## Supplemental data

**Supplemental Table 1|** Forward (F) and reverse (R) primers for molecular cloning, HRM analysis, sequencing and RT-PCR analysis.

| Target | Note | Forward primer sequence (5'->3') | Reverse primer sequence (5'->3') | |
|---|---|---|---|---|
| *asah1a* | Gateway | GGGGACAAGTTTGTACAAAAAAGCAGGC | GGGGACCACTTTGTACAAGAAAGCTGGGT | |
| | cloning | TACCACCATGAAGCTTGTGTTCCGTTAC | cTTACCATGGTGTGCATGGATTTG | |
| *asah1b* | Gateway | GGGGACAAGTTTGTACAAAAAAGCAGGC | GGGGACCACTTTGTACAAGAAAGCTGGGT | |
| | cloning | TACCACCATGAACAACAGATTAAACCTG | cTCACCAGGGCATGCAGGGATTTG | |
| *asah1a* | HRM | GTCTAGACTCGAATAAGTTCATG | TGGGAAACAGTTACCTCTGTG | |
| *asah1b* | HRM | TGCAAAGAGATGTGTTAGATTG | TCCTTCAGATGGCGAGCATG | |
| *asah1a* | Sequencing | TGGGATGTATCCACCTAAAGG | Same as HRM Rev | |
| *asah1b* | Sequencing | CAGCAAGCAAAAGATGGACAG | TACGATTTTGGGAGATTTATCTC | |
| | NCBI code | | | |
| *asah1a* | NM_001006088 | ATTAGGCCTGGTGAACTGAC | CTGCGAGTAAGAAAACCCGTC | 125 bp |
| *asah1b* | NM_200577 | TGGACTGTTCATGGGATGGG | CCGGTCAACATCCCGACATA | 150 bp |
| *gpnmb* | XM_009294247 | GCAAGGGCGTAGAATTGAAA | TGGCAGGGACATGTCAGTAA | |
| *chia.6* | NM_199603 | TCCACGGCTCATGGGAGAGTGTC | AGCGCCCTGATCTCGCCAGT | (73) |
| *catD* | NM_131710 | TGGGTGGAAAGGTCTACTCG | CACTCAGGCAGATGTCGTGT | |
| *il1β* | NM_212844 | TGGACTTCGCAGCACAAAATG | GTTCACTTCACGCTCTTGGATG | (74) |
| *tnfβ** | NM_001024447 | GCATGTGATGAAGCCAAACG | GATTGTCCTGAAGGGTCACC | (75) |
| *apoeb* | NM_131098 | AAACTGACATGACCGACGCT | TAGGTTGCTACGGTGTTGCG | 172 bp |
| *c1qA* | NM_001020527 | CTCTGTTTCCCTTTTCCTTCTG | CTTTCTCTCCTTTTGGTCCTGG | 108 bp |
| *c3a.1* | NM_131242 | CGCTGCACAAAGTACTTCCAC | GCCAGCTCCATGTCCTTGAC | 197 bp |
| *c5aR1* | XM_005159274 | CCGACAAGCTCGCATCCTAT | GCGAATGATGGTTATCGCCC | 163 bp |
| *c5* | XM_001919191 | CAAGGCCACGGTTCAATCAG | TCTTCATGCTTTCGGCAGTCA | 152 bp |
| *th1* | NM_131149 | AGCTTTGTGGACGCTACTGA | GTGGGTTGTCCAGCACTTCT | 112 bp |
| *th2* | NM_001001829 | TACAAGCCATTCGACCCAGC | ATGCTGCAAGTGTAGGGGTC | 173 bp |
| *sncβ* | NM_200969 | GGAGTTTGGTCAGGAAGCCA | CCTCGGGCTCATAATCCTGG | 107 bp |
| *sncγa* | NM_001017567 | TGGAGGGGCTGGAGACTATG | AGCATCATGGGACATTCGGTT | 123 bp |
| *sncγb* | NM_001020652 | ATGGTGAACCCGGGTGACTT | AGGCTTTGGAGCAGAAACGTA | 129 bp |

| | | | | |
|---|---|---|---|---|
| *mcpa* | XM_002665562 | TGGTCATCTATCCTCCTCTCCA | CTTTCTCCCAGGCCCAATAGTTCT | 150 bp |
| *ef1a* | | CTGGAGGCCAGCTCAAACAT | ATCAAGAAGAGTAGTACCGCTAGCATTAC | (76) |
| *rpl13α* | | TCTGGAGGACTGTAAGAGGTATGC | AGACGCACAATCTTGAGAGCAG | (76) |

* Also known as *tnf-α2*

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Human ACase    MPGRSCVALV-LLAAAVSCAVAQHAPPWTEDCRKSTYPPSGPTYRGAVPWYTINLDLPPY      59
Asah1a         ----MKLVFRYNALFISIFIHALYV-QGLEDCRSGMYPPKGPTYRGNVTRYTVNLDLPPS      55
Asah1b         MNNRLNLCFFI-LSYMCMCLSAQYVPPFTEDCRSGMYPPNGPTFKGDVSWYTVDLDLPAS      59
                     : :           * :.    ****.. ***.***::* *  **::****

Human ACase    KRWHELMLDKAPVLKVIVNSLKNMINTFVPSGKIMQVVDEKLPGLLGNFPGPFEEEMKGI      119
Asah1a         ERWTQIIKDKNTELIEMVQTIKDMAKGFF-HGKLVNFVDKELPFIVDTLPNPFNEEIKGI      114
Asah1b         KRWTDVISDKKTEMASMIQAIRDLADAFVPSGKLIQLVDKDLPLMVDTLPYPFNEEIRGI      119
               :** ::: **   :  ;:::::::: . *.  **:::.**:.** ::..:* **:**::**
                          α-subunit helix

Human ACase    AAVTDIPLGEIISFNIFYELFTICTSIVAEDKKGHLIHGRNMDFGVFLGWNINNDTWVIT      179
Asah1a         AAVSGIPLGEIALFNIFYEVFTVCTSLVAEDNNGNIYHGRNLDFGLFMGWDRQNKTWTLT      174
Asah1b         ASVSGVPLGEVVLFNIFYEVFTVCTSLVAEDVNGNLIHARNLDFGLFMGWDLKNRSWVIT      179
               *:*:.:****:  ******:**:***:**** :*:: *.**:***:*:**: :* :*.:*
                                                                β-subunit region

Human ACase    EQLKPLTVNLDFQRNNKTVFKASSFAGYVGMLTGFKPGLFSLTLNERFSINGGYLGILEW      239
Asah1a         EKLKPLVVNINFERKNQTVFKSTSFAGYVGMLTGIRPGELTLTMNERFDFDGGYIGILDW      234
Asah1b         EKLKPLVVNIDFTRNGQTVFKSTNFAGYVGMLTGIHQNSFTLTMNERFSLDGGYIGILEW      239
               *:****.**::* *:.:****::.**********:: . ::**:****.::***:***:*

Human ACase    ILGKKDVMWIGFLTRTVLENSTSYEEAKNLLTKTKILAPAYFILGGNQSGEGCVITRDRK      299
Asah1a         IFGNRDGMWTGFLTRRVLENSTSYEDAKDQLSQTKLLAPVYFILGGNRTGQGCVITRTRI      294
Asah1b         ILGKRDGMWMSFLTRSVLENATSYESAKALLSDTKLLAPAYFILGGNQSGEACIITRSRT      299
               *:*::* ** .**** ****:****.**  *:.**:***.*******::*:.*:*** *

Human ACase    ESLDVYELDAKQGRWYVVQTNYDRWKHPFFLDDRRTPAKMCLNRTSQENISFETMYDVLS      359
Asah1a         NTLDIWELELMLGRWYVLETNYDHWDKPMFLDDRRTPAMKCMNQTTQANISLASIYNVLS      354
Asah1b         QNISPLELNVKNGRWYVLETNYDHWKEPLFLDDRRTPAMKCMNQTTQTNISVKTVYDVLS      359
               :.:.  **:   *****::****:*..*:*********  *:*:*:* ***. ::*:***

Human ACase    TKPVLNKLTVYTTLIDVTKGQFETYLRDCPDPCIGW 395
Asah1a         TKPVLNKLTTYTSLMAVSTGTLESYVRDCPNPCTPW 390
Asah1b         TKPVLNKLTTYTTLMEVSKGTLESFIRDCPNPCMPW 395
               *********.**:*: *:.* :*:::****:**  *
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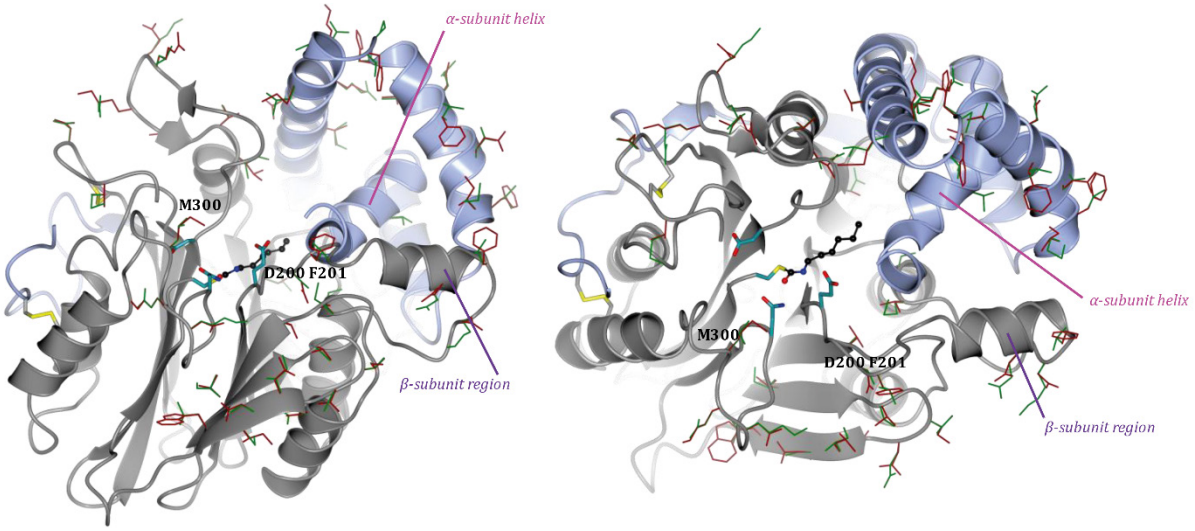
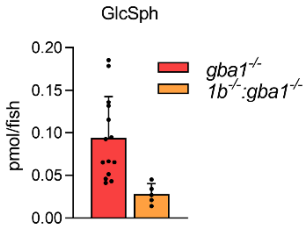
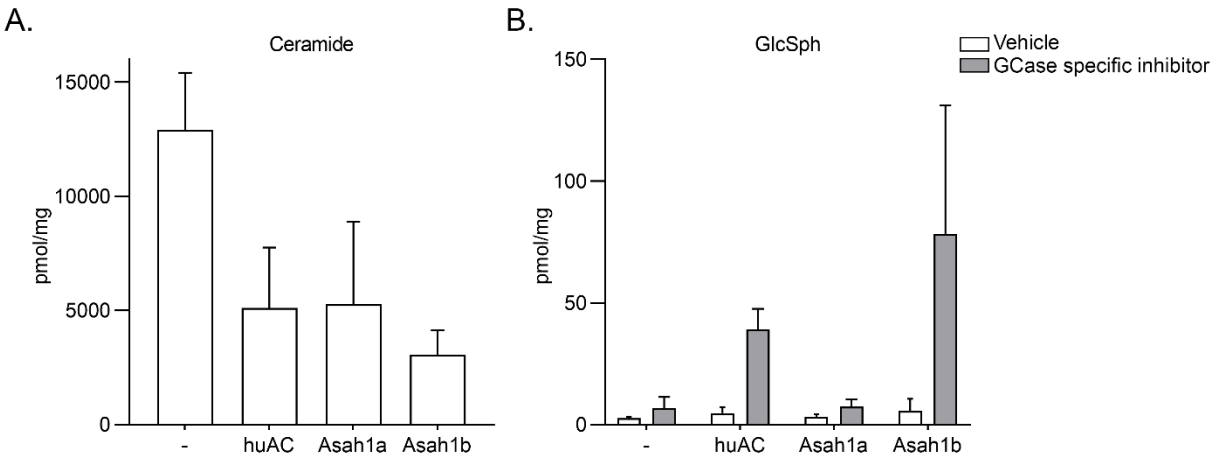
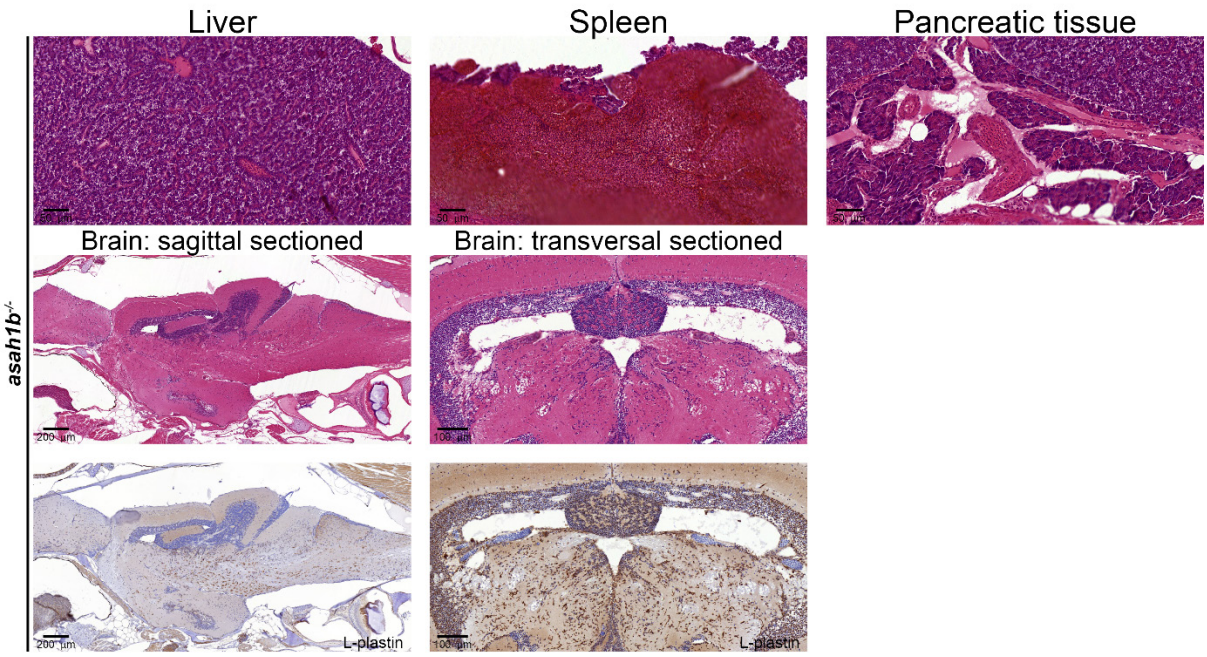



Ribbon diagram of the side (left) or top (right) of human ACase (PDB 6MHM) with α-subunit (light grey), β-subunit (dark grey) and disulfide bonds (yellow) indicated. Inhibitor Carmofur (black, ball and stick) and catalytic residues C143, D162, E225 or N320 (cyan sticks) are also visualized for clarity, as proposed by Dementiev et al.(32). Divergent residues of Asah1a (red) and Asah11b (green) surrounding the catalytic pocket are superimposed on the human structure. Only residues Asp 200, Phe 201 and Met 300 of Asah1a, located on the loops adjacent to the catalytic site, might be close enough to be of significance.

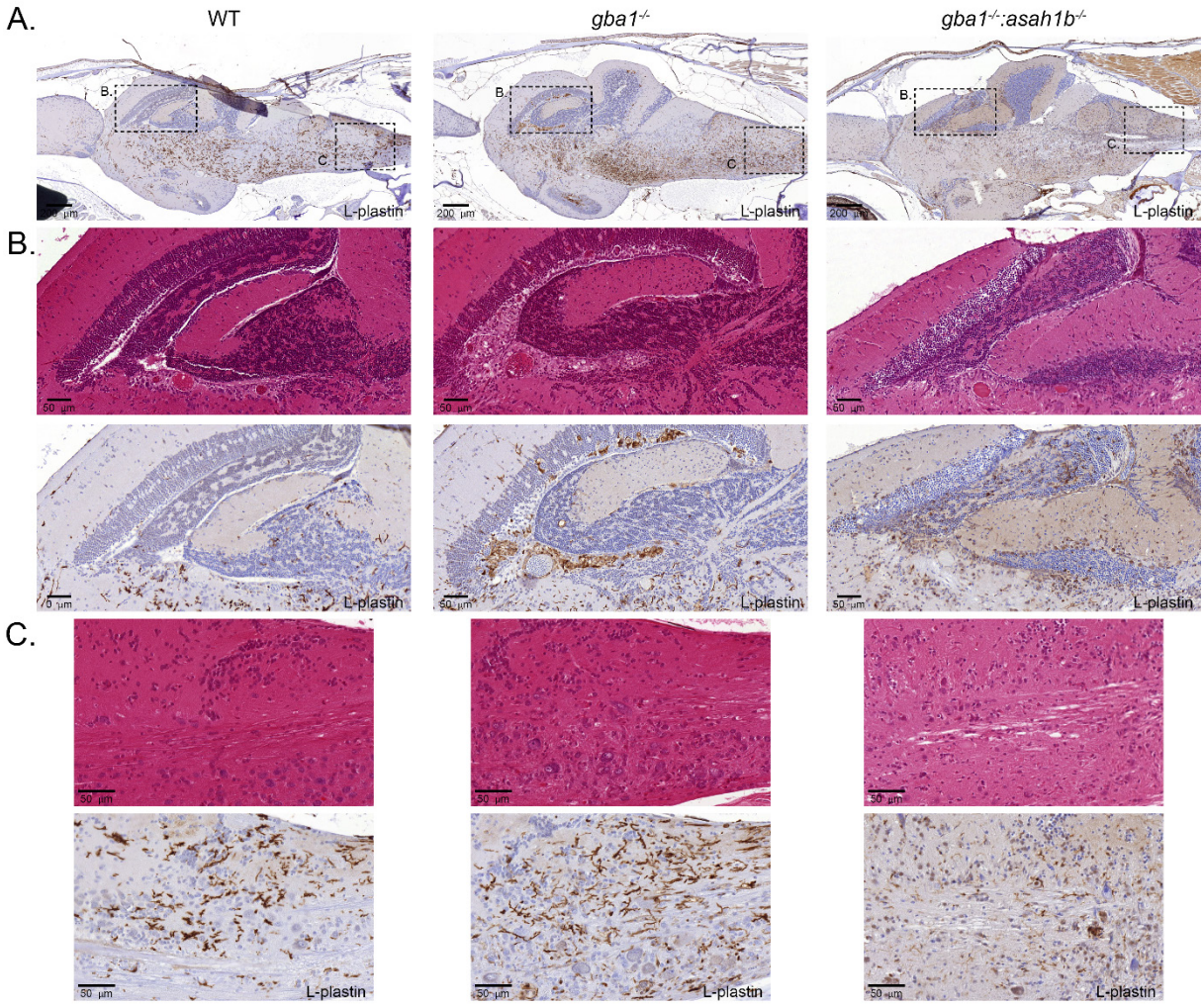



GlcSph levels of *gba1*$^{-/-}$ (n = 14) and *gba1*$^{-/-}$*:asah1b*$^{-/-}$ (n = 5) larvae KO at 5dpf in pmol/fish. Data of *gba1*$^{-/-}$ is obtained from (33). Data is depicted as mean ± SD.

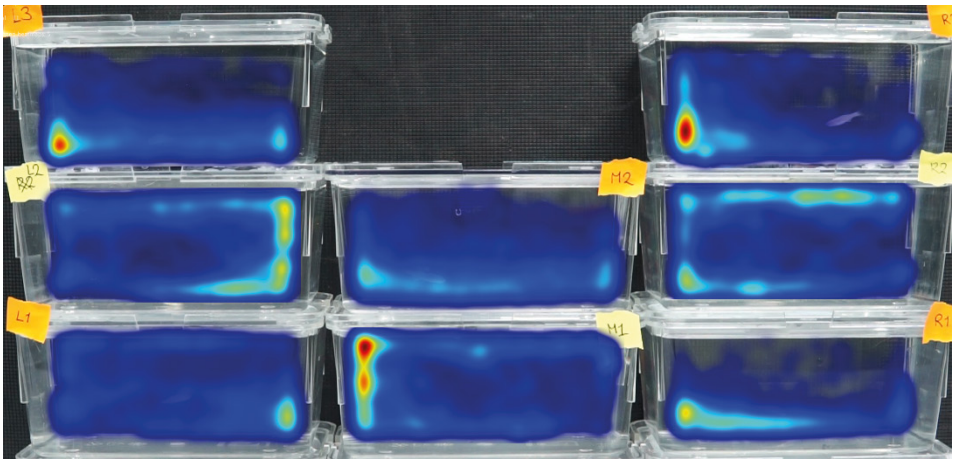

A.
Ceramide
15000
10000
5000
0
pmol/mg
-
huAC
Asah1a
Asah1b
B.
GlcSph
Vehicle
GCase specific inhibitor
150
100
50
0
pmol/mg
-
huAC
Asah1a
Asah1b



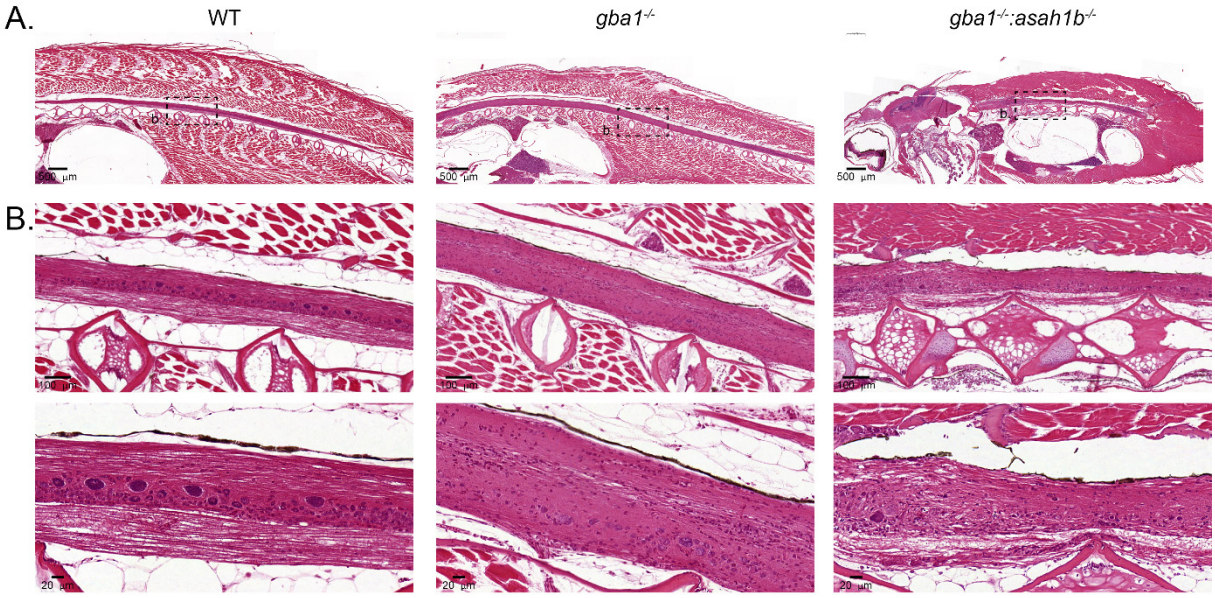

**Supplemental Figure S5:** Histopathology of *asah1b*$^{-/-}$ zebrafish including liver, spleen and pancreatic tissue (top panel) as well as sagittal section (left) and transversal section (right panel) of *asah1b*$^{-/-}$ zebrafish brain using H&E staining. Bottom panel: immunostaining of L-plastin in sagittal section (left) and transversal section (right) of *asah1b*$^{-/-}$ zebrafish brain. Scale bars: 50 µm for top panel, 200 µm and 100 µm for sagital and transversal sectioned brain, respectively.




**Supplemental Figure S6:** Histopathology of WT, *gba1*$^{-/-}$ and *gba1*$^{-/-}$*:asah1b*$^{-/-}$ of sagittal sections of zebrafish brains. **(A)** Immunostaining of L-plastin with low magnification. Scale bars: 200 µm. **(B)** Higher magnification focusing on periventricular grey zone with H&E staining (upper panel) and immunostaining of L-plastin (lower panel). To compare consecutive sections stained with H&E and L-plastin, H&E staining sections were the same as displayed in Figure 6B. Scale bars: 50 µm. **(C)** Higher magnification focusing on the medulla oblongata with H&E staining (upper panel) and immunostaining of L-plastin (lower panel). Scale bars: 50 µm.




**Supplemental Figure S7: Heatmaps of individually filmed *asah1b*$^{-/-}$ and *gba1*$^{-/-}$*:asah1b*$^{-/-}$ zebrafish.** Heatmaps of *asah1b*$^{-/-}$ (yellow stickers) and *gba1*$^{-/-}$*:asah1b*$^{-/-}$ (orange stickers) at 15 wpf, with individual fish starting to show differences in swimming behaviour. Red indicates more time and blue less time spend at that location.




**Supplemental Figure S8: (A)** H&E staining of spinal cords of WT, *gba1*$^{-/-}$ and *gba1*$^{-/-}$*:asah1b*$^{-/-}$ including higher magnifications in **(B)**. Scale bars: 500 µm for **(A)** and 100 µm and 20 µm for the top and bottom panel in **(B)**.

**Supplemental Video 1 |** Short video of WT (white stickers), *gba1*$^{-/-}$ (red), *asah1b*$^{-/-}$ (yellow) and *gba1*$^{-/-}$*:asah1b*$^{-/-}$ (orange stickers).

**Supplemental Video 2 |** Short video of *gba1*$^{-/-}$ (red), *asah1b*$^{-/-}$ (yellow) and *gba1*$^{-/-}$*:asah1b*$^{-/-}$ (orange stickers).

**Supplemental Video 3|** Short video of WT (white stickers), *asah1b*$^{-/-}$ (yellow) and *gba1*$^{-/-}$*:asah1b*$^{-/-}$ (orange stickers).