Supporting Information

Table S1. Mass spectrometry identification of 70 kDa band co-purifying with Cas10-Csm following ultracentrifugation.

Protein	Uniparc accession	Theoretical mass (kDa)	Unique peptide count	Normalized spectral counts
Type III-A CRISPR-associated protein Cas10/Csm1	A0A199C221	88	30	41
Succinate dehydrogenase, flavoprotein subunit	Q5HQ26	66	15	13.5
Phosphoglucomutase	A0A0E2MYC2	61	5	4.5
Glycosyltransferase, group 1 family protein	A0A0E1VH90	59	3	2
Type III-A CRISPR-associated RAMP protein Csm5	A0A199BF65	39	3	2.5
Asparagine--tRNA ligase	A0A0E1VCU9	49	3	2.5
Myosin-cross-reactive antigen	A0A0U5QGJ3	45	2	1.5
4-hydroxy-tetrahydrodipicolinate reductase	A0A017IRS9	29	2	2
Catalase	A0A0E1VL99	58	2	1.5
Polyribonucleotide nucleotidyltransferase	A0A0E1VF71	78	2	1.5
Glycosyl transferase	A0A199KU13	59	2	1.5
Type III-A CRISPR-associated RAMP protein Csm3	A0A199C253	24	2	1.5
Type III-A CRISPR-associated protein Csm2	A0A199KLT6	17	2	1.5

Table S2. Primers used in the construction of p*crispr*/*csm3*^{D32A}.

Name	Sequence (5'-3')	Purpose
F027	GGAGCAATTGCTTCTCCTGTAGTTAG	Gibson assembly, p*crispr-cas csm3*^{D32A}
F014	CGACTGTAAAAAGTACAGTCGGCATTAT	
F028	CAGGAGAAGCAATTGCTCCAATCATAC	Gibson assembly, p*crispr-cas csm3*^{D32A}
F016	ACTGTACTTTTTACAGTCGGTTTTCTAATG	
A426	TTAAATTTTATTATGAAGCAGGACG	Colony PCR confirmation of p*crispr-cas csm3*^{D32A}
F004	AATTTGTCTTGGGTTTGCAACTG	
A414	CAAAGAGCTCGTCTACAAATTTC	Sequencing confirmation of p*crispr-cas csm3*^{D32A}
A415	ACAAGAAACTGATTCAAGTGCTG	
A416	TATTCTGAAAAGGTCAATCAAGG	
A417	GCGATGCTTCATATCGTGCG	
A418	CTACTTTAATAATTGAAAAAGATGG	
A419	GTCTTTTAAATATCAGAACAGTTAC	
A420	TTTAAAGTATATATCAGATTGTTTCG	
A421	GCCGAAGTATATAAATCATCAG	
A422	TTATGGTTATTCAATTCTCAGATC	
A423	ATCAATTTTTGTCCCAATTTTCAG	
A424	TTTTGTATACAGGTGGTGGCC	
A425	CAAATTACTGCTATATATTCAGGC	
A426	TTAAATTTTATTATGAAGCAGGACG	

Table S3. Target RNAs

Name	Method	Sequence (5'-3')	Description
ssRNA-01	Synthetic	CUUUGUACUGAUGAUUUAUAUACUUCG GCAUACGUUCUCUAAA	Analog of *nes* transcript
ssRNA-02	Synthetic	CUUUGUACUGAUGAUUUAUAUACUUCG GCAUACGUUCAGAAAA	Analog of *nes* transcript with no complementarity to the 5' tag region
ssRNA-03	Synthetic	CUUUGUACUGAUGAUUUAUAUACUUCG GCAUACGUGUUCUCGU	Complementary to *spc1* including the 5' tag region
ssRNA-NC	Synthetic	GCUGACAUUAAGAUUACUAUUUAUUAU CCUUGCGAUUUCUACG	Shuffled *nes* analog sequences with no complementarity to *spc1*
ssRNA-500	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU CGGCAUACGUUCUCUAAA	Analog of *nes* transcript generated by IVT
ssRNA-501	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU CGGCAUACGAUCUCUAAA	Analog of *nes* transcript with a mismatch at +1
ssRNA-502	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU CGGCAUACCUUCUCUAAA	Analog of *nes* transcript with a mismatch at +2
ssRNA-503	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU CGGCAUAGGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +3
ssRNA-504	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU CGGCAUUCGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +4
ssRNA-505	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU CGGCAAACGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +5
ssRNA-507	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU CGGGAUACGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +7
ssRNA-508	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU CGCCAUACGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +8
ssRNA-509	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU CCGCAUACGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +9
ssRNA-510	IVT	GGCUUUGUACUGAUGAUUUAUAUACUU GGGCAUACGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +10
ssRNA-511	IVT	GGCUUUGUACUGAUGAUUUAUAUACUA CGGCAUACGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +11
ssRNA-512	Synthetic	GGCUUUGUACUGAUGAUUUAUAUACUU CGGCAUACCUUCUCUAAA	Analog of *nes* transcript with a mismatch at +2
ssRNA-515	Synthetic	GGCUUUGUACUGAUGAUUUAUAUACUU CGGCAAACGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +5
ssRNA-517	Synthetic	GGCUUUGUACUGAUGAUUUAUAUACUU CGGGAUACGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +7
ssRNA-518	Synthetic	GGCUUUGUACUGAUGAUUUAUAUACUU CGCCAUACGUUCUCUAAA	Analog of *nes* transcript with a mismatch at +8

Table S4. Overlap extension PCR primers for generation of IVT templates

Name	Direction	Sequence (5'-3')	Purpose
prJAD500A	F	GAATTCTAATACGACTCACTATAGGCTTTGTACTGATGATTTATATACTTCGGCA	Introduces T7 promoter and templates 5' region of target RNA
prJAD500B	R	TTTAGAGAACGTATGCCGAAGTATATAAATC	Pairs to prJAD500A to generate ssRNA-500
prJAD501	R	TTTAGAGATCGTATGCCGAAGTATATAAATC	Pairs to prJAD500A to generate ssRNA-501
prJAD502	R	TTTAGAGAAGGTATGCCGAAGTATATAAATC	Pairs to prJAD500A to generate ssRNA-502
prJAD503	R	TTTAGAGAACCTATGCCGAAGTATATAAATC	Pairs to prJAD500A to generate ssRNA-503
prJAD504	R	TTTAGAGAACGAATGCCGAAGTATATAAATC	Pairs to prJAD500A to generate ssRNA-504
prJAD505	R	TTTAGAGAACGTTTGCCGAAGTATATAAATC	Pairs to prJAD500A to generate ssRNA-505
prMON507A	F	GAATTCTAATACGACTCACTATAGGCTTTGTACTGATGATTTATATACTTCGGGA	Introduces T7 promoter and templates 5' region of target RNA
prMON507B	R	TTTAGAGAACGTATCCCGAAGTATATAAATC	Pairs to prMON507A to generate ssRNA-507
prMON508A	F	GAATTCTAATACGACTCACTATAGGCTTTGTACTGATGATTTATATACTTCGCCA	Introduces T7 promoter and templates 5' region of target RNA
prMON508B	R	TTTAGAGAACGTATGGCGAAGTATATAAATC	Pairs to prMON508A to generate ssRNA-508
prMON509A	F	GAATTCTAATACGACTCACTATAGGCTTTGTACTGATGATTTATATACTTCCGCA	Introduces T7 promoter and templates 5' region of target RNA
prMON509B	R	TTTAGAGAACGTATGCGGAAGTATATAAATC	Pairs to prMON509A to generate ssRNA-509
prMON510A	F	GAATTCTAATACGACTCACTATAGGCTTTGTACTGATGATTTATATACTTGGGCA	Introduces T7 promoter and templates 5' region of target RNA
prMON510B	R	TTTAGAGAACGTATGCCCAAGTATATAAATC	Pairs to prMON510A to generate ssRNA-510
prMON511A	F	GAATTCTAATACGACTCACTATAGGCTTTGTACTGATGATTTATATACTACGGCA	Introduces T7 promoter and templates 5' region of target RNA
prMON511B	R	TTTAGAGAACGTATGCCGTAGTATATAAATC	Pairs to prMON511A to generate ssRNA-511