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 dSTORM 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 ωSTORM and the SEM image the ωSTORM/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-G), 100 nm (D,E), 250 nm (F,G).
Figure S3. Correlative SEM-dSTORM 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).