



Figure A. Isolation of the *S. cerevisiae* closed DNA damage recognition complex of Rad4/Rad23-TFIH on damaged DNA by glycerol gradient centrifugation. Analysis of the peak fraction (right) shows a stoichiometric complex of the early DNA damage recognition proteins Rad4/Rad23/Rad33 and the multi-subunit, TFIH composed of the helicases Rad3 and Ssl2 and core proteins Tfb1, Tfb2, Ssl1 and Tfb4. Peak fractions were pooled and concentrated for structural determination.

Figure B. Representative image of complex used for structural determination. R2/2 Quantifoil 200 mesh grids were imaged on Titan Krios with Gatan K2 detector at 130,000x magnification with a nominal dose of 42 e⁻/Å.

Figure C. 2D class averages of closed Rad4/Rad23-TFIH complex show Rad4/Rad23 (V shape, green star) positioned between the two helicases of TFIH.

Figure D. Initial 3D reconstruction of Rad4/Rad23-TFIH complex fit with crystal structure of Rad4/Rad23 (green, PDB: 2QSH) and TFIH helicases Rad3 (Red) and Ssl2 (Purple) from *S. cerevisiae* Preinitiation Complex (EMDB-3846, PDB:5OQJ) show DNA positioned between two helicases and primed for DNA unwinding.