

Additional file 4: Fig. S3

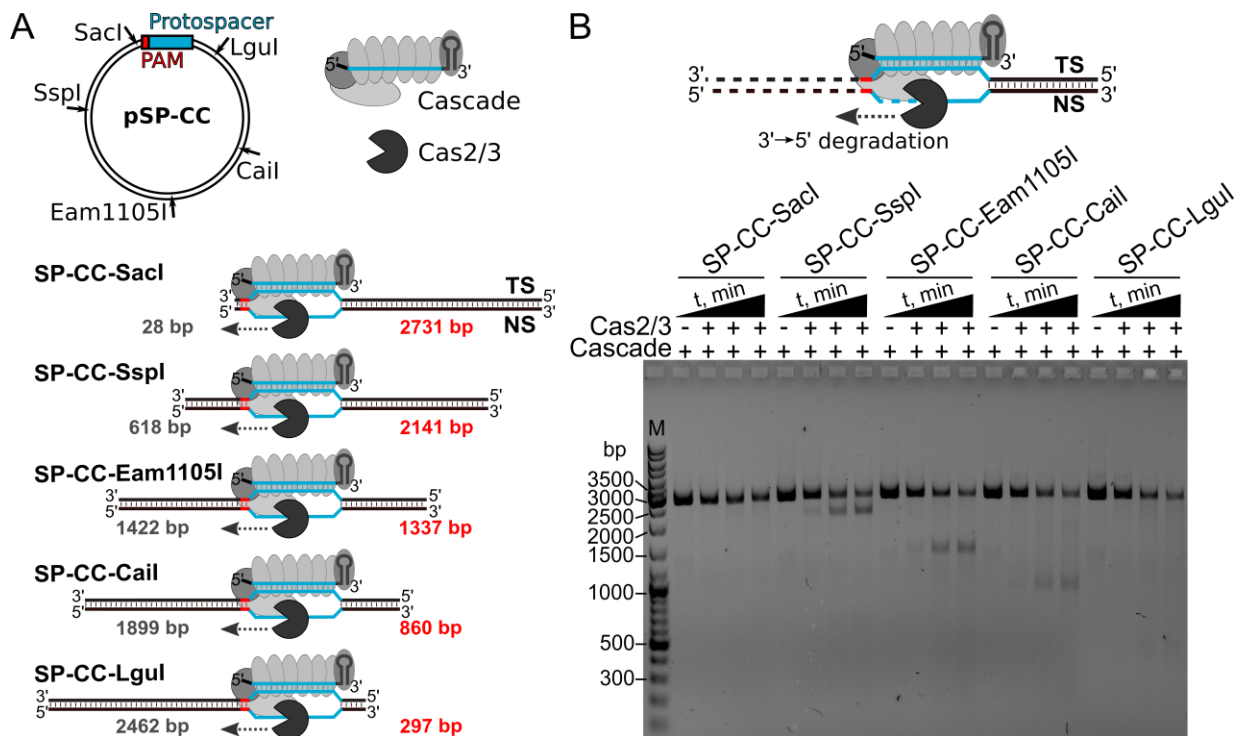


Fig. S3. Cas2/3-mediated DNA degradation. (A) Preparation of linearized plasmid substrates. Plasmid pSP-CC was cleaved using either of Sacl, Sspl, Eam1105I, Cail, or Lgul restriction endonucleases producing long (2759 bp) linear double-stranded DNA molecules that contain protospacer in different positions with the same directionality. A presumable double-stranded break between PAM and protospacer would generate DNA fragments of indicated lengths. **(B) Cas2/3 degradation of Cascade-bound linearized DNA targets.** Five linear DNA fragments pSP-CC-Sacl, pSP-CC-Sspl, pSP-CC-Eam1105I, pSP-CC-Cail, and pSP-CC-Lgul were pre-incubated with WT Cascade complex then treated with Cas2/3 (for 5, 30, and 60 min). Cas2/3 degrades DNA target upstream from the protospacer (in a protospacer-PAM direction) leaving downstream DNA fragment intact (red-coloured numbers in the A).