Supplementary information, Data S1 Materials and Methods

Fish stocks

Wild-type zebrafish (*Danio rerio*) strain TU were used and were maintained as described [1]. Embryos were staged according to Kimmel *et al*. [2]. The experimental protocols were approved by the Institutional Animal Ethics Committee of the Shanghai Institutes for Biological Sciences.

Reverse transcription-PCR

Total RNA was isolated from embryos at different stages of embryogenesis using Trizol (Invitrogen). First-strand cDNA was synthesized from 2 μg of total RNA by SuperScript II RT (Life Technologies) according to the manufacturer's protocol. *fbxl14a*, *fbxl14b* and *β-actin* were amplified by PCR using the primers shown in Supplementary information, Table S1.

cDNA construction for overexpression

The full-length coding sequences of zebrafish *fbxl14a*, *fbxl14b* and murine *fbxl14* (GenBank accession numbers NM_201482 for *fbxl14a*，NM_001015043 for *fbxl14b* and NM_133940 for *fbxl14*). were cloned into the pCS2+ or p3XFLAG-CMV™-14 vector using the primers shown in Supplementary information, Table S1 with the underlined restriction sites. Mutational constructs were generated by PCR using the corresponding constructed plasmid as a template, and the restriction sites were underlined. The primers used were shown in Supplementary information, Table S1.

Cell culture and DNA transfection

HEK 293T and HeLa cells, obtained from ATCC (Manassas, VA), were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37 °C under 5% CO_2 atmosphere. Transient transfection was carried out using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instructions.

Immunofluorescence staining

HeLa cells grown on coverslips in 6-well plates were transfected with Myc-Fbxl14a, Myc-Fbxl14b and Mkp3-HA. After 24 h, cells were washed with PBS three times, fixed in PBS/4% paraformaldehyde buffer for 30 min at 4°C. After washing three times with PBS, cells were permeabilized with 0.2% Triton X-100 for 5 min and wash twice with PBS, following by blocking in 4% BSA buffer for 30 min. Cells were then incubated at 4°C overnight with primary mouse anti-Myc and rabbit anti-HA antibodies. After being washed three times with PBS, cells were incubated with Alexa488 conjugated goat anti-mouse or Cy3 conjugated donkey anti-rabbit secondary antibody for 30 min at room temperature. Cell nuclei were stained with DAPI for 2 min and wash twice with PBS. Fluorescent images were obtained with a Leica TCS SP2 confocal microscope.

Western blotting and immunoprecipitation

Injected embryos at shield stage (early gastrula) were lysed by pipetting in 2 × loading buffer (3 μl per embryo). An equal amount of PBS was added and samples were boiled for 5

min, centrifuged for 2 min, then subjected to SDS-PAGE analysis on a 10% gel (1.5 embryos per lane). After transfer to nitrocellulose membrane, immunoblotting analyses were performed using the anti-Myc antibody.

HEK 293T cells were transiently transfected with various expression plasmids as indicated and incubated for 30 h before analysis. Cells were harvested in IP lysis buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, pH 8.0, 0.2 mM sodium orthovanadate, 10% glycerol, 1% Triton X-100, 10 mM NaF), supplemented with protease and phosphatase inhibitors. 0.5 ml of cell lysate was incubated with the appropriate amount of antibody or corresponding normal immunoglobulin G (IgG) overnight at 4 °C. After incubation, 50 μl of bead slurry (GE Healthcare #12-0780-01) was added to each sample and the mixture was rotated about 4 h at at 4 °C. After centrifugation, the beads were washed four times with IP lysis buffer, eluted by boiling in SDS loading buffer and subjected to Western blotting analysis using the indicated antibodys. The following primary antibodies were used in this study: anti-Flag (Cell Signaling #2368), mouse anti-HA (Sigma #H 9568), rabbit anti-HA (MBL #561), anti-myc-tag (Cell Signaling #2276), anti-ubiquitin (Stressgen #SPA-200), anti-GFP-tag (Abmart #M20004).

Protein ubiquitination assays

In vitro ubiquitylation assay was carried out using SCF^{Fbxl14} complexes immunopurified from HEK 293T cells with beads conjugated with anti-Myc antibody (Cell Signaling #3400). Cells were cotransfected with Skp1, Cul1, Rbx1 and Myc-Fbxl14a/b/-ΔFb. The beads containing the immunocomplexes were washed twice with equilibration buffer (25mM

Tris-HCl, pH 7.5, 50mM NaCl, 1mM EDTA, 0.01% Triton and 10% glycerol) and then added to the following reaction: 0.7 µl E1, 1 µl E2 (UbcH5C),1 µl ATP, 3 µl ubiquitin,5 µl 10× ubiquitination buffer and H_2O in a final volume of 50 µl. The GST-Mkp3 substrate was purified form *Escherichia coli*. The reaction was carried out at 37°C for 90 min. The ubiquitinated Mkp3 proteins were detected by Western blotting. *In vivo* ubiquitylation assay was carried out similar to the immunoprecipitation assay except that, cells were treated with MG132 (Sigma) at a final concentration of 10 µM for 6 h prior to harvesting.

Microinjection of synthetic mRNAs, morpholinos, or plasmid DNAs-

Morpholino oligonucleotides (MOs) against *fbxl14a* and *fbxl14b* and control morpholino were purchased from Gene Tools, LLC. The sequence of the oligonucleotides is indicated in Supplementary information, Table S1.

Synthetic mRNAs encoding Fbxl14a, Fbxl14b and others were transcribed *in vitro* using mMESSAGE mMACHINE kit (Ambion) according to manufacture's instruction. Plasmid DNAs, morpholinos and synthetic mRNAs were dissolved in injection buffer (40 mM Hepes [pH 7.4], 240 mM KCl, and 0.05% phenol red) and were injected into the yolk of one- or two-cell stage zebrafish embryos. For testing the efficacy of MO, the 5'-UTR and a portion of the coding sequence of *fbxl14a* or *fbxl14b* containing morpholino target sites were amplified by PCR and inserted in-frame into pCS2-GFP, resulting in expression plasmid Fbxl14a-5'UTR-GFP and Fbxl14b-5'UTR-GFP, respectively. For rescue experiment, the mRNA of *fbxl14a* or *fbxl14b* was mixed and co-injected with a corresponding MO.

Whole-Mount *In Situ* Hybridization

Digoxigenin-UTP-labeled antisense RNA probes were generated by *in vitro* transcription using a DIG RNA labeling kit (Roche). Whole-mount in situ hybridizations were performed as described [3] with minor modifications.

1. Solnica-Krezel L, Schier AF, Driever W. Efficient recovery of ENU-induced mutations from the zebrafish germline. Genetics 1994; **136** (4):1401-1420.
2. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Dev Dyn 1995; **203** (3):253-310.
3. Jowett T, Lettice L. Whole-mount in situ hybridizations on zebrafish embryos using a mixture of digoxigenin- and fluorescein-labelled probes. Trends Genet 1994; **10** (3):73-74.