

Figure S1. M protein is anchored simultaneously at the mother and daughter septa – analysis of additional populations. (A) Four overnight *S. pyogenes* D471 cultures were diluted 1:100 into TH+Y containing trypsin and pronase, harvested, and resuspended in TH+Y for 30 seconds at 37°C prior to fixation (each sample was processed separately). The fixed cells from the different cultures were mixed and processed together for microscopy. M protein was labeled using specific antibodies, and average intensity 3D-projections were made for numerous DeltaVision images, resulting in a population of 890 cells. The M protein distribution plots were analyzed using MATLAB to determine the presence of newly anchored M protein at the mother and daughter septa (see experimental procedures section). (B) The presence of newly anchored M protein at the mother and daughter septa, was analyzed in the cell population described in Figure 2 (following 2 minutes of protein regeneration), using MATLAB.

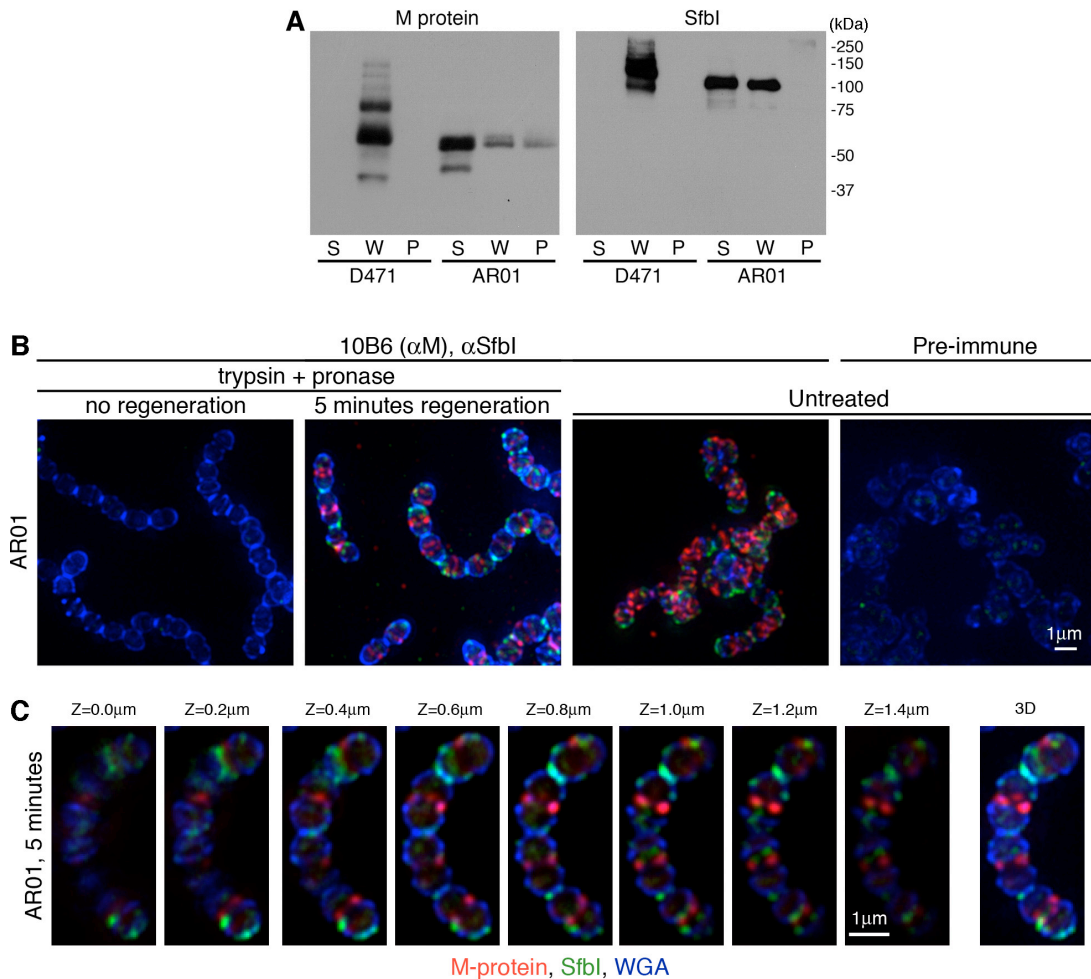


Figure S2. Distribution of M protein and SfbI in the sortase mutant, AR01. (A) Overnight cultures of the sortase deletion mutant AR01, and its parental strain D471, were diluted 1:100 into TH+Y, grown to OD_{600} 0.5, and fractionated into supernatant (S), wall (W), and spheroplast pellet (P). Samples were separated by 10% SDS-PAGE, and examined by Western blot using antibodies specific for M protein or SfbI. (B) Overnight cultures of AR01 were diluted 1:100 into TH+Y or TH+Y containing trypsin and pronase, and grown to OD_{600} 0.5. Protease treated cells were either fixed immediately upon reaching OD_{600} 0.5 (no regeneration), or washed and resuspended in TH+Y without proteases for 5 minutes at 37°C prior to fixation (5 minutes regeneration). Specific antibodies were used to label M protein (red) and SfbI (green), and the cell wall was stained with WGA marina blue (blue). Images are presented as maximum intensity projections. (C) Sequential Z-sections of a representative protease-treated chain following 5 minutes regeneration.