## **Supplemental Results**

## Review of novel SV genes

*Abcg1* is a lipid transporter that appears to be involved in regulation of cholesterol homeostasis at the blood-brain barrier and mutations in Abcg1 have been implicated in impaired monocyte cholesterol clearance in age-related macular degeneration resulting in vision loss (Ban et al., 2018; Kober et al., 2017; Tarling, 2013). Prior to this study, Abca1 expression had not been demonstrated in the cochlea. Malgrange and colleagues have suggested the possibility that cholesterol homeostasis plays a role in the development of SNHL and that genes involved may offer the possibility of therapeutic approaches to treat or prevent SNHL (Malgrange, Varela-Nieto, de Medina, & Paillasse, 2015). Heyl is a downstream effector of the Notch signaling pathway and has been shown to be expressed in the developing organ of Corti, appearing to be responsible for maintaining the fate of cochlear supporting cells (Doetzlhofer et al., 2009; McGovern, Zhou, Randle, & Cox, 2018). However, prior to this study, Heyl expression had not been demonstrated in the adult cochlea, although presumably it plays a role maintaining cell fate. In addition to its role in axon guidance, Nrp2 has been recently demonstrated to be expressed in the cuboidal cells of the avian tegmentum vasculosum, the avian equivalent to the mammalian SV (Coate, Spita, Zhang, Isgrig, & Kelley, 2015; Scott, Yue, Biesemeier, Lee, & Fekete, 2019). A conditional knockout mouse of an Nrp2 paralog, Nrp1, has been associated with enlarged microvessels of the SV and Nrp1 is expressed in the developing SV at postnatal day 5 (Salehi et al., 2017). Kcnj13, encodes a potassium inwardly-rectifying channel known as Kir7.1, has been identified as being expressed by adult cochlear hair cell and supporting cell transcriptome profiles but has not been demonstrated to be expressed in the adult SV (Liu et al., 2018, 2014). Sox8 is a transcription factor involved in initial signaling and maintenance of Sox10 expression in the otic placode during inner ear development (Betancur, Sauka-Spengler, & Bronner, 2011). While Sox8 has been shown to be expressed in the developing chicken otocyst in the region of the tegmentum vasculosum (Sinkkonen et al., 2011), the equivalent to the mammalian SV, it has not been previously demonstrated in the mammalian stria vascularis, much less the adult SV. Nr2f2 is an orphan steroid/thyroid hormone nuclear receptor with an essential role in angiogenesis during development and known expression in embryonic and adult mouse cochlear and vestibular sensory epithelia (Tornari, Towers, Gale, & Dawson, 2014). Expression of Nr2f2 has not been previously demonstrated in the SV. Kcnj16, encodes a potassium inwardly-rectifyng channel known as Kir5.1, has been identified as being expressed by adult cochlear hair cell and supporting cell transcriptome profiles but has not been demonstrated to be expressed in the adult SV (Liu et al., 2018, 2014). P2rx2, encodes the P2X2 ATP-sensitive non-specific cation channel which is known to be expressed in outer sulcus cells but previously described as being absent in the SV (Järlebark, Housley, Raybould, Vlajkovic, & Thorne, 2002; Järlebark, Housley, & Thorne, 2000). We demonstrate *P2rx2* transcript expression in SV spindle cells (Figure 3E, 3F). Finally, Atp13a5 is a P5 ATPase that has been demonstrated to be highly expressed in the adult mouse brain (Schultheis et al., 2004; Weingarten, Dave, Li, & Crawford, 2012). *Atp13a5* has been shown to be expressed in transcriptome profiles

from whole mouse cochlea from early postnatal mice but has not been previously localized within the SV (Son et al., 2012).

## **Bibliography**

- Ban, N., Lee, T. J., Sene, A., Choudhary, M., Lekwuwa, M., Dong, Z., ... Apte, R. S. (2018). Impaired monocyte cholesterol clearance initiates age-related retinal degeneration and vision loss. *JCI Insight*. https://doi.org/10.1172/jci.insight.120824
- Betancur, P., Sauka-Spengler, T., & Bronner, M. (2011). A sox10 enhancer element common to the otic placode and neural crest is activated by tissue-specific paralogs. *Development*. https://doi.org/10.1242/dev.057836
- Coate, T. M., Spita, N. A., Zhang, K. D., Isgrig, K. T., & Kelley, M. W. (2015). Neuropilin-2/semaphorin-3F-mediated repulsion promotes inner hair cell innervation by spiral ganglion neurons. *ELife*. https://doi.org/10.7554/eLife.07830
- Doetzlhofer, A., Basch, M. L., Ohyama, T., Gessler, M., Groves, A. K., & Segil, N. (2009). Hey2 Regulation by FGF Provides a Notch-Independent Mechanism for Maintaining Pillar Cell Fate in the Organ of Corti. *Developmental Cell*. https://doi.org/10.1016/j.devcel.2008.11.008
- Järlebark, L. E., Housley, G. D., Raybould, N. P., Vlajkovic, S., & Thorne, P. R. (2002). ATP-gated ion channels assembled from P2X2receptor subunits in the mouse cochlea. *NeuroReport*.
- Järlebark, L. E., Housley, G. D., & Thorne, P. R. (2000). Immunohistochemical localization of adenosine 5'-triphosphate-gated ion I channel P2X2receptor subunits in adult and developing rat cochlea. *Journal of Comparative Neurology*. https://doi.org/10.1002/(SICI)1096-9861(20000605)421:3<289::AID-CNE1>3.0.CO;2-0
- Kober, A. C., Manavalan, A. P. C., Tam-Amersdorfer, C., Holmér, A., Saeed, A., Fanaee-Danesh, E., ... Panzenboeck, U. (2017). Implications of cerebrovascular ATP-binding cassette transporter G1 (ABCG1) and apolipoprotein M in cholesterol transport at the blood-brain barrier. *Biochimica et Biophysica Acta Molecular and Cell Biology of Lipids*. https://doi.org/10.1016/j.bbalip.2017.03.003
- Liu, H., Chen, L., Giffen, K. P., Stringham, S. T., Li, Y., Judge, P. D., ... He, D. Z. Z. (2018). Cell-Specific Transcriptome Analysis Shows That Adult Pillar and Deiters' Cells Express Genes Encoding Machinery for Specializations of Cochlear Hair Cells. *Frontiers in Molecular Neuroscience*. https://doi.org/10.3389/fnmol.2018.00356
- Liu, H., Pecka, J. L., Zhang, Q., Soukup, G. A., Beisel, K. W., & He, D. Z. Z. (2014). Characterization of transcriptomes of cochlear inner and outer hair cells. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.1690-14.2014
- Malgrange, B., Varela-Nieto, I., de Medina, P., & Paillasse, M. R. (2015). Targeting cholesterol homeostasis to fight hearing loss: A new perspective. *Frontiers in Aging Neuroscience*. https://doi.org/10.3389/fnagi.2015.00003
- McGovern, M. M., Zhou, L., Randle, M. R., & Cox, B. C. (2018). Spontaneous Hair Cell Regeneration Is Prevented by Increased Notch Signaling in Supporting Cells. *Frontiers in Cellular Neuroscience*. https://doi.org/10.3389/fncel.2018.00120
- Salehi, P., Ge, M. X., Gundimeda, U., Michelle Baum, L., Lael Cantu, H., Lavinsky, J., ... Friedman, R. A. (2017). Role of Neuropilin-1/Semaphorin-3A signaling in the functional and morphological integrity of the cochlea. *PLoS Genetics*.

- https://doi.org/10.1371/journal.pgen.1007048
- Schultheis, P. J., Hagen, T. T., O'Toole, K. K., Tachibana, A., Burke, C. R., McGill, D. L., ... Shull, G. E. (2004). Characterization of the P 5 subfamily of P-type transport ATPases in mice. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2004.08.156
- Scott, M. K., Yue, J., Biesemeier, D. J., Lee, J. W., & Fekete, D. M. (2019). Expression of class III Semaphorins and their receptors in the developing chicken (Gallus gallus) inner ear. *Journal of Comparative Neurology*. https://doi.org/10.1002/cne.24595
- Sinkkonen, S. T., Starlinger, V., Galaiya, D. J., Laske, R. D., Myllykangas, S., Oshima, K., & Heller, S. (2011). Serial analysis of gene expression in the chicken otocyst. *JARO Journal of the Association for Research in Otolaryngology*. https://doi.org/10.1007/s10162-011-0286-z
- Son, E. J., Wu, L., Yoon, H., Kim, S., Choi, J. Y., & Bok, J. (2012). Developmental gene expression profiling along the tonotopic axis of the mouse cochlea. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0040735
- Tarling, E. (2013). Expanding roles of ABCG1 and sterol transport. *Current Opinion in Lipidology*. https://doi.org/10.1097/MOL.0b013e32835da122
- Tornari, C., Towers, E. R., Gale, J. E., & Dawson, S. J. (2014). Regulation of the orphan nuclear receptor Nr2f2 by the DFNA15 deafness gene Pou4f3. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0112247
- Weingarten, L. S., Dave, H., Li, H., & Crawford, D. A. (2012). Developmental expression of P5 ATPase mRNA in the mouse. *Cellular and Molecular Biology Letters*. https://doi.org/10.2478/s11658-011-0039-3