

**Supplementary Information for:**

**Involvement of the Wbp pathway in the biosynthesis of *Porphyromonas gingivalis*  
lipopolysaccharide with anionic polysaccharide**

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**Supplemental Figure 1 | Comparison of amino acid sequences among WbpB homologs.**

*Porphyromonas gingivalis* ATCC 33277: PGN\_0168, *Pseudomonas aeruginosa* PAO1: PA3158, *Helicobacter pylori* NCTC 11637: HP0679 and *Bordetella pertussis* Tohama I: BP0093 were compared. The alignment was created using the ClustalW and Boxshade programs hosted at the Swiss EMBnet web site. The red triangle indicates the nonsense mutation in *P. gingivalis* strain HG66.

**Supplemental Figure 2 | Comparison of amino acid sequences among WbpA homologs.**

*Porphyromonas gingivalis* ATCC 33277: PGN\_0613, PGN\_1243, *Pseudomonas aeruginosa* PAO1: PA3159 and *Bordetella pertussis* Tohama I: BP1629, BP3150 were compared. The alignment was created using the ClustalW and Boxshade programs hosted at the Swiss EMBnet web site.

**Supplemental Figure 3 | Comparison of amino acid sequences among WbpE homologs.**

*Porphyromonas gingivalis* ATCC 33277: PGN\_1236, *Pseudomonas aeruginosa* PAO1: PA3155 and *Bordetella pertussis* Tohama I: BP0091 were compared. The alignment was created using the ClustalW and Boxshade programs hosted at the Swiss EMBnet web site. The red triangle indicates the essential residue for WbpE activity.

**Supplemental Figure 4 | Comparison of amino acid sequences among WbpD homologs.**

*Porphyromonas gingivalis* ATCC 33277: PGN\_0002, *Pseudomonas aeruginosa* PAO1: PA3156 and *Bordetella pertussis* Tohama I: BP0092 were compared. The alignment was created using the ClustalW and Boxshade programs hosted at the Swiss EMBnet web site. The red triangles indicate the essential residue for WbpD activity.

**Supplemental Table 1 | Strains used in this study.**

**Supplemental Table 2| Plasmids used in this study.**

**Supplemental Table 3| Primers used in this study.**

## Supplemental Text 1| Construction of *P. gingivalis* mutant strains.

### Construction of *P. gingivalis* mutants

To create *P. gingivalis* PGN\_0168 (*wbpB*::Em<sup>r</sup> (KDP220) and PGN\_0002::Em<sup>r</sup> (KDP222) mutants, the upstream regions were amplified with PGN\_0168upFw/PGN\_0168upRev and PGN\_0002upFw/PGN\_0002upRev primers, respectively, using *P. gingivalis* ATCC 33277 as the template, and cloned into the pCR4 vector or pGEM-T Easy vector. Each upstream and downstream region was swapped with the appropriate restriction region of pKD740<sup>26</sup>, generating pKD892 and pKD893. The resulting targeting vectors pKD892 and pKD893 were linearized with SacI and introduced into *P. gingivalis* ATCC 33277 by electroporation followed by selection on blood agar plates containing Em (10 µg/ ml).

To create a *P. gingivalis* PGN\_1236 (*porR*::Tc<sup>r</sup> (KDP221) mutant, the upstream region was amplified with PGN\_1236upFw/PGN\_1236upRev primers using *P. gingivalis* ATCC 33277 as the template and cloned into the pCR4 vector, yielding pCR4-porRup. The downstream region was amplified with PGN\_1236dwFw/PGN\_1236dwRev primers using *P. gingivalis* ATCC 33277 as the template and cloned into the pCR4 vector, yielding pCR4-porRdw1. To eliminate the EcoRI site that was closest to the NotI site in pCR4-porRdw1, pCR4-porRdw1 was digested with NotI and was then self-ligated, yielding pCR4-porRdw2. The EcoRI-BamHI region of pCR4-porRup was inserted into the same region of pCR4-porRdw2, yielding pCR4-porRupdw2. The *tetQ* gene, which is the BamHI-BglIII region of pKD375<sup>51</sup>, was inserted into the BamHI site of pCR4-porRupdw2, generating pKD894. The resulting targeting vector pKD894, linearized with NotI, was introduced into *P. gingivalis* ATCC 33277 by electroporation and was then selected on blood agar plates containing Tc (0.7 µg/ ml).

## Construction of *P. gingivalis* complemented strains

The promoter region of the *Porphyromonas gulae* catalase gene (accession no. AB083039 in GenBank/EMBL/DDBJ databases) was amplified using p6-34-F-KpnI/p6-34-R-SalI from pKD955<sup>6</sup> and was cloned into the pCR4 vector to yield pCR4-cat pro. A KpnI-EcoRI DNA fragment containing the promoter region of the *Porphyromonas gulae* catalase gene of pCR4-cat pro was swapped into the same region of pKD854<sup>5</sup> to yield pBSSK-cat pro-rgpBter.

To create complementation plasmids for the *wbpB* (PGN\_0168) and PGN\_0002 genes, *P. gingivalis* ATCC 33277 was used as the template for amplification with PGN\_0168compFw/PGN\_0168compRev or PGN\_0002compFw/PGN\_0002compRev primers, respectively. Each PCR product was cloned into pUC118, yielding pUC118-*wbpB* and pUC118-PGN\_0002.

To create a complementation plasmid with the *wbpD* gene from *Pseudomonas aeruginosa*, the coding region of *wbpD* was amplified from *P. aeruginosa* using the *P. a. wbpDFw*/*P. a. wbpDRev* primers. The PCR product was cloned into pUC118, yielding pUC118-*P.a. wbpD*.

The SalI-XbaI DNA fragment containing the coding region of the *wbpB*, PGN\_0002 and *P.a. wbpD* genes from pUC118-*wbpB*, pUC118-PGN\_0002 and pUC118-*P. a. wbpD* was inserted into the same region of pBSSK-cat pro-rgpBter to yield pBSSK-cat pro-*wbpB*-rgpBter, pBSSK-cat pro-PGN\_0002-rgpBter and pBSSK-cat pro-*P.a. wbpD*-rgpBter. The KpnI-NotI DNA fragment of pBSSK-cat pro-*wbpB*-rgpBter, pBSSK-cat pro-PGN\_0002-rgpBter and pBSSK-cat pro-*P.a. wbpD*-rgpBter was inserted into the same region of a pTCB vector<sup>52</sup> to yield pTCB-*wbpB*, pTCB-PGN\_0002 and pTCB-*P.a. wbpD*. The pTCB vector containing *wbpB*, PGN\_0002 or *P. a. wbpD* was introduced into *Escherichia coli* S17-1<sup>53</sup> by electroporation and was then selected on LB agar plates containing Ap (100 µg/ml). The

transformant was mated with strain HG66 or the PGN\_0002 mutant (KDP222) and was then selected on blood agar plates containing Gm (50 µg/ ml) and Tc (0.7 µg /ml), yielding HG66/pWbpB+, PGN\_0002/pPGN\_0002+ (KDP223) and PGN\_0002/p*P. a.*WbpD+ (KDP224).

**Supplemental Text 2| Sequence comparison of the *wbpB* gene in *P. gingivalis* strains.**

	10	20	30	40	50						
<b>W83</b>	ATGCACA	ACT TTGCC	ATCAT	CGGTGT	GGCC GGCT	TATTG	CTCCGC	GCCA			
<b>TDC60</b>	ATGCACA	ACT TTGCC	ATCAT	CGGTGT	GGCC GGCT	TATTG	CTCCGC	GCCA			
<b>HG66</b>	ATGCACA	ACT TTGCC	ATCAT	CGGTGT	GGCC GGCT	TATTG	CTCCGC	GCCA			
<b>33277</b>	ATGCACA	ACT TTGCC	ATCAT	CGGTGT	GGCC GGCT	TATTG	CTCCGC	GCCA			
<b>Consensus</b>	*****	*****	*****	*****	*****	*****	*****	*****			
	60	70	80	90	100						
<b>W83</b>	CCTTCGC	CC ATCAA	GGATA	CAGGCA	AACCG	CTTGGT	ATCG	GCTATG	GATA		
<b>TDC60</b>	CCTTCGC	CC ATCAA	AGATA	CAGGCA	AATCG	CTTGGT	ATCG	GCTATG	GATA		
<b>HG66</b>	CCTTCGC	CC ATCAA	GGATA	CAGGCA	AACCG	CTTGGT	ATCG	GCTATG	GATA		
<b>33277</b>	CCTTCGC	CC ATCAA	GGATA	CAGGCA	AACCG	CTTGGT	ATCG	GCTATG	GATA		
<b>Consensus</b>	*****	*****	****	*****	**	*****	*****	*****	*****		
	110	120	130	140	150						
<b>W83</b>	AGTTCG	ACAG TGTGG	TATC	ATGGAC	AGTT	ACTTCC	CGAA	AGCTGC	CCTTC		
<b>TDC60</b>	AGTTCG	ACAG TGTGG	TATC	ATGGAC	AGTT	ACTTCC	CGAA	AGCTGC	CCTTC		
<b>HG66</b>	AGTTCG	ACAG TGTGG	TATC	ATGGAC	AGTT	ACTTCC	CGAA	AGCTGC	CCTTC		
<b>33277</b>	AGTTCG	ACAG TGTGG	TATC	ATGGAC	AGTT	ACTTCC	CGAA	AGCTGC	CCTTC		
<b>Consensus</b>	*****	*****	*****	*****	*****	*****	*****	*****	*****		
	160	170	180	190	200						
<b>W83</b>	TTTACG	GAGC	AAGAA	CTGTT	CGACCG	TCAC	AACTCC	AAGC	TACGAG	GAAC	
<b>TDC60</b>	TTTACG	GAGC	AAGAA	CTGTT	CGACCG	TCAC	AACTCC	AAGC	TACGAG	GAAC	
<b>HG66</b>	TTTACG	GAGC	AAGAA	CTGTT	CGA	TCGTC	AC	AACTCC	AAGC	TACGAG	GAAC
<b>33277</b>	TTTACG	GAGC	AAGAA	CTGTT	CGA	TCGTC	AC	AACTCC	AAGC	TACGAG	GAAC
<b>Consensus</b>	*****	*****	***	*****	*****	*****	*****	*****	*****	*****	
	210	220	230	240	250						
<b>W83</b>	CGATCAG	GCT ATCG	ACCAC	CG	TGTCCG	TTTG	TACACC	GAAC	TATCTG	CACG	
<b>TDC60</b>	CGATCAG	GCT ATCG	ACCAT	G	TGTCCG	TTTG	TACACC	GAAC	TATCTG	CACG	
<b>HG66</b>	CGATCAG	GCT ATCG	ACCAC	CG	TGTCCG	TTTG	TACACC	GAAC	TATCTG	CACG	
<b>33277</b>	CGATCAG	GCT ATCG	ACCAC	CG	TGTCCG	TTTG	TACACC	GAAC	TATCTG	CACG	
<b>Consensus</b>	*****	*****	*	*****	*****	*****	*****	*****	*****	*****	

	260	270	280	290	300
<b>W83</b>	ATGCTCATTG	TCGCTATGGC	CTCGACTTG	GAGCGGATGT	GATATGCGAG
<b>TDC60</b>	ATGCTCATTG	TCGCTATGGC	CTACGACTTG	GAGCGGATGT	GATATGCGAG
<b>HG66</b>	ATGCTCATTG	TCGCTATGGC	CTCGACTTG	GAGCGGATGT	GATATGCGAG
<b>33277</b>	ATGCTCATTG	TCGCTATGGC	CTCGACTTG	GAGCGGATGT	GATATGCGAG
<b>Consensus</b>	*****	*****	** *****	*****	*****
	310	320	330	340	350
<b>W83</b>	AAGCCTTTGG	TGCTCAATCC	TTGGAATATA	GACGCTTTGC	AGGAGATCGA
<b>TDC60</b>	AAGCCTTTGG	TGCTCAATCC	TTGGAATATA	GACGCTTTGC	AGGAGATCGA
<b>HG66</b>	AAGCCTTTGG	TGCTCAATCC	TTGGAATATA	GACGCTTTGC	AGGAGATCGA
<b>33277</b>	AAGCCTTTGG	TGCTCAATCC	TTGGAATATA	GACGCTTTGC	AGGAGATCGA
<b>Consensus</b>	*****	*****	*****	*****	*****
	360	370	380	390	400
<b>W83</b>	GCGAGAGACG	GGGCATCGGA	TCTATAATAT	TCTTCAACTG	CGACTCCATG
<b>TDC60</b>	GCAAGAGACG	GGGCATCGGA	TCTATAATAT	TCTTCAACTG	CGACTCCATG
<b>HG66</b>	GCAAGAGACG	GGGCATCGGA	TCTATAATAT	TCTTCAACTG	CGACTCCATG
<b>33277</b>	GCAAGAGACG	GGGCATCGGA	TCTATAATAT	TCTTCAACTG	CGACTCCATG
<b>Consensus</b>	** *****	*****	*****	*****	*****
	410	420	430	440	450
<b>W83</b>	AGTCGATCGT	GGCCCTGAAG	AGAAAGATCG	AATCCGGATC	GGCCGACAAG
<b>TDC60</b>	AGTCATCGT	GGCCCTGAAG	AGAAAGATCG	AATCCGGACC	GGCCGACAAG
<b>HG66</b>	AGTCATCGT	GGCCCTGAAG	AGAAAGATCG	AATCCGGACC	GGCCGACAAG
<b>33277</b>	AGTCATCGT	GGCCCTGAAG	AGAAAGATCG	AATCCGGACC	GGCCGACAAG
<b>Consensus</b>	**** *****	*****	*****	*****	* *****
	460	470	480	490	500
<b>W83</b>	ATCTATGATG	TCACGCTTAC	ATATATTACT	TCGCGGGCA	ATTGGTACTA
<b>TDC60</b>	ATCTATGATG	TCACGCTGAC	GTATATTACT	TCGCGGGCA	ATTGGTACTA
<b>HG66</b>	ATCTATGATG	TCACGCTGAC	GTATATTACT	TCGCGTGGCA	ACTGGTACTA
<b>33277</b>	ATCTATGATG	TCACGCTGAC	GTATATTACT	TCGCGTGGCA	ACTGGTACTA
<b>Consensus</b>	*****	*****	** *****	*****	*****
	510	520	530	540	550
<b>W83</b>	TACGAGCTGG	AAGGGAGACG	AGCGTAAGAG	CGGTGGCATA	GCTACGAACA
<b>TDC60</b>	TACGAGCTGG	AAGGGAGACG	AGCGTAAGAG	CGGTGGCATA	GCTACGAACA
<b>HG66</b>	TACGAGCTGG	AAGGGAGACG	AGCGTAAGAG	CGGCGGTATA	GCAACGAATA
<b>33277</b>	TACGAGCTGG	AAGGGAGACG	AGCGTAAGAG	CGGCGGTATA	GCAACGAATA
<b>Consensus</b>	*****	*****	*****	*** ** *	*** ** *



	560	570	580	590	600
<b>W83</b>	TCGGTGTTCA	TTTCTACGAC	ATGCTGTCAT	GGATTTTCGG	CAGTGACGA
<b>TDC60</b>	TCGGTGTTCA	TTTCTACGAC	ATGCTGTCAT	GGATTTTCGG	CAGTGACGA
<b>HG66</b>	TCGGTGTTCA	TTTCTACGAC	ATGCTGTCAT	GGATTTTCGG	CAGTGACGA
<b>33277</b>	TCGGTGTTCA	TTTCTACGAC	ATGCTGTCAT	GGATTTTCGG	CAGTGACGA
<b>Consensus</b>	***** **	*****	***** *	*****	***** **
	610	620	630	640	650
<b>W83</b>	CACAATGTCG	TACATGTGTG	TGTGCATGAC	AGGGCTGCCG	GCTATTTGGA
<b>TDC60</b>	CACAATGTCG	TACATGTGTG	TGTGCATGAC	AGGGCTGCCG	GCTATTTGGA
<b>HG66</b>	CACAATGTCG	TACATGTGTG	TGTGCATGAC	AGGGCTGCCG	GCTATTTGGA
<b>33277</b>	CACAATGTCG	TACATGTGTG	TGTGCATGAC	AGGGCTGCCG	GCTATTTGGA
<b>Consensus</b>	*****	*****	** *****	*****	***** **
	660	670	680	690	700
<b>W83</b>	ATTGGAGCGC	GCCCGTGTGC	GTTACTTCCT	TTCGATCAAT	GAGGAGCTTC
<b>TDC60</b>	ATTGGAGCGC	GCCCGTGTGC	GTTACTTCCT	TTCGATCAAT	GAGGAGCTTC
<b>HG66</b>	ATTGGAGCGC	GCCCGTGTGC	GTTACTTCCT	TTCGATCAAT	GAGGAGCTTC
<b>33277</b>	ATTGGAGCGC	GCCCGTGTGC	GTTACTTCCT	TTCGATCAAT	GAGGAGCTTC
<b>Consensus</b>	*****	*****	*****	*****	** *****
	710	720	730	740	750
<b>W83</b>	TGCCCGAGAA	TGCCGTACAG	GGAGAGAAGC	GAACCTTTCG	TACGATAGAA
<b>TDC60</b>	TGCCCGAGAA	TGCCGTACAG	GGAGAGAAGC	GAACCTTTCG	TACGATAGAA
<b>HG66</b>	TGCCCGAGAA	TGCCGTACAG	GGAGAGAAGC	GAACCTTTCG	TACGATAGAA
<b>33277</b>	TGCCCGAGAA	TGCCGTACAG	GGAGAGAAGC	GAACCTTTCG	TACGATAGAA
<b>Consensus</b>	*****	*****	** *****	*****	*****
	760	770	780	790	800
<b>W83</b>	ATCGACGGCG	AATCATTCGA	GTTCAGTGAA	GGGTTTACGG	AGCTGCACAC
<b>TDC60</b>	ATCGACGGCG	AATCATTCGA	GTTCAGTGAA	GGGTTTACGG	AGCTGCACAC
<b>HG66</b>	ATCGACGGCG	AATCATTCGA	GTTCAGTGAA	GGGTTTACGG	AGCTGCACAC
<b>33277</b>	ATCGACGGCG	AATCATTCGA	GTTCAGTGAA	GGGTTTACGG	AGCTGCACAC
<b>Consensus</b>	*****	*****	*****	*****	*****_
	810	820	830	840	850
<b>W83</b>	CGAGAGTTAT	CGGCCGATTT	TGGCCGGTGA	AGGTTTCGGT	TTGGACGAAG
<b>TDC60</b>	CGAGAGTTAT	CGGCCGATTT	TGGCCGGTGA	AGGTTTCGGT	TTGGACGAAG
<b>HG66</b>	CGAGAGTTAT	CGGCCGATTT	TGGCCGGTGA	AGGTTTCGGT	TTGGACGAAG
<b>33277</b>	CGAGAGTTAT	CGGCCGATTT	TGGCCGGTGA	AGGTTTCGGT	TTGGACGAAG
<b>Consensus</b>	*****	*****	*****	*****	*****

	860	870	880	890	900	
<b>W83</b>	CTCGCAACAG	CATTCAGATC	GTTTCATGACA	TTCGCAATGC	TTCTCCCGTA	
<b>TDC60</b>	CTCGCAACAG	CATTCAGATC	GTTTCATGACA	TTCGCAATGC	TTCTCCCGTA	
<b>HG66</b>	CTCGCAACAG	CATTCAGATC	GTTTCATGACA	TTCGCAATGC	TTCTCCCGTA	
<b>33277</b>	CTCGCAACAG	CATTCAGATC	GTTTCATGACA	TTCGCAATGC	TTCTCCCGTA	
<b>Consensus</b>	*****	*****	*****	*****	*****	
	910	920	930	940	950	
<b>W83</b>	GGA	CTGAAGG	GCGAATACCA	CCCCCTTGCA	CGTTTGCCCC	TGGCCTCTCA
<b>TDC60</b>	GGA	CTGAAGG	GCGAATACCA	CCCCCTTGCA	CGTTTGCCCC	TGGCCTCTCA
<b>HG66</b>	GGA	CTGAAGG	GCGAATACCA	CCCCCTTGCA	CGTTTGCCCC	TGGCCTCTCA
<b>33277</b>	GGA	CTGAAGG	GCGAATACCA	CCCCCTTGCA	CGTTTGCCCC	TGGCCTCTCA
<b>Consensus</b>	*****	*****	****	*****	*****	*****
	960	970				
<b>W83</b>	TCCTTT	CGGA	TGGAGACGCT	GA		
<b>TDC60</b>	TCCTTT	CGGA	TGGAGACGCT	GA		
<b>HG66</b>	TCCTTT	CGGA	TGGAGACGCT	GA		
<b>33277</b>	TCCTTT	CGGA	TGGAGACGCT	GA		
<b>Consensus</b>	*****	*****	**			

Yellow color indicates the same residues between HG66 and other strains.

Red color indicates the HG66 nonsense mutation.

### Supplement Text 3| Abbreviations

GlcNAc, glucosamine; GlcNAcA, 2-acetamido-2,3-dideoxy-D-glucuronic acid;  
3-keto-D-GlcNAcA, 2-acetamido-2-deoxy-D-ribo-hex-3-uluronic acid; GlcNAc3NA,  
2-acetamido-3-amino-2,3-dideoxy-D-glucuronic acid; GlcNAc3NAcA,  
2,3-diacetamido-2,3-dideoxy-D-glucuronic acid; ManNAc3NAcA,  
2,3-diacetamido-2,3-dideoxy-D-mannuronic acid. AcCoA, acetyl-coenzyme A; CoA,  
coenzyme A; 2-HG, 2-hydroxyglutarate;  $\alpha$ -KG,  $\alpha$ -ketoglutarate; NAD<sup>+</sup>, nicotinamide adenine  
dinucleotide; NADH, nicotinamide adenine dinucleotide hydrate; PLP, pyridoxal 5'-phosphate;  
PMP, pyridoxamine 5'phosphate.



# Supplemental Figure 2

BP1629 1 -----  
 BP3150 1 -----  
 PGN\_0613 1 -----  
 PA WbpA 1 -----  
 PGN\_1243 1 **MGQACIGSRPISFLWEVRTCRRFIGSYTLIRRVIVSHVLSGSRHYIPATGRGNSRSLSLDK**

BP1629 1 -----MPKKFSNPADVHL**AVVGLGYVGLPLAVEFGKK**-**RPVIGFDINERRIAALKAG**  
 BP3150 1 -----MRIDDVKL**AVVGLGYVGLPLAVEFGKK**-**RPVIGFDINERRIAALKAG**  
 PGN\_0613 1 -MKPYKALTIINTKEMNNIR**IAIIGLGYVGLPLARLFSTK**-**FPTIGFDINPNRVEELISG**  
 PA WbpA 1 ---MIDVNTVVEKFKSRQAL**IGIVGLGYVGLPLMLRYNAIGFDVLCGIDIDDVKVDKLNAG**  
 PGN\_1243 61 **ASPFYLTFFPKNSISYYNMDIAVVGIGYVGLVSAATCF**FAELGANVRCIDTDRNKIEQLNSG****  
 Consensus .....\*.....\*

BP1629 52 **HDHTEVEVDELARAAQLS---YTAER--EELGKANVFIVTVPTPIDEYKOPDLTPLVKA**  
 BP3150 47 **HDHTEVEVDELARAAQLS---YTAER--EELGKANVFIVTVPTPIDEYKOPDLTPLVKA**  
 PGN\_0613 59 **YDSTGEIGNELLOEALQKG---LLCTTKIEEIRDCNFYIVTVPTPIDHNTPLDRPLAA**  
 PA WbpA 58 **QCYIEHIPQAKTAKARASG---FEATDFSRVSECDA**LILCVPTPLNKYREPDM**SVFINT**  
 PGN\_1243 121 **TIPYEPGLEKMIARNVKA**GRLRFGTEIEQAVPEADII**FI**AVGTPAGE**DGSADMSYV**LD****  
 Consensus .....\*.....\*

BP1629 107 **SETIGAVLKRGDIVIYESTVYPGATEEDCVPVLERVSGLK**ENODFYAGYS**PERINPGDKA**  
 BP3150 102 **SETIGAVLKRGDIVIYESTVYPGATEEDCVPVLERVSGLK**ENODFYAGYS**PERINPGDKA**  
 PGN\_0613 116 **SETIGKVIDQGDVVVYESTVYPGVTEDECIPIIEK**VSGLKYNVDFEAGYS**PERINPGDKV**  
 PA WbpA 115 **TDALKPYLRVCGVVSLESTY**PGTTEELL**PRVQEG-GLVVGRDIYLVV**SPERED**PGNP**  
 PGN\_1243 181 **ARSIGRAMSRVILIVTKSTV**PVGSYRLIRKAIQ**EELDKREVLID**EDIA**SN**PEFL**KEGN**AI****  
 Consensus .....\*.....\*

BP1629 167 **HRVNTIKKVTSGS-TPEVAELVDQLYN-EIITAGTHKASSIRVAEAAKV**IENTORDV**NIA**  
 BP3150 162 **HRVNTIKKVTSGS-TPEVAELVDQLYN-EIITAGTHKASSIRVAEAAKV**IENTORDV**NIA**  
 PGN\_0613 176 **YTVENIKKVTSGS-TPEIADYVDRMYN-TVLVNGTHKASSIRVAEASKI**IENAORDV**NIA**  
 PA WbpA 174 **FETRTIPKVI**GCHTPQC**LEV**GIALYEQA**IDR-VVPV**SSTKAAEMTKL**ENI**HRAV**NI**G****  
 PGN\_1243 241 **DDFMKPD**RVVV**GVDSDRARELITSLYK**PMLLNN**FRVLFMD**IASAEMTKYAN**AML**ATRI**S**  
 Consensus .....\*.....\*

BP1629 225 **LINELALIFNKMGIDTEAVLQ**AAGTKWN**--FLPFRPG-LVGGHCIGVD**PYYLTHKAQA**IG**  
 BP3150 220 **LINELALIFNKMGIDTEAVLQ**AAGTKWN**--FLPFRPG-LVGGHCIGVD**PYYLTHKAQA**IG**  
 PGN\_0613 224 **FMNEIAKIFNAMEIDTREVLEAAATKWN--FLPFOPG-LVGGHCIGVD**PYYLQRAQ**VY**G****  
 PA WbpA 231 **LVNEMKIVADRMGIDIFEVVDAAATK**PF**G-FT**PY**Y**PG**PLGGHCIPID**PFY**L**ITW**KAREY**G****  
 PGN\_1243 301 **FMNDV**ANL**CER**VGAD**VS**MVRLGIG**SDSRIGSKFLY**PGCGY**GGSC**FPKDV**KAL**IRTAED**NG**  
 Consensus .....\*.....\*

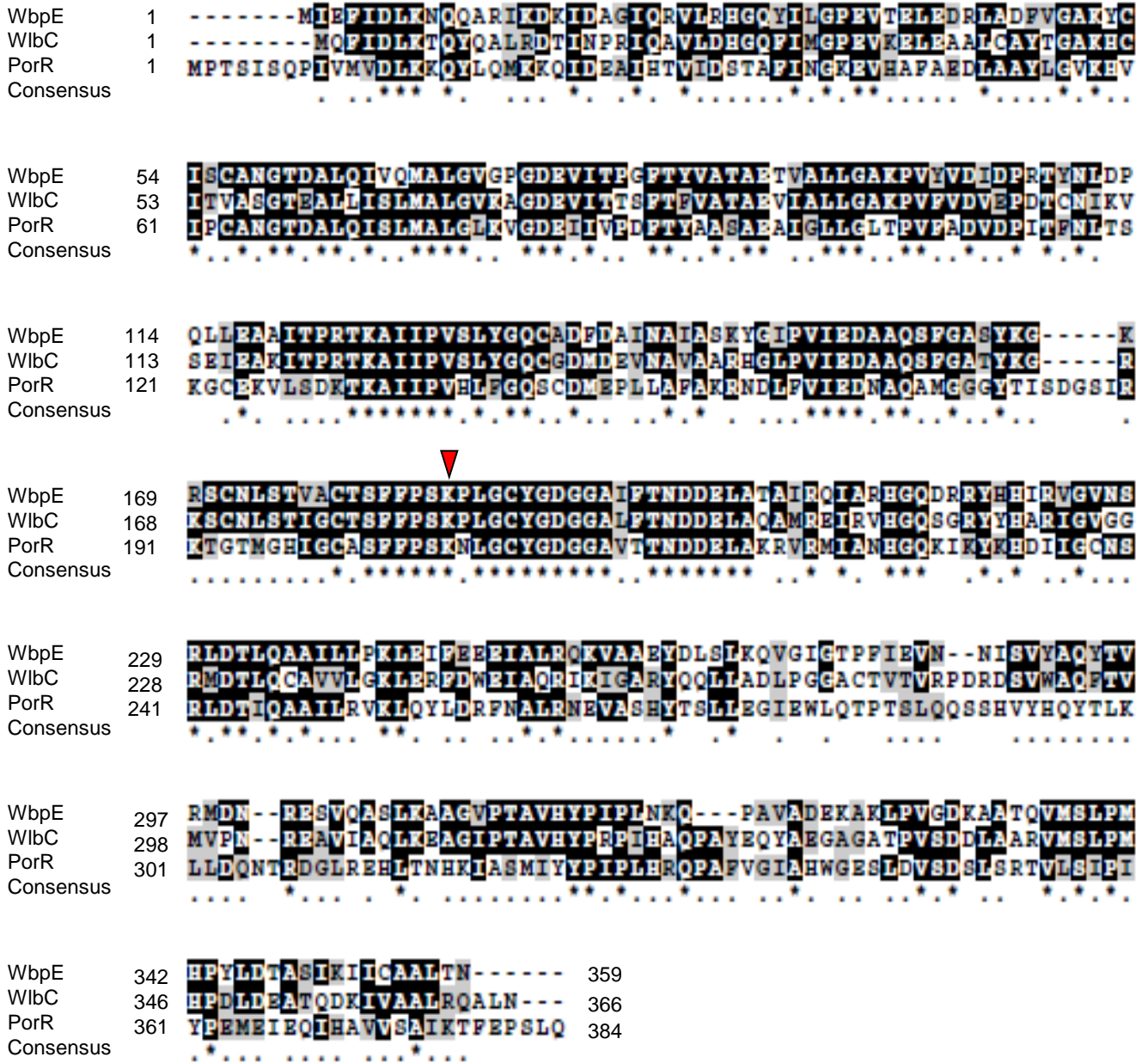
BP1629 282 **YHPEIILAGRR**LNDS**MGYVVSOLVK**AMAKRRIH**VEGARVLL**MGLTFK**ENC**PDLRNTR**RVV**  
 BP3150 277 **YHPEIILAGRR**LNDS**MGYVVSOLVK**AMAKRRIH**VEGARVLL**MGLTFK**ENC**PDLRNTR**RVV**  
 PGN\_0613 291 **VYPRV**LMSARR**LNDSMG**SYLASOTIK**Q**MN**KAGIM**VKDAR**LL**L**G**TFK**ENC**PD**IR**NTK**VI**  
 PA WbpA 290 **L**TRF**IEAKGGV**NQAM**PEY**VLG**KLMD**GLNEA**GRAL**K**SRV**LV**L**GI**AY**KK**N**V**LD**DM**RES**PSV****  
 PGN\_1243 361 **YRMEVLEAVERVNEKQKSILFDKFSTY**YKGN**--V**Q**RCV**AIW**GL**S**FK**PG**T**DD**M**REAP**SL**  
 Consensus .....\*.....\*

BP1629 342 **DIVRELGEYHVDV**DVYDP**WVD**PAAEA**HE**YGI**TP**VAK**PA**AGA**---Y**DAI**ILAV**SH**HQ**FVE****  
 BP3150 337 **DIVRELGEYHVDV**DVYDP**WVD**PAAEA**HE**YGI**TP**VAK**PA**AGA**---Y**DAI**ILAV**SH**HQ**FVE****  
 PGN\_0613 351 **DTYST**LQ**EYTKNI**IV**HDP**WAN**PSIA**EQ**Y**G**IKI**HNE**FP**KGK**---F**DAI**ILAV**SH**HQ**FVE**SN**  
 PA WbpA 350 **E**IM**ELIEAKGG**MVAYS**DP**H**VP**V**FP**KMR**EH**HF**EL**S**E**PL**TAEN**LAR**FD**AV**V**L**AT**D**ED**K**FD**Y****  
 PGN\_1243 418 **V**L**IEK**L**LE**V**GC**R**VR**V**Y**D**EV**AM**KEA**Q**K**R**L**G**DK**V**E**Y**TT**D**MY**D**AV**R**G-**A**E**AL**PH**V**TE**W**KE**ER**M**  
 Consensus .....\*.....\*

BP1629 398 **MGAEAIRK**FGKAEH**ILYDLKYV**L**APE**QAD**LRL-----** 429  
 BP3150 393 **MGAEAIRK**FGKAEH**ILYDLKYV**L**APE**QAD**LRL-----** 424  
 PGN\_0613 407 **IDLEKS--STANTIIY**D**V**K**GIL**P**Q**G**K**Y**HK**L**-----** 435  
 PA WbpA 410 **ELIK**AE**AKLV**VDS**R**G**K**Y**R**S**PA**A**HI**I**KA-----** 436  
 PGN\_1243 477 **PDWS**ALS**Q**AMA**AS**L**VID**G**R**N**V**Y**EL**P**AD**S**D**FT**LL**N**IG**NSA**IES**AS**SK-----** 522  
 Consensus .....\*.....\*



# Supplemental Figure 3





Supplemental Table 1. Bacterial strains used in this study.

<i>P. gingivalis</i> strain	Description	Source or reference
33277	Wild type	ATCC
HG66	Naturally non-pigmented strain	Gift from J. Potempa <sup>a</sup>
HG66/pWbpB+	HG66/pTCB-wbpB	This study
KDP220	<i>wbpB</i> ::Em <sup>f</sup>	This study
KDP221	<i>porR</i> :Tc <sup>f</sup>	This study
KDP222	PGN_0002::Em <sup>f</sup>	This study
KDP223	KDP222/pTCB-PGN_0002	This study
KDP224	KDP222/pTCB-P. a. wbpD	This study
Pugd1	<i>ugdA</i> ::Em <sup>f</sup>	Sato et al. 2009
W83	Wild type	Gift from M. J. Duncan <sup>b</sup>

<i>E. coli</i> strain	Description	Source or reference
S17-1	RP-4-2-Tc::Mu <i>aph</i> ::Tn7 <i>recA</i> , Sm <sup>f</sup>	Simon et al. 1983
XL-1 Blue	General purpose host strain for cloning	Stratagene

<i>P. aeruginosa</i> strain	Description	Source or reference
PAO1	Wild-type	Gift from J. S. Lam <sup>c</sup>



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c Department of Molecular and Cellular Biology, University of Guelph, Ontario, Canada.

Supplemental Table 2. Plasmids used in this study.

Plasmid	Description	Source or reference
pBluescript II SK(-)	Ap <sup>r</sup> , cloning vector	Stratagene
pBSSK-cat pro-rgpBter	Ap <sup>r</sup> , pBSSK-cat pro-rgpBter	This study
pBSSK-cat pro-wbpB-rgpBter	Ap <sup>r</sup> , pBSSK-cat pro-wbpB-rgpBter	This study
pBSSK-cat pro-PGN_0002-rgpBter	Ap <sup>r</sup> , pBSSK-cat pro-PGN_0002-rgpBter	This study
pBSSK-cat pro-P. a. wbpD-rgpBter	Ap <sup>r</sup> , pBSSK-cat pro-P. a. wbpD-rgpBter	This study
pCR4	Ap <sup>r</sup> Km <sup>r</sup> , PCR Topo cloning vector	Invitrogen
pCR4-cat pro	Ap <sup>r</sup> Km <sup>r</sup> , pCR4-cat pro	This study
pCR4-porRup	Ap <sup>r</sup> Km <sup>r</sup> , pCR4-porRup	This study
pCR4-porRdw1	Ap <sup>r</sup> Km <sup>r</sup> , pCR4-porRdw1	This study
pCR4-porRdw2	Ap <sup>r</sup> Km <sup>r</sup> , pCR4-porRdw2 (NotI self-ligated of pCR4-porRdw1)	This study
pCR4-porRupdw2	Ap <sup>r</sup> Km <sup>r</sup> , pCR4-porRup-porRdw2	This study
pGEM-T Easy	Ap <sup>r</sup> , PCR cloning vector	Promega
pKD375	Ap <sup>r</sup> , Tc <sup>r</sup> , pUC19- <i>tetQ</i>	Shi et al. 1999
pKD740	Ap <sup>r</sup> Em <sup>r</sup> , pGEM-T Easy-hbp35up- <i>ermF</i> -hbp35dw	Shoji et al. 2010
pKD854	Ap <sup>r</sup> , pBSSK-sKGP-rgpBter	Sato et al. 2005
pKD892	Ap <sup>r</sup> Em <sup>r</sup> , pGEM-T Easy-PGN_0168up- <i>ermF</i> -PGN_0168dw	This study
pKD893	Ap <sup>r</sup> Em <sup>r</sup> , pGEM-T Easy-PGN_0002up- <i>ermF</i> -PGN_0002dw	This study
pKD894	Ap <sup>r</sup> Km <sup>r</sup> Tc <sup>r</sup> , pCR4-PGN_1236up- <i>tetQ</i> -PGN_1236dw	This study
pKD955	Ap <sup>r</sup> , pBSSK-p-porK+-T	Sato et al. 2010
pTCB	Ap <sup>r</sup> Tc <sup>r</sup> , shuttle vector plasmid	Nagano et al. 2007

pTCB- <i>wbpB</i>	Ap <sup>r</sup> Tc <sup>r</sup> , pTCB-PGN_0168	This study
pTCB-PGN_0002	Apr Tc <sup>r</sup> , pTCB-PGN_0002	This study
pTCB- <i>P. a. wbpD</i>	Ap <sup>r</sup> Tc <sup>r</sup> , pTCB- <i>wbpD</i> from <i>P. aeruginosa</i> PAO1	This study
pUC118	Ap <sup>r</sup> , PCR cloning vector	Takara
pUC118- <i>wbpB</i>	Ap <sup>r</sup> , pUC118-PGN_0168	This study
pUC118-PGN_0002	Ap <sup>r</sup> , pUC118-PGN_0002	This study
pUC118- <i>P.a. wbpD</i>	Ap <sup>r</sup> , pUC118- <i>wbpD</i> from <i>P. aeruginosa</i> PAO1	This study

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Supplemental Table 3. Primers used in this study.

Primer name	Nucleotide sequence (5'-3')	underline	double underline
p6-34-F-KpnI	<u>GGTACCTTCGTCGTC</u> CAATCAGCATCCCAG	KpnI	
p6-34-R-SalI	<u>GTCGACTGTTTTGTCTCTT</u> ATTTAAGTTA	SalI	
PGN_0002upFw	<u>GCATGCTCCAGCAGCGT</u> ACGCTTCAAAGC	SphI	
PGN_0002upRev	<u>GGATCCAAAGACCTGCT</u> ATCAATTGGCGA	BamHI	
PGN_0002dwFw	<u>CTGCAGTCTACACCAA</u> ATAGCGGCATATC	PstI	
PGN_0002dwRev	<u>GAGCTCCGATTACATA</u> CTCGGTTACAACC	SacI	
PGN_0168upFw	<u>GCATGCATGCACAAC</u> TTTGCCATCATCGG	SphI	
PGN_0168upRev	<u>GGATCCCTCGCATAT</u> CACATCCGCTCCAA	BamHI	
PGN_0168dwFw	<u>CTGCAGAAAGGAAT</u> TTCTATAATCTTATC	PstI	
PGN_0168dwRev	<u>GAGCTCCGCTGTGC</u> ACCGATGTCGGACAC	SacI	
PGN_1236upFw	<u>GGTACCGTGAACGAT</u> AGCGAAGGGAAAGCA	KpnI	
PGN_1236upRev	<u>GGATCCAGGCGTGT</u> TGTAGGGATTTCTGGT	BamHI	
PGN_1236dwFw	<u>GGATCCCAAAGAG</u> GAGGGAGGCTCGTCTA	BamHI	
PGN_1236dwRev	<u>GCGGCCGC</u> CAGATTATTGCCCAGACCTTGA	NotI	
PGN_0168compFw	<u>GTCGACATG</u> CACAACTTTGCCATCATCGGT	SalI	Start codon
PGN_0168compRev	<u>TCTAGATTAG</u> CGTCTCCATCCGAAAGGATG	XbaI	Stop codon
PGN_0002compFw	<u>GTCGACATG</u> ATCCATCCCACAGCGATAGTA	SalI	Start codon
PGN_0002compRev	<u>TCTAGATTAG</u> TCCGATACCGGTTCTATTGT	XbaI	Stop codon
<i>P.a. wbpD</i> compFw	<u>GTCGACATG</u> AGTTATTATCAGCACCCCAGCGCGATCGTGGAC	SalI	Start codon
<i>P.a. wbpD</i> compRev	<u>TCTAGATTAC</u> ACGTCCACCTTGCTCAGGAT	XbaI	Stop codon