Supplemental Materials and Methods:

**Figure S1**

**A B**

** **

**C D**

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**Figure S1.** Southern blot analysis of targeted gene deletion mutants generated in the *ku70* background. (A) Restriction map of the *HOG1* region and the position of the fragment used as a probe. (B) Total DNA from the *A. oligospora* *ku70* (control; lane 1) and *hog1* mutant (lane 2) lines was digested using BamHI and EcoRI restriction enzymes and hybridized with the probe. The *ku70* strain generated a 2.52-kb band that matches the expected control banding pattern. The *hog1* mutant displayed a 4.08-kb band matching the expected *hog1*-deletion banding pattern. (C) Restriction map of the *MSB2* region and the position of the fragment used as a probe. (D)Total DNA from the *ku70* (control; lane 1) and *msb2* mutant(lane 2) lines was digested using ApaI and NdeIrestriction enzymes and hybridized with the probe. The *ku70* strain displayed a 2.42-kb band that matches the expected control banding pattern. The *msb2* mutant had a 6.45-kb band matching the expected *msb2*-deletion banding pattern.

Supplementary Table 1. Primer used in this study.

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| Primers | Sequence (5’3’) | Description |
| 298 | CGAGCTCCCAAATCTGTCCA | Amplification of HygB resistance cassette from vector pAN7-1; (forward) |
| 299 | GTGTACCTGTGCATTCTGGG | Amplification of HygB resistance from vector pAN7-1; (reverse) |
| 614 | CGGCATCAGAGCAGATTGTAC | Amplification of clonNAT resistance cassette from vector pRS41N; (forward) |
| 615 | GGTATTTCACACCGCACAGG | Amplification of clonNAT resistance cassette from vector pRS41N; (reverse) |
| 1067 | GTATGGGCTAAATGTACGGG | NAT1 insertion check (reverse) |
| 1068 | GGTCAGGTTGCTTTCTCAGG | NAT1 insertion check (forward) |
| 1069 | TGTCCAAGCAGCAAAGAGTG | HygR insertion check; (forward) |
| 1070 | TTTCATACACCGGGCAAAGC | HygR insertion check; (reverse) |
| 2188 | TCTGGAAGGTGGGAAAGTCG | *KU70* deletion in *A.o* (5' flanking, forward) |
| 2189 | TGGACAGATTTGGGAGCTCGCAAGTTGAGGCTGGTGGAAG | *KU70* deletion in *A.o* (5' flanking, reverse) |
| 2190 | CCCAGAATGCACAGGTACACGGTTGAGCGGATTGAAGAACAC | *KU70* deletion in *A.o* (3' flanking, forward) |
| 2191 | AGAAGGCTATTGCCGGGAAC | *KU70* deletion in *A.o* (3' flanking, reverse) |
| 2192 | GGATATACCGGATGCACCGAG | *KU70* deletion check (internal, forward) |
| 2193 | GCGATGAACCATGCGATACC | *KU70* deletion check (internal, reverse) |
| 2194 | AGCCTCGGTCTGCTTTCTTG | *KU70* deletion check (cassette integration, forward) |
| 1433 | CCTGGGCGATGTTGAGAAATTG | *MSB2* knockout check (internal, forward) |
| 1434 | TCGAAGTAGTTTGCGGAAGGTC | *MSB2* knockout check (internal, reverse) |
| 1435 | GCATACTACGGAAGACGGTTCG | *MSB2* knockout check (cassette integration, forward) |
| 2136 | TGTCAGTAAGTACAGCACACAGC | *MSB2* knockout in *A.o* (5' flanking, forward) |
| 2137 | GTACAATCTGCTCTGATGCCGCCTTACGAGCGACCTTGGAG | *MSB2* knockout in *A.o* (5' flanking, reverse) |
| 2138 | CCTGTGCGGTGTGAAATACCCCAGCGTTTGTTCAGCCTTAA | *MSB2* knockout in *A.o* (3' flanking, forward) |
| 2139 | AACCTGGGCATTCGGTCTAG | *MSB2* knockout in *A.o* (3' flanking, reverse) |
| 2286 | GGAGGACGGTGATTGACGTTG | *HOG1* knockout in *A.o* (5' flanking, forward) |
| 2287 | GTACAATCTGCTCTGATGCCGAGGAGGAGGAGGAGTAGGAAAG | *HOG1* knockout in *A.o* (5' flanking, reverse) |
| 2288 | CCTGTGCGGTGTGAAATACCGACAAGGCGAAAGGGCAAAC | *HOG1* knockout in A.o (3' flanking, forward) |
| 2289 | TGCAGGTTGATGGAATCGAGAG | *HOG1* knockout in A.o (3' flanking, reverse) |
| 2290 | TCACAAGCAGGTTCGTAGTCC | *HOG1* knockout check (internal, forward) |
| 2291 | TAGCGACAAATATCCAATCGGAGG | *HOG1* knockout check (internal, reverse) |
| 2292 | TTGGTGCTAGATGATGGTGACG | *HOG1* knockout check (cassette integration, forward) |

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