SUPPLEMENTARY INFORMATION:

***COS7 cells, transfections and staining***

COS cells were transfected using DEAE-dextran as previously described (Patel et al., 1998). Transfection with Lipofectamine 2000 (Invitrogen) gave similar results. Cells were fixed with 4% paraformaldehyde/ 0.1M phosphate buffer and then stained for actin using Alexa Fluor® 594 phalloidin or cell membranes were labelled with Alexa Fluor® 594 wheat germ agglutinin (WGA). References and dilutions used are provided in supplementary information.

***Neuronal cultures, staining, and imaging***

Hippocampi of rat Wistar embryos were dissected at day E18. Culture medium was Neurobasal (Invitrogen) and 10% horse serum for 24 hours, and then Neurobasal with B27 (Invitrogen) and Ara-C. Cells were plated on 35 mm poly-D-lysine coated plastic dishes (Elvetec, France). Neurons were infected with the Semliki Forest virus containing EYFP or EYFP-TREK1, as previously described (Lauritzen et al., 2003). 20 hours post-infection, cells were fixed with 4% paraformaldehyde/0.1M phosphate buffer. Cells were stained with phalloidin594 (Molecular Probes) and imaged as described for COS cells. Isolation of striatal neurons from wild-type or knock-out mice was carried out as previously described (Heurteaux et al., 2004). Striata were dissected from mouse embryos at day E14. Cells were plated on 35 mm Falcon dishes previously coated with poly-ornithine and 50% fetal calf serum. Culture medium was DMEM (Invitrogen) supplemented with glucose (1.5 g/l) for 24 hours and then with serum-free medium for another 24 hours. After fixation with 4% paraformaldehyde/0.1M phosphate buffer, cells were stained with Alexa Fluor® 594 phalloidin as described above. Some dishes were immuno-labelled with Neuronal specific isotype III beta tubulin (Sigma) in order to verify that the cells used for cell counting were young post-mitotic neurons. For quantitative analysis, cells were counted in blind from randomly selected fields with fluorescence microscopy (Axioplan 2, Carl Zeiss) using a 63X lens objective. Data were accumulated from 4 independent cultures from striata of both wild-type and knock-out mice.

***References and dilutions used for cell biology.***

Alexa Fluor® 594 phalloidin (A12381, Molecular Probes) diluted 1/300 was used to visualize the actin cytoskeleton. Cell membranes were labelled with Alexa Fluor® 594 wheat germ agglutinin (WGA) (W11262, Molecular Probes) at 3 mg/ml. Goat anti-rabbit conjugated to Alexa Fluor® 594 (A11037, Molecular Probes) was diluted 1/800. Ezrin was localized with rabbit polyclonal anti-ezrin (1/700, kindly provided by Monique Arpin, Institut Curie, Paris) and goat anti-rabbit conjugated to Alexa Fluor® 594.

***Plasmid constructs***

Various inserts were generated using PCR with wild-type mTREK-1 (GenBank AY736359) as a template. Point mutations in TREK-1 were also generated by PCR. Fragments were cloned into pEYFP-C1 (BD Biosciences Clontech) creating a chimera between EYFP and TREK-1 or various point mutants in TREK-1. The other $K_{2P}$ channels were similarly amplified by PCR and cloned into the same vector. The chimera between mTREK-1 and rTASK-3 contains mTREK-1 at the 5' end till Ile 292 and rTASK-3 at the 3' end starting at Val 243. The chimera between rTASK-3 and mTREK-1 contains rTASK-3 at the 5' end till Val 242 and mTREK-1 at the 3' end starting at Gly 293.

***Microscopy and quantification.***

After mounting, specimens were observed using an epifluorescence microscope (Axioplan 2, Carl Zeiss) and appropriate combinations of filters. Images were recorded with a cooled CCD camera (Coolsnap HQ, Photometrics) driven by Metavue software. For confocal microscopy, cells were examined with a scanning laser confocal microscope (LEICA) using a 63X oil immersion objective. For fluorescence quantification, randomly selected cells were counted in blind from each transfection or culture, and data were compiled from at least 4 independent experiments. The Dunnet anova test was used for statistical analysis. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

***Genbank accession numbers for $K_{2P}$ channels.***

rTREK-2 (GenBank NM 023096), mTRAAK (GenBank NM 008431), rTWIK-2 (GenBank NM 053806) and rTASK-3 (GenBank NM 053405).

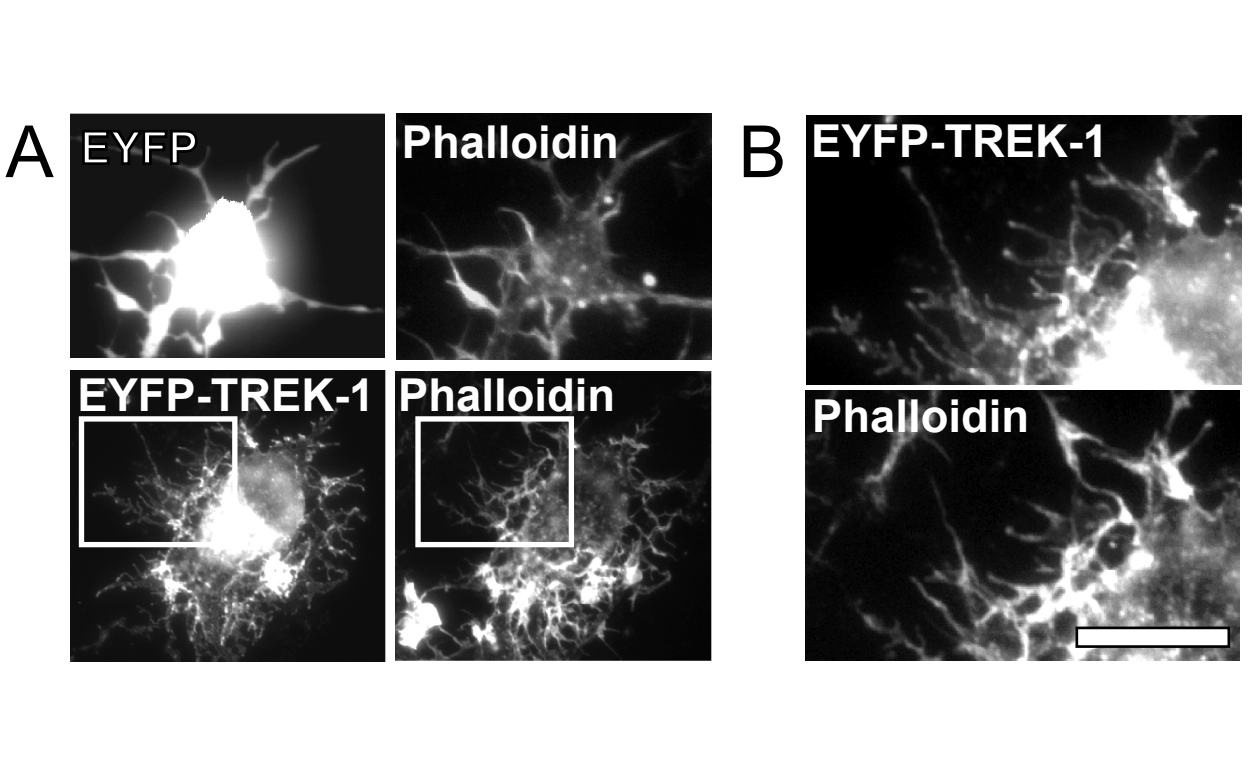
**SUPPLEMENTARY FIGURES :**

**FIGURE SUPP. 1 : A)** Epifluorescence images of SFV infected hippocampal neurons stained with phalloidin. Left panel - images showing EYFP or EYFP-TREK-1 fluorescence and right panel - the same neuron labelled with Alexa Fluor® 594 phalloidin. **B)** Close up of the actin protrusions boxed in A lower panels. The scale bar corresponds to 10 μm in A and 2.5 μm in B. **C)** Microvilli are vizualized with a poly-clonal TREK-1 antibody in COS cells transfected with the untagged TREK-1 channel. The scale bar corresponds to 20 μm.

**FIGURE SUPP. 2 : A)** Comparison of the percentage of expanded growth cones in cultured embryonic striatal neurons from TREK-1 WT and KO mice. Results from 4 independent cultures are illustrated. Numbers of growth cones examined are indicated and the differences are significant ($p<0.001$).

**FIGURE SUPP. 3 : A)** Effect of oleylamin (5 μM) on TREK-1 recorded in the inside-out patch configuration. The patch was stimulated with a voltage ramp of 600 ms duration from –100 mV to 100 mV and the holding potential was –80 mV. An amino-terminal EYFP-tagged mTREK-1 channel was used and no functional difference with non-tagged WT channel was observed, including sensitivity to membrane stretch (Patel and Honoré, 2001). **B)** Dose-effect curve of oleylamin on TREK-1 channel activity in excised inside-out patches (n ranges from 4 to 14). **C)** Induction of filopodia in COS cells expressing TREK-1 in the presence of the TREK-1 inhibitor oleylamin (5 μM) and cultured in a $K^+$-rich solution (75 mM). The morphogenic effect of TREK-1 is independent of channel activity.

Fig. Supp 1


A
EYFP
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EYFP-TREK-1
Phalloidin
B
EYFP-TREK-1
Phalloidin
C
TREK-1

Fig. Supp 2

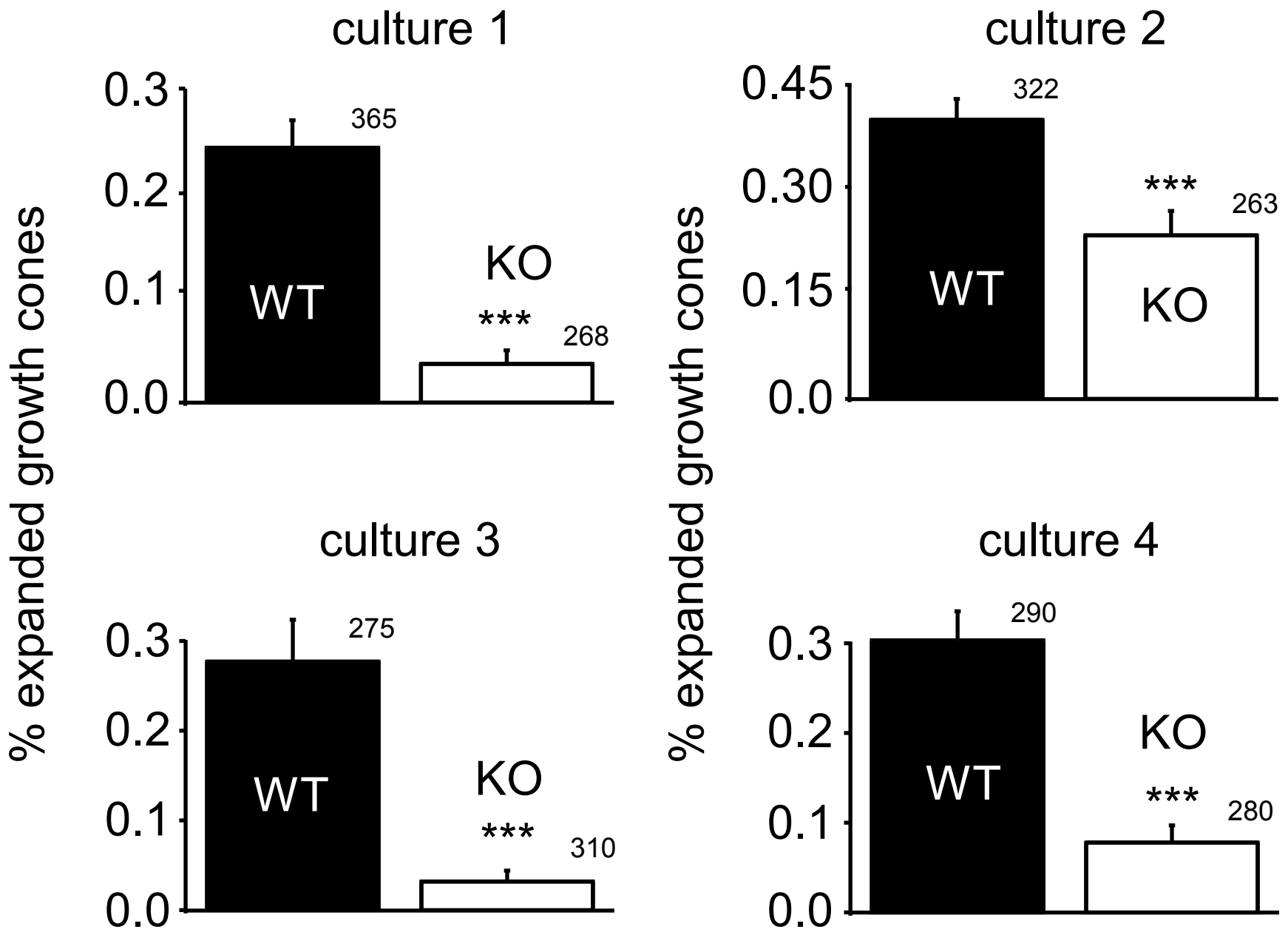
culture 1
culture 2
culture 3
culture 4
% expanded growth cones
% expanded growth cones
0.3
0.2
0.1
0.0
0.45
0.30
0.15
0.0
WT
KO
365
322
275
290
268
263
310
280
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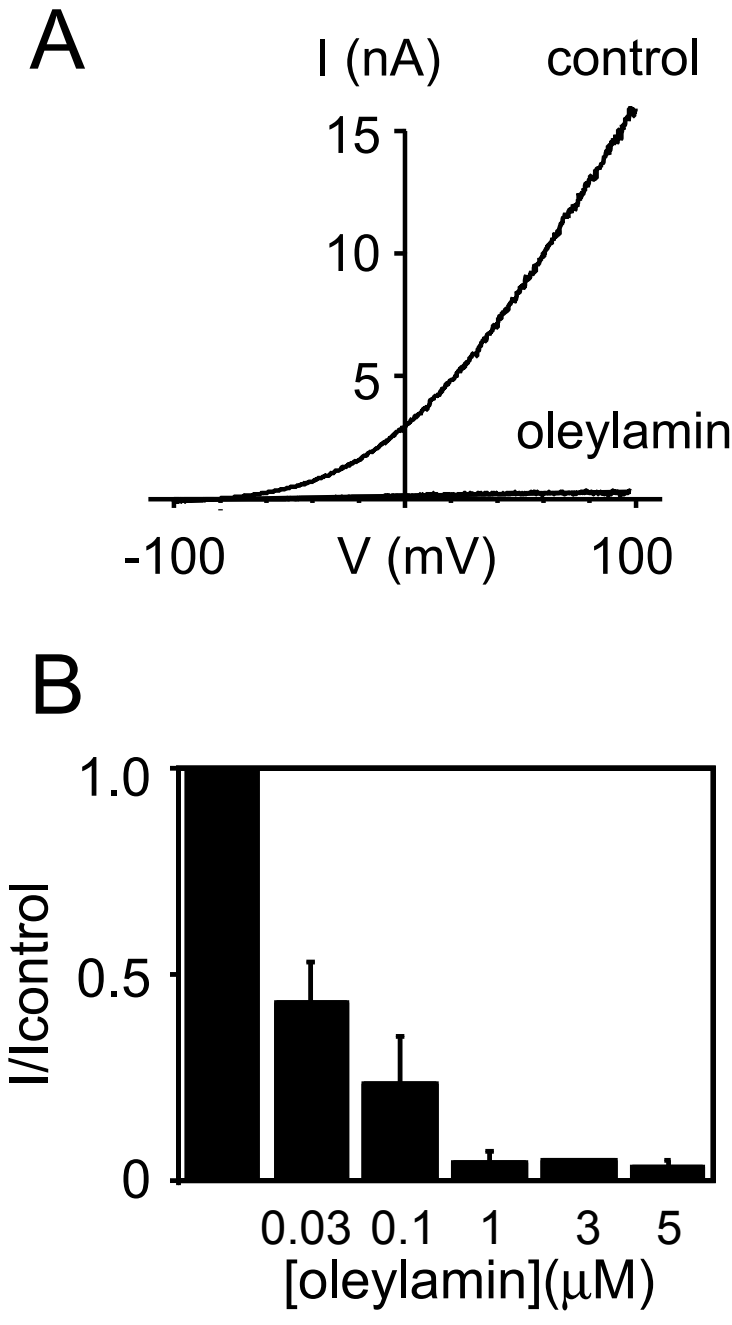
Fig. Supp 3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10
5
oleylamin
-100
V (mV)
100
B
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0.5
0
I/Icontrol
0.03
0.1
1
3
5
[oleylamin](μM)
C
100
80
60
40
20
0
% cells with protrusions
140
140
40
40
control
oleylamin
control
KCl