Supplemental Fig. S4. Insulin and insulin receptors expressed by the cholinergic neurons do not regulate gastrointestinal (GI) motility. (A) Image demonstrates GI tract with charcoal (black) in the lumen. X represents the length between the distal end of pylorus and the most distal end of charcoal traveled within the lumen. Y represents the length between the distal end of pylorus and the distal end of ileum. (B) Graph demonstrates effects of intraperitoneal (i.p.) insulin (0.5 U/kg, blue rectangles, $n=10$) vs. intraperitoneal Dulbecco’s phosphate-buffered saline (DPBS; black circles, $n=10$) on charcoal movement (X/Y). No statistical significance was observed by Mann-Whitney test. (C) Graph demonstrates effects of intracerebroventricular (i.c.v.) insulin (4 mU, blue rectangles, $n=9$) vs. intraperitoneal DPBS (black circles, $n=8$) on charcoal movement (X/Y). No statistical significance was observed by Mann-Whitney test. (D) Graph demonstrates results of the acetaminophen assay in the insulin receptor flox (InsR$^{f/f}$) mice (black circles, $n=8$ in the dark cycle, $n=6$ in the light cycle) and ChATcre/+::InsR$^{f/f}$ mice (blue rectangles, $n=15$ in the dark cycle, $n=14$ in the light cycle). Mice were fed ad libitum in the dark cycle experiments, and were fasted for 18 hours prior to the light cycle experiments. No statistical significance was observed by two-way analysis of variance (ANOVA) with Bonferroni’s multiple comparisons test. ChAT, choline acetyltransferase.