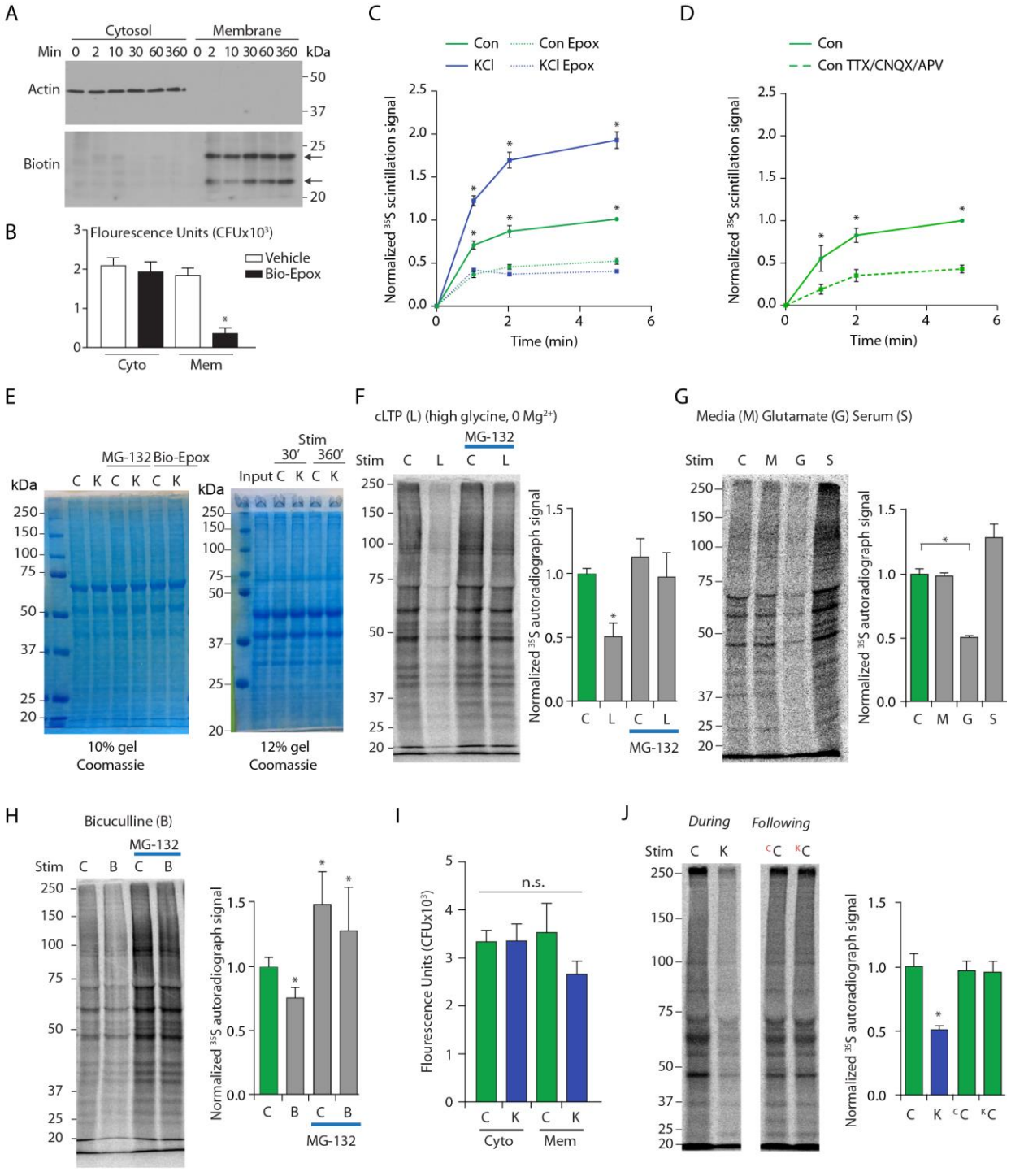


Figure S1. Neuronal stimulation reduces radiolabel incorporation into proteins in a proteasome dependent manner, related to Figure 1.

(**A**) Biotin-epoxomicin exclusively inhibits the NMP and not cytosolic proteasomes. Primary cortical neurons treated with biotin-epoxomicin for indicated times (Min) were subsequently fractionated into Cytosol and Membrane fractions. Samples were immunoblotted using indicated antibodies. Arrows to right indicate biotin signal representing covalent modification of the catalytic proteasome subunits.

(**B**) Concomitant radiolabelling during neuronal stimulation induces NMP-mediated radiolabeled peptide release that is sensitive to epoxomicin treatment. Media collected from neurons concomitantly radiolabeled and treated with control (Con) or KCl stimulation buffer. Epoxomicin (Epox) or Vehicle added following washout of stimulation and radiolabel. Liquid scintillation data for media at the indicated time points are shown normalized to control at the 5-minute time point. Data are presented as mean ± SEM (n = 3). Line graph, *$p < 0.01$ (two-way ANOVA) compared to control, $^{\#}p < 0.01$ (two-way ANOVA) for Control compared to epoxomicin treatment.

(**C**) Suppression of neuronal activity reduces peptide efflux**.** Primary cortical neurons were treated with control (Con) or neuronal activity inhibitors (TTX/CNQX/APV) for 1 hour. ^{35}S-methionine/cysteine radiolabel was incorporated for the last 10 minutes of 1 hour inhibitor treatment. Radiolabel was washed out, and fresh media +/- inhibitor was added. Samples were taken at indicated timepoints over a 5-minute timecourse and radioactivity was counted by liquid scintillation. Data are presented as mean ± SEM (n = 3). Line graph, *$p < 0.01$ (Students t-test) for Control compared to inhibitor cocktail treatment at each time point.

(**D**) Biotin-epoxomicin exclusively inhibits the NMP and not cytosolic proteasomes. Proteasomes were isolated from either cytosolic (Cyto) or Membrane (Mem) fractions from primary cortical neurons treated with DMSO (Vehicle) or Biotin-epoxomicin (Bio-Epox) for 1 hour. Isolated proteasomes were then incubated with SUC-LLVY-AMC, a fluorogenic substrate of the proteasome for 30 minutes to measure proteasome activity. Raw fluorescence values from each condition plotted. Data are presented as mean ± SEM (n = 3). Bar graph, *$p < 0.01$ (two-way ANOVA) for Bio-Epox treatments compared to Vehicle.

(**E**) Gels for Figures 1B and 1C were stained with coomassie dye and dried down onto Whatman filter paper. Note equal loading across conditions.

(**F**) Primary cortical neurons were radiolabelled during either ACSF treatment (C) or chemical LTP induction (L) (as described in Materials and methods). MG132 was added to indicated neurons during stimulation. Autoradiographs quantified by densitometry shown to right. Data are mean and range of $n = 2$ experiments from independent neuronal cultures. Bar graph, *$p < 0.01$ (two-way ANOVA) for treatments compared to controls.

(**G**) Primary cortical neurons were treated with either a Media exchange (M), Glutamate (G), or 5% Fetal Equine Serum (S) and radiolabelled for 10 minutes. Autoradiographs quantified by densitometry shown to right. Data are presented as mean ± SEM (n = 3). Bar graph, *$p < 0.01$ (two-way ANOVA) for treatments compared to controls.

(**H**) Primary cortical neurons were treated with bicuculline (B) or water (C) for one hour. MG132 and radiolabel were added during the final 10 minutes of bicuculline stimulation. Autoradiographs quantified by densitometry shown to right. Data are presented as mean ± SEM (n = 3). Bar graph, *$p < 0.01$ (two-way ANOVA) for treatments compared to controls.

(**I**) Primary cortical neurons stimulated with Control (C) or KCl (K) buffers were separated into Cytosolic (Cyto) and Membrane (Mem) fractions. Proteasomes were purified from each of these samples. Purified proteasomes were incubated for 30 minutes with Suc-LLVY-AMC, a small-molecule proteasome substrate that releases fluorescence when cleaved. Raw fluorescence units are shown. Data are presented as mean ± SEM (n = 3). Bar graph, data were not statistically significantly different across samples (two-way ANOVA).

(**J**) Primary cortical neurons stimulated with either Control (C) or KCl (K) buffers were incubated with ^{35}S methionine/cysteine radiolabel. Radiolabel was either incorporated at the same time as the stimulation (during), or as soon as the stimulation was washed out into media (following). For following experiment, superscript denotes stimulation condition, red lettering indicates

treatment during radiolabelling. Data are presented as mean ± SEM (n = 3). Bar graph, *p < 0.01 (two-way ANOVA) for treatments compared to controls.

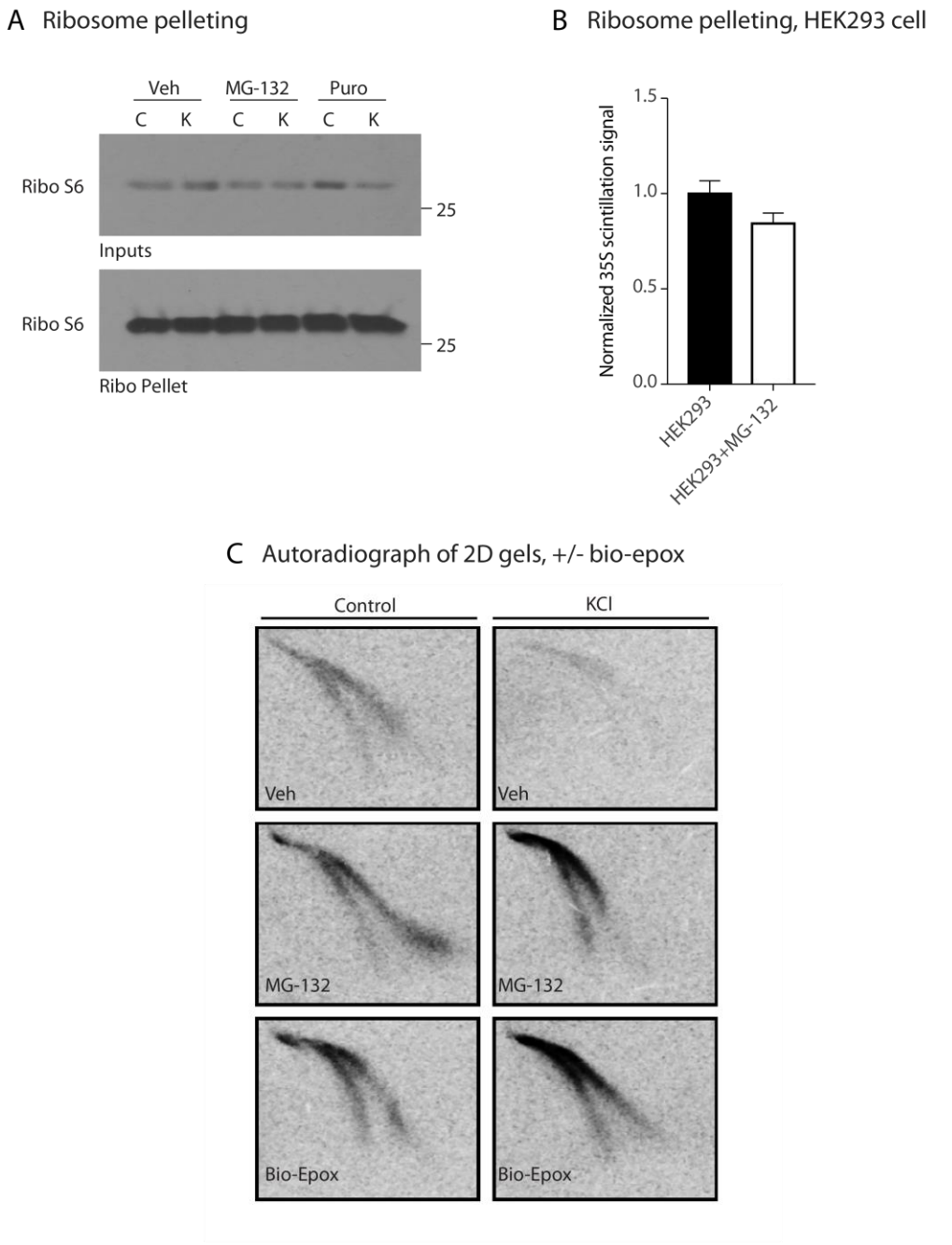


Figure S2. Neuronal stimulation induces NMP-mediated co-translational degradation of ribosome-associated nascent polypeptides, related to Figure 2.

(**A**) Ribosome nascent chain (RNC) complexes were pelleted from primary cortical neurons stimulated with either Control (C) or KCl (K) buffers. DMSO (Veh), MG132 or Puromycin (Puro) were added to neurons where indicated. Samples were immunoblotted with antibodies raised against Ribosomal S6 protein. Immunoblots of inputs are shown above those for pelleted RNC (Ribo pellet).

(**B**) Pelleted RNCs from HEK293 cells, treated with Vehicle (black bar) or MG132 (white bar). Samples analyzed by liquid scintillation. Data are presented as mean ± SEM (n = 3). Scintillation counts normalized to vehicle-treated samples shown.

(**C**) Pelleted RNCs from Control or KCl stimulated primary cortical neurons treated with or without DMSO (Veh), MG132, or biotin-epoxomicin (Bio-Epox). Samples were processed for 2D gel analysis as described in Figure 2.

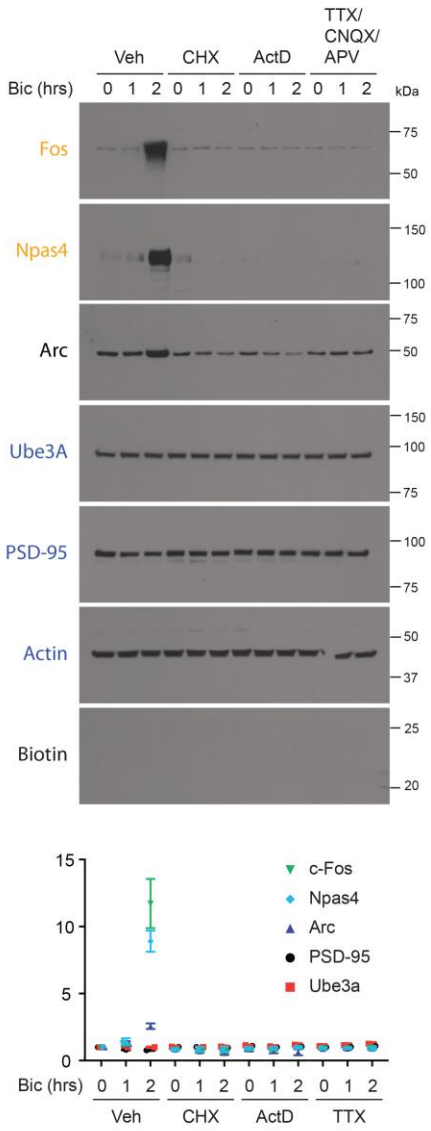
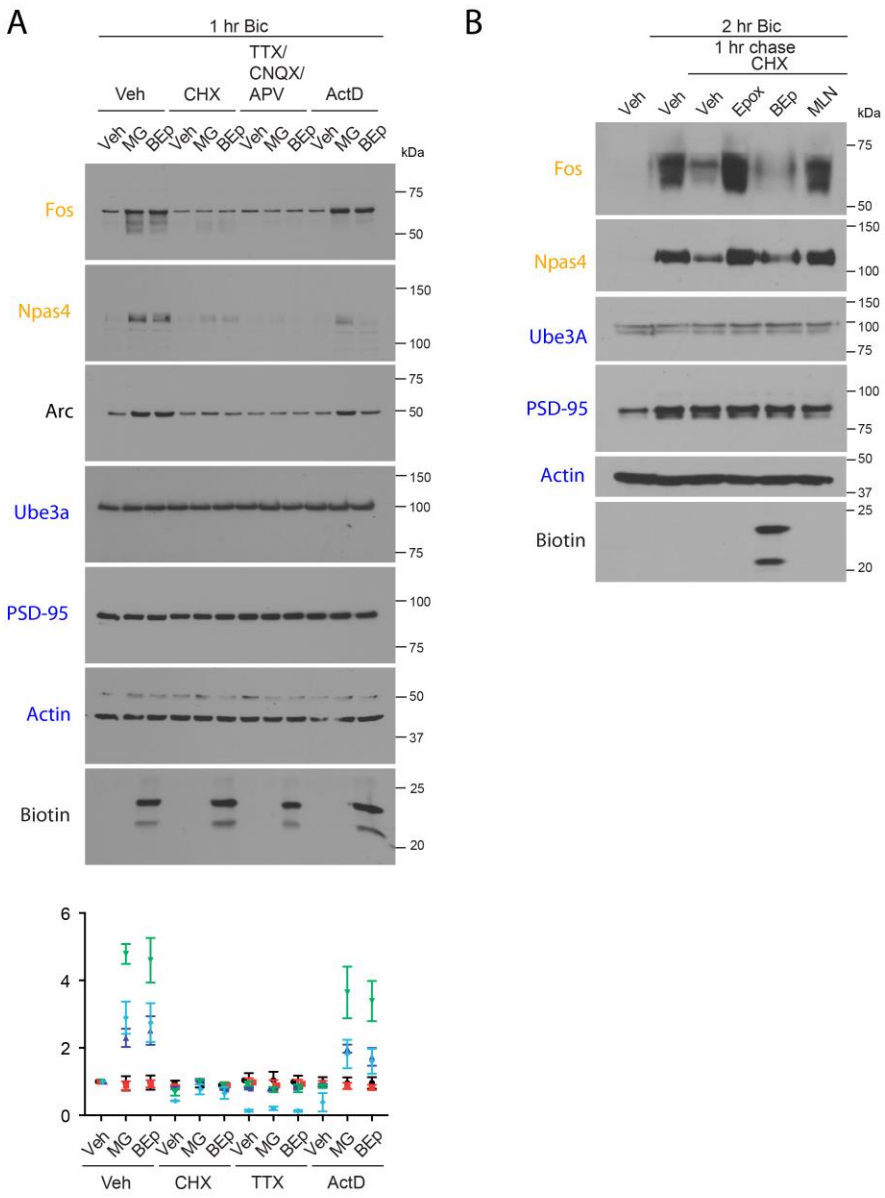


Figure S3. Immediate-early gene products are activity-dependent NMP substrates, related to Figure 3.

Primary cortical neurons were treated with bicuculline (Bic) for indicated times. Treatment conditions above: DMSO (Veh), cycloheximide (CHX), actinomycin D (ActD), neuronal activity inhibitor cocktail (TTX/CNQX/APV). All treatments incubated for indicated times. Neuronal lysates were immunoblotted with antibodies raised against indicated proteins. Protein names in orange classified as NMP targets in mass spectrometry data set (Figure 3), protein names in blue not NMP targets based on MS data set. Representative immunoblots shown. Data are presented as mean ± SEM (n = 3). See also Table S3.



Supplemental Figure S4 related to Figure 4. Ramachandran et al.

Figure S4. Immediate-early gene products are activity-dependent NMP substrates, related to Figure 4.

(**A**) Primary cortical neurons were treated with bicuculline (Bic) for 1 hour. Treatment conditions above: DMSO (Veh), cycloheximide (CHX), actinomycin D (ActD), neuronal activity inhibitors (TTX/CNQX/APV). MG132 (MG) or biotin-epoxomicin (BEp) applied for final 10 minutes of 1 hour Bicuculline stimulation. CHX, ActD, and TTX/CNQX/APV incubated during the whole 1 hour of Bicuculline stimulation. Neuronal lysates were immunoblotted with antibodies raised

against indicated proteins. Protein names in orange classified as NMP targets in mass spectrometry data set (Figure 3), protein names in blue not NMP targets based on MS data set. Representative immunoblots shown. Data are presented as mean ± SEM (n = 3). See also Table S3.

(**B**) Folded IEG protein is not degraded by the NMP, but is turned over by cytosolic proteasomes with the need for ubiquitylation. Following 2 hours of bicuculline (Bic) treatment compared to DMSO alone (Veh), primary cortical neurons were chased into cycloheximide (CHX) for one hour. Neurons treated with either DMSO (Veh), Epoxomicin (Epox), biotin-epoxomicin (BEp), or MLN-7243 (MLN) during chase. Neuronal lysates were immunoblotted with antibodies raised against indicated proteins. For (A) and (B), protein names in orange classified as NMP targets in mass spectrometry data set (Figure 3), protein names in blue are not NMP targets based on MS data set.

Table S3, Related to Figure 4, Figure S3, Figure S4 – Significance tables for all immunoblots.

Figure 4A

Comparison of Vehicle vs treatments	PSD-95	UBE3A	Arc	Fos	Npas4
Veh vs. MG132	0.8761	0.7174	0.0002	<0.0001	0.2734
Veh vs. Bio-epox	0.9300	0.8466	<0.0001	<0.0001	<0.0001
Veh vs. 1 hr Bic	0.8915	0.6953	0.5627	0.3332	0.6694
Veh vs. 1 hr Bic, MG132	0.8133	0.9461	0.8891	0.9787	0.6623
Veh vs. 1 hr Bic, Bio-epox	0.7524	0.7349	0.4909	0.7442	0.6991
Veh vs. 1 hr Bic, CHX	0.9028	0.9398	0.5339	0.8541	0.8487
Veh vs. 1 hr Bic, CHX, MG132	0.9058	0.7299	0.5406	0.4750	0.8393
Veh vs. 1 hr Bic, CHX, Bio-epox	0.9898	0.8292	0.5919	0.5470	0.9932
Veh vs. 1 hr Bic, ActD	0.9655	0.8258	0.7580	0.6559	0.8630
Veh vs. 1 hr Bic, ActD, MG132	0.8895	0.6957	0.0040	<0.0001	0.8640
Veh vs. 1 hr Bic, ActD, Bio-epox	0.9055	0.6030	0.0297	<0.0001	0.8120

Figure 4B

Comparison of Vehicle vs treatments	PSD-95	UBE3A	Arc	Fos	Npas4
0 hr Bic vs. 2 hr Bic	0.7324	0.9419	0.2225	<0.0001	0.0001
0 hr Bic vs. 2 hr Bic, 1 hr chase MG132	0.9693	0.9443	0.2368	<0.0001	0.0001
0 hr Bic vs. 2 hr Bic, 1 hr chase Bio-epox	0.7243	0.9365	0.8221	0.9192	0.5834
0 hr Bic vs. 2 hr Bic, 1 hr chase Veh	0.6935	0.8888	0.9244	0.9601	0.4994
0 hr Bic vs. 2 hr Bic, 1 hr chase CHX, MG132	0.9333	0.8703	0.1762	<0.0001	<0.0001
0 hr Bic vs. 2 hr Bic, 1 hr chase CHX, Bio-epox	0.6792	0.8783	0.9114	0.9699	0.6618
0 hr Bic vs. 2 hr Bic, 1 hr chase CHX	0.6972	0.9056	0.9299	0.9954	0.5447

Figure S3

Comparison of 0 hr Bicuculline vs other treatments	PSD-95	UBE3A	Arc	Fos	Npas4
Bic 0 vs. Bic 1	0.6258	0.9917	0.5060	0.6227	0.2734
Bic 0 vs. Bic 2	0.5684	0.9865	0.0001	<0.0001	<0.0001
Bic 0 vs. Bic 0, CHX	0.9678	0.9273	0.6550	0.6728	0.6694
Bic 0 vs. Bic 1, CHX	0.9760	0.9790	0.4426	0.6071	0.6623
Bic 0 vs. Bic 2, CHX	0.9235	0.9114	0.2819	0.4453	0.6991
Bic 0 vs. Bic 0, ActD	0.8174	0.7129	0.6674	0.6518	0.8487
Bic 0 vs. Bic 1, ActD	0.9752	0.8828	0.5023	0.6689	0.8393
Bic 0 vs. Bic 2, ActD	0.9016	0.6483	0.3338	0.9210	0.9932
Bic 0 vs. Bic 0, TTX/CNQX/APV	0.9815	0.8304	0.7441	0.8485	0.8630

Bic 0 vs. Bic 1, TTX/CNQX/APV	0.9518	0.7140	0.7941	0.8232	0.8640
Bic 0 vs. Bic 2, TTX/CNQX/APV	0.8017	0.5147	0.7868	0.9340	0.8120

Figure S4A

Comparison of Vehicle vs treatments	**PSD-95**	**UBE3A**	**Arc**	**Fos**	**Npas4**
Veh vs. MG132	0.8761	0.7174	0.0002	<0.0001	<0.0001
Veh vs. Bio-epox	0.9300	0.8466	<0.0001	<0.0001	<0.0001
Veh vs. CHX	0.8915	0.6953	0.5627	0.3332	0.0874
Veh vs. CHX, MG132	0.8133	0.9461	0.8891	0.9787	0.4836
Veh vs. CHX, Bio-epox	0.7524	0.7349	0.4909	0.7442	0.3079
Veh vs. TTX/CNQX/APV	0.9028	0.9398	0.5339	0.8541	0.0106
Veh vs. TTX/CNQX/APV, MG132	0.9058	0.7299	0.5406	0.4750	0.0183
Veh vs. TTX/CNQX/APV, Bio-epox	0.9898	0.8292	0.5919	0.5470	0.0100
Veh vs. ActD	0.9655	0.8258	0.7580	0.6559	0.0679
Veh vs. ActD, MG132	0.8895	0.6957	0.0040	<0.0001	0.0154
Veh vs. ActD, Bio-epox	0.9055	0.6030	0.0297	<0.0001	0.0724