# Supplementary Data

## Supplementary Materials and Methods

### *Preparation of expression plasmids of TRα1-ligand-binding domain with mutation*

pCS2$^{+}$-TRα1L plasmid was a generous gift of Dr. Luca Persani (Insituto Auxologico Italiano, Italy). The pcDNA3.1-TRα1L plasmid was generated using the pCS2$^{+}$-TRα1L plasmid as template. The pcDNA3.1-TRαB plasmid (XM_009306720) was purchased by NOVOPRO (Cat. No. NPT 146076). The pcDNA3.1-ThraaPhe404Leufs*22 and pcDNA3.1-ThraaLeu405Glufs*6 mutant plasmids were generated by PCR mutagenesis using pcDNA3.1-TRα1L plasmid as a template. The pcDNA3.1-ThrabGlu394* and pcDNA3.1-ThrabThr393Profs*31 mutant plasmids were generated by PCR mutagenesis method. The pcDNA3.1-TRαB wild-type plasmid was used as a template. All PCR reactions to generate plasmids were performed using Takara LA Taq (Cat. No. HRR002A) per the manufacturer's instructions, and the products were validated by DNA sequencing using sequencing primers (T7, BGH). Primers were as follows:

For pcDNA3.1-TRα1L plasmid:
Forward-5′-CGCATAAGCTTCCACCATGGAAAACAC AGAGCAGGAGCA
Reverse-5′-GCCCTCGAGGCCGCTCACCTTAAGCAG GAACCGTCTTCCT
For pcDNA3.1- ThraaPhe404Leufs*22 mutant plasmid:
Forward-5′-CGCATAAGCTTCCACCATGGAAAACAC AGAGCAGGAGCA
Reverse-5′-GGCCCTCGAGGCCGCTCACCTTAAGCA GGAACCGTCTTCCTGTGCTGCCACTCCAGTGCT TCCCTCCTGATCCTCGAAGACCTCAAGAGCGG CGGGAACAGTT
For pcDNA3.1-ThraaLeu405Glufs*6 mutant plasmid:
Forward-5′-CGCATAAGCTTCCACCATGGAAAACAC AGAGCAGGAGCA
Reverse-5′-GGCCCTCGAGGCCGCTCACCTTAAGCA GGAACCGTCTTCCTGTGCTGCCACTCCAGTGCT TCCCTCCTGACCCTGGGTCACACCTCCTGATCC TCGAAGAGCGGCGGGAACAGTTCTGTTGGACA CTC
For pcDNA3.1-ThrabThr393Profs*31 mutant plasmid:
Forward-5′-AAACTTAAGCTTGGCGCACCATGGAAC ACATGCCCAAGGAGCAGGA
Reverse-5′-TCGGTACCTCACACGTCCTGATCCTCGA AGACCTCCAGGAAAAGTGGGGGAAAGAAGGTG GGCATTCCACCTTCATGTGCAGGAAGCGACTGG CGTGGCAG

For pcDNA3.1-Thrab Glu394* mutant plasmid:
Forward-5′-AAACTTAAGCTTGGCGCACCATGGAAC ACATGCCCAAGGAGCAGGA
Reverse-5′-TCGGTACCTCACACGTCCTGATCCTCG AAGACCTCCAGGAAAAGTGGGGGAAAGAGTTC AAGTGGGGCATTCCACCTTCATGTGCAGGAAGC GACTGGCGTGGCAG

For Sequencing of plasmids
For T7: TAATACGACTCACTATAGGG
For BGH: TAGAAGGCACAGTCGAGG

### *T3-binding assay*

Zebrafish TRα1L, ThraaPhe404Leufs*22, ThraaLeu405-Glufs*6, TRαB, ThrabGlu394*, and ThrabThr393Profs*31 proteins were prepared by *in vitro* transcription/translation (TNT-quick-couple transcription/translation system; Cat. No. L1170; Promega). *In vitro* translated proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by Western blotting using anti-TRα1 antibody to ensure that equal amounts of proteins were used in the binding assays. *In vitro* T3-binding assay was performed as described (S1).

### *Reporter assays*

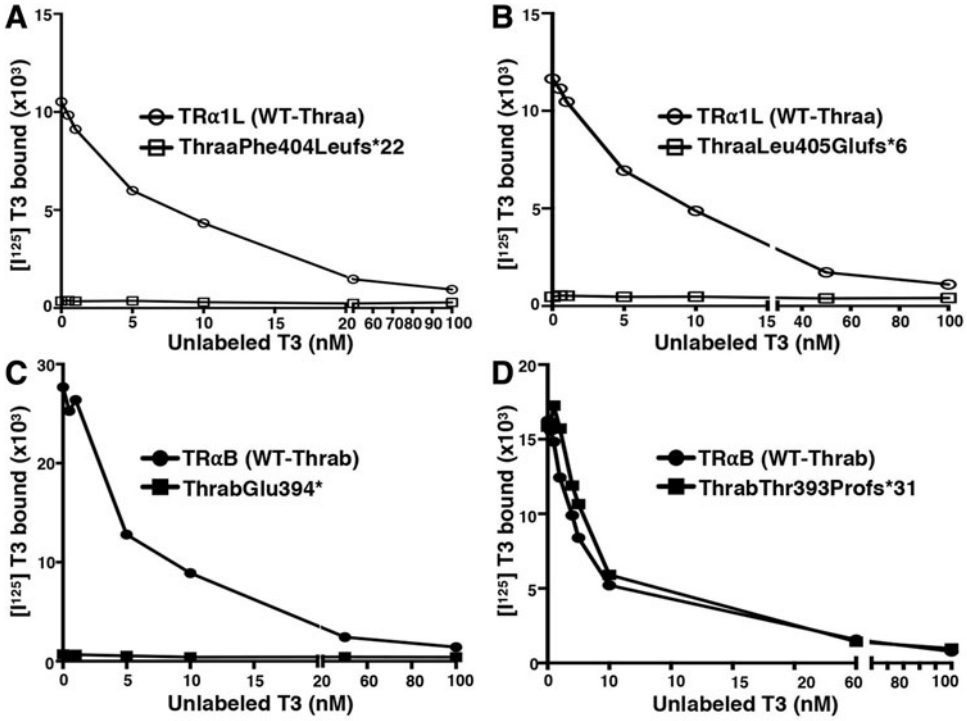
Reporter assays were carried out as described (S2). In brief, 0.2 $\mu$g per well Pal-luciferase reporter plasmids (S3) were transfected with Lipofectamine 2000 (Invitrogen, CA) in CV1 cell with or without T3 treatment according to manufacturer's instructions. Luciferase activity was measured using Victor 3 (PerkinElmer Life and Analytic Sciences, Waltham, MA). Luciferase values were standardized to the ratios of $\beta$-galactosidase activity and protein concentrations. The fold of changes in activity was based on the values of negative controls (i.e., no plasmid transfected cell in the absence of T3) as relative activity of 1.

### *Whole mount* in situ *hybridization*

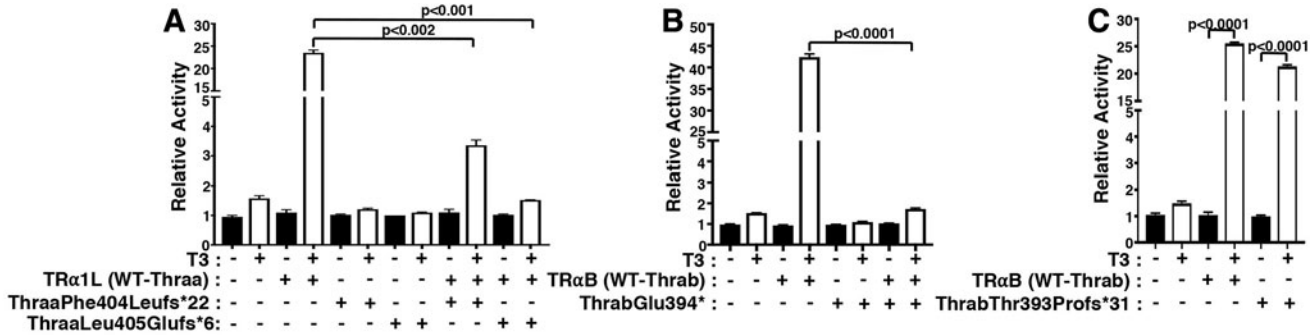
The *gh1* plasmid was a gift from Dr. Alberto Rissone (NIH/NHGRI), *keratin-17* plasmid was a gift from Dr. Wolfgang Driever (Albert-Ludwigs-Universitat Freiburg), and both *keratin-4* and *keratin-18* plasmids were gifts from Dr. Benzamin Feldman (NIH/NICHD). Digoxigene (DIG)-labeled RNA probes for *gh1*, *keratin-4*, *keratin-17*, and *keratin-18* were prepared from linearized template DNAs using T7 or SP6 with RNA polymerase and RNA Labeling Kit (Roche). Whole mount *in situ* hybridization was performed as described (S4). Embryos and larva were treated with 30% hydrogen peroxide to remove pigment. DIG-probes were detected using anti-DIG-alkaline phosphatase and NBT/BCIP (Sigma Aldrich). Embryos and larvae were imaged at from 1 to 5 days postfertilization using Leica TL 5000 with LAS X Imaging Software Suite. In all cases, embryos were genotyped after imaging for identification.

### *Statistical analysis*

All data are expressed as mean ± standard deviation. All tests were two-tail unpaired *t*-test and $p < 0.05$ was considered significant. GraphPad Prism version 7.7 for Mac OS X was used to perform analyses of variances.




**SUPPLEMENTARY FIG. S1.** Three C-terminal truncation mutants of TRα lose T3-binding activity. (**A**) An equal amount of WT-TRα1L, 4-bp deletion ThraaPhe404Leufs*22, (**B**) 8-bp insertion ThraaLeu405Glufs*6, (**C**) WT-TRαB, 1bp-insertion ThrabGlu394*, and (**D**) 4-bp deletion ThrabThr393Profs*31 were used in the competitive T3-binding assay as described in Supplementary Materials and Methods. WT-TRα1L and WT-TRαB bound to T3 with a dissociation constant (Kd) of 3.5 and 3.2 nM, respectively. The three C-terminal truncation mutants of TRα (4-bp deletion ThraaPhe404Leufs*22 (**A**), 8-bp insertion ThraaLeu405Glufs*6 (**B**), and 1 bp-insertion ThrabGlu394* mutant (**C**) did not bind T3. However, the 4-bp deletion ThrabThr393Profs*31 (**D**) mutant bound to T3 with Kd of 3.7 nM, similar to that of WT-TRαB. Data shown are representative of three independent experiments ($n = 3$). bp, base pair; WT, wild-type.




**SUPPLEMENTARY FIG. S2.** Three C-terminal truncation mutants of TRα exhibit dominant negative activity. The reporter plasmid (pPPRE-TK-Luc) and the expression plasmids for (**A**) WT-TRα1-ThraaPhe404Leufs*22 and Thraa-Leu405Glufs*6, (**B**) WT-TRαB and ThrabGlu394*, and (**C**) ThrabThr393Profs*31 were co-transfected into monkey CV-1 cells in absence or presence of T3 (100 nM), as indicated. The transfection of WT receptor plasmids led to T3-dependent activation of luciferase reporter; however, co-transfection of WT and C-terminal truncation mutant receptor plasmids suppressed T3-dependent luciferase reporter activity of the WT receptor (**A**, **B**), but not the 4-bp deletion ThrabThr393-Profs*31 (**C**). Data were normalized against the protein concentration in the lysates. Relative luciferase activity was calculated and shown as fold-induction relative to the luciferase activity of the reporter plasmid (pPPRE-TK-Luc) in the cells in the absence of T3, defined as 1. Data shown are representative of three independent experiments as mean ± standard deviation ($n = 3$). The $p$-values are indicated.

**A Amino acid sequence alignment of the ligand binding domain in human and zebrafish Thraa receptors**

```
(Human) Wild-Type TRα1          PEEWDLIHIATEAHRSTNAQGSHWKQRRKFLPDDIGQSPIVSMPDGDKVDLEAFSEFTKI
(Patient) TRα1F397fs406X        PEEWDLIHIATEAHRSTNAQGSHWKQRRKFLPDDIGQSPIVSMPDGDKVDLEAFSEFTKI
Zebrafish TRαlL                 VSEWELIRMVTEAHRHTNAQGPHWKQKRKFLPEDIGQSPAP-TSDNDKVDLEAFSEFTKI
Zebrafish TRαlS                 VSEWELIRMVTEAHRHTNAQGPHWKQKRKFLPEDIGQSPAP-TSDNDKVDLEAFSEFTKI
ThraaLeu405Glufs*6              VSEWELIRMVTEAHRHTNAQGPHWKQKRKFLPEDIGQSPAP-TSDNDKVDLEAFSEFTKI
                                ..**:**:::***** ***** **** :*****:******     *.**************

(Human) Wild-Type TRα1          ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESDTLTLSGEMA
(Patient) TRα1F397fs406X        ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESDTLTLSGEMA
Zebrafish TRαlL                 ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESETLTLSGEMA
Zebrafish TRαlS                 ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESETLTLSGEMA
ThraaLeu405Glufs*6              ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESETLTLSGEMA
                                **************************************************:*********

(Human) Wild-Type TRα1          VKREQLKNGGLGVVSDAIFELGKSLSAFNLDDTEVALLQAVLLMSTDRSGLLCVDKIEKS
(Patient) TRα1F397fs406X        VKREQLKNGGLGVVSDAIFELGKSLSAFNLDDTEVALLQAVLLMSTDRSGLLCVDKIEKS
Zebrafish TRαlL                 VSREQLKNGGLGVVSDAIFDLGKSLSQFNLDDSEVALLQAVLLMSSDRSGLTCVEKIEKC
Zebrafish TRαlS                 VSREQLKNGGLGVVSDAIFDLGKSLSQFNLDDSEVALLQAVLLMSSDRSGLTCVEKIEKC
ThraaLeu405Glufs*6              VSREQLKNGGLGVVSDAIFDLGKSLSQFNLDDSEVALLQAVLLMSSDRSGLTCVEKIEKC
                                *.*****************:****** ***** :************:***** **:****.

(Human) Wild-Type TRα1          QEAYLLAFEHYVNHRKHNIPHFWPKLLMKVTDLRMIGACHASRFLHMKVECPTELFPPLF
(Patient) TRα1F397fs406X        QEAYLLAFEHYVNHRKHNIPHFWPKLLMKVTDLRMIGACHASRFLHMKVECPTELFPPTL
Zebrafish TRαlL                 QEMYLLAFEHYINHRKHNISHFWPKLLMKVTNLRMIGACHASRFLHMKVECPTELFPPLF
Zebrafish TRαlS                 QEMYLLAFEHYINHRKHNISHFWPKLLMKVTNLRMIGACHASRFLHMKVECPTELFPPLF
ThraaLeu405Glufs*6              QEMYLLAFEHYINHRKHNISHFWPKLLMKVTNLRMIGACHASRFLHMKVECPTELFPPLF
                                ** ********:******* ***********:************************** :

(Human) Wild-Type TRα1          LEVFEDQEV--------------
(Patient) TRα1F397fs406X        PRGL*------------------
Zebrafish TRαlL                 LEVFEDQEGSTGVAAQEDGSCLR
Zebrafish TRαlS                 LEVFEDQEV--------------
ThraaLeu405Glufs*6              EDQEV*-----------------
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**B Amino acid sequence alignment of the ligand binding domain in human and zebrafish Thrab receptors**

```
(Human) Wild-Type TRα1          PEEWDLIHIATEAHRSTNAQGSHWKQRRKFLPDDIGQSPIVSMPDGDKVDLEAFSEFTKI
(Patient) TRα1 E403X            PEEWDLIHIATEAHRSTNAQGSHWKQRRKFLPDDIGQSPIVSMPDGDKVDLEAFSEFTKI
(Patient) TRα1 C392X            PSEWELIRMVTEAHRHTNAQGPHWKQKRKFLPEDIGQSPAP-TSDNDKVDLEAFSEFTKI
Zebrafish TRαB                  -SEWELIRVVTEAHRHTNAQGSHWKQKRKFLPEDIGQSPVAPTSDGDKVDLEAFSEFTKI
ThrabGlu394*                    -SEWELIRVVTEAHRHTNAQGSHWKQKRKFLPEDIGQSPVAPTSDGDKVDLEAFSEFTKI
                                 .**:**:::***** **********:*****:******:.   ****************

(Human) Wild-Type TRα1          ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESDTLTLSGEMA
(Patient) TRα1 E403X            ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESDTLTLSGEMA
(Patient) TRα1 C392X            ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESDTLTLSGEMA
Zebrafish TRαB                  ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESETLTLSGEMA
ThrabGlu394*                    ITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESETLTLSGEMA
                                **************************************************:*********

(Human) Wild-Type TRα1          VKREQLKNGGLGVVSDAIFELGKSLSAFNLDDTEVALLQAVLLMSTDRSGLLCVDKIEKS
(Patient) TRα1 E403X            VKREQLKNGGLGVVSDAIFELGKSLSAFNLDDTEVALLQAVLLMSTDRSGLLCVDKIEKS
(Patient) TRα1 C392X            VKREQLKNGGLGVVSDAIFELGKSLSAFNLDDTEVALLQAVLLMSTDRSGLLCVDKIEKS
Zebrafish TRαB                  VKREQLKNGGLGVVSDAIFDLGKSLAQFNLDDTEVALLQAVLLMSSDRTGLTCVEKIEKC
ThrabGlu394*                    VKREQLKNGGLGVVSDAIFDLGKSLAQFNLDDTEVALLQAVLLMSSDRTGLTCVEKIEKC
                                ******************:*****: ******************:**:** **:****.

(Human) Wild-Type TRα1          QEAYLLAFEHYVNHRKHNIPHFWPKLLMKVTDLRMIGACHASRFLHMKVECPTELFPPLF
(Patient) TRα1 E403X            QEAYLLAFEHYVNHRKHNIPHFWPKLLMKVTDLRMIGACHASRFLHMKVECPTELFPPLF
(Patient) TRα1 C392X            QEAYLLAFEHYVNHRKHNIPHFWPKLLMKVTDLRMIGACHASRFLHMKVE*---------
Zebrafish TRαB                  QEMYLLAFEHYINYRKHNIPHFWPKLLMKVTDLRMIGACHASRFLHMKVECPTELFPPLF
ThrabGlu394*                    QEMYLLAFEHYINYRKHNIPHFWPKLLMKVTDLRMIGACHASRFLHMKVECPT*------
                                ** ********:*:************************************

(Human) Wild-Type TRα1          LEVFEDQEV
(Patient) TRα1 E403X            L*-------
(Patient) TRα1 C392X            ---------
Zebrafish TRαB                  LEVFEDQEV
ThrabGlu394*                    ---------
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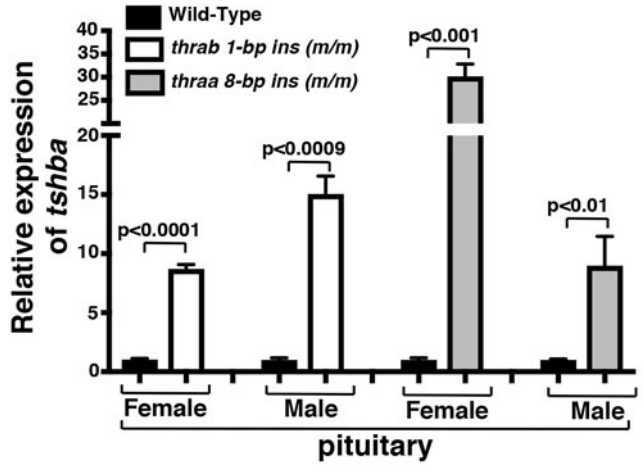
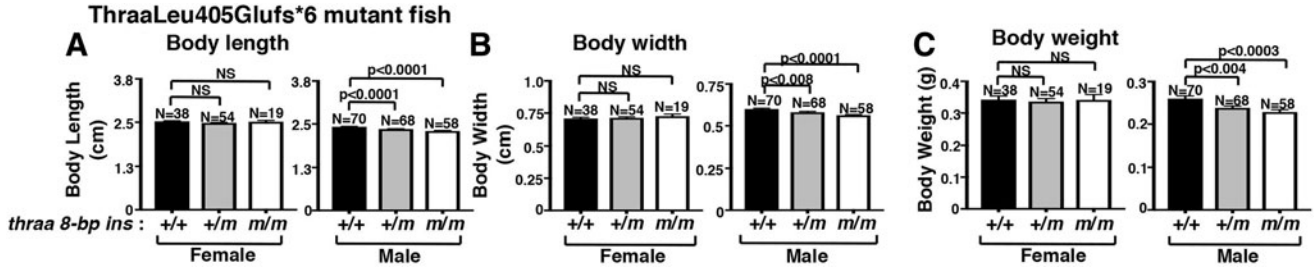
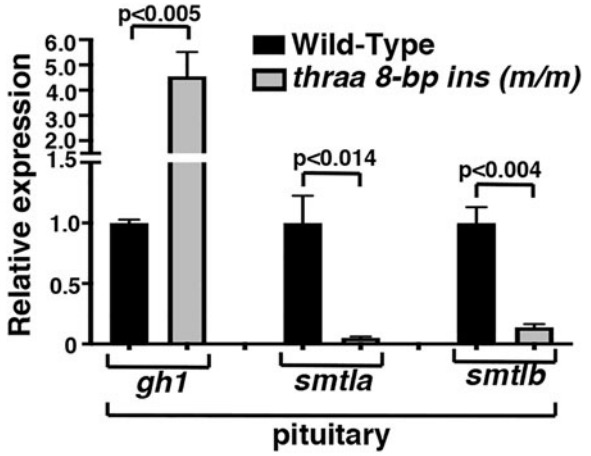
**SUPPLEMENTARY FIG. S3.** Amino acid sequence alignment of the ligand-binding domain in human and zebrafish Thraa receptors (**A**) and Thrab receptors (**B**).



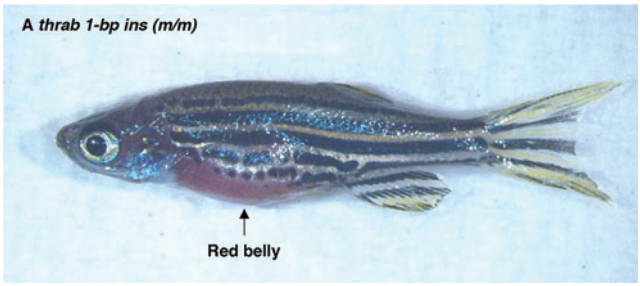

**SUPPLEMENTARY FIG. S4.** The expression of *tshba* gene is upregulated in the pituitaries of *thraa 8-bp insertion (m/m)* and *thrab 1-bp insertion (m/m)* mutant fish. The mRNA expression levels of *tshba* gene of *thraa 8-bp insertion (m/m)* mutant and *thrab 1-bp insertion (m/m)* mutant in the pituitaries of females and males at adult ($N = 10$–$25$ per sample). The mRNA expression levels of *tshba (thyroid stimulating hormone subunit beta a)* gene were measured by RT-qPCR. All expression levels are normalized to that of *efla (elongation factor 1-alpha)* in each well and expressed as the fold changes for each sample relative to WT. This experiment was repeated in triplicate. The data are expressed as mean ± SE ($p < 0.05$). mRNA, messenger RNA; RT-qPCR, real-time quantitative PCR; SE, standard error.



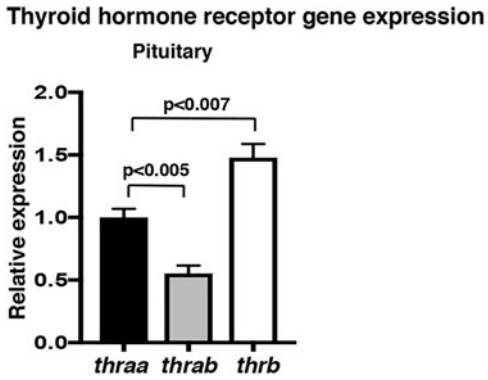

**SUPPLEMENTARY FIG. S5.** Mild impaired growth in adult ThraaLeu405Glufs*6 mutant fish. (**A**) Body length, (**B**) body width, and (**C**) body weight were measured in both females and males WT, heterozygous and homozygous mutant fish. The number of fish (*N*) measured are indicated. The data are shown as mean ± SE with *p*-values to indicate significant changes. NS, not significant.




**SUPPLEMENTARY FIG. S6.** Altered expression of growth-related genes in adult homozygous *thraa 8-bp insertion (m/m)* mutant fish. The mRNA expression of *gh1*, *smtla*, and *smtlb* in pituitary of WT (black bars) and sibling homozygous *thraa 8-bp insertion (m/m)* mutant fish were determined by RT-qPCR as described in Supplementary Materials and Methods ($N = 10$–$24$ per sample). All expression levels are normalized to that of *efla (elongation factor 1-alpha)* in each well and expressed as the fold changes for each sample relative to WT. This experiment was repeated three times, each in triplicates. The data are expressed as mean ± SE ($p < 0.05$).




**SUPPLEMENTARY FIG. S7.** A representative example with enlarged image to show clearly ''red belly'' in a female adult *thrab 1-bp insertion (m/m)* mutant fish.




**SUPPLEMENTARY FIG. S8.** Thyroid hormone receptor gene expression in the pituitary of adult WT fish. The thyroid hormone receptor genes (*thraa*, *thrab*, and *thrb*) mRNA expression in pituitary of WT fish were determined by RT-qPCR as described in Supplementary Materials and Methods ($N = 10$ per sample). All expression levels are normalized to that of *efla (elongation factor 1-alpha)* in each well and expressed as the fold changes for each sample relative to WT. This experiment was repeated three times, each in triplicates. The data are expressed as mean ± SE ($p < 0.05$).

**A** **Sequence analysis of zebrafish thyroid hormone α receptors**

```
Wild type-TRαlL      MENTEQEHNLPEGDETQWPNGVKRKRKNSQCSMNSTSDKSISVPGYVPSYLEKDEPCVVC
Wild type-TRαlS      MENTEQEHNLPEGDETQWPNGVKRKRKNSQCSMNSTSDKSISVPGYVPSYLEKDEPCVVC
8-bp insertion- TRαlL MENTEQEHNLPEGDETQWPNGVKRKRKNSQCSMNSTSDKSISVPGYVPSYLEKDEPCVVC
8-bp insertion- TRαlS MENTEQEHNLPEGDETQWPNGVKRKRKNSQCSMNSTSDKSISVPGYVPSYLEKDEPCVVC
                     ************************************************************

Wild type-TRαlL      GDKATGYHYRCITCEGCKGFFRRTIQKNLHPSYSCKYDSCCIIDKITRNQCQLCRFRKCI
Wild type-TRαlS      GDKATGYHYRCITCEGCKGFFRRTIQKNLHPSYSCKYDSCCIIDKITRNQCQLCRFRKCI
8-bp insertion- TRαlL GDKATGYHYRCITCEGCKGFFRRTIQKNLHPSYSCKYDSCCIIDKITRNQCQLCRFRKCI
8-bp insertion- TRαlS GDKATGYHYRCITCEGCKGFFRRTIQKNLHPSYSCKYDSCCIIDKITRNQCQLCRFRKCI
                     ************************************************************

Wild type-TRαlL      SVGMAMDLVLDDSKRVAKRRLIEENREKRKKEEIVKTLHNRPEPTVSEWELIRMVTEAHR
Wild type-TRαlS      SVGMAMDLVLDDSKRVAKRRLIEENREKRKKEEIVKTLHNRPEPTVSEWELIRMVTEAHR
8-bp insertion- TRαlL SVGMAMDLVLDDSKRVAKRRLIEENREKRKKEEIVKTLHNRPEPTVSEWELIRMVTEAHR
8-bp insertion- TRαlS SVGMAMDLVLDDSKRVAKRRLIEENREKRKKEEIVKTLHNRPEPTVSEWELIRMVTEAHR
                     ************************************************************

Wild type-TRαlL      HTNAQGPHWKQKRKFLPEDIGQSPAPTSDNDKVDLEAFSEFTKIITPAITRVVDFAKKLP
Wild type-TRαlS      HTNAQGPHWKQKRKFLPEDIGQSPAPTSDNDKVDLEAFSEFTKIITPAITRVVDFAKKLP
8-bp insertion- TRαlL HTNAQGPHWKQKRKFLPEDIGQSPAPTSDNDKVDLEAFSEFTKIITPAITRVVDFAKKLP
8-bp insertion-TRαlS HTNAQGPHWKQKRKFLPEDIGQSPAPTSDNDKVDLEAFSEFTKIITPAITRVVDFAKKLP
                     ************************************************************

Wild type-TRαlL      MFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESETLTLSGEMAVSREQLKNGGLGVVSD
Wild type-TRαlS      MFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESETLTLSGEMAVSREQLKNGGLGVVSD
8-bp insertion- TRαlL MFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESETLTLSGEMAVSREQLKNGGLGVVSD
8-bp insertion-TRαlS MFSELPCEDQIILLKGCCMEIMSLRAAVRYDPESETLTLSGEMAVSREQLKNGGLGVVSD
                     ************************************************************

Wild type-TRαlL      AIFDLGKSLSQFNLDDSEVALLQAVLLMSSDRSGLTCVEKIEKCQEMYLLAFEHYINHRK
Wild type-TRαlS      AIFDLGKSLSQFNLDDSEVALLQAVLLMSSDRSGLTCVEKIEKCQEMYLLAFEHYINHRK
8-bp insertion- TRαlL AIFDLGKSLSQFNLDDSEVALLQAVLLMSSDRSGLTCVEKIEKCQEMYLLAFEHYINHRK
8-bp insertion-TRαlS AIFDLGKSLSQFNLDDSEVALLQAVLLMSSDRSGLTCVEKIEKCQEMYLLAFEHYINHRK
                     ************************************************************

Wild type-TRαlL      HNISHFWPKLLMKVTNLRMIGACHASRFLHMKVECPTELFPPLFLEVFEDQEGSTGVAAQ
Wild type-TRαlS      HNISHFWPKLLMKVTNLRMIGACHASRFLHMKVECPTELFPPLFLEVFEDQEV-------
8-bp insertion- TRαlL HNISHFWPKLLMKVTNLRMIGACHASRFLHMKVECPTELFPPLFEDQEV-----------
8-bp insertion-TRαlS HNISHFWPKLLMKVTNLRMIGACHASRFLHMKVECPTELFPPLFEDQEV-----------
                     ************************************************

Wild type-TRαlL      EDGSCLR 428
Wild type-TRαlS      ------- 414
8-bp insertion- TRαlL ------- 410
8-bp insertion-TRαlS ------- 410
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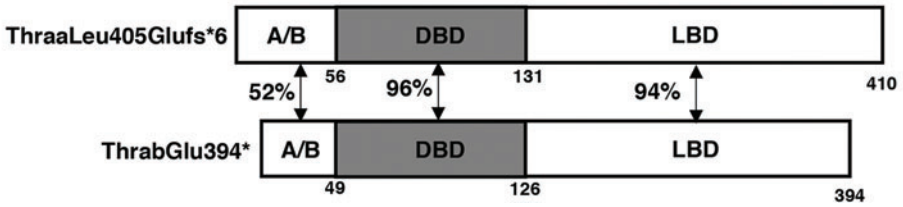
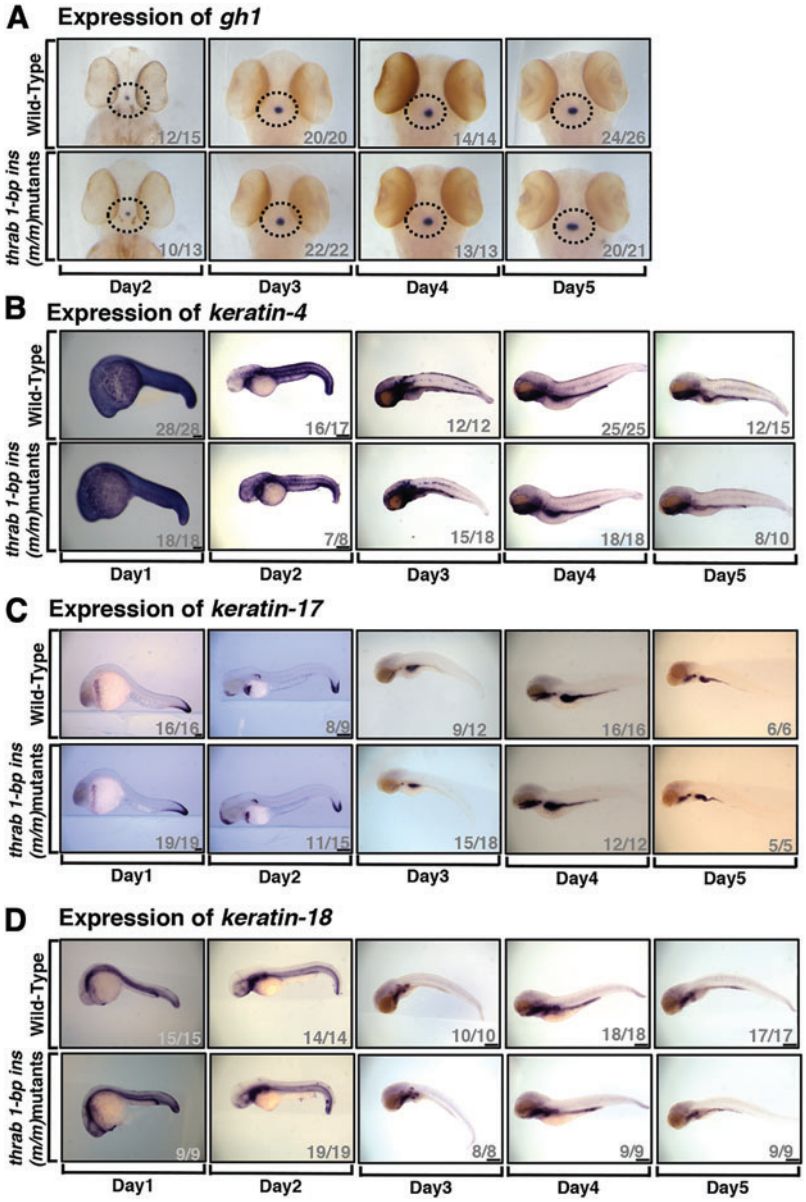
**B** **Amino acid sequence homology in the functional domains of zebrafish mutant receptors**




**SUPPLEMENTARY FIG. S9.** (**A**) Alignment of amino acid sequences among WT TRα1L and TRα1S with the corresponding ThraaLeu405Glufs*6 mutant due to an 8-bp insertion mutation in the *thraa* gene. (**B**) Comparison of the extent of amino acid sequence homology in the functional DBD and LBD between ThraaLeu405Glufs*6 mutant and ThrabGlu394* mutant. DBD, DNA-binding domain; LBD, ligand-binding domain.




**SUPPLEMENTARY FIG. S10.** The expression of the growth hormone and keratin genes was not affected by the expression of the *thrab 1-bp insertion (m/m)* mutant during embryogenesis. (**A**) WISH staining of zebrafish *gh1* expression from 48 to 120 hpf in *thrab 1-bp insertion (m/m)* mutant embryos/larvae and their WT siblings. The *gh1* expression was visualized in the pituitary (indicated with a dotted circle). No apparent differences in the *gh1* expression were detected between WT and mutant embryos/larvae. (**B**) The images of WISH staining of zebrafish *keratin-4* expression. The *keratin-4* is expressed in all cells of the embryos from 24 to 120 hpf. At 24 hpf, the *keratin-4* is expressed in epidermal superficial stratum and epidermis; at 48 hpf, it expressed in the epidermal superficial stratum, epidermis, and pharynx; at 72 hpf, the keratin-4 is expressed in epidermis and pharynx and also intensively expressed in endocrine system; at 96–120 hpf, it was expressed in epidermis, endocrine system, and in pharynx/pharyngeal arch 3–7 skeleton. (**C**) The keratin-17 expression was visualized from 24 to 120 hpf. At 24 hpf, the keratin-17 expression was visualized in head epidermis, hatching gland, and caudal fin epidermis. At 48 hpf, it was expressed in pectoral fin epidermis, nose epidermis, dorsal epidermis, and caudal fin epidermis. At 96 to 120 hpf, the keratin-17 was intensively expressed in epidermis, endocrine system, and pharynx/pharyngeal arch 3–7 skeleton. (**D**) The keratin-18 expression at from day 1 embryos to day 5 larvae in *thrab 1-bp insertion (m/m)* mutant larvae and their WT siblings. At 24 hpf, the keratin-18 was expressed in epidermis, notochord, fin fold, pronephric duct, and axial vasculature. At 48 hpf, it was expressed in nose, branchial arches, pectoral fin bud, metencephalic middle cerebral vein, and pronephric duct. At 72–120 hpf, the keratin-18 expression was intensively visualized in epithelial cells, pronephric duct, digestive tract, dorsal aorta, and fins. Note that keratin-4, keratin-17, and keratin-18 expressions are not changeable in *thrab 1-bp insertion (m/m)* mutant embryos to larvae as compared with their WT siblings from 24 to 120 hpf. The numerators of the fraction in the right corners of each panel represent the embryos/larvae showing identical patterns of staining. The denominators indicate the total number of embryos/larvae analyzed. hpf, hours postfertilization; WISH, whole mount *in situ* hybridization.

Supplementary Table S1. Altered Gene Expression of Hormones and Regulators in the Pituitary of Adult Homozygous *thrab* 1-*bp* *Insertion* Mutant Fish Compared with Wild-Type Fish

| *Gene* | *Fold change* | *FDR* |
|---|---|---|
| *smtlb* | 0.56 | 0.00611 |
| *tshba* | 5.76 | 0.00001 |
| *trhrb* | 4.23 | 0.00967 |
| *gh1* | 0.43 | 0.04472 |
| *pomca* | 0.71 | 0.00005 |
| *lhb* | 0.77 | 0.00632 |

FDR, false discovery rate.

Supplementary Table S2. Real-Time Quantitative PCR Primers Used

| *Target* | *Forward primer (5′-* | *Reverse primer (5′-* |
|---|---|---|
| *Growth hormone (gh1)* | CCTCTGTCGTTCTGCAACTC | ACTCCCAGGATTCAATGAGG |
| *Somatolactin a (smtla)* | TGGTTCAGTCGTGGATGG | AAGATGGTGGAGGATGCC |
| *Somatolactin b (smtlb)* | TCTCGGAGGAAGCCAAGTTG | AGCCATCGGTCGGAAATCTG |
| *Insulin-like growth factor 1a (igf-1a)* | GGTGCTGTGCGTCCTC | GTCCATATCCTGTCGGTTTG |
| *Insulin-like growth factor 1b (igf-1b)* | GGTGGTCCTCGCTCTC | TCTGCTAACTTCTGGTATCG |
| *Insulin-like growth factor 1 receptor a (igf-1ra)* | TCAAGCACACTCATACTCTGGGC | TTTCCGTTGGGAGCGATAGG |
| *Insulin-like growth factor 1 receptor b (igf-1rb)* | TGAACCAGATAAACCCAACGG | CATACACCATACCAAACGACCCC |
| *Insulin receptor a (Ir-a)* | AAGACATAGTGAAAGGAGAGT | TACGGTCAGGTCTTCGGC |
| *Glucose transporter (glut4)* | GTATGATCTCCTCCTTCTGTGTGG | CGATGCCAAACAGTATGCAC |
| *Myostatin (myo)* | ACTCAAGCAGGCTCCAAACAT | GTCGCCTCCTCCGCCGGTCT |
| *Keratin 4 (krt4)* | CTGTAACTGTCAACCAGA ACC | CATCTTGTTCTGCTGTTCC A |
| *Keratin 5 (krt5)* | TGCTTCCTTCATTGACAA | GGTAGTGGTCTGTTCTTG |
| *Keratin 15 (krt15)* | TGGGGTCCGTATCTCCTCTG | CAGGTCCTTGATTGTGGCCT |
| *Keratin 17 (krt17)* | GGAGTGCGTGCTGGTAGTGTCT | GGTAGGAGGCCAGACGATCATT |
| *Keratin 18 (krt18)* | TGACACCAACCTGAACCGCAT | AGGAGCATCAACATCCACCTGC |
| *Collagen, type1, alpha 1b (col1a1b)* | GCTGGTGCTACTGGACCTAAA | GGGCTCCTCTTTGTCCCTCT |
| *Type 1 cytokeratin (cyt1)* | TGGACAAGTCAATGTAGAG | TCATAGTGCTCACGGATA |
| *Type 1 cytokeratin (cki)* | AGACTGGAGATGGAGATT | TACTACTTTGCGTGTTGT |
| *Thyroid stimulating hormone b (tshb)* | CTGTCAACACCACCATCTGC | GTGCATCCCCTCTGAACAAT |
| *Thyrotropin-releasing hormone receptor b (trhrb)* | CTGGTGGTGGTCAACTCCTT | GCTTTCCACCGTTGATGTTT |
| *Proopiomelanocortin a (pomca)* | AGCTCAGTGTTGGGAAAACG | GGTAGACGGGGGTTTCATCT |
| *Luteinizing hormone, beta (lhb)* | CGCTATGAGACCATTAAC | GTAGGTTGATGTGAGTTG |
| *Thyroid hormone receptor apha (thraa)* | CTGATGCCATCTTTGATTTGGG | GTACATCTCCTGACACTTCTCG |
| *Thyroid hormone receptor alpha (thrab)* | TCTGATGCCATCTTCGACTTG | GTACATCTCCTGGCACTTCTC |
| *Thyroid hormone receptor beta (thrb)* | GCTCTGGCTCTTATGACATGG | TCGCTGATATCTCGTGCTTTG |
| *Elongation factor 1 a (ef1a)* | GCCGTCCCACCGACAAG | CCACACGACCCACAGGTACAG |

SUPPLEMENTARY TABLE S3. SOURCES OF ANTIBODIES

| *No.* | *Name* | *Cat. No.* | *Company* | *Reactivity* | *Reference 1* | *Reference 2* |
|---|---|---|---|---|---|---|
| 1 | p-AKT (Ser473) | 4060 | Cell Signaling | H, M, R, Mk, Hm, B, Dm, zebrafish | | |
| 2 | Total-AKT | 9272 | Cell Signaling | H, M, R, Mk, Pg, C, Hm, B, GP, Dm, Dg | Souza *et al*. (S5) | Ciarlo *et al*. (S6) |
| 3 | p-p70S6 (Thr389) | 9205 | Cell Signaling | H, M, R, Mk | Khora *et al*. (S7) | Sasore *et al*. (S8) |
| 4 | Total-p70S6 | 9202 | Cell Signaling | H, M, R, Mk | Wen and Ushio (S9) | |
| 5 | p-S6 (Ser240/244) | 2215 | Cell Signaling | H, M, R, Mk, zebrafish | | |
| 6 | Total S6 | 2217 | Cell Signaling | H, M, R, Mk | Khora *et al*. (S7) | Ding *et al*. (S10) |
| 7 | GAPDH | 2118 | Cell Signaling | H, M, R, Mk, B | Ciarlo *et al*. (S6) | |
| 8 | Keratin-5 | MS-1814-S0 | NeoMarkers | H | Saxena *et al*. (S11) | |
| 9 | Keratin-17 | MS-489-S0 | NeoMarkers | H, R | Saxena *et al*. (S11) | |
| 10 | Keratin-18 | MS-743-S0 | NeoMarkers | H | | |
| 11 | p-STAT3 (Tyr705) | 9131 | Cell Signaling | H, M, R, Mk | Fang *et al*. (S12) | Ogai *et al*. (S13) |
| 12 | Total-STAT3 | 4904 | Cell Signaling | H, M, R, Mk | Fang *et al*. (S12) | Ogai *et al*. (S13) |
| 13 | Cyclin D1 | 9041-P1 | NeoMarkers | H, R, M | Duffy *et al*. (S14) | |
| 14 | CDK6 | SC-7961 | SantaCruz | H, R, M | | |
| 15 | p-Rb (Ser780) | 9307 | Cell Signaling | H, R, Mk | | |
| 16 | Total-Rb | SC-50 | SantaCruz | H, R, M | Zhang *et al*. (S15) | |

Species cross-reactivity key: B, bovine; C, chicken; Dg, dog; Dm, *Drosophila melanogaster*; GP, guinea pig; H, human; Hm, hamster; M, mouse; Mk, monkey; Pg, pig; R, rat; Z, zebrafish.

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