TABLE S1. Bacterial strains, plasmids, and primers used in this study.[a]

Strain/Plasmid	Description	Antibiotic Resistance[b]	Reference
***S. mutans* strains**			
BRSM01	*Streptococcus mutans* UA159 wild-type reference strain	NR	(1)
BRSM05	UA159 Δ*pdrA*::*spec* (ΔSMU_1509::*spec*)	$Spec^R$	This study
BRSM08	UA159 Δ$wgkAB_{N\text{-}term}$::*spec*[c]	$Spec^R$	This study
BRSM38	UA159 Δ*galK*::P_c-*erm*	Erm^R	This study
BRSM49	UA159 *galK*::P_{wgkA}-*luxAB*-P_c-*erm*	Erm^R	This study
BRSM64	UA159 Δ*rgg*::*spec* *galK*::P_{wgkA}-*luxAB*-P_c-*erm* (BRSM05 transformed with *galK*::P_{wgkA}-*luxAB*-P_c-*erm* from BRSM49)	$Spec^R$, Erm^R	This study
BRSM69	UA159 *galK*::P_{shp}-luxAB-P_c*erm*	Erm^R	This study
BRSM73	UA159 Δ*rgg*::*spec* *galK*::P_{shp}-luxAB-P_c-*erm* (BRSM05 transformed with *galK*::P_{shp}-luxAB-P_c-*erm* from BRSM69)	$Spec^R$, Erm^R	This study
BRSM85	UA159 pLZ12Spec (UA159 transformed with pLZ12Spec)	$Spec^R$	This study
GS23	UA159 Δ*pptAB*::*aphA3* pGS101 (JCC263 transformed with pGS101)	Km^R, Erm^R	This study
GS105	UA159 Δ*oppD*::*cat* pJC301 pGS101 (JCC298 was electroporated with pJC301 and pGS101)	Cm^R, $Spec^R$, Erm^R	This study
JCC263	UA159 Δ*pptAB*::*aphA3*	Km^R	(21)
JCC298	UA159 Δ*oppD*::*cat* pLZ12Spec (BRSM85 was transformed with Δ*oppD*::*cat*)	Cm^R, $Spec^R$	This study
MW37	UA159 pWAR307 (UA159 transformed with pWAR307)	Cm^R	This study
Other bacterial strains			
A909	*Streptococcus agalactiae* A909 wild-type isolate	NR	(22)
ATCC 10556	*Streptococcus sanguinis* ATCC 10556 wild-type isolate, also known as SK1	NR	(23)
ATCC 11454	*Lactococcus lactis* ATCC 11454 wild-type isolate	NR	(24)

BH10c	*Escherchia coli* derivative of DH10B	NR	(3)
BL21(DE3)	*Escherchia coli* strain for recombinant plasmid expression	NR	NEB
BL21(DE3) + pRSFDuet-1_6HMBP*wgkA_wgkBC*_DSM20646 + pDB1282	BL21(DE3) deriviative for co-expression of 6HMBPWgkA, WgkB and WgkC from *S. ferus*	Km^R, Amp^R	This study
E. coli BL21(DE3) + pRSFDuet-pRSFDuet-1_6HMBP*wgk*A_*wgkB*-rbs-*wgkC*_UA159 + pDB1282	B21(DE3) deriviative for co-expression of 6HMBPWgkA, WgkB and WgkC from *S. mutans*	Km^R, Amp^R	This study
CCUG 31611	*Streptococcus mitis* CCUG 31611 wild-type isolate, also known as NCTC 12261	NR	(25)
D39	*Streptococcus pneumoniae* D39 wild-type isolate, from M. E. Winkler	NR	(26)
DH5α	*Escherchia coli* strain for cloning	NR	NEB
DSM 20646	*Streptococcus ferus* DSM 20646 wild-type isolate	NR	(6)
JB1	*Streptococcus bovis* JB1 wild-type isolate	NR	(27)
NZ131	*Streptococcus pyogenes* NZ131 wild-type isolate	NR	(2)
S. gordonii Challis	*Streptococcus gordonii* Challis Bt wild-type isolate	NR	(28)
S. oralis 108	*Streptococcus oralis* 108 wild-type isolate, from L. Tao	NR	(29)
V583	*Enterococcus faecalis* V583 wild-type isolate	Van^R	(30)
Plasmids[d]			
pBAD_6HMBPP_HTFLAG	Source of 6HMBP tag	Amp^R	Seyedsayamdost Lab
pDB1282	Vector for *isc* operon expression	Amp^R	(31)
pEVP3	Template plasmid for P_{syncat} chloramphenicol cassette	Cm^R	(32)
pGS101	pWAR303 derivative with P_{wgkA} in front of *luxAB*	Erm^R	This study
pGS103	pWAR303 derivative with P_{shp} in front of *luxAB*	Erm^R	This study
pJC300	pLZ12Spec derivative with P_{syncat} chloramphenicol cassette	Cm^R, $Spec^R$	This study
pJC301	pJC300 derivative, amplified by inverse PCR to leave P_{syncat} in front of multiple cloning site of plasmid	$Spec^R$	This study

pLZ12Spec	Shuttle vector encoding spectinomycin resistance; pWV01 origin		$Spec^R$	(33)
pRSFDuet-1	Co-expression vector		Km^R	Novagen
pRSFDuet-1_6HMBP	pRSFDuet-1 modified with a 6HMBP tag coding sequence		Km^R	This study
pRSFDuet-1_6HMBP*wgkA_wgkBC*_DSM20646	Co-expression of 6HMBPWgkA, WgkB and WgkC		Km^R	This study
pRSFDuet-1_6HMBP*wgkA_wgkB*-rbs-*wgkC*_UA159	Co-expression of 6HMBPWgkA, WgkB and WgkC		Km^R	This study
pWAR303	pFED761 derviative with *luxAB* fragment		Erm^R	(34)
pWAR307	pJC156 derivative with P_{sigX} in front of *luxAB*		Cm^R	(34)
Primers used for strain construction[e]				
Primer	Sequence (5' to 3')[f]	Template	RE Site	Amplicon or Plasmid Product
For construction of BRSM05 (Δ*pdrA*::*spec*)				
GS111	TGGGTATGATTTGCTCTC TGCAGGA	UA159	NotI	5' upstream of *pdrA*
GS112	AAAA**GCGGCCGC**CAATA GTACCTCAATTGTCTATA TGGG			
GS105	AAAA**GCGGCCGC**CATTA GGATCCAGATCTTCCTTC AGGTTATG	pLZ12 Spec	NotI, SalI	Spec cassette
GS106	GCGTG**GTCGAC**GCAAGG GTTTATTGTTTTCTAAAAT CTGAT			
GS113	AAAA**GTCGAC**TAATATGA AATTGTGTATATGGGAT	UA159	SalI	3' downstream of *pdrA*
GS114	AAATAGACGAAATAGCA GCTCCACGAATT			
For construction of BRSM08 (Δ$wgkAB_{N\text{-}term}$::*spec*)				
GS136	GCACGTAAGAGCAACAA TCTAG	UA159	NotI	5' upstream of *wgkA*
GS137	AAAA**GCGGCCGC**AGTAT TTACCTCCTTGATTTGAT AG			
GS105	AAAA**GCGGCCGC**CATTA GGATCCAGATCTTCCTTC AGGTTATG	pLZ12 Spec	NotI, SalI	Spec cassette
GS106	GCGTG**GTCGAC**GCAAGG GTTTATTGTTTTCTAAAAT CTGAT			
GS138	AAAA**GTCGAC**TAAAACAA GGTCTGTAGGTCATTG	UA159	SalI	3' downstream of *wgkA*
GS139	GCTCCAAGAATACCTCC AAATG			
For construction of BRSM38 (Δ*galK*::P_c-*erm*)				

BR94	AGGATATCAACAGCAGT ACGGCCC	UA159	5' upstream of *galK*
BR95	ATCCATTAAAAATCAAAC GGATCCTATTTCCTTTAC TTTTGTTATACTGAAATG AG		
BR96	CTCATTTCAGTATAACAA AAGTAAAGGAAATAGGAT CCGTTTGATTTTTAATGG ATA	P_c-*erm* cassette[g]	P_c-*erm* cassette
BR97	ATCTAGTAATGCTGTCAT CGTTTTATTTCCTCCCGT TAAATAATAGATAACTAT TA		
BR98	TAGTTATCTATTATTTAAC GGGAGGAAATAAAACGA TGACAGCATTACTAGATA CT	UA159	3' downstream of *galK*
BR99	CAGTGTGATGCGGTGTA TCA		
For construction of BRSM49 (*galK*::P_{wgkA}-*luxAB*-P_c-*erm*)			
BR94	AGGATATCAACAGCAGT ACGGCCC	UA159	5' upstream of *galK*
BR192	CTGCTACTGATTTCCTTT ACTTTTGTTATACTG		
BR193	GTAAAGGAAATCAGTAG CAGCAATGAAATTG	pGS101	P_{wgkA}-*luxAB*
BR97	ATCTAGTAATGCTGTCAT CGTTTTATTTCCTCCCGT TAAATAATAGATAACTAT TA		
BR98	TAGTTATCTATTATTTAAC GGGAGGAAATAAAACGA TGACAGCATTACTAGATA CT	BRSM38	P_c-*erm* + 3' downstream of *galK*
BR99	CAGTGTGATGCGGTGTA TCA		
For construction of BRSM69 (*galK*::P_{shp}-luxAB-P_c-*erm*)			
BR94	AGGATATCAACAGCAGT ACGGCCC	UA159	5' upstream of *galK*
BR190	GACTGAATATTTTCCTTT ACTTTTGTTATACTG		
BR191	GTAAAGGAAAATATTCAG TCATCAATAGTACCTC	pGS103	P_{shp}-*luxAB*
BR97	ATCTAGTAATGCTGTCAT CGTTTTATTTCCTCCCGT TAAATAATAGATAACTAT TA		
BR98	TAGTTATCTATTATTTAAC GGGAGGAAATAAAACGA TGACAGCATTACTAGATA CT	BRSM38	P_c-*erm* + 3' downstream of *galK*
BR99	CAGTGTGATGCGGTGTA TCA		

For construction of JCC298 (Δ*oppD*::cat)				
GS309	GCGTG**GCGGCCGC**TCTTTCTTACAAGATGCTTGGCGA	UA159	NotI	5' upstream of *oppD*
GS310	GCGTG**ATCGAT**AACATCGCGAATAGCTTTTA		ClaI	
GS311	GCGTG**ATCGAT**GATGAAAATTTGTTTGATTT	pEVP3	ClaI	Cat cassette
GS312	GCGTG**GTCGAC**TTATAAAAGCCAGTCATTAG		SalI	
GS313	GCGTG**GTCGAC**ATGCTGCTACTTGGCTGTTAGATGA	UA159	SalI	3' downstream of *oppD*
GS314	GCGTG**CTCGAG**AGTACAGTTAAAATAGGACT		XhoI	
For construction of pGS101 (pWAR303 P_{wgk})				
GS203	AAAA**CTGCAG**TACCTCCTTGATTTGATAGAATTA	UA159	PstI	P_{wgk}
GS204	AAAA**GTCGAC**TTTAGTGTTCCTAAAACAACTAAG		SalI	
For construction of pGS103 (pWAR303 P_{shp})				
GS206	AAAA**GTCGAC**ATATTCAGTCATCAATAGTACCTC	UA159	SalI	P_{shp}
GS207	AAAA**CTGCAG**TTCTCCTCACTCACTTTTCTTAT		PstI	
For construction of pJC300 (pLZ12Spec with P_{syncat}-cat cassette)				
JC208	CATG**GAATTC**ATTTGTTTGATTTTTAATGG	pEVP3	EcoRI	P_{syncat}-cat cassette
JC412	CATG**GAATTC**GATGGGTTCCGAGGCTC			
For construction of pJC301 (pJC300 P_{syncat})				
JC413	CATGGCGGCCGCATTAGGATCCAGATCTTCCTTCAGGTTATGACCATC	pJC300		pJC300 P_{syncat} without cat cassette
JC414	CATGGCGGCCGCCCTCCTAAATTTTTATCTAAAGTG			
For construction of pRSFDuet-1_6HMBP*wgkA_wgkBC*_DSM20646				
*wgkA*_DSM20646_F	AGTTCTGTTTCAGGGTCCGGAATTCGGATCCATGTCACCTAAAAAAGAGTTTAATGCTCC	DSM 20646		Cloning *wgkA* into pRSFDuet-1_6HMBP at MCS1
*wgkA*_DSM20646_R	ATGCGGCCGCAAGCTTGTCGACCTGCAGTTAATGCTTACCCCAACTATTAACTTTAGTTG			
*wgkBC*_DSM20646_F	GTTAAGTATAAGAAGGAGATATACATATGAGAGATTATTCGCCATATCCATTATTAGTAG	DSM 20646		Cloning *wgkBC* into pRSFDuet-1_6HMBP at MCS2
*wgkBC*_DSM20646_R	GGTTTCTTTACCAGACTCGAGTTATTTCTCCCTAAAAGGTTTTAACTGTAAGTATATATC			
For construction of pRSFDuet-pRSFDuet-1_6HMBP*wgk*A_*wgkB*-rbs-*wgkC*_UA159				

*wgkA*_UA159_F	ATGATT**GGATCC**ATGCT GACCAAAAAAGAGTTCA GCGTGC	UA159	BamHI	Cloning *wgk*A into pRSFDuet-1_6HMBP at MCS1
*wgkA*_UA159_R	TGAAGA**CTGCAG**TTAAT GTTTGCCCCAGCAGTTC ACTTTGG		PstI	
*wgkB*_UA159_F	ACGGTG**CATATG**CGCGA CTATTCTCCGTATCCG	UA159	NdeI	Cloning *wgkB*-rbs-*C* into pRSFDuet-1_6HMBP at MCS2
wgkB-rbs_UA159	CATGGTATATCTCCTTAT TAATTATCCTCGTCATAG AAGCATGCCAG	UA159		
rbs-*wgkC*_UA159	GACGAGGATAATTAATAA GGAGATATACCATGAAAA ACCATTTCACCATCAAAG GCAAGC	UA159		Cloning *wgkB*-rbs-*C* into pRSFDuet-1_6HMBP at MCS2
*wgkC*_UA159_R	GGGGGG**CTCGAG**TCATA AAGCTTCGCGAAACGGT TTCAGATACAG	UA159	XhoI	
Primers for qRT-PCR and 5' RACE				
Primer	Sequence (5' to 3')	Description		
BR311	GCTAATCATTGCAAGCAGTG GTATCAACGCAGAGTACATG GG	Template switching oligonucleotide (TSO) for 5' RACE		
BR312	CATTGCAAGCAGTGGTATCA AC	TSO annealing primer		
BR315	CTAGTGCTTACCCCAACAGT TT	Reverse primer for *wgkA*		
BR316	CCCCAACAGTTTACCTTAGT TG	Reverse primer for *wgkA*		
GS116	ATTGTTGCTCGGGCTCTT CC	Forward primer for *gyrA* (SMU_1114)		
GS117	TGCGGCTTGTCAGGAGT AAC	Reverse primer for *gyrA* (SMU_1114)		
GS130	GATGTCTGTTGAGAAGC TGGAT	Forward primer for *wgkB* (SMU_1508c)		
GS131	TCTAATAAACGGTTCACC TCCA	Reverse primer for *wgkB* (SMU_1508c)		
GS258	CCCTCAACACACTCTGCT AAGCT	Forward primer for *irvA* (SMU_1397c)		
GS259	CCAAATCATTGGCCAGTT GAA	Reverse primer for *irvA* (SMU_1397c)		
GS260	AATTCTGATACTGTTGCA G CACCTA	Forward primer for *gbpC* (SMU_1396)		
GS261	TTCTGTTGCAGCCGGTT CT	Reverse primer for *gbpC* (SMU_1396)		
GS268	AGGAACAATCCCTTCAG GTAACC	Forward primer for *comEA* (SMU_625)		
GS269	CAGTCGTCTGCGTCTTCT TCTG	Reverse primer for *comEA* (SMU_625)		
GS270	CTTTTTTCTGGACGTCAC GATTT	Forward primer for *comYA* (SMU_1987)		

GS271	TCGCCCCTTGATTTCATTTAA	Reverse primer for *comYA* (SMU_1987)
GS274	CTAGCTGAGAGCGGAATGAAA	Forward primer for *irvR* (SMU_1398)
GS275	TTTGGCAAACTCTTTCCCTTAAC	Reverse primer for *irvR* (SMU_1398)

[a]Strains were constructed as described in *Materials and Methods*.

[b]Antibiotic resistance markers: NR, no resistance markers; Amp^R, ampicillin resistance; $Spec^R$, spectinomycin resistance; Erm^R, erythromycin resistance; Km^R, kanamycin resistance; Cm^R, chloramphenicol resistance; Van^R, vancomycin resistance.

[c]Δ*wgkAB*$_{N\text{-}term}$::*spec* refers to a deletion of the coding region of *wgkA* and the first 992 nucleotides of *wgkB*, replaced by a spectinomycin cassette. See *Materials and Methods* for additional details.

[d]All plasmids were constructed and/or propagated in the *E. coli* BH10c, DH5α, or BL21(DE3) strains.

[e]Genomic DNA of UA159 or indicated plasmid DNA was used as template for PCR or restriction nucleotide reactions.

[f]Bolded and underlined nucleotides indicate engineered restriction sites, if used.

[g]P_c-*erm* cassette was derived from fusion of the synthetic P_c promoter sequence from Land et. al, 2011 (35) with the erythromycin resistance cassette from pWAR303.