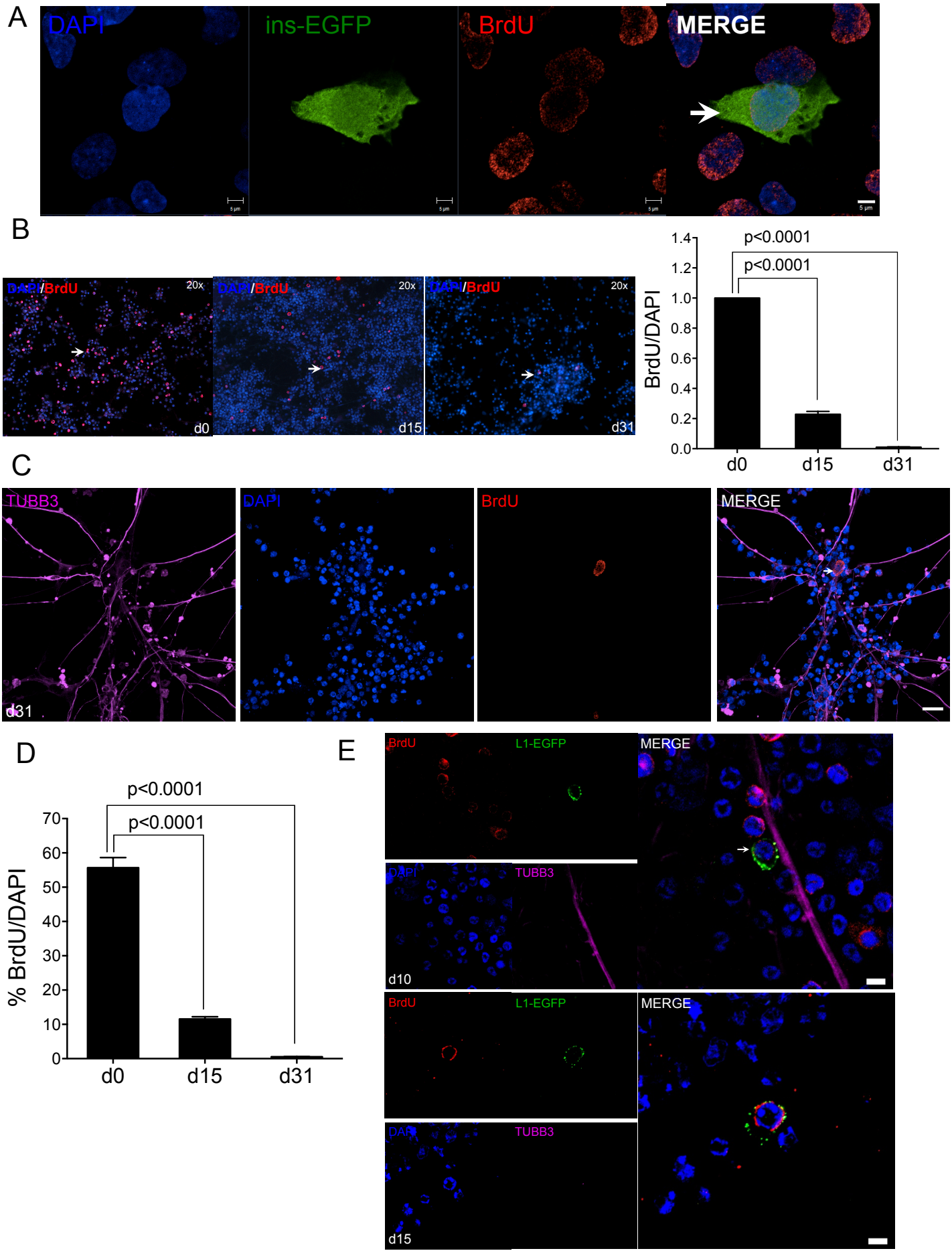
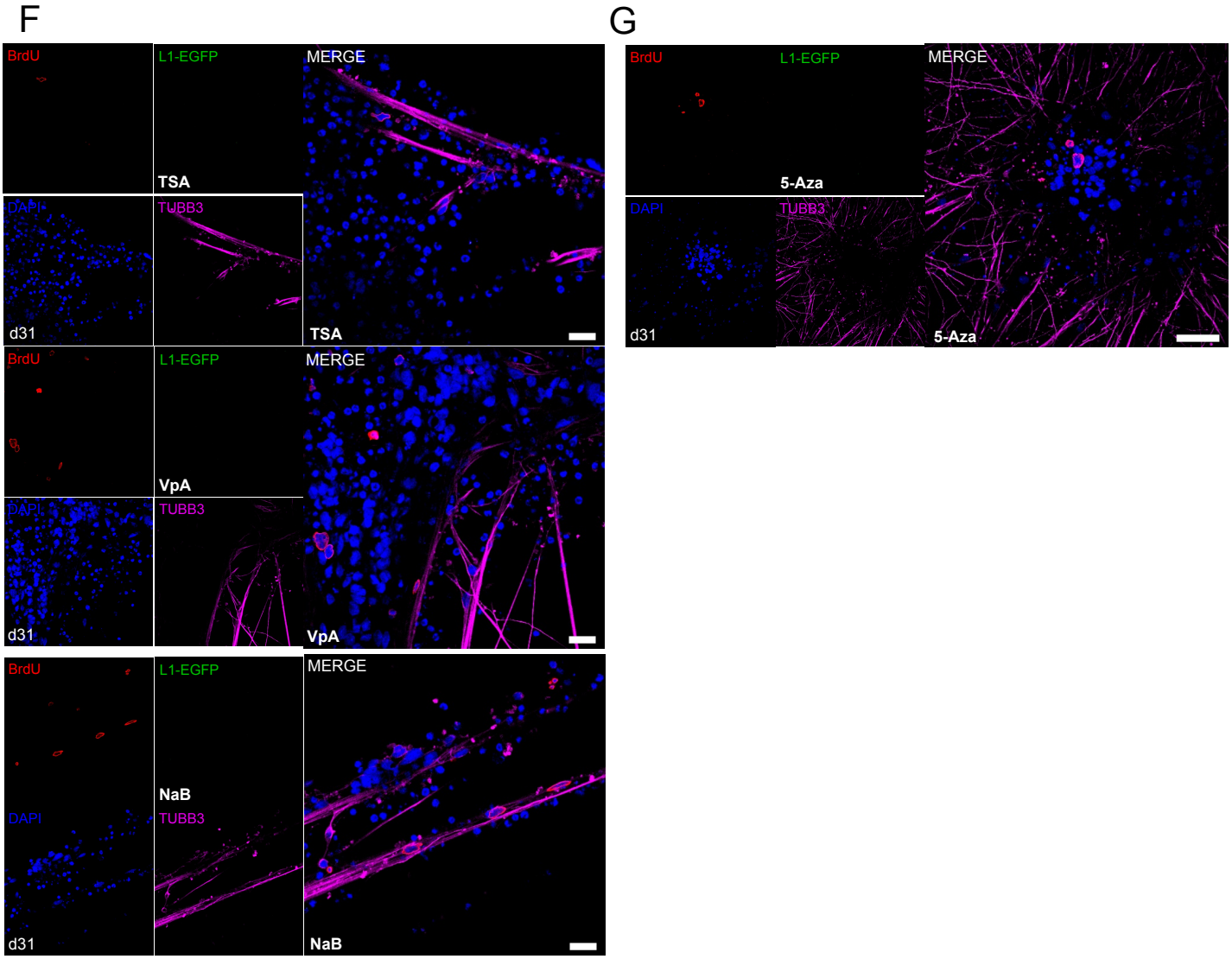

A
DAPI
ins-EGFP
BrdU
MERGE
B
DAPI/BrdU
20x
d0
d15
d31
BrdU/DAPI
p<0.0001
p<0.0001
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
d0
d15
d31
C
TUBB3
DAPI
BrdU
MERGE
d31
D
% BrdU/DAPI
p<0.0001
p<0.0001
70
60
50
40
30
20
10
0
d0
d15
d31
E
BrdU
L1-EGFP
MERGE
DAPI
TUBB3
d10
BrdU
L1-EGFP
MERGE
DAPI
TUBB3
d15



Supplemental Fig. 9. Engineered L1 retrotransposition in NPC-derived mature neuronal cells. (**A**) Dual staining of L1-EGFP and BrdU in cultured cells. Shown is a merged image of cultured pc39 cells stained with an antibody against BrdU (red) and EGFP (green); nuclear DNA was stained with DAPI (blue). Pc39 cells have been described previously (see Methods); it is a clonal cell line that contains at least two silenced L1-EGFP insertions. White bars, 5 mm. (**B**) BrdU labeling in differentiating NPCs. Shown are merged images of differentiating NPCs stained at the indicated time (day 0, 15 and 31) with an antibody against BrdU (red); nuclear DNA was stained with DAPI (blue). The right graph shows a quantification of the ratio of nuclei that stain positive for BrdU at the indicated time. The SEM of the assay is also included and a One-way ANOVA with Tukey was used as a statistical method. The p value is included (<0.0001). (**C**) BrdU incorporation in differentiated NPCs. A merged image of differentiated NPCs (day 31) stained with an antibody against TUBB3 (pink) and BrdU (red); nuclear DNA was stained with DAPI (blue). White bars, 20 mm. (**D**) The graph shows a quantification of the percentage of nuclei that stain positive for BrdU in double DAPI/BrdU staining experiments at the indicated time. The SEM of the assay is also included and a One-way ANOVA with Tukey was used as a statistical method (p value, <0.0001). (**E**) Representative results from retrotransposition assays conducted in differentiating NPCs using the Ad-L1 and analyzed 10 and 15 days after infection. Cells were stained with an antibody against 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 side contains the independent captured images used in the merged picture. White bars, 5 µm. Also indicated in each panel is the day when infection was performed (white letters). (**F&G**) Neither treatment with Histone Deacetylase Inhibitors (F) or 5-Aza (G) resulted in L1-EGFP expression. As in panel E, shown is a staining assay on Ad-L1 infected NPC-derived neurons at day 31 of differentiation. Cells were stained with an antibody against 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 side contains the independent captured images used in the merged picture. White bars, 20 (E) and 50 (F) µm. Also indicated in each panel is the day when infection was performed and the treatment applied (white letters, see Methods).