Supplementary Materials

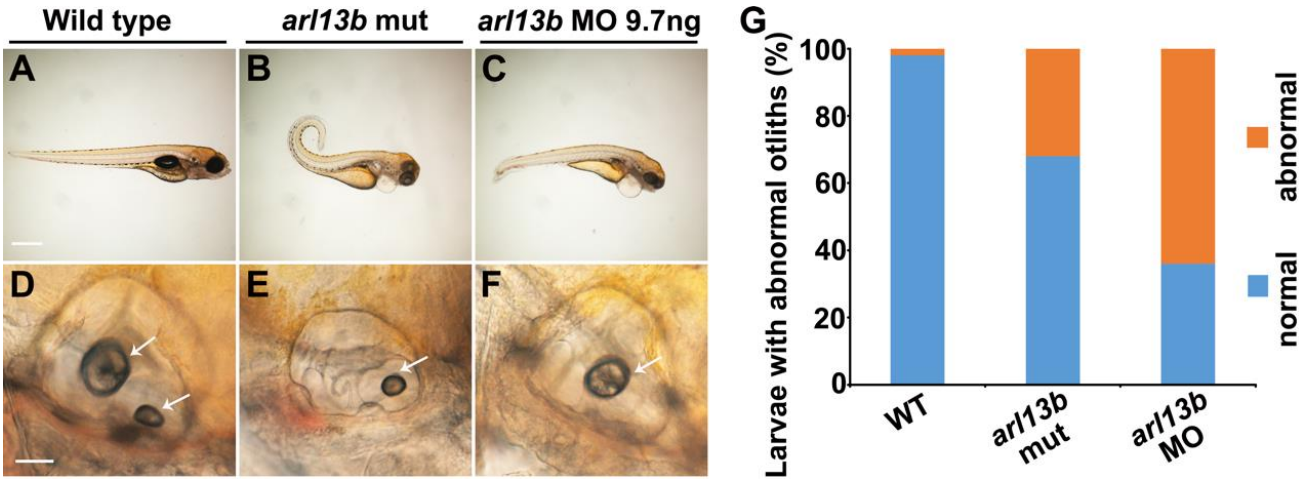

Fig. S1 Disruption of *arl13b* results in morphological defects. **A**–**C** Wild-type embryos are straight at 4 dpf while the bodies of *arl13b* mutants and morphants are curved. **D**–**G** There are usually two otoliths in the inner ear; however, the otoliths of *arl13b*-deficient embryos have defects in number, size, and morphology. A-C, Scale bar 400 μm. F-I'', D-F, Scale bar 100 μm.

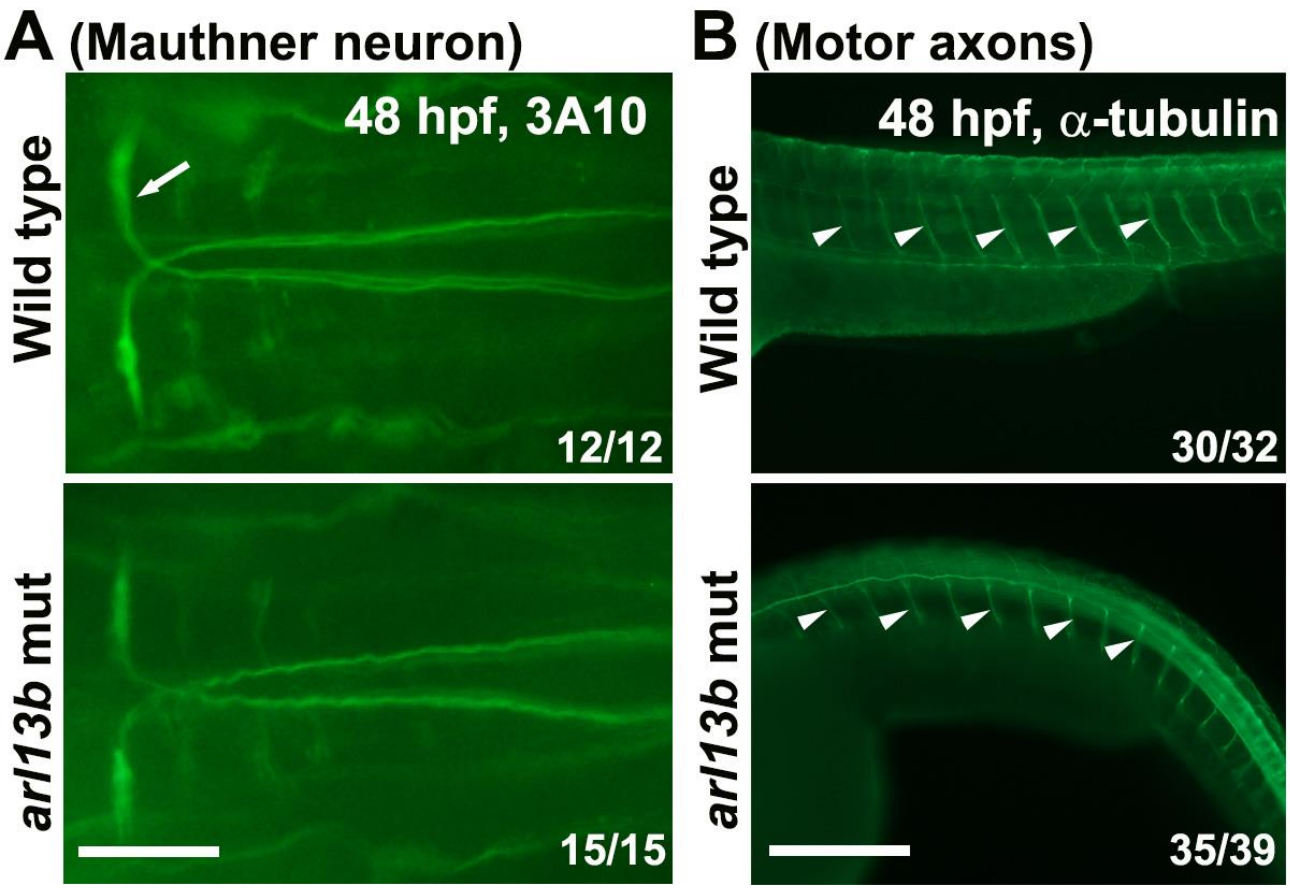

Fig. S2 Locomotion-related neurons remain intact in *arl13b*-deficient embryos. **A** Mauthner neurons still grow out axons, which normally cross the midline and project caudally, in *arl13b*-mutants. **B** No defect of motor axons is detectable in *arl13b*-mutants. A-B, Scale bar 100 μm.

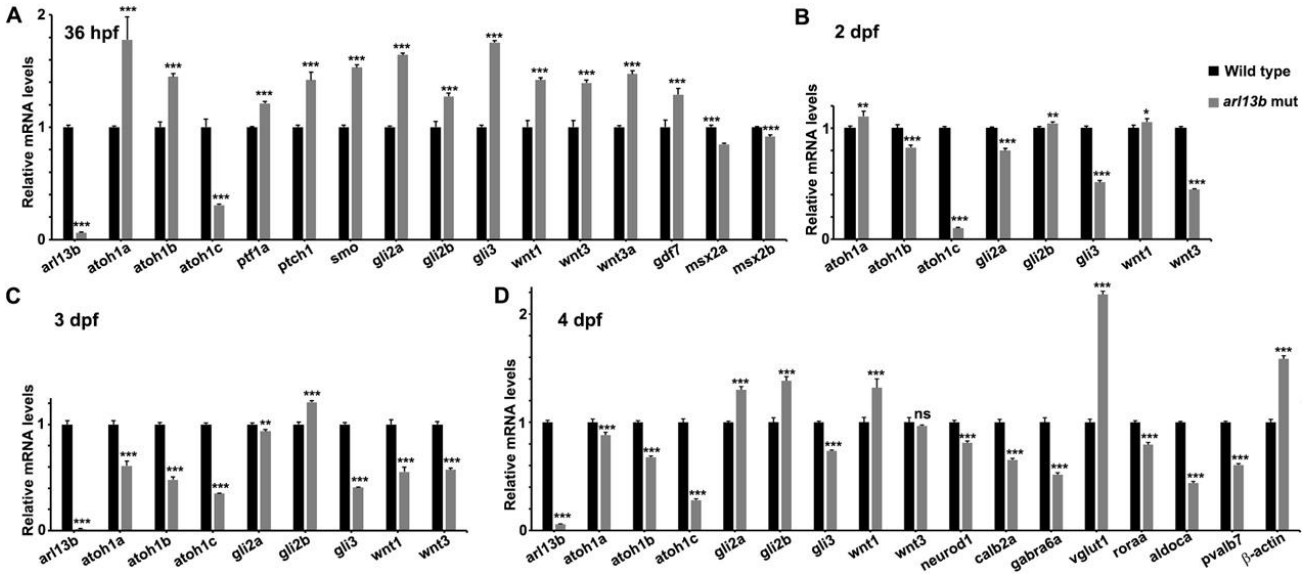

Fig. S3 The expression levels of a network of genes are altered dynamically in *arl13b* mutants. Total RNAs were extracted from wild type and *arl13b* mutant embryos (whole mount) and reversely transcribed. The obtained cDNAs were used as templates for real time quantitative PCR (q-PCR). The expression levels of the genes are normalized to that of elongation factor EF1α. *arl13b* is significantly reduced in mutants, confirming that the embryos picked on the basis of curved tails are virtually *arl13b* mutants with high penetration, consistent with published data. The expression levels of *atoh1a* and *1b* (granule cell precursors) and *ptf1a* (Purkinje cell precursors) are dynamically regulated in *arl13b* mutants. The expression level of *atoh1c* continually declines in *arl13b* mutants at all developmental stages. The expression levels of Shh signaling (*pcht1*, *smo*, *gli2a*, *gli2b*, and *gli3*), Wnt signaling (*wnt1*, *wnt3*, and *wnt3a*), and BMP signaling (*gdf7*, *msx2a*, and *msx2b*) are altered by *arl13b* deficiency. The molecules expressed in differentiated granule cells (*neurod1*, *calb2a*, *gabra6a*, and *vglut1*) and Purkinje cells (*roraa*, *aldoca*, and *pvalb7*) are also altered. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

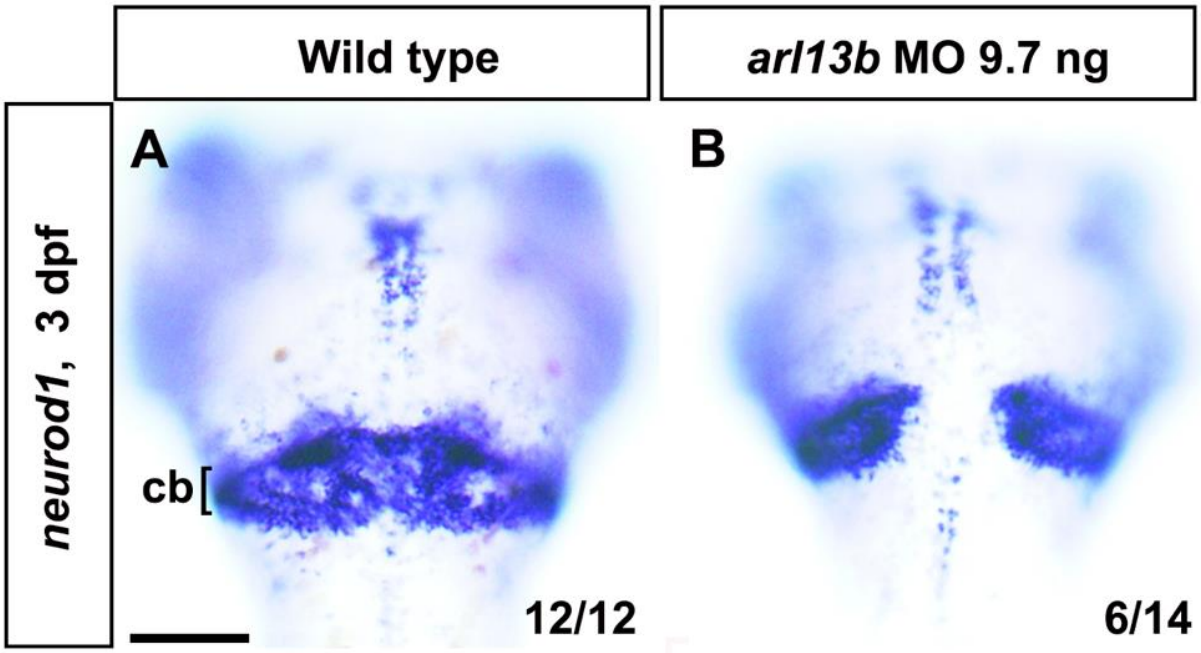

Fig. S4 The expression of granule cell marker *neruod1* is specifically reduced in the dorsomedial clusters. The dorsomedial clusters of cerebellar granule neurons are specifically impaired by *arl13b* disruption. A-B, Scale bar 100 μm.

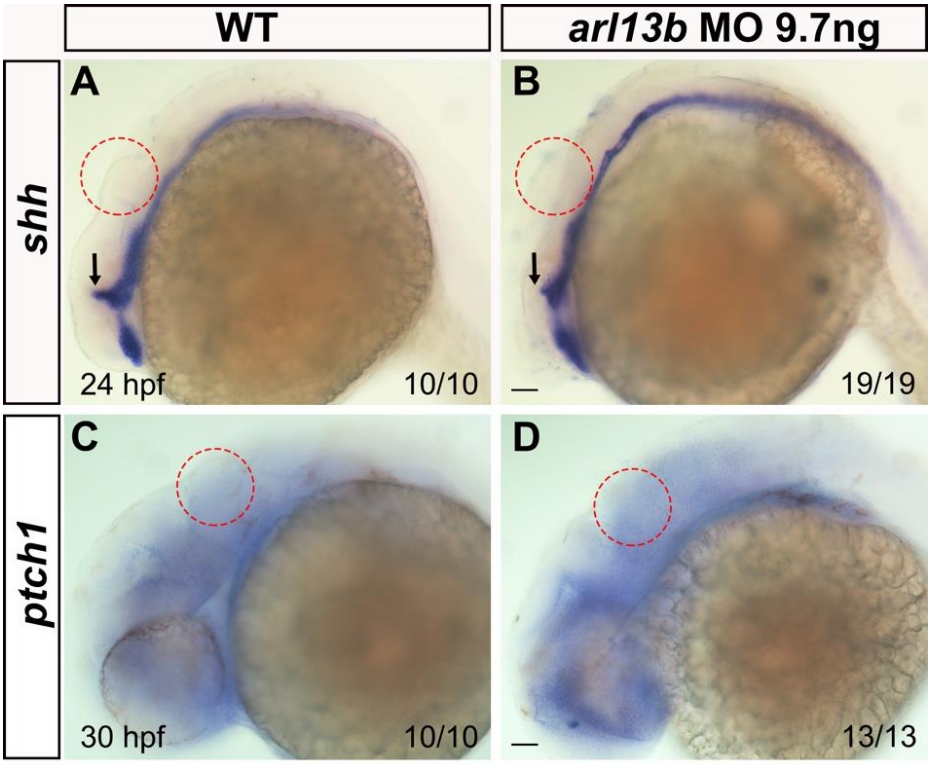

Fig. S5 The expression of *shh* and its receptor, *patched1* (*ptch1*), is not altered by *arl13b* knockdown. **A, C** The expression of Shh signaling pathway components, *shh* and *ptch1*, is barely detectable in the cerebellum (dashed circles) by *in situ* hybridization in wild-type embryos. **B, D** Their expression is not altered by *arl13b* morpholino treatment although the expression of *shh* is changed in the dorsal zona limitans intrathalamica (arrows). A-D, Scale bar 100 μm.

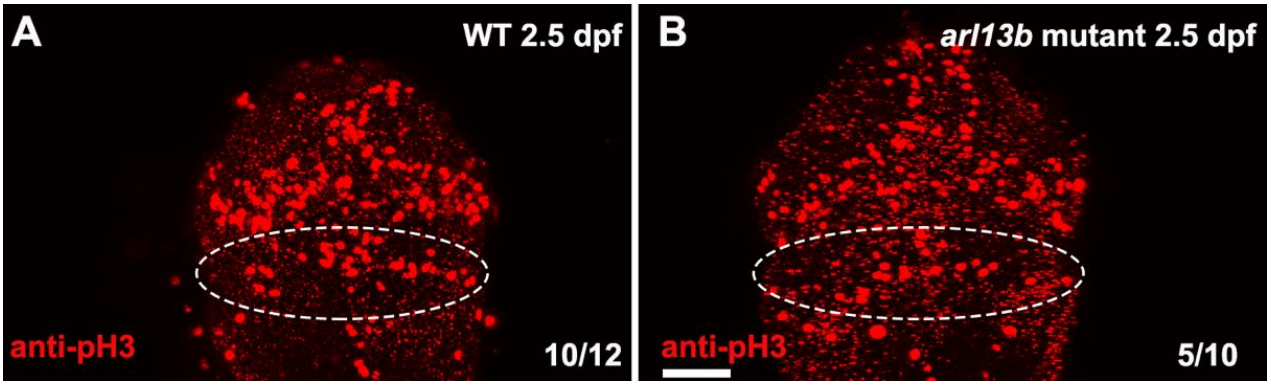

Fig. S6 The proliferation of cells in the cerebellum is impaired in *arl13b* mutants. **A**, **B** Representative images of wild-type and *arl13b* mutant embryos immunostained with anti-pH3 antibody. The cerebellum is outlined by the dashed oval. A-B, Scale bar 100 μm.

Supplemental Tables

Table S1 PCR primers for amplifying gene-specific products

Gene	Primers	Size (bp)
atoh1b	AAGCGTCATTCCTTCATTGGAG ACAAGTATTGACGTGTGCTGAG*	402

atoh1c	ATGCCCCATCCGGACACCCCTTTTGG CTATTTTACACCATTGTTCCTTTCCA*	615
neurod1	TTTGGGCTCTTTCGGAAATCTTG AGACCCGCTGCCTGATAGTG*	469
wnt1	CCCAAACTAACAAGTCAGC AAGCCCTCCCTATTTACCAC*	947
ptch1	ATACCAAGTGTTGCATGGTG AGCTCATTAAACGGCAAGAG*	638
zic1	GAAAATCTGAAAATCCACAAAA GGTGCTGTGGCTGCTCGTGT*	365

* T3 promoter sequence (GGATCCATTAACCCTCACTAAAGGGAA) is added to the 5'-end of the reverse primer for transcription of the gene *in vitro*.

Table S2 PCR primers for quantitative real-time PCR.

Gene	Primer
β-actin	CGGTTTTGCTGGAGATGATGC
	TGGGGTATTTGAGGGTCAGGA
arl13b	GGAGAGAACCTGCAAGGAAGG
	GATTCCTCTGACTGTCGCCG
atoh1a	CCCAGGCAAAATATCCGTCCCT

	CAAGGCTGGTGGGTACTCGCT
atoh1b	CCTCAAGCTGCCTCTGAAGA
	GGGCCATCTGAAGAGTGTCA
atoh1c	CCCGAGAGAGGAGGAGGATG
	GGGTATGGGGTTTCTGGGTC
neurod1	CGAGCAGAGCCAGGAGAT
	AGGGTGGTGTCAAAGAACG
vglut1	CTGTGGAGGGTTTGGAATGGA
	GCGATGTCAAGATGGTTCACA
gabra6b	TCTTGTGTTGGACAGGTGGTG
	AGGTCGGAGTCTGTTGTCATATC
ptf1a	TGCGATCTCACATCCCAACA
	CCGTAGTCTGGGTCATTTGGA
roraa	TGGCCCAGAACATTTCCAAGT
	TGGCAAACTCCACCACATACT
pvalb7	ATCCTGAGTGAGACCGAGCT
	TGTGGTCGAAACTGTCAGCA
calb2a	CCTTACCTTCACTTGGCGGA
	GCTCCTCTCCTCGCAATCTC
aldoca	GCGTCCCGTTTGTCAAGATG
	GGCAAAGTCAGCTCCATCCT
gdf7	AACACGGTCACCAGCTTCAT

	GCAGCTCCACTTCCATCAGT
msx2a	AGGAGCGAGTCAACTTCACG
	CAAGAGCTGAGCAGTGGTGA
msx2b	AGAAGGCGAGAGGATGTGTCCAG
	GATTGGTCTTGTGCTTGCGGAG
wnt1	ATGTGTCCTCCTGGTGTCCTCTC
	CTGGCGGCGACTTAGCAGTG
wnt3	AGCAGCAGCAAACCTTGGAG
	CCCGAGAGCTGGAGAACCAC
wnt3a	CTCCGCTTCTGCCGCAACTAC
	CTGATGCTGACACTCCTGGATGC
gli2a	CAGCACCAGCAGCAGCACTG
	CCTTGGACCGCTTGAAGATCATGG
gli2b	GCGGTCAGCAGCACAGTCAAC
	ACAGTCGTCCATGTCCTCCTTCAG
gli3	TTGCCATGGACCCTCGTTCT
	GGTACTGGCGAGGGTTCGTA
ptch1	TGCGCTGGAGCAGATATCAGAGG
	TCCACGTCCGTCTCCAGATTAGC
smo	TGGCATTTGCTTTGTCGGCT
	CAGCCCTGGATGGTTGCTTTTA