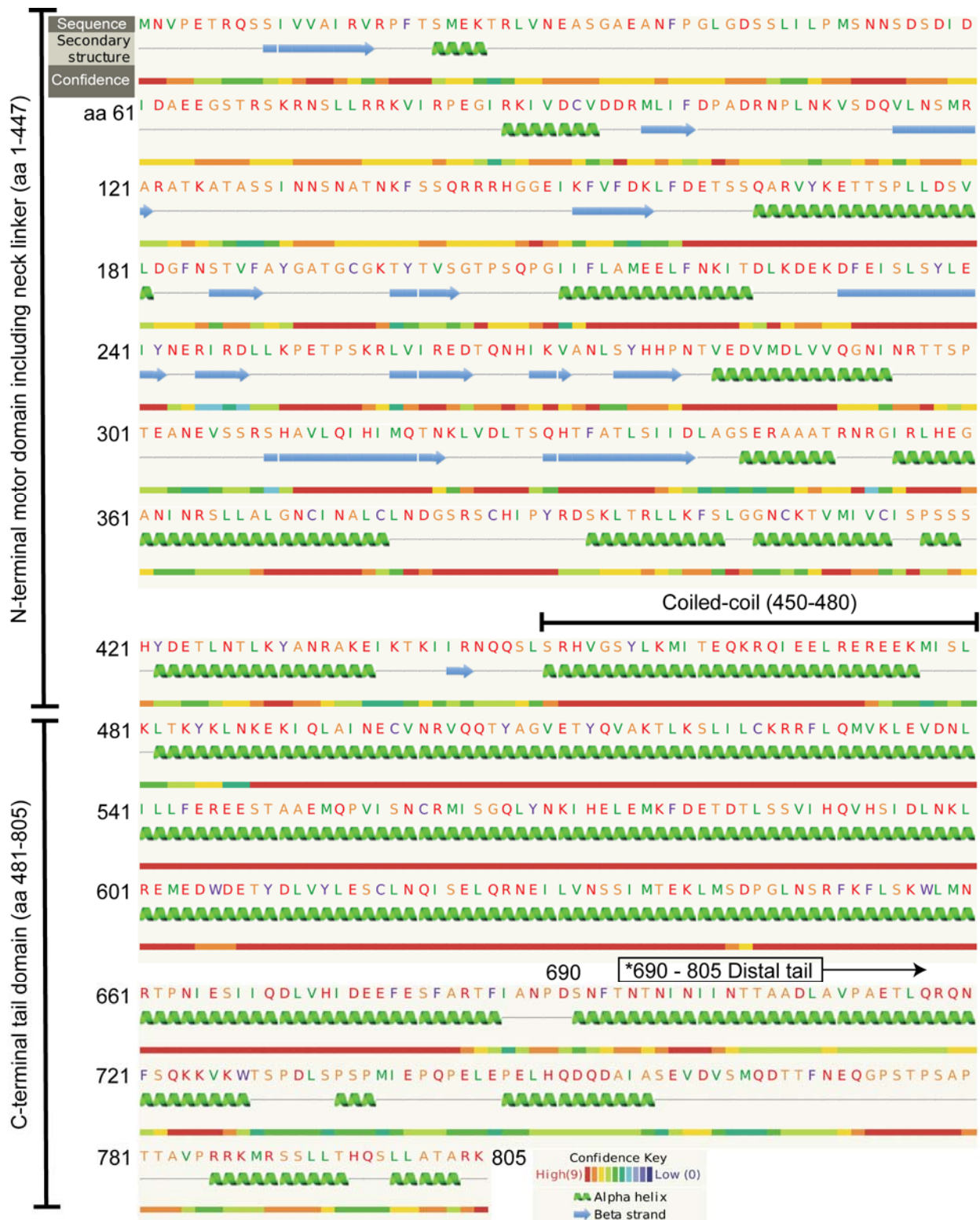


Supplemental Materials

Molecular Biology of the Cell

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Supplementary Figure S1. Predicted secondary structure of Kip3. Major regions are denoted by black brackets. Beyond the motor and coiled-coil regions (1-480), the C-

terminal tail has two distinct regions. The proximal tail region (~481-690) is continuous α -helix, while the distal region (~690-805) is comprised of shorter helices interspersed with less ordered sections. Numbers denote primary sequence (1-805). Structure prediction was performed using Phyre2 (Kelly et al.(2015). Nature Protocols 10(6):845-58).

SupplementalTableS1. Yeast strains and plasmids used in this study.

Strain name	Relevant genotype	Source
MGY50	<i>MATaura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY142	<i>MATaKIP3-13myc-HIS3 ura3-52 leu2-Δ1 trp1-Δ63 his3-Δ200</i>	
MGY249	<i>MATaKIP3-Δdistal-13myc-HIS3ura3-52 leu2-Δ1 trp1-Δ63 his3-Δ200</i>	
MGY256	<i>MATaCFP-TUB1-URA3 KIP3-3YFP-LEU2 his3-Δ200 trp1-Δ63 leu2-Δ1</i>	
MGY258	<i>MATaGFP-TUB1-URA3 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY276	<i>MATaGFP-TUB1-URA3 kip3Δ::KanMX his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY 312	<i>MATαkip3Δ::KanMX ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY316	<i>MATaGFP-TUB1-URA3 KIP3-Δdistal-KanMX his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY345	<i>MATaCFP-TUB1-URA3 KIP3-Δdistal-3YFP-LEU2 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY960	<i>MATaGFP-TUB1-URA3 cdc15-2 his3-Δ200 leu2Δ1 trp1Δ63</i>	Rizk et al.
MGY962	<i>MATaGFP-TUB1-URA3 cdc15-2 kip3Δ::KanMX his3-Δ200 leu2Δ1 trp1Δ63</i>	Rizk et al.
MGY1556	<i>MATaGFP-TUB1-LEU2 dyn1Δ::TRP1 ura3-52 his3-Δ200 trp1-Δ63</i>	Fukuda et al.

MGY1572	<i>MATaGFP-TUB1-LEU2 dyn1Δ::TRP1 2xKIP3ΔT-LZ-KanMX ura3-52 his3-Δ200 trp1-Δ63</i>	Fukuda <i>et al.</i>
MGY1575	<i>MATa GFP-TUB1-LEU2 KIP3ΔT-LZ ura3-52 his3-Δ200 trp1Δ63</i>	Fukuda <i>et al.</i>
MGY1576	<i>MATa GFP-TUB1-LEU2 2xKIP3ΔT-LZ-KanMX ura3-52 his3-Δ200 trp1Δ63</i>	Fukuda <i>et al.</i>
MGY1561	<i>MATaKIP3ΔT-LZ ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	Su <i>et al.</i>
MGY1960	<i>MATaGFP-TUB1-LEU2 cdc15-2-KanMX KIP3-Δdistal-TRP1 ura3-52 his3-Δ200 trp1-Δ63</i>	
MGY2211	<i>MATaGFP-TUB1-URA3 MYO1-GFP-TRP1 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY2209	<i>MATaGFP-TUB1-URA3 MYO1-GFP-TRP1 doc1Δ::NatMX4 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY2210	<i>MATaGFP-TUB1-URA3 KIP3-Δdistal-KanMX MYO1-GFP-TRP1 doc1Δ::NatMX4 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY2213	<i>MATadyn1Δ::KanMX KIP3-Δdistal-3YFP-LEU2 CFP-TUB1-URA3 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY2214	<i>MATadyn1Δ::KanMX KIP3-3YFP-LEU2 CFP-TUB1-URA3his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY2215	<i>MATadyn1Δ::TRP1 ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63</i>	
MGY2216	<i>MATadyn1Δ::TRP1 KIP3-Δdistal-KanMX GFP-TUB1-LEU2 ura3-52 his3-Δ200 trp1-Δ63</i>	

Plasmid name	Description and markers	Source
pAFS125	<i>pTUB1-GFP-TUB1-URA3, ampR</i>	Straight <i>et al.</i>
pMG130	<i>pTUB1-CFP-TUB1-URA3, ampR</i>	
pMG162	<i>KIP3-Δdistal-3YFP-LEU2, ampR</i> (Plasmid containing 500 bp of <i>KIP3</i> coding sequence from aa 524 to 690 fused by a Gly-Ala-Gly-Ala-Gly-Asp-Pro-Val-Ala-Thr linker to 3 tandem copies of YFP followed by a stop codon. Linearization with Swal within the <i>KIP3</i> coding region prior to transformation will direct integration and truncation of endogenous Kip3 at aa 690.)	

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