Supplemental Figure Legends

Supplemental Figure 1. Karyogamy occurs in the basidia and subapical area of basidia. (A) During opposite-sex mating, karyogamy occurs in the basidia (Fig. 3) or subapical area of the basidia. (B) Similar nuclear positions were observed during same-sex mating. Scale bars = 5 μm.

Supplemental Figure 2. Unisexual reproduction of *kar7* mutant in the serotype D, JEC21 strain background. Wild-type JEC21 underwent unisexual mating similar to the XL280 strain. The *kar7* JEC21 mutant did not display any unisexual hyphal growth unlike the *kar7* mutants derived from the XL280 strain (Fig. 4), indicating that early karyogamy may be required to initiate filamentation in the JEC21 strain. V8 medium (pH=7) was used for mating and incubated at room temperature in the dark for three weeks before observation. Scale bar = 50 μm.

Supplemental Figure 3. Disruption of *KAR7* alleles in the diploid MN142.3 strain. (A) One allele of the *KAR7* gene was replaced with the *URA5* gene in the *KAR7*/*KAR7* diploid strain (MN142.3). The probe (red dotted-line) to the 5' region hybridized with 11,089 bp fragments of the wild-type *KAR7* allele, whereas in the heterozygous *KAR7*/*kar7::URA5* mutants, the probe recognized both 11,089 bp fragments of the wild-type *KAR7* allele and 6,486 bp fragments of the *kar7::URA5* allele when the genomic DNAs were digested with KpnI. 1: SL355, 2: SL356, 4: SL357, 5: SL358, 6: SL359, and 7: SL360. (B) The wild-type *KAR7* allele in the *KAR7*/*kar7::URA5* diploid (SL355) strain was replaced with the *HYG* drug resistance marker gene. When the

genomic DNAs were digested with EcoRV, the probe (red-dotted-line) to the 5' region recognized 10,482 fragments of wild-type *KAR7* allele, 1,509 bp for the *kar7::HYG* allele, and 1,700 bp for the *kar7::URA5* allele. 1: SL361, 2: SL362, 3: unknown, 4: SL363, 5: SL364, 6: SL365, and 7: unknown. The strains in lanes 3 and 7 were not chosen for further analysis due to an unknown extra band.

Supplemental Figure 4. Nucleus positioning in a dikaryotic hypha in wild-type and *kar3* crosses. GFP-Nop1 signals indicate that there is no apparent difference in nucleus positioning in wild-type and *kar3* crosses, suggesting that Kar3 is less likely to function in nucleus movement in dikaryotic hyphae in *C. neoformans*. Scale = 5 μm.

Supplemental Figure 5. FACS analyses to determine ploidy of progeny from *KAR4*/*kar4::HYG* diploid strain. Two progeny (SL372 and SL373) were determined to be haploid based on comparison to the haploid control strain XL280, indicating that the *KAR4* gene is not essential for viability.

Supplemental Figure 6. Vegetative yeast growth of *kar7* mutants. *kar7* mutant exhibit abnormal yeast growth pattern: serotype A *kar7* mutants were granulated and some cells showed a pseudo-hyphae-like phenotype and serotype D *kar7* mutant cells were swollen and also granulated compared to wild-type. These results indicate that the *KAR7* gene plays other role(s) during yeast growth in addition to

karyogamy during mating. However, two other meiotic mutants, *dmc11* and *spo11*, did not have any apparent vegetative growth defects. Scale bar = 5 μm.

Supplemental Figure 7. Simplified life cycles of ascomycetes and basidiomycetes. Ascomycetes are predominantly haploid and have a short period of the dikaryotic stage immediately followed by karyogamy (A). Basidiomycetes have a long characteristic dikaryotic stage (B). This major difference in dikaryotic stage length might have resulted in differential evolutionary selection pressure on karyogamy genes.

Supplemental Table 1. Karyogamy genes in the two *Cryptococcus gattii* strains.

	C. gattii	
	WM276	R265
KAR1	none	none
KAR2	*CGB_N2420C*	*CNBG_5018*
KAR3	*CGB_F3540C*	*CNGB_1791*
KAR4	*CGB_H1300W*	*CNGB_2581*
KAR5	none	none
KAR7	*CGB_C2410W*	*CNGB_3798*
KAR8	*CGB_D7630C*	*CNGB_0911*
KAR9	none	none

Supplemental Table 2. Primers used in this study.

	Name	Sequence (5' to 3')
Serotype A *KAR7*	JOHE20336	GCAGGTAGCGTTTGGTCTTC
	JOHE20337	ACTGGCCGTCGTTTTACGATCCTCGTTAGTGGGTTGC
	JOHE20338	GCAACCCACTAACGAGGATCGTAAAACGACGGCCAGT
	JOHE20339	GTGGTGACGCGATAATCATGCAGGAAACAGCTATGAC
	JOHE20340	GTCATAGCTGTTTCCTGCATGATTATCGCGTCACCAC
	JOHE20341	CCAATCAGAGGCTGCAATTT
	JOHE20342	GGAATCCTGCAGAAAATCCA
	JOHE20343	GGAAATGATTATTCGGCGTTA
Serotype D *KAR7*	JOHE19704	CTATCGAAAGCGCAAGACCT
	JOHE19705	ACTGGCCGTCGTTTTACCAATGTGTATTCGGCGTTGT
	JOHE19706	ACAACGCCGAATACACATTGGTAAAACGACGGCCAGT
	JOHE19881	TGACGCGATGATCATGATTCCAGGAAACAGCTATGAC
	JOHE19882	GTCATAGCTGTTTCCTGGAATCATGATCATCGCGTCA
	JOHE19709	AATCATGTCTGGTCGAGGAAA
Serotype D *KAR8*	JOHE19710	ATCACGGAAGCTTGGTCATC
	JOHE19711	ACTGGCCGTCGTTTTACGCAAATAGGGGCGGATAGTT
	JOHE19712	AACTATCCGCCCCTATTTGCGTAAAACGACGGCCAGT
	JOHE19960	CGTGTCACTGCCATTCATTCCAGGAAACAGCTATGAC
	JOHE19961	GTCATAGCTGTTTCCTGGAATGAATGGCAGTGACACG
	JOHE19715	TCACAACTCGATCCCCTTTC
	JOHE19779	TCAGTTTGCTCATTGGTTCG
	JOHE19780	TTCTTGCGCTCCAAAGAAGT
Serotype A *KAR3*	JOHE20352	CTTGCCGACGAGACCATACT
	JOHE20353	ACTGGCCGTCGTTTTACTGATTTGGGACAGGGTCAAT
	JOHE20354	ATTGACCCTGTCCCAAATCAGTAAAACGACGGCCAGT
	JOHE20355	GATGCCAACTCTTCTCCGTCCAGGAAACAGCTATGAC
	JOHE20356	GTCATAGCTGTTTCCTGGACGGAGAAGAGTTGGCATC
	JOHE20357	ACAGCCGTTTGTTCTCTTGG
	JOHE20358	GCAGTGGTGAGCTTGAGGTT
	JOHE20359	CTGAAGCGGACAATGCCTA
Serotype A *KAR4*	JOHE20360	CGAGATAGGTCCAGGAGACG
	JOHE20361	ACTGGCCGTCGTTTTACGTCCATGGCTGTATCCGAGT
	JOHE20362	ACTCGGATACAGCCATGGACGTAAAACGACGGCCAGT
	JOHE20363	CTACACTTGTGAATACCGTCCTGCAGGAAACAGCTATGAC
	JOHE20364	GTCATAGCTGTTTCCTGCAGGACGGTATTCACAAGTGTAG
	JOHE20365	GCCCTTCCTACCAAAGATCC
	JOHE20366	CGTTCCTAAGTGGGAAACGA
	JOHE20367	TCATCACATGGCAACTCCTC

Serotype D *KAR7* for *URA* or *HYG* cassette	JOHE26107	ATGTCCTTCTTCGCTTTGGACAATGTGTATTCGGCGTTGT
	JOHE26108	ACAACGCCGAATACACATTGTCCAAAGCGAAGAAGGACAT
	JOHE26109	TGACGCGATGATCATGATTCGTCATCGAGGAAGACGGAAA
	JOHE26110	TTTCCGTCTTCCTCGATGACGAATCATGATCATCGCGTCA
Serotype D *KAR2*	JOHE19716	CCCTCGTATAATGCAGTCAGC
	JOHE19717	ACTGGCCGTCGTTTTACTATGTAAGGCACGGCAACAG
	JOHE19718	CTGTTGCCGTGCCTTACATAGTAAAACGACGGCCAGT
	JOHE20308	ACTTGCACAGAATTTGGGCTCAGGAAACAGCTATGAC
	JOHE20309	GTCATAGCTGTTTCCTGAGCCCAAATTCTGTGCAAGT
	JOHE19721	TCGCAGTCACAGTTGGTCTC
Serotype D *KAR3*	JOHE19722	GGCAGTGGTGAGCTTGAGAT
	JOHE19723	ACTGGCCGTCGTTTTACCGCGATTTGTTTGTTGTGAT
	JOHE19724	ATCACAACAAACAAATCGCGGTAAAACGACGGCCAGT
	JOHE20310	TATTGGGTGAAGGGAACGTCCAGGAAACAGCTATGAC
	JOHE20311	GTCATAGCTGTTTCCTGGACGTTCCCTTCACCCAATA
	JOHE19727	GTCCTCCTTCTCGCTGAATG
Serotype D *KAR4*	JOHE19728	CTGGTACAGGGGATGCTGAT
	JOHE19729	ACTGGCCGTCGTTTTACGCTCGGAACAGGAAAGAATG
	JOHE19730	CATTCTTTCCTGTTCCGAGCGTAAAACGACGGCCAGT
	JOHE20312	GAAGACATTCGGTCCTCCAACAGGAAACAGCTATGAC
	JOHE20313	GTCATAGCTGTTTCCTGTTGGAGGACCGAATGTCTTC
	JOHE19733	TCATCAGAGTGCCTCAACAGA
Nop1-mCherry for Serotype A	JOHE22274	ACCCTAACCCCAGCAACTCT
	JOHE22275	CTCGCCCTTGCTCACCATAGTGTGTCGTTGGTATATGC
	JOHE22276	GCATATACCAACGACACACTATGGTGAGCAAGGGCGAG
	JOHE22277	GGAGGACATGGAACGCGAATCCAAGCTTGGTACCGAGCTC
	JOHE22278	GAGCTCGGTACCAAGCTTGGATTCGCGTTCCATGTCCTCC
	JOHE22280	AGACGCATTCATGGGAGAAC
GFP-Nop1 for Serotype A	JOHE23127	GAAGATCTGCTATGGCTTTCGGTGACAGAGG
	JOHE23128	GAAGATCTGAGGGGTTTGTCGGTTGATA
GFP-Nop1 for Serotype D	JOHE23129	GAAGATCTGCTATGGCTTTCGGTGACAGAGG
	JOHE23130	GAAGATCTGGAACATGGGGGATATTGTG

Supplemental Materials and Methods

KAR7 gene disruption in the serotype D strains JEC21 and XL280

To disrupt the *KAR7* gene in the JEC21 and XL280 strains, the 5' region of the *KAR7* gene was amplified with primers JOHE19704 and JOHE19705, the *NEO* cassette was amplified with primers JOHE19706 and JOHE19881 from pNATSTM#209 (17), and the 3' region was amplified with primers JOHE19882 and JOHE19709. The three DNA fragments obtained were combined and amplified with primers, JOHE19704 and JOHE19709, and the final overlap PCR products were purified and precipitated onto 0.6 μm gold particles (Bio-Rad, Hercules, CA, USA). Then the wild-type strains were transformed biolistically with the DNAs obtained. The bombarded cells were transferred onto YPD medium containing appropriate selection drugs. Positive transformants were screened by PCR and Southern blot.

To disrupt the *KAR7* gene in the diploid strain, MN143.2, the 5' region of the *KAR7* gene was amplified with primers JOHE19704 and JOHE26170, the *URA5* cassette was amplified with primers JOHE26108 and JOHE26109, and the 3' region was amplified with primers JOHE26110 and JOHE19709. The three DNA fragments obtained were combined and amplified with primers, JOHE19704 and JOHE19709. The *HYG* cassette was amplified with primers JOHE19706 and JOHE19881 from plasmid pJAF15 (17).

KAR8 disruption in the serotype D strains JEC21, JEC20, and XL280

The 5' region of the *KAR8* gene was amplifed with primers JOHE19710 and JOHE19711, the *NEO* or *NAT* cassette was amplified with primers JOHE19712 and

JOHE19960, and the 3' region was amplified with primers JOHE19961 and JOHE19715. The three DNA fragments obtained were combined and amplified with with two internal nested primers, JOHE19779 and JOHE19780, and we proceeded to disrupt the *KAR8* gene as described above or in Materials and Methods.

KAR3 gene disruption in the serotype A strains, KN99α and KN99a

The 5' region of the *KAR3* gene was amplifed with primers JOHE20352 and JOHE20353, the *NEO* or *NAT* cassette was amplified with primers JOHE20354 and JOHE20355, and the 3' region was amplified with primers JOHE20356 and JOHE20357. The three DNA fragments obtained were combined and amplified with with two internal nested primers, JOHE20358 and JOHE20359, and we proceeded as described above or in Materials and Methods.

KAR4 gene disruption in the serotype D strain, MN142.3

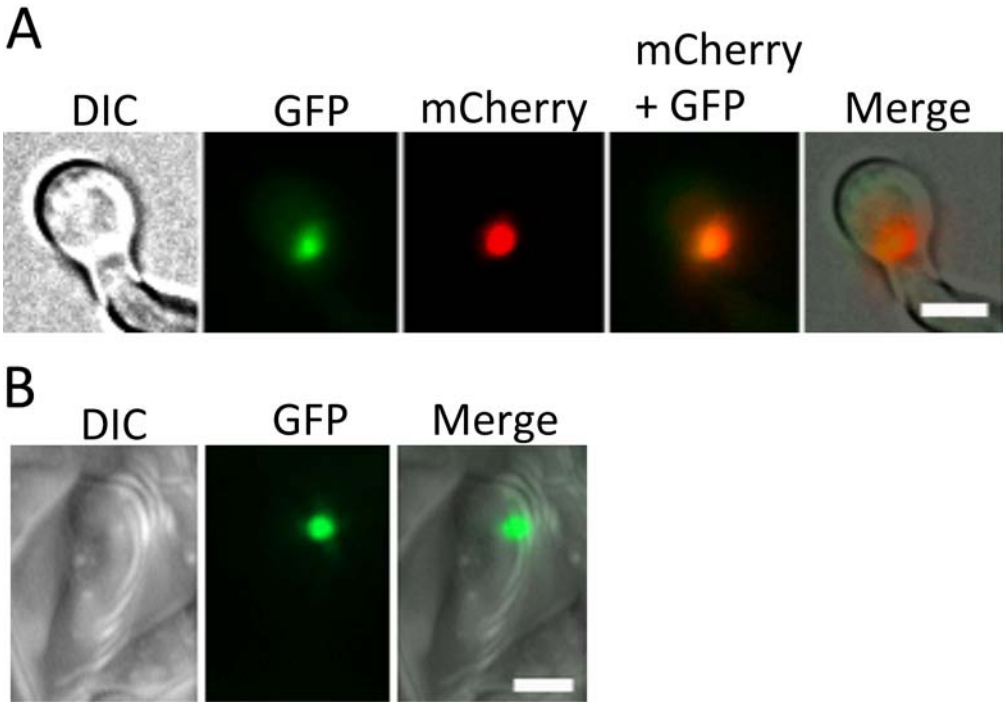
The 5' region of the *KAR4* gene was amplifed with primers JOHE20360 and JOHE20361, the *NEO* or *NAT* cassette was amplified with primers JOHE20362 and JOHE20363, and the 3' region was amplified with primers JOHE20364 and JOHE20365. The three DNA fragments obtained were combined and amplified with with two internal nested primers, JOHE20366 and JOHE20367, and we proceeded as described above or in Materials and Methods.

KAR2 disruption in the serotype D strain, MN142.3

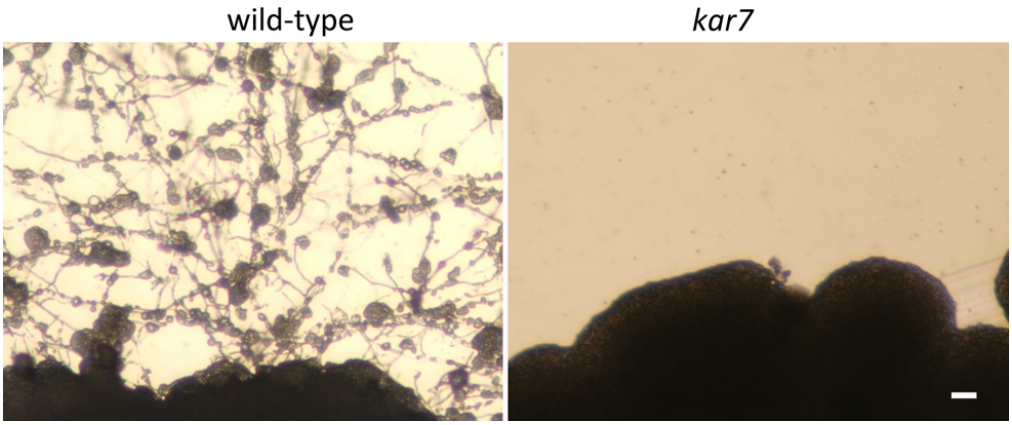
The 5' region of the *KAR2* gene was amplifed with primers JOHE19716 and JOHE19717, the *HYG* cassette was amplified with primers JOHE19718 and

JOHE20308, and the 3’ region was amplified with primers JOHE20309 and JOHE19721. The three DNA fragments obtained were combined and amplified with with JOHE19716 and JOHE19721, and we proceeded as described above or in Materials and Methods.

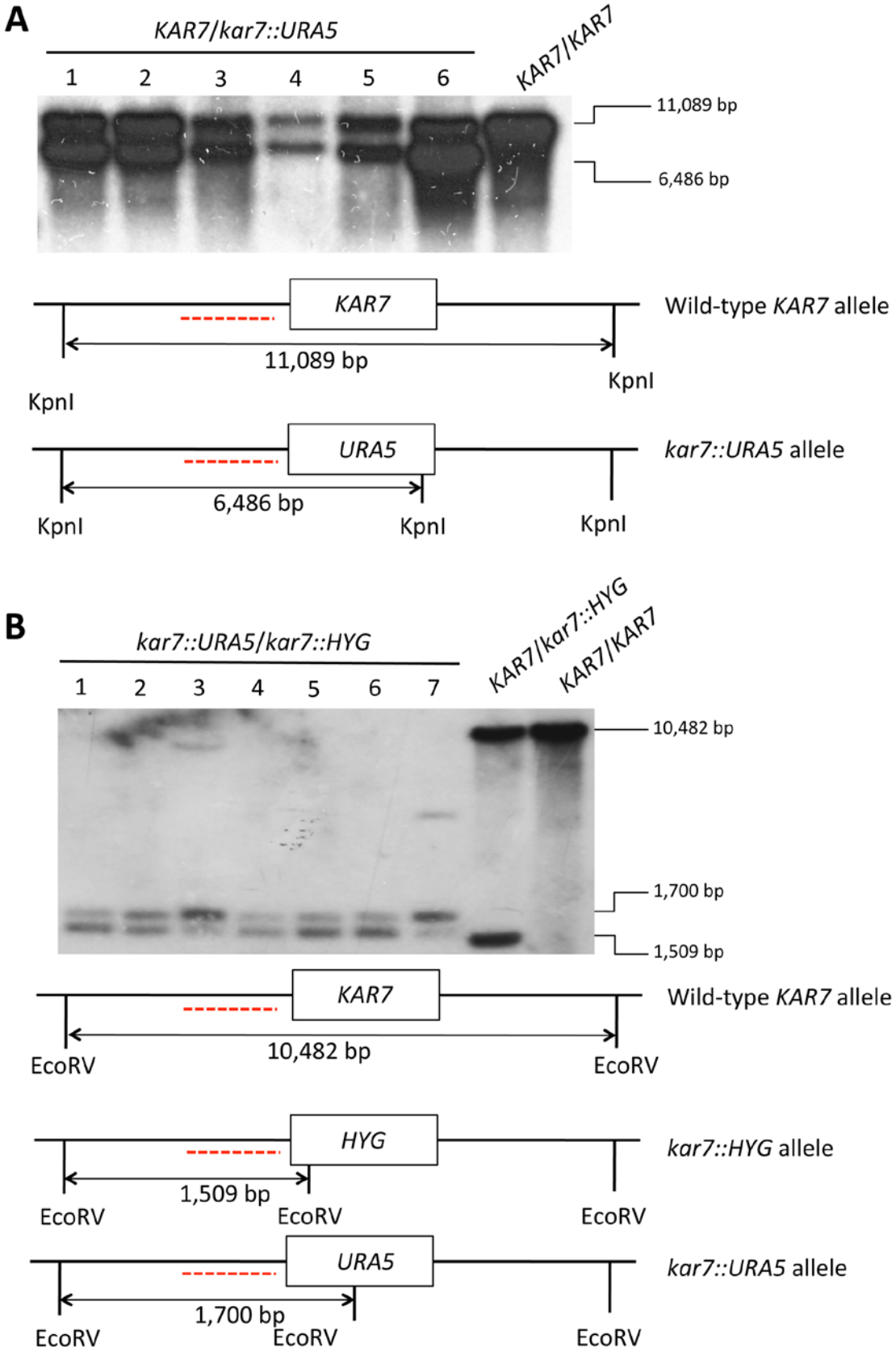
Supplemental Figure 1



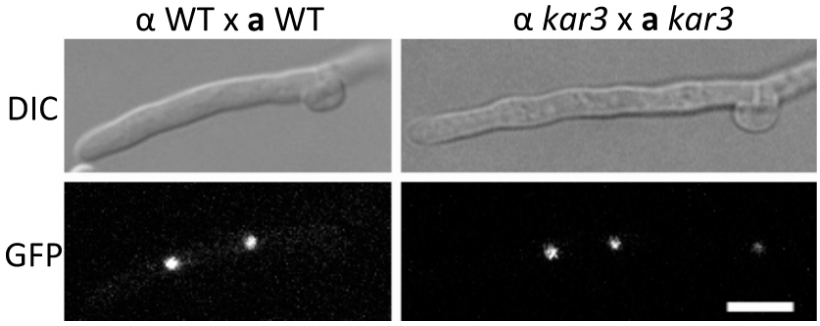
Supplemental Figure 2



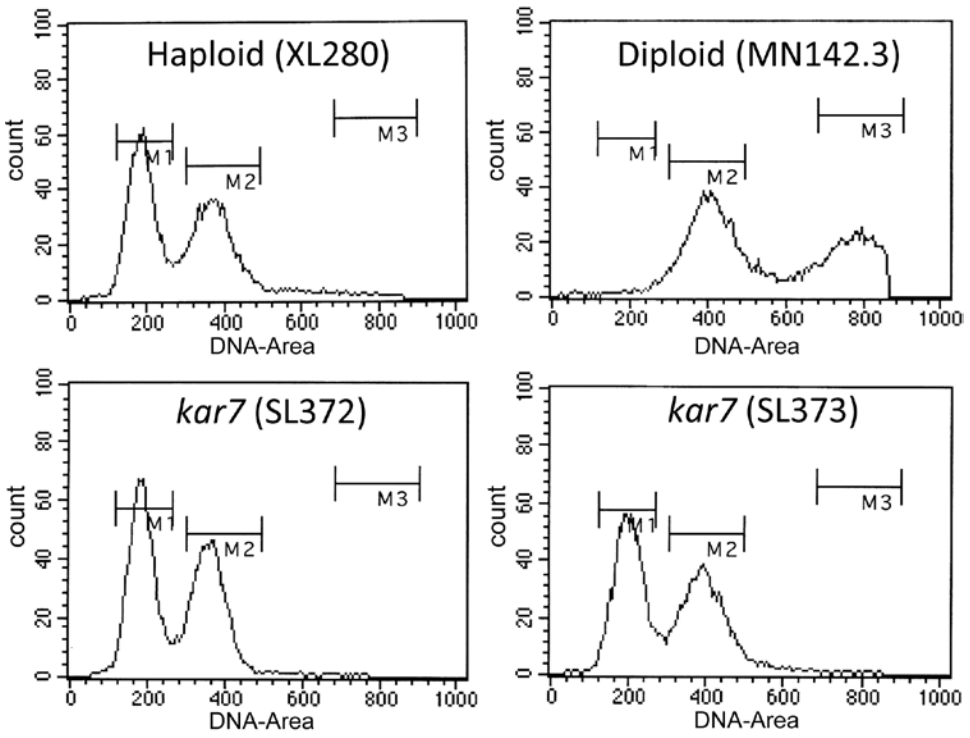
Supplemental Figure 3



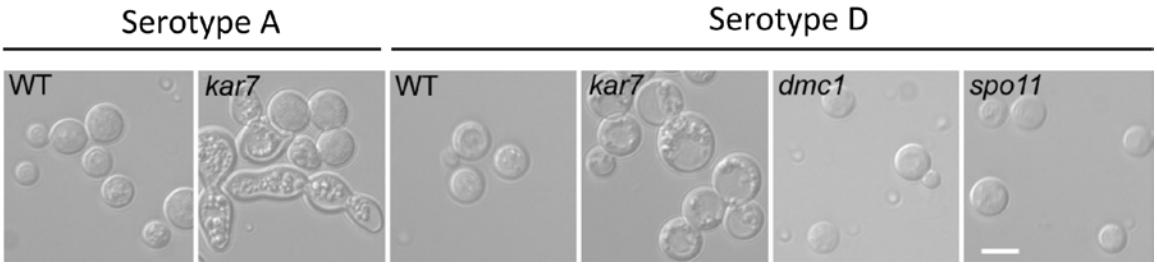
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7