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Supplementary Materials for

Correlative 3D x-ray fluorescence and ptychographic tomography of frozen-hydrated green algae

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/4/11/eaau4548/DC1)

Movie S1 (.mov format). Reconstructed x-ray fluorescence volume of *C. reinhardtii*.

SUPPLEMENTARY FIGURES

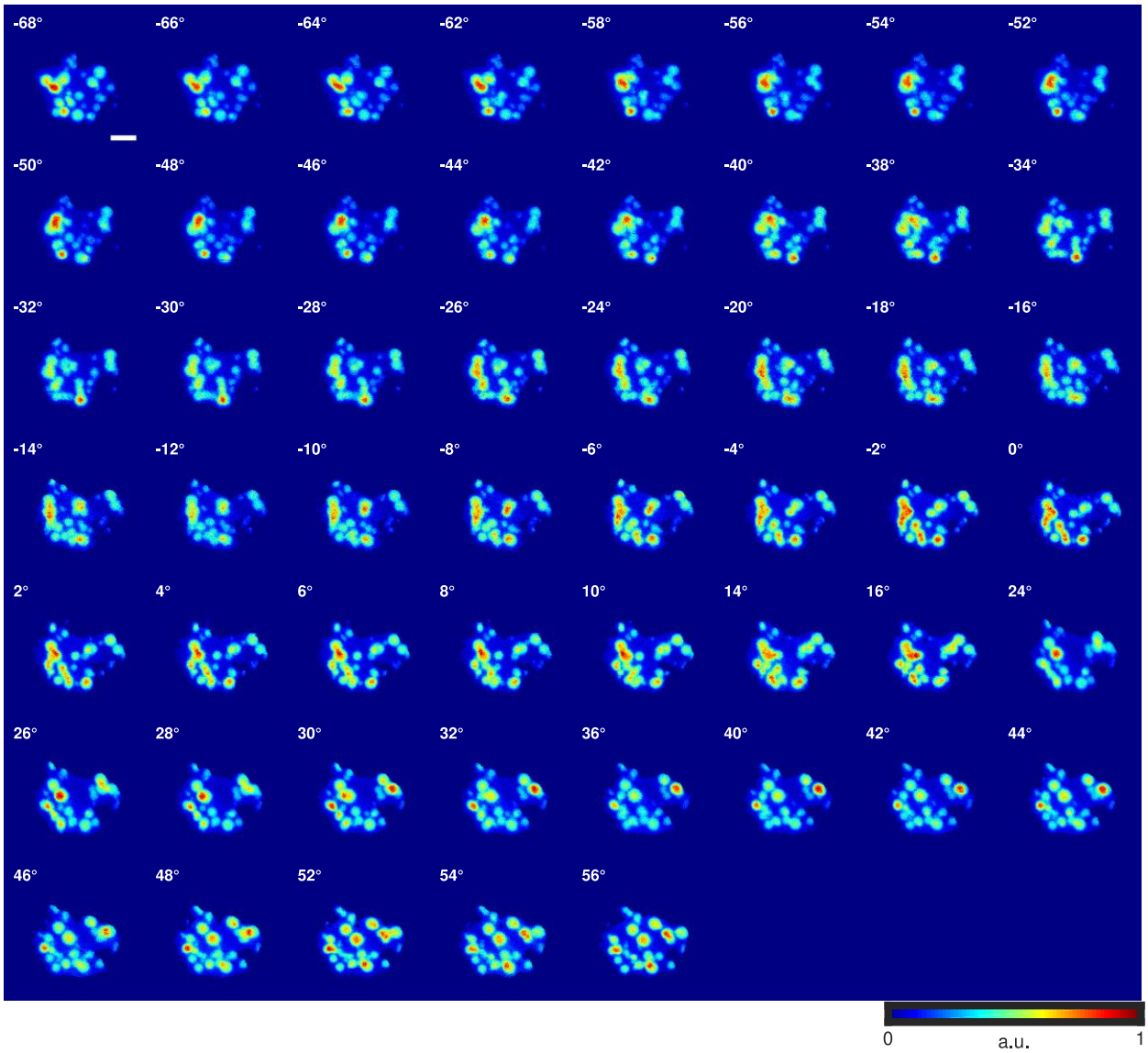

Fig. S1. Experimental phosphorus channel x-ray fluorescence projections. All 53 background-subtracted and aligned phosphorus channel X-ray fluorescence microscopy projections used as input for GENFIRE reconstruction, with tilt range from -68° to 56°. Scale bar, 2 μm.

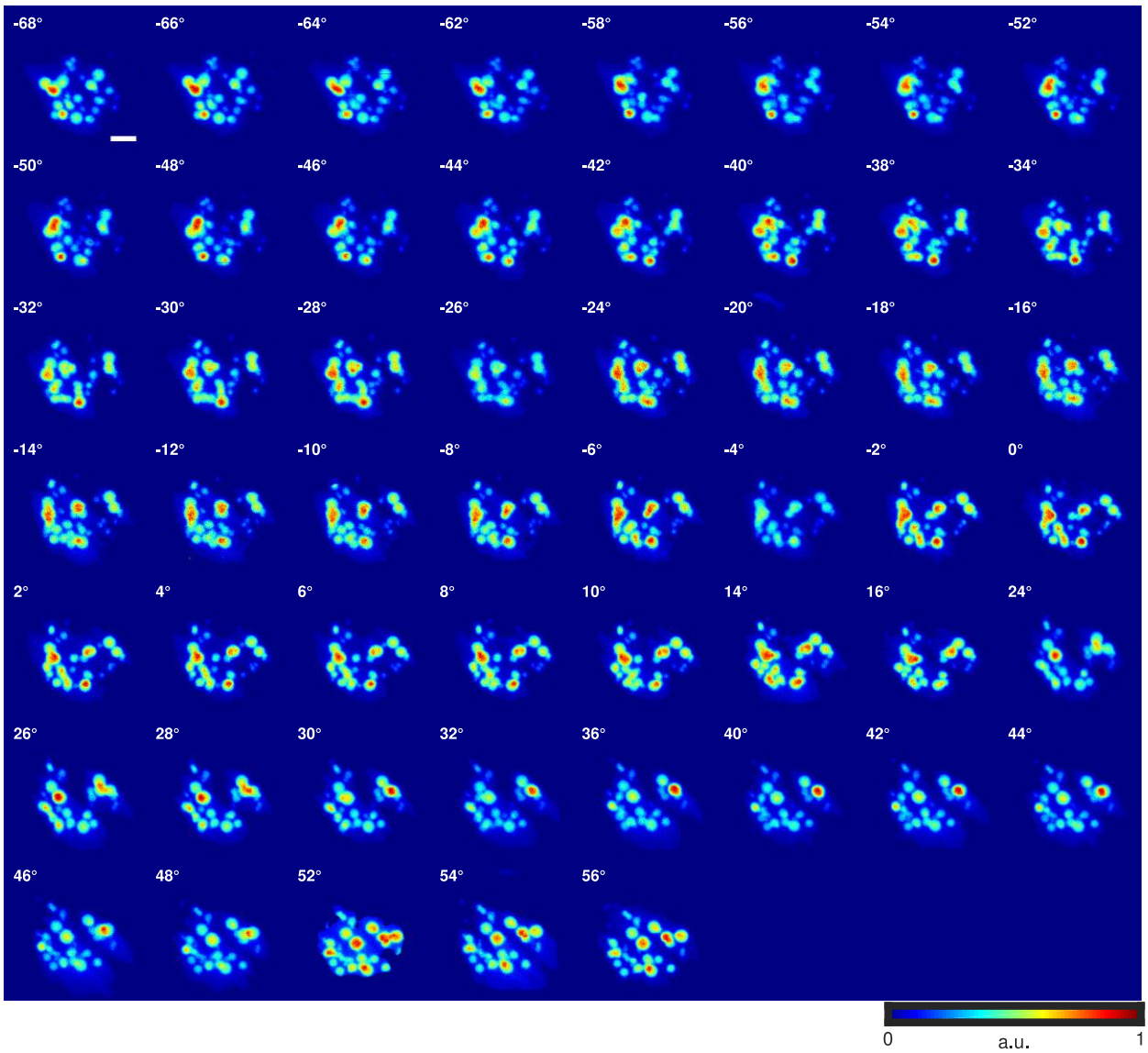

Fig. S2. Experimental calcium channel x-ray fluorescence projections. All 53 background-subtracted and aligned calcium channel X-ray fluorescence microscopy projections used as input for GENFIRE reconstruction, with tilt range from -68° to 56°. Scale bar, 2 µm.

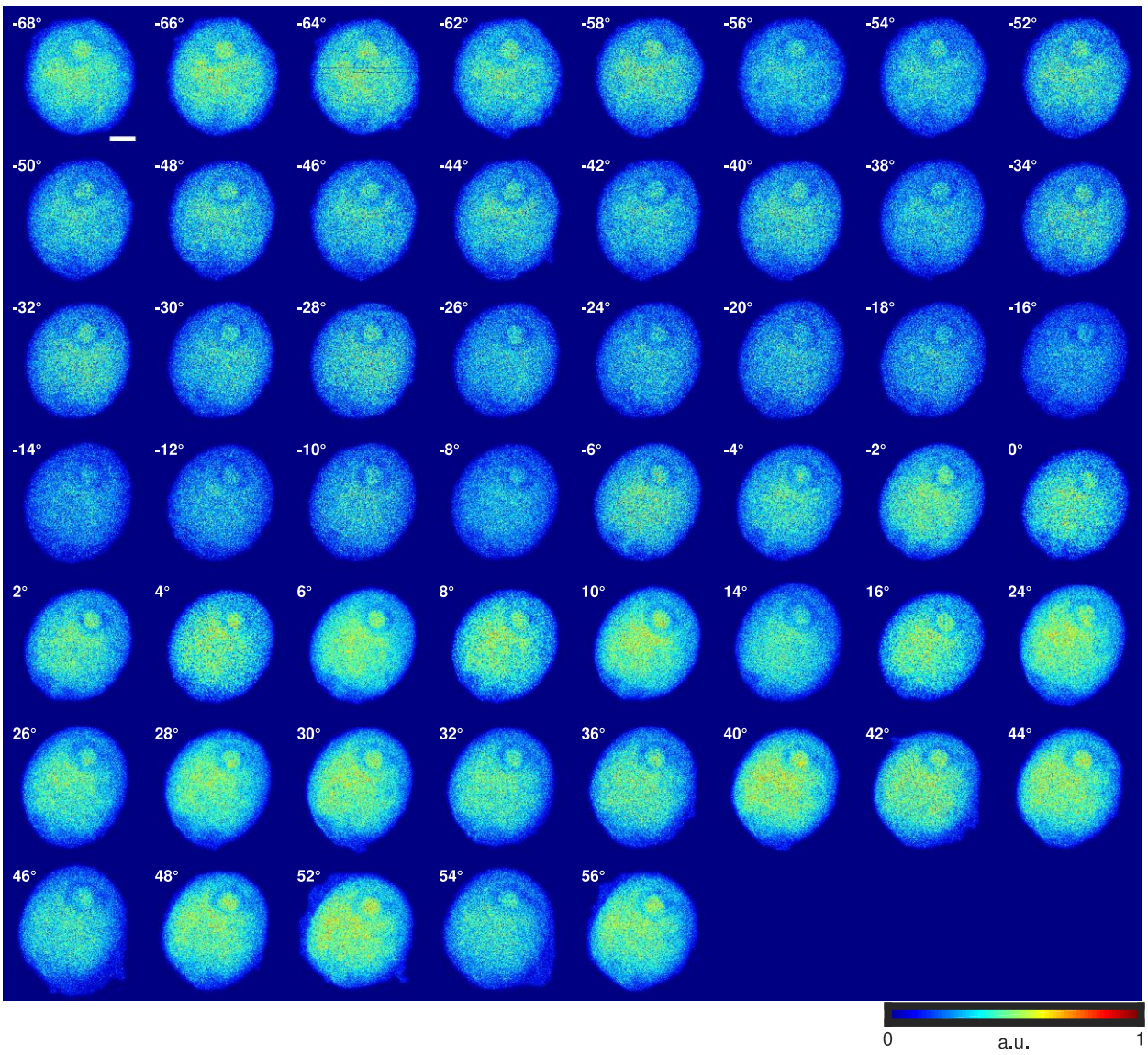

Fig. S3. Experimental sulfur channel x-ray fluorescence projections. All 53 background-subtracted and aligned sulfur channel X-ray fluorescence microscopy projections used as input for GENFIRE reconstruction, with tilt range from -68° to 56°. Scale bar, 2 µm.

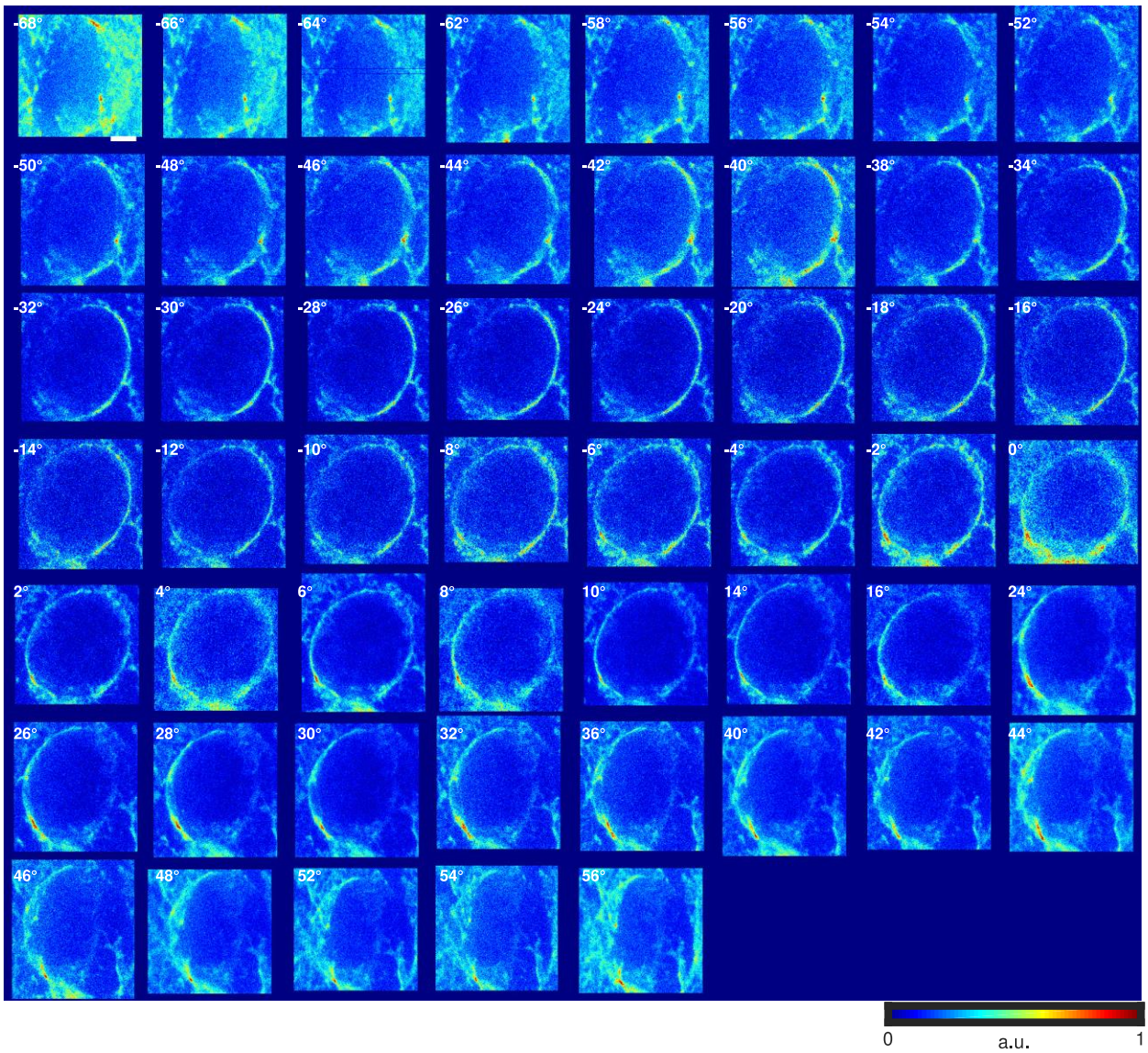

Fig. S4. Experimental chlorine channel x-ray fluorescence projections. All 53 aligned chlorine channel X-ray fluorescence microscopy projections used as input for GENFIRE reconstruction, with tilt range from -68° to 56°. Scale bar, 2 µm.

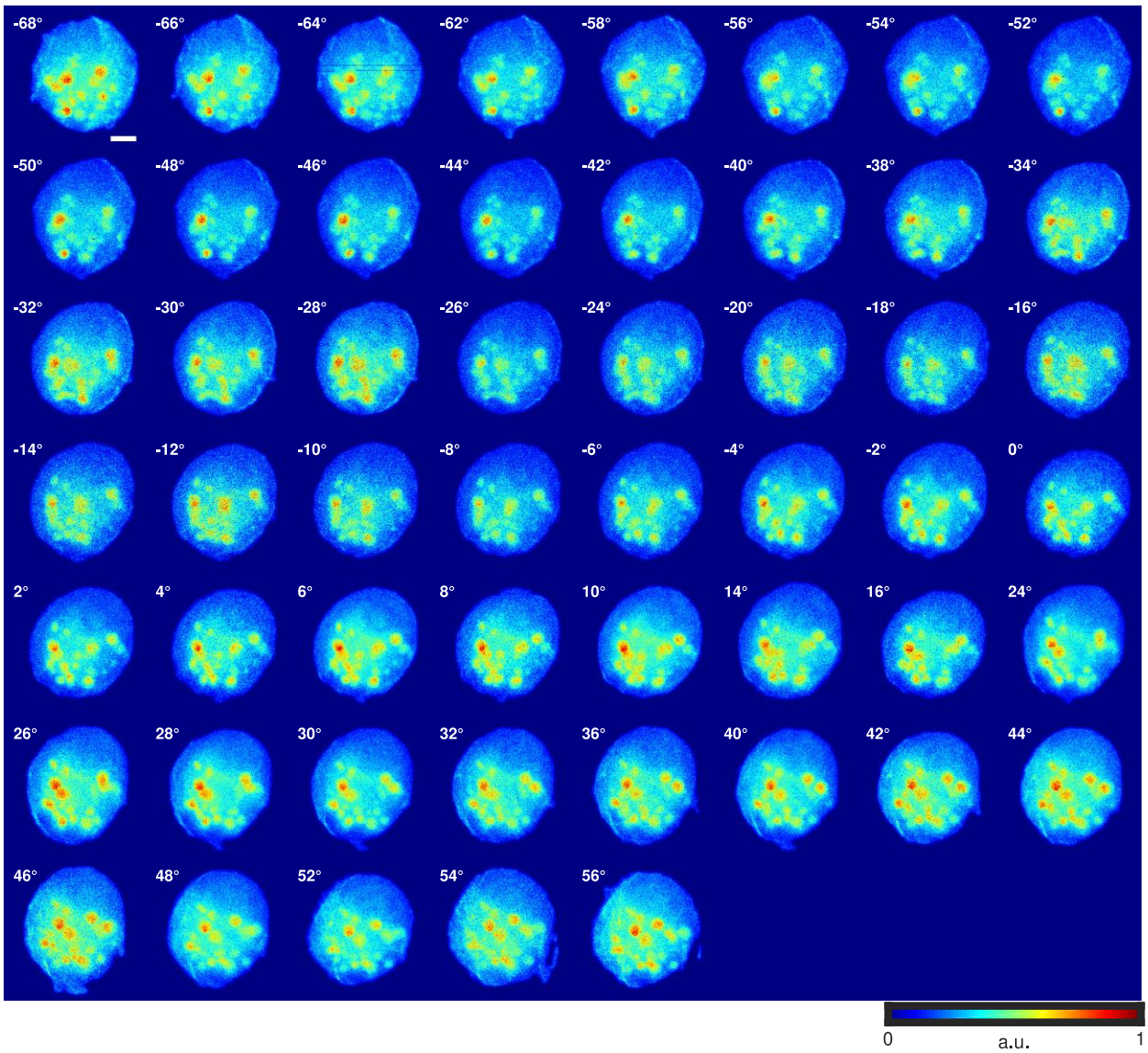

Fig. S5. Experimental potassium channel x-ray fluorescence projections. All 53 background-subtracted and aligned potassium channel X-ray fluorescence microscopy projections used as input for GENFIRE reconstruction, with tilt range from -68° to 56°. Scale bar, 2 μm.

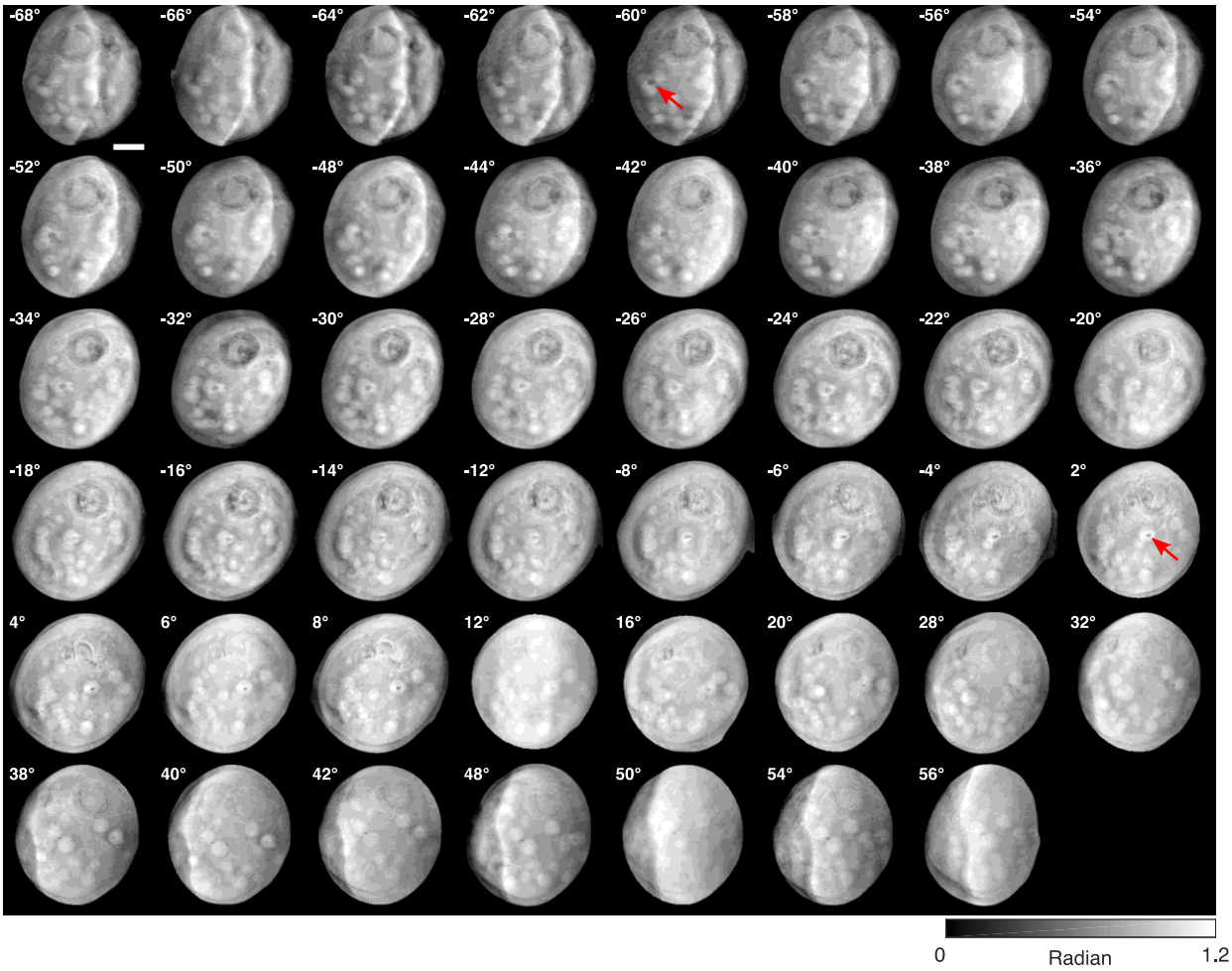

Fig. S6. Experimental ptychographic projections. All 47 background-subtracted and aligned ptychography phase projections used as input for GENFIRE reconstruction, with tilt range from -68° to 56°. The red arrows highlight the X-ray-induced damage from accidental over-exposure at 60° (correspond to same viewing angle as Fig. 2c) and 2° projections. Scale bar, 2 μm.

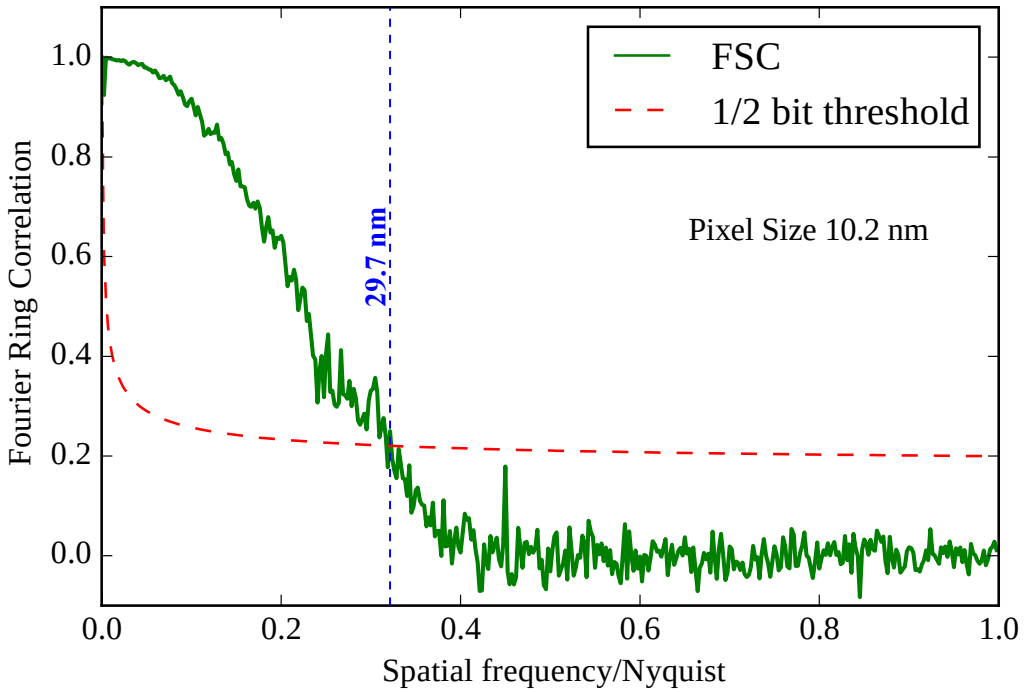

Fig. S7. Resolution of 2D ptychographic projections. Fourier ring correlation (FRC) of two identical ptychography projection measurements at zero degree, indicating a resolution of 29.7 nm using the half-bit criterion.

Movie S1. Reconstructed x-ray fluorescence volume of *C. reinhardtii*. Composite view of the reconstructed cellular volume, containing P (red), Ca (green), S (black), Cl (yellow) and K (blue) fluorescence signals. Distinct localization of fluorescence signals show 3D locations of various organelles such as pyrenoid and acidocalcisomes.