

Supplementary Material

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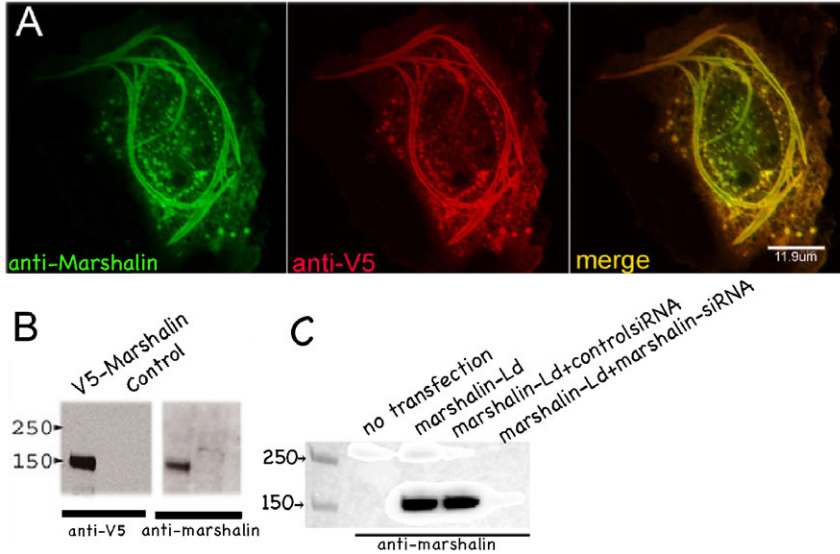


Fig. S1. Specificity of anti-marshalin. *Marshalin-Ld* (~3759 bp) cloned from the mouse cochlea was inserted into a mammalian expression vector with a V5-His epitope downstream of and in-frame with *marshalin-Ld*.

(A) Immunofluorescent images of marshalin-Ld-expressing OK cells labeled with anti-marshalin (left, green) and anti-V5 (middle, red), which recognizes the V5-tag at the C-terminus end of the marshalin protein. The right image superimposes green and red images to indicate that the staining for V5 and marshalin overlap. Scale bar: 10 μ m.

(B) Western blot for marshalin-Ld-V5-His-expressing OK cells (V5-marshalin) vs untransfected cells (control). The marshalin-Ld protein band on SDS-PAGE was recognized by both anti-V5 and anti-marshalin. (C) Marshalin siRNA mediated knockdown of marshalin expression. OK cells were transfected with one of the following combinations: (1) Marshalin-Ld plasmid alone. (2) Marshalin plasmid plus negative control siRNA. (3) Marshalin-Ld plus marshalin siRNA.

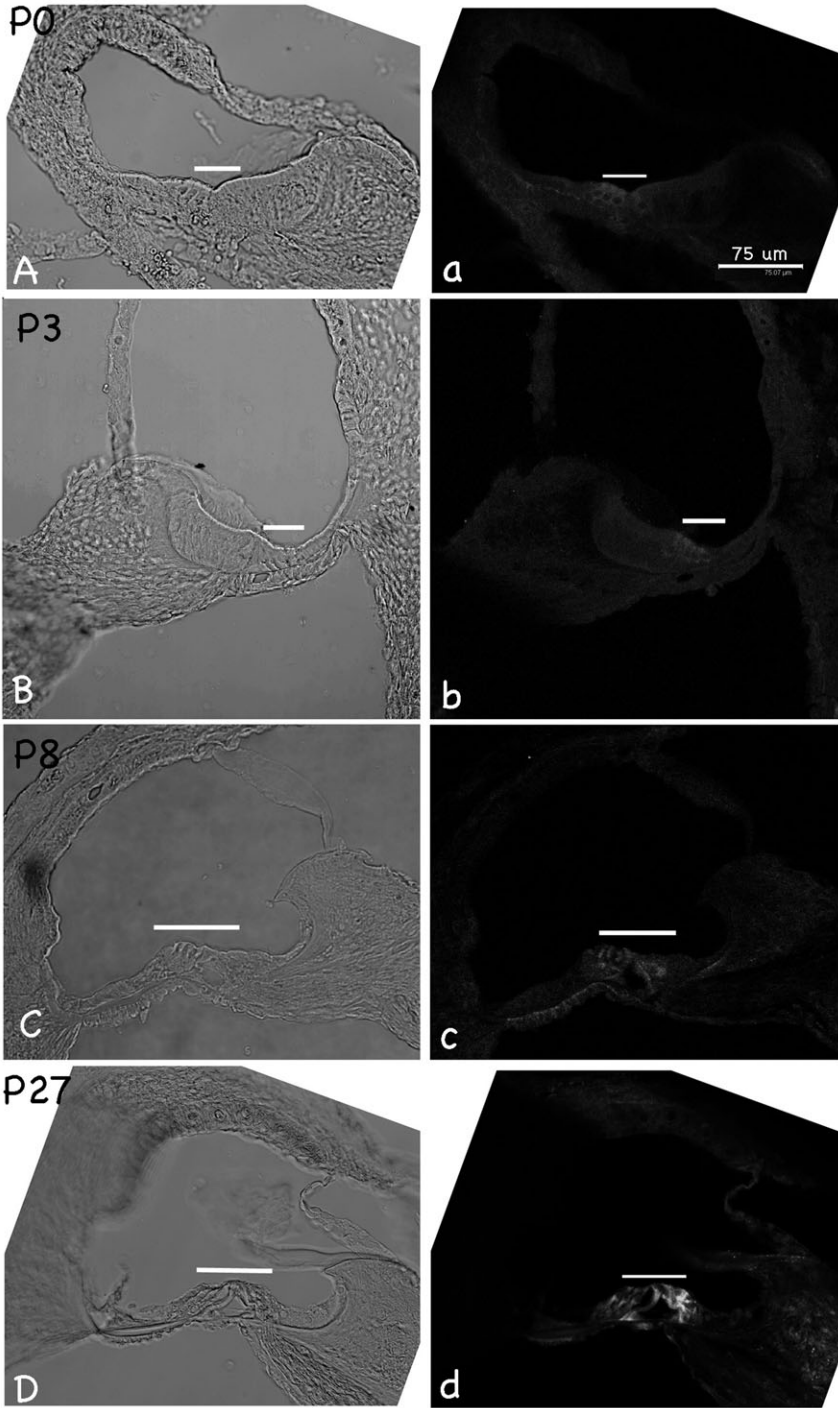


Fig. S2. Marshalin protein expression at different developmental stages. Anti-marshalin immunofluorescent images were taken at low magnification to emphasize that expression is restricted to the organ of Corti. (A) P0; (B) P3; (C) P8; (D) P27. Corresponding light microscope images appear on the left hand side. White bars overlies the OC region. Negative controls using anti-marshalin pre-incubated with antigen peptide or no anti-marshalin both lacked staining. Scale bar: 75 μ m.

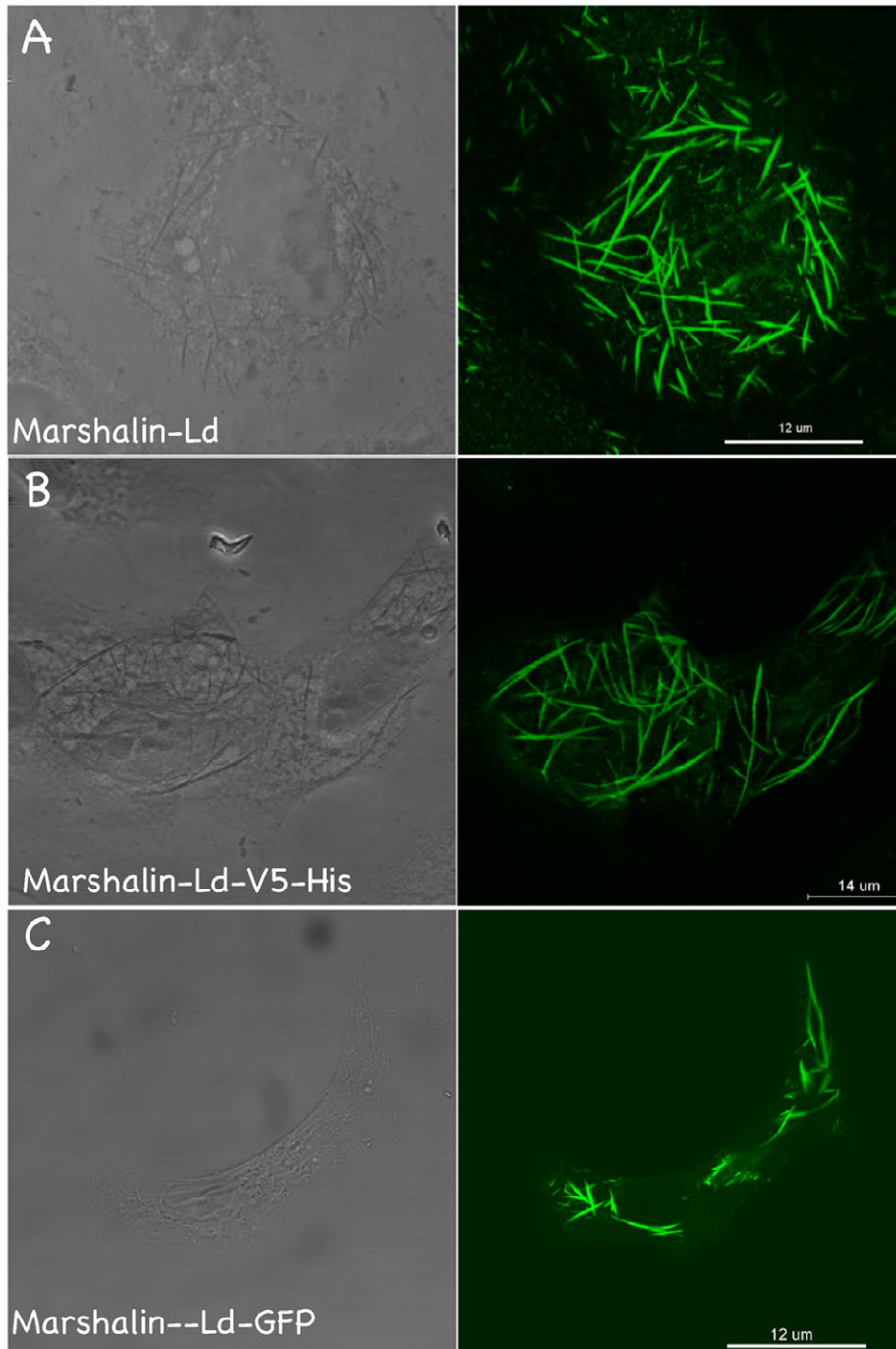


Fig. S3. V5 and GFP tags do not affect MT bundle formations. OK cells were transfected with plasmids encoding (A) *marshalin-Ld* with no tag; (B) *marshalin-Ld* with a V5-His tag; (C) *marshalin-Ld* with a GFP tag. Images were taken 48 hrs after transfection with different plasmids. Scale bars: 12 μm (A,C), 14 μm (B).

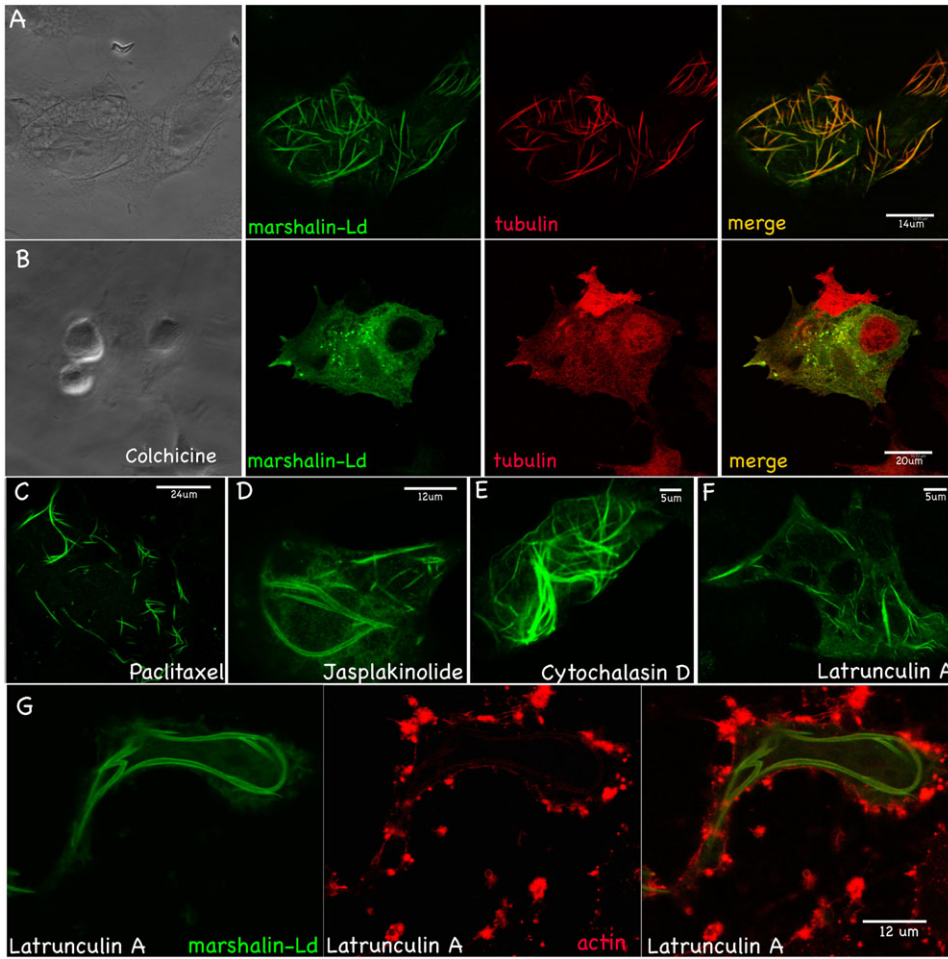
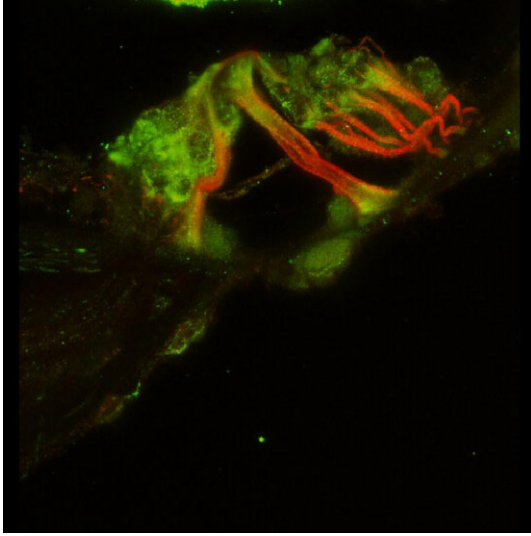


Fig. S4. MT bundles induced by *marshalin-Ld* cDNA. Marshalin-Ld-V5-expressing OK cells stained with anti-V5 for detecting marshalin (green) in panels A–G, anti- α -tubulin for detecting MTs (red) in panels A–F, and Texas Red-X phalloidin for actin filaments in panel G. All images acquired 48 hrs post transfection. (A) Cells without drug treatment. (B) Cells treated with 250 ng/ μ l colchicine, a microtubule polymerization inhibitor, for 24 hrs. (C) Cells treated with 0.5 μ M paclitaxel, a microtubule stabilizer, for 3 hrs. (D) Cells treated with 10 nM jasplakinolide, which increases actin filament assembly, for 24 hrs. (E) Cells treated with 0.5 μ M cytochalasin D, which inhibits actin polymerization, for 24 hrs. (F) Cells treated with 0.25 μ M latrunculin A for 24 hrs, which also inhibits actin polymerization as shown in panel G. Scale bars: 14 μ m (A), 20 μ m (B), 24 μ m (C), 12 μ m (D,G), 5 μ m (E,F).

Table S1. Marshalin isoforms and their accessory numbers.

| Name | Exons | Amino acids | Accession number |
|-------------------|-------------------------------------------|-------------|--------------------------|
| Marshalin-La (v1) | 19 exons: Exon1-19 | 1280 aa | KC426930 |
| Marshalin-La (v2) | 19 exons: Exon1-19 | 1279 aa | KC426931 |
| Marshalin-Lb (v1) | 18 exons: Exon1-4, E6-19 | 1269 aa | KC426932 |
| Marshalin-Lb (v2) | 18 exons: Exon1-4, E6-19 | 1268 aa | KC426933 |
| Marshalin-Lc (v1) | 18 exons: Exon1-5, E7-19 | 1264 aa | KC426934 |
| Marshalin-Lc (v2) | 17 exons: Exon1-5, E7-19 | 1263 aa | KC426935 |
| Marshalin-Ld (v1) | 17 exons: Exon1-4, E7-19 | 1253 aa | KC426936, NM_001163749.1 |
| Marshalin-Ld (v2) | 17 exons: Exon1-4, E7-19 | 1252 aa | KC426937, NM_027171.3 |
| Marshalin-Sa (v1) | 19 exons: Exon1-19 (partial Exon13) | 864 aa | KC426938 |
| Marshalin-Sa (v2) | 19 exons: Exon1-19 (partial Exon13) | 863 aa | KC426939 |
| Marshalin-Sb (v1) | 18 exons: Exon1-4, E6-19 (partial Exon13) | 853 aa | KC426940 |
| Marshalin-Sb (v2) | 18 exons: Exon1-4, E6-19 (partial Exon13) | 852 aa | KC426941 |
| Marshalin-Sc (v1) | 18 exons: Exon1-5, E7-19 (partial Exon13) | 848 aa | KC426942 |
| Marshalin-Sc (v2) | 17 exons: Exon1-5, E7-19 (partial Exon13) | 847 aa | KC426943 |
| Marshalin-Sd (v1) | 17 exons: Exon1-4, E7-19 (partial Exon13) | 837 aa | KC426944 |
| Marshalin-Sd (v2) | 17 exons: Exon1-4, E7-19 (partial Exon13) | 836 aa | KC426945 |



Movie 1. 3D-movie showing the location of marshalin in the organ of Corti. Thirty individual images were collected from a P27 mouse cochlea at intervals of 0.15 μm per section. The movie was created using ImageJ.