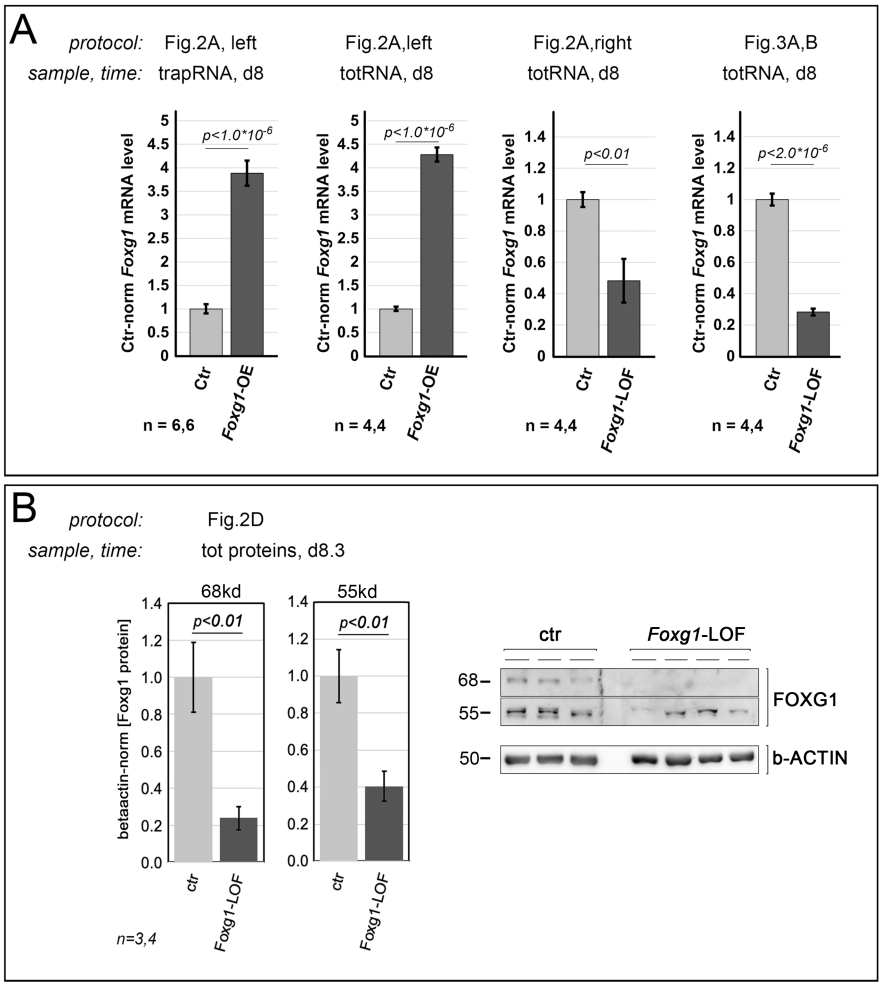
Figures S1-S5

Figure S1



Legend to Figure S1. Evaluation of *Foxg1*-mRNA/FOXG1-protein expression levels in primary neocortical cultures, upon lentiviral delivery of a *Foxg1*-encoding or an anti-*Foxg1*-shRNA-encoding transgene. (A) qRTPCR evaluation of *Foxg1*-mRNA levels. Double-normalized against *Rpl10a* and controls, results refer to samples described in Figures 2A and 3A,B. **(B)** WB evaluation of FOXG1 protein levels. Double-normalized against beta-actin and controls, results refer to samples described in Figure 2D. 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 one-way t-test, one-tailed, unpaired and homoscedastic. Errors bars indicate s.e.m.

Figure S2

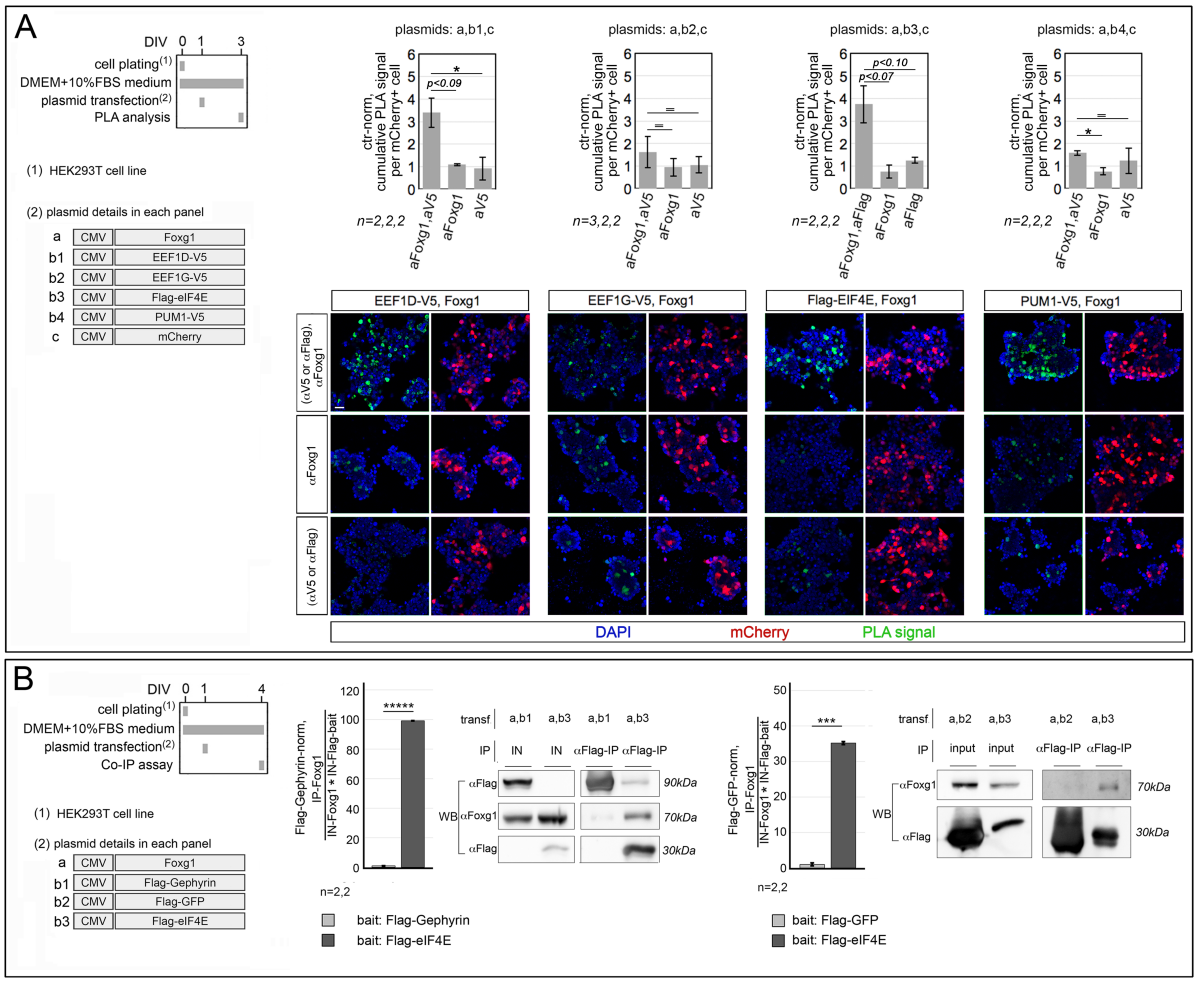
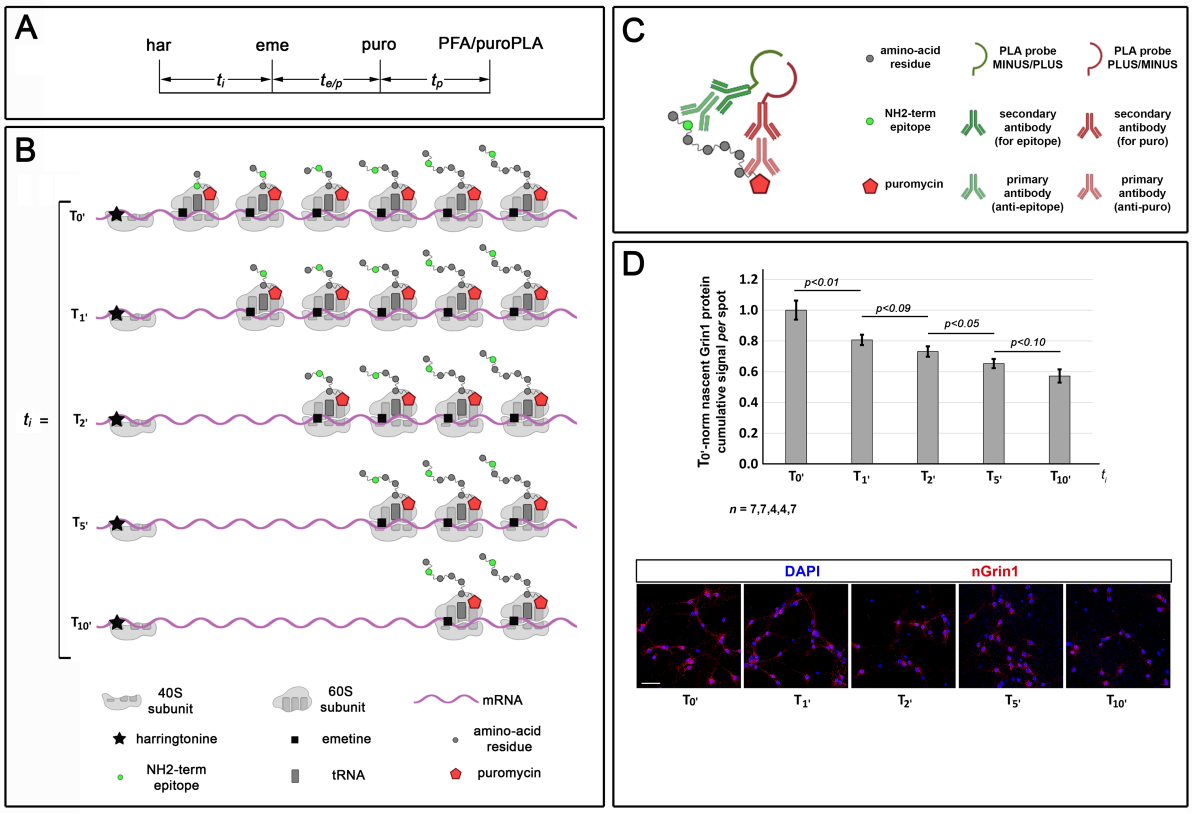


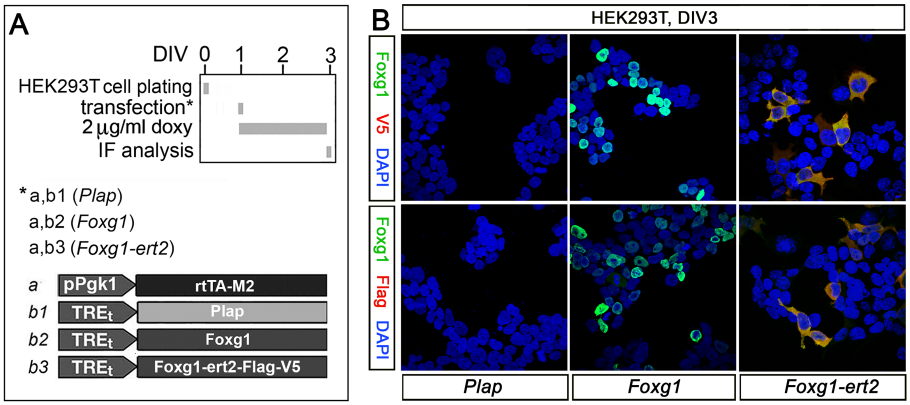
Figure S2. Assessment of FOXG1-protein interaction with selected translation factors in engineered HEK293T cells. (A) Interaction evaluation based on proximity ligation assay (PLA). To left, protocols and lentiviral vectors used, to right, graphical representation of results (upper row) and examples of primary data (lower row). Cells co-transfected, by FOXG1- and Flag-TranslationFactor- or TranslationFactor-V5- encoding transgenes, and further transfected by an mCherry transgene, as an internal control. Interaction signals revealed by anti-FOXG1/anti-Flag- or anti-FOXG1/anti-V5-driven PLAs, mCherry revealed by direct fluorescence. For each co-transfection type, shown is the cumulative "green" PLA signal per transfected mCherry^{+} cell, normalized against the average values of the two corresponding negative controls (each obtained by omitting either primary antibody). **(B)** Evaluation of FOXG1/EIF4E interaction by immuno-precipitation-western-blot (IP-WB) analysis. To left, protocols and lentiviral vectors used, to right, graphical representation of results, with examples of primary data. Cells double transfected, by FOXG1- and Flag-EIF4E-encoding transgenes (negative controls: Flag-GEPHYRIN and Flag-EGFP), IP by anti-Flag, immuno-detection by anti-FOXG1. Densitometric IP-protein values double normalized, against FOXG1 and Flag-protein inputs, and further normalized, against negative controls. Throughout 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 and homoscedastic [panel A (EEF1G-V5 assay)] or heteroscedastic [panel A (EEF1d-V5, Flag-EIF4E, and PUM1-V5 assays) and B]. * p<0.05, *** p<0.001, ***** p<0.00001. Errors bars indicate s.e.m. Scalebars, 50µm.

Figure S3



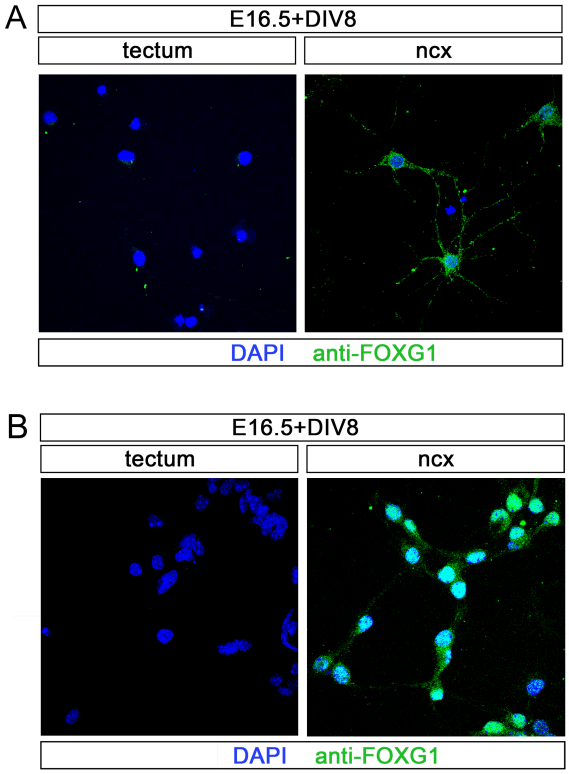
Legend to Figure S3. Puro-PLA run off assay. (**A**) Temporal articulation of the puro-PLA run off assay. (**B**) Idealized representation of ribosomes progression along mRNA, upon harringtonine blockade of translation initiation. (**C**) Revealing puro-labeled polypeptides by PLA. (B) and (C) pictures created in "BioRender.com". (**D**) Graph representing the cumulative signal per spot of nascent GRIN1 (nGRIN1) polypeptide at different time points after harringtonine administration, with examples of primary data. *n* is the number of images analyzed, evenly taken from pairs of independently cultured preparations, in turn originating from a common cell pool. Errors bars indicate s.e.m. Statistical evaluation of results performed by t-test, one-tailed, unpaired and homoscedastic.

Figure S4



Legend to Figure S4. Preliminary assesment of FOXG1-ERT2 confinement to cytoplasm. Shown is the immunoprofiling of HEK293T cultures, alternatively transfected with transgene-sets driving Tet^{ON}-controlled expression of *Plap*-, *Foxg1*- and *Foxg1-Ert2-Flag-V5*-mRNAs. In **(A)** protocol, in **(B)** results.

Figure S5



Legend to Figure S5. Validation of anti-FOXG1 antibodies employed in this study. Shown are immunofluorence specificity assays performed on primary neuronal cultures originating from mesencephalic tectum (*not expressing* FOXG1) and telencephalic neocortex (*expressing* FOXG1), with the two anti-FOXG1 antibodies employed in this study, for generation of Figures 1A and S2B (shown in **A**), and Figures 9E, S1B and S4 (shown in **B**).