Supplemental text S1 Testis-specific expressions of *de novo* miRNA genes

We note that the small RNA libraries of “imaginal discs” in (Lyu et al. 2014) and miRBase are pooled samples that also contain brain and gonad tissues, in addition to imaginal discs. In these data, the *de novo* mirs of this study are detected but the expression should be attributed to the inclusion of testes in the samples. In no other expression studies (published or unpublished) that use testis-free samples did we find these *de novo* miRNAs expressed. We would also like to mention that the fitness analyses carried out in this study do extend beyond the strict association with testicular functions. For example, the long-term population experiments include all fitness components in the life cycle of flies and no unexpected fitness contributions are detected.

Technical details: The pooled disc samples, generated by (Ruby et al. 2007) and used in miRBase, are described as ‘mass isolated imaginal discs’(Berezikov et al. 2011). These samples are collected by the filtering method of Fristrom (Fristrom 1972). In its execution, the ‘mass isolated imaginal discs’ samples are commingled with similarly sized tissues including gonads and brains. Indeed, in the recent publications by the same authors of the miRBase data (Mohammed et al. 2014), these six miRNAs (*mir-973, mir-975, mir-977*,mir-*978 ,mir-983* and *mir-984*) are characterized as testis-restricted. Apparently, the expression levels of these mirs in the imaginal disc data can be accounted for by the testicular contribution.

Finally, we have recently surveyed the expression patterns of these same miRNAs in various male larval tissues by qRT-PCR. Indeed, these miRNAs are expressed only in the gonads (Supplemental Fig.S1)(Lyu et al. 2018).

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