SUPPLEMENTARY INFORMATION

Translocon closure to Ca^{2+} leak in proliferating vascular smooth muscle cells

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SUPPLEMENTARY METHODS

Cells

Ventricular myocytes were obtained from rats killed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.

85-23, revised 1996). Hearts were mounted on a Langendorff apparatus and perfused at 5 $ml.min^{-1}$ at 37 °C with solution of the following composition (mM): NaCl 130, KCl 5.4, $MgCl_2$ 1.4, NaH_2PO_4 0.4, Hepes 5, glucose 10, taurine 20, creatine 10, pH 7.3. The solution first contained 750 μM $CaCl_2$ for 4 min and then was Ca^{2+}-free (containing 100 μM Na_2EGTA) for a further 4 min. Finally, the solution contained 200 μM $CaCl_2$ and collagenase (Worthington type 2; 0.1 mg ml^{-1}) for 9-12 min. The left ventricle was dissected and finely chopped in an enzyme-containing solution with 1 % bovine serum albumin and gently agitated in a water bath at 37 °C. Aliquots of the cell suspension were examined every 5 min until a >80 % yield of rod-shaped cells with a clear striation pattern was obtained. Myocytes were collected by filtration through nylon gauze and gentle centrifugation.

Chemicals and ionic solutions

General salts were from Sigma (UK). Ryanodine was from MP Biomedicals LLC (France) and caffeine was from Acros Organic (USA). Ventricular myocytes were superfused with (mM): NaCl 113, Na acetate 20, KCl 5, $MgSO_4$ 1, Na_2HPO_4 1, $CaCl_2$ 2, Hepes 10 and glucose 10 (pH 7.4).

Ca^{2+} measurement

Ventricular myocytes were incubated with 6 μM fluo-4 AM for 15 min prior to a wash period of 0.5 hr. They were then placed in a chamber on the stage of a Nikon Diaphot Eclipse TE2000 inverted microscope and viewed using a confocal laser-scanning unit (Microradiance, Bio-Rad,

Herts, UK) via a 60x water immersion lens (NA 1.2). Fluo-4 was excited at 488 nm and emitted fluorescence collected at >515nm. Recordings were made at room temperature.

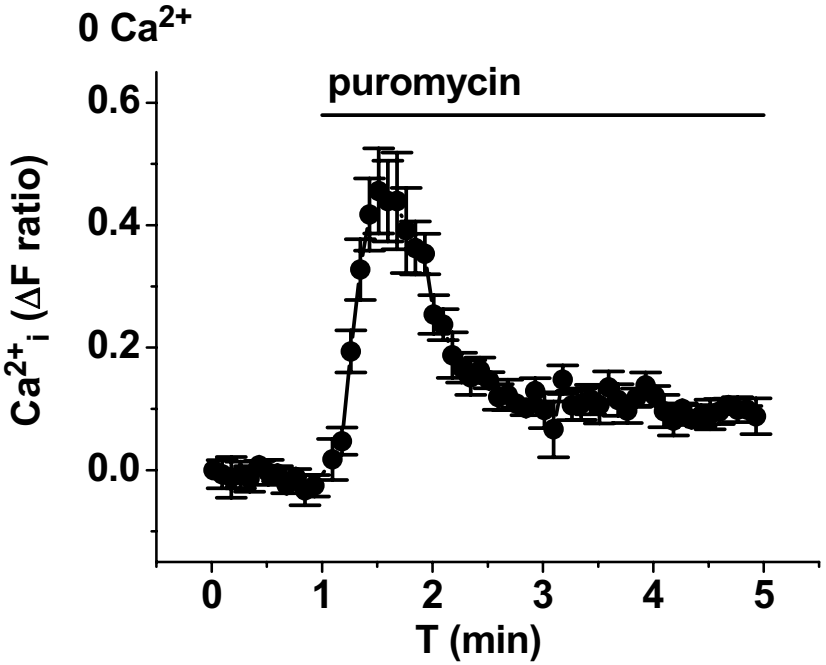
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Puromycin-evoked Ca^{2+}-release in vascular smooth muscle cells (VSMCs) at 37 °C. The graph shows measurement of intracellular Ca^{2+} in the absence of extracellular Ca^{2+} (0 Ca^{2+}) and the response to extracellular puromycin (200 μM).

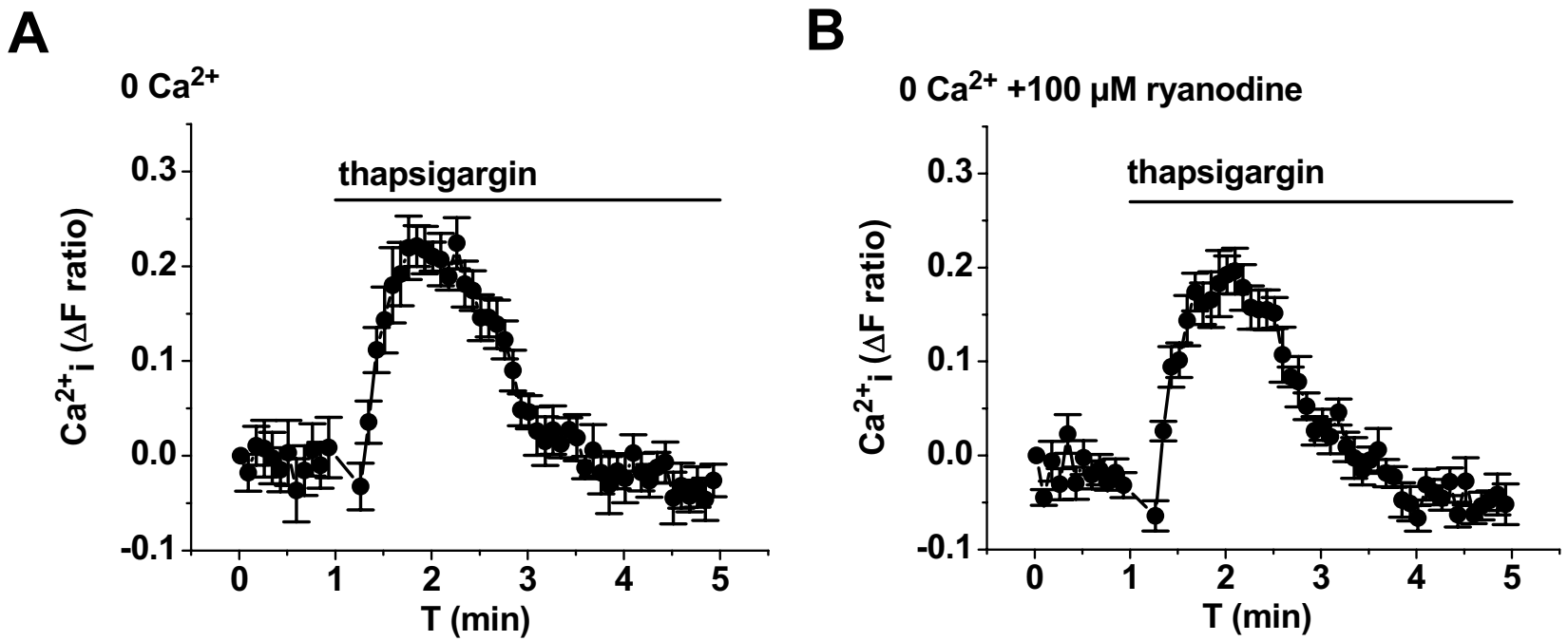
Supplementary Figure 2. Lack of effect of ryanodine on constitutive Ca^{2+} leak. Intracellular Ca^{2+} in VSMCs in the absence of extracellular Ca^{2+} (0 Ca^{2+}) and showing the effect of extracellular thapsigargin (3 μM) after pretreatment with (A) vehicle control and (B) ryanodine (100 μM).

Supplementary Figure 3. Failure of caffeine to evoke Ca^{2+} release in VSMCs. Intracellular Ca^{2+} in VSMCs showing the effect of extracellular caffeine (10 mM) in the presence of (A) 1.5 mM Ca^{2+} and (B) 10 mM Ca^{2+}.

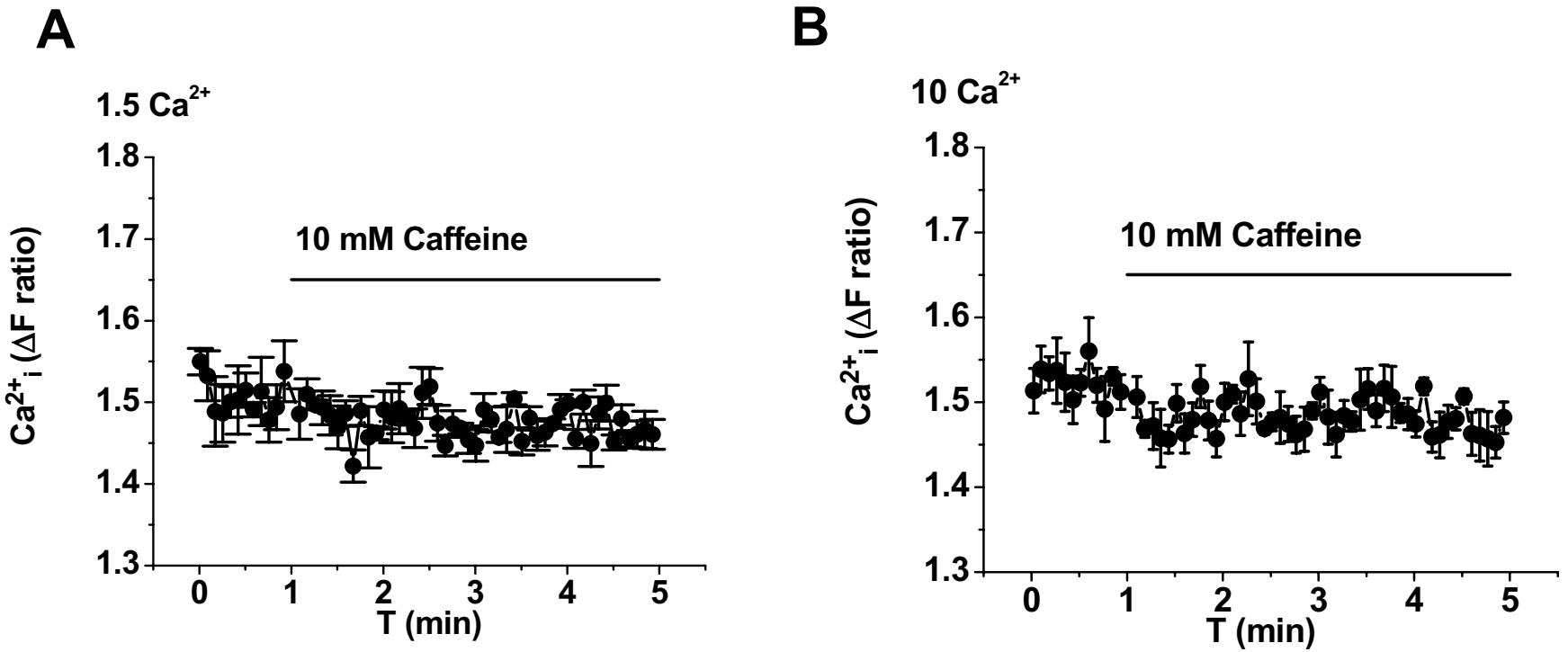
Supplementary Figure 4. Caffeine evoked Ca^{2+} release in cardiac ventricular myocytes. Intracellular Ca^{2+} is indicated by fluo-4 fluorescence at the time of measurement divided by the initial fluorescence (F/F_o). The lower colour trace is a representation of the confocal line-scan across the myocyte where increasing brightness (yellow) indicates increasing Ca^{2+} concentration. Shown are the effects of electrical field stimulation and extracellular caffeine (10 mM).



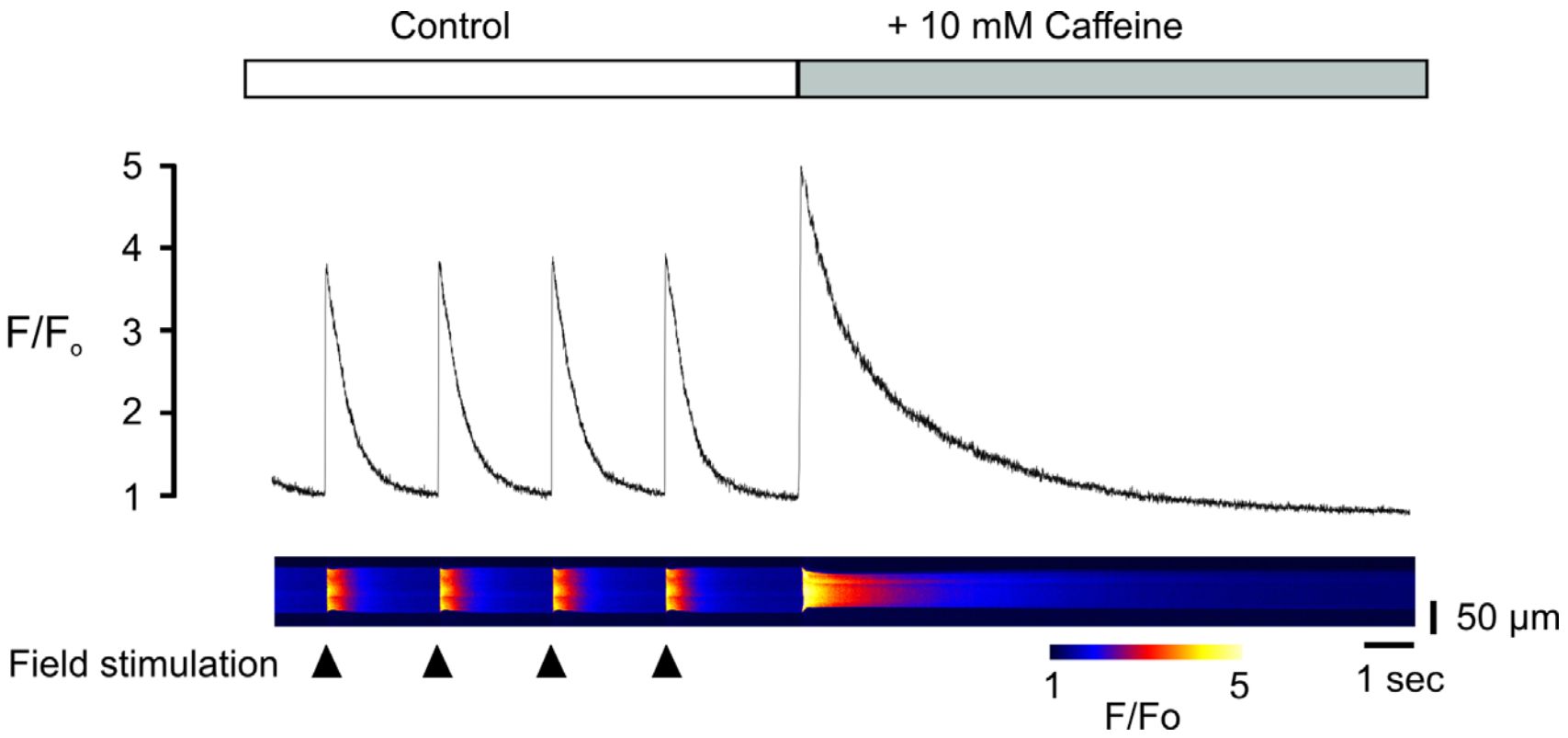
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4