



Figure S7. Construction and characterization of a UAS-/LexAop2-reporter transgene array.

**Figure S7** Construction and characterization of a UAS-/LexAop2-reporter transgene array. (A) Injection and screening strategy to isolate a transgene array: Embryos carrying the primary transgene *attP2[LexAop2-nls-lacZ]* were co-injected with the secondary transgene UAS-tdTomato-nls and an *Int\**-expressing plasmid construct.  $G_0$  adults were crossed to flies homozygous for *attP2[R57C10-Gal4]*, which expresses Gal4 pan-neurally. Progeny were screened for the presence of mini-white (the LexAop2-nls-lacZ marker) and fluorescence. Double-positive flies represented candidate arrays. (B) Molecular characterization of candidate UAS-tdTomato-nls/LexAop2-nls-LacZ reporter arrays: The order of transgenes in an array is determined by whether integration of the additional component occurs at attL or attR. Top – Schematic of the locus that results from integrating the secondary transgene at attL, with the secondary transgene (green) downstream of the primary transgene (purple). Genomic PCR (primers indicated above the schematic) showed that 9/12 candidates were in this orientation. Bottom – Schematic of the locus that results from integrating the secondary transgene at attR. Genomic PCR (primers indicated below the schematic) revealed that candidate 6 is in this orientation. Though candidates 4 and 11 were double-positive for tdTomato-nls and nls-LacZ, they are presumed to reflect off-target integrations and were not further characterized. (C) Expression characteristics of *attP2[LexAop2-nls-lacZ + UAS-tdTomato-nls]*: Top – In the absence of Gal4 and LexA drivers, LacZ and tdTomato are undetectable. Middle – LacZ can be detected in a small number of cells (red arrowheads) when the pan-neural Gal4 driver *C155 (P{GawB}elav<sup>C155</sup>)* is used to drive tdTomato-nls. Bottom – tdTomato-nls can be detected in a small number of cells in the central brain when the pan-neural driver *attP2[R57C10-LexA]* is used to express nls-LacZ.