SUPPLEMENT

Construction of the w*; UAS-eGFP/CyO;P{tubP-GAL4}LL7; P{tubP-GAL80^{ts}}7/TM3 Sb^{1} Ser^{1} flies. *Localization of transgenic insertions in the Drosophila genome*. Since the location and orientation of GAL4 and GAL80^{ts} transgenes on chromosome 3 were not known, they had been mapped by reverse PCR before the crosses were made.

DNA from the fly lines 7018 and 5138 (Bloomington) was isolated according to the standard protocol [1]; 400 ng of DNA from each sample was treated separately with restriction endonucleases *Sau*3A, *Cfo*I, and *Hae*III, and then 10 µl (200 ng of DNA) of each reaction mix was diluted to 200 µl with 1x ligation buffer. T4 ligase (Thermo Fisher, USA) was then added in the amounts recommended by the manufacturer. The reaction was carried overnight at 4°C; 5 µl of the ligation mixture was used for the reverse PCR.

Primers used for reverse PCR (according to [2]).

P{tubP-GAL80^{ts}}7 (7018)

3′-end of P-element *Pry2-Pry1*, *Pry4new-Pry1*

5′-end of P-element *Plac1-Plac4*

P{tubP-GAL4}LL7 (5138)

3′-end of P-element *Pry2-Pry1*

5′-end of P-element *Plac1-Plac4*

PCR products were sequenced after separation in agarose gel and electroelution. The resulting sequences were mapped to the R6 *Drosophila melanogaster* genome using BLAT in the UCSC genome browser (https://genome.ucsc.edu). It was found that *P{tubP-GAL80^{ts}}* in the line 7018 is in chr3R:29806755(+), while *P{tubP-GAL4}* from the line 5138 is in chr3L:21880095(–). The transgenes were far enough from each other and could be easily combined in one chromosome by recombination.

Construction of the recombinant chromosome. The scheme of the recombinant chromosome and the overall design of the *trans*-inactivation experiment are shown in Fig. 1 of the main text.

To create the flies containing both GAL80^{ts} and GAL4 genes on the chromosome 3 and the *UAS-eGFP* reporter gene on the chromosome 2, the following crosses were performed using 7018 (*w*; sna^{Sco}/CyO; P{tubP-GAL80^{ts}}7*) and 5138 (*y^{1} w*; P{tubP-GAL4}LL7/TM3 Sb^{1} Ser^{1}*) lines.

The first cross:

P. *y^{1} w*; P{tubP-GAL4}LL7/TM3 Sb^{1} Ser^{1}* x *w*; sna^{Sco}/CyO; P{tubP-GAL80^{ts}}7*

The *w*; +/CyO;P{tubP-GAL4}LL7/P{tubP-GAL80^{ts}}7* females were selected from the offspring for the second cross:

F1. ♀ *w*;+/CyO; P{tubP-GAL4}LL7/P{tubP-GAL80^{ts}}7* x ♂ *w*; UAS-eGFP/CyO;P{tubP-GAL4}LL7/TM3, Sb^{1} Ser^{1}*

Due to the recombination in *w*; +/CyO; P{tubP-GAL4}LL7/P{tubP-GAL80^{ts}}7* females, all possible allele combinations were present in the offspring. Parent flies were crossed and the offspring was cultivated at 18°C. Under these conditions, the activity of GAL4 was suppressed by the GAL80^{ts} repressor when it was present. From the offspring, were selected flies carrying balancer chromosomes *TM3 Sb^{1} Ser^{1}* and *CyO* and lacking eGFP fluorescence. These flies were then incubated 24 h at 29°C to inactivate GAL80^{ts} and to induce eGFP expression in flies containing GAL4. The flies exhibiting eGFP fluorescence after incubation at elevated temperature have the *w*; UAS-eGFP/CyO; P{tubP-GAL80^{ts}}7 P{tubP-GAL4}LL7/TM3 Sb^{1} Ser^{1}* (hereafter *UAS-eGFP/CyO; GAL80^{ts} GAL4/TM3 Sb^{1} Ser^{1}*) genotype and produced a stable line when crossed to each other. Since the *UAS-eGFP* insertion is viable, there were *UAS-eGFP/CyO* and *UAS-eGFP/UAS-eGFP* flies in the line.

		A		B		C		D		E		F		G		H		I	
		A4	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*
A	*A4*	1.0E+00	9.5E-30	3.5E-17	1.7E-58	4.6E-01	2.3E-03	1.8E-04	1.0E-58	1.2E-05	2.2E-08	7.3E-12	1.3E-44	1.7E-22	3.7E-41	9.9E-19	6.0E-46	1.7E-48	1.1E-70
	A4-		1.0E+00	6.7E-07	2.4E-18	3.8E-30	7.9E-26	1.4E-20	1.5E-42	1.8E-01	2.7E-06	5.6E-06	8.0E-37	2.2E-13	2.5E-35	2.6E-16	1.4E-41	7.8E-45	3.8E-66
B	*A4*			1.0E+00	4.0E-35	5.4E-24	4.9E-15	1.2E-06	3.5E-49	1.0E-02	4.1E-07	3.1E-08	4.8E-40	7.4E-17	8.4E-38	2.7E-17	2.0E-43	2.7E-46	4.0E-68
	A4-				1.0E+00	4.5E-58	9.5E-54	1.5E-52	9.6E-29	2.2E-01	1.1E-04	3.8E-02	5.5E-30	7.3E-07	4.5E-30	3.0E-14	9.3E-38	9.1E-42	3.3E-62
C	*A4*					1.0E+00	3.6E-13	4.4E-07	5.7E-59	7.2E-06	1.9E-08	4.8E-12	1.0E-44	7.7E-23	3.9E-41	8.7E-19	6.0E-46	1.4E-48	1.4E-70
	A4-						1.0E+00	3.6E-02	1.4E-56	5.5E-05	4.0E-08	4.0E-11	1.5E-43	2.1E-21	3.0E-40	2.0E-18	2.9E-45	5.2E-48	7.2E-70
D	*A4*							1.0E+00	3.6E-55	2.4E-04	6.9E-08	1.9E-10	6.7E-43	2.9E-20	6.3E-40	3.5E-18	5.1E-45	1.2E-47	9.2E-70
	A4-								1.0E+00	6.5E-15	5.6E-01	2.7E-08	6.3E-07	6.9E-04	8.1E-10	5.4E-07	8.1E-23	5.0E-31	1.1E-47
E	*A4*									1.0E+00	4.5E-05	1.8E-02	1.7E-24	1.0E-05	2.9E-27	4.9E-15	1.0E-38	1.1E-42	1.5E-64
	A4-										1.0E+00	2.5E-03	6.8E-02	4.4E-02	1.2E-02	1.0E-03	5.9E-08	3.5E-18	1.4E-16
F	*A4*											1.0E+00	2.6E-17	2.9E-02	4.3E-20	3.1E-12	4.9E-32	1.7E-38	2.5E-55
	A4-												1.0E+00	2.3E-12	2.1E-01	1.4E-02	3.3E-10	3.0E-22	9.8E-31
G	*A4*													1.0E+00	2.1E-15	7.2E-10	2.9E-28	2.8E-35	1.8E-53
	A4-														1.0E+00	9.3E-02	1.6E-07	8.2E-20	4.4E-26
H	*A4*															1.0E+00	3.0E-02	3.5E-11	6.4E-10
	A4-																1.0E+00	6.3E-09	3.4E-08
I	*A4*																	1.0E+00	5.6E-02
	A4-																		1.0E+00

Table S1. Matrix of *p*-values of pairwise comparisons of the fluorescence intensity distribution in different temperature treatment variants (based on unpaired two-sample Student's *t*-test). Cells with the *p*-values greater than or equal to 0.05 (i.e., with no significant difference) are highlighted in yellow. Only when the reporter gene was expressed at a high level throughout development (variant I), there was no significant difference between the flies with *trans*-inactivation and the control. The matrix was generated using R (https://cran.r-project.org/).

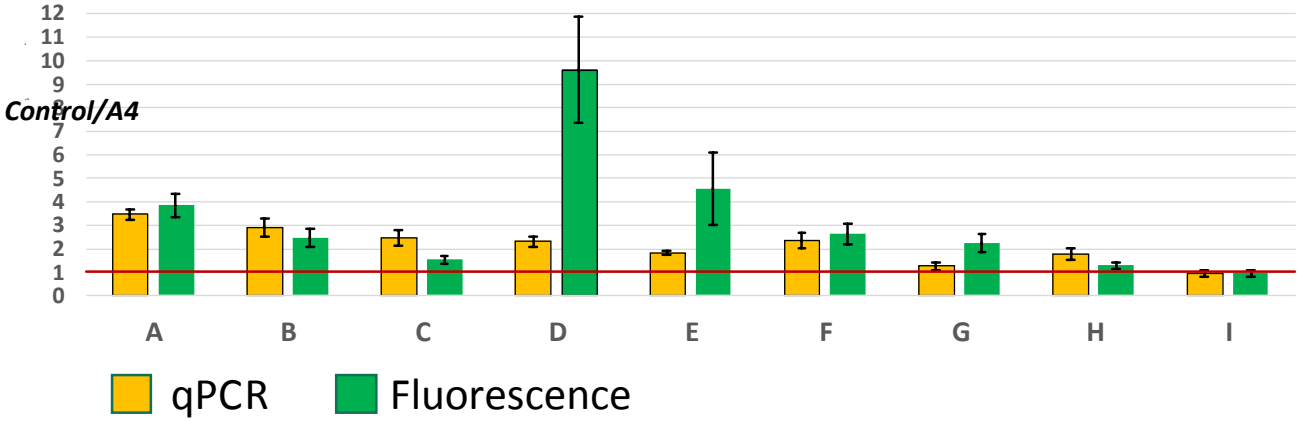

Fig. S1. Degree of gene repression based on the fluorescence measurements (green bars) and *eGFP* mRNA quantification by RT-qPCR (yellow bars). Column height is the ratio between the amount of mRNA or mean fluorescence in the control flies to the amount in the flies with *trans*-inactivation. The higher the column, the greater the level of repression. The red line is the level where the ratio is 1 (no repression). A-I, variants of temperature treatments (see the text of the article).

SD	A		B		C		D		E		F		G		H		I	
FC	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*	*A4*	*A4-*
0-1	0.4	0.3	12.1	0.0	9.1	5.0	2.8	0.0	0.3	0.0	0.0	0.0	0.0	0.0	5.2	0.0	0.0	0.0
1-2	1.9	5.7	8.2	0.0	9.1	0.5	5.7	2.6	24.6	0.2	5.8	0.0	10.7	0.0	0.0	0.0	0.0	0.0
2-4	5.1	4.2	4.8	13.2	0.0	5.4	2.8	2.4	8.1	2.9	4.8	5.2	4.8	0.0	5.1	0.1	0.0	0.0
4-8	2.7	10.2	0.7	11.1	0.0	0.0	5.7	3.5	13.2	14.2	5.4	5.5	2.9	0.0	0.2	2.7	0.2	0.0
8-16	0.0	0.0	7.9	2.1	0.0	0.0	0.0	3.6	2.2	1.9	1.0	6.0	1.6	5.9	3.1	7.2	2.9	5.2
16-32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.7	2.6	14.2	5.0	9.4	7.2	1.1	0.7	6.4	11.0	16.6
32-64	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.6	2.5	2.5	10.0	0.0	4.8	4.2	0.6	12.2	16.8
64-128	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	2.6	0.0	0.0	0.0	2.5	2.0	5.1

Table S2. The quantitative data for standard deviations

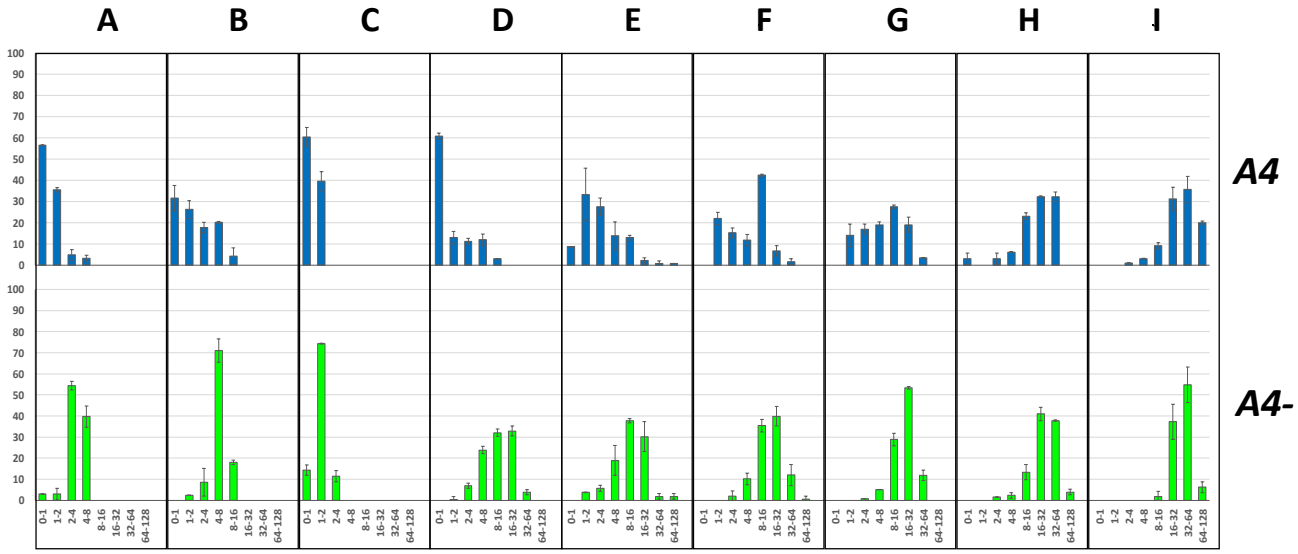

Fig. S2. Standard deviations between the measurements of eGFP fluorescence in the experiment repeats (see Fig. 2C of the article); X-axis, the subranges of fluorescence levels (fold changes relative to the minimal value); Y-axis, percentage of cells that fall into the subrange. Error bars show standard deviation. The quantitative data for standard deviations are presented on Table S2.

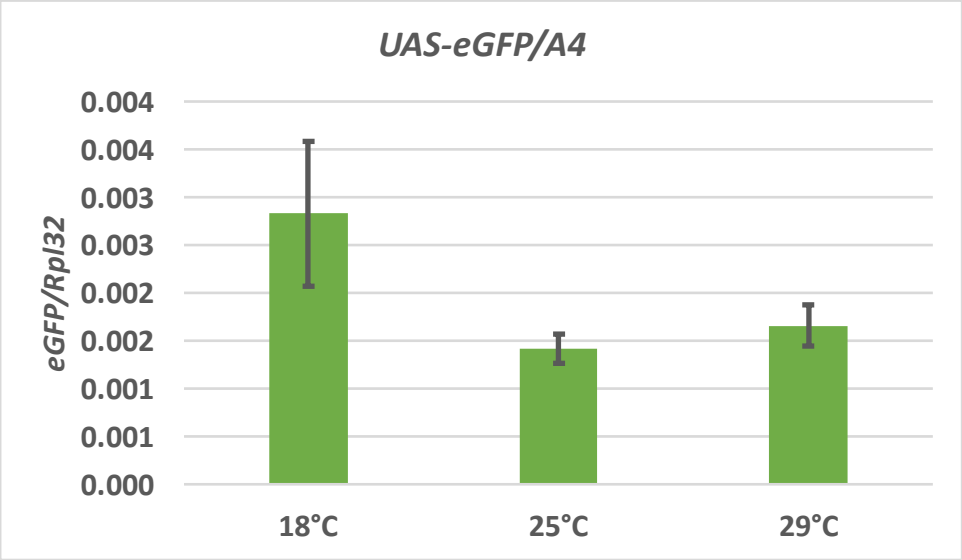

Fig. S3. Quantification of eGFP mRNA in the *UAS-eGFP/A4* entire adult female flies (*trans*-inactivation) at different temperatures (18°C, 25°C, and 29°C) by RT-qPCR using the *ΔCt* method. eGFP mRNA was normalized to the housekeeping *Rpl32* gene mRNA. The flies had no GAL4 gene, so no significant influence of temperature on the *eGFP* expression level was observed

REFERENCES

1. Ashburner, M. (1989) Drosophila, Vol. 2, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory.
2. Huang, A. M., Rehm, E. J., and Rubin, G. M. (2009) Recovery of DNA sequences flanking P-element insertions in Drosophila: inverse PCR and plasmid rescue, *Cold Spring Harb. Protoc.*, **2009**, doi: 10.1101/pdb.prot5199.