SUPPLEMENTARY FIGURE LEGENDS:

FIG. S1. Zebrafish *eif3h* morphants show specific phenotypes compared to uninjected and control morpholino-injected embryos. Shown are representative morphant phenotypes obtained by injecting specific morpholinos (MO) for *eif3ha* observed at 1 dpf (C and F) and for *eif3hb* observed at 2 dpf (I, N and O). In each case, uninjected embryos (panels A and D for *eif3ha* and panels G, J and K for *eif3hb*) are compared with control morpholino-injected embryos (panels B and E for *eif3ha* and panels H, L and M for *eif3hb*) using the same amount of morpholino that give rise to the specific phenotypes for either *eif3ha* or *eif3hb* morpholino. Arrows in panels A – C show normal brain development in uninjected and control morpholino-injected embryos (Panels A and B, respectively), while the brain degeneration phenotype was obtained by loss of *eif3ha* (Panel C). A closer view of the *eif3ha* morphant in the brain region is included in Panel F compared to panels D and E. All of the major brain regions including eyes are severely deformed in these embryos (indicated by the straight line in panel F). The phenotypic manifestation due to loss of *eif3hb* in the form of edema around the heart and the regression of yolk stalk (closed and open arrowheads respectively) are compared in panel I with respect to panels G and H. Higher magnification views of the pericardial edema and the defective cardiac morphogenesis in the *eif3hb* morphants are depicted in panel N (compared with panels J and L). In comparison with the uninjected and control morpholino-injected embryos, in these morphants the cardiac tube is not looped properly resulting in extension of the distance between the atrium (A) and the ventricle (V) (cardiac periphery is represented by the dotted lines). The defects in cardiac morphogenesis obtained by injecting *eif3hb* morpholino was also documented in *cmlc2:gfp* transgenic embryos, which express GFP specifically in the developing heart, shown in panel O compared to panels K and M.

FIG. S2. The brain degeneration phenotype obtained for *eif3ha* morphants is likely not due to off target effects mediated by upregulation of p53.

Panel a. Panels A and B represent wild-type uninjected embryos and embryos injected with 0.75 ng of p53-specific morpholino, respectively. Under these conditions, there is no apparent morphant phenotype. In panel C, wild-type 24 hpf embryos were treated with 500 nM camptothecin (CPT) and cultured for 3 – 4 hours. This drug is known to induce p53-mediated apoptosis in zebrafish (Suppl. Ref: Langheinrich et al., 2002). The apoptosis induced by CPT is clearly visible in these embryos as dark tissue especially in the brain region (arrows). Panel D represents the p53-morphant embryos, which remained in CPT for 3 – 4 hours. In contrast to the wild-type embryos, CPT-mediated apoptosis was not observed (~95 – 100%, n > 30 - 40). This result shows that injection of 0.75 ng of this p53-specific morpholino is sufficient to rescue CPT-mediated apoptosis induced by upregulation of p53. **Panel b.** Panel B shows a representative embryo with brain degeneration phenotype as a result of injecting 4 ng of morpholino (arrowhead). Co-injection of 0.75 ng of the same p53-specific morpholino described above did not rescue this phenotype (compare panel C with panel A). These results suggest that the brain phenotype obtained by loss of *eif3ha* is not due to p53-induced off target effects.

FIG. S3. The splice-blocker morpholino targeting *eif3hb* pre-mRNA retains specificity at high dosage. RT-PCR assays were carried out using total RNA isolated from *eif3hb* morphant embryos targeted with 4 or 5 ng of splice-blocker morpholino to measure the relative levels of both *eif3ha* and *eif3hb* mature mRNAs. Under these conditions, *eif3hb* morphants showed severe brain degeneration phenotypes. A wild-type control (WT) was also included. β-actin (actin) was used as an internal control.

FIG. S4. The loss of function phenotype for a core eIF3 subunit, *eif3c*, is distinct from that obtained for loss of either *eif3ha* or *eif3hb*, affecting the overall features of early embryogenesis. Compared to 24 hpf control uninjected embryos in panel A, *eif3ha* morphants at the same stage lack properly formed brain structures (indicated by an arrowhead in panel B), whereas, stage-matched *eif3c* embryos are severely damaged with an overall grossly defective development. Likewise, loss of *eif3hb* results in a distinct cardiac edema phenotype (represented by an arrow in panel C) at ~2 dpf - a time point when most *eif3c* morphant embryos fail to survive.

Supplementary reference:

Langheinrich U, Hennen E, Stott G, and Vacun G. 2002. Zebrafish as a model organism for the identification and characterization of drugs and genes affecting p53 signaling. Curr Biol 12:2023-2028.