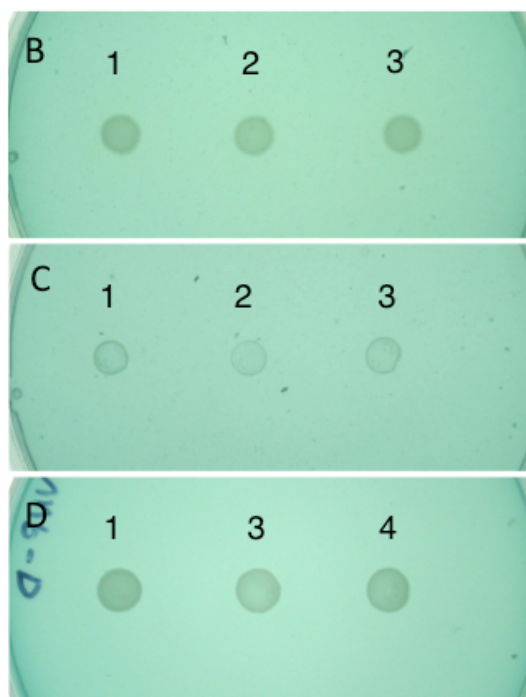
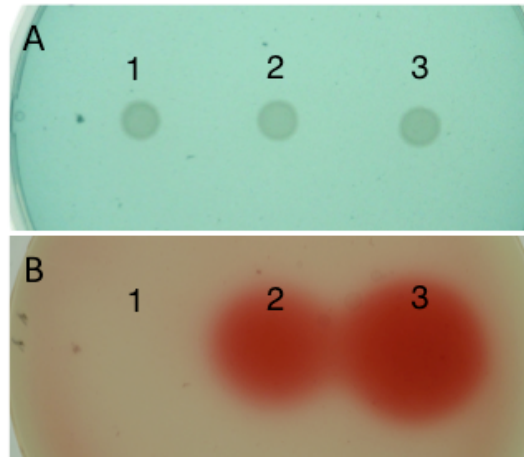


Figure S1



**Figure S1.** Bacterial colonies corresponding to Figure 2, prior to the protease inhibition assay, with **1)** pTet99a empty vector (pDB221), **2)** YebF (pDQ84), **3)** YebF-SFTI (pDQ221) and **4)** YebF<sup>-L<sup>K</sup></sup>SFTI (pDQ44), for panels B, C, and D.

Figure S2

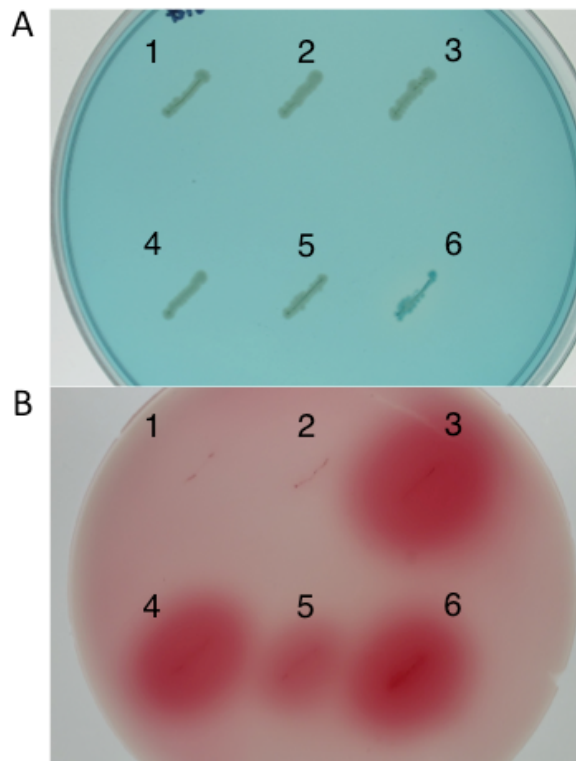


**Figure S2.** Comparison of YebF-SFTI fusions with and without the LK cleavage signal in *E. coli*. YebF-SFTI +/- LK sequence were expressed and compared for protease inhibition using the culture-based method.

**Top Panel:** The bacterial colonies prior to the protease

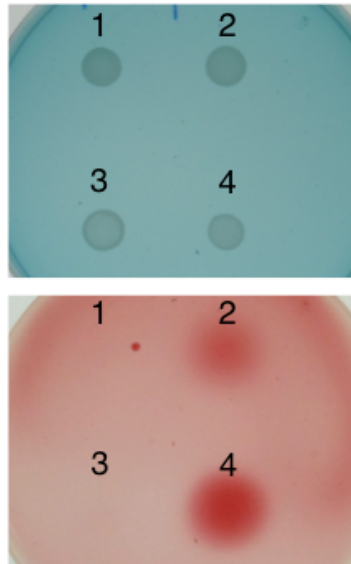
inhibition assay. **Bottom Panel:** The zones of protease inhibition. **1)** The empty vector; no insert cloned into the pTet99a expression site (pDB221). **2)** pTet-YebF-SFTI (pDQ221). **3)** pTet-YebF-LKSFTI (pDQ44)

Figure S3



**Figure S3.** Comparison of hexahistidine fusions with and without the LK cleavage signal in *E. coli*. YebF-SFTI with the hexahistidine C-terminal fusion +/- LK sequence were expressed and compared. **Top Panel:** The bacterial colonies prior to the protease inhibition assay. **Bottom Panel:** The zones of protease inhibition. **1)** The empty vector; no insert cloned into the pTet99a expression site (pDB221). **2)** pTet-YebF (pDQ84). **3)** pTet-YebF-LKSFTI (pDQ44). **4)** pTet-YebF-LKSFTI6H (pDQ92). **5)** pTet-YebF-SFTI6H lacking the LK amino acids (DQ93). **6)** pTet-YebF-LKSFTI-E3LYS (pDQ186).

Figure S4



**Figure S4.** Protease inhibitor production by the polycistronic OTG-PE38K RBS YebF-<sup>LK</sup>SFTI used to demonstrate protection of a therapeutic protein by VNP20009. **Top Panel:** The bacterial colonies prior to the protease inhibition assay. **Bottom Panel:** The zones of protease inhibition. **1)** YebF (pDQ195), **2)** YebF-<sup>LK</sup>SFTI (pDB573), **3)** the chimeric *Pseudomonas* ToxA OTG-PE38K, and **4)** the OTG-PE38K RBS YebF-<sup>LK</sup>SFTI co-expression construct (pDQ169).