Supplementary Material

Mechanics of CRISPR-Cas12a and engineered variants on λ-DNA

Bijoya Paul[1], Loïc Chaubet[2], Dideke Emma Verver[2] & Guillermo Montoya[1]*

[1]Structural Molecular Biology Group, Novo Nordisk Foundation Centre for Protein Research, Faculty of Health and Medical Sciences University of Copenhagen, Blegdamsvej 3-B, Copenhagen, 2200, Denmark.

[2]LUMICKS, Pilotenstraat 41, 1059 CH, Amsterdam, The Netherlands.

*To whom correspondence should be addressed Email: guillermo.montoya@cpr.ku.dk

Supplementary Figure Legends

Supplementary Fig. 1.- Cleavage assays on different λ-DNA target sites. Five different sites were selected on the λ-DNA and the cleavage activity of the endonuclease was tested with the corresponding crRNAs. The site targeted by crRNA1 on λ-DNA displayed the best activity and it was selected for further analysis (Supplementary Table 1).

Supplementary Fig. 2.- Controls for single molecule experiments, mapping of binding profiles to A-T density along λ-DNA and mapping of binding profile to base pairs along λ-DNA. a) 3 kymographs of a single DNA tether which was exposed to 2nM crRNA-1-Cy5/Cas12a (top), then 2nM crRNA-1-Cy5 crRNA only (middle) and finally back into 2nM crRNA-1-Cy5/Cas12a (bottom), all at 50 pN. The binding observed in the top and bottom kymographs is specific to Cas12, as the labelled crRNA alone does not yield any binding. **b)** Representative kymograph illustrating the bead center, which is marked by a dot in the blue channel. This reference was used for localization. **c)** An intensity profile was generated along the entire length of the scanned λ-DNA.

Supplementary Fig. 3.- Sequence analysis for predicted binding locations. **a)** Binding profile of Cas12a showing sequence matches without any PAM considerations on NT-strand (5'→ 3', green lines) and on T-strand (5'→ 3', grey lines). **b)** Binding profile of Cas12a showing sequence matches TTN-PAM on NT-strand (5'→ 3', green lines) and on T-strand (5'→ 3', grey lines). **c)** Scheme showing example search for a sequence match along λ-DNA.

Supplementary Fig. 4.- Bulk activity of Cas12a variants with crRNA-1 and binding of Cas12a variants to λ-DNA. a) Left panel displays 15% urea gel showing enzymatic activity of Cas12a variants with crRNA-6 (See Supplementary Table 1) with the annealed dsDNA formed by TS-6-long and NTS-6-long (See Supplementary table 1 and Materials and Methods). Right panel displays 15% urea gel showing indiscriminate ssDNA activity of Cas12a variants using crRNA-1 and activated by λ-activator (See Supplementary Table 1), with Unspecific ssDNA (See Supplementary Table 1) as the substrate. **b)** The top panel shows representative 120s kymographs of Cas12a variants with increasing forces (n=5). The bottom panel shows the binding profiles of Cas12a variants at increasing forces binned at 120s (n=5). **c)** Binding profiles of wtCas12a and its variants binned at 120s plotted alongside A-T density profile of the λ-DNA. **d)** Top panel in green shows Cas12a_M3 binding to λ-DNA with gradual ramp up in force from 0 to 60 pN. The λ-DNA was co-stained with Sytox orange as a dsDNA marker, and the middle panel in red shows the signal obtained from staining with Sytox orange. Bottom panel shows superimposition of signals from labelled Cas12a-crRNA-1 complex and Sytox orange. The force applied on the λ-DNA vs time is denoted below the figure, as is the distance between the beads vs time (which results in the force applied on the DNA tether).

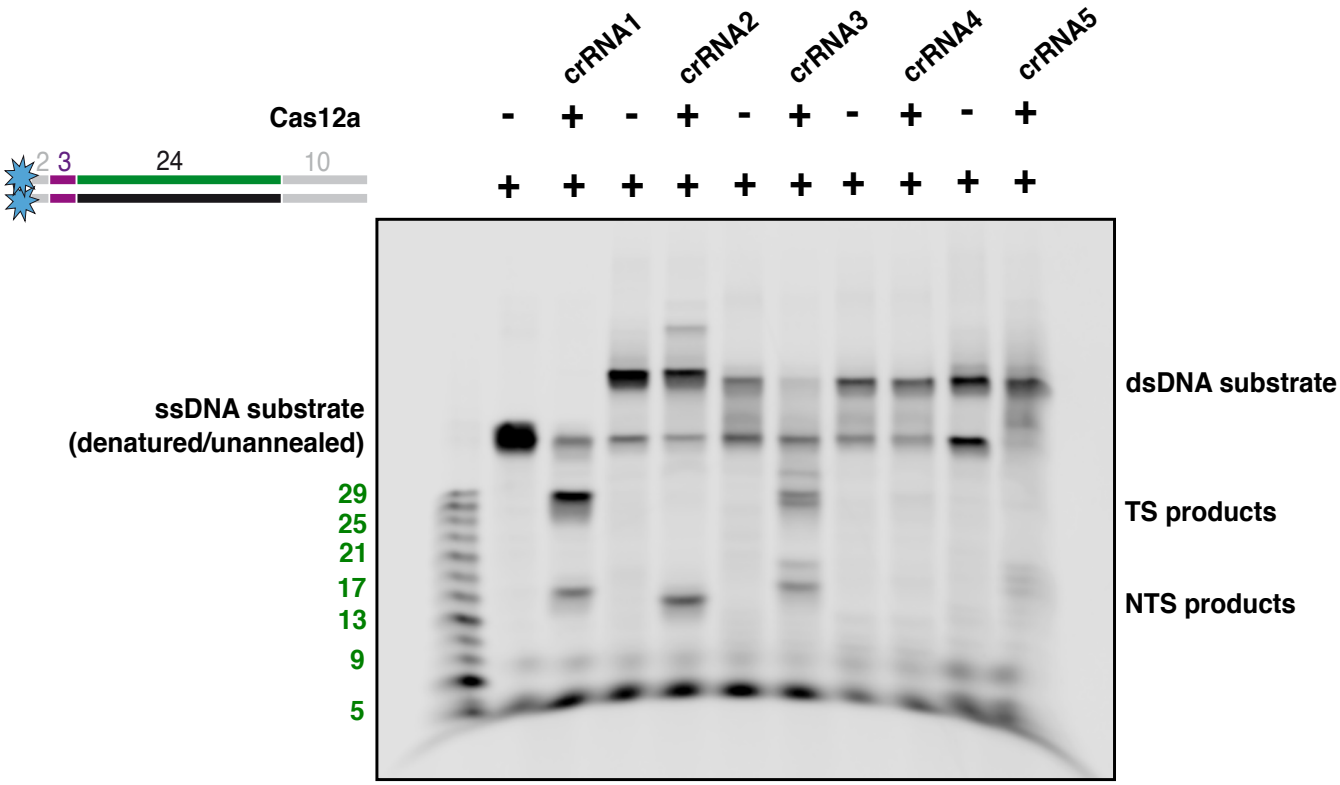
Supplementary Fig. 5.- Kinetics of Cas12a_M2 cleavage on T-strand and NT-strand. **a)** 15% urea gel showing the time-course cleavage assay of the T-strand and NT-strand (n=3). **b)** Quantification and apparent rates of product formation of the T-strand (grey) and NT-strand (green). The experiment was repeated 3 times. The error bars show the SD of each measurement.

Supplementary Table 1: DNA sequences and oligos

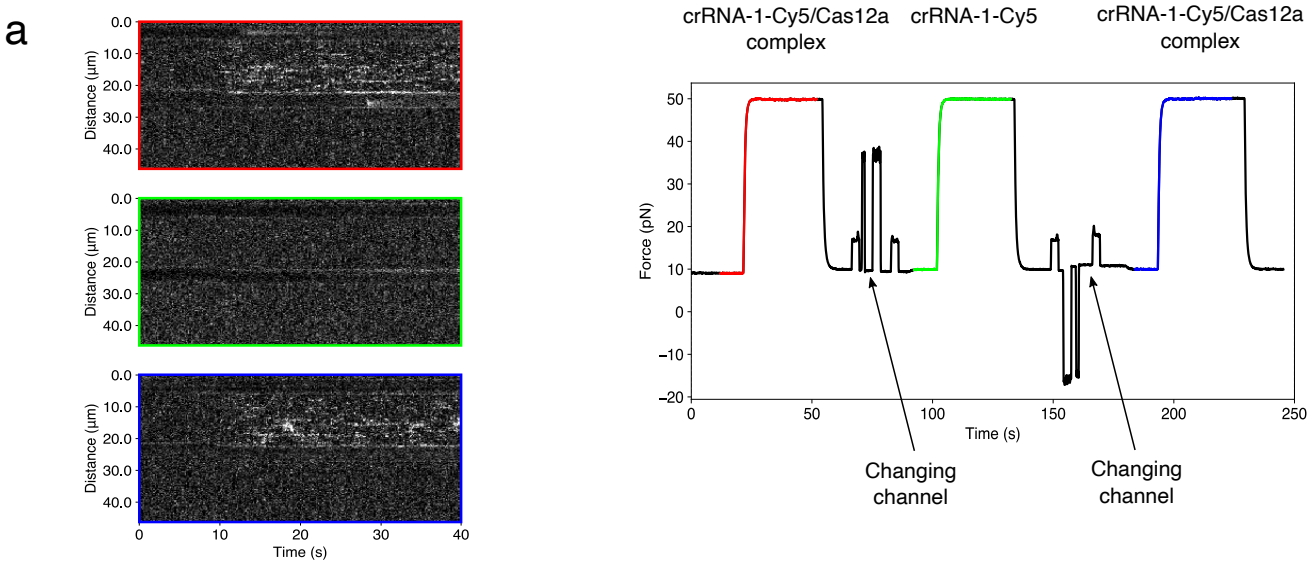
No	Name	Sequence
1	3´-Cy5-labeled crRNA-1	rArArUrUrUrCrUrArCrUrGrUrUrGrUrArGrArUrUrGrUrArArArGrArArArCrArGrUrArArGrArUrArArU/3Cy5Sp/
2	crRNA-1	rArArUrUrUrCrUrArCrUrGrUrUrGrUrArGrArUrUrGrUrArArArGrArArArCrArGrUrArArGrArUrA
3	crRNA-2	rArArUrUrUrCrUrArCrUrGrUrUrGrUrArGrArUrUrGrUrCrGrCrGrGrGrUrArUrUrGrGrCrCrUrCrArGrCrArU
4	crRNA-3	rArArUrUrUrCrUrArCrUrGrUrUrGrUrArGrArUrUrUrGrArCrCrCrGrGrCrArArArCrGrGrGrArArUrGrArArA
5	crRNA-4	rArArUrUrUrCrUrArCrUrGrUrUrGrUrArGrArUrUrCrGrUrArCrArGrUrUrUrUrCrArGrCrUrArUrCrGrUrCrA
6	crRNA-5	rArArUrUrUrCrUrArCrUrGrUrUrGrUrArGrArUrUrCrArArUrUrUrGrUrUrGrCrArArCrGrArArCrA
7	TS-1	ATCGGGTTGAGTATTATCTTACTGTTTCTTTACATAAAC/36-FAM/
8	NTS-1	/56-FAM/GTTTATGTAAAGAAACAGTAAGATAATACTCAACCCGAT
9	TS-2	CGTGTCCTCCATGCTGAGGCCAATACCCGCGACATAATG/36-FAM/
10	NTS-2	/56-FAM/CATTATGTCGCGGGTATTGGCCTCAGCATGGAGGACACG
11	TS-3	AAACATCGGCGTTTCATTCCCGTTTGCCGGGTCAATAAA/36-FAM/
12	NTS-3	/56-FAM/TTTATTGACCCGGCAAACGGGAATGAAACGCCGATGTTT
13	TS-4	CTCAGTAATGTGACGATAGCTGAAAACTGTACGATAAAC/36-FAM/
14	NTS-4	/56-FAM/GTTTATCGTACAGTTTTCAGCTATCGTCACATTACTGAG
15	TS-5	GACTGATAGTGACCTGTTCGTTGCAACAAATTGATAAGC/36-FAM/
16	NTS-5	/56-FAM/GCTTATCAATTTGTTGCAACGAACAGGTCACTATCAGTC
17	TS-1-long	CGTACTCAAACATCGGGTTGAGTATTATCTTACTGTTTCTTTACATAAAC/36-FAM
18	NTS-1-long	/56-FAM/GTTTATGTAAAGAAACAGTAAGATAATACTCAACCCGATGTTTGAGTACG
19	λ-activator	CATCGGGTTGAGTATTATCTTACTGTTTCTTTACATAAAC
20	Unspecific ssDNA	CGATGCATGACCAGTCTTTATCCCCCAGGTCCTCCAAACT/36-FAM/
21	crRNA-6	rArArUrUrUrCrUrArCrUrGrUrUrGrUrArGrArUrGrArGrArArGrUrCrArUrUrUrArArUrArArGrGrCrCrArCrU
22	TS-6	CGATGCATGCAGTGGCCTTATTAAATGACTTCTCTAACG/36-FAM/
23	NTS-6	/56-FAM/CGTTAGAGAAGTCATTTAATAAGGCCACTGCATGCATCG
24	Activator TS	CGATGCATGCAGTGGCCTTATTAAATGACTTCTCTAACG

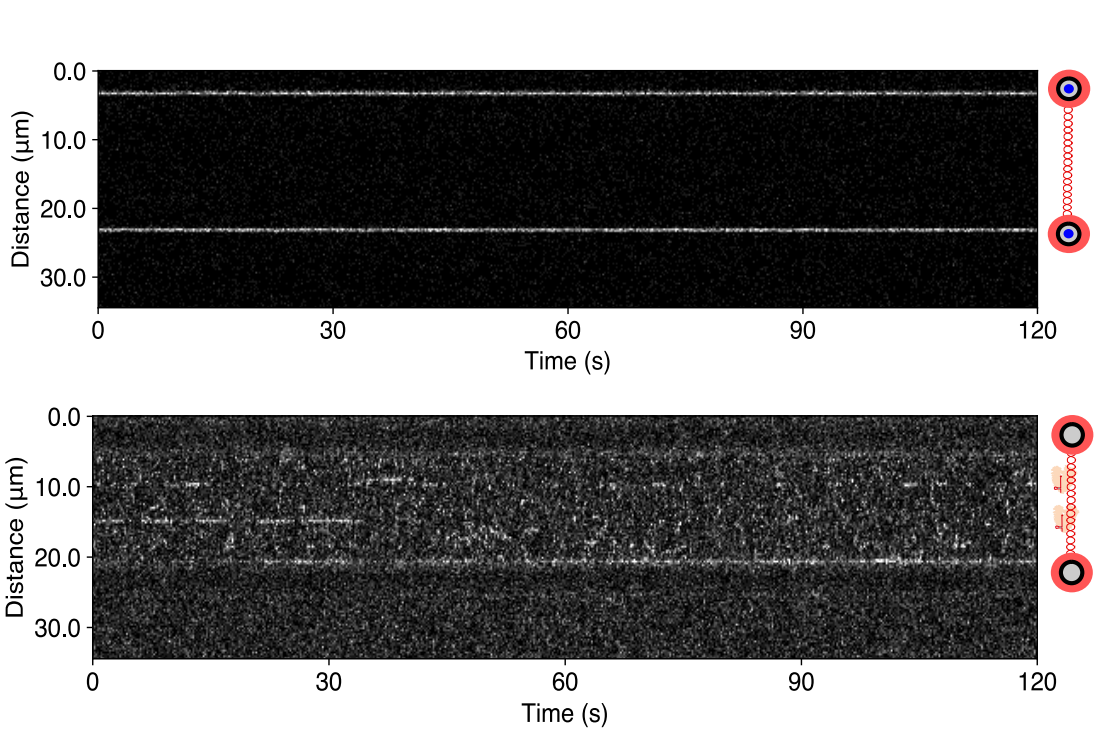
25	Off target	TCCTGCTCCGGATCGGCGTAACTGTTTCCGTTGACGAAGT TCACCGCATCCAGAAAACGGGCGTAAACCTTACGCCGGA CCACCGTTCCGCCGACCAGACTCTGCATATCTTCCGCCAT CCCGGTGACCATACCGTACAGGTTAGAAACCGTCAGCGT GGGGCGCGTACTGGTGCCTTTGCCATTCAGTTCAAAACC GCTCCCCTGAATGGGATACGGCTGATACTGTCGCCCCTG CCAGGTGACCGGCTCACCTTTTTCGTTCTGCTCATTACAG AAAAAATAACGTTCTCCACCGACCTCTGTCAGGTCGATT TCCCAGAGCACCACGCTGGCCGACTGCTCCGCACGG
26	On target	ATGATGTCTGACGCTGGCATTCGCATCAAAGGAGAGTGA GATCGGTTTTGTAAAAGATAACGCTTGTGAAAATGCTGA ATTTCGCGTCGTCTTCACAGCGATGCCAGAGTCTGTAGTG TCAGATGATGACCGTACTCAAACATCGGGTTGAGTATTA TCTTACTGTTTCTTTACATAAACATTGCTGATACCGTTTA GCTGAAACGACATACATTGCAAGGAGTTTATAAATGAGT ATCAATGAGTTAGAGTCTGAGCAAAAAGATTGGGCGTTA TCAATGTTGTGCAGATCCGGTGTCTTGTCTCCATGCAGAC ATCACGAAGGTGTTTATGTAGATGAAGGTATAGAT
27	λ-DNA	https://www.ncbi.nlm.nih.gov/nuccore/J02459.1?report=fasta

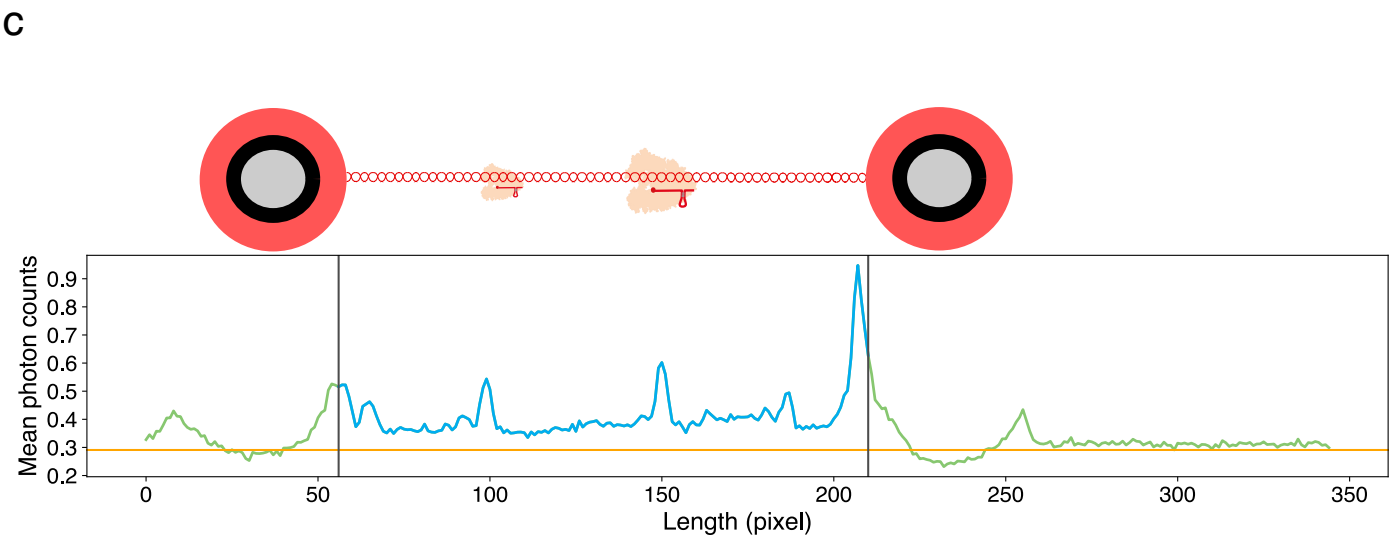
Supp Fig 1


crRNA1
crRNA2
crRNA3
crRNA4
crRNA5
Cas12a
- + - + - + - + - +
2 3 24 10
+ + + + + + + + + +
ssDNA substrate
(denatured/unannealed)
29
25
21
17
13
9
5
dsDNA substrate
TS products
NTS products

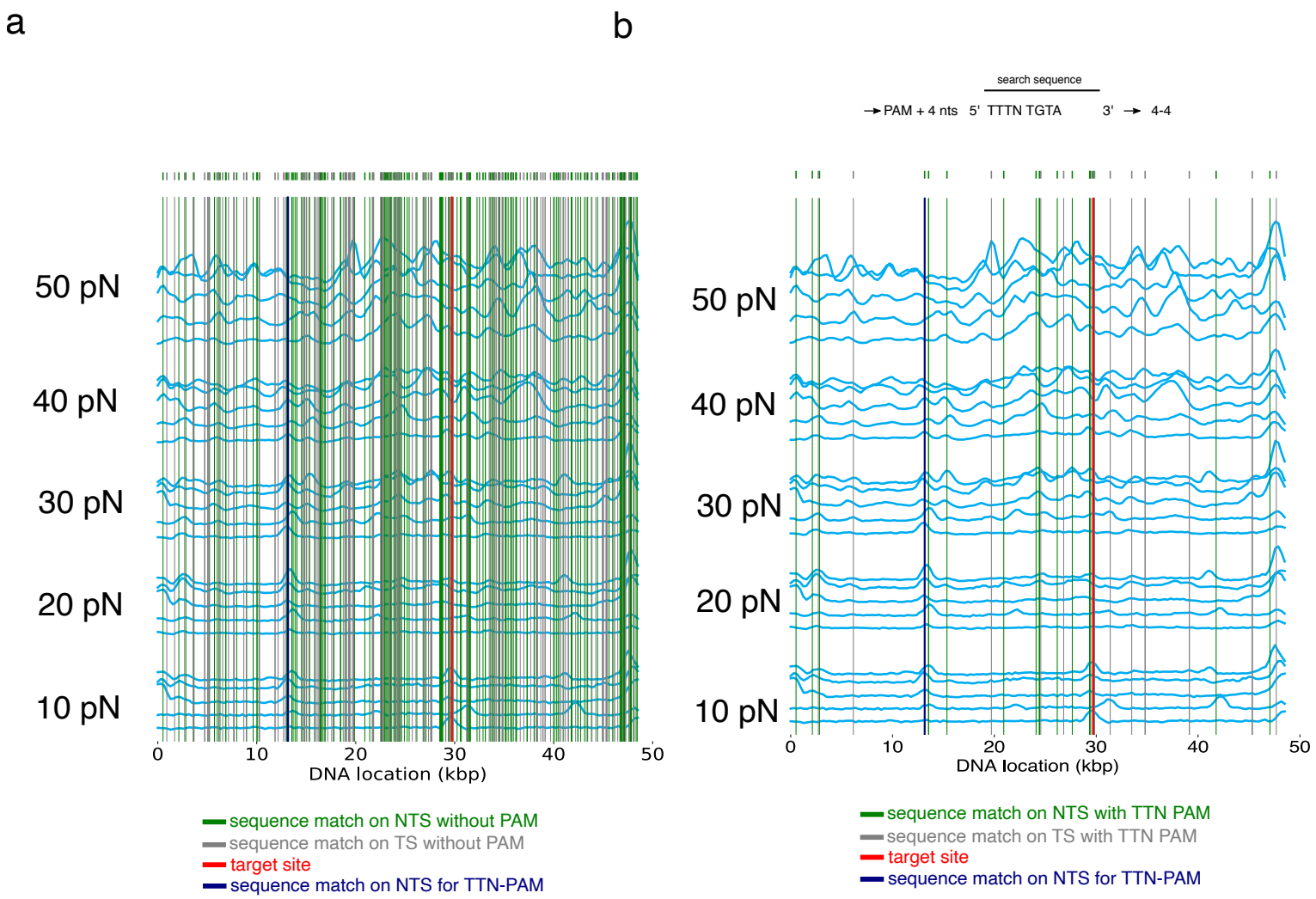
Supp Fig 2

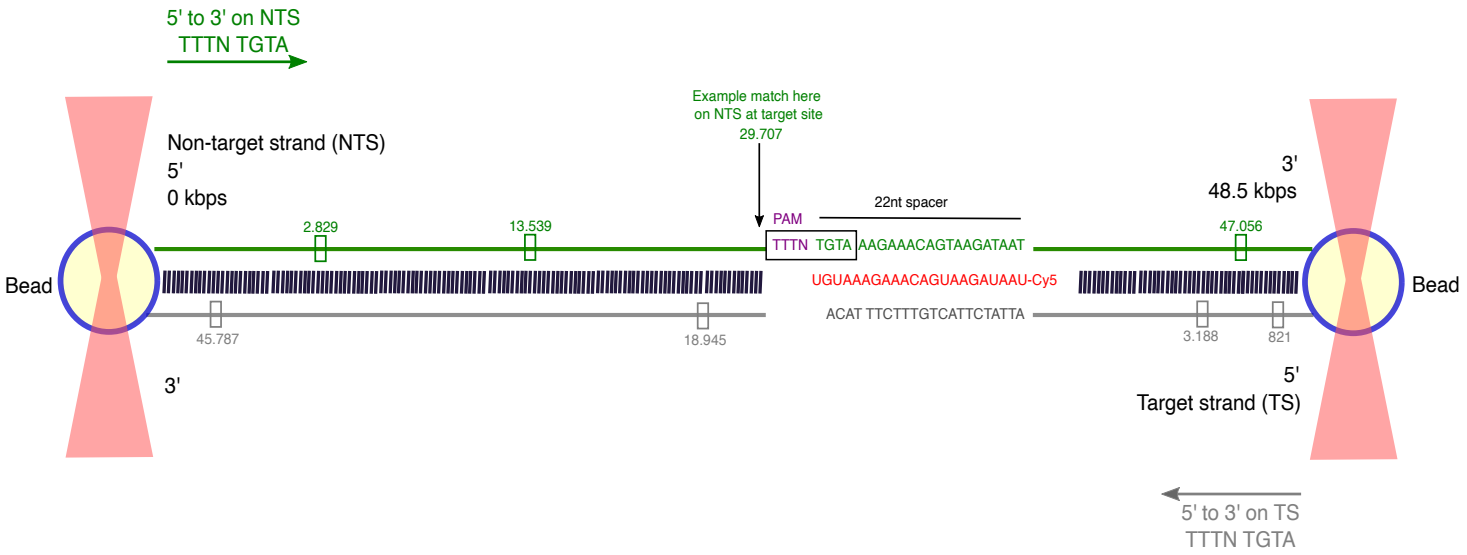

a
Distance (µm)
Time (s)
crRNA-1-Cy5/Cas12a complex
crRNA-1-Cy5
crRNA-1-Cy5/Cas12a complex
Force (pN)
Time (s)
Changing channel
Changing channel


b
Distance (µm)
Time (s)

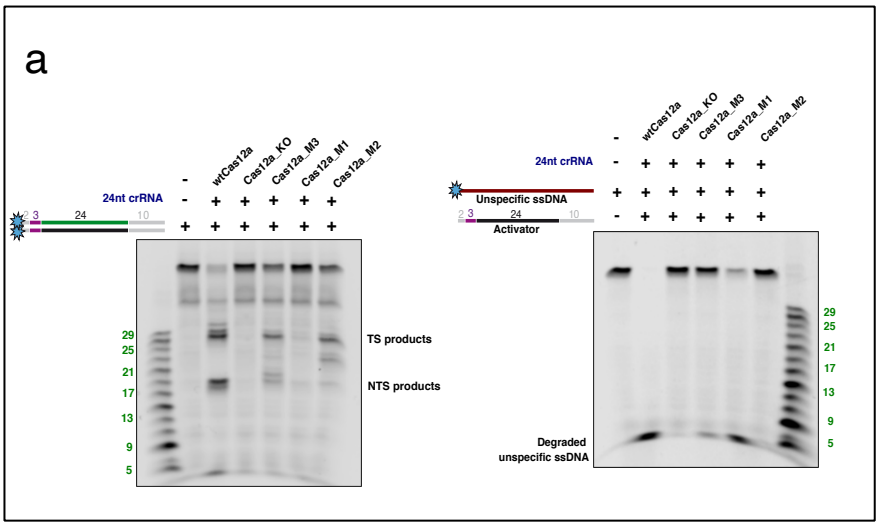

c
Mean photon counts
Length (pixel)

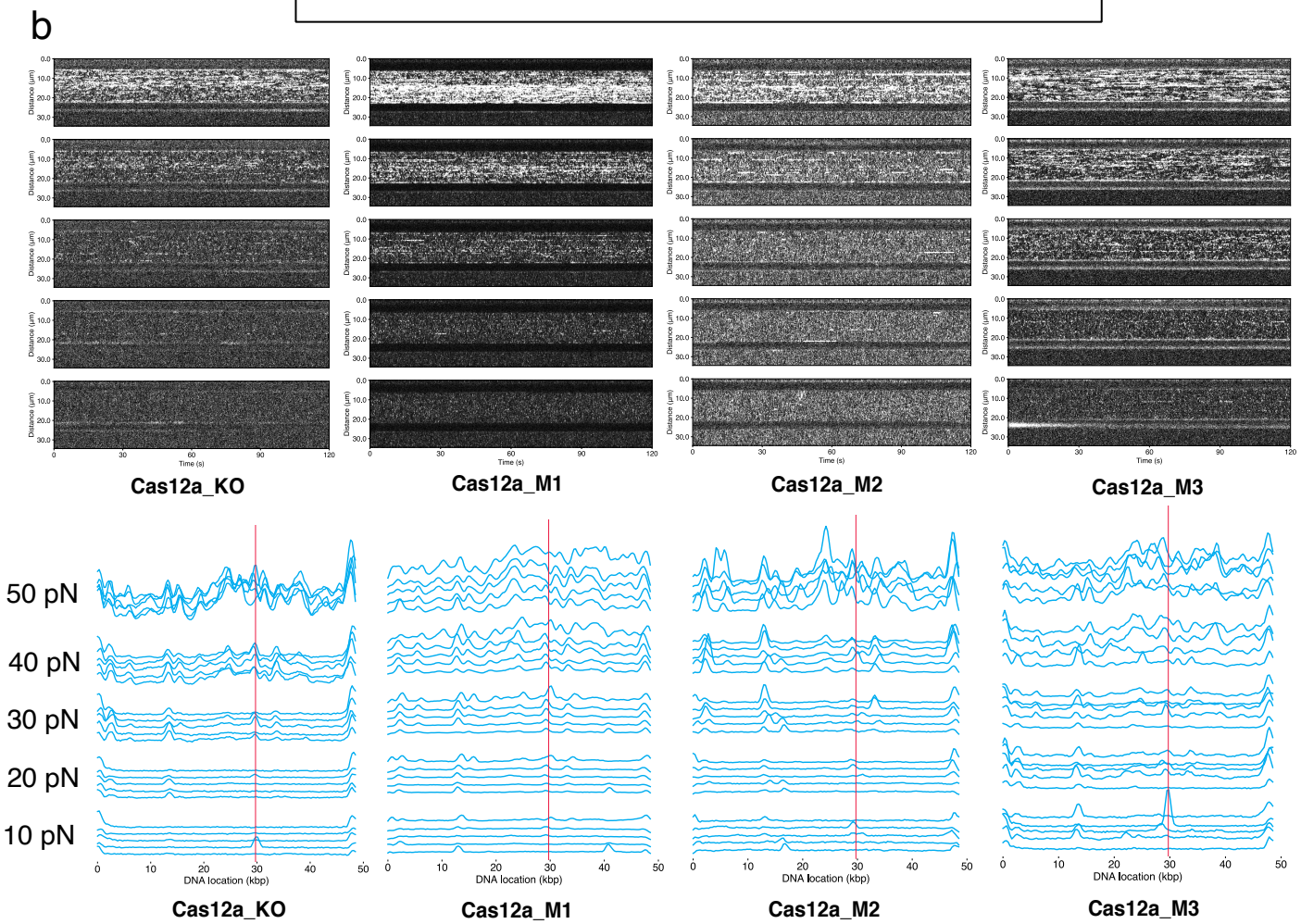
Supp Fig 3

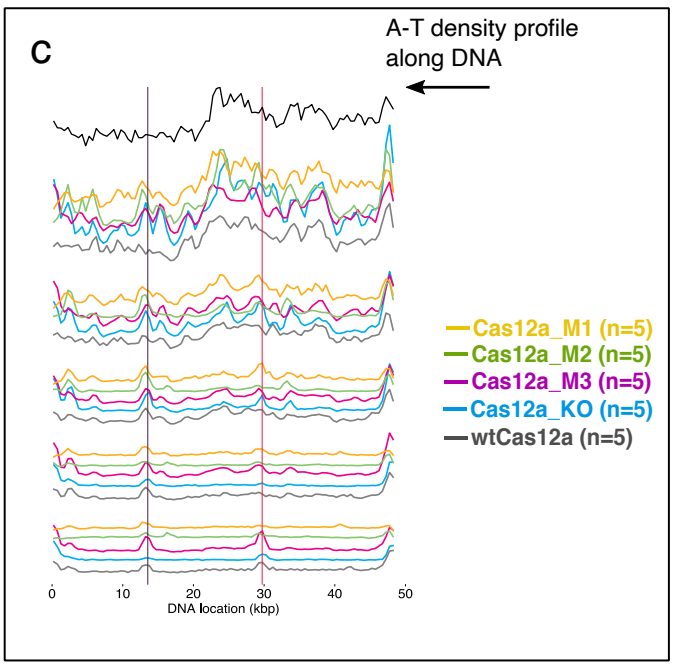

a
b
search sequence
PAM + 4 nts 5' TTTN TGTA 3' 4-4
50 pN
40 pN
30 pN
20 pN
10 pN
0
10
20
30
40
50
DNA location (kbp)
sequence match on NTS without PAM
sequence match on TS without PAM
target site
sequence match on NTS for TTN-PAM
sequence match on NTS with TTN PAM
sequence match on TS with TTN PAM
target site
sequence match on NTS for TTN-PAM

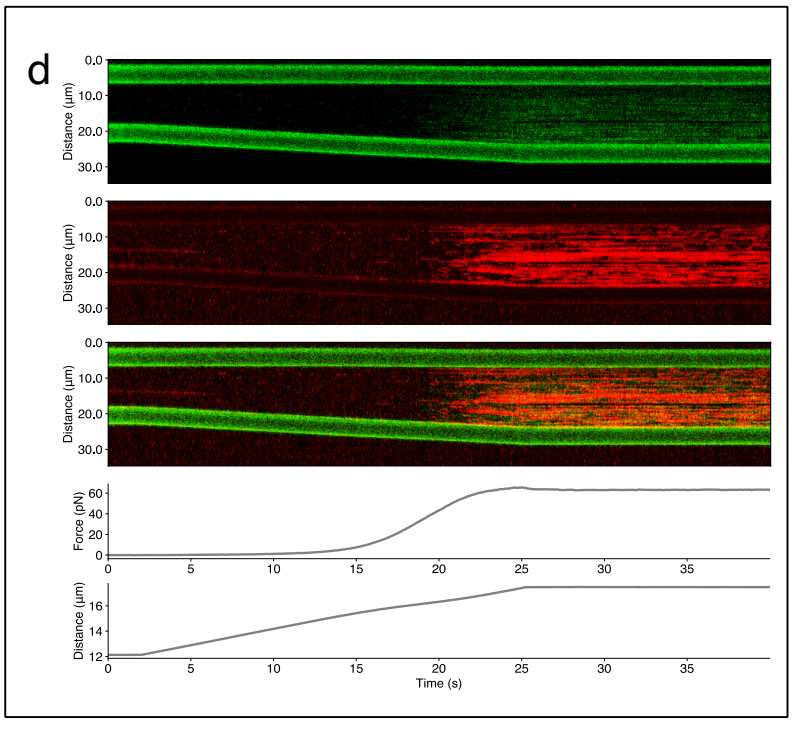

c
5' to 3' on NTS
TTTN TGTA
Example match here
on NTS at target site
29.707
Non-target strand (NTS)
5'
0 kbps
2.829
13.539
PAM
22nt spacer
TTTN TGTA AAGAAACAGTAAGATAAT
47.056
3'
48.5 kbps
Bead
UGUAAAGAAACAGUAAGAUAAU-Cy5
ACAT TTCTTTGTCATTCTATTA
45.787
18.945
3.188
821
Bead
3'
5'
Target strand (TS)
5' to 3' on TS
TTTN TGTA

Supp Fig 4


a
wtCas12a
Cas12a_KO
Cas12a_M3
Cas12a_M1
Cas12a_M2
24nt crRNA
TS products
NTS products
Unspecific ssDNA
Activator
Degraded unspecific ssDNA

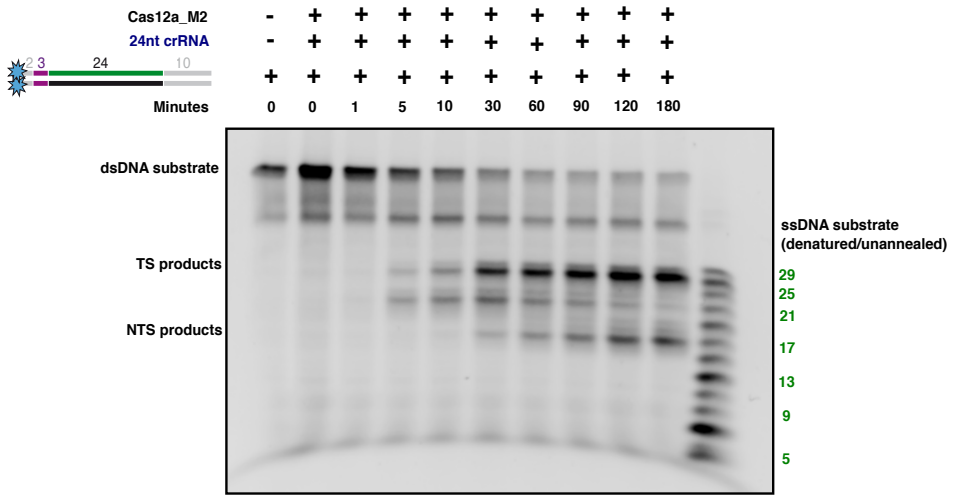

b
Cas12a_KO
Cas12a_M1
Cas12a_M2
Cas12a_M3
50 pN
40 pN
30 pN
20 pN
10 pN
DNA location (kbp)


c
A-T density profile along DNA
Cas12a_M1 (n=5)
Cas12a_M2 (n=5)
Cas12a_M3 (n=5)
Cas12a_KO (n=5)
wtCas12a (n=5)
DNA location (kbp)

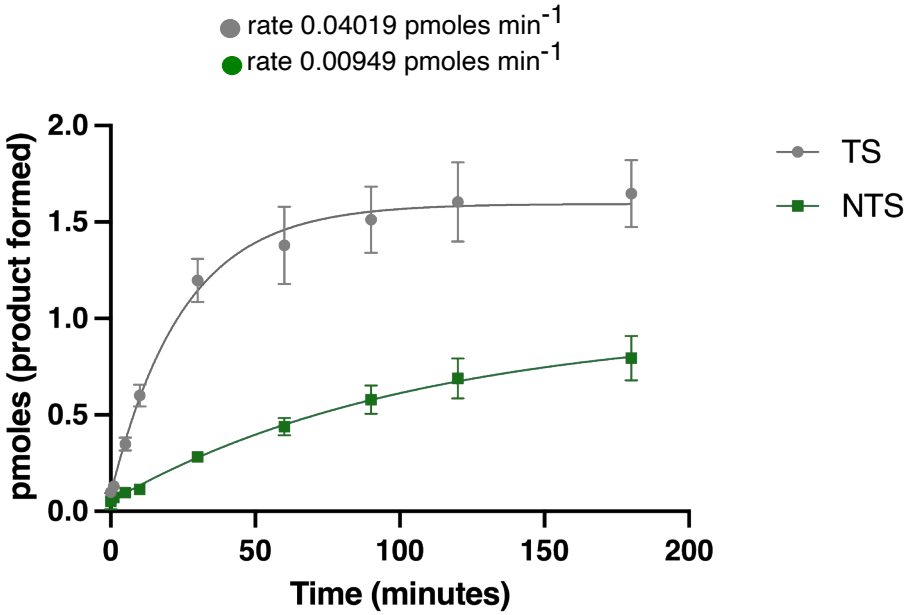

d
Distance (µm)
Force (pN)
Time (s)

Supp Fig 5

a


Cas12a_M2
24nt crRNA
3
24
10
Minutes
0 0 1 5 10 30 60 90 120 180
dsDNA substrate
TS products
NTS products
ssDNA substrate
(denatured/unannealed)
29
25
21
17
13
9
5

b


rate 0.04019 pmoles min-1
rate 0.00949 pmoles min-1
pmoles (product formed)
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Time (minutes)
TS
NTS