supplemental Table S1. Primer sets for construction

Application	Primer Set				
	Primer Name	Sequence	Primer Name	Sequence	Construct Name
SYT1 pomoter cloning	SYT1p F	CACCGGATCCGAAAGATCAGGATGGTGAA	SYT1p R	GGATCCACTAGTTTTGATTTCCGTTTCAGATC	pENTR *SYT1* p
SYT1 cloning/Truncates	TM F	CACCGTCGACACTAGTATGGGCTTTTTCAGTACG	TM R	TACATGCCATGGCGCCGCCACCACCTCCACGATCGAAATCCGG/	pENTR TM
	TM F	CACCGTCGACACTAGTATGGGCTTTTTCAGTACG	SMP R	TACATGCCATGGCGCCGCCACCACCTCCTCCAACAGGCCTTCTG	pENTR TM-SMP
	TM F	CACCGTCGACACTAGTATGGGCTTTTTCAGTACG	C2A R	CCATGGCGCCGCCACCACCTCCTTCAAAGCCTTTGGGCAT	pENTR TM-SMP-C2A
	TM F	CACCGTCGACACTAGTATGGGCTTTTTCAGTACG	C2B R	TACATGCCATGGCGCCGCCACCACCTCCGGCAGTTCGCCACTCC	pENTR SYT1-EmGFP
	SMP F	CACCACTAGTATGGTTGACTGGATAAACAGA	C2B R	TACATGCCATGGCGCCGCCACCACCTCCGGCAGTTCGCCACTCC	pENTR SMP-C2A-C2B
	C2A F	CACCACTAGTATGATTGTCCATGTGAAAGTTGT	C2B R	TACATGCCATGGCGCCGCCACCACCTCCGGCAGTTCGCCACTCC	pENTR C2A-C2B
	C2B F	CACCACTAGTATGACGCAAGCCGTGCAGAAAGC	C2B R	TACATGCCATGGCGCCGCCACCACCTCCGGCAGTTCGCCACTCC	pENTR C2B
SYT5 cloning	SYT5 F	CACCGTCGACATGGGTTTCATAGTCGGCG	SYT5 R	CCATGGCACCTCCTCCACCTCCGGAATCACGATAAATTGATTG	pENTR SYT5
SYT1 mutants	Loop1 F	ATCTTGAATAGGAATTTCACATCCTCAGCCGAATGCAC	Loop1 R	TTAATGGGTAAAGCTGATTCCTTACGTGCGCATCTATT	SYT1 Loop1*/SYT1 Loop1*+Loop3*
	Loop3 F	ATGGGTAAAGCTGATCCTTACGTGCGCATCTATTT	Loop3 R	TAAATCTTGAATAGGAACATCCTCAGCCGAATGCA	SYT1 Loop3*/SYT1 Loop1*+Loop3*

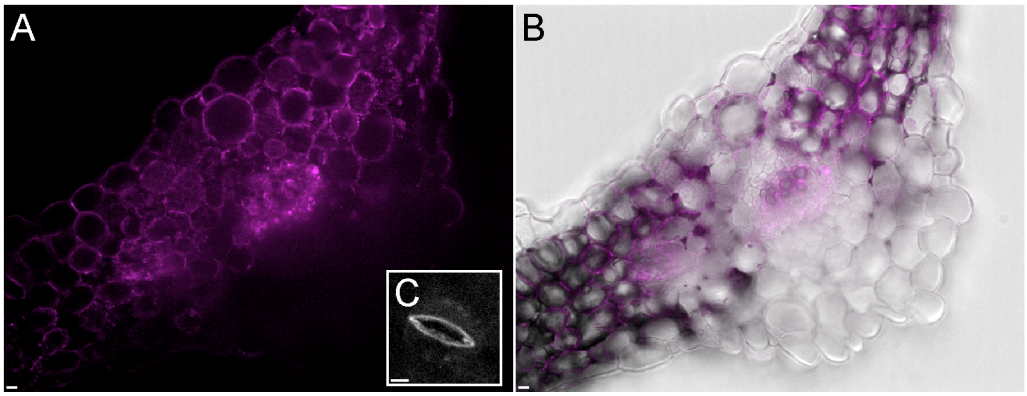
supplemental Table S2. ATPase activity of plasma membrane fraction isolated with aqueous two-phase partitioning system

TX100	ATPase activity (Pi mmol/mg proteins/h)		
	Vanadate		Vanadate sensitive
	-	+	
-	3.29 ± 0.05	0.83 ± 0.03	2.46
+	15.28 ± 0.11	4.75 ± 0.08	10.53

supplemental Table S3. *Syt* genes in angiosperms used in the phylogenetic analysis.

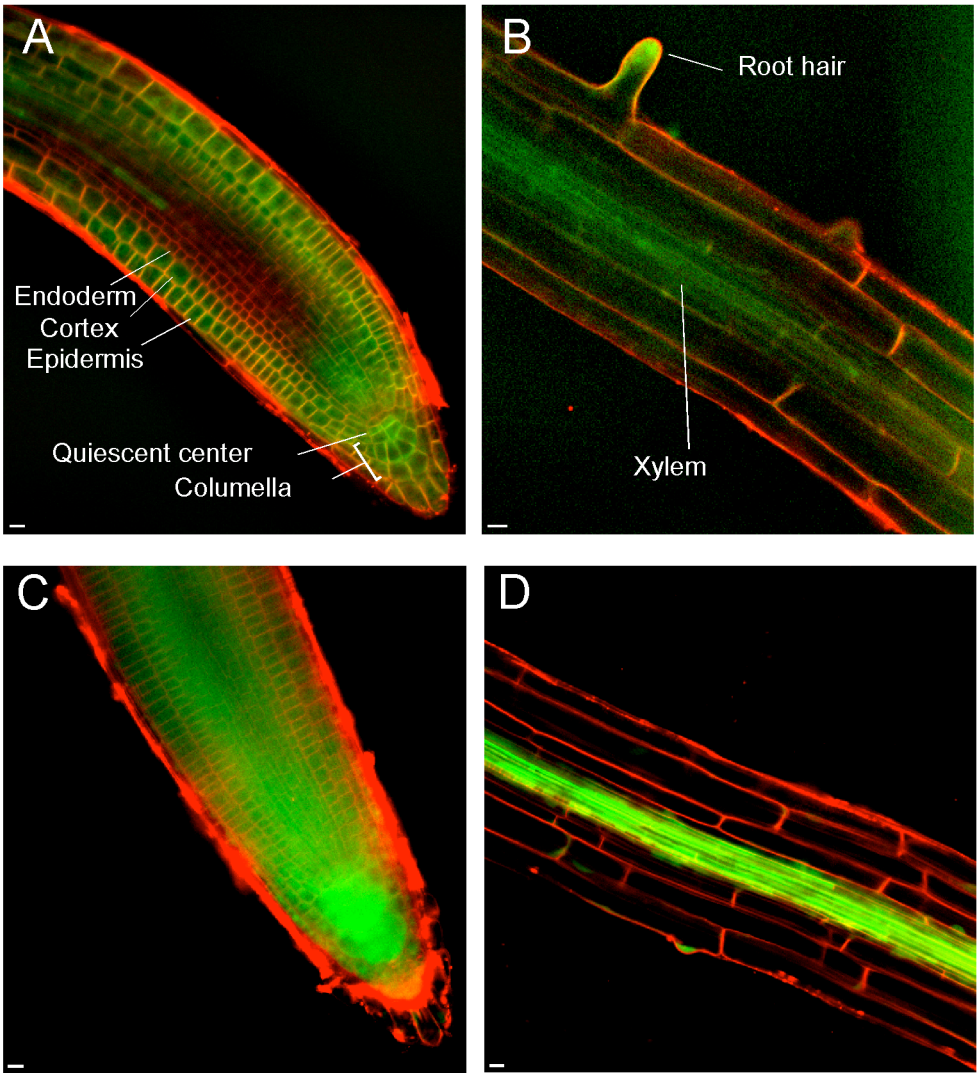
Classification	Species	Gene name	Accession number/Gene ID
monocots	*Oryza sativa*	*OsSyt1/2a*	Os09g0538800
		OsSyt1/2b	Os02g0448400
		OsSyt3	Os06g0685300
		OsSyt4/5	Os08g0300200
	Sorghum bicolor	*SbSyt1/2a*	estExt_fgenesh1_pm.C_chr_10622
		SbSyt1/2b	estExt_Genewise1.C_chr_28638
		SbSyt3	MOL_fgenesh1_pm.C_chr_10000423
		SbSyt4/5	MOL_e_gw1.2.19906.1
		SbSyt5	estExt_Genewise1.C_chr_68072
eudicots	*Vitis vinifera*	*VvSyt1*	GSVIVT00036112001
		VvSyt2	GSVIVT00014388001
		VvSyt3a	GSVIVT00018176001
		VvSyt3b	GSVIVT00023206001
		VvSyt4	GSVIVT00032686001
		VvSyt5	GSVIVT00006418001
	Medicago truncatula	*MtSyt1*	AC121235_39
		MtSyt2	AC149634_14
		MtSyt4	AC148405_51
		MtSyt5	AC144657_3
	Populus trichocarpa	*PtSyt1a*	MOL_estExt_fgenesh4_pg.C_LG_IV1455
		PtSyt1b	MOL_estExt_fgenesh4_pg.C_LG_IX0330
		PtSyt2a	MOL_grail3.0002070502
		PtSyt2b	MOL_estExt_fgenesh4_pg.C_LG_II0188
		PtSyt3a	fgenesh4_pm.C_LG_VIII000119
		PtSyt3b	fgenesh4_pg.C_LG_X002040
		PtSyt4	MOL_gw1.XVIII.1683.1
		PtSyt5a	MOL_MOL_MOL_gw1.XVIII.34.1
		PtSyt5b	MOL_estExt_fgenesh4_pm.C_1520033
	Arabidopsis thaliana	*AtSyt1*	At2g20990
		AtSyt2	At1g20080
		AtSyt4	At5g11100
		AtSyt5	At1g05500
	Carica papaya	*CpSyt1*	EVM_prediction_supercontig_37.147
		CpSyt2	EVM_prediction_supercontig_26.308
		CpSyt4	EVM_prediction_supercontig_217.23
		CpSyt5	EVM_prediction_supercontig_46.58

supplemental Fig. S1



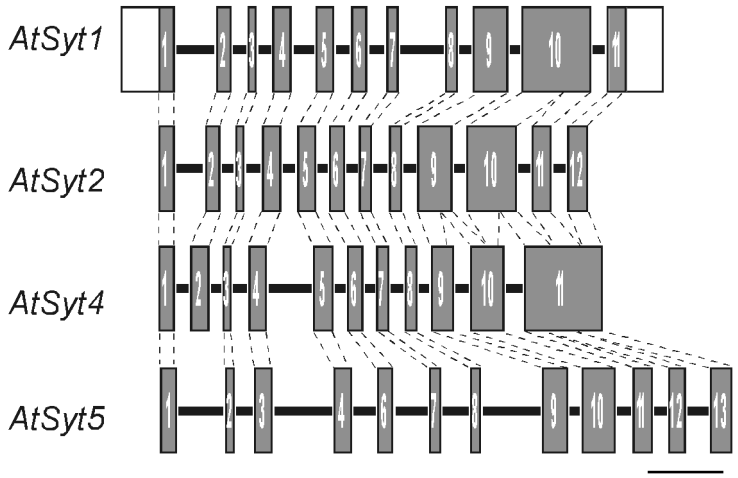
Supplemental Fig. S1. Localization of SYT1-EmGFP in leaf cells. (A, B) Cross-section of living leaf prepared from SYT1-EmGFP expressing plant was observed with confocal fluorescent microscopy. EmGFP emission is pseudo-colored purple (A). Bright field image was merged with EmGFP emission (B). EmGFP emission in the stomata of leaves was also observed (C). Bars indicate 10 μm.

supplemental Fig. S2



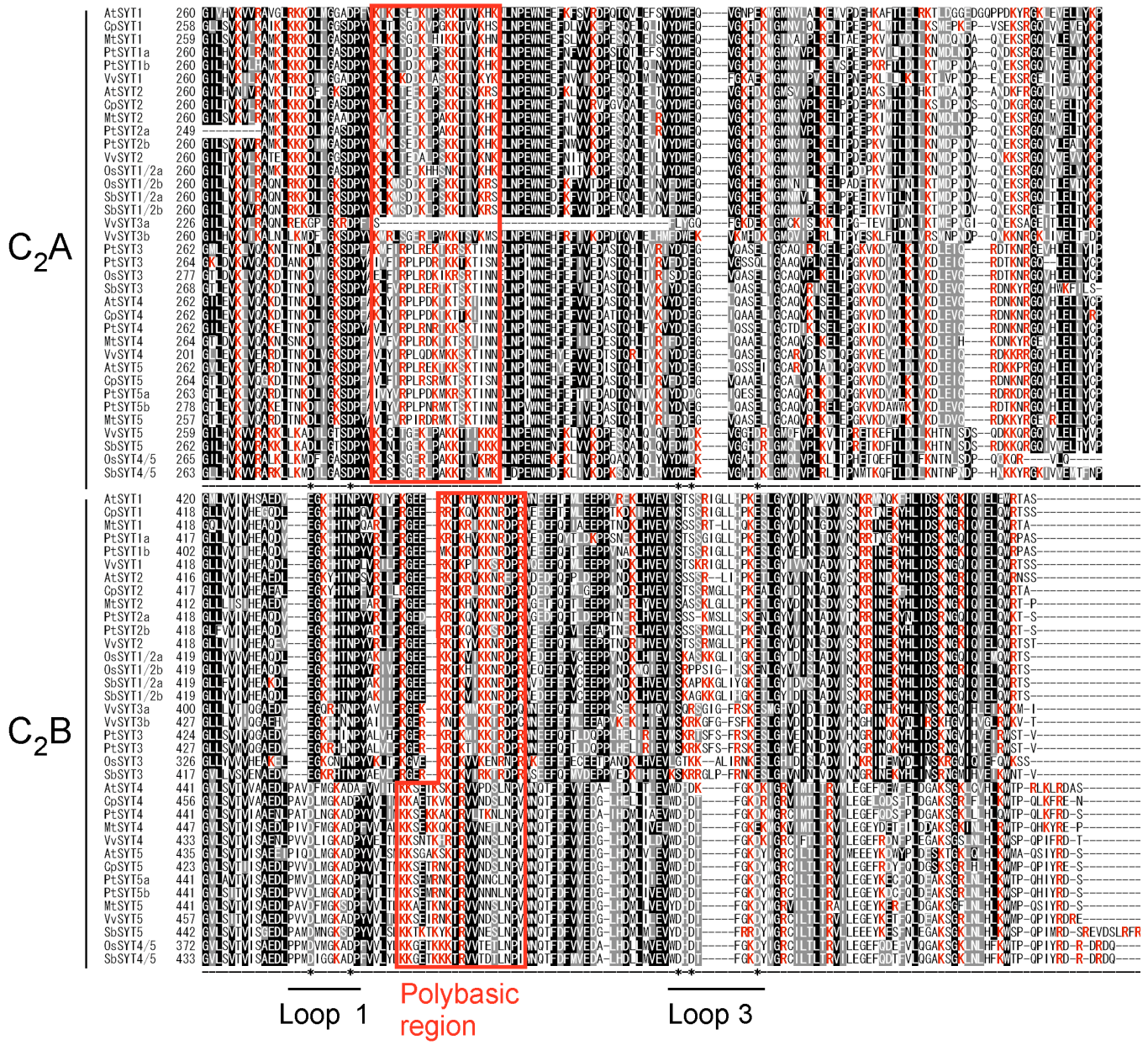
Supplemental Fig. S2. Localization of EmGFP-SYT1 and EmGFP in root cells. Emission of EmGFP (green) and FM4-64 (red) in root cells was observed in transgenic plants expressing SYT1-EmGFP and EmGFP. Fluorescence images of SYT1-EmGFP in the root tip (A) and elongation/differentiation zone (B). Fluorescence images of EmGFP in the root tip (C) and differentiation zone (D). These results of the GFP expression pattern were similar to those of GUS driven by the SYT1 native promoter (Schanpire et al., 2008). Bars indicate 10 μm.

supplemental Fig. S3



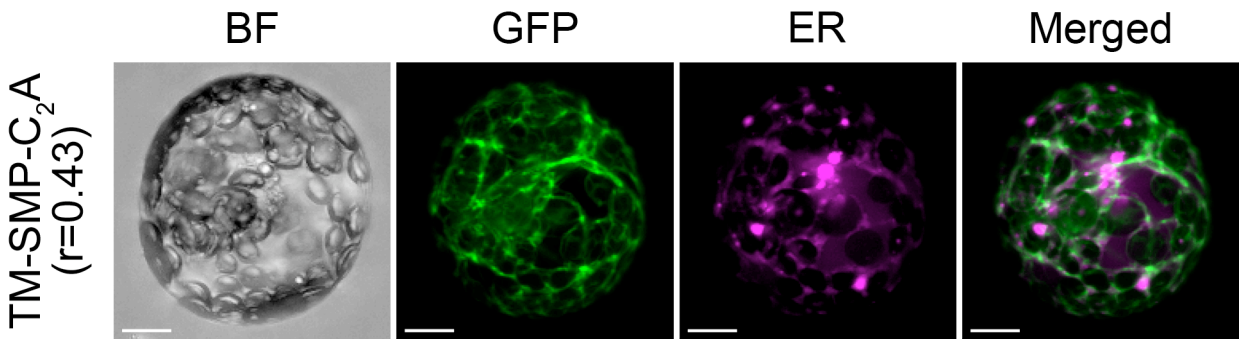
Supplemental Fig. S3. Gene structures of *Arabidopsis Syt* genes. The genomic sequences of *AtSyts* were obtained from TAIR database, and their exon-intron structures were compared. Exon-intron structures were highly conserved among the *AtSyt* genes, with the exon-intron structures from the 1st to the 8th exon being shared by all *AtSyt* genes, except for *AtSyt5*, which lacks the 2nd exon. These results suggest that plant synaptotagmin proteins share a common ancestral gene that existed before the differentiation of eudicots and monocots. The boxes denote exons; the coding regions are shaded gray; and the lines between the boxes represent the introns of each gene. The number in the box indicates the exon number. The conserved regions are connected by dashed lines. Bar, 500 bp.

supplemental Fig. S4



Supplemental Fig. S4. Multiple alignment of C_2A and C_2B domains in plants. The amino acid sequences of the C_2A and C_2B domains in plants were aligned using ClustalW. The names of these genes are presented in supplemental Table S3. Acidic amino acid residues are shown in red. The polybasic region is indicated with red open boxes. Asterisks indicate the amino acid residues of the conserved calcium binding motifs (Loop1 and Loop3) in the C2 domains.

supplemental Fig. S5



Supplemental Fig. S5. Localization of transiently expressed TM-SMP-C_2A in protoplasts stained with ER-Tracker. Projection images generated from optical sections of EmGFP florescence and ER-Tracker in protoplasts expressing the truncated SYT1-EmGFP protein. Emissions of EmGFP and ER-Tracker were observed using a confocal fluorescence microscope. The obtained fluorescence images of each optical section were reconstructed as projection images. TM-SMP-C_2A: TM with SMP and C_2A domains. BF, bright field; GFP, GFP fluorescence; ER, fluorescence of ER-Tracker; Merged, merged image of GFP with ER-Tracker. Fluorescence of GFP, and ER-Tracker were pseudocolored into green and magenta, respectively. Bars indicate 10 μm.