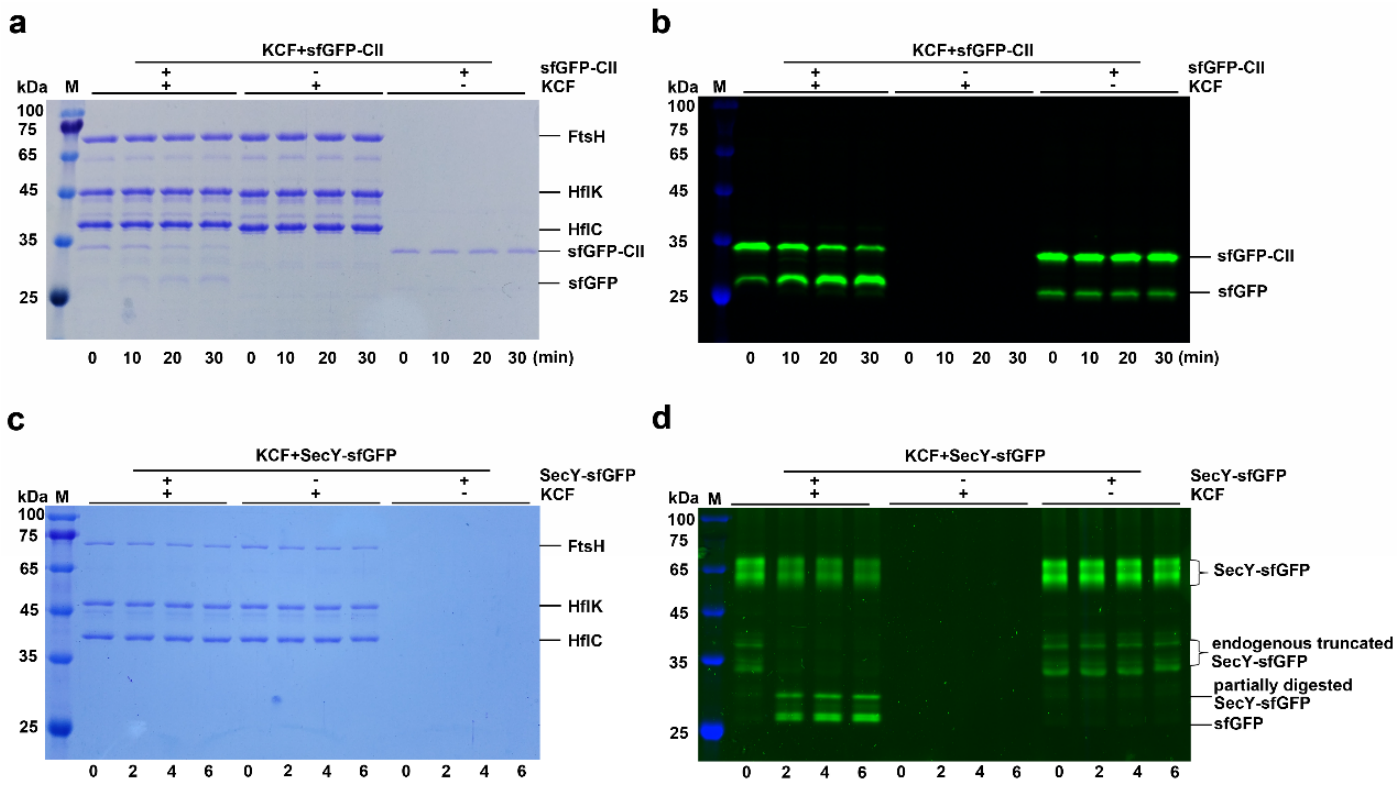

Figure S2. Purified KCF complexes are active in degrading sfGFP-CII and SecY-sfGFP proteins.

a-b Protease activity assay of the KCF complex against sfGFP-CII detected with Coomassie brilliant blue staining (**a**) or GFP imaging (**b**). The sfGFP-CII protein was incubated with the KCF complex in a reaction buffer and sampled every 10 mins (see methods for details). The super-fold GFP (sfGFP) is resistant to the AAA+ unfolding module of FtsH hexamer and the end product of the proteolytic reactions would be sfGFP. **c-d** Protease activity assay of the KCF complex against SecY-sfGFP detected with Coomassie brilliant blue staining (**c**) or imaged for GFP signals (**d**). The SecY-sfGFP protein was incubated with the KCF complex and sampled every 2 h. Note that SecY proteins are resistant to Coomassie brilliant blue staining and thus only faintly appeared in panel **c**. SecY is highly unstable when not in complex with its partners SecE and SecG. Therefore, with detergent solubilization, we obtained a mixture of SecY-sfGFP variants during substrate purification, including full-length fusion proteins and several forms of *in vivo* truncations (Panel **d**, right lanes). These inhomogeneous substrates were subjected to the proteolytic assay, and a complete conversion of endogenous, truncated forms to fast migrating species (indicated by a band of sfGFP and another band of partially digested SecY-sfGFP) was observed after 2-hour incubation. The reactions were kept going on for 6 hours to illustrate the slow degradation of full-length (or near full-length) SecY-sfGFP proteins.