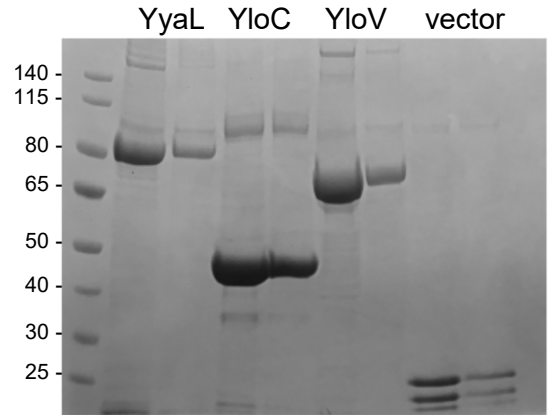

Fig. S1. Purification of 6X His-Sumo-tagged candidate proteins. Samples from two nickel column elution fractions are shown for three candidates. The vector control (pET-57) strain was treated identically to strains carrying the pET-57 vector with cloned CDSs. Migration of size control proteins (kDa) indicated at left.

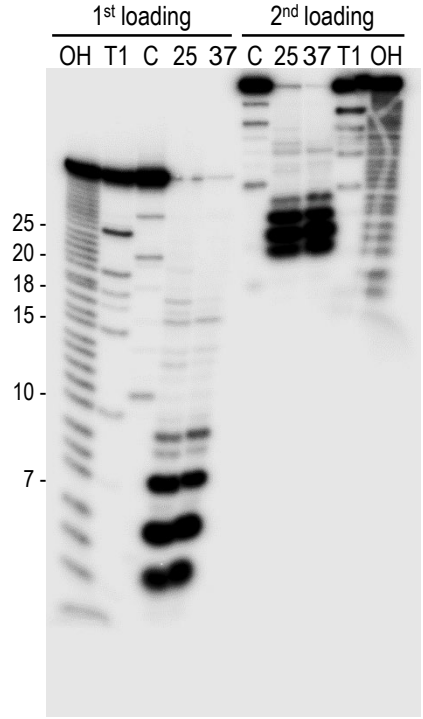

Fig. S2. YloC digestion limit product. YloC digestion limit product. 30 pmol of RNA oligo DHB1866 (labeled + unlabeled) were incubated with 10 pmol YloC and incubated at 25°C for 25 minutes or 37°C for 30 minutes. Two sets of samples were loaded on a 20% denaturing polyacrylamide gel. OH, hydroxyl treatment; T1, T1 RNase digestion; C, no treatment.

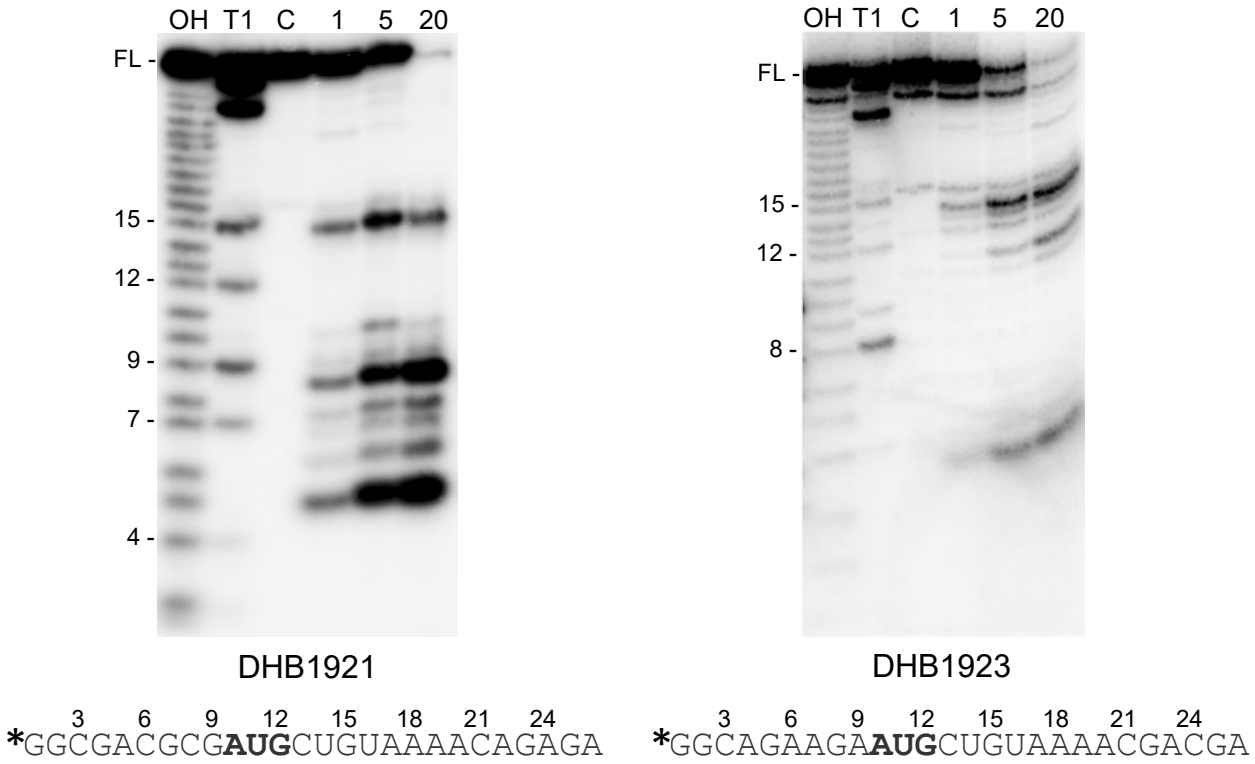

Fig. S3. YloC cleavage of 26-nt RNA oligos. AUG sequence is in bold. 30 pmol of RNA (labeled + unlabeled) were incubated with 10 pmol YloC at 25°C for the indicated times (min). Migration of full-length (FL) RNA and T1 RNase digestion products indicated on the left. OH, hydroxyl digest; T1, RNase T1 digest; C, control with no YloC. Cleavage patterns for these substrates differ from each other and from those of DHB1935 and DHB 1922 (Fig. 5A).

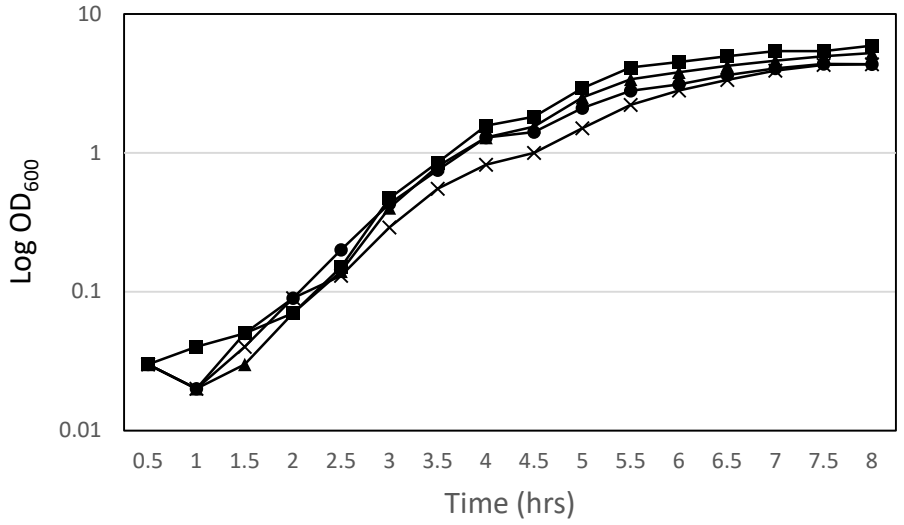

Fig. S4. Growth of *yloC* and *pnpA* mutant strains. Growth curve of wild-type and YloC mutant *B. subtilis* strains in Luria-Bertani medium at 37°C. ▪, wild type; ◆, *yloC* deletion; •, *pnpA* deletion; x *yloC pnpA* double deletion.

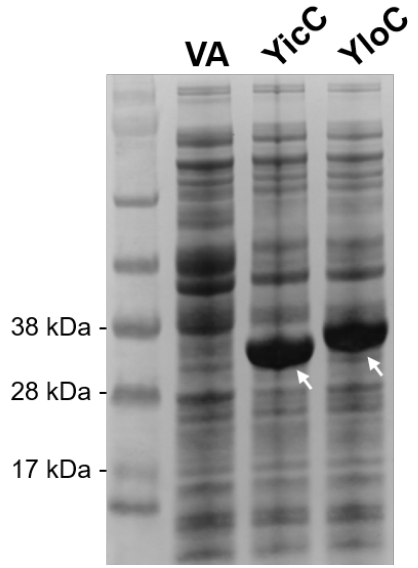

Fig. S5. Coomassie staining of total protein samples prepared from JC1322 transformed with empty vector (pQE80L), pYicC or pYloC and induced, as in Fig. 8D, resolved in NuPAGE 4-12% Bis-Tris protein gel. Induced YicC and YloC proteins are indicated with white arrows.

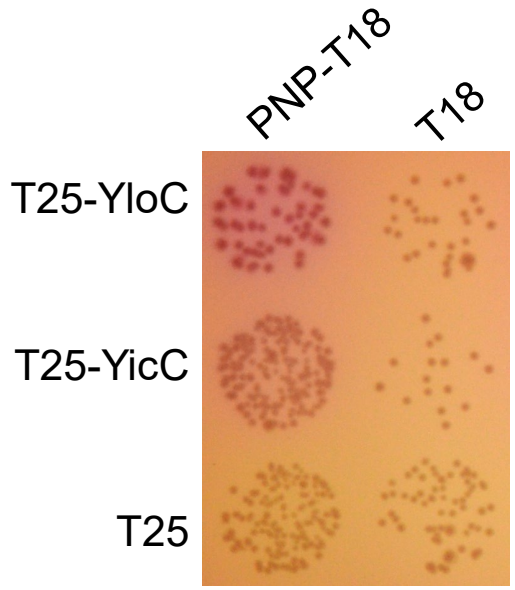

Fig. S6. BACTH reporter strain BTH101 co-transformed with plasmids of T18 and T25 fused to PNPase, YicC or YloC was spotted on MacConkey agar supplemented with ampicillin and kanamycin. Bacterial colonies were imaged after incubation at 30°C for 60 h.

Table S1. *B. subtilis* mutant strains.

Strain designation	Relevant genotype	RNase(s) missing	Source
BG505	*pnpA::cat rph::spec rnr::tet yhaM::phleo*	PNPase, RNase PH, RNase R, YhaM	Oussenko et al. 2005
BG1308	*yloC::erm*	YloC	Bacillus Genetic Stock Center strain 15660
BG1335	*yloC::erm pnpA::neo*	YloC, PNPase	This study
BG1316	*yloC::erm rph::spec rnr::tet yhaM::phleo*	YloC, RNase PH, RNase R, YhaM	This study
BG1317	*yloC::erm pnpA*::neo *rph::spec rnr::tet yhaM::phleo*	YloC, PNPase, RNase PH, RNase R, YhaM	This study

Table S2. Oligonucleotide substrates used in this study.

Oligonucleotide designation	Purpose (description)	Oligonucleotide sequence
DHB1866	RNA sequence for YloC assay (Fig. S2)	GGC GAU GCU GUA ACG CGG CGA UAA GUU CAA AAC ACA
DHB1879	5' IR800-fluorescent-labeled RNA oligo for ribonuclease assay.	IRD800-GGG CAU GCU GUA ACA CGA CGA UAA GUU CAA AAC ACA
DHB1880	DNA oligo used for ligation assay	5'rApp/CTG TAG GCA CCA TCA AT/3'ddC
DHB1921	RNA oligo to test YloC cleavage specificity	GGC GAC GCG AUG CUG UAA AAC AGA GA
DHB1922	RNA oligo to test YloC cleavage specificity	GGC GAC CGC CUG AUG UAA AAC AGA GA
DHB1923	RNA oligo to test YloC cleavage specificity	GGC AGA AGA AUG CUG UAA AAC GAC GA
DHB1935	RNA oligo to test YloC cleavage specificity	GGC GAU GCU GUA ACG CGG CGA UAA GU
DHB1954	DNA version of DHB1935	GGC GAT GCT GTA ACG CGG CGA TAA GT
DHB1955	Reverse complement of DHB1935	ACU UAU CGC CGC GUU ACA GCA UCG CC