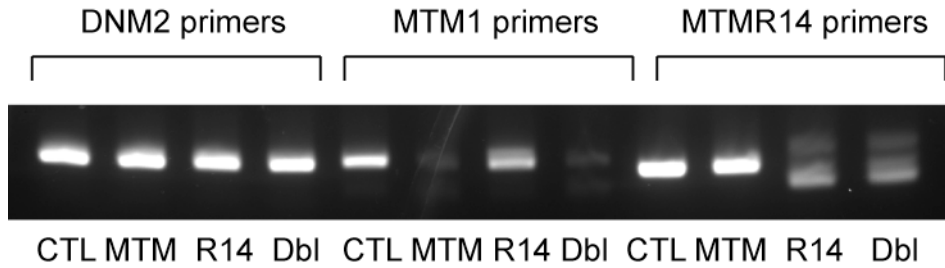
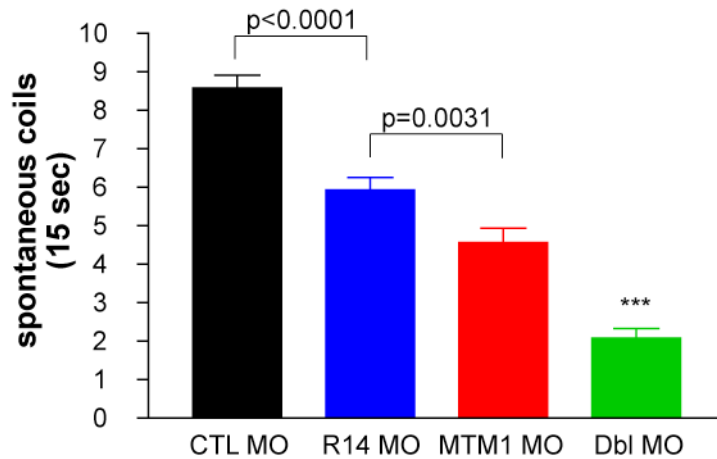


## SUPPLEMENTAL DATA



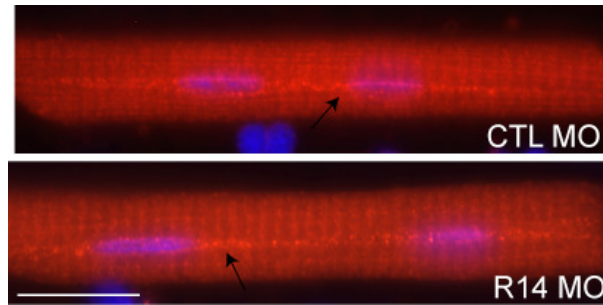
### Supplemental Figure 1: RT-PCR analysis of morpholino injected embryos

PCR analysis on RNA extracted at 48 hpf from control (CTL), MTM1 (MTM), MTMR14 (R14), and MTM1 + MTMR14 (Dbl) morphants. Lanes 1-4: dynamin-2 (DNM2) primers. Lanes 5-8: MTM1 exon 2-4 primers. Lanes 9-12: MTMR14 exon 10-12 primers. MTM1 and MTMR14 morpholino injection results in the predicted loss of the normal PCR product with MTM1 and MTMR14 primers, respectively.



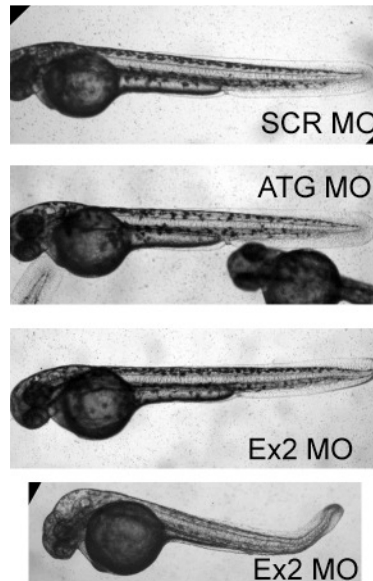
### Supplemental Figure 2: Abnormal motor function in MTMR14, MTM1 and double morphant embryos

Quantitation of spontaneous embryo coiling at 24 hpf. Average number of coiling events per 15 seconds were as follows: control morphants (CTL MO) = 8.58, MTMR14 morphants (R14 MO) = 5.93, MTM1 morphants (MTM1 MO) = 4.56, and MTMR14/MTM1 double morphants (Dbl MO) = 2.08. p value for one-way ANOVA < 0.0001.



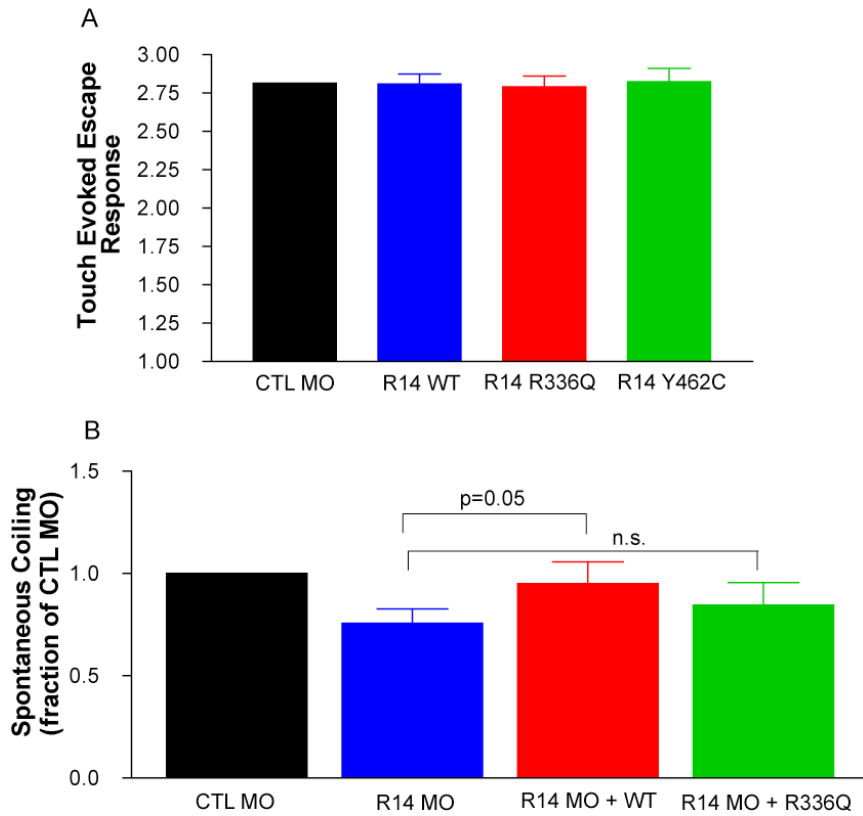
**Supplemental Figure 3: Golgi apparatus appears normal in MTMR14 morphant myofibers**

Myofibers were isolated from control (CTL MO) and MTMR14 (R14 MO) morphant embryos at 72 hpf and then labeled with an antibody to the 58K golgi protein (1:50; Abcam) as previously described (18). No difference in staining was observed between control and MTMR14 morphant myofibers (arrows point to golgi apparatus). Nuclei are labeled with DAPI. Scale bar = 10  $\mu$ m.



**Supplemental Figure 4: MTMR14 morpholinos have a consistent morphologic phenotype**

Embryos were injected with different *mtmr14* morpholinos and analyzed by live microscopy at 48 hpf. Injection of a morpholino with a scrambled exon 11 sequence resulted, as expected, in no phenotypic changes (SCR MO). Injection with morpholinos either to the translation start site (ATG MO) or to the exon 2 splice junction (Ex2 MO) resulted in similar morphologic changes as observed with the exon 11 morpholino (see Figure 3). Many embryos appeared normal, while some (bottom panel) had thinning of the midbody and bending of the tail.



**Supplemental Figure 5: Effect of human MTMR14 RNA on zebrafish control and morphant embryos**

(A) Injection of wild type or mutant human MTMR14 RNA did not affect embryonic motor function, as measured by touch-evoked escape response. Average values were: 2.81 (injection control; CTL MO), 2.81 $\pm$ 0.54 (wild type MTMR14 RNA; R14 WT), 2.79 $\pm$ 2.65 (R336Q MTMR14 RNA; R14 R336Q), 2.82 $\pm$ 0.50 (Y462C MTMR14 RNA; R14 Y462C). (B) Co-injection of human MTMR14 RNA with control or MTMR14 morpholinos. Wild type, but not R336Q, MTMR14 can rescue the motor phenotype associated with MTMR14 knockdown. Average spontaneous coiling measured at 24 hpf, presented as a fraction of average coiling of control morphants (CTL MO): 0.76 $\pm$ 0.07 (MTMR14 morphants; R14 MO), 0.95 $\pm$ 0.10 (MTMR14 morpholino + wild type MTMR14 RNA; R14 MO + WT), and 0.84 $\pm$ 0.11 (MTMR14 morpholino + R336Q MTMR14 RNA; R14 MO + R336Q).