Supplementary File 1. List of protein expression constructs used in this study.

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| --- | --- | --- | --- | --- |
| # | Protein | Vector | Figure | Expression |
| pLM B042 | His6-TEV-2xStrepII-TEV-2xStrepII-TEV-CstF641-198 | 438-E | Fig. 4C,  Fig. 3-Supplement 3A | *Sf9* |
| pLM B043 | His6-TEV-CstF771-717 | 438-B | Fig. 4C,  Fig. 3-Supplement 3A | *Sf9* |
| His6-TEV-CsF501-431 | 438-B |
| pLM B092 | His6-MBP-TEV-CstF7721-549 | 1M | Fig. 3A,  Fig. 3E, Fig. 4A,  Fig. 3-Supplement 2A,  Fig. 3-Supplement 3A | *E. coli* |
| pLM B123 | His6-TEV-CstF7721-559 | 16-B | Fig. 4B,  Fig. 4-Supplement 1A,  Fig. 4-Supplement 1B | *E. coli* |
| pLM B142 | His6-GST-TEV-MmPAP1-504 | 2GT | Fig. 2D | *E. coli* |
| pLM B156 | His6-MBP-TEV-HsPAP1-504 | 1M | Fig. 4B,  Fig. 4C,  Fig. 4-Supplement 1A,  Fig. 4-Supplement 1B | *E. coli* |
| pLM B157 | His6-GFP-TEV-HsPAP1-504 | 2GFP-T | Fig. 1G,  Fig. 2B,  Fig. 4A,  Fig. 2-Supplement 1B | *E. coli* |
| pLM B164 | His6-MBP-TEV-CstF77241-549 | 1M | Fig. 3B | *E. coli* |
| pLM B167 | His6-MBP-TEV-CstF7721-549  R395A/R402A/K431A | 1M | Fig. 3E | *E. coli* |
| pLM B168 | His6-MBP-TEV-CstF7721-549  R395A/R402A/K431A | 1M | Fig. 3E | *E. coli* |
| pLM B170 | His6-TEV-CstF7721-549  R395A/R402A/K431A | 16B | Fig. 4-Supplement 1A | *E. coli* |
| pMC B051 | His6-MBP-TEV-CPSF30118-178 | 1B | Fig. 1B-E | *E. coli* |
| pMC B054 | His6-MBP-TEV-CPSF30118-178 | 16-M | Fig. 1F,  Fig. 1-Supplement 2A,  Fig. 1-Supplement 2B | *E. coli* |
| pMC B055 | His6-MBP-TEV-CPSF30118-178 F131E | 16-M | Fig. 1-Supplement 2A | *E. coli* |
| pMC B056 | His6-MBP-TEV-CPSF30118-178 F155E | 16-M | Fig. 1F,  Fig. 1-Supplement 2A | *E. coli* |
| pMC B057 | His6-MBP-TEV-CPSF30118-178 F131E/F155E | 16-M | Fig. 1-Supplement 2A | *E. coli* |
| pMC B058 | His6-MBP-TEV-CPSF30118-178 Y127E | 16-M | Fig. 1F,  Fig. 1-Supplement 2A | *E. coli* |
| pMC B059 | His6-MBP-TEV-CPSF30118-178 Y127E/F131E | 16-M | Fig. 1-Supplement 2A | *E. coli* |
| pMC B060 | His6-MBP-TEV-CPSF30118-178 Y151E | 16-M | Fig. 1-Supplement 2A | *E. coli* |
| pMC B061 | His6-MBP-TEV-CPSF30118-178 Y151E/F155E | 16-M | Fig. 1-Supplement 2A | *E. coli* |
| pMC B062 | His6-MBP-TEV-CPSF30118-178 Y127E/Y151E | 16-M | Fig. 1-Supplement 2A | *E. coli* |
| pMC B063 | His6-MBP-TEV-CPSF30118-178 Y127E/F155E | 16-M | Fig. 1F,  Fig. 1-Supplement 2A | *E. coli* |
| pMC C011 | His6-GST-TEV-hFip1130-195 | 13S-A | Fig. 1B-E | *E. coli* |
| pMC C015 | His6-GST-TEV-hFip180-195 | 2GT | Fig. 2-Supplement 1B | *E. coli* |
| pMC C030 | His6-TEV-hFip1130-195 | 16-B |  | *E. coli* |
| pMC C049 | His6-TEV-GFP- hFip1130-195 | 16-B | Fig. 1F,  Fig. 1-Supplement 2A,  Fig. 1-Supplement 2B | *E. coli* |
| pMC C050 | His6-GST-TEV-hFip136-80 | 2GT | Fig. 2-Supplement 1B | *E. coli* |
| pMC C059 | His6-GST-TEV-hFip11-35 | 2GT | Fig. 3A,  Fig. 3B,  Fig. 3E,  Fig. 3-Supplement 3A | *E. coli* |
| pMC C060 | His6-GST-TEV-hFip11-195 | 2GT | Fig. 2A,  Fig. 3A | *E. coli* |
| pMC C066 | His6-TEV-GFP-TEV-hFip11-195 W150E | 16-B‡ | Fig. 1-Supplement 2B | *E. coli* |
| pMC C067 | His6-TEV-GFP-TEV-hFip11-195 F161E | 16-B‡ | Fig. 1-Supplement 2B | *E. coli* |
| pMC C068 | His6-TEV-GFP-TEV-hFip11-195 W170E | 16-B‡ | Fig. 1-Supplement 2B | *E. coli* |
| pMC C073 | His6-GST-TEV-hFip136-195 | 2GT | Fig. 3A | *E. coli* |
| pMC C093 | His6-GST-TEV-hFip11-35  E22A + E23A | 2GT | Fig. 3E | *E. coli* |
| pMC C094 | His6-GST-TEV-hFip11-35  W25A + L26A + Y27A | 2GT | Fig. 3E | *E. coli* |
| pMC C096 | His6-GST-TEV-hFip11-35 W25A | 2GT | Fig. 3E | *E. coli* |
| pMC N015 | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2C | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-117 | 438-A |
| pMC N018 | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2C | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-178 | 438-A |
| StrepII-GFP-TEV-hFip1130-195 | 438-RGFP |
| pMC N018A | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2B,  Fig. 2C,  Fig. 2-Supplement 1A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 | 438-A |
| pMC N018C-2 | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2C,  Fig. 2-Supplement 1A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 | 438-A |
| StrepII-GFP-TEV-hFip11-378 | 438-RGFP |
| pMC N018G | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2A,  Fig. 2B,  Fig. 2C,  Fig. 2D,  Fig. 4B,  Fig. 4C,  Fig. 2-Supplement 1A,  Fig. 3-Supplement 2A,  Fig. 4-Supplement 1A,  Fig. 4-Supplement 1B | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018G-0 | His6-TEV-CPSF1601-1443 | 438-B |  | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 | 438-A |
| GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018G-8 | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 F155E | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018G-10 | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 Y127E | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018G-12 | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 Y151E | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018G-14 | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 Y127E/Y151E | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018G-15 | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 Y127E + F155E | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 Y127E/F155E | 438-A |
| GFP-TEV-hFip11-195 | DNA fragment\* |
| pMC N018G-21 | His6-TEV-FLAG-CPSF1601-1443 | 438-B\*\* | Fig. 1G | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018G-22 | His6-TEV-FLAG-CPSF1601-1443 | 438-B\*\* | Fig. 1G | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 Y127E | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018G-23 | His6-TEV-FLAG-CPSF1601-1443 | 438-B\*\* | Fig. 1G | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 Y151E | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018G-24 | His6-TEV-FLAG-CPSF1601-1443 | 438-B\*\* | Fig. 1G | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 Y127E/Y151E | 438-A |
| StrepII-GFP-TEV-hFip11-195 | 438-RGFP |
| pMC N018H | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2C,  Fig. 2-Supplement 1A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 | 438-A |
| StrepII-GFP-TEV-hFip1130-378 | 438-RGFP |
| pMC N018I | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2C,  Fig. 2-Supplement 1A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 | 438-A |
| StrepII-GFP-TEV-hFip1130-195 | 438-RGFP |
| pMC N018J | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2B,  Fig. 2C,  Fig. 4B,  Fig. 2-Supplement 1A,  Fig. 3-Supplement 2A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 | 438-A |
| StrepII-GFP-TEV-hFip136-195 | 438-RGFP |
| pMC N018K | His6-TEV-CPSF1601-1443 | 438-B | Fig. 2B,  Fig. 2C,  Fig. 2-Supplement 1A,  Fig. 3-Supplement 2A | *Sf9* |
| His6-TEV-2xStrepII-TEV-WDR331-410 | 438-D |
| CPSF301-243 | 438-A |
| StrepII-GFP-TEV-hFip180-195 | 438-RGFP |

‡GeneArt Strings: Insert with LIC overhangs ordered as DNA fragment that already contains GFP-hFip1

\*GeneArt Strings: PmeI-digested insert ordered as DNA fragment and directly subcloned

\*\*FLAG-tag inserted by FLAG-overhang in Oligo for PCR.