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Supplementary Materials for

BDNF increases synaptic NMDA receptor abundance by enhancing the local translation of Pyk2 in cultured hippocampal neurons

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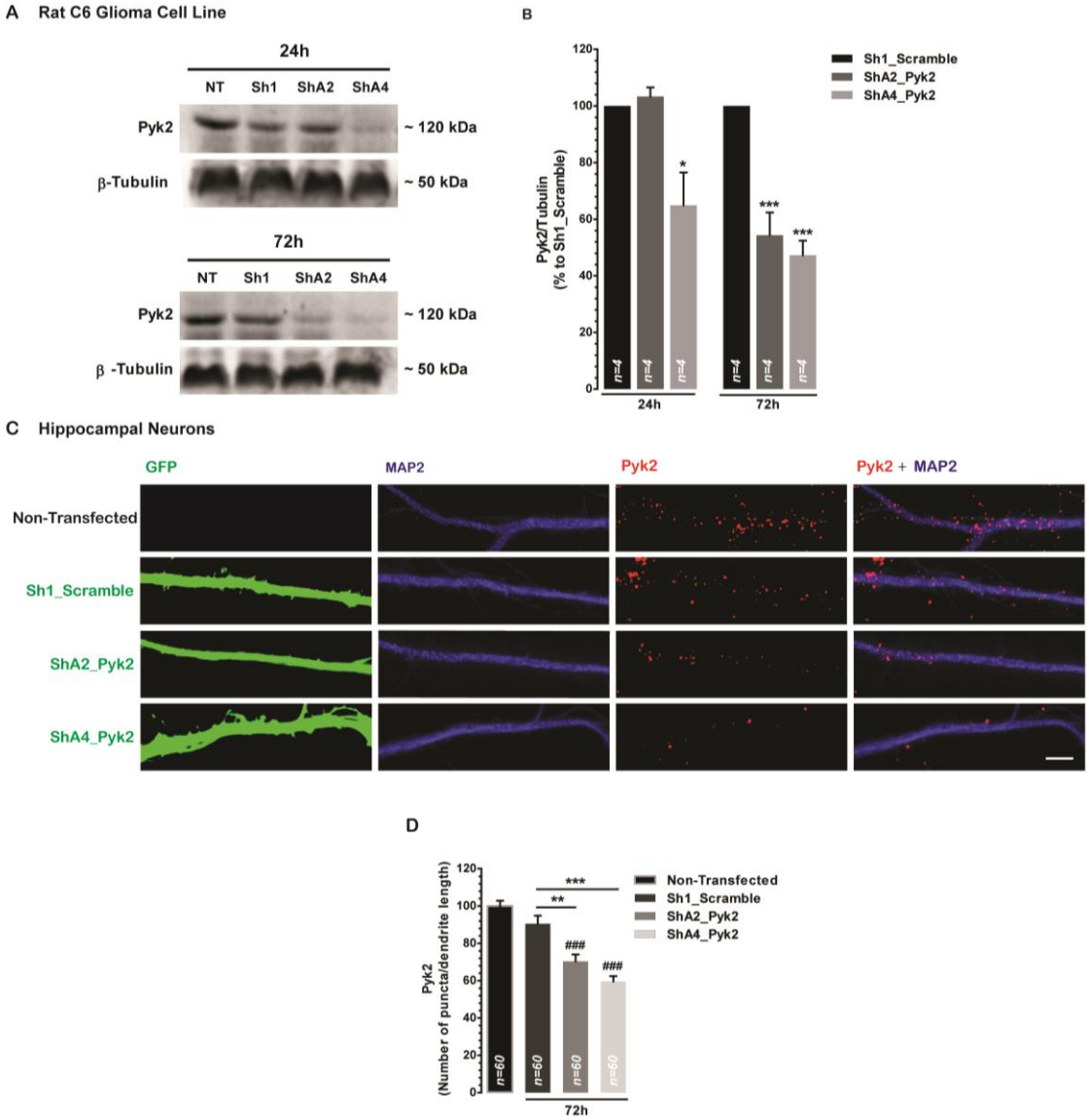

Fig. S1. Evaluation of Pyk2 knockdown efficiency by shRNA. (**A and B)** C6 glioma cells were either unperturbed (non-transfected; NT) or transfected with either scrambled shRNA (Sh1-Scramble; Sh1) or one of 2 shRNAs against Pyk2 [ShA2-Pyk2 (ShA2) or ShA4-Pyk2 (ShA4)]. Protein extracts were prepared 24 or 72 hours after transfection and Pyk2 abundance assessed by immunoblot (represented in A). β-tubulin was used as loading control and the results (mean ± S.E.M.; B) of four independent experiments were calculated as percentage of the results obtained with Sh1-Scramble. Scale bar, 5 µm. **(C and D)** Cultured hippocampal neurons were transfected with Sh1-Scramble, ShA2-Pyk2 or ShA4-Pyk2 at DIV 12. After 72 hours (then DIV 15), neurons were fixed and permeabilized then immunostained for Pyk2, GFP and MAP2. Images (represented in C) were analyzed for the number of Pyk2 puncta per dendritic length and the results are expressed relative to the control, non-transfected neurons (D). Data are presented as means ± S.E.M. from the indicated number of neurons (n) in at least three different experiments, performed in independent preparations. **P*<0.05, ***P*<0.01, ****P*<0.001 (or $^{\#\#\#}P$<0.001 comparing to non-transfected neurons) by a one-way ANOVA with Bonferroni test.

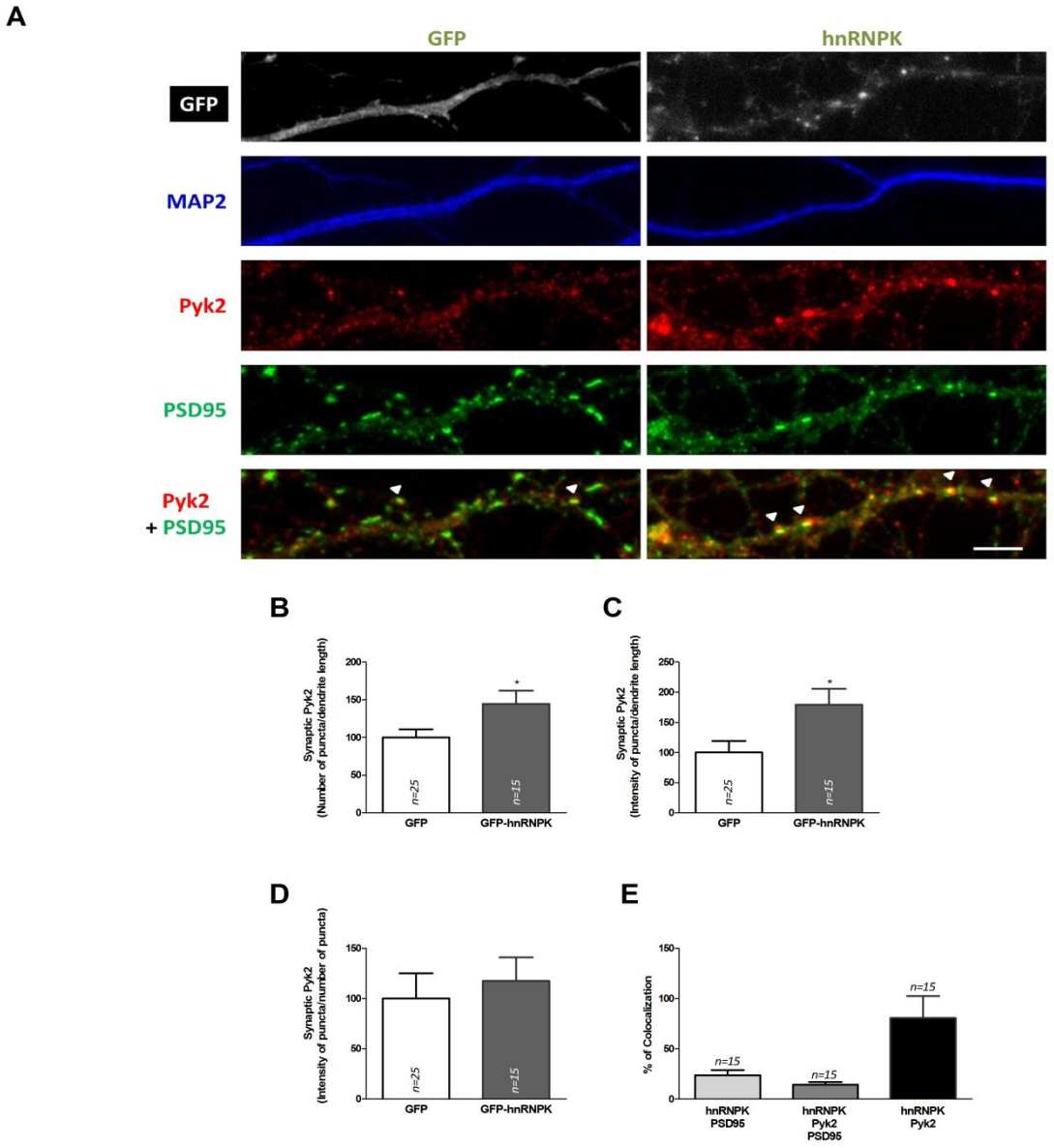

Fig. S2. hnRNP K overexpression increases the synaptic abundance of Pyk2. (A) Representative images of hippocampal neurons (DIV 11-12) that were transfected with control EGFP or hnRNP K-expressing plasmids and immunostained for PSD-95, Pyk2 and MAP2 at DIV 14-15. Scale bar, 5 µm. Arrowheads denote Pyk2-PSD-95 colocalized puncta. **(B to D)** Neurons described in (A) were analyzed for the number of synaptic (PSD-95-colocalized) Pyk2 puncta (B), intensity of Pyk2 puncta per dendrite length (C), Pyk2 immunoreactivity per number of puncta (D), and the percentage of hnRNP K that was colocalized with PSD95, with Pyk2, or with both proteins (E). Data are relative to the EGFP control and are presented as means ± S.E.M. for the indicated number of neurons (n) in three independent experiments performed in different preparations. *$p < 0.05$ and **$p < 0.01$ by an unpaired Student's *t*-test.

Table S1. shRNA sequences. Sequences, 5'-3', of the shRNA oligonucleotides used in this study.

	Target sequence	**Sense oligo**	**Anti-sense oligo**
sh-scramble	none	GATCCCC **GATGAACGCTCTGGATGCG** TTCAAGAGA **CGCATCCAGAGCGTTCATC** TTTTTGGAAA	AGCTTTTCCAAAAA **GATGAACGCTCTGGATGCG** TCTCTTGAA **CGCATCCAGAGCGTTCATC** GGG
sh-hnRNP K	1201-1219 GUAACUAUUCCCAAAGAU U	GATCCCC **GTAACTATTCCCAAAGATT** TTCAAGAGA **AATCTTTGGGAATAGTTAC** TTTTTGGAAA	AGCTTTTCCAAAAA **GTAACTATTCCCAAAGATT** TCTCTTGAA **AATCTTTGGGAATAGTTAC** GGG