Developmental Characterization of Human Induced Neurons

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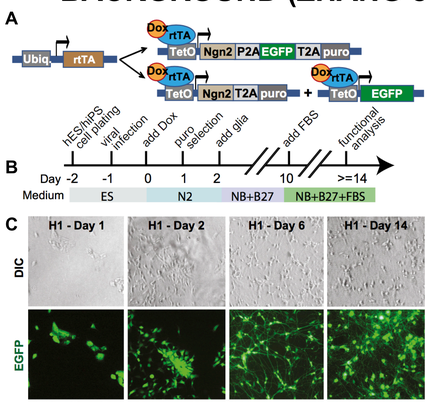
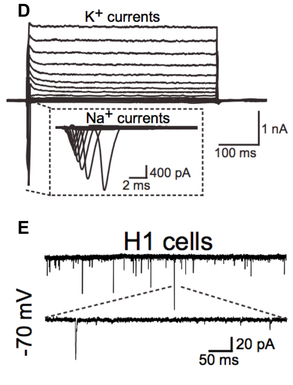
ABSTRACT

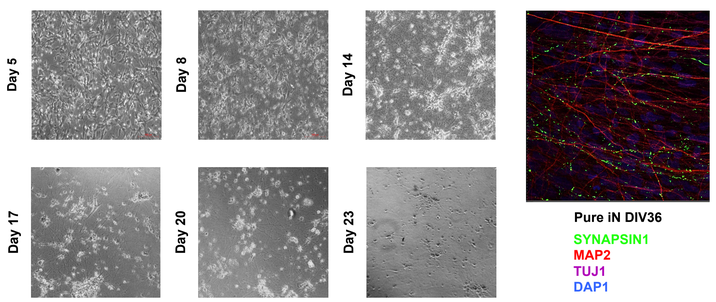
Our lab recently reported that a single transcription factor (Neurogenin-2) can drive the differentiation of human embryonic stem cells (ESCs) into functional induced neurons (iNs) over several weeks. These iNs express synaptic markers at both transcript and protein levels and exhibit electrophysiological properties of excitatory neurons. This reduced system presents many opportunities, but in order to be useful for genetic screens and manipulations, we must understand the transcriptomic and proteomic profiles of these neurons in both immature and mature states. We developed strategies to culture pure iNs that were functionally equivalent to previous iNs grown on mouse glial cells in order to label proteins quantitatively using stable isotope labeling of amino acids (SILAC) and conduct RNAseq.

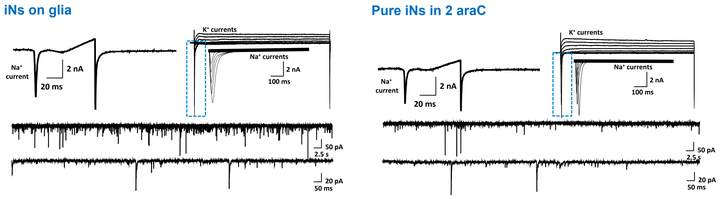
BACKGROUND (ZHANG et al. *Neuron* 2013)



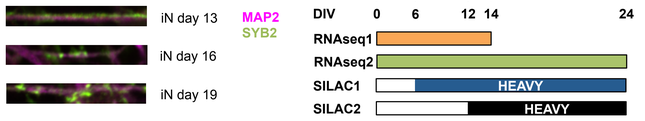


PURE iN CELLS HAVE SYNAPSES



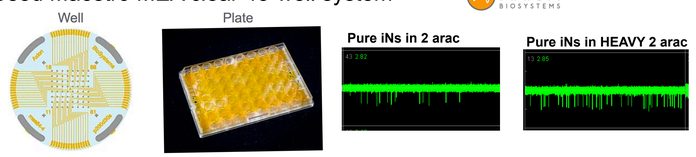


TIME-POINTS FOR SAMPLE COLLECTION

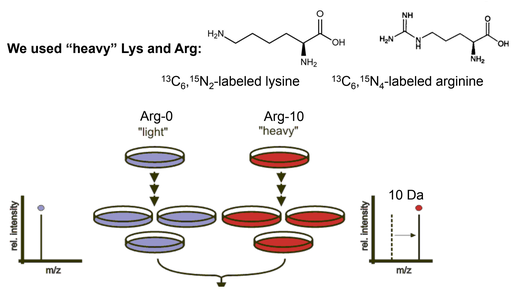


MULTI-ELECTRODE ARRAY (AXION BIOSYSTEMS)

Used Maestro MEA clear 48-well system

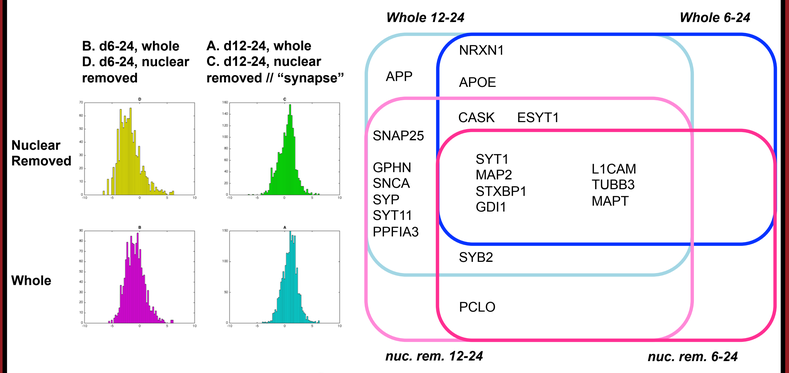


SILAC-MASS SPECTROMETRY (MS) OF PURE iN

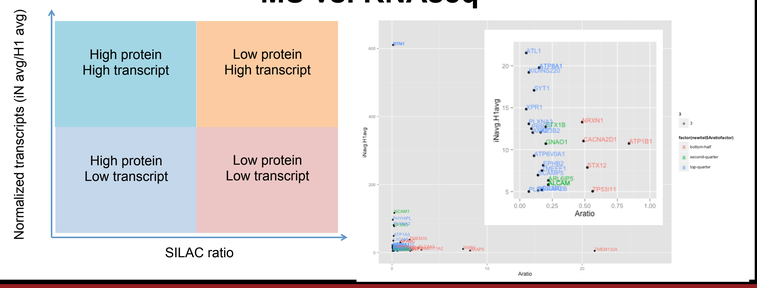


High incorporation ratio (turnover, new synthesis):
Light = 1
Heavy = 10
Log2(1/10) = -3.3

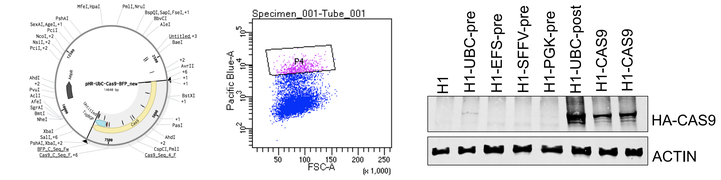
Low incorporation ratio (stable, not synthesized):
Light = 10
Heavy = 1
Log2(10) = 3.3



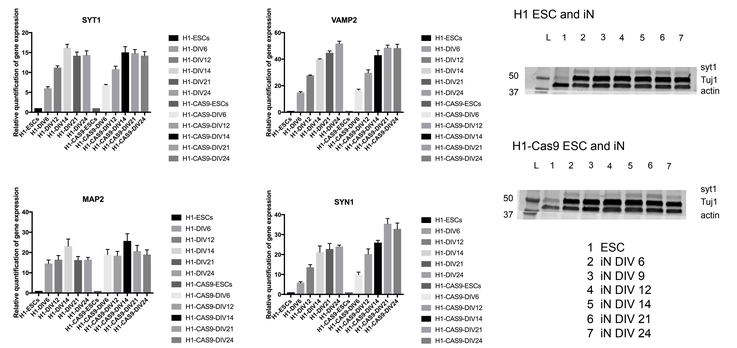
MS vs. RNAseq



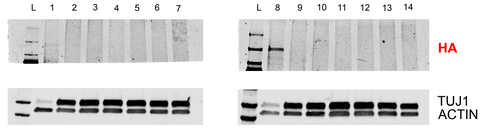
H1-CAS9 ESC LINE



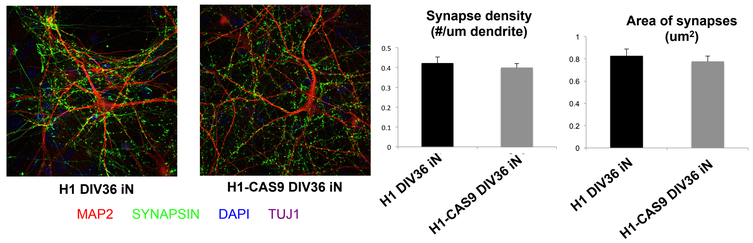
H1-CAS9 iN CELLS



Cas9 expression shuts off after Ngn2 differentiation



1 H1-ESCs	8 H1-CAS9-ESCs
2 H1 DIV6 iN	9 H1-CAS9 DIV6 iN
3 H1 DIV9 iN	10 H1-CAS9 DIV9 iN
4 H1 DIV12 iN	11 H1-CAS9 DIV12 iN
5 H1 DIV14 iN	12 H1-CAS9 DIV14 iN
6 H1 DIV21 iN	13 H1-CAS9 DIV21 iN
7 H1 DIV24 iN	14 H1-CAS9 DIV24 iN



CONCLUSIONS

This set of data acts as an important resource for subsequent investigations of human neurons. Pure iN, to first approximation, are comparable to iN on glia, and critically enable massive unbiased developmental characterization at the protein and transcript levels. Furthermore, the H1-Cas9 ESC line is robust and can be differentiated into iN (whereby Cas9 expression is shut off) and used for genetic screens.

We have since filtered the MS and RNAseq data sets for interesting candidates of little to unknown function and begun a screen to identify molecules that may be involved in synapse formation. Interestingly, a number of genes show phenotypes in immunofluorescence and multi-electrode array assays.

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