Supplementary Data Table of Contents

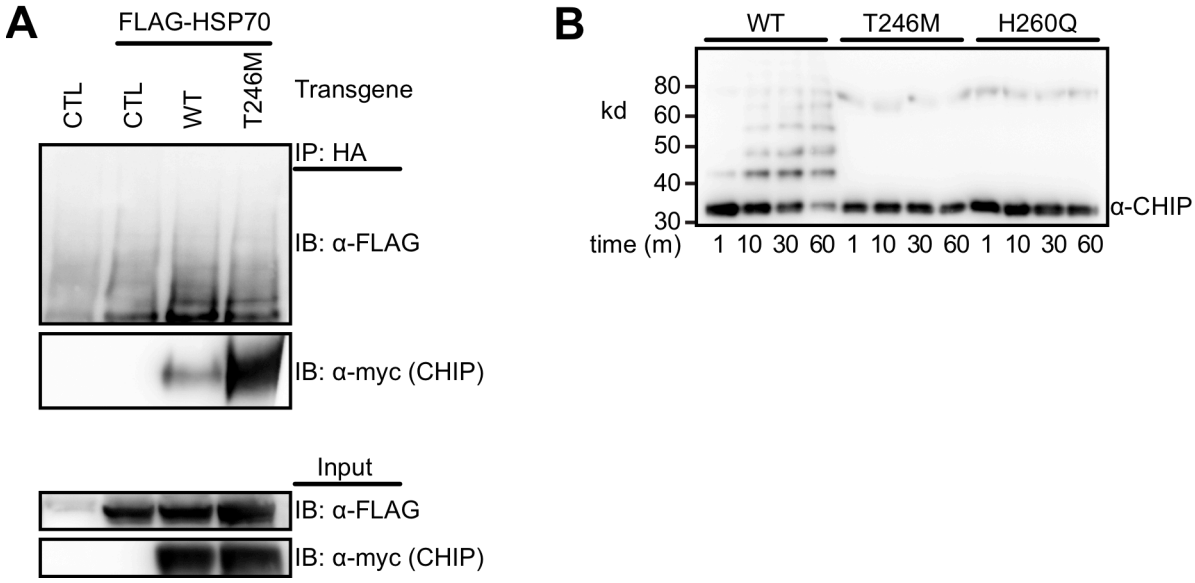

Figure S1: *CHIP-T246M Interacts with Chaperones but Lacks Ubiquitin Ligase Activity.* (**A**) To confirm the effect of CHIP-T246M on the exogenous HSP70 substrate, we performed the reverse IP shown in Figure 3A. COS-7 cells were co-transfected with the indicated vectors (transgenes, CTL = pcDNA3, WT = pcDNA3-CHIP, T246M = pcDNA3-CHIP-T246M) in addition to HA-tagged ubiquitin. Ubiquitinated proteins was immunoprecipitated (IP) with HA beads and the resulting precipitants as well as inputs were immunoblotted (IB) with the indicated antibodies. (**B**) Cell-free ubiquitination reactions from the experiment represented in Figure 3D containing recombinant HSC70 and the indicated CHIP proteins resolved via SDS-PAGE and immunoblotted for an antibody recognizing CHIP.

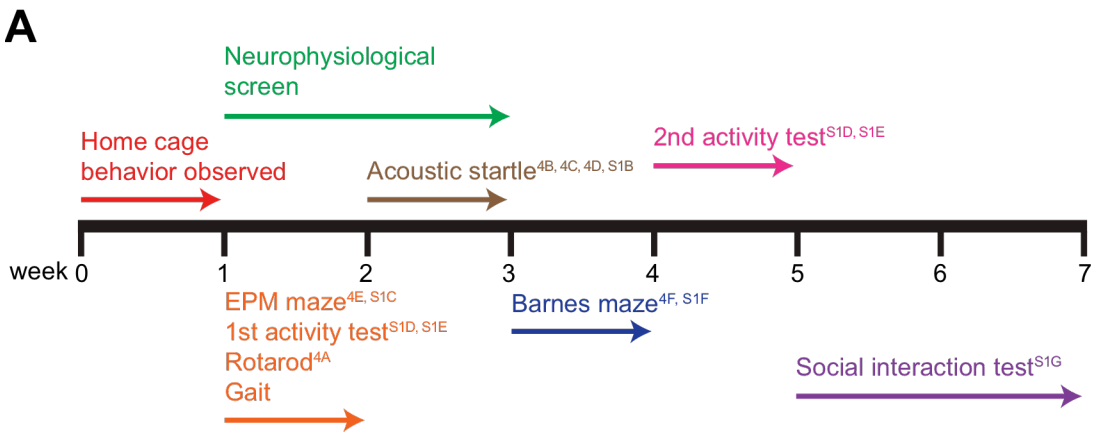

Figure S2A: *Behavioral Assessment Tests of Chip$^{-/-}$ Mice*. The goal of these assessments was to determine the behavioral phenotype of mice with a deficiency in CHIP expression. Subjects were 10 wild-type mice and 10 *Chip*$^{-/-}$ mice with an equal number of males and females per genotype. In all tests, an observer took measures blind to experimental treatment (genotype). Data were analyzed using two-way ANOVAs, looking for effects of genotype (wild-type or *Chip*$^{-/-}$) or sex, or when indicated, repeated measures ANOVAs. Fisher's protected least-significant difference (PLSD) tests were used for comparing group means only when a significant F value was determined. The figure panels representing these data are indicated, if applicable (S = Supplementary Figure).

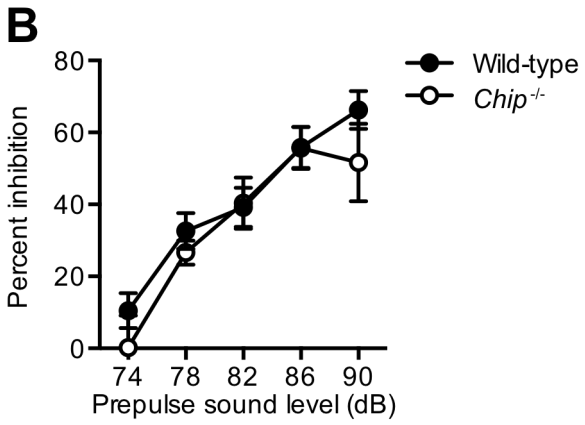

Figure S2B: *Prepulse Inhibition During Acoustic Startle Test, depicted in Figure 4B*. The prepulse inhibition was calculated as 100 - [(response amplitude for prepulse stimulus and startle stimulus together / response amplitude for startle stimulus alone) x 100] and represented by the mean ± SEM for each genotype (n = 6 and 10 for *Chip*$^{-/-}$ and wild-type mice, respectively). Four animals, all *Chip*$^{-/-}$ mice (three males and one female), did not show significant startle responses to the acoustic stimuli, suggesting auditory impairment. Therefore, their data were removed from the analysis. There was no significant effect of genotype on levels of prepulse inhibition, indicating that the Chip deficiency did not lead to impairment in sensory gating. This result also suggested that the remaining knockout mice had normal auditory function, since a prepulse stimulus only 8 dB above background noise (the 78 dB prepulse level) decreased the startle response by more than 25%. These data suggest a possible motoric deficit for the decrease in startle amplitude and reaction time (Fig. 4C and 4D).

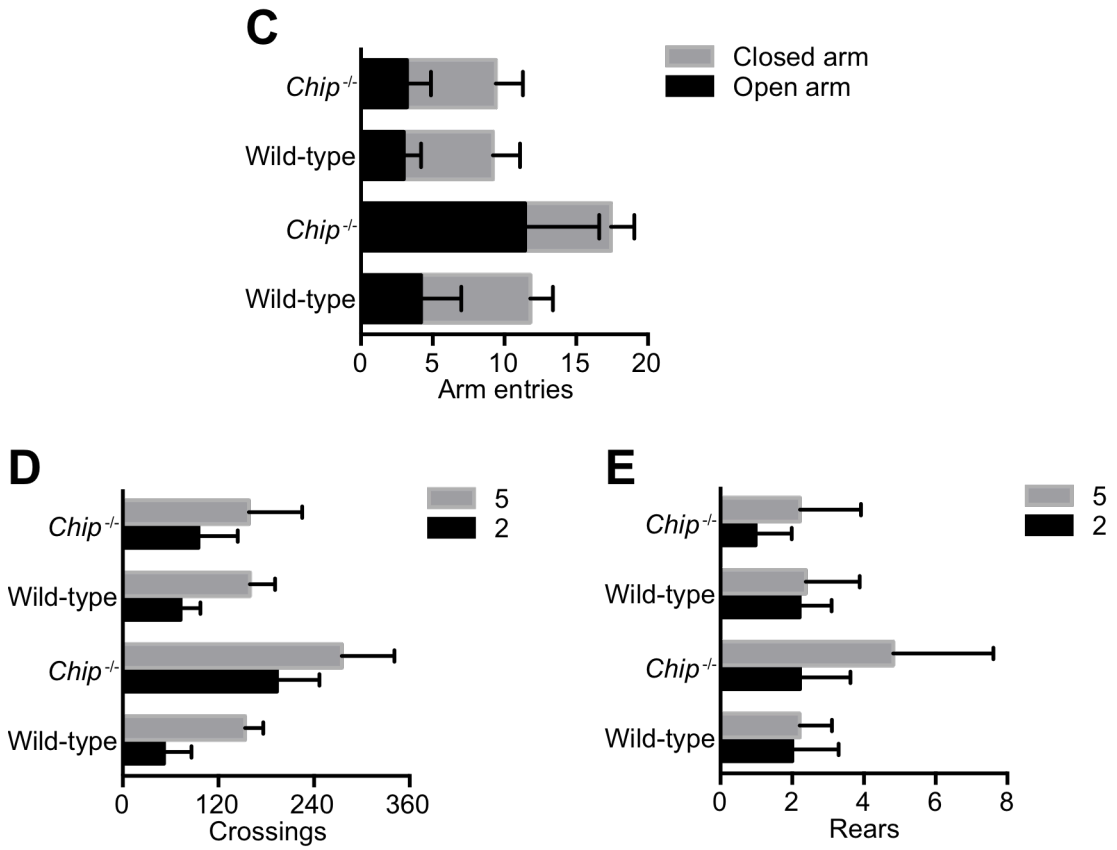

Figure S2C, S2D, and S2E: *Various Measures of Physical Activity Throughout Behavioral Testing*. (**C**) The number of entries into the open and closed arms of mice in the elevated plus maze (EPM). There was no difference between the groups for arm entries, suggesting that wild-type and *Chip*$^{-/-}$ mice have equal levels of activity. (**D** and **E**) The number of crossings and rears in an open-field chamber were counted at two different time points (2 and 5 weeks of testing, Supplementary Material Fig. S2A). For all data shown neither genotype nor sex had a significant impact on the various measures represented by the mean ± SEM for each genotype (n = 10).

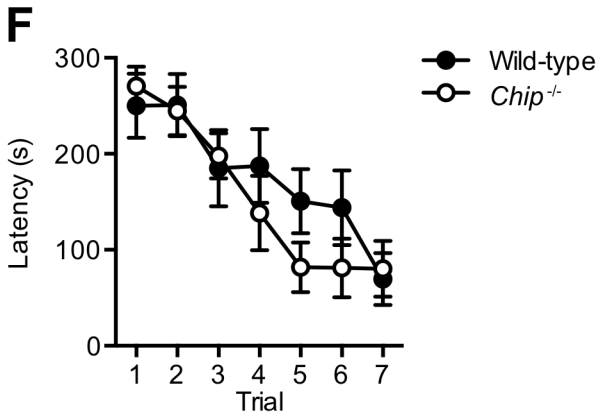

Figure S2F: *Latency in the Barnes Maze*. Each mouse received one trial per day, across seven days. Measures were taken of latency to find the target hole until reaching the target represented by the mean ± SEM for each genotype (n = 10). Both wildtype and *Chip*$^{-/-}$ mice showed high latency scores, reflecting the performance of some animals which never left the center of the maze (especially on the first and second trials). A repeated measures ANOVA did not result in any significant effects for genotype or gender on latency, although the significant effect for trials (the repeated measure) confirmed that some learning occurred in this task [$F(6,108) = 10.99$, $p = .0001$].

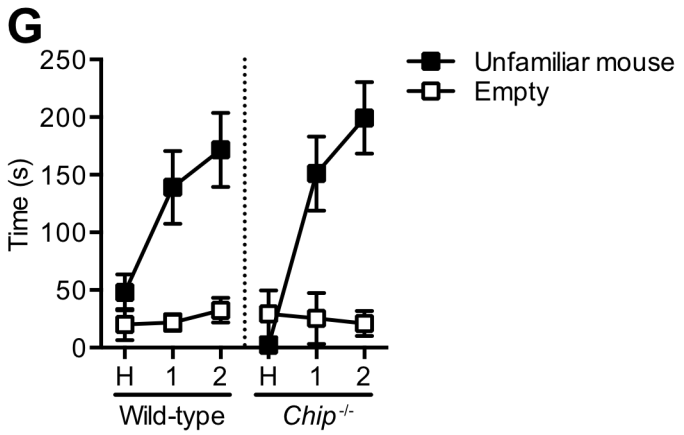

Figure S2G: *Social Affiliation Test*. Animals were tested in a three-chambered apparatus, with the chambers connected by short tubes. Each mouse was first set in the middle chamber and allowed to explore for five minutes in the absence of another mouse (habituation period, H). Then measures were taken of the time each mouse spent in either and empty chamber (open squares) or the chamber containing an unfamiliar mouse (closed squares) for the first (1) and second (2) five minutes of the session. Separate repeated measures ANOVAs were performed for time spent in the side with the unfamiliar mouse and time in the empty side. No significant overall effects of either group (wild-type or *Chip*$^{-/-}$) or gender were detected. One-way ANOVAs indicated that *Chip*$^{-/-}$ mice spent less time exploring during the habituation period (when both sides of the chamber were empty, $p < 0.05$), but *Chip*$^{-/-}$ mice subsequently showed normal social preference during the test period. These data indicate that loss of CHIP protein does not cause deficits in the preference for social affiliation.

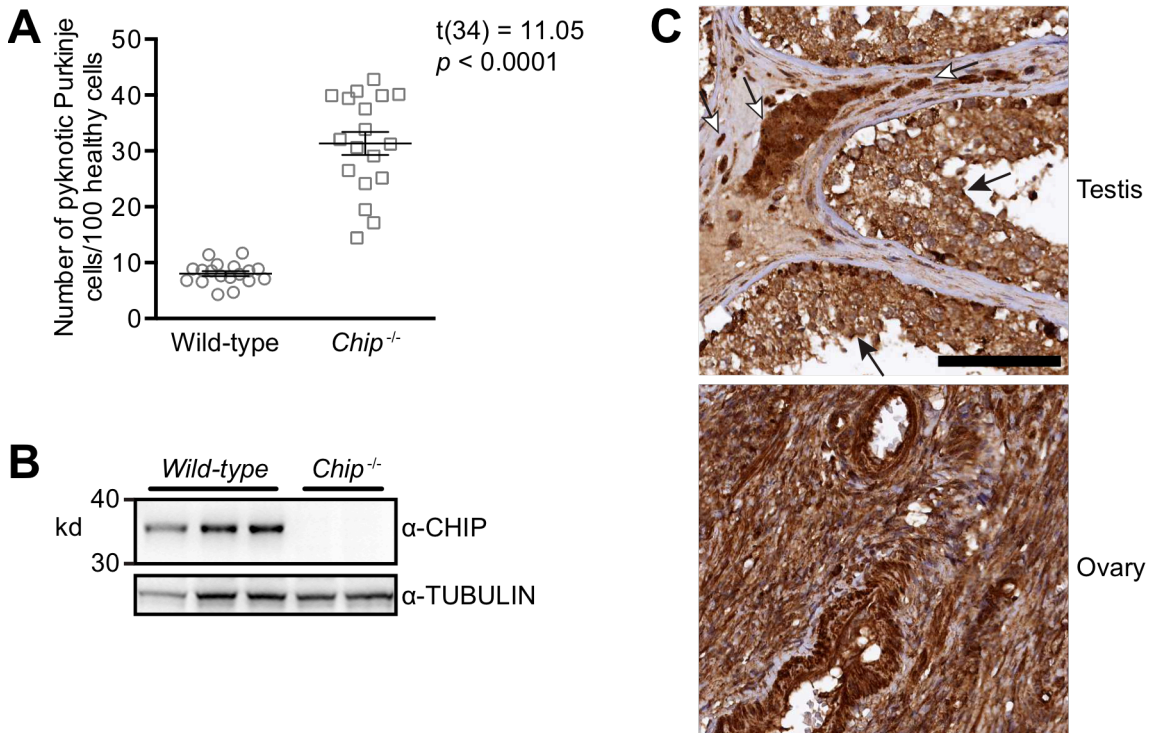

Figure S3: *Increase in Purkinje Cell Pathology in Chip$^{-/-}$ Mice and CHIP Expression in Mouse and Human Gonads*. (**A**) Sagittal sections of whole cerebellums from either wild-type or *Chip*$^{-/-}$ mice were stained with crysel violet (Fig. 5B) to measure Purkinje cell pathology. Data are represented by the number of Purkinje cells with pyknotic nuclei per 100 health Purkinje cells with each data point corresponding to one sagittal section (n = 18). A two-tailed *t* test was used to compare the two genotypes. *Chip*$^{-/-}$ mice had three-fold more pyknotic Purkinje cells compared to wild-type mice (1079 and 324 across all 18 sections from 3 different mice per genotype, respectively) and a decrease in healthy Purkinje cells (3390 and 4051, respectively). (**B**) Immunoblot confirmation of CHIP protein expression in extracts isolated from wild-type testes. (**C**) CHIP is expressed in both human testes (upper) in both Leydig cells (open arrows) and seminiferous ductal cells (closed arrows) and throughout human ovarian stromal cells (lower).

Table S1: *Six Candidate Variants from Exome Sequencing Data*

Chromosome	Position	Reference Allele	Variant Allele	HET/HOM	Gene Name	Substitution	IBD=2	Functional Prediction
chr19	9025639	A	G	HOM	MUC16	N12272S	NO	……
chr1	75037091	G	A	HET	C1orf173	G1435R	NO	……
chr1	75039089	C	G	HET	C1orf173	L769V	NO	……
chr2	97914920	T	A	HET	ANKRD36	C1893S	NO	……
chr2	97915322	T	G	HET	ANKRD36	I1914M	NO	……
chr4	151769986	G	T	HET	LRBA	G94V	YES	TOLERATED
chr4	151356766	G	T	HET	LRBA	S2350I	YES	TOLERATED
chr22	24325095	A	G	HOM	GSTT2	K129E	YES	TOLERATED
chr16	732232	C	T	HOM	STUB1	T246M	YES	DAMAGING

Extended Experimental Procedures

Mouse behavioral assessments

Home cage behavior. In the first week of testing, a bedding nestlet was added to each home cage of the experimental groups. 24 hours later animals were observed to note if nests had been formed from the bedding material and, in the case of multiply-housed mice, if the animals huddled together in the nest. Nests were observed in each cage, and no aberrant behavior was observed.

Elevated plus maze test for anxiety. The elevated plus maze (EPM) test was performed as the first behavioral test to avoid possible confounding effects of extensive handling. Mice were given one five-minute trial on the plus maze, which had two walled arms (the closed arms) and two open arms. The maze was elevated 52 cm from the floor, and the arms were 51 cm long. Animals were placed on the center section (9.5 cm x 9.5 cm), and allowed to freely explore the maze. Measures were taken of time on, and number of entries into, the open and closed arms.

Activity. One day following the EMP test (week two), exploratory activity in a novel environment was further assessed by a five-minute trial in an open field chamber (40 cm x 30 cm). A grid of squares (10 X 6) was drawn on the floor of the chamber, and counts were taken of number of squares crossed and rears during the trial. A second activity test was performed during week five of testing.

Neurophysiological screen and gait testing. The neurophysiological screen consisted of several measures to assay overall appearance and behavior of the mice. Measures included general observations on the animal's appearance, body posture, and normality of gait. Normal reflexive reactions to a gentle touch from a Q-tip to the whiskers on each side of the face, and the approach of the Q-tip to the eyes, were assessed. Each mouse

was placed in a small, empty plastic cage, and ability to remain upright when the cage was moved from side-to-side or up-and-down was noted. Locomotor coordination was assayed by allowing the mouse to walk across an elevated dowel (wrapped in nylon rope to facilitate grasping) and to climb a similar pole. Each subject was also placed on a wire grid and allowed to hang for two minutes. Reaction to 20 seconds of tail-suspension was observed. For the gait test, a footprint record was generated by painting the paws of the mice and letting the animals run down a narrow alley into a small box. Front paws were painted yellow and hind paws were painted blue with a nontoxic poster paint. Each mouse was given two trials, and measures of front paw and hind paw stride lengths, and front paw and hind paw base widths, were taken. In addition, measures were also taken for paw-print overlap.

Rotarod. Mice were tested on an accelerating rotarod (IITC Inc., Woodland Hills, CA) to assess motor coordination. For the first test session, animals were given three trials, with 45 seconds between each trial. Two additional trials were given 48 hours later. RPM (revolutions per minute) was set at an initial value of three, with a progressive increase to a maximum of 30 RPM across three minutes (the maximum trial length). Measures were taken for latency to fall from the top of the rotating barrel.

Acoustic startle. This test is based on the measurement of the reflexive whole-body flinch, or startle response, that follows exposure to a sudden noise. Assessments included startle magnitude and prepulse inhibition, which occurs when a weak prestimulus leads to a reduced startle in response to a subsequent louder noise. Animals were tested with a San Diego Instruments SR-Lab system, using the procedure as described (1). Briefly, mice were placed in a small Plexiglas cylinder within a larger, sound-attenuating chamber (San Diego Instruments). The cylinder was seated upon a piezoelectric transducer, which allowed vibrations to be quantified and displayed on a computer. The chamber included a

houselight, fan, and a loudspeaker for the acoustic stimuli. Background sound levels (70 dB) and calibration of the acoustic stimuli were confirmed with a digital sound level meter (San Diego Instruments). Each mouse was given one session, consisting of 42 trials that began with a five-minute habituation period. There were seven different types of trials: the no-stimulus trials, trials with the acoustic startle stimulus (120 dB) alone, and trials in which a prepulse stimulus (either 74, 78, 82, 86, or 90 dB) occurred 100 ms before the onset of the startle stimulus. Measures were taken of the startle amplitude for each trial, and an overall analysis was performed for each animal's data for levels of prepulse inhibition at each prepulse sound level.

Spatial learning on the Barnes maze. The Barnes maze consisted of a large, brightly lit, circular platform (diameter = 122 cm), elevated 96.5 cm from the floor and positioned like a table, with 40 holes (diameter = 5 cm) drilled along the perimeter. An escape box containing fresh nesting material was placed under one of the holes, and the task required that the animal learn which hole allowed escape from the maze surface. Each mouse was assigned a particular "target" hole, which remained constant across trials, and was different for each subject. At the beginning of each trial, the animal was placed in the center of the maze and allowed 5 minutes to find and enter the escape box. Subjects received one trial per day, across seven days. Measures were taken of latency to find the target hole and number of errors (incorrect holes investigated) until reaching the target.

Social affiliation test. Animals were tested in a three-chambered apparatus, with the chambers connected by short tubes. Each mouse was first set in the middle chamber and allowed to explore for five minutes. After the habituation period, the animal was removed and an unfamiliar male probe mouse (C57BL/6J strain) was set in one of the side chambers (the particular side alternated between trials). The probe mouse was enclosed in a small metal cage, which allowed nose contact between the bars. The test mouse was

then returned to the middle chamber and allowed to freely explore for a ten-minute session. Measures were taken of the amount of time spent in each chamber of the apparatus for the first and second five minutes of the session.

Supplemental References

1 Paylor, R. and Crawley, J.N. (1997) Inbred strain differences in prepulse inhibition of the mouse startle response. *Psychopharmacology*, **132**, 169-180.