

SUPPLEMENTARY ONLINE DATA

Activation of IP₃ receptors requires an endogenous 1-8-14 calmodulin-binding motif

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			1	8	14		
MLCK peptide			RRK	WQKTGHAVRAIGRL			+6
1-8-14 consensus			1	XXX5XX8	XXXXXX14		
rat	IP ₃ R1	46	LNNPP	KKFRDCLF	KLC	PMNRYSAQKQ	71 +4
rat	IP ₃ R2	46	LTNPP	KKFRDCLF	KVC	PMNRYSAQKQ	71 +4
rat	IP ₃ R3	45	LDNPP	KKFRDCLF	KVC	PMNRYSAQKQ	70 +4
chicken	IP ₃ R1	46	LNNPP	KKFRDCLF	KLC	PMNRYSAQKQ	71 +4
chicken	IP ₃ R2	48	LANPP	KKFRDCLF	KVC	PMNRYSAQKQ	73 +4
chicken	IP ₃ R3	45	LDNPP	KKFRDCLF	KVC	PMNRYSAQKQ	70 +4
frog	IP ₃ R1	46	LNNPP	KKFRDCLF	RLC	PMNRYSAQKQ	71 +4
frog	IP ₃ R2	46	LANPP	KKFRDCLF	KVC	PMNRYSAQKQ	71 +4
frog	IP ₃ R3	45	LDNPP	KKFRDCLF	RVC	PMNRYSAQKQ	70 +4
<i>Drosophila</i>	IP ₃ R	49	LSCPP	KKFRDCLI	KIC	PMNRYSAQKQ	74 +4
<i>C. elegans</i>	IP ₃ R	124	PESPP	KKFRDCLF	KVC	PVNRYSAAQKH	149 +4
rabbit	RyR1	59	PP-DL	AICCF	TL	EQSLSV	75 -2
rabbit	RyR2	59	PP-DL	SICTF	VLE	QSLLV	75 -2
rabbit	RyR3	59	PP-DL	CVCNF	VLE	OSLSV	75 -2

Figure S1 A conserved 1-8-14 motif in all IP₃Rs and RyRs

Alignments (with first and last residues numbered) of the N-terminal region of rat IP₃R1–IP₃R3 (SwissProt accession numbers NP_001007236, NP_112308 and NP_037270 respectively), chicken IP₃R1–IP₃R3 (SwissProt accession numbers XP_414438, XP_001235613 and XP_418035 respectively), *Xenopus* IP₃R1–IP₃R3 (SwissProt accession numbers NP_001084015, ABP88141 and ABP88140 respectively), *Drosophila* IP₃R (SwissProt accession number NP_730942), *Caenorhabditis elegans* IP₃R (SwissProt accession number NP_001023170) and rabbit RyR1–RyR3 (SwissProt accession numbers P11716, P30957 and Q9TS33 respectively) highlighting the residues proposed to form a 1-8-14 CaM-binding motif. The consensus sequence for a 1-8-14 motif is shown in the first row, with its three critical (1, 8 and 14 hydrophobic residues) and net charge of +3 to +6. A similar 1-8-14 motif is conserved in all IP₃R, which closely resembles a type A (1-5-8-14) motif, where position 5 is also a large hydrophobic residue. The motif within IP₃Rs differs from a classic 1-8-14 consensus sequence by having a tyrosine residue at position 14. All subtypes of RyR also have a similar 1-8-14 motif within a similar position in the three-dimensional structure, although the sequence lacks the usual net positive charge of a consensus 1-8-14 motif.

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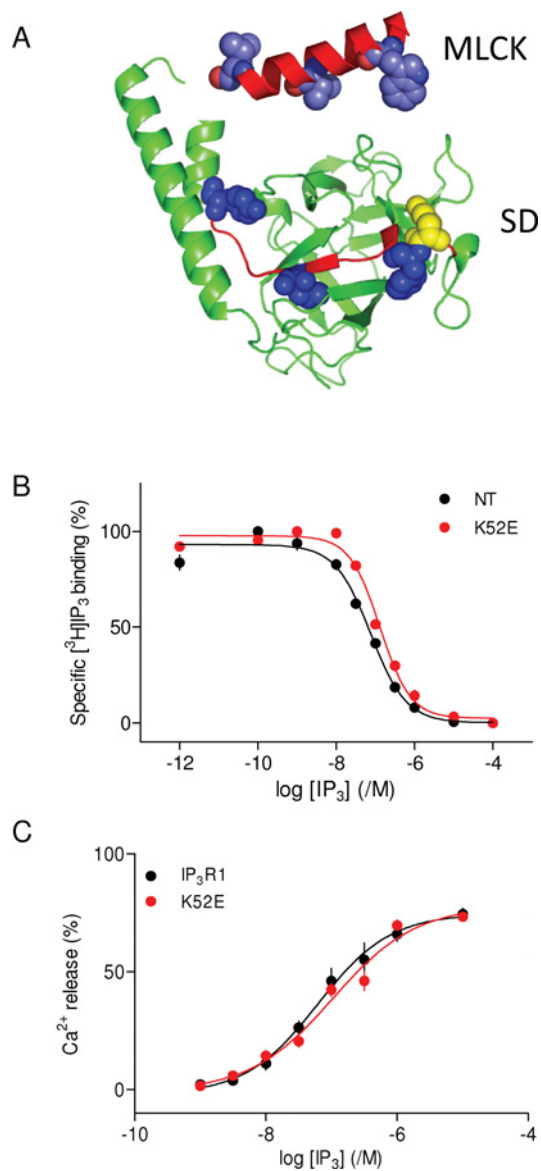


Figure S2 Mutation of a non-critical residue (K52E) within the 1-8-14 motif has no effect on IP_3 binding or IP_3 -evoked Ca^{2+} release

(A) Structure of the SD of $\text{IP}_3\text{R1}$ (PDB code 1XZZ) highlighting the 1-8-14 motif (red), the critical 1-8-14 hydrophobic residues (blue) and Lys^{52} (yellow). (B) Equilibrium competition binding of IP_3 (with 0.75 nM $^3\text{H}]\text{IP}_3$) to native NT and NT^{K52E} . (C) IP_3 -evoked Ca^{2+} release from DT40- $\text{IP}_3\text{R1}$ and DT40- $\text{IP}_3\text{R1}^{\text{K52E}}$ cells. Results are means \pm S.E.M. ($n \geq 3$).

Table S1 Peptides used in the present study

All peptides were synthesised by Sigma or New England Peptide. The isoelectric point (pI) is shown for each peptide calculated from <http://www.innovagen.se/custom-peptide-synthesis/peptide-property-calculator/peptide-property-calculator.asp>. Ac, acetyl.

Peptide	Sequence	Source	pI
MLCK	Ac-RRKWQKTGHAVRAIGRL-NH ₂	Ca ²⁺ –CaM-binding site of smooth muscle MLCK	14.0
1-8-14	Ac-KKFRDALFKLAPMNRV-NH ₂	Fragment of IP ₃ R1 (residues 51–66) containing the 1-8-14 motif	11.6
1-8-14 ^C	Ac-KKERDALFKLAPMNRV-NH ₂	Inactive form of 1-8-14 peptide (mutations highlighted in bold and underlined)	10.8
1-8-14 ^S	Ac-AMRFLKYLKRFDKNA-NH ₂	Scrambled form of 1-8-14 peptide	11.6
1-8-14 ^L	Ac-LNNPPKFRDALFKLAPMNRVSAQKQFWKA-NH ₂	Longer fragment of IP ₃ R1 (residues 46–75) containing the 1-8-14 motif	11.7

Table S2 Primers used in the present study

Primers used for introducing mutations in the N-terminal fragment or full-length IP₃R1. The mutated bases are highlighted.

Primer	Sequence (5'→3')
F53E Forward	GGGGACCTTAACAATCCACCCAAGAAA GAG AGAGACTGCCTCTT
F53E Reverse	AAGAGGCAGTCTCT CTC TTTCTTGGGTGGATTGTTAAGGTCCCC
L60E Forward	GAAATTCAGAGACTGCCTCTTTA GGAG TGTCCTATGAATCGATATTCTGCA
L60E Reverse	TGCAGAATATCGATTCATAGGAC ACTC CTTAAAGAGGCAGTCTCTGAATTC
Y66E Forward	CTCTTTAAGCTATGTCCTATGAATCGA GAG TCTGCACAGAAGCAG
Y66E Reverse	CTGCTTCTGTGCAG ACTC TGATTTCATAGGACATAGCTTAAAGAG
K52E Forward	AACAATCCACCCAAG GAA TTCAGAGACTGCCTC
K52E Reverse	GAGGCAGTCTCTGAAT TTC CTTGGGTGGATTGTT

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