Supplemental Information to

Conservation and divergence in nucleotide excision repair lesion recognition

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Supplemental Figures

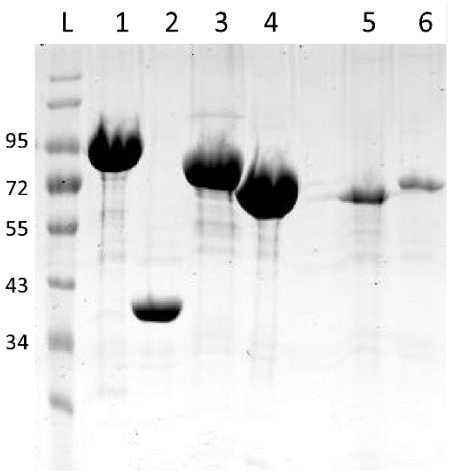

Figure S1: SDS gel of all proteins used in these studies. (1) *ct*XPD, (2) ctp44(1-285), (3) *Bca*UvrB wild type, (4) *Bca*UvrBΔ4, (5) *Bca*UvrC, (6) *Tma*UvrC.

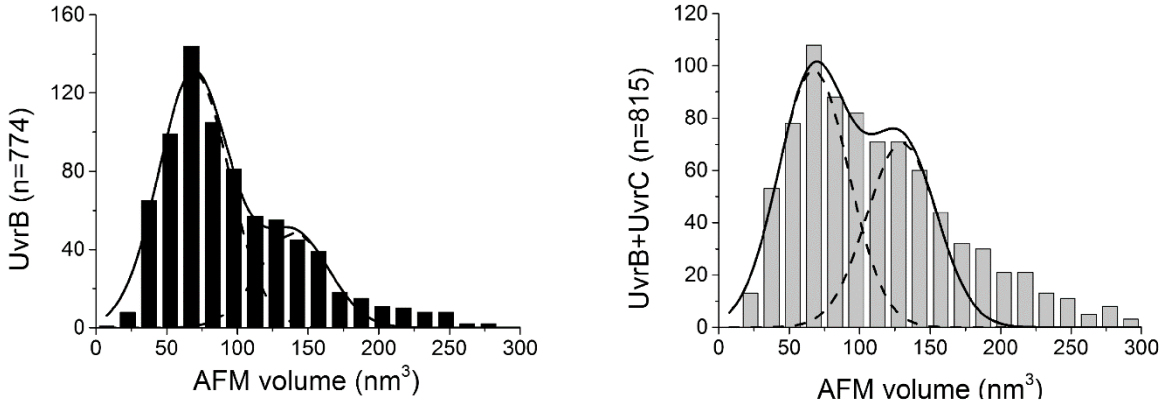

Figure S2: AFM volume distributions of DNA bound complexes. (Left) UvrB and (right) UvrB+UvrC. Gaussian fits to the data show maxima at ~70 nm^3 and ~140 nm^3 for samples of UvrB alone as well as UvrB+UvrC. Volumes of 50-110 nm^3 were classed as monomeric UvrB complexes and volumes of 110-250 nm^3 as potential UvrBC (or dimeric UvrB) complexes. DNA bound peaks in samples containing both UvrB and UvrC show ~45% of volumes in the size range of UvrBC complexes versus ‹30% (representing UvrB dimers) in the absence of UvrC.

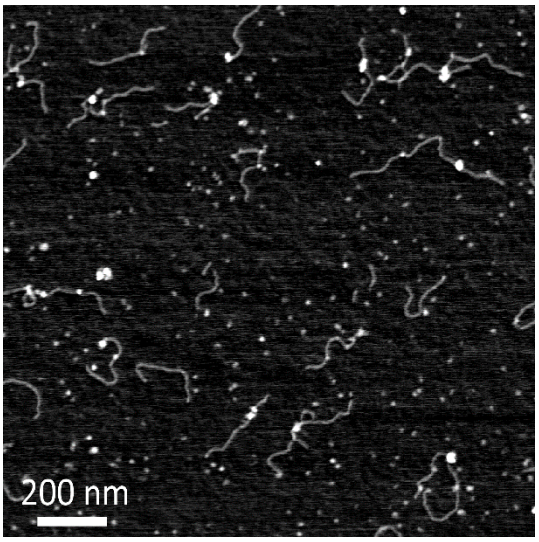

Figure S3: AFM of UvrC aggregates. At high concentrations, UvrC forms aggregates with DNA (large white peaks on the DNA substrates).

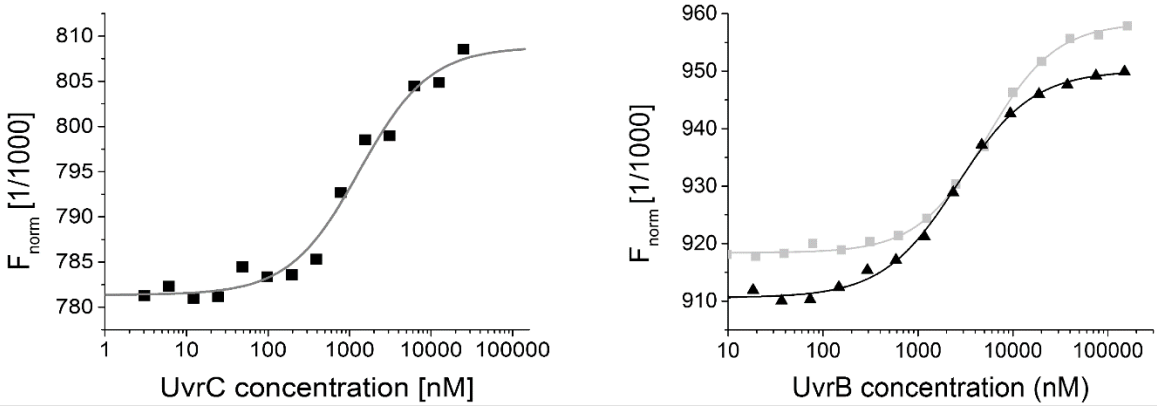

Figure S4: MST of interactions of UvrB with UvrC and with DNA. (Left) UvrB-UvrC interaction, (right) UvrB interactions with DNA substrates. The lines represent Hill equation fits to the data (symbols), giving an approximate K_D of 500 nM for the UvrB-UvrC interaction and K_D's in the low micromolar range for UvrB interactions with dsDNA (grey line) and dsDNA containing an 8 nt unpaired region (DNA bubble, black line).

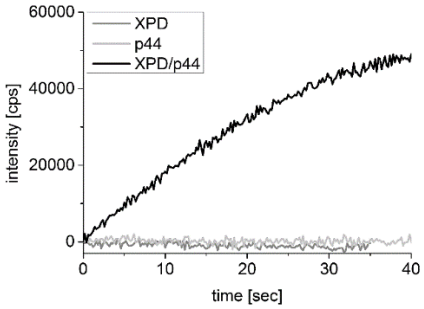

Figure S5: XPD helicase activity. Helicase assay for XPD in the presence (black line) and absence (dark grey) of its activating factor p44. P44 alone was also applied as a control (light grey line).

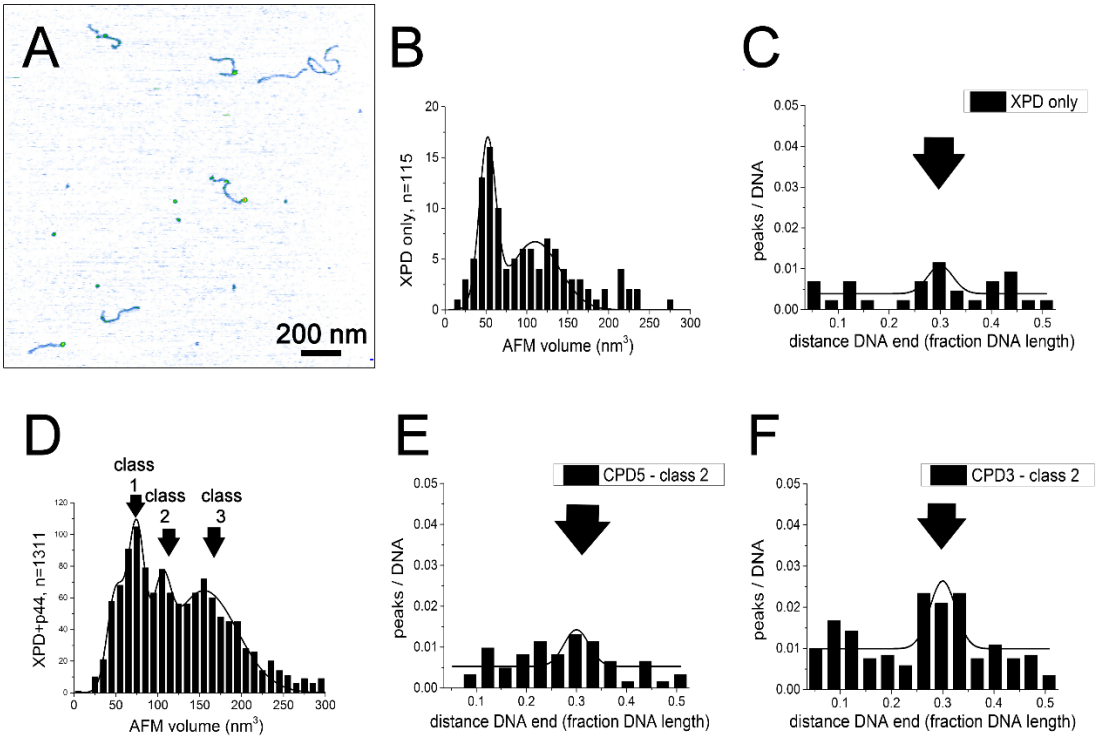

Figure S6: AFM imaging of XPD(/p44)-DNA complexes. (A) Representative image section of DNA sample containing only XPD (no p44). **(B)** Volume and **(C)** position distribution of DNA bound complexes in samples containing only XPD (no p44) including Gaussian fits to the data. **(D)** Volume distribution of DNA bound complexes for XPD+p44 samples. Comparison to volume distribution in the absence of p44 (B) reveals a distinct enhancement of volumes in the range between 140 and 250 nm^3 (class 3). **(E,F)** Position distributions of class 2 peaks (80-140 nm^3, interpreted as monomeric XPD complexes) in XPD/p44 samples with CPD/5'bubble (E) and XPD/3'bubble (F) DNA substrates. Class 2 peaks show clearly less specificity for the lesion position (at ~30% of DNA fragment length, arrows) compared to class 3 peaks (Figure 4).

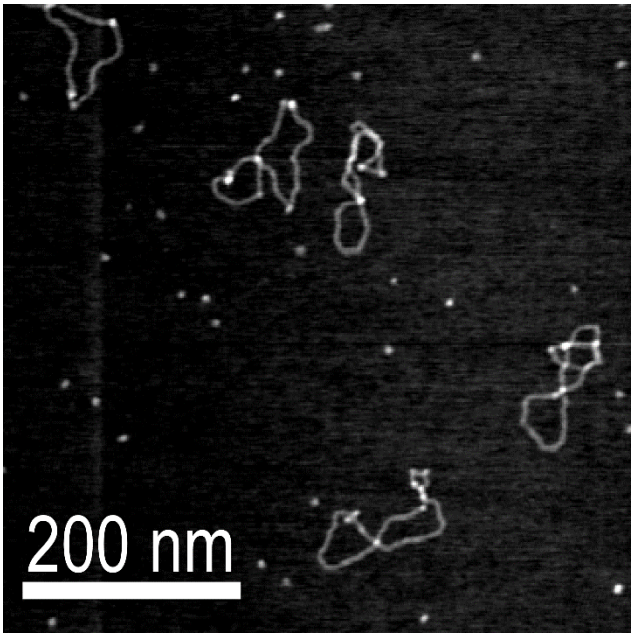

Figure S7: AFM of UvrB binding to closed circular plasmid DNA.

Supplemental Table S1: DNA substrates. Underlined sequences indicate an unpaired region (DNA bubble) in the annealed substrate. Lesions are shown in bold and red: F = fluorescein adducted thymine, T-T = cyclobutane pyrimidine dimer (CPD). The length of the oligonucleotides is given (in units of nucleotides, nts) as well as their experimental application.

	sequence	nts	experiments
1	TACGGCCTCATCTTCAACGCAATC (no damage containing top strand)	24	EMSA, AUC
1'	TACGGCCTCATCFTCAACGCAATC (fluorescein containing top strand)	24	
2	GATTGCGTCTTTTTTGAGGCCGTA(bottom strand containing 6 nts bubble)	24	
3	GGGCGGCCGCGCATTTAACTGAGGTCAGGCACCCGTACGTGACGTCAGT GAGACGAGCFGCATGGAGGCACCGATGCCCTGGATTTAACGCGCGCGC CCG (fluorescein lesion containing top strand)	100	Incision assays with F lesion
4	CGGGCGCGCGCGTTAAATCCAGGGCATCGGTGCCTCCATGCAGCTCGT CTCACTGACGTCACGTACGGGTGCCTGACCTCAGTTAAATGCGCGGCC GCCC (>>no bubble)	100	
5	CGGGCGCGCGCGTTAAATCCAGGGCATCGGTGCCTCCAACATAATCGT CTCACTGACGTCACGTACGGGTGCCTGACCTCAGTTAAATGCGCGGCC GCCC (>> 6 nts central bubble around lesion)	100	
6	CGGGCGCGCGCGTTAAATCCAGGGCATCGGTGCCTCCATGCAGCTCGT CTCACTGACGTCACGTACGGGTGCCTGACCTCAGAATTTAGCG CGG CC GCCC (>> 6 nts bubble 40 nts 5' from lesion)	100	
7	CGGGCGCGCGCGAATTTACCAGGGCATCGGTGCCTCCATGCAGCTCGT CTCACTGACGTCACGTACGGGTGCCTGACCTCAGTTAAATGCG CGG CC GCCC (>> 6 nts bubble 24 bps 3' from lesion)	100	
8	F-AGCTATGACAGCTATGACCATGATT-TACGAATTGCTTGGAATCCTGA CGAACTGTAG (CPD lesion containing top strand)	57	Incision assays with CPD lesions
9	CTACAGTTCGTCAGGATTCCAAGCAATTCGTAAATCATGGTCATAGCTGT CATAGCT (>>no bubble)	57	
10	CTACAGTTCGTCAGGATTCCAAGCAATTCTATTTACATGGTCATAGCTGT CATAGCT (>> 6 nts central bubble around lesion)	57	
11	CTACAGTTCGTCAGGATTCCAAGCAATTCGTAAATCATGGTCATA TAATAAATAGCT (>> 6 nts bubble 13 bps 5' from lesion)	57	
12	CTACAGTTCGTCATTTAAACAAGCAATTCGTAAATCATGGTCATAGCTGT CATAGCT (>> 6 nts bubble 13 bps 3' from lesion)	57	
13	GGTCGACTCTAGAGGATCAGATCTGGTACCTCTAGACTCGAGGCATGC (>> AFM substrate preparation and bottom strand for BLI and MST)	48	AFM, BLI, MST
14	/Phos/GCATGCCTCGTCAAATCTGGTACCAGATCTGATCCTCTAGAGTC GFCC (>> 8 nts bubble 27 bps 5' from fluorescein lesion)	48	AFM with F lesion
15	/Phos/GCAFGCCTCGAGTCTAGAGGTACCAGATCTCTAAAGTAAGAGTCGACC (>> 8 nts bubble 26 bps 3' from fluorescein lesion)	48	
16	/5Phos/GCATGCCTCGTCAAATCTGGTACCAGATCTGATCCTCTAGAT-TCGACC (>> 8 nts bubble 23 bps 5' from CPD lesion)	48	AFM with CPD lesion
17	/5Phos/GCATGCT-TCGAGTCTAGAGGTACCAGATCTCTAAAGTAAGA GTCGACC (>> 8 nts bubble 23 bps 3' from CPD lesion)	48	
18	GCATGCCTCGAGTCTAGAGGTACCAGATCTGATCCTCTAGAGTCGACC	48	EMSA, BLI, MST
19	GCATGCCTCGAGTCTAGACTCTTTCCATCTGATCCTCTAGAGTCGACC (>> 8 nts bubble, no lesion)	48	
19'	GCATGCCTCGAGTCTAGACTCFTTCCATCTGATCCTCTAGAGTCGACC (>> 8 nts central bubble around fluorescein lesion)	48	
19''	GCATGCCTCGAGTCTAGACTCT-TTCCATCTGATCCTCTAGAGTCGACC (>> 8 nts central bubble around CPD lesion)	48	
20	Dabcyl-GCTATGACCATGATTACGAATTGCTT	26	Helicase assay
21	AGCTACCATGCCTGCACGAATTAAGCAATTCGTAATCATGGTCATAGC-Cy3	48	