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Supplementary Information for

Longin R-SNARE is retrieved from the plasma membrane by ANTH domain-containing proteins in *Arabidopsis*

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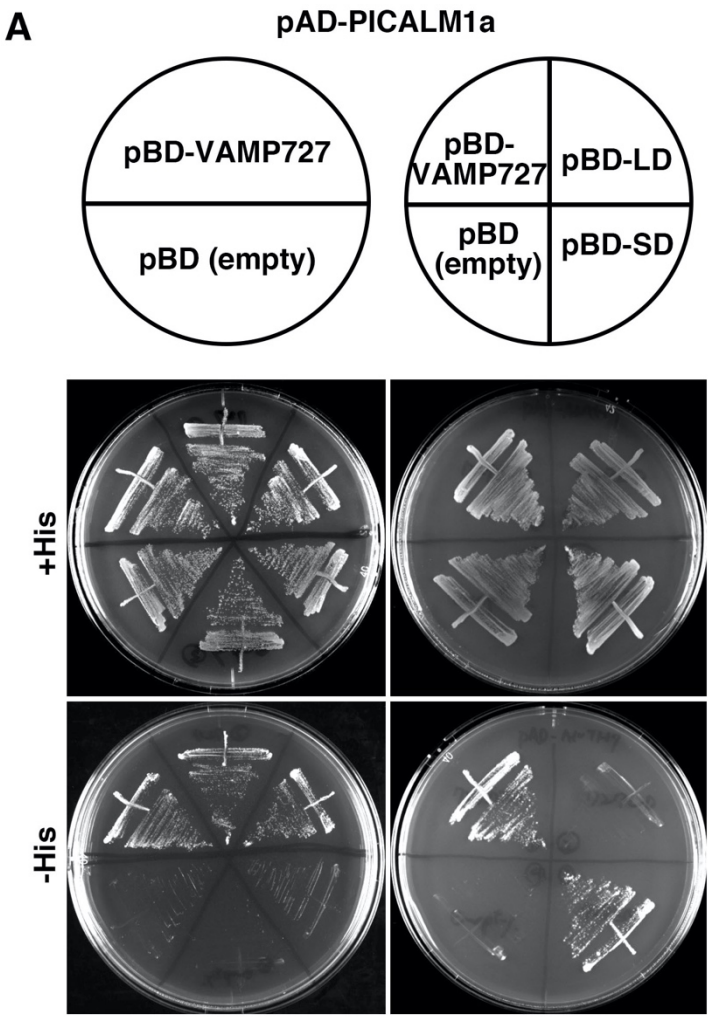

Fig. S1. PICALM1a interacts with the SNARE domain of VAMP727. (A) PICALM1a was expressed as a fusion protein with a transcriptional activation domain (pAD), and the cytoplasmic domain (left) or the longin (LD) or SNARE domain (SD) (right) of VAMP727 was expressed as a fusion protein with the DNA-binding domain (pBD). Interactions between two proteins were tested using the *HIS3* reporter gene.

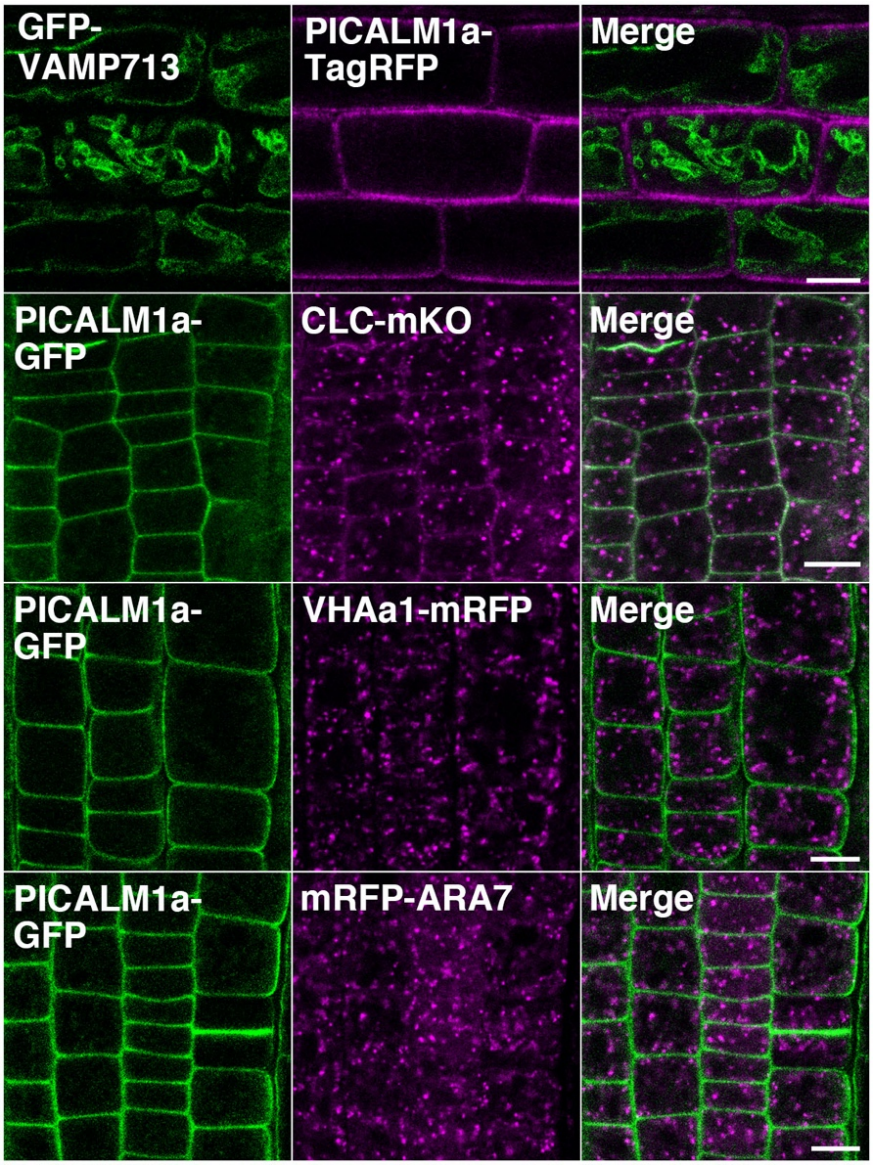

Fig. S2. Comparison of subcellular localization between PICALM1a and organelle markers. The subcellular localization was compared between fluorescently tagged PICALM1a and GFP-VAMP713 (vacuolar membrane), CLC-mKO, VHAa1-mRFP (TGN), or mRFP-ARA7 (multivesicular bodies) in root epidermal cells of 7-day-old plants by confocal microscopy. Scale bars = 10 μm.

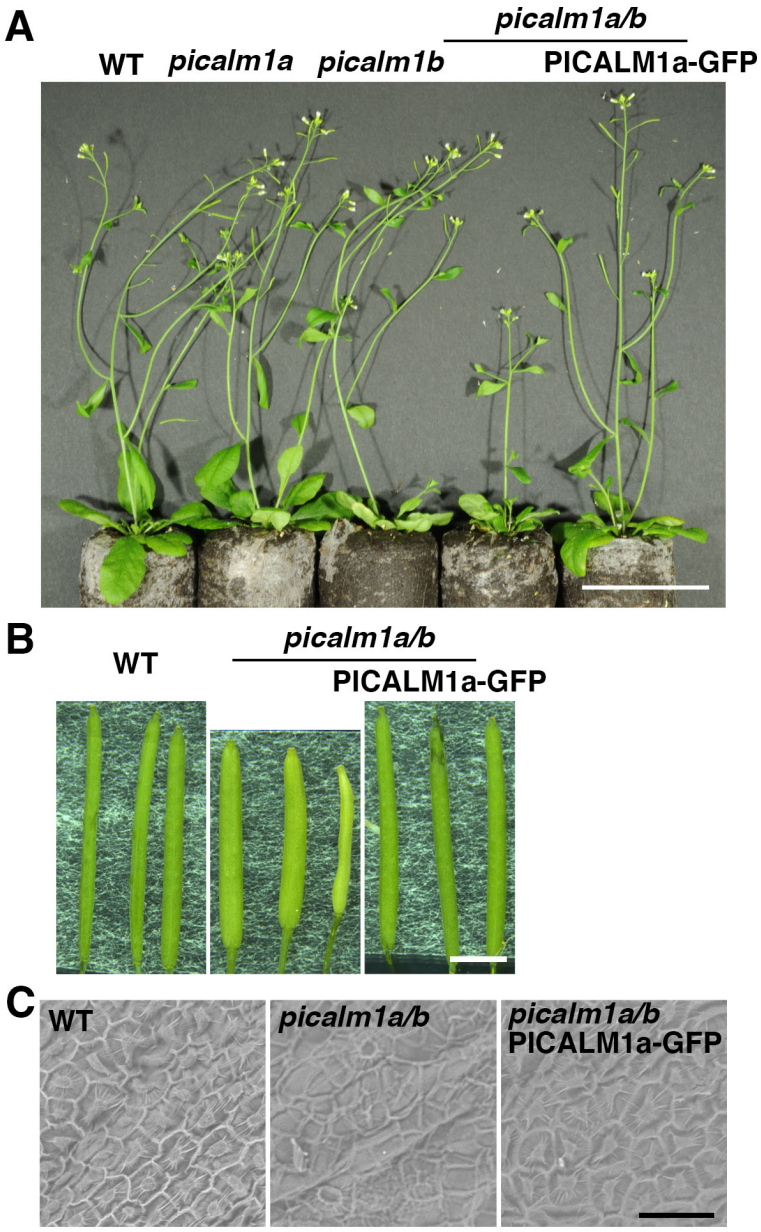

Figure S3. *picalm1* mutants exhibit semi-dwarfism. (A) Forty-day-old *Arabidopsis* plants of wild-type (WT), *picalm1a* and *picalm1b* single mutants, *picalm1a picalm1b* (*picalm1a/b*) double mutant, and double mutant transformed with *PICALM1a*-GFP. (B) Siliques collected from the plants with the indicated genotypes. (C) Images of mucilage secretory cells in the seed coat of dry seeds with indicated genotypes. Scale bars = 5 cm (A), 3 mm (B), or 50 μm (C).

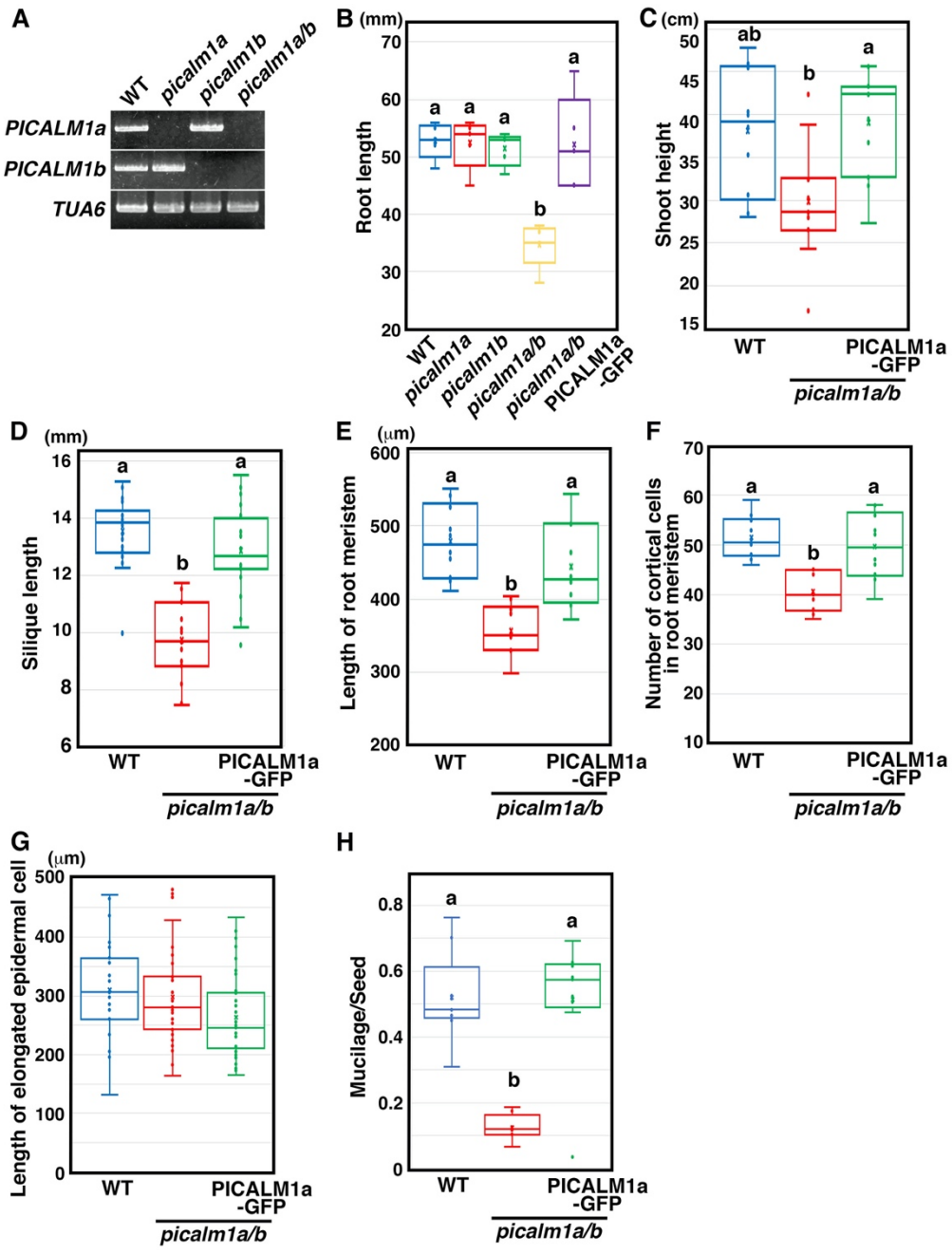

Figure S4. Characterization of *picalm1* mutants. (A) Expression analysis of the *PICALM1* genes by RT-PCR in wild-type and *picalm1* mutant plants. (B) Quantification of root length of 10-day-old *Arabidopsis* seedlings with the indicated genotypes grown on 1/2 MS agar medium. One-way ANOVA followed by Tukey's HSD post-hoc test was used for statistical analyses (n = 5 seedlings). (C) Quantification of the height of fully grown plants with the indicated genotypes. One-way ANOVA followed by Steel–Dwass test was used for statistical analyses (n = 10 plants for WT and 11 plants for *picalm1a/b* and *picalm1a/b* with *PICALM1a-GFP*). (D) Quantification of the length of siliques collected from *Arabidopsis* plants with the indicated genotypes. One-way

ANOVA followed by Steel–Dwass test was used for statistical analysis (n = 9 siliques for WT and *picalm1a/b* and 12 siliques for *picalm1a/b* with *PICALM1a-GFP*). (E and F) Quantification of the meristem length of roots (E) and number of cortical cells in the root meristem (F) of 6-day-old *Arabidopsis* seedlings with the indicated genotypes grown on 1/2 MS agar medium. One-way ANOVA followed by Steel–Dwass test was used for statistical analysis (n = 10 roots for each genotype). (G) Quantification of the length of fully elongated epidermal cells without a root hair of 6-day-old *Arabidopsis* seedlings with the indicated genotypes grown on 1/2 MS agar medium. Significant difference was not detected among the three groups by the one-way ANOVA. (n = 23 cells for WT, 31 cells for *picalm1a/b*, and 34 cells for *picalm1a/b* with *PICALM1a-GFP* for G) (H) Ratio of the area of mucilage to the seed calculated from images of ruthenium red-stained imbibed seeds with the indicated genotypes. One-way ANOVA followed by Steel–Dwass test was used for statistical analysis (n = 9 seeds for WT, 8 seeds for *picalm1a/b*, and 9 seeds for *picalm1a/b* with *PICALM1a-GFP*).

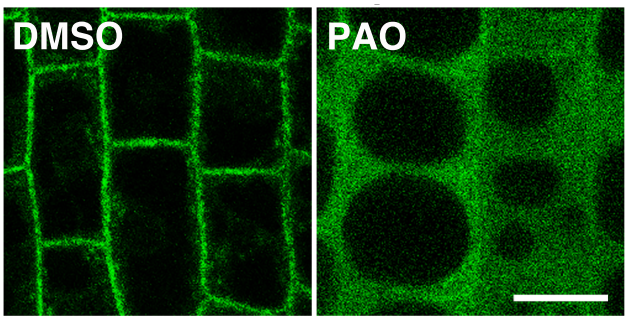

Fig. S5. Effect of phenylarsine oxide (PAO) on PICALM1a-GFP localization. Confocal images of root epidermal cells of 7-day-old *Arabidopsis* plants expressing PICALM1a-GFP treated with DMSO or PAO.

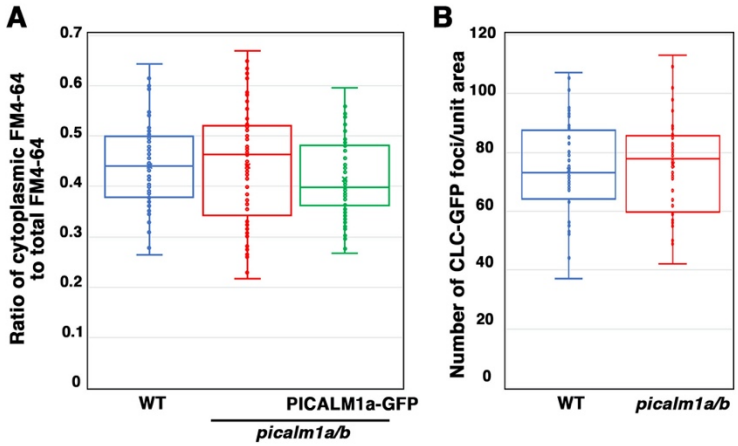

Fig. S6. *picalm1a/b* mutation does not considerably affect general endocytosis. (A) Quantification of the ratio of internalized to total fluorescence from FM4-64 in root epidermal cells of *Arabidopsis* with the indicated genotypes. n = 51 cells in 5 plants for wild type (WT), 81 cells in 8 plants for *picalm1a/b*, and 66 cells in 6 plants for *picalm1a/b* complemented with PICALM1a-GFP. Significant difference was not detected among the three groups by the one-way ANOVA. (B) The number of CLC-GFP foci in 100 μm^2 of PM areas in the root epidermal cells of wild-type (WT) and *picalm1a/b* mutant plants observed by VIAFM. n = 44 unit areas in 4 plants for wild type (WT) and 36 unit areas in 4 plants for *picalm1a/b*. Significant difference was not detected using Wilcoxon rank sum test.

Fig 1C, αHis

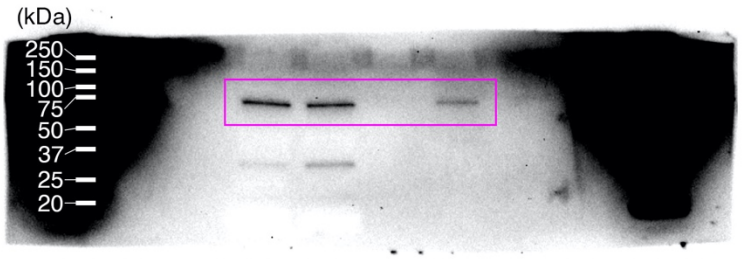

Fig 1C, αGFP

Fig 1D, αCHC

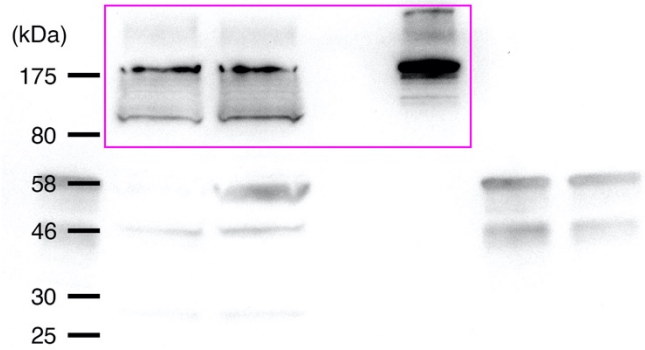

Fig 1D, αGFP

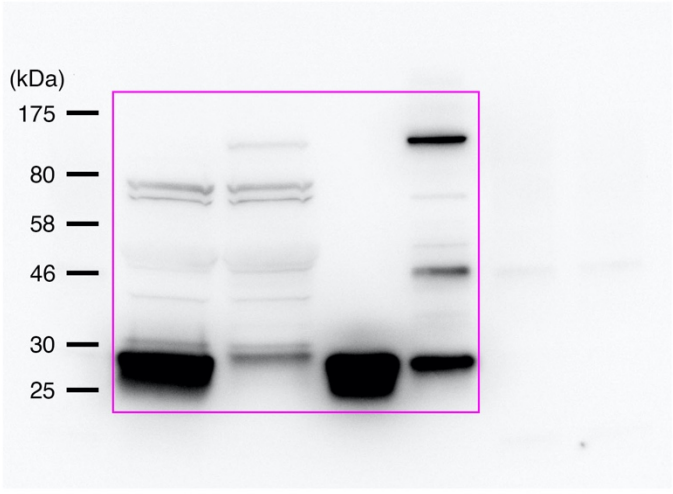

Fig. S7. Full blots.

Table S1. Nucleotide primers used in this study

Genotyping		
PICALM1a	At2g01600-1 for	CTGATCCTCCAACTCCTCATTTTGG
	At2g01600-1 rev	TCAAATGAGGCCAGTGCTACGGA
	LBb1-2	GACCGCTTGCTGCAACTCTCTCA
PICALM1b	026 G05 for2	TGGTACATTAAACTAAACTCGGGG
	026 G05 rev2	TCATCAGGCGTATAGGTCAGAAG
	LB(GABI)	cccatttggacgtgaatgtagacac
Plasmid construction		
PICALM1a genomic / pENTR/D-TOPO	A7g TP fw	caccCAGTGGCCAATTTACGTAGTTAGTG
	ANTH7 del stop rv	AATGAGGCCAGTGCTACGGAAC
PICALM1a genomic sequence	A7g s1f	CGGACAGATATTAAAGCCATGAATG
	A7g s2f	GCGAATACGCCGTACGCTCTC
	A7g s3f	CTCCTTCATTTGTGGGTATATGTG
	A7g s4f	CACAACTGGACACAGTCGGTG
	A7g s5f	CTTTTCTGACAACAATGGAGGAG
	A7g s6f	CTGATCTCTGTGTTTCCAGCTG
	A7g s7f	TGTCTACGGTGCACCAGCTC
PICALM1b genomic / pENTR/D-TOPO	ANTH8 pro TOPO fw	CACCctctctccatagactccgacg
	ANTH8 ORF rvG 2	CAAGAAGCCCGTGCTGCCGAAGGGGTTGTTGCTGTTATG
PICALM1b genomic sequence	A8g sf1	gaattgggcttgatccatac
	A8g sf2	ctaatcaaagttatatattggacgctag
	A8g sf3	ggaaagaaatataagaatgagacg
	A8g sf4	GCGATTAGGCCTCGAGCAGATGTTG
	A8g sf5	CACTTATGCGTTGTTTCTTGAG
	A8g sf6	cataatttacatatatccgctag
	A8g sf7	GCAAAGGATTAGAACTTGCAAGG
	A8g sf8	gtctctgattctctcagtattacacacag
	A8g sf9	CAATCCGTTTGAGGTCCAAGACCTG
mRFP-CESA2 genomic / pENTR/D-TOPO	CACC-pCESA2 f.	caccGACAGATTGTGAGTGCGAAACACACATA
	CESA2 genomic r.	AGCCGTTCGATCAGGTTAAG
	linker-CESA2 f.	GGAGGTAGTGGCGGCAATACTGGTGGTCGGCTCAT

	pCESA2-mRFP r.	CTCGGAGGAGGCCATGATGTCTTCTACACCGA
	mRFP f.	ATGGCCTCCTCCGAGGACGT
	mRFP-linker r.	GCCGCCACTACCTCCTCCGGCGCCGGTGGAGTGGCGGC
CESA2 genomic sequence	CESA2 seq1	ACTCTTACTAGTGCATGCT
	CESA2 seq2r.	GTAAACACATACGAGGTGCAG
	CESA2 seq3	GGGAACGTCGTGCTATGAA
	CESA2 seq4	TATGTGTTTCATGATGGACC
	CESA2 seq5	TTTGGTTTCTGTCTTGTTGA
Y2H Screening		
Library sequence	M5ADLD_scr_amp	ctattcgatgatgaagataccccaccaaacccaaaaaaagag
	M3_ins_scr_amp	AGTGAACTTGCGGGGTTTTTCAGTATCTACGAT
	T7_seq_primer	aatacgactcactatagggc
VAMP721 / pGBKT7	VAMP721 fw for pGBKT7	CATGGAGGCCGAATTCATGGCGCAACAATCGTTGATC
	VAMP721SN rv for pGBKT7	GCAGGTCGACGGATCCTCACATGTTCTGAAGCCACATCTTTC
VAMP727 / pGBKT7	727 fw for pGBKT7	CATGGAGGCCGAATTCATGAGTCAAAAGGGTTTGATATATAGC
	727 SN rv for pGBKT7	GCAGGTCGACGGATCCTCACAGACTCTGTAGCCACATCTTC
sequence	pGBKT7 sf	GAAGAGAGTAGTAACAAAGGTC
	pGBKT7 sr	CGGAATTAGCTTGGCTGCAAG
	pGADT7 sf	CTATTCGATGATGAAGATACCCCAC
	pGADT7 sr	AGATGGTGCACGATGCACAG
Y2H assay		
VAMP721 / pENTR/D-TOPO	VAMP721 ORF TOPO fw2	caccATGGCGCAACAATCGTTGATCTACAGTTTCGTAGCTCGCG
	VAMP721 SN TOPO fw	caccCATCCTGATGAGATTAGCAAG
	VAMP721 SN rv	TCACATGTTCTGAAGCCACATCTTTCTTCTC
	AtVAMP721 LD rv	TTAATCCATGCAATACTGCATATGCTC
VAMP727 / pENTR/D-TOPO	VAMP727 ORF TOPO fw2	caccATGAGTCAAAAGGGTTTGATATATAGC
	727 SN rv	TCACAGACTCTGTAGCCACATCTTC
	727 SN topo fw	caccCATCCAGAAGAGATGAGCAAACTTTC
	727 LD rv	TCAGCTCATACAATACTGCATATGCTC
VAMP713 / pENTR/D-TOPO	TPAt713Fw	caccATGGCGATCATATTTGCGTTG
	TPAt713-TMDRv	TTATGTAAGCTTGACATTTCTC
EPSIN1 / pENTR/D-TOPO	At5g11710 topo fw	caccATGGATTTCATGAAGGTCTTCGATC

	At5g11710 rv	TCACTGCTTAAAGCCACCAGATTG
EPSIN2 / pENTR/D-TOPO	AT2G43160 TOPO fw	caccATGAAGAAAGTCTTCGGACAAACTG
	AT2G43160 rv	TTACCGGTATCCACCACCATAGGATTGTTG
EPSIN3 / pENTR/D-TOPO	At3g59290 topo fw	caccATGAAGAAAGCATTTGGTCAAACTG
	At3g59290 rv	TTACCGGTAACCTCCACCATATGG
PICALM5a/ECA2 / pENTR/D-TOPO	At1g03050 topo fw	caccATGGGTTCGAGTAAGTTTAAACGAG
	At1g03050 rv	TCAATATTGAGGTGTGTAAGAGTAAGGTTG
PICALM5b / pENTR/D-TOPO	At4g02650 topo fw	caccATGGGTTCAAGTAAGCTAAAACGTG
	At4g02650 rv	TCAATAGCGAGGCGTGTAAGAG
PICALM4a/ECA4 / pENTR/D-TOPO	At2g25430 topo fw	caccATGGCTCCGAGTATTCGAAAAGC
	At2g25430 rv	TCAGTAAGGATTGTTGTAGTAATACCCCG
PICALM6/AP180 / pENTR/D-TOPO	At1g05020 topo fw	caccATGCCGAGCAAGCTTAAAAAAGC
	At1g05020 rv	TCAACTCAAGTGCTTGGCTATGATC
PICALM3 / pENTR/D-TOPO	At5g35200 topo fw	caccATGTCAGGTGGTGGTGGATC
	At5g35200 rv	TTACATATACCTTGGATATGGGTTGTAAC
PICALM2b / pENTR/D-TOPO	At4g25940 topo fw	caccATGGCAACGTTTAACAGCTTCC
	At4g25940 rv	CTAGAGGAGCATATGATGATGGTTG
PICALM1a / pENTR/D-TOPO	AT2G01600 TOPO fw	caccATGGGAACGCTACAGTCATGG
	AT2G01600 rv	TCAAATGAGGCCAGTGCTACG
PICALM1b / pENTR/D-TOPO	At1g14910 topo fw	caccATGGGAACGCTACAGTCATGG
	At1g14910 rv	TCACAAGAAGCCCGTGCTGC
PICALM2a / pENTR/D-TOPO	ANTH10_TOPO_fw_3	caccATGGGAACGTTCACGAGCTTTCGAAAAGCCTATG
	ANTH10_TOPO_rv_3	CTAGAGAAGCATATGATTAGGACTGTATTGCTGCTGTGTA
Sequencing	E1 s1f	CTGGTTCCTCAGCTTCATTTG
	E1 s2f	AGCTCCTCCGACGGTTGATC
	E2 s1f	GATTACGGAAGCCGGGATGAAG
	E2 s2f	CTCAGAAAGTTGAGACTTTCGATG
	E2 s3f	GCTACATCTCAGAGTGGTATTG
	E2 s4f	TCATCAGTCTGGGCAGACAC
	E3 s1f	CCGCTATGGAGACAGAGATG
	E3 s2f	GAATCATCTCCTCAGCAAGTTGAG
	E3 s3f	CACCGAACTTATCCGTCTTTCC

	E3 s4f	GACACAGCAACCTTATGGTGTG
	E3 s5f	CAAATCCATTGGCGGATATAGGAG
	A1 s1f	TCTCCACGGCTATCGTGGTC
	A1 s2f	GATATGAACGCAATAAAGGCCTTAC
	A2 s1f	GGAAGATGATCATCGAGGGAC
	A2 s2f	CAAATCTGAGGAGGAGGAGAGC
	A3 s1f	AGAGATGATTTCAGGTCTCCAC
	A3 s2f	GTGTGGCGAGATCGTCTGAG
	A3 s3f	CAGACAGCCGCTTTGGGTG
	A4 s1f	CAGCACCAACTCAACAACAAG
	A4 s2f	CCACGATTGATACGTCGGAG
	A5 s1f	GACACTCCTGATTTGCTCGAG
	A5 s2f	CAGAAGCAGAGAAGCCTGTAG
	A6 s1f	CAAGGATGCTCTCCGATGAAG
	A6 s2f	CCAGCCGCTTATCGAAGAAG
	A7 orf s1	CTTGAGGAACGGCTTGAATGCTTC
	A7 orf s2	GGCCGGATGATGGACTGACTACCG
	A7 orf s3	GAGGGTTAGATACGCTTACACTTAGCAG
	A8 s1f	AAAACTCTTGGAACAGTTGCCAG
	A8 s2f	TCCCAGTCTCCTCCTTCAGTAG
	A8 s3f	GCCACAGCTACAGGTTGCAC
	ANTH10_sf1	AGAAGAGCGGCTTGAATGTTATC
	ANTH10_sf2	AGTACATTAAAGAAGCGCCTC
	ANTH10_sf3	ACAACAATCCTCGTCCCGTTATAG
RT-PCR		
PICALM1a	ANTH7 RT fw	ATGGGAACGCTACAGTCATGGAG
	ANTH7 RT rv	TCAAATGAGGCCAGTGCTACGG
PICALM1b	ANTH8 RT fw	ATGGGAACGCTACAGTCATGGC
	ANTH8 RT rv	TCACAAGAAGCCCGTGCTGC
TUA6	TUA6 RT fw	ATGAGAGAGTGCATTTCGATCCAC
	TUA6 RT rv	TTAGTATTCCTCTCCTTCATCATCCTCATC