



Figure S4: Proof-of-principle of *eve* stripe 2 expression normalization by *eve* stripe 1

(A) Peak *eve* stripe 1 intensity and peak *eve* stripe 2 intensity follow a linear relationship, indicating that stripe 1 is an appropriate measure for normalization. Three different stains of the WT *eve* locus were analyzed (stain 1: $n=10$, stain 2: $n=6$ and stain 3: $n=5$). The r-squared coefficient of the linear relationship is 0.92. The genotypes were as follows: stain 1: $y[1] w[67c23]; attP2\{hbP2-LacZ\}$, stain 2: $y[1] w[67c23]; attP\{eve-TER94 \text{ construct}\}$ (Fujioka *et al.* 2013), stain 3: $y[1] w[67c23]; attP2\{evep-eve37\}$. (B) Normalized peak stripe expression is compared across three stains of the WT *eve* locus. The expression levels do not differ significantly within stripes across multiple stains. Filled circles represent the mean expression level within a stain and open circles are individual embryo peak expressions.