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Supplementary Materials for

Pin1 and PKMζ Sequentially Control Dendritic Protein Synthesis

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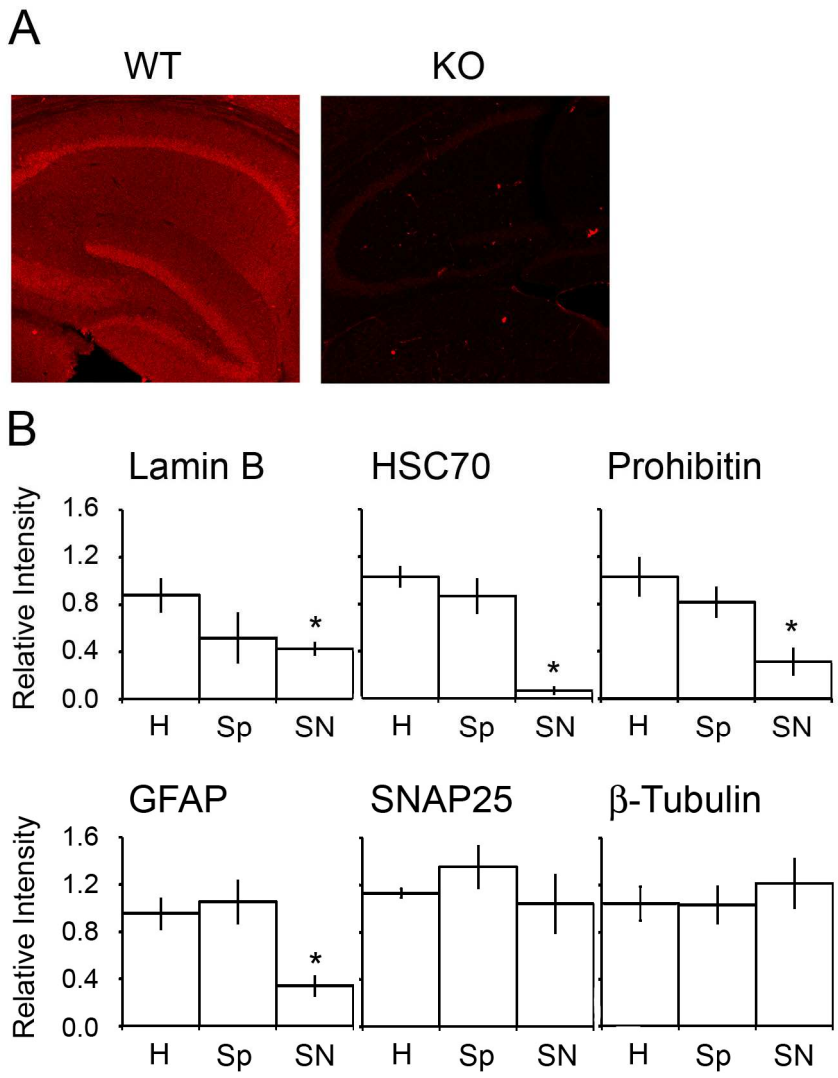


Fig. S1: **SNs are enriched for synaptic markers**. **(A)** Representative 100x confocal images of 30 μm hippocampal slices from 21 to 25 day-old WT and Pin1$^{-/-}$ mice. Slices were immunostained with anti-Pin1-Rhodamine. **(B)** Immunoblots of H (crude cortical homogenate); Sp (supernatant after 1000g spin), and SN from percoll gradients were probed for Lamin B (nuclear), HSC70 (cytoplasmic), Prohibitin (mitochondrial), GFAP (astrocytic), SNAP25 (synaptic), and β-Tubulin. The bands were visualized using a Storm 860 Phosphorimager and normalized to total protein using Ponceau S, n=3, * denotes $p<0.05$.

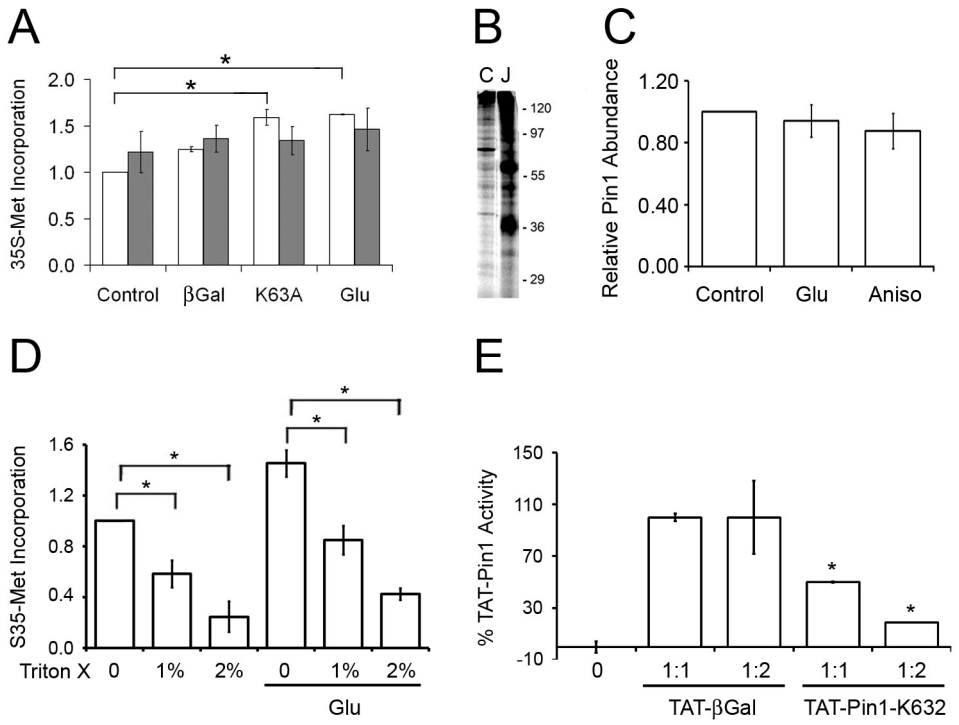
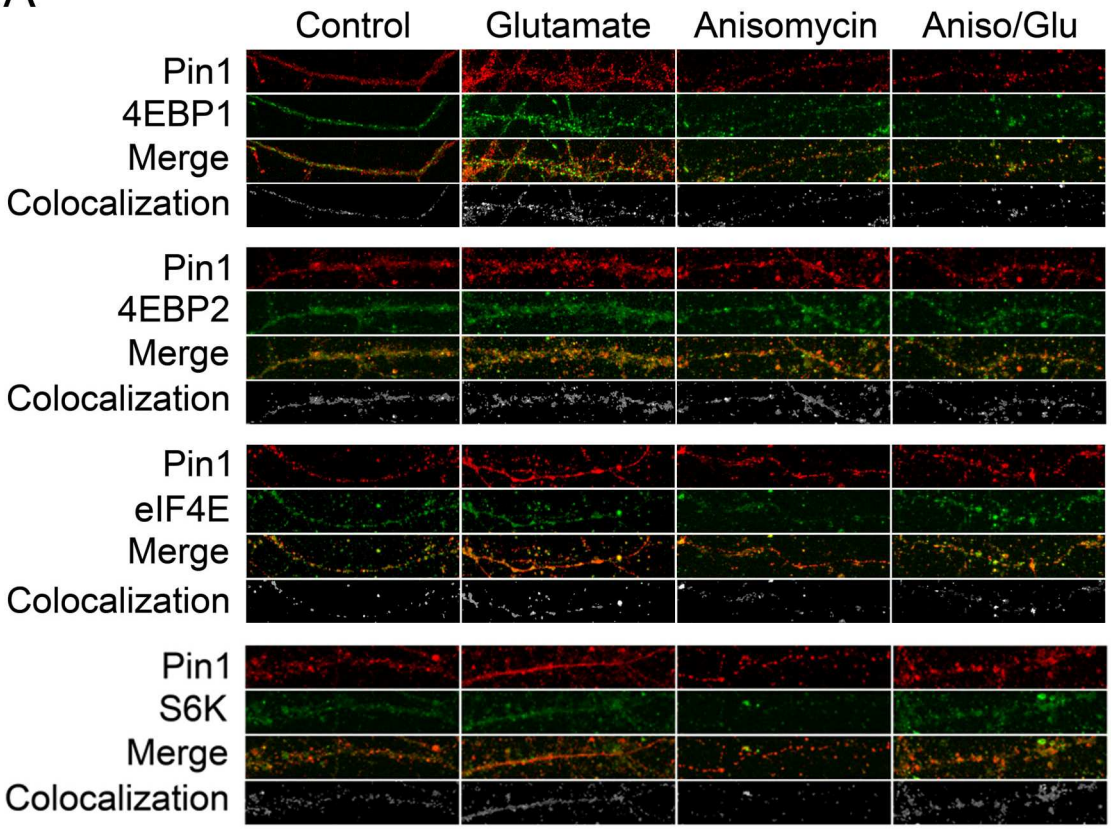
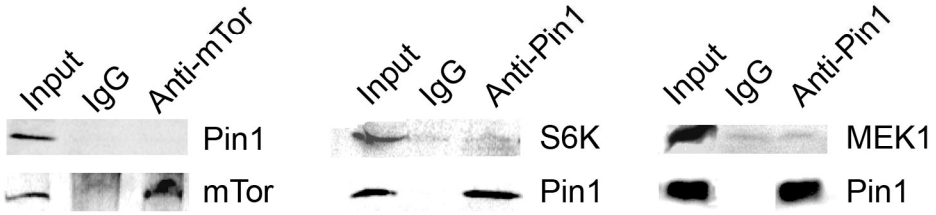


Fig. S2. Pin1 regulates dendritic translation. **(A)** SN were untreated (Control (☐)) or pretreated for 15 min with 5 μM cycloheximide (■) at 37°C. 50 nM βGal, 50 nM K63A, or Glu was added and incubated for 30 min with ^{35}S-Met. Total ^{35}S-Met incorporation was determined, n=3, ± SEM; * = p<0.003. **(B)** SN were untreated (C) or pretreated for 15 min with 1 μM juglone (J) (■) at 37°C and then incubated for 30 min with ^{35}S-Met, representative of n=3, **(C)** SN were untreated (control) or pretreated with Aniso (40 μM) or Glu at 37°C for 10 min and Pin1 abundance determined by immunoblot, n = 3, ± SEM, p>0.1. **(D)** SN were untreated or preincubated for 10 min. with 1 or 2% Triton X-100 prior to addition of ^{35}S-met and incubation for 30 min, lysis, and SDS-PAGE. Total ^{35}S-met incorporation into protein was quantitated by phosphorimaging, n=3, ± SEM, * denotes p<0.02. **(E)** Isomerase activity of recombinant TAT-Pin1 (2 μg) in the presence of TAT-βGal (2 or 4 μg) or TAT-Pin1-K63A (2 or 4 μg), n=3, ±SEM, * denotes p<0.05.

A


Control
Glutamate
Anisomycin
Aniso/Glu
Pin1
4EBP1
Merge
Colocalization
Pin1
4EBP2
Merge
Colocalization
Pin1
eIF4E
Merge
Colocalization
Pin1
S6K
Merge
Colocalization

B


Input
IgG
Anti-mTor
Pin1
mTor
Input
IgG
Anti-Pin1
S6K
Pin1
Input
IgG
Anti-Pin1
MEK1
Pin1

C

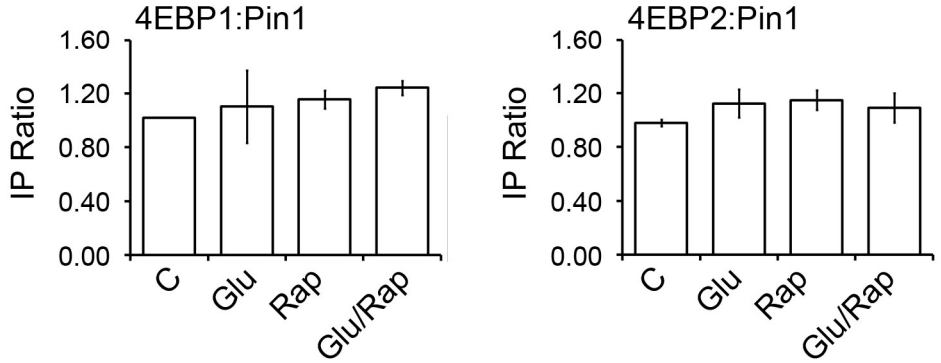

4EBP1:Pin1
IP Ratio
1.60
1.20
0.80
0.40
0.00
C
Glu
Rap
Glu/Rap
4EBP2:Pin1
IP Ratio
1.60
1.20
0.80
0.40
0.00
C
Glu
Rap
Glu/Rap

Fig. S3. Pin1 associates with translational regulators. (A) E17 cortical neurons, DIV 17, from WT or Pin1 KO mice were treated, fixed, stained, and visualized by confocal microscopy. Neurons were untreated or pretreated with anisomycin (40 μM) for 15 min and either not treated further or stimulated with Glu for 30 min. Primary neurons were labeled with anti-4E-BP1-FITC, anti-4E-BP2-FITC, or anti-eIF4E-FITC. Merge panels show colocalized (yellow) and uncolocalized (red and green) points, whereas colocalization panels show only the points of colocalization (white). **(B)** WT brain homogenates (600 μg) were lysed and immunoprecipitated with pre-immune IgG, anti-Pin1, or anti-mTor followed by immunoblot, n=3, with anti-Pin1, anti-S6K, anti-mTor, or anti-MEK1. Homogenate (50-200 μg, input) was used as a positive control. **(C)** WT SN (200 μg) were untreated or preincubated with 100 nM rapamycin for 10 min followed by a 30 min incubation with or without Glu. SN were lysed and immunoprecipitated with pre-immune IgG or anti-Pin1 followed by immunoblot with anti-4EBP1, anti-4EBP2 or anti-Pin1. SN (12.5 μg) was used as the positive control. The ratio of 4EBP1 or 2 abundance to Pin1 abundance, representing protein-protein interaction via IP, was calculated and graphed n=3, ±SEM.

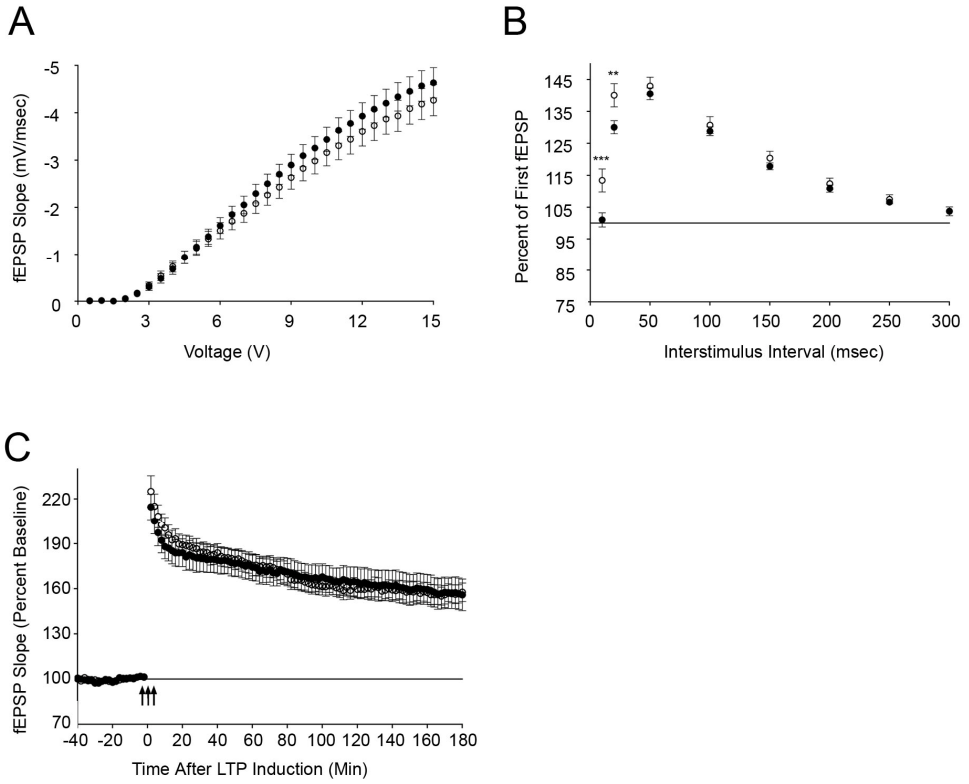


Fig. S4. Pin$^{-/-}$ hippocampal slices show normal, basal synaptic transmission, but enhanced paired-pulse facilitation. (A) The synaptic input-output curve from hippocampal slices shows fEPSP slopes as a function of fiber volley amplitudes for WT (●; n = 25 slices, 6 mice) and KO (O; n = 22 slices, 6 mice, p=not significant). **(B)** Paired-pulse facilitation is shown as the percent of first fEPSP plotted versus the interpulse interval for WT (●; n = 25 slices, 6 mice) and KO (O; n = 22 slices, 6 mice, ***p<0.001 and **p<0.01, respectively for the 10 and 20 ms interstimulus interval). **(C)** L-LTP was induced in Pin1$^{-/-}$ (O; n=11 slices, n= 3 mice) or Pin1$^{+/+}$ (●; n=16 slices, n=3 mice, p=not significant) hippocampal slices with 3 trains of theta burst stimulation separated by 20 sec.

Table S1. Antibody manufacturers and dilutions.

Antibodies	Mfc	Cat#	HostSpecies	W dilution	IF dilution
β-Actin	Sigma	A5441	mouse	1:2000	
4EBP1	Santa Cruz	sc-9977	mouse	1:200	1:10
4EBP1	Santa Cruz	sc-6936	rabbit	1:200	1:100
4EBP2	Cell Signalling	2845	rabbit	1:1000	1:50
eIF4E	Santa Cruz	sc-9976	mouse	1:200	1:100
GABAβ R1	Santa Cruz	sc-14006	rabbit	1:200	
GFAP	Santa Cruz	sc-65343	mouse	1:200	
HSC70	Santa Cruz	sc-7298	mouse	1:200	
mGluR1a	AbCam	ab6439	rabbit	1:1000	
Pin1	R&D	MAB2294	mouse	1:200	
Pin1	Santa Cruz	sc-46660	mouse	1:200	1:100
Pin1	Santa Cruz	sc-15340	rabbit	1:200	1:100
PKC-alpha	AbCam	ab26318-100	mouse	1:100	
PKC-alpha	Cell Signalling	2056	rabbit	1:1000	
PKC-zeta	Santa Cruz	sc-216	rabbit	1:200	1:100
PKC-zeta	Santa Cruz	sc-17781	mouse	1:200	1:100
Prohibitin	Santa Cruz	sc-28259	rabbit	1:200	
PSD-95	Santa Cruz		rabbit	1:200	1:200
PSD-95	Milliore	MAB1596	mouse	5 μg/mL	20 μg/mL
S6K	Cell Signalling	9202	rabbit	1:1000	1:100
SNAP25	AbCam	ab5666-100	rabbit	1:2000	
Synaptophysin	Millipore	MAB368	mouse	1:500	
β-3-Tubulin	AbCam	7751-100	mouse	1:1000	
β-3-Tubulin	Santa Cruz	sc-80016	mouse	1:200	