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Supplemental information

In vitro-derived medium spiny neurons recapitulate human striatal development and complexity at single-cell resolution

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Figure S1

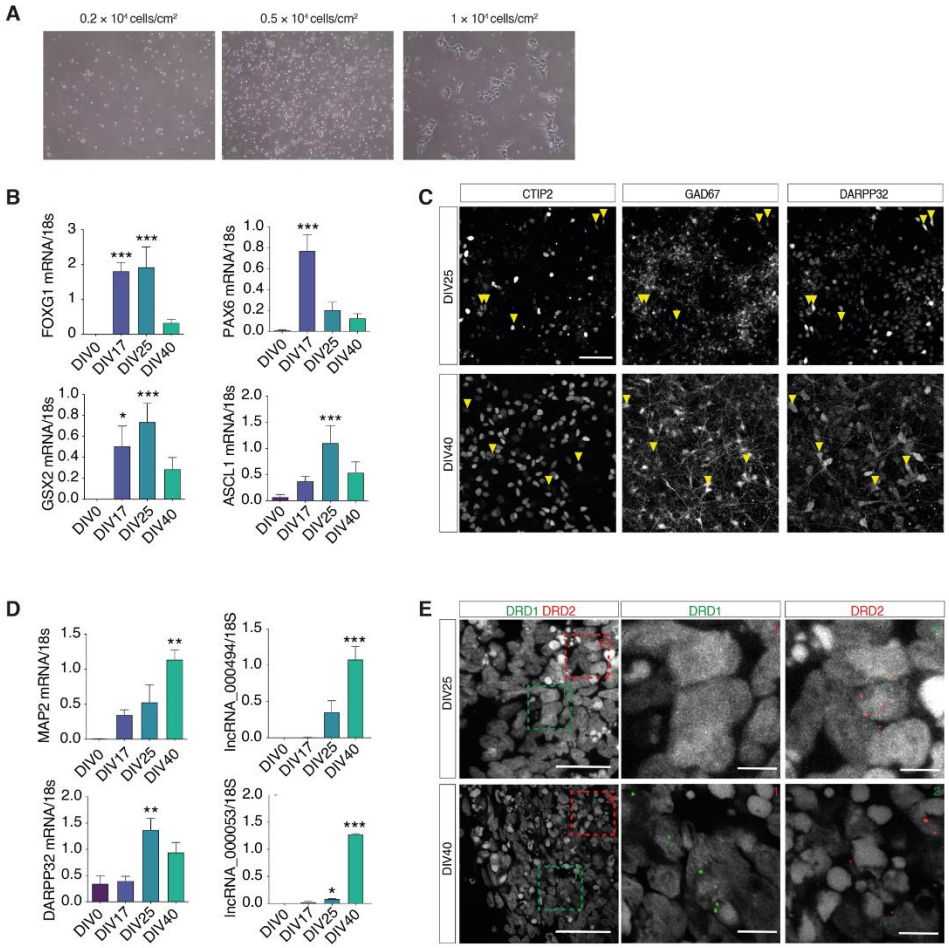

Figure S1 related to Figure 1. Characterization of the protocol to generate MSNs from H9 hES cells.
(A) DIV1 phase contrast images of H9 hES cells plated at different densities. (B) qRT-PCR analysis of FOXG1, PAX6, GSX2 and ASCL1 (n=3 independent biological replicates; error bars represent ± SEM). (C) Single-channel immunofluorescent staining for CTIP2/DARPP32/GAD67; yellow arrows show triple positive cells; Scale bar, 50 μm (D) qRT-PCR analysis of MAP2, DARPP32, lncRNA_000494 and of lncRNA_000053; (n=3 independent biological replicates; error bars represent ± SEM). Anova one way, Tukey's multiple comparison test, *p < 0.05; **p < 0.01, ***p < 0.001 (E) FISH of DRD1 and DRD2. Scale bars, 50 μm.

Figure S2

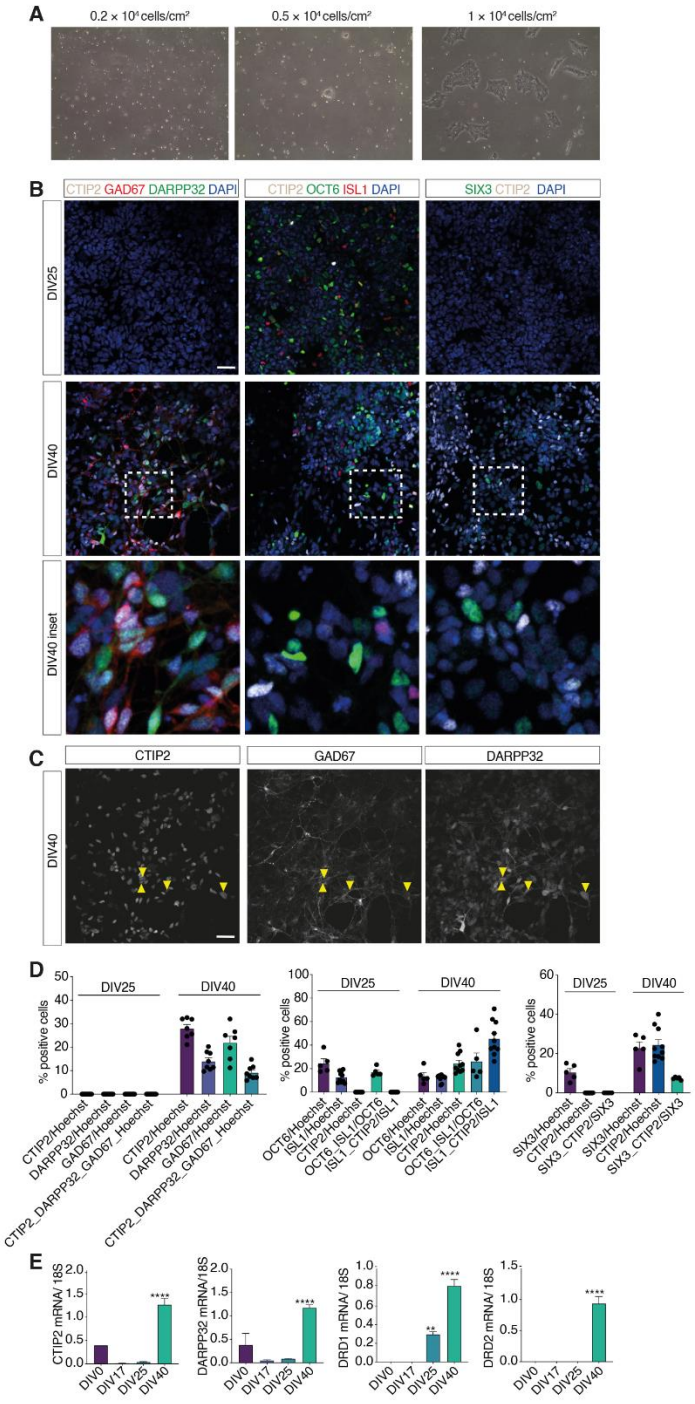

Figure S2 related to Figure 2. Characterization of the protocol to generate MSNs from RUES2 hES cells. (A) DIV1 phase contrast images of RUES2 hES cells plated at different densities. (B) Immunofluorescent staining for CTIP2/DARPP32/GAD67, OCT6/ISL1/CTIP2 and SIX3/CTIP2 in the RUES2 hES cell line. Scale bar, 50 μm. (C) Single-channel immunofluorescent staining for CTIP2/DARPP32/GAD67; yellow arrows show triple positive cells. Scale bar, 50 μm (D) Quantification of the percentage of positive cells at DIVs 25 and 40 (n=3 independent biological replicates). (E) qRT-PCR analysis of *CTIP2, DARPP32, DRD1* and *DRD2*; (n=3 independent biological replicates; error bars represent ± SEM). Anova one way, Tukey's multiple comparison test, **p < 0.01, ****p < 0.0001.

Figure S3

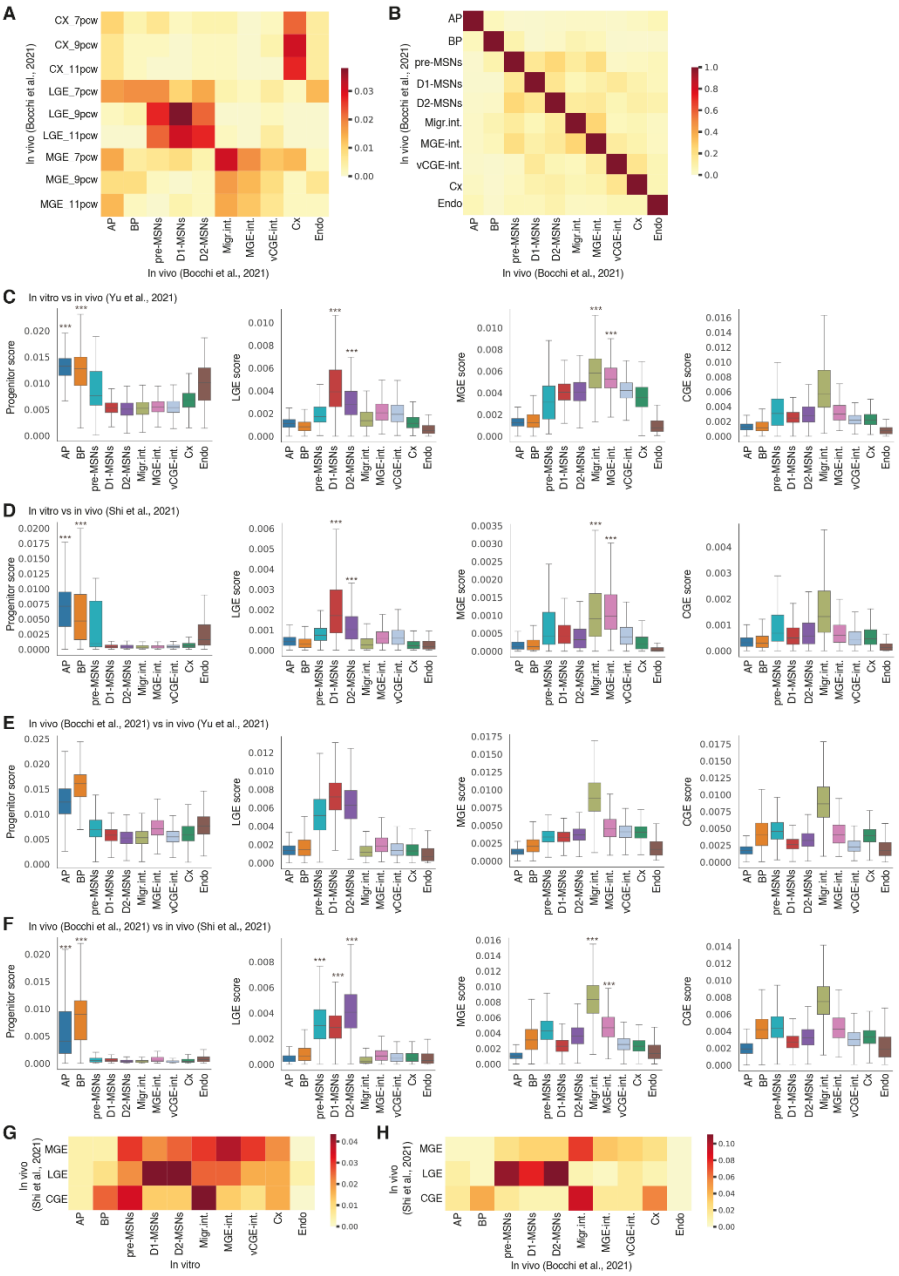

Figure S3 related to Figure 2. Similarity scoring between *in vitro* generated cells and the human fetal brain.

(A, B) Heatmap of the Jaccard index for the comparison between bulk RNA-seq *in vivo* signatures [12] and *in vivo* cell types[12] (A) and between *in vivo* cell-type specific signatures [12] and *in vivo* cell types [12] (B). (C - F) Box plots showing the enrichment scores for each *in vitro* cell type according to a specific *in vivo* signature and the enrichments scores between *in vivo* and *in vivo* signatures. Mann-Whitney rank test and Benjamini-Hochberg correction; ***p < 0.001. (G, H) Heatmaps of the Jaccard index for the comparison between *in vivo* LGE, MGE and CGE branching point signatures with *in vitro* cell types (G) and *in vivo* cell types (H).

Figure S4

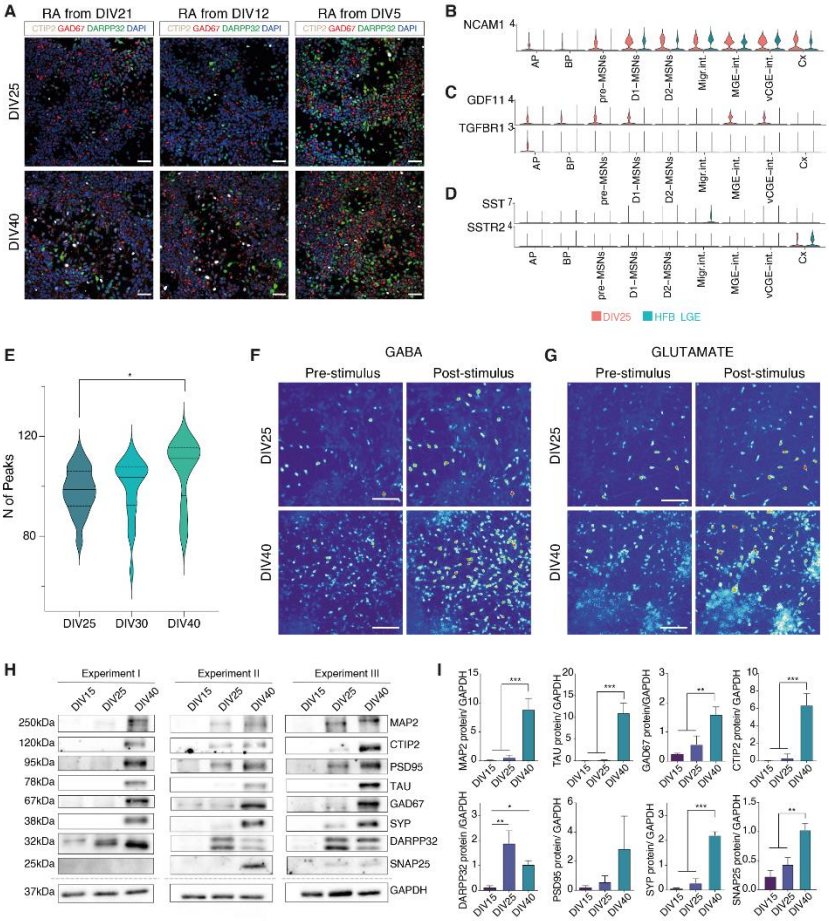

Figure S4 related to Figure 4. Characterization of the active signaling pathways and functional activity of in vitro derived MSNs.

(A) Immunofluorescent staining for CTIP2/DARPP32/GAD67 with RA given from DIV21 of differentiation (standard protocol), from DIV12 or from DIV5. Scale bars, 50 μm. (B-D) Violin plot showing expression of ligand-receptor pairs *in vitro* and *in vivo*[12]. (E) Ca^{++} imaging basal activity. n ≥ 12 from 2/3 independent biological replicates. Krustal-Wallis test with Dunn's post test. *p < 0,05. (F, G) Picture frames of Ca^{++} imaging activity before and after stimulation with GABA (H) or Glutamate (I). (H, I) Western blotting of MAP2, CTIP2, PSD95, TAU, GAD67, SYP, DARPP32, SNAP25 and GAPDH (n=3 independent biological replicates; error bars represent ± SEM); densitometric analysis Anova one way, Tukey's multiple comparison test, *p<0.05, **p < 0.01, ***p < 0.001. The black boxes are aligned outside the blot area for presentation purposes.