Ren et al., https://doi.org/10.1083/jcb.201706027

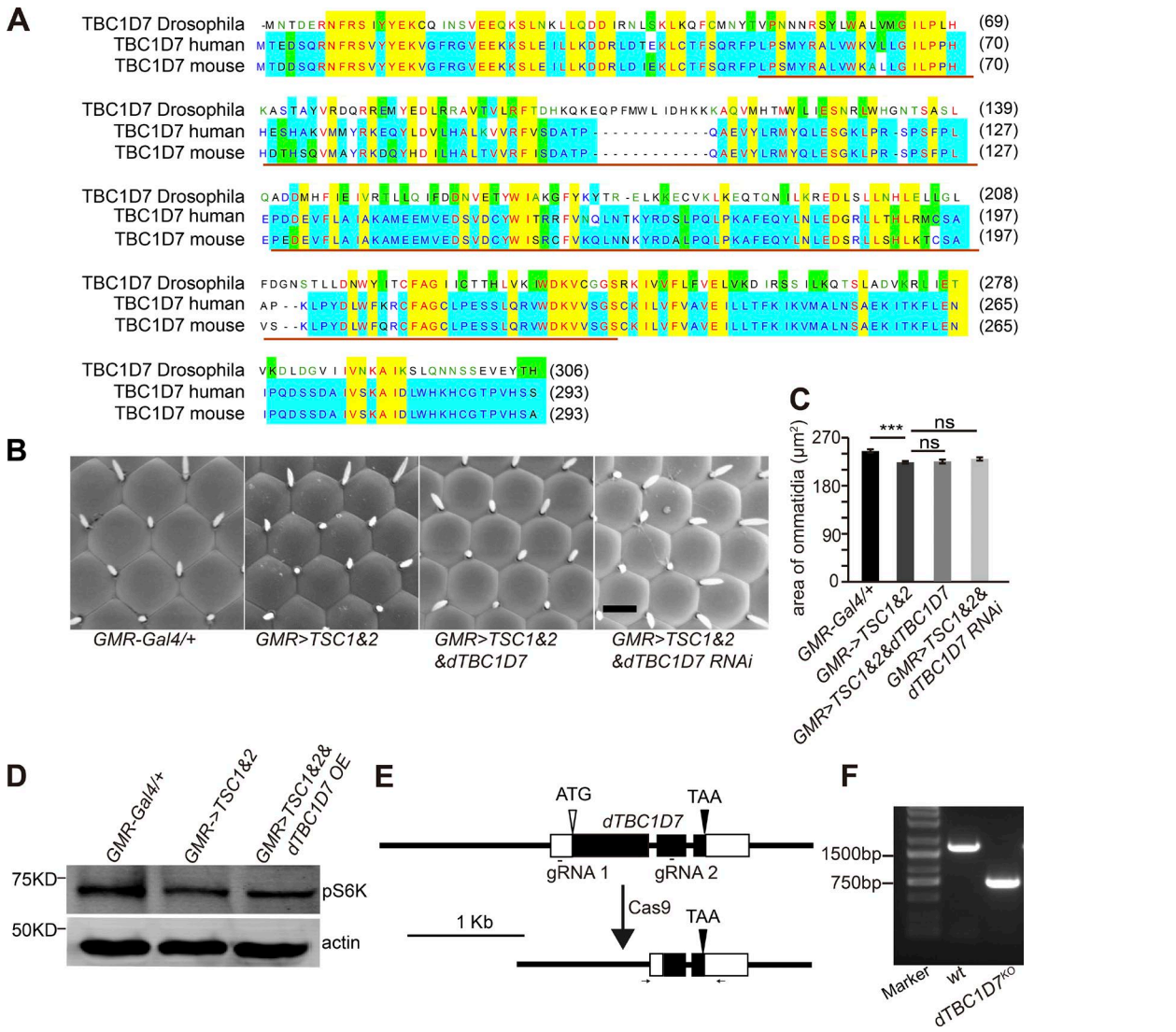

Figure S1. **Generation of the *dTBC1D7* knockout flies. (A)** Alignment of *Drosophila* TBC1D7 amino acid sequence with human and mouse TBC1D7. Identical and conserved residues are enclosed in yellow and green boxes, respectively. The TBC domain is indicated by the red line underneath the corresponding sequence. **(B)** Scanning electron microscopy of the compound eyes. Bars, 100 µm. *dTBC1D7* transcript levels were elevated ~200-fold in heads of *GMR>dTBC1D7* (*GMR-Gal4/UAS-dTBC1D7*) flies compared with control heads. **(C)** Qualification of the ommatidia size. At least 20 male flies of each genotype were assayed. **(D)** Western blot of pS6K with indicated genotypes. **(E)** Schemes for generation of $dTBC1D7^{KO}$ allele by sgRNA targeting. The organization of the *dTBC1D7* locus and the sgRNA (1 and 2) primer pair, which was used to generate the $dTBC1D7^{KO}$ mutation, are shown. The positions of the DNA primers used for knockout test PCR in panel F are indicated with arrows. **(F)** The PCR results from genomic DNA of homozygous $dTBC1D7^{KO}$ flies showed deletion of an 800-bp fragment, including the first exon and part of the second exon of the *dTBC1D7* gene.

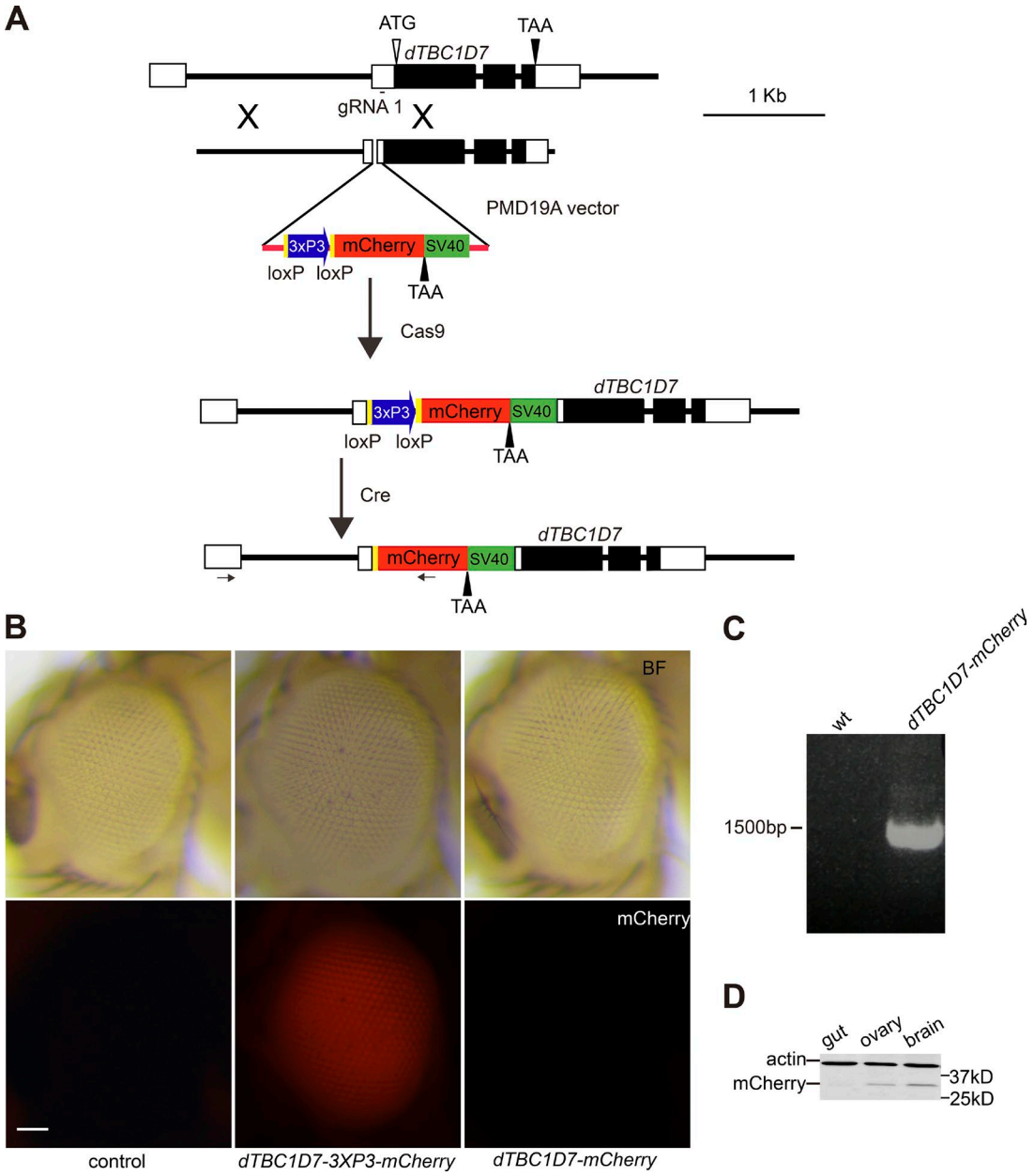

Figure S2. **Generation for *dTBC1D7-mcherry* flies, in which *mcherry* was integrated into the 5′ UTR translational region of *dTBC1D7* locus. (A)** The *mcherry* DNA with an eye-specific *3XP3* promoter was inserted into the genomic locus of *dTBC1D7* by CRISPR/Cas9-mediated homologous recombination, and the knock-in flies were selected by red fluorescence in compound eyes. Subsequently, the *3XP3* promoter region was removed by Cre recombinase. The primer pair (arrows) designed for molecular analysis is indicated. **(B)** The image shows flies inserted with the *3XP3-mcherry* eye-marker into the *dTBC1D7* locus (*dTBC1D7-3XP3-mcherry*) and the *dTBC1D7-mcherry* flies after Cre/LoxP-mediated recombination. Bar, 100 µm. **(C)** Verification of final knock-in flies by PCR from genomic DNA of wild-type and *dTBC1D7-mcherry* flies. **(D)** The expression of mcherry in the *dTBC1D7-mcherry* flies was detected by Western blot.

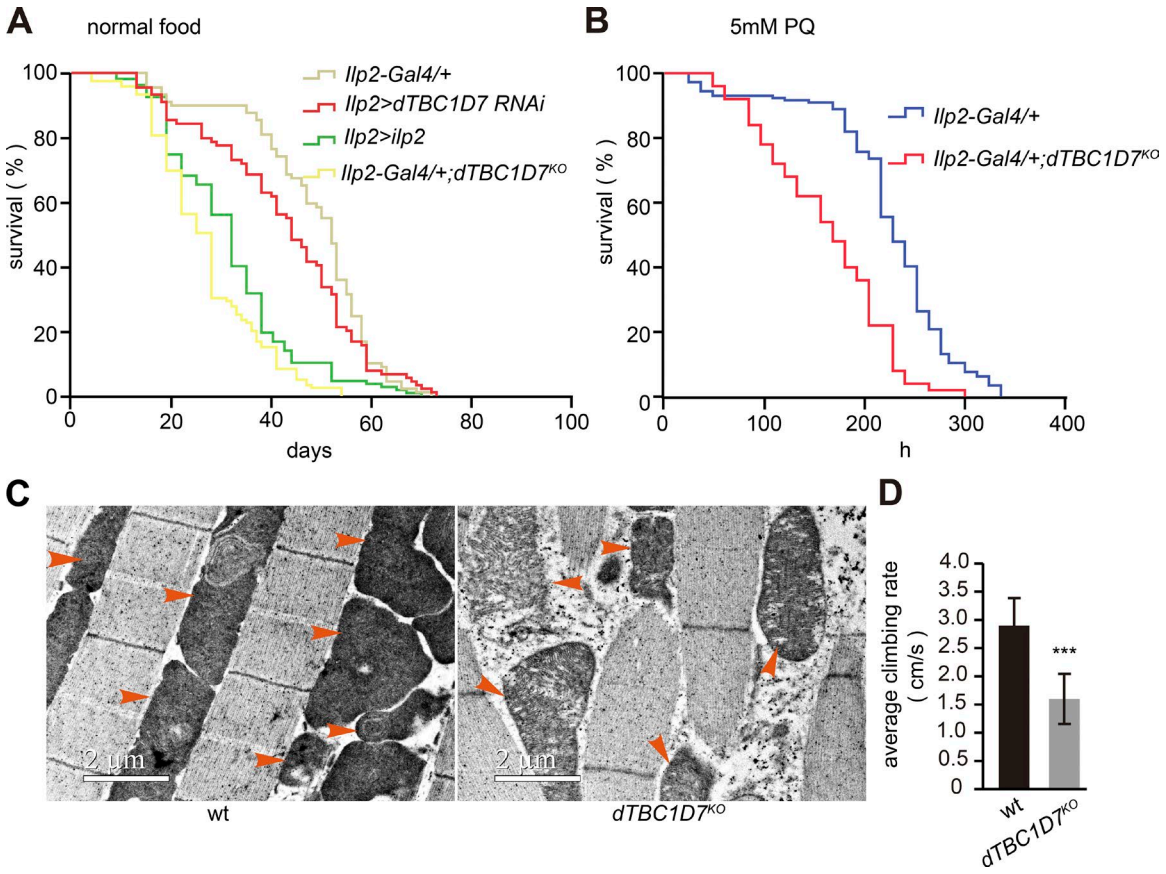

Figure S3. ***dTBC1D7* regulated longevity and protected against oxidative stress. (A)** The life span of different *dTBC1D7* alleles compared with control flies (*Ilp2-Gal4/+*). The *dTBC1D7*^{KO}, *Ilp2>dTBC1D7RNAi*, and *Ilp2>ilp2* flies had shortened life spans. **(B)** The survival curves for 3-d-old control (*Ilp2-Gal4/+*) and *dTBC1D7* mutant (*dTBC1D7*^{KO}*; Ilp2-Gal4/+*) flies after exposure to 5 mM paraquat. *dTBC1D7* mutant flies are more sensitive under oxidative stress. At least 200 male flies were quantified for each genotype. Analysis of life span was performed with SPSS software. **(C)** TEM imaging of indirect flight muscle from 30-d-old wild-type and *dTBC1D7*^{KO} flies. Bars, 2 μm. Red arrowheads indicate mitochondria. **(D)** The climbing activity of the 30-d-old wild-type and *dTBC1D7*^{KO} flies. Five groups of flies were quantified for each genotype. Significant differences were determined with Student's *t* test. ***, $P < 0.001$. Error bars indicate SD.