SUPPLEMENTAL MATERIAL

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   1 ATGGAAGACG CCAAAAACAT AAAGAAAGGC CCGGCGCCAT TCTATCCGCT GGAAGATGGA ACCGCTGGAG AGCAACTGCA TAAGGCTATG
  91 AAGAGATACG CCCTGGTTCC TGGAACAATT GCTTTTACAG ATGCACATAT CGAGGTGGAC ATCACTTACG CTGAGTACTT CGAAATGTCC
 181 GTTCGGTTGG CAGAAGCTAT GAAACGATAT GGGCTGAATA CAAATCACAG AATCGTCGTA TGCAGTGAAA ACTCTCTTCA ATTCTTTATG
 271 CCGGTGTTGG GCGCGTTATT TATCGGAGTT GCAGTTGCGC CCGCGAACGA CATTTATAAT GAACGTGAAT TGCTCAACAG TATGGGCATT
 361 TCGCAGCCTA CCGTGGTGTT CGTTTCCAAA AAGGGGTTGC AAAAAATTTT GAACGTGCAA AAAAAGCTCC CAATCATCCA AAAAATTATT
 451 ATCATGGATT CTAAAACGGA TTACCAGGGA TTTCAGTCGA TGTACACGTT CGTCACATCT CATCTACCTC CCGGTTTTAA TGAATACGAT
 541 TTTGTGCCAG AGTCCTTCGA TAGGGACAAG ACAATTGCAC TGATCATGAA CTCCTCTGGA TCTACTGGTC TGCCTAAAGG TGTCGCTCTG
 631 CCTCATAGAA CTGCCTGCGT GAGATTCTCG CATGCCAGAG ATCCTATTTT TGGCAATCAA ATCATTCCGG ATACTGCGAT TTTAAGTGTT
 721 GTTCCATTCC ATCACGGTTT TGGAATGTTT ACTACACTCG GATATTTGAT ATGTGGATTT CGAGTCGTCT TAATGTATAG ATTTGAAGAA
 811 GAGCTGTTTC TGAGGAGCCT TCAGGATTAC AAGATTCAAA GTGCGCTGCT GGTGCCAACC CTATTCTCCT TCTTCGCCAA AAGCACTCTG
 901 ATTGACAAAT ACGATTTATC TAATTTACAC GAAATTGCTT CTGGTGGCGC TCCCCTCTCT AAGGAAGTCG GGGAAGCGGT TGCCAAGAGG
 991 TTCCATCTGC CAGGTATCAG GCAAGGATAT GGGCTCACTG AGACTACATC AGCTATTCTG ATTACACCCG AGGGGGATGA TAAACCGGGC
1081 GCGGTCGGTA AAGTTGTTCC ATTTTTTGAA GCGAAGGTTG TGGATCTGGA TACCGGGAAA ACGCTGGGCG TTAATCAAAG AGGCGAACTG
1171 TGTGTGAGAG GTCCTATGAT TATGTCCGGT TATGTAAACA ATCCGGAAGC GACCAACGCC TTGATTGACA AGGATGGATG GCTACATTCT
1261 GGAGACATAG CTTACTGGGA CGAAGACGAA CACTTCTTCA TCGTTGACCG CCTGAAGTCT CTGATTAAGT ACAAAGGCTA TCAGGTGGCT
1351 CCCGCTGAAT TGGAATCCAT CTTGCTCCAA CACCCCAACA TCTTCGACGC AGGTGTCGCA GGTCTTCCCG ACGATGACGC CGGTGAACTT
1441 CCCGCCGCCG TTGTTGTTTT GGAGCACGGA AAGACGATGA CGGAAAAAGA GATCGTGGAT TACGTCGCCA GTCAAGTAAC AACCGCGAAA
1531 AAGTTGCGCG GAGGAGTTGT GTTTGTGGAC GAAGTACCGA AAGGTCTTAC CGGAAAACTC GACGCAAGAA AAATCAGAGA GATCCTCATA
1621 AAGGCCAAGA AGGGCGGAAA GATCGCCGTG (***)
1687 CTGCAGTATC CGTATGACGT CCCGGACTAT GCAGGTTCCT ATCCATATGA CGTTCCAGAT TACGCTTCTA GATATCCGTA TGACGTCCCG
1777 GACTATGCAT GATGACACCG ATTATTTAAA GCTACAACAT ACGATATATA TACATGTGTA TATATGTATA CCTATGAATG TCAGTAAGTA
1867 TGTATACGAA CAGTATGATA CTGAAGATGA CAAGGTAATG CATCATTCTA TACGTGTCAT TCTGAACGAG GCGCGCTTTC CTTTTTTCTT
1957 TTTGCTTTTT CTTTTTTTTT CTCTTGAACT CGAGAAAAAA AATATAAAAG AGATGGAGGA ACGGGAAAAA GTTAGTTGTG GTGATAGGTG
2047 GCAAGT
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K12      (***) = AAGAAGAAAA AGAAGAAAAA GAAGAAAAAG AAGAAA

RZ       (***) = CCTGTCACCG GATGTGTTTT CCGGTCTGAT GAGTCCGTGA GGACGAAACA GGGG

RZ*      (***) = CCTGTCCAAG GATGTGTTTT CCGGTCTGAT GAGTCCGTGA GGACGAAACA GGGG
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Figure S1. Luciferase reporters. Nucleotide sequences of *LUC, LUC-K12-3HA*, *LUC-RZ-3HA*, *LUC-RZ*-3HA,* and *LUC-RZt*. Shown is the luciferase *orf*, the three *HA* sequences (blue), the stop codon (red), and the 3´-*UTR*. The position at which *K12*, *RZ*, or *RZ** is inserted is indicated (***). The nucleotide sequence of *K12*, *RZ*, or *RZ** is given below. Numbering of the nucleotide sequence refers to *LUC-K12-3HA*. Cleavage of *LUC-RZ-3HA* occurs 3´ of *CCTCTG* within the *RZ* sequence (red arrow head). The position of the Northern probe is highlighted in yellow.

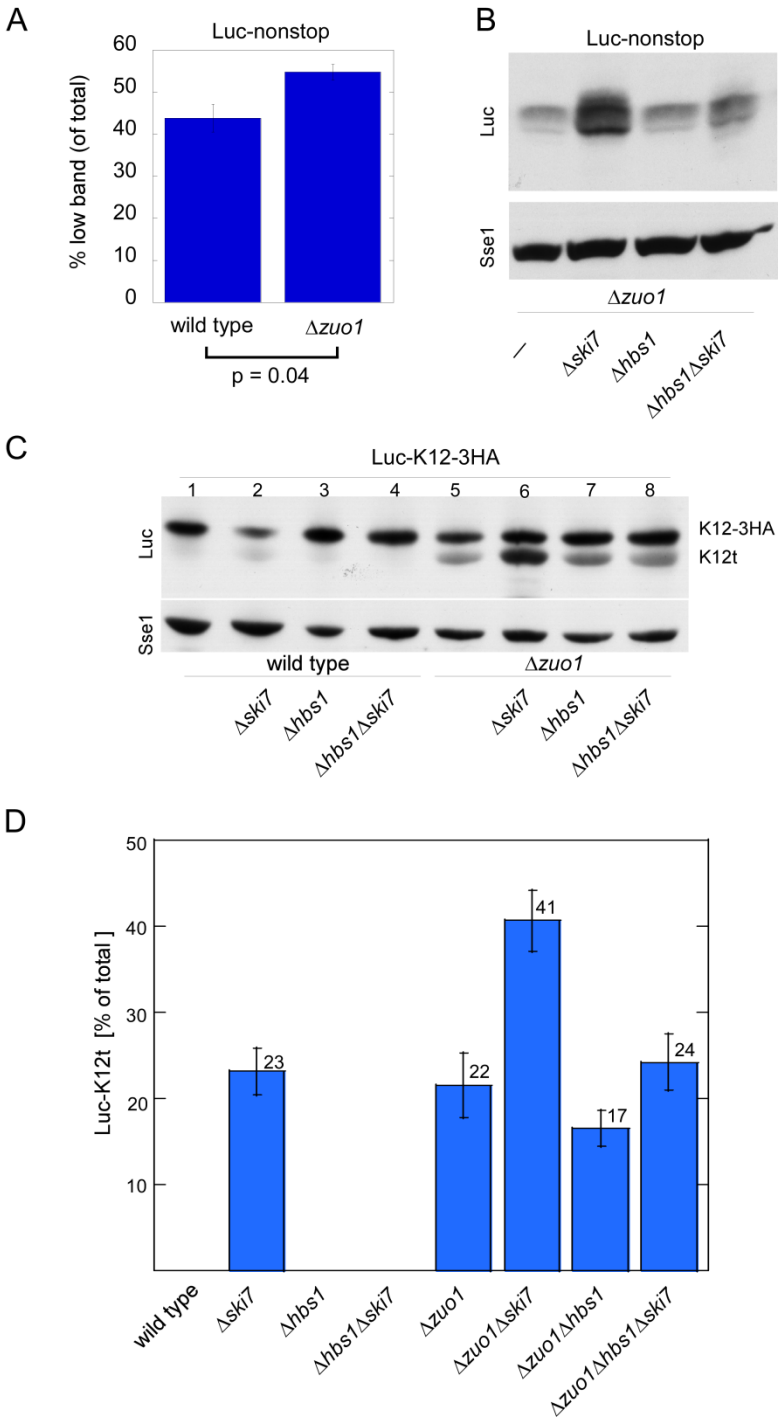

Figure S2. Additive effects of Δ*ski7* and Δ*zuo1* in respect to the generation of truncated protein species. (A) The Δ*zuo1* mutation exerted a minor effect on the expression of truncated nonstop protein species. Shown is the quantification of 3 independent experiments, performed as in Fig. 2B. Error bars represent the standard error of the mean. The p value was calculated via Student´s T-test. (B) Expression of Luc-nonstop depends mainly on Ski7 and Hsb1•Dom34. Luc-nonstop was expressed in Δ*zuo1*, Δ*zuo1*Δ*ski7,* Δ*zuo1*Δ*hbs1,* and Δ*zuo1*Δ*hbs1*Δ*ski7* strains. Total extracts of strains expressing Luc-nonstop were analyzed via immunoblotting with antibodies recognizing luciferase (Luc) and, as a loading control, Sse1. (C) The Δ*zuo1 and* Δ*ski7* mutations exerted additive effects on the expression of Luc-K12t. The indicated strains harboring the Luc-K12-3HA reporter were analyzed as described in B. The position of Luc-K12-3HA (K12-3HA) and Luc-K12t (K12t) is indicated. (D) Quantification of the data presented in C. The quantification was performed as described in Fig. 5B. Shown are results of at least 3 independent experiments; error bars represent the standard error of the mean.

Explanatory text

Hbs1•Dom34 and RAC/Ssb affect nonstop and polylysine protein expression via distinct mechanisms.

The data presented in this manuscript revealed that truncated luciferase species can be generated by two distinct mechanisms, which differ in ribosome-release of the truncated polypeptide (Fig. 10). One mechanism was dependent on Sup35, which induced premature termination on polylysine-stalled ribosomes (Fig. 9). Sup35-dependent premature termination occurred at a sense codon and was independent of whether or not the mRNA contained a stop codon. Premature termination was enhanced in the absence of RAC/Ssb (Fig. 1). The second mechanism was dependent on Hbs1•Dom34, which induced polypeptide release from ribosomes stalled at the 3´-end of a stop codon-less mRNA (Fig. 3F and Fig. 4). Because Ski7 is involved in the efficient degradation of stop codon-less mRNA, Hbs1•Dom34-dependent expression was enhanced in a Δ*ski7* strain (Fig. 3D and 3F). This mechanism was independent of Sup35 and RAC/Ssb (Fig. 3D and 3G). For details and references compare Results and Discussion.

The origin of truncated protein species may be complex, because both mechanisms can contribute to the expression of a single reporter construct. For example, Luc-nonstop and Luc-K12-3HA contain polylysine segments (Fig. 1A and 1B), which cause ribosome stalling followed by endonucleolytic cleavage (Fig. 1A and Fig. 10). As a result, expression of Luc-nonstop and Luc-K12-3HA reporters gave rise to mixed mRNA populations, from which truncated proteins were generated either by Hbs1•Dom34-dependent release (stop codon-less templates), which was enhanced in the absence of Ski7, or Sup35-dependent release (full length templates), which was enhanced in the absence of RAC/Ssb (Fig. S2).

Hbs1•Dom34-dependent release dominates upon Luc-nonstop expression

The Δ*zuo1* mutation exerted a rather minor effect on the production of truncated Luc-nonstop protein fragments (Fig. 2B and S2A). This suggested that Sup35-dependent release played only a minor role for the expression of truncated Luc-nonstop species. Consistently, the high level of Luc-nonstop expression in Δ*zuo1*Δ*ski7* (Fig. S2B) was strongly reduced in a Δ*zuo1*Δ*hbs1*Δ*ski7* strain (Fig. S2B). The finding indicated that the bulk of Luc-nonstop was derived from Hbs1•Dom34-dependent translation of stop codon-less mRNA species.

Hbs1•Dom34 and Sup35 contribute equally to Luc-K12t expression

Luc-K12-3HA expression was analyzed using the same set of strains employed for the analysis of the Luc-RZ-3HA reporter (Fig. 3D). In the wild type background (Fig. S2C lane 1-4, Fig. S2D) a fragment corresponding in size to Luc-K12t was detected in the Δ*ski7* strain (Fig. S2C lane 2), but was not detected in the Δ*hbs1*Δ*ski7* strain (Fig. S2C, lane 4). This indicated the presence of stop codon-less mRNA fragments, which were stabilized by the Δ*ski7* mutation and were expressed in an Hbs1•Dom34-dependent manner. In the Δ*zuo1* background (Fig. S2C lane 5-8, Fig. S2D) the effect of Δ*ski7* and Δ*hbs1* was similar to that in the wild type background: when compared to the Δ*zuo1* strain, expression of Luc-K12t was increased by ~ 20% in Δ*zuo1*Δ*ski7*, and was similar to Δ*zuo1* in the Δ*zuo1*Δ*hbs1*Δ*ski7* strain (Fig. S2C lane 5, 6, and 8). On top of that, Luc-K12t was generated in a process that was enhanced by the Δ*zuo1* mutation and was independent of Hbs1•Dom34 and Ski7:

expression of Luc-K12t in the Δ*ski7* and Δ*zuo1* strains was additive (Fig. S2C, lane 2, 5, and 6) and Luc-K12t expression in the Δ*zuo1* strain was not affected by the Δ*hbs1* mutation (Fig. S2C, lane 1, 3, 5, and 7). This is consistent with a model in which a significant fraction of Luc-K12t was generated by Sup35-dependent release.