SUPPLEMENTAL MATERIAL

Genetic interactions of hypomorphic mutations in the m^7G cap binding pocket of yeast nuclear cap binding complex: an essential role for Cbc2 in meiosis via splicing of *MER3* pre-mRNA

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Figures S1, S2, S3, S4 and S5.

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Yeast    MSLEEFDEVKYDHSTKRLDTPSRYLLRKARRNPNGLQELRESMKSSTIYVGNLSFYTSEEQIYE  64
Human        MSGGLLKALRSDSYVELSQYRDQHFRGD-NEEQE-KLLKKSCTLYVGNLSFYTTEEQIYE  58

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Yeast    LFSKCGTIKRIIMGLDRFKFTPCGFCFIIYSCPDEALNALKYLSDTKLDEKTITIDLDPGFEDG  128
Human    LFSKSGDIKKIIMGLDKMKKTACGFCFVEYYSRADAENAMRYINGTRLDDRIIRTDWDAGFKEG  122

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Yeast    RQFGRGKSGGQVSDELRFDFDASRGGFA 156
Human    RQYGRGRSGGQVRDEYRQDYDAGRGGYG 150
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Figure S1. **Primary structure alignment of yeast Cbc2 and human CBP20**. Positions of amino acid side chain identity/similarity are denoted by ●. Conserved amino acids of the cap binding pocket of human CBP20 that have been subjected to alanine mutation in yeast Cbc2 are highlighted in cyan.

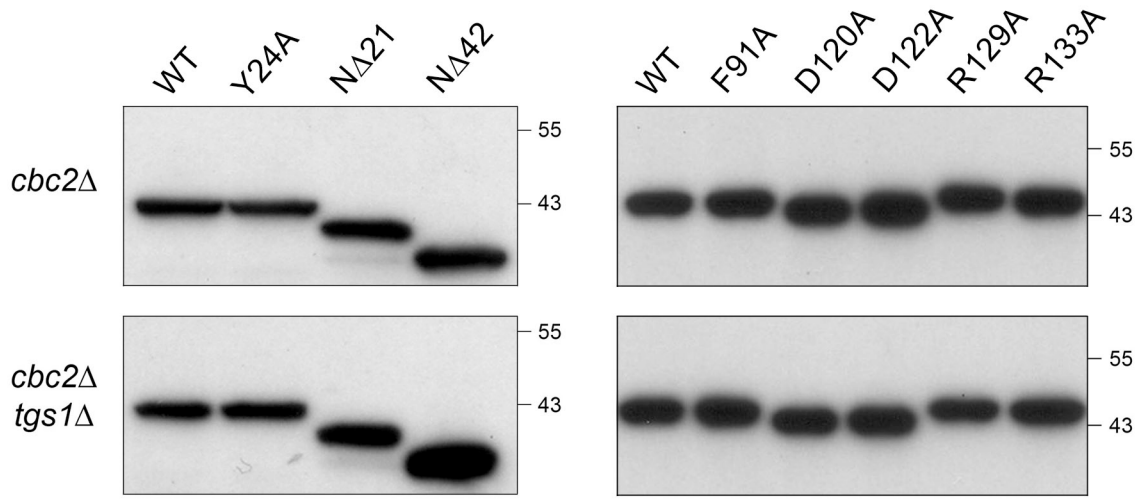

Figure S2. **Western blot analysis of Cbc2 levels**. Yeast *cbc2*Δ or *cbc2*Δ *tgs1*Δ cells harboring *CEN LEU2* plasmids for expression of wild-type Cbc2-TAP or the Cbc2-TAP mutant variants as specified were maintained in exponential growth in SD-Leu medium at 30°C. Aliquots of the cultures (containing equivalent A_{600} units) were harvested and whole-cell extracts were resolved by SDS-PAGE. The polypeptides were transferred to a membrane and probed with anti-TAP antibodies (Thermo Scientific). The positions and sizes (kDa) of marker proteins are indicated at *right*.

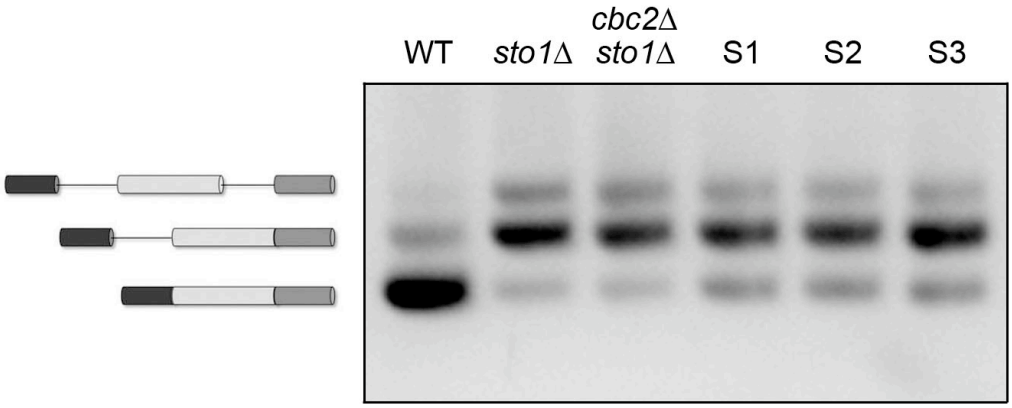

Figure S3. ***SUS1* mRNA splicing in *sto1*Δ *cbc2*Δ suppressors strains**. RNAs isolated from *STO1 CBC2* (WT), *sto1*Δ, *sto1*Δ *cbc2*Δ, and "suppressed" S1, S2 and S3 *sto1*Δ *cbc2*Δ cells were reverse transcribed with an oligo(dT) primer and the cDNAs were PCR-amplified with primers in the first and third exons of chromosomal *SUS1* gene. The PCR products were resolved by native agarose gel electrophoresis and visualized by staining with ethidium bromide (a negative image is shown). The positions of the RT-PCR products of unspliced, partially spliced, and fully spliced *SUS1* transcripts are indicated at *left.*

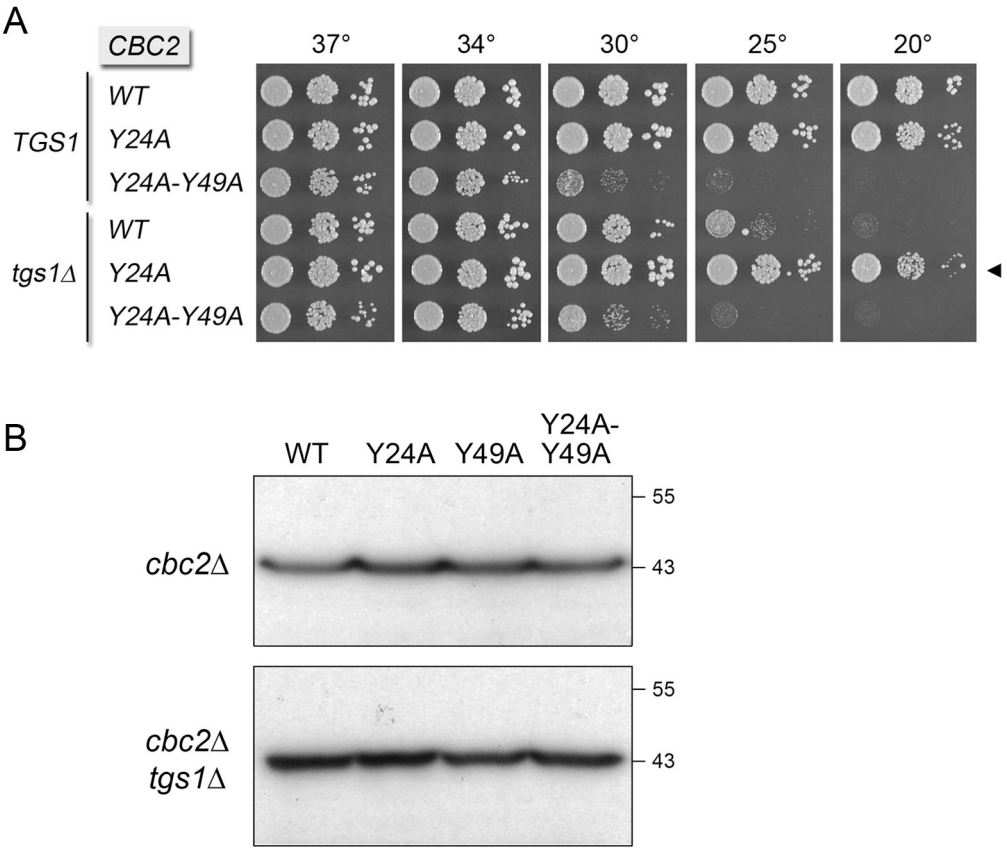

Figure S4. ***cbc2-(Y24A-Y49A)* phenocopies *cbc2Δ***. (A) The chromosomal *CBC2* locus of *TGS1* or *tgs1*Δ cells was replaced by the *CBC2-TAP* wild-type, *Y24A* or *Y24A-Y49A* alleles as specified. The integrated alleles express Cbc2 proteins with a C-terminal TAP tag and are marked by a hygromycin-resistance gene. Log-phase cultures of cells grown in YPD medium at 34°C were adjusted to A_{600} of 0.1 and aliquots (3 µl) of serial 10-fold dilutions were spotted to YPD agar. The plates were photographed after 2 d (30, 34 and 37°C), 3 d (25°C) or 5 d (20°C). Suppression of the *tgs1*Δ *cs* phenotype is denoted by the ◀ symbol at right. (B) Western blotting. Whole-cell extracts from equivalent A_{600} units of *cbc2*Δ or *cbc2*Δ *tgs1*Δ cells harboring plasmids for expression of Cbc2-TAP proteins as specified (above the lanes) were resolved by SDS-PAGE, transferred to a membrane and probed with anti-TAP antibodies. The positions and sizes (kDa) of marker proteins are indicated at *right*.

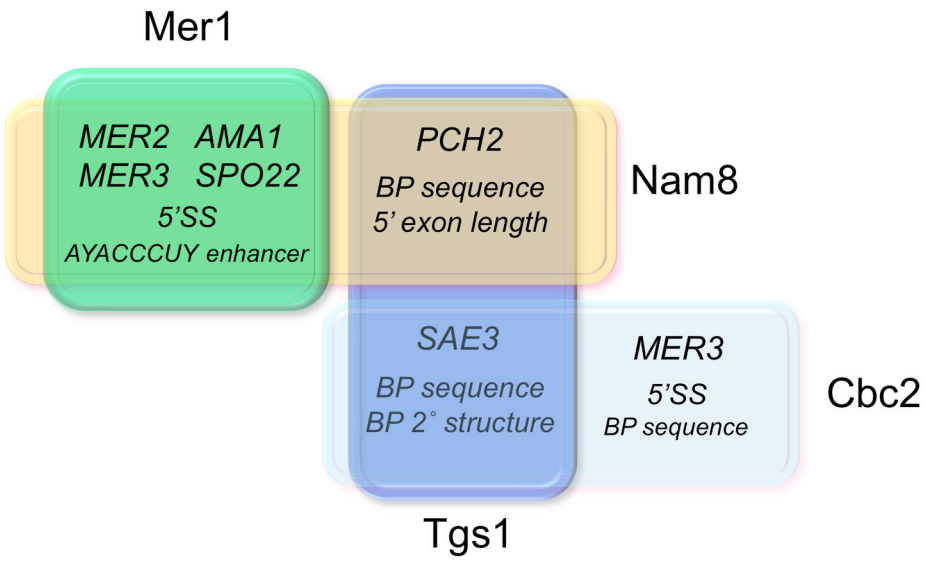

Figure S5. **Four genetically distinct meiotic splicing regulons**. The Venn-like diagram illustrates the meiotic pre-mRNAs that comprise the Mer1/Nam8-codependent, Nam8/Tgs1-codependent, Tgs1-dependent, and Cbc2-dependent splicing regulons. The pre-mRNA features that govern each regulon are listed below the target genes. The features listed for *SAE3* pertain to the Tgs1-dependence of *SAE3* splicing. (The *SAE3* determinants of *cbc2-NΔ42* sensitivity are uncharted).