Expanded View Figures

Figure EV1. Identification of the CRISPR-Cas systems in *T. onnurineus* and *in vitro* assembly of the *To*Csm complex.

A Six CRISPR loci identified in the genome of *T. onnurineus* NA1 from the CRISPR database (http://crispr.u-psud.fr/).

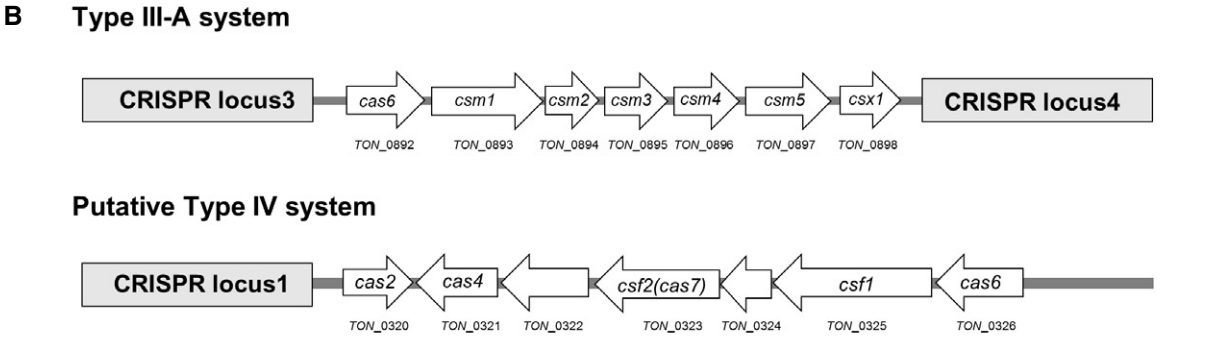
B The organization of the *cas* locus in the Type III and the putative Type IV systems. CRISPR loci 1, 3 and 4 are labelled (grey box). White arrows indicate the ORFs.

C The dotted lines and the blue lines correspond to the elution profile of the *To*Csm complex in the absence or presence of the crRNA, respectively. SDS–PAGE analyses of the SEC fractions are shown in the red boxes. The pooled fractions are indicated.

A

CRISPR_id	Start position	End position	# of spacer	CONSENSUS	
				sequence	size
NC_011529_1	294116	295760	24	GTTTCAATTCTCCTAGAGTCTTATTGCAAC	30
NC_011529_2	728608	730178	23	GTTTCAATAAGACTCTAAGAGAATTGAAAG	30
NC_011529_3	818816	819904	16	GTTCCAGTAGGACAGAATTGTGTGGAAAG	29
NC_011529_4	828033	828820	11	GTTTCAGTAGGACAGAATTGTGTGGAAA	28
NC_011529_5	994457	994969	7	GTTTCAATTCTCTTAGAGTCTTATTGCAAC	30
NC_011529_6	995057	997976	43	GTTTCAATTCTCTTAGAGCTTATTGCAAC	29

B



C

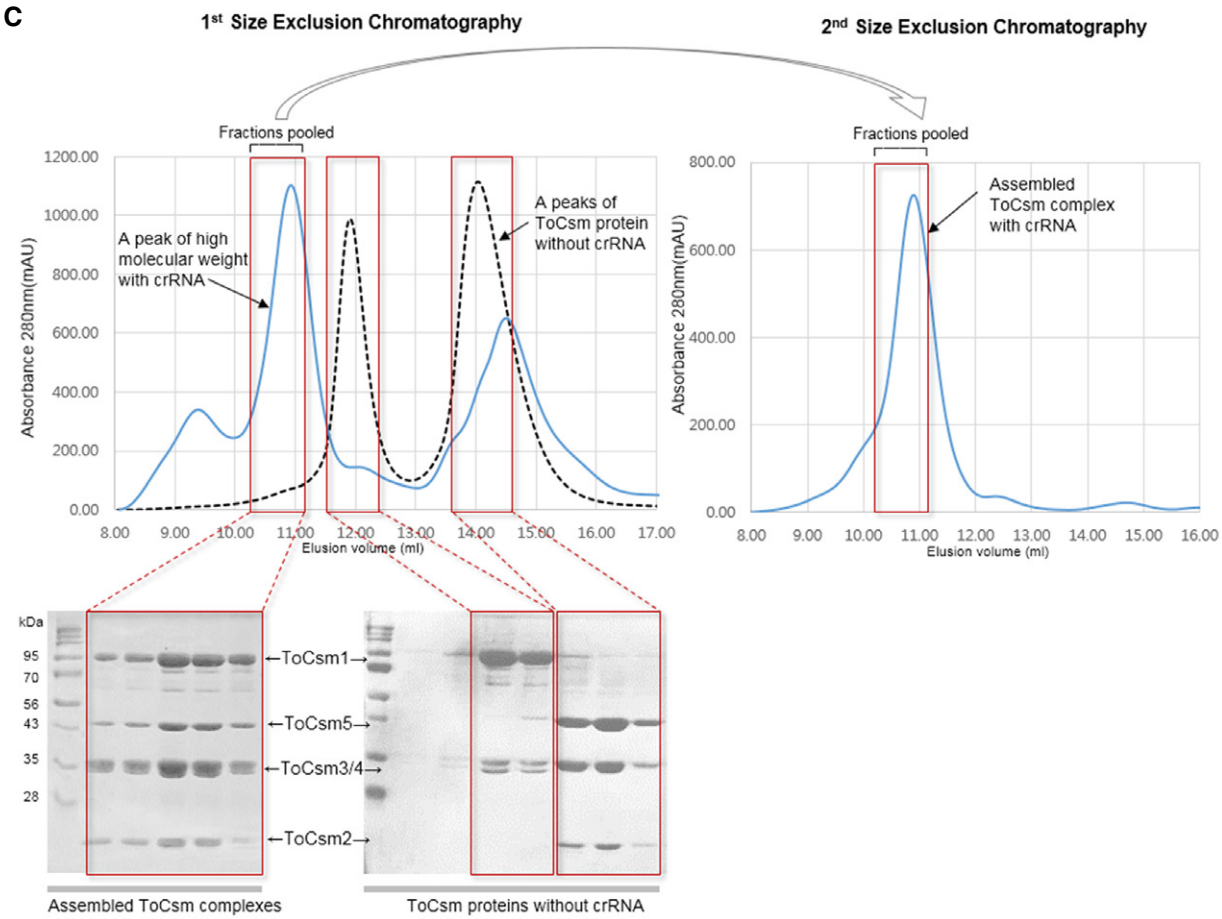


Figure EV1.

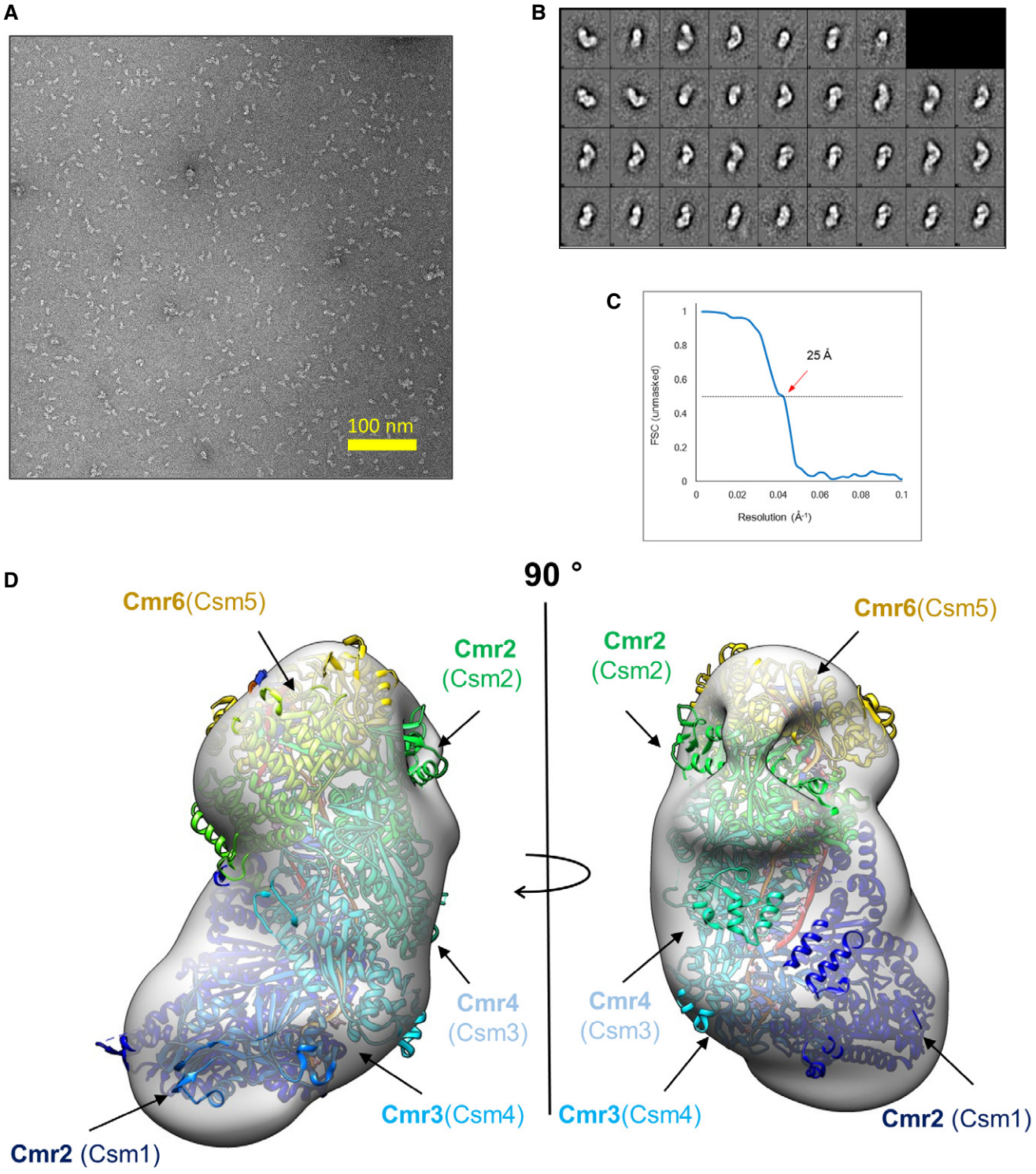


Figure EV2. EM reconstruction of the *To*Csm complex.

A Raw micrograph.
B Free 2D image classification.
C Fourier shell correlation (FSC) plot. The resolution was estimated at an FSC = 0.5 cut-off.
D Fit of the Cmr complex structure (PDB ID: 3X1L) into the EM map of the *To*Csm complex (EMD-3454). The Cmr subunits are shown in different colours. The corresponding Csm subunits are indicated in brackets.

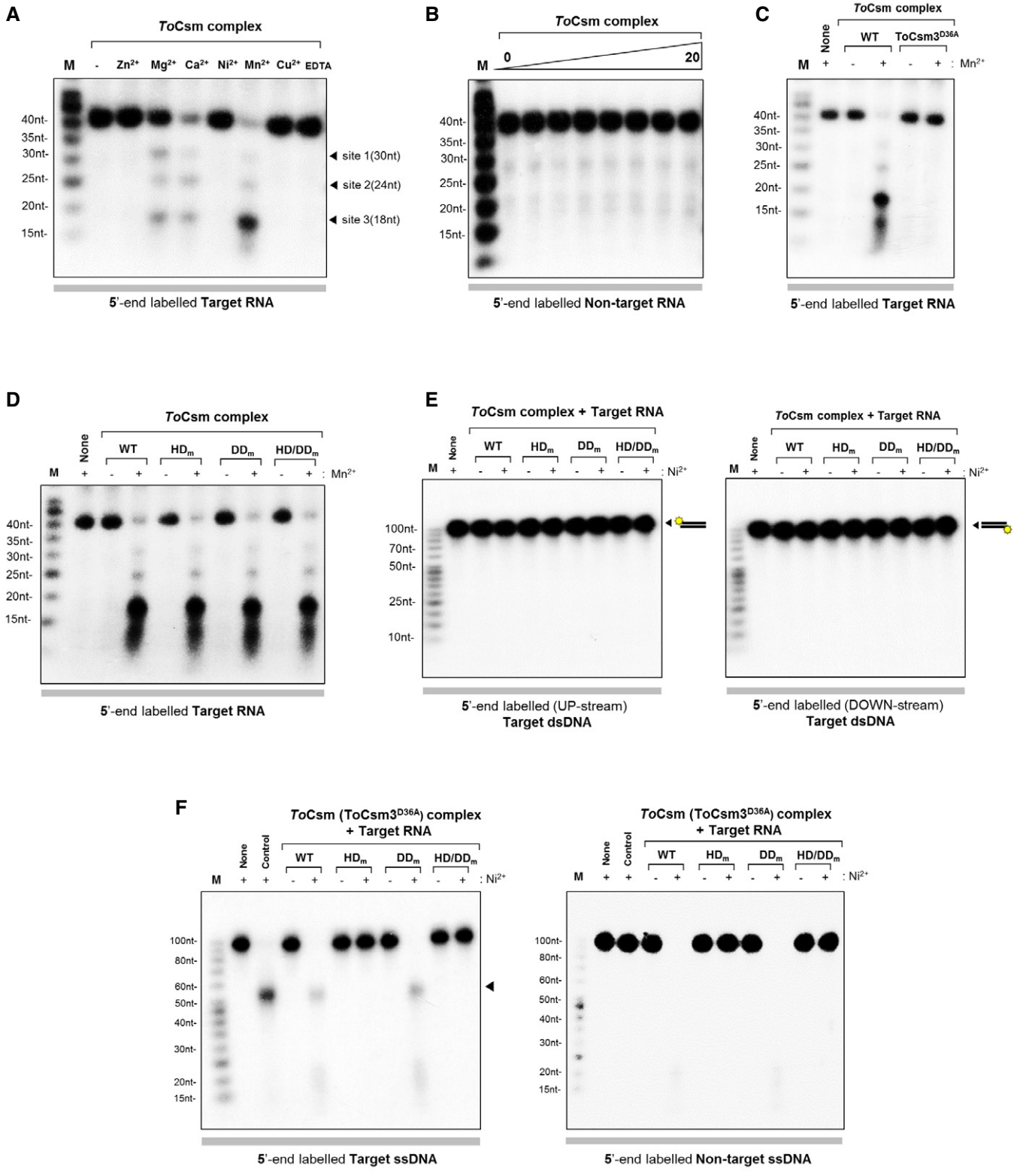


Figure EV3.

Figure EV3. RNase activity and target RNA-activated ssDNase activity.

A Metal ion-dependent RNase activity. The RNase activity was measured in the presence of 5 mM $ZnSO_4$, $MgCl_2$, $CaCl_2$, $NiSO_4$, $MnCl_2$, $CuSO_4$ or EDTA.

B Cleavage assay. Non-target RNA was reacted with the *To*Csm complex in the presence of 5 mM Mn^{2+}.

C Cleavage of target RNA by wild-type (WT) and mutant *To*Csm complex (*To*Csm3^{D36A}). The metal ion cofactor Mn^{2+} was included in all of the reactions.

D Cleavage of target RNA by the wild-type *To*Csm complex and the mutant *To*Csm complex containing a *To*Csm1 mutant (HD_m, *To*Csm1$^{H14A/D15N}$; DD_m, *To*Csm1$^{D587A/D588A}$; or HD/DD_m, *To*Csm1$^{H14A/D15N/D587A/D588A}$).

E Cleavage assay. Target dsDNA was reacted with the wild-type or mutant *To*Csm complex containing the *To*Csm1 mutant (HD_m, *To*Csm1$^{H14A/D15N}$; DD_m, *To*Csm1$^{D587A/D588A}$; or HD/DD_m, *To*Csm1$^{H14A/D15N/D587A/D588A}$) in the presence of the target RNA. Two dsDNA substrates were labelled at one of the two 5′ ends, respectively.

F Cleavage assay. Target ssDNA (left) and non-target ssDNA (right) were incubated with the *To*Csm complex containing *To*Csm3^{D36A} and wild-type or mutant *To*Csm1 (HD_m, DD_m or HD/DD_m) in the presence of the target RNA. "Control" represents the reaction with the wild-type *To*Csm complex in the absence of the target RNA. The arrow in the left panel indicates the largest cleavage fragment.

Source data are available online for this figure.

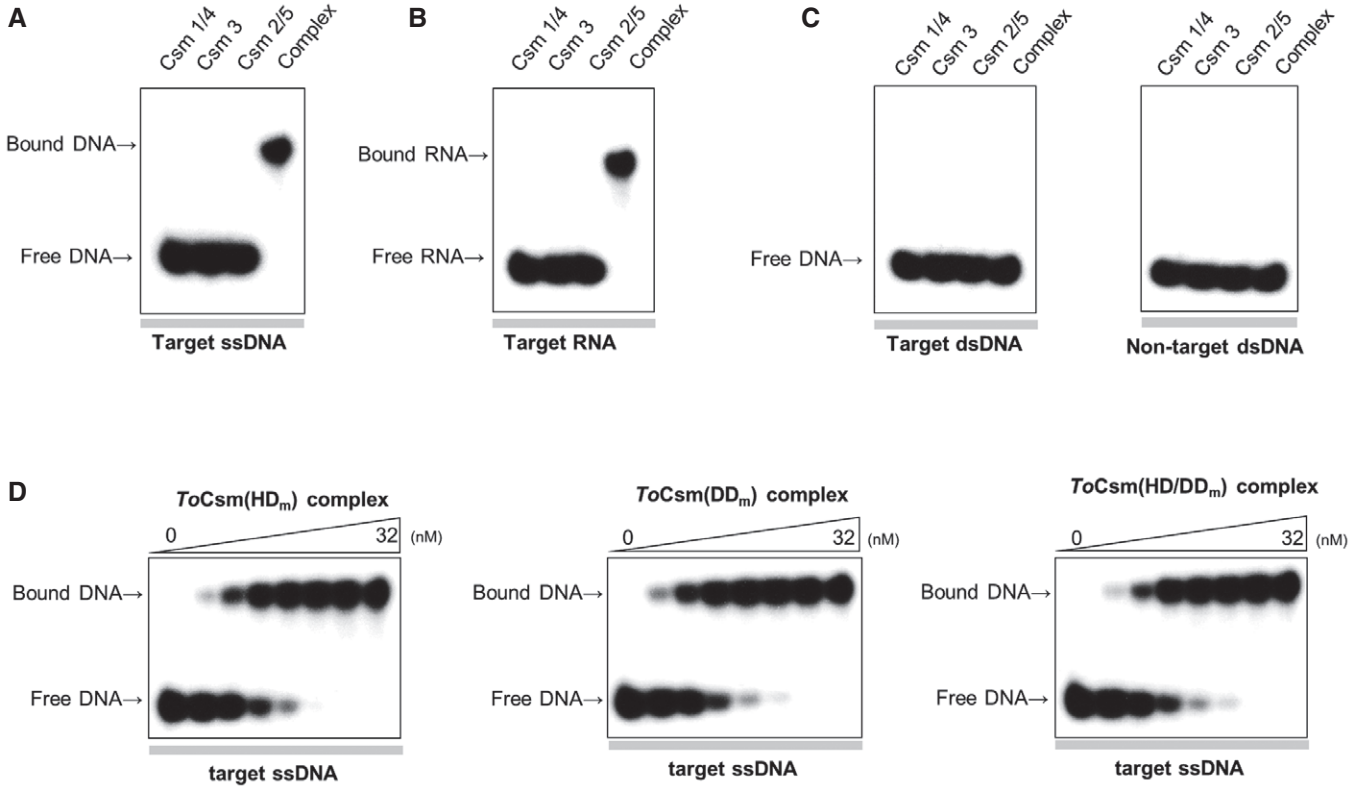


Figure EV4. EMSA analysis of the binding properties of the *To*Csm complex.

A–C Analysis of the interaction between the *To*Csm protein and target ssDNA (A), target RNA (B) and dsDNA (C).

D Analysis of the interaction between the mutant *To*Csm complex and target ssDNA. Mutant *To*Csm complex containing the *To*Csm1 mutant (HD_m, DD_m and HD/DD_m).

Source data are available online for this figure.

Figure EV5. DNA cleavage by the *To*Csm complex.

A Size analysis of the major fragment generated from the target ssDNA. WT represents the fragment produced by the wild-type *To*Csm complex. "ssDNA size" indicates synthetic oligonucleotides (57, 55 and 53 nt) analysed by 15% denaturing urea–PAGE. The dotted line indicates the size of the major product.

B Cleavage assay. 5′ radio-labelled non-target ssDNA was reacted with the *To*Csm complex in a time course.

C Cleavage assay. Two target dsDNA substrates (with identical sequence), each radio-labelled at one of the two 5′ ends, were reacted with the *To*Csm complex.

D, E *Trans*-cleavage of non-target ssDNA by the *To*Csm-target ssDNA (RNA) complex. *To*Csm complex was incubated with target ssDNA or RNA (mixed molar ratio; 1:1) and then complex with increasing concentration react circular non-target ssDNA. The *To*Csm complexes containing a *To*Csm1 mutant are indicated as in Fig 2B. The designed 40-nt target ssDNA (RNA) contains a 30-nt target sequence in the centre (D) and designed 30-nt target ssDNA (RNA) only contains a 30-nt target sequence (E).

F Schematic drawing of an artificial DNA duplex bound to the *To*Csm complex. The designed duplex contains 25-nt complementary sequence at both ends. The target strand contains a 30-nt target sequence in the centre, and the non-target strand contains an 80-nt sequence not complementary to the target strand. The two DNA strands were annealed and analysed on a 2% agarose gel. Lane 1: 130-nt target ssDNA, lane 2: 130-bp annealed DNA and lane 3: 130-bp annealed DNA containing a 5′-handle-complementary sequence. The target strand and the non-target strand correspond to the non-template strand and the template strand of a transcription bubble, respectively.

Source data are available online for this figure.

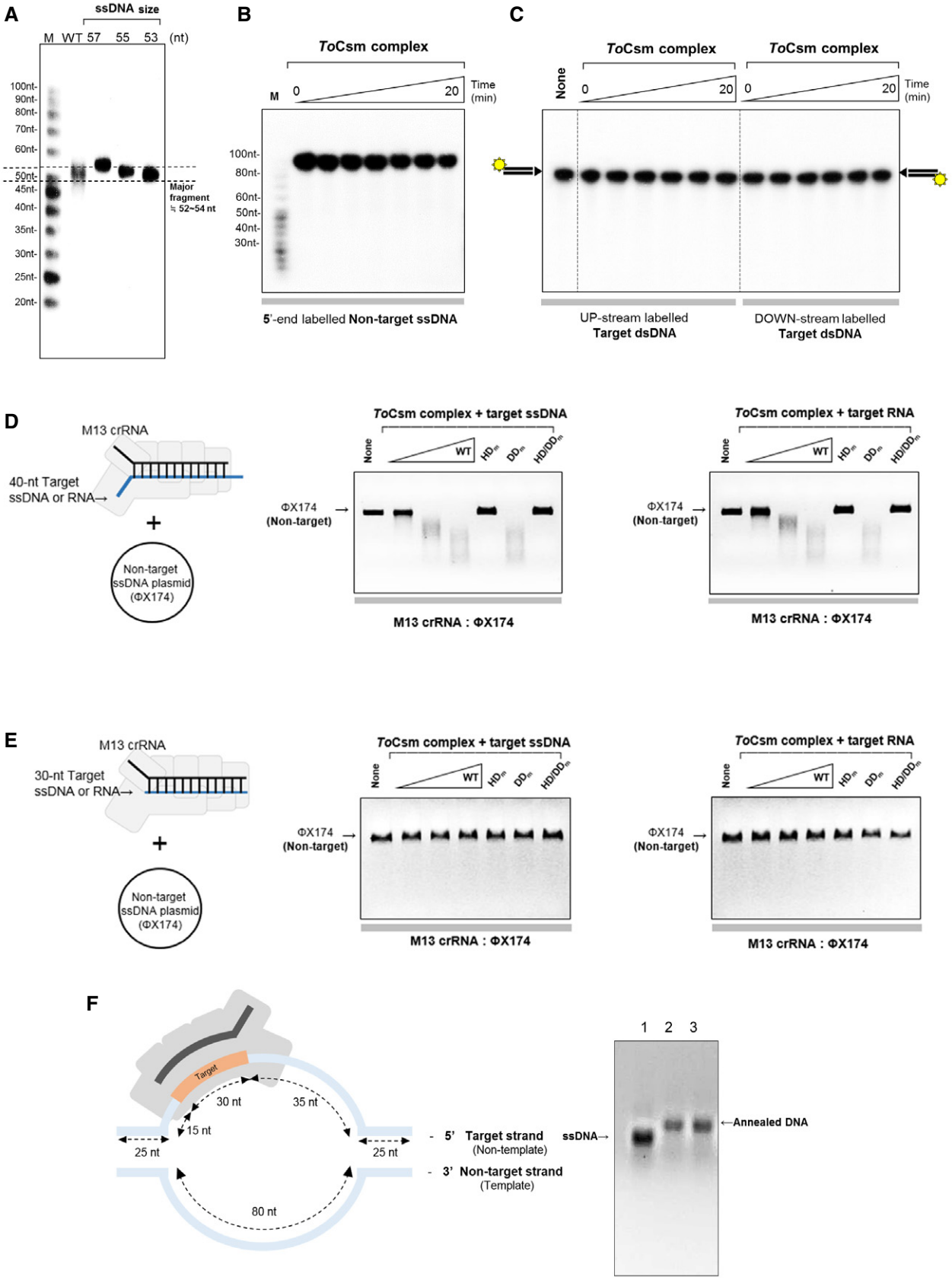


Figure EV5.