Supplementary information

Figure S1- Adcy5 and Adcy6 expression in the sciatic nerve. Relative expression of Adcy5 and Adcy6 mRNA normalized to Cyclophilin A (*CycloA*) in 8-month-old WT mice (Adcy5: 9.781± 1.702, n=3; Adcy6: 15.65± 0.5373, n=3). Bars represent the mean±SEM and each plot represents the number of mice analyzed (*n*).

Figure S2- Motor nerve conduction analysis of the sciatic nerve-gastrocnemius muscle pathway. Following sciatic nerve stimulation, compound muscle action potential (CMAP) amplitude was recorded. **(A)** CMAP amplitude in 2-month-old mice (control: 20.04± 3.146, n=8; *Adcy5*$^{-/-}$: 20.03± 3.250, n=4; *Adcy6*$^{-/-}$: 17.87± 3.407, n=7; *Adcy5*$^{-/-}$*Adcy6*$^{-/-}$: 10.28± 1.984, n=5). Statistical analyses were performed using the one-way ANOVA test. **(B)** CMAP amplitude in 2-month-old mice (control: 20.04± 3.146, n=8; *Adcy5*$^{-/-}$*Adcy6*$^{-/-}$: 10.28± 1.984, n=5; *Adcy5*$^{-/-}$ *Adcy6*$^{lox/lox}$ *MPZ*$^{cre+}$: 22.08± 1.589, n=9; p= 0.0274 (*Adcy5*$^{-/-}$*Adcy6*$^{-/-}$ *vs. Adcy5*$^{-/-}$ *Adcy6*$^{lox/lox}$ *MPZ*$^{cre+}$)). Statistical analyses were performed using the using the Kruskal-Wallis test. Bars represent the mean±SEM and each plot represents the number of mice analyzed (*n*).

Figure S3- Relative mRNA expression of Adcy6 normalized to Cyclophilin A in the sciatic nerve and the brain of WT and *Adcy5*$^{-/-}$ *Adcy6*$^{lox/lox}$ *MPZ*$^{cre+}$ adult mice analyzed by RT-qPCR. A significant decrease in Adcy6 expression was observed in the sciatic nerve of *Adcy5*$^{-/-}$ *Adcy6*$^{lox/lox}$ *MPZ*$^{cre+}$ 8-month-old mice (control: n=3; *Adcy5*$^{-/-}$ *Adcy6*$^{lox/lox}$ *MPZ*$^{cre+}$: n=4; p <0.0001) but not in the brain (control: n=4; *Adcy5*$^{-/-}$ *Adcy6*$^{lox/lox}$ *MPZ*$^{cre+}$: n=4). Statistical analyses were performed using the unpaired *t*-test. Bars represent the mean±SEM and each plot represents the number of mice analyzed (*n*).

Figure S4- Genotype proportion in zebrafish at 3 dpf and in adults. At 3 dpf : *adcy6a*$^{-/-}$*; adcy6b*$^{-/-}$: 23% ; *adcy6a*$^{-/-}$*; adcy6b*$^{+/-}$: 43% ; *adcy6a*$^{-/-}$*; adcy6b*$^{+/}$: 14% ; n=80. Adults : *adcy6a*$^{-/-}$*; adcy6b*$^{-/-}$: 3% ; *adcy6a*$^{-/-}$*; adcy6b*$^{+/-}$: 34% ; *adcy6a*$^{-/-}$*; adcy6b*$^{+/}$: 32% ; n=69.

Figure S5- Quantification of apoptotic cells in the posterior lateral line ganglion in zebrafish larvae at 48hpf. (A) Representative confocal images of AO+ cells (white arrows) within the PLLg in WT and *adcy6a*$^{-/-}$*; adcy6b*$^{-/-}$ embryos at 48 hpf. PLLg are delimited with white dashed lines. **(B)** The number of acridine orange-positive cells (AO+) was counted in the

PLLg (WT: 0.3750± 0.1548, n=16; *adcy6a*$^{-/-}$*; adcy6b*$^{-/-}$**:** 0±0, n=10). Statistical analyses were performed using the Mann-Whitney test. Bars represent the mean±SEM and each plot represents the number of embryos analyzed (*n*).

Figure S6- Quantification of apoptotic cells in the spinal cord of zebrafish larvae at 48hpf. **(A)** Representative confocal images of AO+ cells (white arrows) within a defined region of the spinal cord in WT and *adcy6a*$^{-/-}$*; adcy6b*$^{-/-}$ embryos at 48 hpf. The number of acridine orange-positive cells (AO+) was counted within a defined region (bottom white dashed lines to top) in the spinal cord (WT: 0.7857± 0.1869, n=14; *adcy6a*$^{-/-}$*; adcy6b*$^{-/-}$: 1.200± 0.5735, n=10). Statistical analyses were performed using the Mann-Whitney test. Bars represent the mean±SEM and each plot represents the number of embryos analyzed (*n*).

Movie S1- External phenotypic comparison of control and *Adcy5*$^{-/-}$*Adcy6*$^{-/-}$ mice at P5.

Movie S2- Tail suspension test performance.

Movie S3- Pole test performance.

Movie S4- Zebrafish swimming behavioral recording.

Movie S5- Real-time imaging of mitochondria along the PLLn of a WT embryo at 48hpf. Video presents 45 seconds of *mito::gfp* live imaging. White arrows depict an anterograde movement of a single mitochondria. Lateral view; anterior to the left and dorsal to the top. N= 32 mitochondria from 10 different embryos.

Movie S6- Real-time imaging of mitochondria along the PLLn of an *adcy6a*$^{-/-}$*; adcy6b*$^{-/-}$ embryo at 48hpf. Video presents 39 seconds of *mito::gfp* live imaging. White arrows depict an anterograde movement of a single mitochondria. Lateral view; anterior to the left and dorsal to the top. N= 24 mitochondria from 7 different embryos.

Movie S7- Real-time imaging of mitochondria along the PLLn of a WT embryo at 48hpf. Video presents 19 seconds of *mito::gfp* live imaging. Red arrows depict a retrograde movement

of a single mitochondria. Lateral view; anterior to the left and dorsal to the top. N= 29 mitochondria from 10 different embryos.

Movie S8- Real-time imaging of mitochondria along the PLLn of an *adcy6a*$^{-/-}$; *adcy6b*$^{-/-}$ embryo at 48hpf. Video presents 15 seconds of *mito::gfp* live imaging. Red arrows depict a retrograde movement of a single mitochondria. Lateral view; anterior to the left and dorsal to the top. N=23 mitochondria from 6 different embryos.

Movie S9- Real-time imaging of mitochondria along the PLLn of an *adcy6a*$^{-/-}$; *adcy6b*$^{-/-}$ embryo at 48hpf. Video presents 54 seconds of *mito::gfp* live imaging. Yellow arrows depict anterograde while red arrows depict retrograde movements respectively of a single mitochondria moving in both directions. Lateral view; anterior to the left and dorsal to the top.

Supplementary 1- PCR-RFLP primers for mice genotyping

Target Gene	**Control/Knock-out**	**Primers (5'→ 3')**	
Adcy5	Control	Forward	GTCCGAGGATGGAGGCAGTT
		Reverse	CACCACTGCAATGAGCGCATA
	Knock-out	Forward	ACCGTCGAGGATGGAGACGG
		Reverse	TGTCCATCTGCTGCACGAGACTA
Adcy6	Control	Forward	GGAGACCTAGAGATGGAGTG
		Reverse	GCCACTTGTGTAGCGCCAAG
	Knock-out	Forward	AAGATCTGCTTTGTGGGTGC
		Reverse	AGCCACTGGCTCGATTCGCGTGGCG
Adcy6$^{lox/lox}$	Knock-out	Forward	CAGCTCCTTGTGTTCCCATA
		Reverse	AGCACAGTGACCAGCAACAG
MPZ^{cre+}	Control	Forward	CTAGGCCACAGAATTGAAAGATCT
		Reverse	GTAGGTGGAAATTCTAGCATCATCC
	Knock-out	Forward	CCACCACCTCTCCATTGCAC
		Reverse	ATGTTTAGCTGGCCCAAATG

Supplementary 2- DNA extraction from zebrafish tail biopsies and embryos

Zebrafish tail biopsies:

1. Place a piece of fish tail into 50 µL of 50mM NaOH in tubes
2. Incubate for 45 minutes to 1 hour at 95℃ to digest the tissue
3. Cool at room temperature for 5 minutes
4. Add 7.5 µL of 1M TrisHCl pH8
5. Shortly vortex
6. Centrifuge at 14500 rpm for 5 minutes to collect remaining debris at the bottom
7. Store at -20°

Embryos:

1. Place the embryos (or a portion of the embryo) in tubes
2. Add an equal volume (1 :1) of 2X TE (20 mM TrisHCl ph7.5-8, 2 mM EDTA) and proteinase K (10 mg/ml)
3. Incubate for at least 1 hour at 55℃ to digest the tissue
4. Centrifuge at 14500 rpm for 5 minutes
5. Inactivate the Proteinase K by heating the tubes at 95℃ for 12 minutes
6. Centrifuge again at 14500 rpm for 5 minutes
7. Store at -20°

Supplementary 3- PCR-RFLP primers for zebrafish genotyping

Target gene	**Primers (5'→ 3')**	
adcy6a	Forward	CTCCATGTGGTGAGGAGGTG
	Reverse	TGCTCACCTTCCACTGCATT
adcy6b	Forward	GATCCACCATCAACAGGCC
	Reverse	AAGGGCGATGTTGGTCTGA
gfp	Forward	TCCATGGCCAACACTTGTCA
	Reverse	CTGGTAAAAGGACAGGGCC

Supplementary 4- *In situ* hybridization solutions

Hybridization Buffer (HB)		
Reagents	Initial Concentration	Final Concentration
Formamide	99%	50%
SSC	20X	5X
Heparin	50 mg/ml	50 µg/ml
tRNA	50 mg/ml	500 µg/ml
Tween-20	100X	0.1%
Citric Acid	1 M	9.2 mM
Water	-	Bring to final volume
Post-hybridization Washes		
Solutions	Composition	Time
Solution 1	100% HB	1 x 5 minutes
Solution 2	75% HB - 25%2XSSCTw	1 x 5 minutes
Solution 3	50% HB - 50%2XSSCTw	1 x 5 minutes
Solution 4	25% HB - 75%2XSSCTw	1 x 5 minutes
Solution 5	100% 2XSSCTw	2 x 30 minutes
Solution 6	100% 0.2XSSCTw	2 x 30 minutes
AP Buffer		
Reagents	Initial Concentration	Final Concentration
TrisHCl (PH=9.5)	1 M	100 mM
MgCl2	1 M	50 mM
NaCl	5 M	100 mM
Tween-20	100X	0.1%
Water	-	Bring to final volume
Blocking Solution		
Reagents	Initial Concentration	Final Concentration
BSA	-	2 mg/ml
Sheep Serum	100%	1.5%
PBSTw (0.1%)	-	Bring to final volume

Supplementary 5- Immunohistochemistry antibodies

Primary antibodies	Dilution	References	Target in this study

Anti-HUC (Mouse)	1:500	Invitrogen, A21271	Neurons
Anti-EGFP (Chicken)	1:500	Abcam, AB13970	Schwann cells
Anti-Phospho-Histone H3 (Rabbit)	1:500	EMD Millipore Corp, 06-570	Proliferating cells
Anti-NaCh (Mouse)	1:500	Sigma, clone K38/35	Sodium Channels (NaCh)
Anti-Acetylated Tubulin (Mouse)	1:500	Sigma, T7451-25UL	Posterior lateral line nerve (PLLn)

Secondary antibodies	**Dilution**	**References**
Alexa 488 goat anti-mouse	1:500	Invitrogen, A21121, A11001
Alexa 488 goat anti-chicken	1:500	abcam, AB150169
Alexa 568 goat anti-mouse	1:500	Invitrogen, A21144
Alexa 568 goat anti-rabbit	1:500	Invitrogen, A11036

Supplementary 6- *Solutions for TEM experiment*

Fixative solution 1 – Sciatic nerve		
Reagents	Initial Concentration	Final Concentration
Sodium cacodylate buffer	1 M	0.1 M
Glutaraldehyde	25%	2.5%
Sucrose	30%	3.5%
Fixative solution 2 – Zebrafish embryos		
Reagents	Initial Concentration	Final Concentration
Sodium cacodylate buffer	1 M	0.1 M
Glutaraldehyde	25%	2%
PFA	10%	2%

Resin composition			
Mixtures	Reagents	Volume	References
Mixture A	Embed 812	20 ml	no. 14900, Electron Microscopy Sciences
	DDSA	31 ml	no. 13710, Electron Microscopy Sciences
Mixture B	Embed 812	20 ml	no. 14900, Electron Microscopy Sciences
	NMA	17 ml	no. 45347-250ML-F, Sigma-Aldrich
Mixture A		13 ml	
Mixture B		15 ml	

DMP-30	0.5 ml	no. 13600, Electron Microscopy Sciences

Supplementary 7- RT-qPCR primers

Target gene	Concentration	Primers (5'→ 3')	
Adcy5	50 μM	Forward	GCTGCTCTCGGCTTAGGCA
		Reverse	GCCGGACGCAGAGATGTC
Adcy6		Forward	GGTGAGGGAGAATCACTGTCTGA
		Reverse	ATGTTCACGTTCACACCTGTTACCT
Sox10		Forward	CAACCACCCCAAAGACAGAGC
		Reverse	TATTGGTCCAGCTCAGTCACATC
Krox20		Forward	GCCCCTTTGACCAGATGAAC
		Reverse	CAGGGTACTGTGGGTCAATGG
Oct6		Forward	GGTGAGGTGGGAACCATGTAAATA
		Reverse	GTTTAGTCGGGCATACAAACCCT
Mpz		Forward	AGACTACAGTGACAACGGCACTTTC
		Reverse	AGAAGAGCAACAGCAGCAACAG
Pmp22		Forward	GTTCCTGTTCTTCTGCCAGCTC
		Reverse	GCGAAGCCATAGGAGTAGTCAGT
Mbp		Forward	GTACAAGGACTCACACACGAGAACTAC
		Reverse	TTGAAGAAATGGACTACTGGGTTTT
CycloA		Forward	GTCAACCCCACCGTGTTCTT
		Reverse	CTGCTGTCTTTGGGACCTTGT