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Supplemental information

Neuropeptidergic regulation of neuromuscular signaling in larval zebrafish alters swimming behavior and synaptic transmission

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SUPPLEMENTAL MATERIAL

Supplemental Figures

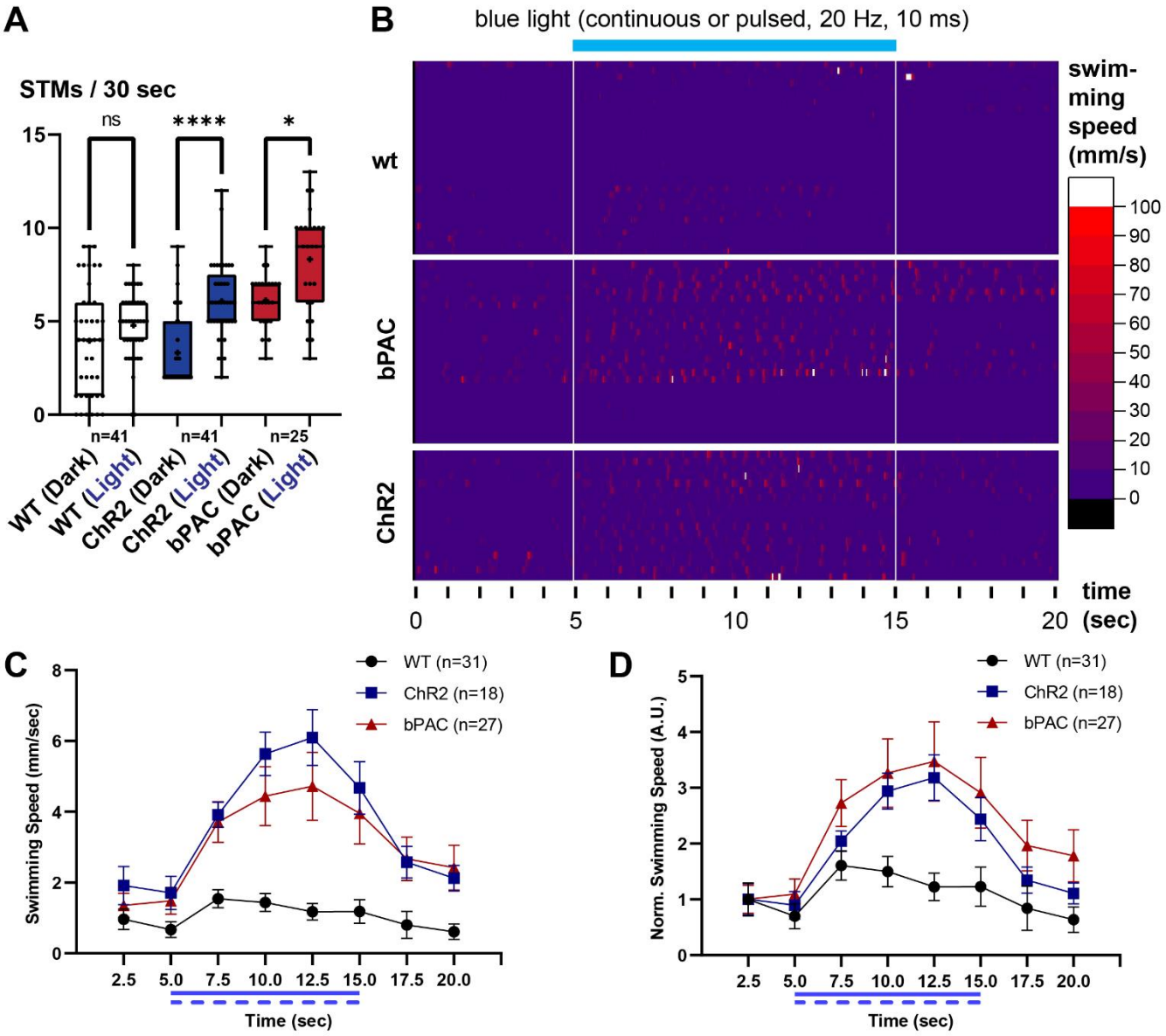

Figure S1: Locomotion during ChR2 or bPAC stimulation, related to Figure 1. (A) Spontaneous (Dark) and evoked (Light) tail movements (STMs / ETMs) in 24 hpf embryos. Kruskal-Wallis test with Dunn post-hoc-test. **(B)** Raster plot of swimming speeds, extracted from videos, at 30 Hz sampling frequency, color coded as indicated on the right, and for the respective animals expressing no transgene (WT) or bPAC or ChR2 in cholinergic motor neurons. Each line represents one animal. **(C)** Swimming speed as measured with and without light stimulation. Swimming speed was measured for wild type, ChR2 transgenic or bPAC transgenic individuals 4 dpf before, during and after blue light stimulation. **(D)** Swimming speed as measured with and without blue light stimulation and normalized to the first 2.5 second time interval. Animals as in C. Statistical significance given as ****p<0.0001, *p<0.05; n.s. – non significant.

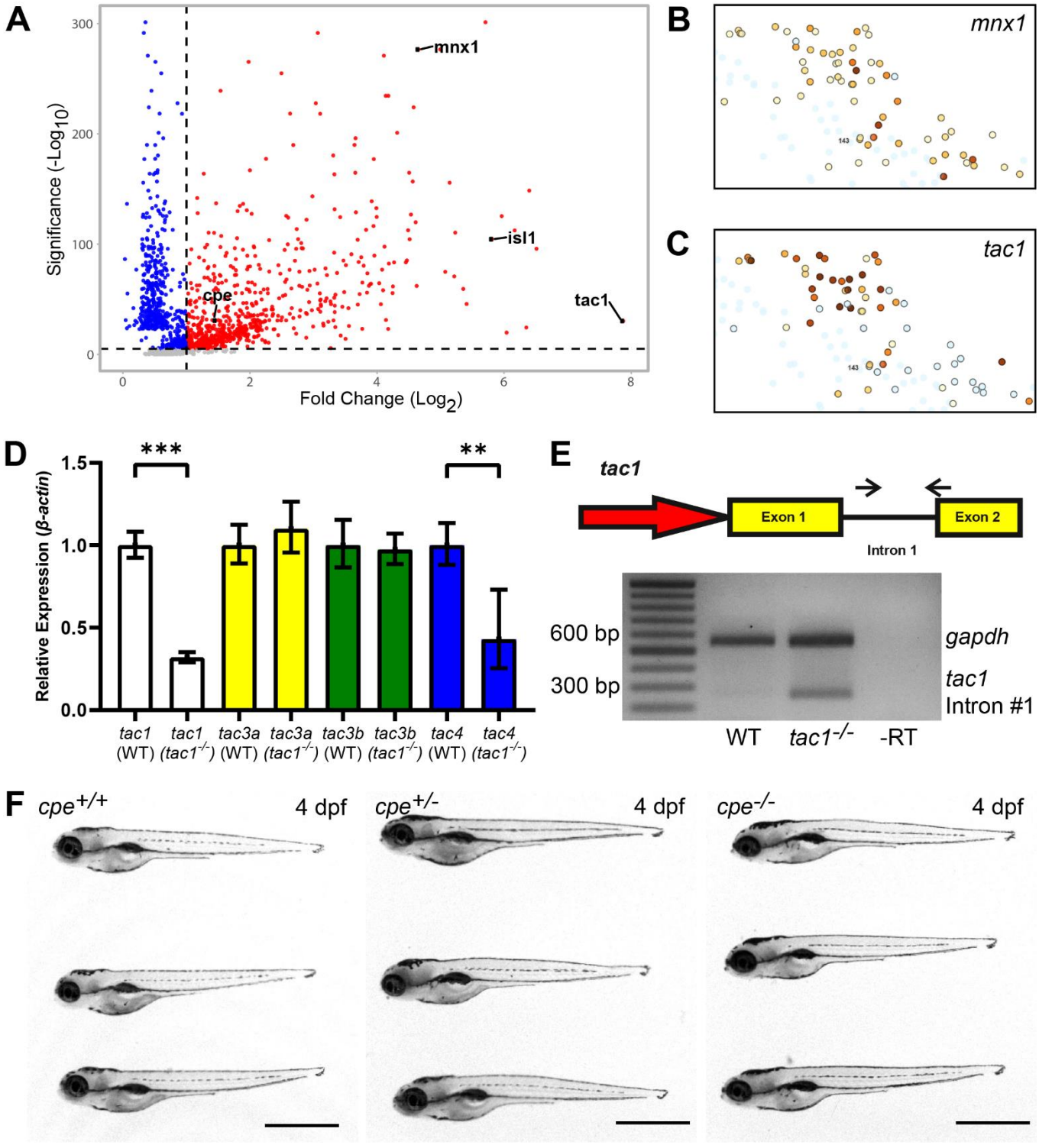

Figure S2: Genes encoding carboxypeptidase E (*cpe*) and the neuropeptide *tac1* are expressed in larval motor neurons, related to Figure 2. (A) Gene expression profile of the motor neuron cluster #143 obtained by single cell RNAseq. *mnx1* and *isl1*: Specific markers for motor neurons used to identify the cell type. **(B – C)** Magnified view of a motor neuron cluster shows high expression levels of the marker gene *mnx1* as well as the candidate gene *tac1*. (**A – C**) adapted from {Farnsworth, 2020 #8139} and the respective publicly available data sets (http://cells.ucsc.edu/?ds=zebrafish-dev). **(D)** qPCR analysis showing *tac3a*, *tac3b* and *tac4* mRNA levels in *tac1*$^{-/-}$ animals (relative to *β-actin*). Means ± standard deviation. Student's t-Test; statistical significance: **p<0.01, ***p<0.001. **(E)** Non-quantitative RT-PCR analysis of wild type (WT) and mutant (*tac1*$^{-/-}$) *tac1* transcripts and an unaffected housekeeping gene (*gapdh*). Primers specific for amplification of the first intron of the *tac1* mRNA are indicated as arrows. **(F)** Bright-field images of representative groups of *cpe* wild type, heterozygous and homozygous siblings 4 dpf. No obvious morphological differences were observed. Scale bar represents 1 mm.

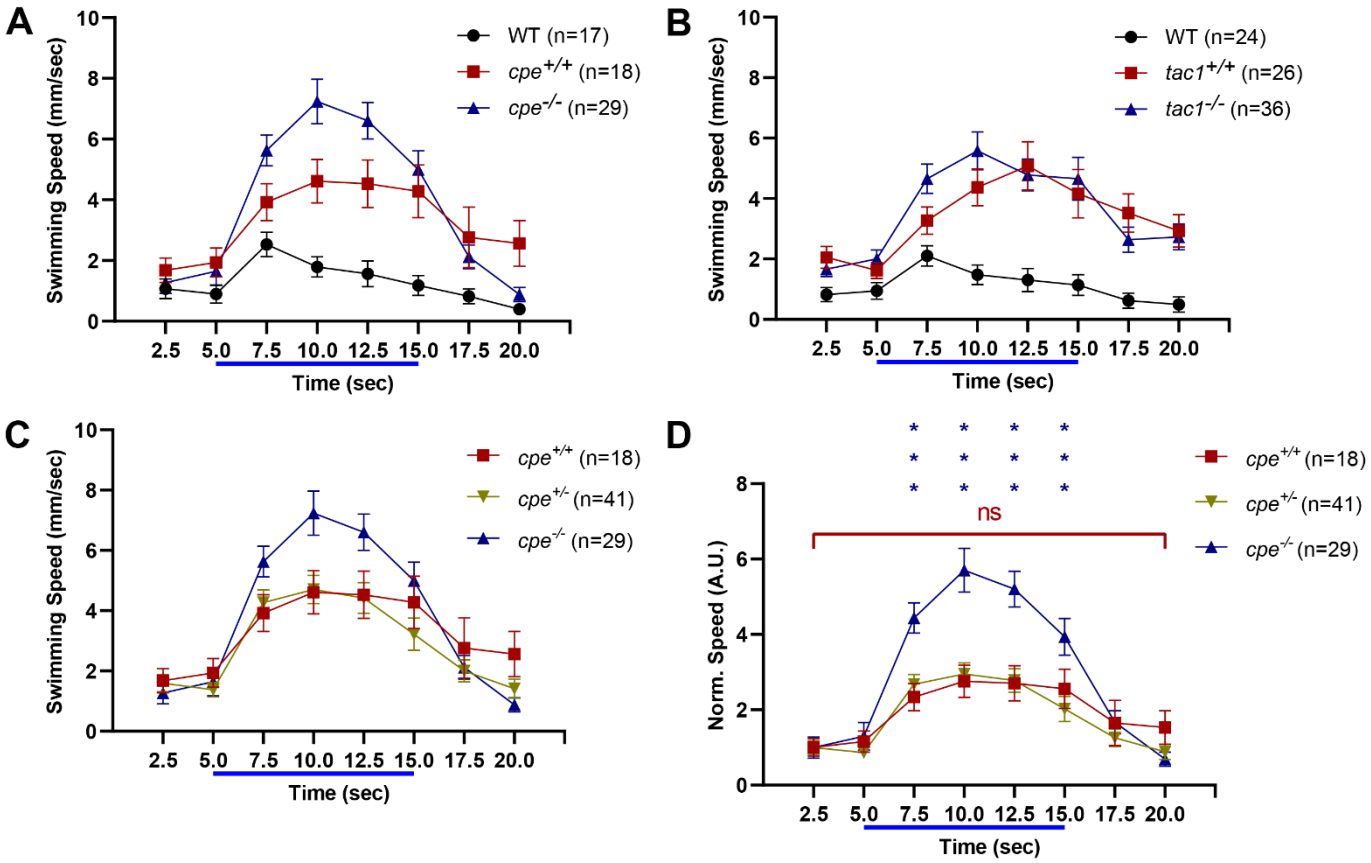

Figure S3: Locomotion behavior of *cpe* and *tac1* mutants, related to Figure 3. (A) Swimming speed was measured for wild type animals as well as *cpe* homozygous knockouts and wild type siblings (4 dpf) in the Tg[*mnx1:Gal4*] / Tg[*UAS:bPAC-V2A-mCherry*] transgenic background. **(B)** Swimming speed of wild type, *tac1*$^{-/-}$ homozygous knockouts and respective wild type siblings (4 dpf) carrying the Tg[*mnx1:Gal4*] / Tg[*UAS:bPAC-V2A-mCherry*] transgenes before, during and after bPAC activation. **(C)** Swimming speed of *cpe*$^{-/-}$ homozygous larvae as well as heterozygous and wild type siblings (4 dpf) carrying the Tg[*mnx1:Gal4*] / Tg[*UAS:bPAC-V2A-mCherry*] transgenes was analyzed before, during and after bPAC photo-activation. **(D)** Data in C are shown as normalized dataset. Two-Way-ANOVA with Tukey multiple comparisons of means. Statistical significance given as ***p<0.001.

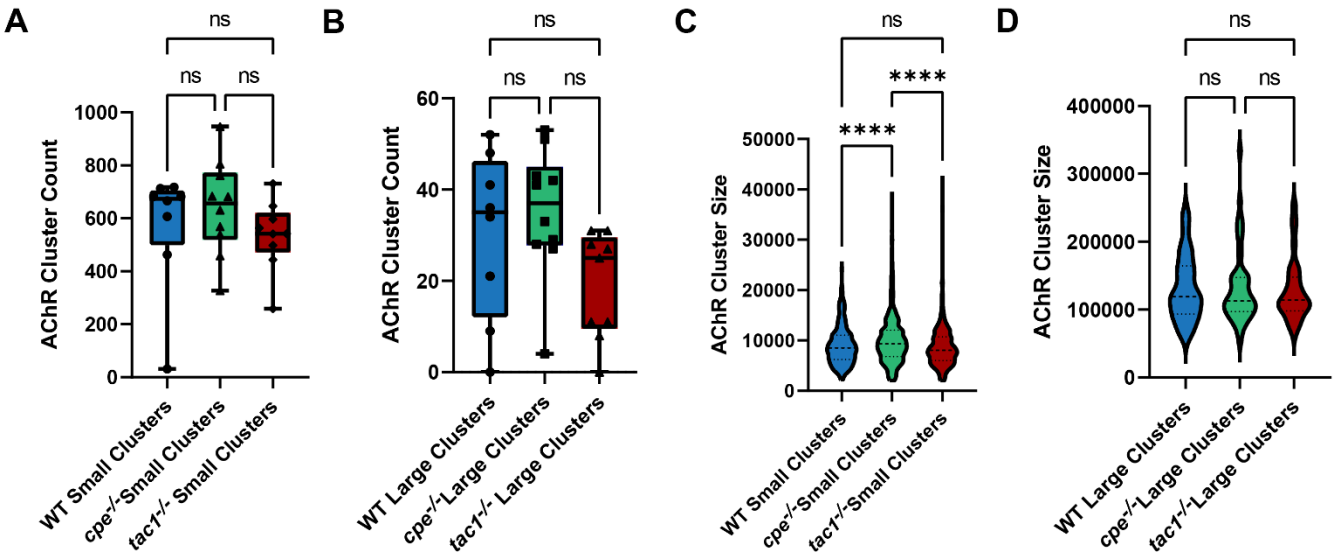

Figure S4: Analysis of nAChR cluster properties on muscle cells, related to Figure 5. (A – B) Quantification of the number of small and large receptor clusters in wild type animals and *cpe* or *tac1* homozygous mutants. **(C – D)** Quantification of small and large receptor cluster sizes in wild type animals and *cpe* or *tac1* homozygous mutants. One-Way-ANOVA with Tukey multiple comparisons of means. Statistical significance given as ****p<0.0001; n.s. – non significant.

Oligonucleotides		
bPAC-attB1-F (GGGGACAAGTTTGTACAAAAAAGCAGGCTGCGCCACCATGATGAAGCGGCTGGTGTA)	This paper	N/A
bPAC-attB2-R (GGGGACCACTTTGTACAAGAAAGCTGGGTAGTACGTCCGCGGCTTGTCGTTT)	This paper	N/A
cpe_F_2 (CCCATCTCAAACGCCTCTGT)	This paper	N/A
cpe_R_2 (ATAAGTCTGGACGCAGTGCC)	This paper	N/A
tac1_F (GATGGGGAAACGGTCCTCTG)	This paper	N/A
tac1_R (GCGCAGGACTGTCGGTATTA)	This paper	N/A
cpe_Oligo_1-1 (TAGGACAGCGCAGAAAACAGGA)	This paper	N/A
cpe_Oligo_1-2 (AAACTCCTGTTTTCTGCGCTGT)	This paper	N/A
cpe_Oligo_3-1 (TAGGGTCGCGAGCTGCTCGTGC)	This paper	N/A
cpe_Oligo_3-2 (AAACGCACGAGCAGCTCGCGAC)	This paper	N/A
tac1_Oligo_1-1 (TAGGAAGTAACTAAAGTTTAGA)	This paper	N/A
tac1_Oligo_1-2 (AAACTCTAAACTTTAGTTACTT)	This paper	N/A
tac1_Oligo_2-1 TAGGATTTATTTAACATGCTTA)	This paper	N/A
tac1_Oligo_2-2 (AAACTAAGCATGTTAAATAAAT)	This paper	N/A
cpe_Geno_F (CAAATATATGTGACCCGTTCGTC)	This paper	N/A
cpe_Geno_R (GGCGATCCTCCATTATTGATTGG)	This paper	N/A
tac1_Geno_F3 (GCTCACCTCCTCTGACGTAA)	This paper	N/A
tac1_Geno_R2 (TGTGAAATGTCACTAACTTTGTTGC)	This paper	N/A
tac1_Intron1_F (TTGACATTGCGGGTTGGAAG)	This paper	N/A
tac1_Intron1_R (TGAATCCACTCATCCTGCGA)	This paper	N/A
gapdh_F (TGTTCCAGTACGACTCCACC)	This paper	N/A
gapdh_R (GCCATACCAGTAAGCTTGCC)	This paper	N/A
cpe_qPCR_F2 (GGTCAACTACATAGAGCAGGTTCA)	This paper	N/A
cpe_qPCR_R2 (CCAACAAGCGCCAGTAGTCA)	This paper	N/A
tac1_qPCR_F2 (ATCGGTCTGATGGGGAAACG)	This paper	N/A
tac1_qPCR_R2 (ACGACTCTGGCTCTTCTTGG)	This paper	N/A
gapdh_qPCR_F (CAGGCATAATGGTTAAAGTTGGTA)	This paper	N/A
gapdh_qPCR_R (CATGTAATCAAGGTCAATGAATGG)	This paper	N/A
bactin_qPCR_F (GATCTTCACTCCCCTTGTTCA)	This paper	N/A
bactin_qPCR_R (GGCAGCGATTTCCTCATC)	This paper	N/A

Table S1: Oligonucleotides used in this study, related to the STAR Methods.