1 **Supplementary Information**

2 **Supplementary Figure 1:**

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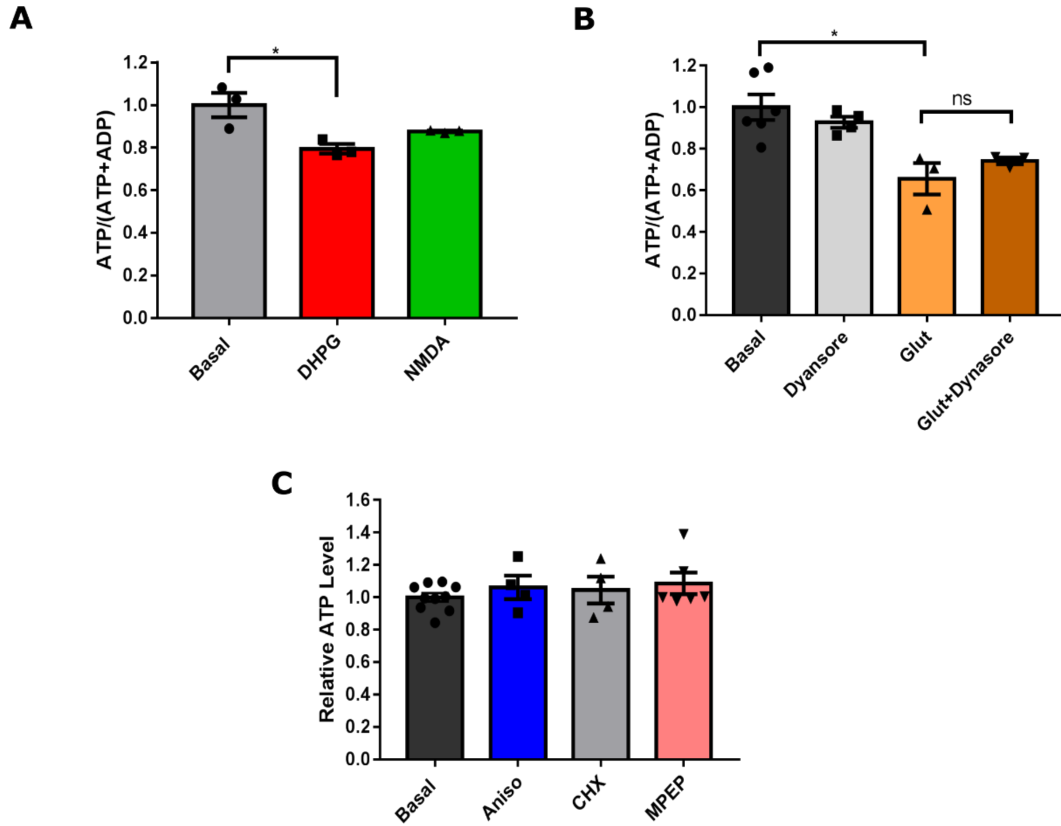

14 **Quantifying Activity Dependent Changes in Energy Level.** (Related to Figure 1)

15 **(A)** Bar graph representing normalized average value of neuronal ATP/ATP+ADP ratio in basal
16 condition and on DHPG (50µM) and NMDA (20µM) treatment for 5 minutes. *p<0.05, n=3
17 independent plating. One-way ANOVA followed by Bonferroni's multiple comparison test.

18 **(B)** Bar graph representing normalized average value of neuronal ATP/ATP+ADP ratio in basal
19 condition, on dynasore (100µM) treatment for 30 minutes, on Glutamate (25µM) treatment for
20 5 minutes and on glutamate treatment along with dynaosre pre-treatment. *p<0.05, n=3-6
21 independent plating. One-way ANOVA followed by Bonferroni's multiple comparison test.

22 **(C)** Quantification showing the effect of treatment of anisomycin, cycloheximide and MPEP on

23 synaptic ATP level measured from cortical synaptoneurosomes. Data = mean ± SEM with

24 scattered data points.

25 **Supplementary Figure 2:**

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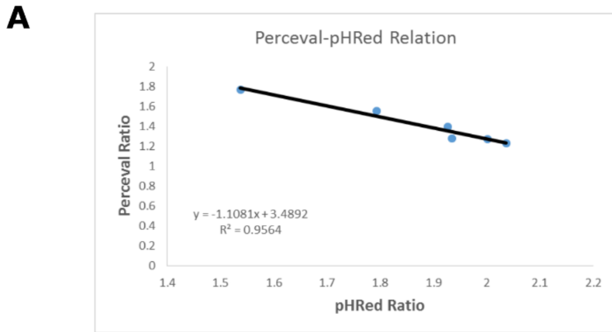

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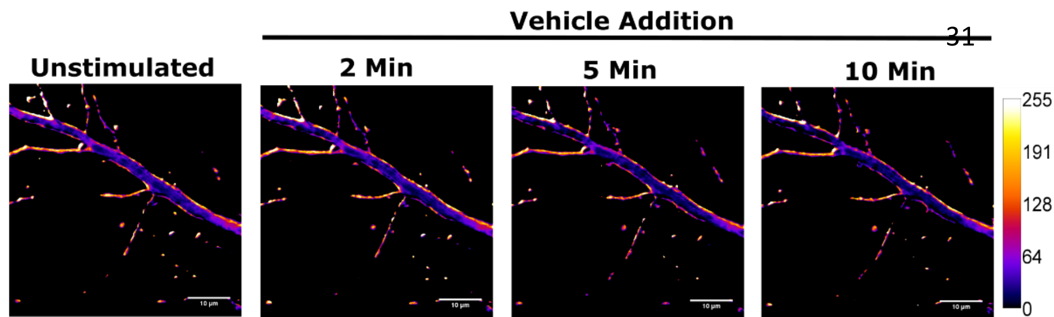

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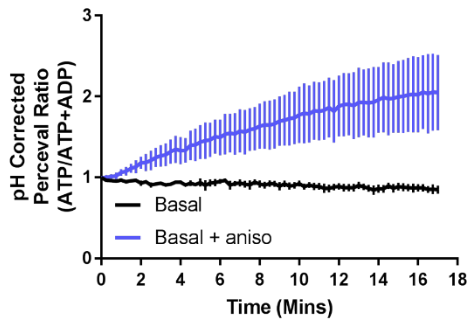

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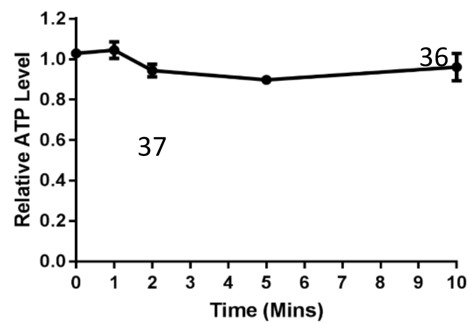

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Sensor Calibration and Basal ATP Measurement. (Related to Figure 2)

(A) Graph depicting a linear relationship was established between the PercevalHR fluorescence ratio and the pH-Red fluorescence ratio using NH_4Cl pre-pulse method following method established previously (Pederson et. al, 1998, Tantama et. al, 2013). Briefly a gradient of 5-15mM NH_4Cl solution was applied to allow the changes in intracellular pH in a short period of time to avoid any metabolic stress. Changes in PercevalHR fluorescence was measured along with pH-Red fluorescence and the linear relation was used to remove pH bias approximately.

(B) Representative dendrite showing the temporal profile of PercevalHR fluorescence (ATP/ADP) in unstimulated condition. Scale=10μM.

(C) Average time traces showing the change in the dendritic ATP/ADP ratio on unstimulated condition in presence or absence of anisomycin. Data= mean ± SEM. n≥3 cells from independent platings per group.

(D) Quantification showing the changes in synaptic ATP level on unstimulated conditions. Data= mean ± SEM. n= 4 animals.

64 Supplementary Figure 3:

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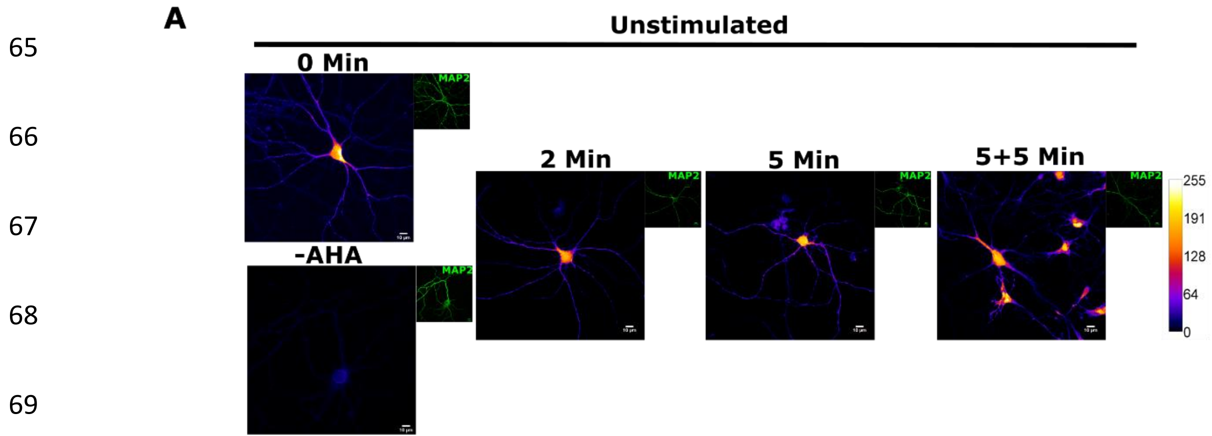

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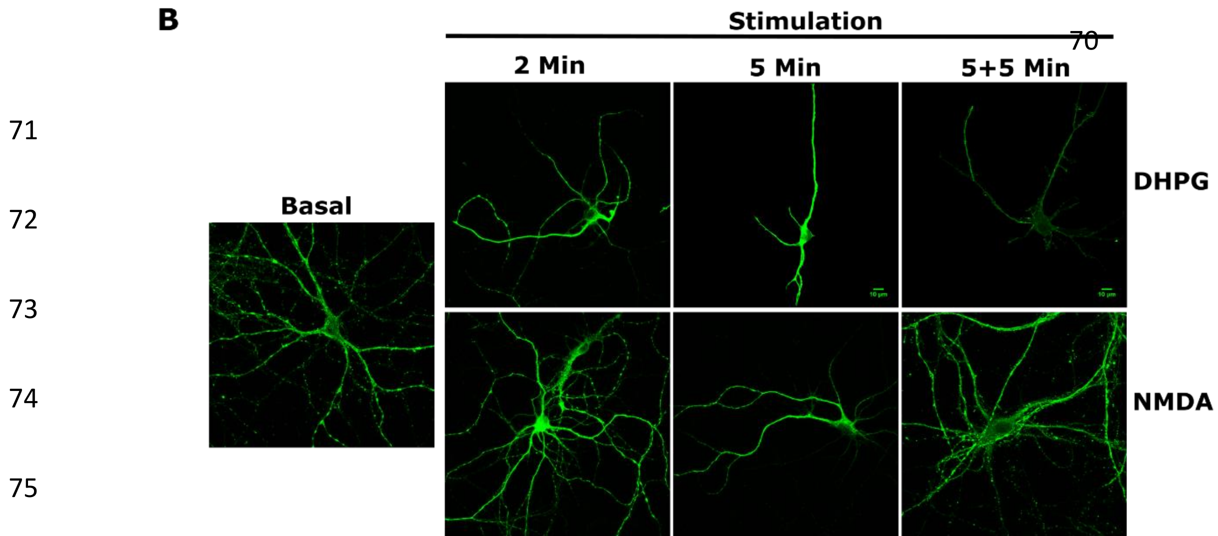

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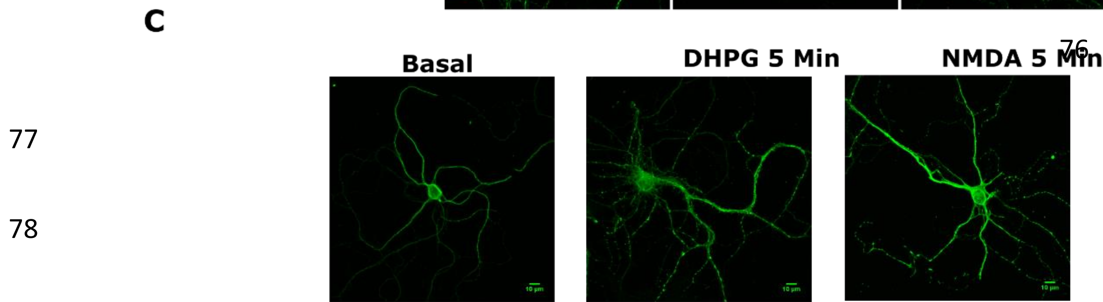

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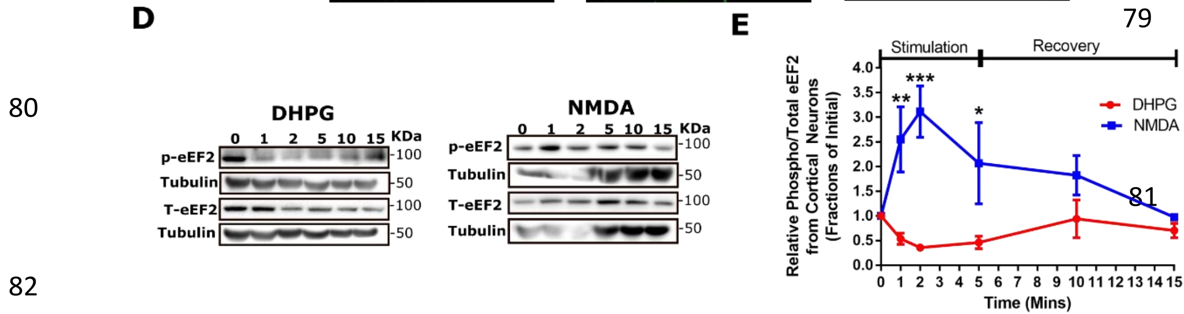

83 **Translation Kinetics.** (Related to Figure 3)

84 **(A)** Representative images showing newly synthesized proteins visualized through FUNCAT
85 metabolic labeling (pseudo-colored) in cortical neurons at various time points in unstimulated
86 condition. Absence of AHA while metabolic labeling was used as a negative control. MAP2B
87 immunolabeling (green, inset) was used for identifying neurons and intensity was used for
88 normalization. The average trace quantification and data point distribution for unstimulated
89 cells have been presented in Figure 3C and 3D. Scale=10μM.

90 **(B)** Representative images showing immunolabeling of MAP2B (green) in cortical neurons at
91 various time points following DHPG and NMDA treatment for the cells used for FUNCAT intensity
92 quantification. Scale=10μM.

93 **(C)** Representative images showing immunolabeling of MAP2B (green) in cortical neurons in
94 basal condition and DHPG and NMDA treatment for 5 minutes for the cells used for p-eEF2
95 quantification. Scale=10μM.

96 **(D)** Representative immunoblots describing changes in phospho-eEF2 and total-eEF2 levels at
97 various time points after DHPG (50μM) and NMDA (20μM) treatment to cultured cortical neurons.
98 Note in each case phospho and total eEF2 levels were normalized individually to tubulin before
99 calculating the ratio.

100 **(E)** Line graph showing average value for neuronal phospho/total ratio of eEF2 at various time
101 points after DHPG and NMDA treatment and 5 minutes and 10 minutes after recovery. Data:
102 mean +/- SEM, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, n= 3 animals per group. Two Way ANOVA followed
103 by Bonferroni's multiple comparison test.

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106 **Supplementary Figure 4:**

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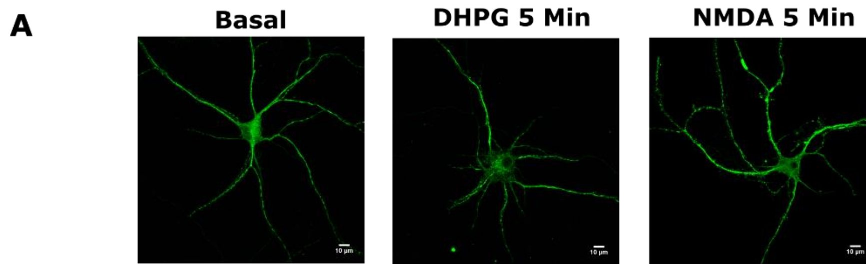

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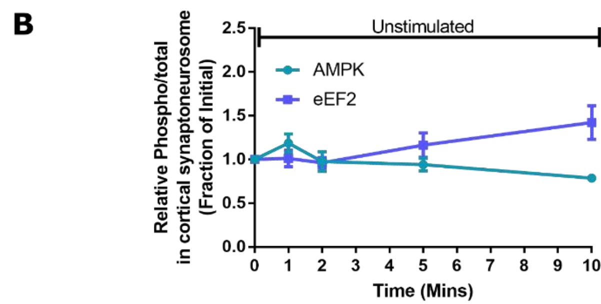

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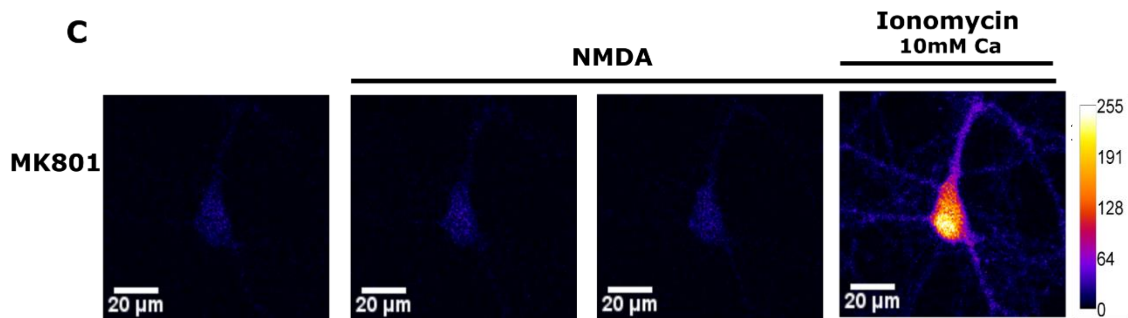

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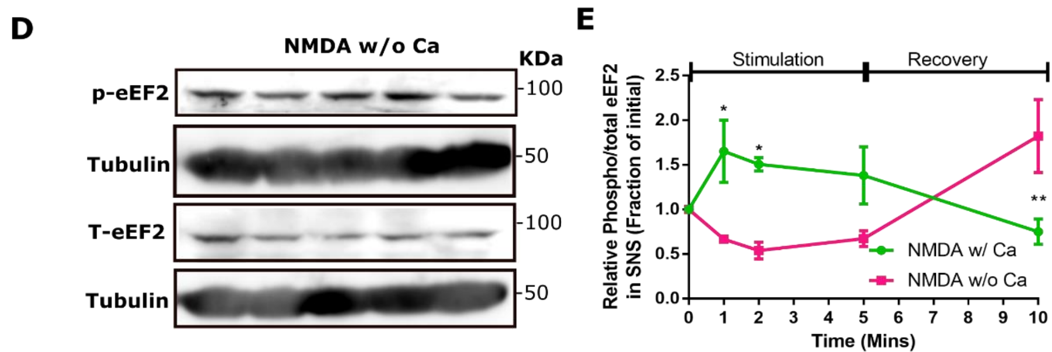

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125 **Baseline eEF2 phosphorylation and Characterization of NMDAR dependent Ca^{2+} entry.**
126 (Related to Figure 4 and Figure 5)

127 **(A)** Representative images showing immunolabeling of MAP2B (green) in cortical neurons in
128 basal condition and DHPG and NMDA treatment for 5 minutes for the cells used for p-AMPK
129 quantification. Scale=10µM.

130 (B) Line graph showing average value for synaptic phospho/total ratio of eEF2 and AMPK at
131 various time points after DHPG and NMDA treatment and 5 minutes after recovery in cortical
132 synaptoneurosomes. Data: mean +/- SEM, n≥ 2 animals per group. Two Way ANOVA followed by
133 Bonferroni's multiple comparison test.

134 **(C)** Representative images depicting intracellular Ca^{2+} levels (Pseudo-colored) of a cortical
135 neuron before stimulation, immediately (15s) after NMDA (20µM) treatment, at a later time
136 point (300sec) after NMDA treatment and after ionomycin treatment along with 10mM Ca^{2+} in
137 presence MK-801. Scale=20µM.

138 **(D)** Representative immunoblots describing changes in phospho-eEF2 and total-eEF2 levels at
139 various time points after NMDA (40µM) treatment to cortical synaptoneurosomes in presence or
140 absence of extracellular Ca^{2+}. Note in each case phospho and total AMPK levels were normalized
141 individually to tubulin before calculating the ratio.

142 **(E)** Line graph showing average value for synaptic phospho/total ratio of eEF2 at various time
143 points after NMDA treatment and 5 minutes after recovery in presence or absence of Ca^{2+}. Data:
144 mean +/- SEM, *$p<0.05$, **$p<0.01$, n≥ 4 animals per group. Two Way ANOVA followed by
145 Bonferroni's multiple comparison test.

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148 **Supplementary Figure 5:**

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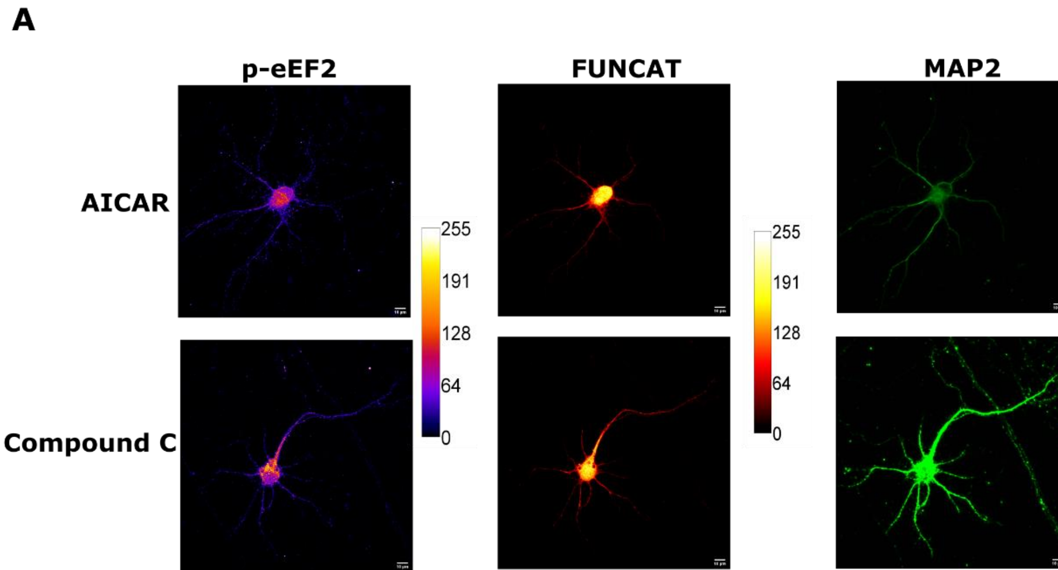

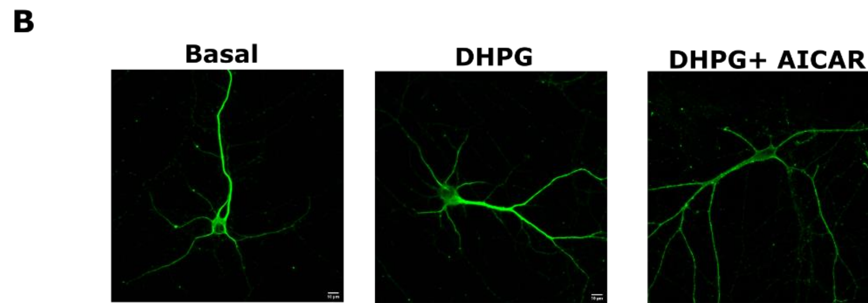

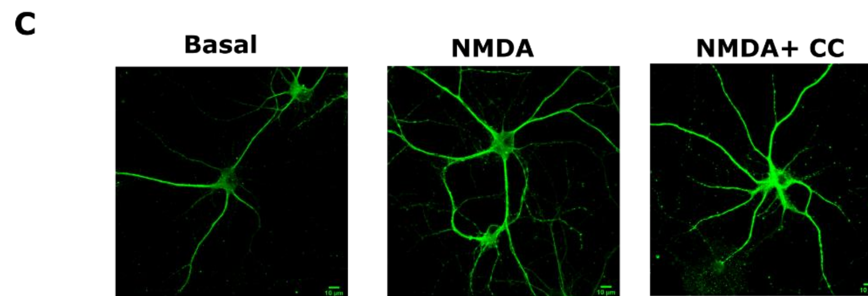

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165 **AMPK perturbations: (related to figure 6)**

166 **(A)** Representative images showing phospho-eEF2 immunolabeling (Pseudo-colored), newly
167 synthesized protein through FUNCAT metabolic labeling and MAP2B immunolabeling in cortical

168 neurons on AICAR (1mM) treatment and Compound C (10µM) treatment for 1 hour. MAP2B
169 immunolabeling was used for identifying neurons and intensity was used for normalization.
170 Scale=10µM.

171 **(B)** Representative images showing immunolabeling of MAP2B (green) in cortical neurons in
172 basal condition and on DHPG treatment for 5 minutes in presence or absence of AICAR for the
173 cells used for p-eEF2 and FUNCAT quantification. Scale=10µM.

174 **(C)** Representative images showing immunolabeling of MAP2B (green) in cortical neurons in
175 basal condition and on NMDA treatment for 5 minutes in presence or absence of Compound C
176 for the cells used for p-eEF2 and FUNCAT quantification. Scale=10µM.

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