



Fig. S5. Experimental determination of the midgut closure timing. (a) Experimental set-up of re-inoculation of the *Burkholderia* symbiont. An RFP-labelled symbiont was orally administered at the 2nd instar stage. After an interval of 6h, 12h, 15h, 18h or 3 days, a GFP-labelled symbiont was fed to the insects. In the 3-day-interval group, insects molted to 3rd instar during the interval, and the molted insects were fed with the GFP-labelled symbiont. Three days after infection of the GFP-labelled symbiont, insects were dissected and fluorescence signals were observed by fluorescence microscopy. (b) Detection rate of GFP signals at the symbiotic region (M4B+M4) after the re-inoculation test. RFP signals were detected in all tested individuals. The ratio of GFP-positive insects to all tested insects is depicted in the histograms. Different letters above the bars indicate a significant difference between groups by the Fisher's exact test after the Bonferroni correction ($P < 0.05$). Raw data (pictures) are shown in Supplementary Fig. S5.