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17 mutants.
- 18

19 Table S1. List of primers used in this study.

20

	<i>Target</i>	<i>Sequence (5'→3')</i>
<i>qRT-PCR</i>	Glycogen synthase (CC2G_008991)	F TCTACCTCTTCACGGCTGGA R CGAAGGCGACGATAGTGACA
	Glycogen phosphorylase (CC2G_002698)	F TCCTCGTCTCGATGTCACCT R CAACGACTTCCTGTCCACCA
	Alpha, alpha-trehalose-phosphate synthase (CC2G_007168)	F CGATTAGGGCGTCAAGGT R CAGCCAGTTGGACCAAAACG
	RasGAP (CC2G_009974)	F ACGATGACCCCTGAGATGGA R TGCTCATCTGCCGGACTTT
	DNA mismatch repair protein msh6 (CC2G_013873)	F TCCACAAACTAGCGACCCAC R CGGC GGATGTTGGGATGATA
	CMGC/MAPK protein kinase (CC2G_005966)	F TGCGATCCCTCGCTTACATC R TCGCAGAGCTTCAAGACACC
	Atypical/Alpha protein kinase (CC2G_008473)	F AGGGCTCAATGAAGGTCGTG R ATCGCTCGTAGAACAGCTCG
	Adenylate cyclase (CC2G_009607)	F GTCTGCGAGACGGATTGGAT R ATGATCTGCCCTCCTTGTGC
	AGC/PKA protein kinase (CC2G_008645)	F TAGCACCCGAGATTGTCAGC R TTGGTGATATGGCGGTAGCC
	RasGAP (CC2G_007150)	F CTGCAGACTCGTTCCCTGT R CTCTGTTGCCGTCTCGAACT
	18S rRNA	F GCCTGTTGAGTGTCAATTAAATTCTC R CTGCAACCCCCACATCCA
<i>Genetic variant validation</i>	DNA mismatch repair protein msh6 (CC2G_013873, scaffold_8:1037634C>T)	F AAGTCAGCGAGGATAAGGCG R TAGACTCAGGCACACTCGGT
	Atypical/Alpha protein kinase (CC2G_008473, scaffold_4:542515G>A)	F AGCTGTTCTACGAGCGATGTG R TGTCAATGCCCTTTGTCCA
	RasGAP (CC2G_009974, scaffold_5:492578_492644del)	F CAGCGGTCAGCTATGTCTGT R ACCAGGGTATCGCTCTATCCA

21

22 Table S2. Comparison of length of contigs between *C. cinerea* strain #130 and #326.

23

Okayama-7 #130		<i>A43mut B43mut pab1-1</i> #326	
Contigs from the longest to the smallest			
	(bp)		(bp)
AACS02000001.1	4,146,986	4,326,744	scaffold_1
AACS02000002.1	3,743,637	3,984,596	scaffold_2
AACS02000003.1	3,623,902	3,740,466	scaffold_3
AACS02000004.1	3,559,201	3,721,636	scaffold_4
AACS02000005.1	3,468,139	3,444,717	scaffold_5
AACS02000007.1	2,959,814	2,822,236	scaffold_6
AACS02000008.1	2,513,103	2,745,734	scaffold_7
AACS02000009.1	2,486,401	2,567,019	scaffold_8
AACS02000010.1	2,252,722	2,382,659	scaffold_9
AACS02000012.1	2,097,972	2,316,625	scaffold_10
AACS02000006.1	2,020,770	2,197,563	scaffold_11
AACS02000011.1	1,954,623	2,176,702	scaffold_12
AACS02000013.1	982,921	1,109,935	scaffold_13
Subtotal	35,810,191	37,536,632	Subtotal
(mt) AACS02000068.1	42,448	116,032	scaffold_14
AACS02000016.1	23,510	103,986	scaffold_15
AACS02000017.1	23,135	99,945	scaffold_16
AACS02000018.1	9,579	96,933	scaffold_17
AACS02000019.1	9,441	92,116	scaffold_18
AACS02000020.1	9,343	78,712	scaffold_20
AACS02000021.1	9,280	75,690	scaffold_21
AACS02000022.1	9,252	70,843	scaffold_22
AACS02000023.1	9,208	69,949	scaffold_23
AACS02000024.1	9,182	62,405	scaffold_24
AACS02000025.1	9,181	61,197	scaffold_25
AACS02000026.1	9,180	57,039	scaffold_26
AACS02000027.1	9,155	52,398	scaffold_27
AACS02000028.1	9,057	50,293	scaffold_28
AACS02000029.1	9,031	42,417	scaffold_19 (mt)
AACS02000032.1	8,670	12,442	scaffold_29
AACS02000035.1	7,876	11,170	scaffold_30
AACS02000036.1	7,480	7,943	scaffold_31
AACS02000037.1	7,452		
AACS02000038.1	7,407		

24

25 Table S2. Continued.

Okayama-7 #130	<i>A43mut B43mut pab1-1</i> #326
Contigs from the longest to the smallest	
(bp)	(bp)
AACS02000039.1	7,405
AACS02000040.1	6,705
AACS02000041.1	6,624
AACS02000042.1	6,618
AACS02000045.1	6,415
AACS02000048.1	5,668
AACS02000033.1	5,317
AACS02000030.1	5,246
AACS02000049.1	4,951
AACS02000052.1	4,610
AACS02000053.1	4,495
AACS02000054.1	4,220
AACS02000055.1	4,187
AACS02000056.1	4,138
AACS02000057.1	4,117
AACS02000058.1	3,902
AACS02000046.1	3,725
AACS02000031.1	3,458
AACS02000059.1	3,446
AACS02000060.1	3,422
AACS02000043.1	3,390
AACS02000061.1	3,368
AACS02000062.1	3,319
AACS02000063.1	3,303
AACS02000034.1	3,279
AACS02000064.1	3,193
AACS02000065.1	3,148
AACS02000066.1	3,120
AACS02000015.1	3,102
AACS02000044.1	3,095
AACS02000067.1	2,862
AACS02000014.1	2,584
AACS02000050.1	2,476
AACS02000047.1	2,416
AACS02000051.1	2,208
Assemble size	36,192,590
	38,698,142
	Assemble size

26

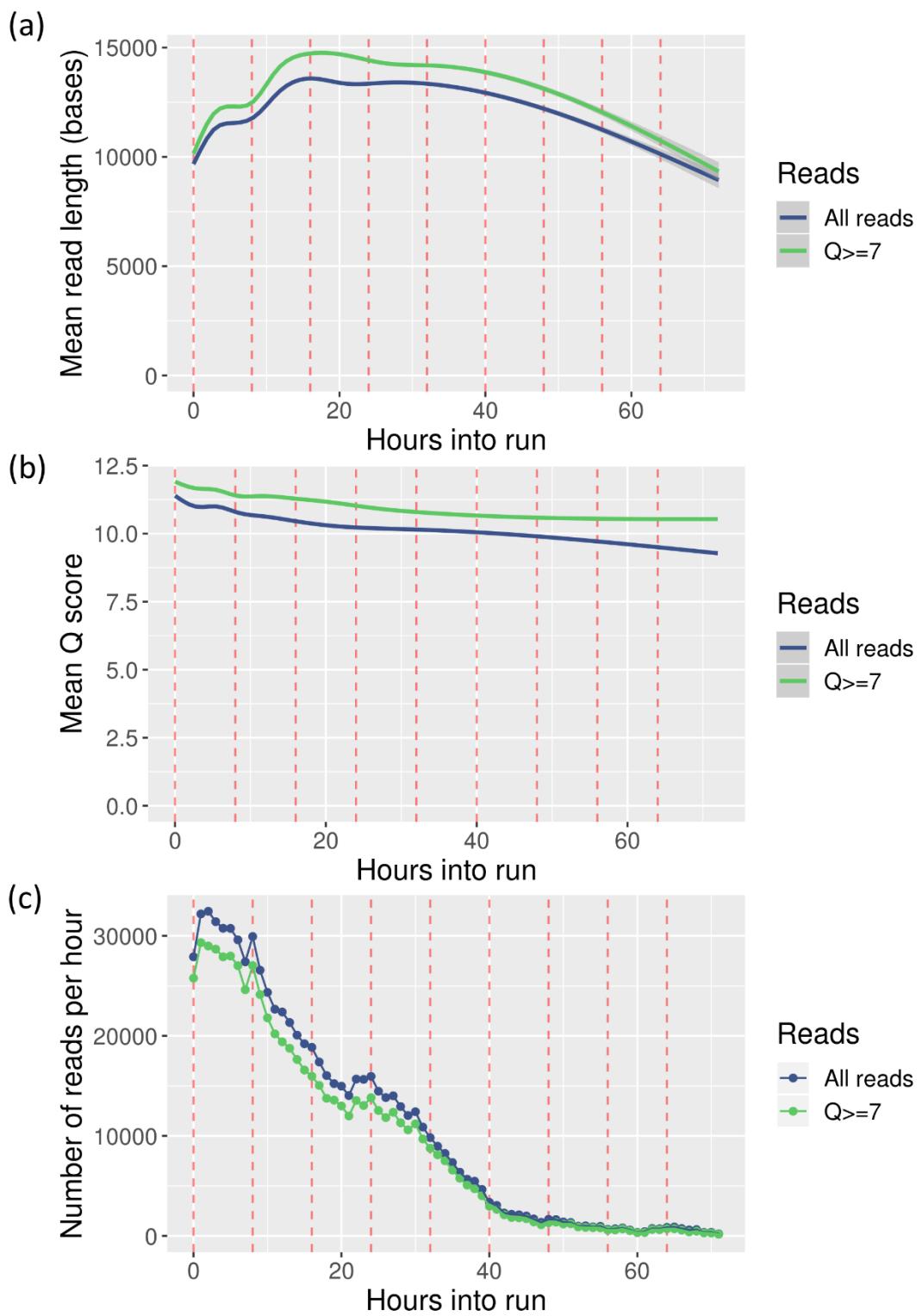
27

28 Table S3. Sequencing and alignment summary of mutants.

Reference genome			Nanopore + Illumina (this study)			Illumina (Muraguchi et al., 2015)		
Isolate	Clean reads	Clean bases	Mapping rate (%)	No. of SV	No. of SNP	Mapping rate (%)	No. of SV	No. of SNP
UV423	254,905	689,150,300	99.98	2	107	97.46	1,016	1,306
UV433	174,129	449,282,787	99.98	2	79	97.22	767	1,076
UV506	446,094	892,039,439	99.05	1	82	95.28	987	1,087
UV512	191,598	436,634,540	99.97	1	53	96.44	766	1,062
UV554	1,044,025	942,362,673	99.33	2	95	95.76	901	1,118
UV663	368,993	554,092,307	99.48	2	80	97.53	909	1,433

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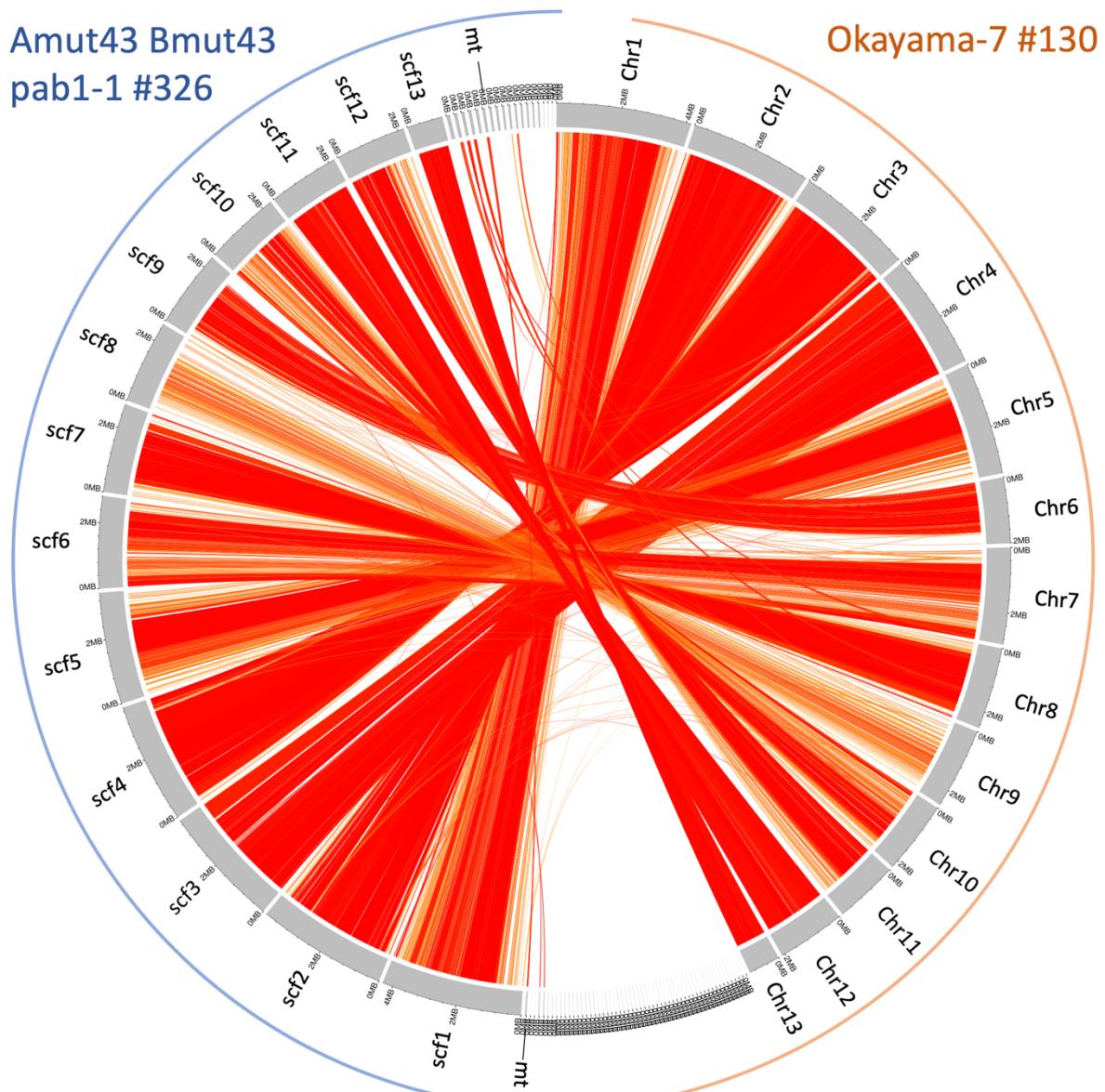
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32 Fig. S1. Long-read sequencing summary. (a) Mean read length by hour; (b) Mean Q score by
33 hour; (c) Yield of reads by hour.

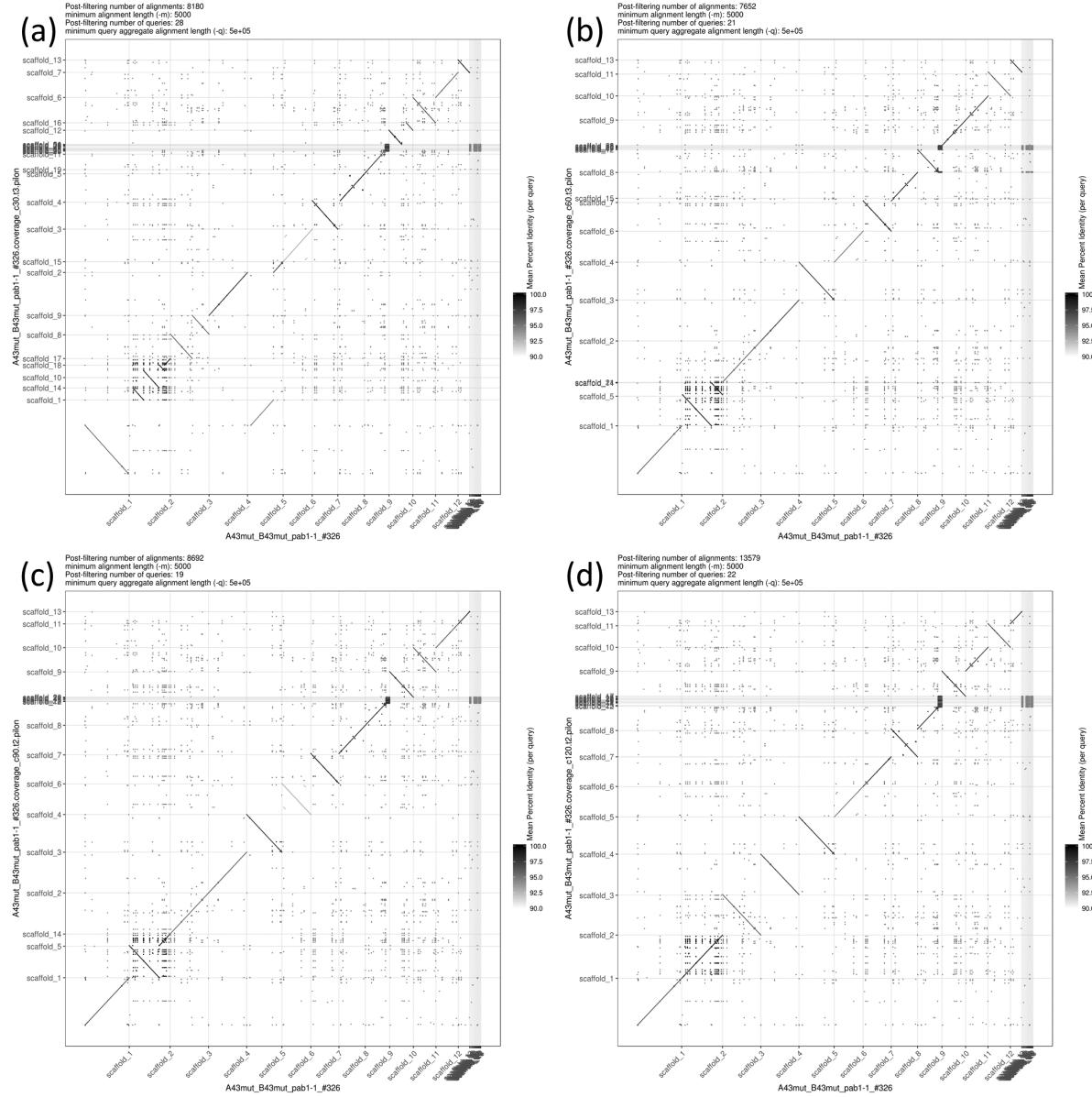
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35

36 Fig. S2. Sequence similarity between *A43mut B43mut pab1-1 #326* hybrid assembly draft
 37 genome and Okayama-7 #130 reference genome. Alignment length > 10 kb were shown.
 38 Identical matches range from 90 to 100 %. The higher ratio of identical matches, the sharper
 39 the red color.

40

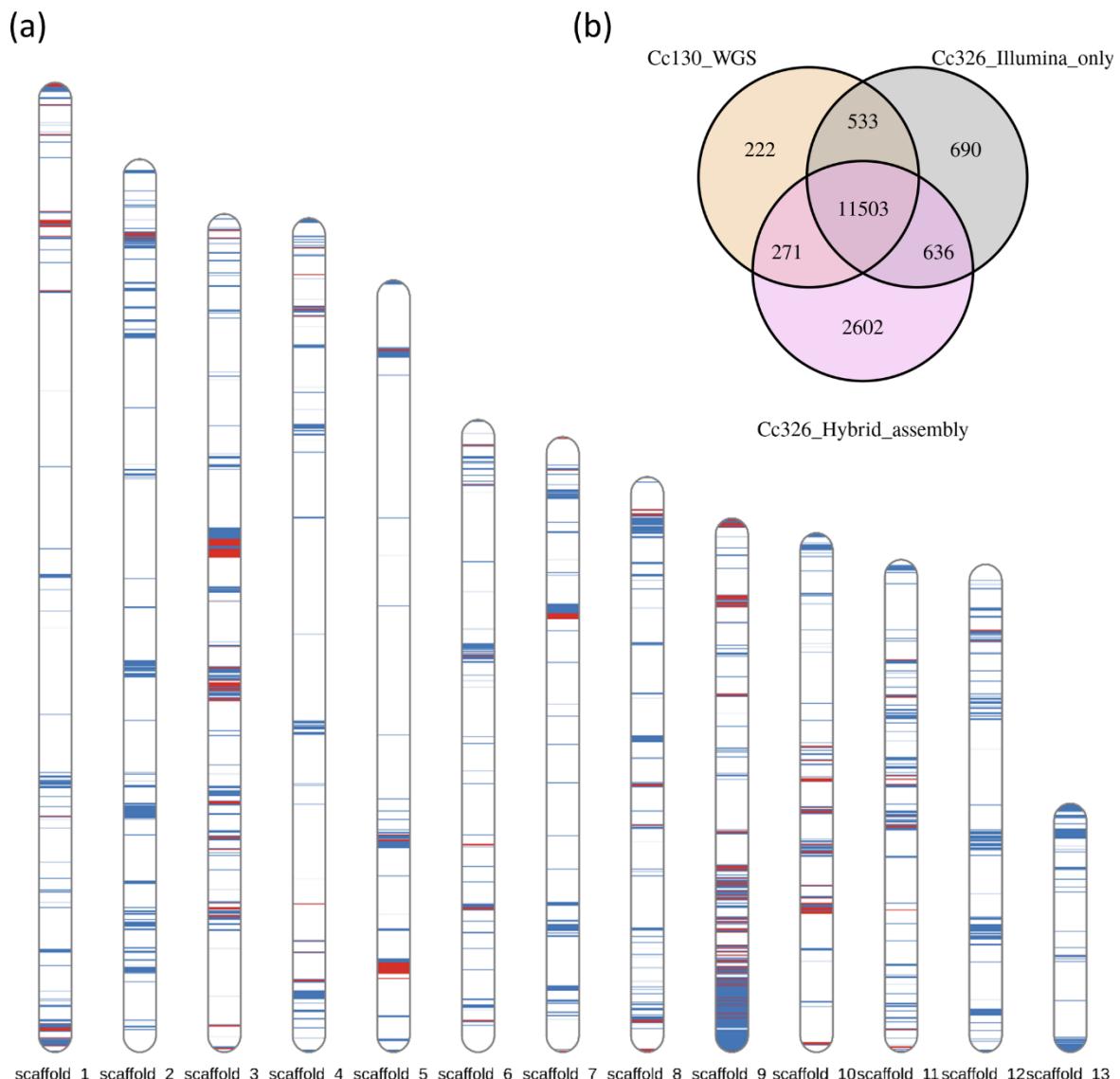


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42 Fig. S3. Genome contiguity of assemblies constructed with gradient sequencing coverage.

43 Genome alignment results were generated by MUMmer package, minimum alignment length
44 set to 5 Kbp. (a) 30-fold coverage; (b) 60-fold coverage; (c) 90-fold coverage; (d) 120-fold
45 coverage.

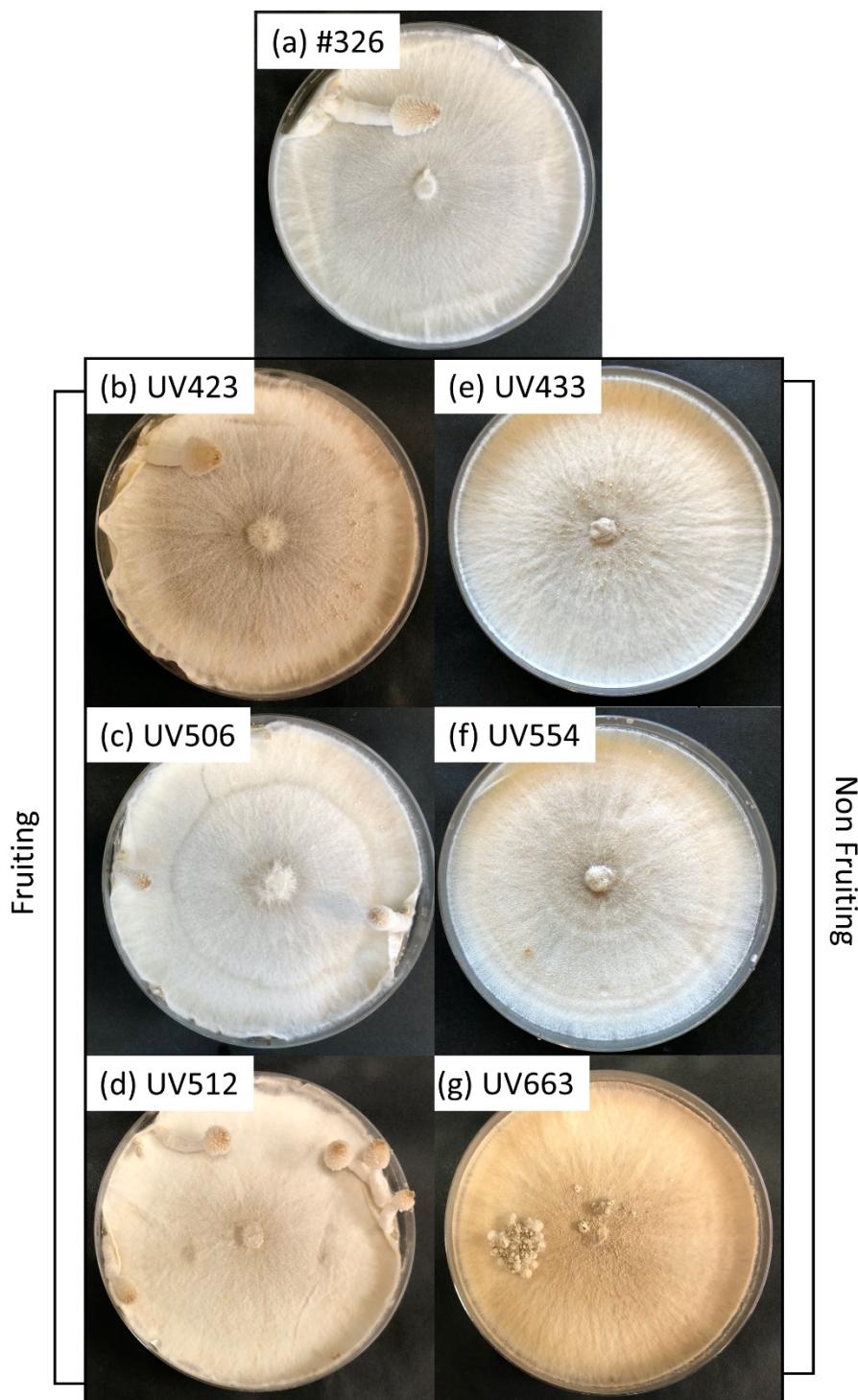
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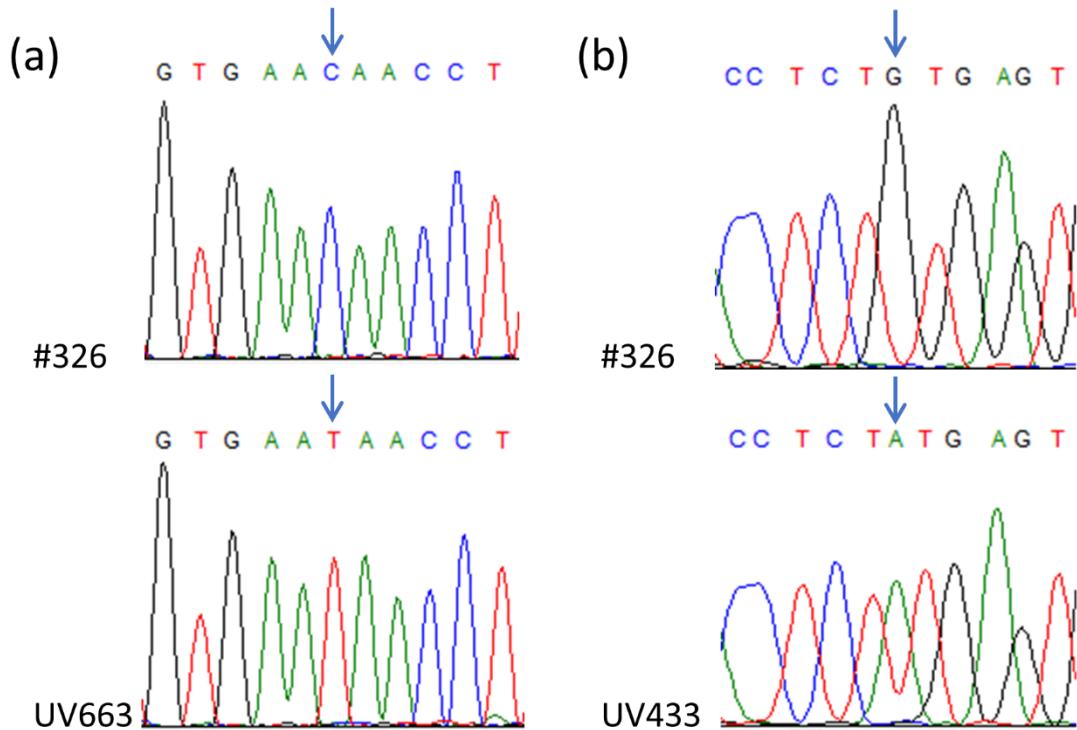
48 Fig. S4. Summary plot of additional sequences and genes found in the hybrid assembly. (a)
49 Chromosomal distribution of new sequences (blue colour) found in hybrid assembly against
50 short read assembly of strain #326. Annotated genes are further indicated in red colour. (b)
51 Number of commonly and specifically annotated protein coding genes in three *C. cinerea*
52 genomes. Overlapping areas show the number of genes that hit against each other by crossed
53 blast searches.

54



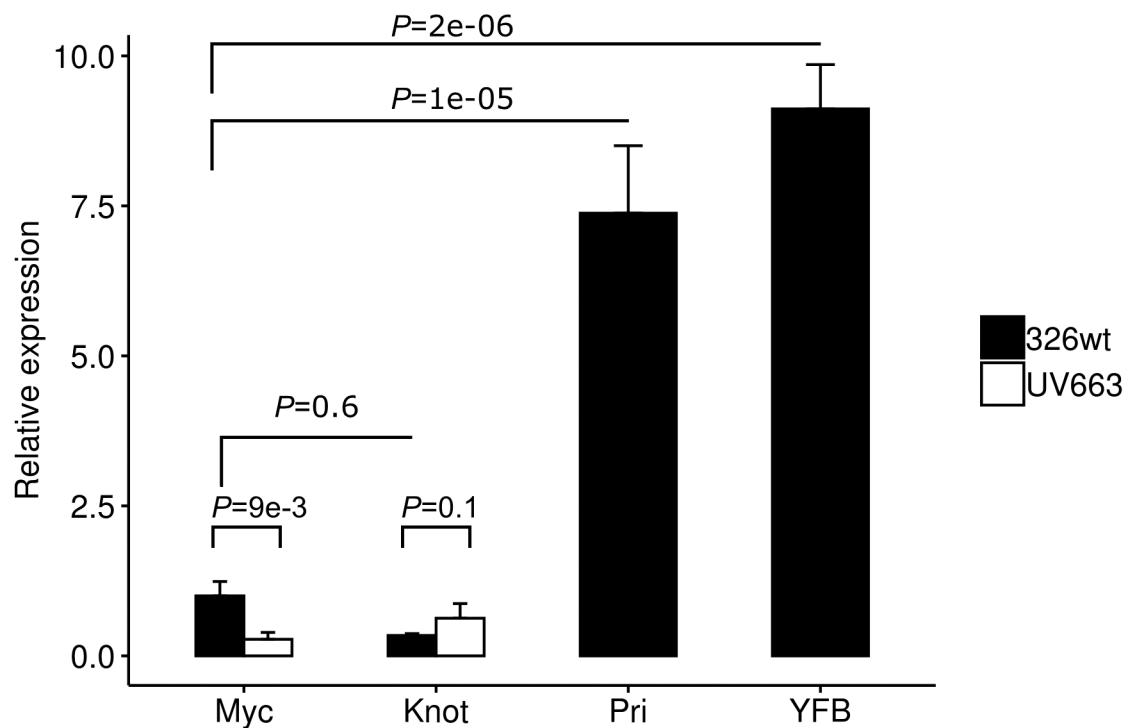
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56 Fig. S5. Morphology and fruiting test of mutated isolates. Fruiting tests were repeated for
57 three times at different timeslots and observation continued until the media were dried out.



58

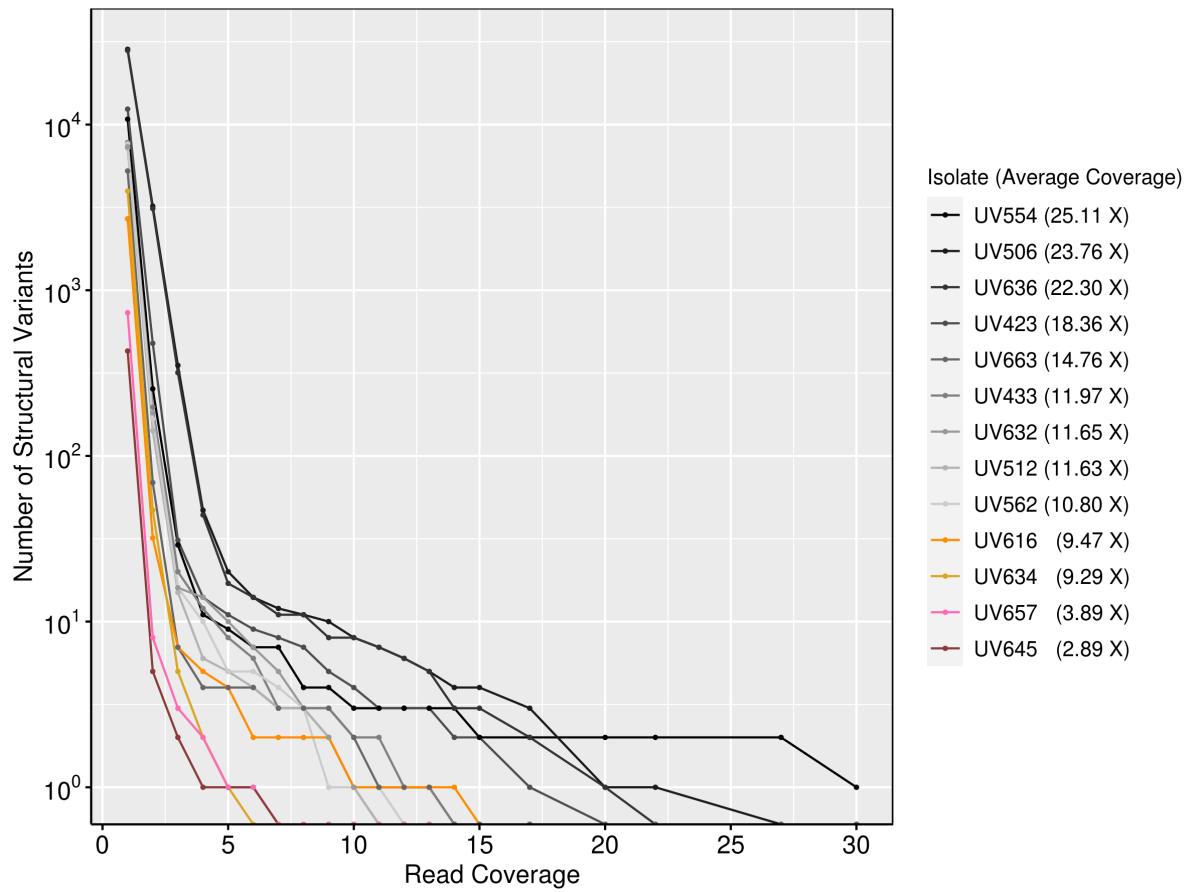
59 Fig. S6. Validation of single nucleotide variants. (a) DNA mismatch repair protein *msh6*
 60 (CC2G_013873-T1 c.1483C>T, scaffold_8:1037634C>T) of isolate UV663. (b)
 61 Atypical/alpha protein kinase (CC2G_008473-T1 c.55+1G>A, scaffold_4:542515G>A) of
 62 isolate UV433.



63

64 Fig. S7. qPCR validated gene expression levels of DNA mismatch repair protein *MSH6*
 65 (CC2G_013873) in #326 and UV663. Expression levels are normalized and compared to
 66 strain #326 at vegetative mycelia stage.

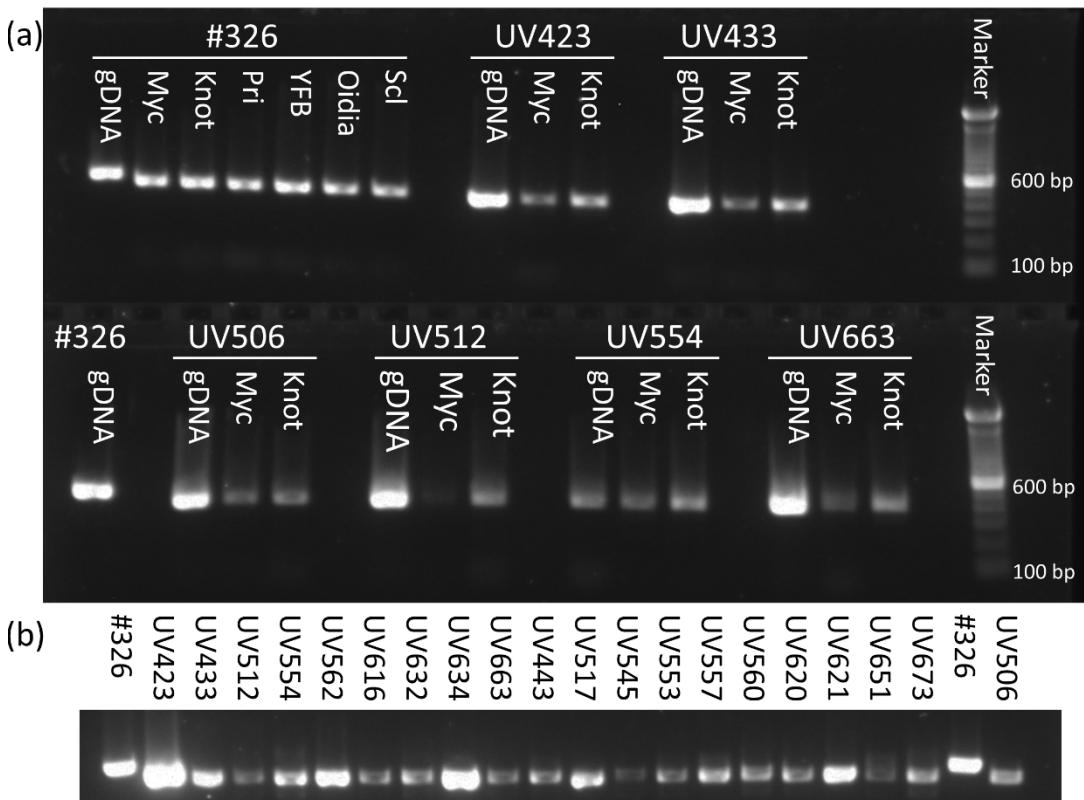
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69 Fig. S8. Navigation on the structural variant calling cutoffs.

70



(c)

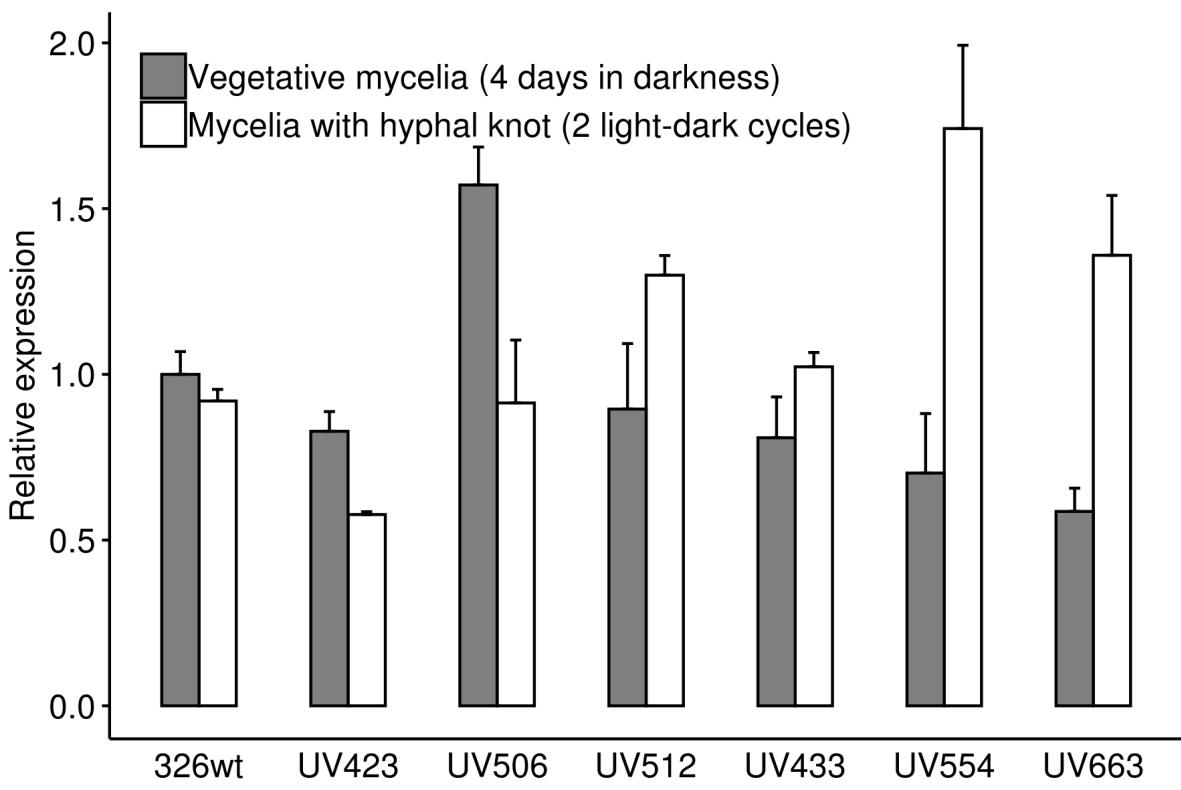
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>#326-gDNA
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CTCCAATTGATGGCATACTCGAACCTGGATCGTCACTACTTGTCACTTCACCGTGAGAACATTATTGTACCTACCGTC
GGTAGCCTTCTGACGACGCTTAGATGTCAGTTCAATTACTGGTCAACATGGGACGT
>UV433-gDNA
ATGAATTGGCCTGTAGTTACCACAGGCTAAGGGCCAAGATTGATTCCCTGGGAGGTACGGCAGATGACCCGACCACGTTGAC
CTCCAATT-----ACCTACCGTC
GGTAGCCTTCTGACGACGCTTAGATGTCAGTTCAATTACTGGTCAACATGGGACGT
>UV512-gDNA
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CTCCAATT-----ACCTACCGTC
GGTAGCCTTCTGACGACGCTTAGATGTCAGTTCAATTACTGGTCAACATGGGACGT
>UV636-gDNA
ATGAATTGGCCTGTAGTTACCACAGGCTAAGGGCCAAGATTGATTCCCTGGGAGGTACGGCAGATGACCCGACCACGTTGAC
CTCCAATT-----ACCTACCGTC
GGTAGCCTTCTGACGACGCTTAGATGTCAGTTCAATTACTGGTCAACATGGGACGT
>#326-cDNA
ATGAATTGGCCTGTAGTTACCACAGGCTAAGGGCCAAGATTGATTCCCTGGGAGGTACGGCAGATGACCCGACCACGTTGAC
M N W P V V Y H R L R A K I R F L G G T A D D D P D H V D
CTCCAATTGATGGCATACTCGAACCTGGATCGTCACTACTTGTCACTTCACCGTGAGAACATTCTAACG-----
L Q L M A Y S N L D R P L L V S I L N -----
-----ATGTCAGTTCAATTACTGGTCAACATGGGACGT
-----D V S S L L V N M G R
>RasGAP_del-cDNA
ATGAATTGGCCTGTAGTTACCACAGGCTAAGGGCCAAGATTGATTCCCTGGGAGGTACGGCAGATGACCCGACCACGTTGAC
M N W P V V Y H R L R A K I R F L G G T A D D D P D H V D
CTCCAATT-----ACCTACCGTC
L Q L -----P T V
GGTAGCCTTCTGACGACGCTTAGATGTCAGTTCAATTACTGGTCAACATGGGACGT
G S L L T T L * M S V H Y W S T W D V

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72 Fig. S9. Validation of the 66bp deletion in *RasGAP*. (a) Deletion on DNA and cDNA of
73 selected strains shown by gel electrophoresis. (b) Deletion in RasGAP of several isolates
74 shown by gel electrophoresis. (c) Deletion on DNA and cDNA confirmed by Sanger
75 sequencing, with prediction of protein sequences.



76

77 Fig. S10. qPCR validated gene expression levels of *RasGAP* (CC2G_009974) in #326 and
78 mutants. Expression levels are normalized and compared to strain #326 at vegetative mycelia
79 stage.

80