**The PorX/PorY system is a virulence factor of *Porphyromonas gingivalis* and mediates the activation of the Type IX secretion system**

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Running Title: Gene regulation of the PorX/PorY system in *P. gingivalis*
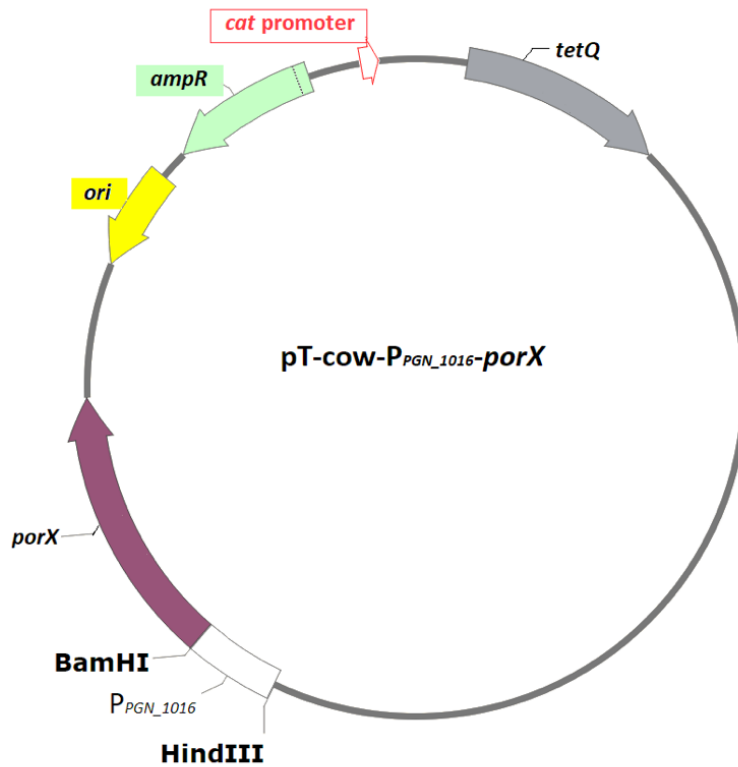
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Figure S1 to S3

**Fig. S1**

**Fig. S1. Map of the *porX* complementation plasmid.** The plasmid pT-COW-P$_{PGN\_1016}$-*porX* contained a wild-type copy of the *porX* coding region (marked as the *purple curved arrow*). Transcription of this gene was initiated by a promoter located upstream of the *PGN_1016* gene. Both *PGN_1016* and *porX* genes were predicted to be members of an operon on *P. gingivalis* genomes.
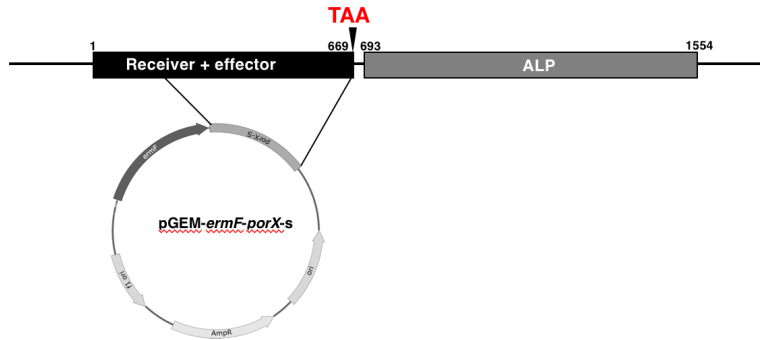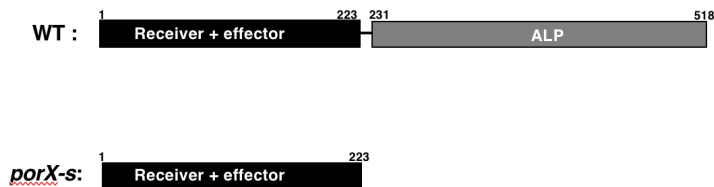
**A**

porX coding region:



**B**



# Fig. S2

F

**Fig. S2. Schematic representatives of the strategy to construct the *porX-s* strain. (A)** The *porX* coding region. The *black box* represents the DNA region encoding the first 223 amino acid residues with receiver + DNA-binding regions, and the *grey box* represents the DNA region encoding the ALP region. Suicide plasmid pGEM-*ermF-porX-s* was inserted into the *porX* coding region to introduce a TAA stop codon between the 669th-670th nucleotides of the *porX* coding sequence (indicated by an *arrowhead*). *Numbering* represents the nucleotides of the coding sequence. **(B)** Structures of the PorX proteins. The full-length wild-type PorX protein (*upper*) and truncated PorX-s protein (*bottom*). The first 223 amino acid residues (*black box*) represent receiver + DNA-binding regions shared by both WT PorX and PorX-s proteins. The ALP sequence (*grey box*) was deleted in PorX-s protein. *Numbering* represents codon position.
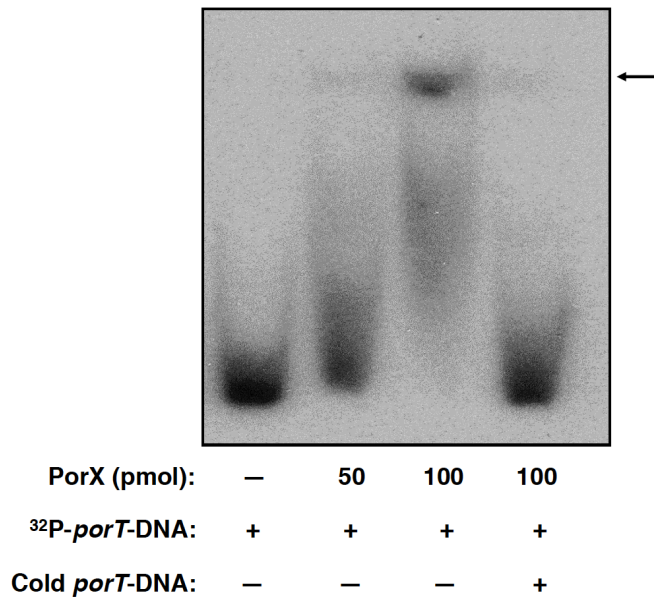
| PorX (pmol): | — | 50 | 100 | 100 |
| $^{32}$P-*porT*-DNA: | + | + | + | + |
| Cold *porT*-DNA: | — | — | — | + |

# Fig. S3

**Fig. S2. The PorX response regulator binds to the *porT* promoter region.** EMSA analysis for binding of PorX to the *porT* promoter. $^{32}$P-labeled *porT* DNA fragment was incubated with PorX-c-His$_6$ protein at the indicated amount. The 4th lane is the same as the 3rd lane but supplemented with non-labeled (cold) *sigP* DNA fragment. The PorX/DNA mixtures were subjected to 5% PAGE. Location of DNA migration was detected by autoradiography. *Arrow* indicates the shifted bands after DNA fragments were associated with the PorX-His$_6$ protein.