Supplementary data

A pseudaminic acid or a legionaminic acid derivative transferase is strain-specifically implicated in the general protein *O*-glycosylation system of the periodontal pathogen *Tannerella forsythia*

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Supplementary data: Supplementary Figures 1-3

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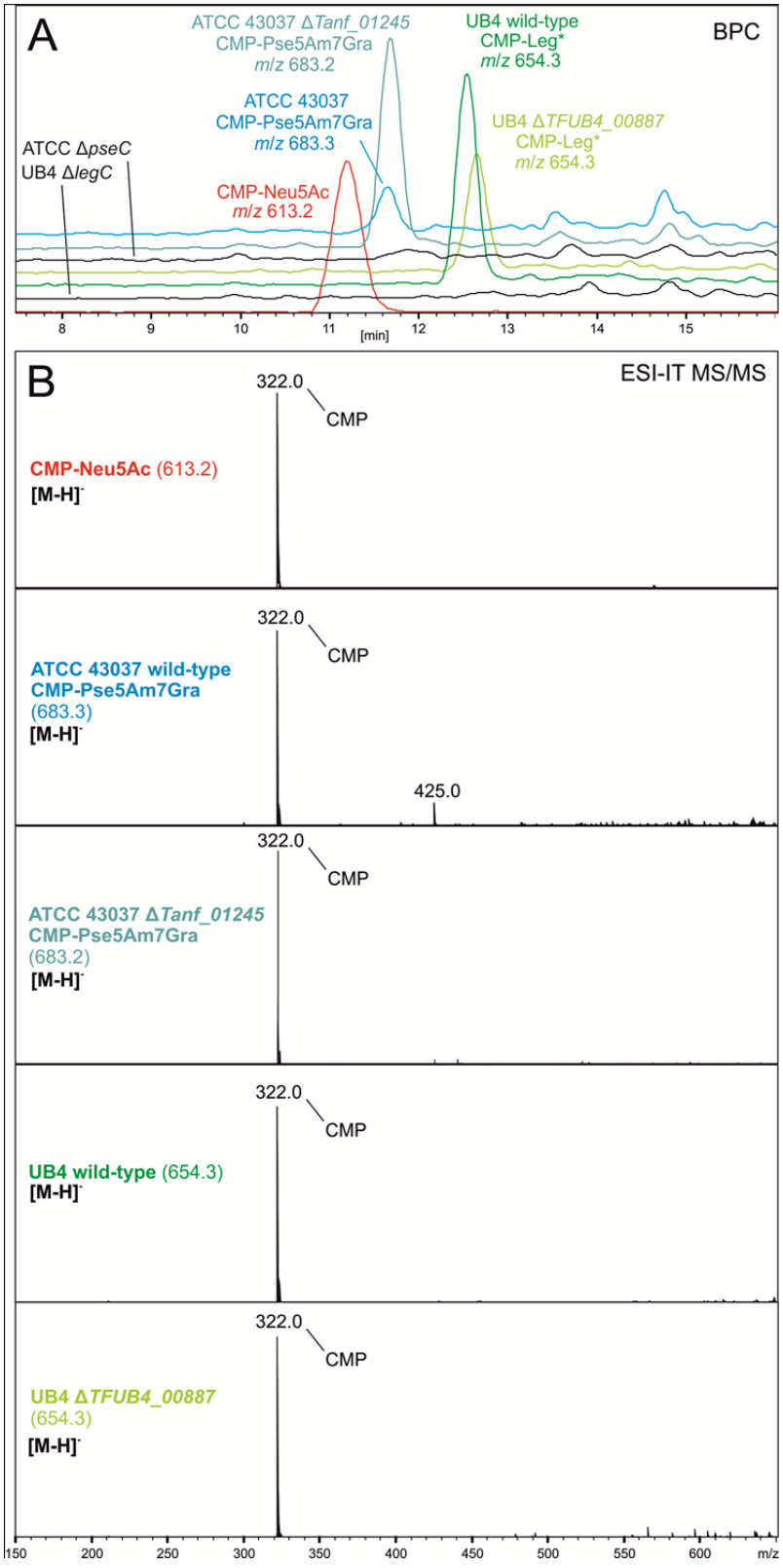
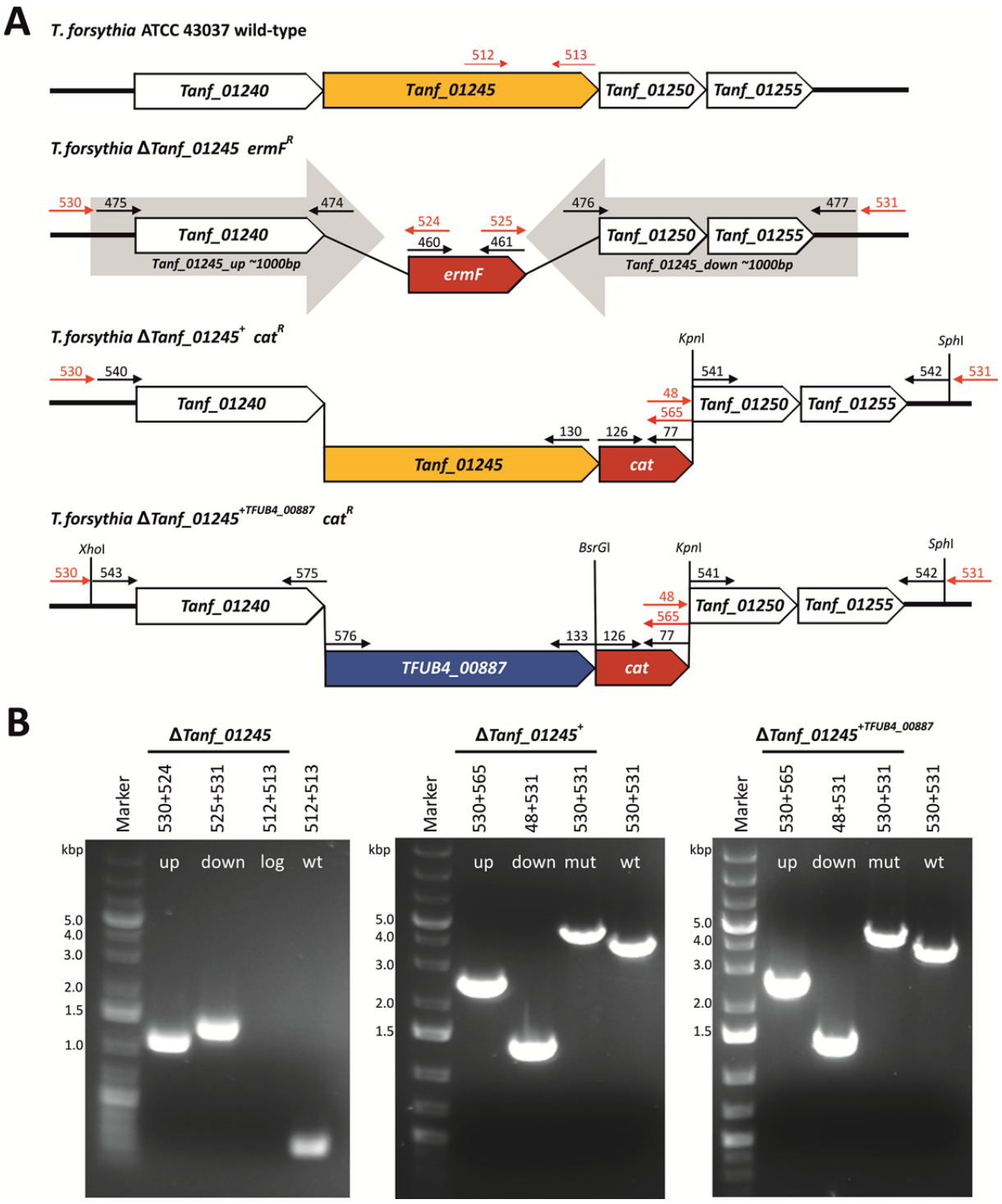


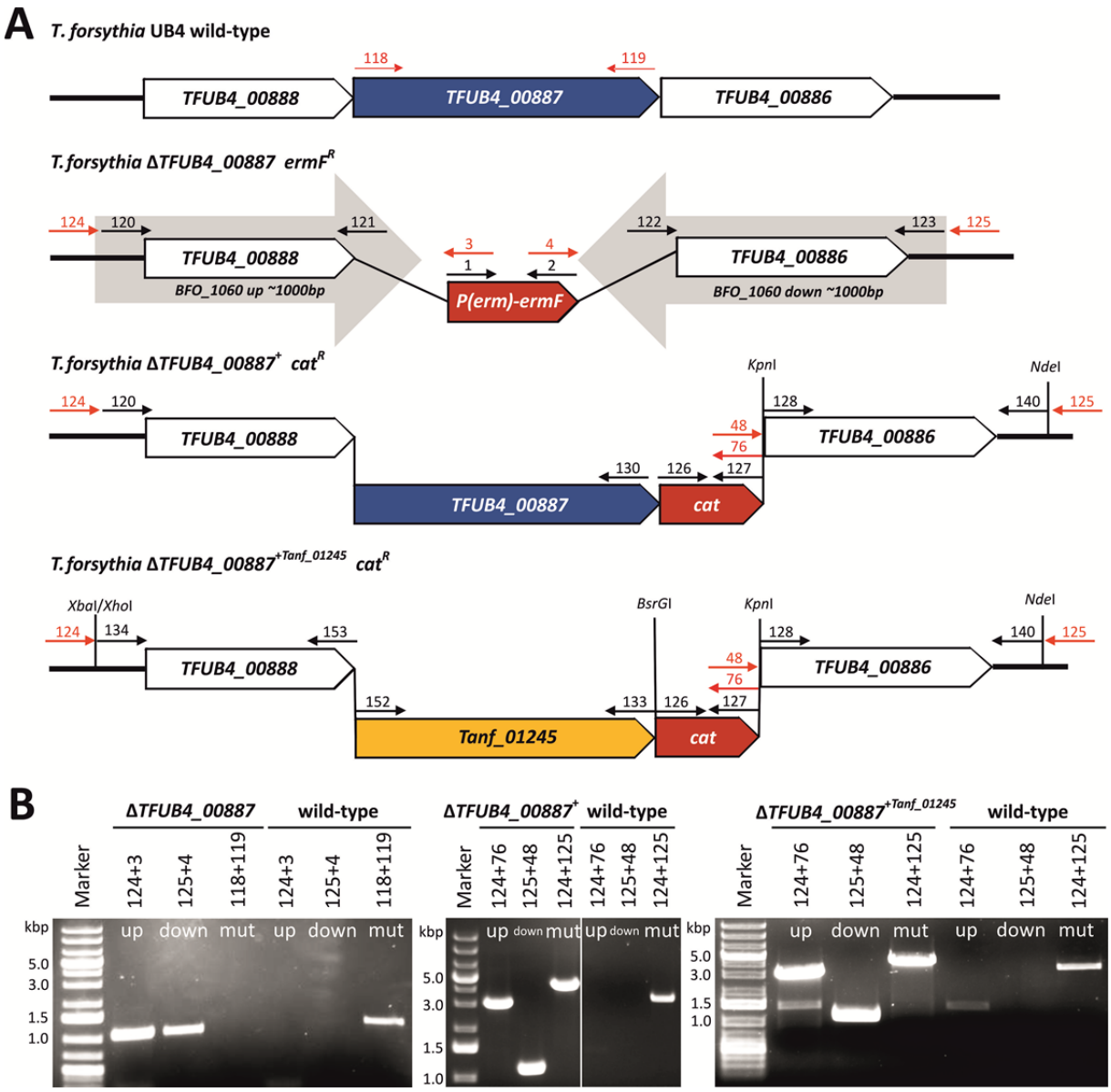
Supp. Fig. 1. ESI-MS analyses of CMP-activated sugars from *T. forsythia* ATCC 43037 and *T. forsythia* UB4. (**A**) Overlay of the PGC column elution profile of CMP-Neu5Ac serving

as standard (*m*/*z* 613.2; red) and the known CMP-Pse5Am7Gra unit from ATCC 43037 (*m*/*z* 683.3; dark blue) and the Δ*Tanf_01245* mutant (*m*/*z* 683.2; light blue) in comparison to the uniquely found UB4 mass of *m*/*z*=654.3, present in the parent strain (dark green) and the Δ*TFUB4_00887* mutant (light green), shown in a base peak chromatogram (BPC). (**B**) Confirmation of the presence of the CMP-activator in all of the analyzed samples by CID-fragmentation, where all CMP-activated sugars show the typical *m*/*z*=322.0 peak originating from CMP. The sugar part did not ionize well and, therefore, could not be detected. Relative intensities of occurring peaks are given on the *y* axis.



Supp. Fig. 2. Strategy for the generation of *T. forsythia* ATCC 43037 mutants at the *Tanf_01245* locus and confirmation by PCR. (**A**) The genomic organization of the

Tanf_01245 locus is shown for the parent strain *T forsythia* ATCC 43037, the Δ*Tanf_01245* mutant, the reconstituted mutant Δ*Tanf_01245*$^{+}$ and the cross-complemented mutant Δ*Tanf_01245*$^{+TFUB4\_00887}$. Black colored arrows represent primers used for PCR amplification of genes and homologous regions, red colored primers represent those used to screen for correct integration of the knock-out, reconstitution and cross-complementation cassettes; restriction sites used for cloning are indicated (not drawn to scale). (**B**) Agarose gel electrophoresis (left) confirms the deletion of *Tanf_01245* using the up-stream primers 530/524 (1105 bp) and down-stream primers 525/531 (1314 bp) on genomic DNA of *T. forsythia* ATCC 43037 Δ*Tanf_01245* mutant with integrated *ermF* cassette. Primers 512/513 yield in a 239-bp PCR fragment when using *T. forsythia* wild-type genomic DNA, whereas this fragment is absent on genomic DNA of the Δ*Tanf_01245* mutant confirming the loss of the gene (log). The agarose gel electrophoresis (middle) confirms the reconstitution of the deleted *Tanf_01245* gene using up-stream primers 530/565 (2442 bp) and down-stream primers 48/531 (1337 bp). Screening primers 530/531 yield in a 4374-bp PCR product on genomic DNA of the reconstituted mutant Δ*Tanf_01245*$^{+}$ with integrated *cat* resistance gene, whereas the same primer pair results in a 3700-bp product on genomic DNA of the *T. forsythia* wild-type. Agarose gel electrophoresis (right) confirms the cross-complementation with *TFUB4_00887* using up-stream primers 530/565 (2433 bp) and down-stream primers 48/531 (1337 bp) on genomic DNA of Δ*Tanf_01245*$^{+TFUB4\_00887}$. Screening primers 530/531 yield in a 4365-bp PCR product on genomic DNA of the cross-complemented mutant Δ*Tanf_01245*$^{+TFUB4\_00887}$, whereas the same primer pair results in a 3700-bp product on genomic DNA of the *T. forsythia* wild-type. O´Gene Ruler 1 kb Plus DNA Ladder (Thermo Fisher Scientific) was used as a gene ladder.



Supp. Fig. 3. Strategy for the generation of *T. forsythia* UB4 mutants at the *TFUB4_00887* locus and confirmation by PCR. (**A**) The genomic organization of the *TFUB4_00887* locus is shown for the parent strain, the UB4 Δ*TFUB4_00887* mutant, the reconstituted UB4 Δ*TFUB4_00887*$^{+}$ mutant and the cross-complemented UB4 Δ*TFUB4_00887*$^{+Tanf\_01245}$ strain. Black colored arrows represent primers used for PCR amplification of genes and homologous regions, red colored represent those used to screen for correct integration of the knock-out, reconstitution and cross-complementation cassettes; restriction sites used for cloning are

indicated (not drawn to scale). (**B**) Agarose gel electrophoresis (left) confirms the deletion of *TFUB4_00887* using the up-stream primers 124/3 (1079 bp) and down-stream primers 4/125 (1090 bp) on genomic DNA of *T. forsythia* UB4 Δ*TFUB4_00887*mutant with integrated *ermF* cassette and on UB4 wild-type genomic DNA. Primers 118/119 yield in a 1278-bp PCR fragment when using *T. forsythia* UB4 wild-type genomic DNA, whereas this fragment is absent on genomic DNA of the Δ*TFUB4_00887* mutant (mut), confirming the loss of the gene. The agarose gel electrophoresis (middle) confirms the reconstitution of the deleted *TFUB4_00887* gene using up-stream primers 124/76 (2987 bp) and down-stream primers 48/125 (1102 bp). Screening primers 124/125 yield in a 4048-bp PCR product on genomic DNA of the reconstituted mutant Δ*TFUB4_00887*$^{+}$ with integrated *cat* resistance gene, whereas the same primer pair results in a 3395-bp product on genomic DNA of the *T. forsythia* UB4 wild-type. Agarose gel electrophoresis (right) confirms the cross-complementation with *Tanf_01245* using up-stream primers 124/76 (3047 bp) and down-stream primers 48/125 (1102 bp) on genomic DNA of Δ*TFUB4_00887*$^{+Tanf\_01245}$. Screening primers 124/125 yield in a 4118-bp PCR product on genomic DNA of the cross-complemented mutant Δ*TFUB4_00887*$^{+Tanf\_01245}$, whereas the same primer pair results in a 3395-bp product on genomic DNA of the *T. forsythia* UB4 wild-type. O´Gene Ruler 1 kb Plus DNA Ladder (Thermo Fisher Scientific) was used as a gene ladder.