SUMMARY

Trim32 is ubiquitously expressed throughout the nervous system, however functional roles for the E3 ligase in the developed brain are speculative at best. Here, using LC-M/MS, we report that Trim32 interacts with Kv1.2, an ion channel critically involved with establishing and maintaining membrane excitability in neurons. In the cerebellum particularly, we show Trim32 localizes in regions of high Kv1.2 density that provide inhibitory input to the cerebellums communication with other brain regions. From the tissue homogenates that identified the Kv1.2:Trim32 interaction, we additionally identified ubiquitylation sites within Kv1.2. \textit{In Vitro}, we show that Trim32 is catalytically equipped to ubiquitylate Kv1.2 and further demonstrate that presence of the E3 ligase greatly influences surface trafficking of the channel. Finally, using a combination of quantitative LC-MS/MS, mutagenesis, and flow cytometry, we provide a 2 mechanistic model of Trim32 modulation of Kv1.2: Trim32 can increase surface Kv1.2 through channel ubiquitylation while, separately, can decrease surface Kv1.2 independent of channel ubiquitylation, with both mechanisms involving cross-talk with particular phosphorylation sites.