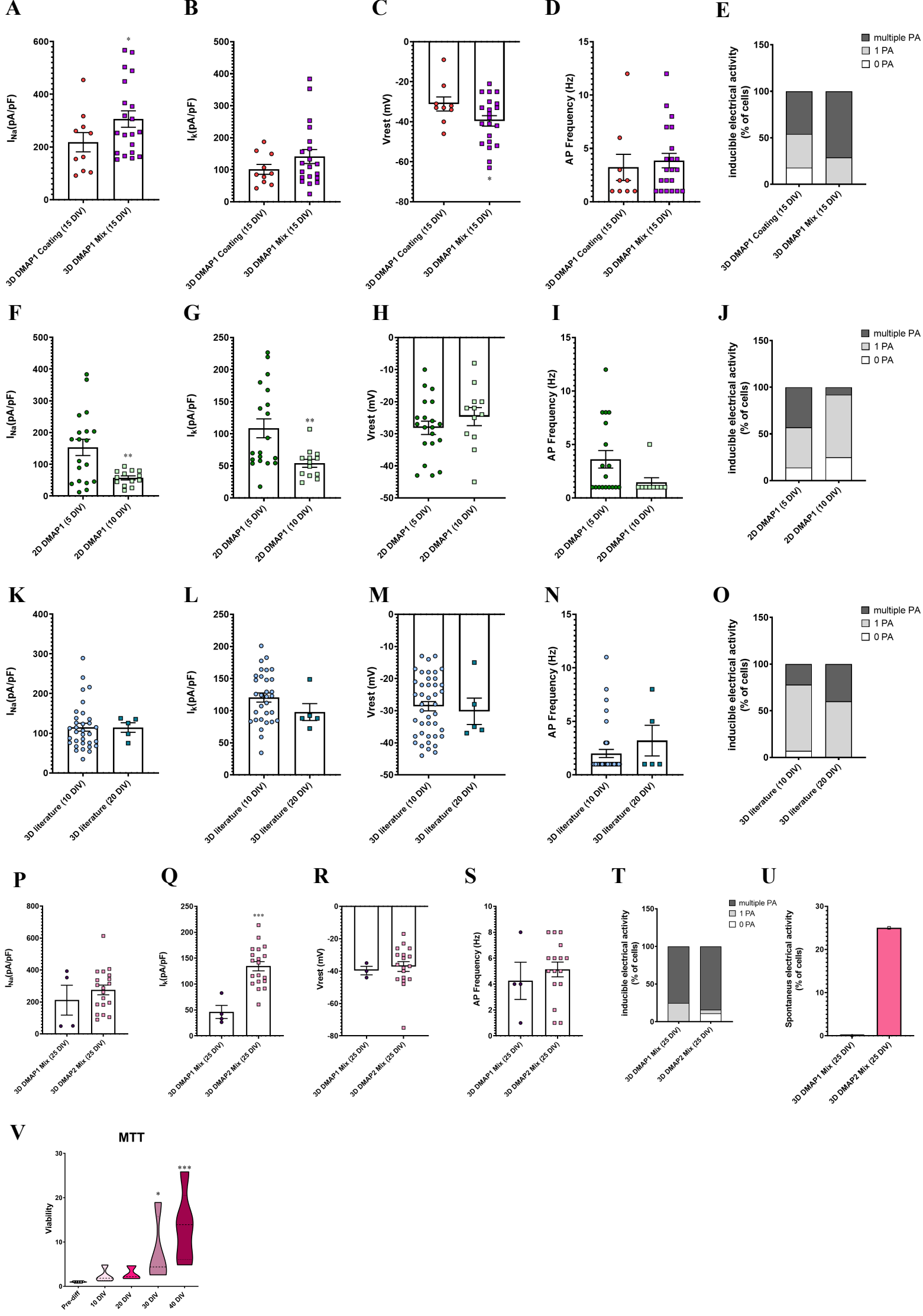
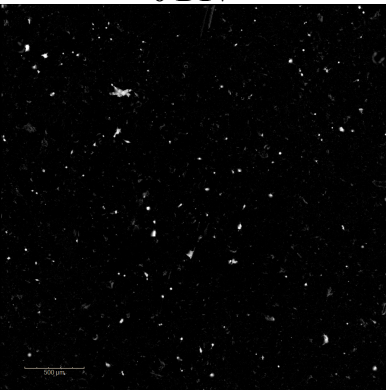
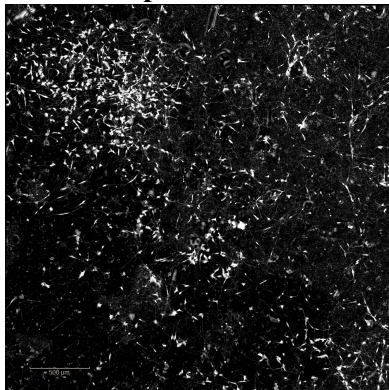
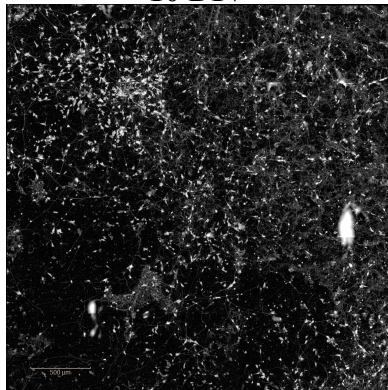
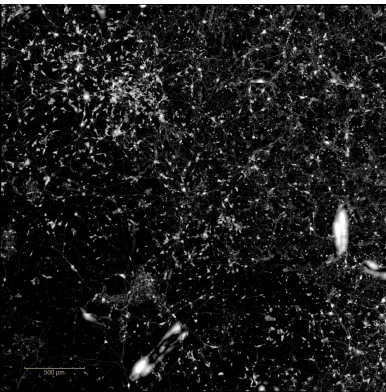
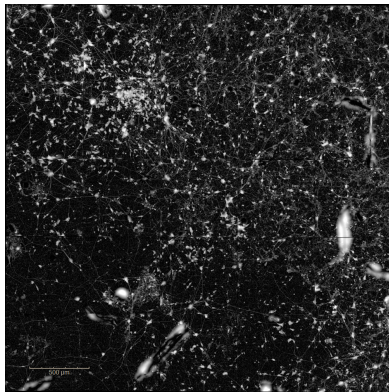
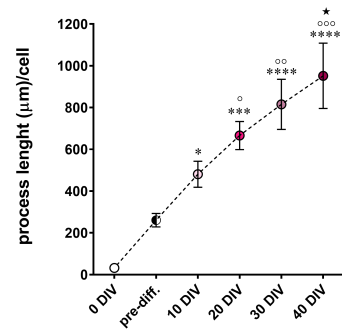
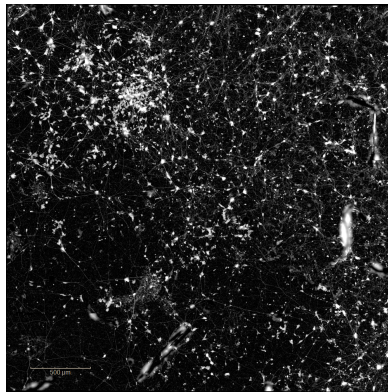
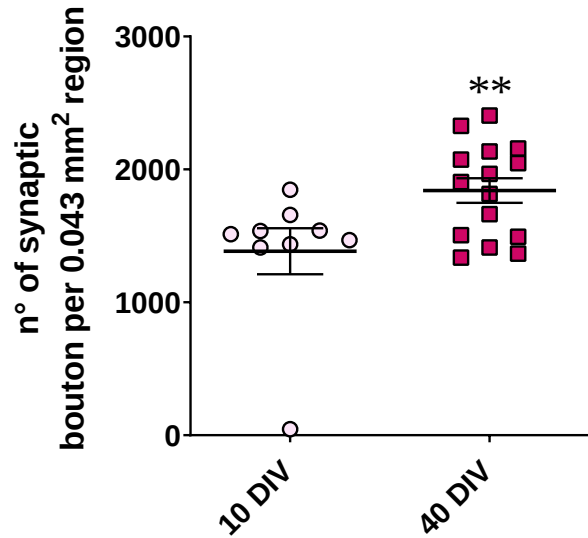
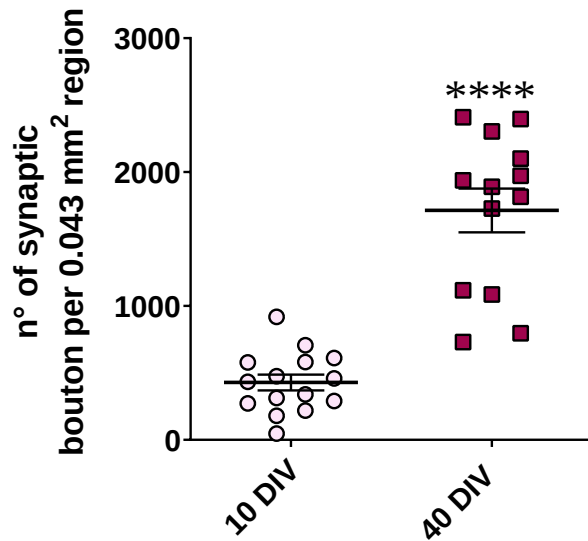
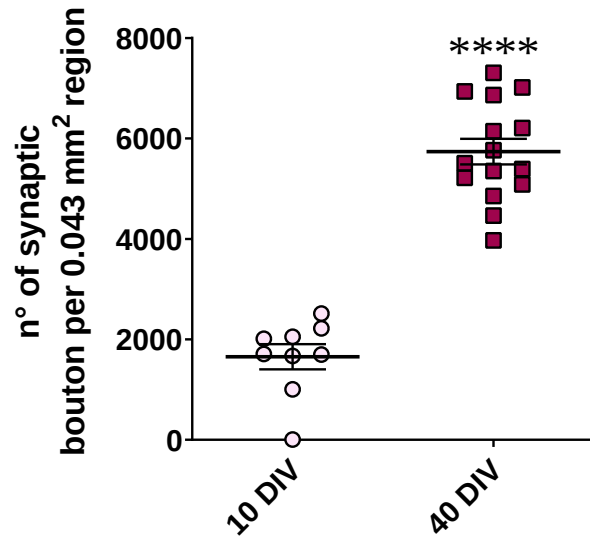




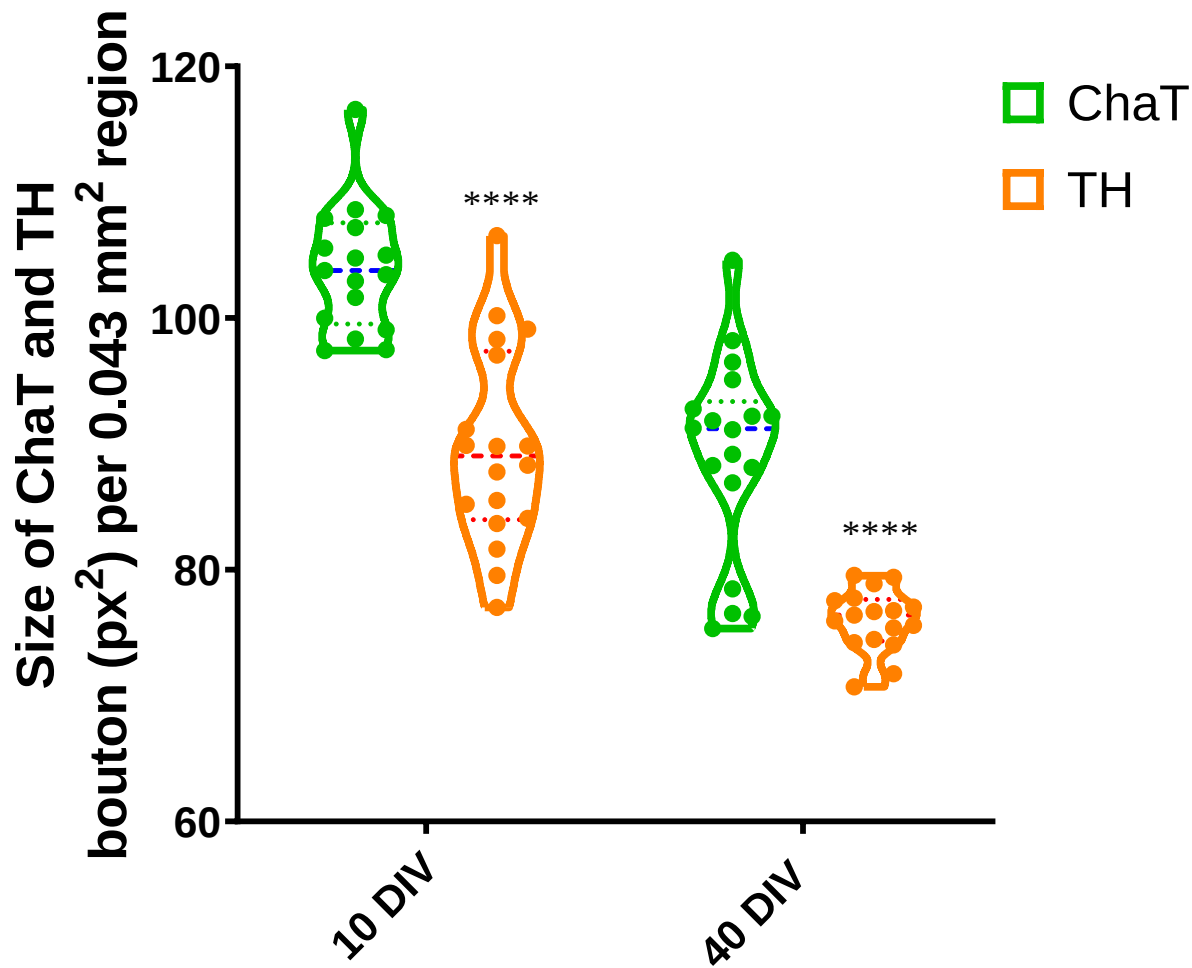
Supplementary Figure 1. Functional properties and viability of SH-SY5Y cells cultured in different conditions at different days of differentiation. We tested sodium and potassium current densities (A, B), resting membrane potential (C), and action potential firing frequency (D, E) of cells cultured in 3D DMAP1 Coating and 3D DMAP1 Mix conditions at 15 DIV. The same parameters were analyzed for the 2D DMAP1 at 5 and 10 DIV (F, G, H, I, J), for the 3D Literature condition at 10 and 20 DIV (K, L, M, N, O), and for the 3D DMAP1 Mix and 3D DMAP2 Mix at 25 DIV (P, Q, R, S, T). The 3D DMAP2 Mix condition showed a higher percentage of cells with spontaneous activity compared to the 3D DMAP1 Mix condition (U). (V) Cell viability (MTT assay) of cells cultured in 3D DMAP2 Mix for 7 days of pre-differentiation, and 10, 20, 30, and 40 days of differentiation (DIV). The number of total samples analyzed for each time point described above were: 5 for Pre-diff., 4 for 10 DIV, 4 for 20 DIV, 4 for 30 DIV, and 5 for 40 DIV. Data are expressed as the ratio between different groups (10, 20, 30, and 40 DIV) and Pre-diff. Differences among groups were tested for significance by: Unpaired t-test, in panels A, C, G, and Q; Chi-Square test, in panel U; Kruskal-Wallis test followed by Dunn's multiple comparisons test, in panel V. Significance was set as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

0 DIV**pre-diff.****10 DIV****20 DIV****30 DIV****40 DIV**

Supplementary Figure 2. Characterization of neuronal arborization. SH-SY5Y cells were plated and differentiated as described in the Material and Methods section (3D DMAP2 Mix). Live cell images were taken at 0, pre-differentiation (7 days), 10, 20, 30, and 40 DIV using Operetta CLS™ equipped with 20× immersion objective in Digital Phase Contrast (DPC) to detect neurites. Large images (25 fields) are shown in the left panel. Scale bar: 500 μm. Quantification of process length per cell (using Fiji software) is shown in the right panel. 0 DIV, pre-diff., 20 DIV, 30 DIV, and 40 DIV (n=6 large images); 10 DIV (n= 5 large images). One-way ANOVA followed by Tukey's multiple comparisons test was used to determine the significance of differences among the conditions.* indicates significance compared to 0 DIV; ° indicates significance compared to pre-diff; ★ indicates significance compared to 10 DIV. Significance was set as *p<0.5, **p<0.01, ***p< 0.001, ****p<0.0001.

A**Synapsin 1****B****Synaptophysin****C****Complexin 1/2**

Supplementary Figure 3. Pre-synaptic markers evaluation. We analyzed the number of pre-synaptic boutons per 0.043 mm² region positive for (A) synapsin 1 (10 DIV, n=9 fields, and 40 DIV, n=15 fields), (B) synaptophysin (10 DIV, n=15 fields, and 40 DIV, n=13 fields), and (C) complexin 1/2 (10 DIV, n=9 fields, and 40 DIV, n=15 fields). a), The statistical test used to determine the significance of differences among the conditions was the Unpaired t-test. Significance was set as *p< 0.5, **p<0.01, ***p< 0.001, ****p<0.0001.



Supplementary Figure 4. Size quantification of ChaT and TH vesicles per 0.043 mm² region at 10 DIV (ChaT: n=17 fields, TH: n=18 fields) and 40 DIV (ChaT: n=18 fields, TH:=17 fields). The statistical test used to determine the significance of differences among the conditions was Multiple t-tests. Significance was set as *p<0.5, **p<0.01, ***p< 0.001, ****p<0.0001.