



Supplemental Fig. 10. Plasmid DNA-transfection experiments in NPC-derived mature neuronal cells. (A) Scheme of plasmid 99-gfp-LRE3-UB* (see also Figure 6). (B) Representative retrotransposition assays in HeLa cells transfected with plasmid 99-gfp-LRE3-UB* and analyzed 7 days post transfection. In the experiment, transfected cells were selected with puromycin. The FACS histogram also indicates the level of L1-EGFP-expressing cells as determined in triplicate (including the SD of the assay). (C&D) Representative results from retrotransposition assays conducted on differentiating NPCs transfected at day 31 with plasmid 99-gfp-LRE3-UB*(C) or 99-gfp-JM111-UB*(D) in the presence of BrdU and stained with TUBB3 (pink) and BrdU (red); nuclear DNA was stained with DAPI (blue) and L1-EGFP expression was visualized using an EGFP polyclonal antibody (green). The left panels are the independent captured images used in the merged picture, right. White bars, 10 μ m. (E) Secondary-only antibody controls for the staining shown in Figure 6A and panels C and D of this figure. (F & G) Representative results from the

EGFP intron PCR assay conducted on gDNA isolated from transfected hESC-NPCs (G) and hESC-NPCs-d31 (F) 7 after transfection. In the PCR assay shown in panel F, pk87 cells were used as a positive amplification control of spliced L1-EGFP (see Methods). Shown are the size of the amplification products. Both cell types were transfected with a plasmid containing a very active human LINE-1 (LRE3 lanes, plasmid 99-gfp-LRE3-UB*) or with a plasmid containing a mutant LINE-1 (JM111 lanes, plasmid 99-gfp-JM111-UB*). Only those samples transfected with plasmid 99-gfp-LRE3-UB* revealed amplification of the 342bp long spliced EGFP.