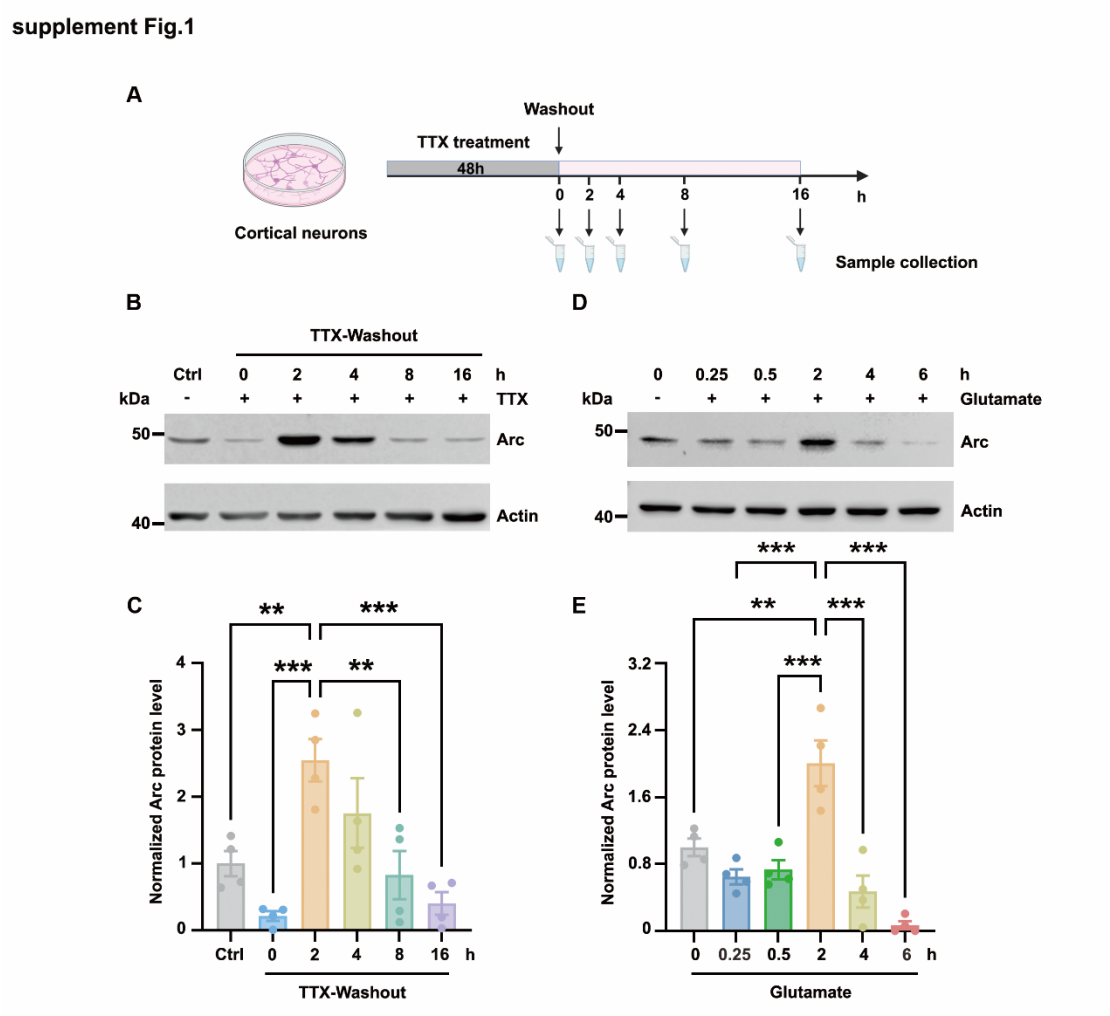
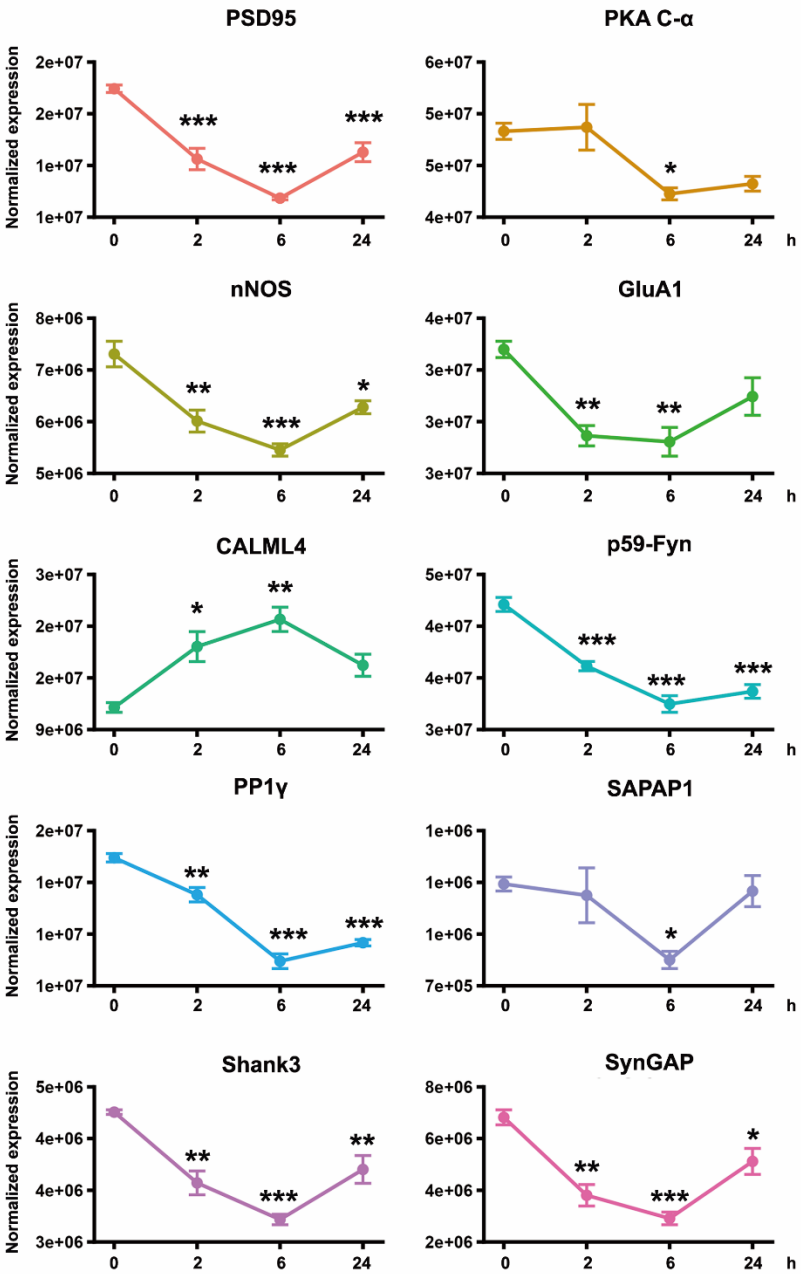


Supplementary Figure 1. Diverse excitatory stimuli consistently induce dynamic changes of Arc protein levels in cultured cortical neurons. (A) Schematic of the TTX washout assay. This panel was created using BioRender.com. (B, C) TTX washout induced time-dependent changes in Arc protein levels. Cultured cortical neurons were pretreated with TTX for 48 h before washout and subsequent assessment at the indicated time points (0, 2, 4, 8, and 16 h; control group indicates the untreated group), and then subjected to Western blot analysis. All values are normalized to the control group and represent mean ± s.e.m. from n = 4 independent experiments. $^{**}p = 0.0098$ (Ctrl), $^{***}p = 0.0002$ (0 h), $^{**}p = 0.0042$ (8 h), $^{***}p = 0.0005$ (16 h) versus 2 h group; one-way ANOVA with Dunnett's post hoc test. (D, E) Acute exposure to glutamate rapidly altered Arc protein levels. Cultured cortical neurons were acutely treated with glutamate for 5 min, returned to the conditioned culture medium for the indicated time points (0, 0.25, 0.5, 2, 4, and 6 h), and then subjected to Western blot analysis. All

Values are normalized to the 0 h group and represent mean ± s.e.m. from n = 4 independent experiments. **p = 0.0011 (0 h), ***p < 0.001 versus 2 h group; one-way ANOVA with Dunnett's post hoc test.

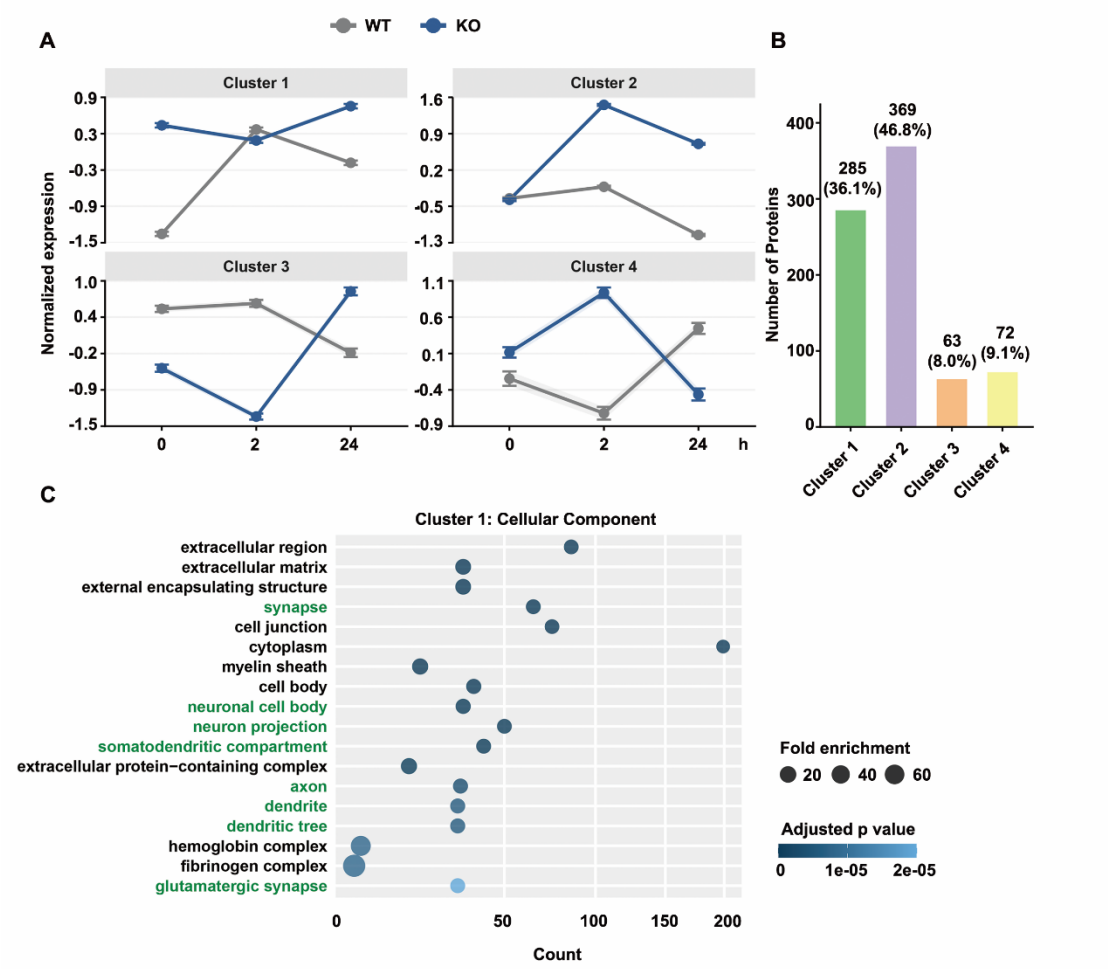
supplement Fig.2



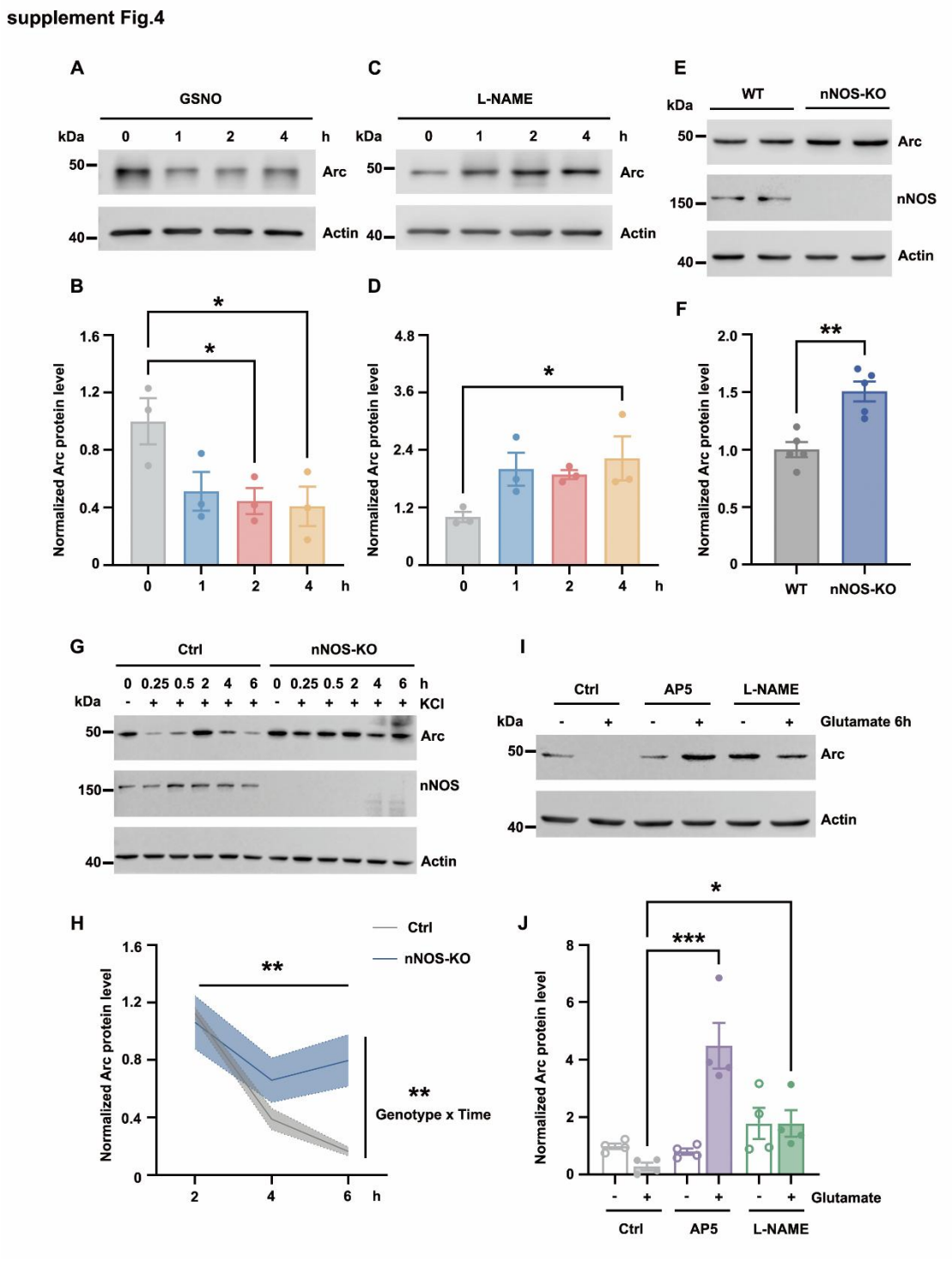
Supplementary Figure 2, related to Figure 2. Temporal profiles of the top 10 RWR-ranked candidate proteins. The charts illustrate the actin-normalized expression of individual proteins at 0, 2, 6, and 24 h following NMDA stimulation. All values represent mean ± s.e.m. from n = 3 independent experiments. PSD95, ***p = 0.0006 (2 h), ***p < 0.001 (6 h), ***p = 0.0008 (24 h); PKA C-α, *p = 0.0234; nNOS, **p = 0.0029 (2 h), ***p = 0.0003 (6 h), *p = 0.0110 (24 h); GluA1, **p = 0.0049 (2 h), **p = 0.0035 (6 h); CALML4, *p = 0.0132 (2 h), **p = 0.0014 (6 h); p59-Fyn, ***p = 0.0006 (2 h), ***p < 0.001 (6 h), ***p < 0.001 (24 h); PP1γ, **p = 0.0048 (2 h), ***p

< 0.001 (6 h), ***p < 0.001 (24 h); SAPAP1, *p = 0.0307; Shank3, **p = 0.0020 (2 h), ***p < 0.001 (6 h), **p = 0.0071 (24 h); SynGAP, **p = 0.0011 (2 h), ***p = 0.0001 (6 h), *p = 0.0314 (24 h); one-way ANOVA with Dunnett's post hoc test.

supplement Fig.3

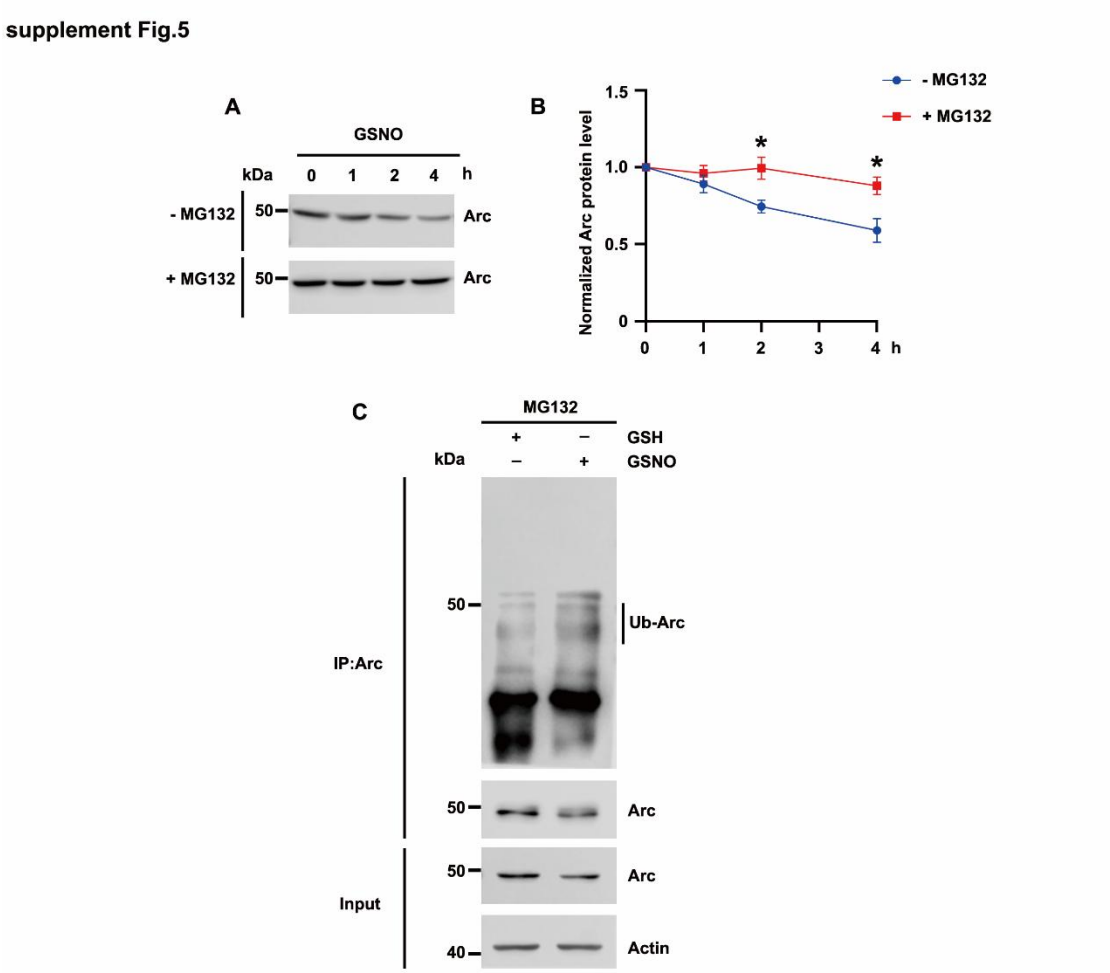


Supplementary Figure 3, related to Figure 3. Temporal clustering of the FC-stimulated proteome identifies distinct trajectories associated with nNOS deficiency. (A) Trajectory plots illustrating four distinct temporal expression patterns (Clusters 1–4) of DEPs in the hippocampus at 0 h (baseline), 2 h and 24 h following FC stimulation between wild-type (WT, grey) and nNOS-KO (KO, blue) mice. (B) Bar chart presents number of proteins and their relative percentages assigned to each of the four identified temporal clusters. (C) Cellular Component (CC) functions analysis for the proteins of Cluster 1. Green-labeled terms represent enriched functional categories associated with synapses and neurons.



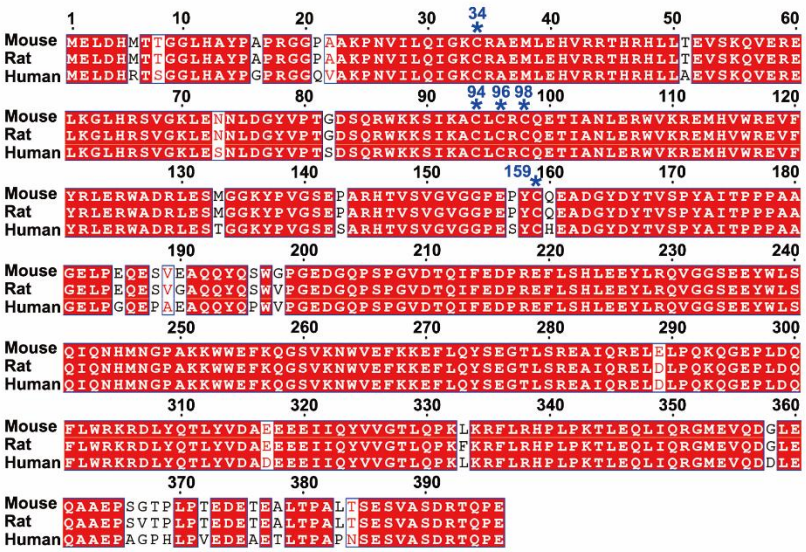
Supplementary Figure 4, related to Figure 4. NO regulates Arc degradation under basal and stimulated conditions. (A, B) GSNO-derived NO downregulates Arc protein expression. (A) Cultured hippocampal slices were treated with the NO donor GSNO for the indicated time points, and then subjected to Western blot analysis. (B) Quantitative analysis of Arc protein expression. All values are normalized to the 0 h groups and shown as mean ± s.e.m. from n = 3 independent experiments. *$p = 0.0450$

(2 h), *p = 0.0334 (4 h); one-way ANOVA with Dunnett's post hoc test. (C, D) Inhibition of endogenous NO production increases Arc protein levels. (C) Cultured hippocampal slices were treated with the NOS inhibitor L-NAME for the indicated time points, and then subjected to Western blot analysis. (D) Quantitative analysis of Arc protein expression. All values are normalized to the 0 h groups and shown as mean ± s.e.m. from n = 3 independent experiments. *p = 0.0466; one-way ANOVA with Dunnett's post hoc test. (E, F) Arc is highly expressed in nNOS-KO mice. (E) Hippocampal lysates prepared from WT and nNOS-KO mice were subjected to Western blot analysis. (F) Quantitative analysis of Arc protein expression. Values are normalized to WT and shown as mean ± s.e.m. from 5 animals of three independent experiments. **p = 0.0016; unpaired two-tailed Student's t-test. (G, H) NO mediates KCl-induced late-phase degradation of Arc. Representative Western blot images (G) and quantitative analysis (H) showing Arc protein levels in control neurons (nNOS wild-type and heterozygous) and nNOS-KO neurons at the indicated time points (2, 4, and 6 h). Values are presented as mean ± s.e.m. from n = 3 independent experiments. **p = 0.0042 for the Genotype × Time interaction (two-way ANOVA). Within-group significance was determined by two-way ANOVA with Dunnett's post hoc test (**p = 0.0015 for 2 h versus 6 h in the control group). No significant temporal alterations were observed in nNOS-KO group (2 h versus 6 h). (I, J) NO mediates the glutamate-induced late-phase degradation of Arc. (I) Cultured cortical neurons were pretreated with L-NAME or the NMDA receptor inhibitor AP5 prior to acute exposure to glutamate, and then subjected to Western blot analysis. (J) Quantitative analysis of Arc protein expression. All values are normalized to the untreated control group and shown as mean ± s.e.m. from n = 4 independent experiments. *p = 0.0256, ***p = 0.0003; Kruskal-Wallis test with Dunn's post hoc test.

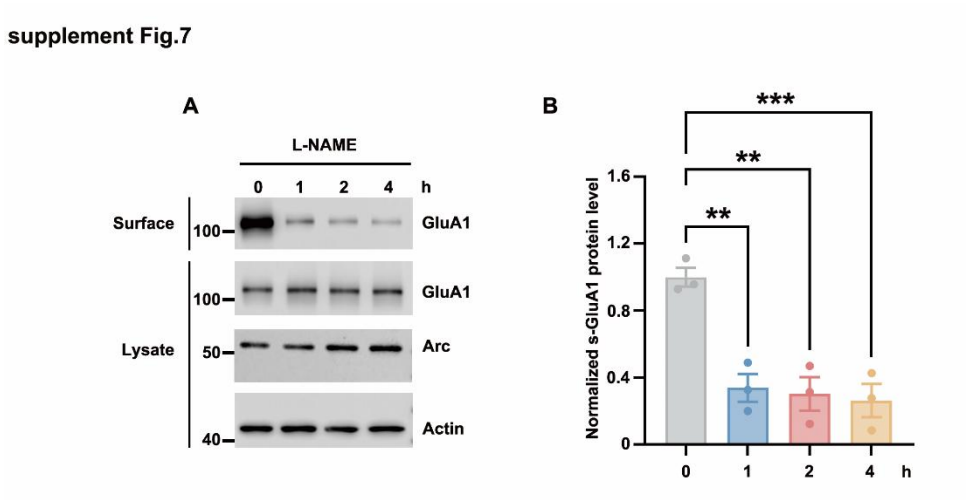


Supplementary Figure 5. NO promotes Arc degradation in a proteasome-dependent manner. (A, B) GSNO decreased Arc protein levels in a proteasome-dependent manner. Neuro2a cells were treated with GSNO in the presence or absence of the proteasome inhibitor MG132 for the indicated times and then subjected to Western blot analysis. (B) Values are normalized to the 0 h group and shown as mean ± s.e.m. from n = 5 independent experiments. *p = 0.0221 (2 h), **p = 0.0184 (4 h); unpaired Welch's t-test. (C) GSNO increased the ubiquitination of Arc in neurons. Cultured cortical neurons were treated with GSNO or GSH in the presence of the proteasome inhibitor MG132, followed by the assessment of Arc ubiquitination.

supplement Fig.6



Supplementary Figure 6, **related to Figure 5. Sequence analyses of Arc reveal highly conserved cysteine residues.** Sequence alignment of Arc orthologs across diverse species reveals five highly conserved cysteine residues.



Supplementary Figure 7, related to Figure 6. Nitric oxide regulates surface AMPA receptor trafficking. (A, B) Inhibition of NO production rapidly decreases surface GluA1 levels. (A) Cultured cortical neurons were treated with L-NAME for the indicated time points (0, 1, 2, and 4 h), and then subjected to the surface biotinylation assay. (B) Quantitative analysis of surface GluA1 (s-GluA1) protein levels. Values are normalized to the 0 h group and shown as mean ± s.e.m. from n = 3 independent experiments. $^{**}p = 0.0017$ (1 h), $^{**}p = 0.0012$ (2 h), $^{***}p = 0.0008$ (4 h); one-way ANOVA with Dunnett's post hoc test.