

Conclusions

1) Ai14 has leaky tdTom expression in medial cochlea (seen consistently in all injected *and uninjected* Ai14 ears; *not seen* in wild-type controls)

Unfortunately, this where we expect LNP-induced cells

2) 1/12 surgery animal (#147) might have induced medial cells

Different cell type, more apical than leaky cells, higher R26 expression

Not ideal but quantification perhaps doable if effective LNP/delivery was achieved in the future

Of note: #147 is first id number= first injected animal? may suggest LNP unstable or not stored properly during procedure?

Experiment outline

- 1) LNP injected in 12 Ai14 animals (left inner ear; #147-152; #155-160)
(Surgery: Lutz team)
- 2) Uninjected: 2 Ai14 animals (#161-162)
- 3) Added: 2 C57BL/6J control animals (#14443-14444; 6 week-old)

5 day chase; temporal bones fixed 1h PFA (4C) (Murray team)
right cochleae serve as control

(Dissection, immunolabeling, mounting and analysis: Tarchini team)

2 x 12

2 x 2

2 x 2 >> 32 cochleae for immunolabeling (MYO7A; SOX2; Hoechst)

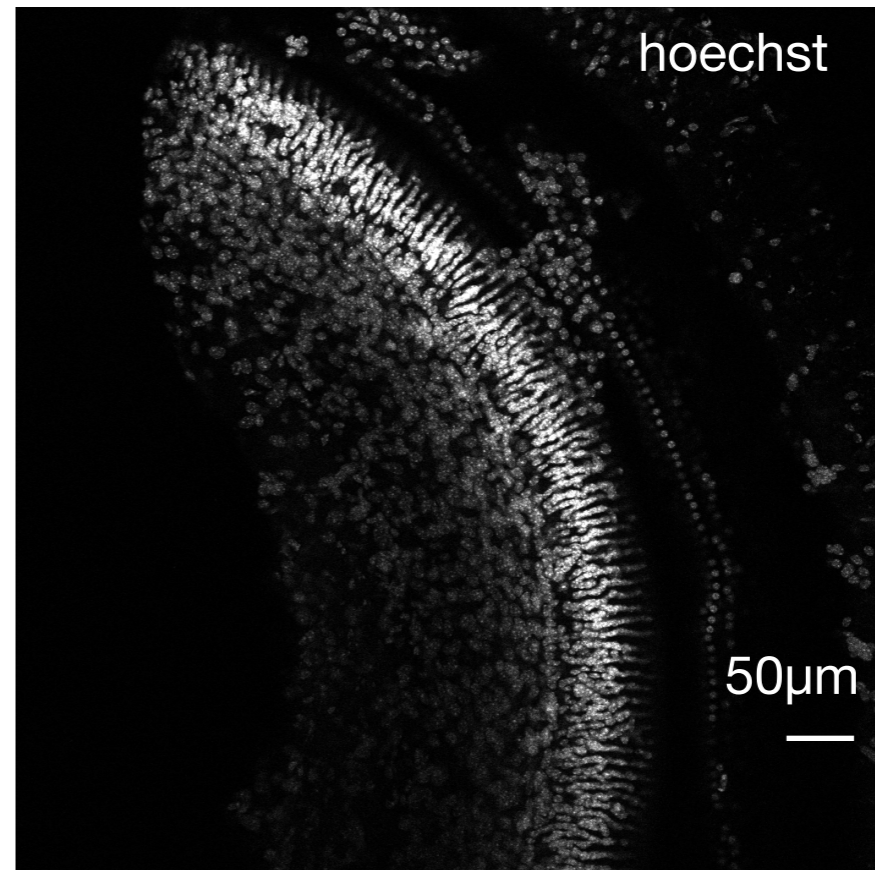
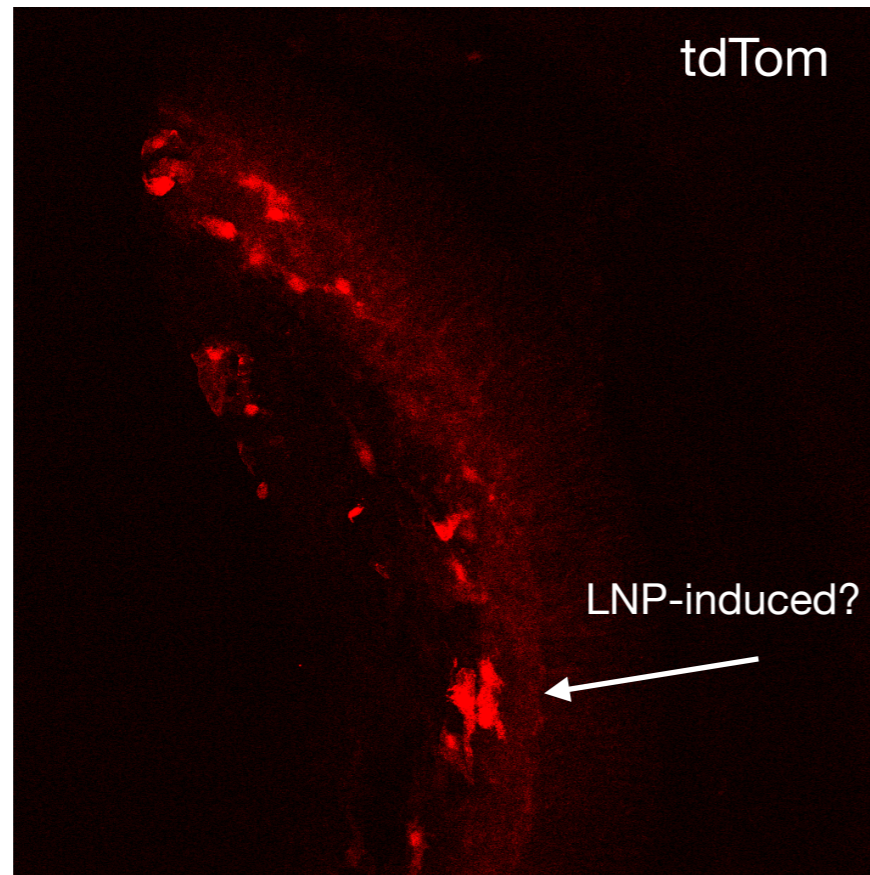
Each cochlea is mounted in 3 pieces (base, mid, apex) >> 96 samples

EXPERIMENTAL

cochlear BASE; chosen single Z slice

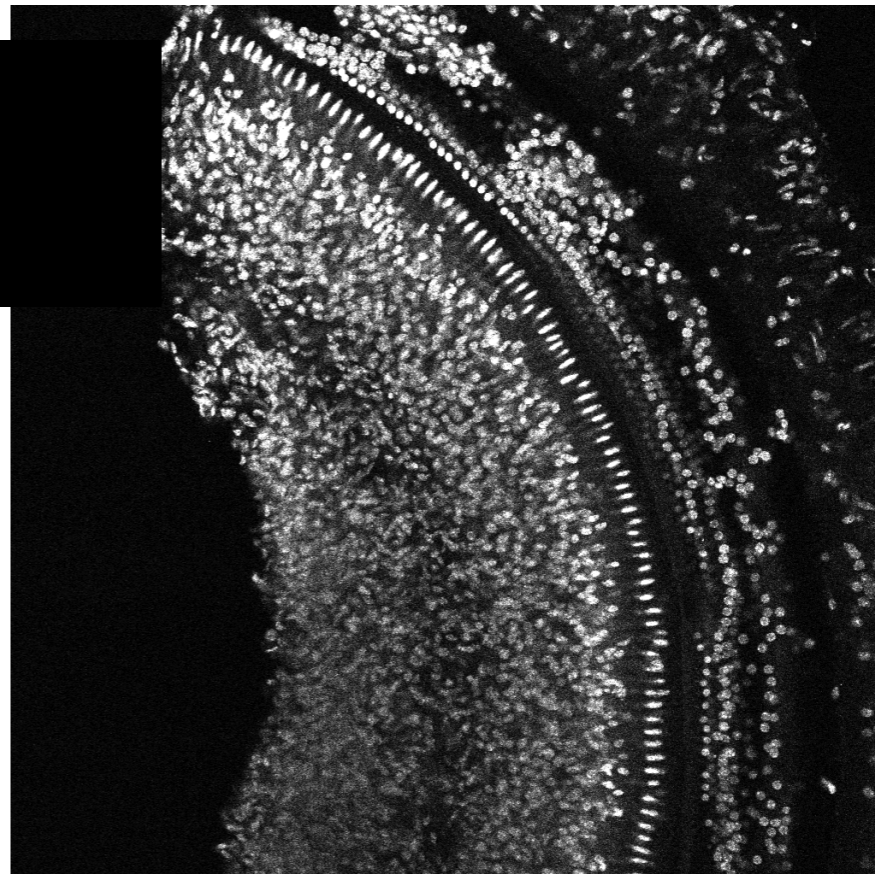
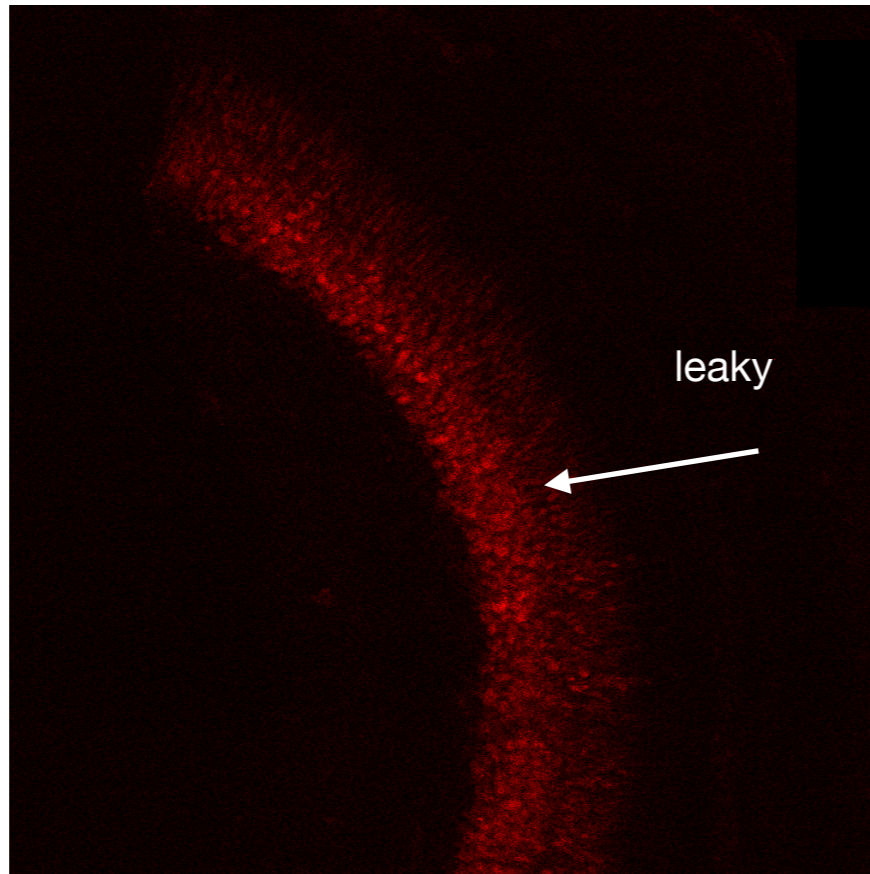
Sample 147
left inner ear
injected

apical Z



Sample 147
left inner ear
injected

basal Z (-10 µm)

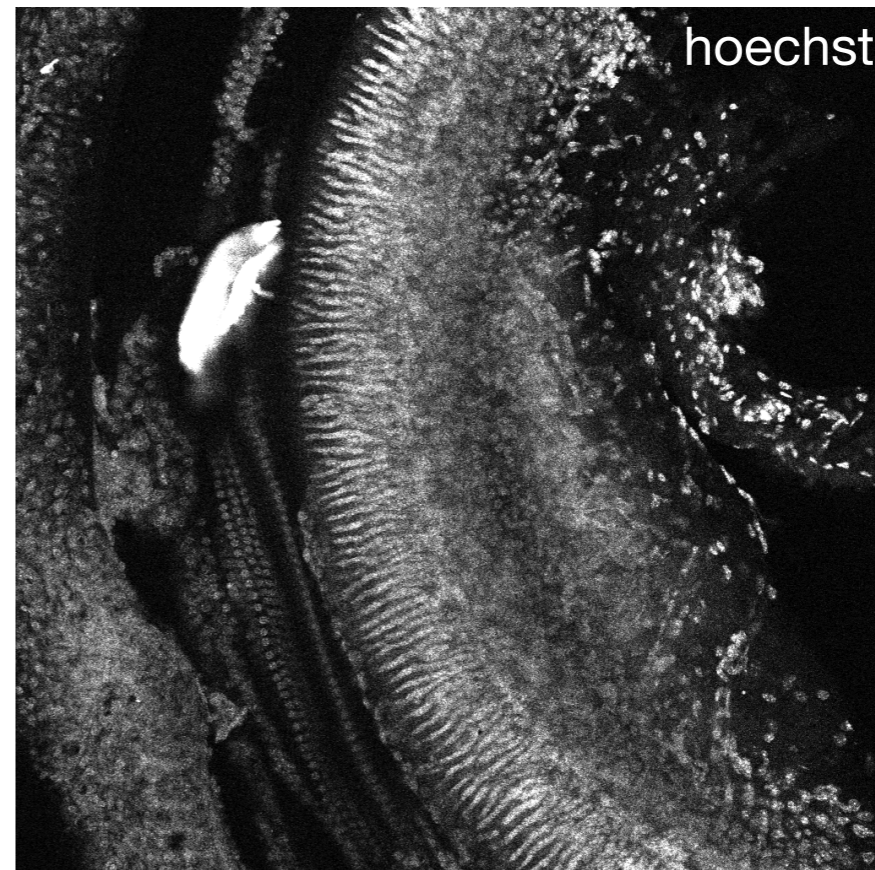
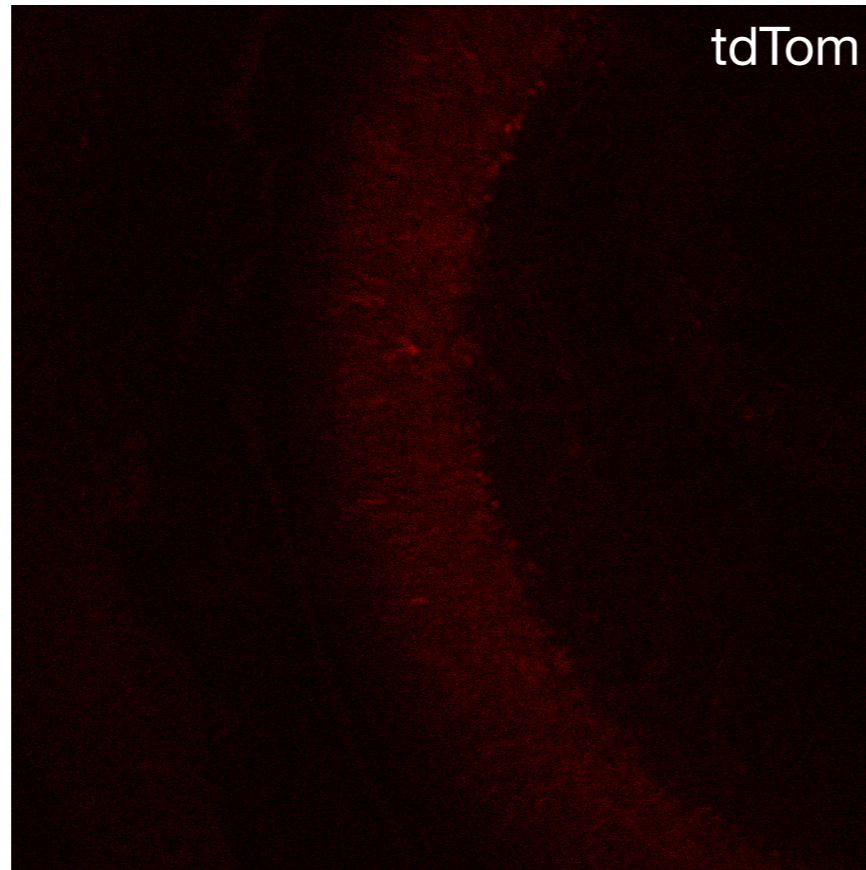


CONTROL

cochlear BASE; chosen single Z slice

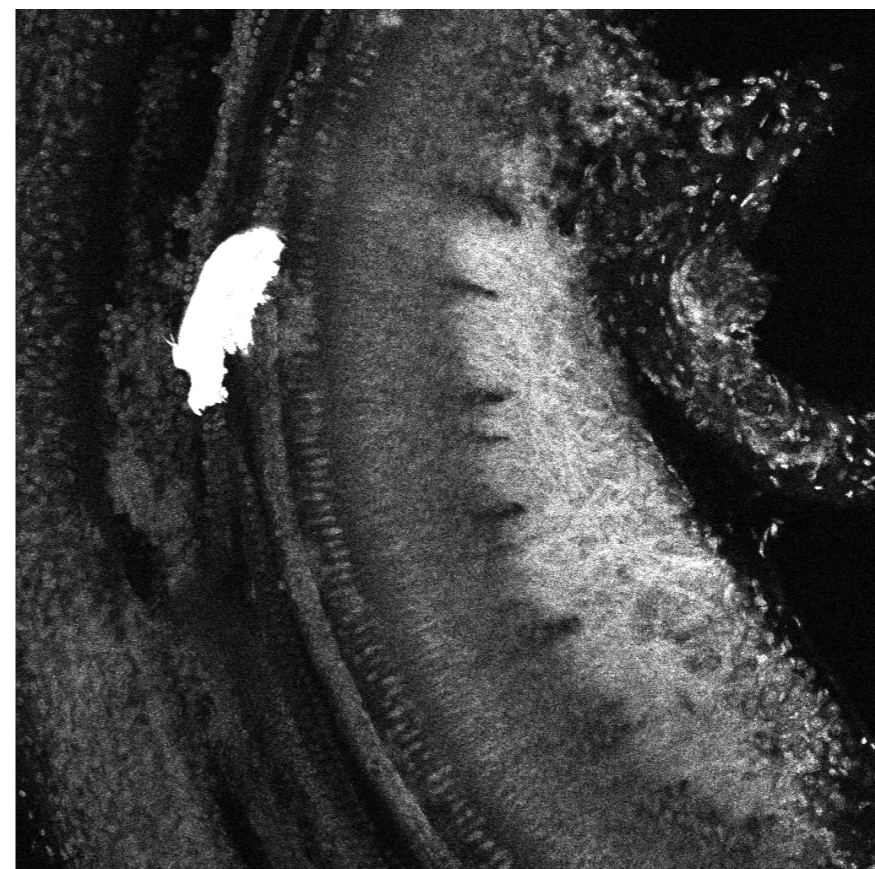
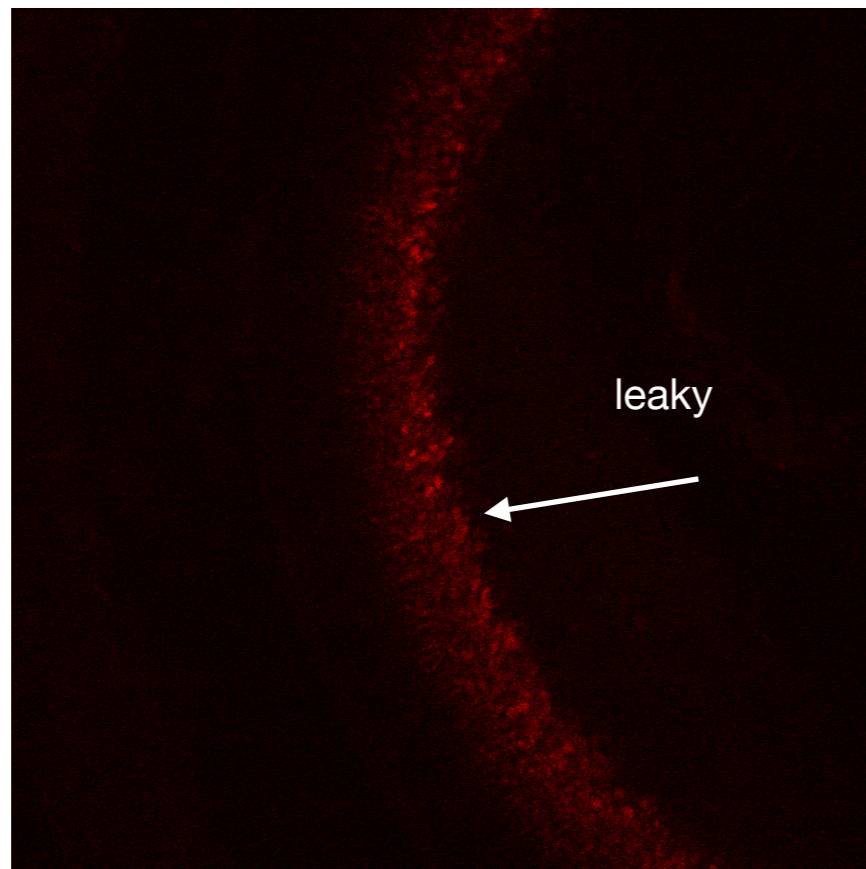
Sample 147
right inner ear
control
(not injected)

apical Z



Sample 147
right inner ear
control
(not injected)

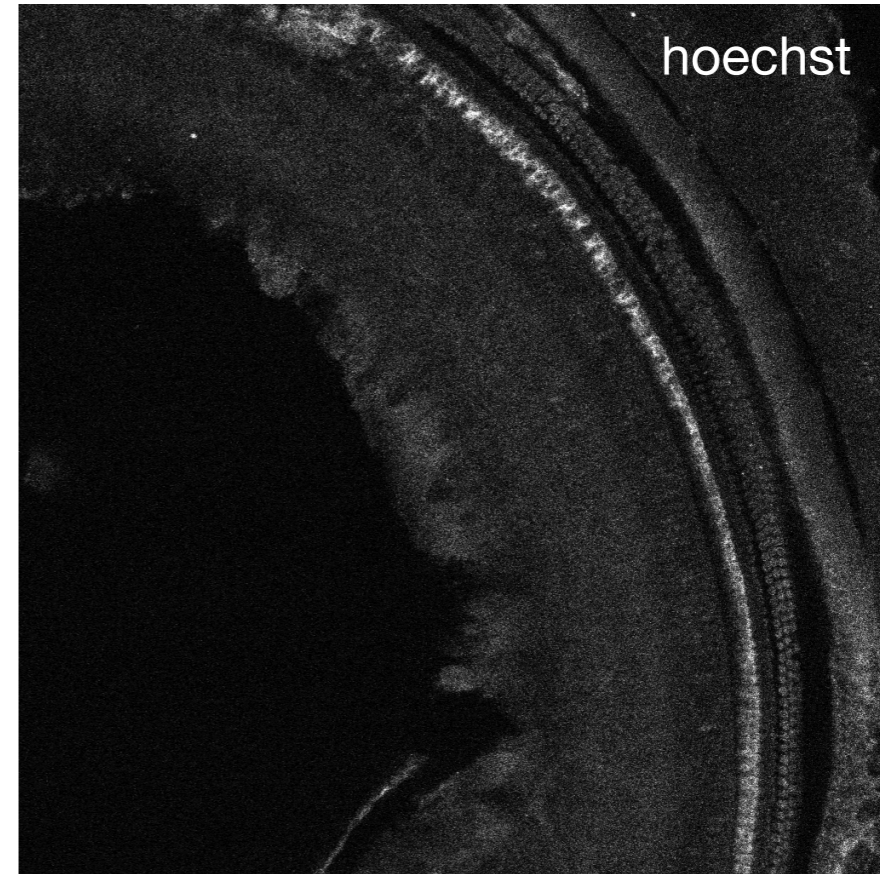
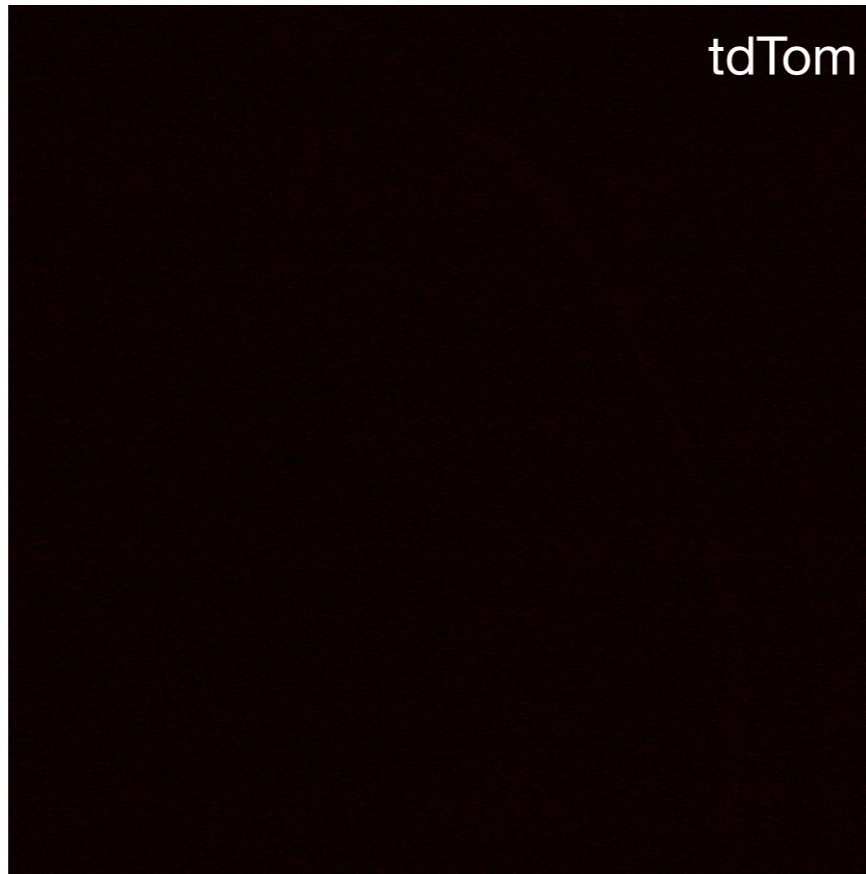
basal Z (-10 μm)



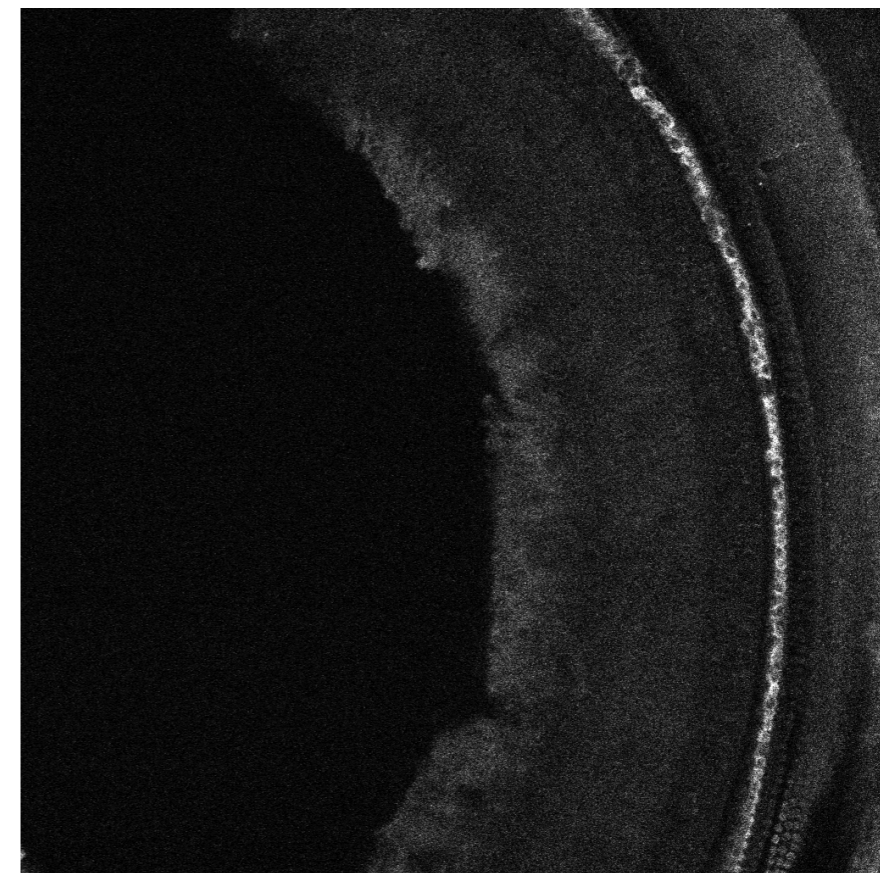
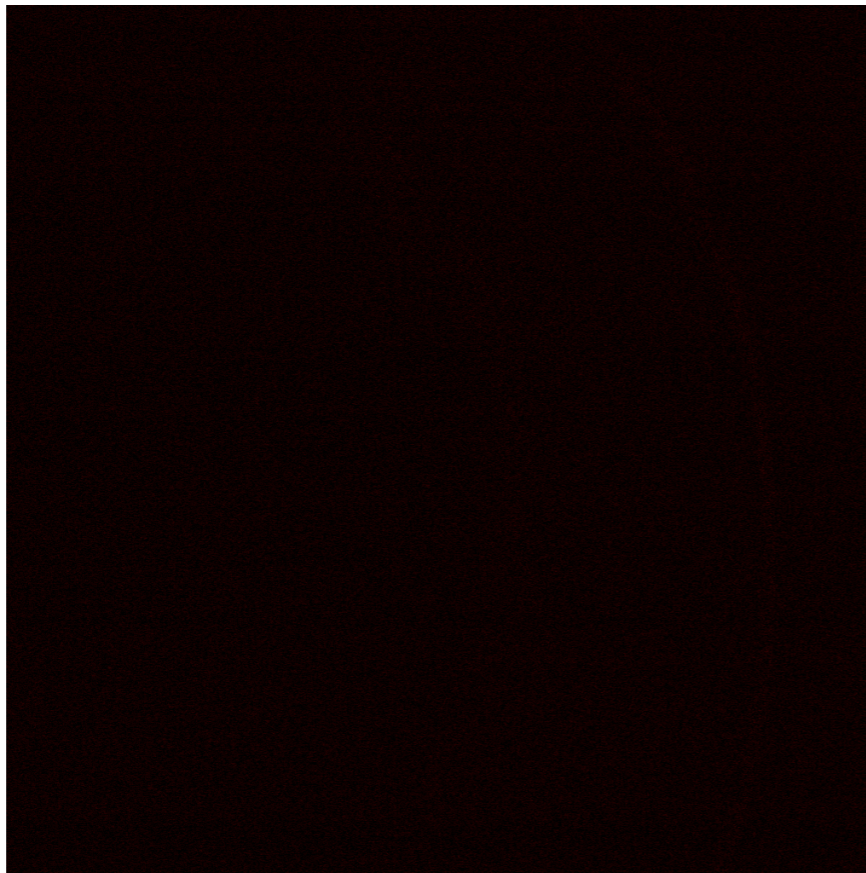
CONTROL

cochlear BASE; chosen single Z slice

Sample 14444
left inner ear
unrelated C57BL/6J
control
(non injected)



Sample 14443
left inner ear
unrelated C57BL/6J
control
(non injected)

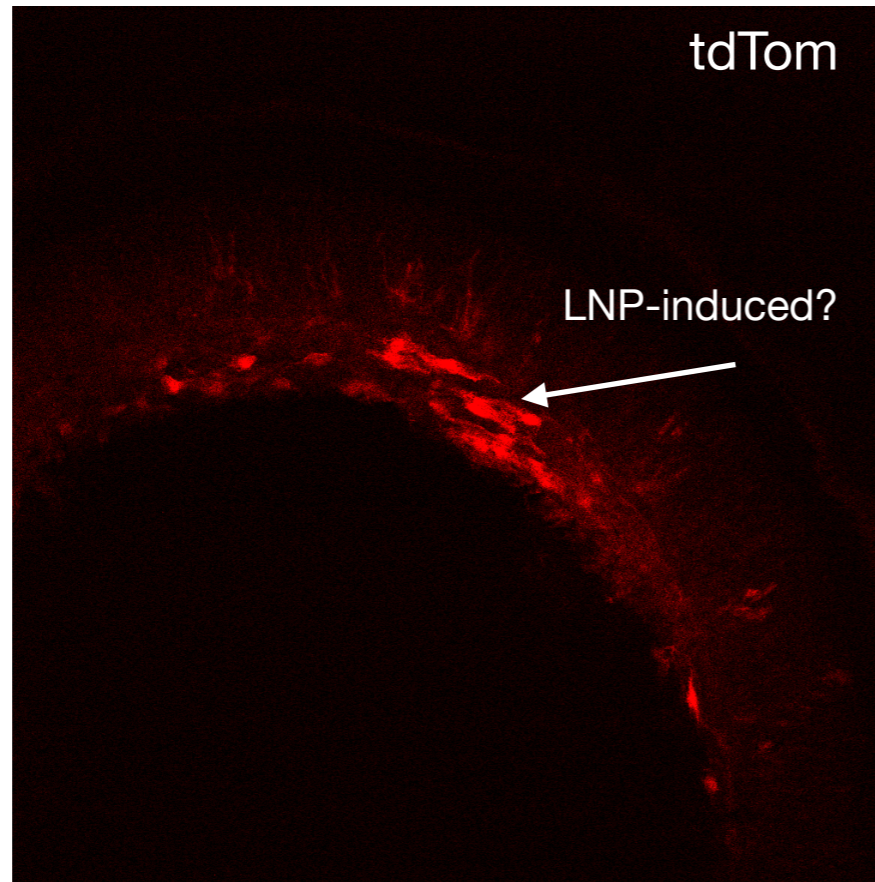


EXPERIMENTAL

cochlear MID chosen single Z slice

Sample 147
left inner ear
injected

apical Z



Sample 147
left inner ear
injected

basal Z (-10 μ m)

