Foxg1 regulates translation of neocortical neuronal genes, including the main NMDA receptor subunit gene, Grin1

Osvaldo Artimagnella1,3†, Elena Sabina Maftei1†, Mauro Esposito2,4, Remo Sanges2 and Antonello Mallamaci1*

Abstract

Background  Mainly known as a transcription factor patterning the rostral brain and governing its histogenesis, FOXG1 has been also detected outside the nucleus; however, biological meaning of that has been only partially clarified.

Results  Prompted by FOXG1 expression in cytoplasm of pallial neurons, we investigated its implication in translational control. We documented the impact of FOXG1 on ribosomal recruitment of Grin1-mRNA, encoding for the main subunit of NMDA receptor. Next, we showed that FOXG1 increases GRIN1 protein level by enhancing the translation of its mRNA, while not increasing its stability. Molecular mechanisms underlying this activity included FOXG1 interaction with EIF4E and, possibly, Grin1-mRNA. Besides, we found that, within murine neocortical cultures, de novo synthesis of GRIN1 undergoes a prominent and reversible, homeostatic regulation and FOXG1 is instrumental to that. Finally, by integrated analysis of multiple omic data, we inferred that FOXG1 is implicated in translational control of hundreds of neuronal genes, modulating ribosome engagement and progression. In a few selected cases, we experimentally verified such inference.

Conclusions  These findings point to FOXG1 as a key effector, potentially crucial to multi-scale temporal tuning of neocortical pyramid activity, an issue with profound physiological and neuropathological implications.

Keywords  Foxg1, Translation, Grin1, NMDAR, Neuronal activity

Background  FOXG1 is an evolutionarily ancient transcription factor mastering a number of developmental processes that take place in the rostral brain. These include early activation of pan-telencephalic [1], subpallial [2], and paleo-neopallial [3] programs, promotion of neural precursors self-renewal [4], balance between neuronogenesis and gliogenesis [5–8], and laminar specification of neocortical neurons [9–13]. Later, it promotes morphological maturation of glutamatergic [5, 14, 15] and gabaergic [16] telencephalic neurons. Moreover, FOXG1 sustains activity and excitability of these neurons [7, 16, 17], exerting a complex impact on the transcription of specific gene-sets [17, 18]. Besides, its expression is in turn stimulated by neuronal activity [17, 19]. Finally, FOXG1 promotes hippocampal plasticity, by enhancing NMDA receptor-mediated currents [15]. As a result of such a pleiotropic impact on brain development and neuronal function, Foxg1 mutations result in complex, cognitive,
and behavioral phenotypes, in both mutant mouse models and human patients. In the mouse, loss of Foxg1 leads to defective social interaction and impaired spatial learning and memory [15, 20]. Moreover, a co-misregulation of Foxg1 in postnatal excitatory as well as inhibitory neurons is necessary and sufficient to evoke the emergence of ASD-like phenotypes [21]. In humans, >120 distinct FOXG1 mutations result into a complex series of neuropathologies, collectively referred to as FOXG1 syndrome, including brain dysmorphologies, epilepsy, and ASD-like symptoms [22–29]. Moreover, a specific FOXG1 upregulation has been detected in brain organoids originating from ASD-patient iPSCs [30].

Albeit mainly known as a transcription factor [31], FOXG1 was also previously reported to be in the cytoplasm of olfactory placode and early born neocortical neurons [32], as well as in the cytoplasm and mitochondria of a hippocampal neuronal line and whole brain homogenates [33]. Next, three high throughput screenings in HEK293T, yeast, and N2A cells [34–36] showed that FOXG1 may interact with a number of factors implicated in post-transcriptional gene regulation, including translation. In addition, we noticed that murine FOXG1 harbors a YATHHLT motif (at 366–372 position), conserved among vertebrates and reminiscent of the EIF4E-binding motif detectable in EIF4E-BP, eIF4G, and other effectors [37]. These observations suggest a possible involvement of FOXG1 in the control of mRNA translation.

To address this issue, we interrogated primary neocortical cultures via a variety of complementary experimental approaches. We found that—in neuronal soma as well as in neurites—FOXG1 promotes translation of Grin1, encoding for the main subunit of the NMDA receptor and playing a pivotal role in neuronal plasticity. Interestingly, this requires FOXG1 interaction with EIF4E. We also demonstrated that FOXG1 promotes fast homeostatic tuning of GRIN1, an issue of potential relevance to the etiopathogenesis of FOXG1-linked neurological disorders. Finally, we got evidence that FOXG1 modulates ribosomal recruitment of dozens of other mRNAs encoding for effectors of neuronal activity, and it also affects ribosome progression. In this way, beyond their “slow” and cell-wide impact on gene expression, originating from transcription regulation, fluctuations of FOXG1 levels might be implicated in a far more complex control on neuronal functions, at different timescales, and in distinctive regions of cell cytoplasm.

**Results**

**Subcellular FOXG1 localization in pallial neurons**

To corroborate previous reports about non-nuclear FOXG1 localization, first, we profiled murine, E16.5 + DIV8 neocortical neurons (Fig. 1A) for subcellular distribution of anti-FOXG1 immunoreactivity. In addition to the nucleus, we found FOXG1 in TUBB3-positive soma and neurites (Fig. 1A, a,b), as well as—more specifically—in punctate-PSD95+ dendrites (Fig. 1A, c,d) and SMI312+ axons (Fig. 1A, d,e). Then, to get further information about partition of non-nuclear FOXG1 between mitochondria and cytosol, we transduced murine P0 + DIV3 hippocampal precursors by a TetON-controlled transgene encoding for a FOXG1-EGFP chimera, we activated it at DIV5, and, upon pre-terminal mitochondria staining by MitoTracker, at DIV8, we profiles living neurons for fluorescence distribution by confocal microscopy and Volocity analysis (Fig. 1B). For simplicity, we restricted the analysis to mid-distal neurites. Specifically, we quantified (1) the MitoTrackerON and MitoTrackerOFF fractions of the EGFPON space, (2) the average intensity of EGFP signal peculiar to each MitotrackerON/OFF fraction, and (3) the cumulative EGFP signal ending in each fraction. We found that mitochondria occupied almost 30% of neurite volume...
Fig. 1 (See legend on previous page.)
and that EGFP density was almost three times higher in mitochondria than in cytoplasm. That resulted in a substantial, cumulative equipartition of the FOXG1-EGFP chimera between the former and the latter. Intriguingly, large patches of non-mitochondrial EGFP could be specifically detected at distal ends of neuritic processes, including lamellipodia and filopodia (Fig. 1C).

**FOXG1 promotes Grin1-mRNA translation in neocortical neurons**

To explore FOXG1 implication in translation, we selected a small sample of genes undergoing translational regulation and/or being implicated in fine-tuning of neuronal activity (Grid1, Grin1, Slc17a6, Gria1, Gabra1, Bdnf - 2c and 4 isoforms -, Ptd95, and Foxg1) [38, 39], and we evaluated the impact of Foxg1 expression level on ribosomal engagement of their mRNAs. For this purpose, we used neocortical neurons obtained from E16.5 Rpl10a E−/− mouse embryos [40] to conditionally overexpress Foxg1 (Foxg1-OE) or a PLAP control (Additional file 1: Figure S1). Four days after transgenes activation, at DIV8, we analyzed them by translating ribosome affinity purification (TRAP)-qRTPCR (Fig. 2A, to left). Specifically, by means of an anti-EGFP antibody, we purified RNA associated to EGFP-tagged ribosomes (IP component) and supernatant RNA (SN component). Then, we scored these RNAs by qRT-PCR, for transcripts (IP component) and supernatant RNA (SN component).

To corroborate this finding and explore its biological meaning, we focused our attention on Grin1 gene, encoding for the main subunit of NMDA receptor, whose activity is impaired upon conditional Foxg1 ablation in the murine hippocampus [15]. For this gene, we evaluated the protein-to-mRNA ratio upon artificial modulation of Foxg1 expression. Tests were run in cultures of E16.5+DIV8 murine neocortical neurons, engineered to conditionally overexpress Foxg1 (Additional file 1: Figure S1; Fig. 2A, to left) or reduce its level (Foxg1-LOF) (Additional file 1: Figure S1; Fig. 2A, to right).

GRIN1 protein was quantified via WB, by a monoclonal antibody recognizing an epitope encoded by Grin1-exon 20. Grin1-mRNA was measured via qRT-PCR, by two oligonucleotide pairs, detecting all Grin1 isoforms (pan-Grin1) or exon20-containing ones (ex20-Grin1) (Fig. 2C). Normalized against betaACTIN, GRIN1 protein was decreased by 36.7 ± 4.7% (with $p < 0.01$ and $n=4.4$) and increased by 12.8 ± 3.8% (with $p < 0.02$ and $n=4.4$), following down- (Fig. 2D) and upregulation (Fig. 2E) of Foxg1, respectively. Opposite trends were displayed by pan-Grin1-mRNA (+6.9 ± 2.0%, with $p < 0.04$ and $n=4.4$, in Foxg1-LOF samples; $-13.7 ± 4.7%$, with $p < 0.04$ and $n=4.4$, in Foxg1-OE ones). Remarkably, such trends were even more pronounced in the case of ex20-Grin1-mRNA (+31.5 ± 13.0%, with $p < 0.07$ and $n=4.4$, in Foxg1-LOF samples; $-17.7 ± 7.0%$, with $p < 0.05$ and $n=4.4$ in Foxg1-OE ones) (Fig. 2D, E). Finally, to get a comprehensive index of the post-transcriptional impact exerted by Foxg1 on Grin1 expression, we calculated the “GRIN1-protein/Grin1-mRNA” ratios peculiar to Foxg1-misexpressing cultures and normalized them against their controls. Such ratios ranged from 0.59 (Foxg1-LOF) to 1.31 (Foxg1-OE), referring to pan-Grin1-mRNA, and from 0.48 (Foxg1-LOF) to 1.37 (Foxg1-OE), referring to ex20-Grin1-mRNA (Fig. 2F). All suggests that Foxg1 plays a robust positive impact on post-transcriptional tuning of GRIN1-protein levels.

Next question was (1) does Foxg1 enhance the translation of Grin1-mRNA and/or (2) does it diminish the degradation of GRIN1 protein?

As for (1), we assessed Grin1 translation rates in E16.5+DIV8 neocortical cultures made Foxg1-LOF by RNAi (Additional file 1: Figure S1). To this aim, we terminally pulsed these cultures with puromycin and we measured levels of nascent GRIN1 protein, (n)GRIN1, via anti-GRIN1/anti-puromycin-driven proximity ligation assay (PLA) (Fig. 3A). To distinguish among translation of all Grin1-mRNA isoforms (pan-Grin1) and exon20-containing ones (ex20-Grin1), two anti-GRIN1 antibodies were alternatively used in addition to anti-puromycin (Fig. 3A, a and b). The former, anti-GRIN1-NH2-term, recognizes the amino-terminal protein region shared by all isoforms (hereafter collectively referred to as “pan-GRIN1”). The latter, anti-GRIN1-COOH-term, interacts with a more carboxyterminal ex20-encoded epitope, restricted to a smaller isoform set (hereafter collectively referred as “ex20-GRIN”) (Fig. 2C). Moreover, the analysis was firstly run on whole neurons and then limited to neurites. In case of whole neurons, two indices of (n) GRIN1 levels were evaluated, the cumulative PLA signal per cell and the cumulative PLA signal per spot. In case of neurites, the first parameter was hard to evaluate, and the measure was restricted to the cumulative PLA signal per spot. Compared to controls, whole neuron (n) pan-GRIN1 signal was reduced in Foxg1-LOF samples, by 6.7 ± 1.8% per cell ($p < 0.039$, $n=7.8$), and 15.6 ± 2.9% per spot ($p < 0.003$, $n=7.8$). In a similar way, neurite (n) pan-GRIN1 signal per spot was decreased by 11.0 ± 3.5%
Fig. 2 Impact of Foxg1 manipulation on ribosomal allocation and protein output of selected neuronal transcripts. A Protocols and lentiviral vectors used to engineer neocortical cultures to conditionally overexpress Foxg1 (Foxg1-OE, left panel) or reduce its level (Foxg1-LOF, right panel). B Comparative translating ribosome affinity purification (TRAP) quantification of ribosome-associated mRNA fraction (TRAP-mRNA) and its supernatant fraction (SN-mRNA), referring to selected neuronal transcripts, in Foxg1-OE cultures. mRNA levels measured by qRT-PCR, and double normalized, against Rpl10a-mRNA and controls. C Grin1 gene locus with the main polypeptide-encoding transcripts originating from it. The top polygon represents the protein epitope recognized by the anti-Grin1 antibody used in Western blot assays. Arrowheads indicate oligos used to quantify Grin1-mRNA, distinguishing between ex20-containing Grin1 (ex20-Grin1) isoforms and pan-Grin1 isoforms. D, E Western blot analysis of Grin1 protein and qRT-PCR quantification of pan-Grin1 and ex20-Grin1 mRNA isoforms, upon Foxg1-LOF (D) and Foxg1-OE (E) manipulations. Protein levels double normalized against bAct and controls, mRNA levels against Rpl10a-mRNA and controls. F Progression of "normalized Grin1-protein" to "normalized Grin1-mRNA" ratio upon Foxg1 manipulation, referring to pan-Grin1 mRNA (left graph) or ex20-Grin1-mRNA (right graph). Throughout figure, n is the number of biological replicates, i.e., independently cultured and engineered preparations, originating from a common neural cell pool. Statistical evaluation of results was performed by t-test, two-tailed (B, D), or one-tailed (E), unpaired and homoscedastic. * p < 0.05, ** p < 0.01. Errors bars indicate s.e.m.
GRIN1 signal per spot was also decreased by 7.7 ± 3.2% \( (p<0.003, n=8,8) \) (Fig. 3A, a). As for (n)ex20-GRIN1, its signal was also reduced in Foxg1-LOF samples, by 20.9 ± 6.1% per cell \( (p<0.013, n=8,8) \) and 14.4 ± 3.6% per spot \( (p<0.003, n=8,8) \). In a similar way, neurite (n)ex20-GRIN1 signal per spot was also decreased by 7.7 ± 3.2% \( (p<0.003, n=8,8) \) (Fig. 3A, a). In a few words, dampening Foxg1 reduces GRIN1 synthesis, in soma as well as in neurites. Interestingly, this is peculiar to GRIN1, and it does not apply to all translactome, as shown by anti-puro immunofluorescence (IF) run on Foxg1-LOF neural cultures terminally treated by emetine and puromycin (Fig. 3B). Taking into account the 6.9% and 31.5% \( (p<0.043, n=8,8) \) decreases undergone by ex20-Grin1 respectively, upon Foxg1 downregulation (see Fig. 2D), these data suggest that Foxg1 specifically promotes Grin1-mRNA translation, with particular emphasis on its ex20-containing isofoms.

As for (2), we evaluated GRIN1 degradation rates in similar Foxg1-LOF neocortical samples. To this aim, we blocked translation by cycloheximide and monitored time course progression of previously synthesized GRIN1 protein over 14 h (Fig. 3C, to left). Remarkably, GRIN1 degradation rate resulted to be not increased, but—rather—it displayed a slight decreasing trend upon Foxg1 downregulation. Specifically, the GRIN1(ti)/GRIN1(t0) ratio equalled \( e^{-[(-0.039/h)t](t0)} \) and \( e^{-[(-0.067/h)t](t0)} \) in Foxg1-LOF cultures and controls, respectively (with \( p<0.093, n=3,3,3,3,3 \)) (Fig. 3C, to right). This result rules out that the increase of “GRIN1-protein/GRIN1-mRNA ratio” evoked by higher Foxg1 expression (Fig. 2F) may be enhanced by FOXG1 impact on GRIN1 protein degradation.

Finally, concerning the process through which FOXG1 promotes Grin1-mRNA translation, we reasoned that the cumulative translation gain peculiar to a given mRNA is a function of both the rate of ribosomes engagement to such mRNA and the speed at which they progress along its cds. We have shown that FOXG1-dependent promotion of GRIN1 translation firstly reflects an improved recruitment of ribosomes to Grin1-mRNA (Fig. 2B). We wondered if FOXG1 is also able to stimulate ribosome progression along Grin1-cds. To address this issue, we set a dedicated puro-PLA run-off assay (Additional file 1: Figure S3A-C) and implemented it in neocortical neuronal cultures. Specifically, upon blockade of de novo ribosome recruitment to mRNA cap by harringtonine \([41, 42]\), ribosomes were allowed to continue ongoing translations for a time presumptively close to that required for full Grin1-mRNA translation. At the end of this time, unfinished GRIN1 polypeptides were labeled by terminal puromycin supplementation and revealed by anti-Grin1/puro-PLA. The PLA signal was subtracted from its \( t=0 \) counterpart (evaluated prior to harringtonine treatment), and the resulting difference, normalized against \( t=0 \) value, was employed as an index positively correlated to ribosome progression speed along Grin1-cds (Additional file 1: Figure S3; Fig. 4A, top). Such run-off assay was performed on Foxg1-LOF samples and their “wild type” controls, driving PLA by anti-GRIN1-NH2-term (which recognizes all GRIN1 polypeptides).

As expected, 11 min after harringtonine supplementation, \( (n)\text{pan-GRIN1 signal underwent a substantial decline compared to its}\ t=0\ value (almost \( \sim40\% \)); however, no difference was detected between Foxg1-LOF samples and controls (Fig. 4A, bottom). This suggests that no generalized change of Grin1-mRNA translation speed occurs upon Foxg1 manipulation.

(See figure on next page.)

**Fig. 3** Quantification of nascent Grin1 protein (A) and nascent proteome (B) and evaluation of Grin1-protein degradation rate (C) in Foxg1-LOF neurons. A To the top, protocols (including lentiviruses employed and operational details of proximity ligation assay (PLA) analysis), to the bottom, results. Graphs represent quantitative confocal immunofluorescence (IF) assessment of nascent Grin1 protein, \( (n)\text{Grin1}, \) performed upon Foxg1 down-regulation, terminal (5') puromycin administration, and subsequent anti-Grin1/anti-puromycin-driven PLA. Two anti-Grin1 antibodies were alternatively used, recognizing (a) amino-terminal (anti-Grin1-NH2-term) and (b) carboxyterminal (anti-Grin1-COOH-term) protein regions. Neuron cell silhouettes were identified by direct EGFP fluorescence, driven by the Map2\textsuperscript{GFP} transgene. PLA signal was quantified throughout the whole neuron or restricted to neurites. As indices of (n)Grin1 levels, shown are the average cumulative IF signals per cell and the average cumulative IF signals per spot. B To the left, protocols (including lentiviruses employed), to the right, results. Graph represents quantitative confocal immunofluorescence (IF) evaluation of nascent total puromycilated proteins, performed upon Foxg1 down-regulation, terminal emetine (25') and puromycin (5') supplementation, and final anti-puromycin-driven IF. In both A and B, results were normalized against controls, error bars indicate s.e.m., and statistical evaluation of results was performed by one-way t-test, one-tailed, unpaired, and homoscedastic \( (*p<0.05, **p<0.01) \). In both A and B, included are examples of primary data referred to by the corresponding graphs. Scale bars, 50 μm. C To the left, protocols and lentiviruses employed for this analysis, to the right, results. Graph represents progression of Grin1-protein levels at different time points, evaluated by western blot, upon Foxg1 down-regulation and subsequent 50 μg/ml cycloheximide (CHX) blockade of translation. For each genotype, results double normalized against \( \text{g/bAct} \) protein levels and \( t0\)average values. Superimposed, exponential trendlines and y(t) functions. Statistical evaluation of results performed by ANCOVA test. Included are examples of primary data referred to by the corresponding graphs. Throughout the figure, \( n \) is the number of biological replicates, i.e., independently cultured and engineered preparations, originating from a common neural cell pool.
FOXG1 physically interacts with selected translation factors

We have shown that FOXG1 enhances translation of GRIN1. Next question was does FOXG1 stimulate translation (1) acting as a canonical nuclear transcription factor (i.e., tuning transcription of translation factor genes) or (2) working as a proper “translation...
modulator"? Results of previous FOXG1 interaction screenings [34–36], our detection of a EIF4E-binding motif-like string within FOXG1 as well as limited responsivity of translation factors’ mRNA levels to Foxg1 overexpression (Additional file 2: Table S1) suggested that type (2) mechanisms might be prevailing.

To preliminarily corroborate this prediction, we engineered HEK293T cells to overexpress FOXG1 and selected translation factors putatively interacting with it (EIF4E, EEF1D, EEF1G, PUM1), and we evaluated their interaction with FOXG1 by proximity ligation assays (PLA). We got evidence that FOXG1 interacts with two of them, EIF4E and EEF1D, implicated in translation initiation and polypeptide elongation, respectively. In case of EIF4E, these results were confirmed by co-immunoprecipitation assays (co-IP). (Additional file 3: Supplementary Results; Additional file 1: Fig. S2A,B).

Next, to assess the biological plausibility of these findings and their relevance to neural genes tuning, we measured the interaction occurring between endogenous FOXG1 and endogenous EIF4E and EEF1D within primary neocortical cultures, by qPLA (Fig. 5A, B, protocols to left). Compared with technical controls (“anti-FOGX1 only” and “anti-EF4E only”), the FOXG1/EIF4E assay gave a moderate, however, statistically robust signal. Normalized against controls’ average, the number of PLA spots per cell equalled 3.6 ± 0.6 (with \( p_{\text{vs-anti-FOGX1-only}} < 0.01, p_{\text{vs-anti-EF4E-only}} < 0.02 \) Fig. 4 Evaluation of endogenous pan-GRIN1 elongation rate by run-off assay. To the top, protocols (including lentiviruses employed, and operational details of the translational run-off assay), to the bottom, results. Graph represents progression of nascent GRIN1 levels evaluated by anti-GRIN1-NH2-term/anti-Puromycin-driven PLA, upon Foxg1 down-regulation, in basal conditions (T0) and 11 min after 2 μg/ml harringtonine (har) blockade of translation initiation (T11'). In both cases, ribosome progression was subsequently inhibited by 208 μM emetine (eme), and nascent polypeptides were terminally labeled by 10 μg/ml puromycin (puro). For each genotype, results normalized against the corresponding average T0’ values. Superimposed, linear trendlines. Statistical evaluation of results performed by t-test, one-tailed, unpaired, homoscedastic. n is the number of biological replicates, i.e., independently cultured and engineered preparations, originating from a common neural cell pool. =, not statistically significant. Included are examples of primary PLA data referred to by the corresponding graphs. Scale bars, 50 μm.
was obtained when restricting the analysis to neurites only (PLA signal was obtained when restricting the analysis to neurites only, with $p_{\text{vs-anti-FOXG1-only}}<0.001$, $p_{\text{vs-anti-EIF4E-only}}<0.02$ and $n=4,4,4$) (Fig. 5A, graph b). Evaluated against the corresponding controls (“anti-FOXG1 only” and “anti-EEF1D only”), the FOXG1/EEF1D assay gave an even stronger signal. Normalized against controls’ average, the number of PLA-spots per cell equalled 25.1 ± 3.6 (with $p_{\text{vs-anti-FOXG1-only}}<0.0003$, $p_{\text{vs-anti-EEF1D-only}}<0.0003$ and $n=4,4,4$) (Fig. 5B, graph a), and a similar result was obtained when restricting the analysis to neurites only (PLA signal =3.2 ± 0.4, with $p_{\text{vs-anti-FOXG1-only}}<0.0001$, $p_{\text{vs-anti-EEF1D-only}}<0.0001$ and $n=4,4,4$) (Fig. 5A, graph b). This suggests that within neocortical neurons, FOXG1 genuinely interacts with both EIF4E and EEF1D.

Finally, to assess the relevance of FOXG1/EIF4E interaction to GRIN1 translation, we outcompeted such interaction by a fragment of the FOXG1 protein and evaluated consequences of this manipulation on GRIN1 synthesis rates. Specifically, by means of a lentiviral vector, we transduced neuronal cultures with a transgene encoding for the mmu-FOXG1 aa357-381 polypeptide, harboring the putative, EIF4E-binding YATHHLT motif. Then, we quantified FOXG1/EIF4E interaction as well as nascent-GRIN1, (n)GRIN1, levels. As expected, compared to a scrambled control, the FOXG1/EIF4E PLAs were lowered, specifically by $-29.9\%\pm 2.7\%$ decrease of (n)GRIN1 ($p<0.02$ and $n=4,4,4$) (Fig. 5B, graph b). All this suggests that within neocortical neurons (including their neurites), FOXG1 physically interacts with both EIF4E and EEF1D.

**FOGX1 physically interacts with Grin1-mRNA**

To further support the hypothesis that Foxg1 promotes Grin1 translation as a translation factor, we investigated if FOXG1 interacts with Grin1-mRNA. To this aim, we quantified the fraction of endogenous Grin1-mRNA immunoprecipitated by an anti-FOXG1 antibody in lysates of E16.5+DIV8 neocortical neurons, by RNA immunoprecipitation (RIP)-qRT-PCR (Fig. 6A). This fraction exceeded the IgG background by 17.6 ± 7.4-folds (with $p<0.05$, $n=4,5$) (Fig. 6A, graph a). Upon Foxg1 knockdown, such fraction also showed a declining trend compared to “wild type” control; however, this was not statistically significant (Fig. 6A, graph b). Then, as an anti-Foxg1 antibody independent control, we scored RNA extracted from neurons overexpressing a FOXG1-EGFP chimera and immunoprecipitated by an anti-EGFP antibody, for Grin1-mRNA enrichment. Remarkably, such enrichment equalled 6.1 ± 0.8, upon normalization against PLAP expressing controls (with $p<0.05$, $n=2,2$) (Fig. 6A, graph c). Altogether, these results indicate that within neocortical neurons, endogenous Foxg1 protein interacts with endogenous Grin1-mRNA.

Next, to identify Grin1-mRNA domains needed to bind Foxg1 protein, we co-transduced murine neocortical neurons with TetON-controlled, intronless transgenes, encoding for the *Rattus norvegicus* Grin1-203 transcript (including exon 20 and orthologous to the *Mus musculus* Grin1-201 isoform) and artificially deleted variants of it. (Within these transgenes, to prevent toxicity induced by chronic *Grin1* overexpression and potential artifacts due to differential protection of rnoGrin1-mRNA by translating ribosomes, a stop codon was inserted between codons 30 and 31 (rnoGrin1.2035)). Then, we immunoprecipitated RNA originating from these cultures by

(See figure on next page.)

**Fig. 5** Assessment of FOXG1 interaction with EIF4E and EEF1D, and functional relevance of FOXG1-EIF4E interaction to Grin1 translation, in primary cultures of neocortical neurons. **A** PLA assessment of endogenous-FOXG1/endogenous-EIF4E interaction ($\text{mmuFOXG1}^{-}\text{md}$$\text{EIF4E}$), in whole neurons, or restricted to neurites. To the left, protocols and lentiviral vectors used, to the right, results. Assays run on cultures of *MaptEGFP* neocortical neurons, interaction signals revealed by anti-FOXG1- and anti-EIF4E-driven PLA, performed on whole neurons (graph a) or restricted to their neurites (graph b), according to graphically displayed criteria. Here, graphs report the numbers of spots/cell, normalized against the average of the two corresponding negative controls (each obtained by omitting either primary antibody). **B** PLA assessment of endogenous-FOXG1/endogenous-EEF1D interaction ($\text{mmuFOXG1}^{-}\text{md}$$\text{EEF1D}$), in whole neurons, or restricted to neurites. To the left, protocols and lentiviral vectors used, to the right, results. Assays run on cultures of wild-type neocortical neurons, interaction signals were revealed by anti-FOXG1- and anti-EEF1D-driven PLA, and results quantification was performed on whole NeuN-immunoreactive neurons (graph a) or restricted to their neurites (graph b), according to graphically displayed criteria. Here, graphs report the numbers of spots/cell, normalized against the average of the two corresponding negative controls (each obtained by omitting either primary antibody). **C** PLA assessment of endogenous-FOXG1/endogenous-EIF4E interaction (graph a) and nascent GRIN1 (nGRIN1) levels (graph b) in whole neurons, upon lentivirus-mediated over-expression of a tagged polypeptide including aa357-381 of murine FOXG1 protein (LV_b). A scrambled version of this polypeptide was used as a control (LV_a). To the left, protocols and lentiviral vectors used, to the right, results. Here, shown are cumulative PLA signals per cell, normalized against controls. Throughout the figure, $n$ is the number of biological replicates, i.e., independently cultured and engineered preparations, originating from a common cell pool. Statistical evaluation of results performed by $t$-test, one-tailed, unpaired, heteroscedastic (panel **A**), or homoscedastic (panels **B** and **C**), $p<0.05$, **$p<0.01$**, ***$p<0.001$*. Errors bars indicate s.e.m. Throughout the figure, included are examples of primary data referred to by the adjacent graphs. Scale bars, 50 μm.
anti-Foxg1 and normalized the IP-Grin1-mRNA fraction peculiar to each deletion against the IP fraction of full-length rnoGrin1.203*d0. Finally, we critically evaluated the relevance of distinct Grin1-mRNA segments to anti-Foxg1 immuno-precipitability (Fig. 6B). We observed that the two variants missing the AccIII-PshAI fragment at the Grin1-cds 3’ end, rnoGrin1.203*d3 and rnoGrin1.203*d5, specifically displayed a normalized IP
fraction far below 1 (0.53 ± 0.04 with \( p < 0.005 \) and \( n = 4 \), and 0.30 ± 0.19, with \( p < 0.005 \) and \( n = 2 \), respectively), pointing to a pivotal role of this fragment in the interaction with Foxg1. On the other side, the removal of the whole 3'UTR, peculiar to rnoGrin1.203.*d4, increased the IP fraction up to 2.39 ± 0.13 (with \( p < 10^{-5} \) and \( n = 2 \)), suggesting that such domain may normally antagonize Foxg1 recruitment to Grin1-mRNA (Fig. 6B). Despite the relatively low number of biological replicates scored, altogether, these results corroborate the specificity of FOXG1/Grin1-mRNA interaction and provide a coarse-grained, tentative framework for reconstruction of its regulation. Of course, they do not allow us to make any inference about the topology of such interaction, direct or mediated by an (unknown) bridging effector.

Fig. 6  Evaluation of Foxg1-protein/Grin1-mRNA interaction in neocortical neurons, by RNA immunoprecipitation (IP) qPCR (qRIP-PCR) assays. A Immuno-precipitation of Foxg1-bound, endogenous Grin1-mRNA in neocortical neurons. To the left, protocols and lentiviral vectors used, to the right, results. Anti-Foxg1-IP fraction of endogenous Grin1-mRNA in neurons expressing naive (a) or decreased (b) levels of Foxg1-mRNA. Results double normalized, against input-RNA and IgG-IP samples. Anti-EGFP-IP fraction of endogenous Grin1-mRNA in neurons expressing a lentivector-driven, Foxg1-EGFP transgene or a Plap control (c). Results double normalized, against input-RNA and control samples. B Mapping determinants of Foxg1-protein binding on a heterologous rno-Grin1-mRNA, encoded by a lentiviral transgene. To the left, protocols and lentiviral vectors used, to the right, results. Here, a number of partially overlapping deletions were generated starting from the full-length cDNA (d0), by standard molecular cloning techniques, so giving rise to five distinct mutants (d1–d5). To prevent toxicity originating from chronic, exaggerated Grin1 expression and potential artifacts stemming from differential protection of rnoGrin1-mRNA by translating ribosomes, in all constructs a stop codon was inserted in a fixed position, between codons 30 and 31, so resulting into modified transcripts (rno-Grin1-203* and derivatives). To quantify the impact of each deletion, neuronal cultures were co-transduced with lentiviral mixes encoding for different combinations of full-length (d0) and mutant (dx) transgenes. Anti-Foxg1-IP fractions of mutant rno Grin1-mRNAs, primarily normalized against the corresponding inputs, were diminished by the corresponding IgG-IP backgrounds and finally renormalized against the average full-length fraction. At the bottom, a color-coded cartoon summarizes the positive or negative impact that distinct transcript domains apparently exert on Grin1-mRNA/Foxg1-protein interaction. Throughout the figure, \( n \) is the number of biological replicates, i.e., independently cultured and engineered preparations, originating from a common neural cell pool. Statistical evaluation of results was performed by t-test, two-tailed, unpaired, and homoscedastic. **\( p < 0.01 \), ****\( p < 0.0001 \). Errors bars indicate s.e.m.
FOXG1 is needed to achieve proper homeostatic tuning of neuronal Grin1-mRNA translation

Grin1 is a key player implicated in neuronal plasticity and, in turn, it is the subject of intricate, activity-dependent post-transcriptional regulation [38, 39, 43, 44]. We previously observed that exposing E16.5+DIV8 neocortical cultures to 55 mM KCl resulted into a dramatic drop of (n)GRIN1 level that was partially rescued upon transferring the same cultures to a low K+-containing medium. This points to a dedicated mechanism taking care of homeostatic translation tuning (our unpublished results).

To evaluate the relevance of FOXG1 levels to such tuning, we compared the impact of high extracellular K+ on GRIN1 translation in Foxg1-LOF vs wild-type neural cultures (Fig. 7, left). As expected, in wild-type neurons, we confirmed the previously observed collapse of (n)GRIN1 evoked by acute 55 mM K+ (to 4.4 ± 0.9% of unstimulated wild-type samples, with p vs-wt-ctr < 0.0005 and n = 3,3), as well as the partial rebound of (n)GRIN1 levels upon retransferring cultures to a low K+ medium (to 59.0 ± 4.3%, with p vs-wt-K5' < 0.0002, p vs-wt-ctr < 0.02, n = 3,3,3). Conversely, when Foxg1 was knocked-down, (a) basal GRIN1 translation was reduced to 49.9 ± 3.7% (with p vs-wt-ctr < 0.0006 and n = 3,4), (b) the exposure of Foxg1-LOF cultures to high K+ reduced (n)GRIN1 to 9.1 ± 0.8% (normalized against wt砼), with p vs-Foxg1-LOF-ctr < 0.0003 and n = 3,4, and (c) the subsequent re-transfer of these cultures to standard potassium allowed (n)GRIN1 to rebound to 27.3 ± 7.2% (again normalized against wt砼), with p vs-Foxg1-LOF-K5' < 0.03, p vs-Foxg1-LOF-ctr < 0.03, and n = 3,4,4 (Fig. 7, right). In other words, compared to controls, Foxg1 knock-down dampened the early homeostatic response to high K+ by about fourfolds (4.4% vs 100.0% and 9.1% vs 49.9%, respectively, with p(genotype/K+)interaction < 0.002, as assessed by two-way ANOVA).

To sum up, we found that GRIN1 de novo synthesis undergoes a prominent and reversible, homeostatic regulation, and FOXG1 is instrumental to that.

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**Fig. 7** Foxg1 relevance to homeostatic Grin1-mRNA translational tuning. To the left, protocols (including lentiviruses employed, and operational details of transient neuronal stimulation), to the right, results. Impact of Foxg1-down-regulation on (n)Grin1 levels, following acute exposure of neocortical neurons to high extracellular potassium (K5') and their return to not-K+-supplemented medium (K10'-noK25'). Foxg1 knockdown elicited via shRNA-encoding lentivirus. (n)Grin1 evaluated by anti-Grin1-COOH-term/anti-puromycin-driven proximity ligation assay (PLA). Results normalized against unstimulated controls (wt砼). Included are examples of primary data. n is the number of biological replicates, i.e., independently cultured and engineered preparations, originating from a common cell pool. Scale bars, 50 μm. Statistical evaluation of results performed by t-test, one-tailed, unpaired, and homoscedastic, and two-way ANOVA =, not statistically significant, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Errors bars indicate s.e.m.
Widespread impact of FOXG1 on mRNA engagement to ribosomes

We wondered if Foxg1 impact on translation is peculiar only to a few genes including Grin1 or is it a pervasive phenomenon. To get an insight into this issue, we systematically sequenced ribosome-engaged-mRNA (trapRNAseq) purified from Foxg1-OE and control cultures (as in Fig. 2A, to left) and compared it to total-mRNA originating from corresponding sister cultures (totRNAseq) [17].

For simplicity’s sake, we took into account trapRNAseq and totRNAseq reads belonging to the only principal isoform of each gene (according to APPRIS annotation) [45]. We calculated log2 “expression fold change” values (log2FC) peculiar to trapRNA and totRNA samples and evaluated statistical significance of results by DESeq2 software [46]. Next, we scored each gene on the basis of the “log2FC(trapRNAseq)-log2FC(totRNAseq)” difference (hereafter Δlog2FC), as a measure of FOXG1-dependent stimulation of ribosomal mRNA engagement and a presumptive index of FOXG1-driven promotion of its translation. Finally, we evaluated statistical significance of results by Ribodiff software [47].

Upon filtering out low-expressed genes as well as those with \( p_{\text{adj}} \geq 0.1 \), we found 183 genes with \( \Delta \log_{2} \text{FC} > 0.5 \) (i.e., with FOXG1 presumptively promoting their translation) and 175 genes with \( \Delta \log_{2} \text{FC} < -0.5 \) (i.e., with FOXG1 presumptively antagonizing their translation). Categorized by \( \Delta \log_{2} \text{FC} \) value, these genes largely fell within the “1.0 to 1.5” and the “−1.5 to −1.0” intervals (66 and 72 genes, respectively) (Fig. 8A; Table 1; Additional file 4: Table S2A). As shown by GO analysis, such genes preferentially encode for proteins (a) involved in synaptic signaling, behavior, memory, and fatty acid catabolism, (b) localized at the plasma membrane and synapses, and (c) acting as channels, neurotransmitter receptors, transmembrane transporters, and transcription factors (Additional file 4: Table S2B).

Next, we further classified these genes as for their Foxg1-driven totRNA dynamics. We found that among 183 genes with increased ribosomal engagement, as many as 118 displayed reduced totRNA and only 14 increased totRNA. Symmetrically, among 175 genes with decreased ribosomal engagement, 117 and 5 had upregulated and downregulated totRNA, respectively (Fig. 8B). All that results in a variegated scenario, as shown in Fig. 8C.

Next, to exclude possible artifactual results originating from FOXG1-dependent alteration of pre-mRNA maturation, we re-analyzed primary totRNA data [18] by CASH [48] and ROAR [49] softwares. Interestingly, we found that, upon FOXG1 overexpression, only \((7 + 14) = 21\) of the \((183 + 175) = 358\) genes “with altered ribosomal engagement” mentioned above displayed altered splicing and polyadenylation, respectively (Table 1; Additional file 4: Table S2A).

Then, to further corroborate our findings, we systematically interrogated mRNAs “with altered ribosomal engagement” for a possible interaction with the FOXG1 protein. For this purpose, we relied on sequencing of RNA extracted from E16.5 +DIV8 pallial cultures and immunoprecipitated by an anti-FOXG1 antibody, taking selectively into account exonic reads representative of mature mRNAs (here we referred to the principal splicing isoform, according to APPRIS annotation [45]). We monitored the distribution of these reads by Sicer software, comparing anti-FOXG1-IP samples with IgG-treated controls. FOXG1/mRNA interaction peaks with anti-FOXG1/IgG_enrichment ≥ 2 and fdr < 0.05 resulting from this analysis were further taken into account, and mRNAs sharing ≥ 1 peak in ≥ 2 out of 3 biological replicates were considered as interacting with the FOXG1 protein. Specifically, 2857 distinct mRNAs fulfilled this requirement and, interestingly, among the 358 genes “with altered ribosomal engagement” mentioned above, as many as 138 encoded for them (Table 1; Additional file 4: Table S2A).

PuroPLA validation of presumptive translational targets of FOXG1

To validate the bioinformatic procedure described above, we selected Sgk1 and Homer1, namely two genes presumptively undergoing a robust Foxg1-OE-driven translational enhancement (\( \Delta \log_{2} \text{FC} \) equaling +1.89, with \( p_{\text{adj}} < 0.04 \), and +1.56, with \( p_{\text{adj}} < 0.01 \), respectively) in the face of a significative downregulation of the corresponding mRNAs (−24.94%, with \( p < 10^{-4} \), and −46.24%, with \( p < 10^{-21} \), respectively), and we monitored the synthesis rate of their protein products in Foxg1-OE neurons by puro-PLA. We found that, compared to controls, such rate was increased in the case of Sgk1 (by \( 3.09 \pm 0.54 \)-folds, with \( p < 0.012 \) and \( n = 4,4 \)), and barely shifted upward in the case of Homer1 (1.12 ± 0.28, \( p = 0.28 \), \( n = 5,5 \)). Taking into account the underlying declining mRNA dynamics, these results unambiguously point to a positive Foxg1 impact on both Sgk1 and Homer1 translation gains. Conversely, the translation rate of NMT1, displaying no statistically significant \( \Delta \log_{2} \text{FC} \) value or variation in \( \text{totmRNA} \) level, was not affected upon Foxg1 overexpression (Fig. 9A-C).

Moreover, to confirm that the differences observed among protein and mRNA dynamics upon Foxg1 manipulation were due to a direct FOXG1 impact on translation, we considered to overexpress a cytoplasm-confined variant of FOXG1 and assay its impact on neuronal rates of SGK1 translation. For this purpose, we generated a Tet\(^{\text{ON}}\)-controlled Foxg1-Ert2-Flag-V5 transgene encoding...
for a chimeric polypeptide, which included FOXG1, the estrogen receptor-derivative ERT2 module (confining the polypeptide to cytoplasm [50, 51]), as well as Flag and V5 epitopes (for immunolocalization) (Additional file 1: Figure S4A, B). We delivered this transgene (as well as its Plap and Foxg1 controls) to primary neocortical cultures, by lentiviral vectors (Fig. 9D). As expected, we observed a confinement of V5 immunofluorescence to cytoplasm, which was specifically abolished upon 4-hydroxytamoxifen (4OHT) supplementation (Fig. 9E). Interestingly, we found that, while not affecting expression levels of two mRNAs which are highly sensitive to wild-type FOXG1 (Gad1 and Arc) (Fig. 9F, G), Foxg1-Ert2-Flag-V5 stimulated SGK1 translation (Fig. 9F, H, I). Compared to Plap controls, it increased the cumulative PuroPLA-SGK1 signal per neuron by 1.92 ± 0.14 (p < 0.008, n = 3,4),
Table 1 Distribution of Foxg1-sensitive splicing, Foxg1-sensitive polyadenylation, and mRNA interaction with Foxg1 protein, among gene transcripts characterized by altered ribosomal engagement and/or progression upon Foxg1 over-expression

| Genes | Total | With altered splicing | With altered polyadenylation | With Foxg1-interacting mRNA |
|-------|-------|-----------------------|-----------------------------|-----------------------------|
| with altered ribosomal engagement | 358   | 7                     | 14                          | 138                         |
| with altered ribosomal engagement | 328   | 6                     | 17                          | 93                          |

1 Identified by integrated evaluation of totRNA-Seq and TRAP-seq data; satisfying Δlog2FC ≥ 0.1
2 Identified on the basis of distribution of TRAP-seq reads along every transcript; satisfying Δψ ≥ 3
3 Identified by cash software, with -0.1 ≤ Δpsi ≥ 0.1; fdr < 0.05
4 Identified by roar software, with 1.12 ≥ Δr ≥ 1.2; fdr < 0.05
5 Identified by aFoxg1RIP-seq, based on the occurrence of Foxg1-protein/mRNA interaction peaks with aFoxg1/lgs enrichment ≥ 2 and fdr < 0.1 (mRNAs taken into account sharing ≥ 1 peak in ≥ 2 out of 3 biological replicates; calculations restricted to the main isoform of each mRNA)

log2FC(rpi) ≤ −1 and p < 0.05 (boi.downs), we similarly obtained an alternative, gene-specific index of Foxg1-dependent inhibition of ribosomal progression (f_{boi.down}).

Similarly to what was achieved by its Foxg1 counterpart (2.25 ± 0.30 folds, with p < 0.005, n = 3,4). This confirms a direct impact of FOXG1 on translation, independent from its transcription factor activity.

FOXG1 impact on ribosome progression along mRNAs

We further mined our TrapSeq data, aiming at unveiling a possible impact of Foxg1 expression levels on ribosomal progression along mRNAs. For this purpose, we assumed that because of random mechanical fragmentation undergone by “ribo-trapped” mRNA during the immunoprecipitation procedure, reads location should provide information about the position occupied by the 60S subunit along the mRNA-cds. Specifically, for each gene, we took into account the principal isoform (according to APPRIS annotation) [45], and, for each transcript, we allotted reads to adjacent 125 base-wide cds bins. Next, considering each bin/bin boundary as a potential bottleneck for ribosome advancement, we calculated the corresponding ribosome progression index (rpi), as the ratio among reads falling downstream and upstream of such boundary (Fig. 10A). For each boundary, we averaged rpis of the three Foxg1-OE replicates and those of the four controls, and we annotated boundaries with log2FC(rpi) ≥ 1 and p < 0.05 as “boundaries of interest, up” (boi.ups). Then, we evaluated the frequency of such boundaries over the full cds (f_{boi.ups}), as a global, gene-specific index of Foxg1-dependent promotion of ribosomal progression. In parallel, referring to boundaries with
Fig. 9  Experimental validation of FOXG1 impact on translation rates, upon its generalized or cytosol-confined overexpression in primary neocortical cultures. A–C Validation of FOXG1 impact on translation rates upon generalized FOXG1 overexpression. In A, protocol, including lentiviral vectors for TetON-controlled overexpression of the Foxg1 transgene and its Plap control. In B, quantification of nascent NMT1, SGK1, and HOMER1 proteins in engineered cultures, as revealed by anti-protein/anti-puromycin-driven, proximity ligation assay (PLA), and confocal immunofluorescence (IF). Specifically, shown are average cumulative signals per cell. In C, examples of primary data referred to in B. Scale bar, 50 μm. D, E Assaying restriction of the FOXG1-V5-FLAG-ERT2 chimera to cytoplasm of neocortical neurons harboring a Foxg1-V5-Flag-Ert2 transgene. In D, protocol, including lentiviral vectors driving TetON-controlled expression of the Foxg1-V5-Flag-Ert2 transgene (abbreviated as Foxg1-ert2) or its controls (Plap and Foxg1). In E, co-profiling of engineered cultures for FOXG1 and V5 (as expected, the V5 signal is basically confined to cytoplasm, and a displacement of it to nucleus takes place upon 4-hydroxytamoxifen (4OHT) medium supplementation). Scale bar, 50 μm. F–I Validation of FOXG1 impact on translation rates upon cytosol-confined FOXG1 overexpression. In F, protocols, including lentiviral vectors for TetON-controlled overexpression of the Foxg1-ert2 transgene and its Plap and Foxg1 controls. In G, H results. The engineered cultures were profiled for G Gad1- and Arc-mRNA levels, by qRT-PCR, as well as for H SGK1 translation rates, by PuroPLA and IF. Results were normalized against G Gapdh and Plap controls and H Plap controls. In I, examples of primary data referred to in H graphs. Scale bar, 50 μm. In B, G, H, n is the number of biological replicates, i.e., independently cultured and engineered preparations, originating from a common cell pool. Statistical evaluation of results performed by t-test, one-tailed, unpaired, and homoscedastic (panels B (NMT, HOMER1), G (Gad1), H (right)) or heteroscedastic (panels B (SGK1), G (Arc), H (left)). Error bars indicate s.e.m.
Fmr1, namely two boi.up-rich genes (with \(3.0 \leq f_{\text{boi.up}} \text{ z-score} < 4.5\) and \(f_{\text{boi.up}} \text{ z-score} > 4.5\), respectively), characterized by diversified reads distributions along their cds in Foxg1-OE vs control samples (Fig. 10C) and mRNA expression levels not affected upon Foxg1 manipulation (Additional file 5: Table S3B). For this purpose, we employed a dedicated puro-PLA run-off assay, similar to the one used for GRIN1 (Figs. 4 and 11A; Additional file 1: Figure S3). In the case of CAMK2B, upon setting the \(t_t\) time to 4.5 min, we found that the
t0-normalized decline of the PLA signal (−22.2 ± 0.2% in controls) was remarkably exacerbated in Foxg1-OE samples (−47.2 ± 3.5% with p < 0.002 and n = 3,3) (Fig. 11B, D). This points to an overt positive impact exerted by FOXG1 overexpression on ribosome progression along Camk2b-mRNA. It provides a first positive assessment of the predictive power of the bioinformatic strategy we employed. Vice versa, in the case of FMR1, upon setting the t0 time to 6 min, we found that the t0-normalized decline of the PLA signal (−54.5 ± 8.7% in controls) was reduced in Foxg1-OE samples (−27.3 ± 8.8% with p < 0.027 and n = 6,6) (Fig. 11C, E). It is possible that in this case, rather than simply originating from faster holo-ribosome progression through the very body of the cts, the preferential clustering of trapmRNA reads detectable in the 3’ half of it upon Foxg1-OE might reflect some pre-terminal holoribosome accumulation, due to an alternative, 3’-terminal bottleneck evoked by this treatment (Fig. 10C).

Discussion
Here, inspired by the detection of FOXG1 protein in neuritic cytoplasm of pallial pyramids (Fig. 1; Additional file 1: Figure S5), we investigated its potential implication in the translation of selected neuronal genes, and we documented an impact of Foxg1 on ribosomal engagement of Grin1-mRNA (Fig. 2A, B). Next, we showed that FOXG1 increases GRIN1 protein level by enhancing translation of its mRNA, while not ameliorating its stability (Figs. 2C–F and 3). Such enhancement
was apparently due to increased translational initiation (Fig. 4). Mechanisms underlying these phenomena included FOXG1 protein interaction with EIF4E (Fig. 5) and, possibly, Grin1-mRNA (Fig. 6). Moreover, we found that Grin1-mRNA translation undergoes a prominent (and reversible) homeostatic regulation and FOXG1 is instrumental to that (Fig. 7). Finally, a dedicated TRAP-seq survey showed that functional FOXG1 implication in translation control (both initiation and ribosome progression) is a pervasive phenomenon, affecting hundreds of neuronal genes. In selected cases, we experimentally verified such implication (Figs. 8, 9, 10, and 11).

The localization of FOXG1 in early-born neocortical glutamatergic neurons outside of the nucleus, had been already reported [32]. Here we showed that FOXG1 is specifically detectable in soma, dendrites, and axons of the majority of pallial pyramids, including the mitochondria as well as the cytoplasm (Fig. 1).

Based on higher Grin1-mRNA levels detectable in ribosome-engaged compared to not-ribosome-engaged mRNA of Foxg1-OE neurons (Fig. 2B), we inferred a likely ribosome-engaged compared to not-ribosome-engaged (Fig. 2F) in the absence of GRIN1-protein stabilization (Fig. 3C). Moreover, we found that they resulted in increased puromycin-tagged, nascent GRIN1 (Fig. 3A).

All that allowed us to definitively validate the aforesaid inference. Intriguingly, a substantial fraction of nascent GRIN1 was detected in neurites (Fig. 3A), consistently with previously reported localization of the corresponding mRNA in these structures [52–55]. To notice, Foxg1 did not drive any appreciable, generalized enhancement of translation (Fig. 3B).

The synthesis rate of a given polypeptide does not depend only on the initiation of its translation, but it also reflects the speed at which it is elongated. In this respect, combined use of harringtonine and puromycin had already been employed to assay cumulative, proteome-wide polypeptide elongation rates [42]. Here, by means of PLA, we re-adapted this method to evaluate elongation rates of specific polypeptides in distinctive sub-cellular locales (Additional file 1: Figure S3). Albeit technically working (Fig. 11), this approach did not allow us to document any Foxg1-driven change of this rate in the case of GRIN1 (Fig. 4).

As for molecular mechanisms underlying FOXG1 impact on Grin1 translation, we achieved multiple pieces of evidence pointing to it as a “translation modulator”. In fact, beyond its detection in neuronal cytoplasm (Fig. 1), we found that FOXG1 interacts with EIF4E, and partial inhibition of its interaction with the latter resulted in a substantial decline of Grin1 translation (Fig. 5). Moreover, FOXG1 binds to Grin1-mRNA (Fig. 6).

To note, whereas our PLA-based investigation of FOXG1/EEF1D association confirmed in pallial neurons results achieved by means of high-throughput mass spectrometry (MS) screenings previously run in HEK293T and N2A cells [34, 36], conversely, the FOXG1 interaction with EIF4E, which we proved by both IP-WB analysis and PLA (Fig. 5A; Additional file 1: Figure S2), is novel. Moreover, while an interaction of FOXG1 with ncRNAs (miRNA precursors) had been previously reported [36], FOXG1 interaction with mRNA has been only recently described, however as a retrotransposition-related phenomenon [56]. Needless to say, FOXG1 association to EIF4E and EEF1D resonates with presumptive FOXG1 implication in translation initiation (Fig. 8) and polypeptide elongation (Fig. 10), respectively [57, 58].

It has been shown that acute stimulation of hippocampal pyramids by high extracellular potassium may evoke a fast increase of cap-dependent translation [59]. Moreover, Grin genes—which encode for subunits of the heteromeric NMDA receptor—undergo an intricate, multi-step regulation needed for proper setting of integrative properties of neocortical pyramids [44]. In this context, specific and reversible high K+–driven downregulation of GRIN1 translation (Fig. 7) might represent the experimental correlate of specific physiological mechanisms contributing to homeostatic scaling of neuronal response to glutamate [39].

Next, Foxg1 has been recently shown to promote activity and excitability of neocortical neurons, largely via a profound impact on their transcriptome [17]. Consistently, FOXG1-depleted hippocampal neurons display reduced NMDA currents and defective long-term potentiation (LTP) [15]. In this respect, Foxg1-dependent modulation of GRIN1 translation (Figs. 3 and 7) might be a key mechanism concurring to both these effects.

Finally, we have recently shown that Foxg1 is transiently upregulated by neuronal hyperactivity [17, 19]. In this way, delayed FOXG1-mediated promotion of GRIN1 translation, following episodes of intense electrical activity, might contribute to normal dynamic shaping of pyramidal excitability, and its absence might impair neuronal plasticity, contributing to major cognitive deficits of FOXG1-haploinsufficient patients [22, 27, 29].

The involvement of a neurodevelopmental transcription factor in the control of mRNA translation is not
novel. It has already been reported in a few cases, including those of Bicoid [60], EMX2 [37], and EN2 [61] homeoproteins. In our case, we found that FOXG1 implication in translation is not limited to Grin1 only, but it likely is a pervasive phenomenon, affecting hundreds of genes (Figs. 8 and 10), among which a large subset encoding for proteins involved in neuronal metabolism and activity (Additional file 4: Table S2). In a subset of cases, we got robust evidence of physical interaction between mRNAs subject of Foxg1-dependent translational control and the FOXG1 protein (Table 1), suggesting that—at least in such cases—the latter may work as a “translational factor”. To note, the number of mRNA interactors of FOXG1, 2857, largely exceeded the number of those specifically undergoing FOXG1 control of their translation, $138+46+47=231$, pointing to a likely FOXG1 involvement in other aspects of post-transcriptional gene tuning.

Remarkably, albeit our quantification of ribosome engagement and progression was intentionally restricted to the principal isoform of each polypeptide-encoding transcript, as such isoform often shares a large subset of its exon/intron architecture with minor ones, a number of reads originating from the latter were likely misattributed to the principal isoform of each polypeptide-encoding transcript, as such isoform often shares a large subset of its exon/intron architecture with minor ones. However, while binning of trap-RNA reads may unveil FOXG1 control over ribosomes engagement and progression parameters. To address this issue, we re-analyzed primary totRNA-Seq data from Foxg1-OE and control cultures by CASH and ROAR softwares. It turned out that only a minority of presumptive translational targets of FOXG1 regulation underwent Foxg1-dependent modulation of splicing and/or polyadenylation patterns (Table 1; Additional file 4: Table S2A, C), therefore allowing us to fix this concern. To note, while running these controls, we detected an additional impact of FOXG1 on two steps of pre-mRNA maturation, i.e., splicing and polyadenylation. We will address these novel aspects of FOXG1 biology in a forthcoming dedicated study.

As said above, we have shown that integrated mining of trap- and total RNA data can provide evidence of FOXG1 control over ribosomes engagement to mRNA, while binning of trap-RNA reads may unveil FOXG1 control of ribosomes progression along it. However, the interpretation of results originating from such approaches deserves caution. This applies firstly to the evaluation of the Δlog2FC parameter. For example, rather than simply reflecting enhanced translation initiation, Δlog2FC values above 0 might also alternatively originate from pronounced ribosome stalling by the kozak motif. Consistently with this prediction, we found that 8 transcripts out of 183 ones with Δlog2FC > 0 (see Fig. 8B) were also characterized by “average_log2FC(rpi) > 0 and $f_{\text{boi,down}}$ z-score > 3”. In such cases, FOXG1 could actually limit baseline translation (possibly paving the way to subsequent, prompt completion of it, upon the arrival of due inputs). In a symmetrical way, Δlog2FC values below 0 might originate from extremely fast ribosome progression along the cds and anticipated detachment from it. Again, consistently with this prediction, we found that 2 transcripts out of 175 ones with Δlog2FC < 0 (see Fig. 8B) were also characterized by “average_log2FC(rpi) < 0 and $f_{\text{boi,up}}$ z-score > 3”. Here, an increase of FOXG1 levels might elicit an extremely fast upregulation of translation, just by relieving ribosomal stalling. Finally, beyond Δlog2FC issues, even the rpi (Fig. 10A) has an intrinsically limited predictive power, similar to the corresponding Ribo-seq parameters [62]. In fact, it provides only a static snapshot of presumptive ribosome distribution along mRNA and no direct information about the actual speed at which ribosomes move. For all these reasons, TRAP-seq data mandatorily require to be integrated by experimental investigation of the actual rate at which polypeptides of interest are synthesized.

Prompted by these considerations, we challenged results of our total/TRAP-seq analyses, firstly by assessing translation rates of Sgk1- and Homer1-mRNA, namely two transcripts apparently undergoing FOXG1-driven promotion of ribosome engagement. In both cases, integrated evaluation of puro-PLA results and tot-mRNA dynamics pointed towards an overt translational gain increase evoked by Foxg1 overexpression (Fig. 9A-C). Remarkably, a comparable increase of Sgk1 translation was also evoked upon overexpression of a cytoplasm-confined FOXG1-ERT2 chimera (Fig. 9D-I), ruling out that this phenomenon may trivially reflect an impact of FOXG1 on transcription of translation factor genes. Next, we focused our attention on Camk2b- and Fmr1-mRNA, namely two transcripts showing 3'-shifted distributions of trapmRNA-reads upon Foxg1 overexpression. We measured the temporal decline rate of their translation upon harringtonin blockade of translation initiation, as an index of ribosome progression along their cds. For this purpose, we employed a “puro-PLA run-off” assay, i.e., a novel method we developed to evaluate ribosome advancement speed along specific mRNA-cds (Additional file 1: Figure S3). As expected, this method provided evidence of faster ribosomal progression through Camk2b-cds, upon Foxg1-OE (Fig. 11A, B, D). In case of Fmr1, it conversely pointed to the alternative emergence of a novel ribosomal pausing site, likely evoked by Foxg1-OE towards the 3’ end of Fmr1-cds (Fig. 11A, C, E). Of course, the assays described in Figs. 9 and 11 represent...
a small-scale experimental validation of our procedure, which needs to be corroborated by further experimental work. This will be the subject of dedicated follow-up studies.

To note, albeit providing us with only coarse-grained information about ribosome location along mRNA, our reanalysis of “cheap” TRAP-seq data allowed us to identify as many as >300 genes characterized by a robustly diversified ribosome association to distinctive mRNA regions, dependent on Foxg1 expression levels (Fig. 10B, C). This suggests that when interested in the control of ribosomal progression rate, mining publicly available TRAP-seq data might be an advisable first approach, prior to moving to more expensive, state-of-art Ribo-seq profiling.

Intriguingly, in a number of cases including Grin1, we also found that a large subset of genes characterized by statistically significant Δlog2FC > 0 displayed a robust downregulation of their total-mRNA (118/183) and vice versa for those with Δlog2FC > 0 (117/175) (Fig. 8B, C; Additional file 5: Table S3). In the former case, reminiscent of activity-driven regulation of NPAS4 and ARC [63, 64], the very same effector, FOXG1, might promote a rapid upregulation of the protein, while however limiting the temporal duration of its overexpression. In the latter, FOXG1 could conversely elicit a slow protein upregulation followed by a delayed fast decrease of it. Evolutionarily speaking, multilevel target gene regulation by a single multitask effector is a rare and thermodynamically demanding phenomenon. Such phenomenon could ease the portability/selectability of temporally structured expression programs (in the minutes/hours range). In this way, FOXG1, mainly known as a transcription factor patterning the terminal brain and ruling its histogenesis, could also act as a multi-scale, temporal modulator of neocortical pyramid plasticity. Interesting per se as well as for its profound neuropathogenic implications, this issue will be specifically investigated in a future, dedicated follow-up study.

Conclusions
In this study, we showed that Foxg1, a transcription factor mastering telencephalic development, stimulates the translation of Grin1, encoding for the main subunit of the NMDA receptor. We found that this is associated to increased ribosome engagement to Grin1-mRNA and requires physical Foxg1 interaction with EIF4E. Moreover, we discovered that Foxg1 is needed for proper homeostatic response of Grin1 translation to neuron depolarization.

We further reported that Foxg1 impact on translation is a pervasive phenomenon, affecting hundreds of genes, many of which deeply implicated in neuronal physiology. Depending on cases, Foxg1 may promote or dampen translation, modulating ribosome engagement to mRNA and/or their later progression through cds. Instrumental to these phenomena may be physical Foxg1 interaction with key translation factors EIF4E and EEF1D and target mRNA.

In this way, Foxg1 adds to a small set of transcription factors (including Emx2, En2, and Bcd) which are also implicated in the direct tuning of translation gain. We speculate that orthogonal control of gene transcription and translation exerted by the same polypeptide effector may ease the evolutionary portability of temporally structured expression programs, an issue of paramount relevance to the philogenesis of neuronal excitability dynamics.

Methods
Animal handling
In this study, the following rodent models were employed:

- Wild-type (wt) CD1 strain mice (purchased from Envigo Laboratories, Italy);
- Transgenic Gt(Rosa)26Sgrtm1.1(CAG–EGFP/Rpl10a–biArp)Wtp/J mice, throughout the text referred to as Rpl10aEGFP–Rpl10a/+ [40] (founders purchased from Jackson Laboratories, USA, Jax #002386; transgenic line maintained according to Jackson’s instructions);
- Transgenic MaptEGFP/+ mice [65] (founders purchased from Jackson Laboratories, USA, Jax #004779; transgenic line transferred to CD1 background (> 20 backcrossing generations));
- wt Wistar rats (generated at the SISSA animal facility starting from founders purchased from Envigo Laboratories, Italy).

Mutant mouse embryos were obtained by crossing wt females to mutant or wt males and were staged by timed breeding and vaginal plug inspection. Pregnant dams were killed by cervical dislocation. Rpl10aEGFP–Rpl10a/+ and MaptEGFP/+ mouse embryos were distinguished from their wt littermates by UV lamp inspection.

Rat pups were anesthetized with CO2 and sacrificed by decapitation.

Mouse and rat neural tissues were dissected out in sterile ice-cold 1×-phosphate-buffered saline (PBS) supplemented with 0.6% D-glucose (Sigma) under sterile conditions.

Plasmids and lentiviruses
Plasmids employed in this study include:

- LV_pU6-shFoxg1 (Sigma SHCLND-NM_008241, TRCN0000081746); see Figs. 2, 3C, 4B, and 7A.
LV_pU6-shFoxg1-DPuroR (built by removing the SacII/SacII fragment, including the 5’ end portion of puromycin resistance cds and its upstream hPGK-promoter, from “LV_pU6-shFoxg1”; annotated as “LV_pU6-shFoxg1” in Figs. 3A-B, 4A, 8, and 11).

- LV_pU6-shCtrl [14].
- LV_pPgk1-rTA2S-M2 [66].
- LV_TREt-Foxg1 [67].
- LV_TREt-PLAP [6].
- LV_pPgk1-mCherry [6].
- LV_pPgk1-3xF-wt.mmuFoxg1aa357-381-V5 (built by replacing the AgeI/SalI EGFP-cds fragment of LV_pPgk1-EGFP, by the AgeI/SalI wt.mmuFoxg1aa357-381-V5 module (as detailed in Additional file 6: Table S4)).
- LV_pPgk1-3xF-scr.mmuFoxg1aa357-381-V5 (built by replacing the AgeI/XhoI scr.mmuFoxg1aa357-381-V5 module (as detailed in Additional file 6: Table S4)).
- LV_TREt-Foxg1-EGFP (built by replacing the SrfI/ApaI fragment of LV_TREt-Foxg1 (including the last 161nt of Foxg1-cds) with the “SrfI-Foxg1(cds-3’term)-EGFP-ApaI” fragment, detailed in Additional file 6: Table S4).

- LV_CMV-Flag-eIF4E (lentivirus of second generation; Addgene plasmid #38239).
- CMV-Flag-GFP (Addgene plasmid #60360).
- CMV-Flag-Gephyrin (a gift from E.Cherubini’s Lab).
- LV_CMV-EEF1G-V5 (DNASU Plasmid Repository, HsCD00434091).
- LV_CMV-EEF1D-V5 (DNASU Plasmid Repository, HsCD00444454).
- LV_CMV-PUM1-V5 (DNASU Plasmid Repository, HsCD00438817).

Starting from a subset of these plasmids, self-inactivating lentiviral vectors (LV) were generated and titrated as previously described [5].

**Primary neural cell cultures**

Cortical (or tectal) tissue from E16.5 mice or hippocampal tissue from P1 mouse pups were chopped to small pieces for 5 min, in the smallest volume of ice-cold 1×PBS—0.6% D-glucose—5mg/ml DNaseI (Roche #10104159001) solution. After chemical digestion in 2.5×trypsin (Gibco #15400054)—2 mg/ml DNaseI (Roche) for 5 min and trypsin inhibition with DMEM-gluatMAX (Gibco)—10% FBS (Euroclone)—1×Pen-Strep (Invitrogen), cells were spun down and transferred to differentiative medium (Neurobasal-A (Gibco), 1×Glumax (Gibco), 1×B27 supplement (Invitrogen), 25 μM L-glutamate (Sigma), 25 μM β-Mercaptoethanol (Gibco), 2% FBS (Euroclone), 1×Pen/Strept (Invitrogen), and 10 pg/ml fungizone (Invitrogen)). Cells were counted and plated as follows:

(a) in case of RNA profiling (totalRNA-, TRAP-, and RIP-qRTPCR assays) and western blot experiments, cells were plated onto 0.1 mg/ml poly-L-Lysine (Sigma #P2636) pre-treated 12-multiwell plates (Falcon) at 8×10⁵ cells/well in 0.6–0.8 ml differentiative medium;

(b) in case of immunofluorescence and PLA assays, cells were plated onto 0.1 mg/ml poly-L-lysine pre-treated 12 mmØ glass coverslips in 24-multiwell plates (Falcon) at 1×10⁵ cells/well in 0.6–0.8 ml differentiative medium;

(c) in case of live imaging, cells were plated onto 0.1 mg/ml poly-L-lysine pre-treated 35 mmØ glass dishes (Ibidi), at 0.8×10⁵ cells/dish, in 2 ml differentiative medium/dish.
In general, when required and as indicated in each figure: (a) lentiviral infection was done at DIV1-3; (b) TetON-regulated transgenes were activated by 2 μg/ml doxycycline (Clontech #631311) administration; and (c) 10 μM cytosine β-D-arabinofuranoside (AraC; Sigma #C6645) was acutely added to the medium at DIV1. Cells were kept in culture for 8 days.

Live imaging of primary hippocampal cell culture
Hippocampal cultures, set as described above and engineered as in figure legend, were analyzed at DIV8 as follows. Cultures were supplemented by 50 nM Mitotracker dye (Life Technologies #M7512) for 30 min, medium was replaced by PBS, and confocal images were immediately acquired. Live fluorescent imaging was done with a confocal microscope (NIKON A1R) equipped with 488 nm and 594 nm laser excitation light and a 60× oil immersion objective (N.A. 1.40), keeping samples at 37 °C, 5% CO2, and 95% humidity.

HEK293T cell cultures
HEK293T cells were used for lentivirus production, lentivirus titration (Brancaccio et al. 2010), as well as to evaluate protein–protein interactions via co-immunoprecipitation (co-IP) and proximity ligation assay (PLA). HEK293T cells were cultured in DMEM-glutaMAX—10% FBS—1× Pen-Strep, on 6-multiwell plates at 1.2×10^6 cells/well (for co-IP assays) or on 0.1 mg/ml poly-L-lysine pre-treated 12 mmØ glass coverslips in 24-multiwell plates at 3×10^5 cells/well (for PLA assays). In all cases, cells were transfected by LipoD293 (SignaGen laboratories #SL100668) at DIV1, according to manufacturer's instructions. Cells were further kept in culture for 3 and 2 days, for co-IP and PLA assays, respectively, and finally analyzed.

Immunofluorescence assays
Neural cell cultures were fixed by ice-cold 4% PFA for 15–20 min and washed three times in 1× PBS. Samples were subsequently treated with blocking mix (1× PBS; 10% FBS; 1mg/ml BSA; 0.1% Triton X-100) for at least 1 h at room temperature (RT). After that, incubation with primary antibodies was performed in blocking mix, overnight at 4°C. The day after, samples were washed three times in 1× PBS—0.1% Triton X-100 for 5 min and then incubated with secondary antibodies in blocking mix, for 2 h at RT. Samples were finally washed three times in 1× PBS—0.1% Triton X-100 for 5 min, and subsequently counterstained with DAPI (4',6'-diamidino-2-phenylin- dole) and mounted in Vectashield Mounting Medium (Vector).

The following primary antibodies were used:
- Anti-Flag, mouse monoclonal, clone M2, Sigma #F1804, 1:1000;
- Anti-FOXG1 ChIP-grade, rabbit polyclonal, Abcam #ab18259, 1:500 [Fig. 9E, S4B, S5B];
- Anti-FOXG1, rabbit polyclonal, gift from G.Corte, 1:200 [Fig. 1A, S5A];
- Anti-NEUN, guinea pig polyclonal, affinity purified, Merck #ABN90P, 1:800.
- Anti-PSD95, mouse monoclonal, clone 6G6-1C9, Abcam #ab2723, 1:500;
- Anti-Puromycin, mouse monoclonal, clone 12D10, Millipore #MABE343, 1:4000;
- Anti-SMI312, mouse monoclonal, Abcam #ab24574, 1:1000;
- Anti-TUBB3, mouse monoclonal, clone Tuj1, Covance #MMS-435P, 1:1000;
- Anti-V5, mouse monoclonal, clone SV5-Pk1, Abcam #ab27671, 1:800.

Secondary antibodies were conjugates of Alexa Fluor 488 and Alexa Fluor 594 (Invitrogen, 1:600).

Proximity ligation assays (PLAs), puro-PLAs, puro-PLA-run-off assays
PLA assays were performed according to manufacturer’s instructions (Duolink™ PLA Technology, Sigma). Briefly, cells were fixed for 15–20 min in ice-cold 4% PFA, washed three times in 1× PBS, permeabilized in 1× PBS×0.1% Triton X-100 for 1h at RT, blocked for 1 h at 37°C in Duolink blocking buffer and incubated for 3 h/overnight at RT with mouse and rabbit primary antibodies (as indicated in the corresponding Figures). Afterwards, samples were washed three times for 5 min in Duolink buffer A and then incubated for 1 h at 37°C with Duolink anti-mouse MINUS and anti-rabbit PLUS probes, both co-diluted 1:5 in Duolink antibody dilution buffer. Next, samples were washed three times for 5 min in buffer A, incubated for 30 min at 37°C in Duolink ligase diluted 1:40 in 1× ligation buffer, washed again three times in buffer A, and incubated for 100 min at 37°C in Duolink polymerase diluted 1:80 in 1× green or red amplification buffer. Finally, samples were washed two times for 10 min in Duolink buffer B and 1 time in 1:100 buffer B for 1 min and mounted in Duolink mounting medium with DAPI. Then, by 48 h, confocal images were acquired.

Puro-PLA samples [68] were prepared as indicated in the corresponding figures and schematized in Additional file 1: Figure S3. Briefly, cortico-cerebral cells were pulsed for 5 min with 3 μM puromycin (Sigma #P8833) or with 1× PBS (negative control) and, immediately afterwards,
fixed in ice-cold 4% PFA for 15 min. Then, they were processed by standard PLA, as above.

Puro-PLA-run-off DIV8 samples were prepared as indicated in the corresponding figures. In particular, before terminal puromycin labeling, cells were cumulatively exposed to 2 μg/ml harringtonine (Abcam #ab141941) for 20' or (20+x)’ depending on the “T0” or the “Tx” branch of the protocol and 208 μM emetine (Sigma #E2375) for 20 min. Finally, during the last 5’ of harringtonin/emetin treatment, unfinished polypeptides were labeled via further medium supplementation by 10 μg/ml puromycin. Immediately afterwards, samples were fixed in ice-cold 4% PFA for 15 min and processed for standard PLA, as above.

The following primary antibodies were used:

- Anti-CAMK2B, rabbit polyclonal, GeneTex #GTX133072, 1:500;
- Anti-EF1D, mouse monoclonal, clone 3B1B11, Proteintech #60085-1-Ig, 1:200;
- Anti-EF1E, mouse monoclonal, clone 5D11, Ther-mofisher #MA1-089, 1:100;
- Anti-Flag, mouse monoclonal, clone M2, Sigma #F1804, 1:1000.
- Anti-FMR1, rabbit monoclonal, Huabio #ET1703-70, 1:500;
- Anti-FOXG1, rabbit polyclonal, ChIP-grade, Abcam #ab18259, 1:500;
- Anti-GRIN1 COOH-term, rabbit monoclonal, clone EPR2481(2), Abcam #ab109182, 1:500;
- Anti-GRIN1 NH2-term, rabbit polyclonal, Alomone #AGC-001, 1:500;
- Anti-HOMER1, rabbit polyclonal, GeneTex #GTX103278, 1:300;
- Anti-NMT1, rabbit polyclonal, GeneTex #GTX130852, 1:500;
- Anti-puromycin, mouse monoclonal, clone 12D10, Millipore #MABE343, 1:1000;
- Anti-SGK1, rabbit polyclonal, GeneTex #GTX54726, 1:200;
- Anti-V5, mouse monoclonal, clone SV5-Pk1, Abcam #ab27671, 1:1000.

Neuronal stimulation assays
Cortico-cerebral cultures were set up as described above (to see “Primary neural cell cultures”) and as detailed in Fig. 7. Specifically, their terminal DIV8 manipulation was as follows. “K5” samples were pulsed with 55 mM KCl-supplemented medium for 5 min. “K10-noK25” samples were firstly pulsed with 55 mM KCl-supplemented medium for 10 min and then transferred to a conditioned medium, taken from unstimulated sister cultures, for 25 min. “Ctr” samples were kept in standard, not KCl-supplemented medium. Next, “K5”, “K10-noK25,” and “Ctr” cells were all pulsed by 3 μM puromycin for 5 min and, immediately afterwards, fixed in ice-cold 4% PFA for 15 min.

Photography and image analysis
Basic immunofluorescence
αFoxg1-, αTubb3-, αPSd95-, αSmi312-, and αPuro-immunoprofiled cells were photographed by a Nikon C1 confocal system equipped with 40×oil objective (Figs. 1A–F and 3B). Photos were collected as 3 μm Z-stacks (step=0.3 μm). Upon Z-stack flattening (max version), pictures were imported into Adobe Photoshop CS6, for subsequent processing. αFlag- and αHA-immunoprofiled cells were photographed by a Nikon Eclipse TI microscope, equipped with a 40×objective through the Hamamatsu 1394 ORCA-285 camera (Fig. 4B). Collected as 1024×1024 (case Fig. 3B) and 1344×1024 pixel images (case Fig. 4B), photos were imported in Volocity 6.5.1 for analysis (Figs. 3B and 4B). Here, for each individual neuron, an ROI was outlined by an operator blinded of sample identity and background-subtracted, average αFlag, and αHA, non-nuclear signals, and total-cell αPuro signal were collected.

PLA analysis
PLA-profiled cells were photographed by a Nikon C1 confocal system equipped with 40×oil objective (Figs. 3A, 6, 7, 9, and 11; Additional file 1: Figure S2A). Photos were collected as 2 μm Z-stacks (step=1μm) and 3 μm Z-stacks (step=1μm) of 1024×1024 pixel images, for Additional file 1: Figure S2A and Figs. 3A, 5, 7, 9, and 11, respectively. All primary images were generally analyzed with Volocity 6.5.1 software (here, positive spots were 3D clusters including ≥1 voxels, each voxel corresponding to 0.1 μm³ and displaying a signal above 90 background standard deviations; for cumulative PLA signal calculation, only voxels above this threshold were taken into account). Limited to Fig. 5A (b), files originating from flattened Z-stacks (max version) were imported into Adobe Photoshop CS6 and 2D-spots counting was performed manually by an operator blind of sample identity. When appropriate (Figs. 3A and 5A), spot counting and/or cumulative signal evaluation was restricted to specific cell compartments (highlighted in gray, in idealized neuron silhouettes).

Common
Results of numerical image analysis were imported into Microsoft Excel for subsequent processing. Finally, representative photos were edited for figure preparation by ImageJ-Fiji and Adobe Photoshop CS6 softwares.
**Total RNA extraction**

Total RNA was extracted from cells (Fig. 2D, E) using TRIzol Reagent (Thermofisher) according to the manufacturer’s instructions, with minor modifications. Briefly, for each biological replicate, a pellet including 300,000–800,000 cells was dissolved in 250–500 μl of Trizol. RNA was precipitated using isopropanol and GlycoBlue (Ambion) overnight at −80°C. After two washes with 75% ethanol, the RNA was resuspended in 20 μl sterile nuclease-free deionized water. Agarose gel electrophoresis and spectrophotometric measurements (NanoDrop ND-1000) were employed to estimate its concentration, quality, and purity.

**Translating ribosome affinity purification (TRAP) assay: RNA preparation**

The TRAP assay was performed as previously described [52, 69] with minor modifications. For each TRAP reaction, 10 μg of anti-GFP antibody, purchased from the Monoclonal Antibody Core Facility at the Memorial Sloan-Kettering Cancer Center (purified form of HtzGFP-19C8), was covalently bound to 1 mg magnetic epoxy beads (Dynabeads Antibody Coupling kit, Life Technologies #14311D), according to manufacturer’s protocols, followed by BSA treatment to reduce non-specific binding. Antibody-coupled beads were resuspended at the concentration of 1 mg/100μl. Cortico-cerebral cells, derived from Rpl10αEGFP–Rpl10α/+ embryos, were set up as described above (see “Primary neural cell cultures”) and as detailed in Fig. 2A. At DIV8, cells were treated by supplementing the medium with 0.1mg/ml cycloheximide (CHX; Sigma #C7698) at 37°C for 15 min. Then, cells were washed two times with ice-cold 1× PBS containing 0.1mg/ml CHX; 75 μl ice-cold lysis buffer (see below) was added to each cell-containing well (12-multiwell plate) for 10 min on ice. Afterwards, cells were scraped and lysed by vigorously pipetting them up and down without creating bubbles. The lysate derived from two wells (about 1.6×10⁶ cells; corresponding to one biological replicate) was pooled. Upon addition to each replicate sample of 1/9 volume of 300 mM 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC, Avanti Polar Lipids #850305), such sample was firstly centrifuged at 2000 × g for 10 min at 4°C. The resulting supernatant (about 150 μl) was incubated with 100 μl antibody-coupled beads for 1 h at 4°C on a rotating wheel at 10 rpm. After incubation, beads were collected with a magnet; the immunoprecipitated component (TRAP-IP) bound to beads was washed four times with 1 ml of ice-cold high-salt buffer (see below); the supernatant component (TRAP-SN) of each sample was stored on ice. (Lysis buffer: 20 mM HEPES (Ambion), 150 mM KCl (Ambion), 10 mM MgCl2 (Ambion), 1%(vol/vol) NP-40 (Thermo Fisher Scientific), 1× EDTA-free protease inhibitors (Roche), 0.5 mM DTT (Invitrogen), 0.1 mg/ml cycloheximide, 10 μl/ml RNAsin (Promega), and 10 μl/ml Superasin (Applied Biosystems). High-salt buffer: 20 mM HEPES, 350 mM KCl, 10 mM MgCl2, 1%(vol/vol) NP-40, 1× EDTA-free protease inhibitors, 0.5mM DTT, 0.1 mg/ml cycloheximide). For each sample, RNA of TRAP-SN and TRAP-IP fractions were extracted with Trizol® LS reagent (Thermofisher) according to manufacturer’s instructions, with minor modifications. The extraction procedure was repeated to improve RNA sample purity. RNA was finally precipitated using NaOAc, isopropanol, and GlycoBlue overnight at −80°C, according to standard protocols. After two washes with 75% ethanol, the RNA was resuspended in 10 μl sterile nuclease-free deionized water. Agarose gel electrophoresis and spectrophotometric measurements (NanoDrop ND-1000) were employed to estimate quantity, quality, and purity of the resulting preparation.

**RNA immunoprecipitation (RIP) assay: RNA preparation**

Cortico-cerebral cells were set up as described above (see “Primary neural cell cultures”) and as detailed in Fig. 6. For each RIP reaction, 10 μl of protein A/G Dynabeads (Thermofisher #492024) were coupled with 10 μg of anti-protein of interest (POI; anti-FOXG1 ChIP-grade, rabbit polyclonal, Abcam #ab18259; anti-GFP, rabbit polyclonal, Abcam #ab290), or 10 μg of rabbit IgG (Millipore #12370) as control, according to manufacturer’s protocols. Pre-clearing beads were prepared omitting antibody coupling. DIV8 cells were washed once with ice-cold 1× PBS; 75 μl ice-cold lysis buffer (see below) was added to each cell-containing well (12-multiwell plate) for 10 min on ice. Afterwards, the cells were scraped and lysed by vigorously pipetting them up and down without creating bubbles. The lysate derived from 10 wells (about 8×10⁶ cells; to be employed for one set of paired anti-POI/IgG assays) was pooled, pipetted up and down, and kept 10 min on ice. Pipetting and incubation on ice were repeated. Next, each sample was centrifuged at 2000g for 10 min at 4°C. Then, the supernatant was re-centrifuged, at 16,000g for 10 min at 4°C. The resulting supernatant was incubated with pre-clearing beads (pre-equilibrated in lysis buffer, see below) for 30 min at 4°C on a rotating wheel, at 10rpm. Then, the pre-clearing beads were removed with a magnet, and the supernatant was incubated with antibody-coupled beads (pre-equilibrated in lysis buffer), overnight at 4°C on a rotating wheel, at 10 rpm; 10% of supernatant (Input, RIP-IN) was stored at −80°C. The day after, the beads were collected with a magnet and the immunoprecipitated material bound to beads was harvested by washing them five times...
with 0.5 ml of ice-cold high-salt buffer. (Lysis buffer: 25 mM TRIS-HCl, 150 mM KCl (Ambion), 10 mM MgCl2 (Ambion), 1%(vol/vol) NP-40 (Thermo Fisher Scientific), 1×EDTA-free protease inhibitors (Roche), 0.5 mM DTT (Invitrogen), 10 μl/ml rRNasin (Promega), and 10 μl/ml Superscin (Applied Biosystems). High-salt buffer: 25 mM TRIS-HCl, 350 mM KCl (Ambion), 10 mM MgCl2 (Ambion), 1%(vol/vol) NP-40 (Thermo Fisher Scientific), 1×EDTA-free protease inhibitors (Roche), and 0.5 mM DTT (Invitrogen)). For each sample, immunoprecipitated RNA (RIP-IP) and input (RIP-IN) were extracted with Trizol® LS reagent according to manufacturer's instructions, with minor modifications. The extraction procedure was repeated to improve RNA sample purity. RNA samples were precipitated using isopropanol and GlycoBlue overnight at −80°C, according to standard protocols. After two washes with 75% ethanol, the RNA was resuspended in 10 μl sterile nuclease-free deionized water. Agarose gel electrophoresis and spectrophotometric measurements (NanoDrop ND-1000) were employed to estimate quantity, quality, and purity of the resulting RNA.

**RNA quantitation:** **DNase treatment, reverse transcription, and real-time quantitative PCR**

DNA contaminants were removed from total RNA, TRAP-SN, RIP-IN, and RIP-IP samples by treating them with TURBO™ DNase (2U/μl) (Ambion) for 1 h at 37°C, following manufacturer's instructions. cDNA was produced via reverse transcription (RT) of the resulting preparations by Superscript™III (Invitrogen), primed by random hexamers, according to manufacturer's instructions. For RT reactions, the following aliquots of RNA preparations were used: 1/10 TRAP-IP, 1/10 (DNA-free) TRAP-SN, 1/6 (DNA-free) IP- and IN-RIP, and 0.5 μg (DNA-free) total RNA. Following Superscript™III thermo-inactivation, the RT reaction (20 μl) was diluted 1:3 (in case of TRAP samples) or 1:5 (in case of RIP and total RNA samples), and 1–2 μl of the resulting cDNA solution was used as substrate of any subsequent quantitative PCR (qPCR) reaction. Limited to intronless amplicons and/or TRAP-IP, RIP-IN, and RIP-IP samples, negative control PCRs were run on RT(-) RNA preparations. qPCR reactions were performed by the SsoAdvanced SYBR Green Supermix™ platform (Biorad), according to manufacturer's instructions, on a CFX Bio-Rad thermocycler. For each transcript under examination and each sample (i.e., biological replicate), cDNA was qPCR-analyzed in technical triplicate and results averaged. In case of total RNA and TRAP-IP and TRAP-SN samples, mRNA levels were normalized against Rpl10a-mRNA [70]. In addition, in case of TRAP samples, IP/IN ratios were further calculated per each sample, as indices of mRNA engagement to holoribosomes. In case of RIP samples, IP values were strictly normalized against IN values. Final results were averaged and the corresponding sems calculated using Excel software.

The following oligonucleotides have been employed in this study:

- **Psf95/F:** GCC GTG GCA GCC CTG AAG AAC ACA
- **Psf95/R:** GCT GCT ATG ACT GAT CTC ATT GTC CAGG
- **Foxg1(cds)/F:** GAC AAG AAG AAC GGC AAG TAC GAG AAGC
- **Foxg1(cds)/R:** GAA CTC ATA GAT GCC ATT GAG CGT CAGG
- **Foxg1(5utr)/F:** TAG AAG CTG AAG AGG AGG TGG AGT GC
- **Foxg1(5utr)/R:** CAG ACCCAAACAGTCCCCGAAA TAAAGC
- **Gria1/F:** TCC ATG TGA TCG AAA TGA AGC ATG ATG GAA TCC
- **Gria1/R:** CGA TGT AGG TTC TAT TCT GGA CGC TTG AGT TG
- **pan-Grin1/F:** CGA GGA TAC CAG ATG TCC ACC AGA CTA AAG A
- **pan-Grin1/R:** CTT GAC AGG GTC ACC ATT GAC TGT GAACT
- **ex20-Grin1/F:** CCG TGA ACG TGT GGA GGA AGA ACC T
- **ex20-Grin1/R:** GTG TCT TTG GAG GAC CTA CGT CTC TTG
- **Grid1/F:** AAG GAC TGA CTC TCA AAG TGG TGA CTG TCT T
- **Grid1/R:** CCT TAC TCC TCA AAG TGG TGA CTG TCT T
- **Gabra1/F:** AAA CCA GTA TGA CCT TCT TGG ACA AAC AGT TGAC
- **Gabra1/R:** GTG GAA GTG AGT CGT CAT AAC CAC ATA TTC TC
- **Slc17a6/F:** TTT TGC TGG AAA ATC CCT CGG ACA GAT CTA CA
- **Slc17a6/R:** CTT ACC TGTC ATC ACC AGT GCC AAC GAT CTA CA
- **Bdnf2c/F:** CTT TGG GAA ATGCAA GTG TTT ATC ACC AGG AT
- **Bdnf4/F:** CTG CCT TGA TGT TTA CTT TGA CAA GTA GTG ACTG
- **Bdnf(2c,4)/R:** GCC TTC ATGCAA CGT GCC AGT GCC AGT GCC AGT GCC
- **Rpl10a/F:** CAG CAG CAC TGT GAT GAA GCC AAG G
- **Rpl10a/R:** GGG ATC TGC TTA ATC AGA GAC TCA GAGG
\[ F\text{-}rnoGrin1\text{-}H/F: \quad ACCTCCACCCCTGCGCTCC \]
\[ F\text{-}rnoGrin1\text{-}H/R: \quad GGGTAGCCAGCGTAATC \]
\[ rnoGrin1.d/F1: \quad AGTCGCGCCTCGACTCGAGAG \]
\[ rnoGrin1.d/F2: \quad GTCGCGCCTCGAGCAACCC \]
\[ rnoGrin1.d/F3: \quad CCCAAAGATCGTCAAATCGGC \]
\[ rnoGrin1.d/F4: \quad CTTGCGCTGTGAATTCAATG \]
\[ rnoGrin1.d/F5: \quad TGGAGATAAGATGGAATGTA \]
\[ rnoGrin1.d/F6: \quad TGCAGGATAGAAGATGGA \]
\[ rnoGrin1.d/R1: \quad CAGTGTTGTAGCCTAAAGGA \]
\[ rnoGrin1.d/R2: \quad ACTTCTGTGAAGCCTCAAAC \]
\[ rnoGrin1.d/R3: \quad TCCTCCTCTCTAAGCG \]
\[ rnoGrin1.d/R4: \quad TGAGTGGCGACAGAAGTGCGT \]
\[ rnoGrin1.d/R5: \quad GGGCGACACAGATGCGT \]

NB. rnoGrin1.d oligos employed for assays referred to by Fig. 6B were associated as follows:

- \( d0/d1 \) assay: \( rnoGrin1.d/F1, rnoGrin1.d/F2, rnoGrin1.d/R1 \)
- \( d0/d2 \) assay: \( rnoGrin1.d/F3, rnoGrin1.d/R2, rnoGrin1.d/R4 \)
- \( d0/d3 \) assay: \( rnoGrin1.d/F4, rnoGrin1.d/F6, rnoGrin1.d/R5 \)
- \( d0/d4 \) assay: \( rnoGrin1.d/F5, rnoGrin1.d/F6, rnoGrin1.d/R5 \)
- \( d0/d5 \) assay: \( rnoGrin1.d/F3, rnoGrin1.d/R3, rnoGrin1.d/R4 \)

**Ribosome engagement analysis**

Transcripts whose ribosome engagement was affected by Foxg1 overexpression were identified as follows. First, \( Mus\ var textrm{musculus} \) mRNA sequences (GRCm38.p6 reference genome version) were retrieved by Ensembl Biomart [71], selecting the principal isoform of each gene according to APPRIS annotations [45] (if more transcripts were indexed at the highest level, then the longest one was selected). The resulting reference transcriptome included 22,442 transcripts. On this transcriptome, total RNA-seq FASTQ reads [17] as well as TRAP-seq FASTQ reads, originating from sister primary neural cultures, were mapped using Bowtie2 [72] (in “very-sensitive-local” configuration). Finally, the number of reads mapped to each transcript was computed by means of featureCounts [73] (with “primaryOnly=TRUE” and “minMQS=10” settings).

Next, for both total RNA-seq and TRAP-seq assays, differential gene expression analysis was performed using the \( R \) package DESeq2 software [46]. Then, for each gene, the difference between log2FC(trapRNAseq) and log2FC(totalRNAseq) (named \( D\log2FC \)) was primarily calculated, as an index of Foxg1-OE impact on mRNA engagement to ribosomes. Moreover, statistical significance of \( D\log2FC \) values was evaluated with Python package Ribodiff software (default parameters) [47]. Finally, genes were filtered out if not satisfying “\( p\text{adj} < 0.1 \)” conditions, as well as if not reaching the “\( \text{baseMean} \)” DESeq2 value of 200 in case of both RNA-seq and TRAP-seq profiling (Artimagnella and Mallamaci, doi: temporarily restricted).

**Ribosome progression analysis**

This analysis was performed, taking advantage of the reference transcriptome generated for ribosome engagement analysis. TRAP-seq FASTQ reads were mapped on it using Bowtie2 (in “very-sensitive-local” configuration) and those falling within the cds further taken into account. Hence, for each transcript, the cds was divided in 125-nt bins and the number of reads mapping to each bin was computed by featureCounts.
were considered significant (here, Δψsi is the difference in percentage of "spliced-in transcripts" between Foxg1-OF and control samples).

In the case of polyadenylation analysis, ROAR software [49] was used. Genes with 1/1.2 ≥ r ≥ 1.2 and padj < 0.05 were considered significant (here, being the m/M the ratio between the shortest and the longest polyA isoform, r is the ratio between Foxg1-OF and control m/M parameters).

**RIP-seq profiling**
Produced as described in “RNA immunoprecipitation (RIP) assay: RNA preparation” section, RIP samples were sequenced by IGA Technology Services Srl. Libraries were produced using retrotranscribed cDNA previously amplified by Ovation Ultralow Library System V2 (NuGEN Technologies, Inc.). Library size and integrity were assessed using the Agilent Bioanalyzer (Santa Clara, CA) or Caliper GX (PerkinElmer, MA) apparatus. Sequencing was performed by Illumina HiSeq 2500 (Illumina, San Diego, CA); 10 M paired-end reads (2 × 125nt) per replicate were generated; 3 anti-Foxg1 and 3 IgG-Ctr paired samples were profiled. Quality control of the sequenced reads was performed by a commercial operator (Sequentia, Barcelona, Spain). Reads were processed with the FASTQC v0.11.5 software, then low-quality bases and adapters were removed with the software BBduk version 35.85, setting a minimum base quality of 30 and a minimum read length of 35 bp. So-filtered high-quality reads were used in the following analyses.

**RIP-seq analysis and identification of FOXG1-protein-interacting transcripts with Foxg1-sensitive ribosomal engagement and progression rates**
Foxg1 protein-bound transcripts were identified as follows (steps 1–3 executed by Sequentia, Barcelona, Spain).

First, a reference transcriptome was generated. The web-based tool Biomart [71] was used to extract GenElDs, TranscriptIDs, cDNA sequences, APPRIS annotations [45], and transcript support levels (TSLs) of mouse genome GRCm38.6. To select a unique representative transcript per gene, these rules were sequentially implemented: (1) the transcript with the highest APPRIS annotation level was chosen; (2) if multiple transcripts with the same annotation level were available, the transcript with the highest TSL was chosen; (3) if more than one transcript had the same TSL, one of them was randomly selected; and (4) if a gene had no transcripts with either an APPRIS annotation or a TSL, one transcript was also randomly chosen. The final reference transcriptome consisted of 55,647 unique transcripts.

Second, prior to mapping the reads to transcripts, the reference transcriptome was indexed with STAR (version
2.7.9a), using the genomeGenerate function. The parameter "genomeChrBinNbits" was set according to the formula:

\[
\min(18, \log_2(\max(\text{GenomeLength}/\text{AmountOfReferences}, \text{ReadLength})))
\]

RIP-seq reads were mapped in local alignment mode, with maximum intron size set to 1, so that the resulting BAM files did not actually include reads mapped on introns.

Third, Foxg1 protein/mRNA interaction peaks were identified by SICER2 (version 1.0.2) [75]. The SICER2/sicer/lib/GenomeData.py file was manually edited in the SICER2 repository, to include a list of our reference transcriptome transcripts IDs, and a Python dictionary that maps these IDs to their lengths. Moreover, SICER was run setting its parameters as follows: “fragment_size=median read length”, “redundancy_threshold=1”, “window_size=200”, “gap_size=200”, and “effective_genome_fraction=1”. In this way, 8352, 8851, and 7120 peak islands with fdr < 0.1 were identified in samples 1, 2, and 3, respectively.

Fourth, peak islands were filtered out if not satisfying “aFoxg1/IgG_enrichment ≥ 2” and “fdr < 0.05”. Next, transcripts sharing ≥1 peak island in ≥2 out of 3 biological replicates were considered as interacting with the Foxg1 protein. A total of 2857 transcripts satisfied this requirement.

Fifth, to estimate the magnitude of the geneset undergoing direct Foxg1 regulation of translation, these 2857 transcripts were intersected with the 358 and 328 ones resulting from our “Ribosome engagement analysis” and “Ribosome progression analysis” pipelines, respectively.

### Co-immunoprecipitation (co-IP) assay
HEK293T cell lines were cultured and transfected as described in “HEK293T cell cultures” section and as detailed in Additional file 1: Figure S3B. After 3 days, cells were washed in 1× PBS and lysed with 500 μl of CHAPS buffer, supplemented with 1× protease inhibitors (Roche). Next, lysates were processed for co-IP analysis by the FLAG Immunoprecipitation Kit (Sigma), according to manufacturer’s instructions. Specifically, total cell lysates were centrifuged at 12,000 g for 10 min at 4°C to remove debris. For each sample, the 4% of supernatant was saved as input (IN). The remaining part was incubated with anti-Flag-conjugated resin for 3h at 4 °C, on a rotating wheel. Next, the immuno-precipitated resin (IP) was resuspended and washed four times in 1× wash buffer. Finally, IP and IN samples were denatured at 95 °C for 5 min in 1× sample buffer (supplemented with 0.5% β-Mercaptoethanol), prior to subsequent western blot analysis.

### Protein degradation assay
Cortico-cerebral cells were set up as described above (see “Primary neural cell cultures”) and as detailed in Fig. 3C. At DIV8, cells were treated with 50 μg/ml cycloheximide (CHX). Cells were analyzed at four different time points, 0, 3, 6, 10, and 14 h after CHX administration. For each point, samples were lysed in CHAPS buffer, supplemented with 1× protease inhibitors (Roche), and stored at −80°C. Upon thawing, samples were centrifuged at 12,000g for 10 min at 4°C, to remove debris, and then processed for western blot analysis.

### Western blot analysis
Western blot analysis was performed according to standard methods. Total cell lysates in CHAPS buffer were quantified by BCA protein assay kit (Fisher Scientific #10678484) (except for co-IP samples) and denatured at 95°C for 5 min, prior to loading; 20–30μg of proteins were loaded per each lane on a 10% acrylamide—0.1% SDS gel. Afterwards, proteins were transferred to nitrocellulose membrane. Membranes were incubated 1 h in 1×TBS-Tween containing 5% non-fat dry milk before to be exposed to primary antibodies at 4°C overnight. Then, membranes were washed three times in 1×TBS-Tween, incubated 1 h with HRP-conjugated secondary antibodies (DAKO, 1:2000) in 1×TBS-Tween containing 5% non-fat dry milk, at room temperature, washed again three times, and finally revealed by an ECL kit (GE Healthcare #GERPN2109). The following primary antibodies were used: anti-FOXG1, rabbit polyclonal, ChIP-grade, Abcam #ab18259, 1:1000 (Additional file 1: Fig. S1B); anti-FOXG1, rabbit polyclonal, a gift from G.Corte, 1:2000 (Additional file 1: Fig. S2B); anti-Flag, mouse monoclonal, clone M2, Sigma #F1804, 1:1000; anti-GRIN1-COOH-term, rabbit monoclonal, clone EPR2481(2), Abcam #ab109182, 1:5000; anti-beta-ACTIN, mouse monoclonal, HRP-conjugated, Sigma #A3854, 1:20000. Images were acquired by an Alliance LD2–77.WL apparatus (Uvitec, Cambridge) and analyzed by Uvitec Nine-Alliance software. Finally, protein levels were normalized against β-actin. Uncropped pictures of western blot assays are reported in Additional file 7: Fig. S6.

### Numerical and statistical analysis
Full details of numerical and statistical analysis of data (including normalization criteria, number and definition of biological replicates, statistical tests employed for
result evaluation) are provided in the figures and their legends.

Full primary data referred to in Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 and Additional file 1: Figures S1-5, as well as full details of their statistical evaluation, are reported in Additional file 8: Table S5.

Abbreviations
4OHt  4-Hydroxytamoxifen
AA  Amino acid
AMP  Adenosine monophosphate
APPRIS  Annotating principal splice isoforms software
ARC  Activity-regulated cytoskeleton-associated protein
ASD  Autism spectrum disorder
BAC  Bicinchoninic acid assay
BCD  Bicoid protein
Bdnf  Brain-derived neurotrophic factor
CA3H  Comprehensive alternative splicing hunting software
cds  Coding sequence
CHAPS  3-[3-Cholamidopropyl] dimethylammonio)-1-propanesulfonate
COOH-term  Carboxy terminal
DeSeq2  Differentially expressed RNA Sequences software, version 2
DIV  Day in vitro
E  Embryonic day
EEF1D  Eukaryotic elongation factor 1D
EEF1G  Eukaryotic elongation factor 1G
EGFP  Enhanced green fluorescent protein
EIF4E  Eukaryotic initiation factor 4E
EIF4E-BP  EIF4E-binding protein
EIF4G  Eukaryotic initiation factor 4G
EMX2  Empty spiracles homeobox 2 protein
EN2  Engrailed homeobox 2 protein
ERT2  Estrogen receptor tamoxifen-binding domain, version 2
ex20  Exon 20
Flag  Flag epitope (DYKDDDDK)
Fmr1  Fragile X messenger ribonucleoprotein 1
FOXG1  Forkhead box G1 protein
Gabra1  Gamma-aminobutyric acid type A receptor subunit alpha1
GAD1  Glutamate decarboxylase 1
GO  Gene Ontology
Gria1  Glutamate ionotropic receptor AMPA type subunit 1
Grin1  Glutamate ionotropic receptor NMDA type subunit 1
HOMER1  Homer scaffold protein 1
IF  Immunofluorescence
IP  Immunoprecipitated
iPSC  Induced pluripotent stem cells
LOF  Loss of function
LTP  Long-term potentiation
Mapt  MAPT-microtubule associated protein tau
MS  Mass spectrometry
muu  Mus musculus
(n)  Nascent
NH2-term  Amino-terminal
NMlDA  N-Methyl-D-aspartic acid
NMlDAR  NMlDA receptor
NMT1  N-Myristoyltransferase 1
NPSA4  Neuronal PAS domain protein 4
OE  Over-expressing
PLAP  Placental alkaline phosphatase (encoded by human ALPP gene)
PSD95  Postsynaptic density protein 95 (encoded by Dlg4 gene)
PlmU1  Pumilio RNA-binding family member 1
Puro-PLA  Puromycin proximity ligation assay
qRTPCR  Quantitative retrotranscription polymerase chain reaction
RiboDiff  Ribosomal (vs total) mRNA differential analysis software
RIP  RNA immunoprecipitation
ROAR  Ratio of a ratio software
Rpi  Ribosome progression index
Rpl10a  Ribosomal protein L10a
SGK1  Serum/glucocorticoid regulated kinase 1
SICER  Spatial clustering for identification of ChiP-enriched regions
Slc17a6  Solute carrier family 17 member 6 (aka VGLUT2, vesicular glutamate transporter 2)
SMI312  Neurofilament medium chain antigen, recognized by the SMI312 antibody
SN  Supernatant
STAR  Spliced transcripts alignment to a reference software
TetON  Tetracycline-activated
totrRNAseq  Total RNA reads
TRAP  Translating ribosome affinity purification
trapRNAseq  TRAP RNA reads
TSL  Transcript support level
TUBB3  Tubulin beta 3 class II
V5  V5 epitope (GFPfP[NPLG]L)ST
WB  Western blot

Supplementary Information
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Authors’ contributions
O.A. performed experiments, analyzed data (including bioinformatic processing of tot-, trap- and rip-Seq data), and contributed to writing the manuscript. E.S.M. performed experiments and analyzed data, with particular emphasis on revision of the original manuscript. M.E. took care of bioinformatic analysis. A.M. designed the study, supervised its execution, prepared figures, wrote the manuscript, and revised them. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article; its supplementary information files, and publicly available repositories. In particular: (a) primary numerical values referred to by figure graphs are in Additional file 7_Table S5; (b) uncropped pictures of western blot assays reported in this study are in Additional file 7_Figure S6; and (c) raw totRNA, trapRNA, and ripRNA data employed for ribosome engagement and progress analysis are in Additional file 8_Table S5; (b) uncropped pictures of western blot assays reported in this study are in Additional file 7_Figure S6; and (c) raw totRNA, trapRNA, and ripRNA data employed for ribosome engagement and progress analysis are in Additional file 8_Table S5; (b) uncropped pictures of western blot assays reported in this study are in Additional file 7_Figure S6; and (c) raw totRNA, trapRNA, and ripRNA data employed for ribosome engagement and progress analysis are in Additional file 8_Table S5; (b) uncropped pictures of western blot assays reported in this study are in Additional file 7_Figure S6; and (c) raw totRNA, trapRNA, and ripRNA data employed for ribosome engagement and progress analysis are in Additional file 8_Table S5.

Declarations

Ethics approval and consent to participate
Animal handling and subsequent procedures were in accordance with European and Italian laws (European Parliament and Council Directive of 22 September 2010 (2010/63/EU); Italian Government Degree of 04 March 2014, no.26). Experimental protocols were approved by SISSA OpBA (Institutional SISSA Committee for Animal Care) and authorized by the Italian Ministry of Health (Auth. No 22DABN-4GJ).

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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