative transcript level between two samples was calculated by using the $2^{-\Delta\Delta CT}$ method.

Protein preparation and western blot.

Protein extracts from rat VSMC were prepared using Promokine Mammalian Whole Cell Extraction kit (PromoCell GMBH, Heidelberg, Germany) and phosphatase inhibitors (Sigma-Aldrich, Saint-Quentin Fallavier, France).The total protein concentration was determined with the Bio-Rad assay (Bio-Rad, Marne-la-coquette, France). Fifty µg of total protein were separated by 10% SDS-PAGE followed by immunoblotting with a Trans-Blot Semi-Dry (Bio-Rad), following the manufacturer's protocol. The following antibodies were used: rabbit polyclonal antibody to GAPDH (ab9485, 1/2500, Abcam, Paris, France), mouse anti cyclinD1 (556470, 1/1000, BD Biosciences (Le Pont de Claix, France)), rabbit anti calumenin (1/500) was a generous gift of Dr Kim do [2], rabbit anti STIM1 c-terminal (S6197,1/1000, Sigma-Aldrich).Western blots were revealed with the ECL+ reagent (GE Healthcare, Diegem, Belgique) and scanned with an Ettan-DIGE Imager (GE Healthcare). Densitometric analysis was performed with NIH Image/ImageJ, and the expression level of the various proteins was normalized to GAPDH.

Luciferase reporter constructs and miRNA target validation by luciferase assay.

PsiCHECK-2 vector (Promega, Charbonnieres, France) containing both firefly and renilla luciferase genes was used to introduce 3'UTR sequence immediately downstream the stop codon of the Renilla luciferase gene. Various constructs of cyclinD1, calumenin and STIM1 3'UTR (see oligonucleotides list in **supplementary table 3**) surrounding the predicted miRNA binding sites were inserted into

psiCHECK-2 using XhoI and NotI restriction enzyme (New England Biolabs, Evry, France). miRNA target reporter assays were performed in quadruplicate in 96-well plates. 200 ng of each target construct and Pre-322 or PreNeg (30 nM final) were mixed with 2 µl Lipofectamine 2000 in 200 µl Opti-MEM according to the recommended protocol. Cell suspension in 10% FBS medium (200 000 cells- in 100µl) was added to 50 µl mix per well. After 24 hours, cells were washed twice in PBS (Life technologies) and fresh medium was added. After 48h of incubation, firefly and renilla luciferase activities were sequentially measured using the Dual-Glo Luciferase Assay system (Promega) as recommended.

Ca^{2+} transients measurements

Pre-322 or PreNeg (30 nM final) were transfected in rat VSMC by Amaxa nucleofector using D-033 program. 3 days post transfection cells were loaded with 6 µM FURA-2AM (Life Technologies) and SOCE was measured using standard "Ca^{2+} off/ Ca^{2+} on" protocol [3]. Briefly, cells were cultured on 30 mm glass coverslips. Three days after transfection coverslips with cells attached were mounted in a Teflon chamber. Cells were then incubated at 37°C for 60 min in culture medium containing 6 µM Fura-2/AM. Upon completion of incubation, cells were washed 3 times and bathed in HEPES-buffered saline solution (140 mM NaCl, 1.13 mM $MgCl_2$, 4.7 mM KCl, 2 mM $CaCl_2$, 10 mM D-glucose, and 10 mM HEPES; pH 7.4) for at least 7 min before Ca^{2+} measurements were done. For Ca^{2+} measurements, a digital fluorescence imaging system (InCyt Im2; Intracellular Imaging Inc., Cincinnati, OH) was used and fluorescence images of several cells were recorded and analyzed. Fura-2 dye was excited alternately at 340 and 380 nm and emission fluorescence was collected at the 510 nm. The 340/380 nm ratio images were obtained on pixel-by-pixel basis. Ca^{2+} traces shown are an average from several cells from one coverslip and are representative of several independent recordings as mentioned in the figure legend.

Generation of adenovirus vectors overexpressing miR-322

The recombinant adenovirus vector was created using the AdEasy Adenoviral vector system (Agilent technologies, Massy, France) according to the manufacturer's protocol. A miR-322 precursor DNA consisting of the mature miRNA with flanking sequences (400 bp upstream and 250 bp downstream miR-322) was PCR-amplified from rat genomic DNA using the following primers: SalI-705bp-miR-322 gene-forward:AATAgtcgacACCCGGGTCAATAAATGAAA, XhoI-705bp-miR-322gene-reverse: CTGGctcgagTGGGCTGAGTTCAGGATACC. The purified PCR product was subcloned into a pShuttle-CMV vector (Life technologies) using SalI and XhoI restriction sites. Overexpression of miR-322 by this vector was verified by transfection into Hek cells. This plasmid shuttle vector was then linearized by PmeI, treated with calf intestinal phosphatase (New England Biolabs) and gel-purified using the Qiaquick Gel extraction kit (Qiagen). The linearized pCMV-miR-322 shuttle vector was then electroporated in BJ5183-AD-1 electroporation competent cells (Agilent Technologies)

allowing recombination of an adenoviral plasmid including miR-322 gene. This pAd-miR-322 was amplified after transformation in XL10-Gold ultracompetent cells (Agilent technologies). Five µg of PacI-linearised pAd-miR-322 was then transfected in Hek 293A cells using Lipofectamine 2000. After few rounds of amplifications, virus titer was assayed by serial dilutions of viral stock. AdCMV-miR-322 efficiency was tested on VSMC and showed a strong overexpression of mature miR-322. An adenovirus control encoding beta-galactosidase under the CMV-promoter was similarly generated (AdCMV-BGal).

***In vivo* experiments**

Adult male Wistar rats (Janvier, France) weighing 350 to 400 g were anesthetized with sodium pentobarbital (50mg/kg, one intraperitoneal injection) and simultaneously received Meloxicam (1.5 mg/kg, one subcutaneous injection). Anesthesia was monitored by periodic observation of respiration and pain response. The left external carotid artery was injured using a 2F Fogarty embolectomy catheter (Baxter Healthcare, Maurepas, France) as reported [4]. Carotids were infused 30 min with physiological serum. After surgery, the animals received Furosemide (5mg/kg, IP). All surgical procedures have been approved (Ministère de l'Agriculture, France, authorization for surgery C-75-665-R). Animals were sacrificed 1, 2, 4, 7 and 30 days after surgery (as described in rat VSMC culture paragraph). The left and right carotid arteries were retrieved and flushed with physiological serum. Part of the carotids was frozen in liquid nitrogen for qRT-PCR analysis and part included in cryomatrix (Thermo Fisher Scientific, Illkirch, France) for immunohistochemical analysis.

For adenovirus infections, carotids were infused 30 min with a viral mix containing 1×10^{10} pfu of AdCMV-322 or AdCMV-Bgal diluted to a total volume of 100 µL in physiological serum. Adenovirus infected animals were sacrified at 14 days and carotid arteries were included in cryomatrix. Hematoxylin/eosin staining was performed on cross sections. The area of the media and intima layers were measured by using Lucia G computer software (Laboratory imaging sro, Prague, Czech Republic) on Leica microscope (Microsystems, Nanterre, France). Genomic DNA was extracted from carotid mounted slices and adenovirus infection of each animal was verified by PCR as previously described[5]. Oligonucleotides used to detect Ad-CMV-miR-322 were: Ad322-F: 5'-GGTCTATATAAGCAGAGCTG-3'and Ad322-R: 5'-GCACTCCAAGTTGGTGGACT-3'.

MiRNA in situ hybridization

Fluorescent in situ hybridizations (FISH) of miR-322 were performed on 5-µM cryomatrix embedded arterial sections according to Exiqon's protocol and recommandations for miRcury LNA™ microRNA ISH (Exiqon, Vedbaek , Denmark) and TSA™ Plus Fluorescence system (Perkin-Elmer, Waltham, USA). Briefly carotid sections were submerged in 10%, Neutral buffered formalin (Sigma-Aldrich) for 15 minutes and washed with PBS. Sections were then incubated with 15µg/ml proteinase

K for 10 min at 37°C for permeabilization and treated with 3% H2O2 3 minutes to block endogenous peroxidase activity. Sections were then dehydrated in a series of increasing ethanol solutions (70-90-100%). MiR-322 (39520-15, Exiqon) 5' and 3'-DIG labelled LNA mercury probes were used at 40nM and U6 5'-DIG labelled probes (positive control) at 1nM and hybridized for 1h 30°C below their RNA Tm. After post-hybridization washes in decreasing SSC baths (5X, 1X, 0.2X), an anti-digoxigenin-POD antibody (Roche Diagnostics) was added at 1/400 for 1h at room temperature. Signal was amplified with a TSA plus Cy3 substrate (Perkin-Elmer). Carotid sections were then mounted with SlowFade Gold antifade reagent with DAPI (Life technologies). Cy3-fluorescence (em: 550nm, exc: 570nm) was observed on an Olympus BX51 microscope using a 20X objective and an Hamamatsu camera orca03G and HCimage live software.

Confocal microscopy

Immunohistochemistry was performed on methanol-fixed and 0.1% Triton-PBS-permeabilized sections according to a standard protocol. The following antibodies were used: anti-cyclin D1 (Ab7958, 1/50, Abcam) and anti-STIM1 (S6197,1/1000, Sigma-Aldrich). Proteins were visualized by using secondary antibodies conjugated to Alexa 594 (Life technologies). Slides were examined with a Leica SP2 AOBS AOTF confocal scanning laser microscope using a 40X objective (NA 1.40, oil immersion). Green fluorescence was observed with a 505-550 nm band-pass emission filter under 488 nm laser illumination. Red fluorescence was observed under 594 nm laser illumination. Pinholes were set at 1.0 Airy units. Stacks of 6 images were collected every 0.4 μm along the z-axis. All settings were kept constant to allow comparison.

Statistical Analysis

Data are expressed as means ± SEM. Experiments with 2 groups were analysed with the non parametric Mann-Whitney test or two-sample t-test when indicated. Time course experiments were analysed with Kruskall Wallis corrected by Conover Imam test. When p values were below 0.05, differences were considered significant.

Supplemental figure 1: Proliferative VSMC express less differentiation markers. ACTA2, CNN1 and MYH11 mRNA were quantified by qRT-PCR in quiescent and serum-induced proliferative VSMC. ACTA2, CNN1 and MYH11 mRNA expression in human VSMC (n=5) (**A**) and rat VSMC (n=4) (**B**) (*, $p<0.05$; **, $p<0.01$). **C.** Comparison of hsa-miR-424 (from human) sequence with rno- miR-322 (from rat).

Supplemental figure 2: miR-503, but not miR-16, is upregulated during restenosis. Time course of miR-16 and miR-503 level after balloon injury of the rat left carotid artery compared to the level in right non-injured carotid artery. Rats were sacrificed at 4 and 14 days post-angioplasty (n=4; *, $p<0.05$).

Supplemental figure 3: cyclin D1 mRNA is rapidly and durably induced by serum in VSMC *in vitro* and *in vivo*. A. qRT-PCR was performed to detect cyclin D1 mRNA in quiescent or serum-induced proliferative VSMC for 24h to 72h. Cyclin D1 expression was normalized to RPL32 mRNA. Ratio of cyclin D1 in proliferative cells compared to quiescent cells is shown (n=5; *, $p<0.05$). **B.** Cyclin D1 mRNA was detected in left and right carotid artery of rats at 1-2-4-7-14 and 30 days post-angioplasty of left carotid arteries (n= 4 or 5 for each time point). Ratio of cyclin D1 expression between left injured carotids and right carotids are presented (*, $p<0.05$; **, $p<0.01$).

Supplemental figure 4: miR-322 inhibition increase proliferation of VSMC. VSMC were transfected or not with 100nM anti-Neg (control) or anti-322 for 72 hours. **A.** miR-322 expression is decreased by antimiR-322 transfection. MiR-322 expression was normalized to U87 and expressed as percentage of the value observed in anti-Neg transfected cells (n=5; **, $p<0.01$). **B.** Proliferation was assessed by measurement of BrDU incorporation in rat VSMC after 24 hours serum stimulation. Results are expressed as percentage of Anti-Neg + 10 % FBS (n=4; *, $p<0.05$).

Supplemental figure 5: miR-322 has no effect on apoptosis. VSMC transfected with Pre-Neg or Pre-322 for 72 hours and cultured in 0% or 10% serum for 24 hours were incubated 30 minutes with NucView 488 caspase-3 Substrate. Representative area of cells (scale bar: 50µm) under visible and 488/540 nm fluorescence are shown. Apoptotic nuclei showed a strong green fluorescence. Cells treated 2h with 0.5µM staurosporine were used as positive control.

Supplemental figure 6: **Alignment of miR-322 sequence and 3'UTR sequence of cyclin D1 (A), calumenin (calu) (B) and STIM1 (C)** as predicted by bioinformatic program TargetScan. miR-322 seed sequence and its complementary sequence in 3'UTR are presented in bold.

Supplemental figure 7: AdmiR-322 allows miR-322 overexpression in VSMC *in vitro* and in the carotid *in vivo* and decrease cyclin D1 mRNA in VSMC. A. In vitro overexpression of miR-322 in VSMC using adenovirus decrease cyclin D1 mRNA level. Cells were infected at an MOI of 200

with recombinant adenovirus encoding either the Beta-galactosidase gene (AdBgal= control) or miR-322 gene (AdmiR-322). Medium was changed 24 hours later. 72 hours after infection RNA were extracted. QRT-PCR results are shown: miR-322 level was normalized to U87 and cyclin D1 mRNA level was normalized to RPL32. Each values were compared to AdBgal transfected cells (n=4, *, $p<0.05$). **B. MiR-322 is still overexpressed in carotid infected with AdmiR-322 14 days post-infection**. RNAs were prepared from slices of left carotid 14 days post-injury and infection with either AdBgal or AdmiR-322. QRT-PCR results are shown: miR-322 level was normalized to U87 (n=4, *, $p<0.05$).

Supplemental figure 8: A. Cyclin D1 and STIM1 expression detected in rat carotid 14 days post-injury and infection. Immunolabelling of slices of injured carotid infected with AdBgal or AdmiR-322 with an anti-cyclin D1 antibody (red) and DAPI (blue) upper panel or an anti-STIM1 antibody (red) lower panel. Green autofluorescence of elastin indicates the media. Media (m), neointima (ni) and adventitia (ad) are indicated (scale bars: 50µM). **B. In situ hybridization of U6 in carotid 14 days post-injury and infection with AdmiR-322.** U6 was detected as a control for miRNA in situ hybridization using 1nM 5'-DIG labeled Probe and TSA plus Cy3 substrate. Representative image showing: U6 (red), green autofluorescence of the elastic lamina (green), and cell nuclei (DAPI, blue). Media (m), neointima (ni) and adventitia (ad) are indicated (scale bars: 50µM).

Supplemental table 1: Fold change of expression between proliferation and quiescence in human and rat VSMC determined by qRT-PCR.

8 miRNA with fold change >1.3 in microarray experiments were tested. n=5 for human VSMC and n=5 for rat VSMC; **, p<0.01, NS: non significant.

	human VSMC proliferation/quiescence			**rat VSMC proliferation/quiescence**		
microRNA	**fold change**	**SEM**		**fold change**	**SEM**	
miR-30c	0,89	0,06	NS			
miR-31	**1,7**	0,27	**	1,21	0,13	NS
miR-138	1,1	0,14	NS			
miR-143	**0,59**	0,09	**	**0,58**	0,07	**
miR-221	**1,75**	0,09	**	**1,62**	0,14	**
miR-320	1,12	0,09	NS			
miR-424	**0,53**	0,03	**	**0,65**	0,08	**
miR-494	1,19	0,05	NS			

Supplemental table 2: list of primers used for qRT-PCR

ACTA2 rat F : 5'-TGGTAGTGCCCCCCGAGA-3'

ACTA2 rat R : 5'- CCCTCTTCCAGCCATCTTTCAT-3'

CALU rat F: 5'-TGGTCACCGAATGAGAATTGG -3'

CALU rat R: 5'-GGGAATCCCCTTCGGTATCA -3'

CCND1 rat F : 5'-GAACTGCTTCTGCTGAACAAGC-3'

CCND1 rat R : 5'-CTTCGCGGATGCCACTACTTG-3'

CNN1 rat F : 5'-ATGTCTTCCGCACACTTTAAC-3'

CNN1 rat R : 5'-GCTCAAATCTCCGCTCTT-3'

MYH11 rat F : 5'-TGGAAAGCAAGCCTGCATT-3'

MYH11 rat R : 5'-CCAGGACTCCCGTACGAAAG-3'

RPL32 rat F: 5'-CCAGAGGCATCGACAACA-3'

RPL32 rat R: 5'-GCACTTCCAGCTCCTTGACAT-3'

STIM1 rat F : 5'-TGGAAGAAAGTGATGAGTTCCT -3'

STIM 1 rat R : 5'-TCATCCACGGTCCAGTTATAC -3'

ACTA2 human F : 5'-CAACCGGGAGAAAATGACTC-3'

ACTA2 human R : 5'-GCGTCCAGAGGCATAGAGAG-3'

CCND1 human F : 5'-CTTCCTCTCCAAAATGCCAG- 3'

CCND1 human R : 5'-AGAGATGGAAGGGGGAAAGA- 3'

CNN1 human F : 5'-GAGTGTGCAGACGGAACTTCAGCC-3'

CNN1 human R : 5'-GTCTGTGCCCAACTTGGGGTC-3'

MYH11 human F : 5'-CTCCGTGCTACACAACCTGA-3'

MYH11 human R : 5'-CGAGTAGATGGGCAGGTGTT-3'

RPL32 human F: 5'-CCCAAGATCGTCAAAAAGA-3'

RPL32 human R: 5'-TCAATGCCTCTGGGTTT-3'

Supplemental table 3: list of oligonucleotides used for luciferase assays

XhoI- 3'UTR cyclinD1 miR-322-site1-f:

tcgagGGCCGCGGCTCCTTTCACAGC**GCTGCTA**CCAATGACTCCCAGGATCCCgc

NotI-3'UTR cyclinD1 miR-322-site1-rev:

ggccgcGGGATCCTGGGAGTCATTGG**TAGCAGC**GCTGTGAAAGGAGCCGCGGCCc

XhoI- 3'UTR cyclinD1 miR-322-site2-f:

tcgagGTATCTTTCATGTTGTTTTC**GCTGCTA**TTGGAGGGTCAGTTTTATTTgc

NotI-3'UTR cyclinD1 miR-322-site2-rev:

ggccgcAAATAAAACTGACCCTCCAA**TAGCAGC**GAAAACAACATGAAAGATACc

XhoI- 3'UTR cyclinD1 miR-322-site1+2-f:

tcgagGGCTCCTTTCACAGC**GCTGCTA**CCAATGACTCCCAGGATCCCCGGCGTTCGGAACCAGATTCACGTTGCT
TTGTATCTTTCATGTTGTTTTC**GCTGCTA**TTGGAGGGTCAGTTTTgc

NotI-3'UTR cyclinD1 miR-322-sites1+2-rev:

ggccgcAAAACTGACCCTCCAA**TAGCAGC**GAAAACAACATGAAAGATACAAAGCAACGTGAATCTGGTTCCGAA
CGCCGGGGATCCTGGGAGTCATTGG**TAGCAGC**GCTGTGAAAGGAGCCc

XhoI -3'UTR-Calu site miR-322-f:

tcgagGGCTGGTTTCTAAAACCTCA**TGCTGCT**GATTTGACCAGGGATCCTCAgc

NotI- 3'UTR- Calu site miR-322-rev:

ggccgcTGAGGATCCCTGGTCAAATC**AGCAGCA**TGAGGTTTTAGAAACCAGCCc

XhoI -3'UTR-Calu site miR-322 mut-f:

tcgagGGCTGGTTTCTAAAACCTCA**TCGACGA**GATTTGACCAGGGATCCTCAgc

NotI- 3'UTR- Calu site miR-322 mut-rev:

ggccgcTGAGGATCCCTGGTCAAATC**TCGTCGA**TGAGGTTTTAGAAACCAGCCc

XhoI -630bp-3'UTR-Calu-f:

AAGTctcgagAGGGCATGCATCTCATTCTTAGTG

NotI- 630bp-3'UTR-Calu -rev:

TAATgcggccgcGGCCCATCAGTAAGAGACCAAAGA

Internal oligonucleotides to mutate Calu miR-322 binding site :

f: GGTTTCTAAAACCTCA**TCGACGA**GATTTGACCAGGGATCC

rev : GGATCCCTGGTCAAAT C**TCGTCGA** TGAGGTTTTAGAAACC

XhoI-1300bp-3'UTR STIM1-f:

TGGGctcgagGCCTTCACTTCCCTTCCTTT

NotI-1300bp-3'UTRSTIM1-rev :

AAATgcggccgcAAAACCCATGTGAGGGTGTC

Supplemental References

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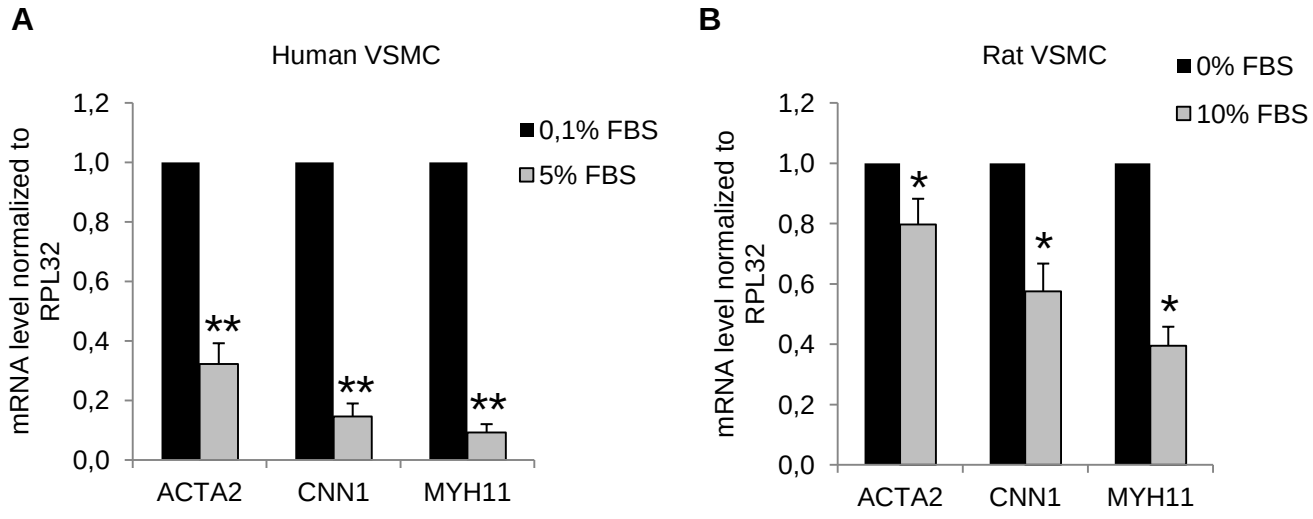
5. Merlet E, Lipskaia L, Marchand A, Hadri L, Mougenot N, Atassi F, Liang L, Hatem SN, Hajjar RJ, Lompre AM. A calcium-sensitive promoter construct for gene therapy. *Gene Ther.*2012.

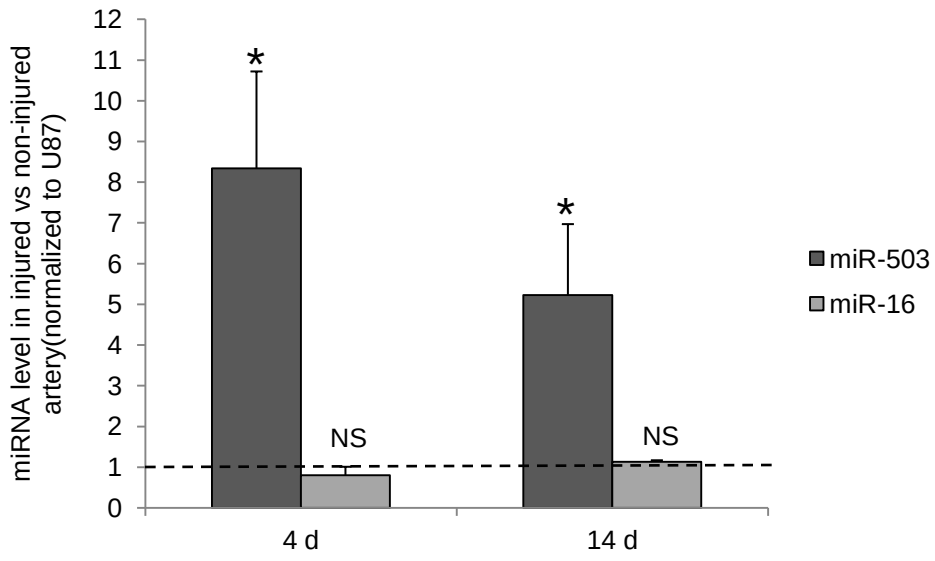
Supplemental figure 1



C

hsa-miR-424: CAGCAGCAAUUCAUGUUUUG**A**A
rno-miR-322: CAGCAGCAAUUCAUGUUUUG**G**A

Supplemental figure 2



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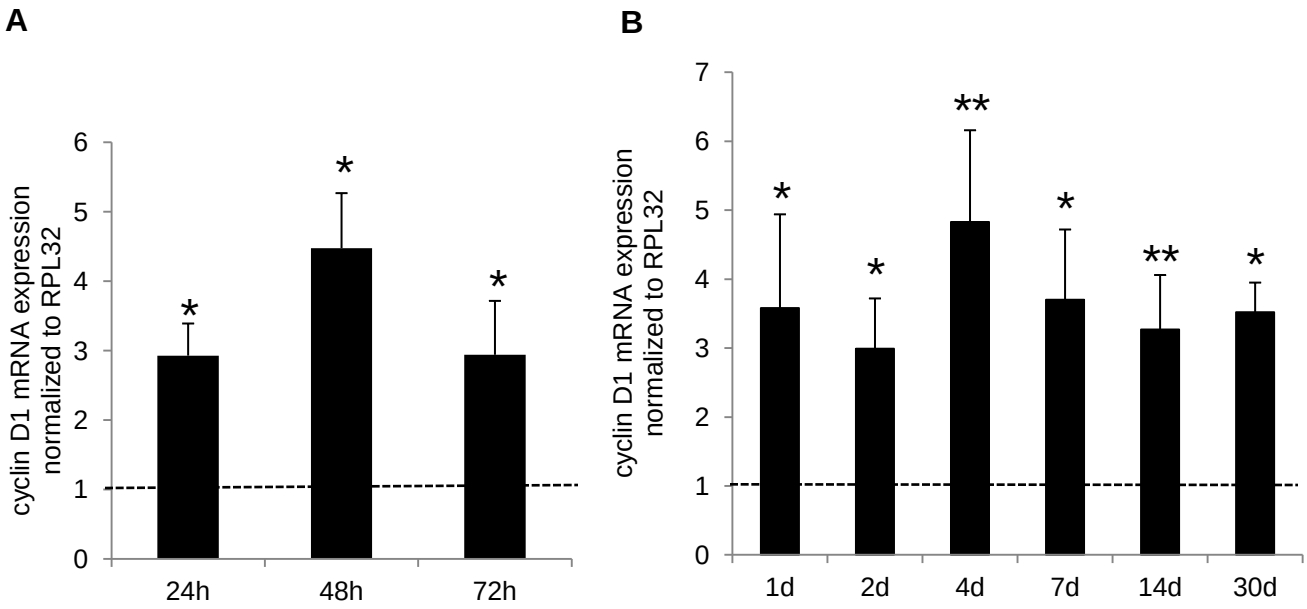
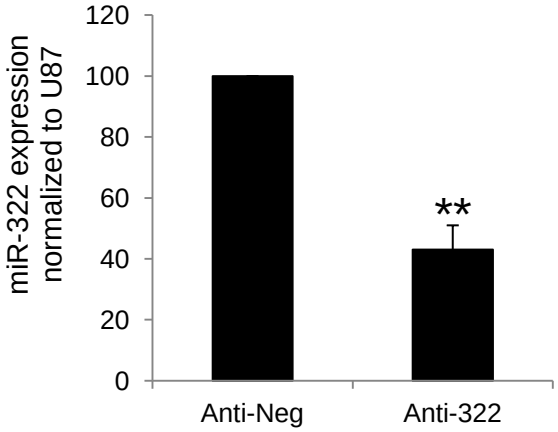


Supplemental figure 4

A



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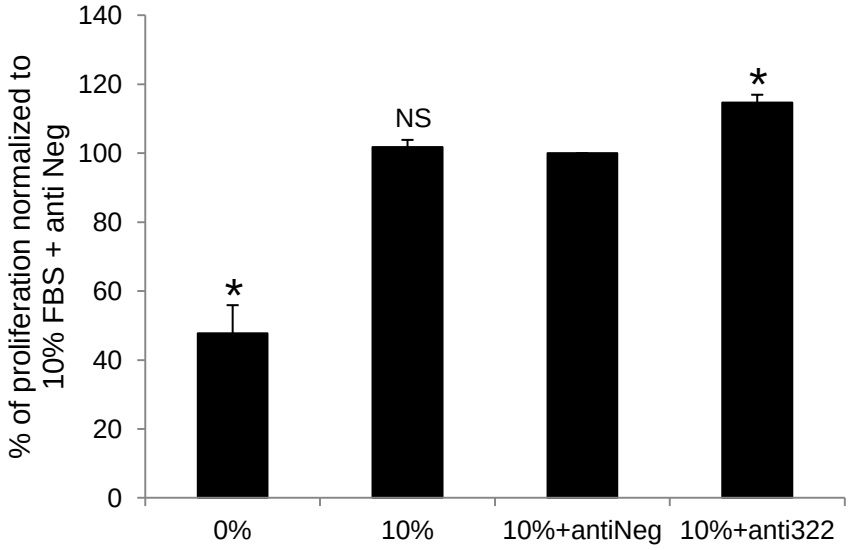
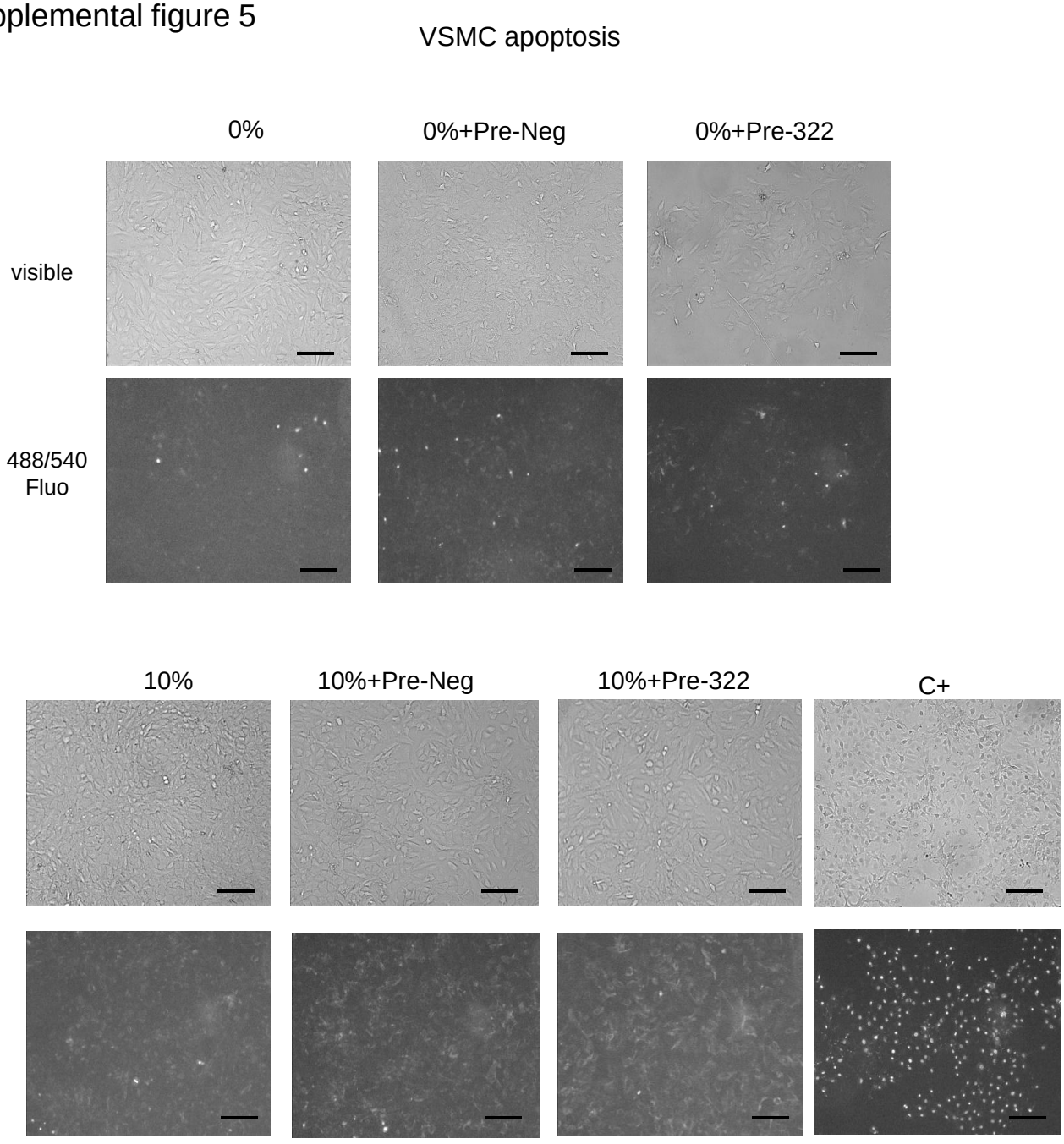


Supplemental figure 5



Supplemental figure 6

A

site 1
1787-1793

Rat **cyclin D1** 3'UTR : …GGCTCCTTTCACAGC**GCTGCT**ACCAAT

rno-miR-322 3'-5': AGGUUUUGUACUUAA**CGACGA**C

site 2
1864-1870

Rat **cyclin D1** 3'UTR : …TGTATCTTTCATGTTGTTTTC**GCTGCT**ATTGG...

rno-miR-322 3'-5': AGGUUUUGUACUUAA**CGACGA**C

B

1138-1144

Rat **calu** 3'UTR : …GGCTGGTTTCTAAAACCTCA**TGCTGCT**GATTTGACCAGGGATCCTC

rno-miR-322 3'-5': AGGUUUUGUACUUA**ACGACGA**C

C

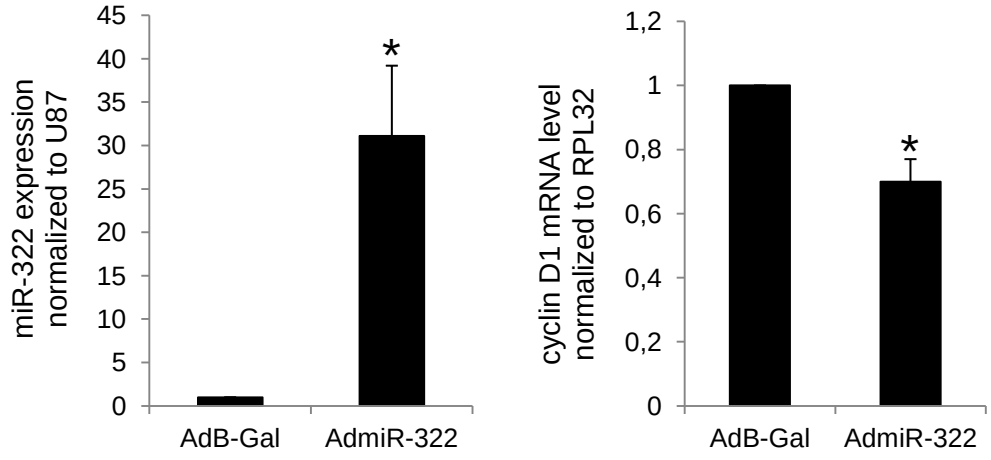
226-232

Rat **STIM1** 3'UTR :…AAATCCTTCCAACCACGGGC**TGCTGCT**GTCACATCCTCTCCACTTCA

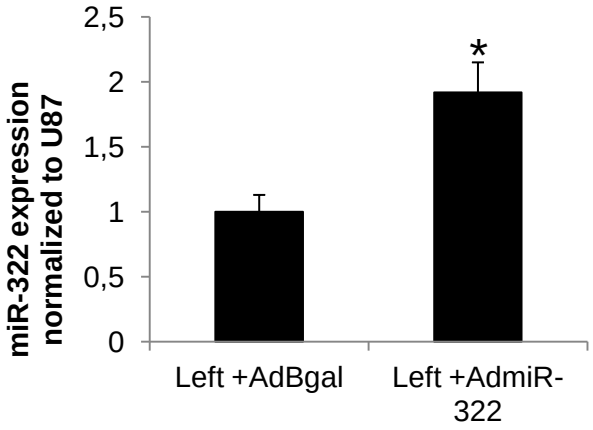
rno-miR-322 3'-5': AGGUUUUGUACUUA**ACGACGA**C

Supplemental figure 7

A

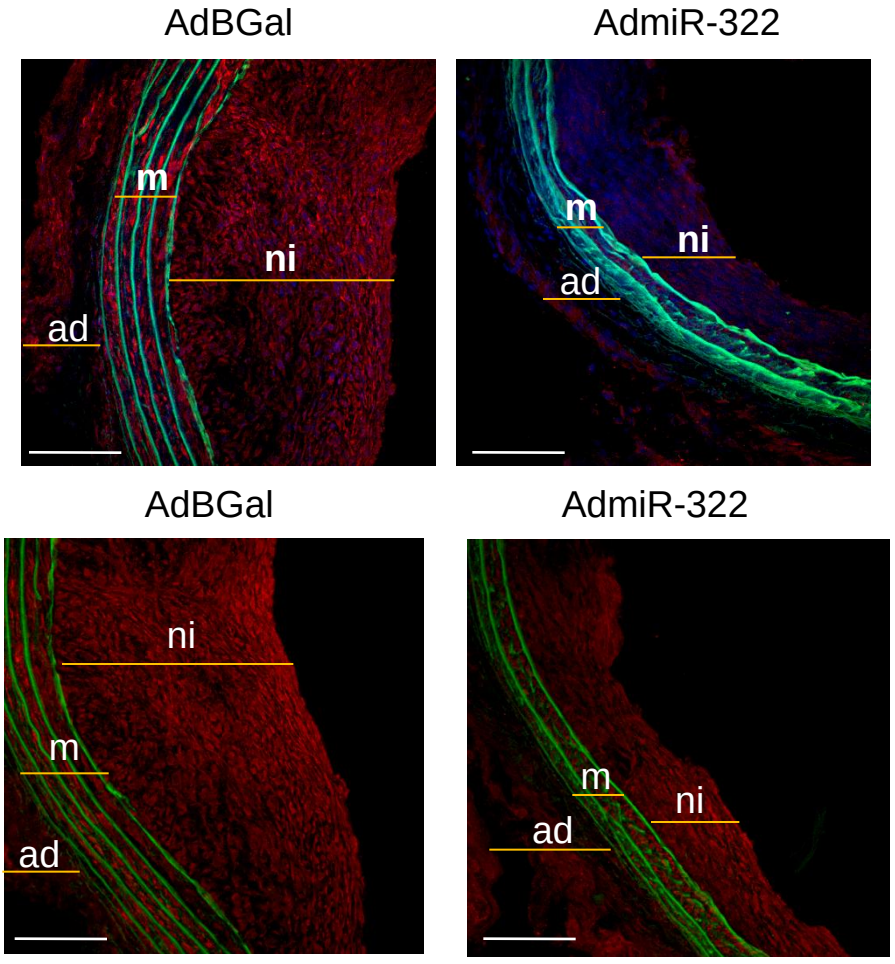


B



Supplemental figure 8

A



B