Supplementary Material 2: Quantification of CtBP2 and GluA2/3 puncta number and intensity

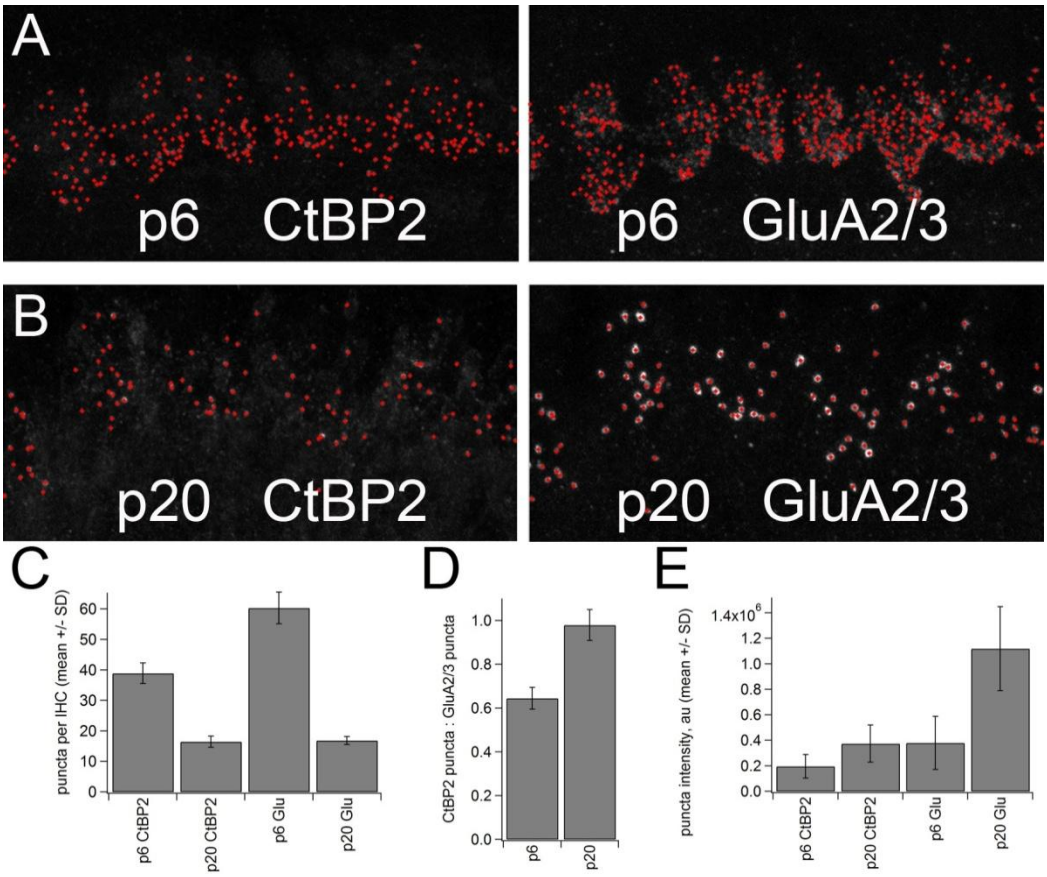

Figure S2. Quantification of CtBP2 and GluA2/3 puncta number and intensity

(A-B) Projection of confocal images across approximately 6 IHCs in the organ or Corti whole-mount at p6 (A) and p20 (B). The same images are presented in merged color in Figure 1 of the main text. Images are single-channel gray scale immunofluorescence for RIBEYE/CtBP2 (left) and GluA2/3 (right). With custom procedures in Matlab software, the locations of synapses in confocal image stacks were defined as the centers of mass of fluorescent spots after thresholding by a subjective intensity criterion for each channel. The background (defined as the average voxel intensity in the entire synaptic region excluding voxels that exceeded the threshold value) was subtracted. Gaussian functions were fitted in all three dimensions to determine the center of mass of each cluster. Each red symbol marks the center of mass, in 3D, of a ribbon or a punctum of

glutamate receptors. Immunofluorescence intensity was measured as the integral of the voxel values within a defined region of interest (13 × 13 × 5 voxels in the *X*, *Y*, and *Z* direction, which has the volume of ~0.7 × 0.7 × 2.0 μm) centered on the voxel where the center of mass of each punctum was located.

(C) Puncta per IHC (mean±s.d.) for 24 hair cells from 4 different image stacks. From p6 to p20 ribbons are reduced by ~50% and GluA2/3 puncta are reduced to ~30% in number.

(D) Ratio of ribbons to glutamate receptor puncta per IHC (mean±s.d.).

(E) Summed voxel intensity in the defined region around each center of mass. Per puncta intensity approximately doubled for ribbons and approximately tripled for GluA2/3.