

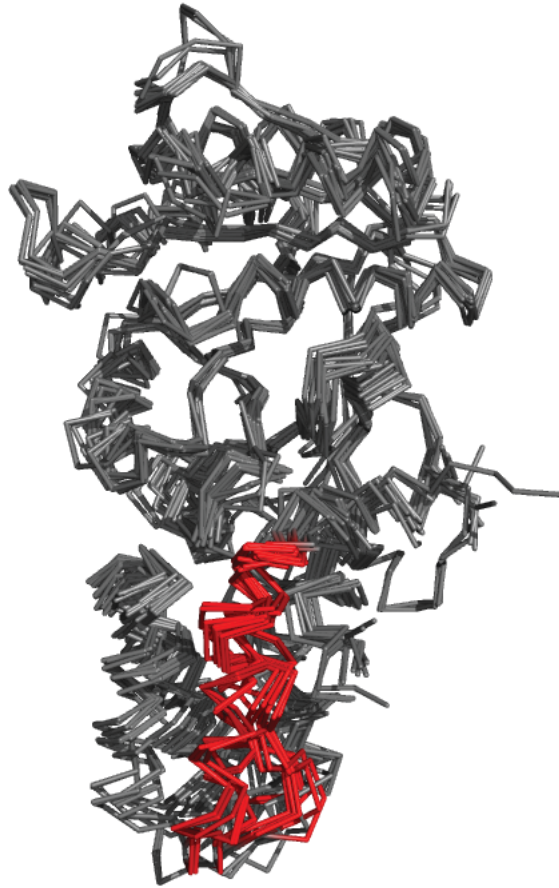
**Supplementary figures for:**

**SRP RNA controls a conformational switch regulating the SRP-SRP  
receptor interaction**

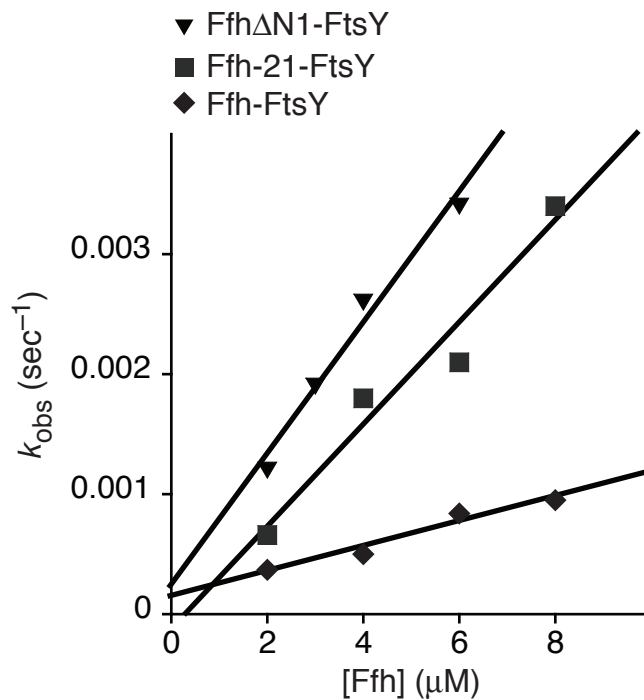
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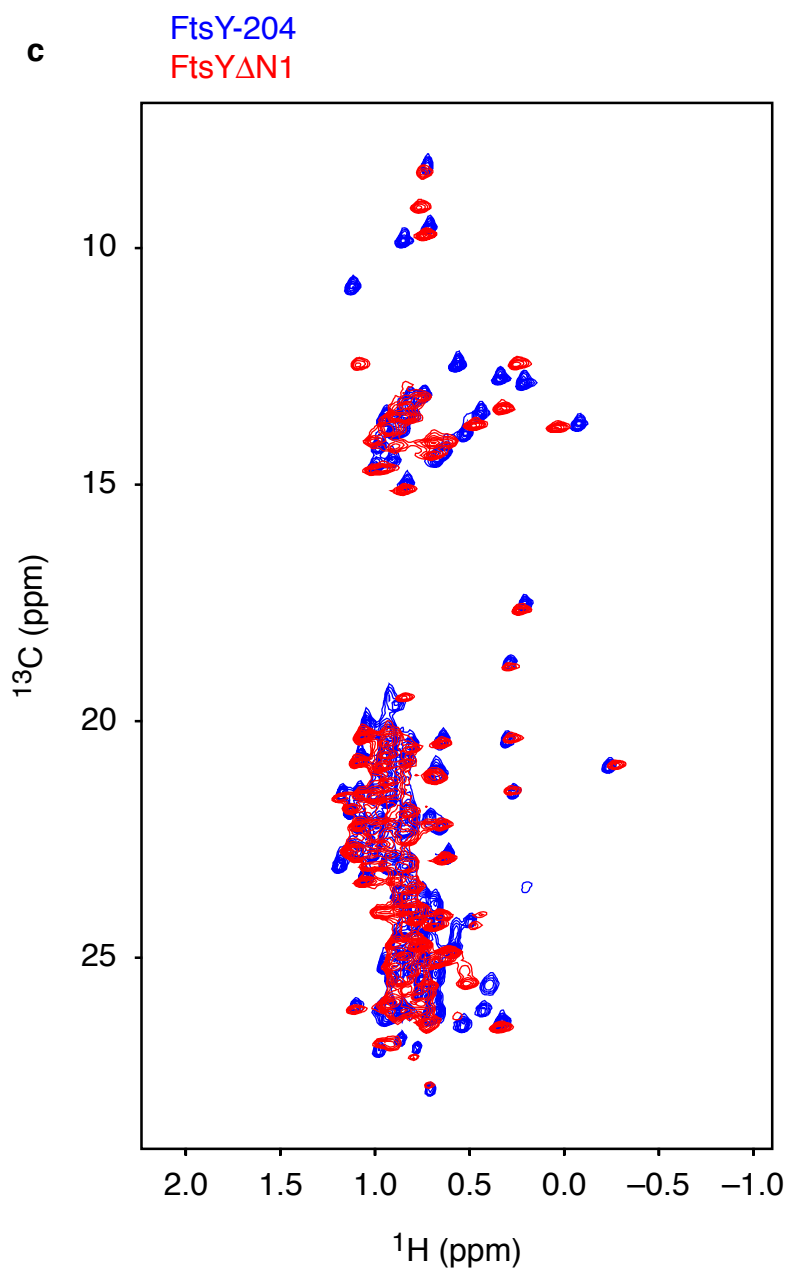
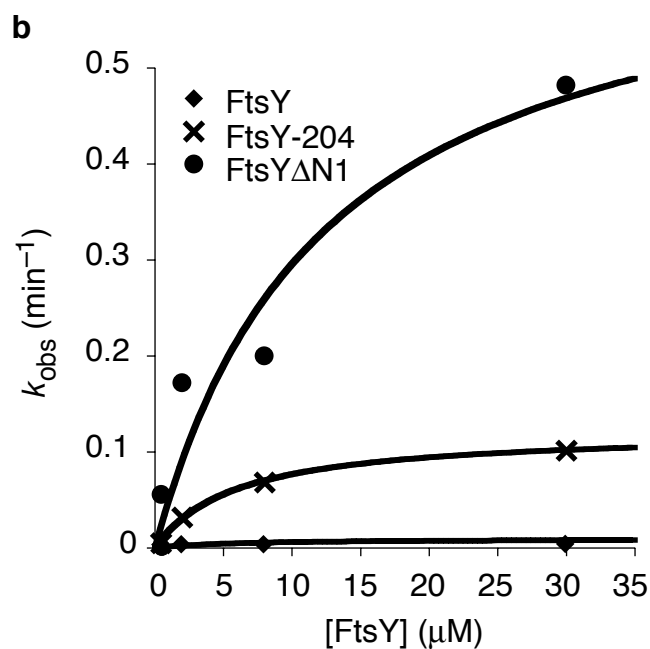
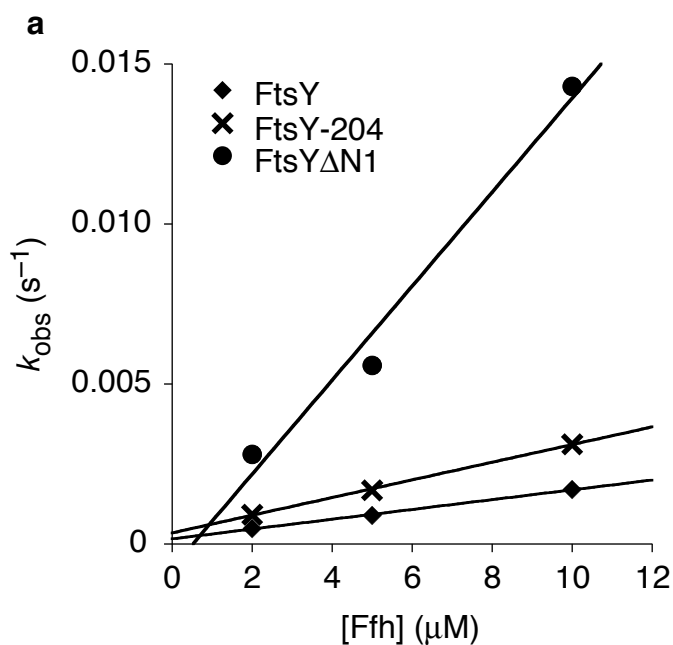
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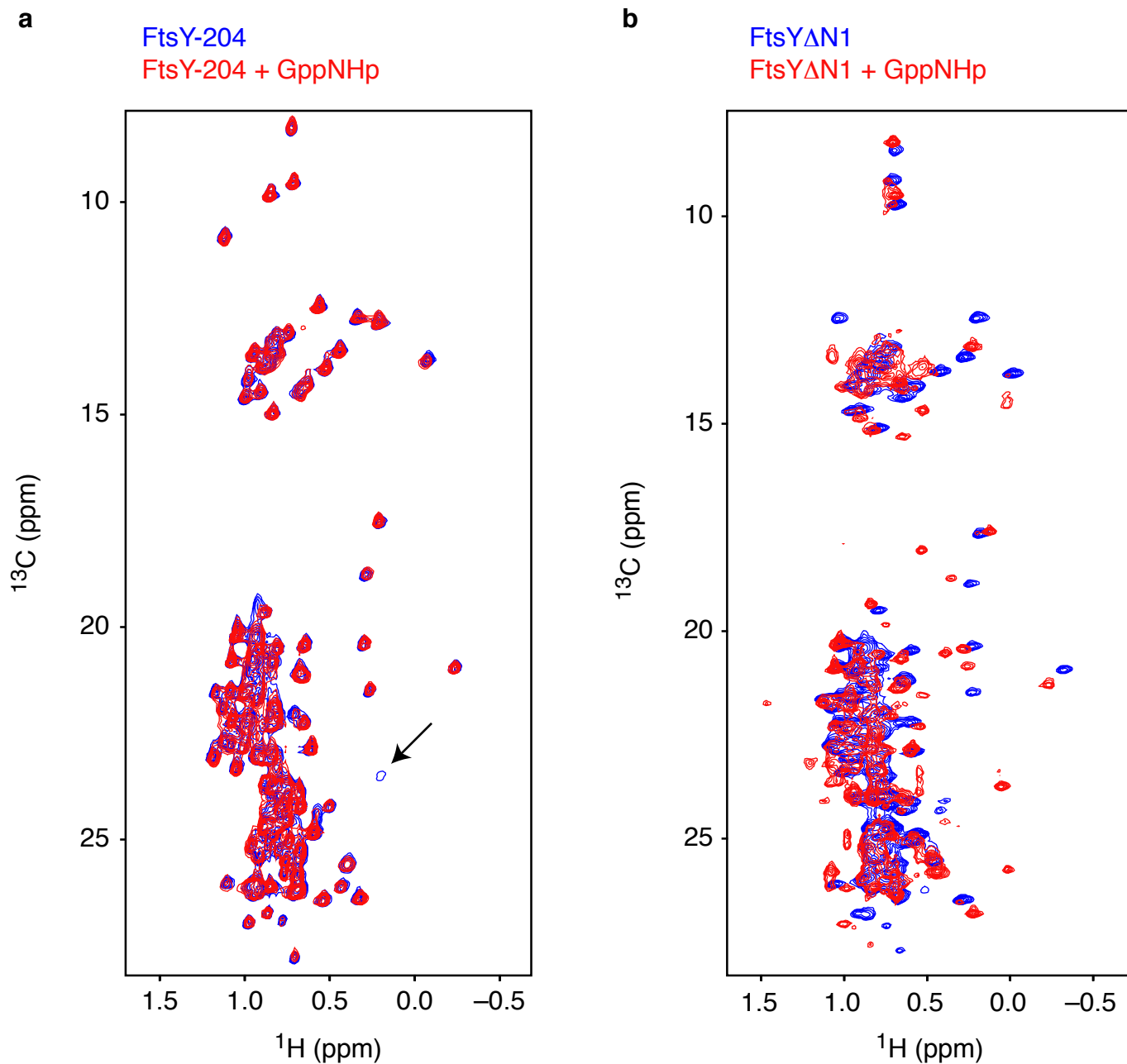
**Supplementary Figure 1:** Helix N1 is present in structures of uncomplexed FtsY. Alignment of FtsY structures from PDB files 1FTS, 2QY9, 1ZU4, 1ZU5, 2Q9C, 2Q9B, 2Q9A, 1VMA, 3B9Q, and 2OG2. Residues homologous to *E. coli* residues 204-221 of helix N1 are shown in red.



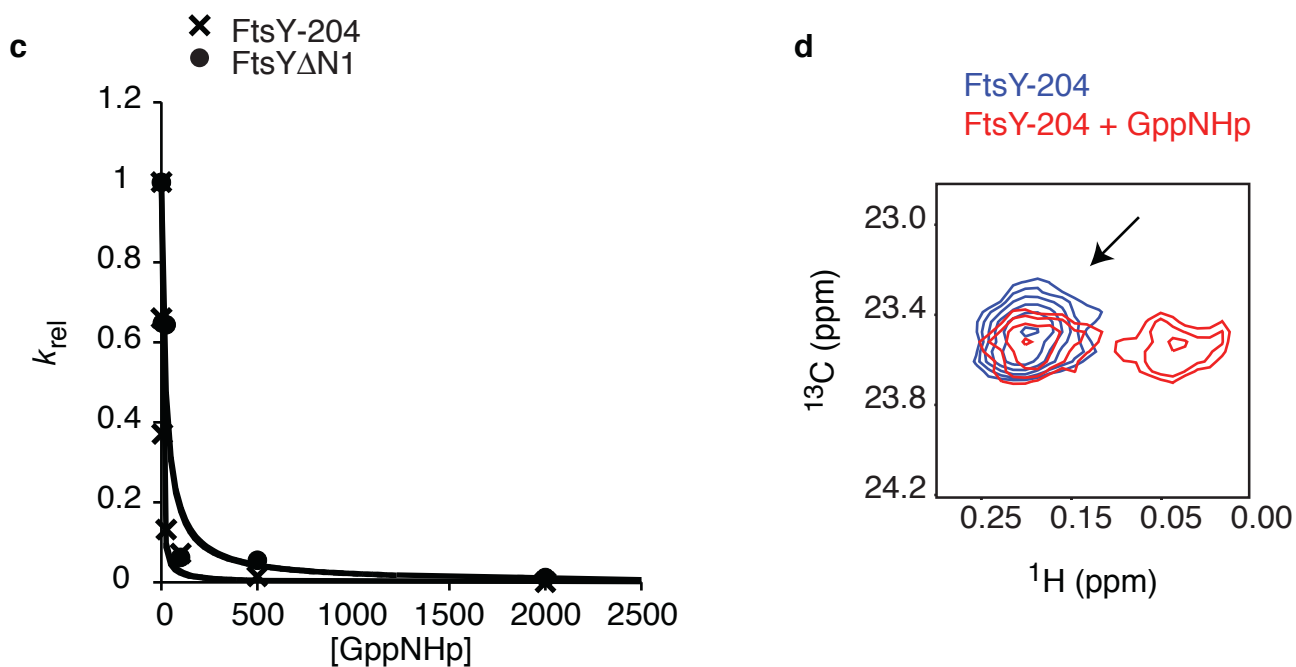
**Supplementary Figure 2:** Effect of length of Ffh N-terminal truncation. Truncation of the entire Ffh helix N1 (amino acids 1-20, called Ffh-21 here) is functionally equivalent to truncation of the first 8 amino acids (FfhΔN1). Observed binding rates are plotted as a function of Ffh concentration for FfhΔN1-FtsY –RNA (▼), Ffh-21-FtsY –RNA (■), and Ffh-FtsY –RNA (◆). Lines are fits to the equation  $k_{\text{obs}} = k_{\text{on}}[\text{Ffh}] + k_{\text{off}}$ .



**Supplementary Figure 3:** Comparison of FtsY-204 and FtsY $\Delta$ N1. a. Observed binding rates are plotted as a function of Ffh concentration for Ffh-FtsY –RNA (◆), Ffh-FtsY-204 –RNA (✕), and Ffh-FtsY $\Delta$ N1 –RNA (●). Lines are fits to the equation  $k_{\text{obs}}=k_{\text{on}}[\text{Ffh}]+k_{\text{off}}$ . b. Plot of observed rates from single turnover GTPase assays measuring GTP hydrolysis rate as a function of FtsY $\Delta$ N1 (●), FtsY-204 (✕), or FtsY (◆) concentration. Lines are fits to the equation  $k_{\text{obs}}=k_{\text{cat}}[\text{FtsY}]/(K_{\text{M}}+[\text{FtsY}])$ . c. 2D CHSQC NMR spectrum for FtsY $\Delta$ N1 (red) is overlaid on the spectrum of FtsY-204 (blue).



**Supplementary Figure 4:** FtsY $\Delta$ N1 but not FtsY-204 undergoes a GppNHp dependent conformational change. a. 2D CHSQC spectrum for FtsY-204+GppNHp (red) is overlaid on the spectrum of FtsY-204 (blue). A peak that broadens in the FtsY-204 spectrum +GppNHp is marked with an arrow. b. 2D CHSQC spectrum for FtsY $\Delta$ N1+GppNHp (red) is overlaid on the spectrum of FtsY $\Delta$ N1 (blue).



**Supplementary Figure 4.** FtsY $\Delta$ N1 but not FtsY-204 undergoes a GppNHp dependent conformational change. c. The affinity of GppNHp for FtsY $\Delta$ N1 and FtsY-204 was measured by GTPase inhibition assays. Relative rates of GTP hydrolysis are plotted as a function of concentration of GppNHp. Lines are fits to the equation  $k_{rel} = K_I / (K_I + [\text{GppNHp}])$  d. A region of the 2D CHSQC spectrum containing the peak marked in a is magnified with decreased contour cutoff.