Supplemental material

Supplemental MATERIALS AND METHODS

Southern blot analysis. Southern blot analysis was performed using a probe amplified with the primers (attB4)Aolah-up-F and (attB1)Aolah-up-R for verification of *Aolah* gene disruption. For confirmation of *Aowsc* gene disruption, Southern blot analysis was performed using a probe amplified with the primers (attB4)Aowsc-up-F and (attB1)Aowsc-up-R. After electrophoresis, genomic DNAs digested with restriction enzymes *Eco*T22I or *Apa*I were transferred onto Hybond N+ membrane (GE Healthcare, Buckinghamshire, UK). The ECL (enhanced chemiluminescence) Direct Nucleic Acid Labeling and Detection system (GE Healthcare) and a LAS-1000plus luminescent image analyzer (Fuji Photo Film, Tokyo, Japan) were used for detection.

Protein extraction and Western blot analysis The *A. oryzae* strains expressing full-length AoLAH (AoLAH-3×HA) or middle-region deleted AoLAH (AoLAH[(1-2039)+(4710-5727)]-3×HA) were grown in DPY liquid nutrient medium as shaking cultures for 24 h at 30°C. Cell extracts were prepared by homogenizing the mycelia using liquid nitrogen in an elution buffer (50 mM Tris/HCl, pH 7.5, 1 mM PMSF, and 1:100 protease inhibitor cocktail [Sigma-Aldrich, St. Louis, MO]). Total cell lysates were centrifuged at 500×*g* for 3 min to remove cell debris, and the obtained supernatants were further centrifuged (10,000×*g*, 10 min, 4°C). The resulting pellet fraction was used for detection of middle-region deleted AoLAH. To prepare a fraction

highly enriched with Woronin body proteins, the 10,000×*g* pellet fraction was re-suspended in the elution buffer supplemented with 0.5% Triton X-100, and then centrifuged at 20,000×*g*, 4°C for 10 min, of which the pellet fraction was used for detection of full-length AoLAH. The pellet fractions were dissolved in sampling buffer and analyzed by Western blotting. NuPAGE® Novex 3%-8% Tris-Acetate Gel, NuPAGE®LDS Sample Buffer (4X), and HiMark™ Pre-Stained Protein marker (Invitrogen Life Technologies, Carlsbad, CA) were used for Western blotting analysis. The primary antibodies used was mouse anti-HA monoclonal antibody (12CA5; Roche, Mannheim, Germany), and the secondary was peroxidase-conjugated anti-mouse antibody (Vector Laboratories, Burlingame, CA). Protein bands were detected and analyzed by the ECL detection reagents (Pierce, Rockford, IL) and a LAS-1000plus luminescent image analyzer.

Table S1 Primers used in this study

Name	Sequence (5'-3')
(attB4)Aolah-up-F	GGGGACAACTTTGTATAGAAAAGTTGGGAGGATTGCCTCCGCATACAATAC
(attB1)Aolah-up-R	GGGGACTGCTTTTTTGTACAAACTTGGCCTTGATCGCTCTCTGCCCACT
(attB2)Aolah-down-F_2	GGGGACAGCTTTCTTGTACAAAGTGGGATGACTATGATGACTGCCAACATCTCC
(attB3)Aolah-down-R_2	GGGGACAACTTTGTATAATAAAGTTGCGATATGGAACCGATTCAAAGTCAACTCC
(attB1) Aolah_1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGATGTTTAAGGCCTTATTGGCCGGGGGCCGT
(attB2) Aolah_6243-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCCAGCCCGCCATCGTCTTTTACACCGA
Aolah-Cter-F (14254)	GGGGACAAGTTTGTACAAAAAAGCAGGCTATGGAATGGCCTTTGATAGATTGGGAGAAGGAA
Aolah-Cter-R (17400)	GGGGACCACTTTGTACAAGAAAGCTGGGTCAATCACATTGCTCATGTCCATGGTCGACGA
Aolah_N-R-fusion	TATCAAAGGCCATTCCTCCAGCCCGCCATCGTCTTTTACACCGAC
Aolah_C-F-fusion	GATGGCGGGCTGGAGGAATGGCCTTTGATAGATTGGGAGAAGGAA
(attB4) Aowsc-up-F	GGGGACAACTTTGTATAGAAAAGTTGAGATGAGAGCATAGCGCGGTACC
(attB1) Aowsc-up-R	GGGGACTGCTTTTTTGTACAAACTTGGATGGCGGTTGATGCGGTTGCGT
(attB2) Aowsc-down-F	GGGGACAGCTTTCTTGTACAAAGTGGATAGCGTTACGACCAACGTCGCG
(attB3) Aowsc-down-R	GGGGACAACTTTGTATAATAAAGTTGTGCCTCCAAGAGGCGAAAGTCAGT
(attB1)-DsRed-M-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGGACAACACCGAGGACGTCATC
(attB2)-PTS1-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTCATAATTTGGACTGGGAGCCGGAGTGGCG

Supplemental figure legends

FIG. S1 Amino acid sequence alignment of N-terminal regions from *Aspergillus* LAH proteins. Based on comparison with other LAH proteins, the *Aolah* gene was re-predicted to contain additional 1,198 amino acids at N-terminus (indicated by an arrow) of the original ORF AO090011000895 from the *A. oryzae* genome database (DOGAN: Database of the Genomes Analyzed at NITE [National Institute of Technology and Evaluation, Japan]; http://www.bio.nite.go.jp/dogan/Top). Amino acid sequence data of LAH proteins from *A. fumigatus* (AfLAH) and *A. nidulans* (AnLAH) were taken from the *Aspergillus* Genome Database (AspGD; http://www.aspgd.org/).

FIG. S2 Amino acid sequences of poly-lysine regions in the middle region of LAH proteins.

FIG. S3 Southern blot analysis of the *Aolah* disruptant. Genomic DNAs of the parent strain (NSRKu70-1-1; P) and *Aolah* disruptant (NSK-Δlah2; Δ) were digested with *Eco*T22I, and then subjected to Southern blot analysis. Filled bars indicate the used probe.

FIG. S4 Domain prediction of WSC proteins. Amino acid sequence data of WSC proteins from *A. fumigatus* and *A. nidulans* were taken from the *Aspergillus* Genome Database (AspGD; http://www.aspgd.org/). SMART (http://smart.embl-heidelberg.de/) was used for prediction of domains. ClustalW program (http://www.genome.jp/tools/clustalw/) was used for the sequence identity analysis.

FIG. S5 Southern blot analysis of the *Aowsc* disruptant. Genomic DNAs of the parent strain (NSRKu70-1-1; P) and *Aowsc* disruptant (NSK-Δwsc1; Δ) were digested with *Apa*I, and then subjected to Southern blot analysis (lower). Filled bars indicate the used probe.

FIG. S6 Western blot analysis of full length (A) and middle-region deleted (B) AoLAHs. Arrowheads indicate the bands corresponding to each protein. Lanes: C, wild-type control strain (NSRKu70-1-1A); FL, AoLAH full-length expressing strain; ΔM: strain expressing middle-region deleted AoLAH.

FIG. S7 Disorder prediction analysis for AoLAH. The disorder probability of AoLAH was predicted by PrDOS (Protein DisOrder prediction System; http://prdos.hgc.jp/cgi-bin/top.cgi). The plot of disorder probability of each residue along the sequence is shown. Residues beyond the black threshold line in this plot are predicted to be disordered. Note that most of the AoLAH N-terminal and middle regions is predicted to be disordered, and that part of the AoLAH C-terminal region is predicted to be ordered.

FIG. S8 Amino acid content of the middle region of LAH protein. The amino acid content of the LAH proteins was calculated and the three most abundant amino acids are listed. Important amino acids are highlighted by different colors; E and K are highlighted by green and red, respectively.

Supplemental Video S1 Time-lapse movie of Woronin body localization in the wild-type strain. Woronin bodies were visualized by expressing AoLAH[1-2039]-EGFP and asterisks indicate the septum.

Supplemental Video S2 Time-lapse movie of Woronin body localization in the *Aolah* disruptant. Woronin bodies were visualized by expressing AoLAH[1-2039]-EGFP and asterisks indicate the septum.

Supplemental Video S3 Time-lapse movie of Woronin bodies tethered to the septtum in strain expressing full-length AoLAH. Woronin bodies were visualized by expressing AoLAH[1-2039]-EGFP.

Supplemental Video S4 Time-lapse movie of Woronin bodies tethered to the septum in strain expressing middle-region deleted AoLAH. Woronin bodies were visualized by expressing AoLAH[1-2039]-EGFP.

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A. oryzae AoLAH	*A. fumigatus* LAH	*A. nidulans* LAH
2316 KKKKKDKKKK 2325	2345 KKKKKKNKKK 2354	2670 SKKAKKKKKK 2679
2945 KKGKKKKKNR 2954	2473 KKSKKKKKKK 2482	2823 KASKKKKKNK 2832
3067 KKNKKKKKKK 3076	2738 KKNKKKNKRK 2747	2988 KKSKKKNKKK 2997
3155 KKKTKKEQKK 3164	2874 KKKAKKKKNR 2883	3272 KNAKKKKKKK 3281
3491 KKDKKKKKKQ 3500	3003 KKSKKNKKKK 3012	3445 KKKNKKKKKK 3454
3593 KKKAKKDKKK 3602	3089 KKTKKEKKKK 3098	3575 KKKVKKDKKK 3584
3819 KDKKKKKKRK 3828	3330 KKKDKKKKKK 3339	3734 KKRAKKEKKR 3743
3942 KKAKKKERKR 3951	3438 KKKAKKDKKK 3447	4273 KKKNKKAKKQ 4282
4443 KKQKKKAKKQ 4452	3642 KSKKNKKKKK 3651	4443 SKKEKKKLKK 4452
	3718 KKKAKKKDKK 3727	
	4246 SKKKSKKAKK 4255	

FIG. S2 Amino acid sequences of poly-lysine regions in the middle region of LAH proteins.

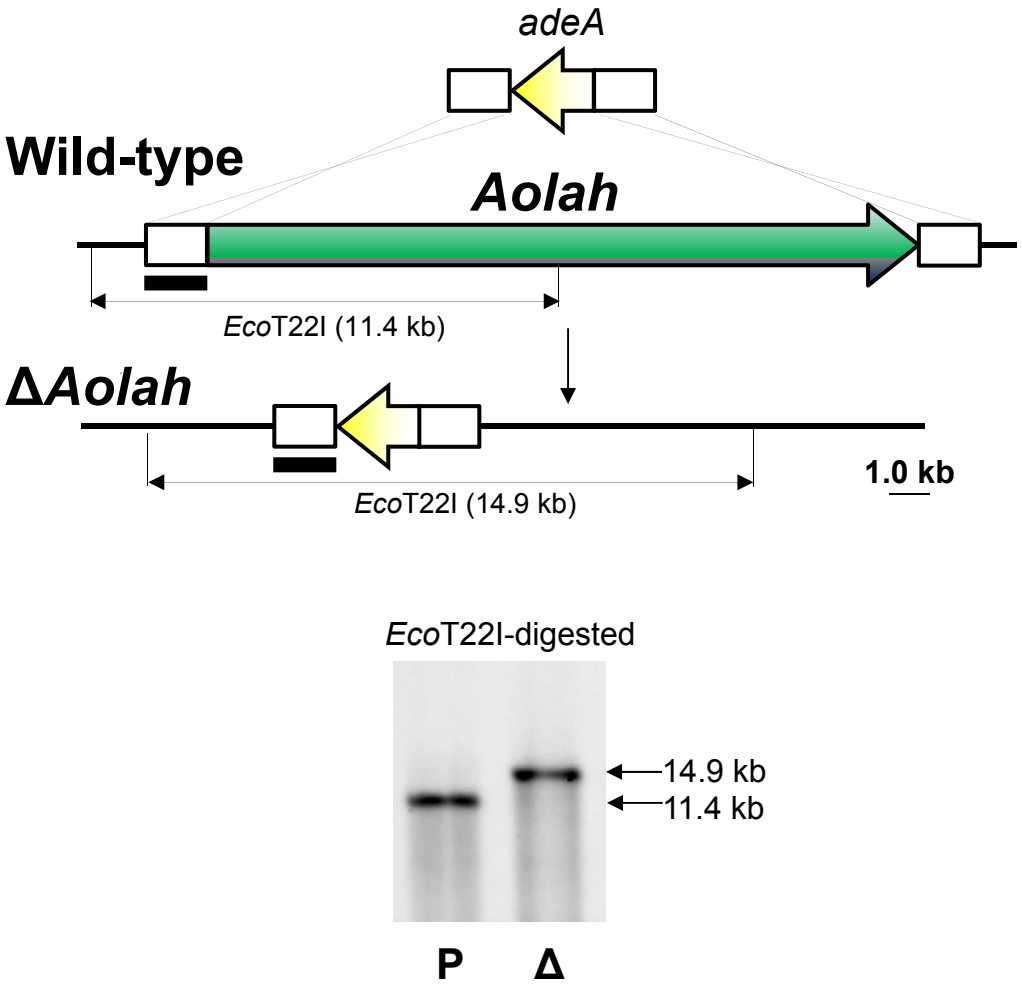
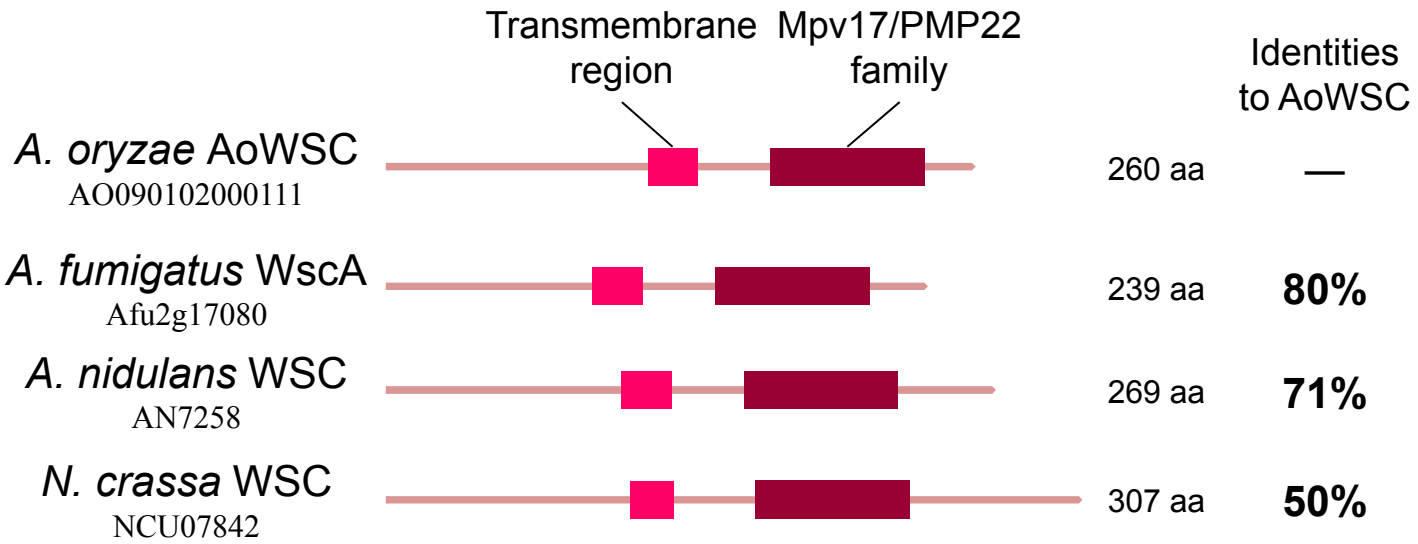


FIG. S3 Southern blot analysis of the *Δolah* disruptant. Genomic DNAs of the parent strain (NSRKu70-1-1; P) and *Δolah* disruptant (NSK-Δlah2; Δ) were digested with *Eco*T22I, and then subjected to Southern blot analysis. Filled bars indicate the used probe.



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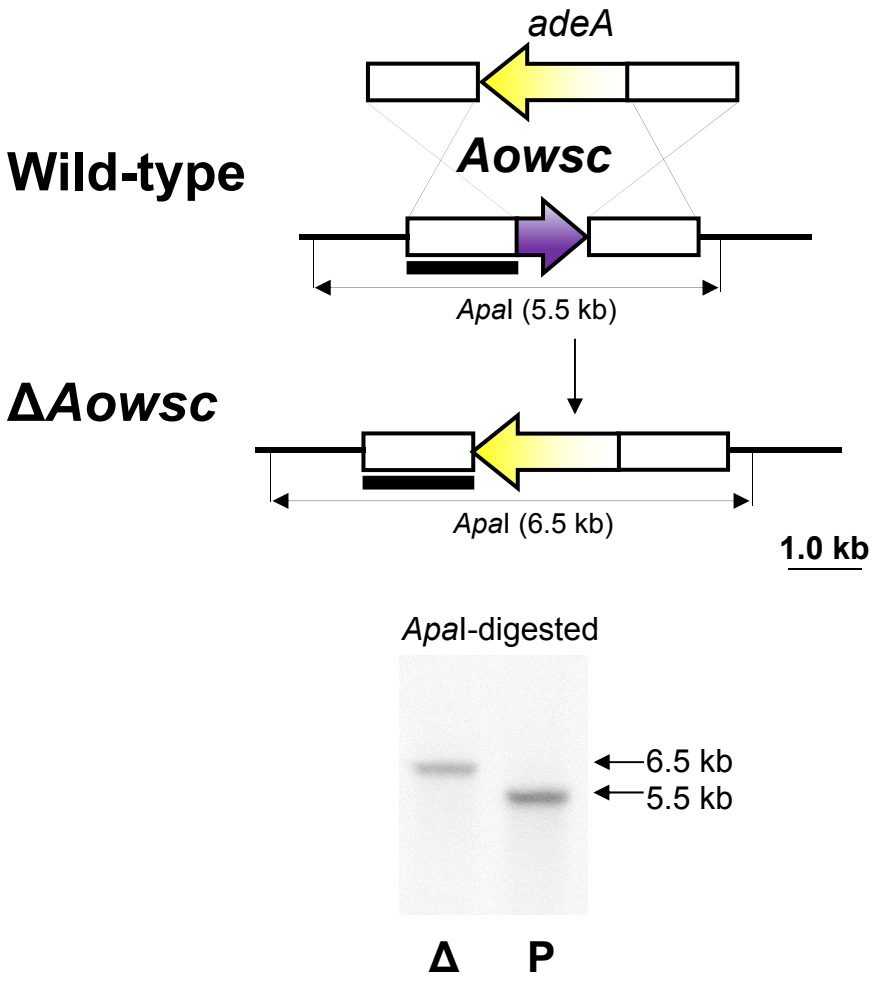
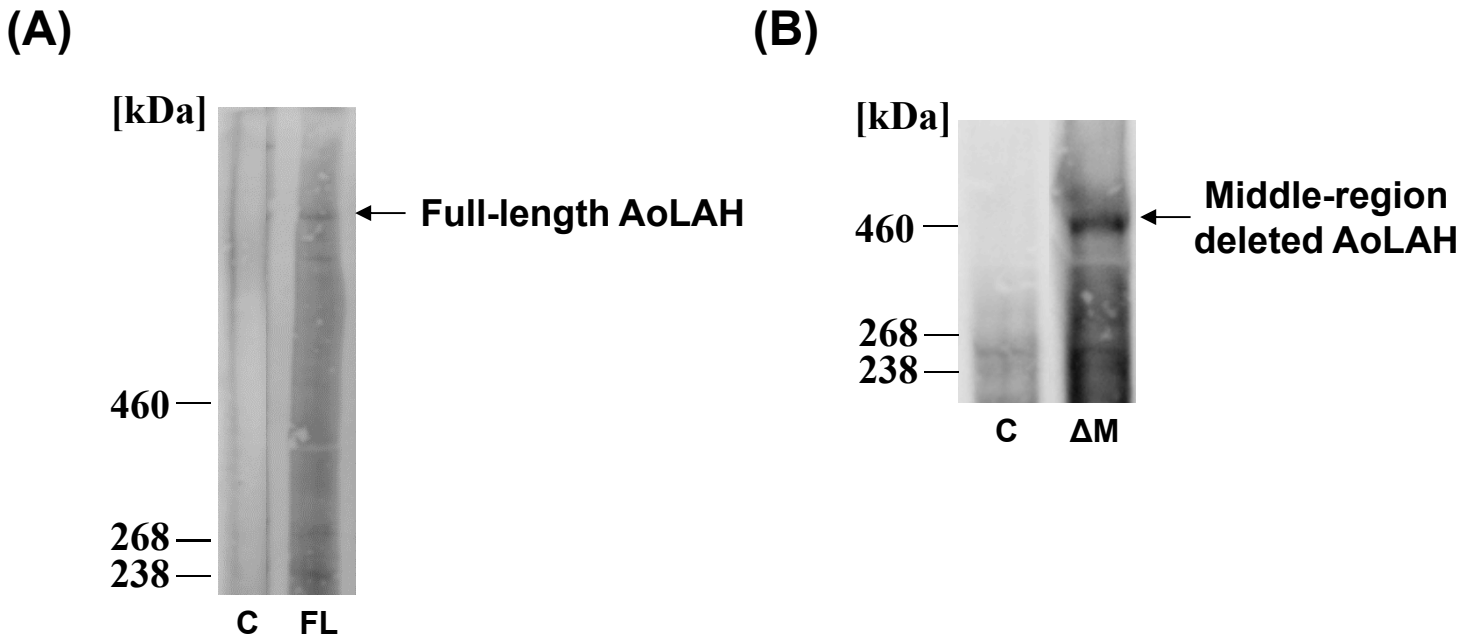


FIG. S5 Southern blot analysis of the *Aowsc* disruptant. Genomic DNAs of the parent strain (NSRKu70-1-1; P) and *Aowsc* disruptant (NSK-Δwsc1; Δ) were digested with *Apa*I, and then subjected to Southern blot analysis (lower). Filled bars indicate the used probe.



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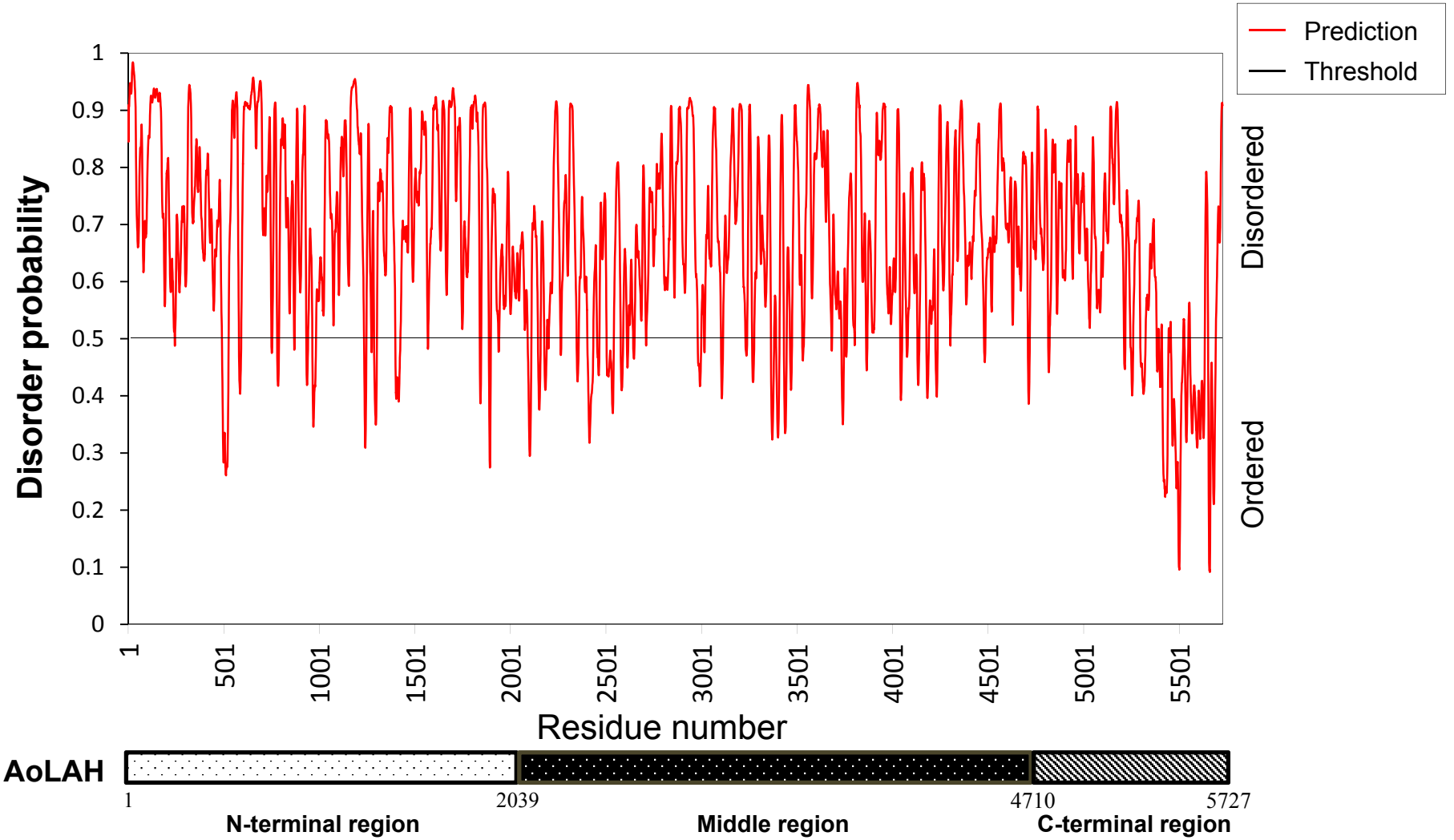

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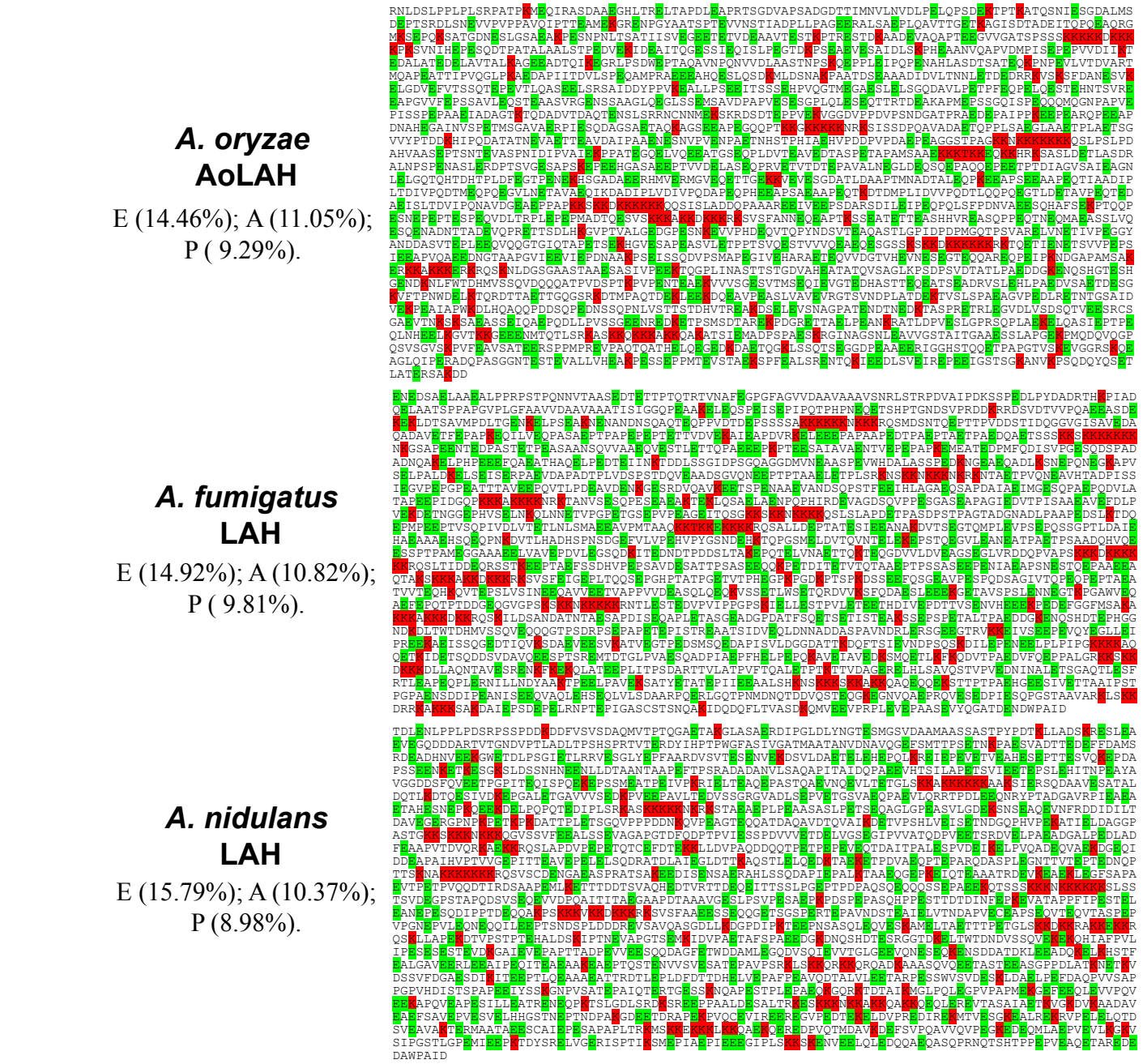

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