

**A**

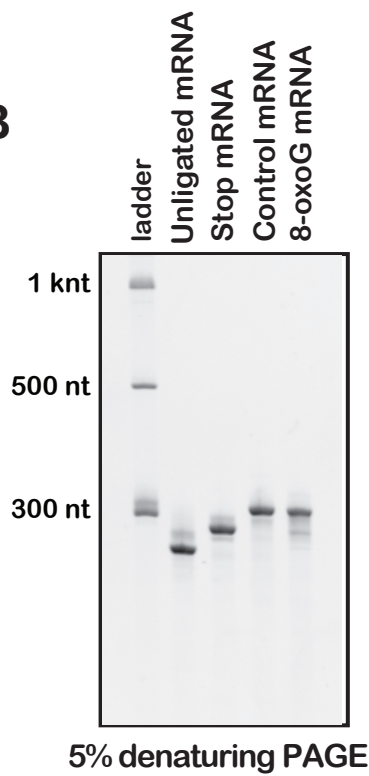
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Q  U  D  G  F  K  U  L  A  E  G  Q  R  V  E  F  E  I  U  D  G  A  K  G  P  S  A
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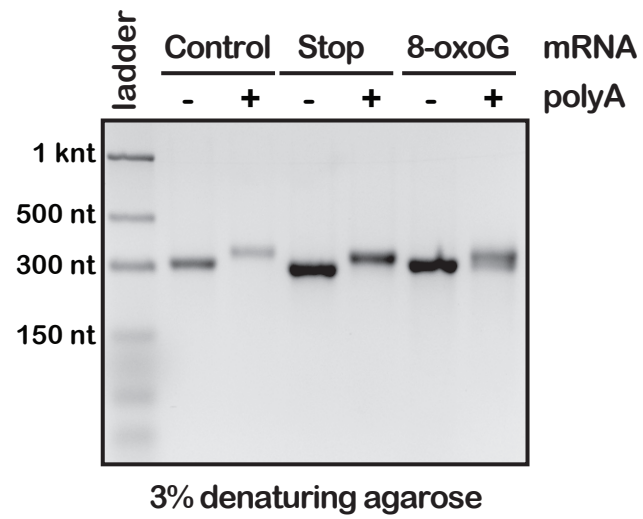
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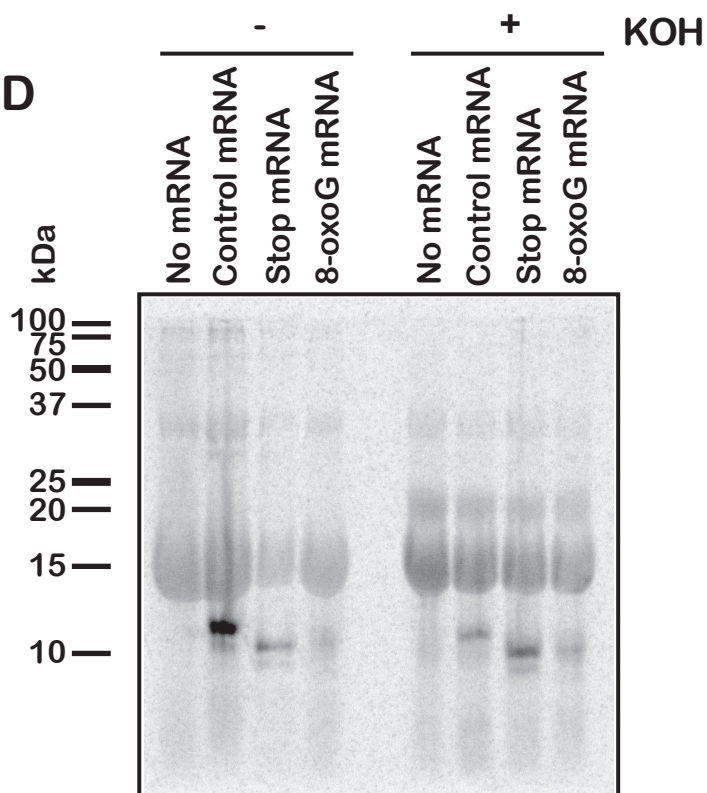
**B**



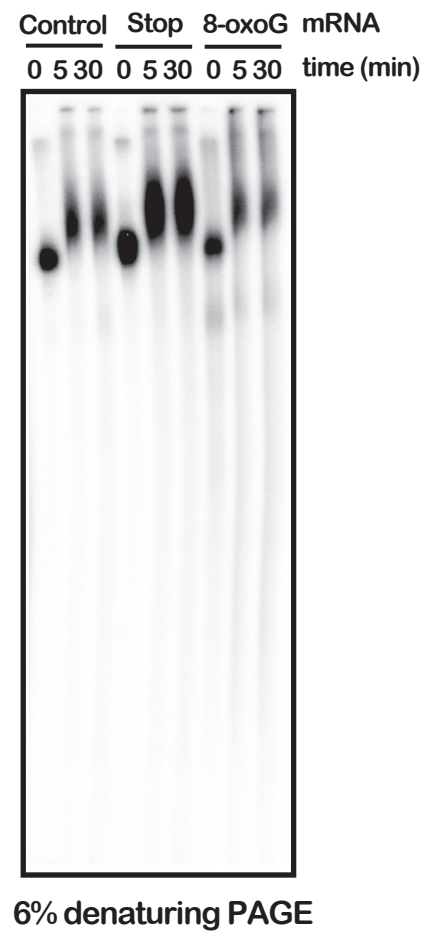
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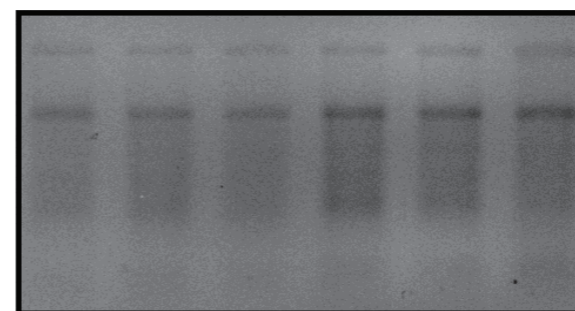
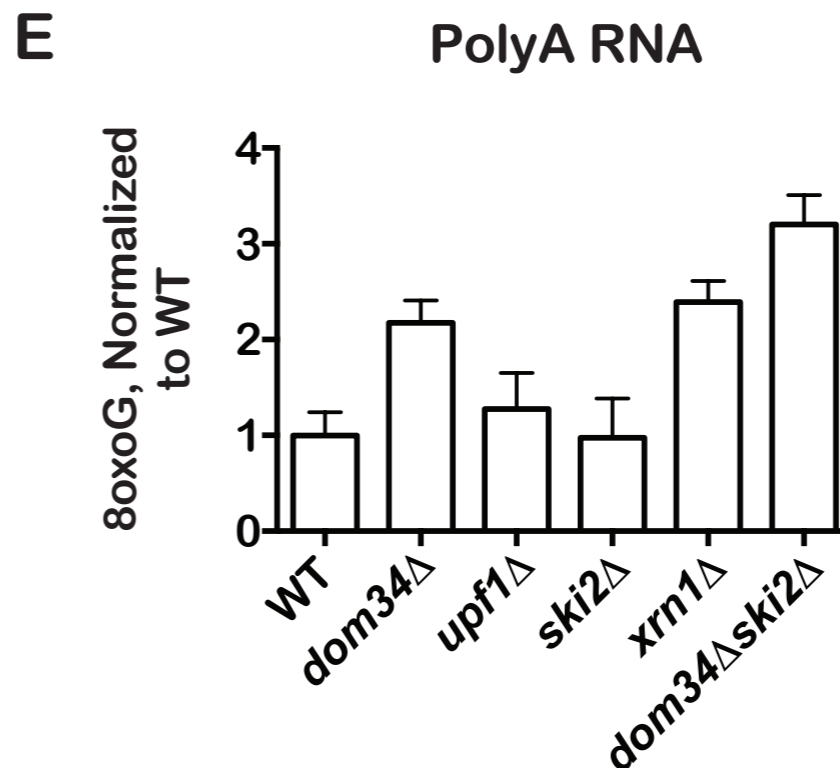
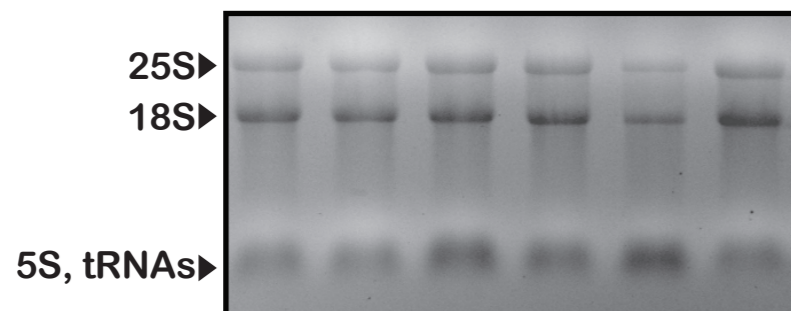
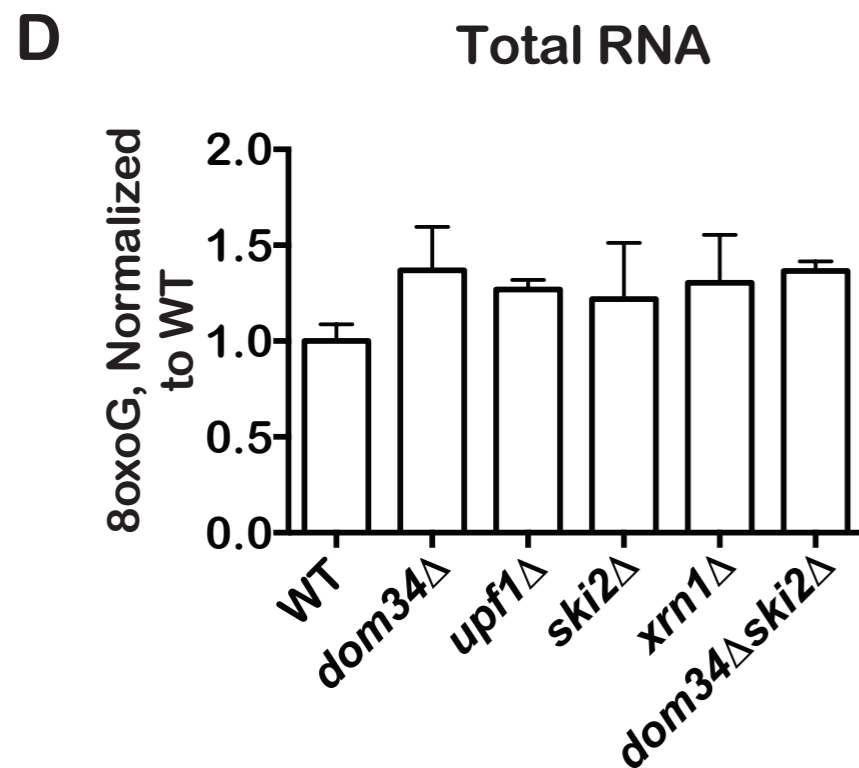
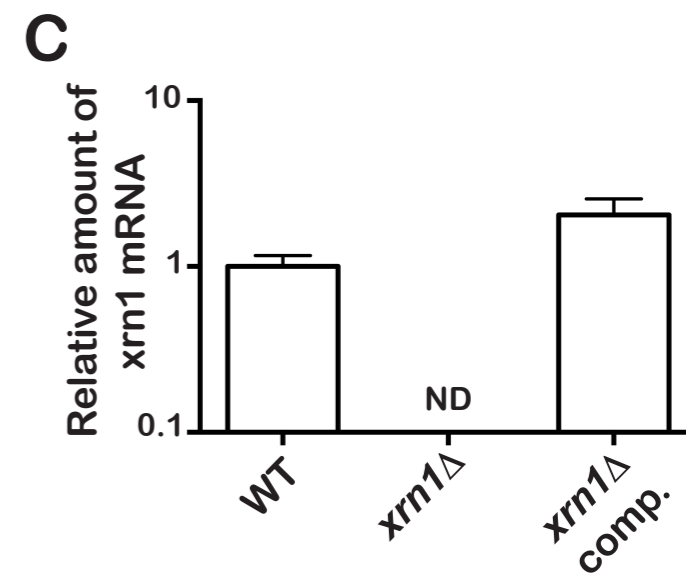
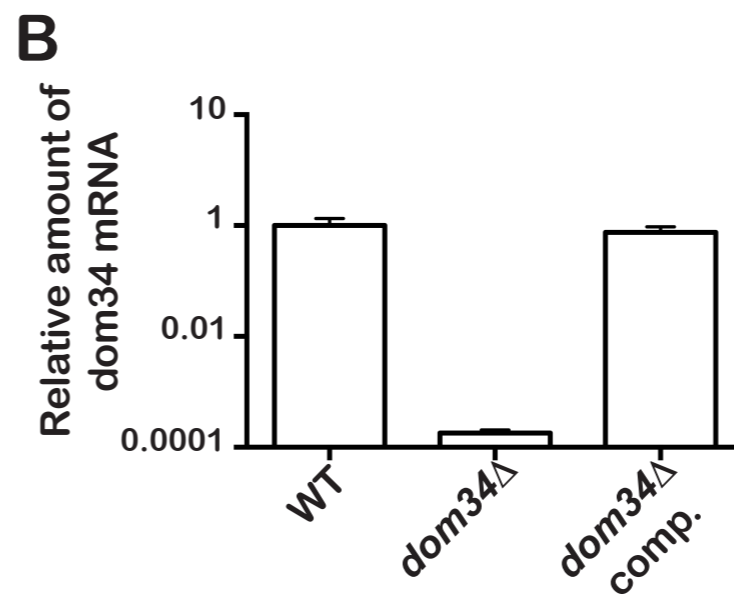
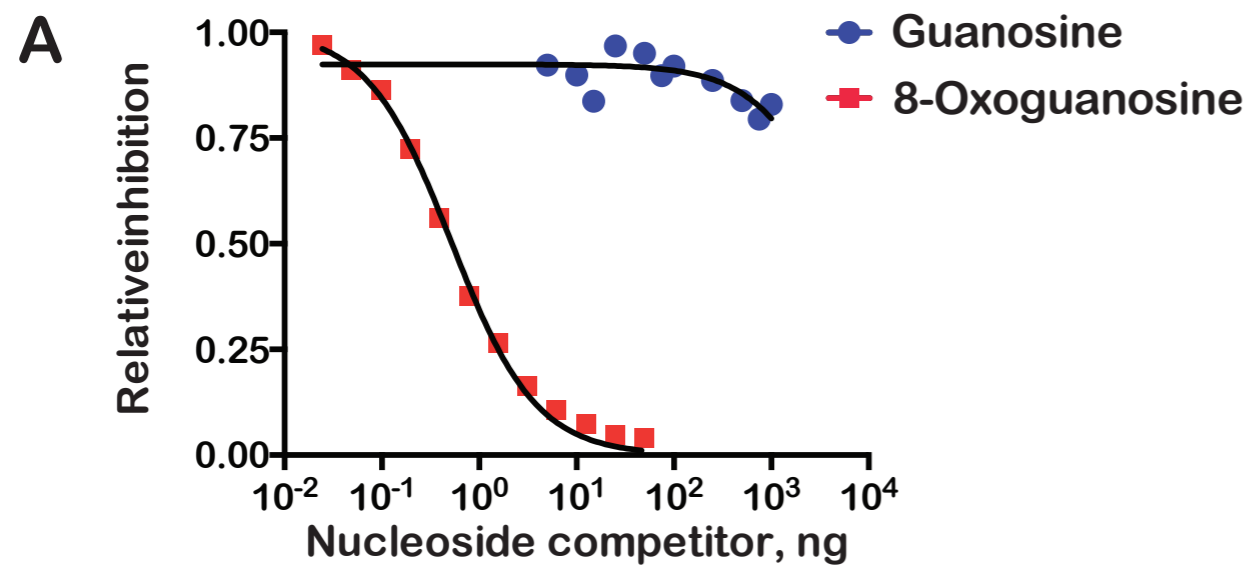


**D**



**E**





## Supplemental Figure Legends

**Figure S1, related to Figure 1: Reactivities of initiation complexes with ternary complexes in our reconstituted system.** An autoradiograph of an electrophoretic TLC used to follow the reaction of initiation complexes encoding for the indicated dipeptides with the corresponding ternary complex.

**Figure S2, related to Figure 1: Reactivity survey of additional native and oxidized complexes for Met-Val, Met-Gly, and Met-Glu.** Schematic representation of initiation complexes for Met-Val (**A**), Met-Gly (**E**), or Met-Glu (**I**). The Met-Val pair of complexes display either a GUU codon or an <sup>8oxo</sup>GUU in the A site, where the intact complex is cognate for Val-tRNA<sup>Val</sup><sub>GAC</sub> and the oxidized complex is predicted (if 8-oxoG pairs with A, or G at the wobble position) to pair with Phe-tRNA<sup>Phe</sup><sub>GAA</sub> (**B**). The Met-Gly complexes display a GGC codon or a G<sup>8oxo</sup>GC codon in the A site and were expected to react with the cognate Gly-tRNA<sup>Gly</sup><sub>GCC</sub> and near cognate Val-tRNA<sup>Val</sup><sub>GAC</sub>, respectively (**F**). The Met-Glu guanosine complex displays a GAG codon in the A site and the 8-oxoguanosine complex displays a GA<sup>8oxo</sup>G in the A site, and are predicted to pair with cognate Glu-tRNA<sup>Glu</sup><sub>UUC</sub> and near cognate Asp-tRNA<sup>Asp</sup><sub>GUC</sub> (**J**). Autoradiographs of electrophoretic TLCs used to follow the formation of dipeptides between the initiation complexes and the indicated ternary complexes (**C**), (**G**), (**K**). Green asterisk represents the cognate dipeptide, whereas the red one represents the near-cognate dipeptide. (**D**), (**H**), (**L**) Quantification of the dipeptide yield in (**C**), (**G**), and (**K**) respectively. The predicted codon-anticodon interaction is shown below the x-axis and the corresponding dipeptide is shown above the bars.

**Figure S3, related to Figure 3: Construction of mRNA reporters for translation assays and translation in rabbit reticulocyte lysates.** (**A**) Nucleotide sequence of the reporters. No stop mRNA (not shown) contains the full upstream fragment but is lacking the underlined region. (**B**) An image of ethidium-bromide-stained denaturing PAGE used to follow the construction of the different indicated mRNAs (judged by their sizes). (**C**) An image of ethidium-bromide-stained formaldehyde agarose gel used to follow the addition of the polyA to the different mRNA constructs. (**D**) Autoradiograph of Tris-Tricine gel of rabbit reticulocyte *in vitro* translation assays with and without addition of KOH. Proteins were labeled by the addition of [<sup>35</sup>S]-Methionine to the reactions. The 8-oxoG mRNA produces a truncated protein product of a similar size to that observed in the presence of

stop mRNA. **(E)** To examine the stability of mRNA in translation extracts, mRNAs were separated on 6% denaturing PAGE after incubation in translation reactions with wheat germ extracts for 0, 5, and 30 min.

**Figure S4, related to Figure 4:** **(A)** Sensitivity and selectivity of the 8-oxoG competitive ELISA assay. Inhibition curves of anti-8OHG antibody binding to 8oxo-G-conjugated BSA in the presence of varying concentrations of guanosine (blue circles) or 8-oxoguanosine (red squares). **(B,C)** quantitative RT-PCR of *dom34* and *xrn1* genes in the indicated strains. ND (not determined): levels were too low to measure. Data were normalized to *act1*. **(D,E)** 8-oxoG levels in total and polyA-enriched RNAs, respectively, isolated from different yeast strains. Shown below the graphs are images of ethidium-bromide-stained formaldehyde agarose gels of the samples. Values shown are the mean of at least three measurements  $\pm$ SEM.

### Supplemental Experimental Procedures

**Materials.** 70S tight-couple ribosomes were purified from *Escherichia coli* MRE600 (ATCC29417) as described (Moazed and Noller, 1986). His-tagged Initiation factors 1 and 3 were cloned into *Bam*HI and *Xho*I sites of pPROEXHtb1. The proteins were purified over Ni-NTA resin. After removal of the His tag by tobacco etch virus (TEV) protease, the proteins were passed one more time over Ni-NTA resin and the flowthrough was collected. Finally the proteins were dialyzed against storage buffer composed of 40 mM Tris-HCl pH 7.5, 10 mM MgCl<sub>2</sub>, 100 mM KCl, 1 mM DTT and 50% glycerol. His-tagged RF1 and the 20 aminoacyl-tRNA synthetases were expressed and purified on Ni-NTA resin as previously described (Shimizu et al., 2001). His6-tagged EF-Tu and EF-G were purified using protocols published earlier (Blanchard et al., 2004). Overexpressed His-tagged RF2 was purified as described (Dincbas-Renqvist et al., 2000). mRNA templates were generated from double-stranded DNA templates by runoff transcription using T7 RNA polymerase (Zaher and Unrau, 2004) followed by PAGE purification. Sequences of mRNA transcripts used for initiation complexes were: GGGCAGAGGAGGUAAAAAA AUG X UUG UAC AAA where X represents the codon occupying the A-site. RNA oligos with modified bases were purchased from Midland Scientific, Thermoscientific (Dharmacon) and Chemgenes.

Oligo	Sequence	Source
8-oxoVal	CAGAGGAGGUAAAAAA AUG (8-oxo-rG)UU UUG UAC AAA	Dharmacon
8-oxoArg	CAGAGGAGGUAAAAAA AUG C(8-oxo-rG)C UUG UAC AAA	Dharmacon
8-oxoGlu	CAGAGGAGGUAAAAAA AUG GA(8-oxo-rG) UUG UAC AAA	Dharmacon
8-oxo Stop	CAGAGGAGGUAAAAAA AUG U(8-oxo-rG)A UUG UAC AAA	Dharmacon
8-oxoGly	CAGAGGAGGUAAAAAA AUG G(8-oxo-rG)C UUG UAC AAA	Midland Sci.
8-oxoHZ1	GGACUACAAAGAC (8-oxo-rG)AC GACGACAAGUAAUCUCU	Chemgenes

*E. coli* tRNA<sup>fMet</sup>, tRNA<sup>Phe</sup>, tRNA<sup>Val</sup>, tRNA<sup>Tyr</sup>, tRNA<sup>Glu</sup>, tRNA<sup>Lys</sup>, tRNA<sup>Arg</sup> and tRNA<sup>Met</sup> were purchased from ChemBlock. Total *E. coli* tRNA was purchased from Roche. BSA and all chemicals, except when noted, were purchased from Sigma-Aldrich. 8-hydroxyguanosine was purchased from VWR (manufacturer: Enzo Lifesciences). Mouse monoclonal anti 8-OHG (15A3) was purchased from Santa-Cruz Biotechnology.

Yeast knockout strains were obtained from Thermo Openbiosystems, on background BY4741 (MATa (his3Δ1 leu2Δ0 met15Δ0 ura3Δ0). Double knockout strains were obtained by crossing the two mutants followed by dissection of the tetrad.

Yeast Strain	Genotype
BY4741 (WT)	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0
dom34Δ	BY4741 dom34Δ::kanMX
xrn1Δ	BY4741 xrn1Δ::kanMX
dom34Δ xrn1Δ	BY4741 dom34Δ::kanMX xrn1Δ::kanMX
ski2Δ	BY4741 ski2Δ::kanMX
dom34Δ ski2Δ	BY4741 dom34Δ::kanMX ski2Δ::kanMX
upf1Δ	BY4741 upf1Δ::kanMX
dom34Δ comp	BY4741 dom34Δ::kanMX his3::DOM34 hisMX
xrn1Δ comp	BY4741 xrn1Δ::kanMX his3::XRN1 hisMX

**Ribosome complexes.** Initiation complexes were prepared by incubating 70S ribosomes (2 μM) with f-[<sup>35</sup>S]-Met-tRNA<sup>fMet</sup>, IF1, IF2, IF3 (each 3 μM), mRNA (6 μM) and GTP (2 mM) in polymix buffer (95 mM KCl, 5 mM NH<sub>4</sub>Cl, 5 mM Mg(OAc)<sub>2</sub>, 0.5mM CaCl<sub>2</sub>, 8 mM putrescine, 1 mM spermidine, 5 mM potassium phosphate pH7.5, 1 mM DTT) (Jelenc and Kurland, 1979) at 37°C for 15 min. These were layered over a 700 mL sucrose cushion (1.1 M sucrose, 20 mM Tris-HCl pH 7.5, 500 mM NH<sub>4</sub>Cl, 10 mM MgCl<sub>2</sub>,

0.5 mM EDTA) and spun in a MLA-130 rotor at 69,000 rpm for 2 hours to remove unincorporated factors. The pellet was resuspended in polymix buffer and stored at -80°C. The fraction of radioactivity that pelleted with the complex was used to calculate the concentration of the complex.

Ternary complexes were formed by initially incubating EFTu (final concentration 30 μM) with 2 mM GTP at 37°C for 15 minutes to exchange the bound GDP for GTP. The appropriate aa-tRNA (charged from total tRNA) was added to a final concentration of ~2 μM, and the mixture was further incubated for an additional 15 minutes.

**tRNA aminoacylation.** Formylation/aminoacylation of tRNA<sup>fMet</sup> was carried out as described (Walker and Fredrick, 2008). Pure tRNAs were charged by incubating the tRNA at 10 mM together with the appropriate tRNA synthetase (~1 mM), amino acid (400 mM), and 2 mM ATP in the following buffer: 20mM Tris-HCl pH 7.5, 20 mM MgCl<sub>2</sub>, 1 mM DTT. After 30 min of incubation at 37°C, tRNAs were phenol/chloroform extracted and ethanol precipitated before resuspending in 20 mM potassium acetate (pH 5.2) buffer supplemented with 1 mM DTT. A similar procedure was used to charge individual tRNAs from total tRNA mix by adding the appropriate tRNA synthetase and the amino acid and increasing the total tRNA concentration to 100 mM. For consistency, we used aa-tRNAs from the bulk total tRNA for dipeptide reactivity surveys. In contrast, except for the Gly-tRNA<sup>Gly</sup> and Leu-tRNA<sup>Leu</sup> - which are not available commercially, pure aa-tRNAs were used to determine the rates of peptide bond formation.

**Bacterial and eukaryotic in vitro translation.** RNA used to generate templates for cell free assays was prepared using runoff transcription on PCR-amplified DNA templates and T7 RNA polymerase, followed by PAGE purification. To eliminate heterogeneity at the ligation site, the 3' end contained a self-cleaving hammerhead ribozyme sequence, which results in a 2'-3' cyclic phosphate after cleavage. The ends were dephosphorylated using T4 polynucleotide kinase (NEB) in 100 mM morpholinoethanesulfonate (MOPS)-NaOH [pH 5.5], 10 mM MgCl<sub>2</sub>, 10 mM β-mercaptoethanol, 300 mM NaCl (Schurer et al., 2002) leaving a 3'-OH group; the reaction was incubated at 37°C for 5 hours, extracted with phenol/chloroform and ethanol precipitated.

An RNA oligo with a modified guanosine was purchased from Chemgenes (5'-GGACUACAAAGAC (8-oxo-rG) ACGACGACAAGUAAUCUCU); the 5' end of the oligo



was phosphorylated using T4 PNK (NEB) in the presence of ATP. The oligo was then ligated to the 3' end of the RNA template using T4 RNA ligase 2 (NEB) and a DNA splint at a molar ratio of (1:1.5:1.2). The RNA and DNA were combined, heated to 80°C, and slowly cooled to room temperature before addition of buffer and ligase followed by incubation at 37°C overnight. The ligated products were purified on denaturing PAGE, eluted in 300 mM NaCl overnight and ethanol precipitated.

For assays in eukaryotic extracts, both the ligated RNA and transcribed RNA controls were capped using the *Vaccinia* capping system (NEB) followed by addition of a 3' polyA sequence using *E coli* polyA polymerase (NEB). Reactions were assembled per the manufacturer's instructions and incubated for 30 min at 37°C followed by phenol/chloroform extraction and ethanol precipitation.

In vitro translation assays were carried out in S30 extracts, rabbit reticulocyte lysates and wheat germ extracts (Promega) per the manufacturer's instructions in the presence of [<sup>35</sup>S]-methionine (1175 Ci/mmol) at 0.5 mCi/mL. S30 reactions were incubated at 37°C for one hour and stopped by chilling on ice. Rabbit reticulocyte and wheat germ extract reactions were incubated for 90 min at 30°C and 25°C, respectively, and were stopped either by chilling on ice or by addition of 100 mM KOH. RNase A was added to samples to observe elimination of peptidyl tRNA. Samples were acetone precipitated, pellets were resuspended in Tricine sample buffer and radiolabeled proteins resolved on 16% bis-Tris PAGE or 16% Tris-tricine PAGE. To assess protein stability in the presence of proteasome inhibitor, 20 μM MG132 was included in the reaction.

A set of mRNAs was capped and polyadenylated as above, except the polyA reaction included [ $\alpha$ -<sup>32</sup>P]-ATP to label the 3' polyA sequence. These were added to translation reactions in wheat germ extracts to monitor the stability of the mRNAs during the incubation.

**8-hydroxyguanosine conjugation to BSA.** 8-hydroxyguanosine (8-oxoG) was conjugated to BSA as described earlier (Erlanger and Beiser, 1964) using the following procedure: Equal volumes of 10 mg/mL 8-oxoG and 0.2 M NaIO<sub>4</sub> were combined and incubated for 20 min with shaking. Excess periodate was quenched with ethylene glycol (to 5%) followed by addition of 17 mg/mL BSA in 20 mM Na<sub>2</sub>CO<sub>3</sub> (pH 9.5). After shaking the mixture for 45 min, 50 μL of 5 mg/mL NaBH<sub>3</sub>CN was added and the mixture was incubated overnight at room temperature. The conjugate was dialyzed two times against

PBS before storing at 4°C. The conjugate was characterized by measuring the UV spectra, which indicated that ~25-30 molecules of 8-oxoG was conjugated to one molecule of BSA.

**RNA purification** Total RNA was collected from yeast cultures at mid-log phase using the hot phenol method (Kohrer and Domdey, 1991). polyA-enriched RNA was isolated from each sample using polyd(T) sepharose. polyd(T) sepharose was generated by coupling ~300 nmole of amino-modified d(T)<sub>25</sub> DNA oligo (IDT) to 1 g of CNBr-activated sepharose (GE lifesciences) as per (Chockalingam et al., 2001). Briefly, CNBr-sepharose was washed in 1mM HCl followed by incubation with the oligo overnight in 0.1 M boric acid pH 8. The next day, excess reactive groups were blocked using 0.5 M glycine in 0.1 M boric acid pH 8 and finally the resin was washed and stored in 20% ethanol.

For polyA purification, total RNA was heated to 65°C for 5 minutes, cooled briefly and added to polyd(T) sepharose in binding buffer (10 mM Tris-HCl pH 7.5, 0.4 M NaCl, 1mM EDTA, 0.1% SDS). The resin was washed with 5 column volumes wash buffer (10mM Tris-HCl pH7.5, 0.04M NaCl, 1mM EDTA, 0.1% SDS). Bound RNA was eluted with 20 mM KOH, followed by immediate neutralization with 300 mM sodium acetate pH 5.1. The RNA was concentrated using ethanol precipitation. For competitive ELISA assays, total and polyA-enriched RNA was digested with P1 nuclease (USBio) at 50°C overnight, and dephosphorylated using CIP (NEB) for 1 hour before adding to pre-treated 96-well plates.

**ELISA.** The ELISA plate pretreatment procedure was as follows: 1) Plates were coated by adding 100 µL of 0.5 µg/mL of 8-oxoG-conjugated BSA in 0.1 M carbonate buffer pH 9.5 to each well; the plates were then incubated at room temperature for 4 to 8 hours before washing two times with 200 µL of PBST. 2) Plates were then blocked by adding 200 µL of 1% BSA in PBS to each well and incubating them at 4°C overnight. 3) After washing the plate two times with PBST, 50 µL of digested RNA sample (corresponding to 25 µg total RNA or 3 µg polyA RNA) in 0.1% BSA in PBS was added in triplicates to the wells followed by the addition of 50 µL of 1/2000 mouse anti-8-OHG primary antibody; the mixture was incubated at room temperature for 3-5 hours before washing 3 times with PBST. 4) 100 µL of 1/3000 HRP-conjugated secondary antibody (Pierce) in PBS was added to each well and the mixture was incubated at room temperature for 1-2

hours. 5) After washing with PBST three times, 75  $\mu$ L of room-temperature-equilibrated TMB substrate (Pierce) was added to each and the color was allowed to develop for 5-30 minutes before stopping the reaction with 2M H<sub>2</sub>SO<sub>4</sub>. Absorbance readings were collected at 450 nm using a plate reader (Tecan).

**Polysomes analysis.** Yeast cultures were grown to mid-log phase before adding 100  $\mu$ g/mL cyclohexamide, chilling and pelleting the cells at 4°C. Cells were then resuspended in polysome lysis buffer (20 mM Tris pH 8, 15 mM KCl, 1 mM MgCl<sub>2</sub>, 0.5 mM DTT, 0.1  $\mu$ g/mL cyclohexamide, 0.2  $\mu$ g/mL heparin), washed once and lysed with glass beads using a FastPrep (MP Biomedical). Supernatant from cleared lysate corresponding to 20 mg of total RNA was layered over a 10-50% sucrose gradient and centrifuged at 26,000 rpm for 270 min in an SW28 (Beckman) swinging bucket rotor. Gradients were fractionated using a Brandel tube-piercing system combined with continuous absorbance reading at A<sub>280nm</sub>. RNA was isolated using phenol/chloroform extraction in the presence of 1% SDS followed by ethanol precipitation. The extraction/precipitation protocol was repeated to get pure RNA samples. polyA-enrichment and competitive ELISA was conducted as described earlier.

**Quantitative RT-PCR.** Total RNA from different yeast strains was DNase treated and cDNA was amplified using M-MuLV reverse transcriptase (NEB). qRT-PCR reactions were assembled using ~50ng of cDNA, random hexamer and Evagreen SYBR reaction mix (Lambda Biotech) together with probe primers specific for *dom34*, *xrn1* and *act1*. Data was normalized using *act1* and relative fold change was calculated using the  $\Delta\Delta$ Ct method.

qRT-PCR Probe	Sequence (5'-3')
act1.1	ATTTGGCCGGTAGAGATTTG
act1.2	TCCAAGGCGACGTAACATAG
dom34.1	GGCTGAGTATGGCGCAATTA
dom34.2	CCTCCATTGCTCTCTACGGA
xrn1.1	ATCGCGGTATCTTTGGAGTC
xrn1.2	ACAGCCTCACGAGGAAC TTT

## Supplemental References

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