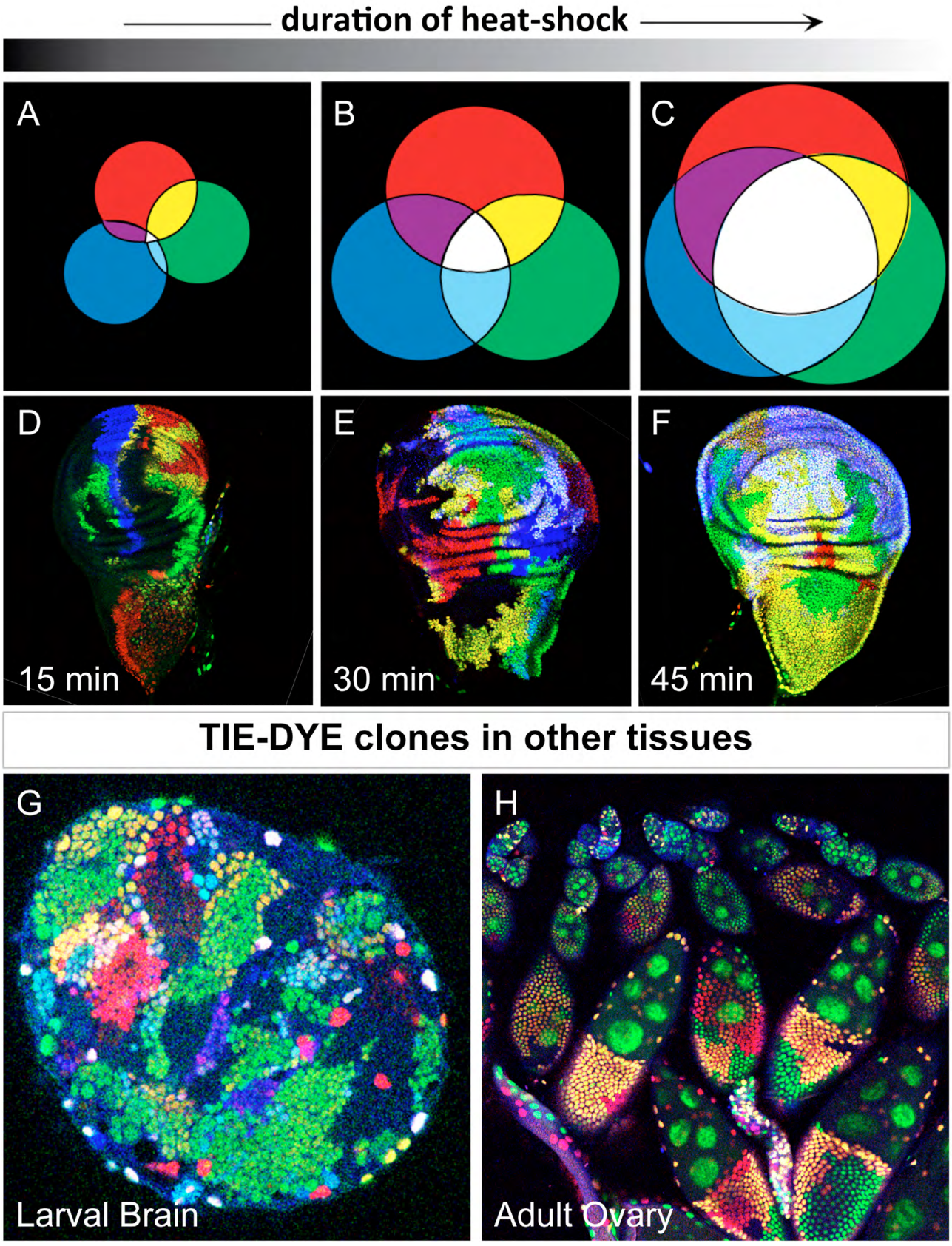


Fig S1. The *Drosophila* TIE-DYE system (**A-F**) The duration of the heat-shock effects the number of cells that are marked by one, two or three of the independent flip-out constructs. (A-C) With a longer heat shock, the proportion of cells are doubly (yellow, teal, purple) or triply labeled (white) increases. (D-F) Third instar wing imaginal discs from animals that were heat-shocked for (D)15, (E) 30 or (F) 45 minutes at 24±2 AEL. There are more yellow and white clones with longer heat shocks. (**G**,**H**) The TIE-DYE method has been tested for generating all the different colors in other tissues, including (G) the larval brain and (H) the adult ovary. *UAS-his2A::RFP* is not expressed in the germ line (because it is not a UASp construct); therefore, the germ line cells only appear green, blue or teal.

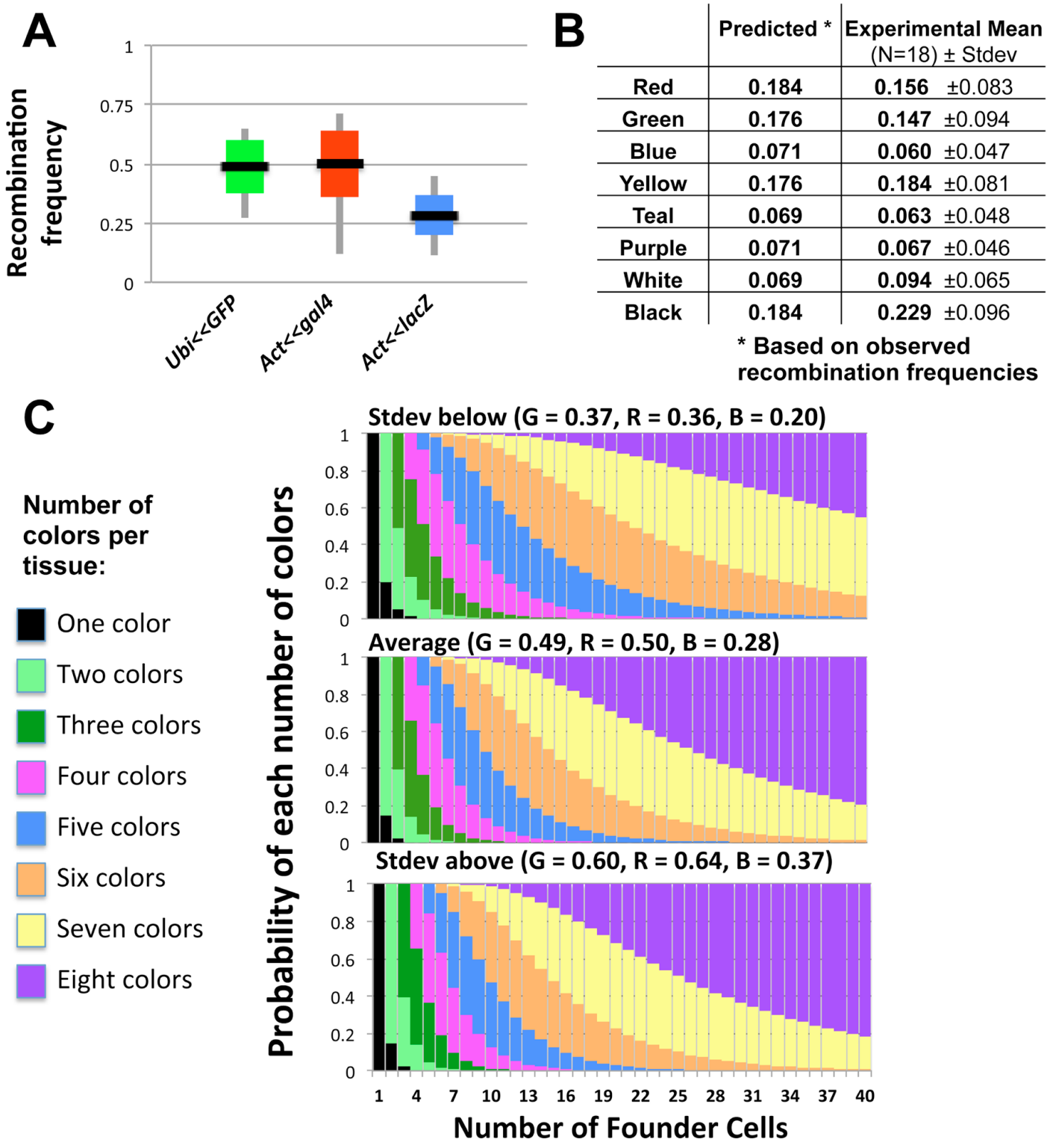
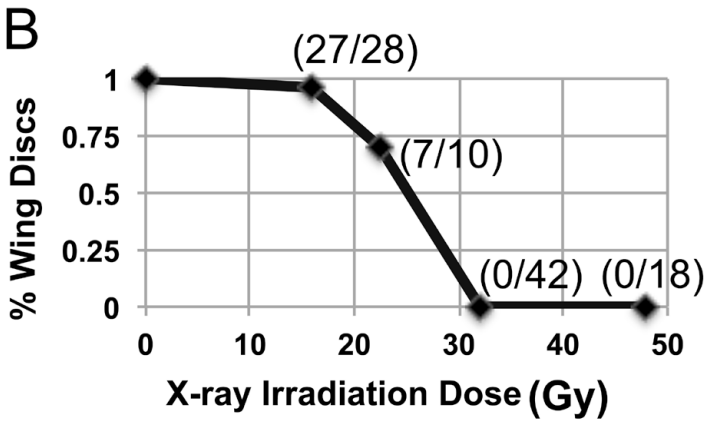


Fig. S2. Modeling the number of colors in a tissue versus the number of founder cells. (**A**) Experimentally calculated recombination frequencies for a 30-minute heat shock at 16±1 hours AEL under non-irradiated conditions. The frequencies were based on the ratio of the area of GFP, RFP and β-gal-positive tissue to total area in third instar wing discs. The black bars show the average (mean), the colored rectangles show a s.d. above and below the mean, and the vertical gray lines show the extremes (*n*=18 discs). (**B**) The predicted versus experimentally observed color frequencies, which are generated by unique marker combinations from the three FLP-out cassettes. The predicted color frequencies were generated using equations that assume the FLP-out events occur independently (see Materials and methods). The predicted color frequencies are all within a s.d. of the experimental color frequencies. (**C**) The modeling of the number of colors predicted to be in a disc based on the number of founder cells using three recombination frequencies (listed above the graphs: G = *Ubi<<GFP*, R = *Act<<GAL4*, *UAS-RFP* and B = *Act << lacZ*). The upper graph represents the model generated with the recombination frequencies a standard deviation below average; the lower graph a standard deviation above the mean. The center graph shows a representation of the model generated by the average recombination frequency (also shown in Fig. 2B) for comparison.

A

IR Dose	% Viable*
12.8 Gy	17 % (N = 183)
19.2 Gy	0 % (N = 183)
25.6 Gy	0 % (N = 97)

*adults



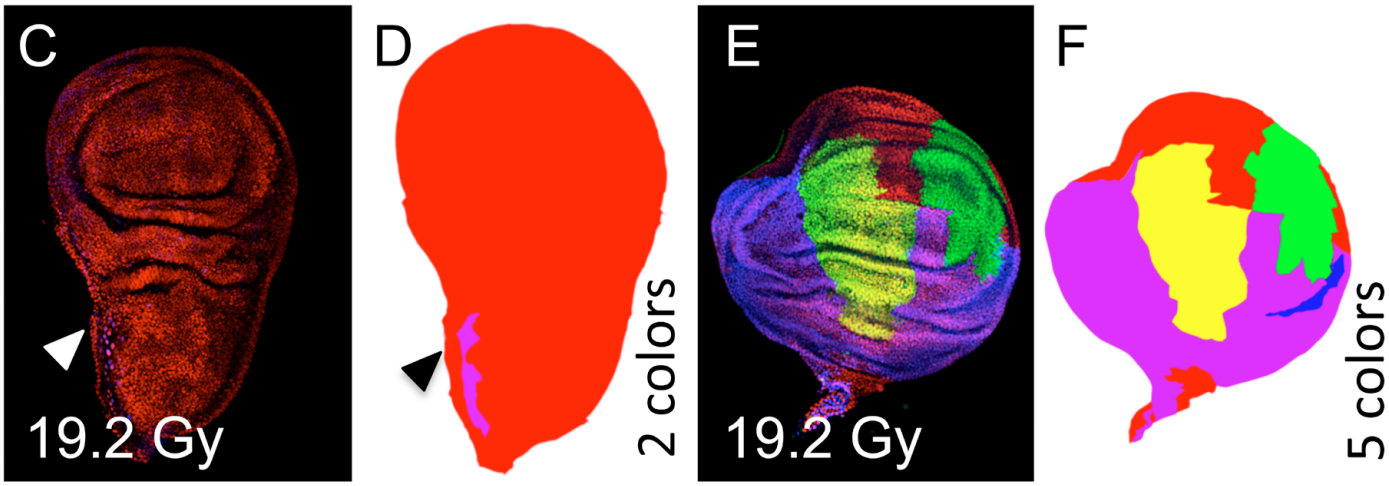


Fig. S3. Additional information on the X-ray irradiation experiments. (**A**) The number of animals that eclosed from their pupal cases compared with the total number of pupal cases observed at different doses of X-ray irradiation. Animals survived to adulthood only with the lowest dose of irradiation. (**B**) The number of wing discs recovered during dissection of third instar larvae at different doses of X-ray irradiation at 16±1 hours AEL. Higher X-ray dose produced larvae without any recognizable imaginal discs. (**C-F**) Additional examples of wing imaginal discs from the intermediate X-ray dose of 19.2 Gy, where C and D show an example of a morphologically normal wing disc that is only made up of two colors, and E and F show an example of a disc with abnormal morphology, with lost or underdeveloped notum, made up of five colors.

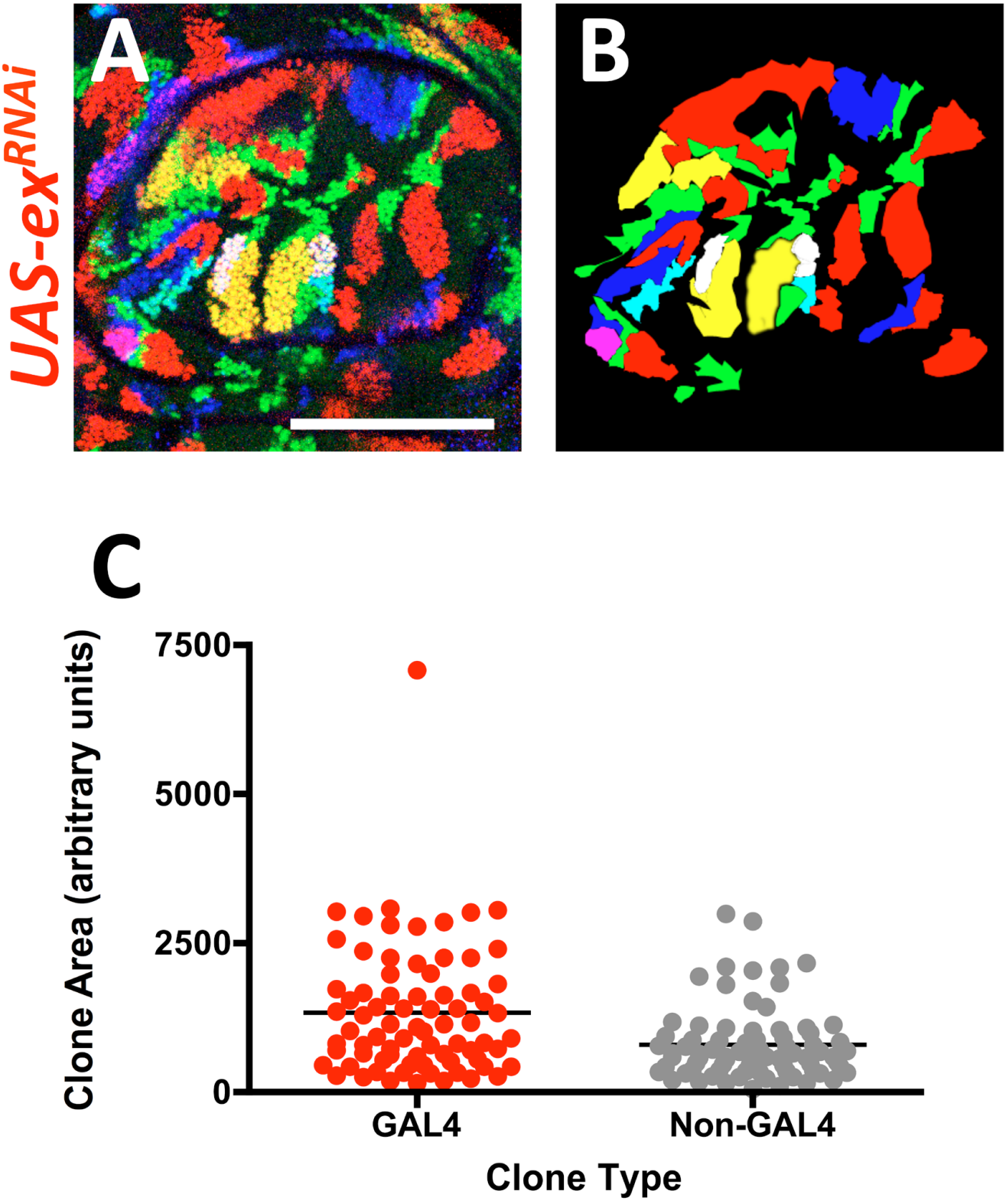


Fig. S4. GAL4(+) clone expressing *UAS-ex*^{RNAi} versus GAL4(–) clone sizes. (**A**) Example of wing pouch expressing RNAi to *ex* (same image as in Fig. 4). (**B**) The clone area tracing to show different color clones. (**C**) The clone area was measured for GAL4 and non-GAL4 clones from three separate wing discs. GAL4(+) clones include red, yellow, purple and white clones. Non-GAL4 clones include blue, green and teal. Each dot is the area of a single clone. The population of GAL4(+) clones are larger than the population of non-GAL4 clones. This difference is statistically significant as assessed by the Mann Whitney test, two-tailed (P=0.0003).