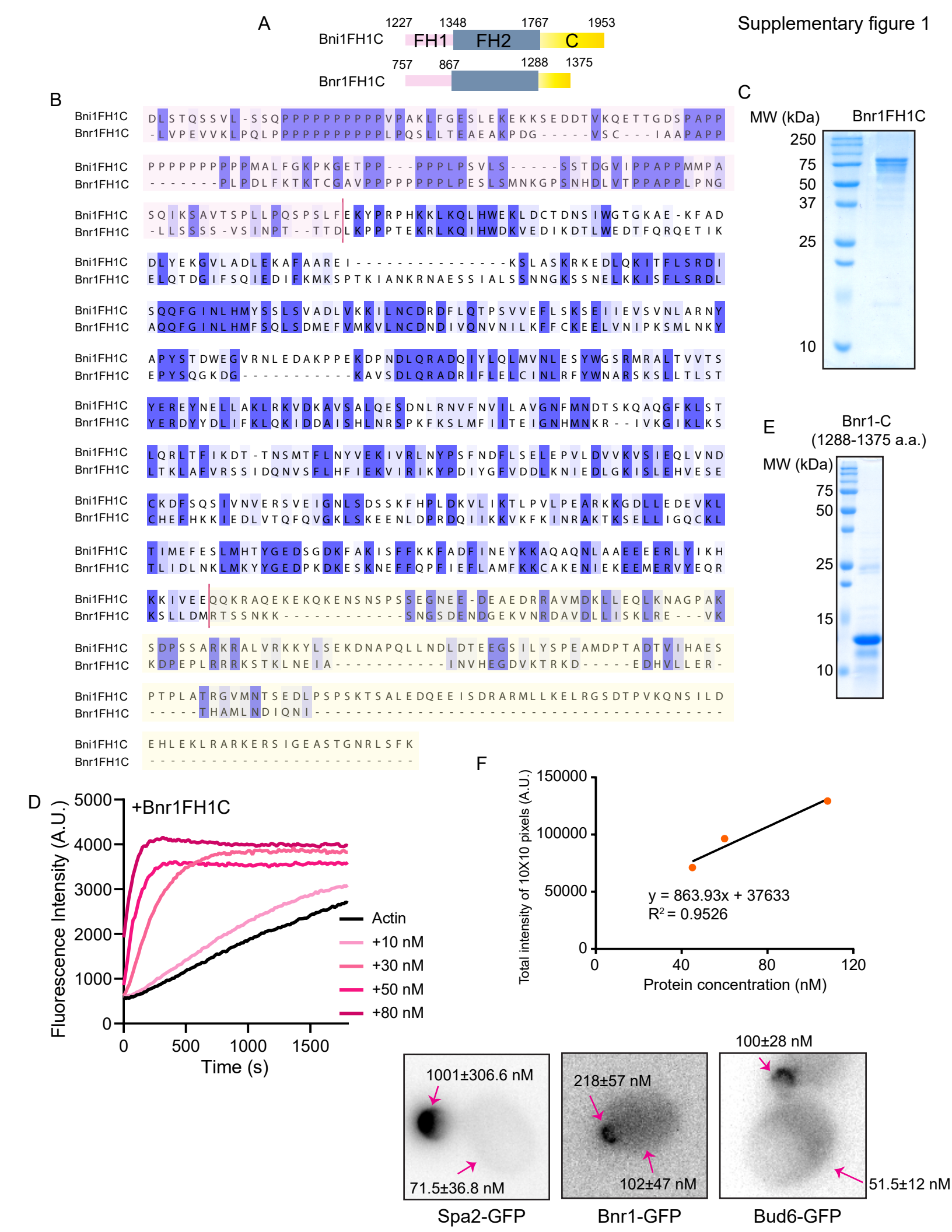
Supplemental Materials

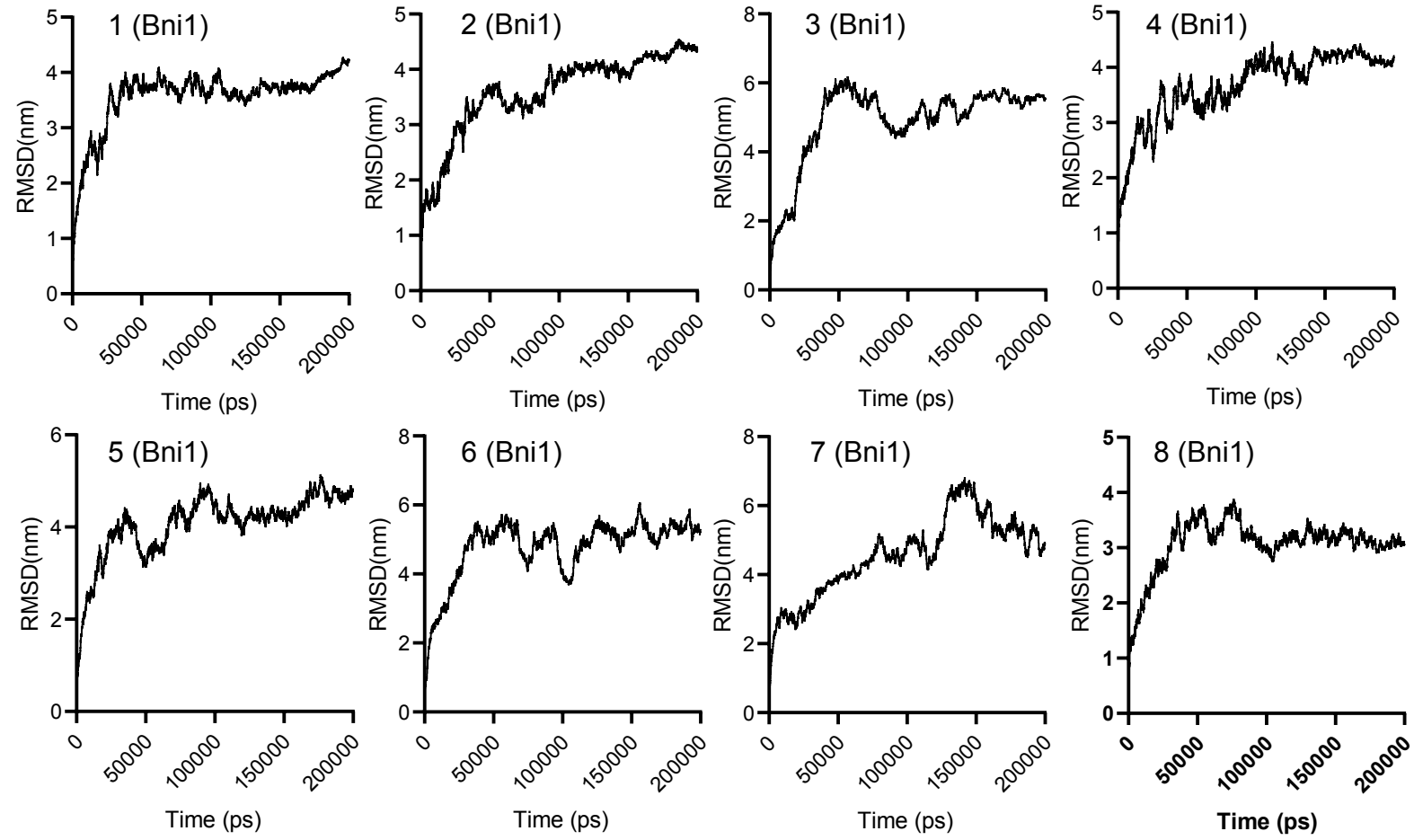
Molecular Biology of the Cell

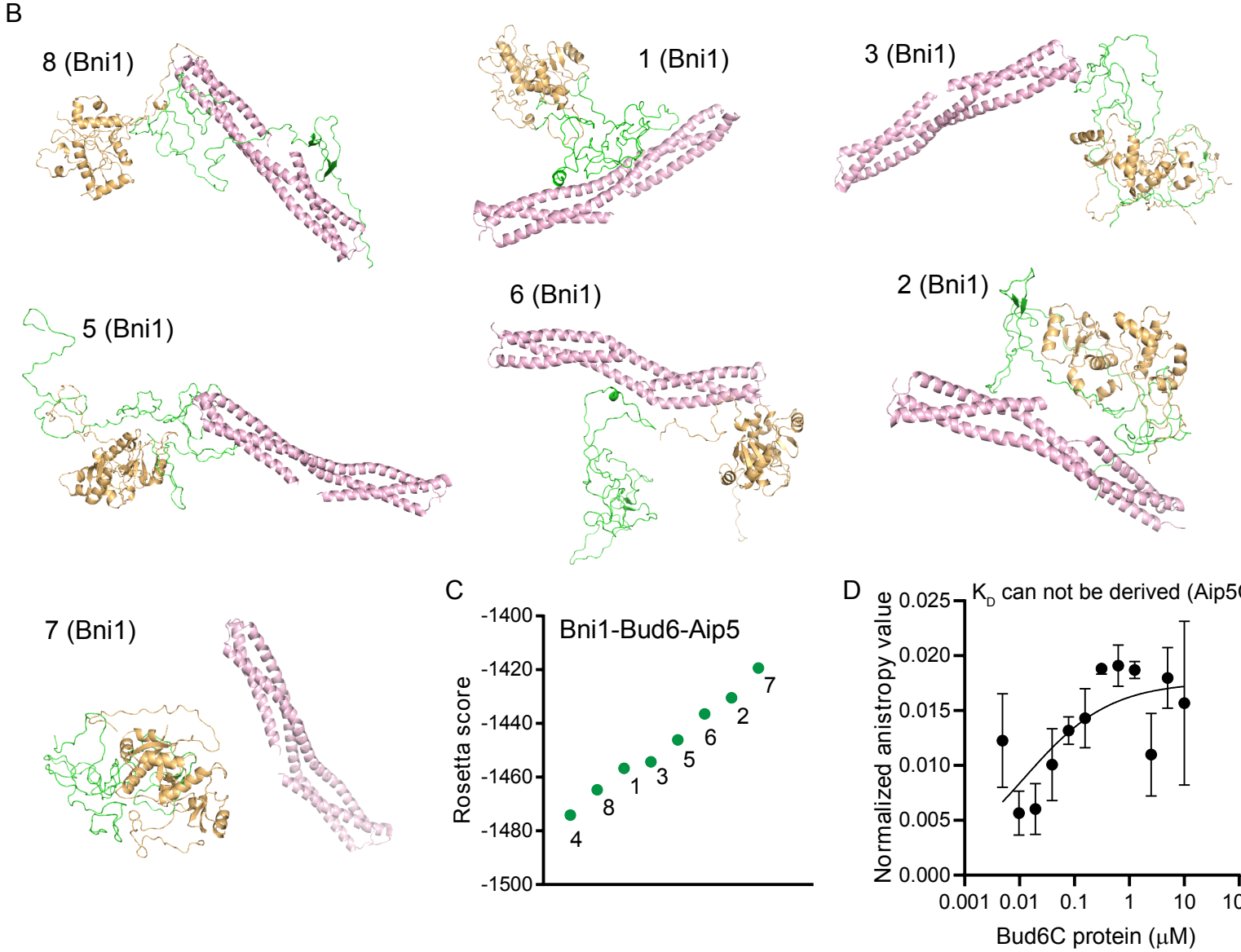
Xie *et al*.

Supplementary figure 1



Supplementary figure 1. Conservative analysis of formin sequences in *S. cerevisiae*
(A) Domain schematic of yeast formins (FH domain, formin homolog domain). (B) Protein sequence alignment of Bni1FH1C and Bnr1FH1C. The FH1 domain is highlighted by pink color, whereas the C terminal region is highlighted by yellow color. (C) Coomassie-blue staining of purified recombinant protein Bnr1FH1C by SDS-PAGE (D) Pyrene actin polymerization of 2 μM monomeric actin with the indicated concentrations of Bnr1FH1C. (E) SDS-PAGE gel of purified recombinant protein Bnr1-C. (F) In vivo concentration of Bud6 and Bnr1 in the cytosol and concentrated bud-tip or bud-neck using signal intensity measurements compared with reference proteins based on the previously described method (see Method). The protein concentrations standard curve was generated.

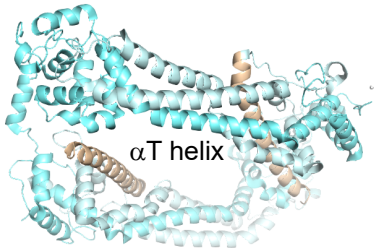

A
1 (Bni1)
2 (Bni1)
3 (Bni1)
4 (Bni1)
5 (Bni1)
6 (Bni1)
7 (Bni1)
8 (Bni1)
RMSD(nm)
Time (ps)


B
8 (Bni1)
1 (Bni1)
3 (Bni1)
5 (Bni1)
6 (Bni1)
2 (Bni1)
7 (Bni1)
C
Bni1-Bud6-Aip5
Rosetta score
D
K_D can not be derived (Aip5C)
Normalized anistropy value
Bud6C protein (μM)

Supplementary figure 2. The RMSD plots and Rosetta scoring of the Bni1-Bud6-Aip5 complexes from MD simulations

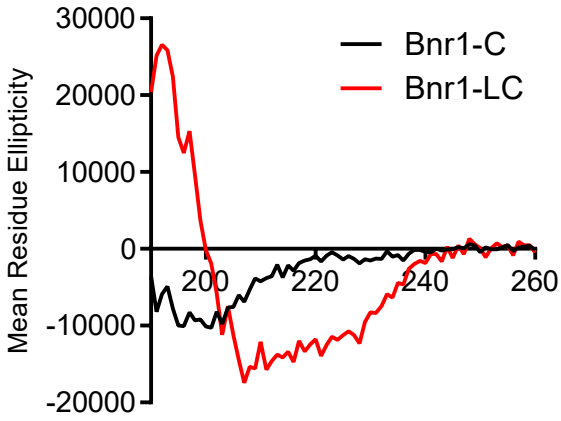
(A) The RMSD plots of eight groups that maintain stable complex conformations after MD simulations. (B) The conformations of the most stable assembly of the Bni1-Bud6-Aip5 tri-protein complex for each set. (C) The Rosetta scores of the eight sets of tri-protein complex after MD simulations. (D) Fluorescence anisotropy binding measurements of 30 nM Alexa-488 labelled Aip5C titrated by a serial concentration of Bud6C, respectively.

A



B

C



D

	Alpha helix	Beta sheet	Random coil
Bnr1-C	0.06	0.46	0.48
Bnr1-LC	0.45	0.23	0.32

Supplementary figure 3. Secondary structure analysis of Bnr1 protein varients
(A) The structure conformation of Bni1-FH2 domain (PDB: 1UX5), the αT helix region is highlighted by orange colour. (B) Sequence alignment of αT helix region between Bni1and Bnr1 proteins. (C) Circular dichroism spectrum of Bnr1-LC and Bnr1-C. (D) Percentage analysis of secondary structures from the circular dichroism spectrum.

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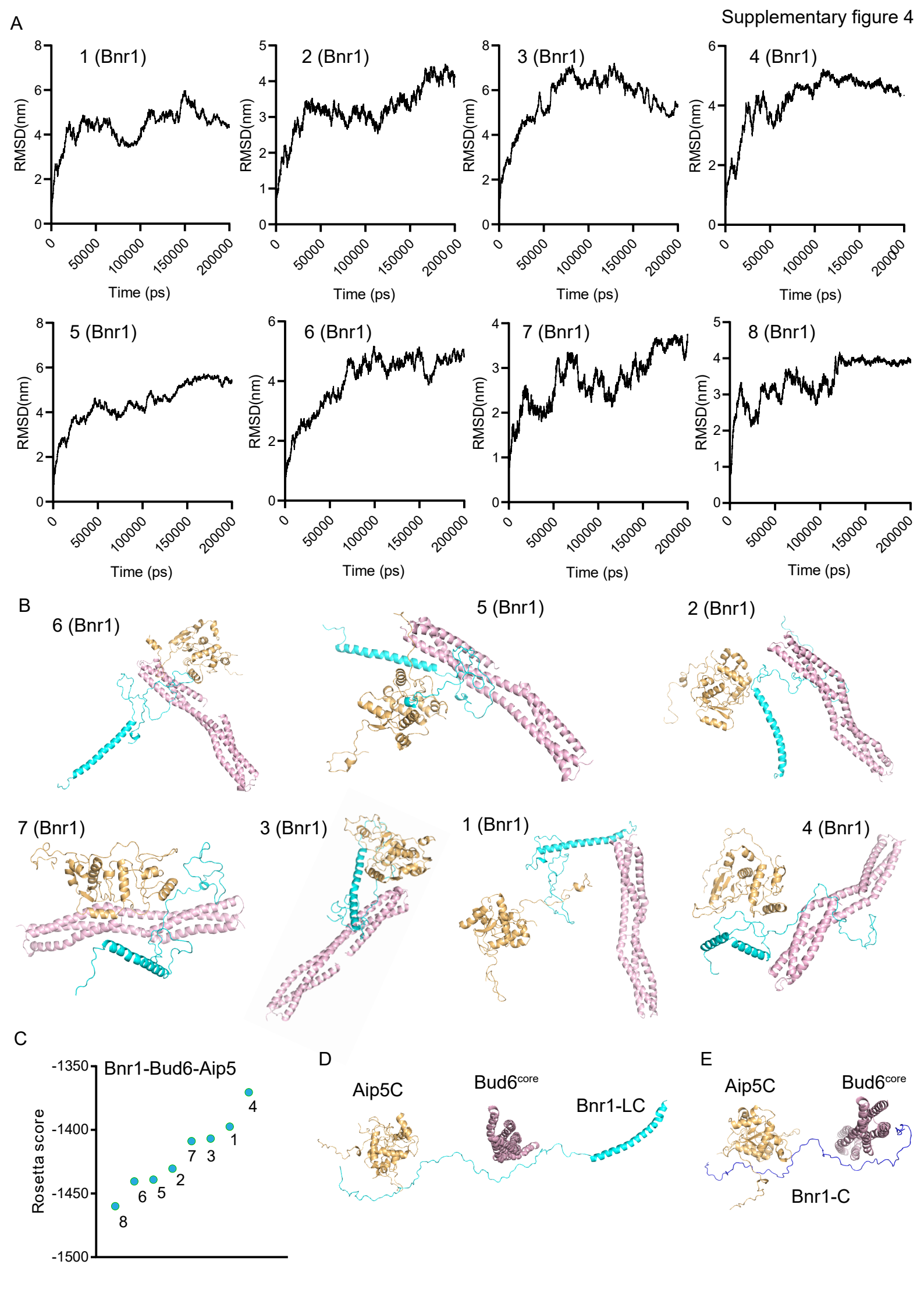
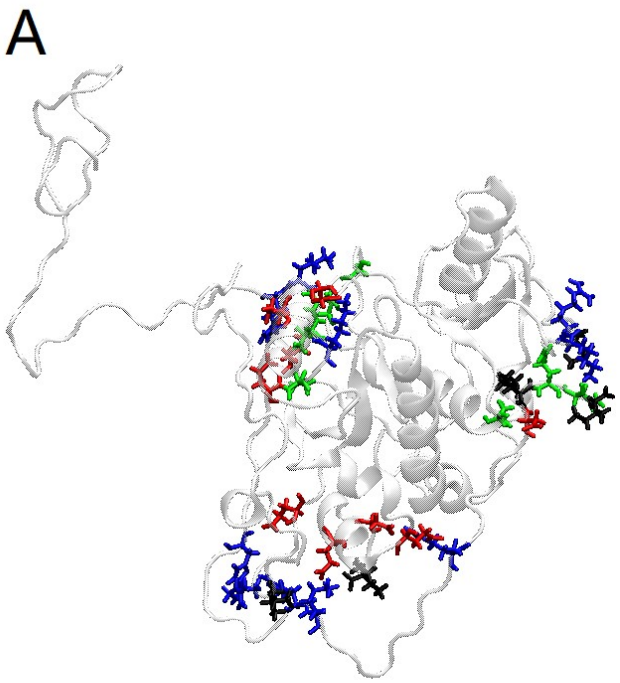
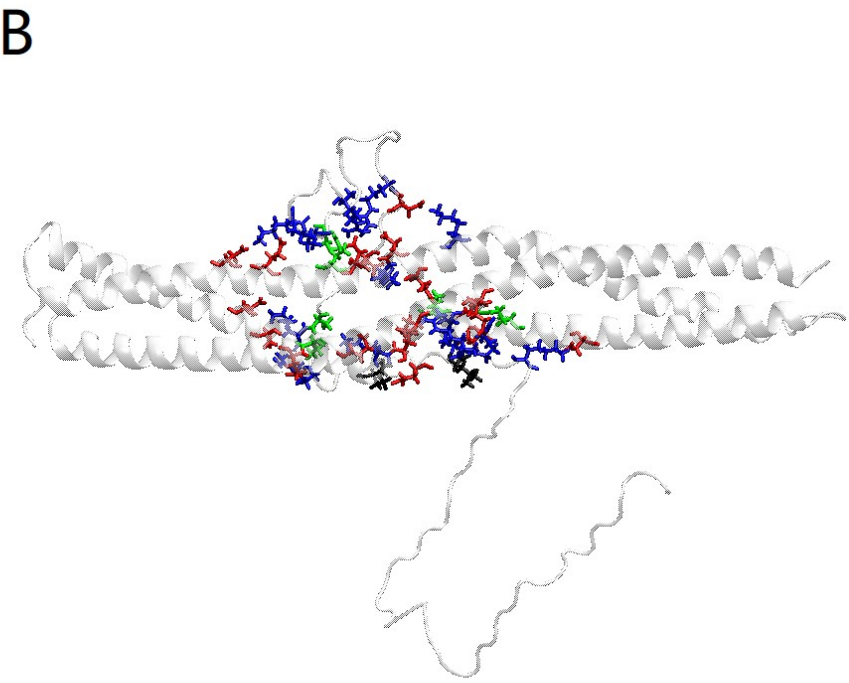

A
1 (Bnr1)
2 (Bnr1)
3 (Bnr1)
4 (Bnr1)
5 (Bnr1)
6 (Bnr1)
7 (Bnr1)
8 (Bnr1)
RMSD(nm)
Time (ps)
B
6 (Bnr1)
5 (Bnr1)
2 (Bnr1)
7 (Bnr1)
3 (Bnr1)
1 (Bnr1)
4 (Bnr1)
C
Bnr1-Bud6-Aip5
Rosetta score
-1350
-1400
-1450
-1500
4
1
7
3
2
6
5
8
D
Aip5C
Bud6core
Bnr1-LC
E
Aip5C
Bud6core
Bnr1-C

Supplementary figure 4. The RMSD plots and Rosetta scoring of the Bnr1-Bud6-Aip5 complexes from MD simulations

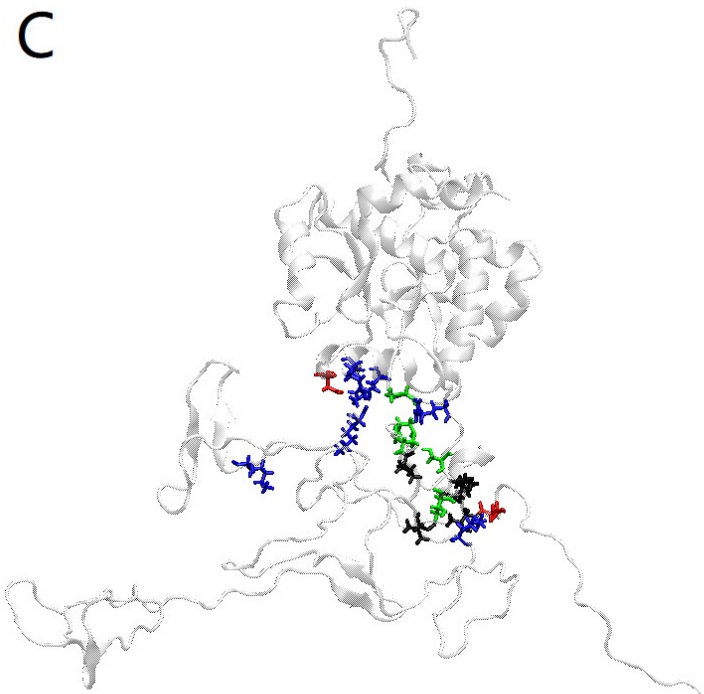
(A) The RMSD plots of eight groups that maintain stable complex conformations after MD simulations. (B) The conformations of the most stable assembly of the Bnr1-Bud6-Aip5 tri-protein complex for each set. (C) The Rosetta scores of the eight sets of tri-protein complex after MD simulations. (D, E) The initial structures of Bnr1 complex before subjecting to the MD simulation.



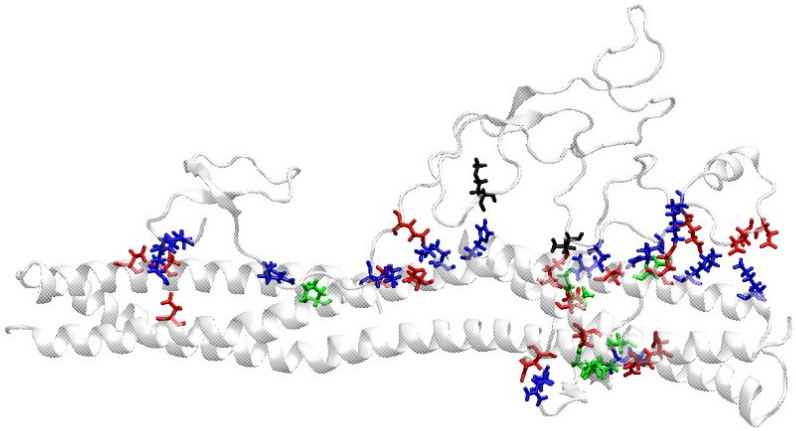
Aip5C-Bnr1C



Bud6-Bnr1C



Aip5C-Bni1C

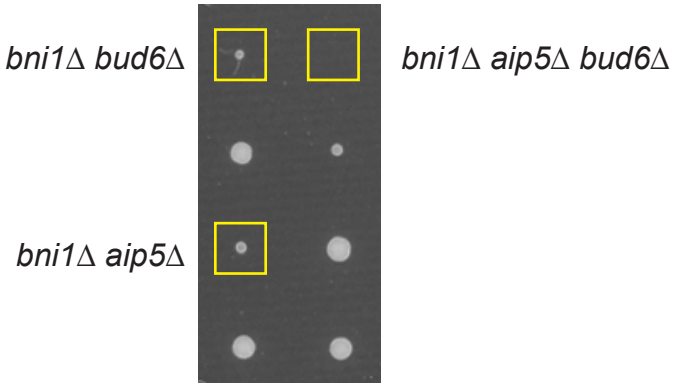


Bud6-Bni1C

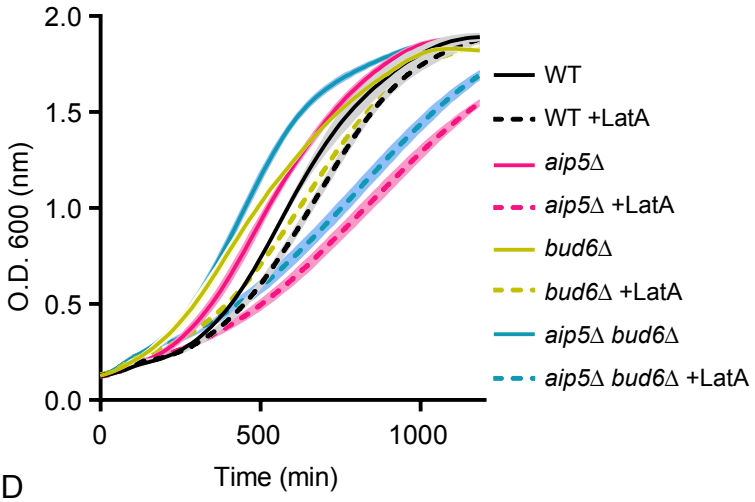
Supplementary figure 5. Types of protein–protein interactions between each NF-NPF pair. The interactions of Bud6core and Aip5C with the Bnr1C and Bni1C were determined via the Rosetta scoring. The negatively charged residues ASP and GLU are colored in red, the positively charged residues ARG, LYS and HIS are colored in blue, the polar residues SER, THR, ASN and GLN etc are colored in green, whereas the hydrophobic residues ALA, VAL, LEU, ILE etc are colored in black.

Supplementary figure 5

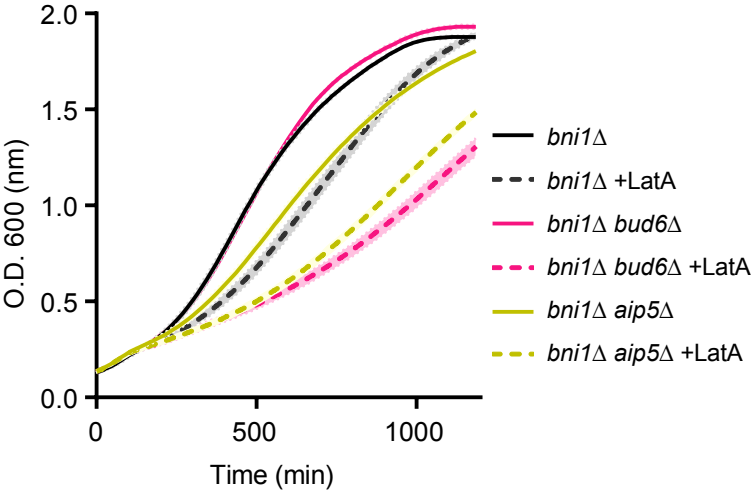
A



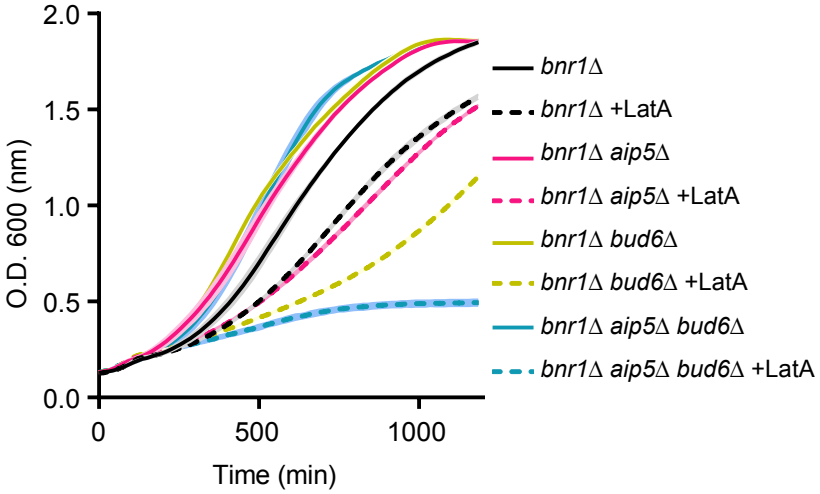
B



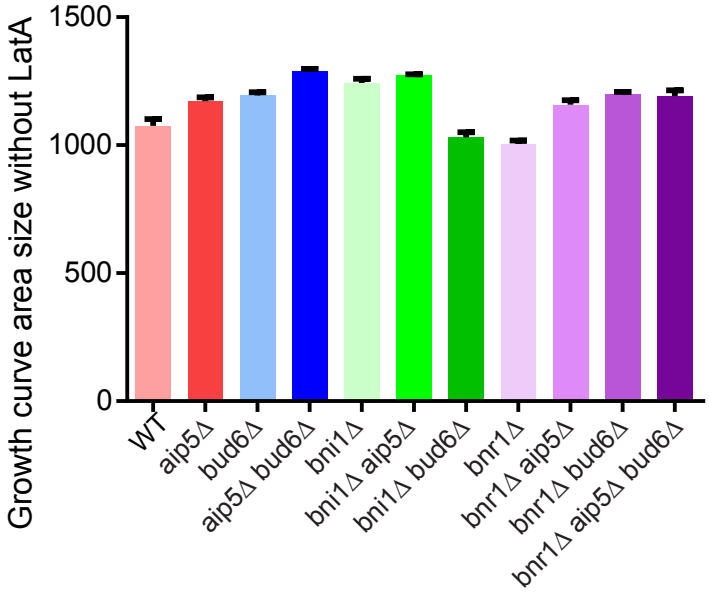
C



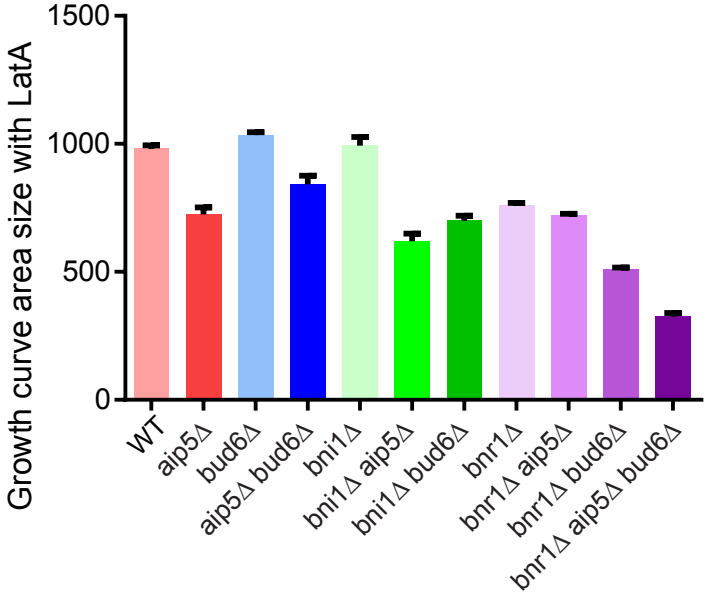
D



E



F



Supplementary figure 6. Yeast cell growth with Latrunculin A treatment.
(A) Tetrads dissection of the indicated diploid. The triple mutant *bni1*Δ *bud6*Δ *aip5*Δ is lethal. (B-D) Growth curve of the indicated yeast mutants that were treated with or without 1 μM Latrunculin A. The data were averaged from four technical replicates and showed with an error bar of S.D. (E, F) Quantification of the indicated strains for growth curve area size without Latrunculin A treatment (E) and with Latrunculin A treatment (F).

Movie S1. TIRF imaging of actin filament assembly. Reactions contain 1 µM actin (10% Oregon green 488 labelled actin and 0.5% biotin-actin), 3 µM yeast Pfy1, 10 nM Bni1FH1C, 2 nM Bnr1FH1C,5 nM Bud6C, or 20 nM Aip5C, as indicated. Images were acquired every 20 sec. Scale Bar, 5 µm. The playback is at 100x speed of the original movie.