Expanded View Figures

Figure EV1. ELYS and SEC13 knockdown lead to smaller nuclei and the formation of cytoplasmic lamin aggregates.

A Representative images from the screen are shown. To the far right, ELYS knockdown nuclei were cropped to highlight cytoplasmic lamin aggregates indicated with arrows.

B Cells with and without cytoplasmic lamin B1 aggregates were counted, and the percentage of cells with aggregates was calculated. For each experiment, 698–1,213 cells were examined per condition. Data are shown for two siRNA sequences for each gene and two biological replicates.

C, D Cells were transfected with control or ELYS siRNA and stained with ELYS and lamin B1 antibodies as indicated. Representative images are shown. Some examples of cytoplasmic lamin aggregates are indicated with arrows. For each experiment, nuclear ELYS staining intensity was quantified for 90–143 nuclei per condition and normalized to the negative control. Data from two biological replicates are shown. ELYS knockdown was greater in cells with lamin aggregates.

E Nuclear cross-sectional areas were quantified for 70–762 nuclei per condition, averaged, and normalized to the negative control. Six biological replicates, data from one representative experiment shown. While not all ELYS and SEC13 knockdown cells exhibited cytoplasmic lamins, those that did had smaller nuclei compared to knockdown cells without cytoplasmic lamin accumulations. This suggests that accumulation of lamins in the cytoplasm is associated with smaller nuclei.

F MCF-10AT1k.cl2 cells were transfected with control siRNA or with siRNA against the indicated genes. Cells were stained with DAPI, and quantification of DNA staining intensity was used to estimate the fraction of cells in various stages of the cell cycle by high-throughput imaging as previously described [123] (see Materials and Methods). The stacked bars represent the means of the fractions for each cell cycle phase calculated over 3 biological replicates. The control data are the same shown in Fig. 6D. Median cell number *z*-scores were 0.35 (*P*-value 0.62) and 0.41 (*P*-value 0.34) for ELYS and SEC13 knockdown, respectively, indicating no significant effect on cell numbers.

G Cell lysates from control and ELYS siRNA-transfected cells were analyzed by Western blot and probed for ELYS and tubulin. One representative Western blot is shown. ELYS band intensity was normalized to tubulin. Quantification from two biological replicates is shown.

Data information: Two-tailed Student's *t*-tests assuming equal variances: ***$P < 0.005$; **$P < 0.01$. Scale bars are 20 μm. Error bars represent SD.

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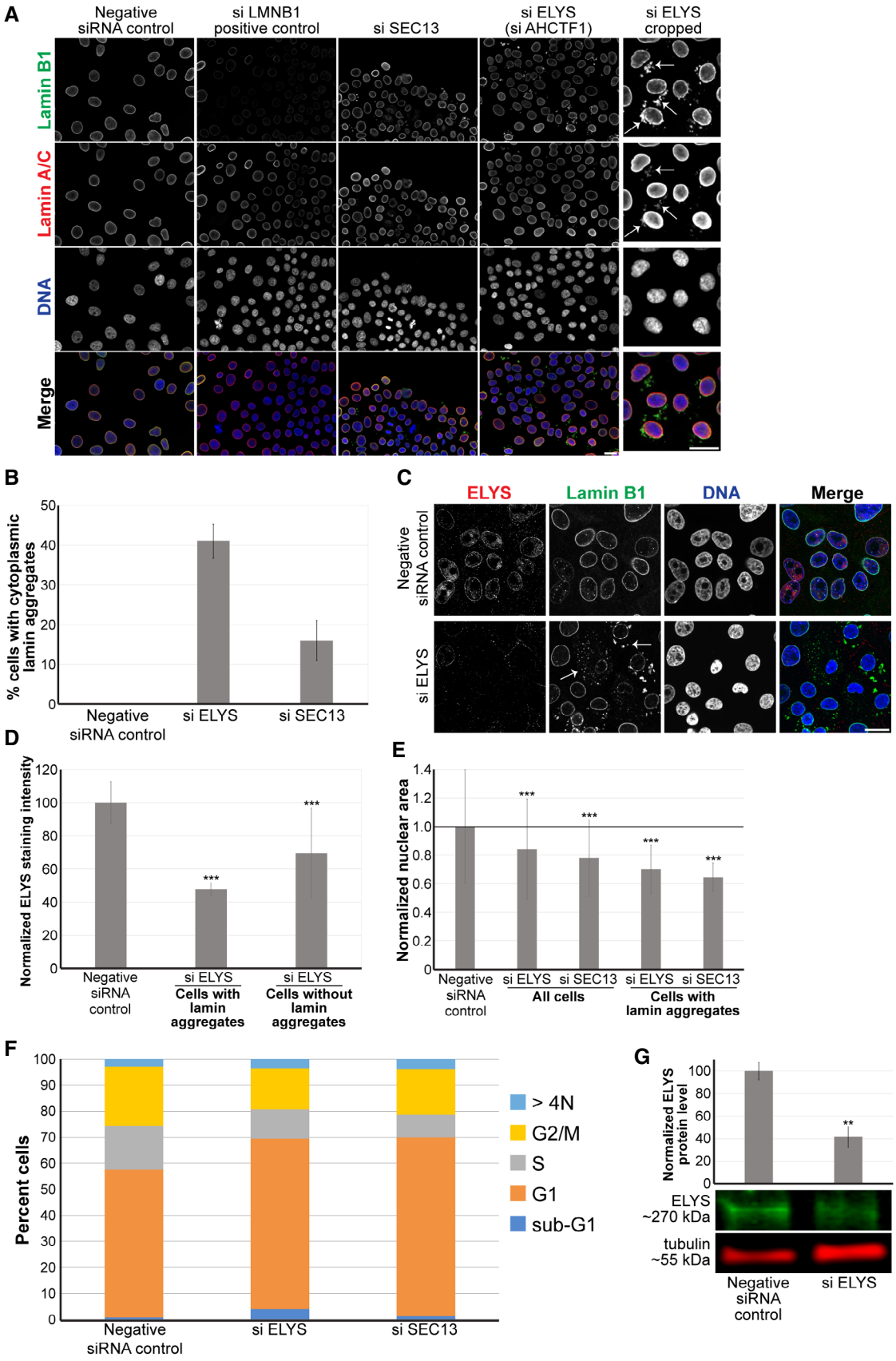

Figure EV1.

Figure EV2. Protein phosphatase PP1α knockdown rescues the lamin aggregation phenotype in ELYS knockdown cells.

A To confirm that lamin puncta were not an artifact of fixation, we performed live cell imaging. MCF-10AT1k.cl2 cells were co-transfected with control or ELYS siRNA and with plasmids expressing mCherry-lamin B2 and H2B-GFP. Representative images of live cells are shown. Also see Movies EV1–EV3. Cytoplasmic lamin puncta were apparent in ELYS knockdown cells but not in control cells. In addition, time-lapse imaging showed that cytoplasmic lamin aggregates exhibited dynamic movements, often appeared immediately after mitosis regardless of whether or not the mother cell contained lamin puncta, and coalesce or dissolve during interphase. In some extreme cases, cells contained abundant cytoplasmic lamin foci but no NE-localized mCherry-lamin B2.

B Because lamins are known to be regulated by phosphorylation [32,125–127], we asked whether the appearance of cytoplasmic lamin aggregates might depend on the lamin phosphorylation state. To test this hypothesis, we knocked down two putative lamin protein phosphatases, PPP1CA (protein phosphatase 1 catalytic subunit alpha) and PPP2R4 (protein phosphatase 2A activator regulatory subunit 4) [65]. MCF-10AT1k.cl2 cells were transfected with control siRNA, ELYS siRNA, ELYS siRNA plus PPP1CA siRNA, or ELYS siRNA plus PPP2R4 siRNA and stained with antibodies against lamin B1 and B2. Representative images are shown.

C Cells with and without cytoplasmic lamin B aggregates were counted and the percentage of cells with aggregates was calculated. For each experiment, 28–120 cells were examined per condition. Two biological replicates, data from one representative experiment shown. While over 40% of ELYS knockdown cells exhibited cytoplasmic lamins, less than 10% of cells co-transfected with siRNA against ELYS and PPP1CA showed cytoplasmic lamins. Knockdown of PPP2R4 had no impact on the appearance of cytoplasmic lamin accumulations.

D For each experiment, nuclear cross-sectional areas were quantified for 70–184 nuclei per condition (117 nuclei on average) and averaged. Two biological replicates, data from one representative experiment shown. Dissolving cytoplasmic lamin aggregates through PPP1CA knockdown did not result in a significant increase in nuclear size in ELYS knockdown cells. Thus, cytoplasmic lamin aggregates formed in ELYS knockdown cells are not sufficient to cause reduced nuclear size.

Data information: Two-tailed Student's *t*-tests assuming equal variances: ***$P < 0.005$; NS, not significant. Scale bars are 10 μm. Error bars represent SD.

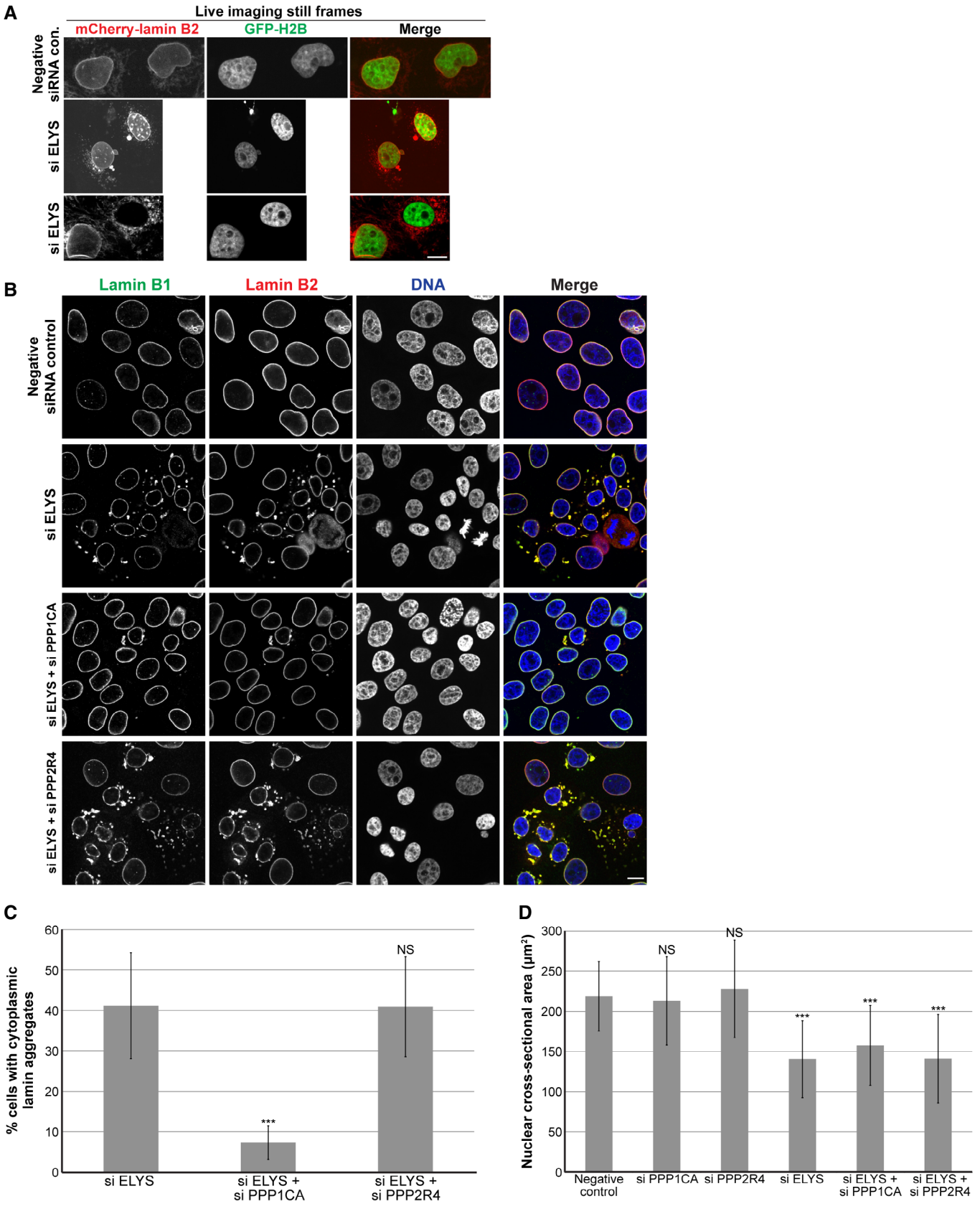

Figure EV2.

Figure EV3. ELYS overexpression, Nup153 and Nup107 knockdowns, and importin α overexpression.

A MCF-10AT1k.cl2 cells were transfected with a plasmid expressing eGFP-ELYS. Cell lysates were analyzed by Western blot and probed for ELYS. The signal from the ectopically expressed eGFP-ELYS band was divided by the signal from the endogenous ELYS band, indicated below the blots. Ectopically expressed eGFP-ELYS was expressed 1.9 ± 0.9-fold (average ± SD) above endogenous ELYS levels, based on four biological replicates.

B MCF-10AT1k.cl2 cells were transfected with plasmids expressing eGFP alone or eGFP-ELYS. Cells were stained with a Ran antibody. Representative images are shown.

C MCF-10AT1k.cl2 cells were transfected with plasmids expressing eGFP alone or eGFP-ELYS. Cells were stained with an NTF2 antibody. Representative images are shown.

D MCF-10AT1k.cl2 cells were transfected with control, Nup153, or Nup107 siRNA and stained with an antibody against FG-Nups (mAb414). Cells were co-transfected with BLOCK-iT Alexa Fluor red fluorescent control to identify transfected cells. Representative images are shown. Confocal imaging was performed through nuclear cross-sections as well as on the surface of nuclei. Cytoplasmic staining for FG-Nups apparent in ELYS knockdown cells (Fig 2D) was not evident upon knockdown of Nup153 or Nup107.

E Nuclear cross-sectional areas were quantified for 36–49 nuclei per condition (41 nuclei on average), averaged, and normalized to the siRNA negative control. Nuclear mAb414 staining intensity was quantified for 36–49 nuclei per condition (41 nuclei on average), averaged, and normalized to the siRNA negative control. Data from three biological replicates are shown.

F MCF-10AT1k.cl2 cells were transfected with plasmids expressing mCherry-importin α2 or mCherry-importin α2-E [30]. Cell lysates were analyzed by Western blot and probed for importin α and β-actin. The signal from the ectopically expressed mCherry-importin α2 band was divided by the signal from the endogenous importin α band, indicated below the blots. Ectopically expressed mCherry-importin α2 was expressed at 81% ± 35% (average ± SD) of endogenous importin α levels, based on three biological replicates.

Data information: Two-tailed Student's *t*-tests assuming equal variances: ***$P < 0.005$; NS, not significant. Scale bars are 20 μm. Error bars represent SD.

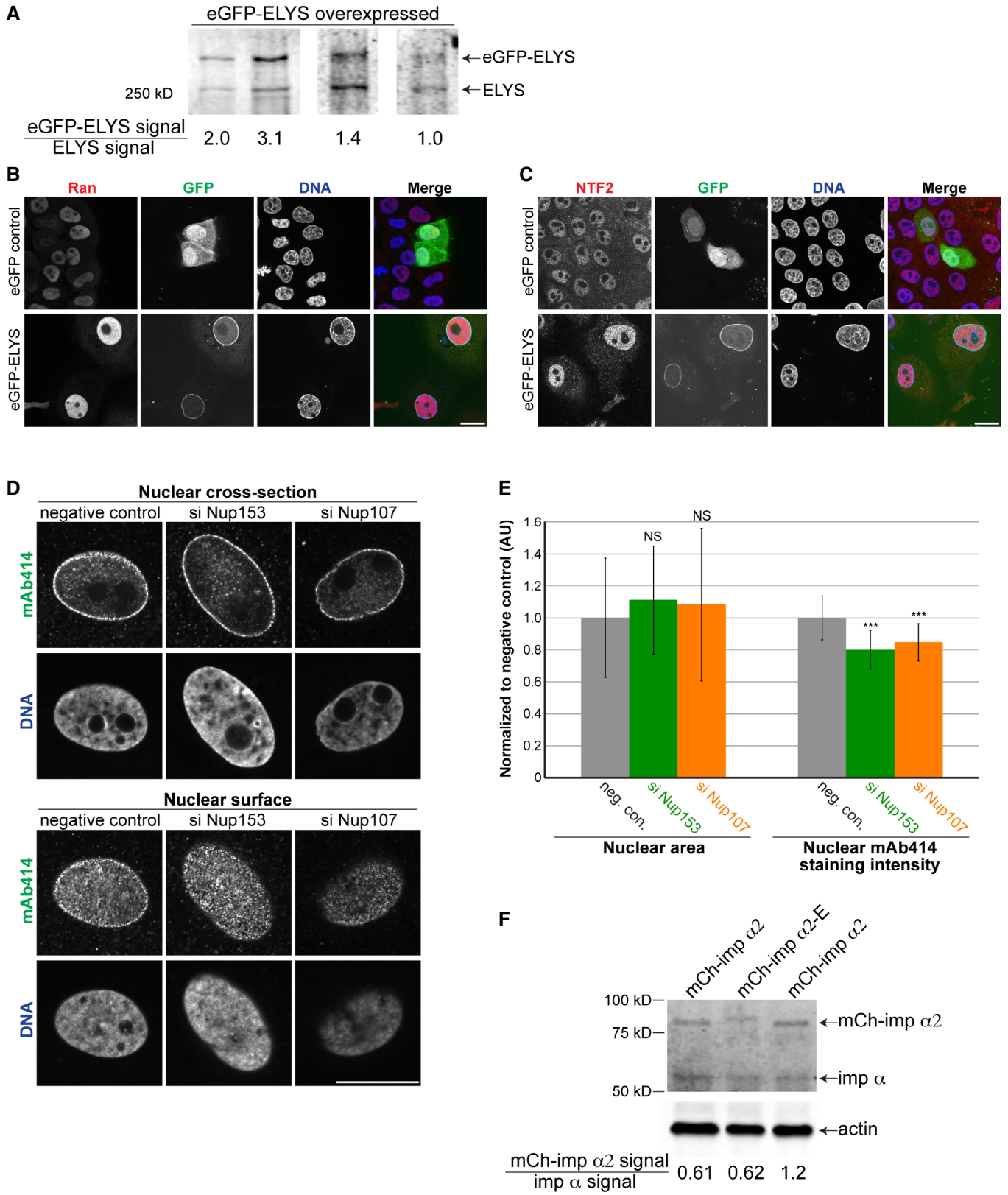

Figure EV3.

Figure EV4. ELYS, SEC13, and XPO1 knockdown in different cell lines.

The indicated cell lines were transfected with a negative siRNA control or siRNA against ELYS, SEC13, or XPO1. Cells were stained with DAPI and with antibodies against lamin B1 and lamin A/C.

A Representative images for hTERT immortalized CRL-1474 cells, roughly normal human skin fibroblasts.
B Representative images for MCF7 cells, breast adenocarcinoma cells.
C Representative images for MCF-10A cells, roughly normal breast epithelial cells.
D Representative images for MCF-10AT1k.cl2 cells, breast epithelial cells with atypical hyperplasia.
E Nuclear cross-sectional areas were quantified, and z-scores were calculated as described in Materials and Methods. For each cell line and condition, > 500 cells were quantified and the average *z*-score and SD from three biological replicates are plotted.
F Cell lysates were analyzed by Western blot and probed for ELYS and histone H3. Band intensities were quantified, and ELYS signal was normalized to the histone H3 signal to obtain the relative ELYS protein levels in each cell line. Average data from three biological replicates are plotted. Notably, ELYS levels are lowest in MCF7 cells and ELYS was efficiently knocked down by siRNA treatment, eliminating high ELYS expression or poor ELYS knockdown as reasons for why ELYS knockdown minimally affected nuclear size in MCF7 cells.

Data information: *absolute value of the *z*-score is greater than 1.5. Scale bars are 25 μm. Error bars represent SD.

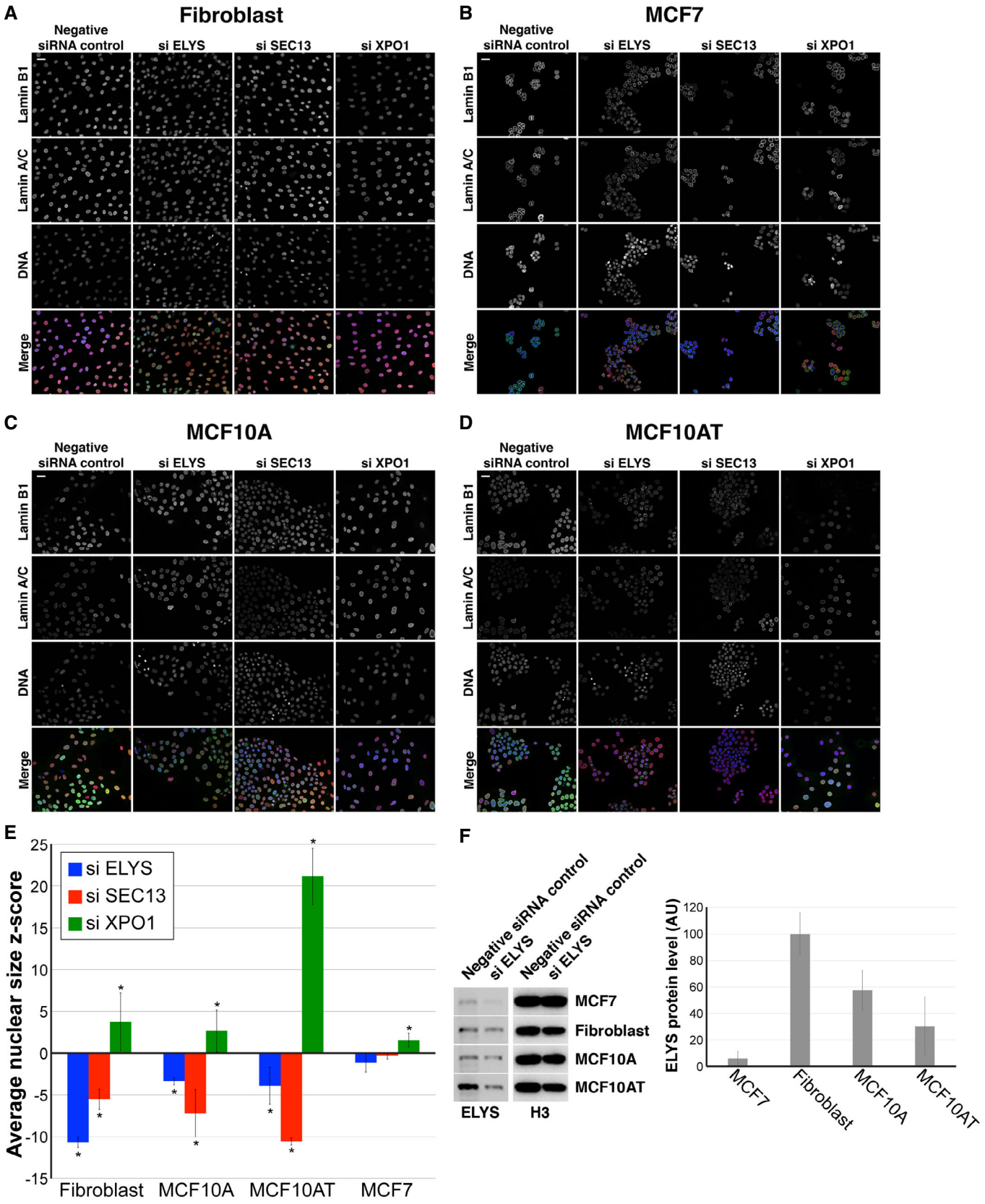

Figure EV4.

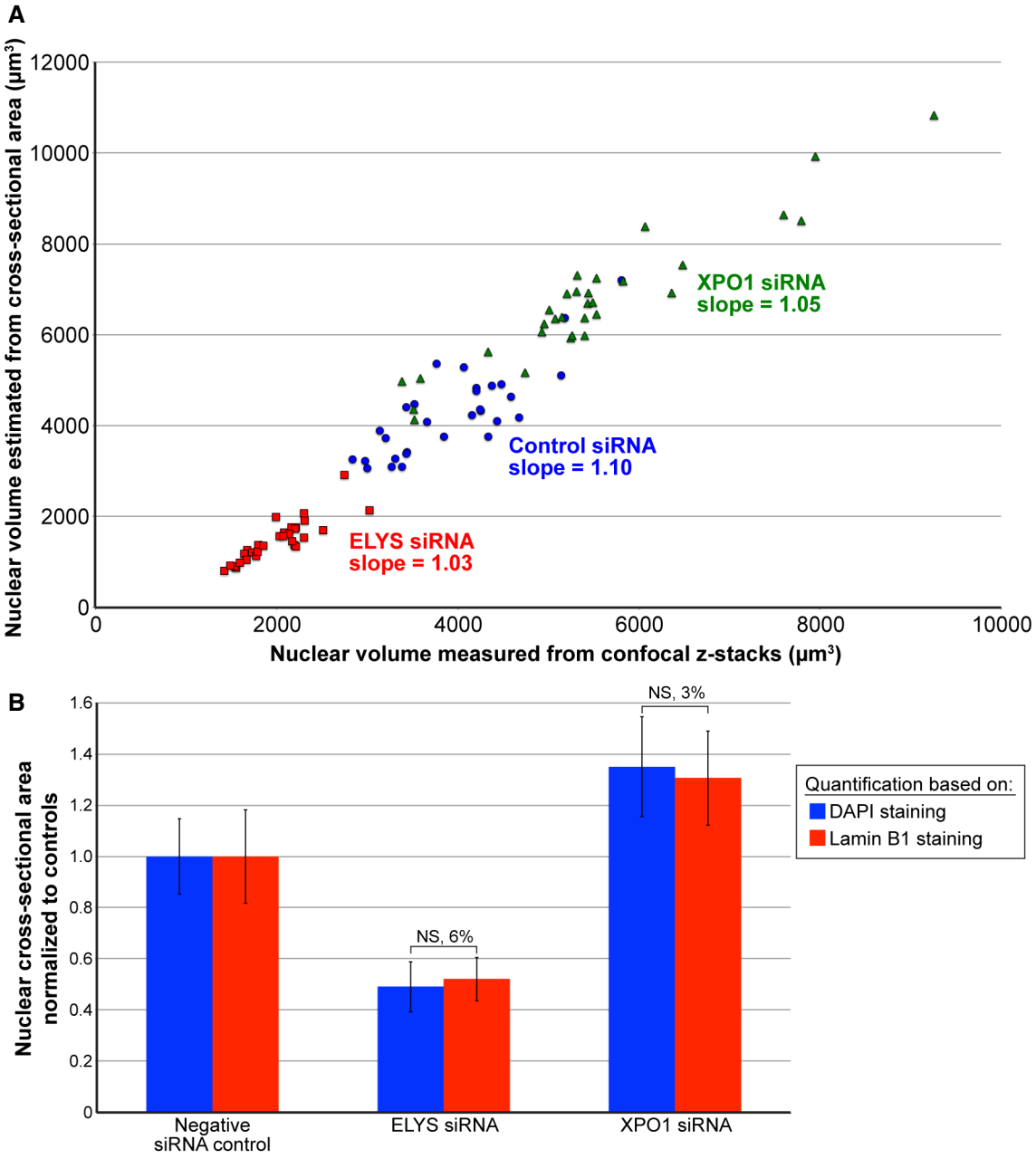

Figure EV5. Validation of nuclear size quantification methods.

A MCF-10AT1k.cl2 cells were transfected with control siRNA, ELYS siRNA, or XPO1 siRNA and stained with DAPI and an antibody against lamin B1. Confocal *z*-stacks were acquired with a *z*-slice thickness of 1 µm and a total of 21 *z*-slices. Nuclear volume was quantified from the DAPI confocal *z*-stacks and plotted on the *x*-axis. For the same DAPI-stained nuclei, nuclear volume was estimated from the maximum cross-sectional nuclear area assuming a spherical nucleus and plotted on the *y*-axis. Data from three biological replicates are shown. For each experimental condition, 30 nuclei were quantified, linear regression analysis was performed, and the slope of the fitted line is indicated. A *t*-test calculated by comparing all measured nuclear volumes to estimated nuclear volumes returned a non-significant *P*-value of 0.25.

B Maximum nuclear cross-sectional areas were quantified from the confocal *z*-stacks described in (A) for both the DAPI and lamin B1 channels. For each experimental condition, three biological replicates were performed and 30 nuclei were quantified. For a given staining method, nuclear areas were normalized to the negative siRNA control. The percent difference between the DAPI and lamin B1 staining methods is indicated above each pair of bars.

Data information: Two-tailed Student's *t*-tests assuming equal variances: NS, not significant. Error bars represent SD.