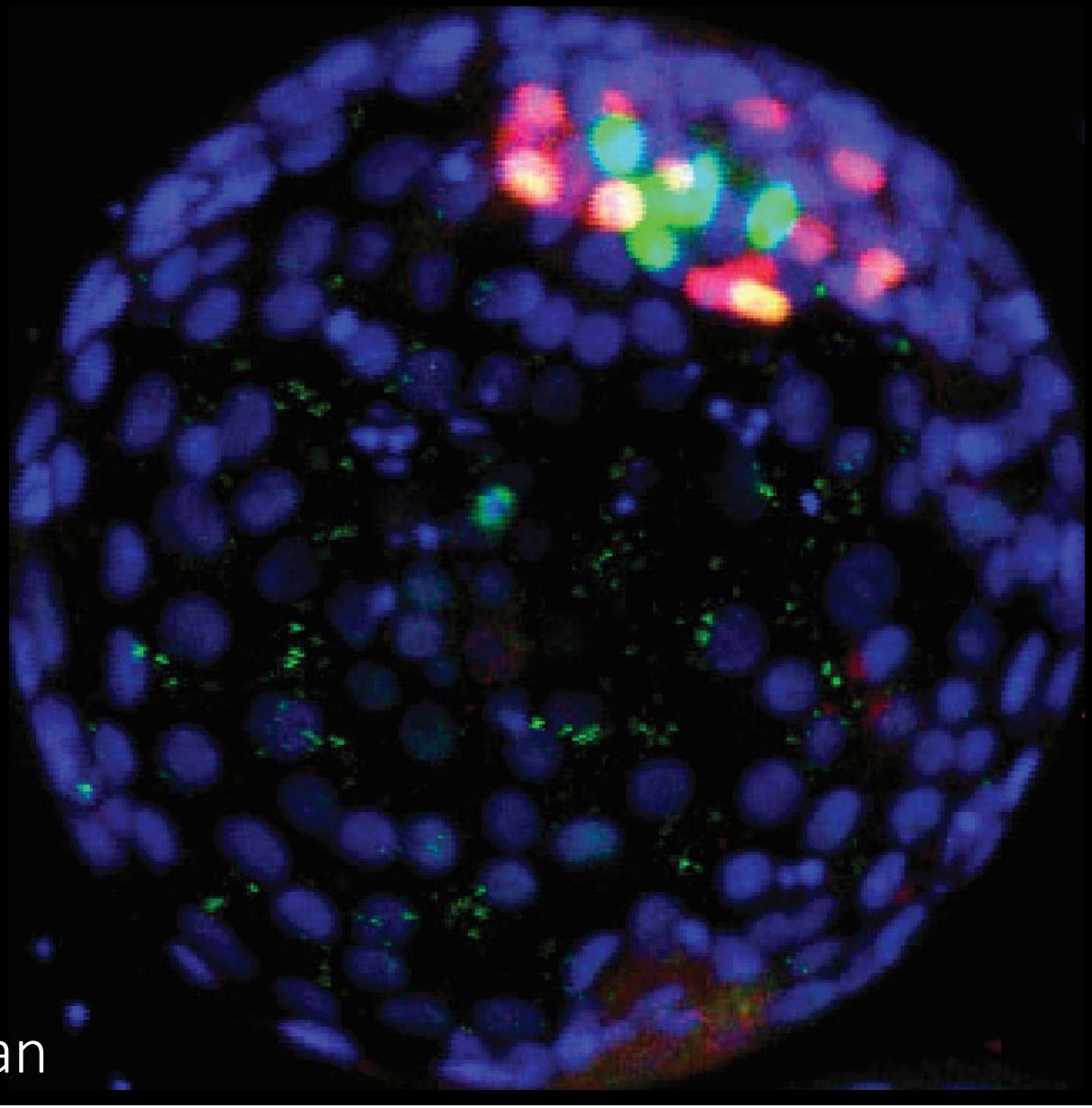
Gene editing of human embryos - understand mechanisms of lineage specification

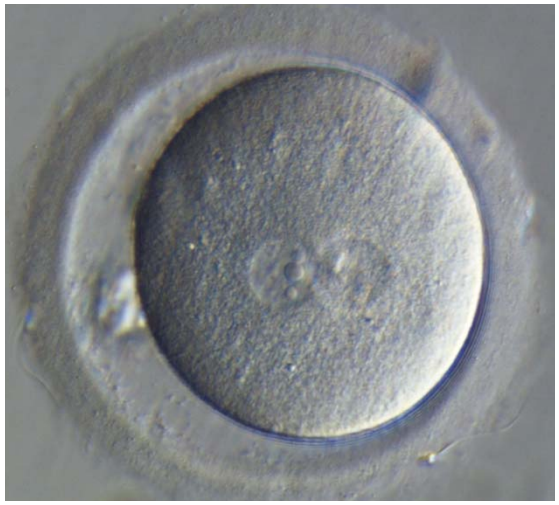


OCT4
CDX2
SOX17

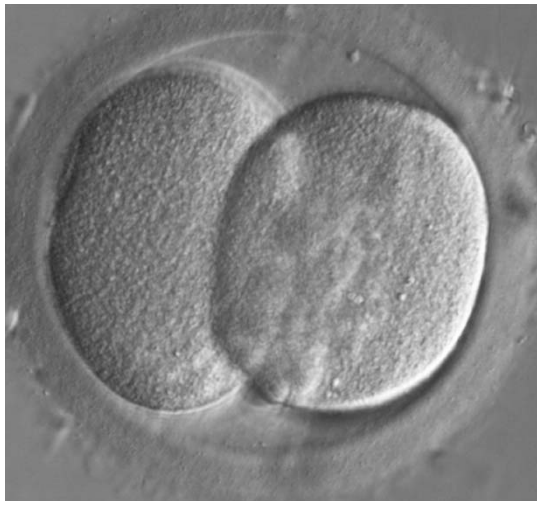
THE FRANCIS CRICK INSTITUTE

Kathy Niakan

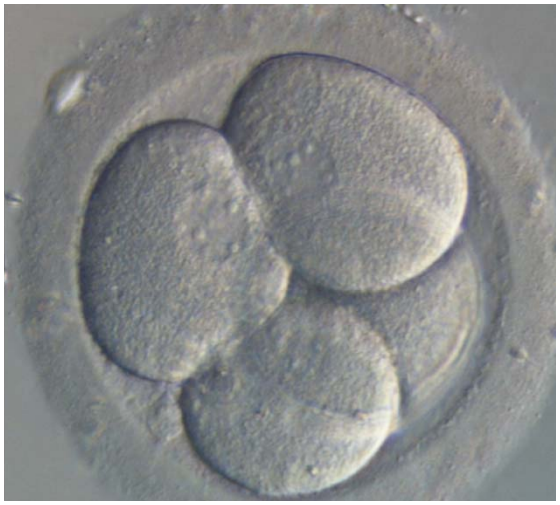
Human pre-implantation development



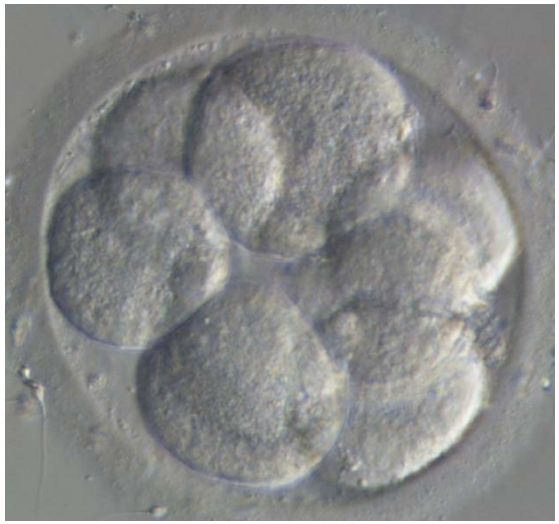
Day 1
1 cell (zygote)



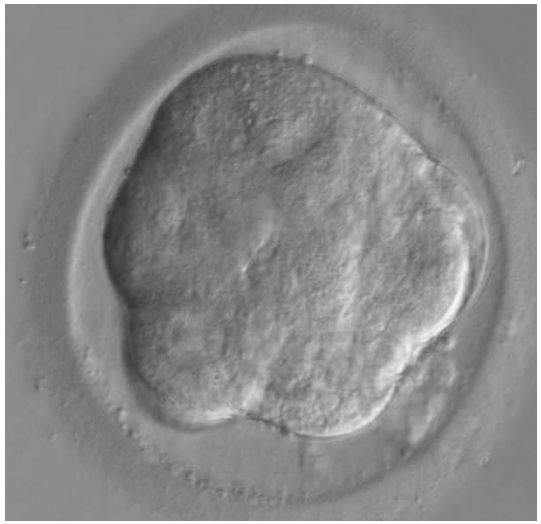
Day 2
2 cell



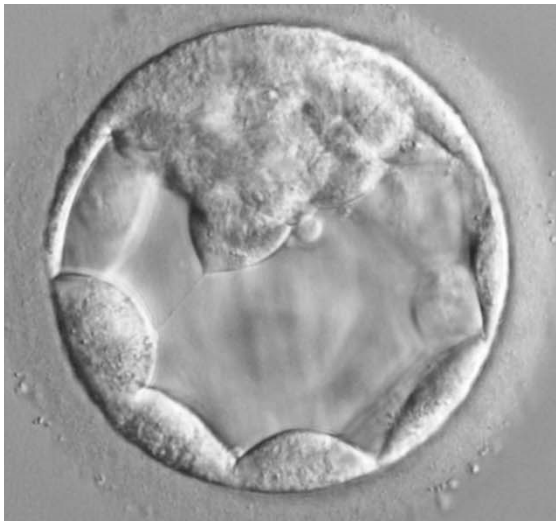
Day 3
4 cell



Day 3
8 cell

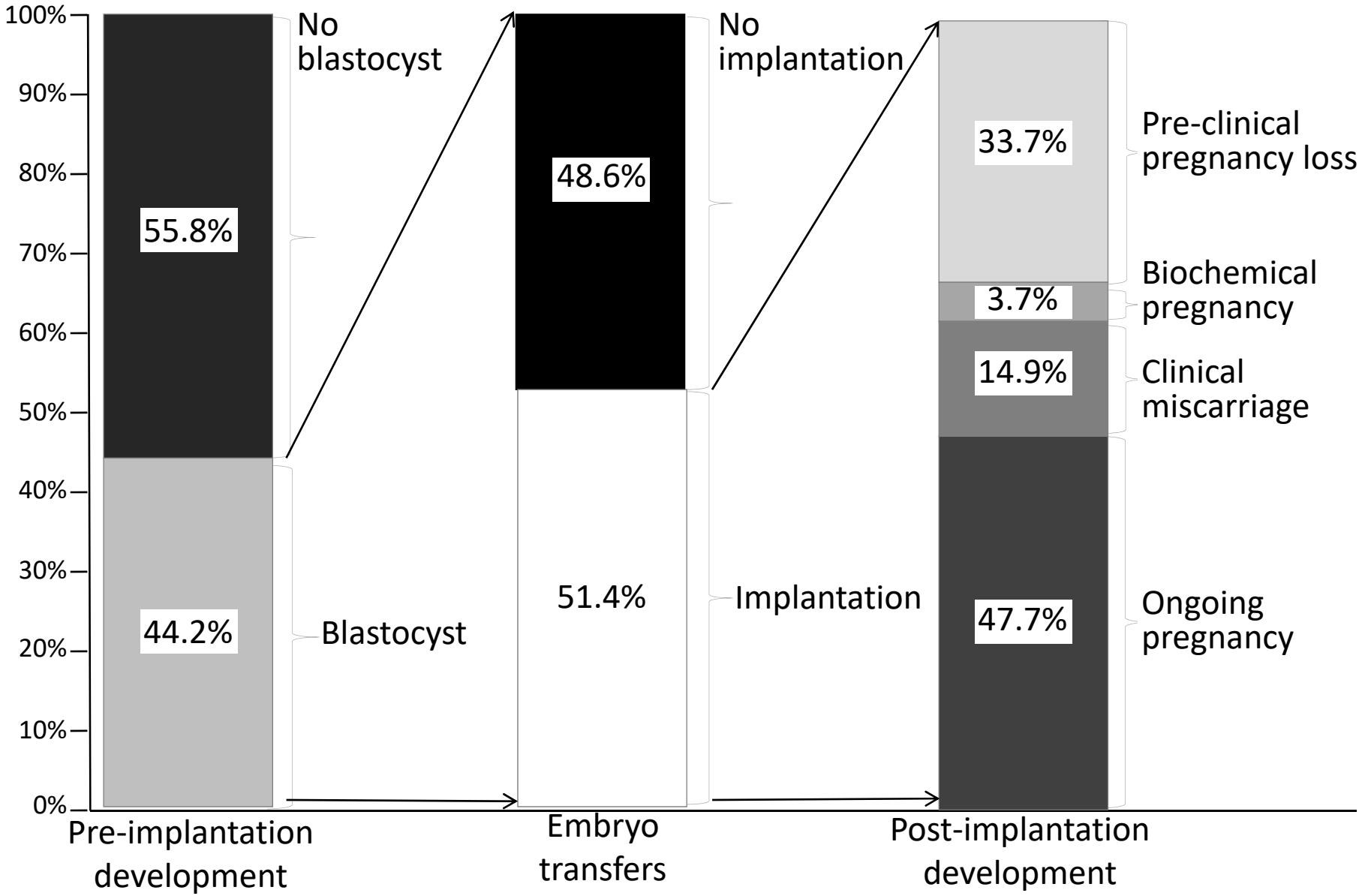


Day 4
16 - 32 cell (morula)



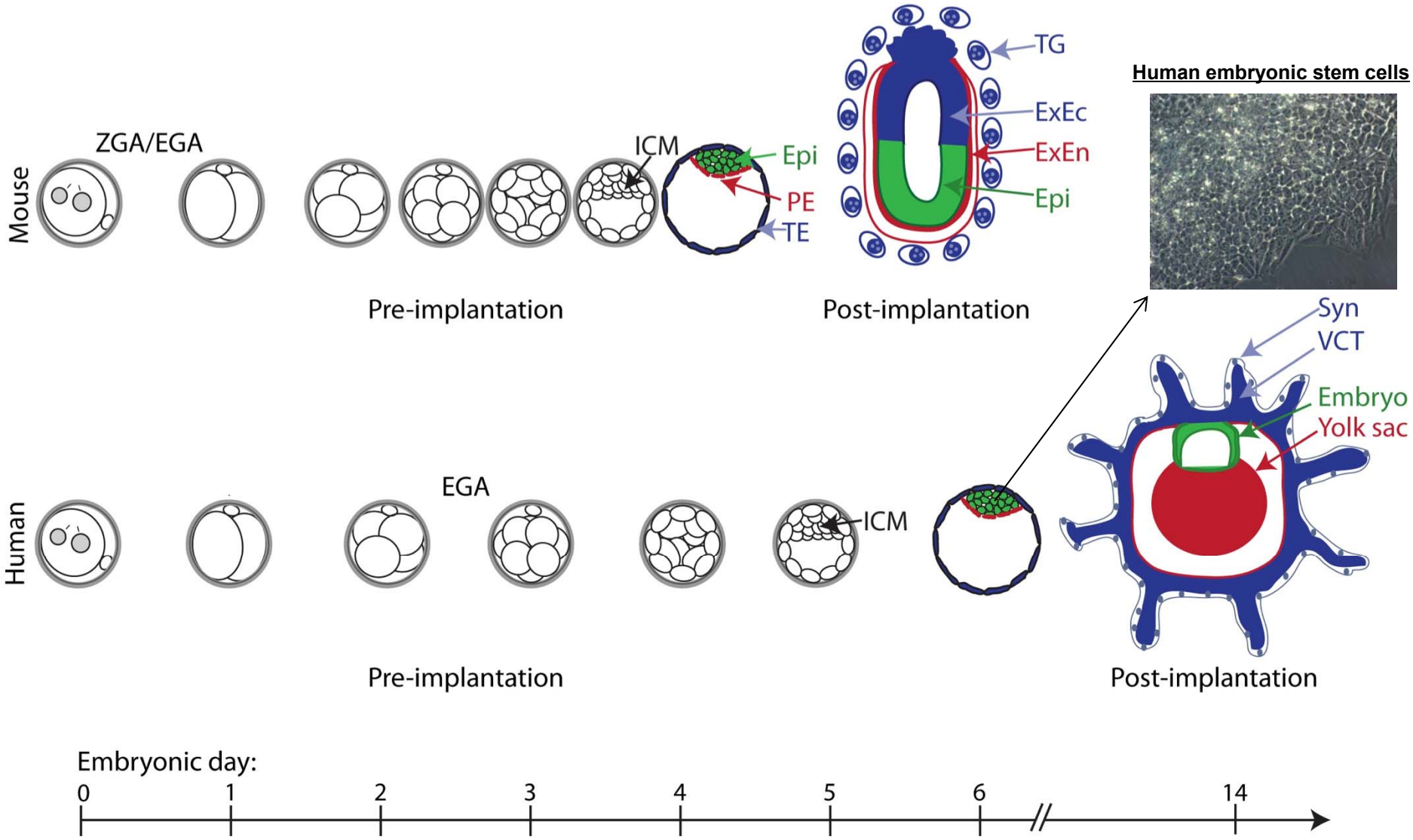
Day 5 - 7
64 - 256 cell (blastocyst)

Early development of human embryos



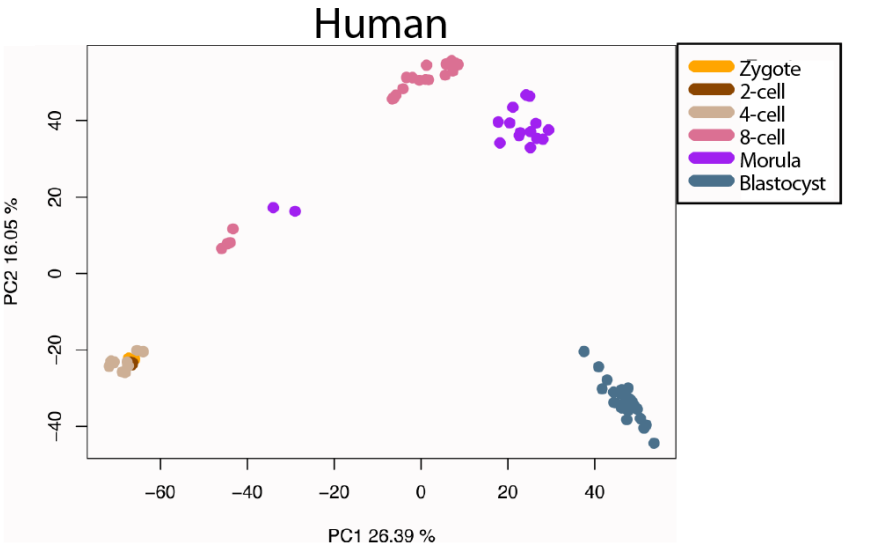
Adapted from Koot *et al., Biochimica et Biophysica Acta* 2012

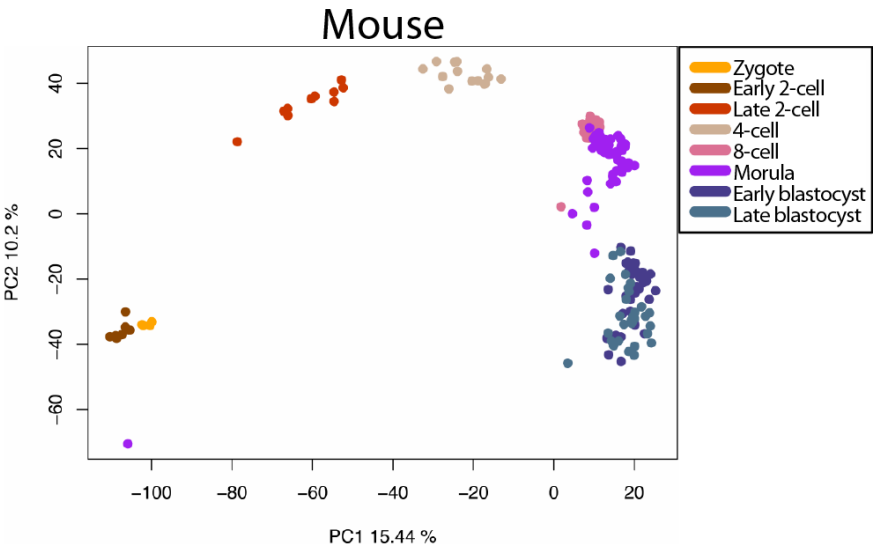
There are important differences between mouse and human development before implantation

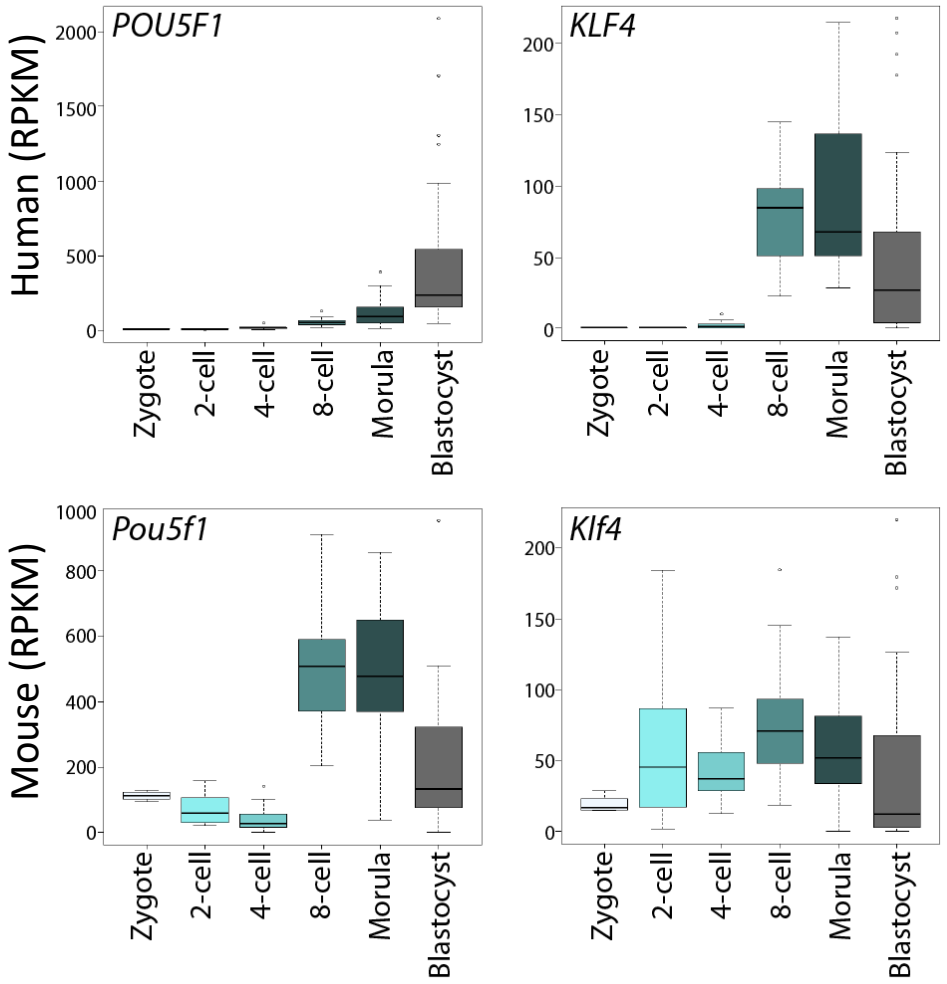


Niakan *et al.*, *Development* 2012

Distinct expression dynamics in human versus mouse embryos

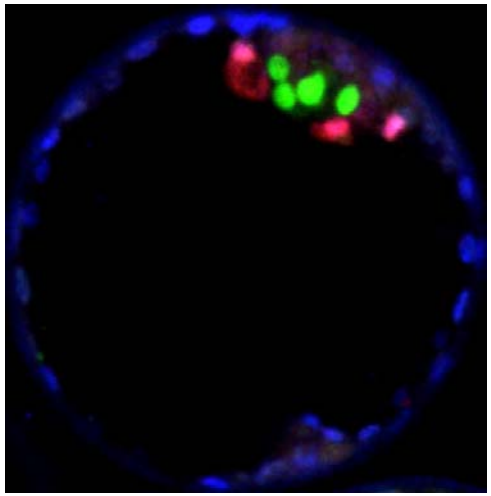


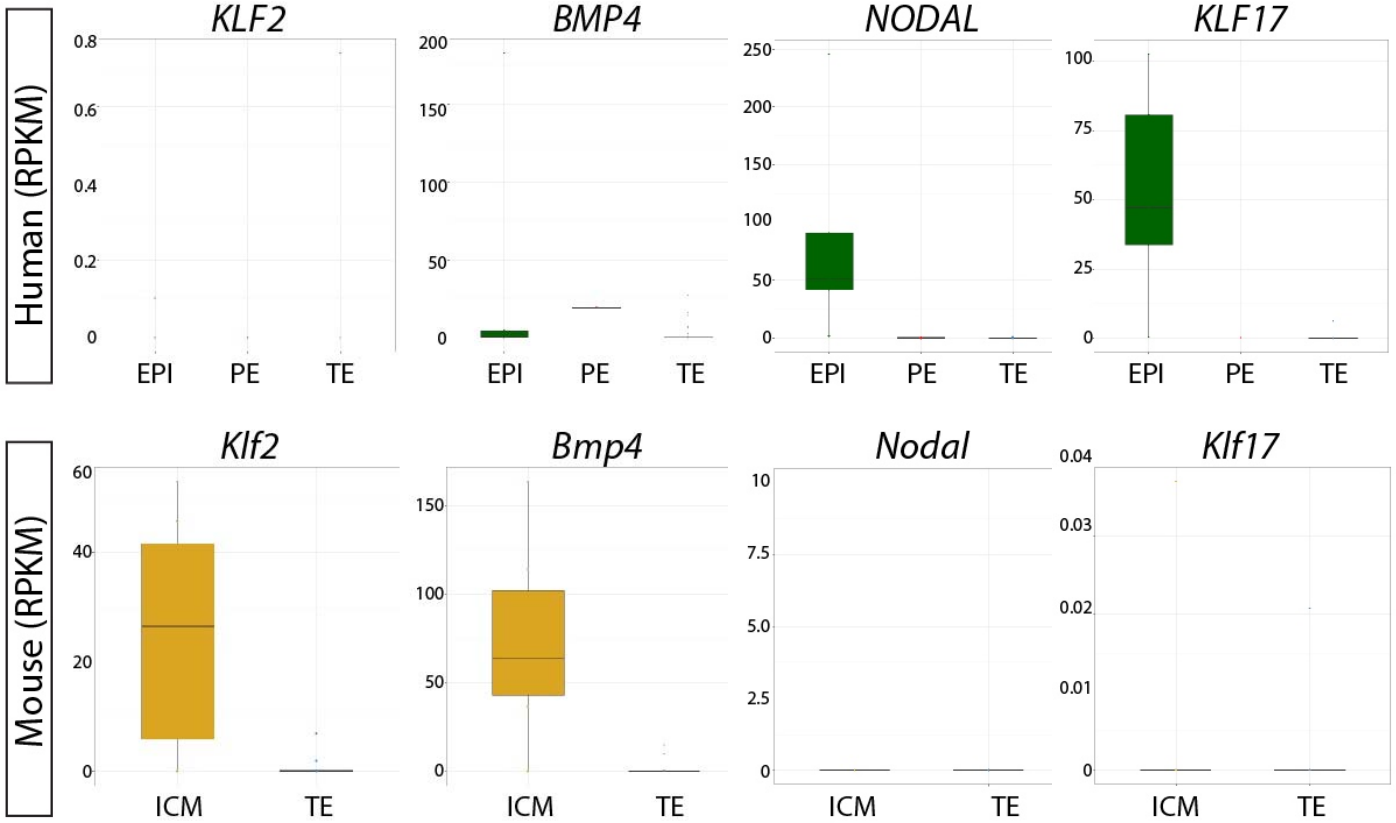




Blakeley *et al., Development* 2015

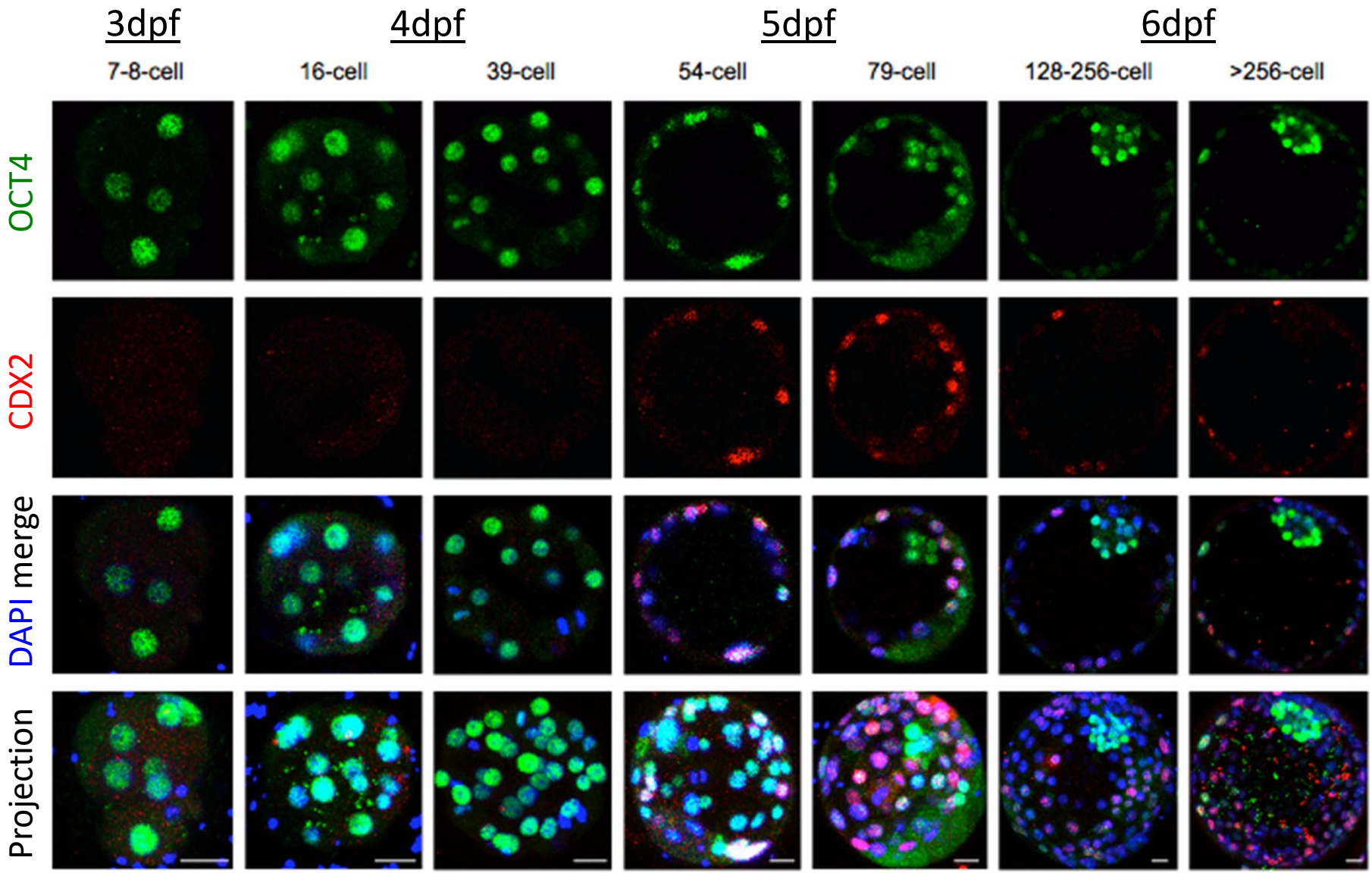
Epiblast-specific expression in human embryos





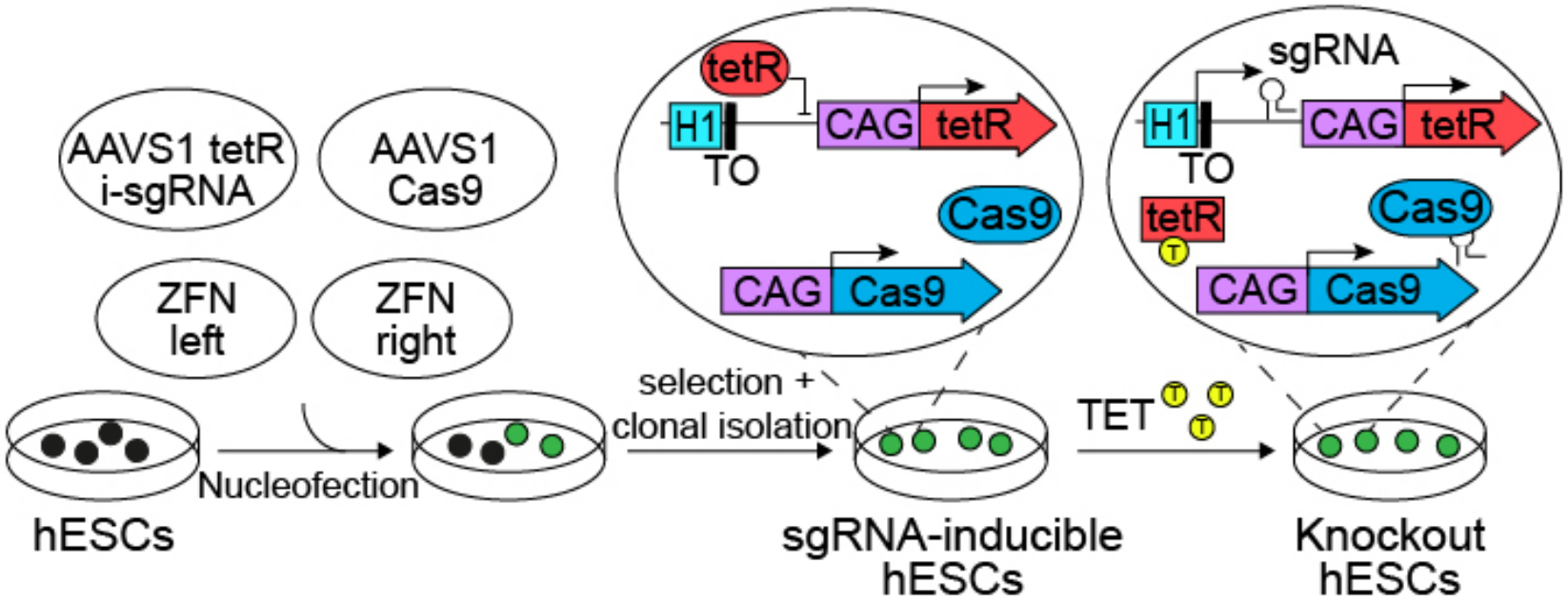
Blakeley, *et al., Development* 2015

Targeting OCT4 as a proof of principle



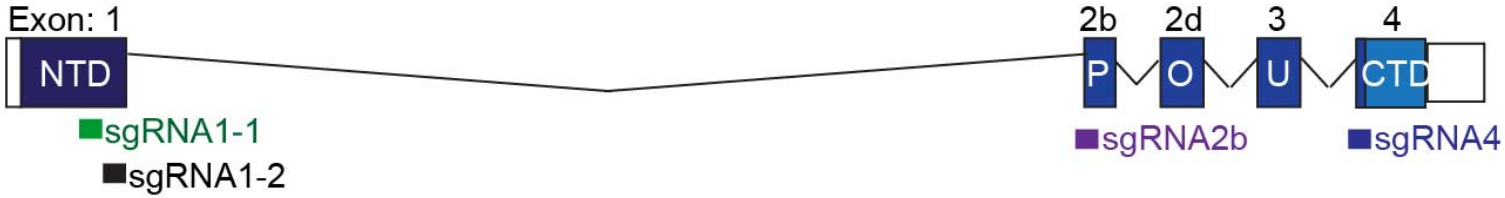
Chen *et al.*, *Cell Stem Cell* 2009; Niakan and Eggan, *Dev Bio* 2013

Identifying the most efficient method to inactivate OCT4

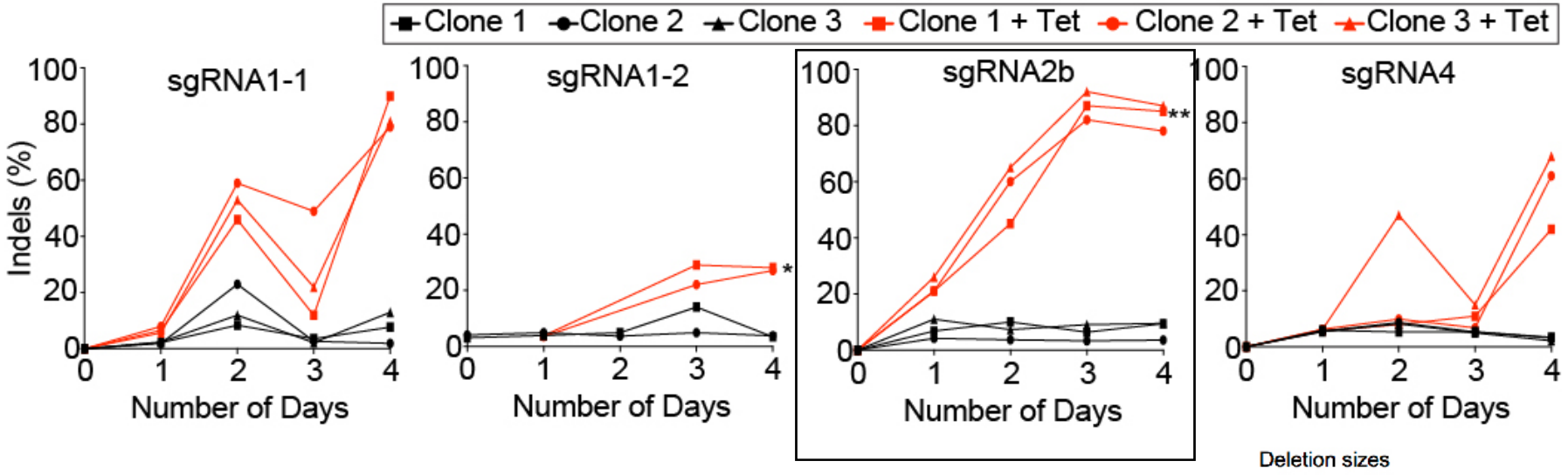


Bertero et al., *Development* 2016

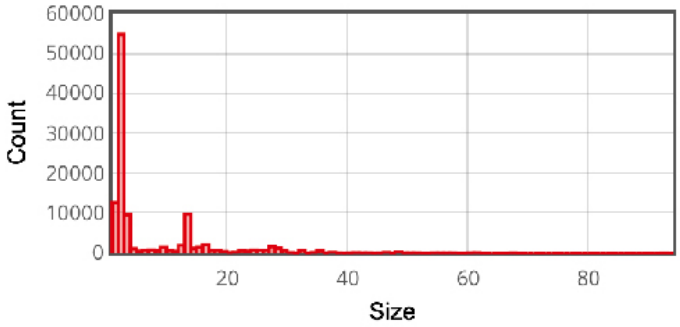
Stereotypic pattern to on-target indel mutations



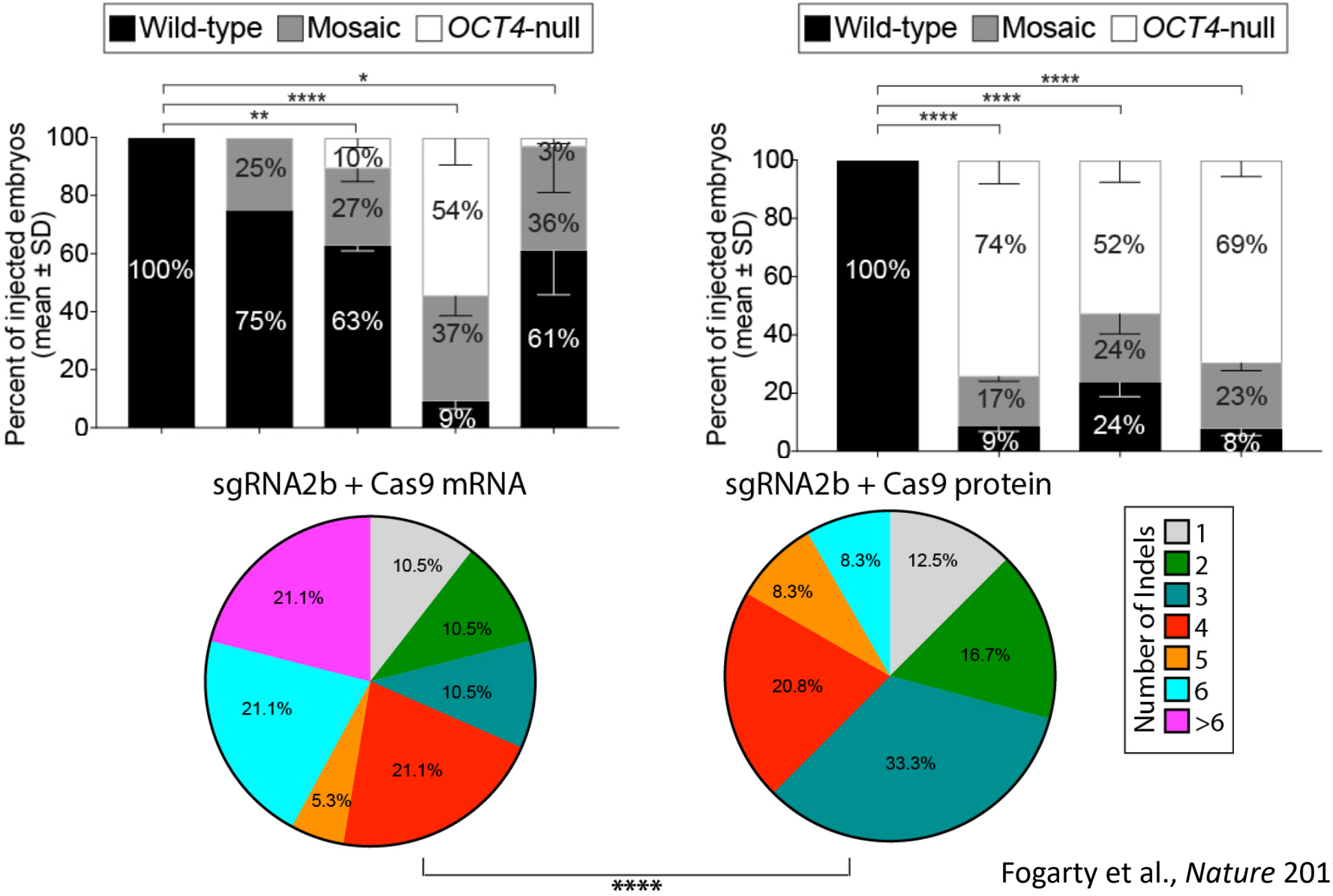
	sgRNA1-1	sgRNA1-2	sgRNA2b	sgRNA4
Human	CTTCACGGCACCAGGGGTGACGG	GCACTAGCCCCACTCCAACCTGG	acccacCAAATAGAACCCCCAGG	acttgcagGTGGTCCGAGTGTGG
Mouse	CTTCACGGCATTGGGGCGGTCGG	GCAAAGTCTCCACGCCAACTTGG	acccacCAAAGAGAACGCCCAGG	catcctagGTGGTTCGAGTATGG



Reference: AGGGGAACCCACCAAATAGAACCCCCAGGGTGAGC
2 bp deletion: AGGGGAACCCACCAAATAGAACC--CAGGGTGAGC
1 bp deletion: AGGGGAACCCACCAAATAGAACC-CCAGGGTGAGC
3 bp deletion: AGGGGAACCCACCAAATAGAACC---AGGGTGAGC

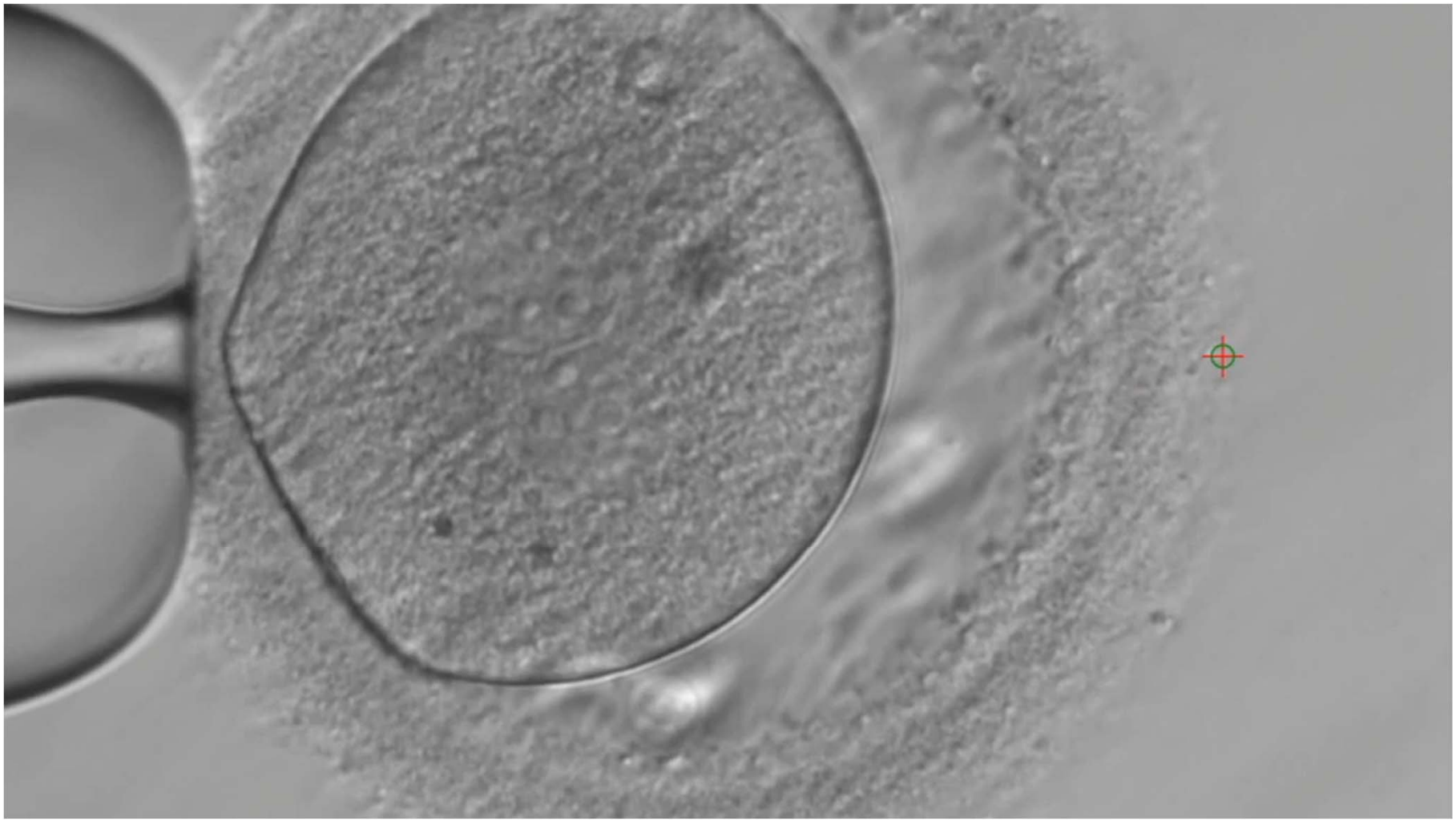


Assessing sgRNA activity and optimizing microinjection methodologies using mouse as a model system



Fogarty et al., *Nature* 2017

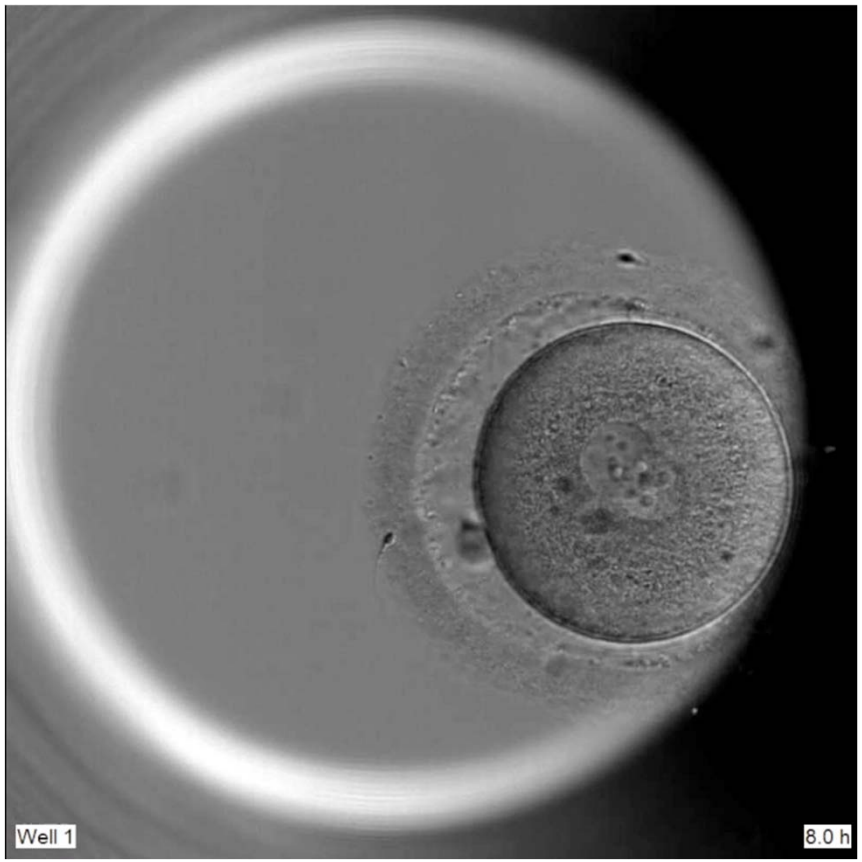
Microinjection of the CRISPR/Cas9 components to inactivate OCT4 in human embryos



Fogarty et al., *Nature* 2017

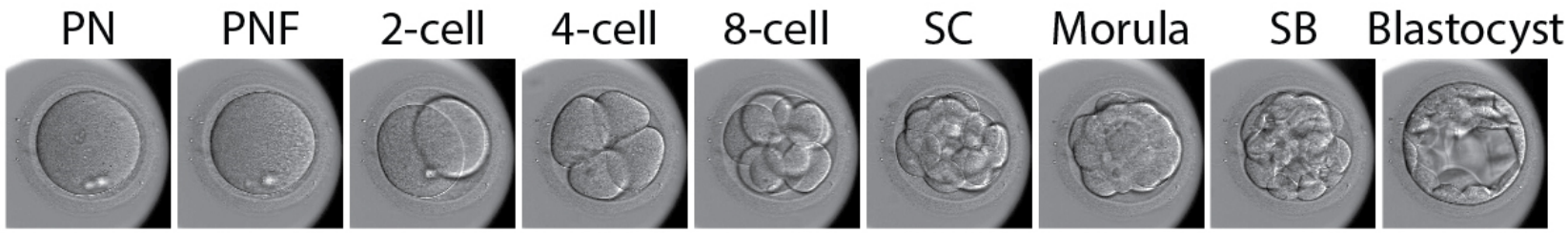
Human embryos need OCT4 to correctly form a blastocyst

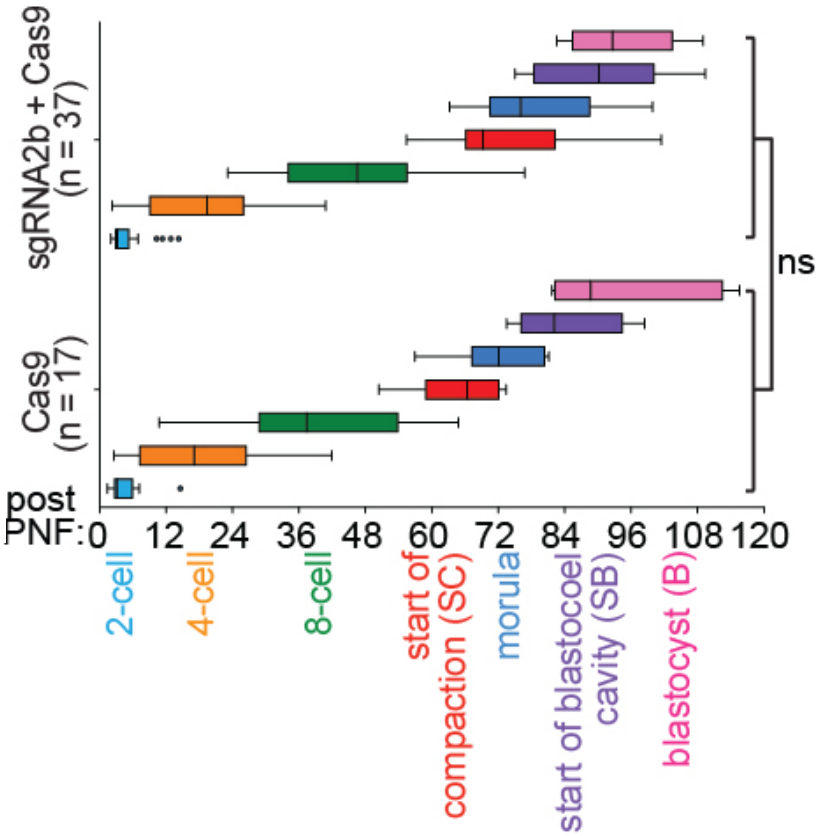
Cas9 protein control

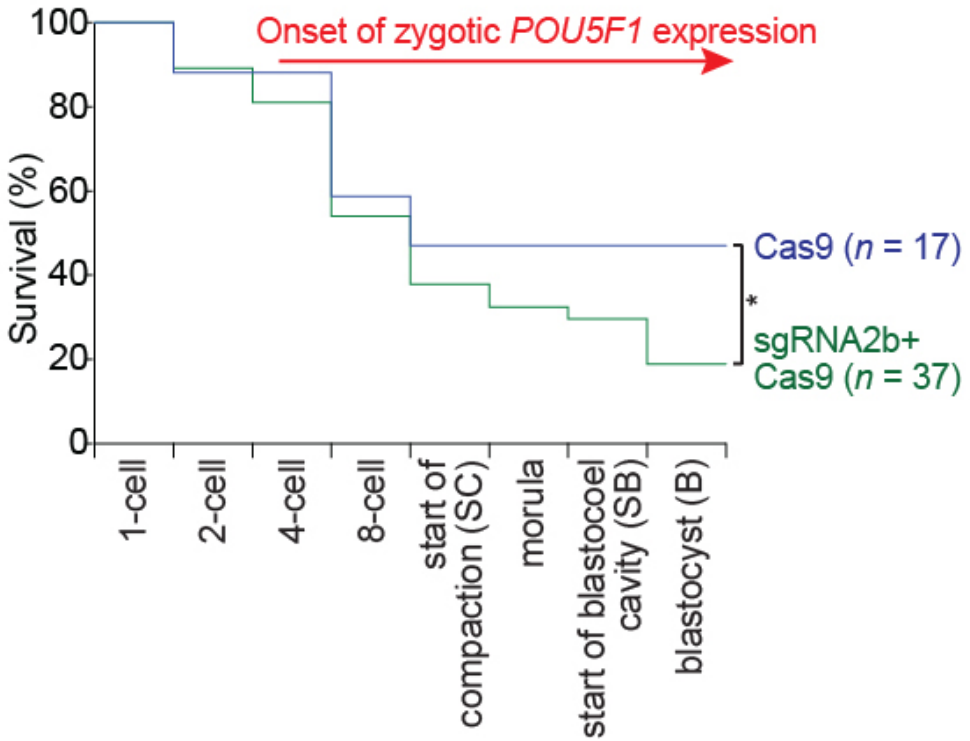


sgRNA2b-Cas9 ribonucleoprotein complex

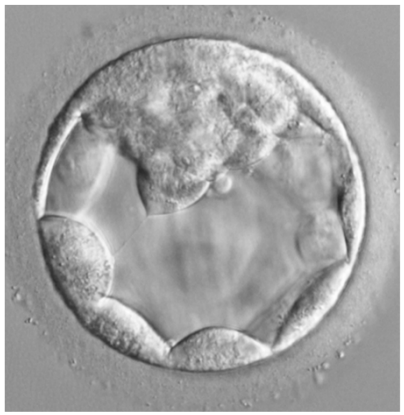
Human embryos need OCT4 to reach the blastocyst stage



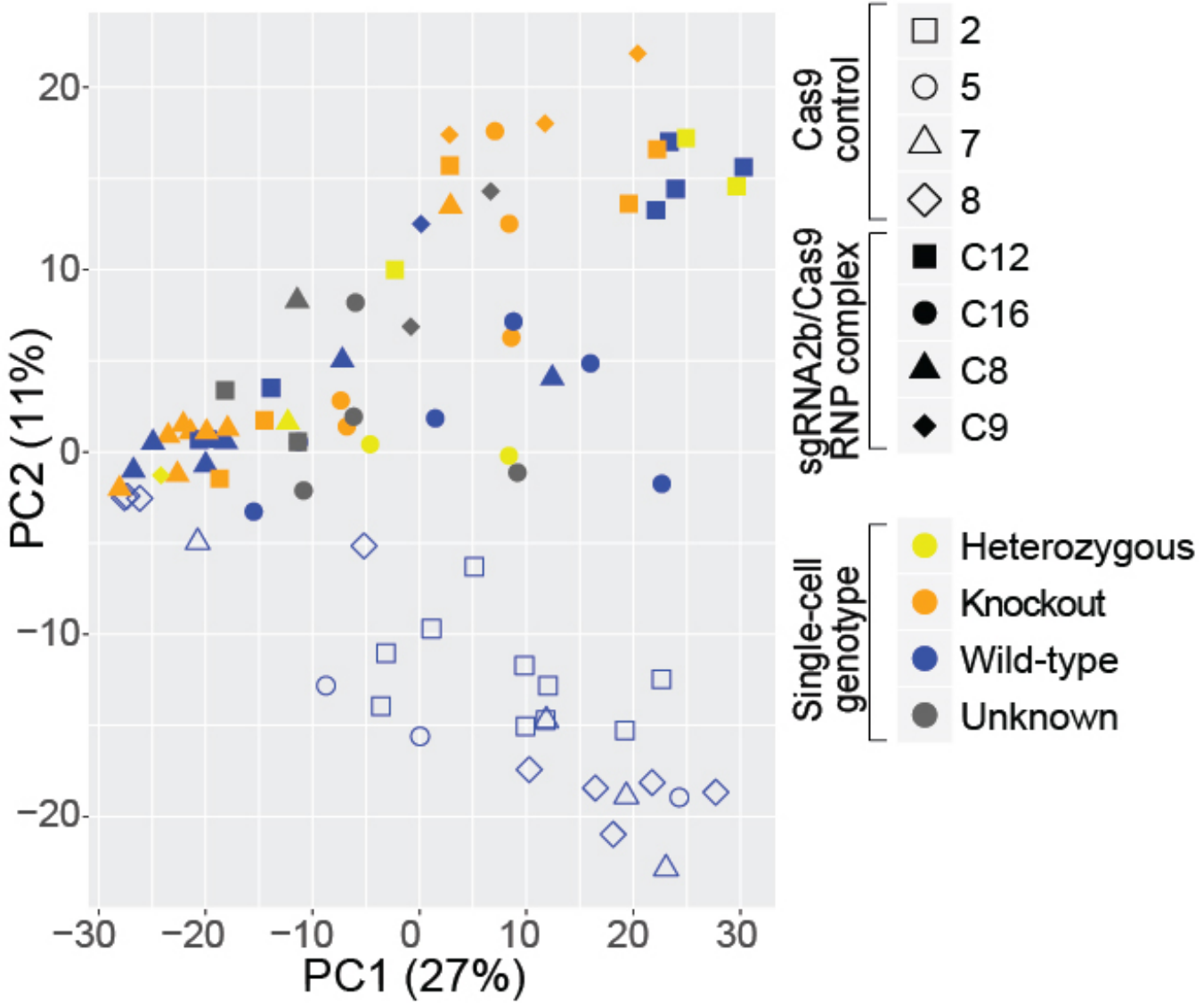




G&T-seq: loss of OCT4 is associated with gene mis-expression

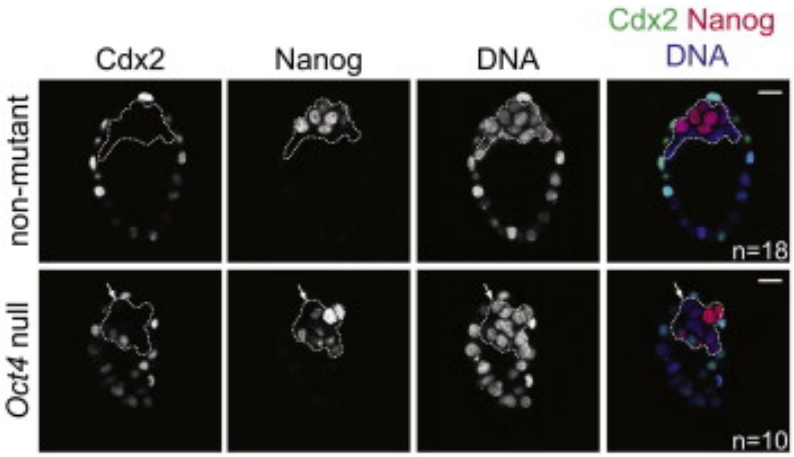


RNA DNA

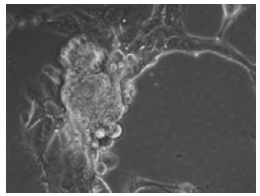


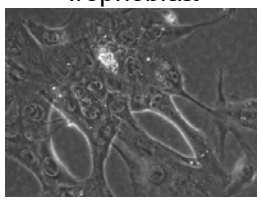
Trophectoderm development is compromised in OCT4-targeted human (but not mouse) embryos

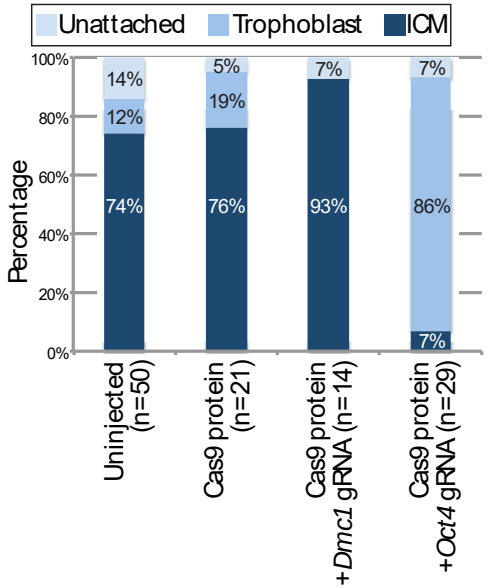
Mouse



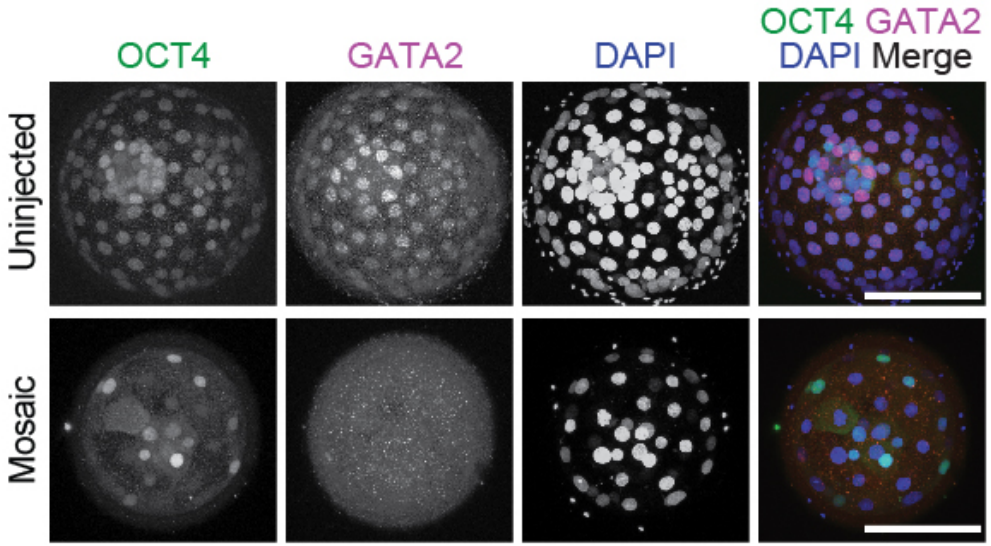
Frum *et al.*, *Dev Cell* 2013

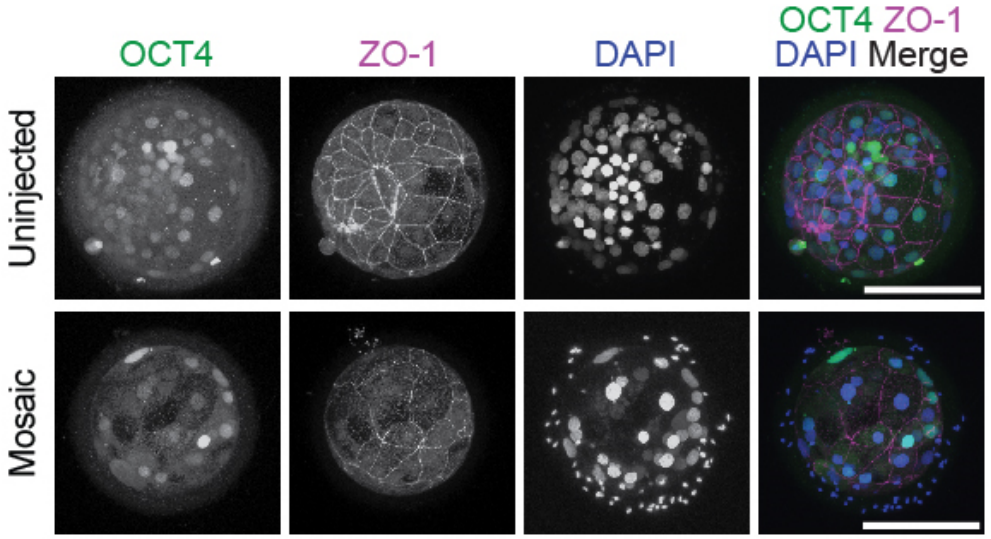




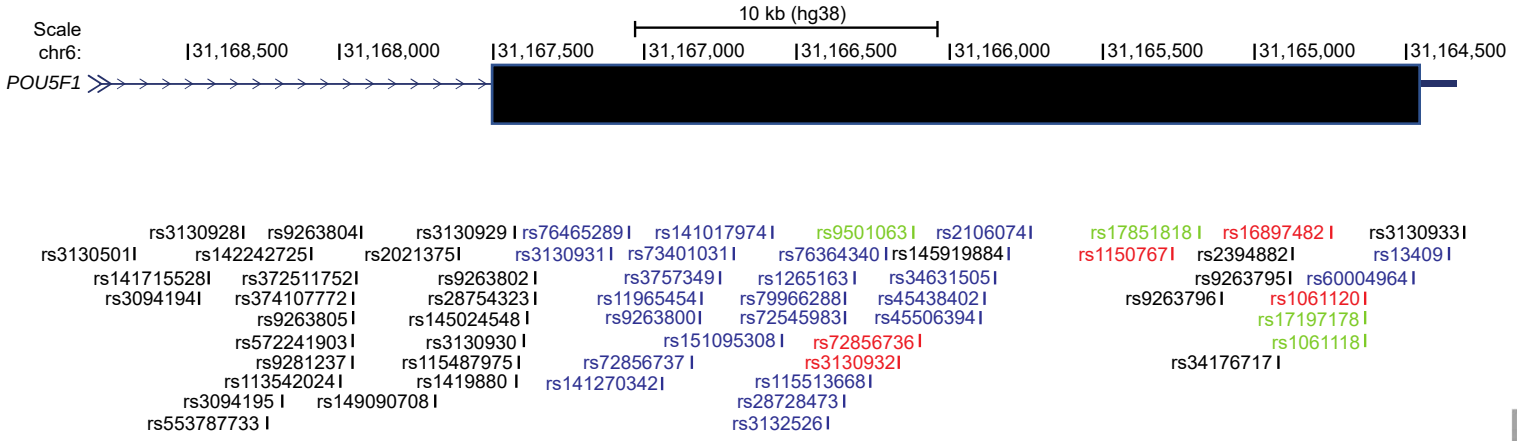


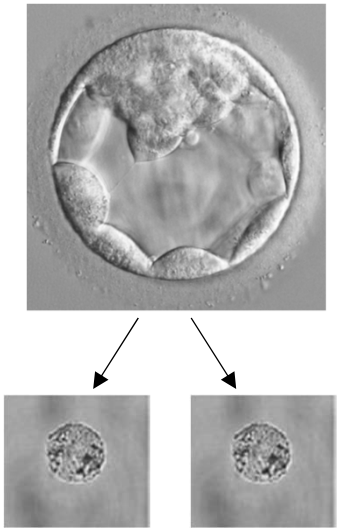
Human



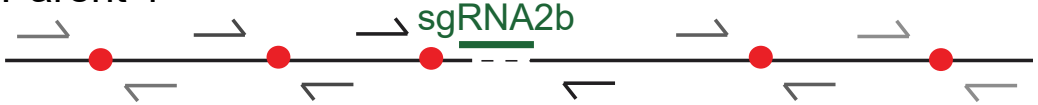


Loss of heterozygosity or large deletions in human embryos following CRISPR/Cas9 genome editing





Parent 1



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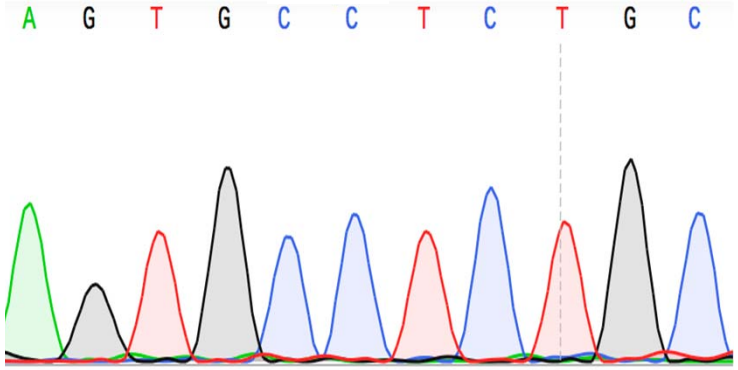
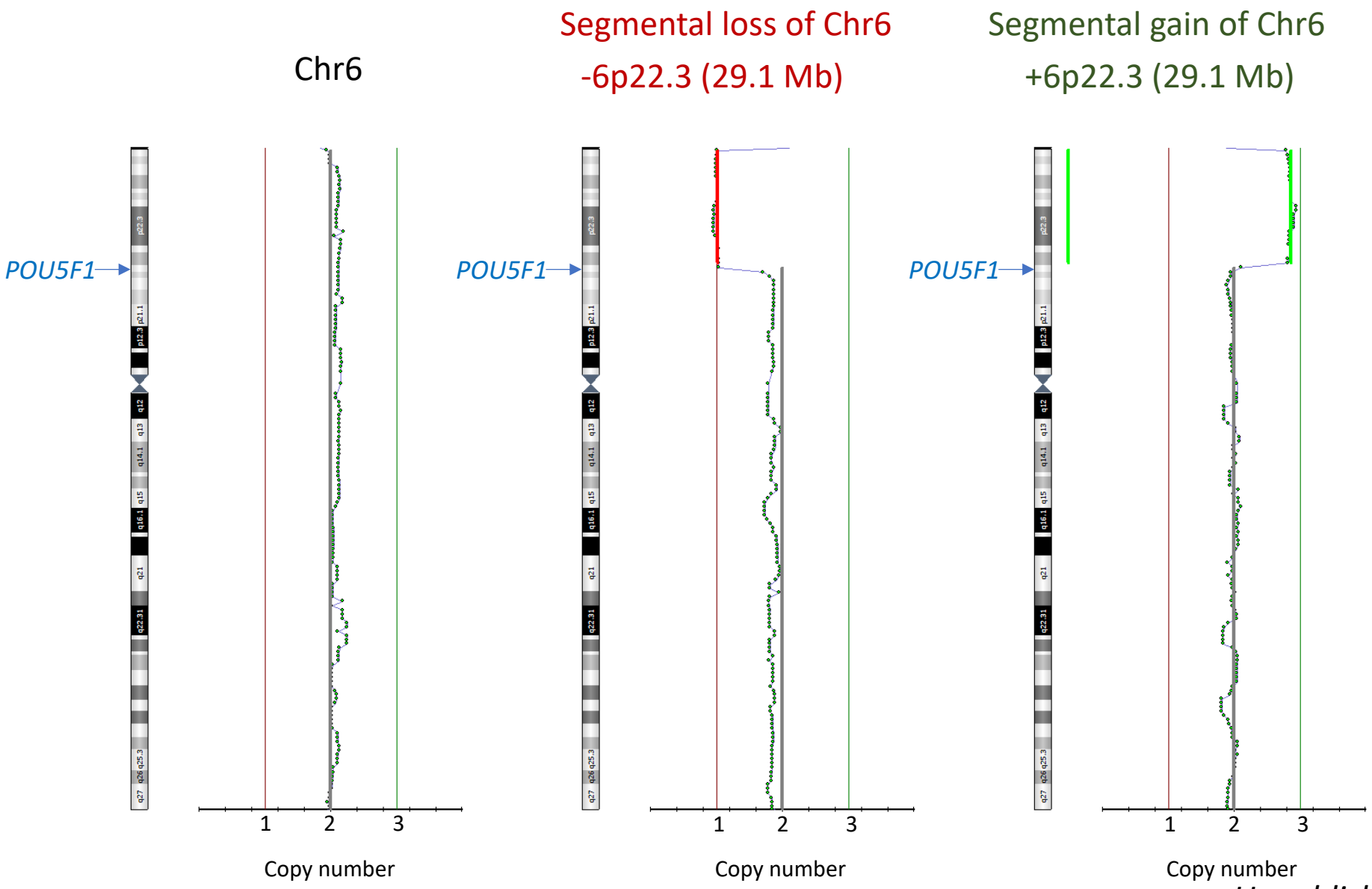


SNPs

Unpublished

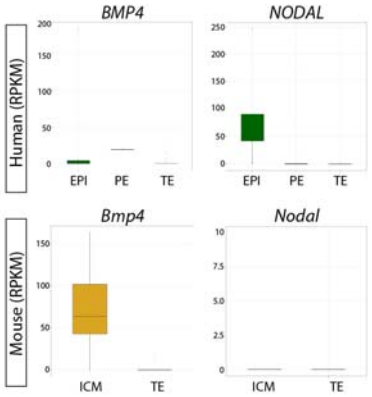
Segmental loss or gain of Chr6 in human embryos following CRISPR/Cas9 genome editing

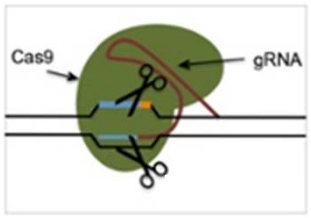


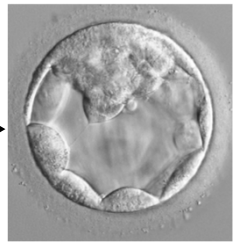
Unpublished

Summary and future aims

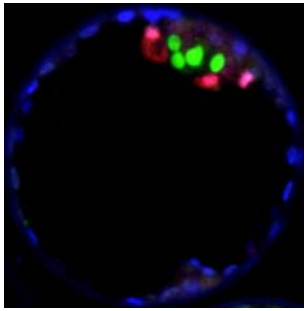
Understand early human development

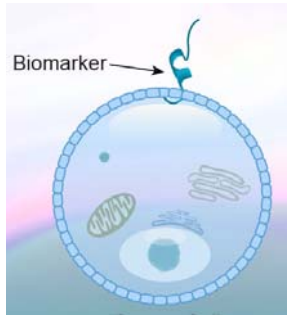


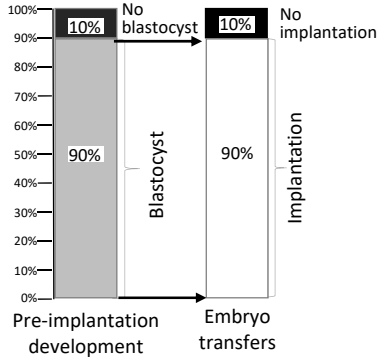




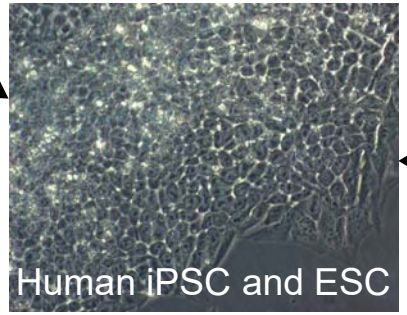
Identify biomarkers of successful embryo development
Improve IVF culture conditions

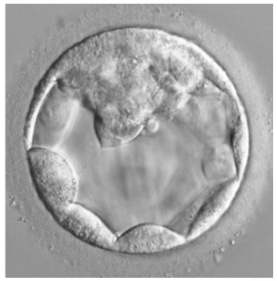


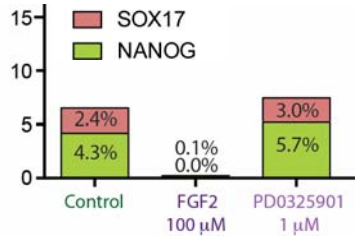




Defining requirements for human iPSCs and hESCs







Acknowledgements

Lab members
Norah Fogarty
Sissy Wamaitha
Paul Blakeley
Afshan McCarthy
Rebecca Lea

Collaborators
Kay Elder and Phil Snell (Bourn Hall)
Ludovic Vallier, Kirsten Snijders and Alessandro Bertero (Cambridge University)
James Turner, Benjamin Powell, Valdone Maciulyte, Jens Kleinjung (Francis Crick Institute)
Dagan Wells and Nada Kubikova (Oxford University)
Daesik Kim and Jin-Soo Kim (Seoul National University)
Ricardo Baptista and Iris Valero (Cell and Gene Therapy Catapult)

Rosa Beddington Fund

MRC Confidence in Concept

Evaluating putative off-target mutations

ACCCACCAAATAGAACCCCCAGG *POU5F1* Exon2b sgRNA

CCTTCCCAAATAGAACCCCCAGG *POU5F1P3*

CCTTCCCAAATAGAACCCCCAGG *POU5F1B*

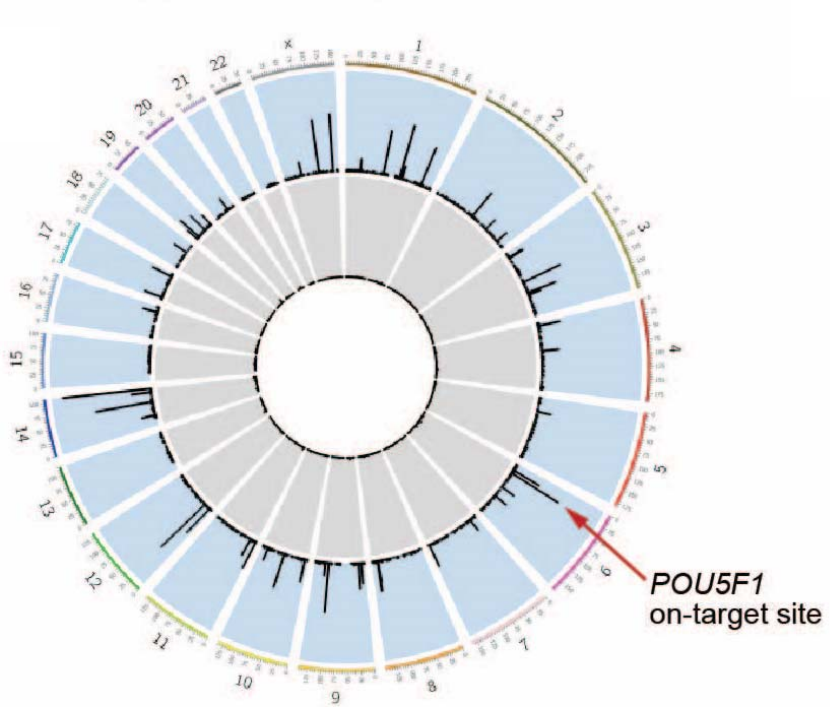
CCTTCCCAAATAGAACCCCCAGG *POU5F1P4*

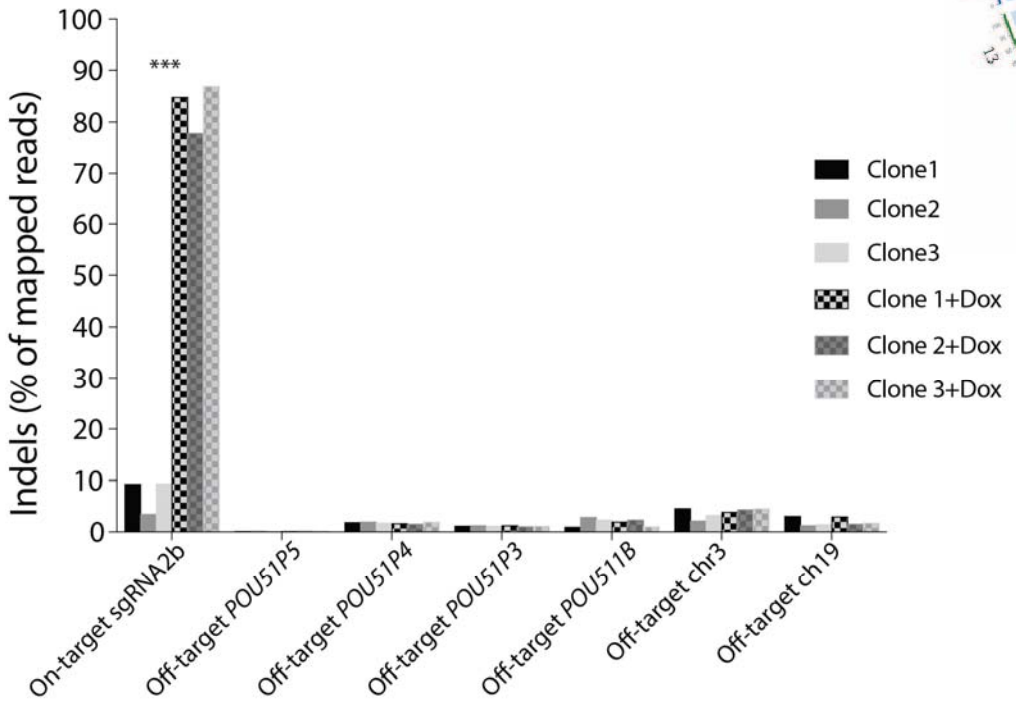
TATTCCCAAATAGAACCCCCAGG *POU5F1P5*

ACCCATCAAATACAACCCCCCAG chr 19

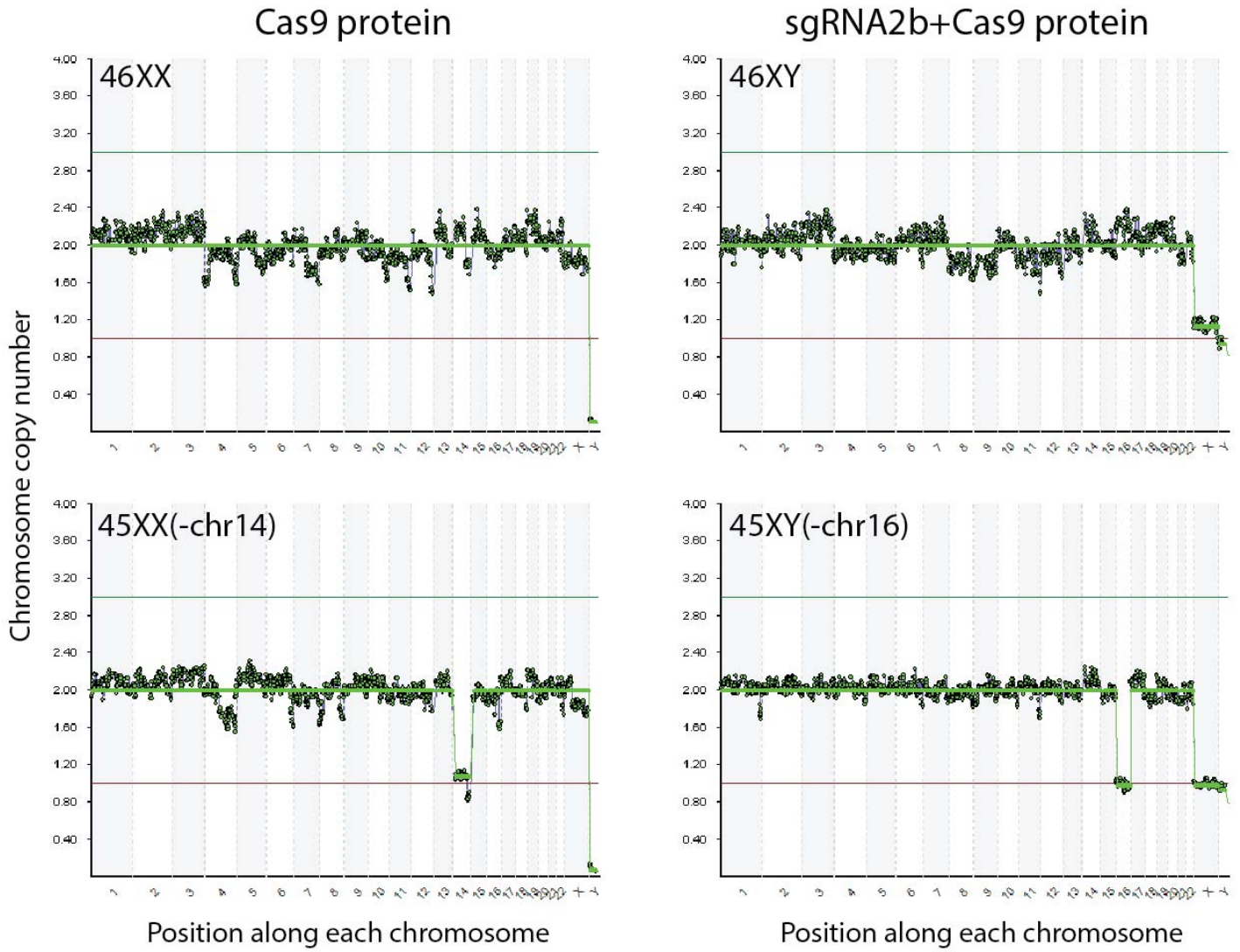
AGCCACCAGGTAGAACCCCCAAG chr 3

Putative off-target sites





Aneuploidy rates are equivalent in sgRNA2b-Cas9 protein injected embryos compared to controls



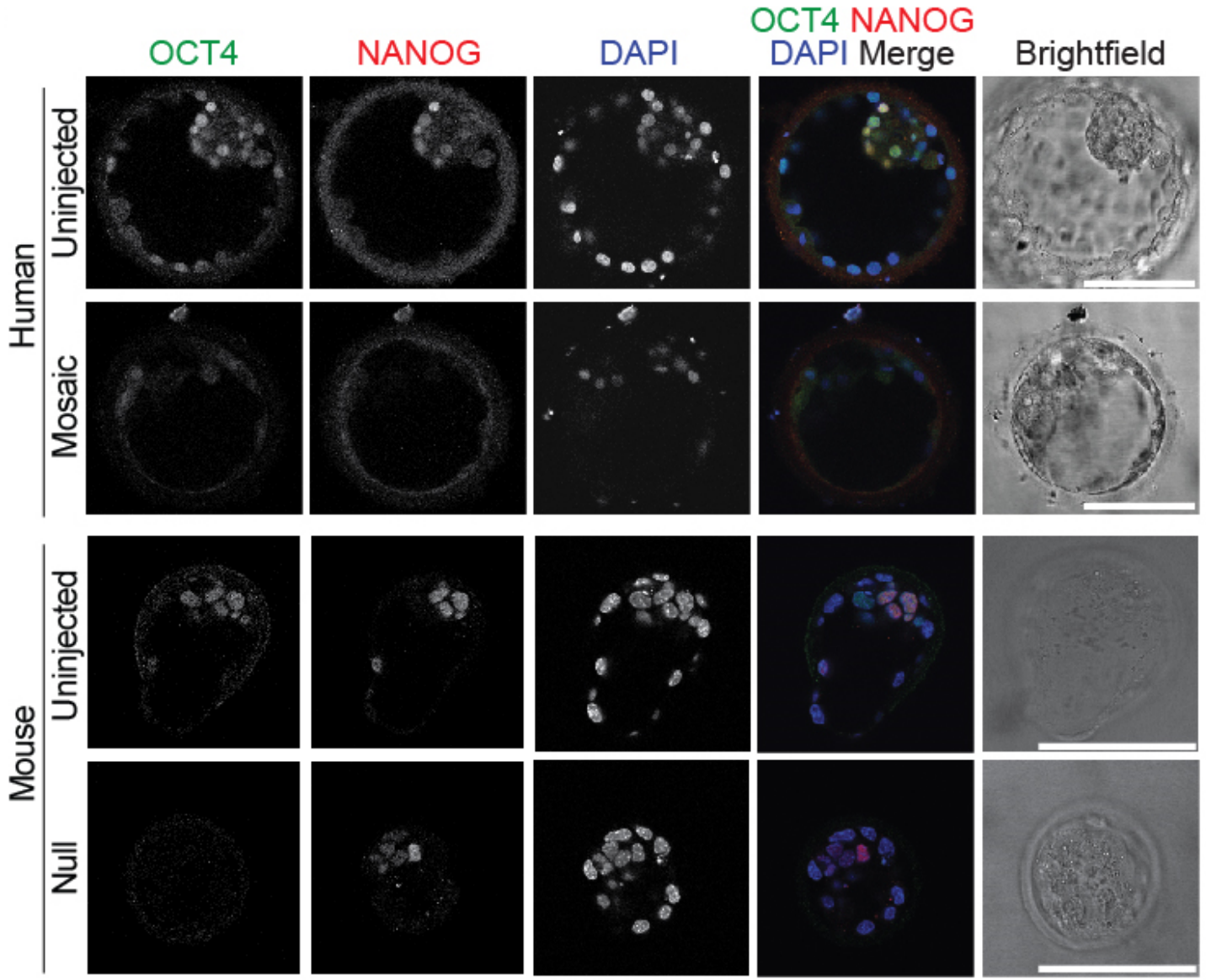
Dagan Wells and Nada Kubikova

HFEA – Scientific and Clinical Advances Advisory Committee, Horizon Scanning Panel and the Ethics and Law Advisory Group

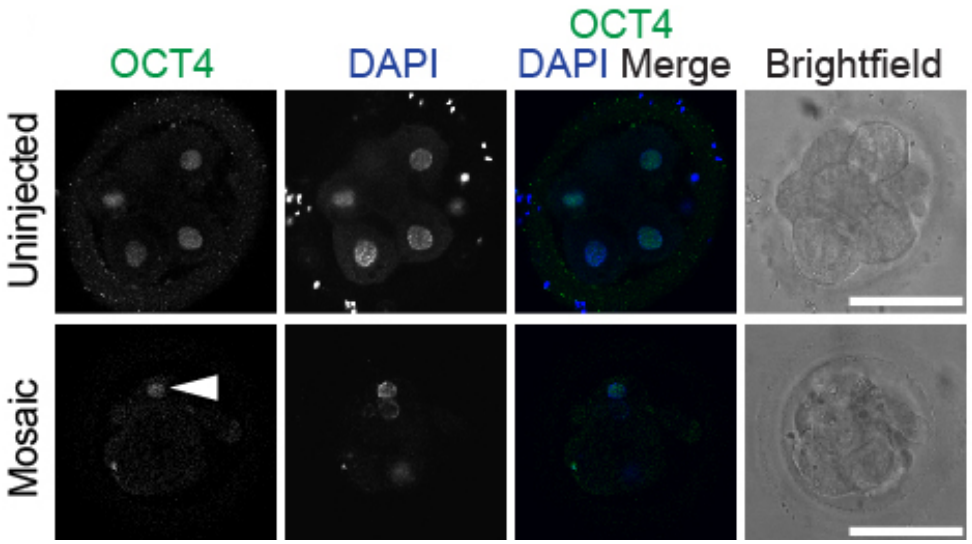
1. Research into human embryo development – specifically the roles of genes and growth factors involved in early development
2. The development of more objective criteria for embryo selection – by investigating gene function in early embryogenesis
3. Research into the genetic background of adverse medical conditions – genetically modified ES cells could be create to model medical conditions
4. Research into the fate of cells during embryo development
5. Introducing a gene to increase the efficiency of stem cell derivation

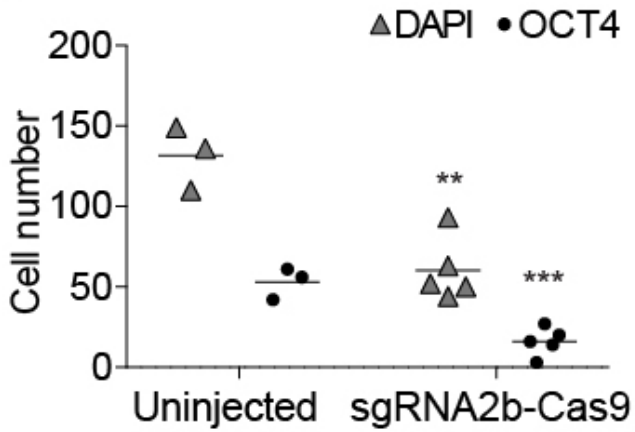
"Genetic modification of embryos: information for Research Licence Committee" paper SCAAC(01/09)04 January 27th 2009

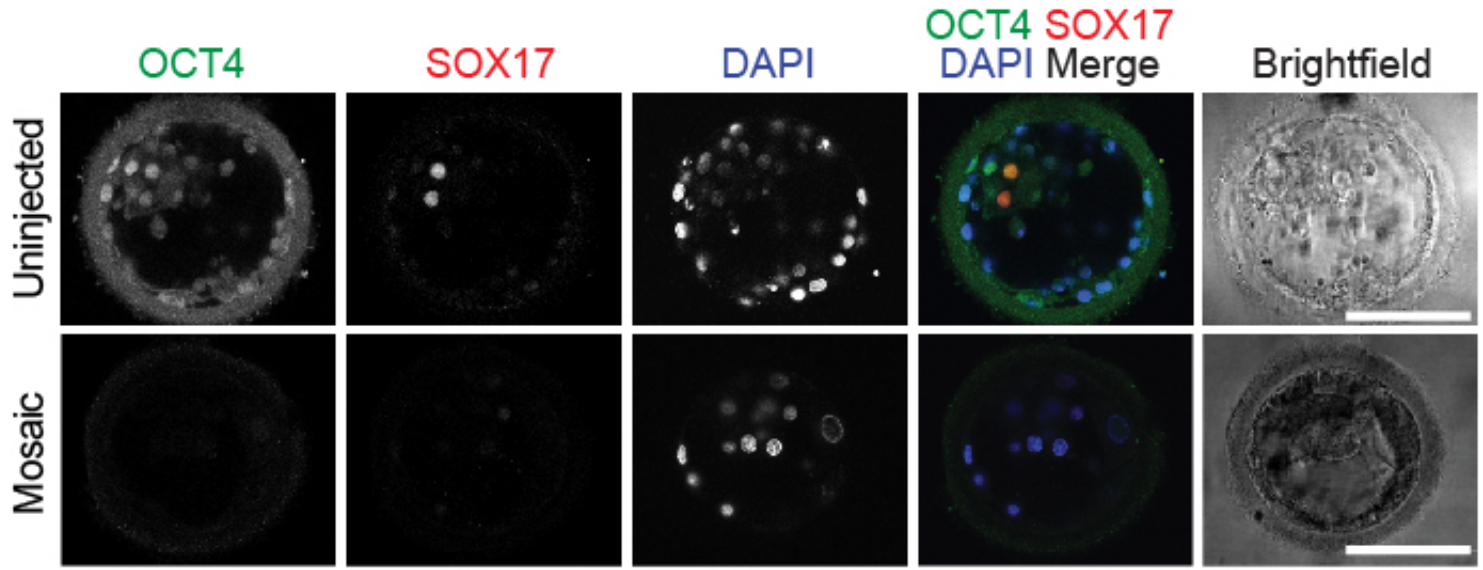
In human embryos OCT4 is required to correctly form all of the cell types of the blastocyst

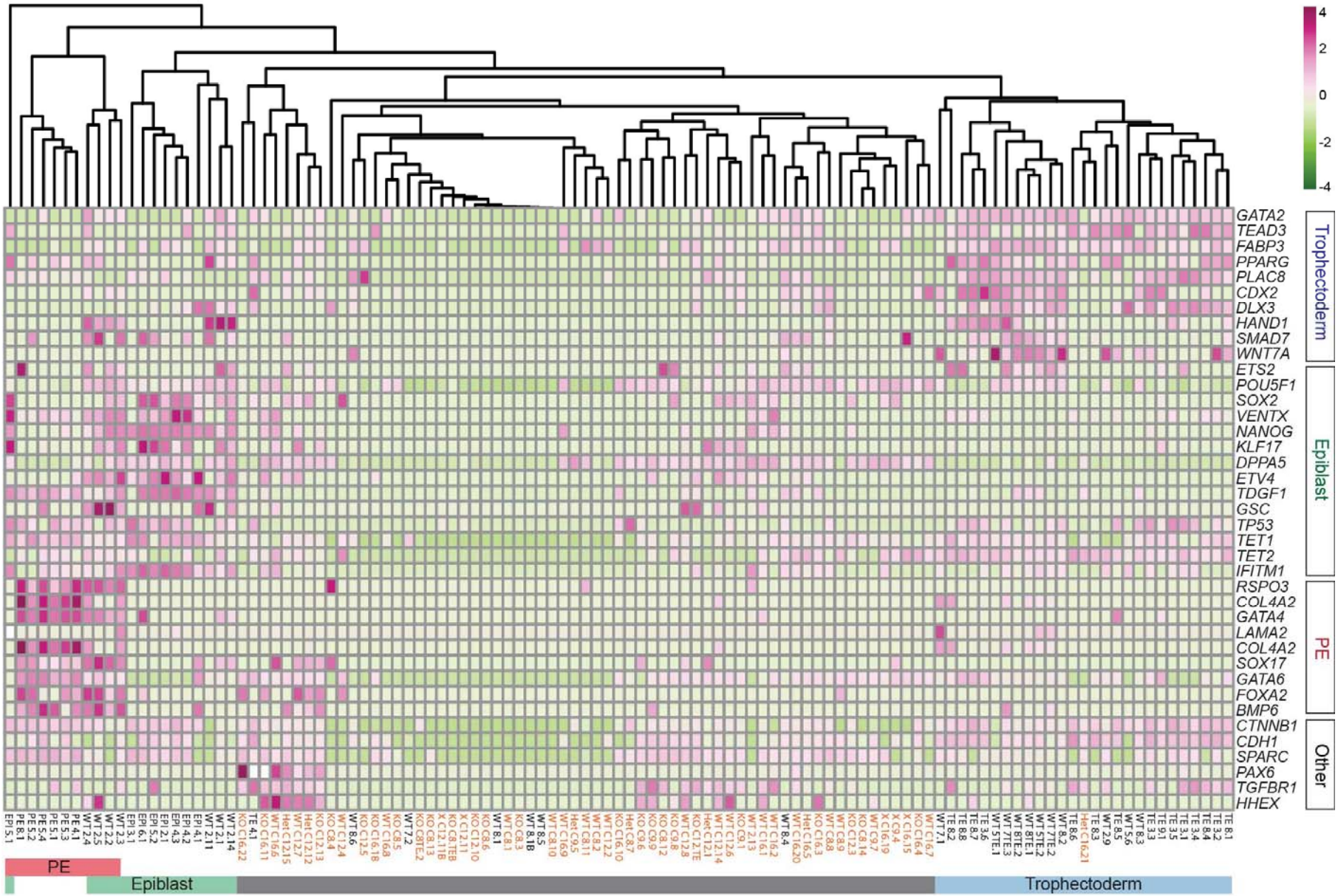


OCT4 expression in cleavage and blastocyst stage embryos following CRISPR/Cas9 genome editing





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2
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-2
-4
GATA2
TEAD3
FABP3
PPARG
PLAC8
CDX2
DLX3
HAND1
SMAD7
WNT7A
ETS2
POU5F1
SOX2
VENTX
NANOG
KLF17
DPPA5
ETV4
TDGF1
GSC
TP53
TET1
TET2
IFITM1
RSPO3
COL4A2
GATA4
LAMA2
COL4A2
SOX17
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FOXA2
BMP6
CTNNB1
CDH1
SPARC
PAX6
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Epiblast
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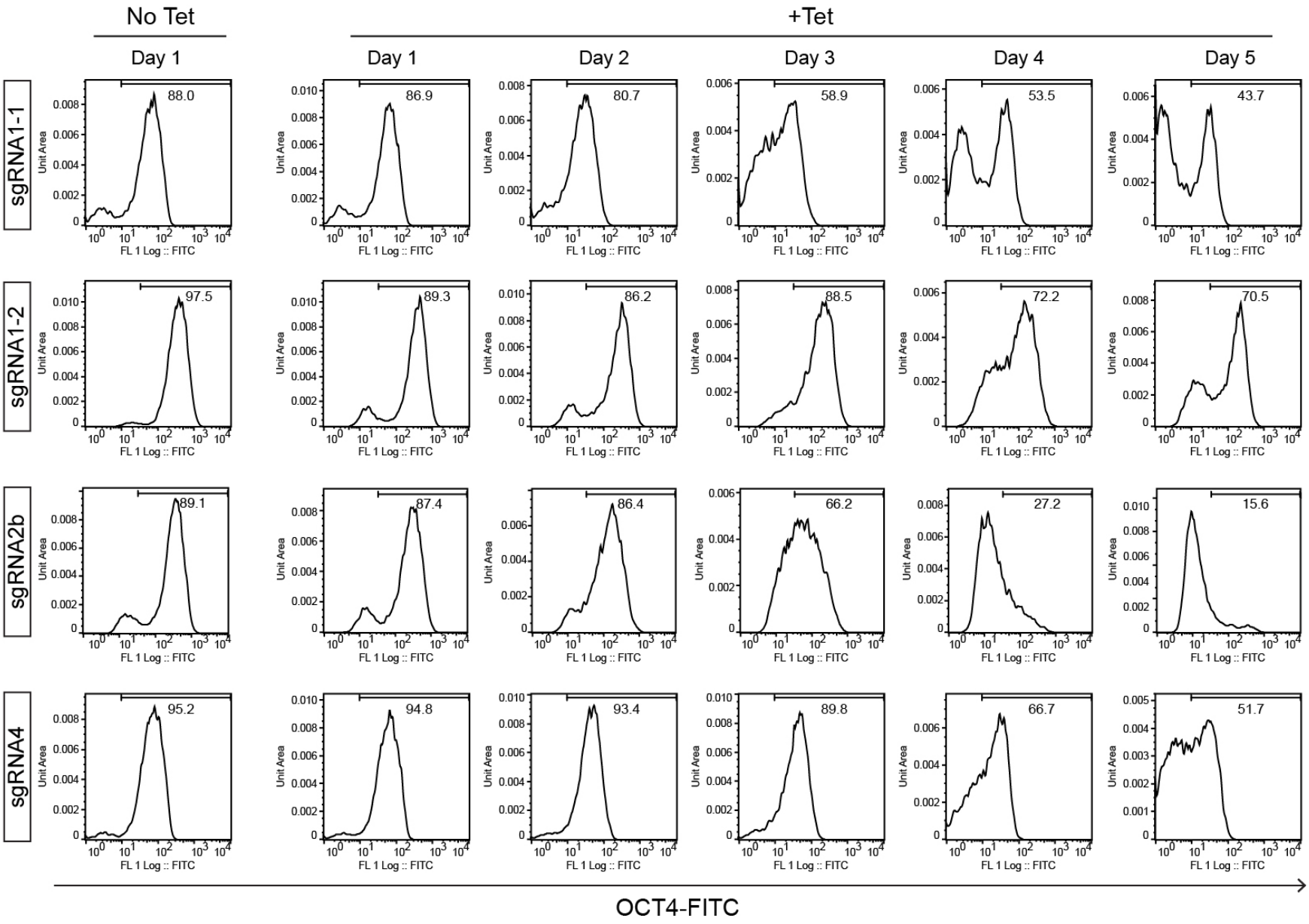
Other
PE
Epiblast
Trophectoderm

A highly-efficient sgRNA targeting *POU5F1* in hESCs



Efficient targeting of OCT4 in human embryos