**Supplemental Results**

**Fat Body**

Clusters of fat body cells are found throughout the body of insects, including two discrete lateral abdominal clusters that surround the spermatheca and parovaria (and to a lesser extent to the gut) of *D. melanogaster* females. These FRT-associated fat body likely have specialized functions related to their spatial distribution, as demonstrated for both female abdominal fat bodies, which produce lipids for oocyte development (Bownes 1982; Keeley 1985), and male head fat bodies which are involved in courtship behaviors (Fujii *et al.* 2008). Although the potential function of the FRT-associated fat body in post-copulatory reproductive functions of *Drosophila* is unknown, in reduviid bugs this cluster of fat body cells have been implicated in sperm storage (Nascimento *et al.* 2019).We included the FRT-associated fat body in our survey of FRT tissues for two reasons: first, to investigate its putative contributions to reproductive events within the FRT. Second, to account for any potential “contamination” of fat body cells to the transcriptome of the spermatheca and parovaria as has been suggested in previous studies (Allen and Spradling 2007; Prokupek *et al.* 2009).

The FB had a distinct transcriptome compared to the FRT tissues. Notably, it possessed a significantly larger number of tissue-specific genes relative to FRT tissues, despite having a smaller number of expressed genes (χ2= 1085.30, df = 5, *p* = 2.08e-232). FRT-associated fat body-specific genes exhibited a range of enriched GO categories including the production of lipid proteins (*lipid particles*, *p* = 3.28e-6), immune response (*innate immune response*, *p* = 2.95e-13; *response to bacterium*, *p* = 6.74e-15), oxidation-reduction processes (*p* = 3.19e-11), and proteolysis (*p* =8.48e-13; Table S3). These enrichments correspond to the established role of the FRT-associated fat body in metabolism, immunity, and the production of vitellogenins that contribute to oocyte maturation (Keeley 1985). As expected given its distinct transcriptomic characteristics, post-mating expression changes in the FB had a weak, albeit significant, correlation with all FRT tissues (average unmated-to-6 hrs post-mating R2 = 0.03 $\pm 0.02$; average 6 hrs-to-24 hrs post-mating R2 = 0.05 $\pm 0.02$; Fig S4). The unique expression patterns of the FRT-associated fat body confirm minimal contamination of FRT tissues.

To further investigate the FRT-associated fat body we examined the relationship of gene expression in FRT tissues to the array of somatic and reproductive tissues available from FlyAtlas2 (Leader *et al.* 2018). We first compared the correlation in expression across all tissue samples for the genes expressed in the FRT (8,337 genes; Fig S2A). We found that our fat body sample was most similar to the general abdominal fat body sample of both males and females from FlyAtlas2. To compare the expression patterns of the FRT-associated fat body to other fat body samples while mitigating the effect of technical difference, we compared the log2 fold change between our FRT-associated fat body and the FlyAtlas2 male and female fat body samples (Fig S2B). The differences between the FRT-associated fat body and the flyatlas2 male and female fat bodies were highly correlated (R2 = 0.2, *p* < 0.001). Notably, the male fat body differed from both female fat body samples in the greater expression of SFPs. In addition, we found that our FRT-associated fat body sample had even greater expression of female-biased and FRT-biased genes than the FlyAtlas2 fat body sample. To look at the expression patterns of the fat body more broadly, we examined the correlation matrix for only FlyAtlas2 tissues including all genes with available expression data (13,678 genes; Fig S2C). In this analysis we found that female and male fat body samples did not group together due to the correlation among the male fat body with the male carcass, male whole body and accessory glands and the female fat body with the female carcass and spermathecae. Together these analyses suggest that fat bodies, particularly those that are closely associated with the reproductive tract, have sex-specific expression patterns including genes predominantly expressed in the female and male reproductive glands.

In total, the data presented here suggests that the FB is mating responsive tissue that may have specific reproductive functions in relation to the FRT. However, more in-depth study, particularly of defined fat body clusters or single cells, is required to for definitive assessment of heterogeneity in fat body expression. The FRT-associated fat body-specific genes identified here represent an initial step in characterizing this cluster of cells and could be used to develop fat body-expression drivers to directly assess fat body functions. The potential contributions of the FRT-associated fat body to the female-ejaculate interactions that occur within the FRT is an intriguing possibility in need of further study.

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