

Supplemental material

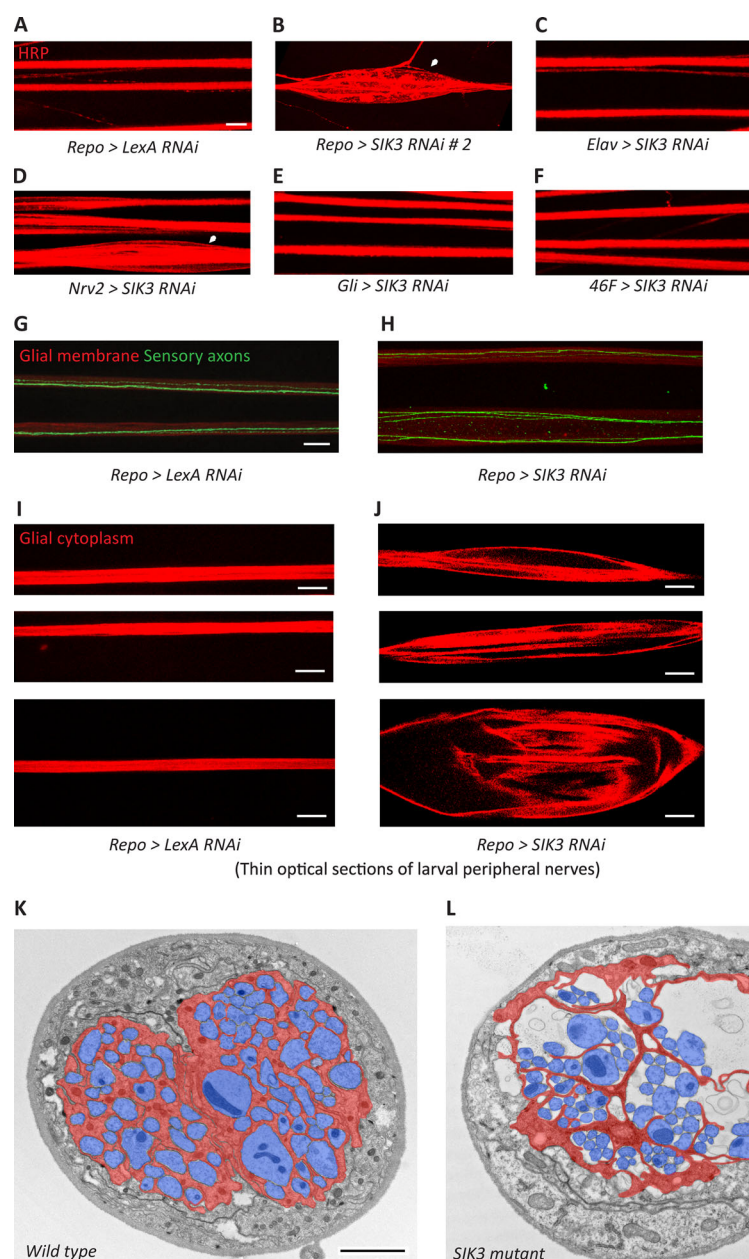
Li et al., <https://doi.org/10.1083/jcb.201907138>

Figure S1. **SIK3 mainly functions in wrapping glia of larval peripheral nerves and is not required for the maintenance of axon integrity.** Related to Fig. 1. (A–F) Representative images of peripheral nerves in larvae stained for the nerve membrane marker HRP, with RNAi-mediated knockdown of SIK3 in neurons or glial cells. Scale bar, 15 μ m. (A) Pan-glial expression of a control UAS-LexA RNAi does not affect peripheral nerve morphology. (B) Pan-glial knockdown of SIK3 using a nonoverlapping RNAi line (*Repo>SIK3 RNAi no. 2*) recapitulates the dramatic localized nerve swellings (arrow) shown in Fig. 1 A. (C) Loss of SIK3 in neurons (*Elav>SIK3 RNAi*) does not have notable effects on peripheral nerve morphology. (D–F) Depletion of SIK3 in wrapping glia (*Nrv2>SIK3 RNAi*) causes nerve swellings, but its loss in subperineurial glia (*Gli>SIK3 RNAi*) or perineurial glia (*46F>SIK3 RNAi*) does not cause significant changes in nerve width. A minimum of 30 nerves from at least 10 larvae were examined for each genotype. (G and H) Representative images of larval peripheral nerves with glial-specific expression of membrane-targeting UAS-RFP (red) and GFP-labeled sensory axons (green). Larvae that express a control RNAi in glia (*Repo>LexA RNAi*) and those with SIK3 RNAi-expressing glia (*Repo>SIK3 RNAi*) both have intact sensory axons. A minimum of 30 nerves from at least 10 larvae were examined for each genotype. Scale bar, 15 μ m. (I and J) Representative thin optical sections of peripheral nerves with glial-specific expression of cytoplasmic UAS-RFP (red). Nerves with SIK3 RNAi-expressing glia show dramatic unstained regions that are indicative of extracellular swellings. Scale bars, 15 μ m. (K and L) Representative EM sections from Fig. 1. The axons and their surrounding glia are color-coded in blue and red, respectively, to highlight that with glial SIK3 knockdown swellings occur between the glial membrane and the axons. Scale bars, 2 μ m.

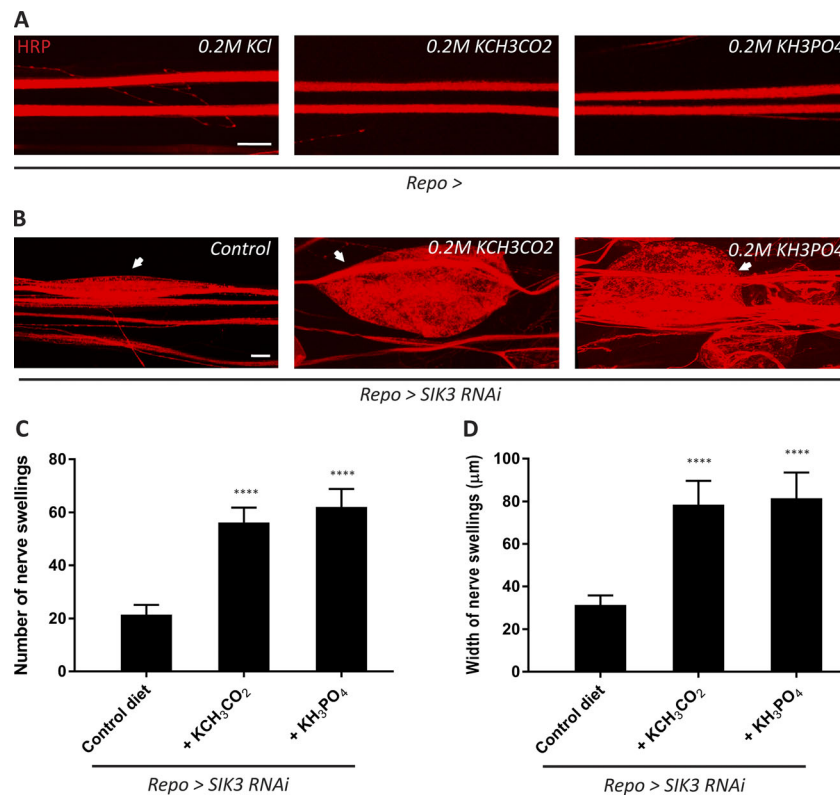


Figure S2. **SIK3 LOF-induced nerve swellings are sensitive to high K⁺ diets.** Related to Fig. 2. **(A)** Representative images of peripheral nerves of control larvae expressing a UAS-GFP transgene in glia raised on high K⁺ diets containing 200 mM of KCl, KCH₃CO₂ or KH₃PO₄; swellings were not observed in ≥30 peripheral nerves from ≥10 larvae examined for each condition. Scale bar, 20 μm. **(B)** Representative images of larval peripheral nerves with pan-glial knockdown of SIK3 fed high K⁺ diets. Swellings are denoted by arrows. Scale bar, 20 μm. **(C and D)** Quantification of nerve swellings in larvae with pan-glial knockdown of SIK3 raised on control or a high K⁺ diet. Similar to a KCl-rich diet, both KCH₃CO₂- and KH₃PO₄-rich diets significantly enhance the number (C) and the size (D) of swellings in larval peripheral nerves caused by SIK3 depletion in glia. *n* ≥ 15. One-way ANOVA with Tukey's multiple comparisons; ****, *P* < 0.0001. Data are mean ± SEM.

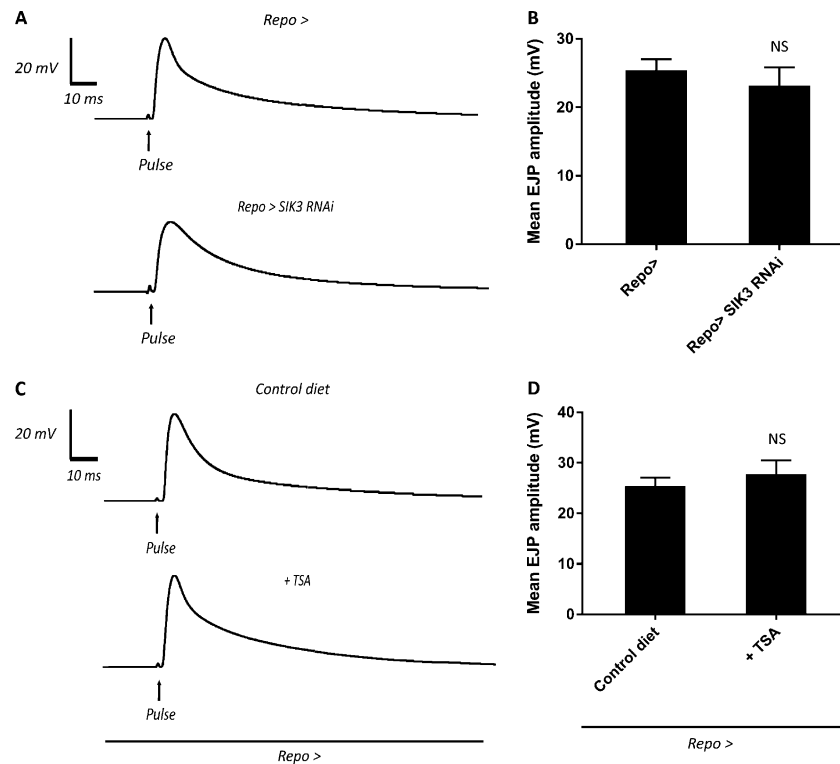


Figure S3. **Genetic depletion of SIK3 and pharmacological inhibition of HDAC do not disrupt evoked synaptic transmission at larval NMJ.** Related to Figs. 2 and 5. **(A)** Representative averaged EJP traces recorded following a stimulus (arrow) from larval NMJs at muscle 6 of control larvae expressing a UAS-GFP transgene (*Repo>*) and those depleted of SIK3 in glia (*Repo>SIK3 RNAi*). Each trace shown is the averaged recording of 75 consecutive evoked events from the same cell. **(B)** Quantification of mean EJP amplitude for genotypes in A, in which 75 consecutive evoked events were averaged per cell, and cell amplitudes were then averaged per genotype. Loss of SIK3 does not affect mean EJP amplitude, despite the subsequent supernumerary responses (shown in Fig. 2 F). $n = 7$ cells for *Repo>*; $n = 10$ cells for *Repo>SIK3 RNAi*. Two-tailed Student's t test; NS, $P > 0.05$. **(C)** Representative averaged EJP traces following a stimulus recorded from NMJs at muscle 6 of control larvae raised on control diet or diet enriched with TSA ($10 \mu\text{M}$). **(D)** Quantification of mean EJP amplitude for conditions described in C. The mean EJP amplitudes are not significantly different between larvae raised on control diet and those fed TSA during the bulk of the developmental period. $n = 7$ cells for *Repo>* on control diet; $n = 7$ cells for *Repo>* on TSA-rich diet. Two-tailed Student's t test; NS, $P > 0.05$. Data are mean \pm SEM.

Provided online is one table containing the list of genes tested in the *in vivo* glial-specific RNAi screen.