Supplemental figure legends.

Supplemental figure S1. (**A**) Analysis of crRNAs associated with the wild-type and *csm3*^{D32A} Cas10-Csm complex. RNA was extracted from purified complexes, radiolabeled, separated by denaturing PAGE and visualized by gel autoradiography. (**B**) ssDNA and dsDNA substrates used in to determine the cleavage properties of the Cas10-Csm complex in the absence of transcription. (**C**) Radiolabeled substrates shown in panel B were incubated with the Cas10-Csm complex in the absence of transcription for 0, 10, 30, 60 and 120 minutes. (**D**) Transcription of the substrate shown in Fig. 1D followed as the extension of a radiolabeled RNA primer. Transcription is shown for both the assay of top (Fig. 1E) and bottom (Fig. 1F) strand cleavage. Lanes (a) and (b) differ in the order of addition of the Cas10-Csm complex with respect to the initiation of transcription. In lane (a) the Cas10-Csm complex was added to the elongation complex and incubated for 10 minutes prior to the initiation of transcription by the addition of rNTPs. In lane (b) the Cas10-Csm complex was added 10 minutes after transcription initiation by the addition of rNTPs to the elongation complex. Related to Fig. 1.

Supplemental figure S2. (**A**) Target:crRNA configuration that licenses CRISPR-Cas immunity. crRNAs annealing to the coding strand of a transcribed region of the phage can provide robust immunity. The most abundant crRNA produced by the *S. epidermidis* type III-A CRISPR-Cas system in staphylococci contains 35 nt of spacer sequence, which acts as the guide for the Cas10-Csm complex (dark green). At the 5' end of the guide sequence there are 8 nt of repeat sequence known as the crRNA tag (light green). (**B**) A crRNA guide complementary to the template strand of a transcribed phage does not support immunity. (**C**) The presence of an anti-tag sequence (complementary to the crRNA tag sequence) immediately upstream of the target prevents effective immunity, even if the crRNA guide anneals to the coding strand of a transcribed viral region. Related to Fig. 3.

Supplemental figure S3. (**A**) Mapping of the RNA cleavage products shown in Fig. 3C. The Cas10-Csm cleavage products (black arrowheads) were compared to those of the RNases A (5' of adenine and uridine residues, orange arrowheads) and T1 (5' of guanosine residues, red arrowheads). (**B**) Analysis of the cleavage sites detected in panel A in relation with the ssRNA substrate sequence. (**C**) Sequence of a ssRNA substrate with a scrambled, non-specific sequence. (**D**) Cleavage of the radiolabeled substrate shown in panel C by the Cas10-Csm complex; incubation times: 0, 5, 10, 20, 30, 60, 120, 180 and 240 minutes. Related to Fig. 4.

Supplemental tables.

Supplemental Table S1. DNA oligonucleotides used in this study (5'-3').

Name	Sequence
A248	CCTCCTTATAAAATTAGTATAATTATAGCAC
A67	GTGACCTCCTTGCCATTGTC
NP36	TATACTTCGGCATACGTGTTCTCGTTATCTTGTTCATATTTATC
NP37	GATAAATATGAACAAGATAACGAGAACACGTATGCCGAAGTATA
oGG164	TGAGACCAGTCTCGGAAGCTCAAAGGTCTCTTAAATCTAACAACACTCTAAAAAATTG
oGG165	GTTCTCGTCCCCTTTTCTTCGGGGTGGGTATCGATCCGATACTTTAACAAATGCCATC
oGG250	GAACATTCGTCATCTTCAAGTAATGCCTCTAAATCAATA
oGG251	GATCTATTGATTTAGAGGCATTACTTGAAGATGACGAAT
PS153	GGTAAATCAAAACTAACTAACAAATACATTAGTTTCCCACCTCTATCATC
PS154	GATGATAGAGGTGGGAAACTAATGTATTTGTTAGTTAGTTTTGATTTACC
PS171	TATTTAGAGAACGTATGCCGAAGTATATAAATCATCAGTACAAAGGTAAGAATCA
PS172	TGATTCTTACCTTTGTACTGATGATTTATATACTTCGGCATACGTTCTCTAAATA
PS364	GCGGTAATTTTAATGAGATATTTAGAGAACGTATGCCGAAGTATATAAATCATCAGTACAAAGGTAAGAATCACAGTAAACAGCGCGCGG
PS365	CCGCGCGCTGTTTACTGTGATTCTTACCTTTGTACTGATGATTTATATACTTCGGCATACGTTCTCTAAATATCTCATTAAAATTACCGC
PS392	GCGGCGTAGAGAACGTATGCCGAAGTATATAAATCATCAGTACAAAGGTAAGGCGGCG
PS393	CGCCGCGAATGGCCCTTTGACTACCCCCCCCCCCCAAGCCGCCCTTTAGAGATCGCCGC
PS396	GCGCGGCTTACCTTTGTACTGATGATTTATATACTTCGGCATACGTTCTCTAGCGGCG
PS397	CGCCGCATCTCTTTTCCCCGGCTTCCCCCCCCCCGTAGTCTTTCCCCCATTCCCGCGC
PS465	GAATCTAGTATGATTGGAGCAATTGCTTCTCCTGTAGTTAGAGATTTGCAAACC
PS466	GGTTTGCAAATCTCTAACTACAGGAGAAGCAATTGCTCCAATCATACTAGATTC
PS532	GCGGTAATTTTAATGAGATAACGAGAACACGTATGCCGAAGTATATAAATCATCAGTACAAAGGTAAGAATCACAGTAAACAGCGCGCGG
PS533	CCGCGCGCTGTTTACTGTGATTCTTACCTTTGTACTGATGATTTATATACTTCGGCATACGTGTTCTCGTTATCTCATTAAAATTACCGC
PS556	CTGCTATATATTCAGGCGGTGCCGCTTTATTTTTAATCGGTGCATGG
PS557	CCATGCACCGATTAAAAATAAAGCGGCACCGCCTGAATATATAGCAG
PS558	GGCGAATTTTCAGGTTCAGGTATAAAAACAAGCTTAGG
PS559	CCTAAGCTTGTTTTTATACCTGAACCTGAAAATTCGCC
W1020	TGATAAATATAATACTCTAACGCTG
W1021	ACAGCGTTAGAGTATTATATTTATC
W1022	AATAACATCTTTCATTTTTCCATCC
W494	GGGATGGAAAAATGAAAGATGTTA
W845	CTTCGGGGTGGGTATCGATCAGAGACCTTTGAGCTTCCGAGAC
W846	AAAAGGGGACGAGAACTAAATCTAACAACACTCTAAAAAATTG

Supplemental Table S2. RNA oligonucleotides used in this study (5'-3').

Name	Sequence
*nes*_target	UGAUUCUUACCUUUGUACUGAUGAUUUAUAUACUUCGGCAUACGUUCUCUAAAUA
NS_ssRNA	GCUGUUAAGUUACUCGAGCACAUCAGUGAUAGCCUUAUUCCCGCUGUGCCUAUAC
nes anti-tag target	UGAUUCUUACCUUUGUACUGAUGAUUUAUAUACUUCGGCAUACGUGUUCUCGUUA
EC Primer 1	GUUUACUGUG
EC Primer 2	UUAAUGAGAU

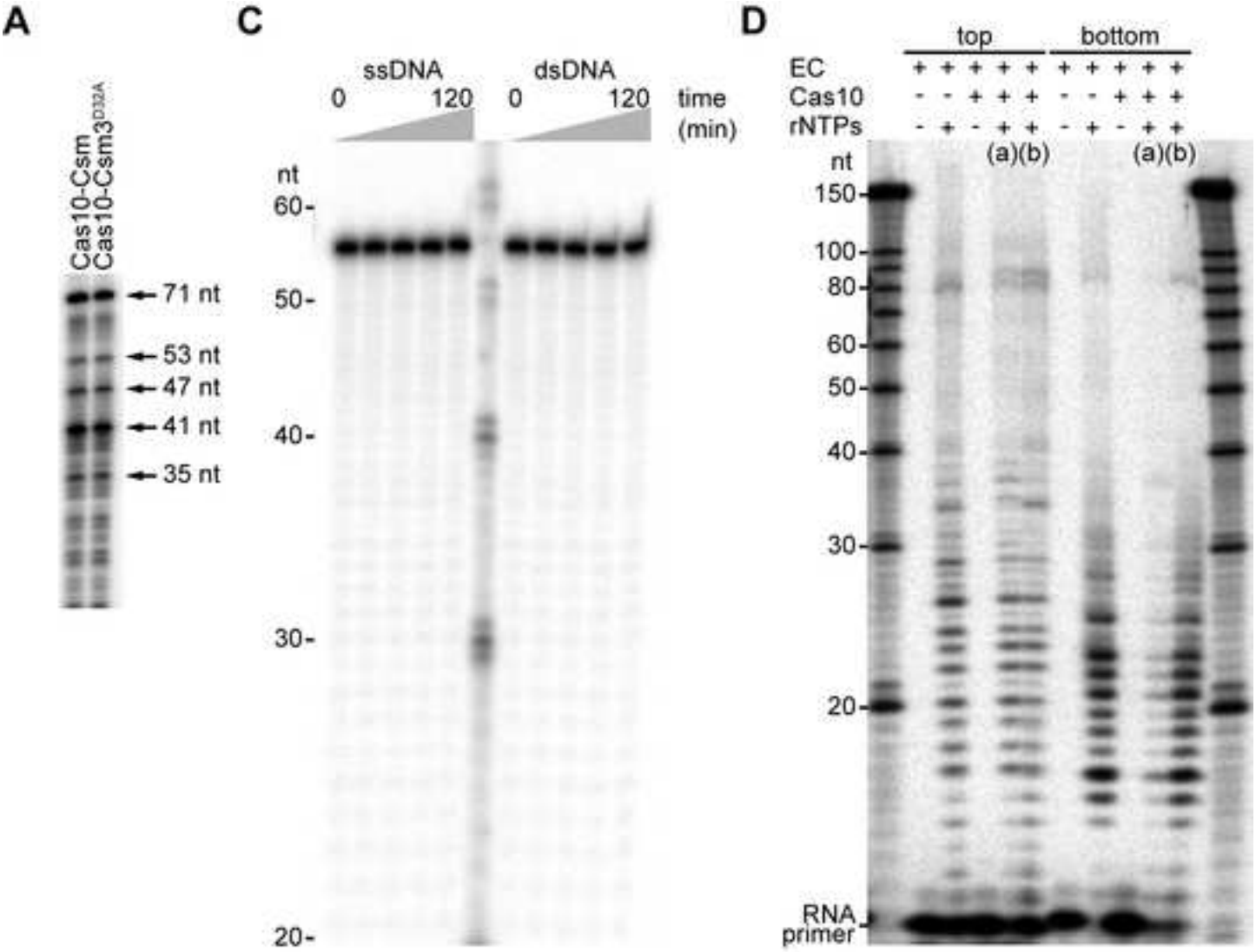
Extended experimental procedures.

Purification of recombinant Cas10-Csm complex from *E.coli*. The pPS22 and pPS plasmids were transformed into *E. coli* BL21 (DE3) Rosetta 2 cells (Merck Millipore). Cultures (10 liters) were grown at 37 °C in Terrific Broth medium (Fisher Scientific) containing 100 µg/ml ampicilin and 34 µg/ml chloramphenicol until the A_{600} reached 0.6. The cultures were adjusted to 0.3 mM isopropyl-1-thio-β-d-galactopyranoside and incubation was continued for 16 h at 17 °C with constant shaking. The cells were harvested by centrifugation and the pellets stored at −80 °C. All subsequent steps were performed at 4 °C. Thawed bacteria were resuspended in 75 ml of buffer A (50 mM Tris–HCl, pH 7.5, 350 mM NaCl, 200 mM Li_2SO_4, 20% sucrose, 10 mM Imidazole) containing two complete EDTA free protease inhibitor tablet (Roche). Triton X-100 and lysozyme were added to final concentrations of 0.1 % and 0.1 mg/ml, respectively. After 1 hr, the lysate was sonicated to reduce viscosity. Insoluble material was removed by centrifugation for 30 min at 15,000 rpm in a Beckman JA-3050 rotor. The soluble extract was mixed for 1 hr with 5 ml of Ni^{2+}-Nitrilotriacetic acid-agarose resin (Qiagen) that had been pre-equilibrated with buffer A. The resin was recovered by centrifugation, then first washed with 50 ml of buffer A, followed by washing with 50 ml of IMAC buffer (50 mM Tris-HCl pH 7.5, 250 mM NaCl, 10% glycerol) containing 15 mM imidazole. The resin was subsequently resuspended in 10 ml of IMAC buffer containing 50 mM imidazole, and then poured into a column. The column was then eluted step-wise with 10 ml aliquots of IMAC buffer (50 mM Tris-HCl pH 7.5, 250 mM NaCl, 10% glycerol) containing 100, 200, 350 and 500 mM imidazole. The 200 mM imidazole elutes containing the complex was pooled together and dialyzed against 50 mM Tris-HCl pH 7.5, 50 mM NaCl, 10% glycerol. Subsequently the complex was purified using a 1 ml Source 15Q column (GE Life Sciences), eluting with a linear gradient of 50 mM - 2 M NaCl. The peak fraction from the Source 15Q column was further purified by size exclsuion chromatography using Superdex 200 10/300 GL (GE Healthcare) using buffer B (50 mM Tris-HCl pH 7.5, 5% glycerol, 150 mM NaCl).

Transcription coupled DNA cleavage assay in the presence of a transcription elongation inhibitor. The Darst Laboratory (The Rockefeller University) kindly provided the CBR703 inhibitor. Elongation complexes (ECs) were reconstituted as described in the main experimental procedure. Assembled ECs were either incubated with CBR703 (final concentration of 1 µM) or 10% DMSO at 37°C for 10 minutes. In all cases, Cas10-Csm complex was added to a final concentration of 15 ng/µl. Transcription was initiated with the addition of 2.5 mM of RNTPs. All the following steps were performed at 37°C. For reactions containing a labeled RNA primer, rNTPs were added to the EC and the Cas10-Csm complex was added 10 minutes after transcription start. Samples were taken at 30, 60 and 120 minutes. Likewise, for the DNA cleavage time course experiments, rNTPs were added to the EC prior to the addition of Cas10-Csm complex. After addition of Cas10-Csm, the samples were collected at timed intervals of 30, 60, 90 and 120 minutes, and in control experiments where each

of the components of the reaction were omitted, a single time point was taken at 120 minutes. The reactions were quenched by mixing with Proteinase K (NEB) and 20 mM EDTA. The DNA/RNA samples were then extracted using phenol-chloroform-isoamyl alcohol (25:24:1), ethanol precipitated and resuspended into loading buffer (90% formamide). The DNA products were heater at 95°C for 5 min before loading onto the gel. Cleavage products were resolved on a 12% denaturing polyacrylamide gels containing 7 M urea and visualized by phosphorimaging (Typhoon, GE Life Sciences).

Supplemental Figure 1
Click here to download Supplemental Figure: Fig S1.tif



B

ssDNA 5'-TGATTCTTACCTTTGTACTGATGATTTATATACTTCGGCATACGTTCTCTAAATA-3'

dsDNA 5'-TGATTCTTACCTTTGTACTGATGATTTATATACTTCGGCATACGTTCTCTAAATA-3'
3'-ACTAAGAATGGAAACATGACTACTAAATATATGAAGCCGTATGCAAGAGATTTAT-5'

Figure S1. Samai *et al.*

Supplemental Figure 2
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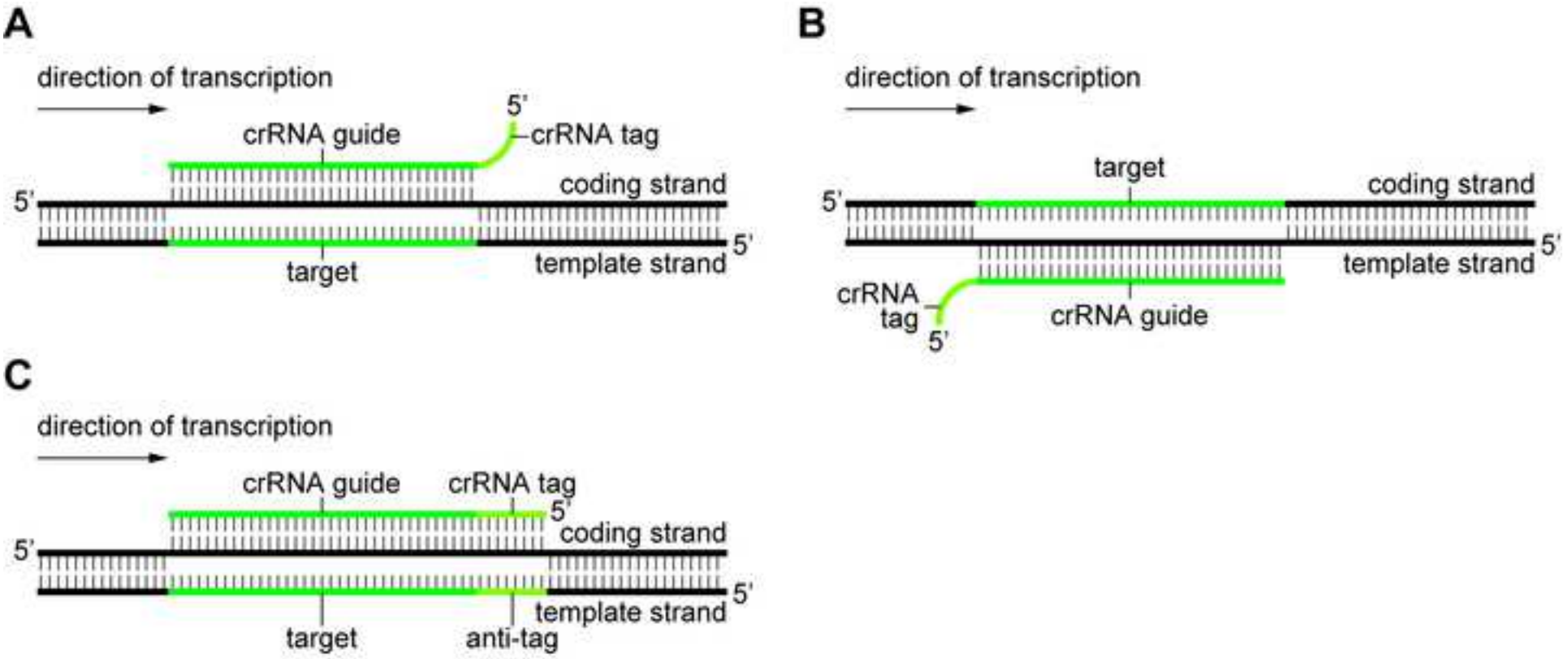

Figure S2. Samai *et al.*

Supplemental Figure 3
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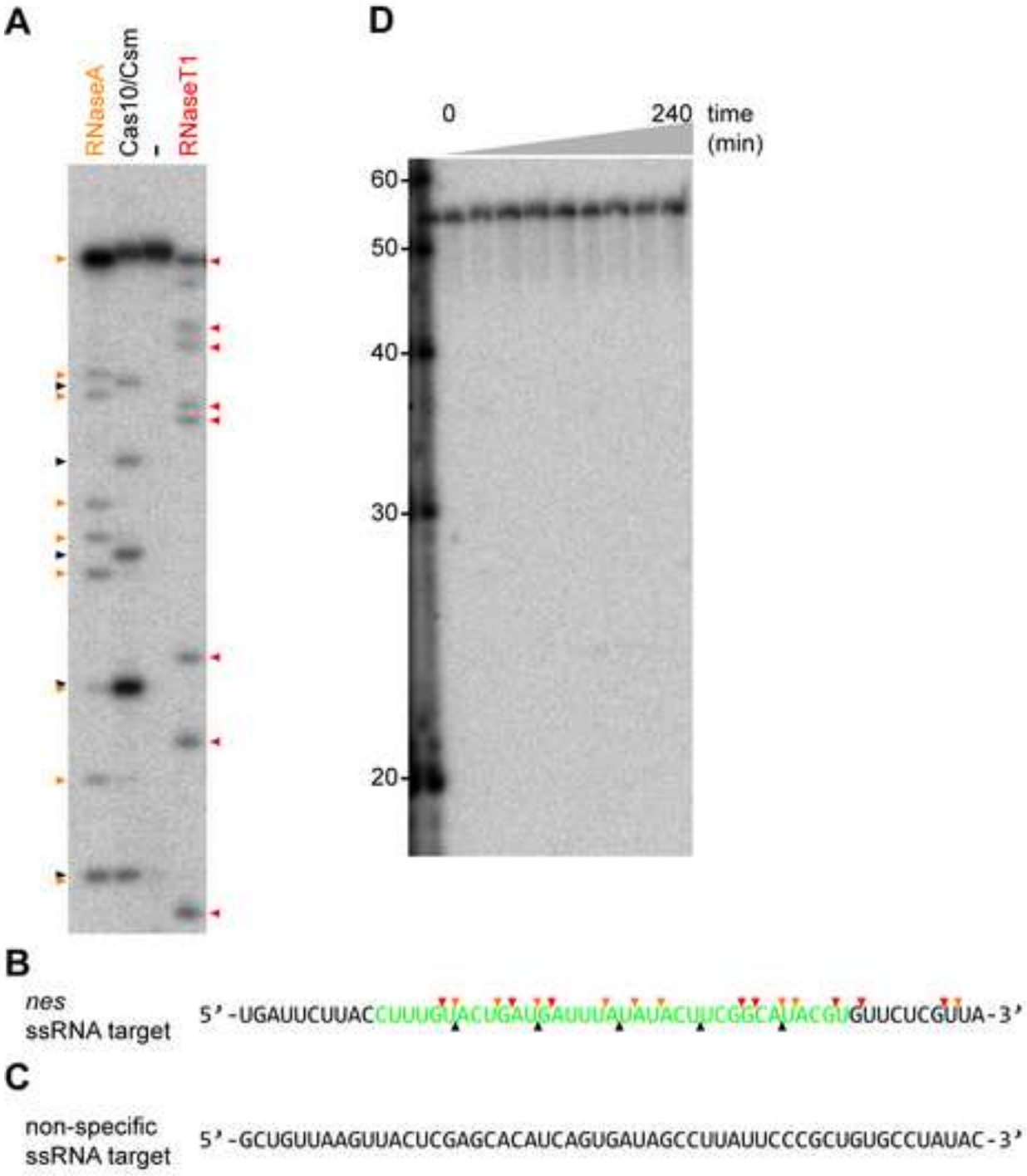

Figure S2. Samai *et al.*