Supporting Information

The bacterial SRP receptor, FtsY, is activated on binding to the translocon

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**Fig. S1.** Protection against proteinase K digestion of the N-terminal SAS of Lep75-RNC by SecYEG. Lep75-RNC carrying a Bpy label at the N-terminal methionine was digested with proteinase K in the absence or presence of SecYEG or SecYEG(MDCC111) in nanodiscs (Methods). Digestion products were analyzed on Tricine-SDS-PAGE. N-terminal peptides were visualized by the fluorescence of Bpy.
Fig. S2. Binding of FtsY-A207 to SecYEG in the presence of FtsY-NG. Titration of SecYEG(MDCC) with FtsY-A207(Bpy) in the absence or presence of FtsY-NG (2 µM), monitoring MDCC fluorescence.
Fig. S3. Two-dimensional NMR spectra of full-length FtsY and FtsY-A207. The $^{15}\text{N} \, ^1\text{H}$-TROSY-HSQC spectra of $^2\text{H}^{15}\text{N}^{13}\text{C}$-labeled full-length FtsY (A) or FtsY-A207 (B) were measured at 700 MHz (Methods).
**Fig. S4.** Binding of 70S ribosomes to SecYEG in the presence of FtsY or FtsY domains. MDCC-labeled 70S ribosomes were titrated with SecYEG(Alx488) in the presence of increasing concentrations of FtsY (A) or the FtsY domain constructs FtsY-A196 (B), FtsY-A207 (C), FtsY-NG+1 (D) and FtsY-NG (E). Final values of relative donor (MDCC) fluorescence at saturation are plotted in Fig. 6. $K_d$ values were calculated by nonlinear fitting (Methods).