SUPPLEMENTAL MATERIAL

Supplemental data

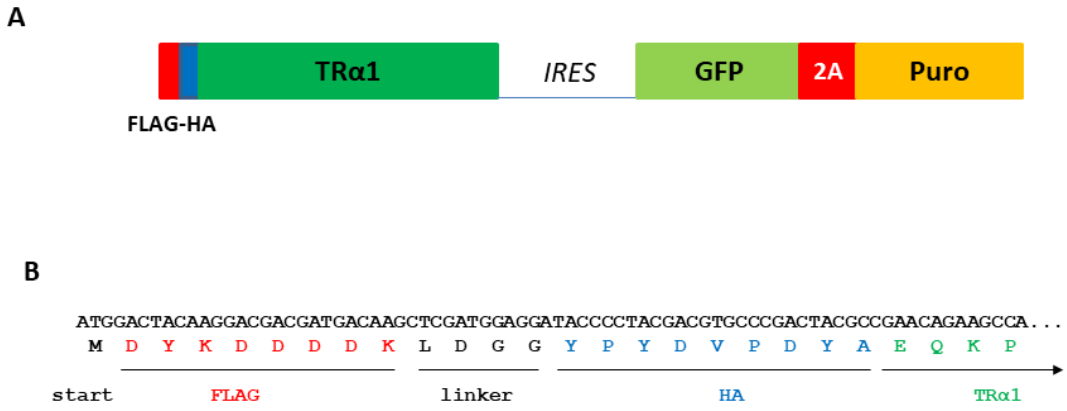

Figure S1. Structure of expression cassette of the FLAG-HA tagged TRα1 (FHTRα1) construct. The second codon of the full length coding sequence of TRα1 is fused at the 5'-end to consecutive sequences encoding the FLAG and hemagglutinin (HA) epitope tags (*B*). To select cells expressing FHTRα1, the construct is expressed from a bicistronic messenger RNA, due to the inclusion of an internal ribosome entry site (IRES) sequence, together with a selection marker consisting of the sequences encoding the puromycine resistrance gene (PURO) and green fluorescent protein (GFP), fused by the sequence encoding the 2A self-cleaving peptide (*A*).

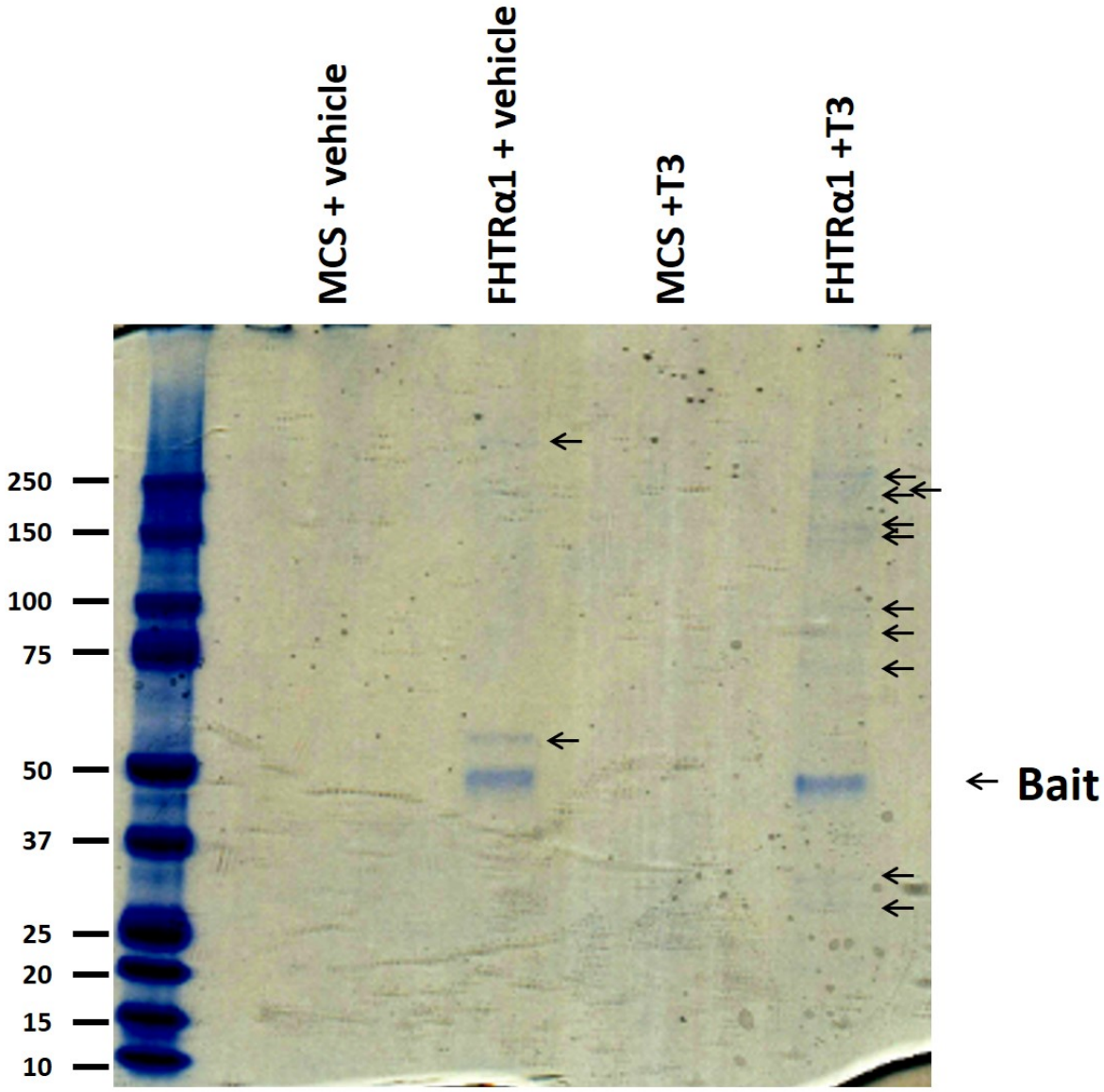

Figure S2. SDS-PAGE of FHTRα1-containing protein complexes from HepG2 cells. HepG2 cells expressing FHTRα1 or empty vector (MCS) as control were stimulated for 4 hrs with vehicle or 100 nM T3 and subjected to TAP purifications. One third of the final eluates were separated on a 4-12% Bis-Tris gel and protein bands visualized by Colloidal Coomassie staining.

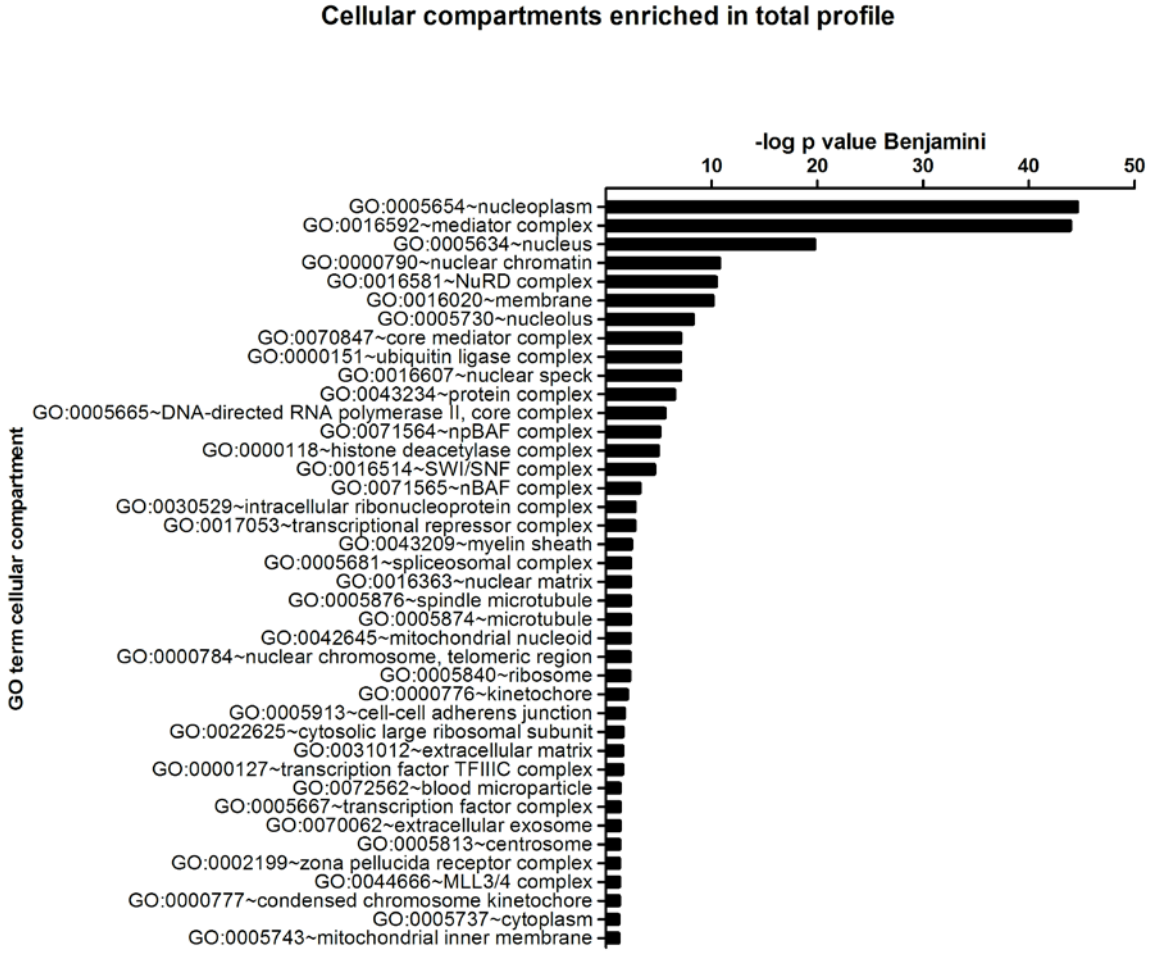

Figure S3. Enrichment for cellular compartments of the total profile. Proteins that specifically co-purified with FHTRα1 in any purification (supplemental table 1) were analysed for enrichment of cellular compartment using DAVID (minimum count 2, ease 0.1). The top 40 most significant GO terms are displayed. P values were adjusted for false discovery rates using the Benjamini correction.

Supplemental methods

Construction of lentiviral vectors pWCAGpCasC-MCS-IRESPuro2AGFP and pWCAGpCasC-FHTRα1-IRESPuro2AGFP

To add the sequence encoding the FLAG and HA epitope to the 5'-side of TRα1, the full length coding sequence for TRα1 was amplified using Pfu polymerase (Thermofisher) by which the start codon was replaced with consecutive sequences for the FLAG and HA epitope tag (fig. S1). The resulting fragments was cloned into the pCR2.1TOPO vector (Invitrogen) and subsequently excised and ligated into the HindIII and BamHI sites of pENTL1-MCS-R5 to yield the plasmid pENTL1-FHTRα1-R5. The correct sequence was confirmed by Sanger sequencing. The MCS (multiple cloning site) or FHTRα1 fragments were next fused to the IRESPuro2AGFP-fragment from pENTL5-IRESPuro2AGFP-R2 and inserted into pWCAGpCasC by multiple gateway cloning using the LR II Clonase Plus (Invitrogen) according to the manufacturers protocol.

Tandem affinity purification

Throughout the procedure, buffers were supplemented with 100 nM T3 or vehicle, and cOmplete™ protease inhibitors cocktail (Roche). Cells were washed twice with ice-cold DPBS, scraped into 2 ml DPBS per plate, pelleted in a tabletop centrifuge at 1200 rpm for 5 min at 4 °C and resuspended in 5 ml buffer A (10 mM Hepes.NaOH, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, pH 7.9) . After 15 min on ice, 0.6% NP40 was added and the cells vortexed for 30" to lyse the cells. Phosphatase inhibitors (5 mM NaF and 1 mM NaPPi) were added and nuclei pelleted by centrifugation at 2000 rpm for 10 min at 4 C. Nuclear proteins were extracted by nutating the nuclei in 1.88 ml buffer C (20 mM HEPES.NaOH, 400 mM NaCl, 1 mM EDTA, 1 mM DTT, 5 mM NaF, 1 mM NaPPi, pH 7.9) for 20 min at 4 °C. The nuclei were spun down in a microfuge at 15000 rpm. The supernatant was transferred to a 15 ml conical tube, the salt concentration reduced by addition of 3.12 ml buffer D (20 mM HEPES.NaOH, 1 mM EDTA, 5 mM NaF, 1 mM NaPPi, pH 7.9) and NP40 added to a final concentration of 0.1%. Next, FHTRα1-containing protein complexes were bound overnight at 4 °C to 20 µl bedvolume of anti-FLAG

agarose (clone M2; Sigma), washed 1x with 10 ml and 4x with 1 ml of ice-cold washbuffer (20 mM HEPES.NaOH, 150 mM NaCl, 1 mM EDTA, 5 mM NaF, 1 mM NaPPi, 0.1% NP40, pH 7.9) and eluted twice with 50 µl of 200 µg/ml FLAG peptide (Sigma) in washbuffer for 30' at 4 °C in spin columns (Biorad). Eluates were combined, 500 µ of washbuffer was added to the eluates and FHTRα1-containing protein complexes were bound overnight at 4 °C to 20 µl bedvolume of anti-HA agarose (Sigma), after which the resin was washed 4x with 1 ml ice-cold washbuffer. For the first purification from HepG2 cells, the resin was subsequently washed 1x with 1 ml washbuffer w/o NP40, transferred into a spin column and protein complexes eluted once at 4 °C and once at room temperature with 25 µl 400 µg/ml HA peptide (Sigma), after which the eluates were pooled. For the other purifications, 0.1% RapiFest SF (Waters) was added to the final wash buffer and elution buffer, to improve bead handling. Elutes were pooled and subjected to LC-MS/MS for protein identification. For the first purification from HepG2 cells, 1/3 part of the eluate was denatured in 1x NuPAGE LDS sample buffer (Thermo Fisher Scientific) supplemented with 10 mM DTT at 70 °C for 10 minutes, separated by SDS-PAGE on a 4-12% Bis-Tris gel (Novex, Invitrogen) and bands visualized with a Colloidail Blue Staining Kit (Invitrogen).

In-solution digestion

Eluates from two independent purifications from each cell line were precipitated using acetone and then resuspended in 0.1% Rapigest in 50mM NH_4HCO_3. The solution was reduced with 100 mM dithiothreitol (DTT) at 60 °C for 30 min. After the mixture was cooled down to room temperature, it was alkylated in the dark with 300 mM iodoacetamide at ambient temperature for 30 min, and digested overnight with 0.4 µg trypsin (Promega, Madison, WI). Five percent trifluoroacetic acid was added, to obtain a final concentration of 0.5% trifluoroacetic acid (pH < 2). After 45 min of incubation at 37 °C the samples were centrifuged at 13,000 g for 10 minutes.

NanoLC data dependent mass spectrometry measurements

Digested samples were subjected to LC-MS/MS analysis. Samples were analyzed by nano-LC (Ultimate 3000RS, Thermo Fisher Scientific, Germering, Germany). After pre-concentration and washing of the samples on a C18 trap column (1 mm × 300 µm i.d., Thermo Fisher Scientific), they were loaded onto a C18 column (PepMap C18, 75 mm ID × 150 mm, 2 µm particle and 100 Å pore size, Thermo Fisher Scientific) using a linear 15 minutes gradient (4-38% ACN/H20; 0.1% formic acid) at a flow rate of 250 nL/min. The separation of the peptides was monitored by a UV detector (absorption at 214 nm). The integrated area of the UV chromatogram is used to determine the maximum injection volumes for the LC-MS analyses. For the LC-MS the same type of LC system is used connected to a nanospray source. The LC system was equipped with a PepMap C18 column (75 mm ID × 250 mm, 2 µm particle and 100 Å pore size, Thermo Fisher Scientific) and a 30 or 90 minutes gradient (4-38% ACN/H20; 0.1% formic acid) at a flow rate of 300 nL/min coupled to either an Orbitrap Fusion or to a Orbitrap Fusion Lumos (Thermo Fisher Scientific, San Jose, CA, USA). The Orbitrap Fusion Lumos was operated in the data dependent acquisition (DDA) mode. Full scan MS spectra (m/z 375-1,500) in profile mode were acquired in the orbitrap with a resolution of 120,000 after accumulation of an AGC target of 400,000. A top speed method with a maximum duty cycle of 3 seconds was used. In these 3 seconds the most intense peptide ions from the full scan in the orbitrap were fragmented by HCD (normalized collision energy 28%) and measured in the iontrap with a AGC target of 10,000. Maximum fill times were 50 ms for the full scans and 50 ms for the MS/MS scans. Precursor ion charge state screening was enabled and only charge states from 2-7 were selected for fragmentation. The dynamic exclusion was activated after the first time a precursor was selected for fragmentation and excluded for a period of 60 seconds using a relative mass window of 10 ppm. Lock mass correction was activated to improve mass accuracy of the survey scan. In the Orbitrap Fusion the following settings were used; DDA mode, Full scan MS spectra (m/z 400-1,600) in profile mode with a resolution of 120,000 and an AGC target of 400,000. A top speed method with a maximum duty cycle of 3 seconds was used. In these 3 seconds the most intense peptide ions from the full scan

in the orbitrap were fragmented by CID (normalized collision energy 30%) and measured in the iontrap with a AGC target of 10,000. Maximum fill times were 100 ms for the full scans and 40 ms for the MS/MS scans. The rest of settings were the same as for the measurements on the Orbitrap Fusion Lumos. From the data files of both systems the MS/MS spectra were extracted and converted into mgf files by using MSConvert of ProteoWizard (version 3.0.06245). All mgf files were analyzed using Mascot (version 2.3.02; Matrix Science, London, UK). Mascot was used to perform database searches against the human subset of either uniprot_sprot download November 2015 (20194 entries) or download September 2014 (20196 entries), using Mascot version 2.3.02. Monoisotopic fragment tolerance was set to 0.50 Da and parental tolerance at 10 ppm. Carbamidomethylation of cysteine was specified as fixed modification and oxidation of methionine as variable modification for all, and n-terminal acetylation for the first HepG2 purification. Version 4.8.3 of the Scaffold platform was used to validate proteins and peptides. Stringency settings allowed FDRs of 1% for proteins and peptides when screened against a decoy database. This allowed probability scores of 5% and 78% for proteins and peptides respectively with a minimum of 2 peptides for the first purification from HepG2, and 99% and 91% for proteins and peptides with a minimum of 1 peptide for the other purification. Proteins that were present in any of the control purifications were excluded from our final profile. The uniprot_sprot database contains the TRα isoform 2 (P10827-1) instead of our bait TRα1 (P10827-2). These isoforms differ in their C-terminal domain (371-410 in TRα1), which has high homology between TRα1 and TRβ. Therefore, a peptide (sequence MIGACHASR; position 376-384 in TRα1, position 440-448 in TRβ1) that is conserved between TRα1 and TRβ was scored as an exclusive unique peptide for the C-terminus of TRβ. Apart from one peptide with a probability score of 85% (sequence KLIEENR; position 190-196 in TRβ1), in HepG2 purification 1 from vehicle treated cells, we did not find any peptides that are unique for TRβ as well as TRα2. As such, we decided that all peptides are derived from TRa1 and assigned the C-terminal peptide and spectra to TRα1 in Table 2 and Supplemental Table 1. Gene enrichment analysis was performed using the Database for Annotation, Visualization and Integrated

Discovery (DAVID), version 6.8 (https://david.ncifcrf.gov/) using default settings (count 2, ease 0.1) and p-values adjusted using the Benjamini correction. Existing protein interaction networks were searched using STRINGS (https://strings-db.org) with minimum required interaction scores set at medium confidence (0.400).