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ARTICLE

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

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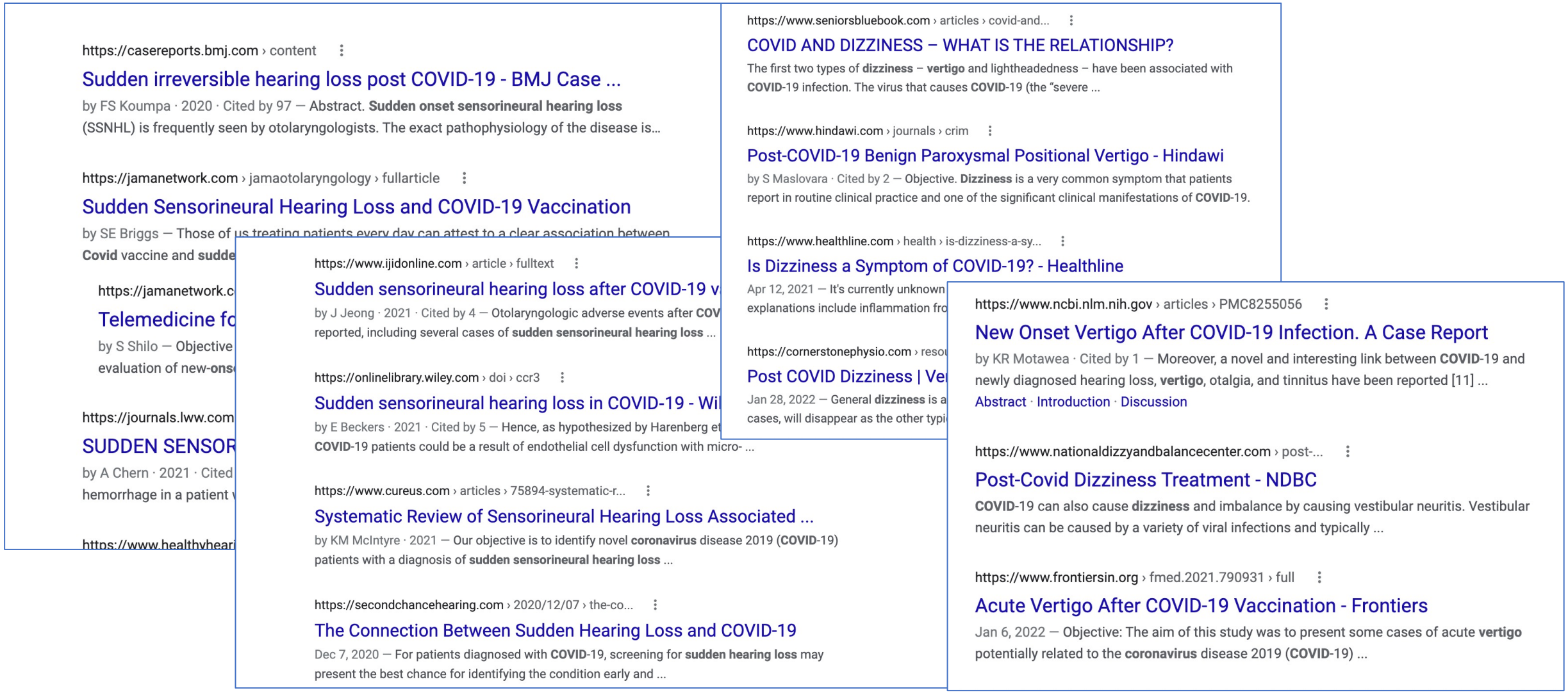
Judith Kempfle

02/09/22

Is COVID-19 infection causing audiovestibular dysfunction?



Potential access routes to the inner ear

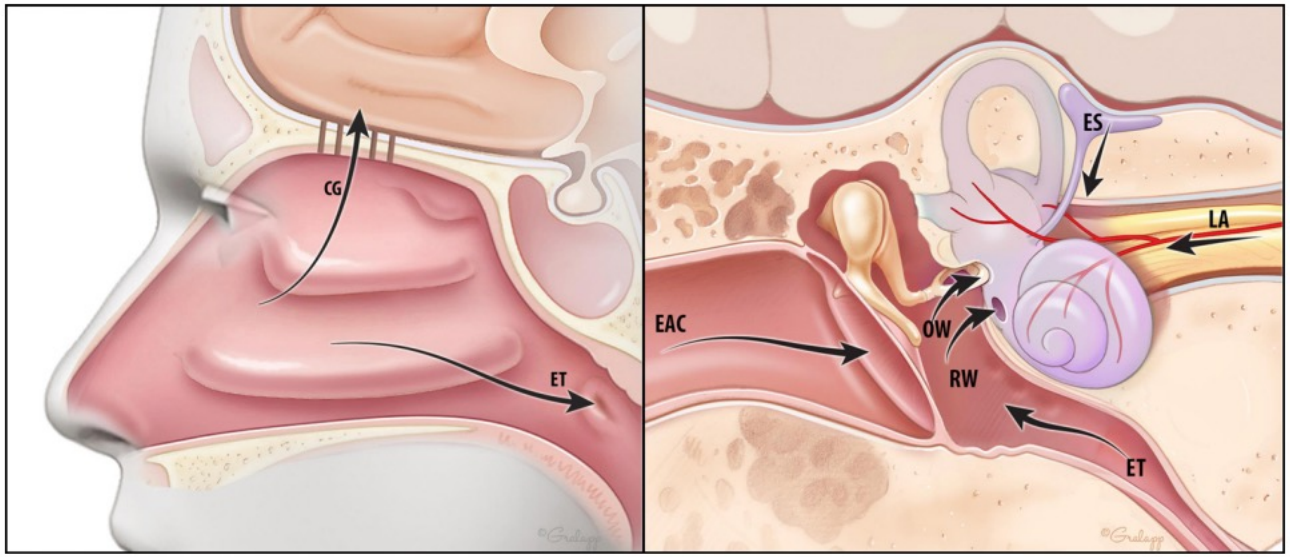


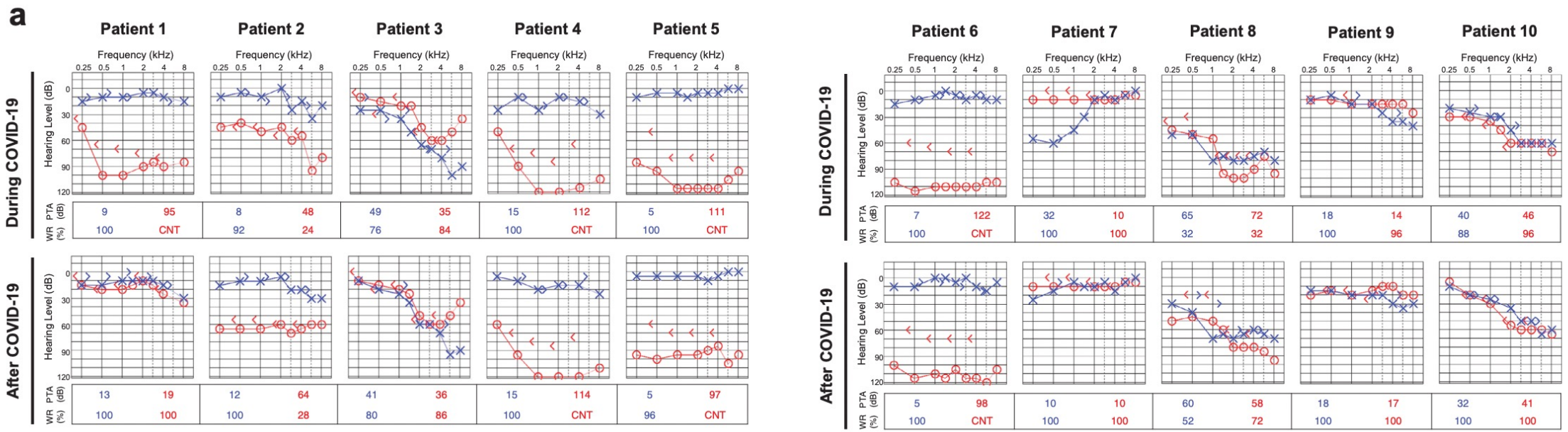
Fig. 7 Potential paths for SARS-CoV-2 entry into the inner ear. Arrows indicate potential paths via the nose and olfactory foramina (OF) into the central nervous system; via the endolymphatic sac (ES); via labyrinthine artery (LA) to ultimately reach stria vascularis; via round window (RW) and oval window (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

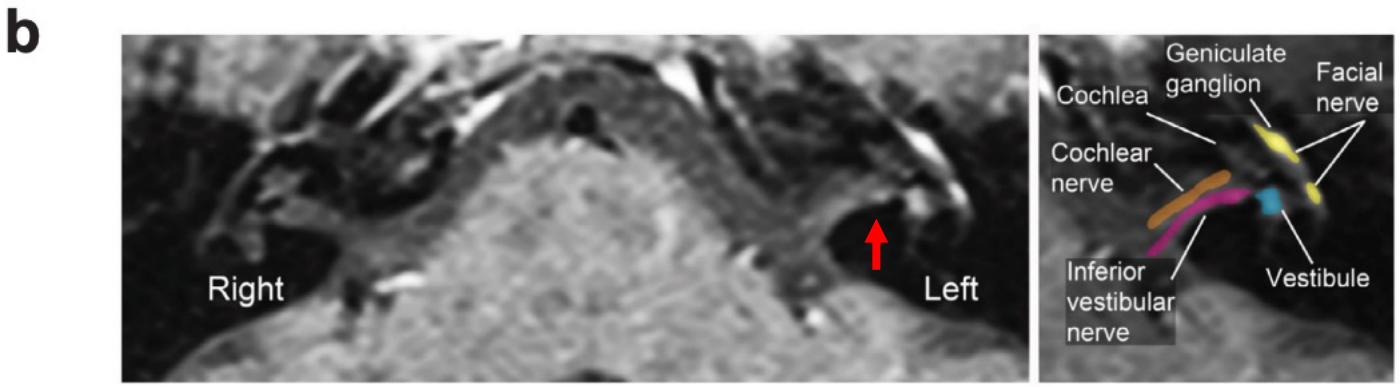
Otoacoustic emissions to assess inner ear hair cell function

Table 1 Otoacoustic emission test results reflecting cochlear outer hair cell function.

Patient	Hearing loss	Otoacoustic emission test results by frequency										
		Ear	2 kHz	2.5 kHz	3 kHz	3.5 kHz	4 kHz	5 kHz	6 kHz	7 kHz	7.5 kHz	8 kHz
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	Absent	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

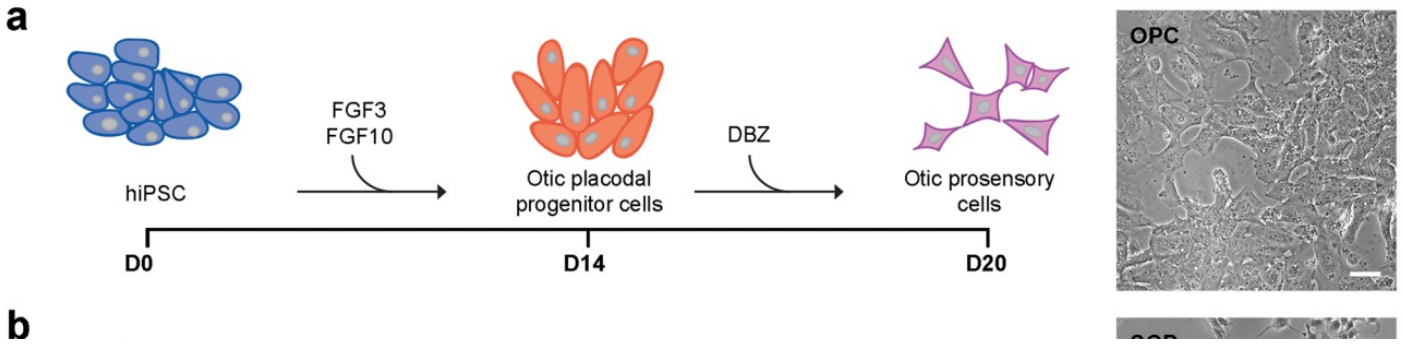
- Pluripotent, can be differentiated into many different cell types
- Can be propagated in culture for a long time – nearly unlimited supply of cells
- Can be created from patients with a specific disease to be used as a model to understand the mechanisms of diseases.

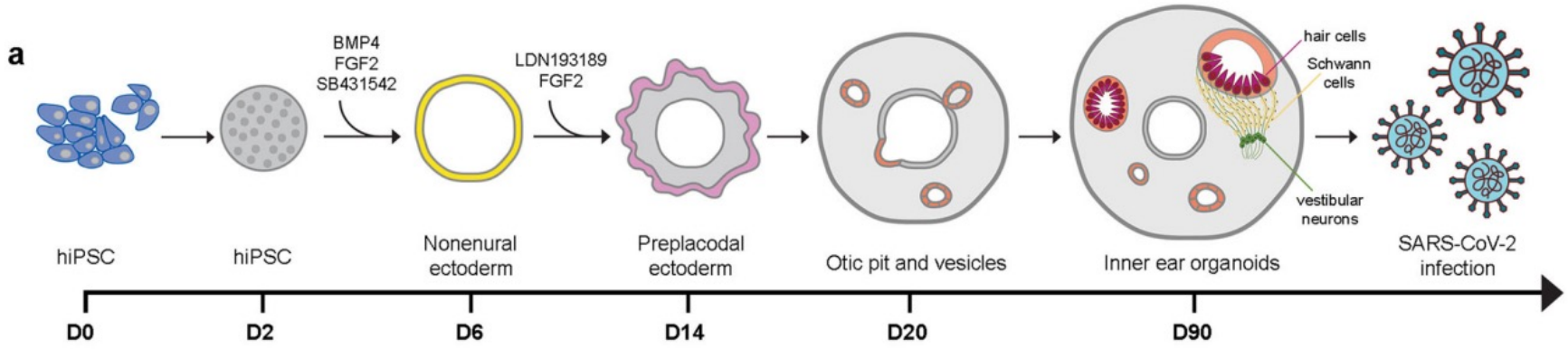
Disadvantages

- 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)



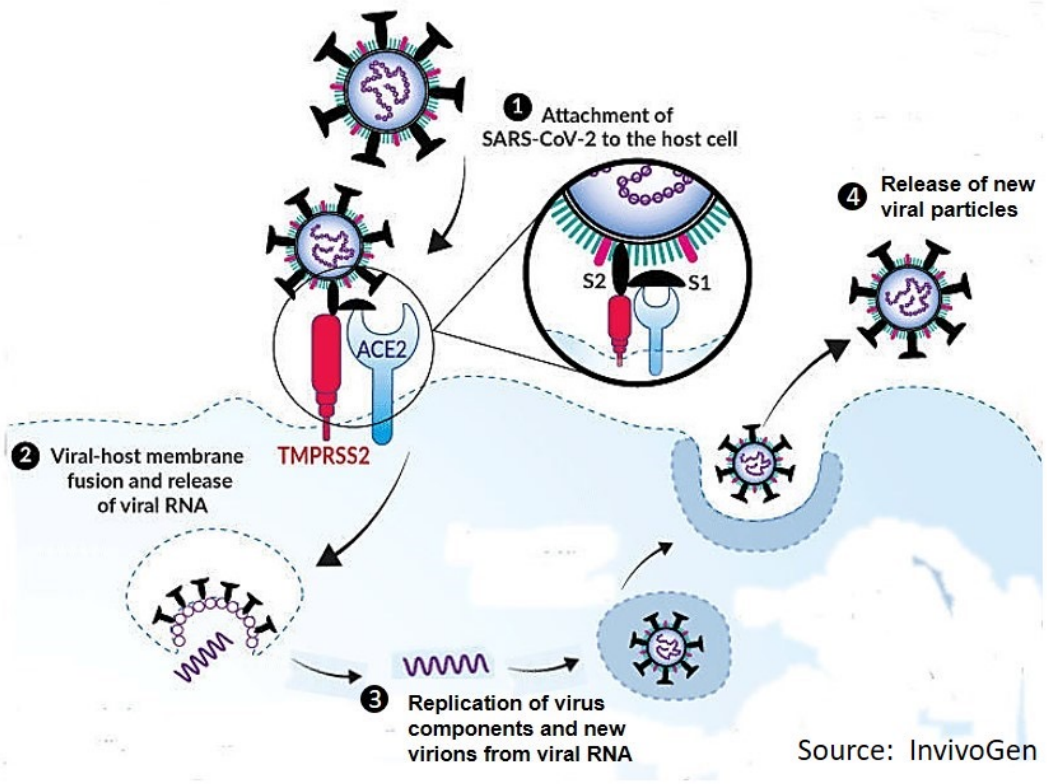


EXPRESSION ANALYSIS

- 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?

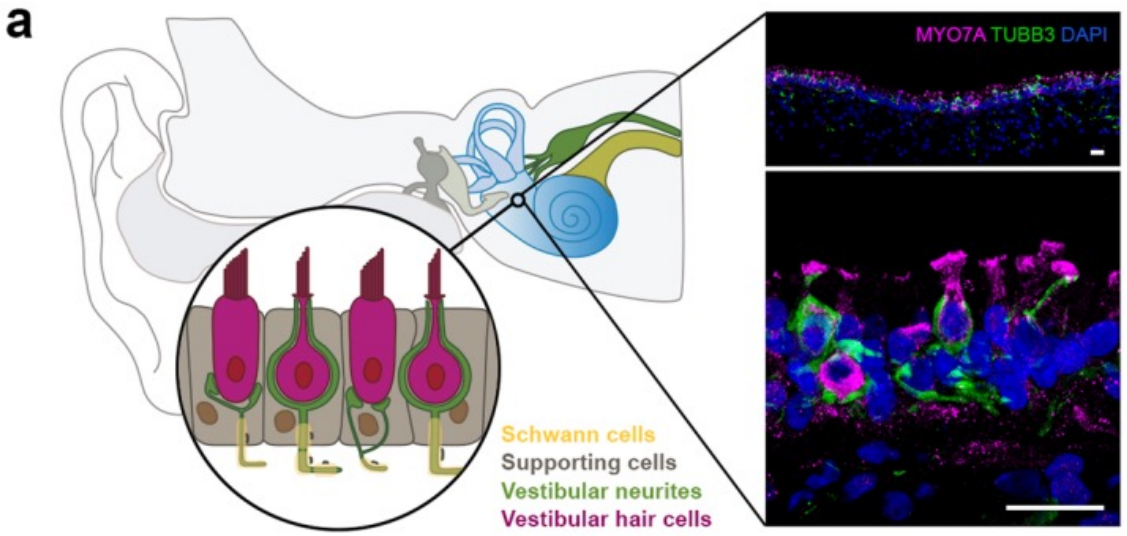


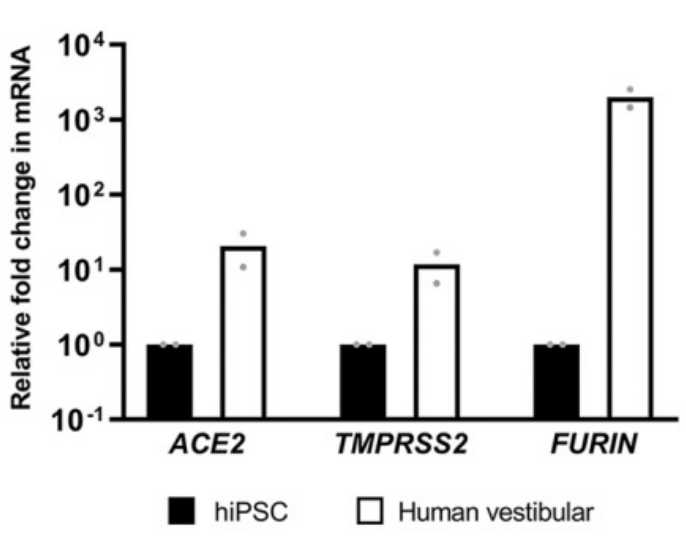
- Angiotensin-converting enzyme 2 (ACE2) – Entry receptor for SARS-CoV-2 spike protein – widely expressed in multiple tissues

- 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

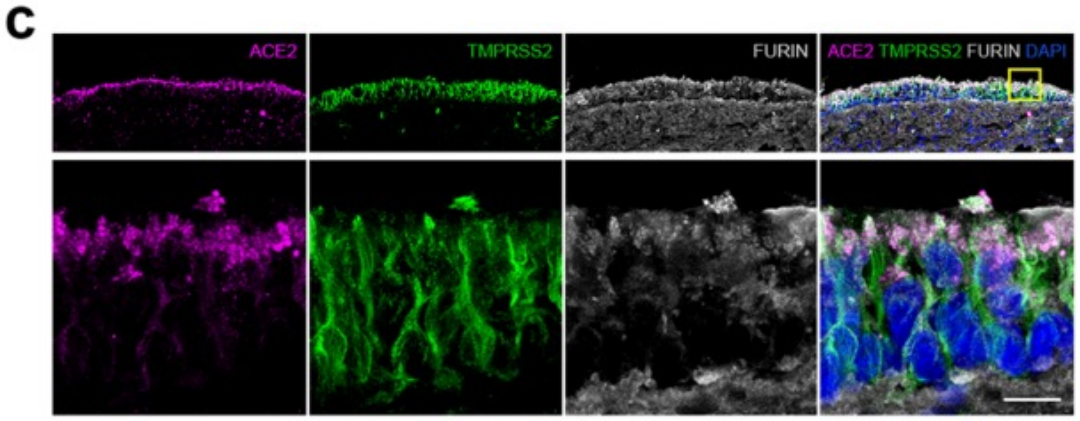


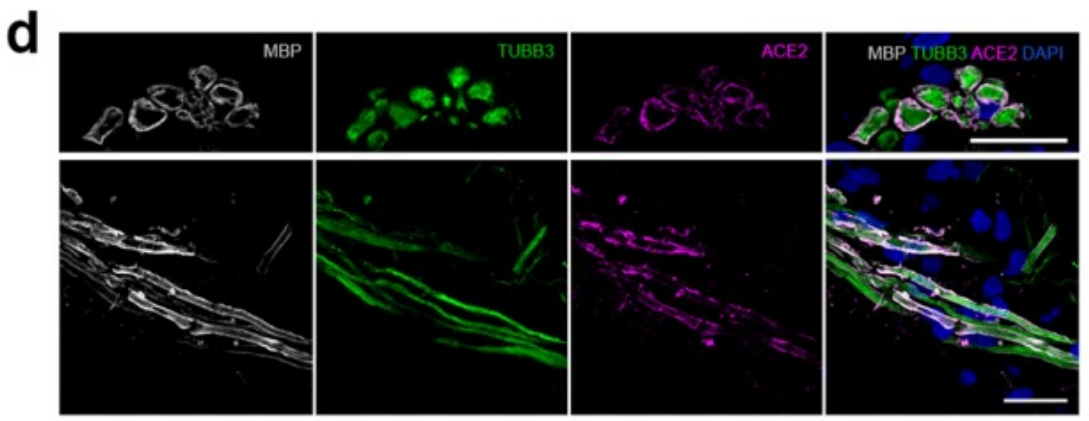


qPCR just demonstrates PRESENCE of ACE2, TMPRSS2 and FURIN on mRNA level in human vestibular tissue and to a very low degree in human iPSCs

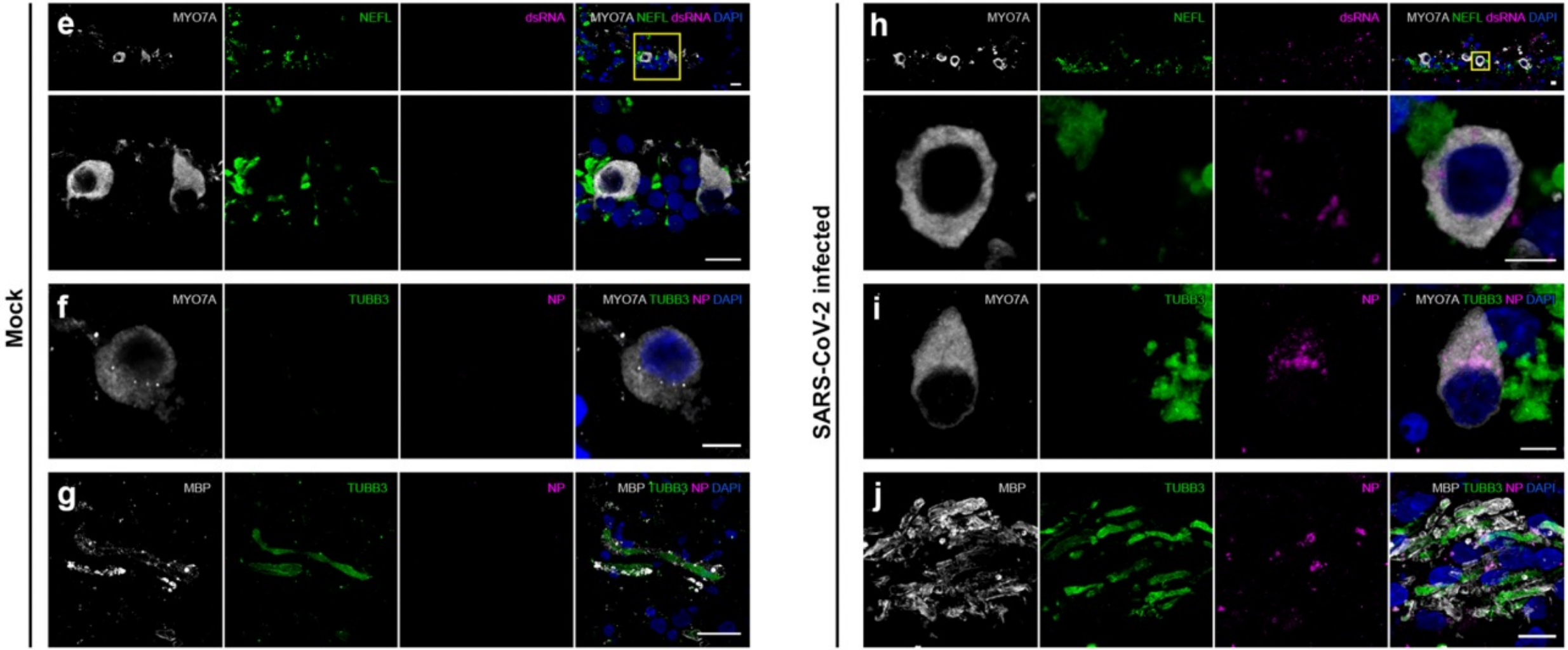
Immunohistochemistry (IHC) of human vestibular tissue demonstrates presence of virus related entry genes

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

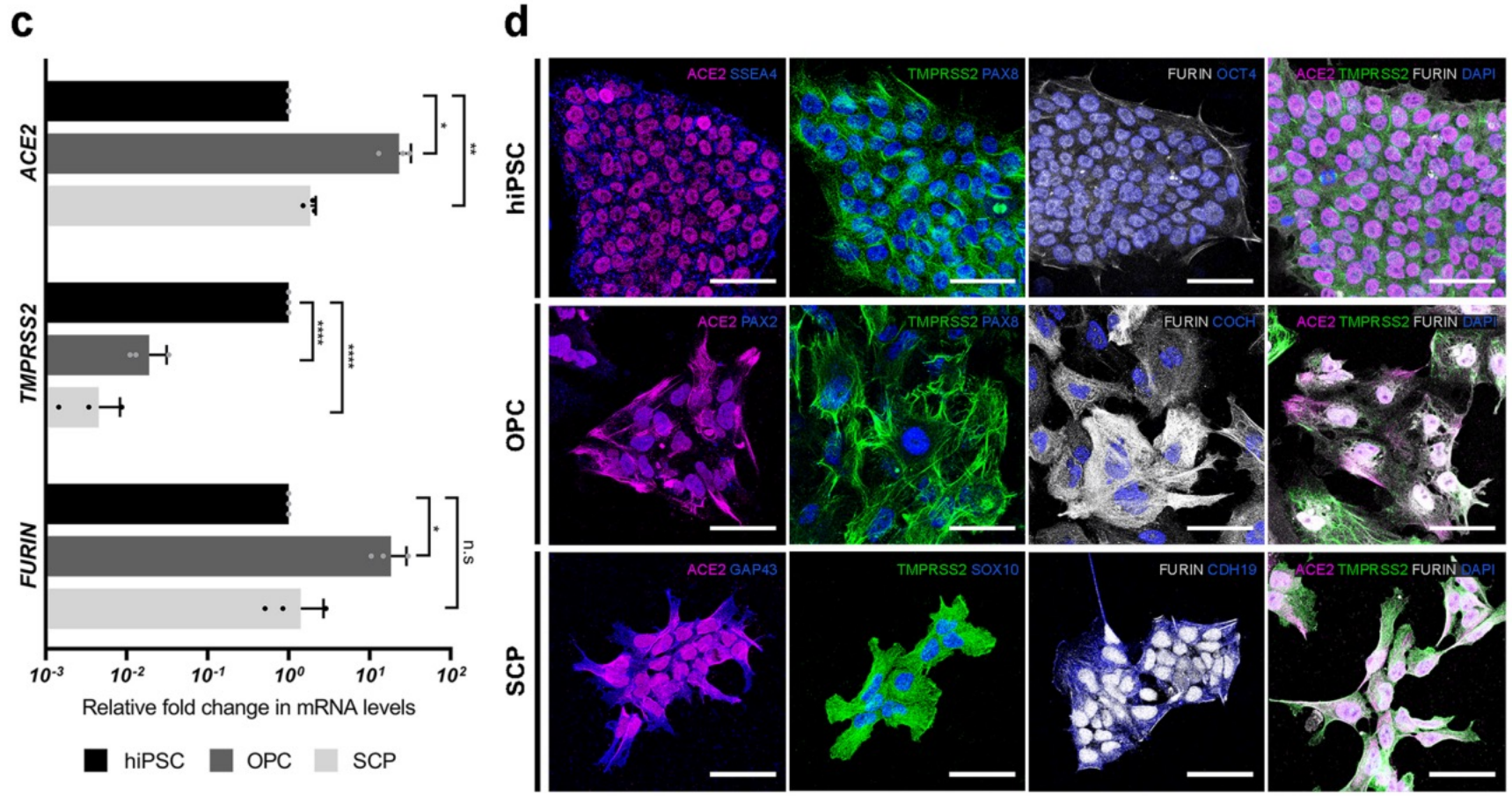




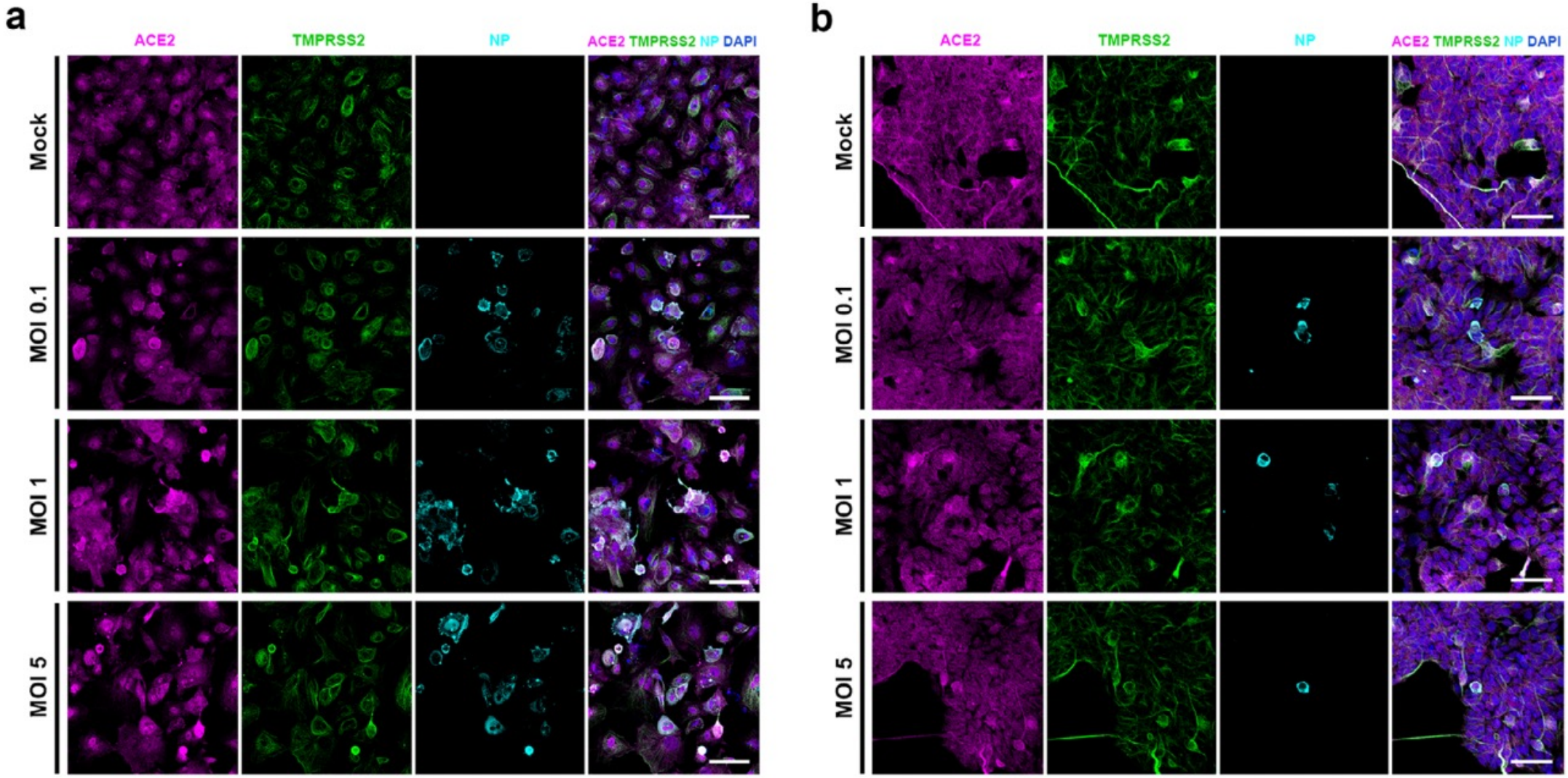
IHC demonstrates virus nucleoprotein (NP) and double-stranded RNA (dsRNA)in hair cells



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

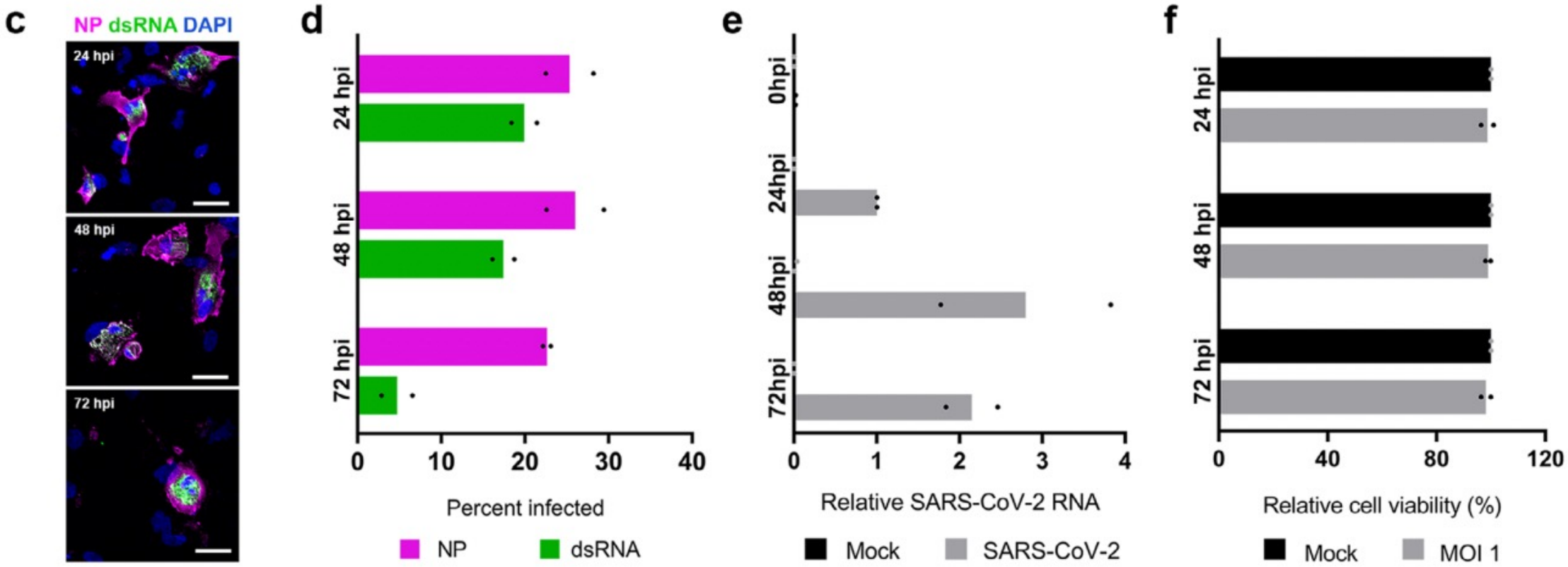


OPC and SCP infection with SARS-CoV-2: SCP infection rate is < 1%

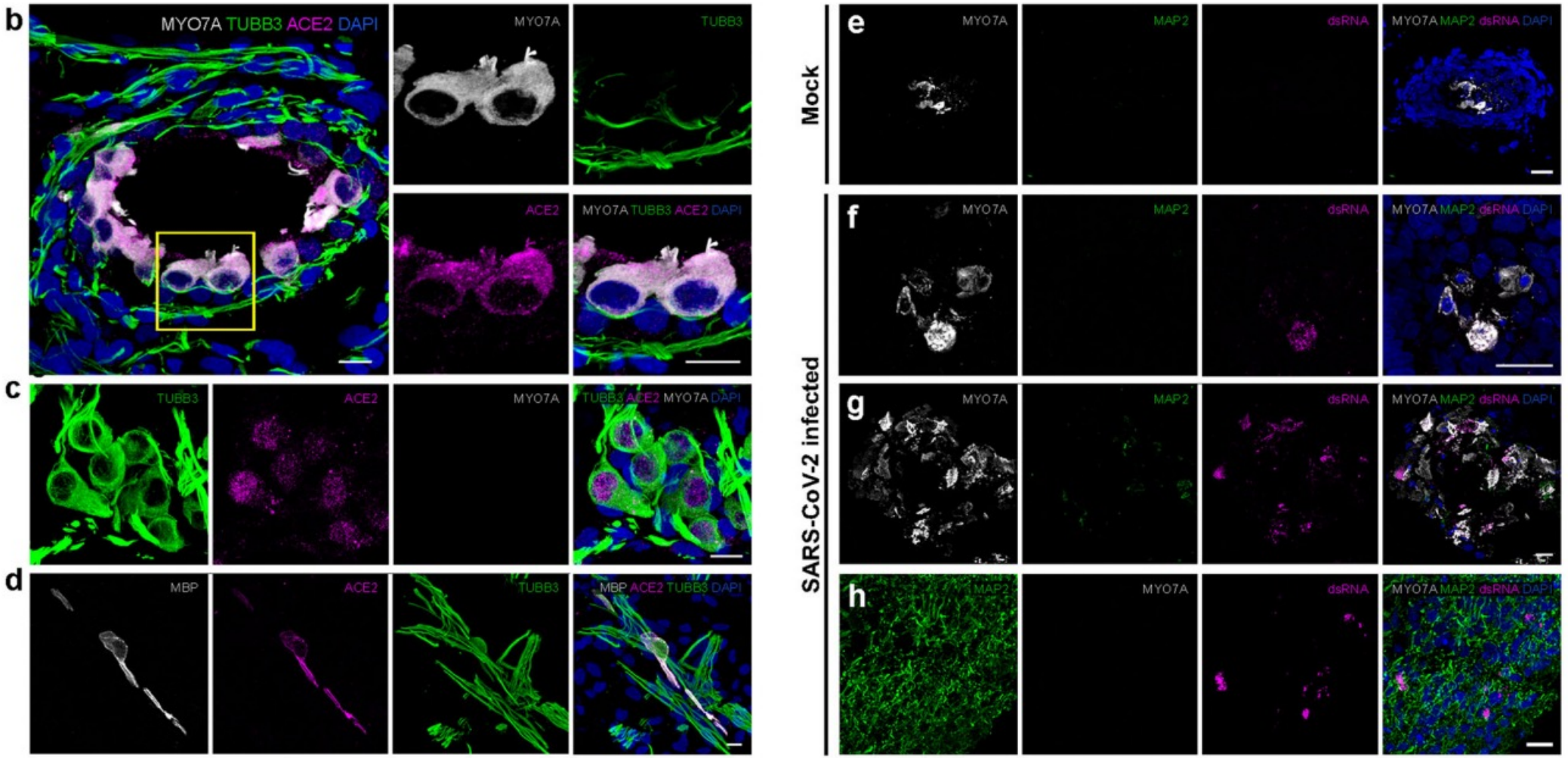


Multiplicity of infection or MOI represents **the ratio of the numbers of virus particles to the numbers of the host cells** in a given infection medium. A value of MOI = 1 implies that on an average there is a single host cell for a single phage particle.

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!

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