

## Supplementary Figure Legends:

**Supplementary Figure S1.** Structure-function analysis of Kuk in the cellularizing *Drosophila* embryo: additional constructs.

A- B: Nuclear morphology of *kuk* $\Delta$ 15 and wt fly embryos in late cellularization. The nuclear morphology of these embryos was used as a reference for describing the effect of the injected constructs shown in Figure 4 and Figure S1 C-F'. C-F': Nuclear morphology of *kuk* $\Delta$ 15 embryos at late cellularization stage, injected with mRNA of different Kuk constructs. The invaginating membrane front, stained by Slam (blue) indicates the embryonic stage. Lamin Dm0 (red) is used as a marker of the NM. Kuk staining is shown in green. C: Kuk- $\Delta$ C328 injected embryo, side view. C': Surface view. D: Kuk-C567S injected embryo, side view. D': Surface view. E: Kuk- $\Delta$ N185 injected embryo, side view. E': Surface view. F: Kuk- $\Delta$ 453-473 injected embryo, side view. F': Surface view. Scale bar throughout the figure: 10  $\mu$ m.

**Supplementary Figure S2.** Localization and activity on nuclear shape of Kuk and lamin Dm0 constructs in mouse fibroblasts.

NIH-3T3 cells, transiently transfected with Kuk and lamin Dm0 deletion constructs. Cells were stained 24 h post transfection with the exception of E where the cells were incubated for 72 h. The constructs used were the following: Kuk-C567S (A), Kuk- $\Delta$ 353-404 (B), Kuk- $\Delta$ 453-473 (C), GFP-NLS-C-term (D) and GFP-NLS-CaaX (E). In A-C, Lamin A/C staining (green) is used as a marker of the NM and Kuk staining is shown in red. In D and E, nuclear pore staining (mAb414) in green marks the NM and GFP marking the transfected cells is shown in red. Scale bar: 10  $\mu$ m.

**Supplementary Figure S3.** Kuk and lamin Dm0 localize at the NM independently of a group of selected INM proteins.

Localization of Kuk and lamin Dm0 after depletion of different INM proteins by RNAi in *Drosophila* S2 cells. A-B: Kuk staining (green) shows Kuk localization in RNAi treated cells. In red, the staining for the respective depleted protein is shown (dMAN1 in A, lamin C in B). C: Lamin Dm0 staining (green) shows lamin Dm0 localization in Kuk RNAi treated cells. In red, Kuk staining is shown. D: S2 cells treated with control RNAi against eGFP. Lamin Dm0 staining is shown in red and Kuk staining in green. Scale bar: 5  $\mu$ m.

**Supplementary Figure S4.** Protein amounts of the different constructs used for the liposome deformation assays.

A: Coomassie blue stained SDS-gel showing the protein amounts used for the liposome deformation assays. Approximately 2  $\mu$ g of protein were loaded for each construct. The bands representing the corresponding proteins are indicated by black boxes. The amounts shown here refer to the 1x protein amount described in Table 2. Considering that the 1x protein amount was 2  $\mu$ g and taking into account the MW of each protein we estimated that the protein concentration in the final liposome-protein mixture was approximately 1-4  $\mu$ M. For GFP-LaminDm0 $\Delta$ N a 5x amount, corresponding to the protein amount used for the EM analysis of liposome deformation, was also loaded on the gel. For the Kuk constructs the protein amount shown here was the highest protein amount that could be used for the liposome assays, due to the low protein concentration of the samples. For FT the two subunits of the enzyme, 75 kDa (GST-tagged large

subunit) and 45 kDa (His-tagged small subunit) respectively can be visualized in the last lane of the gel.

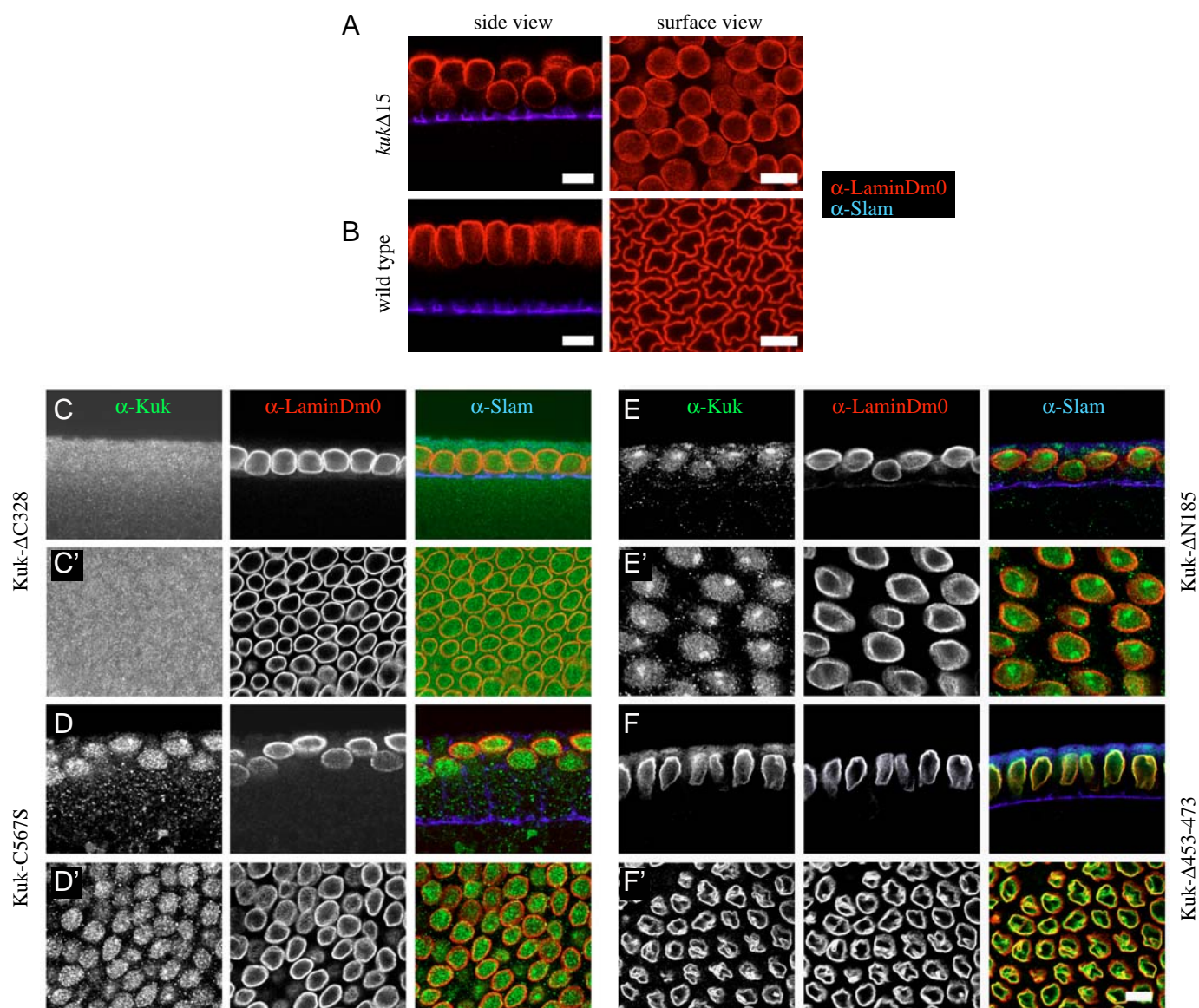
**Supplementary Figure S5.** Liposome morphology upon addition of the different proteins.

A: Fluorescence microscopy images showing the morphology of liposomes upon addition of the different protein constructs used. The data are summarized in Table 2. -F= no addition of FPP. +F= FPP was added to the mixture of liposome and protein. The protein amount used was 1x for all constructs except LaminDm0ΔN (0,1x) and GFP (10x).

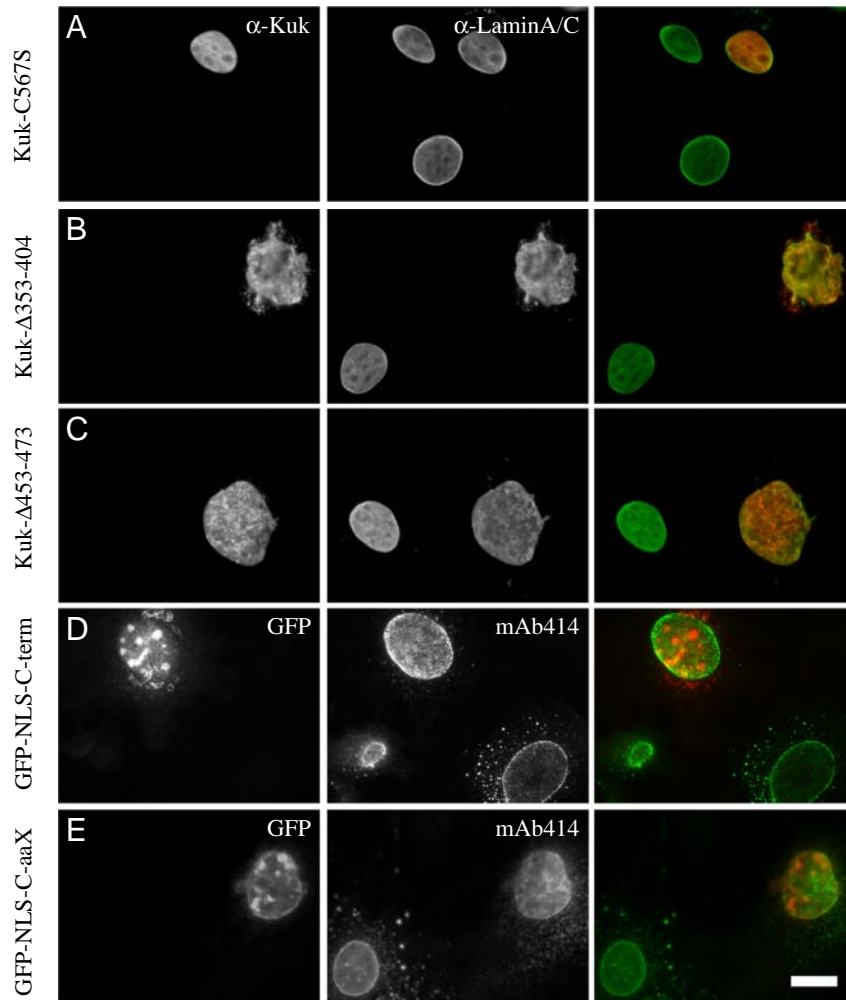
B. Morphology of liposomes upon addition of 10x GFP protein together with the components of the farnesylation reaction. Rhodamine-PE labels the liposomes. The GFP channel shows that there is no colocalization of protein and liposomes. Scale bar throughout the figure: 15 μm.

**Supplementary Figure S6.** Quantification of tubule length.

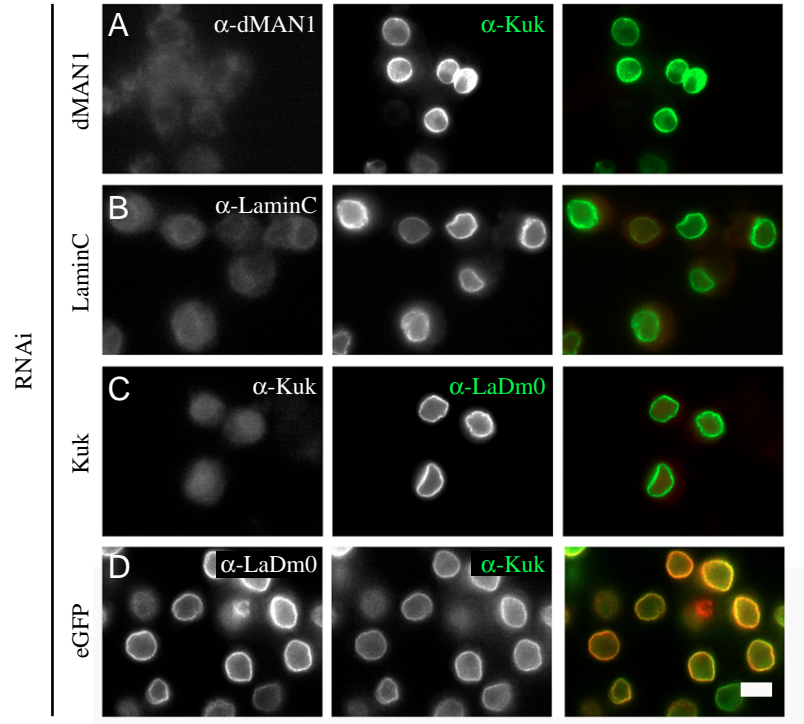
The length of the tubules formed after incubation of spherical liposomes with farnesylated LaminDm0ΔN was measured and plotted against the number of liposomes showing this length. Structures with a length up to 200 nm were excluded from the measurements, since they might represent collapsed liposomes. The majority of the tubules had an average length of 400 nm. When a sphere of a given diameter is deformed so as to give rise to a cylindrical shape, the length of the cylinder depends on the diameter of the sphere, since the volume of the shape is not expected to change. The volume of the sphere ( $\frac{4}{3}\pi r^3$ ) would theoretically be equal to the volume of the cylinder ( $\pi(r')^2 l$ ). ( $r$ = radius of the sphere and  $r'$ = radius of the cylinder) The expected length ( $l$ ) of the transformation of a 100nm spherical liposome to a tubule with a diameter of ~30 nm according to the above calculations is ~300 nm. Therefore the measured average is close to the theoretically expected length. (Only tubules with a diameter of ~30 nm were counted and included in the quantification.)



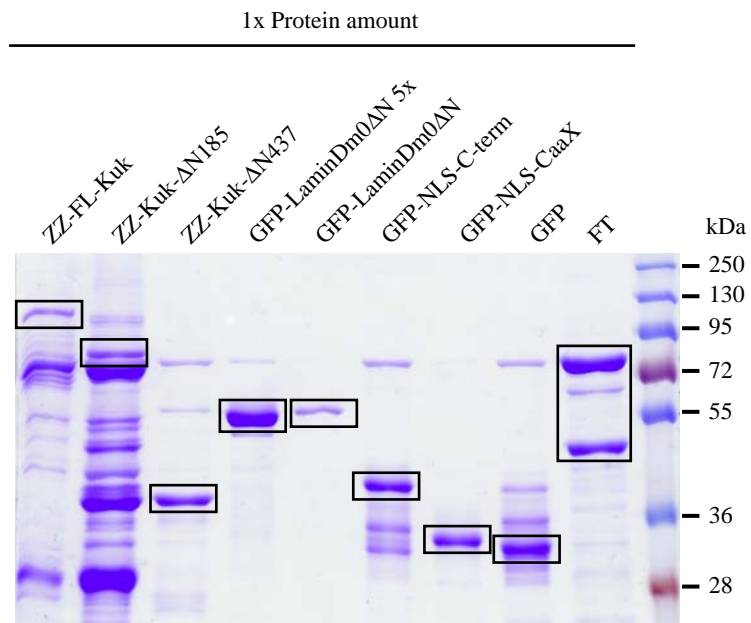
**Supplementary Figure S1**  
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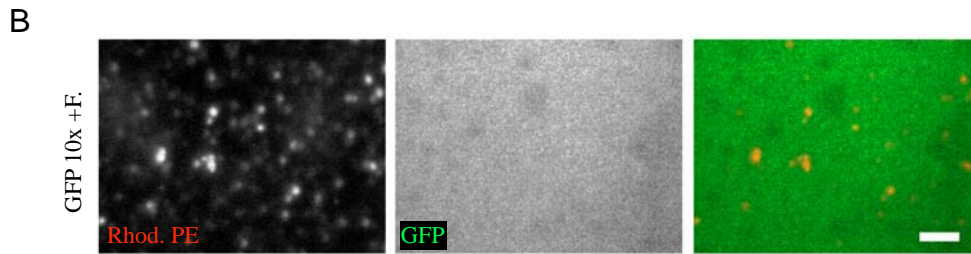
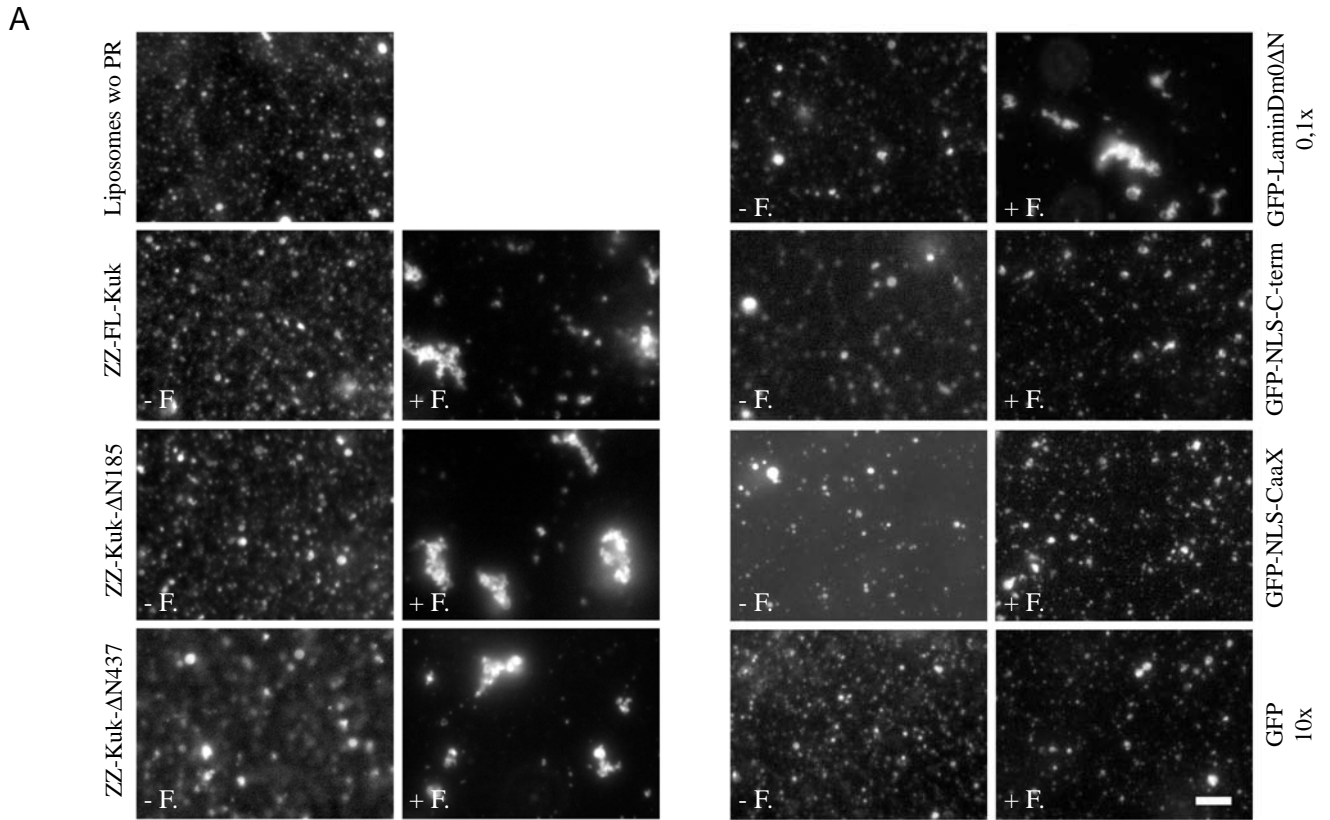
**Supplementary Figure S2**  
**Polychronidou et al.**



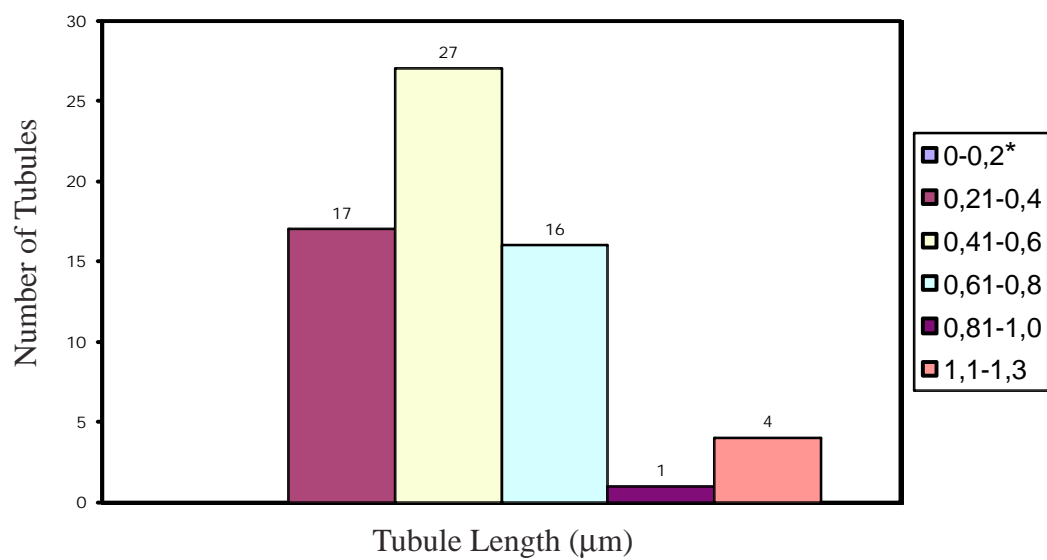
**Supplementary Figure S3**  
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**Supplementary Figure S4**  
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**Supplementary Figure S5**  
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**Supplementary Figure S6**  
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