SUPPLEMENTAL DATA

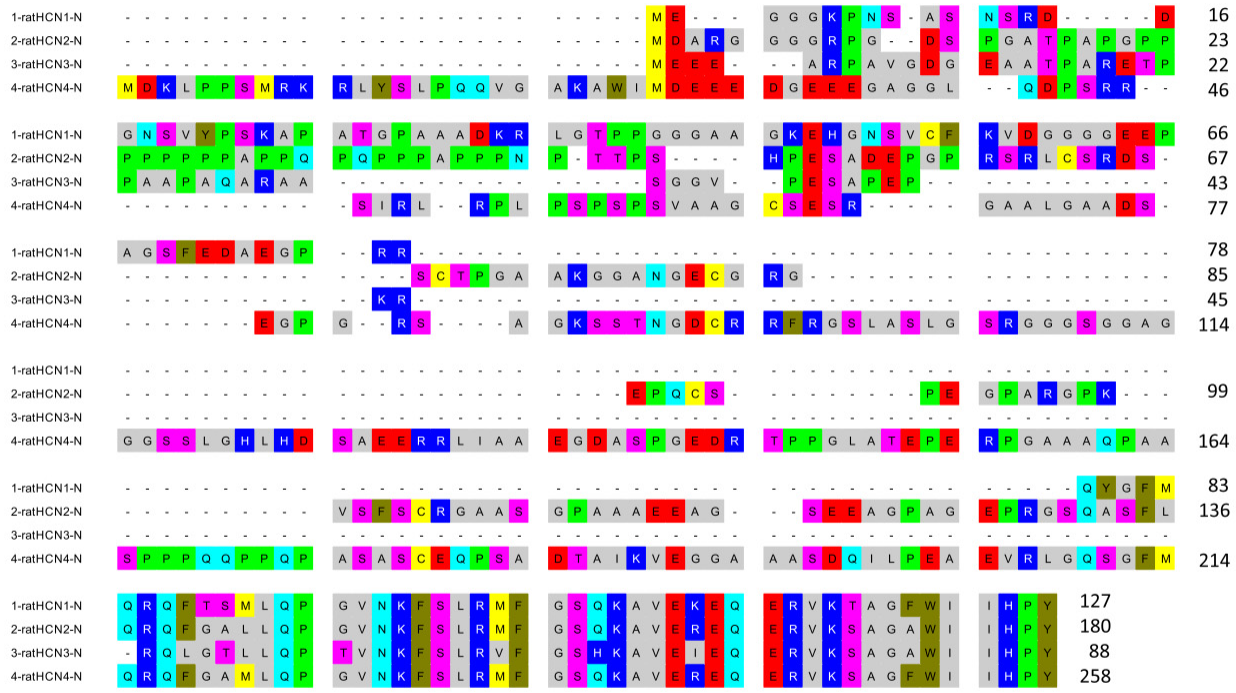



FIGURE 1. **Alignment (with OMIGA 2.0, Oxford Molecular/Accelrys) of the specific and conserved sequences for the amino termini of HCN isoforms.** HCN isoform-specific N-terminus sequence for rat organ of Corti HCN1 (aa 1-78), and rat HCN2 (aa 1-131), rat HCN3 (aa 1-45) and rat HCN4 (aa 1-209) (GenBank Accession Numbers NP_446136, NP_446137, NP_067690, respectively). Amino terminal sequence conserved across rat HCN isoforms is shown for organ of Corti HCN1 (aa 79-127), HCN2 (aa 132-180), HCN3 (aa 46-88), and HCN4 (aa 210-258).

**HCN2 N-TERMINAL AMINO ACID SEQUENCE**

M D A R G G G G R P G D S P G A T P A P G P P P P P P P P A P P Q P Q P P P A P P P N P T T P S H P E S A D E P G P R S R L C S R D S S C T P G A
A K G G A N G E C G R G E P Q C S P E G P A R G P K V S F S C R G A A S G P A A A E E A G S E E A G P A G E P R G S Q

**HCN2 N-TERMINAL OPTIMIZED NUCLEOTIDE SEQUENCE WITH ALTERNATE CODONS**

ATG GAT GCA AGA GGT GGT GGT GGA AGA CCT GGA GAT AGT CCA GGT GCA ACA CCT GCT CCA GGA CCA CCT CCA CCT CCA CCA CCT CCT
GCA CCT CCT CAA CCT CAG CCA CCA CCT GCA CCA CCA CCT AAC CCT ACA ACA CCT TCA CAT CCA GAG TCA GCA GAC GAG CCT GGA CCT AGA
TCT AGA CTC TGC AGC CGA GAC AGC TCC TGC ACT CCT GGA GCT GCA AAG GGT GGA GCA AAT GGT GAG TGC GGA CGA GGA GAG CCT CAG
TGT AGC CCT GAG GGA CCT GCA CGA GGT CCA AAG GTT TCG TTC TCA TGT CGA GGT GCA GCT TCG GGA CCT GCA GCT GCA GAG GAG GCA
GGT AGC GAG GAG GCA GGT CCT GCA GGT GAG CCA CGA GGA AGC CAG TGA

**PRIMERS**

| | |
|---|---|
| rHCN2-1 | ATGGATGCAAGAGGTGGTGGTGGAAGACCTGGAGATAGTCCAGGT |
| rHCN2-2 | TGCAGGAGGTGGTGGAGGTGGAGGTGGTCCTGGAGCAGGTGTTGCACCTGG |
| rHCN2-3 | CCTCCTGCACCTCCTCAACCTCAGCCACCACCTGCACCACCACCTAACC |
| rHCN2-4 | AGATCTAGGTCCAGGCTCGTCTGCTGACTCTGGATGTGAAGGTGTTGTAGGGTTAGG |
| rHCN2-5 | GGAGCTGTCTCGGCTGCAGAGTCTAGATCTAGG |
| rHCN2-6 | AGCCGAGACAGCTCCTGCACTCCTGGAGCTGCAAAGGGTGGAGCAAATGGT |
| rHCN2-7 | TCCCTCAGGGCTACACTGAGGCTCTCCTCGTCCGCACTCACCATTTGCTCC |
| rHCN2-8 | CCTGAGGGACCTGCACGAGGTCCAAAGGTTTCGTTCTCATGTCGAGGTGCAGCT |
| rHCN2- 9 | ACCTGCCTCCTCGCTACCTGCCTCCTCTGCAGCTGCAGGTCCGAAGCTGCACC |
| rHCN2-10 | GAGGCAGGTCCTGCAGGTGAGCCACGAGGAAGCCAGTGAGAATTCGAC |
| rHCN2-11 | AGCCGAGACAGCTCCTGCACTCC |
| rHCN2-12 | GTCGAATTCTCACTGGCTTCCT |
| rHCN2-13 | GATGGATCCGAATGGATGCAAGAGGTGGTGGT |

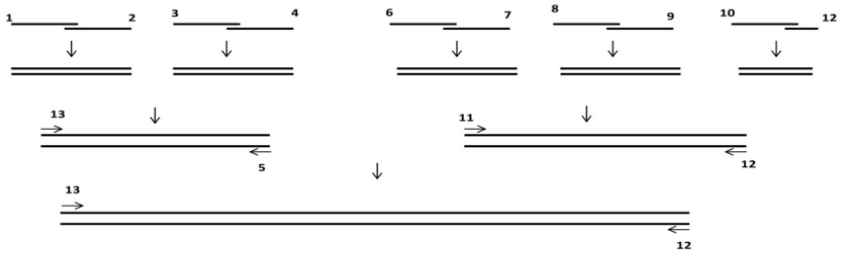
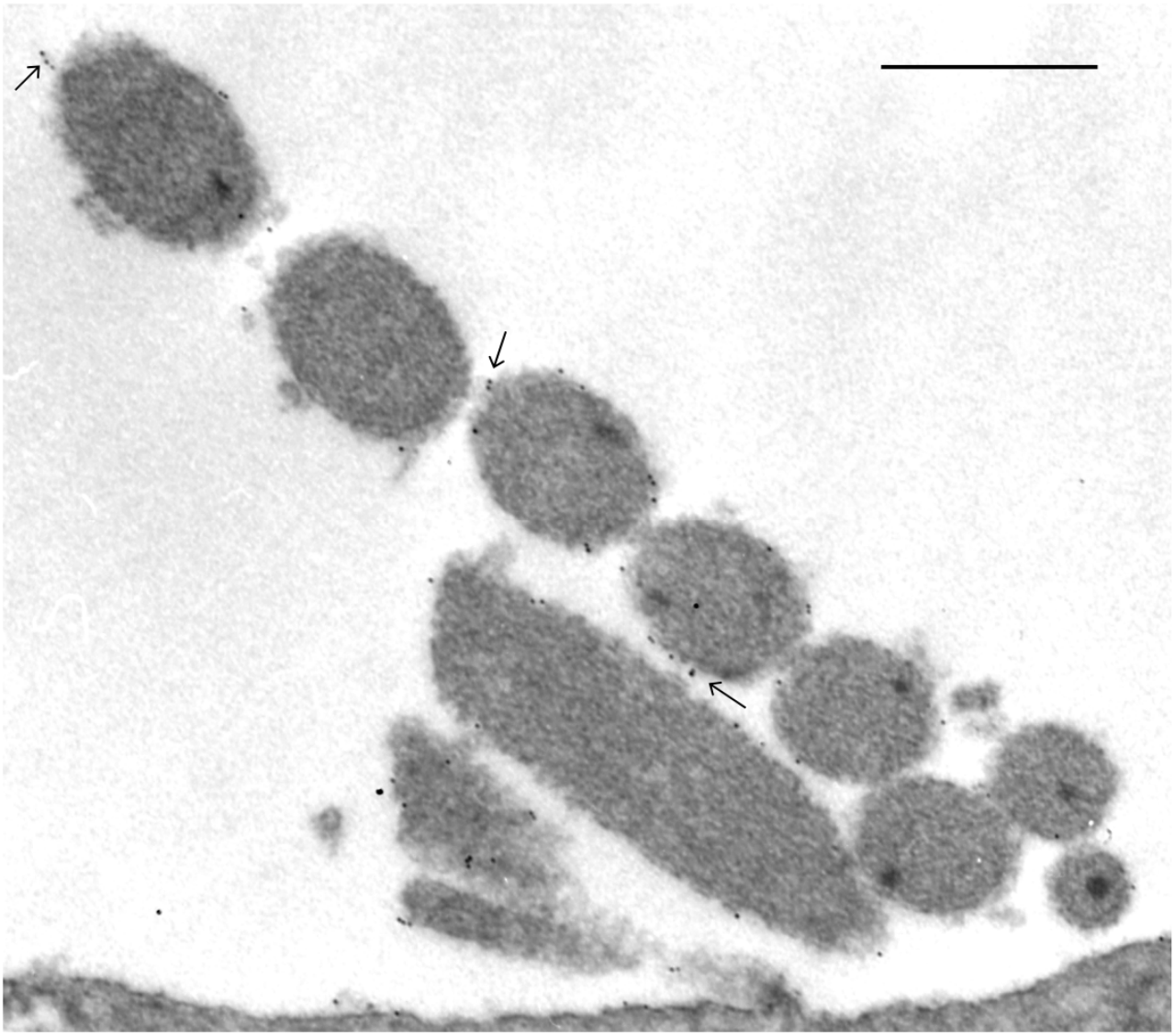



FIGURE 2. **Alternate codon usage reducing cDNA GC content for synthesis of the HCN2-specific N-terminus.** Primers, as listed, were employed for recursive PCR (Prodromou and Pearl (1992) *Protein Eng*. 5, 827-829). Single-stranded oligonucleotides of 40-50 bp (Invitrogen) containing overlapping sequences were paired and extended by PCR amplification, as indicated. Two fragments containing overlapping sequences, 204 bp and 237 bp in length, were synthesized in separate reactions, gel-purified and mixed in a fresh amplification reaction using upstream and downstream primers 12 and 13, then cloned in pGEMTeasy vector. The desired sequence was cloned in expression vector pRSETA for his-tag fusion protein expression, and sequence-verified. Altered nucleotides are indicated in red with no change of amino acids. Stop and start codons in primers 12 and 13, respectively, are underlined.



**Magnification of immunogold EM microphotographs.**

FIGURE 3A. Full magnification of Fig. 6C (primary article) illustrating HCN1 immunogold (arrows) on stereocilia of IHC. Scale bar = 300 nm.

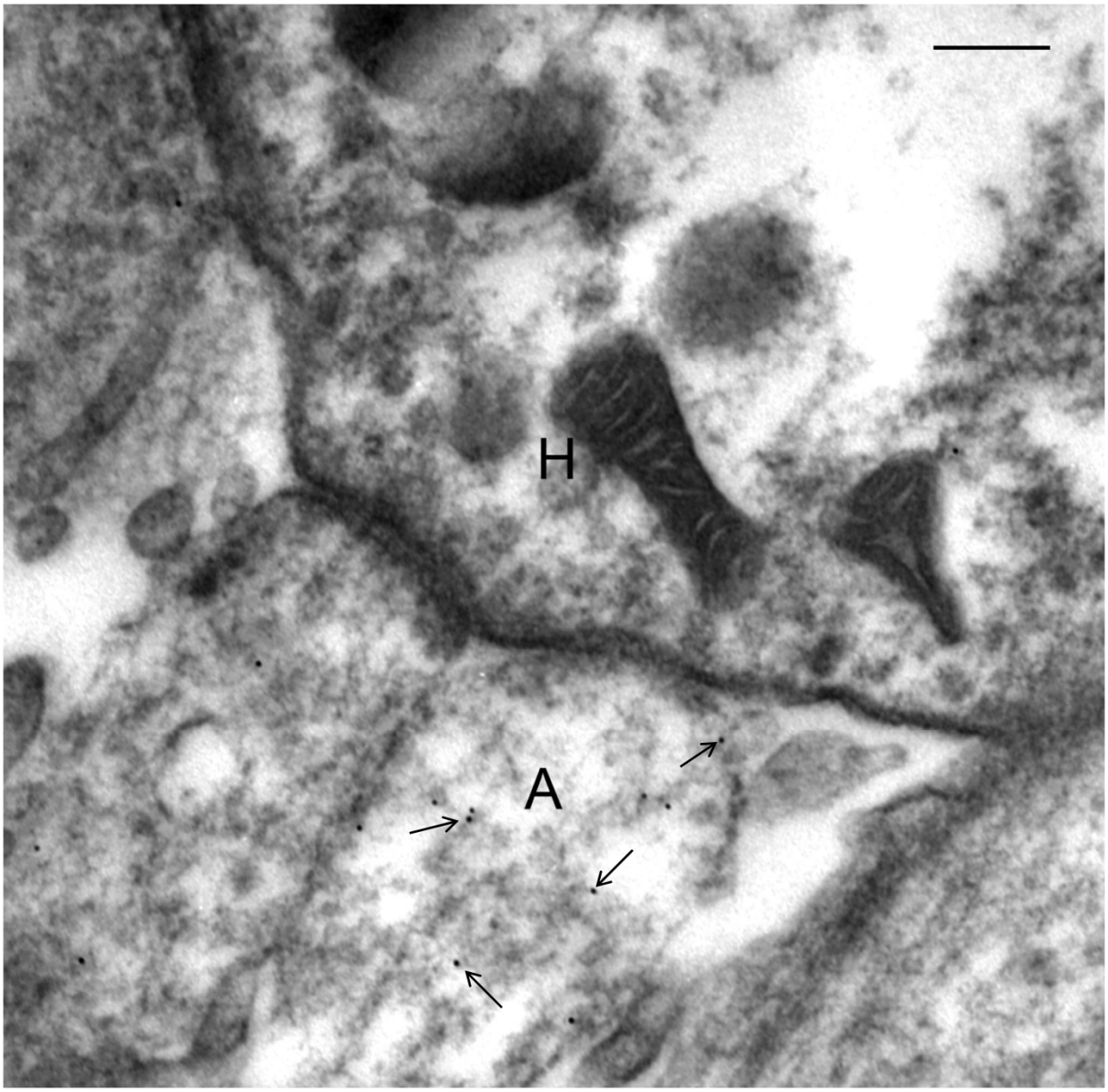



FIGURE 3B. Full magnification of Fig. 6D (primary article) illustrating specific localization of HCN1 immunogold (arrows) to type II afferents (A) in synaptic contact with cochlear outer hair cell (H). Scale bar = 200 nm.

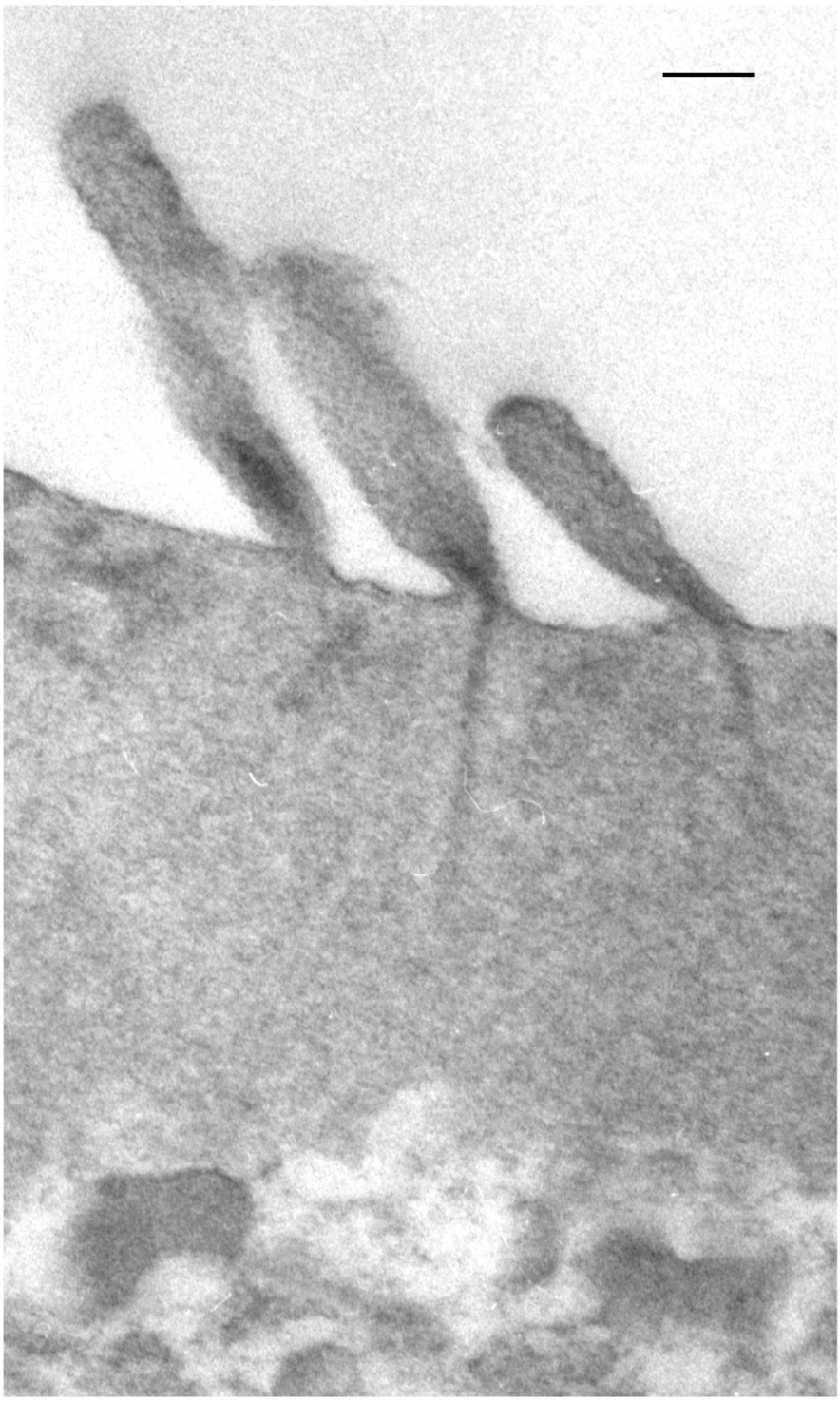


FIGURE 3C. Negative control for an equivalent amount of rabbit IgG used for immunogold resolution of HCN1 and HCN2 protein localization in rat cochlear outer hair cell. No gold was detected on hair cell stereocilia. Scale bar = 150 nm.

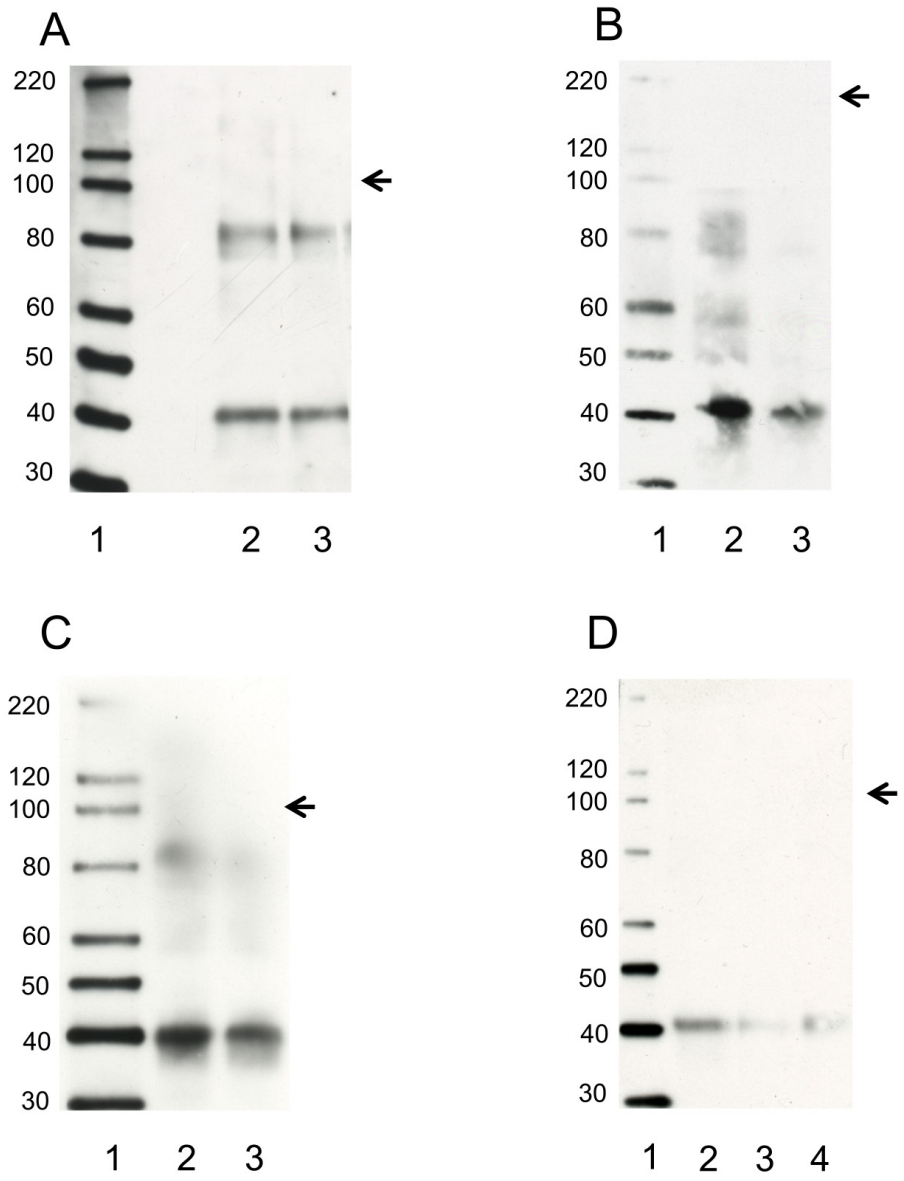



FIGURE 4. **IgG immunoprecipitation negative controls.** *A, B,* negative immunoprecipitation controls for filamin-A (mouse primary antibody). *A,* lane 1, standards; lane 2, mouse IgG immunoprecipitation (negative control) of brain lysate + beads + HCN1 goat primary (1:50), donkey anti-goat secondary; lane 3, mouse IgG (no lysate) + beads, primary and secondary antibodies. HCN1 is not detected in any negative control (arrow indicates position of HCN1 if present). *B,* lane 1, standards; lane 2, mouse IgG immunoprecipitation (negative control) of brain lysate + beads + protocadherin 15 CD3 chick primary antibody (1:10,000), bovine anti-chick secondary; lane 3, mouse IgG (no lysate) + beads + protocadherin 15 CD3 primary antibody, bovine anti-chick secondary. Protocadherin 15 CD3 at 189 kDa (arrow) is not present in any negative control. *C,* rabbit IgG immunoprecipitation negative control. Lane 1, standards; lane 2, rabbit IgG immunoprecipitation (negative control) of brain lysate + beads + HCN1 goat primary (1:50) + donkey anti-goat secondary; lane 3, rabbit IgG + beads + primary and secondary antibodies. HCN1 is not present in negative controls. (Arrow indicates position of HCN1 if it were present.) *D*, negative controls for fascin-2 immunoprecipitation. Lane 1, standards; lane 2, goat IgG immunoprecipitation (negative control) of brain lysate + beads + HCN2 rabbit primary antibody (1:200), donkey anti-rabbit secondary; lane 3, goat IgG (no lysate) + beads + primary and secondary antibodies; lane 4, goat denatured IgG directly electrophoresed, no beads + primary and secondary antibodies. There was no HCN2 in the negative controls. (Arrow indicates position of HCN2 if it were present.)