Supplemental Figures

Figure S1. Quantification of [^{35}S]-Met-labeled protein bands of indicated Figures in Figure 2 and 3 by ImageJ.

Figure S2. (A) [^{35}S]-Met-labeled total translation products of WT *Luc* mRNA showing that the polypeptide species are stable after the addition of CHX. (B) Top panel: A diagram showing the stop-codon-less mRNA (StoplessNLUC), which contains N-terminus luciferase sequence (223 aa) with no stop codon followed by a 30 nt poly(A) tail. Bottom panel: [^{35}S]-Met-labeled total translation products of the *StoplessNLUC* mRNA. .After 10 min of translation, reactions were terminated by addition of cycloheximide or RNase A. Ribosome-associated nascent peptides were separated by sucrose cushion centrifugation. Sup: supernatant.

Figure S3. Expression level of eGFP-2A-LUC reporter constructs. (A) A long exposure of western blot result in Figure 4D. (B) Relative mRNA levels from eGFP-2A-OPT and eGFP-2A-WT reporter constructs measured by RT-qPCR. Data are means ± SD. *P < 0.05. **P < 0.01.

Figure S4. [^{35}S]-Met-labeled total translation products of WT or OPT *Luc* mRNA in cell-free translation extracts prepared from the wild-type and *dom34*^{KO} strains.

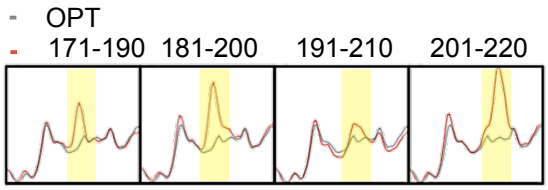
Figure S5. (A) [^{35}S]-Met-labeled in vitro translation products of the WT *Luc* mRNA using the WT *Neurospora* cell extracts in the presence of different concentrations of WT or mutant eRF1 proteins. 1x indicates 0.6 μM of eRF1. A short exposure of result in

Figure 6C is shown. (B) [^{35}S]-Met-labeled translation products of the WT *Luc* mRNA with the addition of GGQ mutant eRF1. RNase A (1 μg/μl) was added after 15 min of translation. (C) Quantification of the relative total levels of the full-length peptide and the full-length peptidyl-tRNA from independent experiments described in Figure 6C. Data are means ± SD. **P < 0.01. **** P < 0.0001. ns: not significant.

Figure S6. (A) RT-qPCR results showing the relative *eRF1* mRNA levels from the wild-type or eRF1-KD strains. Data are means ± SD. ***P < 0.001. (B) Real-time luciferase activity of WT *Luc* mRNA in cell-free translation extracts prepared from the wild-type or eRF1 knock-down strains. Recorded relative light units (RLUs) were plotted versus translation reaction time in 1-min intervals. Times of first appearance are indicated by arrows. Data are represented as mean ± SD. (C) Relative mRNA levels from OPT and M-WT reporter construct strains measured by RT-qPCR. Data are means ± SD. *ns: not significant.

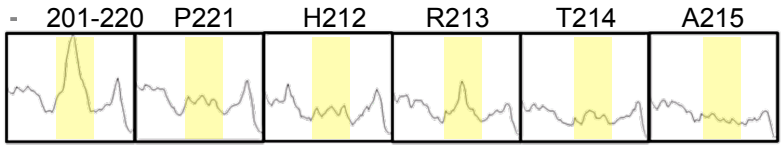
Figure S1

A



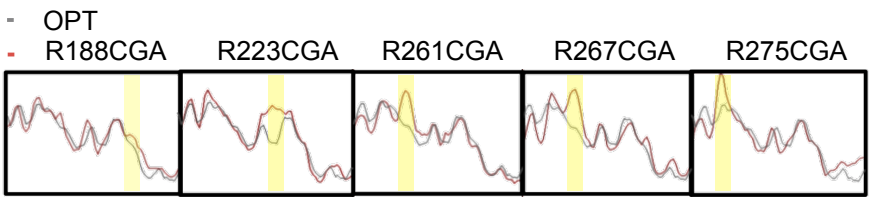
Related to Figure 2B

B



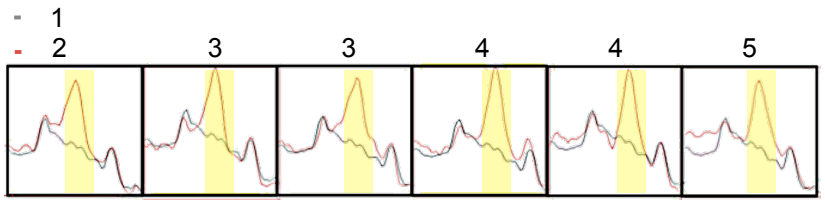
Related to Figure 2C

C



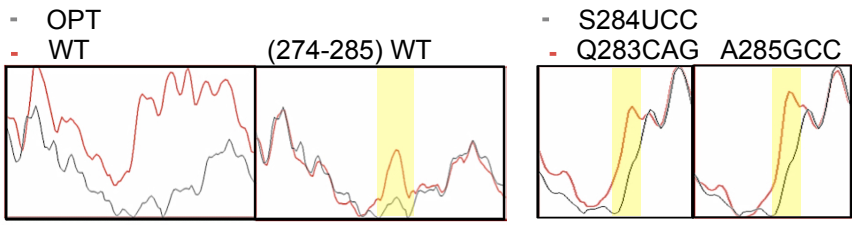
Related to Figure 2D

D



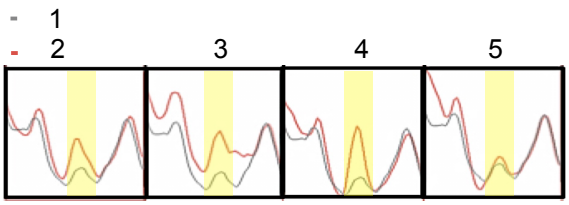
Related to Figure 2E

E



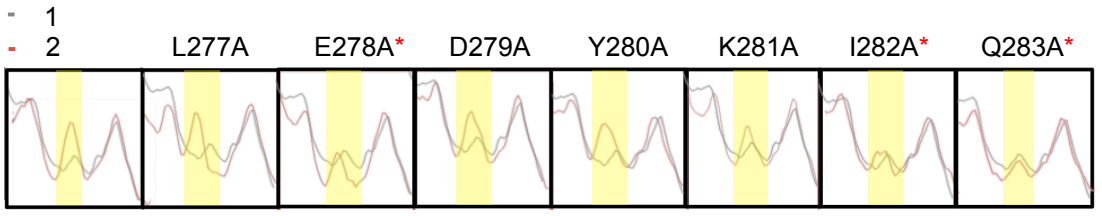
Related to Figure 3A

F



Related to Figure 3B Left panel

G



Related to Figure 3B Right panel

Figure S2

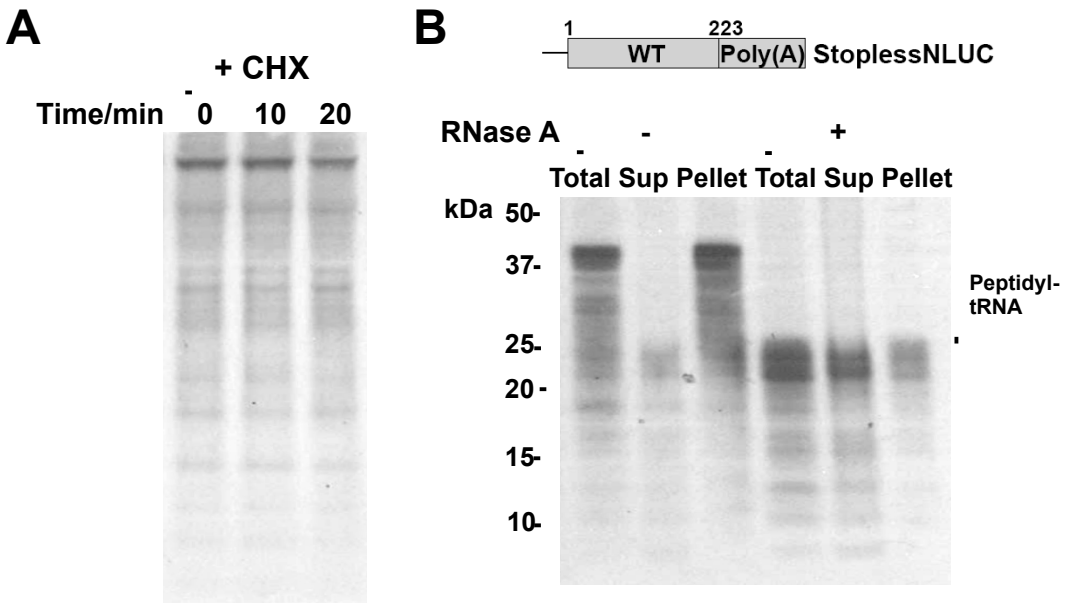

A
+ CHX
-
Time/min 0 10 20
B
1
223
WT
Poly(A)
StoplessNLUC
RNase A - -
+
Total Sup Pellet Total Sup Pellet
kDa
50-
37-
25-
20-
15-
10-
Peptidyl-
tRNA

Figure S3

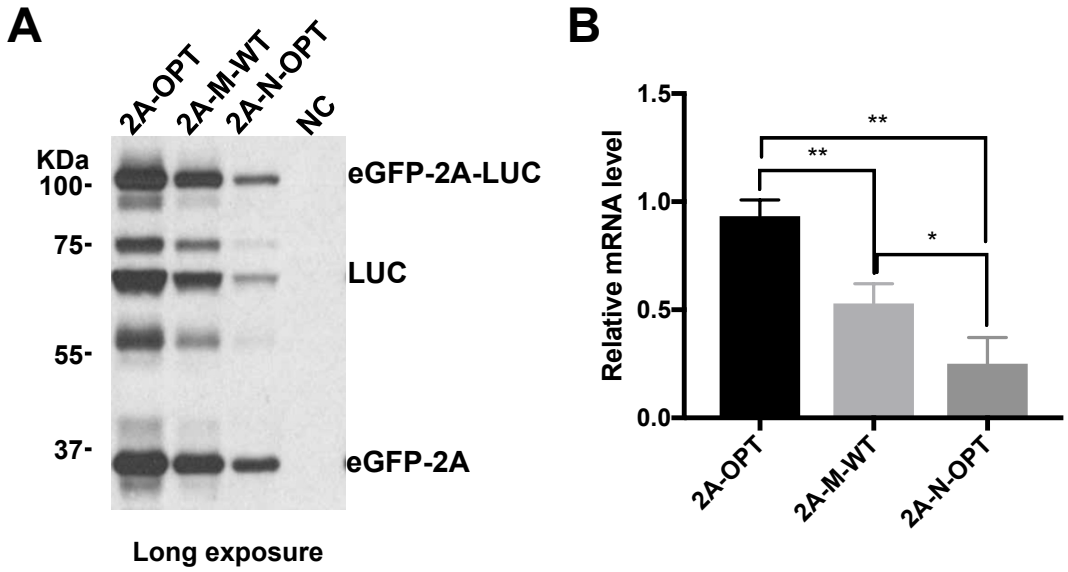

A
2A-OPT
2A-M-WT
2A-N-OPT
NC
KDa
100-
75-
55-
37-
eGFP-2A-LUC
LUC
eGFP-2A
Long exposure
B
Relative mRNA level
1.5
1.0
0.5
0.0
**
**
*
2A-OPT
2A-M-WT
2A-N-OPT

Figure S4

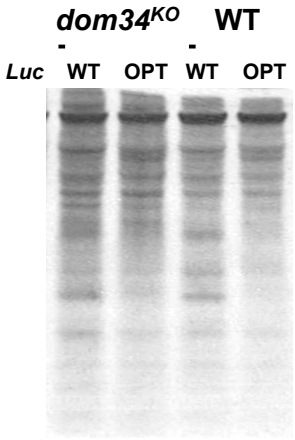


Figure S5

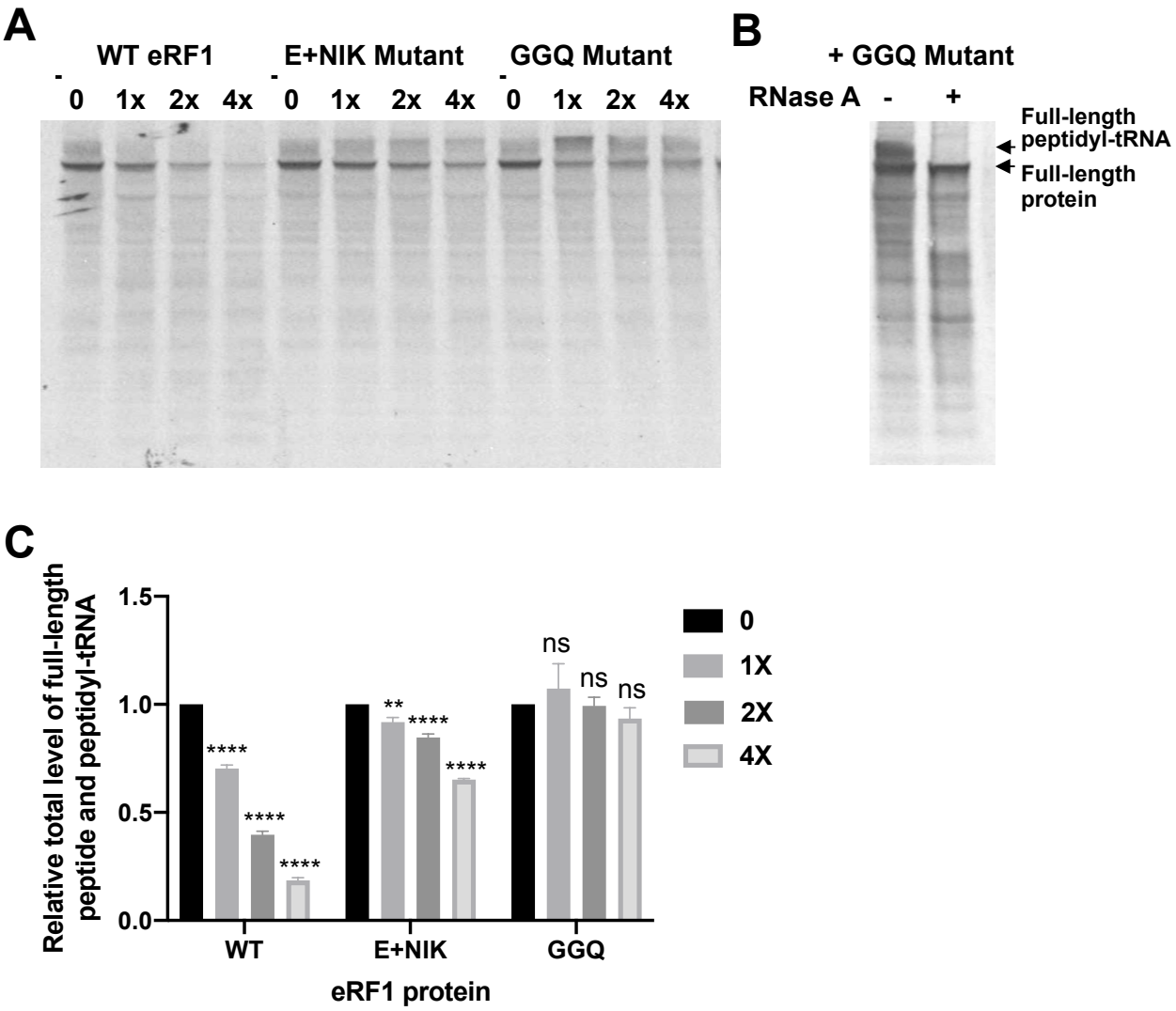
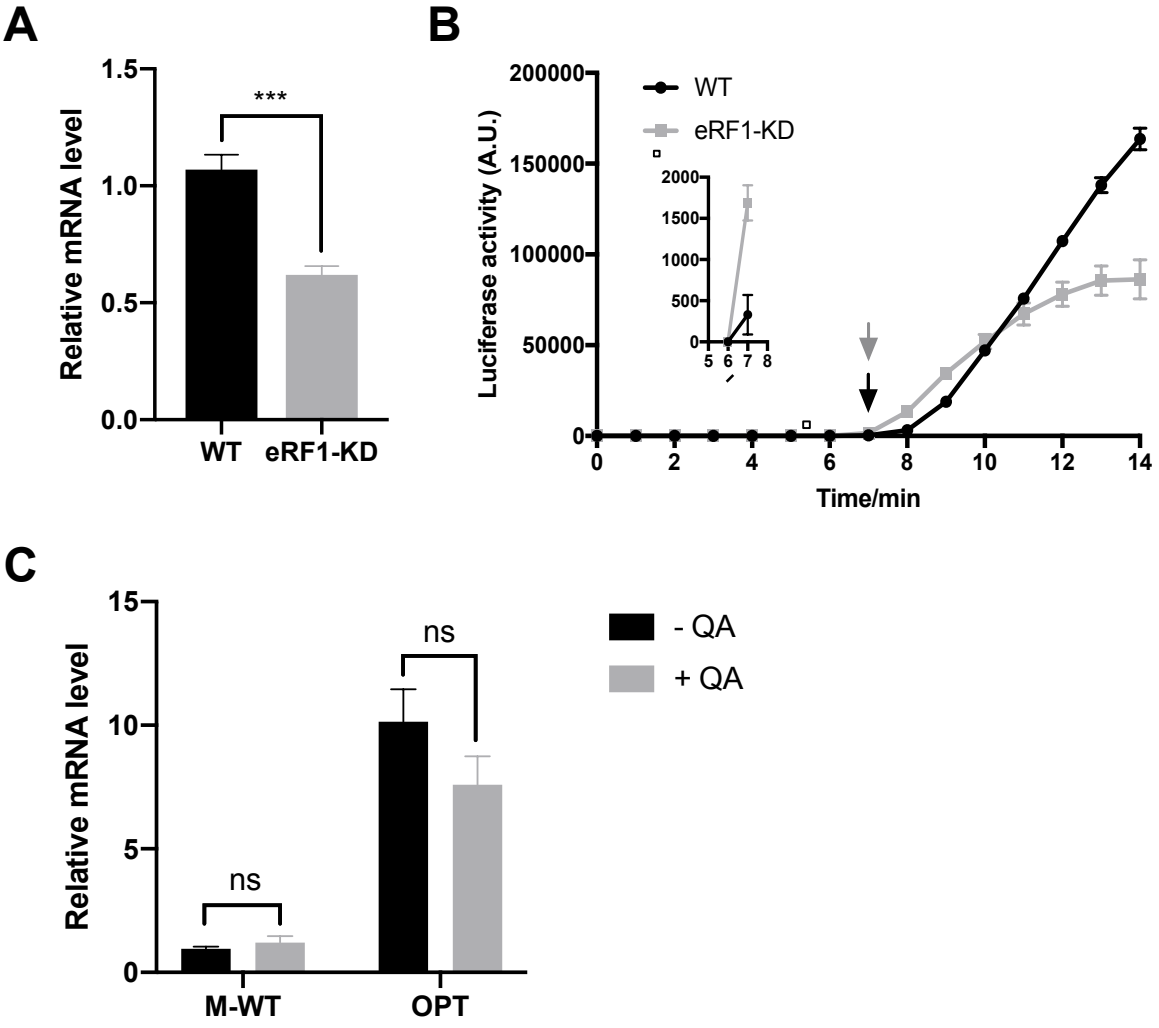

A
WT eRF1
E+NIK Mutant
GGQ Mutant
0 1x 2x 4x 0 1x 2x 4x 0 1x 2x 4x
B
+ GGQ Mutant
RNase A - +
Full-length peptidyl-tRNA
Full-length protein
C
Relative total level of full-length peptide and peptidyl-tRNA
1.5
1.0
0.5
0.0
****
****
****
**
****
****
ns
ns
ns
WT
E+NIK
GGQ
eRF1 protein
0
1X
2X
4X

Figure S6


A
Relative mRNA level
1.5
1.0
0.5
0.0
***
WT
eRF1-KD
B
Luciferase activity (A.U.)
200000
150000
100000
50000
0
WT
eRF1-KD
2000
1500
1000
500
0
5 6 7 8
0 2 4 6 8 10 12 14
Time/min
C
Relative mRNA level
15
10
5
0
ns
ns
M-WT
OPT
- QA
+ QA