Expanded View Figures

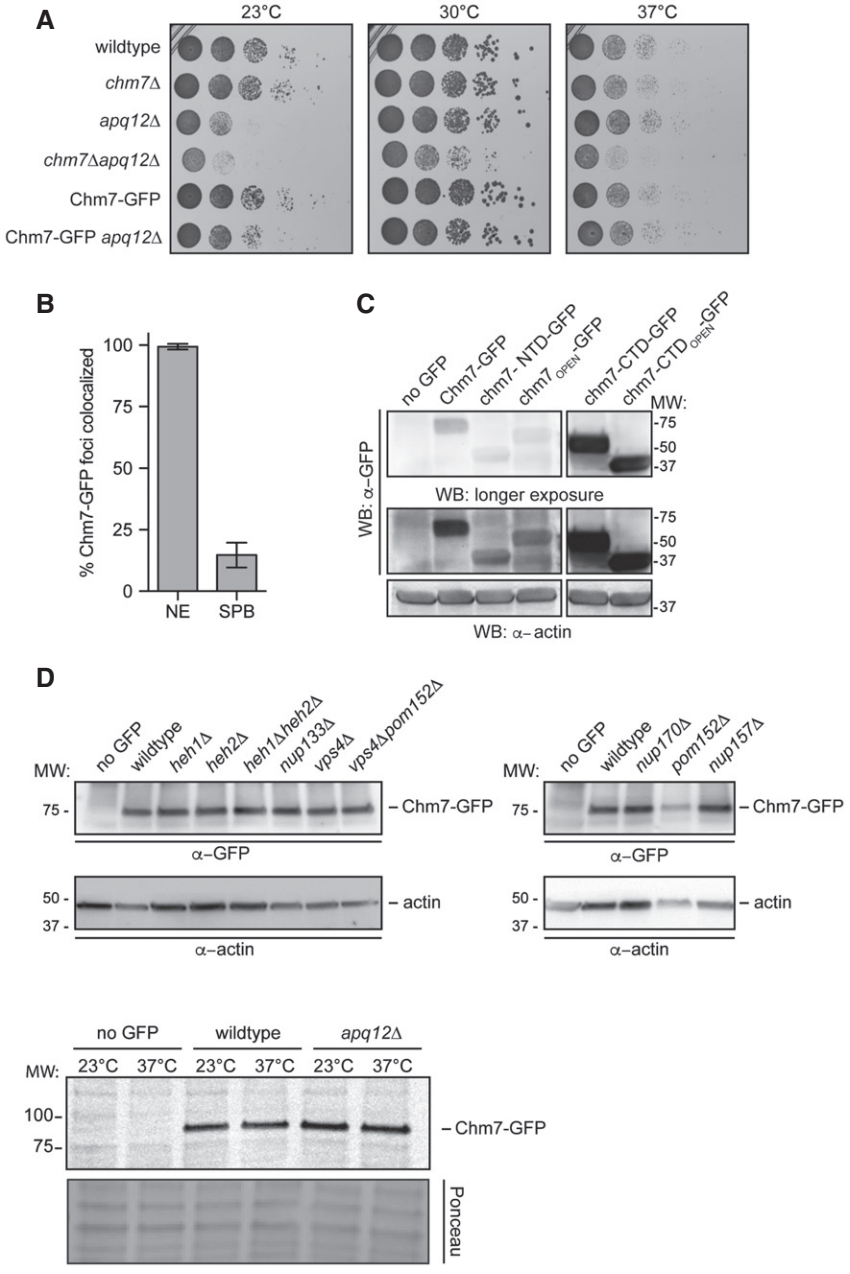

Figure EV1. Expression levels of Chm7-GFP and truncations.

A Tenfold serial dilutions of the indicated yeast strains grown at 23, 30 or 37°C for 2–4 days.

B Plot of the percentage of Chm7-GFP foci that colocalize with the NE or with Mps3-mCherry-labeled SPBs. Error bars are SD from the mean from three independent experiments where > 50 Chm7-GFP foci were counted.

C Relative levels of GFP fusions of Chm7 and truncations assessed by Western blot with anti-GFP antibody and ECL detection; anti-actin is a loading control. Left and right panels are from same nitrocellulose membrane at same exposure. Note that constructs in left panel are expressed from the endogenous *CHM7* promoter, whereas right panel constructs are expressed behind the *GPD* promoter. Numbers reflect positions of molecular weight (MW) standards.

D Western blots (anti-GFP) detecting Chm7-GFP levels in the indicated strains with anti-actin loading control or Ponceau-stained membrane to assess relative protein loads.

Source data are available online for this figure.

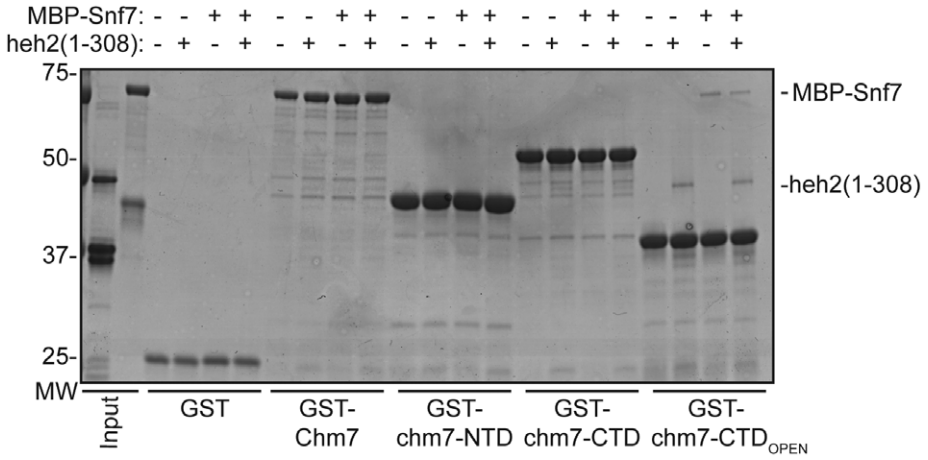

Figure EV2. Co-incubation of Snf7 and Heh2 does not alter their binding to chm7-CTD$_{OPEN}$.
GST and GST fusions of Chm7 and truncations were immobilized on GT-resin and incubated with buffer (—), recombinant heh2(1-308)-His6, or MBP-Snf7. Bound proteins were separated by SDS–PAGE and visualized by Coomassie stain. Numbers on the side of the gel show position of molecular weight (MW) markers.

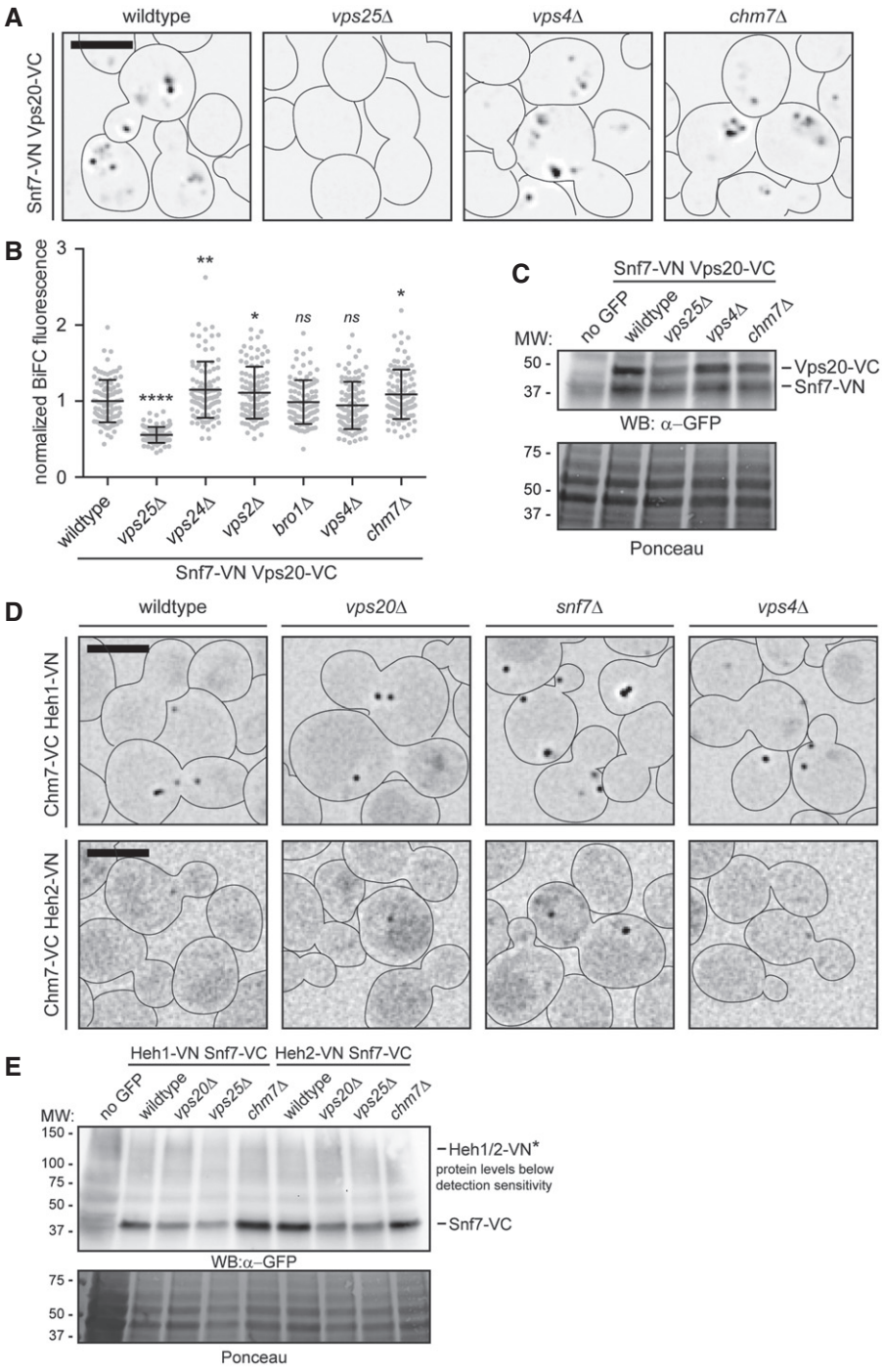

Figure EV3. Chm7 BiFC interactions.

A Deconvolved inverted fluorescence micrographs of BiFC signal of Snf7-VN and Vps20-VC in the indicated genetic backgrounds. Cell borders are outlined. Scale bar is 5 μm.

B Plot of total cellular BiFC fluorescence of Snf7-VN and Vps20-VC in the indicated strains. Data are from three independent replicates where 100 cells per strain were quantified. Error bars are SD from the mean of each replicate. *P*-values from unpaired Student's *t*-test where ns is $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ****$P \leq 0.0001$.

C Western blot using polyclonal anti-GFP antibodies to detect both VN and VC fusions of Snf7-VN and Vps20-VC in the indicated strains; detection by ECL. Bottom panel shows Ponceau-stained nitrocellulose membrane as a total protein load reference. Numbers at the side show positions of molecular weight (MW) markers.

D Deconvolved inverted fluorescence micrographs of BiFC signal in the indicated yeast strains expressing Chm7-VC and either Heh1-VN or Heh2-VN. Cell borders are outlined. Scale bar is 5 μm.

E Western blot of VN and VC fusions in the indicated strains detected using a polyclonal anti-GFP antibody and ECL. To assess relative protein loads, bottom panel shows corresponding Ponceau-stained nitrocellulose membrane.

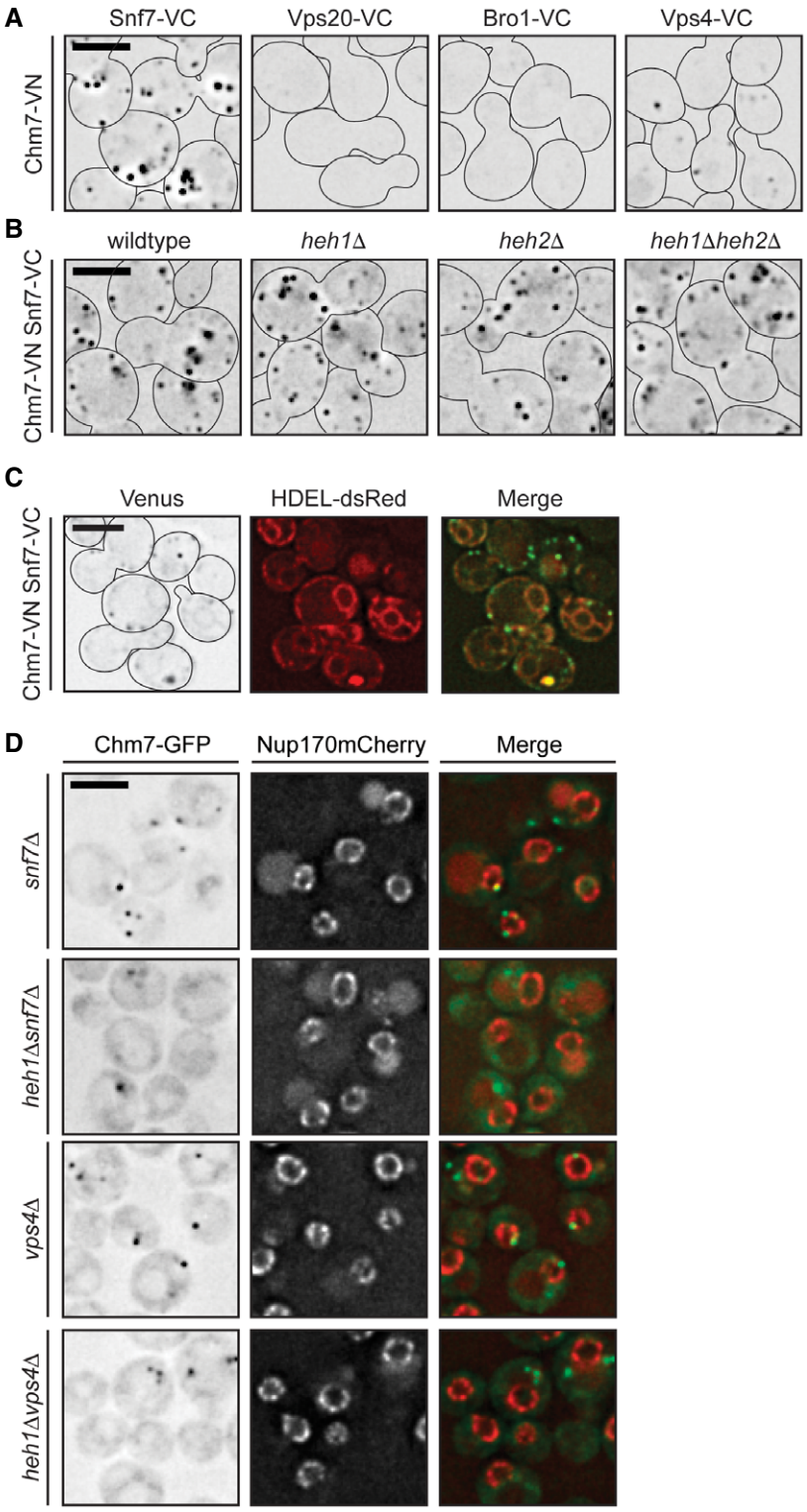

Figure EV4. Chm7-Snf7 interactions can occur outside of the NE.

A Deconvolved inverted fluorescence micrographs of BiFC signal in wild-type yeast strains expressing Chm7-VN and the indicated VC fusions. Cell borders are outlined. Scale bar is 5 μm.

B Deconvolved inverted fluorescence micrographs of BiFC signal of Chm7-VN and Snf7-VC in the indicated null backgrounds. Cell borders are outlined. Scale bar is 5 μm.

C Deconvolved fluorescence micrographs of BiFC signal of Chm7-VN and Snf7-VC with NE/ER marker HDEL-dsRED and merge. Note there is a small amount of bleed-through of the HDEL-dsRED into the Venus channel. Cell borders are outlined. Scale bar is 5 μm.

D Deconvolved fluorescence micrographs of Chm7-GFP and Nup170-mCherry (with merge) in the indicated strains. Scale bar is 5 μm.

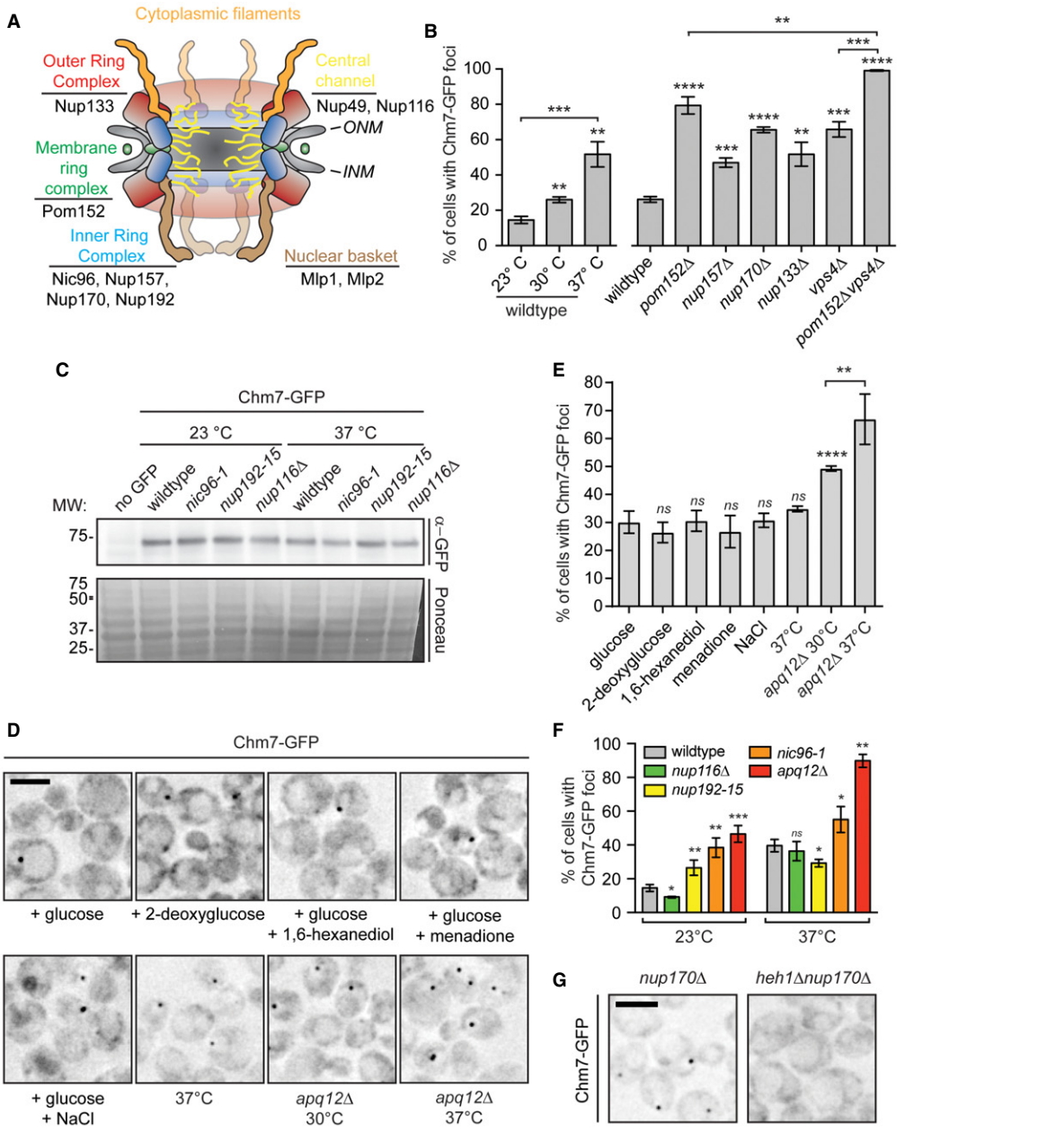

Figure EV5. Chm7-GFP analysis in NPC-defective strains.

A Schematic of the yeast NPC depicting distinct nup subcomplexes and corresponding architectural units. Listed nups are referred to in the text.

B Plot of the percentage of cells with Chm7-GFP foci in the indicated genetic backgrounds and temperatures (23 and 37°C temperature shifts were for 5 h; where not indicated, cells grown at 30°C). Error bars are SD from the mean from three independent experiments where > 175 cells per strain or condition were assessed. *P*-values from unpaired Student's *t*-test. $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; $^{****}P \leq 0.0001$.

C Western blot using an anti-GFP antibody and ECL to detect Chm7-GFP levels in the indicated genetic backgrounds. Cells were grown at the indicated temperatures for 5 h prior to protein harvesting or 3 h for *nup116*Δ cells. Bottom panel is Ponceau-stained nitrocellulose membrane to show relative protein loads.

D Deconvolved inverted fluorescence micrographs of Chm7-GFP in wild-type or *apq12*Δ strains treated as indicated for 45 min. Scale bar is 5 μm.

E Plot of the percentage of cells with Chm7-GFP foci in wild-type cells or *apq12*Δ cells treated as indicated. Data are from three independent replicates of > 150 cells per condition. Error bars represent SD from the mean. *P*-values are from unpaired Student's *t*-test where ns is $P > 0.05$; $^{**}P \leq 0.01$; $^{****}P \leq 0.0001$.

F Plot of the percentage of cells with Chm7-GFP foci in the indicated strains after 5 h at the indicated temperature or 3 h for *nup116*Δ. Data are from three independent replicates of > 100 cells. Error bars represent SD from the mean. *P*-values from unpaired Student's *t*-test where ns is $P > 0.05$; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

G Deconvolved inverted fluorescence micrographs of Chm7-GFP in the indicated strains. Scale bar is 5 μm.