



Supplementary Figure 1: Expression and induction of β -actin-GFP transgene in hair cells. a-d, Mosaic expression of the β -actin-GFP transgene in hair cells. Atoh1-Cre, constitutively expressed during hair cell development²³, induced β -actin-GFP expression in a subset of hair cells in (a-b) the organ of Corti and (c-d)

the utricle. In the merged image, β -actin-GFP is green and phalloidin stained F-actin is red. **e-j**, Tamoxifeninducible, ubiquitously expressed CreER induced expression of β -actin-GFP in hair cells . **e**, Organ of Corti. β actin-GFP was not detected in mice expressing CreER but not dosed with tamoxifen. **f**, Seven days after tamoxifen administration at P21, mosaic β -actin-GFP expression was detected in IHCs and supporting cells, but not in OHCs. **g-j**, Utricular hair cells. There was sporadic expression of β -actin-GFP at P28 without tamoxifen treatment (**g**,**i**) and robust induction of β -actin-GFP 7 days after tamoxifen treatment at P21 (**h**,**j**). In the merged image, β -actin-GFP is green and phalloidin stained F-actin is red.



Supplementary Figure 2: β -actin-GFP fluorescence recovery is limited to stereocilia tips. a,b, Stacks of confocal images were used to generate 3D image reconstructions (a) and surface renderings (b) of photobleached IHC stereocilia after 400 minutes or 24 hours or recovery. c The size of the compartment that recovered β -actin-GFP fluorescence was measured in the surface renderings (n=109 and 98 stereocilia at 400 minutes and 24 hours, respectively). The length of the compartment did not increase between 400 minutes and 24 hours.