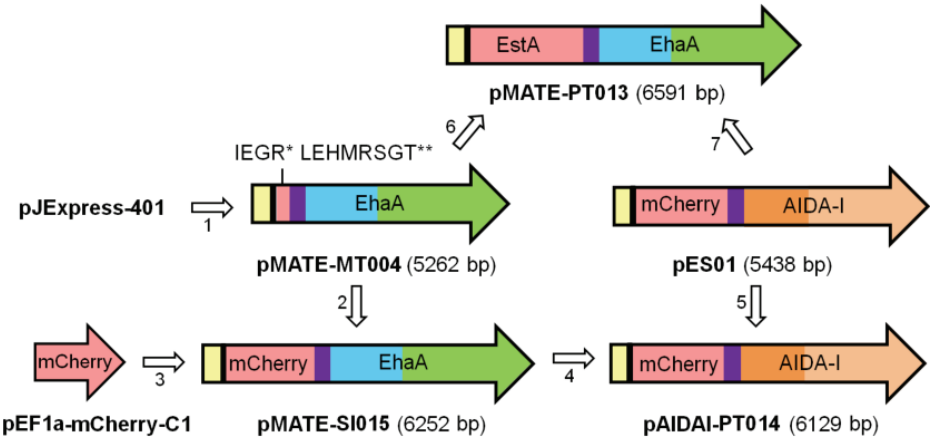
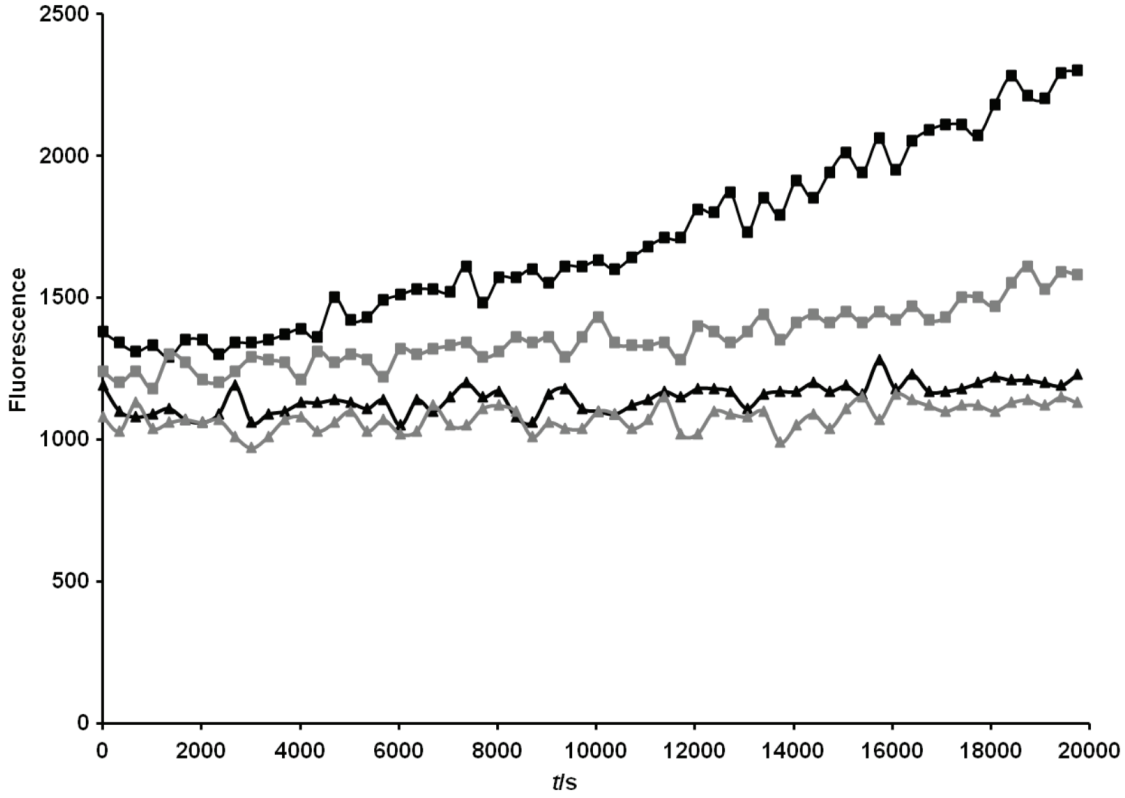
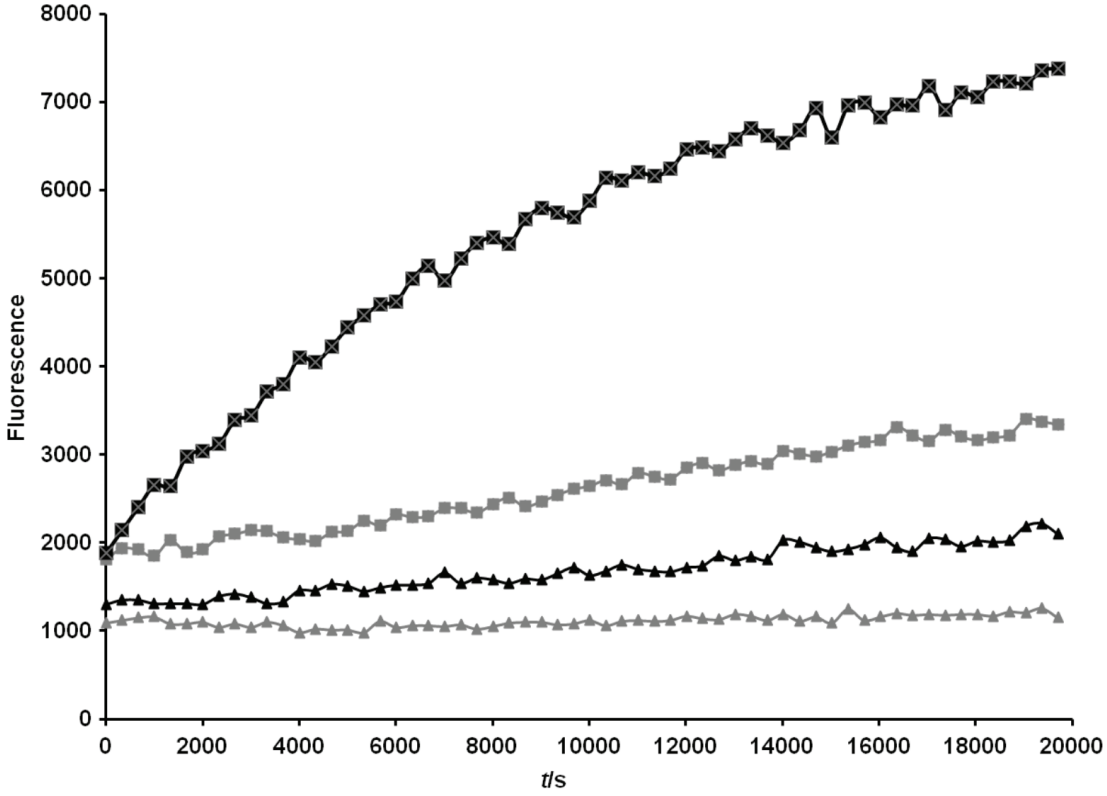


Supplementary Fig. 1. Structure of encoded fusion proteins and plasmid construction scheme used in this study. Plasmid sizes are depicted below the plasmid names. Structure of the encoded fusion proteins are shown schematically, with CtxB signal peptide in yellow, 6xHis epitope in black, passenger domains in light red, epitope and protease cleavage site in violet, EhaA autotransporter in blue/green, and AIDA-I autotransporter in orange/pink. FXa protease recognition site is indicated with an asterisk (*). Two asterisks (**) indicate the amino acid sequence encoded by the multiple cloning site. 1=Commercial synthesis in the pJExpress backbone by DNA2.0 (Menlo Park, CA, USA), 2=amplification of pMATE-MT004 backbone using SI020 and PQ019 primers, 3=amplification of mCherry from pEF1a-mCherry-C1 using SI021 and PQ024 primers, 4=amplification of pMATE-SI015 backbone using PT-MCE-7f and PT-MCE-8r primers, 5=amplification of AIDA-I β-barrel and linker region from pES01 using PT-MCE-5f and PT-MCE6r primers, 6=amplification of pMATE-MT004 backbone using PT-MCE-3r and PT-MCE-4f primers, 7=amplification of *estA* from pES01 using PT-MCE1f and PT-MCE2r primers. The respective PCR fragments were combined using In-Fusion cloning technique (Clontech Laboratories Inc, Mountain View, CA, USA). pES01 has been described previously by Schultheiss *et al.* (*39*)



Supplementary Fig. 2. OmpT activity in whole cells of *E. coli*. Cells were grown to $A_{600\,nm}$=0.5 as described in the Materials and Methods. A volume of 100 μL of protein solution was added to the Abz-Ala-Arg-Arg-Ala-Tyr(NO_2)-NH_2 substrate to a final substrate concentration of 0.16 mM. Fluorescence was recorded over 5 h at 37 °C in intervals of 5 min with an excitation wavelength of 320 nm and an emission wavelength of 420 nm. Designations: outer membrane proteins from OmpT-positive *E. coli* UT2300 cells with substrate (■), and without substrate (▲); outer membrane proteins from OmpT-negative *E. coli* UT5600 cells with substrate (■), and without susbstrate (▲). LB medium was inoculated with a pre-culture of *E. coli* UT2300 or *E. coli* UT5600 as described above. Cells were grown at 37 °C to $A_{600\,nm}$=0.5. After a centrifugation step, cells were washed in assay buffer (0.5 M Tris/HCl, pH=7.0) and subsequently resuspended to $A_{600\,nm}$=0.5



Supplementary Fig. 3. OmpT activity in isolated outer membrane proteins of *E. coli*. Outer membrane proteins were isolated from a 40-mL culture of cells at $A_{600\,nm}$=0.5 as described in the Materials and Methods and resuspended in 1 mL of assay buffer. A volume of 100 μL of protein solution was added to the Abz-Ala-Arg-Arg-Ala-Tyr(NO_2)-NH_2 substrate to a final substrate concentration of 0.16 mM. Fluorescence was recorded over 5 h at 37 °C in intervals of 5 min with an excitation wavelength of 320 nm and an emission wavelength of 420 nm. Designations: outer membrane proteins from OmpT-positive *E. coli* UT2300 cells with substrate (■), and without substrate (▲); outer membrane proteins from OmpT-negative *E. coli* UT5600 cells with substrate (■), and without substrate (▲)

Supplementary Table 1. Sequences of primers used for the construction of plasmids pMATE-MT004, pMATE-SI015, pMATE-PT013 and pAIDAI-PT014

Primer name	Primer sequence (5′ to 3′)
SI020	GCTCGTCGTGCTATTGAGGGCCGCATCCC
PQ019	ATGATGGTGATGGTGGTGGGTGATGTTCTG
PQ024	CACCATCACCATCATATGGTGAGCAAGGGCGAGGAGGATAACATG
SI021	AATAGCACGACGAGCCTTGTACAGCTCGTCCATGCCGCCGGTGG
PT-MCE-1f	CTCGAGGGCGGCGGTGACGAC
PT-MCE-2r	GGTACCCTTGGTGACGCCGGC
PT-MCE-3r	ACCGCCGCCCTCGAGATGATGGTGATGGTGGTGGGTGATGTTCTG
PT-MCE-4f	GTCACCAAGGGTACCGCTCGTCGTGCTATTGAGGGCCGC
PT-MCE-5f	CTTAATCCTACAAAAGAAAGTGCAGG
PT-MCE-6r	GAAGCTGTATTTTATCCCCAGTGCTC
PT-MCE-7f	GATAAAATACAGCTTCCTCGAGCCCCAAGGGCGACACCCCCTAAT
PT-MCE-8r	CTTTTGTAGGATTAAGTTTAAAGTATTCCGGGATGCGGCCCTCAATAG