

1 **Supporting Text**

2 **Strain construction**

3 A diagram of the general strain construction protocol is included in S4 Fig. *S. cerevisiae* Y55
4 and *S. paradoxus* N17 strains were constructed starting from haploid prototrophs (Y55:
5 MAT α = YDP0302, MAT α = YDP1275; N17: MAT α = YDP1276, MAT α = YDP0742). To
6 allow selection for the integration of the spore-autonomous fluorescent protein expression
7 constructs, we first knocked out the endogenous *LEU2* and *URA3* loci in one or both mating
8 types: *LEU2* was replaced with *HYGMX* amplified from plasmid p167 using primers
9 0899/0900 (Y55: generating MAT α strain YDP1307 and MAT α strain YDP1303) or
10 0897/0898 (N17: generating MAT α strain YDP1295) while *URA3* was replaced with *KANMX*
11 from plasmid p161 using primers 0889/0890 (Y55: generating MAT α strain YDP1277 and
12 MAT α strain YDP1285) or primers 881/882 (N17: generating MAT α strain YDP1289 and
13 MAT α strain YDP1281). For *S. paradoxus* N44 and YPS138, we started with MAT α haploids
14 generated as part of the *Saccharomyces* Genome Resequencing Project (SGRP; [S8]) that
15 already had *URA3* replaced with *KANMX* and *HO* replaced with *HYGMX* (N44 = NCYC3687;
16 YPS138 = NCYC3684). To allow selection of diploids formed with N17 and Y55, we swapped
17 the *ura3::KANMX* marker with *NATNT2* from plasmid p30346 (EUROSCARF) using primers
18 1057/1058 (N44: generating strain YDP1493) or 1018/1019 (YPS138: generating strain
19 YDP1487). We subsequently replaced the *ho::HYGMX* markers in these strains with *KANMX*
20 using primers 0255/0256 from plasmid p161 in both N44 (generating strain YDP1500) and
21 YPS138 (generating strain YDP1470). For *S. cerevisiae* S288C, we started with MAT α
22 *ura3::HYGMX* haploid strain YDP0972, and swapped with *HYGMX* marker with *NATMX*
23 using primers 0255/256 from plasmid p178 to generate strain YGL3.

24 Fluorescent proteins were placed under the expression of two different promoters. We first
25 replaced the YKL050c ORF in Y55 and N17 with promoterless fluorescent constructs:
26 GFP_*URA3* was amplified from plasmid pSK726 and RFP_*LEU2* was amplified from plasmid
27 pSK691 (both gifts from Scott Keeney) as described in S1 Table. Genomic DNA extracted
28 from these strains was then used as a template to transfer fluorescent constructs under the

29 endogenous YKL050c promoter ($P_{YKL050c}$ -GFP_URA3 and $P_{YKL050c}$ -RFP_LEU2) to a different
30 chromosome (either III, VII, VIII or IX, see panel A in S1 Table). Although fluorescent protein
31 expression under endogenous promoters was improved compared to heterologous
32 promoters, it remained difficult to score in *S. paradoxus* and interspecific hybrids.
33 Consequently, we switched to the *DIT1* (YDR403w) promoter, which we found could be
34 scored with much greater reliability. The *DIT1* ORF in Y55, S288C, N17, N44, and YPS138
35 was replaced with a promoterless fluorescent construct as described in panel B of S1 Table.
36 Genomic DNA from these strains was then used as a template to transfer fluorescent
37 constructs under the *DIT1* promoter to each chromosome (except for S288C and YPS138
38 where we only used chromosomes I, VII, and XII). Loci chosen for integration on each
39 chromosome were located near the centromere (but were not directly apposed to minimize
40 influence of the fluorescent construct on centromere function), had no annotated function
41 related to viability, meiosis, or chromosome segregation, and were not associated with
42 reduced sporulation or germination efficiency [S9,S10]. Correct integration of fluorescent
43 constructs at desired loci was confirmed by diagnostic PCR (primer sequences available on
44 request).

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46 **Anti-recombination**

47 We created meiotic null mutants of *SGS1* by replacing the endogenous promoter with the
48 meiotically silent *CLB2* promoter, as described in [S11]. The NATNT2 cassette from plasmid
49 p30346 (EUROSCARF) was inserted upstream of the *CLB2* promoter in Y55 strain YDP1275
50 (creating strain YDP1478) and in N17 strain YDP1276 (creating strain YDP1479) using
51 primers P1013/1015 and P1012/1014, respectively. Strains YDP1478 and YDP1479 were
52 then used as species-specific templates to replace the endogenous *SGS1* promoter with the
53 appropriate NATNT2-linked *CLB2* promoter, using primers P1020/1021 (for Y55) and
54 P1022/1038 (for N17), in strains with fluorescent-marked chromosomes (panel B in S1
55 Table). Strains of both mating types in N17 and Y55 with fluorescent markers on
56 chromosomes II, VII, XII, and XIII (YDP1405, YDP1392, YDP1430, YDP1418, YDP1410,

57 YDP1397, YDP1402, YDP1389, YDP1427, YDP1415, YDP1411, YDP1398, YDP1435,
58 YDP1436, YDP1424) were converted to *pCLB2_SGS1* (YDP1494, YDP1495, YDP1496,
59 YDP1497, YGL13, YGL12, YDP1501, YDP1502, YDP1503, YDP1504, YDP1509, YDP1510,
60 YGL14, YDP1511, YDP1512, respectively).

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62 **Genome alignments and % identity**

63 Genomes for *S. cerevisiae* strains S288C and SK1 and *S. paradoxus* strains CBS432 and
64 N44 were downloaded from https://yjx1217.github.io/Yeast_PacBio_2016/data/ and full
65 genome alignments were performed using Mauve [S12] implemented in Geneious 10.2.3.
66 Distances of the *S. cerevisiae* genomes from the 100-genomes project [S2] to S288C
67 described in S2 Fig panel C and S1 Data were calculated using Geneious 10.2.3 after being
68 aligned using REALPHY [S5].

69 A. *YKL050c* promoter-driven spore-autonomous fluorescence

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chr	locus	Y55α		Y55a		N17α		N17a
		YDP1307	YDP1277	YDP1303	YDP1285	YDP1295	YDP1289	YDP1281
III	<i>YCR007c</i>	YDP1373 0948/0910 YDP1344			YDP1369 0948/0910 YDP1350	YDP1365 0947/0912 YDP1342		YDP1361 0947/0912 YDP1348
VII	<i>YGL006-7</i>	YDP1374 0935/0936 YDP1344			YDP1370 0935/0936 YDP1350	YDP1366 0933/0934 YDP1342		YDP1362 0933/0934 YDP1348
VIII	<i>YHR007cA</i>	YDP1375 0950/0908 YDP1344			YDP1371 0950/0908 YDP1350	YDP1367 0949/0906 YDP1342		YDP1363 0949/0906 YDP1348
IX	<i>YIL002wA</i>	YDP1376 0939/0940 YDP1344			YDP1372 0939/0940 YDP1350	YDP1368 0937/0938 YDP1342		YDP1364 0937/0938 YDP1348
XI	<i>YKL050c</i>		YDP1350 0920/0921 pSK726	YDP1344 0920/0921 pSK691	YDP1352 0920/0921 pSK726	YDP1342 0922/0923 pSK691	YDP1346 0922/0923 pSK726	YDP1348 0922/0923 pSK726

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73 B. *DIT1* (*YDR403W*) promoter-driven spore-autonomous fluorescence

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chr	locus	S288Ca	Y55α	Y55a		N17α		N17a	N44a	YPS138a
		YGL3	YDP1307	YDP1285	YDP1303	YDP1295	YDP1289	YDP1281	YDP1500	YDP1470
I	<i>YAL018c</i>	YGL8 1030/1031s YGL5	YDP1491 1030/1031 YDP1343	YDP1490 1030/1031 YDP1351		YDP1489 1024/1025 YDP1341		YDP1488 1024/1025 YDP1347	YDP1526 1067/1025 YDP1499	YDP1513 1024/1025 YDP1498
II	<i>YBL029w</i>		YDP1389 0958/0959 YDP1343	YDP1402 0958/0959 YDP1351		YDP1415 0956/0957 YDP1341		YDP1427 0956/0957 YDP1347	YDP1527 1068/1069 YDP1499	
III	<i>YCR007c</i>		YDP1390 0961/0910 YDP1343	YDP1403 0961/0910 YDP1351		YDP1416 0960/0912 YDP1341		YDP1428 0960/0912 YDP1347	YDP1528 1070/1071 YDP1499	
IV	<i>YDR403w</i> <i>DIT1</i>	YGL5 0916/0917 pSK726	YDP1349 0916/0917 pSK726	YDP1351 0916/0917 pSK726	YDP1343 0916/0917 pSK691	YDP1341 0918/0919 pSK691	YDP1345 0918/0919 pSK726	YDP1347 0918/0919 pSK726	YDP1529 1059/1060 pSK726	YDP1498 1036/1037 pSK726
V	<i>YER004w</i>		YDP1391 0963/0932 YDP1343	YDP1404 0963/0932 YDP1351		YDP1417 0962/0930 YDP1341		YDP1429 0962/0930 YDP1347	YDP1530 1072/0930 YDP1499	
VI	<i>YFR006w</i>		YDP1482 0966/0967 YDP1343	YDP1483 0966/0967 YDP1351		YDP1480 0964/0965 YDP1341		YDP1481 0964/0965 YDP1347	YDP1559 1097/1099 YDP1499	
VII	<i>YGL006-7</i>	YGL9 0969/0936 YGL5	YDP1392 0969/0936 YDP1343	YDP1405 0969/0936 YDP1351		YDP1418 0968/0934 YDP1341		YDP1430 0968/0934 YDP1347	YDP1531 1073/0934 YDP1499	YDP1521 1047/0934 YDP1498
VIII	<i>YHR007cA</i>		YDP1393 0971/0908 YDP1343	YDP1406 0971/0908 YDP1351		YDP1419 0970/0906 YDP1341		YDP1431 0970/0906 YDP1347	YDP1532 1074/1075 YDP1499	
IX	<i>YIL002wA</i>		YDP1394 0973/0940 YDP1343	YDP1407 0973/0940 YDP1351		YDP1420 0972/0938 YDP1341		YDP1432 0972/0938 YDP1347	YDP1533 1076/1077 YDP1499	
X	<i>YLO16w</i>		YDP1395 0976/0975 YDP1343	YDP1408 0976/0975 YDP1351		YDP1421 0974/0975 YDP1341		YDP1433 0974/0975 YDP1347	YDP1534 1078/0975 YDP1499	
XI	<i>YKR005c</i>		YDP1396 0978/0997 YDP1343	YDP1409 0978/0997 YDP1351		YDP1422 0977/0996 YDP1341		YDP1434 0977/0996 YDP1347	YDP1535 1079/1080 YDP1499	
XII	<i>YLR004c</i>	YGL11 0981/0980 YGL5	YDP1397 0981/0980 YDP1343	YDP1410 0981/0980 YDP1351		YDP1423 0979/0980 YDP1341		YDP1435 0979/0980 YDP1347	YDP1536 1081/1082 YDP1499	YDP1522 1053/0980 YDP1498
XIII	<i>YMR003w</i>		YDP1398 0984/0983 YDP1343	YDP1411 0984/0983 YDP1351		YDP1424 0982/0983 YDP1341		YDP1436 0982/0983 YDP1347	YDP1537 1083/0983 YDP1499	
XIV	<i>YNL018c</i>		YDP1399 0987/0988 YDP1343	YDP1412 0987/0988 YDP1351		YDP1484 0985/0986 YDP1341		YDP1485 0985/0986 YDP1347	YDP1538 1084/0986 YDP1499	
XV	<i>YOL014w</i>		YDP1400 0990/0998 YDP1343	YDP1413 0990/0998 YDP1351		YDP1425 0989/0998 YDP1341		YDP1437 0989/0998 YDP1347	YDP1539 1085/0998 YDP1499	
XVI	<i>YPR003c</i>		YDP1401 0993/0992 YDP1343	YDP1414 0993/0992 YDP1351		YDP1426 0991/0992 YDP1341		YDP1438 0991/0992 YDP1347	YDP1540 1086/0992 YDP1499	

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76 **S1 Table.** Spore-autonomous fluorescent protein expression strains under (A) the *YKL050c*

77 promoter or (B) the *DIT1* promoter. Strains are listed in red if RFP-expressing and green if

78 GFP-expressing and the genes into which these cassettes were integrated are listed in the
79 locus column. *YGL006-7* indicates the intergenic region between *YGL006W* and *YGL007W*.
80 Primers (see S3 Table below) used for each transformation are listed below each strain
81 number, and the source of the template DNA used in each transformation is listed below the
82 primer numbers.

83	YDP0302	Y55	MAT α	ho			
84	YDP0972	S288C	MATa	ho	ura3::HYGMX		
85	YGL3	S288C	MATa	ho	ura3::NATMX		
86	YGL5	S288C	MATa	ho	ura3::HYGMX		dit1::GFP_URA3
87	YDP1307	Y55	MAT α	ho		leu2::HYGMX	
88	YDP1275	Y55	MATa	ho			
89	YDP1285	Y55	MATa	ho	ura3::KANMX		
90	YDP1303	Y55	MATa	ho		leu2::HYGMX	
91	YDP1277	Y55	MAT α	ho	ura3::KANMX		
92	YDP1349	Y55	MAT α	ho	ura3::KANMX		dit1::GFP_URA3
93	YDR1343	Y55	MATa	ho		leu2::HYGMX	dit1::RFP_LEU2
94	YDP1285	Y55	MATa	ho			
95	YDP1351	Y55	MATa	ho	ura3::KANMX		dit1::GFP_URA3
96	YDP1276	N17	MAT α	ho			
97	YDP1295	N17	MAT α	ho		leu2::HYGMX	
98	YDP1341	N17	MAT α	ho		leu2::HYGMX	dit1::RFP_LEU2
99	YDP1289	N17	MAT α	ho	ura3::KANMX		
100	YDP1345	N17	MAT α	ho	ura3::KANMX		dit1::GFP_URA3
101	YDP0742	N17	MATa	ho			
102	YDP1281	N17	MATa	ho	ura3::KANMX		
103	YDP1347	N17	MATa	ho	ura3::KANMX		dit1::GFP_URA3
104	NCYC3687	N44	MATa	ho::HYGMX	ura3::KANMX		
105	YDP1493	N44	MATa	ho::HYGMX	ura3::NATNT2		
106	YDP1499	N44	MATa	ho::HYGMX	ura3::NATNT2		dit1::GFP_URA3
107	YDP1500	N44	MATa	ho::KANMX	ura3::NATNT2		
108	YDP1529	N44	MATa	ho::KANMX	ura3::NATNT2		dit1::GFP_URA3
109	NCYC3684	YPS138	MATa	ho::HYGMX	ura3::KANMX		
110	YDP1487	YPS138	MATa	ho::HYGMX	ura3::NATNT2		
111	YDP1470	YPS138	MATa	ho::KANMX	ura3::NATNT2		
112	YDP1469	YPS138	MATa	ho::HYGMX	ura3::NATNT2		dit1::GFP_URA3
113	YDP1498	YPS138	MATa	ho::KANMX	ura3::NATNT2		dit1::GFP_URA3
114	YDP1478	Y55	MATa	ho			NATNT2_CLB2
115	YDP1479	N17	MAT α	ho			NATNT2_CLB2
116							
117							

118 **S2 Table.** Starting strains used to generate spore-autonomous fluorescent protein
119 expression strains.

primer	sequence (5' to 3')
P0255	cgtacgctgcaggtcgac
P0256	atcogatgaattcgagctcg
P0881	TGTGGCTGGGTTTTCAGGGTCCACTACTTTTCTTCCcgtacgctgcaggtcgac
P0882	TTTTCGTCATTATAAAAAATCATACGACCGAGATCCCGGatcgatgaattcgagctcg
P0889	GACCATCAAAGAGGTTAATGTGGCTGTGGTTTCAGGGTTCcgtacgctgcaggtcgac
P0890	TTTTCGTCATTATAAAAAATCATACGACCGAGATCCCGGatcgatgaattcgagctcg
P0897	GCTATTTGGATTTTATATATGACTTTCATTTAACATGATcgtacgctgcaggtcgac
P0898	GCTCTACCCATGAACATATCCATTTTGTAAATTCGTGTatcgatgaattcgagctcg
P0899	GCTATTTGGATTTTATATATGACTTTCGTGTACATATGATcgtacgctgcaggtcgac
P0900	CGTCTACCCATGAACATATCCATTTTGTAAATTCGTGTatcgatgaattcgagctcg
P0906	CCTCTCGCTTTTTCGGGATACCTTGGCCTGTACACTACATTCgtttcggtgatgacggtgaaaa
P0908	TCCCTCGCTTCTACGTTACTTGGCCTGTGCACTACATTCgtttcggtgatgacggtgaaaa
P0910	GAAAATATCACTTTACCTGAAGACACCTTTAAATCATATAcgtttcggtgatgacggtgaaaa
P0912	GAAAATATCACTTACCTGTAGACACCTTTAAATCATATAcgtttcggtgatgacggtgaaaa
P0916	TATCCTAATTCGGTAAAGCTTTGTGAGACATTAACAAAAtggtgagcaagggcgagga
P0917	AAAGAACAAAAGGTAGACCAATGTAGCGCTTACTTTAcgtttcggtgatgacggtgaaaa
P0918	TAATATTTTACTCGGAAAACCTTTGAAATATAGCAAAAAtggtgagcaagggcgagga
P0919	AGGACAAAAGTAAAGCGCTAGCGCTTCTGCTTTATCCcgtttcggtgatgacggtgaaaa
P0930	TCCCAGAAGGATTCACGCCAAATGAACACCAACTTTCCcgtttcggtgatgacggtgaaaa
P0932	TCCCAGAAGGATTCACGCCAAATGAACACCAACTTTCCcgtttcggtgatgacggtgaaaa
P0934	AAAAAGCCTCCATCGGTTTCTAAACGGGCTATACATGCcgtttcggtgatgacggtgaaaa
P0936	AAAAAGCCTCCATCGGTTTCTAAACGGGCTATACATGCcgtttcggtgatgacggtgaaaa
P0938	CGCCAAATCGTTGCTGCTTGTGACAGTCCCTAAGTACggtttcggtgatgacggtgaaaa
P0940	TGCCAAATCATTTGTTGTCCTGTGTAGATCTTCTAAGTACggtttcggtgatgacggtgaaaa
P0956	ACATCAACGTTAATGCTTATGGTACGAAAATTTAAGCTAaggagggacaataatcgcaagg
P0957	TGTAATTCGGGGTACTTTACCCTTACTCGTTCGTGACAGTTGcgtttcggtgatgacggtgaaaa
P0958	ACATCAACGTTAATGCTTATGGTACGAAAATTTAAGCTAagggtggacaataatcgcaagg
P0959	TGTAATTCGGGGTACTTTACCCTTACTCGTTCGTGACAGTTGcgtttcggtgatgacggtgaaaa
P0960	CCCTTTCACAAGGAATCTGAAGAATTTGTAGCACCTTTCaaggagggacaataatcgcaagg
P0961	TTCTTTTACAAGGAATCTGAAGAATTTGTAGCATCTTTCaagggtggacaataatcgcaagg
P0962	CAAAGCGGATTTGGTGGCAATCTGACTGCTGCTTTGGGCAaggagggacaataatcgcaagg
P0963	CAAAGCGGATTTGGGCGCAATCTGACTGCTGCTTTGGGCAagggtggacaataatcgcaagg
P0964	TTAAATGATGACGGCGAGAATGGAGACAAATACAAAGTAgggagggacaataatcgcaagg
P0965	GATTACCACCAACGCTGCTGATCATCAAGCCCAACATATGcgtttcggtgatgacggtgaaaa
P0966	TTAAATGATGACGGTGCAGAATGGAGACAAATACAAAGTAggggtggacaataatcgcaagg
P0967	GGTTACCACCAACGCTGCTGATCATCAAGCCCAACATATGcgtttcggtgatgacggtgaaaa
P0968	ATGCCGCTATATAGAGCAATGATGACTTAATACGCAGTAgggagggacaataatcgcaagg
P0969	ATGCCGCTATATAGAGCAATGATGACTTAATACGCAGTAggggtggacaataatcgcaagg
P0970	TTACGACGTAATACCTTTCACAATTTACGGTTGGAACAATaggagggacaataatcgcaagg
P0971	TTACGACCTTAATGCTTTTACAATTTATCGGTTGGAACAATagggtggacaataatcgcaagg
P0972	ATGACTCGTGATACCCCTGAAGATGTCAGCACTGACAGTAgggagggacaataatcgcaagg
P0973	ATGACTCGTGATACACCCGAAGATGTCAGTACTGACAGGCGagggtggacaataatcgcaagg
P0974	TCCCTGTACAGTGCAGACGCTGGCCATCATAGATTCcaggagggacaataatcgcaagg
P0975	AATAATCCACTGAGGCAATTTAGGTAATCCAAACATTTAcgtttcggtgatgacggtgaaaa
P0976	TCCTCTGTACAAGTGCAGACGCTGGCCATCATAGATTCcagggtggacaataatcgcaagg
P0977	GACGTGATTAAGGTGCTCCTTGTTCGGTTTCTAGTCTTAGaggagggacaataatcgcaagg
P0978	GACGTGATTAAGGTGCTCCTTGTTCAGTATCAGTCTTGGagggtggacaataatcgcaagg
P0979	TAATGACACGAAACCACTGTAAGCAAGTACAGAGAAAaggagggacaataatcgcaagg
P0980	GGTCAATCACACTATAACCACTATGTATATTTCCAGcgtttcggtgatgacggtgaaaa
P0981	TAATGACACGAAACCACTGTAAGCAAGTACAGAGAAAagggtggacaataatcgcaagg
P0982	AGCAGTGCAAAACAAGAGGTTGAAATTTACAGGTCGAAaggagggacaataatcgcaagg
P0983	GATTCCTGAGGTTCCAGGTTCCACCAAGACACGATAAACcgtttcggtgatgacggtgaaaa
P0984	AGCAGTGCAAAACAAGAGGTTAAAATTTACAGGTCGAAagggtggacaataatcgcaagg
P0985	TCAAATGTTCTTTGTACCTCTTAGGAAGAGTGGACAGTATTagggagggacaataatcgcaagg
P0986	AAGTCACTGCTTTGATTTCTGCCGCACCAAGGACTCCAGcgtttcggtgatgacggtgaaaa
P0987	TCAAATGTTCTTTGTACTTCTCAGGAAGAGTGGACAGTATTaggggtggacaataatcgcaagg
P0988	AAGTGACTTGTCTTATTTCCGCCGCACCAAGGACTCCAGcgtttcggtgatgacggtgaaaa
P0989	ACCACATTTCTCAGTGCATGACATGCTACTTCTTCTTGGGATAaggagggacaataatcgcaagg
P0990	ACCACATTTCTCAGTGCATGACATGCTACTTGGCATGagggtggacaataatcgcaagg
P0991	TGTTGAGGCAAGCTTACTAAAAATTTGTCTAGCTTTAAATTagggagggacaataatcgcaagg
P0992	CCACTTGGCCTATCGTACACAACCTCAATTGCACATGCTCCcgtttcggtgatgacggtgaaaa
P0993	TGTTGAGGCAAGCTTACTAAAAATTTGTCTAGCTTTAAATTaggggtggacaataatcgcaagg
P0996	CTGCTATTTAAGCTTTAGCCATAATCAAACACTAcgtttcggtgatgacggtgaaaa
P0997	TCTGCACTATTAACCTCTTTAACCAATAGTCAAAGACTAcgtttcggtgatgacggtgaaaa
P0998	CATAATTCCTGGCATATGTTTACCACAATTCCAATAATTCggtttcggtgatgacggtgaaaa
P1012	CCCACTATTTACCTCGGCCAAGAAATAATAGAAATTTATTTTcgtacgctgcaggtcgac
P1013	CCTACGTTATTTCTGTAAGAAATGGCAGAAATTTCTGTcgtacgctgcaggtcgac
P1014	GACACGATAACACTTGGCCCTGTTTTTGAATAAGATTTGcattacaacaggtgtgtcctc
P1015	GATAACAAAACACTTGCACCTATTTTTTGAATAAAAACCGcattacaacaggtgtgtcctc
P1018	TGTGGCTGTGGTTTTTCAGGGTCCACTACTTTTCTTTTCcgtacgctgcaggtcgac
P1019	TTTTCGTCATTATAAAAAAGCATACGACCGAGACTCCCGGcattacaacaggtgtgtcctc
P1020	GGAAAAATACAGATTTATTTGTTGATATATTTAAAAATCATACAGTACACCAAGCGGTAcgtacgctgcaggtcgac
P1021	TCGCCGTTTCTTTAACCATTTGTGCTCCTTTCTTAAGTTATGTGACGGCTTCGTACCATctataagatcaatgaagagagagagggg
P1022	GGAAAACTTATAAGTTFCAGTGTATATATTTAAAGTACACGCATACCGCAAGGAGTAcgtacgctgcaggtcgac
P1024	TCACGCTTTGTATGTTGTCGAAAATTTGTAAACGTAAAAaggagggacaataatcgcaagg
P1025	CAAGGACGCATTCACAGGAGGTCGTTTCATGTACCGGTTTCggtttcggtgatgacggtgaaaa
P1030	TCACGCTTTGTATGTTGTCGAAAATTTGTAAACGTAAAAagggtggacaataatcgcaagg
P1031	CAAAGACGCATTCACAGGAGGTCATTCATGTACCGGTTTCggtttcggtgatgacggtgaaaa
P1031s	CAAAGATGCATTCACAGGAGGTCATTCATGTACCGGTTTCggtttcggtgatgacggtgaaaa
P1036	CGTAAAAATAAACAGTCTGTTAATAATTTTACTCGGAAAAGCTTAAAAATTTAGCAAAAAtggtgagcaagggcgagga
P1037	ACTAATAATTTAAGTAAAGACAAAAGTAAAGCGGCTTACGCTGCTGCTCATACcgtttcggtgatgacggtgaaaa
P1038	TCGCCGTTTCTTCAACCATTTGTGCTCCTTTCTTAAGTTATGTGAGGGCTTCGTACCATctataaaatcaatcaaaagggggatagggg
P1047	ATGCCGCTATATAGAGCAAAATGATGACTTAATACGCAATaggagggacaataatcgcaagg
P1053	TAATGACACAGGAACCACTGTAAGCAGGTACAGAGAAAaggagggacaataatcgcaagg
P1057	TGTGGCTGTGGTTTTTCAGGATCCACTACTTTTCTTTTCcgtacgctgcaggtcgac
P1058	TTTTCGTCATTATAAAAAATCATACGACCGAGATCCCGGcattacaacaggtgtgtcctc
P1059	TAATATTTTACTCGGAAAAGCTTTGAAATATAGCAAAAAtggtgagcaagggcgagga
P1060	AAGACAAAAGTAAAGCGGCTGGCGCTGCTGTTTATCCcgtttcggtgatgacggtgaaaa
P1067	TCACGCTTTGTATGTTGTCGAAAATTTGTAAACGTAAAAaggagggacaataatcgcaagg

P1068	ACATCAACGTTAATGCTTATGGTGACGAAAAATTAACGTAaggagggacaattatcgcaaagg
P1069	TGTATTCGGGGTACTTTACCTTTACTCGTTCGTGCAGTTGcgtttcggtgatgacggtgaaaa
P1070	CCCTTTCACAAGGAATCTGAAGAATTTGTAGCACCTTTCAaggagggacaattatcgcaaagg
P1071	GAAAAATATCACCTTTACCTGTAGACACCTTTAAATCATATAcgtttcggtgatgacggtgaaaa
P1072	CAAAGCGGATTTGGTGGCAATCTGACTGCTGCTTTGGGCAaggagggacaattatcgcaaagg
P1073	ATGCGCGTATATAGAGCAATTGATTGACTTAATACGCAGTtaggagggacaattatcgcaaagg
P1074	TTACGACAGTAATACCTTTACAATATCGGTTGGAACAATaggagggacaattatcgcaaagg
P1075	CCTCTCGCCTTTTGGCTTACTTGGCCTGTACACTACATTCgtttcggtgatgacggtgaaaa
P1076	ATGACTCGTGATACCCCTGAAGATGTCAGCACTGCAGGTGaggagggacaattatcgcaaagg
P1077	CGCCAAATTTGTTGCTGTCCTTGTGCAGATCCTTAACCTGAcgtttcggtgatgacggtgaaaa
P1078	TCCTCTGTACGAGTCGCAGACGTGGCCATCATAGATTCCcaggagggacaattatcgcaaagg
P1079	GACGTGATTAGGGTGGTCTTGTTCAGTTTCAGTCTTAGaggagggacaattatcgcaaagg
P1080	TCTGCATTATTAACCTCTTTAGCCAATGATCAAAACACTAcgtttcggtgatgacggtgaaaa
P1081	TAATGACACAGGAACCACGTGTAAGCAAGTACAGAGAAAAaggagggacaattatcgcaaagg
P1082	GGTCAATCACACTCATAACCACATCTATGTATATCCAGcgtttcggtgatgacggtgaaaa
P1083	AGCAGTCAAAAACAAGAGGGTTGAAATTATCAGGTCGCAaggagggacaattatcgcaaagg
P1084	TCAATGTTCTTTGTACCTCTTAGGAAGAGTGGCAGTATTaggagggacaattatcgcaaagg
P1085	ATCACATTCTCAGTGCATGCCTACTTCTTCTGGGATAaggagggacaattatcgcaaagg
P1086	TGTTGAGGCAAGCTTACTAAAAATTTGTCTAGCTTAAATTaggagggacaattatcgcaaagg
P1097	TGTGGACATTACACTACGTCAAGAATCCGAAGATATCAaggagggacaattatcgcaaagg
P1099	CACTTGTATATTGTCTCCATTTGACACCTGCATCAATTAacgtttcggtgatgacggtgaaaa

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S3 Table. Primers used in this study.

Supporting References

S1. Hou J, Friedrich A, De Montigny J, Schacherer J. Chromosomal rearrangements as a major mechanism in the onset of reproductive isolation in *Saccharomyces cerevisiae*. *Curr Biol* 2014;24:1153–9.

S2. Strobe PK, Skelly DA, Kozmin SG, Mahadevan G, Stone EA, Magwene PM, et al. The 100-genomes strains, an *S. cerevisiae* resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen. *Genome Res* 2015;125:762–74.

S3. Pérez-Ortín JE, Querol A, Puig S, Barrio E. Molecular characterization of a chromosomal rearrangement involved in the edaptive evolution of yeast strains. *Genome Res* 2002;1533–9.

S4. Liti G, Barton DBH, Louis EJ. Sequence diversity, reproductive isolation and species concepts in *saccharomyces*. *Genetics* 2006;174:839–50.

S5. Bertels F, Silander OK, Pachkov M, Rainey PB, Van Nimwegen E. Automated reconstruction of whole-genome phylogenies from short-sequence reads. *Mol Biol Evol* 2014;31:1077–88.

S6. Ma C, Mortimer RK. Empirical equation that can be used to determine genetic map distances from tetrad data. *Mol Cell Biol* 1983; 3:1886-87.

- 144 S7. Thacker D, Lam I, Knop M, Keeney S. Exploiting spore-autonomous fluorescent
145 protein expression to quantify meiotic chromosome behaviors in *Saccharomyces*
146 *cerevisiae*. *Genetics* 2011;189:423–39.
- 147 S8. Cubillos FA, Louis EJ, Liti G. Generation of a large set of genetically tractable haploid
148 and diploid *Saccharomyces* strains. *FEMS Yeast Res* 2009;9:1217–25.
- 149 S9. Marston AL, Tham WH, Shah H, Amon A. A genome-wide screen identifies genes
150 required for centromeric cohesion. *Science* 2004;303:1367–70.
- 151 S10. Enyenihi AH, Saunders WS. Large-scale functional genomic analysis of sporulation
152 and meiosis in *Saccharomyces cerevisiae*. *Genetics* 2003;163:47–54.
- 153 S11. Amin AD, Chaix ABH, Mason RP, Badge RM, Borts RH. The roles of the
154 *Saccharomyces cerevisiae* RecQ helicase SGS1 in meiotic genome surveillance.
155 *PLoS One* 2010;5:e15380.
- 156 S12. Darling ACE, Mau B, Blattner FR, Perna NT. Mauve: Multiple alignment of conserved
157 genomic sequence with rearrangements. *Genome Res* 2004;14:1394-1403.