SUPPLEMENTARY RESULTS

Preliminary assesment of physical FOXG1 interaction with selected translation factors in engineered HEK293T cells.

Previous FOXG1 interaction screening reports [34–36], our detection of a EIF4E-binding motif-like within FOXG1, as well as limited responsivity of translation factors' mRNA levels to *Foxg1* overexpression (**Table S1**) suggested us that, in addition to its canonical impact on transcription, FOXG1 may also act as a translation factor.

To preliminarily assay the goodness of this prediction, we engineered HEK293T cells to overexpress FOXG1 and selected translation factors putatively interacting with it, each tagged by V5 or Flag. We focused on EIF4E, EEF1D, EEF1G, PUM1, and evaluated their interaction with FOXG1 by proximity ligation assay (PLA), driven by anti-FOXG1 and anti-tag antibodies, and confocal immunofluorescence (IF) (**Figure S2A**, to left). Compared with their technical controls ("anti-FOXG1 only" and "anti-tag only"), EEF1D and EIF4E gave stronger PLA signals. Normalized against averages of such controls, these signals equalled 3.4±0.6 (with $p_{\text{vs-anti-FOXG1-only}}<0.09$, $p_{\text{vs-anti-tag-only}}<0.05$ and n=2,2,2), and 3.7±0.8 (with $p_{\text{vs-anti-FOXG1-only}}<0.07$, $p_{\text{vs-anti-tag-only}}<0.10$ and n=2,2,2), respectively. Conversely, EEF1G and PUM1 signals hardly overcame the corresponding controls (**Figure S2A**, to right). As for FOXG1/EIF4E interaction, we also interrogated HEK293T cells cotransfected with FOXG1- and Flag-EIF4E-encoding transgenes, via immunoprecipitation-western blot (IP-WB) assays. In this case, negative controls were set replacing *Flag-EIF4E* by *Flag-Gephyrin* and *Flag-GFP* transgenes (**Figure S2B**, to left). Immunoprecipitated by anti-Flag and probed upon WB separation by anti-FOXG1, lysates of *Foxg1/Flag-EIF4E*-transduced cells specifically gave a robust Foxg1 enrichment. Normalized against the product of FOXG1 and Flag-protein inputs, and further normalized against *Flag-Gephyrin*- and *Flag-GFP*-transduced controls, IP-FOXG1 equalled 99.1±0.1 (with $p<2.0*10^{-6}$ and n=2,2) and 35.2±0.4 (with $p<2.0*10^{-4}$ and n=2,2), respectively (**Figure S2B**, to right). To sum up, within HEK293T cells overexpressing them, FOXG1 seems to interact with key factors implicated in both translation initiation (EIF4E) and polypeptide elongation (EEF1D).