Supplementary Material

Structure of a double-stranded DNA (6-4) photoproduct in complex with the 64M-5 antibody Fab

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Table S1

Double-stranded DNA with the (6-4) photoproduct

Sequence	Base pair	Complex formation	Crystal
5′- GCGTGAT(6-4)TATGGAC -3′ 3′- CGCACTA ATACCTG -5′	14 bp	-	
GCGAGTGAT(6-4)TATGGACGG CTCACTA ATACCTGCCCG	16 bp	-	
GCGAGTGAT(6-4)TATGGACGG GCTCACTA ATACCTGCCC	17 bp	+	+*
CGGAGTGAT(6-4)TATGGACGG CCTCACTA ATACCTGCCG	17 bp	+	+
GCGAGTGAT(6-4)TATGGACGG GCTCACTA ATACCTGCCG	17 bp	+	-
GCGAGTGAT(6-4)TATGGACGG CGCTCACTA ATACCTGCC	18 bp	+	-
TGCGAGTGAT(6-4)TATGGACGGC CGCTCACTA ATACCTGCCGT	19 bp	+	-
TGCGAGTGAT(6-4)TATGGACGGC CGCTCACTA ATACCTGCCGA	19 bp	+	-

*Crystal used for structural analysis.

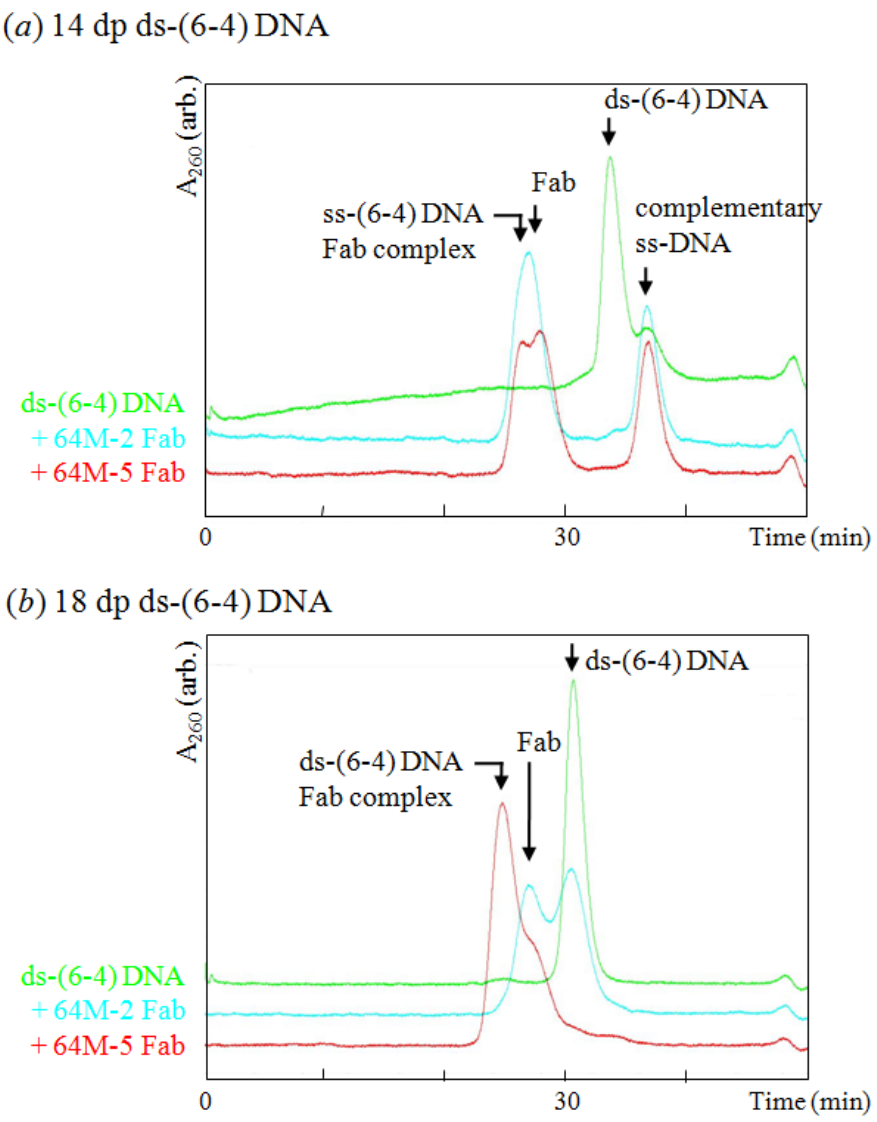


Figure S1. Gel-filtration analyses of the complex formed between the double-stranded (ds) (6-4) DNA and Fabs 64M-2 and 64M-5. A mixture of 1 μM ds-(6-4) DNA and 5 μM Fab was kept on ice for 20 min, and then applied to a Superdex-75 (10/300, GE Healthcare) column equilibrated with 20 mM Tris-HCl and 0.4 M NaCl (pH 7.0). (*a*) Complex formed with the blunt-end 14-bp ds-(6-4) DNA (its nucleotide sequence is shown in Table S1). Both the 64M-2 and 64M-5 Fabs unraveled the complementary strand and formed a complex with the resultant single-stranded (ss)-(6-4) DNA. (*b*) Complex formed with the blunt-end 18-bp ds-(6-4) DNA (Table S1). The 64M-2 Fab showed no interaction with the 18-bp ds-(6-4) DNA, while the 64M-5 Fab, which exhibits an affinity constant at least 10-fold higher than that of 64M-2 (Mori *et al.*, 1991; Kobayashi *et al.*, 1999), forms a complex with the 18-bp ds-(6-4) DNA.

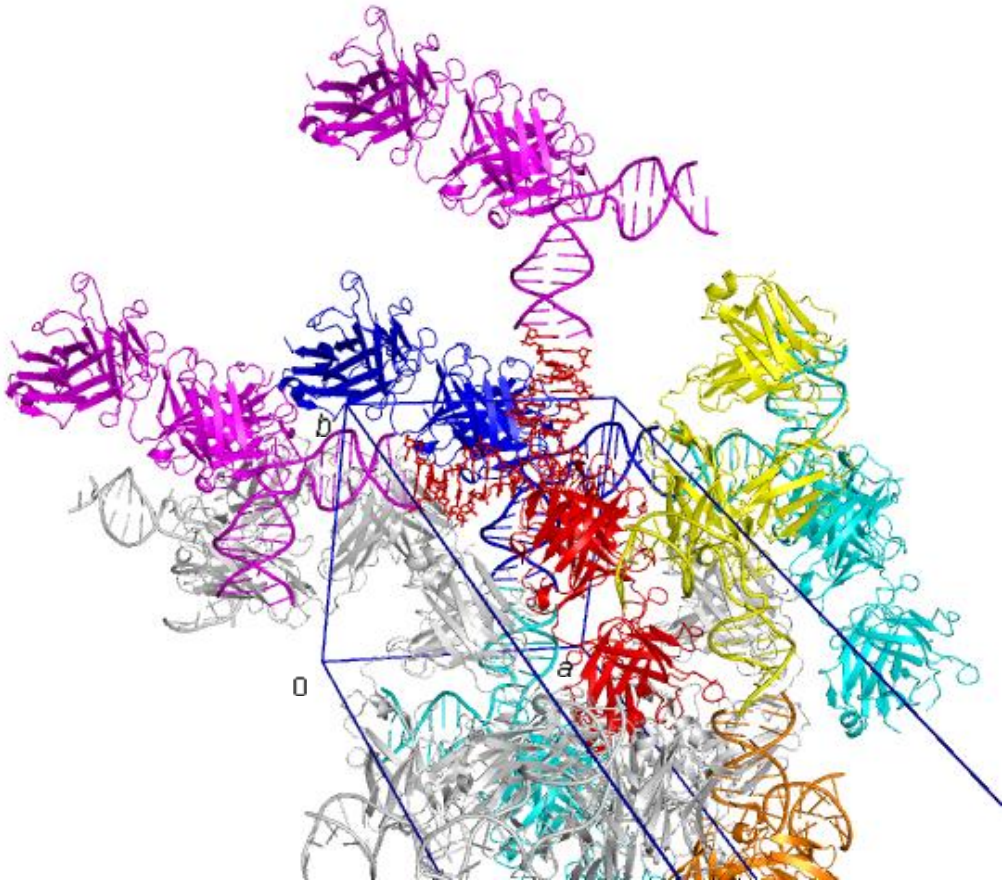
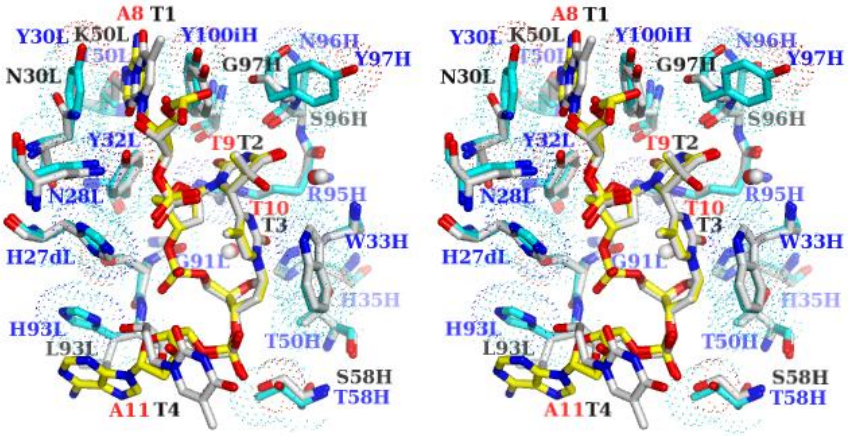


Figure S2. Crystal packing of the double-stranded (6-4) DNA in complex with the 64M-5 Fab. One DNA/Fab complex is drawn in red with a unit cell box. DNA strands of this complex are shown as sticks. Other complexes symmetry-related to the complex at the origin are shown in cartoon representation and differentiated with colors. Overhang bases at the 5'-end of both strands interact with the other overhangs related by the crystallographic symmetry.

(*a*)



(*b*)

V_L domain

	24	27 a b c d e	28	30	32	34	50	56	89	93	95	97
64M-5	R	S S Q N I V	**H**	S	**N**	G	**Y**	T	**Y**	L	E	
64M-2	-	- - - - S - - - - - -		**N**	- - - -		**K**	- - - - - - -		- Q - -	**L**	- - -
		L1					L2			L3		

V_H domain

	31	35	50	52 a	53	58	65	95	97	100 i	j	k	102
64M-5	N Y	**W** M	**H**	**T** I Y P G N S D T		**T**	Y S Q K F K G		**RN**	**Y** G S S	**Y**	A M D	Y
64M-2	S F	- - -		- - - - - - - - -		**S**	- N - - - - -		**-S**	**G** Y K Y	- -	L	- -
		H1				H2				H3			

Figure S3. Comparison of 64M-5 and 64M-2 Fabs in complex with the (6-4) photoproducts. (*a*) The structures of the 64M-5 Fab - double-stranded (6-4) DNA and the 64M-2 Fab - dTT(6-4)TT (PDB ID: 1keg) (Yokoyama *et al.*, 2012) are shown as stick models with Fab variable domains superposed. The 64M-5 Fab is shown in cyan (labeled in blue), and dAT(6-4)TA in the double-stranded (6-4) DNA is shown in yellow (labeled in red). The 64M-2 Fab and dTT(6-4)TT are shown in grey, and the dTT(6-4)TT and the residues different from 64M-5 are labeled in black. The side chains of the 64M-5 Fab are also shown as dots. Two water molecules involved in the interactions are in almost the same location, and are shown as red (64M-5) and grey (64M-2) spheres. (*b*) Amino acid sequences of CDR residues of 64M-5 and 64M-2.