

## Supplementary Material 4 Evaluation of specificity of $\text{Ca}_v1.3$ immunolabeling

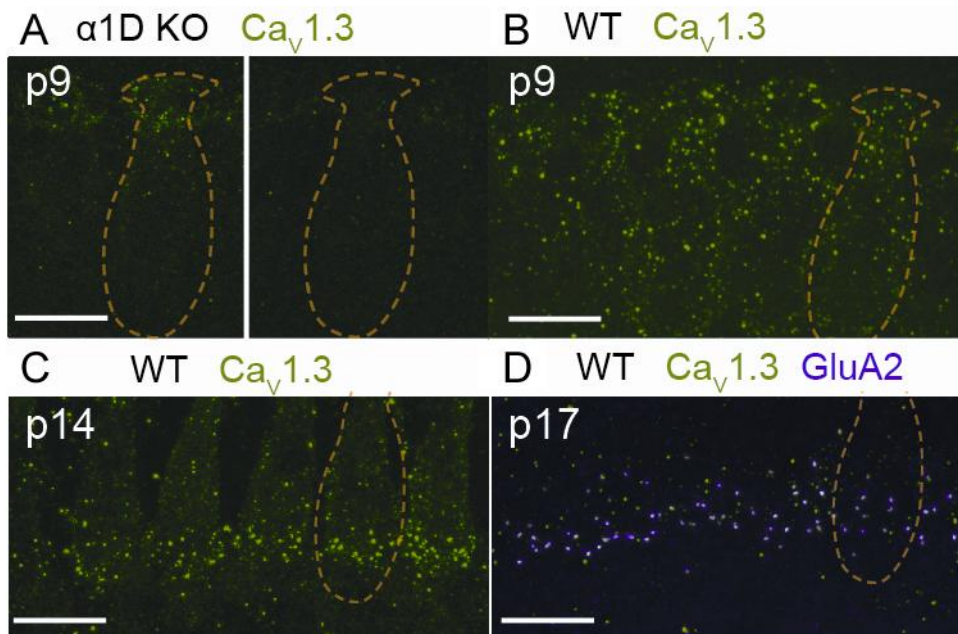


Figure S4. Evaluation of specificity of  $\text{Ca}_v1.3$  immunolabeling

(A) Control experiments probed for  $\text{Ca}_v1.3$  immunoreactivity at p9 in the  $\alpha 1D$  knockout mouse<sup>1</sup>, which lacks the channel's pore-forming  $\alpha$ -subunit recognized by the antibody. The non-specific staining pattern was somewhat selective for the hair cell apical plate and neck region. However, there were no presynaptic  $\text{Ca}_v1.3$  clusters and the basolateral membrane was not immuno-positive relative to surrounding tissue. (B) Same as in panel (A) but with wild-type mice at p9. (C)  $\text{Ca}_v1.3$  in wild-type IHCs at p14. In addition to synaptic clusters, extrasynaptic  $\text{Ca}_v1.3$  labeling highlights the IHC plasma membrane against the surrounding tissue in (B) and (C). (D) By p17 the IHC membrane is no longer discernible by diffuse  $\text{Ca}_v1.3$  stain, however, some extrasynaptic  $\text{Ca}_v1.3$  puncta persist. Each postsynaptic patch of glutamate receptors (GluA2, magenta) is juxtaposed to a presynaptic punctum of  $\text{Ca}_v1.3$  that appears colocalized (white). All scale bars: 10  $\mu\text{m}$ .