Supplementary figures

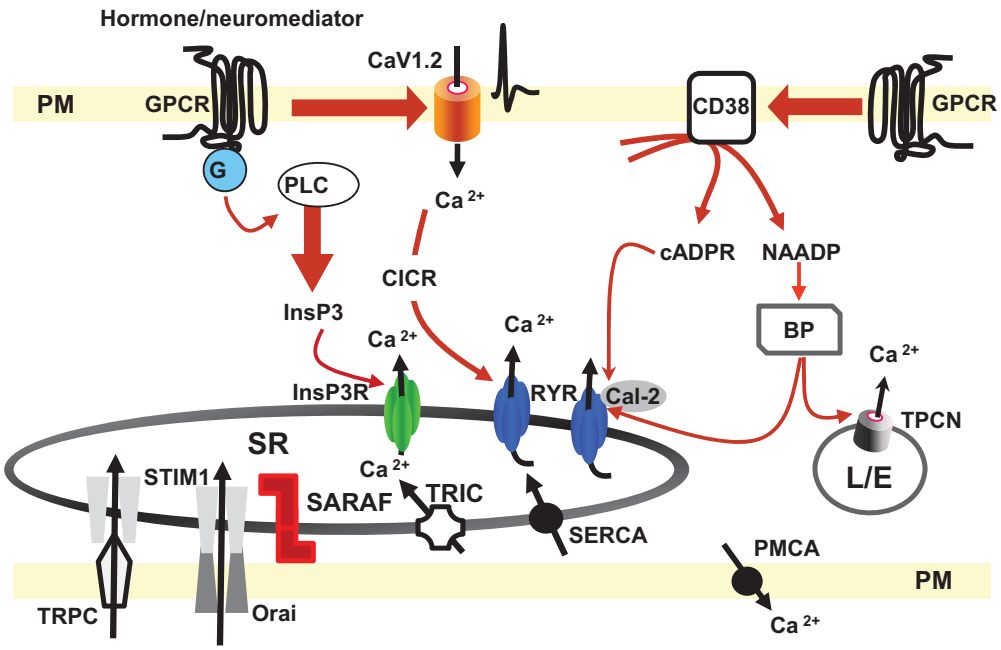

Figure S1: Ca^{2+} channels and pumps implicated in Ca^{2+} signalling in VSMC. GPCR (G-protein coupled receptors) located in plasma membrane (PM) activate transduction pathways to produce InsP3 (via activation of phospholipase C, PLC) or cADP (via CD38 activation) evoking a Ca^{2+} signal by the binding on their receptors (InsP3R and RyR respectively). They can also activate the production of NAADP that binds on a specific binding protein (BP) to activate RyR and TPCN located in SR (sarcoplasmic reticulum) and L/E (lysosome or endosome) membranes, respectively. The voltage-gated Ca^{2+} channels (CaV1.2) activated by GPCR or depolarization evoked a Ca^{2+} entry responsible for the Ca^{2+}-induced Ca^{2+} release mechanism (CICR). After activation of Ca^{2+} signals, the return to the basal level is due to the activation of SERCA and PMCA, two Ca^{2+} ATPases located in SR and plasma membrane (PM). The SR Ca^{2+} refilling is thus the sum of SERCA activation and store operated Ca^{2+} entry (SOCE) due to Orai and TRPC channels, as well as STIM, SARAF and TRIC proteins located in PM and SR, respectively.

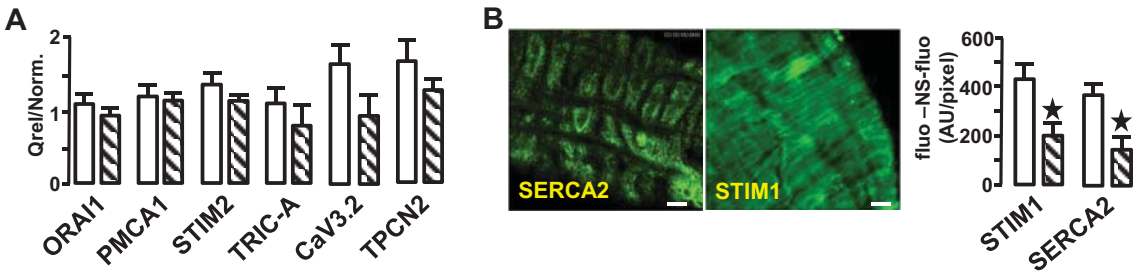

Figure S2: **(A)** Mean of gene expression levels quantified after RT-q-PCR in posterior cerebral arteries (PCA) from young (open bars) and old (hatched bars) mice. **(B)** Evaluation of protein expression by immunolabelling. Immunohistofluorescence obtained in PCA from mice and mean fluorescence in young (open bars) and old mice (hatched bars). Means calculated with 6 different mice for each age; scale bar: 5 µm.

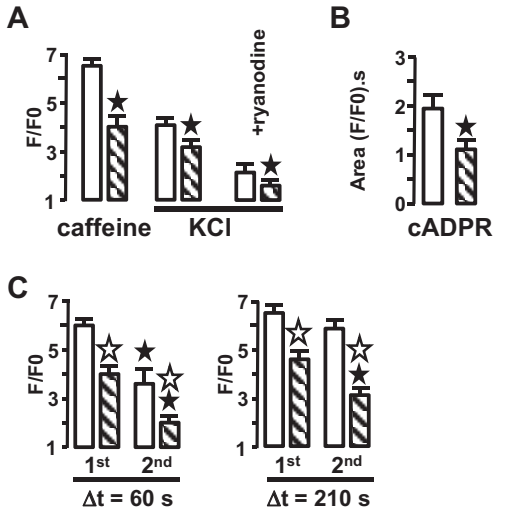

Figure S3: **(A)** Means of amplitude of Ca^{2+} responses induced by caffeine and KCl in control condition and in presence of ryanodine. **(B)** Means of area of cADPR-induced Ca^{2+} response. **(C)** Means of amplitude of two successive caffeine-evoked calcium responses separated by 60 s (left) and 210 s (right). ☆ p<0,05 between young (open bars) and old (hatched bars) mice. ★ p<0,05 between the 1st and the 2nd responses.

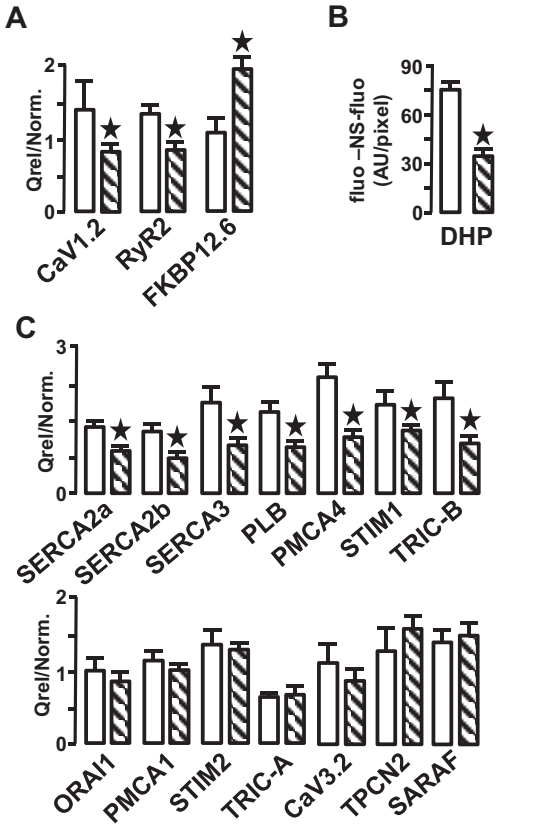

Figure S4: **(A, C)** Means of expression of pumps and channels responsible for Ca^{2+} signals measured by RT-qPCR in young (open bars) and old (hatched bars) mice. **(B)** Means of fluorescence emitted by ST-bodipy(-)-DHP in middle cerebral arteries (MCA). Data are expressed as mean ±sem, ★ p < 0.05.

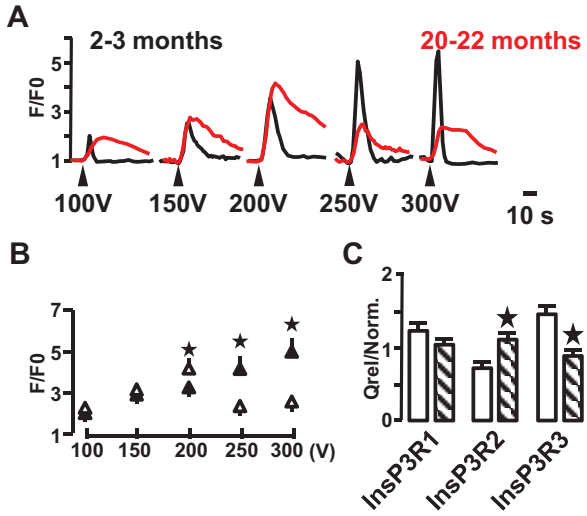

Figure S5: **(A)** Typical InsP3-induced Ca^{2+} response observed in middle cerebral arteries (MCA). **(B)** Means of amplitude of Ca^{2+} responses induced by InsP3 photolysis observed in young (▲) and old mice (△). **(C)** Means of expression of InsP3R subtypes measured by RT-qPCR in young (open bars) and old (hatched bars) mice. Data are expressed as mean ±sem, ★ p < 0.05.

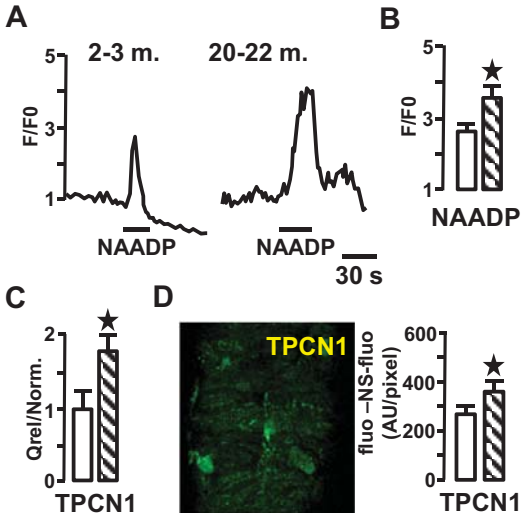

Figure S6: **(A)** Typical NAADP-induced Ca^{2+} response observed in middle cerebral arteries (MCA). **(B)** Means of amplitude of Ca^{2+} responses induced by NAADP. **(C)** Means of TPCN1 expression measured by RT-qPCR. **(D)** Mean fluorescences emitted by immunostaining with anti-TPCN1 antibody in young (open bars) and old (hatched bars) mice. Data are expressed as mean ±sem, ★ p < 0.05.

Supplementary methods

Table of primers: The efficiency, optimal Tm and dimerization of primers were tested and verified before qPCR experiments.

Gene of interest	Primer (5' -> 3')	Tm	Accession Number
CaV1.2	For : GACGTTCCCCCAGGCTGTGTTACT	60	NM_001256002.1
	Rev : GTGATGGGGACCGAGGATAGACC		
RyR2	For : CATGGACAGCTTCCCCTGAA	60	NM_023868.2
	Rev : GTGTGACTGCCGTGCTTGG		
FKBP12.6	For: CCCCAGGAGACGGAAGGACA	60	NM_016863.3
	Rev : GTGGGGATGATTAAATGGCTG		
IP3R1	For : TGGCAGAGATGATCAGGGAAA	59	NM_010585.5
	Rev : GCTCGTTCTGTTCCCCTTCAG		
IP3R2	For : GCTCAGATGATCACGGAGAAG	59	NM_010586.1
	Rev : ATCTCATTTTGCTCACTGTCACCT		
IP3R3	For : TCATTGTACTGGTCCGAGTCAAGA	59	NM_080553.3
	Rev : GCGGGAACCAGTCCAGGT		
SERCA2a	For : TCATGGATGAGACGCTCAAG	60	NM_001110140.3
	Rev : AGGGAGCAGGAAGATTTGGT		
SERCA2b	For : TTGGGTTTCCTGAGGCTTTA	61	NM_009722.3
	Rev : GTCCAGGTCTGGAGGATTGA		
SERCA3	For : TCTCGAATCGTGGAGAACCT	60	NM_016745.3
	Rev : CCGATCTCTGCCTTCTTCAG		
PLB	For : CGAAGCCAAGGTCTCCTAAA	60	NM_023129.5
	Rev : TAGCCGAGCGAGTGAGGTAT		
PMCA1	For : TTAGTCTGGGAAGCATTACAAGATGTCAC	60	NM_026482.2
	Rev : CTTCTTCCCCAACAGAAACTTCTCC		
PMCA4	For : ACGTCTTCCCACCCAAGGTTC	60	NM_213616.4
	Rev : CCAGCAGCCCACACTCTGTC		
STIM1	For : GCTCTCAATGCCATGCCTTCCAAT	60	NM_009287.4
	Rev : TCTAGGCCATGGTTCAACGCCATA		
STIM2	For : AGGGCAACTTGACACAGACAGGAT	60	NM_001081103.2
	Rev : ATCAGGGTTGTTGGAAGTCG		
ORAI-1	For : TCCACGGTCATCGGGACGCT	60	NM_175423.3
	Rev : GTCGCTGTGGTTGGCGACGA		

TPCN1	For : ACCTCGCTCTGTCTTCCTGA	60	NM_145853.2
	Rev : GAGGGCTTCCAGAGTTTTCC		
TPCN2	For : ATGAAGCACAGGACCAGGAG	60	NM_146206.4
	Rev: ATCAGGGTTGTTGGAAGTCG		
SARAF	For : CTTGAGCTAGGTGGCTTTGG	60	NM_026432.3
	Rev : AGTAGTCGGCACTGGGCTTA		
TRIC-A	For : GTGTCCAAGGCCAGCCTCAT	60	NM_144534.1
	Rev : CCAAACAGCACTGGGCAGAT		
TRIC-B	For : AAGGTGATGAATGGCTGAAGATGTC	60	NM_028053.2
	Rev : ATGCTTTGAGATCGCCAGGTG		

Immunohistofluorescence staining: Cerebral arteries were fixed during 15 minutes in 4% (g per 10^{-3} L) paraformaldehyde solution in 0.1 mol.L^{-1} phosphate buffer (PB, pH = 7.4). Fixed arteries were rinsed 5 times during 10 minutes each in PB. Vessels were transferred in a saturation and permeabilization solution (SPS: PB containing 1% bovine serum albumin (BSA), 2% donkey serum and 0.2% triton X100) during 60 minutes and the primary antibody (1:100, 48h, 4°C) was added. After 4 rinses (10 minutes each) in SPS, vessels were placed in SPS containing a secondary antibody (1:200, 2h, 22°C) for 2 hours at room temperature. Arteries were rinsed 3 times (10 minutes each) in PB. Samples were mounted in Fluoromount G medium and observed with SP5. All parameters of the SP5 were kept constant to evaluate the fluorescence levels and compare the immunostaining obtained in arteries from yound and old animals.

Antibodies: anti-FKBP12 (PA1-026A, Thermo Scientific, Brebieres France), anti-RyR2 (AB9080, Merck-Millipore, Nottigham, UK), anti-STIM1 (4119, ProSci Inc, Interchim, Montluçon) were produced in rabbits; anti-SERCA2 (F-1, sc-376235, Santa Cruz Biotechnology, Tebu-bio, Le-Perray-en-Yvelines France) was produced in mice and anti TPCN1 (C-14, sc-67973) was produced in goats. Secondary antibodies were from FluoProbes (Interchim) and were against rabbit, mouse, and goat IgG (FP-SA5110, FPDAMOTTGX546 and FP-DAGOTTGX488, respectively.