SUPPLEMENTARY INFORMATION

Cwc21p promotes the second step conformation of the spliceosome and modulates 3' splice site selection.

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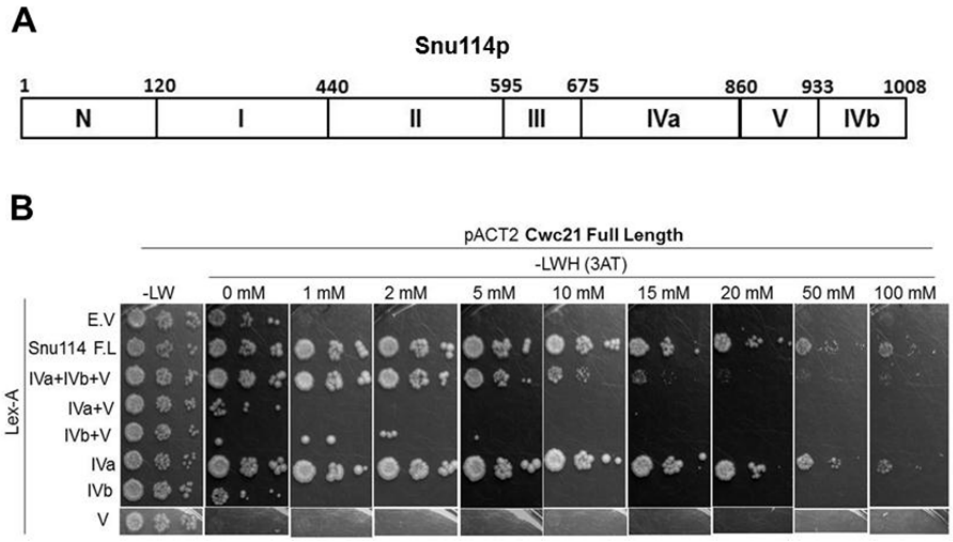
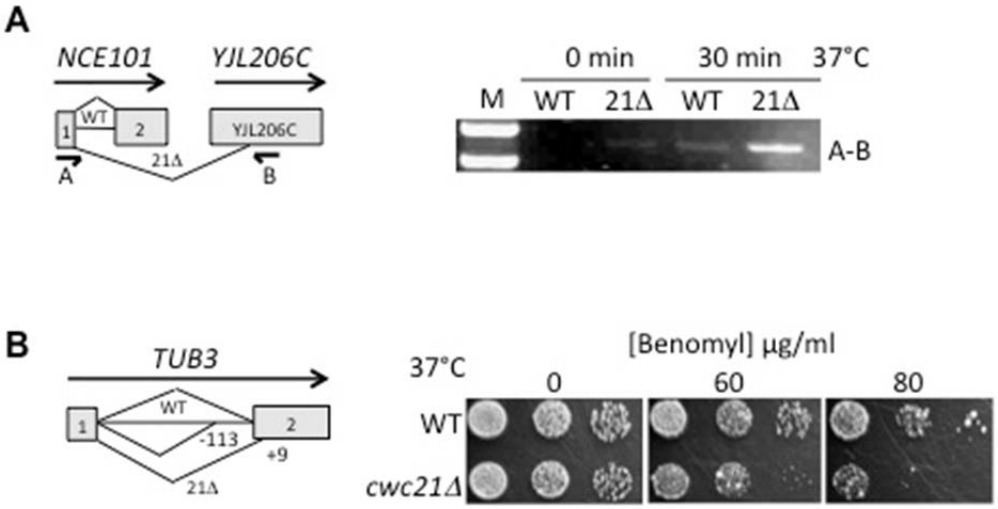
Supplementary Table S1. List of yeast strains and plasmids.

Strain	**Genotype**	**Source/reference**
BY4741 (WT)	*MAT**a**, his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	Euroscarf
Y04316-cwc21Δ	*MAT**a**, his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0, cwc21Δ::KanMX4*	Euroscarf
L40ΔG	*MAT**a** his3Δ200 trp1-901 leu2-3,112 ade2 LYS2::(4lexAop-HIS3) URA3::(8lexAop-lacZ) Δgal4::KAN*	(1)
yJU75-8+	*MAT**a**, ade2 cup1Δ::ura3 his3 leu2 lys2 prp8**Δ**::LYS2 trp1, pMK8-1 (PRP8-wt TRP CEN ARS)*	(2)
yJU75-8-WT-21Δ	*MAT**a**, ade2 cup1Δ::ura3 his3 leu2 lys2 prp8**Δ**::LYS2 trp1, pMK8-1 (PRP8-wt TRP CEN ARS), cwc21Δ::HPH-NT1*	This work
U5KO	*MAT**a**; ura3-52; trp1Δ63; leu2Δ1; his3Δ200; GAL2; snr7Δ::kanMX6; pRS416-U5*	(3)
U5KO-21Δ	*MAT**a**; ura3-52; trp1Δ63; leu2Δ1; his3Δ200; GAL2; snr7Δ::kanMX6; pRS416-U5, cwc21Δ::HPH-NT1*	This work
PRP16KO	*Mat**a**, prp16Δ:Hph-NT1, leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*, pRS416-P_{met25}-PRP16,	(4)
PRP16KO -21Δ	*Mat**a**, prp16Δ:Hph-NT1, leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*, pRS416-P_{met25}-PRP16, *cwc21Δ::NAT-NT2*	This work
yTB23	*MATa, lys2Δ, his3Δ, leu2Δ, ura3Δ, met15, snu114::KanMX, pTB1*	(5)
yRG23	*MATa, lys2Δ, his3Δ, leu2Δ, ura3Δ, met15, snu114::KanMX, pTB1, cwc21::HPH-NT1*	This work
Plasmid	**Description**	**Source/reference**
pFA6a-NatNT2	Nourseothricin knockout vector	PCR toolbox, Euroscarf
pFA6a-HphNT1	Hygromycin knockout vector	PCR toolbox, Euroscarf
p*PRP8* series	pRS314-*PRP8-WT, TRP, CEN, ARS*	(2)
	pRS314-*prp8-R1753K, TRP, CEN, ARS*	(2)
	pRS314-*prp8-162, TRP, CEN, ARS*	(2)
	pRS314-*prp8-syf77, TRP, CEN, ARS*	(2)
	pRS314-*prp8-161, TRP, CEN, ARS*	(2)
	pRS314-*prp8-156, TRP, CEN, ARS*	(2)
p*SNU114* series	pRS315-*SNU114, LEU2, ARS, CEN*	(5)
	pRS315-*snu114- 40, LEU2, ARS, CEN*	(5)
pBTM116	2μ, *TRP1*, lexA(1-202)-BD	(6)
pBTM116-Snu114	2μ, *TRP1*, lexA(1-202)-BD-Snu114 (Full length)	This work
pBTM116-Snu114 – IVa+IVb+V	2μ, *TRP1*, lexA(1-202)-BD-Snu114 (domains IVa (aa692-aa860) +IVb+V	This work
pBTM116-Snu114 – IVa+V	2μ, *TRP1*, lexA(1-202)-BD-Snu114 (domains IVa (aa692-aa860) +V	This work
pBTM116-Snu114 – IVb+V	2μ, *TRP1*, lexA(1-202)-BD-Snu114 (domains (IVb+V)	This work
pBTM116-Snu114 –IVa	2μ, *TRP1*, lexA(1-202)-BD-Snu114 domains IVa	This work
pBTM116-Snu114 –IVb	2μ, *TRP1*, lexA(1-202)-BD-Snu114 domains IVb	This work
pBTM116-Snu114 –V	2μ, *TRP1*, lexA(1-202)-BD-Snu114 domains V	This work
pACTII	2μ, LEU2, Gal4-AD	(7)
pACTII-Cwc21	2μ, *LEU2*, Gal4-AD-*CWC21* (full length)	(8)
pU5 snRNA-series	pRS416-*U5-WT, URA3, CEN, ARS*	(3)
	pRS314-*U5-1, TRP1, CEN, ARS*	(3)
	pRS314-*U5-2, TRP1, CEN, ARS*	(3)
	pRS314-*U5-3, TRP1, CEN, ARS*	(3)
	pRS314-*U5-Δ94/95, TRP1, CEN, ARS*	(3)
	pRS314-*U5-Ins 1U 94/95, TRP1, CEN, ARS*	(3)
	pRS314-*U5-Δ96/97, TRP1, CEN, ARS*	(3)
	pRS314-*U5-ΔG93, TRP1, CEN, ARS*	(3)
	pRS314-*U5-Ins 1U 93/94, TRP1, CEN, ARS*	(3)
pPrp16 series	pRS416-P_{met25}-*PRP16*, *URA3, CEN, ARS*	(4)
	pRS314-*PRP16*, *TRP1, CEN, ARS*	(4)
	pRS314-*prp16-R686I*, *TRP1, CEN, ARS*	(4)

	pRS314-*prp16-L335F*, *TRP1, CEN, ARS*	This work
	pRS314-*prp16-K379R*, *TRP1, CEN, ARS*	This work
	pRS314-*prp16-Q685H*, *TRP1, CEN, ARS*	(4)
	pRS314-*prp16-302*, *TRP1, CEN, ARS*	(4)
	pRS314-*prp16-R686Q*, *TRP1, CEN, ARS*	This work
	pRS314-*prp16-Y386D*, *TRP1, CEN, ARS*	This work
	pRS314-*prp16-C862Y*, *TRP1, CEN, ARS*	This work
	pRS314-*prp16-201*, *TRP1, CEN, ARS*	(4)
	pRS314-*prp16-202*, *TRP1, CEN, ARS*	(4)
Copper reporters	pMA-WT	(2)
	pMA-A3C	(2)
	pMA-BS-G	(2)
	pMA-BS-C	(2)
	pMM-WT	(2)
	pMM-3'UuG	(2)



Supplementary Figure S1. Yeast two-hybrid interaction of Snu114p and Cwc21p. **(A)** Domain structure of Snu114p. **(B)** Interaction of full-length Cwc21p as a pACT2 fusion (pACT2-Cwc21) with different Snu114p LexA-DNA binding domain constructs on drop-out (lacking leucine (L), tryprophan (W) and/or histidine (H)) plates with different concentrations of 3-aminotriazole (3-AT). The cells were diluted to OD = 0.3 and two tenfold serial dilutions, spotted and grown at 30ºC for at least 2 days. F.L is full length; roman numeral indicate the domains of Snu114p present, as in Figure 1A.



Supplementary Figure S2. Examples of unusual splicing events in *cwc21Δ*.
(A) RT-PCR validation of splicing of *NCE101* exon 1 to a non-canonical 3'SS in YJL206C. Left: schematic of the genes as in Fig. 7. Arrows A and B indicate the positions of PCR primers. Right: agarose gel electrophoresis of RT-PCR products from WT and *cwc21Δ* (*21Δ*) cells grown at 30°C (0 min) or shifted to 37°C for 30 min.
(B) Left: schematic of *TUB3* alternative splicing, showing the 3'SS detected by microarray analysis (lower; 9 bases downstream of the canonical 3'SS) and an additional 3'SS identified by cDNA sequencing (113 bases upstream of the canonical 3'SS), Right: consistent with a defect in splicing *TUB3* transcripts, sensitivity to the microtubule toxin benomyl is increased in *cwc21Δ* relative to WT at 37°C.

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