Supplementary Information

Supplementary Materials and Methods

**Generation of zebrafish-specific Trappc11 N-terminal antibody**

A polyclonal antibody against the N-terminus of zebrafish Trappc11 was developed in rabbits by immunizing with the epitope KVLPGDHEYPKCRTKR, corresponding to amino acids 54 to 69 of the zebrafish Trappc11 protein. This epitope is encoded within exon 2, and is expected to detect the full length protein (129 kDa) in addition to other splice variants, and also to detect a predicted ~59 kDa product from the truncated protein expressed by the trappc11\textsuperscript{hi1532b} mutant allele.

**Immunofluorescence**

HeLa cells transfected with siRNA targeting TRAPPC11, or non-targeting control siRNA, were collected 48 hours after transfection and stained with antibodies recognizing TRAP\(\alpha\), the Golgi complex protein p115 (kind gift from Dennis Shields, Albert Einstein College of Medicine, New York, USA) and the ER chaperone calnexin (kind gift from John Bergeron, McGill University, Montreal, Canada) and counterstained with DAPI to label the nucleus.

**Live imaging**

Live 5 dpf trappc11 mutants or their phenotypically WT siblings with Tg(fabp10:CAAX-GFP) were mounted in 0.5% low melt agarose and imaged at 30 second intervals for 5 minutes using a 60X oil immersion objective with a 1.5X zoom on a Zeiss LSM-780 confocal microscope. Movies are representative of the 3-4 embryos observed per genotype.

**Spatial Analysis of Functional Enrichment (SAFE)**

The data measuring UPR regulation were downloaded from the Supplementary Table 1 of the original publication (Jonikas et al., 2009) in the form of average GFP/RFP reporter ratios. The chemical genomic data for Tm were downloaded from the Supplementary Table 7 of the original publication (Parsons et al., 2006) in the
form of raw log2 barcode hybridization ratios. Spatial Analysis of Functional Enrichment (SAFE) was performed with default parameters as described in (Baryshnikova, 2015).

**Supplementary Figure Legends**

**Figure S1. Trappc11 protein is affected in trappc11 mutants**

WT control, siblings (sibs) and trappc11 mutant whole larvae were collected at 5 dpf, and lysates were separated by SDS-PAGE and probed using an antibody against the N-terminal region of zebrafish Trappc11. The full length (129 kDa) Trappc11 protein and the foigr protein (predicted to be ~56 kDa) from trappc11 mutants are indicated. Molecular weight standard sizes are shown on left. Tubulin was used as loading control.

**Figure S2. Tm induces a stressed UPR.**

WT zebrafish embryos were treated with DMSO (n=6) or 1 μg/ml Tm (n=7) between 3 to 5 dpf, and livers were collected at 5 dpf for qPCR analysis. A gene panel of 8 UPR responsive genes was analyzed and demonstrates induction of a stressed UPR following Tm exposure.

**Figure S3. UPR induction persists in trappc11 mutants during development.**

(A) WT and trappc11 mutant transgenic zebrafish harboring a liver-specific fluorescent marker (Tg(fabp10:dsRed)) were collected at 3, 4 and 5 dpf and analyzed for overt morphological abnormalities. At 3 dpf, trappc11 mutants are comparable to WT siblings, but at 4 and 5 dpf progressive hepatomegaly is observed in trappc11 mutants. Genotyping PCR results are shown with WT and trappc11 indicated. (B) qPCR analysis of the UPR response gene panel reveals that at 3 dpf, a stressed UPR is present in both WT and trappc11 mutants, that is resolved in WT siblings but persists in trappc11 mutants at 4 and 5 dpf (Figure 1). Box and whisker plots are shown for 3 clutches.
Figure S4. Spatial Analysis of Functional Enrichment (SAFE) of the UPR program in yeast.
(A) The yeast genetic interaction similarity network connects genes that share similar genetic interaction profiles and likely act in related biological processes (Costanzo et al., 2010). Spatial Analysis of Functional Enrichment (SAFE) determines which regions of the network are overrepresented for a quantitative phenotype of interest and therefore uncovers statistical associations between phenotypes and underlying biological processes (Baryshnikova, 2015). Using the data from a quantitative genome-wide screen that examined UPR activity in ~5,000 yeast deletion mutants (Jonikas et al., 2009), SAFE showed that mutations in protein folding and glycosylation, as well as vesicle-mediated transport pathways, cause an up-regulation of the UPR program. Network localization of members of the TRAPP protein complex and the N-glycosylation pathway is shown with respect to the affected network regions. (B) Chemical genomic data from genome-wide mutant screens (Parsons et al., 2006) were examined with SAFE in a similar manner. Mutants that show resistance or sensitivity to a chemical compound are highly indicative of the compound’s mode-of-action (Ho et al., 2011; Roemer et al., 2012). SAFE showed that, consistently with the up-regulation of UPR activity, mutations in protein folding and glycosylation were the most sensitive to Tm and some of the genes involved in protein trafficking were also synthetically lethal with Tm.

Figure S5. trappc11 mutants have reduced glycosylation.
(A) Livers from Tg(fabp10:Gc-EGFP) WT and trappc11 mutants at 5 dpf were collected and analyzed by Western blot for glycosylation of Gc-EGFP. Samples were subjected to Endo H or PNGase F digestion to detect high-mannose and all N-glycan modifications, respectively. Differing exposure times were necessary to clearly show digestions from both WT sibling and trappc11 mutant samples. (B) N-glycans were prepared by PNGase F digestion, from 5 dpf WT and trappc11 mutants, or WT embryos treated with DMSO or 1 μg/ml Tm, and subjected to FACE analysis. Two
WT and 3 trappc11 mutant samples are shown, and a single sample for DMSO and Tm-treatment. Decreased total N-glycans are found in trappc11 mutants, compared to WT siblings, a pattern similar to that seen with Tm treatment. Glucose standards (G4, G5, G6 and G7) were used for structure sizing and identification. Typically, G6 standard runs slightly slower than the released N-linked heptasaccharide Man5GlcNAc2.

Figure S6. Terpenoid pathway response in trappc11 mutants.
(A and B) Livers from WT and trappc11 mutant zebrafish (6 clutches) were collected at 5 dpf, pooled and analyzed by qPCR for genes involved in the terpenoid biosynthetic pathway (A) and glycosylation and cholesterol synthesis (B). dCt values, relative to rpp0, are shown. Asterisks indicate a statistically significant change in dCt compared to WT siblings (p<0.05, Student’s t-test). (C) WT and trappc11 mutant transgenic zebrafish (Tg(actb2:CAAX-GFP)) were analyzed at 5 dpf for membrane localization in the gut of a CAAX-GFP prenylation marker. trappc11 mutants have increased cytosolic mislocalization of CAAX-GFP away from the plasma membrane. Samples were counterstained for DAPI (blue) and Cy5-Streptavidin (red). Scale bars are 10 μm.

Figure S7. Atorvastatin induces the UPR in zebrafish liver.
WT zebrafish larvae were treated with DMSO or 5 μM atorvastatin (Atv) between 3 to 5 dpf, and livers were collected at 5 dpf and analyzed by qPCR for a panel of 8 UPR response genes (7 clutches).

Figure S8. TRAPPC11 depletion causes fragmentation of the GC and does not affect localization of TRAP alpha.
(A and B) Fluorescence microscopy of HeLa cells transfected with a non-specific siRNA (NS) or treated with 60 nM siRNA targeting TrappC11 (C11 KD) for 48 hours, fixed and stained with anti-TRAP® and anti-p115 (A) or anti-calnexin and anti-p115
(B). The merged images include DAPI staining to reveal the nucleus. Scale bars are 20 μm.

**Supplemental References**

Baryshnikova, A. (2015). Systematic Functional Annotation and Visualization of Biological Networks. bioRxiv.

Costanzo, M., Baryshnikova, A., Bellay, J., Kim, Y., Spear, E.D., Sevier, C.S., Ding, H., Koh, J.L., Toufighi, K., Mostafavi, S., Prinz, J., St Onge, R.P., VanderSluis, B., Maknevych, T., Vizeacoumar, F.J., Alizadeh, S., Bahr, S., Brost, R.L., Chen, Y., Cokol, M., Deshpande, R., Li, Z., Lin, Z.Y., Liang, W., Marback, M., Paw, J., San Luis, B.J., Shuteriqi, E., Tong, A.H., van Dyk, N., Wallace, I.M., Whitney, J.A., Weirauch, M.T., Zhong, G., Zhu, H., Houry, W.A., Brudno, M., Ragibizadeh, S., Papp, B., Pal, C., Roth, F.P., Giaever, G., Nislow, C., Troyanskaya, O.G., Bussey, H., Bader, G.D., Gingras, A.C., Morris, Q.D., Kim, P.M., Kaiser, C.A., Myers, C.L., Andrews, B.J., and Boone, C. (2010). The genetic landscape of a cell. Science 327, 425-431.

Ho, C.H., Piotrowski, J., Dixon, S.J., Baryshnikova, A., Costanzo, M., and Boone, C. (2011). Combining functional genomics and chemical biology to identify targets of bioactive compounds. Curr Opin Chem Biol 15, 66-78.

Jonikas, M.C., Collins, S.R., Denic, V., Oh, E., Quan, E.M., Schmid, V., Weibezaehn, J., Schwappach, B., Walter, P., Weissman, J.S., and Schuldiner, M. (2009). Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum. Science 323, 1693-1697.

Parsons, A.B., Lopez, A., Givoni, I.E., Williams, D.E., Gray, C.A., Porter, J., Chua, G., Sopko, R., Brost, R.L., Ho, C.H., Wang, J., Ketela, T., Brenner, C., Brill, J.A., Fernandez, G.E., Lorenz, T.C., Payne, G.S., Ishihara, S., Ohya, Y., Andrews, B., Hughes, T.R., Frey, B.J., Graham, T.R., Andersen, R.J., and Boone, C. (2006). Exploring the mode-of-action of bioactive compounds by chemical-genetic profiling in yeast. Cell 126, 611-625.

Roemer, T., Davies, J., Giaever, G., and Nislow, C. (2012). Bugs, drugs and chemical genomics. Nat Chem Biol 8, 46-56.
Supplemental Figure S1

5 dpf, whole larvae

ctrl  sib  trappc11

anti-Trappc11

full length
129 kDa

truncated
56 kDa

anti-tubulin
Supplemental Figure S2

A. 

$2^{\Delta \Delta Ct}$ (Target - CtRef)

- $bip$
- $ddit3$
- $dnjc3$
- $edem1$
- $atf4$
- $atf6$
- $xbp1s$
- $xbp1t$

DMSO
Tm 1 μg/ml
Supplemental Figure S3

A.

| Tg(fabp10:dsRed) | 3 dpf | 4 dpf | 5 dpf |
|------------------|-------|-------|-------|
| WT               | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| trappc11         | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |

B.

3 dpf

![Graph showing gene expression levels for trappc11 and WT](image7.png)

4 dpf

![Graph showing gene expression levels for trappc11 and WT](image8.png)
**Supplemental Figure S5**

**A.** 5 dpf, liver extracts

|                | WT   | trappc11 | WT   | trappc11 |
|----------------|------|----------|------|----------|
| DMSO           | -    | -        | +    | -        |
| Endo H         | -    | -        | +    | -        |
| PNGase F       | -    | -        | +    | -        |

**FACE analysis, N-glycan, 5 dpf**

| DMSO | Tm | WT | trappc11 | WT | trappc11 | trappc11 | std |
|------|----|----|----------|----|----------|----------|-----|
|      |    |    | glycosylated |   | hypoglycosylated | unglycosylated | anti-GFP |

**B.**

- 5 dpf, liver extracts
- 40 sec exposure
- 5 min exposure
Supplemental Figure S6

A. 

B. 

C. 

*Tg(actb2:CAAX-GFP), DAPI, Cy5-Streptavidin; 5 dpf*
Supplemental Figure S7

The figure shows a box plot comparison of two conditions: WT and Atv 5 μM. The x-axis represents different genes: bip, ddit3, dnajc3, edem1, atf4, atf6, xbp1s, xbp1t. The y-axis represents the value of $2^{-\Delta\text{Ct}_{\text{target}}}$, where $\Delta\text{Ct}_{\text{target}}$ is the difference in the threshold cycle between the target gene and the reference gene. The box plot indicates the distribution of the data, with the median, quartiles, and outliers shown for each condition.
Supplemental Figure S8

A. 

| NS | TRAPα | p115 (Golgi Complex) | merge |
|----|-------|----------------------|-------|
| NS  | ![Image](image1) | ![Image](image2) | ![Image](image3) |
| C11 KD | ![Image](image4) | ![Image](image5) | ![Image](image6) |

B. 

| NS | Calnexin (ER) | p115 (Golgi Complex) | merge |
|----|-------------|----------------------|-------|
| NS | ![Image](image7) | ![Image](image8) | ![Image](image9) |
| C11 KD | ![Image](image10) | ![Image](image11) | ![Image](image12) |
| Gene name                                      | Primer name/gene abbreviation | sequence – 5'-3'          |
|-----------------------------------------------|-------------------------------|---------------------------|
| Asparagine-linked glycosylation 2 homolog     | alg2-F                        | GTGTTCCCTGACCCCTGATCT     |
| Asparagine-linked glycosylation 5 homolog     | alg5-F                        | TCTAAATGGCTATGCTGACG      |
| Asparagine-linked glycosylation 9 homolog     | alg9-F                        | GTTCTGTGCTGCTGTTTGGAA     |
| Activating transcription factor 4             | atf4-F                        | TTAGCGATTGCTCCGATAGC      |
| Activating transcription factor 6             | atf6-F                        | CTGTGGTGAAACCTCCACCT      |
| Glucose-regulated protein, 78kD (also called bip) | bip-F                        | AAGAGGCGCAAGAGAAGGAC      |
| DNA-damage-inducible transcript 3            | ddit3-F                       | AAGGAAAGTGCAGAGCCTGTA     |
| Dehydroadolichyl diphosphate synthase         | dhdds-F                       | AAATGGCGATGGGAGTGCAGG     |
| DnaJ homolog, subfamily C, member             | dnajc3-F                      | TCCCCATGCATCTGAGATC       |
| Dolichol kinase                               | dolk-F                        | GAGAGAAACGTTCTAACTGATG    |
| Dolichyl-diphosphatase 1                      | dolpp1-F                      | TCGCGATCTGCTGACCTGCC      |
| Dolichyl-phosphate N-acetylglicosaminephosphotransferase 1 | dpag1-F                       | CTCCGCCACTTTAGCAAGAC      |
| Dolichyl-phosphate mannosyltransferase 1, catalytic subunit | dpm1-F                      | CACGCATAAAACATGCAC       |
| Dolichyl-phosphate mannosyltransferase 2, regulatory subunit | dpm1-R                      | GTTTGCCCTGACTGATA        |
| Dolichyl-phosphate mannosyltransferase 3      | dpm2-F                        | CACGTTCTGTAATGGCAACC      |
| ER degradation enhancer, mannosidase alpha-like 1 | edem1-F                      | ATCCAAAGGAAGATGCATGG     |
| Farnesyl-diphosphate farnesyltransferase 1    | fdf1-F                        | TGAGGACCTGCTACGGCTAT      |
| Farnesyl diphosphate synthase                 | fdps-F                        | AGCTAGAGGCATTTAGCAGCA     |
| Glucosidase 1                                 | gcs1-F                        | CGCAGCAAAGTACCATCTGA     |
| GDP-mannose pyrophosphorylase B               | gmppb-F                       | CCAAAACACTGCTGGAGTAT      |
| 3-hydroxy-3-methylglutaryl-Coenzyme A reductase a | hmgcra-F                     | CTQAGGGCTCTGGAGACCTG      |
| 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 | hmgca-R                      | CGCCACGCTACGGTGGAGG       |
| Mannose phosphate isomerase                   | mpi-F                         | AGAAGCGCAAGGTAAGACAGA     |
|                                               | mpi-R                         | AACACGCCATACACTCCACA     |
| Protein Name                                      | Forward Primer | Reverse Primer         |
|--------------------------------------------------|----------------|------------------------|
| Mevalonate (diphospho) decarboxylase a           | mvda-F         | CGAGCCACCTTCTGAAGTACA  |
|                                                  | mvda-R         | TAGGTGGGTAGGTGTCCAGG   |
| Mevalonate kinase                                | mvk-F          | CACACTTTTGCGACCAGAGA   |
|                                                  | mvk-R          | CAGCAGCAATGAGCATCTGT   |
| Phosphomannomutase 2                             | pmm2-F         | GCAGCCACAGGAAAAAGAATC  |
|                                                  | pmm2-R         | CCAGCCTTTCTGGAACACAT   |
| Phosphomevalonate kinase                         | pmvk-F         | GCAGCCCGTCTGGAATAATCA  |
|                                                  | pmvk-R         | TCGGACTCAGCGTCTGTAT    |
| Ribosomal protein/large/p0                       | rpp0-F         | CTGAACATCTGCCCCTTCTC   |
|                                                  | rpp0-R         | TAGCCGATCTGACAGACAC    |
| DnaJ homolog, subfamily C, member                 | dnajc3-F       | TCCCCATGGGTCTGAGACTC   |
|                                                  | dnajc3-R       | CTCTGTGTGTGAGGGGTCT    |
| Steroid 5 alpha-reductase 3                      | srd5a3-F       | GGACTGACAGCTCAGTGTTAAGT|
|                                                  | srd5a3-R       | AACGTACAGCACCACCATCC   |
| X box binding protein-1 (spliced; xbp1-s)        | xbp1s-F        | TGTTGCGGACAAAGAGCA    |
|                                                  | xbp1s-R        | CCTGCACCTGCGGACT       |
| X box binding protein-1 (total; xbp1-t)          | xbp1t-F        | GAGGAGCCACAAAGTCCTCTC  |
|                                                  | xbp1t-R        | CGAAGTGCTTTTCTCTTG     |