



Protein extracts from embryonic wild type and Kidins220^{-/-} astrocytes (**A**), from wild type embryonic and postnatal astrocytes (**B**) and from Kidins220^{lox/lox- Δ Cre} and Kidins220^{lox/lox-Cre} astrocytes (**C**) were analyzed by western blotting with anti-p75^{NTR} antibodies. Representative immunoblots are shown on the left; quantification of immunoreactive bands is on the right. The intensity of bands from Kidins220^{-/-}, postnatal and Kidins220^{lox/lox-Cre} samples were normalized to the corresponding control samples within the same nitrocellulose membrane. p>0.05, one sample Student's *t*-test, n=7 independent cultures of both genotypes in (A), n=5 in (B) and (C).



Fig. 2. Activation of signaling pathways upon administration of 1 ng/ml BDNF in embryonic astrocytes.

(A) Wild type and Kidins220^{-/-} embryonic astrocyte cultures were treated with 50 ng/ml BDNF for 60 min and 120 min or left untreated (time **(B)**) Wild type and Kidins220^{-/-} embryonic astrocyte cultures were treated with 1 ng/ml BDNF for 5 and 30 min or left untreated (time 0). In both (A) and (B), lysates were analyzed for phosphorylated MAPK1/2 (Thr202/Tyr204) and Akt (Ser473). PLC γ did not show any reliable activation at 1 ng/ml BDNF concentration (not shown). Membranes were subsequently stripped and re-probed for the total amount of the same protein. *Left*: Representative immunoblots. *Right*: Time dependence of MAPK1/2 and Akt phosphorylation upon BDNF stimulation in wild type and Kidins220^{-/-} astrocytes. The graphs express the fold change activation of MAPK1/2 and Akt compared to the untreated phosphorylation levels for each genotype, set to

1 (dashed line in all graphs). For further details, see legend to Fig. 1 and Methods. For MAPK, we report the sum of MAPK1 and MAPK2 immunoreactivity. p<0.05, p<0.01, unpaired Student's *t*-test, n=5 in (A) n=4-5 in (B) for both wild type and Kidins220^{-/-} cultures. Values are expressed as means \pm S.E.M.



Fig. S3. Activation of signaling pathways upon administration of 1 ng/ml BDNF in wild type postnatal astrocytes.

Experiments as in Suppl. Figure 1, but for wild type postnatal astrocytes cultures. p<0.05, one sample Student's *t*-test compared to baseline, n=10 independent cultures. Values are expressed as means \pm S.E.M.



Fig. S4. Activation of signaling pathways upon administration of 1 ng/ml BDNF in Kidins220-deficient postnatal astrocytes.

Experiments as in Suppl. Figure 2, but for Kidins $220^{lox/lox-Cre}$ and Kidins $220^{lox/lox-\Delta Cre}$ astrocytes cultures. p>0.05, unpaired Student's *t*-test, n=15 independent cultures. In all experiments, GAPDH was used as a loading control. Values are expressed as means \pm S.E.M.

Table S1. List of primers u	used for the RT-qPCR analysis.
-----------------------------	--------------------------------

Gene	GenBank Accession	Forward primer (5' → 3')	Reverse primer $(5' \rightarrow 3')$
Slc1a2 (GLT-1)	NM_001077514.4	ACTGGCTGCTGGATAGAATGA	AATGGTGTCCAGCTCAGACT
Kcnj10 (Kir4.1)	NM_001039484.1	GCCCCGCGATTTATCAGAG	TCCATTCTCACATTGCTCCG
Aqp4	NM_009700.3	CTGTGGCAGCGAGATAATGG	GCCTTTCTGGGAACTCACAC
Gja1 (Cnx43)	NM_010288.3	CTTTGACTTCAGCCTCCAAGG	GGGCACCTCTCTTTCACTTAAT
Housekeeping			
genes			
TBP	NM_013684.3	ACTTCGTGCAAGAAATGCTGAAT	CAGTTGTCCGTGGCTCTCTTATT
TRFR	NM_011638.4	AGACCTTGCACTGTTTGGACATG	GGTGTGTATGGATCACCAGTTCCTA
TUBB2	NM_009450.2	CAAGGCTTTCCTGCACTGGT	AACTCCATCTCGTCCATGCC