Pervasive behavioural effects of microRNA regulation in *Drosophila*

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- Supplemental Figures -
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Figure Supplementary 1. Association between miRNA expression level and time to SR. (A) Schematic representation of the 108 miRNA expression levels from 12h-24h of embryogenesis (see S2 File; (Chung et al. 2008)). (B) Plot showing the association between the expression level of the 108 miRNAs studied at 12h-24h of embryogenesis (y-axis) and time to self-right (x-axis). Linear regression ($R^2=0.0045$) and 95% confidence interval in red line and dotted line, respectively. The Spearman coefficient ($r_s$) and $p$ value are shown. There is no significant correlation between miRNA expression and the SR delay ($r_s=0.0735$; $p=0.5146$).
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Figure Supplementary 2. Protein expression of Hox genes Ubx, Abd-A and Abd-B in miR-1003 and miR-310c mutant embryos (A-C, left) Protein expression of Ubx (A, green), Abd-A (B, red) and Abd-B (C, yellow) in ventral nerve cords of wild-type and mutants for miR-1003 and miR-310c embryos at late 16 stage. (A-C, right) Profile quantification along the A-P axis for the three Hox proteins in the wild-type (mean in black line and SEM in grey) and in the miRNA mutants (mean in red line and SEM in lighter red). These miRNA mutants did show any significant protein expression level difference for any of the three Hox proteins. N=10 embryos per genotype for each immunostaining. DAPI in blue. Anterior is to the left.
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Figure Supplementary 3. Overexpression of Abd-B disrupts SR behaviour (A) Expression pattern of Abd-B<sub>LDN</sub>-GAL4 driver (GFP, magenta), in respect to the endogenous pattern of Abd-B protein expression (yellow) in dissected embryonic ventral nerve cord. DAPI in blue and anterior is to the left. (B) Quantification of Abd-B expression profile along the A-P axis in dissected embryonic nerve cords of wild-type (w<sup>1118</sup>, mean in black and SEM in grey) and Abd-B overexpression (Abd-B<sub>LDN</sub> &gt; Abd-B mean in magenta and SEM in light magenta) (N = 9 embryos per genotype). (C) Significant delay in time to SR in larvae overexpressing Abd-B (Abd-B<sub>LDN</sub> &gt; Abd-B, yellow bars) in comparison with wild-type (w<sup>1118</sup>) and parental lines (Abd-B<sub>LDN</sub>-GAL4/+, light grey bar, and UAS-Abd-B/+ in dark grey) (mean ± SEM; an average of 20 larvae per genotype were analysed; Mann-Whitney U test with Bonferroni correction, *** p < 0.001).
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Figure Supplementary 4. Whole embryo Abd-B protein expression in miR-980, miR-8, miR-iab4/iab8 and miR-278 mutants. (A-D) Abd-B protein expression (yellow) in whole embryos of wild-type and mutants for miR-980, miR-8 and miR-278 at late 16 stage counterstained with DAPI in blue (A-D). The increase of Abd-B in the ventral nerve cord (vnc) of these mutants is accompanied by increase in muscle and epiderm (m & ep) of the most posterior segments (indicated with brackets). (E) Schematic representation of miR-980 (green), miR-8 (blue) and miR-278 (red) predicted binding sites to the longest Abd-B 3’UTR annotated (BDGP6, see Materials and Methods), according to the PITA algorithm to predict miRNA target sites (Kertesz et al.2007). Each individual site ΔΔG score from PITA is indicated. Note that the more negative a ΔΔG score is, the more probable is the targeting of a miRNA to a site in the 3’UTR. The two alternative polyadenylation signals (PAS) are represented with black arrowheads.
Figure Supplementary 5. Whole embryo expression pattern of Abd-B-Gal4 drivers. (A) Schematic representation of the ventral and lateral views of late 16 stage embryos. The embryonic central nervous system is represented in grey. (B-C) Expression pattern of Abd-B^{LDN}-GAL4 (B) and Abd-B^{199}-GAL4 (C) drivers (GFP, green) of whole mounted embryos in respect to the endogenous pattern of Abd-B protein expression (red) counterstained with DAPI (blue). (B) Abd-B^{LDN}-GAL4 drives expression in parasegment (p.s.) 13 of the embryonic ventral nerve cord (vnc), muscle (m) and epidermis (ep). (C) Abd-B^{199}-GAL4 drives high expression in p.s. 13 of the embryonic vnc, m and ep, and lower expression in p.s 12 of the same tissues. Anterior is to the left.
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Figure Supplementary 6. SR time of controls for miR-sponges experiment (Fig. 5B). (A) Time to self-right in seconds (s) of the two genetic background lines (w¹¹¹⁸ and yw, black bars), as well as all the parental lines (GAL4/+ and UAS-miR-SP/+), used to obtained the experimental larvae (GAL4>UAS-miR-SP, shown in Fig. 5B). Since these crosses and the developing embryos were maintained at 29°C for maximal Gal4 activity, the miRNA KO mutants were re-analysed at this temperature. At 29°C the three SR times were slightly decreased compared when embryonic development was conducted at 25°C (Fig. 1B) but still within the same magnitude of effect. Gal4 drivers (GAL4+/+) are represented with white bars. All genotypes related with miR-980 are represented in light grey, miR-8 in medium grey, and miR-278 in dark grey. Scramble-SP related genotypes are the darkest grey bars. Bars represent mean ± SEM; an average of 35 larvae per genotype were analysed; Mann-Whitney U test; (ns) non-significant $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).