

Supplementary Figure S1. Localization of FlhF-GFP in cells. (A) Cells of strain LPN1 ($\Delta flhF$ mutant) harboring a plasmid—pAK325 (WT) or the mutant derivative (Q419ochre, or Q391amber)—induced with 0.02% arabinose were examined under a fluorescence microscope. The rate of polar dot formation was determined by calculating the ratio of the number of cells that have a fluorescent dot at the cell pole to the total number of cells seen in the image. (B) Immunoblotting was performed using the anti-FlhF antibody to detect the truncated mutant proteins and the GFP fused proteins.



Supplementary Figure S2. Fractionation of the FlhF mutant proteins. Cells of strain LPN1 ($\Delta flhF$ mutant) harboring plasmid "WT" (pAK322) or the mutant plasmid (K311A, G445W, D470A) induced by means of 0.02% arabinose were separated into a membrane fraction (ppt) and soluble fraction (sup) by ultracentrifugation. before UC is the sample before ultracentrifugation. To detect the interaction of FlhF and the membrane, the membrane fraction was solubilized with Triton X-100 and then fractionated into the supernatant and the pellet by ultracentrifugation.