Supplementary Materials

**Supplementary File 1. Housekeeping genes identified by Corrales et al.**

This .csv file includes the gene names, the dm6 release coordinates, and the FlyBase numbers (FBgns) that I matched to the list of housekeeping gene names and coordinates kindly provided by Dr. Corrales (Corrales et al., 2017). Some genes have since been withdrawn and are noted as such in the FBgn column. Others have more than one matching FBgn because their coordinates overlap with both. In this case, both FBgns are included.

**Supplementary File 2. Enhancer-gene pairs collected from a variety of sources**

This .csv file includes the dm6 release coordinates of the collected enhancer-promoter pairs and the names of their target genes. Enhancer-genes pairs were identified from multiple databases—(1) REDfly & CRM Activity Database 2, or CAD2, which consists of non-redundant experimentally characterized enhancers identified through enhancer trapping experiments (Bonn et al., 2012; Halfon, Gallo, & Bergman, 2008); (2) Vienna Tile (VT) enhancers, which were identified through functional characterization of ~7000 enhancer candidates using high throughput *in situ* hybridization and were limited to those expressed during stages 4-6 (Kvon et al., 2014); and (3) 4C-seq performed by Ghavi-Helm et al. and include enhancers that are active 3-4 hours after egg laying (stages 6-7) (Ghavi-Helm et al., 2014)⁠. Those that are part of the smallest, minimally overlapping set of enhancers with an allowed base pair overlap of 25% of the median length of the enhancers in this set (249bp) were identified by using SelectSmallestFeature.py available at the Halfon Lab GitHub (<https://github.com/HalfonLab/UtilityPrograms>) and are indicated in the column “non-overlapping enhancer” with a 1.

**Supplementary Files 3 – 17. Plasmid maps of cloned MS2 reporter constructs as GenBank files**

The MS2 reporter constructs have been cloned into the pBphi vector, and the enhancers, promoters, MS2 sequences, and when relevant, mutations or substitutions, have been annotated.

**Supplementary Files 18 – 35. Representative videos of the transcriptional dynamics of each transgenic reporter measured here**

The movies show the maximum projection of transcription driven by the transgenic reporter in hemizygous embryos in the middle of nc14. Elapsed time of movie is shown in upper left corner. For the anterior-p1 reporters and the locus reporters imaged in the anterior, the imaging region is centered at approximately 22% egg length on the ventral side of the embryo, and for the remaining enhancer-promoter reporters and the locus reporters imaged in the posterior, the imaging region is centered at approximately 63% egg length on the lateral side of the embryo.