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SI Guide

Lesion recognition by XPC, TFIIH and XPA in DNA Excision Repair

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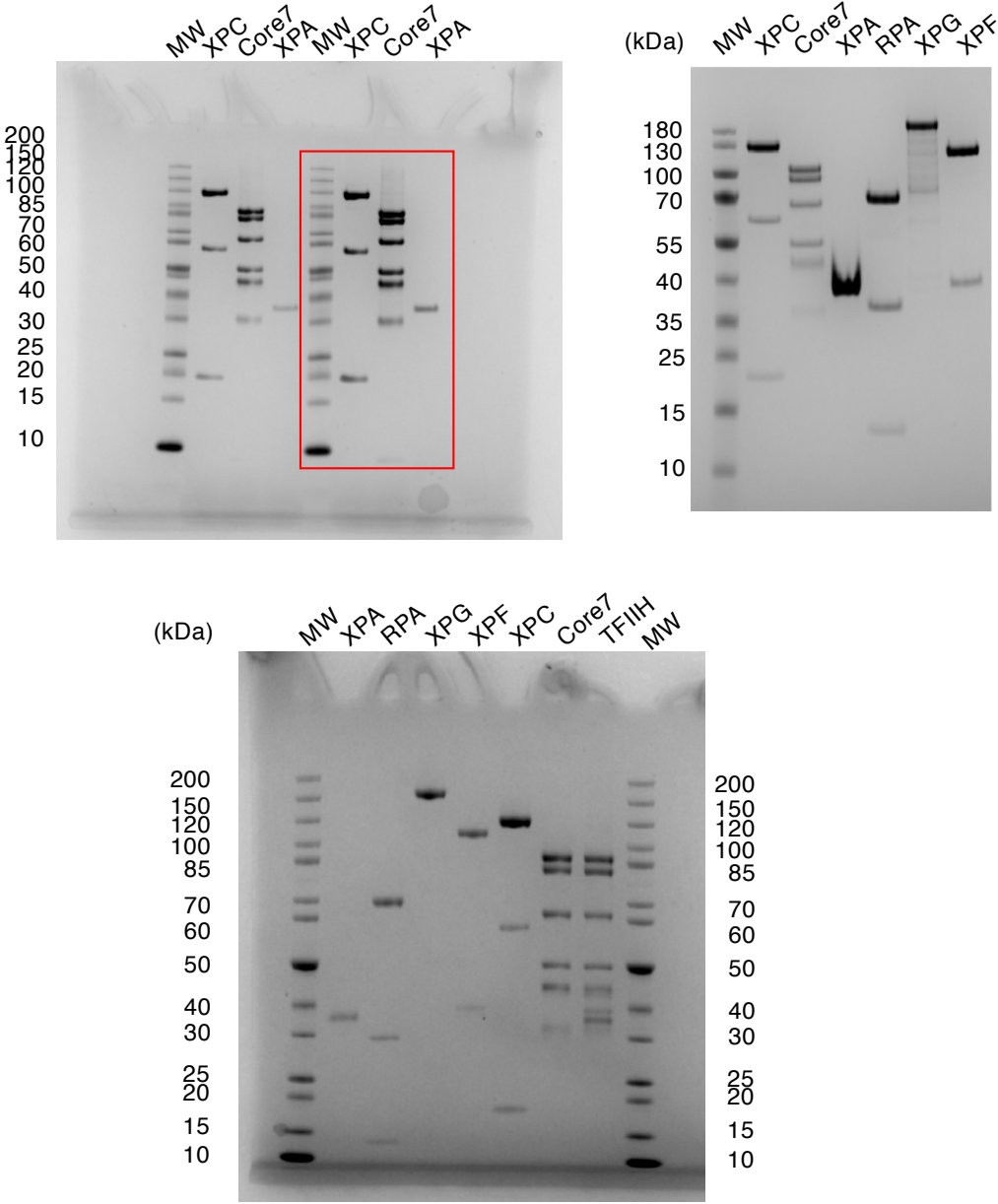
1. **Supplementary Table 1** DNA oligos used in this study
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7. **Video 1** Movement of XPC between flipping out 3 and 2 nt. The movie was generated based on Cy5_C7CD and AP_C7CD (conf2). Protein subunits are color-coded according to Fig. 1a. The trimeric XPC translocates downstream of the lesion by 1 bp, which involves ~36° rotation and ~3.4 Å translation.
8. **Video 2** Movement of Core7 relative to XPC-DNA upon XPA binding. The movie was generated based on Cy5_C7CD (C7CD) and Cy5_C7CAD. The attachment of the C-terminus of XPC to Core7 (XPB-p52-p8) prevents Core7 and XPC from dissociation. XPA is included in the last frame.

- 40 9. **Video 3** Structural changes between Cy5_C7CAD and AP_C7CAD. AP-DNA is more bent
41 at the lesion site than Cy5, but it is more relaxed (straight) upstream of the lesion, where
42 XPB and XPA bind.
- 43 10. **Video 4** Conformational changes from the U-shaped Core7 in C7CD to the σ shape in
44 C7CAD. The XPB and XPD arms move relatively to the unchanged p52-p34-p44 interface
45 at the U-turn.
- 46 11. **Video 5** An orthogonal view of the XPB and XPD movement between C7CD and C7CAD.
47 This viewpoint shows the crossing of XPB and XPD subunits when forming the closed σ
48 shape in C7CAD.
- 49 12. **Video 6** Movement of the XPD arm from C7CD to C7CAD. The flexible joint formed by
50 p34-p44 in the XPD arm is stabilized by the p62 helical bundle.
- 51 13. **Video 7** Movement of the XPB arm from C7CD to C7CAD. The extensive domain
52 interface between p52 and XPB allows XPB to undergo precession-like movement.
- 53

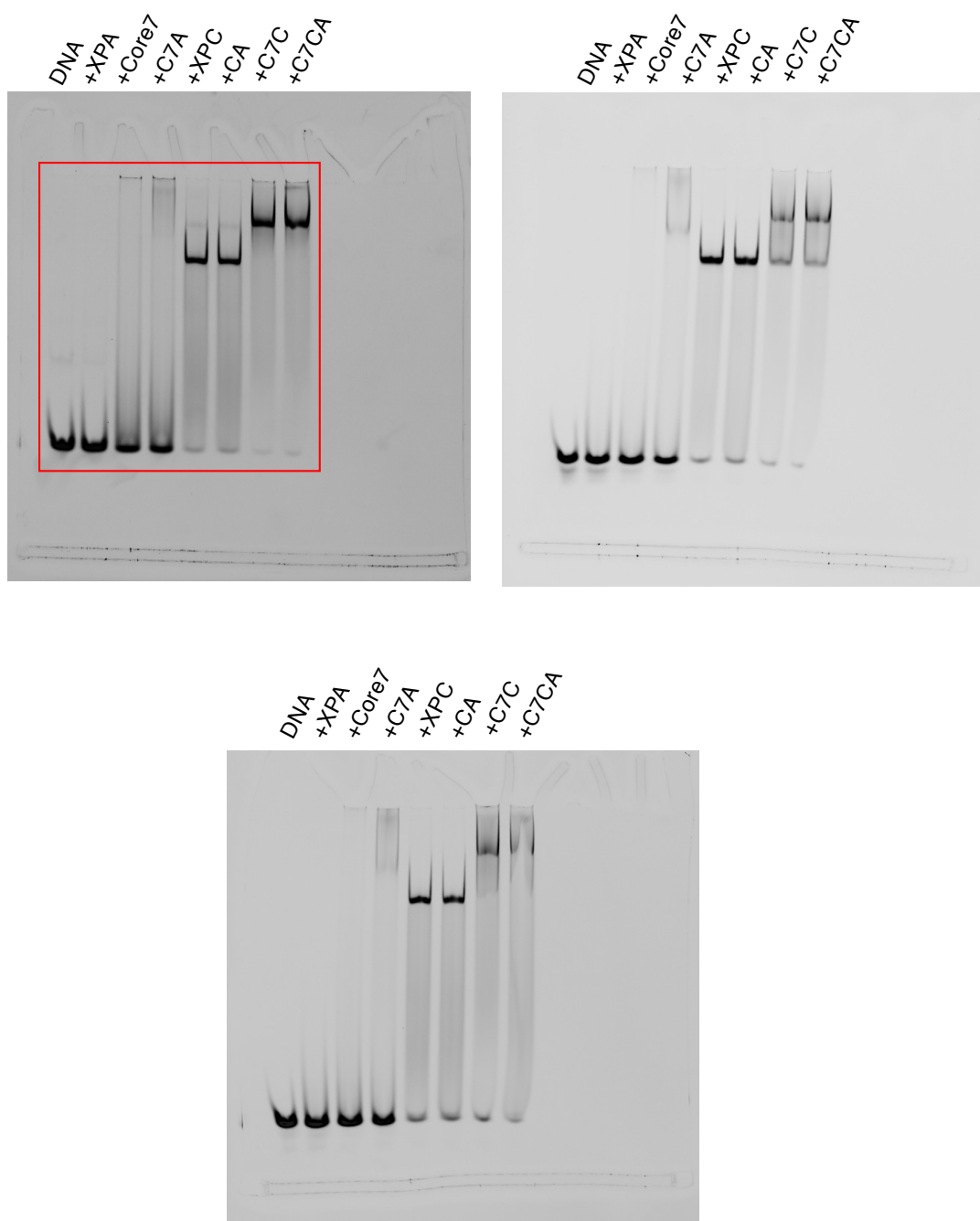
Supplementary Table 1. Oligonucleotide sequences used in EMSA, cryoEM and dual incision assay

Oligo Name	Sequence (5'→3')
Top56_iCy5	5'-AGGTAGTCACAGCTGATTGCGCTGAGGAT- iCy5 -TAGACGTGCGATATCTATGCCACTT
Top56_2AP	5'-AGGTAGTCACAGCTGATTGCGCTGAGGAT- ApAp -TAGACGTGCGATATCTATGCCACTT
Bot56	5'-AAGTGGCATAGATATCGCACGTCTA TT TATCCTCAGCGCAATCAGCTGTGACTACCT
Top_F1_57	5'-ACTGGGAACAGCTGCGGTCCGTTGACGACATACAGCTCAACGATGCCACTTCAGCAT
Top_F1_47	5'-GCTGCGGTCCGTTGACGACATACAGCTCAACGATGCCACTTCAGCAT
Top_F1_37	5'-GTTGACGACATACAGCTCAACGATGCCACTTCAGCAT
Top_F2_iCy5	/5Phos/TGCACTCATGTTCTG- iCy5 -CACTAGTACTACGACTACGC
Top_F3_10_5P	/5Phos/CTCGGCTCGA
Top_F3_20_5P	/5Phos/CTCGGCTCGAGTGTTCGATC
Top_F1_53	5'-GCTGCGGTCCGTTGACGACATACAGCTCAACGATGCCACTTCAGCATTGCACT
Top_F2_AP	³² p-5'-CATGTTCTG- ApAp -CACTAGTAC
Top_F3_21_5P	/5Phos/TACGACTACGCCTCGGCTCGA
Cy5_72/20_top	Top_F1_57+Top_F2_iCy5
Cy5_62/30_top	Top_F1_47+Top_F2_iCy5+Top_F3_10_5P
Cy5_52/40_top	Top_F1_37+Top_F2_iCy5+Top_F3_20_5P
AP_62/30_top	Top_F1_53+Top_F2_AP+Top_F3_21_5P
Bot_F1_74	5'-CGAACACTCGAGCCGAGGCGTAGTCGTAGTACTAGTGTTTCAGAACATGAGTGCAATGCTGA AGTGGCATCGTTG (a template to ligate top_F1, F2 and F3)
mm94_Bot_72/20	5'-GCGTAGTCGTAGTACTAGTG TT TCAGAAATATGAGTACAATACTGAAGTGGCATCGTTGAGCTG TATGTCGTCAACGGACCGCAGCTGTTCCCACT
mm94_Bot_62/30	5'-TCGAGCCGAGGCGTAGTCGTAGTACTAGTG TT TCAGAAATATGAGTACAATACTGAAGTGGCAT CGTTGAGCTGTATGTCGTCAACGGACCGCAGC
mm94_Bot_52/40	5'-GATCGAACACTCGAGCCGAGGCGTAGTCGTAGTACTAGTG TT TCAGAAATATGAGTACAATACT GAAGTGGCATCGTTGAGCTGTATGTCGTCAAC
Annealed Substrate (Diagram)	
Cy5-DNA	Top56_iCy5 5' _____ Cy5 _____
	Bot56 3' _____ 29 _____ TT _____ 25 _____
AP-DNA	Top56_2AP 5' _____ AP _____
	Bot56 3' _____ 29 _____ TT _____ 25 _____
Cy5- or AP-DNA in dual incision	Top94 (F1+F2 (+F3)) 5' _____ XX _____
	mm94_Bot (3 variation) 3' _____ 52 / 62 / 72 _____ TT _____ 40 / 30 / 20 _____

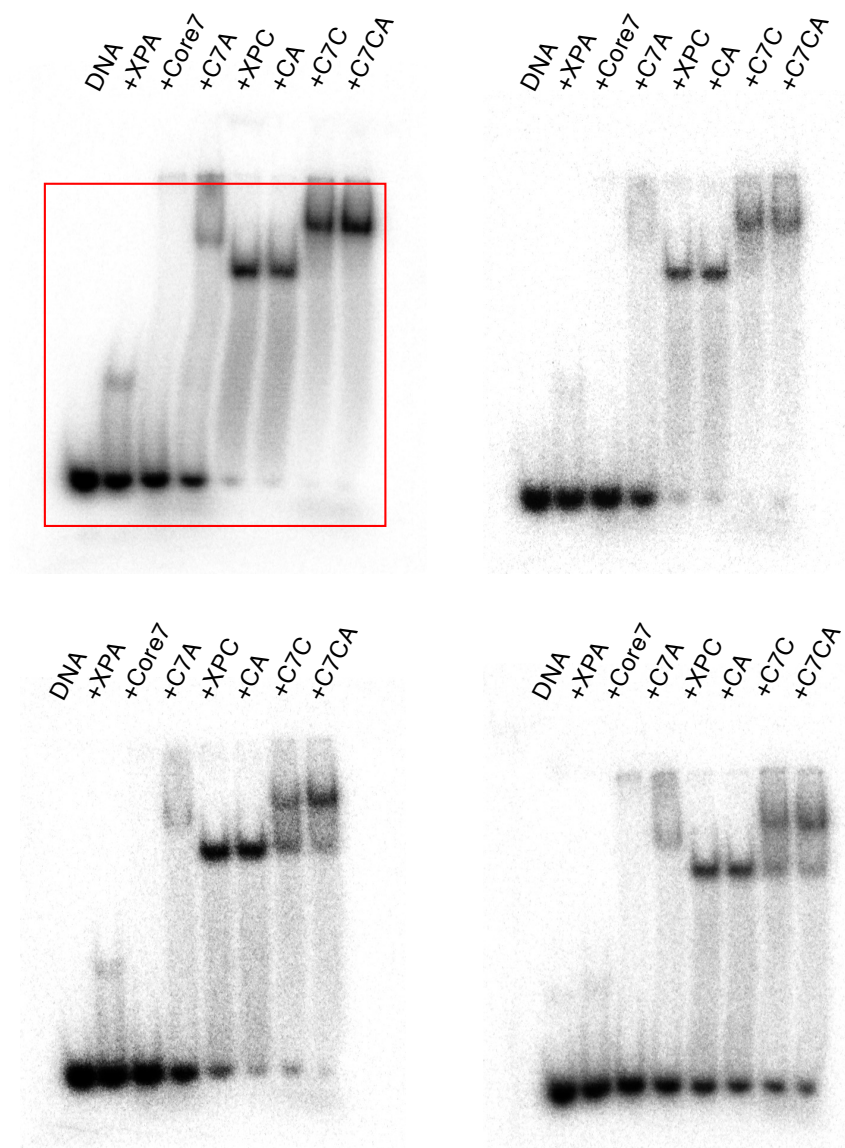
Supplementary Figure 1 Uncropped SDS gels related to Fig. 1b



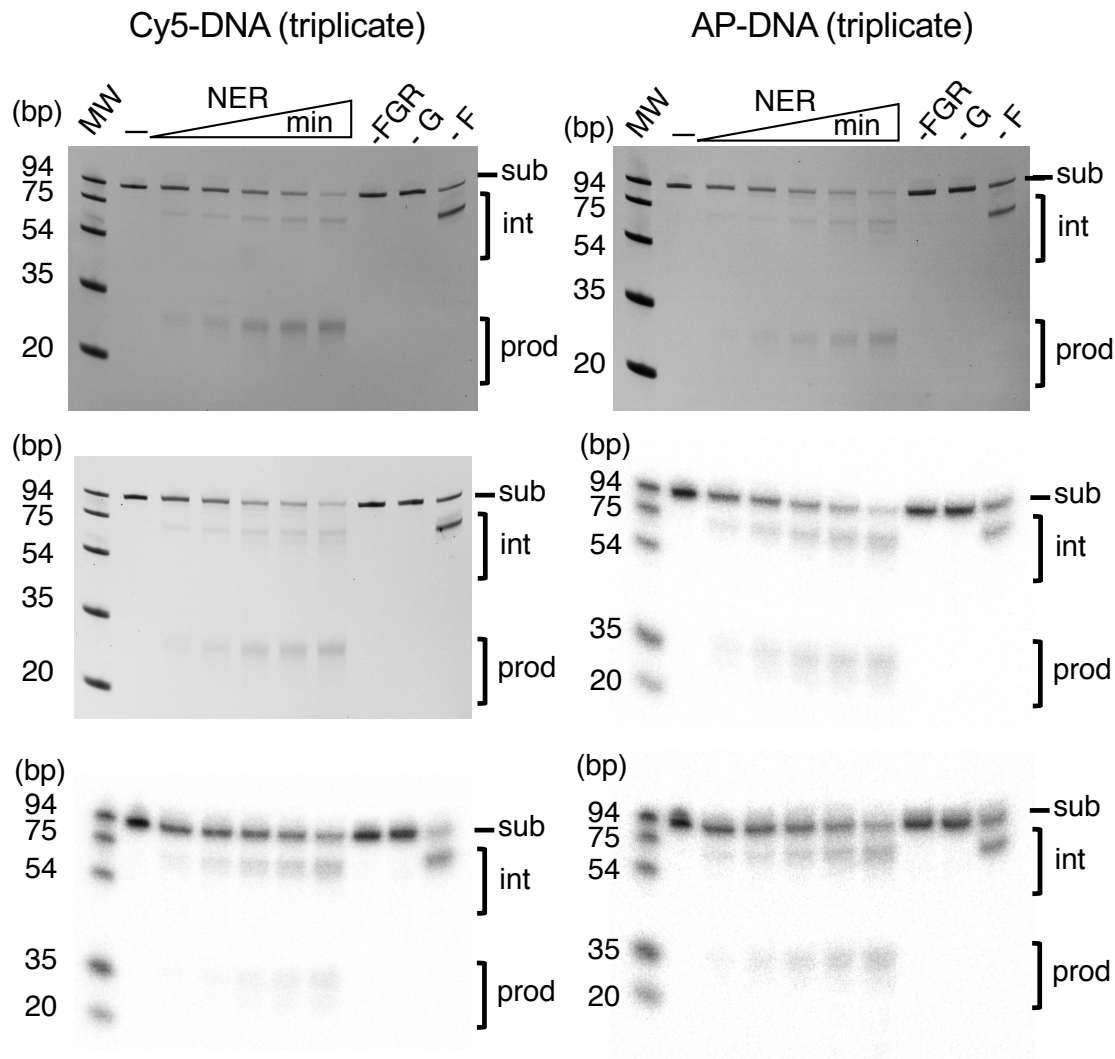
Supplementary Figure 2 Uncropped EMSA gels related to Fig. 1c



Supplementary Figure 3 Uncropped EMSA gels related to ED Fig. 3a



Supplementary Figure 4 Gels of DNA dual incision assay used to generate Fig. 4c



NER prod (%)							
	Cy5-DNA			AP-DNA			
min	run 1	run 2	run 3	run 1	run 2	run 3	
0	0	0	0	0	0	0	
5	14.48	14.44	12.21	1.79	3.63	1.18	
10	27.41	24.72	27.72	4.42	7.94	2.46	
20	41	45.15	47.08	9.27	14.92	7	
30	50.77	60.46	51.03	14.8	20.28	9.38	
60	63.16	75.52	62.46	24.47	28.53	13.6	

Supplementary Figure 5 Gels of DNA dual incision assay used to generate ED Fig. 10d

