

Male fertility assay:

F0 ♀	Reference female (<i>w¹¹¹⁸</i>) <i>w¹¹¹⁸/FM7C;+/+;+/+</i>			♂	Experimental male <i>miRNA⁻,w¹¹¹⁸;+/+;+/+</i>			
	X							
	↓							
F1	Days	1	2	3	4	5	6	7
	No. of eggs (x)	✓	✓	✓		✓		✓
	No. of 1 st larvae (y)	✓	✓	✓		✓		✓

Male fertility in days 1-2 : $y_1 + y_2$ (N~12)

Male fertility in days 3-7 : $y_3 + y_5 + y_7$ (N~12)

Fig. S3. Experimental procedure for the male fertility assays.

Similar crosses are set up for miRNA KO and control lines. For convenience, we use the miRNA KO flies to represent the experimental flies in the cross schemes.