UNIVERSITY OF CAMBRIDGE

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Cambridge Stem Cell Institute

Train MALTA Horizon 2020

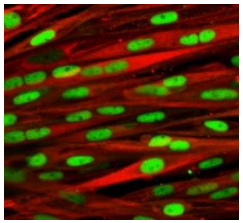
Inducible modulation of gene function in human pluripotent stem cells and their derivatives

Daniel Ortmann

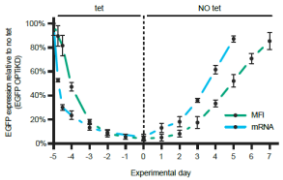
1.6.2017 Train Malta

Laboratory for Regenerative Medicine
Stem Cell Institute, Cambridge UK

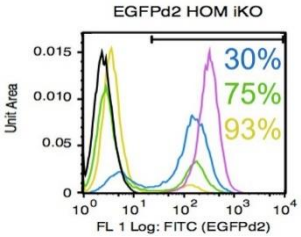
Structure of the talk



Inducible over-expression and forward programming

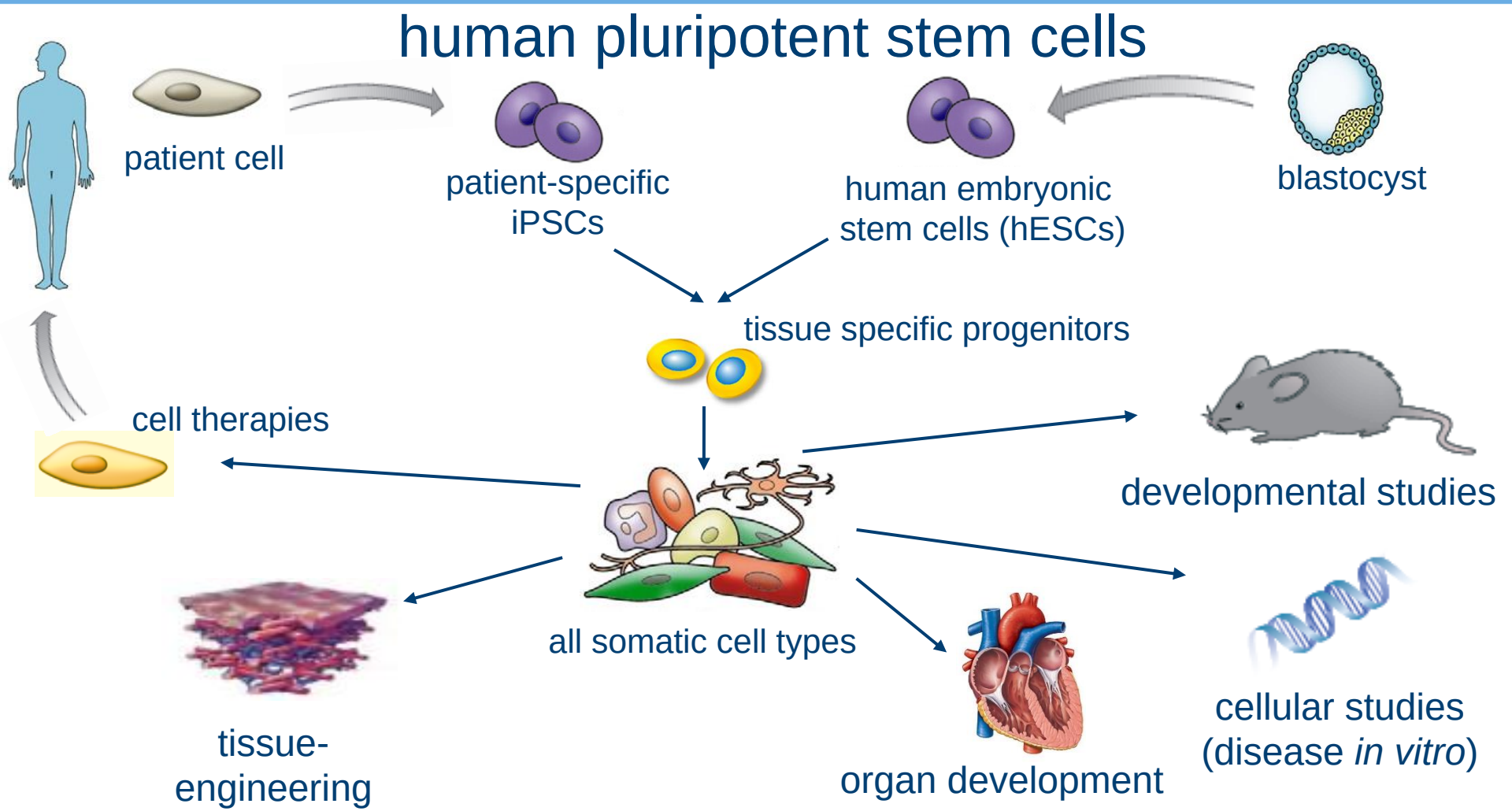


Inducible knock-down using shRNAs



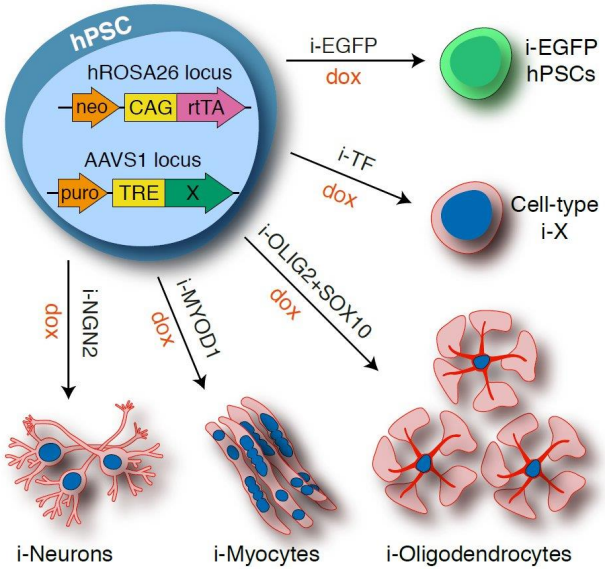
Inducible knock-out using CRISPR/CAS9 and gRNAs

Stem cells in medical research



Inducible gene expression and forward programming

Dr. Alessandro Bertero



Dr. Matthias Pawlowski

STEM CELL REPORTS

Volume 8, Issue 4, 11 April 2017, Pages 803–812

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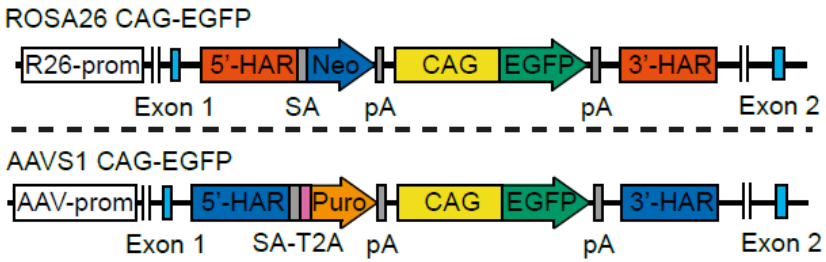
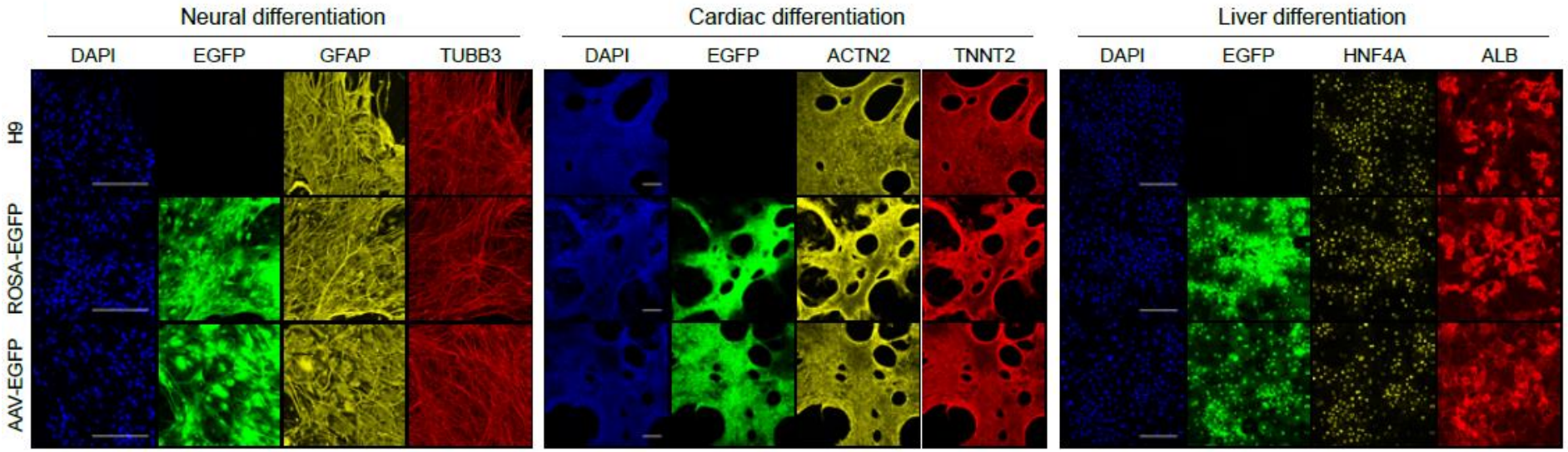
Report

Inducible and Deterministic Forward Programming of Human Pluripotent Stem Cells into Neurons, Skeletal Myocytes, and Oligodendrocytes

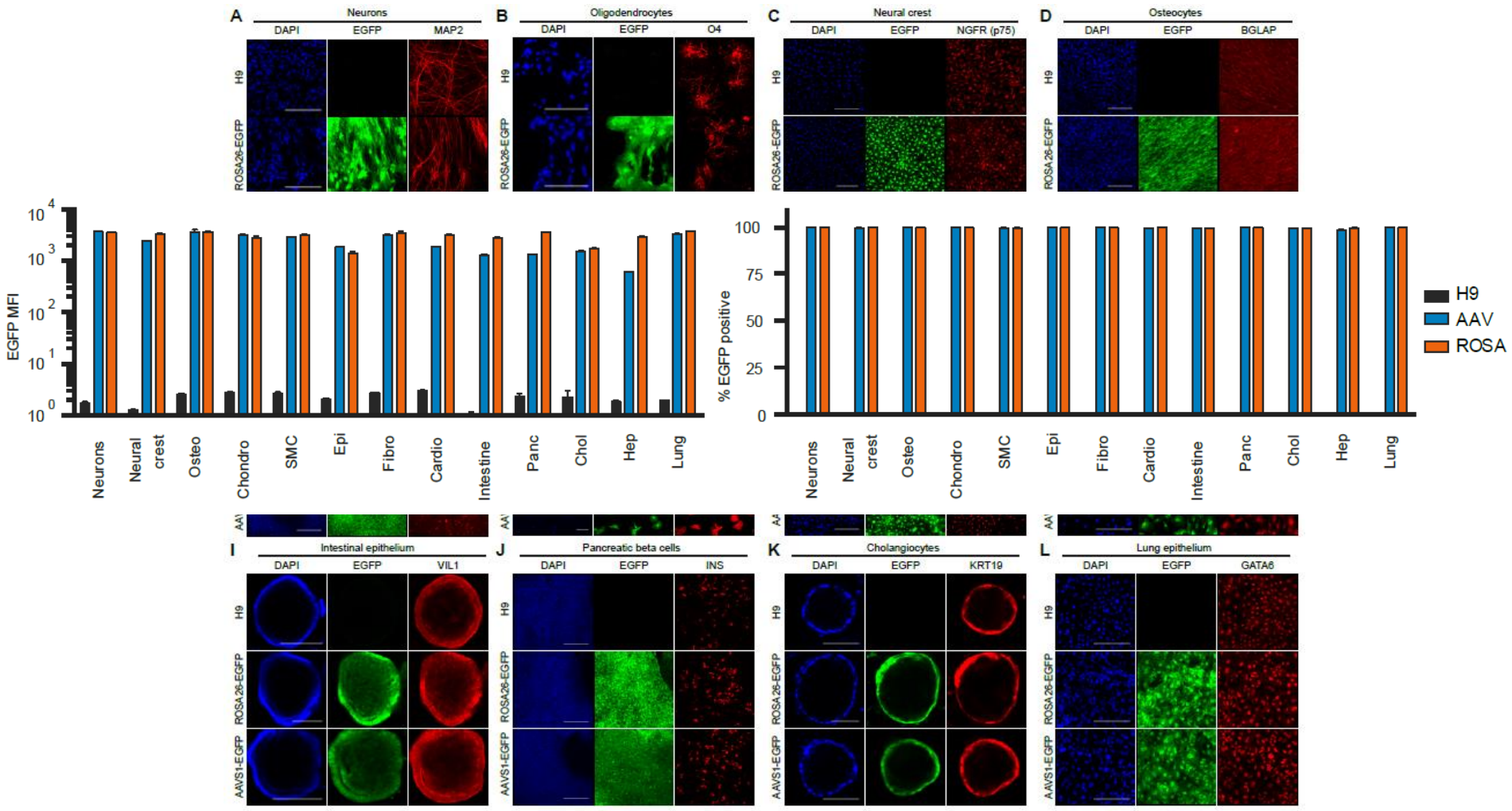
Matthias Pawlowski[1, 2, 6, 7], Daniel Ortmann[1, 3, 6], Alessandro Bertero[1, 3, 6, 8], Joana M. Tavares[2], Roger A. Pedersen[1, 5], Ludovic Vallier[1, 3, 4], Mark R.N. Kotter[1, 2]

Expression from Genomic Safe Harbours (GSHs)

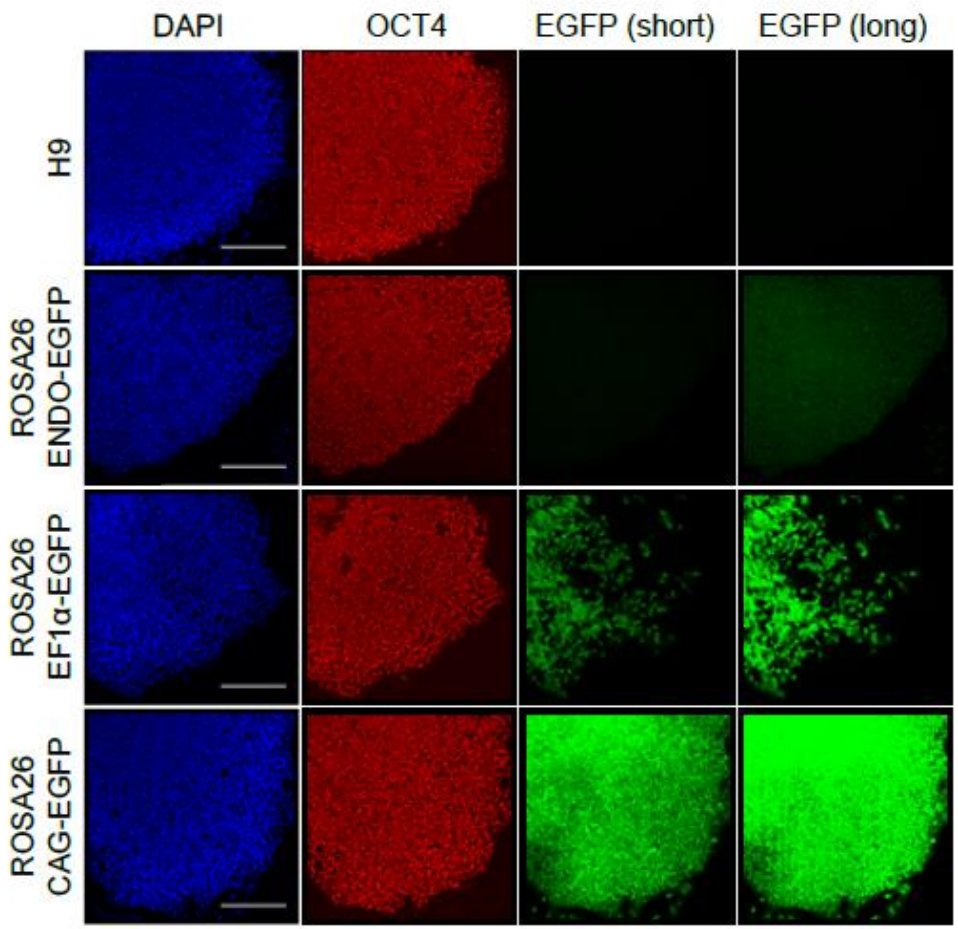


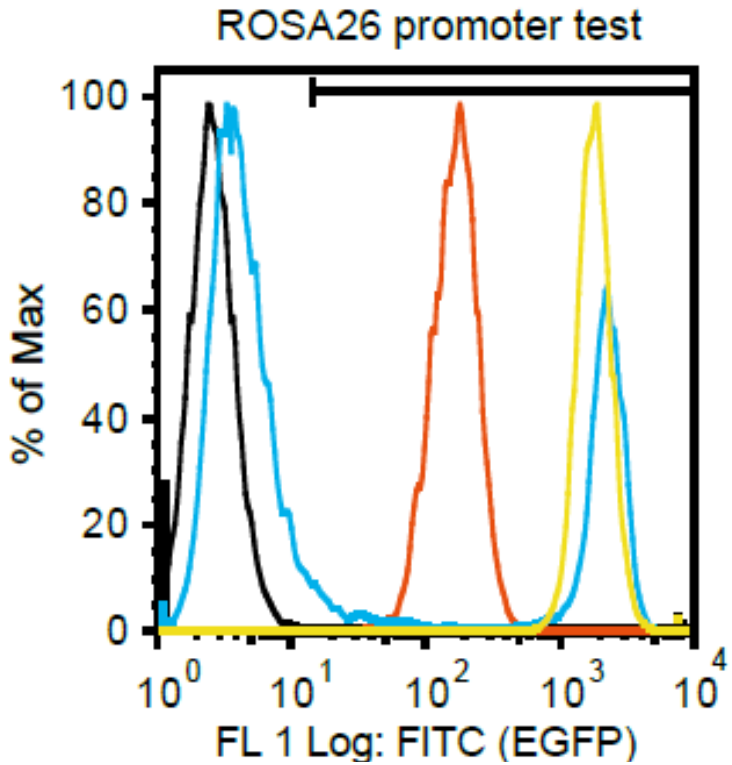


Expression from GSHs is stable in all cell lineages tested



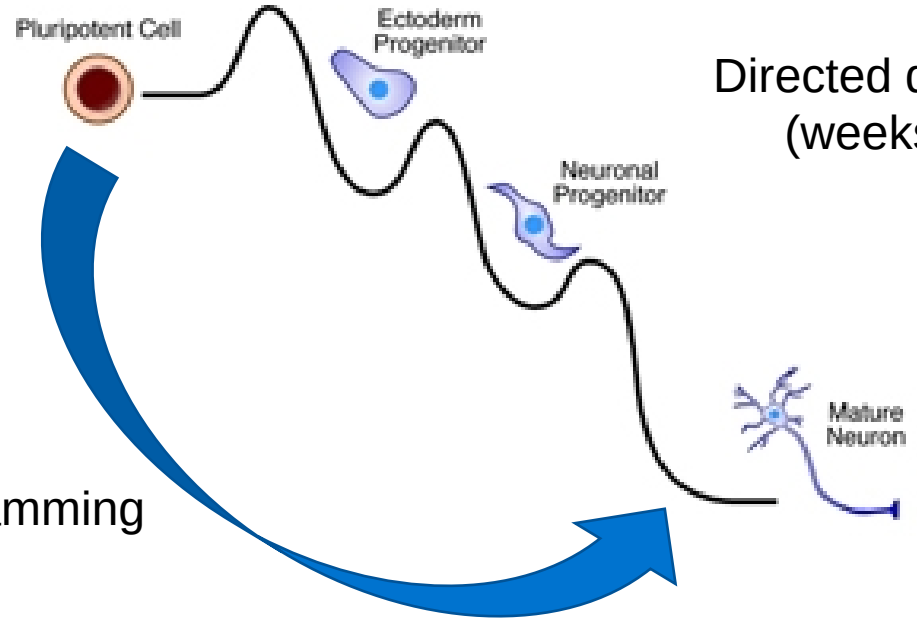
Choice of Promoter





Sample	%EGFP+	EGFP MFI
H9 (CTR)	0.0177	2.47
ENDO-EGFP	99.7	168
EF1α-EGFP	31.9	5.22
CAG-EGFP	100	1835

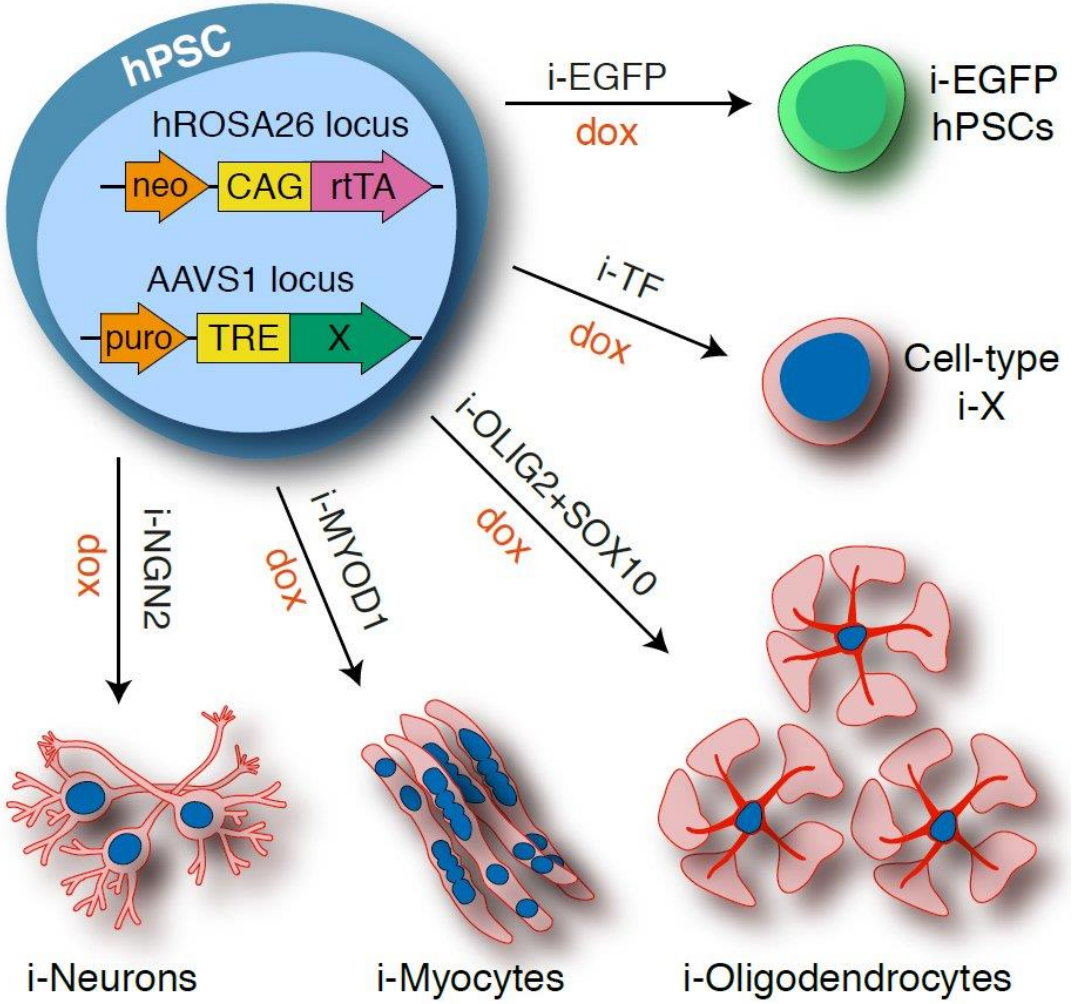
Directed differentiation vs Forward programming



Directed differentiation (weeks-months)

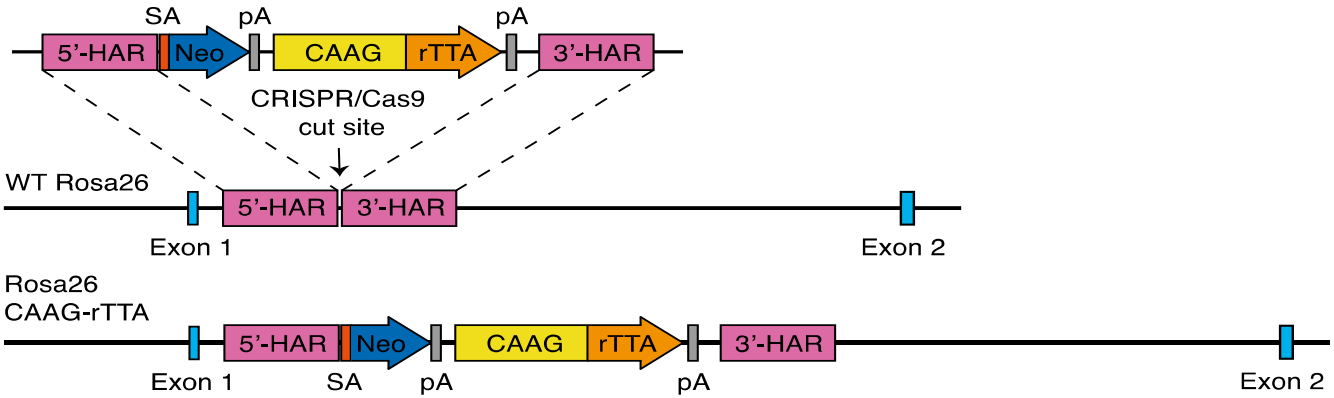
Forward programming (days)

Inducible over-expression (iOX) and forward programming

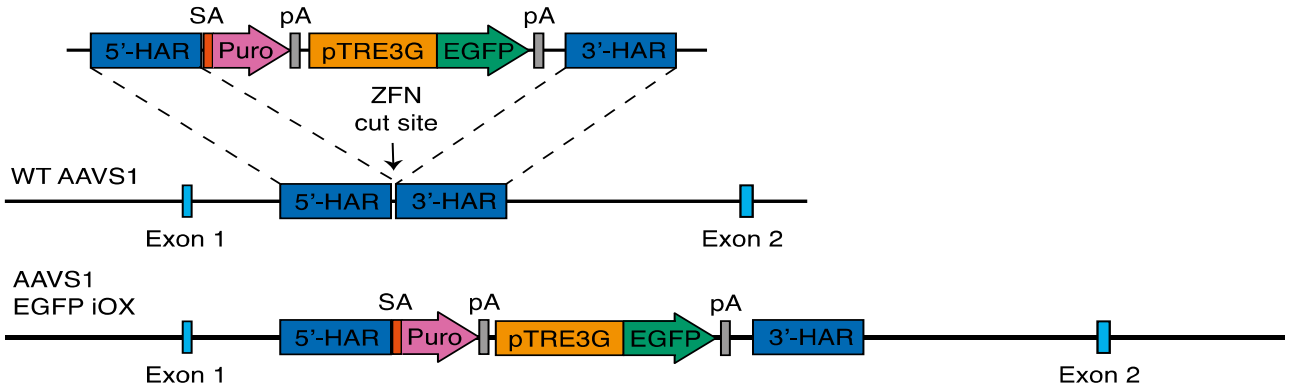


iOX design

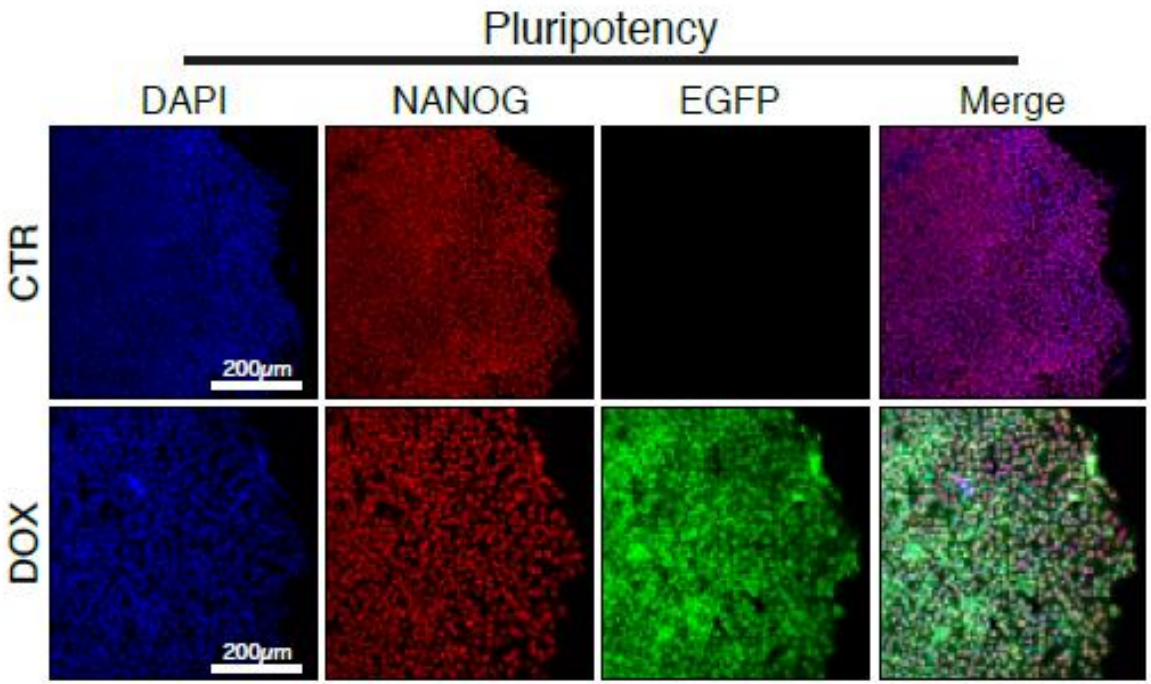
ROSA26: activator

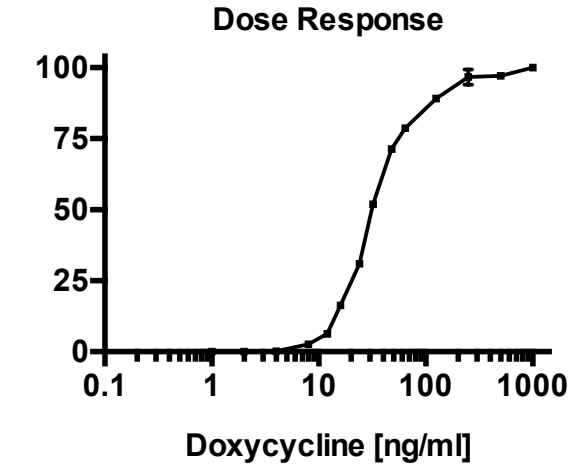


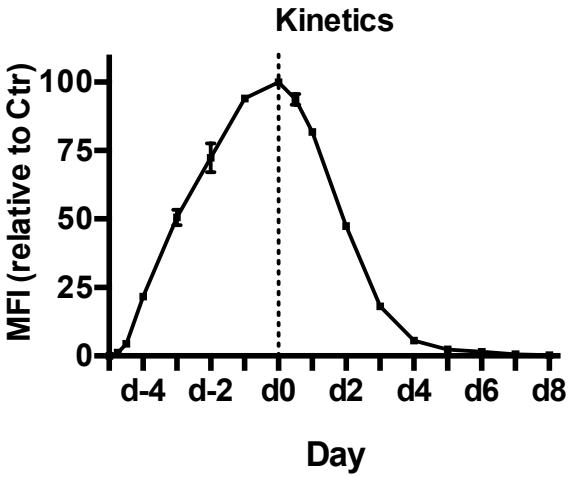
AAVS1: inducible transgene



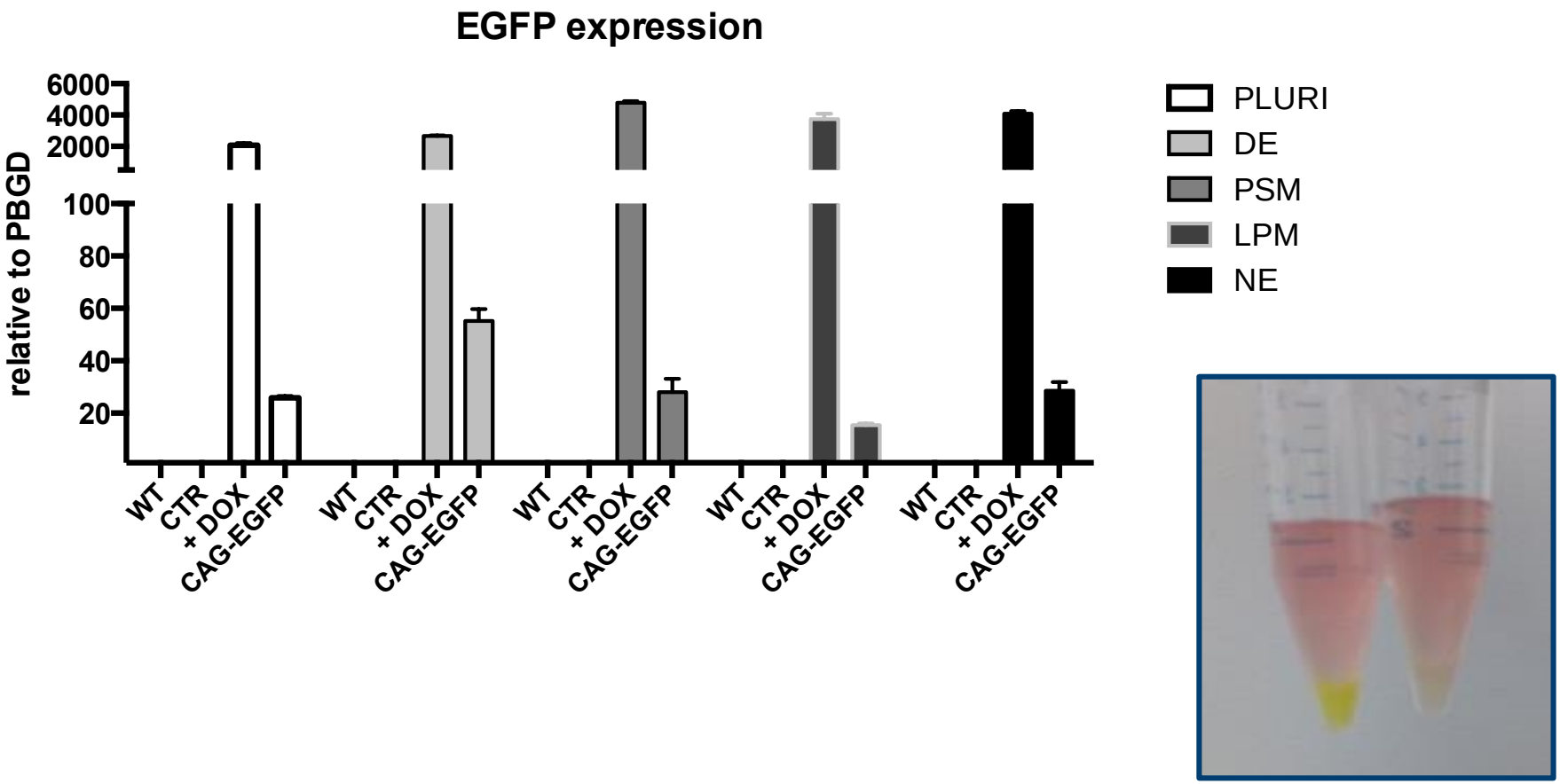
Proof of principle - inducible EGFP



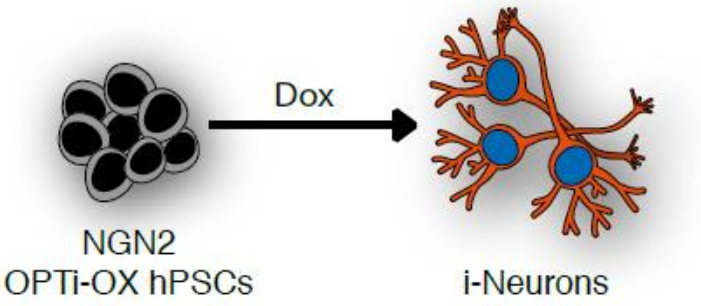


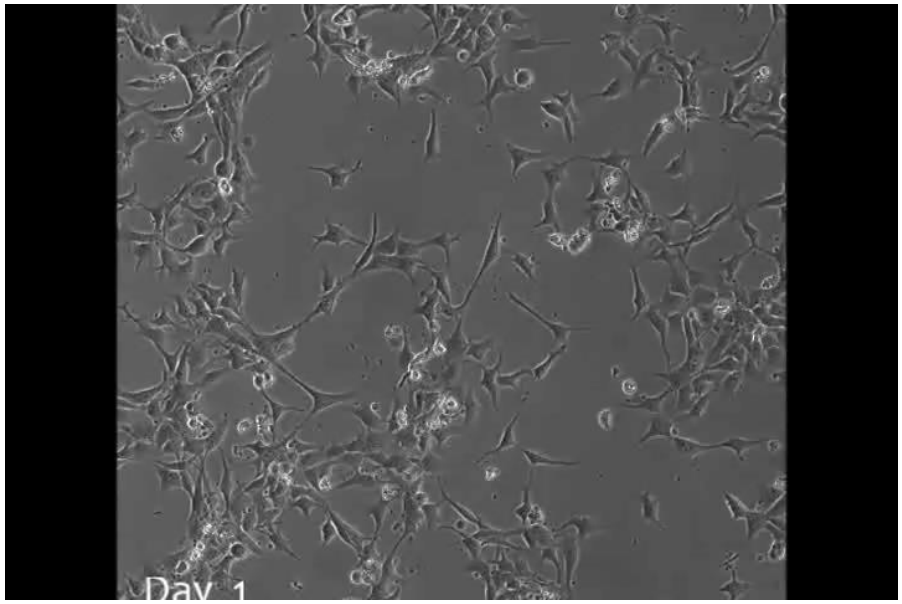


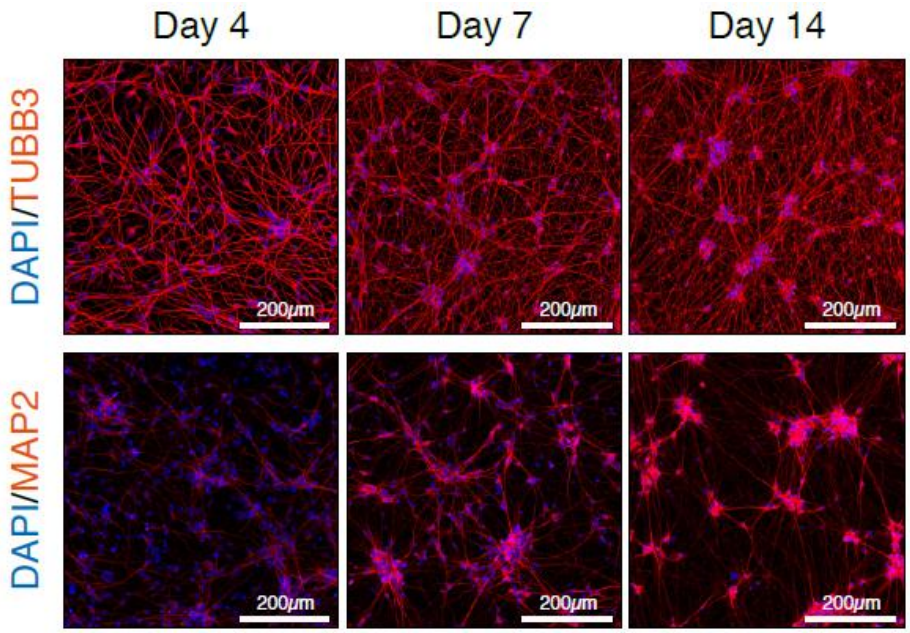
Proof of principle - inducible EGFP



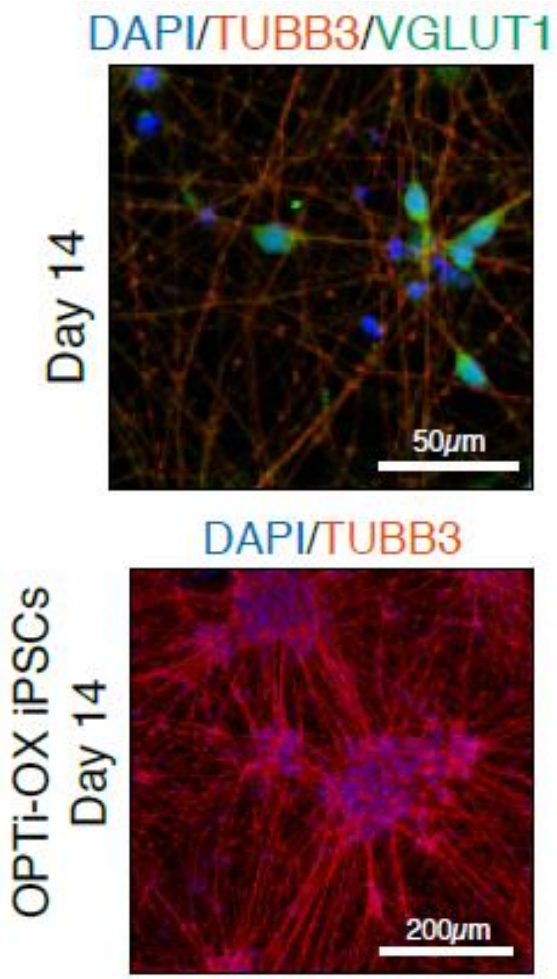
Forward programming of neurons using iNGN2

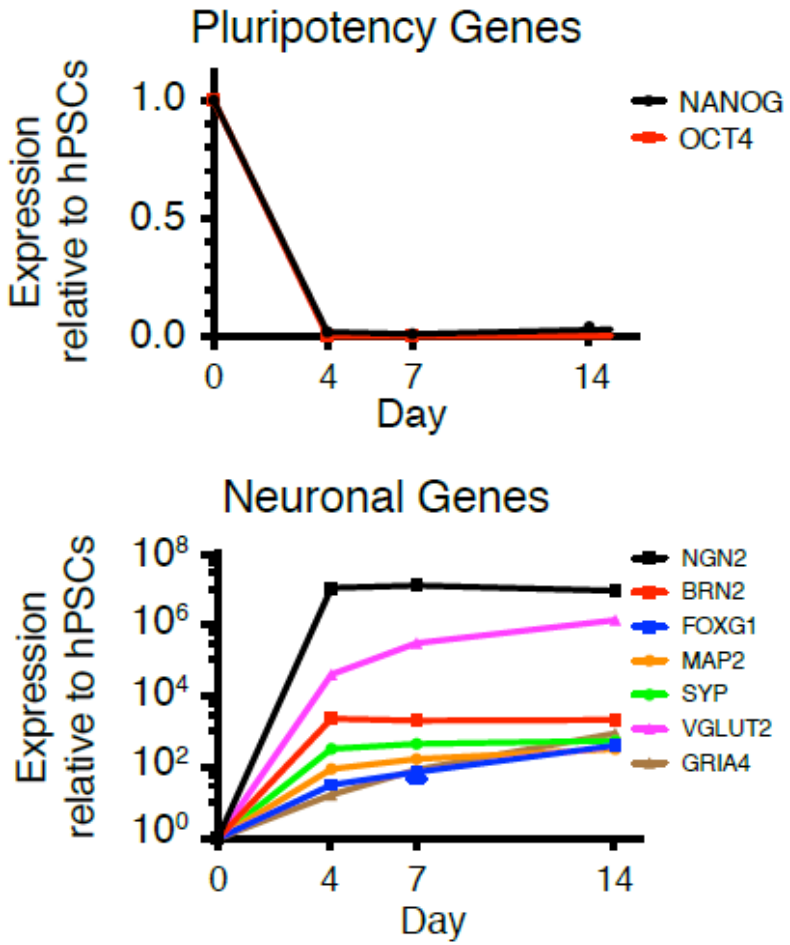




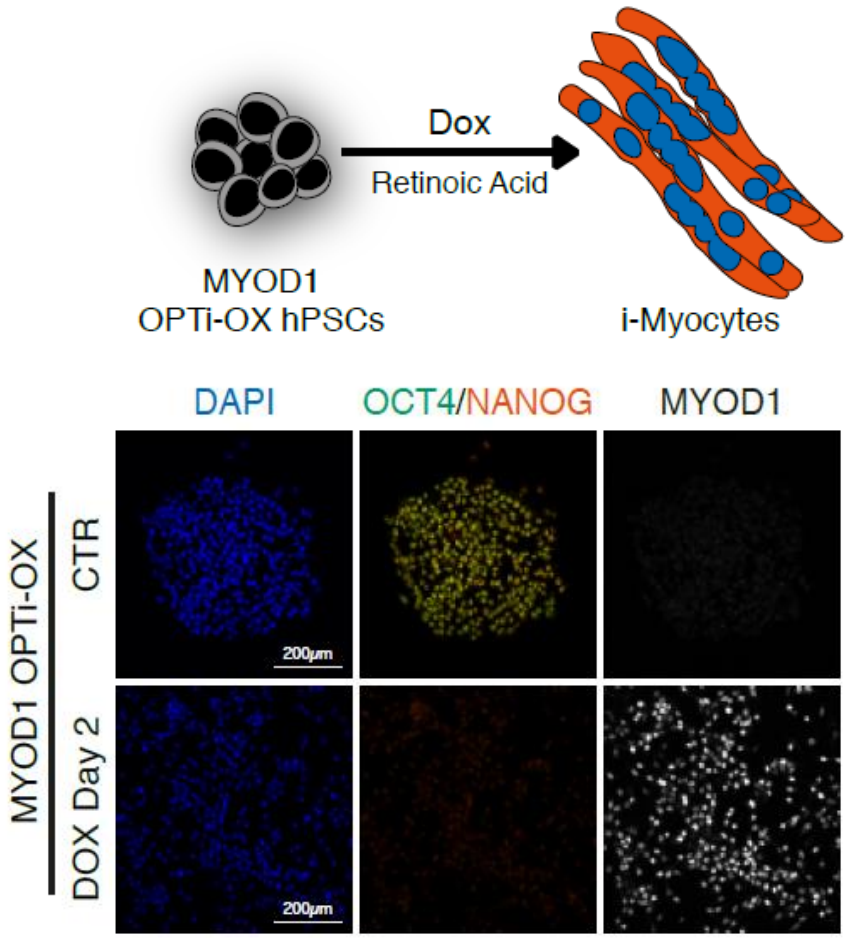


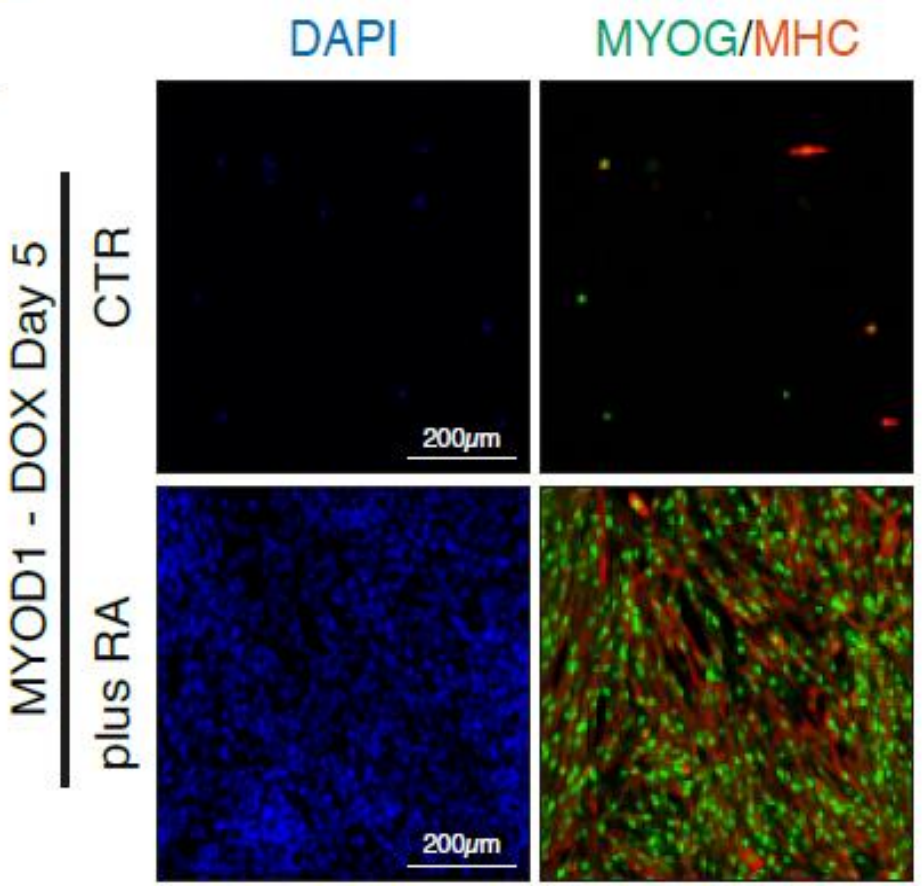
Induced Neurons



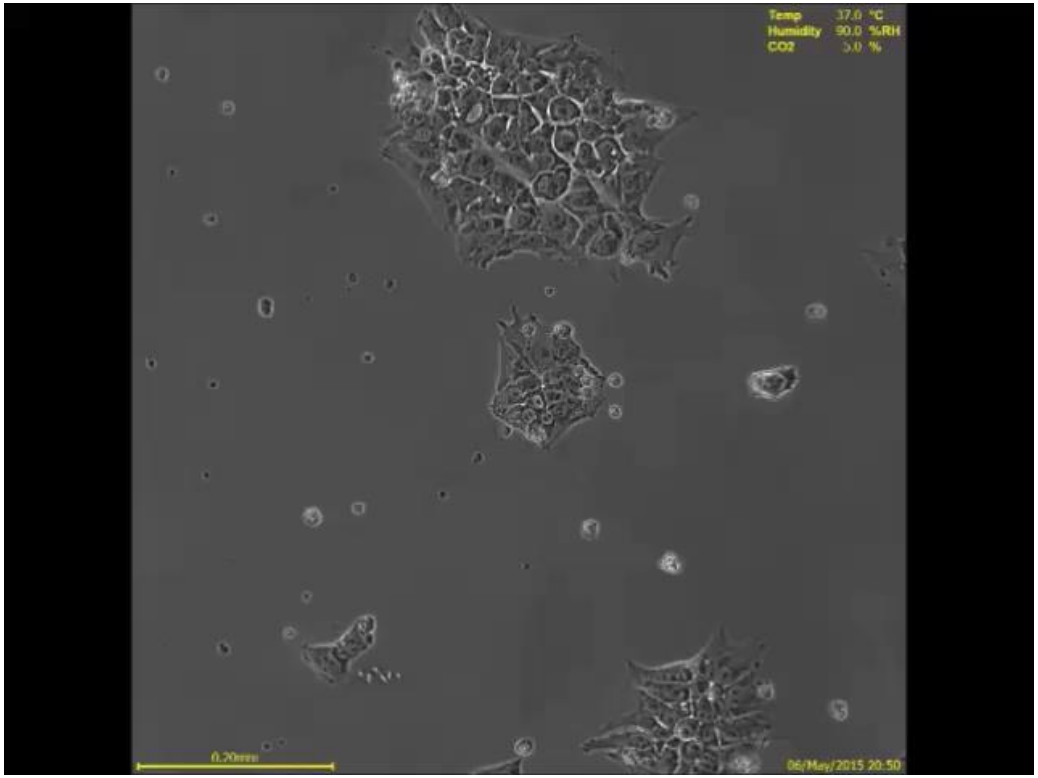


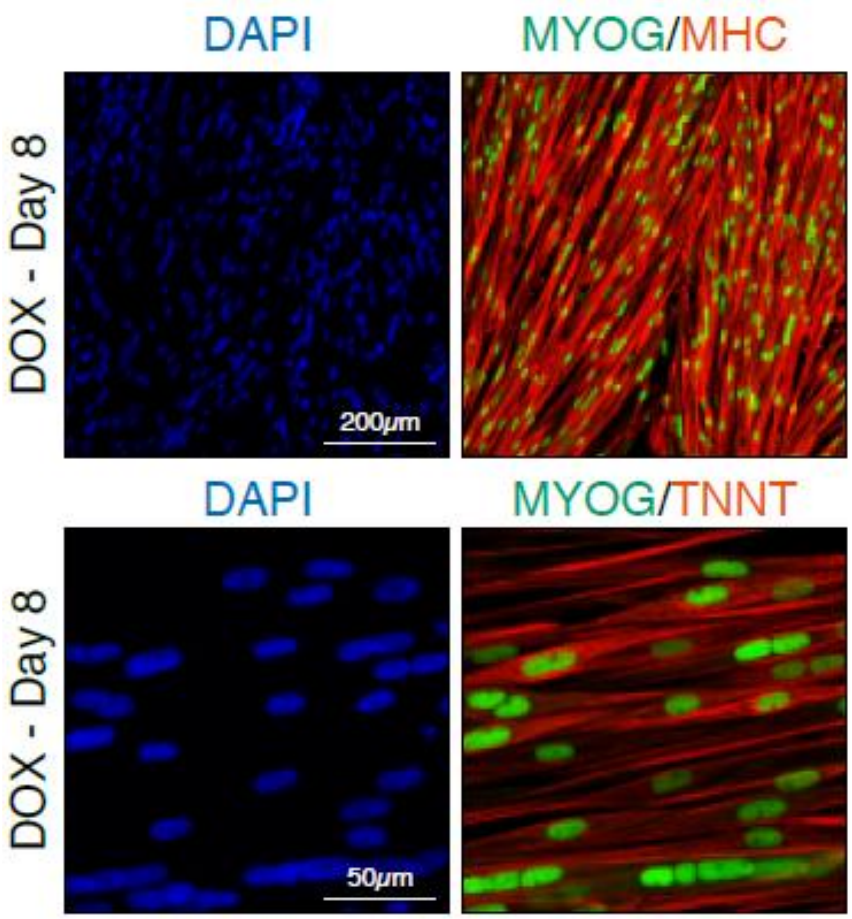
Forward programming of skeletal muscle using iMYOD1



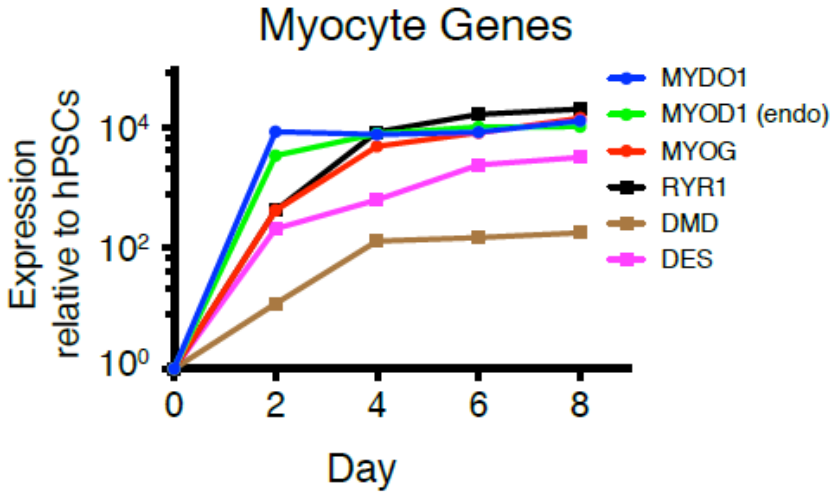


Induced skeletal Muscle – RA

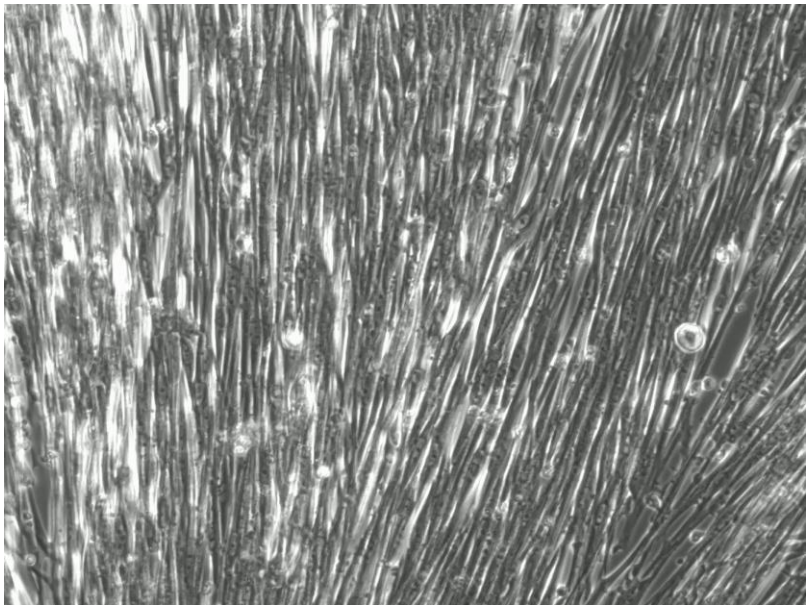




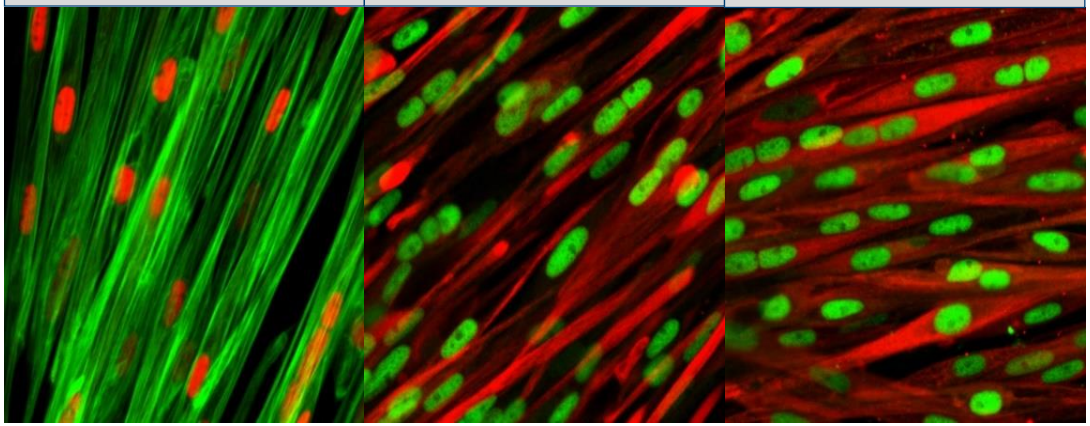
Induced skeletal Muscle – functionality



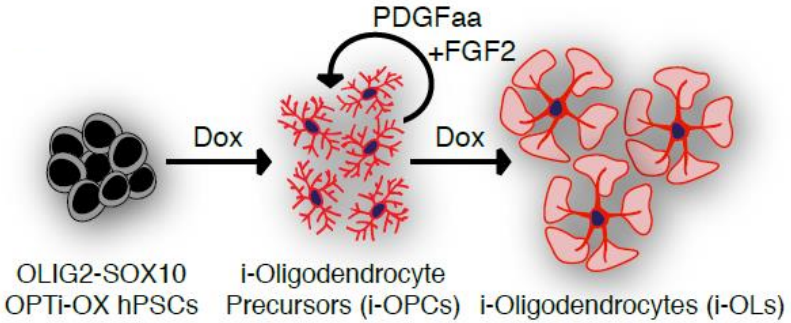
Stimulation with Acetylcholine

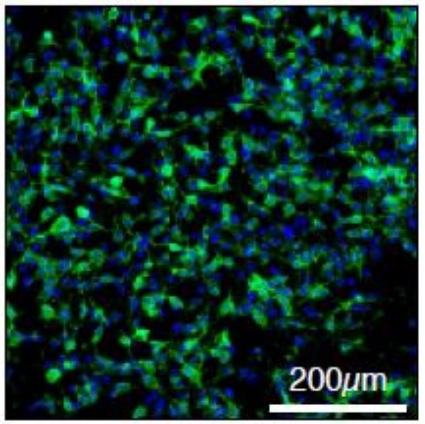


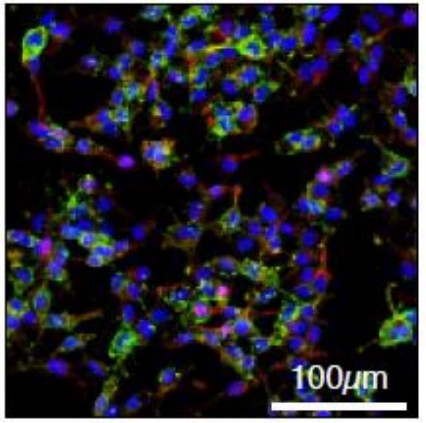
F-Actin / MYOG	MYOG / Desmin	MYOG / Myosin

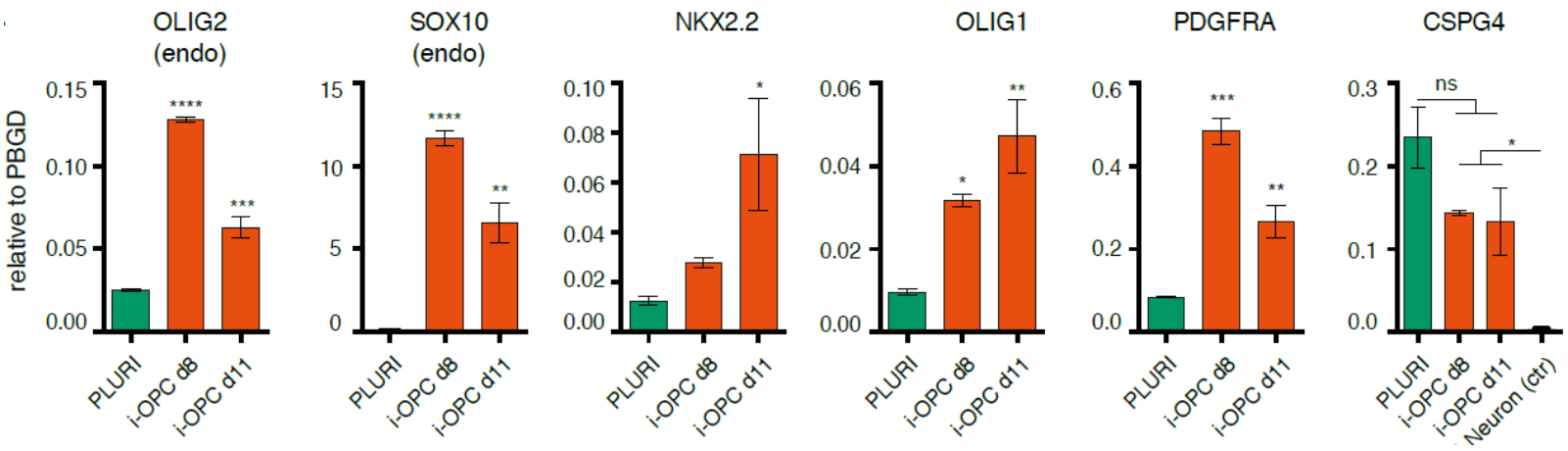


Forward programming of Oligodendrocytes using iOLIG2/SOX10

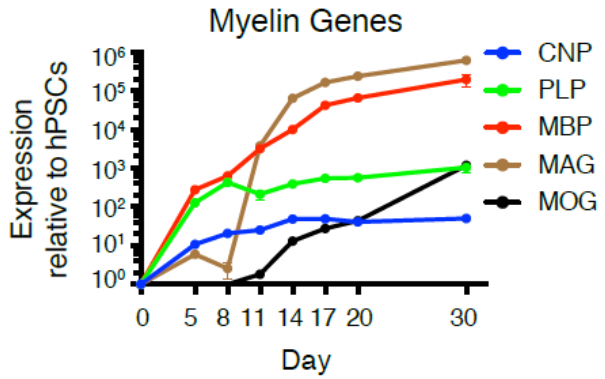


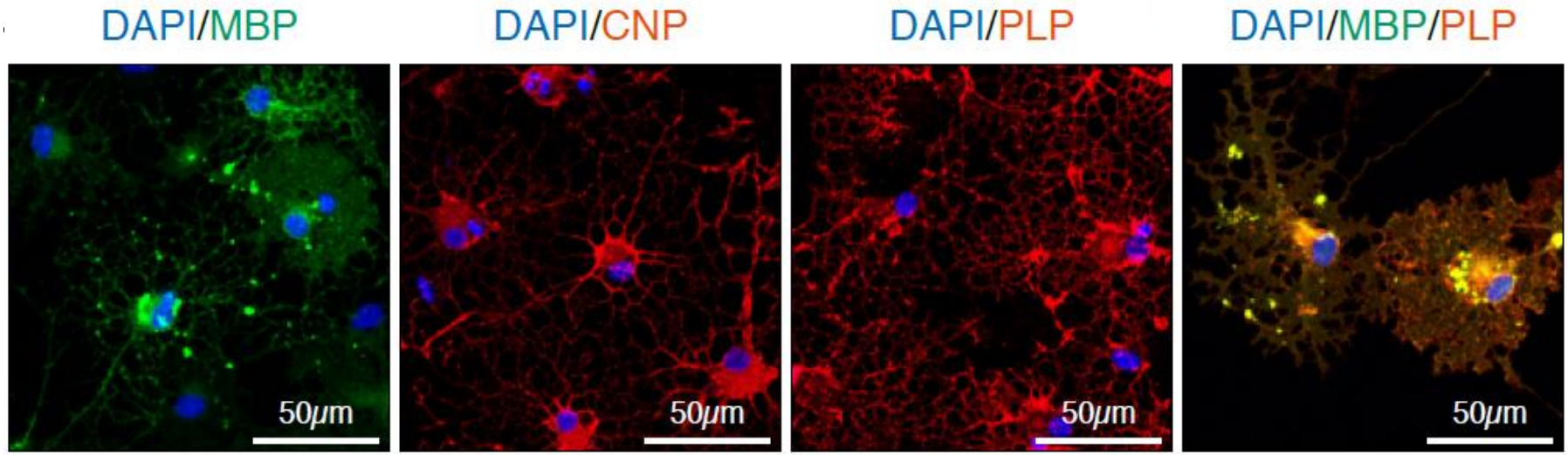






Induced Oligodendrocytes



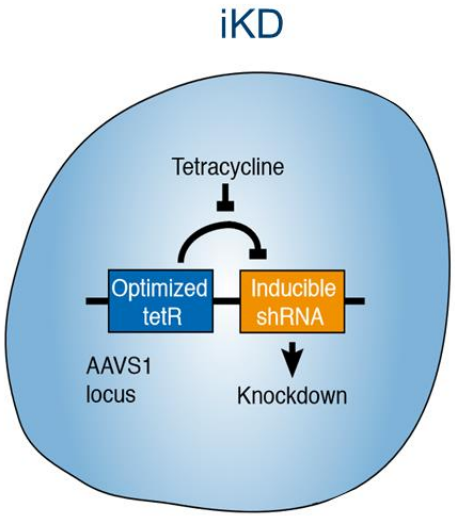


Conclusions iOX forward programming

- This inducible system can robustly achieve very high levels of overexpression.
- Overexpression of lineage determining transcription factors can be used for forward programming.
- This strategy enables the rapid, reproducible and scalable generation of mature cell types from a renewable source.

Inducible gene knock-down and knock-out

Dr. Alessandro Bertero



Dr. Matthias Pawlowski

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The Company of Biologists

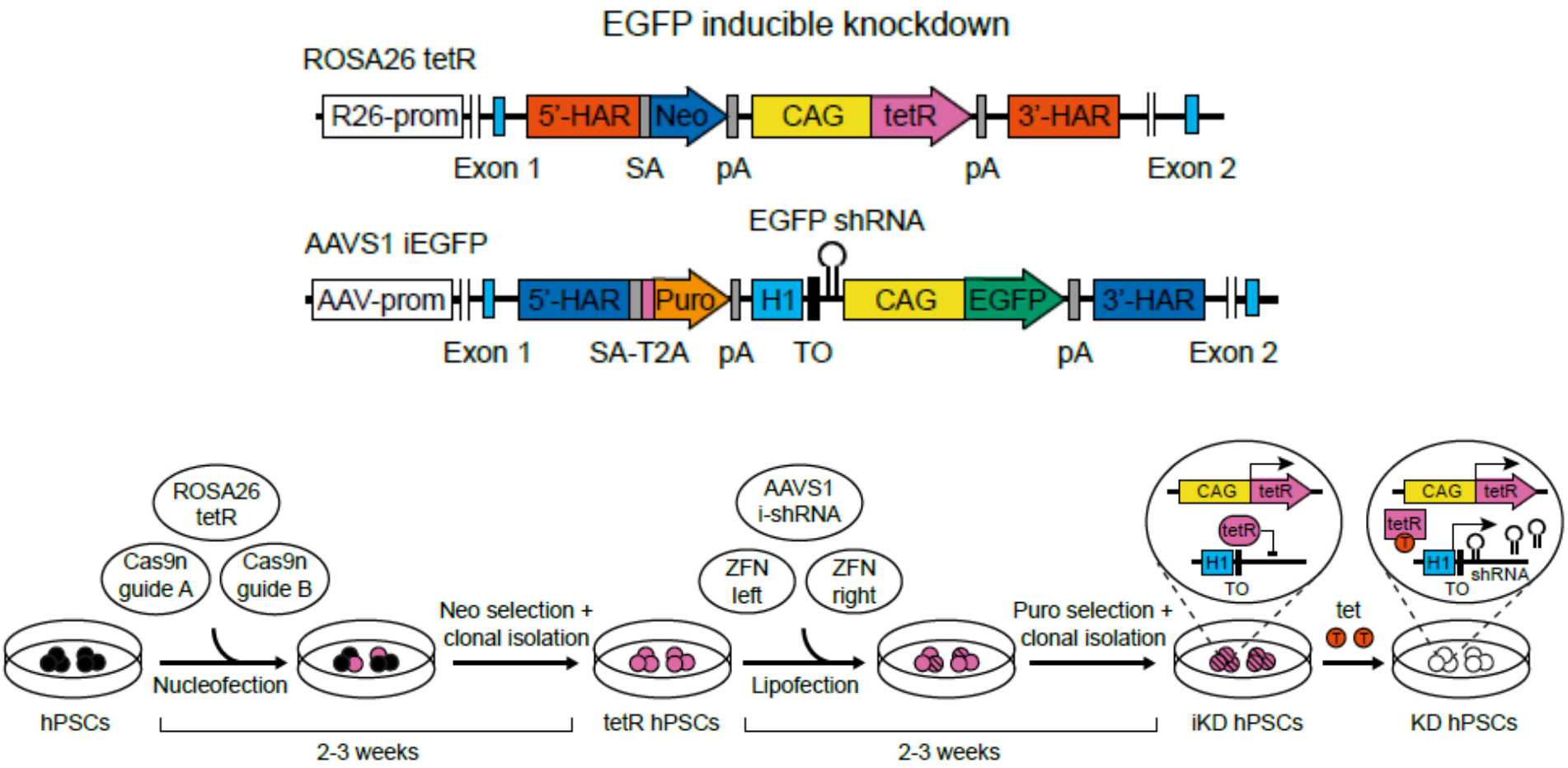
STEM CELLS AND REGENERATION

TECHNIQUES AND RESOURCES ARTICLE

Optimized inducible shRNA and CRISPR/Cas9 platforms for *in vitro* studies of human development using hPSCs

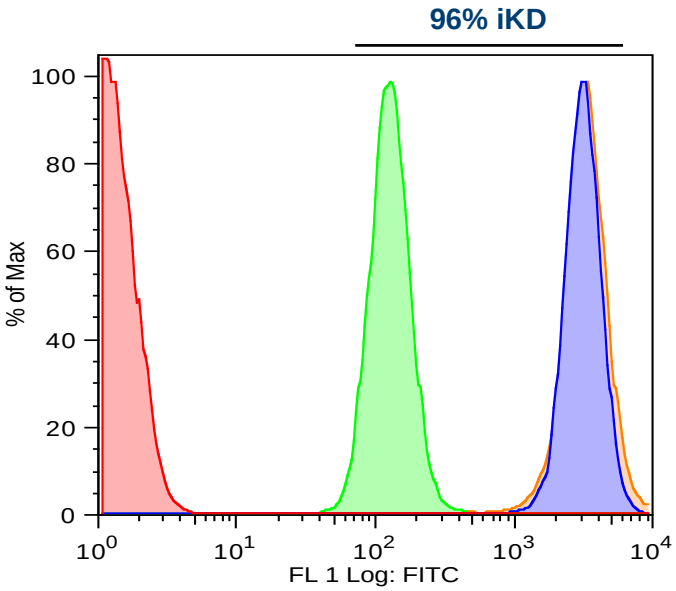
Alessandro Bertero[1,2,‡,§,¶], Matthias Pawlowski[1,3,§], Daniel Ortmann[1,2,§], Kirsten Snijders[1,2], Loukia Yiangou[1,4], Miguel Cardoso de Brito[1,2], Stephanie Brown[1,2], William G. Bernard[1,4], James D. Cooper[1,4], Elisa Giacomelli[1,2], Laure Gambardella[1,4], Nicholas R. F. Hannan[1,2,*], Dharini Iyer[1,4], Fotios Sampaziotis[1,2], Felipe Serrano[1,4], Mariëlle C. F. Zonneveld[1,2], Sanjay Sinha[1,4], Mark Kotter[1,3] and Ludovic Vallier[1,2,5,¶]

Inducible knock-down system (iKD)

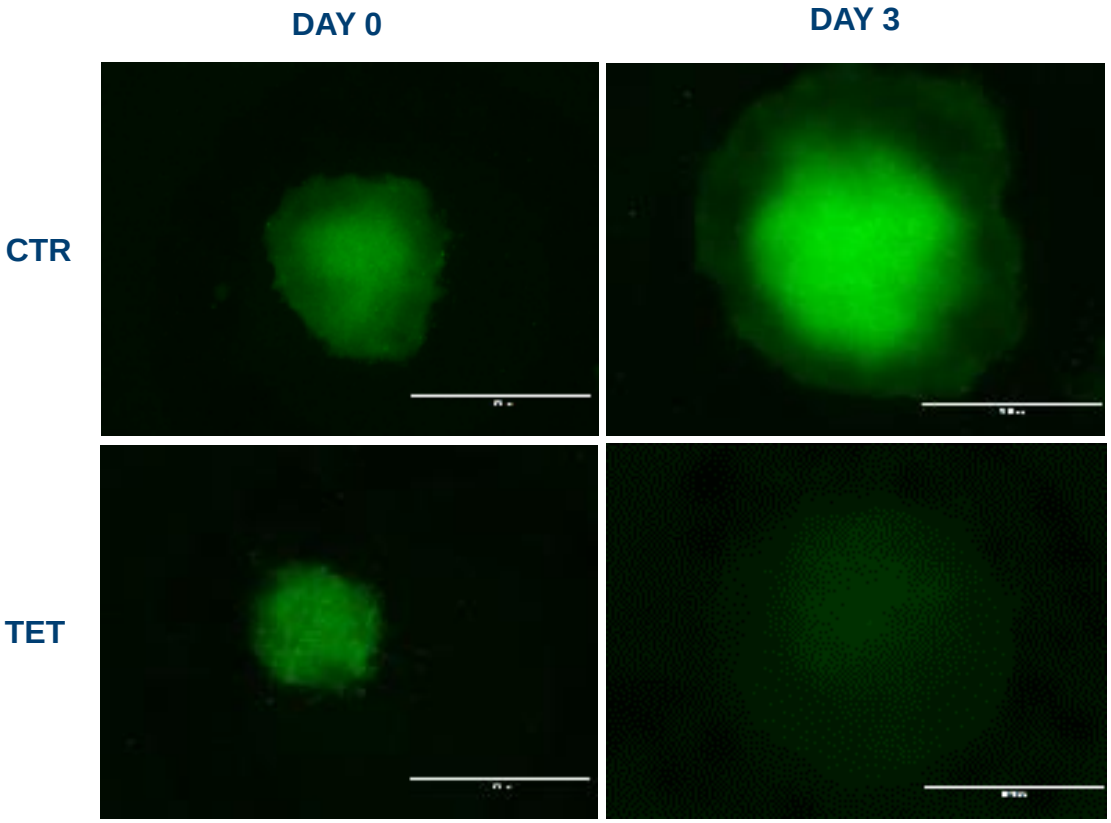


iKD of EGFP

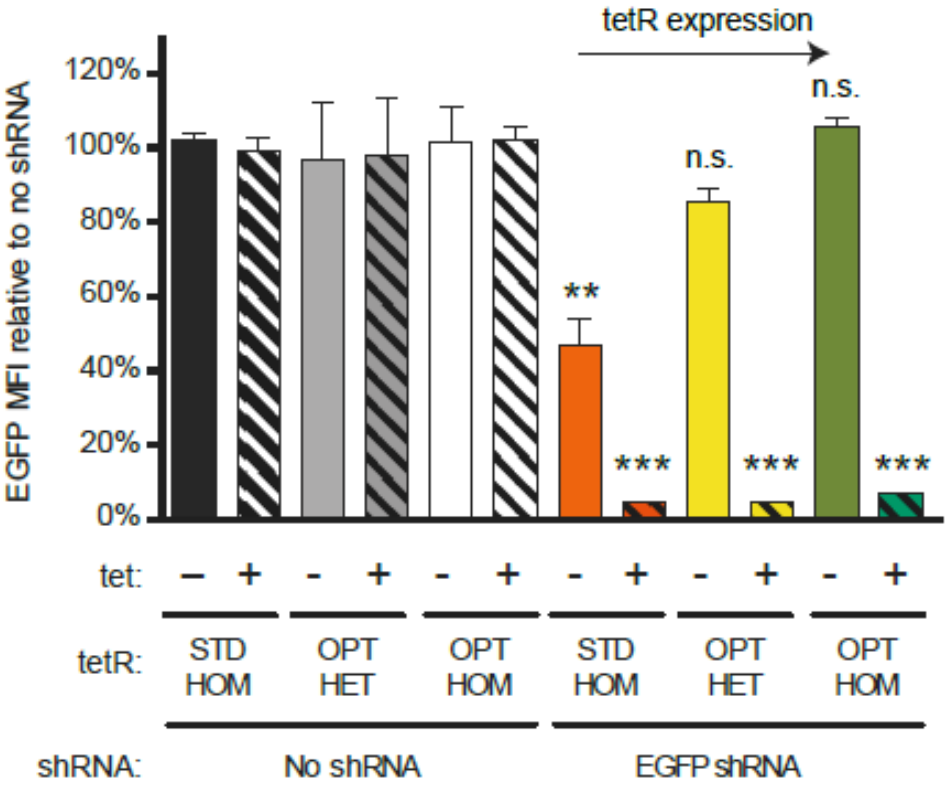
EGFP

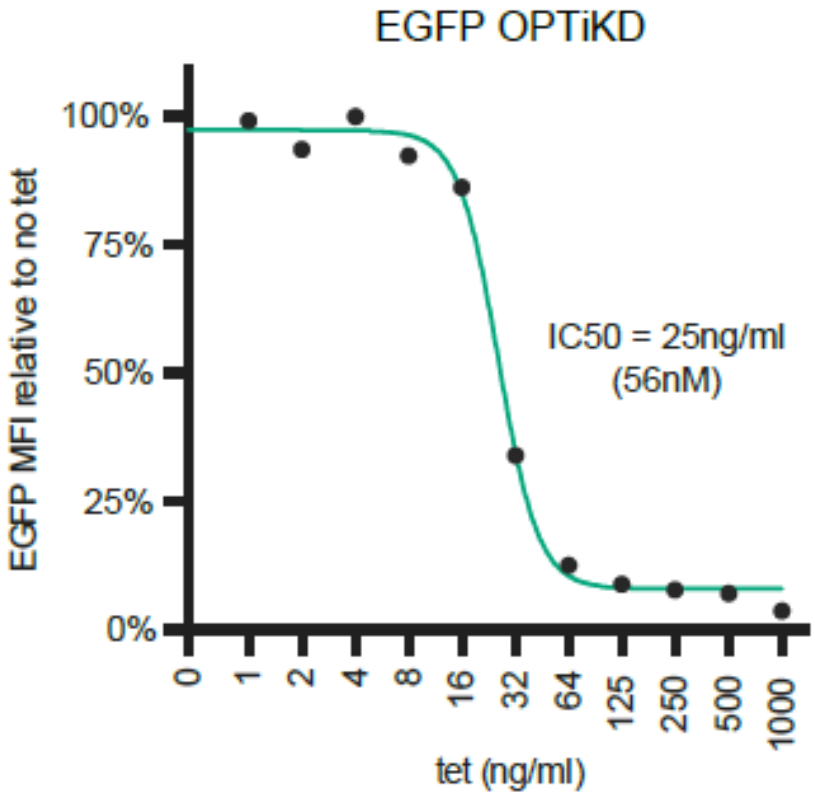


Sample	Median:FL 1 Log
H9 iKD.fcs	1.04
OPTtetR-TOshRNA CTR.fcs	3286
OPTtetR-TOshRNA TET.fcs	129
NO shRNA.fcs	3460



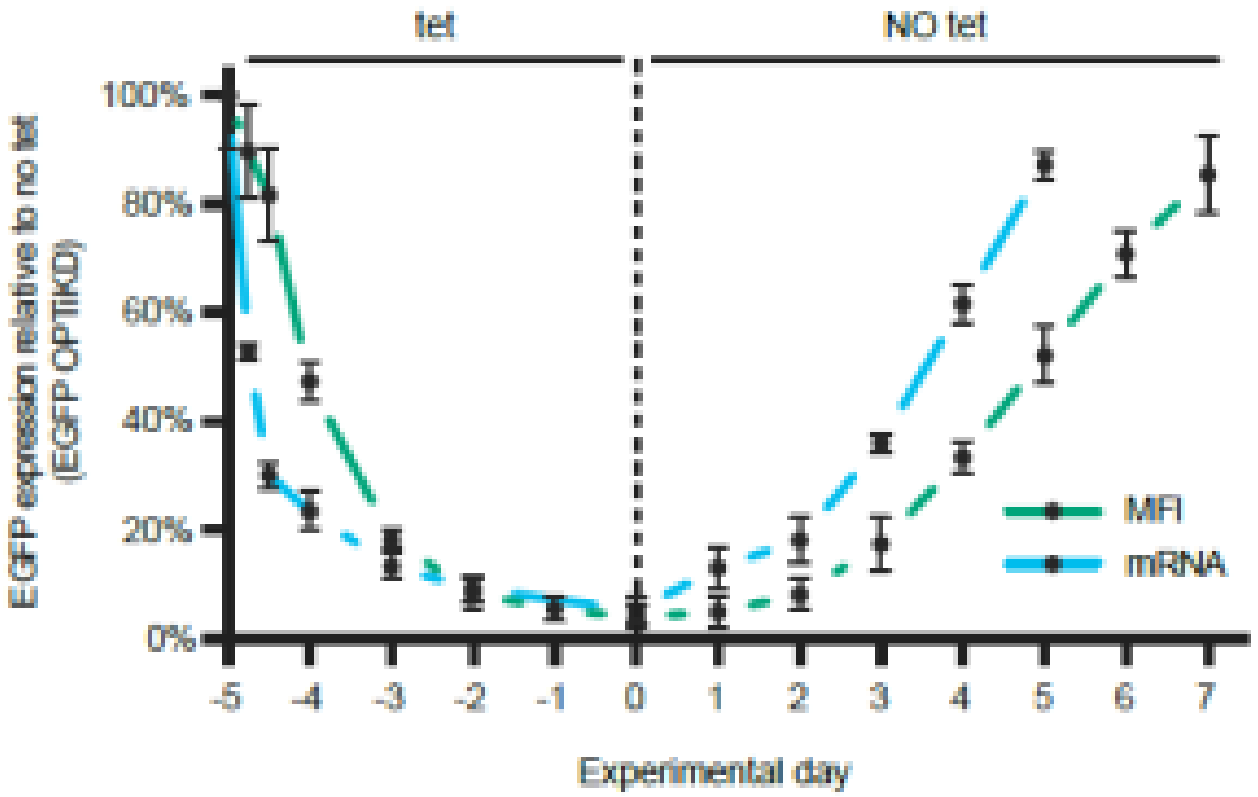
TetRepressor optimization and dose response



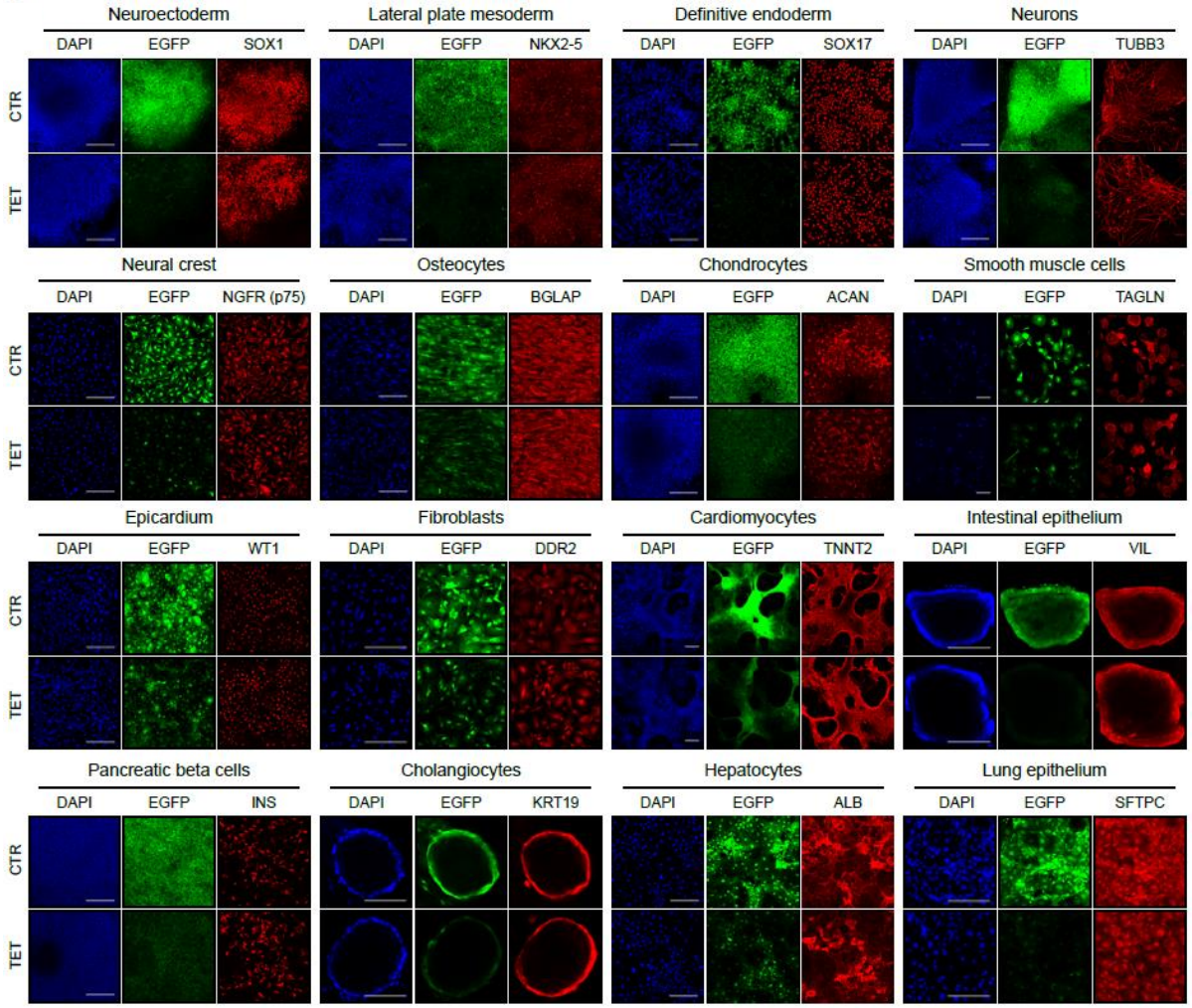


Codon-optimised for expression in hPSCs

Kinetics of KD and recovery

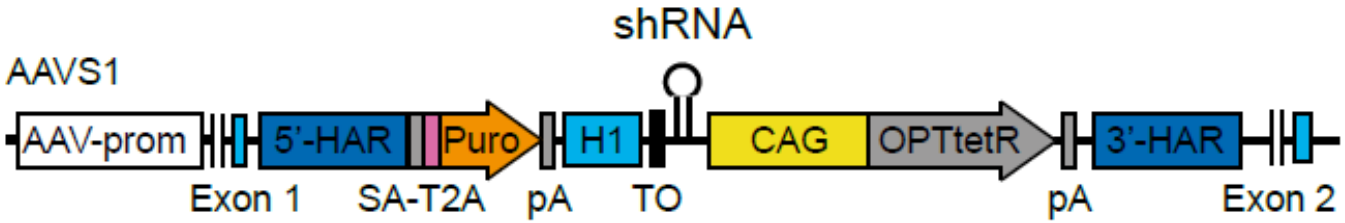


iKD also works in differentiated cells

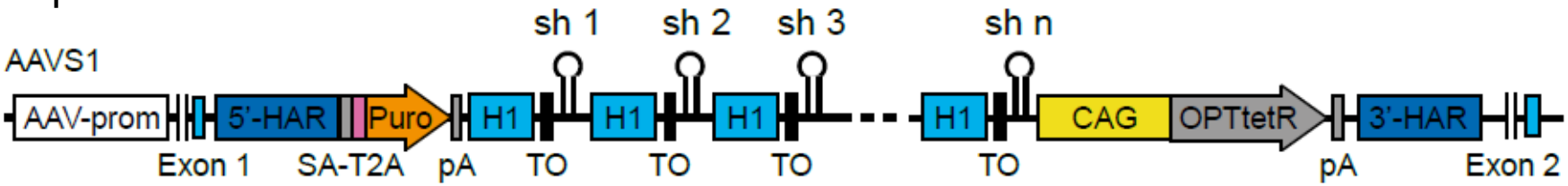


Single vector and multiple shRNAs

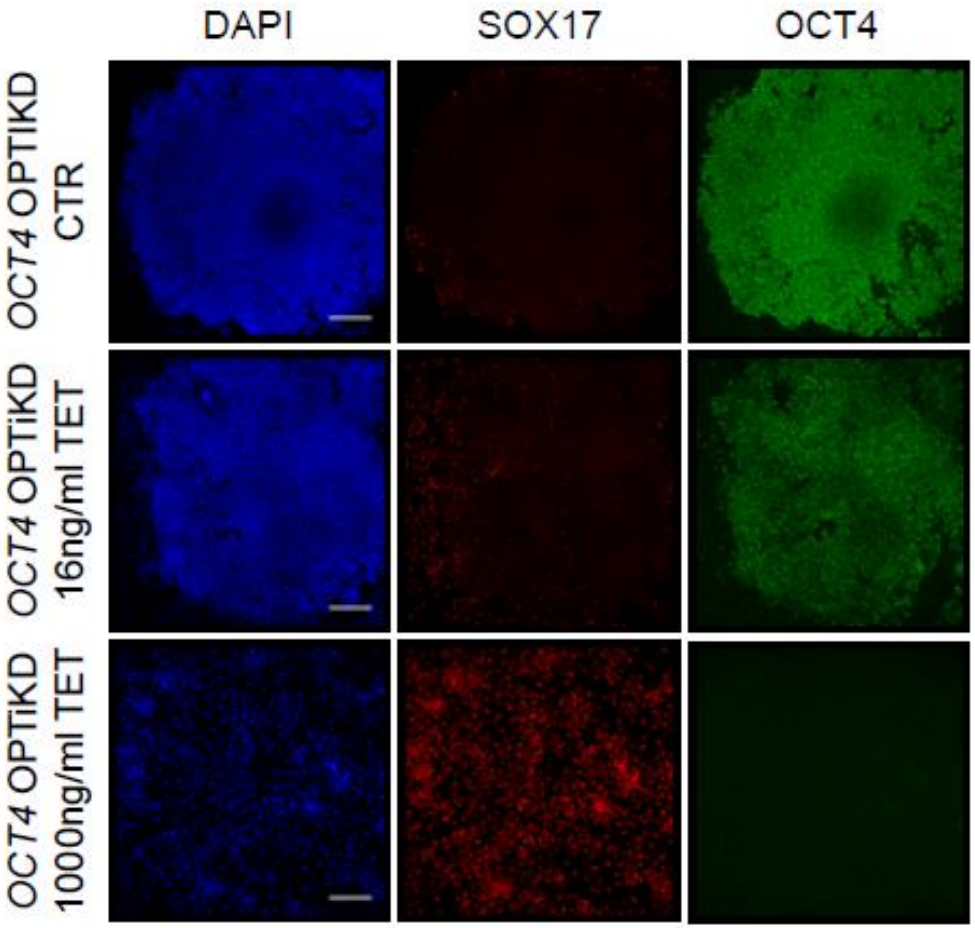
Single vector design

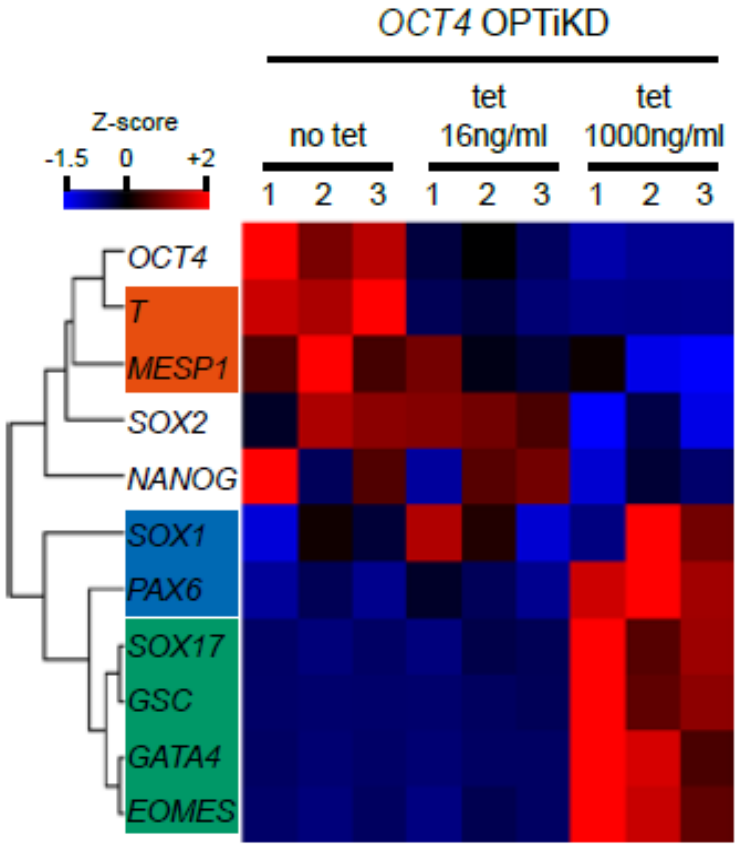


Multiple shRNAs

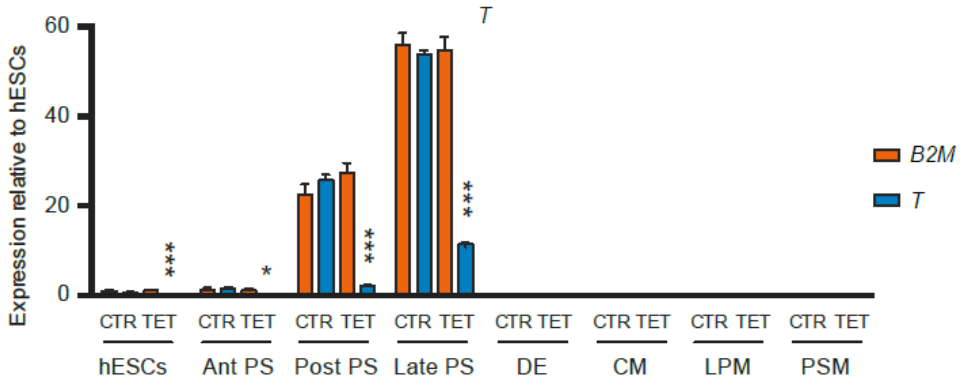


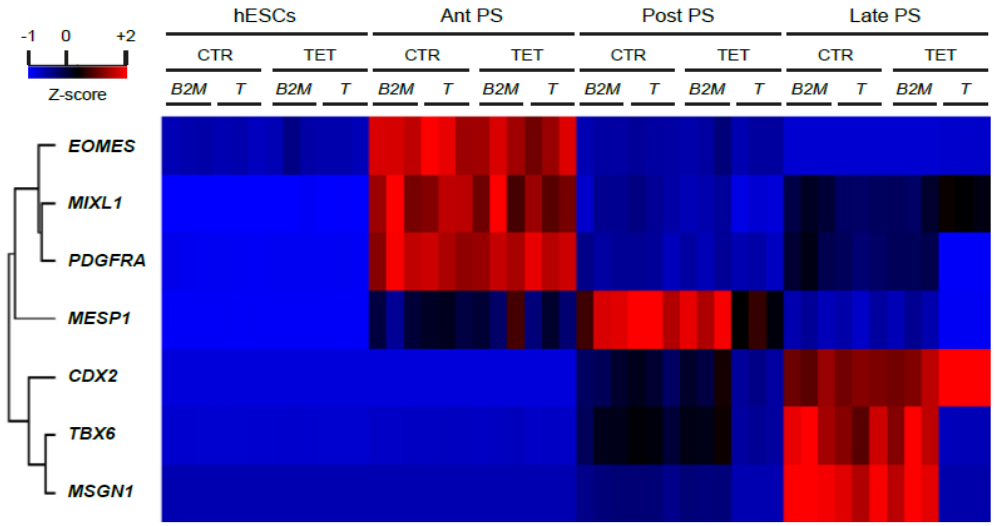
OCT4 iKD

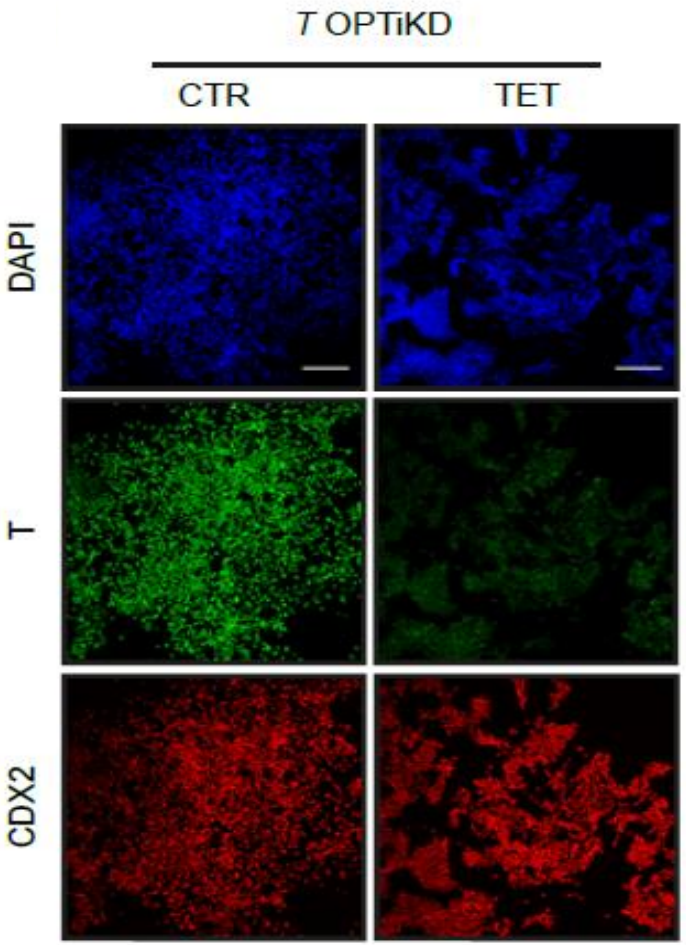

DAPI
SOX17
OCT4
OCT4 OPTiKD CTR
OCT4 OPTiKD 16ng/ml TET
OCT4 OPTiKD 1000ng/ml TET


OCT4 OPTiKD
Z-score
-1.5 0 +2
no tet
tet 16ng/ml
tet 1000ng/ml
1 2 3 1 2 3 1 2 3
OCT4
T
MESP1
SOX2
NANOG
SOX1
PAX6
SOX17
GSC
GATA4
EOMES

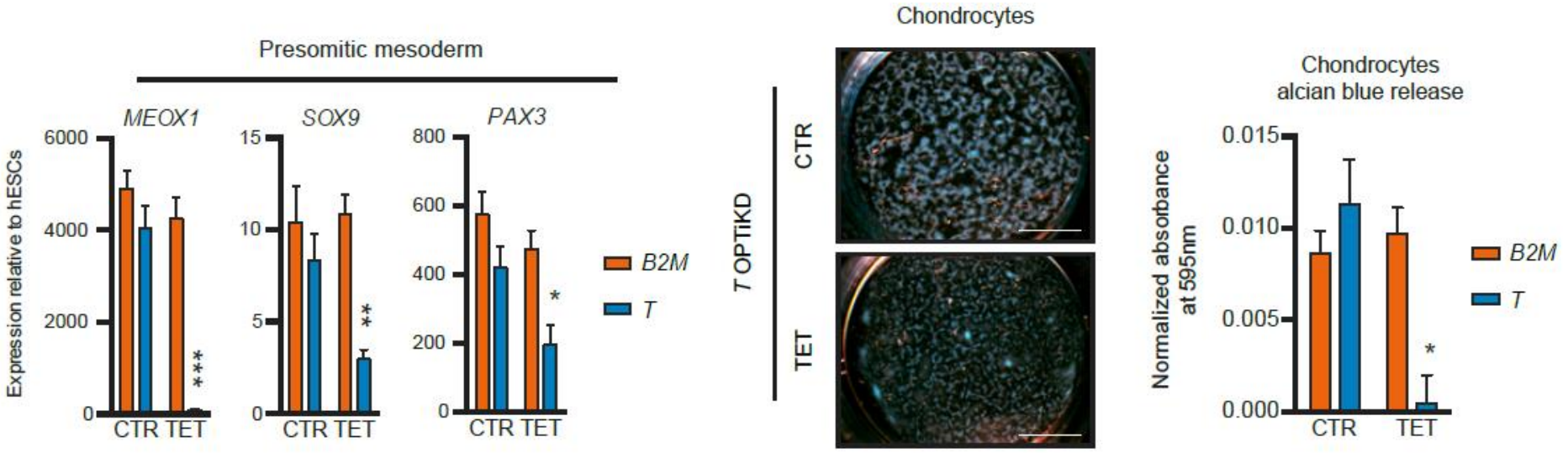
T(Bachyury) iKD



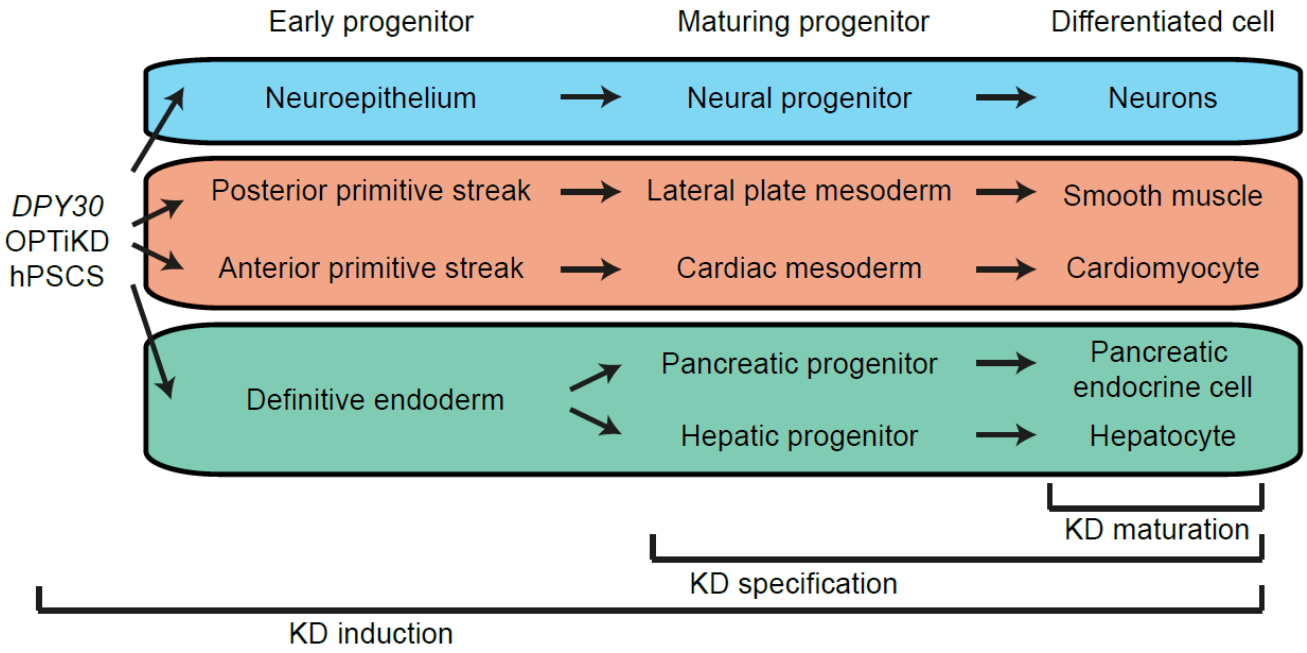




T(Bachyury) iKD

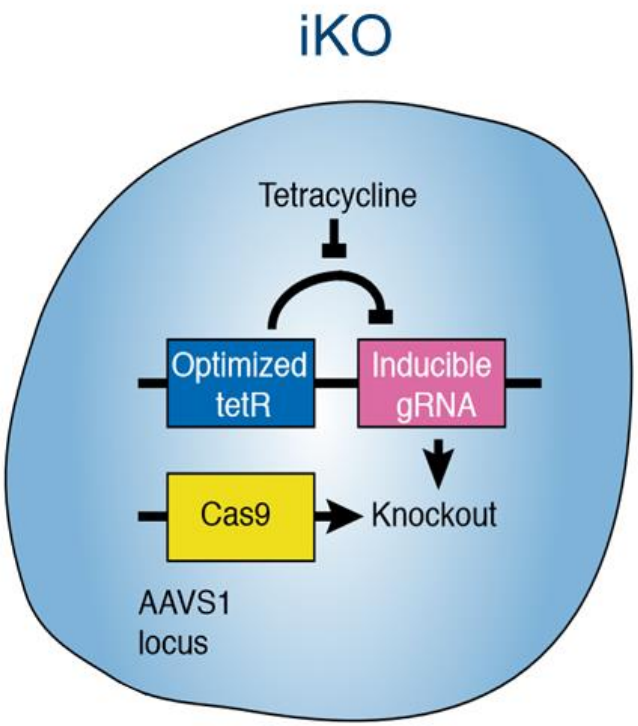


Stage specific iKD

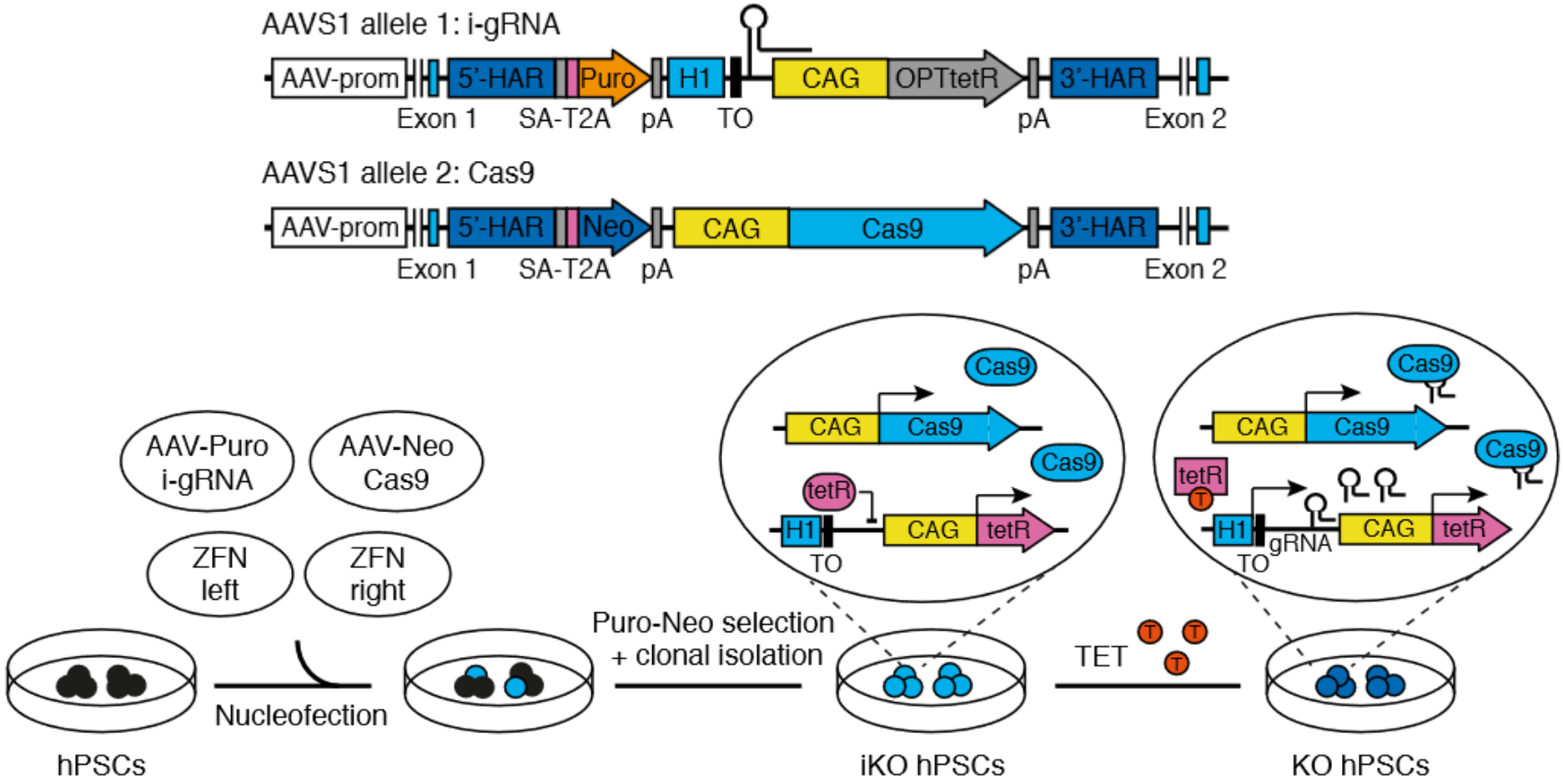


System allows for iKD during specific stages of differentiation/development

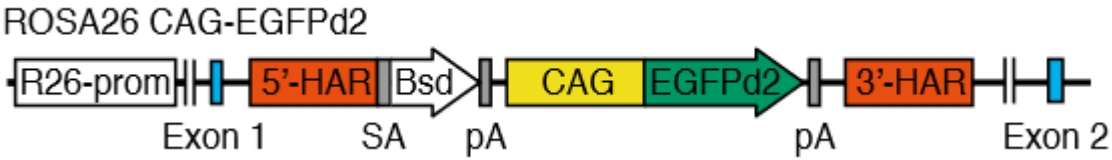
Inducible Knock-Out (iKO)

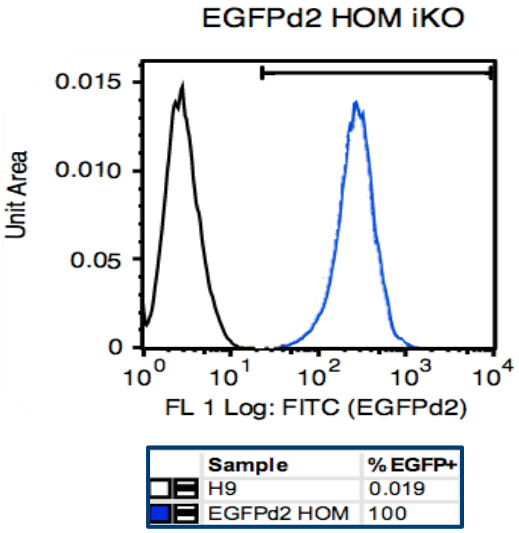


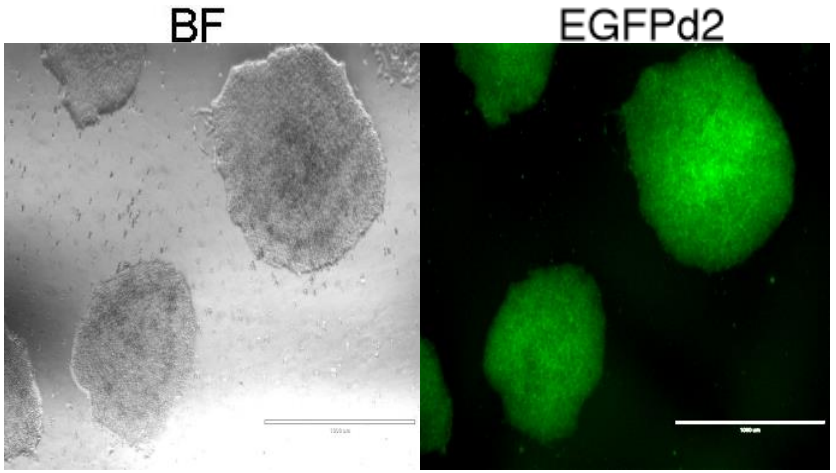
Inducible Knock-Out (iKO)



EGFPd2 iKO

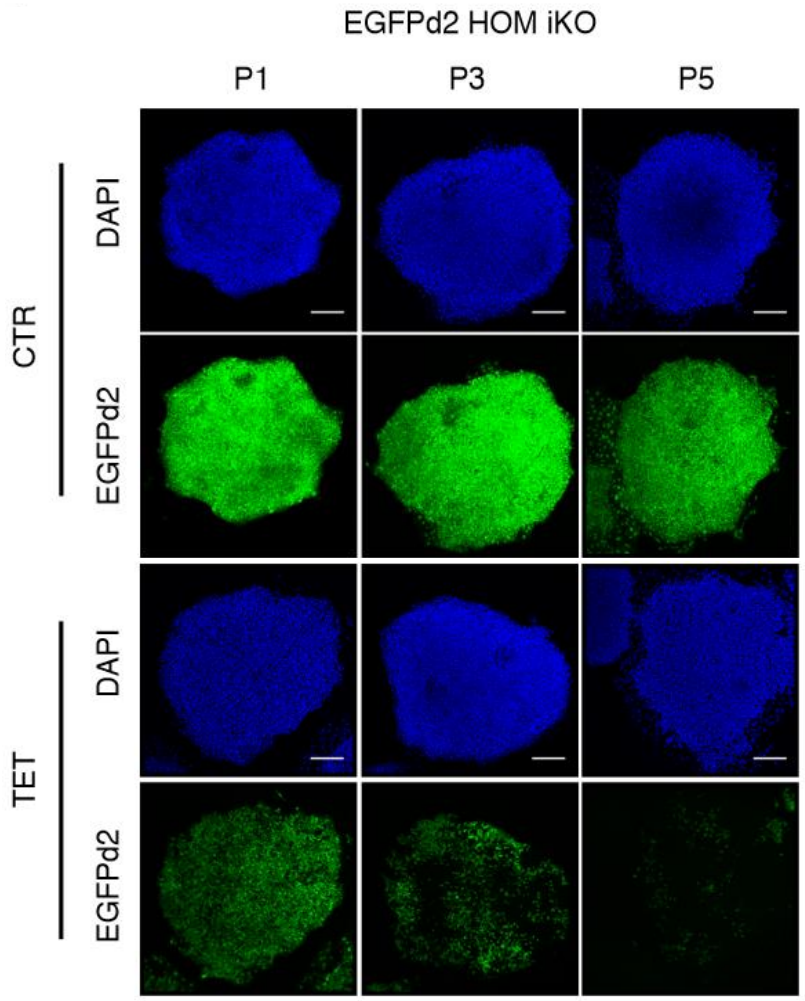


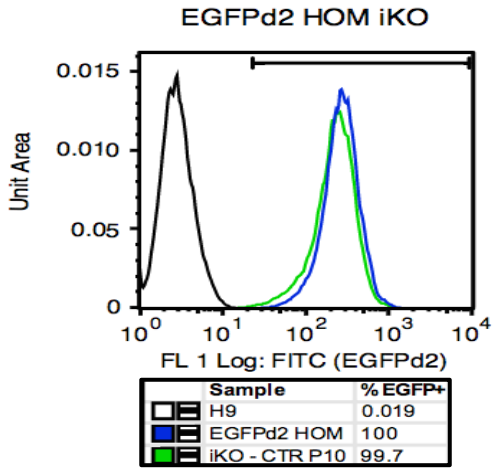


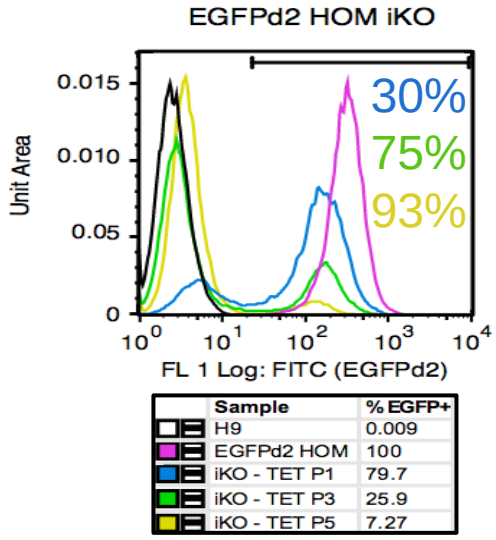


Choosing guide RNAs using in silico predictions

EGFPd2 iKO

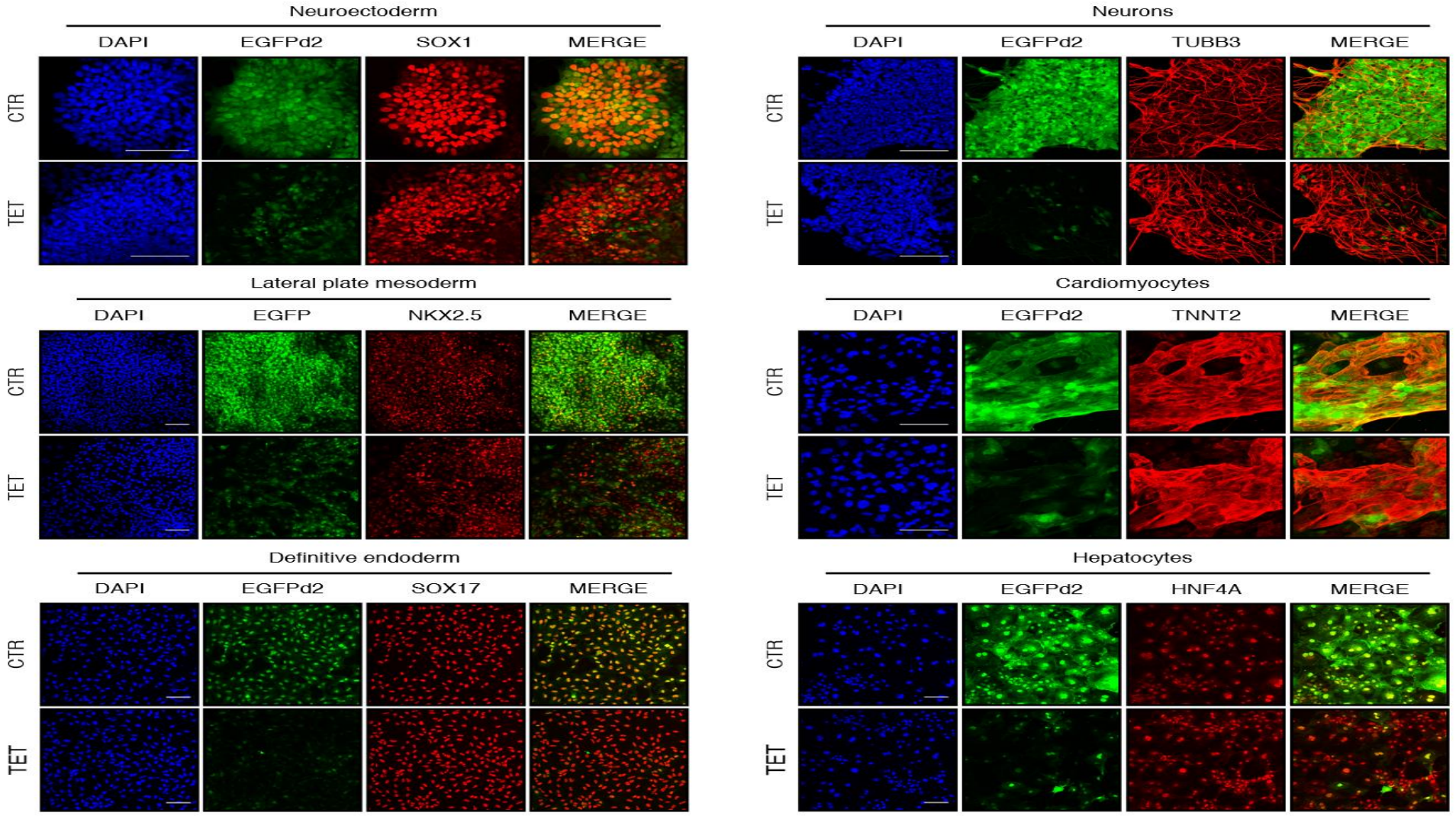




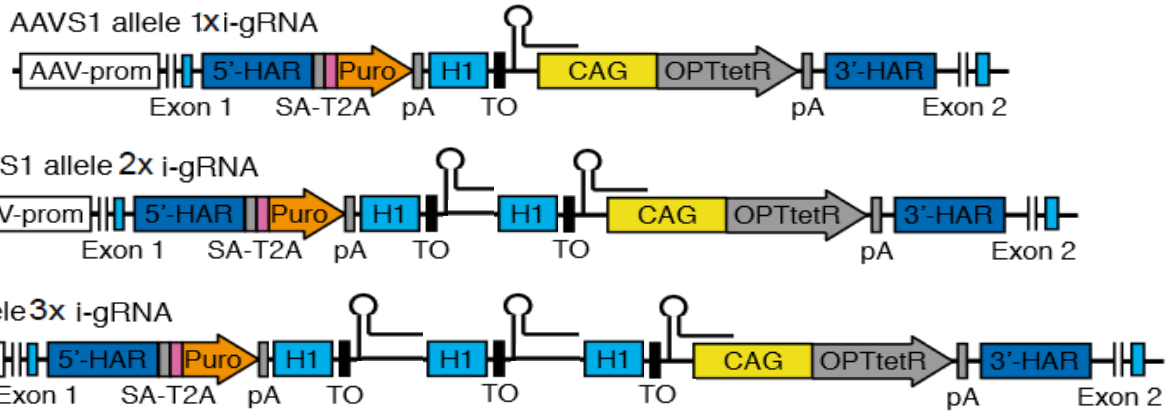


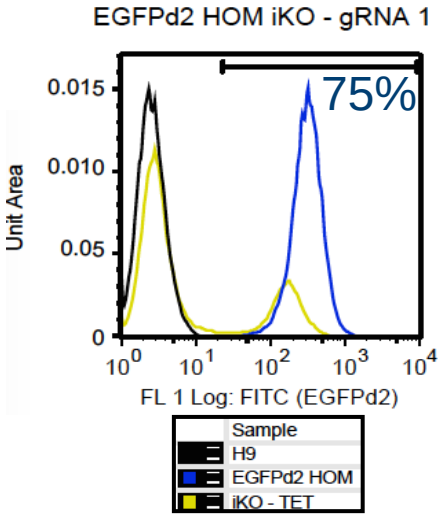
Efficient KO after 5 passages

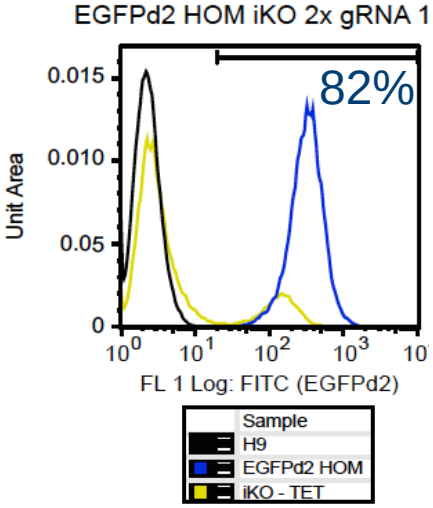
iKO during differentiation

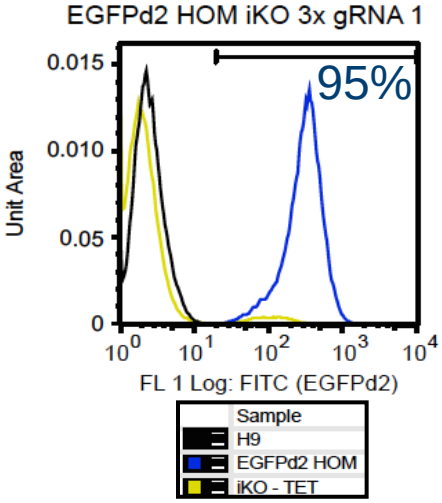


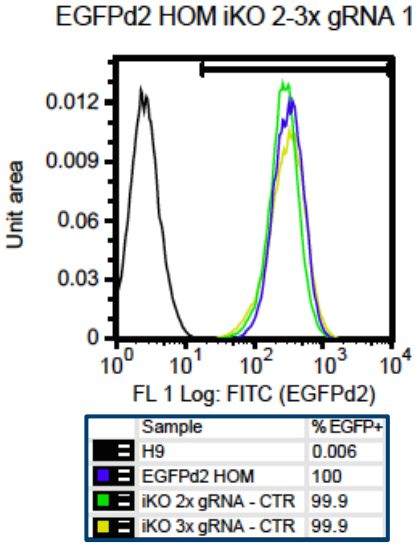
Improving iKO - More of the same guides?









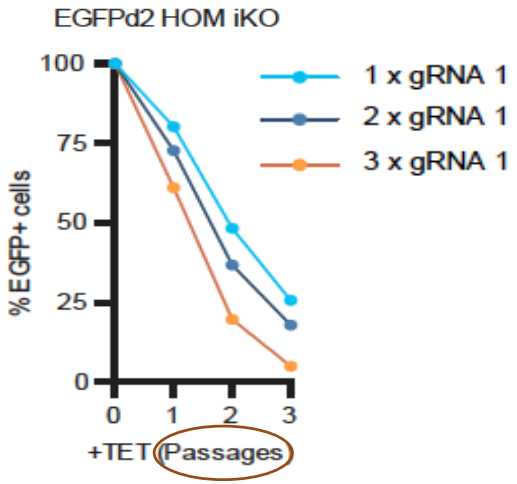


KO after p3

Improving iKO – Different guides?

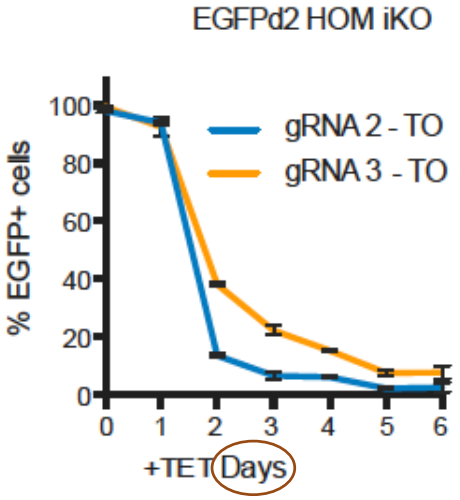
After 3 passages (~18 days):

- 1x guide 1= 75% KO
- 2x guide 1 = 82% KO
- 3x guide 1 = 95% KO



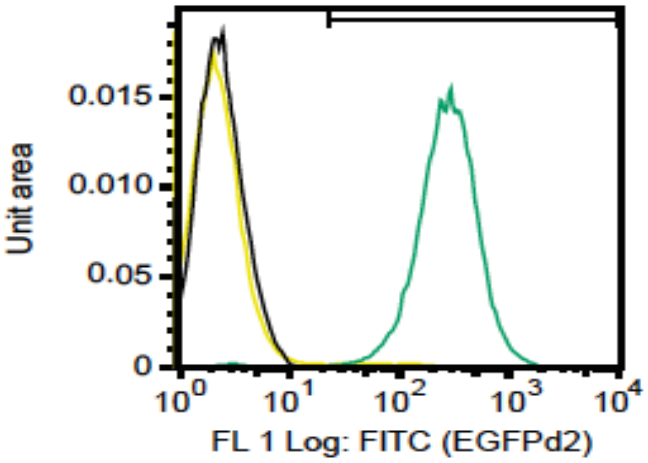
After 1 passage (~6 days):

- Guide 2 = 98% KO
- Guide 3 = 92% KO



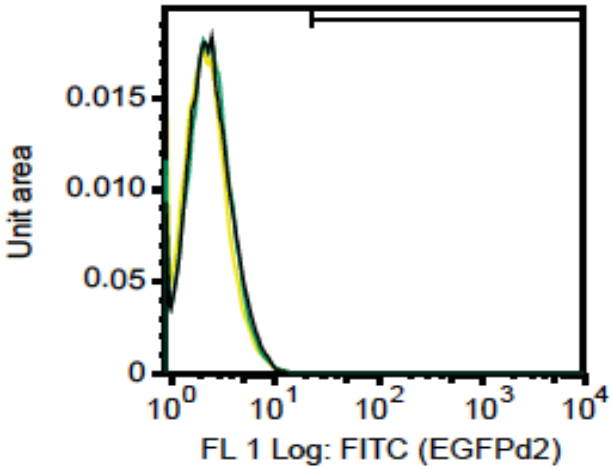
Some gRNAs show problems with leakiness

EGFPd2 HOM iKO
gRNA 2 - TO promoter



Sample	% EGFP+	EGFP+ MFI
H9	0.003	-
iKO CTR	98.7	299
iKO TET - 5 days	2.91	81.5

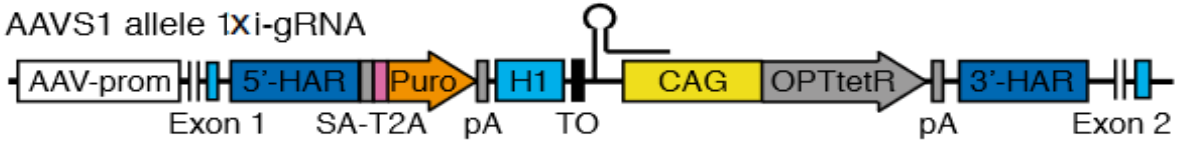
EGFPd2 HOM iKO
gRNA 3 - TO promoter



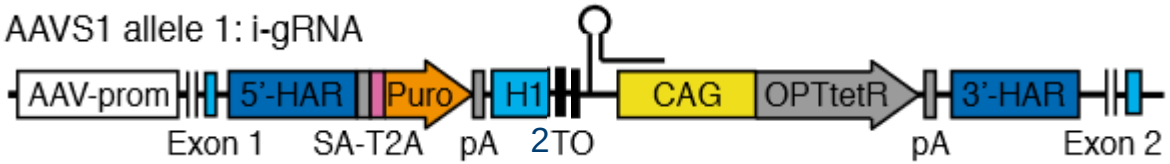
Sample	% EGFP+	EGFP+ MFI
H9	0.003	-
iKO CTR	0.29	151
iKO TET - 5 days	0.17	53

Counteracting leakiness of strong gRNAs

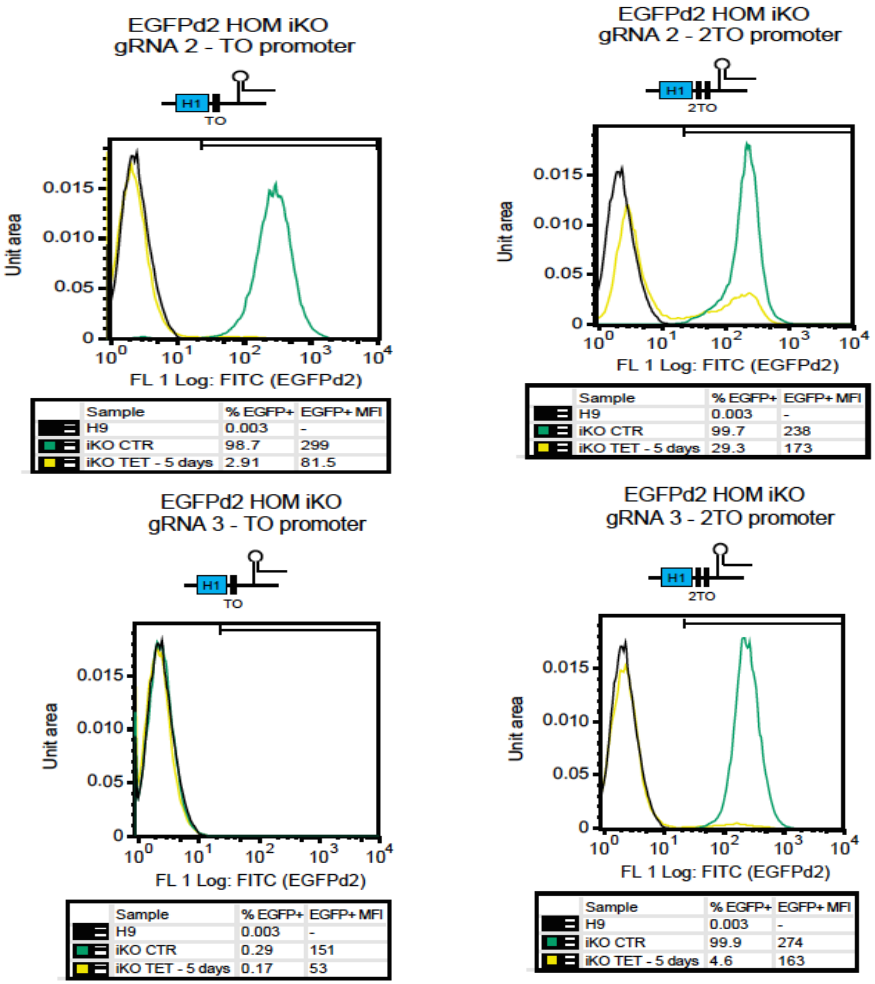
1 TO



2 TO

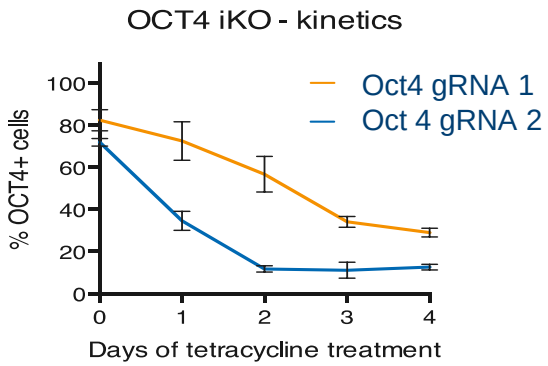


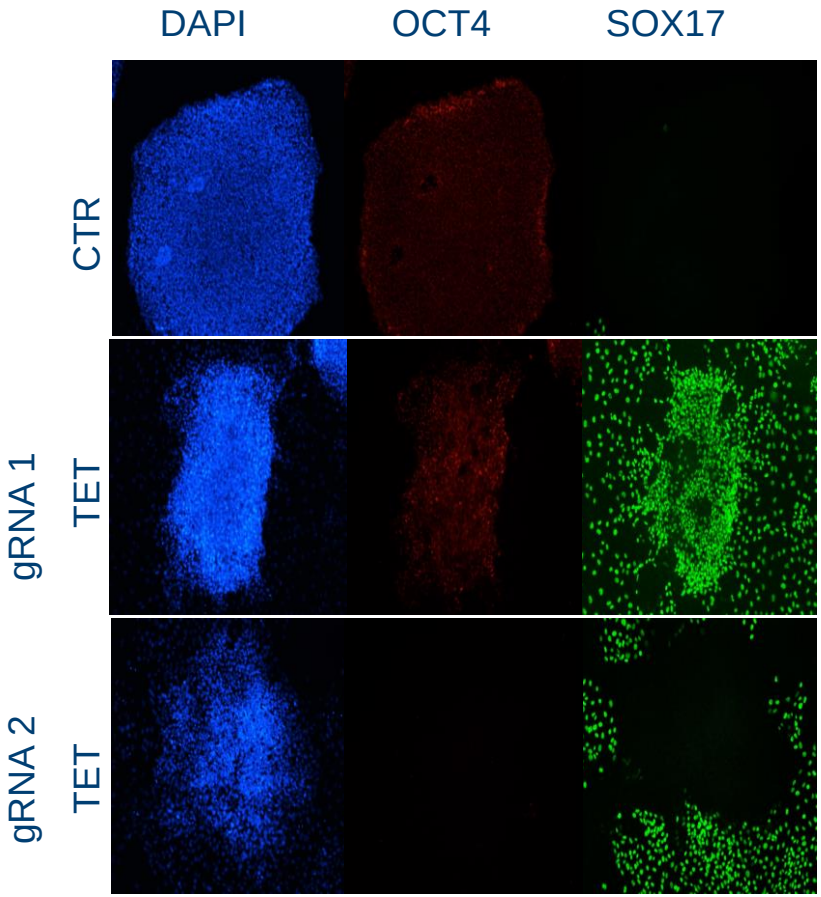
Counteracting leakiness of strong gRNAs



OCT4 KO shows expected phenotype

- Guide 1 = 70% KO
- Guide 2 = 88% KO





Conclusions iKD and iKO

- Inducible expression of shRNAs can achieve efficient and reproducible KD in pluripotency and differentiation
- Stage specific KD allows stage specific studies
- CRISPR/CAS9 and inducible gRNAs allow efficient iKO
- Useful especially for genes essential for pluripotency and early stages
- iKD and iKO are dependent on the quality of shRNAs/gRNAs

Thank you for your attention!
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