Supplementary Materials:

"ATP activates P2X receptors to mediate gap junctional coupling in the cochlea"

Yan Zhu and Hong-Bo Zhao

Dept. of Otolaryngology, University of Kentucky Medical Center, Lexington, KY 40536-0293

Methods and Materials:

Immunofluorescent staining

The detailed method of immunofluorescent staining has been described by our previous reports (Zhao and Yu, 2006; Liu and Zhao, 2008). The cochlear cells were fixed with 4% paraformaldehyde for 30 min. After washing out, the cells were incubated in a blocking solution (10% goat serum and 1% BSA in PBS) with 0.1% Triton X-100 for 20 min, following incubation with primary antibodies to P2X and P2Y receptors (1:200; P2X2, P-7982; P2X7, P-8232; P2Y4, P-1231; Sigma-Aldrich, St. Louis, USA) in the blocking solution at 4^{o}C overnight. In control experiments, the primary antibody was omitted. After washing out, the cells were reacted to the second antibody Alexa Fluor 488 or 568 (1:500; Molecular Probes) in the blocking solution at room temperature for 1 hr and observed under a Leica confocal microscope (Leica TCS SP2) equipped with 40x and 100x apochromatic oil objectives. All images were saved in the TIFF format.

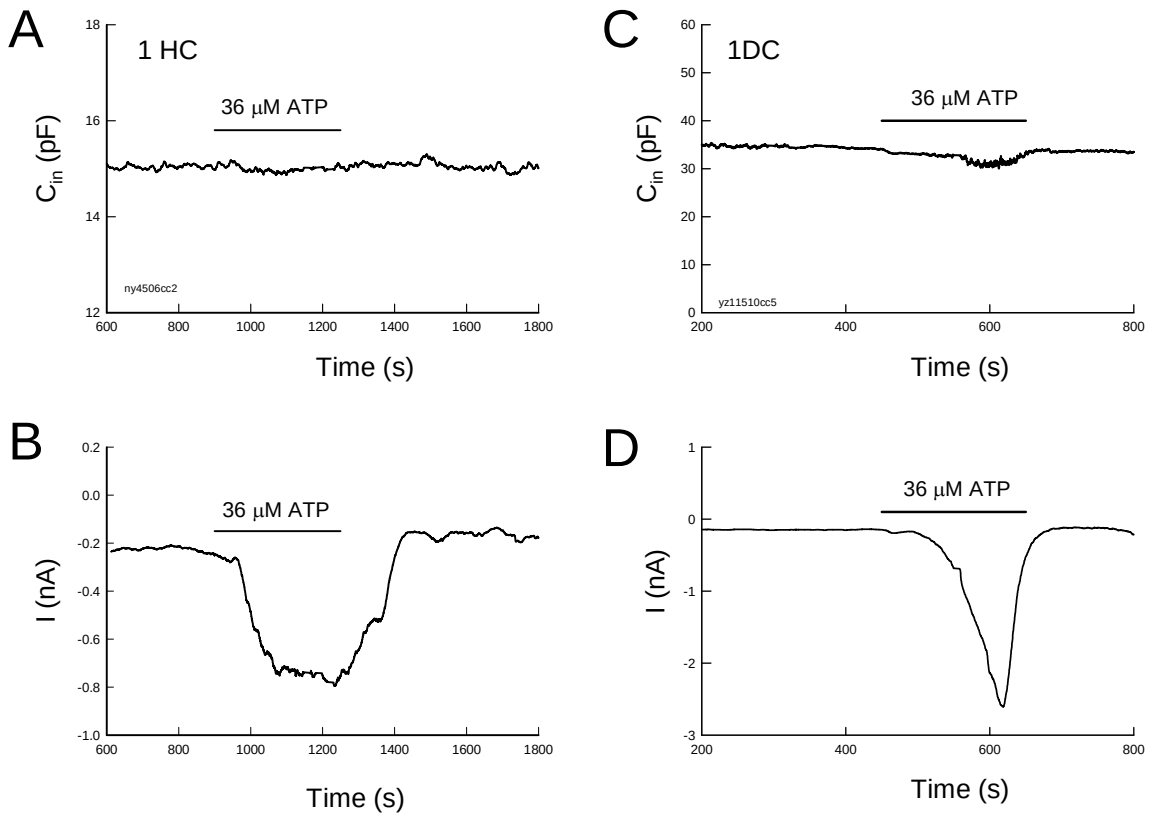

Fig. S1. Inward current evoked by ATP does not reduce C_{in} in single supporting cell. A-B: ATP evoked an inward current but did not reduce C_{in} in single HC. The cell was held at -80 mV. C-D: ATP evoked an inward current but did not reduce C_{in} in single DC.

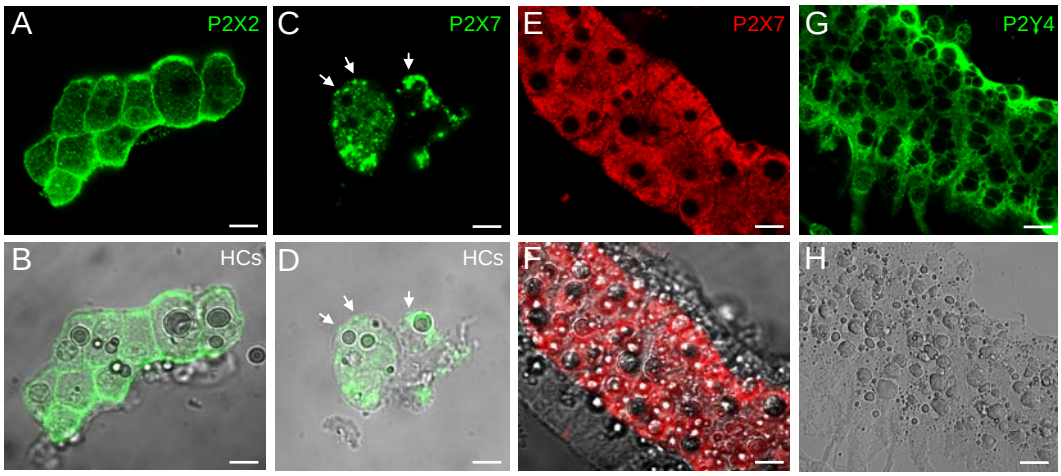

Fig. S2. Immunofluorescent staining for P2X and P2Y receptors in the cochlear sensory epithelium. A-B: Immunofluorescent staining of HCs for P2X2. C-F: Immunofluorescent staining for P2X7 in HCs and whole-mounting epithelium. Arrows in panel C-D indicate punctate labeling on the cell surface. G-H: Immunofluorescent staining for P2Y4 in the cochlear sensory epithelium. Scale bars: 10 μm in A-D, 20 μm in E-H.

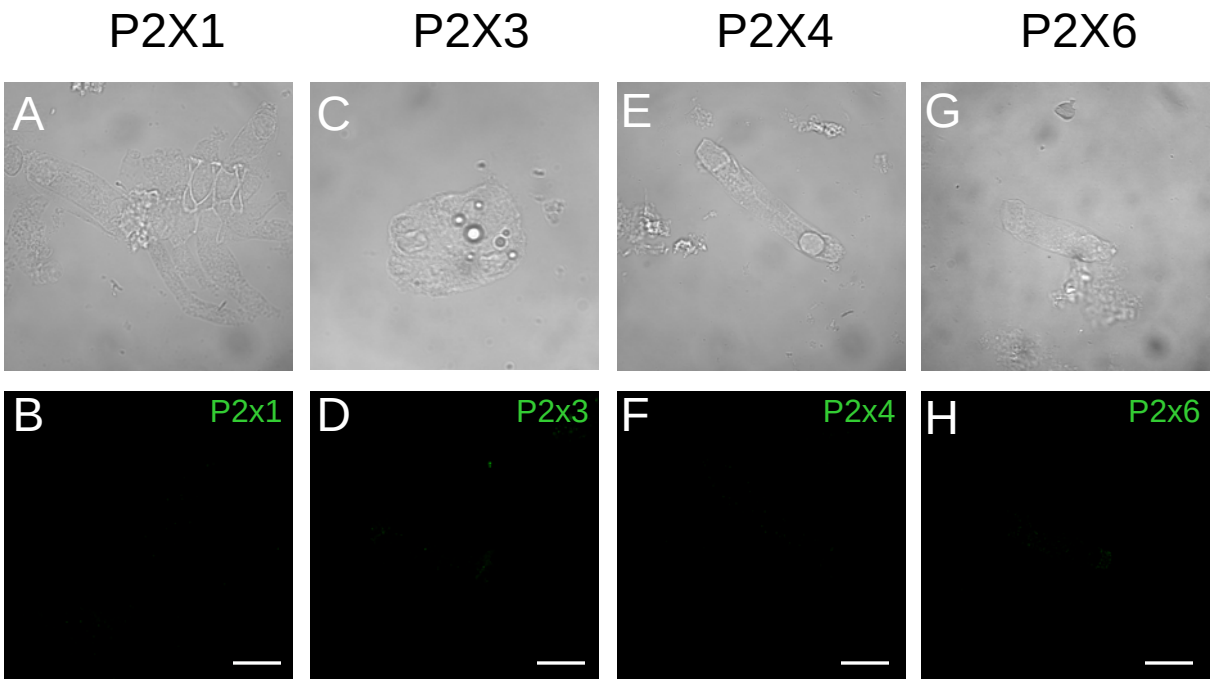

Fig. S3. Negative staining for P2X1, P2X3, P2X4, and P2X6 in cochlear cells. Scale bar: 15 μm