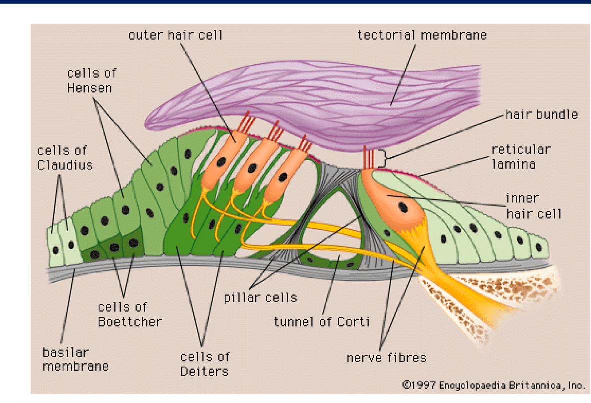
459. An Integrated Adeno-Associated Vector Development Platform for Inner Ear Disorders

Charlene Vaux, Sandra Pierredon, Guillaume Olivier, Christophe Tran Van Ba, Lise Barrot, Pierre Rambeau, Julie Duron Dos Reis, Anais Pages, Pauline Liaudet, Audrey Broussy, Anne Gabrielle Harrus, Selma Dadak, Arnaud Giese, Rafik Boudra, Patrice Vidal, Laurent Desire
Sensorion SA, Montpellier, France Presenting Author

Abstract

- Half of the congenital non-syndromic deafness cases have a genetic cause, and 80% are inherited in an autosomal recessive fashion;
- The inner ear is a closed bony labyrinth that contains a small and non-expandable volume with a diverse mosaic of highly specialized cells;
- The production of an AAV-based gene therapy product, especially for the inner ear, requires the development of novel tissue-targeted capsids and cell-specific expression cassettes aiming to reach a therapeutic effect using a minimal dose;
- Sensorion AAV platform allows fast and affordable screening of therapeutic candidates for inner ear indications using an optimized "in house" small scale AAV production protocol;
- AAV productions are injected in mouse inner ear through the round window membrane to assess transduction profiles;
- Gene therapy candidate vectors are assessed by Sensorion animal pharmacology platform which includes auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) recordings, supported by morphological and immunohistochemical examination as well as behavioral evaluation and early tolerability and biodistribution.
- Models for testing include genetic models, noise-induced hearing loss, synaptopathy, and vestibular disorders.



Locus (OMIM)	Gene (OMIM)	Expression profile
DFNB1A	*GJB2*	SC, Stria Vascularis
DFNB1B	*GJB6*	SC
DFNB2	*MYO7A*	IHC,OHC
DFNB3	*MYO15A*	IHC,OHC
DFNB4	*SLC26A4*	Endol. sac, cochlea, vestibule
DFNB6	*TMIE*	IHC,OHC
DFNB7/11	*TMC1*	IHC,OHC-stereocilia
DFNB8/10	*TMPRSS3*	IHC,OHC, SGN
DFNB9	*OTOF*	IHC-synaptic vesicles
DFNB12	*CDH23*	IHC,OHC-stereocilia
DFNB16	*STRC*	OHC-stereocilia
USH2A	*Ush2A*	IHC,OHC-stereocilia
DFNB21	*TECTA*	Tectorial membrane
DFNB23	*PCDH15*	IHC,OHC

Development of an "in house" small scale AAV production protocol

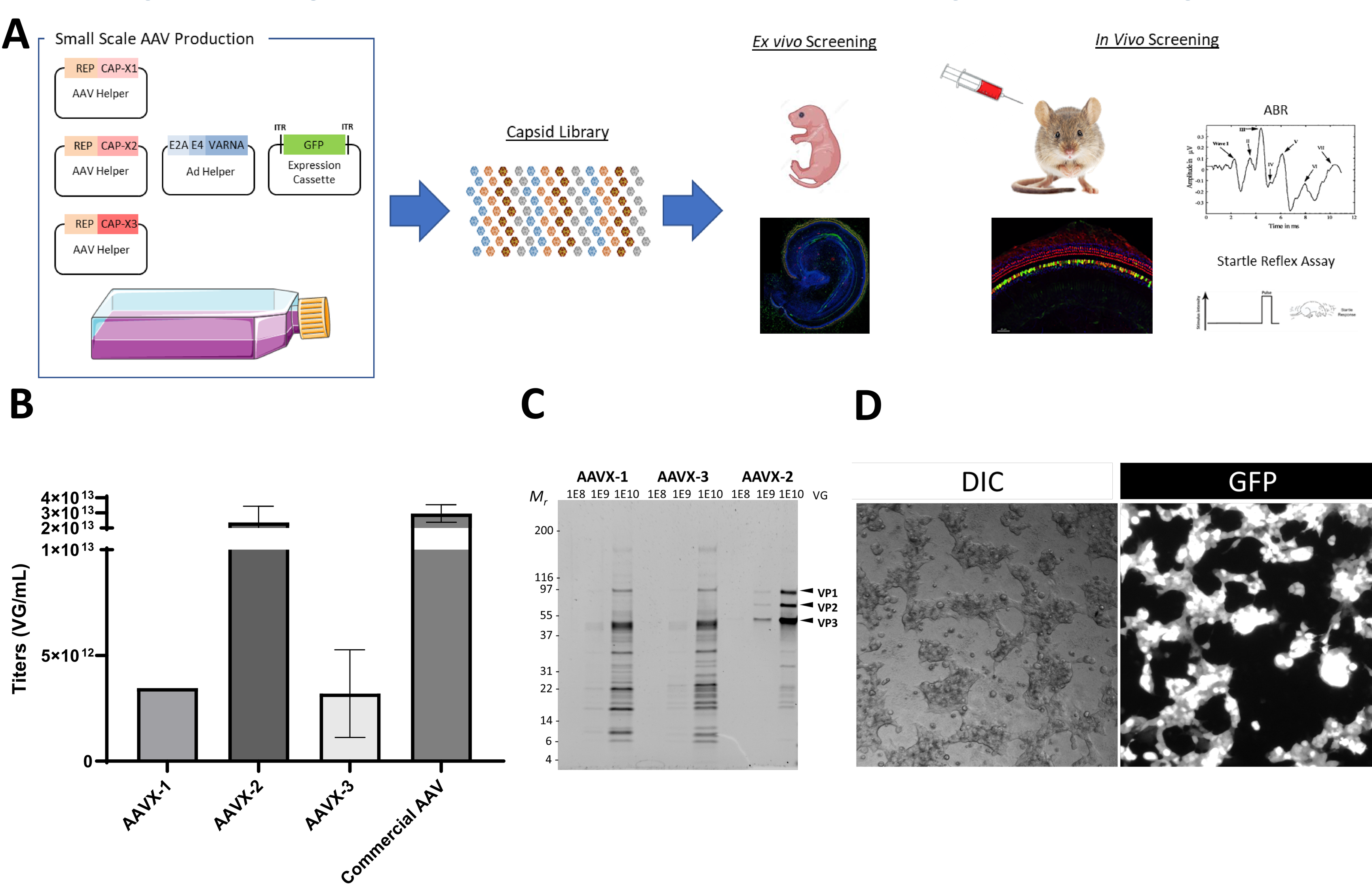


Figure 1. *Development of an in house small scale AAV production protocol.* **A.** HEK293T cells are transfected with a classic tri-vector system and AAV are produced for 72 hours. Cells are lysed and AAV are separated from other biomolecules. Purified AAV are then concentrated to reach final titers of ~3E+12-5E+13 Vector Genome (VG) per mL (VG/mL). Generated AAV libraries are then screened *ex* vivo and *in* vivo, using GFP fluorescence to track transduced cells. *In* vivo screenings are complemented with functional audiology tests (ABR, DPOAE, Startle reflex assay) to verify local tolerability of the AAV. **B.** Purified AAV are digested and vector DNA is titrated by qPCR using primers targeting the transgene (eGFP). **C.** SDS-PAGE of the indicated VG copy number of AAVX-1, AAVX-2 and AAVX-3 are stained with Sypro Ruby. The main band visible for all three serotype corresponds to VP3. VP1 and VP2 are clearly visible. **D.** HEK293T cells were transduced with AAVX-1 (Multiplicity of Infection: 10000) during 24 hours before imaging for GFP fluorescence.

In vivo injection and audiology platform

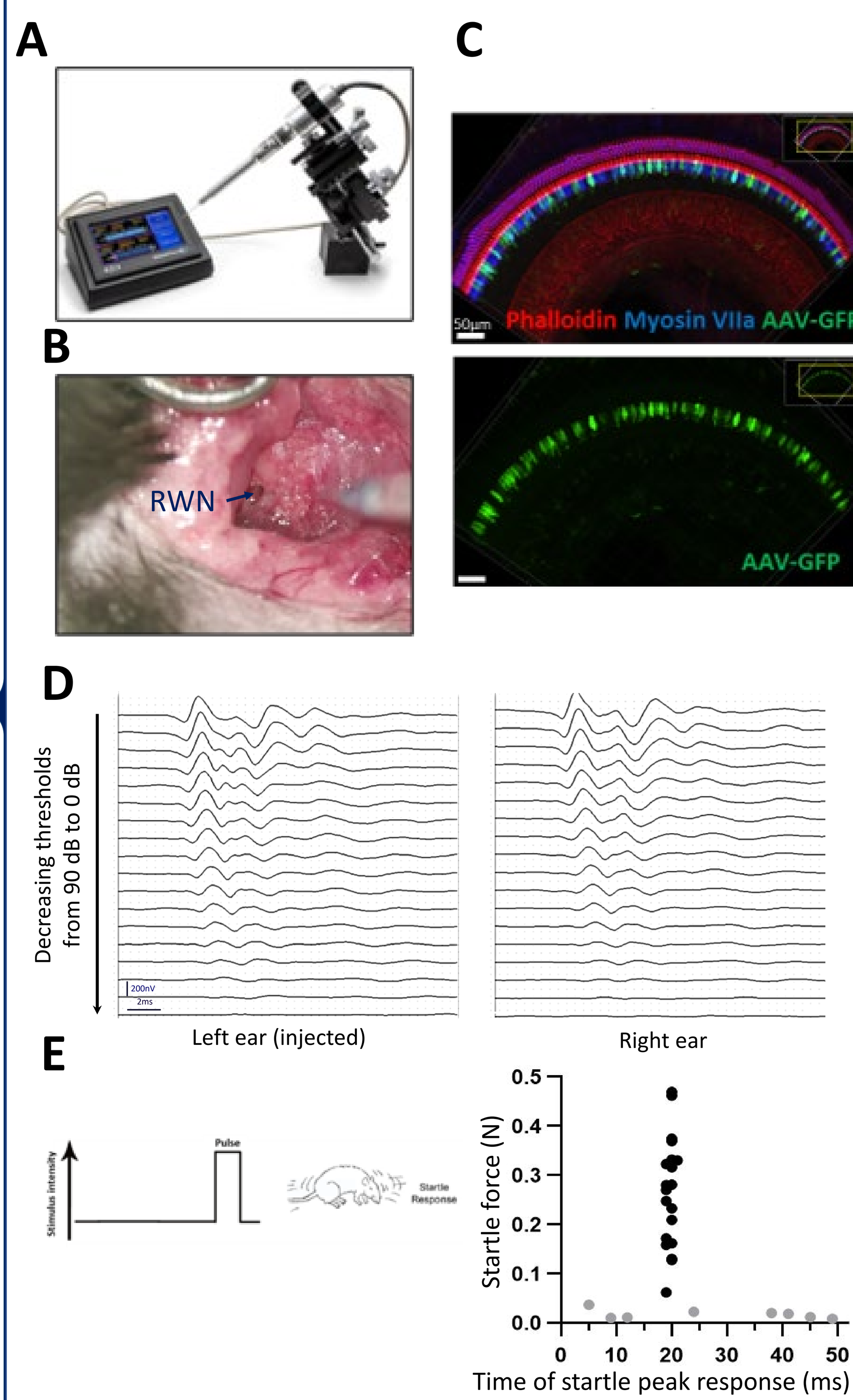
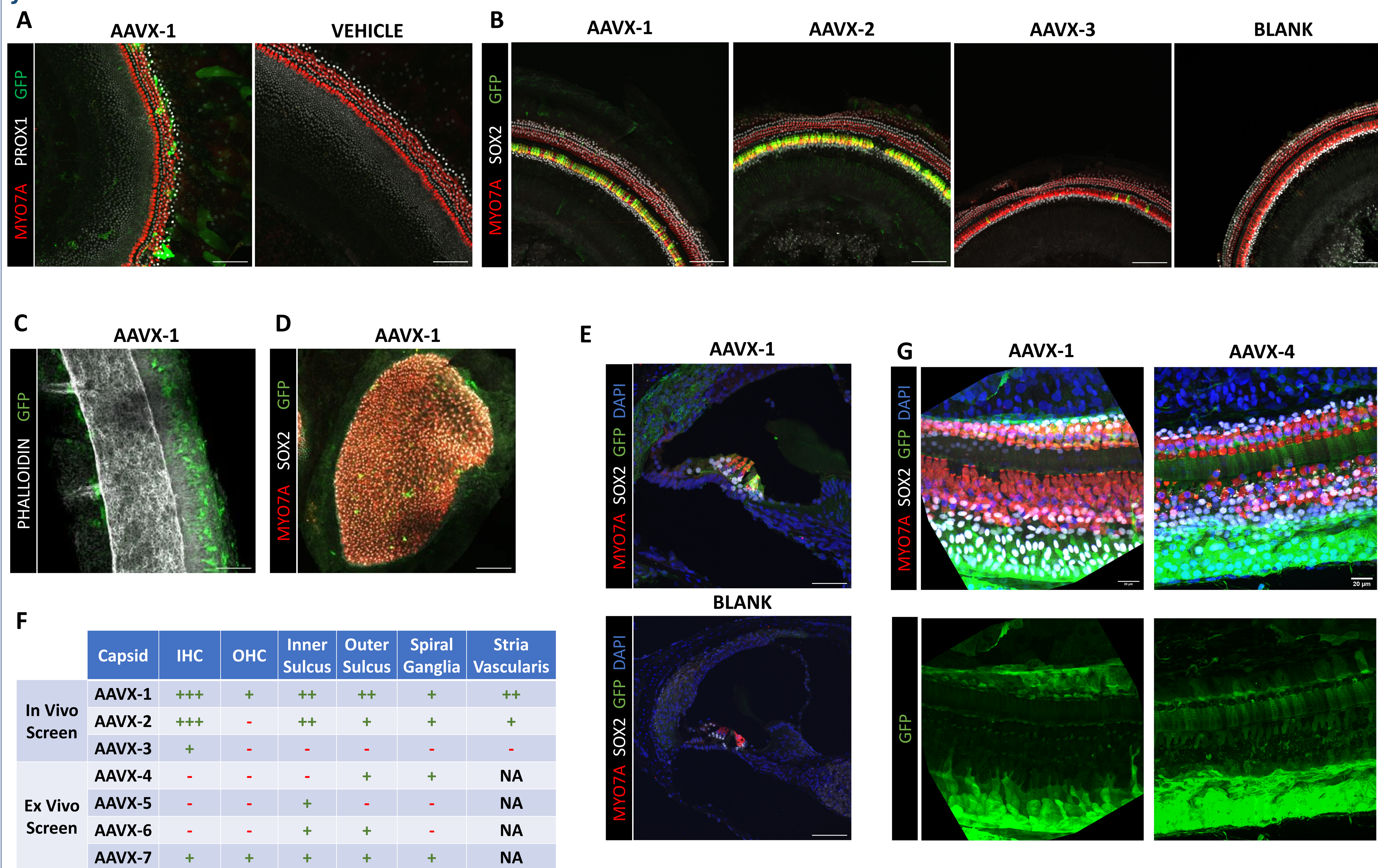


Figure 2. *In Vivo injection of AAV through the RWM is efficient and safe in rodents*. **A.** Nanoliter2020 Injector apparatus used for precise nanoliter-volume injections along with a with a SMARTouch™ controller. **B.** An injection of AAV into the round window membrane of the adult mouse inner ear is performed. RWN: round window niche. **C.** Imaging of the cochlea of a mouse that received an injection of commercial AAV-GFP (green) into the round window membrane at P14. Hair cells are immunostained with anti-MyoVIIa antibody (blue), anti-GFP (Green) and counterstained with phalloidin (red). GFP shows cells transduced by the AAV. **D.** *In vivo* validation of surgery procedure safety with normal pure-tone ABR traces and thresholds recorded at T+16d after AAV injection into WT mouse RWM. **E.** Schematic explanation of the acoustic startle reflex (ASR) assay (adapted from Turner & Parrish, 2008 and Kraus KS *et al.*). Startle reflexes are shown for a wild type mouse (Black) and a deaf mouse (Grey) in response to a white noise stimulus (2-20kHz) at 115 dB.

In vitro, ex vivo and in vivo screen of AAV to identify new synthetic capsids with cell specific tropism in the inner ear: from mice to Non-Human Primate



	Capsid	IHC	OHC	Inner Sulcus	Outer Sulcus	Spiral Ganglia	Stria Vascularis
In Vivo Screen	AAVX-1	+++	+	++	++	+	++
	AAVX-2	+++	-	++	+	+	+
	AAVX-3	+	-	-	-	-	-
Ex Vivo Screen	AAVX-4	-	-	-	+	+	NA
	AAVX-5	-	-	+	-	-	NA
	AAVX-6	-	-	+	+	-	NA
	AAVX-7	+	+	+	+	+	NA

Figure 3. *In house production protocol generates AAV suitable for in vitro, ex vivo and in vivo transduction.* **A**. Organ of Corti explants from p2 mice were transduced with 1.2E+10 VG of AAVX-1 during 96 hours before imaging for GFP fluorescence. **B-D.** p14 mice were injected with 1µL of AAVX-1 (7.5E+12 VG/mL), AAVX-2 (4.45E+13 VG/mL), AAVX-3 (3.45E+12 VG/mL) or blank through the RWM. Animals were euthanized 15 days later and cochlea (**B**), stria vascularis (**C**) and utricule (**D**) whole mounts were stained with the indicated antibodies. **E.** p14 mice were injected with 1µL of AAVX-2 (4.45E+13 VG/mL) or blank through the RWM. Animals were euthanized 15 days later. Cross-sections of the cochlea were stained with the indicated antibodies. **B-E**. Blank productions are generated using the purification protocol (Fig1A) on untransfected cells. **F.** Summary of AAV tropism after injection in p14 mice inner ear through the RWM (top rows). Additional AAV serotypes are being pre-screened *ex vivo* (bottom rows) on cochlear explants from p2 mice before testing tropism *in vivo*. NA: Not applicable, as Stria Vascularis is not co-cultured with cochlear explants. **G.** 22-month old Cynomolgus monkey were injected with 40 µL of AAVX-1 or AAVX-4 (both at 1.0E+13 VG/mL) through the RWM. Animals were euthanized 3 weeks later and cochlea whole mounts were stained with th indicated antibodies. Bottom panel show GFP staining only, illustrating the different cell tropis of the 2 capsids. Scale bar: 20 µm.

Conclusions

- High AAV Productivity (comparable with commercial productions)
- High AAV purity assessed by SDS-PAGE using Sypro Ruby staining
- Low endotoxin content (≤ 10 EU/mL)
- *In vitro* lack of toxicity
- High transduction efficiency *in vitro* and *in vivo*
- *In vivo* safety

- **Sensorion has invested in CMC process development and scale up to 50L in-house, with development & qualification of specific-product analytical methods**
- **Here, for agile, lower AAV vector scales, we developed an integrated AAV production protocol for screening purposes**
- **This small scale AAV production generates AAVs with high titers, high transduction efficiency and low endotoxin contamination making it suitable for in vivo screening up to evaluation in non-human primate**
- **This platform allows to identify and validate novel AAV variants of suitable tropism and cell-specific expression cassettes for inner ear disorder therapies.**

ASGCT 26TH ANNUAL MEETING Los Angeles