



Figure 1 . Single molecule nanoscopy of the septin Cdc11 in budding yeast. (a) Reconstructed superresolution images of the yeast septin Cdc11-GFP and widefield images of the GFP-fluorescence signal overlaid over the phase contrast image and the reconstructed superresolution image of the Cdc11-GFP positive structure. Cdc11-GFP localizes to a characteristic hourglass-shaped and later ring-like structure around the mother-bud neck. Dot-like structures are emphasized by arrowheads. (b) Dual color reconstructed superresolution images of a population of yeast cells expressing Cdc11-GFP stained with AF647 anti-GFP nanobodies (red) and the cell wall labeled with AF700-ConA (green). (c) Dual-color reconstructed superresolution images visualizing the different organizational stages of Cdc11-GFP structures during the cell cycle. (d) Reconstructed 3D-superresolution images of the development of the bud-neck yeast septin structure over time. The central opening is clearly visible in 200 nm thick z-sections. Depth is color-coded from 0 to 600 nm. (e) Quantification of the diameter of the opening of the septin structure in the different (n = 9, 36, 20, 18; \pm s.d., *: $P < 0.05$, ***: $P < 0.001$, Dunn's multiple comparison test). Scale bars: single line 1 μ m, double line 100 nm.