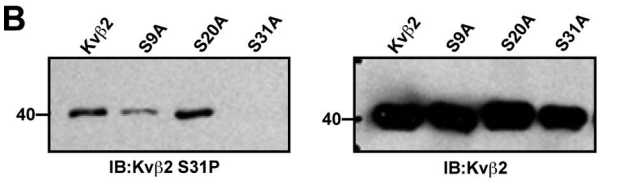
Vacher et al., http://www.jcb.org/cgi/content/full/jcb.201007113/DC1

A

Phosphorylation site	Heterologous cells	
	HEK 293	COS-1
pS9	+	+
pS20	+	-
pS31	-	-
pS112	+	+

+, identified phosphorylation site; -, phosphorylation site not identified



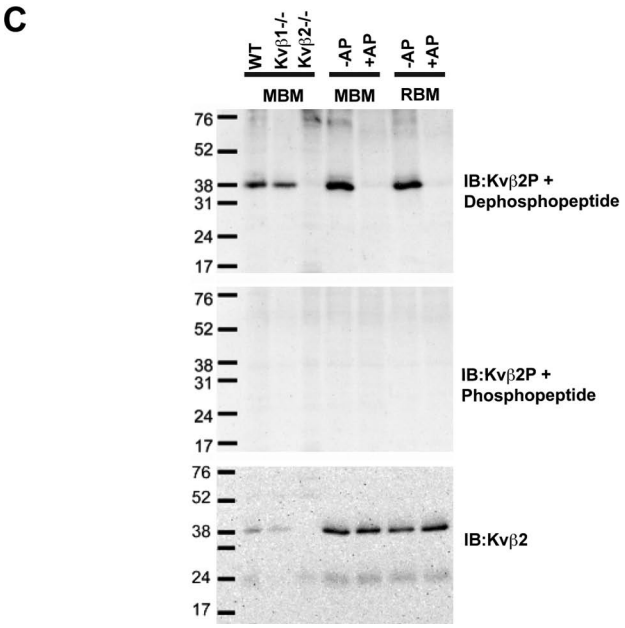


Figure S1. **Kvβ2 phosphorylation in heterologous cells and in mammalian brain.** (A) Identification of phosphosites present on recombinant rat Kvβ2 heterologously expressed in two mammalian kidney cell lines: HEK293 and COS-1. Using LC-MS/MS, phosphosites at S9, S20, and S112 on WT Kvβ2 expressed in HEK293 cells, and S9 and S112 on WT Kvβ2 expressed in COS-1 cells were identified. (B) Immunoblots of lysates of WT Kvβ2 or Kvβ2 mutants expressed in COS-1 cells using the Kvβ2P phosphospecific Ab or anti-Kvβ2 mAb K25/73. Robust Kvβ2P immunoreactivity was observed against WT Kvβ2, S9A, and S20A, whereas no immunoreactivity was observed against S31A. (C) Immunoblots on equal amounts (30 µg) of brain membrane samples. MBM: WT, Kvβ1$^{-/-}$, and Kvβ2$^{-/-}$ (samples courtesy of Dr. Geoff Murphy, University of Michigan, Ann Arbor, MI). RBM and MBM$^{+/-}$ AP treatment. Immunoblots were probed with Kvβ2P Ab preincubated overnight with 5 µg/ml of Kvβ2P dephosphopeptide (top), Kvβ2P Ab preincubated overnight with 5 µg/ml of Kvβ2P phosphopeptide (middle), or anti-Kvβ2 mAb K25/73.

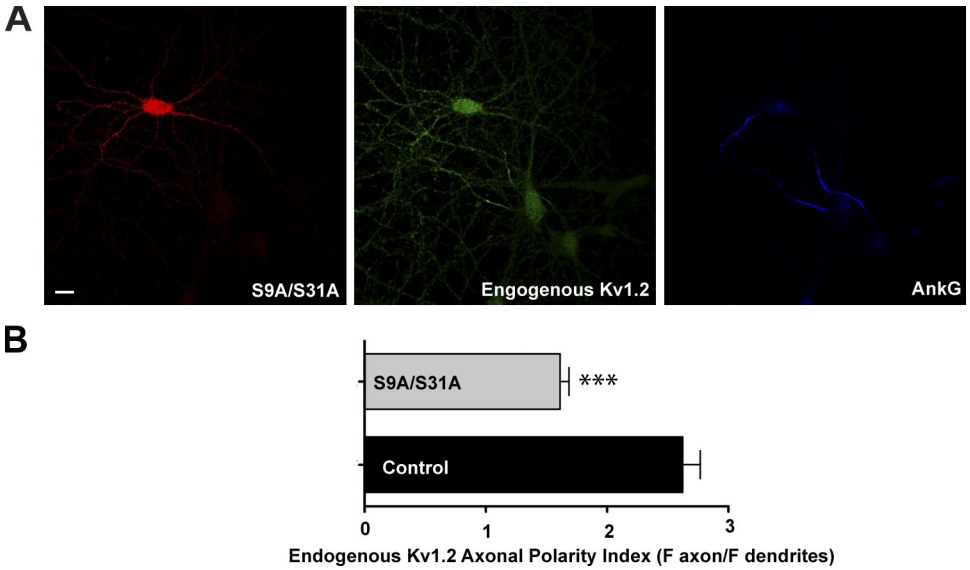


Figure S2. **Effect of Kvβ2 S9A/S31A mutant on endogenous Kv1.2 axonal distribution.** (A) Cultured hippocampal neurons (10 DIV) were transfected with Kvβ2 S9A/S31A. 5 d after transfection, neurons were immunostained with anti-Kvβ2, anti-Kv1.2, and anti-AnkG Abs. Bar, 25 µm. (B) Endogenous Kv1.2 axonal polarity index was determined by quantifying the immunofluorescence intensity profiles of the AIS versus three dendritic branches of either transfected or nontransfected (control) neurons using NIH Neuron/J. Control Kv1.2 API = 2.62 ± 0.14, n = 25; S9A/S31A Kv1.2 API = 1.61 ± 0.07, n = 24. ***, P < 0.0001 two-tailed t test.

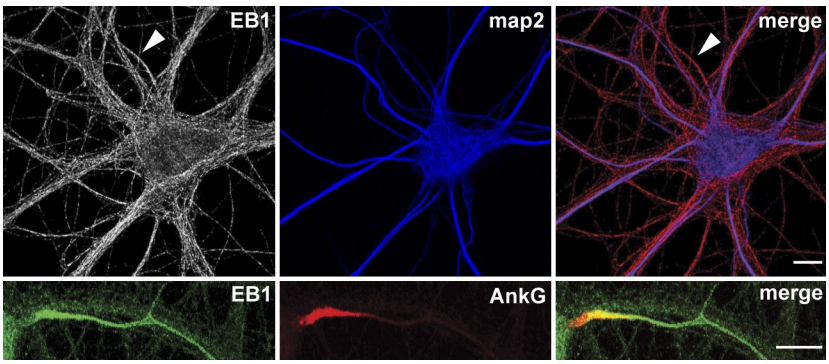


Figure S3. **Subcellular distribution of EB1 in cultured hippocampal neurons at 21 DIV.** Neurons were immunostained with anti-EB1 Ab. MAP2 immunostaining marks the somatodendritic compartment, and Ank-G immunostaining marks the AIS. EB1 exhibits a preferential axonal localization with enrichment in the AIS. Bars: 20 µm (top) and 10 µm (bottom).

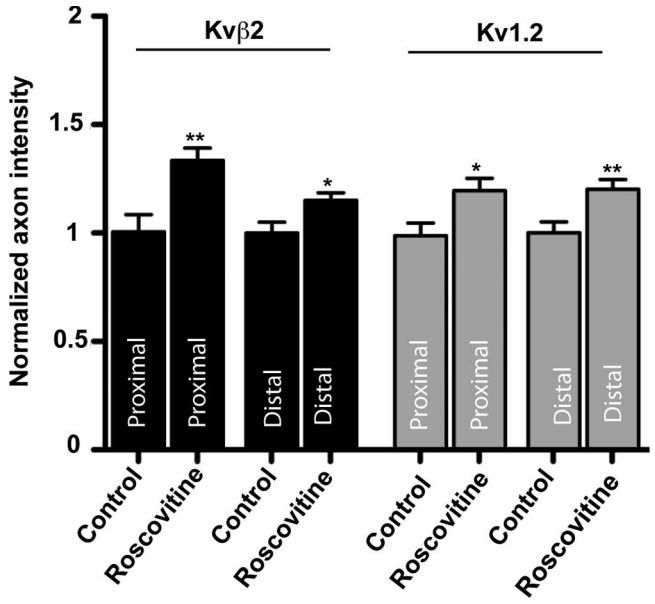


Figure S4. **Effect of Cdk inhibition on Kv1 channel and Kvβ2 distribution in proximal and distal axons.** Hippocampal neurons (21 DIV) with or without 10 μM roscovitine treatment for 24 h were multiple immunofluorescence stained for Kvβ2 and Kv1.2, with Ank-G as a specific marker of the AIS. The average fluorescence intensity for a 50-μm-long section located to the proximal axon (just after the AIS) or in the distal axon (at least 200 μm from the AIS) was quantified using NIH Neuron/J and subjected to statistical analysis using PRISM 5 (n = 15). *, $P < 0.05$; **, $P < 0.01$.

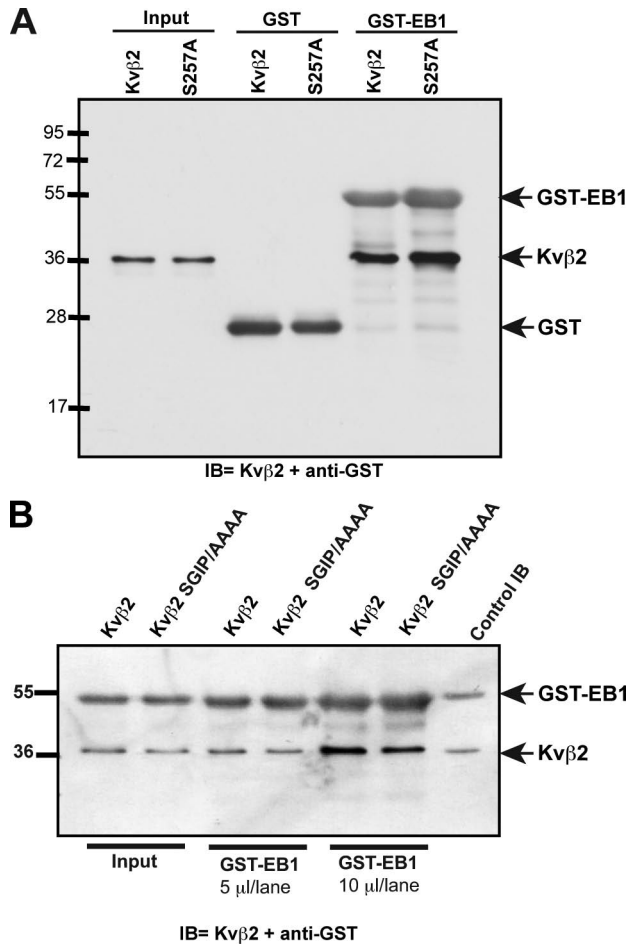


Figure S5. **Effect of the mutagenesis of Kvβ2 SGIP motif on its interaction with EB1.** (A) Input and products of GST pull-down reactions performed with GST-EB1 on Kvβ2 or Kvβ2 S257A COS-lysates. GST was used as a negative control. The gel was blotted at the same time with anti-Kvβ2 (K25/73) and anti-GST (rabbit polyclonal E93) Abs. (B) Input and products of GST pull-down reactions performed with GST-EB1 on purified Kvβ2 or Kvβ2 SGIP/AAAA proteins (bacterially expressed and precision protease cleaved). The gel was simultaneously blotted with anti-Kvβ2 mAb K25/73 and anti-GST Ab E93.