Figure S1: Maximum TPM per AG *de novo* Gene. AG de novo genes show primarily low expression, though ~14% show a maximum expression ≥ TPM 5.

Figure S2: Coding Potential of *D. melanogaster* Protein Coding Genes. The coding potential for protein coding genes from FlyBase are shown binned into 100bp windows. Most AG *de novo* genes, which had a 300bp minimum size filter, were assigned a coding probability less than the cutoff for Drosophila of 0.39, unlike the protein coding genes which had an average coding potential of ≥ 0.8 for genes 300bp or greater.

Table S1: Reads per Tissue. This table contains the number of reads sequenced for each library.

Table S2: AG-expressed *de novo* Gene Information. This table contains information about the location, expression and other properties of the observed *de novo* genes.

Table S3: Screened Out Testis *de novo* Genes. This table indicates the testis *de novo* genes from Zhao *et al.* 2014 that were screened out from our analysis due to matches to transcripts in other species.

Table S4: AG-biased ncRNA. This table includes information about signal sequence and matches to the *D. melanogaster* protein database for AG-biased ncRNA.

Table S5: AG-biased ncRNA ORFs. This table has open reading frames for ncRNA that either have predicted signal sequence or match the *D. melanogaster* protein database.

Table S6: Comparison of AG-biased ncRNA vs. *de novo* Genes in 500kb Windows. This table contains the 500kb window analysis for AG-biased ncRNA vs. *de novo* genes.

Table S7: Comparison of Tissue Biased vs. *de novo* Genes in 100kb Windows. This table contains the 100kb window analysis for both the AG and testis *de novo* genes.

Table S8: Regulatory Mechanisms for *de novo* Genes. This table contains the predicted regulatory mechanisms for both AG and testis *de novo* genes.