

Fig. S1. Construction of Donor DNA

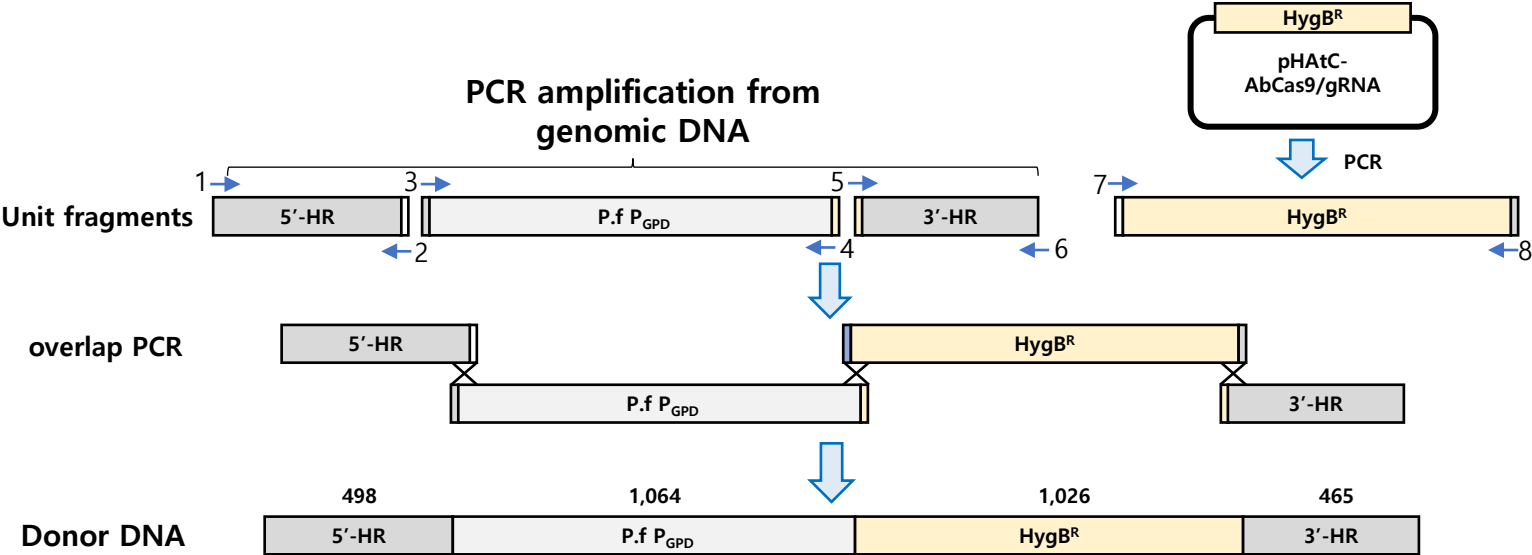


Fig. S2. sgRNA efficiency test for Cas9-sgRNA RNP

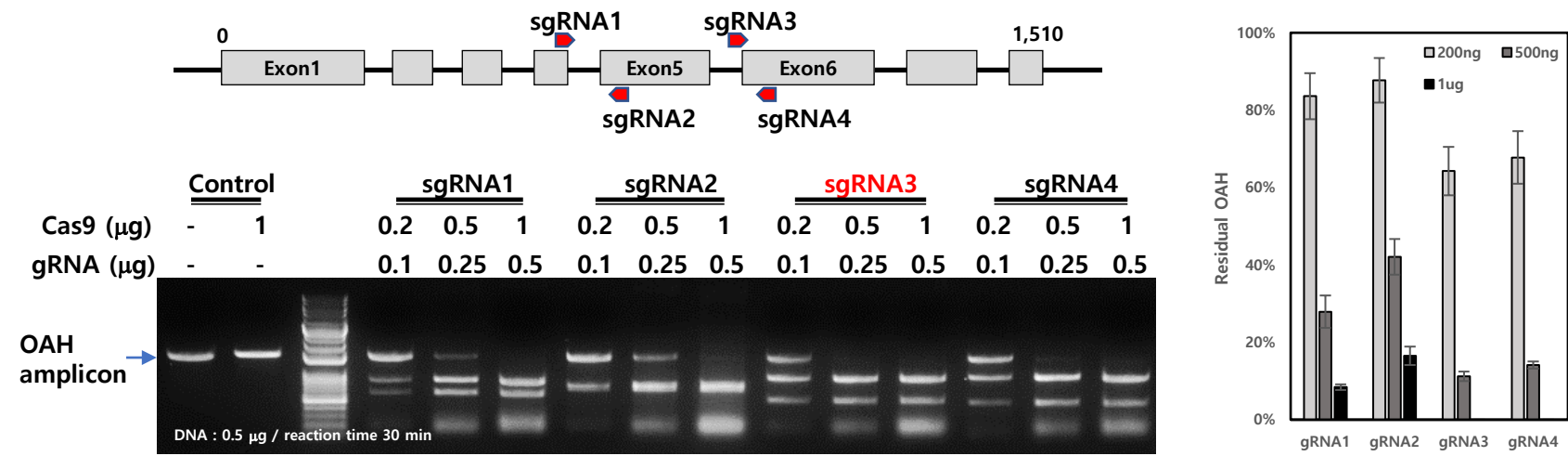


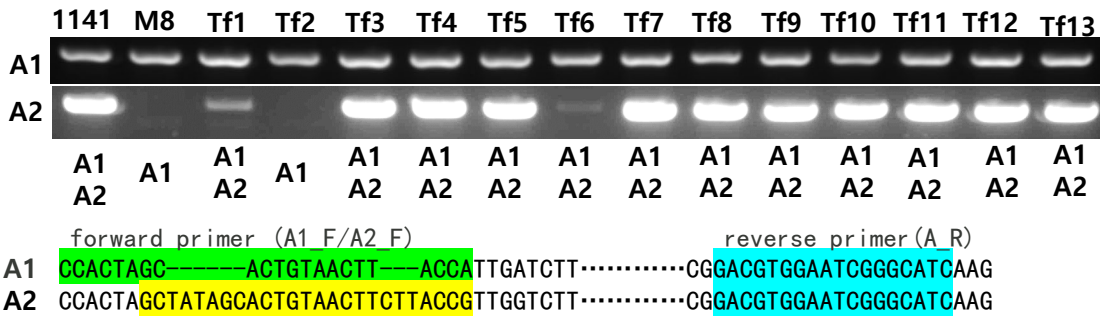
Fig S3. Identification of nuclear types. Karyotyping of the transformants using sequence variations in *homoaconitase* gene and SNPs in *OAH* gene in genomes of A1 or A2 nucleus, respectively. The nucleus-specific primer sequences in *homoaconitase* are shaded in green and yellow for A1 .and A2, respectively.

Homoaconitase gene in A1 and A2

A1 GTCGA **CCACTAGC**-----**ACTGTAACCTACCA**TTGATCTTTGACTACTTCCTGCCCTTCCTCCCACTCGACCAATGCGTCTACCAACAAGATGGGGCCATAGCGGTCTTTCTTCTCAGGGGGTTTGCACGGGTGTGAATACTCCTCGGATACCGCAGACGGTGATTGAA
A2 GTCGACCACTAG**GCTATAGCACTGTAACCTCTTACCG**TTGGTCTTTGACTACTTCCTGCCCTTCCTCCCACTCGACCAATGCGTCTACCAACGAGATGGGGCCATAGCGGTCTTTCTTCTCAGGGGGTTTGCACGGGTGTGAATACTCCTCGGATACCGCAGACGGTGATTGAA

A1 AAGGTTGTGCAGCGGTATGCGGTGCACCTACCTGCTGGAAGCCGTTAAGGCGGGGGACTATGTGATGATACGTCCGAGCACGTTATGACGCACGACAACACGGGTCCCGTTATCTCCAA**GTGAGTGACTACGTGTTGAATTGTGAATCTCATT**CATGATTCTCAG**GTTCAAG**
A2 AAGGTTGTGCAGCGGTATGCGGTGCACCTACCTGCTGGAAGCCGTTAAGGCGGGGGACTATGTGATGATACGTCCAGAGCACGTTATGACGCACGACAACACGGGTCCCGTTATCTCCAA**GTGAGTGACTACGTGTTGAATTGTGAATCTCATT**CATGATTCTCAG**GTTCAAG**

A1 TCGATAGGTGCTACGAGGATACATAACCCGAAGCAACTCGTCTTACCCTCGACCACGATGTGCAGAAAGTACCTAAGAACTTGTCTAAATACGCTTCGATTGAGGCTTTTGCGCGACAACACGGCGTGGACTTCTACCTGCCG**GACGTGGAATCGGGCATCAAGTCTTGGTC**
A2 TCGATAGGTGCTACGAGGATACATAACCCGAAGCAACTCGTCTTACCCTCGACCACGATGTGCAGAAAGTACCTAAGAACTTGTCTAAATACGCTTCGATTGAGGCTTTTGCGCGACAACACGGCGTGGACTTCTACCTGCCG**GACGTGGAATCGGGCATCAAGTCTTGGTC**



SNPs in OAH

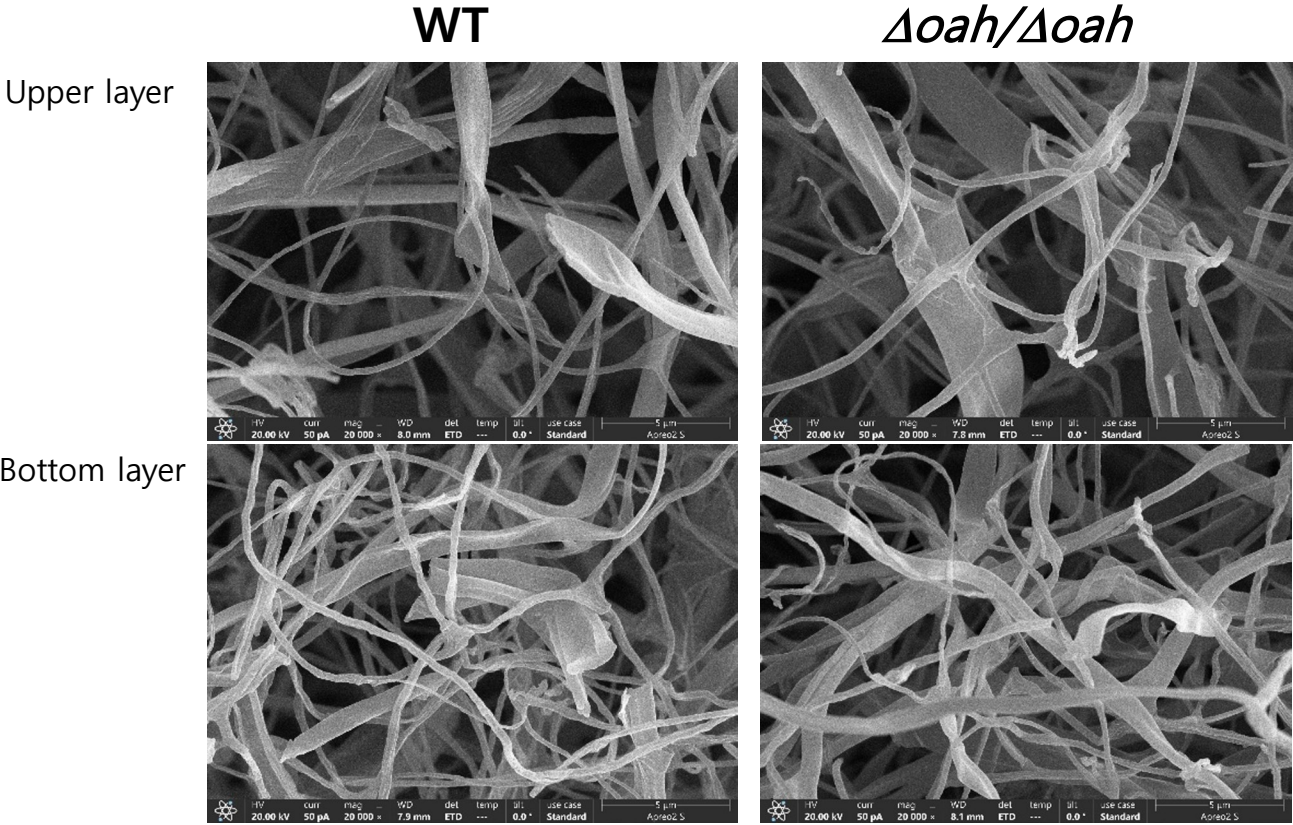
836 839 866 869

A1 ccgcgaggagtt**cg**ttaccgcatccgtgctgccgtcat**cg**acgcgattcgatacctggcggctccgactttgtaagcgaccgttctgctcgcttcctacattcacgaatgcttatcccgatcccgtaggtgatcatcgggcgaacagactcggccc
A2 ccgcgaggaa**tt**gttaccgcatccgtgctgccgtcat**cg**acgcgattcgatacctggcggctccgactttgtaagcgaccgttctgctcgcttcctacattcacgaatgcttatcccgatcccgtaggtgatcatcgggcgaacagactcggccc

1116 1126 1144
A1 agccact**tg**gcatggaagaagcccttatccgtctccgcctcgccgcgagcaggtgcagacgtgtgcttcacgaaggtgtgctaatgcggaactcctcacgccacagtcgaggcactcgcgccca**ag**gccggtgct**cg**tgaacgtcatctcagg**tg**g
A2 agccact**tg**gcatggaagaagcccttatccgtctccgcctcgccgcgagcaggtgcagacgtgtgcttcacgaaggtgtgctaatgcggaactcctcacgccacagtcgaggcactcgcgccca**ag**gccggtgct**cg**tgaacgtcatctcagg**cg**g

1211
A1 cctcactccctcgttcaccacccaggaggtgaggcgctcggtgctaaaatcatcagtagct**ct**ttctgctgtcatatttggttatcacgtgatactgaaactgtcctggcagtccttctcgctagtgagcagcgtggctg
A2 cctcactccctcgttcaccacccaggaggtgaggcgctcggtgctaaaatcatcagtagct**ct**ttctgctgtcatatttggttatcacgtgatactgaaactgtcctggcagtccttctcgctagtgagcagcgtggctg

Supplementary Fig. S4. Measurements of hyphal number and thickness. Number of hyphae and hyphal width were measured using ImageJ software.



	Layer	hyphal type	Number of hypha per 287 μm ²		Width of hypha (μm)
WT	upper	Large	11.2±3.1	23.8±3.9	1.66±0.51
		Small	12.6±5.4		0.25±0.02
	bottom	Large	8.0±1.9	56.6±3.8	0.97±0.29
		Small	48.6±11.0		0.25±0.04
<i>Δoah/Δoah</i>	upper	Large	15.3±4.6	23.3±5.0	1.71±0.47
		Small	8.0±3.4		0.21±0.03
	bottom	Large	15.0±5.4	51.2±6.5	1.03±0.20
		Small	36.2±12.8		0.21±0.03

Supplementary Fig. S5. Coloration of mycelial mats. Mycelia mats were produced in PDB by static culture for 2 weeks at 30°C.

