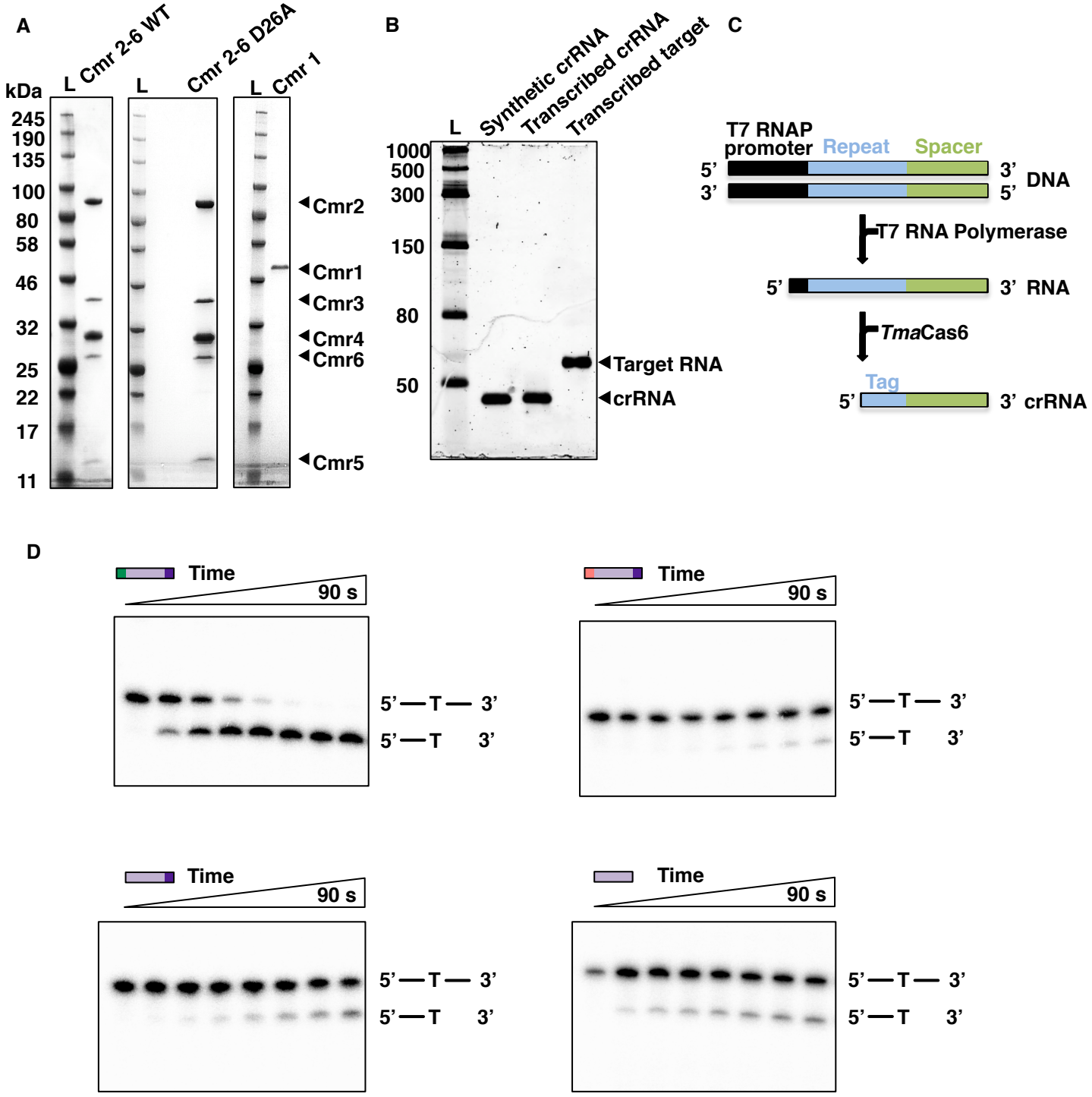

Figure S1. *(A)* SDS-PAGE gels of recombinant *Tma*Cmr2-6 WT, *Tma*Cmr2-6 D26A, and *Tma*Cmr1. "L" indicates ladder (NEB P7712). (*B*) Urea-PAGE gel of crRNA and target RNA. "L" indicates ladder (NEB N0364). Synthetic crRNA was included as a control to confirm appropriate size after crRNA transcription and processing by *Tma*Cas6. (*C*) Schematic of crRNA production. A double-stranded DNA was first transcribed by T7 RNA Polymerase and the resulting RNA was processed by *Tma*Cas6 to produce a mature crRNA consisting of an 8 nt tag and 37 nt spacer. (*D*) PAGE of DNA cleavage products by the *Tma*Cmr complex over time upon activation with RNA targets containing various 3' and 5' flanks. Quantification of these gels is shown in **Fig. 1C**.

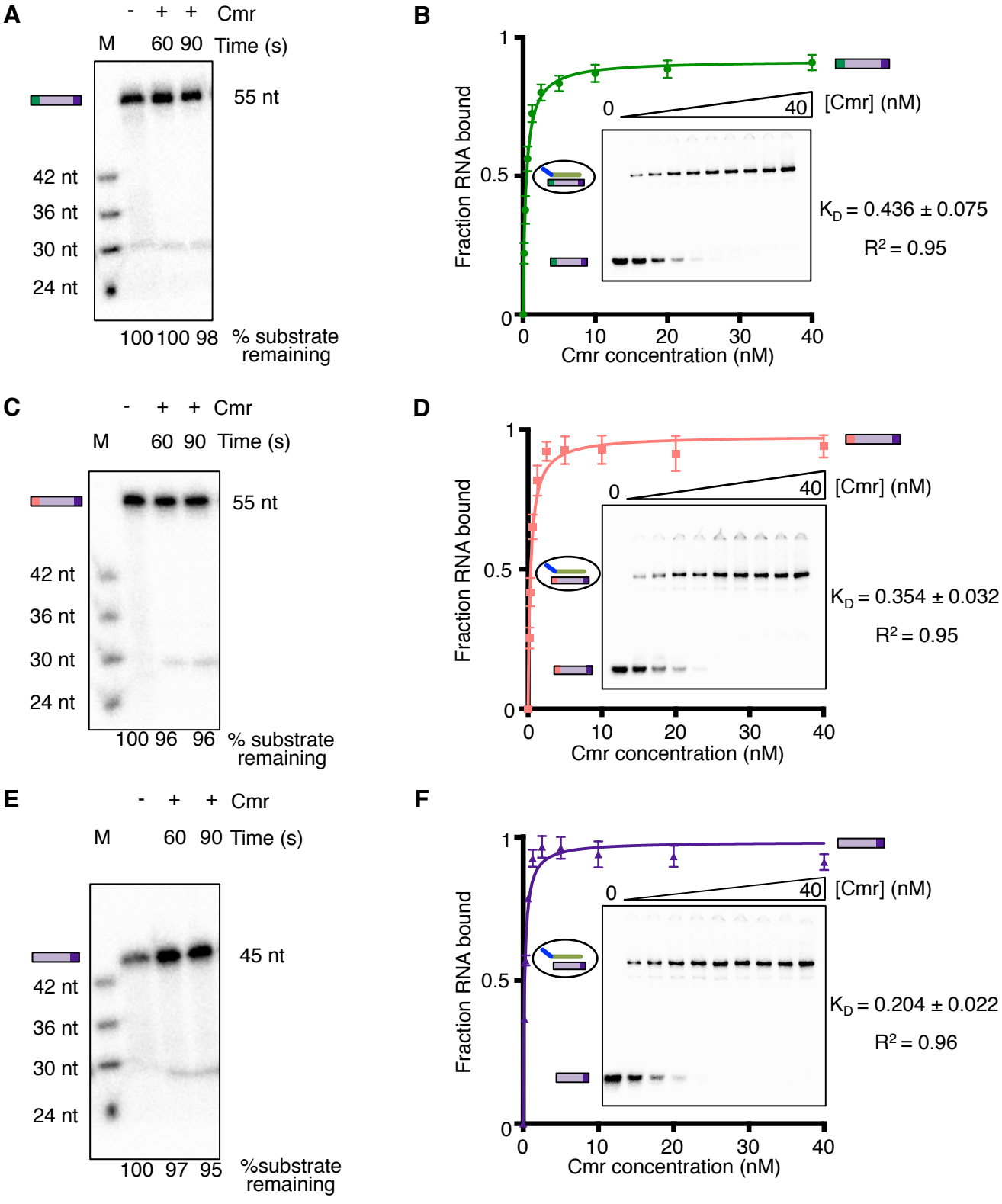

Figure S2. Target binding and persistence. Green (*A, B*) indicates a non-complementary PFS, pink (*C, D*) indicates an anti-tag PFS, and purple (*E, F*) indicates no PFS. (*A,C,E*) Representative Urea-PAGE showing persistence of RNA target after incubation with *Tma*Cmr for 60 and 90 seconds under DNA cleavage conditions. "M" indicates marker generated by 5' radiolabeling expected cleavage products (synthetic) of a 55 nt substrate. *(B, D, F)* Binding curve with representative EMSA and binding constants of *Tma*Cmr with RNA targets.

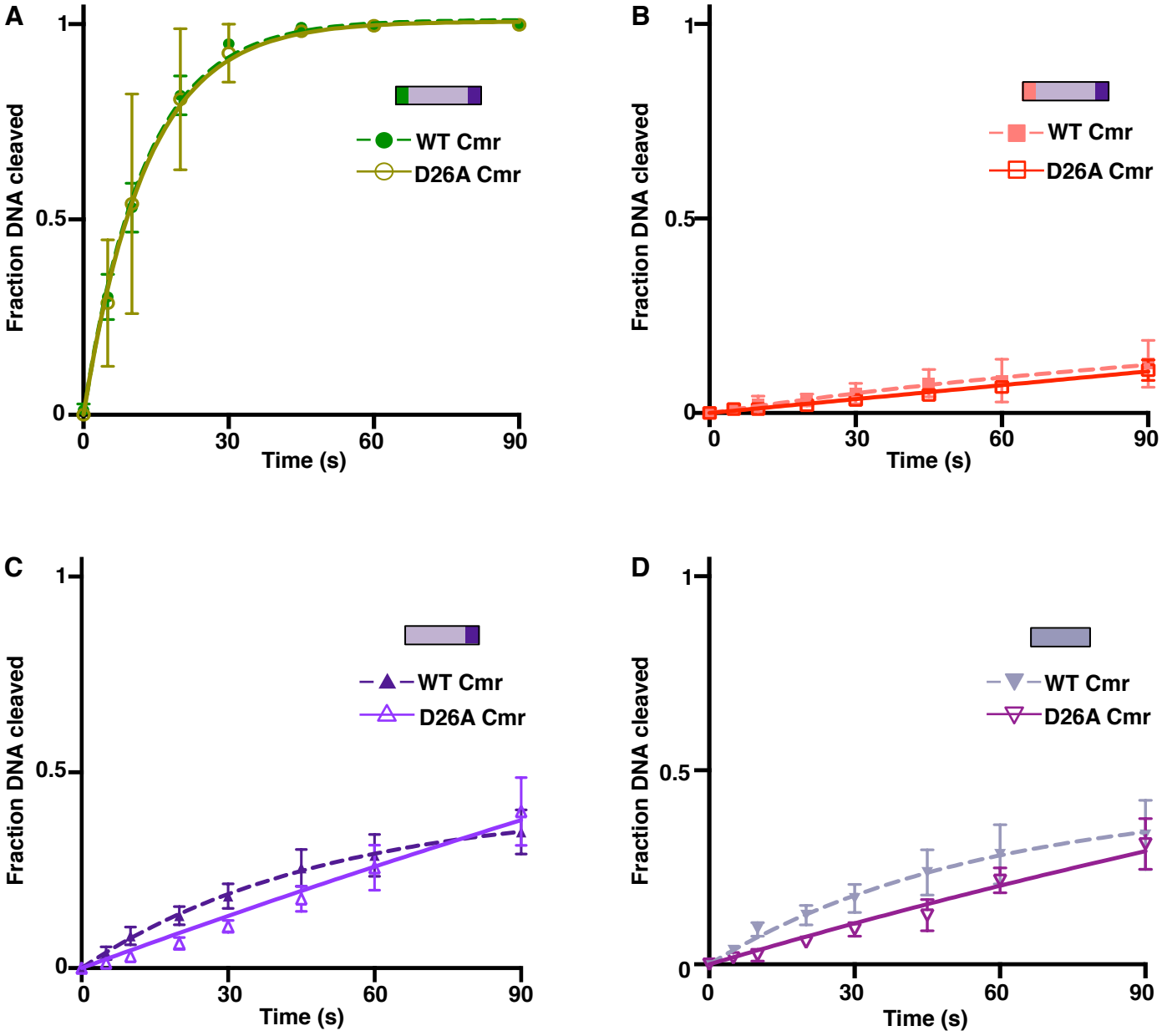

Figure S3. Time courses of DNA cleavage with wild-type (dashed lines and filled shapes) and RNA cleavage mutant D26A (solid lines, open shapes) *Tma*Cmr complexes upon activation with targets with various PFSs. (*A*) Target contains noncomplementary PFS. *(B)* Target contains anti-tag PFS. *(C)* Target lacks PFS. (D) Target lacks PFS and 5' flank.

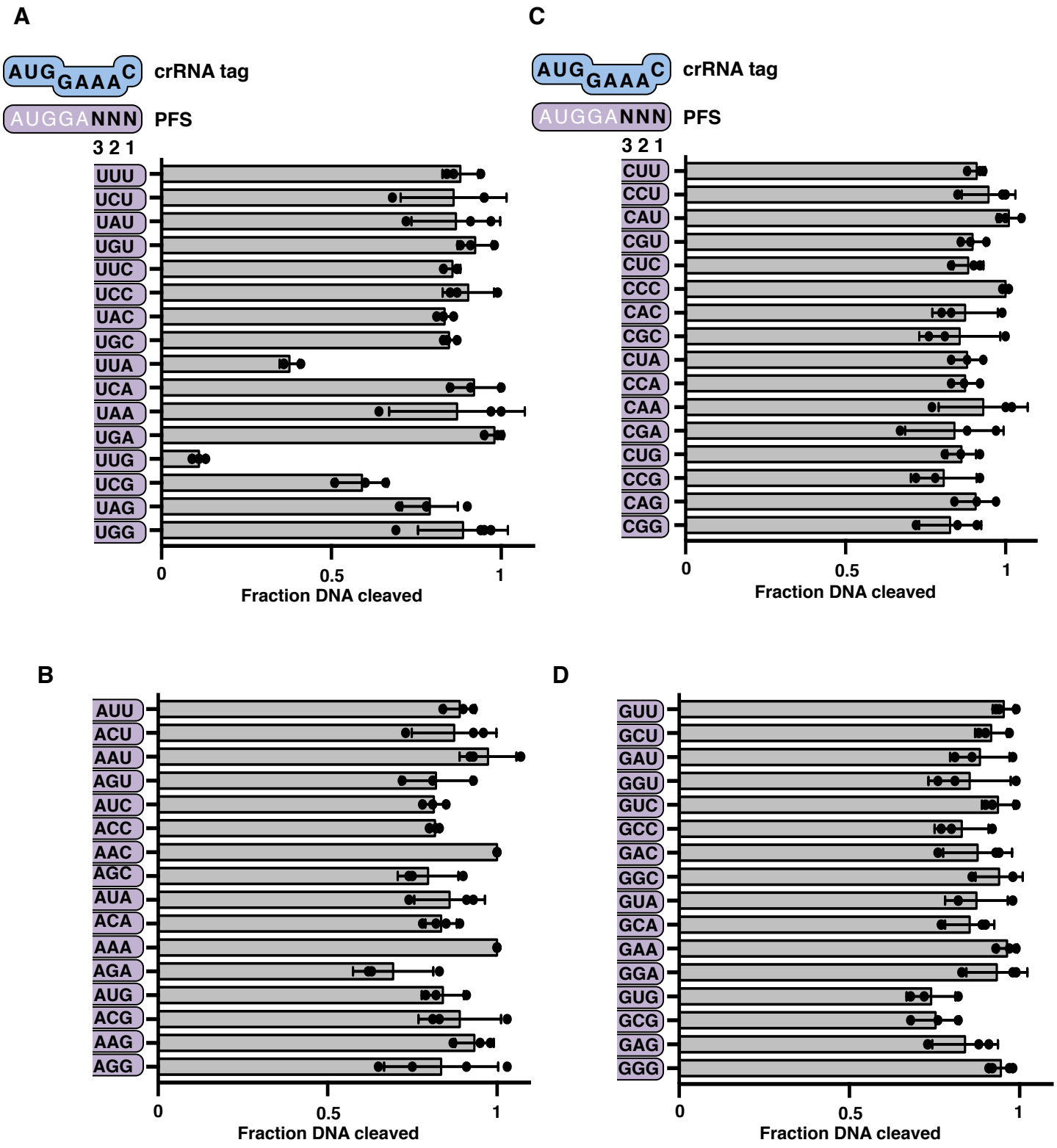

Figure S4. Data from Fig. 2B plotted as individual bars with errors. (*A,B,C,D*) Quantification of DNA cleavage by *Tma*Cmr complex upon activation with targets containing all possible sequences in positions -1 to -3. All targets contain noncomplementary sequence of 5'-AGGUA-3' in positions -4 to -8.

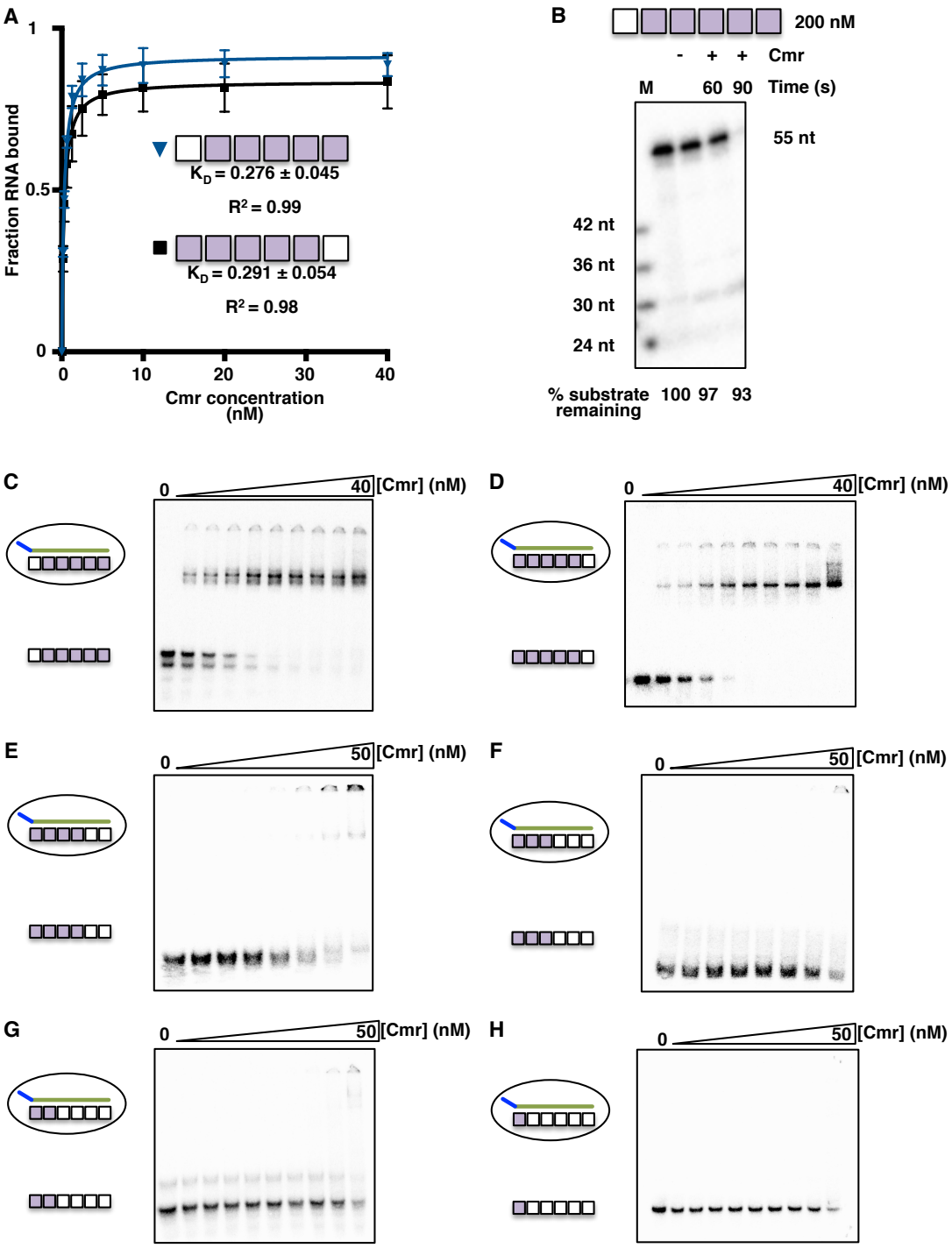

Figure S5. Binding and persistence of mismatched targets. *(A)* Binding curves and binding constants for *Tma*Cmr with Segment 1 and Segment 6 mismatched targets. *(B)* Representative Urea-PAGE showing persistence of RNA target after incubation with *Tma*Cmr for 60 and 90 seconds under DNA cleavage conditions. M indicates marker generated by 5' radiolabeling expected cleavage products (synthetic) of a 55 nt substrate. *(C-H)* Representative EMSAs of *Tma*Cmr with targets containing mismatched segment 1 (C), segment 6 (D), segments 5-6 (E), segments 4-6 (F), segments 3-6 (G), or segments 2-6 (H).