2023 CHEMISTRY OF THE YEAR

nature chemical biology

Article

https://doi.org/10.1038/s41589-022-01170-9

Velcrin-induced selective cleavage of $tRNA^{Leu}(TAA)$ by SLFN12 causes cancer cell death

Wenting Li

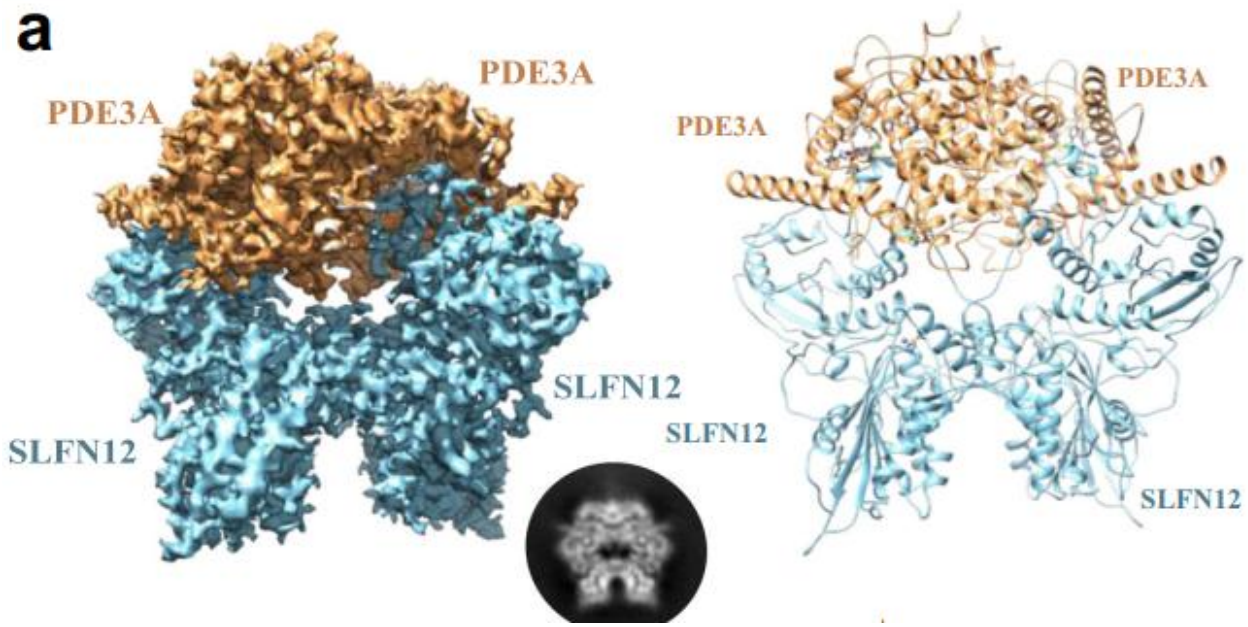
Jan. 14, 2023

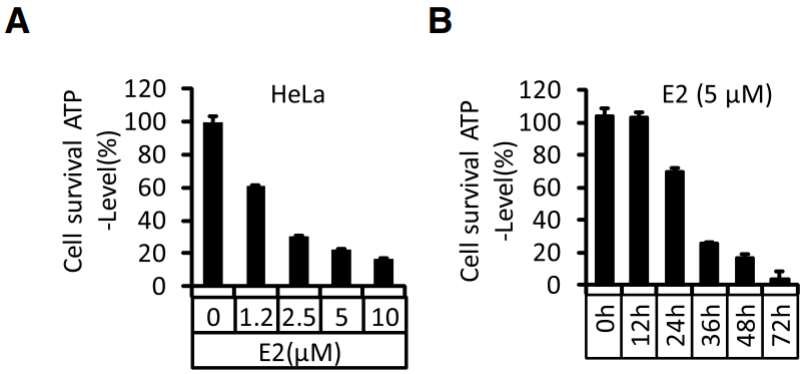
■ Background

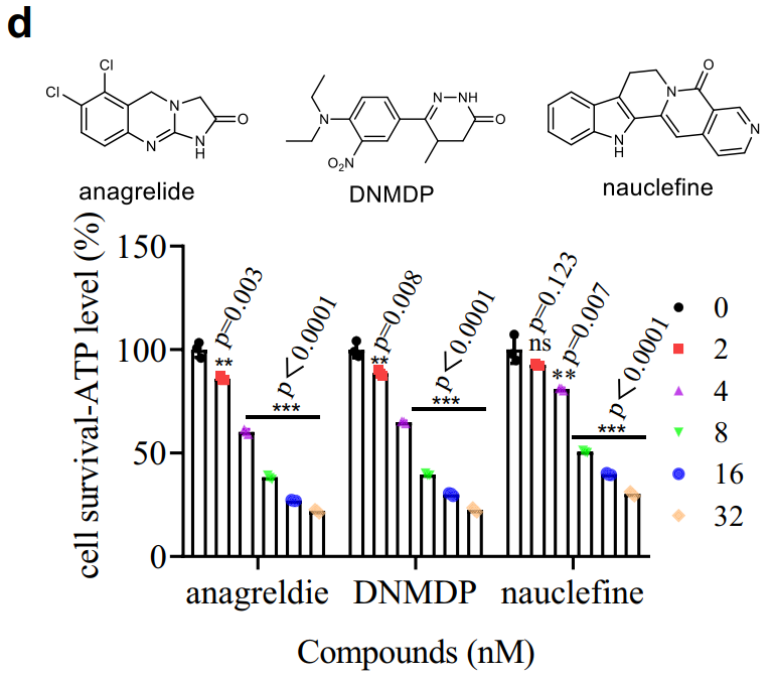
Velcrins family compounds:

PDE3A inhibitors, such as DNMDP;

Estradiol (Estradiol) and progesterone-related compounds.







■ Background

BIOCHEMISTRY
including biophysical chemistry & molecular biology

Article

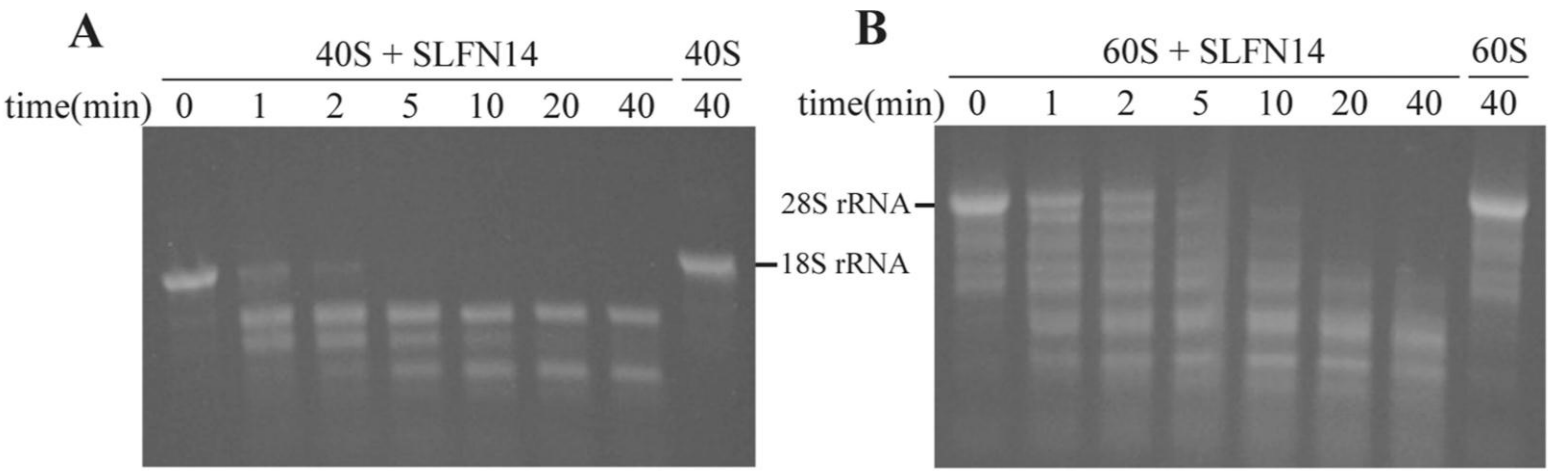
pubs.acs.org/biochemistry

Characterization of Novel Ribosome-Associated Endoribonuclease SLFN14 from Rabbit Reticulocytes

Vera P. Pisareva,*,† Ilham A. Muslimov,‡ Andrew Tcherepanov,‡ and Andrey V. Pisarev*,†

†Department of Cell Biology and ‡Department of Physiology and Pharmacology, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York 11203, United States

Supporting Information



SLFN14 binds ribosomes and ribosomal subunits and cleaves different types of RNA in a Mg^{2+}- and Mn^{2+}- dependent and ATP-independent manner.

■ Background

nature communications

Explore content ▾ About the journal ▾ Publish with us ▾

nature › nature communications › articles › article

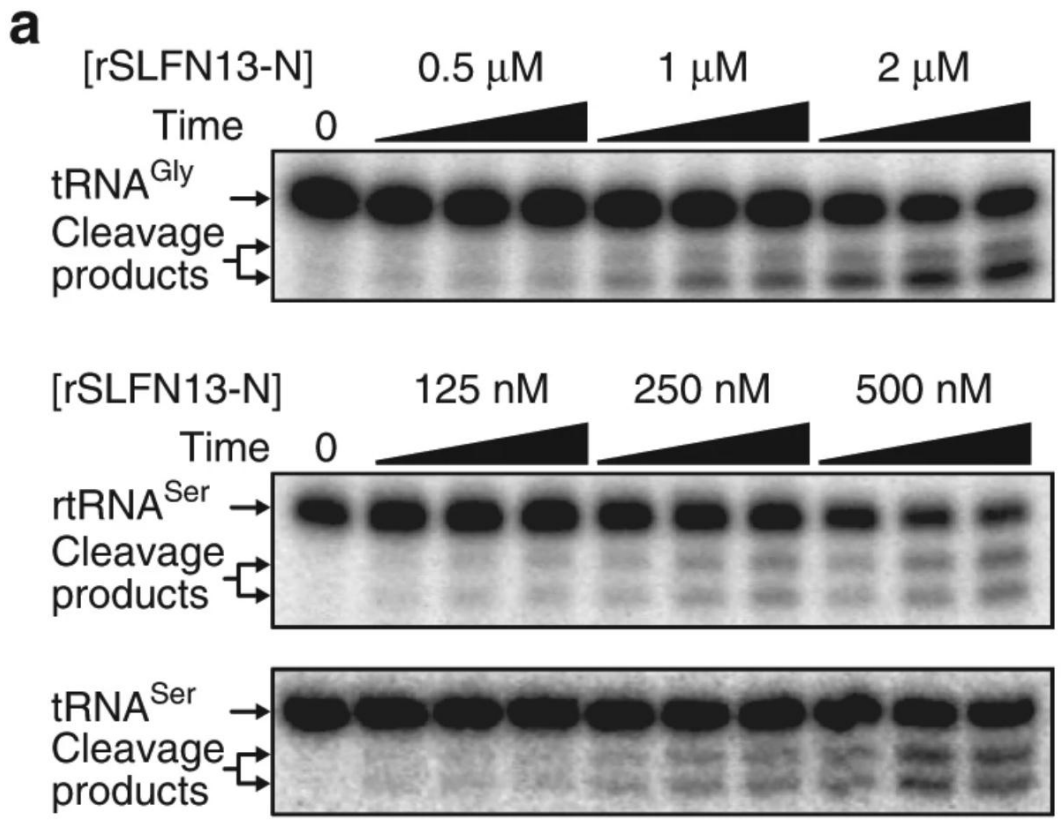
Article | Open Access | Published: 21 March 2018

Structure of Schlafen13 reveals a new class of tRNA/rRNA- targeting RNase engaged in translational control

Jin-Yu Yang, Xiang-Yu Deng, Yi-Sheng Li, Xian-Cai Ma, Jian-Xiong Feng, Bing Yu, Yang Chen, Yi-Ling Luo, Xi Wang, Mei-Ling Chen, Zhi-Xin Fang, Fu-Xiang Zheng, Yi-Ping Li, Qian Zhong, Tie-Bang Kang, Li-Bing Song, Rui-Hua Xu, Mu-Sheng Zeng, Wei Chen, Hui Zhang, Wei Xie ✉ & Song Gao ✉

Nature Communications **9**, Article number: 1165 (2018) | Cite this article

7569 Accesses | **50** Citations | **14** Altmetric | Metrics



SLFN13 N'-domain cleaves tRNA in vitro in a dose- and time-dependent manner

■ Background

nature communications

Explore content ⌄ About the journal ⌄ Publish with us ⌄

nature › nature communications › articles › article

Article | Open Access | Published: 16 July 2021

Structure of PDE3A-SLFN12 complex reveals requirements for activation of SLFN12 RNase

Colin W. Garvie, Xiaoyun Wu, Malvina Papanastasiou, Sooncheol Lee, James Fuller, Gavin R. Schnitzler, Steven W. Horner, Andrew Baker, Terry Zhang, James P. Mullahoo, Lindsay Westlake, Stephanie H. Hoyt, Marcus Toetzl, Matthew J. Ranaghan, Luc de Waal, Joseph McGaunn, Bethany Kaplan, Federica Piccioni, Xiaoping Yang, Martin Lange, Adrian Tersteegen, Donald Raymond, Timothy A. Lewis, Steven A. Carr, … Heidi Greulich ✉ + Show authors

Nature Communications **12**, Article number: 4375 (2021) | Cite this article

6728 Accesses | **14** Citations | **19** Altmetric | Metrics

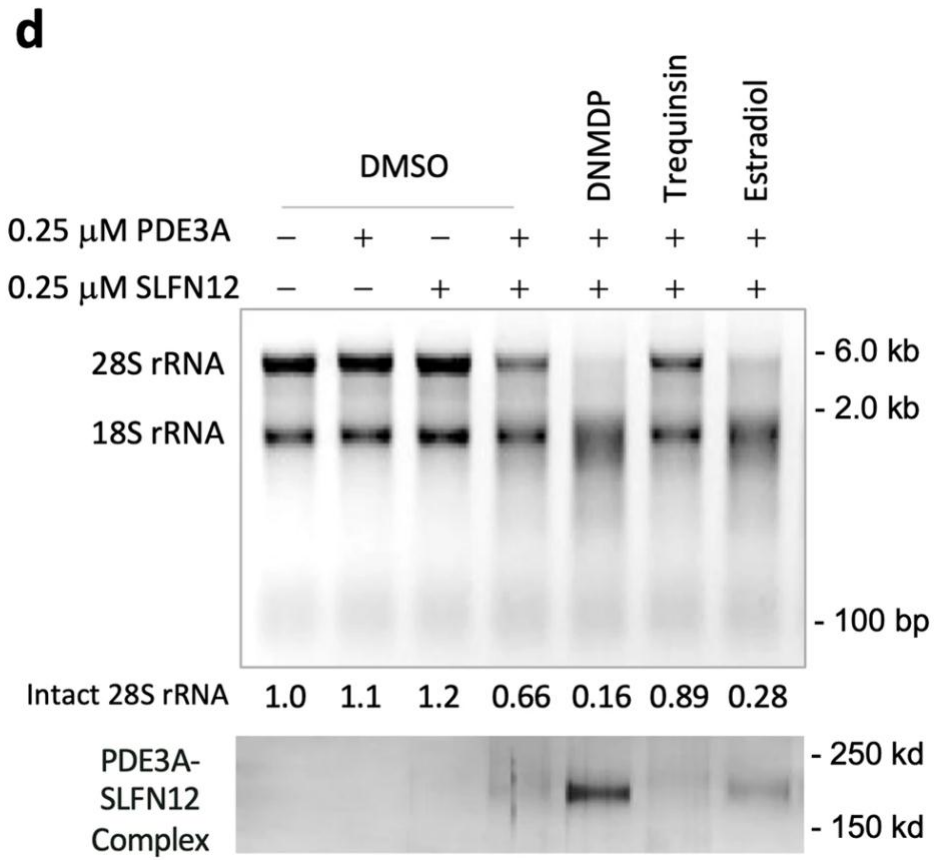
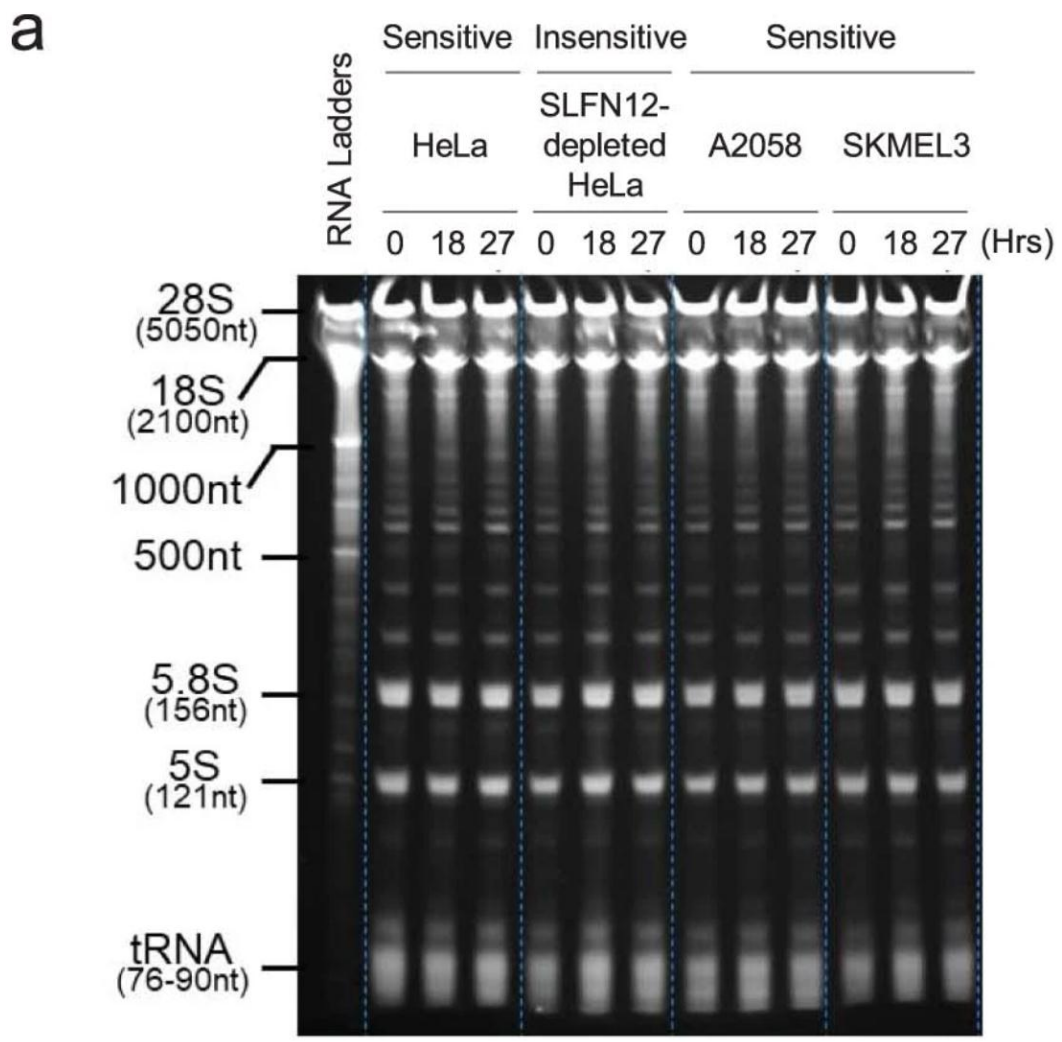


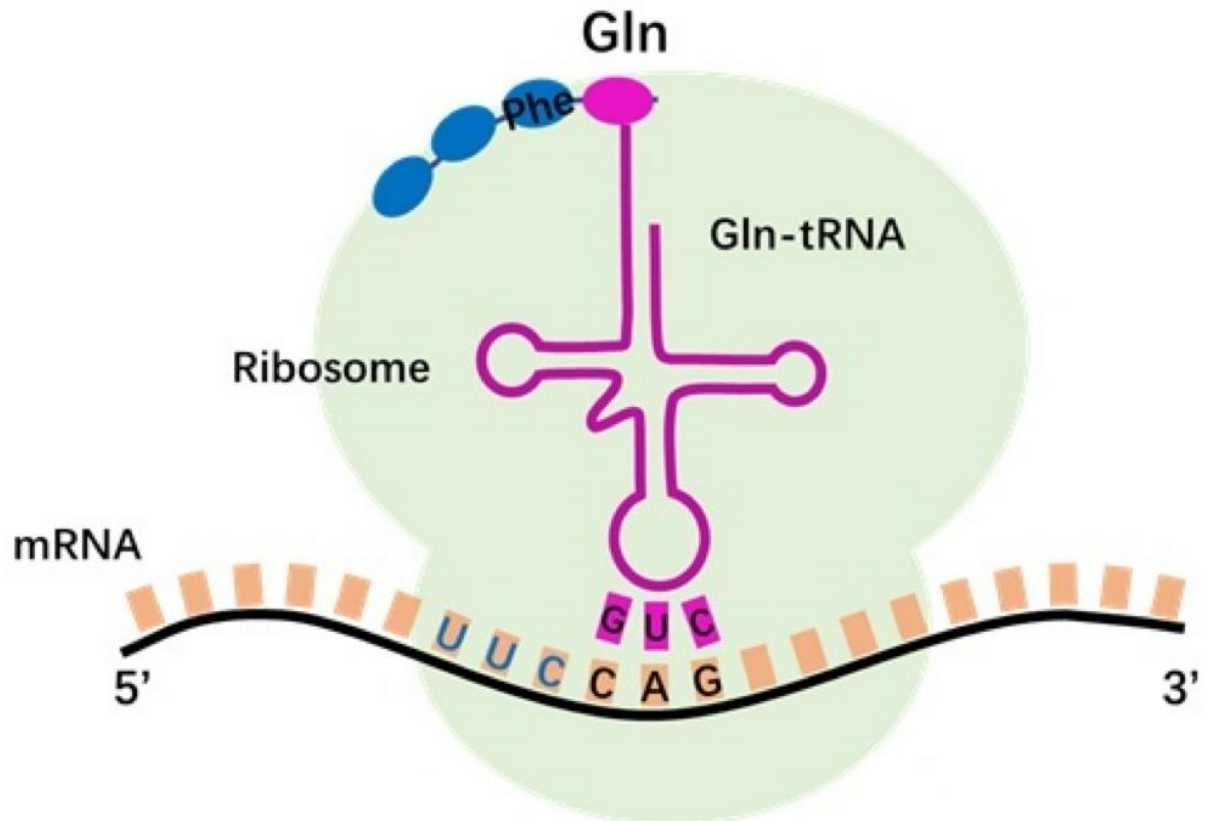
Fig. 7: SLFN12 RNase activity is required for sensitivity to DNMDP.
d Induction of SLFN12 RNase activity by PDE3A-SLFN12 complex formation. 0.25 μM PDE3A and SLFN12 proteins were incubated with DMSO, 12.5 μM DNMDP, 12.5 μM trequinsin, or 12.5 μM estradiol at room temperature for 30 min prior to a 1:10 dilution of complex and incubation with rRNA.

■ Results: SLFN12–PDE3A complex formation downregulates tRNALeu(TAA)



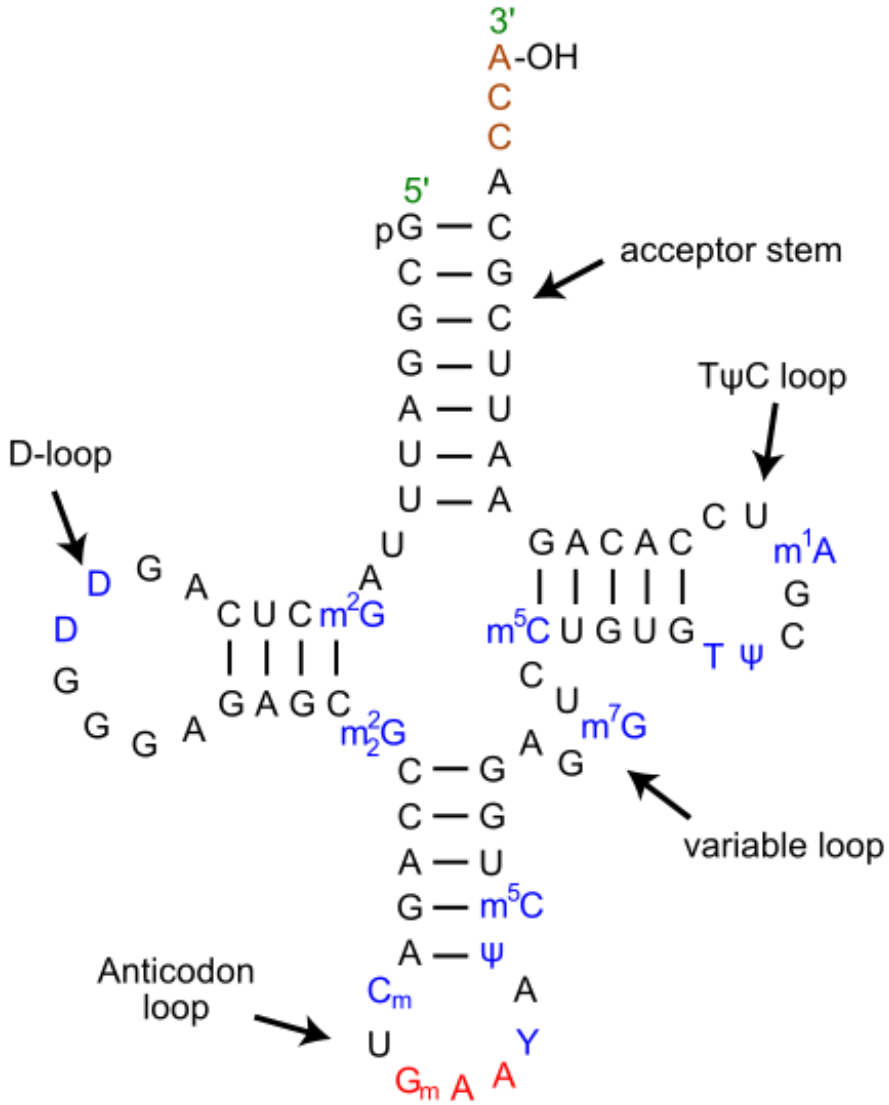
Extended Data Fig. 1: Down-regulation of tRNA-Leu-TAA upon DNMDP treatment.
(a) The effect of DNMDP on ribosomal RNA integrity. Three velcrin-sensitive cell lines (HeLa, A2058, and SKMEL3) and insensitive SLFN12-depleted HeLa were treated with 3 μM DNMDP for the indicated time. SLFN12-depleted HeLa cells were generated by prolonged incubation with DNMDP2, and serve as a negative control in this experiment. Total RNA purified from these cell lines was analyzed on a 6% urea-polyacrylamide gel with RNA ladders (n = 4 replicates for HeLa and n = 2 for others).

■ Background



■ Background

三叶草



TTA	(Leu/L) 亮氨酸
TTG	
CTT	
CTC	
CTA	
CTG	

■ Results: SLFN12–PDE3A complex formation downregulates tRNALeu(TAA)

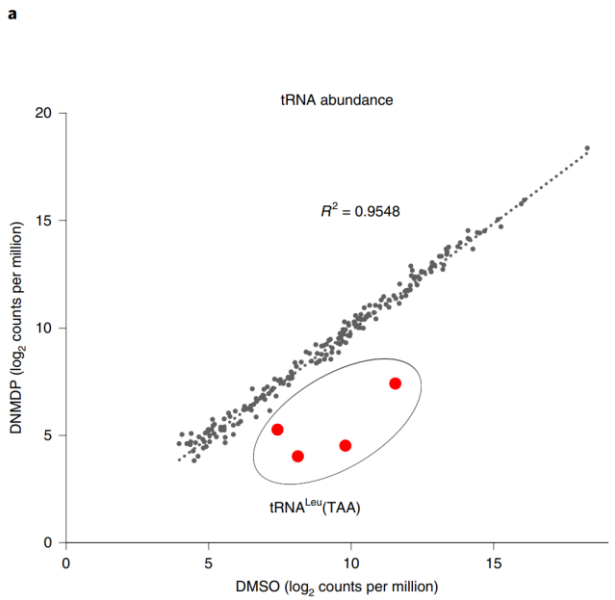
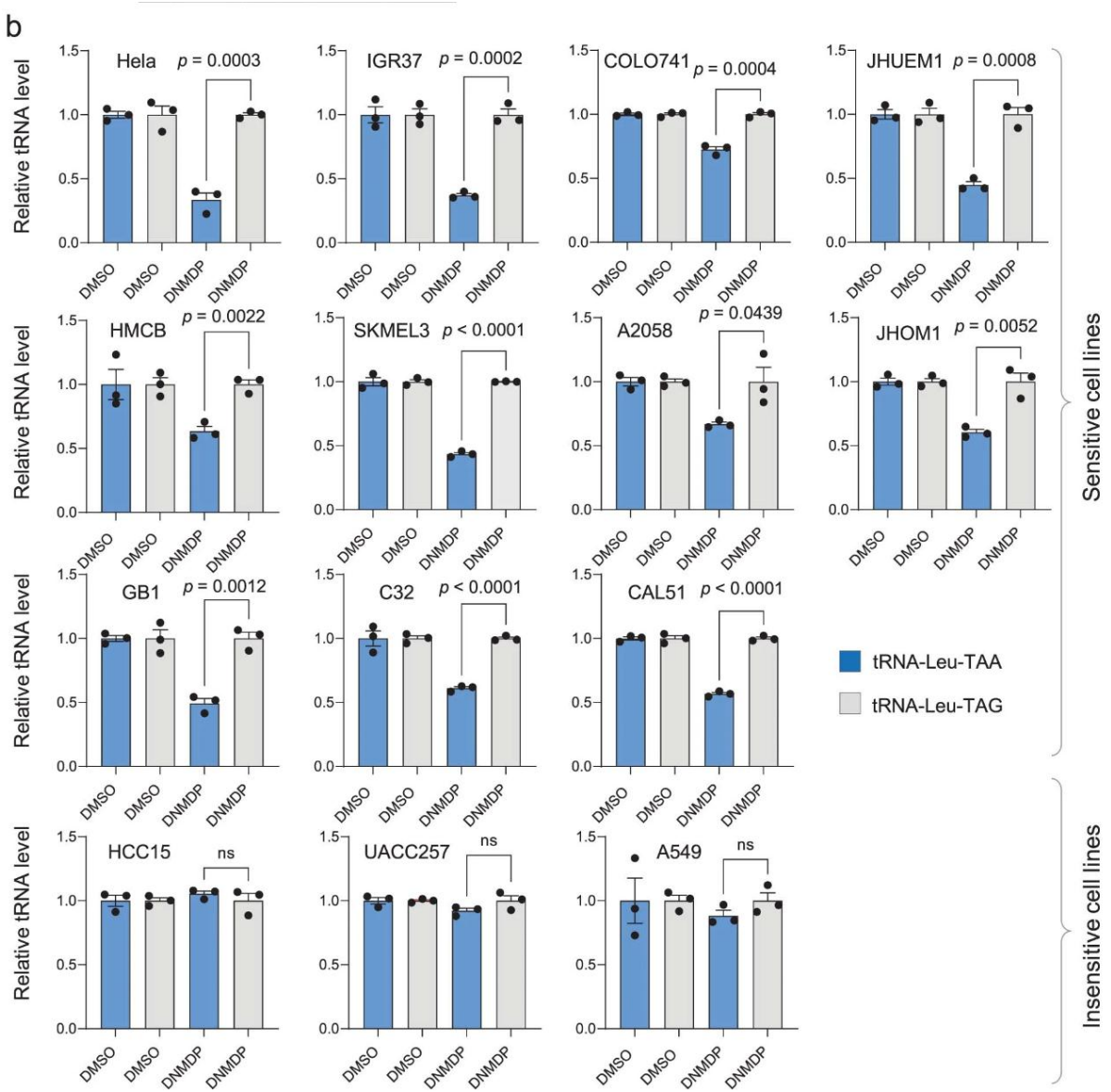


Fig. 1 | Downregulation of tRNALeu(TAA) following DNMDP treatment.
a, Sequence analysis of tRNAs extracted from HeLa cells treated with DMSO or 1 μM DNMDP for 18 h (n = 2 replicates), based on the mapping to the genomic tRNA database, GtRNAdb35. Scatter plot (left), tRNA abundance in DMSO-treated (X axis) and DNMDP-treated cells (Y axis). Red dots, tRNALeu(TAA) isodecoders.



Extended Data Fig. 1: Down-regulation of tRNA-Leu-TAA upon DNMDP treatment.
(b) Leucine tRNA abundance in a panel of cancer cell lines treated with DMSO or 1 mM DNMDP3. After demethylation of tRNA, cDNA was synthesized and leucine tRNA levels were measured using qPCR. tRNA-Leu-TAA-1 level is plotted relative to tRNA-Leu-TAG-2 as a control.

■ Results: SLFN12 RNase preferentially cleaves tRNALeu(TAA)

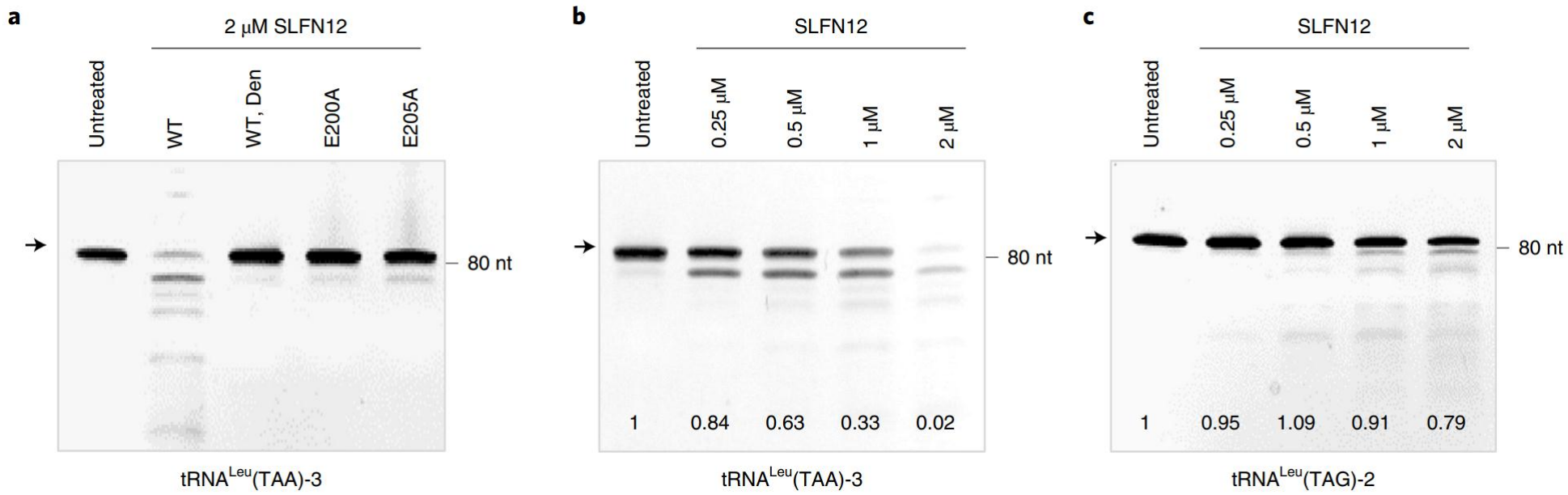


Fig. 2 | SLFN12 cleaves the 3′ acceptor stem of tRNALeu(TAA) and SLFN12–PDE3A complex formation upregulates SLFN12 RNase activity.
a, Rnase activity of SLFN12 on tRNALeu(TAA) isodecoder 3. 2 μM of WT, catalytically inactive mutant (E200 or E205 substituted with alanine)2 or heat-denatured (WT, Den) SLFN12 proteins were incubated with 0.5 μg of tRNALeu(TAA)-3, in vitro transcribed from DNA templates containing a T7 promoter sequence and tRNA genes by T7 RNA polymerase. b,c, The indicated concentrations of WT SLFN12 protein were incubated with (b) tRNALeu(TAA)-3 or (c) tRNALeu(TAG)-2.

■ Results: SLFN12 RNase preferentially cleaves tRNALeu(TAA)

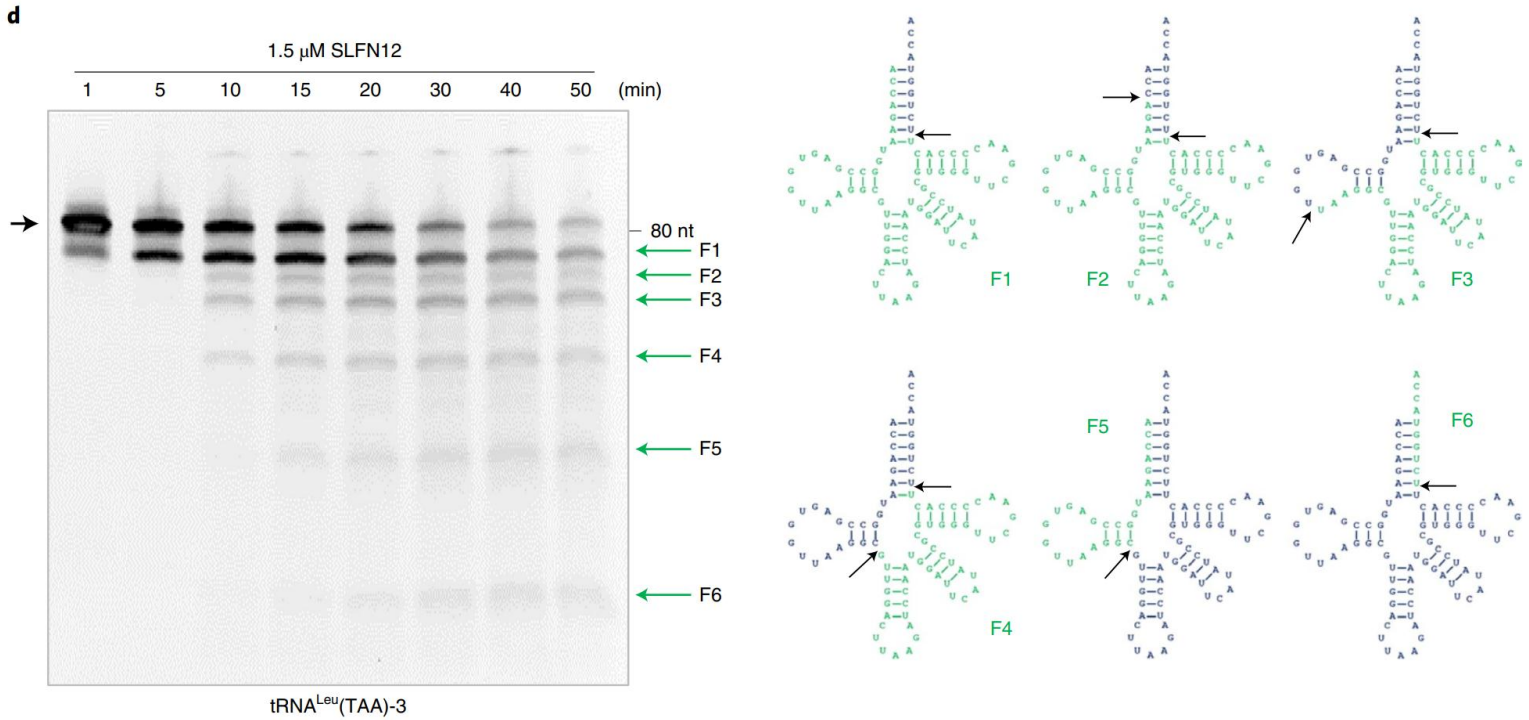


Fig. 2 | SLFN12 cleaves the 3′ acceptor stem of tRNALeu(TAA) and SLFN12–PDE3A complex formation upregulates SLFN12 RNase activity. d, Six tRNA fragments indicated by green arrows were generated from timecourse cleavage of tRNALeu(TAA)-3 by 1.5 μM SLFN12. Each tRNA fragment was extracted from a 15% TBE-urea gel (left) and converted into sequencing libraries using the TruSeq small RNA Library Prep Kit (Illumina). Fragments identified by Sanger sequencing, corresponding to bands isolated from the TBE-urea gel, are shown in green. Cleavage sites are indicated by black arrows.

■ Results: SLFN12 RNase preferentially cleaves tRNALeu(TAA)

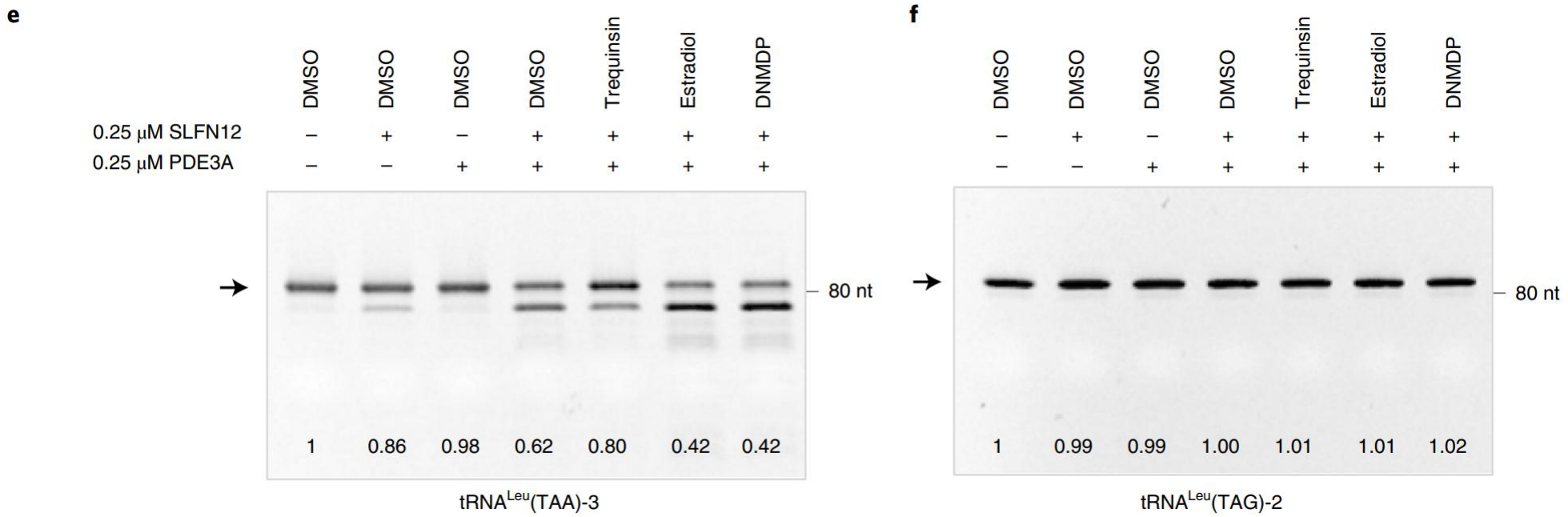


Fig. 2 | SLFN12 cleaves the 3′ acceptor stem of tRNALeu(TAA) and SLFN12–PDE3A complex formation upregulates SLFN12 RNase activity. e,f, Upregulation of SLFN12 RNase by PDE3A complex formation. 0.25 μM PDE3A and SLFN12 proteins were preincubated with DMSO, 1.25 μM DNMDP, 1.25 μM trequinsin, or 1.25 μM estradiol before incubation with (e) tRNALeu(TAA)-3 or (f) tRNALeu(TAG)-2. Digested tRNA was analyzed on a denaturing polyacrylamide gel. Intact tRNA is indicated with an arrow. The relative amount of intact tRNA, quantified using ImageJ software, is shown at the bottom. The results were reproducible (n = 2 replicates). Representative figures are shown in source data.

■ Results: tRNALeu(TAA) acceptor stem and variable loop are required for cleavage

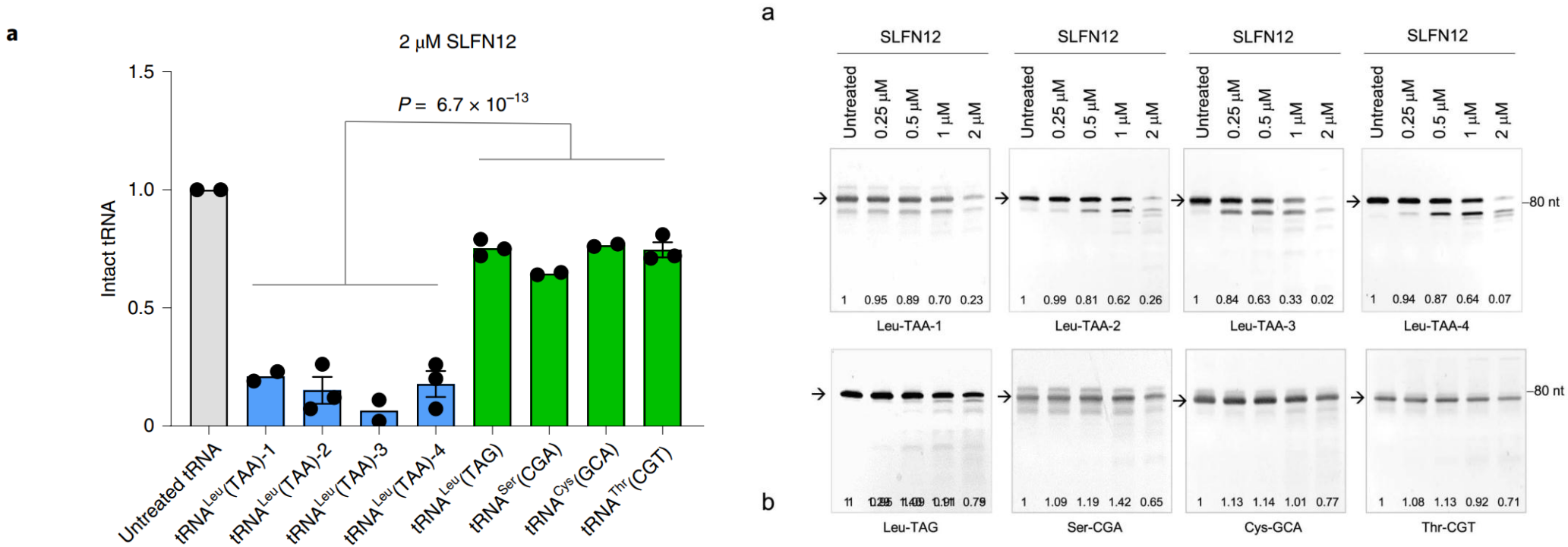


Fig. 3 | SLFN12 preferentially digests tRNALeu(TAA) in vitro and this selective cleavage requires distinct sequences in the variable loop and the acceptor stem regions.
a,b, Preferential digestion of tRNALeu(TAA) by SLFN12. 0.5 μg of the indicated tRNAs were digested with (a) 2 μM SLFN12 (n = 2 replicates for tRNALeu(TAA)-1, tRNALeu(TAA)-3, tRNASer(CGA) and tRNACys(GCA), and n = 3 replicates for others) or (b) 0.25 μM PDE3A and SLFN12 proteins preincubated with 1.25 μM DNMDP (n = 3 replicates for tRNALeu(TAA)-1 and tRNALeu(TAA)-3, and n = 2 replicates for others). The relative amounts of intact tRNAs were measured and plotted.

Extended Data Fig. 4: SLFN12 selectively digests tRNA-Leu-TAA in vitro.
(a) Indicated tRNAs were synthesized using a T7 RNA polymerase-mediated transcription reaction. Synthetic tRNAs were treated with the indicated concentrations of SLFN12 at 37 ° C for 40 minutes (n = 2 replicates for Leu-TAA-1, Leu-TAA-3, Ser-CGA and Cys-GCA; n = 3 replicates for others).

■ Results: tRNALeu(TAA) acceptor stem and variable loop are required for cleavage

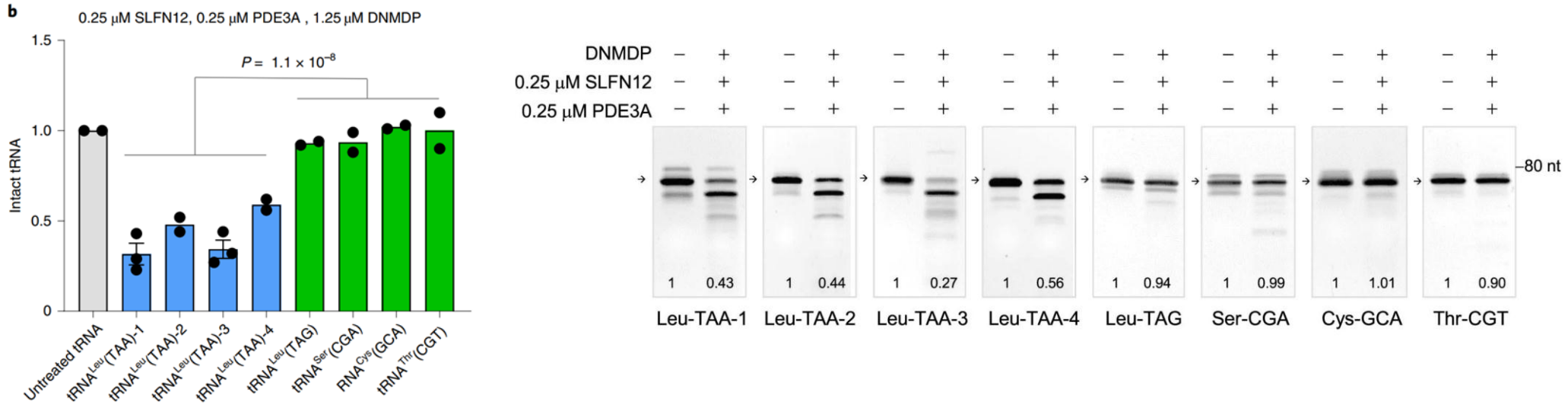


Fig. 3 | SLFN12 preferentially digests tRNALeu(TAA) in vitro and this selective cleavage requires distinct sequences in the variable loop and the acceptor stem regions.
a,b, Preferential digestion of tRNALeu(TAA) by SLFN12. 0.5 μg of the indicated tRNAs were digested with (a) 2 μM SLFN12 (n = 2 replicates for tRNALeu(TAA)-1, tRNALeu(TAA)-3, tRNASer(CGA) and tRNACys(GCA), and n = 3 replicates for others) or (b) 0.25 μM PDE3A and SLFN12 proteins preincubated with 1.25 μM DNMDP (n = 3 replicates for tRNALeu(TAA)-1 and tRNALeu(TAA)-3, and n = 2 replicates for others). The relative amounts of intact tRNAs were measured and plotted.

Extended Data Fig. 4: SLFN12 selectively digests tRNA-Leu-TAA in vitro.
(b) 0.25 μM PDE3A and SLFN12 proteins pre-treated with 1.25 μM DNMDP were incubated with synthetic tRNAs at 37 ° C for 40 minutes (n = 3 replicates for Leu-TAA-1 and Leu-TAA-3; n = 2 replicates for others).

■ Results: tRNALeu(TAA) acceptor stem and variable loop are required for cleavage

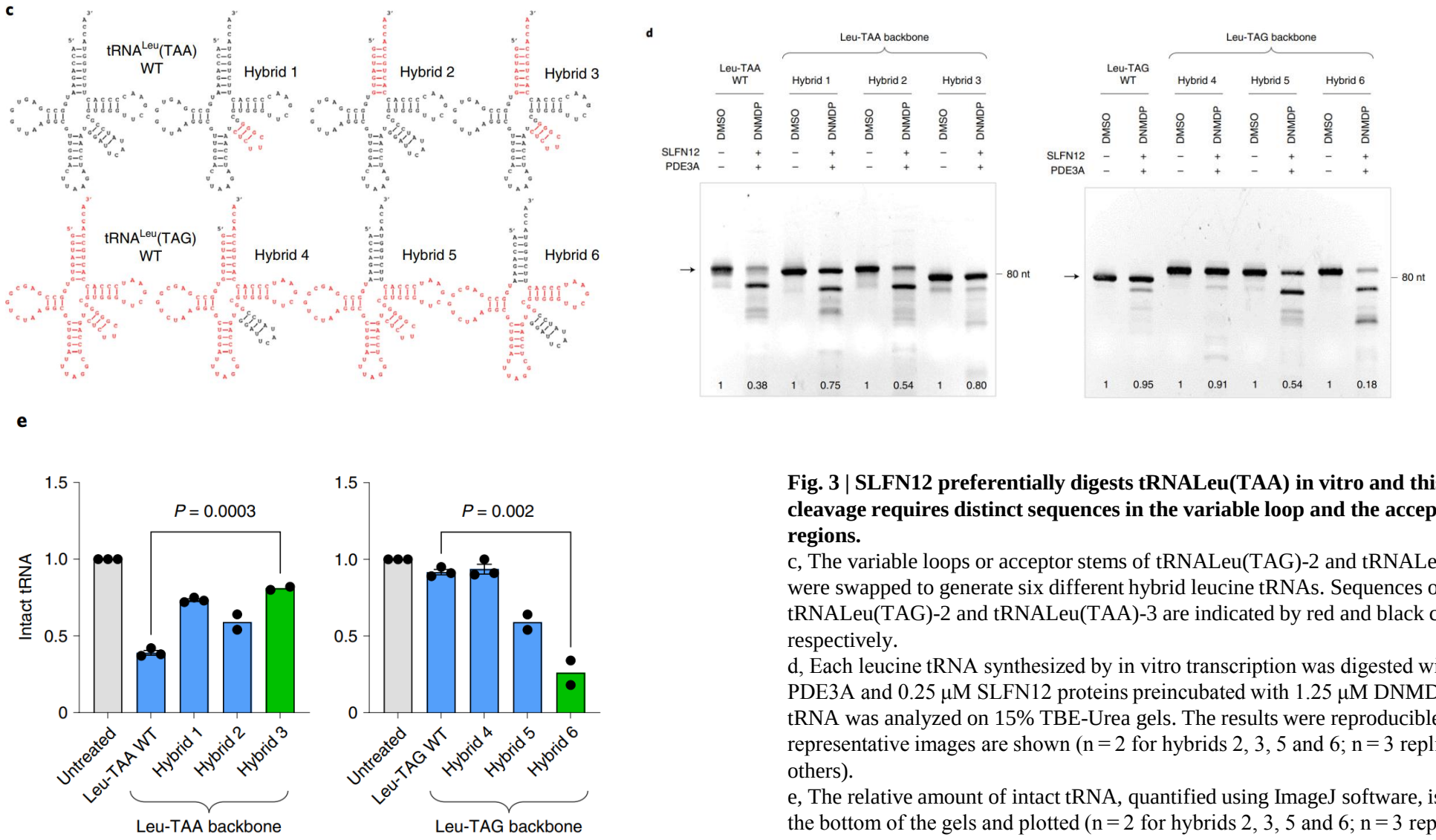


Fig. 3 | SLFN12 preferentially digests tRNALeu(TAA) in vitro and this selective cleavage requires distinct sequences in the variable loop and the acceptor stem regions.

c, The variable loops or acceptor stems of tRNALeu(TAG)-2 and tRNALeu(TAA)-3 were swapped to generate six different hybrid leucine tRNAs. Sequences of tRNALeu(TAG)-2 and tRNALeu(TAA)-3 are indicated by red and black colors, respectively.

d, Each leucine tRNA synthesized by in vitro transcription was digested with 0.25 μM PDE3A and 0.25 μM SLFN12 proteins preincubated with 1.25 μM DNMDP. Cleaved tRNA was analyzed on 15% TBE-Urea gels. The results were reproducible and representative images are shown (n = 2 for hybrids 2, 3, 5 and 6; n = 3 replicates for others).

e, The relative amount of intact tRNA, quantified using ImageJ software, is shown at the bottom of the gels and plotted (n = 2 for hybrids 2, 3, 5 and 6; n = 3 replicates for others). Data are represented as mean ± s.e.m. P values were calculated by the unpaired two-tailed Student's t-test.

■ Results: Depletion of tRNALeu(TAA) pauses ribosomes at TTA codons

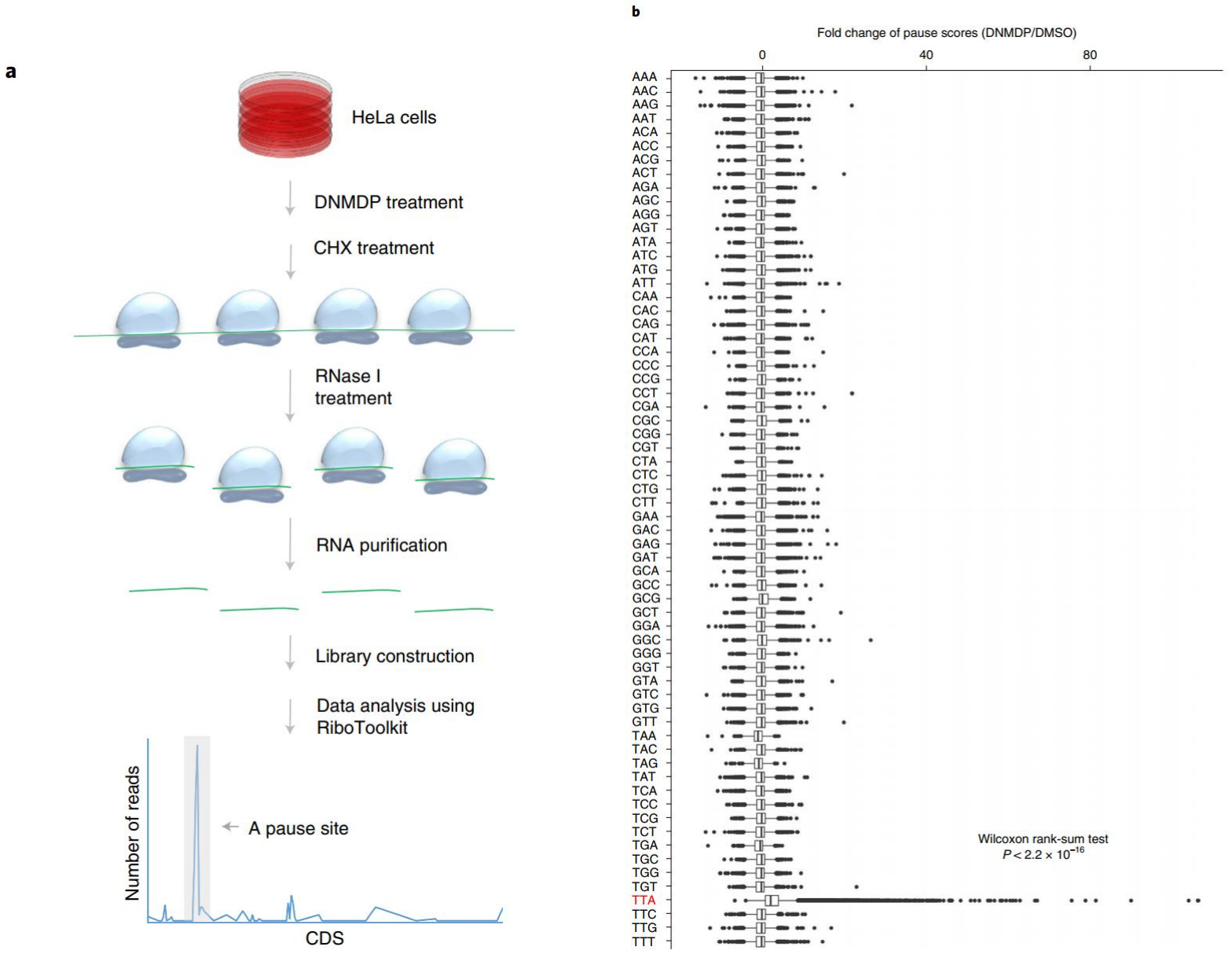


Fig. 4 | SLFN12 RNase activation by DNMDP causes ribosomal pausing at TTA codons.

a, Experimental procedure for Ribo-seq in HeLa cells treated with DMSO or 1 μM DNMDP for 18 h.

b, Fold change of pause scores from DNMDP- and DMSO-treated samples for each codon was plotted in a box plot (center line, median; box limits, upper and lower quartiles;

■ Results: Depletion of tRNALeu(TAA) pauses ribosomes at TTA codons

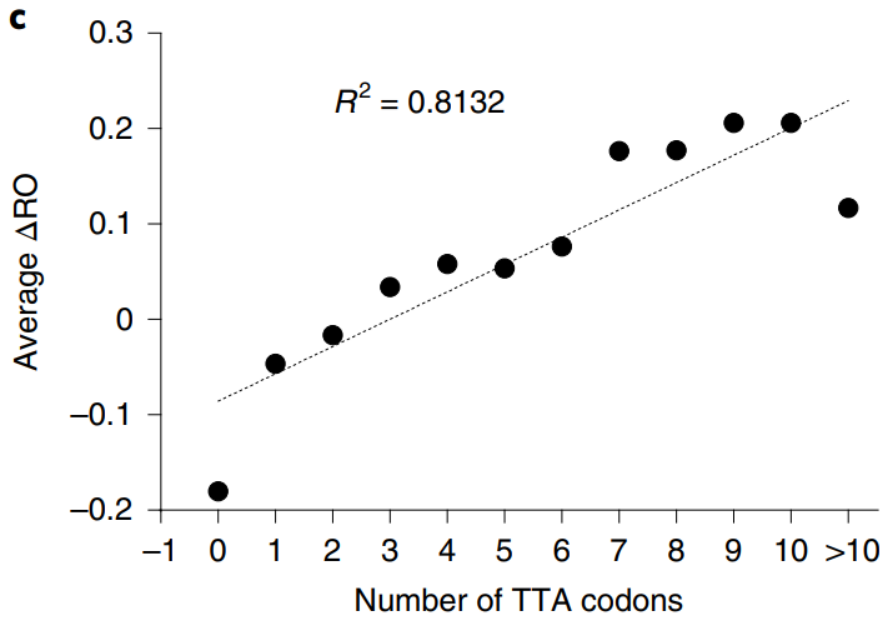
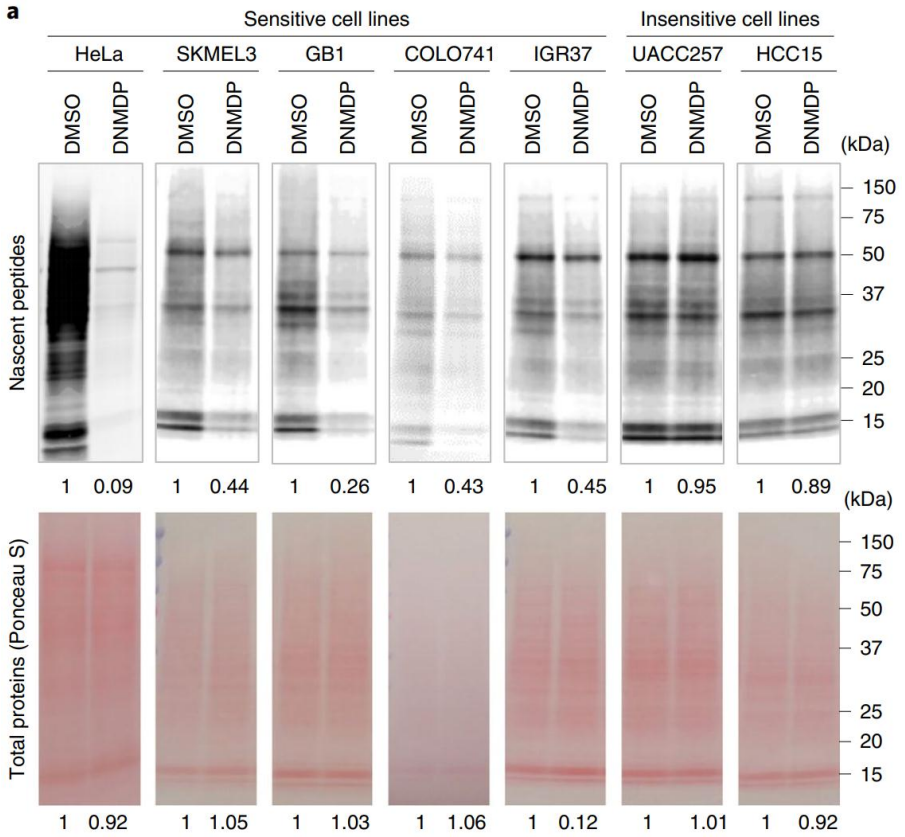


Fig. 4 | SLFN12 RNase activation by DNMDP causes ribosomal pausing at TTA codons.
c, Scatter plot of the correlation between the change in ribosome occupancy or ΔRO (RPF counts divided by the RNA read counts for a given gene) in response to DNMDP treatment, and the number of TTA codons per gene. The Pearson correlation coefficient was measured to assess the strength of a linear correlation between ΔRO and the number of TTA codons.

■ Results: DNMDP inhibits global translation in sensitive cells



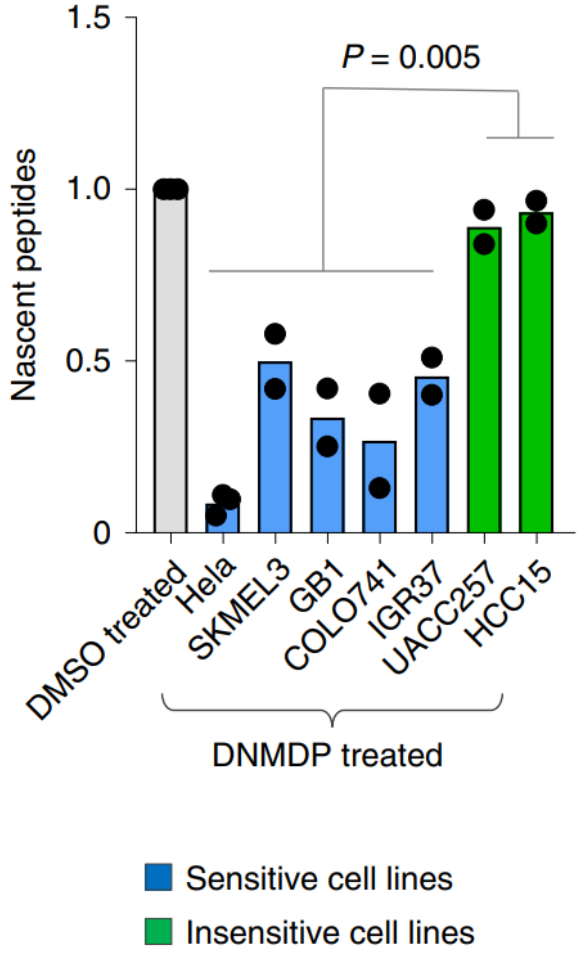


Fig. 5 | DNMDP treatment or ectopic expression of SLFN12 inhibits global translation in sensitive cancer cell lines.
a, The effect of DNMDP treatment on global translation in a panel of cancer cell lines treated with DMSO or 1 μM DNMDP for 18 h. To label nascent peptides, cells were incubated with 50 μM AHA in methionine-free medium. The AHA-labeled peptides were biotinylated using click chemistry and analyzed by SDS–PAGE. Biotinylated nascent peptides were visualized using fluorescent dye-labeled streptavidin (top) and total protein was stained by Ponceau S (bottom).
b, The relative intensity of biotinylated peptides to total protein was quantified and plotted using ImageJ (1.8.0) .

■ Results: DNMDP inhibits global translation in sensitive cells

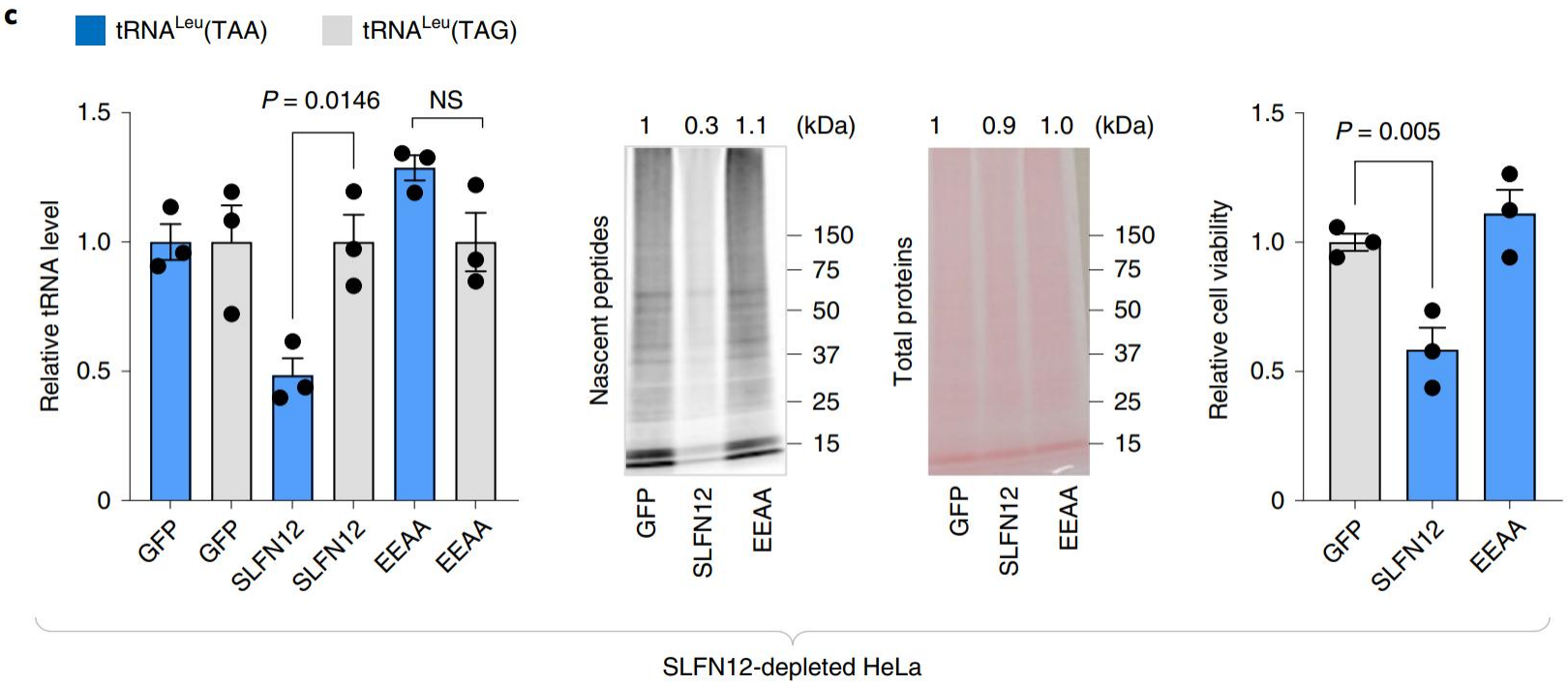


Fig. 5 | DNMDP treatment or ectopic expression of SLFN12 inhibits global translation in sensitive cancer cell lines.
c, Ectopic expression of WT SLFN12 downregulates tRNALeu(TAA)-1, decreases global translation and decreases cell survival in SLFN12-depleted HeLa cells made resistant to velcrin treatment by prolonged incubation with DNMDP2. However, ectopic expression of catalytically inactive SLFN12 (with amino acids E200 and E205 substituted with alanine, EEAA)2 or GFP does not. tRNALeu(TAA)-1 and tRNALeu(TAG)-2 levels were measured by qRT–PCR (left, n = 3 replicates). Nascent peptides and total protein were measured as described above (middle, n = 2 replicates). Viable cells were measured by CellTiter-Glo and viability relative to GFP control (right, n = 3 replicates).

■ Results: DNMDP mediates apoptosis via cleavage of tRNALeu(TAA)

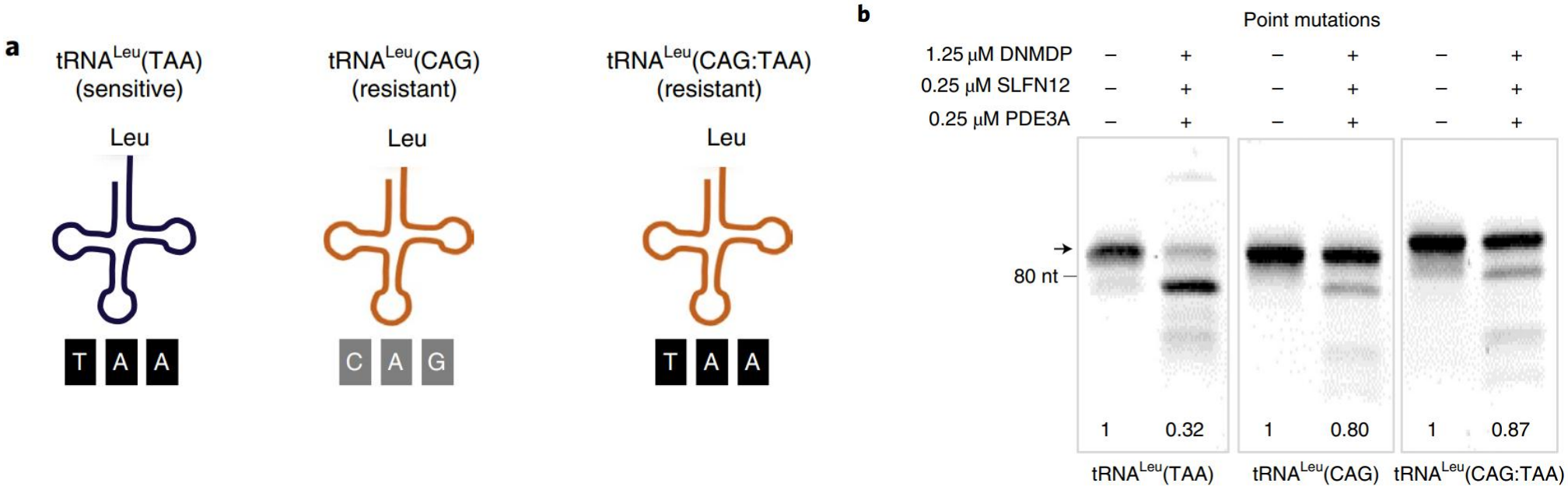


Fig. 6 | Expression of leucine tRNA resistant to SLFN12 RNase rescues cells from DNMDP-mediated apoptosis.
a, Construction of resistant tRNALeu(CAG:TAA) by mutating the anticodon of tRNALeu(CAG)-1 to TAA. b, Indicated tRNAs were digested with 0.25 μM PDE3A and 0.25 μM SLFN12 proteins preincubated with 1.25 μM DNMDP. Intact tRNAs are marked with an arrow and were quantified by ImageJ (1.8.0), as reported at the bottom of each lane (n = 2 replicates).

■ Results: DNMDP mediates apoptosis via cleavage of tRNALeu(TAA)

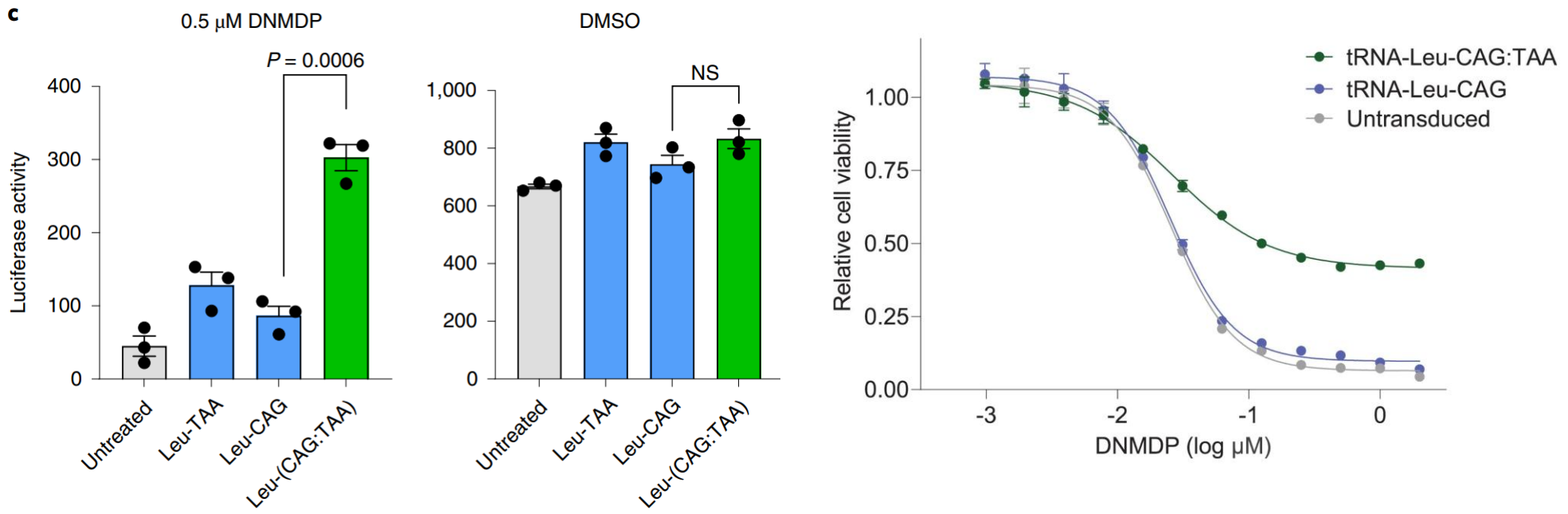


Fig. 6 | Expression of leucine tRNA resistant to SLFN12 RNase rescues cells from DNMDP-mediated apoptosis.
c, HeLa cells were transfected with plasmids expressing tRNALeu(CAG)-1, tRNALeu(TAA)-3 or variant tRNALeu(CAG:TAA) followed by DMSO or 0.5 μM DNMDP treatment. Forty-eight hours after drug treatment, cell viability was assessed by CellTiter-Glo (n = 3 replicates). Data are represented as mean ± s.e.m. P values were calculated by the unpaired two-tailed Student's t-test (NS, not significant).

Extended Data Fig. 9: Effects of leucine tRNA ectopic expression on DNMDP response.
HeLa cells stably expressing wild-type leucine tRNA-Leu-CAG or hybrid tRNA-Leu-[CAG:TAA] were treated with the indicated DNMDP concentrations for 2 days. Cell viability was assessed by CellTiter-Glo (n = 3 replicates). Data are represented as mean ± SEM.

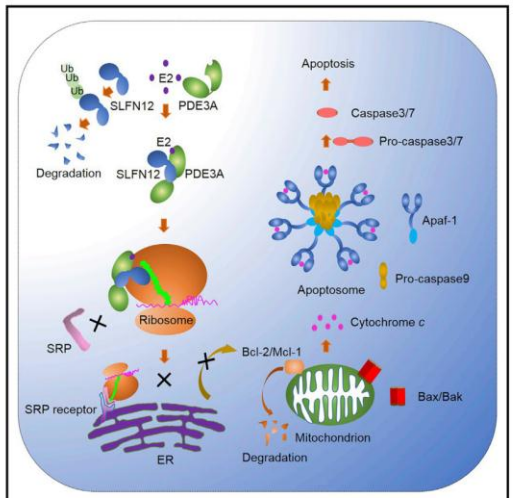
■ Discussion

Article

Molecular Cell

Estrogen-Related Hormones Induce Apoptosis by Stabilizing Schlafen-12 Protein Turnover

Graphical Abstract



Authors

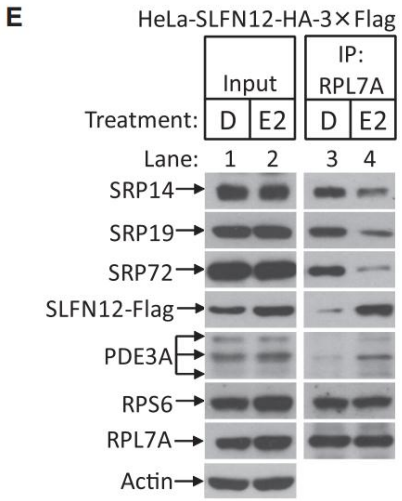
Dianrong Li, Jie Chen, Youwei Ai, ..., Yang Cao, Xiangbin Qi, Xiaodong Wang

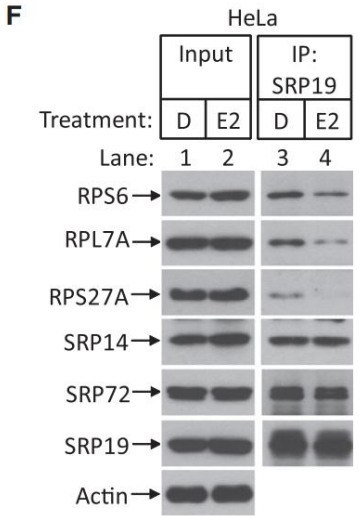
Correspondence

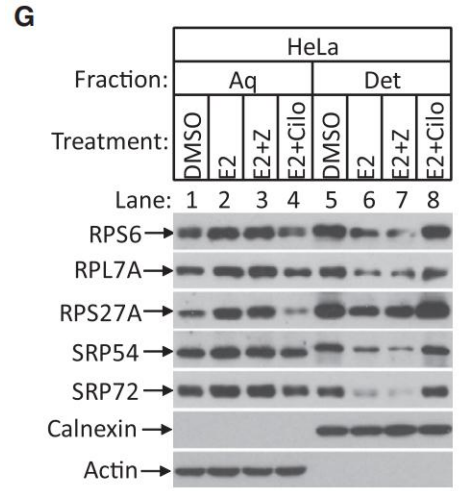
wangxiaodong@nibs.ac.cn

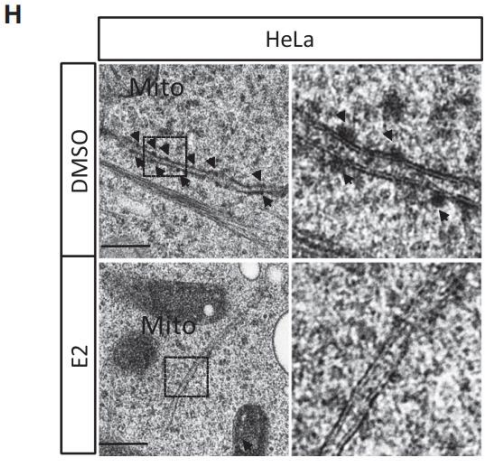
In Brief

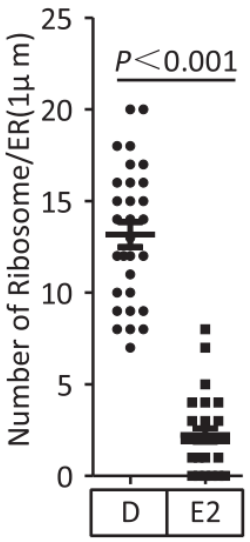
Estrogen-like female sex hormones at concentrations present in human placenta induce apoptosis. They do so by binding to phosphodiesterase 3A, which in turn recruits and stabilizes Schlafen 12 protein, whose elevated level stops the protein translation on ER, resulting in downregulation of anti-apoptotic proteins Bcl-2 and Mcl-1 and subsequent apoptosis.

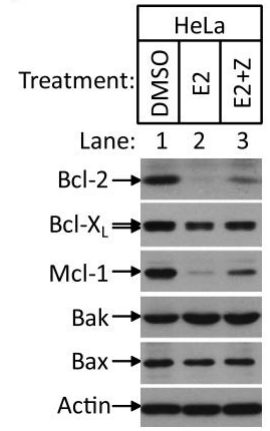




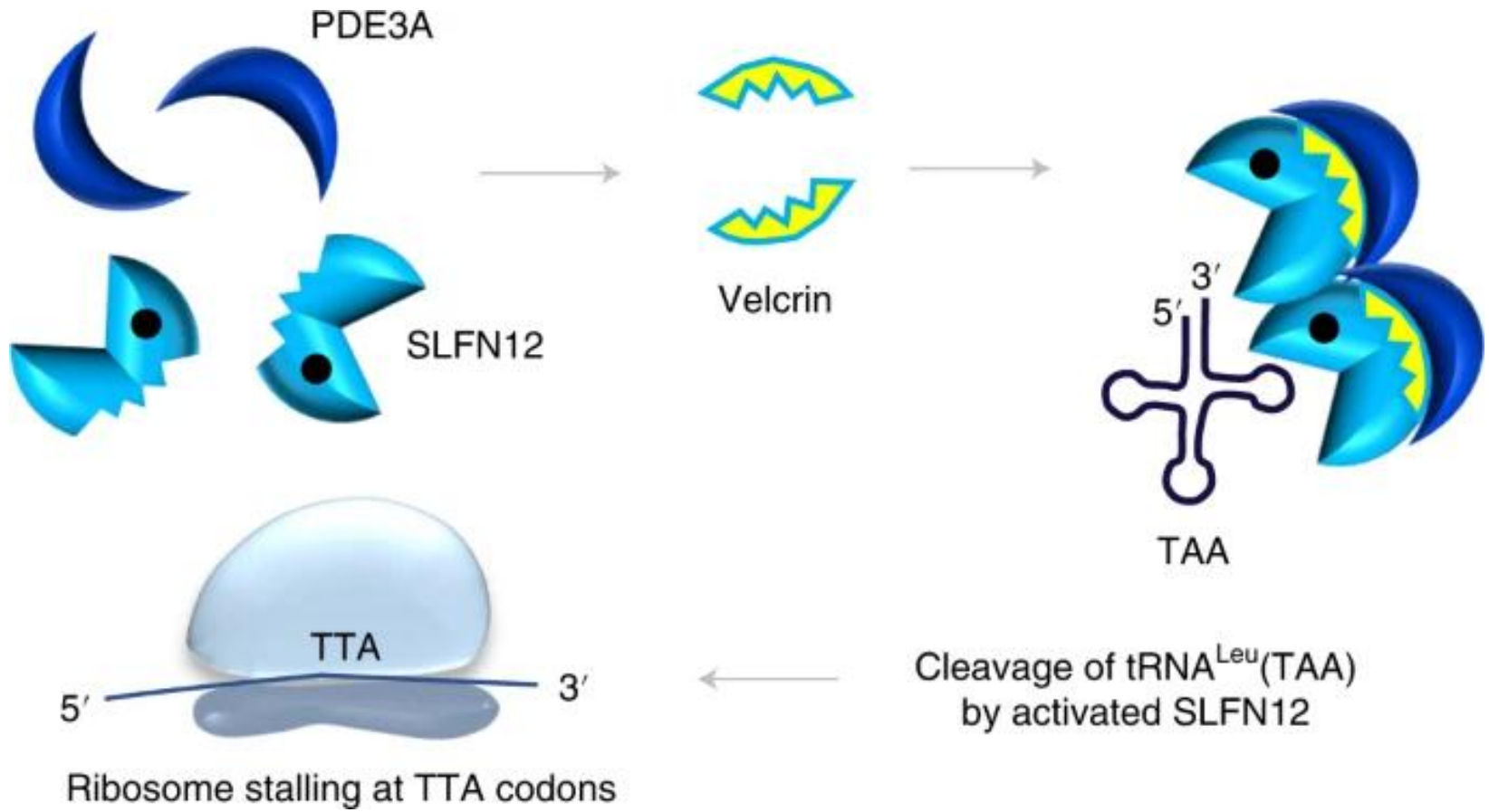








■ Discussion


PDE3A
SLFN12
Velcrin
3′
5′
TAA
Cleavage of tRNA^{Leu}(TAA)
by activated SLFN12
TTA
5′
3′
Ribosome stalling at TTA codons

“转运”RNA