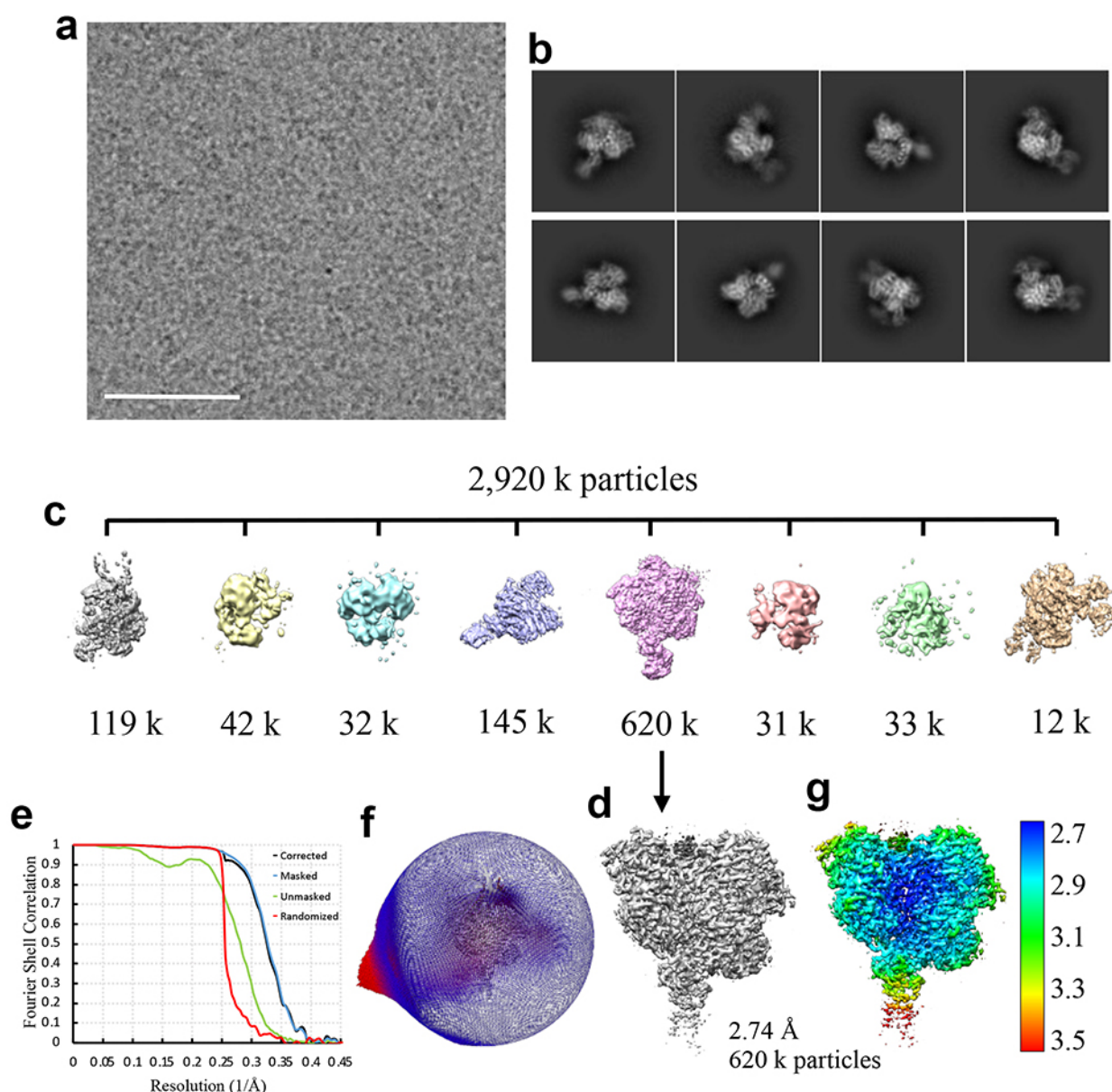
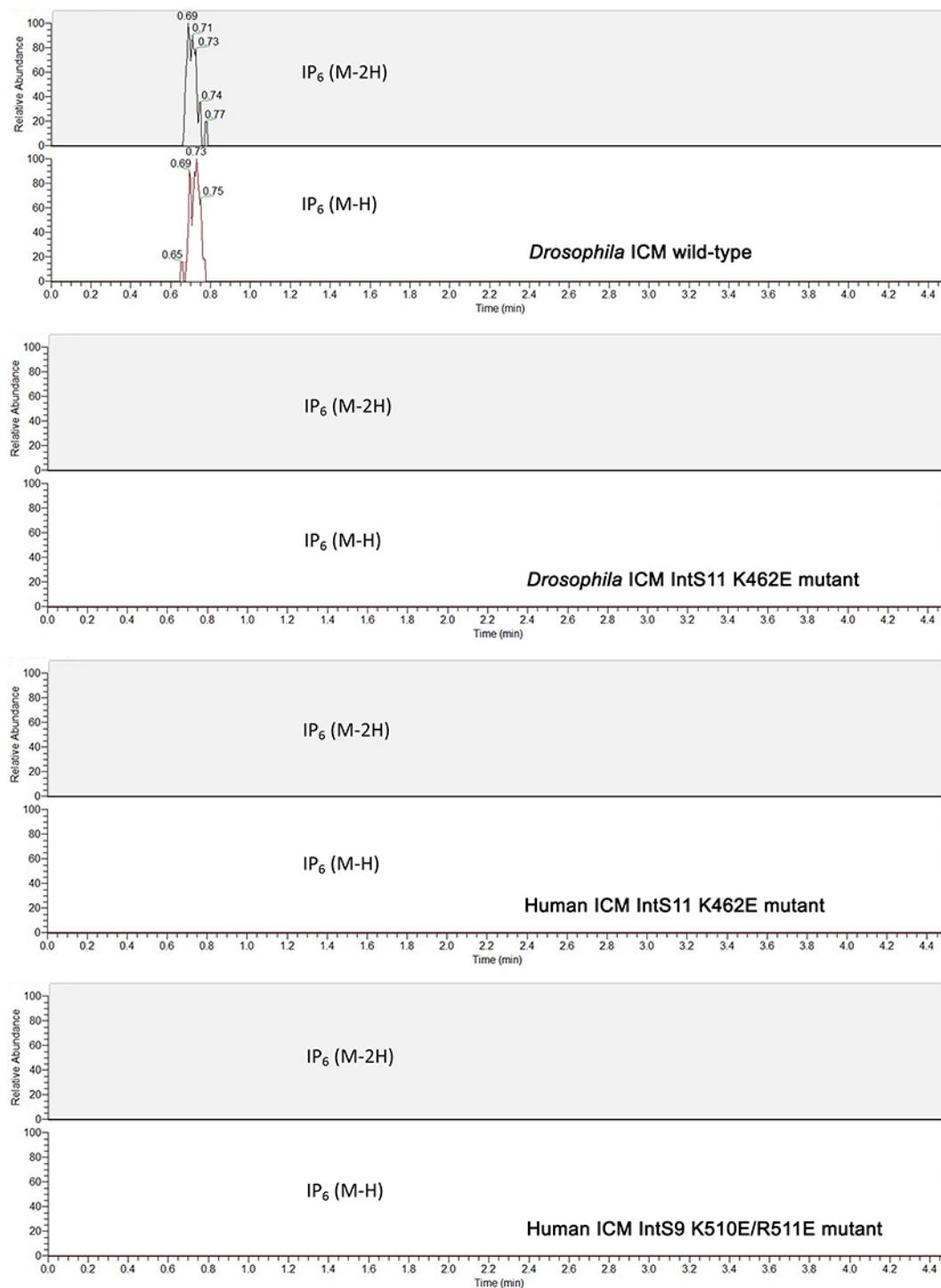
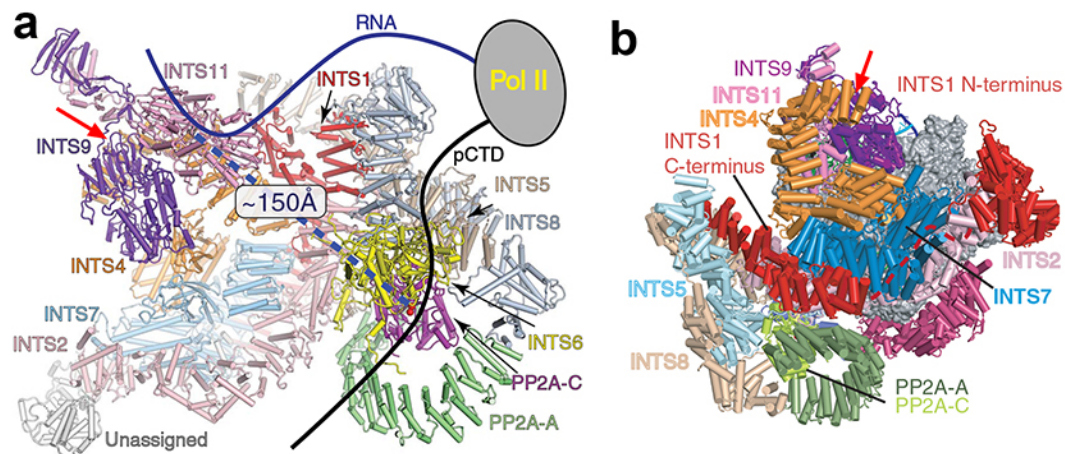




Supplementary Fig. 1. Single-particle cryo-EM analysis of the *Drosophila* IntS4-IntS9-IntS11 complex. (a). A region of a cryo-EM micrograph of the IntS4-IntS9-IntS11 complex. Scale bar: 50 nm. One grid was examined. Most of the image stacks contain particles of similar quality. (b). Ten 2D class averages from the cryo-EM micrographs. (c). 3D classification (hetero refinement) of particles identified from 2D class averages. (d). Final cryo-EM map after Bayesian polishing and refinement in RELION. (e). Fourier shell correlation curves for the final model. (f). Orientations of particles used in the refinement for the final cryo-EM reconstruction. (g). Local resolution map for the final cryo-EM reconstruction.



Supplementary Fig. 2. No IP₆ binding to mutant ICM samples was detected by mass spectrometry. LC-MS spectra of wild-type and mutant *Drosophila* ICM and mutant human ICM samples for [M-2H] and [M-H] ions of IP₆ are shown. While clear signals are observed for IP₆ with wild-type *Drosophila* ICM, no signals were observed for the mutant samples.



Supplementary Fig. 3. The IP₆ binding site is far away from other modules of Integrator and Pol II.

(A). The IP₆ binding site in the structure of human Integrator is indicated with the red arrow. Modified from Ref. 21. (B). The IP₆ binding site in the structure of human Integrator-Pol II complex is indicated with the red arrow. Modified from Ref. 26.

Supplementary Table 1. Primer sequences

Primer	5'>3' sequence
Site-directed mutagenesis of INTS11	
Drosophila K462E (F)	CCCTGCTTGAAGCAGAGGCC
Drosophila K462E (R)	GGCCTCTGCTTCAAGCAGGG
Site-directed mutagenesis of INTS9	
Drosophila R504D (F)	GGCGAGATCATAGATCTGCCATTAACCGG
Drosophila R504D (R)	CCGTTTAAATGGCAGATCTATGATCTCGCC
Drosophila K508E (F)	CGTCTGCCATTAGAACGGAAGCTGG
Drosophila K508E (R)	CCAGCTTCCGTTCTAATGGCAGACG
Drosophila R509E (F)	CGTCTGCCATTAAGAGAAAGCTGGATCG
Drosophila K509E (R)	CGATCCAGCTTCTCTTTAATGGCAGACG
Drosophila R2E (F)	GGATCCAGAATTGTATTGTCTCAGCGGGGACC
Drosophila R2E (R)	GCGGCCGCCTAAAACTCTGTAAGCATTTTCATGATGC
Primers used for luciferase cloning	
KpnI Renilla luciferase (F)	GGCCGGTACCATGGCTTCCAAGGTGTACGA
MfeI Renilla luciferase (R)	GGCCCAATTGCGCCTAGAATTACTGCTCGT
Drosophila qPCR amplicons	
U4 snRNA misprocessing (F)	GGTGGCAATACCGTAACCAAT
U4 snRNA misprocessing (R)	GGCTAAGACAACCGTCATATTAA
Tiggrin (F)	GCAACATATGGGTGCGGTGAC
Tiggrin (R)	CTTGTTGCGGATCAGGTAGC
Bj1 (F)	CGCAGAAAGGCCCTGACTAAT
Bj1 (R)	AGCTCCAGATGAAAAGCGATTC