



Figure S1. Spadin does not alter K⁺ conductance in *TWIK-1* or both *TREK-1* and *TWIK-1* knock-down astrocytes. (A) K⁺ current densities were recorded in primary astrocytes transfected with scrambled shRNA (sc sh) or *TWIK-1* shRNA before and after 10 μ M spadin (SP) treatment. (B) Summary histogram showing averaged current densities in each group in (A) at -150 mV and +50 mV. Number on each bar indicates *n* for each condition. All values are mean \pm s.e.m. *P*-values were obtained with one-way ANOVA followed by Turkey's post hoc test. n.s.: not significant, **P* < 0.05 and ****P* < 0.01. (C) Spadin-sensitive currents were calculated by differences between currents before and after spadin treatment in (A). (D) Summary histogram indicating averaged spadin-sensitive currents from the plot in (C) at +50 mV and -150 mV. All values are mean \pm s.e.m. *P*-values were obtained with Student's *t*-test. n.s.: not significant, ****P* < 0.001. (E) Current densities were measured in astrocytes transfected with scrambled control shRNA (Sc shRNA) or *TREK-1* shRNA/*TWIK-1* shRNA before and after

spadin administration. (F ~ H) Summary plot and bar graphs showing the same parameters as in (B ~ D), respectively. Number on each bar indicates *n* for each condition. All values are mean \pm s.e.m. *P*-values were obtained with one-way ANOVA followed by Turkey's post hoc test or Student's *t*-test. n.s: not significant, **P* < 0.05 and ****P* < 0.001.



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