

Description of Additional Supplementary Files

File Name: Supplementary Data 1

Description: BLAST alignment of rDNA sequences from the Dm.nT2T genome assembly to the R6 genome.

File Name: Supplementary Data 2

Description: BLAST alignment of histone gene clusters from the Dm.nT2T genome assembly to the R6 genome.

File Name: Supplementary Data 3

Description: Public omics data of *D. melanogaster* used in this study, downloaded from the ENA database (originally sourced from NCBI).

File Name: Supplementary Data 4

Description: *D. melanogaster* resequencing samples from multiple populations (data from Lu et al.)

File Name: Supplementary Data 5

Description: The list of genes in the Dm.nT2T genome assembly.

File Name: Supplementary Data 6

Description: Statistics of different types of misannotation for genes.

File Name: Supplementary Data 7

Description: Public RNA-seq data of *D. melanogaster* used in this study, downloaded from the ENA database (originally sourced from NCBI).

File Name: Supplementary Data 8

Description: Summary of differential gene expression analysis (Chr2R.722).

File Name: Supplementary Data 9

Description: Summary of differential gene expression analysis (Chr3L.2449).