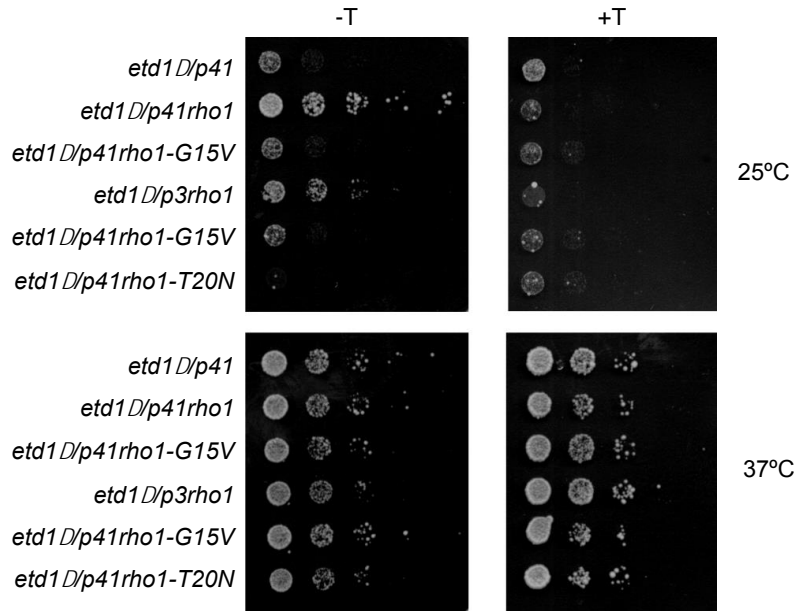
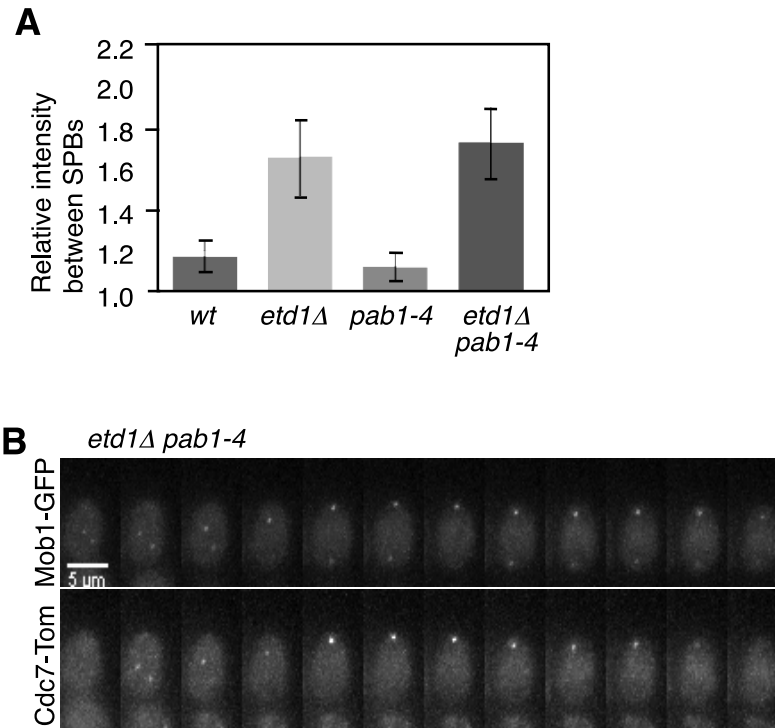


**Figure S1** Characterization of the *sid2-as* allele. (A) Growth assay (serial dilution drop tests on plates) of *sid2-as* cells in YES media + DMSO (left panels) and YES with 10µM 1NMPP1 (right panels). The wild type strain was used as controls. Cells were grown at 25°C for and 10-fold dilutions (starting with 10<sup>4</sup> cells) were spotted and incubated at 32°C. Similar results were obtained upon incubation at 25°C and 35°C (not shown). (B) Increase in cell number (determined by direct counting cell/ml under de microscope in a counting chamber) of the *sid2-as* strain incubated at 32°C in YES with DMSO (control) or with 10µM 1NMPP1. The wild type strain was used as control. (C) Dapi staining of *sid2-as* cells incubated at 32°C in YES with DMSO (left panel) or with 10µM 1NMPP1 (right panels). (D) SPB localization of Cdc7-Tom expressed in *sid2-as* cells incubated at 32°C in YES with DMSO (control) or with 10µM 1NMPP1.



**Figure S2** Suppression of growth defects of the *etd1Δ* strain. Growth assay (serial dilution drop tests on plates) of *etd1Δ* cells transformed with the plasmid pREP41x empty (p41) or expressing *rho1<sup>+</sup>*, *rho1-T20N* and *rho1-G15V* alleles as indicated. Expression of *rho1<sup>+</sup>* with the pREP3x plasmid (*p3rho1*) and was also used. Cells were spotted in EMM with (+T) or without (-T) thiamine and incubated at the indicated temperature during 3 days.



**Figure S3** Influence of Etd1 in the symmetric localization of Sid2-Mob1 at the SPBs. (A) Mob1-GFP fluorescence intensity at the two SPBs was quantified (arbitrary units) at their maximal levels (reaching maximal spindle elongation) in ten cells of each strain, and the average values of the related intensity between SPBs (Intensity of the Brighter SPB/intensity of the partner) are represented. Error bars indicate SEM. (B) Mob1-GFP and Cdc7-Tom were imaged in live *etd1Δ pab1-4* cells by time-lapse microscopy at 5 min intervals at 25°C, restrictive conditions for *etd1Δ* mutants. Given that Spg1 is activated at the new SPB (SPBd), co-expression of Mob1-GFP and Cdc7-GFP in *etd1Δ pab1-4* cells allows identifying the SPB at which Mob1 is specifically enriched in Etd1-depleted cells.

**Table S1 Strains used in this study.**

Strain code	Genotype	Source
RD 312	<i>ade6-216 leu1-32 ura4-D18</i>	R. Daga
RD 313	<i>ade6-216 leu1-32 ura4-D18</i>	R. Daga
JJ 900	<i>h+ leu1-32 ura4-D18 etd1::ura4</i>	Lab. Stock
JJ 2034	<i>h- leu1-32 ura4-D18 etd1::ura4</i>	This study
JJ 1165	<i>h- leu1-32/p41:EGFP:etd1-D1 (1-66aa)</i>	This study
JJ 1166	<i>h- leu1-32/p41:EGFP:etd1-D2 (1-133aa)</i>	This study
JJ 1167	<i>h- leu1-32/p41:EGFP:etd1-D3 (1-200aa)</i>	This study
JJ 1168	<i>h- leu1-32/p41:EGFP:etd1-D4 (1-300aa)</i>	This study
JJ 1169	<i>h- leu1-32/p41:EGFP:etd1-D5 (66-300aa)</i>	This study
JJ 1170	<i>h- leu1-32/p41:EGFP:etd1-D6 (133-300aa)</i>	This study
JJ 1171	<i>h- leu1-32/p41:EGFP:etd1-D7 (200-300aa)</i>	This study
JJ 1197	<i>h- leu1-32/p41:EGFP:etd1-D12 (66-133aa)</i>	This study
JJ 1198	<i>h- leu1-32/p41:EGFP:etd1-D8 (66-391aa)</i>	This study
JJ 1199	<i>h- leu1-32/p41:EGFP:etd1-D9 (133-391aa)</i>	This study
JJ 1200	<i>h- leu1-32/p41:EGFP:etd1-D10 (200-391aa)</i>	This study
JJ 1231	<i>h- leu1-32/p41:EGFP:etd1-D12N (66-100aa)</i>	This study
JJ 1232	<i>h- leu1-32/p41:EGFP:etd1-D12C (100-133aa)</i>	This study
JJ 1233	<i>h- leu1-32/p41:EGFP:etd1</i>	Lab. Stock
JJ 1235	<i>h- leu1-32/p41:EGFP</i>	Lab. Stock
JJ 1264	<i>h- leu1-32 /p41:EGFP:etd1-D11 (300-391aa)</i>	This study
JJ 1359	<i>ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1-D1 (1-66aa)</i>	This study
JJ 1360	<i>ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1-D2 (1-133aa)</i>	This study
JJ 1361	<i>ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1-D3 (1-200aa)</i>	This study
JJ 1362	<i>ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1 D4 (1-300aa)</i>	This study
JJ 1363	<i>ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1 D5 (66-300aa)</i>	This study
JJ 1364	<i>ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1 D6 (133-300aa)</i>	This study
JJ 1365	<i>ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1 D7 (200-300aa)</i>	This study
JJ 1366	<i>ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1-D12 (66-133aa)</i>	This study
JJ 1367	<i>h- ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1 D8 (66-391aa)</i>	This study
JJ 1368	<i>h+ ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1 D9 (133-391aa)</i>	This study
JJ 1369	<i>h- ade6-216 leu1-32 ura4-D18 etd1::ura4/ p41:EGFP:etd1-D10 (200-391aa)</i>	This study
JJ 1370	<i>h- ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1</i>	Lab. Stock
JJ 1372	<i>ade6-216 leu1-32 ura4-D18 etd1::ura4/p41:EGFP</i>	Lab. Stock
RD 701	<i>h+ ade6- leu1-32 ura4-D18 cdc7::Tom:Nat</i>	Fred Chang

JJ 1705	<i>h- ade6- leu1-32 ura4-D18 cdc7:Tom:Nat</i>	This study
JJ 1692	<i>ade6- leu1-32 ura4-D18 cdc7:Tom:Nat etd1::ura4</i>	This study
JJ 1421	<i>ade6- leu1-32 ura4-D18 cdc7:Tom:Nat pab1-4</i>	This study
JJ 1690	<i>ade6- leu1-32 ura4-D18 cdc7:Tom:Nat etd1::ura4 pab1-4</i>	This study
JJ 1723	<i>h- ade6- leu1-32 ura4-D18 cdc7:Tom:Nat rlc1:GFP:Kan</i>	This study
JJ 1724	<i>h+ leu1-32 ura4-D18 cdc7:Tom:Nat rlc1:GFP:Kan</i>	This study
RD 702	<i>h+ leu1-32 ura4-D18 rlc1:Tom:Nat</i>	Fred Chang
JJ 774	<i>h- leu1-32 ura4-D18 mob1:GFP:kan</i>	Lab. Stock
JJ 1838	<i>ade- leu1-32 ura4-D18 mob1:GFP:kan etd1::ura4 nmt81:etd1</i>	This study
JJ 1839	<i>leu1-32 ura4-D18 mob1:GFP:kan etd1::ura4 pab1-4</i>	This study
JJ 1782	<i>h- leu1-32 ura4-D18 rlc1:Tom:NatR mob1:GFP:Kan</i>	This study
JJ 1784	<i>h- ade6- leu1-32 ura4-D18 rlc1:Tom:NatR mob1:GFP:Kan pab1-4</i>	This study
JJ 1785	<i>h+ leu1-32 ura4-D18 rlc1:Tom:NatR mob1:GFP:Kan pab1-4</i>	This study
JJ 1840	<i>leu1-32 ura4-D18 rlc1:Tom:NatR mob1:GFP:Kan pab1-4 etd1::ura4</i>	This study
JJ 1372	<i>ade6- leu1-32 ura4-D18 etd1::ura4/p41:EGFP</i>	This study
JJ 1370	<i>h- ade6- leu1-32 ura4-D18 etd1::ura4/p41:EGFP:etd1</i>	This study
JJ 1273	<i>ade6- leu1-32 ura4-D18 etd1:ura4/p41:HA:rho1</i>	This study
JJ 1750	<i>leu1-32 ura4-D18 ade6- cdc7:Tom:Nat/p41:HA:rho1</i>	This study
JJ 1752	<i>leu1-32 ura4-D18 ade6- cdc7:Tom:Nat etd1::ura4/p41:HA:rho1</i>	This study
JJ 1139	<i>h- ade6- leu1-32 HA:rho1:ura4</i>	Pilar Pérez
JJ 1201	<i>etd1::ura4 HA:rho1:ura4</i>	Lab. Stock
JJ 1157	<i>h- leu1-32 ade6- HA:rho1:ura4 pab1-4</i>	Lab. Stock
JJ 1184	<i>leu1-32 ade6- HA:rho1:ura4 pab1-4 etd1::ura4</i>	Lab. Stock
JJ 1310	<i>h+leu1-32 ade6- HA:rho1:ura4 /p41:EGFP:etd1</i>	This study
JJ 1879	<i>h+leu1-32 ade6- HA:rho1:ura4 /p3:pab1</i>	This study
JJ 1541	<i>leu1-32 ade6- HA:rho1:ura4 /p41:EGFP</i>	This study
JJ 2034	<i>h- leu1-32 ura4-D18 etd1::ura4 nmt81:etd1</i>	Lab. Stock
JJ 2035	<i>h+ leu1-32 ura4-D18 etd1::ura4 nmt81:etd1</i>	Lab. Stock
JJ 1847	<i>leu1-32 ura4-D18 ade- rga1::ura4</i>	Pilar Pérez
JJ 1849	<i>h- leu1-32 ura4-D18 rga5::ura4</i>	Pilar Pérez
JJ 1850	<i>h+ leu1-32 ura4-D18 rga5::ura4</i>	Pilar Pérez
JJ 2042	<i>h- leu1-32 ura4-D18 rga8::Kan</i>	Pilar Pérez
JJ 2020	<i>leu1-32 ura4-D18 rga1::ura4 etd1::ura4</i>	This study
JJ 2000	<i>leu1-32 ura4-D18 rga5::ura4 etd1::ura4</i>	This study
JJ 2061	<i>leu1-32 ura4-D18 rga8::Kan etd1::ura4</i>	This study
JJ 2119	<i>leu1-32 ura4-D18 cdc7:Tom:Nat rga5::ura4</i>	This study

JJ 2113	<i>leu1-32 ura4-D18 cdc7:Tom:Nat rga5::ura4 etd1::ura4 nmt81:etd1</i>	This study
JJ 2151	<i>ade6- leu1-32 ura4-D18 etd1:ura4 /p41:YFP:rgf1</i>	This study
JJ 2152	<i>ade6- leu1-32 ura4-D18 etd1:ura4 /p41:YFP:rgf2</i>	This study
JJ 2173	<i>ade6- leu1-32 ura4-D18 etd1:ura4/p41:rgf3</i>	This study
JJ 1311	<i>h+ leu1-32 ade6- HA:rho1:ura4 /p41:EGFP:etd1-D8 (66-391aa)</i>	This study
JJ 1312	<i>h+ leu1-32 ade6- HA:rho1:ura4 /p41:EGFP:etd1-D10 (200-391aa)</i>	This study
JJ 1799	<i>h+ leu1-32 cut11:GFP:ura4 cdc7:Tom:Nat</i>	This study
JJ 1802	<i>h- ura4-D18 sid2-as</i>	This study
JJ 1862	<i>h+ ade6- leu1-32 ura4-D18 sid2-as</i>	This study
JJ 1857	<i>h+ ade6- leu1-32 ura4-D18 sid2-as cdc7:Tomato:Nat</i>	This study
RD 1407	<i>h- ade6- leu1-32 ura4-D18 hob3::ura4</i>	Pilar Pérez
JJ 1906	<i>h+ leu1-32 ura4-D18 cdc7:Tom:Nat rlc1:GFP:Kan hob3::ura4</i>	This study
JJ 1917	<i>leu1-32 ura4-D18 cdc7:Tom:Nat rlc1:GFP:Kan drc1-191</i>	This study
JJ 2268	<i>rho1::ura4/p41xRho1 ade6- leu1-32 ura4-d18</i>	Pilar Pérez
JJ 2305	<i>cdc7:Tom:Nat rlc1:GFP:Kan drc1-191 rho1::ura4 p41rho1</i>	This study
JJ 2306	<i>cdc7:Tom:Nat rlc1:GFP:Kan drc1-191 sid2-as</i>	This study

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