FUNCTIONAL STUDIES OF SPLIT ARABIDOPSIS Ca^{2+}/H^{+}

Supplemental Figure S1. Topoplogy and Design of CAX half proteins.

(A and B) Schematic representation of the procedure for making split half proteins. Topology was predicted using transmembrane domain hidden Markov model (TMHMM version 2.0). Rectangles represent the vacuolar membrane. In, cytosol side; Out, vacuolar lumen side. N-terminal truncated versions of CAX1 (sCAX1) **(A)** and CAX3 (sCAX3) **(B)** were cut into N-terminal and C-terminal halves by using a PCR cloning strategy and dividing the proteins at the acidic motif (as shown by arrow, see Supplemental Table S2). A stop codon was added to the N-terminal ORF and a start codon added to the C-terminal ORF. The ORF halves of sCAX1 and sCAX3 were subcloned into yeast expression vectors, by adding a stop codon and start codon to the end of N-terminal half and the start of the C terminus half.

(C-F) Schematic representation of the topology of full-length CAX1 and CAX3, and CAX1 and CAX3 N- and C-terminal half proteins with or without an N-terminal ubiquitin (Nub) or C-terminal ubiquitin (Cub) fusion. Topology was predicted using transmembrane domain hidden Markov model (TMHMM version 2.0). Rectangles represent the vacuolar membrane. In, cytosol side; Out, vacuolar lumen side. **(C)** CAX1 full-length protein (CAX1) without or with Nub and Cub fusions at the N terminus (Nub-CAX1 or Cub-CAX1) or at the C terminus (CAX1-Nub or CAX1-Cub). **(D)** CAX3 full-length protein (CAX3) without or with Nub fusions at the N terminus (Nub-CAX3). **(E)** CAX1 N-terminal half protein (N-CAX1; amino acids 1 to 272) without or with Nub or Cub fusions at the N terminus (Nub-N-CAX1) or at the C terminus (N-CAX1-Nub or N-CAX1-Cub). **(F)** CAX3 C-terminal half protein (amino acids 275-459) without or with Nub fusion at the N-terminus (Nub-C-CAX3).

Supplemental Figure S2. Functionality of epitope tagged CAX1 and CAX3 N-terminal and C-terminal half protein complexes.

Suppression of Ca^{2+} sensitivity of K667 yeast co-expressing: sCAX1 N-terminal half protein (N-sCAX1)+c-Myc-tagged CAX1 C-terminal half protein (C-CAX1-Myc), N-sCAX1+GFP-tagged CAX3 C-terminal half protein (C-CAX3-GFP), GFP-tagged N-sCAX1 (N-sCAX1-GFP)+C-CAX1, or N-sCAX1+RFP-tagged C-CAX3 (C-CAX3-RFP). sCAX1 and empty vectors were used as controls. Yeast growth was performed as described in Fig. 1.

Supplementary Table S1. PCR primers used in this study

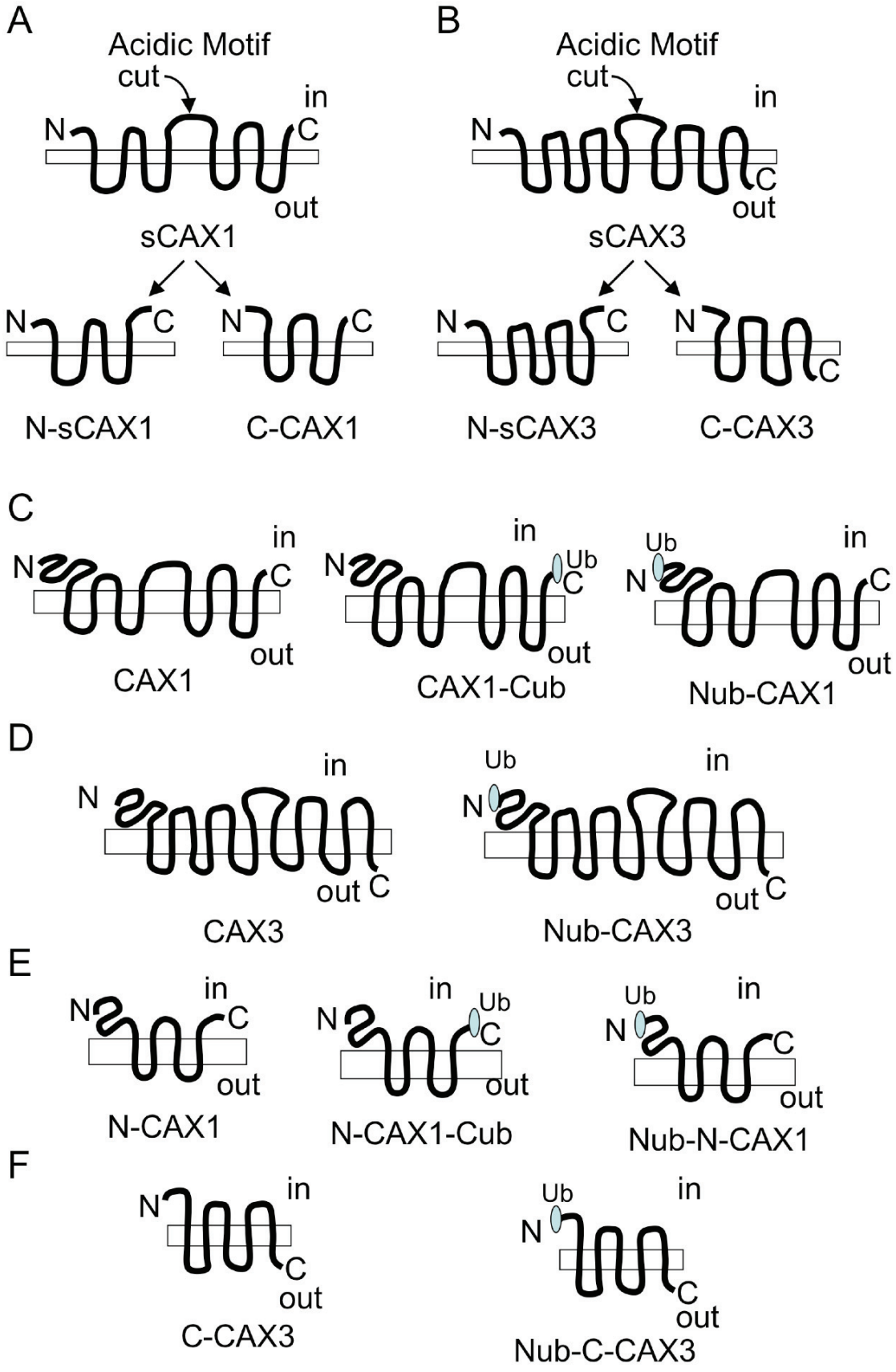
Primer name	Primer sequence with introduced restriction enzyme site (underlined)	Enzyme site
N-sCAX1F	5′-GGCCAAATCGGCCATGTCTTCTTCTTCTTTGAGG-3′	SfiI
N-sCAX1R	5′-GGCCCTTATGGCCTTACTCATCTTCCTGTTCTTGTG-3′	SfiI
C-CAX1F	5′-GGCCAAATCGGCCATGTATGATGACGATGTGGAGCAAG-3′	SfiI
C-CAX1R	5′-CGGCCCTTATGGCCTTAAGATGAGAAAACTCCTCC-3′	SfiI
N-sCAX3F	5′-GGCCAAATCGGCCATGTCTTCATCGTCGCTAAG-3′	SfiI
N-sCAX3R	5′-GGCCCTTATGGCCTTATGCATCATCATCATCCTG-3′	SfiI
C-CAX3F	5′-GGCCAAATCGGCCATGTATGATGATGAGGTTAG-3′	SfiI
C-CAX3R	5′-CGGCCCTTATGGCCTTAAGCTGAGAAAACTTCTC-3′	SfiI
N-sCAX1HAF	5′-GAGCGGCCGCATGTCTTCTTCTTCTTTGAGG-3′	NotI
N-sCAX1HAR	5′-GGGATCCTTACTCATCTTCCTGTTCTTG-3′	BamHI
C-CAX3GFPF	5′-GGTACCACTAGTATGTATGATGATGAGGTTAG-3′	SpeI
C-CAX3GFPR	5′-CGAATTCGCGGCCGCGCGAGCTGAGAAAACTTCTC-3′	NotI
C-CAX3RFPF	5′-GGTACCACTAGTATGTATGATGATGAGGTTAG-3′	SpeI
C-CAX3RFPR	5′-CGAATTCGCGGCCGCTAAGCTGAGAAAACTTCTC-3′	NotI
N-sCAX1BiFCF	5′-AAAGAGCTCATGTCTTCTTCTTCTTTGAGG-3′	SacI

N-sCAX1BiFCR	5′-AAAGGATCCTTACTCATCTTCCTGTTCTTGTG-3′	BamHI
C-CAX1BiFCF	5′-AAAGAGCTCATGTATGATGACGATTGTGAGCAAG-3′	SacI
C-CAX1BiFCR	5′-AAAGGATCCTTAAGATGAGAAAACTCCTCC-3′	BamHI
C-CAX3BiFCF	5′-AAAGAGCTCATGTATGATGATGAGGTTAG-3′	SacI
C-CAX3BiFCR	5′-AAAGGATCCTTAAGCTGAGAAAACTTCTC-3′	BamHI
CAX1BiFCF	5′-AAAGAGCTCATGGCGGGAATCGTGACAGAG-3′	SacI
CAX1BiFCR	5′-AAAGGATCCTTAAGATGAGAAAACTCCTCC-3′	BamHI
CAX3BiFCF	5′-AAAGAGCTCATGGGAAGTATCGTGGAGCCATG-3′	SacI
CAX3BiFCR	5′-AAAGGATCCTTAAGCTGAGAAAACTTCTC-3′	BamHI
CAX2BiFCF	5′-AAAGAGCTCATGAGTTGTTGTAAAG-3′	SacI
CAX2BiFCR	5′-AAAGGATCCTTATATACCATCTTGG-3′	BamHI
ACT8BiFCF	5′-AAATCTAGAATGGCCGATGCTGATGACATTC-3′	XbaI
ACT8BiFCR	5′-AAAGGTACCGAAGCATTTTCTGTGGACAATG-3′	KpnI
CAX3BamHIF[a]	5′-GAATTCCGTCTCGTTGCGTGGAT**A**CTGGGAATAAAAATG-3′	BsmBI
CAX3BamHIR	5′-GAATTCCGTCTCCGCAACGATAACACTCAATGGGACCAC-3′	BsmBI

[a]The mutation site to abolish the BamHI restriction site is indicated in bold on the CAX3BamHIF primer.

Supplemental Table S2. Yeast plasmid constructs used in this study

Constructs	Vector, Promoter, and replication origin	selection marker	References
CAX1, aa1 -aa 463	pHGpd, GPD, CEN	His	Shigaki *et al*, 2001
CAX1, aa1 -aa 463	pUGpd, GPD, CEN	Ura	Shigaki *et al*, 2001
CAX3, aa1 -aa 459	pHGpd, GPD, CEN	His	Shigaki *et al*, 2001
CAX3, aa1 -aa 459	pUGpd, GPD, CEN	Ura	Shigaki *et al* 2001
sCAX1, aa 37- aa 463	pUGpd, GPD, CEN	Ura	Shigaki *et al*, 2001
sCAX1, aa 37- aa 463	pHGpd, GPD, CEN	His	Shigaki *et al*, 2001
sCAX1H338N, aa 37-aa 463, H338N	pHGpd, GPD, CEN	His	Shigaki *et al*, 2005
sCAX3, aa 37- aa 459	pUGpd, GPD, CEN	Ura	Shigaki *et al*, 2001
sCAX3, aa 37- aa 459	pHGpd, GPD, CEN	His	Shigaki *et al*, 2001
N-sCAX1, aa37-aa 236	pUGpd, GPD, CEN	Ura	This study
N-sCAX1, aa37-aa 236	pHGpd, GPD, CEN	His	This study
HA-N-sCAX1, aa37-aa 236	pHGpd, GPD, CEN	His	This study
HA-N-sCAX1, aa37-aa 236	pUGpd, GPD, CEN	Ura	This study
N-sCAX1-GFP, aa37-aa 236	pHGpd, GPD, CEN	His	This study
C-CAX1, aa 237-aa 463	pUGpd, GPD, CEN	Ura	This study
C-CAX1, aa 237-aa 463	pHGpd, GPD, CEN	His	This study
C-CAX1-c-Myc, aa 237-aa 463	pHGpd, GPD, CEN	His	This study
C-CAX1H338N, aa237-aa463, H338N	pUGpd, GPD, CEN	Ura	This study
N-sCAX3, aa 37-aa 238	pUGpd, GPD, CEN	Ura	This study
N-sCAX3, aa 37-aa 238	pHGpd, GPD, CEN	His	This study
C-CAX3, aa 239- aa 459	pUGpd, GPD, CEN	Ura	This study
C-CAX3, aa 239- aa 459	pHGpd, GPD, CEN	His	This study
C-CAX3-GFP, aa 239- aa 459	pHGpd, GPD, CEN	His	This study
C-CAX3-RFP, aa 239- aa 459	pUGpd, GPD, CEN	Ura	This study
Split Ubiquitin Assay			
CAX1-Cub, aa1 -aa 463	pMetYCgate, Met25, CEN/ARS	Leu	This study
Nub-CAX1, aa1 -aa 463	pNXgate 33, ADH, CEN	Trp	This study
Nub-CAX3, aa1-aa 459	pNXgate 33, ADH, CEN	Trp	This study
N-CAX1-Cub, aa1-aa 237	pMetYCgate, Met25, CEN/ARS	Leu	This study
Nub-N-CAX1, aa1-aa 237	pNXgate 33, ADH, CEN	Trp	This study
Nub-C-CAX1, aa 237- aa 459	pNXgate 33, ADH, CEN	Trp	This study
KAT1-Cub	pMetYCgate, Met25, CEN/ARS	Leu	Obrdlik *et al*, 2004; Ludewig *et al*, 2003
Nub-KAT1	pNXgate 33, ADH, CEN	Trp	Obrdlik *et al*, 2004; Ludewig *et al*, 2003
NubWTgate	pNXgate 33, ADH, CEN	Trp	Obrdlik *et al*, 2004; Ludewig *et al*, 2003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C
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B
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C
out
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C-CAX3
C
CAX1
CAX1-Cub
Ub
Nub-CAX1
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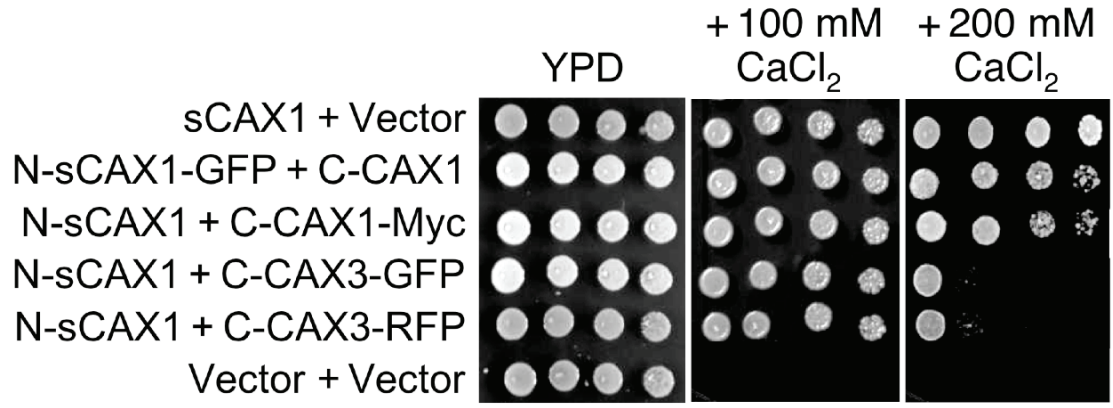
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+ 100 mM CaCl2
+ 200 mM CaCl2
sCAX1 + Vector
N-sCAX1-GFP + C-CAX1
N-sCAX1 + C-CAX1-Myc
N-sCAX1 + C-CAX3-GFP
N-sCAX1 + C-CAX3-RFP
Vector + Vector

S2