

Supplementary Materials:

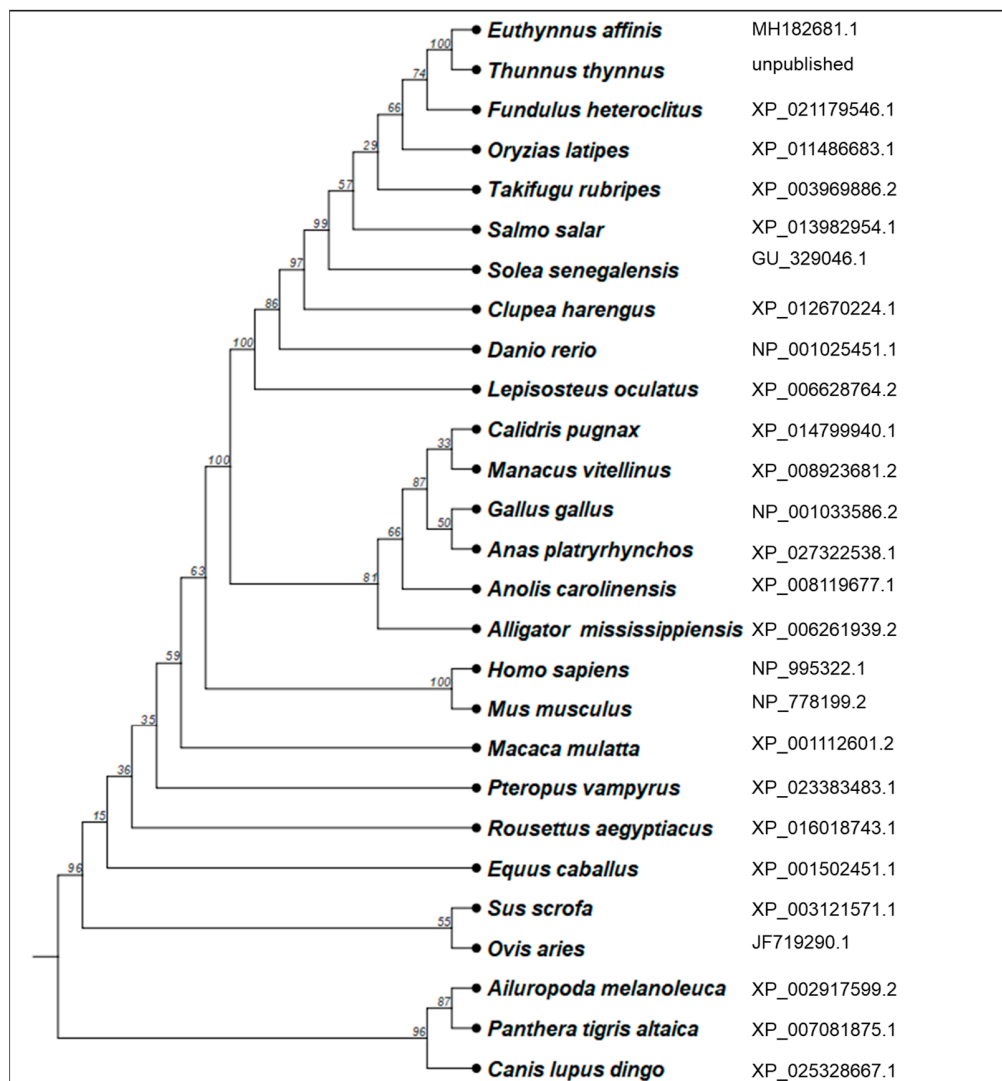


Figure S2. Dendrogram showing *slc24a5* relationships between kawakawa with those of other selected species based on comparisons of their polypeptide sequence deduced from cDNA. Numbers adjacent to each node represent bootstrap values (100 bootstraps replicates). Reference amino acid sequence of *slc24a5* used in this study from different species, and their GenBank accession numbers are showing on the right-hand side.

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Kawakawa      1  -----MNKVAALQKKKRKDFIPYFLGFVLFLYCTVHLVSFTAKTTQETHSVRVRRA
Tuna          1  -----MNKVTALQKKKRKDFIPYFLGFVLFLYCTVHLVSFTAKTAQETHFVRVRRA
Zebrafish     1  -----MRTDVFLQRRRRRDVLLSIALLLLLIFAIVHLVFCAGLSFQGSSSARVRRD
Chicken       1  -----MQARAGLWRRRRS-----ALLLPLLLLLALWGWKPRG-----ARAQRPPTD
Wall_lizard   1  -----MHR--GGSQRRRSRGIRVVAAALLFFFATVVPLGK----LPVASAQEIPGD
Mouse         1  -----MRTKWGPTWTRRV-----LLLGIFWVSAYLPVRG-----VSLPRRLPRA
Frog          1  MERGSVALNVGRRPIGKRLPCRAPLGSFALFLIVCGTVYLVNQVATSLVVTGTQRIRRD

Kawakawa      52  LENEEECILPOSSSEFPDGFFTVQERKDGGLVIYFMLIFYMLLAVAIACDDYFLPSLEVIS
Tuna          52  LENEEECILPOSSSEFPDGFFTVQERKDGGLVIYFMLIFYMLLAVAIACDDYFLPSLEVIS
Zebrafish     52  LENASECVQPOSSSEFPEGFFTVQERKDGGLLIYFMIIFYMLLSVSIVCDEYFLPSLEVIS
Chicken       40  TENRTDCVP-PSSEFPEGFFTVQERKDGGIVIYFLIILYMFLASIVCDYFLPSLEIIT
Wall_lizard   46  SENKTCCVASPSSEFPEGFFTVQEREDGGIIIYFLIILYMFLASIVCDYFLPSLEIIS
Mouse         40  TGNSTCAVPSPSEFPEGFFTVQESTDGGIVIYFLIILYMCMASIVCDKYFLPSLEIIS
Frog          61  VENEETLCIASPSSEFPEDDFFTVQERKQGGLIIHFLIILYMFLAVIAVICESSYFIPSLEVIS

Kawakawa      112  DRLGLSQDVAGATFMAAGSSAPELVTAFLGVFVTKDGIGVSTIVGSAVYNLLGICAACGL
Tuna          112  DRLGLSQDVAGATFMAAGSSAPELVTAFLGVFVTKDGIGVSTIVGSAVYNLLGICAACGL
Zebrafish     112  ERLGLSQDVAGATFMAAGSSAPELVTAFLGVFVTKDGIGVSTIVGSAVYNLLCICAACGL
Chicken       99  ECLGLSQDVAGATFMAAGSSAPELVTAFLGVFVTKDGIGVSTIVGSAVYNLLGICAACGL
Wall_lizard   106  DALGLSQDVAGATFMAAGSSAPELVTAFLGVFVTKDGIGVSTIVGSAVYNLLGICAACGL
Mouse         100  DSLGLSQDVAGATFMAAGSSAPELVTAFLGVFVTKDGIGVSTIVGSAVYNLLGICAACGL
Frog          121  ERLGLSQDVAGATFMATGSSAPEFVTVFLGVFVTKDGIGVSTIVGSAVYNLLGICAACGL

Kawakawa      172  LASMAGRLTCWPLFRDCLAYAISVAAVAIAIISDNKVYWYEAASLLLVYSVYIVLCFDLR
Tuna          172  LASMAGRLTCWPLFRDCLAYGISVAAVAIAIISDNKVYWYEAASLLLVYGVYIVLCFDLR
Zebrafish     172  LSSAVGRLSCWPLFRDCVAYAISVAAVAIAIISDNRVYWYDGACLLLVYGVYVAVLCFDLR
Chicken       159  LSTVVSRLSCWPLFRDCLAYTISAAVLAMISDSRIYWYESASLLLIYGCYILVCFDIK
Wall_lizard   166  LSGMVSRLSCWPLFRDCLAYAVSAAVLAMITDNKVYWYESASLLLIYGVYILVCFDIK
Mouse         160  LSNMVSTLSCWPLFRDCAVYAVSGAVFGIIFDNRIYWYESAGLLLIYGYVLLCFDTT
Frog          181  LSLSVSRLTCWPLFRDCVAYAISVAAVAIAITFDNRIYWYESASLLLIYGYIVTMCFDIR

Kawakawa      232  ISEFVLRKLSPCCTCLGKGSGEKTETOPLMGWNDDTSLRVHSRSRTDSGIFQDDSGYSHL
Tuna          232  ISEFVLRKVSPCCCTCLGRGSGEKTETOPLMGWNDDTSLRVHSRSRTDSGIFQDDSGYSHL
Zebrafish     232  ISEYVMQRFSPCCWCLKPRRDSSGEQOPLVGWSDDSSLRVQRRRSRNDSGIFQDDSGYSHL
Chicken       219  INRCLMKKLSPCCSCFTKATEQSGEQOPLAGWREERGPLIRQQSRTDSGIFQDELDYSQL
Wall_lizard   226  INQYIMKKFSRCCTCFTVTAET-EQOPLMGWKEESGPLLRROSRADSGIFHDESDYAOL
Mouse         220  ISRHVMKTCSPCCECLARAMERIEQOTLLGWEDESQLFIRRQSRTDSGIFQEDSGYSQL
Frog          241  INQYIMRRFSPCCTCCREAMVGNTEHEPLLGWKEDSLPVIRRHSRSDSGIF-----

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Figure S3. Deduced amino acid sequence alignment of kawakawa *slc24a5* (MH_182681.1) with other species; Tuna (Atlantic bluefin tuna, unpublished data), Zebrafish (NP_001025451), Chicken (NP_001033586.2), Wall Lizard (XP_028561132.1), Mouse (NP_778199.2), and Frog (XP_002936612.2). Identical residues in each species of peptides are marked in white font on a black background while less similar peptide shown in black font on a gray background. Non-identical peptides are shown in black font on a white background.

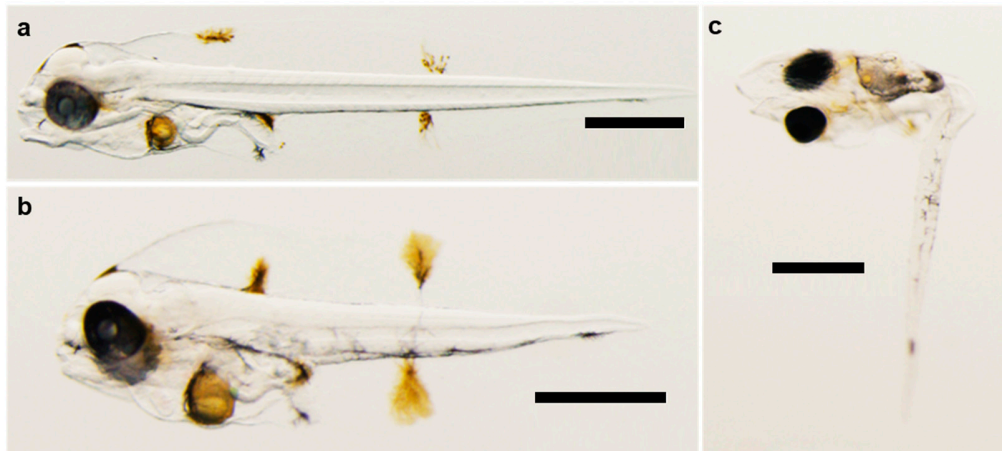


Figure S4. *slc24a5* TALEN induced mutants and their body development (sign of toxicity): a mutant with normal development at 72 hpf (a); a dwarf body at 72 hpf (b), and a twisted body at 120 hpf (c). Scale: 1mm.

Table S1. Phenotype and appearance ratio in F0 *slc24a5* genetically modified by TALEN.

Group	Stages of embryo injected	Dose (ng/μl)	<u>Survival rate</u>		Total no. of embryos	<u>Classification according to phenotype</u>			
			Cont. (%)	Exp. (%)		Type I (low)	Type II (moderate)	Type III (severe)	Type IV (abnormal)
<i>slc24a5</i> 47A	1-2 cell	150	14 (93%)	4 (67%)	4	4 (100%)	0 (0%)	0 (0%)	0 (0%)
	1-2 cell	150	24 (80%)	27 (100%)	27	25 (93%)	2 (7%)	0 (0%)	0 (0%)
	1-2 cell	150	29 (97%)	16 (89%)	16	13 (81%)	2 (13%)	1 (6%)	0 (0%)
	1-2 cell	150	175 (88%)	21 (91%)	21	21 (100%)	0 (0%)	0 (0%)	0 (0%)
<i>slc24a5</i> 63A	1-2 cell	150	57 (95%)	48 (94%)	48	26 (54%)	11 (23%)	5 (10%)	6 (13%)
	1-2 cell	150	30 (100%)	18 (95%)	18	7 (39%)	7 (39%)	1 (6%)	3 (17%)
	1-2 cell	150	26 (81%)	22 (93%)	22	7 (32%)	3 (14%)	7 (32%)	5 (23%)
	1-2 cell	150	29 (97%)	42 (91%)	42	22 (52%)	7 (17%)	7 (17%)	6 (14%)
<i>slc24a5</i> 47B	1-2 cell	150	25 (100%)	10 (91%)	10	4 (40%)	2 (20%)	4 (40%)	0 (0%)
	1-2 cell	150	28 (93%)	18 (86%)	18	13 (72%)	3 (17%)	2 (11%)	0 (0%)
	1-2 cell	150	29 (97%)	11 (92%)	11	8 (73%)	3 (27%)	0 (0%)	0 (0%)
	1-2 cell	150	46 (92%)	22 (96%)	22	10 (45%)	4 (18%)	7 (32%)	1 (5%)
<i>slc24a5</i> 63B	1-2 cell	150	23 (92%)	12 (63%)	12	0 (0%)	1 (9%)	4 (33%)	7 (58%)
	1-2 cell	150	30 (100%)	11 (73%)	11	1 (9%)	1 (9%)	3 (27%)	6 (55%)
	1-2 cell	150	57 (95%)	48 (78%)	48	27 (56%)	3 (6%)	7 (15%)	11 (23%)
	1-2 cell	150	175 (88%)	16 (72%)	16	7 (44%)	1 (6%)	4 (25%)	4 (25%)