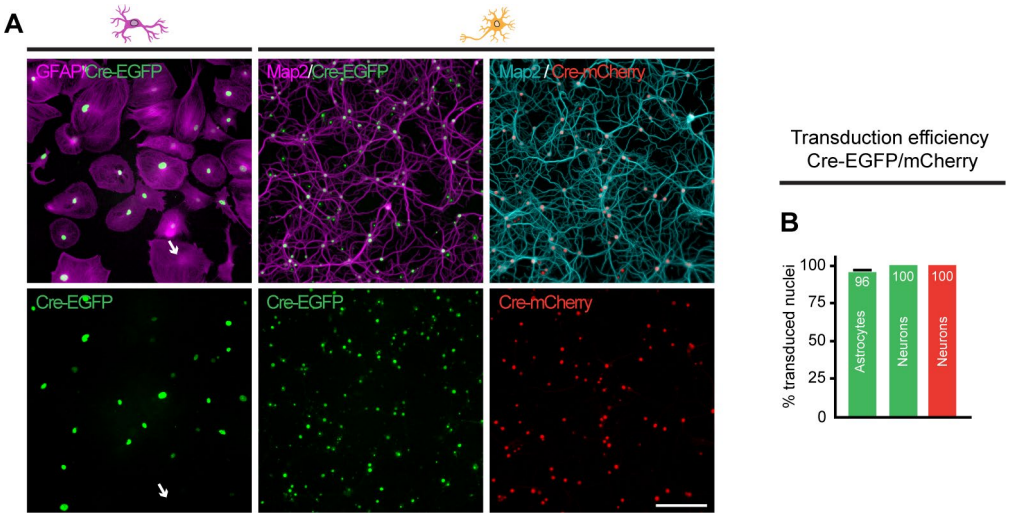
Appendix

Neurons shift translational control to secure proteostatic resilience during ER stress

Wolzak et al.

Content



Appendix Figure S1: Transduction efficiency of lentivirus expressing Cre-recombinase fused to EGFP or mCherry in primary astrocytes and neurons - Related to Figure 1

A Representative images obtained by high-content microscopy. Nuclei (Cre-EGFP, green; Cre-mCherry, red), astrocytic soma (GFAP, magenta), dendrites (MAP2, magenta/cyan). Scale bar: 200 µm. Arrow: Untransduced cell.

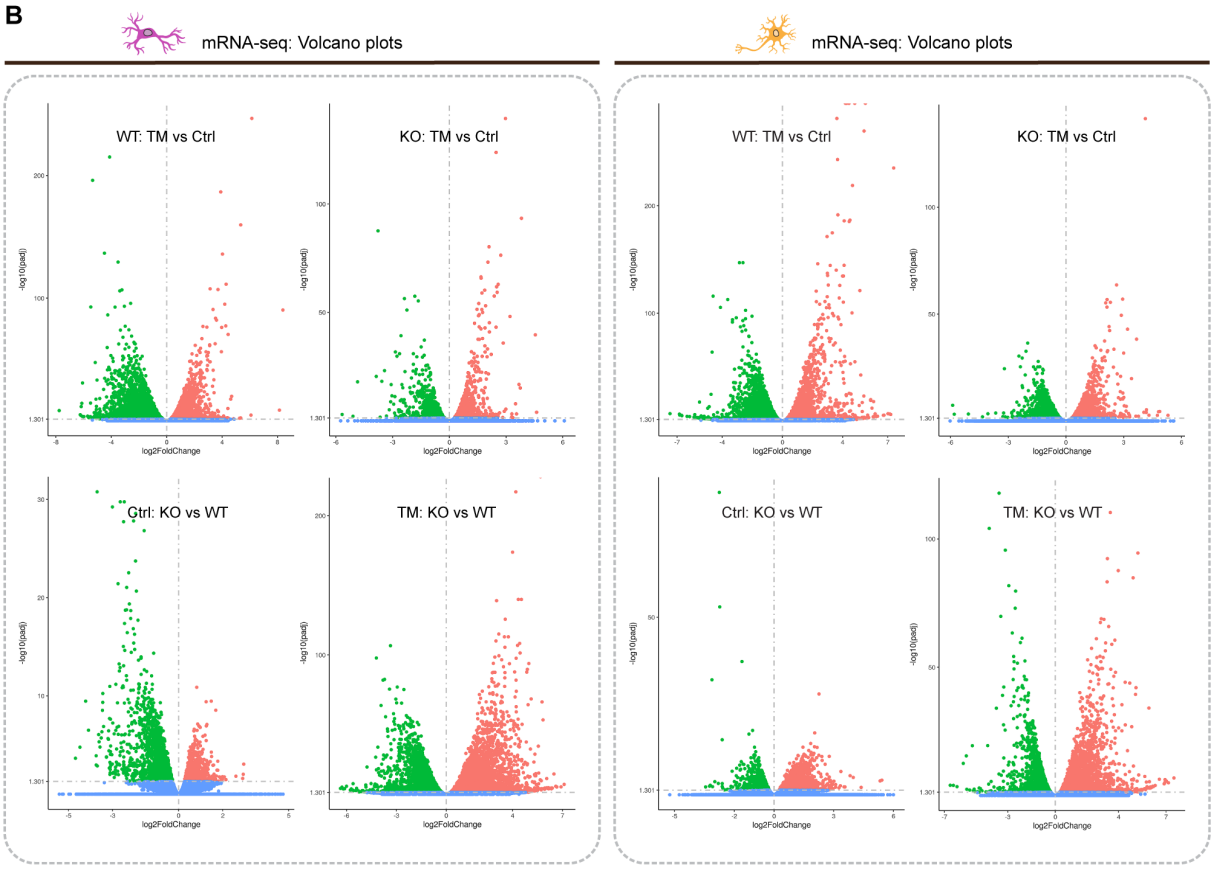
B Quantification of percentage of tranduced cells (Astrocytes n = 10k cells/18 wells, Neurons 14k cells/12 wells). Data are presented as mean ± SEM. Source data is available online.

A

mRNA-seq: Transcripts and DEGs

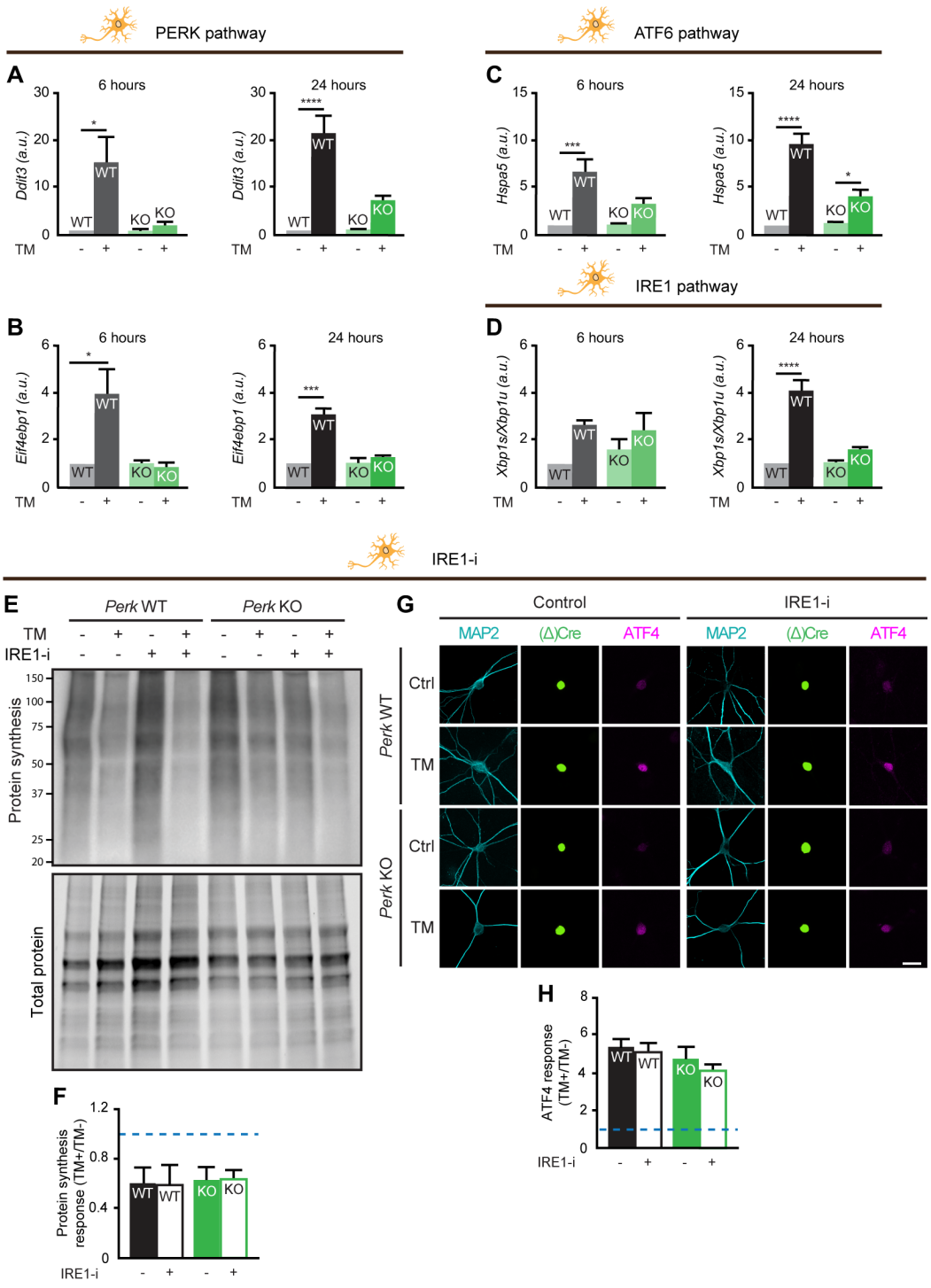
Comparison	Not	Up	Down
WT: TM vs Ctrl	16956	3808	4127
KO: TM vs Ctrl	23305	997	889
Ctrl: KO vs WT	22171	1040	1923
TM: KO vs WT	15475	4772	4772
WT: TM vs Ctrl	18453	3897	3960
KO: TM vs Ctrl	24756	2092	1966
Ctrl: KO vs WT	24417	2108	1354
TM: KO vs WT	21154	3421	2974

B



Appendix Figure S2: The effect of PERK deficiency and ER stress on the transcriptome of astrocytes and neurons - Related to Figure 2, 4 and 7

A, B WT and *Perk* KO neurons and astrocytes ± 20-24 hours of TM-induced ER stress were analysed by mRNA-seq (N=3). (A) The number of genes of which expression is not altered (Not), higher (Up) or lower (Down) in the comparisons as indicated. (B) Volcano plots of DEGs in the comparisons as indicated. X-axis: fold change in expression. Y-axis: adjusted p-values of changes in gene expression levels. Dots represent genes; blue: no significant difference, red: upregulated differentially expressed genes (DEGs), green: downregulated DEGs. N: Biological replicate.

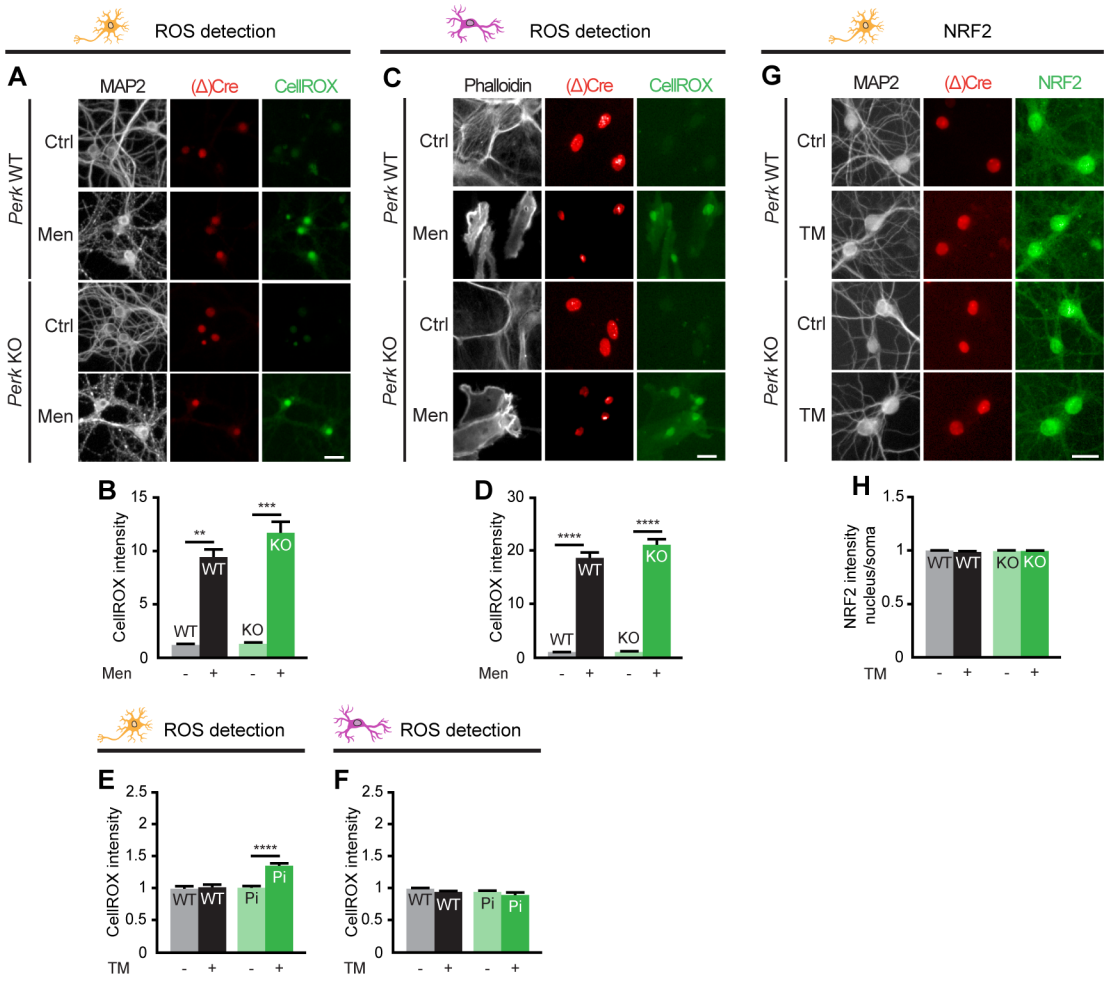

PERK pathway
ATF6 pathway
IRE1 pathway
IRE1-i
A
6 hours
24 hours
Ddit3 (a.u.)
B
Eif4ebp1 (a.u.)
C
Hspa5 (a.u.)
D
Xbp1s/Xbp1u (a.u.)
WT
KO
TM
E
Perk WT
Perk KO
IRE1-i
Protein synthesis
Total protein
F
Protein synthesis response (TM+/TM-)
G
Control
IRE1-i
MAP2
(Δ)Cre
ATF4
Ctrl
TM
H
ATF4 response (TM+/TM-)

Appendix Figure S3: PERK deficiency in neurons is not compensated by the ATF6 and IRE1 arm of the UPR

A-D mRNA expression of *Ddit3* (CHOP), *Eif4ebp1*, *Hspa5* (BiP/GRP78) and splicing of *Xbp1* (spliced/unspliced; *Xbp1s/Xbp1u*) in WT and *Perk* KO neurons ± 6 or 20-24 hours of TM-induced ER stress, determined by qPCR (N=3-4). Data are normalised to untreated WT.

E-H Protein synthesis and ATF4 expression in WT and *Perk* KO neurons ± 20-24 hours of TM-induced ER stress in the absence or presence of IRE-I (N=3). (E) Representative WB showing puromycinilated proteins. (F) quantification of the ER stress-induced protein synthesis response. (G) Representative confocal images of ATF4 immunofluorescence. Dendrites (MAP2, cyan), nuclei ((Δ)Cre, green), ATF4 (magenta). Scale bar: 25 µm. (H) Quantification of the ER stress-induced ATF4 response. (F, H) Data are related to untreated cells of the same genotype. Baseline level (without ER stress) is depicted by a dashed line.

Data information: Data are presented as mean ± SEM. N: Biological replicate. Relevant P-values are indicated: *p<0.05, *** p<0.001, ****p<0.0001. Statistical analysis: Two-way ANOVA with Tukey's post hoc test. Source data is available online.

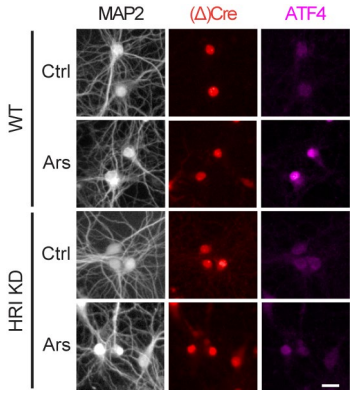


Appendix Figure S4: ER stress induces the accumulation of reactive oxygen species in PERK-deficient neurons, but not nuclear NRF2 – Related to Figure 4

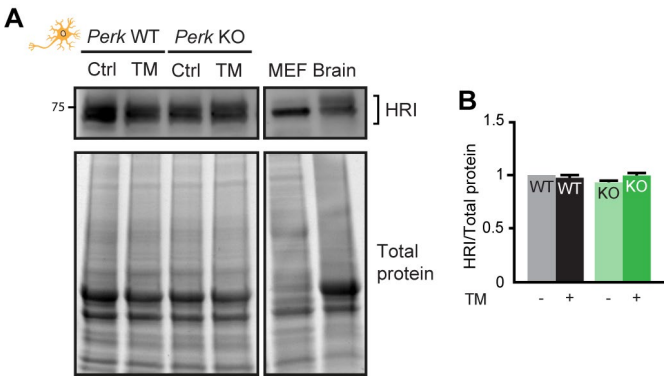
A-D ROS accumulation in WT and *Perk* KO neurons and astrocytes ± 4 hours of Menadione (Men)-induced ROS (positive control) and (E, F) neurons and astrocytes ± 20-24 hours of TM-induced ER stress ± PERK-i, determined by nuclear CellROX green intensity (N=3). (A, C) Representative images obtained by high-content microscopy. Dendrites (MAP2, white), astrocytic soma (Phalloidin, white), nuclei ((Δ)Cre, red), ROS (CellROX green, green). Scale bar: 25 µm. (B, D, E, F) Quantification of CellROX green intensity.

G, H NRF2 translocation in WT and *Perk* KO neurons ± 20-24 hours of TM-induced ER stress, determined by immunofluorescence (N=4). (G) Representative images obtained by high-content microscopy. Dendrites (MAP2, white), nuclei ((Δ)Cre, red), NRF2 (green). Scale bar: 25 µm. (F) Ratio of NRF2 intensity in nucleus/soma.

Data information: Data are normalised to untreated WT and presented as mean ± SEM. N: Biological replicate. Relevant P-values are indicated: ** p<0.01, *** p<0.001, **** p<0.0001. Statistical analysis: Two-way ANOVA with Tukey's post hoc test. Source data is available online.



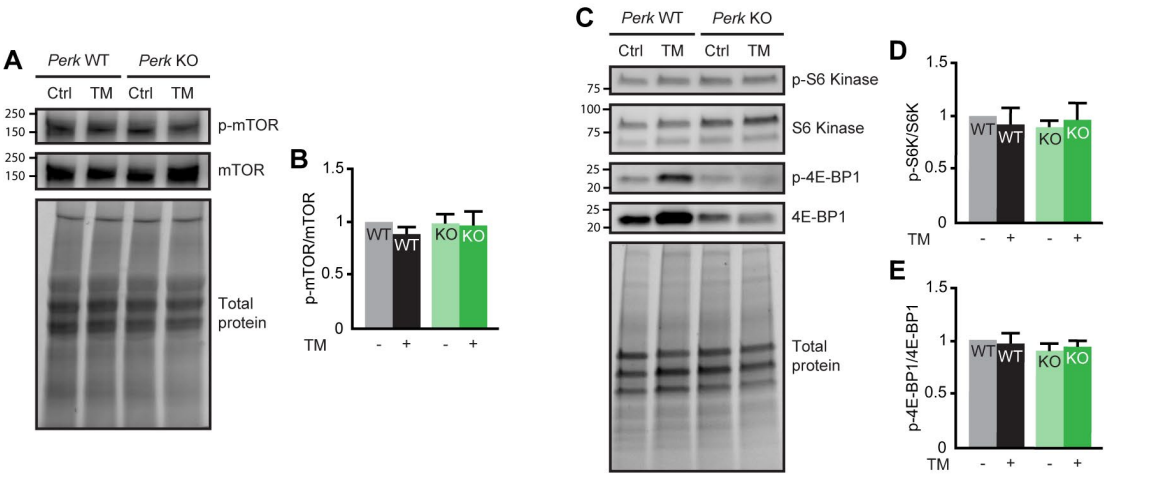
Appendix Figure S5: Representative images for Figure 4l
Representative images obtained by high-content microscopy. Dendrites (MAP2, white), nuclei (ΔCre, red), ATF4 (magenta). Scale bar: 25 µm.

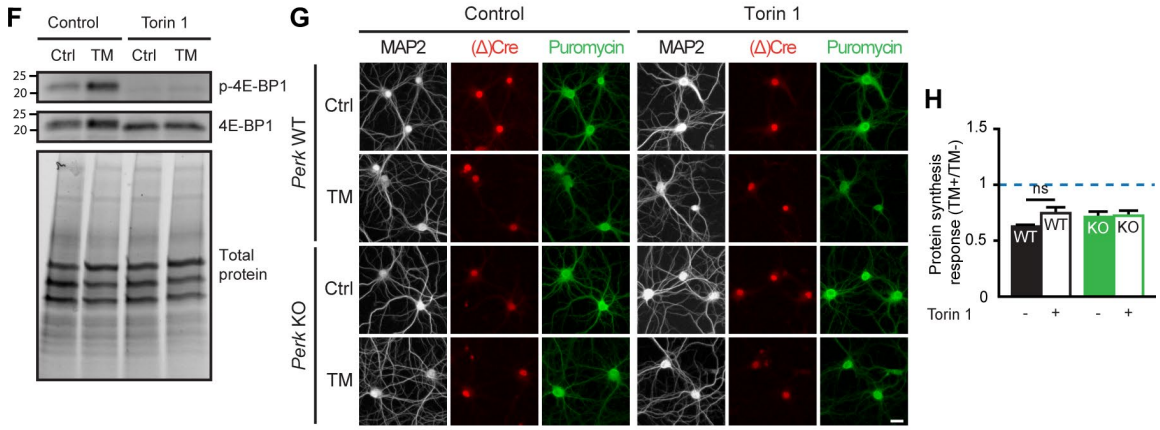


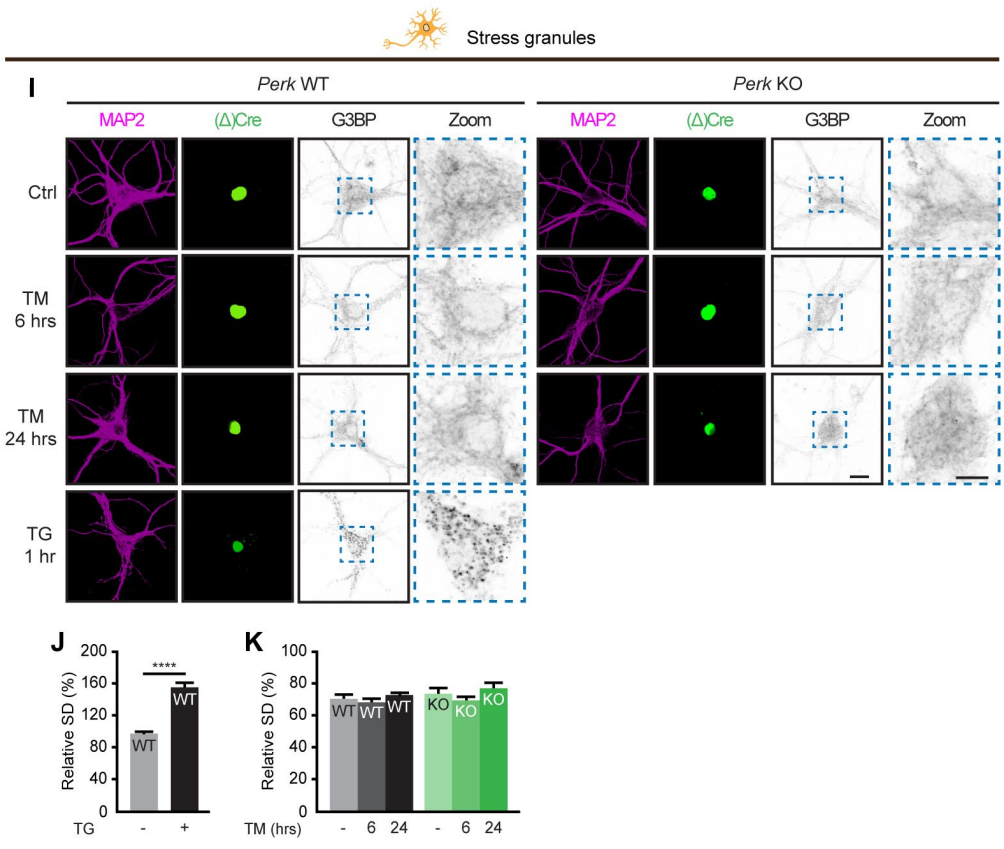
Appendix Figure S6: HRI protein levels are not altered in PERK-deficient neurons

A-B Analysis of HRI protein levels in WT and *Perk* KO neurons ± 20-24 hours of TM-induced ER stress (N=3). (A) Representative WB and (B) quantification of HRI protein level. All lanes are from the same gel/blot.

Data information: Data are normalised to untreated WT and presented as mean ± SEM. N: Biological replicate. Statistical analysis: Two-way ANOVA with Tukey's post hoc test. Source data is available online.


A
Perk WT
Perk KO
Ctrl
TM
Ctrl
TM
250
150
p-mTOR
mTOR
Total protein
B
p-mTOR/mTOR
WT
WT
KO
KO
TM
C
Perk WT
Perk KO
Ctrl
TM
Ctrl
TM
p-S6 Kinase
S6 Kinase
p-4E-BP1
4E-BP1
Total protein
D
p-S6K/S6K
E
p-4E-BP1/4E-BP1


F
Control
Torin 1
Ctrl
TM
Ctrl
TM
p-4E-BP1
4E-BP1
Total protein
G
Control
Torin 1
MAP2
(Δ)Cre
Puromycin
Perk WT
Perk KO
Ctrl
TM
H
Protein synthesis response (TM+/TM-)
ns
Torin 1


Stress granules
I
Perk WT
Perk KO
MAP2
(Δ)Cre
G3BP
Zoom
Ctrl
TM 6 hrs
TM 24 hrs
TG 1 hr
J
Relative SD (%)
****
TG
K
Relative SD (%)
TM (hrs)

Appendix Figure S7: mTOR signalling and stress granule assembly are not involved in ER stress-induced translational control in PERK-deficient neurons

A-H Analysis of mTORC1 activity in WT and *Perk* KO neurons ± 20-24 hours of TM-induced ER stress. (A-E) Phosphorylation of mTOR (N=5), p70 S6 Kinase (N=4) and 4E-BP1 (N=4). (A, C) Representative WB and (B, D, E) quantification of phosphorylated mTor, S6K and 4E-BP1 level (p-/total). Data are normalised to untreated WT.

F Representative WB showing inhibition of phosphorylation of 4E-BP1 WT neurons ± 20-24 hours of TM-induced ER stress in the presence of Torin 1.

G-H Protein synthesis in WT and *Perk* KO neurons ± 20-24 hours of TM-induced ER stress in the absence or presence of Torin 1 (N=3). (G) Representative images obtained by high-content microscopy. Dendrites (MAP2, white), nuclei (ΔCre, red), *de novo* synthesized proteins (Puromycin, green). Scale bar: 25 µm. (H) Quantification of the ER stress-induced protein synthesis response, relative to untreated cells of the same genotype. Baseline level (without ER stress) is depicted by dashed line.

I-K Stress granule assembly in WT and *Perk* KO neurons ± 6 or 20-24 hours of TM-induced ER stress. (I) Representative confocal images and zooms. Dendrites (MAP2, magenta), nuclei ((Δ)Cre, green), stress granules (G3BP, grey). Scale bar: 10 µm; Scale bar zoom: 5 µm. (J, K) Quantification of the standard deviation (SD) of the intensity of the G3BP immunofluorescence signal. One hour TG-treatment was used as positive control. (J) WT Ctrl: N= 83, WT TM 6 hrs: N= 84, WT TM 24 hrs: N= 87, KO Ctrl: N= 83, WT TM 6 hrs: N= 83, WT TM 24 hrs: N= 86. (K) Ctrl: N=29, TG: N=6.

Data information: Data are presented as mean ± SEM. N: Biological replicate. Relevant P-values are indicated: **** $p<0.0001$, ns: not significant. Statistical analysis: Two-way ANOVA with Tukey's post hoc test (B, D, E, H, K); Mann–Whitney U-test (J). Source data is available online.