ALTERATIONS IN MICRORNA-124 AND AMPA RECEPTORS CONTRIBUTE TO SOCIAL BEHAVIORAL DEFICITS IN FRONTOTEMPORAL DEMENTIA

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Neurodegenerative diseases, such as frontotemporal dementia (FTD), are often associated with behavioral deficits, but the underlying anatomical and molecular causes remain poorly understood. Here we show that forebrain-specific expression of FTD-associated mutant CHMP2B in mice causes several age-dependent neurodegenerative phenotypes, including social behavioral impairments. The social deficits were accompanied by a change in AMPA receptor (AMPA) composition, leading to an imbalance between Ca2+-permeable and Ca2+-impermeable AMPARs. Expression of most AMPAR subunits was regulated by the brain-enriched microRNA miR-124, whose abundance was markedly decreased in the superficial layers of the cerebral cortex of mice expressing the mutant CHMP2B. We found similar changes in miR-124 and AMPAR levels in the frontal cortex and induced pluripotent stem cell–derived neurons from subjects with behavioral variant FTD. Moreover, ectopic miR-124 expression in the medial prefrontal cortex of mutant mice decreased AMPAR levels and partially rescued behavioral deficits. Knockdown of the AMPAR subunit Gria2 also alleviated social impairments. Our results identify a previously undescribed mechanism involving miR-124 and AMPARs in regulating social behavior in FTD and suggest a potential therapeutic avenue.

Different neurodegenerative diseases are characterized by progressive dysfunction and loss of specific neuronal populations, often resulting in various behavioral abnormalities1–3. The molecular mechanisms of these impairments, and the neural circuits involved, are poorly understood. FTD is the second most common cause of dementia before 65 years of age. More than 50% of subjects with FTD have the behavioral variant, characterized by marked changes in personality and social behavior4. FTD has a strong genetic component; about 40% of subjects with FTD have the behavioral variant, characterized by marked changes in personality and social behavior4. FTD is linked clinically, pathologically and molecularly to amyotrophic lateral sclerosis6,7.

Mutations in several seemingly unrelated genes cause familial FTD6,7. However, the clinical outcome in subjects with FTD carrying different mutations is often similar, suggesting that the same neural circuits are affected. Mutations in CHMP2B, encoding charged multivesicular body protein 2B, are implicated not only in FTD and amyotrophic lateral sclerosis8,9 but also in early-onset Alzheimer’s disease10. CHMP2B encodes a component of the endosomal sorting complexes required for transport-III (ESCRT-III), which functions in the endosomal-lysosomal and autophagy pathways11,12. The mutation that causes FTD results in a CHMP2B protein that is truncated at the carboxy terminus (CHMP2BINtron5)9, and several studies suggest a gain of toxic function unique to the CHMP2BINtron5 isoform13–16.

Although the molecular basis of the social deficits associated with FTD is unknown, microRNAs (miRNAs), a class of small noncoding RNAs, are important contributors to neurodegeneration17,18. miR-124 is evolutionarily conserved and one of the most abundant miRNAs in the brain. Although miR-124 has well-established functions during neuronal development19, its specific roles in neurodegeneration are poorly understood17,18. Here, using a new mouse model of FTD with the CHMP2BINtron5 mutation and studies on induced pluripotent stem cell (iPSC)-derived human neurons and brain tissues from subjects with behavioral variant FTD (bvFTD), we found miR-124 and AMPARs contribute to FTD-associated social impairments.

RESULTS

A new mouse model of FTD exhibits deficits in sociability

To examine the molecular basis of behavioral abnormalities in FTD, we generated a new transgenic mouse model expressing, under

Received 22 July; accepted 11 September; published online 17 November 2014; doi:10.1038/nm.3717
the control of the tetracycline promoter, CHMP2B<sup>Intron5</sup> (Fig. 1a). When crossed with a reporter line (TetO-GFP), tetracycline-controlled transactivator (tTA), whose expression is under the control of the Camk2a promoter, drives GFP expression only in forebrain neurons just as the reporter line (TetO-GFP), tetracycline-controlled transactivator (tTA), whose expression is under the control of the TetO promoter. The resulting double-transgenic mice are named tTA:CHMP2B<sup>WT</sup> and tTA:CHMP2B<sup>Intron5</sup> mice, respectively. These transgenes were expressed in forebrain neurons just as the reporter GFP was (Supplementary Fig. 1b).

Compared to endogenous Chmp2b, transgene expression was modest in tTA:CHMP2B<sup>WT</sup> and tTA:CHMP2B<sup>Intron5</sup> mice at both the mRNA level (Supplementary Fig. 1c) and the protein level (Supplementary Fig. 1d). We selected tTA:CHMP2B<sup>WT</sup> line 3 and tTA:CHMP2B<sup>Intron5</sup> line 3 for further analysis in most experiments because of their equal transgene expression at the mRNA level (Supplementary Fig. 1c) and because CHMP2B<sup>Intron5</sup> and CHMP2B<sup>WT</sup> proteins are equally stable<sup>15</sup>. Moreover, a doxycycline-containing diet repressed transgene transcription (Supplementary Fig. 1d).

To determine whether the mice had disease-relevant behavioral deficits, we first used the resident-intruder test in which social interaction in the home cage was measured<sup>20</sup>. Although tTA:CHMP2B<sup>Intron5</sup> mice performed similarly to tTA:CHMP2B<sup>WT</sup> mice at the beginning of the test, their interaction times decreased at the last two time points (Supplementary Fig. 2a), suggesting specific deficits in this social behavior. To further characterize the social behavioral deficits, we used a modified version of the three-chamber social paradigm<sup>21</sup>. After a habituation trial where the test mouse is allowed to explore all three empty chambers, in the next three trials, a social partner (stranger 1) is placed inside a cage in one side of the apparatus, and an identical empty cage is placed in the other compartment. In the final trial (trial 5), a novel social partner (stranger 2) is introduced into the previously empty cage. This task quantifies not only the sociability but also social memory and social recognition. In trial 2, both tTA:CHMP2B<sup>WT</sup> and tTA:CHMP2B<sup>Intron5</sup> mice spent more time in the target chamber containing stranger 1 than in the empty chamber (Fig. 1b), indicating that CHMP2B<sup>Intron5</sup> expression does not perturb the ability to recognize an animal of the same species.

To quantify sociability, we measured the time test mice spent in close interaction with social partners across trials. In trial 2, both tTA:CHMP2B<sup>WT</sup> and tTA:CHMP2B<sup>Intron5</sup> mice showed equal sociability, which gradually decreased in later trials, suggesting progressive loss of social interest in the familiar mouse (Fig. 1c). However, tTA:CHMP2B<sup>Intron5</sup> mice had a more pronounced, age-dependent decrease in sociability, particularly in trial 5, when both social partners were present (Fig. 1c). We also observed a similar age-dependent decrease in thigmotaxis in the elevated-plus-maze test (Supplementary Fig. 2c). In trial 5, the proportion of time tTA:CHMP2B<sup>Intron5</sup> mice spent interacting with stranger 2 was similar to that of tTA:CHMP2B<sup>WT</sup> mice, suggesting that they distinguished stranger 1 from stranger 2 equally well (Fig. 1d). Moreover, time spent in each chamber during trial 5 did not differ between tTA:CHMP2B<sup>Intron5</sup> and tTA:CHMP2B<sup>WT</sup> mice (Fig. 1e), suggesting that social behavior, but not exploration pattern, is specifically compromised in tTA:CHMP2B<sup>Intron5</sup> mice. Moreover, in an object recognition task in which exploration can be assessed independently of social cues, the times spent exploring identical objects during the familiarization phase as well as the familiar and novel object during the test phase were similar in tTA:CHMP2B<sup>Intron5</sup> and tTA:CHMP2B<sup>WT</sup> mice (Supplementary Fig. 2d), confirming the specificity of the social deficits.
The selective effect of CHMP2B\textsuperscript{Intron5} is also supported by the lack of a major deficiency in locomotion in \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice (Supplementary Fig. 2e). Moreover, it is unlikely that the social deficits were due to perturbed olfaction, as we did not observe gross morphological defects in the olfactory bulb (Supplementary Fig. 3a,b). However, \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice showed no differences from \textit{tTA:CHMP2B\textsuperscript{WT}} mice at 8 months of age in the time spent with either two supposedly preferred scents (cinnamon and vanilla) or an aversive scent (2-methyl butyrate; Supplementary Fig. 2f), suggesting that their olfactory discrimination is not disrupted.

Thus, the \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice have a selective impairment in sociability. Moreover, 7 of 36 aged \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice (age 14–20 months) had cutaneous lesions compatible with excessive grooming, suggesting an obsessive-compulsive–like behavior (Supplementary Fig. 2b). These results indicate that expression of CHMP2B\textsuperscript{Intron5} in the mouse forebrain causes age-dependent behavioral deficits that recapitulate some of the clinical symptoms of behavioral variant FTD.

**AMPA composition is altered in the cortex of \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice**

To investigate the cellular and molecular mechanisms of the behavioral deficits, we found that at 8 months of age, forebrain structures of \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice were similar to those of \textit{tTA:CHMP2B\textsuperscript{WT}} mice (Supplementary Fig. 3a,b) and showed no obvious neuronal loss (Supplementary Fig. 3c,d). However, \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice had some FTD-like histopathological features, including astroglia-, ubiquitin deposits and an increase of p62 in insoluble fraction (Supplementary Fig. 4a-d). In addition, in pyramidal neurons in the superficial layers (II and III) of the medial prefrontal cortex (mPFC)—which are most vulnerable in subjects with FTD\textsuperscript{22,23}—spine number and density were greater in \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice than in \textit{tTA:CHMP2B\textsuperscript{WT}} mice (Supplementary Fig. 3c,e,f). This increase was largely due to ‘thin’ spines, which are considered immature\textsuperscript{24–26}.

We did not observe spine changes in hippocampal CA3 pyramidal neurons (Supplementary Fig. 3f), consistent with a greater susceptibility of certain neuronal populations in the mPFC.

To further examine synaptic defects, we first analyzed glutamate receptor levels and composition. Quantitative RT-PCR analysis of the various NMDA and kainate subunits showed no apparent differences between \textit{tTA:CHMP2B\textsuperscript{Intron5}} and \textit{tTA:CHMP2B\textsuperscript{WT}} mice (Fig. 2a). However, in \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice, mRNAs encoding AMPAR subunits Gria2, Gria3 and Gria4, but not Gria1, were upregulated at 4 and 8 months of age (Fig. 2a), time points that are associated with age-dependent defects in sociability. We then analyzed postsynaptic density (PSD) fractions from the cortex of \textit{tTA:CHMP2B\textsuperscript{Intron5}} and \textit{tTA:CHMP2B\textsuperscript{WT}} mice. NMDA subunits and Gria1 were expressed at similar levels in \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice at all time points examined, whereas Gria2, Gria3 and Gria4 levels increased substantially in mice aged 4 months and older (Fig. 2b and Supplementary Fig. 5a). Moreover, \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice at 8 months of age had a higher number of Gria2\textsuperscript{+}PSD95\textsuperscript{+} (Fig. 2c) and Gria4\textsuperscript{+}PSD95\textsuperscript{+} (Supplementary Fig. 5b) puncta in the mPFC, confirming the increased synaptic content of these subunits in situ. Thus, an age-dependent dysregulation of AMPAR subunit composition is associated with the onset of behavioral deficits in \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice.

**AMPA function in the mPFC is altered in \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice**

We next sought to link altered postsynaptic AMPARs to social defects in \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice. In 8-month-old \textit{tTA:CHMP2B\textsuperscript{WT}} mice, intraperitoneal injection of 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX; 50 mg kg\textsuperscript{-1}), a generic AMPAR antagonist\textsuperscript{27}, did not modify sociability (Fig. 3a). In 8-month-old \textit{tTA:CHMP2B\textsuperscript{Intron5}} mice, however, NBQX increased sociability, suggesting that abnormal AMPAR activity might underlie the social deficits in these mice (Fig. 3a). Injections of saline or the NMDA receptor antagonist AP5 (10 mg kg\textsuperscript{-1}) did not affect sociability.
Intron5 expression was turned off in npg © 201

Figure 3 Functional consequences of altered AMPAR composition in tTA:CHMP2B<sup>introns5</sup> mice. (a) Behavioral consequences of AMPAR inhibitor NBQX in tTA:CHMP2B<sup>WT</sup> and tTA:CHMP2B<sup>introns5</sup> mice at 8 months of age (n = 10 for all groups except n = 13 for tTA:CHMP2B<sup>WT</sup> saline; *P < 0.05 by two-sided Kruskal-Wallis test corrected for multiple comparisons). (b) Whole-cell patch-clamp recordings of mEPSCs in pyramidal neurons in the mPFC. The amplitude and frequency of these miniature events were compared between tTA:CHMP2B<sup>WT</sup> mice (n = 11) and tTA:CHMP2B<sup>introns5</sup> mice (n = 12; P > 0.1 by two-sided t-test). (c) The rectification index, calculated as EPSC<sub>–60 mV</sub>/EPSC<sub>+60 mV</sub>, for AMPAR-mediated EPSCs (n = 10 for tTA:CHMP2B<sup>WT</sup> mice and n = 12 for tTA:CHMP2B<sup>introns5</sup> mice; **P < 0.01 by two-sided t-test). (d) Decreased sensitivity of evoked EPSCs to Naspm (200 µM) inhibition in pyramidal neurons expressing CHMP2B<sup>introns5</sup>. Representative EPSC traces at –60 mV before (control) and after Naspm application are shown on the left (n = 8 mice for tTA:CHMP2B<sup>WT</sup> and n = 9 for tTA:CHMP2B<sup>introns5</sup>; *P < 0.05 by two-sided t-test). Mice used in b–d were 15–16 weeks of age. Error bars represent mean ± s.e.m.

(d) suggest that Gria2-lacking AMPARs exist in mPFC neurons of adult tTA:CHMP2B<sup>WT</sup> mice and that these native Ca<sup>2+</sup>-permeable receptors are replaced by Gria2-containing, Ca<sup>2+</sup>-impermeable AMPARs in tTA:CHMP2B<sup>introns5</sup> mice.

miR-124 level is decreased in the cortex of tTA:CHMP2B<sup>introns5</sup> mice

We next examined mechanisms for the upregulation of AMPARs in tTA:CHMP2B<sup>introns5</sup> mice. We focused on miRNAs, as they control gene expression at the post-transcriptional level<sup>33</sup> and are essential regulators of synaptic function<sup>34</sup>. Furthermore, each miRNA can regulate multiple mRNA targets that may contribute to complex processes, such as sociability. We examined the expression levels of several brain-enriched miRNAs in the cortex in tTA:CHMP2B<sup>introns5</sup> mice at 1 month and at 1 year of age. At both time points, many miRNAs were expressed in the cortex at similar levels in tTA:CHMP2B<sup>introns5</sup> mice and tTA:CHMP2B<sup>WT</sup> mice (Supplementary Fig. 6b). However, at 1 year, some miRNAs were downregulated in tTA:CHMP2B<sup>introns5</sup> mice, especially miR-124, which is one of the most abundant miRNAs in the brain (Supplementary Fig. 6a,b). miR-124 downregulation was specific to expression of the FTD-causing CHMP2B<sup>introns5</sup>, as these miRNAs remained unchanged in the cerebellum, where the transgene was not expressed (Supplementary Fig. 6a). miR-124 was also downregulated to a lesser extent in the cortex of two other independent transgenic lines expressing lower levels of CHMP2B<sup>introns5</sup> (Supplementary Fig. 6c). In contrast, variable levels of CHMP2B<sup>WT</sup> expression in the other mouse lines did not affect miR-124 abundance (Supplementary Fig. 6c). Moreover, when CHMP2B<sup>introns5</sup> expression was turned off in tTA:CHMP2B<sup>introns5</sup> mice fed a diet containing doxycycline (Supplementary Fig. 1d), their cortical miR-124 expression was identical to that in tTA:CHMP2B<sup>WT</sup> mice fed a regular diet or a diet containing doxycycline (Supplementary Fig. 7a). These results support a specific association between CHMP2B<sup>introns5</sup> expression and miR-124 reduction.

Computer algorithms (Targetscan, PicTar, miRanda) indicate that three AMPAR subunits, Gria2, Gria3 and Gria4, are potential targets
Figure 4 miR-124 regulates AMPAR subunits in HEK293 cells, and its expression is reduced in the cortex of tTA:CHMP2BIntron5 mice. (a) Alignment of potential binding sites for miR-124 in the 3’ UTRs of Gria2, Gria3 and Gria4 mRNAs. (b) Direct interaction between 3’ UTRs of Gria2, Gria3 and Gria4 mRNAs and miR-124 (n = 4 assays *P < 0.05, **P < 0.001 by two-sided t-test). (c) The effects of miR-124 on the expression of luciferase-containing subunit 3’ UTRs in which we mutated the miR-124 binding site (n = 4 assays; *P < 0.05, **P < 0.001 by two-sided t-test). (d) Northern blot analysis of miR-9 and miR-124 expression levels in the cortex of tTA:CHMP2BWT and tTA:CHMP2BIntron5 mice at different ages. (e) Quantification of the northern blots in d (n = 3 mice per group; *P < 0.05, **P < 0.01 by two-sided t-test). (f) Fluorescence in situ hybridization of miR-124 in brain sections from tTA:CHMP2BWT and two tTA:CHMP2BIntron5 mice at 8 months of age. (g) Double-fluorescence in situ hybridization staining of miR-124 (red) in CHMP2BIntron5-expressing cells (green) in the cortex at 8 months of age. Scale bars, 150 μm in f and g. (h) Higher magnifications of the boxed areas in g. Scale bars, 20 μm. Error bars represent mean ± s.e.m. in b and c and mean ± s.d. in e.

Figure 5 miR-124 and AMPARs are dysregulated in a subset of subjects with bvFTD. To confirm that our findings in mice are relevant to human disease, we next examined miR-124 and AMPAR expression in subjects with FTD. As FTD is a heterogeneous disorder, we focused on the frontal cortex in

of miR-124 (Fig. 4a). Thus, downregulation of miR-124 might contribute to the upregulation of these AMPARs in tTA:CHMP2BIntron5 mice. Indeed, in human HEK293 cells, transfected miR-124 suppressed the expression of the reporter gene with the 3’ UTRs of Gria2, Gria3 and Gria4, but not Gria1 (Fig. 4b). Their expression was not suppressed when we mutated the miR-124 binding site in their 3’ UTRs (Fig. 4c). Similarly, in the cortex of tTA:CHMP2BIntron5 mice, miR-9 levels remained unchanged, whereas miR-124 was downregulated in an age-dependent manner (Fig. 4d,e), which is associated with the age-dependent increase in Gria2, Gria3 and Gria4 levels (Fig. 2) and the social behavioral deficits (Fig. 1).

Consistent with previous studies, miR-124 was widely expressed in all layers of the cortex of 2-month-old mice (Supplementary

Figure 5). In contrast, miR-124 expression was markedly decreased in the cortex of tTA:CHMP2BIntron5 mice at 8 months of age, and this decrease seemed to be most pronounced in layers II and III (Fig. 4f). Most neurons expressing miR-124 at low levels in the superficial layers were also strongly positive for CHMP2BIntron5 (Fig. 4g,h). Thus, expression of CHMP2BIntron5 triggers a progressive loss of miR-124 that is greater in the superficial layers.

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Figure 5 miR-124 and AMPAR expression are altered in the frontal cortex of subjects with bvFTD and in cortical neurons derived from iPSC lines from three subjects with bvFTD. (a) Quantification of the abundance of miR-124, miR-9 and AMPAR transcripts by RT-PCR in the frontal cortex of subjects with bvFTD and controls. We normalized the content of AMPAR mRNAs against the geometric mean of four different neuronal-specific reference genes (encoding MAP2, enolase 2, GAP43 and PSD95; n = 5 subjects per group, *P < 0.05 by Mann-Whitney test). (b) Quantification of miR-124, miR-9 and AMPAR transcripts in 2-week-old human neurons derived from three iPSC lines from two control individuals and four iPSC lines from three subjects with bvFTD (***P < 0.1 by two-sided t test). (c) Quantification of miR-124, miR-9 and AMPAR transcripts in 8-week-old human neurons derived from the same lines as in b (*P < 0.05, **P < 0.01 by two-sided t test). Error bars represent mean ± s.e.m.
miR-124 expression and Gria2 knockdown in the mPFC partially rescue social deficits in tTA:CHMP2B<sup>introns</sup> mice. (a) Double staining for GFP and miR-124 in tTA:CHMP2B<sup>introns</sup> mice (8 months) that received an intracranial injection of AAV-GFP (left) or AAV-GFP-miR-124 (right). To facilitate assessment of differences in miR-124 levels, we took pictures at the periphery of the injection site so infected and noninfected cells are present in the same field. (b) Quantitative RT-PCR analysis of Gria transcripts in the mPFC of tTA:CHMP2B<sup>introns</sup> mice 2 months after AAV injection (n = 5 AAV-GFP–infected mice and n = 4 AAV-GFP–miR-124–infected mice; \( P < 0.05 \) by two-sided t-test). (c) Quantification of the number of Gria2-positive puncta in tTA:CHMP2B<sup>introns</sup> mice 2 months after intracranial injection of AAV-GFP or AAV-GFP-miR-124 (n = 3 mice per genotype; \( P < 0.05 \) by two-sided t-test). (d) Quantification of miR-124–dependent rescue of social deficits in tTA:CHMP2B<sup>introns</sup> mice 1 and 2 months after bilateral injection of AAV vectors in mPFC of 7-month-old mice (n = 10 per AAV vector; \( * P < 0.05 \), ** P < 0.01, *** P < 0.001 by two-sided repeated-measures two-way analysis of variance (ANOVA) corrected for multiple comparison, Bonferroni test). (e) Quantification of the rescue effects of in vivo downregulation of Gria2 expression after bilateral injection on social deficits in tTA:CHMP2B<sup>introns</sup> mice (n = 7 per group; \( * P < 0.05 \), by two-sided repeated-measures two-way ANOVA followed by Fisher’s least significant difference test). Scale bars in a and c, 20 µm. Error bars represent mean ± s.e.m.

the subset of subjects with bvFTD, whose clinical presentation is closest to the phenotypes observed in our mice. In samples from two brain banks, we found a decrease in miR-124 expression, but not miR-9, and a concomitant upregulation of Gria2 and Gria4 in the frontal cortex of five bvFTD cases compared with five age-matched controls (Fig. 5a).

We then studied miR-124 and AMPARs in previously established iPSC lines derived from three subjects with bvFTD\(^{36,37}\). The expression of miR-124 and AMPARs in 2-week-old neurons derived from four iPSC lines from three subjects with bvFTD were the same as those in controls (Fig. 5b). However, in 8-week-old neurons from subjects with bvFTD, miR-124 levels were substantially reduced and some AMPAR subunit mRNAs were upregulated (Fig. 5c). This change is not due to the culture conditions, as the percentages of different neuronal subtypes remained the same as those in cultures derived from controls (Supplementary Fig. 8a). As controls, the miR-9 level was not decreased (Fig. 5c), and we did not detect a change in NMDA or kainate receptor transcripts or neuronal subtype-specific markers (Supplementary Fig. 8a,b).

To further confirm this finding, we surveyed published genome-wide studies of miRNAs in frontal cortex samples from subjects with FTD\(^{38}\). Reanalysis of the data showed a decrease in miR-124 expression in the frontal cortex of subjects with progranulin deficiency (FTD-GRN) (Supplementary Fig. 9a). Another study of a small number of subjects also showed a trend toward a decrease\(^{39}\). Similarly, reanalysis of published transcriptome profiling data\(^{40}\) revealed an increase in some AMPAR subunits, but not NMDA receptor subunits, in the frontal cortex but not the cerebellum of six subjects with FTD-GRN (Supplementary Fig. 9b,c). These data are relevant to our study, as most subjects with FTD-GRN have bvFTD\(^{41}\). These findings suggest that the dysregulation of miR-124 and AMPARs discovered in our mouse model also occurs in the frontal cortex in a subset of subjects with bvFTD.

Partial rescue of social deficits in tTA:CHMP2B<sup>introns</sup> mice

To determine whether downregulation of miR-124 contributes to FTD-associated social deficits in tTA:CHMP2B<sup>introns</sup> mice, we engineered an adeno-associated vector (AAV) to express miR-124 in vitro (Supplementary Fig. 10a) and in vivo (Fig. 6a). The mPFC controls most social behaviors in mice\(^{42}\), and we detected synaptic defects in that region in tTA:CHMP2B<sup>introns</sup> mice (Fig. 2). Therefore, we injected an AAV expressing miR-124 or a control AAV-GFP vector into the mPFC of 7-month-old tTA:CHMP2B<sup>introns</sup> mice.

AAV-mediated miR-124 expression reduced mRNA levels of Gria2 and Gria4 in the mPFC of tTA:CHMP2B<sup>introns</sup> mice (Fig. 6b). The decrease was not due to the surgical procedure, as mRNA levels did not differ in the occipital cortex of the same mice (Supplementary Fig. 10b). Although we could not obtain enough material from these experiments for PSD fractionation, we did detect a decrease in the number and the intensity of Gria2 synaptic puncta in brain sections (Fig. 6c). Notably, AAV-GFP injection did not alter sociability of tTA:CHMP2B<sup>introns</sup> mice at either 1 or 2 months (Fig. 6d). In contrast, 1 month after injection of AAV-GFP-miR-124, the mice showed a substantial increase in sociability in trial 5. These mice showed a further improvement at 2 months that was significantly better than the preinjection level (\( P < 0.001 \)), suggesting that the deficits were reversed (Fig. 6d). Finally, the time spent with stranger 2 did not differ between AAV-GFP–injected mice and those injected with AAV-GFP-miR-124 (Supplementary Fig. 10c), arguing for a role of miR-124 in specifically regulating sociability but not novelty recognition.

To further demonstrate that a decrease in Ca\(^{2+}\)-impermeable AMPARs contributes to the social deficits in tTA:CHMP2B<sup>introns</sup> mice, we constructed lentiviral vectors encoding scrambled shRNA or shRNA directed against mouse Gria2 (Supplementary Fig. 11a). After lentivirus injection into the mPFC of 7-month-old tTA:CHMP2B<sup>introns</sup> mice, downregulation of Gria2 expression partially rescued the social deficits (Fig. 6e). Of note, recognition of the new mouse (stranger 2) during this trial was not affected (Supplementary Fig. 11b), suggesting that Gria2 levels are critical for sociability but not all aspects of social behavior. These results support the notion that alterations in AMPAR composition in the mPFC contribute to social deficits in tTA:CHMP2B<sup>introns</sup> mice.
DISCUSSION
In a new mouse model of FTD harboring the CHMP2B<sub>Intron5</sub> mutation, we found that downregulation of miR-124 causes a dysregulation in AMPAR composition and a selective impairment in sociability. AAV-mediated ectopic expression of miR-124 in the mPFC reduced the levels of AMPAR subunits and partially rescued the social behavioral deficits. Knockdown of Gria2 also alleviated the social deficits in tTA:CHMP2B<sup>Intron5</sup> mice, suggesting a key role for Ca<sup>2+</sup>-impermeable AMPARs. We also found similar alterations in miR-124 and AMPAR levels in human cortical neurons derived from iPSCs of subjects with bvFTD and in the frontal cortex of the subset of subjects with bvFTD and age-matched controls that we were able to obtain for this study. Thus, the mechanisms uncovered in our mouse model of FTD may have general implications for understanding behavioral abnormalities in humans with the disease.

Marked changes in social behavior such as social withdrawal and obsessive-compulsive behaviors are common in people with bvFTD<sup>4,43</sup>. Social deficits similar to those in our tTA:CHMP2B<sup>Intron5</sup> mice were also reported in progranulin-haploinsufficient mice<sup>44</sup>, another mouse model of FTD. In addition, several FTD histopathological hallmarks were present in our mice and in published mouse models<sup>14,44</sup>, suggesting that the anatomical and behavioral abnormalities in people with FTD can be reproduced in mice. Despite widespread forebrain expression of mutant CHMP2B<sup>Intron5</sup> protein in our mouse model of FTD, only sociability was impaired at an early age, indicating that specific circuits are particularly vulnerable. Consistent with this notion, neurons in some regions of the human prefrontal cortex are most susceptible in FTD<sup>45</sup>. Moreover, ectopic miR-124 expression or Gria2 knockdown in this cortical area partially rescued the social behavioral deficits.

Our findings suggest that AMPAR composition is regulated by miR-124 and altered in tTA:CHMP2B<sup>Intron5</sup> mice. Furthermore, we also found miR-124 downregulation and a concomitant increase in AMPAR levels in the frontal cortex and iPSC-derived cortical neurons from subjects with bvFTD. AMPARs have been implicated in social behaviors. For instance, Gria3-knockout mice show increased aggression<sup>46</sup>. Modulating AMPAR activity in the mPFC can result in changes in the social hierarchy<sup>42</sup>. Our electrophysiological analysis suggested that FTD-related social behaviors are linked to an increase in Ca<sup>2+</sup>-impermeable AMPARs at excitatory synapses of PFC pyramidal neurons. These abnormally inserted receptors may interfere with synaptic Ca<sup>2+</sup> signaling or impair synaptic efficacy and plasticity<sup>47</sup>, leading to structural alterations in dendritic spines and behavioral deficits in social interaction. We speculate these molecular changes may also contribute to the early behavioral abnormalities in subjects with FTD.

miRNAs are deregulated in many neurodegenerative disorders<sup>18</sup>. The exact molecular link between CHMP2B<sup>Intron5</sup> mutation and miR-124 reduction remains to be determined. However, previous works implicated a role for ESCRTs in the miRNA pathway<sup>48,49</sup>, raising the possibility that CHMP2B<sup>Intron5</sup> mutation may affect miR-124 stability. Only 6 of 91 neurotransmitter receptor subunit miRNAs are predicted targets of miR-124, including three of four known AMPAR subunits (Supplementary Table 1). Thus, miR-124 may have a unique role in fine-tuning glutamate neurotransmission by controlling the expression and composition of AMPARs, consistent with the inverse correlation between miR-124 and Gria2 levels reported here and by others<sup>50</sup>. Moreover, our finding that ectopic expression of miR-124 or Gria2 knockdown partially rescues behavioral deficits in early stages of FTD may suggest a potentially beneficial therapeutic approach before neuronal cell loss becomes apparent and irreversible.

METHODS
Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS
We thank S. Ordway and Gao lab members for comments, Y. Li and A. Philbrook for help with some experiments, A. Tapper for sharing behavioral test equipment, the Digital Light Microscopy Core at the University of Massachusetts Medical School (UMMS) for assistance with Golgi staining, the UMMS Viral Vector core for help with AAV vectors, and the University of California–San Francisco Neurodegenerative Disease Brain Bank for some human brain tissues. We also thank R. Remakers for genotyping some human samples in a previous work<sup>31</sup> that we used in the current study and A. Chen-Plotkin for sharing published array data<sup>46,47</sup>. This work was supported by a UMMS startup fund (F.-B.G.), The Consortium for Frontotemporal Dementia Research (W.W.S.) and the US National Institutes of Health (NS057553, NS066586 and NS079725 to F.-B.G.; DA032283 to W.-D.Y.; MH086509 to S.A.; AG023501 and AG19724 to W.W.S.; and AG016574 to D.W.D. and L.P.).

AUTHOR CONTRIBUTIONS
E.G., K.L., S.A. and H.Z. performed most experiments. H.R. and W.-D.Y. carried out the electrophysiology analysis and wrote the relevant sections. J.M.V., D.S. and A. Kargi performed most experiments. E.G. and F.-B.G. analyzed the data and wrote the manuscript. F.-B.G. conceived and supervised the project.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Mice. All procedures involving mice were approved by the University of Massachusetts Medical School Institutional Animal Care and Use Committee. We cloned CHMP2BWT and CHMP2Bflhomo into the NotI and Sall sites of the pTRE-Tight-Bi-ZsGreen1 mammalian expression vector (Clontech). We linearized pTRE-CHMP2BWT vector with BsrBI and purified DNA for microinjection. As no adequate restriction enzymes were present on the pTRE-CHMP2Bflhomo plasmid, we amplified the cassette by PCR (TREsalIIFwd, 5′-CCGCGGCCACCTGACGTCGGAGTGAA-3; TRESacIIRV, 5′-GCTCGTTTAGTGAACCGTCAG-3′); and subcloned into pCR2.1 TOPO (Invitrogen). We linearized this construct with SacI and Apal enzymes and purified DNA for microinjection. For both CHMP2BWT and CHMP2Bflhomo constructs, ZsGreen coding sequence was lost after linearization. Injected eggs were transferred to female recipients, and F1 litters analyzed for transgene insertion by PCR with two specific sets of primers (forward primer, 5′-GCTCATTAGTGAAACCGTCAG-3′ and reverse primer, 5′-AGCTCGGGGAGTGGAGCAGG-3′; or forward primer, 5′-ATGGGCTGCTCCTTCCTAAAGAAATG-3′ and reverse primer, 5′-GGGCCGCAGTCATGGGGAGGAGAAGCTC-3′).

We maintained germline-transmitting founders (three for CHMP2BWT and three for CHMP2Bflhomo) as hemizygous lines by backcrossing to wild-type C57BL/6 mice. To drive transgene expression in the forebrain, we crossed CHMP2BWT and CHMP2Bflhomo transgenic mice with Camk2a-tTA mice (Jackson Laboratory, 007004). We used both male and female double-transgenic offspring for all experiments.

Behavioral tests. We conducted all experiments between 9 a.m. and 5 p.m. We housed mice under specific-pathogen–free conditions in an animal facility with a regular 12-h light and dark cycle (light on at 7:00 a.m.) under constant conditions (21 ± 1 °C; 60% humidity). Food and water were supplied ad libitum.

We conducted all behavioral studies using both male and female mice and analyzed data blinded to genotypes. For all tasks, each mouse was selected randomly and then tested on the same day. We tested the first cohort of mice for social interactions (21 ± 1 °C; 60% humidity). Food and water were supplied ad libitum. For trials 2–5, the mouse was placed in the middle chamber and left to explore the area containing the empty wire cages for 5 min (habituation).

For trials 2–4, the mouse was placed in the middle chamber, but an unfamiliar mouse (stranger 1) was placed into a wire cage in one of the side chambers (the wire cage in the other side chamber remains empty; sociability, social learning acquisition).

For trial 5, a novel stranger mouse (stranger 2) was placed in the previously empty wire cage and again the test mouse is left to explore for 5 min (sociability, social recognition memory).

We recorded and analyzed all the trials post hoc. We measured the time spent in each chamber and the time spent in close interaction with stranger mice for each trial. The following parameters were considered: (i) social recognition, defined as the ability to identify a conspecific (stranger 1 versus the empty cage) in trial 2 (spending more time in the side-chamber containing stranger 1); (ii) social novelty, defined as the ability to discriminate between a novel mouse (stranger 2) and a familiar mouse (stranger 1) in trial 5 (spending more time interacting with stranger 2); and (iii) sociability, which reflects the motivation of the test mouse to spontaneously interact with target mice in trials 2–5.

Social interaction in home cage (resident-intruder test). We assessed social interaction in the home cage by standard protocols52. Briefly, we housed individual test mice for 1 week before the test. Then, they were habituated for 30 min to the test room. In this task, a mouse in his home cage is allowed to freely roam in the absence of the cage top for 1 min. A novel juvenile (4-week-old) male intruder is then placed in the opposite corner as the resident subject and allowed to roam freely for 5 min. The task is recorded, and total physical interaction between the two mice is quantified visually; social interaction is scored as the time during which the resident mouse actively explores the intruder. We did not observe fighting, biting or attacking in this task.

Olfactory testing. After habituation to empty cages with no bedding, we tested mice for odor discrimination or sensitivity, as described53. In the discrimination task, mice are challenged with a filter paper embedded with an attractive scent (vanilla or cinnamon), an aversive scent (2-methyl butyrate) or a neutral scent (water). For the sensitivity task, dilutions of the same scent (vanilla) are used. The mice are videotaped, and the time spent sniffing the filter paper during a 3-min period is calculated post hoc.

Novel object recognition task. We performed the object recognition task as described54 following the no-habituation paradigm. The testing set-up was the same as for open field experiment. We placed mice in the testing room for at least 30 min for habituation. Then, mice were placed into the setup containing two identical plastic sample objects (cubes) placed 5 cm from one of the walls and −12 cm apart. The mice were allowed to explore the environment for 10 min, during which we recorded their movements. After this familiarization session, we cleaned the objects and open-field with 70% ethanol to eliminate any olfactory cues and returned the mice to their home cage for 2 min. We then placed the mice again in the open field, but this time a novel object of similar size and complexity (cylinder instead of a cube) replaced one of the objects present during the familiarization session. The mice were allowed to explore the environment for 10 min (test session), after which we returned them to their cages. For both sessions, we placed mice into the open field, with heads positioned opposite the objects. Object exploration, defined as the duration of time in which the head of the mouse faced less than 2 cm from the object, was measured during the first 5 min of each session. We calculated the recognition index as the percentage of time spent exploring the novel object versus the total time spent exploring the objects.

Drug injections. We diluted NBQX and AP5 (both from Tocris) in 0.9% saline solution and intraperitoneally injected them 10 min before the start of the test. Doses were 50 mg kg−1 for NBQX and 10 mg kg−1 for AP5. We injected at least 10 mice per genotype with each drug, but each mouse received only a single injection. We quantified the total interaction time with both stranger 1 and stranger 2 (trial 5). To reverse transgene expression, we fed mice with a diet containing doxycycline (200 mg kg−1; Bioserv). To ensure early repression, we maintained breeder mice on a doxycycline-containing diet. After weaning and genotyping, we fed mice with this diet until 8 months of age.
Golgi staining. We processed mouse brains for morphological assessment with the FD Rapid Golgi Stain Kit (FD NeuroTechnologies) according to the manufacturer’s protocol. We submerged mouse brains in impregnation solution for 14 d and then flash froze them in isopentane at –70 °C. Next, we cut 200-μm-thick cryosections with a cryostat (Leica), mounted on gelatin-coated slides, rinsed in dH2O, and incubated in developing solution for 10 min. Sections were dehydrated with increasing concentrations of ethanol, cleared with xylene and fixed coverslips with Permount mounting medium.

We examined the slides with a Nikon inverted microscopy at the imaging core facility (University of Massachusetts Medical School). We took image stacks of 40- to 120-μm segments of apical dendrites on pyramidal neurons of layer II-III of the mPFC or in the CA3 region with a 100x oil-immersion lens (N.A. 1.45). For spine analysis, we quantified 15–25 dendritic segments (n = 3 mice per genotype). Total dendritic length was 1,897 μm for tTA:CHMP2B^diffract mice and 1,702 μm for tTA:CHMP2B^Wildtype mice. All image stacks were first deconvolved (with the iterative three-dimensional [3D] deconvolution plug-in for ImageJ) using a point-spread function as a reference (point-spread function plug-in for ImageJ). We imported images into Neuron Studio for 3D analysis of spine density and spine size55 and measured spines in 3D from the z-stacks. We calculated density by dividing the total number of spines per 100 μm of dendrite. A spine was labeled thin if its head was < 0.6 μm in diameter and had a maximum length of at least twice the head diameter. We classified a spine as dendrite. A spine was labeled thin if its head was < 0.6 μm in diameter and had a maximum length of at least twice the head diameter. We classified a spine as ‘other’. AxoSom neurons were identified by ThinSOM (Molecular Devices) under infrared differential interference contrast microscopy. We identified pyramidal neurons by their morphology and delivered presynaptic stimuli (0.033 Hz, 200 μs) with a concentric bipolar electrode (FHC) placed at layers II and III of the mPFC cortex. For recordings of evoked EPSCs and mEPSCs, neurons were voltage clamped at –60 mV unless indicated otherwise.

We recorded evoked EPSCs every 30 s. The superfusion medium contained picrotoxin (100 μM) to block GABA_B receptor–mediated synaptic responses. We added tetrodotoxin (1 μM, Sigma) and AP5 (2-amino-5-phosphonopentanoic acid, 50 μM, Abcam Biochemicals) during recordings of mEPSCs. We filled recording pipettes with solution containing 142 mM Cs-gluconate, 8 mM NaCl, 10 mM HEPES, 0.4 mM EGTA, 2.5 mM QX-314 [N-(2,6-dimethylphenylcarba moyethyl)triethylammonium bромide], 2 mM Mg-ATP and 0.25 mM GTP-Tris, pH 7.25. For all recordings, temperature was maintained at 32 °C with a temperature controller (Warner Instruments). For the Naspin (1-naphthylacetyl spermine trihydrochloride) sensitivity assay, after stable EPSCs were obtained for 10 min as baseline, we delivered the drug to the bath for 10–15 min with a gravity-driven perfusion system (Harvard Apparatus). We acquired data with a Digitida 1440A and pClamp software (version 10.2; Molecular Devices). Signals were filtered at 1 kHz, digitized at 10–20 kHz, and analyzed with Clampfit. We analyzed mEPSCs with Mini Analysis 6 (Synaptosoft).

**In situ hybridization and immunofluorescence.** Mouse brains were fixed in 4% paraformaldehyde. Brains were extracted, postfixed in the same fixative overnight at 4 °C, transferred to a 30% sucrose solution for cryoprotection, frozen and stored at –80 °C. We sectioned brain samples with a standard cryostat.

We used standard protocols for *in situ* hybridization and immunofluorescence61. For detection of miR-124, we purchased digoxigenin-labeled LNA probes from Exiqon. For transgene expression, a biotin- or digoxigenin-labeled RNA probe was synthesized with gene-specific PCR primers (forward, 5′-AA ATATACGCTGACAATGGGATTCTTGTAGA AATTTGATAAGA AT-3′ and reverse, 5′-GCGATGTGAATAAAATGCTTATTGT-3′) and cDNA templates from transgenic mouse brains. Probes were hybridized overnight at 55 °C, and the slides were incubated with horseradish peroxidase–conjugated anti-digoxigenin antibody (Roche, 11633716001; 1:500) and streptavidin linked to the peroxidase (Roche). FITC or Cy3 TSA Plus kits (PerkinElmer) were used for final detection.

For immunofluorescence, we diluted primary antibodies in PBS containing 10% donkey serum (Sigma), 3% bovine albumin (Sigma) and 0.3% Triton X-100, and incubated overnight at 4 °C. Corresponding donkey anti-rabbit or anti-mouse Alexa 488 or 555 (Invitrogen) was used for secondary detection. The primary antibodies were anti-NeuN (Millipore, MAB377 1:1,000), PSD95 (NeuroMab, 52-028; 1:5,000), GFAP (Dako, Z0334; 1:2,000), Gria2 (Abcam, ab52932; 1:2,000), Gria4 (Abcam, ab77407; 1:1,000), ubiquitin (Enzo Life Sciences, BML-PW8810; 1:1,000) and anti-GFP (Life Technologies, A1128; 1:3,000).

**Human brain samples.** We obtained five FTD samples (from 3 men and 2 women, mean age at death 68 ± 6 years) and five age-matched normal control samples (from 2 men and 3 women, mean age at death 79 ± 5 years) from the Mayo Clinic Jacksonville and the University of California—San Francisco Neurodegenerative Disease Brain Bank. Informed consent was obtained and approved by the Mayo Clinic Institutional Review Board and UCSC Institutional Review Board for postmortem studies. Sex and postmortem interval to autopsy were not different between cases and controls. FTD cases had sporadic bvFTD due to FTLD-TDP type B. Control brains had no evidence of neurological disease either clinically or neuropathologically. We used frozen tissue (100–150 mg) from gray matter of the middle frontal gyrus for RNA extraction and subsequent RT-PCR analysis.

**Image acquisition and processing.** For immunofluorescence and *in situ* hybridization, we acquired images with an Eclipse 80i confocal microscope and software (Nikon). We quantified the number of NeuN neurons from fluorescence images of four tTA:CHMP2B^Wildtype mice and four tTA:CHMP2B^diffract mice at 8 months of age; observers were blinded to genotype. For NeuN counting, we counted three to four sections of the mPFC with the ImageJ cell-counter plug-in.
For Gria puncta quantification, we used confocal z-stacks (159 × 159 × 10 µm). We analyzed three to four sections (n = 3 mice per genotype) or two sections (n = 6 mice per AAV vector). Following z-projection images, we quantified puncta using Particle Analyzer plug-in. We carried out GFAP coverage quantification as described44.

**Northern blot.** We extracted RNA from specific brain regions with Trizol (Invitrogen) according to the manufacturer's instructions. We loaded 5–10 µg total RNA into a well-containing 12.5% acrylamide gel (SequraGel-UreaGel, National Diagnostics) and electrophoresed in standard TBE (1×) buffer. We then transferred RNA to a positively charged nylon membrane (Amersham) and hybridized overnight with digoxigenin-labeled LNA probes (Exiqon). We used anti-digoxigenin antibody linked to alkaline phosphatase (Roche, 11 093 274 910; 1:10,000) and CDP-star reagent (Roche) for detection.

**Western blot.** We obtained cortical extracts62 and cortical PSD fractions21 as described. PSD or cortical samples (20 µg) were subjected to SDS-PAGE and probed with specific primary antibodies. After incubation with the appropriate secondary antibody linked to horseradish peroxidase (Jackson ImmunoResearch), SuperSignal West Pico Chemoluminescent Substrate (Thermo) was used for detection. Antibodies used in these experiments included rabbit antibodies against CHMP2B (Abcam, ab 33174; 1:2,000), Gria1 (Abcam, ab109450; 1:5,000), Gria2 (Abcam, ab52932; 1:2,000), Gria3 (Abcam, ab78366; 1:1,000), Gria4 (Abcam, ab77407; 1:1,000), Gria1 (Upstate Biotechnology, 05–4220; 1:1,000), Gria2x (Upstate Biotechnology, 07–632; 1:2,000), Gria2b (Upstate Biotechnology, 0–600; 1:1,000), p62 (Cell Signaling Technology, 5114; 1:2,000) and β-actin (Sigma, A-5316; 1:10,000) as a loading control.

**Luciferase assays.** We cultured HEK cells in DMEM containing 10% FCS and split them the day before transfection to achieve ~50% confluence at the time of transfection. We amplified 3′ UTRs from Gria1, Gria2, Gria3 and Gria4 transcripts by PCR from mouse brain cDNA and cloned downstream from the Renilla luciferase coding sequence of the psiCheck2 vector (Promega). We obtained 3′ UTRs containing mutated miR-124 binding sites (wild-type binding site GUGCCUA; mutated binding site GUGCAAA) by PCR with specific primers including the mutated nucleotide sequence. We transfected cells with a plasmid containing an AMPAR subunit 3′ UTR together with a vector driving the expression of miR-124, miR-9 or an empty vector (pSuper, Oligoengine). For transfection, we used FuGene (Roche) according to the manufacturer’s instructions. After 24 h, we lysed the cells and measured luciferase activity with the Dual Luciferase Reporter Assay (Promega), and normalized results to firefly luciferase activity.

**Quantitative RT-PCR.** We extracted RNA with the miRNeasy kit (Qiagen) according to the manufacturer's instructions and digested with DNase for 30 min on-column. We performed first-strand synthesis with 500 ng of total RNA, random hexamers and TaqMan reverse transcription reagents (Applied Biosystems). Reactions without reverse transcription were always included.

For quantitative PCR, we designed and tested specific primers (Supplementary Table 2) at different cDNA dilutions, and calculated their efficiency. We only used primers showing 95–105% efficiency for further analysis. We performed real-time quantitative PCR with a StepOnePlus system (Applied Biosystems). We carried out reactions (in triplicate) with SYBR Green PCR Master Mix (Applied Biosystems). Each SYBR Green reaction (total volume, 20 µl) contained 1 µl of cDNA as template and each primer at 0.25 µM. Controls without template DNA (reverse transcription minus reaction) were always negative. We incubated the reactions at 95 °C for 10 min to activate the HotStar Taq polymerase followed by 40 cycles at 95 °C for 15 s (denaturation) and at 60 °C for 1 min (annealing and extension). We used β-actin or GAPDH as internal control. Because SYBR Green indiscriminately binds to double-stranded DNA, other products in the PCR such as primer dimers may be detected along with the target gene. To verify that the SYBR Green dye detected only one PCR product, we subjected the samples to the heat dissociation protocol after the final cycle of PCR to check for the presence of only one peak.

Given the limited amount of human samples available, we used TaqMan miRNAs assays to quantify miRNA abundance in brain tissues and iPSC-derived neurons (Applied Biosystems, assay 001182 for miR-124 and assay 000469 for miR-9). We extracted RNA as described for mouse samples. Then, we used specific primers for each miRNA for both reverse transcription and subsequent TaqMan quantitative PCR as specified by the manufacturer. We used U6 as internal reference gene for those experiments.

**Mouse cortical cultures and iPSC-derived neuronal cultures.** To test the efficiency of Gria2 shRNAs, we prepared neuronal cortical cultures from newborn mice as described10. At 2 d after plating, 250 µl of lentiviral supernatant was added to the medium. We allowed cells to grow for 3 d, lysed cells for protein extraction and assessed Gria2 levels by immunoblottting.

For iPSC-derived neuronal cultures, we used four different lines from three subjects with bvFTD. Two of these lines carry the progranulin S116X mutation36; the other two lines were derived from two subjects with bvFTD caused by C9ORF72 repeat expansion23. We used two iPSC lines from one healthy subject36 as controls and performed neuronal differentiation as described26,37.

**Statistical analyses.** We performed all statistical analyses with Prism GraphPad 6.0. We compared tTA:CHMP2BWT and tTA:CHMP2Bmut mice for each behavioral task. We used the Student’s t-test to detect genotype differences in electrophysiological experiments, qPCR, NeuN counting, biochemical analysis and some behavioral tests. For shRNA efficiency, we used one-way ANOVA. We also used repeated-measure two-way ANOVA to detect differences in the AAV injections. We used Bonferroni’s or Fisher’s least significant difference test after ANOVA results and analyzed the results of antagonist injections with the Kruskal-Wallis test corrected for multiple comparisons. We used Mann-Whitney test for comparisons of brain human samples.

**Accession numbers.** Microarray and deep sequencing data39,40 are available at the NCBI Gene Expression Omnibus database under series accession numbers GDS3459 and GPL10999.

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