SUPPLEMENTARY INFORMATION

Nascent peptide assists the ribosome in recognizing chemically distinct small molecules

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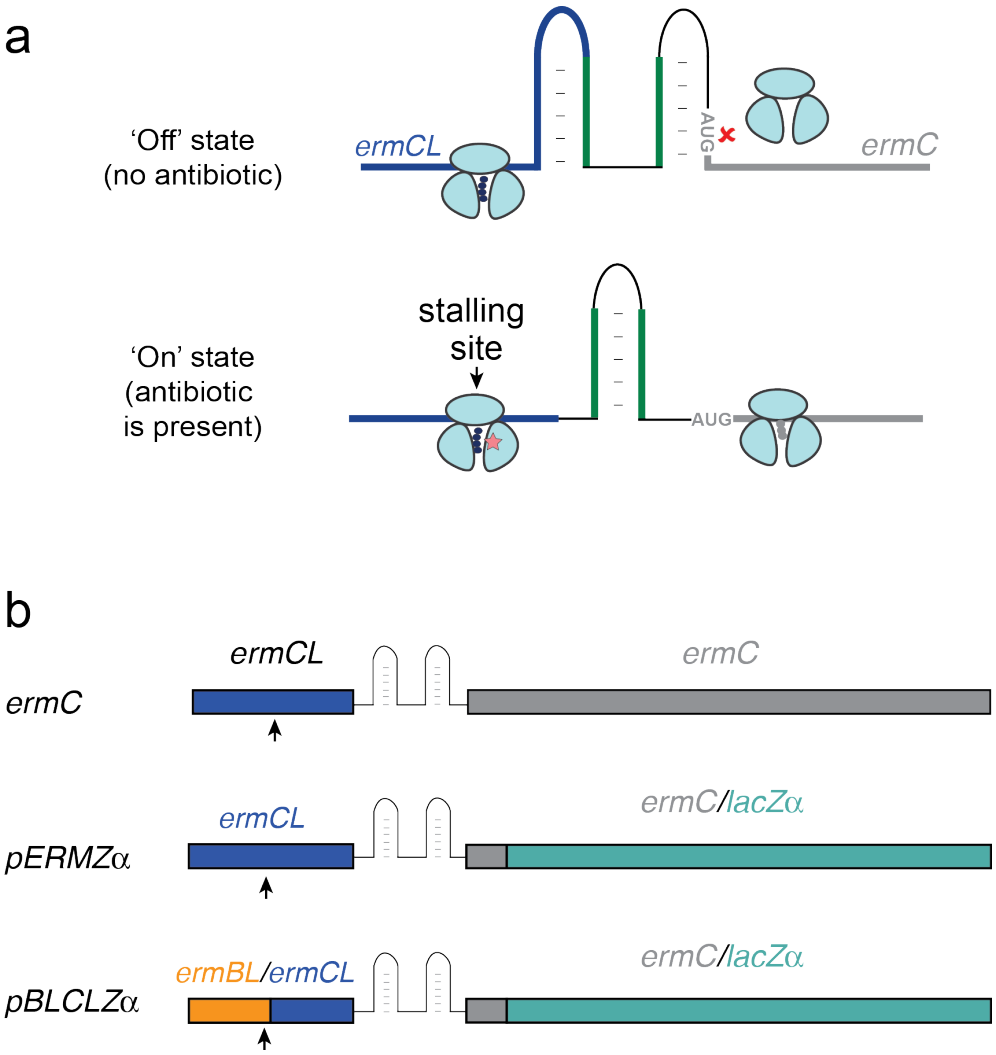
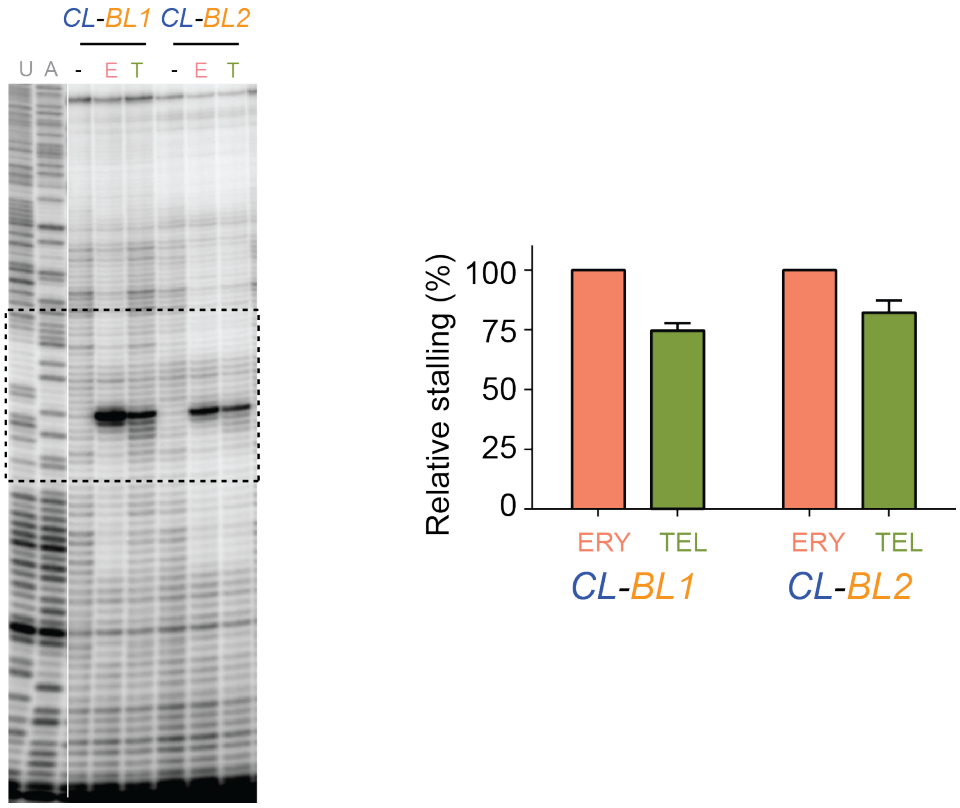
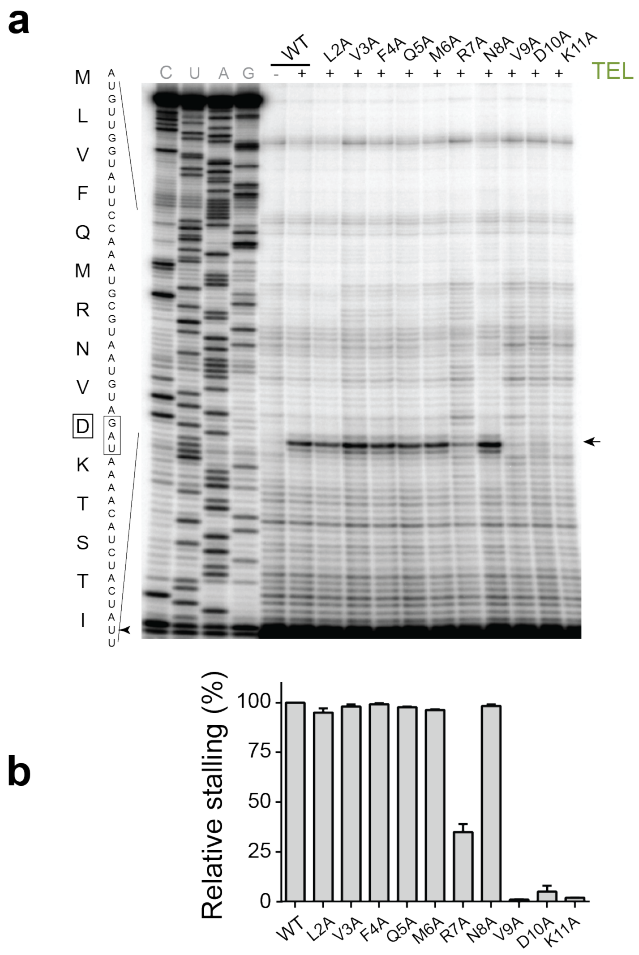
SUPPLEMENTARY RESULTS



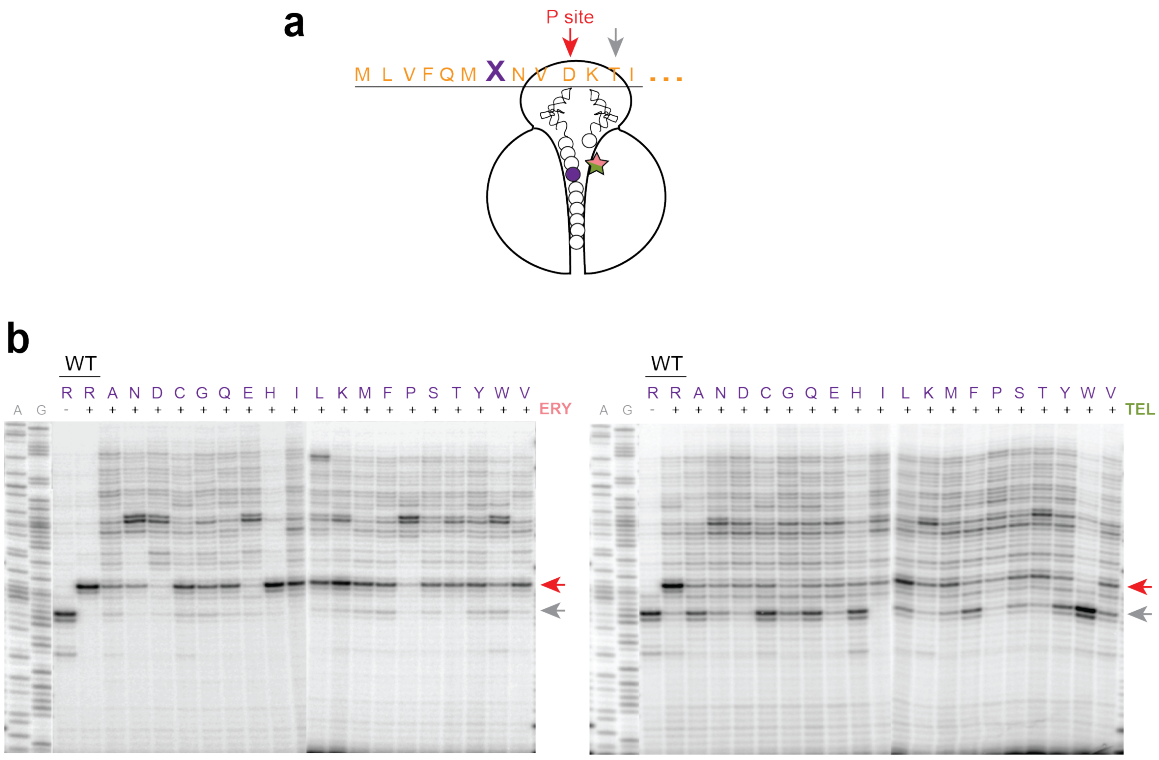
Supplementary Figure 1. Antibiotic mediated activation of the resistance gene *ermC*. (**a**), The general scheme of antibiotic- and nascent peptide-controlled induction of *erm* genes (using the example of the *ermC* operon). In the absence of antibiotic ('Off' state), the leader ORF is continuously translated whereas translation of *ermC* is attenuated due to sequestration of the Shine-Dalgarno sequence and initiator codon ('AUG') in the mRNA secondary structure. At limiting concentrations of the inducer, binding of the antibiotic to the ribosome promotes translation arrest at a specific codon ('stalling site') of the leader *ermCL* ORF. The stalled ribosome induces isomerization of the mRNA structure liberating the ribosome binding site and activating expression of the *erm* gene ('On' state). (**b**), The organization of the wt *ermC* operon (top), the previously engineered pERMZα reporter[1] (middle), and the hybrid pBLCLZα reporter (bottom). Arrows indicate the schematic location of the programmed arrest site in the leader ORFs.



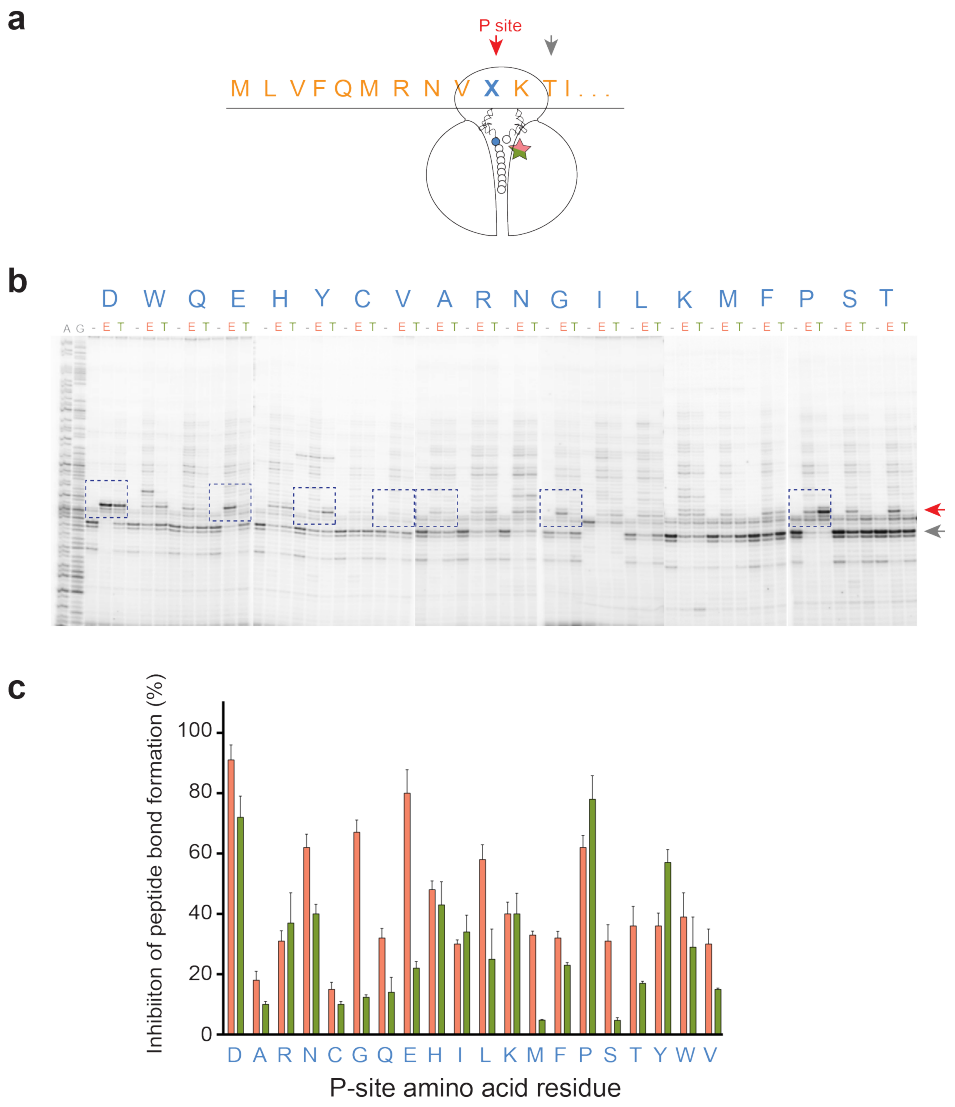
Supplementary Figure 2. Antibiotic mediated arrest in hybrid templates *CL-BL1* and *CL-BL2*. *Left:* Full gel of the toeprinting analysis of the CL-BL1 and CL-BL2 templates (the portion of the gel shown in Fig. 1d of the main text, is boxed). *Right*: Bar graph of the stalling efficiency (%) with ERY or TEL, quantified from the intensities of the toeprint bands generated by the ribosomes stalled at codon 10 of the templates. Efficiency of stalling with ERY in each sample was set at 100%. Error bars show deviation from the mean in two independent experiments.



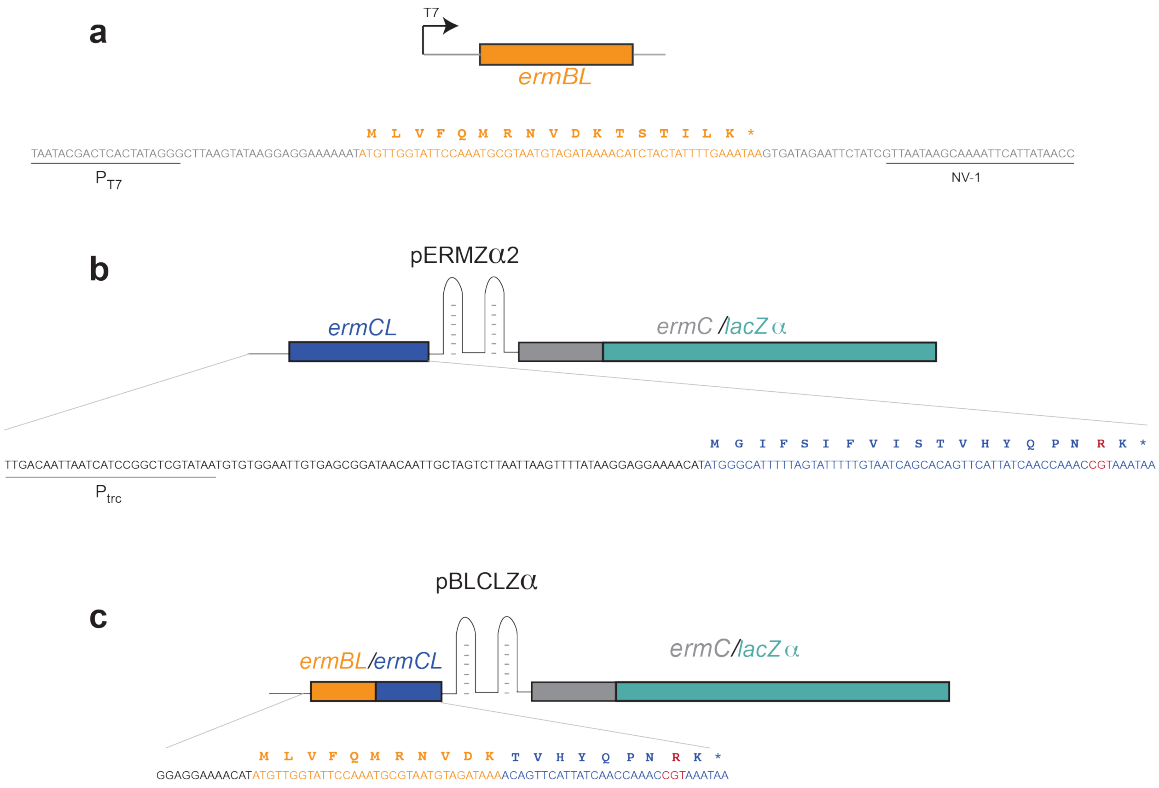
Supplementary Figure 3. The ErmBL residues critical for TEL-promoted stalling reside in the C-terminal segment of the stalled nascent peptide. (a) Alanine scanning mutagenesis reveals that the identity of Arg7, Val9, Asp10 and Lys11, but not that of Asn8 of ErmBL is critical for TEL-dependent stalling. Alanine substitution mutants of ErmBL were translated in a cell-free system in the absence (only wt) or in the presence of TEL. Translation arrest was monitored by toeprinting. The arrow indicates the site of TEL-induced ribosome stalling. The Asp10 codon, located in the P-site of the stalled ribosomes, and the encoded amino acid are boxed. The observation that the identities of Arg7, Val9, Asp10 and Lys9, but not of Asn8, are critical for TEL directed arrest, matches the results obtained previously with ERY-directed stalling of ErmBL [2]. (**b**) Bar graph of the relative stalling efficiency (%) with TEL, estimated from the intensities of the toeprint bands generated by the ribosomes stalled at codon 10 (indicated with an arrowhead) of the corresponding templates. Efficiency of stalling in the wt ErmBL template was set at 100%. Error bars show deviation from the mean in two independent experiments.



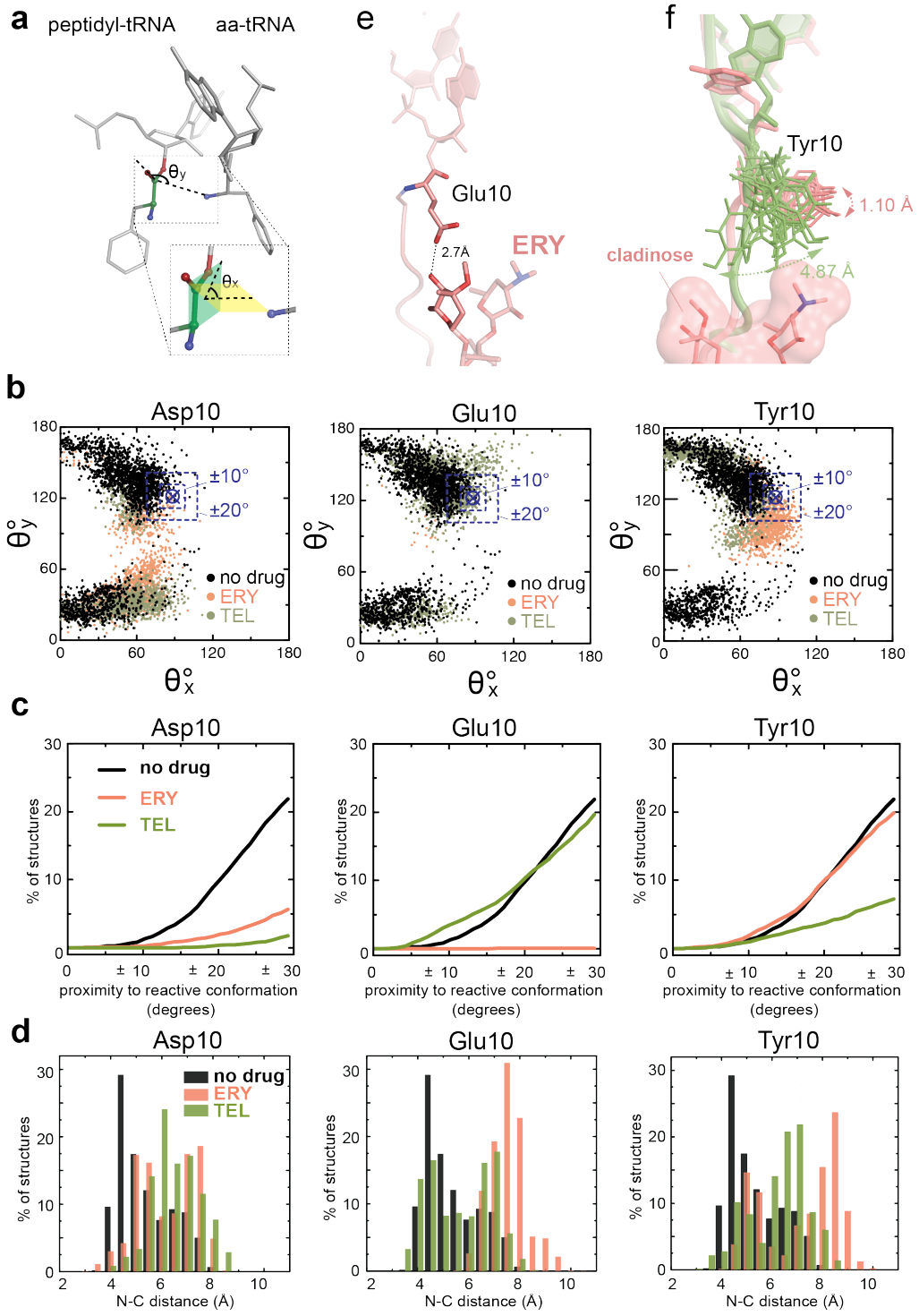
Supplementary Figure 4. Antibiotic mediated arrest in wt *ermBL* or its codon 7 mutants. (**a**) Cartoon representation of the stalled ribosome associated with ERY or TEL (represented by a star) with codon Asp10 of *ermBL* occupying its P-site (indicated with a red arrow). The mutagenized codon 7 of *ermBL* is indicated with 'X'. The Thr12 'catch' codon preceding the 'hungry' Ile 13 codon is marked with a gray arrowhead. (**b**) Examples of representative toeprinting analysis gels used to generate the bar graph shown in Fig. 2b of the main text. The toeprint band generated by ribosomes stalled at codon Asp10 because of the presence of ERY or TEL is marked with a red arrow. Ribosomes, which fail to stall at codon 10, are trapped at the subsequent codon Thr12 (indicated with a gray arrowhead) because presence of mupirocin in the reaction mixture depletes the system from charged Ile-tRNA.



Supplementary Figure 5. Antibiotic mediated arrest in wt *ermBL* or its codon 10 mutants. (a) Cartoon representation of the arrested ribosome with ERY or TEL (represented by a star), with codon 10 (shown by 'X') of *ermBL* occupying its P-site (red arrow). The Thr12 codon of *ermBL* is marked with a gray arrowhead. (**b**) Representative full-size toeprinting analysis gels used to generate the bar graph shown in Fig. 3b of the main text. The boxed areas represent the gel segments shown in Fig. 3c. The toeprint band representing ribosomes stalled at codon 10 of the templates because of the presence of ERY (E) or TEL (T) is marked with a red arrow. Ribosomes, which fail to stall at codon 10, are trapped at the subsequent codon Thr12 (indicated with a gray arrowhead) because presence of mupirocin in the reaction mixture depletes the system from charged Ile-tRNA. The reaction with template ErmBL(Ile10) contained borrelidin instead of mupirocin (to deplete the reaction from Thr-tRNA^{Thr}). (**c**) Bar graph of the stalling efficiency estimated by quantifying the relative intensities of the toeprint bands of codon 10 (red arrow) vs. codon 12 (gray arrow). Error bars show deviation from the mean in two independent experiments.



Supplementary Figure 6. The nucleotide sequences of the genes and amino acid sequences of the encoded proteins in the constructs used in this work. (**a**) The *ermBL* template used for toeprinting analysis. The T7 promoter region and site of annealing of the toeprinting primer NV-1 are underlined. (**b**) The sequence of the *ermCL* gene in the pERMZα2 reporter plasmid. The Ptrc promoter is underlined. The penultimate *ermCL* Lys codon (AAA) of the original pERMZα plasmid[1] was mutated to an Arg codon (CGT) in order to disrupt the slippery sequence and prevent the reporter induction via ketolide-induced frameshifting[3]. (**c**) The sequence of the pBLCLZα reporter containing the ten 5'-terminal codons of *ermBL* followed by nine 3'-terminal codons of *ermCL* with the mutated slippery sequence. *See also Supplementary Fig. 1*



Supplementary Figure 7. MD simulations provide insights into the mechanisms of peptide- and cofactor-dependent translation arrest. (**a**) The orientation of the donor and acceptor substrates in the peptidyl transferase active site of the elongating ribosome [4] (PDB accession number 4V5D). The definitions of angles θ_x and θ_y characterizing the direction of the nucleophilic attack [5,6] are indicated. In the crystal structure, the θ_x and θ_y

angles are equal to 88° and 122°, respectively. (**b**) and (**c)** The proximity of the structures along the MD simulation trajectories to the reactive conformation of the donor and acceptor substrates. (**b)** The θ_x and θ_y angles observed in snapshots during the simulation sampling are indicated by color dots (green for TEL, salmon for ERY and black for the 'no drug' wt complex). The point with the attack angles observed in the crystal structure 4V5D is indicated by a circled cross. The increasing size of boxes was drawn around that point and the fraction of all the simulation snapshots fitting within the box of a defined size were plotted in graphs shown in (**c)**. Boxes corresponding to ±10° and ±20° boundaries are shown for illustration. The plots are based only on the snapshots of the structures in which the distance between the attacking α-amino group nitrogen atom of the aminoacyl-tRNA and the carbonyl carbon atom of the ester bond of peptidyl-tRNA is ≤ 6Å. The percentage of the total structure satisfying this criterion were: ASP10 (no drug): 73%, ASP10(ERY): 46%, Asp10(TEL): 33%; Glu10(ERY): 0.5%; Glu10(TEL): 54%; Tyr10(ERY): 31%; Tyr10(TEL): 33%. (**d**) Distributions of the distances between the nucleophilic nitrogen of the amino acid in the A site and the electrophilic carbonyl carbon of the P site amino acid (N-C distance) from MD simulations. The first peak of N-C distance distribution of "no drug" system (6 Å) is used as the cutoff N-C distance in calculations used for generation of panels **b** and **c**. (**e**) The extended glutamate side chain may allow for a direct interaction between Glu10 of the ErmBL(Glu10) mutant peptide and the cladinose hydroxyl of ERY offering an explanation for 'ERY only' specific stalling. (**f**) The increased conformational freedom of Tyr10 in the ErmBL(Tyr10) mutant may allow the donor substrate to deviate from the productive conformation when cladinose-lacking TEL is bound. The arrows indicate the range of conformation motion of Tyr10 side chain in the TEL complex (green) or ERY complex (salmon); the radii of gyration calculated from the MD simulation snapshots are indicated. ERY molecule is shown as sticks and semi-translucent surface.

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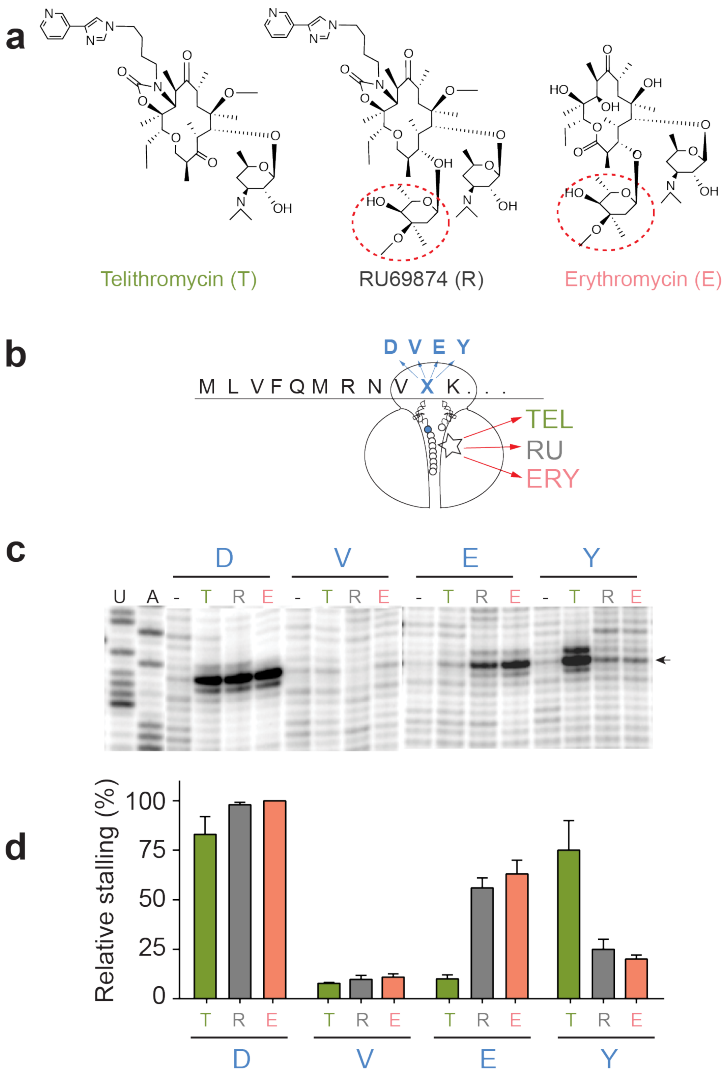
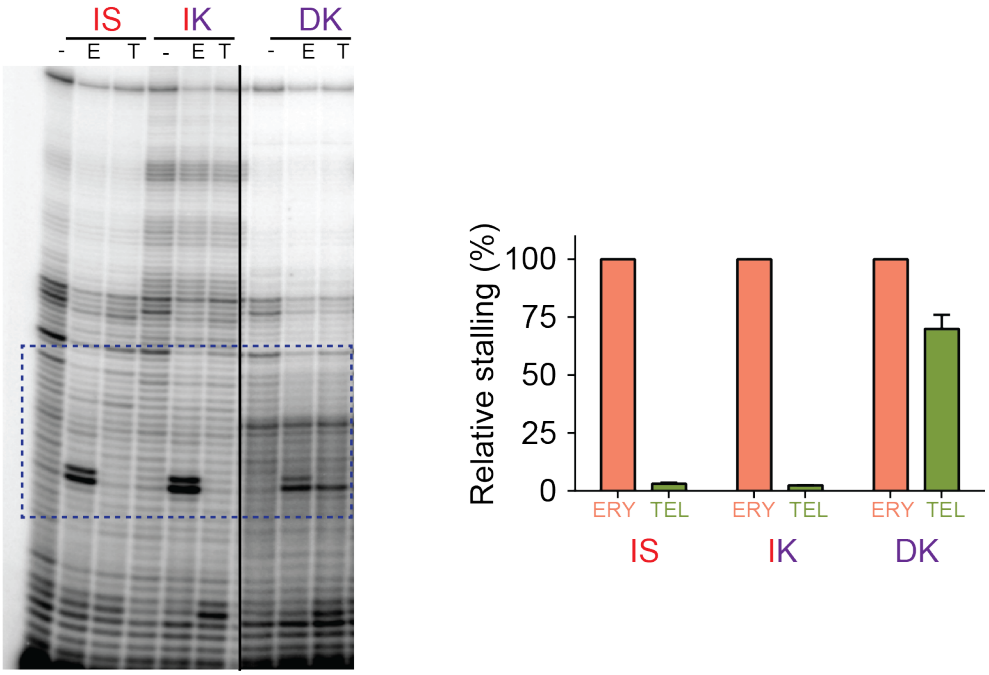


Supplementary Figure 8. The C-terminal residue of the ErmBL stalling peptide helps to distinguish macrolide antibiotics based on the nature of the C3 substituent in the macrolactone ring. (**a**) The structures of C3-keto drug TEL (T), its isostructural C3 cladinose analog RU69874 (R), and ERY (E). C3 cladinose sugars in the structures of RU69874 and ERY are circled**.** (**b**) Cartoon representing the four ErmBL codon 10 variants with distinct response to TEL and ERY (Fig. 3 in main text), tested for their ability to induce ribosome stalling in response to RU69874 (RU). (**c**) Toeprinting analysis showing translation arrest promoted by TEL (T), RU69874 (R), or ERY (E). (**d**) Bar graph of relative stalling (%) in the wt (D) or mutant *ermBL* templates (V, E, or Y) promoted by ERY (E), RU69874 (R), or TEL (T), estimated from the intensities of the toeprint bands generated by the ribosomes stalled at codon 10 (indicated with an arrowhead) of the corresponding templates. Efficiency of stalling with ERY in the wt ErmBL template was set at 100%. Error bars show deviation from the mean in two independent experiments.



Supplementary Figure 9. Antibiotic-directed translation arrest in wt *ermCL* or its codons 9 and 10 mutant variants. *Left:* Full-size gel of the toeprinting analysis of ermCL and its mutants (the segment of the gel shown in Fig. 4b of the main text is boxed). *Right*: Bar graph of the relative stalling efficiency (%) with ERY or TEL, quantified from the intensities of the toeprint bands generated by the ribosomes stalled at codon 9 of the templates. Efficiency of stalling with ERY at the wt (IS) or mutant templates (IK or DK) was set at 100%. Error bars show deviation from the mean in two independent experiments.

Supplementary Table 1: DNA oligonucleotides used in this study

Primer Name	Primer sequence
ermCL18(AAA:CGT)	CAGCACAGTTCATTATCAACCAAACCGTAAATAAGTGGTTATAATGAATCGTT
ermCL (IS:DK)	TAAGGAGGAAAACATATGGGCATTTTTAGTATTTTTGTAGATAAGACAGTTCATTATCAAC CAAACCGTAAATAAGTG
T7-ermCL	ATTAATACGACTCACTATAGGGATATAAGGAGGAAAACATATGGGCATTTTT
NV1	GGTTATAATGAATTTTGCTTATTAAC
T7	TAATACGACTCACTATAGGG
ptac-ermBL	GTGAGAGGAGGAAAACATATGTTGGTATTCCAAATGCGTAATGTAGATAAAACAATTACT ATTTT
Ptac	ACGGATCCTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGAGGAGGAAA ACATATG
ermBL-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCGTAATGTAGATAAAACAATTACTATTTT
ermBL-R	GGTTATAATGAATTTTGCTTATTAACGATAGAATTCTATCACTTATTTCAAAATAGTAATT GTTTTATCTACATT
ermBL(A7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GGCAAATGTAGATAAAACAATTACTATTTT
ermBL(N7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GAATAATGTAGATAAAACAATTACTATTTT
ermBL(D7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GGATAATGTAGATAAAACAATTACTATTTT
ermBL(C7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GTGTAATGTAGATAAAACAATTACTATTTT
ermBL(G7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GGGCAATGTAGATAAAACAATTACTATTTT
ermBL(Q7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCAGAATGTAGATAAAACAATTACTATTTT
ermBL(E7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GGAGAATGTAGATAAAACAATTACTATTTT
ermBL(H7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCACAATGTAGATAAAACAATTACTATTTT
ermBL(I7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GATTAATGTAGATAAAACAATTACTATTTT
ermBL(L7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GTTGAATGTAGATAAAACAATTACTATTTT
ermBL(K7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GAAGAATGTAGATAAAACAATTACTATTTT
ermBL(M7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GATGAATGTAGATAAAACAATTACTATTTT
ermBL(F7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GTTTAATGTAGATAAAACAATTACTATTTT
ermBL(P7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCCTAATGTAGATAAAACAATTACTATTTT
ermBL(S7)F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GTCTAATGTAGATAAAACAATTACTATTTT
ermBL(T7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GACAAATGTAGATAAAACAATTACTATTTT
ermBL(Y7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GTACAATGTAGATAAAACAATTACTATTTT
ermBL(W7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GTGGAATGTAGATAAAACAATTACTATTTT
ermBL(V7)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GGTAAATGTAGATAAAACAATTACTATTTT
ermBL(A10)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCGTAATGTAGCCAAAACAATTACTATTTT
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ermBL(C10)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCGTAATGTATGTAAAACAATTACTATTTT
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ermBL(G10)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCGTAATGTAGGCAAAACAATTACTATTTT
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ermBL(T10)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCGTAATGTAACGAAAACAATTACTATTTT
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ermBL(Y10)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCGTAATGTATACAAAACAATTACTATTTT
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ermBL(V10)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCAAAT GCGTAATGTAGTGAAAACAATTACTATTTT
ermBL(V10)-R	GGTTATAATGAATTTTGCTTATTAACGATAGAATTCTATCACTTATTTCAAAATAGTAATT GTTTTCACTACATT
ermCL-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGGGCATTTTTAGTAT TTTTGTAATC
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ermCL(D9)-F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGGGCATTTTTAGTAT TTTTGTAGAT
ermCL(D9,K10)-R	GGTTATAATGAATTTTGCTTATTAACGATAGAATTCTATCACTTAATGAACTGTTTTATCTA CAAAAATACTAAAAATG
ermCL(D9,S10)-R	GGTTATAATGAATTTTGCTTATTAACGATAGAATTCTATCACTTAATGAACTGTGCTATCT ACAAAAATACTAAAAATG
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BL(Y:V)-CL	TATTCCAAATGCGTAATGTAGTGAAAACAGTTCATTATCAACC

Supplementary Information References

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