SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURES

MATERIALS AND METHODS

Animals. A mouse line carrying a microdeletion of mouse chromosome 7qF3, the syntenic region of human chr16p11.2, was generously provided by Professor Alea Mills from the Cold Spring Harbor Laboratory prior to publication[8]. In the present study F3 heterozygous mutant male mice on a mixed 129/C57BL/6 background were backcrossed 5-10 generations to C57BL/6J mice from Charles River Laboratory. Mice were group-housed on a 12 hour on/12 hour off light/dark cycle. All experiments were conducted in accordance with the rules and regulations of The Institutional Animal Care and Use Committee at MIT. During backcrossing we observed a steady decline in the percentage of heterozygous mutant mice (Supplemental Figure 1), indicating a non-Mendelian transmission of the mutant allele. All experiments were performed by an experimenter blind to genotype.

Reagents. S-3,5-dihydrozyphenylglycine (S-DHPG) was purchased from Sigma-Aldrich. Aliquots of DHPG were prepared in H_2O as 1000x stock (50 mM) and used within 7 days of preparation. Cycloheximide (CHX) was purchased from Sigma-Aldrich, prepared fresh in H_2O as 1000x (60 mM) stock and used on the same day of preparation. CTEP, [2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine], was synthesized at Hoffmann-La Roche, formulated as a micro-suspension prepared in 0.9% saline/0.3% Tween-80, and administered by oral gavage at a dose of 2 mg/kg.

Hippocampal electrophysiology. Electrophysiological experiments were performed at the Schaffer collateral-CA1 synapse of dorsal hippocampal slices (400 μm thick) prepared from p28-35 male mice using experimental protocols previously described[1]. Input-output functions were determined by incrementally (10 μA to 100 μA) stimulating the Schaffer collaterals and recording the resulting fEPSP response. Paired-pulse facilitation was conducted by applying two stimulus pulses (stimulus 1 and 2) at varying inter-stimulus-intervals (ISI). Facilitation was measured by taking the ratio of the fEPSP slope in response to stimulus 2 to that of stimulus 1. Long-term

potentiation (LTP) was induced using theta-burst stimulation (TBS) delivered in two trains with a 30 sec interval. Each train was composed of 50 ms bursts of stimuli (100 Hz) delivered at 5 Hz for 1 sec. NMDA receptor dependent long-term depression (LTD) was elicited with low-frequency stimulation (LFS) comprised of 900 pulses at 1 Hz. For DHPG-LTD, slices were incubated in artificial cerebrospinal fluid (ACSF) in the presence or absence of the protein synthesis inhibitor cycloheximide (± CHX, 60 μM, 40 min), and mGluR5 was activated by bath application of DHPG (50 μM, 5 min). Synaptic responses were followed for an additional 60 min following DHPG application. Paired pulse facilitation was assessed 30 min prior to, and 60 min following DHPG application in all slices used in DHPG-LTD experiments (see Figure 1 C, D). For paired-pulse low frequency stimulation (PP-LFS), slices were incubated in ACSF containing APV (50 μM) ± CHX for 30 min mGluR5-LTD was then induced by application of 1200 paired-pulse stimulation (50 ms ISI) at 1Hz, and synaptic responses were recorded for an additional 60 min in the presence of APV. Statistical significance was determined using repeated measures two-way ANOVA and *post hoc* Bonferroni tests.

Metabolic Labeling. Metabolic labeling of new protein synthesis was performed as previously described[16]. Male P28-P32 littermate (WT) and 16p11.2 *df/+* mice were anesthetized with isoflurane and the hippocampus was rapidly dissected into ice-cold artificial cerebral spinal fluid (ACSF) (in mM: 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 dextrose, 1 $MgCl_2$, 2 $CaCl_2$, saturated with 95% O_2 and 5% CO_2). Hippocampal slices (500 μm) were prepared using a Stoelting Tissue Slicer and transferred into 32.5°C ACSF (saturated with 95% O_2 and 5% CO_2) within 5 min. Slices were incubated in ACSF undisturbed for 3.5-4 h to allow for recovery of basal protein synthesis. Actinomycin D (25 μM) was then added to the recovery chamber for 30 min to inhibit transcription after which slices were transferred to fresh ACSF containing ~10 mCi/ml [^{35}S] Met/Cys (Perkin Elmer) for an additional 30 min. Slices were then homogenized, and labeled proteins isolated by TCA precipitation. Samples were read with a scintillation counter and subjected to a protein concentration assay (Bio-Rad). Data was analyzed as counts per minute per microgram of protein, normalized to the [^{35}S] Met/Cys ACSF used for incubation and the average incorporation of all samples analyzed and then normalized to percent WT for each experiment. Statistical significance was determined using unpaired t-tests.

Immunoblotting. Immunoblotting was performed according to established methods using primary antibodies to Arc (Synaptic Systems #156002), p-ERK1/2 (Thr202/Tyr204 #9101) (Cell

Signaling Technology), ERK1/2 (Cell Signaling Technology #9102), or MVP (Abcam #ab97311). Arc, MVP, ERK1 and ERK2, as well as ERK1 and ERK2 phosphorylation were measured by densitometry (Quantity One), and quantified as the densitometric signal of each protein divided by the total protein signal (determined by Memcode staining) in the same lane. Statistical significance was determined using unpaired Student's t-tests.

Contextual fear conditioning (CFC). CFC was performed in a Freezeframe Chamber (Coulbourn Instrument) as previously described[1]. Briefly**,** 6 to 12 week-old wildtype and mutant male mice were fear conditioned on day 1 and the subsequent percentage of time spent freezing in either the familiar or a novel context was determined 24 hr later. On the day of conditioning, animals were allowed to explore the behavioral chamber for 3 min, followed by delivery of a 2 sec, 0.8 mA foot-shock. Mice remained in the context for 15 sec after the shock before returning to their home cages. Fear response was assessed 24 hr later. To determine context specificity of the conditioned response, mice trained on day 1 were separated into two groups on day 2: one group was tested in the same training context (familiar context), and the other tested in a novel context. The novel context was created by varying spatial cues, floor material, and lighting of the testing chamber. The percentage of time a mouse spent freezing during a testing session of 4 min on day 2 was the behavioral readout. To determine if WT and mutant mice had the same sensitivity to foot-shock, the distance traveled by each subject during the 2 sec foot-shock and 1 sec immediately following was recorded. Statistical significance was determined using repeated measures two-way ANOVA and *post hoc* Student's t-tests.

Inhibitory avoidance (IA) test. IA tests were performed with a passive avoidance apparatus (Ugo Basile Passive Avoidance Apparatus, Step-through model for mouse) as previously described with modification[18]. In all IA tests, a mouse was subjected to one training session (0 hr) and three subsequent testing sessions (6, 24 and 48 hr). In each session, a mouse was first placed in a LED-illuminated "START" compartment of a two-compartment test apparatus for 30 sec prior to the partition door opening. Upon entering the dark compartment the door was closed immediately. The subject remained in the dark compartment for 60 sec before being taken out. During the training session, a 2 sec foot-shock of 0.4 mA was delivered. No shock was delivered in the three testing sessions. The latency to enter the dark compartment was recorded to assess baseline level (0 hr), acquisition of fear memory (6 hr), and memory extinction (24 hr and 48 hr). Male mice of age 6-10 weeks of age were used in IA tests without drug treatment. In IA tests with CTEP treatment, 4-6 week old male mice were divided into four groups according to

genotype (WT or mutant) and drug treatment (vehicle or CTEP). Administration of vehicle or CTEP (2 mg/kg) was initiated at the age of 4-6 weeks, given as one dose every 48 hr by oral gavage, and continued for 4 weeks prior to IA testing by an experimenter blind to both genotype and drug condition. Statistical significance was determined using repeated measures two-way ANOVA and *post hoc* Student's t-tests.