Supplementary Materials for

Phosphorylation of XPD drives its mitotic role independently of its DNA repair and transcription functions

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The PDF file includes:

Figs. S1 to S7
Key Resources Table
Legend of source data file

Other Supplementary Material for this manuscript includes the following:

Source data file
Figure S1

A

DAPI  XPD  p44  DAPI  XPD  p44

DAPI  XPD  CYCH  DAPI  XPD  CYCH

DAPI  XPD  MMS19  DAPI  XPD  MMS19

A.1  A.2  A.3  A.4  A.5  A.6  A.7  A.8  A.9  A.10  A.11  A.12

B

XPD/WT cells in metaphase

DAPI  XPD  MMS19  DAPI  XPD  MMS19

B.1  B.2  B.3  B.4

C

XPD  HD1  HD2

XPD  444-760

Eg5  +  -  +  -  +  -  -  +  -  -  +  -

XPD  1  2  3  4  5

D

input  IP Eg5

Eg5  +  +  +  +  +  +  +

XPD  +  +  +  +  +  +  +

ssDNA  -  -  -  -  -  -  -

Eg5  130 kDa

XPD  80 kDa

XPD  444-760

1  2  3  4  5

10 kDa

15 kDa
Fig. S1. Immunolocalization of XPD and partners in XPD/WT cells.

(A) Localization of XPD, p44, Cyclin H and MMS19 during cytokinesis. XPD/WT cells were synchronized by double thymidine block, released in mitosis and analyzed by confocal immunofluorescence microscopy. Cells in telophase were identified according to their DAPI staining. The arrows point to the localization of XPD at the midbody. Scale bar is 5µm.

(B) Localization of XPD and MMS19 during metaphase. XPD/WT cells were synchronized by double thymidine block, released in mitosis and analyzed by confocal immunofluorescence microscopy. Cells in metaphase were identified according to their DAPI staining. Scale bar is 5µm.

(C) Schematic representation of entire 760-aa XPD protein and the truncated form corresponding to the C-terminal part of XPD (444-760). Immunoprecipitated Eg5 (IP Eg5) was incubated with either entire Flag-XPD or Flag-XPD (444-760) fragment (as indicated, +). After washes, the coimmunoprecipitated proteins were resolved by SDS-PAGE and blotted with anti-Flag and Eg5. The results are representative of two independent experiments.

(D) Recombinant Eg5 and XPD were incubated in the presence of single-strand DNA (ssDNA, 7.5nM) and immunoprecipitated with anti-Eg5 (IP Eg5). After washes, coimmunoprecipitated proteins were resolved by SDS-PAGE and blotted with anti-Eg5 and -XPD. The results are representative of two independent experiments.
Figure S2

A. Images show DAPI, XPD, and NEK6 staining in various cell cycle phases. Cells with XPD/WT, XPD/T425A, and XPD/T425D are compared.

B. Western blot analysis of MMS19 and XPD in different conditions. MMS19 expression is detected at 110 kDa and XPD at 80 kDa.

C. Similar analysis for XPG showing bands at 200 kDa.

D. Western blot for core-IIH, Flag-XPD WT, Flag-XPD/T425A, and Flag-XPD/T425D. XPD is detected at 89 kDa and 81 kDa.
Fig. S2. Interaction of MMS19, XPG and core-TFIIH with XPD, depending on the XPD/T425 phosphorylation state.

(A) Immunolocalization of XPD and NEK6 during different mitotic phases. Human XPD/WT cells were synchronized by double thymidine block, released in mitosis and analyzed by confocal microscopy at prometaphase, metaphase, anaphase and telophase. The arrows point to the co-localization of XPD and NERK6 at the mitotic spindle and spindle poles in prometaphase and metaphase. Scale bar is 5µm.

(B-C) Immunoprecipitated MMS19 (IP MMS19, panel B) or XPG (IP XPG, panel C) was incubated with either XPD/WT, /T425A or /T425D. After washes, the co-immunoprecipitated proteins were resolved by SDS-PAGE and blotted with anti-XPD and either anti-MMS19 or XPG. Graph shows the ratio XPD/MMS19 (B) or XPD/XPG (C) (n=3, means±s.d.) in arbitrary units (au).

(D) Recombinant core-TFIIH (containing the p8, p34, p44, p52, p62, XPB) was incubated with either immunoprecipitated Flag-XPD/WT, /T425A or /T425D. After washes, the co-immunoprecipitated proteins were resolved by SDS-PAGE and blotted with anti-XPB and XPD. Graph shows the ratio XPD/XPB (n=3, means±s.d.) in arbitrary units (au).
Figure S3

A

interphase

prophase

prometaphase

metaphase

anaphase

telophase/cytokinesis

B

input XPD-GFP IP XPD-GFP IP XPD-GFP

Eg5 XPD-GFP

123456

130 kDa

110 kDa
Fig. S3. Immunolocalization of XPD and Eg5 in XPD/R683W cells.

(A) Immunofluorescence of XPD/R683W cells synchronized with double thymidine block and collected 9h after release. XPD and Eg5 localization was monitored through all the cell cycle stages (cells from each mitotic phase were identified according to their DAPI staining). The arrows point to irregular nuclear shapes and chromosome segregation errors. Scale bar is 5µm.

(B) Immunoprecipitation of either XPD (IP XPD-GFP) or Eg5 (IP Eg5) from cells overexpressing (when indicated, +) C-terminally GFP-tagged-XPD. After washes, the co-immunoprecipitated proteins were resolved by SDS-PAGE and blotted with antibodies targeting XPD and Eg5.
Figure S4

A

| XPD/WT cells | XPD/R683W cells |
|---------------|-----------------|
| + Flag-XPD/WT | + Flag-XPD/T425A | + Flag-XPD/T425D |

A.1 | A.6 | A.11 | A.16 | A.21 |
A.2 | A.7 | A.12 | A.17 | A.22 |
A.3 | A.8 | A.13 | A.18 | A.23 |
A.4 | A.9 | A.14 | A.19 | A.24 |
A.5 | A.10 | A.15 | A.20 | A.25 |
Fig. S4. Localization of XPD, Eg5 and α-Tubulin in anaphase

(A) Unmerged images obtained with antibodies targeting the Flag-Tag, Eg5 and α-Tubulin in XPD/WT and XPD/R683W cells overexpressing either Flag-XPD/WT, /T425A or /T425D in anaphase. Chromosomes were stained with DAPI. The arrows point to DNA bridges. Scale bar is 5µm.
Fig. S5. CREST and BubR1 in XPD/WT and XPD/R683W cells in prometaphase.
(A) Wild-type and XPD mutated cells were synchronized in prometaphase with Taxol (16h, 1µM). Chromosomes were stained with DAPI. The arrows point to XPD/R683W anaphase-like cells that escaped prometaphase arrest in the presence of chromosome segregation errors. Scale bar is 5µm.
(B) Unmerged images for Flag-Tag, CREST and BubR1, from which merges presented Fig. 6E were made.
Figure S6

A.1 A.2 A.3 A.4 A.5 A.6 A.7 A.8 A.9 A.10 A.11 A.12 A.13 A.14 A.15 A.16 A.17 A.18 A.19 A.20

B.1 B.2 B.3 B.4 B.5 B.6 B.7 B.8 B.9 B.10 B.11 B.12 B.13 B.14 B.15 B.16 B.17 B.18 B.19 B.20

C.1 C.2 C.3 C.4 C.5 C.6 C.7 C.8 C.9 C.10 C.11 C.12 C.13 C.14 C.15 C.16 C.17 C.18 C.19 C.20

Figure S6

A.1 A.2 A.3 A.4 A.5 A.6 A.7 A.8 A.9 A.10 A.11 A.12 A.13 A.14 A.15 A.16 A.17 A.18 A.19 A.20

B.1 B.2 B.3 B.4 B.5 B.6 B.7 B.8 B.9 B.10 B.11 B.12 B.13 B.14 B.15 B.16 B.17 B.18 B.19 B.20

C.1 C.2 C.3 C.4 C.5 C.6 C.7 C.8 C.9 C.10 C.11 C.12 C.13 C.14 C.15 C.16 C.17 C.18 C.19 C.20

Figure S6

A.1 A.2 A.3 A.4 A.5 A.6 A.7 A.8 A.9 A.10 A.11 A.12 A.13 A.14 A.15 A.16 A.17 A.18 A.19 A.20

B.1 B.2 B.3 B.4 B.5 B.6 B.7 B.8 B.9 B.10 B.11 B.12 B.13 B.14 B.15 B.16 B.17 B.18 B.19 B.20

C.1 C.2 C.3 C.4 C.5 C.6 C.7 C.8 C.9 C.10 C.11 C.12 C.13 C.14 C.15 C.16 C.17 C.18 C.19 C.20

Figure S6

A.1 A.2 A.3 A.4 A.5 A.6 A.7 A.8 A.9 A.10 A.11 A.12 A.13 A.14 A.15 A.16 A.17 A.18 A.19 A.20

B.1 B.2 B.3 B.4 B.5 B.6 B.7 B.8 B.9 B.10 B.11 B.12 B.13 B.14 B.15 B.16 B.17 B.18 B.19 B.20

C.1 C.2 C.3 C.4 C.5 C.6 C.7 C.8 C.9 C.10 C.11 C.12 C.13 C.14 C.15 C.16 C.17 C.18 C.19 C.20

Figure S6

A.1 A.2 A.3 A.4 A.5 A.6 A.7 A.8 A.9 A.10 A.11 A.12 A.13 A.14 A.15 A.16 A.17 A.18 A.19 A.20

B.1 B.2 B.3 B.4 B.5 B.6 B.7 B.8 B.9 B.10 B.11 B.12 B.13 B.14 B.15 B.16 B.17 B.18 B.19 B.20

C.1 C.2 C.3 C.4 C.5 C.6 C.7 C.8 C.9 C.10 C.11 C.12 C.13 C.14 C.15 C.16 C.17 C.18 C.19 C.20

Figure S6

A.1 A.2 A.3 A.4 A.5 A.6 A.7 A.8 A.9 A.10 A.11 A.12 A.13 A.14 A.15 A.16 A.17 A.18 A.19 A.20

B.1 B.2 B.3 B.4 B.5 B.6 B.7 B.8 B.9 B.10 B.11 B.12 B.13 B.14 B.15 B.16 B.17 B.18 B.19 B.20

C.1 C.2 C.3 C.4 C.5 C.6 C.7 C.8 C.9 C.10 C.11 C.12 C.13 C.14 C.15 C.16 C.17 C.18 C.19 C.20

Figure S6
Fig. S6. Localization in XPD/R683W cells of Eg5/WT, /S1033A and /S1033E in telophase

(A) Images obtained from XPD/WT and XPD/R683W cells overexpressing either the mCherry-Eg5/WT, /S1033A or /S1033E in telophase. Immunofluorescence analyses were performed with antibodies targeting the mCherry Tag and the mitotic spindle marker α-Tubulin. Chromosomes were stained with DAPI. Arrows point to DNA bridges. Scale bar is 5µm.

(B) Whole cell extracts were isolated from XPD/R683W cells overexpressing (when indicated, +) either mCherry-Eg5/WT, /S1033A or /S1033D. After immunoprecipitation with anti-mCherry antibody, the co-immunoprecipitated proteins were resolved by SDS-PAGE and blotted with anti-Eg5.

(C) When indicated (+) immunoprecipitated Eg5/WT, Eg5/S1033A or Eg5/S1033E was incubated with purified either XPD/WT or XPD/R683W. After washes, the co-immunoprecipitated proteins were resolved by SDS-PAGE and blotted with anti-Eg5 and XPD. Graph shows the ratio XPD/Eg5 (n=3, means ± s.d.) in arbitrary units (au).
Figure S7

A

NER factors
core IIH+CAK
XPD/WT
Eg5
34nt
26nt

B

viability (%)

UV-C (J/cm²)

C

fold change in RARB2 gene expression

D

RNAPII+GTF
core-IIH
XPD
CAK
Eg5
load

RPB2

ILP5

XPB

XPD

CDK7

Eg5

140 kDa

34 kDa

89 kDa

80 kDa

40 kDa

130 kDa
Fig. S7. Incidence of Eg5 and XPD/R683W in DNA repair and transcription.

(A) Increasing amounts of Eg5 were added to an incision/excision assay using purified recombinant NER factors (XPC, XPA, RPA, XPF/ERCC1 and XPG), the core-IIH, the CAK and XPD/WT. The reaction was analyzed by electrophoresis followed by autoradiography.

(B) Wild-type (XPD/WT) and XPD/R683W cells overexpressing either XPD/WT, XPD/R683W, XPD/R683W-T425A or XPD/R683W-T425D were treated with increasing UV-C doses and cells survival was determined 48h later. Data were normalized to the unexposed cells (as value of 100%). The results are the mean of 2 independent experiments performed in triplicates ± s.d. Significant statistical difference between XPD/R683W + XPD/WT cells and XPD/R683W, XPD/R683W + XPD/R683W, XPD/R683W + XPD/R683W-T425A or XPD/R683W + XPD/R683W-T425D at 10, 20 and 30J/cm² (p<0.0001, Student’s t test).

(C) XPD/WT and XPD/R683W cells overexpressing either XPD/WT, XPD/R683W, XPD/R683W-T425A or XPD/R683W-T425D were treated 8h with t-RA (5µM) and relative RARβ2 gene expression has been measured by RT-PCR. The mRNA levels were normalized to the GAPDH RNA amount. The RARβ2 mRNA expression is presented as n-fold induction relative to non-treated cells. The results represent the mean of two independent experiments performed in triplicates. Bars 1-3 correspond to the values presented Fig. 8G.

(D) Biotinylated AdMLP bound to streptavidin magnetic beads was incubated with RNAPII, TFIIA,-B,-D (TBP),-E, and -F, in the presence of core-IIH, CAK, XPD and Eg5 as indicated at the top of the panel. After washes, the sequential binding of different factors (RPB2, TFIIΕβ, XPB, XPD, CDK7, Eg5) in the PIC formation, was evaluated by immunoblotting.
## Key Resources Table

| REAGENTS and RESOURCES | SOURCE | IDENTIFIER |
|------------------------|--------|------------|
| **Antibodies** | | |
| mouse monoclonal anti-rabbit light chain-HRP | Jackson Immunoresearch | 211-032-171 |
| goat anti-mouse kappa-HRP | Southern Biotech | 1050-05 |
| rabbit polyclonal anti-Aurora B (immunoblotting) | Abcam | ab2254; AB_302923 |
| rabbit polyclonal anti BubR1 (immunoblotting) | Invitrogen | 720297; AB_2610165 |
| mouse monoclonal anti CDK7 (immunoblotting) | IGBMC Antibody Facility | clone 2F8 |
| human polyclonal anti CREST (immunostaining) | Antibodies Inc. | 15-234; AB_2687472 |
| rabbit polyclonal anti CCNB1 (immunoblotting) | GeneTex | GTX100911; AB_1949886 |
| mouse monoclonal Cyclin H (immunoblotting) | IGBMC Antibody Facility | clone 2D4 |
| rabbit polyclonal anti-Eg5 (immunoblotting) | Abcam | ab72413; AB_1268734 |
| rabbit polyclonal anti-Eg5 (immunoblotting) | Abcam | ab61199; AB_941397 |
| mouse monoclonal anti-Eg5 (immunoprecipitation) | Abcam | ab51976; AB_941398 |
| mouse monoclonal anti-Eg5 (immunostaining) | Abcam | ab51976; AB_941398 |
| rabbit polyclonal anti-Eg5 PhosphoT926 | Abcam | ab61104; AB_942236 |
| mouse monoclonal anti-Flag tag | Sigma-Aldrich | F1804; AB_262044 |
| rabbit polyclonal anti-Flag tag | Sigma-Aldrich | F7425; AB_439687 |
| rabbit polyclonal anti-GAPDH (immunoblotting) | Sigma-Aldrich | G9545; AB_796208 |
| mouse monoclonal anti-GFP (immunoblotting) | Abcam | ab3277; AB_308705 |
| rabbit polyclonal anti H3-pS10 (immunoblotting) | Cell Signaling | 9701; AB_331535 |
| normal mouse IgG (immunoprecipitation) | Santa Cruz | Sc-205; AB_737182 |
| normal rabbit IgG (immunoprecipitation) | Cell Signaling | 2729S; AB_1031062 |
| rat monoclonal anti mCherry (immunoprecipitation) | Fisher Scientific SAS | M11217; AB_2536611 |
| rabbit monoclonal MMS19 (immunoblotting) | Cell Signaling | #90637; DS1B8 |
| mouse monoclonal MMS19 (immunostaining) | IGBMC Antibody Facility | 3MM3H10 |
| mouse monoclonal anti NEK6 (immunostaining) | GeneTex | GTX84058; AB_10727078 |
| rabbit monoclonal anti NEK6 (immunoblotting) | Abcam | ab109177; AB_10863726 |
| rabbit monoclonal anti-phosphothreonine | Sigma-Aldrich | MABS499; clone RM102 |
| rabbit monoclonal anti-PLK1 (immunoblotting) | Cell Signaling | 4513S; AB_2167409 |
| chicken polyclonal anti-α Tubulin (immunostaining) | Abcam | ab89984; AB_10672056 |
| mouse monoclonal anti-α Tubulin (immunostaining) | Sigma-Aldrich | T9026; AB_477593 |
| mouse monoclonal anti XBP (immunoblotting) | IGBMC Antibody Facility | clone 1B3 |
| mouse monoclonal anti-XPD (immunoprecipitation) | Abcam | ab54676; AB_946174 |
| rabbit monoclonal anti-XPD (D3Z6I) (immunoblotting) | Cell Signaling | 11963; AB_2797781 |
| rabbit polyclonal anti-XPD (immunoblotting) | Abcam | ab111596; AB_10863985 |
| rabbit polyclonal anti-XPD [N2C2] (immunostaining) | GeneTex | GTX105357; AB_10616680 |
| mouse monoclonal anti-XPG (immunoprecipitation) | IGBMC Antibody Facility | clone 1XPG1B5 |
| rabbit polyclonal anti-XPG (immunostaining) | IGBMC Antibody Facility | 3328 |
| **Chemicals and Commercial Assays** | | |
| Anti-Flag M2 Affinity Gel | Sigma-Aldrich | Cat#A2220 |
| ATP | GE Healthcare Europe | 27-2056-61 |
| CDK1/CCNB1 (recombinant) | Abcam | ab104618 |
| Chymotrypsin | Promega | V1061 |
| CTP | Life Technologies SAS | R0451 |
| DAPI (4',6-Diamidino-2-phenylindole dihydrochloride) | Sigma-Aldrich | D8417 |
| Dynabeads M-280 Streptavidin | Invitrogen | Cat#11206D |
| Item                                      | Supplier                        | Cat. No. |
|-------------------------------------------|---------------------------------|----------|
| Dynabeads Protein G                       | Invitrogen                      | Cat#10004D |
| Monastrol                                 | Sigma-Aldrich                   | M8515    |
| Mowiol                                    | Calbiochem                      | 475904   |
| NEK6 (recombinant)                        | Sigma-Aldrich                   | N4662    |
| Nocodazole                                | Sigma-Aldrich                   | M1404    |
| Paraformaldehyde 16%                     | Thermo Fisher                   | Cat#50-980-487 |
| Prolong gold antifade mountant with DAPI  | Fisher Scientific               | P36935   |
| TALON metal affinity resin                | Clonetech                       | Cat#635501 |
| Taxol (Paclitaxel)                        | Sigma-Aldrich                   | T7191    |
| Thymidine                                 | Sigma-Aldrich                   | T1895    |
| Trypsin                                   | Promega                         | V5111    |
| X-treme GENE9                             | Roche Diagnostics               | 6365809001 |

**Cell Lines**

- **HeLa (control cell line for HD2)**: IGBMC Cell Culture Facility
- **HD2 (bearing XPD/R683W)**: IGBMC Cell Culture Facility (29)

**Recombinant DNA**

- **AdMLP DNA**: (55)
- **mCherry-Kinesin11-N-18**: Michael Davidson's lab, Addgene plasmid # 55067
- **pAK309**: (55)
- **p-Flag Eg5/WT, /T926A, /T926D, /S1033A, /S1033E, /T926A-S1033A, /T926D-S1033E**: this paper, N/A
- **p-mCherry Eg5/S1033E**: this paper, N/A
- **pE-GFP Eg5 and pE-GFP XPD**: this paper, N/A
- **pSK278-Flag MMS19/WT**: this paper, N/A
- **pSK278-Flag XPD/WT, /R112H, R683W, /R722W, /T425A, and /T425D**: this paper, N/A

**Primers**

- **RARβ2**: CCAGCAAGCCTCACATGTTT CCA, TACACGCTCTGCACCTTTAG CACT
- **GAPDH**: ACAACTTTGCTATCGTGGA AGG, GCCATCAGCCACAGTTC

**Software**

- **ImageJ 2.1.0**: NIH, https://imagej.nih.gov/ij/
- **Prism 9.1.0**: Graphpad
- **Proteome Discoverer 2.4 software**: Thermo Fisher Scientific

**Other**

- **Amersham Imager 600**: GE Healthcare LifeSciences, 29-0834-61
- **Cytospin 4 centrifuge**: Thermoscientific, TH-CYTO4
- **Rotary Mixer**: LABINCO, LD76
- **Thermomixer C**: Eppendorf, 035963
Caption for the Source Data File
This supplementary data file in Microsoft Excel format contains all the raw data and uncropped versions of any gels and blots presented in the figures.