Supplementary methods

Western blotting

Protein extracts were prepared from 20 wild-type or $z^{\nu 77h}$ mutant larvae ($z^{\nu 77h}w^{67c23}$, Bloomington Center #1385). Larvae were homogenized in HEMGN buffer (25mM Hepes-KOH pH 7.6, 0.1 mM EDTA, 12.5 mM MgCl2, 10% glycerol, 0.1% NP40, 1 mM DTT, 0.1 mM PMSF, 0.3 M KCl). An equal volume of 2X SDS reducing buffer was added and then boiled for 5 minutes. The extract was centrifuged at 12 000 g for 2 min, supernatant was used for SDS-PAGE. After electrophoresis proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane. The membranes were incubated for 1 h with the indicated primary antibody, followed by incubation for 1 h with the secondary antibody (GE Healthcare Amersham), which was conjugated to horseradish peroxidase. Proteins were detected using chemiluminescence reagents. As a control anti-tubulin mouse antibody (Abcam ab44928) was used.

Preparation of the nuclear extracts and immunoprecipitation

The nuclear extracts were obtained from Drosophila Sg4 cells. For this purpose, 10⁸ cells were washed twice in 10 mL of ice cold PBS and resuspended in in 10 mL of ice cold IP-Sucrose buffer (10 mM Tris, pH 7.5; 10 mM NaCl, 10 mM MgCl2, 1mM EDTA, 1 mM EGTA, 1 mM DTT, 250 mM sucrose, EDTA-free protease inhibitor cocktail), incubated on ice for 10 min, and homogenized with a Dounce loose pestle. The nuclei were then pelleted by centrifugation at 3000 g, 40C for 10 min. The pellet was resuspended in 1 mL RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 2mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, EDTA-free protease inhibitor cocktail). Lysates were cleared by centrifugation at 18 000 g, + 4°C for 10 min. Immunoprecipitation experiments involved incubation with corresponding antibody (rabbit antibody against PH, Sfmbt or nonimmune rabbit IgG) and A-Sepharose beads (Thermo Scientific). Antibody–protein complexes were collected by centrifugation. The beads were

washed three times with RIPA buffer. After the last washing step, the beads were resuspended in SDS-PAGE loading buffer, boiled, and analyzed by Western blotting.