SUPPLEMENTAL MATERIAL

Regulation of Blood Pressure by Targeting $Ca_V1.2$-Galectin-1 Protein Interaction

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Supplemental Methods

DNA constructs

$Ca_V1.2$-77wt, β_{2a} subunit in pIRES2-EGFP vector, $\alpha_2\delta$ subunit, and Galectin-1 in pIRES2-DsRed vector have been described in our previous study[1]. pIR-β_3-GFP was kindly provided by Dr David Yue from Johns Hopkins University, USA. β_3 subunit was then subcloned into pIRES2-EGFP vector with digestion sites of EcoRI and BamHI. Galectin-1 was also subcloned into pcDNA3.1(-) vector following digestion by XhoI and BamHI. Galectin-1 with C-terminal fused DsRed was generated by subcloning into pDsRed-N1 vector with restriction enzyme sites of XhoI and BamHI. GFP-$Ca_V1.2$ and pGW-β_{2a} subunit without GFP tag were kindly provided by Dr Terry Snutch from the University of British Columbia, Canada. Rat HA-$Ca_V1.2$ channel was from Dr Emmanuel Bourinet from Institut de Génomique Fonctionnelle, France. For GST-tagged exon 9 construct, exon 9 was amplified by PCR and then ligated into pGEM-T Easy vector. The fragment was digested using EcoRI and SalI and then sub-cloned into the pGEX4T-1 vector to generate the various GST fusion constructs. Reverse primers containing mutation sites were used to generate the GST-exon 9 mutants. For HA-tagged constructs, HA tag was attached to the C-terminus of the fragments using the reverse primers containing HA sequences. The HA-tagged fragments were cloned into pIRES2-EGFP vector with digestion sites of EcoRI and BamHI. Additionally, the mutations within $Ca_V1.2$ HA-I-II loop were generated by site-directed mutagenesis using HA-I-II loop in the pGEM-T easy vector as the template and then subcloned into pIRES2-EGFP vector. For generating mutations into the full length $Ca_V1.2$ channel, exon 1-12 subcloned in pGEM-T Easy vector was used as the template for site-directed mutagenesis experiments or as template for overlapping PCR experiments to introduce mutations into the ER export signal. The required DNA fragments were then ligated into pcDNA3-$Ca_V1.2$ after restriction enzyme digestion with HindIII and SgrAI. Chimeric $Ca_V3.1$ channels were kindly provided by Dr Henry Colecraft from Columbia University, USA. All HA-Ubiquitin mutants were gifts from Dr Kah Leong Lim from National Neuroscience Institute, Singapore. ERoxBFP was a gift from Dr. Erik Snapp from Albert Einstein College of Medicine, USA (Addgene plasmid #68126)[2]. Details of cDNA constructs and primers for PCR used in this study are listed in Table S1-S3.

Cell culture and transfection

HEK 293 or A7r5 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco) containing 10 % fetal bovine serum (Gibco) and 1 % penicillin–streptomycin and maintained at 37 °C in a humidified atmosphere containing 95 % air and 5 % CO_2. Cells were transiently transfected using the calcium phosphate method.

Vascular Smooth Muscle Cells (VSMCs) were isolated from 200 to 250 g male Wistar rats and maintained in 10 % calf serum and DMEM. Passage 3 to 6 VSMCs at 70 % to 80 % confluence in 60-mm or 35-mm dishes were used to study the subcellular localizations of $Ca_V1.2$ and Gal-1, and the effect of Tat-e9c peptide in $Ca_V1.2$-Gal-1 interactions.

Human artery collection

Human internal mammary arteries were collected from patients who had coronary artery bypass surgeries and pulmonary arteries were collected from patients who had heart transplant surgeries at the National Heart Center, Singapore. This study was approved by the SingHealth Centralised Institutional Review Board (Reference No.:2004/033/C). The arteries from both hypertensive and non-hypertensive patients were used for detection of total Gal-1 and $Ca_V1.2$ channel.

Purification of Galectin-1 protein

The supernatant containing Gal-1 was applied to Glutathione Sepharose 4B column (GE) at flow-rate of 1 ml/min. Followed by washing with at least 10 column volume of STE buffer (mM: 500 NaCl, 10 Tris, 1 EDTA, 2 DTT and 10% glycerol, pH 8.0), the bound proteins

were eluted by freshly prepared 20 mM reduced glutathione. The fractions containing Gal-1 were confirmed using SDS-PAGE gel and then pooled together. ProScission protease was applied to remove GST at 4 °C overnight under gentle rotation. To isolate GST from Gal-1, the mixture containing both GST tag and Gal-1 were dialyzed in 100 mM NaCl and 25 mM Tris (pH 7.5), and then loaded onto Hiload 26/60 column (GE) with gel filtration buffer (mM: 500 NaCl, 25 Tris, 0.5 EDTA, 4 DTT, pH 7.5) at flow-rate of 2.5 ml/min. After 1.5 column volume elution, fractions containing Gal-1 will be collected following identification by SDS-PAGE gel.

GST pull-down assay

As previously described[1], Glutathione sepharose 4B GST beads (GE Healthcare) were used to purify GST-fusion proteins of different mutants of exon 9. The GST beads were then incubated with A7r5 cell lysates overnight at 4 °C with gentle rotation. After washing the beads 3 times by cold PBS, GST-tagged proteins were eluted by boiling at 95 °C in 2XSDS sample loading buffer for 10 minutes. Western blot was performed to detect the binding of different exon 9 mutants to Gal-1 using anti-Gal-1 (1:1000) or anti-GST (1:5000).

Co-immunoprecipitation

Co-immunoprecipitation was performed as described previously with brief modification. Proteins harvested from transfected HEK 293 cells, A7r5 cells or lysed human vessels were incubated with different primary antibodies overnight at 4 °C with gentle rotation, followed by the incubation with 20 µl of protein A/G agarose (Pierce) for another 1 h at 4 °C. The beads were washed 3 times using cold PBS and then denatured in 2XSDS sample loading buffer. Proteins were used for western blot analysis.

Surface protein biotinylation

For surface biotinylation of $Ca_V1.2$ channels in transfected HEK 293 cells or A7r5 cells, $Ca_V1.2$ channels were biotinylated using an EZ-Link Sulfo-NHS-Biotinylation Kit (Thermo Fisher Scientific). Briefly, cells were incubated with 0.25 mg/ml Biotin for 1 h at 4 ^{o}C. Unbound biotin was removed by incubation with quenching buffer for 20 min and then washing by PBS buffer. Following measurement of protein concentration, NeutrAvidin (Pierce) was used to pull down the biotinylated surface proteins by incubating with cell lysates overnight. Avidin-bound proteins were eluted by boiling in 2X sample loading buffer and then loaded on SDS-PAGE gel.

For surface biotinylation of $Ca_V1.2$ channels obtained from freshly isolated arteries, arteries with connective tissues removed were incubated with a mixture of 1 mg/mL EZ-Link Sulfo-NHS-LC-LC-Biotin (21338) and Maleimide-PEG2-Biotin (21901BID, Thermo Fisher Scientific) reagents for 1 h at room temperature, and then washed using 100 mM glycine in PBS for 20 min to remove the unbound biotin. After removing the glycine solution using ice-cold PBS buffer, the arteries were pulverized and dissolved in lysis buffer for Western blot assays.

Ubiquitination assay

HEK 293 cells were transiently transfected with plasmids coding for the studied substrates alone or with WT or mutant HA-tagged ubiquitin. About 24 hr post-transfection, MG132 (3 µM) was added overnight, and the cells were then harvested using lysis buffer (1% SDS, 1 mM EDTA in PBS). Cell lysates were boiled for 5 mins at 95 °C, votexed for 10 sec, and then boiled for another 3 mins at 95 °C. Ubiquitinated substrates in the supernatant were immunoprecipitated with anti-$Ca_V1.2$ or anti-HA, washed 3 times with cold PBS buffer, and resolved by SDS-PAGE.

Western blot

Cells were harvested using lysis buffer (mM: Tris 50, NaCl 150, EDTA 1, Triton X-100, 1 %, pH 7.4) including protease inhibitor cocktails (Roche) at 48 h after transfection. After

determining protein concentration, proteins were loaded onto 8 % or 12 % SDS-PAGE gel and then electrophoresed for 45 min at 150 V. The proteins were electro-transferred to PVDF membrane overnight at 4 °C at a constant voltage of 30 V in a transfer buffer with 10 % methanol. After blocking for 1 h at room temperature with 5 % non-fat milk in TBS-T buffer, the membranes were then incubated overnight at 4 °C with the primary antibodies: rabbit anti-$Ca_V1.2$ (1:1000), anti-$Ca_V3.1$ (1:1000), rabbit anti-β_2 (1:1000), mouse anti-Gal-1 (1:2000), mouse anti-ubiquitin (1:1000), rabbit anti-HA (1:1000) or mouse anti-β-actin (1:5000). The membranes were washed three times with TBS-T buffer and then incubated with corresponding HRP-conjugated secondary antibodies (1:5000) for 1 h at room temperature. Signals were detected using West Pico or Femto Chemiluminescent Substrate (Pierce). The blots were scanned and then quantified using ImageJ software (NIH). Information of antibodies used is listed on Table S4.

Galectin-1 siRNA

To silence Gal-1 in A7r5 cells, we used the ON-TARGETplus SMART pool rat Gal-1 siRNAs synthesized by Dharmacon (L-090699-02). Transfection of A7r5 cells with the siRNA oligonucleotides was performed using DharmaFECT 2 transfection reagent according to the manufacturer's instructions. Briefly, A7r5 cells were grown to 80 % confluence in DMEM not supplemented with antibiotics. Gal-1 siRNAs (100 nmol/L) were added to the medium. The efficiency of gene suppression was monitored at 48 hr after transfection by detecting Gal-1 protein level, followed by 8-hour exposure to 1% O_2. ON-TARGETplus Non-targeting Control siRNAs (D-001810-02) was used as a negative control of Gal-1 siRNAs.

Quantitative RT-PCR

Total RNA of hypertensive human arteries was extracted with TRIzol and cDNA was obtained with SuperScript™ III Reverse Transcriptase (ThermoFiainedsher). A 20 μl fluorescence qPCR amplification reaction system was performed with SYBR Green Master Mix (ThermoFisher). Primer sequences were as follows: *CACNA1C* forward, 5'-GAAGCGGCAGCAATATGGGA-3'; reverse, 5'-TTGGTGGCGTTGGAATCATCT-3'; *CACNB2* forward, 5'-GGTTCGGCAGACTCCTACAC-3'; reverse, 5'-GCAAATGCAACGGGCTTTGT-3'; *CACNB3* forward, 5'-TCCGCCATCTCTAGCCAAG-3', reverse, 5'-TGCCCGGATTGTTGAGCAC-3'; *LGALS1 (Gal-1)* forward, 5'-AACCCTCGCTTCCATACCAC-3', reverse, 5'- TCCTCATTCCCGAAGAGAAAGAG-3'; and *CAPDH* forward, 5'-GAGTCCACTGGCGTCTTCA-3', reverse, 5'-TCTTGAGGCTGTTGTCATACTTC-3'. Each sample was loaded in three replicates and *GAPDH* was used as the internal control. The qPCR amplification conditions followed standard protocol with denaturation step at 95 ^oC and annealing at 60 ^oC in a total of 40 cycles. Fluorescence data were calculated to determine the relative mRNA expression according to $2^{-\Delta\Delta C}{}_T$ Method.

Electrophysiological recordings

As previously described[1], patch-clamp recordings were performed at 24-72 h after transfection using an Axopatch 200B amplifier (Molecular Device). The external solution contained (in mM): 144 TEA-$MeSO_3$, 10 HEPES, 1.8 $CaCl_2$ or 5 $BaCl_2$, pH 7.4 adjusted with CsOH and osmolarity 300-310 mOsm with glucose). The internal solution contained (in mM): 138 Cs-$MeSO_3$, 5 CsCl, 0.5 EGTA, 10 HEPES, 1 $MgCl_2$, 2 mg/ml Mg-ATP, pH 7.3 adjusted with CsOH and osmolarity 300-310 mOsm with glucose). The cells were activated by a 6 ms test pulse of variable voltage family from -90 to 70 mV with a holding potential of -90 mV, and then tail currents were measured after repolarization to -50 mV for 10 ms. The tail currents were normalized to the peak currents before fitting with a dual Boltzmann equation:

$$G/G_{max} = F_{low}/\{1 + \exp((V_{1/2,low} - V)/k_{low}) + (1-F_{low})/\{1 + \exp((V_{1/2,high} - V)/k_{high})\}$$

where, G is the tail current and G_{max} is the peak tail current, F_{low} is the fraction of low

threshold component; $V_{1/2,low}$, $V_{1/2,high}$, k_{low}, and k_{high} are the half-activation potentials and slope factors for the low and high threshold components.

To determine the whole cell current-voltage (*I-V*) relationships in A7r5 cells, currents were recorded by holding the cell at -70 mV before stepping to various potentials from -60 to 60 mV over 900 ms in an external solution containing 5 mmol/L Ba^{2+}. The *I-V* curve was fitted with the equation:

$$I_{Ba} = G_{max}(V - E_{rev})/(1 + \exp(V - V_{1/2})/k)$$

where G_{max} is the maximum conductance; E_{rev} is the reversal potential; $V_{1/2}$ is the half-activation potential; and k is the slope

Imaging of cell surface- and ER-localized $Ca_V1.2$ channels

As previously described[3-5], for staining of surface $Ca_V1.2$ channels, HA-$Ca_V1.2$, β_{2a} subunit without GFP tag, and Gal-1-DsRed were co-transfected in HEK 293 cells cultured in 35 mm dish using calcium phosphate method. 48 h after transfection, cells were passaged to 2 wells with coated coverslips in 24-well plate, followed by 10 μM MG132 treatment for 2 h with cells in one of the wells. After that, cells were washed with cold PBS containing 10 % FBS and fixed in 4 % paraformaldehyde for 15 min. Following blocking by 10 % FBS/PBS for 20 min, Cell staining with mouse anti-HA (Pierces, 1:100) was conducted at 37 °C for 90 min. Then Alexa Fluor 488-conjugated goat α-mouse IgM antibody (Molecular Probes, 1:500) was used as secondary antibody to incubate with cells for 60 min in room temperature.

For staining of ER-localized $Ca_V1.2$ channels and Gal-1, GFP-$Ca_V1.2$, ERoxBFP, β_{2a} subunit without GFP tag, $\alpha_2\delta$ and pDsRed-Gal-1were co-transfected into HEK 293 cells cultured in 35 mm dish. 48 h after transfection, cells were passaged to 2 wells with coated coverslips in 24-well plate, followed by 10 μM MG132 treatment for 2 h with cells in one of the wells. After that, cells were washed with cold PBS containing 5 % FBS and fixed in 4 % paraformaldehyde for 15 min.

For staining of ER-localized $Ca_V1.2$ channels in VSMCs, ERoxBFP was transfected into VSMCs cultured in 35 mm dish using Lipofectomine. 48 h after transfection, cells were passaged to 2 wells with coated coverslips in 24-well plate, followed by 10 μM MG132 treatment for 2 h with cells in one of the wells. After that, VSMCs were washed with cold PBS containing 5 % FBS and fixed in 4 % paraformaldehyde for 15 min, followed by permeabilization by 0.2 % Tween-20/PBS for 15 min and blocking by 5 % FBS/PBS for 20 min. The mixture of rabbit anti-$Ca_V1.2$ and mouse anti-Gal-1(1:100 dilution for each antibody) was used to incubate cells at 37 °C for 60 min. After washing with PBS, the mixture of Alexa Fluor 594-conjugated goat α-rabbit IgG antibody and Alexa Fluor 488-conjugated goat α-mouse IgM antibody (Molecular Probes, 1:500) was used as secondary antibodies to incubate with cells for 60 min in room temperature.

Cells were imaged using a Zeiss LSM-510 Meta confocal microscope with a 63×1.4NA oil immersion lens in the inverted position. AF-488 antibody or GFP was visualized by excitation with an argon laser (488 nm) and emission detected using a long-pass 530-nm filter. oxBFP was visualized by excitation with 405 nm diode laser and emission detected using a 415–505 nm bandpass filter. AF-594 antibody or DsRed was visualized by excitation with a HeNe laser (543 nm) and emission detected using a 585–615 nm bandpass filter. Image acquisition was performed with identical gain, contrast, laser excitation, pinhole aperture and laser scanning speed for each round of cultures. Images were processed using ImageJ.

Immunohistochemistry

Aortas isolated from WT or *Lgals1*$^{-/-}$ (Jackson Laboratory, stock number: 006337) mice were fixed with 4 % paraformaldehyde and then incubated with 30 % sucrose. Frozen tissues were

cryosectioned (10μm) in the transverse axis and incubated with blocking buffer (5 % goat serum in 0.1 % Triton X-100 in PBS). The sections were then incubated with primary antibodies diluted in blocking buffer overnight at 4 °C, followed by washing with PBS on the next day and incubation with secondary antibodies for 1h. Primary antibodies used were rabbit anti-$Ca_V1.2$ (1:50), anti-Gal-1 (1:50), anti-SM-actin (1:50) and the detailed antibody information is listed in Table S4. The secondary antibodies were conjugated to Alexa Fluor 594 or 647 (1:100, Invitrogen). Staining with DAPI (1:1000) was used to visualize the nuclei. The autofluorescence of elastin was used to show the outline of artery. The intensity of $Ca_V1.2$ channel was analyzed by the NIKON Elements AR software and normalized to DAPI intensity.

Mesenteric artery preparation and organ culture

As previously described[6], Male Wistar rats (5~7-week-old) or *Lgals1*$^{-/-}$ mice (9~10-week-old) were decapitated after anesthetization using CO_2. The main branch of the superior mesenteric artery was quickly isolated under sterile conditions. After removal of fat and adventitia in physiological saline solution (PSS, in mmol/L: 119 NaCl, 4.7 KCl, 1.8 $CaCl_2$, 1.2 $MgSO_4$, 24 $NaHCO_3$, 0.2 KH_2PO_4, 10.6 glucose), the mesenteric artery was cultured in 400 μl Dulbecco's Modified Eagle Medium (DMEM) with 10 % FBS (FBS) supplemented with 1 % penicillin-streptomycin in 24-well plates and maintained at 37 °C in an atmosphere of 95 % air and 5 % CO_2 for 24 h. Following peptide treatment and pressure myography, the remaining arteries were used to determine the protein levels of total Gal-1 and $Ca_V1.2$ channels, and the level of $Ca_V1.2$-Gal-1 protein interactions by co-IP following treatment with MG132 (10 μmol/L). All arteries used for measurement of isometric tension in this study were denuded of endothelium by perfusing 1 ml of air through the artery lumen. Animal care and treatment were approved by the Institutional Animal Care and Use Committee at National University of Singapore.

Pressure myography

Cultured mesenteric arterial segments were treated with 10 μmol/L Tat-e9c or Tat-e12c for 24 h and then cannulated on glass micropipettes mounted in a 5 ml myograph chamber (Living System, University of Vermont Instrumentation and Model Facility) as described previously[1, 7]. Only secondary or third branches of mesenteric arteries (MA) were used in this experiment. Arterial diameter was measured with video edge detection equipment and recorded using data acquisition software (Dataq Instruments, Akron, OH). To assess myogenic tone, MAs were subjected to stepwise increases of pressure from 20 to 80 mmHg, and spontaneous myogenic tone was allowed to develop at each step until a stable diameter was achieved, approximately 2 min. After completion of the pressure–response curve, intraluminal pressure was maintained at 20 mmHg, and MAs were superfused with Ca^{2+}-free PSS (in mmol/L: 119 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 24 $NaHCO_3$, 0.2 KH_2PO_4, 10.6 glucose and 3 EGTA). The pressure–response curve was repeated under Ca^{2+}-free conditions to obtain passive responses. Myogenic tone was calculated as the percent difference in diameter observed for Ca^{2+}-replete versus Ca^{2+}-free PSS at each pressure, which was as follows: Myogenic tone (%) = [(luminal diameter in Ca^{2+}-free PSS – luminal diameter in Ca^{2+}-replete PSS)/luminal diameter in Ca^{2+}-free PSS]×100. For depolarization-induced arterial constriction, following cannulation, arteries were pressurized at 20 mmHg and continuously superfused with aerated PSS at 37 °C and pH 7.4 for 30 min to allow equilibration. Then, arteries were exposed to PSS containing elevated $[K^+]$ ranging from 6 to 120 mmol/L (in details, mmol/L: 6, 10, 15, 20, 25, 30, 35, 40, 50, 60, 80 and 120) for 4 min individually, made by isoosmotic replacement of NaCl with KCl. Arterial constriction was expressed using the following equation: % Constriction = $[1 - (D - D_{min}/D_{max} - D_{min})]\times100$, where D_{max} is the maximum diameter obtained in Ca^{2+}-free PSS and D_{min} is the minimum diameter obtained with the Ca^{2+} ionophore ionomycin (Sigma, 10 μmol/L) at the end of each experiment. Half-maximal effective concentration (EC_{50}) was determined from each $[K^+]_O$ concentration-response curve. Arteries not achieving > 70 % constriction in response to ionomycin were not used for analysis.

Tail-cuff blood pressure measurements
Blood pressure was measured using a non-invasive tail-cuff system (BP-2000, Visitech Systems) as previously described[8].

For experiments in spontaneously hypertensive rats (SHR), Wistar and SHR rats (15-16-week-old) purchased from Charles River and *Lgals1*$^{-/-}$ mice purchased from Jackson Lab were maintained at the Comparative Medicine Animal Vivarium at National University of Singapore. Briefly, rats or mice were trained to accustom the tail-cuff procedure, and then measurements of blood pressure were performed by the same investigator on 2-3 consecutive days. After confirmation of hypertension in SHR rats, rats were anesthetized using CO_2, and then the thoracic aortas and mesenteric arteries were quickly isolated. After removing the fat tissues and adventitia, the arteries were pulverized and dissolved in lysis buffer for western blot assays. These studies were approved and performed in accordance with the guidelines of the Institutional Animal Care and Use Committee at National University of Singapore.

For experiments of peptide infusion in rats, the Wistar rats (180-230g) were ordered from In Vivos (Singapore) and maintained at Animal Vivarium at National Neuroscience Institute (For experiments in Figure 7 and Figure S19) or National University of Singapore (For experiments in Figure S20), and randomized to two different peptide groups. Briefly, rats were trained to accustom the tail-cuff procedure for 3 days. Following these baseline recordings, the osmotic mini-pumps (Alzet Model 2001, DURECT) filled with control Tat-e12c or Tat-e9c (400 pmol/kg/min) were implanted into jugular vein. Blood pressure was monitored once every day. As previously described[9, 10], echocardiography was performed with Vevo 2100 from Visualsonics at 1 day before pump implantation and at day 2 and 9 after pump implantation. Cardiac output was calculated according to: Cardiac output = Stroke volume × Heart rate. All assessments were made at 9 days after surgery. 6 animals were used in each group. This study was approved and performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Neuroscience Institute and National University of Singapore.

Adeno-associated viral constructs of Galectin-1
The AAV plasmid construct is shown in Figure 8. The WPRE/SV40 polyA fragment was synthesized and inserted into pBluescript II KS+ vector with BamH1/Xba1 and Not1. Sequentially, the IRES-GFP fragment was cut from Not1 blunted pIRES2-AcGFP1 vector and inserted with BamH1 and Xba1. The full-length Flag-tagged human Gal-1 cDNA was inserted with Hind III and BamH1 and the chimeric smooth muscle-specific enhancer/promoter (EnSM22α) was generated as reported and inserted with Sal1/Not1 and Hind III to obtain final Not1 flanked Gal-1 cassette (EnSM22α/Gal-1/Flag/IRES/GFP/WPRE/SV40 polyA). For control GFP cassette, the Gal-1/Flag/IRES/GFP fragment was replaced with GFP through Hind III and BamH1 restriction enzyme digestion sites. Both Gal-1 and control GFP cassette were transferred with Not1 to CMV-MCS Vector (Agilent Technologies, AAV Helper-Free System) and packaged into AAV5 by Biowit Technologies Ltd.

Telemetric recordings of blood pressure in AAV5-Galectin-1-injected SHR
Animal experiments were performed in accordance with guidelines and protocols approved by the Institutional Animal Care and Use committee at Southwest Medical University in China. SHR (11~12-week-old) were implanted with telemetry devices (TRM54PB. Telemetry Research, Auckland, New Zealand). Rats were randomized to AAV5-Gal-1 or AAV5-GFP injection. Briefly, rats were anesthetized with Ketamine and Xylazine. Body temperature was maintained with heating pad. Following an abdominal incision, the pressure sensor was inserted into abdominal aorta below the level of the renal artery and sealed in position with medical glue. The body of the transmitter was placed into the abdominal cavity and sutured to the abdominal wall. Animals were allowed to recover and individually housed for at least 7 days in a temperature-controlled room with 12 h light-12 h dark cycle. During the period, the healthy state of rats was tightly monitored by body weight weighing and activity observation.

Recording of arterial systolic and diastolic (SBP and DBP) blood pressure were performed in conscious, unrestrained and healthy rats for up to 3 days. Mean arterial pressure (MAP) was calculated according to: MAP=(SBP+2DBP)/3. Rats were then divided into two groups: those that were injected with variable amounts of either (1) AAV5-Gal-1 (1×10^{13} vg/kg, n=3) or (2) AAV5-GFP (1×10^{13} vg/kg, n=4). Data were recorded for a further 30 days.

Supplemental References

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Supplemental Figures

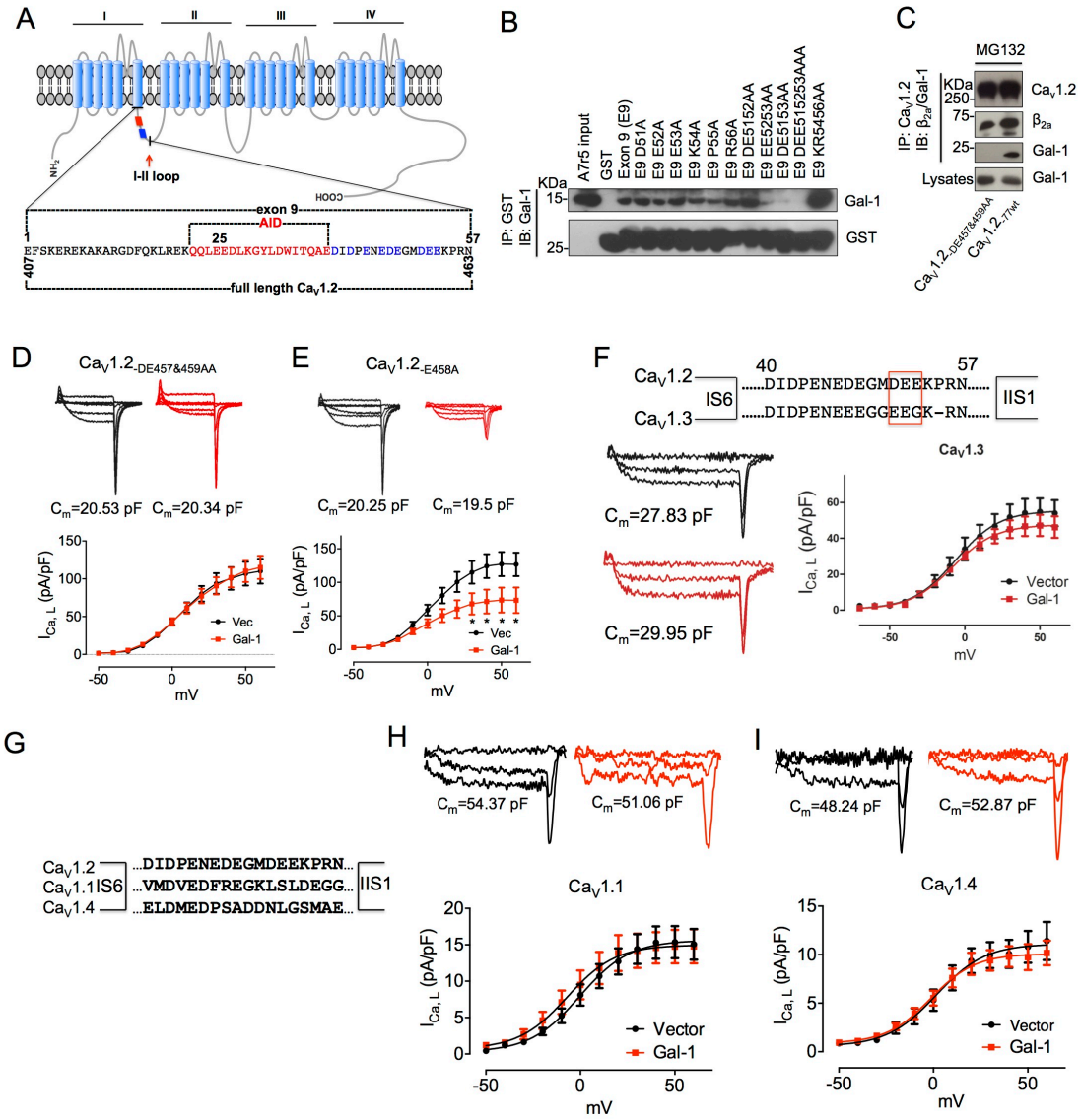

Figure S1. Gal-1 interacts with DEE motif of $Ca_V1.2$ channels. (**A**) Schematic diagram of human $Ca_V1.2$ channel shows the amino acid sequences of exon 9 in I-II loop with AID domain highlighted in red and di-acidic motifs of ER export signal in blue. (**B**) Western blot of GST pull-down assay show the binding of Gal-1 to different exon 9 mutants with GST tag. The mutants of exon 9 were incubated with A7r5 cell lysates at 4 °C for 16 h (n=4). (**C**) Co-immunoprecipitations of $Ca_V1.2$ or $Ca_V1.2_{-DE457\&459AA}$, β_{2a} or Gal-1 from transfected HEK 293 cells (n=3). (**D, E**) $I_{Ca,L}$ was recorded by *Tail* protocol in HEK 293 cells co-transfected with $Ca_V1.2_{-DE457\&459AA}$ (Vec, n=10,; Gal-1, n=11) or $Ca_V1.2_{-E458A}$ (Vec, n=9, Gal-1, n=14), $\alpha_2\delta$ and β_{2a} subunit, vector, or Gal-1 in 1.8 mM Ca^{2+} external solution. (**F**) Alignment of exon 9 C-terminus of I-II loop of $Ca_V1.2$ and $Ca_V1.3$ channels and recordings of *Tail* current of $Ca_V1.3$ channels co-transfected with Gal-1 in external solution with 1.8 mM Ca^{2+} (Vec, n=9; Gal-1, n=10). (**G**) Alignment of exon 9 C-terminus of I-II loop of $Ca_V1.2$, $Ca_V1.1$ and $Ca_V1.4$ channels. (**H, I**) Recordings of *Tail* current of $Ca_V1.1$ (Vec, n=11; Gal-1, n=13) and $Ca_V1.4$ (vec, n==7; Gal-1, n=8) channels co-transfected with Gal-1 in external solution with 5 mM Ca^{2+}. Data were shown as mean ± SEM. * p<0.05 versus Vec group.

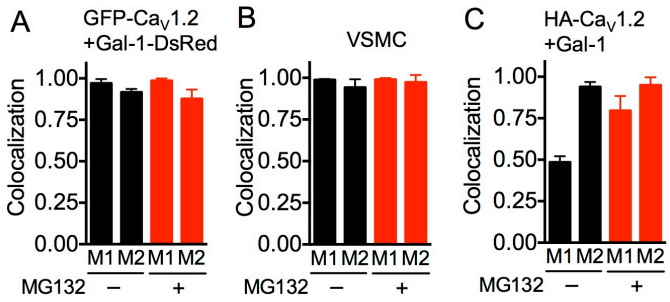

Figure S2. Colocalization analysis of $Ca_V1.2$ and Gal-1. Mander's coefficient was measured (Imaris 9.1.0, BITPLANE) for GFP-$Ca_V1.2$ and Gal-1-DsRed colocalization in transfected HEK 293 cells (**A**, n=18-20 cells) and $Ca_V1.2$ and Gal-1 colocalizaiton in A7r5 smooth muscle cells (**B**, n=10-12 cells) and in transfected HEK 293 cells (**C**, n=20-24 cells) with or without MG132. M1 indicates the correlation of Gal-1 overlapping $Ca_V1.2$, while M2 signifies the overlap coefficient of $Ca_V1.2$ to Gal-1.

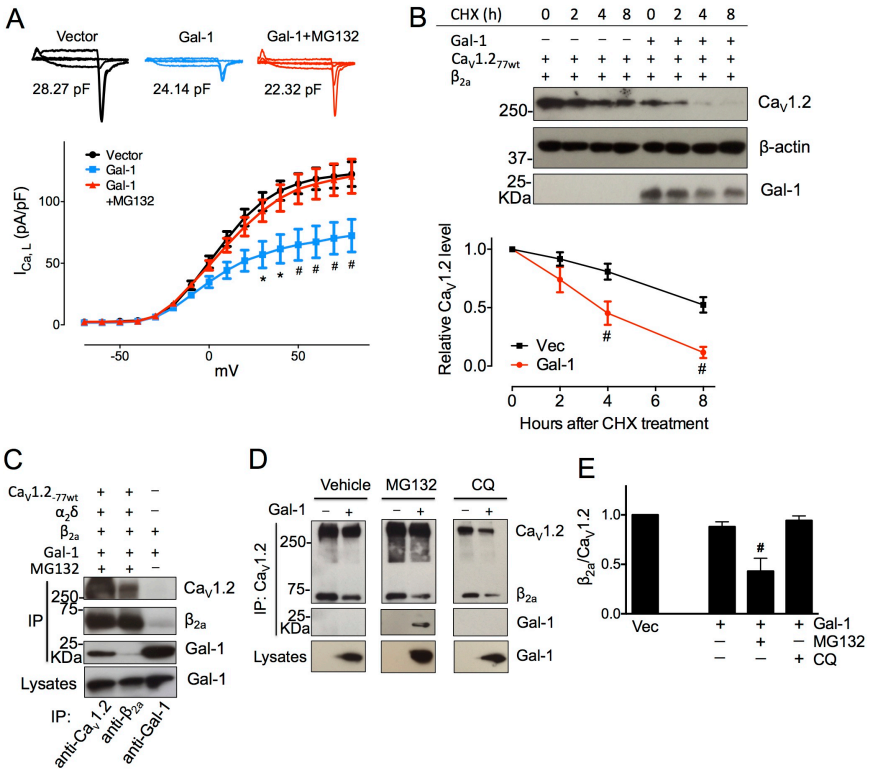

Figure S3. Gal-1 promotes the proteasomal degradation of $Ca_V1.2$ channels by disrupting the $Ca_V1.2$-$Ca_V\beta$ subunits. (**A**) $I_{Ca,L}$ was recorded by *Tail* protocol in HEK 293 cells co-transfected with $Ca_V1.2$, $\alpha_2\delta$ and β_{2a} subunit, vector (black, n=12), or Gal-1 (Gal-1:$Ca_V1.2$=1:1), with (red, n=14) or without (blue, n=14) treatment with MG132 (1 μM) for 16 h before recording in 1.8 mM Ca^{2+} external solution. *p<0.05, # p<0.01 versus Vector group. (**B**) Western blots and quantifications of total $Ca_V1.2$ channels co-expressed with or without Gal-1 in HEK 293 cells with treatment by a protein translation inhibitor, cycloheximide (CHX, 100 μg/ml). Cell lysates were collected at different time points and then immunoblotted with anti-$Ca_V1.2$ (n=3). (**C**) Co-IP that showed the interactions among $Ca_V1.2$ channels, β_{2a} subunit and Gal-1 overexpressed in HEK 293 cells in the presence or absence of MG132 (1 μM, n=3). (**D, E**) Western blots and quantifications of the ratio of β_{2a} subunit to

$Ca_V1.2$ channels co-expressed with or without Gal-1 in HEK 293 cells under 16 h treatment by vehicle, MG132 (1 μM) or CQ (40 μM, n=4). # p<0.01 versus Vector group.

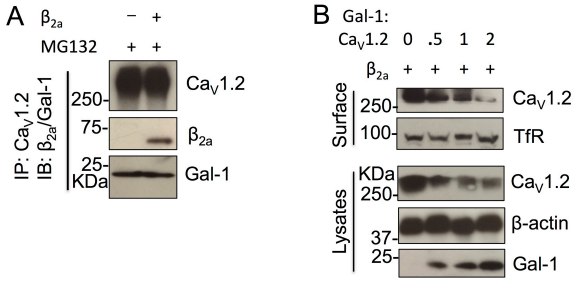

Figure S4. Gal-1 dose-dependently reduces the total and surface level of $Ca_V1.2$ channels. (**A**) Co-IP of $Ca_V1.2$ channels and Gal-1 in the presence or absence of β_{2a} subunit in transfected HEK 293 cells. The results indicated that β_{2a} subunit did not affect the $Ca_V1.2$-Gal-1 interactions (n=3). (**B**) Western blots of total and surface biotinlyated $Ca_V1.2$ channels co-transfected with Gal-1 at different amounts (n=3).

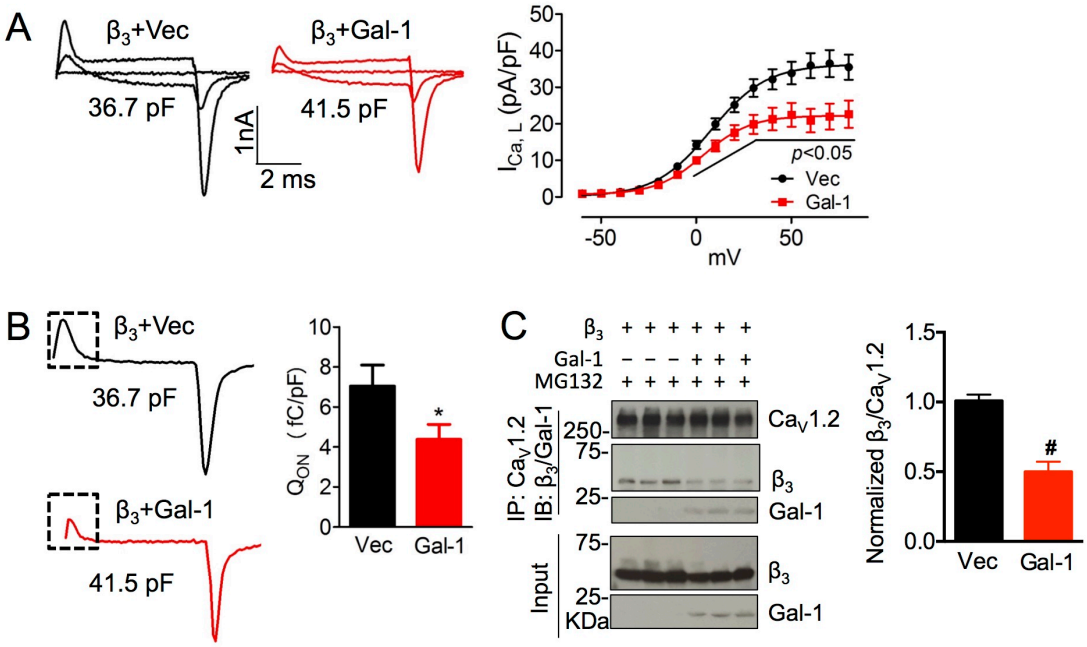

Figure S5. Effect of Gal-1 on the function of $Ca_V1.2$ channel co-transfected with β_3 subunit. (**A**) $I_{Ca,L}$ was recorded by *Tail* protocol in HEK 293 cells co-transfected with $Ca_V1.2_{-77wt}$, $\alpha_2\delta$ and β_3 subunit, vector (n=43) or Gal-1 (n=30) in 1.8 mM Ca^{2+} external solution. (**B**) Q_{ON} measured by *Tail* protocol in HEK 293 cells co-transfected with $Ca_V1.2_{-77wt}$ and vector (n=43) or Gal-1 (n=30) when holding at V_{rev}. Q_{ON} recordings were performed in 1.8 mmol/L Ca^{2+} external solution. (**C**) Western blots and quantifications of the ratio of β_3 subunit to $Ca_V1.2$ channels co-expressed with or without Gal-1 in HEK 293 cells (n=6) under 16 h treatment by MG132 (1 μM). Data were shown as mean ± SEM. *p<0.05, # p<0.01 versus Vector group.

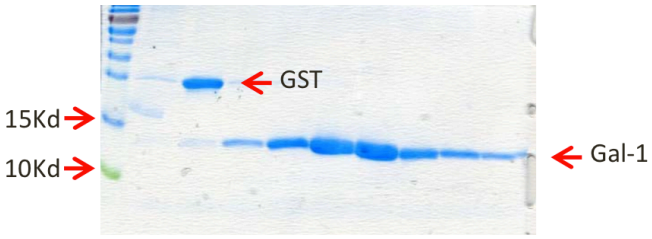

Figure S6. Purification of Gal-1 protein using gel filtration column. Fractions eluted by gel filtration column were pooled together and then concentrated to 10 mg/ml.

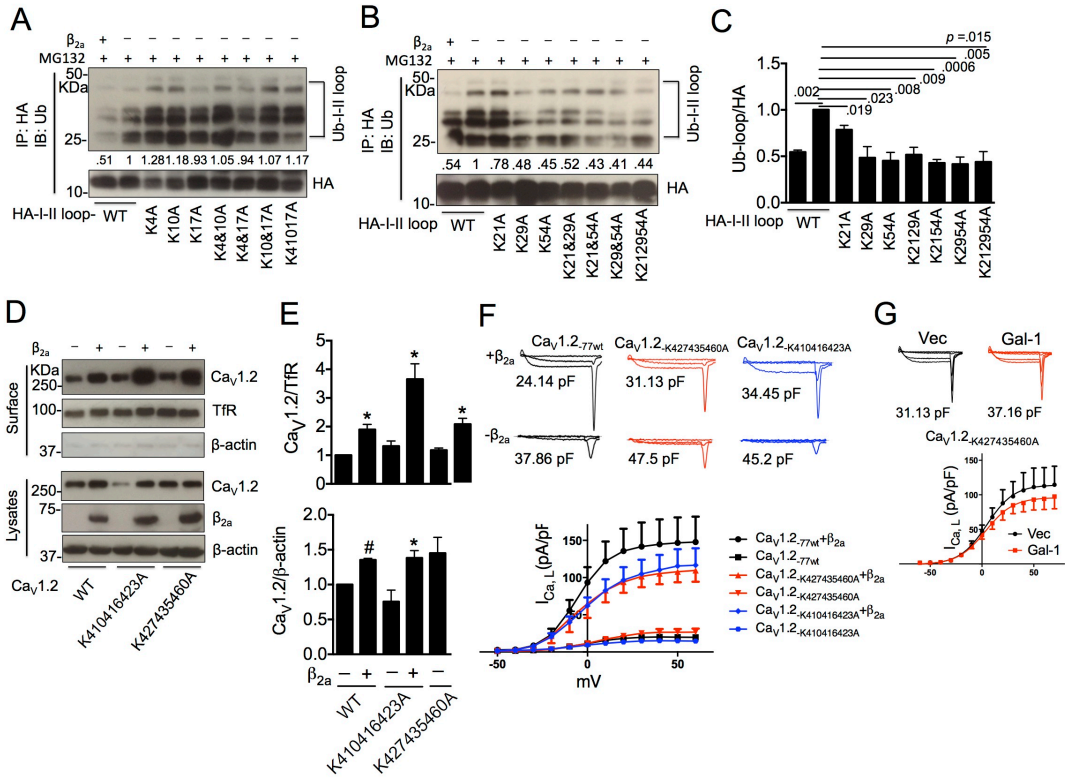

Figure S7. Mutations of K21, K29 and K54 into alanines in $Ca_V1.2$ I-II loop down-regulates the ubiquitination level of $Ca_V1.2$ channels. (**A**) Western blots of ubiquitinated HA-tagged I-II loop in HEK 293 cells co-transfected with various I-II loops with mutations of K4, K10 and/or K17 into alanines in the presence or absence of β_{2a} subunit (n=4). Cell lysates were harvested after MG132 (1 μM) treatment for 16 h. (**B, C**) Western blots of ubiquitinated HA-tagged I-II loop in HEK 293 cells co-transfected with various I-II loops with mutations of K21, K29 and/or K54 into alanines in the presence or absence of β_{2a} subunit (n=4). Cell lysates were harvested after MG132 (1 μM) treatment for 16 h. Data were shown as mean ± SEM. (**D, E**) Western blots and quantifications of total and biotinylated surface $Ca_V1.2_{-77wt}$, $Ca_V1.2_{-K410416423A}$ and $Ca_V1.2_{-K427435460A}$ channels in the presence or absence of β_{2a} subunits in tranfected HEK 293 cells treated with MG132 (1 μM) for 16 h (n=3). (**F**) $I_{Ca,L}$ was recorded by *Tail* protocol in HEK293 cells co-transfected with $Ca_V1.2_{-77wt}$ (n=10), $Ca_V1.2_{-K427435460A}$ (n=15) or $Ca_V1.2_{-K410416423A}$ (n=14), in the presence or absence of β_{2a} subunit in 1.8 mM Ca^{2+} external solution. (**G**) $I_{Ca,L}$ was recorded by *Tail* protocol in HEK293 cells transfected with $Ca_V1.2_{-K427435460A}$, in the presence (n=9) or absence (n=8) of Gal-1 in 1.8 mM Ca^{2+} external solution. Data were shown as mean ± SEM. *p<0.05, #p<0.01 versus control group.

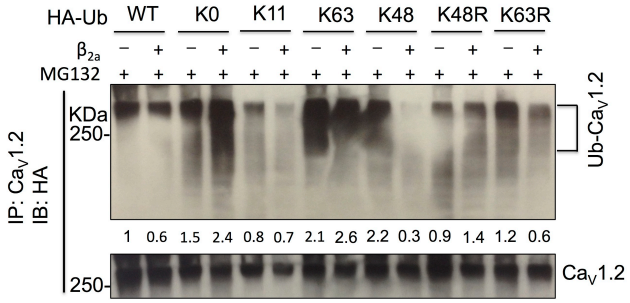

Figure S8. The K48 linkage is involved in increased ubiquitination of $Ca_V1.2$ channels without $Ca_V\beta$ subunit. Western blots of ubiquitinated $Ca_V1.2$ channels in HEK 293 cells co-transfected with various HA-tagged ubiquitin mutants (K0 means that all the lysines are mutated. K48 means only K48 was kept while K48R means that only K48 is mutated) in the presence or absence of β_{2a} subunit (n=3). Cell lysates were harvested after MG132 (1 μM) treatment for 16 h. The numbers between blots indicated the average grey intensity of each group.

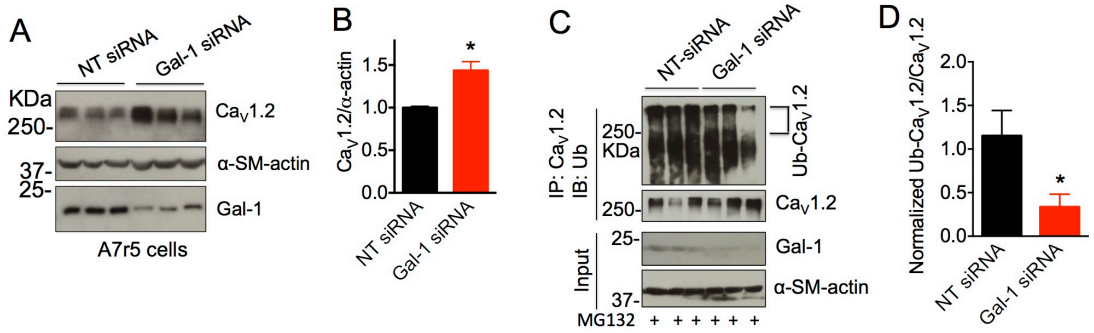

Figure S9. Knock-down of Gal-1 in A7r5 cells increased total $Ca_V1.2$ channels by reducing $Ca_V1.2$ ubiquitination. (**A, B**) Western blots and quantifications of total $Ca_V1.2$ channels in A7r5 cells transfected with non-target (NT) siRNA or ON-TARGETplus rat Gal-1 siRNA (n=6, 100 nM, Dharmacon). (**C, D**) Western blots and quantifications of ubiquitinated $Ca_V1.2$ channels in A7r5 cells transfected with non-target (NT) siRNA or ON-TARGETplus rat Gal-1 siRNA (n=6). Data were shown as mean ± SEM. *p<0.05 versus NT siRNA group.

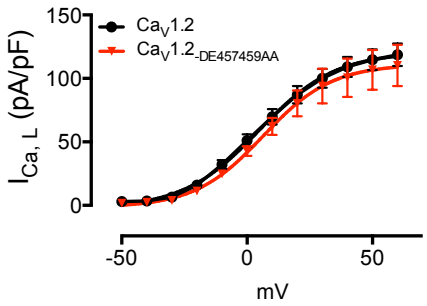

Figure S10. Mutations of D457 and E459 into alanines did not affect the current density of $Ca_V1.2$ channels. $I_{Ca,L}$ was recorded by *Tail* protocol in HEK 293 cells transfected with $Ca_V1.2_{-DE457459AA}$ (n=10) or $Ca_V1.2$ (n=11) channels in external solution with 1.8 mM Ca^{2+}.

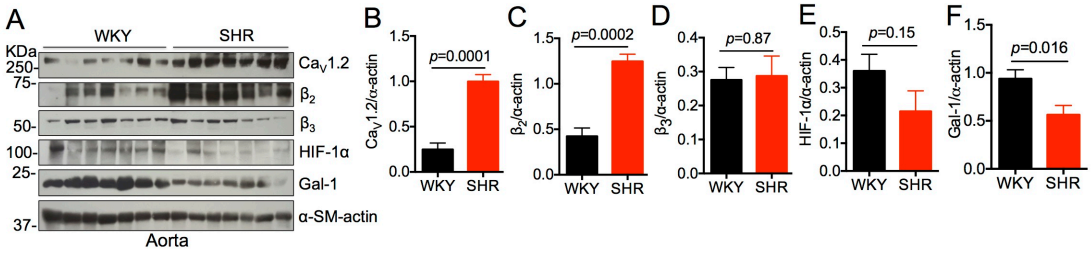

Figure S11. Protein expression level of $Ca_V1.2$, β_2, β_3, HIF-1α and Gal-1 in aorta of WKY rats and SHR. (**A-F**) Western blots and quantifications of total $Ca_V1.2$ channels in aorta in WKY rats and SHR (n=7). α-smooth muscle actin was used as the loading control. Data were shown as mean ± SEM.

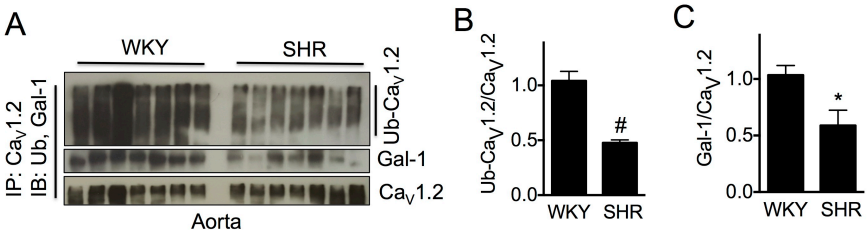

Figure S12. The level of ubiquitinated $Ca_V1.2$ channels in aorta of WKY rats and SHR. (**A-C**) Western blots and quantifications of the ubiquitinated $Ca_V1.2$ channels and the Gal-1 bound to $Ca_V1.2$ in aorta in WKY rats and SHR (n=7). α-smooth muscle actin was used as the loading control. Data were shown as mean ± SEM. * $p<0.05$, # $p<0.01$ versus WKY group.

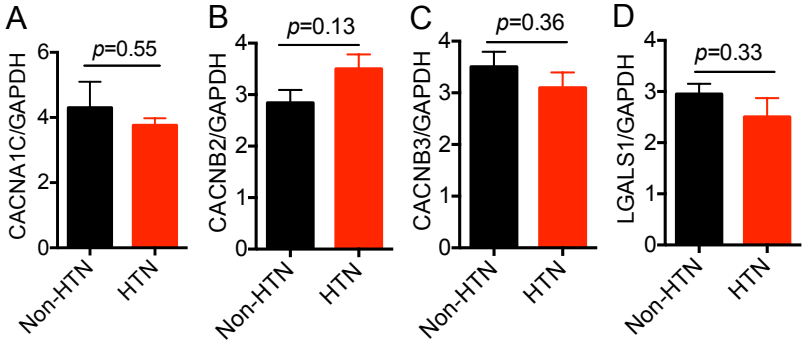

Figure S13. The mRNA expression levels of *CACNA1C*, *CACNB2*, *CACNB3* and *LGALS1* in human non-hypertensive (Non-HTN) or hypertensive (HTN) arteries. (**A-D**) mRNA levels of *CACNA1C*, *CACNB2*, *CACNB3* and *LGALS1* were measured by quantitative real time PCR from the non-hypertensive or hypertensive human arteries (n=4). The relative expression was normalized by internal expression of human *GAPDH* mRNA. Data were shown as mean ± SEM.

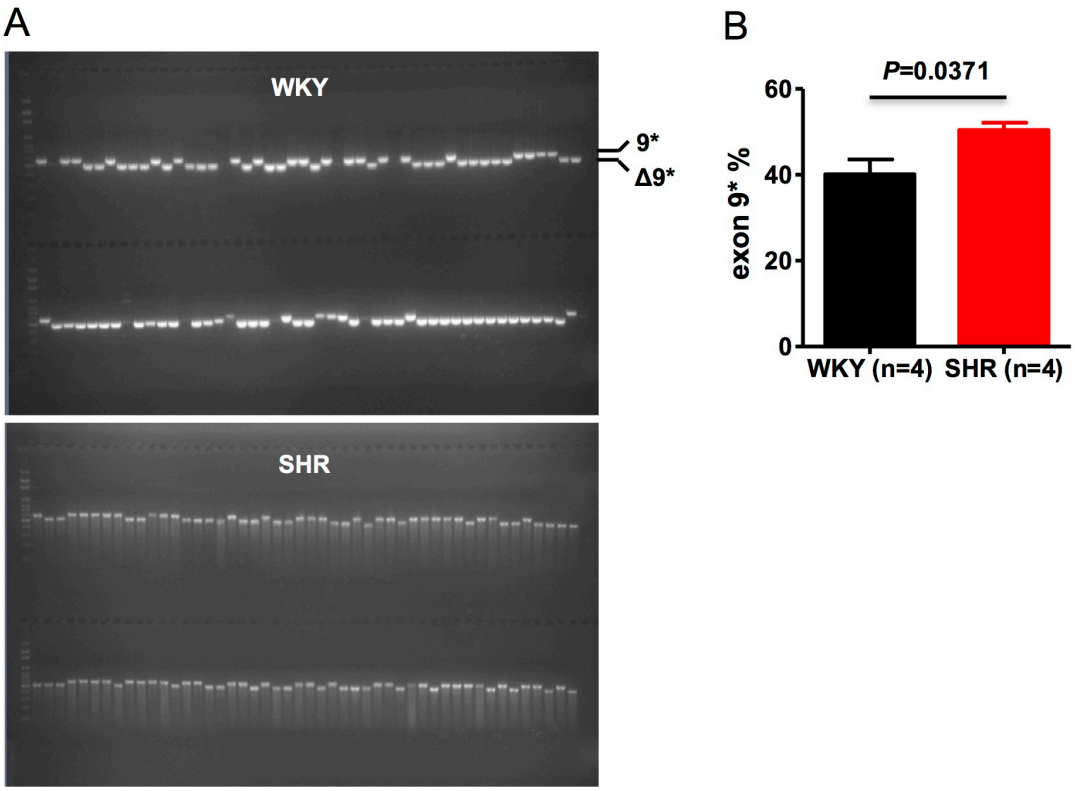

Figure S14. The inclusion level of exon 9* in $Ca_V1.2$ channel is significantly increased in the mesenteric arteries of SHR. **(A)** The primers were designed to amplify and detect rat $Ca_V1.2$ I-II loop inclusive of or in the absence of exon 9*. PCR products were separated in 2% agarose gel, the upper band shows the inclusion of exon 9*, and the low band shows the exclusion of exon 9*. **(B)** The MAs from 4 rats each group were used for colony PCR (n=196 each rat), *p*=0.0371 vs WKY rats.

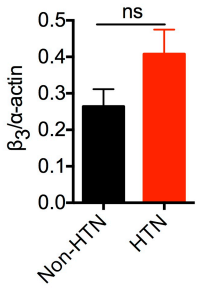

Figure S15. Quantification of β_3 subunit in non-hypertensive or hypertensive human pulmonary arteries.

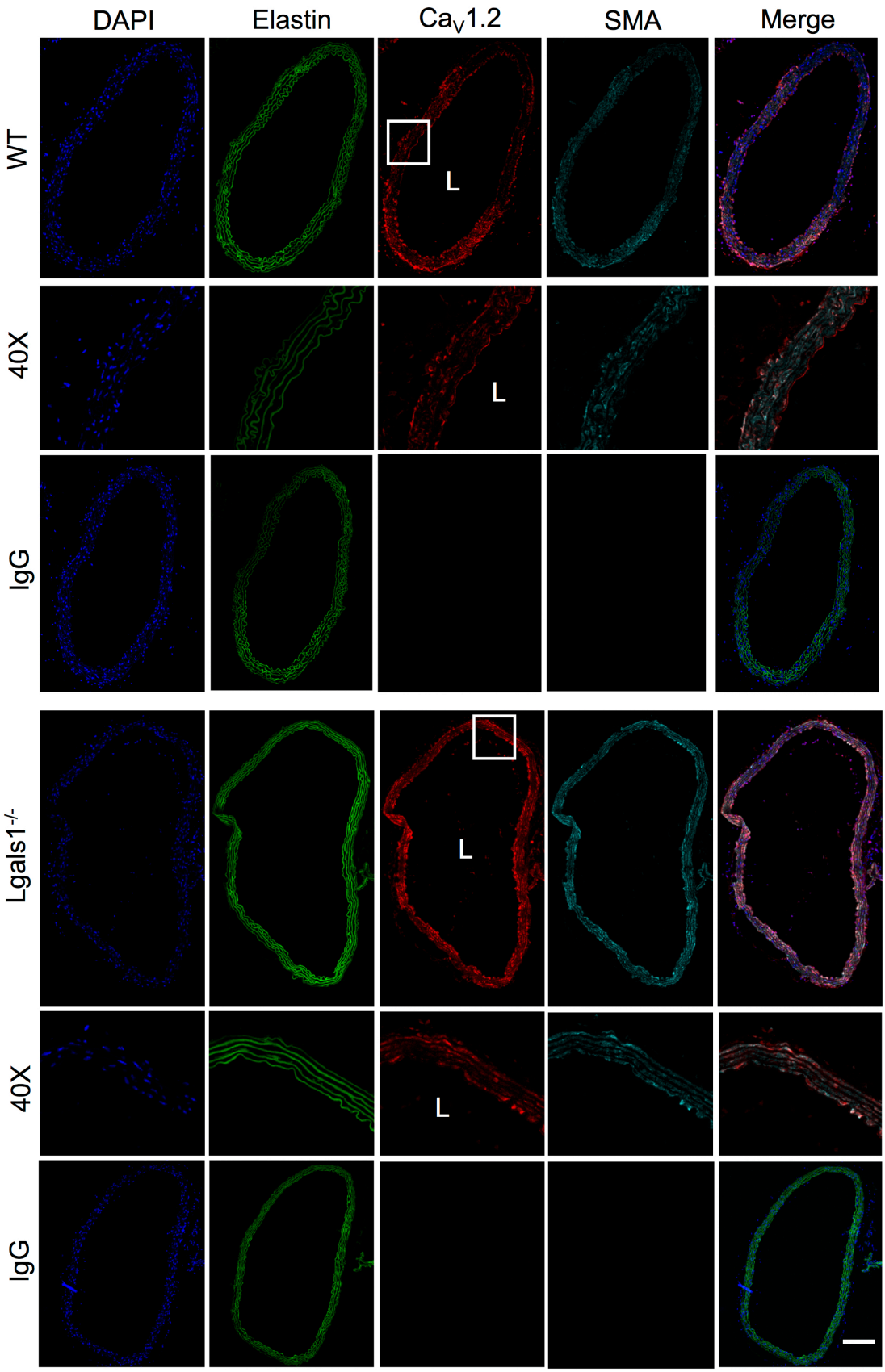

Figure S16. Immunostaining of $Ca_V1.2$ channel and smooth muscle actin in aorta from WT or *Lgals1*$^{-/-}$ mice. n=3 mice each group and 6 sections were used for analysis. SMA: smooth muscle actin. Scale bar: 20 μm.

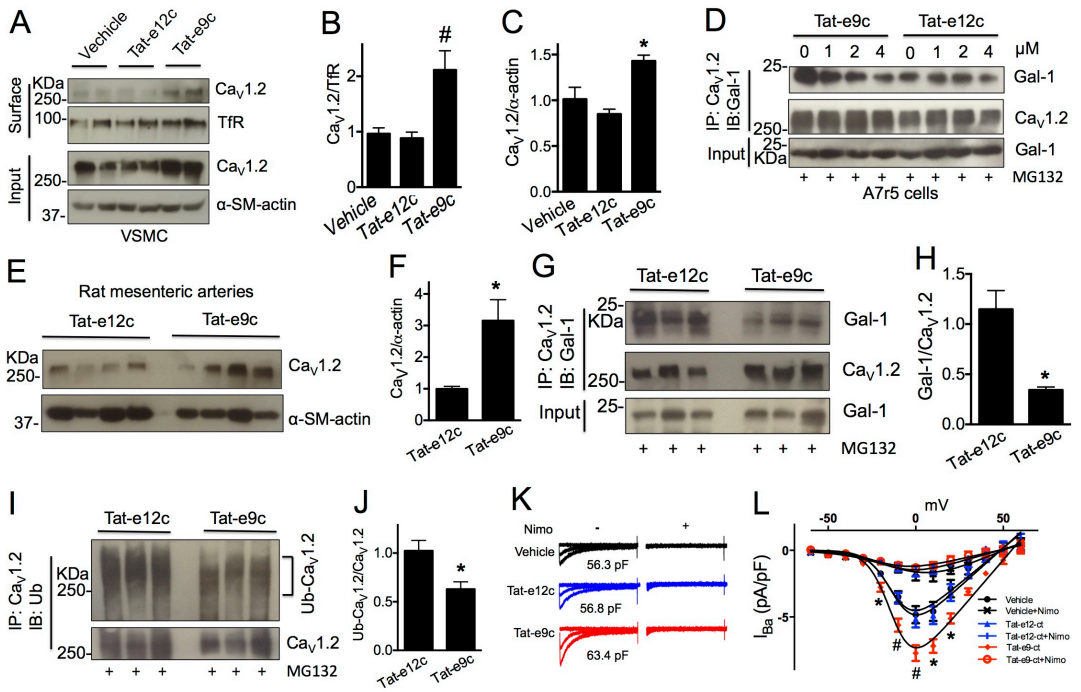

Figure S17. Tat-e9c peptide decreases the total and surface expression level in cultured rat aortic smooth muscle cells and the $Ca_V1.2$ current in A7r5 cells. (**A-C**) Western blots of total and surface biotinylated $Ca_V1.2$ channels in cultured rat aortic smooth muscle cells treated with Tat-e9c peptide (4 μM) for 24 h (n=4). (**D**) Co-IP of $Ca_V1.2$ channels and Gal-1 in A7r5 cells treated with Tat-e9c peptide at different concentrations for 24 h, followed by 4 h MG132 (5 μM) treatment (n=3). Data were shown as mean ± SEM. (**E, F**) Western blots and quantifications of total $Ca_V1.2$ channels in rat mesenteric arteries treated with Tat-e12c (4 μM) or Tat-e9c (4 μM) for 24 h (n=8). (**G-J**) Western blots and quantifications of $Ca_V1.2$-Gal-1 interactions and poly-ubiquitinated $Ca_V1.2$ channels in rat mesenteric arteries treated with Tat-e12c or Tat-e9c for 24 h, followed by MG132 (5 μM) treatment for 4 h (n=3). (**K, L**) Effects of Tat-e9c peptide on $Ca_V1.2$ current density in A7r5 cells treated with Tat-e9c peptide (4 μM) for 24 h. I-V curves were obtained in an external solution containing 1.8 mM Ca^{2+}. Nimodipine (5 μM) was used to block the L-type calcium current in A7r5 cells. * $p<0.05$, # $p<0.01$ versus Vehicle group.

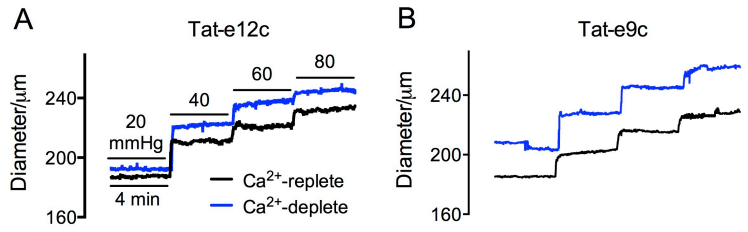

Figure S18. Effect of Tat-e9c peptide on the myogenic tone of isolated rat mesenteric arteries. (**A, B**) Representative traces of Tat-e12c or Tat-e9c-treated rat mesenteric arteries responding to gradually increasing intraluminal pressure in Ca^{2+}-replete or deplete PSS solution (n=10).

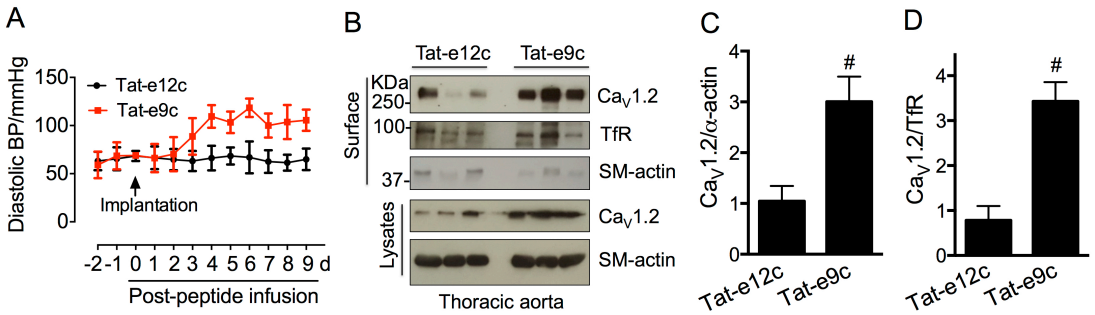

Figure S19. Effect of Tat-e9c on diastolic blood pressure in rats. (**A**) Daily diastolic blood pressures in rats before, during or after 9-day Tat-e9c infusion (n=6 for each group). Osmotic mini-pumps were implanted via jugular vein at day 0. Blood pressure was measured by non-invasive tail-cuff method. Data were shown as mean ± SD. Data were analyzed by two-way repeated measures ANOVA ($F(1, 10)=163.382$, $p<0.0001$ for treatment; $F(11, 110)=8.178$, $p<0.0001$ for time; $F(11, 110)=8.136$, $p<0.0001$ for interaction). (**B-D**) Western blots and quantifications of biotinylated surface and total $Ca_V1.2$ channels in thoracic aorta in Tat-e12c- or Tat-e9c-treated rats (n=6). α-smooth muscle actin was used as the loading control. Data were shown as mean ± SEM. # $p<0.01$ versus Tat-e12c group.

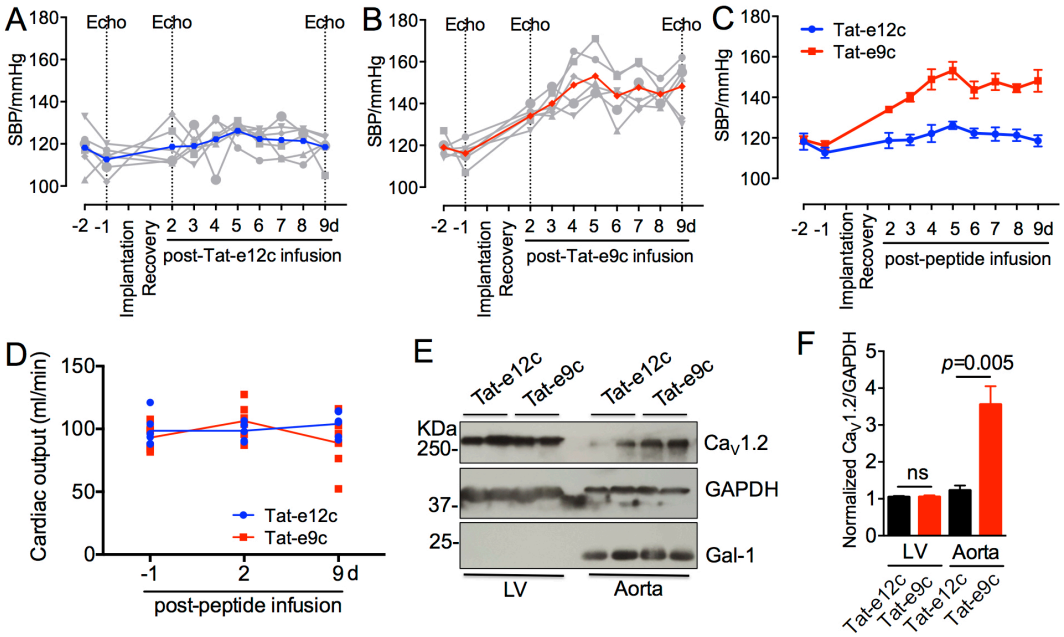

Figure S20. Effect of Tat-e9c on $Ca_V1.2$ expression in left ventricle and cardiac output of rats. (**A, B**) Representative traces of blood pressure demonstrating the time course of Tat-e12c (**A**) or Tat-e9c (**B**) treatment in individual rat are shown as lighter gray traces. The blue or red trace is the average of the lighter traces that represent the single-animal blood pressure. (**C**) Daily systolic blood pressures in rats before, during or after 9-day peptide infusion (n=6 for each group). Osmotic mini-pumps were implanted via jugular vein at day 0. Blood pressure was measured by non-invasive tail-cuff method. Data were analyzed by two-way repeated measures ANOVA ($F(1, 10)=58.048$, $p<0.0001$ for treatment; $F(9, 90)=13.697$, $p<0.00011$ for time; $F(9, 90)=5.441$, $p<0.0001$ for interaction). (**D**) Cardiac output of rats measured at day -1, 2 and 9 post-pump implantation by echocardiography (n=6). Data were analyzed by two-way repeated measures ANOVA ($F(1, 10)=0.658$, $p=0.436$ for treatment; $F(2, 20)=1.076$, $p=0.360$ for time; $F(2, 20)=2.700$, $p=0.092$ for interaction). (**E, F**) Western blots and quantifications of total $Ca_V1.2$ channels in left ventricle and aorta in Tat-e12c- or Tat-e9c-treated rats (n=6). α-smooth muscle actin was used as the loading control.

Supplemental Tables

Table S1. cDNA constructs used in this study

Constructs	Description	Source
$Ca_V1.2$-77wt	Wild-type human $Ca_V1.2$	Our Lab
Galectin-1 (in pIRES2-DsRed, pcDNA3.1(-) and pDsRed-N1 vector)	Cloned from human aorta library	
β_{2a} and β_3 in pIRES2-EGFP	β subunits of $Ca_V1.2$	
$\alpha_2\delta$	$\alpha_2\delta$ subunit of $Ca_V1.2$	David T. Yue
HA-$Ca_V1.2$	Full length $Ca_V1.2$ with extracellular HA tag	Emmanuel Bourinet
GFP-$Ca_V1.2$	Full length $Ca_V1.2$ with GFP tag	Terry Snutch
pGW-β_{2a}	β_2 subunit without GFP tag	
$Ca_V3.1$-GCGGG -GGCGG -GGGGC	Chimeric $Ca_V3.1$ channels featuring the substitution of $Ca_V1.2$ I-II loop, II-III loop or C-terminus into $Ca_V3.1$ channels	Henry Colecraft
HA-ubiquitin HA-Ub-K0 HA-Ub-K11 HA-Ub-K48 HA-Ub-K63 HA-Ub-K48R HA-Ub-K63R	Wild type HA-tagged ubiquitin, and HA-Ub with mutations of one or more lysines	Kah Leong LIM
ERoxBFP	ER-localized oxidizing-optimized oxBFP	Erik Snapp
GST-exon 9-D51A, E52A, E53A, K54A, P55A, R56A, DE5152AA, EE5253AA, DE5153AA, DEE515253AAA, KR5456AA	GST-tagged exon 9 with mutations of residue 51-56 into alanines in different combinations	This study
$Ca_V1.2$-K457&459A, $Ca_V1.2$-K458A	$Ca_V1.2$ mutant lacking Gal-1-binding sites	This study
HA-I-II loop HA-II-III loop HA-C terminus	HA-tagged intracellular loops of $Ca_V1.2$ channels	This study
HA-I-II loop-K4A, K10A, K17A, K410A, K417A, K1017A, K41017A; HA-I-II loop-K21A, K29A, K54A, K2129A, K2154A, K2954A, K212954A	HA-tagged I-II loop with mutations of single, double and triple lysines into alanines	This study
$Ca_V1.2$-K410416423A, $Ca_V1.2$-K427A, K435A, K460A, K427435A, K427460A, K435460A, K427435460A, and $Ca_V1.2$-K410416423A/ K427435460A-ER	Full length $Ca_V1.2$ channels with mutations of lysines or both lysines and ER export signals within I-II loop	This study

Table S2: Primers used in this study

Targets	Primers	Oligonucleotides (5'→3')
GST-exon 9 mutants	E9 FOR	G*GAATTC*AGTTTTCCAAAGAGAGGGAGAAGGC CAAGGC
	E9-D51A REV	G*GTCGAC***TCA**GTTTCGGGGCTTCTCCTCAGCCA TGCCTTC
	E9-E52A REV	G*GTCGAC***TCA**GTTTCGGGGCTTCTCCGCATCCT CCATGC
	E9-E53A REV	G*GTCGAC***TCA**GTTTCGGGGCTTCGCCTCATCC
	E9-K54A REV	G*GTCGAC***TCA**GTTTCGGGGCGCCTCCTCATCCA TGC
	E9-P55A REV	G*GTCGAC***TCA**GTTTCGGGCCTTCTCCTCATCCA TGC
	E9-R56A REV	G*GTCGAC***TCA**GTTTGCGGGCTTCTCCTCATCCA TGC
	E9-DE5152AA REV	GGTCGAC**TCA**GTTTCGGGGCTTCTCCGCAGCC ATGC
	E9-EE5253AA REV	G*GTCGAC***TCA**GTTTCGGGGCTTCGCCGCATCCA TGC
	E9-DE5153AA REV	G*GTCGAC***TCA**GTTTCGGGGCTTCGCCTCAGCCA TGC
	E9-DEE515253AAA REV	G*GTCGAC***TCA**GTTTCGGGGCTTCGCCGCAGCCA TGC
	E9-KR5456AA REV	G*GTCGAC***TCA**GTTTGCGGGCGCCTCCTCATCCA TGC
Template for site-directed mutagenesis	ClaI-FOR	*ATCGAT*CCTGAGAATGAGGACGAAGGCATGG
	SgrAI-REV	*CACCGGCG*CCAGTAGCGGCTGAACTTTGAC
$Ca_V1.2$-DE457&459AA	1C-457459 FOR	GCATGGCTGAGGCGAAGCCCCGAAAC
	1C-457459 REV	GTTTCGGGGCTTCGCCTCAGCCATGC
$Ca_V1.2$-E458A	1C-458 FOR	GCATGGATGCGGAGAAGCCCCGAAAC
	1C-458 REV	GTTTCGGGGCTTCTCCGCATCCATGC
HA-I-II loop	loop1 FOR	GGAATTCATGGAGTTTTCCAAAGAGAGGGAGA AGGCCAAGGC
	loop1-HA REV	GGGATCCTCAAGCGTAATCTGGAACATCGTAT GGGTAGCTGAACTTTGACTTGGAGATCCG
HA-II-III loop	loop2 FOR	GGAATTCATGGACAACCTGGCTGATGCTGAGA GCC
	loop2-HA REV	GGGATCCTCAAGCGTAATCTGGAACATCGTAT GGGTACGTGTCATTGACAATGCGGTGGC
HA-C-terminus	CT FOR	GGAATTCATGGACAACTTTGACTACCTGACAA GGGACTGGTCC
	CT-HA REV	GGGATCCTCAAGCGTAATCTGGAACATCGTAT GGGTACAGGCTGCTGACGTAGACCC

Table S3: Primers used in this study

Targets	Primers	Oligonucleotides (5'→3')
HA-loop1-K4A	K4A FOR	GAGTTTTCCGCAGAGAGGGAGAAGG
	K4A REV	CCTTCTCCCTCTCTGCGGAAAACTC
HA-loop1-K10A	K10A FOR	GAGAAGGCCGCGGCCCGGGGAGATTTC
	K10A REV	GAAATCTCCCCGGGCCGCGGCCTTCTC
HA-loop1-K17A	K17A FOR	GAGATTTCCAGGCGCTGCGGGAG
	K17A REV	CTCCCGCAGCGCCTGGAAATCTC
HA-loop1-K41017A (or $Ca_V1.2_{-K410416423A}$)	K41017A-FOR	GTTTTCCGCAGAGAGGGAGAAGGCCGCGGCCC GGGGAGATTTCCAGGCGCTGCGG
	K41017A-REV	CCGCAGCGCCTGGAAATCTCCCCGGGCCGCGG CCTTCTCCCTCTCTGCGGAAAAC
HA-loop1-K21A (or $Ca_V1.2_{-K427A}$)	K21A FOR	GAGATTTCCAGAAGCTGCGGGAGGCGCAGCAG CTAGAAGAG
	K21A REV	CTCTTCTAGCTGCTGCGCCTCCCGCAGCTTCTG GAAATCTC
HA-loop1-K29A (or $Ca_V1.2_{-K435A}$)	K29A FOR	GAGAAGCAGCAGCTAGAAGAGGATCTCGCAG GC
	K29A REV	GCCTGCGAGATCCTCTTCTAGCTGCTGCTTCTC
HA-loop1-K54A (or $Ca_V1.2_{-K460A}$)	K54A FOR	GAAGGCATGGATGAGGAGGCGCCCCGAAAC
	K54A REV	GTTTCGGGGCGCCTCCTCATCCATGCCTTC
Template for $Ca_V1.2$ with mutant ub sites and ER export signals	-65 FOR (vector sequence)	GGAGACCCAAGCTTGCTTGTTC
	1635 REV	GTGAGCCAGTTGGGCTGGTTGTAG
$Ca_V1.2_{-K410416423A-ER}$	For	GAATGCGGCCGCAGGCATGGATGCGGAGAAGC CCCG
	Rev	CGGCCGCATTCGCAGGAGCGATGGCTTCGGCC TGAG
$Ca_V1.2_{-K427435460A-ER}$	For	GAATGCGGCCGCAGGCATGGATGCGGAGGCGC CCCG
	Rev	CGGCCGCATTCGCAGGAGCGATGGCTTCGGCC TGAG
Gal-1 in pDsRed-N1	For	GCTCGAGATGGCTTGTGGTC
	Rev	GGGATCCGTCAAAGGCCACAC

Table S4. Antibodies used in this study

Antibodies against:	Catalogue#	Source
$Ca_V1.2$	ACC-003	Alomone
$Ca_V\beta_2$	ACC-105	
$Ca_V\beta_3$	ACC-008	
$Ca_V3.1$	ACC-021	
Galectin-1	437400	Invitrogen
HA tag	71-5500	
Ubiquitin	13-1600	
Transferrin receptor (TfR)	13-6800	
α-SM-actin	A5228	Sigma
β-actin	A1978	
GST tag	sc-138	Santa Cruz
Goat Galectin-1	ab21027	Abcam
Goat α-SM-actin	AF1245	R&D system

Table S5. Prediction of ubiquitination sites (lysines) in full length $Ca_V1.2$ channels

Domain	lysine position	Score	Confidence
I-II loop (407-525)	410	0.79	M
	416	0.74	
	423	0.82	
	427	0.91	H
	435	0.76	M
	460	0.9	H
II-III loop (754-900)	767	0.81	M
	773	0.71	
	787	0.82	
	793	0.74	
	800	0.78	
	803	0.78	
	807	0.86	H
	820	0.9	
	834	0.68	L
	868	0.84	H
	870	0.82	M
	1211	0.62	
C-terminus (478-2138)	1681	0.75	M
	1684	0.8	
	1732	0.94	H
	1747	0.96	
	1840	0.9	
	1876	0.78	M
	1917	0.79	
	1981	0.94	H

Legend: Low confidence (L), 0.62 < score < 0.69; Medium confidence (M), 0.69 < score < 0.84; High confidence (H), 0.84 < score < 1.00.

Table S6. Information of hypertensive patients

	Specimen code	Age/years	Non-cardiac diseases	Anti-hypertension medicines
Non-hypertension (IMA)	2013153	48		
	2013158	66		
	2013165	69		
	2013168	55		
	2013174	63		
	2013178	69		
	2013180	59		
	2013185	57		
	2013238	38	HLD	
	2013246	67	HLD	
	2013248	65	HLD, DM	
Hypertension (IMA)	201349	75	HTN, HLD	ATENOLOL 50MG OM, LOSARTAN 100MG OM (selective β1 receptor antagonist)
	201357	50	HTN, HLD, DM	BISOPROLOL 2.5MG OM, ENALAPRIL 5MG OM (beta-blockers)
	201373	70	HTN	BISOPROLOL2.5MG OM PERINDOPRIL 2MG OM (beta-blockers)
	201384	65	HTN, DM	LISINOPRIL 2.5MG OM, BISOPROLOL 5MG OM (angiotensin converting enzyme inhibitor)
	201388	59	HTN, DM	VALSARTAN 40mg OM (angiotensin receptor blockers)
	201392	70	HTN, HLD, DM	BISOPROLOL 2.5MG OM, ENALAPRIL 10MG BD (beta-blockers)
	2013113	64	HTN, HLD, DM	BISOPROLOL 1.25MG OM, ENALAPRIL 2.5MG BD (beta-blockers)
	2013232	57	HTN, HLD, Smoker	
	2013233	66	HTN, HLD	
	2013234	63	HTN, HLD, DM	
	2013237	63	HTN, HLD, DM	

HTN, Hypertension; HLD, Hyperlipidemia; DM, Diabetes Mellitus

Table S7. Information for pulmonary arteries from heart transplant patients

Samples	Age	Diagnosis	PA collection date	LVAD	Pre-LVAD PASP/mmHg	Pre-Transplant PASP/mmHg
HTN01 (Recipient)	50	Dilated CMP	26/08/2013	2011	68	25
Non-HTN01 (Donor)				NA		
HTN02 (ecipient)	51	Dilated CMP	22/02/2016	2011	44	19
Non-HTN02 (Donor)				NA		
HTN03 (Recipient)	50	Ischemic CMP	08/05/2016	2014	64	35
Non-HTN03 (Donor)				NA		

Note: All donors have normal hearts, so no pulmonary pressure assessment on echocardiography as all would not have tricuspid regurgitation. HTN, Hypertension; CMP, Cardiomyopathy; LVAD, Left ventricular assist device; PASP, Pulmonary artery systolic pressure.