Expanded View Figures

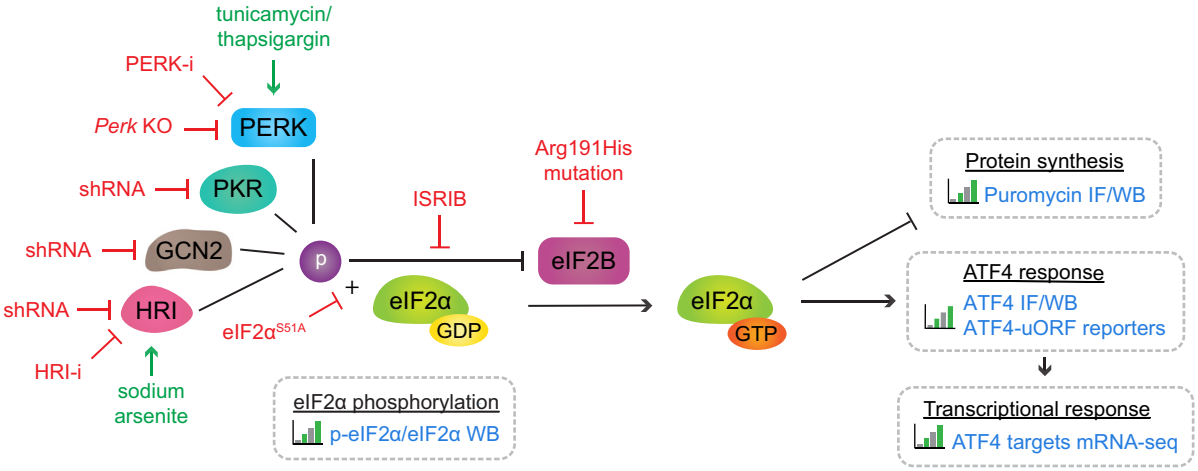

Figure EV1. ISR interventions and experimental readouts used in this study.

Schematic overview of the interventions inhibiting (red) and activating (green) the ISR and experimental readouts (blue text) used in this study. WB: Western blot; IF: Immunofluorescence.

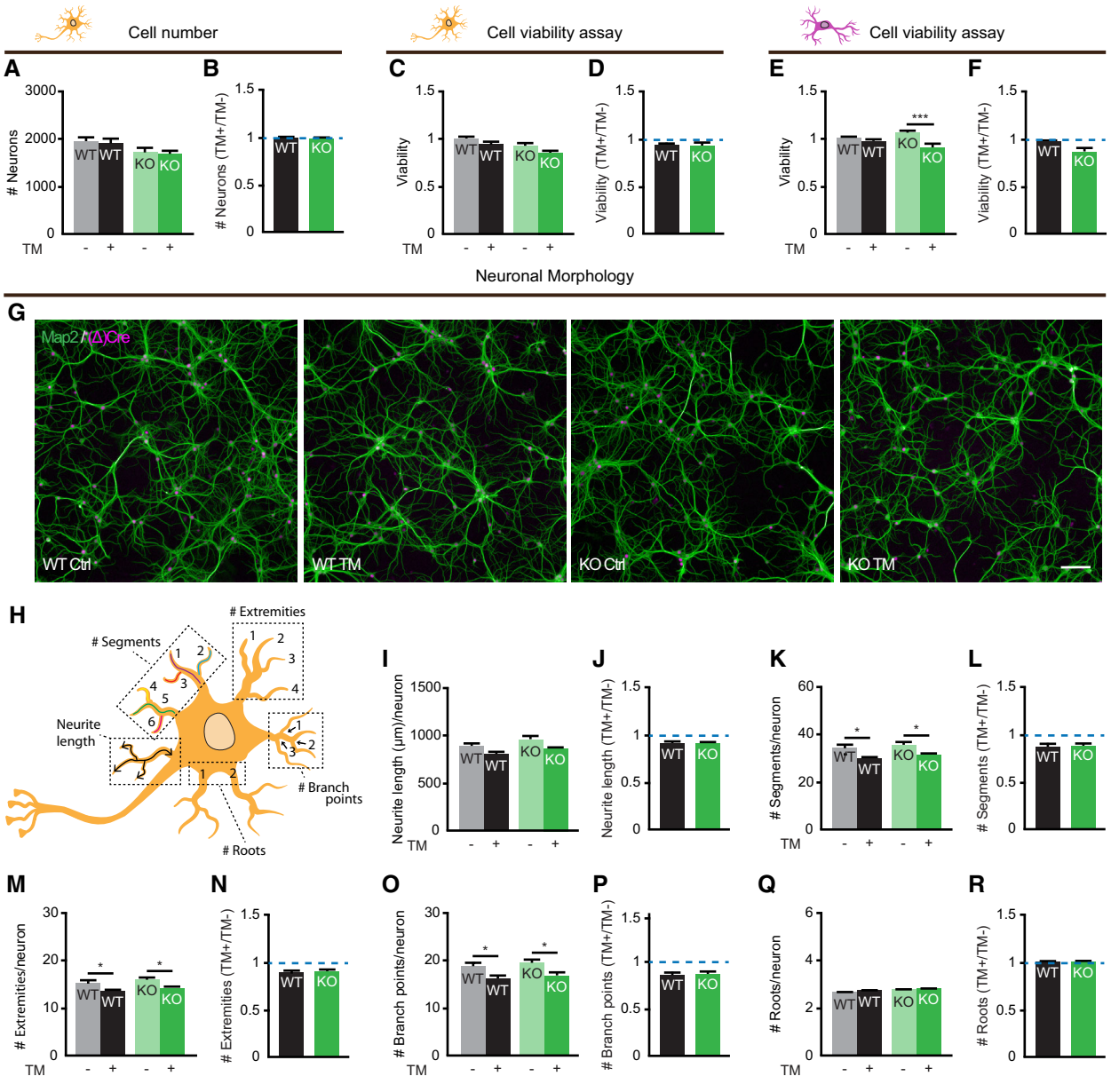

Figure EV2. Neurons *in vitro* are tolerant to PERK dysfunction.

A–F Viability of WT and *Perk* KO neurons (A-D) and astrocytes (E-F) ± 20–24 h of TM-induced ER stress. (A) Absolute cell number and (B) the change in cell number in response to ER stress ($N = 7$). (C, E) Absolute metabolic activity and (D, F) the change in metabolic activity in response to ER stress, determined by the CellTiter-Glo Luminescent Cell Viability Assay (Promega) (E-D, $N = 6$; E-F, $N = 3$). Baseline level (without ER stress) is depicted by dashed line.

G–R Neuronal morphology of WT and *Perk* KO neurons ±20–24 h of TM-induced ER stress ($N = 7$). (G) Representative images obtained by high-content microscopy. Dendrites (MAP2, green), nuclei ((Δ)Cre, magenta). Scale bar: 100 µm. (H) Schematic representation of morphological measures. Absolute morphological measures ((I) neurite length, (K) # segments, (M) # extremities, (O) # branch points and (Q) # roots) and the change in neuronal morphology in response to ER stress (J, L, N, P, R). Baseline level (without ER stress) is depicted by dashed line.

Data information: Data are presented as mean ± SEM. *N*: Biological replicate. Relevant *P*-values are indicated: $*P < 0.05$ and $***P < 0.001$. Statistical analysis: Two-way ANOVA with Tukey's *post-hoc* test (A, C, E, I, K, M, O, Q); nested *t*-test (B, D, F, J, L, N, P, R).
Source data are available online for this figure.

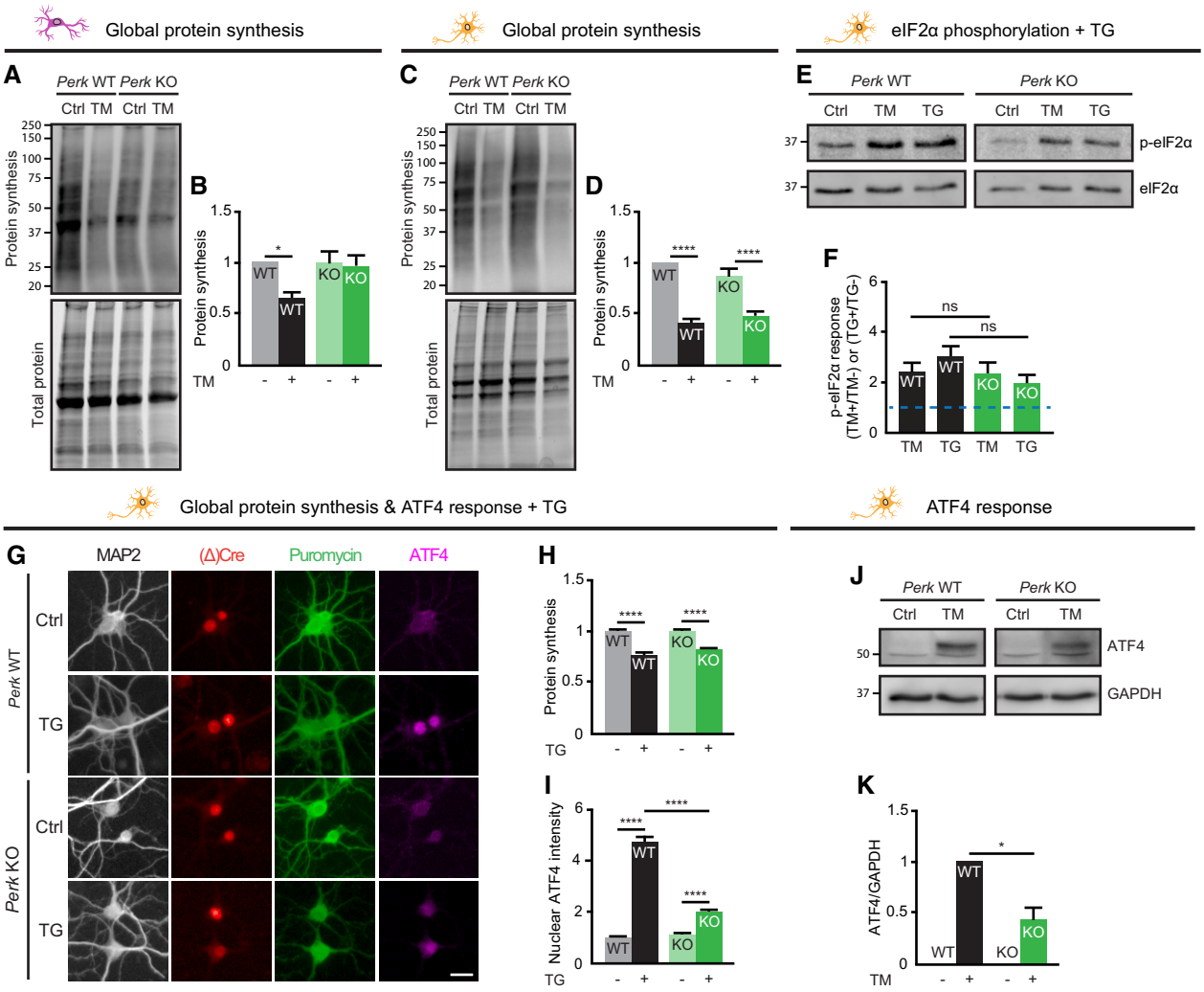

Figure EV3. ER stress-induced reduction in protein synthesis is preserved in PERK-deficient neurons—Related to Figs 1 and 2.

A–D Protein synthesis in WT and *Perk* KO astrocytes (A, B, $N = 4$) and neurons (C, D, $N = 7$) ± 20–24 h of TM-induced ER stress. Representative WB (A, C) and quantification (B, D) of puromycinilated proteins as a measure for *de novo* protein synthesis. Data are normalised to untreated WT.

E–I eIf2α phosphorylation ($N = 4$), protein synthesis ($N = 3$) and ATF4 expression ($N = 3$) in WT and *Perk* KO astrocytes and neurons ±20–24 h of TG-induced ER stress. Representative WB (E) and quantification (F) of the ER stress-induced p-eIf2α response, relative to untreated cells of the same genotype. Baseline level (without ER stress) is depicted by dashed line. (E) All lanes are from the same gel/blot. (G) Representative images obtained by high-content microscopy showing puromycinilated proteins and ATF4 immunofluorescence. Dendrites (MAP2, white), nuclei ((Δ)Cre, red), *de novo*-synthesised proteins (puromycin, green) and ATF4 (magenta). Scale bar: 25 μm. Quantification of (H) puromycin intensity as a measure for *de novo* protein synthesis and (I) absolute nuclear ATF4 intensity. Data are normalised to untreated WT.

J, K Representative WB (J) and quantification (K) of ATF4 expression in WT and *Perk* KO neurons ±20–24 h of TM-induced ER stress ($N = 4$). ATF4 level is corrected for GAPDH level. Data are normalised to TM-treated WT. (J) All lanes are from the same gel/blot.

Data information: Data are presented as mean ± SEM. *N*: Biological replicate. Relevant *P*-values are indicated: $*P < 0.05$, $****P < 0.0001$ and ns: not significant Statistical analysis: two-way ANOVA with Tukey's *post-hoc* test (B D, F, H, I); one-sample *t*-test with a hypothetical value of 1 (K).
Source data are available online for this figure.

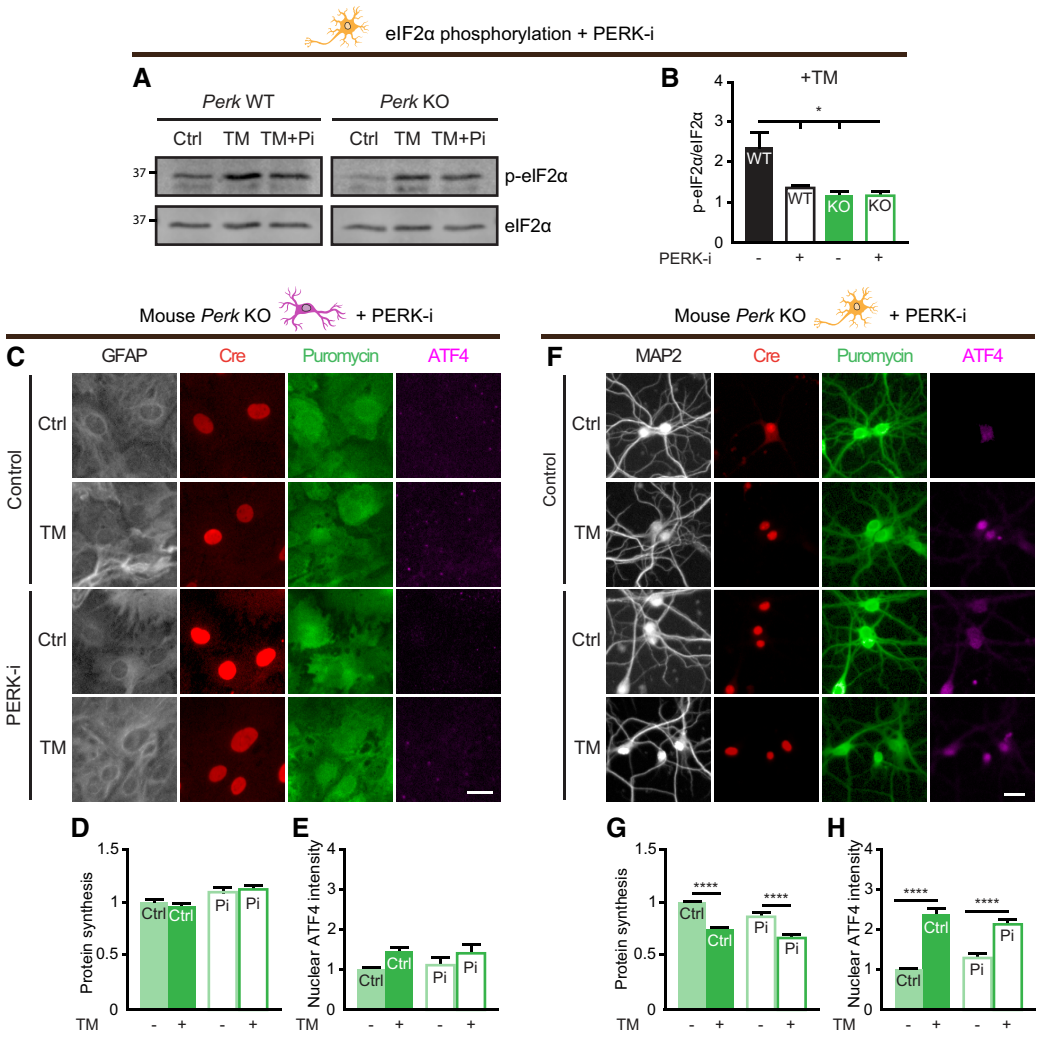

Figure EV4. Preservation of ER stress-induced translational control in *Perk* KO neurons is not caused by low level residual PERK—Related to Fig 3.

A, B eIf2α phosphorylation in WT and *Perk* KO astrocytes and neurons ±20–24 h of TM-induced ER stress in the presence or absence of PERK-i ($N = 3$). Representative WB (A) and quantification (B) of phosphorylated eIF2α level (p-eIF2α/eIF2α). Data are normalised to untreated WT. (A) All lanes are from the same gel/blot.

C–H Protein synthesis and ATF4 expression in *Perk* KO astrocytes and neurons ±20–24 h of TM-induced ER stress in the presence of PERK-i. (C, F) Representative images obtained by high-content microscopy. Astrocytic soma (GFAP, white), dendrites (MAP2, white), nuclei (Cre, red), *de novo*-synthesised proteins (puromycin, green) and ATF4 (magenta). Scale bar: 25 µm. (D, $N = 4$; G, $N = 4$) Quantification of puromycin intensity as a measure for *de novo* protein synthesis. (E, $N = 5$; H, $N = 4$) Quantification of absolute nuclear ATF4 intensity. Data are normalised to untreated *Perk* KO cells. See for data WT cells Fig 3A and D (representative images are of the same experiment).

Data information: Data are presented as mean ± SEM. *N*: Biological replicate. Relevant *P*-values are indicated: *$P < 0.05$, ****$P < 0.0001$ and ns: not significant. Statistical analysis: two-way ANOVA with Tukey's *post-hoc* test.
Source data are available online for this figure.

Figure EV5. HRI mediates part of the ER stress-induced translational control in PERK-deficient neurons—Related to Fig 4.

A–C ATF4 expression in WT and *Perk* KO neurons ±20–24 h of TM-induced ER stress in the absence or presence of HRI-I ($N = 3$). (A) Representative images obtained by high-content microscopy. Dendrites (MAP2, white/cyan), nuclei ((Δ)Cre, green/red), ATF4 (magenta). Scale bar: 25 μm. (B) Quantification of the ER stress-induced ATF4 response. Data are normalised to WT without HRI-i. Baseline level (without ER stress) is depicted by dashed line. (C) Quantification showing the percentage change in the ATF4 response induced by HRI-i. Calculated from data in (B): $\left[\left(\mathrm{HRI{-}i^{+}}-\mathrm{HRI{-}i^{-}}\right)/\mathrm{HRI{-}i^{-}}\right]*-100$.

D–I Validation of PKR and GCN2 KD. (D, G) mRNA expression of (D) *Eif2ak2* (PKR) in PKR KD neurons and (G) *Eif2ak4* (GCN2) in GCN2 KD neurons, determined by RT–qPCR ($N = 3$). Data are normalised to WT transduced with a scrambled construct (represented by a dashed line). (E, H) Quantification of the increase in nuclear ATF4 intensity in WT neurons ± PKR KD ± 6 h of BEPP treatment ($N = 3$) or in WT neurons ± PKR KD ± 6 h of Halofuginone (Halo) treatment ($N = 4$). (F, $N = 3$; I, $N = 4$) The remaining ATF4 response (%) upon PKR KD and GCN2 KD, respectively. Related to ATF4 response of WT transduced with shRNA control (100%, represented by a dashed line). (F) Calculated from data in (E): $[[(\mathrm{KD\_BEPP^{+}}-\mathrm{KD\_BEPP^{-}})/\mathrm{KD\_BEPP^{-}}]/[(\mathrm{WT\_BEPP^{+}}-\mathrm{WT\_BEPP^{-}})/\mathrm{WT\_BEPP^{-}}]]*100$. (I) Calculated from data in (H): $\left[\left[(\mathrm{KD\_Halo^{+}}-\mathrm{KD\_Halo^{-}})/\mathrm{KD\_Halo^{-}}\right]/\left[(\mathrm{WT\_Halo^{+}}-\mathrm{WT\_Halo^{-}})/\mathrm{WT\_Halo^{-}}\right]\right]*100$.

J–L Effects of PKR and GCN2 KD in WT and *Perk* KO neurons ±20–24 h of TM-induced ER stress. (J) Representative images of ATF4 immunofluorescence obtained by high-content microscopy and quantification of the ER stress-induced ATF4 response in PKR KD (K) and GCN2 KD (L) neurons ($N = 5$). Dendrites (MAP2, white/cyan), nuclei ((Δ)Cre, green/red) and ATF4 (magenta). Scale bar: 25 {m. Data are related to untreated (scrambled) 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: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ and ns: not significant. Statistical analysis: Two-way ANOVA with Tukey's *post-hoc* test (B, E, H, K, L); nested *t*-test (C); and one-sample *t*-test with a hypothetical value of 100 (D, F, G, I).
Source data are available online for this figure.

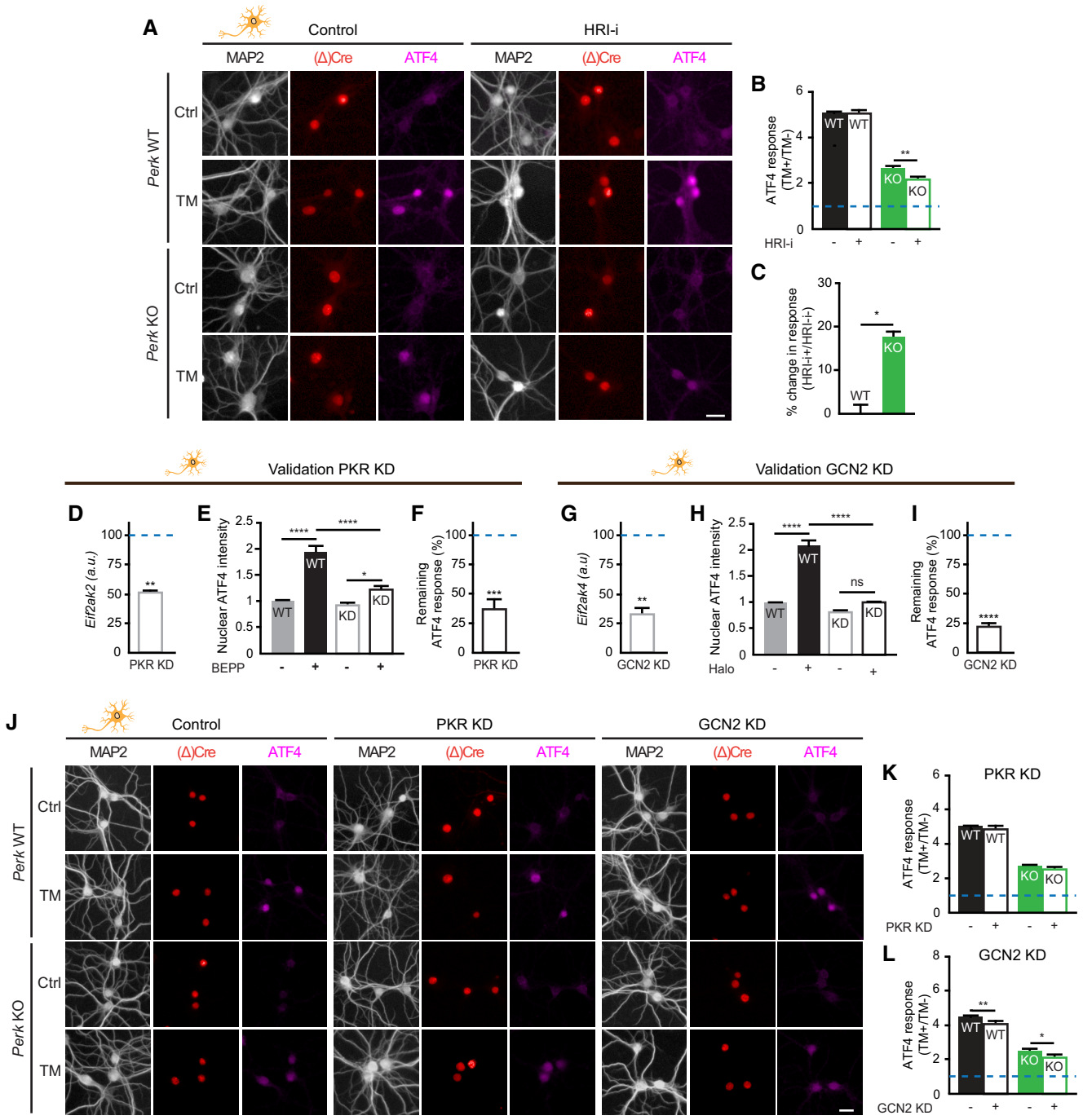

Figure EV5.