

Title: Selective inhibition of human translation termination by a drug-like compound

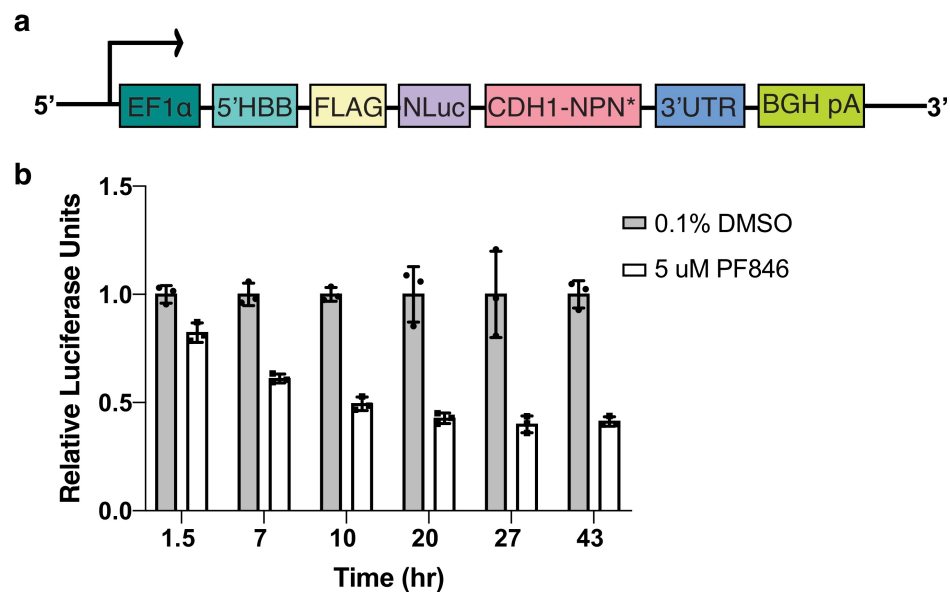
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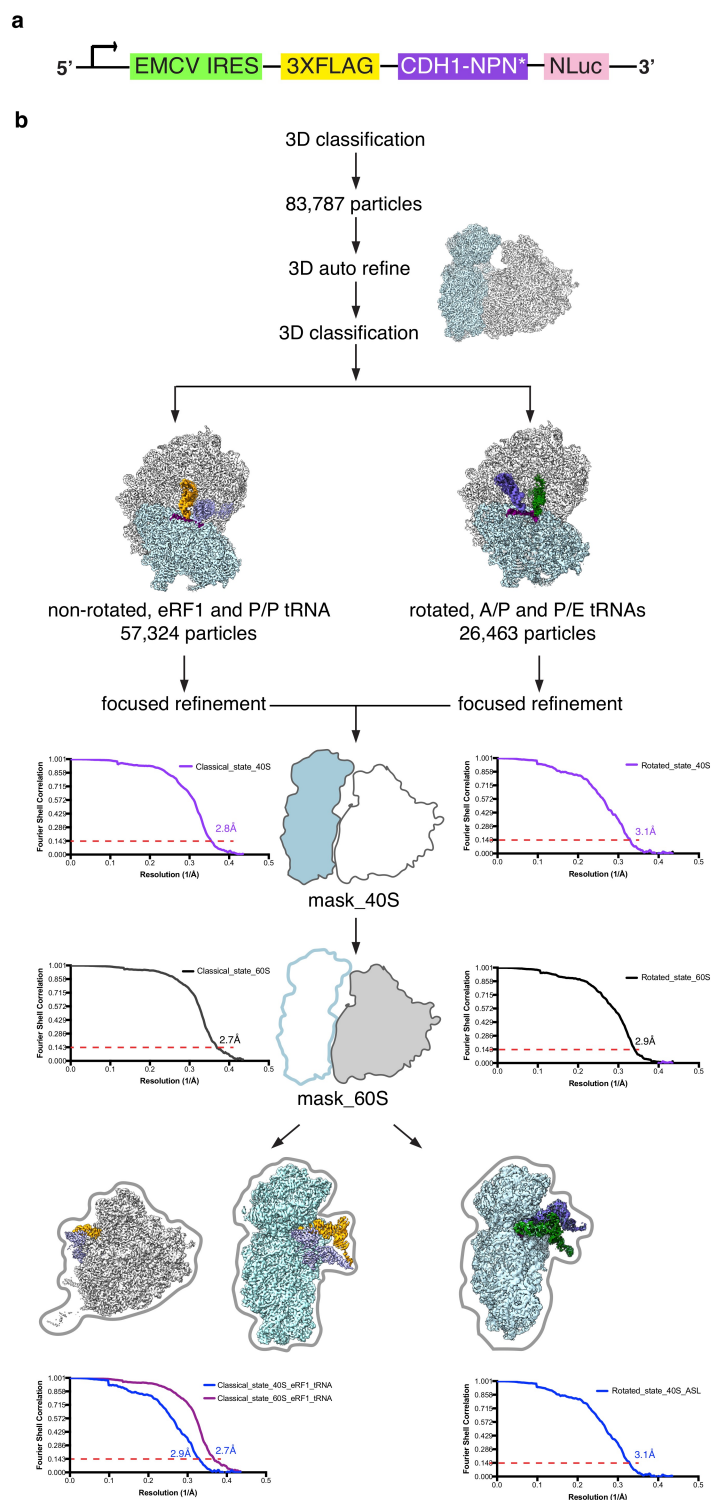
Supplementary Information



Supplementary Fig. 1 | Inhibition of translation termination by PF846 in cells. (a)

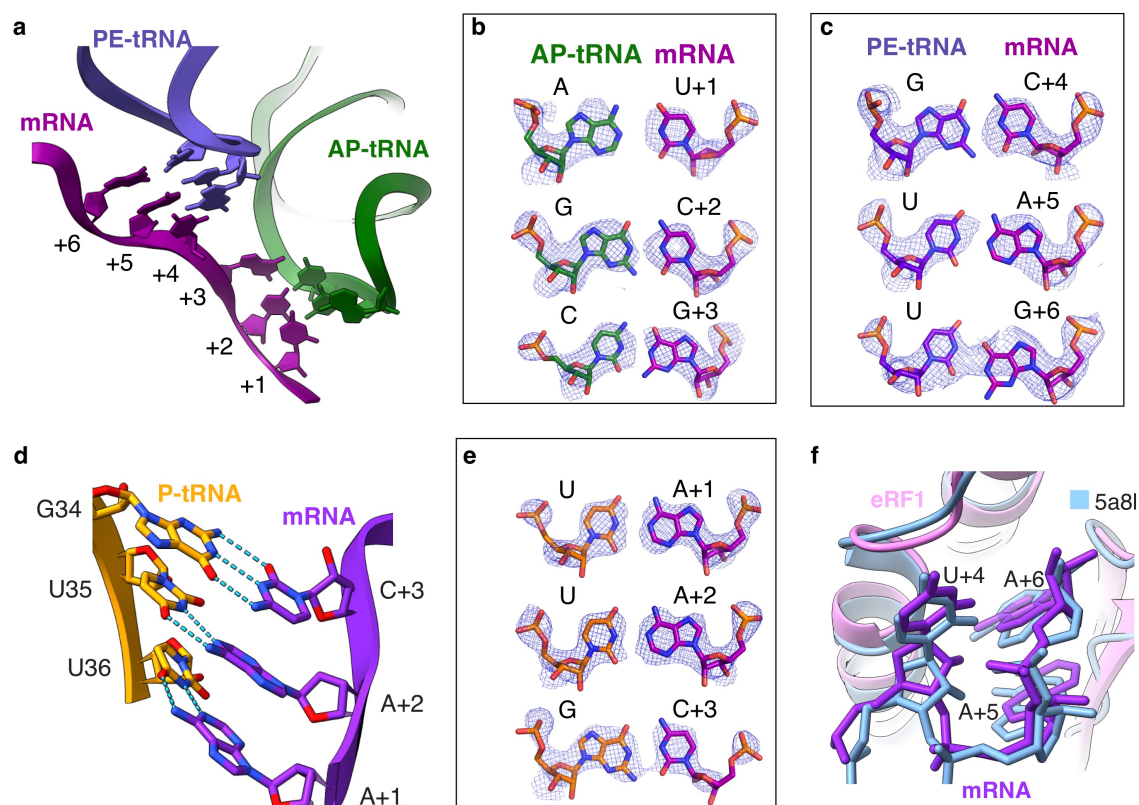
Schematic representation of the CDH1-NPN* lentiviral construct used for making stable cell lines. **(b)** Luciferase reporter assays using the CDH1-NPN* cell line at different time points after treatments with 0.1% DMSO (grey bars) or 5 μM PF846 (white bars). Bars show mean $\pm$ s.d., $n = 3$ independent experiments. Source data for **(b)** is available in

Supplementary Data Set 1.



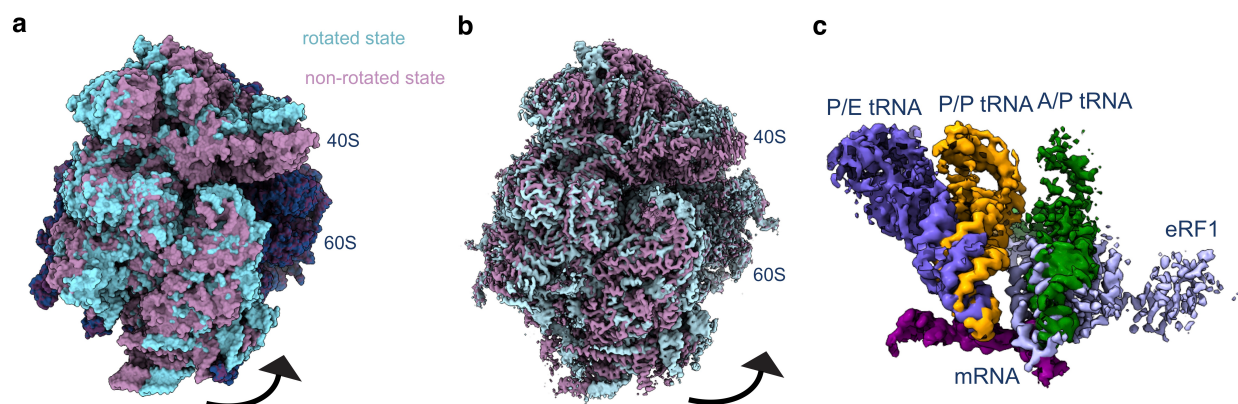
Supplementary Fig. 2 | Cryo-EM data processing of PF846-stalled termination complexes. (a) Schematic representation of the DNA construct used to prepare PF846-

stalled termination complexes. **(b)** Cryo-EM data processing workflow. Particle numbers at different steps are indicated. Two main populations were obtained after 3D classification. For the non-rotated state, focused refinements involved masking of the 40S subunit or 60S subunit individually, or the 40S subunit with eRF1, mRNA and tRNA, or the 60S subunit with eRF1 and tRNA. For the rotated state, focused refinements involved masking of the 40S subunit or 60S subunit individually, or the 40S subunit with mRNA and tRNA ASLs (anticodon stem loops). Final FSC curves of stalled RNCs in the non-rotated state and rotated state are presented, with the “gold standard” value of 0.143 used to define the resolution.

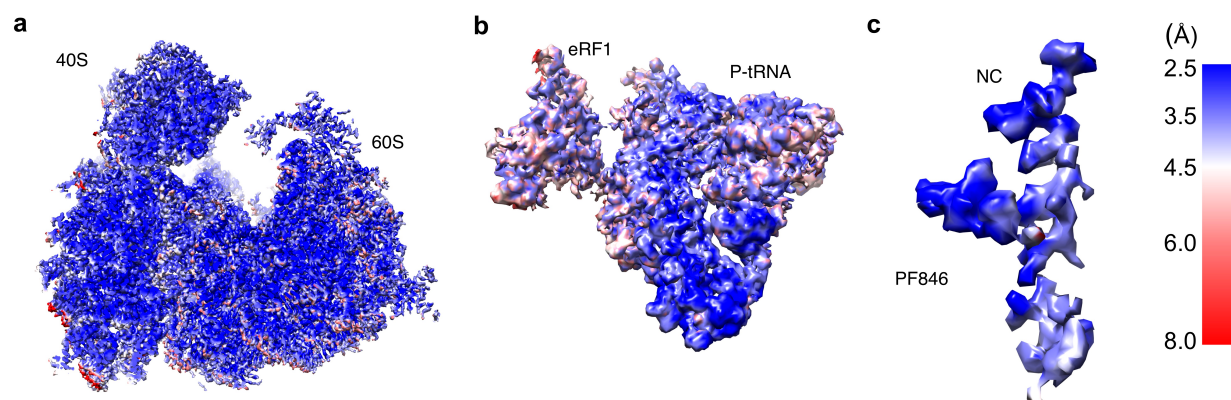


Supplementary Fig. 3 | Analysis of the mRNA codon-tRNA anticodon stem loop

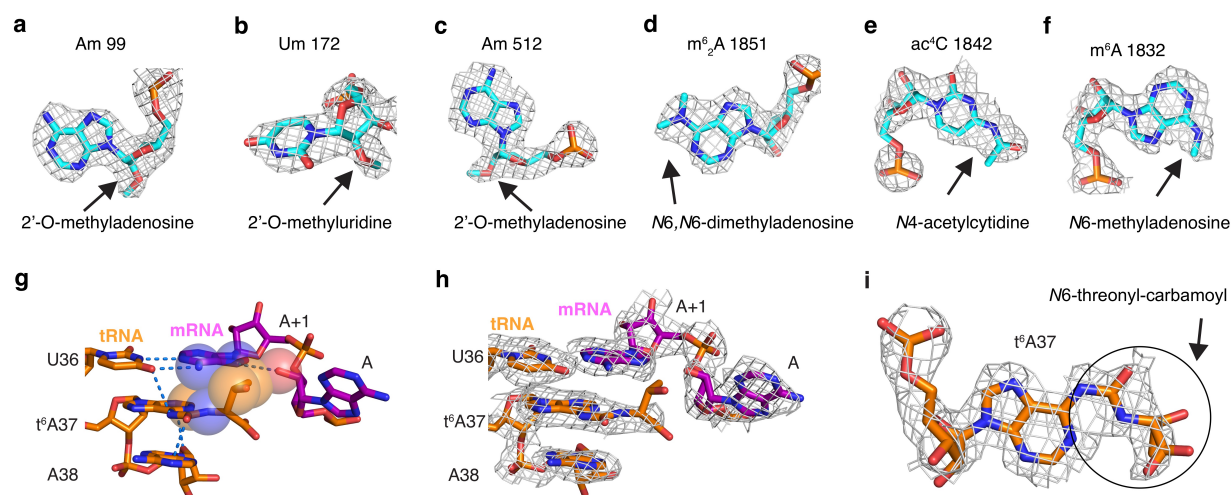
(ASL) interactions. (a) Model of the ASLs from the rotated RNC, with mRNA colored in magenta, P/E-site tRNA in dark purple and A/P-site tRNA in green. (b-c) Models of the base pairs between A/P-site tRNA anticodon nucleotides and mRNA codon nucleotides. Note that the individual nucleotides cannot be modeled with either pyrimidine or purine bases. The base pairs were refined into the cryo-EM density of the rotated RNC using Phenix. (d) Model of the P-site tRNA ASL (orange) from the non-rotated state RNC, with mRNA in magenta. The dashed lines indicate base pairing interactions. (e) Cryo-EM density of the P-site tRNA and mRNA in the non-rotated state, colored as in (d). (f) Superposition of the UAA stop codon with a previously-reported human termination structure (light blue) ¹¹.



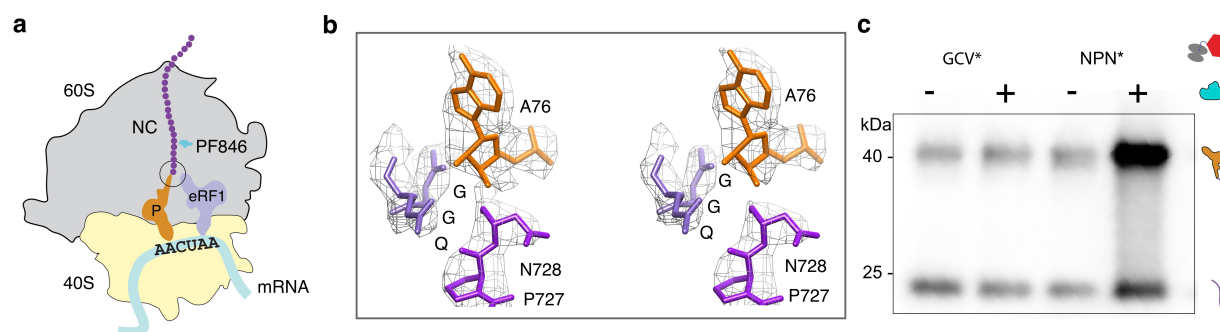
Supplementary Fig. 4 | Comparison of the two different states of PF846-stalled termination RNCs. (a) Comparison of the atomic model (shown with surface in ChimeraX⁶⁹) of the 40S subunit in the rotated state (light cyan) with the non-rotated state (dark purple). Alignments were done using the model or map with 60S subunit as the reference. (b) Comparison of the cryo-EM density map of the 40S subunit in the rotated state (light cyan) with the non-rotated state (dark purple). (c) Comparisons of the ribosomal A, P, and E sites in both structural models, with A/P-site tRNA (dark green) and P/E-site tRNA (purple) from the rotated state; eRF1 (slate blue) and P/P-site tRNA (orange) from the non-rotated state.



Supplementary Fig. 5 | Local resolution of cryo-EM maps of PF846-stalled termination RNCs. (a-c) Local resolution estimation of the non-rotated RNC with (a) 40S subunit and 60S subunit, (b) 40S subunit plus tRNA plus eRF1 and (c) NC plus PF846. Resolution scale in angstroms (Å) is shown to the right.

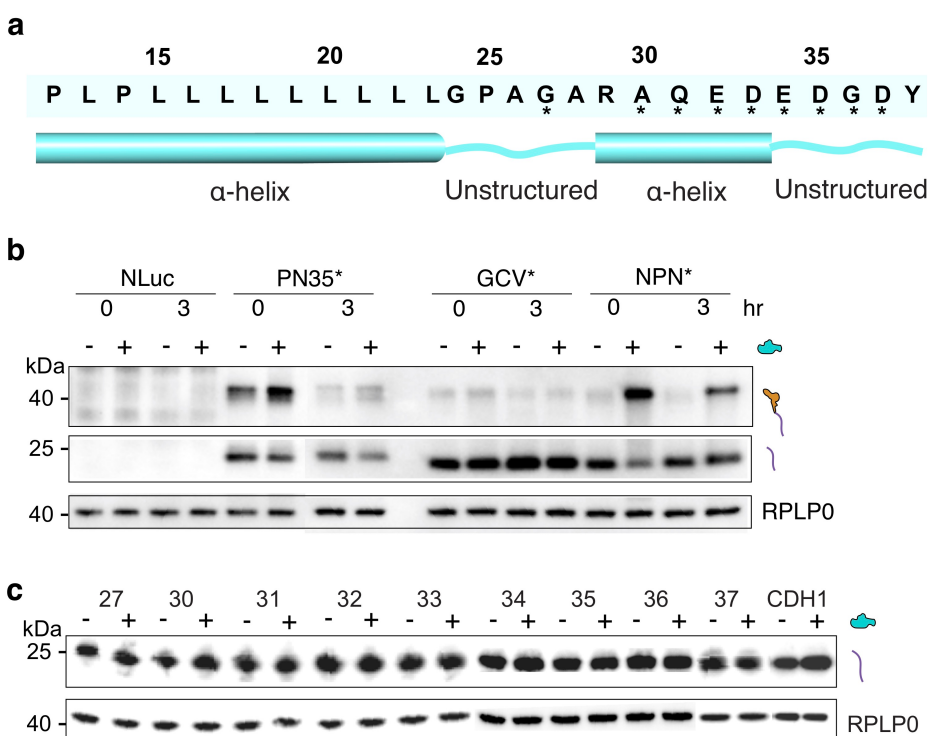


Supplementary Fig. 6 | Representative rRNA and tRNA modifications from the non-rotated state. (a-c) Representative 18S rRNA modifications found in the non-rotated RNC, observed in mass spectrometry experiments¹⁶ but not in previous cryo-EM structures. (d-f) Representative 18S rRNA modifications observed in this study. (g) Interactions between mRNA codon nucleotides A and A+1 (magenta) with P-site tRNA nucleotides 36-38 (orange). Hydrogen bonds are indicated with dashed lines and van der Waals radii are shown with spheres. (h) Density for model shown in (g). (i) Model and cryo-EM density for *N*6-threonyl-carbamoyl adenosine at nucleotide 37 (*t*⁶A37), a universal tRNA modification of almost all tRNAs decoding ANN codons (N = A,U,C or G)¹⁷. The *N*-threonylcarbamoyl group is highlighted with a circle.



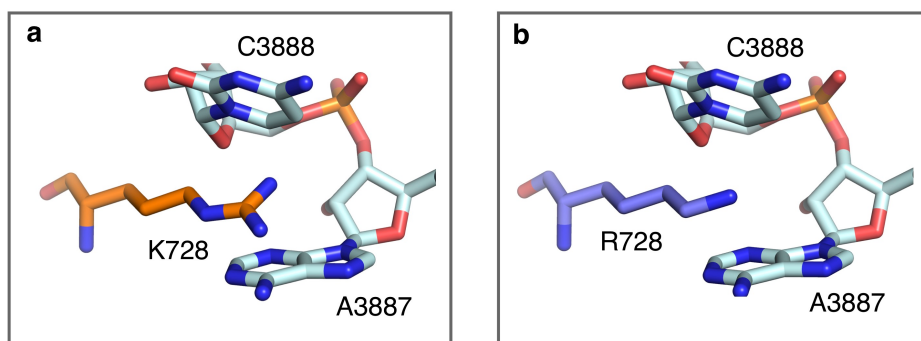
Supplementary Fig. 7 | Analysis of NC-tRNA in PF846 stalled CDH1-NPN*

complex. (a) RNC model for PF846-stalled ribosome nascent chain complex, with ester bond region indicated with a circle. (b) Zoom in view of the ester bond region with low (left) and high (right) contour levels. The model for the GGQ motif of eRF1, A76 of P-site tRNA and the C terminus of the nascent chain is shown, with mesh indicating the cryo-EM density. A single threshold is used in visualizing the density map for different ligands in each panel. (c) Affinity purification of CDH1-GCV* and CDH1-NPN* in the absence (–) or presence (+) of PF846. Pelleted RNCs were loaded on the gel with the same volume. Experiment shown in (c) was repeated 3 times independently with similar results. Uncropped gel image for (c) is available in **Supplementary Data Set 3**.

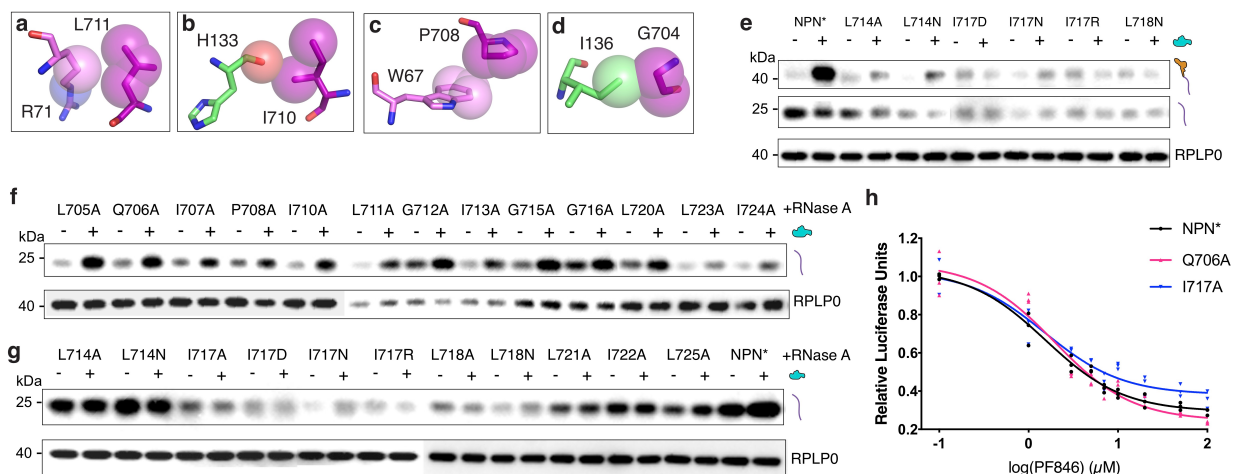


Supplementary Fig. 8 | Analysis of PCSK9-derived nascent chains in PF846-stalled termination complexes *in vitro*. (a) Secondary structure prediction of the PCSK9 nascent chain sequence required for PF846-dependent stalling of elongation, using Phyre2⁶⁷, with α -helices represented by cylinders and unstructured regions as curved lines. The stalling sequence of PCSK9 is shown. A break is observed from G24 to A28 which would prevent the PCSK9 sequence from forming a complete α -helix as observed for the CDH1-NPN sequence. Asterisks indicate the beginning amino acid for NPN* replacements used in translation assays. (b) Time course analysis of pelleted RNCs assembled with different nascent chains from *in vitro* translation reactions in the presence (+) or absence (–) of 50 μ M PF846. The positions of tRNA-bound and free nascent chains are shown, with RPLP0 serving as a loading control. (c) Western blot

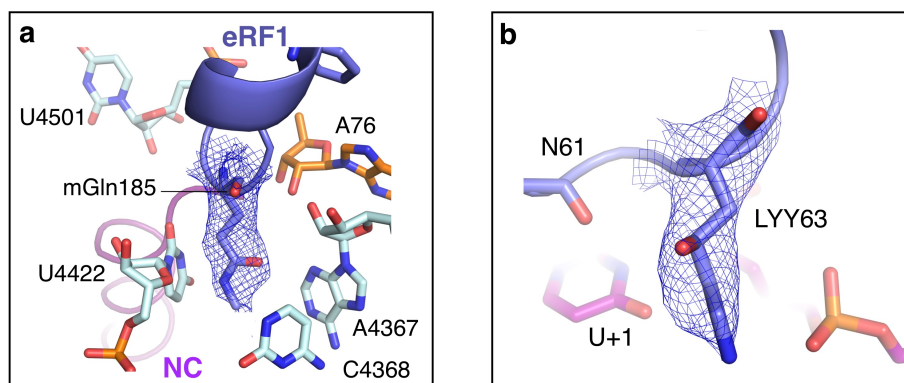
showing the effects of NPN* insertions at different positions in the PCSK9 nascent chain. *In vitro* translation reactions with DMSO (–) or with 50 μ M PF846 (+) are shown. Samples were treated with RNase A, and the stalled PCSK9 nascent chains were detected by blotting with an anti-FLAG antibody, with RPLP0 serving as a loading control. Experiments shown in (b-c) were repeated 3 times independently with similar results. Uncropped gel images for (b-c) are available in **Supplementary Data Set 3** and (d) is available in **Supplementary Data Set 4**.



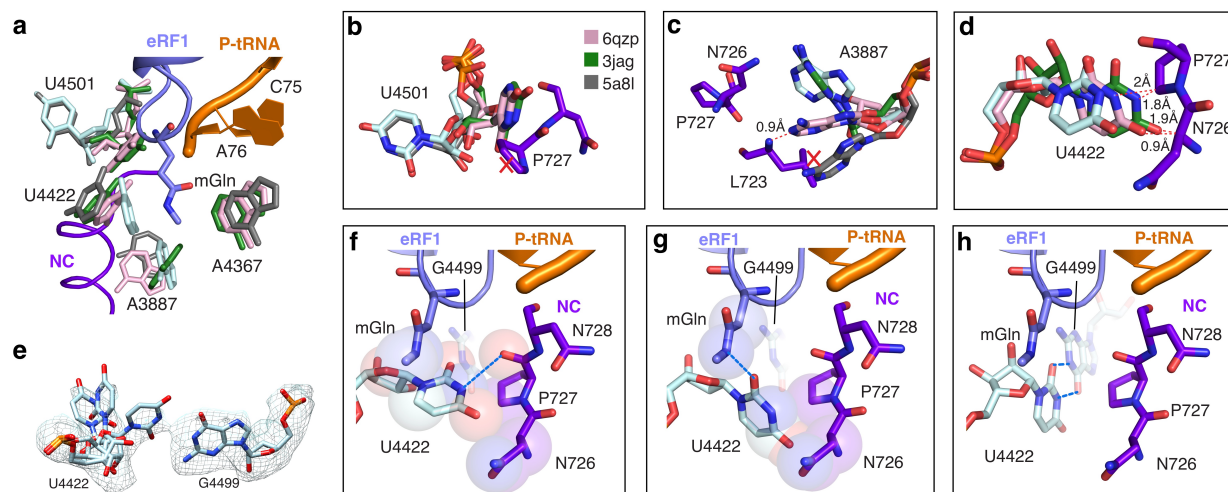
Supplementary Fig. 9 | Models of different amino acids at the CDH1-NPN C-terminal position 728. (a-b) The pocket formed by 28S rRNA nucleotides A3887 and C3888 can accommodate different sizes of amino acids, with Lys (K728 in **a**) and Arg (R728 in **b**) as examples.



Supplementary Fig. 10 | Effects of mutations in the CDH1-NPN nascent chain on PF846-dependent inhibition of termination. (a-d) Interactions of ribosomal protein uL22 (light green) and uL4 (hot pink) with the nascent chain, with van der Waals surfaces shown. (e) Western blots of FLAG-tagged CDH1-NPN nascent chains containing single mutations, from *in vitro* translation reactions in the presence (+) or absence (–) of 50 μ M PF846. The positions of tRNA-bound and free nascent chains are shown, with RPLP0 serving as a loading control. RNCs were assembled as in **Figure 3e**. (f-g) Western blots of CDH1-NPN nascent chains containing single mutations after treatment with RNase A. (h) IC₅₀ values for PF846-dependent inhibition of translation termination, using stable cell lines expressing the CDH1-NPN* nascent chain (black dots) or NCs with mutations Q706A (red dots) and I717A (blue dots). Data in (h) show mean $\pm$ s.d., $n = 3$ independent experiments. Experiment shown in (e-g) was repeated 3 times independently with similar results. Source data for (h) are available in **Supplementary Data Set 1**. Uncropped gel images for (e) are available in **Supplementary Data Set 2** and (f-g) are available in **Supplementary Data Set 5**.

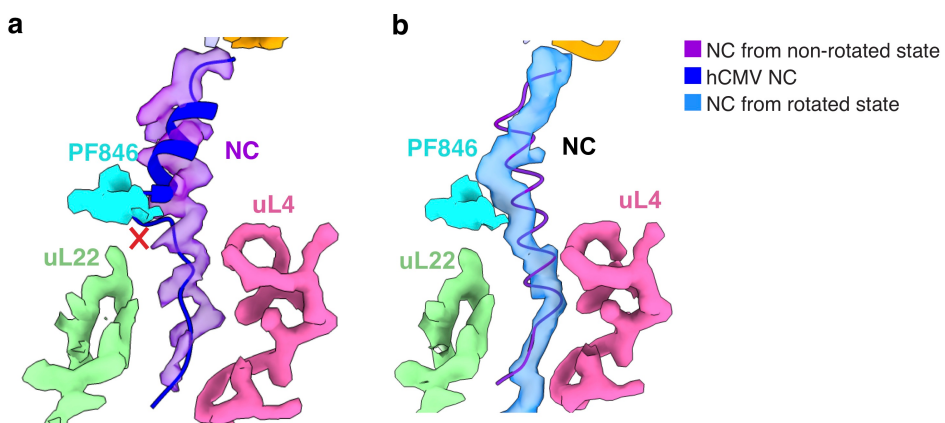


Supplementary Fig. 11 | Posttranslational modifications of eRF1 observed in the cryo-EM density map. (a) Close-up view of the GGQ motif within the PTC. Cryo-EM density for the methylated Gln (mGln185, slate blue) of the GGQ motif, positioned next to A76 of P-site tRNA and surrounded by PTC rRNAs, is represented with mesh. The map was sharpened with a B-factor of $-40 \text{ \AA}^2$. (b) The cryo-EM density for the C4 hydroxylysine 63 (LYY63) is shown in mesh. The map was sharpened with a B-factor of $-40 \text{ \AA}^2$.



Supplementary Fig. 12 | Conformation of the PTC in eukaryotic ribosome

termination complexes. (a) Overall alignment of the PTC from different eukaryotic ribosome structures (6qzp¹⁵ colored in pink is an apo-80S ribosome, 3jag¹⁰ colored in forest green is a termination complex with tRNA and eRF1, and 5a8l¹¹ colored in grey is the hCMV stalled translation termination complex). Positions of the eRF1 GGQ motif (slate blue), the P-site tRNA (orange) and nascent chain (purple) are shown. (b-d) Positions of (b) U4501, (c) A3887 and (d) U4422 aligned within the PTC. Potential steric clashes are highlighted with a red “X” or with labeled atomic distances. (e) Cryo-EM density for U4422 and G4499, with U4422 modeled in three different conformations based on the observed density. (f) The major conformation of U4422 observed in the cryo-EM density, which makes multiple interactions with the nascent chain and mGln185 of eRF1. Dashed lines indicate hydrogen bonds and spheres represent van der Waals radii. (g) Conformation showing U4422 turned outward from the nascent chain, with H-bonds and van der Waals radii as in (g). (h) Conformation showing U4422 flipped inward to base pair with G4499.



Supplementary Fig. 13 | Comparisons of NCs within ribosomes stalled at termination. (a) Superposition of the hCMV nascent chain model (blue, PDB: 5a8l) ¹¹ with the non-rotated CDH1-NPN RNC stalled by PF846. The potential steric clash of the hCMV NC with PF846 is indicated. (b) Superposition of the CDH1-NPN NC model from the non-rotated RNC with the cryo-EM density of the rotated RNC.

Supplementary Table 1 | DNA primers used for NC mutagenesis in this study.

Primers for NC mutations	Sequence (5'-3') with mutations in CAPS
P700A- Forward	gcacagGCTgtcgaagcaggattgc
P700A-Reverse	tgcttcgacAGCctgtgccttctacagacg
V701A- Forward	cacagcctGCTgaagcaggattgcaaattcct
V701A-Reverse	aatcctgcttcAGCaggctgtgccttctacaga
E702A- Forward	agcctgtcGCTgcaggattgcaaattcctgcc
E702A-Reverse	caatcctgcAGCgacaggctgtgccttcc
L705A-Forward	aagcaggaGCTcaaattcctgccattctgg
L705A-Reverse	aggaatttgAGCtctgtctcgacaggctg
Q706Q-Forward	caggattgCGAattcctgccattctgggga
Q706Q-Reverse	ggcaggaatCTGcaatcctgtctcgacagg
I707A-Forward	gattgcaaGCTcctgccattctggggattc
I707A-Reverse	aatggcaggAGCttgcaatcctgtctcgac
P708A-Forward	tgcaaattGCTgccattctggggattctt
P708R-Reverse	agaatggcAGCaatttgcaatcctgtctcg
I710A-Forward	ttcctgccGCTctggggattcttggaggaatt
I710A-Reverse	atccccagAGCggcaggaatttgcaatcctgc
L711A-Forward	ctgccattGCTgggattcttggaggaattctt
L711A-Reverse	caagaatcccAGCaatggcaggaatttgcaatcc
G712A-Forward	ccattctgGCTattcttggaggaattcttg
G712A-Reverse	ccaagaatAGCcagaatggcaggaatttgc
I713A-Forward	ttctggggGCTcttggaggaattcttgcttg
I713A-Reverse	cctccaagAGCccccagaatggcaggaatttg
L714N-Forward	tggggattAATggaggaattcttgcttgcta
L714N-Reverse	ttcctccATTaatccccagaatggcaggaa
L714A-Forward	tggggattGCTggaggaattcttgcttgcta
L714A-Reverse	ttcctccAGCaatccccagaatggcaggaa
G715A-Forward	ggattcttGCTggaattcttgcttgctaa
G715A-Reverse	agaattccAGCaagaatccccagaatggca
G716V-Forward	ttcttgaGTCattcttgcttgctaattctg
G716V-Reverse	agcaagaatGACtccaagaatccccagaatg
I717A-Forward	cttggaggaGCTcttgcttgctaattctg
I717A-Reverse	aaagcaagAGCtctccaagaatccccaga
I717R-Forward	cttggaggaCGGcttgcttgctaattctg
I717R-Reverse	aaagcaagCCGtctccaagaatccccaga
L718A-Forward	gaggaattGCCgcttggctaattctgattctga
L718A-Reverse	agcaaagcGGCaattcctccaagaatccccaga

A719N-Forward	ggaattcttAATtgctaattctgattctgaat
A719N-Reverse	aattagcaaATTaagaattcctccaagaatc
L720A-Forward	ttcttgctGCTctaattctgattctgaatcca
L720A-Reverse	cagaattagAGCagcaagaattcctccaagaa
L721A-Forward	ttgctttgGCTattctgattctgaatccaaacta
L721A-Reverse	atcagaatAGCcaaagcaagaattcctccaag
I722A-Forward	ctttgctaGCTctgattctgaatccaaactaa
I722A-Reverse	gaatcagAGCtagcaaagcaagaattcctccaa
L723A-Forward	tgctaattGCTattctgaatccaaactaatttcttc
L723A-Reverse	ttcagaatAGCaattagcaaagcaagaattcctcc
I724A-Forward	ctaattctgGCTctgaatccaaactaatttcttc
I724A-Reverse	ggattcagAGCcagaattagcaaagcaagaatt
L725A-Forward	ttctgattGCTaatccaaactaatttctcgagg
L725A-Reverse	gtttggattAGCaatcagaattagcaaagcaagaa
Primers for PCSK9 NPN scan	
Primers	Sequence (5'-3')
Nluc-F	GTCTTCACACTCGAAGATTTCGTT
PCSK9_27NPN-Reverse	ATCTTCGAGTGTGAAGACGTCCTCgtcctcctgtagttgg attcgcgggaccag
PCSK9_30NPN-Reverse	ATCTTCGAGTGTGAAGACGTCCTCtagtttgattacggg cgcccgcgggga
PCSK9_31NPN-Reverse	ATCTTCGAGTGTGAAGACGTCtagtttgattcgacgggc gccccgcgga
PCSK9_32NPN-Reverse	ATCTTCGAGTGTGAAGACtagtttgattctgcgcacgggcg ccgc
PCSK9_33NPN-Reverse	ATCTTCGAGTGTGAAGACtagtttgattctcctgcgcacggg cgccc
PCSK9_34NPN-Reverse	ATCTTCGAGTGTGAAGACtagtttgattgtcctcctgcgcacg ggcgc
PCSK9_35NPN-Reverse	ATCTTCGAGTGTGAAGACtagtttgattCTCgtcctcctgcg cacgggc
PCSK9_36NPN-Reverse	ATCTTCGAGTGTGAAGACtagtttgattGTCCTCgtcctcc tgcgac
PCSK9_37NPN-Reverse	ATCTTCGAGTGTGAAGACtagtttgattGCCGTCCTCgt cctcctgcgcac

PCSK9_38NPN-Reverse	ATCTTCGAGTGTGAAGACtagtttggattGTCGCCGTCC TCgtcctcctgcgcacgg
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Supplementary Table 2 | Primers and gene blocks used for cell line construction.

Sequence motif	Sequence
NPN*	AATCCAAACTAA
GCV*	ggatgtgtctaa
gBlocks from IDT	
gBlock1: 5HBB_FLAG_Nluc	CCTCAAACAGACACCATGGACTACAAGGACGA CGACGACAAGATGGTCTTCACACTCGAAGATT TCGTTGGGGACTGGCGACAGACAGCCGGCTA CAACCTGGACCAAGTCCTTGAACAGGGAGGTG TGTCCAGTTTGTTCAGAATCTCGGGGTGTCC GTA ACTCCGATCCAAAGGATTGTCCTGAGCGG TGAAAATGGGCTGAAGATCGACATCCATGTCA TCATCCCGTATGAAGGTCTGAGCGGCGACCAA ATGGGCCAGATCGAAAAAATTTTAAAGGTGGT GTACCCTGTGGATGATCATCACTTTAAGGTGAT CCTGCACTATGGCACACTGGTAATCGACGGGG TTACGCCGAACATGATCGACTATTTCCGACGG CCGTATGAAGGCATCGCCGTGTTGACGGCAA AAAGATCACTGTAACAGGGACCCTGTGGAACG GCAACAAAATTATCGACGAGCGCCTGATCAAC CCCGACGGCTCCCTGCTGTTCCGAGTAACCAT CAACGGAGTGACCGGCTGGCGGCTGTGCGAA CGCATTCTGGCG

gBlock2: CDH1-NPN*_3'UTR_bGH	TCTGATGTGAATGACAACGCCCCCATACCAGA ACCTCGAACTATATTCTTCTGTGAGAGGAATCC AAAGCCTCAGGTCATAAACATCATTGATGCAGA CCTTCCTCCCAATACATCTCCCTTCACAGCAGA ACTAACACACGGGGCGAGTGCCAACTGGACCA TTCAGTACAACGACCCAACCCAAGAATCTATCA TTTTGAAGCCAAAGATGGCCTTAGAGGTGGGT GACTACAAAATCAATCTCAAGCTCATGGATAAC CAGAATAAAGACCAAGTGACCACCTTAGAGGT CAGCGTGTGTGACTGTGAAGGGGCGCTGGC GTCTGTAGGAAGGCACAGCCTGTCTGAAGCAG GATTGCAAATTCCTGCCATTCTGGGGATTCTTG GAGGAATTCTTGCTTTGCTAATTCTGATTCTGct gctcttgctgTTTCTTCGGAGGAGAGCGGTGGTCAA AGAGCCCTTACTGCCCCCAGAGGATGACACCC GGGACgggactcgagagaggcgggccccagacccatgtgctg ggaaatgcagaaatcacgttgctaCTCGAGcgactgtgccttcta gttgccagccatctgtgtttgcccctccccgtgccttcttgaccctg gaaggtgccactcccactgtccttcctaataaaatgaggaaattgca tcgcattgtctgagtaggtgtcattctattctggggggtgggggtggggc aggacagcaagggggaggattgggaagacaatagcaggcatgc tggggatgcggtgggctctatggCTCGAG
For NPN* stable cell line	
Primers	Sequence (5'-3')
1. Use gBlock1 as template to make fragment: FLAG (overlap 5'UTR of HBB) and NLuc	
Forward	CCTCAAACAGACACCCCATGGACTACAAGGAC GACGA
Reverse	CGCCAGAATGCGTTCGCACA
2. Use 1 as template to make fragment: HBB_FLAG_Nluc	
Forward	ACATTTGCTTCTGACACAACTGTGTTCCTAGC AACCTCAAACAGACACCATGGACT
Reverse	CGCCAGAATGCGTTCGCACA
3. Use gBlock2 as template to make fragment: Nluc_CDH1_bGH	
Nluc_CDH1_F	CTGTGCGAACGCATTCTGGCGTCTGATGTGAA TGACAACGCC
bGH_R	ATTGTCGACTCGAGCCATAGAGCCCAC
4. Fuse Fragments 2 and 3	

5. Use 4 as template to make fragment from 5HBB to bGH, with overlap with EF1	
EF1 α _5HBB_F	TTTCAGGTGTCGTGAGTCGAACATTTGCTTCTG ACACAACCTG
bGH_WPRE_R	ATCCAGAGGTTGATTGTCTGACTCGAGCCATAG AGCCCAC
6. Cut CD813 vector with Sal1	
7. Ligate fragment 5 with CD813 vector	
For GCV* stable cell line	
Primers	Sequence (5'-3')
Use NPN* construct as the template	
1. Fragment: 5HBB (overlap with EF1 α)_FLAG_Nluc_CDH1_GCV	
EF1 α _5HBB_F	TTTCAGGTGTCGTGAGTCGAACATTTGCTTCTG ACACAACCTG
Lenti_GCV_R	CACCGCTCTCCTCCGAAGAAAttagacacatccCAG AATCAGAATTAGCAA
2.Fragment: GCV_3UTR_bGH	
CDH1-730F	TTTCTTCGGAGGAGAGCGGTGGTCAA
bGH_WPRE_R	ATCCAGAGGTTGATTGTCTGACTCGAGCCATAG AGCCCAC
Fuse fragment 1 and 2 to CD813 vector	
Make construct for Q706A stable cell line	
Primers	Sequence (5'-3')
Use NPN* construct as the template	
1. Fragment: 5HBB (overlap with EF1 α)_FLAG_Nluc_CDH1_Q706A	
EF1 α _5HBB_F	TTTCAGGTGTCGTGAGTCGAACATTTGCTTCTG ACACAACCTG

Lenti_Q706A_R	CAGGAATagcCAATCCTGCTTCGACAGGCTG
2.Fragment: 706A_3UTR_bGH	
Lenti_Q706A_F	GCAGGATTGGCTATTCCTGCCATTCTGGGG
bGH_WPRE_R	ATCCAGAGGTTGATTGTCTGACTCGAGCCATAG AGCCCAC
Fuse fragment 1 and 2 to CD813 vector	
Make construct for L714A stable cell line	
Primers	Sequence (5'-3')
Use NPN* construct as the template	
1. Fragment: 5HBB (overlap with EF1α)_FLAG_Nluc_CDH1_Q70 6A	
EF1α_5HBB_F	TTTCAGGTGTCGTGAGTCGAACATTTGCTTCTG ACACAACCTG
Lenti_L714A_R	ttcctccAGCaatccccagaatggcaggaa
2.Fragment: 706A_3UTR_bGH	
Lenti_L714A_F	tggggattGCTggaggaattcttgctttgcta
bGH_WPRE_R	ATCCAGAGGTTGATTGTCTGACTCGAGCCATAG AGCCCAC
Fuse fragment 1 and 2 to CD813 vector	

Supplementary data set 1 | Source data for the bar graphs presented in the main text.

Supplementary data set 2 | The uncropped images for gels shown in Fig. 3e, Supplementary Fig. 10e and g.

Supplementary data set 3 | The uncropped images for gels shown in Fig. 2c, Supplementary Fig. 7c and 8b-c.

Supplementary data set 4 | The uncropped images for gels shown in Supplementary Fig. 8c.

Supplementary data set 5 | The uncropped images for gels shown in Supplementary Fig. 10f and Fig. 3e.