Additional file 1: Tables

Additional file 1: Table S1. gRNA gene-target sequences, primers for PCR genotyping and RT-qPCR probes used in this study

Item	Exon/Intron	Sequence (5'-3')	Amplicon length (bp)	Annealing temp (°C)	Ensemble gene ID
gRNA target: ***shank3b***	ex2	GGGCGTGTTGTTGCCACGGCCGG			ENSDART00000145613
PCR genotyping	in1	ATATGGGTCTTGCTTGTATACAGGCGA	500	60	
	in2	GAATCAACAACTCGGTCTGTACCAATCA		60.3	
RT-qPCR					
shank3b	ex2	CGGCCGTGGCAACAACAC	245	59	ENSDART00000145613
	ex3	TTAAGCACATCGGTCAGGCTTTGT		59	
β-actin	ex2	CGAGCTGTCTTCCCATCCA	102	60	
	ex3	TCACCAACGTAGCTGTCTTTCTG		60	
shank3a	ex5/6	GGCACTTATTACGCTGCTGGATCTG	191	60	ENSDART00000091805.5
	ex6/7	CATGACGGCAAGCCTGGTGAAT		60.7	
RFP		CATGAAGCTGTACATGGAGGGCAC	219	59.4	
		GACTGCTTAAAGAAGTCGGGGATG		59	

Additional file 1: Table S2. SHANK family gene sequences used in this study

Gene	NCBI reference protein number	Amino acid	Species
SHANK3	NP_277052.1	1731	Human
shank3a	XP_017207498.1	1933	Zebrafish
shank3b	XP_009298459.1	1643	Zebrafish
SHANK1	NP_057232.2	2161	Human
shank1	NP_036441.2	1849	Zebrafish
SHANK2	NP_036441	1849	Human
shank2	XP_021326187.1	1810	Zebrafish

Additional file 1: Table S3. Protein homology analysis of zebrafish shank3a and shank3b and human SHANK3

Gene	NCBI reference mRNA number	NCBI reference protein number	Amino acid	vs. human SHANK3 protein		
				Query cover (%)	Identity (%)	Accession (human SHANK3)
shank3a	XM_017352009.2	XP_017207498.1	1933	95	59	NP_277052.1
shank3b	XM_009300184.3	XP_009298459.1	1643	97	55	NP_277052.1

Additional file 1: Table S4. Protein homology comparison between zebrafish shank3a and shank3b

Gene	NCBI reference mRNA number	NCBI reference protein number	Amino acid	shank3a VS. shank3b	
				Query cover(%)	Identity (%)
shank3a	XM_017352009.2	XP_017207498.1	1933	95	59
shank3b	XM_009300184.3	XP_009298459.1	1643		

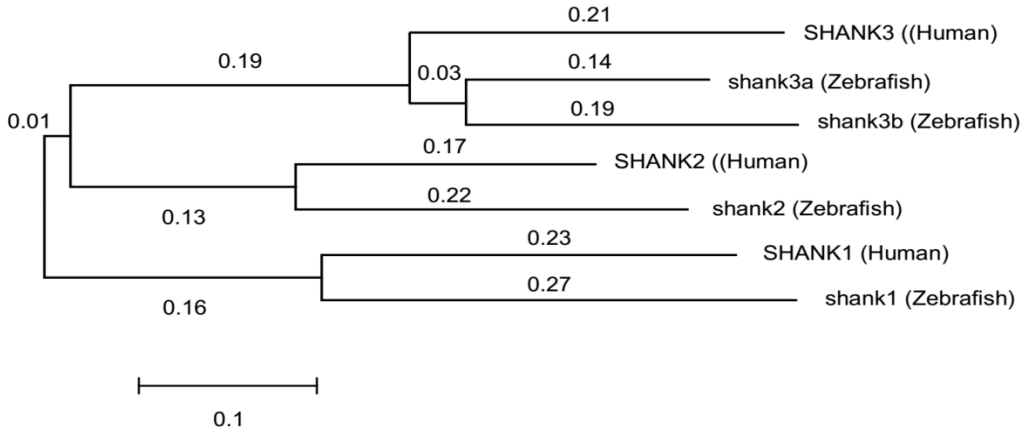
Additional file 1: Table S5. Repetitive behaviors of *shank3b*$^{-/-}$ adult male zebrafish (3.5 mpf)

	Figure "8"		Big circling		Small circling		Walling	
*No.	#shank3b$^{+/+}$	shank3b$^{-/-}$	shank3b$^{+/+}$	shank3b$^{-/-}$	shank3b$^{+/+}$	shank3b$^{-/-}$	shank3b$^{+/+}$	shank3b$^{-/-}$
1	0	15	0	2	0	2	4	2
2	2	1	0	8	0	0	3	4
3	0	1	0	6	0	0	0	0
4	0	2	0	6	1	2	2	2
5	0	14	0	5	0	1	4	8
6	0	8	1	1	0	2	2	0
7	1	6	1	0	2	2	2	0
8	0	8	0	2	0	7	0	2
9	1	2	0	4	0	0	0	8
10	0	4	0	0	0	0	3	3
11	2	18	2	2	0	1	0	2
12	0	2	0	0	1	0	0	7
13	0	1	1	3	1	0	0	3

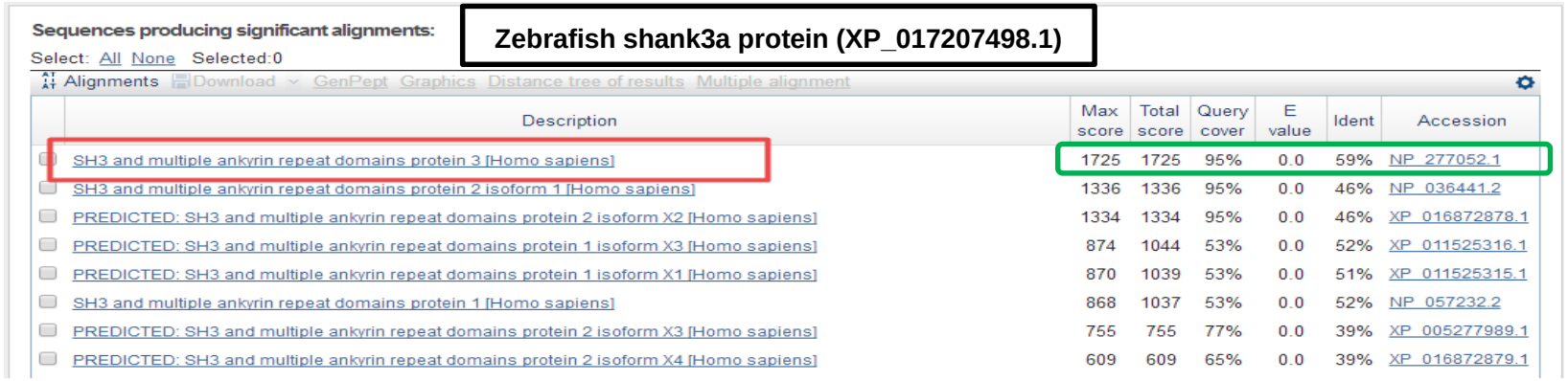
*, Tested adult (3.5 mpf) male zebrafish in this study.

#, Number of each type of repetitive behavior occurring in the examination window.

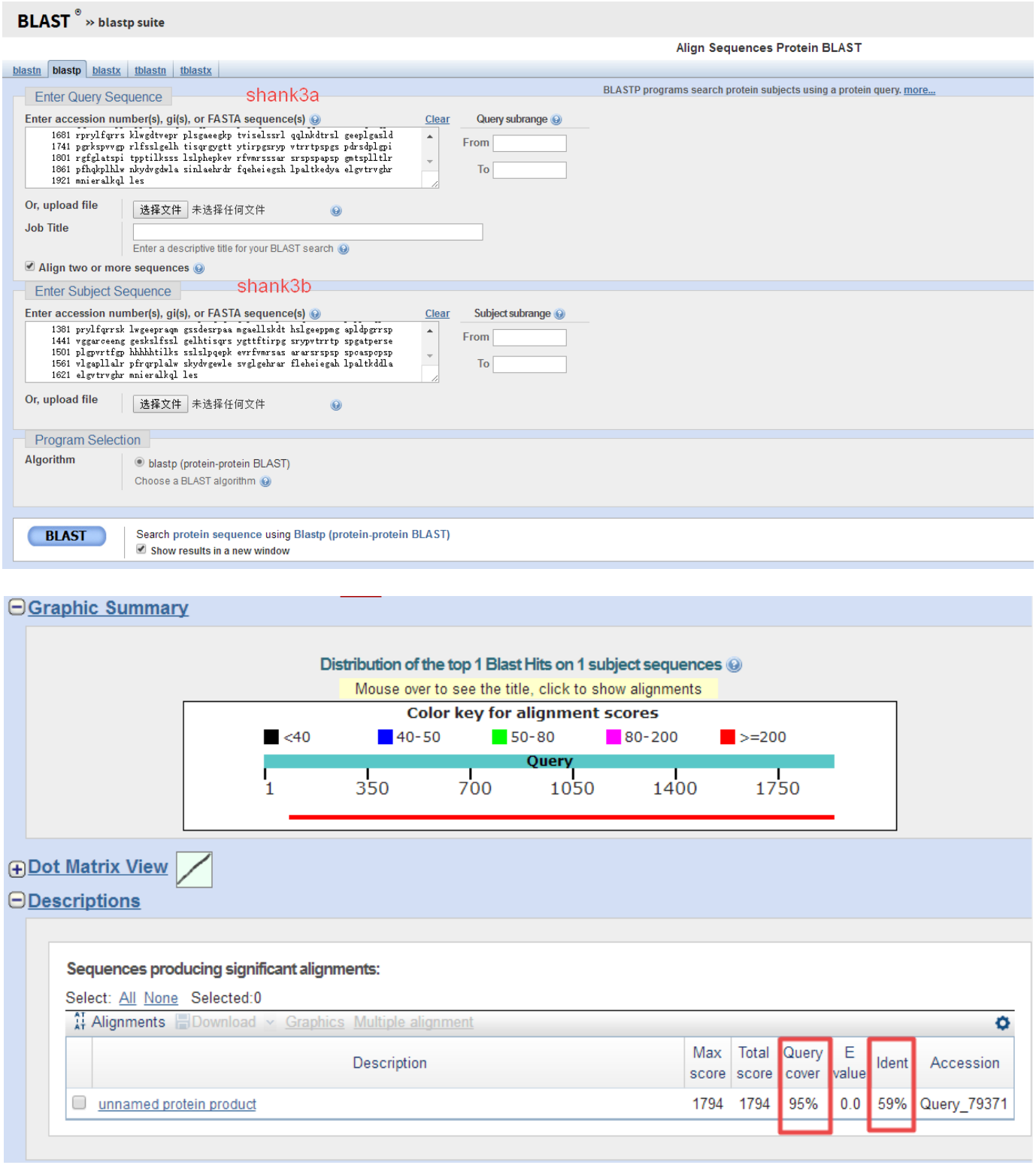
Additional file 1: Figures



Additional file 1: Figure S1. Phylogenetic tree of evolutionary relationship of SHANK family proteins.

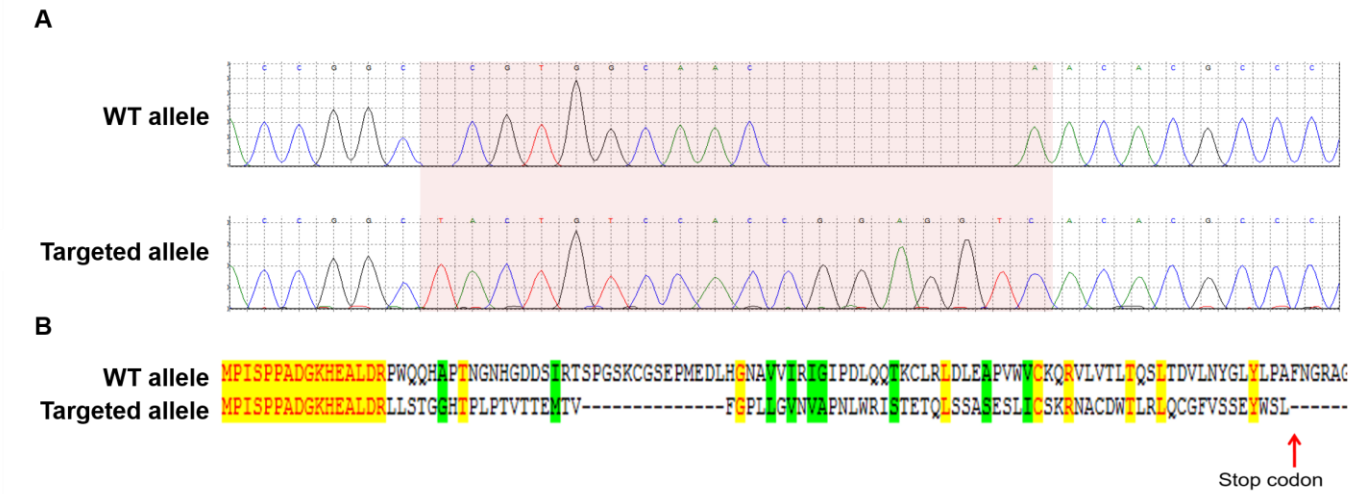


Additional file 1: Figure S2. Homology comparison of zebrafish shank3a and shank3b with human SHANK3

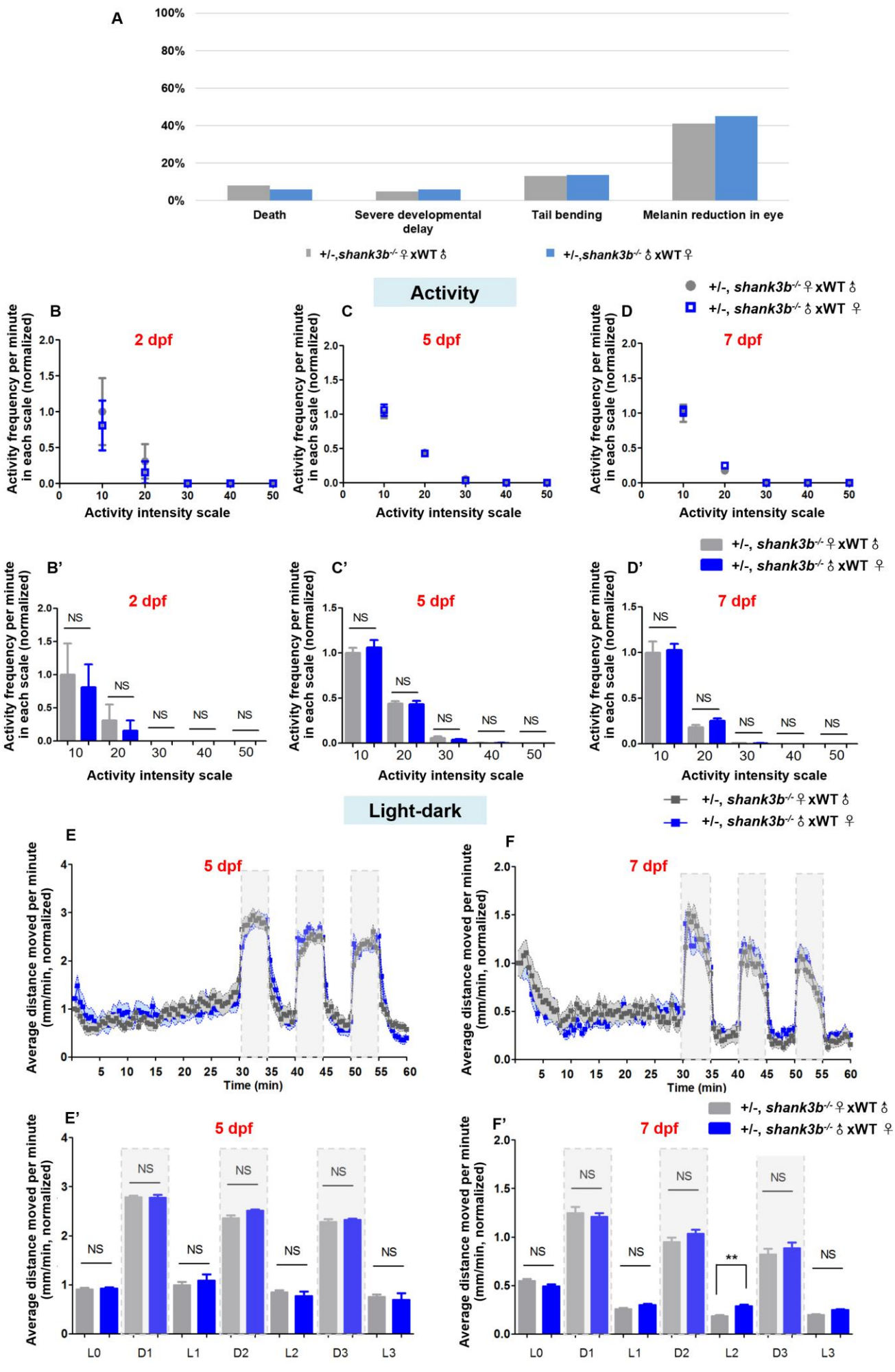


Additional file 1: Figure S3. Homology comparison between zebrafish shank3a and shank3b.

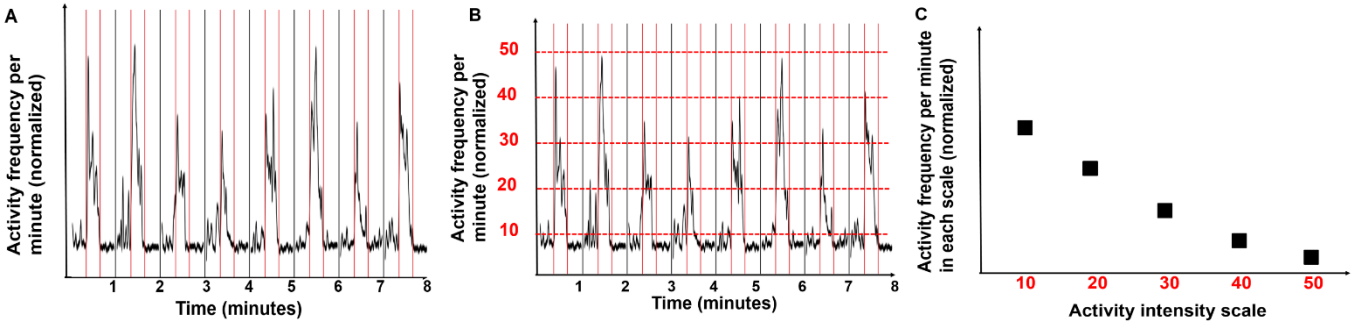
We used the “Protein BLAST” tool of NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS) to compare the homology of zebrafish shank3a and shank3b.



Additional file 1: Figure S4. *shank3b* target-mutation in zebrafish via CRISPR-Cas9 system. (A) Sanger sequencing result of WT and *shank3b* site mutations in zebrafish. *shank3b*$^{-/-}$ zebrafish has a 5-base deletion and a 13-base insertion. **(B)** shank3b protein of WT zebrafish and *shank3b*$^{-/-}$ predicted translated protein sequences are shown. Protein spatial structure prediction results of WT.


A
100%
80%
60%
40%
20%
0%
Death
Severe developmental delay
Tail bending
Melanin reduction in eye
+/-,shank3b-/- ♀ xWT ♂
+/-,shank3b-/- ♂ xWT ♀
Activity
B
2 dpf
C
5 dpf
D
7 dpf
Activity frequency per minute in each scale (normalized)
Activity intensity scale
+/-, shank3b-/- ♀ xWT ♂
+/-, shank3b-/- ♂ xWT ♀
B'
C'
D'
NS
Light-dark
E
F
Average distance moved per minute (mm/min, normalized)
Time (min)
E'
F'
L0
D1
L1
D2
L2
D3
L3
**

Additional file 1: Figure S5. Examinations of maternal or paternal origin effects on the morphological and behavioral phenotypes. (A) Morphological examination of the *shank3b*$^{-/-}$ ♀ and *shank3b*$^{-/-}$♂ offspring at 1 dpf. **(B-D)** Activity of *shank3b*$^{-/-}$ ♀ and *shank3b*$^{-/-}$♂ offspring at 2 dpf, 5 dpf and 7 dpf. **(E-F)** Light/dark response of *shank3b*$^{-/-}$ ♀ and *shank3b*$^{-/-}$♂ offspring at 5 dpf and 7 dpf. *shank3*$^{+/-}$ ♀ were obtained from crosses of *shank3b*$^{-/-}$ ♀ with WT♂ zebrafish. *shank3*$^{+/-}$♂ were obtained from crosses of *shank3b*$^{-/-}$♂ with WT♀ zebrafish. There were no significant differences between the two groups in **(B'-D')** activity test and **(E'-F')** light/dark response test (except in the second light response). N=24 for each group.



Additional file 1: Figure S6. Analysis of activity frequency at different activity intensity scale. Viewpoint's zebrabox was used to track the larvae and ViewPoint's zebralab system was used to analyze the larvae activity, as reported before [1, 2]. **(A)** The X-axis shows the time, and the Y-axis shows the activity frequency traveled by larvae in each 1-minute time bin. **(B)** To further analyze the variances of different activity intensity scales among WT, *shank3b*$^{+/-}$ and *shank3b*$^{-/-}$ zebrafish, we divided the activity equally into five levels (10, 20, 30, 40 and 50). **(C)** Next, we calculated the activity frequency of different activity intensity scales.

[1]. Rihel, J., et al., Zebrafish Behavioral Profiling Links Drugs to Biological Targets and Rest/Wake Regulation. Science, 2010.

[2]. Kokel, D., et al., Rapid behavior-based identification of neuroactive small molecules in the zebrafish. Nature Chemical Biology, 2010.