Supplementary Materials for

Structure of the hexameric fungal plasma membrane proton pump in its autoinhibited state

Sabine Heit, Maxwell M. G. Geurts, Bonnie J. Murphy, Robin A. Corey, Deryck J. Mills, Werner Kühlbrandt, Maike Bublitz*

*Corresponding author. Email: maike.bublitz@bioch.ox.ac.uk

Published 10 November 2021, *Sci. Adv.* 7, eabj5255 (2021)
DOI: 10.1126/sciadv.abj5255

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Fig. S1: Topology diagram and $E1/E2$ scheme of Pma1. (A) Overall Pma1 topology. Nucleotide-binding (N) domain red, actuator (A) domain yellow, phosphorylation (P) domain blue, regulatory (R) domain cyan, N-terminal extension (green), M1-2 pink, M3-4 gold, and M6-10 grey. (B) Canonical $E1$-$E2$ catalytic cycle for proton pumping by Pma1 with transient phosphorylation.
Fig. S2: Overview of the refinement of the Pma1 hexamer and monomer cryo-EM maps.
(A) Schematic representation of the refinement workflow resulting in the final hexamer map H3 and the final focused monomer map M. Masks for 3D classification and refinement are shown in white. (B) Angular distribution plot of all particles that contributed to the final hexamer map H3. The height of the bars is proportional to the number of particles in those views. (C) Local resolution of the final hexamer map H3 and (D) for the final monomer map M. Resolution estimates from Relion 3Drefine (51), maps shown are H3-DE and M-DE.
Fig. S3: Structural state comparison of Pma1 with related P-type ATPases. RMSD (root mean square deviation) calculated over all C-alpha atoms of Pma1 (E1) compared to the crystal structures of AHA2 (E1) and SERCA in different states along the catalytic cycle using PyMOL(82). PDB entries: 5KSD (AHA2 E1), 4HW1 (SERCA MgE1), 3N8G (SERCA Ca2E1), 1T5T (SERCA Ca2E1~P), 3BA6 (SERCA Ca2E1P), 3B9B (SERCA E2P), 3N5K (SERCA E2~P), 1WPJ (SERCA E2.P), 3NAL (SERCA E2).
Fig. S4: Nucleotide-binding site and assignment of the R domain to its respective monomer.

(A) ADP is bound at the nucleotide-binding site between the P (blue) and N (light red) domains. ADP, residues involved in its coordination and Asp378 are shown as sticks (spheres for glycine) with C-atoms coloured in green (ADP) or according to their domain, a Mg$^{2+}$ ion is shown as a light green sphere. The cryo-EM map (M-DM) is shown as blue or black mesh for MgADP and the protein, respectively, with a higher contour level for MgADP. Polar contacts are indicated by yellow dashes. (B) The distance from the helical part of the R domain to M10 and M10’ (shown as cartoon) is very similar, the assignment is based on a short extension at the N terminus of the R-helix that points towards M10. The cryo-EM map (M-DM) is shown as grey mesh, residues of the non-helical part of R and M10 are shown in stick representation.
Fig. S5. Membrane lipid composition used in the coarse grained MD simulations of Pma1. For details on the lipids, refer to Table S6.
Fig. S6. Fractional interaction times of all lipids with Pma1 residues surrounding (A) site I and (B) site II, defined as the number of frames in which a lipid is within 0.6nm of a given residue. For details on the lipids, refer to Table S6.
Fig. S7: Monomerisation of Pma1 with OGNG. (A) Size-exclusion chromatography of Pma1 in n-dodecyl-β-D-maltopyranoside (DDM) (blue; highlighted fractions used for cryo-EM) or octyl glucose neopentyl glycol (OGNG) (orange). SEC column: Superose 6 Increase 10/300 (GE Healthcare). (B) Native PAGE of Pma1 in DDM or OGNG (samples in 0.2 M ammonium acetate buffer for native MS). Unlabelled arrows indicate uncharacterised Pma1 oligomers (potentially dimers and tetramers). Protein ladder (M): NativeMark™ (Invitrogen), gel: 3-12% NativePAGE™ (Invitrogen).
Fig. S8. Radial distribution functions of PIPC and DIPC in the coarse grained simulation of a PIPC/DIPC-only membrane. The distribution confirms a preferential binding of Pma1 to double-unsaturated lipids. For details on the lipids, refer to Table S6.
Fig. S9. Lipid density maps for PIPC, DIPA, Ergosterol, PIPE, PIPA, PIPS, PVSM, XNSM, DPCE, and PNCE. Values correspond to average numbers of molecules per nm³ and do not account for the respective membrane composition fraction. For details on the lipids, refer to Table S6.
Fig. S10. Quantitative bilayer deformation analysis. Average z-height position of lipid headgroup-phosphates in (A) the outer leaflet, and (B) the inner leaflet. Values represent the z-height difference in Ångström relative to the value at coordinate -50, 110 (assumed to represent a membrane region unperturbed by protein or boundary effects). (C) Zoom of (B) on the region of one Pma1 monomer. (D) Average leaflet thickness between phosphates (inner leaflet minus outer leaflet) at each x, y coordinate. Scale is in Ångström. (E) Zoom of (D) on the region of one monomer.
Fig. S11. Proposed model of the proton transport mechanism in Pma1, based on homology models with SERCA. In the $E1$ state, a proton enters from the cytosol and binds between D730 and N154, facilitated by a local membrane depression. The proton gets occluded by a concerted upward movement of M1-2. The side chain of R695 in M7 appears to shield the protonated D730 from the former ion entry region. A large conformational change is expected to follow phosphoryl transfer to the P domain, leading to the transient opening of the extracellular proton exit pathway in the $E2P$ state. In this state, N154 in M2 could interact with Q125 at the M1 kink to stabilise the bundle, and R695 can form a salt bridge with D730, favouring its deprotonation. A cluster of negatively charged residues (red shaded area) facilitates proton exit. In the subsequent dephosphorylation reaction, the exit pathway closes, leading to the $E2$ state, from which the pump cycles back to open up to the cytosol once again. Residues shown as coloured circles are: D (orange): protonated D730; D (red): deprotonated D730, N (purple): Asn154; Q (purple): Q125; R (blue): Arg695.
Fig. S12: Proton exit funnel in the open-to outside E2P homology model. Aqueous cavity representing the proton exit funnel between M1, M4 and M6. Important residues for proton transport are shown as sticks. M1-2 are coloured pink, M3-4 gold and M5-10 grey. The proton acceptor/donor Asp730 (labelled in red) at the inner end of the funnel lies in bonding distance to Arg695 (C-alpha distance: 7.3 Å; indicated bond: 4.9 Å), presuming a small side chain rotation of the latter. The E1-interaction partner of Asp730, Asn154 (labelled in red), has moved away and forms a putative hydrogen bond with Gln125 (2.8 Å). Putative bonds indicated as orange dashes. There is a clustering of negatively charged residues (Glu139, Asp140, Asp143, Glu324 and Glu720) at the extracellular end of the exit funnel.
Fig. S13: Distance between M10 and the R helix, and hexamer homology model diameters

(A) Distance between the C-alpha atoms of the last residue of M10 (Asp880) and the first residue of the R-helix (Gln897) in the autoinhibited E1 structure and homology models throughout the catalytic E1/E2 cycle. The R-helix was placed into the homology models in its relative position to the P domain as observed in E1. The homology models were generated with SWISS-MODEL (64) based on a structural alignment and SERCA crystal structures with the PDB entries 1T5T (E1~P), 3B9B (E2P), 3N5K (E2~P), and 3NAL (E2).

(B) Maximal outer diameter of the autoinhibited Pma1 E1 structure and homology models in states E1P, E2P, E2~P, and E2.
Fig. S14: Compound docking into Pma1. (A) Chemical structure of the docked tetrahydrocarbazole compounds. (B) Tetrahydrocarbazole compounds docked into Pma1 in the autoinhibited $E_1$ state giving estimated affinities stronger than -9 kcal/mol (only one representative mode with the highest affinity score shown per compound): 6/S (green), 7/R (blue), 7/S (pink), 8/R (yellow). The protein surface is coloured according to conservation with proton pumps from human- and plant-pathogenic fungi from purple (conserved) to bluegreen (variable) (calculated with ConSurf (42)). (C) Enlarged view of the putative inhibitor binding site with the protein shown as grey cartoon. Residues involved in binding of most compounds are shown as sticks.
Table S1. Cryo-EM data collection, refinement and validation statistics.

| Data collection                  | hexamer          | monomer          |
|---------------------------------|------------------|------------------|
| Instrument                      | FEI Titan Krios / Gatan K3 |
| Magnification                   | 105’000          |
| Voltage (kV)                    | 300              |
| Electron dose (e-/Å²)           | 42               |
| Defocus range (μm)              | -1.3 to -2.5     |
| Calibrated pixel size (Å)       | 0.837            |

| Map values                      | hexamer          | monomer          |
|---------------------------------|------------------|------------------|
| Map ID                          | H3               | M                |
| EMDB ID                         | EMD-12644        | EMD-12638        |
| No. of particles                | 59’511           | 293’999          |
| Map symmetry                    | C6               | C1               |
| Map resolution (Å)              | 3.28             | 3.21             |
| FSC threshold 0.143             |                  |                  |

| Model                           |                  |                  |
|---------------------------------|------------------|------------------|
| PDB ID                          | 7NY1             | 7NXF             |
| Protein residues                | 4'974            | 829              |
| Ligands (no.)                   | K (6), Mg (6), ADP (6) | K (1), Mg (1), ADP (1) |

| Model validation                |                  |                  |
|---------------------------------|------------------|------------------|
| Map CC (ligands)                | 0.76 (0.70)      | 0.80 (0.79)      |
| MolProbity score                | 2.08             | 1.96             |
| Clash score                     | 12.01            | 8.99             |
| Bond length rmsd (Å)            | 0.007            | 0.006            |
| Bond angle rmsd (°)             | 1.142            | 0.816            |
| B factor (Å²) (min/max/mean)    |                  |                  |
| Protein                         | 19.28/155.87/82.25 |
| ligands                         | 61.94/109.49/107.30 |
| Rotamer outliers (%)            | 0                |                  |
| Cβ outliers (%)                 | 0                |                  |
| Ramachandran Plot               |                  |                  |
| Favoured/allowed/outliers (%)   | 92.1 / 7.9 / 0   |                  |
Table S2: Residues involved in intra- and intermolecular contacts mediated via the R domain.

|       | R – P   | R – P’  | R – R’/ R” – R |
|-------|---------|---------|----------------|
| Pro893| G589    | Val562  | Leu902         |
| Lys 894| M592    | Gly563  | Ser892         |
| Arg900| G594    | Arg566  | Arg893         |
| Glu903| Ser595  | Asn577  |                |
| Asp904| Tyr598  | Ile578  |                |
| Val907| Asp599  | Tyr579  |                |
| Arg911| Glu602  | Arg583  |                |
|       | Arg625  | Asp500  |                |
|       |         | Phe600  |                |
Table S3: Residues involved in the intermolecular contact within the M domain.

| M3 / M4  | M7 / L7-8 | M10     |
|----------|-----------|---------|
| Thr295   | Ile772    | Ile862  |
| Ile299   | Thr775    | Phe863  |
| Ile302   | Thr776    | Cys869  |
| Leu306   | /         | Ile870  |
| Trp309   | Gly784    | Tyr876  |
| Val310   | Gly785    |         |
| Phe313   | Ile786    |         |
| Tyr314   | Gln788    |         |
|          | /         |         |
| Pro318   |           |         |
| Ile319   |           |         |
Table S4: Alignment of Neurospora crassa Pma1 with the plasma membrane proton pumps of human-pathogenic (above black line) and plant-pathogenic (below black line) fungi.

| Neurospora crassa | Sporothrix schenckii | Histoplasma capsulatum | Coccidioides immitis | Blastomyces dermatitidis | Acremonium chrysogenum | Talaromyces marneffei | Syncephalastrum racemosum | Rhizopus stolonifer | Cryptococcus gattii | Cryptococcus neoformans | Claviceps purpurea | Colletotrichum gloeosporioides | Magnaporthe oryzae | Fusarium oxysporum | Botrytis cinerea | Fusarium graminearum | Aspergillus niger | Blumeria graminis | Sclerotinia sclerotiorum | Mycosphaerella graminicola | Cochliobolus heterostrophus | Rhizoctonia solani | Ustilago maydis | Puccinia graminis |
|-------------------|---------------------|------------------------|---------------------|------------------------|------------------------|----------------------|-------------------------|------------------|----------------|----------------|----------------|----------------|----------------|------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| N                   | Y                   | S                   | D                   | E                   | D                   | E                   | D                     | S               | D             | E             | D              | S              | D                | S                | D                 | S              | D            | E              | D               | D               | D              | D                | S               | D              | D               | D              |
| Q                   | K                   | P                   | K                   | E                   | K                   | K                   | P                     | K               | E             | K             | E              | K               | K              | P                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| K                   | K                   | E                   | K                   | E                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| P                   | C                   | I                   | N                   | C                   | I                   | N                   | C                     | I               | N             | C             | I              | N               | C              | I                | N               | C                 | I               | N            | P              | C               | I               | N              | I                | N               | C              | I               | N              |
| Y                   | Q                   | K                   | P                   | E                   | K                   | K                   | P                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| P                   | C                   | I                   | N                   | C                   | I                   | N                   | C                     | I               | N             | C             | I              | N               | C              | I                | N               | C                 | I               | N            | P              | C               | I               | N              | I                | N               | C              | I               | N              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| Q                   | K                   | P                   | E                   | K                   | E                   | K                   | E                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| Y                   | Q                   | K                   | P                   | E                   | K                   | K                   | E                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| P                   | C                   | I                   | N                   | C                   | I                   | N                   | C                     | I               | N             | C             | I              | N               | C              | I                | N               | C                 | I               | N            | P              | C               | I               | N              | I                | N               | C              | I               | N              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| Q                   | K                   | P                   | E                   | K                   | E                   | K                   | E                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| Y                   | Q                   | K                   | P                   | E                   | K                   | K                   | E                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| P                   | C                   | I                   | N                   | C                   | I                   | N                   | C                     | I               | N             | C             | I              | N               | C              | I                | N               | C                 | I               | N            | P              | C               | I               | N              | I                | N               | C              | I               | N              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| Q                   | K                   | P                   | E                   | K                   | E                   | K                   | E                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| Y                   | Q                   | K                   | P                   | E                   | K                   | K                   | E                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| P                   | C                   | I                   | N                   | C                   | I                   | N                   | C                     | I               | N             | C             | I              | N               | C              | I                | N               | C                 | I               | N            | P              | C               | I               | N              | I                | N               | C              | I               | N              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| Q                   | K                   | P                   | E                   | K                   | E                   | K                   | E                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| Y                   | Q                   | K                   | P                   | E                   | K                   | K                   | E                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| K                   | E                   | K                   | E                   | K                   | K                   | K                   | K                     | K               | E             | K             | E              | K               | K              | K                | K               | E                 | K               | K            | Q              | K               | K               | K              | K                | K               | K              | K               | K              |
| P                   | C                   | I                   | N                   | C                   | I                   | N                   | C                     | I               | N             | C             | I              | N               | C              | I                | N               | C                 | I               | N            | P              | C               | I               | N              | I                | N               | C              | I               | N              |
| Database  | Taxonomy | Species | Table S4: continued |
|-----------|----------|---------|---------------------|
| Neurospora crassa | VDELKLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 42 |
| Sporothrix schenckii | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Histoplasma capsulatum | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Coccioides immitis | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Blastomyces dermatitidis | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Acremonium chrysogenum | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Trichophyton rubrum | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Candida glabrata | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Candida auris | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Candida albicans | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Pneumocystis jirovecii | VDELKTLADAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 22 |
| Aspergillus fumigatus | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 7 |
| Talaromyces marneffei | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 23 |
| Syncphalastrum racemosum | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 146 |
| Rhizopus stolonifer | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 48 |
| Lichtheimia corymbifera | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 21 |
| Cryptococcus gattii | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 23 |
| Cryptococcus neoformans | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 33 |
| Claviceps purpurea | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Colletotrichum gloeosporioides | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Magnaporthe oryzae | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Fusarium oxysporum | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Botrytis cinerea | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Fusarium graminearum | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Aspergillus niger | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Blumeria graminis | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Sclerotinia sclerotiorum | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Mycosphaerella graminicola | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Cochliobolus heterostrophus | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Rhizoctonia solani | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Ustilago maydis | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
| Puccinia graminis | VASLGDIAKAVLVRGSKTEIAKRYVPQSGDLVVEEGIIIPAGKIVTE | 223 |
# Table S4: continued

| Yeast | Molecular Biology and Pathogenesis | Table S4: described in the text |
|-------|----------------------------------|---------------------------------|
| Neurospora crassa | Q C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Sporothrix schenckii | Q C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Histoplasma capsulatum | T C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Coccidioides immitis | T C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Blastomyces dermatitidis | T C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Acremonium chrysogenum | N C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Trichophyton rubrum | H C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Candida glabrata | Q F S T Y E A A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Candida auris | S Y S T Y E A A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Candida albicans | S Y S T Y E A A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Pneumocystis jirovecii | S Y S T Y V E A A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Aspergillus fumigatus | T C Y T T G K K A T A I V A A K Q F Y G A A T V L V A G Q D H D D Q V Q L V A R C V F L U S G E | 34 |
| Talaromyces marneffei | T C Y T T G K K A T A I V A A K Q F Y G A A T V L V A G Q D H D D Q V Q L V A R C V F L U S G E | 34 |
| Syncyphalastrum racemosum | 35 |
| Rhizopus stolonifer | E Y S T Y E A A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Lithichaeum corymbifera | E Y S T Y E A A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Cryptococcus gattii | E Y S T Y E A A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Cryptococcus neoformans | E Y S T Y E A A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Claviceps purpurea | N C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Colletotrichum gloeosporioides | N C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Magnaporthe oryzae | Q C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Fusarium oxysporum | N C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Botrytis cinerea | T C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Fusarium graminearum | N C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Aspergillus niger | V C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Blumeria graminis | V C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Sclerotinia sclerotiorum | V C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Mycosphaerella graminicola | V C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Cochliobolus heterostrophus | V C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Rhizoctonia solani | V C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Ustilago maydis | O C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Puccinia graminis | O C Y A S K E A F Y V I A R G N D T Y G A A L L N A A S S G G T G E V L G G I T L I L I L Y V | 34 |
| Organism                          | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
|----------------------------------|----------------------------------------------------------------------------|
| Neurospora crassa                | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Sporothrix schenckii             | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Histoplasma capsulatum           | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Coccidioides immitis             | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Blastomyces dermatitidis         | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Acremonium chrysogenum           | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Trichophyton rubrum              | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Candida glabrata                 | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Candida auris                    | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Candida albicans                 | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Pneumocystis jirovecii           | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Aspergillus fumigatus            | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Talaromyces marneffei            | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Syncephalasrum racemosum         | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Rhizopus stolonifer              | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Lichtheimia corymbifera          | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Cryptococcus gattii              | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Cryptococcus neoformans          | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Claviceps purpurea               | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Colletotrichum gloeosporioides    | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Magnaporthe oryzae               | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Fusarium oxysporum               | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Botrytis cinerea                 | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Fusarium graminearum             | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Aspergillus niger                | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Blumeria graminis                | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Sclerotinia sclerotiorum          | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Mycosphaerella graminicola       | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Cochliobolus heterostrophus      | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Rhizoctonia solani               | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Ustilago maydis                  | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |
| Puccinia graminis                | AAYLAKKAIYVGLAIcirculated AYVLGII7RMLCKKLGKeligic7086a = GYVPKDLILX 46 |

Table S4: continued
| Table S4: continued |
|---------------------|
| **Neurospora crassa** | **Sporothrix schenckii** | **Histoplasma capsulatum** | **Coccidioides immitis** | **Blastomyces dermatitidis** | **Acremonium chrysogenum** | **Trichophyton rubrum** | **Candida glabrata** | **Candida auris** | **Candida albicans** | **Pneumocystis jirovecii** | **Aspergillus fumigatus** | **Talaromyces marneffei** | **Syncyphalastrum racemosum** |
| Neurospora crassa | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Sporothrix schenckii | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Histoplasma capsulatum | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Coccidioides immitis | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Blastomyces dermatitidis | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Acremonium chrysogenum | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Trichophyton rubrum | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Candida glabrata | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Candida auris | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Candida albicans | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Pneumocystis jirovecii | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Aspergillus fumigatus | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Talaromyces marneffei | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Syncyphalastrum racemosum | - | - | - | - | - | - | - | - | - | - | - | - | - |

| **Claviceps purpurea** | **Colletotrichum gloeosporioides** | **Magnaporthe oryzae** | **Fusarium oxysporum** | **Botrytis cinerea** | **Fusarium graminearum** | **Aspergillus niger** | **Buchnera glaucescens** | **Sclerotinia sclerotiorum** | **Myxococcus xanthus** | **Cochliobolus heterostrophus** | **Rhizoctonia solani** | **Ustilago maydis** | **Puccinia graminis** |
|------------------------|-------------------------------|-----------------------|-------------------|---------------------|---------------------|-------------------|---------------------|-------------------|---------------------|---------------------|-------------------|-------------------|
| Claviceps purpurea | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Colletotrichum gloeosporioides | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Magnaporthe oryzae | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Fusarium oxysporum | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Botrytis cinerea | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Fusarium graminearum | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Aspergillus niger | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Buchnera glaucescens | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Sclerotinia sclerotiorum | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Myxococcus xanthus | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Cochliobolus heterostrophus | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Rhizoctonia solani | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ustilago maydis | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Puccinia graminis | - | - | - | - | - | - | - | - | - | - | - | - | - |
Table S4: continued

| Organism                        | Sensitivity | Specificity |
|---------------------------------|-------------|-------------|
| Neurospora crassa               | 0.97        | 0.97        |
| Sporothrix schenckii            | 0.97        | 0.97        |
| Histoplasma capsulatum          | 0.97        | 0.97        |
| Coccioides immitis              | 0.97        | 0.97        |
| Blastomyces dermatitidis        | 0.97        | 0.97        |
| Acremonium chrysogenum          | 0.97        | 0.97        |
| Trichophyton rubrum             | 0.97        | 0.97        |
| Candida glabrata                | 0.97        | 0.97        |
| Candida auris                   | 0.97        | 0.97        |
| Candida albicans                | 0.97        | 0.97        |
| Pneumocystis jirovecii          | 0.97        | 0.97        |
| Aspergillus fumigatus           | 0.97        | 0.97        |
| Talaromyces marneffei           | 0.97        | 0.97        |
| Syncphalariae racemosum         | 0.97        | 0.97        |
| Rhizopus stolonifera            | 0.97        | 0.97        |
| Lichtheimia corymbifera         | 0.97        | 0.97        |
| Cryptococcus gattii             | 0.97        | 0.97        |
| Cryptococcus neoformans         | 0.97        | 0.97        |
| Claviceps purpurea              | 0.97        | 0.97        |
| Colletotrichum gloeosporioides   | 0.97        | 0.97        |
| Magnaporthe oryzae              | 0.97        | 0.97        |
| Fusarium oxysporum              | 0.97        | 0.97        |
| Botrytis cinerea                | 0.97        | 0.97        |
| Fusarium graminearum            | 0.97        | 0.97        |
| Aspergillus niger               | 0.97        | 0.97        |
| Blumeria graminicola            | 0.97        | 0.97        |
| Sclerotinia slerotiorum          | 0.97        | 0.97        |
| Mycosphaerella graminicola      | 0.97        | 0.97        |
| Cochliobolus heterostrophus     | 0.97        | 0.97        |
| Rhizoctonia solani              | 0.97        | 0.97        |
| Ustilago maydis                 | 0.97        | 0.97        |
| Puccinia graminis               | 0.97        | 0.97        |
| Table S4: continued |
|---------------------|

- **Neurospora crassa**
- **Sporothrix schenckii**
- **Histoplasma capsulatum**
- **Coccidioides immitis**
- **Blastomyces dermatitidis**
- **Acremonium chrysogenum**
- **Trichophyton rubrum**
- **Candida glabrata**
- **Candida auris**
- **Candida albicans**
- **Pneumocystis jirovecii**
- **Aspergillus fumigatus**
- **Talaromyces marneffei**
- **Syncephalastrum racemosum**
- **Rhizopus stolonifer**
- **Lichtheimia corymbifera**
- **Cryptococcus gattii**
- **Cryptococcus neoformans**

- **Claviceps purpurea**
- **Colletotrichum gloeosporioides**
- **Magnaporthe oryzae**
- **Fusarium oxysporum**
- **Botrytis cinerea**
- **Fusarium graminearum**
- **Aspergillus niger**
- **Blumeria graminis**
- **Sclerotinia sclerotiorum**
- **Mycosphaerella graminicola**
- **Cochliobolus heterostrophus**
- **Rhizoctonia solani**
- **Ustilago maydis**
- **Puccinia graminis**

| Neurospora crassa | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
|-------------------|----|----|----|----|----|----|-----|----|----|----|----|----|----|----|----|
| Sporothrix schenckii | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Histoplasma capsulatum | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Coccidioides immitis | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Blastomyces dermatitidis | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Acremonium chrysogenum | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Trichophyton rubrum | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Candida glabrata | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Candida auris | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Candida albicans | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Pneumocystis jirovecii | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Aspergillus fumigatus | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Talaromyces marneffei | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Syncephalastrum racemosum | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Rhizopus stolonifer | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Lichtheimia corymbifera | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Cryptococcus gattii | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Cryptococcus neoformans | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |

- **Claviceps purpurea**
- **Colletotrichum gloeosporioides**
- **Magnaporthe oryzae**
- **Fusarium oxysporum**
- **Botrytis cinerea**
- **Fusarium graminearum**
- **Aspergillus niger**
- **Blumeria graminis**
- **Sclerotinia sclerotiorum**
- **Mycosphaerella graminicola**
- **Cochliobolus heterostrophus**
- **Rhizoctonia solani**
- **Ustilago maydis**
- **Puccinia graminis**

- **Claviceps purpurea** | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Colletotrichum gloeosporioides | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Magnaporthe oryzae | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Fusarium oxysporum | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Botrytis cinerea | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Fusarium graminearum | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Aspergillus niger | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Blumeria graminis | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Sclerotinia sclerotiorum | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Mycosphaerella graminicola | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Cochliobolus heterostrophus | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Rhizoctonia solani | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Ustilago maydis | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
| Puccinia graminis | V V | V L | G W | V L | V V | V V | A Q | R E | E C | V O | P K | N T | P E | 7 9 |
### Table S4: continued

| **Neurospora crassa** | **Sporothrix schenckii** | **Histoplasma capsulatum** | **Coccidioides immitis** | **Blastosomyces dermatitidis** | **Acremonium chrysogenum** | **Trichophyton rubrum** | **Candida glabrata** | **Candida auris** | **Candida albicans** | **Pneumocystis jirovecii** | **Aspergillus fumigatus** | **Talaromyces marneffei** | **Syncephalastrum racemosum** | **Rhizopus stolonifer** | **Lichtheimia corymbifera** | **Cryptococcus gattii** | **Cryptococcus neoformans** |
|------------------------|--------------------------|----------------------------|--------------------------|------------------|-------------------------|------------------------|---------------------|-------------------|------------------------|-------------------------|-----------------------------|--------------------------|-----------------------------|-------------------------|--------------------------|------------------------|--------------------------|
| Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... | Table 1 continued... |
| Pathogen Name                  | Codon Sequence |
|--------------------------------|----------------|
| Neurospora crassa              | NN...           |
| Sporothrix schencki            | GN...           |
| Histoplasma capsulatum         | KN...           |
| Coccidioides immitis           | KG...           |
| Blastomyces dermatitidis       | KN...           |
| Acremonium chrysogenum         | KS...           |
| Trichophyton rubrum            | KS...           |
| Candida glabrata               | TK...           |
| Candida auris                  | RK...           |
| Candida albicans               | RN...           |
| Pneumocystis jirovecii         | RM...           |
| Aspergillus fumigatus          | RN...           |
| Talaromyces marneffi           | AT...           |
| Syncphalastrum racemosum       | AQ...           |
| Rhizopus stolonifer            | AAAAAA          |
| Lichtheimia corymbifera        | AAAA            |
| Cryptococcus gattii            | CC...           |
| Cryptococcus neoformans        | CC...           |
| Claviceps purpurea             | RRA             |
| Colletotrichum gloeosporioides | RRA             |
| Magnaporthe oryzae             | RRA             |
| Fusarium oxysporum             | RRA             |
| Botrytis cinerea               | RRA             |
| Fusarium graminearum           | RRA             |
| Aspergillus niger              | RRA             |
| Rhizoctonia solani             | SRRRRRRR        |
| Ustilago maydis                | SRRRRRRR        |
| Puccinia graminis              | LKLVDAIGFLRR    |
| Neurospora crassa              |       |
| Sporothrix schencki            |       |
| Histoplasma capsulatum         |       |
| Coccidioides immitis           |       |
| Blastomyces dermatitidis       |       |
| Acremonium chrysogenum         |       |
| Trichophyton rubrum            |       |
| Candida glabrata               |       |
| Candida auris                  |       |
| Candida albicans               |       |
| Pneumocystis jirovecii         |       |
| Aspergillus fumigatus          |       |
| Talaromyces marneffi           |       |
| Syncphalastrum racemosum       |       |
| Rhizopus stolonifer            |       |
| Lichtheimia corymbifera        |       |
| Cryptococcus gattii            |       |
| Cryptococcus neoformans        |       |
| Claviceps purpurea             |       |
| Colletotrichum gloeosporioides |       |
| Magnaporthe oryzae             |       |
| Fusarium oxysporum             |       |
| Botrytis cinerea               |       |
| Fusarium graminearum           |       |
| Aspergillus niger              |       |
| Rhizoctonia solani             |       |
| Ustilago maydis                |       |
| Puccinia graminis              |       |
Table S5: Alignment of *Neurospora crassa* Pma1 with plasma membrane proton pumps of selected plants.

Sequences are sorted in descending order according to their identity with Pma1. Accession codes: *N. crassa* (sp|P07038), *Coffea eugenioides* (XP_021716212.1), *Spinacia oleracea* (XP_021865157.1), *Cucumis sativus* (XP_004152192.1), *Hordeum vulgare* (KAE8805265.1:29-858), *Jatropha curcas* (XP_012068768.1:37-846), *Triticum aestivum* (P83970.1:29-858), *Ananas comosus* (XP_02090190.1), *Chenopodium quinoa* (XP_021755229.1:32-855), *Carica papaya* (XP_021899224.1), *Ricinus communis* (XP_015572514.1:34-875), *Punica granatum* (XP_031378860.1), *Nicotiana tabacum* (NP_001312285.1), *Brassica napus* (XP_022556197.1), *Gossypium hirsutum* (KAA3489374.1:33-874), *Arabidopsis thaliana* (NP_194748.1), *Manihot esculenta* (XP_021598156.1:34-875), *Malus domestica* (XP_008372282.1:35-876), *Camellia sinensis* (XP_028098451.1:32-870), *Zea mays* (AQK46772.1:25-866), *Theobroma cacao* (EOY29625.1), *Sesamum indicum* (XP_011084025.1), *Hevea brasiliensis* (XP_021654241.1:37-846), *Glycine max* (XP_003549696.1:32-903)

```
Neurospora crassa Pma1
Coffea eugenioides
Spinacia oleracea
Cucumis sativus
Hordeum vulgare
Jatropha curcas
Triticum aestivum
Ananas comosus
Chenopodium quinoa
Carica papaya
Ricinus communis
Punica granatum
Nicotiana tabacum
Brassica napus
Gossypium hirsutum
Arabidopsis thaliana
Manihot esculenta
Malus domestica
Camellia sinensis
Zea mays
Theobroma cacao
Sesamum indicum
Hevea brasiliensis
Glycine max
```

Table S5: Alignment of *Neurospora crassa* Pma1 with plasma membrane proton pumps of selected plants.
| Table S5: continued |
|---------------------|

**Neurospora crassa**

| Coffea eugenioides |
|---------------------|
| Spinacia oleracea |
| Cucumis sativus |
| Hordeum vulgare |
| Jatropha curcas |
| Triticum aestivum |
| Arabidopsis thaliana |
| Manihot esculenta |
| Malus domestica |
| Camellia sinensis |
| Zea mays |
| Theobroma cacao |
| Sesamum indicum |
| Hevea brasiliensis |
| Glycine max |

| Neurospora crassa |
|---------------------|
| Coffea eugenioides |
| Spinacia oleracea |
| Cucumis sativus |
| Hordeum vulgare |
| Jatropha curcas |
| Triticum aestivum |
| Ananas comosus |
| Chenopodium quinoa |
| Carica papaya |
| Ricinus communis |
| Punica granatum |
| Nicotiana tabacum |
| Brassica napus |
| Gossypium hirsutum |
| Arabidopsis thaliana |
| Manihot esculenta |
| Malus domestica |
| Camellia sinensis |
| Zea mays |
| Theobroma cacao |
| Sesamum indicum |
| Hevea brasiliensis |
| Glycine max |

| Neurospora crassa |
|---------------------|
| Coffea eugenioides |
| Spinacia oleracea |
| Cucumis sativus |
| Hordeum vulgare |
| Jatropha curcas |
| Triticum aestivum |
| Ananas comosus |
| Chenopodium quinoa |
| Carica papaya |
| Ricinus communis |
| Punica granatum |
| Nicotiana tabacum |
| Brassica napus |
| Gossypium hirsutum |
| Arabidopsis thaliana |
| Manihot esculenta |
| Malus domestica |
| Camellia sinensis |
| Zea mays |
| Theobroma cacao |
| Sesamum indicum |
| Hevea brasiliensis |
| Glycine max |

| Neurospora crassa |
|---------------------|
| Coffea eugenioides |
| Spinacia oleracea |
| Cucumis sativus |
| Hordeum vulgare |
| Jatropha curcas |
| Triticum aestivum |
| Ananas comosus |
| Chenopodium quinoa |
| Carica papaya |
| Ricinus communis |
| Punica granatum |
| Nicotiana tabacum |
| Brassica napus |
| Gossypium hirsutum |
| Arabidopsis thaliana |
| Manihot esculenta |
| Malus domestica |
| Camellia sinensis |
| Zea mays |
| Theobroma cacao |
| Sesamum indicum |
| Hevea brasiliensis |
| Glycine max |
Table S5: continued

| Neurospora crassa | Coffea eugenioides | Spinacia oleracea | Cucumis sativus | Hordeum vulgare | Jatropha curcas | Triticum aestivum | Arabidopsis thaliana | Manihot esculenta | Malus domestica | Camellia sinensis | Zea mays | Theobroma cacao | Sesamum indicum | Hevea brasiliensis | Glycine max |
|-------------------|-------------------|------------------|----------------|----------------|----------------|------------------|---------------------|-------------------|----------------|----------------|----------|----------------|-----------------|-----------------|------------|
Table S5: continued

| Plant Name | Neuron | Correlation Coefficient |
|------------|--------|-------------------------|
| Neurospora crassa |  | 5.7 |
| Coffea eugenioides |  | 7.14 |
| Spinacia oleracea |  | 7.15 |
| Cucumis sativus |  | 7.15 |
| Hordeum vulgare |  | 6.83 |
| Jatropha curcas |  | 6.83 |
| Triticum aestivum |  | 6.83 |
| Arabidopsis thaliana |  | 6.83 |
| Chenopodium quinoa |  | 6.83 |
| Carica papaya |  | 7.14 |
| Ricianus communis |  | 7.17 |
| Punica granatum |  | 7.17 |
| Nicotiana tabacum |  | 7.17 |
| Brassica napus |  | 7.17 |
| Gossypium australis |  | 6.83 |
| Ananas comosus |  | 7.75 |
| Manihot esculenta |  | 6.83 |
| Malus domestica |  | 6.83 |
| Zea mays |  | 6.83 |
| Theobroma cacao |  | 6.83 |
| Sesamum indicum |  | 6.82 |
| Hevea brasiliensis |  | 6.82 |
| Glycine max |  | 6.83 |

| Plant Name | Neuron | Correlation Coefficient |
|------------|--------|-------------------------|
| Neurospora crassa |  | 7.98 |
| Coffea eugenioides |  | 7.01 |
| Spinacia oleracea |  | 7.01 |
| Cucumis sativus |  | 7.01 |
| Hordeum vulgare |  | 7.01 |
| Jatropha curcas |  | 7.01 |
| Triticum aestivum |  | 7.01 |
| Arabidopsis thaliana |  | 7.01 |
| Chenopodium quinoa |  | 7.01 |
| Carica papaya |  | 7.01 |
| Ricianus communis |  | 7.01 |
| Punica granatum |  | 7.01 |
| Nicotiana tabacum |  | 7.01 |
| Brassica napus |  | 7.01 |
| Gossypium australis |  | 7.01 |
| Ananas comosus |  | 7.01 |
| Manihot esculenta |  | 7.01 |
| Malus domestica |  | 7.01 |
| Zea mays |  | 7.01 |
| Theobroma cacao |  | 7.01 |
| Sesamum indicum |  | 7.01 |
| Hevea brasiliensis |  | 7.01 |
| Glycine max |  | 7.01 |

| Plant Name | Neuron | Correlation Coefficient |
|------------|--------|-------------------------|
| Neurospora crassa |  | 8.55 |
| Coffea eugenioides |  | 8.21 |
| Spinacia oleracea |  | 8.35 |
| Cucumis sativus |  | 8.35 |
| Hordeum vulgare |  | 8.35 |
| Jatropha curcas |  | 8.35 |
| Triticum aestivum |  | 8.35 |
| Arabidopsis thaliana |  | 8.35 |
| Chenopodium quinoa |  | 8.35 |
| Carica papaya |  | 8.35 |
| Ricianus communis |  | 8.35 |
| Punica granatum |  | 8.35 |
| Nicotiana tabacum |  | 8.35 |
| Brassica napus |  | 8.35 |
| Gossypium australis |  | 8.35 |
| Malus domestica |  | 8.35 |
| Theobroma cacao |  | 8.35 |
| Sesamum indicum |  | 8.35 |
| Hevea brasiliensis |  | 8.35 |
| Glycine max |  | 8.35 |
Table S5: continued

| Species                        | Amino Acid Sequence | Alignments |
|--------------------------------|---------------------|------------|
| Neurospora crassa              |                     |            |
| Coffea eugenioides             |                     |            |
| Spinacia oleracea              |                     |            |
| Cucumis sativus                |                     |            |
| Hordeum vulgare                |                     |            |
| Jatropha curcas                |                     |            |
| Triticum aestivum              |                     |            |
| Ananas comosus                 |                     |            |
| Chenopodium quinoa             |                     |            |
| Carica papaya                  |                     |            |
| Ricinus communis               |                     |            |
| Punica granatum                |                     |            |
| Nicotiana tabacum              |                     |            |
| Brassica napus                 |                     |            |
| Gossypium australe             |                     |            |
| Arabidopsis thaliana           |                     |            |
| Manihot esculenta              |                     |            |
| Malus domestica                |                     |            |
| Camellia sinensis              |                     |            |
| Zea mays                       |                     |            |
| Theobroma cacao                |                     |            |
| Sesamum indicum                |                     |            |
| Hevea brasiliensis             |                     |            |
| Glycine max                    |                     |            |

| Species                        | Amino Acid Sequence | Alignments |
|--------------------------------|---------------------|------------|
| Neurospora crassa              |                     |            |
| Coffea eugenioides             |                     |            |
| Spinacia oleracea              |                     |            |
| Cucumis sativus                |                     |            |
| Hordeum vulgare                |                     |            |
| Jatropha curcas                |                     |            |
| Triticum aestivum              |                     |            |
| Ananas comosus                 |                     |            |
| Chenopodium quinoa             |                     |            |
| Carica papaya                  |                     |            |
| Ricinus communis               |                     |            |
| Punica granatum                |                     |            |
| Nicotiana tabacum              |                     |            |
| Brassica napus                 |                     |            |
| Gossypium australe             |                     |            |
| Arabidopsis thaliana           |                     |            |
| Manihot esculenta              |                     |            |
| Malus domestica                |                     |            |
| Camellia sinensis              |                     |            |
| Zea mays                       |                     |            |
| Theobroma cacao                |                     |            |
| Sesamum indicum                |                     |            |
| Hevea brasiliensis             |                     |            |
| Glycine max                    |                     |            |
Table S6. Lipid composition used in the coarse-grained molecular dynamics simulations.

| Lipid name | Head group          | Tail                  | Net charge | Content in inner leaflet (%) | Content in outer leaflet (%) |
|------------|---------------------|-----------------------|------------|-----------------------------|-----------------------------|
| PIPC       | Phosphatidylcholine | C16:0/18:2            | 0          | 11                          | 28                          |
| DIPC       | Phosphatidylcholine | di-C16:2-C18:2        | 0          | 5                           | 14                          |
| PIPE       | Phosphatidylethanolamine | C16:0/18:2         | 0          | 8                           | 13                          |
| DIPE       | Phosphatidylethanolamine | di-C16:2-C18:2      | 0          | 4                           | 7                           |
| PIPA       | Phosphatidic acid   | C16:0/18:2            | -2         | 11                          | 11                          |
| DIPA       | Phosphatidic acid   | di-C16:2-C18:2        | -2         | 5                           | 5                           |
| PIPS       | Phosphatidylerine   | C16:0/18:2            | -1         | 17                          | 0                           |
| DIPS       | Phosphatidylerine   | di-C16:2-C18:2        | -1         | 9                           | 0                           |
| PIPI       | Phosphatidylinositol| C16:0/18:2            | -1         | 8                           | 0                           |
| XNSM       | Sphingomyelin       | C(d24:1/24:1)         | 0          | 2                           | 2                           |
| PVSM       | Sphingomyelin       | C(d18:1/18:1)         | 0          | 4                           | 4                           |
| DPCE       | Ceramide            | C(d18:1/18:0)         | 0          | 3                           | 3                           |
| PNCE       | Ceramide            | C(d18:1/24:1)         | 0          | 8                           | 8                           |
| ERGO       | Ergosterol          | –                     | 0          | 5                           | 5                           |