

Supplementary Information:

Splice-specific deficiency of the PTSD-associated gene PAC1 leads to a paradoxical age-dependent stress behavior

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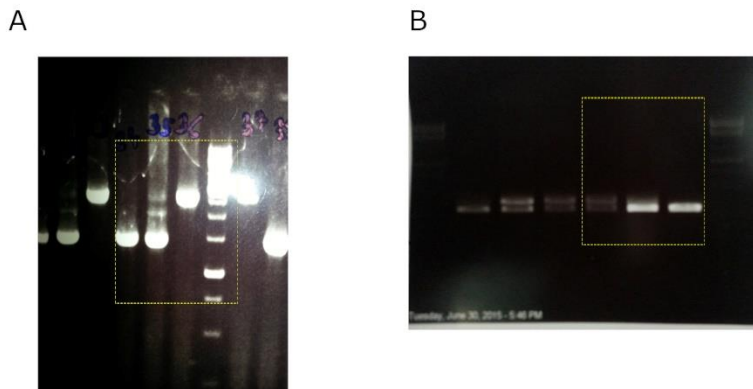
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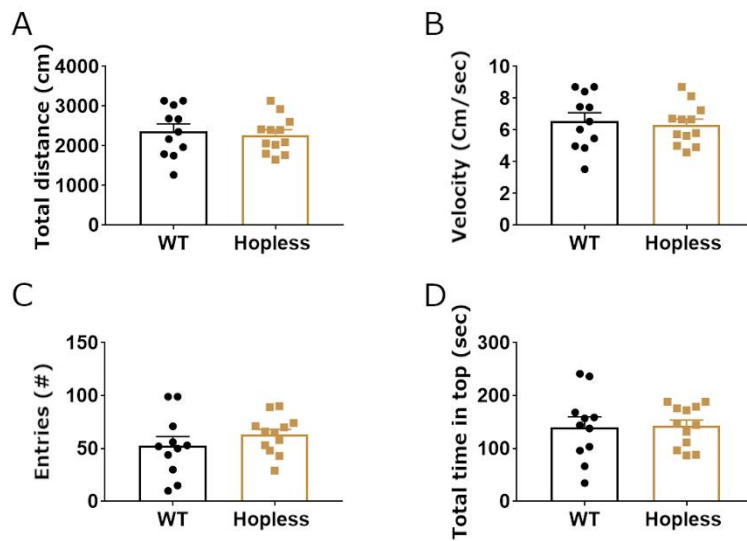
Running title: Stress modulation by PAC1 splice isoform

Key words: adcyap1r1, gpcr, adcyap1, hypothalamus, stress, post-traumatic stress disorder, alternative splicing

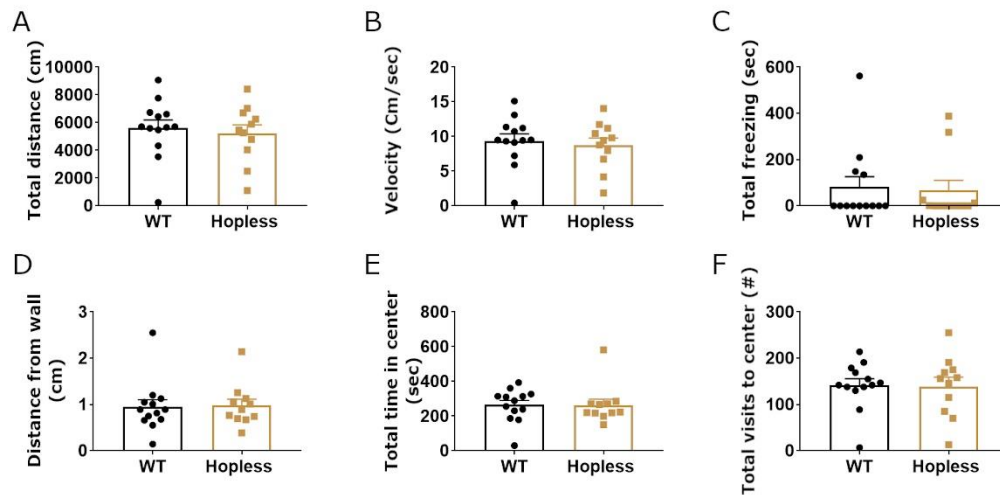
Supplementary Figures



Supplementary Figure 1. Full length electrophoresis gel images of genomic DNA (A) and cDNA (B) in Figure 1. Images were cropped to the yellow dashed rectangle and color inverted.



Supplementary Figure 2. Naïve *hopless* adults display normal behavior in novel tank diving assay. The behavioral response of adult *hopless* mutants (n=12) and WT siblings (n=11) to a novel tank was measured. The two genotypes exhibited a similar activity level, as seen in their total swimming distance swum and average swimming velocity during the 10 min test (A and B). Both genotypes displayed similar exploration of the top half of the tank (C and D). Data was analyzed using students-t test.



Supplementary Figure 3. Naïve adult *hopless* mutants display normal behavior in the open field assay. The behavioral response of adult *hopless* mutants (n=11) and WT siblings (n=13) in a novel open field arena was measured. Similarly to the novel tank diving assay, the two genotypes exhibited a similar activity level, as seen in their mean swimming distance and velocity, which significantly increased with time (A and B). In addition, both genotypes displayed significantly decreased freezing, distance from walls and time spent in the center of the arena along the test (C-E) and a slight but significant increase in their visits to the center (F), suggesting increased exploration activity over time. Data were analyzed using Student’s *t*-test.

Supplementary Table 1. Oligonucleotides used for gRNA transcription and RT-PCRs

Primer	Sequence	Length (nt)
Hop genotyping 1F	CAAAGGTTGTTGGCGTCATA	20
Hop genotyping 2697R	ATTCCCCACGTTTGTCTCAG	20
Upstream hop gDNA	ATTTAGGTGACACTATAGTGTGAAAAGGAACAGTGTGTGTTTTAGAGCTAGAAATAGCAAG	60
Downstream hop gDNA	ATTTAGGTGACACTATATGGTGTGGACATTAAGGAGGTTTTAGAGCTAGAAATAGCAAG	60
Constant gDNA	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTATTTTAACTTGCTATTCTAGCTCTAAAAC	80
PAC1 cDNA – F	TGATAAACTGCCCCTGGTG	21
PAC1 cDNA – R	CCAGGCCGAGCTCAAAGACC	20