SUPPLEMENTARY INFORMATION FOR SELA ET AL., "Neddylation and CAND1 independently stimulate SCF ubiquitin ligase activity in *Candida albicans*"

Supplementary methods

Plasmids and strains construction

Ca*RUB1* (orf19.330.1) deletion plasmids KB1477-1, -2 were generated by cloning the CaRUB1 -800 to -1 and +300 to +740 sequences (relative to the translation start site) into KB985, KB986 (1), carrying the hisG-Ca*URA3*-hisG fragment in two orientations. The Ca*JAB1* (orf19.3371) deletion plasmids 1474-1, -2 were similarly constructed, using sequences -610 to -10 and +621 to +1470 of Ca*JAB1*. The Ca*TIP120* (orf19.6729) open reading frame was reassessed (see Fig. S1 below) and found to start 139 nt beyond the described start site, i.e. at Chr.3 position 1735569 of assembly 21 of the *C. albicans* genome; the following coordinates are relative to the new start site. The Ca*TIP120* deletion plasmids were constructed in two steps: first, the Ca*TIP120* genomic region (-867 to +3725) was cloned as a SacI-SalI fragment into pBSII-SK+ (Stratagene). The resulting plasmid KB1640 was then digested with BclI, which cuts at positions -67 and +3007 of the 3585 nt-long Ca*TIP120* ORF. The hisG-Ca*URA3*-hisG blaster fragment (3) was introduced in BclI-digested KB1640, to generate KB1657-1, -2 (both orientations).The long version of Ca*TIP120* open reading frame (position -139 to +3725) was constructed by cloning the BamH1-Sal1 PCR fragment into p415GAL1 (8) to generate KB1586. CaTip120 fused to 6xMyc at the C-terminus was created by replacing *SOL1* in plasmid KB1798 (described below) with Ca*TIP120* (position -1000 to +3581). The Ca*TIP120* overexpression plasmid KB1958 was created by transferring the Ca*TIP120*(-1 to +3581)-6xMyc construct as a SmaI-SalI fragment into p415GAL1. KB1960 was generated by cloning Ca*TIP120*(-1 to +3725) as a BamHI-SalI PCR fragment into KB1947, a vector based on p415-GAL1 (8) containing a Myc epitope tag sequence in the polylinker (our collection). KB1962 was generated by transferring a Xba1-Kpn1 Myc-Ca*TIP120*(-1 to +3726) fragment into KB1317 (1) digested with SpeI-KpnI. KB2029 was constructed by cloning Not1-Kpn1 Ca*TIP120*-6xMyc fragment (position -1000 to +3726) into pBES116 (2). A *Candida albicans* construct for C-terminal 3xHA epitope-tagging was generated by fusing the Ca*URA3* BamHI-PmeI fragment of pFA-URA3-MAL2p (5) to BglII-PmeI-digested pFA6a-3HA-TRP1 (7), to generate pFA6a-3HA-CaURA3 (KB1570). KB1734 contains the Ca*CDC53* region, cloned

as a -910 - +2500 SacI-XhoI PCR fragment into p413MET25 (8). KB1735, KB1847 were generated by site-directed mutagenesis of KB1734 to introduce mutations [Lys699, 701, 702 to Arg] to generate Ca*cdc53*^{RRR}, and [Arg462 to Cys] to generate Ca*cdc53-1*, respectively. The PstI-XhoI fragments of KB1734, KB1735, 1847, extending from positions +135 to +2500 of Ca*CDC53*, were cloned into pBES116 (2) to generate KB1782, KB1783 and KB1848, respectively. Plasmid KB1869 was generated by cloning Ca*CDC53* (positions -889 to +2500) as a SpeI-XhoI fragment into pBES116 (2) digested with SpeI-SalI. Plasmids KB1192 (Ca*MAL2p*-Ca*GCN4*-6xMyc) and KB1578 (Ca*MAL2p-SOL1*-6xMyc) were described before (1, 4). Plasmids KB1697 (Ca*MAL2p*-Ca*CLN3*-6xMyc) and KB1698 (Ca*MAL2p-CCN1*-6xMyc) were constructed by substituting the *TEC1* open reading frame of KB1610 (1) with either a Ca*CLN3* PstI-HindIII or a CCN1 PstI-ApaI PCR product. For KB1799 (Ca*GRR1*-6xMyc), first the *SOL1*-6xMyc PstI-KpnI fragment of KB1578 (1) was cloned into pBES116 (2) to generate KB1798, and then a Ca*GRR1* NotI-ApaI PCR product (-1500 to +2340) was substituted for *SOL1* in KB1798. KB1869 is a Ca*CDC53* (-889 to +2500) SpeI-XhoI PCR fragment cloned into pBES116 digested with SpeI and SalI. KB1897 (Ca*CUP1p*-Myc-Ca*CDC53*) consists in a Ca*CDC53* BglII-XhoI fragment (+1 to +2500) cloned into KB1321 (1) digested BglII-SalI. To generate KB2152 (*MAL2*p-Ca*CLN3*-3xHA), an EcoRV-HindIII PCR fragment of the Ca*CLN3* open reading frame was cloned into KB2083, carrying the 3xHA sequence under the *MAL2* promoter of pBES118 (2).

The *S. cerevisiae* strains were all derived from the EUROSCARF BY4741 (**a** *ura3Δ0 met15Δ0 leu2Δ0 his3Δ1*) and BY4742 (α *ura3Δ0 lys2Δ0 leu2Δ0 his3Δ1*) background strains, except for the *cdc53-1* mutant (MTY740, obtained from M. Tyers), which is in the W303 background. Strains KY1293 (BY4742 *CDC53p-CaCDC53 HIS3*) and KY1294 (BY4742 *CDC53p-CaCDC53*^{RRR} *HIS3*), carrying the Ca*CDC53* open reading frame integrated instead of the *CDC53* open reading frame, were built by oligonucleotide-directed site-specific recombination of a Ca*CDC53 HIS3* fragment into the *CDC53* locus, using plasmids KB1734 and KB1735, respectively, as templates. KY1352 (BY4742 *CDC53p*-FLAG-Ca*CDC53 HIS3*) was constructed similarly, but using a FLAG-tag-encoding oligonucleotide for amplification and integration (primer sequence 5’-CAAAGAATAACAACAAAATCGCAAGCAGCAAAGCATGGACTACAAGGACGAC

GACGACAAGTCATCTACTCTTCCACCA). KY1310 (*ura3Δ0 lys2Δ0 leu2Δ0 his3Δ1 rub1Δ::KANr CDC53p-CaCDC53 HIS3*) is the product of a cross between KY1293 and BY4741 *rub1Δ::KANr* (EUROSCARF). KY1309 *(ura3Δ0 met15Δ0 leu2Δ0 his3Δ1 jab1Δ::KANr CDC53p-CaCDC53 HIS3*) is the product of a cross between KY1293 and BY4741 *jab1Δ::KANr* (EUROSCARF). KY1306 (*ura3Δ0 lys2Δ0 leu2Δ0 his3Δ1 dcn1Δ::KANr CDC53p-CaCDC53 HIS3*) is the product of a cross between KY1293 and BY4741 *dcn1Δ::KANr* (EUROSCARF).

C. albicans deletions of Ca*RUB1,* Ca*JAB1* and Ca*TIP120* were achieved by sequential deletion of both alleles using plasmid sets KB1477, KB1474, KB1657 respectively. C-terminal epitope tagging of Ca*CDC53* was achieved by site-specific targeting of a 3xHA-Ca*URA3* PCR product amplified from KB1570, using additional 65nt homology at each end to the Ca*CDC53* 3' end. The Ca*cdc53* knock-in alleles were generated by deleting one copy of Ca*CDC53* in strain SN78, using the described method (9). The second allele was generated by integration of plasmids KB1782, KB1783 and KB1848 - digested with EcoRV that cuts within the Ca*CDC53* coding sequence - at the remaining Ca*CDC53* allele, generating KC326, KC327, KC362. These strains have one copy of the plasmid-borne allele under the chromosomal Ca*CDC53* promoter, followed by Ca*URA3* and vector sequences, and by one promoterless N-terminal truncation allele, Ca*cdc53ΔN*. KC363 was generated by 5-fluoroorotic acid selection of KC362 for $ura3^-$ clones that have "popped out" the cointegrate by homologous recombination, and screening of the resulting clones for those that have retained the mutant allele.

Supplementary figures

Fig. S1. Assignment of the translation start site of CaTip120 (orf19.6729). A. Western blot of 6xMyc-tagged orf19.6729 expressed from the *GAL1* promoter in *S. cerevisiae*. 1: *C. albicans* no-tag control; 2. orf19.6729-6xMyc expressed in *C. albicans* under its native promoter; 3, 4: orf19.6729-6xMyc expressed in *S. cerevisiae* under the Sc*GAL1* promoter. 5 *S. cerevisiae* no-tag control. B. Alignment by MAFFT (6) of the N-terminal region of several fungal Tip120 homologs, as well as of human CAND1. Arrow indicates the site of presumed actual start of CaTip120 translation.

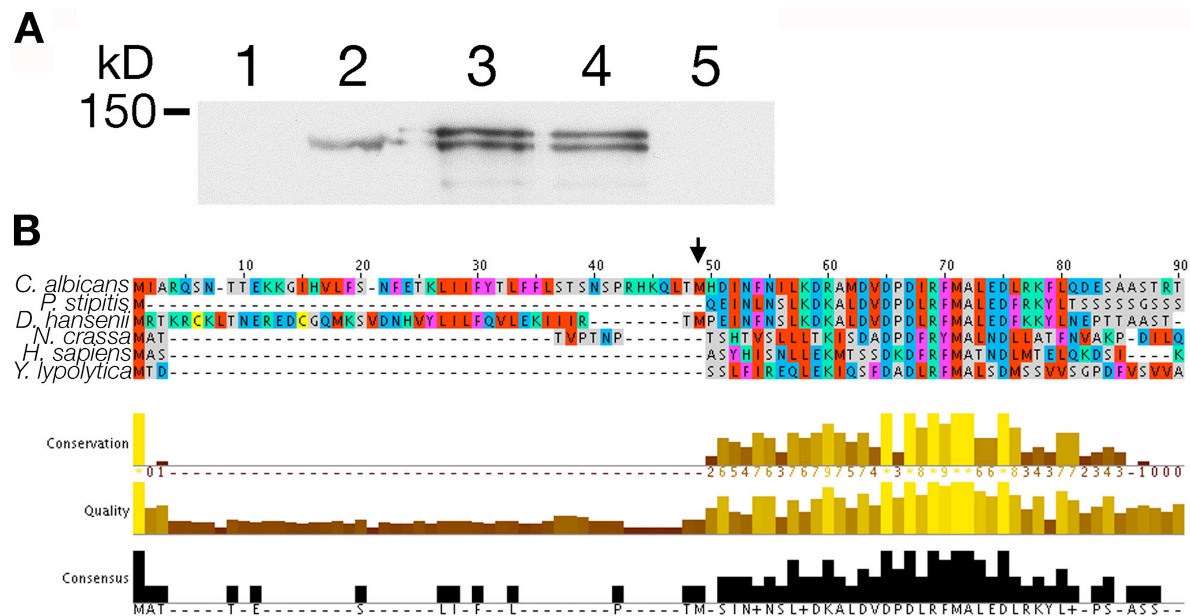


Fig. S2. CaCdc53 is specifically sensitive to CaTip120. 5-fold dilutions of overnight cultures of the indicated strains, transformed with either vector plasmid or with plasmid KB1586 (*GAL1p-CaTIP120*), were spotted on an SC-LEU, 2% galactose plate, and the plate was incubated for 3 days at 30°C.

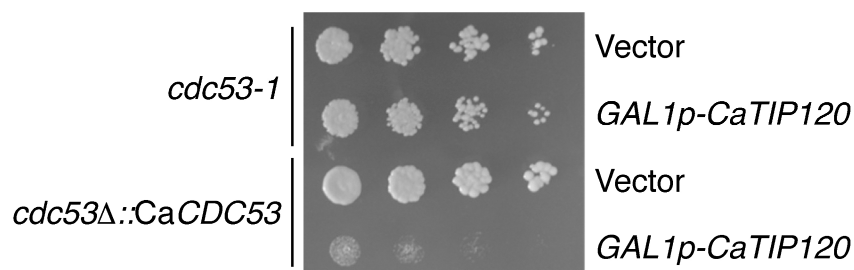


Fig. S3. Morphology of cell lawns (left column) and microscopic cell morphology (right column) of indicated strains. For cell lawns, 500 cells were spotted on YPD agar and the plates were incubated for 2 days at 30°C. For microscopic morphology, cells were grown to mid-log phase (O.D. 600 of 1-2) and photographed with a 10X objective. The strains, uniformly in KC274 background and all URA+, are KC471, KC326, KC327, KC395, KC424 and KC427, respectively.

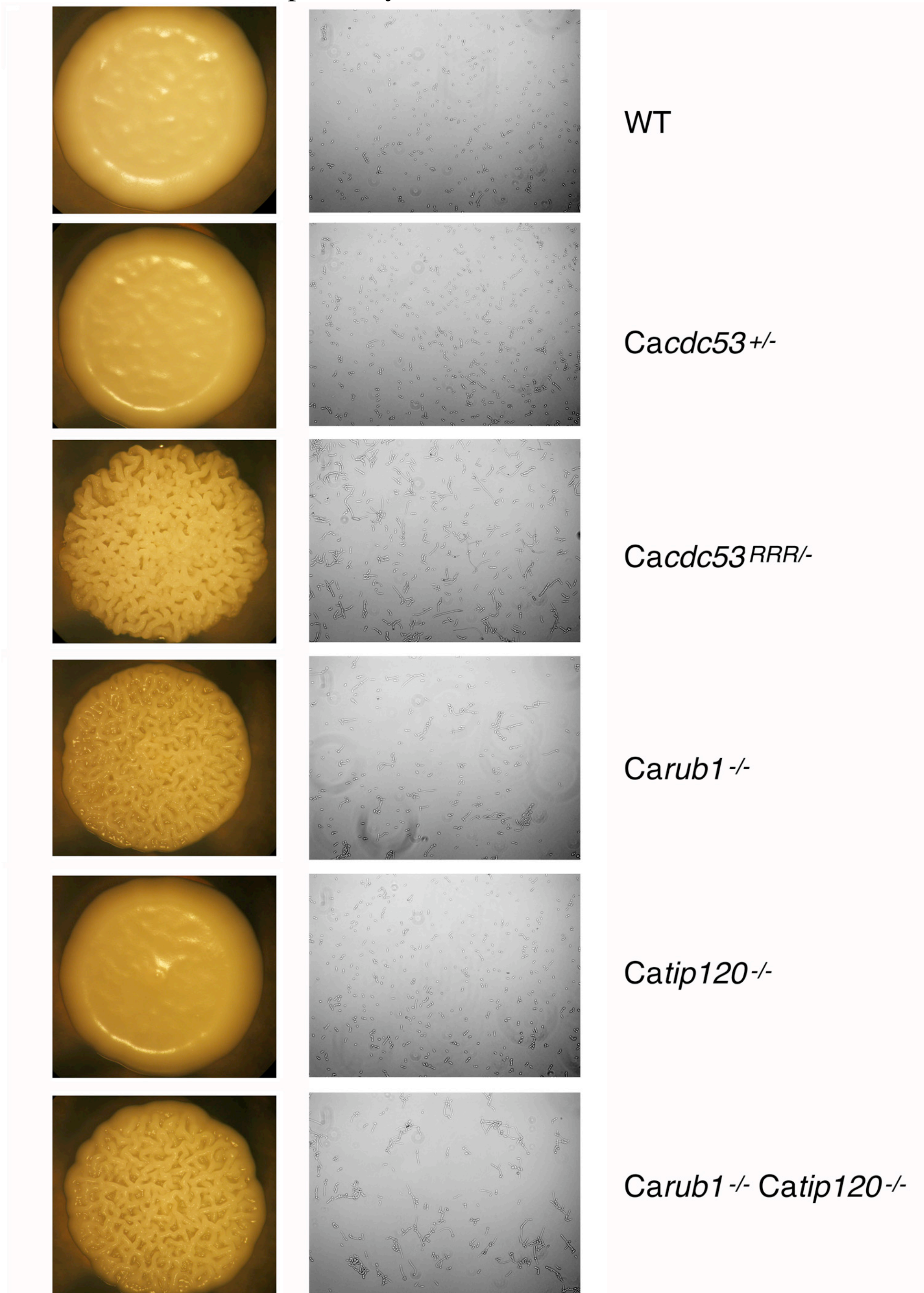


Fig. S4. Complementation of the *S. cerevisiae cdc53Δ* allele with Myc-CaCdc53 (plasmid KB1824) or CaCdc53-3xHA (plasmid KB1828) under the *MET25* promoter , vs. vector plasmid. A *cdc53Δ* deletion strain, kept alive with a *CDC53 URA3* 2μ plasmid (strain KY484) was transformed with the indicated plasmids, then grown overnight in SC – TRP, and spread on a SC –TRP plate containing 1 mg/ml 5-fluoroorotic acid. The plate was incubated for 2 days at 30°C.

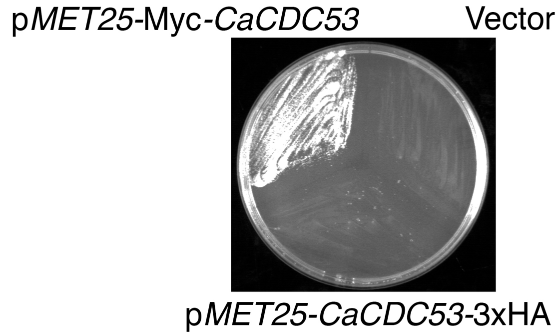
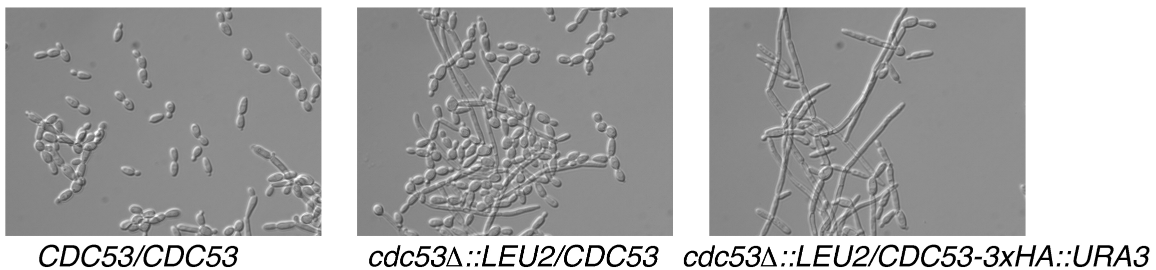


Fig. S5. Phenotype of the *C. albicans* strain expressing only CaCdc53-3xHA. Cultures of strains with the indicated genotypes (KC271, KC325, KC329, respectively), were diluted in the morning, grown to logarithmic phase and photographed.



Supplementary references

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