

# Otology Journal Club

## communications medicine








ARTICLE



<https://doi.org/10.1038/s43856-021-00044-w>

OPEN

## Direct SARS-CoV-2 infection of the human inner ear may underlie COVID-19-associated audiovestibular dysfunction

Minjin Jeong<sup>1,2,12</sup>, Karen E. Ocwieja<sup>3,4,5,13</sup>, Dongjun Han<sup>1,2,12,13</sup>, P. Ashley Wackym <sup>6</sup>, Yichen Zhang <sup>7</sup>, Alyssa Brown <sup>2</sup>, Cynthia Moncada<sup>2</sup>, Andrea Vambutas <sup>8</sup>, Theodore Kanne<sup>9</sup>, Rachel Crain<sup>10</sup>, Noah Siegel<sup>1,2</sup>, Valerie Leger<sup>5</sup>, Felipe Santos<sup>1,2</sup>, D. Bradley Welling <sup>1,2</sup>, Lee Gehrke<sup>5,7,11,14</sup>  & Konstantina M. Stankovic<sup>1,2,12,14</sup> 

Judith Kempfle

02/09/22

# Is COVID-19 infection causing audiovestibular dysfunction?

<https://casereports.bmj.com/content> ⋮

## [Sudden irreversible hearing loss post COVID-19 - BMJ Case ...](https://casereports.bmj.com/content)

by FS Koumpa · 2020 · Cited by 97 — Abstract. **Sudden onset sensorineural hearing loss** (SSNHL) is frequently seen by otolaryngologists. The exact pathophysiology of the disease is...

<https://jamanetwork.com/jamaotolaryngology/fullarticle> ⋮

## [Sudden Sensorineural Hearing Loss and COVID-19 Vaccination](https://jamanetwork.com/jamaotolaryngology/fullarticle)

by SE Briggs — Those of us treating patients every day can attest to a clear association between **Covid** vaccine and sudden...

<https://jamanetwork.com>

## [Telemedicine for](https://jamanetwork.com)

by S Shilo — Objective evaluation of new-ons...

<https://journals.lww.com>

## [SUDDEN SENSOR](https://journals.lww.com)

by A Chern · 2021 · Cited hemorrhage in a patient v...

<https://www.healthhear>

<https://www.ijdonline.com/article/fulltext> ⋮

## [Sudden sensorineural hearing loss after COVID-19 v](https://www.ijdonline.com/article/fulltext)

by J Jeong · 2021 · Cited by 4 — Otolaryngologic adverse events after **COV** reported, including several cases of **sudden sensorineural hearing loss** ...

<https://onlinelibrary.wiley.com/doi/ccr3> ⋮

## [Sudden sensorineural hearing loss in COVID-19 - Wi](https://onlinelibrary.wiley.com/doi/ccr3)

by E Beckers · 2021 · Cited by 5 — Hence, as hypothesized by Harenberg et **COVID-19** patients could be a result of endothelial cell dysfunction with micro- ...

<https://www.cureus.com/articles/75894-systematic-r...> ⋮

## [Systematic Review of Sensorineural Hearing Loss Associated ...](https://www.cureus.com/articles/75894-systematic-r...)

by KM McIntyre · 2021 — Our objective is to identify novel **coronavirus** disease 2019 (**COVID-19**) patients with a diagnosis of **sudden sensorineural hearing loss** ...

<https://secondchancehearing.com/2020/12/07/the-co...> ⋮

## [The Connection Between Sudden Hearing Loss and COVID-19](https://secondchancehearing.com/2020/12/07/the-co...)

Dec 7, 2020 — For patients diagnosed with **COVID-19**, screening for **sudden hearing loss** may present the best chance for identifying the condition early and ...

<https://www.seniorsbluebook.com/articles/covid-and...> ⋮

## [COVID AND DIZZINESS – WHAT IS THE RELATIONSHIP?](https://www.seniorsbluebook.com/articles/covid-and...)

The first two types of **dizziness** – **vertigo** and lightheadedness – have been associated with **COVID-19** infection. The virus that causes **COVID-19** (the “severe ...

<https://www.hindawi.com/journals/crim> ⋮

## [Post-COVID-19 Benign Paroxysmal Positional Vertigo - Hindawi](https://www.hindawi.com/journals/crim)

by S Maslovara · Cited by 2 — Objective. **Dizziness** is a very common symptom that patients report in routine clinical practice and one of the significant clinical manifestations of **COVID-19**.

<https://www.healthline.com/health/is-dizziness-a-sy...> ⋮

## [Is Dizziness a Symptom of COVID-19? - Healthline](https://www.healthline.com/health/is-dizziness-a-sy...)

Apr 12, 2021 — It's currently unknown explanations include inflammation fro...

<https://cornerstonephysio.com/resources>

## [Post COVID Dizziness | Ve](https://cornerstonephysio.com/resources)

Jan 28, 2022 — General **dizziness** is a cases, will disappear as the other typi...

<https://www.ncbi.nlm.nih.gov/articles/PMC8255056> ⋮

## [New Onset Vertigo After COVID-19 Infection. A Case Report](https://www.ncbi.nlm.nih.gov/articles/PMC8255056)

by KR Motawea · Cited by 1 — Moreover, a novel and interesting link between **COVID-19** and newly diagnosed hearing loss, **vertigo**, otalgia, and tinnitus have been reported [11] ...  
[Abstract](#) · [Introduction](#) · [Discussion](#)

<https://www.nationaldizzyandbalancecenter.com/post-...> ⋮

## [Post-Covid Dizziness Treatment - NDBC](https://www.nationaldizzyandbalancecenter.com/post-...)

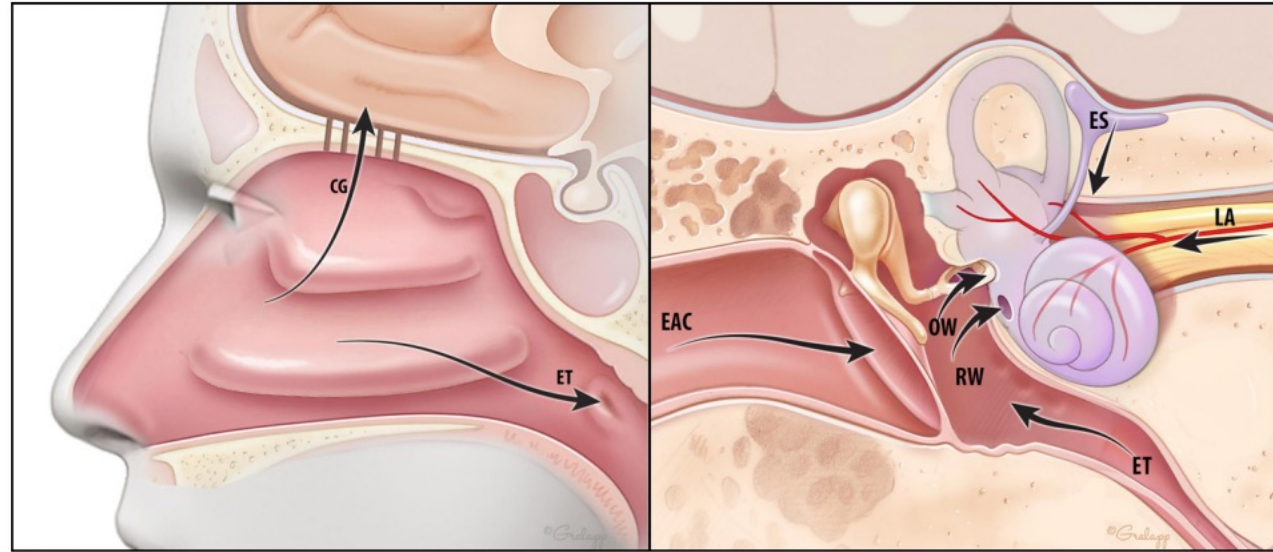
**COVID-19** can also cause **dizziness** and imbalance by causing vestibular neuritis. Vestibular neuritis can be caused by a variety of viral infections and typically ...

<https://www.frontiersin.org/fmed.2021.790931/full> ⋮

## [Acute Vertigo After COVID-19 Vaccination - Frontiers](https://www.frontiersin.org/fmed.2021.790931/full)

Jan 6, 2022 — Objective: The aim of this study was to present some cases of acute **vertigo** potentially related to the **coronavirus** disease 2019 (**COVID-19**) ...

# Potential access routes to the inner ear

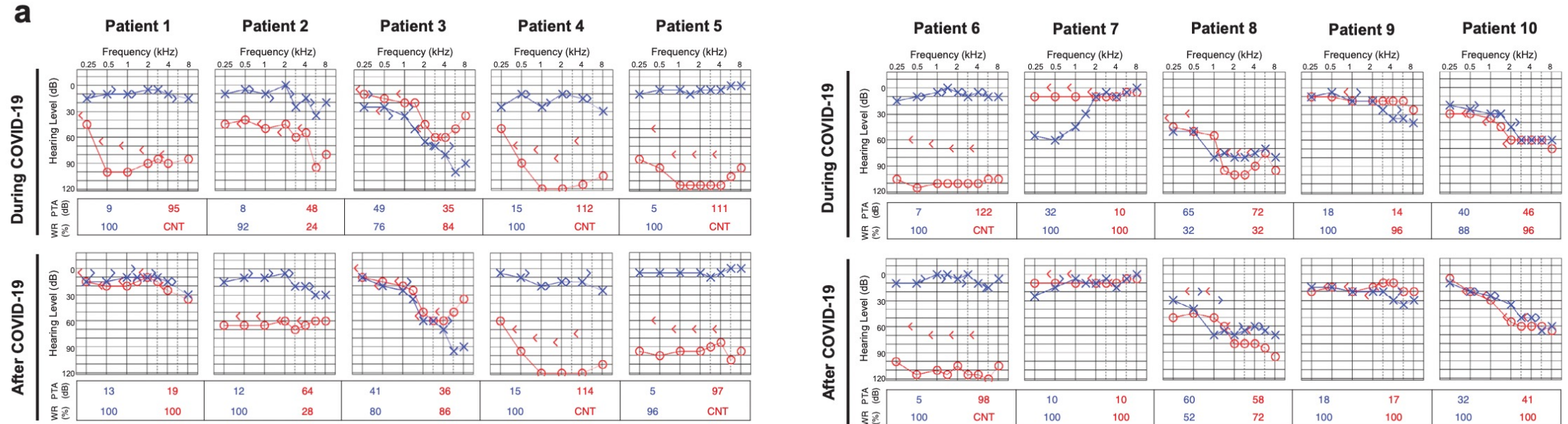


**Fig. 7 Potential paths for SARS-CoV-2 entry into the inner ear.** Arrows indicate potential paths via the nose and olfactory foramina (OF) into the central nervous system; via the endolymphatic sac (ES); via labyrinthine artery (LA) to ultimately reach stria vascularis; via round window (RW) and oval window (OW) membranes which the virus could reach through the Eustachian tube (ET) or external auditory canal (EAC), middle ear and mastoid. The diagram within this figure was drawn by Chris Gralapp and is being reproduced with permission.

# Study methods

- 10 COVID-19 patient audiograms and MRIs
- Human inner ear tissue analysis
- Human inner ear in vitro cellular models
- Mouse inner ear tissue

# Part 1: Hearing loss in patients with COVID-19



9 out of 10 patients experienced classic COVID-19 symptoms (fever, cough, dyspnea) between 21 days before and 14 days after onset of hearing loss, tinnitus or vertigo.

Complete (1,7), partial (8), or no recovery (2,3,4,5,6,9,10) after steroids (oral and/or IT)

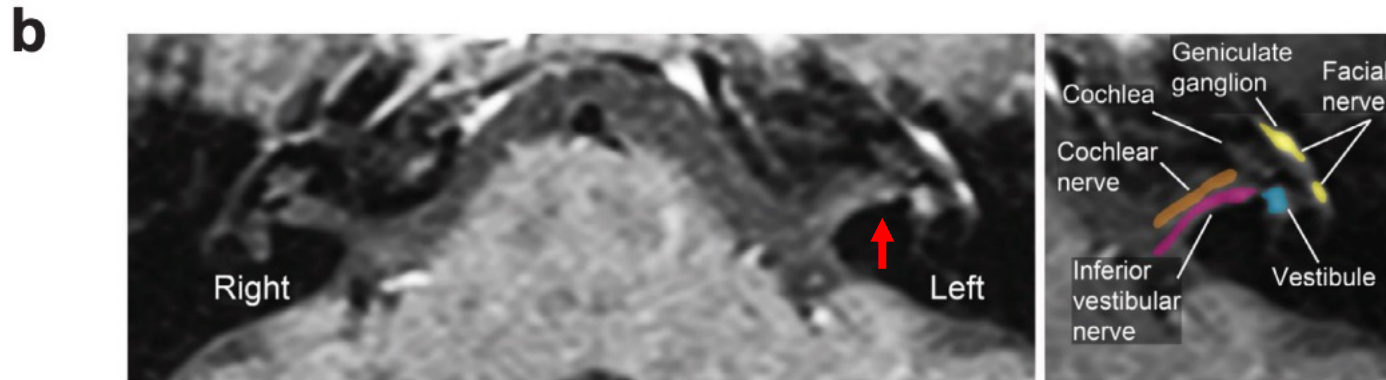
**BUT: no pre-COVID audiograms available- audiograms do not demonstrate causality**

# Otoacoustic emissions to assess inner ear hair cell function

<b>Table 1 Otoacoustic emission test results reflecting cochlear outer hair cell function.</b>												
<b>Patient</b>	<b>Hearing loss</b>	<b>Otoacoustic emission test results by frequency</b>										
		<b>Ear</b>	<b>2 kHz</b>	<b>2.5 kHz</b>	<b>3 kHz</b>	<b>3.5 kHz</b>	<b>4 kHz</b>	<b>5 kHz</b>	<b>6 kHz</b>	<b>7 kHz</b>	<b>7.5 kHz</b>	<b>8 kHz</b>
5	R profound SNHL	Right	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Left	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
6	R profound SNHL	Right	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Left	Present	Present	Present	Present	Present	Present	Present	Present	Present	Absent
7	L moderate low-frequency SNHL	Right	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
		Left	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
8	B severe to profound SNHL	Right	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Left	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
9	L mild high-frequency SNHL	Right	Present	Present	Present	Present	Present	Present	Present	Present	Absent	Absent
		Left	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
10	B moderate SNHL	Right	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
		Left	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

*L left, R right, B both, SNHL sensorineural hearing loss.*

# MRI to rule out retrocochlear pathology



- All 10 patients underwent MRI: MRIs were normal except for patient 7 – patient demonstrated diffuse enhancement consistent with inflammation “related to virus infection”
- “These data suggest a direct correlation between hearing loss, as quantified by audiometric data, and COVID-19”
- **Unspecific, not confirming COVID-19 infection!**

# CELL LINES TO STUDY SARS-CoV-2



# Generation of human iPS cell line

- What are induced pluripotent stem cells (iPSC)?
- Viral reprogramming of fibroblasts (from healthy patients) into pluripotent cells via 4 factor method (Klf4-Oct3/4-Sox2-cMyc)

## Advantages

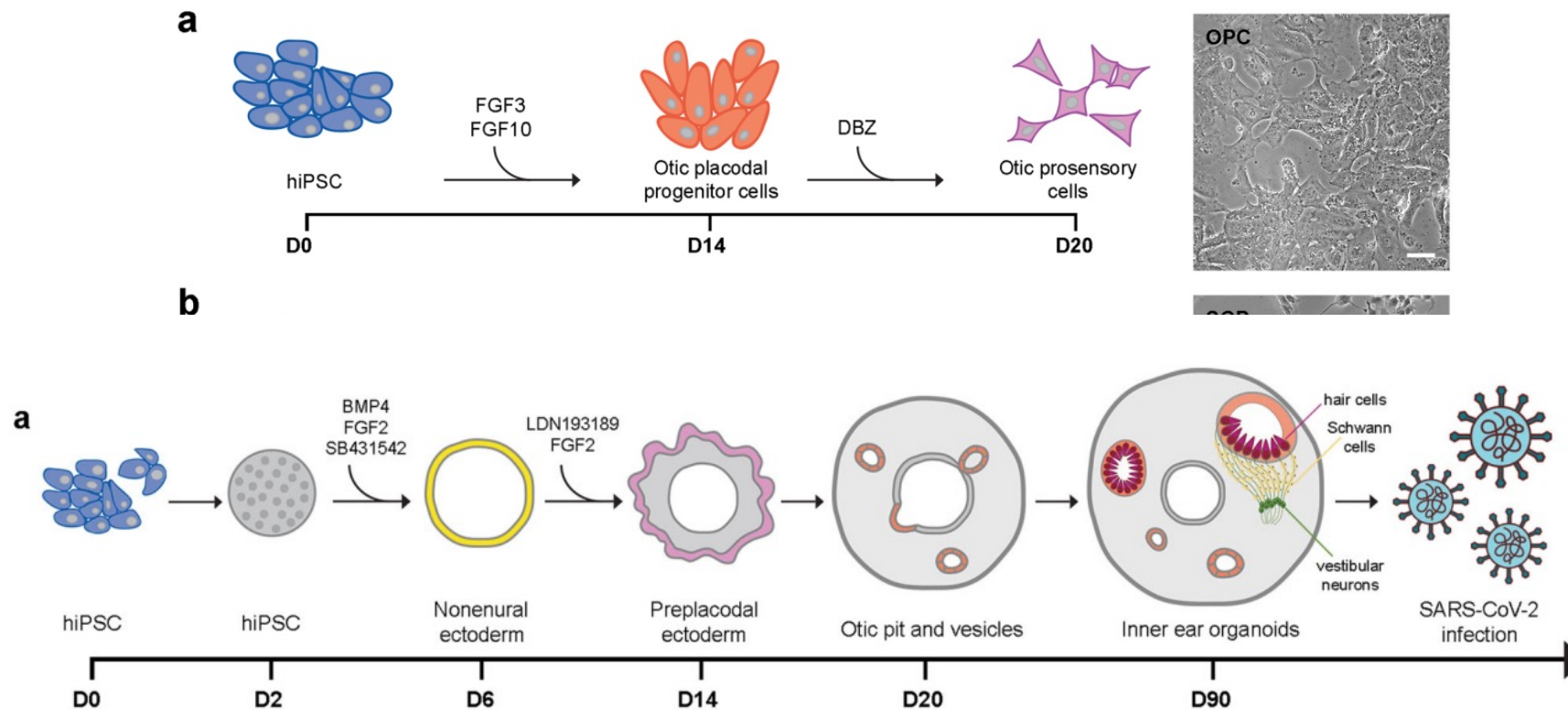
- Pluripotent, can be differentiated into many different cell types
- Can be propagated in culture for a long time – nearly unlimited supply of cells
- Can be created from patients with a specific disease to be used as a model to understand the mechanisms of diseases.

## Disadvantages

- Tumorigenesis from viral reprogramming
- Low reprogramming efficiency, ~ 0.02%
- Genetic and epigenetic abnormalities from in vitro culture
- Differentiation does not create mature, perfect inner ear cells!

# Generation of a human iPS cell line

- Differentiation into otic progenitor cells (OPC)
- Differentiation into Schwann cell progenitors (SCP)
- Generation of inner ear organoids (3D model of inner ear tissue)

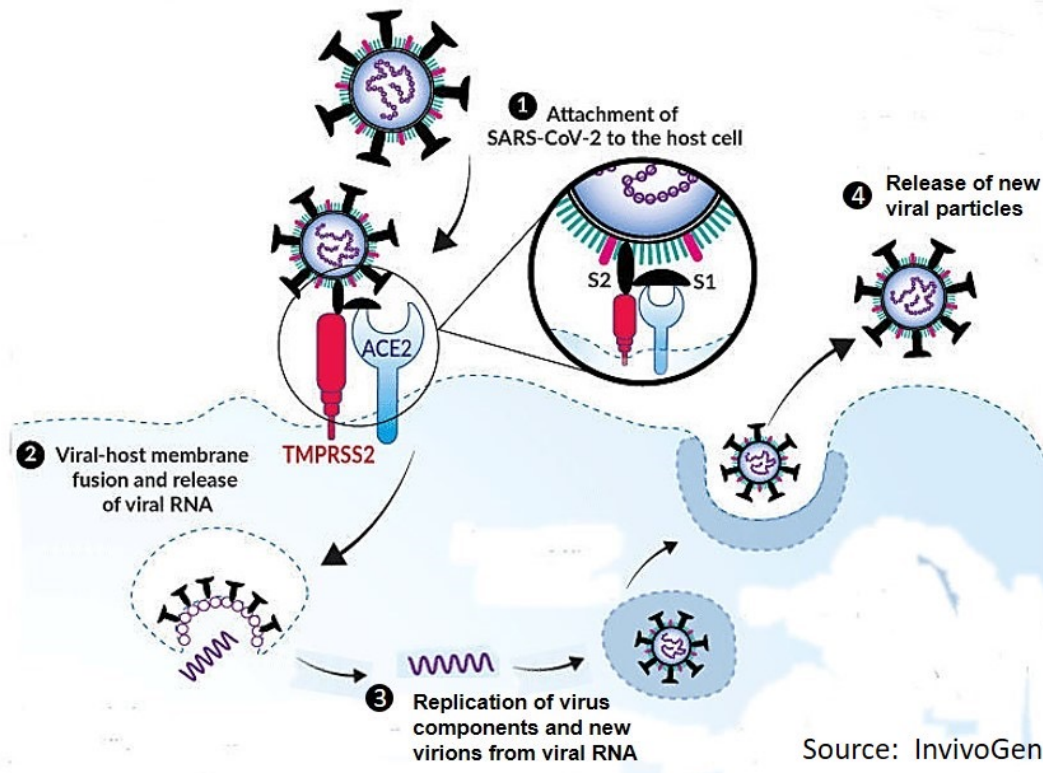


# EXPRESSION ANALYSIS

- Expression of mRNA or protein in a cell or tissue just demonstrates the presence on RNA or protein level
- Co-expression does not mean functional connection of different mRNAs or proteins
- No mechanistic information!

# Part 2: Human inner ear tissue analysis –

Are inner ear cells equipped to allow for SARS-CoV-2 entry?

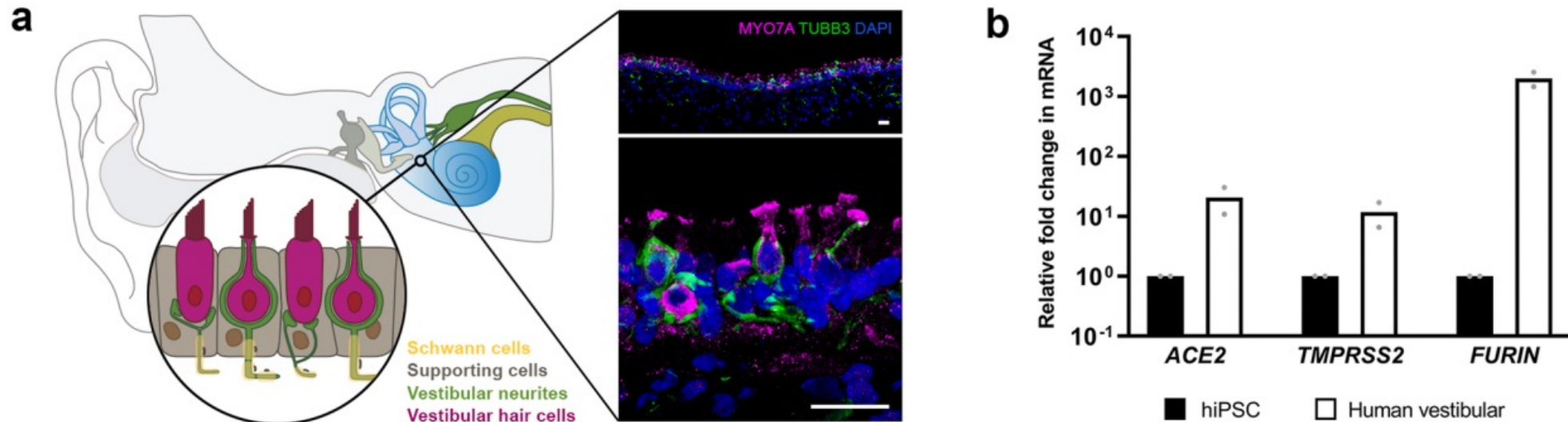


- **Angiotensin-converting enzyme 2 (ACE2)** – Entry receptor for SARS-CoV-2 spike protein – **widely expressed in multiple tissues**

- **Transmembrane protease serine 2 (TMPRSS2)** - Cleavage primes spike protein to allow entry into host cell - **widely expressed in multiple tissues**

- **FURIN** (Protease, **ubiquitously expressed** in all cells at low levels, well known to cleave polybasic viral sites)

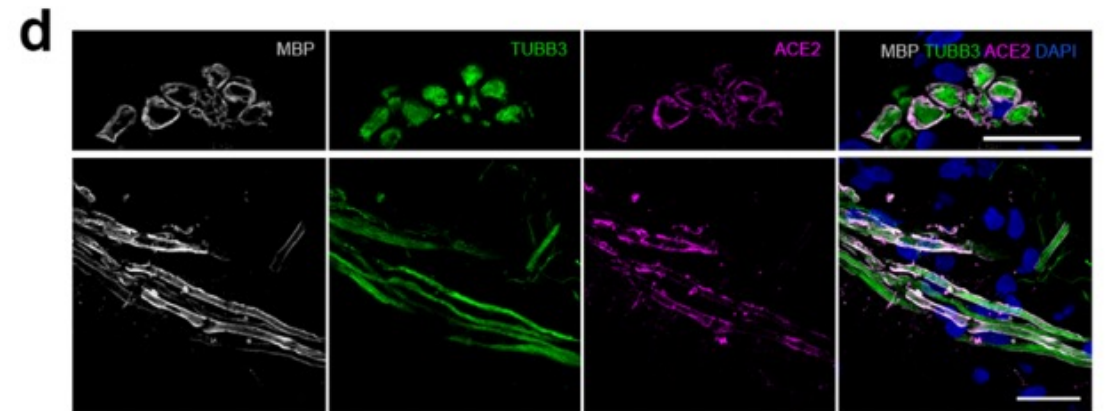
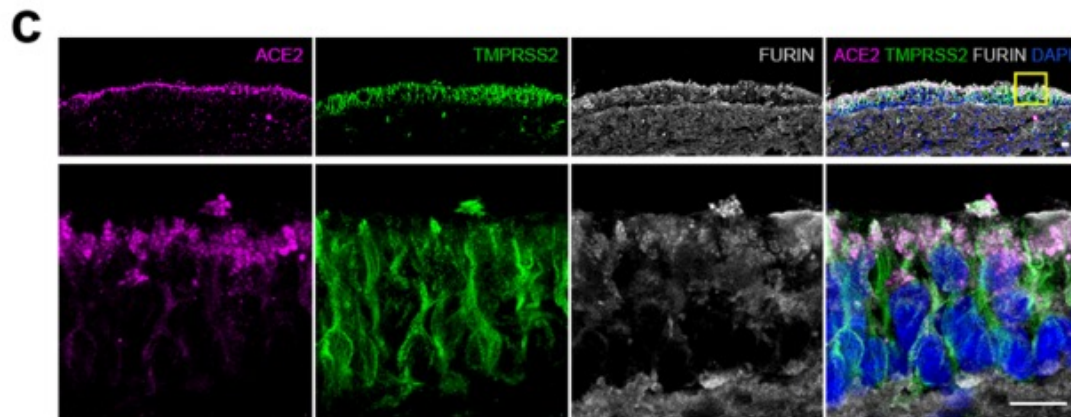
# mRNA and protein expression in normal human peripheral vestibular tissue (mixed cells) and hiPSC



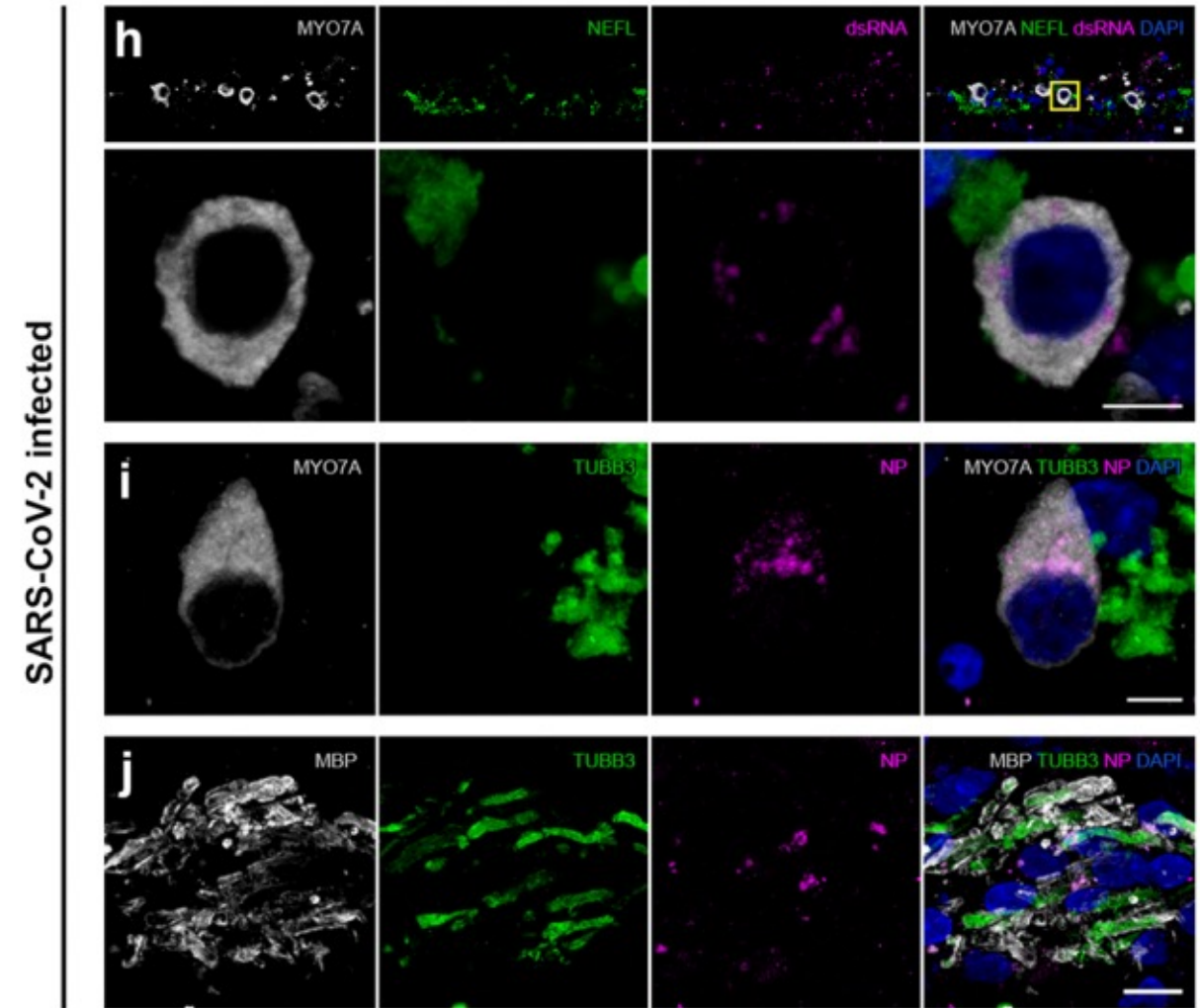
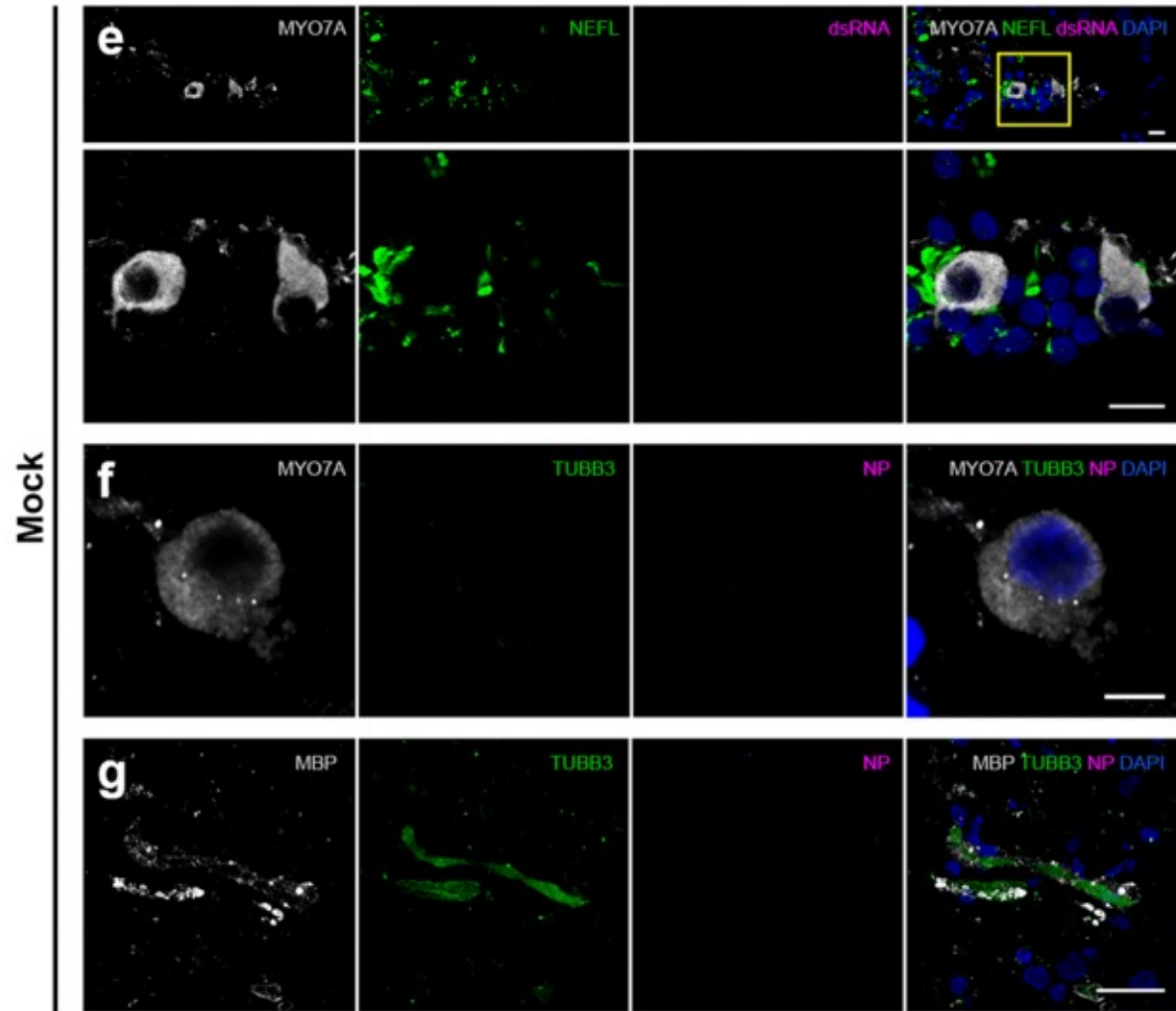
qPCR just demonstrates PRESENCE of ACE2, TMPRSS2 and FURIN on mRNA level in human vestibular tissue and to a very low degree in human iPSCs

# Immunohistochemistry (IHC) of human vestibular tissue demonstrates presence of virus related entry genes

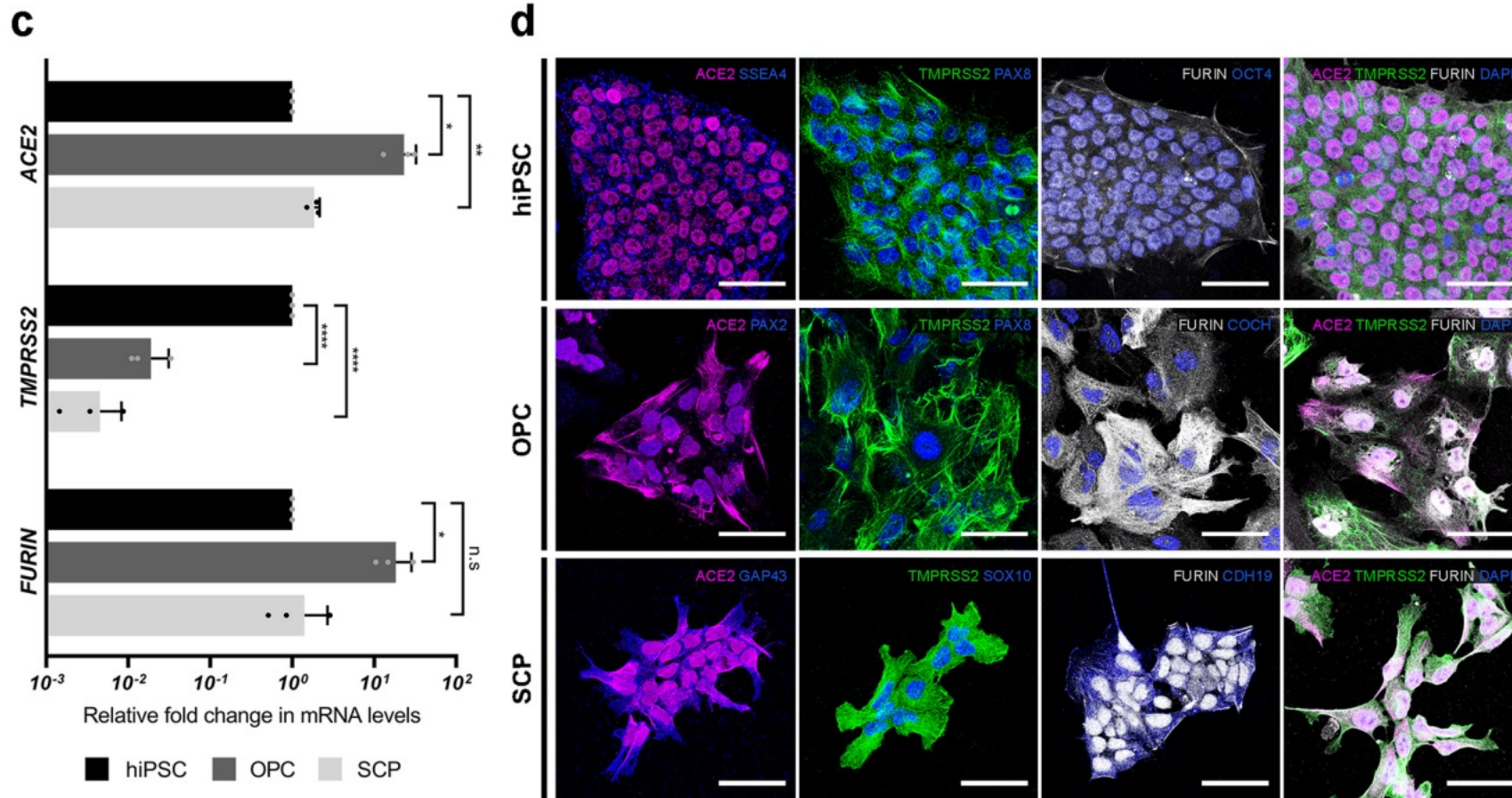
- Co-expression of ACE, TMPRSS2 and FURIN in vestibular hair cells
- ACE2 expression in Schwann cells



# IHC demonstrates virus nucleoprotein (NP) and double-stranded RNA (dsRNA) in hair cells

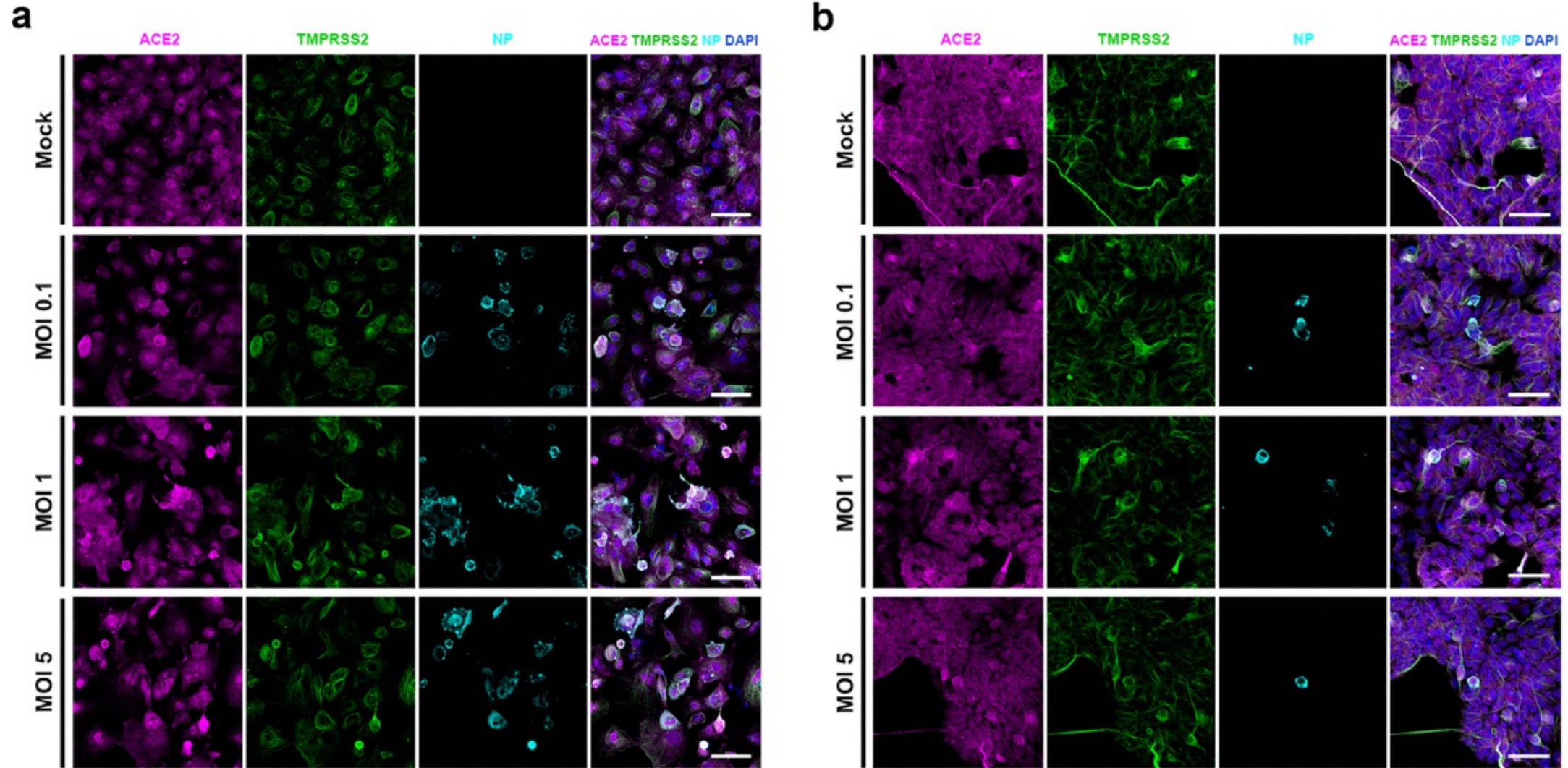


# ACE2, TMPRSS2 and FURIN are expressed in SCP, OCP and hiPSC



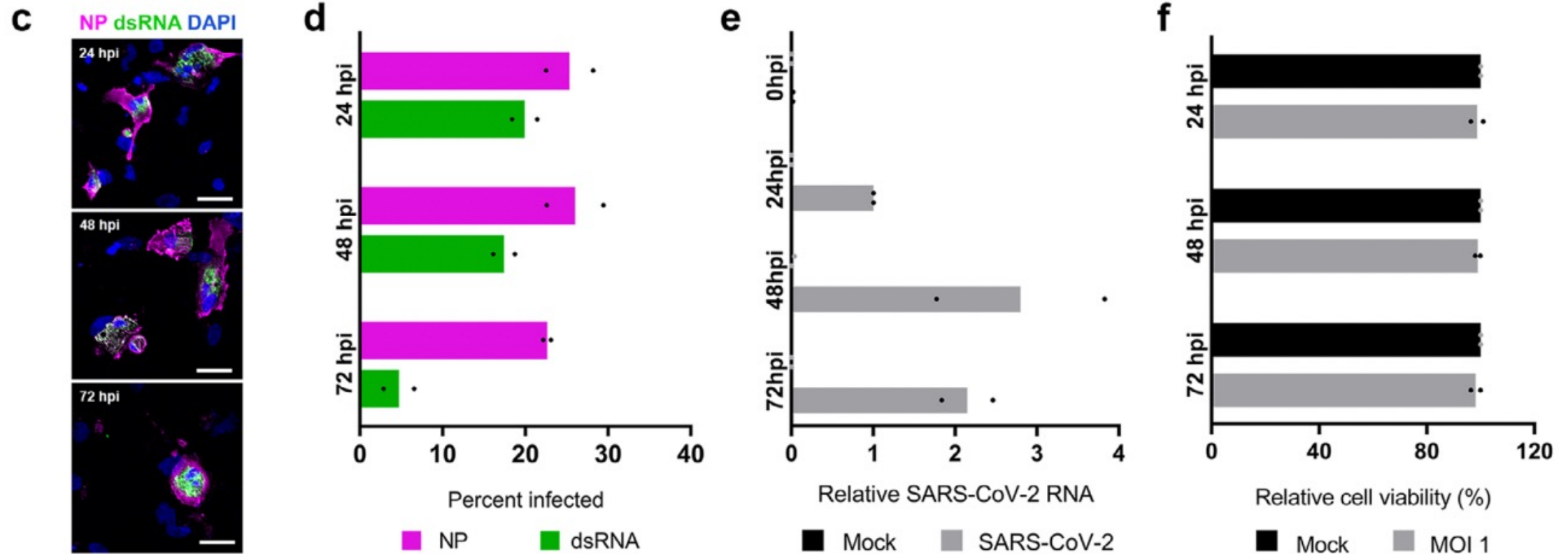


# OPC and SCP infection with SARS-CoV-2: SCP infection rate is < 1%

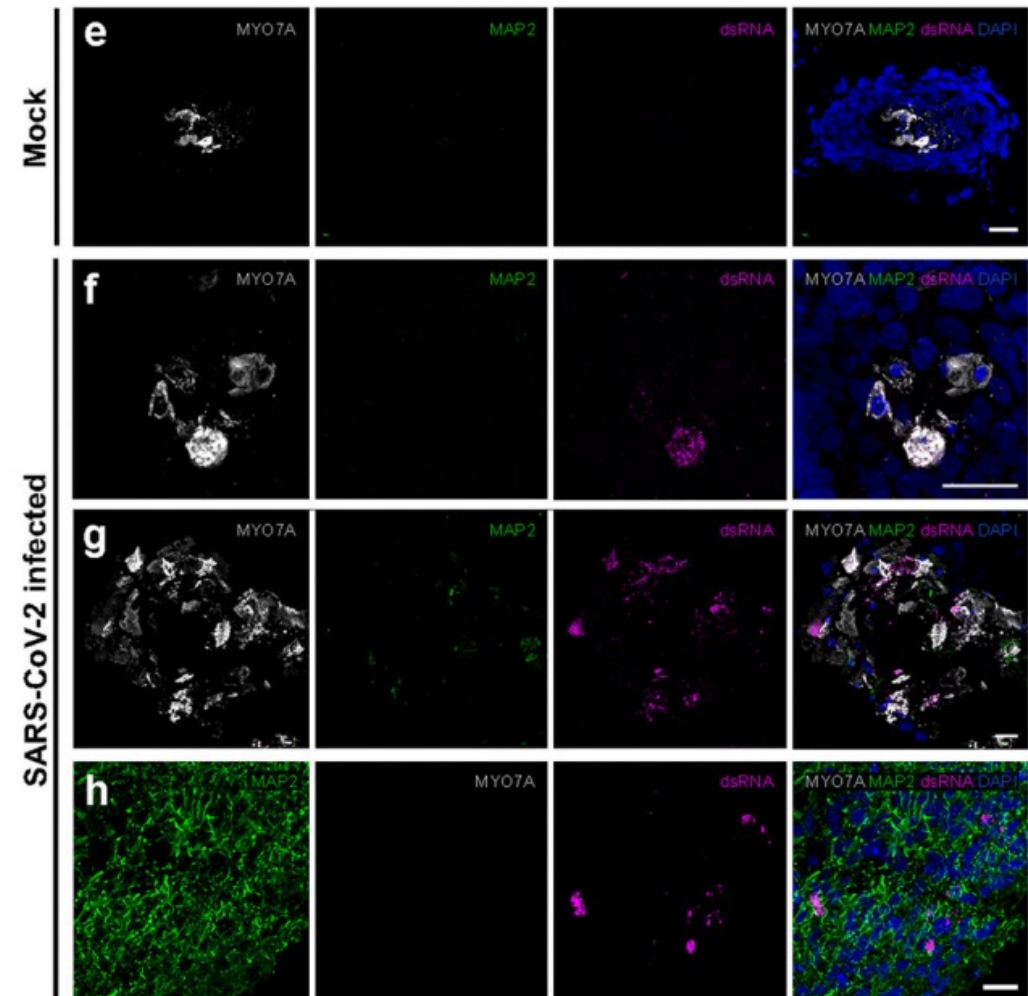
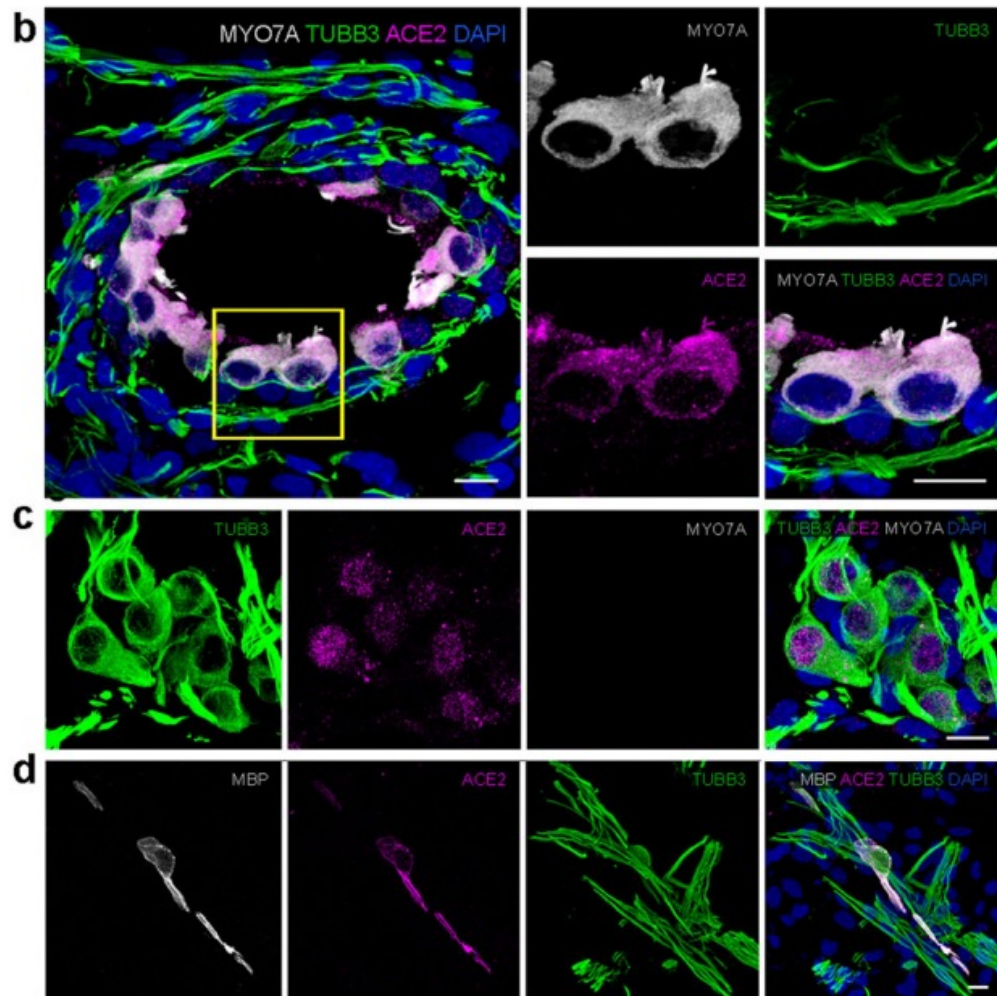


Multiplicity of infection or MOI represents **the ratio of the numbers of virus particles to the numbers of the host cells** in a given infection medium. A value of MOI = 1 implies that on an average there is a single host cell for a single phage particle.

# Quantification of virus infected OPC cells by IHC and levels of viral RNA in supernatant



3D organoid culture demonstrates ACE2 in neurons, glia and hair cells. Viral infection reveals viral dsRNA in hair cells and ?neurons



# Bottom line

- Virus related entry genes are expressed in vestibular hair cells and to some degree in Schwann cells
- SARS-CoV-2 does not infect Schwann cell progenitors easily, but may infect hair cells and otic progenitors
- No convincing data suggesting neural infection
- Models are not directly comparable to native inner ear tissue
- Viral entry genes are expressed widely throughout the body, but this does not indicate tropism (e.g. ACE2 expression in testes)
- SARS-CoV-2 infection in vitro cannot explain auditory vestibular dysfunction!

# communications medicine







ARTICLE



<https://doi.org/10.1038/s43856-021-00044-w>

OPEN

## Direct SARS-CoV-2 infection of the human inner ear may underlie COVID-19-associated audiovestibular dysfunction

Minjin Jeong<sup>1,2,12</sup>, Karen E. Ocwieja<sup>3,4,5,13</sup>, Dongjun Han<sup>1,2,12,13</sup>, P. Ashley Wackym <sup>6</sup>, Yichen Zhang <sup>7</sup>, Alyssa Brown <sup>2</sup>, Cynthia Moncada<sup>2</sup>, Andrea Vambutas <sup>8</sup>, Theodore Kanne<sup>9</sup>, Rachel Crain<sup>10</sup>, Noah Siegel<sup>1,2</sup>, Valerie Leger<sup>5</sup>, Felipe Santos<sup>1,2</sup>, D. Bradley Welling <sup>1,2</sup>, Lee Gehrke<sup>5,7,11,14</sup>  & Konstantina M. Stankovic<sup>1,2,12,14</sup> 