DNA Manipulation and Strain Construction

The strains used in this study are described in Supplementary Materials Table S1. Phusion DNA polymerase, restriction enzymes, and T4 DNA ligase were all purchased from New England Biolabs. The PCR primers used in this study are listed in Table S2.

Creation of hdrRM operon luciferase reporter strains

To create the markerless replacement of the *hdrRM* ORFs with that of luciferase, we first created an allelic replacement of the *hdrRM* ORFs with the counterselectable IFDC2 cassette (Xie et al. 2011). Using UA140 genomic DNA as a template, two fragments corresponding to the upstream region and downstream regions of the *hdrRM* operon were amplified with the primer pairs hdrRupF/hdrRupR-ldh and hdrMdnF-erm/hdrMdnR, respectively. The IFDC2 cassette was amplified using the primer pair ldhF/ermR. The three fragments were mixed and used as template for overlap extension PCR (OE-PCR) with the primer pair hdrRupF/hdrMdnR. The resulting OE-PCR product was transformed into UA140 and selected on medium containing erythromycin to obtain strain RMIFDC2. Next, a DNA fragment containing the *hdrR* upstream region and luciferase ORF was amplified with the primer pair hdrRupF/lucR-1856 and strain LZ89-luc (Merritt et al. 2007) as a template. Using strain UA140 as a template, a fragment corresponding to the *hdrM* downstream region was amplified with the primer pair 1856F-luc/hdrMDnR. The two fragments were mixed and assembled with OE-PCR using the primer pair hdrRupF/hdrMdnR. The OE-PCR amplicon was transformed into strain RMIFDC2 and selected on medium containing *p*-chlorophenylalanine (4-CP) to obtain strain RpLuc. To create strains Rp+1luc and Rp-10mluc, the upstream and downstream regions of the *hdrRM* operon were amplified from strain UA140 with the primer pairs hdrRupF/(luc)hdrRp-R or hdrRupF/(luc)hdrRp-10-R and (lucR)hdrMdn-F/hdrMDn-R, respectively. The luciferase ORF was amplified from strain RpLuc with the primer pair lucF/lucR. The three fragments were mixed and used as template for OE-PCR with the primer pair hdrRupF/hdrMdnR. OE-PCR products were transformed into RMIFDC2 and selected on medium containing 4-CP to obtain the strains Rp+1luc and Rp-10mluc. Strains Rp+1luc and Rp-10mluc were both transformed with the plasmid pHdrRoe (Okinaga et al. 2010) to create the strains Rp+1lucROE and Rp+1lucROE-10. Using the strain RpLuc as a template, two fragments were amplified with the primer pairs hdrRupF/(repeat-m)hdrR-LR and (repeat-m)hdrR-RF/hdrMDnR. The two PCR amplicons were mixed with hybridized EMSA-hdrRpm-F/R primers and assembled using OE-PCR with the primer pair hdrRupF/hdrMdnR. The OE-PCR amplicon was transformed into strain RMIFDC2 and selected on medium containing 4-CP to create the strain RpDRmluc. The suicide vector construct pJYROEflag was transformed into RpDRmluc to create the strain RpmLucROEflag.

To insert the luciferase ORF downstream of the *hdrRM* ORFs, a DNA fragment containing the *hdrR* upstream region and IFDC2 were PCR amplified from strain RMIFDC2 with the primer pair hdrRupF/ermR-lucf. Using the genomic DNA of RpLuc as a template, the luciferase ORF was amplified with the primer pair lucF-erm/lucmR. The two amplicons were assembled using OE-PCR and the primer pair hdrRupF/lucmR. The resulting overlapping PCR products were transformed into RpLuc strain and selected on medium containing erythromycin to obtain the strain RMlucIFDC2. Next, two fragments encompassing the *hdrRM* locus were amplified from strain UA140 with the primer pair hdrRupF/MterR-luc, while the luciferase ORF was amplified from strain RpLuc with the primer pair lucF-Mter/lucmR. The PCR amplicons were mixed and

assembled by OE-PCR using the primer pair hdrRupF/lucmR. The resulting OE-PCR amplicon was transformed into strain RMlucIFDC2 and selected on plates supplemented with 4-CP to obtain the strain hdrRMluc. To mutate *hdrM* in strain hdrRMluc, three fragments were amplified from this strain using the primer pairs hdrRupF/(spec)smu1853R, (spec)smu1853-hdrR-LF2/hdrM(TAA)R, and hdrM(TAA)F/lucmR. The spectinomycin resistance cassette *aad9* was amplified from the *E. coli-Streptococcus* shuttle vector pDL278 using the primer pair specF/specR. The four amplicons were mixed and assembled by OE-PCR using the primer pair hdrRupF/lucmR. The resulting OE-PCR amplicon was transformed into strain hdrRMluc to obtain the strain ΔhdrMluc. To mutate the direct repeats upstream of the *hdrRM* promoter in strain ΔhdrMluc, two fragments were amplified from this strain using the primer pair hdrRupF/(repeat-m)hdrR-LR and (repeat-m)hdrR-RF/lucmR. The two PCR amplicons were mixed with hybridized EMSA-hdrRpm F/R primers and assembled using OE-PCR and the primers hdrRupF/lucmR. The resulting OE-PCR amplicon was transformed into strain hdrRMluc to obtain the strain ΔhdrMΔDRluc.

Creation of hdrR *and* hdrM *epitope tagged ectopic expression strains*
Using pHdrRoe as a template, the *ldh_p-hdrR* transcription fusion was amplified with the primer pair ldhF-bamHI/hdrR3xFlagOL. The resulting amplicon was subsequently amplified with the primer pair ldhF-bamHI/hdrR3xFlag-hindIII, then digested with *Bam*HI/*Hin*dIII, and ligated to the suicide vector pJY4164 to obtain pJYROEflag. To create the plasmid pJYROE, a fragment containing *hdrR* ORF driven by the *ldh* promoter was amplified with the primer pair ldhF-bamHI/hdrRR-hindIII from pHdrRoe. The PCR product was digested with *Bam*HI and *Hin*dIII, and ligated to pJY4164 to obtain the suicide vector pJYROE. The plasmid pJYROEflag was transformed into strain RpLuc and selected on plates supplemented with erythromycin to obtain the epitope tagged *hdrR* expression strain ROEflag. To create the *hdrM* HA epitope tagged expression plasmid pMOEha, an *ldh-hdrM* transcription fusion was first assembled by creating two PCR amplicons with the primer pairs ldhF-BamHI/ldhR-SpeI and hdrMF-SpeI/hdrMR-EcoRI and UA140 gDNA as a template. The fragments were digested with (*Bam*HI/*Spe*I) and (*Spe*I/*Eco*RI) and ligated to the *E. coli-Streptococcus* shuttle vector pDL278 after cutting with (*Bam*HI/*Eco*RI). The resulting plasmid pMOE was then used as a template and amplified with the primer pair ldhF-bamHI/hdrMR-linker2. The resulting PCR amplicon was further amplified with the primer pair ldhF-bamHI/linker2-HAR-EcoRI, then digested with *Bam*HI/*Eco*RI, and ligated to the shuttle vector pDL278 to create the plasmid pMOEha. The plasmids pMOE and pMOEha were transformed into the strain ROEflag to obtain RMOEflag or the epitope tagged *hdrRM* expression strain RMOEflagha. pJYROE and pMOEha were both sequentially transformed into strain RpLuc to create the strain RMOEha.

Using pJYROEflag as a template, two DNA fragments were PCR amplified with the primer pairs JYmF/ala5R and ala5F/JYmR. The amplicons were assembled by POE-PCR to create the suicide vector pJYAla5flag. The suicide vector pJYAla6flag was constructed using the same strategy except the primers ala6R and ala6F are used. For the construction of pJYAla5, two DNA fragments were PCR amplified from pJYROE with the primer pairs JYmF/ala5R and ala5F/JYmR. The amplicons were assembled by POE-PCR to create the suicide vector pJYAla5. The suicide vector pJYAla6 was constructed using the same strategy except the primers ala6R and ala6F were used. The suicide vector pJYAla5flag and the shuttle vector pMOEha were transformed into the strain RpLuc to obtain the strain Ala5MOEflagha. The

suicide vector pJYAla6flag and the shuttle vector pMOEha were transformed into the strain RpLuc to obtain the strain Ala6MOEflagha.

Creation of HdrR and HdrM fluorescent protein fusion strains

The HdrR-mCherry and HdrM-GFP protein fusion strains were created as follows. Using pHdrRoe as a template, the *ldh$_P$-hdrR* transcription fusion was amplified with the primer pair ldhF-bamHI/hdrRR-linker2. The ORF for mCherry was amplified from the plasmid pWUC3 (Wu and Ton-That 2010) using the primer pair mcherryF-linker2/mcherryR-HindIII. The two amplicons were assembled using OE-PCR, then digested with *Bam*HI/*Hin*dIII, and ligated to the suicide vector pJY4164 to create the plasmid pJYROEmChe. The plasmid was then transformed into strain RpLuc to create the strain ROEmChe. Strain ROEmChe was also transformed with the plasmid pMOE to create the strain ROEmCheM. Using pMOEha as a template, the *ldh$_P$-hdrM* transcription fusion was amplified with the primer pair ldhF-bamHI/linker2R-L1, while the *gfp* ORF was amplified from the plasmid pldhGFP (unpublished plasmid) with the primer pair GFPF-linker1/GFPR-HindIII. The resulting amplicons were next assembled using OE-PCR with the primer pair ldhF-bamHI/GFPR-HindIII, then digested with *Bam*HI/*Hin*dIII, and ligated to the shuttle vector pDL278 to create the plasmid pMOEgfp. This plasmid was transformed into the strain RpLuc to create the strain MOEgfp. Using pMOEgfp as a template, two fragments were amplified with the primer pairs pDLmF/DTMR and pDLmR/DTMF and assembled using prolonged overlap extension PCR (POE-PCR) (Xie et al. 2013) to create the plasmid pMtmOEgfp. This plasmid was transformed into strain RpLuc to create the strain MtmOEgfp.

The HdrR and HdrM BiFC YFP fusion strains were created as follows. Using pJYROE as a template, the *ldh$_P$-hdrR* transcription fusion was amplified with the primer pair ldhF-bamHI/hdrRR-linker2. The N-terminal fragment of YFP was amplified from the plasmid pEYFP (unpublished plasmid) using the primer pair cYNF-L2/YN155R-HindIII. The two fragments were assembled using OE-PCR, then digested with *Bam*HI/*Hin*dIII, and ligated to the suicide vector pJY4164 to create the plasmid pJYRYNOE. This plasmid was then used as a template for amplification with the primer pairs ldhR-L2/JYmF and L2F-ldh/JYmR and assembled by POE-PCR to create the plasmid pJYYNOE. Using the plasmid pMOE as a template, the *ldh$_P$-hdrM* transcription fusion was amplified with the primer pair ldhF-bamHI/hdrMR-linker2, while the C-terminal YFP fragment was amplified from pEYFP with the primer pair YC155F-L2/YCR-HindIII. The two fragments were assembled by OE-PCR, then digested with *Bam*HI/*Hin*dIII, and ligated to the shuttle vector pDL278 to create the plasmid pMYCOE. This plasmid was then used as a template for amplification with the primer pairs ldhR2-L2/pDLmF and L2F-ldh2/pDLmR and assembled by POE-PCR to create the plasmid pYCOE. The plasmids pJYRYNOE and pYCOE were both sequentially transformed into the strain RpLuc to create the strain RYNOE. The plasmids pJYYNOE and pMYCOE were both sequentially transformed into strain RpLuc to create the strain MYCOE. The plasmids pJYRYNOE and pMYCOE were both sequentially transformed into strain RpLuc to create the strain RYNMYCOE.

Creation of HdrR alanine scanning mutants

To perform alanine scanning mutagenesis of HdrR, blocks of six consecutive alanine mutations were introduced into HdrR starting at amino acid #2 and ending at the final amino acid #133 for a total of 22 unique mutant HdrR proteins (see Fig. S3A). Using the plasmid pJYROE as a template, *ldh$_P$-hdrR* transcription fusion was amplified with the primer pair ldhF-DpDL/HdrRR-

DpDL, while the pVA380 streptococcal replicon (LeBlanc et al. 1992) was amplified from the shuttle vector pDL278 (Chen and LeBlanc 1992) with the primer pair DpDLF-ldh/DpDLR-hdrR. The two amplicons were assembled by POE-PCR to create the plasmid pRwt. This plasmid then served as a template for all subsequent alanine scanning mutagenesis constructs. Plasmid pRwt was transformed into strain RpLuc to create the strain RpLucROE. The first alanine mutant ectopic expression plasmid (pRAla1) was created by amplifying pRwt with the primer pairs pDLmR/Ala1R and pDLmF/Ala1F. The two amplicons were assembled by POE-PCR and transformed into strain RpLuc to create the strain RpLucAla1. Subsequent *hdrR* mutant ectopic expression plasmids were assembled similarly except the corresponding alanine mutagenesis primers were substituted for Ala1R and Ala1F in the POE-PCR reactions to create the plasmids pRAla2 – pRAla22. Each of the plasmids was transformed into strain RpLuc to create the strains RpLucAla2 – RpLucAla22. The plasmids pRAla5 – pRAla16 and pRAla18 – pRAla20 were transformed into strain hdrRMluc to create the strains RMlucAla5 – RMlucAla16 and RMlucAla18 – RMlucAla20. The empty vector pVA380 was also transformed into strain hdrRMluc to obtain the negative control strain Rmluc-mock.

Creation of hdrR *recombinant expression vectors*

The *hdrR* ORF was amplified from strain UA140 using the primer pair hdrRF-NdeI/HdrRR-Hind. The amplicon was digested with *Nde*I/*Hin*dII and ligated to the expression vector pET29b to create the plasmid pEcROE. Using pEcROE as a template, the primer pairs Ala5F/pETmF and pETmR/Ala5R were used to create two amplicons that were subsequently assembled with POE-PCR to create the vector pEcAla5. The same strategy was used to create the vector pEcAla6, except the primers Ala6F and Ala6R were used for POE-PCR.

Creation of S. anginosus *and* S. pneumoniae *luciferase reporter strains*

Using *S. anginosus* OUP10 as a template, two fragments were amplified with the primer pairs SAIN319LF/(luc)SAIN319luc-LR and (erm)SAIN319-luc-RF/SAIN319RR. The green renilla luciferase ORF was amplified from the strain ldhRenGSm (Merritt et al. 2016) with the primer pair (SAIN319L)luc-F/(erm)luc-R, while the erythromycin resistance gene was amplified from the suicide vector pJY4164 with the primer pair (luc)Erm-F/(SAIN319R)erm-R. The four amplicons were mixed and assembled using OE-PCR and the primer pair SAIN319LF/SAIN319RR. The OE-PCR amplicon was transformed into *S. anginosus* OUP10 and selected on medium supplemented with erythromycin to create the strain ZZ318luc. Using *S. anginosus* OUP10 as a template, two fragments were amplified with the primer pairs SAIN319LF/(luc)SAIN319LR and (erm)SAIN319RF/SAIN319RR. The green renilla luciferase ORF was amplified from strain ldhRenGSm with the primer pair (318L)lucF/(erm)luc-R, while the erythromycin resistance gene was amplified from the suicide vector pJY4164 using the primer pair (luc)Erm-F/(319R)ermR. The four amplicons were mixed and assembled using OE-PCR with the primer pair SAIN319LF/SAIN319RR. The OE-PCR amplicon was transformed into *S. anginosus* OUP10 and selected on medium supplemented with erythromycin to create the strain ZZd319luc.

Using *S. pneumoniae* R6 as a template, two fragments were amplified with the primer pairs spr1730-F/(luc)1730-lucL-R and (spec)spr1730-luc-R/spr1730R-R. The green renilla luciferase ORF was amplified from strain ldhRenGSm with the primer pair (1730-lucL)luc-F/(spec)Luc-R, while the spectinomycin resistance cassette was amplified from the shuttle vector pDL278 using the primer pair specF/(1730-lucR)spec-R. The four amplicons were mixed together and

assembled using OE-PCR with the primer pair spr1730-F/spr1730R-R. The OE-PCR amplicon was transformed into *S. pneumoniae* R6 and selected on medium containing spectinomycin to create the strain ZZ1731luc. Using *S. pneumoniae* R6 as a template, two fragments were amplified with the primer pairs spr1730-F/(luc)spr1730-R and (spec)spr1730R-F/spr1730R-R. The green renilla luciferase ORF was amplified from strain ldhRenGSm with the primer pair (1730L)luc-F/(spec)Luc-R, while the spectinomycin resistance cassette was amplified from the shuttle vector pDL278 using the primer pair specF/(spr1730R)specR. The four amplicons were mixed together and assembled using OE-PCR with the primer pair spr1730-F/spr1730R-R. The OE-PCR amplicon was transformed into *S. pneumoniae* R6 and selected on medium containing spectinomycin to create the strain ZZd1730luc.

Creation of SANR_RS01820 and SANR_RS01825 epitope tagged ectopic expression strains
Using pJYROEflag as a template, the vector backbone and 3×FLAG tag sequence was amplified with the primer pair Ldhp-R/pJY-flag-F. Using *S. anginosus* OUP10 as a template, the SANR_RS01820 ORF was amplified with the primer pair (pJY)sain318F/(pJY-flag)sain318R. The two amplicons were assembled by POE-PCR to create the suicide vector pJYZZ318OEflag. The plasmid pJYZZ318OEflag was transformed into strain OUP10 to create the strain ZZ318OEflag. Using pMOEha as a template, the vector backbone and HA tag sequence was amplified with the primer pair Ldhp-R/pDL-HA-F. Using *S. anginosus* OUP10 as a template, the SANR_RS01825 ORF was amplified with the primer pair (pDL)sain319F/(pDL-HA)sain319-R. The two amplicons were assembled by POE-PCR to create the shuttle vector pZZ319OEha. The plasmid pZZ319OEha was transformed into strain OUP10 to create the strain ZZ319OEha. Strain ZZRMOEflagha was created by transforming both pJYZZ318OEflag and pZZ319OEha into strain OUP10.

HdrR molecular weight determination via mass spectrometry
Strains RMOEflag and ROEflag were each grown to an optical density of OD_{600} 0.3 – 0.4, collected by centrifugation, washed with TBS buffer (pH 7.4), and then sonicated in the same buffer containing 1 mM PMSF. The cell lysate was collected by centrifugation at 16,000 x g for 30 min. and then the clarified lysates were further was centrifuged at 105,000 x g for 1.5 h at 4 ^{o}C to pellet the cell membranes. The resulting supernatant was used as the cytoplasmic fractions, while the membrane protein pellets were solubilized with TBS containing 1% Triton X-100. HdrR proteins were immunopurified from cytoplasmic fractions of strain ROEflag and membrane fractions of RMOEflag using α-FLAG M2 Affinity Gel (Sigma-Aldrich) according to the manufacturer's protocol. Eluted HdrR proteins were separated in duplicate with 15% SDS-PAGE. The resulting gels were negatively stained with the imidazole-zinc procedure (Castellanos-Serra and Hardy 2006). Protein bands corresponding to HdrR proteins were excised from the gel and directly eluted from the gel slices by passive diffusion (Castellanos-Serra and Hardy 2006). The eluted proteins were concentrated and then injected onto a 1.0 × 250-mm C4 column (214 MS C4; Vydac, Hesperia, CA). Masses were determined by electrospray ionization mass spectrometry on a model LTQ Velos Pro ion trap instrument (ThermoFinnigan, San Jose, CA). The flow rate was 20 μl min.$^{-1}$ and used a mobile phase A containing water and mobile phase B containing acetonitrile, both with 0.1% formic acid. The sample was loaded at 2% B for 5 min. onto a 1 × 8 mm Opti-Trap protein trap (Optimize Technologies, Oregon City, OR), then separated using a 1 min. gradient to 7.5% B, 30 min. to 60% B, 4 min. to 100% B, 5 min. at 100% B, 1 min to 2% B, and equilibration for 14 min. at 2% B. Mass spectra of 350-2000 m/z were collected in profile mode while averaging 20 μscans,

using an automatic gain control of 3×10^4, and a maximum ion time of 200 msec. Mass spectra were deconvoluted using Protein Deconvolution 4.0 software (Thermo Scientific).

Construction of LRS phylogenetic trees

To generate phylogenetic trees of the 5 *S. mutans* LRS, sequences were aligned using Clustal Omega (Sievers et al. 2011) and ambiguously aligned regions were removed using Gblocks (Talavera and Castresana 2007). The evolution model GTR+I+G (General Time Reversible plus Invariant sites plus Gamma distribution) used for all trees was selected using jModelTest2 (Darriba et al. 2012). Bayesian trees were constructed using MrBayes as implemented in Geneious (Huelsenbeck and Ronquist 2001; Kearse et al. 2012). A chain length of 1,000,000 was used with a burn-in fraction of 25% and sampling every 100 trees.

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Figure S1

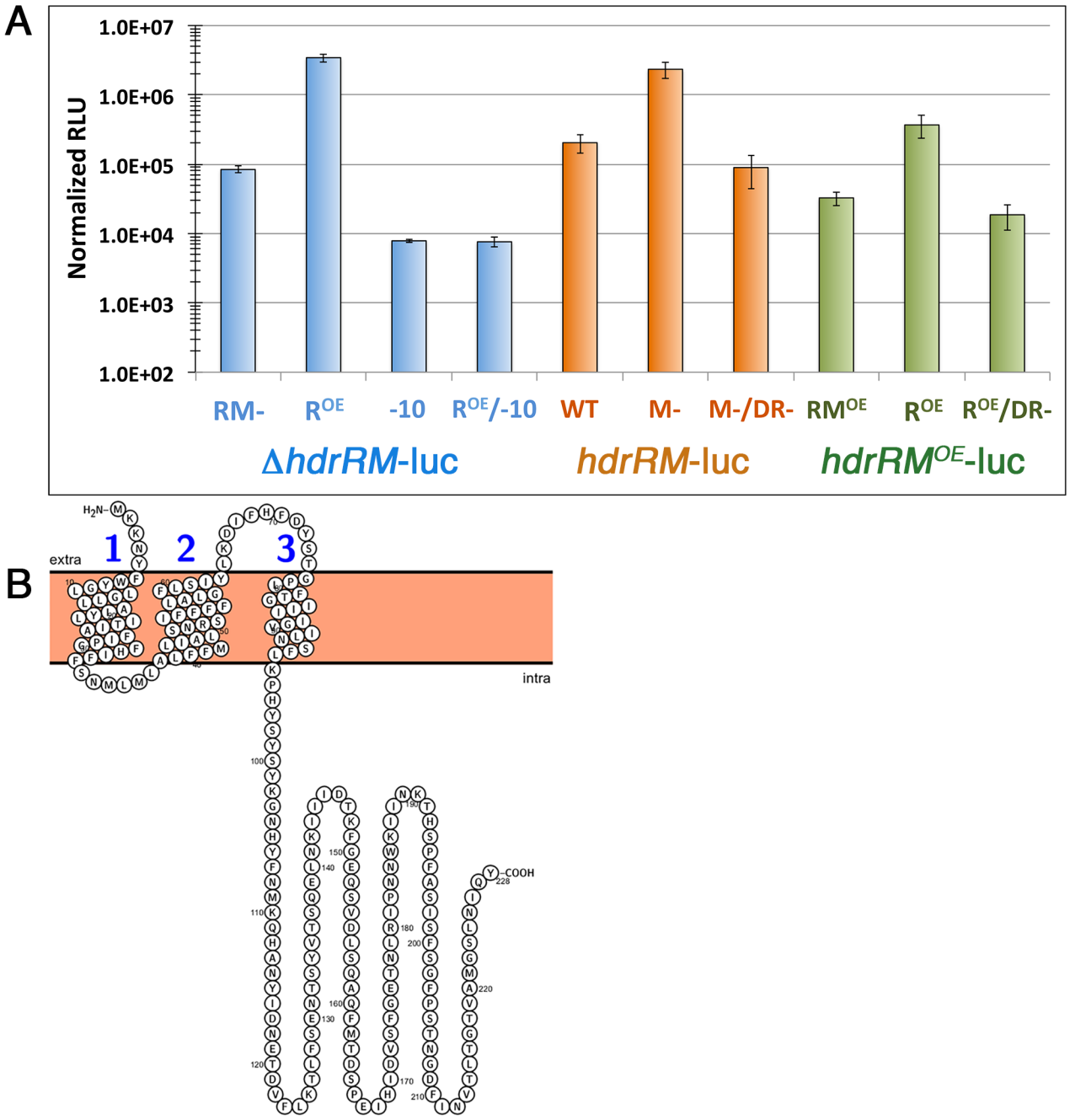


Fig. S1. Role of HdrR and HdrM in *hdrRM* operon autoregulation. A) A comparison of normalized luciferase values is presented for three different *hdrRM* transcription fusion luciferase reporter strains. Blue bars correspond to the results obtained using a luciferase ORF replacement of the *hdrR* and *hdrM* ORFs (Δ*hdrRM*-luc). Strains from left to right: (RM-) parental reporter strain with a luciferase replacement of *hdrR* and *hdrM*, (R^{OE}) parental strain with *hdrR* ectopically expressed on a multi-copy plasmid, (-10) parental strain containing an *hdrRM* operon promoter mutation in the -10 sequence, and (R^{OE}/-10) parental strain with *hdrR* ectopically expressed on a multi-copy plasmid and containing an *hdrRM* operon promoter

mutation in the -10 sequence. Orange bars correspond to the results obtained using a luciferase ORF insertion after the *hdrR* and *hdrM* ORFs (*hdrRM*-luc). Strains from left to right: (WT) parental reporter strain with luciferase insertion after the *hdrRM* ORFs, (M-) parental strain with an *hdrM* mutation, and (DR-/M-) parental strain with mutations in the direct repeats upstream of the *hdrRM* operon promoter and a mutation in *hdrM*. Green bars correspond to the results obtained using a luciferase ORF replacement of the *hdrRM* ORFs in addition to having *hdrR* and *hdrM* separately ectopically overexpressed (*hdrRM*^{OE}-luc). Strains from left to right: (RM^{OE}) parental reporter strain with *hdrR* and *hdrM* separately ectopically overexpressed (Note: *hdrR* is overexpressed in a single copy), (R^{OE}) parental strain lacking *hdrM*, and (R^{OE}/DR-) parental strain lacking *hdrM* and containing mutant direct repeats upstream of the *hdrRM* operon promoter. B) Predicted topology of HdrM. The image was created using the Protter webserver (http://wlab.ethz.ch/protter/#).

Figure S2

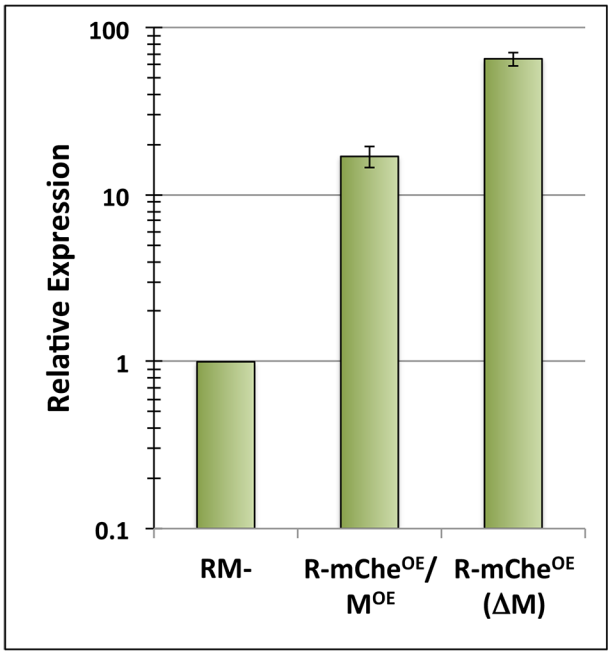


Fig. S2. Assessment of HdrR-mCherry functionality. An *hdrRM* luciferase reporter was created by replacing the *hdrRM* ORFs with that of luciferase. The luciferase activity of this parent reporter strain (RM-) was then compared after ectopic overexpression of HdrR-mCherry & HdrM (R-mChe^{OE}/M^{OE}) as well as HdrR-mCherry alone [R-mChe^{OE} (ΔM)]. Data are presented relative to the parent reporter strain, which was arbitrarily assigned a value of 1.

Figure S3

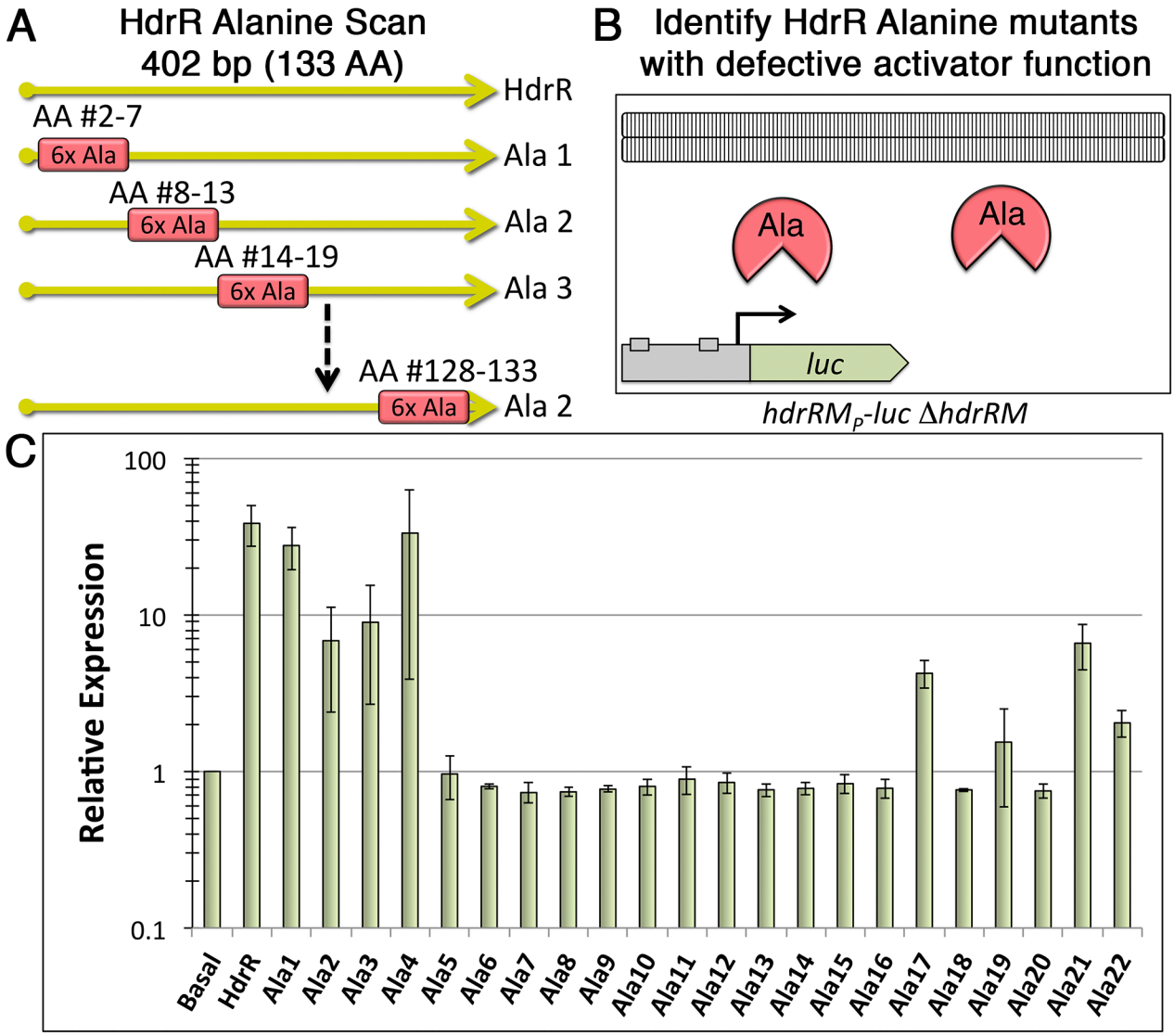


Fig. S3. Creation of mutant HdrR proteins with defects in transcription activation. A) Illustration of the alanine scanning mutagenesis scheme. Blocks of 6 consecutive alanine mutations were engineered throughout the entire length of HdrR for a total of 22 separate mutants. The mutants were named sequentially starting with Ala1, which contained alanine substitutions in amino acids #2 – 7. B) Illustration of the luciferase screening procedure to identify mutant proteins defective in *hdrRM* operon transcription activation. C) Each of the 22 alanine mutant *hdrR* genes was ectopically expressed to assess its ability to activate gene expression from a reporter strain containing a luciferase ORF replacement of the *hdrRM* ORFs.

Figure S4

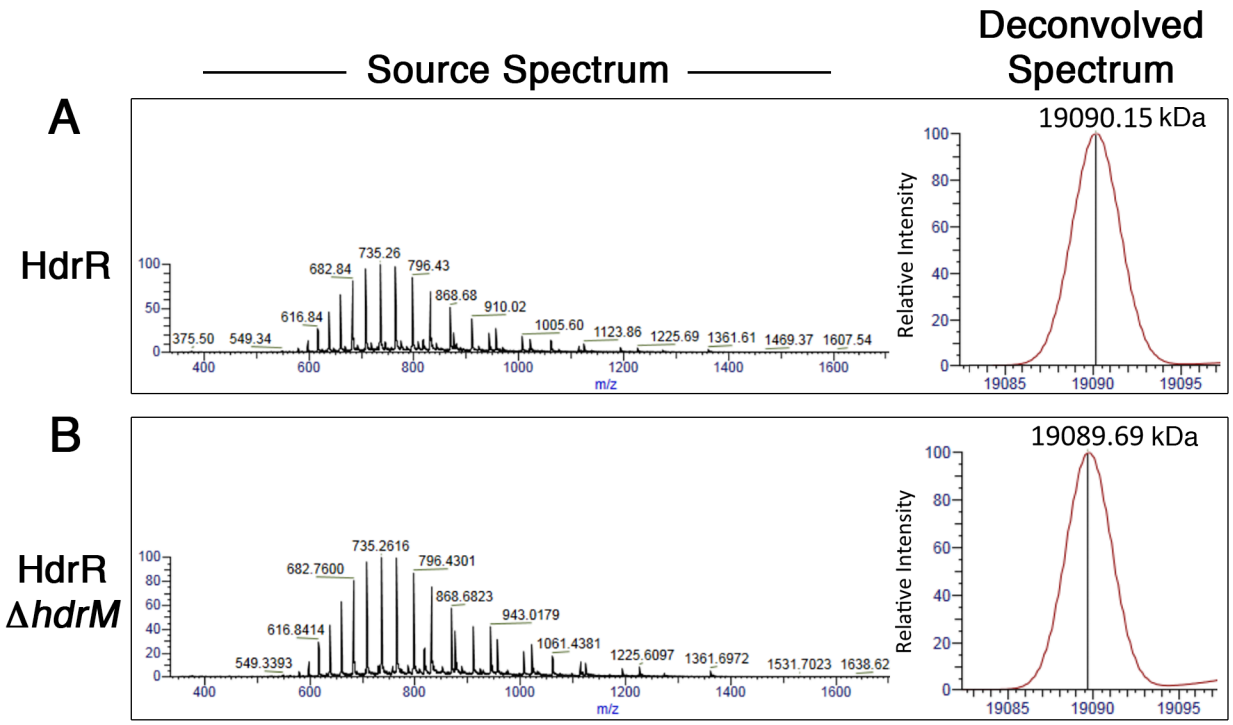
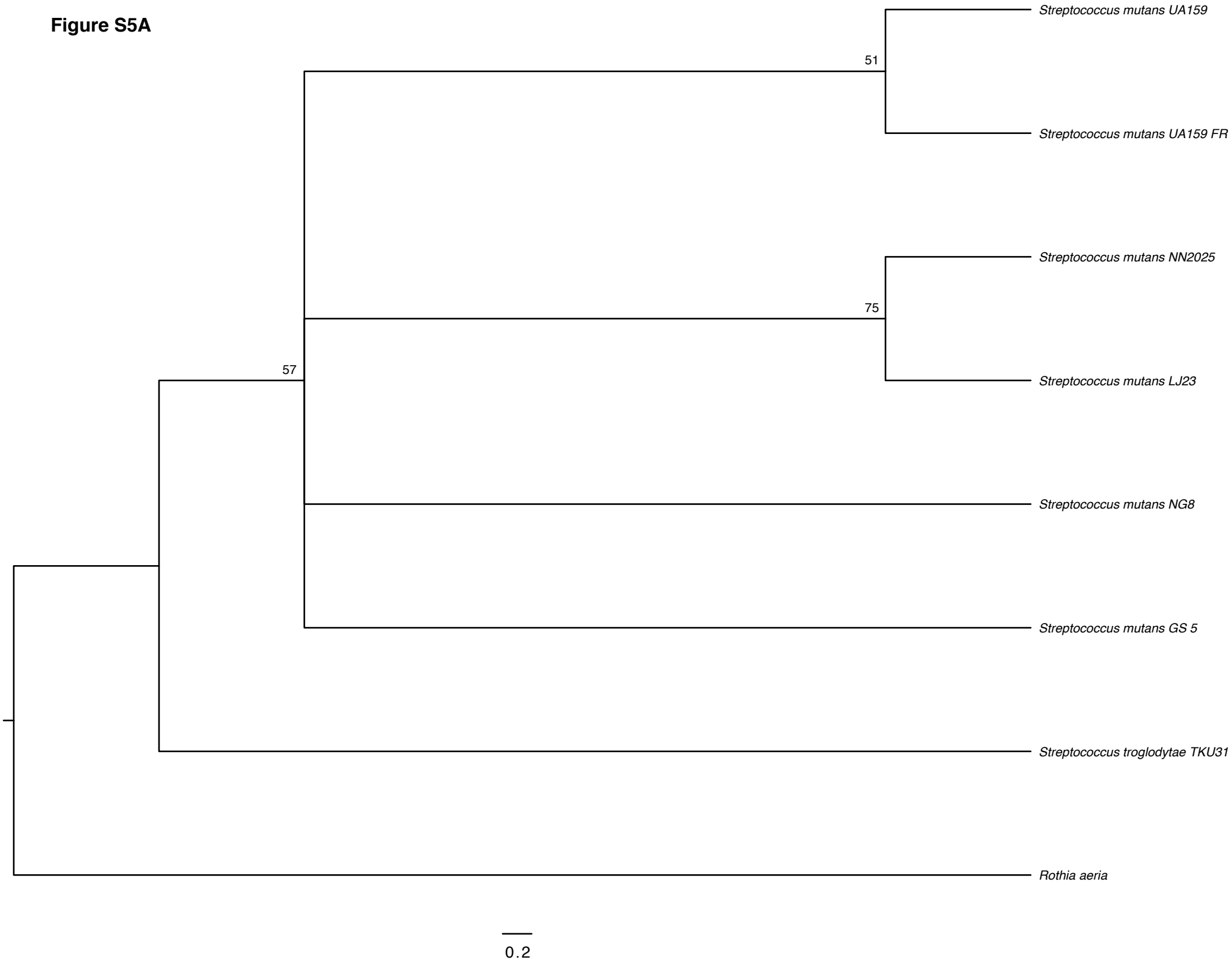


Fig. S4. HdrR molecular weight determination using mass spectrometry. FLAG epitope tagged HdrR was directly purified from *S. mutans* strains either (A) encoding or (B) lacking *hdrM* and then subjected to electrospray ionization mass spectrometry as described in Supplemental Materials and Methods. Mass spectra of 350 – 2000 m/z were collected and then deconvoluted with Protein Deconvolution 4.0 software.

Figure S5A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51
Streptococcus mutans UA159 FR
Streptococcus mutans NN2025
75
57
Streptococcus mutans LJ23
Streptococcus mutans NG8
Streptococcus mutans GS 5
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Rothia aeria
0.2

Figure S5B

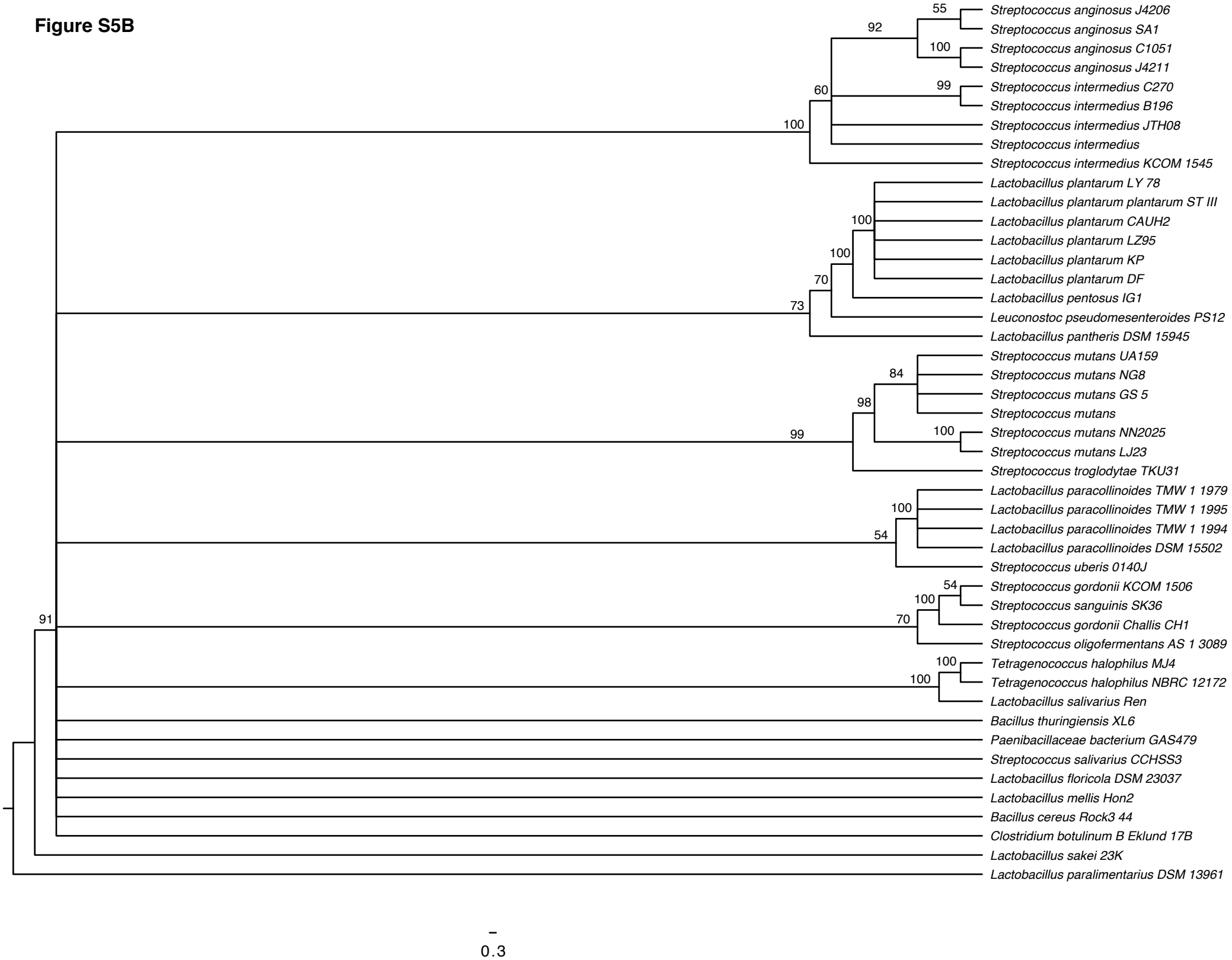
Streptococcus anginosus J4206
Streptococcus anginosus SA1
Streptococcus anginosus C1051
Streptococcus anginosus J4211
Streptococcus intermedius C270
Streptococcus intermedius B196
Streptococcus intermedius JTH08
Streptococcus intermedius
Streptococcus intermedius KCOM 1545
Lactobacillus plantarum LY 78
Lactobacillus plantarum plantarum ST III
Lactobacillus plantarum CAUH2
Lactobacillus plantarum LZ95
Lactobacillus plantarum KP
Lactobacillus plantarum DF
Lactobacillus pentosus IG1
Leuconostoc pseudomesenteroides PS12
Lactobacillus pantheris DSM 15945
Streptococcus mutans UA159
Streptococcus mutans NG8
Streptococcus mutans GS 5
Streptococcus mutans
Streptococcus mutans NN2025
Streptococcus mutans LJ23
Streptococcus troglodytae TKU31
Lactobacillus paracollinoides TMW 1 1979
Lactobacillus paracollinoides TMW 1 1995
Lactobacillus paracollinoides TMW 1 1994
Lactobacillus paracollinoides DSM 15502
Streptococcus uberis 0140J
Streptococcus gordonii KCOM 1506
Streptococcus sanguinis SK36
Streptococcus gordonii Challis CH1
Streptococcus oligofermentans AS 1 3089
Tetragenococcus halophilus MJ4
Tetragenococcus halophilus NBRC 12172
Lactobacillus salivarius Ren
Bacillus thuringiensis XL6
Paenibacillaceae bacterium GAS479
Streptococcus salivarius CCHSS3
Lactobacillus floricola DSM 23037
Lactobacillus mellis Hon2
Bacillus cereus Rock3 44
Clostridium botulinum B Eklund 17B
Lactobacillus sakei 23K
Lactobacillus paralimentarius DSM 13961
0.3

Figure S5C

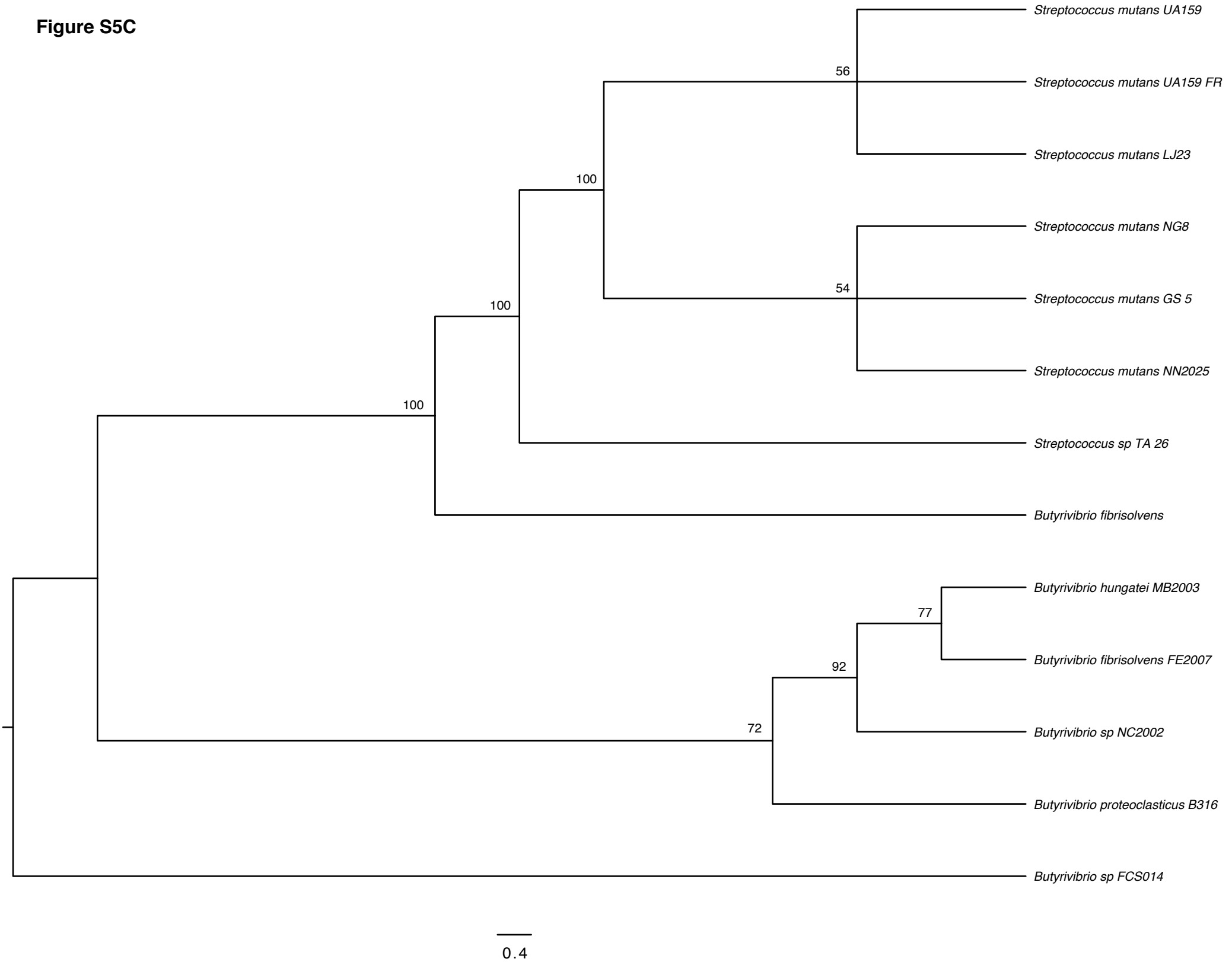
Streptococcus mutans UA159
Streptococcus mutans UA159 FR
Streptococcus mutans LJ23
Streptococcus mutans NG8
Streptococcus mutans GS 5
Streptococcus mutans NN2025
Streptococcus sp TA 26
Butyrivibrio fibrisolvens
Butyrivibrio hungatei MB2003
Butyrivibrio fibrisolvens FE2007
Butyrivibrio sp NC2002
Butyrivibrio proteoclasticus B316
Butyrivibrio sp FCS014
56
100
54
100
100
77
92
72
0.4

Figure S5D

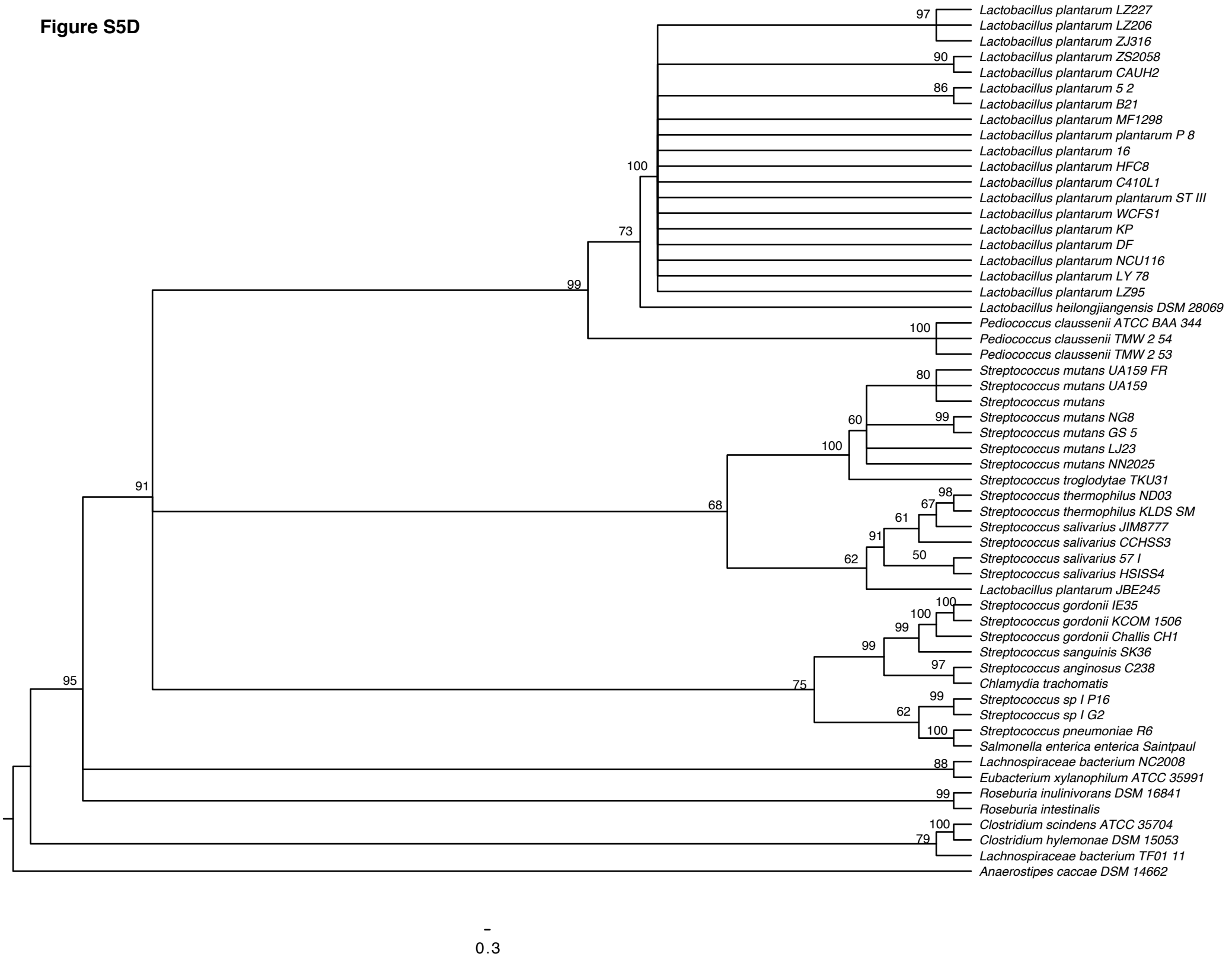
Lactobacillus plantarum LZ227
Lactobacillus plantarum LZ206
Lactobacillus plantarum ZJ316
Lactobacillus plantarum ZS2058
Lactobacillus plantarum CAUH2
Lactobacillus plantarum 5 2
Lactobacillus plantarum B21
Lactobacillus plantarum MF1298
Lactobacillus plantarum plantarum P 8
Lactobacillus plantarum 16
Lactobacillus plantarum HFC8
Lactobacillus plantarum C410L1
Lactobacillus plantarum plantarum ST III
Lactobacillus plantarum WCFS1
Lactobacillus plantarum KP
Lactobacillus plantarum DF
Lactobacillus plantarum NCU116
Lactobacillus plantarum LY 78
Lactobacillus plantarum LZ95
Lactobacillus heilongjiangensis DSM 28069
Pediococcus claussenii ATCC BAA 344
Pediococcus claussenii TMW 2 54
Pediococcus claussenii TMW 2 53
Streptococcus mutans UA159 FR
Streptococcus mutans UA159
Streptococcus mutans
Streptococcus mutans NG8
Streptococcus mutans GS 5
Streptococcus mutans LJ23
Streptococcus mutans NN2025
Streptococcus troglodytae TKU31
Streptococcus thermophilus ND03
Streptococcus thermophilus KLDS SM
Streptococcus salivarius JIM8777
Streptococcus salivarius CCHSS3
Streptococcus salivarius 57 I
Streptococcus salivarius HSISS4
Lactobacillus plantarum JBE245
Streptococcus gordonii IE35
Streptococcus gordonii KCOM 1506
Streptococcus gordonii Challis CH1
Streptococcus sanguinis SK36
Streptococcus anginosus C238
Chlamydia trachomatis
Streptococcus sp I P16
Streptococcus sp I G2
Streptococcus pneumoniae R6
Salmonella enterica enterica Saintpaul
Lachnospiraceae bacterium NC2008
Eubacterium xylanophilum ATCC 35991
Roseburia inulinivorans DSM 16841
Roseburia intestinalis
Clostridium scindens ATCC 35704
Clostridium hylemonae DSM 15053
Lachnospiraceae bacterium TF01 11
Anaerostipes caccae DSM 14662
0.3

Figure S5E

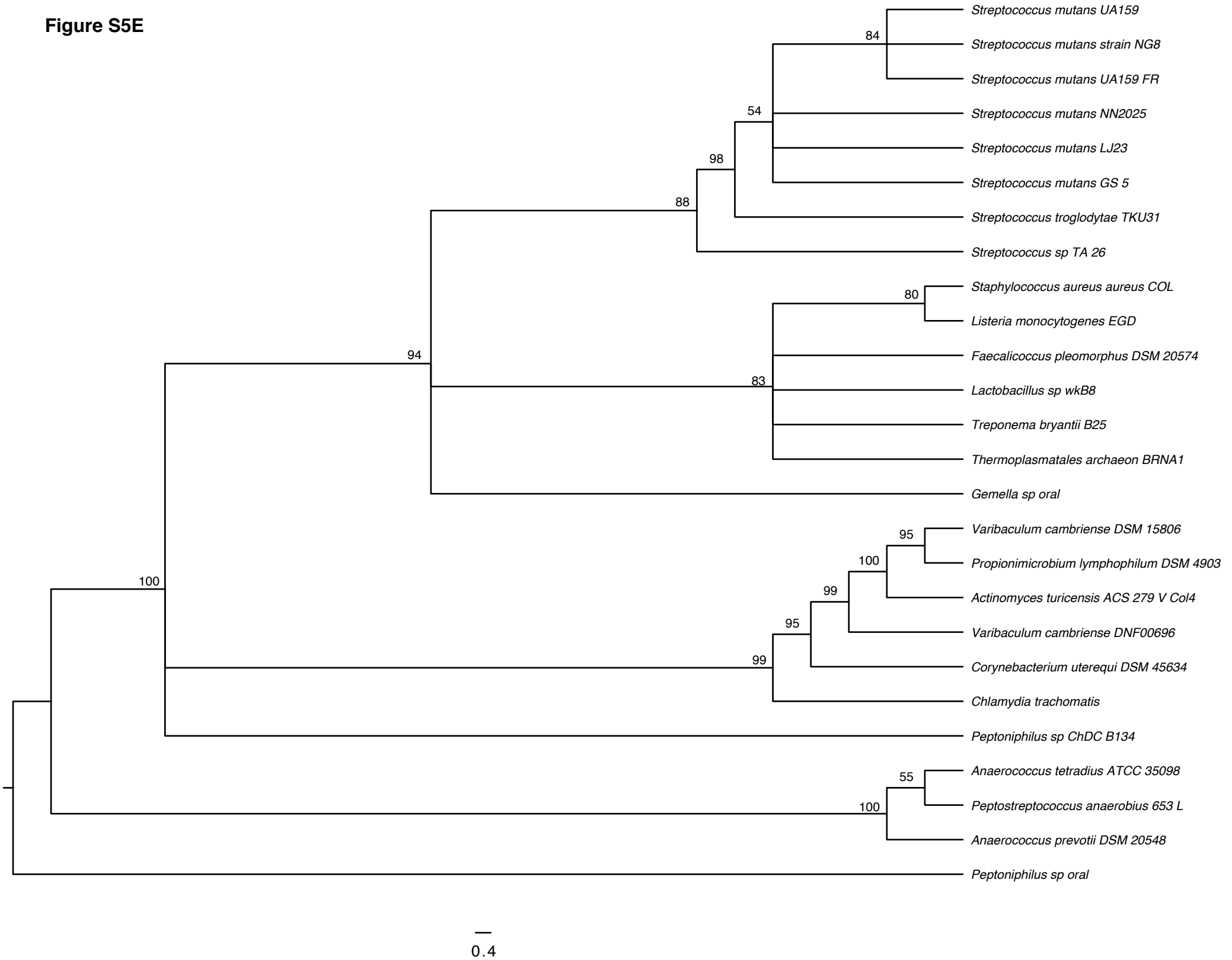
Streptococcus mutans UA159
Streptococcus mutans strain NG8
Streptococcus mutans UA159 FR
Streptococcus mutans NN2025
Streptococcus mutans LJ23
Streptococcus mutans GS 5
Streptococcus troglodytae TKU31
Streptococcus sp TA 26
Staphylococcus aureus aureus COL
Listeria monocytogenes EGD
Faecalicoccus pleomorphus DSM 20574
Lactobacillus sp wkB8
Treponema bryantii B25
Thermoplasmatales archaeon BRNA1
Gemella sp oral
Varibaculum cambriense DSM 15806
Propionimicrobium lymphophilum DSM 4903
Actinomyces turicensis ACS 279 V Col4
Varibaculum cambriense DNF00696
Corynebacterium uterequi DSM 45634
Chlamydia trachomatis
Peptoniphilus sp ChDC B134
Anaerococcus tetradius ATCC 35098
Peptostreptococcus anaerobius 653 L
Anaerococcus prevotii DSM 20548
Peptoniphilus sp oral
84
54
98
88
80
94
83
95
100
99
95
99
100
55
100
0.4

Figure S5. Phylogeny estimation of LytTR Regulatory Systems encoded by *S. mutans*. Bayesian phylogenies were constructed for LytTR Regulatory Systems using homologs of following LRS membrane proteins: (A) SMU_295, (B) SMU_434, (C) SMU_1069c, (D) SMU_1855 (HdrM), and (E) SMU_2081 (BrsM). Posterior probability values >50 are indicated at the nodes.