Constitutive Regulation of the Glutamate/Aspartate Transporter EAAT1 by Calcium/Calmodulin-Dependent Protein Kinase II

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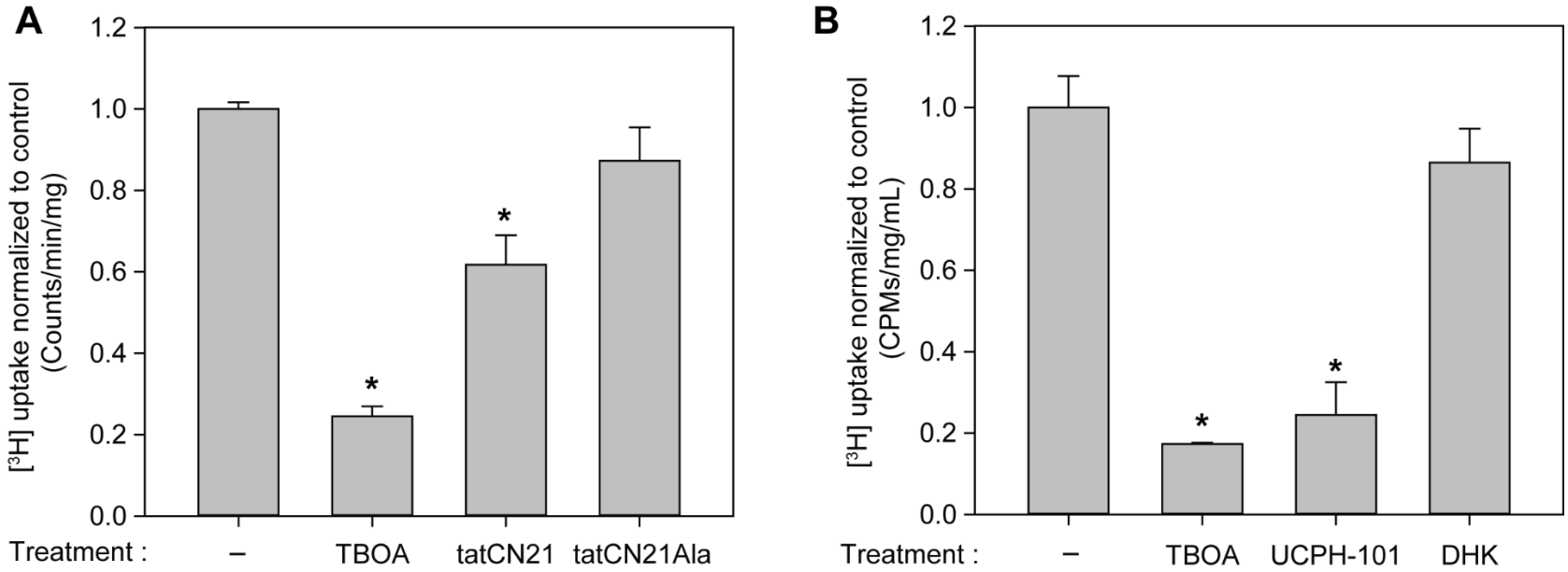
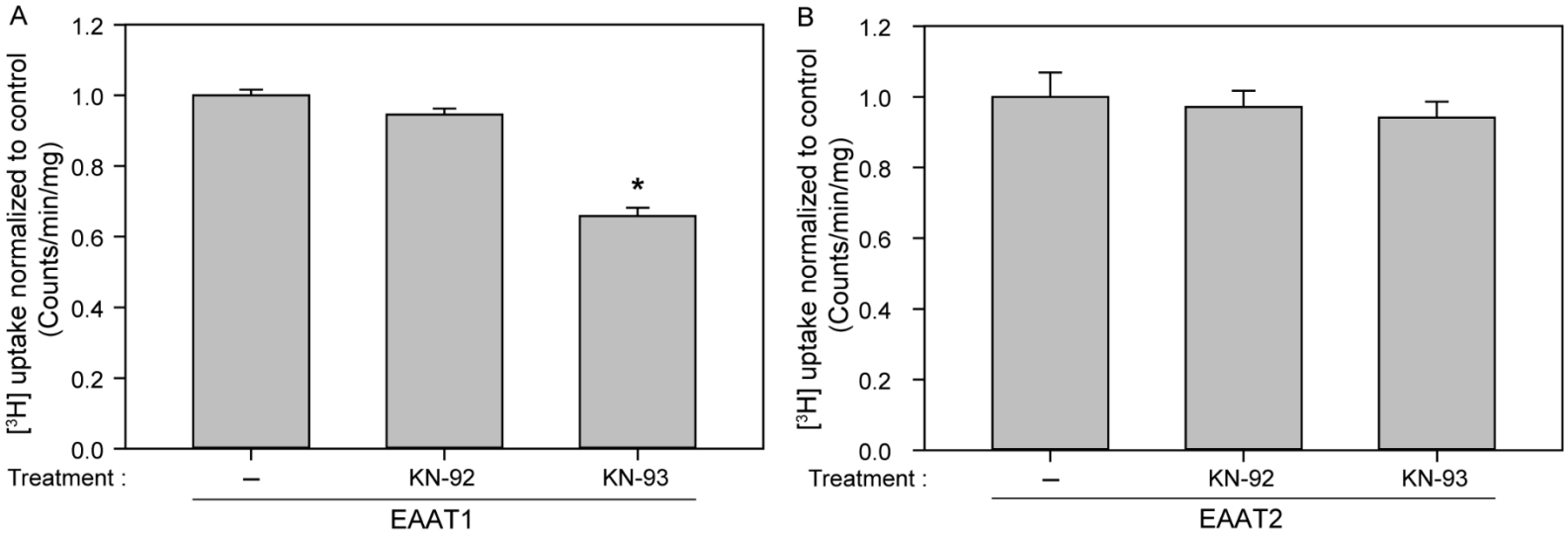
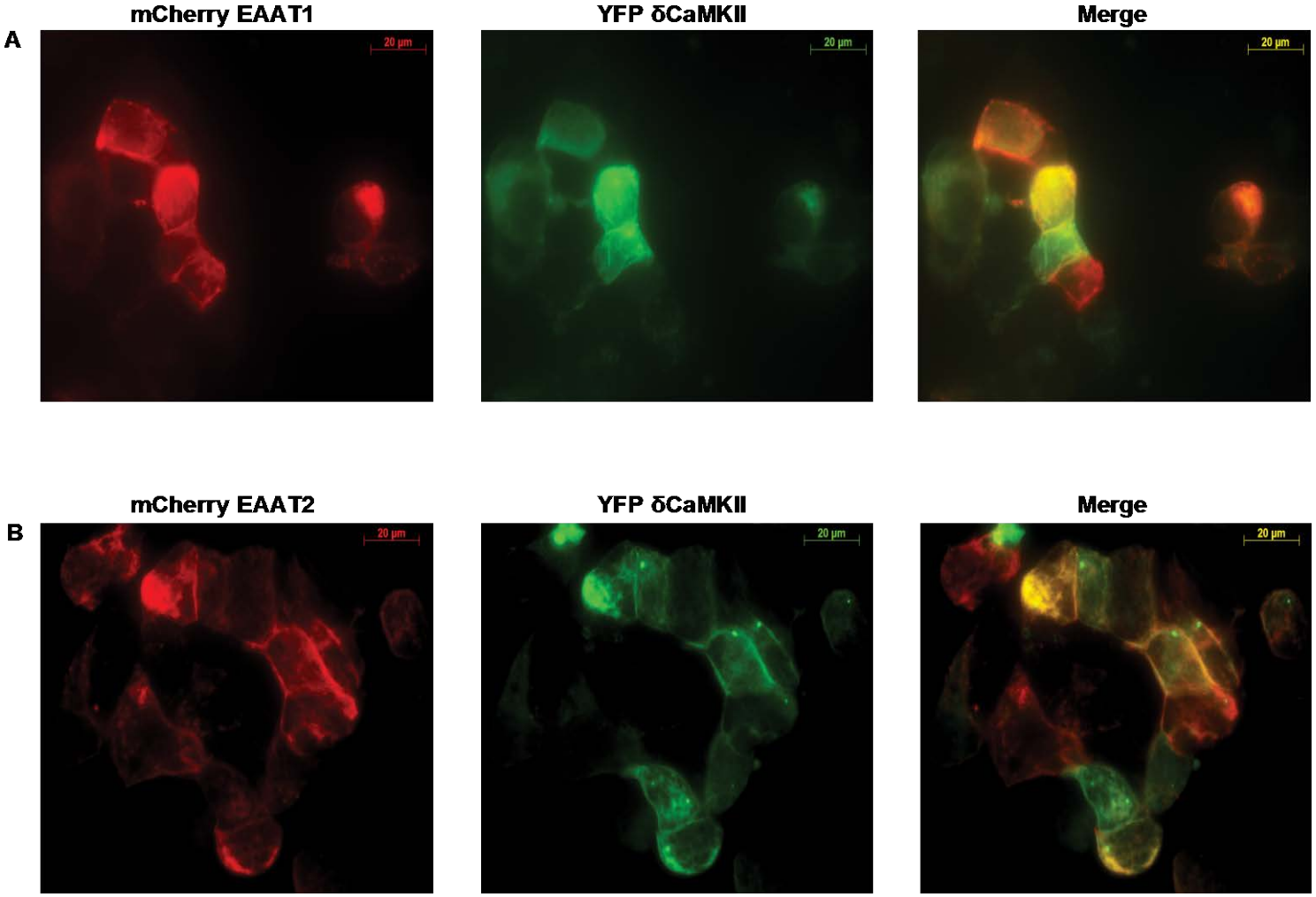
Supplementary figure 1: CaMKII signaling regulates glutamate uptake in rodent cultured cortical astrocytes predominantly expressing EAAT1. (A) Average [^{3}H]-glutamate uptake following CaMKII inhibition by tat-CN21 in cultured cortical astrocytes (n=3, ± S.D.) normalized to untreated control. Tat-CN21 (10 μM) and the non-active inhibitor tat-CN21 Ala (10 μM) were applied to cells for 20 minutes prior to a 20 minute [^{3}H]-glutamate measurement protocol. (B) Average [^{3}H]-glutamate uptake normalized to untreated control following inhibition of glutamate transporters in cultured cortical astrocytes (n=3, ± S.D.). Glutamate transport inhibitors TBOA (100 μM), UCPH-101 (10 μM) and DHK (10 μM) were applied to cells for 20 minutes prior to a 20 minute [^{3}H]-glutamate uptake measurement protocol. The asterisk indicates significant difference compared to untreated control (*, p≤0.05, one-way ANOVA, *post-hoc* Dunnett's test).



Supplementary figure 2: CaMKII inhibition reduces EAAT1 but not EAAT2 mediated [^{3}H]-glutamate uptake in A172 glioblastoma cells. (A) Average [^{3}H]-glutamate uptake in mCherry-EAAT1 (n=9, ± S.E.M) and (B) mCherry-EAAT2 transfected cells (n=6, ± S.D.) normalized to mCherry transfected untreated A172 glioblastoma cells. The CaMKII inhibitors KN-93 (5μM) and its inactive analog KN-92 (5μM) were applied to cells for 20 minutes prior to a 20 minute [^{3}H]-glutamate uptake measurement protocol. The asterisk indicates significant difference compared to transfected untreated control (*, p≤0.05, one-way ANOVA, *post-hoc* Dunnett's test).



Supplementary figure 3: Subcellular localization of transfected wild-type CaMKII HEK293T cells with EAAT1 or EAAT2. Representative images of YFP wild-type δCaMKII in A, mCherry wild-type EAAT1 transfected and B, mCherry wild-type EAAT2 transfected HEK293T cells. Images are viewed using the red channel (excit. 595 nm/ emis. 620 nm) (left), green channel (excit. 490 nm/emis. 525 nm) (center) and merged (right). HEK293T cells were co-transfected with 6 μg of δCaMKII cDNA and with 6 μg of either EAAT1 or EAAT2 cDNA.



Supplementary table 1: Primers used for site-directed mutagenesis. Specific primer sequences resulting in a mutation are bolded and underlined.

cDNA	Mutation	Primer Sequence (5' to 3')
δCaMKII	Thr287Asp	atttcttcaagcagtcta**cat**cctcctgtctgtgcatcatgg
δCaMKII	Thr287Asp	ccatgatgcacagacaggaggatgta**gac**tgcttgaagaaat
δCaMKII	Asp136Asn	attctcaggcttcag**gtt**cctgtgaactatgccat
δCaMKII	Asp136Asn	atggcatagttcacagg**aac**ctgaagcctgagaat
EAAT1	Thr26Ala	ttcttggccaaaag**tgc**gcgtttacggactccc
EAAT1	Thr26Ala	gggagtccgtaaacgc**gca**cttttggccaagaa
EAAT1	Thr26Asp	cactttcttcttggccaaaag**atc**gcgtttacggactccctgctg
EAAT1	Thr26Asp	cagcagggagtccgtaaacgc**gat**cttttggccaagaagaaagtg
EAAT1	Thr37Ala	gtaacttttaacatcctcctt**tgc**aatgttctgcactttcttcttgg
EAAT1	Thr37Ala	ccaagaagaaagtgcagaacatt**gca**aaggaggatgttaaaagttac
EAAT1	Thr37Asp	cacacttttggccaagaagaaagtgcagaacatt**gat**aaggaggatgttaaaagtta
EAAT1	Thr37Asp	taacttttaacatcctccttatcaatgttctgc**act**ttcttcttggccaaaagtgtg

Supplementary table 2: Antibodies tested for Western Blotting mCherry-EAAT1 and mCherry-EAAT2

Primary antibody	Company	Catalog Number	Target	Dilution	Detection
Rabbit anti-EAAT1 (H-50)	Santa Cruz Biotechnology	sc-15316	EAAT1 (N-terminus)	1:500	-
Rabbit anti-EAAT1	Abcam	ab416	EAAT1 (C-terminus)	1:500	+
Rabbit anti-EAAT2 (H-85)	Santa Cruz Biotechnology	sc-15317	EAAT2 (N-terminus)	1:500-1:1000	-
Goat anti-EAAT2 (N-19)	Santa Cruz Biotechnology	sc-7760	EAAT2 (N-terminus)	1:200	-
Rabbit anti-EAAT2	Cell Signaling	3838	Human EAAT2	1:500-1:1000	-
Rabbit anti-EAAT2	Abcam	41621	EAAT2 (C-terminus)	1:1000	+