A new in vivo and in vitro single-cell atlas of developing medium spiny neurons to guide future improvements for Huntington disease cell replacement therapies and disease modelling

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Summary
Use of single-cell transcriptomics to measure how well medium spiny projection neurons, derived from human pluripotent stem cells, recapitulate human striatal development in vivo.

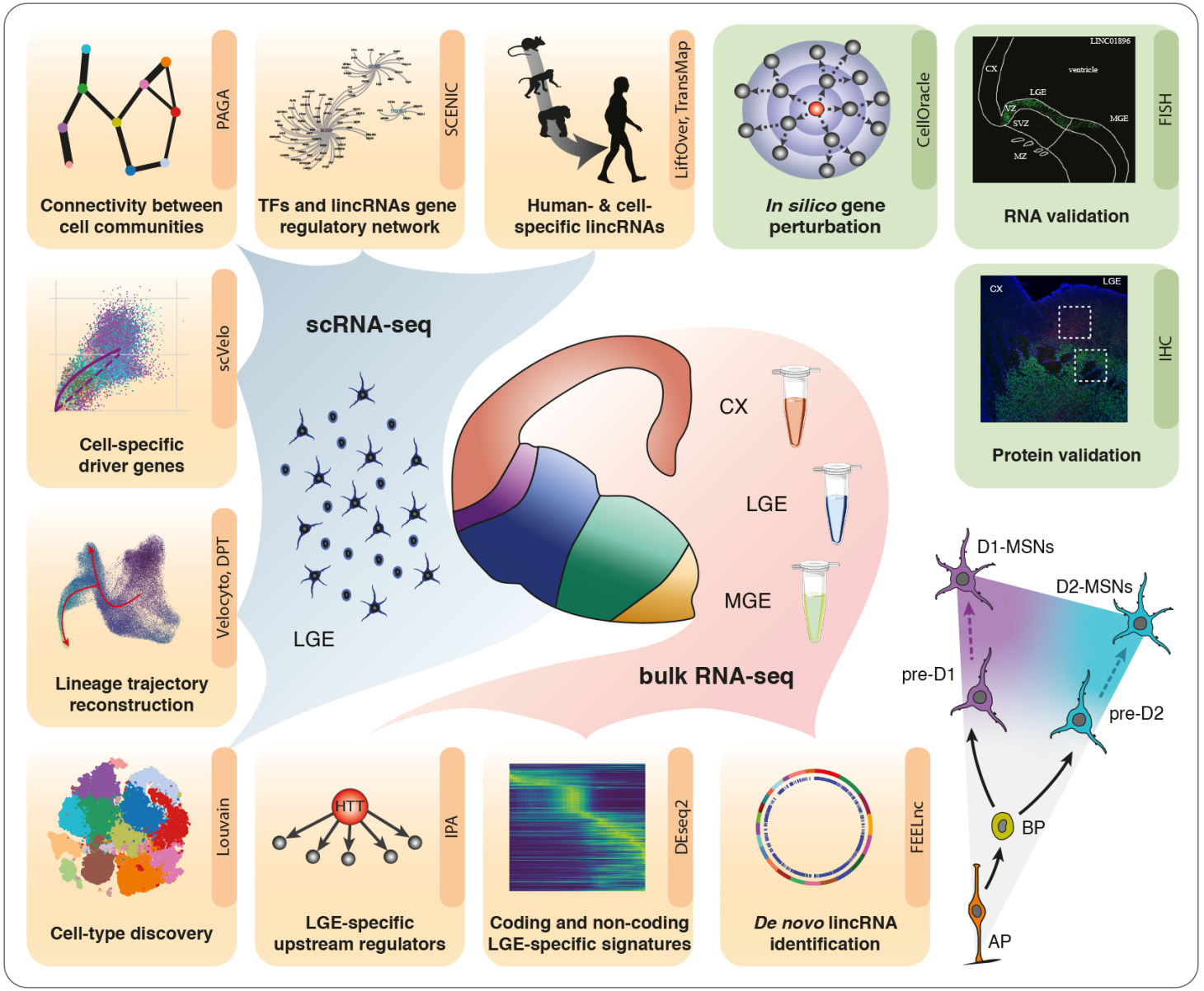
Background
Stem cell engineering and grafting of striatal medium spiny projection neurons (MSNs) is a promising strategy to understand the molecular mechanisms underlying Huntington Disease (HD) and for future cell-replacement strategies. However, optimal recapitulation of MSN development requires a deep knowledge of how MSNs form during early fetal striatal development. In our recent work (Bocchi et al., Science 2021) we were able to map the developmental landscape of individual cell states transitioning from early progenitors of the LGE to MSNs and then classify how subtype-specific heterogeneity progresses through the expression of specific combinations of gene regulatory networks, transcription factors and cardinal genes including uniquely human specific lincRNAs identified de novo.

Aims and Methods
Here we aim to measure the fidelity of MSNs derived from human pluripotent stem cells with our recently developed protocol, by comparing the transcriptome of each cell generated in vitro to the in vivo counterpart.

Results
By comparing our in vivo MSN cell-atlas to our new in vitro derived MSNs cell-atlas, we show that our protocol is able to mimic cell-fate acquisition steps seen in vivo, in terms of gene and cell type composition. Furthermore, we show that we are able to produce, for the first time, both D1 and D2-MSNs after 25 days of differentiation. Thus, stem cells under these new culture conditions recapitulate key stages of in vivo striatal development.

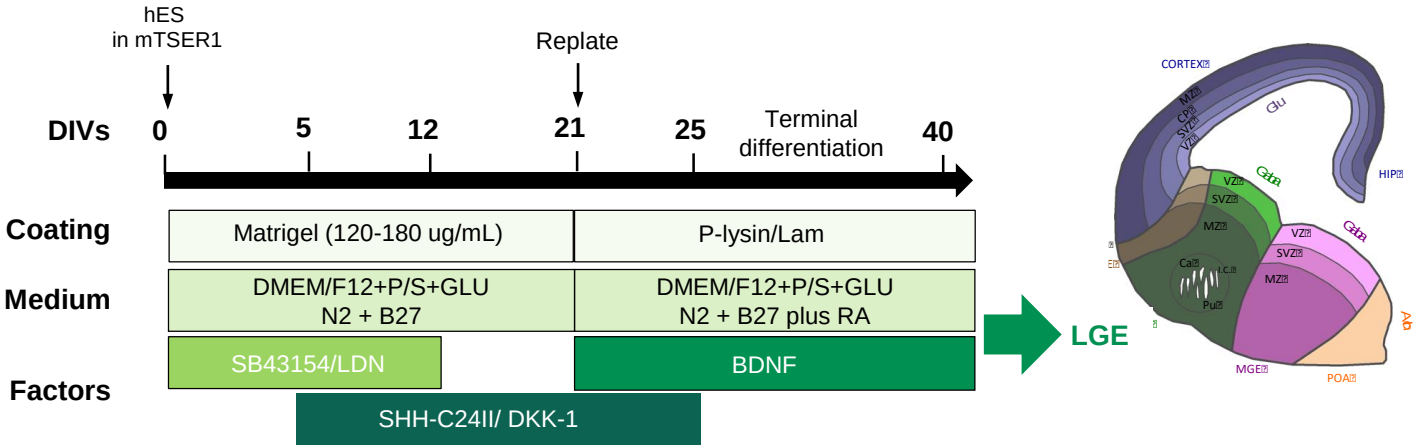
Conclusions
Overall, we expect that our in vivo and in vitro single-cell datasets will act as benchmarks to quantify and refine current stem cell engineering protocols and to achieve a deeper understanding of the HD pathophysiology to accelerate the development of effective therapies.

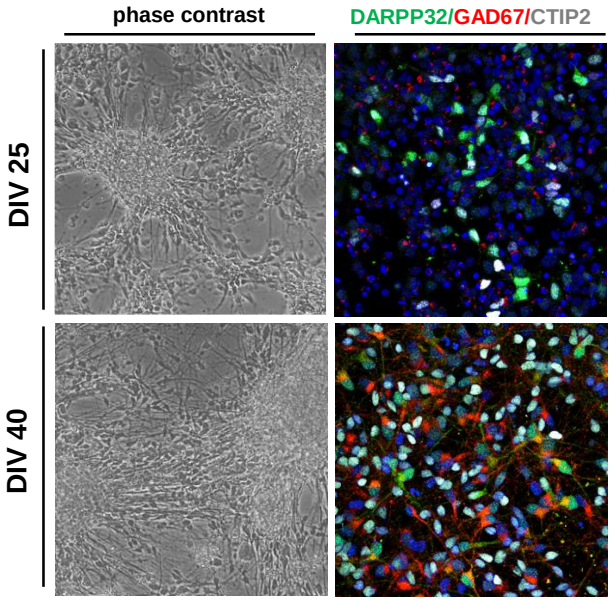
The human fetal striatum single-cell atlas as a reference

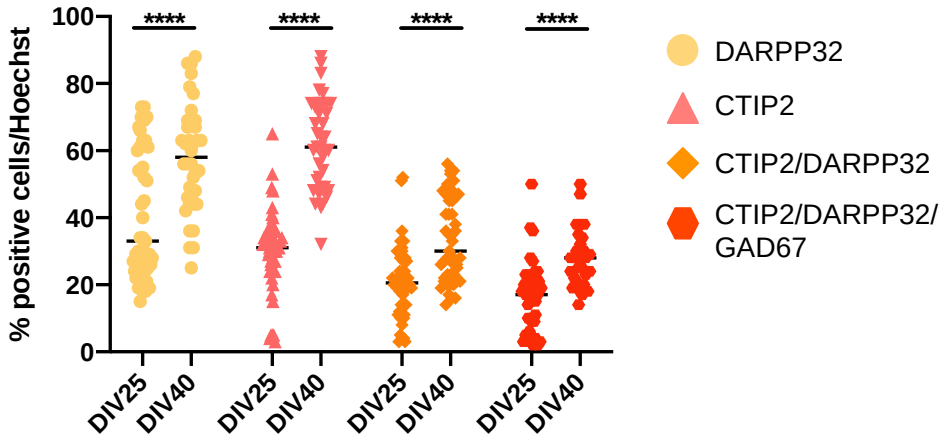


Bocchi et al., Science, 2021

Low density 2D MSN protocol





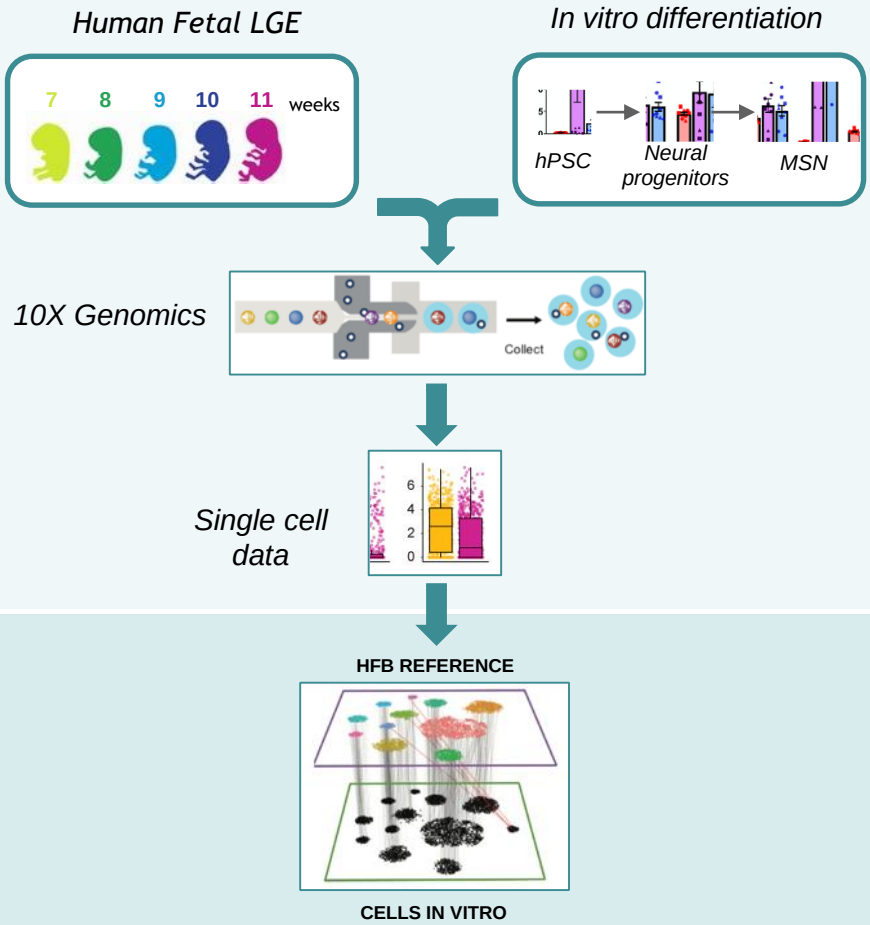


LGE progenitors efficiently differentiate into striatal MSNs starting from DIV25

N=9 differentiation experiments. Counts by Cell Profile.
Images counted for DARPP32 DIV25 n=48, DIV40 n=37; CTIP2 DIV25 n=48, DIV40 n=37; DARPP32/CTIP2 DIV25 n47, DIV40 n=36; CTIP2/DARPP32/GAD67 DIV25 n=41, DIV40 n=33.
Darpp32: Abcam (Rabbit, 1:250)/ Ctip2: Abcam (Rat, 1:2500)/ Gad67: Millipore (Mouse, 1:2500)

Unpublished, Conforti et al., in preparation

Single-Cell RNA-seq analysis to correlate *in vivo* development with *in vitro* differentiation



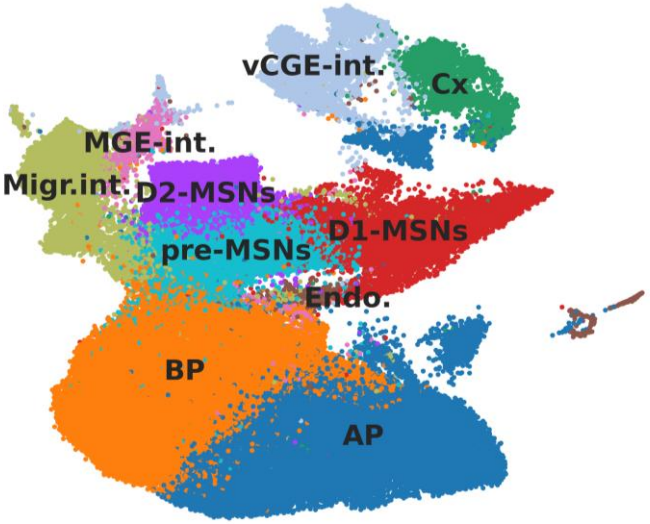
On the basis of the successful implementation of the striatal differentiation protocol in H9 hESCs, we have performed Single-Cell RNA-Seq to characterize the transcriptomic signature of the subpopulations present in the culture dish at different time points (DIVs 15, 25).

We compare results with single cell data obtained from the human fetal striatal samples (7, 8, 9 and 11 weeks).

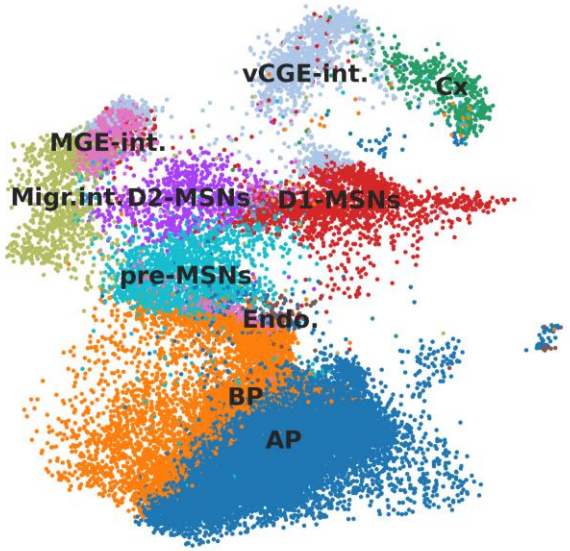
Exploiting this high-throughput method/approach we are able to understand which *in vivo* cell types and developmental stages our derivatives recapitulated.

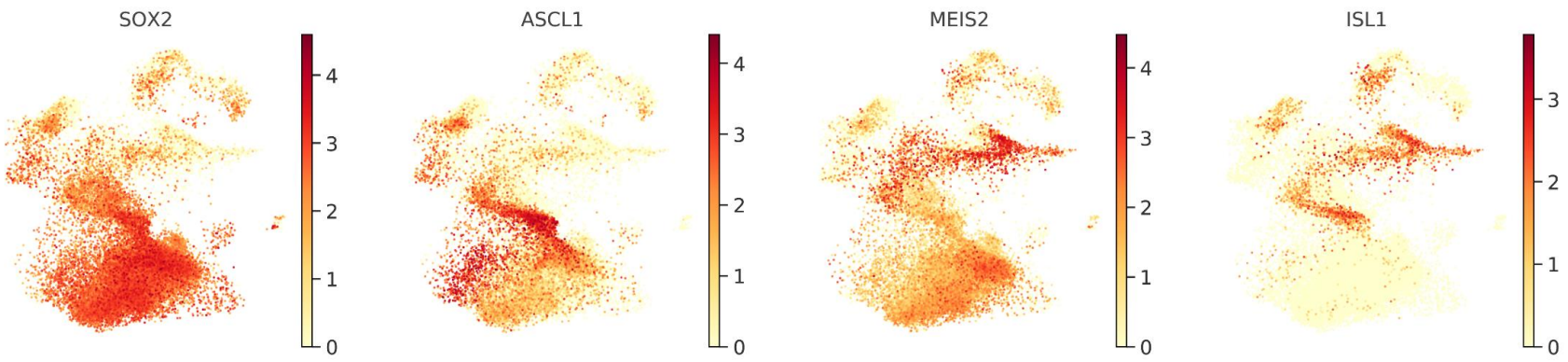
Mapping 2D in vitro data on *in vivo* striatal data

HUMAN STRIATAL IN VIVO DATA



MSN IN VITRO DATA – DIV15 & 25





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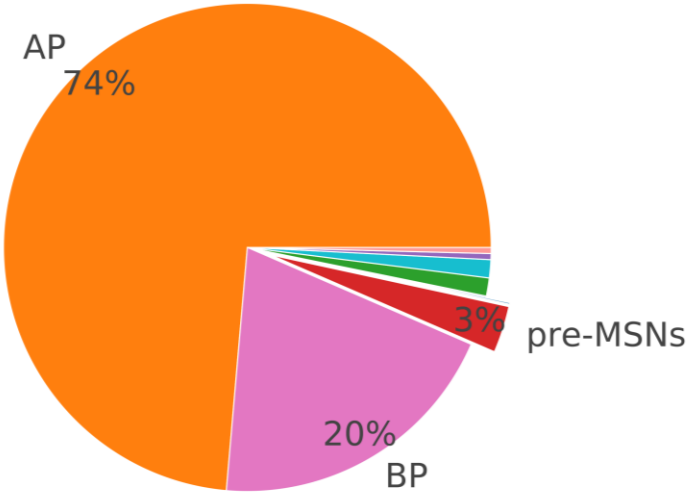
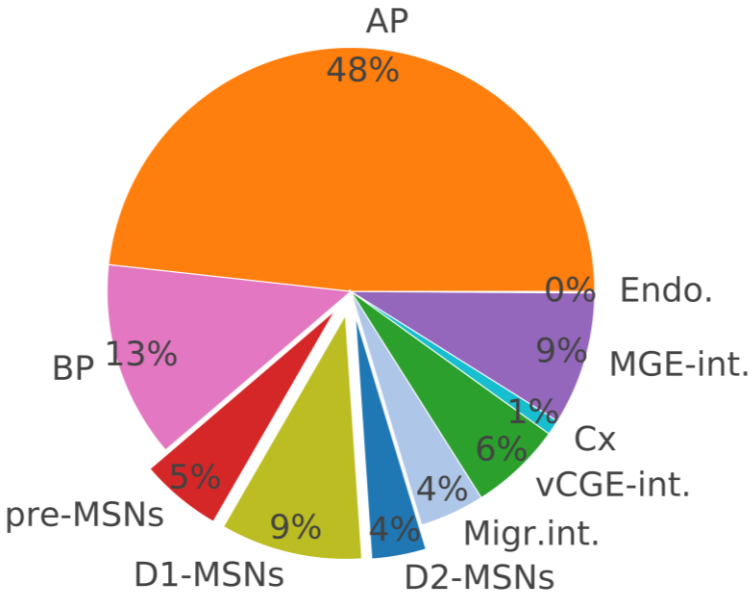
Mapping 2D in vitro data on *in vivo* striatal data

DIV 15



DIV 25



As differentiation progress the number of progenitors decreases and the number of neurons increases, as expected.

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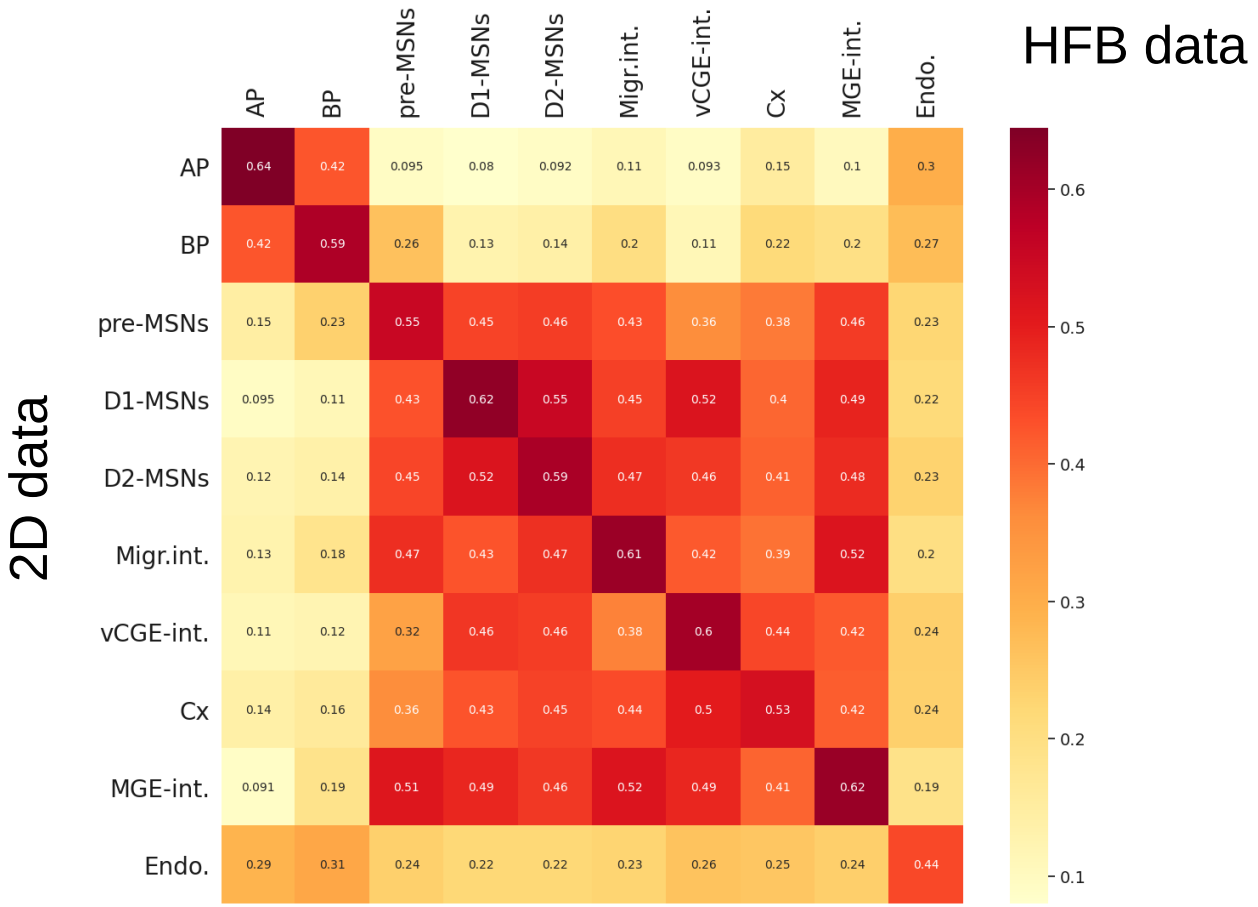
Mapping 2D in vitro data on *in vivo* striatal data



We have a good overlap of cell specific DEGs between *in vivo* and *in vitro* data confirming that our protocol is able to mimic MSN differentiation *in vitro*.

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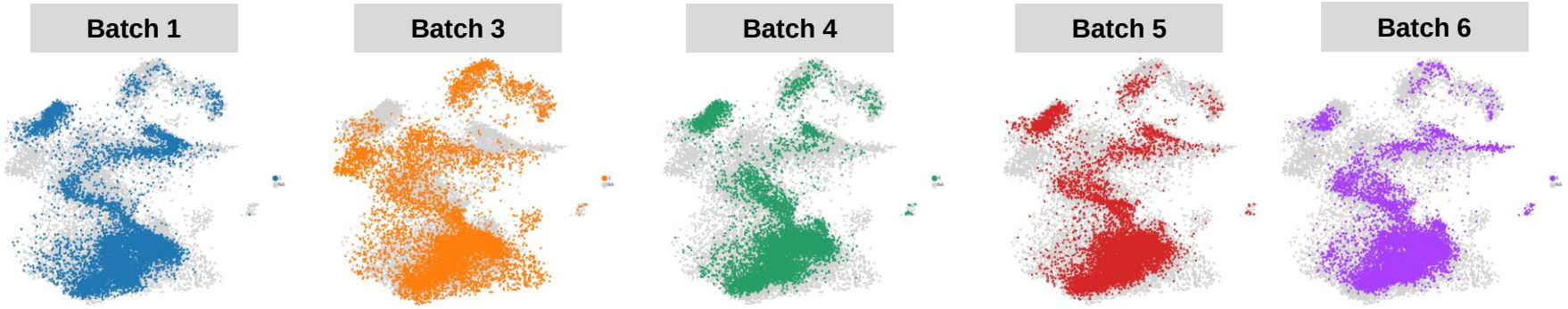
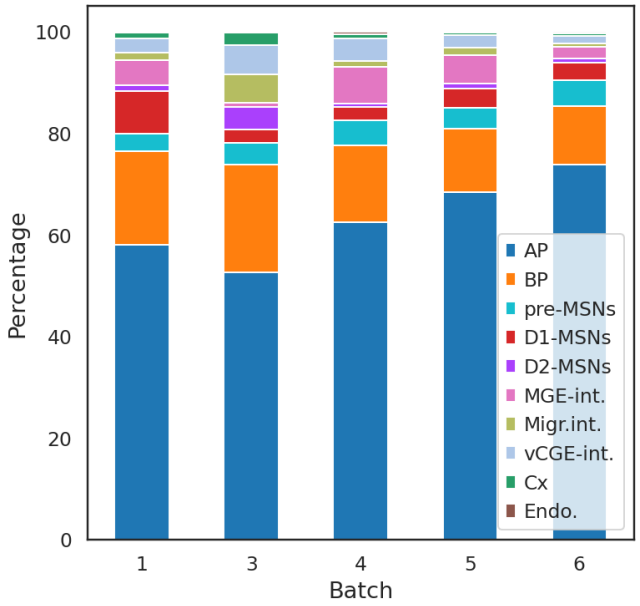
Protocol Reproducibility





Our protocol shows a high level of reproducibility with all batches producing both D1 and D2-MSNs

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Conclusions

1) IC, WB, QPCR and scRNA-seq analyses indicate that LGE progenitors efficiently differentiate into striatal MSNs starting from DIV25.

2) At DIV25 we reach 19%±5 MSNs

3) Single-cell RNA-seq data show that the new protocol mimics a ventral identity and is able to produce both D1 and D2 MSNs together with different subtypes of interneurons.

4) We show that our HFB reference data (Bocchi et al., Science 2021) and our 2D *in vitro* cells share similarities in terms of DEGs and average expression of top genes.

5) We show that our protocol is highly reproducible giving similar cell types in each batch

Overall, we expect that our in vivo and in vitro single-cell datasets will act as benchmarks to quantify and refine current stem cell engineering protocols and to achieve a deeper understanding of the HD pathophysiology to accelerate the development of effective therapies.