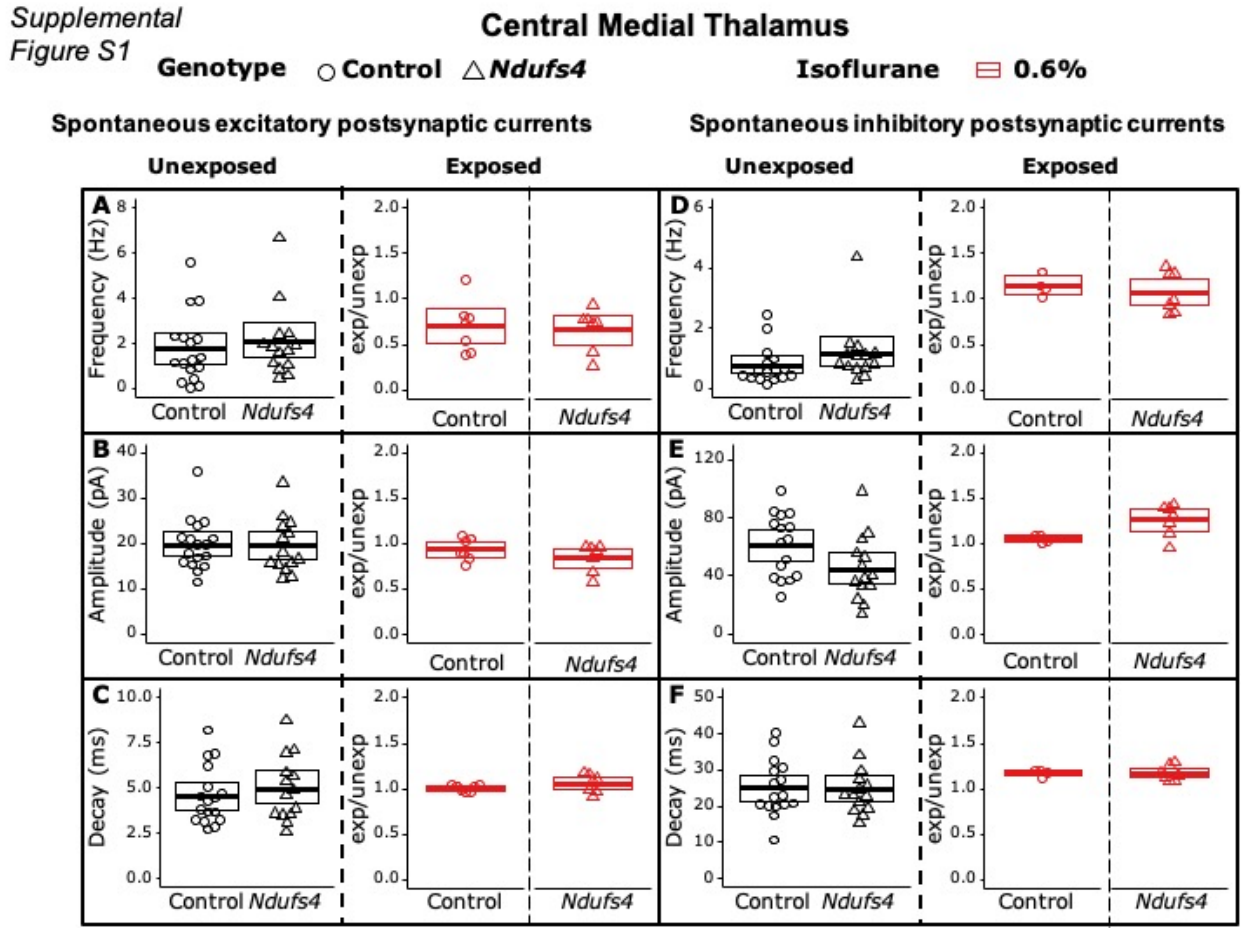
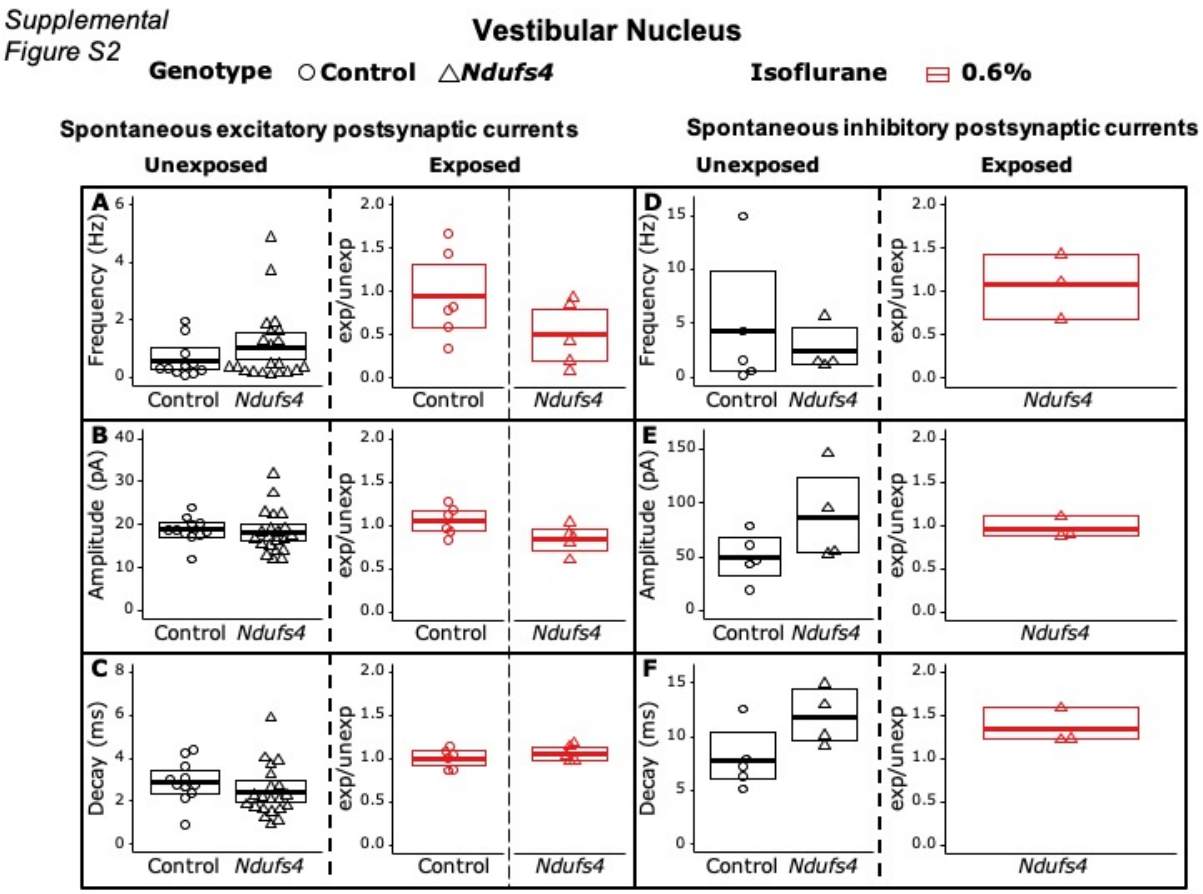
**Supplemental Figures.**



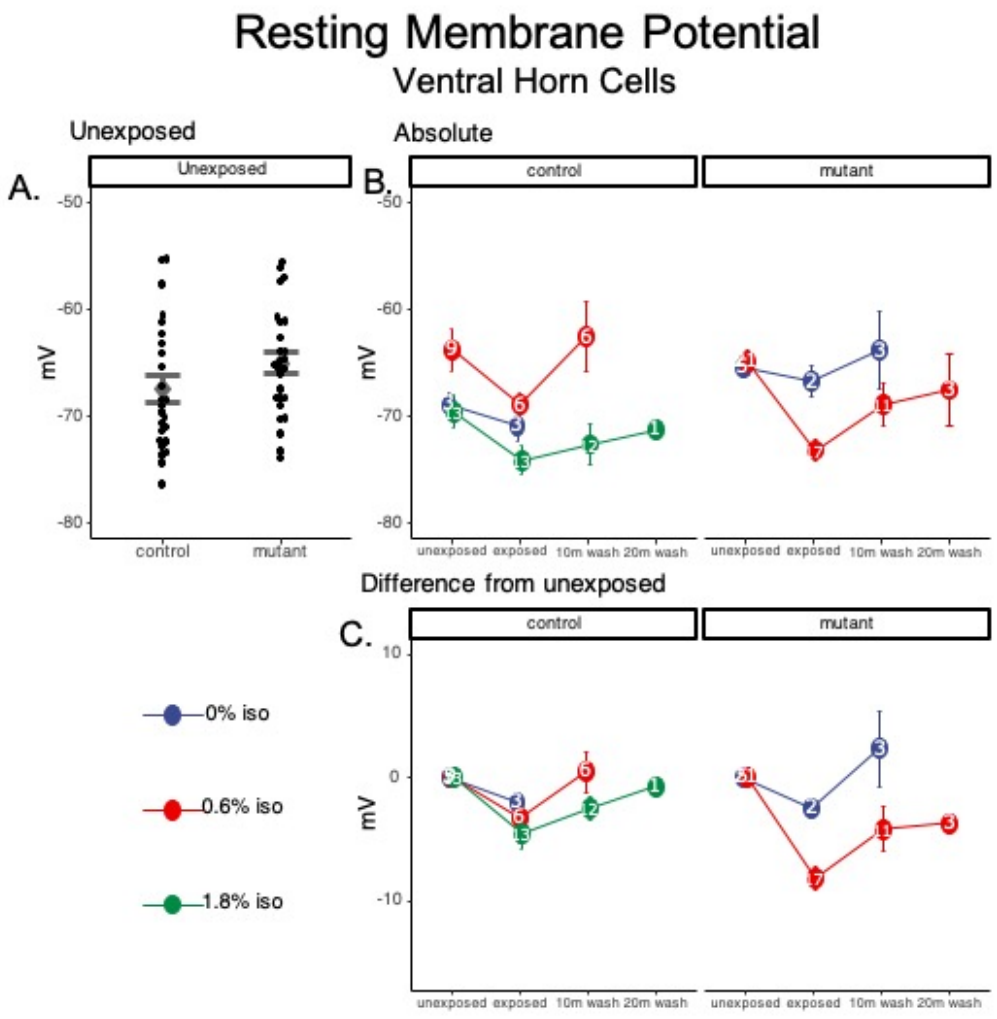

**Supplemental Figure S1. A –F.** Spontaneous synaptic activity in the central medial thalamus. Synaptic event properties of frequency, amplitude, and decay are quantified and compared between wildtype controls (circles) and mutant (triangles) mice. Spontaneous excitatory postsynaptic currents are shown in A-C; Spontaneous inhibitory postsynaptic currents are shown in D-F. Black and white columns are mean absolute values of the five minutes before isoflurane is applied to the slice. Red columns are mean normalized values of the last five minutes of 0.6% isoflurane exposure. Normalized mean values were calculated for each cell by dividing the exposed mean by the unexposed mean. Crossbars are mean and 95% confidence interval.

*Supplemental Figure S2*




**Supplemental Figure S2. A –F.** Spontaneous synaptic activity in the vestibular nucleus. Synaptic event properties of frequency, amplitude, and decay are quantified and compared between wildtype controls (circles) and mutant (triangles) mice. Spontaneous excitatory postsynaptic currents are shown in A-C; Spontaneous inhibitory postsynaptic currents are shown in D-F. Black and white columns are mean absolute values of the five minutes before isoflurane is applied to the slice. Red columns are mean normalized values of the last five minutes of isoflurane exposure. Normalized mean values were calculated for each cell by dividing the exposed mean by the unexposed mean. Crossbars are mean and 95% confidence interval.

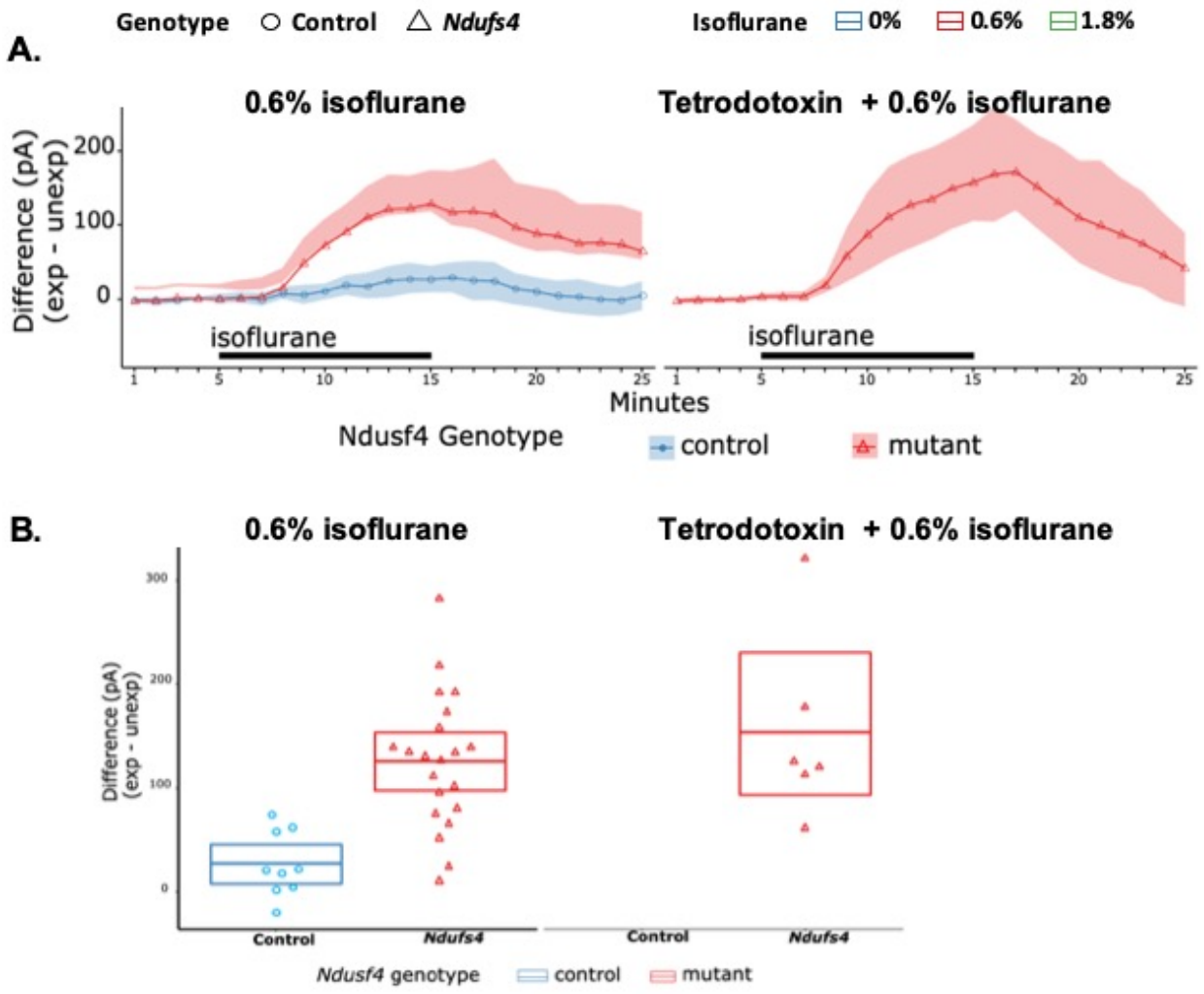
*Supplemental Figure S3*




**Supplemental Figure S3**. **A–C.** Resting membrane potentials in cells of the ventral spinal cord. **A.** Resting membrane potentials in control (left) and mutant (right) cells in 0% isoflurane (unexposed). No difference was detected between genotypes. **B.** Absolute resting membrane potentials upon isoflurane exposure. Absolute resting membrane potentials prior to, during), and following isoflurane exposure in spinal cord neurons. Left plots are from control cells; right plots are from mutant cells. Blue circles are values for cells in 0% isoflurane (a time control); red circles are values for cells exposed to 0.6% isoflurane; green circles are values for cells exposed to 1.8% isoflurane (control cells only). **C.** Difference in resting membrane potentials s caused by isoflurane

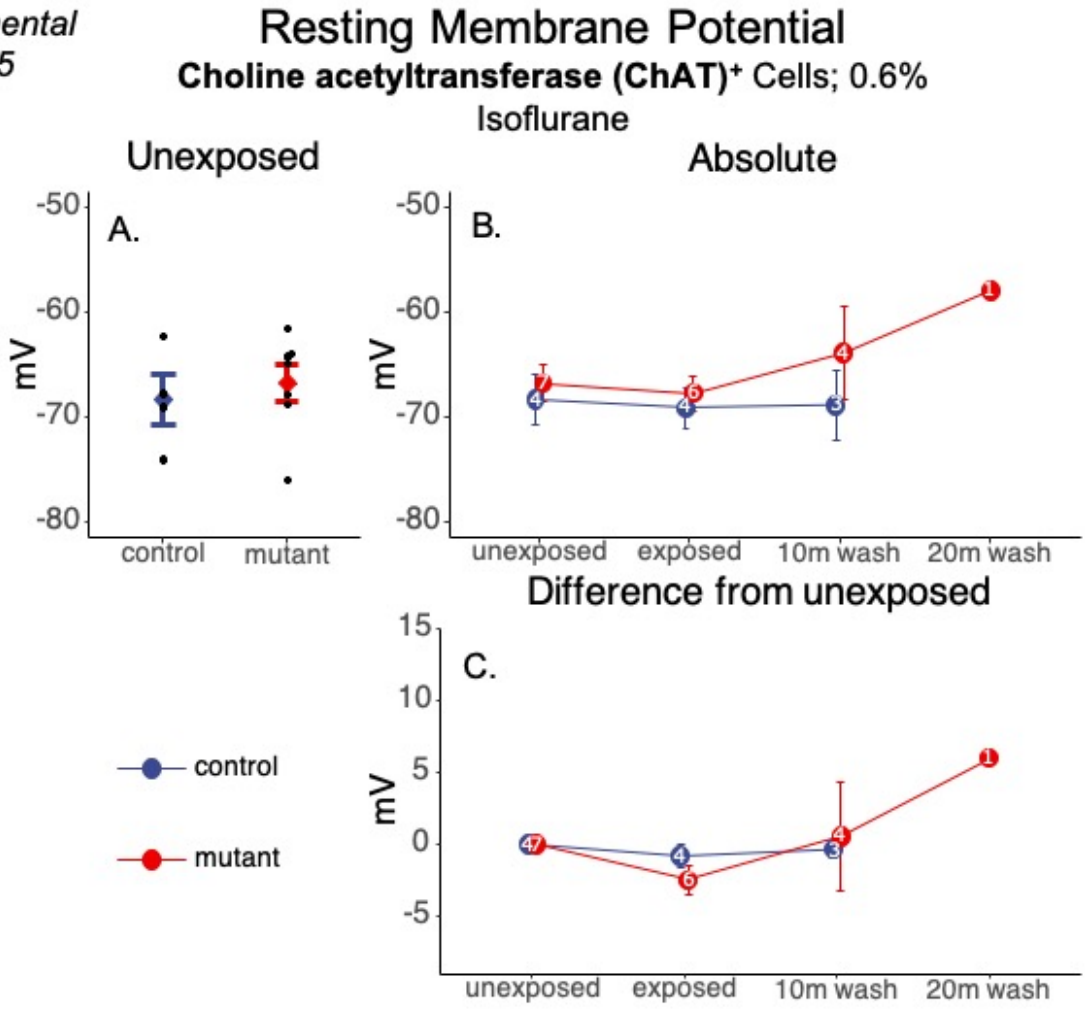
exposure. Resting membrane potentials are subtracted for each cell from its value prior to anesthetic exposure). Resting membrane potentials before, during, and following isoflurane exposure in spinal cord cells. Left plots are from control cells; right plots are from mutant cells. Blue circles are values for cells in 0% isoflurane; red circles are values for cells exposed to 0.6% isoflurane; green circles are values for cells exposed to 1.8% isoflurane (control cells only). Numbers within circles represents n for each group.

*Supplemental*
*Figure S4*




**Supplemental Figure S4.** Comparison of the effect of synaptic input on change in holding current caused by isoflurane in *Ndufs4(KO)* spinal cord slices. The data in the left sides of A and B are from Figure 2 for comparison. The data on the right sides of A and B are after treatment of slices with tetrodotoxin and show no difference from the untreated slices.

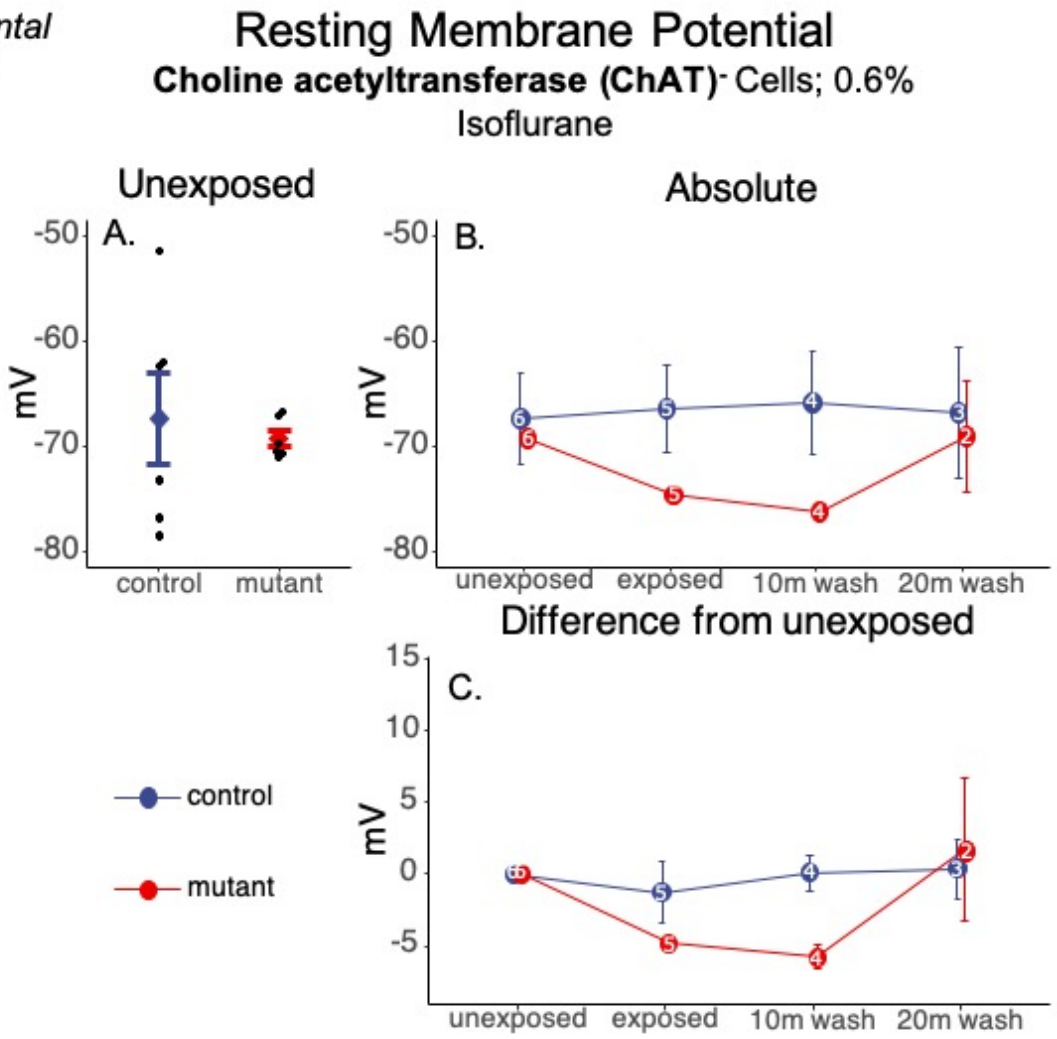
*Supplemental Figure S5*




**Supplemental Figure S5.** **A–C.** Resting membrane potentials in ChAT$^+$ (cholinergic) ventral spinal cord cells. **A**. Resting membrane potentials in unexposed control (blue) and mutant (red) cells. No difference was detected between genotypes. **B**. Absolute resting membrane potentials upon isoflurane exposure. Absolute resting membrane potentials s prior to, during, and following 0.6% isoflurane exposure in spinal cord neurons. Blue plot is from control cells; red plot is from mutant cells. **C.** Difference in resting membrane potentials upon isoflurane exposure**.** Resting membrane potentials are subtracted for each cell from its value prior to anesthetic exposure. Resting membrane potentials before, during and following isoflurane exposure in ChAT$^+$ spinal cord

neurons. Blue plot is from control cells; red plot is from mutant cells. No significant difference was noted between genotypes and no change in resting membrane potentials were seen in either genotype with 0.6% isoflurane.

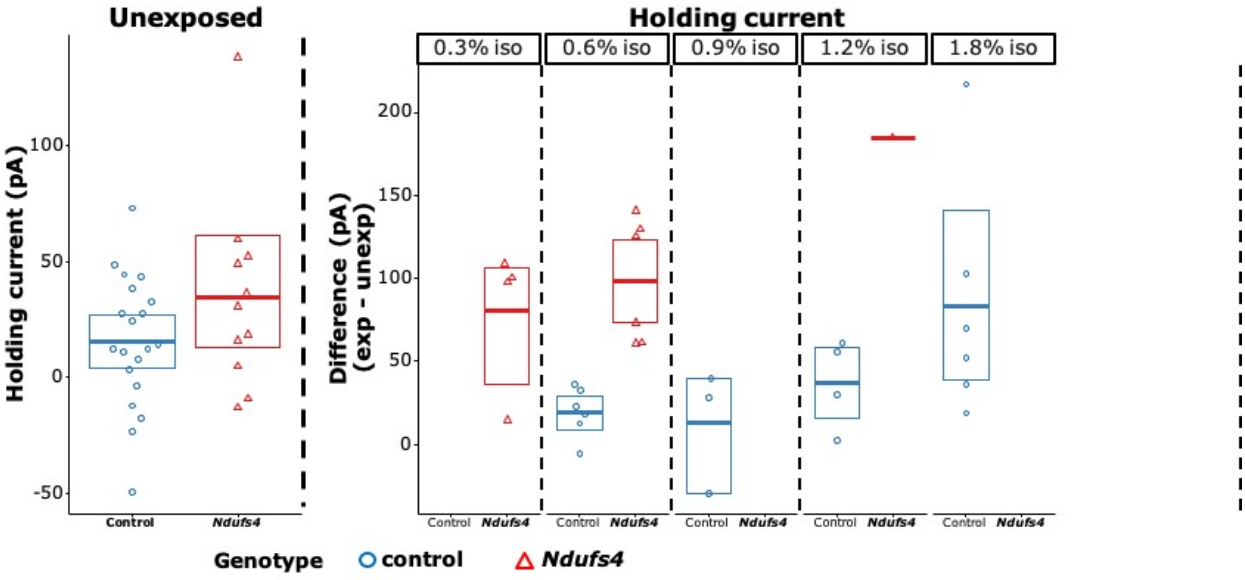
*Supplemental Figure S6*




**Supplemental Figure S6. A–C.** Resting membrane potentials in ChAT$^-$ ventral spinal cord cells. **A.** Resting membrane potentials in unexposed control (blue) and mutant (red) cells. No difference was detected between genotypes. **B.** Absolute resting membrane potentials upon isoflurane exposure. Absolute resting membrane potentials prior to, during and following 0.6% isoflurane exposure in spinal cord cells. Blue plot is from control cells; red plot is from mutant cells. **C**. Difference in resting membrane potentials upon isoflurane exposure**.** Resting membrane potentials are subtracted for each cell from its value prior to anesthetic exposure. Resting membrane potentials during, and following isoflurane exposure in ChAT$^-$ spinal cord cells. Blue

plot is from control cells; red plot is from mutant cells. In both B and C, a significant difference was noted between genotypes in cells exposed to 0.6% isoflurane. There was a significant decrease in resting membrane potentials (hyperpolarization) seen in the mutant neurons which was not seen in the control neurons. A significant decrease in resting membrane potentials were seen in $ChAT^{-}$ control cells at 1.8% isoflurane (not shown).

*Supplemental Figure S7*




**Supplemental Figure S7.** Holding current at intermediate concentrations of isoflurane (<$EC_{95}$ for control or KO animals) in non-cholinergic ($ChAT^-$) cells. **A, Left.** As seen in Figure 6A, left, at baseline, non-cholinergic cells for both genotypes require similar holding current to voltage clamp at -60 mV. The data here includes those cells shown in Figure 6 plus additional cells later exposed to intermediate concentrations of isoflurane. **Right.** The median holding current of control noncholinergic cells did not reach a significant change from baseline with intermediate concentrations (0.9% and 1.2%) of isoflurane. Mutant cells also did not significantly increase their holding currents with 0.3% isoflurane (red triangles) (p=0.001). Data for control and KO cells at their respective $EC_{95}$ concentrations are the same as in Figure 6 and shown here for comparison. Numbers; unexposed; n=19(wildtype; 7M, 5F), n=11(*Ndufs4(KO)*; 4M, 3F): exposed; n=4(*Ndufs4(KO)* (0.3%); 2M, 2F); n=1(*Ndufs4(KO)* (1.2%);1M); n=6(wildtype(0.6%);3M, 3F); n=4(wildtype (0.9%); 2M, 2F); n=4(wildtype(1.2%) 2M. 2F).