



Figure S4 Crosses to introduce *B* chromosomes into a wild-type stock. (A) To analyze the *B* chromosomes in a wild-type genetic background by CID foci in ovaries, *mtrm¹²⁶/TM3* flies were crossed to either females or males from a wild-type stock. From this cross, their *TM3 Sb, Ser* progeny were collected and crossed to each other. The *TM3* balancer ensured the *mtrm¹²⁶* mutation was no longer present, and the balancer was lost from the stock after a few generations. From this cross a WT+B stock was created. (B) For the larval neuroblast experiments, male and female *mtrm¹²⁶/TM3* flies from the *B* chromosome-containing stock were crossed to either females or males from a wild-type stock. From this cross, their *TM3 Sb, Ser* progeny were collected and crossed to each other as described in (A). From these crosses, two stocks were created: a wild-type stock with paternally-derived *B* chromosomes (Set 1) and a wild-type stock with maternally-derived *B* chromosomes (Set 2). Each set of crosses for the neuroblast experiment was started twice. (C) Crosses were initiated as in (B) but then non-*mtrm¹²⁶* progeny were again crossed to wild-type flies and progeny were subsequently outcrossed to wild type for four additional generations. For each outcross set, the crosses were initiated four times and the data pooled.