Supporting Information

Tu et al. 10.1073/pnas.1203035109

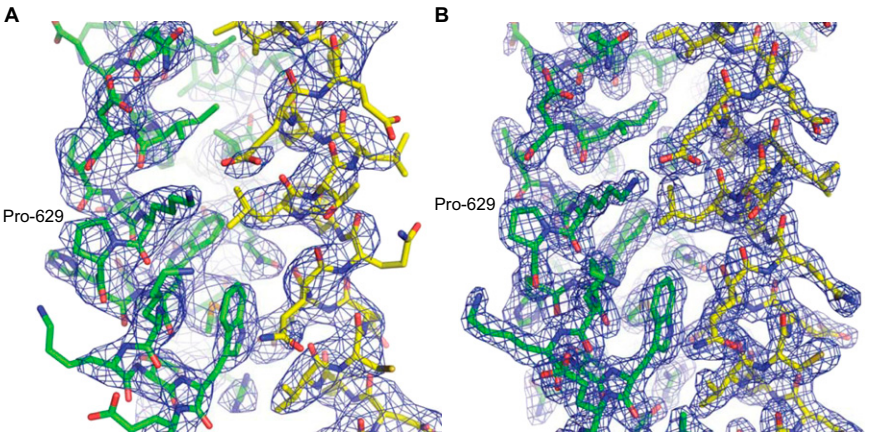

Fig. S1. Electron density maps of Bud6 core. (*A*) Solvent flattened experimental map at 2.9 Å, contoured at 1σ. The dimer interface around the proline residue, Pro-629, is illustrated. Two chains are shown in different colors. (*B*) σ_A-weighted $2F_o - F_c$ map at 2.04 Å, contoured at 1.3 σ. The viewing angle is the same as in *A*.

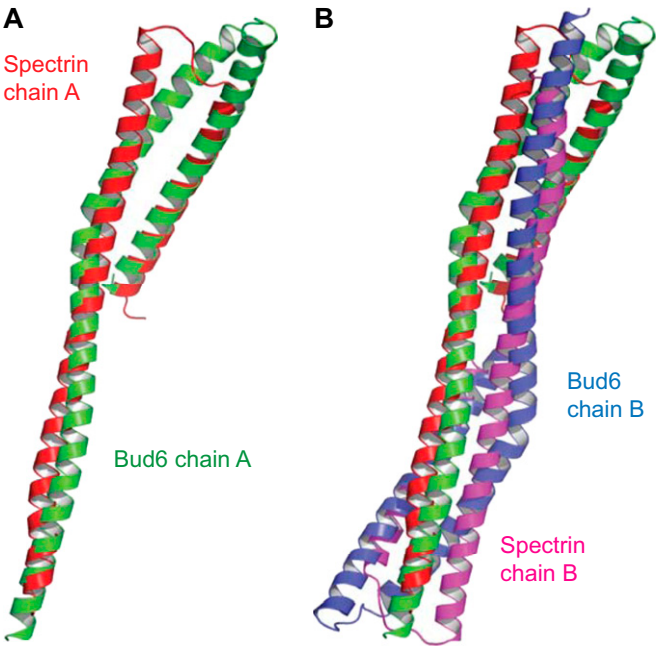

Fig. S2. Superposition of Bud6 core with a domain-swapped form of spectrin repeat using the overlay matrix determined by distance matrix alignment (DALI). (*A*) One chain of Bud6 (residues 553–672, colored green) superimposes with the 14th segment of *Drosophila* α-spectrin (Protein Data Bank ID code 2SPC; chain A, colored red) with an rmsd deviation of 3.1 Å for 96 aligned Cα atoms and a Z-score of 7.1. (*B*) Entire Bud6 core dimer is shown together with the spectrin dimer, which is not thought to be physiological.

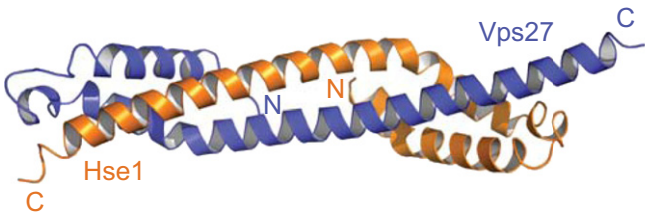

Fig. S3. Crystal structure of a Vps27/Hse1 complex (Protein Data Bank ID code 2PJW) has a topology resembling that of the Bud6 core dimer.

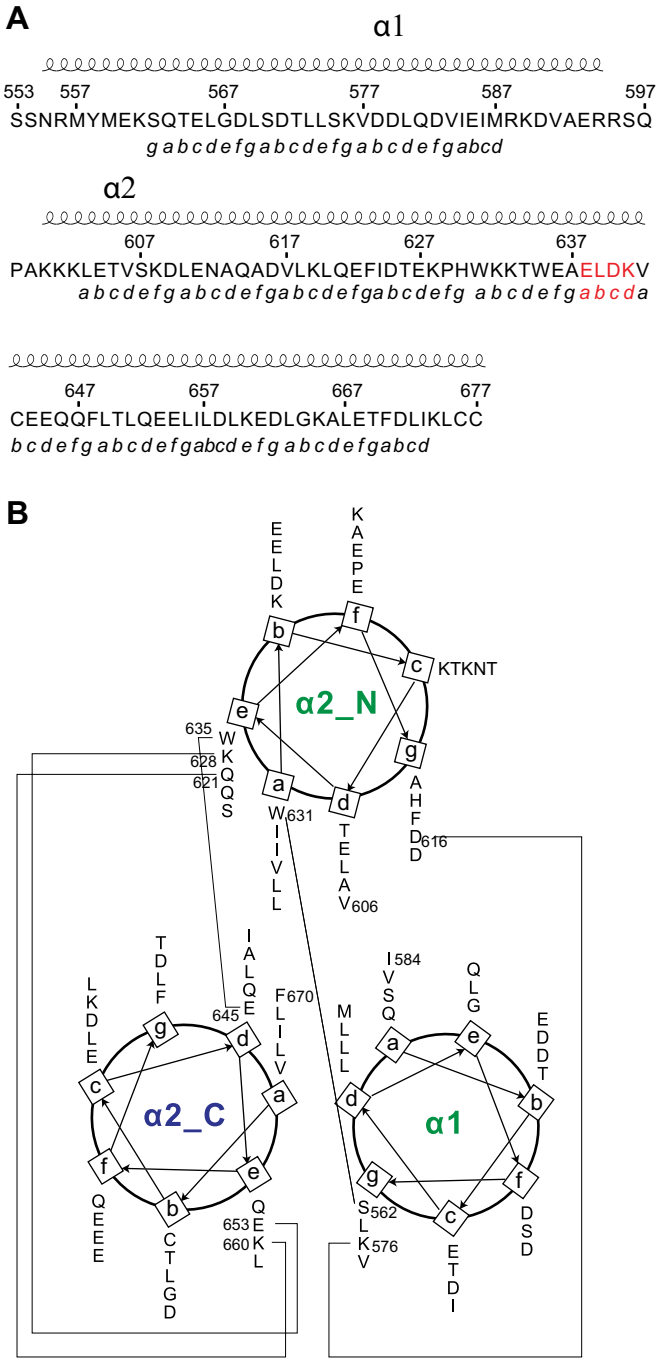

Fig. S4. Coiled-coil interface of Bud6^{core}. (*A*) Heptad register according to the knobs-into-holes criterion is displayed below the primary sequence. The secondary structure is on top. We noted a change in the phasing of the heptad pattern of helix α2, where the structure changes momentarily from a three-helix to two-helix coiled-coil and then reenters the three-helix coiled-coil. This type of discontinuity has been classified as a stutter (1), in which a heptad repeat is shortened to four residues, leaving only residues *a*–*d*. This stutter in Bud6^{core} is colored red. (*B*) Helical wheel diagram of the three-helix coiled-coil. α2_N stands for the N-terminal portion of helix α2 before it enters the stutter. α2_C stands for the C-terminal portion of helix α2 after the stutter. Note that the green α1 and α2_N are from one subunit and the blue α2_C is from the other subunit. From the N terminus to the C terminus, helix α1 is coming out of the page toward the reader, whereas helix α2_N is going into the page. Helix α2_C is also coming out of the page toward the reader. Selected residues are numbered, and polar interactions are indicated by connecting lines.

1. Brown JH, Cohen C, Parry DA (1996) Heptad breaks in alpha-helical coiled coils: stutters and stammers. *Proteins* 26(2):134–145.

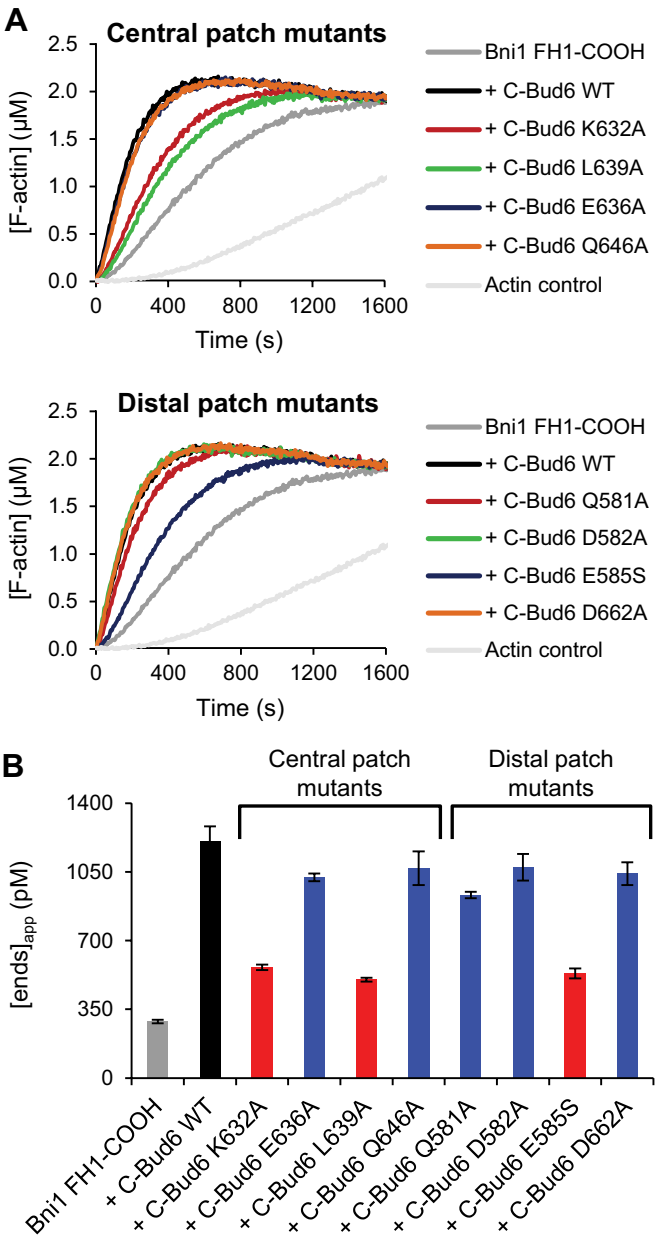

Fig. S5. Effects of C-terminal fragment of Bud6 (c-Bud6) polypeptides on Bni1-mediated filament nucleation. (*A*) G-actin (2 mM) was assembled in the presence of Bni1 formin homology-1 (FH1)–COOH (10 nM) and WT or mutant c-Bud6 (200 nM) as indicated. (*B*) Quantitation of the raw curves in *A*. Concentrations of filament ends were calculated from the slopes of the raw curves measured at 50% polymerization (a detailed description of the calculation is provided in *Materials and Methods*). Each bar represents an average of three independent trials. Red bars indicate point mutants that impair Bud6 NPF activity; blue bars indicate pseudo-WT mutations. Error bars indicate SEM.

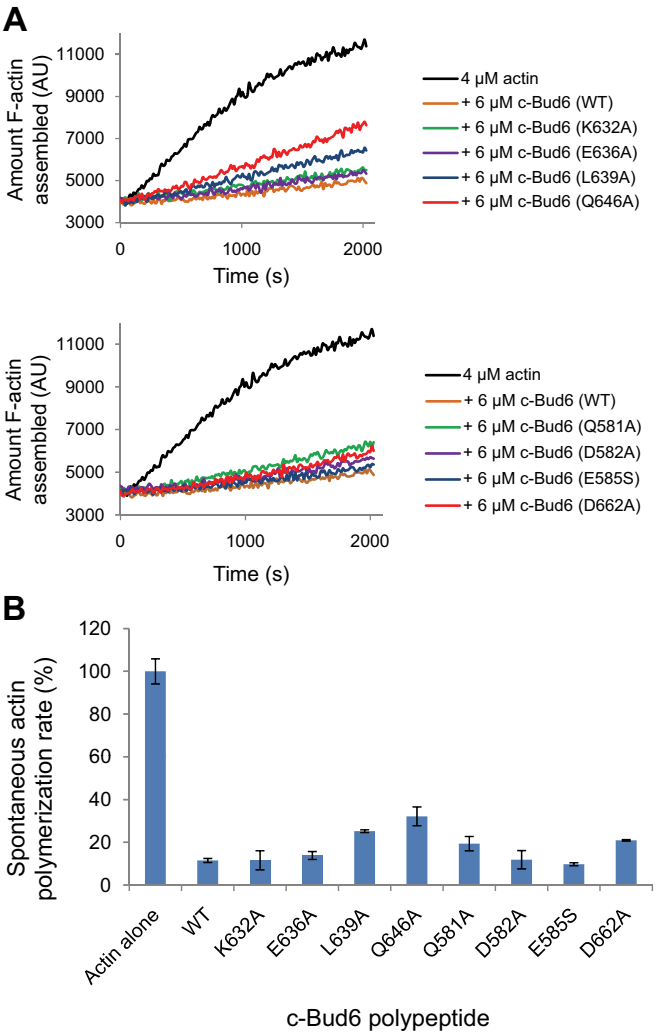

Fig. S6. Direct binding of C-terminal fragment of Bud6 (c-Bud6) polypeptides to monomeric actin via suppression of spontaneous nucleation. (*A*) Monomeric actin (4 μM, 2.5% pyrene-labeled) was polymerized alone or in the presence of the indicated c-Bud6 polypeptide (6 μM). (*B*) Quantification of reactions in *A*. The rate of spontaneous polymerization in reactions containing actin alone was normalized to 100%. Rates of reactions containing the indicated c-Bud6 polypeptides were scaled relative to reactions containing actin alone. Each bar represents an average of at least two trials. AU, arbitrary units; These are the raw fluorescence readings.

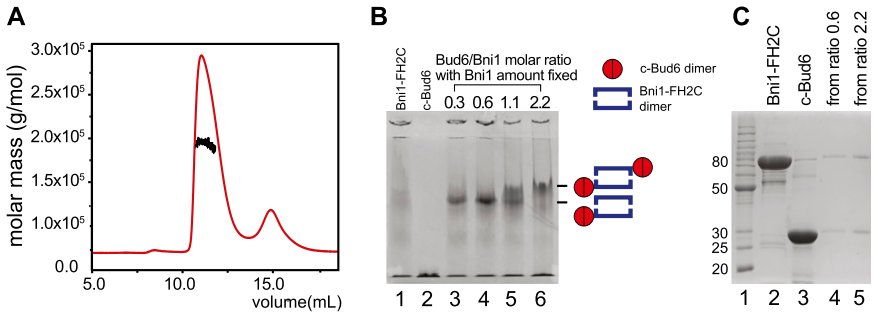

Fig. S7. Stoichiometry of Bni1/Bud6 complexes. (*A*) Analysis of the Bud6^{core}/Bni1–formin homology-2 (FH2C) complex by size exclusion chromatography (SEC) multiangle light scattering (MALS) revealed a molar mass of ~196 kDa, consistent with a 2:1 complex of Bud6^{core} and the Bni1 construct encompassing its FH2 domain and C-terminal tail region (Bni1-FH2C) dimer, which has an expected molar mass of 208 kDa. (*B*) Native-PAGE analysis of C-terminal fragment of Bud6 (c-Bud6) and Bni1-FH2C. The Bni1-FH2C protein alone (lane 1) or with increasing molar ratios of c-Bud6 as indicated (lanes 3–6) was resolved on a 7.5% native gel. Bni1 alone does not form a well-defined band (lane 1), and c-Bud6 alone migrates at the dye front (lane 2), but the complex forms clear bands that migrate in the center of the gel (lanes 3–6). Two species are apparent: a faster migrating (*Lower*) band seen with substoichiometric amounts of Bud6 and a more slowly migrating (*Upper*) band that predominates at a 2.2:1 molar ratio of c-Bud6 to Bni1. As indicated by the schematic, this is the expected banding pattern for binding of one (*Lower*) or two (*Upper*) c-Bud6 dimers to noninteracting sites on the Bni1-FH2C dimer. (*C*) SDS/PAGE analysis of excised native-PAGE bands of the Bud6/Bni1 complex. The bands from Bud6/Bni1 molar ratios of 0.6 and 2.2 in *B* were exercised and resolved on a 12% SDS/PAGE denaturing gel.

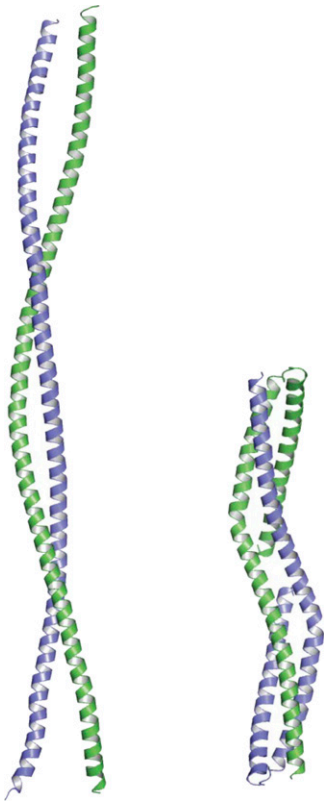

Fig. S8. Comparison of structures of p160^{rock} (residues 535–700) and Bud6^{core}. The structures are displayed at the same scale.

Other Supporting Information Files

Dataset S1 (PDF)