



Fig. S9. Cas2/3 cleavage within protospacers of different length. (A and B) Denaturing polyacrylamide gels of Cas2/3-cleaved DNA targets. DNA duplexes PS-WT, PS-M6, PS-T6 and PS-T30 were ³²P-labelled on the non-target DNA strand then pre-incubated with WT or +30 Cascade complex in the absence (A) or presence (B) of ATP and cleavage was initiated by introducing the Cas2/3. The boundaries for the expected 32 and 62 bp length R-loops are indicated beside the gels. (C) The

sequence of the non-target strands. The protospacer region and PAM are coloured in red and blue, respectively. Nucleotides mismatching the PS-WT protospacer are coloured in cyan. Triangles of different colours on the right and left of the gels (**A** and **B**) and within non-target strand sequences (**C**) indicate cleavage positions of the Cas2/3. The height of the triangles correlates with a relative amount of cleavage product after 1-hour incubation with 500 nM Cas2/3 in the absence (-ATP) or presence (+ATP) of ATP. The expected lengths of the R-loops formed upon binding of WT or +30 Cascade complexes (specified in parentheses) to DNA target are indicated beside the sequences.