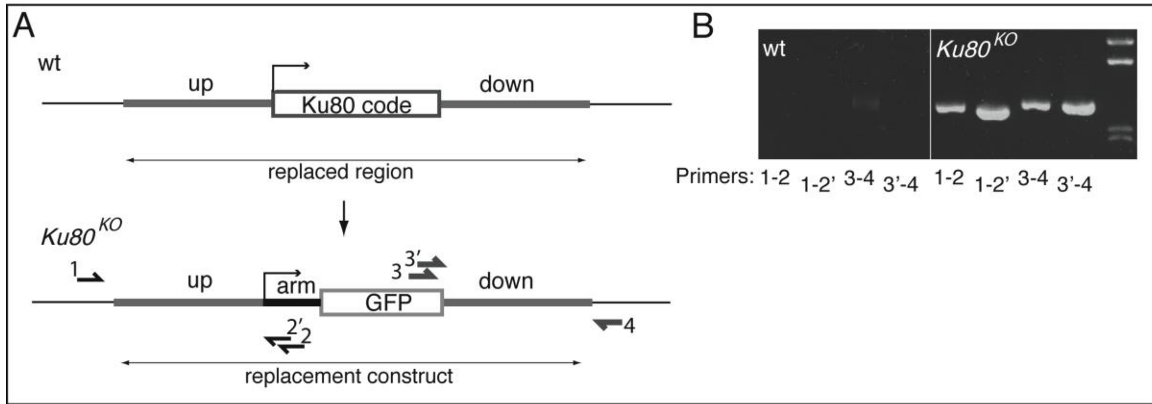

Figure S1 Molecular characterization of Ku80 deletion

A. Schematics of Ku80 region. Top: the wt Ku80 region with upstream (up) and downstream (dn) sequences (~2.5 kb each) that flank the coding region; “replacement region” designates sequences replaced by ends-out targeting. Bottom: the Ku80 region in Ku80Δ mutant; the up and dn flanking fragments were used in ends-out gene targeting, in which the Ku80 coding sequence was replaced by an arm-GFP marker gene; “replacement construct” designates sequences brought in by ends-out targeting. The flanking sequences are unaltered from wt. Half-arrows indicate primers used for the PCRs in B. Note that primer 1 and 4 reside outside the Ku80 region. Primers 2 and 2’ reside within armadillo promoter sequences, while primer 3 and 3’ are within GFP.

B. PCR products from four test PCRs for wt and Ku80Δ lines. Primer pairs used for each PCR are indicated bellow the image, and their locations are specified within the schematic in A. For exact primer sequences see the Supplemental Methods.

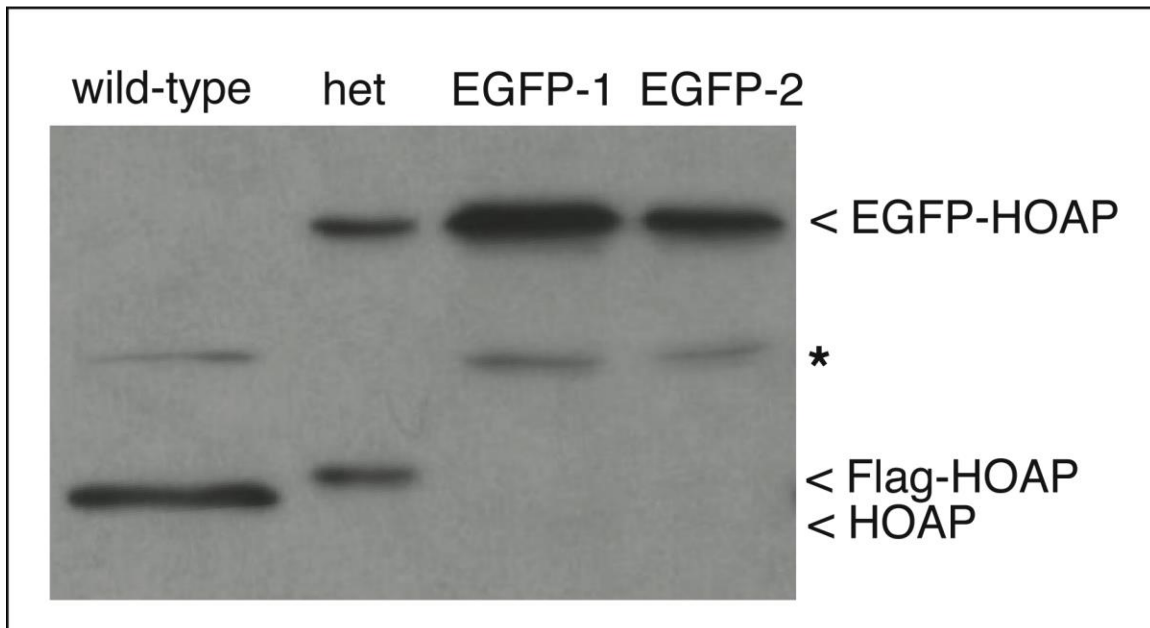

Figure S2 EGFP-HOAP is produced at normal level
Western Blot was performed on embryos extracts from flies of the indicated genotype and probed using rabit anti-HOAP antibody. Lanes are labeled at the top: 1) wild-type; 2) het: heterozygous line, which carries both EGFP-HOAP and a Flag-tagged HOAP (accounting for slightly larger HOAP band); 3) EGFP-1 and EGFP-2: two independent EGFP-HOAP lines which carry only the tagged version of HOAP. The major band corresponds to a cumulative size of HOAP plus EGFP. A non-specific band is labeled with an asterisk (*).

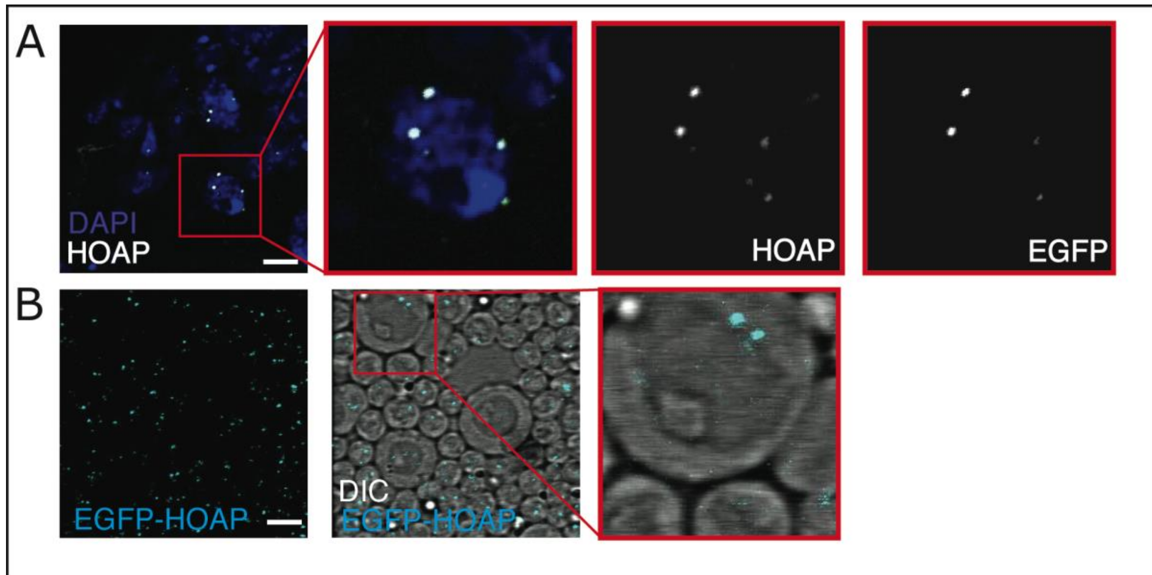

Figure S3 Telomere distribution in larval brain

A. Confocal projection showing immunostaining in larval brain nuclei; HOAP or EGFP in white as indicated, DAPI in blue. Panels in order: 1st, merge; 2nd, zoom-in of area in red square; 3rd, HOAP antibody staining alone for zoom-in; 4th, EGFP fluorescence alone for zoom-in.

B. Confocal projections collected from live larval brain; EGFP in cyan, DIC in grayscale. Panels in order: 1st, EGFP alone; 2nd, DIC and EGFP; 3rd, zoom-in of the area in the red square.

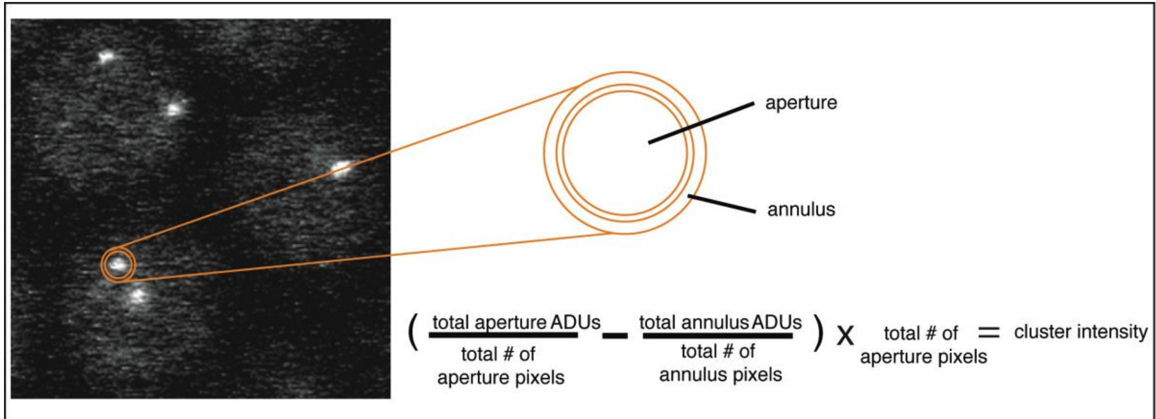

Figure S4 The measurement method used to quantify EGFP-HOAP signal in embryos
A schematic of an aperture superimposed over a Z-section. Aperture is the area of the inner circle, whereas annulus is the area of the surrounding ring. The measurement of spot intensity is obtained according to the formula presented, i.e. the average pixel intensity within the aperture was adjusted for background by subtracting average pixel intensity within the annulus and then multiplying by the number of aperture pixels.

File S1

Supporting Methods

Generating the construct for Ku80 replacement

Vector pBS(arm-GFP), previously described in Gong et al. (2005) was used for generating the construct for Ku80 replacement. A 2.5 kb fragment immediately upstream of the start codon of Ku80 was PCR-amplified and cloned into the HindIII and KpnI sites in pBS(arm-GFP). A 2.5 kb fragment downstream of the stop codon of Ku80 was PCR-amplified and cloned into the NotI and XbaI sites in pBS(arm-GFP). The final 7.8 kb targeting fragment that contained the Ku80-upstream and downstream fragments as well as the arm-GFP marker was cloned into the NotI and XhoI sites of the ends-out targeting vector pW30, which carries the white+ (w+) marker gene (Gong and Golic, 2003). Primers used for the test PCRs on targeted lines (see Supplemental Figure 1): 1– Ku80-2394d - CAGAGACCCACTCACAAATG, 2 – arm288up - CGATAACTCCTCTATCGCAG, 2' – arm31up-GCAGTCGTAGAAGTGGGTTC, 3 – eGFP-1269f - GACAACCACTACCTGAGCAC, 3'– eGFP1330f - CACATGGTCCTGCTGGAGTTC, 4 – Ku80-10334u - GTAAGCAGTTACAATGCCATCAC.