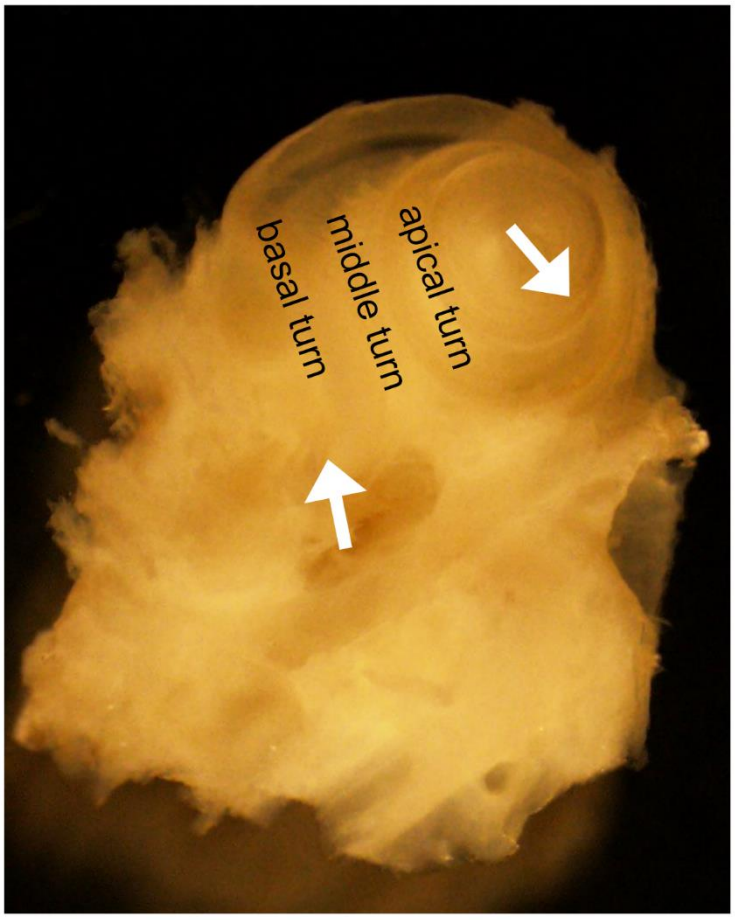
Distinct Expression Patterns Of Causative Genes Responsible For Hereditary Progressive Hearing Loss In Non-Human Primate Cochlea

Makoto Hosoya[1], **Masato Fujioka**[1], **Kaoru Ogawa**[1], **and Hideyuki Okano**[2,*]

1 Keio University School of Medicine, Department of Otorhinolaryngology, Head and Neck Surgery, 35 Shinanomachi Shinjyuku-ku Tokyo, 160-8582, Japan

2 Keio University School of Medicine, Department of Physiology, 35 Shinanomachi Shinjyuku-ku Tokyo, 160-8582, Japan

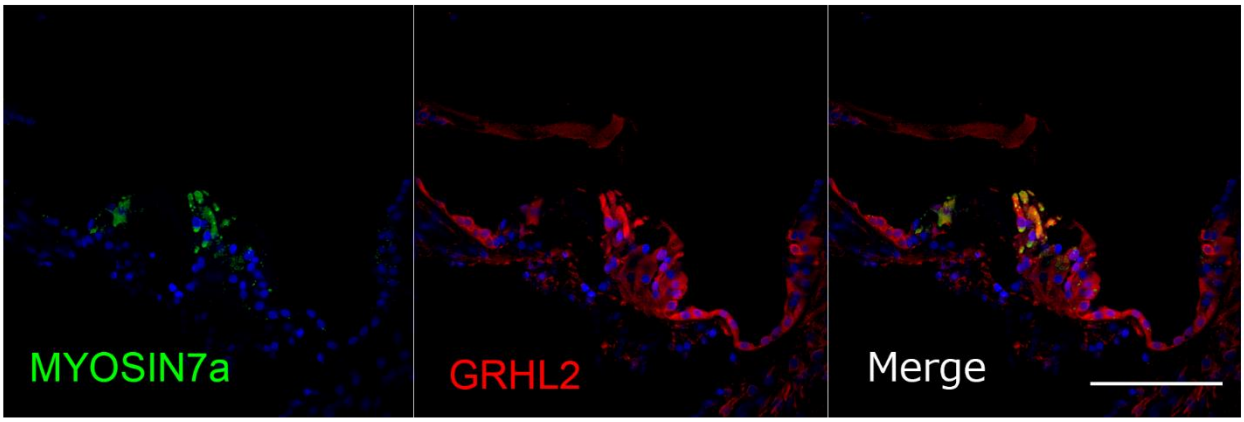
*hidokano@a2.keio.jp



Supplementary Figure S.1

The common marmoset cochlea

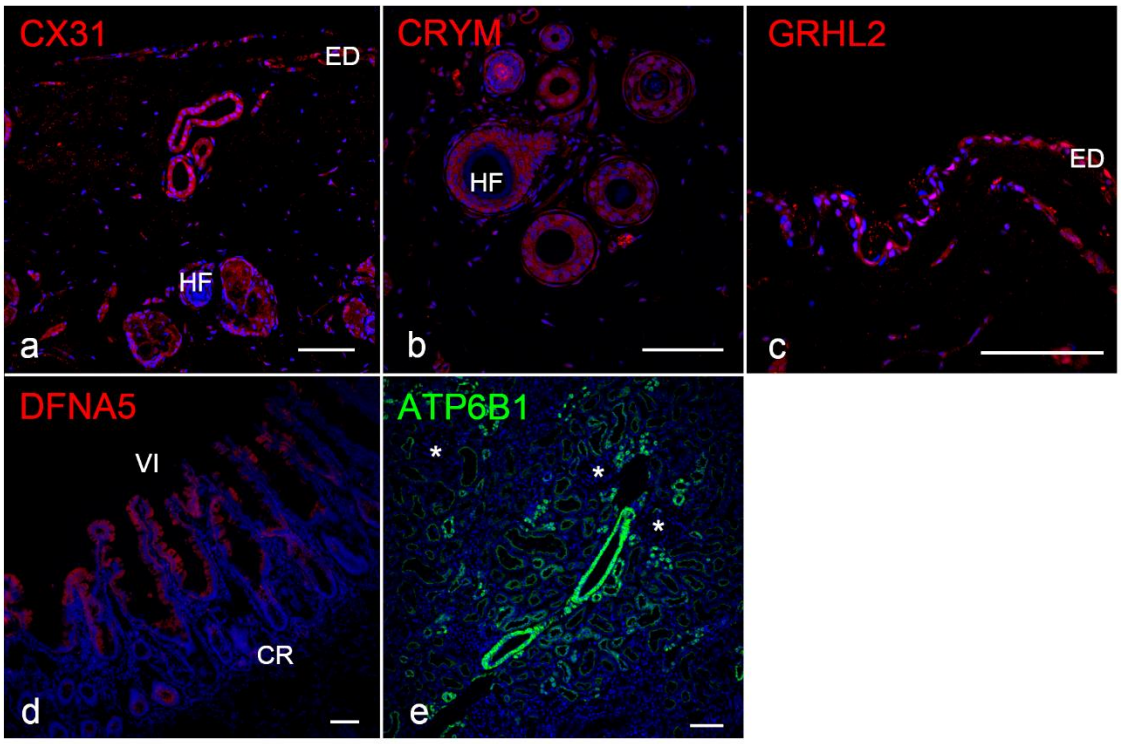
The common marmoset cochlea has 2 and 3/4 turns (between allows) with apical, middle and basal turns as similar to that of human. Note that the cochlear bone capsule was removed.



Supplementary Figure. S2

Cytoplasmic expression of GRHL2

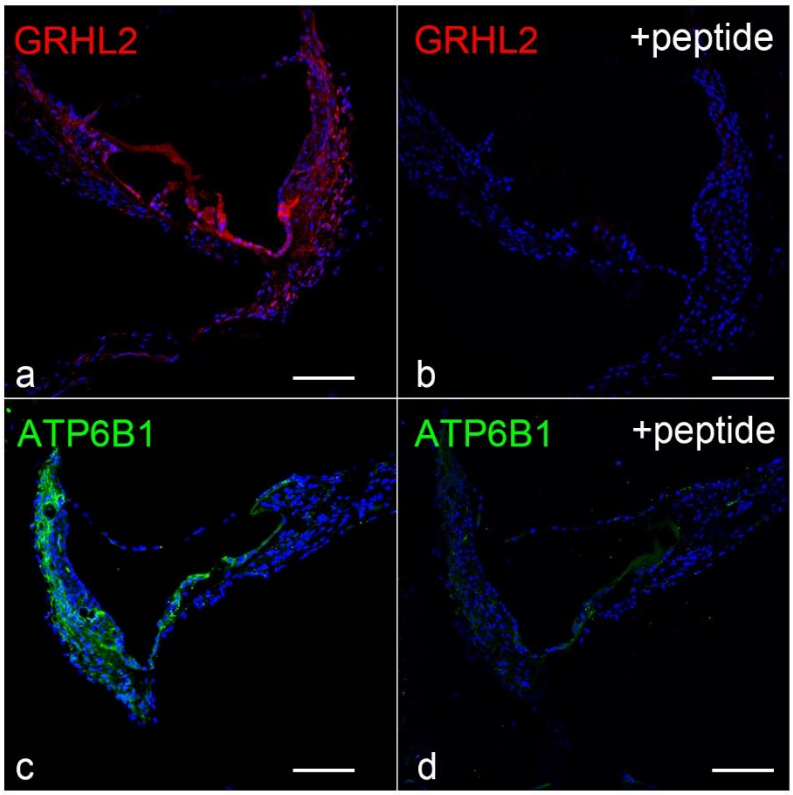
Immunostaining for GRHL2 was observed in the nuclear and cytoplasm. We confirmed the localization by using two different antibodies. Like as shown in Fig.4 (using antibody from Sigma-Aldrich), the immunoreactivity was also detected in cytoplasm with another anti GRHL2 antibody from LSBio. The nuclei were counterstained with Hoechst (blue). Scale bar: 100 µm.



Supplementary Figure. S3

Expressions of CX31, CRYM and GRHL2 in the skin, DFNA5 in the small intestine and ATP6B1 in the kidney

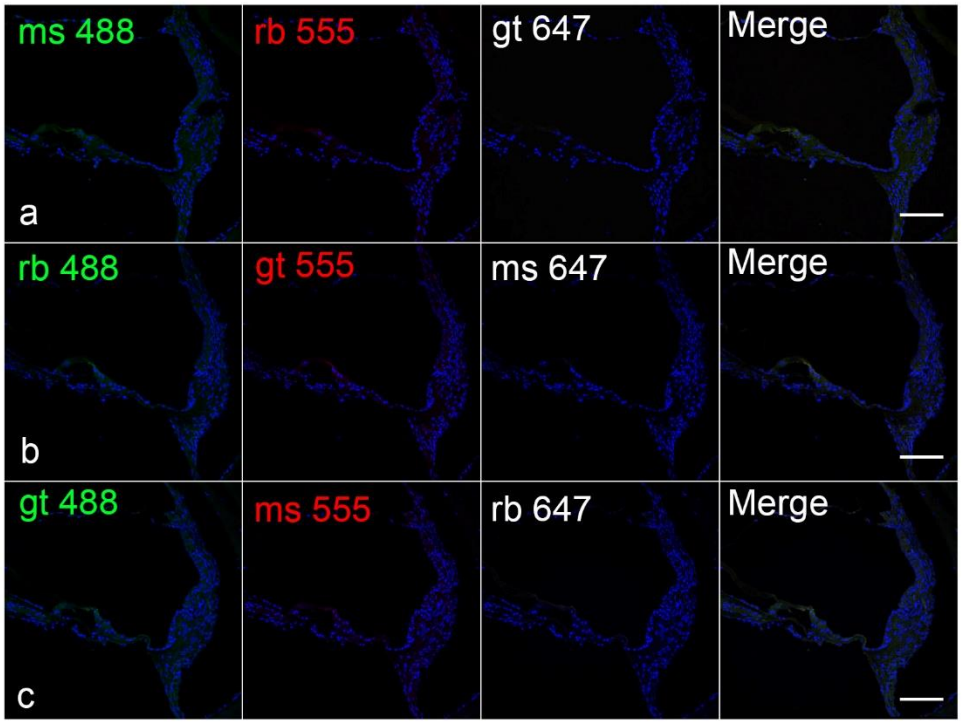
For validating antibodies specificities, we performed immunostaining of the skin, the small intestine and the kidney of common marmoset. (a-c) CX31, CRYM and GRHL2 immunoreactivities were detected in hair follicles (HF) or epidermal layers (ED) and not detected in surrounding connective tissues. (d) DFNA5 immunoreactivities were detected in villus (VI) and not detected in crypt (CR) of the small intestine. (e) ATP6B1 immunoreactivities were detected in collecting ducts and tubules and no immunoreactivities was detected in glomeruli (*). The nuclei were counterstained with Hoechst (blue). Scale bar: 100 µm.



Supplementary Figure. S4

Antibody absorption test by antigen peptide

For validating antibodies specificities, we performed antibody absorption test by antigen peptide. Immunoreactivities (a and c) were abolished by incubations with antigen peptide (b and d). The nuclei were counterstained with Hoechst (blue). Scale bar: 100 μm.



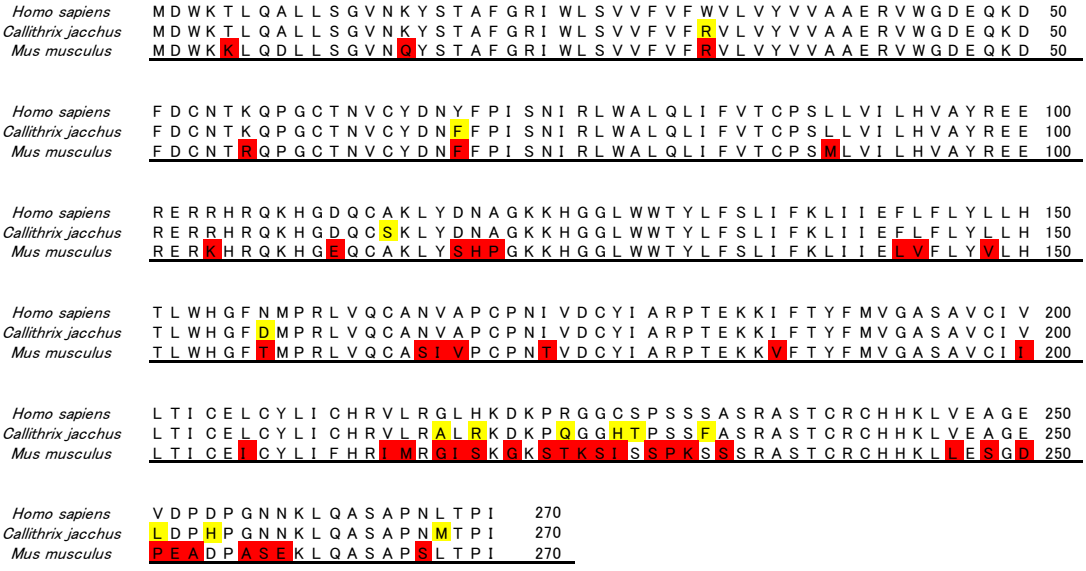
Supplementary Figure. S5

Validations of secondary antibodies.

For validating secondary antibodies' specificities, we performed immunostaining without primary antibody incubations. No immunoreactivities were observed. The nuclei were counterstained with Hoechst (blue). Scale bar: 100 μm. ms: anti-mouse IgG rb: anti-rabbit Ig G gt: anti-goat IgG, 488: Alexa 488, 555: Alexa 555, 647: Alexa 647

Supplementary Figure. S6

a. CX31



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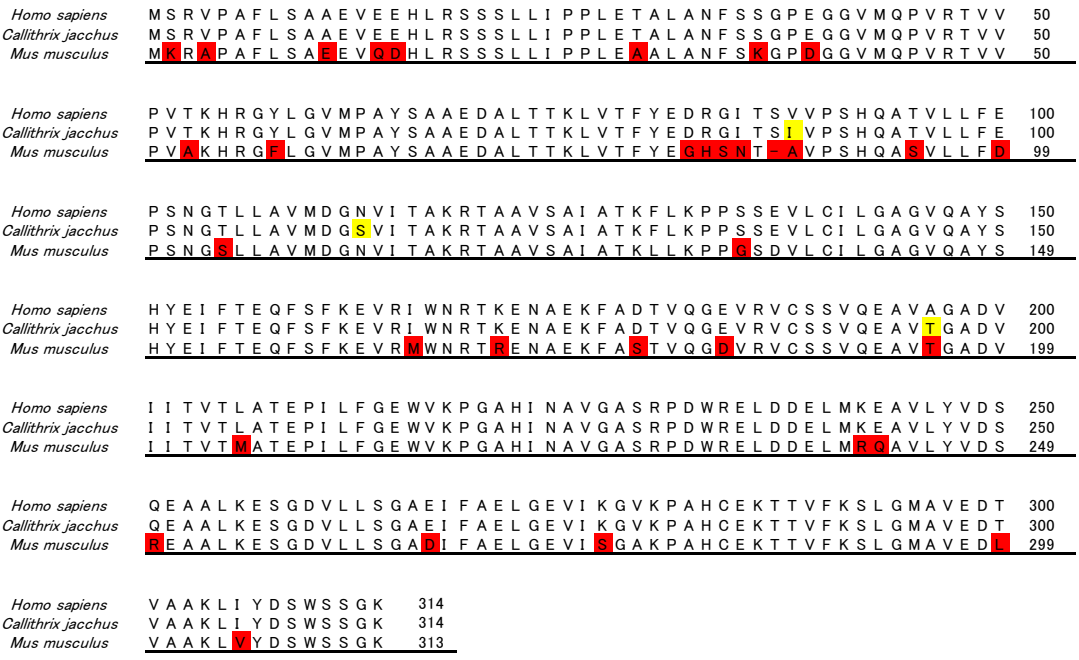
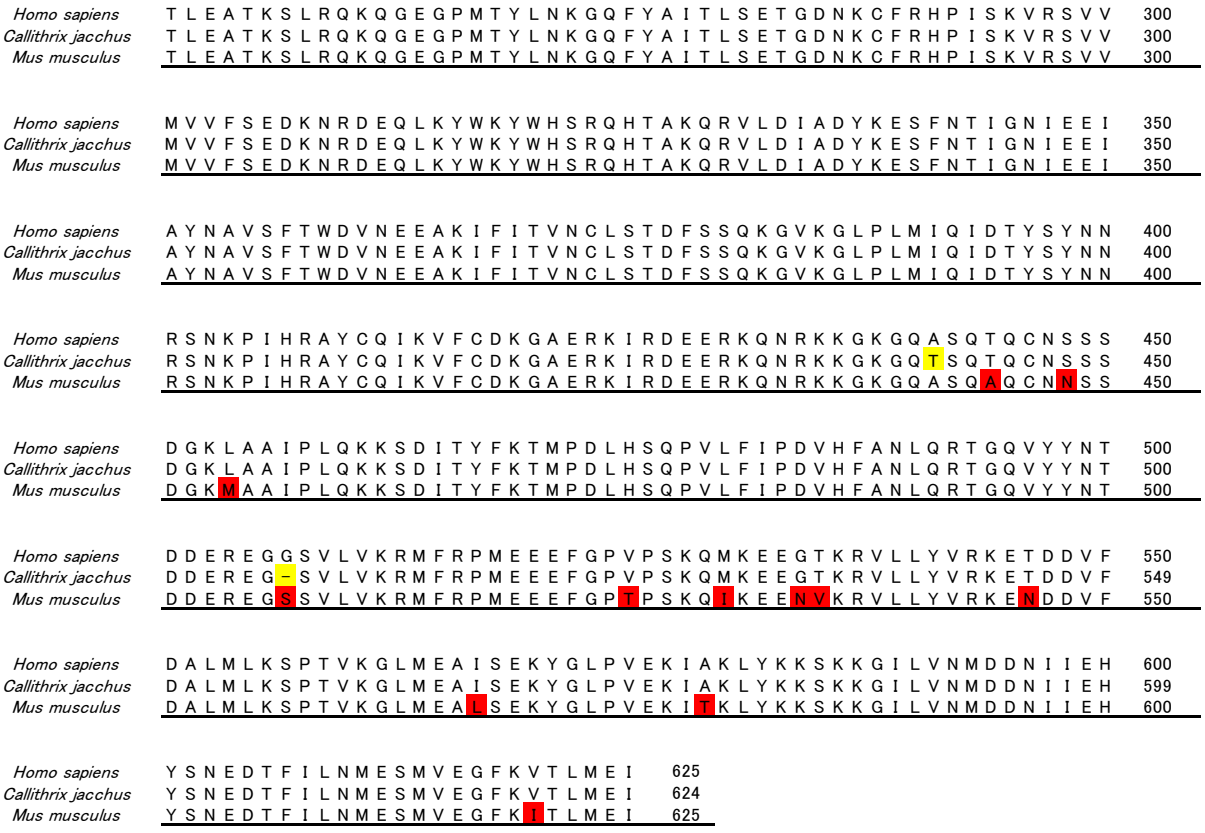


c. GRHL2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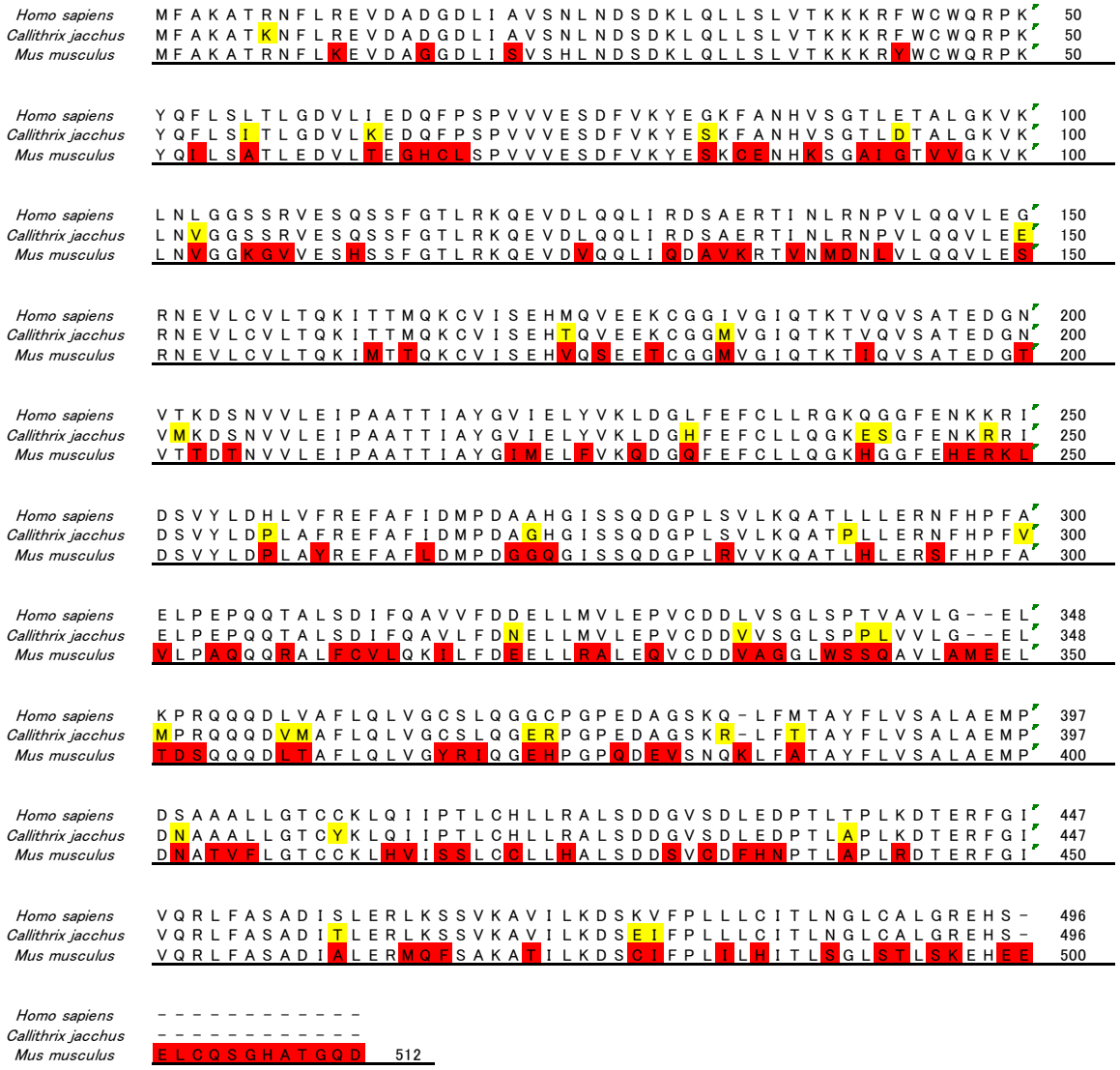
d. DFNA5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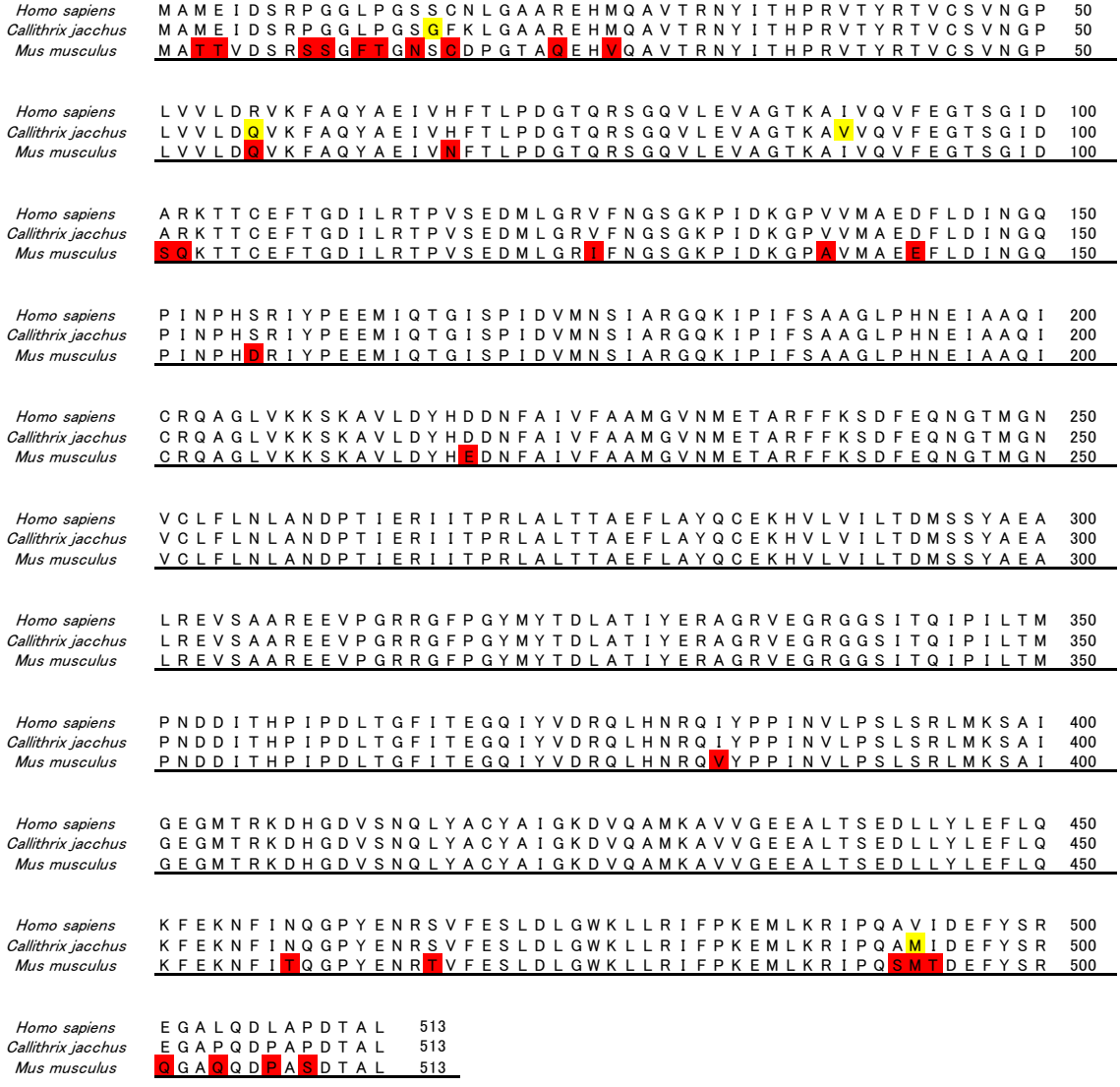
e. ATP6B1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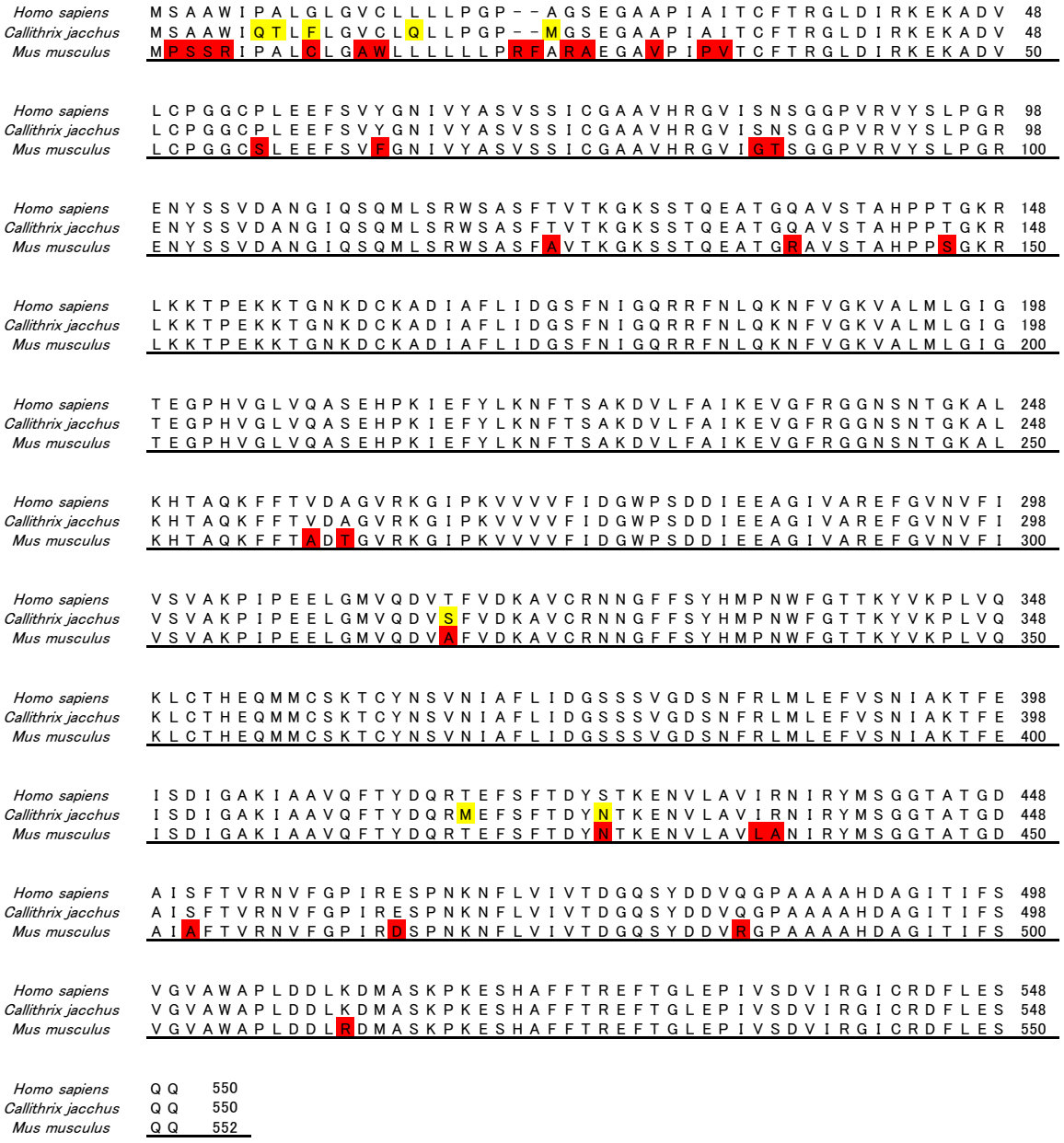
f. COCH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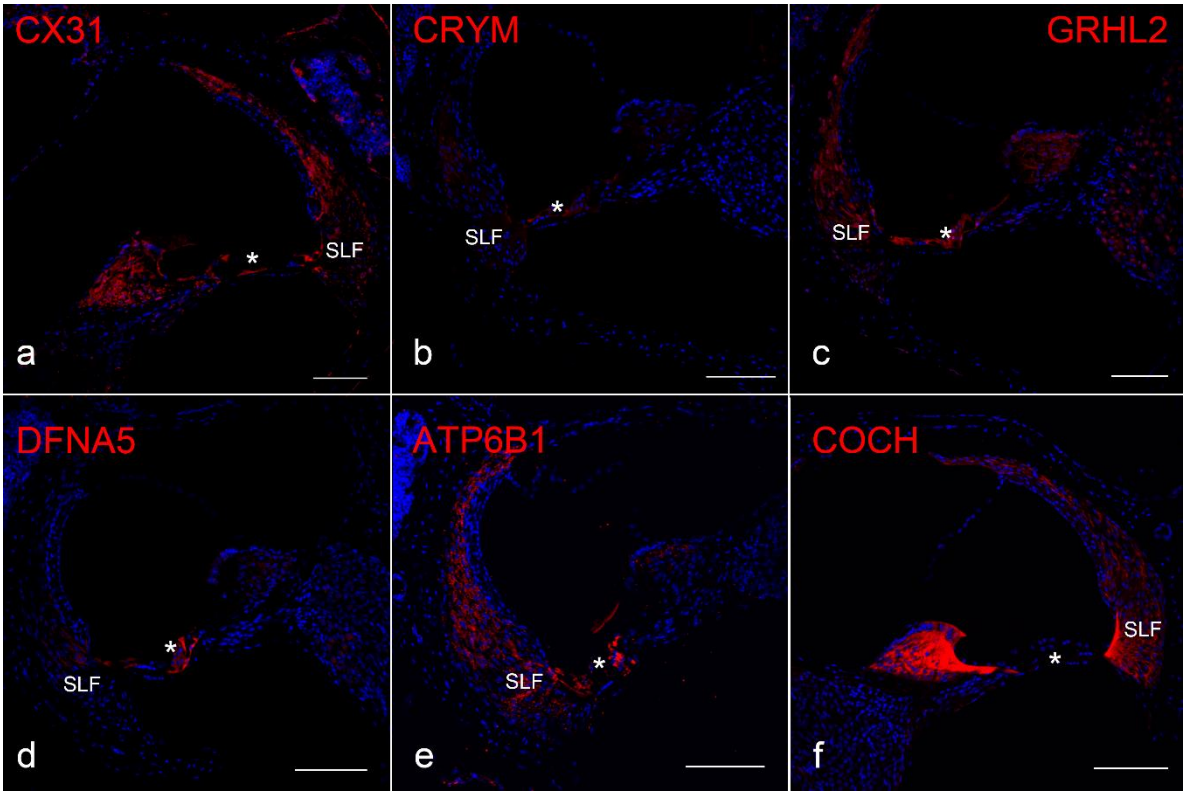
Comparisons of amino-acid sequences between human (*Homo sapience*), common marmoset (*Callithrix jaccus*) and mouse (*Mus musculus*)

We compared amino-acid sequences of these three animals and we showed in these six deafness genes amino-acid sequences of common marmoset is more similar to human than mouse. (a) Homology of amino-acid sequences of CX31 was shown. Human vs common marmoset: 95.19%, human vs mouse 82.22% (b) Homology of amino-acid sequences of CRYM was shown. Human vs common marmoset: 99.04%, human vs mouse 88.50% (c) Homology of amino-acid sequences of GRHL2 was shown. Human vs common marmoset: 98.88%, human vs mouse 94.72% (d) Homology of amino-acid sequences of DFNA5 was shown. Human vs common marmoset: 92.14%, human vs mouse 71.37% (e) Homology of amino-acid sequences of ATP6B1 was shown. Human vs common marmoset: 98.44%, human vs mouse 94.72% (f) Homology of amino-acid sequences of COCH was shown. Human vs common marmoset: 98.55%, human vs mouse 93.45%



Supplementary Figure. S7

Immunostaining of mouse specimens.

(a) Anti-CX31 antibody recapitulated previous report and strong immunoreactivities were detected in the spiral ligament fibrocytes of mouse and no immunoreactivities was detected in the organ of Corti. (b) Anti-CRYM antibody did not work well against mouse specimens. (c) Anti-GRHL2 antibody used in this report partially recapitulated previous report and immunoreactivities were detected in the organ of Corti. (d) Anti-DFNA5 antibody used in this report partially recapitulated previous report and immunoreactivities were detected in the organ of Corti. (e) Anti-ATP6B1 antibody recapitulated previous report and strong immunoreactivities were detected in the spiral ligament fibrocytes of mouse.

(f) Anti-COCH antibody recapitulated previous report and strong immunoreactivities were detected in the spiral ligament fibrocytes of mouse and no immunoreactivities was detected in the organ of Corti. SLF: Spiral Ligament Fibrocytes, *. Organ of Corti. The nuclei were counterstained with Hoechst (blue). Scale bar: 100 μm.