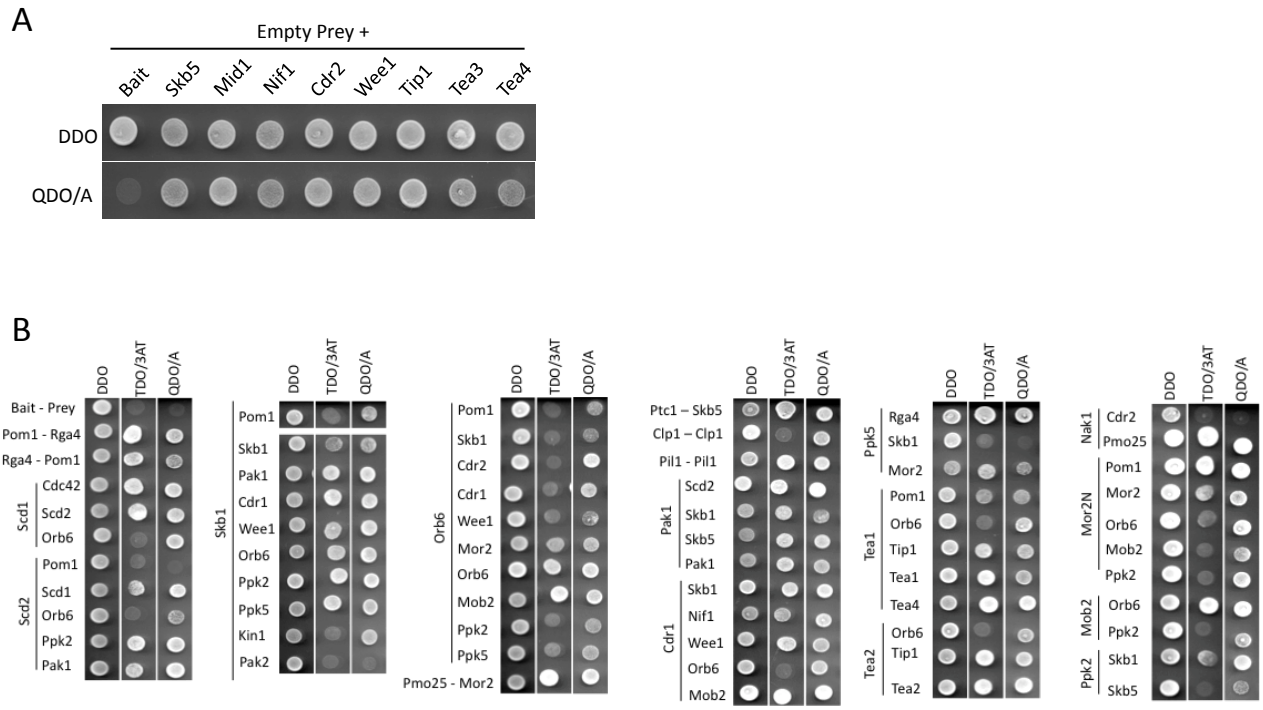


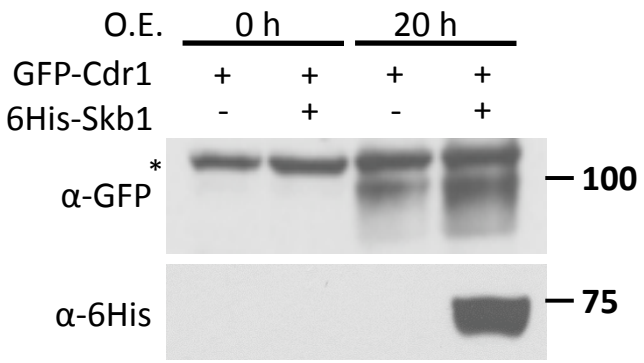
Supplementary Figure S1. Comprehensive pair-wise yeast two-hybrid screen. (A) Bait vectors showing auto-activation with empty prey vector. Empty bait vector was used for negative control, these auto-activating bait vectors were eliminated from the screen. (B) Verification and scoring of protein-protein interactions. Transformants were selected on double dropout (DDO) plates, and interactions were tested by growth on triple dropout + 3AT (TDO/3AT) and quadruple dropout + aureobasidin A (QDO/A) plates.

Strain	Length of cells with 2 SPB (µm)
sid4-mCherry	14.3 ± 0.8
sid4-mCherry skb1Δ	12.9 ± 0.6

Supplementary Figure S2. Length of cells from the indicated genotypes with separated SPBs, as marked by Sid4-mCherry (mean ± s.d.; n > 50 cells for each value).

Strain	Length at division (μm)
WT	13.7 ± 1.0
skb1-3GFP	13.8 ± 1.0
skb1(E422A,E431A)-3GFP	13.7 ± 0.7

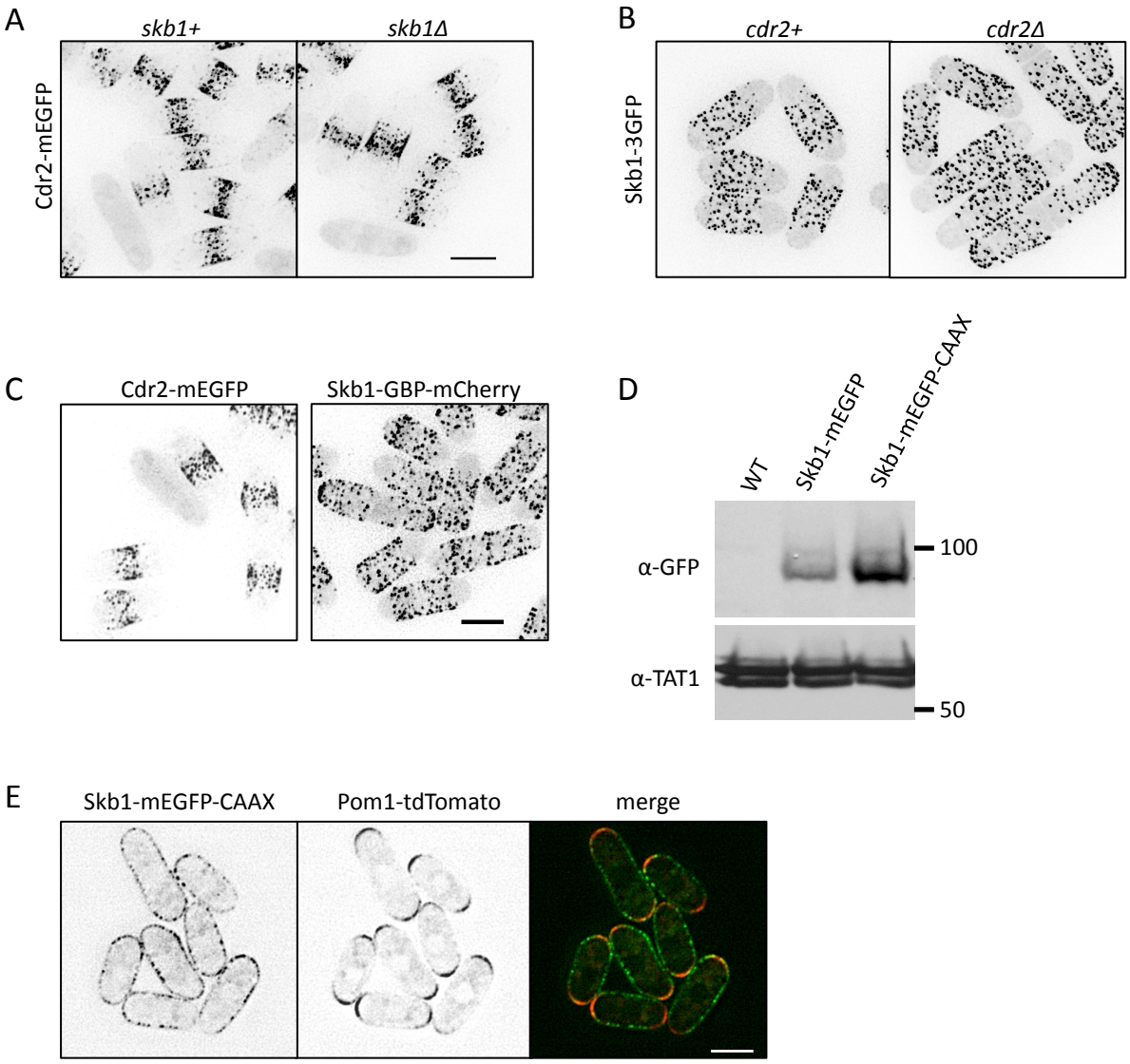
Supplementary Figure S3. Length of dividing, septated cells of the indicated genotypes (mean ± s.d.; n > 50 for each value). Note that cell length at division is not affected by Skb1-3GFP tag or by methyltransferase dead mutant *skb1(E422A,E431A)-3GFP*.



Supplementary Figure S4. GFP-Cdr1 levels are not reduced by co-overexpression of Skb1. Cells were induced as in Figure 3B, and whole-cell extract was probed by Western blot using the indicated antibodies. Asterisk marks nonspecific band.

strain	overexpression	Length at division (μm)
WT	*none*	13.5 ± 1.1
	cdr1	8.1 ± 0.9
	skb1	18.9 ± 2.7
nif1Δ	*none*	12.0 1.2
	cdr1	7.9 ± 1.0
	skb1	19.1 ± 2.0
pom1Δ	*none*	11.7 ± 0.9
	cdr1	7.9 ± 0.9
	skb1	19.8 ± 2.3
cdr1Δ	*none*	17.5 ± 1.5
	cdr1	8.2 ± 1.0
	skb1	24.1 ± 3.5
cdr2Δ	*none*	18.5 ± 1.3
	cdr1	8.2 ± 1.0
	skb1	22.1 ± 3.4
wee1Δ	*none*	8.3 ± 1.3
	cdr1	8.3 ± 1.1
	skb1	8.4 ± 1.1

Supplementary Figure S5. The indicated strains were induced to over-express the indicated genes for 20 hours at 32˚C using a multicopy pREP3 plasmid. Values for cell length at division are mean ± s.d.; n > 50 for each value.



Supplementary Figure S6. (A) Cdr2 localization is independent of Skb1. Images are inverted maximum projections from deconvolved Z-series. (B) Skb1 localization is independent of Cdr2. Images are inverted maximum projections from deconvolved Z-series. (C) Localization of Cdr2-mEGFP and Skb1-GBP-mCherry in separate strains. Note that Skb1-GBP-mCherry localization mimics Skb1-3GFP. These tags were combined in the same strain for Figure 7B. Images are inverted maximum projections from deconvolved Z-series. (D) Western blot to compare expression levels of Skb1-mEGFP versus Skb1-mEGFP-CAAX. Whole-cell extracts were resolved by SDS-PAGE, transferred to nitrocellulose, and probed with anti-GFP antibody. Anti-TAT1 was used as a loading control. (E) Skb1-mEGFP-CAAX does not disrupt Pom1 localization at cell tips. Images are from a single deconvolved focal plane. Scale bar, 5 μm.

Table S1. Summary of protein-protein interactions obtained from two-hybrid screen. Numbers indicate previously known interactions and their references below.

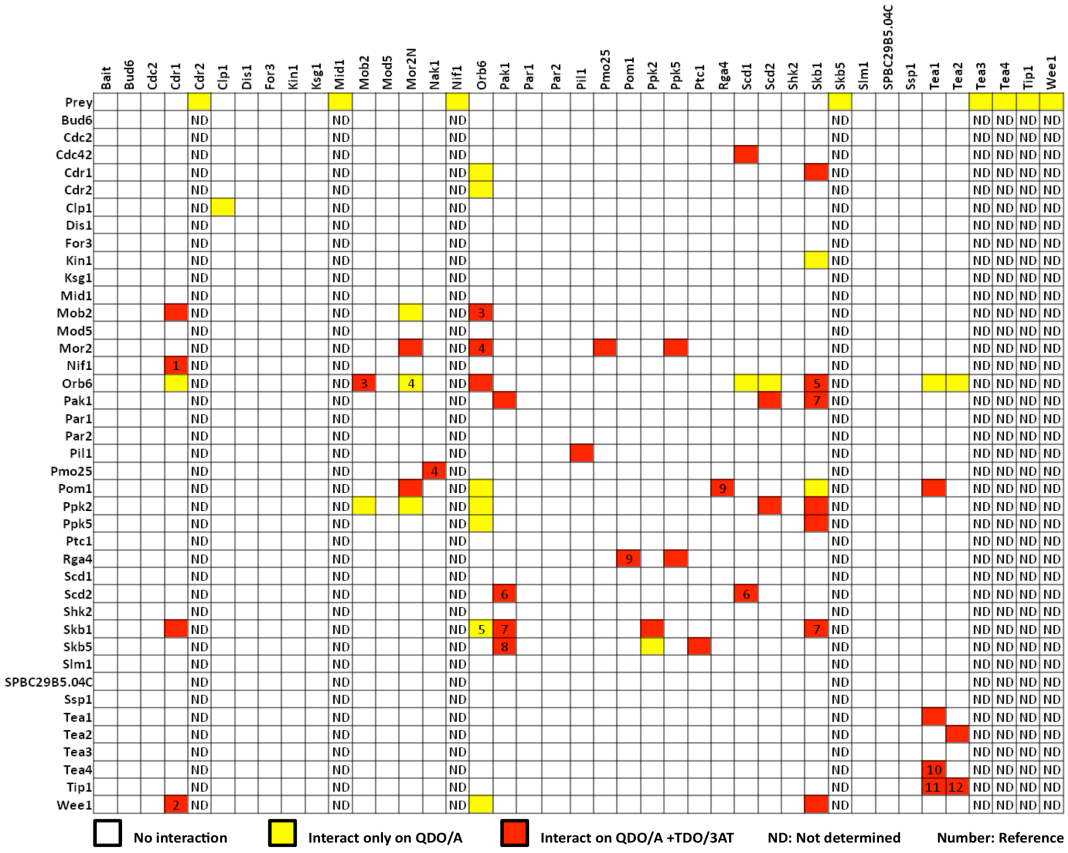


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Table S2. Plasmids used in this study

Plasmid	Description	Note	Selection
pJM587	pGBKT7	-	Kan, TRP
pJM588	pGBKT7-pom1	-	Kan, TRP
pJM589	pGBKT7-rga4	-	Kan, TRP
pJM591	pGBKT7-scd1	-	Kan, TRP
pJM592	pGBKT7-scd2	-	Kan, TRP
pJM594	pGBKT7-skb1	-	Kan, TRP
pJM595	pGBKT7-skb5	-	Kan, TRP
pJM596	pGBKT7-pak1	-	Kan, TRP
pJM597	pGBKT7-shk2	-	Kan, TRP
pJM598	pGBKT7-mid1	-	Kan, TRP
pJM599	pGBKT7-cdr2	C488T (L163P)	Kan, TRP
pJM600	pGBKT7-cdr1	-	Kan, TRP
pJM601	pGBKT7-nif1	-	Kan, TRP
pJM602	pGBKT7-wee1	-	Kan, TRP
pJM603	pGBKT7-cdc2	-	Kan, TRP
pJM604	pGBKT7-mor2N	aa 1-1100	Kan, TRP
pJM605	pGBKT7-pmo25	-	Kan, TRP
pJM606	pGBKT7-nak1	-	Kan, TRP
pJM607	pGBKT7-orb6	-	Kan, TRP
pJM608	pGBKT7-mob2	-	Kan, TRP
pJM609	pGBKT7-ppk2	aa 1-649	Kan, TRP
pJM610	pGBKT7-ppk5	-	Kan, TRP
pJM611	pGBKT7-kin1	-	Kan, TRP
pJM612	pGBKT7-ssp1	-	Kan, TRP
pJM613	pGBKT7-pil1	-	Kan, TRP
pJM614	pGBKT7-slm1	-	Kan, TRP
pJM616	pGBKT7-tip1	-	Kan, TRP
pJM617	pGBKT7-mod5	-	Kan, TRP
pJM618	pGBKT7-tea1	-	Kan, TRP
pJM619	pGBKT7-tea2	-	Kan, TRP
pJM620	pGBKT7-tea3	-	Kan, TRP
pJM621	pGBKT7-tea4	-	Kan, TRP
pJM622	pGBKT7-for3	-	Kan, TRP
pJM623	pGBKT7-bud6	-	Kan, TRP
pJM624	pGBKT7-par1	-	Kan, TRP
pJM626	pGBKT7-SPBC29B5.04C	-	Kan, TRP
pJM627	pGBKT7-clp1	-	Kan, TRP
pJM628	pGBKT7-ptc1	-	Kan, TRP
pJM629	pGBKT7-ksg1	-	Kan, TRP
pJM630	pGADT7	-	Amp, LEU
pJM631	pGADT7-pom1	-	Amp, LEU
pJM632	pGADT7-rga4	-	Amp, LEU
pJM633	pGADT7-cdc42	-	Amp, LEU
pJM634	pGADT7-scd1	-	Amp, LEU

pJM635	pGADT7-scd2	-	Amp, LEU
pJM637	pGADT7-skb1	-	Amp, LEU
pJM638	pGADT7-skb5	-	Amp, LEU
pJM639	pGADT7-pak1	-	Amp, LEU
pJM640	pGADT7-shk2	-	Amp, LEU
pJM641	pGADT7-mid1	-	Amp, LEU
pJM642	pGADT7-cdr2	-	Amp, LEU
pJM643	pGADT7-cdr1	C749A (P250Q)	Amp, LEU
pJM644	pGADT7-nif1	-	Amp, LEU
pJM645	pGADT7-wee1	-	Amp, LEU
pJM646	pGADT7-cdc2	-	Amp, LEU
pJM647	pGADT7-mor2	A5075G (T1692A)	Amp, LEU
pJM648	pGADT7-pmo25	-	Amp, LEU
pJM650	pGADT7-orb6	-	Amp, LEU
pJM651	pGADT7-mob2	-	Amp, LEU
pJM652	pGADT7-ppk2	-	Amp, LEU
pJM653	pGADT7-ppk5	-	Amp, LEU
pJM654	pGADT7-kin1	-	Amp, LEU
pJM655	pGADT7-ssp1	-	Amp, LEU
pJM656	pGADT7-pil1	-	Amp, LEU
pJM657	pGADT7-slm1	-	Amp, LEU
pJM658	pGADT7-dis1	-	Amp, LEU
pJM659	pGADT7-tip1	-	Amp, LEU
pJM660	pGADT7-mod5	-	Amp, LEU
pJM661	pGADT7-tea1	-	Amp, LEU
pJM662	pGADT7-tea2	-	Amp, LEU
pJM663	pGADT7-tea3	-	Amp, LEU
pJM664	pGADT7-tea4	-	Amp, LEU
pJM665	pGADT7-for3	-	Amp, LEU
pJM666	pGADT7-bud6	-	Amp, LEU
pJM667	pGADT7-par1	-	Amp, LEU
pJM668	pGADT7-par2	-	Amp, LEU
pJM669	pGADT7-SPBC29B5.04C	-	Amp, LEU
pJM670	pGADT7-clp1	-	Amp, LEU
pJM671	pGADT7-ptc1	-	Amp, LEU
pJM672	pGADT7-ksg1	-	Amp, LEU
pJM705	pGADT7-cdr1 (1-258)	-	Amp, LEU
pJM706	pGADT7-cdr1 (259-593)	-	Amp, LEU
pJM210	pREP3x	-	Amp, LEU
pJM416	pREP3x-6His-Cdr1	-	Amp, LEU
pJM482	pREP3x-6His-Skb1	-	Amp, LEU
pJM684	pJK148-Pskb1-skb1-3GFP-Tskb1-KanR	-	Amp
pJM763	pJK148-Pskb1-skb1(E422A,E431A)-3GFP-Tskb1-KanR	SDM of pJM684	Amp

Table S3. Strains used in this study

Strain	Genotype	Source
JM14	*cdc25-22 h+*	lab collection
JM186	*pom1Δ::ura4+ ura4-D18 h+*	lab collection
JM199	*cdr2Δ::ura4+ ura4-D18 h+*	lab collection
JM228	*wee1-50 h+*	lab collection
JM366	*972 h-*	lab collection
JM454	*cdr1-3GFP::kanMX6 h-*	lab collection
JM482	*nim1Δ::kanMX6 leu1-32 h+*	lab collection
JM488	*skb1-3GFP::kanR h-*	This study
JM496	*kanR-P41-nmt1-GFP-cdr1 ULA- h-*	This study
JM497	*kanR-P81-nmt1-GFP-cdr1 ULA-*	This study
JM499	*skb1-3GFP::kanR cdr2-mCherry::natR ade6-M216*	This study
JM504	*skb1-3GFP::kanR cdr2Δ::ura4+ ura4-D18*	This study
JM548	*kanR-P41nmt1-GFP-wee1 leu1-32 ura4-D18 h-*	This study
JM554	*cdr2Δ::kanR h-*	lab collection
JM570	*cdr1Δ::natR h-*	This study
JM618	*cdc25-22 pom1Δ::ura4-D18 ura4-D18*	lab collection
JM623	*cdr2-mEGFP::kanR skb1Δ::ura4+ ura4-D18 leu1-32 ade6-M21X*	This study
JM636	*skb1Δ::ura4+ cdr1Δ::natR ura4-D18*	This study
JM673	*cdr2-mEGFP::kanR leu1-32 h-*	This study
JM777	*wee1Δ::ura4+ ura4-D18 leu1-32 h-*	lab collection
JM837	*leu1-32 h-*	lab collection
JM906	*skb1Δ::kanMX6 h+*	This study
JM909	*skb1Δ::ura4+ ura4-D18 leu1-32 h+*	This study
JM918	*nif1Δ::natR h+*	This study
JM923	*cdc25-22 skb1Δ::kanR*	This study
JM937	*pom1Δ::kanMX6 ura4-D18 leu1-32 ade6-M216 h+*	lab collection
JM963	*cdc25-22 nif1Δ::natR h+*	This study
JM964	*cdc25-22 nif1Δ::natR skb1Δ::kanR h+*	This study
JM1005	*skb1-3GFP::kanR pom1Δ::ura4+ ura4-D18*	This study
JM1006	*skb1-3GFP::kanR tea1Δ::ura4+ ura4-D18*	This study
JM1691	*cdc25-22 skb1Δ::kanR pom1Δ::ura4-D18 h+*	This study
JM1721	*skb1Δ::natR h-*	This study
JM1862	*skb1Δ::natR pom1Δ::ura4+ ura4-D18*	This study
JM1872	*skb1Δ::natR cdr2Δ::ura4+ ura4-D18*	This study
JM1932	*skb1-3GFP::natR pAct1-Lifeact-mCherry::leu+*	This study
JM1974	*skb1-GBP-mCherry::hphR h+*	This study
JM1995	*cdr2-mEGFP::kanMX6 skb1-GBP-mCherry::hphR h+*	This study
JM2025	*skb1(E422,431A)-3GFP::kanR ura4-D18 leu1-32 h+*	This study
JM2072	*cdr2Δ::ura4+ ura4-D18 leu1-32 h+*	lab collection
JM2325	*skb1-mEGFP::kanR h-*	This study
JM2337	*skb1-mEGFP-caax::natR h-*	This study
JM2399	*skb1-mEGFP-caax::natR cdr2-mCherry::natR*	This study
JM2622	*pom1-tdTomato::natR skb1-mEGFP-caax::natR ura4-D18*	This study

JM2624	*skb1Δ::natR wee1-50*	This study
JM2625	*nif1Δ::natR leu1-32*	This study