

Rha *et al.* (HMG-2016-TWB-00166) Supplementary Material

Supplementary Material Figure Legends

Table S1. General behavioral tests. Twelve male *Zc3h14*^{+/+} (+/+) and twelve *Zc3h14* ^{Δ ex13/ Δ ex13} (Δ 13/ Δ 13) mice were evaluated using several paradigms as described in Materials and Methods to assess general activity in open field, exploratory behavior in novel cage, anxiety in light-dark box, and fear conditioning.

Table S2. Proteomic data. The results of the proteomic analysis of four *Zc3h14*^{+/+} (+/+) hippocampi and four *Zc3h14* ^{Δ ex13/ Δ ex13} (Δ 13/ Δ 13) hippocampi are provided as a searchable excel file.

Fig. S1. Mass spectrometry analysis of *Zc3h14* ^{Δ ex13/ Δ ex13} truncation products. A schematic of ZC3H14 with individual exons denoted is shown. The functional domains are indicated on the bottom of the schematic. The top shows peptide spectrum matches (PSM) for immunoprecipitation using an N-terminal ZC3H14 antibody of ZC3H14 isolated from *Zc3h14*^{+/+} (green) or *Zc3h14* ^{Δ ex13/ Δ ex13} (red) mouse whole brain lysate. The total number of identified PSMs for ZC3H14 is indicated. The location of the peptides identified is indicated by the position on the schematic. A scale bar to the left indicates the number of times fragment was identified. ZF, zinc finger.

Fig. S2. Statistical values for body weight. (A) Mean weight (grams) of animals measured from juvenile to adult age is shown. M, male. F, female. For individual data points on mouse body weight reported, the following tables show (B) n values, (C) standard error of the means (SEMs), and (D) *p* values. +/+, wildtype. Δ 13/ Δ 13, *Zc3h14* ^{Δ ex13/ Δ ex13}.

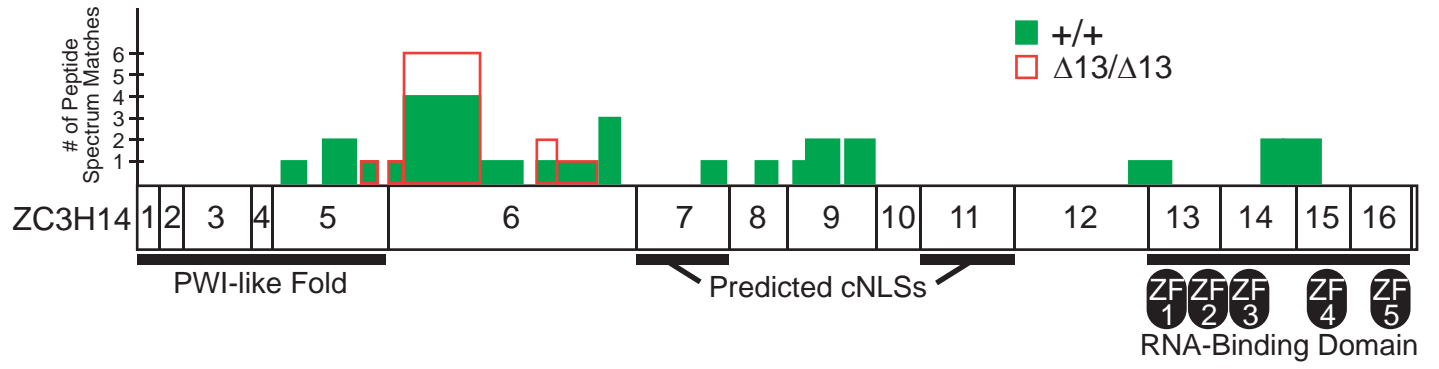
Fig. S3. *Zc3h14* ^{Δ ex13/ Δ ex13} mice exhibit normal visual and motor function. To rule out possible visual or motor coordination deficits that could interfere with performance during water radial arm maze, we tested the visual and motor capabilities of the mice. When compared to controls (+/+), mutant (Δ 13/ Δ 13, *Zc3h14* ^{Δ ex13/ Δ ex13}) mice performed at least as well on all visual and motor coordination assays. (A) Illustration of Optokinetic apparatus and visual cues. Visual acuity was measured by the ability of the mice to discern small spatial frequencies of white and black bars (91-93). Contrast sensitivity was measured by the ability of the mice to discern alternating bars of similar shades of gray (91-93). (B) Average of lowest spatial frequencies able to be detected by mice by genotype is shown. (C) Average of lowest percent

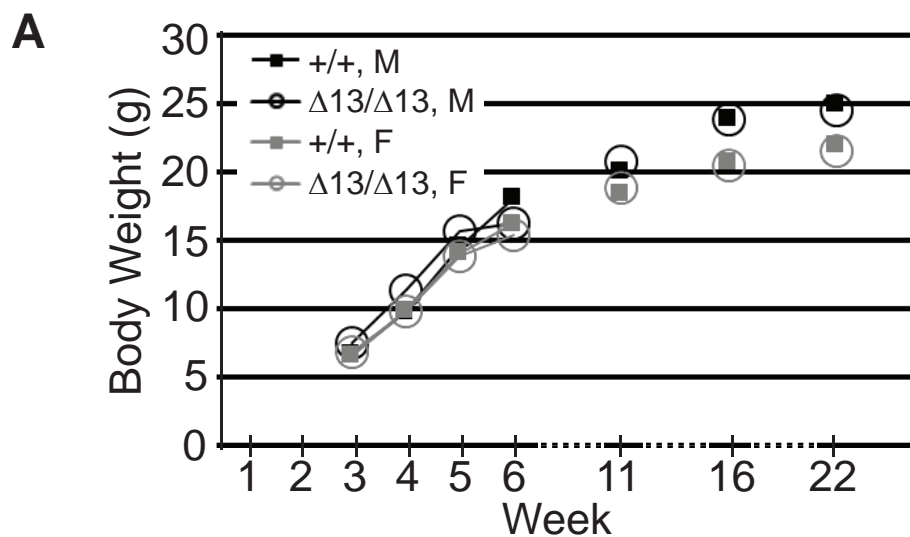
contrast able to be detected by mice by genotype is shown. For (B) and (C) *Zc3h14*^{+/+}, n=4; *Zc3h14* ^{Δ ex13/ Δ ex13}, n=4. (D) Illustration of wire-hang assay setup. (E) Latency to fall is the average time in seconds for mice, by genotype, to fall from a wire mesh when placed upside down against gravity. *Zc3h14*^{+/+}, n=9; *Zc3h14* ^{Δ ex13/ Δ ex13}, n=14. Comparing by genotype, $p=0.1828$. (F) Illustration of rotarod apparatus, which measures the ability of mice to balance on a rotating rod, tested across three days. (G) Latency to fall is the average time for mice to fall from the rotating rod, grouped by genotype. *Zc3h14*^{+/+}, n=9; *Zc3h14* ^{Δ ex13/ Δ ex13}, n=10. Differences between *Zc3h14*^{+/+} and *Zc3h14* ^{Δ ex13/ Δ ex13} on a given test day were not statistically significant after applying the Bonferroni correction for repeated measures. Error bars indicate SEM for all panels in figure.

Fig S4. Heatmap with hierarchical clustering of significantly different proteins. Proteins identified as significantly different between *Zc3h14*^{+/+} (+/+) and *Zc3h14* ^{Δ ex13/ Δ ex13} (Δ 13/ Δ 13) are presented in a heatmap with hierarchical clustering. Clustering discriminates between *Zc3h14*^{+/+} and *Zc3h14* ^{Δ ex13/ Δ ex13} mice. Blue represents low protein levels and red represents high proteins levels. The names of the proteins are indicated to the left with ZC3H14 shown at the bottom.

Supplemental Table 1

Test	Measurement	Genotype	Average	SEM	<i>P</i>
Open Field	Time in center (sec)	+/+	35.10	5.18	0.03
		$\Delta 13/\Delta 13$	77.00	17.54	
	Number of entries into the center	+/+	18.20	9.38	0.05
		$\Delta 13/\Delta 13$	27.70	12.37	
	Total distance traveled (cm)	+/+	36.60	2.67	0.62
		$\Delta 13/\Delta 13$	34.50	3.28	
Average speed (cm/sec)	+/+	0.10	0.00	0.61	
	$\Delta 13/\Delta 13$	0.10	0.01		
Novel Cage	Time spent rearing (sec)	+/+	133.00	14.05	0.38
		$\Delta 13/\Delta 13$	117.40	10.27	
	Time spent digging (sec)	+/+	44.50	10.96	0.45
		$\Delta 13/\Delta 13$	34.30	7.38	
	Time spent grooming (sec)	+/+	13.80	1.81	0.81
		$\Delta 13/\Delta 13$	13.00	2.48	
Light-Dark Box	Latency to first dark entry (sec)	+/+	15.70	4.28	0.56
		$\Delta 13/\Delta 13$	21.20	8.34	
	Number of transitions between light/dark	+/+	23.80	1.79	0.22
		$\Delta 13/\Delta 13$	26.80	1.63	
	Time in dark (sec)	+/+	393.60	19.63	0.86
		$\Delta 13/\Delta 13$	386.60	33.20	
Time in light (sec)	+/+	206.30	19.62	0.86	
	$\Delta 13/\Delta 13$	386.60	33.20		
Fear Conditioning	Percent Freezing (%)	+/+	67.76	67.76	0.32
		$\Delta 13/\Delta 13$	61.14	61.14	





B

N values for Body Weight							
Week	3	4	5	6	11	16	22
Male $+/+$	7	7	8	5	7	8	6
Male $\Delta 13/\Delta 13$	3	9	8	4	5	10	6
Female $+/+$	6	6	8	7	6	9	5
Female $\Delta 13/\Delta 13$	4	13	9	7	8	13	9

C

SEM for Body Weight							
Week	3	4	5	6	11	16	22
Male $+/+$	0.46	0.96	0.93	0.32	1.07	0.40	0.60
Male $\Delta 13/\Delta 13$	1.63	0.94	0.81	1.23	1.66	0.91	1.84
Female $+/+$	0.33	0.43	0.33	0.32	0.44	0.44	0.73
Female $\Delta 13/\Delta 13$	0.98	0.63	0.65	0.61	0.73	0.49	0.56

D

<i>P</i> values for Body Weight							
Week	3	4	5	6	11	16	22
Male	0.49	0.26	0.41	0.16	0.87	0.97	0.82
Female	0.73	0.94	0.75	0.26	0.67	0.7	0.63

