SUPPLMENTAL METHODS

Assessment of YlxM conservation among bacterial *taxa*. The prevalence of YlxM and its genetic context, as depicted in Fig. S12 and Fig. S2, are from information gathered on the ncbi.nlm.nih.gov/protein and ncbi.nlm.nih.gov/gene web sites using YlxM as the search term. When required, BLAST searches were used to confirm non-annotated genes.

Western analysis of antisera. 30 mL each of mid-log cells of Wild-type, Δ*ffh,* Δ*ftsY,* and Δ*ylxM* were resuspended to 10 % their original volume in HM buffer and lysed by glass bead breakage in a Mini-bead Beater 8 apparatus (BioSpec Products, Inc., Bartlesville, OK). 10 µl of each cell extract was analyzed by Western blot as described in the main text with anti-Ffh, FtsY, and YlxM antisera. Serum dilutions were 1:5000, 1:2000, and 1:250, respectively. As a loading control total protein staining was performed using Colloidal Gold (Bio-Rad, Hercules, Ca).

Western analysis of Ffh and FtsY in *ylxM* mutants 30 mL each of mid-log cells of Wild-type, Δ*ylxM*, and +YlxM were resuspended to 10 % their original volume in HM buffer and lysed by glass bead breakage in a Mini-bead Beater 8 apparatus (BioSpec Products, Inc., Bartlesville, OK). 10 µl of each cell extract was analyzed by Western blot using anti-Ffh, FtsY, and YlxM antisera. Dilutions were 1:5000, 1:2000, and 1:500 respectively for Western analysis. As a loading control total protein staining was performed using Colloidal Gold (Bio-Rad, Hercules, Ca).

Capture ELISA. 96-well polystyrene plates (Costar, Corning, NY) were coated with 200 ng of purified recombinant FtsY. Wells were blocked with PBST (phosphate buffered saline containing 0.3 % Tween 20). Two-fold serial dilutions of FFh or YlxM, beginning at 200 ng, were added to the wells and incubated for 2 hours at 37 °C. Unbound proteins were removed by washing with PBST. Bound Ffh or YlxM was detected with specific rabbit antisera at dilutions of 1:5000 or 1:1000, respectively, followed by HRP-labeled goat anti-rabbit IgG, and development with o-phenylenediamine.

Protein translocation machinery mutants. The single mutants utilized were those generated previously (1,2). Genomic DNA obtained from the Δ*ylxM* mutant was used to transform Δ*ftsY,* Δ*yidC1,* or Δ*yidC2* single mutants. The Δ*ylxM*-Δ*ffh* strain was generated by allelic replacement with a kanamycin-resistance gene cassette. Splice overlap extension (SOE) PCR (3) was utilized to combine PCR-amplified fragments upstream and downstream of *ylxM-ffh* from NG8 genomic DNA with an intervening kanamycin resistance gene amplified from pALH124 (1). Primer pairs used were MW4F (CGCGAATTCCCTACAGCAATTTATCAGATTGGA) and MW25B (TTTAAGCTTAGGCTCTATTTTACCATATTTA), MW25C (ATAGAGCCTAAGCTTAAAAGAGGAAGGAAAT) and MW25D (GTCACTCTTTTCTACTAAACAATTCATCCAG), MW28E (TAGAAGCTTCAAATCACTTTATATCATTATG) and MW3R (CGCCTGCAGGATTACCTGACAAAAACTGTGATA). Primers MW4F and MW25D were used to combine the *ylxM-ffh* upstream and Kanamycin amplicons. The product was combined with the *ylxM-ffh* downstream fragment by SOE PCR using primers MW4F and MW3R. This SOE PCR product was used to transform wild-type NG8 *S. mutans.* All constructs were verified via sequencing.

Growth curves. Cultures of *S. mutans* strains grown for 16 h were diluted 1 : 20 in THYE, pH 7.0, without antibiotics and grown to OD_{600} 0.4–0.45. Strains then were diluted 1 : 10 into THYE buffered to pH 7.0 and 300 µl was loaded in triplicate wells on a 100-well Bioscreen C plate and overlaid with mineral oil. Growth was monitored in the Bioscreen C Machine (Growth Curves USA) at 37 °C for 18 h with OD_{600} recorded every 30 min.

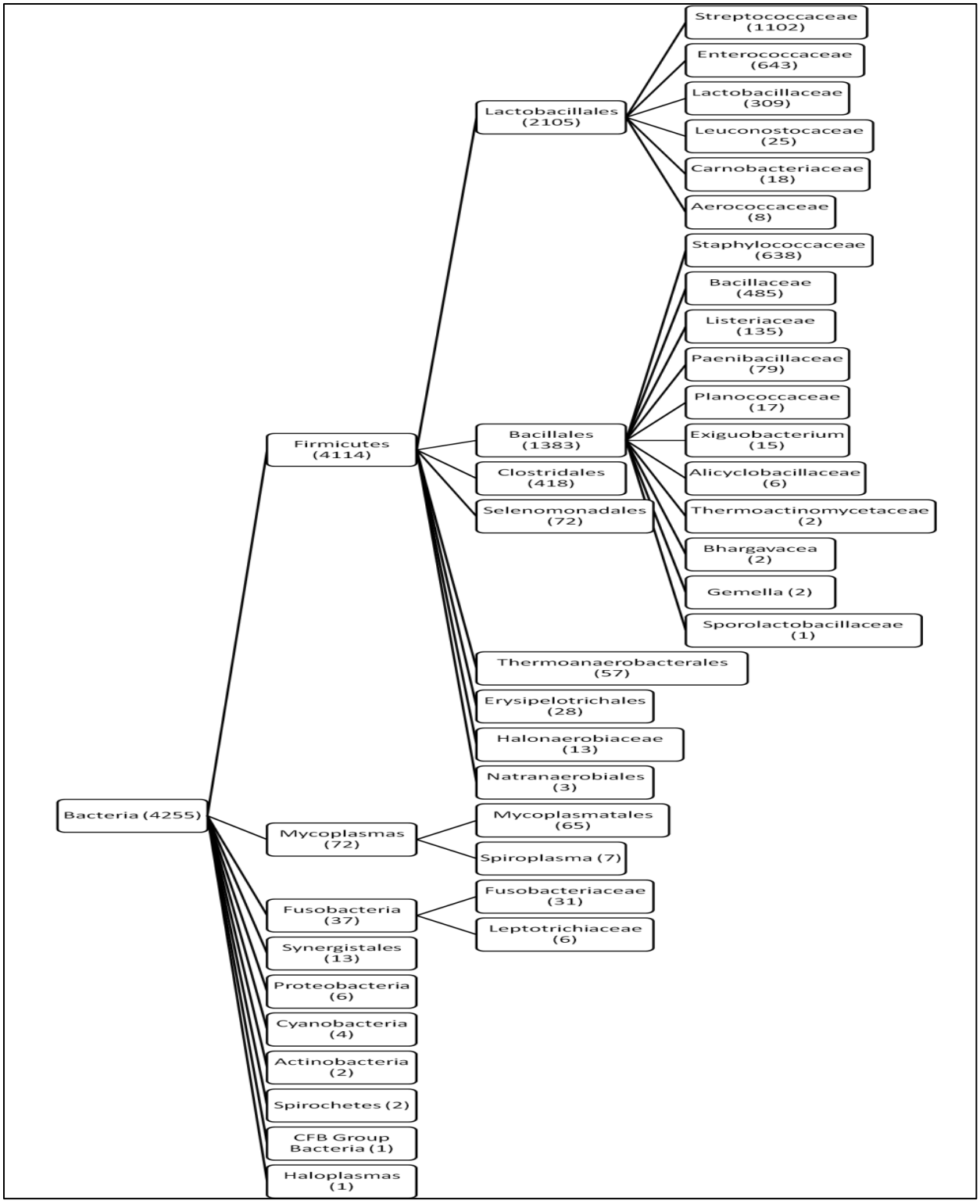

Fig. S1. Prevalence of *ylxM* among bacterial *taxa.* Schematic illustration of the known presence of YlxM within given bacterial classifications. Numbers represent the number of positive hits for proteins annotated as YlxM within *taxa* with sequenced genomes available in the NCBI database.

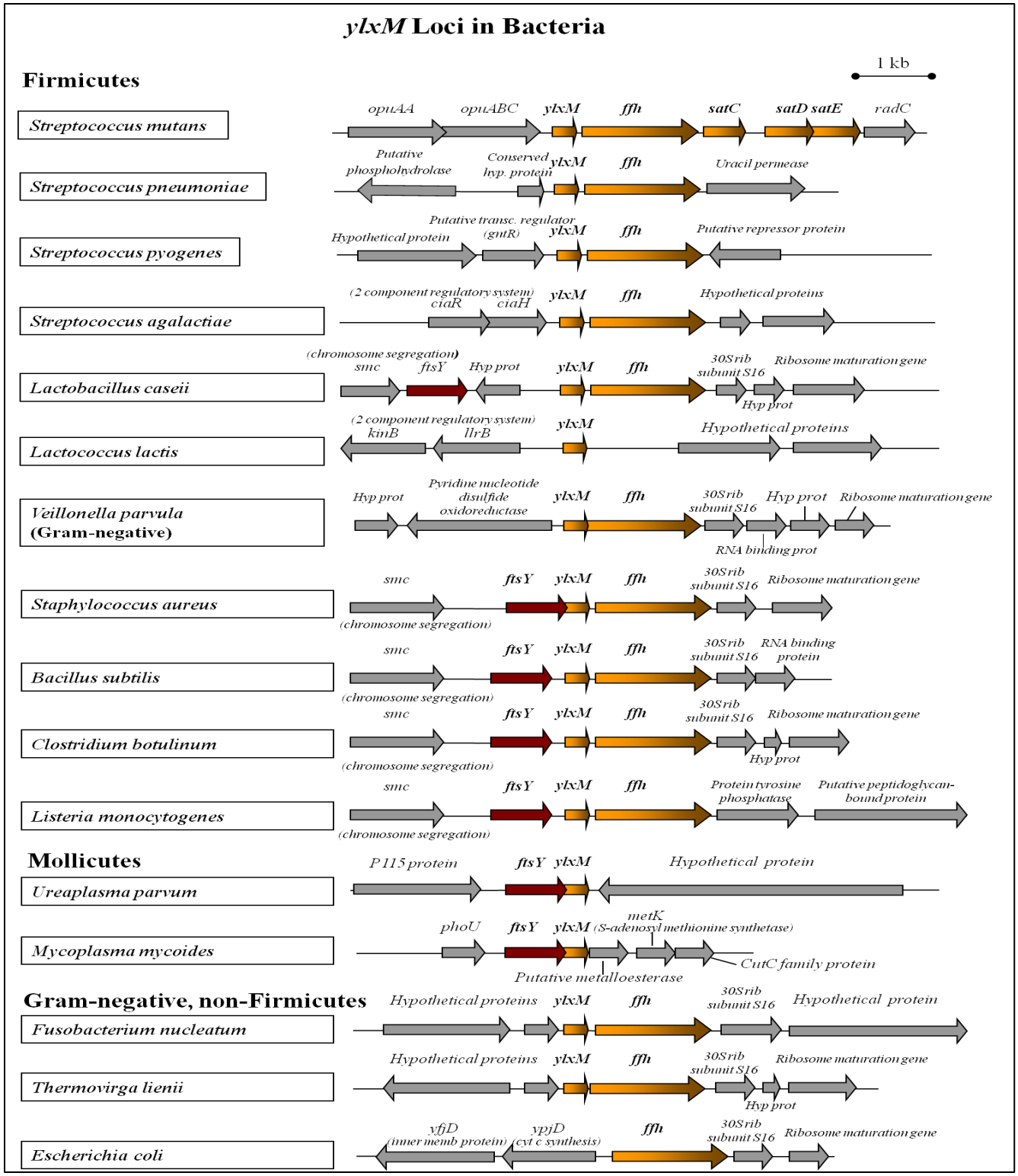

Fig. S2. Genetic context of *ylxM* among representative species. Illustration of representative genetic loci containing *ylxM*. The model Gram-negative organism *Escherichia coli*, which lacks *ylxM*, is included for comparison.

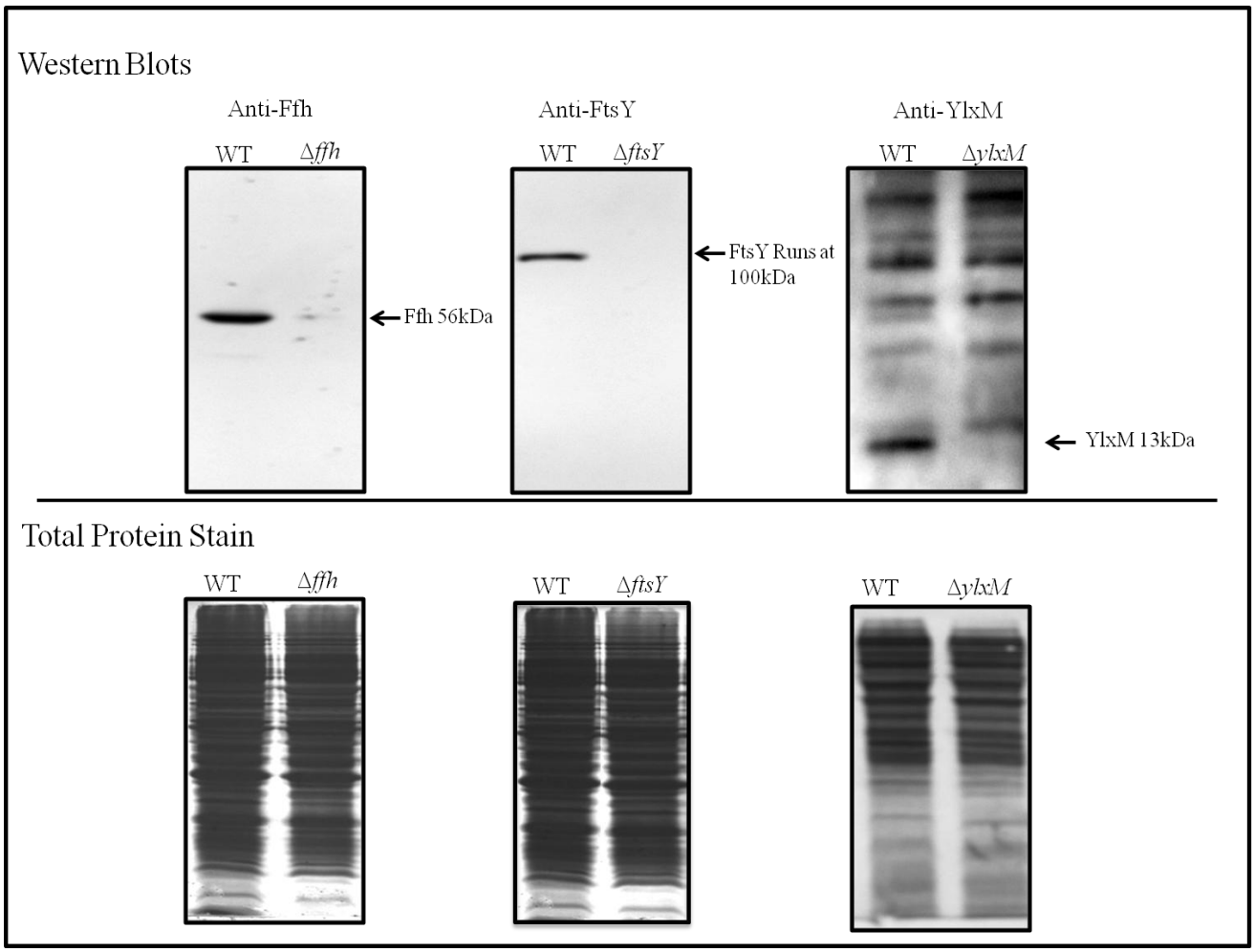

Fig. S3. Specificity of antisera against SRP pathway components. Western blot analysis of wild-type *S. mutans* and each respective mutant, reacted with anti- Ffh, FtsY, or YlxM. Dilutions were 1:5000, 1:2000, and 1:250, respectively. Colloidal Gold total protein stains are show as a loading control.

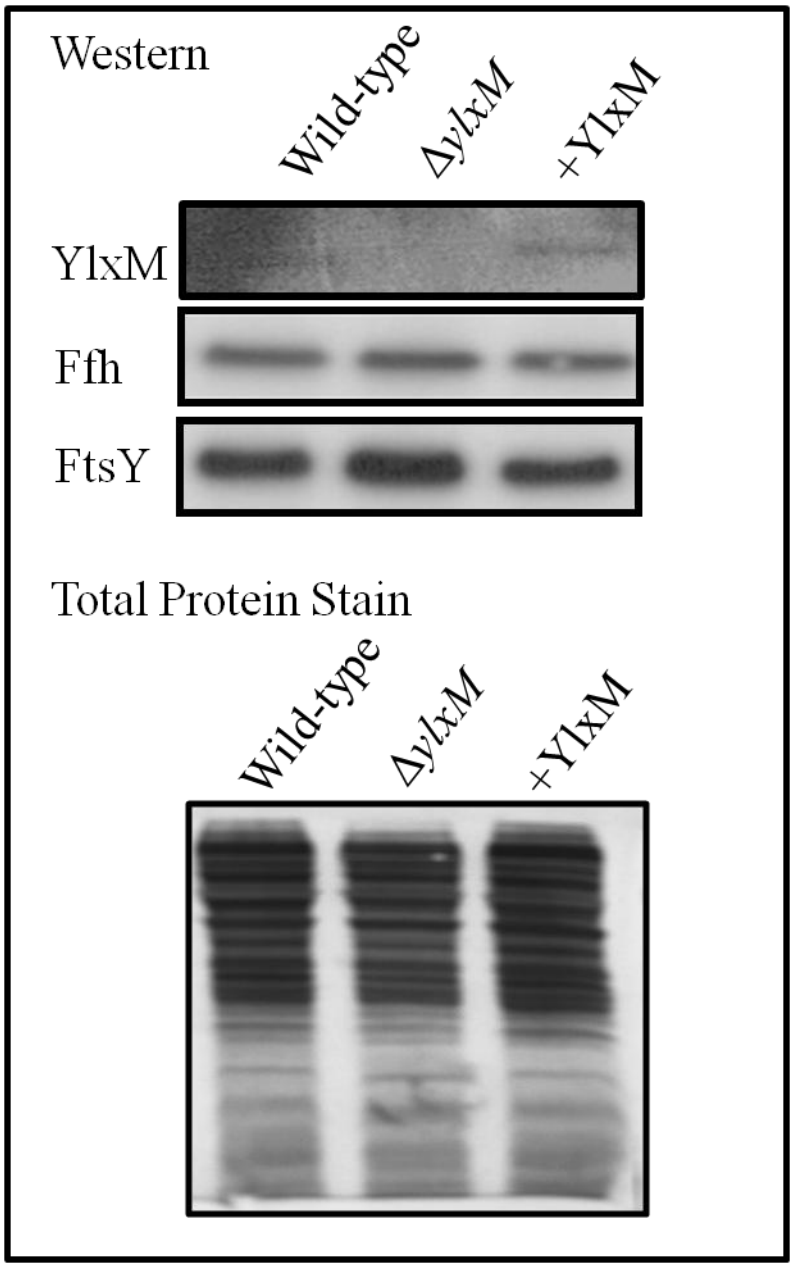

Fig. S4. Deletion of *ylxM* does not alter Ffh or FtsY production in *S. mutans.* Western blot analysis of YlxM, Ffh, and FtsY in whole cell lysates of *S. mutans* wild-type, the Δ*ylxM* mutant, and the complemented +YlxM strain.

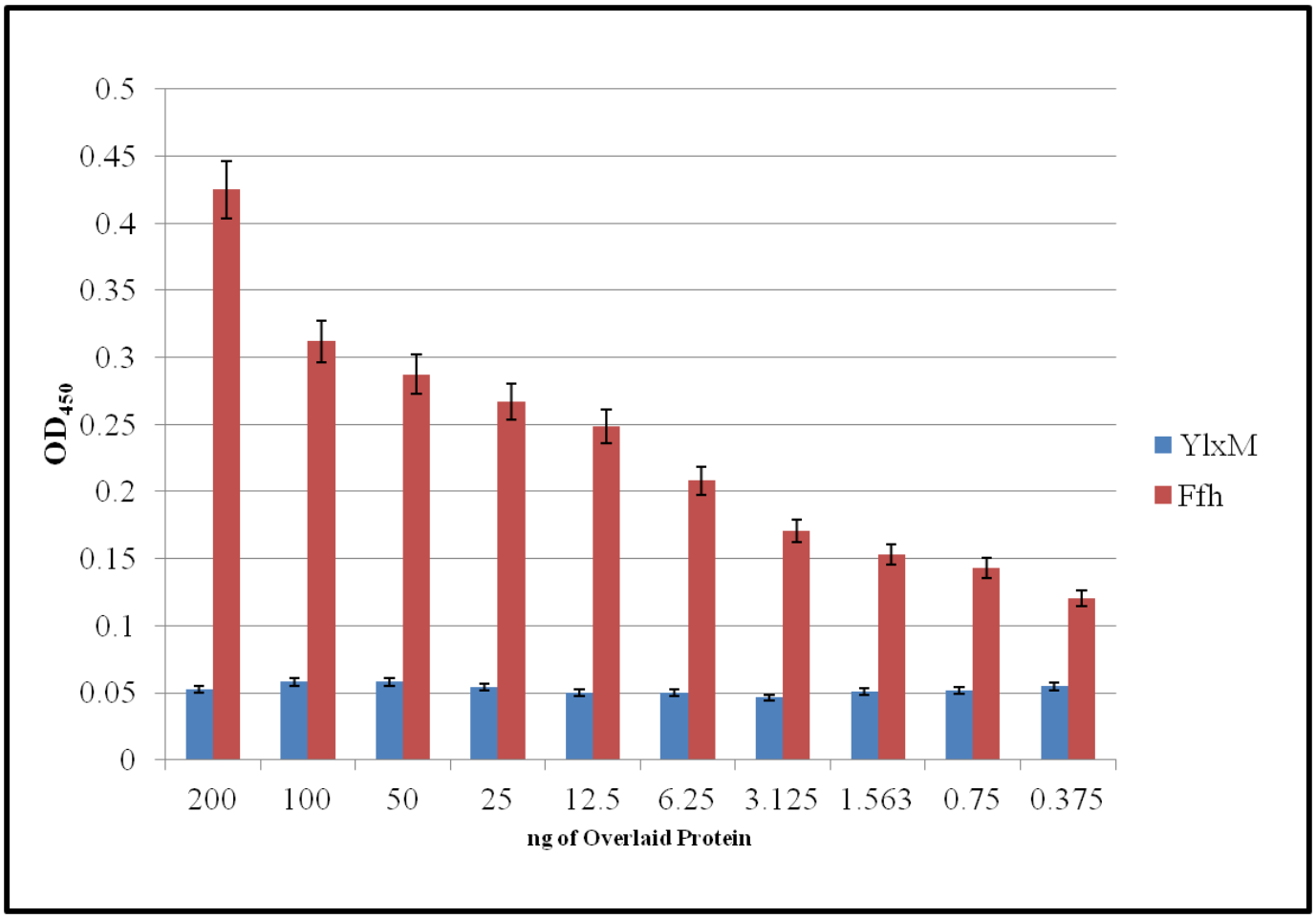

Fig. S5. Ffh, but not YlxM, interacts with FtsY. A capture ELISA was utilized to investigate interactions between purified recombinant FtsY and Ffh or YlxM. Antisera specific to each overlaid protein was used to detect protein bound to 200ng FtsY used to coat the plate.

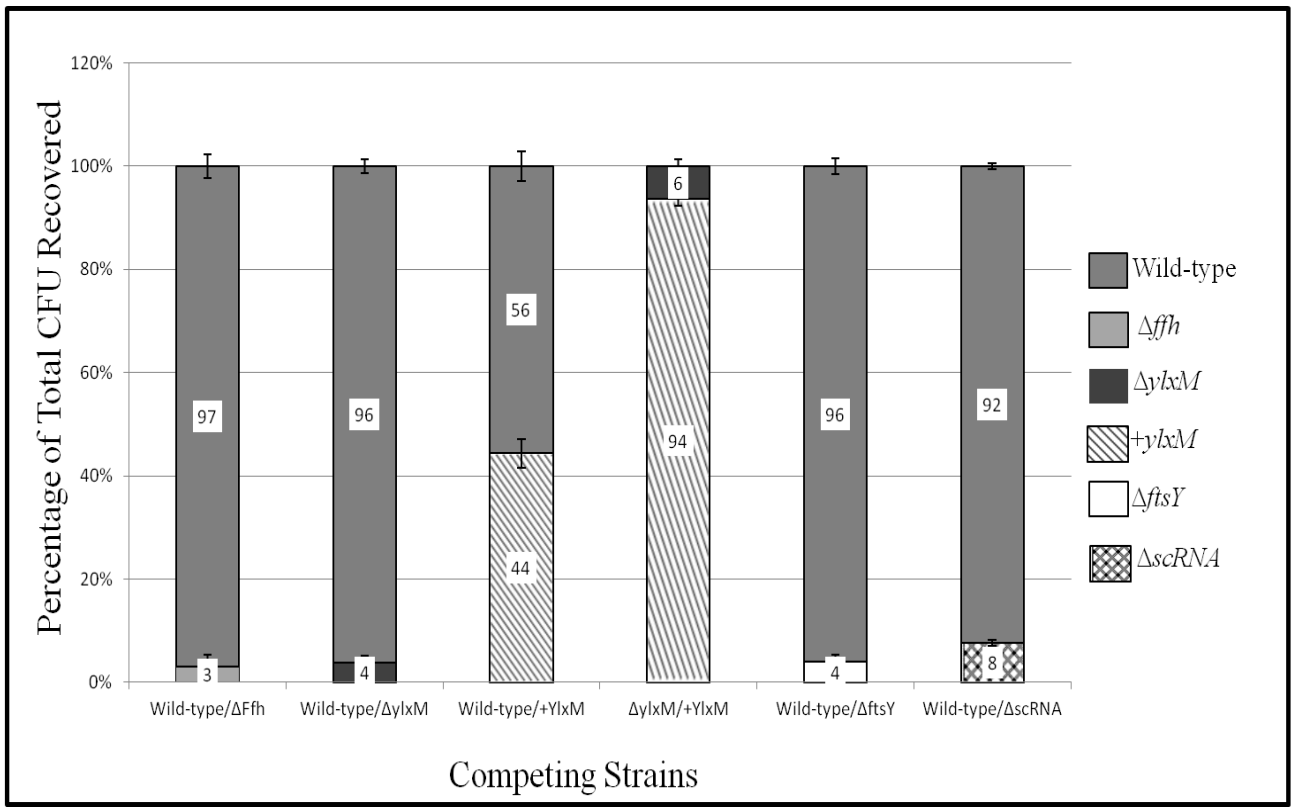

Fig. S6. YlxM confers a competitive advantage to *S. mutans* under acid-stress conditions. Wild-type and SRP mutant strains were mixed at a 1:1 ratio and grown to stationary phase under acid-stress conditions (pH 5.0) before plating onto THYE agar (pH 5.0). The experiments were performed in triplicate and the data are reported as the percentage of total colonies of each strain recovered. Significant differences in the recovery of wild-type compared to the SRP mutant strains were detected as follows: Δ*ffh* $p<0.01$, Δ*ylxM* $p<0.01$, Δ*ftsY* $p<0.01$, Δ*scRNA* $p<0.01$. There was no significant difference between the wild-type compared to +YlxM ($p=0.09$).

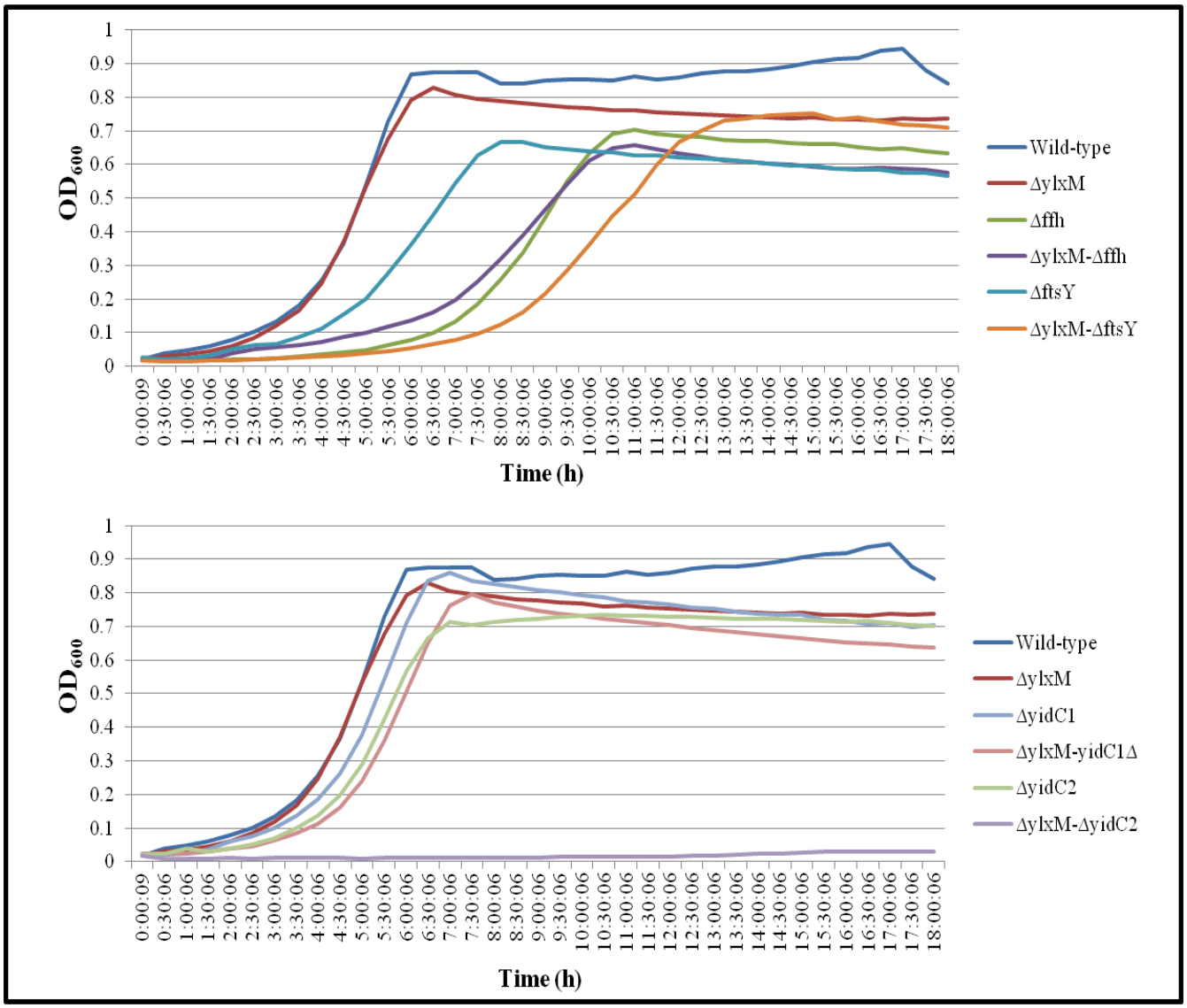

Fig. S7. Growth curves of protein translocation machinery mutants. Top panel shows growth of wild type compared to SRP protein single and double Δ*ylxM* mutants. Bottom panel shows growth of wild type compared to YidC1 and YidC2 single and double Δ*ylxM* mutants.

REFERENCES

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