Appendix for

Structural centrosome aberrations promote non-cell-autonomous invasiveness

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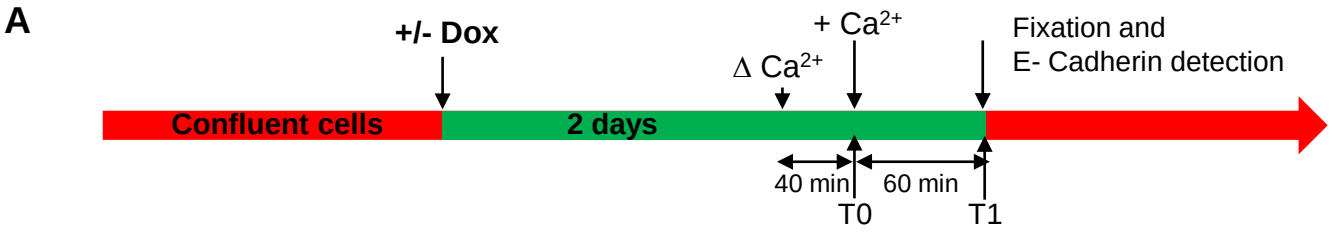
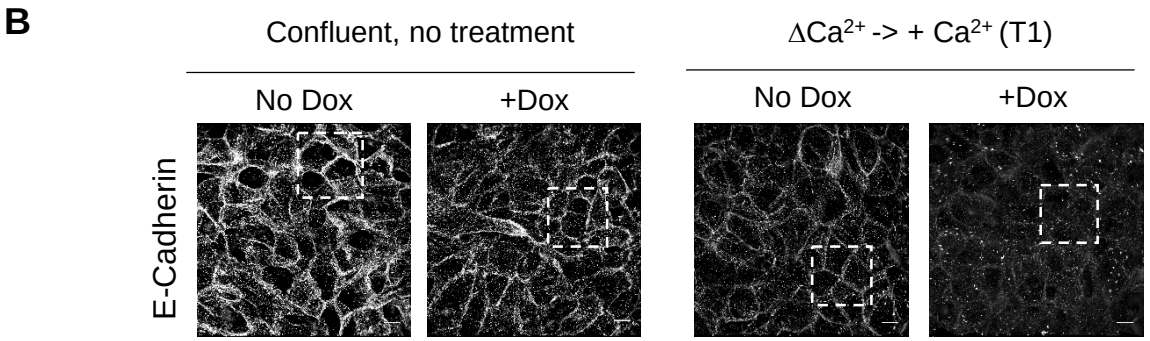
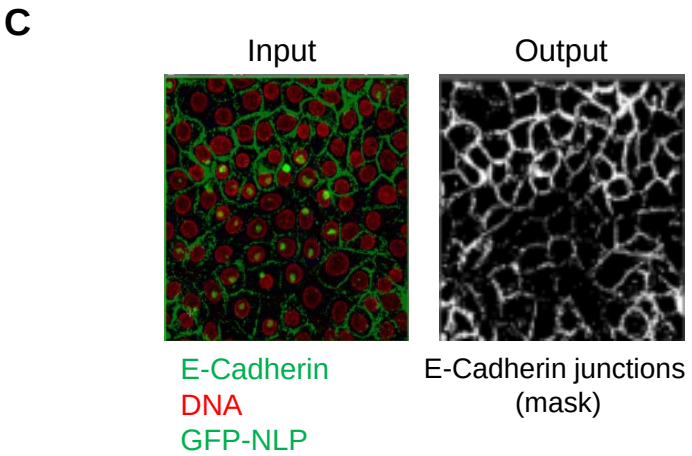
1. Appendix Figures S1-S4
2. Appendix Figures legends

Appendix Fig S1

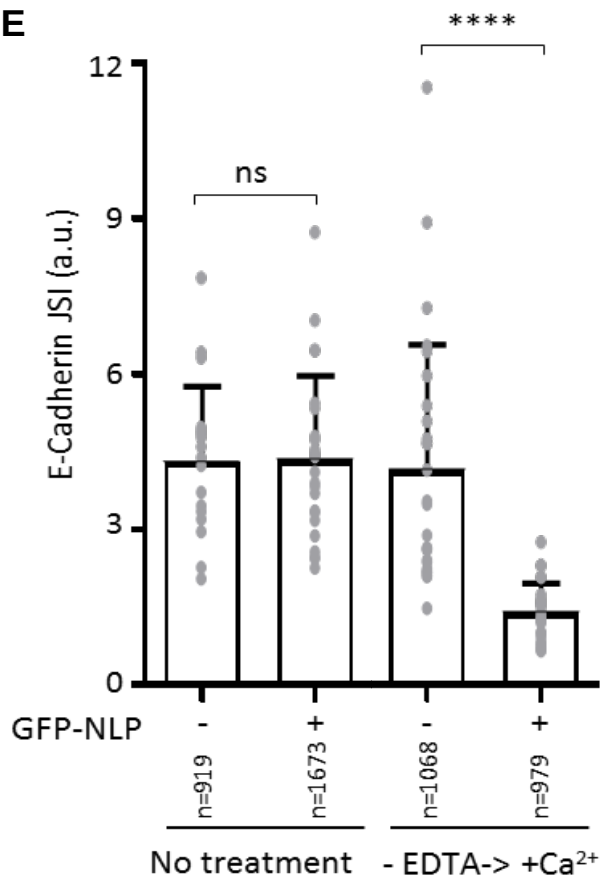




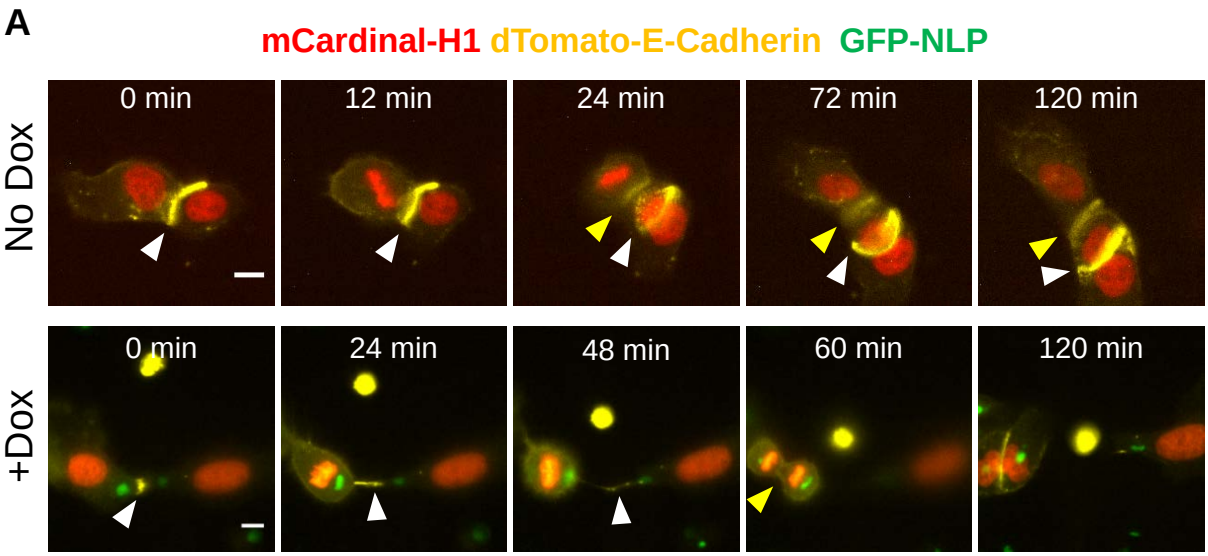


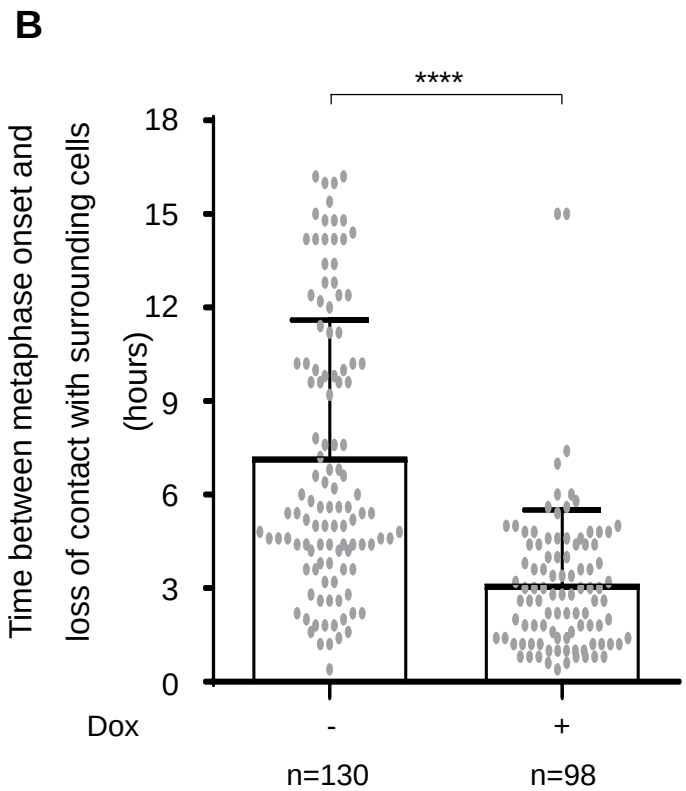
D

$$\text{E-Cadherin Junction Strength Index} = \frac{\text{Area of the signal}}{\text{Entropy of the signal}}$$

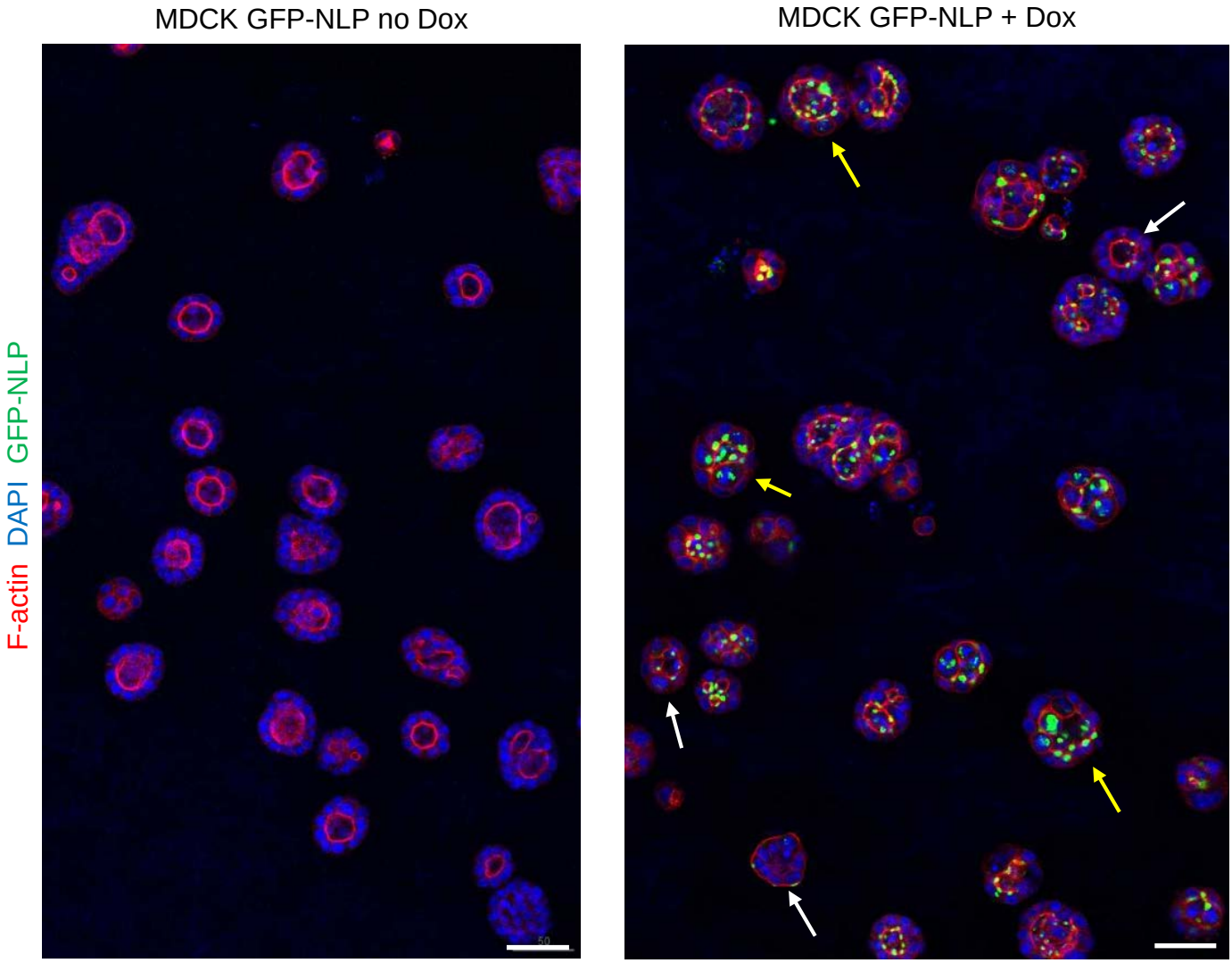


Appendix Fig S2

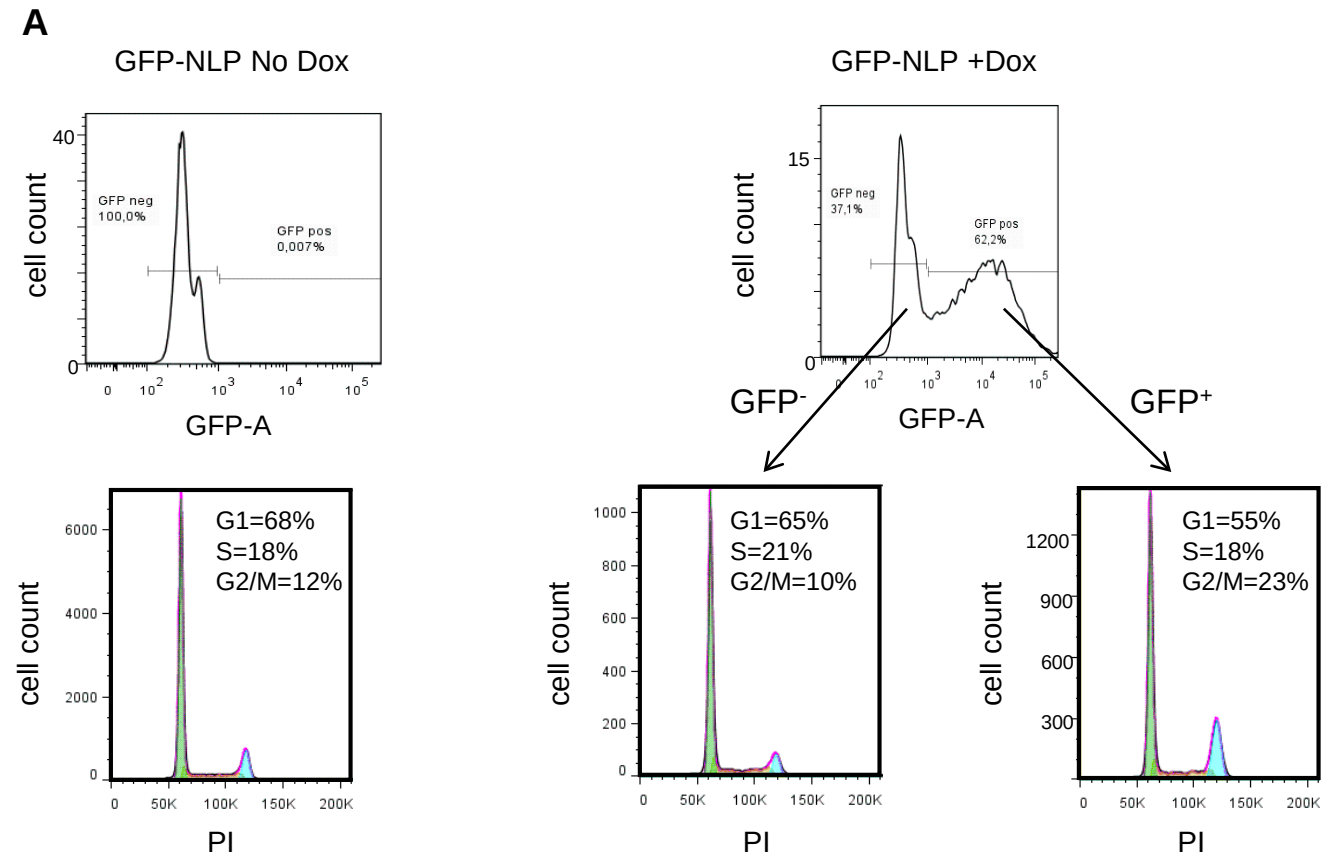


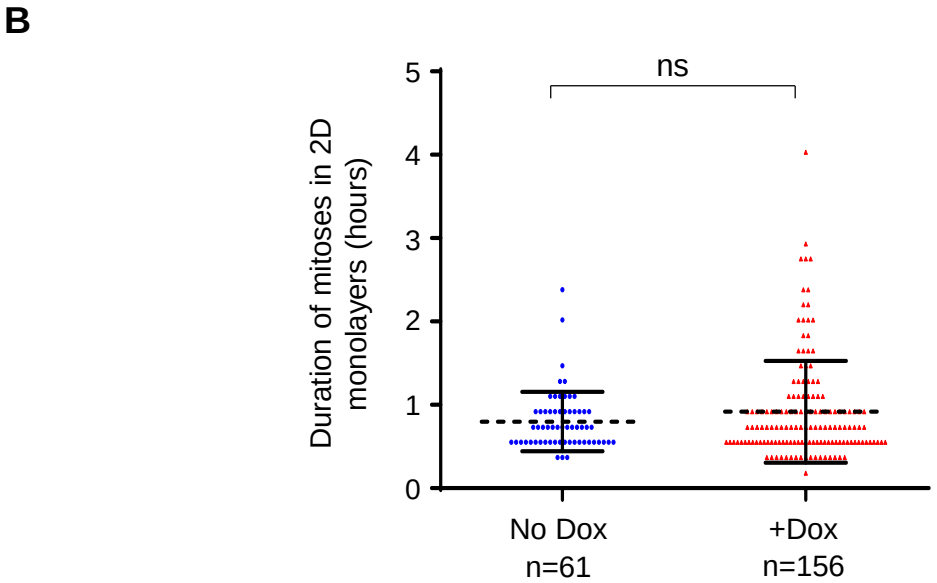


Appendix Fig S3



Appendix Fig S4





Appendix Figures Legends

Appendix Figure S1: <u>Calcium repletion experiments and definition of E-Cadherin junction strength index</u>

A. Schematic description of calcium repletion experiments. Confluent MDCK cells were induced to express GFP-NLP (+Dox) or no transgene (No Dox) for 2 days, before medium was deprived of Ca^{2+} by addition of 2.5 mM EDTA, to destabilize E-Cadherin junctions. After 40 minutes without Ca^{2+} (referred to as ΔCa^{2+} (T0)), cells were washed with PBS and fresh medium containing Ca^{2+} was added for 60 minutes (referred as ΔCa^{2+} -> + Ca^{2+} (T1)) to allow re-formation of E-Cadherin junctions.

B. Representative images obtained by E-Cadherin immunofluorescence microscopy were used to determine an E-Cadherin junction strength index (JSI). Images are z-projections of the entire heights of cells. Blow ups of parts of these overview images (marked by dashed squares) are shown in main Fig 3C. Scale bars = 10 µm.

C. Screen shot of Icy software showing the contour of E-Cadherin signals at the junctions (right, Output), as used to calculate the E-Cadherin junctions' strength index, and the corresponding raw image (left, Input).

D. Formula used to calculate the E-Cadherin junction strength index (JSI). This ratio implies that the JSI will be reduced in response to either a disorganization of the E-Cadherin signal, which increases the entropy of the signal, or a decrease in signal intensity, which decreases the area parameter.

E. Histogram shows evolution of the E-Cadherin JSI during calcium repletion experiments performed on MDCK cells expressing GFP-NLP (+) or not (-). Error bars represent the s.d. of the means from 3 independent experiments, with at least 6 independent and randomly chosen

fields analyzed for each condition; n indicates the numbers of cells analyzed and the values obtained for each field are plotted on the graph. P-values were derived from unpaired, two-tailed Student's t-test. (****) indicates P-value < 0.0001 and ns, not significant.

Appendix Figure S2: NLP overexpression decreases cell-cell adhesion following division

A. Still series from time lapse experiments performed on dividing MCF10A cells (2D cultures) stably expressing mCardinal-histone H1 (red) and dTomato-E-Cadherin (yellow), after cells had been induced (+Dox) or not (No Dox) to express GFP-NLP. Images were acquired every 12 minutes and time stamps are indicated (min); scale bars = 10 µm. The yellow arrowheads point to newly created junctions between two emerging daughter cells, and the white arrowheads point to junctions between mitotic cells and adjacent cells.

B. Histogram represents the time (in hours) between the metaphase onset and the loss of contact between just divided cell and adjacent cells. Error bars represent the s.d. of the means and n indicates the numbers of analyzed adjacent cells in 4 independent experiments; the values obtained for each mitosis are plotted on the graph. P-values were derived from Mann-Whitney test;(****) indicates a P-value <0.0001.

Appendix Figure S3: Induction of GFP-NLP expression results in mosaic cysts

Representative overviews of MDCK cysts without (left panel, no Dox) or after induction of GFP-NLP expression (right panels, +Dox). Cysts were fixed and stained for F-actin (red) and DNA (blue). White arrows show cysts containing a low proportion of cells expressing NLP-GFP while yellow arrows point to cysts with a high proportion of GFP-NLP^{+} cells (green). Scale bars=50 µm.

Appendix Figure S4: <u>NLP overexpression causes a minor delay at G2/M and mitosis</u>

A. FACS profiles of MCF10A cell cultured for 3days without (GFP-NLP No Dox, left panels) or with induction of GFP-NLP expression (GFP-NLP + Dox, right panels). Upper panels show cell counting as a function of GFP intensity, revealing a broad peak corresponding to GFP-NLP+ cells, as expected (right panel). Lower panels show the cell cycle profiles as determined by PI incorporation. Note that GFP-NLP+ cells (broad peak in right panel) show a slightly increased proportion of G2/M phase cells, as compared to GFP-NLP- cells from the same cell population (middle panel) and the non-induced cells (left panel), in line with previous results (Casenghi et al., 2003). To obtain these FACS profiles, cells were trypsinized, washed twice with PBS, fixed with PFA 4% for 10 minutes at room temperature (RT), permeabilized for 2 minutes with 0.2% Triton X-100 and then treated with 50 $\mu g.ml^{-1}$ RNAse A (Sigma, R6513) and stained with 25 $\mu g.ml^{-1}$ propidium iodide (PI) for 30 minutes at 37°C. Cells were analyzed with a BD FACS Canto II cytometer and an approximate evaluation of the different cell cycle populations were obtained using the software FlowJo (Tree Star Inc, Ashland, Oregon, U.S.A.).

B. Graph shows the mitotic duration (time between metaphase onset and cytokinesis), as measured by time lapse microscopy in 2D cultures of MCF10A cells induced for GFP-NLP (+Dox, red triangles) or not (No Dox, blue circles). Error bars represent the s.d. of the means of 3 independent experiments and n indicates the numbers of mitoses analyzed. Statistical significance was tested using a Mann-Whitney test. ns indicates not significant.