Supporting Information

Rigort et al. 10.1073/pnas.1201333109

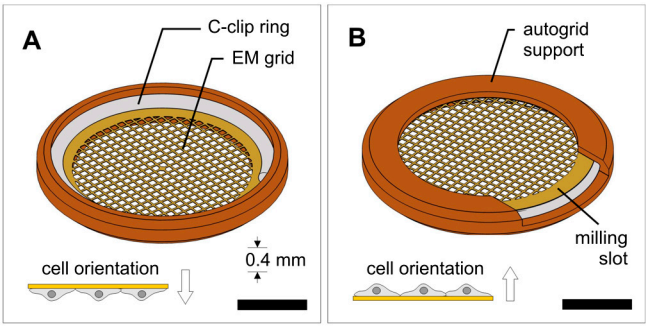

Fig. S1. Schematic drawings of the EM grid mounted into the modified autogrid support frame structure. (*A*) The frozen-hydrated EM grid is held by clamping a C-shaped clip ring into the autogrid support. The cells are facing the bottom side as indicated in (*A*). (*B*) View from the top side, exhibiting the cutout modification (milling slot) which enables FIB-milling under grazing angles of incidence. The cells are facing upwards. [Scale bars, (*A* and *B*) 1 mm].

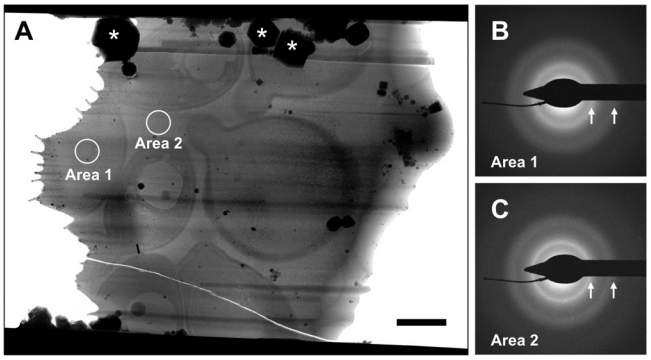

Fig. S2. Electron diffraction patterns of the lamella displaying its vitreous state. (*A*) Lamella obtained by cryoFIB micromachining and imaged under low-dose conditions in the transmission electron microscope. Various cross-sectioned *Saccharomyces cerevisiae* cells can be discerned. At the upper side, large surface frost particles can be recognized (asterisks). Stitched overview image optimized for contrast using Adobe Photoshop CS4. (*B* and *C*) Selected area electron diffraction patterns of the spots highlighted in (*A*). The pair of diffuse rings (arrows) is characteristic for amorphous ice and indicates the vitreous state of the lamella. [Scale bar, (*A*) 2 μm].

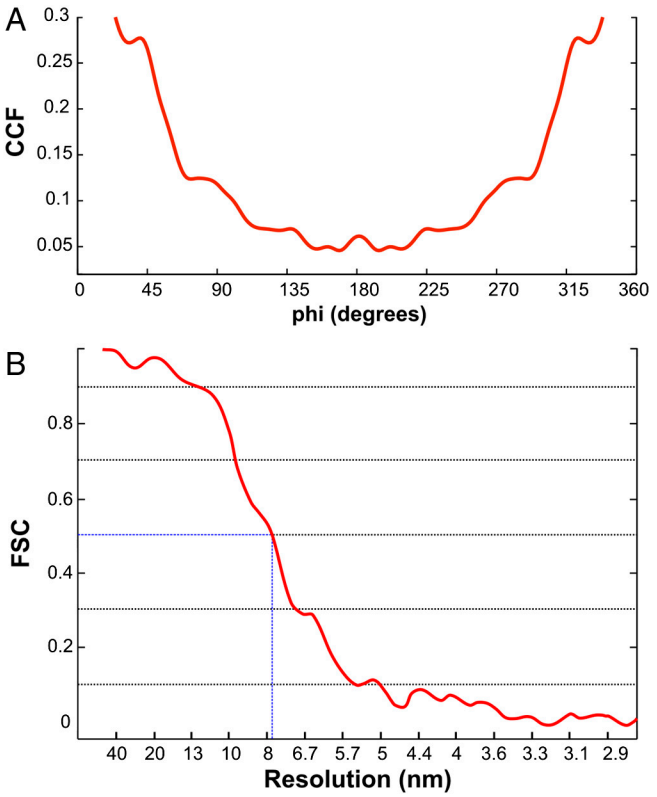

Fig. S3. Determination of symmetry and resolution of the nuclear pore complexes (NPC). (*A*) Averaged rotational correlation function of the three full particles (aligned without imposing symmetry). The local maxima at intervals of 45 degrees indicate an eightfold rotational symmetry. (*B*) Fourier shell correlation of the NPC protomer. The resolution as determined by the widely used 0.5-Fourier shell correlation (FSC) criterion is 7.9 nm.

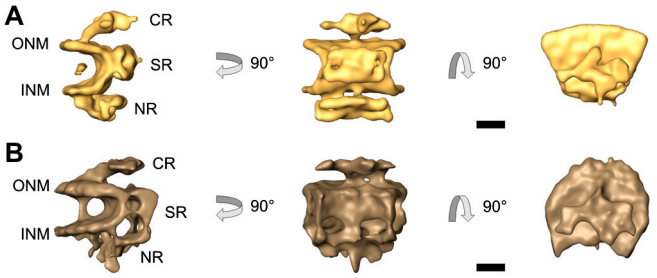

Fig. S4. Comparison of the *Dictyostelium discoideum* NPC structures obtained from a single tomogram of a FIB lamella (*A*) and multiple isolated nuclei (*B*); reported in ref. 1. ONM: outer nuclear membrane; INM: inner nuclear membrane; CR: cytoplasmic ring; SR: spoke ring; NR: nuclear ring. [Scale bar: (*A* and *B*) 25 nm].

1 Beck M, Lucic V, Forster F, Baumeister W, Medalia O (2007) Snapshots of nuclear pore complexes in action captured by cryo-electron tomography. *Nature* 449:611–615.

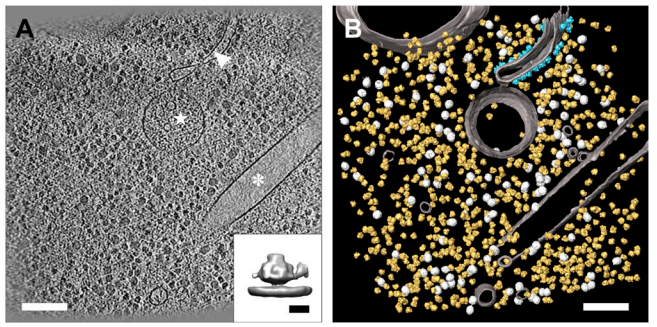

Fig. S5. Visualization of the crowded cytoplasm. (*A*) Tomographic slice showing the dense packing of macromolecular complexes in the cytoplasm (endoplasmic reticulum: white arrowhead; endosomal compartment: white asterisk; mitochondrion: white star). (*B*) Corresponding surface rendering color coded for membrane-bound ribosomes (blue), cytoplasmic ribosomes (yellow), and putative storage vesicles (white), obtained by subtomogram classification [inset in (*A*) shows the class average for membrane-bound ribosomes]. The displayed 200 nm thick tomographic volume covers 0.31 μm^3 of cellular space. 3.24% of this volume is occupied by ribosomes and 1.7% by larger macromolecular complexes of similar size (e.g. putative storage vesicles). Membrane-bound ribosomes account for only 0.41% of the mapped volume and 1.44% space is taken by the rough endoplasmic reticulum (cross-section). [Scale bar, (*A*) 200 nm].