

Fig. S2. *R-loop formed by WT Cascade.* (**A**). *Denaturing polyacrylamide gel of oligoduplex footprints*. DNA oligoduplexes SP-CC, SP-AC, SP-GC, and SP-GC bearing the protospacer and PAM variant (CC, AC, GC, and GC, respectively) were ³³P-5'-labelled either on the target strand (TS) or on the non-target strand (NT) then treated with P1 nuclease in the absence (-) or presence (+) of WT Cascade complex. C and M represent ddCTP and mix of sequencing reactions of SP-CC non-target strand by ³³P-5'-labelled-oligonucleotide TS166, respectively. (**B**) *R-loop within SP-CC oligoduplex sequence*. R-loop is formed when crRNA of Cascade complex base-pairs with the target strand of the protospacer displacing the non-target strand as ssDNA, which is a substrate for P1 nuclease. A dashed line represents P1-sensitive region within 18th - 25th nt of the non-target strand of the protospacer. The protospacer region is indicated by a solid line and marginal nucleotides (1 and 32). The PAM is coloured in red.