Supplementary Data for

Conformational dynamics of CasX (Cas12e) in mediating DNA cleavage revealed by single-molecule FRET

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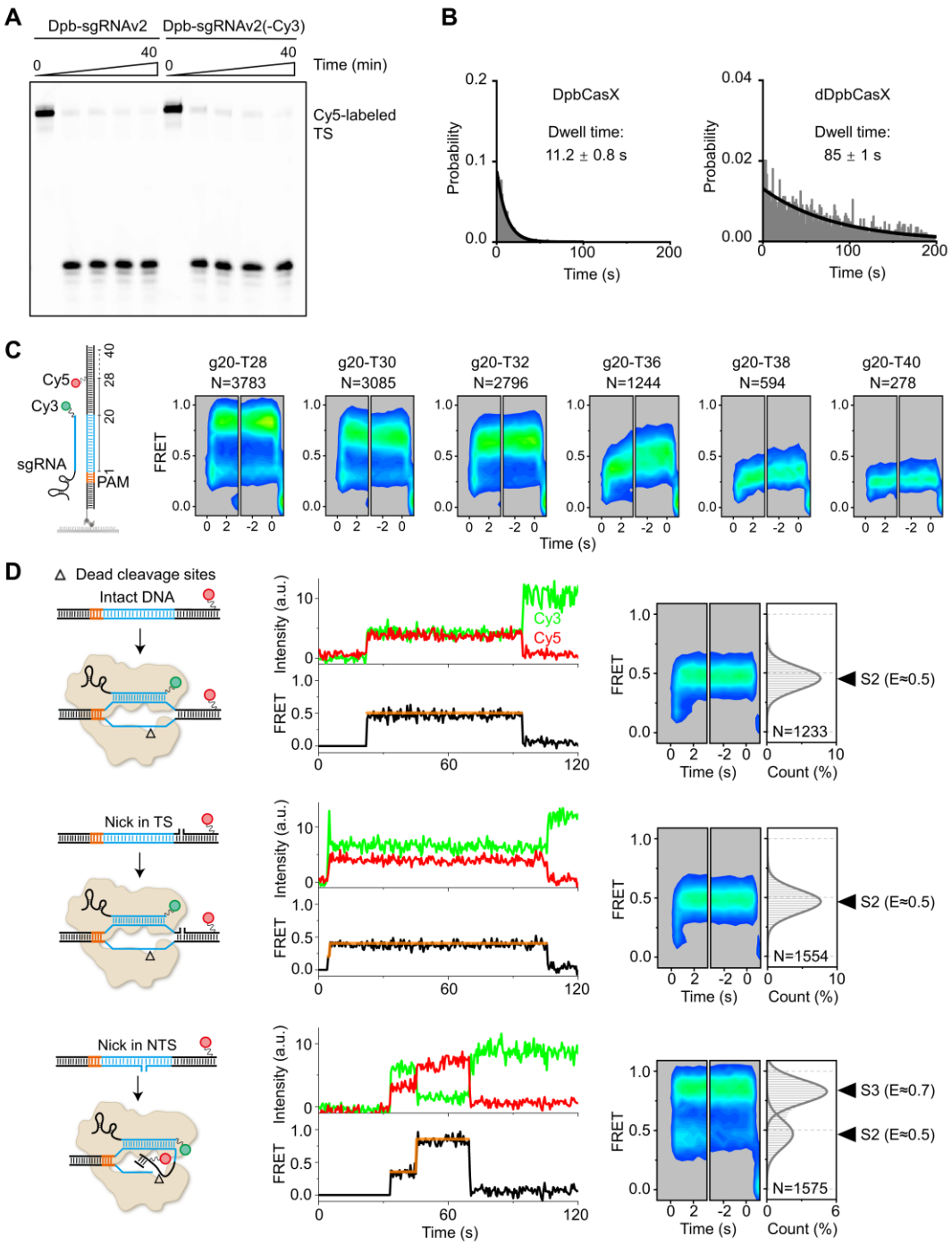

Figure S1. Cleavage activity of labeled sgRNA and single-molecule FRET measurements of different designs.

(A) Cleavage activity of DpbCasX on cognate DNA using unlabeled or Cy3-labeled sgRNAs at time points of 0, 5, 10, 20, and 40 min. Three repeats show consistent results. **(B)** Dwell time distributions of DpbCasX and dDpbCasX on cognate DNA. **(C)** Schematic of FRET pair design and time-dependent FRET probability density plots of DpbCasX-sgRNAv2 on cognate DNA with different labeling sites on the TS, ranging from 28 to 40 nt from the PAM (T28 – T40). *N* represents the number of events. Three independent experiments show consistent results. **(D)** Schematic, representative single-molecule FRET traces and time-dependent FRET probability density plots of dDpbCasX-sgRNAv2 on intact DNA, DNA with a nick in the TS, and DNA with a nick in the NTS.

Three repeats were performed. Mean and SEM are shown where appropriate.

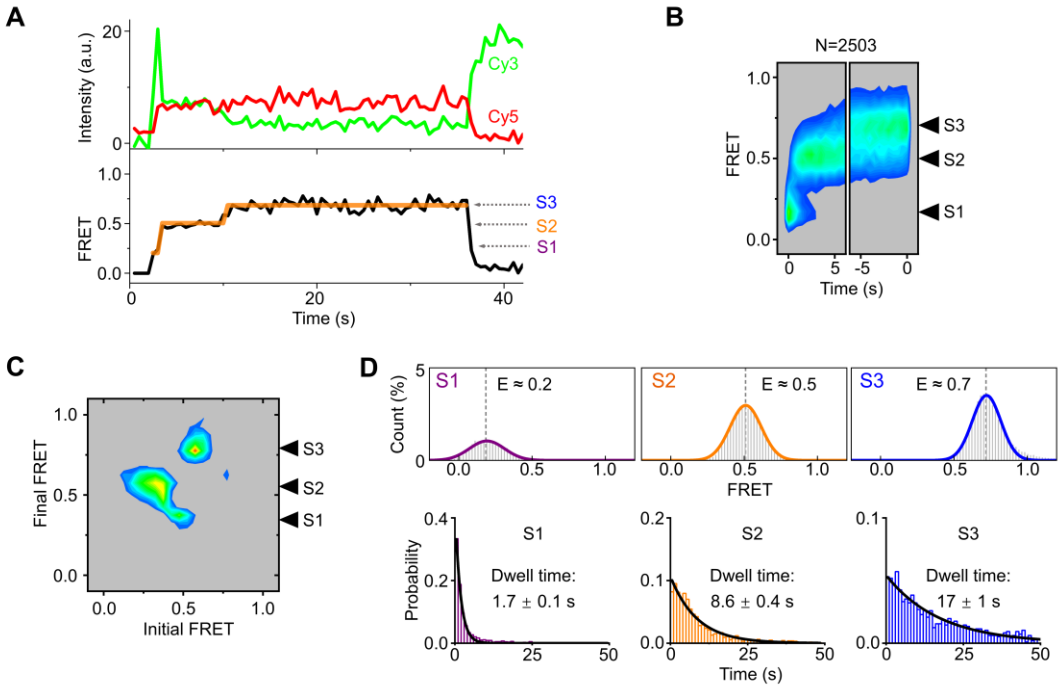

Figure S2. Conformational dynamics of DpbCasX cleavage at 25℃.

(A) Representative single-molecule FRET trajectories of DpbCasX-sgRNAv2 on cognate DNA at 25℃. Three distinct FRET states are indicated and assigned as S1, S2 and S3, ranging from low to high values. **(B)** Time-dependent FRET probability density plot of DpbCasX/sgRNAv2 on cognate DNA at 25℃. **(C)** Transition density plot reflecting the transition frequencies between different FRET states. **(D)** FRET histograms and dwell time distributions of individual states determined by a Hidden Markov Model-based software HaMMy. FRET values for each state were obtained by fitting the FRET histograms to a single Gaussian function, and dwell time were fitted by single exponential decay curves.

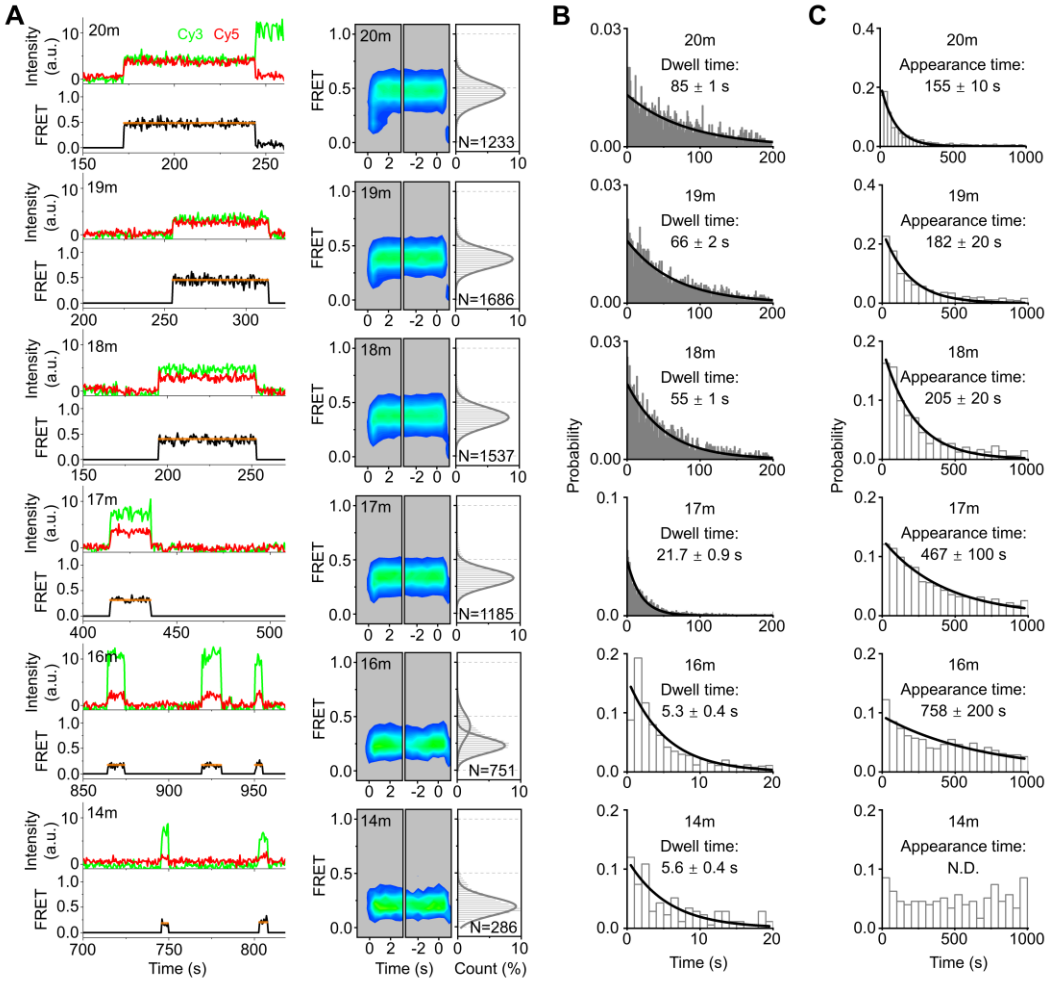

Figure S3. Conformational dynamics of dDpbCasX on DNA.

(A) Representative single-molecule FRET trajectories and time-dependent FRET probability density plots of dDpbCasX-sgRNAv2 on DNAs. **(B)** Distributions of dwell time of dDpbCasX on DNAs. The photobleaching rate, calculated from the dwell time of dDpbCasX on 20m-DNA, was used to estimate the corrected dwell times of 14m-, 16m- and 17m-DNAs, as shown in Figure 2B. **(C)** Distributions of appearance time of dDpbCasX on DNAs, related to Figure 2C. The appearance time of dDpbCasX on 14m-DNA exceeded the detection limit of our experimental condition, as our observation length was 1000 s. Three repeats were performed. Mean and SEM are shown where appropriate.

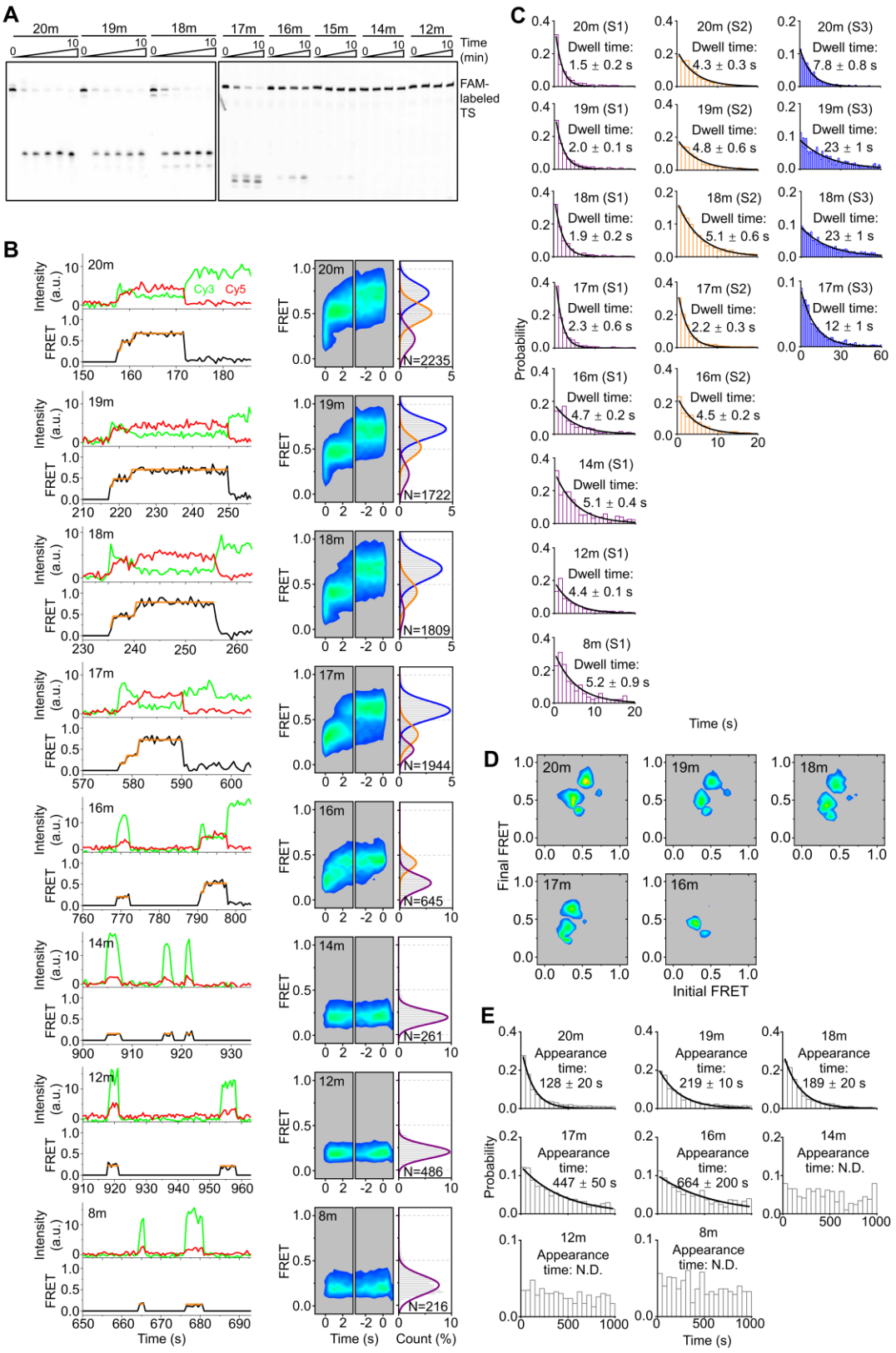

Figure S4. Cleavage activity and conformational dynamics of DpbCasX on DNA.

(A) Gel images of DpbCasX cleavage on mismatched DNAs, related to Figure 2D. Time points were 0, 0.5, 1, 2, 5, and 10 min for 20m-, 19m-, and 17m-DNA, and 0, 2, 5, and 10 min for the remaining DNA. **(B)** Representative single-molecule FRET trajectories and time-dependent FRET

probability density plots of DpbCasX-sgRNAv2 on DNAs. **(C)** Dwell time distributions of individual states of DpbCasX on DNAs, related to Figure 2F. **(D)** Transition density plots reflecting the transition frequencies between different FRET states of DpbCasX on 20m-, 19m-, 18m-, 17m- and 16m-DNA, respectively. **(E)** Distribution of appearance time of DpbCasX on mismatched DNAs related to Figure 2C. The appearance time of DpbCasX on 14m-DNA exceeded the detection limit of our experimental conditions, as our observation length was 1000 s. Three repeats were performed. Mean and SEM are shown where appropriate.

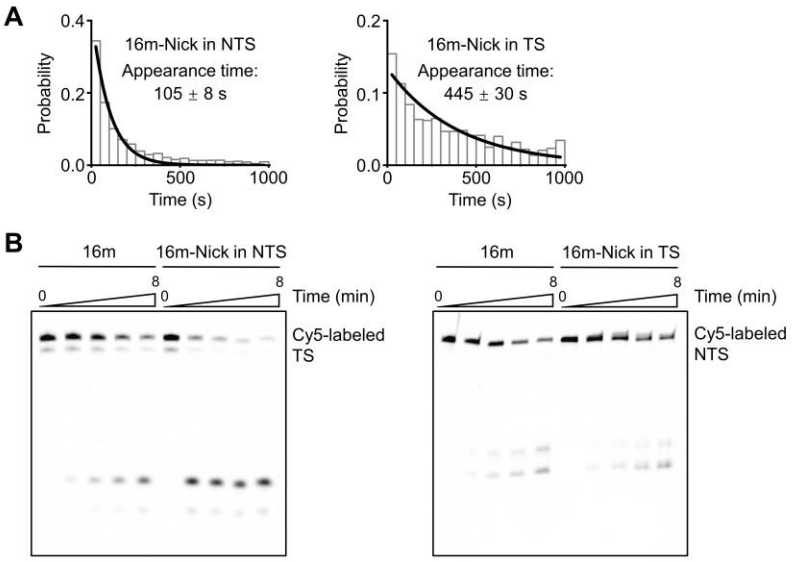

Figure S5. Appearance time and cleavage activity of DpbCasX on 16m-DNA with nick in TS or NTS

(A) Distributions of appearance time of DpbCasX on 16m-DNA with a nick in TS or NTS, related to Figure 2G. **(B)** Gel images of DpbCasX cleavage on 16m-DNA with a nick in TS (NTS was labeled with Cy5) or NTS (TS was labeled with Cy5), related to Figure 2H. Time points were 0, 1, 2, 4, and 8 min. Three repeats were performed. Mean and SEM are shown where appropriate.

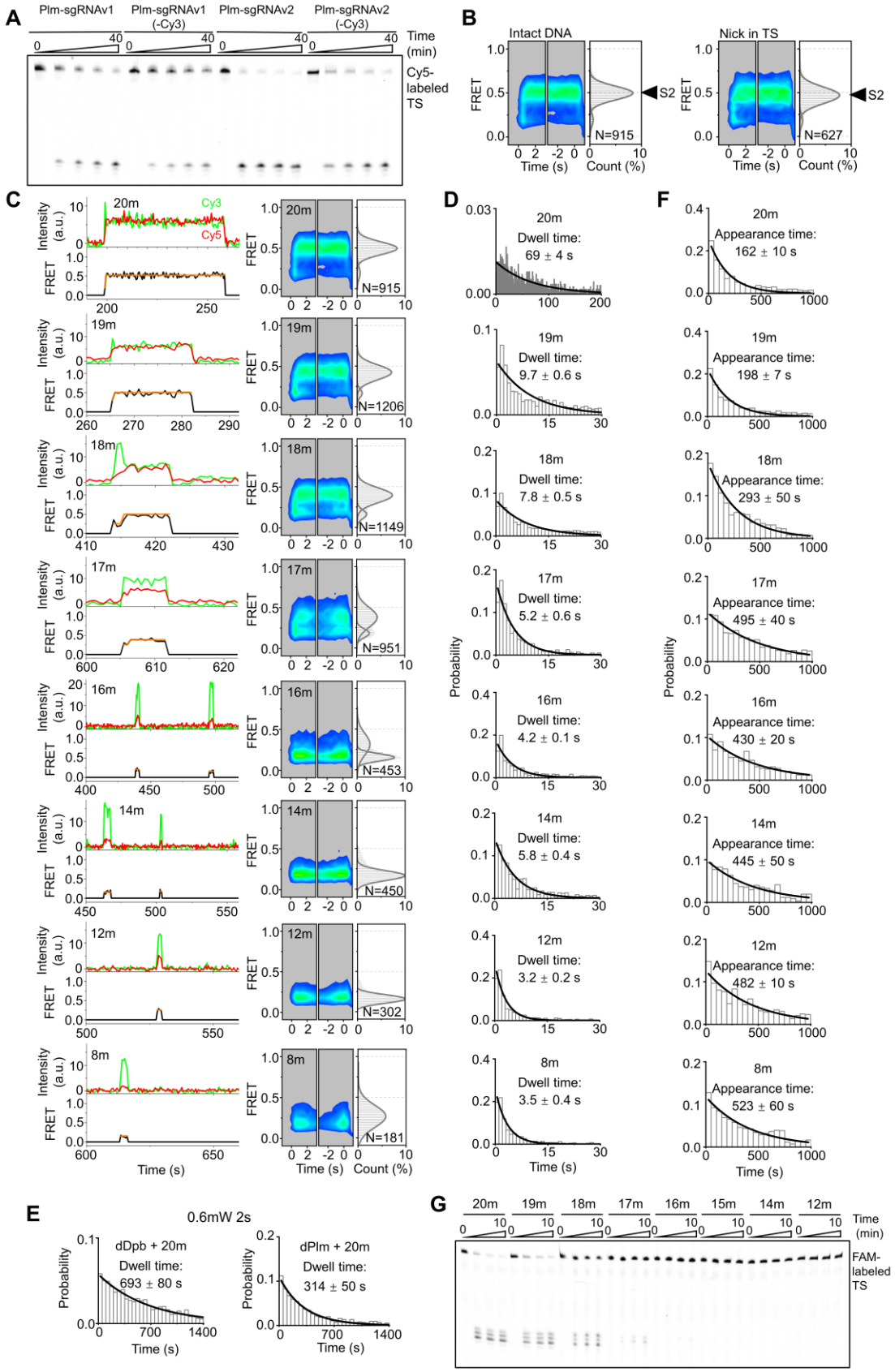

Figure S6. The conformational dynamics of dPlmCasX on DNA and the cleavage activity of PlmCasX

(A) Cleavage activity of PlmCasX on cognate DNA using unlabeled or Cy3-labeled sgRNAs. Time

points were 0, 5, 10, 20, and 40 min. Three repeats show consistent results. **(B)** Time-dependent FRET probability density plots of dPlmCasX-sgRNAv2 on cognate DNA and DNA with a nick in TS. **(C)** Representative single-molecule FRET trajectories and time-dependent FRET probability density plots of dPlmCasX-sgRNAv2 on DNAs. **(D)** Distribution of dwell time of dPlmCasX on DNAs. The photobleaching rate measured using dDpbCasX on 20m-DNA, as presented in Figure S3B, was used to calculate the corrected dwell times shown in Figure 3E. **(E)** Distributions of dwell time of dDpbCasX and dPlmCasX on cognate DNA at an exposure time of 2 s are presented. To further reduce the contribution of photobleaching in quantifying the dwell time of stable dPlmCasX-sgRNAv2 binding on cognate DNA, we reduced the laser power to 0.6 mW and increased the exposure time to 2 s per frame. Dwell time of dDpbCasX on 20m-DNA was used to calculate photobleaching rate under such experimental condtion. Corrected dwell time of dPlmCasX on 20-DNA was shown in Figure 3E. **(F)** Distributions of appearance time of dPlmCasX on DNAs, related to Figure 3F. **(G)** Gel images of the cleavage of PlmCasX on mismatched DNA, related to Figure 3G. Time points were 0, 2, 5, and 10 min. Three repeats were performed. Mean and SEM are shown where appropriate.

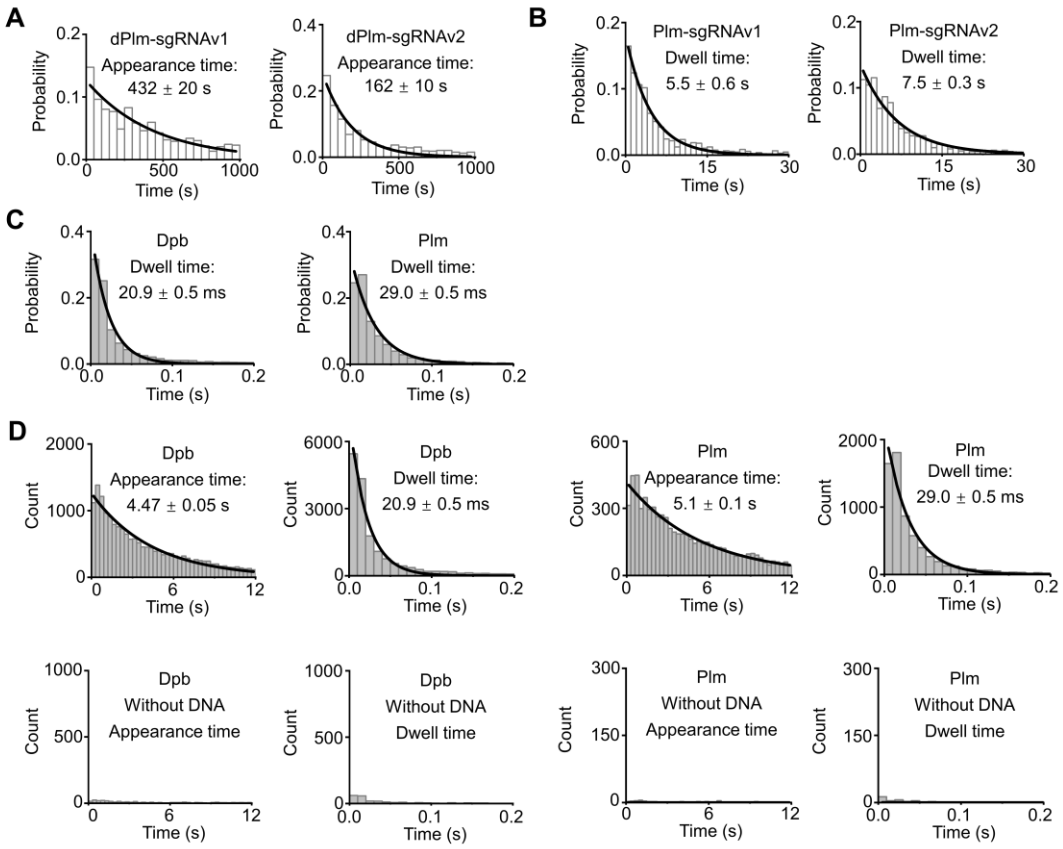

Figure S7. Distributions of appearance time and dwell time of CasX on cognate or nonspecific DNAs.

(A) Distributions of appearance time of dPlmCasX-sgRNAv2 and dPlmCasX-sgRNAv1 on cognate DNA, related to Figure 4C. **(B)** Distributions of dwell time of PlmCasX-sgRNAv2 and PlmCasX-sgRNAv1 on cognate DNA. **(C)** Distributions of dwell time of DpbCasX-sgRNAv2 and PlmCasX-sgRNAv2 on nonspecific DNA with an exposure time of 2 ms/frame, related to Table S3. **(D)** The original distributions of appearance time and dwell time. DpbCasX-sgRNAv2 and PlmCasX-sgRNAv2 were introduced into the channel with nonspecific DNA or without DNA at the exposure time of 2 ms/frame. Almost no nonspecific transient binding events were observed in the absence of DNA. Three repeats were performed. Mean and SEM are shown where appropriate.

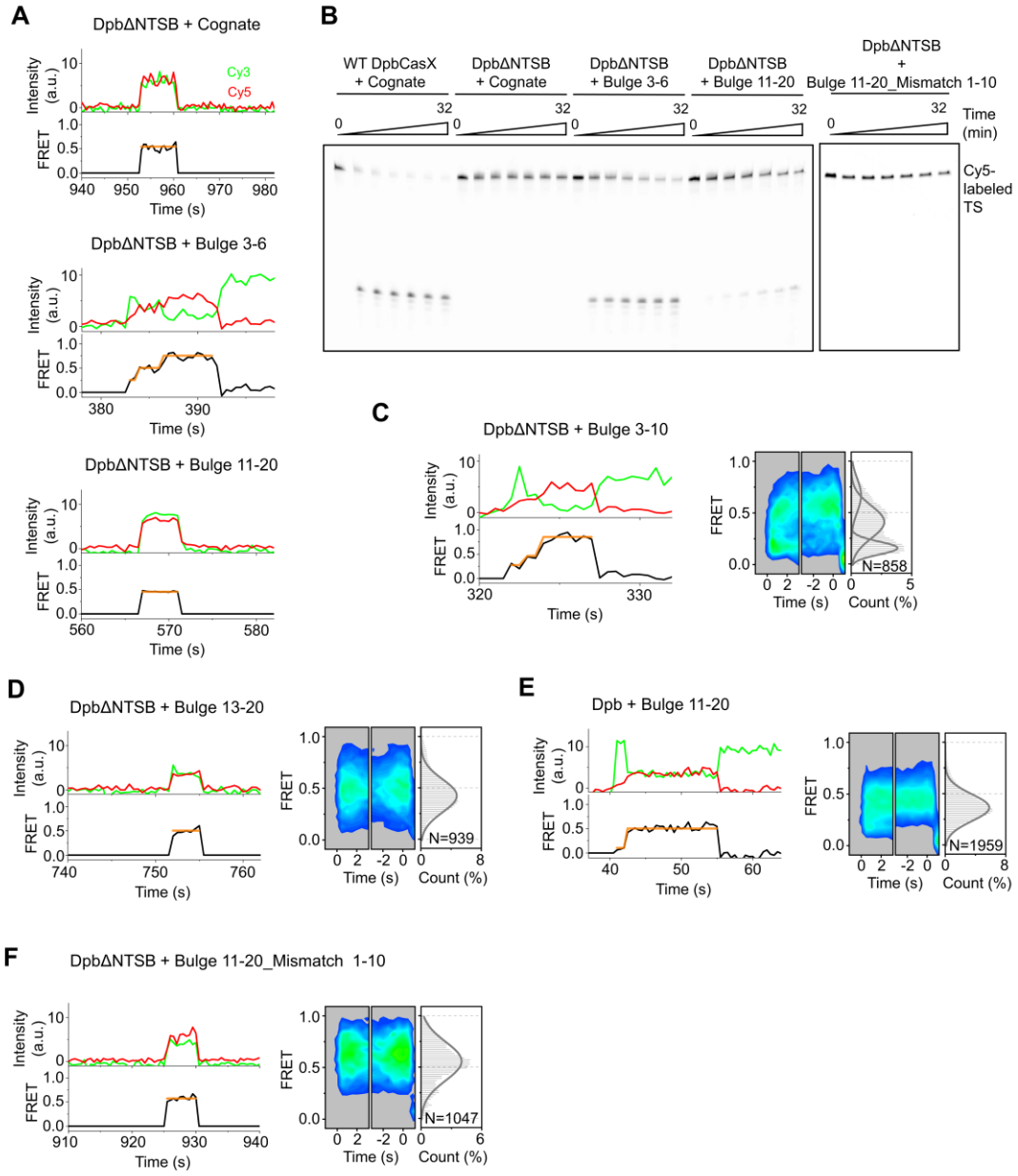

Figure S8. Cleavage activity and conformational dynamics of DpbCasXΔNTSB on cognate and bulged DNA.

(A) Representative single-molecule FRET trajectories of DpbCasXΔNTSB-sgRNAv2 on cognate and bulged DNAs. **(B)** Gel images of DpbCasXΔNTSB cleavage on cognate DNA, bulged DNA, and bulged DNA with mismatches, compared to DpbCasX on cognate DNA, related to Figure 5B. Time points were 0, 1, 2, 4, 8, 16, and 32 min. **(C)-(F)** Representative single-molecule FRET trajectory and time-dependent FRET probability density plot of DpbCasXΔNTSB-sgRNAv2 on Bulge 3-10 DNA **(C)**, DpbCasXΔNTSB-sgRNAv2 on Bulge 13-20 DNA **(D)**, DpbCasX-sgRNAv2 on Bulge 11-20 DNA **(E)**, DpbCasXΔNTSB-sgRNAv2 on Bulge 11-20_Mismatch 1-10 DNA **(F)**.

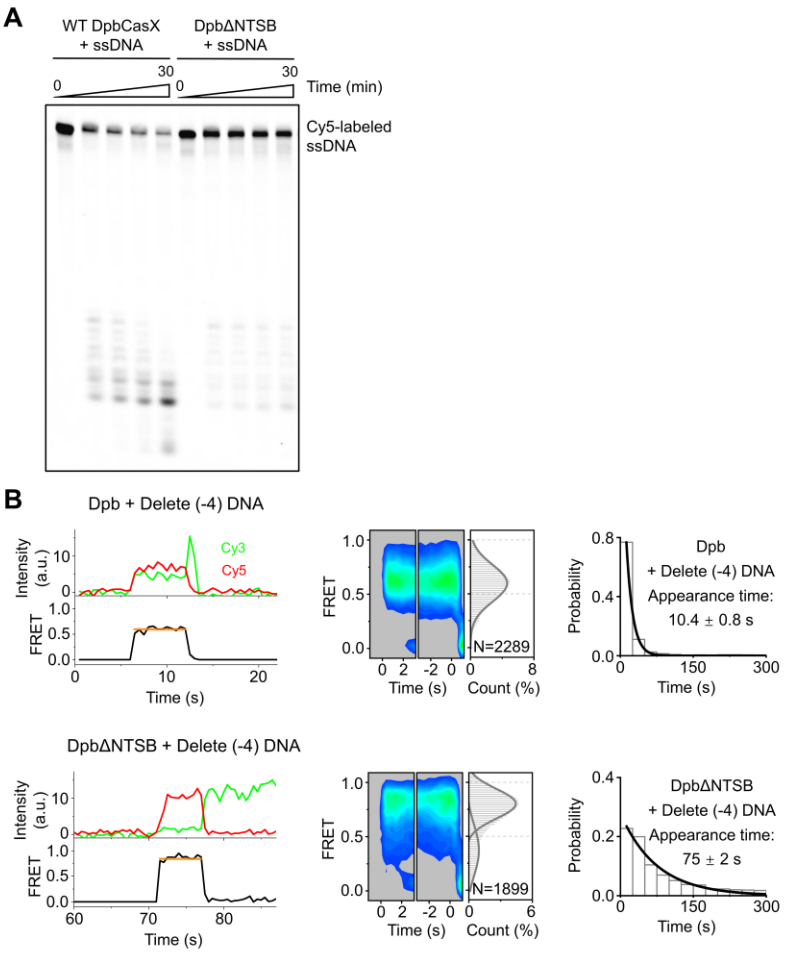

Figure S9. Cleavage activity and conformational dynamics of DpbCaX and DpbCasXΔNTSB on ssDNA.

(A) Gel images showing the cleavage of DpbCasX and DpbCasXΔNTSB on ssDNA, related to Figure 5F. Time points were 0, 2, 5, 10, and 30 min. **(B)** Representative single-molecule FRET trajectories, time-dependent FRET probability density plots and distributions of appearance time of DpbCasX-sgRNAv2 and DpbCasXΔNTSB-sgRNAv2 on ssDNA. Three repeats were performed. Mean and SEM are shown where appropriate.

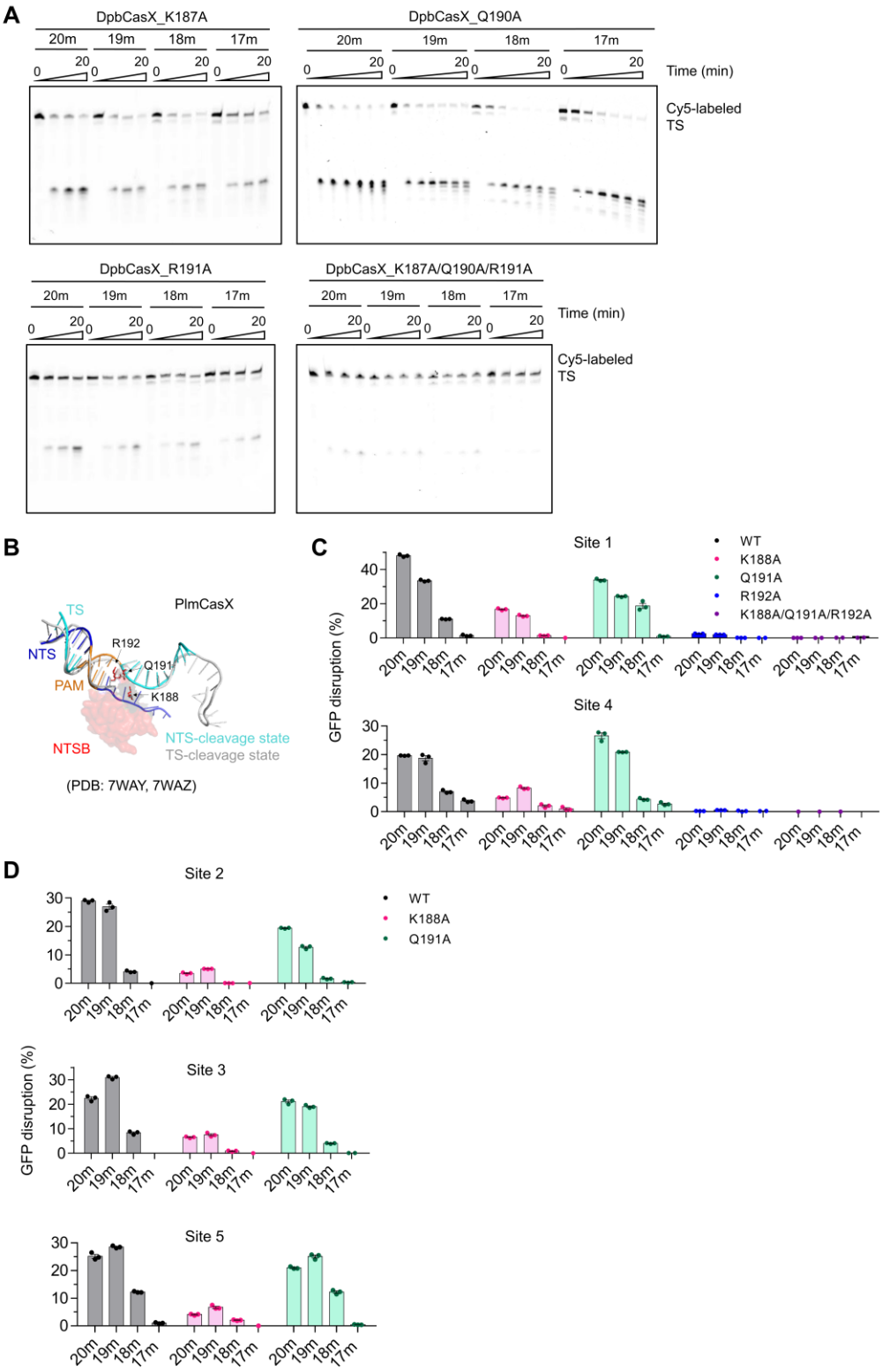

Figure S10. DNA cleavage and genome editing of engineered CasX.

(A) Gel images showing the cleavage of DpbCasX-K187A, DpbCasX-Q190A, DpbCasX-R191A, DpbCasX-K187A/Q190A/R191A on 20m-, 19m-, 18m- and 17m-DNA, related to Figure 5H. Time points were 0, 0.5, 1, 2, 5, 10, and 20 min for DpbCasX_Q190A, and 0, 5, 10, and 20 min for the remaining mutants. **(B)** Three residues (K188, Q191 and R192 of PlmCasX) within the NTSB domain closely interact with TS in both the NTS-cleavage state and the TS-cleavage state of

PlmCasX. **(C)** GFP disruption percentage using WT PlmCasX and PlmCasX mutants (K188A, Q191A, R192A, K188A/Q191A/R192A) targeting site 1 and site 4 within GFP. **(D)** GFP disruption percentage using WT PlmCasX and PlmCasX mutants (K188A, Q191A) targeting site 2, site 3, and site 5 within GFP. Three repeats were performed. Mean and SEM are shown where appropriate.

Table S1. RNA sequences used for smFRET assay and cleavage assay

smFRET assay (Cy3 is labeled at the 3' end of RNAs)	
Description	Sequence (5' to 3')
sgRNAv2	GGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUG UCGUAUGGGUAAAGCGCUUAUUUAUCGGAGAAACCGAUAAAUAAG AAGCAUCAAAG AGG GCG ACA CCC UGG UGA AC(-Cy3)
sgRNAv1	GGCGCGUUUAUUCCAUUACUUUGGAGCCAGUCCCAGCGACUAUGU CGUAUGGACGAAGCGCUUAUUUAUCGGAGAGAAACCGAUAAGUAA AACGCAUCAAAG AGG GCG ACA CCC UGG UGA AC(-Cy3)
Cleavage assay	
Description	Sequence (5' to 3')
sgRNAv2-20m	GGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUG UCGUAUGGGUAAAGCGCUUAUUUAUCGGAGAAACCGAUAAAUAAG AAGCAUCAAAG AGG GCG ACA CCC UGG UGA AC
sgRNAv2-19m	GGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUG UCGUAUGGGUAAAGCGCUUAUUUAUCGGAGAAACCGAUAAAUAAG AAGCAUCAAAG AGG GCG ACA CCC UGG UGA AU
sgRNAv2-18m	GGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUG UCGUAUGGGUAAAGCGCUUAUUUAUCGGAGAAACCGAUAAAUAAG AAGCAUCAAAG AGG GCG ACA CCC UGG UGA GU
sgRNAv2-17m	GGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUG UCGUAUGGGUAAAGCGCUUAUUUAUCGGAGAAACCGAUAAAUAAG AAGCAUCAAAG AGG GCG ACA CCC UGG UGG GU
sgRNAv2-16m	GGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUG UCGUAUGGGUAAAGCGCUUAUUUAUCGGAGAAACCGAUAAAUAAG AAGCAUCAAAG AGG GCG ACA CCC UGG UAG GU
sgRNAv2-15m	GGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUG UCGUAUGGGUAAAGCGCUUAUUUAUCGGAGAAACCGAUAAAUAAG AAGCAUCAAAG AGG GCG ACA CCC UGG CAG GU
sgRNAv2-14m	GGCGCUUUUAUCUCAUUACUUUGAGAGCCAUCACCAGCGACUAUG UCGUAUGGGUAAAGCGCUUAUUUAUCGGAGAAACCGAUAAAUAAG AAGCAUCAAAG AGG GCG ACA CCC UGA CAG GU

Table S2. DNA sequences used for smFRET assay and cleavage assay

smFRET assay and cleavage assay	
Description	Sequence (5’ to 3’)
Biotin handle	CCCTGGTCCGGTGGTCCGCCTGCTGGTCCC-biotin
Cognate DNA (20m)-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
Cognate DNA (20m)-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TGG TGA AC CGT AGA TAG CCG GAC TCG GTC
20m-TS-(28)	GAC CGA GTC TGT CT(-Cy5)A TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
20m-NTS-(28)	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TGG TGA AC CGT AGA TAG ACA GAC TCG GTC
20m-TS-(30)	GAC CGA GTC TGT(-Cy5) CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
20m-TS-(32)	GAC CGA GTC T(-Cy5)GT CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
20m-TS-(36)	GC GCC GCT CTC TGT(-Cy5) GTC CGG CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
20m-NTS-(36)	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TGG TGA AC CGT AGA TAG CCG GAC ACA GAG AGC GGC GC
20m-TS-(38)	GC GCC GCT CTC T(-Cy5)GT GTC CGG CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
20m-TS-(40)	GC GCC GCT CT(-Cy5)C TGT GTC CGG CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
19m-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG CT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
19m-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TGG TGA AG CGT AGA TAG CCG GAC TCG GTC
18m-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG CA TCA CCA GGG TGT CGC CCT C GAA ATC CCG
18m-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TGG TGA TG CGT AGA TAG CCG GAC TCG GTC
17m-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG CA ACA CCA GGG TGT CGC CCT C GAA ATC CCG
17m-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TGG TGT TG CGT AGA TAG CCG GAC TCG GTC
16m-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG CA AGA CCA GGG TGT CGC CCT C GAA ATC CCG
16m-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TGG TCT TG CGT AGA TAG CCG GAC TCG GTC
14m-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG CA AGT GCA GGG TGT CGC CCT C GAA ATC CCG

14m-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TGC ACT TG CGT AGA TAG CCG GAC TCG GTC
12m-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG CA AGT GGT GGG TGT CGC CCT C GAA ATC CCG
12m-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC ACC ACT TG CGT AGA TAG CCG GAC TCG GTC
8m-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG CA AGT GGT CCC AGT CGC CCT C GAA ATC CCG
8m-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACT GGG ACC ACT TG CGT AGA TAG CCG GAC TCG GTC
4m-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG CA AGT GGT CCC ACA GCC CCT C GAA ATC CCG
4m-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GGC TGT GGG ACC ACT TG CGT AGA TAG CCG GAC TCG GTC
16m-Nick in NTS_NTS (A)	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC
16m-Nick in NTS_NTS (B)	TGG TCT TG CGT AGA TAG CCG GAC TCG GTC
16m-Nick in TS_TS (A)	GAC CGA GT(-Cy5)C CGG CTA TCT A
16m-Nick in TS_TS (B)	CG CA AGA CCA GGG TGT CGC CCT C GAA ATC CCG
Nick in NTS (12,13)-NTS (A)	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC
Nick in NTS (12,13)-NTS (B)	TGG TGA AC CGT AGA TAG CCG GAC TCG GTC
Nick in NTS (14,15)-NTS (A)	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TG
Nick in NTS (14,15)-NTS (B)	G TGA AC CGT AGA TAG CCG GAC TCG GTC
Nick in TS-TS (A)	GAC CGA GT(-Cy5)C CGG CTA TCT A
Nick in TS-TS (B)	CG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
Nonspecific DNA-TS	TCT ACG GT TCA CCA GGG TGT CGC CCT G ACC ATC CCG
Nonspecific DNA-NTS	CGG ACC ACC GGA CCA GGG CGG GAT GGT C AGG GCG ACA CCC TGG TGA AC CGT AGA

Bulge 3-6-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
Bulge 3-6-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGT TAT ACA CCC TGG TGA AC CGT AGA TAG CCG GAC TCG GTC
Bulge 3-10-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
Bulge 3-10-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGT TAT CAC ACC TGG TGA AC CGT AGA TAG CCG GAC TCG GTC
Bulge 11-20-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
Bulge 11-20-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CAA GTT GTC CA CGT AGA TAG CCG GAC TCG GTC
Bulge 13-20-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
Bulge 13-20-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC GTT GTC CA CGT AGA TAG CCG GAC TCG GTC
Delete (-4)-TS	GAC CGA GT(-Cy5)C CGG CTA TCT ACG GT TCA CCA GGG TGT CGC CCT C GAA ATC CCG
Delete (-4)-NTS	CGG ACC ACC GGA CCA GGG CGG GAT TTC G AGG GCG ACA CCC TG
Cleavage assay	
Description	Sequence (5’ to 3’)
Cleavage sites DNA-TS	(FAM-)GCCGCTGCGCCGAAATATTCATTGTCGTTCACCAGGGTGTC GCCCTTGAAATCCCGCGG
Cleavage sites DNA-NTS	(Cy5-)CCGCGGGATTTCAAGGGCGACACCCTGGTGAACGACAATGA ATATTTCGGCGCAGCGGC

The dsDNAs used in cleavage assays were annealed with the corresponding TS and NTS, while the ssDNA used in cleavage assays was the TS alone. For the smFRET assays, dsDNAs were annealed with the corresponding TS, NTS, and a biotin handle.

In the mismatch DNA cleavage assays of DpbCasX and PlmCasX, the same DNA target (Cleavage sites DNAs) was used, but the sequence of the sgRNA was altered to produce mutations from 20m to 14m.

Table S3. The nonspecific binding rate, effective target search rate and nonspecific dissociation rate of different Cas proteins.

	Nonspecific binding rate ($\mu M^{-1}s^{-1}$)	Effective target search rate ($\mu M^{-1}s^{-1}$)	Nonspecific dissociation rate (s^{-1})
DpbCasX	22.4 ± 0.3	2.6 ± 0.3	48 ± 1
PlmCasX	19.6 ± 0.4	2.1 ± 0.2	34 ± 1
SpCas9	~ 2.5(1)	2(1), 4(2)	~ 89(1)
AsCas12a	~ 85(3)	~ 40(3)	~ 238(3)
LbCas12a	~ 98(3)	~ 50(3)	~ 192(3)
FnCas12a	~ 50(3)	~ 5.6(3)	~ 769(3)

Table S4. Fully-matched and partially-matched sgRNA spacer sequences used in cellular assays

GFP site	crRNA spacer sequences (from 5' to 3')
1	AGGGCGACACCCTGGTGAAC (20m)
	AGGGCGACACCCTGGTGAAT (19m)
	AGGGCGACACCCTGGTGAGT (18m)
	AGGGCGACACCCTGGTGGGT (17m)
2	GCCGCTACCCCGACCACATG (20m)
	GCCGCTACCCCGACCACATA (19m)
	GCCGCTACCCCGACCACACA (18m)
	GCCGCTACCCCGACCACGCA (17m)
3	AGGAGGACGGCAACATCCTG (20m)
	AGGAGGACGGCAACATCCTA (19m)
	AGGAGGACGGCAACATCCCA (18m)
	AGGAGGACGGCAACATCTCA (17m)
4	GGGTCAGCTTGCCGTAGGTG (20m)
	GGGTCAGCTTGCCGTAGGTA (19m)
	GGGTCAGCTTGCCGTAGGCA (18m)
	GGGTCAGCTTGCCGTAGACA (17m)
5	TCTGCACCACCGGCAAGCTG (20m)
	TCTGCACCACCGGCAAGCTA (19m)
	TCTGCACCACCGGCAAGCCA (18m)
	TCTGCACCACCGGCAAGTCA (17m)

Table S5. GFP disruption percentage using PlmCasX-WT or PlmCasX mutants targeting site 1 to site 5 located within GFP.

site 1_GFP disruption percentage (%)					
	WT	188A	191A	192A	188/191/192/A
NT	0.5 ± 0	0.8 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.1
20m	48.5 ± 0.4	17.5 ± 0.2	34.3 ± 0.3	2.8 ± 0.2	0.5 ± 0.0
19m	33.9 ± 0.3	13.7 ± 0.3	24.7 ± 0.2	2.4 ± 0.0	0.4 ± 0.0
18m	11.5 ± 0.1	2.2 ± 0.2	19 ± 1	0.5 ± 0.0	0.5 ± 0.1
17m	1.9 ± 0.2	0.6 ± 0.1	1.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
site 4_GFP disruption percentage (%)					
	WT	188A	191A	192A	188/191/192/A
NT	0.7 ± 0.0	1.0 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.1
20m	20.4 ± 0.0	5.8 ± 0.0	27.2 ± 0.9	0.6 ± 0.0	0.6 ± 0.0
19m	19.5 ± 0.9	9.3 ± 0.3	21.4 ± 0.1	0.9 ± 0.0	0.9 ± 0.0
18m	7.7 ± 0.3	3.0 ± 0.3	4.9 ± 0.3	0.6 ± 0.1	0.6 ± 0.1
17m	4.5 ± 0.3	2.0 ± 0.3	3.3 ± 0.3	0.5 ± 0.1	0.5 ± 0.1

site 2_GFP disruption percentage (%)			
	WT	188A	191A
NT	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
20m	29.2 ± 0.3	3.8 ± 0.2	19.5 ± 0.1
19m	27.1 ± 0.8	5.3 ± 0.1	12.8 ± 0.4
18m	4.3 ± 0.1	0.3 ± 0.0	1.7 ± 0.1
17m	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.1
site 3_GFP disruption percentage (%)			
	WT	188A	191A
NT	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
20m	22.6 ± 0.6	6.8 ± 0.2	21.3 ± 0.6
19m	31.1 ± 0.4	7.8 ± 0.4	19.1 ± 0.4
18m	8.6 ± 0.4	1.1 ± 0.2	4.1 ± 0.1
17m	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
site 5_GFP disruption percentage (%)			
	WT	188A	191A
NT	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
20m	25.3 ± 0.7	4.3 ± 0.1	21.1 ± 0.2
19m	28.7 ± 0.2	7.0 ± 0.3	25.2 ± 0.5
18m	12.4 ± 0.1	2.3 ± 0.1	12.3 ± 0.4
17m	1.2 ± 0.1	0.1 ± 0.0	0.5 ± 0.0

PlmCasX WT and PlmCasX mutants (188A and 191A) targeting site 1 to site 5; PlmCasX mutants (192A and 188/191/192/A) targeting site 1 and site 4.

Supplementary References

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