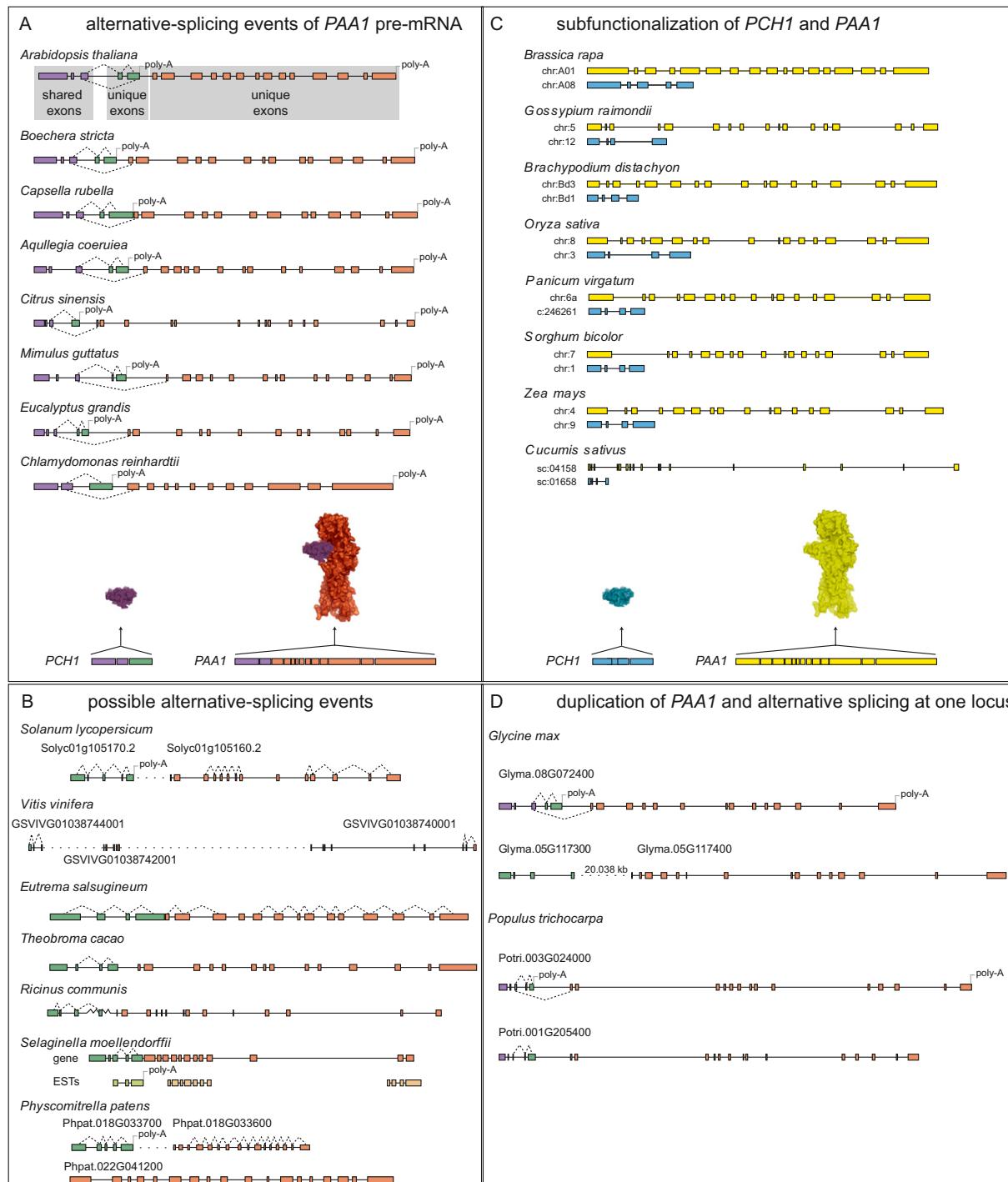
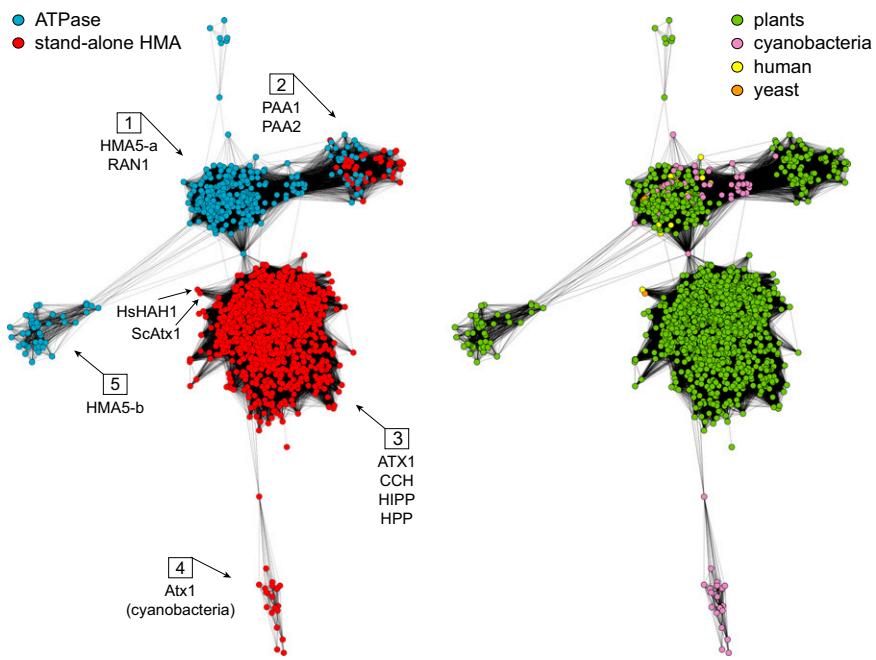


# Supporting Information

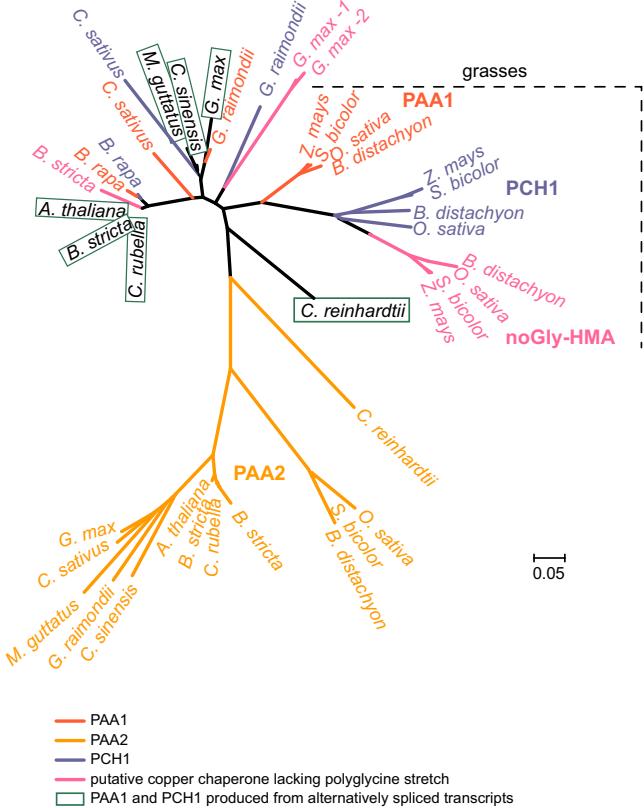
Blaby-Haas et al. 10.1073/pnas.1421545111



**Fig. S1.** Schematics of alternative-splicing events and gene duplications. (A) Gene models presented in Fig. 1A (Upper) and predicted structural models of PCH1 and PAA1 (Lower). The N-terminal HMBD of PAA1 is colored purple to highlight identity with PCH1, also colored purple. (B) Gene models not presented in the main text, where alternative-splicing events producing both PCH1 and PAA1 are likely (because EST support for PCH1 is available). (C) Gene models of PAA1 and PCH1 presented in Fig. 1B (Upper) and predicted structural models of PCH1 and PAA1 (Lower). (D) Gene models from *G. max* and *P. trichocarpa*, where the presence of two genes with alternative splicing is unclear. chr, chromosome; sc, scaffold.



**Fig. S2.** PAA1/PAA2 protein cluster uniquely contains soluble proteins. The protein similarity network of the heavy metal associated (HMA) domains (CDD cl00207) in Cu<sup>+</sup>-ATPases and Cu<sup>+</sup> chaperones from plants, cyanobacteria, *Saccharomyces cerevisiae* (ScAtx1), and *Homo sapiens* (HsHah1) is shown. Nodes that represent the HMA domain from the Cu<sup>+</sup>-ATPases are colored blue, whereas the nodes that represent the HMA domain from identified and predicted chaperones are colored red. Proteins that are more similar to one another than to other proteins in the network will cluster, allowing the delineation of protein subgroups based on amino acid similarity. Each distinct cluster is given a number designation, and representative *A. thaliana* proteins from each cluster are shown. AtHMA5 contains two HMA domains, one in cluster 2 and the other in cluster 3. The same network, but colored by taxonomic group, is provided to the right. Atx1, antioxidant 1; CCH, Copper chaperone; HIPP, heavy metal associated isoprenylated plant proteins; HPP, heavy metal associated plant proteins; RAN1, responsive-to-antagonist 1.

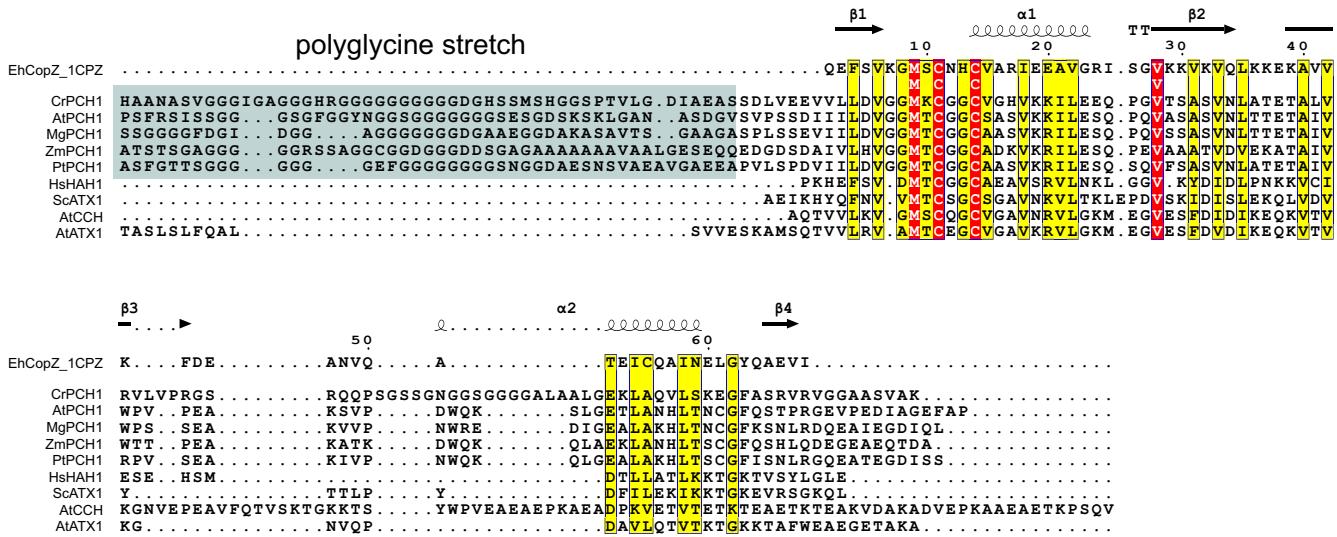


**Fig. S3.** Phylogenetic reconstruction (neighbor-joining tree) of the HMA domain from selected Cu<sup>+</sup>-ATPases (PAA1 in orange and PAA2 in yellow), PCH1 (lilac), and no-glycine stretch protein (pink) from the PAA1/PAA2 cluster identified in Fig. S2. The clade that represents grass-specific sequences is highlighted with a dashed line.

EhCopZ\_1CPZ A.....

CrPCH1 MQSVAQR.....GVVARP.....ATTHFVQCPRRARPALVTLAVSCPNNNTSLQSOPQHASWAQKFVRATVVATTLQFFAAAS  
 AiPCH1 MESTLSAFSTVKATAMARSSGGPSLPLLTISKALNRHFTGARHLHPLLARCSVRLGGFHGSR..FTSSNSA..LRSLGAAVLPIVRHRLECLSSSS  
 MgPCH1 MESTLLSV.....GASTMSIAALSRSLSNH..STPIIAHLHRRFSTTLPSEQIALLQLSRRVELRRFGPHLVRG.HDSV.DLRRRNRIQFSVRSA  
 ZmPCH1 MEAVVAT.....TTTRVSFPLPTAGRPLLP.....PVLRPWRGFAVASV  
 PtPCH1 MESAL AI.....SATTVPLFILSKALNRHFTNTTRSLLRISSTQLVTRRLSSIYSRNSDFLPSSPS..LKGLRVGV..PLVRLRLECVSSSA  
 HsHAF1 M.....  
 ScATX1 M.....  
 AtCCH M.....  
 AtATX1 M.....

LKDLFQAVSYQN



**Fig. S4.** Amino acid alignment of plant PCH1 sequences with the sequences of known Atx1-like chaperones. A cartoon representation of the aligned CopA (*Enterococcus hirae* Protein Data Bank ID code 1CPZ) secondary structure is shown above. The polyglycine stretch found near the N terminus of PCH1 is highlighted with a gray-blue box. At, *A. thaliana*; Cr, *C. reinhardtii*; Hs, *H. sapiens*; Mg, *M. guttatus*; Pt, *P. trichocarpa*; Sc, *S. cerevisiae*; Zm, *Z. mays*.

1 10 20 30 40 50 60 70 80 90 100 110 120

AlPAA1 MESTLSAFSTVKATAMARSSGGPSLPLLTISKALNRHFTGARHLHPLLARCSVRLGGFHGSRFTSSNSALRSLGAAVLPIVRHRLECLSSSSPSFRSISSSGGSGFGGYNNGSGGGGGGG  
 AiPCH1 MESTLSAFSTVKATAMARSSGGPSLPLLTISKALNRHFTGARHLHPLLARCSVRLGGFHGSRFTSSNSALRSLGAAVLPIVRHRLECLSSSSPSFRSISSSGGSGFGGYNNGSGGGGGGG

130 140 150 160 170 180 190 200 210 220 230

AlPAA1 ESGDSKSKLGANASDCVSVPSDDIIILDVGGMPCGCCSASVVKKILESOPOQVASASVNLTTEATAIVWPVPEAKSVPDWOKSICGETLANHTNCFOSTPR.....DLVTENFFKVFET  
 AiPCH1 ESGDSKSKLGANASDCVSVPSDDIIILDVGGMPCGCCSASVVKKILESOPOQVASASVNLTTEATAIVWPVPEAKSVPDWOKSICGETLANHTNCFOSTPRGEVPEDIAGEFAP.....

240 250 260 270 280 290 300 310 320 330 340 350 360

AlPAA1 KTKDKQARLKESGRELAVALCAVCLVGHLLTHFLGVNAPWIHAHSTGFHVSLLCLITLLGPGRKLVLGDIKSLLKGSPNMNTLVGLGALSSFSVSSLAAMIPKLGWKTFFEEPVMILAFVLLGR  
 AiPCH1 .....

370 380 390 400 410 420 430 440 450 460 470 480

AlPAA1 NLEQRAKIKATSDMTGLLSVLPSKARLLLDGLLQNNTVEVPCNSLSVGDLVVLPGDRVPADGVVKSGRTIDESSTGTPELPTVKESGSQVAAGSINLNGLTLEVHRSGETAVGDIIRLVEE  
 AiPCH1 .....

490 500 510 520 530 540 550 560 570 580 590 600 610

AlPAA1 AQSREAPVQQQLWDKVAGRFTYGVMAAATFTFWNLFGAHLVPSALHNGSPMSLALQLSCSVLVAACPCALGLATPTAMLVGTSLGARRGLLRGGDILEKFSLVDTVVFDTGTLTKGHPVVTE  
 AiPCH1 .....

620 630 640 650 660 670 680 690 700 710 720 730

AlPAA1 VIIPENPRHNLLNDTWSSEVEVIMLAAEVESNTTHPVGKAIVKAARARNQTMKAEDGTFTEEPGSGAVAIVNKKRVTGTLLEWVKRHGATGNSLLALEEEHEINNQSVVYIGVDNTLAAVIRFEDKV  
 AiPCH1 .....

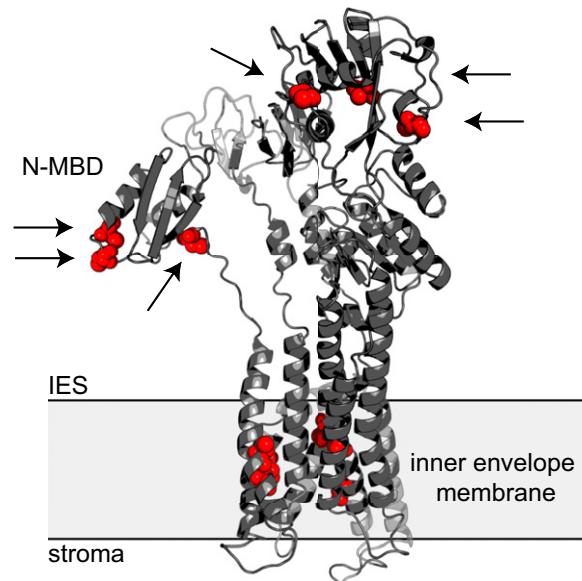
740 750 760 770 780 790 800 810 820 830 840 850 860

AlPAA1 REDAAQVVENLTRQGIDVYMLSGDKRNAANYVASVVGINSHERVIAGVKPAEKKNFINELKQKNKKIVAMVGDGINDAAALASSNVGVAMGGAGAASEVSPVVLGMNRLTQLLDAMELSRQTMKTV  
 AiPCH1 .....

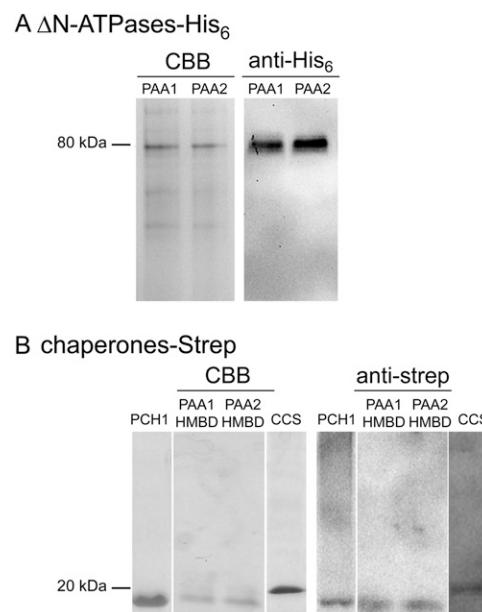
870 880 890 900 910 920 930 940

AlPAA1 KQNLWWAFGYNIVGIPIAAGVLLPTGMLTPSMAGALMGVSSLGVMTNSLLLRYRFFNSRNKDKNVKEPEKGETKQPHENTRWKQSS  
 AiPCH1 .....

**Fig. S5.** Amino acid alignment of full-length AtPAA1 and AtPCH1. Identical amino acid residues are highlighted with a red background.



**Fig. S6.** Predicted structural model of the pea PAA1 ortholog. Each cysteine is shown as a red space-filled model.



**Fig. S7.** Coomassie brilliant blue (CBB) staining and immunoblot of recombinantly expressed and purified  $\Delta$ N-AtPAA1 and  $\Delta$ N-AtPAA2 (A) and AtPCH1, AtPAA1-HMBD, AtPAA2-HMBD, and AtCCS (B).

**Table S1. Primers**

Primer name	R.E. site	Sequence 5' → 3'
PAA1_His_For	Ndel	aaaaaaacatatgCCTCGAGATTTGGTGACAGAGAA
PAA1_His_Rev	Sall	aaaaaaagtgcacAGAGCTTGCTTCATCTG
PAA2_His_For	Ndel	aaaaaaacatatgAAATGGAAAGAAATGGTTAGC
PAA2_His_Rev	Sall	aaaaaaagtgcacCAGAGAATTGGCTGGTT
AtPCH1_strep_For	Ndel	aaaaaaacatatgTCAAGTGGCGCCGGTTCT
AtPCH1_strep_Rev	Sall	aaaaaaagtgcacAGGTGCAAACCTCCCTGCAA
CCS_strep_For	AseI	atgcataatATGGTGGATATGACATGTGAGG
CCS_strep_Rev	Sall	atgcgtcgacAACTTACTGGCCACGAAATC
PAA2HMBD_strep_For	Ndel	aaaaaaacatatgATCGAAAGCGTAAAAAGTATTACG
PAA2HMBD_strep_Rev	Sall	aaaaaaagtgcacGGATTGACCAGCAGGTCTTC
PAA1HMBD_strep_For	Ndel	aaaaaaacatatgTCAAGTGGCGCCGGTTCT
PAA1HMBD_strep_Rev	Sall	aaaaaaagtgcacGCGAGCTGCTTGTCTTTG
PhoLacZ_For	BamHI	atcgatccCTGTTCTGGAAAACCGG
PhoLacZ_Rev	XmaI	atccccccggGCATTCGCCATTCAAGGCT
PAA1_PhoLac_For	Ncol	atgcctatggAGTCTACACTCTCAGCTTTC
PAA1_PhoLac_Rev	Nhel	atgcgttagCTAAGAGCTTGCTTCATCTTG
PAA1_PhoLac_1Rev	BamHI	atgcgtatccAACAGAGACACCATCACTTGCA
PAA1_PhoLac_1For	AgeI	atgcacccggtCCGTCGGCAGATATCATTATTCTC
PAA1_PhoLac_2Rev	BamHI	atgcgtatccGGGAGCATTAACCCCTAGAAA
PAA1_PhoLac_2For	AgeI	atgcacccgggTGGATTATGCGATCCATT
PAA1_PhoLac_3Rev	BamHI	atgcgtatccACCTTTAAAAGACTCTTGATACCATC
PAA1_PhoLac_3For	AgeI	atgcacccgggTCCCCAACATGAACACG
PAA1_PhoLac_4Rev	BamHI	atgcgtatccTGTCTTCCAGCCAAATTG
PAA1_PhoLac_4For	AgeI	atgcacccgggTTTTGAGGAACCAAGTTATGTTAA
PAA1_PhoLac_5Rev	BamHI	atgcgtatccACCATAAGCAGAAGGCG
PAA1_PhoLac_5For	AgeI	atgcacccgggTATCTGGAGAATTCAACTGTTGA
PAA1_PhoLac_6Rev	BamHI	atgcgtatccAACATGTGCACCAAATAGATTCC
PAA1_PhoLac_6For	AgeI	atgcacccgggCTTCCTCTGCCTTGCATAA
PAA1_PhoLac_7Rev	BamHI	atgcgtatccCCCATTATGCAAGGCAGAAG
PAA1_PhoLac_7For	AgeI	atgcacccgggTAGCCAAATGTCTTGGCC
PAA1_PhoLac_8Rev	BamHI	atgcgtatccATTGTGCTTGGATTTCAGGAA
PAA1_PhoLac_8For	AgeI	atgcacccgggTTGAATGATACTTGTCAAGAAGTAGA
PAA1_PhoLac_9Rev	BamHI	atgcgtatccTGTGGACAATTACGAGGCC
PAA1_PhoLac_9For	AgeI	atgcacccgggATGAAGGCAGAGGATGGAAC

R.E., restriction enzyme.

## Other Supporting Information Files

[Dataset S1 \(XLSX\)](#)