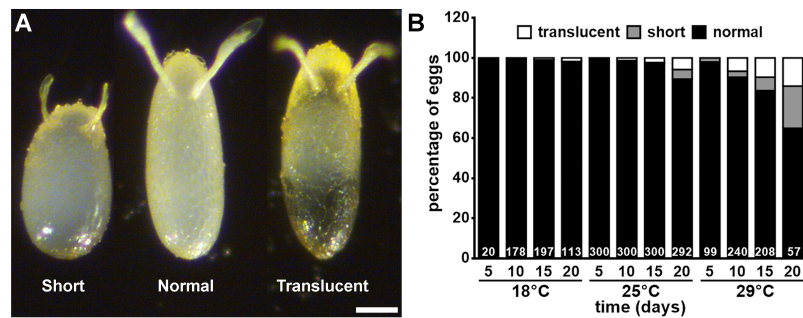
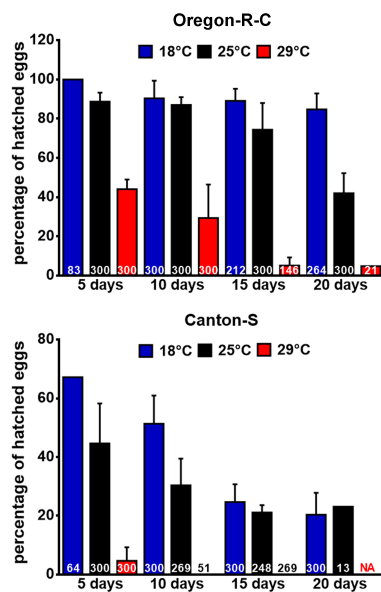


**Fig. S1. Egg production decreases in response to chronic exposure of adult Oregon-R-C and Canton-S females to suboptimal temperatures. (A,B)**

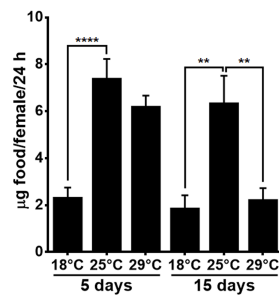
Representative graph showing daily temperature and humidity measurements for three separate 20-day experiments performed at different times of the year. (C,D) Average number of eggs laid per female per day over time upon chronic exposure of adult Oregon-R-C (C) or Canton-S (D) females to 18°C (cold) or 29°C (warm) compared to 25°C controls. Data shown as mean  $\pm$  s.e.m. from six replicates. \*\* $p < 0.01$ ; \*\*\*\* $p < 0.0001$ , F-test of third order polynomial fitted curves using 25°C as control.



**Fig. S2. Chronic exposure of females to 29°C has a small effect on eggshell morphology.** (A) Examples of eggshell phenotypes observed in eggs laid by *y w* females maintained at 29°C for 20 days. Scale bar, 100 µm. (B) Frequency of eggs showing normal, short, or translucent eggshell phenotypes as in (A) laid by *y w* females maintained at 18°C, 25°C, or 29°C for five, 10, 15, or 20 days. Numbers of eggs analyzed are shown inside bars. Data represent one experiment.

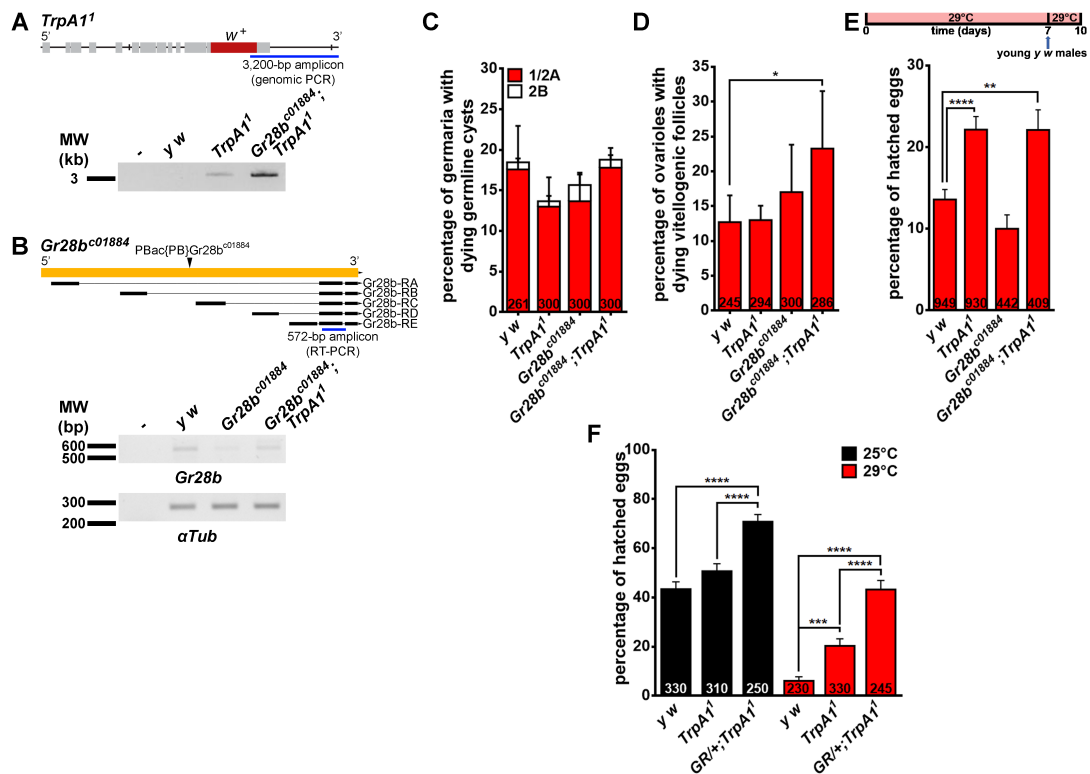


**Fig. S3. Hatching rates of eggs laid by Oregon-R-C and Canton-S females maintained at suboptimal temperatures are affected.** Percentage of hatched eggs laid by Oregon-R-C (top), or Canton-S (bottom) females maintained at 18°C, 25°C, or 29°C for five, 10, 15, or 20 days. No eggs were obtained from Canton-S females incubated at 29°C for 20 days (NA). Data shown as mean  $\pm$  s.d. for one experiment with three replicates.



**Fig. S4. Food consumption by adult females is influenced by temperature.**

Graph showing total food consumption per female per day for y w females incubated at 18°C, 25°C, or 29°C for five or 15 days, based on the Consumption-Excretion assay. (See text for details.) Data shown as mean  $\pm$  s.e.m. from three independent experiments.  $**p < 0.01$ ;  $****p < 0.0001$ , Unpaired two-tailed t-test using 25°C as control.



**Fig. S5. Canonical warm temperature sensors do not play a major role in mediating the effects of 29°C exposure on oogenesis.** (A) Verification of *TrpA1*<sup>1</sup> allele (originally generated by ends-out homologous recombination (Kwon *et al.*, 2008)) by genomic PCR. The 3,200-bp diagnostic PCR product is indicated in blue. (See Methods for details.) (B) Verification of *Gr28b*<sup>c01884</sup> allele by RT-PCR from female heads (Thorne and Amrein, 2008). The 572-bp RT-PCR product, which reflects expression of all *Gr28b* isoforms (black lines and bars), is indicated in blue. (See Methods for details.) (C) Percentage of germaria containing Apoptag-positive dying cysts in Region 1/2A or Region 2B from *y w* control, *TrpA1*<sup>1</sup> homozygous, *Gr28b*<sup>c01884</sup> homozygous, or *Gr28b*<sup>c10084</sup>; *TrpA1*<sup>1</sup> double homozygous females incubated with *y w* males for 10 days at 29°C. Numbers of germaria analyzed are shown inside bars. Data shown as mean  $\pm$  s.e.m. from three independent experiments. No statistically significant differences, Chi-square test. (D) Frequencies of ovarioles containing dying vitellogenic follicles in same females as in (C). Numbers of ovarioles analyzed are shown inside bars. Data shown as mean  $\pm$  s.e.m. from three independent experiments. \* $p < 0.05$ , Chi-Square test using *y w* as control. (E) Quantification of effect of 29°C on oocyte quality in females of same

genotypes as in (C) and (D). Control and experimental females (with *y w* males) were incubated at 29°C for seven days, then two-day-old *y w* males replaced original males followed by incubation for three additional days at 29°C prior to collection of eggs laid within the last 24 hours and hatching rate quantification. Numbers of eggs analyzed shown inside bars.  $**p < 0.01$ ,  $****p < 0.0001$ , Unpaired two-tailed t-test using *y w* as control.(F) Quantification of oocyte quality in *y w* or homozygous *TrpA1*<sup>1</sup> females with or without a *TrpA1* genomic rescue (GR) incubated with *y w* males for 10 days at 29°C as in (E) or maintained at the 25°C control temperature. These results show that the *TrpA1*<sup>1</sup> mutation is not responsible for the observed phenotype. It is conceivable that remaining genetic background differences cause the observed differences in hatching rates (despite the fact that the *TrpA1*<sup>1</sup> mutant and rescue transgenes were backcrossed into the isogenized *y w*). Numbers of eggs analyzed shown inside bars.  $***p < 0.001$ ,  $****p < 0.0001$ , Unpaired two-tailed t-test.