ACA12 Is a Deregulated Isoform of Plasma Membrane Ca^{2+}-ATPase of *Arabidopsis thaliana*

PLANT MOLECULAR BIOLOGY

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Online Resource 1

DNA sequences of plasmid constructs

Plasmid Stock 391, *35s::ACA12-GFP*. The ACA12 coding sequence (blue italics) begins downstream of a unique *Asc1* site, with the sequence *ATGAGGGACCTC* and ends with *CTCAAGAAACCT*. Immediately downstream is a GFP coding sequence (green italics) starting with the sequence ATGGTGAGCAAG and ending with the sequence *CATCACCATCACCATCACGGATCCTGA*, encoding a 6xHis tag followed by a stop codon in red highlight. This plant expression vector is based on a pBIN101.2 vector and harbors a kanamycin resistance marker for selection in bacteria and plants.

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TGCCTGTGGACAGCCCCTCAAATGTCAATAGGTGCGCCCCTCATCTGTCAGCACTCTGCCCCTCAAGTGTCAAGG
ATCGCGCCCCTCATCTGTCAGTAGTCGCGCCCCTCAAGTGTCAATACCGCAGGGCACTTATCCCCAGGCTTGTCC
ACATCATCTGTGGGAAACTCGCGTAAAATCAGGCGTTTTCGCCGATTTGCGAGGCTGGCCAGCTCCACGTCGCCG
GCCGAAATCGAGCCTGCCCCTCATCTGTCAACGCCGCGCCGGGTGAGTCGGCCCCTCAAGTGTCAACGTCCGCCC
CTCATCTGTCAGTGAGGGCCAAGTTTTCCGCGAGGTATCCACAACGCCGGCGGCCGCGGTGTCTCGCACACGGCT
TCGACGGCGTTTCTGGCGCGTTTGCAGGGCCATAGACGGCCGCCAGCCCAGCGGCGAGGGCAACCAGCCCGG

Plasmid Stock 688, ***ACA9promoter::ACA12-TAP2(YFP)***.The *ACA9* promoter (gray shading) starts immediately after a unique *Sbf1* site and ends just before a *Sal1* site. The *ACA12* coding sequence (blue shading and italics) begins downstream of a unique *Asc1* site, with the sequence *ATGAGGGACCTC* and ends with the sequence *CTCAAGAAACCT*. Immediately downstream is a sequence encoding aYFP (yellowshading and italics) starting with the sequence ATGGTGAGCAAG. Following the YFP is a sequence encoding a tandem affinity tag, which ends with the stop codon (red highlight) at the end of the sequence *AAGTCAACCTGA*.This plant expression vector is based on a pGreenII backbone and harbors a kanamycin resistance marker for selection in bacteria and a hygromycin resistance marker for selection in plants. The *ACA9* promoter provides expression primarily in pollen.

TTTTTATCCCCGGAAGCCTGTGGATAGAGGGTAGTTATCCACGTGAAACCGCTAATGCCCCGCAAAGCCTTGATT
CACGGGGCTTTCCGGCCCGCTCCAAAAACTATCCACGTGAAATCGCTAATCAGGGTACGTGAAATCGCTAATCGG
AGTACGTGAAATCGCTAATAAGGTCACGTGAAATCGCTAATCAAAAAGGCACGTGAGAACGCTAATAGCCCTTTC
AGATCAACAGCTTGCAAACACCCCTCGCTCCGGCAAGTAGTTACAGCAAGTAGTATGTTCAATTAGCTTTTCAAT
TATGAATATATATATCAATTATTGGTCGCCCTTGGCTTGTGGACAATGCGCTACGCGCACCGGCTCCGCCCGTGG
ACAACCGCAAGCGGTTGCCCACCGTCGAGCGCCAGCGCCTTTGCCCACAACCCGGCGGCCGGCCGCAACAGATCG
TTTTATAAATTTTTTTTTTTGAAAAAGAAAAAGCCCGAAAGGCGGCAACCTCTCGGGCTTCTGGATTTCCGATCC
CCGGAATTAGAGATCTTGGCAGGATATATTGTGGTGTAACGTTATCGATCTGGATTTTAGTACTGGATTTTGGTT
TTAGGAATTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCTTATATGCTCAA
CACATGAGCGAAACCCTATAAGAACCCTAATTTCCCTTATCGGGAAACTACTCACACATTATTTATGGAGAAAAT
AGAGAGAGATAGATTTGTAGAGAGAGACTGGTGATTTCAGCGTACCGAATTAATTCTCCCGCGACCAGCCGAGCG
AGCTTAGCGAACTGTGGACGAGAACTGTGCCACCAAGCGTAAGGCCGTTCTCTCGCATTTGCCTTGCTAGGCTCG
CGCGAGTTGCTGGCTGAGGCGTTCTCGAAATCAGCTCTTGTTCGGTCGGCATCTACTCTATTCCTTTGCCCTCGG
ACGAGTGCTGGGGCGTCGGTTTCCACTATCGGCGAGTACTTCTACACAGCCATCGGTCCAGACGGCCGCGCTTCT
GCGGGCGATTTGTGTACGCCCGACAGTCCCGGCTCCGGATCGGACGATTGCGTCGCATCGACCCTGCGCCCAAGC
TGCATCATCGAAATTGCCGTCAACCAAGCTCTGATAGAGTTGGTCAAGACCAATGCGGAGCATATACGCCCGGAG
GCGTGGCGATCCTGCAAGCTCCGGATGCCTCCGCTCGAAGTAGCGCGTCTGCTGCTCCATACAAGCCAACCACGG
CCTCCAGAAGAAGATGTTGGCGACCTCGTATTGGGAATCCCCGAACATCGCCTCGCTCCAGTCAATGACCGCTGT
TATGCGGCCATTGTCCGTCAGGACATTGTTGGAGCCGAAATCCGCGTGCACGAGGTGCCGGACTTCGGGGCAGTC
CTCGGCCCAAAGCATCAGCTCATCGAGAGCCTGCGCGACGGACGCACTGACGGTGTCGTCCATCACAGTTTGCCA
GTGATACACATGGGGATCAGCAATCGCGCATATGAAATCACGCCATGTAGTGTATTGACCGATTCCTTGCGGTCC
GAATGGGCCGAACCCGCTCGTCTGGCTAAGATCGGCCGCAGCGATCGCATCCATAGCCTCCGCGACCGGCTGAAG
AACAGCGGGCAGTTCGGTTTCAGGCAGGTCTTGCAACGTGACACCCTGTGCACGGCGGGAGATGCAATAGGTCAG
GCTCTCGCTGAACTCCCCAATGTCAAGCACTTCCGGAATCGGGAGCGCGGCCGATGCAAAGTGCCGATAAACATA
ACGATCTTTGTAGAAACCATCGGCGCAGCTATTTACCCGCAGGACATATCCACGCCCTCCTACATCGAAGCTGAA
AGCACGAGATTCTTCGCCCTCCGAGAGCTGCATCAGGTCGGAGACGCTGTCGAACTTTTCGATCAGAAACTTCTC
GACAGACGTCGCGGTGAGTTCAGGCTTTTTCATAGGGATCAGCTTGGGCTGTCCTCTCCAAATGAAATGAACTTC
CTTATATAGAGGAAGGGTCTTGCGAAGGATAGTGGGATTGTGCGTCATCCCTTACGTCAGTGGAGATGTCACATC
AATCCACTTGCTTTGAAGACGTGGTTGGAACGTCTTCTTTTTCCACGATGCTCCTCGTGGGTGGGGGTCCATCTT
TGGGACCACTGTCGGCAGAGGCATCTTGAATGATAGCCTTTCCTTTATCGCAATGATGGCATTTGTAGGAGCCAC
CTTCCTTTTCTACTGTCCTTTCGATGAAGTGACAGATAGCTGGGCAATGGAATCCGAGGAGGTTTCCCGAAATTA
CCCTTTGTTGAAAAGTCTCAATAGCCCTTTGGTCTTCTGAGACTGTATCTTTGACATTTTTGGAGTAGGGGTACG
ATAACATTAACGTTTACAATTTCGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGG
GCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTT
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GCAGGAAGCTTGAACTGAGAAACCATTGGAAGCAAGAAAGGATCTCAAACAAAAACAGTTGTTATCATTTCATTT
GATGATGGAATCGAATCTGAGTTGTGTTTAGGGTTTCTCTGGGTTAATCTCAGTCTATGTGGGCACATTTTAGTC
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TCTCTCATCTGTGTGTTTGATTATATTTTCAGGCAGAAAACTTTTTTTTGTTCTTGTATTTGATTACAATTAGTT
AAATATTTAAGACTTAGTAGAGATAATGCAATCTATGTATAAAAATAGAAACAATGATTGGTTATGTCGTGCAAC
CCAAGTGACCTTAATGTCGTACACACGTTCATCAGGTTAAAACGAAATTTCTCAGCAACTTCACTATTCTCTCTG
TCACATTGTTTTATTCTTTTCCATTATCTTTATTGGAAACTTGATTAGAAAGAACTGATTAAGGTATGAAGATAA

TTAGTGTACCATTCTTCACATGATACGAGGTGAACAATAGTATATCAGAATACTAGAATAATTTTATAAGTTATA
AAGATGAATGGTATACCACTTATTACAAAACGAAAACATAGTTAGATCTGAGAAGAAGGAAAGATTTTTGTTTTA
AAAAATGAGATGGTTAGATATATGCCATGTTAAGAGATGCCACCATGTTCTTCTTCTTCTTCTTACCCGGAAAAA
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AAAAATAGTAATGATCTCATTCAATTTGAACCCTCTCTCCATTTTCTTTCATAGTTTTTTTTTAGGGTAAATCAA
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AAAAAAGAGAGAAAGTGTTTCGGTTTTTGTTGCCTTCTCTCGAGTTAGGCCTTCTCTCTTCCGTCACACCATCAT
TTTTTTTCTCCATAACGACCAAATCATAAAACCGGTCGTTCCTATTTTTTCTTTCTCTGCGTTTTTTGTTTCGAA
TTCTTCAATAGAATTTTAAAAGAAAGAAGAAAGCTGGGTGAGACGAAGGAAGGAAGAGTCGACGGCGCGCCCGGT
ATGAGGGACCTCAAGGAATATGATTACAGTGCACTTTTGCTCAACCTTACCACCTCCAGCCTCAACAAGGCCCAG
AGGCGTTGGCGTTTTGCCTACGCTGCCATCTACTCTATGAGGGCTATGCTCTCTCTTGTTAAGGAGATAGTTCCC
GCAAGGATTGATCCCAAAACCTCAGATGCCTCTCTCTCTCTCCTACACAGCCCTCGAGTCCGGTGAGGGAGCA
AAGATCAACTCTATGCCTCTCTCTTACGTTCCTGCCATTGATCAAGAACAACTTGTGGAGATCATGAAGGGTAAG
GACTTACCGGGCATCCAAGCGCTGGGCGGCGTGGAGGGTGTCGCCGCTTCCCTTAGGACTAACCCCACCAAAGGG
ATCCACGGGAATGAGCAAGAAGTCAGTAGACGCCGTGACCTCTTTGGCTCTAACACCTACCATAAGCCACCGCCT
AAAGGACTTCTCTTCTTTGTGTATGAAGCTTTCAAAGACCTAACCATCTTGATCTTGTTGGTCTGCGCTATTTTC
TCCCTTGGCTTCGGTATCAAAGAACATGGCATCAAAGAAGGTTGGTATGAAGGCGGAAGCATCTTTGTAGCAGTC
TTCTTGGTCATAGTTGTCTCTGCTCTCAGCAACTTTAGGCAGGAGAGACAGTTCGACAAGCTGTCCAAGATAAGC
AATAACATCAAAGTGGAAGTCCTTCGGGACAGCAGGCGGCAACATATCTCCATCTTTGACGTTGTTGTTGGTGAT
GTTGTCTTCTTGAAGATCGGAGATCAGATTCCCGCTGATGGTCTGTTCTTGGAAGGGCATTCACTTCAGGTGGAC
GAGTCTAGTATGACAGGAGAGAGTGACCATCTTGAAGTGGACCACAAGGATAATCCCTTCTTGTTCTCAGGGACA
AAGATAGTCGATGGGTTTGCTCAAATGCTCGTTGTCTCTGTGGGTATGAGTACAACCTGGGGACAGACGATGAGC
TCCATAAATCAAGATTCCAGCGAGAGAACACCTTTGCAAGTCCGTCTTGACACGCTGACCTCCACCATCGGAAAA
ATTGGTCTTACGGTGGCAGCACTTGTTCTGGTAGTTCTATTAGTCCGTTACTTCACTGGGAACACAGAGAAAGAG
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TGCACAGACAAAACAGGTACTTTAACACTGAACGAGATGAAGGTTACCAAGTTTTGGCTTGGCCAAGAGTCAATC
CATGAAGACTCTACCAAAATGATCTCACCAGACGTTCTTGATCTGCTTTACCAAGGCACCGGTCTGAACACAACG
GGAAGTGTCTGTGTGTCAGACTCAGGATCAACGCCTGAGTTCTCAGGCAGTCCAACAGAGAAGGCCCTCTTGTCT
TGGACTGTGCTAAATCTGGGTATGGATATGGAGTCAGTAAAGCAGAAACATGAAGTTCTCCGCGTTGAAACTTTC
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GCCGCTGAAATGGTCCTAGCTATGTGTTCTCACTACTACACAAGCACTGGGTCTGTTGACTTAATGGACTCCACC
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GTTTTCACTGCAAAAGCTATCGCTTTTGAATGCGGAATCCTCGACCACAATGACAAAGATGAAGAAGATGCTGTT
GTAGAAGGTGTTCAATTCAGAAATTATACGGACGAAGAGAGAATGCAGAAAGTTGATAAGATCCGGGTCATGGCA
AGGTCCTCTCCCTCCGACAAGCTTCTAATGGTCAAGTGTCTGAGACTTAAAGGCCATGTGGTAGCCGTCACAGGG
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GCAAAAGAAAGCTCAGACATTGTAATTCTAGATGATAACTTCGCATCCGTTGCCACAGTCTTAAAATGGGGAAGG
TGTGTCTACAACAATATCCAGAAATTCATTCAGTTTCAGCTAACAGTGAACGTTGCAGCTCTTGTGATCAATTTT
ATCGCAGCAATTTCAGCCGGTGAGGTCCCTTTGACAGCAGTTCAACTGCTGTGGGTAAACCTCATCATGGACACA
TTGGGAGCTCTGGCTCTCGCCACGGAGCGACCCACTAACGAGCTCCTGAAGAGAAAGCCAGTTGGCCGAACAGAG
GCCCTGATAACAAATGTCATGTGGAGGAATCTCCTGGTTCAGTCATTATATCAAATAGCCGTACTCTTGATCTTA
CAATTCAAGGGTATGTCAATATTCAGTGTTCGCAAGGAAGTGAAGGACACGCTCATATTCAACACTTTCGTGCTC
TGTCAAGTTTTTAACGAATTCAATGCGAGGGAGATGGAGAAGAAAAATGTGTTCAAAGGCCTTCACAGAAACAGG
TTGTTCATTGGAATAATAGCGATAACTATTGTGCTTCAAGTCATTATGGTGGAATTCCTAAAGAAGTTTGCGGAT
ACAGTAAGGCTTAACGGGTGGCAATGGGGAACTTGCATAGCACTTGCATCCCTTTCATGGCCGATCGGCTTTTTC
ACAAAATTCATACCTGTTTCTGAGACACCATTCCTCAGTTACTTTAAGAATCCAAGATCCTTATTTAAGGGTTCA
AGAAGCCCATCTCTCAAGAAACCTGGACTAGTGATGGTGAGCAAGGGCGAGGAGCTTTTCACTGGAGTTGTCCCA
ATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACA
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TTCGGCTACGGCGTGCAGTGCTTCGCGCGTTATCCGGACCATATGAAGCGGCACGACTTCTTCAAGAGCGCCATG

CCTGAGGGATACGTGCAGGAGAGGACCATCTTCTTCAAGGACGACGGGAACTACAAGACACGTGCTGAAGTCAAG
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CACAAGTTAGAATACAACTACAACTCCCACAACGTATACATCATGGCCGACAAGCAAAAGAACGGCATCAAAGCC
AACTTCAAGACCCGCCACAACATCGAAGACGGCGGCGTGCAACTCGCTGATCATTATCAACAAAATACTCCAATT
GGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGTACCAGTCCGCCCTTTCGAAAGATCCCAACGAA
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GAACAACGAAACGCCTTCATCCAAAGTTTAAAAGATGACCCAAGCCAAAGCGCTAACCTTTTAGCAGAAGCTAAA
*AAGCTAAATGGTGCTCAGGCGCCGAAAGTAGACGCGAATTCCGCGGGGAAGTCAACCTGA*GAGCTCGAATTTCCC
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ATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTAT
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CTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTA
TTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTC
ACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGAAGGCCTTGACAGGAT
ATATTGGCGGGTAAACTAAGTCGCTGTATGTGTTTGTTTGAGATCTCATGTGAGCAAAAGGCCAGCAAAAGGCCA
GGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGAC
GCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGC
GCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTC
ATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCG
TTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCAC
TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAAGAG
TTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGC
GCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCAC
GTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTA
AATCAATCTAAAGTATATATGTGTAACATTGGTCTAGTGATTAGAAAAACTCATCGAGCATCAAATGAAACTGCA
ATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGA
GGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTA
ATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCA
AAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAA
CCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAA
CAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTT
CTAATACCTGGAATGCTGTTTTCCCTGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAAT
GCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACAACATTGGCAA
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ATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTTG
AGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTG
TTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGTGGCTTTGTTGAATAA
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AGTTCAAAATCACCAACTGGTCCACCTACAACAAAGCTCTCATCAACCGTGGCTCCCTCACTTTCTGGCTGGATG
ATGGGGCGATTCAGGCGATCCCCATCCAACAGCCCGCCGTCGAGCGGGCT

Plasmid Stock 585, *ACA9promoter::ACA2-TAP2(GFP).* The *ACA9* promoter (gray shading) starts immediately after a unique *Sbf1* site and ends just before a *Sal1* site. The *ACA2* coding sequence (blue shading and italics) begins downstream of the *Sal1* site, with the sequence *ATGGAGAGTTAC* and ends with the sequence *AAGACGATTCCG.* Immediately downstream is a GFP coding sequence (greenshading and italics) starting with the sequence *ATGGTGAGCAAG*.Following the GFP is a sequence encoding a tandem affinity tag, which ends with the stop codon (red highlight) at the end of the sequence AAGTCAACCTGA.This plant expression vector is based on a pGreenII backbone and harbors a kanamycin resistance marker for selection in bacteria and a hygromycin resistance marker for selection in plants.The *ACA9* promoter provides expression primarily in pollen.

TTTTTATCCCCGGAAGCCTGTGGATAGAGGGTAGTTATCCACGTGAAACCGCTAATGCCCCGCAAAGCCTTGATT
CACGGGGCTTTCCGGCCCGCTCCAAAAACTATCCACGTGAAATCGCTAATCAGGGTACGTGAAATCGCTAATCGG
AGTACGTGAAATCGCTAATAAGGTCACGTGAAATCGCTAATCAAAAAGGCACGTGAGAACGCTAATAGCCCTTTC
AGATCAACAGCTTGCAAACACCCCTCGCTCCGGCAAGTAGTTACAGCAAGTAGTATGTTCAATTAGCTTTTCAAT
TATGAATATATATATCAATTATTGGTCGCCCTTGGCTTGTGGACAATGCGCTACGCGCACCGGCTCCGCCCGTGG
ACAACCGCAAGCGGTTGCCCACCGTCGAGCGCCAGCGCCTTTGCCCACAACCCGGCGGCCGGCCGCAACAGATCG
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TTAGGAATTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCTTATATGCTCAA
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ATCGTTGTGGTTGCGGTTCCTGAAGGATTGCCTTTAGCTGTGACTCTAAGTCTTGCGTTTGCCATGAAGAAAATG
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TCTGGCTGGATGATGGGGCGATTCAGGCGATCCCCATCCAACAGCCCGCCGTCGAGCGGGCT

Plasmid Stock pYES2 *ACA12*, *GAL1::ACA12*. The ACA12 coding sequence (blue italics) was cloned into the pYES2 vector downstream a unique KpnI restriction site; *ACA12* begins with the sequence *ATGAGGGACCTC* and ends with the sequence *CTCAAGAAACCT*. Downstream is a *GGTCTAGAGGGCCGCATCATGTAA* sequence containing pYES2 polilinker terminator and stop codon (highlighted in red). The yeast expression pYES2 vector harbors: GAL1 promoter for galactose-inducible protein expression in yeast, *ura3* gene for selection in yeast by uracil prototrophy and ampicillin resistance marker for selection in bacteria.

ACGGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCTCCGTGCGTCCTCGTCTTCACCGG
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GGCTAGGGCGGTGGGAAGTGTAGGGGTCACGCTGGGCGTAACCACCACACCCGCCGCGCTTAATGGGGCGCTACA
GGGCGCGTGGGGATGATCCACTAGT

Plasmid Stock pYES2 *ACA12-GFP*, *GAL1::ACA12-GFP*. The ACA12 coding sequence (blue italics) was cloned into the pYES2 vector downstream a unique KpnI restriction site; *ACA12* begins with the sequence *ATGAGGGACCTC* and ends with the sequence *CTCAAGAAACCT*. Immediately downstream is a GFP coding sequence (green italics) starting with the sequence *ATGGTGAGCAAG* and ending with the sequence *CATCACCATCACCATCACGGATCCTGA*, encoding a 6xHis tag followed by a stop codon (highlighted in red). Single N211D and R334E mutants and the N211D-R334E double mutant were produced by site-directed mutagenesis using pYES2 *ACA12-GFP* construct as a template. Bases highlighted in yellow are those modified for generating the specified mutations. The yeast expression pYES2 vector harbors: GAL1 promoter for galactose-inducible protein expression in yeast, *ura3* gene for selection in yeast by uracil prototrophy and ampicillin resistance marker for selection in bacteria.

ACGGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCTCCGTGCGTCCTCGTCTTCACCGG
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ACA12 Is a Deregulated Isoform of Plasma Membrane Ca^{2+}-ATPase of *Arabidopsis thaliana*

PLANT MOLECULAR BIOLOGY

Margherita Limonta, Shawn Romanowsky, Claudio Olivari, Maria Cristina Bonza, Laura Luoni, Alexa Rosenberg[2], Jeffrey F. Harper and Maria Ida De Michelis

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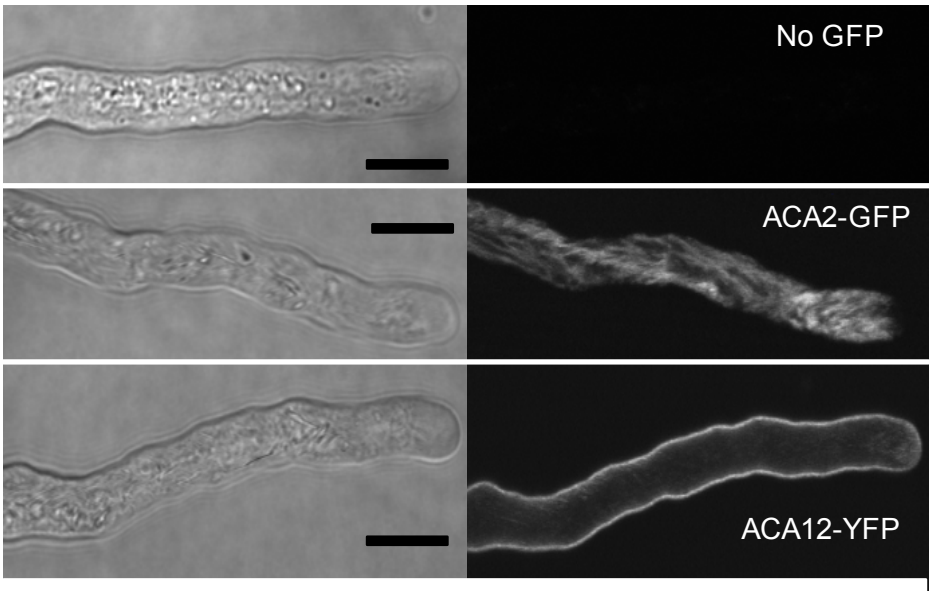
Online Resource 2



Confocal microscopy showing PM localization for ACA12-YFP. Pollen were germinated *in vitro* and imaged. DIC (Differential Interference Contrast) images are shown to the left, and corresponding confocal fluorescence micrographs to the right. A) A negative control showing a wild type pollen tube without any GFP. B), ACA2-GFP in *aca9-2 -/-* showing endomembrane localization. Image shown is from transgenic line ss-2106, transformed with *ACA9promoter::ACA2-TAP2(GFP)*. Equivalent results were observed for 3 other independent transgenic lines, ss 2103, 2104, and 2105. C) ACA12-YFP in *aca9-2 -/-* showing PM (plasma membrane) localization. Image shown is from transgenic line ss-2100, transformed with *ACA9promoter::ACA12-TAP2(YFP)*. Equivalent results were observed for 3 other independent transgenic lines, ss2099, 2101, 2102. Scale bar = 10 µm.

Images of XFP fluorescence were collected on a Olympus confocal system (FluoView FV10-ASW 2.1; Olympus) attached to an Olympus microscope (Inverted IX81) using a 60X objective (N.A. = 1.42) and an argon gas laser for generating a 488-nm excitation line. Emission was detected with band pass between 510 and 530 nm. Differential interference contrast (DIC) images were collected on the same system by using a single transmitted light detector. Images were processed by using FluoView software.

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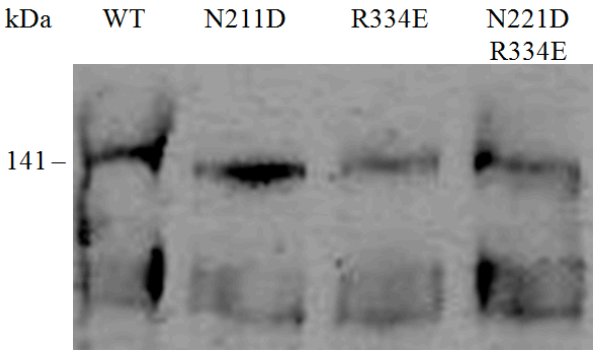
Online Resource 3



Expression of ACA12-GFP mutants in yeast strain K616. K616 yeast transformed with WT or mutant ACA12-GFP was galactose-induced at 20 °C for 24 h; solubilized microsomal proteins (40 μg) were subjected to SDS-PAGE, blotted and decorated with the HisProbe-HPR.