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## Supplemental Information

# Structure-Function Relationship of the Bik1-Bim1 Complex

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# Table S1

**Table S1, related to Figure 1, 5 and 6. $K_d$ Values determined by ITC.**
Various experiments were repeated to confirm the $K_d$ values.

| Cell | Syringe | $K_{d\ Exp1}$ [µM] | $K_{d\ Exp2}$ [µM] |
|---|---|---|---|
| Bim1C | Bik1CG | 1.2 ± 0.3 | 1.0 ± 0.3 |
| Bik1CG | ETF | 5.6 ± 0.8 | 4.7 ± 0.6 |
| Bim1C ΔETF | Bik1CG | n.b. | - |
| Bim1C | Bik1CG K46E | n.b. | - |
| Bik1cc | Stu2Ctail | 0.6 ± 0.1 | 0.8 ± 0.1 |
| Bim1C | Stu2Ctail | n.b. | - |
| Bim1C | SxIP | 11.6 ± 0.2 | n.d. [a] |
| Bim1C-Bik1CG | SxIP | 13.0 ± 0.6 | n.d. |
| Bim1C-SxIP | Bik1CG | 2.0 ± 0.6 | n.d. |
| Bim1C | LxxPTPh | 2.2 ± 0.6 | 2.2 ± 0.5 |
| Bim1C-Bik1CG | LxxPTPh | 2.7 ± 0.5 | n.d. |

n.b., no binding; n.d., not determined

[a] A $K_d$ value of 13.8 µM for this interaction is reported in (Kumar et al., 2017) using ITC.

± refers to the standard error of the least square fit (i.e., assuming a one set of site model) versus the data points.

# Table S2

## Table S2, related to Figure 7. Summary of the yeast strains that has been used in this study.

| Yeast strain number (yYB) | Mating type | Genotype | Source |
|---|---|---|---|
| 11069, 11070 | a | Bik1-3xGFP::hphNT1 Spc72-GFP::HIS ura3-52 his3Δ200 leu2 lys2-801 trp1Δ63 Ade2+ | This study |
| 11068 | alpha | Bik1-3xGFP::hphNT1 Spc72-GFP::HIS ura3-52 his3Δ200 leu2 lys2-801 trp1Δ63 Ade2+ | This study |
| 11077, 11078, 11079 | a | Bik1-3xGFP::hphNT1 Spc72-GFP::HIS bim1::hphNT1 ura3-52 his3Δ200 leu2 lys2-801 trp1Δ63 Ade2+ | This study |
| 13751, 13752, 13753 | a | Bik1-3xGFP::hphNT1 Spc72-GFP::HIS Bim1-ΔETF ura3-52 his3Δ200 leu2 lys2-801 trp1Δ63 Ade2+ | This study |
| 14945, 14946, 14947 | alpha | Bik1-K46E-3xGFP::hphNT1 Spc72-GFP::HIS ura3-52 his3Δ200 leu2 lys2-801 trp1Δ63 Ade2+ | This study |
| 11263 | a | bik1::NatMX Spc72-GFP::HIS ura3-52 his3Δ200 leu2 lys2-801 trp1Δ63 Ade2+ | This study |
| 8597, 8600, 8602 | a | CFP-Tub1:Trp1 kar9::His3 ura3-52 leu2 lys2-801 Ade2+ | Manatschal et al., 2016 |
| 8461, 8462, 8463 | a | CFP-Tub1:Trp1 kar9::Kar9-wt-3xGFP:KanMX ura3-52 his3Δ200 leu2 lys2-801 ade2-101 | Manatschal et al., 2016 |
| 12656, 12657 | a | CFP-Tub1:Trp1 kar9::Kar9-wt-3xsfGFP:KanMX ura3-52 his3Δ200 leu2 lys2-801 Ade2+ | Manatschal et al., 2016 |
| 14940, 14941 | a | CFP-Tub1:Trp1 kar9::Kar9-wt-3xsfGFP:KanMX Bik1-K46E ura3-52 his3Δ200 leu2 lys2-801 Ade2+ | This study |
| 14942 | alpha | CFP-Tub1:Trp1 kar9::Kar9-wt-3xsfGFP:KanMX Bik1-K46E ura3-52 his3Δ200 leu2 lys2-801 Ade2+ | This study |
| 14491, 14831 | a | CFP-Tub1::Trp1 kar9::Kar9-wt-3xsfGFP:KanMX Bim1-ΔETF ura3-52 his3Δ200 leu2 lys2-801 Ade2+ | This study |
| 14492, 14830 | alpha | CFP-Tub1::Trp1 kar9::Kar9-wt-3xsfGFP:KanMX Bim1-ΔETF ura3-52 his3Δ200 leu2 lys2-801 Ade2+ | This study |

# Figure S1

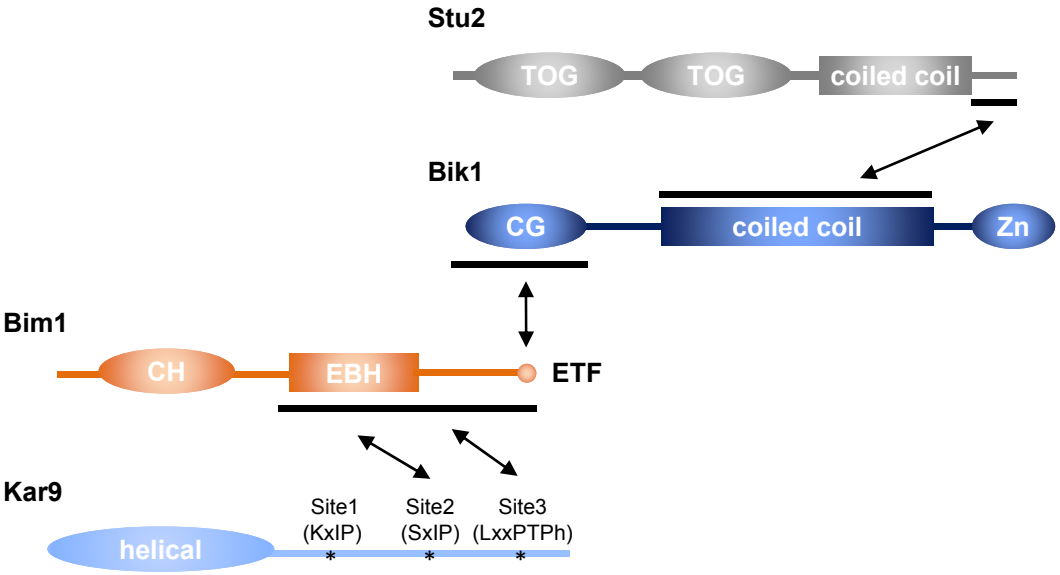


**Figure S1, related to Figures 1, 5 and 6. Schematic representation of +TIP network formed between Bik1, Stu2, Bim1 and Kar9.**

The interactions highlighted by double arrows are reported in (Wolyniak et al., 2006; Blake-Hodek et al., 2010; Manatschal et al., 2016). Note that not in all instances it is clear whether the interactions are direct or indirect; see also Introduction. The protein fragments and domains reporting the interactions are indicated by horizontal black lines.

# Figure S2

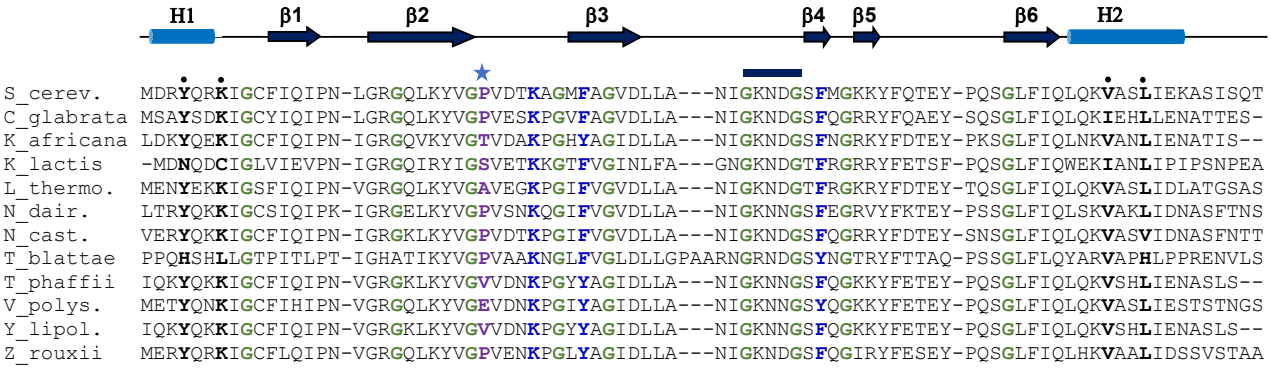


**Figure S2, related to Figure 2. Multiple sequence alignment of budding yeast Bik1 CAP-Gly domains.**

The characteristic glycine residues and GKNDG motif of CAP-Gly domains are indicated in green and with a black horizontal bar on top of the alignment, respectively. The position of the β2-β3 loop residue that in the case of p150CG interacts with the EBH domain of EB1 (Ala49; Honnappa et al., 2006) is highlighted with an asterisk and the corresponding residues are shown in bold and in purple. Key residues of the exposed hydrophobic cavity of CAP-Gly are shown in bold and dark blue. Conserved residues in both the N- and C-terminal helices are highlighted in bold and with a dot on the top of the alignment.