Hayakawa et al., http://www.jcb.org/cgi/content/full/jcb.201108124/DC1



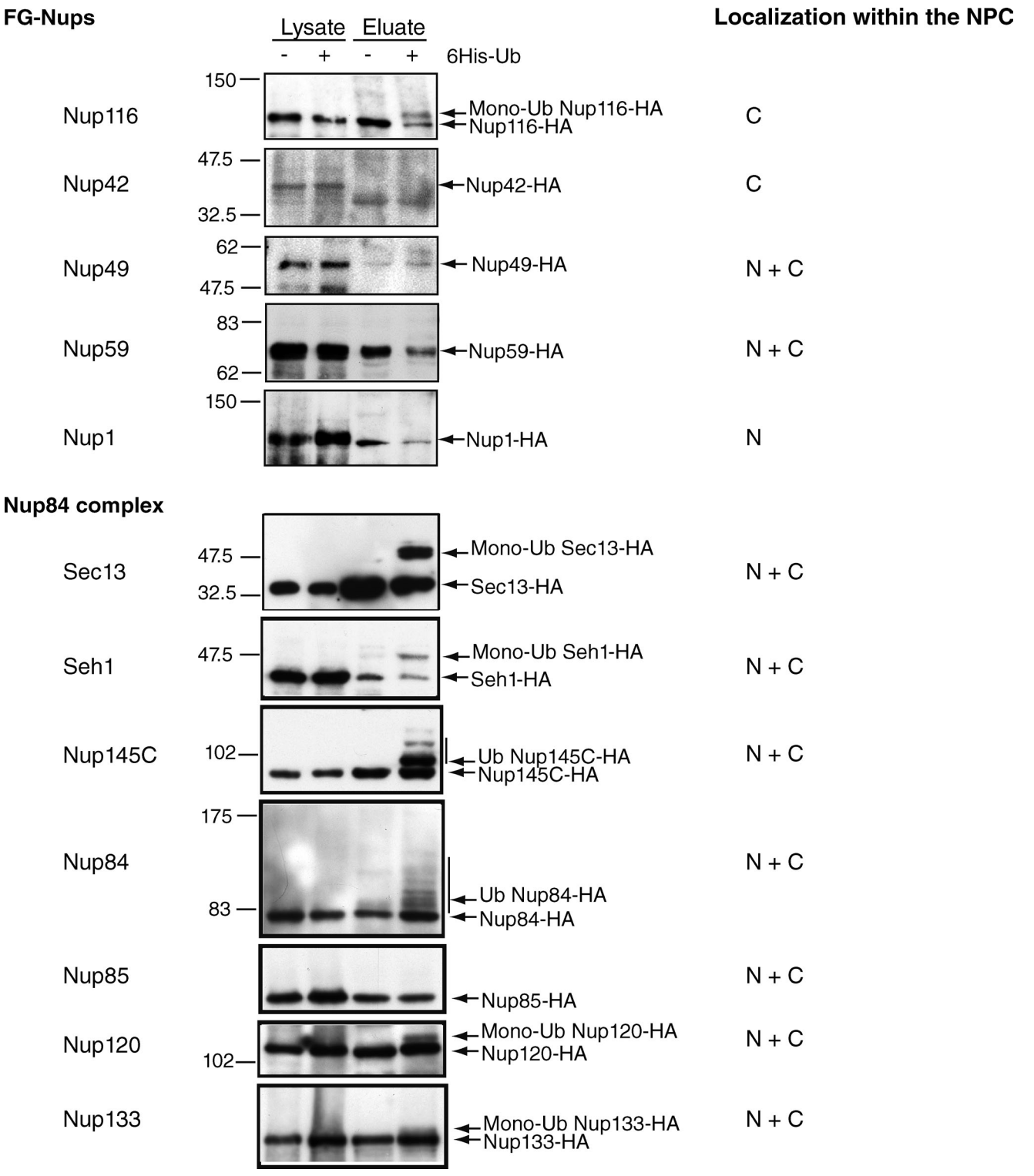


Figure S1. **Ubiquitylation of FG-Nups and Nup84 subcomplex.** Ni-purified 6His-ubiquitin (Ub)–conjugated forms of genomically HA-tagged Nups were extracted from cells transformed (+) or not transformed (−) with a plasmid encoding 6His-ubiquitin under the control of the *CUP1* promoter. Cell lysates and Ni-purified material were examined by Western blotting with an anti-HA antibody. Ubiquitin expression and efficiency of purification were controlled using an anti-6His antibody (not depicted). Localization of Nups as previously determined (Rout et al., 2000) is indicated on the right (N, nuclear side of the NPC; C, cytoplasmic side of the NPC). Molecular markers are given in kilodaltons.

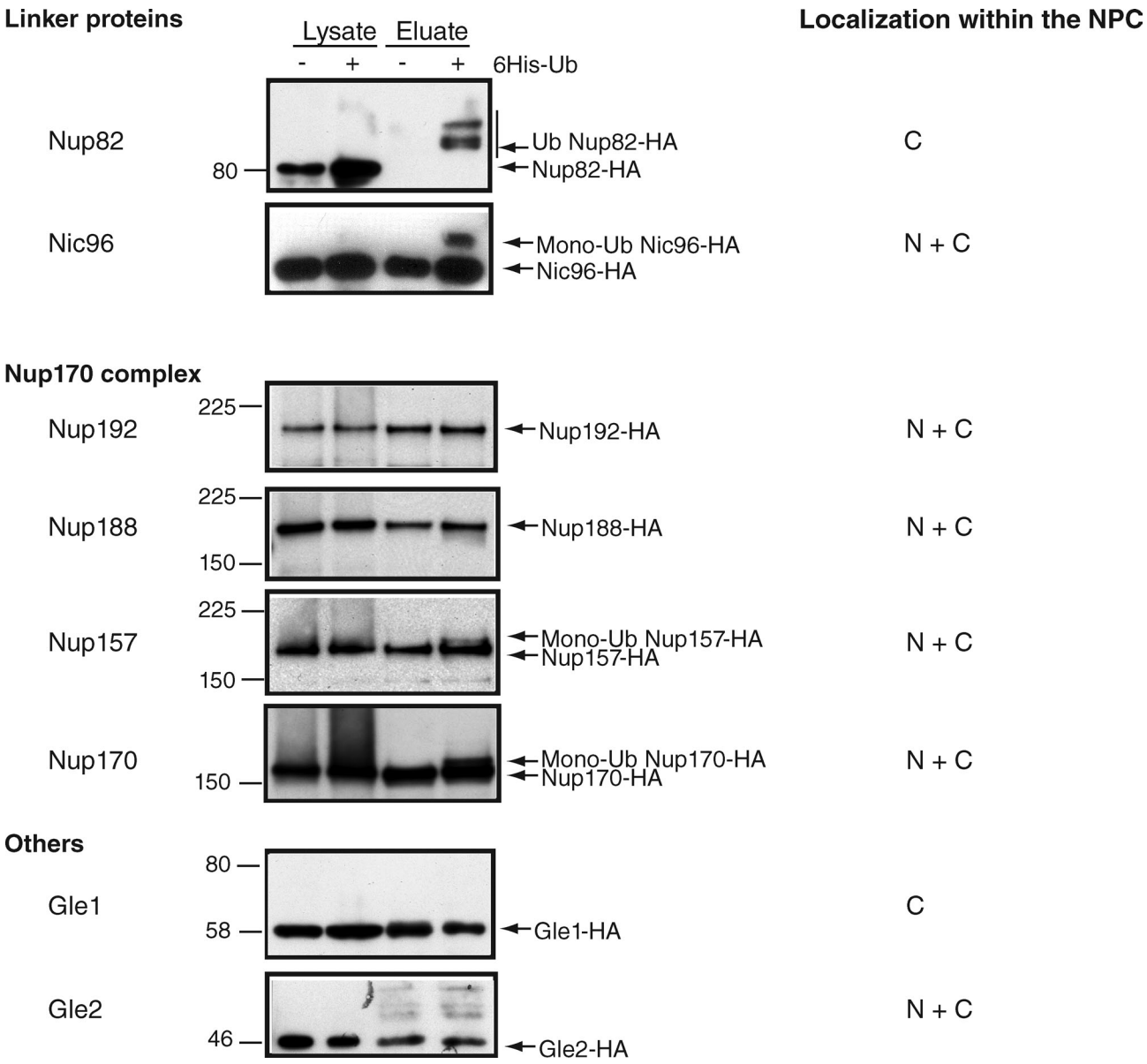


Figure S2. **Ubiquitylation of linker Nups, Nup170 subcomplex, Gle1, and Gle2.** Ni-purified 6His-ubiquitin (Ub)–conjugated forms of genomically HA-tagged Nups were analyzed as in Fig. S1. Localization of Nups as previously determined (Rout et al., 2000) is indicated on the right (N, nuclear side of the NPC; C, cytoplasmic side of the NPC). Molecular markers are given in kilodaltons.

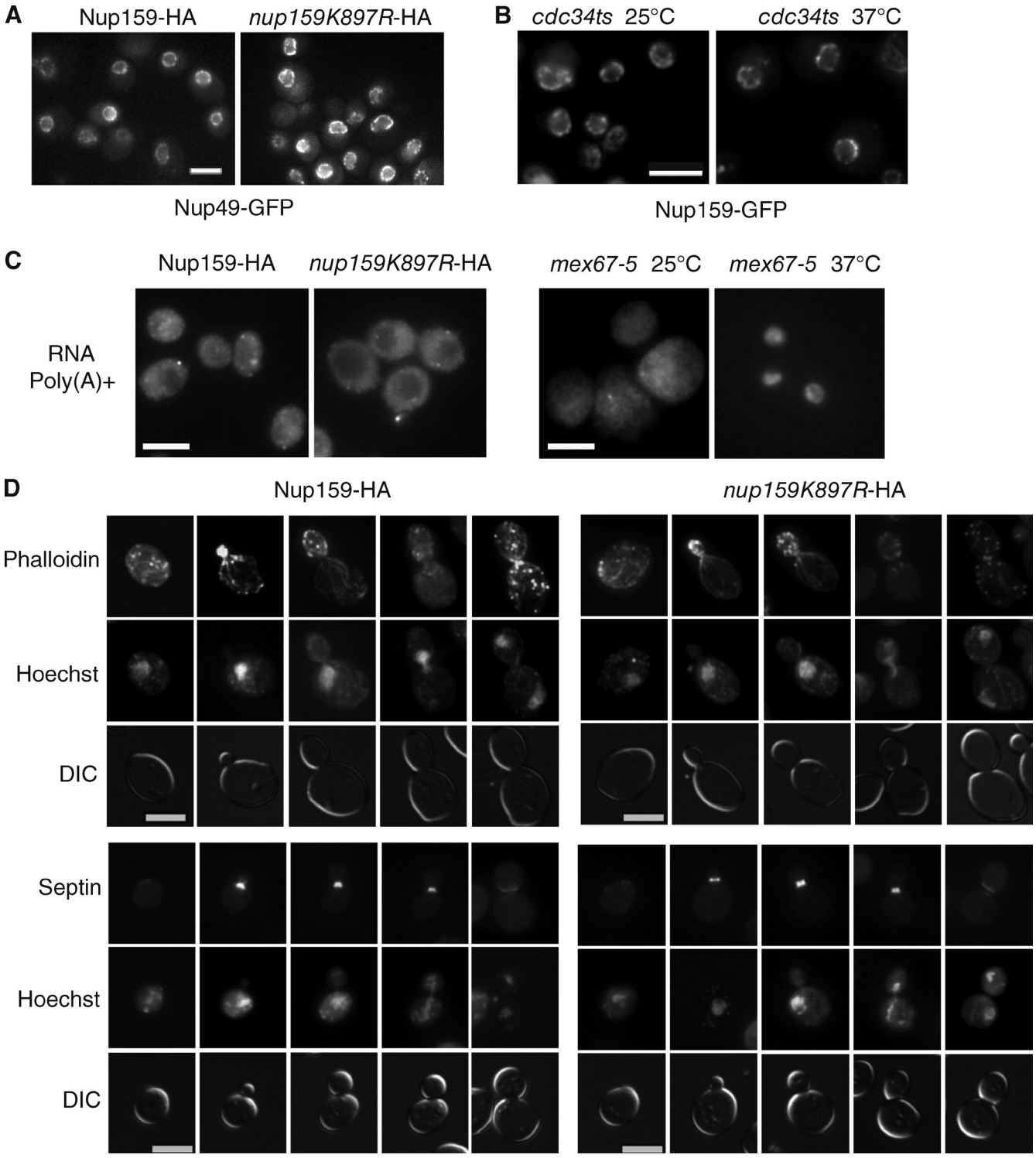


Figure S3. **Ubiquitylation of Nup159 does not control NPC distribution nor mRNA nuclear export.** (A and B) Distribution of NPC was analyzed by fluorescence microscopy in Nup159-HA and *nup159K897R*-HA cells expressing Nup49-GFP (A) or in *cdc34ts* cells expressing Nup159-GFP at 25 or 37°C (B). (C) Subcellular localization of poly(A)$^+$ RNA was analyzed by FISH using Cy3-labeled oligo-dT probe in Nup159-HA and *nup159K897R*-HA but also in mex67-5 thermosensitive cells at 25 and 37°C as a positive control. (D) Localization of actin filaments and septins were analyzed by fluorescence microscopy in Nup159-HA and *nup159K897R*-HA cells using phalloidin–Alexa Fluor and genomically tagged Cdc10-mCherry, respectively. DIC, differential interference contrast. Bars, 5 µm.

## Reference

Rout, M.P., J.D. Aitchison, A. Suprapto, K. Hjertaas, Y. Zhao, and B.T. Chait. 2000. The yeast nuclear pore complex: composition, architecture, and transport mechanism. *J. Cell Biol.* 148:635–651. http://dx.doi.org/10.1083/jcb.148.4.635