SUPPLEMENTAL DATA

Proteomic analysis of NCK1/2 adaptors uncovers paralog-specific interactions that reveal a new role for NCK2 in cell abscission during cytokinesis.

Kévin Jacquet[1,2,3], Sara L. Banerjee[1,2,3], François J. M. Chartier[1,2,3], Sabine Elowe[2,3,4,5] , Nicolas Bisson[1,2,3,6*]

[1] Centre de recherche du Centre Hospitalier Universitaire (CHU) de Québec-Université Laval, Axe Oncologie, Québec, QC, Canada

[2] Centre de recherche sur le cancer de l'Université Laval, Québec, QC, Canada

[3] PROTEO-Quebec Network for Research on Protein Function, Engineering, and Applications

[4] Centre de recherche du Centre Hospitalier Universitaire (CHU) de Québec-Université Laval, Axe Reproduction, santé de la mère et de l'enfant, Québec, QC, Canada

[5] Department of Pediatrics, Université Laval, Québec, QC, Canada.

[6] Department of Molecular Biology, Medical Biochemistry and Pathology, Université Laval, Québec, QC, Canada.

* To whom correspondence should be addressed (nick.bisson@crchudequebec.ulaval.ca)

RUNNING TITLE: NCK2 but not NCK1 is required for cytokinesis

Contents

Data availability: The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository, with the dataset identifier PXD008824.

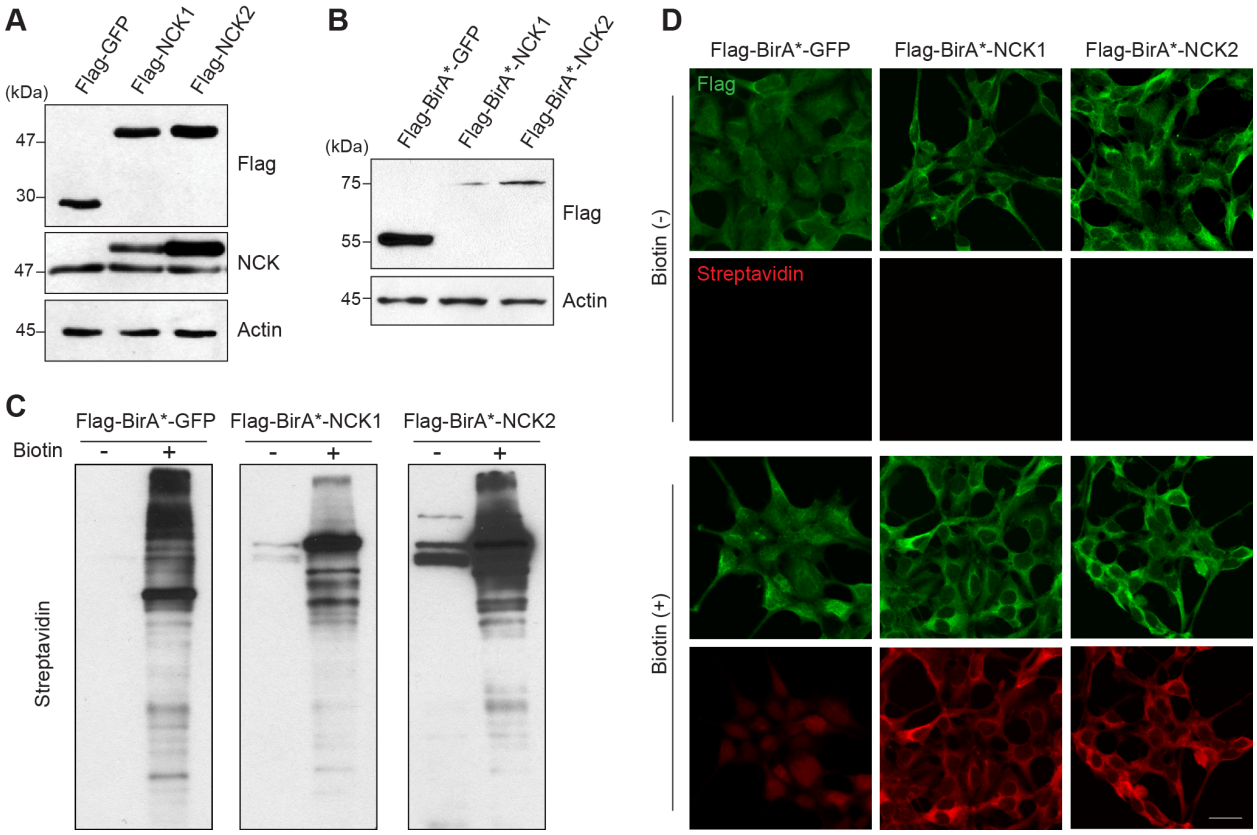

Figure S1. Validation of the expression and localisation of Flag-NCK1/2 or Flag-BirA*-NCK1/2 in HEK293 cells.

(A) HEK293T cells stably expressing Flag-GFP (control), Flag-NCK1 or Flag-NCK2 were analyzed by Western blotting to compare exogenous Flag-NCK1/2 protein expression to endogenous NCK1/2. (B) Flp-In T-REx HEK293 cells stably expressing Flag-BirA*-GFP (control), Flag-BirA*-NCK1 or Flag-BirA*-NCK2 were analyzed by Western bloting to confirm protein expression. (C) Biotinylation assay to validate BirA* activity of the GFP and NCK1/2 chimeras. (D) Validation of the subcellular localization and biotinylation activity of the Flag-BirA* constructs (scale bar: 20 μm).

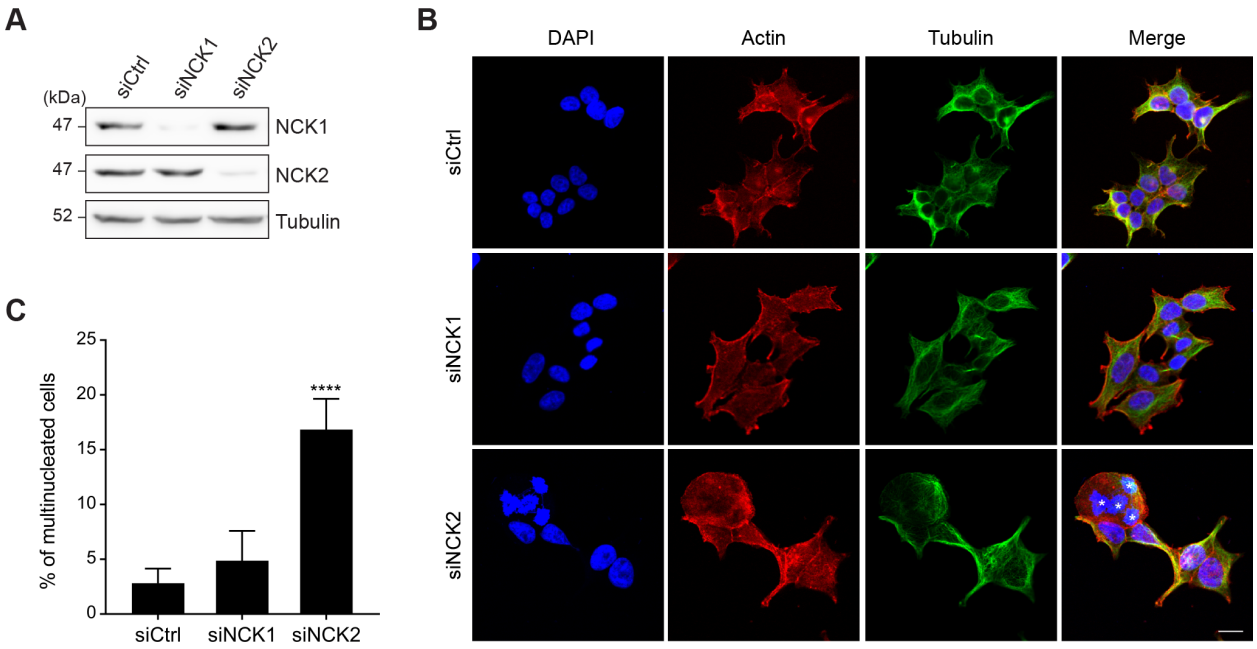

Figure S2. NCK2-depleted HEK293T cells are multi-nucleated and display long protrusions.

(A) Endogenous NCK1- and NCK2-depleted 293T cells were analyzed by Western blot to confirm NCK1/2 protein depletion. (B) Control, siNCK1 and siNCK2 transfected 293T cells were analyzed by immunofluorescence for actin (red), tubulin (green) and DAPI (blue) to assess cellular morphology. Multi-nucleation is indicated with asterisks. Representative images are presented (scale bar: 20 µm). (C) The penetrance of the multi-nucleation phenotype was calculated for each condition. Mean values and standard deviation from four independent experiments with >90 cells each are presented (siCtrl, n=308; siNCK1, n=435; siNCK1, n=372) (**** p≤0.0001; Fisher's exact test).

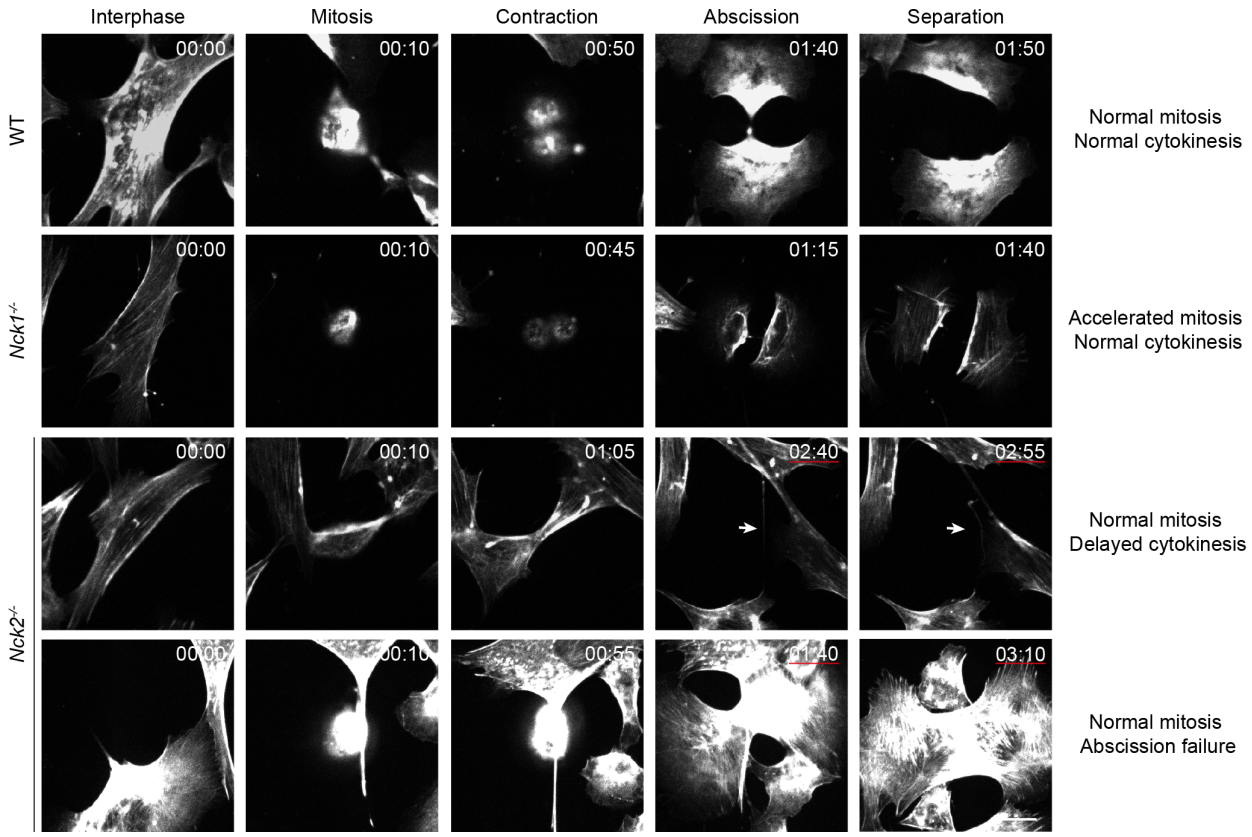

Figure S3. Cytokinesis delay and abscission failure in *Nck2*$^{-/-}$ cells.

Wild-type, *Nck1*$^{-/-}$ and *Nck2*$^{-/-}$ MEFs were stained with SIR-ACTIN and analyzed by live imaging. Images were taken every 5 minutes for 24 hours. Representative images from 3 experiments are presented (scale bar: 20 μm).

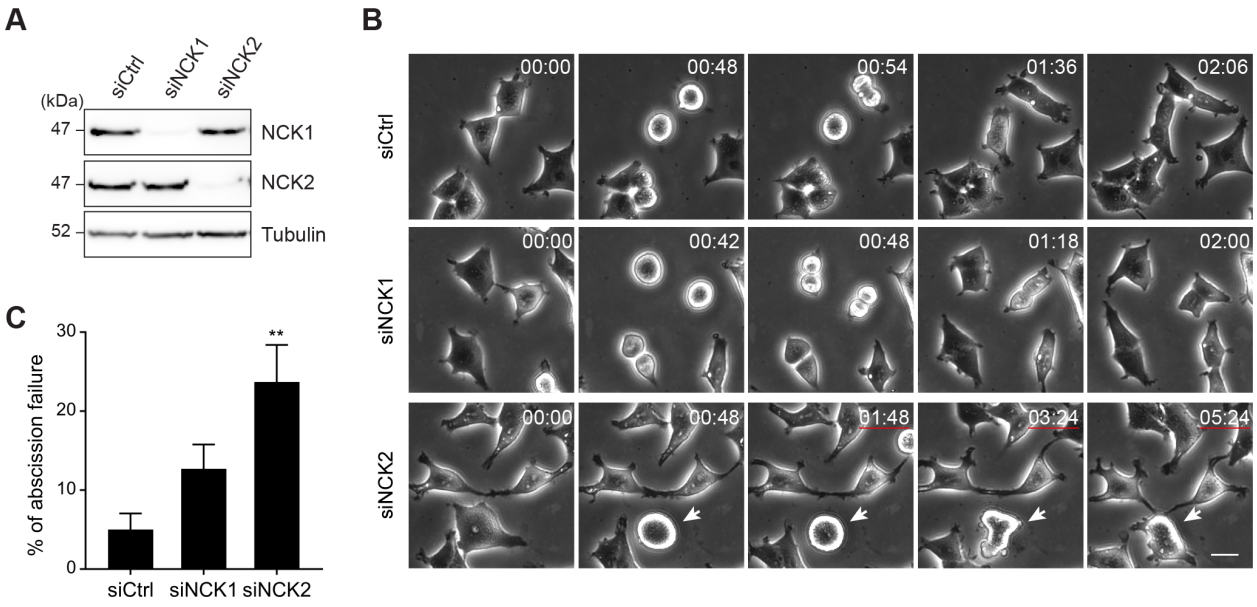

Figure S4. NCK2-depleted 293T cells fail to complete abscission.

(A) Endogenous NCK1- and NCK2-depleted 293T cells were analyzed by Western blot to confirm NCK1/2 protein depletion. (B) Control, siNCK1 and siNCK2 transfected 293T cells were analyzed by live cell imaging. Images were taken every 5 minutes for 24 hours. Representative images from 3 experiments are presented (scale bar: 20 μm). Abscission failure is indicated with arrows. (C) Average abscission failure was calculated for each condition, from 2 independent experiments with >30 cells each (siCtrl, n=87; siNCK1, n=123; siNCK2, n=119) (**$p \leq 0.01$; Fisher's exact test).

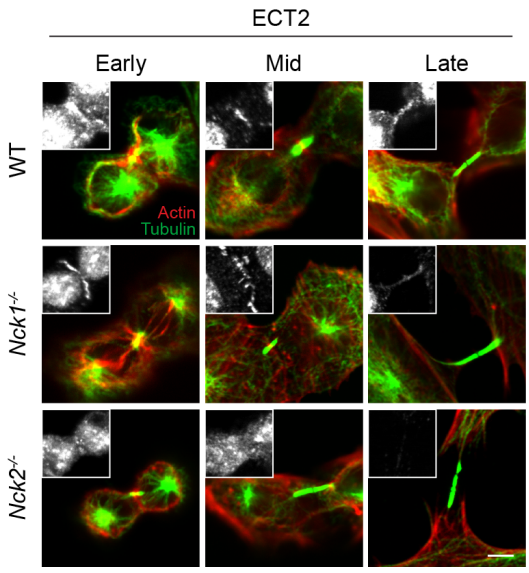

Figure S5. ECT2 cytokinesis localization is altered in *Nck2*$^{-/-}$ cells.

Wild-type, *Nck1*$^{-/-}$ and *Nck2*$^{-/-}$ MEFs were fixed at different stages of cytokinesis and analyzed by immunofluorescence for actin (red), tubulin (green), and ECT2 (insert). Representative images from four experiments are shown (scale bar: 5 µm).