Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors

Marc Ehrlich, et al. 2017

Journal club presentation by
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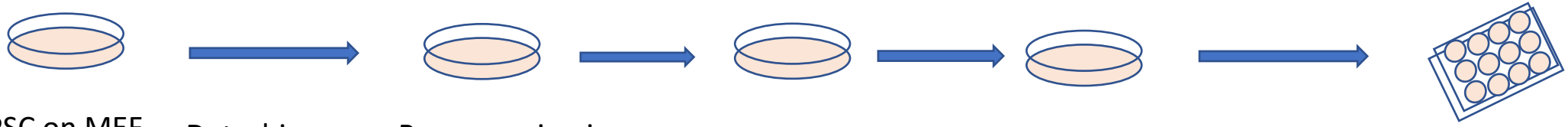
Purpose of This Study

- To generate oligodendrocytes (OLs) from human pluripotent stem cells (hPSC).
- To use OLs for disease modeling for multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and multiple system atrophy (MSA) .
- For drug discovery through high-throughput screening (HTS) format to test myelination of neurons by OLs in response to pro-myelinating drugs.
- To utilize for therapeutic OL transplantation.

Generation of human NPC



hPSC on MEF

Detaching
& collecting
pieces of
colonies

Day 0

Resuspension in
ESC medium in
petri dish.
- FGF
+ SB-431542
+ Dorsomorphin
+ CHIR99021
+ Purmorphamine

Day 2

N2B27 medium
+ SB-431542
+ Dorsomorphin
+ CHIR99021
+ Purmorphamine

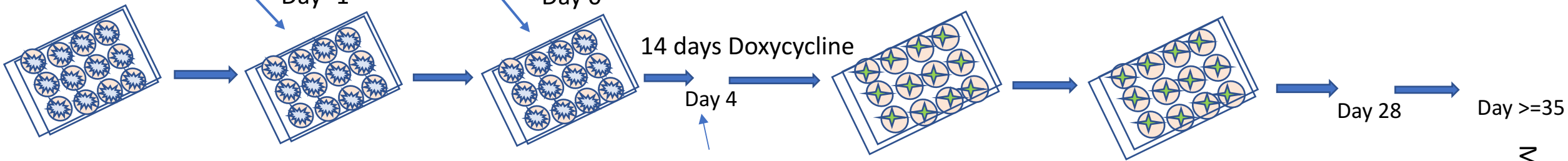
Day 4

N2B27 medium
+ CHIR99021
+ Purmorphamine
+ Ascorbic Acid

Day 6

Disintegrated EBs
Plated on matrigel.
NPCM medium:
N2B27 medium
+ CHIR
+ SAG
+ AA

Oligodendrocyte Differentiation



hNPCs seeding at $1X10^5$ cells/well

NPCM medium
Protamine sulfate
Lenti-rtTA
pLV-TetO-TF:
ASCL1
MYT1
NKX2.2
NKX6.1
NKX6.2
OLIG2
SOX10

GIM:
N2B27 medium
SAG
PDGF
NT3
IGF-1
AA
Trace Elements B
T3

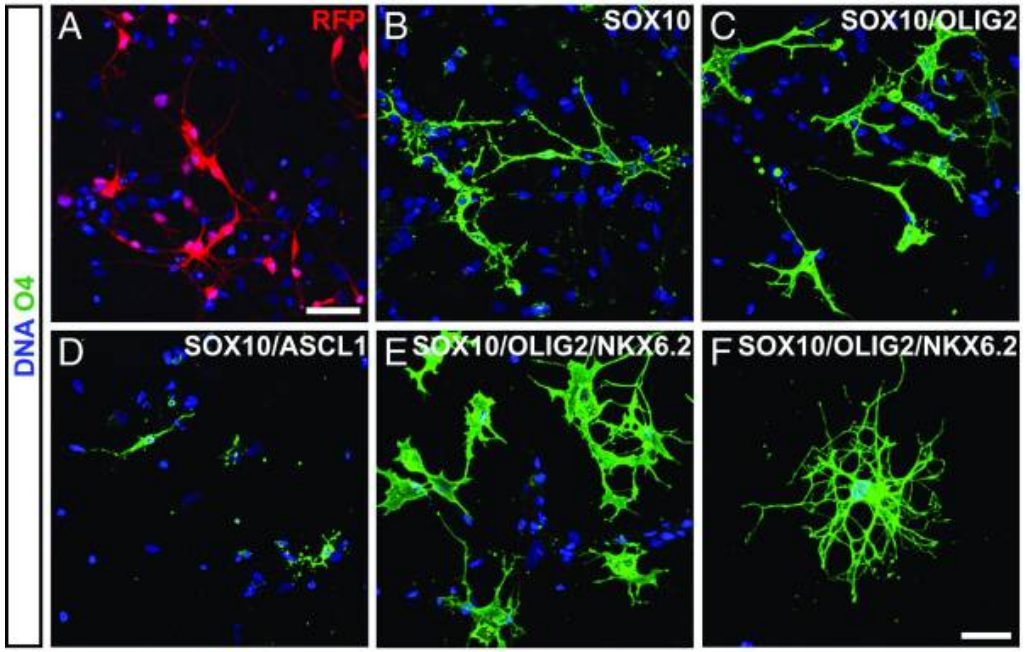
DM:
DMEM-F12
N2
B27 - Vitamin A
Pen/Strep/Glutamine
T3
NT3
IGF-1
AA
Trace Element B
dbcAMP

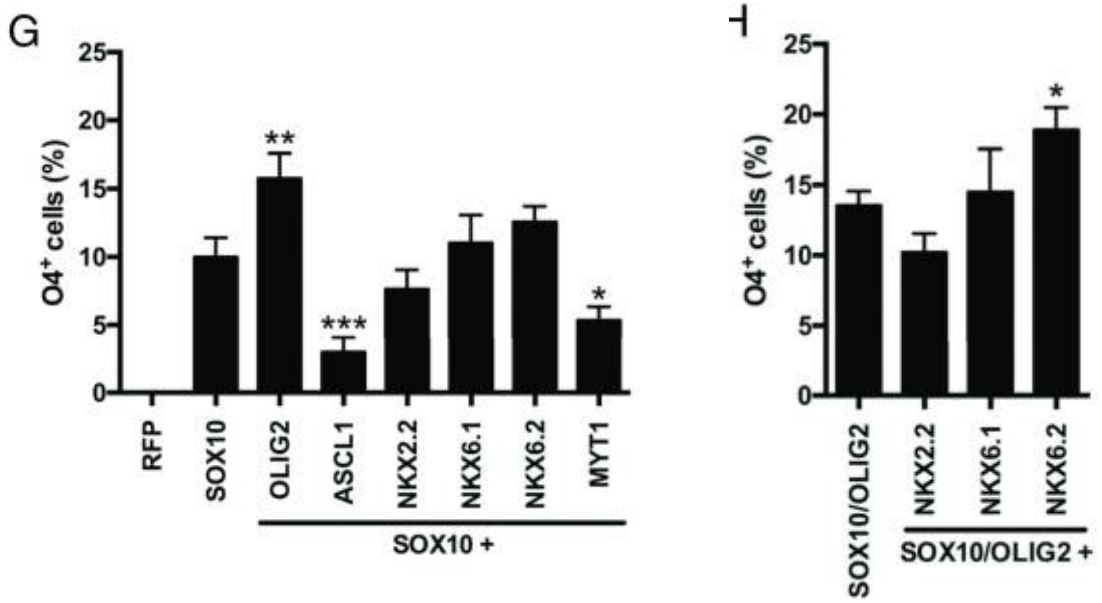
OLs

Accutase
Single cells seeding at $2.5X10^5$ cells/well

Gene expression profiling
Compound screening

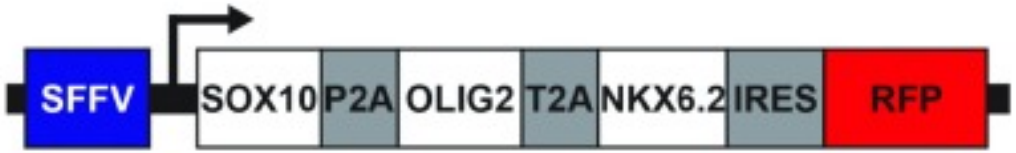
Screening for TFs that induce oligodendroglial lineage



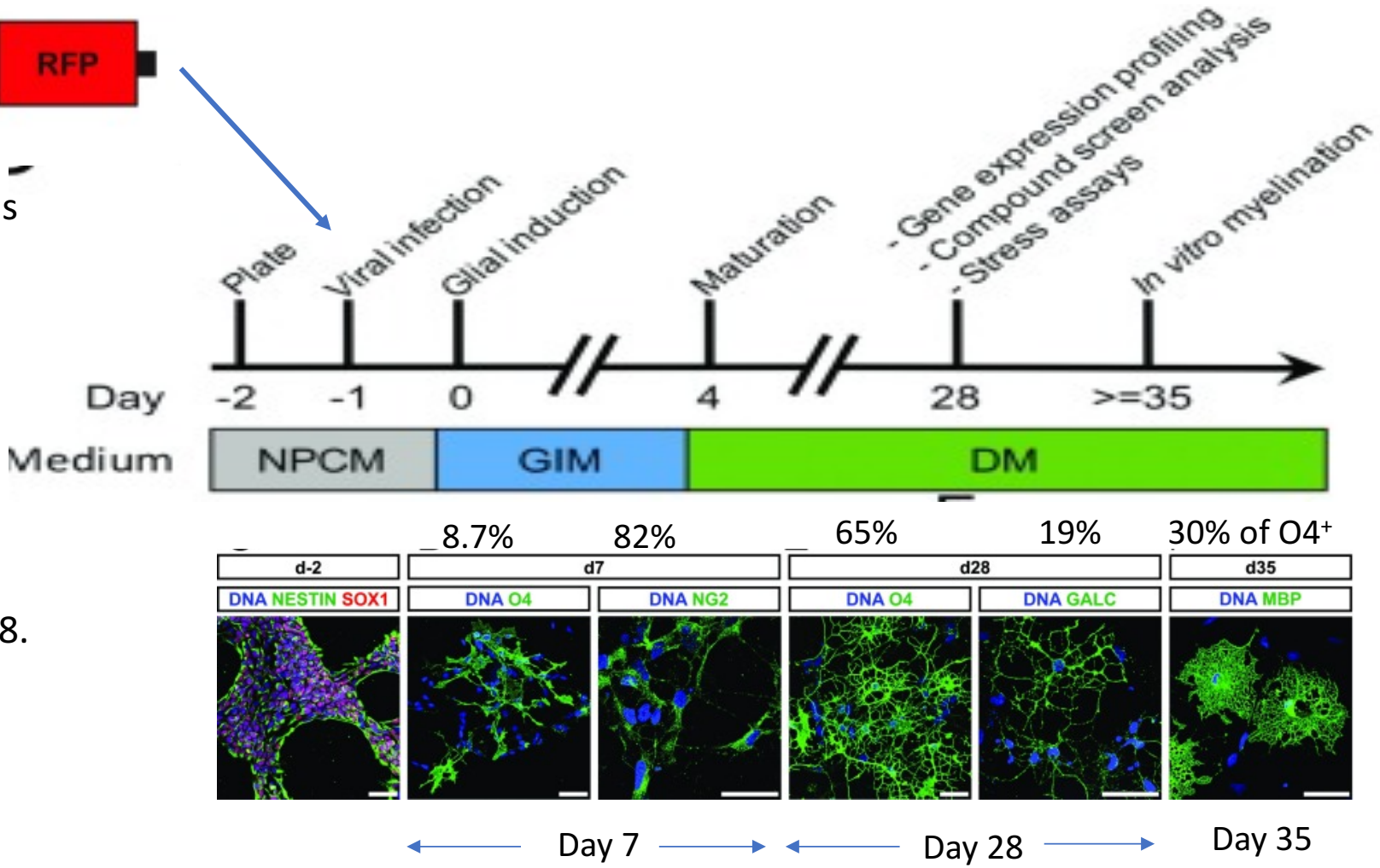


Immunostaining for $O4^+$ OLs at day 14 of TF induction shows coexpression of SOX10, OLIG2, and NKX6.2 (SON) is the most efficient combination to induce the OL-lineage commitment.

SON induce a rapid and efficient oligodendroglial lineage commitment.



Polycistronic expression of SON in Lentivirus

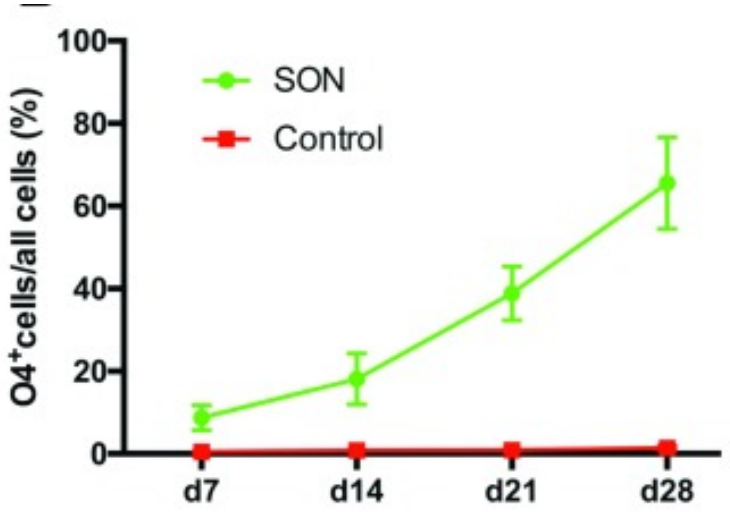


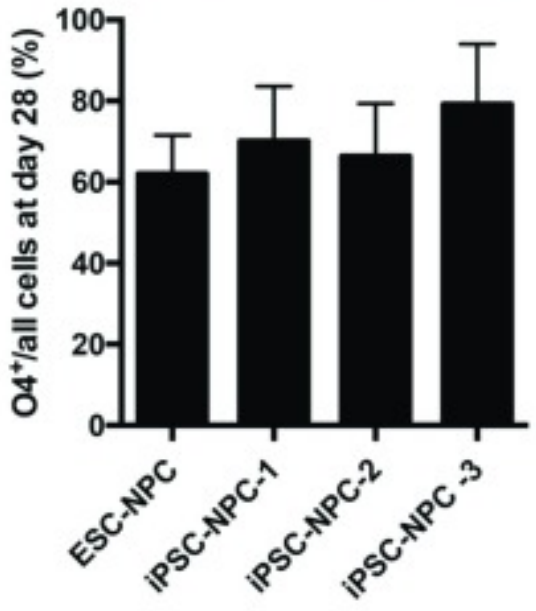
7 days after transgene induction, NG2$^+$ and O4$^+$ oligodendroglial lineage cells are detected.

Mature OL marker GALC appears by day 28.

MBP$^+$ marker for mature iOL is expressed by day 35.

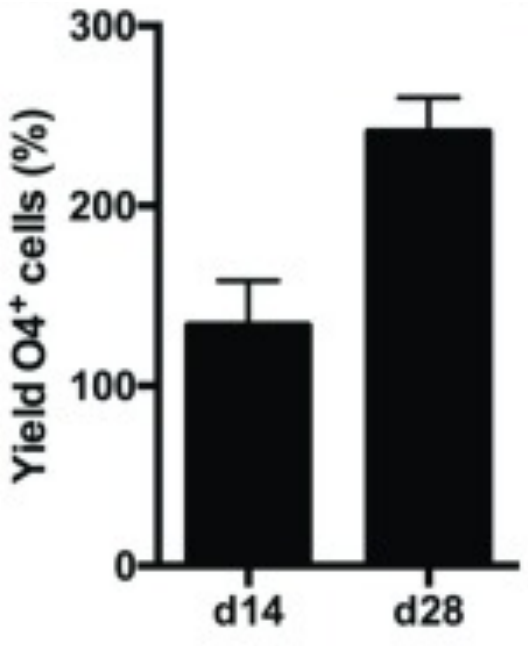
Quantification of FACS analysis after SON induction



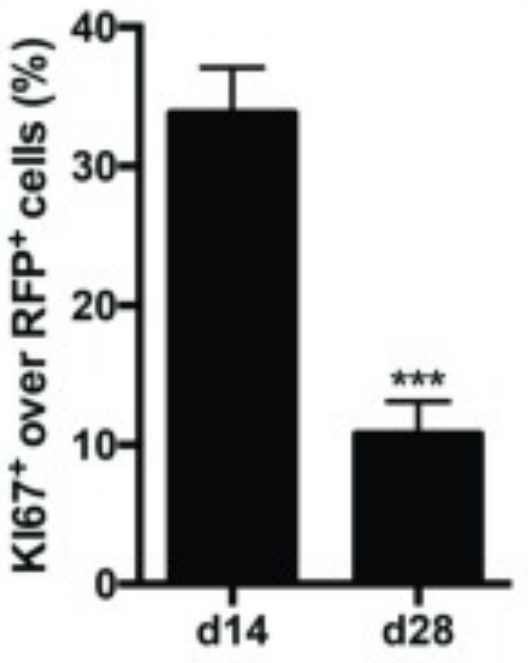


All three independent NPC derived from different iPSC lines, and one NPC line derived from ESC yielded similar results for ~65% $O4^+$ cells on day 28.

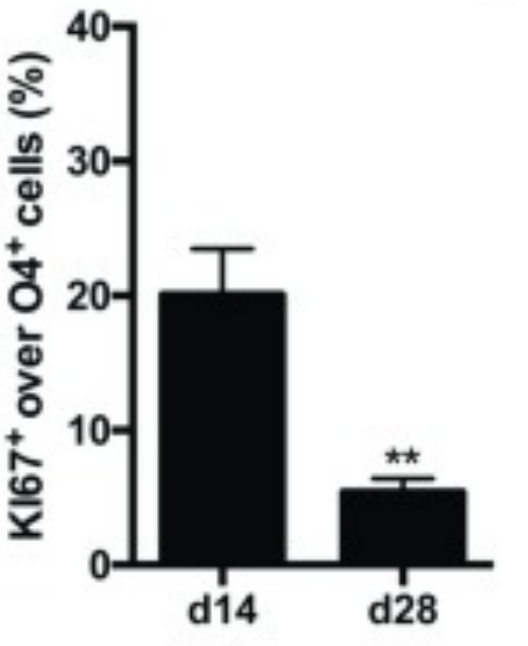
Transgene-expressing cell population expands during differentiation.



Total $O4^+$ iOL/starting NPC cell numbers ranged from 133-241% from day 14-28.

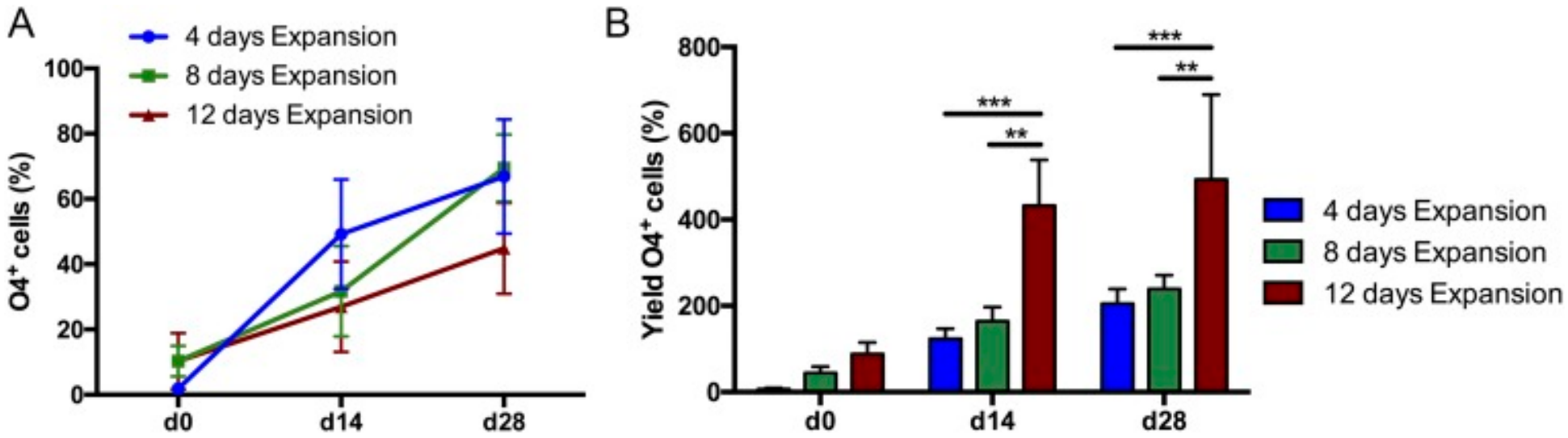


Proliferative Ki-67^+ cells changed from 35% to 10% among RFP^+ population.



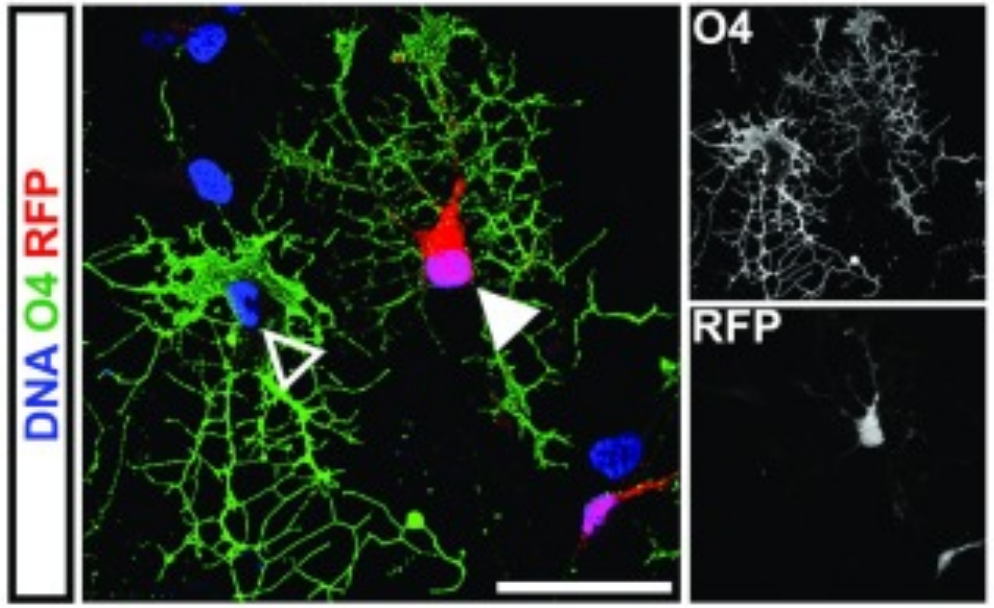
Proliferation of $O4^+$ cells diminished from 20% to 5% from day 14-28.

Yield of $O4^+$ iOL significantly increases after prolonged exposure to GEM.



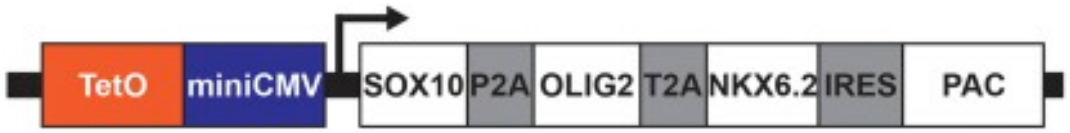
Glial expansion medium containing FGF2 rather than IGF-1 was added postlentiviral transduction for 4, 8, or 12 days. Transferred to differentiation medium for additional 28 days.
FACS analysis at day 0, 14, and 28 shows the differentiation efficiency decreased but $O4^+$ yield increased.

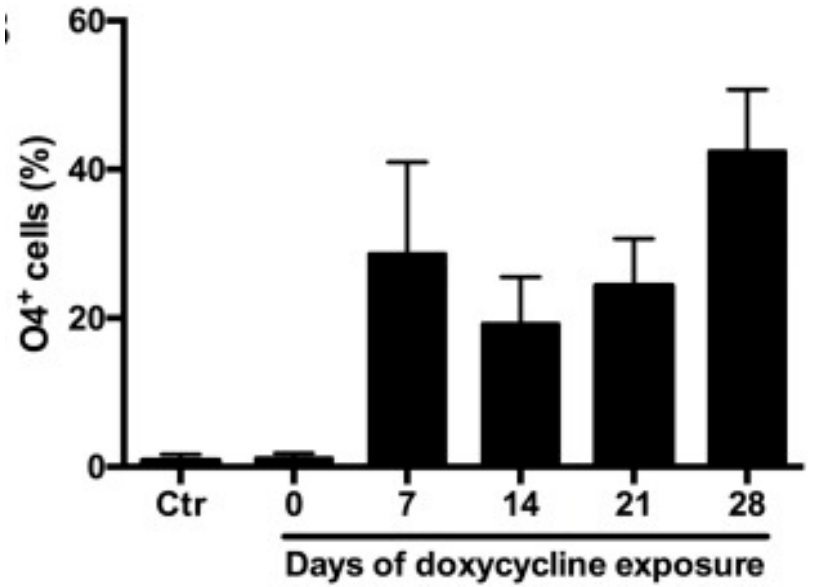
Transgene silencing in a subset of iOL cells



The presence of an $O4^{+}/RFP^{-}$ cell population in SON-transduced cultures 28 days after transduction.

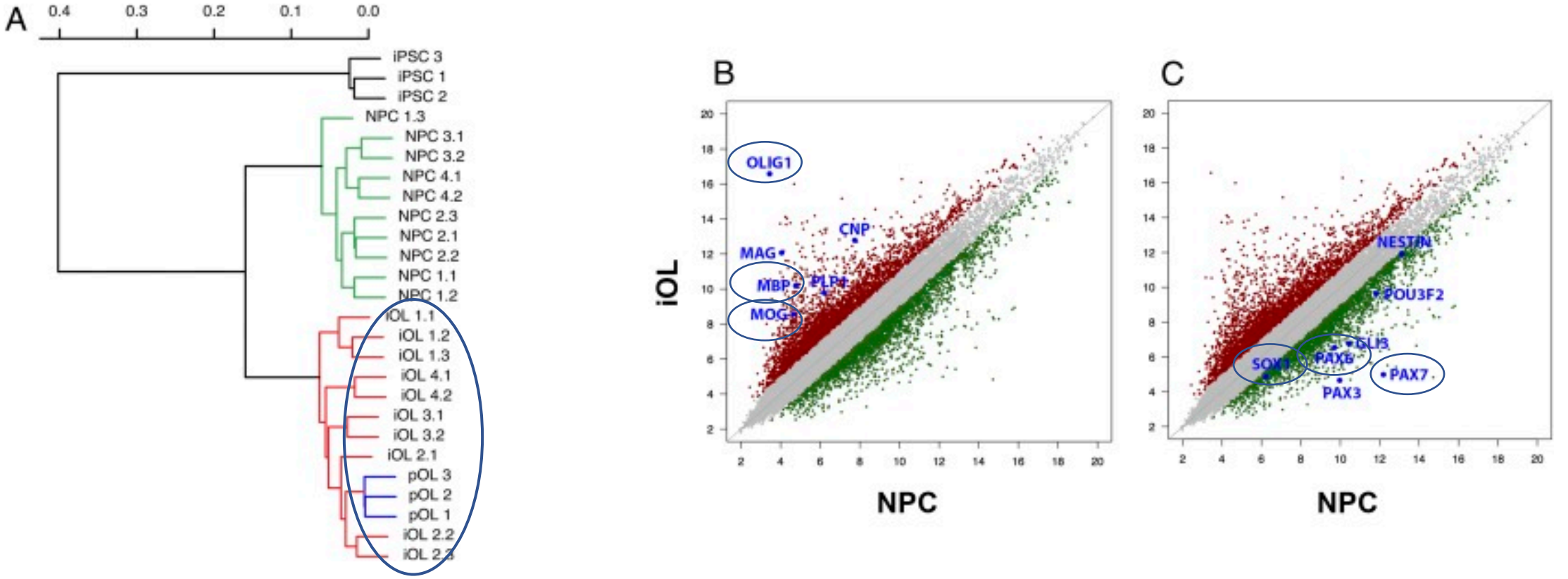
A subpopulation of O4^{+} iOL remains dependent on the ectopic expression of SON.





FACS analysis shows induced expression of SON for 7 days is sufficient to obtain a stable and transgene independent Oligodendroglial lineage commitment.

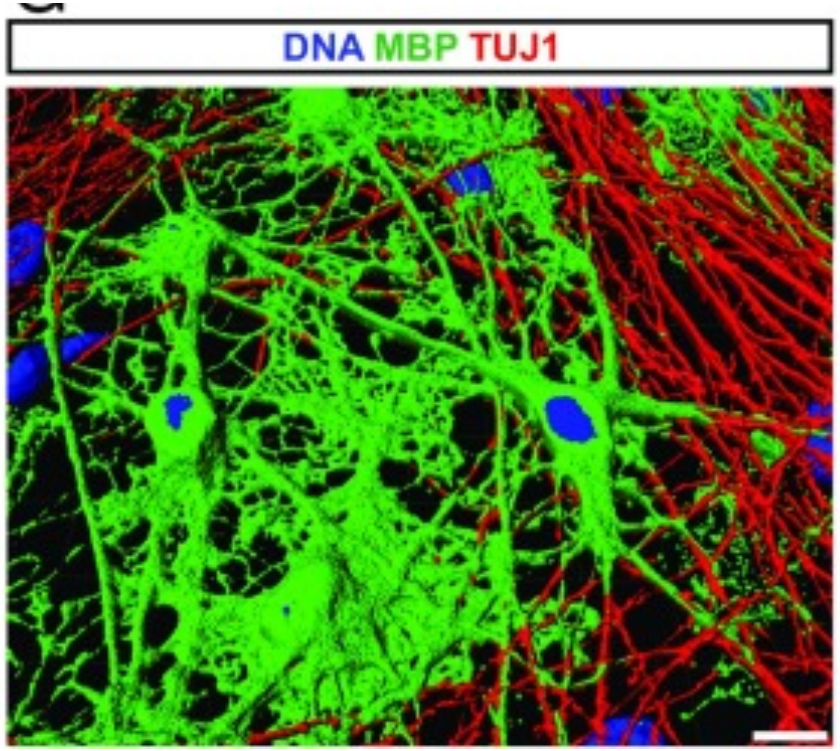
Global gene-expression profiling demonstrates that iOLs resemble primary human adult OL.



Up-regulation of OL-specific genes OLIG1, MOG, and MBP and down-regulation of NPC-related genes SOX1, PAX6, and PAX7 in $O4^+$ iOL compared with NPC.

Induced OLs mature and ensheath iPSC-derived neurons in vitro.

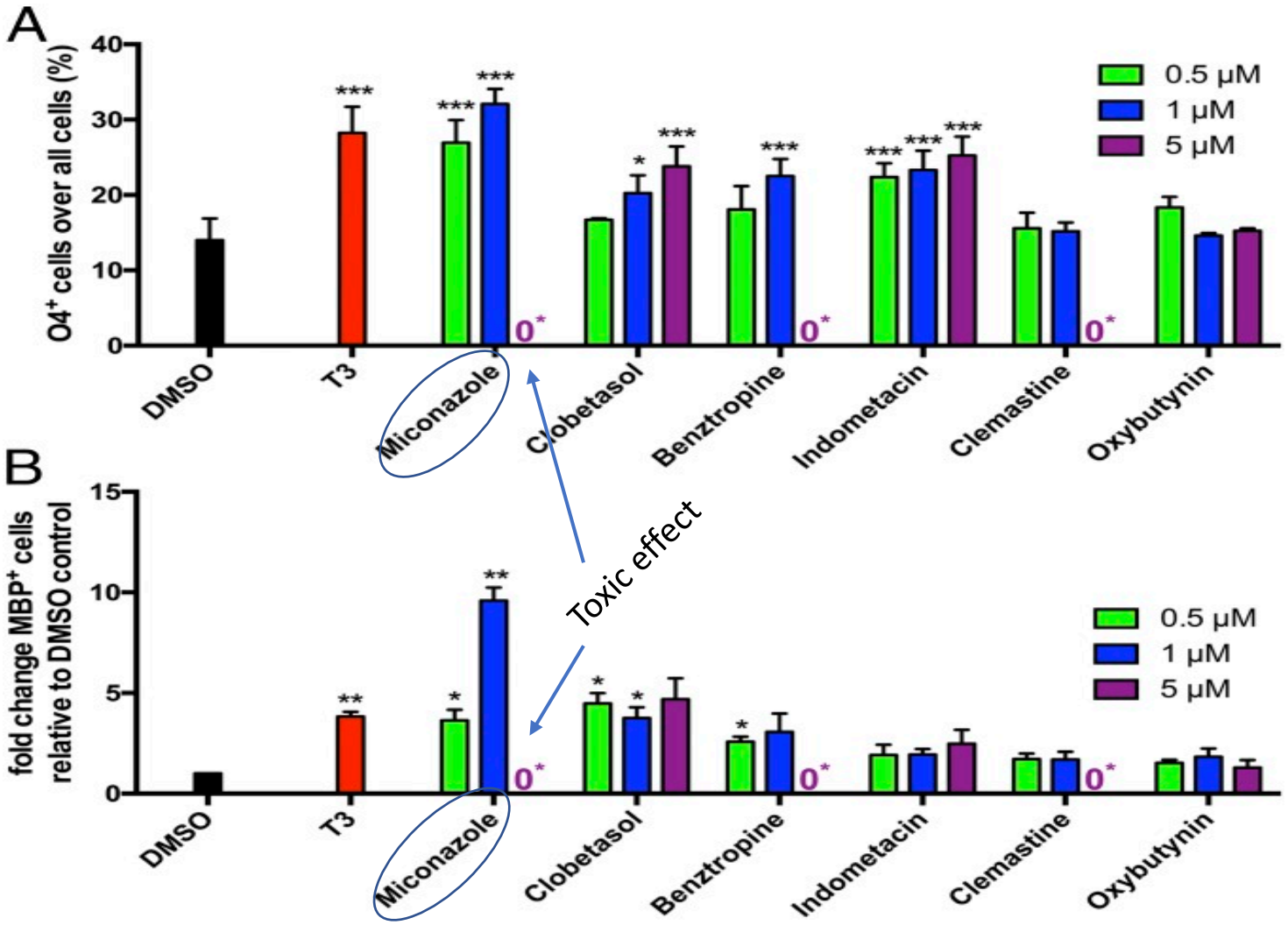
Human in vitro myelination assay:
Co-culture of $O4^+$ iOL purified at day 21 by MACS with iPSC-derived neurons for 3 weeks suggests wrapping of $Tuj1^+$ axons with MBP^+ mature OLs.



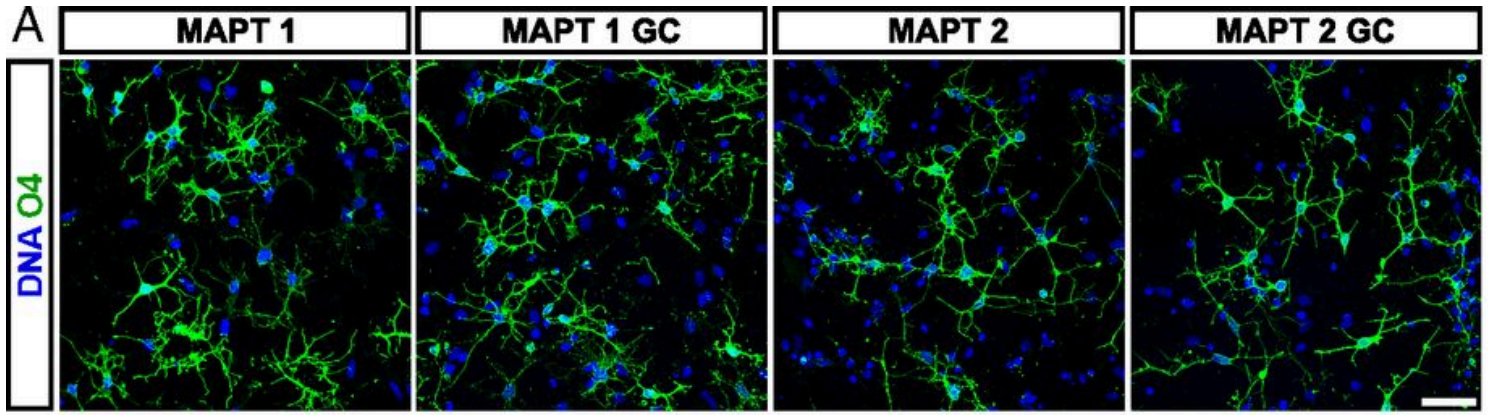
The iOLs are suitable for pharmacological screens.

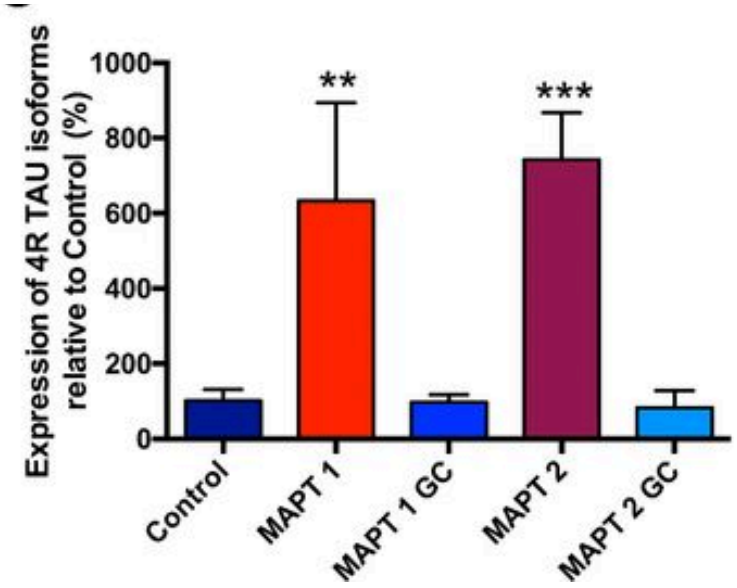
The compounds affect different stages of oligodendroglial differentiation.

Only a subset of compounds enhanced the maturation of iOL.



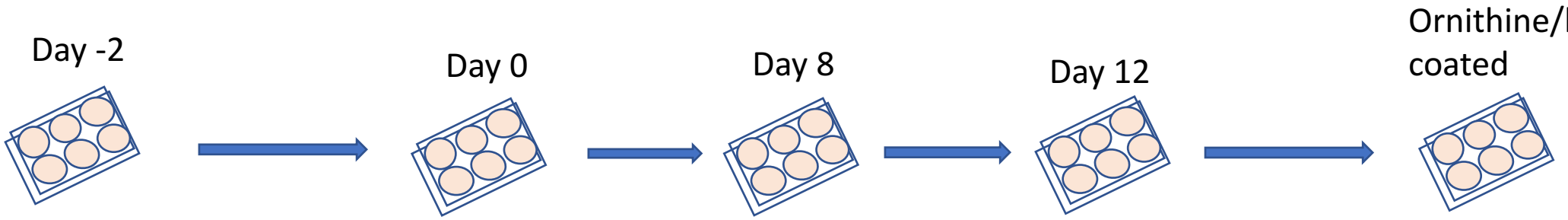
The iOLs are suitable for disease modeling.





RT-qPCR analysis of iOL from patients with a N279K mutation in the MAPT gene exhibits increased levels of the 4R tau isoform.

Generation of human NPC (Garcia-Leon)



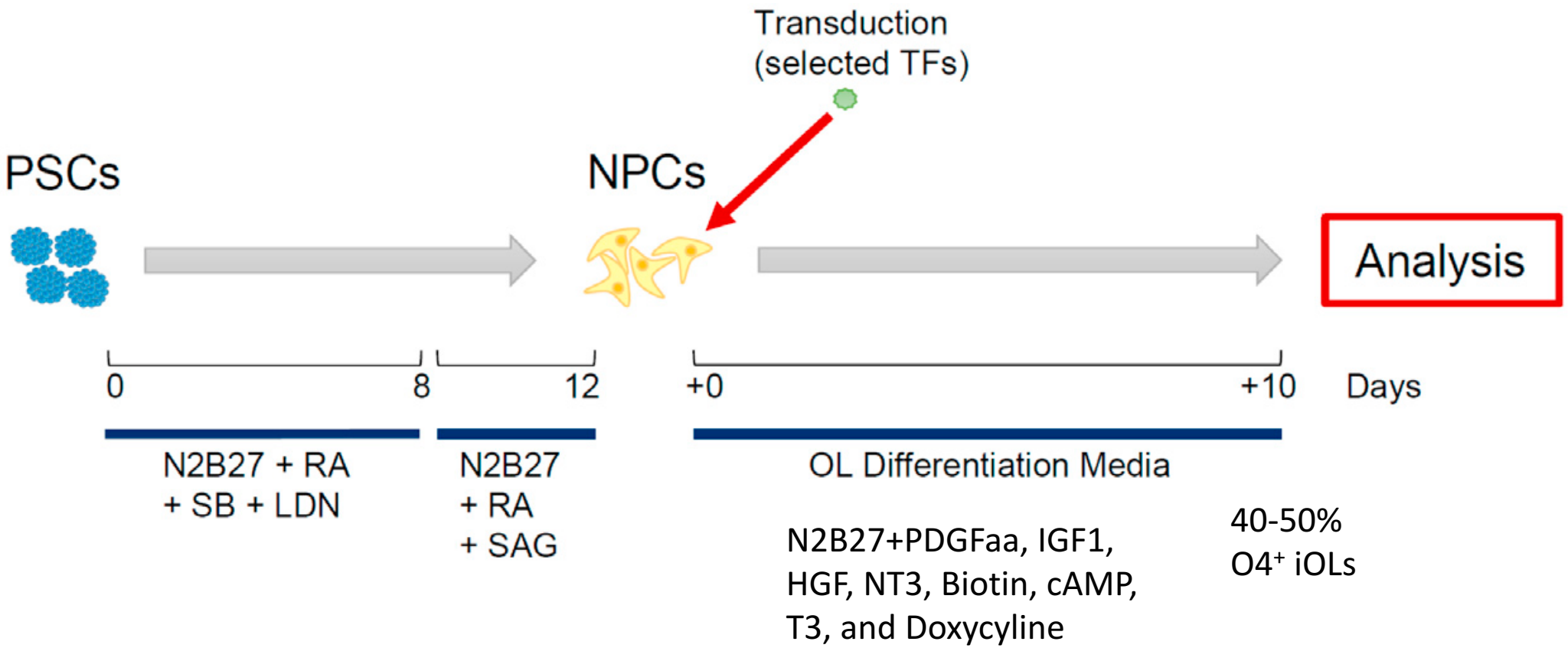
hPSCs seeded at 20,000 cells/cm^2 on matrigel.

N2B27 medium:
DMEM/F12, N2, B27, glutamax, NEAA, beta-mercaptoethanol, Pen/Strep, and Insulin.
SB-431542
LDN193189
Retinoic acid (RA)

N2B27 medium
Insulin
SAG
RA

Dissociated & plated at 50,000 cells/cm^2 for transduction & differentiation to OL lineage

Oligodendrocyte Differentiation



TFs screened by Garcia-Leon (2018) vs Ehrlich (2017)

16 Transcription Factors were selected for transduction into NPC.

ASCL

AXIN2

MYRF

MYT1

NKX2-2, NKX6-1, NKX6-2

OLIG1, OLIG2

SOX2, SOX8, SOX9, SOX10, ST18

ZEB2, ZNF536

7 Transcription Factors were selected for transduction into NPC.

ASCL1

MYT1

NKX2.2

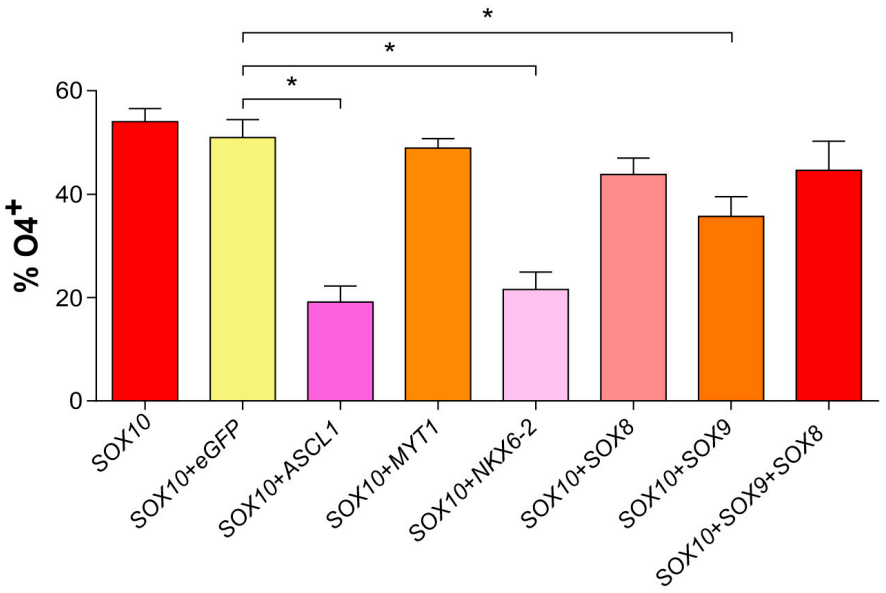
NKX6.1

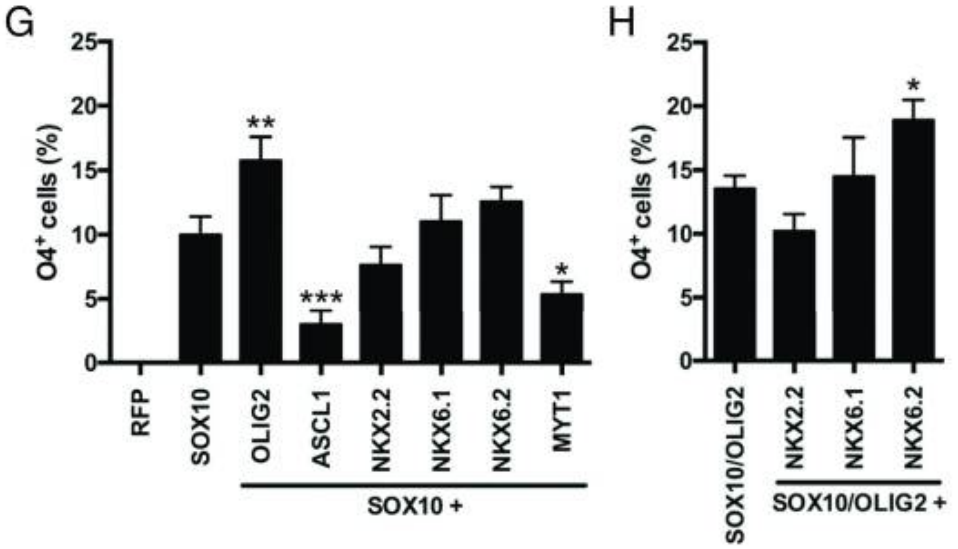
NKX6.2

OLIG2

SOX10

Conclusion Garcia-Leon vs Ehrlich





No further increase in $O4^+$ cells was seen with any TF combination over SOX10 alone. SOX10 alone induces functional OLs from hPSCs in 22 days with 40-50% $O4^+$ OLs.

Combination of SOX10, OLIG2, and NKX6.2 is the most efficient to induce the OL-lineage commitment in 28 days from NPC stage with 65.5% $O4^+$ OLs.

Using Piggybac to deliver transcription factors to NPCs