Supplementary Tables and Figures

Table S1. DNA oligonucleotides used in this study.

Primer	Sequence (5'-3')	Purpose
A17	5'-Biotin-CTTTGTACTGATGATTTATATACTTCGGCATACGT	*spc1* crRNA capture and protein affinity purification
F003	GCAAGAGTAACAAGAGGATCTGAGTTTG	inverse PCR: p*crispr/csm3*^{E140A}
F004	AATTTGTCTTGGGTTTGCAACTG	
F005	TCACTTATTAGAGAATGCCTATCTTGGTGG	inverse PCR: p*crispr/csm3*^{D179A}
F006	ATCGCTTTTTCAATATTCTCAAAATCATCC	
AA001	AACAAAGCCATTTATAGCAAAATTAAAGTCAGC	inverse PCR: p*crispr/csm5*^{D153A}
AA002	TTTTTCATTATTATATTTTGACACGAGAGTTGTTTTG	
F033	GAATTGTCAGATAGGCCTAATGACTGG	Gibson assembly of p*crispr* mutants
F034	GGCCTATCTGACAATTCCTGAATAG	
F118	CGGGCTTACAACAAGCGCGTTAGCAAATTTAATGGAGCAGG	Gibson assembly: p*crispr/csm2*^{K47A,R49A}
F119	CCTGCTCCATTAAATTTGCTAACGCGCTTGTTGTAAGCCCG	
F194	GAAGCAGGACGAGCAAAAAGCGTTGATG	Gibson assembly: p*crispr/csm2*^{E92A}
F195	CATCAACGCTTTTTGCTCGTCCTGCTTC	
F011	ATTATTGTAAATACTTTGCAGCTTTAGTTGCATAC	Gibson assembly: p*crispr/csm2*^{E128A}
F030	CAACTAAAGCGTCAAAGTATTTACAATAATCTAAG	
F031	CAAAAGGAGGCTTAATATGTATTCAAAAATTAAAATTTC	Gibson assembly: p*crispr/csm2*^{D141A}
F032	CATATTAAGCCTCCTTTTGATAATATTTAGCG	
F120	GGAGGATTAATATGTATTCAGCAATTAAAATTTCAGGAACAATTGAAG	Gibson assembly: p*crispr/csm3*^{K4A}
F121	CTTCAATTGTTCCTGAAATTTTAATTGCTGAATACATATTAATCCTCC	
F122	CCTATCATACCTGGCAGTTCAATCGCAGGAGCAATGGCAAATTTATTAGCAAAAC	Gibson assembly: p*crispr/csm3*^{K52A,K54A,R56A}
F123	GTTTTGCTAATAAATTTGCCATTGCTCCTGCGATTGAACTGCCAGGTATGATAGG	
F128	GGTCAAGTTATGAAGAAGCAAGCTTACATTTATGAC	Gibson assembly: p*crispr/csm5*^{D28A}
F129	GTCATAAATGTAAGCTTGCTTCTTCATAACTTGACC	
F196	CAAGTTATGAAGAAGCAAGATTACATTTATGCCTTTTATAATTC	Gibson assembly: p*crispr/csm5*^{D32A}
F197	GAATTATAAAAGGCATAAATGTAATCTTGCTTCTTCATAAC	
F035	CCGCTAAATGCTTTACACTTAATGGTAAG	Gibson assembly: p*crispr/csm5*^{D115A}
F036	CATTAAGTGTAAAGCATTTAGCGGTTTAGG	
F037	TAAGAGCCGGTCAAAATAAAGTGTATCTTC	Gibson assembly: p*crispr/csm5*^{D122A}
F038	GATACACTTTATTTTGACCGGCTCTTACC	
F130	GCGATTTATCAAAAAATAGCCATTAATAAAAGTGAAAAATCAATG	Gibson assembly: p*crispr/csm5*^{D178A}
F131	GATTTTTCACTTTTATTAATGGCTATTTTTTGATAAATCGC	
F039	CAATGCCTTTATATAGAGCGTGCATAGATGTAAATACCG	Gibson assembly: p*crispr/csm5*^{E191A}
F040	CATCTATGCACGCTCTATATAAAGGCATTGATTTTTCAC	
F078	GGAGAATACGATAGTACAAATCTTAAAATTAAGTAGCTCGAGCACCACCACCACCAC	pET28b-His$_{10}$Smt3-*csm3*^{K4A} and *csm3*^{D179A} construction: pET28b amplification
F079	GTGGTGGTGGTGGTGCTCGAGCTACTTAATTTTAAGATTTGTACTATCGTATTCTCC	pET28b-His$_{10}$Smt3-*csm3*^{K4A} and

		csm3^{D179A} construction: *csm3* amplification
F088	CAGAGAACAGATTGGTGGATCCATGTATTCAAAAATTAAAATTTCAGGAACAATTGAAG	pET28b-His$_{10}$Smt3-*csm3*^{D179A} construction: *csm3* amplification
F089	CTTCAATTGTTCCTGAAATTTTAATTTTTGAATACATGGATCCACCAATCTGTTCTCTG	pET28b-His$_{10}$Smt3-*csm3*^{D179A} construction: pET28b amplification
F146	CAGAGAACAGATTGGTGGATCCATGTATTCAGCAATTAAAATTTCAGGAACAATTGAAG	pET28b-His$_{10}$Smt3-*csm3*^{K4A}: *csm3* amplification
F147	CTTCAATTGTTCCTGAAATTTTAATTGCTGAATACATGGATCCACCAATCTGTTCTCTG	pET28b-His$_{10}$Smt3-*csm3*^{K4A} construction: pET28b amplification
A159	ACAGAGAACAGATTGGTGGATCCATGACAATAAAAAATTATGAAGTCGTTATTAAAAC	pET28b-His$_{10}$Smt3-*csm5* and pET28b-His$_{10}$Smt3-*csm5*^{D162A} construction: *csm5* amplification
A160	GAGTTTTCAAGAGTTAAATAATGAGGTGCTATAACTCGAGCACCACCACCACCACCAC	
A161	CAAGAGTTAAATAATGAGGTGCTATAACTCGAGCACCACCACCACCACCACTGAG	pET28b-His$_{10}$Smt3-*csm5* and pET28b-His$_{10}$Smt3-*csm5*^{D162A} construction: pET28b amplification
A162	GCTCACAGAGAACAGATTGGTGGATCCATGACAATAAAAAATTATGAAGTCG	
F134	GCGTCACATAAAGTATTAGAGCAATCTAAAAATTAACTCGAGCACCACCACCACCACCAC	pET28b-His$_{10}$Smt3-*pnpase* construction*:* pET28b amplification
F137	CAGTTTTAAAAACTTTCTTCTCTTGAGACATGGATCCACCAATCTGTTCTCTG	
F135	GTGGTGGTGGTGGTGGTGCTCGAGTTAATTTTTAGATTGCTCTAATACTTTATGTGACGC	pET28b-His$_{10}$Smt3-*pnpase* construction: *pnpase* amplification
F136	CAGAGAACAGATTGGTGGATCCATGTCTCAAGAGAAGAAAGTTTTTAAAACTG	
F139	CATTTCTTCGATGGATTGCTTTAAATTCATGGATCCACCAATCTGTTCTCTG	pET28b-His$_{10}$Smt3-*rnaser* construction: *pET28b* amplification
F140	GAAATCGCGTCGAAAGAAAAAATAGCTCGAGCACCACCACCACCACCACTG	
F138	CAGAGAACAGATTGGTGGATCCATGAATTTAAAGCAATCCATCGAAGAAATG	pET28b-His$_{10}$Smt3-*rnaser* construction: *rnaser* amplification
F141	GGTGGTGGTGGTGGTGCTCGAGCTATTTTTTCTTTCGACGCGATTTC	
A146	CGCGGATCCATGAGAAATGTAGAAAAATTAAATCCCGGCG	pET28b-His$_{10}$Smt3-*cbf1* construction
A147	CGCCTCGAGTTAATCAAGTGTTGTTGGATTATAAAATTGTCTATTTTC	
A414	CAAAGAGCTCGTCTACAAATTTC	Sequencing confirmation: p*crispr*-based vectors
A415	ACAAGAAACTGATTCAAGTGCTG	
A416	TATTCTGAAAAGGTCAATCAAGG	
A417	GCGATGCTTCATATCGTGCG	
A418	CTACTTTAATAATTGAAAAAGATGG	
A419	GTCTTTTAAATATCAGAACAGTTAC	
A420	TTTAAAGTATATATCAGATTGTTTCG	
A421	GCCGAAGTATATAAATCATCAG	

A422	TTATGGTTATTCAATTCTCAGATC	
A423	ATCAATTTTTGTCCCAATTTTCAG	
A424	TTTTGTATACAGGTGGTGGCC	
A425	CAAATTACTGCTATATATTCAGGC	
A426	TTAAATTTTATTATGAAGCAGGACG	
T7P	TAATACGACTCACTATAGGG	Sequencing confirmation: pET28b-based vectors
T7T	TATGCTAGTTATTGCTCAG	

Table S2. Mass spectrometry analysis of CRISPR-associated proteins that co-purify with Csm2^{H6N}.

CRISPR-associated protein	Theoretical Mass (KDa)	Unique peptide count	Normalized spectral counts[a]
Cas 10	88	61	807.65
Csm2^{H6N}	17	17	336.37
Csm3	24	17	419.80
Csm4	34	17	315.96
Csm5	39	27	227.21
Csm6	50	5	7.99
Cas6	29	3	6.21

a. Values reflect relative protein abundance.

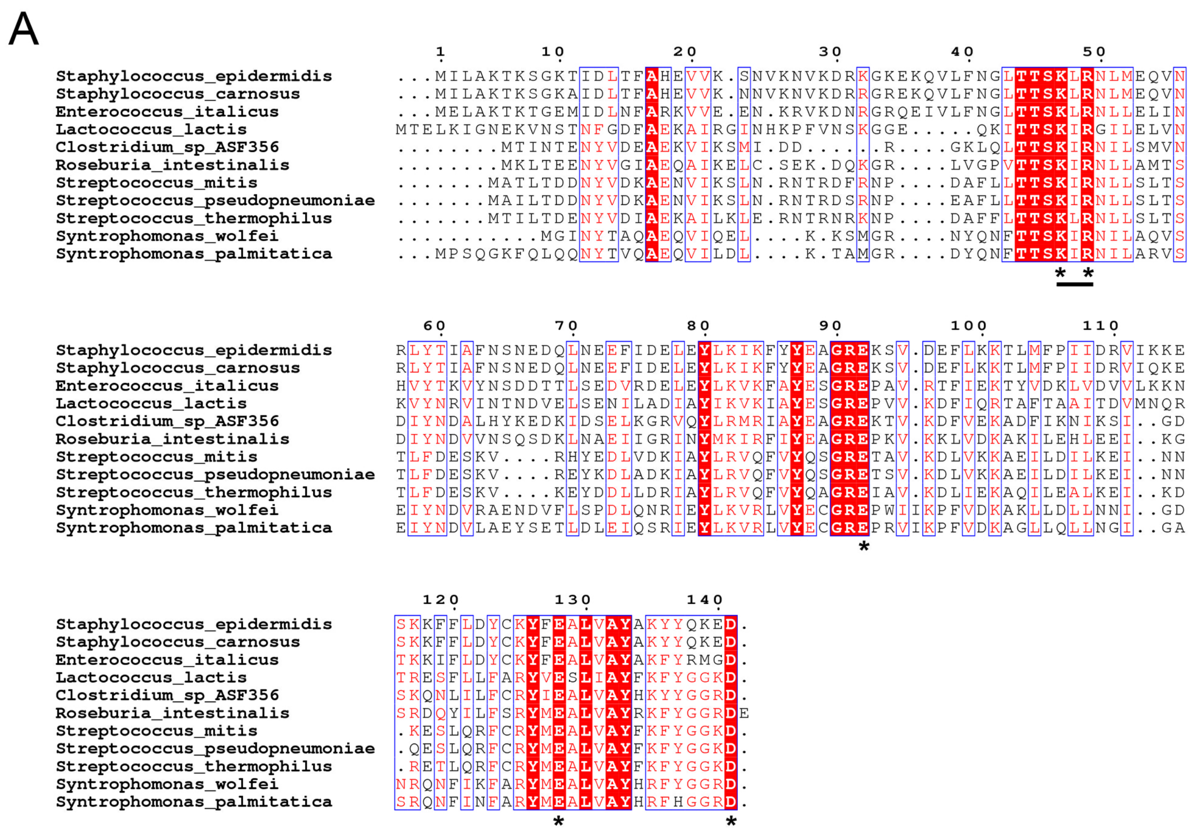
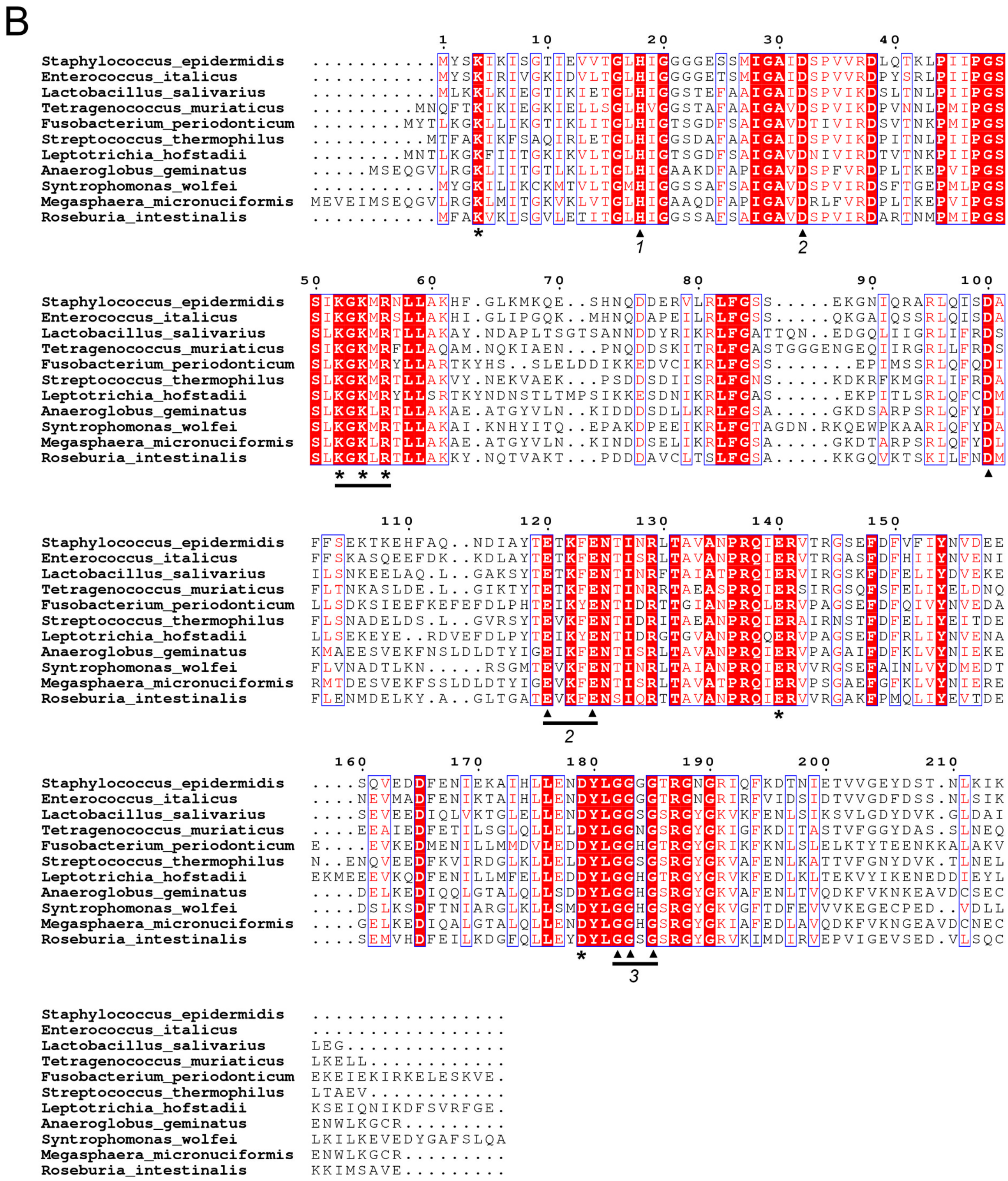


Figure S1. BLAST sequence alignments of Csm2 (A), Csm3 (B), and Csm5 (C) in indicated organisms. Related to Figure 1.

Conserved residues are highlighted in red. Residues mutated in this study (asterisks, *) and previous studies (arrows) are indicated. Underlying bars connect residues that were mutated together. Numbers in italics (*1-3*) represent references in which previously-characterized mutants were reported as follows: *1*, Hatoum-Aslan *et al*, *J Biol Chem*, 2013; *2*, Samai *et al*, Cell, 2015; *3*, Hatoum-Aslan et al, *J Bact*, 2014.


B
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Enterococcus_italicus ...........MYSKIRIVGKIDVLTGLHIGGGGETSMIGAIDSPVVRDPYSRLPIIPGS
Lactobacillus_salivarius ...........MLKKLKIEGTIKIETGLHIGGSTEFAAIGAIDSPVIKDSLTNLPIIPGS
Tetragenococcus_muriaticus .........MNQFTKIKIEGKIELLSGLHVGGSTAFAAIGAVDSPVIKDPVNNLPMIPGS
Fusobacterium_periodonticum ........MYTLKGKLLIKGTIKLITGLHIGTSGDFSAIGAVDTIVIRDSVTNKPMIPGS
Streptococcus_thermophilus ..........MTFAKIKFSAQIRLETGLHIGGSDAFAAIGAIDSPVIKDPITNLPIIPGS
Leptotrichia_hofstadii ........MNTLKGKFIITGKIKVLTGLHIGTSGDFSAIGAVDNIVIRDTVTNKPIIPGS
Anaeroglobus_geminatus .....MSEQGVLRGKLIITGTLKLLTGLHIGAAKDFAPIGAVDSPFVRDPLTKEPVIPGS
Syntrophomonas_wolfei ...........MYGKILIKCKMTVLTGMHIGGSSAFSAIGAVDSPVIRDSFTGEPMLPGS
Megasphaera_micronuciformis MEVEIMSEQGVLRGKLMITGKVKLVTGLHIGAAQDFAPIGAVDRLFVRDPLTKEPVIPGS
Roseburia_intestinalis ...........MFAKVKISGVLETITGLHIGGSSAFSAIGAVDSPVIRDARTNMPMIPGS
*
1
2
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Enterococcus_italicus SIKGKMRSLLAKHI.GLIPGQK..MHNQDAPEILRLFGSS.....QKGAIQSSRLQISDA
Lactobacillus_salivarius SLKGKMRTLLAKAY.NDAPLTSGTSANNDDYRIKRLFGATTQN..EDGQLIIGRLIFRDS
Tetragenococcus_muriaticus SIKGKMRFLLAQAM.NQKIAEN...PNQDDSKITRLFGASTGGGENGEQIIRGRLLFRDS
Fusobacterium_periodonticum SLKGKMRYLLARTKYHS..SLELDDIKKEDVCIKRLFGSS.......EPIMSSRLQFQDI
Streptococcus_thermophilus SLKGKMRTLLAKVY.NEKVAEK...PSDDSDIISRLFGNS.....KDKRFKMGRLIFRDA
Leptotrichia_hofstadii SLKGKMRYLLSRTKYNDNSTLTMPSIKKESDNIKRLFGAS......EKPITLSRLQFCDM
Anaeroglobus_geminatus SLKGKLRTLLAKAE.ATGYVLN..KIDDDSDLLKRLFGSA.....GKDSARPSRLQFYDL
Syntrophomonas_wolfei SLKGKMRTLLAKAI.KNHYITQ..EPAKDPEEIKRLFGTAGDN.RKQEWPKAARLQFYDA
Megasphaera_micronuciformis SLKGKLRTLLAKAE.ATGYVLN..KINDDSELIKRLFGSA.....GKDTARPSRLQFYDL
Roseburia_intestinalis SLKGKLRTLLAKKY.NQTVAKT...PDDDAVCLTSLFGSA.....KKGQVKTSKILFNDM
* * *
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Enterococcus_italicus FFSKASQEEFDK..KDLAYTETKFENTISRLTAVANPRQIERVTRGASFDFHIIYNVENI
Lactobacillus_salivarius ILSNKEELAQ.L..GAKSYTETKFENTINRFTAIATPRQIERVIRGSKFDFELIYDVEKE
Tetragenococcus_muriaticus FLTNKASLDE.L..GIKTYTETKFENTINRRTAEASPRQIERSIRGSQFSFELIYELDNQ
Fusobacterium_periodonticum LLSDKSIEEFKEFEFDLPHTEIKYENTIDRTTGIANPRQLERVPAGSEFDFQIVYNVEDA
Streptococcus_thermophilus FLSNADELDS.L..GVRSYTEVKFENTIDRITAEANPRQIERAIRNSTFDFELIYEITDE
Leptotrichia_hofstadii LLSEKEYE..RDVEFDLPYTEIKYENTIDRGTGVANPRQQERVPAGSEFDFRLIYNVENA
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Syntrophomonas_wolfei FLVNADTLKN.....RSGMTEVKFENTINRLTAIANPRQIERVVRGSEFAINLVYDMEDT
Megasphaera_micronuciformis RMTDESVEKFSSLDLDTYIGEVKFENTISRLTAVATPRQIERVPSGAEFGFKLVYNIERE
Roseburia_intestinalis FLENMDELKY.A..GLTGATEVKFENSIQRTTAVANPRQIERVVRGAKFPMQLIYEVTDE
*
2
160 170 180 190 200 210
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Enterococcus_italicus ....NEVMADFENIKTAIHLLENDYLGGGGTRGNGRIRFVIDSIDTVVGDFDSS.NLSIK
Lactobacillus_salivarius ....SEVEEDIQLVKTGLELLENDYLGGSGSRGYGKVKFENLSIKSVLGDYDVK.GLDAI
Tetragenococcus_muriaticus ....EEAIEDFETILSGLQLLELDYLGGNGSRGYGKIGFKDITASTVFGGYDAS.SLNEQ
Fusobacterium_periodonticum E....EVKEDMENILLMMDVLEDDYLGGHGTRGYGRIKFKNLSLELKTYTEENKKALAKV
Streptococcus_thermophilus N..ENQVEEDFKVIRDGLKLLELDYLGGSGSRGYGKVAFENLKATTVFGNYDVK.TLNEL
Leptotrichia_hofstadii EKMEEEVKQDFENILLMFELLEDDYLGGHGTRGYGRVKFEDLKLTEKVYIKENEDDIEYL
Anaeroglobus_geminatus ....DELKEDIQQLGTALQLLSDDYLGGHGSRGYGKVAFENLTVQDKFVKNKEAVDCSEC
Syntrophomonas_wolfei ....DSLKSDFTNIARGLKLLSMDYLGGHGSRGYGKVGFTDFEVVVKEGECPED..VDLL
Megasphaera_micronuciformis ....GELKEDIQALGTALQLLENDYLGGHGSRGYGKIAFEDLAVQDKFVKNGEAVDCNEC
Roseburia_intestinalis ....SEMVHDFEILKDGFQLLEYDYLGGSGSRGYGRVKIMDIRVEPVIGEVSED.VLSQC
*
3
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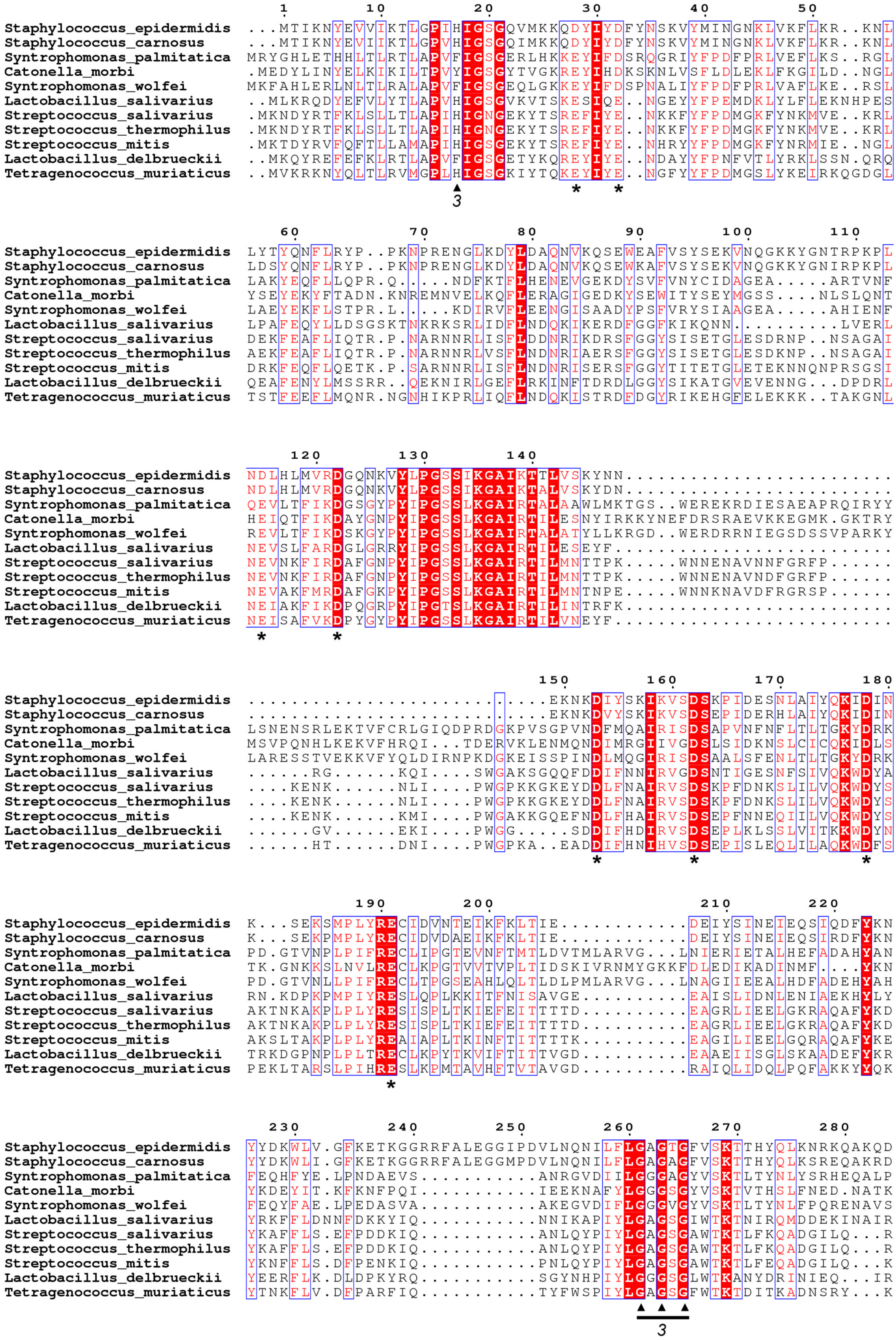
Enterococcus_italicus ....
Lactobacillus_salivarius LEG..............
Tetragenococcus_muriaticus LKELL............
Fusobacterium_periodonticum EKEIEKIRKELESKVE.
Streptococcus_thermophilus LTAEV............
Leptotrichia_hofstadii KSEIQNIKDFSVRFGE.
Anaeroglobus_geminatus ENWLKGCR.........
Syntrophomonas_wolfei LKILKEVEDYGAFSLQA
Megasphaera_micronuciformis ENWLKGCR.........
Roseburia_intestinalis KKIMSAVE.........

C



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Catonella_morbi .MEDYLINYELKIKILTPVYIGSGYTVGKREYIHDKSKNLVSFLDLEKLFKGILD..NGL
Syntrophomonas_wolfei MKFAHLERLNLTLRALAPVFIGSGEQLGKKEYIFDSPNALIYFPDFPRLVAFLKE..RSL
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Streptococcus_thermophilus .MKNDYRTFKLSLLTLAPIHIGNGEKYTSREFIYE..NKKFYFPDMGKFYNKMVE..KRL
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Lactobacillus_delbrueckii ..MKQYREFEFKLRTLAPVFIGSGETYKQREYIYE..NDAYYFPNFVTLYRKLSSN.QRQ
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3
* *
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Catonella_morbi YSEYEKYFTADN.KNREMNVELKQFLERAGIGEDKYSEWITYSEYMGSS....NLSLQNT
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Streptococcus_mitis DRKFEQFLQETK.P.SARNNRLISFLNDNRISERSFGGYTITETGLETEKNNQNPRSGSI
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Syntrophomonas_wolfei REVLTFIKDSKGYPYIPGSSLKGAIRTALATYLLKRGD..WERDRRNIEGSDSSVPARKY
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Streptococcus_salivarius NEVNKFIRDAFGNPYIPGSSLKGAIRTILMNTTPK.....WNNENAVNNFGRFP......
Streptococcus_thermophilus NEVNKFIRDAFGNPYIPGSSLKGAIRTILMNTTPK.....WNNENAVNDFGRFP......
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Catonella_morbi TK.GNKKSLNVLRECLKPGTVVTVPLTIDSKIVRNMYGKKFDLEDIKADINMF....YKN
Syntrophomonas_wolfei PD.GTVNLLPIFRECLTPGSEAHLQLTLDLPMLARVG...LNAGIIEEALHDFADEHYAH
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Streptococcus_thermophilus AKTNKAKPLPLYRESISPLTKIEFEITTTTD..........EAGRLIEELGKRAQAFYKD
Streptococcus_mitis AKSLTAKPLPLYREAIAPLTKINFTITTTTK..........EAGILIEELGQRAQAFYKE
Lactobacillus_delbrueckii TRKDGPNPLPLTRECLKPYTKVIFTITVGD...........EAAEIISGLSKAADEFYKR
Tetragenococcus_muriaticus PEKLTARSLPIHRESLKPMTAVHFTVTAVGD..........RAIQLIDQLPQFAKKYYQK
*
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Staphylococcus_carnosus YYDKWLI.GFKETKGGRRFALEGGMPDVLNQNILFLGAGAGFVSKTTHYQLKSREQAKRD
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Catonella_morbi YKDEYIT.KFKNFPQI..........IEEKNAFYLGGGSGYVSKTVTHSLFNED.NATK
Syntrophomonas_wolfei FEQYFAE.LPEDASVA..........AKEGVDIFLGGVGYVSKTLTYNLFPQRENAVS
Lactobacillus_salivarius YRKFFLDNNFDKKYIQ..........NNIKAPIYLGAGSGIWTKTNIRQMDDEKINAIR
Streptococcus_salivarius YKAFFLS.EFPDDKIQ..........ANLQYPIYLGAGSGAWTKTLFKQADGILQ...R
Streptococcus_thermophilus YKAFFLS.EFPDDKIQ..........ANLQYPIYLGAGSGAWTKTLFKQADGILQ...R
Streptococcus_mitis YKNFFLS.DFPENKIQ..........PNLQYPIYLGAGSGAWTKTLFEQADGILQ...K
Lactobacillus_delbrueckii YEERFLK.DLDPKYRQ..........SGYNHPIYLGGGSGLWTKANYDRINIEQ...IR
Tetragenococcus_muriaticus YTNKFLV.DFPARFIQ..........TYFWSPIYLGAGSGFWTKTDITKADNSRY...K
3

```
                                   290       300       310                             320
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Staphylococcus_carnosus     SFDI.LSKKFR..RTYGKMKEIPSN.VPVALKG..............TTNQSRRTSYQQG
Syntrophomonas_palmitatica  LVSKIMLKQF.PKH..G.......HHRDV........KEHHVSPHILKTTLYKDEYYQMG
Catonella_morbi             VVSEILNEVFTQKSKPS.......ANKDD........EVLGVSPHTLKCTYYLENLCQMG
Syntrophomonas_wolfei       LAAKILTKQFSPKH..G.......HSKDA........SQYKVSPHILKTTMYAGEYYHMG
Lactobacillus_salivarius    RKQY.VKMRMKDKGVMK.LTKYPTN....................VDSKIVKTKNFYEMG
Streptococcus_salivarius    RYSR.MKTKMVKKGVLK.LTKAPLKTVKIPSG.............NHSLIKNHESFYEIG
Streptococcus_thermophilus  RYSR.MKTKMVKKGVLK.LTKAPLKTVKIPSG.............NHSLVKNHESFYEMG
Streptococcus_mitis         RYSR.MKTKMVGKGVLK.LTKAPDKSIKTNKG.............ERKLIINSDSFYEMG
Lactobacillus_delbrueckii   SRTP.RKMKMTGNGVFK.LTKAKRQSYRLKIKGVIEERELVGKNGGAADTKDGIGFYEMG
Tetragenococcus_muriaticus  K..R.GKMSMKGKGVLK.LTKAPMVKYRLNGK.............SRQLIENSENLYEMG

                              330       340
Staphylococcus_epidermidis  MCKVSFQELNNEVL
Staphylococcus_carnosus     MCKLSFQELNNEVL
Syntrophomonas_palmitatica  RCELVFD.......
Catonella_morbi             LCRIVD........
Syntrophomonas_wolfei       KCELIITR......
Lactobacillus_salivarius    KCNFEVKKKS....
Streptococcus_salivarius    KANFMIKEIDK...
Streptococcus_thermophilus  KANFMIKEIDK...
Streptococcus_mitis         KANFMIREILQ...
Lactobacillus_delbrueckii   KCCFTIKEKK....
Tetragenococcus_muriaticus  KCVFSLKEGEK...
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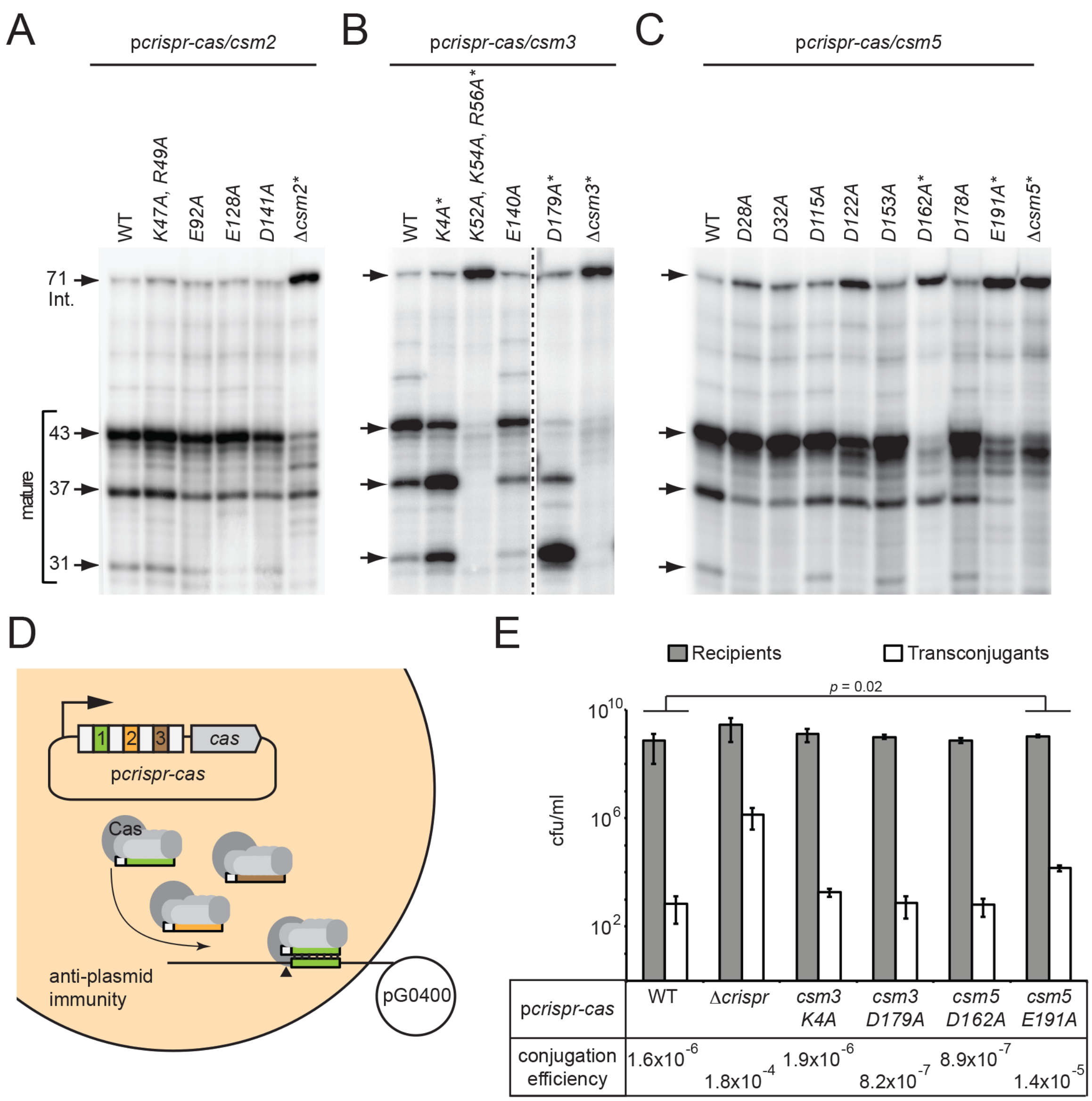

Figure S2. Conserved residues in Cas10-csm impact crRNA length distribution, maturation efficiency, and anti-plasmid immunity. Related to Figures 2 and 3.

Spc1 crRNAs in the presence of mutations in Csm2 (A), Csm3 (B), and Csm5 (C) are shown. Mutations were introduced into p*crispr-cas*, expressed in *S. aureus* RN4220, and confirmed in *S. epidermidis* LM1680 if a maturation defect was observed (*). *Spc1* crRNAs were captured from total RNA extracts using a biotinylated oligonucleotide probe antisense to *spc1*, radiolabeled on their 5' ends, and resolved using denaturing PAGE. The dotted line in (B) separates non-contiguous lanes in the same gel. (D) The conjugation assay used to measure anti-plasmid immunity in which p*crispr-cas* provides *spc1*-mediated immunity against the conjugative plasmid pG0400. (E) Efficiency of pG0400 transfer in the presence of indicated p*crispr-cas* constructs in *S. epidermidis* LM1680 (used as recipients). Conjugation was carried out in triplicate; the values (in cfu/ml; mean +/- S.D.) obtained for recipients and transconjugants are shown. Conjugation efficiency is calculated as the average numbers of transconjugants/recipients. The significant difference between conjugation efficiencies observed for wild-type CRISPR-cas and the *csm5*^{E191A} mutant variant (t-test, p=0.02) is indicated.

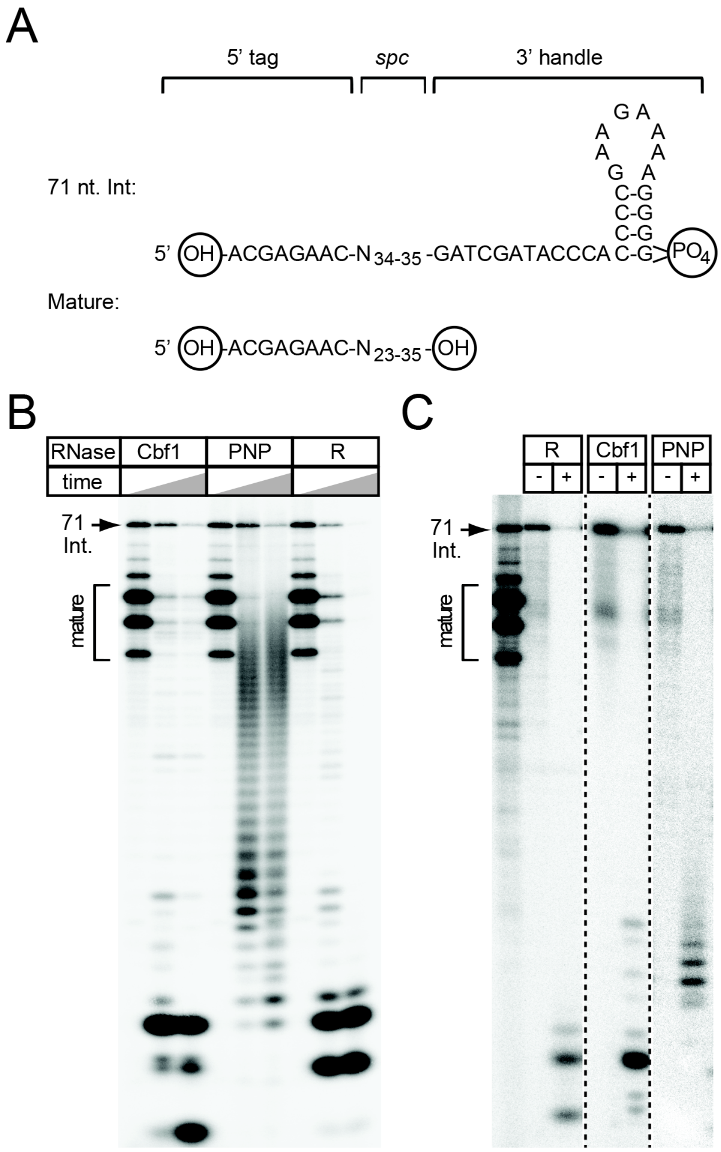

Figure S3. Cellular nucleases degrade both intermediate and mature crRNAs. Related to Figure 4.

(A) Sequences and end-features of intermediate and mature crRNAs are shown. Intermediate crRNAs possess a "3' handle" consisting of structured repeat sequence and a 2',3'-cyclic phosphate group, both of which might pose a barrier to 3'-5' exonucleases. (B) The activities of cellular exonucleases Cbf1, PNPase (PNP) and RNase R (R) against intermediate and mature crRNAs extracted from wild-type Cas10-Csm complexes are shown. CrRNAs were radiolabeled on their 5' ends, and used as substrates in nuclease assays containing Mg^{2+} (PNPase and RNase R) or Mn^{2+} (Cbf1). Indicated nucleases (1 pmol) were added, and the reaction was allowed to proceed at 37°C for 0, 5, or 10 minutes. (C) Intermediate crRNAs purified from Cas10-Csm/ΔCsm5 complexes were challenged with cellular exonucleases as described for panel B. Reactions were allowed to proceed at 37°C for 10 minutes. The leftmost lane contains uncut, wild-type crRNAs as a reference. RNAs were resolved using denaturing PAGE. Dotted lines separate lanes derived from different gels.

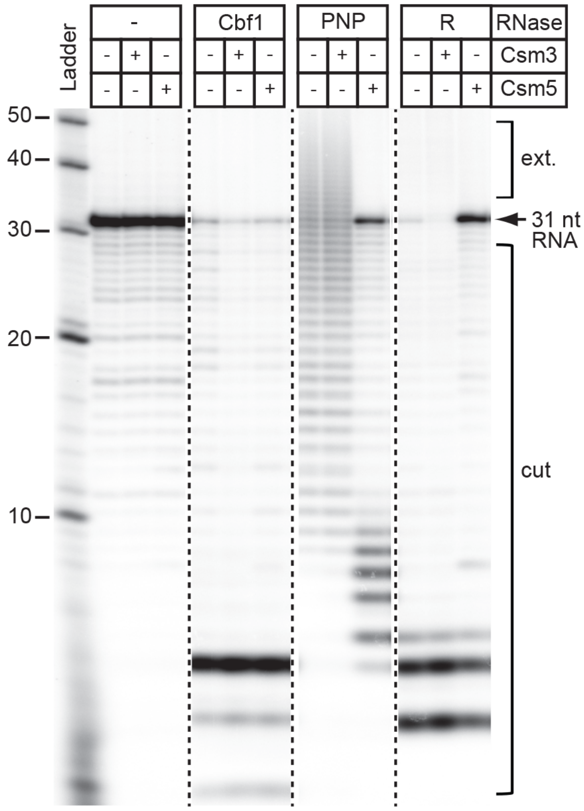

Figure S4. Csm3 does not impact the activity of cellular nucleases. Related to Figure 4.

A 31-nucleotide RNA substrate radiolabeled on the 5'-end (Fig. 3E) was pre-incubated for 2 minutes with Csm3 or Csm5 (4 pmols) where indicated in a buffer containing Mg^{2+} (PNPase and RNase R) or Mn^{2+} (Cbf1). Indicated nucleases were then added (1 pmol), and the reaction was allowed to proceed at 37°C for 10 minutes. RNAs were resolved using denaturing PAGE. Dotted lines separate non-contiguous lanes in the same gel.

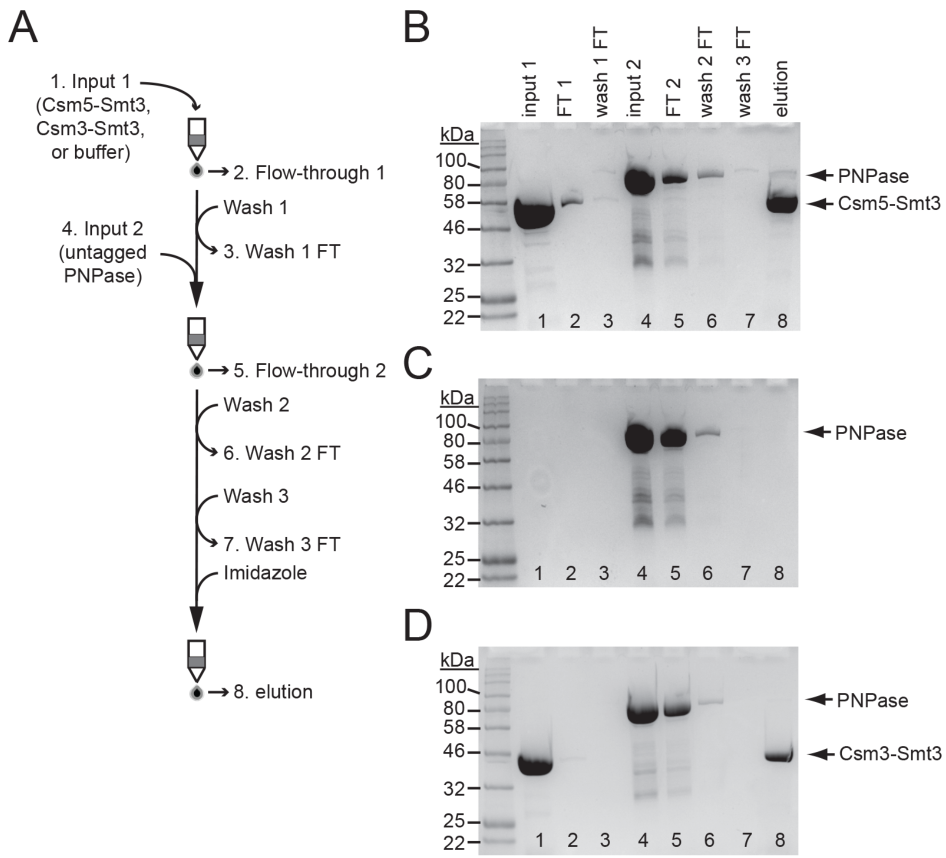

Figure S5. PNPase associates with Csm5, but not Csm3. Related to Figure 5.

(A) Illustration of the experimental flow of a pull-down assay in which 10-His Smt3-tagged Csm proteins (Csm3-Smt3 or Csm5-Smt3) or dialysis buffer are loaded onto a column containing Ni^{2+}-agarose beads, and their interaction with untagged PNPase is assessed by their ability to bind and retain PNPase in the column after thorough washing. (B-D) Csm5-Smt3 (1 nmol, panel B), dialysis buffer (panel C), or Csm3-Smt3 (0.5 nmol, panel D) were applied to the column in the first step as "input 1". For all experiments, PNPase (0.7 nmol) was applied as "input 2". Samples were collected at each numbered step outlined in the experimental flow (panel A), and resolved using SDS-PAGE. Shown is a representative of three (Csm3-Smt3), four (Csm5-Smt3) or eight (dialysis buffer) replicates. Signal intensities of PNPase in the final elutions (+/- S.D.) are as follows: Panel B, 5.9% +/- 2.4; Panel D, 1.2% +/- 0.8. FT, flow-through.

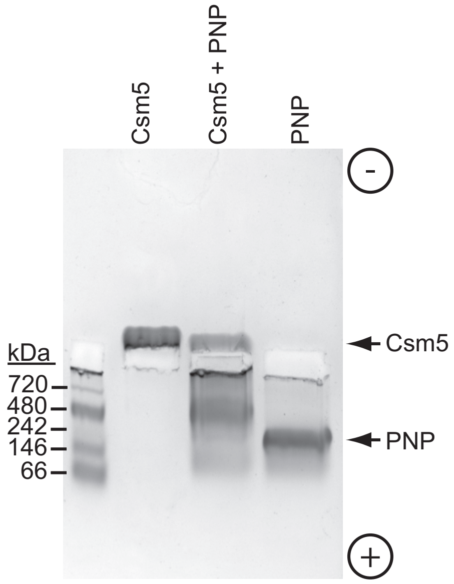

Figure S6. PNPase associates with Csm5. Related to Figure 5.

Csm5 and PNPase (100 pmols each) were resolved on a 5% horizontal native gel with wells cast in the center of the gel. The position of (+) and (-) electrodes are indicated. NativeMark Protein Standard (Thermo Fisher Scientific) was used to estimate molecular weight for proteins migrating toward the (+) electrode. Proteins were visualized with Coomassie G-250. Shown is a representative of four independent trials.