Supplementary Methods

Protein homology searches

A variety of publicly available and commercial databases were used to search for homologues to the *RMI1* gene and protein sequences, including NCBI (http://www.ncbi.nlm.nih.gov); SGD (Saccharomyces Genome Database, (Christie et al., 2004)); Ensembl (http://www.ensembl.org, (Hubbard et al., 2002)) genome assemblies; Celera (http://www.celera.com, (Kerlavage et al., 2002)) human and mouse genome assemblies; DOE Joint Genome Institute fugu genome assembly (http://www.jgi.doe.gov/fugu/index.html); tetraodon (Tetraodon nigroviridis) reads and genome assembly at GENOSCOPE (http://www.genoscope.cns.fr/externe/tetraodon/); and the sea squirt (*Ciona savignyi*) genome at the Center for Genome Research at Whitehead institute (http://www-genome.wi.mit.edu/annotation/ciona/background.html) and at the DOE Joint Genome Institute (http://www.jgi.doe.gov/programs/ciona.htm).

Programs used for homology searches were: BLAST (local generic and on the Paracel Blaster system (Paracel, Inc.), and web implementations), Smith-Waterman algorithm for identifying remote homologues (implemented at Paracel GeneMatcher2), and BLAT (web implementation). GeneMatcher2 was also used for Hidden Markov Model searches. Alignments were produced using ClustalW (Chenna et al., 2003) and ClustalX, and shaded using BOXSHADE. The OB-fold nucleic acid binding domain family is Pfam accession number PF01336.

REFERENCES

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G. and Thompson, J.D. (2003) Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res*, **31**, 3497-3500.

Christie, K.R., Weng, S., Balakrishnan, R., Costanzo, M.C., Dolinski, K., Dwight, S.S., Engel, S.R., Feierbach, B., Fisk, D.G., Hirschman, J.E., Hong, E.L., Issel-Tarver, L., Nash, R., Sethuraman, A., Starr, B., Theesfeld, C.L., Andrada, R., Binkley, G., Dong, Q., Lane, C., Schroeder, M., Botstein, D. and Cherry, J.M. (2004) Saccharomyces Genome Database (SGD) provides tools to identify and analyze sequences from Saccharomyces cerevisiae and related sequences from other organisms. *Nucleic Acids Res*, **32**, D311-314.

Hubbard, T., Barker, D., Birney, E., Cameron, G., Chen, Y., Clark, L., Cox, T., Cuff, J., Curwen, V., Down, T., Durbin, R., Eyras, E., Gilbert, J., Hammond, M., Huminiecki, L., Kasprzyk, A., Lehvaslaiho, H., Lijnzaad, P., Melsopp, C., Mongin, E., Pettett, R., Pocock, M., Potter, S., Rust, A., Schmidt, E., Searle, S., Slater, G., Smith, J., Spooner, W., Stabenau, A., Stalker, J., Stupka, E., Ureta-Vidal, A., Vastrik, I. and Clamp, M. (2002) The Ensembl genome database project. *Nucleic Acids Res*, **30**, 38-41.

Kerlavage, A., Bonazzi, V., di Tommaso, M., Lawrence, C., Li, P., Mayberry, F., Mural, R., Nodell, M., Yandell, M., Zhang, J. and Thomas, P. (2002) The Celera Discovery System. *Nucleic Acids Res*, **30**, 129-136.

Table S1. *Saccharomyces cerevisiae* strains used in this study.

Strain	Genotype	Source
Y5646	*MATα rmi1Δ::natR lyp1Δ can1Δ::MFA1pr-HIS3-MFα1pr-LEU2 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2*	This study
BY4741	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	(Brachmann et al. 1998)
BY4742	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	(Brachmann et al. 1998)
MCY304	BY4742 with *MATa rmi1Δ::kanMX6*	This study
MCY16	*MATα rad53-11-URA3 can1Δ::MFApr-HIS3 his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	(Chang et al. 2002)
MCY352	BY4742 with *SGS1-3HA-LEU2*	This study
MCY312	BY4741 with *RMI1-TAP-HIS3*	This study
MCY353	BY4741 with *MATα SGS1-3HA-LEU2 RMI1-TAP-HIS3*	This study
MCY348	BY4742 with *TOP3-V5-VSV-kanMX6*	I. Stagljar
MCY355	BY4741 with *MET15 TOP3-V5-VSV-kanMX6 RMI1-TAP-HIS3*	This study
MCY356	BY4742 with *LYS2 TOP3-V5-VSV-kanMX6 RMI1-TAP-HIS3 sgs1Δ::kanMX6*	This study
MCY365	BY4741 with *MATα SGS1-3HA-LEU2 TOP3-TAP-HIS3 rmi1Δ::kanMX6*	This study
MCY367	BY4741 with *MATα SGS1-3HA-LEU2 RMI1-TAP-HIS3 top3Δ::kanMX6*	This study
MCY372	BY4741 with *sgs1::kanMX6* (pRS415)	This study
MCY373	BY4741 with *sgs1::kanMX6* (pSM100-HA)	This study
MCY374	BY4741 with *sgs1::kanMX6* (pSM100-hd-HA)	This study
MCY375	MCY356 (pSM100-HA)	This study
MCY376	MCY356 (pSM100-hd-HA)	This study
MCY377	BY4742 with *LYS2 TOP3-V5-VSV-kanMX6 rmi1Δ::natMX6 sgs1Δ::kanMX6* (pSM100-HA)	This study
MCY378	BY4742 with *LYS2 TOP3-V5-VSV-kanMX6 rmi1Δ::natMX6 sgs1Δ::kanMX6* (pSM100-hd-HA)	This study
MCY379	BY4742 with *LYS2 top3Δ::natMX6 RMI1-TAP-HIS3 sgs1Δ::kanMX6* (pSM100-HA)	This study
MCY380	BY4742 with *LYS2 top3Δ::natMX6 RMI1-TAP-HIS3 sgs1Δ::kanMX6* (pSM100-hd-HA)	This study
MCY357	BY4741 (pWJ1344)	This study
MCY358	MCY304 (pWJ1344)	This study
MCY328	BY4741 with *top3Δ::kanMX6*	This study
MCY359	MCY328 (pWJ1344)	This study
MCY360	BY4741 with *sgs1Δ::kanMX6* (pWJ1344)	This study
RDY9	*MATa mfa1::MFA1pr-HIS3 can1Δ::natR leu2ΔEcoRI::URA3-HOcs::leu2ΔBstII leu2Δ0 his3Δ0 ura3Δ0 met15Δ0 lyp1Δ*	This study
RDY10	*MATa sgs1Δ::kanMX6 mfa1::MFA1pr-HIS3 can1Δ::natR leu2ΔEcoRI::URA3-HOcs::leu2ΔBstII leu2Δ0 his3Δ0 ura3Δ0 met15Δ0 lyp1Δ*	This study
RDY14	*MATa rmi1Δ::kanMX6 mfa1::MFA1pr-HIS3 can1Δ::natR leu2ΔEcoRI::URA3-HOcs::leu2ΔBstII leu2Δ0 his3Δ0 ura3Δ0 met15Δ0 lyp1Δ*	This study
RDY15	*MATa top3Δ::kanMX6 mfa1::MFA1pr-HIS3 can1Δ::natR leu2ΔEcoRI::URA3-HOcs::leu2ΔBstII leu2Δ0 his3Δ0 ura3Δ0 met15Δ0 lyp1Δ*	This study
CZY106	*MATa mfa1::MFA1pr-HIS3 hxt13Δ::URA3 his3Δ1 ura3Δ0 lyp1Δ leu2Δ0 met15Δ0*	This study
CZY211	*MATa sgs1Δ::kanMX6 mfa1::MFA1pr-HIS3 hxt13Δ::URA3 his3Δ1 ura3Δ0 lyp1Δ leu2Δ0 met15Δ0*	This study
CZY232	*MATa sgs1Δ::natR mfa1::MFA1pr-HIS3 hxt13Δ::URA3 his3Δ1 ura3Δ0 lyp1Δ leu2Δ0 met15Δ0*	This study

CZY212	*MATa top3Δ::kanMX6 mfa1::MFA1pr-HIS3 hxt13Δ::URA3 his3Δ1 ura3Δ0 lyp1Δ leu2Δ0 met15Δ0*	This study
CZY213	*MATa rmi1Δ::kanMX6 mfa1::MFA1pr-HIS3 hxt13Δ::URA3 his3Δ1 ura3Δ0 lyp1Δ leu2Δ0 met15Δ0*	This study
MCY340	*BY4741 with can1Δ::MFA1-HIS3 rmi1Δ::natMX6*	This study
MCY323	*BY4741 with lyp1Δ rmi1Δ::natMX6 sgs1Δ::kanMX6*	This study
MCY335	*BY4741 with sgs1Δ::kanMX6 top3Δ::natMX6*	This study
MCY345	*BY4741 with sgs1Δ::kanMX6 top3Δ::kanMX6 rmi1Δ::natMX6*	This study
MCY297	*MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 MET15/met15Δ0 LYS2/lys2Δ0*	This study
MCY370	*MATa/MATα rmi1Δ::kanMX6/rmi1Δ::natMX6 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 LYS2/lys2Δ0 LYP1/lyp1Δ0*	This study
GBY635	*SGS1-3HA-LEU2 TOP3-V5-VSV-kanMX6 RMI1-TAP-HIS3 leu2Δ0 his3Δ1 ura3Δ0*	This study

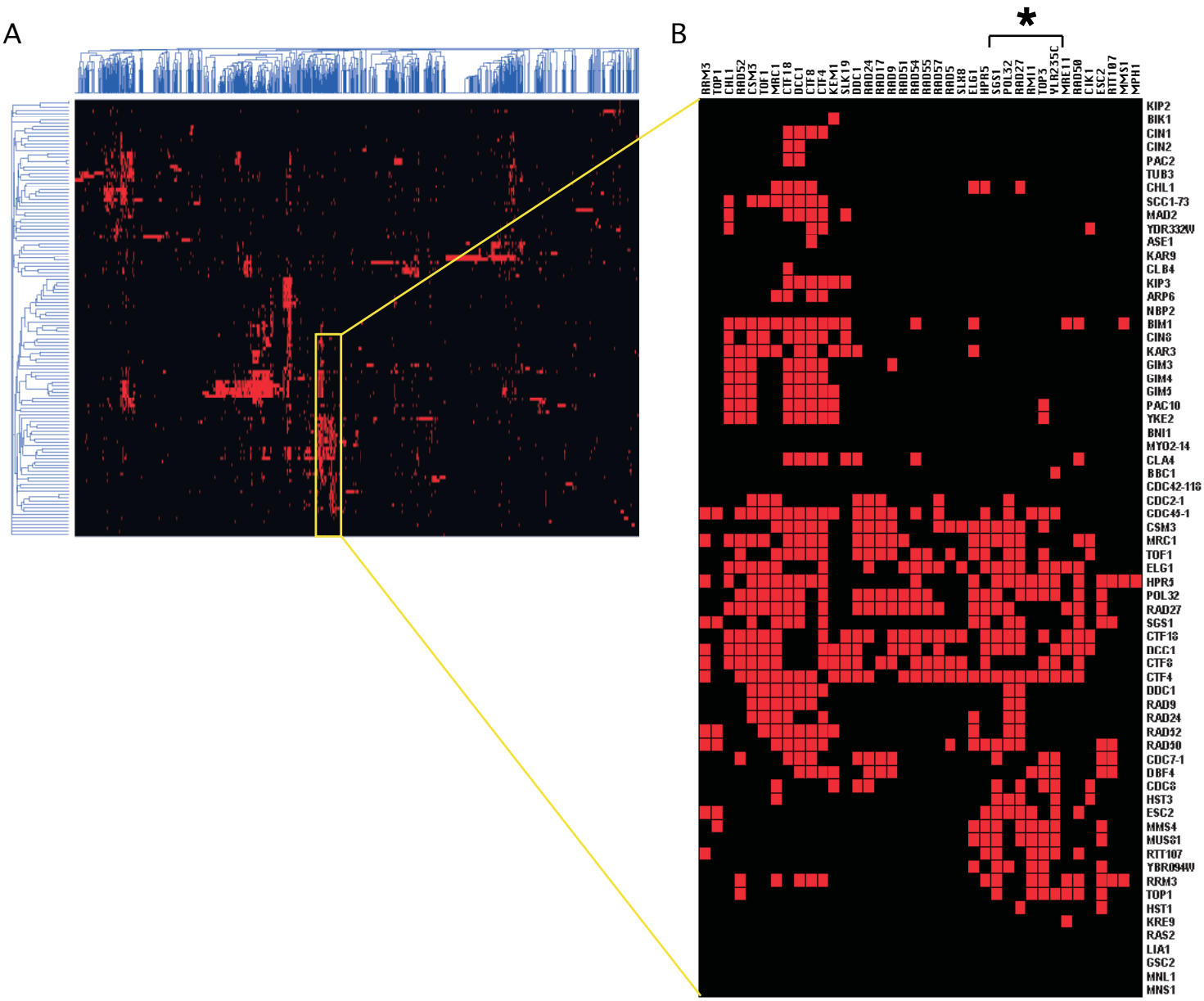

Figure S1. Two-dimensional hierarchical clustering of synthetic genetic interactions determined by SGA analysis (Tong et al., 2004). (*A*) Synthetic genetic interactions are represented as red lines. Rows, 132 query genes; columns, 1007 array genes. The cluster trees organize query (y-axis) and array genes (x-axis) that show similar patterns of genetic interactions. (*B*) The relevant section (yellow outline in *A*) is expanded to allow visualization of the *RMI1/SGS1/TOP3* array gene cluster. Synthetic genetic interactions are represented as red squares.

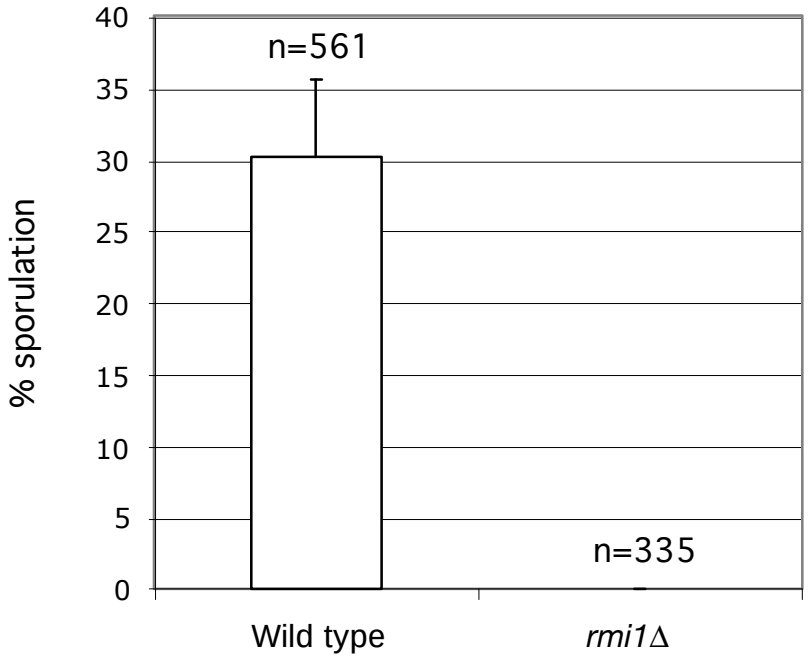

Figure S2. *rmi1Δ/rmi1Δ* diploids are sporulation defective. Sporulation efficiency was determined following 5 days in sporulation medium at 30°C. The value presented for each strain is the average of three trials.

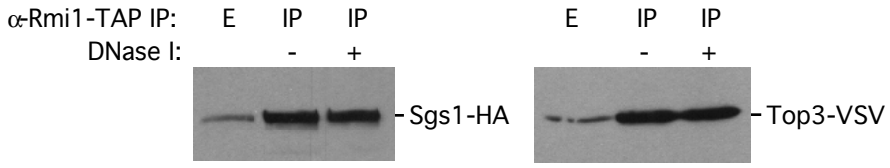

Figure S3. The Rmi1-Sgs1 and Rmi1-Top3 interactions are not mediated by DNA. Extract from a yeast strain expressing Sgs1-HA, Top3-VSV, and Rmi1-TAP was immunoprecipitated with IgG agarose following incubation for 15 minutes in the presence (+) or absence (-) of 10U of DNase I and 10 mM $MgCl_2$. Ten percent of the input extract (E) and the entire immunoprecipitate (IP) were fractionated by SDS-PAGE. Immunoblots were probed with anti-HA antibody to detect Sgs1 and with anti-VSV antibody to detect Top3.

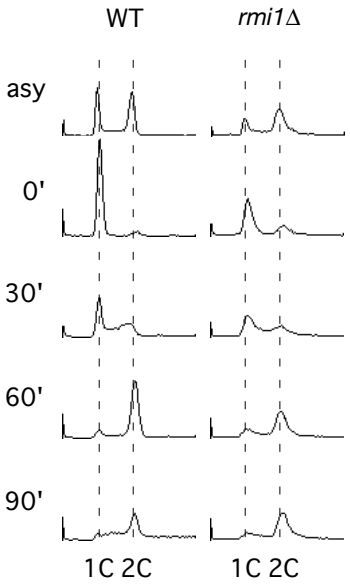

Figure S4. Wild-type or *rmi1*Δ cells were arrested in G1 (t = 0) and released synchronously into fresh YPD medium. Samples were removed at the indicated times and analyzed by flow cytometry. The positions of cells with 1C and 2C DNA contents are indicated.

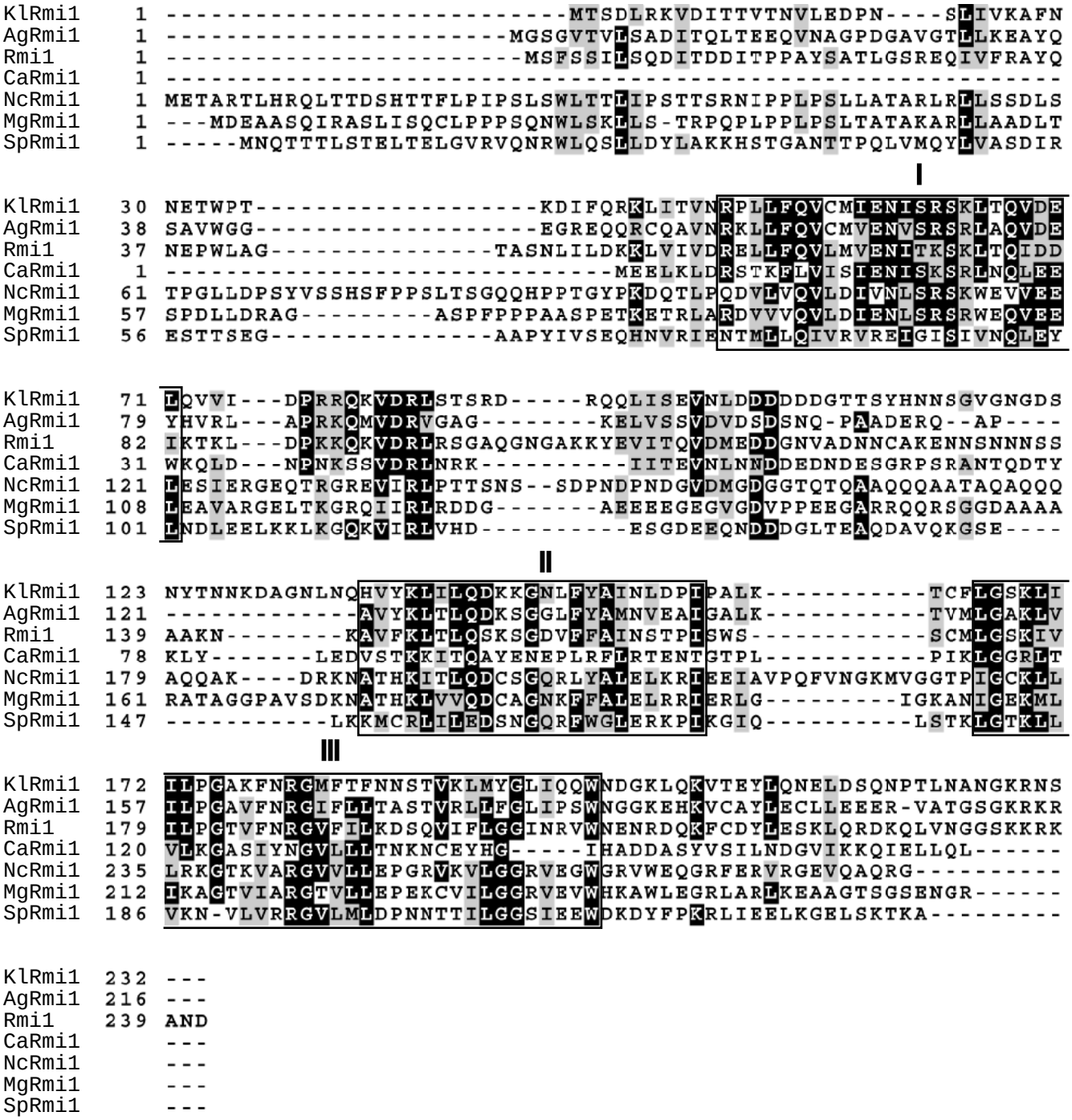

Figure S5. ClustalW alignment of yeast Rmi1 homologues. *Saccharomyces cerevisiae* Rmi1 (ScRmi1, Accession number NP_015301) is aligned with Rmi1 homologues from *Kluveromyces* lactis (KlRmi1, XP_453604), *Ashbya gossypii* (AgRmi1, AAS53829), *Candida albicans* (CaRmi1, EAK98148), *Neurospora crassa* (NcRmi1, EAA29355), *Magnaporthe grisea* (MgRmi1, EAA51673), and *Schizosaccharomyces pombe* (SpRmi1, CAA93226). Identical amino acids are shaded black and similar amino acids are shaded grey, at positions where the identity or similarity is shared by at least four of the homologues. Regions of extensive sequence similarity are designated I, II, and III.

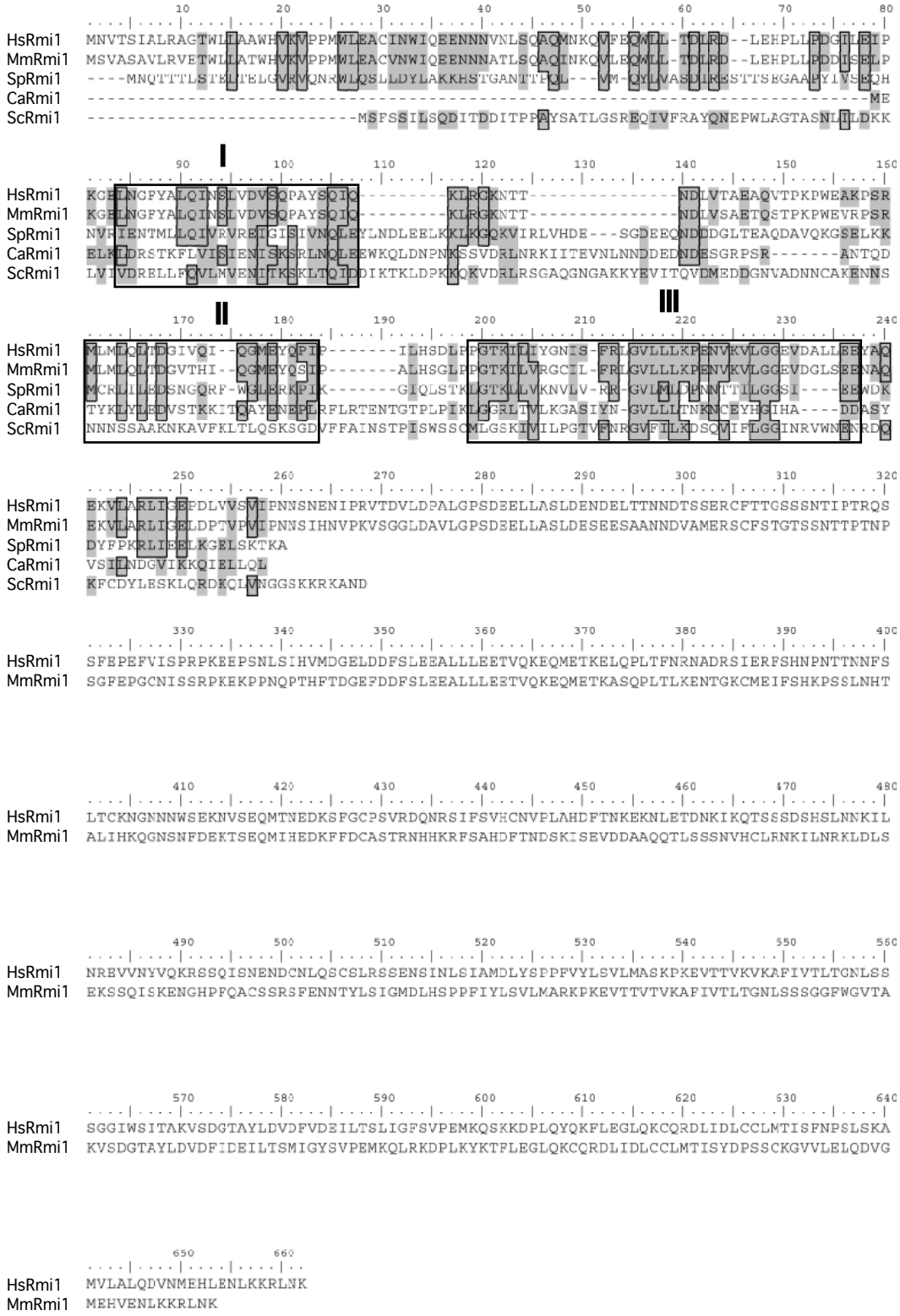

Figure S6. Alignment of Rmi1 homologues. *Saccharomyces cerevisiae* Rmi1 (ScRmi1, Accession number NP_015301) is aligned with Rmi1 homologues from *Candida albicans* (CaRmi1, EAK98148), *Schizosaccharomyces pombe* (SpRmi1, CAA93226), human (HsRmi1, NP_079221), and mouse (MmRmi1, NP_0833180. Identical amino acids are boxed in black and similar amino acids are shaded grey, at positions where the identity or similarity is shared by at least three of the homologues. Regions of extensive sequence similarity from the yeast analysis are designated I, II, and III.