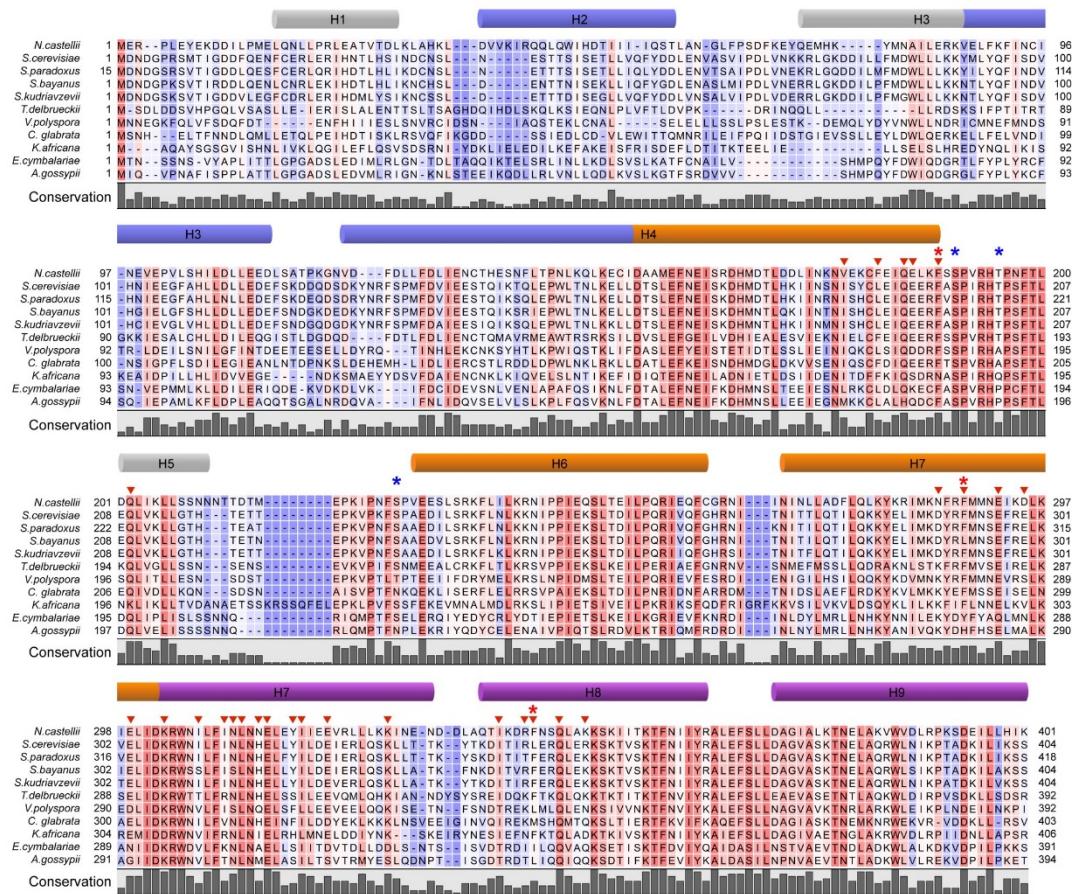


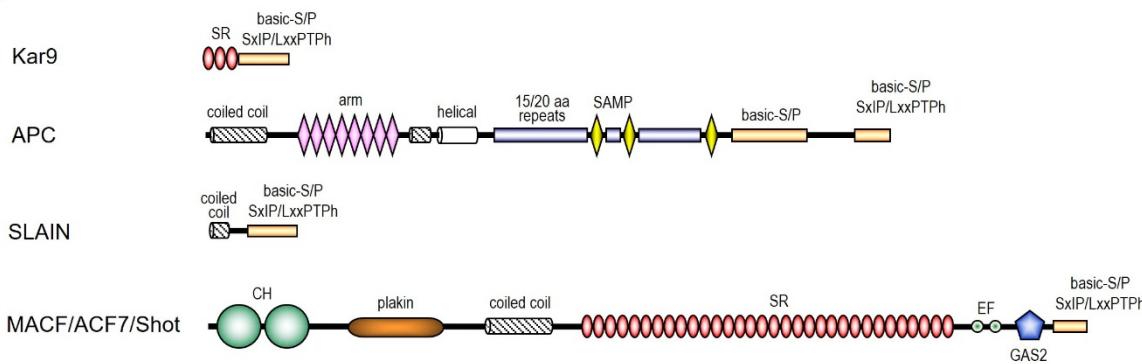
## SUPPLEMENTARY MATERIALS

## Supplementary Figures and Legends

A



B

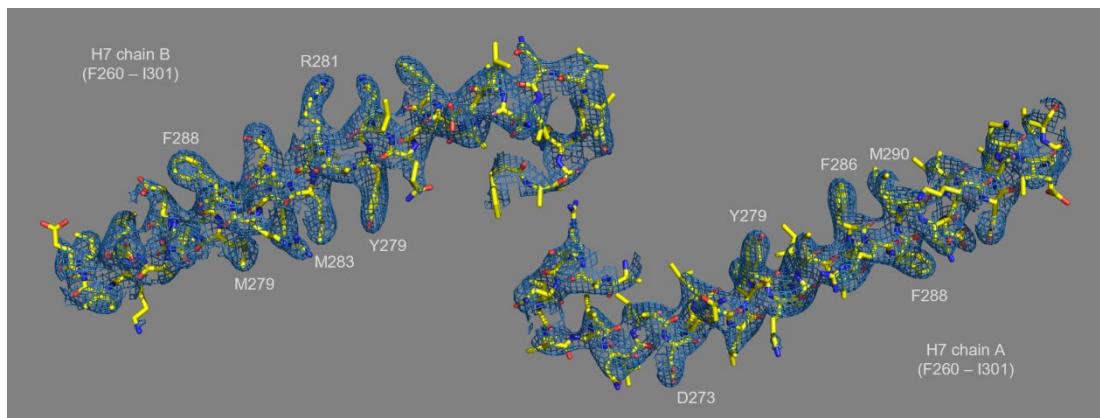


**Figure S1.** Multiple sequence alignment of selected budding yeast Kar9 orthologues and domain comparison of selected +TIPs. Related to Figure 1.

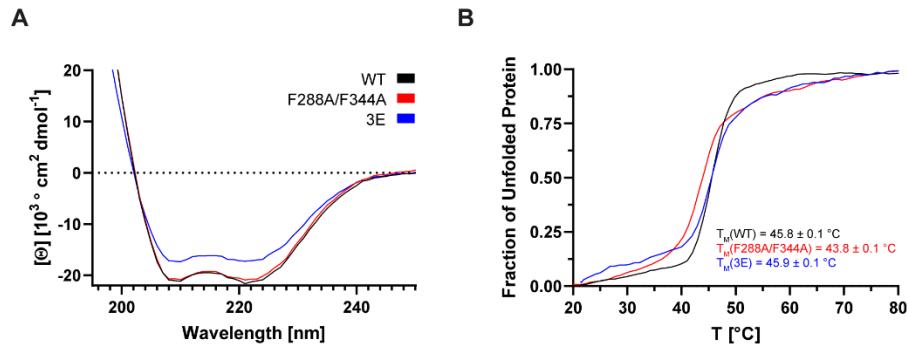
A, Sequence alignment of N-terminal domains of Kar9 orthologues including all constructs used for crystallization plus those of *S.paradoxus*, *S.bayanus*, *T. delbrueckii*, *C. glabrata* and *A. gossypii* for comparison. Conservation is indicated by background color from blue (low) to

red (high). The color of helices H1-9 on top indicate to which spectrin repeat (SR1-3; Figure 1A) they belong. Red triangles indicate residues that contribute to the dimerization interface. Red stars indicate residues mutated to perturb the dimerization interface (ScF195 only in *S. cerevisiae*), blue stars indicate position of Cdk1 site of interest. All features are aligned to the *N.castellii* sequence.

B, Kar9 domain comparison to APC, SLAIN and MACF/ACF7.



**Figure S2.** Section of the electron density of the NcKar9N structure. Secondary structural elements and selected residues are labeled. Related to Figure 1.



**Figure S3.** CD analysis of NcKar9N variants. Related to Figure 2.

A, CD spectra as mean residue molar ellipticity at 20°C, 4  $\mu\text{M}$  protein concentration, 150 mM NaCl, 20 mM Tris-HCl at pH 7.5, 1 mM DTT. Shown are the average of three replicates.

B, Thermal denaturation from 20°C to 80°C plotted as unfolded protein fraction based on CD signal at 222 nm. Shown are single runs.

His-NcKar9N WT (black), His-NcKar9N-F288A/F344A (red), and His-NcKar9N-3E (blue).

## Supplementary Tables

**Table S1.** Detailed numbers of analyzed cells and clones (cells/clones). Related to Figures 3-6.

Figure 3		Sckar9-wt	NcKar9-wt						
D	319/3	193/3	241/3	174/3					
E	361/3	262/3	308/3	209/3					
F-G	285/3	199/3	262/3	268/3	172/3				
Figure 4		Sckar9-wt	Sckar9ΔN	ATF1-Sckar9ΔN	NcKar9-dim-				
C, F-G	560/3	52/3		89/2	172/3 (F-G)				
D	113/3	77/3	114/2						
E	171/3	52/3	89/2						
Figure 5		Sckar9-wt	Sckar9-dim-	Sckar9-Gcn4-m-dim-	Sckar9-Gcn4-e-dim-	Sckar9-S197E	Sckar9-3E	Sckar9-Gcn4-m-3E	Sckar9-Gcn4-e-3E
D, F-G	560/3	415/3	156/3	136/3	181/3	229/3	134/3	113/3	172/3 (F-G)
E	549/3	256/3	114/3	93/3	171/3	198/3	143/3	133/3	
Figure 6		Sckar9-wt	Sckar9-dim-	Sckar9-Gcn4-m-dim-	Sckar9-Gcn4-e-dim-	Sckar9-S197E	Sckar9-3E	Sckar9-Gcn4-m-3E	Sckar9-Gcn4-e-3E
B	171/3	170/3	155/3	136/3	181/3	138/3	133/3	113/3	
	Sckar9ΔN		ATF1-Sckar9ΔN	Sckar9-3E					
C	83/1	33/2	24/2	41/2					