Supporting Information

The FtsK-like motor TraB is a DNA-dependent ATPase that forms higher-order assemblies

Eric Amado[1], **Gunther Muth**[2], **Ignacio Arechaga**[1,*] **and Elena Cabezón** [1,*]

This document includes 3 figures (Figure 1S, Figure 2S and Figure 3S) with their respective legends

Figure 1S

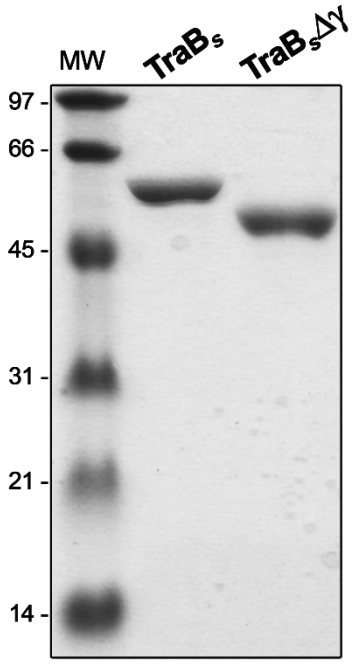

FIGURE 1S. SDS-PAGE of $TraB_s$ and $TraB_s\Delta\gamma$ proteins. Both proteins were purified to homogeneity following the protocol described in Experimental procedures, consisting of a HisTrap-HP column followed by a HiTraP Q-Sepharose column. (MW) Molecular weight markers (SDS-PAGE Standards, Low Range, BioRad).

Figure 2S

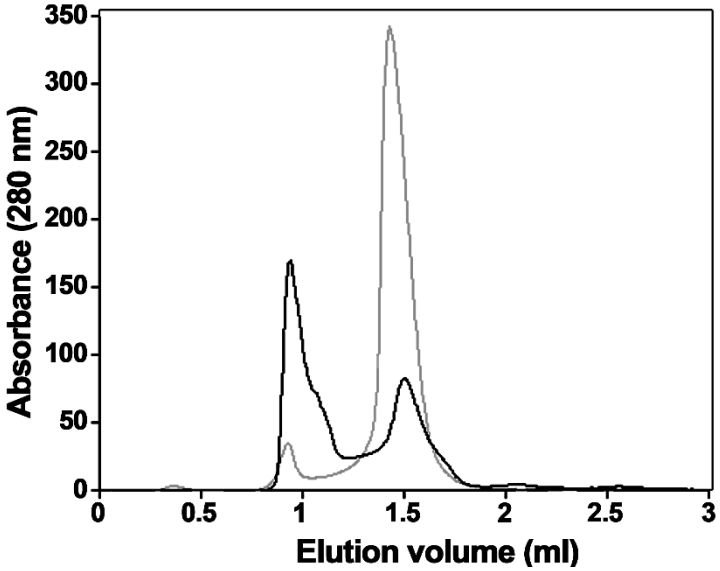

FIGURE 2S. Double-peak elution pattern of $TraB_s$ obtained by size-exclusion chromatography. $TraB_s$ elutes from a Hi-Load Superdex-200 3.2/300 column in two peaks of estimated MWs of > 800 KDa (peak 1) and ~70 KDa (peak 2), respectively (Figure 5). Both peaks contain pure $TraB_s$ protein. Differences in buffer composition associated to the pH value (pH 7.5 and pH 6.2 for black and grey traces, respectively) or glycerol content (5 versus 10 %) induce changes in mobility profiles. Thus, at pH 6.2 most of the protein elutes at a MW compatible with the size of monomeric $TraB_s$, whereas at pH 7.5 an increase in higher MW species can be observed.

Figure 3S

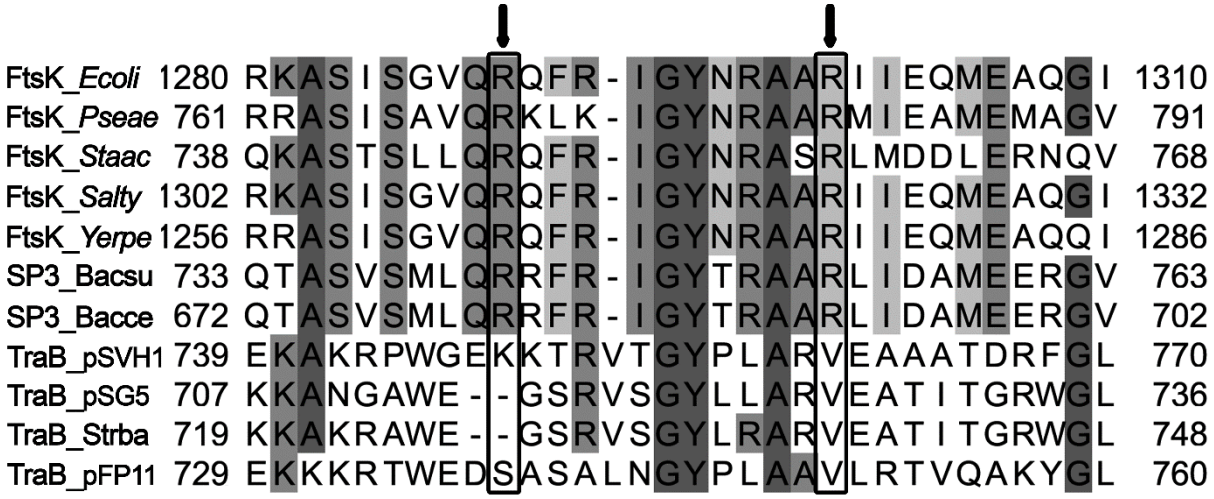

FIGURE S3. **Sequence alignment of FtsK family of proteins**. Members of the FtsK family of proteins include FtsK, SpoIIIE and TraB proteins. There is a high degree of conservation between these proteins. However, some differences are observed at their C-termini. In particular, there are two arginine residues (i.e. R1289 and R1300 in *E. coli* FtsK and R770 and R781 in *P. aeruginosa* FtsK, or R742 and R753 in *B. subtilis* SpoIIIE; boxed and pointed with arrows) that have been shown to play an essential role in DNA dependent ATPase stimulation (Besprozyannaya et al., 2013) which are absent in TraB. The sequences shown in the figure correspond to (Uniprot code): FtsK_*Escherichia coli* (sp_P46889), FtsK_*Pseudomonas aeruginosa* (sp_Q9I0M3), FtsK_*Staphylococcus aureus* (sp_Q5HGF5), FtsK_ *Salmonella typhimurium* (sp_Q8ZQD5), FtsK_*Yersinia pestis* (sp_Q8ZGC7), SpoIIIE_*Bacillus subtilis* (sp_P21458), SpoIIIE_*Bacillus cereus* (tr_A0A0K6K636), TraB_*Streptomyces venezuelae pSVH1* (tr_Q3LAJ2), TraB_*Streptomyces viridosporus* (tr_O70031), TraB_*Streptomyces badius* (tr_A0A328JNL9), TraB_*Streptomyces sp. pFP11* (tr_Q58IQ9).