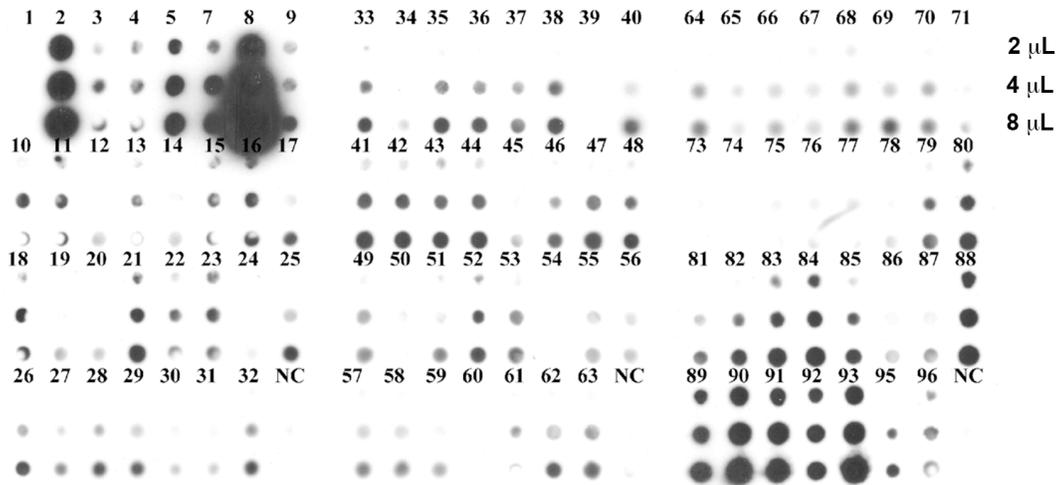
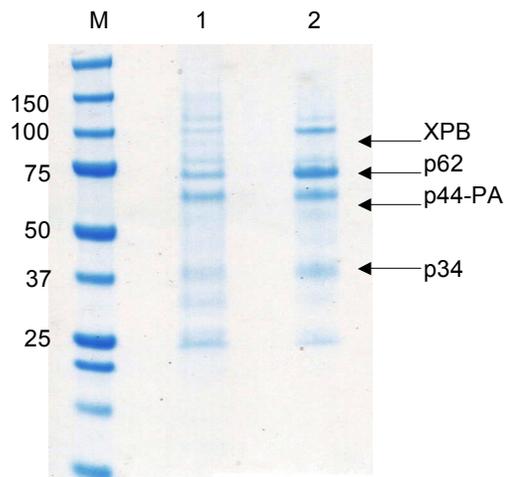


## Supplementary Figures



Supplementary Fig. 1

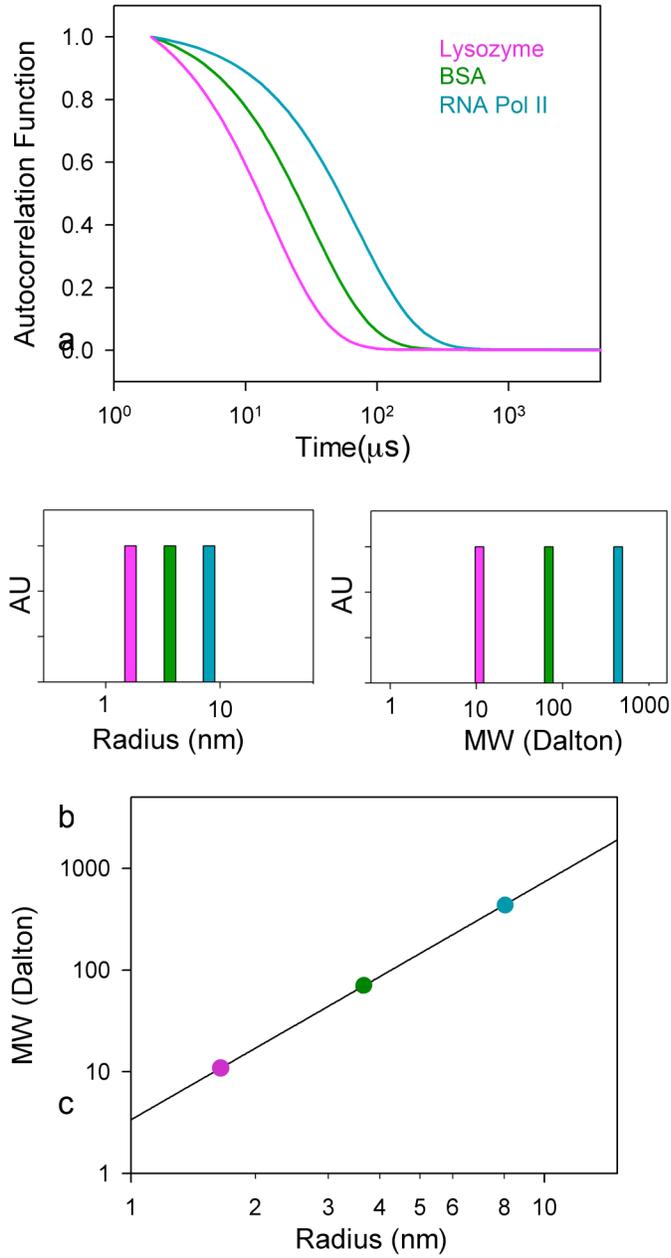
96 well-format detergent extraction using of the human single-pass trans-membrane protein CD3- $\delta$  quantified using immuno-blots. Each number corresponds to individual detergent. NC denotes sample solubilization in buffer with no detergent.



Supplementary Fig. 2

Sf9 cells (0.5 gms) co-infected with four viruses expressing 10His-XPB, 10His-p62, p44-PA and 10His-p34 were resuspended in 5mls of buffer (400 mM  $C_2H_3O_2K$  pH7.5, 50 mM Hepes pH 7.5, 2mM BME and protease inhibitors). Cells were sonicated without detergents or in the presence of Brij-58 and  $C_{13}E_8$ . Clarified lysate was obtained after centrifugation and incubated with  $Ni^{2+}$  beads (200 $\mu$ L) for an hour and washed with the same buffer with or without detergents and 40 mM imidazole. After removal of wash buffer beads were eluted with 300 mM imidazole and the elution was boiled with sample buffer and ran on a SDS-PAGE gel for analysis. The following

conclusion can be drawn from this figure: the sample purified without detergent (lane 1) shows no enrichment of the 10His-XPB while the sample with detergents (lane 2) shows enrichment of all components.

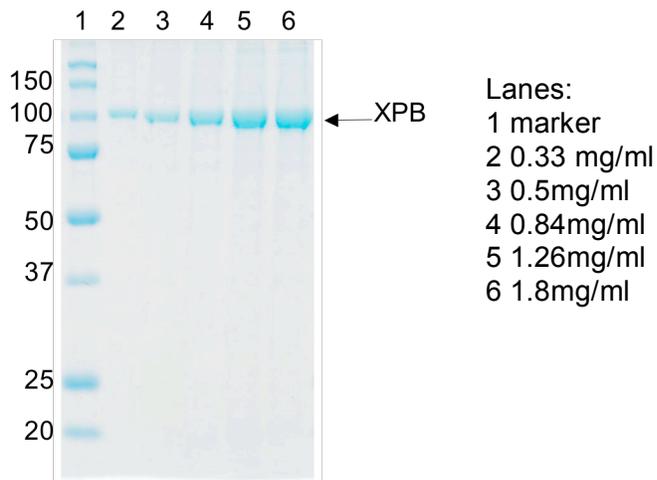
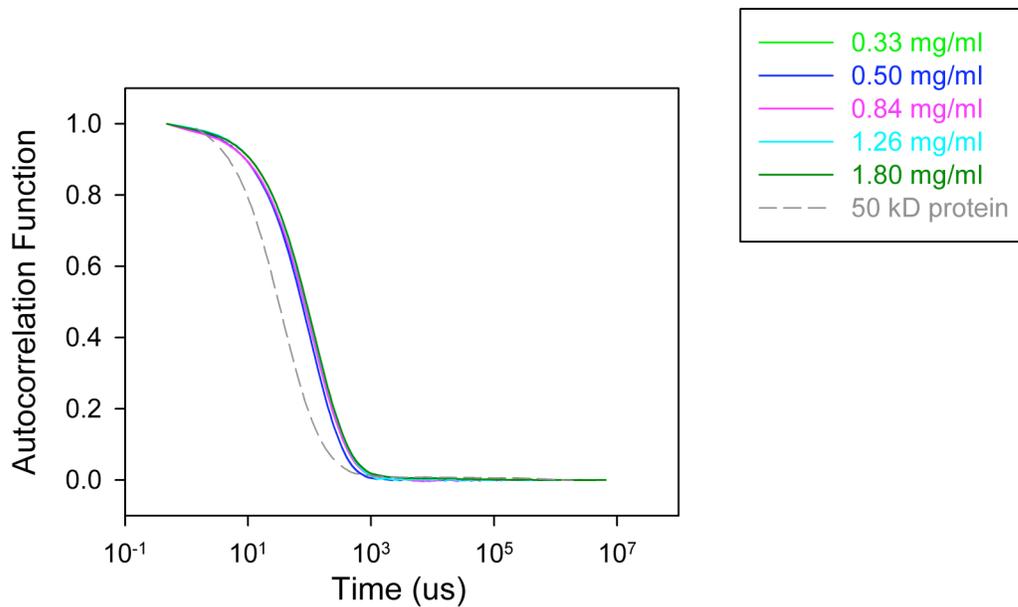


Supplementary Fig. 3A DLS measurements for lysozyme, Bovine Serum Albumin (BSA) and RNA Polymerase II (RNA Pol II) in buffer A. The molecular weights (MW) of these proteins range from 10 to 550 kDa.

a) Correlograms

b) Left panel. Average particle size measured with the DynaPro for lysozyme, BSA and RNA Pol II are 1.7 nm, 3.6 nm and 8.1 nm, respectively. Right Panel. The calculated (predicted) MW (using Dynamics DynaPro software) corresponding to such particle radii are 12 (14) kDa, 69 (67) kDa and 450 (500) kDa respectively.

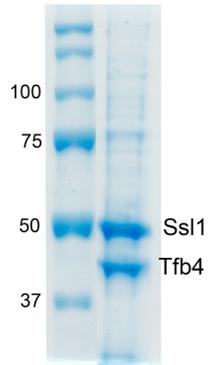
c) Log-log plot of particle radii vs molecular weight.



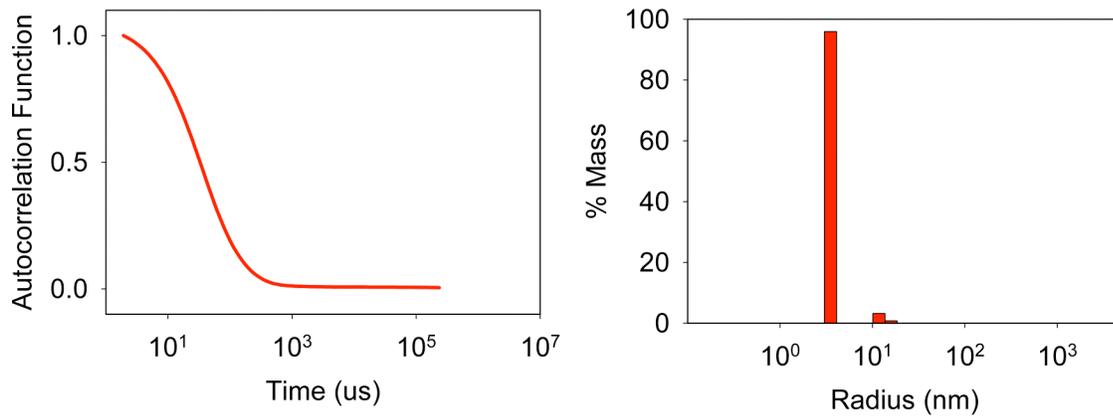
Supplementary Fig. 3B

Top Panel: Comparison of DLS of XPB samples at several concentrations. Continuous colored lines represent correlograms of samples with XPB concentration ranging from 0.33 to 1.8 mg/ml. As a comparison, gray dashed line represent the correlogram of a 50 kD protein. Note that the variation range in the correlograms ensemble is very small.

Bottom Panel: Equal volumes (10  $\mu$ L) from XPB samples in the top panel ran on a SDS-PAGE gel.



Supplementary Fig. 4  
 cTFIIH disruption in the presence of detergents. The use of sucrose monolaureate and  $C_{12}E_8$  during purification of cTFIIH (using a TAP-tag on the core's subunit Tfb4) removed most subunits from cTFIIH.



Supplementary Fig. 5  
 DLS measurements of purified Fzd4. The particle size of the purified proteins corresponds to a monomer of 55 kDa.

**a. Pre-initiation templates**

5' TGT CTA CCG CCG AGC AGC ACG ATC CTC TCG ATG CCC CTT TTA TAG GCG CCC 3'  
3' ACA GAT GGC GGC TCG TCG TGC TAG GAG AGC TAC GGG GAA AAT ATC CGC GGG 5'

TEMPLATE STRAND

NON-TEMPLATE STRAND

**b. Transcription bubble**

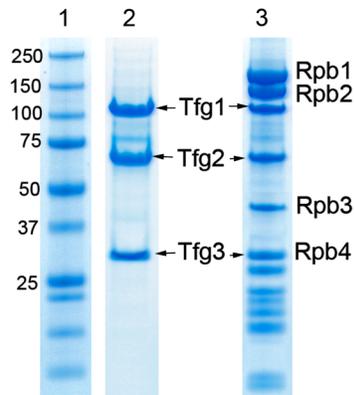
5' CTA CCG ATA AGC AGA CGA TCC TCT CGAT GC ATT GAC TCA TCG ACG 3'  
3' GAT GGC TAT TCG TCA CGA TCC TCT CGAT GC TAA CTG AGT AGC TGC 5'  
**Non-complimentary**

RNA

TEMPLATE STRAND

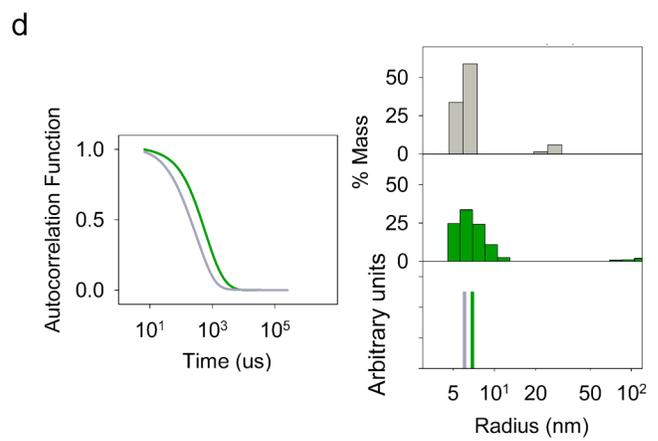
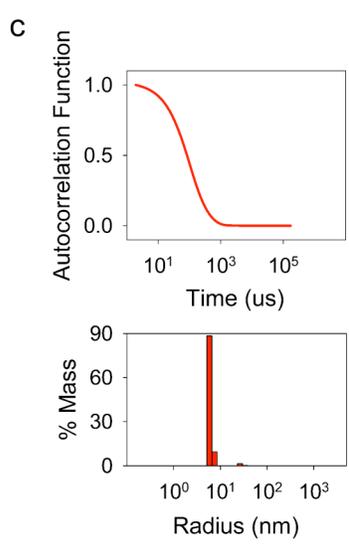
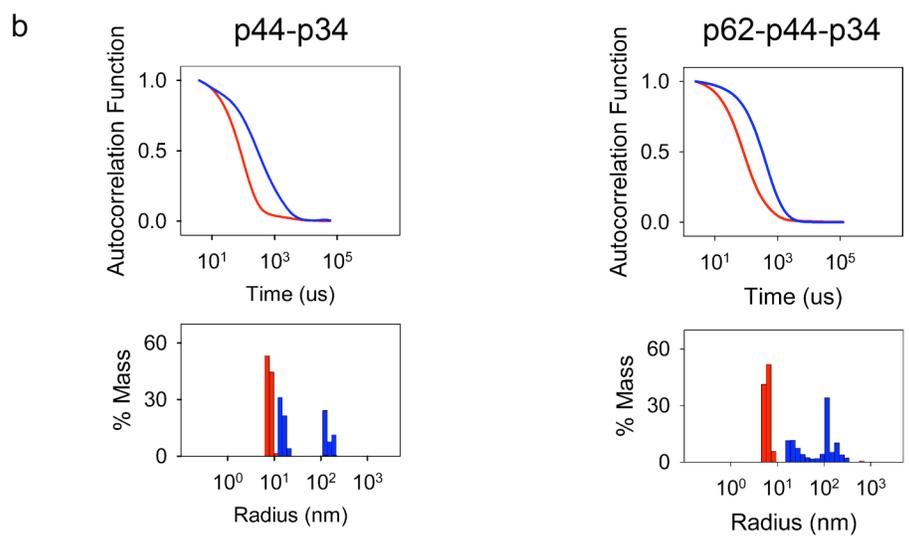
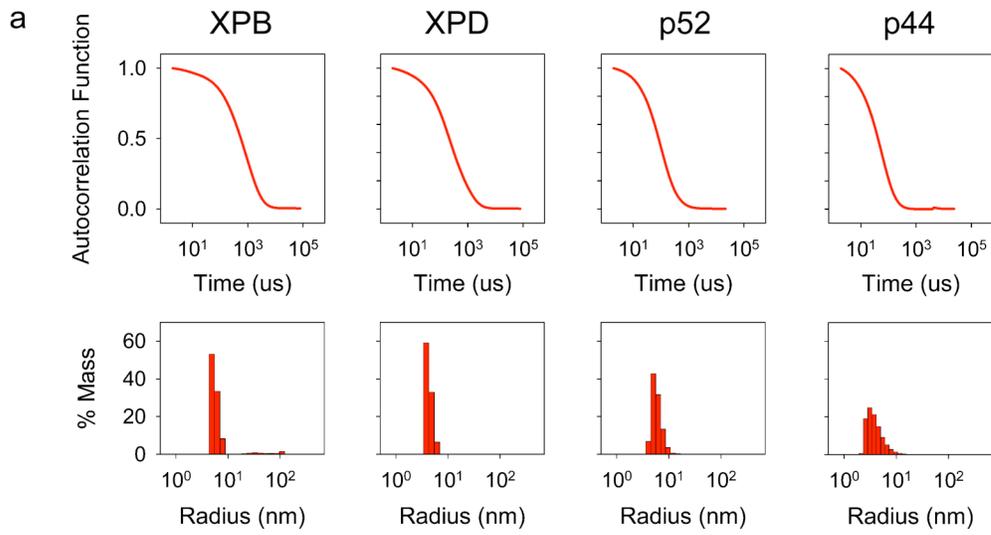
NON-TEMPLATE STRAND (15 MER BUBBLE)

Supplementary Fig. 6. Nucleotide sequences used for transcriptional complex assembly. a. Minimal pre-initiation complex (mPIC). b. Elongation complex.



Supplementary Fig. 7

SDS-PAGE of TFIIH (lane 2) purified in the presence 0.2% Z3-10 and a Pol-TFIIF complex used to reconstitute mPIC, and most other transcriptional complexes. Labels for Pol II subunits Rbp5-Rbp12 in lane 3 are omitted for clarity.



Supplementary Fig. 8:

a. DLS measurements of individual cTFIIH subunits solubilized in the presence of detergent.

b. (I) DLS measurements of p44-p34. The heterodimer was successfully purified without detergent, however DLS experiments revealed the presence of large particle aggregates that were partially refractory to detergent solubilization (particle size in the presence of n-tridecyl- $\beta$ -D maltopyranoside decreased from 18 nm to 8 nm (1800 kDa to 400 kDa, blue and red respectively). (II) DLS measurements of p62-p44-p34 heterotrimer in buffer only conditions (blue) and in the presence of 0.5 mM Brij-58 (red).

c. DLS measurements of the heterotrimeric complex formed by XPB-p52-p8-GST purified in the presence of 0.5 mM Brij-58

d. DLS measurement of the complex formed by XPB-P62-P44-P34 before (gray) and after addition of p52 and p8-GST (green). Predicted molecular weights for average mass distributions are  $\approx$ 240 kD for the heterotetramer (gray) and  $\approx$ 300 kD (green) for the 6-subunit complex.

## Supplementary Material

### Notes on the use of detergents

1. Stocks and buffers: It is highly recommended to keep all detergent stock solutions and buffers at a temperature no lower than 4°C. It is important to not keep Sarkosyl containing buffers on ice because it may precipitate out of solution below 4°C. Certain protease inhibitors such as PMSF may also precipitate Sarkosyl out of solution. Preparing the buffer with inhibitors and then adding Sarkosyl can help to avoid this problem.

2. Concentrating a sample with detergent: If a sample (bearing a 10-His affinity tag) containing detergent is eluted at a low protein concentration, then as it is concentrated the detergent CMC may increase several fold. Dialysis may deplete the excess detergent and lower the imidazole in order to perform a second binding of the sample to a small volume of Ni<sup>2+</sup> beads. After binding to the affinity beads the extensive washes with buffer and detergent at 1.5 CMC to further decrease detergent concentration. The importance of the small volume of affinity beads (100-200µL) is to keep the sample concentrated and be able to elute in a small buffer volume. Then, protein concentration can be followed by DLS without the risk of increasing detergent concentration.

### Supplementary Protocols 1

#### 1. Membrane Proteins and Membrane associated proteins

##### Purification of Fzd4

Insect cells expressing His-Fzd4 were collected 48 hours after infection. Cells were sonicated in a buffer containing: 200 mM KCl, 30 mM Tris-HCl pH 7.5, 2 mM BME, protease inhibitors (buffer A). The sample was centrifuged at 100000 RCF for 1 hour. The pellet was resuspended in buffer A with 20 mM Fos-Choline-12 and 8 mM Sarkosyl. The sample was left rocking at 4°C for 2 hours and then it was centrifuged at 100000 RCF for 1 hour. The supernatant was diluted 1 to 4 with buffer A and it was loaded into a column packed with Nickel beads (Sigma). The beads were washed with buffer A with 5 mM Fos-Choline-12 and increasing amounts of imidazole and finally eluted at 150 mM and 300 mM concentrations. The elution fractions were diluted to bring the KCl and

imidazole concentrations, to 100 mM and 60 mM respectively. The sample was loaded into a 5 ml Heparin column (Sigma) and a gradient of 20 column volumes was performed from 100 mM KCl to 600mM KCl keeping the rest of the components the same (30 mM Tris pH7.5, 2 mM BME, protease inhibitor and 5 mM Fos-Choline-12). Initially, Fzd4 was extracted with Fos-Choline-12. Then, a combination with Sarkosyl and Fos-Choline-12 was tested (since Sarkosyl was another detergent shown by dot-blot analysis to be able to extract the target protein). The use of two detergents for extraction was effective and the protein yield was improved by 50%. The pellet after detergent extraction in the presence of Sarkosyl is smaller and clearer. The increase in yield may suggest that the presence of Sarkosyl in solution helps disrupting cell membranes leading to a higher protein yield.

#### Purification of PTHR1

Bacteria cell pellets expressing PTHR1 were homogenized in buffer P consisting of 150 mM NaCl, 25 mM Tris pH 8.0, and 1 X Protease Inhibitor Cocktail (10 µg/ml pepstatin, leupeptin, chymostatin) plus 100 µM phenylmethylsulfonyl fluoride (PMSF) and 0.5 mM ethylenediaminetetraacetic acid (EDTA). Lysis was performed by sonication. Lysate was cleared by centrifuged at 5,000 RCF for 15 mins. The membrane fraction was isolated by centrifugation at 100,000 RCF for 30 mins. Membrane pellets were extracted in 25 mM sarcosine, 5 mM Fos-choline 14 for 2 hrs before being spun down at 100,000 RCF for 30 mins. The supernatant was diluted 1:3 in Buffer P and was at 0.1 mL/min onto 4 mLs of His-Select Fast Flow Resin (Sigma). Resin was washed in stepwise gradients with 15 column volumes of buffer P + 25, 40 and 60 mM imidazole and eluted with 90 mM imidazole in buffer P. Elutant was pooled and loaded onto a HiPrep 26/10 desalting column (GE healthcare).

#### Purification of Wnt5A-Protein A

Insect cells expressing Protein A-Wnt5A were separated from media 48 hours after infection. To the media we add 100 mM NaCl, 30 mM HEPES pH 7.5, 5 mM DDMAP and kept stirring for 1 hour at 4°C. The media was loaded in 25 ml of IgG beads (GE) at 1 ml/min. Column was washed with 120 mM NaCl, 30 mM HEPES pH 7.5, 5 mM

DDMAP (buffer A) for 20-30 column volume. In order to exchange detergent from DDMAP to Brij-58, it was run a gradient from buffer A to 120 mM NaCl, 30 mM HEPES pH 7.5, 0.5 mM Brij-58 (buffer B) in 5 column volume. After washing the column with buffer B for additional 10 columns volume TEV enzyme was added to the beads in order to cut the Protein A tag and kept overnight at 4°C. The next day Wnt5A was eluted from the column. The sample was loaded in a Resource Q column and washed with buffer B. To elute the protein a gradient was performed from buffer B to 600 mM NaCl, 30 mM HEPES pH 7.5, 0.5 mM Brij-58.

#### Purification of G $\alpha$ S $\beta$ $\gamma$

Insect cells expressing His-G $\alpha$ S $\beta$  $\gamma$  were collected 48 hours after infection. Cells were sonicated in a buffer containing: 250 mM KCl, 30 mM HEPES pH 7.5, 5 mM MgCl<sub>2</sub>, 2 mM BME, protease inhibitors (buffer A) and 5 mM C12E8. The sample was centrifuged at 100000 RCF for 45 minutes. The supernatant was diluted 1 to 3 with buffer A in order to drop C12E8 concentration. The diluted sample was loaded into a column packed with Nickel beads (Sigma) and the beads were washed with buffer A with 0.6 mM C12E8. In order to exchange detergent from 0.6 mM C<sub>12</sub>E<sub>8</sub> to 0.5 mM Brij-58 it was run a gradient in 5-10 column volume. The beads were washed with increasing amounts of imidazole and the protein was eluted with 90 mM and 300 mM concentrations. Elution was diluted up to 100 mM KCl maintaining the same concentration for all other components and the sample was loaded in a Q column. Beads were washed for 10 columns volume with 100 mM KCl, 30 mM HEPES pH 7.5, 2 mM BME and 0.5 mM Brij-58 (Buffer B). For eluting the protein a gradient was run from 100 mM to 600 mM KCl.

#### Purification of CD3 subunits

Bacteria cells expressing each of the CD3 subunits ( $\delta$  and  $\zeta$ ) were sonicated with a buffer containing: 20 mM KCl, 10 mM HEPES pH 7.5, 10 mM MgCl<sub>2</sub>, 0.1 mM PMSF and 2x protease inhibitors. After removing cell debris and unbroken cells with low speed centrifugation at 2800 RCF, the supernatant was then subjected to a high speed of centrifugation at 100000 RCF for 30 minutes. The resulting membranes were solubilized with 25 mM Sarcosyl and 2.5 mM Fos-Choline-14 in a buffer containing 100 mM NaCl, 25 mM Tris-HCl pH8.0 and incubated overnight on a rocking platform at 4°C. The supernatant from further high speed centrifugation was diluted (1:3) and loaded onto a column packed with Nickel beads (Sigma). The column was subsequently washed with a

buffer containing 100 mM NaCl, 25 mM Tris-HCl pH 8.0 and 0.5 mM C<sub>12</sub>E<sub>8</sub> supplemented with 20 mM imidazole. The protein was eluted with 90 mM imidazole in the same washing buffer.

### cTFIIH components

#### Purification of p44

Insect cells expressing p44-Protein A were collected 48 hours after infection. Cells were sonicated in a buffer containing: 200 mM NaCl, 50 mM HEPES pH 7.5, 2 mM BME, protease inhibitors and 2 mM Fos-Choline-14. The sample was centrifuged at 100000 RCF for 45 minutes. The supernatant was diluted to have the detergent in a concentration of 0.5 mM Fos-Choline-14. The diluted sample was incubated with IgG beads (GE) overnight. The next day the beads were transferred to a carcass and washed. TEV enzyme was added overnight to the beads in order to cut the Protein A tag. The next day p44 was eluted from the column. The elution was passed through a HiTrap Q HP column to further clean the p44 sample.

#### Purification of p44 and p34

Insect cells expressing His-XPB, His-p62, p44-ProteinA and His-p34 were collected 48 hours after infection. Cells were sonicated in a buffer containing: 250 mM NaCl, 50 mM HEPES pH 7.5, 2 mM BME and protease inhibitors. The sample was centrifuged at 100000 RCF for 45 minutes and loaded into a column packed with Nickel beads (Sigma). The beads were washed with increasing amounts of imidazole and the proteins were eluted with 80 mM and 300 mM concentrations. The elution was incubated overnight with IgG beads and the next day the beads were washed and TEV enzyme was added at room temperature for a few hours on a rotating platform to remove the Protein A tag on p44. The beads were separated from the supernatant containing the eluted p44 with p34.

#### Purification of p62, p44 and p34

Insect cells expressing His-p62, p44-ProteinA and His-p34 were collected 48 hours after infection. Cells were sonicated in a buffer containing: 400 mM C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>K pH 7.5, 50 mM HEPES pH 7.5, 2 mM BME and protease inhibitors. The sample was centrifuged at

100000 RCF for 45 minutes. The supernatant was incubated with pre-equilibrated Nickel beads (Sigma) overnight or for 6 hours. The beads were transferred to a carcass and washed with increasing amounts of imidazole and the proteins were eluted with 80 mM and 300 mM concentrations. The Protein A tag on p44 was cleaved with TEV enzyme overnight in a dialysis cassette.

#### Purification of XPB, p62, p44 and p34

Insect cells expressing His-XPB, His-p62, p44-ProteinA and His-p34 were collected 48 hours after infection. Cells were sonicated in a buffer containing: 300mM  $C_2H_3O_2K$  pH 7.5, 50 mM HEPES pH 7.5, 2 mM BME, protease inhibitors, 5 mM Brij58 and 1 mM  $C_{13}E_8$ . The sample was centrifuged at 100000 RCF for 45 minutes. The supernatant was diluted to have the detergents in a concentration of 1 mM Brij58 and 0.25 mM ANAPOE  $C_{13}E_8$ . The diluted sample was loaded into a column packed with Nickel beads (Sigma). The beads were washed with increasing amounts of imidazole and finally eluted at 80 mM and 300 mM concentrations. The Protein A tag on p44 was cleaved with TEV overnight in a dialysis cassette.

#### Purification of p52

Insect cells expressing p52-Protein A were collected 48 hours after infection. Cells were sonicated in a buffer containing: 300 mM  $C_2H_3O_2K$  pH 7.5, 50 mM HEPES pH 7.5, 2 mM BME, protease inhibitors and 5 mM Brij58. The sample was centrifuged at 100000 RCF for 45 minutes. The supernatant was diluted to have the detergent in a concentration of 1 mM Brij58. The diluted sample was incubated with IgG beads (GE) overnight. The next day the beads were transferred to a carcass and washed. TEV enzyme was added overnight to the beads in order to cut the Protein A tag. The next day p52 was eluted from the column.

#### Purification of XPB

Insect cells expressing His-XPB were collected 48 hours after infection. Cells were sonicated in a buffer containing: 200 mM KCl, 50 mM HEPES pH 7.5, 2 mM BME, protease inhibitors and 5 mM Brij58. The sample was centrifuged at 100000 RCF for 45 minutes. The supernatant was diluted to have Brij58 to a final concentration of 1 mM. The diluted sample was loaded into a column packed with Nickel beads (Sigma). The

beads were washed with increasing amounts of imidazole and the protein was eluted with 80 mM and 300 mM concentrations.

#### Purification of XPB and p52

Insect cells expressing His-XPB and p52-Protein A were collected after 48 hours of infection. Cells were sonicated in a buffer containing: 300 mM  $C_2H_3O_2K$ , 50 mM HEPES pH 7.5, 2 mM BME, protease inhibitors and 5 mM Brij58. The sample was centrifuged at 100000 RCF for 45 minutes. The supernatant was diluted to have Brij58 to a final concentration of 1mM. The diluted sample was loaded into a column packed with Nickel beads (Sigma). The beads were washed with increasing amounts of imidazole and the proteins were eluted with 80 mM and 300 mM concentrations. The elution fractions were concentrated and passed again through a packed His-Trap column (GE) in order to clean the sample. The His-Trap column was washed with increasing amounts of imidazole and the proteins were eluted with 80 mM and 300 mM.

#### Purification of XPD

Insect cells expressing His-XPD were collected 48 hours after infection. Cells were sonicated in a buffer containing: 300 mM  $C_2H_3O_2K$ , 50 mM HEPES pH 7.5, 2 mM BME, protease inhibitors and 5 mM ANAPOE  $C_{12}E_8$ . The sample was centrifuged at 100000 RCF for 45 minutes. The supernatant was diluted to have ANAPOE  $C_{12}E_8$  to a final concentration of 1 mM. The diluted sample was loaded into a column packed with Nickel beads (Sigma). The beads were washed with increasing amounts of imidazole and finally eluted at 80 mM and 300 mM concentrations. The elution fractions were concentrated and passed again through a packed His-Trap column (GE) in order to clean the sample. The His-Trap column was washed with increasing amounts of imidazole and the protein was eluted with 80 mM and 300 mM.

#### Purification of GST-p8

*E. coli* cells (Rosetta) were transformed with pGEX-4T-1 (GE) containing p8. The cells were grown and induced with IPTG for 2 hours. Cells were collected and sonicated with a buffer containing: 200 mM KCl, 50 mM HEPES pH 7.5, 2 mM BME and protease inhibitors. The sample was centrifuged at 100000 RCF for 45 minutes. The supernatant was loaded into a packed column of glutathione sepharose beads (GE). The column was washed and then eluted with buffer containing 10 mM reduced glutathione.

## Purification of XPG

Insect cells expressing a double tag XPG were collected 48 hours after infection. The N-terminus has a PreScission site after the His tag and the C-terminus has a TEV site before the Protein A tag. Cells were sonicated in a buffer containing: 25 mM Tris-HCl pH 7.5, 200 mM KCl, 2 mM BME, protease inhibitors, and 0.15 mM Anapoe-80. The sample was centrifuged at 100000 RCF for 45 minutes. The supernatant was diluted to have the concentration of Anapoe-80 to 0.05 mM. The diluted sample was loaded into a column packed with IgG conjugated agarose beads (GE) and loaded twice. The column was washed and the protein was eluted after an overnight incubation with TEV enzyme. The eluted protein was then loaded on a second column- HisTrap from GE. The column was washed with increasing amounts of imidazole and eluted with 350mM.

## In-vitro reconstitution of *s. cerevisiae* Pol II-TFIIF-TFIIB-TBP-DNA complex

Complex subunits were expressed in yeast (Pol II and TFIIF) and *E. coli* (TFIIB and TBP) systems. For yeast-expressed Pol II we designed forward and reverse primers to: 1) insert the protein-A region of pBS1479 at the C-terminal end of RPB1; 2) exclude the CBP-tag from the template plasmid pBS1479 [1]. The resulting PCR products were used to transform the yeast strain CB010 {*Mata, pep4::HIS3/prb1::LEU2, prc1::HISG, can1, Ade2, trp1, ura3, his3.leu2-3,112*} as previously described [2]. Transformants were screened by PCR and confirmed by immunoblotting as described in (ref avital jbc). PA-tagged Pol II was purified over IgG resin (GE Healthcare).

Detergent screen to identify extracting conditions.

(1) Purification of 3-subunit TFIIF (tap-tagged, protein-A tag plus calmodulin-binding-peptide (CBP) tag) had consistently shown low protein yields due to aggregation. We performed detergent screens in an attempt to extract TFIIF from the pellet and analyzed the results using immuno-dot blot (to detect PA tag on TFG2). Clear detergent candidates were identified and 2.5XCMC detergent concentration was added to cell lysates during purification. TFIIF was successfully extracted and purified with a yield of 5-10mgs/Kg of cells. DLS experiments revealed a particle of 6 nm, equivalent to an average radius of MW 250 kDa. Addition of detergent during purification allowed us to obtain for the first time milligram (mg) quantities of TFIIF. (2) Mixing Pol II and TFIIF

under detergent and high salt (400 mM ammonium sulfate), and dialyzing them into a buffer containing no detergent and low salt (120 mM KCl) allowed purification of a – transcriptionally active– Pol II-TFIIF complex (using the CBP tag on TFIIF) over calmodulin resin. (3) Addition of 2 x molar excess TFIIB-TBP-DNA complex (purified on Ni<sup>2+</sup> beads using a His-tag on TFIIB, ref) to Pol II-TFIIF complex, (4) allowed us to purify (over calmodulin beads, using the CBP-tag on TFIIF) a minimum PIC consisting of Pol II, TFIIF, TBP, TBP and a double stranded 51-mer DNA oligonucleotide.

## **Supplementary Protocols 2. Fluorescence Anisotropy (FA) Measurements**

The assays were performed using a Cary Eclipse fluorescence spectrophotometer equipped with automated polarizers by exciting 6-carboxyfluorescein (6-FAM) at 485 nm and monitoring its emission at 520 nm (Varian). The reactions were performed in triplicate at 37°C in a reaction buffer of 400 μL.

The FA measurements for human XPG [3] were carried out using a Y shaped substrate containing FAM positioned at the 5' end of the duplex region produced by annealing of the 5' labeled 6-FAM TOP 39mer and BOTTOM 39mer:

3'-TT TTT TTT TTT TTT TTT TTG GAA CTG TTC GAA CGC GTG A-FAM-5'

5'-TT TTT TTT TTT TTT TTT TTT CTT GAC AAG CTT GCG CAC T-3'

The binding buffer contained 20mM potassium acetate, 50mM HEPES pH7.5, 2m BME, 1mM EDTA, with / without 50 mM anapoe-80

The FA measurements for human XPD [4] were carried out using a single strand DNA containing 6-FAM positioned at its 5' end noted as TOP39mer:

3'-TT TTT TTT TTT TTT TTT TTG GAA CTG TTC GAA CGC GTG (A-FAM)-5'

The binding buffer contained 20mM Tris pH7.0, 5mM MgCl<sub>2</sub>, 1mM DTT and 100 mM potassium acetate

Each protein addition was allowed 5 minutes for equilibration prior to reading the anisotropy values on each of three separate cuvettes. These three anisotropy values were averaged for each protein addition. Each binding isotherm consisting of the mean of these three values. At the end of each titration, the binding isotherm was shown to be fully reversible by the addition of 1M final concentration of potassium acetate. XPD data

were fitted to the equation below using Kaleidagraph assuming the reaction is simple 1:1 interaction:

$$r = r_{\min} + \frac{(C_D + C_P + K_D) - \sqrt{(C_D + C_P + K_D)^2 - 4 C_D C_P}}{2 C_D} (r_{\max} - r_{\min})$$

where  $r$ , measured anisotropy;  $r_{\min}$ , anisotropy of free DNA;  $r_{\max}$ , anisotropy of DNA-protein complex;  $C_D$ , total DNA concentration; and  $C_P$ , total protein concentration; and  $K_D$ , dissociation constant.

## References

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