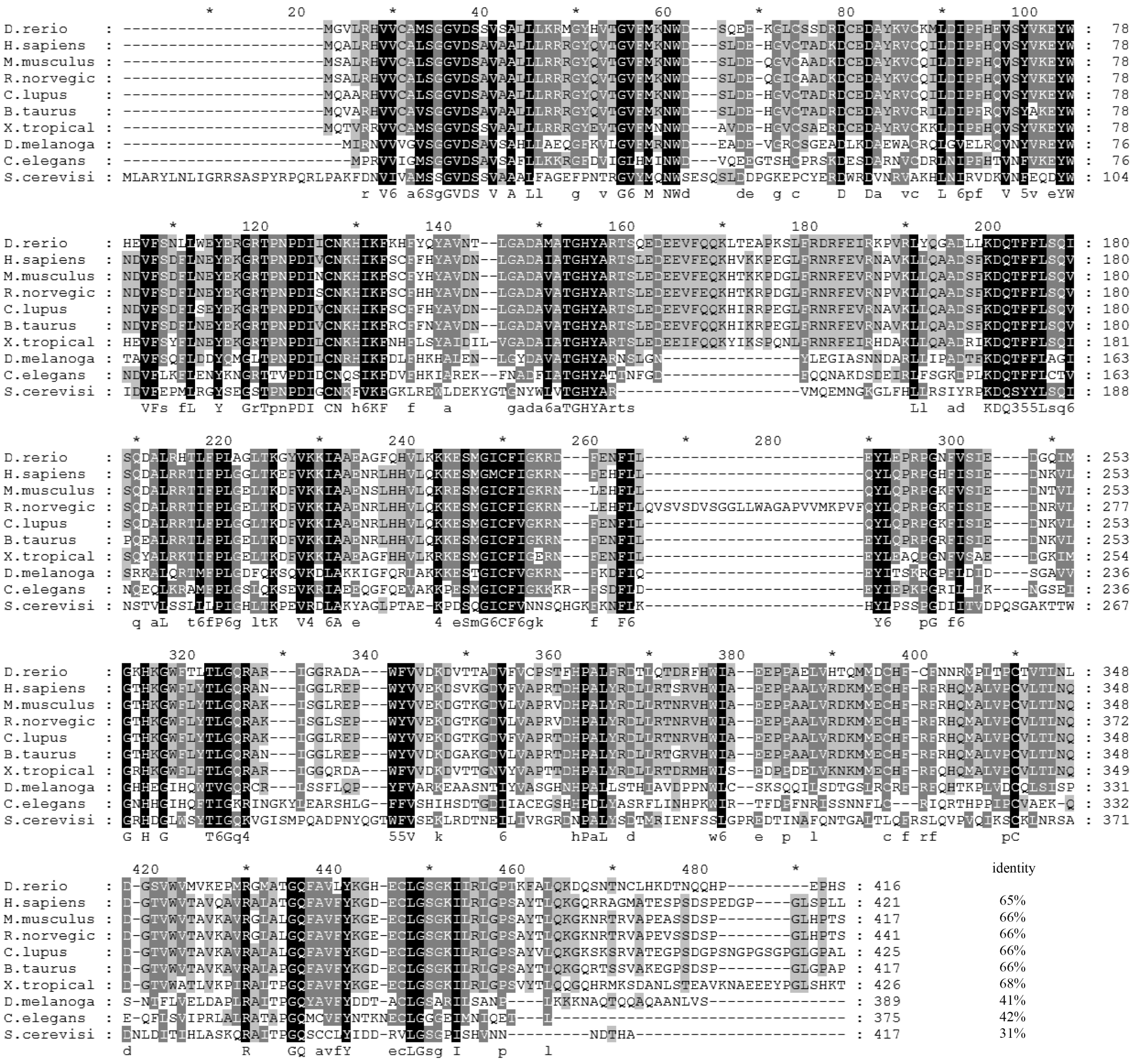
Supplemental Data

Table S1
Figure S1
Figure S2
Figure S3
Figure S4

Supplementary Table S1. The sequence of tRNA probes

tRNA Probes	Sequence
mt-tRNA^{Lys}	TCACTAAGGGTGGTCGGTAAGCACCAAGTT
mt-tRNA^{Glu}	AATTCTTACTCAGACTTTAACTGAGACCGG
mt-tRNA^{Gln}	TTAGAAAGAAGGGGTCGAACCCATGCCCA
mt-tRNA$^{Leu（UUR）}$	GGCCTTTTGCAATTACCGAGCTCTGCCATC
mt-tRNA^{Trp}	TCTACTGAGAGCTTTGAAGGCTCTTGGTCT
mt-tRNA^{His}	GGGGCTTTAACCCCTATCTTTAGAGTCACG
mt-tRNA^{Met}	TGATGAAGGAGGGACTTTAACCATCATGTT
ct-tRNA^{Glu}	TTCCCTGACCGGGAATCGAACCCGGGCCGC
ct-tRNA^{Ala}	TGGAGATGCCGGGGATTGAACCCGGGGCCT
ct-tRNA^{Gly}	TGCATTGGCCGGGAATCGGACCCGGGTCAC
5S rRNA	GCAACCTAGTTTTCCCATGTGGTCTCCCAT

Supplementary Figure S1



```
                      *        20         *        40         *        60         *        80         *       100
D.rerio    : ----------------------MGVLRHVVCAMSGGVDSSVSALLLKRMGYHVTGVFMKNWD---SQEE-KGLCSSDRDCEDAYKVCKMLDIPFHEVSYVKEYW :  78
H.sapiens  : ----------------------MQALRHVVCALSGGVDSAVAALLLRRRGYQVTGVFMKNWD---SLDE-HGVCTADKDCEDAYRVCQILDIPFHQVSYVKEYW :  78
M.musculus : ----------------------MSALRHVVCALSGGVDSAVAALLLRRRGYQVTGVFMKNWD---SLDE-QGVCAADKDCEDAYKVCQILDIPFHQVSYVKEYW :  78
R.norvegic : ----------------------MSALRHVVCALSGGVDSAVAALLLRRRGYQVTGVFMKNWD---SLDE-QGICAADKDCEDAYKVCQILDIPFHQVSYVKEYW :  78
C.lupus    : ----------------------MQAARHVVCALSGGVDSAVAALLLRRRGYQVTGVFMKNWD---SLDE-HGVCTADRDCEDAYRVCQILDIPFHQVSYVKEYW :  78
B.taurus   : ----------------------MQVARHVVCAVSGGVDSAVAALLLRRRGYQVTGVFMKNWD---SLDE-HGVCTADRDCEDAYRVCRILDIPFRQVSYAKEYW :  78
X.tropical : ----------------------MQTVRRVVCAMSGGVDSSVAALLLRRRGYEVTGVFMNNWD---AVDE-HGVCSAERDCEDAYRVCKKLDIPFHQVSYVKEYW :  78
D.melanoga : ------------------------MIRNVVVGVSGGVDSAVSAHLLAEQGFKVLGVFMRNWD---EADE-VGRCSGEADLKDAEWACRQLGVELRQVNYVREYW :  76
C.elegans  : -------------------------MPRVVIGMSGGVDSAVSAFLLKKRGFDVIGLHMINWD---VQEEGTSHCPRSKDESDARNVCDRLNIPFHTVNFVKEYW :  76
S.cerevisi : MLARYLNLIGRRSASPYRPQRLPAKFDNVIVAMSSGVDSSVAAALFAGEFPNTRGVYMQNWSESQSLDDPGKEPCYERDWRDVNRVAKHLNIRVDKVNFEQDYW : 104
                                       r V6 a6SgGVDS V A Ll   g  v G6 M NWd     de  g c    D  Da  vc  L 6pf  V 5v eYW

                  *       120         *       140         *       160         *       180         *       200
D.rerio    : HEVFSNLLWEYERGRTPNPDIICNKHIKFKHFYQYAVNT--LGADAMATGHYARTSQEDEEVFQQKLTEAPKSLFRDRFEIRKPVRLYQGADLLKDQTFFLSQI : 180
H.sapiens  : NDVFSDFLNEYEKGRTPNPDIVCNKHIKFSCFFHYAVDN--LGADAIATGHYARTSLEDEEVFEQKHVKKPEGLFRNRFEVRNAVKLLQAADSFKDQTFFLSQV : 180
M.musculus : NDVFSDFLNEYEKGRTPNPDINCNKHIKFSCFYHYAVDN--LGADAVATGHYARTSLEDEEVFEQKHTKKPDGLFRNRFEVRNPVKLLQAADSFKDQTFFLSQV : 180
R.norvegic : NDVFSDFLNEYEKGRTPNPDISCNKHIKFSCFHHYAVDN--LGADAVATGHYARTSLEDEEVFEQKHTKRPDGLFRNRFEVRNPVKLLQAADSFKDQTFFLSQV : 180
C.lupus    : NDVFSDFLSEYEKGRTPNPDIVCNKHIKFSCFFHYAVDN--LGADAVATGHYARTSLEDEEVFQQKHIRRPEGLFRNRFEVRNAVKLLQAADSFKDQTFFLSQV : 180
B.taurus   : NDVFSDFLNEYEKGRTPNPDIVCNKHIKFRCFFNYAVDN--LGADAVATGHYARTSLEDEEVFQQKHIKRPEGLFRNRFEVRNAVKLLQAADSFKDQTFFLSQV : 180
X.tropical : HEVFSYFLNEYEKGRTPNPDILCNKHIKFNHFLSYAIDIL-VGADAIATGHYARTSLEDEEIFQQKYIKSPQNLFRNRFEIRHDAKLLQAADRIKDQTFFLSQI : 181
D.melanoga : TAVFSQFLDDYQMGLTPNPDILCNRHIKFDLFHKHALEN--LGYDAVATGHYARNSLGN---------------YLEGIASNNDARLLIPADTFKDQTFFLAGI : 163
C.elegans  : NDVFLKFLENYKNGRTTVPDIDCNQSIKFDVFHKIAREK--FNADFIATGHYATTNFGD---------------FQQNAKDSDEIRLFSGKDPLKDQTFFLCTV : 163
S.cerevisi : IDVFEPMLRGYSEGSTPNPDIGCNKFVKFGKLREWLDEKYGTGNYWLVTGHYAR--------------------VMQEMNGKGLFHLLRSIYRPKDQSYYLSQI : 188
               VFs fL  Y  GrTpnPDI CN h6KF  f   a      gada6aTGHYArts                              Ll  ad  KDQ355Lsq6

              *       220         *       240         *       260         *       280         *       300         *
D.rerio    : SQDALRHTLFPLAGLTKGYVKKIAAEAGFQHVLKKKESMGICFIGKRD---FENFIL------------------------EYLEPRPGNFVSIE----DGQIM : 253
H.sapiens  : SQDALRRTIFPLGGLTKEFVKKIAAENRLHHVLQKKESMGMCFIGKRN---FEHFLL------------------------QYLQPRPGHFISIE----DNKVL : 253
M.musculus : SQDALRRTIFPLGELTKDFVKKIAAENSLHHVLQKRESMGICFIGKRN---LEHFLL------------------------QYLQPRPGKFVSIE----DNTVL : 253
R.norvegic : SQDALRRTIFPLGELTKDFVKKIAAENRLHHVLQKKESMGICFIGKRN---LEHFLLQVSVSDVSGGLLWAGAPVVMKPVFQYLQPRPGKFISIE----DNRVL : 277
C.lupus    : SQDALRRTLFPLGGLTKDFVKKIAAENRLHHVLQKKESMGICFVGKRN---FENFIL------------------------QYLQPRPGKFISIE----DNKVL : 253
B.taurus   : PQEALRRTLFPLGELTKDFVKKIAAENRLHHVLQKKESMGICFIGKRN---FENFIL------------------------EYLQPRPGRFISIE----DNKVL : 253
X.tropical : SQYALRKTIFPLGELTKDFVKKIAAEAGFHHVLKRKESMGICFIGERN---FENFIL------------------------EYLEAQPGNFVSAE----DGKIM : 254
D.melanoga : SRKALQRTMFPLGDFQKSQVKDLAKKIGFQRLAKKKESTGICFVGKRN---FKDFIQ------------------------EYITSKRGPFLDID----SGAVV : 236
C.elegans  : NQEQLKRAMFPLGSLQKSEVKRIAEEQGFQEVAKKPESMGICFIGKKKR--FSDFLD------------------------EYIEPKPGRIL-LK----NGSEI : 236
S.cerevisi : NSTVLSSLLLPIGHLTKPEVRDLAKYAGLPTAE-KPDSQGICFVNNSQHGKFKNFLK------------------------HYLPSSPGDIITVDPQSGAKTTW : 267
              q aL  t6fP6g ltK  V4 6A e        4 eSmG6CF6gk     f  F6                          Y6   pG f6

                  320         *       340         *       360         *       380         *       400         *
D.rerio    : GKHKGWFTLTLGQRAR---IGGRADA---WFVVDKDVTTADVFVCPSTFHPALFRDTIQTDRFHWIA--EEPPAELVHTQMMDCHF-CFNNRMPITPCTVTINL : 348
H.sapiens  : GTHKGWFLYTLGQRAN---IGGLREP---WYVVEKDSVKGDVFVAPRTDHPALYRDLIRTSRVHWIA--EEPPAALVRDKMMECHF-RFRHQMALVPCVLTINQ : 348
M.musculus : GTHKGWFLYTLGQRAK---ISGLREP---WYVVEKDGTKGDVLVAPRVDHPALYRDLIRTNRVHWIA--EEPPAALVRDKMMECHF-RFRHQMALVPCVLTINQ : 348
R.norvegic : GTHKGWFLYTLGQRAK---ISGLSEP---WYVVEKDGTKGDVLVAPRVDHPALYRDLIRTNRVHWIA--EEPPAALVRDKMMECHF-RFRHQMALVPCVLTINQ : 372
C.lupus    : GTHKGWFLYTLGQRAK---IGGLREP---WYVVEKDGTKGDVFVAPRTDHPALYRDLIRTNRVHWIA--EEPPAALVRDKMMECHF-RFRHQMALVPCVLTINQ : 348
B.taurus   : GTHKGWFLYTLGQRAN---IGGLREP---WYVVDKDGAKGDVLVAPRTDHPALYRDLIRTGRVHWIA--EEPPAALVRDKMMECHF-RFRHQMALVPCVLTINQ : 348
X.tropical : GRHKGWFLFTLGQRAR---IGGQRDA---WFVVDKDVTTGNVYVAPTTDHPALYRDLIRTDRMHWLS--EDPPDELVKNKMMECHF-RFQHQMALVPCVLTINQ : 349
D.melanoga : GHHEGIHQWTVGQRCR---LSSFLQP---YFVARKEAASNTIYVASGHNHPALLSTHIAVDPPNWLC--SKSQQILSDTGSLRCRF-RFQHTKPLVDCQLSISP : 331
C.elegans  : GNHHGIHQFTIGKRINGKYLEARSHLG--FFVSHIHSDTGDIIACEGSHHPDLYASRFLINHPKWIR--TFDPFNRISSNNFLC---RIQRTHPPIPCVAEK-Q : 332
S.cerevisi : GRHDGLWSYTIGQKVGISMPQADPNYQGTWFVSEKLRDTNEILIVRGRDNPALYSDTMRIENFSSLGPREDTINAFQNTGALTLQFRSLQVPVQIKSCKINRSA : 371
             G H G    T6Gq4               55V  k      6       hPaL  d        w6   e  p  l       c f rf       pC

              420         *       440         *       460         *       480         *                          identity
D.rerio    : D-GSVWVMVKEPMRGMATGQFAVLYKGH-ECLGSGKIIRIGPTKFALQKDQSNTNCLHKDTNQQHP---------EPHS : 416
H.sapiens  : D-GTVWVTAVQAVRALATGQFAVFYKGD-ECLGSGKIIRIGPSAYTLQKGQRRAGMATESPSDSPEDGP----GLSPLL : 421        65%
M.musculus : D-GTVWVTAVKAVRGIALGQFAVFYKGE-ECLGSGKIIRIGPSAYTLQKGKNRTRVAPEASSDSP--------GLHPTS : 417        66%
R.norvegic : D-GTVWVTAVKAVRGIALGQFAVFYKGE-ECLGSGKIIRIGPSAYTLQKGKNRTRVAPEVSSDSP--------GLHPTS : 441        66%
C.lupus    : D-GTVWVTAVKAVRAIALGQFAVFYKGD-ECLGSGKIIRIGPSAYVLQKGKSKSRVATEGPSDGPSNGPGSGPGLGPAL : 425        66%
B.taurus   : D-GTVWVTAVKAVRAIAPGQFAVFYKGD-ECLGSGKIIRIGPSAYTLQKGQRTSSVAKEGPSDSP--------GLGPAP : 417        66%
X.tropical : D-GTVWATLVKPIRALTPGQFAVFYKGE-ECLGSGKIIRIGPSVYTLQQGQHRMKSDANLSTEAVKNAEEEYPGLSHKT : 426        68%
D.melanoga : S-NTFLVELDAPLRAITPGQYAVFYDDT-ACLGSARIISANP----IKKKNAQTQQAQAANLVS--------------- : 389        41%
C.elegans  : E-QFLSVIPRLALRATAPGQMCVFYNTKNECLGGGEIMNIQET---I-------------------------------- : 375        42%
S.cerevisi : DNLDITIHLASKQRAITPGQSCCLYIDD-RVLGSGPISHVNN---------NDTHA----------------------- : 417        31%
             d            R    GQ avfY    ecLGsg I    p    l
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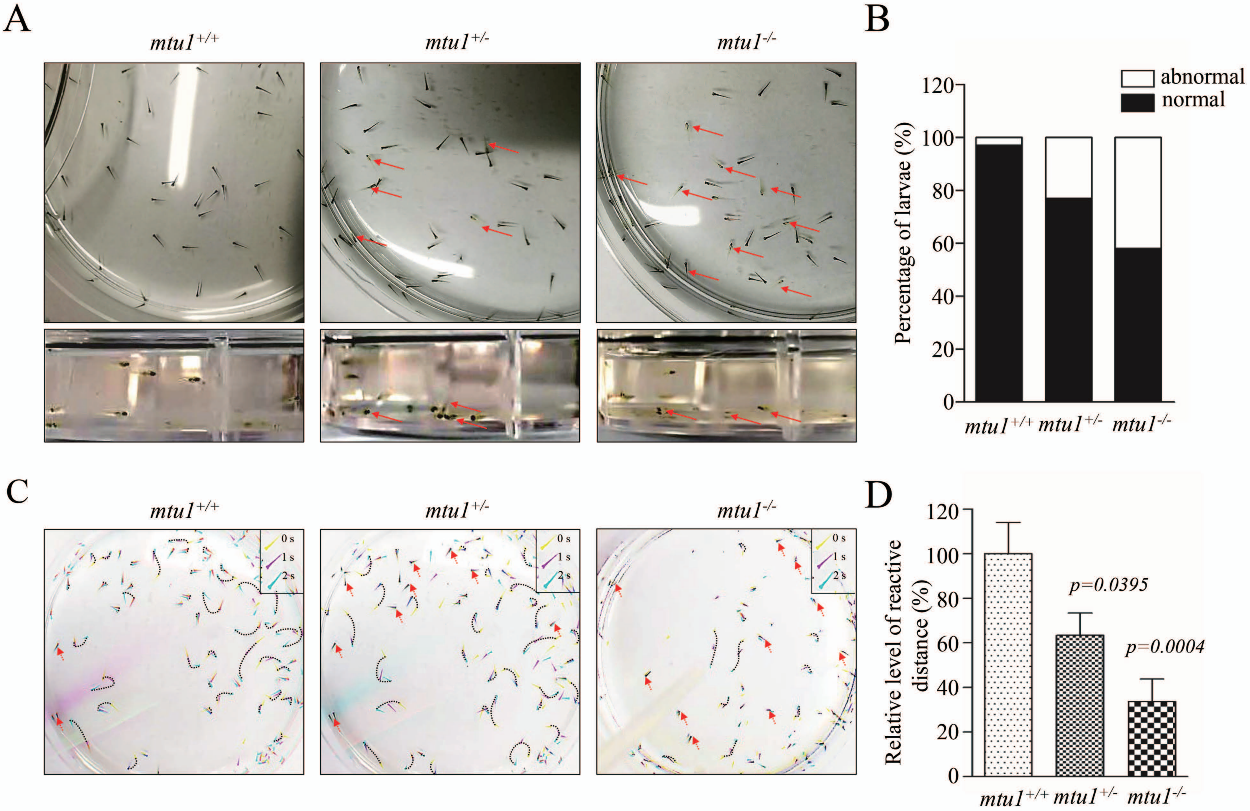
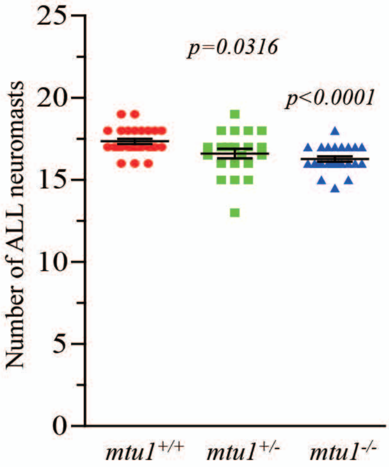
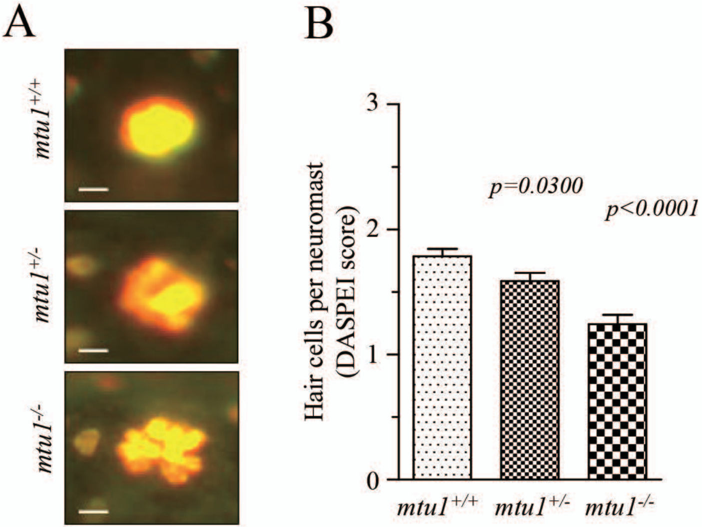
Supplementary Figure S1 :Amino acid sequence multiple alignment of the TRMU family encompassing *D.rerio, H.sapiens, M.musculus, R.Norvegicus, C.lupus, B.taurus, X.tropicalis, D.melanogaster, C.elegans,* and *S.cerevisiae* were performed using Clustal W. zebrafish Trmu shares about 65% sequence identity with human TRMU . (*Danio rerio* : NP_001018435.1 ; *Homo sapiens* : NP_060476.2 ; *Mus musculus* : NP_082339.1 ; *Rattus norvegicus* : XP_006242226.1 ; *Canis lupus* : XP_538325.2 ; *Bos Taurus* : NP_001156660.1 *; Xenopus tropicalis* : XP_002932238.1 ; *Drosophila melanogaster* : NP_652639.1 ; *Saccharomyces cerevisiae* : NP_010251 ; *Caenorhabditis elegans*：NP_502120)



Supplementary Figure S2. (A) Balance/orientation in the *mtu1*$^{-/-}$, *mtu1*$^{+/-}$ and wildtype fish at 5 dpf is shown. The red arrow indicates those abnormally swimming zebrafish, such as frequently sanking to the bottom of the dishes in the head down position and slowly settled their lateral side. (B) Quantification of the levels of abnormal swimming behavior in the *mtu1*$^{-/-}$ (n=196), *mtu1*$^{+/-}$ (n=200) and *mtu1*$^{+/+}$ (n=189) zebrafish. (C) Zebrafish larvae corresponding to the artificial startle stimulus. Images were captured right before (0 s, yellow) and after (1 s, purple; 2 s, cyan) the stimulus, and then overlapped using Image J software. The red arrows indicated representative non-responders. The motion curves were indicated by dashed lines. (D) Quantification of the average movement distance in the *mtu1*$^{-/-}$ (n=30), *mtu1*$^{+/-}$ (n=40) and *mtu1*$^{+/+}$ (n=45). The error bars indicate standard errors; *p* indicates the significance, according to Student's t test, of the difference between mutant and wild type.



Supplementary Figure S3. The number of FM1-43FX+ hair cells in the neuromases of All system were counted in the *mtu1*$^{+/+}$ (n=13) and *mtu1*$^{+/-}$ (n=15) mutants and wildtype (n=16) fishes at 5dpf.



Supplementary Figure S4. (A) Representative image of DASPE labeled hair cells from the *mtu1*$^{+/+}$ and *mtu1*$^{+/-}$ mutants and wildtype fishes at 5dpf . (B) Selected the SO1 SO2 OC1 D1 and the L1 to assess states of the neuromsts at 5dpf zebrafish larvae.