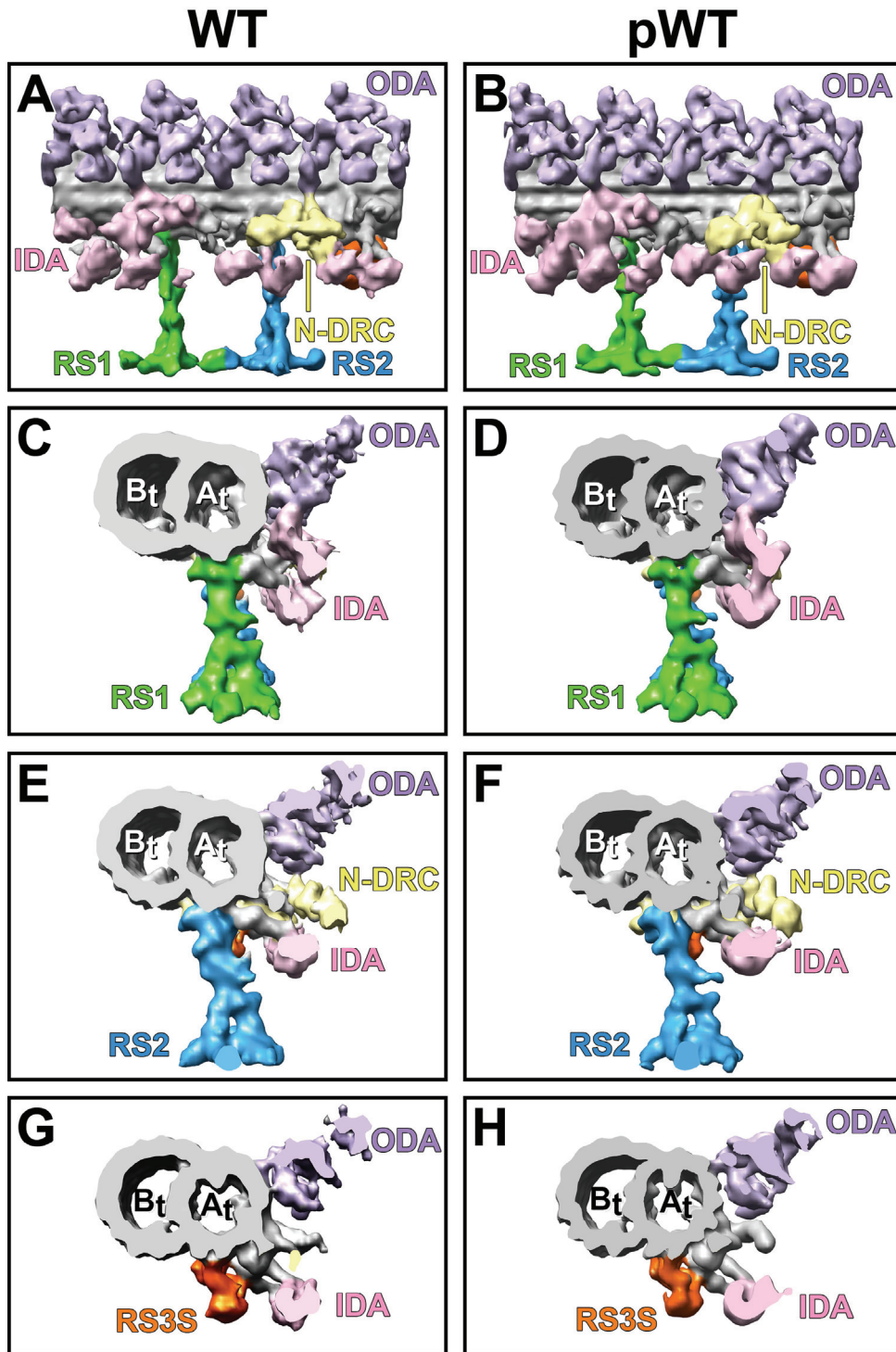
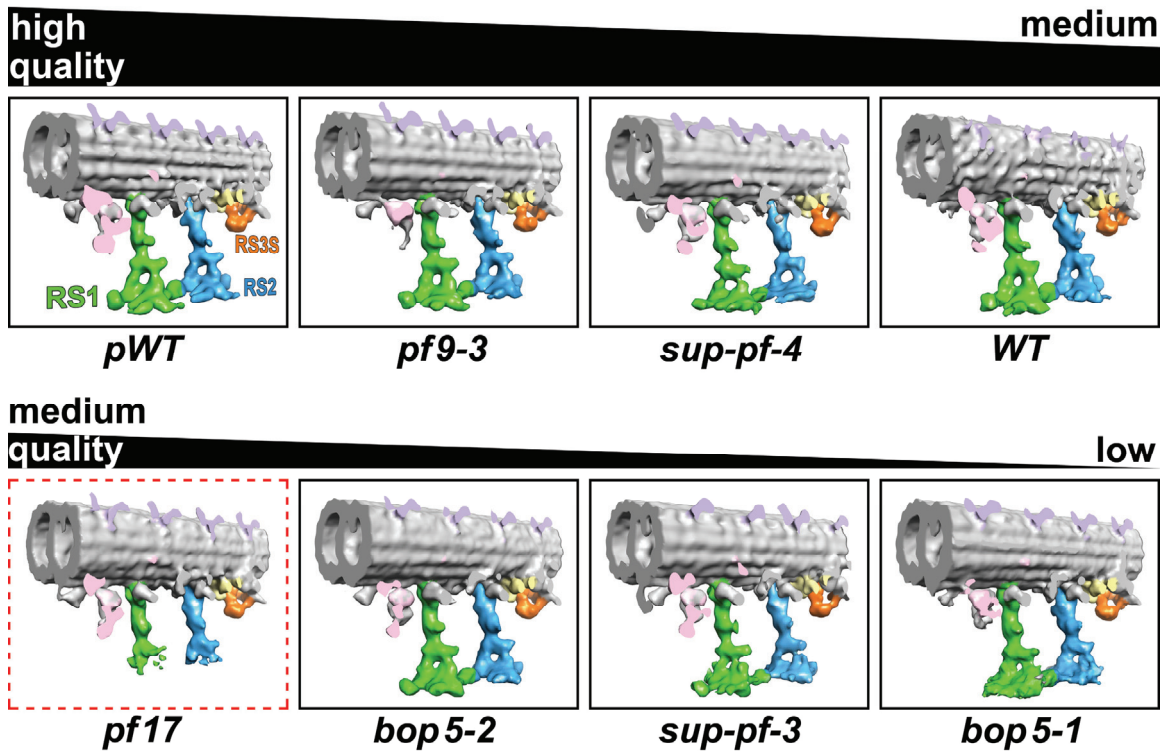


SUPPLEMENTAL MATERIAL



Supplemental Figure S1. Comparison of the RS structure between WT and pWT.

Longitudinal (A, B) and cross-section (C-H) views of isosurface renderings from the averaged repeats of WT and pWT axonemes are highly consistent and show almost identical RS and RS3S structures between the two strains. Greater detail is visible in the pWT average due to the better resolution. Labels: A-tubule (A_t), B-tubule (B_t).



Supplemental Figure S2. Quality comparison of averages from axonemal repeats of different *Chlamydomonas* strains. Isosurface renderings of averaged axonemal repeats from strains with wild type phenotype (*pWT* and *WT*), I1-dynein mutants (*pf9-3*, *bop5-2*, *bop5-1*), N-DRC mutants (*sup-pf-4*, *sup-pf-3*) and the RS head-less mutant *pf17* (highlighted by a dashed red box) are arranged according to average quality with the best average shown top left and the worst bottom right. The comparison of all strains (except for *pf17*) shows that the main features of RS and RS3S structure (see text for details) are highly consistent between different averages. Therefore, the missing RS heads in *pf17*, which has a medium quality structure, are not averaging artifacts or caused by poor resolution, but rather represent missing electron density due to structural defects. (The N-DRC and I1-dynein mutants were previously published in Heuser *et al.*, 2009 and Heuser *et al.*, under review).

Supplemental Video 1. The comparison of the 3D structure of RS1, RS2 and RS3S of an averaged pWT axonemal repeat reveals that RS3S is distinct from RS1 and RS2. RS1 and RS2 share a similar overall shape, but red arrows and circles highlight differences between the spokes.

Supplemental Video 2. Animated visualization of an averaged axonemal repeat from pWT shows the 3D radial spoke structure and attachment to the doublet microtubule. Connections of the N-DRC to RS2 and RS3S are highlighted. Related to Figure 2.

Supplemental Video 3. Serial cross-sectional slices from proximal to distal through the averaged axonemal repeat of pWT show the connections of IDAs to the bases of the radial spokes and RS3S: the tails of dynein inner arms IA2, IA3 and IA6 connect to RS1, RS2 and RS3S, respectively. Related to Figure 2.

Supplemental Video 4. A comparison of the 3D structures of the radial spokes from WT and the head-less spoke mutant *pf17* reveal the extent of the spoke heads (red colored mesh). While both spoke heads are missing in *pf17*, RS3S as well as all other key axonemal structures are unaffected. Related to Figure 3.