Supplementary Material

Correlative super-resolution fluorescence and scanning electron microscopy of the nuclear pore complex with molecular resolution

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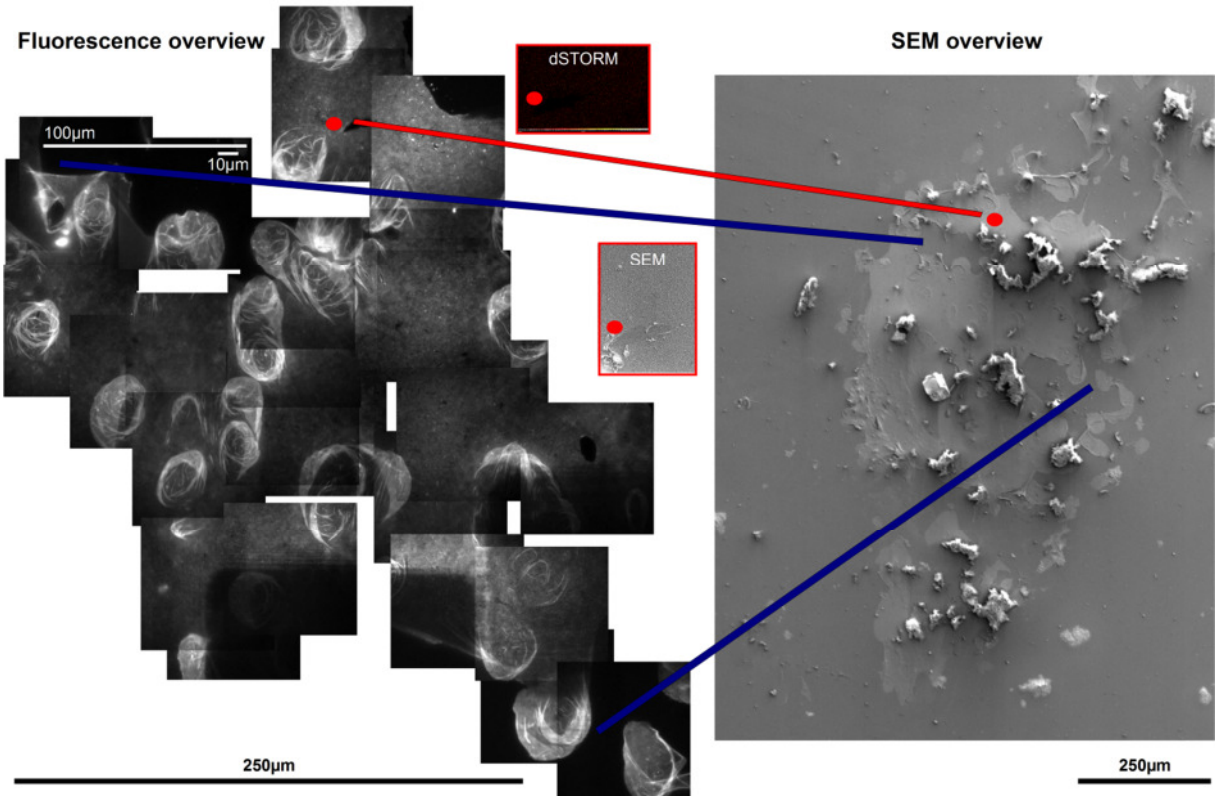

Figure S1. **Correlative imaging procedure**. Wide-field fluorescence and SEM overviews of nuclear envelopes of *Xenopus laevis* oocytes were used to find the region of interest (red dot) for SEM after *d*STORM imaging. Blue and red lines serve as orientation. Scale bars, see image.

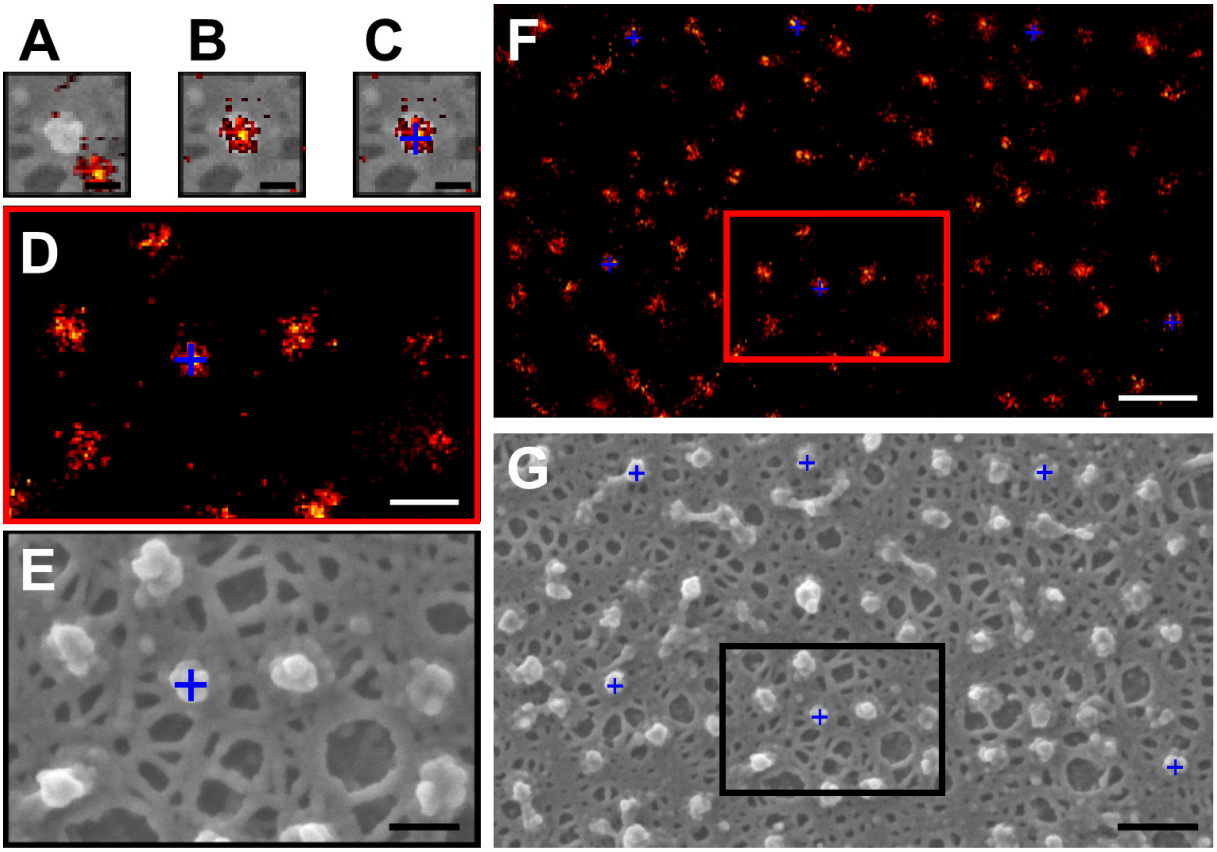

Figure S2. Landmark selection for correlative images of WGA Alexa Fluor 647 labeled NPCs of *Xenopus laevis* oocytes. (**A-G**) After adjusting the pixel size of the *d*STORM and the SEM image the *d*STORM/SEM overlay image is moved manually to a corresponding SEM pore (**A,B**). Corresponding pores are landmarked (+) (**C-E**). Only nuclear pores that match each other very precisely are chosen for future landmarks (**F,G**). Images were finally transformed with bUnwarpJ. Scale bars, 50 nm (A-C), 100 nm (D,E), 250 nm (F,G).

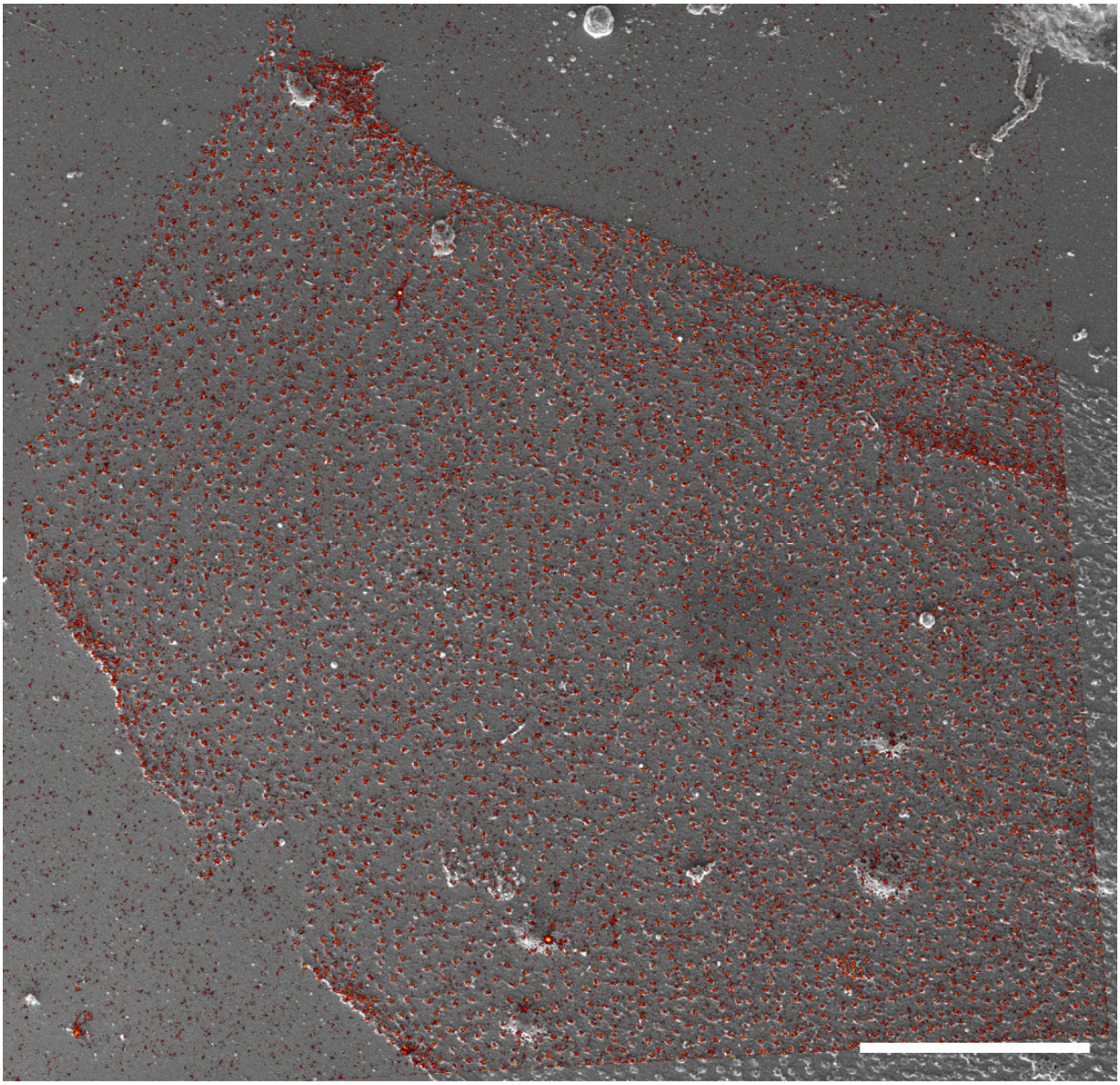

Figure S3. **Correlative SEM-*d*STORM image**. The majority of Alexa 647 WGA labeled NPCs perfectly fit the SEM image. Scale bar, 5 µm.

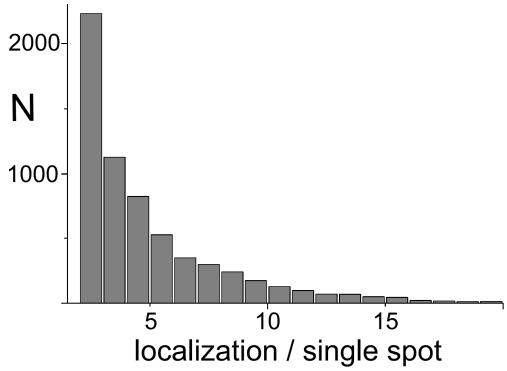

Figure S4. **Localization statistics of Alexa 647 labeled F(ab')$_2$ fragments in the absence of primary antibody directed against gp210 proteins.** Single-molecule experiments (sample size 5.000 isolated fluorescence signals) with Alexa 647 labeled F(ab')$_2$ fragments non-specifically adsorbed on nuclear envelopes show a similar distribution of localizations identified per single spatially isolated fluorescence signal than obtained in our quantification experiments (Fig. 3C). The mean localization number is determined to 5.4 ± 0.1 (s.e.) localizations per Alexa 647 labeled F(ab')$_2$ fragment (median: 3.5).