SUPPLEMENTARY INFORMATION

Interactions, functions and independence of plasma membrane

STIM1 and TRPC1 in vascular smooth muscle cells

Jing Li1,2, Piruthivi Sukumar‡1,2, Carol J Milligan‡1,2, Bhaskar Kumar1, Zhi-yong Ma1,2, Christopher M Munsch4, Lin-Hua Jiang1, Karen E Porter1,3 & David J Beech*1,2

1Multidisciplinary Cardiovascular Research Centre and 2Institute of Membrane & Systems Biology, Faculty of Biological Sciences and 3Faculty of Medicine & Health, University of Leeds, Leeds, LS2 9JT, UK. 4Yorkshire Heart Centre, General Infirmary at Leeds, Leeds, LS1 3EX, UK.

‡These authors contributed equally

*Author for correspondence: Prof David J Beech, Faculty of Biological Sciences, Garstang Building, Mount Preston Street, University of Leeds, Leeds, LS2 9JT, England, UK; d.j.beech@leeds.ac.uk; Tel +44 (0) 113 34 34323/4727; Fax +44 (0) 113 34 34228.

SUPPLEMENTARY MATERIALS AND METHODS

RNA isolation and quantitative RT-PCR

Total RNA was extracted from saphenous vein smooth muscle cells using a Tri-reagent protocol followed by DNase I (Ambion) treatment1. 1 μg of total RNA was used for reverse transcription (RT) based on oligo-dT primers and AMV RT enzyme. The specificity of PCR was verified by reactions without RT (-RT) and by melt-curve analysis of PCR products. Sequences of PCR primers are in Supplementary Table I. PCR products were electrophoresed on 2 % agarose gels
containing ethidium bromide. No PCR products occurred in the absence of RT. With RT there was only a single product of the correct size, giving a single peak in the melt-curve. All PCR products were sequenced to confirm identity (Lark, UK). Real-time PCR was carried out using a Lightcycler (Roche) largely as described previously. Relative abundance of target RNA was normalized to β-actin RNA, which showed no difference between samples. PCR efficiency (E) was $10^{(-1/slope)}$. Relative abundance of target RNA was calculated from $(E_{β-actin}^{Cp}) / (E_{target}^{Cp})$, where PCR cycle crossing-points ($C_p$) were determined by fit-points methodology. PCR reactions were at least in duplicate.

**Anti-TRPC5 antibody**

Antibody targeted to the C-terminus of TRPC5 (T5C3) has been described. Permeabilisation of cells was achieved with 0.1 % Triton X-100 in 1 % bovine serum albumin for 2 h.

**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure I.** Effectiveness and specificity of siRNA probes. (a-c) siRNA knock-down of STIM1 mRNA. (a) Using STIM1 PCR primers, a melt-curve analysis of PCR product showing change in SYBR Green I fluorescence (F) with temperature (t). When there was reverse transcriptase (+RT) reaction a single peak occurred, corresponding to the single band on the gel of Fig 1a. (b) PCR amplification curves following treatment with scrambled siRNA (control) or siRNA targeted to STIM1 mRNA (black circles). The rightward shift shows reduced abundance of STIM1 mRNA caused by STIM1 siRNA. (c) Relative abundance of mRNA species after STIM1 siRNA treatment and normalization to paired control data (only one bar is indicated as
'scrambled' but each data set was normalized to its own scrambled siRNA control data; \( n \geq 3 \) in each case). (d) Similar to the data of (e) but showing the effectiveness of each TRPC siRNA against its own mRNA species. (e) Lack of effect of the TRPC1+4+5 siRNA cocktail on STIM1 mRNA (\( n \geq 3 \)).

**Supplementary Figure II.** Background Ca\(^{2+}\)-entry was small compared with Ca\(^{2+}\)-entry after store-depletion. Shown is a typical example of the Ca\(^{2+}\)-add back response with (+) or without (-) store-depletion evoked by 1 \( \mu \)mole/L thapsigargin (TG) (16 wells per data point).

**Supplementary Figure III.** Lack of effect of STIM1 siRNA on agonist-evoked Ca\(^{2+}\)-release. (a) Summary data for paired experiments exploring peak agonist-evoked Ca\(^{2+}\)-release after treatment with STIM1 siRNA normalized to control. The agonists tested were adenosine triphosphate (ATP 10 \( \mu \)mole/L: 20 wells) and angiotensin II (Ang II 0.1 \( \mu \)mole/L: 24 wells); data are for two independent experiments. (b) Agonist responses were not lost in Ca\(^{2+}\)-free solution. Responses were compared in paired experiments: in the presence of 1.5 mmole/L extracellular Ca\(^{2+}\); in the absence of Ca\(^{2+}\) and presence of 0.4 mmole/L EGTA to chelate residual Ca\(^{2+}\). ATP, \( n = 12 \) wells per column; Ang II, \( n = 24 \) wells per column.

**Supplementary Figure IV.** Confirmation that intracellular targets were inaccessible in non-permeabilised cells. Antibody to the TRPC5 C-terminus (intracellular) labeled protein (FITC, green) in permeabilised but not non-permeabilised cells. Labeling with anti-TRPC5 antibody was absent when it was preadsorbed to its antigenic peptide (+peptide). Where FITC fluorescence was not apparent, the presence of cells was detected by DAPI fluorescence (not shown). Scale bar, 20 \( \mu \)m.
**SUPPLEMENTARY TABLE**

**Supplementary Table I:** PCR primer pairs (F, forward direction; R, reverse direction), PCR amplicon sizes, and siRNA probes sequences.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer 5’-3’</th>
<th>Predicted amplicon (bp)</th>
<th>siRNA sequence (5’-3’)</th>
</tr>
</thead>
</table>
| STIM1 | F  CTCTCTTGACTCGCCA  
R  GCTTAGCAAGGTTGATCT | 276 | CAAUUCGGCAAAACUCUGCtg  
GCAGAGUUUUGCCGAAUUUGtt |
| TRPC1 | F  TTAGCGCATGTGGCAA  
R  CCACCTACTGAGGCTACTAAT | 303 | GCCCGGAAUUUCUGAAUUt  
AUUCACGAGAAUUCCGCGGt |
| TRPC4 | F  ATTAGCTTCACGGGGT  
R  CTTCGTGGGTGACTGT | 241 | GGAGGUACUCUGCCUACUTt  
GAGUAGGCAGAGUACCUCt |
| TRPC5 | F  ACATTTTAAGTTCGTTGCG  
R  ACATCGGATCCCTTG | 218 | GCAACCUPGGGCUUGUCAUt  
AUGAACAGCCCAAGGUUGCtc |
SUPPLEMENTARY REFERENCES


**Figure Legends**

**Supplementary Figure I**

**a**

Graph showing fluorescence intensity as a function of temperature for STIM1.

**b**

Graph showing fluorescence intensity as a function of PCR cycle for scrambled and STIM1 siRNA.

**c**

Bar graph showing normalised RNA levels for STIM1, TRPC1, and β-actin for scrambled and STIM1 siRNA.

**d**

Bar graph showing normalised RNA levels for TRPC1, TRPC4, and TRPC5 mRNAs for scrambled and TRPC1, 4, or 5 siRNA.

**e**

Bar graph showing normalised RNA levels for STIM1 mRNA for scrambled and TRPC1+4+5 siRNA.

*Li et al* Supplementary Figure I
Supplementary Figure II

Li et al
Ang II

ATP / Ang II

Ca^{2+} / EGTACa^{2+}

ATP

Normalised peak Ca^{2+}

Li et al  Supplementary Figure III