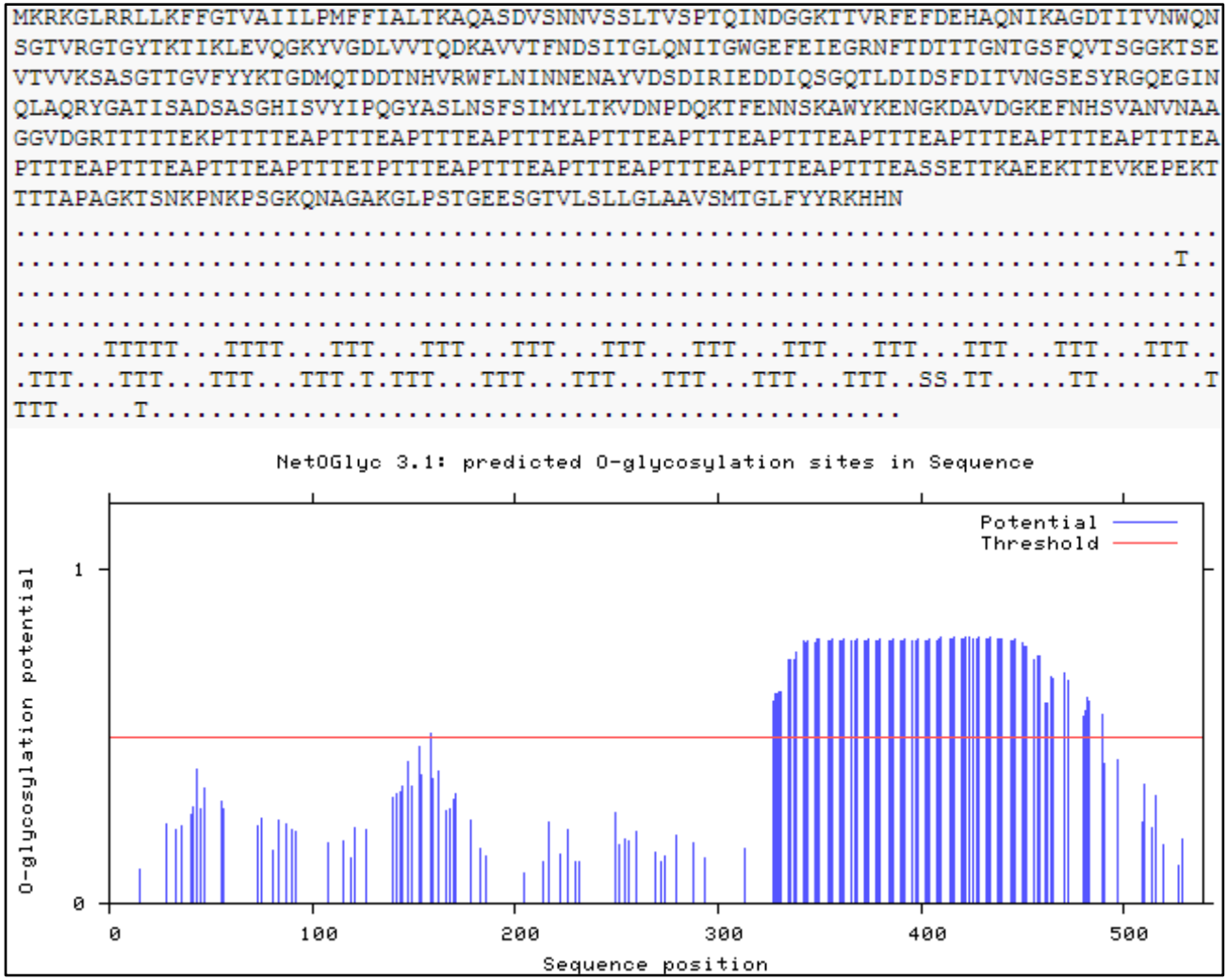

Figure S1 NetOGlyc 3.1 *In silico* analysis for *O*-glycosylation sites in Cnm. The prediction shows that the B-domain of Cnm has a sequence characteristic of mucin-like *O*-linked glycoproteins. Dotted residues represent sites not predicted to undergo glycosylation while serine and threonines predicted to be glycosylated appear as S or T, respectively. Amino acid positions predicted to be glycosylated are above the horizontal red line in the lower panel.

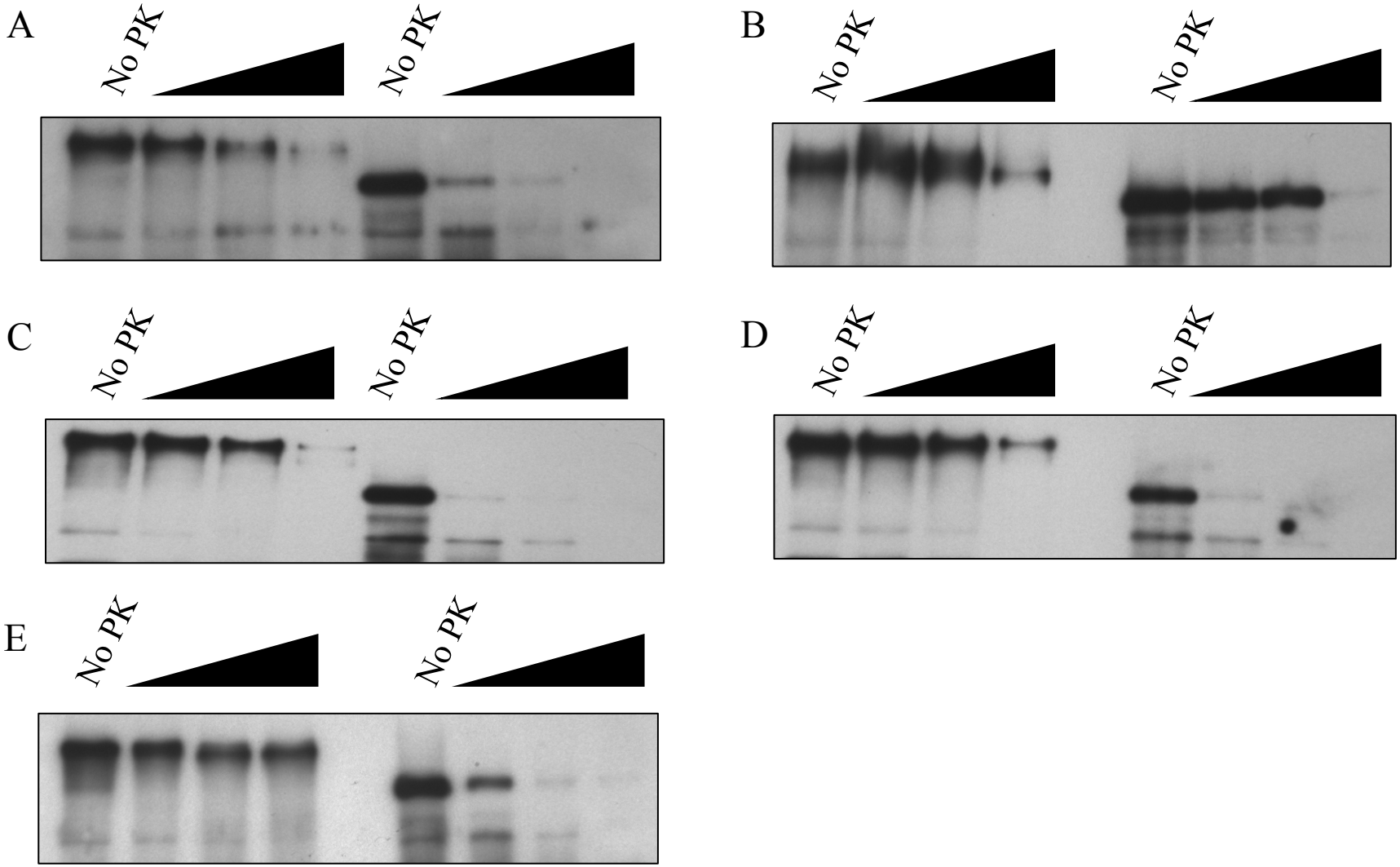

Fig S2 PgfS modification of Cnm is conserved among different strains of *S. mutans.* Detection of Cnm in *S. mutans* (A) NCTC11060, (B) B14, (C) LM7, (D) OM50E, and (E) UA159+*cnm* from whole cells treated with increasing concentrations of proteinase K (0, 0.3, 3 or 30 μg ml^{-1}) for 30 min. Whole cell lysates were prepared and Cnm degradation was monitored by Western blotting using anti-rCnmA. Western blot analysis shows a band at 120 kDa corresponding to mature Cnm. Shift in size to 90 kDa and increased susceptibility of Cnm in Δ*pgfS* strains compared to their respective parent strain indicates PgfS-dependent modification.

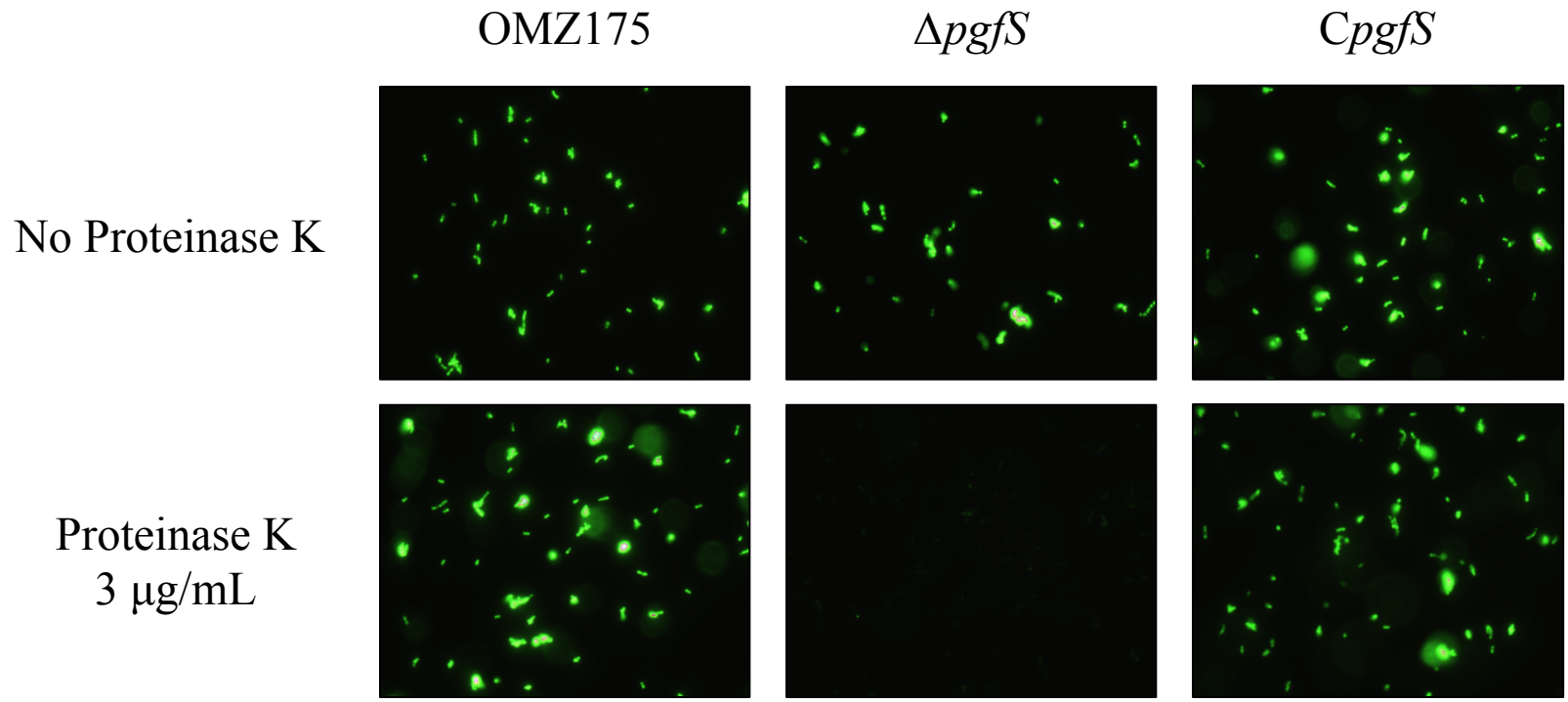

Fig S3 Immunofluorescence analysis of proteinase K treated *S. mutans.* Cnm degradation *in situ* by proteinase K in *S .mutans* strains (OMZ175, Δ*pgfS, CpgfS*) was monitored by immunofluorescent labeling using anti-rCnmA and anti-rabbit IgG coupled with Alexa-488 dye followed by visualization using a fluorescent microscope at 1000x magnification.

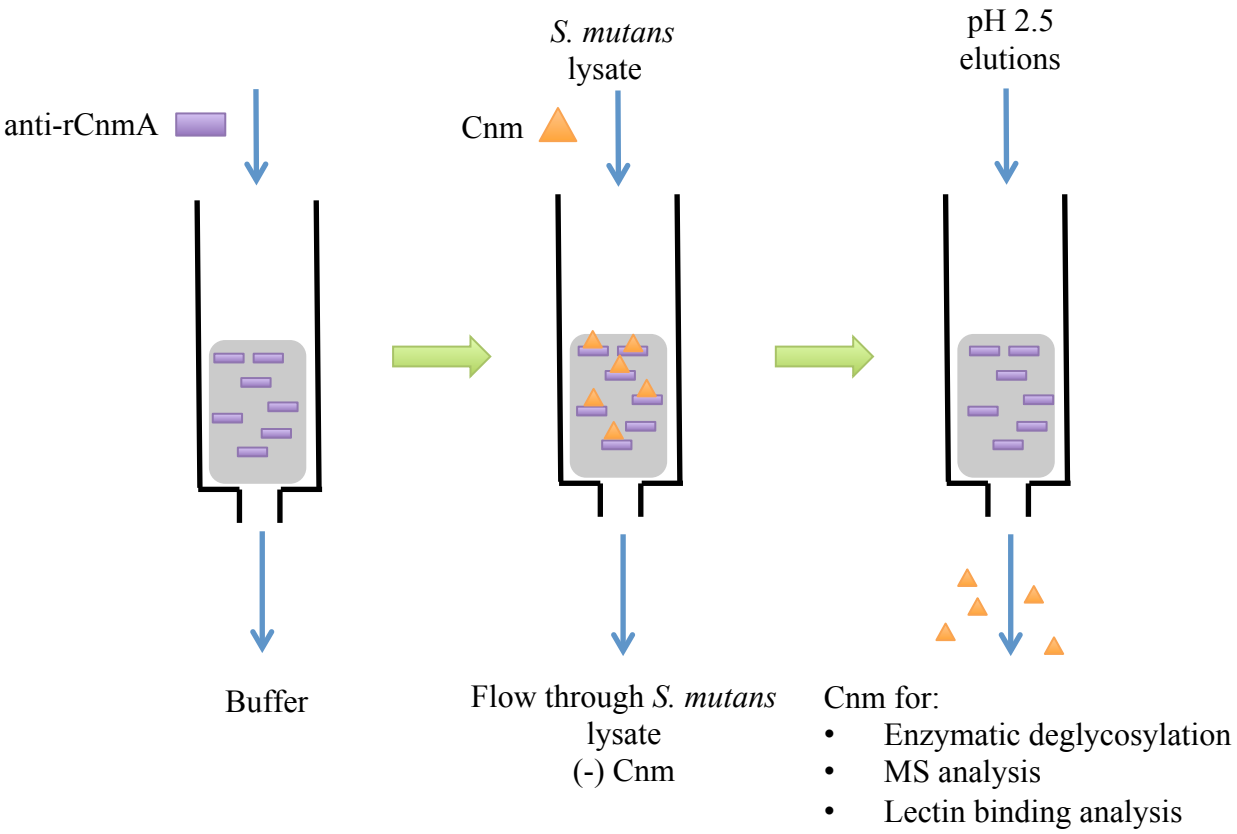

Fig S4 Diagram of Cnm affinity purification from *S. mutans* OMZ175 and Δ*pgfS* using anti-rCnmA. Purification of native Cnm was accomplished using a NHS-resin coupled with purified anti-rCnmA antibodies, which are specific for the A-domain of Cnm. Whole cell lysates from *S. mutans* OMZ175 and *ΔpgfS* were prepared in PBS and incubated with resin for 18 h. After washing the resin, Cnm was eluted of the column using 0.1M glycine pH 2.5. Purified Cnm was then concentrated and dialyzed against PBS or 100mM ammonium bicarbonate for subsequent analysis.

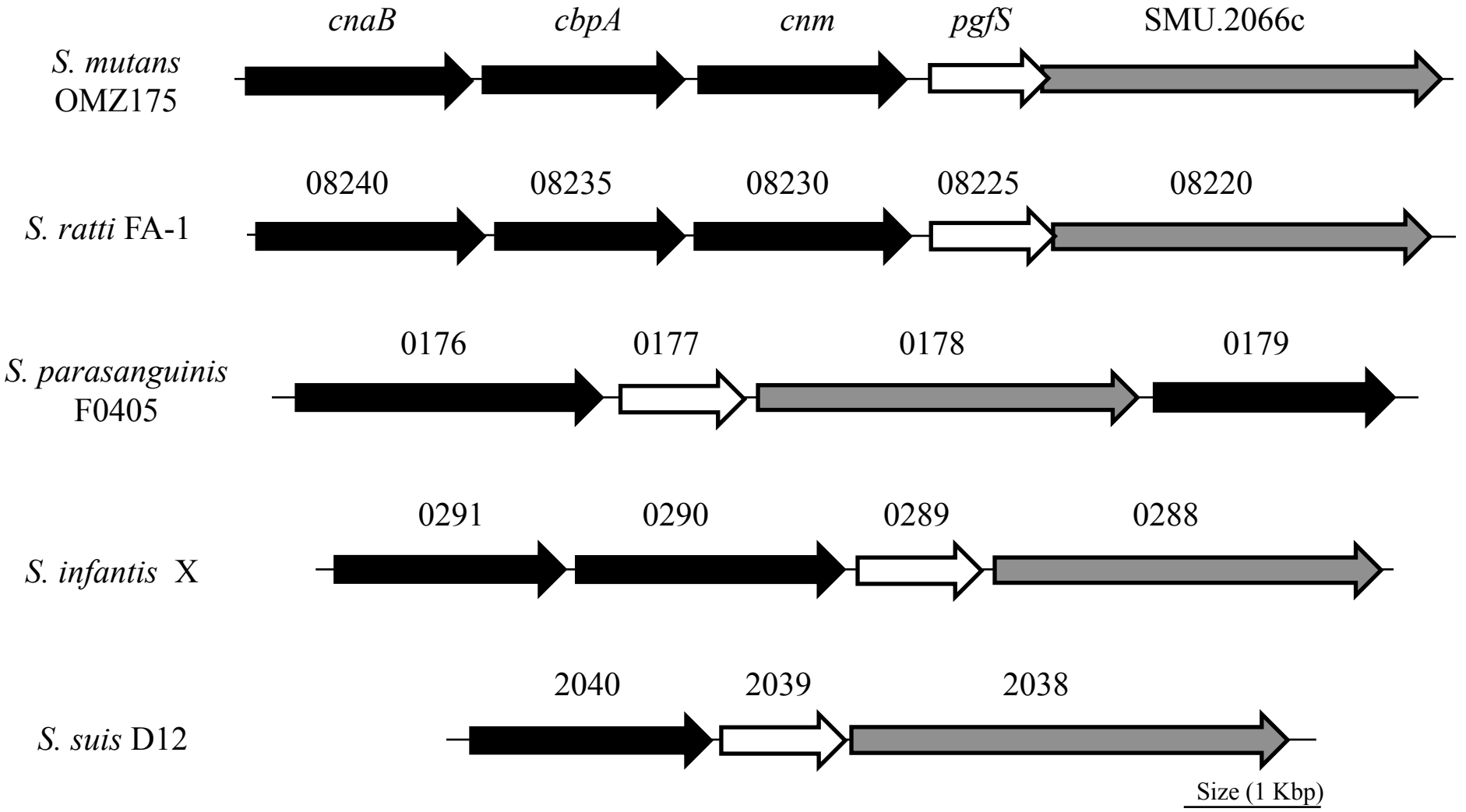

Fig S5 Threonine-rich CBPs and PgfS genomic arrangement in other streptococci. Conservation of CBPs adjacent to PgfS homologs in streptococcal species. Genes coding for threonine-rich collagen binding protein with LPXTG motif are shown in black, genes coding for *pgfS*-like putative glycosyltransferase are shown in white, and genes coding for SMU.2066c-like integral membrane protein are shown in gray.