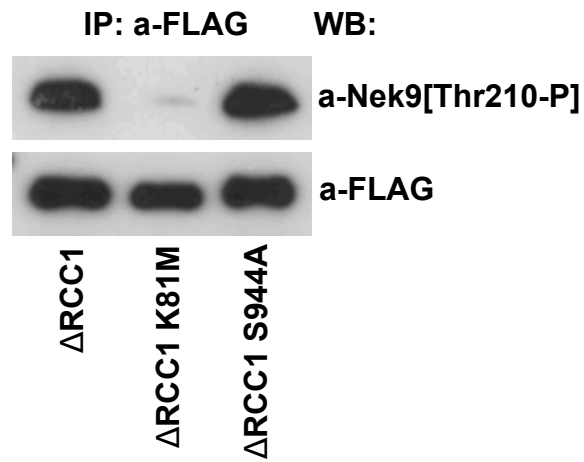**Supplementary Data**
Supplementary data consists of two figures.

Supplementary Figure 1. Nek9[Δ346-732, S944A] activity. (A) anti-FLAG immunoprecipitates from HEK293T cells expressing the indicated forms of FLAG-Nek9[Δ346-732] (*ΔRCC1*), were immunobloted with antibodies against FLAG and active Nek9 (*a-Nek9[Thr210-P]*) (B) The indicated forms of FLAG-Nek9 [Δ346-732] (*ΔRCC1*), were immunoprecipitated from HEK293T cells, washed and incubated for the indicated times at 30ºC in phosphorylation buffer with [γ-^{32}P]ATP and MBP. The reaction was stopped by addition of SDS sample buffer plus boiling, followed by SDS-PAGE. ^{32}P incorporation into MBP as measured by PhosphoImager is shown together with MBP Coomassie staining (*Coomassie*) and ^{32}P incorporation (*^{32}P autorad.*).

Supplementary Figure 2. Structural model for the interaction between LC8 (*surface*) and Nek9 recognition motif (*sticks*) based on PDB ID 3E2B. Nek9[Ser944] and Nek9[Q948] are labeled.

A

IP: a-FLAG   WB:

a-Nek9[Thr210-P]

a-FLAG

ΔRCC1

ΔRCC1 K81M

ΔRCC1 S944A

B