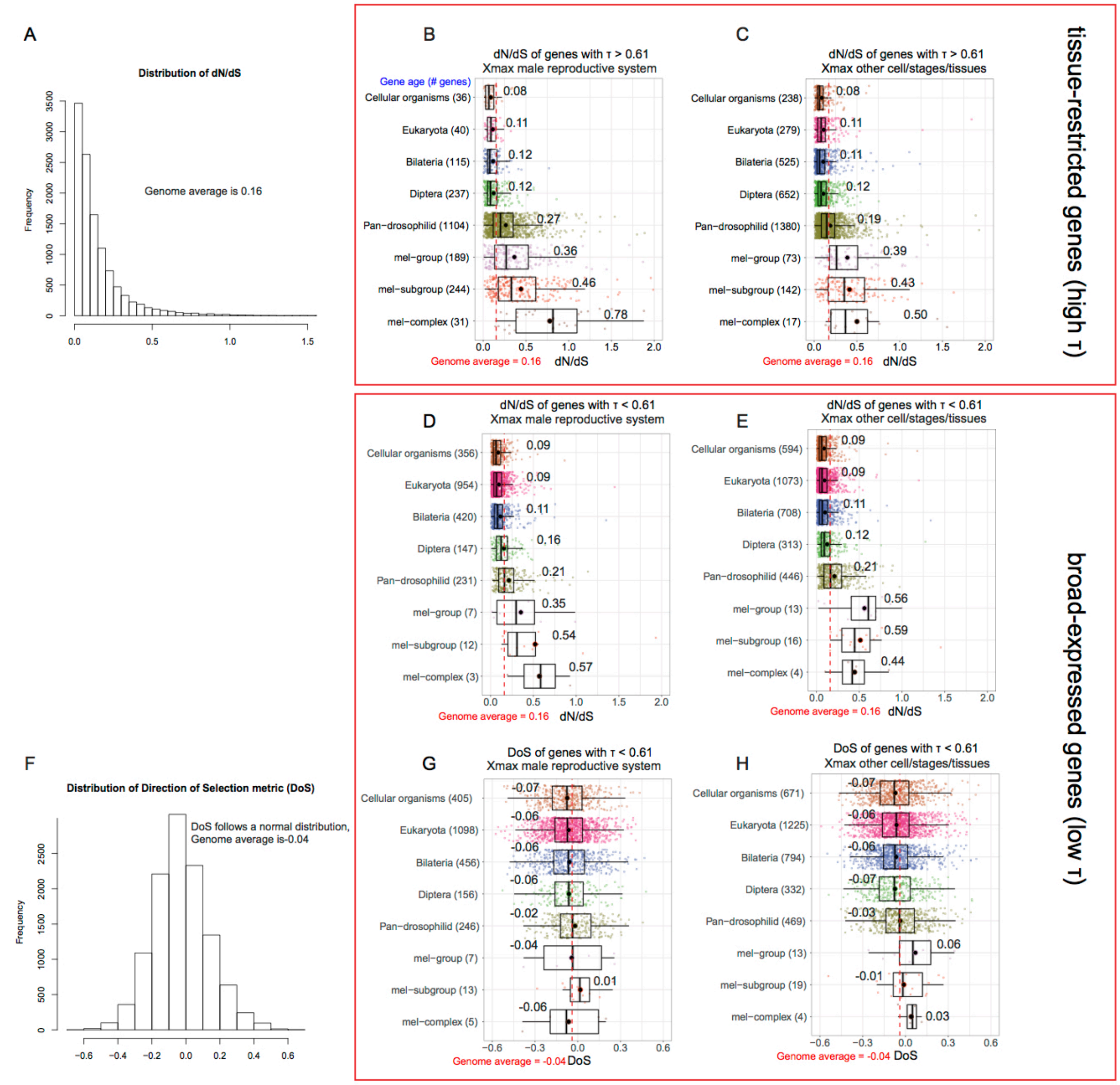

Supplementary Figure 16: Summary of dN/dS analyses and DoS statistics

(A) Genomewide distribution of dN/dS for D. melanogaster genes. The distribution is skewed to the left with a genome average of 0.16. (B, C) dN/dS ratios of tissue-restricted genes (τ>0.61, see Figure 1C) were calculated for loci that were highest expressed in the male reproductive system (B) or in other tissues/stages/cell types (C). Genome average of dN/dS is indicated with a dotted red line, the dots in each box-plot represent means and values are indicated; the median is shown as a midline in the box plots. dN/dS for older genes are much below the genome average indicating purifying selection, while genes in the younger age groups show higher dN/dS relative to genome average, a pattern indicative of positive selection. However, high dN/dS ratio can result from either positive selection or relaxed selective constraint; and dN/dS cannot distinguish these two alternatives due to the lack of within-species polymorphism data in the analysis, which is a limitation of this analysis. While dN/dS has limitations to detect positive selection, synonymous changes inferred from dN/dS do not confound patterns of purifying selection.
(D) dN/dS ratios according to gene age for broadly expressed genes (τ <=0.614) that happen to be highest (Xmax) in male reproductive system. (E) dN/dS ratios for broadly expressed genes with Xmax outside of the male reproductive system. While the dN/dS ratios of younger genes tends to be higher than the genome average in both (D) and (E), there are relatively few genes in this categories and the skew is lower than for young genes with high tissue-restriction to the male reproductive system (B). On the other hand, highly conserved, ubiquitously expressed older genes tend to show a strong signature of purifying selection, as the average dN/dS ratio for these age categories is well below the genome average.

(F) Genomewide distribution of DoS values for D. melanogaster genes. The distribution follows a normal distribution with an average of -0.04. For DoS statistics divided according to gene age classes, we observe the average DoS of different classes of broadly expressed young genes with Xmax male reproductive system (G) vs Xmas in other cells/tissues (H) are mostly below the genome average, indicative of purifying selection. Two exceptions are mel-subgroup in Panel G, and mel-group in Panel H for which DoS is above the genome average, but still close to neutral evolution (0.01 and 0.06, respectively). Overall, the trend of DoS in broadly expressed genes is consistent with purifying selection. This contrasts with the behavior of young genes with high tissue-restriction to the male reproductive system exhibits strongly positive DoS (see main Figure 3).

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Supplementary Figure 16