

1 **Supplemental material**

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4 **Supplemental MATERIALS AND METHODS**

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

15

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

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

37 **Table S1** Primers used in this study

| 38 Name | 39 Sequence (5'-3') |
|--------------------------|---|
| 40 (attB4)Aolah-up-F | GGGGACAACCTTGTATAGAAAAGTTGGGAGGATTGCCTCCGCATAAACATAC |
| 41 (attB1)Aolah-up-R | GGGGACTGCTTTTGACAAACTTGGCCTTGATCGCTCTGCCCACT |
| 42 (attB2)Aolah-down-F_2 | GGGGACAGCTTCTTGTACAAAGTGGATGACTATGATGACTGCCAACATCTCC |
| 43 (attB3)Aolah-down-R_2 | GGGGACAACCTTGTATAATAAAGTTGCGATATGGAACCGATTCAAAGTCAACTCC |
| 44 (attB1) Aolah_1-F | GGGGACAAGTTGTACAAAAAAGCAGGCTTGTATTTAAGGCCTTATTGGCCGGGGCCGT |
| 45 (attB2) Aolah_6243-R | GGGGACCACCTTGTACAAGAAAGCTGGGTCCAGCCGCCATCGTCTTTACACCGA |
| 46 Aolah-Cter-F (14254) | GGGGACAAGTTGTACAAAAAAGCAGGCTATGGAATGGCCTTGTATAGATTGGGAGAAGGAA |
| 47 Aolah-Cter-R (17400) | GGGGACCACCTTGTACAAGAAAGCTGGGTCAATCACATTGCTCATGTCCATGGTCGACGA |
| 48 Aolah_N-R-fusion | TATCAAAGGCCATTCCCTCCAGCCCCCATCGTCTTTACACCGAC |
| 49 Aolah_C-F-fusion | GATGGCGGGCTGGAGGAATGGCCTTGATAGATTGGGAGAAGGAA |
| 50 (attB4) Aowsc-up-F | GGGGACAACCTTGTATAGAAAAGTTGAGATGAGAGCATAGCGCGGTACC |
| 51 (attB1) Aowsc-up-R | GGGGACTGCTTTTGACAAACTTGGATGGCGGTGATCGGTTGCGT |
| 52 (attB2) Aowsc-down-F | GGGGACAGCTTCTTGTACAAAGTGGATAGCGTTACGACCAACGTCGCG |
| 53 (attB3) Aowsc-down-R | GGGGACAACCTTGTATAATAAAGTTGCGCTCCAAGAGGGCGAAAGTCAGT |
| 54 (attB1)-DsRed-M-F | GGGGACAAGTTGTACAAAAAAGCAGGCTCTATGGACAAACACCGAGGACGTCATC |
| 55 (attB2)-PTS1-R | GGGGACCACCTTGTACAAGAAAGCTGGGTTCATATTGGACTGGAGGCCGGAGTGGCG |

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58 **Supplemental figure legends**

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

68

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

71

72 **FIG. S3** Southern blot analysis of the *Aolah* disruptant. Genomic DNAs of the parent
73 strain (NSRKu70-1-1; P) and *Aolah* disruptant (NSK- Δ lah2; Δ) were digested with
74 EcoT22I, and then subjected to Southern blot analysis. Filled bars indicate the used
75 probe.

76

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

82

83 **FIG. S5** Southern blot analysis of the *Aowsc* disruptant. Genomic DNAs of the parent
84 strain (NSRKu70-1-1; P) and *Aowsc* disruptant (NSK- Δ wsc1; Δ) were digested with
85 *Apa*I, and then subjected to Southern blot analysis (lower). Filled bars indicate the used
86 probe.

87

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

92

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

100

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

105

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

109

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

113

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

117

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

| | | | |
|-------|---|-------|---|
| AoLAH | MFKALLAGGRSS-DARSSSS-TSSSSRRR---TESKASSTVSRKPS-RGDDDRDGLGD | AoLAH | TAIADSTTHRSRRRTGEVLIKEPPTPLRGYSSSNTNDAWEDVDSGDQSSSSVSSAALFG |
| AfLAH | MFRALMGCGGRSS-DSRSTRSSSSSSRRR---TNSKASSTVSRKSS-RGDDDRDGLGD | AfLAH | TALAATSHRSRRRAGEILVAKETRSRHSYDASVNTDEGWEGLDSDQGSSSSVSSAALFG |
| AnLAH | MFKALMGCRSSSDVRSRSTSSSSKSGRSRKSHGRHSSASSTVSRKSSRGDRDGLGD | AnLAH | TALAASSPGRSRSRGEVFGVGQSRSRSDTYSSASNDWEEDMDSREGO-ASSVSSALAFG |
| | *** : * . * : * : * : * . * : * . * : * : * : * : * : * : * : * : * | | *** : * . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | LSAYPTSGNRSK--RYAPSAAGDSVASSYATAE PQS VIE PDRN VIERA PRR RD TDSERR | AoLAH | GSGLYGNTASPSSDGTSLW GWRWG NR --KGKQKRTRSNASES RFTNA ALAAG ALGTA |
| AfLAH | LSAYS FSGRSK--RYAPSAAGDSVASSYATAE PGIA VEHDRII TERTL KRRD TD EESGR | AfLAH | DTGLFGSDESQSSDGTSGK WGRWG SKS --PKQGFPTGA ALAAG ALGTA |
| AnLAH | LSYAA PAAGSRSARYAE SAAGESVASSYATAR PN NDS -VDRV YTERE PRKEESE YERDS | AnLAH | TSGHFAREDHS SSS DSS GWSK WGWGSKS KTKN KS KRPSSPKDRF PAGT A VMTG ALG |
| | *** : * . * : * * : * : * : * : * : * : * : * : * : * : * : * : * : * | | * : * . * : * * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | DRYSDSDGSDDKP RRRNRLSRSQSRERTR--ERPDNTELDNGN RGS RHR RRRR TQP D | AoLAH | ALA HRYNN QGR RTSE GAGSGAGN LQHV APVPT DS P QFD AVN VPPSP -POP VPI RPR GHIP |
| AfLAH | DRYSE I DRDGRG SSS RDR QSR ER RER P SER IERA QLE DAVES DR D RR RER SRT QP D | AfLAH | ALAST QDRDRS RL P RQHASS SSS LQHV APVPT DFTL DV A VR SLP HA FAV FVR GP P |
| AnLAH | DRYR D DRDGRD PDI D YER R KAR PD ---REH S RER DR D R RER RER RER V SG | AnLAH | AASSG YHR GDK ---ES STS SAS LQHV APVPT DS P A RY DA PI SS F PSE QL VR GA IP |
| | *** : * . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | * : * . * : * * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | ELPS VPIS GAE ADLAP KVGT FDYD PQFP FP FH NT HV PVT SMP SPN VP GY DP H VQQ QFP GQ | AoLAH | LQQP QPVAP VS QAVY TS QSG ETI PPY T VPS R PPF ANTL SHY DY QAH GSG LRE HEI PLY RD |
| AfLAH | DT YL P FVS ---PS M P I QP NS P L V Y DP H VQQ QFP GQ | AfLAH | LQQP QPM T FV S QAVY AT QG ASI PT Y A P M P A F P -PT S YI PY RE -----Q |
| AnLAH | DNY YPI ASSA V PAP --FD P I QAT QGP QFP FM NS V SVP AA P A P T Y D P H VQQ QFP GQ | AnLAH | LQQP QPV FV S QAVY TS QSG AP I QP FT A P T F V P T S E N P F P S Y E S Q S R D A D R R I S G Y G D |
| | : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | ***** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | FPA FVA EPN -PPN PAGA AADYY DQG QSV AQQ P GVR PE PP K II P N T QAH LMP A S P H N P P | AoLAH | FTDIRS NVN RP P RRS D S P F V H T E PLV STS VF --S AKR RST MKD QG -S V Q F D L T R E Q A E K |
| AfLAH | FPA YAE PYR PNP PAGE AADYY DQG QSV A D Q P G V R P K P P P I P S S Q A H L M T A S P V A N P P | AfLAH | I QN V ALG F N R P H H R S D S S P F V H T E P L E G V P A P --G L K R R S T S K D Q S -S V Q F D L T K E Q A D K |
| AnLAH | FPA S YAE PYR PNP PAGE AADYY DQG QSV Q E Q P G V R P K P P L V I P N S Q A H L M T A S F T A N P P | AnLAH | G T S D V F -V V K S H K R S G S T F V I R E T P I Q S A E P F G L G I K H R F T A K D Q T S I V Q F D L T R E Q E D K |
| | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | QE PSS MG Q T PAA D Y Y ADD T D P E I Q -A P E Q S S K P P A G P ---T P K P P R T I Q T E G V L G P | AoLAH | ERRAD QF EH Q K R D H G -S Q G V E L I D R E A H -----ADKN R S R Y Y E G H R D S D F G |
| AfLAH | PE PSS MG Q V G A A A A Y F A D D A E L E V D P A P G R P D R P T A G T ---T S E P P K P S N T T F G I S G I | AfLAH | E R M R D L R E Q L K R D A E R A S G V Q L I D R D H E P T V R D D D R R S G R Y E D R G Y V D R R Q D E F R D Y A |
| AnLAH | PE PSS L GE V G A A A F P A N D P A L D I E N S G H S S G P E P R P P K P S N S Q Q P Y H A Q A Q A G M A | AnLAH | E R R A E D L E R K R D L R S G D R I E L I D R E G D -----S Q Y A P A Y D P P R D S Q P D |
| | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | A A A T A T H D E G --R P Q G I G S G S P M L E S P T P T S V P L T G T S P S K P N A Q D I G T A V G A A A A T A | AoLAH | PQEG Y G R E P R G K R S P A S W D D L G T S A G S -V L S G Q S F N G N P S E Q Q R S H Q E R S E K R A E R R |
| AfLAH | A A G A A Y G A G G S L P L P A S P T S P F E V P T T A P Y A P P V T S T T K P P H T H G I G A S V G A A A A G A | AfLAH | M D S G K D K D S F S R V G A V A A G S I G A A A A T V L S G R S S V D E S S E T S Q R H E R R Q Q R A E R R |
| AnLAH | A A A T A T Y G V G N Y S P E H V I L T E S P -----A A Y V P P T S T N G N K P P H T Q P H S H G V A V G L G A | AnLAH | W R R E R E R E R E R E R E R E Y L E R -----E R Q R E R E Q E R D R S E R R E R R E R Q R A E R R |
| | ** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | A A G Y V G M H H H Q S S L N -V E H L S Q G N H N N E E G F P N V I L G P A G P S A Y S Q D L N A P P L N T A G A E T | AoLAH | AS G S E -I S S G L P M P E R A Y D V D Q R P N P -----V P Q A E H F K T S V F R D I P R K P V H |
| AfLAH | A A G Y M L G H H H Q S M S S A D H L S Q T Y M Q N D E S S Q Y G L G I P Q P T V Y N A P A N A H E W A G T G A | AfLAH | G S E P E V A S S R S K S E R A Q E T T D Y L P E -----E R Q P E T K P P S P R S R H K Y |
| AnLAH | A A G Y M L N H H H H H S S S -S D H I S Q Y T L Q N P D D V S Q I C P G Y S S A F N P T L Y T A G A A G V G A A G | AnLAH | A S G S A L S S L A E P P K P R D P E L P E H A E P R E I T E R A S S T S P S N D V H S A F R N V S R K S V Y |
| | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | T A Y A A D P S H P H A A L Y H G A P F Q S G S M A F Q O R Q G P L D K F I D F W R D A E G V G M F E D Y T E T I G | AoLAH | D D Y A Q F A P K E L R Y S P D A Y A R R E P A S T P T I I E A E P A S -----O K I K A T E E H H P -----E Y |
| AfLAH | V P Y A A S P L H F H A A V H H G A P F P G S L A F Q Q R Q R G P L D K F I D F W R D P E G V G M F E E Y T E A I G | AfLAH | D D Y A E F F A P E E L R Y S P D T Y K Q R A P T S M P T I V E I F A S E R Q S R E A L L P A E E S H P -----G Y |
| AnLAH | A P Y A A S P L H F H A A Y I H A S P F Q G G A M A F Q Q R Q R G P L D K F I D F W R D P E G V G M F E D Y T E A I G | AnLAH | D D Y A T F Y P E E L R H S P D H S R Q E T P M T I V E I V P A S E R A E P P P N P N A D Y E P P T D F K D F |
| | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | V C R H C F E P G T S S R D A P R R H Y Y R R R S S D R Y R S S R V G K P S R Y S S S E D E G R R R K K T S I S S | AoLAH | R G L P W P V P K L N V V P T P P Q S Q G S V R D I A S P I P S P P -----E V L D D D R K S K R T T G S R V S |
| AfLAH | V C Y K C F E P G T S S R D A P R K H Y Y R R S S G E R Y A S G S R V G K A S R Y T S S E D E G R R R K K T S S N S | AfLAH | R D L P W P V P V K L I L E P T P P Q S V G S V R D A S P V G N P R D L P P H E E E D V K P A R Q T G S R V S |
| AnLAH | V C K Y C F E P G T S S R D A P R T H Y Y R R K R R -S S D R L S G G S R V D K F S R Y T S S E D E G R R K R R -S K S S | AnLAH | D R L P W P V P V L R V I E T P P H P S S G S V R G A P S V I T P S -----E S A E A K E L E R P N A S R V S |
| | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | ***** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | W L P G M I A G --Y T P F I K K D F E N T Y S D R S G R P A S S P D N E N L S T L E K Q S H T S R G V C G R S P | AoLAH | W G K D E T R E Y E V P S T S S E L D S A D H D I V A D R Q G K E Q K N D S E A T R E I A A I Q A D L P K A A G N Y P D |
| AfLAH | W L P L G M I G A K A L P N K N D F E D T Y S V R S G R -V M T V K D T E S V T A R S Q T S R G V Y R R R S Q | AfLAH | W G E H K T H E Y E V P S T S S E L D S A D H D I V A D R Q G K E Q K N D S E A T R E I A A I Q A D L P K A A G N Y P D |
| AnLAH | W L P A I L G -Y V K A S L F D S K D F D D S Y V S K G R V A G A F S E S D S I S D K -R S K T S R G V Y K Q L H R | AnLAH | W G E H H T H E Y E V P S T S S E R S S E R V D L H E R -Q H G F L P K A S V H D D I G Y S I A P S E A H V E D V N E D |
| | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * |
| AoLAH | R R S Y E G -----L R D N E S R R L S R S T S R S S S K S E K H C A H R D V G T A I T G V S ----- | AoLAH | FIG. S1 Amino acid sequence alignment of N-terminal region of LAH proteins. Based on comparison with other LAH protein was re-predicted to contain additional 1,198 amino acids at 1 (indicated by an arrow) of the original ORF AO0900110008 [National Institute of Technology and Evaluation, Japan]; http://www.bio.nite.go.jp/dogan/Top). Amino acid sequence |
| AfLAH | S R D R E S I I Y S D S K S Q Y E D K R H R S R S R H S S S R N R R H S A L D R A A V G A V G S A T A L A K S R | | |
| AnLAH | S H S R D S F G Y R D Q R P S R Y E G S R I N G R V Q S R S R S S R A N R H T L R D A A I G A I T A A S V V K S | | |
| | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | |
| AoLAH | -----E N Q P R R S R S P R K T K S R K S S S S E S S F V D I S R P S M K S V G G -L S S F T A S -E N R R K -R | | |
| AfLAH | D R S R S R S R S R S P R K T K S R K S S S T D S S S F L D I S Q P A R K S V G G I A S F T A S S E N R K -R | | |
| AnLAH | P G E R S R S R G R S P S K K S R G K S S S S G S S Y E N I S P R K K I A G G -F G S F T A S S E N R S K K R | | |
| | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | |
| AoLAH | Q S K K R S I F S F N N S S S S L D A D L A F G N G Y A K R P P G K S K R S K K D Q D D V A A L L G I G A A A | | |
| AfLAH | R V K K R R S I F S F N N S S S S L D A D L A F G T G F A R K P A R K S T K K S K K D R D V D A A L L G I G A A A | | |
| AnLAH | Q S K K R S I F S F N N S S S S V N D L A F G S S Y A K R L I G K S K R S R S K E K -K D V D K L L A L G A T A | | |
| | *** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | | |

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/>).

A. oryzae
AoLAH

2316 KKKKKDKKKK 2325
2945 KKGKKKKKNR 2954
3067 KKNKKKKKKK 3076
3155 KKTKKEQKK 3164
3491 KKDKKKKKQ 3500
3593 KKKAKKDKKK 3602
3819 KDKKKKKKRK 3828
3942 KKAKKKERKR 3951
4443 KKQKKKAKKQ 4452

A. fumigatus
LAH

2345 KKKKKKNKKK 2354
2473 KKSKKKKKKK 2482
2738 KKNKKKNRK 2747
2874 KKKAKKKKNR 2883
3003 KKSCKNKKKK 3012
3089 KKTKEKKKK 3098
3330 KKKDKKKKKK 3339
3438 KKKAKKDJKK 3447
3642 KSCKNKKKKK 3651
3718 KKKAKKKDKK 3727
4246 SKKSKKAKK 4255

A. nidulans
LAH

2670 SKKAKKKKKK 2679
2823 KASKKKKKNK 2832
2988 KKSCKKNKKK 2997
3272 KNAKKKKKKK 3281
3445 KKKNKKKKKK 3454
3575 KKKVKKDKKK 3584
3734 KKRAKKEKRR 3743
4273 KKKNKKAKKQ 4282
4443 SKKEKKKLKK 4452

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

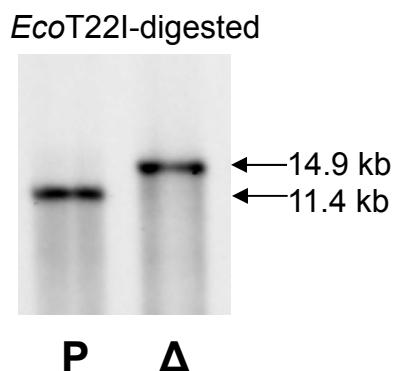
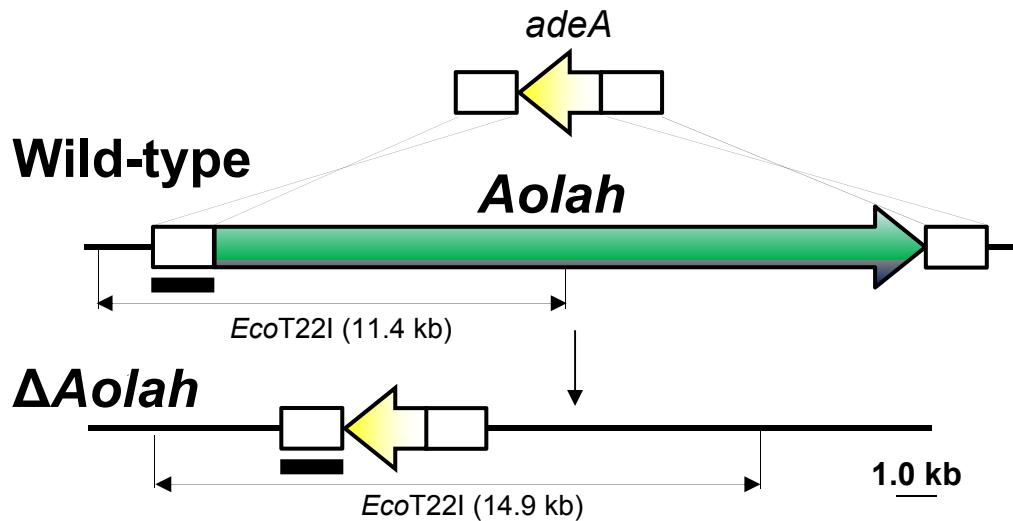


FIG. S3 Southern blot analysis of the *Aolah* disruptant. Genomic DNAs of the parent strain (NSRKu70-1-1; P) and *Aolah* disruptant (NSK- Δ lah2; Δ) were digested with *EcoT22I*, and then subjected to Southern blot analysis. Filled bars indicate the used probe.

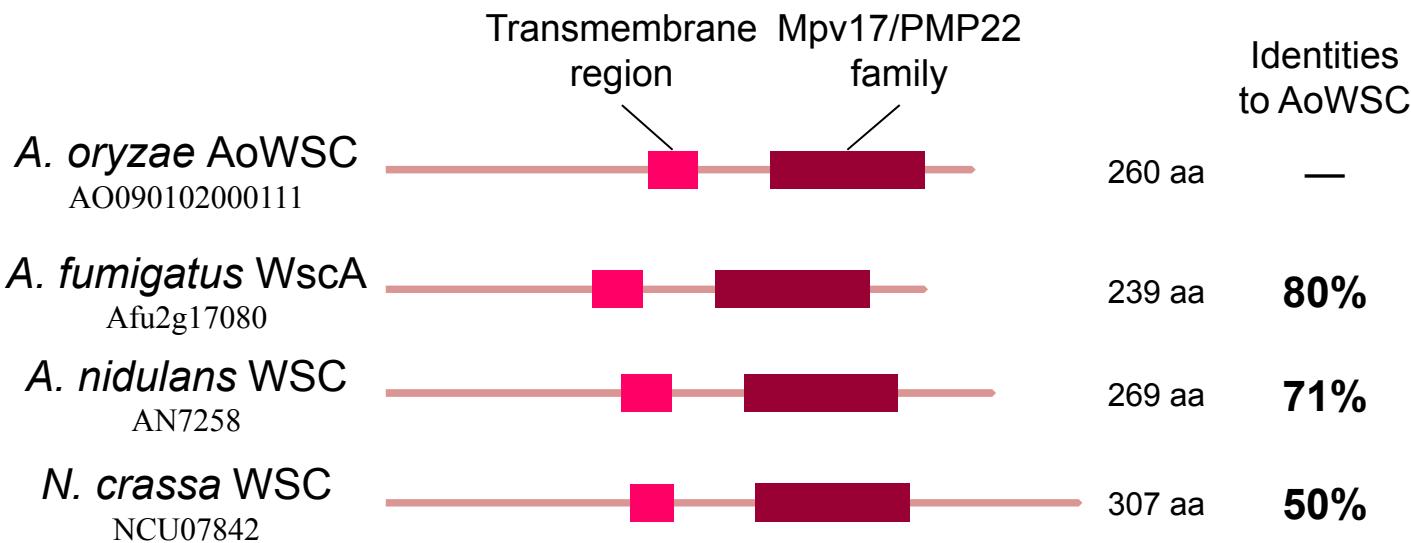


FIG. S4 Domain prediction of WSC proteins. Amino acid sequence data of WSC proteins from *A. fumigatus* and *A. nidulans* were taken from the *Aspergillus* Genome Database (AspGD; <http://www.aspgd.org/>). SMART (<http://smart.embl-heidelberg.de/>) was used for prediction of domains. ClustalW program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

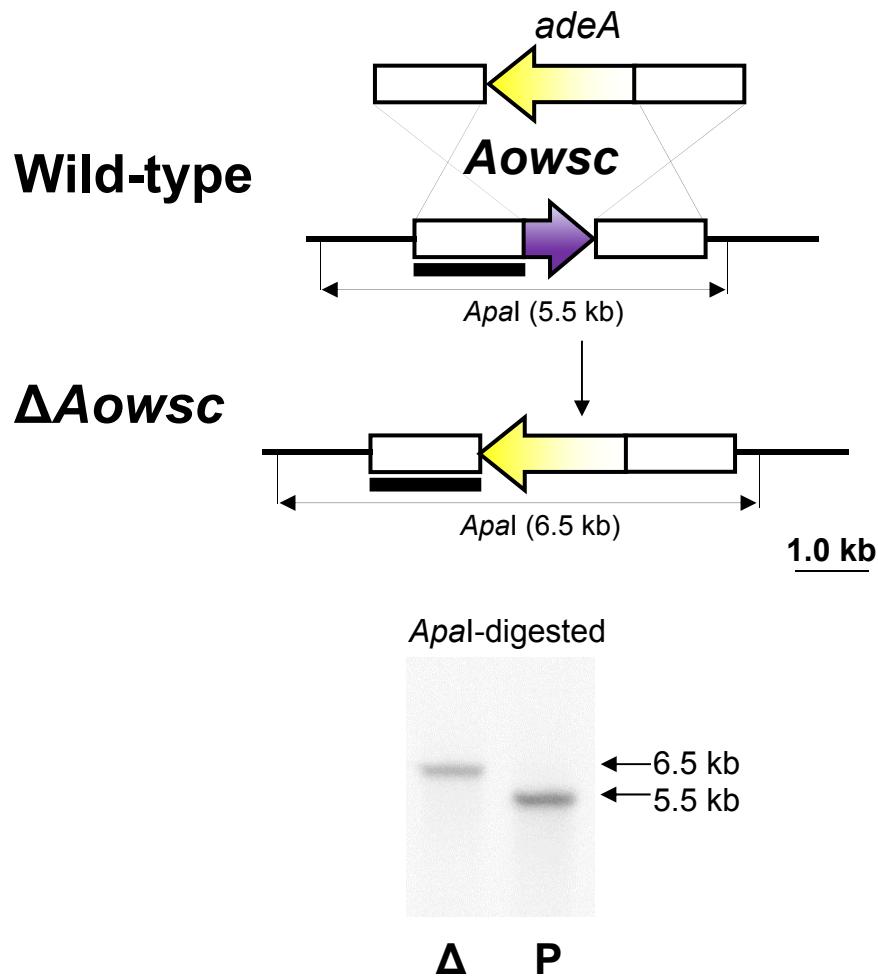


FIG. S5 Southern blot analysis of the *Aowsc* disruptant. Genomic DNAs of the parent strain (NSRKu70-1-1; P) and *Aowsc* disruptant (NSK- Δ wsc1; Δ) were digested with *Apa*I, and then subjected to Southern blot analysis (lower). Filled bars indicate the used probe.

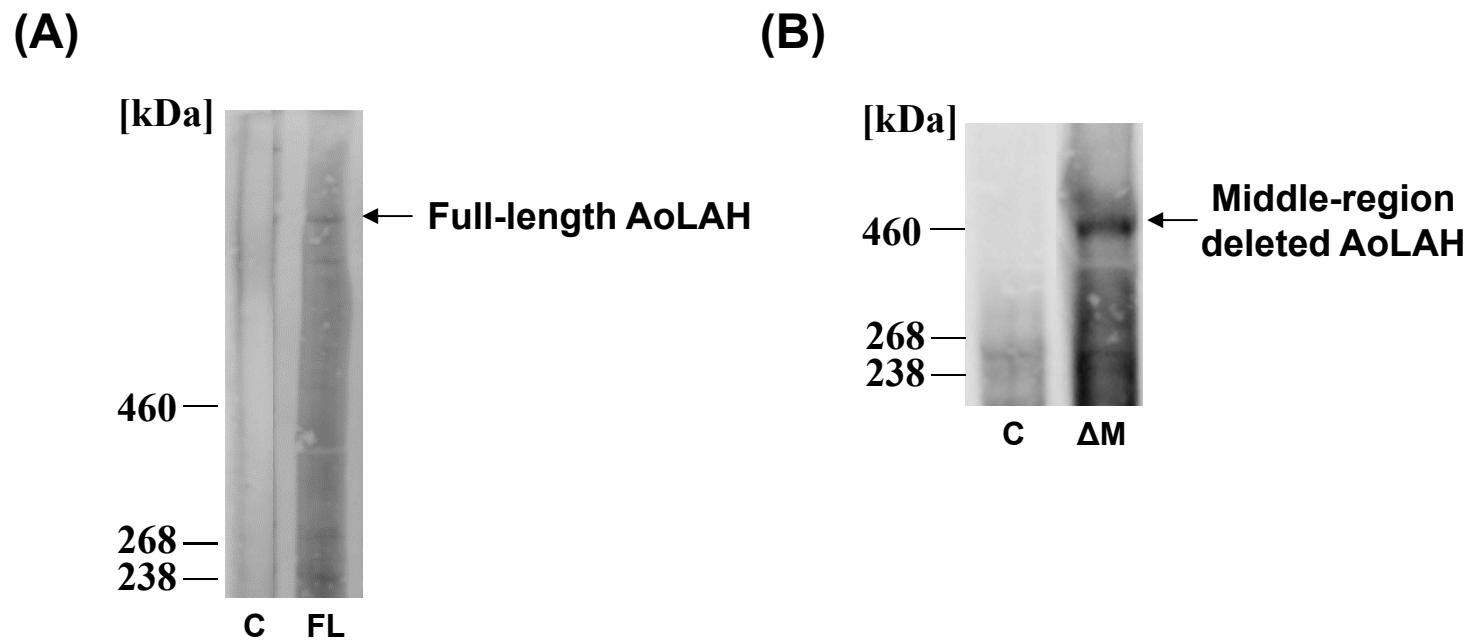


FIG. S6 Western blot analysis of full length (A) and middle-region deleted (B) AoLAHs. Arrowheads indicate the bands corresponding to each protein. Lanes: C, wild-type control strain (NSRKu70-1-1A); FL, AoLAH full-length expressing strain; ΔM : strain expressing middle-region deleted AoLAH.

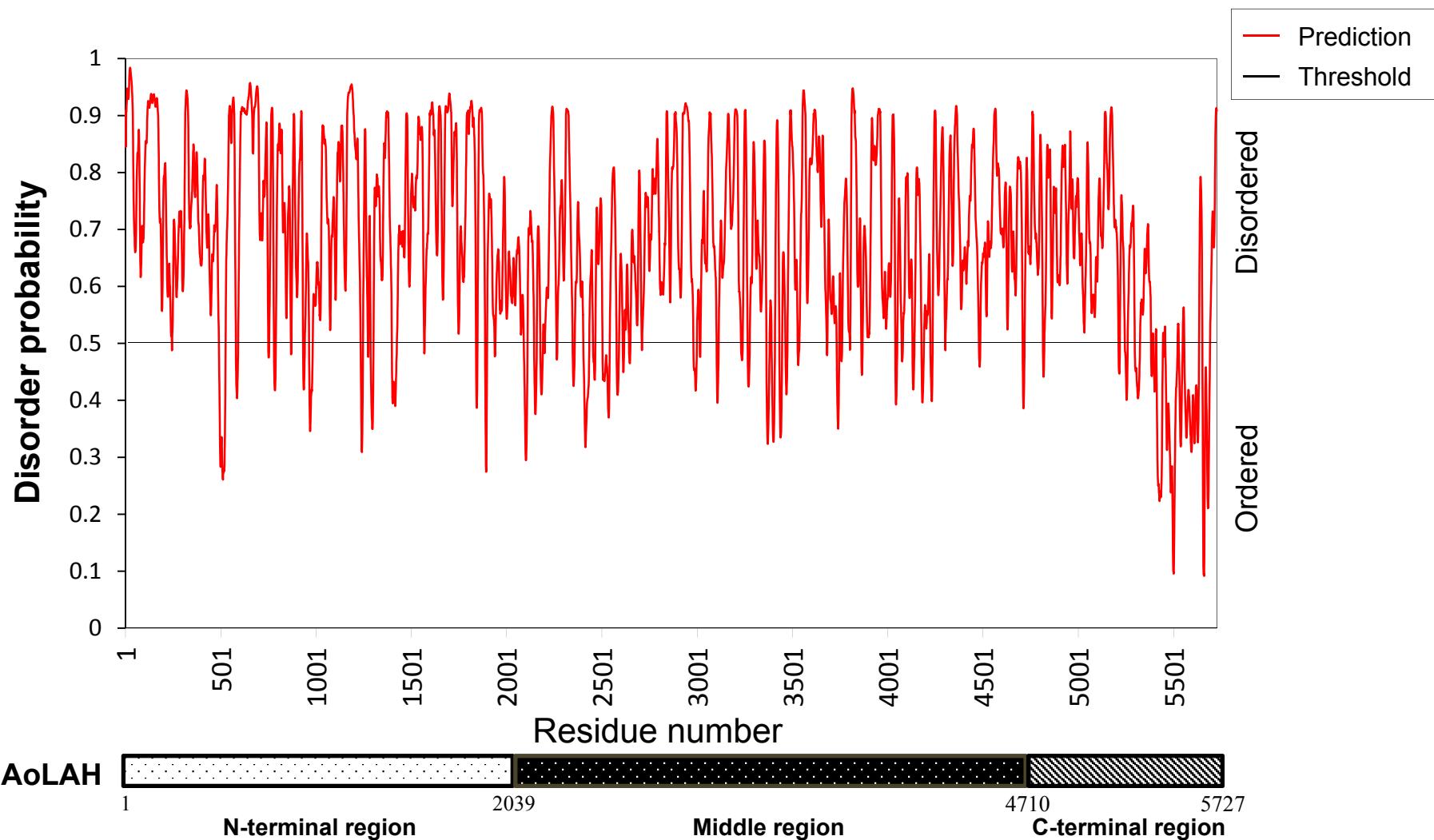
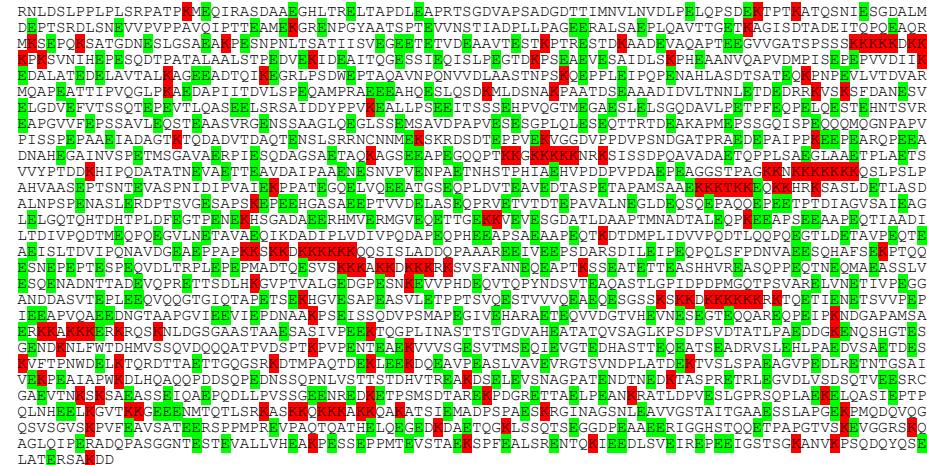


FIG. S7 Disorder prediction analysis for AoLAH. The disorder probability of AoLAH was predicted by PrDOS (Protein DisOrder prediction System; <http://prdos.hgc.jp/cgi-bin/top.cgi>). The plot of disorder probability of each residue along the sequence is shown. Residues beyond the black threshold line 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.

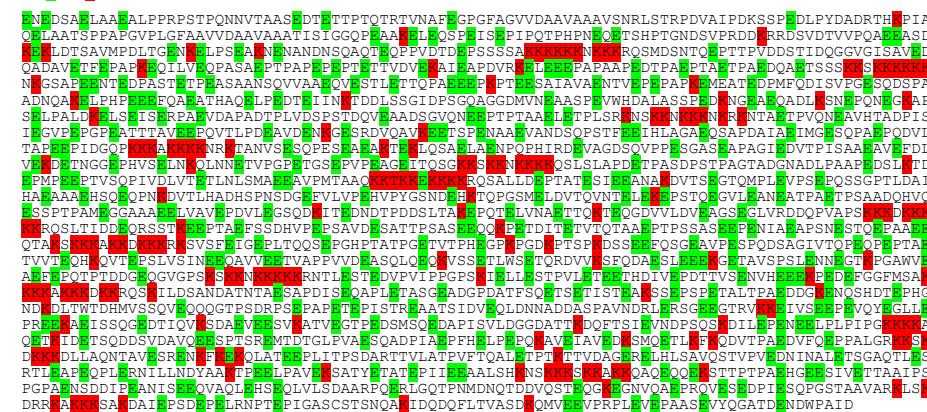
A. oryzae
AoLAH

E (14.46%); A (11.05%);
P (9.29%).



A. fumigatus
LAH

E (14.92%); A (10.82%);
P (9.81%).



A. nidulans
LAH

E (15.79%); A (10.37%);
P (8.98%).

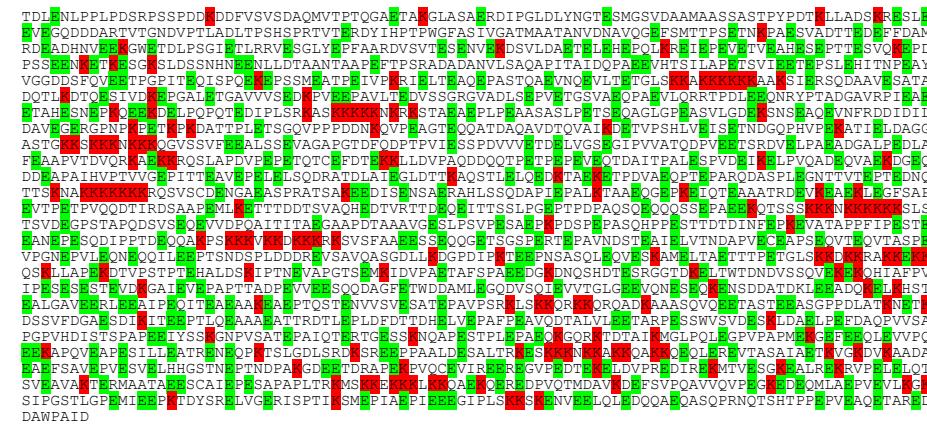


FIG. S8 Amino acid content of the middle region of LAH protein. The amino acid content of the LAH proteins was calculated and the three most abundant amino acids are listed. Important amino acids are highlighted by different colors; E and K are highlighted by green and red, respectively.