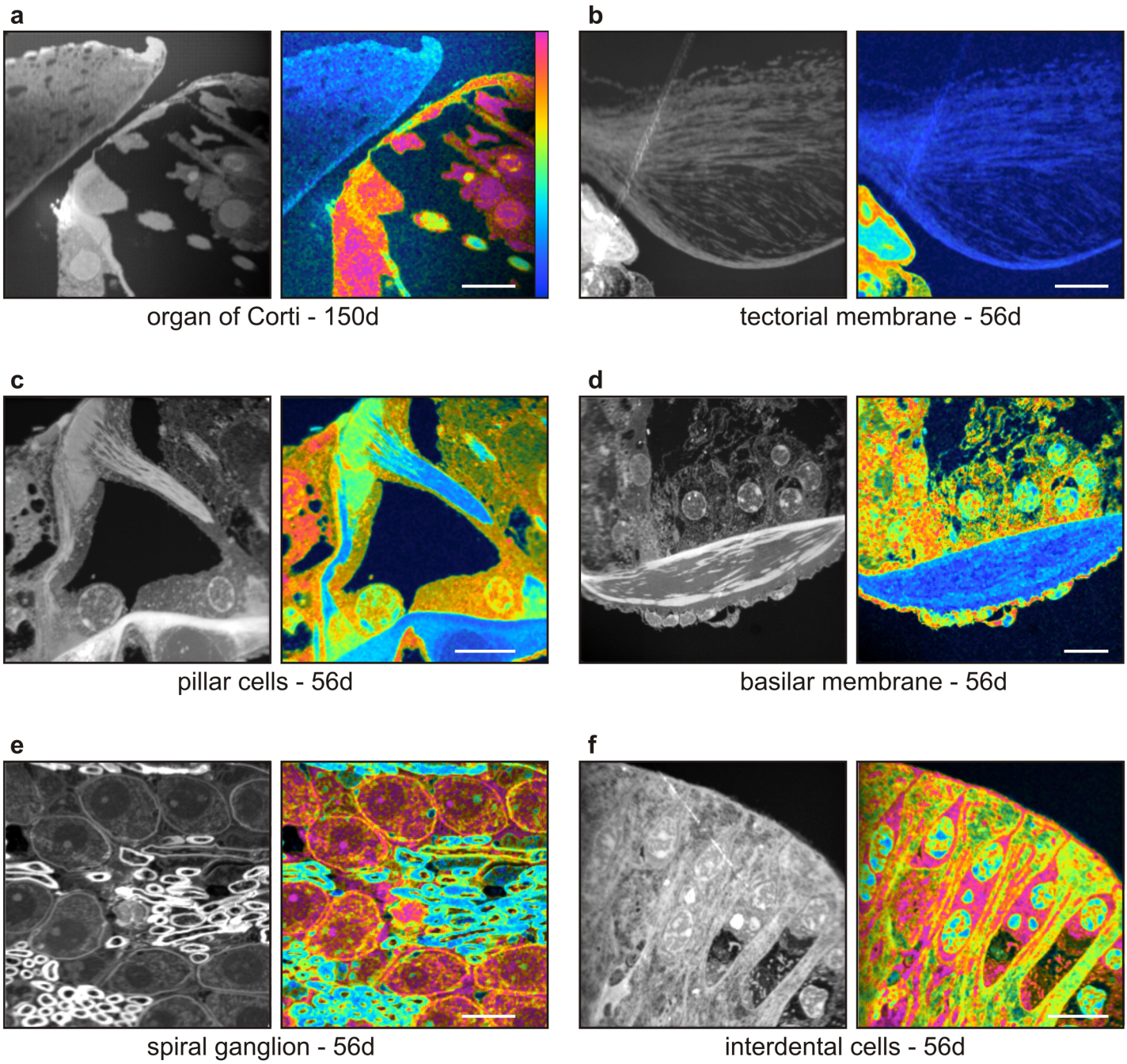


Supplementary Figure 1. Turnover in various tissues of adult cochlea

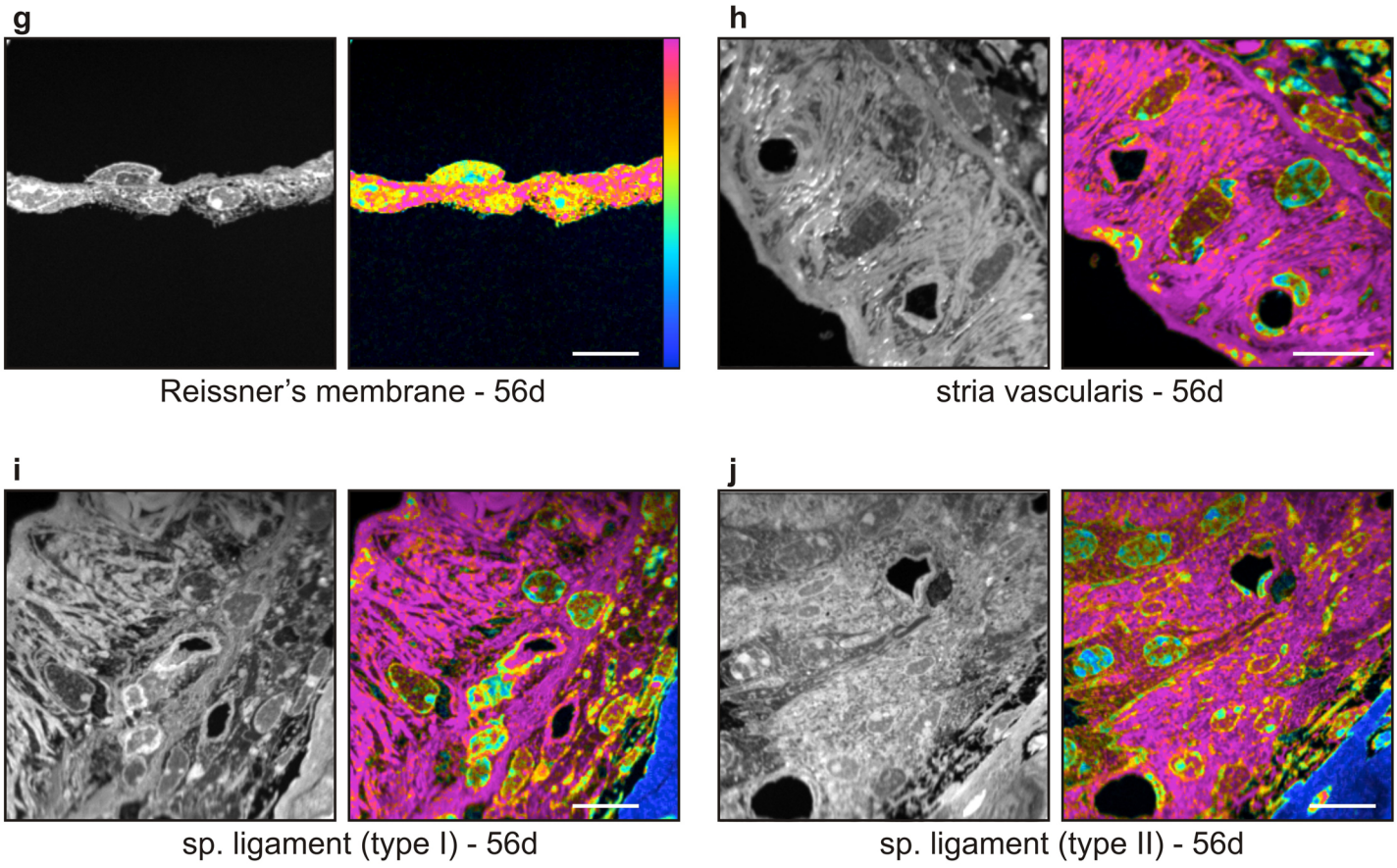
a, b. Tectorial membrane and organ of Corti. The tectorial membrane showed very little incorporation, even after five months. There was slightly more new protein at the surface of the tectorial membrane, in zones that are morphologically distinct from the core (the basal layer and cover net; refs 1,2), but even these showed less incorporation than any cellular element. The slow turnover of protein within the tectorial membrane is consistent with the large reduction, in adult mice, of mRNA for α-tectorin, a major protein component of the tectorial membrane[3]. Color scale is 0-100% incorporation for all HSI panels.

c,d. Pillar cells and basilar membrane. Shafts of pillar cells showed low incorporation, but the heads and the membrane regions of the shafts were higher. Fibrous regions of basilar membrane cells had low incorporation.

e. Spiral ganglion. Neuronal cell bodies showed high incorporation, but myelin was conspicuously low.

f. Interdental cells.

Scale bars = 10 μm.



Supplementary Figure 1 (cont). Turnover in various tissues of adult cochlea

g. Reissner's membrane.
h. Stria vascularis showed the highest incorporation in the cochlea.
i. Spiral ligament, in the region of type-I fibrocytes.
j. Spiral ligament, in the region of type-II fibrocytes.

Color scale is 0-100% incorporation for all HSI panels. Scale bars = 10 μm.

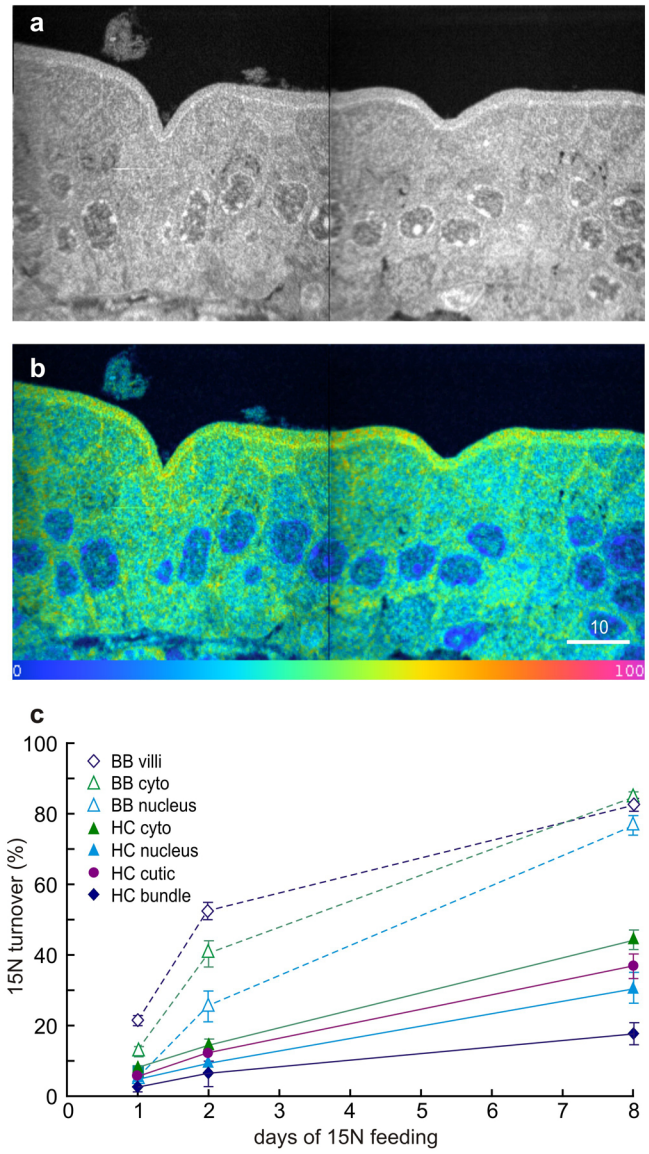


Figure S2. Incorporation of 15N into adult mouse intestinal brush border and utricular hair cells.
a, Intestine, mass 26 image, showing total protein. **b**, Intestine after two days 15N feeding, mass 27/mass26 ratio, showing high incorporation of 15N in microvilli. Color scale represents 0-100%; scale bar in micrometers. **c**, Incorporation after 1, 2 or 8 days 15N feeding, for different regions. Utricle data from Fig. 2. Mean +/- SD. In brush border, protein in microvilli turns over more rapidly than in cytoplasm; in hair cells, protein in stereocilia turns over more slowly than in cytoplasm.

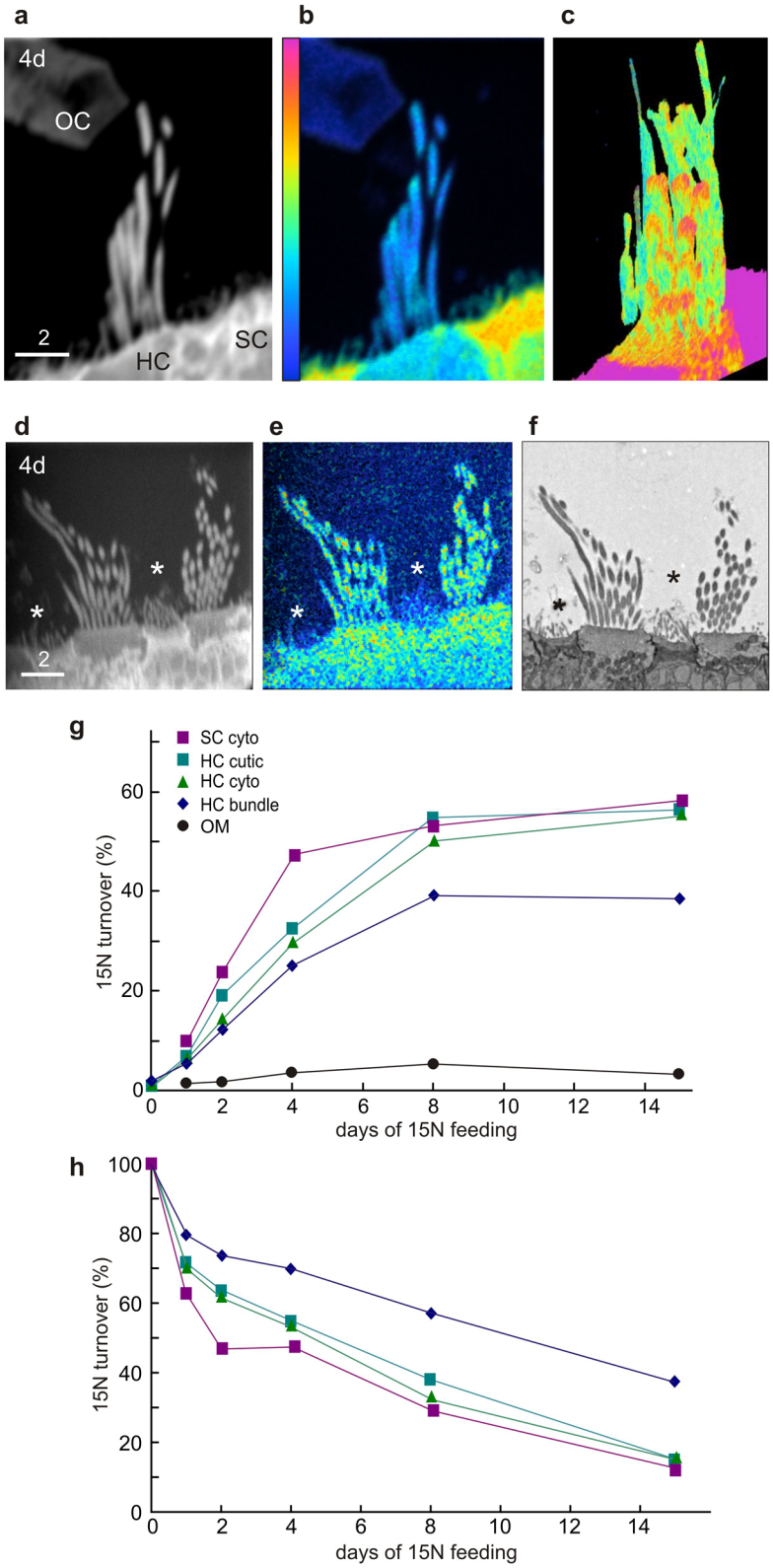
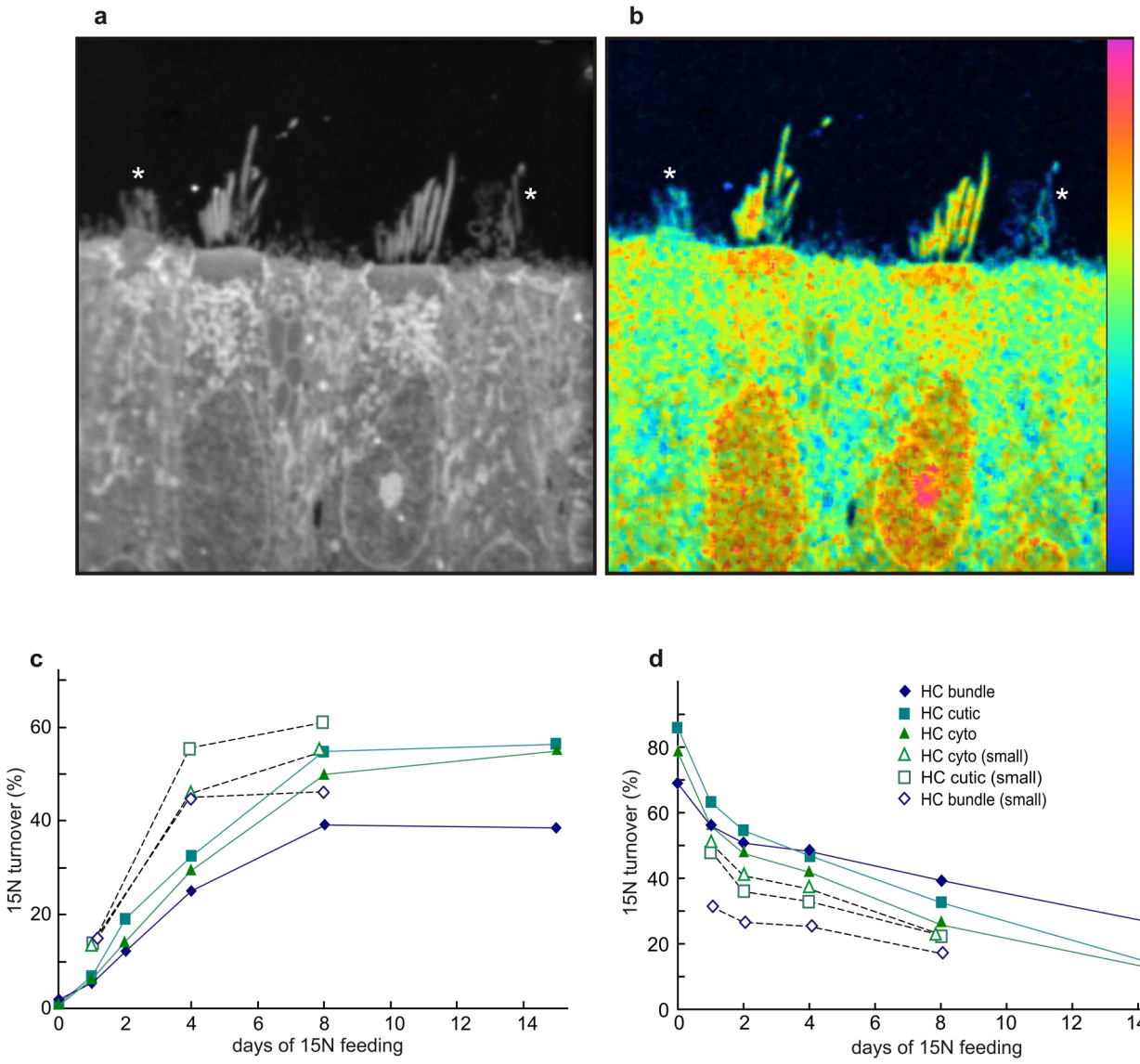
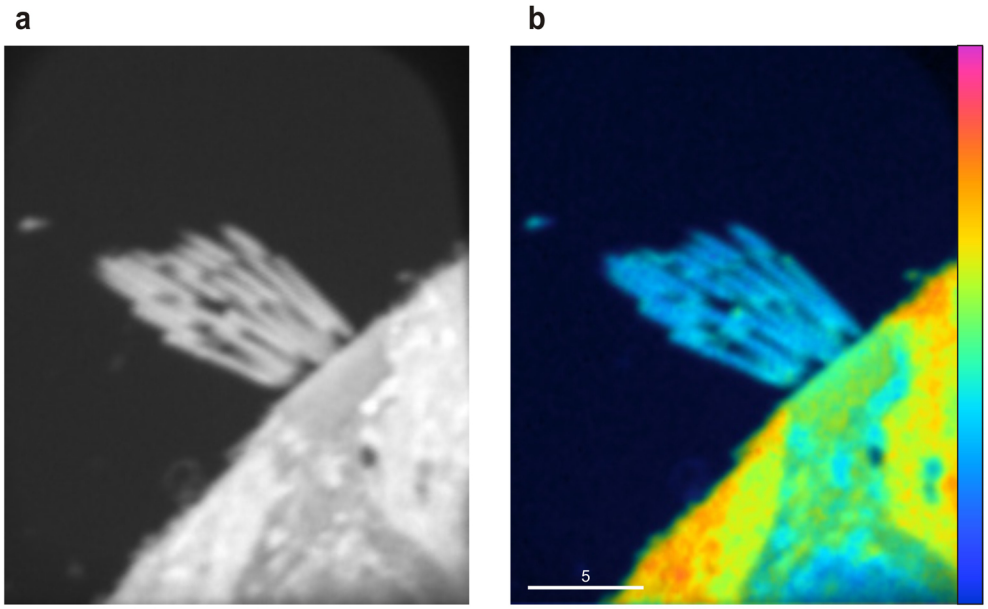


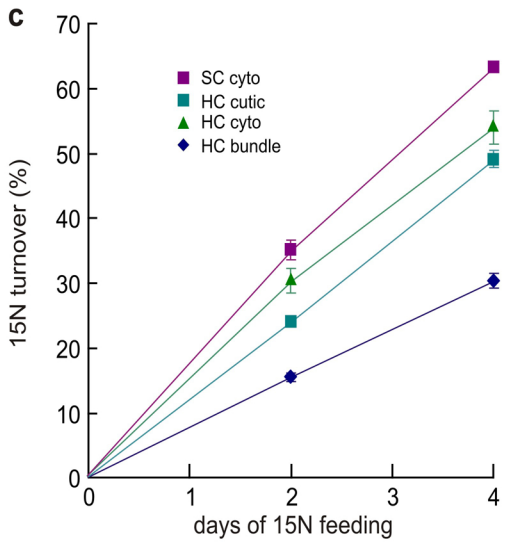
Figure S3. Turnover in neonatal mouse utricle. **a,** Hair cell from a pup nursed on 15N milk from birth, after 4 days; mass 26. **b**, Ratio image reveals lower incorporation in stereocilia than in hair-cell (HC) or supporting cell (SC) cytoplasm. Otoconia (OC) had very low incorporation. Color scale 0-100%. **c**, 3D volume rendering of the same cell, rotated; 0-45% scale. Within stereocilia, the highest labeling was in the top micrometer. **d**, Four hair cells from a pup born to a 15N mother and nursed on 14N milk from birth, after 4 days; mass 26. **e**, Ratio image illustrating retention of label in mature stereocilia at levels higher than cytoplasm. Small bundles (*), presumably postnatal, showed no label. **f**, Electron micrograph of an adjacent section. **g**, Plot of incorporation during 16 days for pups on 15N milk. Hair bundles and the otolithic membrane (OM) had the lowest incorporation. **h**, Loss of label for 15N pups fed 14N milk, normalized to level at birth. Stereocilia retained >50% of label for 8 days. Both stereocilia and cytoplasm showed fast and slow phases of 15N loss and were fitted with a sum of exponentials: In cytoplasm, about 33% had a τ=1 day and the remainder dissipated at τ=10 day. Stereocilia were slower, with just ~20% of 15N dissipating quickly (τ=1 day) and the remainder dissipating at τ=20 day.



Supplementary Figure 4. Turnover in postnatal bundles of neonatal mouse utricle
The number of hair cells in the mouse utricular macula, identified by the presence of hair bundles, increases from about 1800 to 3500 in the first two postnatal weeks, although the total number of cells does not change[4]. Thus 1700 committed hair cells grow bundles in those two weeks. We confirmed the increase in number of bundles in the postnatal mouse utricle, using phalloidin labeling (D-S.Z, not shown).

a, Mass-26 image, showing two small bundles (*) that were presumably still growing, and two mature bundles. This section was from a pup born to a 15N mother and fed 14N milk for 1 day.
b, Ratio image, showing that small bundles have low 15N incorporation. They may have developed mostly with protein synthesized from 14N milk. In contrast, the mature bundles retained much more 15N from the 15N birth mothers.
c, Increase in 15N labeling with days of feeding from 15N nurse mothers. Hair cells with small bundles (dashed lines) show more label; they may have developed entirely after the pups were born, and would be expected to have higher 15N than mature bundles.
d, Decrease in 15N labeling with days of feeding from 14N nurse mothers, as in **b**. Hair cells with small bundles had less label. Bundles may have very low 15N if they develop after birth.

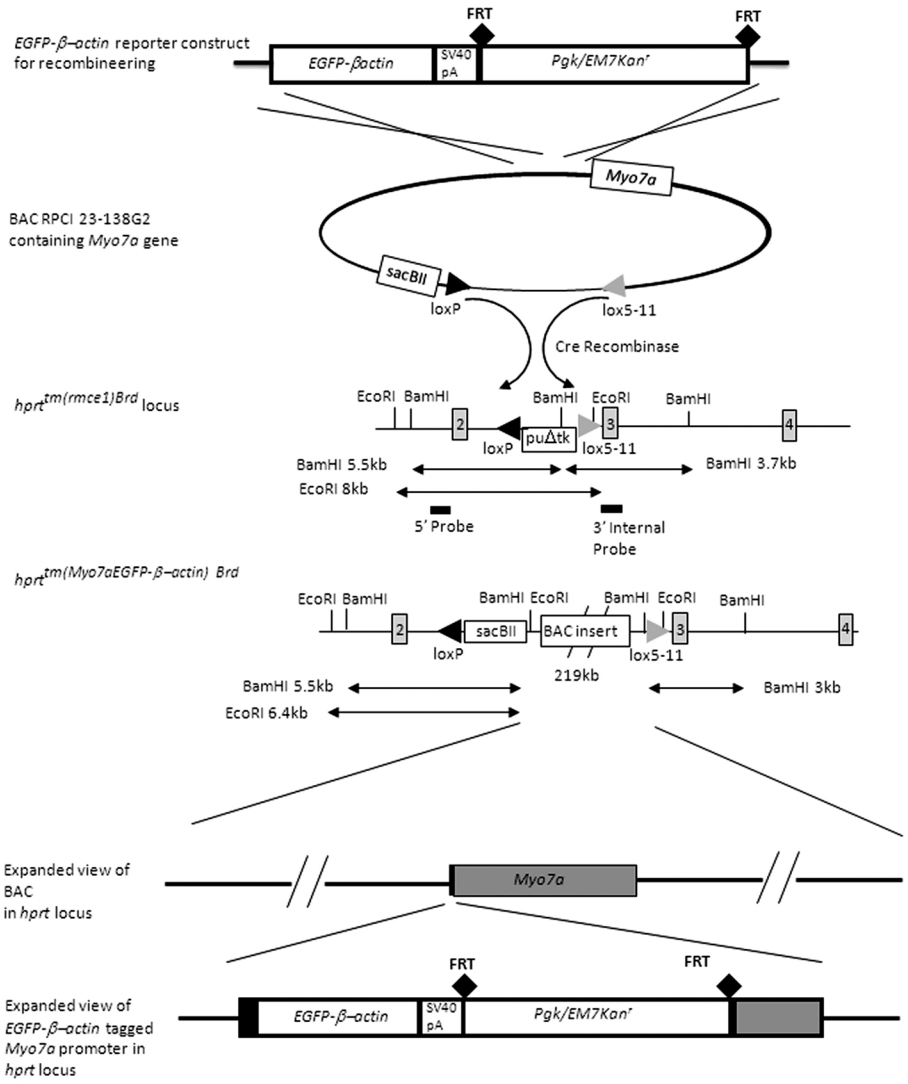




Supplementary Figure 5. Turnover in cultured mouse utricle.
Utricles from P0 pups born to control-fed (14N) mothers were dissected and placed in organotypic culture. One day after dissection and plating, the medium was replaced with one containing 15N-leucine. Both experimental and control (without 15N-leucine) cultures were maintained for up to four more days. As in vivo, utricles in the first postnatal week had a mix of mature and small hair bundles. We measured turnover only in mature bundles and in the cytoplasm of those cells.

a, A hair cell and two supporting cells from mouse utricle in culture; mass 26 image. **b**, Image of mass27/mass26 ratio, showing incorporation. Scale is 0-100%. **c**, Plot of incorporation during 4 days in culture with 15N-leucine medium (with 1% fetal bovine serum). Hair bundles had the lowest incorporation.

Rzadzinska et al.[5] observed rapid treadmilling of GFP-actin in neonatal cultures maintained in 7% fetal bovine serum (FBS). As FBS is a metabolic enhancer, we wondered whether the 1% FBS medium might have inhibited treadmilling. We repeated the 15N-leucine experiments in 6% FBS, but still observed slow incorporation of new protein into stereocilia. Apparently the FBS concentration is not the determinant of rapid treadmilling.



Supplementary Figure 6. Construction of the pEGFP-β-actin mouse.
The EGFP-β-actin-SV40pA was isolated as a 2.1kb HindIII/MluI fragment from a modified pEGFP-β-actin vector (Clontech). A plasmid was constructed in which a EGFP-β-actin-SV40pA PGK-EM7-Neo cassette was flanked by Myo7a-specific homology arms (clone pHP_CXG). The homology arms were PCR amplified from BAC clone RPCI23-138G2 using primers for the 5' arm (Myo7a pr1 AATCCATCTCGAGCCTATTCCTGGGTCCAG and Myo7a pr2 CCCAAGCTTACGTCAGCACACCTGCCGGAGTGACC) and 3' arm (Myo7a pr3 CGCGG ATCCCTGCATGTCCTCCTCTCTATGTGCTTAGCC and Myo7a pr4 TCCCCGCGGAACTCGTGGCTGAGCCCCAGT AGCACCCGG). The EGFP-β-actin-SV40pA fragment was inserted into the BAC clone RPCI23-138G2 by homologous recombination in E.coli deleting the Myo7a translation initiation codon as previously described (ref 6). The modified RPCI23-138G2AB1 BAC was integrated by recombinase-mediated cassette exchange into the AB1-derived (129 mouse strain) ES-cell clone CCI18#1.6G (ref 6). Myo7a:: EGFP-β-actin BAC transgenic ES cell clones were microinjected giving rise to germline-transmitting male chimeras that were bred with C57BL/6-Tyrc-Brd females for one generation. The line Myo7a:: EGFP-β-actin was then backcrossed for one generation to C3H and intercrossed for expansion (129 x C57Bl/6 x C3H mixed background).

For genotyping BAC transgenes integrated as the hprt locus, three oligonucleotide primers each at 2 μM were used to amplify purified tail DNA, annealing at 65 °C for 30 cycles (ref 6). Two primers flank the insertion site (Hprt-F: GACAAGTAAAAATCA CTGGTCAAGG and Hprt-R: CTGGACTGTAATCATAATCCTTGTCTCTAC). The third primer is specific for the BAC vector sequence (BAC-R: GTTAGCAATTTAACTGTGATAAACTACCG). Genomic coordinates for primer Hprt-L are X-chromosome 49246013bp to 49246037bp (NCBI m36; Ensembl v42). BAC-L primer position are 4744bp to 4772bp of pBACe3.6; 6595bp to 6623bp of pTARBAC1). PCR product sizes are 473bp for a wild type Hprt allele and 364bp for a BAC transgene allele.

Supplementary References

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