# Supporting Information

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## SI Materials and Methods

**Plasmid Constructs.** Plasmids p29SEN, p29-SecB and SecB(E77K) (23), pSE380ΔNcoI (32), pET15b-SecB (33), pMPMK6, pK6-*Mycobacterium tuberculosis* (*Mtb*)-HigB1, pK6-*Mtb*-HigBA1, pK6-*Mtb*-HigA1$^{Strep}$, pET15b-*Mtb*-HigA1, pET15b-*Mtb*-SecB$^{TA}$ (9), pK6-*Mtb*-HigBA1$^{W108A/W137A}$, pK6- *Geobacter lovleyi* (*Glov*)-HigBA, pK6-*Glov*-HigBA-*Mtb*-ChAD, pK6-*Vibrio cholerae* (*Vcho*)-MqsRA, pK6-*Vcho*-MqsRA-*Mtb*-ChAD, pK6-*Methylomonas methanica* (*Mmet*)-HicAB, and pK6-*Mmet*-HicAB-*Mtb*-ChAD (11) were described previously. The plasmid pSE-SecB$^{NHis}$ (encoding SecB with an N-terminal $His_6$ tag) was constructed by PCR using primers SecBNH-For (5′-gaggaattcatgggcagcagccat-catc-3′) and SecBNH-Rev (5′-gagaagctttcaggcatcctgatgtt-cttc-3′) and pET15b-SecB as templates. The PCR products were digested with EcoRI and HindIII enzymes, and ligated into pSE380ΔNcoI previously digested with the same enzymes. To construct plasmid pK6-SecA$^{FLAG}$ (SecA with the N-terminal FLAG tag "GSDYKDDDDK"), the SecA gene was amplified from W3110 genomic DNA using primers SecAF-For (gacaattgatggccagcgactacaaagatgacgacgataaaagcggcatgcta atcaaattgttaactaaag) and SecAF-Rev (gaggatccttattgcaggcggccatggcactg), digested with MfeI (partial digestion) and BamHI, and ligated into pMPMK6 digested with EcoRI and BglII. Plasmids pSE-SecB (I114Y)$^{NHis}$ and pET15b-SecB(E77K), -SecB(I114Y), -SecB(T10A), or -SecB(R15H), as well as all of the p29-SecB suppressor mutants, were constructed by Quick Change mutagenesis (Stratagene) using appropriate primers. To construct plasmids pK6-SecB and pK6-SecB(I114Y), *secB* genes were subcloned from p29-SecB and p29-SecB(I114Y), respectively, as EcoRI/HidIII fragments into plasmid pMPMK6 digested with the same enzymes. Plasmids pSE-*Mtb*-HigA1 and pSE-*Mtb*-HigA1$^{Strep}$ were obtained by subcloning the corresponding ORFs as EcoRI/BglII fragments from pK6-*Mtb*-HigBA1 and pK6-*Mtb*-HigA1$^{Strep}$, respectively, into plasmid pSE380ΔNcoI digested with the same enzymes. Plasmid pK6-*Mtb*-HigBA1Δ$^{104–119}$ was obtained by PCR using pK6-*Mtb*-HigBA1 as a template and primers higA del-ForW1 (GACCACCAGGTCCGGGTG)/higA del-RevW1 (GCGAAGCGTGGGAACTTC) to delete nucleotides 310–357 of *higA1* (corresponding to amino acids 104–119 of HigA1). PCR product was digested by DpnI, phosphorylated with T4 polynucleotide kinase during the ligation step, and transformed into competent *Escherichia coli*. All plasmids were confirmed by sequencing.

**Isolation of Protein Aggregates in Vivo.** Prevention of protein aggregation by overexpressed SecB in vivo in the absence of TF and DnaK was performed as described (23). Cultures of fresh transformants of MC4100 Δ*tig*::Cm$^R$ Δ*dnaKdnaJ*::Kan$^R$ (32) containing plasmid pSE380ΔNcoI, pSE-SecB$^{NHis}$, or pSE-SecB (I114Y)$^{NHis}$ obtained at 25 °C were diluted to an $OD_{600}$ of 0.05 in LB/ampicillin (50 µg/mL) and incubated at 25 °C to an $OD_{600}$ of 0.3, and expression was induced with 0.5 mM IPTG for 1 h. Cultures were then transferred at 33 °C in a water bath under shaking for 1 h, and protein aggregates were isolated as described (34).

**In Vivo Pull-Down Assay.** *E. coli* strain W3110 Δ*secB*::Cm$^R$ was cotransformed with pK6-*Mtb*-HigA1$^{Strep}$ and p29SEN, p29-SecB, or p29-SecB$^{TA}$ variant as indicated. Overnight cultures of transformants were diluted to an $OD_{600}$ of 0.05 and grown at 37 °C to an $OD_{600}$ of about 0.4 in LB supplemented with ampicillin and kanamycin. Expression of the SecB chaperones was then induced with 50 µM IPTG; 20 min later, *Mtb*-HigA1 expression was induced with 0.5% arabinose for 1 h. The cells were collected by centrifugation (6,000 × *g* for 5 min at 4 °C) and resuspended in 1 mL of cold PD buffer [150 mM NaCl, 50 mM Tris (pH 7.5), 1 mg/mL lysozyme, and protease inhibitors from Roche] supplemented with Benzonase (3 units/mL culture volume; Invitrogen). Crude cell extracts were obtained by sonication and centrifugation (14,000 × *g* for 30 min). Pull-down assays were performed with 400-µL aliquots of cell extracts and 50 µL of streptactin Sepharose resin (IBA) in PD buffer. The suspensions were gently rocked at 4 °C for 1 h, and the beads were pelleted by spinning for 30 s at 375 × *g*. The beads were then washed six times with 1 mL of PD buffer. Proteins were eluted in PD buffer containing 5 mM desthiobiotin. Samples were separated by SDS/PAGE on 4–20% Mini-Protean TGX gels (Bio-Rad) and were further analyzed by SYPRO orange coloration (Sigma).

**Pulse-Chase Analysis.** Strain W3110 Δ*secB*::Cm$^R$ was transformed with plasmid p29SEN, p29-SecB, or p29-SecB(I114Y) and grown overnight at 30 °C in M9 minimal medium supplemented with 0.2% glycerol, ampicillin (50 µg/mL), and 0.2% casamino acids. Cultures were diluted to an $OD_{600}$ of about 0.05 in the same medium supplemented with 0.2% maltose and grown to an $OD_{600}$ of ∼0.3 at 30 °C. Cells were centrifuged (5 min at 5,000 × *g*); washed once with M9 containing 0.2% glycerol, ampicillin (50 µg/mL), 0.2% maltose, and a methionine/cysteine-free amino acid mix; and resuspended in the same medium. No IPTG inducer was added because expression of the chaperones was sufficient to observe complementation under these conditions. After 1 h at 30 °C, cells were pulse-labeled for 1 min at 30 °C with 5 µCi/mL [$^{35}$S]methionine/cysteine and chased with 15 mM unlabeled methionine/cysteine for the indicated times. Samples were then precipitated in 10% (wt/vol) trichloroacetic acid on ice, washed, and immunoprecipitated with anti–maltose-binding protein (New England Biolabs), anti-OmpA, or anti-OmpF antibodies as described (21).

The experiment of competition with *Mtb*-HigA1 was carried out in strain W3110 Δ*secB*::Cm$^R$ transformed with plasmid pMPMK6, pK6-SecB, or pK6-SecB(I114Y) and with plasmid pSE380 or pSE-*Mtb*-HigA1$^{Strep}$. Cells were grown overnight at 30 °C in M9 minimal medium supplemented with 0.2% glycerol, kanamycin (50 µg/mL), ampicillin (100 µg/mL), and 0.2% casamino acids. Cultures were diluted to an $OD_{600}$ of about 0.05 in the same medium supplemented with 0.2% maltose and grown to an $OD_{600}$ of ∼0.3 at 30 °C. Cells were centrifuged; washed once with M9 containing 0.2% glycerol, kanamycin (50 µg/mL), ampicillin (100 µg/mL), 0.2% maltose, and a methionine/cysteine-free amino acid mix; and resuspended in the same medium supplemented with 50 µM IPTG inducer. After 30 min at 30 °C, 0.002% arabinose was added, and cultures were allowed to grow for an additional 30 min at 30 °C. Cells were then pulse-labeled for 1 min at 18 °C with 5 µCi/mL [$^{35}$S]methionine/cysteine and chased with 15 mM unlabeled methionine/cysteine for the indicated times. Samples were then precipitated in 10% (wt/vol) trichloroacetic acid on ice, washed, and immunoprecipitated with anti-OmpA or anti-OmpF antibody as described (21).

**Protein Purification.** *Mtb*-HigA1 and *Mtb*-SecB$^{TA}$ were purified from a BL21(λDE3) strain harboring plasmid pET15b-*Mtb*-HigA1 or pET15b-*Mtb*-SecB$^{TA}$, respectively, as described (9). SecB and the SecB$^{T10A}$, SecB$^{R15H}$, SecB$^{E77K}$, and SecB$^{I114Y}$ variants were overexpressed from pET15b-SecB–based plasmids in *E. coli* strain BL21(λDE3) as previously described (33), except that following the nickel-nitrilotriacetic acid (Ni-NTA) column, the suspension

was applied to a HiLoad 16/60 Superdex 200 prep-grade column (GE Healthcare) preequilibrated with buffer GF [20 mM Tris·HCl (pH 7.5), 200 mM NaCl, 1 mM DTT, and 10% glycerol]. Finally, the peak fractions containing SecB and variants were pooled, concentrated in buffer GF with Vivaspin 20 (Sartorius), and stored at −80 °C. Strain W3110 harboring pK6-SecA$^{FLAG}$ was used to purify SecA as a fusion protein containing an N-terminal FLAG tag. Cells (500 mL) were grown to an $OD_{600}$ of 0.4 in LB medium containing kanamycin at 37 °C, and SecA expression was induced with 1% arabinose for 14 h at 20 °C. Cells were harvested and resuspended in buffer A [50 mM Tris·HCl (pH 7.5), 400 mM NaCl, and 10% glycerol] supplemented with 1 mg/mL lysozyme, protease inhibitors (Roche), and 1 µL/mL Benzonase. Following sonication, lysate was cleared by centrifugation at 30,000 × *g* for 30 min at 4 °C. The supernatant was incubated with anti-Flag M2 affinity gel (Sigma) preequilibrated with buffer A. The resin was washed with 20 mL of buffer A, and elution was achieved with 100 µg/mL Flag peptide (Sigma) in buffer A. Fractions containing the SecA protein were pooled, concentrated in Amicon 30 (Sigma), and stored at −80 °C until further use.

**DSF.** The thermal stability of *E. coli* SecB and SecB$^{I114Y}$ mutant was assayed by DSF alone or in the presence of peptides derived from the *Mtb*-HigA1 sequence as previously described (11). Purified SecB or SecB$^{114Y}$ (10 µM monomer) and SYPRO orange (Invitrogen) were mixed with or without 600 µM 13-mer peptide in a final volume of 20 µL of DSF buffer [20 mM Tris (pH 7.5), 200 mM NaCl, and 1 mM DTT] and loaded into a 96-well PCR plate (Bio-Rad). A temperature gradient from 20 to 90 °C with a 0.3 °C increment was then applied. DSF experiments were performed using a CFX96 real-time PCR system (Bio-Rad), and $T_m$ was given by the inflection point of the curve of the fluorescence intensity as a function of temperature. All measurements were performed in triplicate.

**In Vitro Aggregation Assays.** Denaturation of 60 µM *Mtb*-HigA1 or 25 µM L-MDH from pig heart (Roche) was carried out in 6.5 M urea and 10 mM DTT at room temperature for at least 2 h. Denatured protein was diluted to 4 µM (*Mtb*-HigA1) or 2 µM (L-MDH) in PBS alone or containing the indicated concentration of chaperones, and light scattering was measured in a fluorimeter (Fluoromax-4; Horiba Scientific) set as follows: 30 °C temperature, 350 nm excitation wavelength, 355 nm emission wavelength, 1.5-nm slits, 0.5-s integration time, maximal level of stirring. Measurements were done in triplicate, and the average was fitted to the Hill1 sigmoidal equation.

**In Vitro Pull-Down Assay.** Purified SecA (0.5 µM dimer) and SecB variants (0.5 µM tetramer) were mixed in a final volume of 100 µL of buffer AB [20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM DTT, and 20 mM imidazole]. After 15 min of incubation at room temperature, 30 µL of Ni-NTA resin (previously equilibrated with buffer AB) was added, and suspensions were gently rocked for 1 h at 4 °C. The resin was then washed with 5 mL of buffer AB, and proteins were eluted with buffer AB containing 200 mM imidazole. Samples were analyzed by SDS/PAGE using instant blue staining (CBS Scientific).

**Chymotrypsin Proteolysis.** Partial α-chymotrypsin proteolysis of SecB and SecB$^{I114Y}$ was performed as follows. SecB or SecB$^{I114Y}$ (0.2 mg/mL) was incubated with α-chymotrypsin (10:1 wt/wt, C4129; Sigma) in reaction buffer [20 mM Tris (pH 7), 5–200 mM NaCl, and 1 mM DTT] for 10, 30, 60, 120, or 180 min at 12 °C. Samples were migrated on 4–20% SDS/PAGE and stained with Coomassie blue.

**Circular Dichroism.** Far-UV (185–260 nm) circular dichroism spectra were recorded at room temperature with a Jasco J-815 spectropolarimeter and a 0.01-mm path-length cuvette for SecB and SecB$^{I114Y}$ proteins at 600 µM in 20 mM Tris·HCl, 200 mM NaCl, 1 mM DTT, and 10% (vol/vol) glycerol (pH 7.5).

**Cell-Free Protein Synthesis and Aggregation Assay.** A cell-free transcription/translation-coupled in vitro assay using the protein synthesis using recombinant elements (PURE) system was carried out as described (11). In brief, template DNA of *Mtb*-HigA1 was added at a final concentration of 7.2 ng/µL to the PURE system, with or without purified SecB or SecB$^{I114Y}$. Protein synthesis was performed at 37 °C for 60 min. An aliquot was withdrawn as the total fraction, the remaining reaction was centrifuged at 20,600 × *g* for 30 min, and the supernatant fraction was collected. Both fractions were separated on 4–20% Mini-Protean TGX gels (Bio-Rad) and visualized by Western blot using anti–*Mtb*-HigA1 antibodies. Quantification of solubilized *Mtb*-HigA1 in the presence or absence of SecB or SecB$^{TA}$ was performed from Western blots using Image Lab Software from Bio-Rad based on three independent experiments.

**Data Availability.** All relevant data are available from the authors.

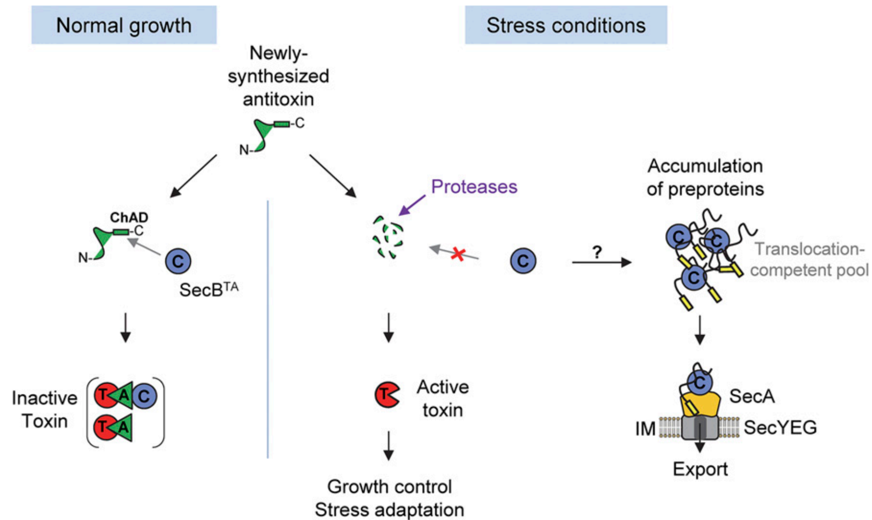


**Fig. S1.** Working model for SecB$^{TA}$ function. The different proteins are depicted as follows: toxin (T), red; antitoxin (A), green; chaperone (C), blue; SecA, orange; SecYEG, gray; signal sequence of presecretory proteins, yellow. IM, inner membrane. (*Left*) Under normal growth conditions, SecB$^{TA}$ binds to the C-terminal ChAD region of the antitoxin, stabilizes it, and facilitates its interaction with the toxin, leading to toxin inactivation. The brackets indicate that it is not known yet whether the chaperone is part of the inactive toxin complex. (*Right*) Under specific stress conditions affecting Sec-dependent protein export, the SecB$^{TA}$ chaperone could be hijacked by accumulating presecretory proteins, exposing the unstable antitoxin to yet unidentified proteases, thus leading to transient activation of the toxin until normal conditions are resumed. Both the reversible growth inhibition induced by the RNase toxin and an accumulated pool of SecB$^{TA}$-bound translocation-competent preproteins could represent a fined-tuned response to export stress in *M. tuberculosis*. To date, there is no direct evidence for such a link between TA control and export stress.

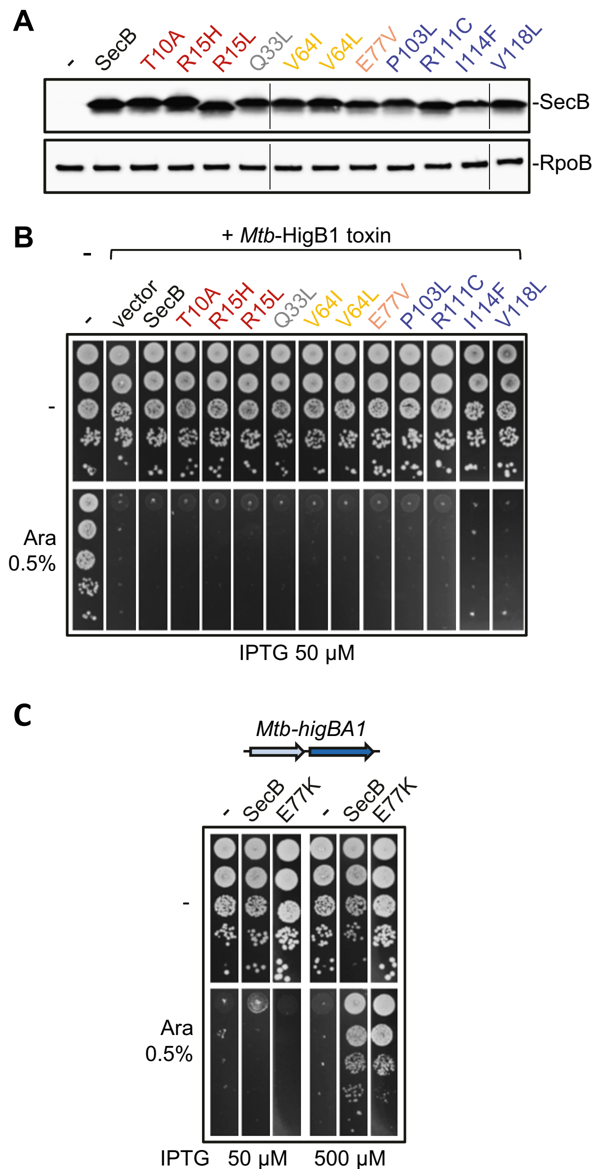


**Fig. S2.** (*A*) Steady-state expression of SecB$^{TA}$ variants. Overnight cultures of strain W3110 Δ*secB* containing plasmid p29SEN(−), p29-SecB, or p29-SecB$^{TA}$ variant were diluted at an $OD_{600}$ of 0.05 and grown at 37 °C to an $OD_{600}$ of ∼0.3, and expression was induced with 50 μM IPTG for 2 h. Whole-cell extracts were analyzed by immunoblot using either anti-SecB (*Top*) or anti-RpoB (*Bottom*) antibody. (*B*) SecB$^{TA}$ variants do not suppress *Mtb*-HigB1 toxicity in the absence of antitoxin. Midlog-phase cultures of strain W3110 Δ*secB* containing plasmids pMPMK6 and p29SEN(−) or pK6-*Mtb*-HigB1 and p29SEN, p29-SecB, or p29-SecB$^{TA}$ variant were serially diluted and spotted on agar plates containing the indicated concentrations of arabinose and IPTG inducers, and incubated at 37 °C. (*C*) SecB$^{E77K}$ is not a SecB$^{TA}$. Midlog-phase cultures of strain W3110 Δ*secB* containing plasmids pK6-*Mtb*-HigBA1 and p29SEN(−), p29-SecB, or p29-SecB(E77K) were serially diluted and spotted on agar plates containing the indicated concentrations of arabinose and IPTG inducers, and incubated at 37 °C.

A

B

**Fig. S3.** Mapping of the TA-directed SecB variants on the SecB structure. *(A)* Ribbon representation and semitransparent molecular surface of the NMR structure of SecB in complex with PhoA [Protein Data Bank (PDB) ID code 5jtl]. The A, B, C, and D SecB subunits are colored, respectively, in yellow, cyan, violet, and green, whereas the main-chain trace of PhoA is black. The SecB tetramer results from the association of two β-dimers. This association involves helix α1 from each subunit. (*B*) Superimposition of the SecB-PhoA NMR structure and the crystal structure of SecB alone (PDB ID code 1qyn, colored in gray) is shown for β-dimers AB (*Top*) and CD (*Bottom*). For the sake of clarity, only the main-chain trace of PhoA within 5 Å of SecB has been represented. Residues for which variants have been identified are shown with carbon atoms in orange and labeled. Residue R15 from one subunit makes cation-π interaction with F32 from the cognate subunit (also displayed as gray sticks). Superposition of the two SecB structures reveals that binding of PhoA mainly affects the cross-over loop and helix α2, whereas minor and local perturbations are found in helix α1 and the β-sheet. These features reflect the intrinsic flexibility of SecB. Most variants are localized in the β-sheet and helix α1 halves close to the cross-over loop. The corresponding mutations might further increase protein dynamics in these regions, which could result in the productive interaction with the *Mtb*-HigA1 antitoxin.

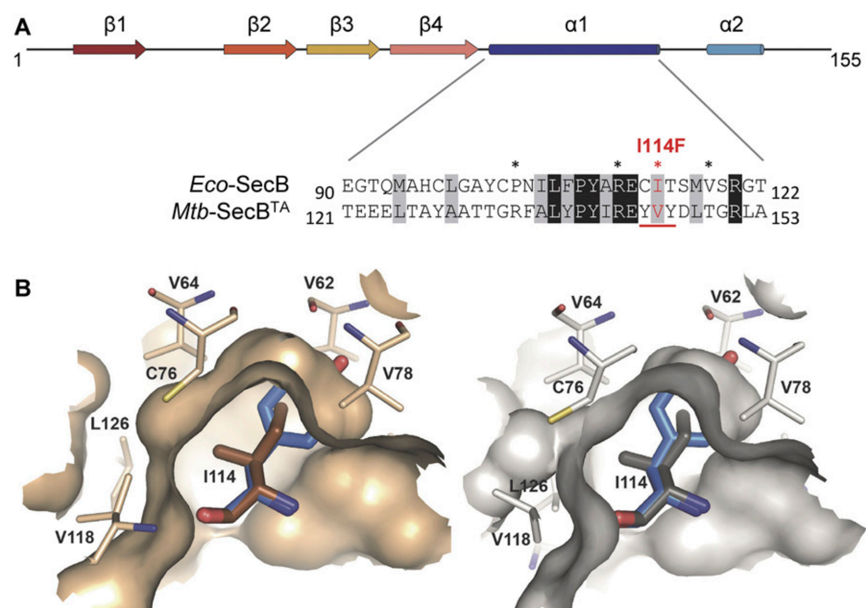


**Fig. S4.** SecB$^{TA}$ variants in helix α1 and SecB$^{TA}$ interaction with *Mtb*-HigA1. (*A*) Alignment of helix α1 of SecB with the corresponding region of *Mtb*-SecB$^{TA}$. Identical and similar residues are colored in black and gray, respectively. Positions of residues in helix α1 isolated during the genetic selection are shown with an asterisk. I114 is depicted in red, and the "YVY" triplet in *Mtb*-SecB$^{TA}$ is underlined. (*B*) Zoom-in view of the environment of residue I114 in the crystal structure of SecB alone (PDB ID code 1qyn, colored in maroon; *Left*) and the SecB-PhoA NMR structure (PDB ID code 5jtl, colored in gray; *Right*). Residue I114 is shown as enlarged sticks in brown or gray according to SecB structure. Residues directly surrounding I114 are shown as sticks and the van der Waals surface, which delineates a hydrophobic pocket. Both PDB ID code 1qyn and 5jtl structures of SecB have been used for modeling the I114Y variant in SWISS-MODEL (35). Residue Y114 of each generated model is shown as blue enlarged sticks in the corresponding panel. According to both models, replacement of isoleucine by a tyrosine residue would generate steric clashes, exemplified by the phenol hydroxyl group protruding over the hydrophobic pocket, and thus requires structural rearrangements in both SecB and SecB-PhoA structures.

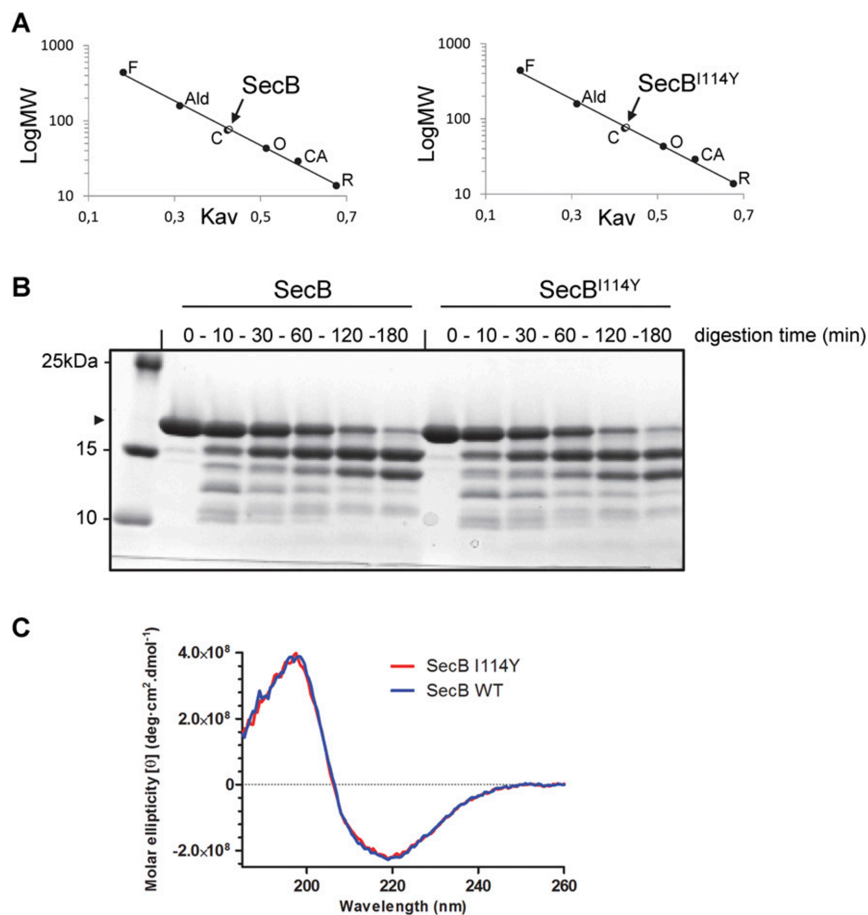


**Fig. S5.** SecB$^{I114Y}$ in vitro properties. (*A*) Gel filtration analysis of SecB$^{I114Y}$ revealed that it forms a tetramer. The molecular mass (MMs) of SecB and SecB$^{I114Y}$ were determined by gel filtration chromatography on a HiLoad 16/60 Superdex 200 pg (GE Healthcare) with protein markers (●) of ribonuclease A (R; 13.7 kDa), carbonic anhydrase (CA; 29 kDa), ovalbumin (O; 43 kDa), conalbumin (C; 75 kDa), aldolase (Ald; 158 kDa), and ferritin (F; 440 kDa). Proteins were subjected to size exclusion chromatography and the elution volume (Ve) was measured. The distribution coefficient ($K_{av}$) was given by $K_{av}$ = (Ve − V0)/(Vt − V0), where Vt and V0 are the total bed volume and the void volume, respectively. The calibration curve was obtained by plotting LogMW (LogMM) versus $K_{av}$. The $K_{av}$s of SecB and SecB$^{I114Y}$ are indicated (○). Calculated MMs for SecB and SecB$^{I114Y}$, obtained from the linear regression of MM = 1415.3e − 6.805 $K_{av}$ ($R^2$ = 0.9967), are 77.4 and 77.3 kDa, respectively. (*B*) Partial α-chymotrypsin proteolysis of SecB and SecB$^{I114Y}$. SecB or SecB$^{I114Y}$ (0.2 mg/mL) was incubated with α-chymotrypsin (10:1 wt/wt) for 10, 30, 60, 120, or 180 min at 12 °C. Samples were migrated on 4–20% SDS/PAGE and stained with Coomassie blue. The black arrowhead on the left indicates full-length SecB and SecB$^{I114Y}$. (*C*) Circular dichroism spectra of SecB (blue curve) and SecB$^{I114Y}$ (red curve). Far-UV (185–260 nm) circular dichroism spectra were recorded at room temperature at protein concentrations of 10 mg/mL. No difference is observed between the two spectra, indicating that the secondary structure elements of SecB wild type are maintained in the I114Y variant.

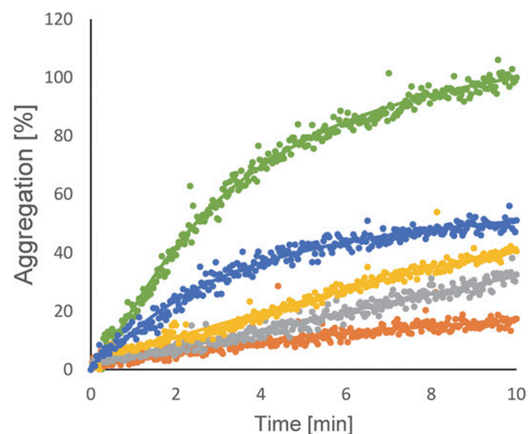


**Fig. S6.** SecB$^{I114Y}$ and *Mtb*-SecB$^{TA}$ prevent *Mtb*-HigA1 aggregation in vitro. Aggregation kinetics of denatured *Mtb*-HigA1 (4 μM) were followed at 30 °C by light scattering at 350 nm without (green) or with 0.125 μM or 0.250 μM purified SecB$^{I114Y}$ (blue and gray, respectively) or *Mtb*-SecB$^{TA}$ (yellow and orange, respectively) tetramer. A representative result of three independent experiments is shown.

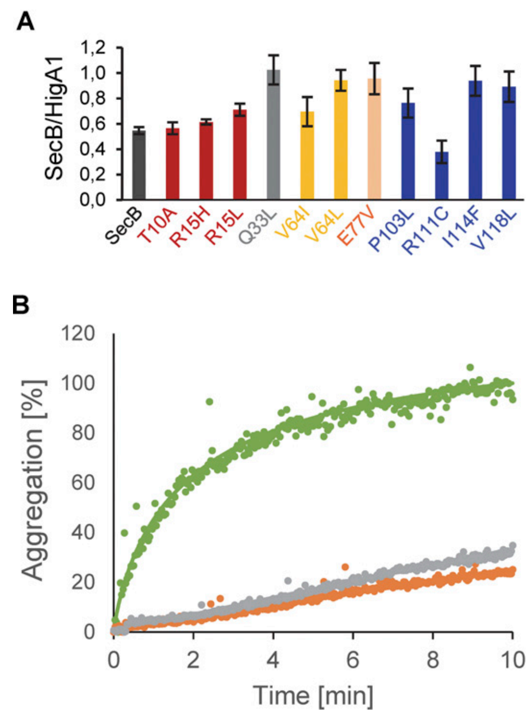


**Fig. S7.** Interaction between *Mtb*-HigA1 and other $SecB^{TA}$ variants. (*A*) Interaction in vivo. Strain W3110 Δ*secB* cotransformed with plasmids pMPMK6-$HigA1^{Strep}$ and p29-SecB or p29-$SecB^{TA}$ variant was grown to midlog phase. SecB expression was induced with 50 μM IPTG, and *Mtb*-HigA1 expression was induced 20 min later with 0.5% arabinose for 1 h. Crude cell extracts were mixed with streptactin Sepharose resin, and protein complexes were eluted with 5 mM desthiobiotin. Quantification of SecB and *Mtb*-HigA1 in elution fractions was performed after coloration with SYPRO orange, and the ratio of SecB/*Mtb*-HigA1 was calculated for each $SecB^{TA}$. The results are given as fold change normalized to wild-type SecB, and are the mean of three independent experiments. Error bars indicate SD. (*B*) $SecB^{T10A}$ and $SecB^{R15H}$ variants efficiently prevent *Mtb*-HigA1 aggregation in vitro. Aggregation kinetics of denatured *Mtb*-HigA1 (4 μM) were followed at 30 °C by light scattering at 350 nm without (green) or with 1 μM purified $SecB^{T10A}$ (orange) or $SecB^{R15H}$ (gray) tetramer. A representative result of three different experiments is shown.

**Fig. S8.** SecB$^{I114Y}$ is directed toward the ChAD region. (*A*) Control plates with no arabinose inducer added from Fig. 3*A* of W3110 Δ*secB* containing the pK6-*Glov*-HigBA, pK6-*Mmet*-HicAB, or pK6-*Vcho*-MqsRA native TA pair (*Top*) or their chimeric derivatives with their respective ChAD sequences being replaced by *Mtb*-ChAD (*Bottom*). (*B*) Melting profiles of SecB and SecB$^{I114Y}$ alone (light blue and gray curves, respectively) or in the presence of C5 peptide at a ratio of 60:1 of peptide/chaperone (red and green curves, respectively). $T_m$ values deduced from each profile are indicated. (*C*) Thirteen-mer peptides covering the ChAD sequence are depicted on top of the *Mtb*-HigA1 amino acid sequence.

**Fig. S9.** Generic chaperone function of TA-directed $SecB^{TA}$ variants. (*A*) Midlog-phase cultures of strain W3110 Δ*secB* containing plasmid p29SEN(−), p29-SecB, or p29-$SecB^{TA}$ variant were serially diluted and spotted on agar plates in the absence of IPTG inducer, and incubated at the indicated temperatures. (*B*) Midlog-phase cultures of strain W3110 Δ*secB* containing plasmid p29SEN(−), p29-SecB, or p29-$SecB^{TA}$ variant were serially diluted and spotted on agar plates with 60 μg/mL novobiocin, and incubated at 37 °C. (*C*) MC4100 Δ*tig*::$Cm^R$ Δ*dnaKdnaJ*::$Kan^R$ strain transformed with p29SEN(−), p29-SecB, or p29-$SecB^{TA}$ variant was grown to midlog phase at 25 °C; serially diluted; spotted on agar plates containing IPTG inducer as indicated; and incubated at 25 °C or 37 °C.

**Fig. S10.** *Mtb*-HigA1 delays export of proOmpF in the presence of $SecB^{I114Y}$. Pulse-chase analysis showing the processing of radiolabeled proOmpF in the strain W3110 Δ*secB* expressing SecB or $SecB^{I114Y}$ in the presence of coexpressed *Mtb*-HigA1. Cells were grown at 30 °C to midlog phase, and induced with 50 μM IPTG and 0.002% arabinose 30 min later. After 30 min, cultures were switched to 18 °C, pulse-labeled, and chased for the indicated times. Samples were immunoprecipitated with anti-OmpF antibodies, separated by SDS/PAGE, and analyzed by autoradiography.