Two different kinds of interaction modes of deaminase APOBEC3A with single-stranded DNA in solution detected by nuclear magnetic resonance

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Supplemental figures and tables

Figure S1

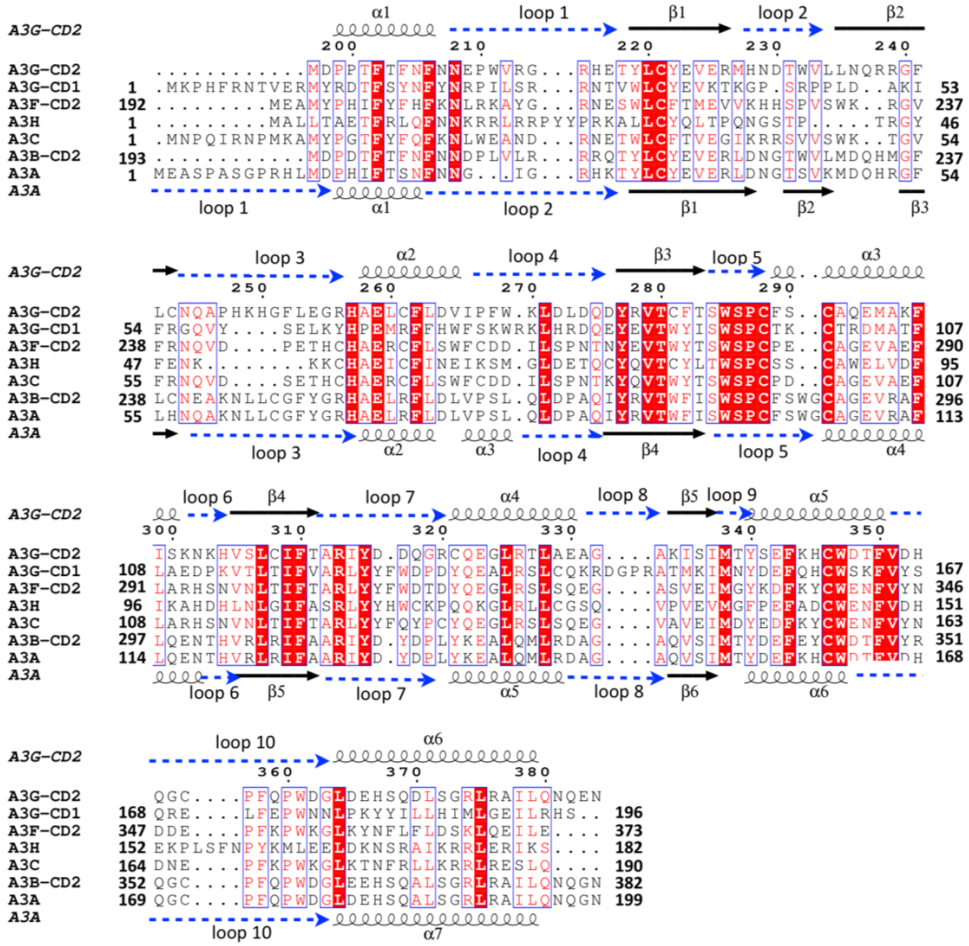

Figure S1 Sequence alignment of A3 members indicates the conserved sites for specific identification of target motifs TC and CC[1, 2].

Figure S2

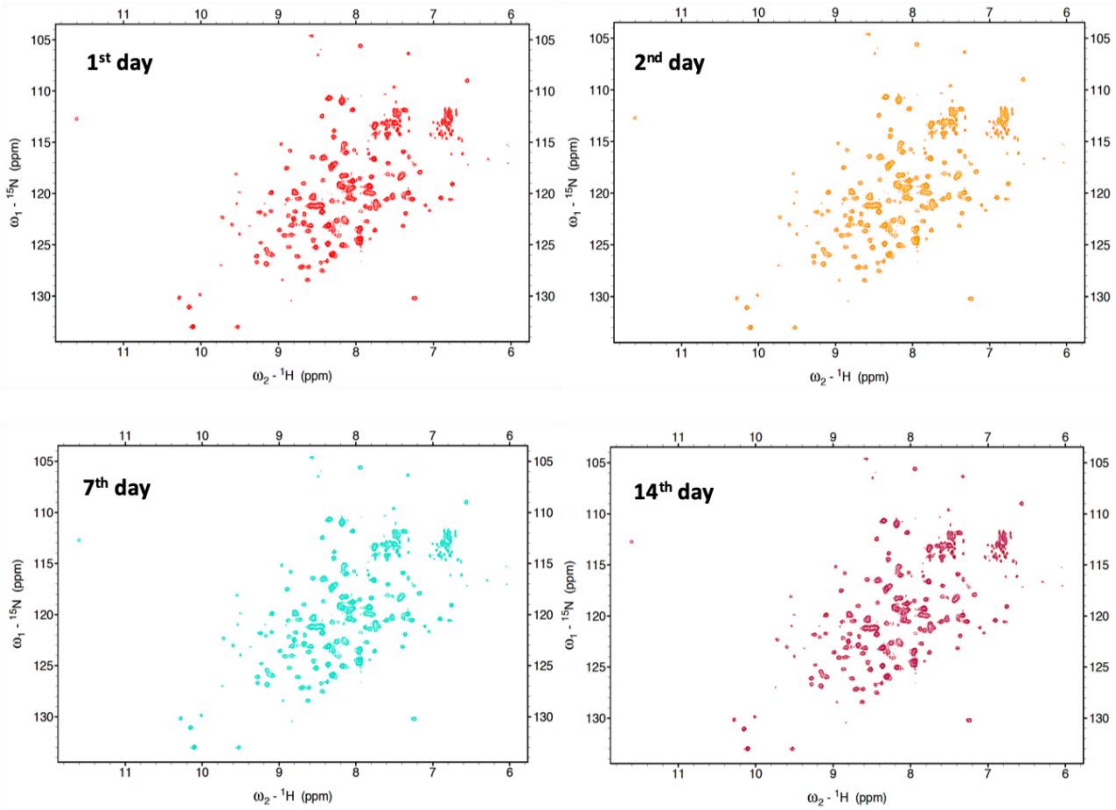

Figure S2 $A3A_{4m}$ is stable for NMR studies within 14 days tested by running ^{1}H-^{15}N HSQC spectra, in which there was only one set of ^{1}H-^{15}N cross-peaks.

Figure S3

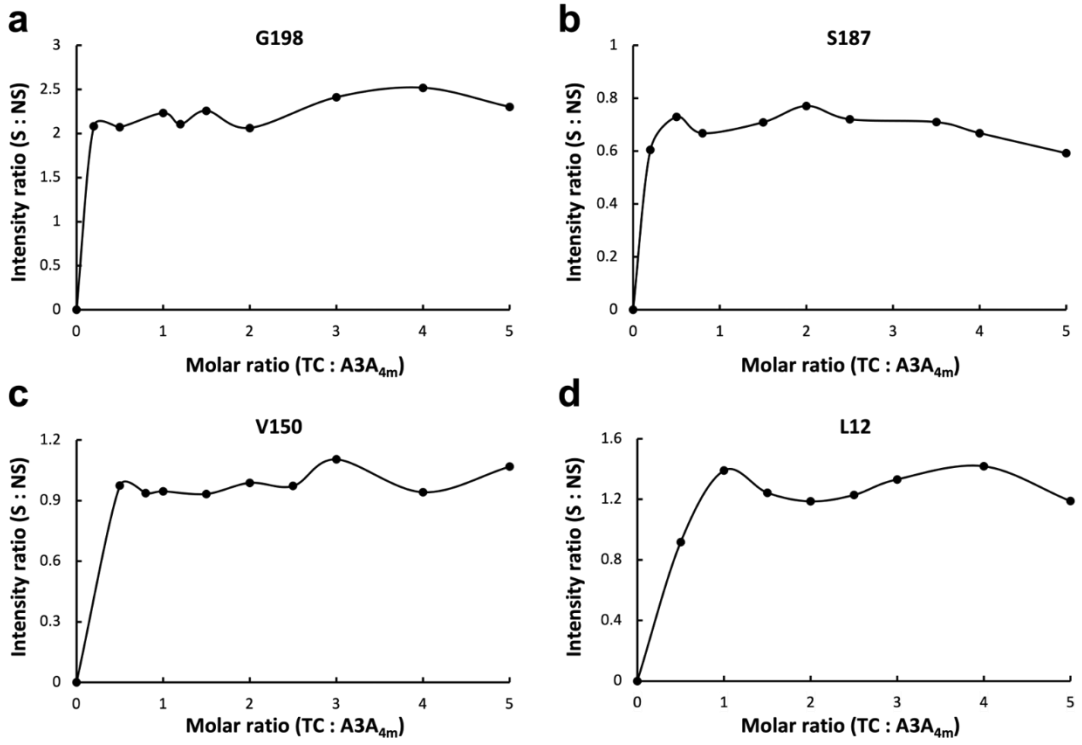

Figure S3 The ratios of specific complex (*i.e.* S in vertical axis) vs non-specific complex (*i.e.* NS in the abscissa axis) in solution, measured by intensity ratios of the cross-peaks belonging to residues (a) G198, (b) S187, (c) V150 and (d) L12 in HSQC spectra acquired at different molar ratios of TC DNA vs $A3A_{4M}$.

Figure S4

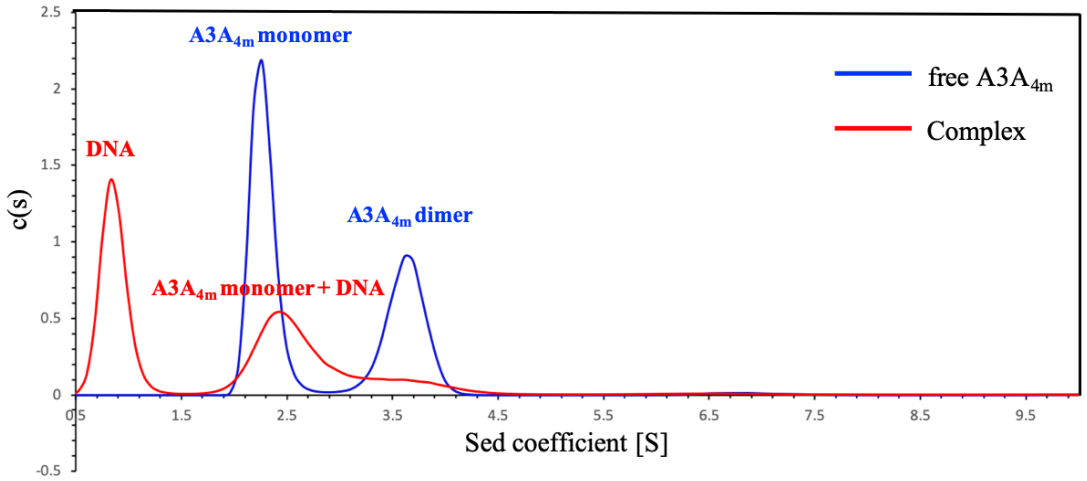

Figure S4 The aggregation states of free A3A$_{4M}$ (in blue) and its complex with TC DNA (in red) determined by ultracentrifugation assay. Based on the fact that the theoretical molecular weights of A3A$_{4M}$ and of free TC DNA are 23.02KDa and 2.99KDa, respectively, we deduced that free A3A$_{4M}$ was a mixture composed by monomer and dimer in solution, but its complex with TC DNA was mainly made of A3A monomer plus TC DNA. In this figure, the complex sample of A3A with DNA was prepared at a molar ratio equal to 2 (TC DNA *vs* A3A).

Figure S5

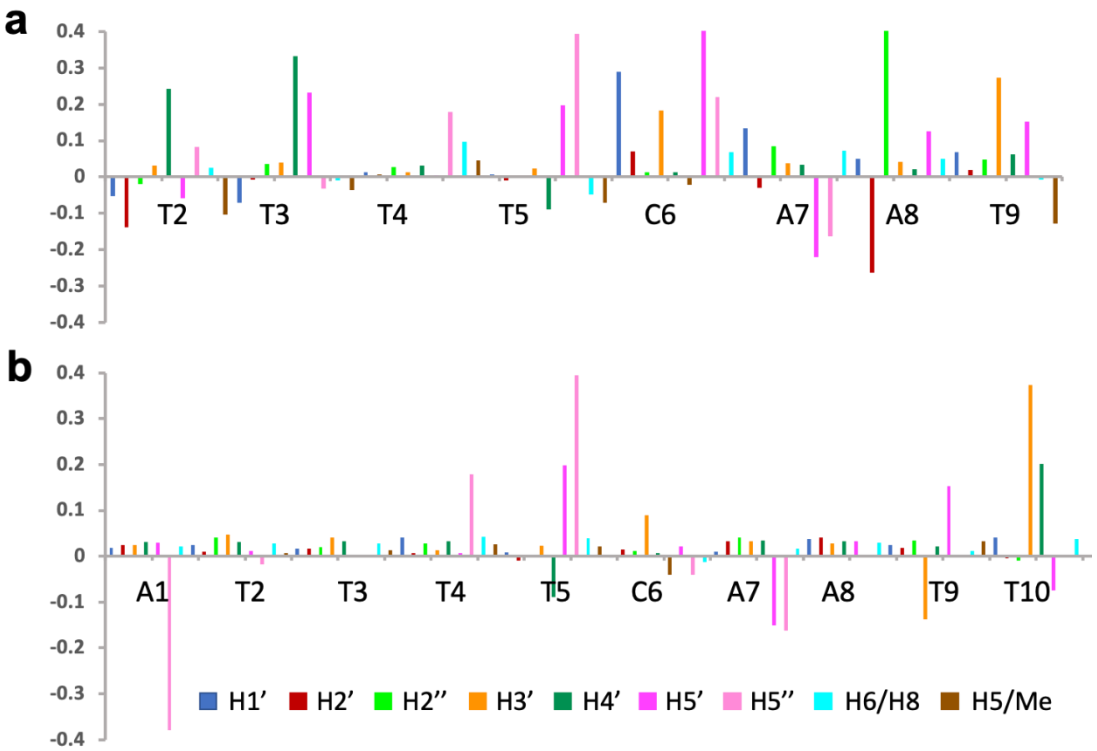

Figure S5 Chemical shift changes of the protons in two kinds of TC DNA conformers upon it interaction with $A3A_{4M}$. (a) DNA^{S} and (b) DNA^{NS}.

Figure S6

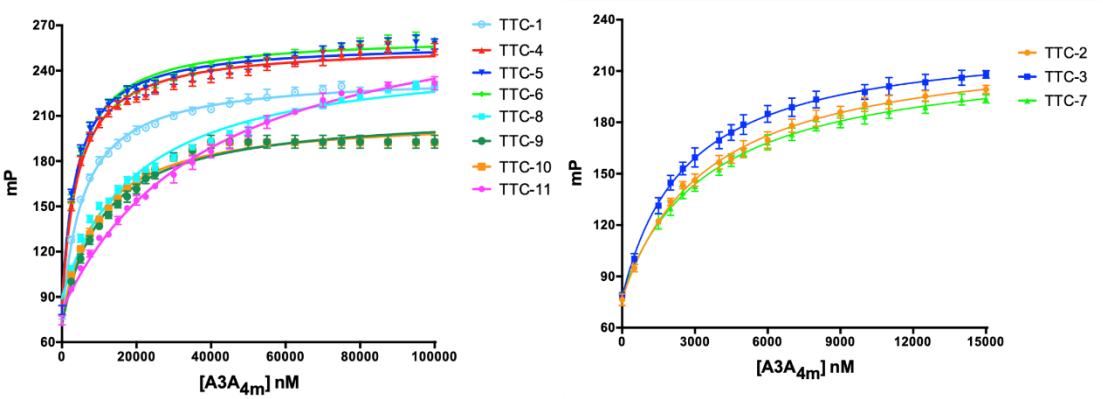

Figure S6 The effects on A3A$_{4M}$ binding affinities by adding bases into 3'- end (*i.e.*, TTC-4 DNA, TTC-5 DNA and TTC-6 DNA) or 5'- end (*i.e*, TTC-8 DNA, TTC-9 DNA, TTC-10 DNA and TTC-11 DNA), compared to TTC-2. The binding affinities were measured by fluorescent polarization (FP) assay were listed in Table S1.

Figure S7

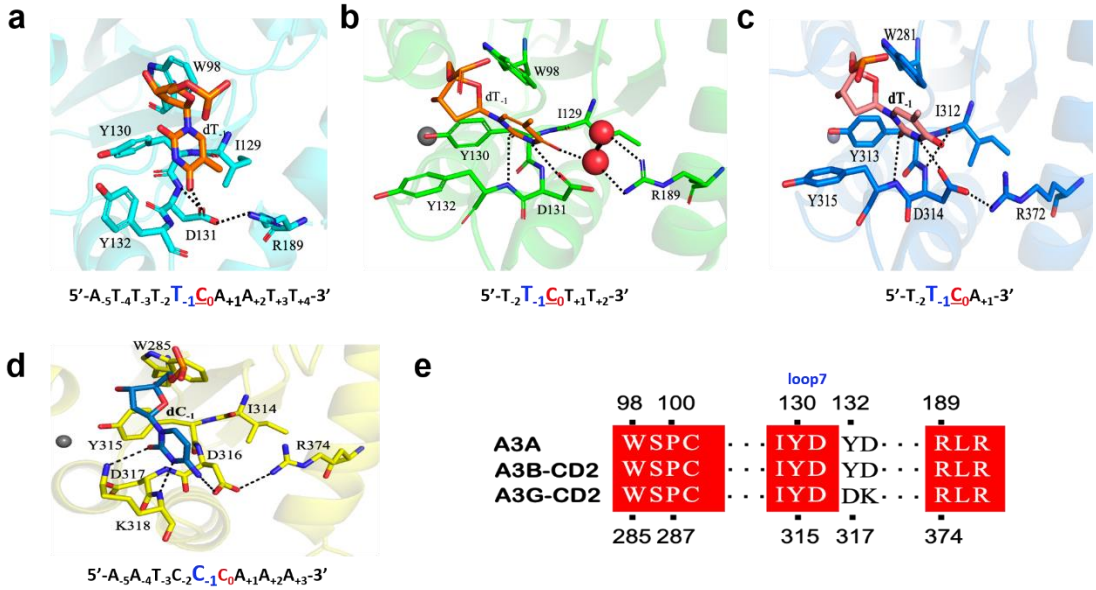

Figure S7 The base dT_{-1} or dC_{-1} in DNA was specifically identified by the conserved residues in A3 members (shown in figure S1) in different A3-DNA structures. (a) $A3A^{S}$-DNA^{S} complex, (b) A3A-DNA complex (PDB: 5KEG); (c) A3Bctd*-DNA complex (PDB: 5TD5), (d) A3G-CTD2* complex (PDB: 6BUX); In (a-d), DNA sequences were listed in each structure. (e) The conserved residues among A3A, A3B and A3G interact with dT_{-1} or dC_{-1}. The dashed lines represent hydrogen-bonds.

Figure S8

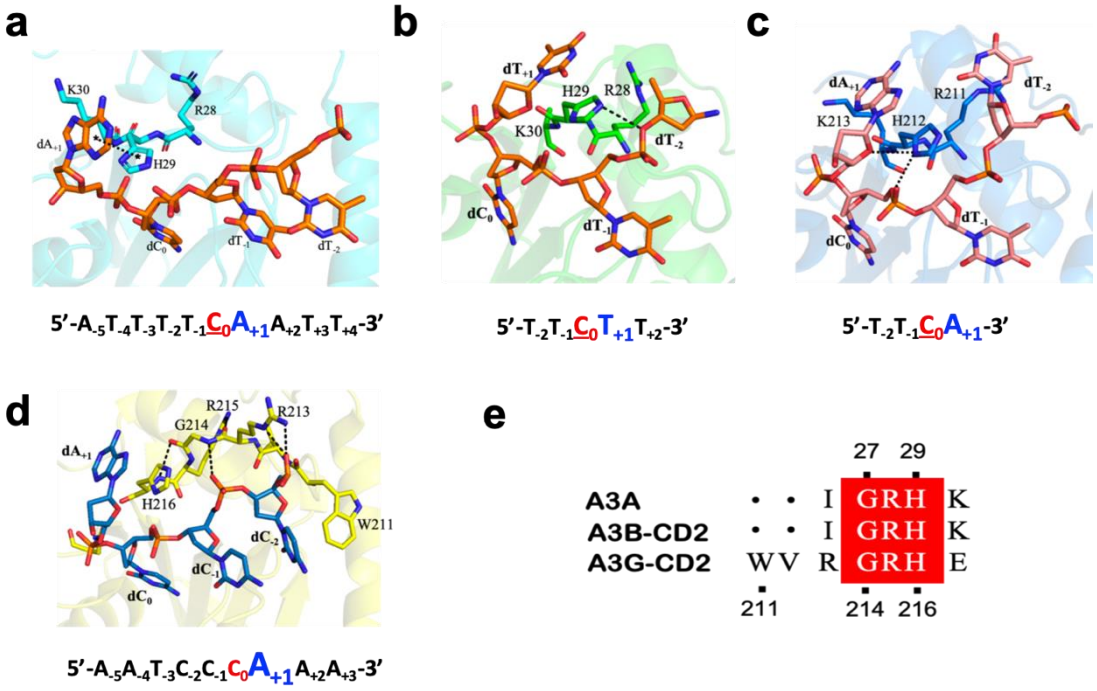

Figure S8 The base dA_{+1} in DNA was specifically identified by the conserved residues in loop 1 of A3 members in different A3-DNA structures. (a) $A3A^{S}$-DNA^{S} complex, (b) A3A-DNA complex (PDB: 5KEG); (c) A3Bctd*-DNA complex (PDB: 5TD5), (d) A3G-CTD2* complex (PDBB: 6BUX); In (a-d), DNA sequences were listed in each structure. (e) The conserved residues in loop 1 among A3A, A3B and A3G interact with dA_{+1}. The dashed lines with stars stands for stacking interactions, while dashed lines without starts represent hydrogen-bonds.

Figure S9

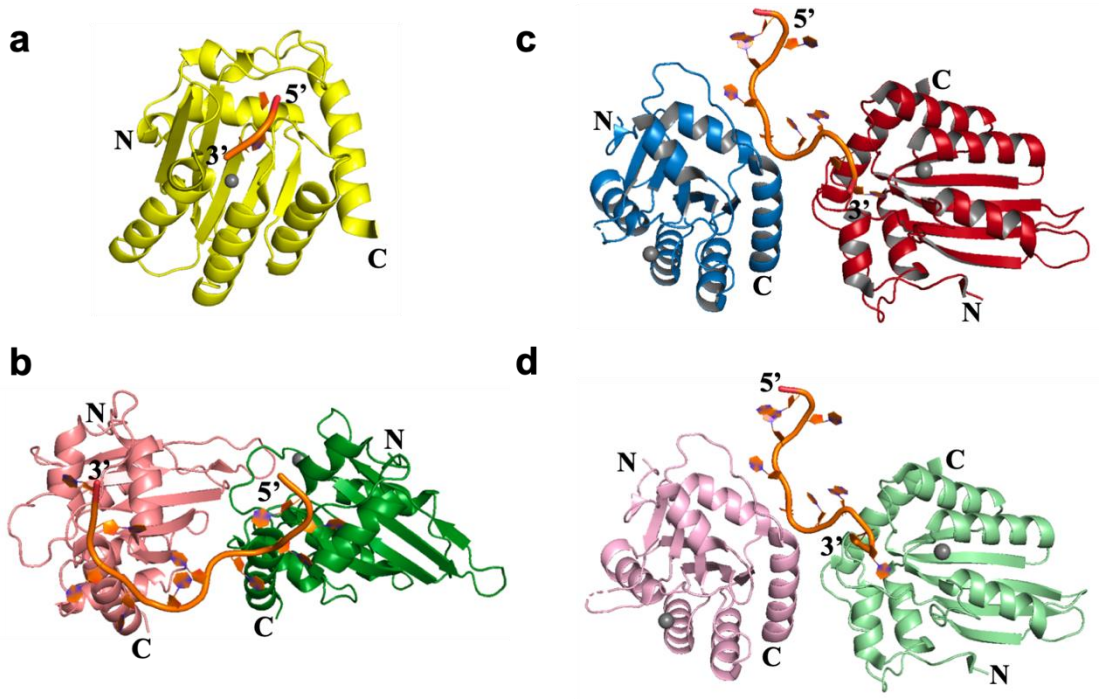

Figure S9 DNA non-specific binding is involved in its interactions with different A3 members. (a) rA3G-CD1 with poly dT ssDNA (PDB: 5K83); (b) hA3F-CD2 dimer with one poly dT ssDNA (PDB: 5W2M); (c and d) hA3Fc-CD2 dimer with one DNA strand containing two TC motifs with PDB codes of 5ZVB and 5ZVA, respectively. The N- and C-termini of A3 proteins, and the 3'- and 5'- ends of DNA were labeled. DNA and A3 proteins were shown in cartoon modes.

Table S1 Intermolecular NOEs used in structural determination of $A3A^{NS}$-DNA^{NS}.

Atom 1 (in DNA^{NS})	**Atom 2 (in $A3A^{NS}$)**
A1- H1’	Q 184-Hγ1
	Q 184-Hγ2
	A185-β-CH_3
A1-H3’	A185-β-CH_3
A1-H4’	A185-Hα
	A185-β-CH_3
A1-H5’’	A185-Hα
A1-H8	E181-Hα
	E181-Hβ1
	E181-Hβ2
	Q184-Hγ1
	Q184-Hγ2
T2-H4’	A185-β-CH_3
T2 -H5’	A185-Hα
T2 -H5’’	A185-Hα
	A185-β-CH_3
T2-H6	G25-Hα1
	G25-Hα2
	L186- δ2- CH_3
T2-5-CH_3	E181-Hα
	E181-Hβ1
	E181-Hβ2
	H182-Hα
T3-H1’	N24-Hα
T3-H6	I26- δ1-CH_3
	I26- γ2-CH_3
T3-5-CH_3	G25-Hα1
	G25-Hα2
	I26- δ1-CH_3
	A185-β-CH_3
T4-H4’	G27-Hα1
	G27-Hα2
T4-H5’’	I26- δ1-CH_3
T4-H6	A185-Hα
T4-5-CH_3	I26- δ1-CH_3
	K30-Hβ1
	K30-Hβ2
T5-H6	G25-Hα1
	G25-Hα2

	I26- Hα
	I26- Hβ
	I26- γ2-CH_3
T5-5-CH_3	G25-Hα1
	G25-Hα2
	I26- Hα
C6-H6	I26- HN
	G27-Hα1
	G27-Hα2
C6-H5	I26- γ2-CH_3
	K30- Hα
A7-H1’	Q58-Hα
A7-H3’	K30- Hβ1
	K30- Hβ2
A7-H4’	Q58-Hα
	Q58-Hγ1
	Q58-Hγ2
A7-H8	N57- Hβ1
	N57- Hβ2
A8-H1’	N57- Hβ1
	N57- Hβ2
	Q58- Hβ1
	Q58- Hβ2
	F66- Hβ1
	F66- Hβ2
A8-H8	A59-β-CH_3
T9-H6	L62-Hβ1
	L62-Hβ1
	L62- δ1-CH_3
T9-5-CH_3	A59- Hα
T10-H1’	N63- Hα

Table S2 Intermolecular NOEs used in structural determination of $A3A^S$-DNA^S.

Atom 1 (in DNA^S)	**Atom 2 (in $A3A^S$)**
T2-5-CH_3	H182- Hβ1
	H182-Hβ2
T3-H1’	I26- δ1-CH_3
T3-H2’	I26- δ1-CH_3
T3-H6	I26- γ2-CH_3
T3- 5-CH_3	I26- γ2-CH_3
	A185-β-CH_3
	L186- δ2-CH_3
T4-H1’	I26- γ2-CH_3
T4-H6	I26- γ2-CH_3
T4- 5-CH_3	I26- Hβ
	I26- δ1-CH_3
	I26- γ2-CH_3
T5-H6	I26- Hβ
	I26- γ2-CH_3
	W98-Hβ1
	W98-Hβ2
	D131- Hα
T5- 5-CH_3	I26- Hβ
	G27-Hα1
	G27-Hα2
	W98-Hα
	Y132-Hβ1
	Y132-Hβ2
C6-H1’	H70-Hβ1
	H70-Hβ2
	W98-Hα
C6-H2’	N57- Hβ1
	N57- Hβ2
C6-H2’’	T31-Hα
	N57- Hβ1
	N57- Hβ2
	H70-Hβ1
	H70-Hβ2
	P100-Hβ1
	P100-Hβ2
	P100-Hγ1
	P100-Hγ2
C6-H3’	H70-Hδ2
C6-H4’	H29-Hβ1
	H29-Hβ2

	H70-Hδ2
C6-H5’	N57- Hβ1
	N57- Hβ2
C6-H5’’	H29-Hβ1
	H29-Hβ2
C6-H6	K30-Hα
	S97-Hβ1
	S97-Hβ2
	W98-Hα
	W98-Hβ1
	W98-Hβ2
	P100-Hα
	P100-Hγ1
	P100-Hγ2
	C101-Hβ1
	C101-Hβ2
	Y130- Hα
C6-H5	H70-Hδ2
	H70-Hε1
	W98-Hα
	W98-Hδ1
	P100-Hα
	Y130- Hα
	Y130- Hδ1
	Y130- Hδ2
	Y132-Hβ1
	Y132-Hβ2
A7-H1’	K30-Hα
	R69-Hβ1
	R69-Hβ2
A7-H2’	K30-Hα
	K30-Hβ1
	K30-Hβ2
	H70-Hδ2
A7-H2’’	K30-Hα
	K60-Hε1
	K60-Hε2
A7-H3’	K30-Hα
	K30-Hβ1
	K30-Hβ2
	K60-Hβ1
	K60-Hβ2
	K60-Hδ1

	K60-Hδ2
	K60-Hε1
	K60-Hε2
A7-H5’	K30-Hβ1
	K30-Hβ2
A7-H8	K30-Hα
	A59-Hα
	A59-β-CH_3
	K60-Hα
A8-H1’	R69- Hα
A8-H2’	K60-Hε1
	K60-Hε2
A8-H2”	K60-Hα
	K60-Hε1
	K60-Hε2
A8-H3’	Q58-Hβ1
	Q58-Hβ2
	K60-Hα
	L62-δ1-CH_3
A8-H8	K60-Hα
	K60-Hδ1
	K60-Hδ2
	K60-Hε1
	K60-Hε2
T9-H3’	L62- δ1-CH_3
T9- 5-CH_3	K60-Hα
	K60-Hδ1
	K60-Hδ2
	K60-Hε1
	K60-Hε2
	L62-Hα
	L62-Hβ1
	L62-Hβ2
	L62- δ1-CH_3
	L62- δ2-CH_3
T10-H6	L62- δ2-CH_3
T10- 5-CH_3	L62-Hβ1
	L62-Hβ2
	L62- δ1-CH_3
	L62- δ2-CH_3

Table S3 Experimental restraints and structural statistics for $A3A_{4M}$ in complexes with different ssDNA.

	$A3A^{NS}$-DNA^{NS}	$A3A^{S}$-DNA^{S}
Number of restraints		
Distance restraints from NOEs		
Total NOE	3346	3409
Intra-residue(i-j=0)	1613	1606
Sequential(|i-j|=1)	654	678
Medium range((1<|i-j|≤5)	375	369
Long range(|i-j|>5)	654	661
Intermolecular (protein-DNA)	50	95
Inter-monomers		
Hydrogen bonds	170	174
Total dihedral angle restraints	369	369
Φ	184	184
Ψ	185	185
Structural statistics		
r.m.s.d versus the mean structure(Å)		
All backbone atoms	1.57 ± 0.23 Å	1.46 ± 0.46 Å
All heavy atoms	1.91 ± 0.19 Å	1.79 ± 0.38 Å
Backbone atoms (2^{nd} structure)	0.55 ± 0.11 Å	0.54 ± 0.07 Å
Heavy atoms (2^{nd} structure)	1.13 ± 0.09 Å	1.16 ± 0.13 Å
Rms Deviations from the experimental restraints		
NOE distance(Å)	0.035 ± 0.005	0.032 ± 0.001
Dihedral angels (deg)	1.37 ± 0.098	0.96 ± 0.11
Rms Deviations from idealized geometry		
Bonds(Å)	0.0025 ± 0.00017	0.0027 ± 0.00010
Angels(deg.)	0.38 ± 0.013	0.42 ± 0.029
Impropers(deg.)	0.31 ± 0.018	0.32 ± 0.013
Ramachandran Analysis		
residues in most favored regions	89.0%	86.6%
residues in additionally allowed regions	9.8%	11.7%
residues in generously allowed regions	1.2%	1.7%
residues in disallowed regions	0.0%	0%

Supplemental References

1. Kouno, T.; Silvas, T. V.; Hilbert, B. J.; Shandilya, S. M. D.; Bohn, M. F.; Kelch, B. A.; Royer, W. E.; Somasundaran, M.; Kurt Yilmaz, N.; Matsuo, H.; Schiffer, C. A., Crystal structure of APOBEC3A bound to single-stranded DNA reveals structural basis for cytidine deamination and specificity. *Nature communications* **2017,** *8*, 15024.

2. Shi, K.; Carpenter, M. A.; Banerjee, S.; Shaban, N. M.; Kurahashi, K.; Salamango, D. J.; McCann, J. L.; Starrett, G. J.; Duffy, J. V.; Demir, O.; Amaro, R. E.; Harki, D. A.; Harris, R. S.; Aihara, H., Structural basis for targeted DNA cytosine deamination and mutagenesis by APOBEC3A and APOBEC3B. *Nature structural & molecular biology* **2017,** *24* (2), 131-139.