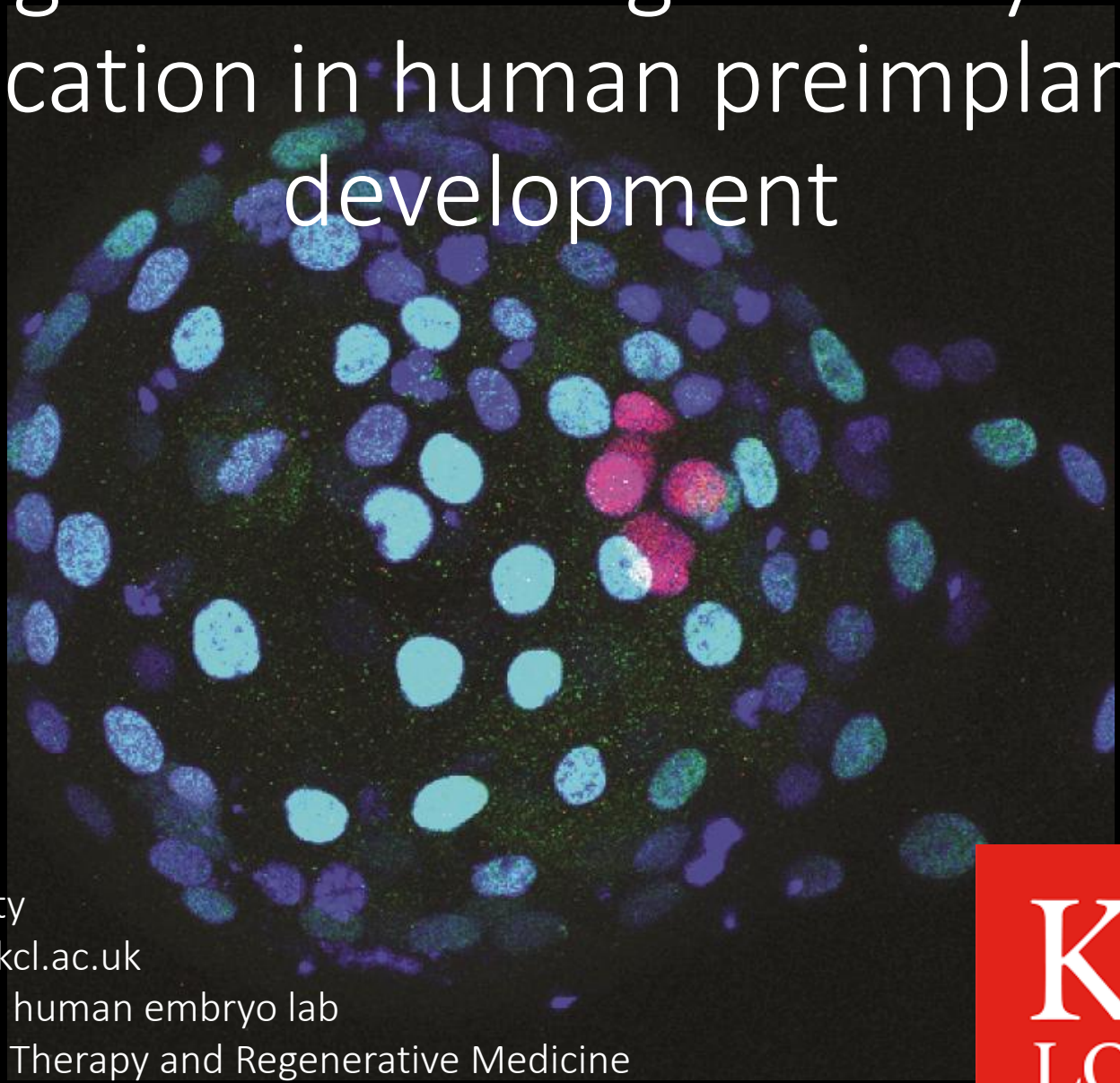
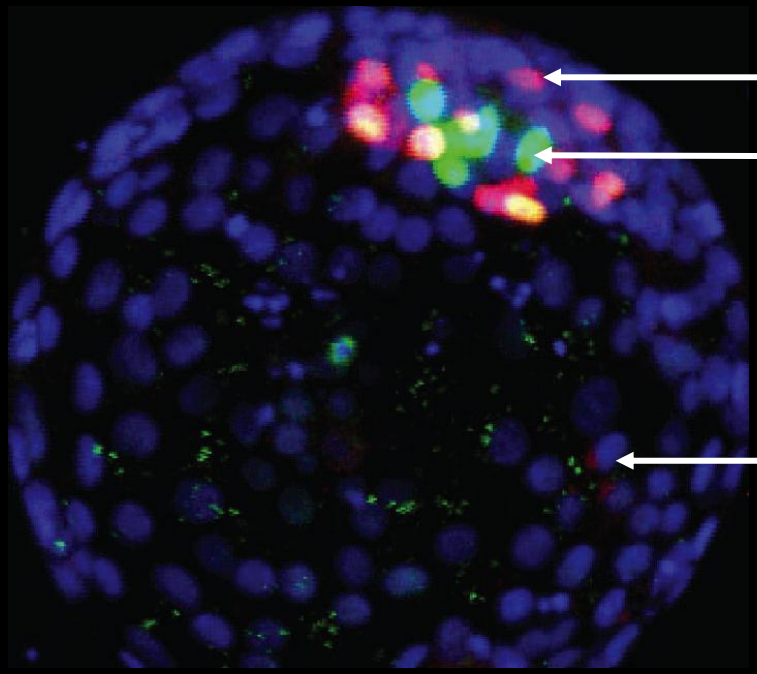
Using genome editing to study lineage specification in human preimplantation development



Dr. Norah Fogarty
norah.fogarty@kcl.ac.uk
Trophoblast and human embryo lab
Centre for Gene Therapy and Regenerative Medicine
King's College London

KING'S College LONDON

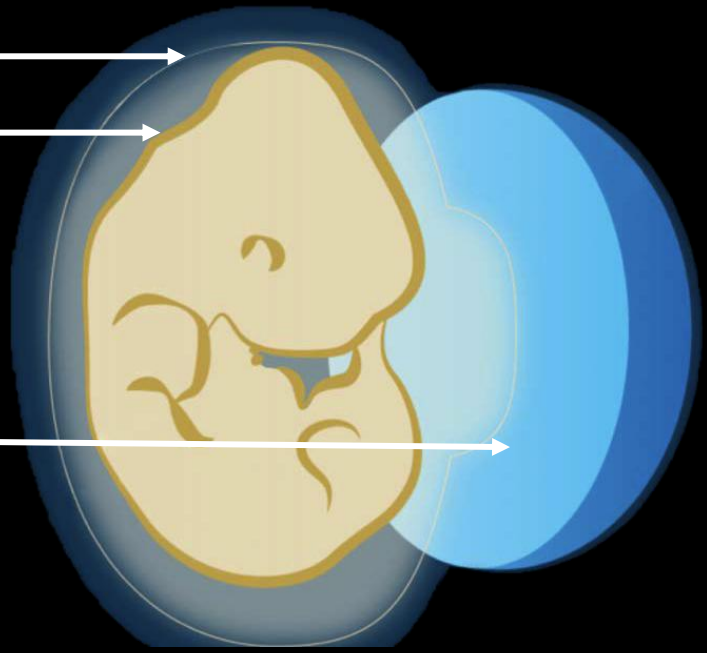
We seek to uncover molecular mechanisms regulating lineage specification in the human embryo



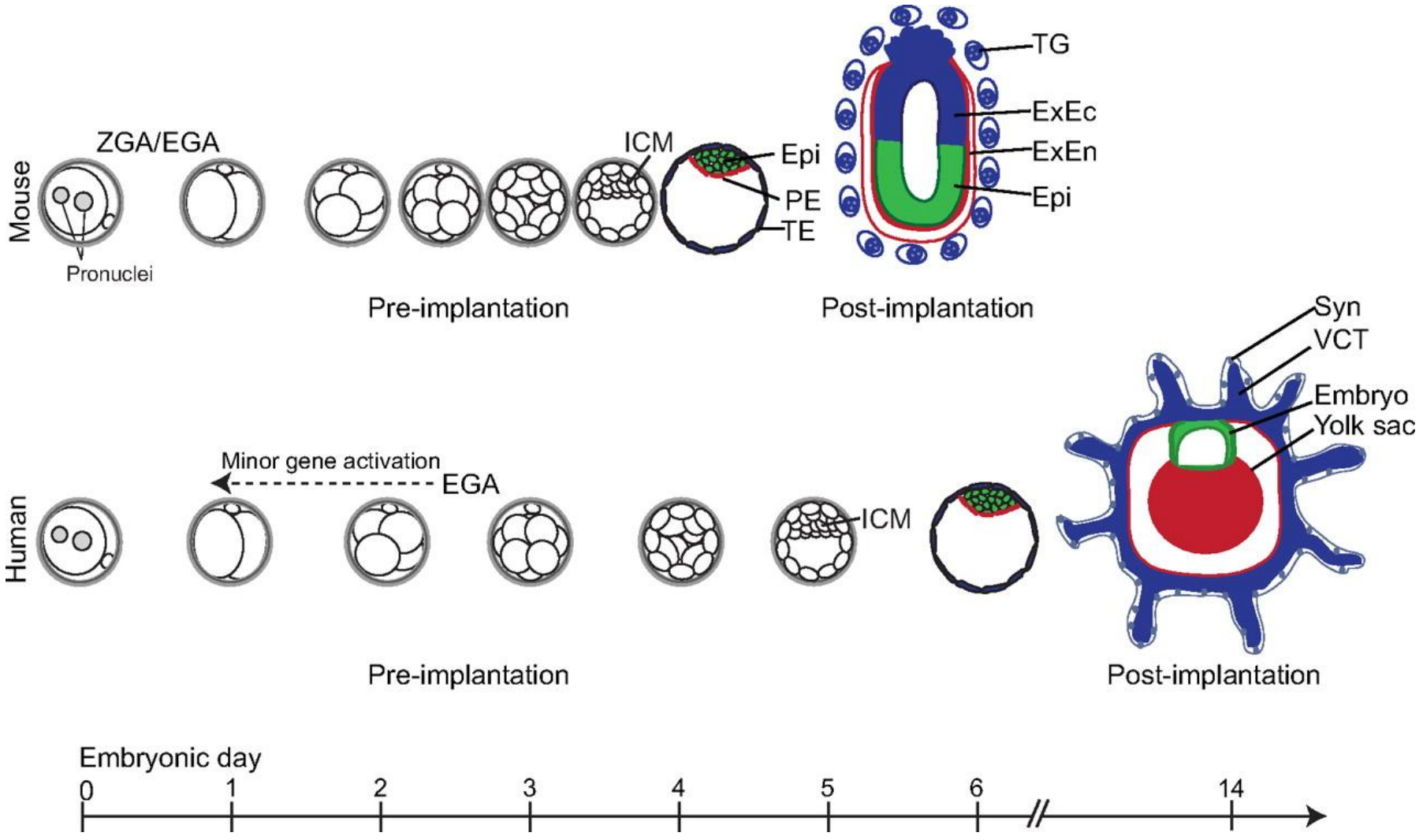
Primitive endoderm
Yolk sac

Epiblast
Embryo proper

Trophectoderm
Placenta

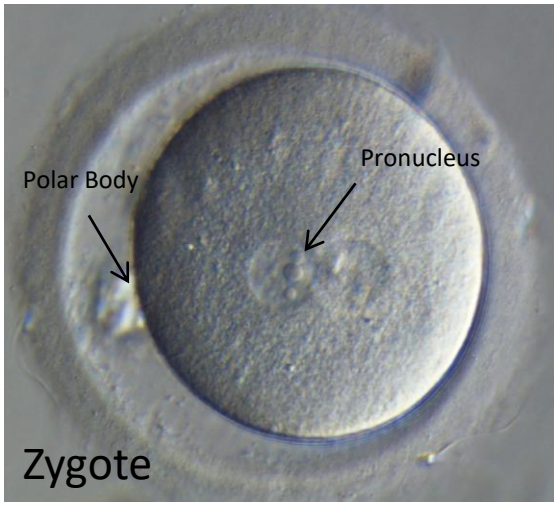


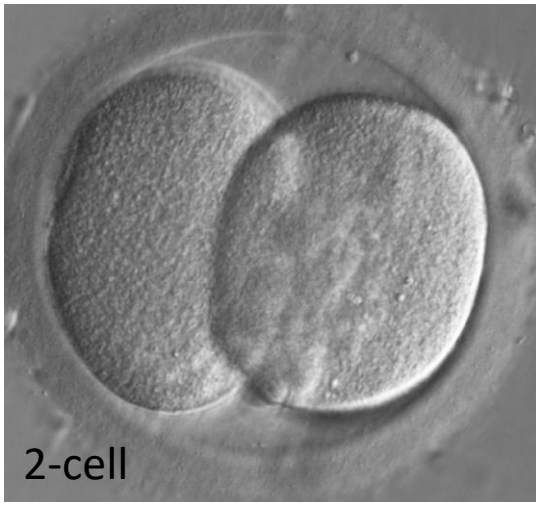
Cell fate decisions and their timing in mouse versus human early embryo development

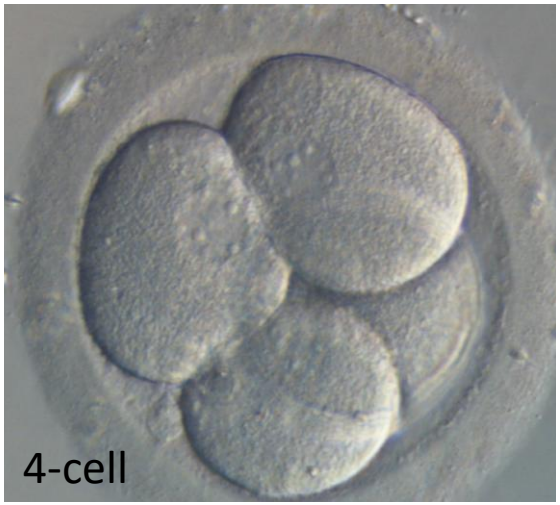


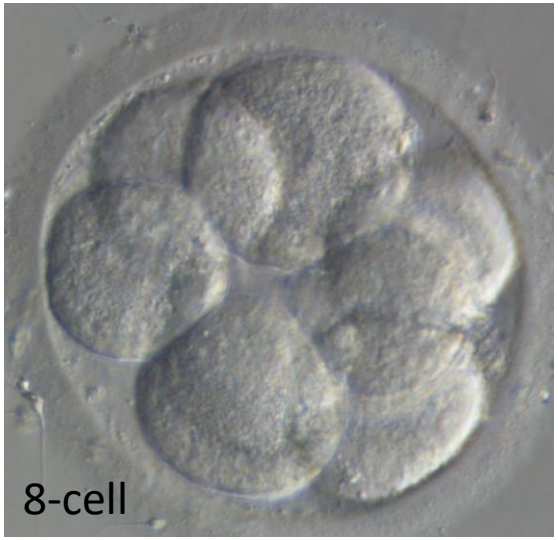
Niakan et al. Development 2012

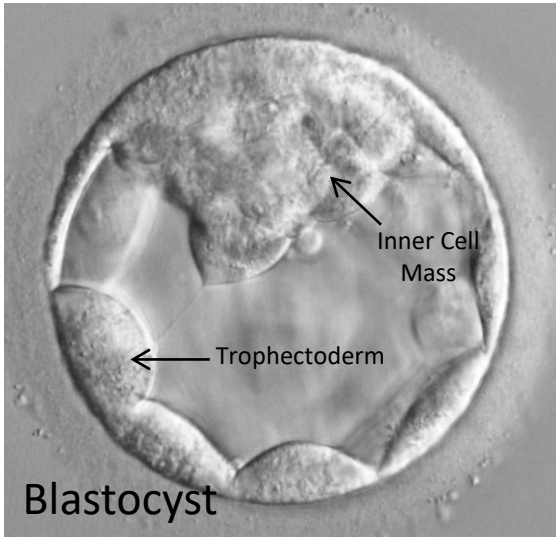
RNA-sequencing analysis of human preimplantation embryos provides a resource for identifying candidate developmental regulators







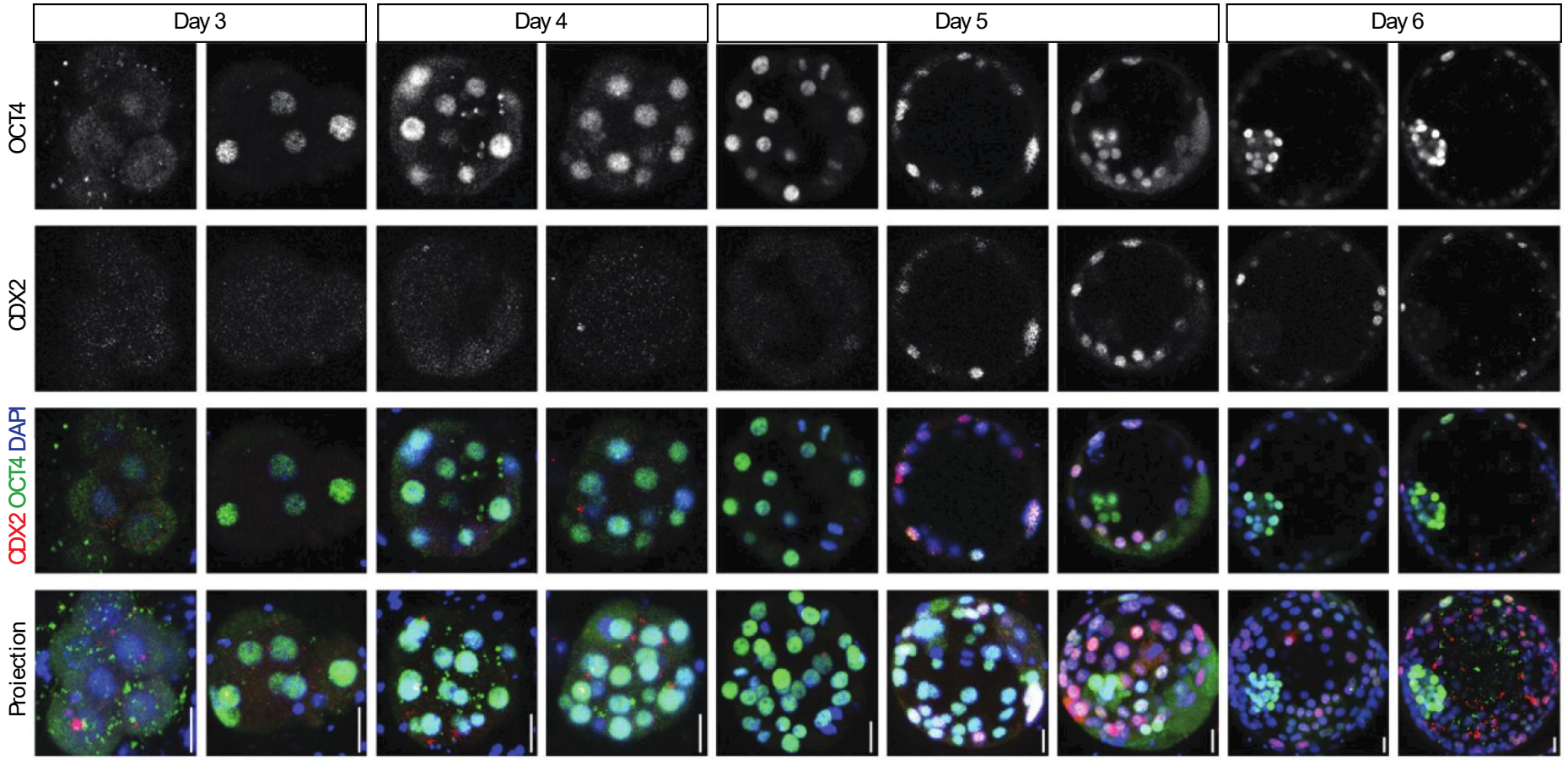




Blakeley, Fogarty* et al. Development 2015*

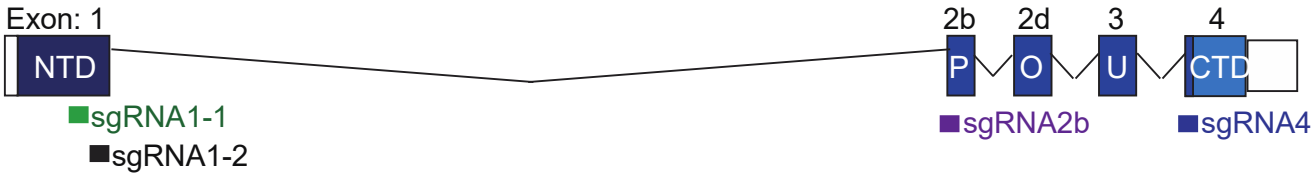
OCT4: Candidate for CRISPR/Cas9-mediated genome editing proof-of-principle experiments

Expressed from 8-cell stage in human embryos and required for pluripotency



Adapted from Niakan et al. (2013)

Selection criteria for candidate guide RNA design

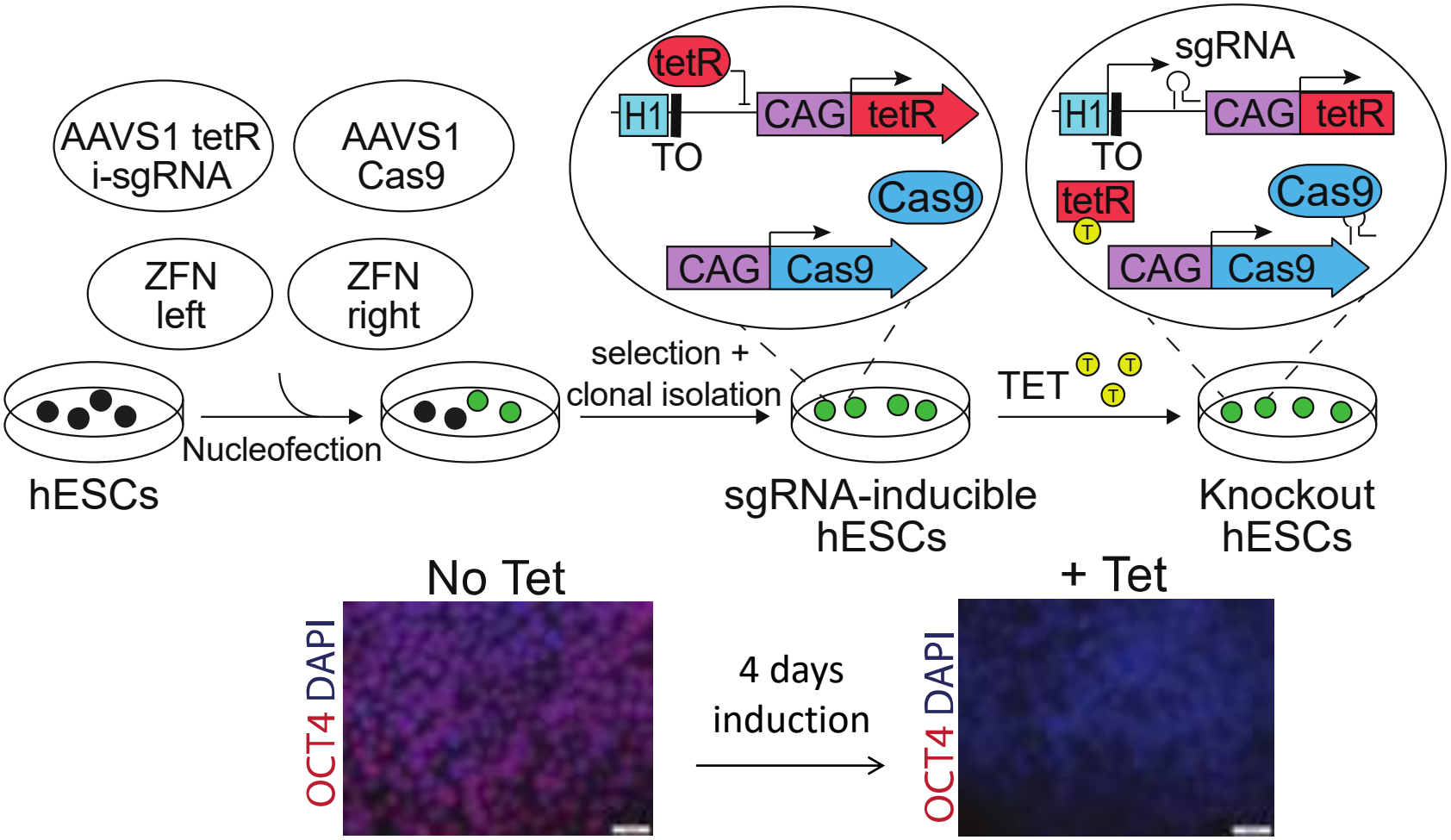


	sgRNA1-1	sgRNA1-2	sgRNA2b	sgRNA4
Human	CTTCACGGCACCAGGGGTGA CGG	GCACTAGCCCCACTCCAACC TGG	acccacCAAATAGAACCCCC AGG	acttgcagGTGGTCCGAGTG TGG
Mouse	CTTCACGGCATTGGGGCGGT CGG	GCAAAGTCTCCACGCCAACT TGG	acccacCAAAGAGAACGCCC AGG	catcctagGTGGTTCG AGTA TGG

Design Considerations:

- Target key motif or the N-terminal domain
- Sequence conservation of PAM and seed region
- Polymorphisms e.g. SNPs
- Pseudogenes
- Isoforms

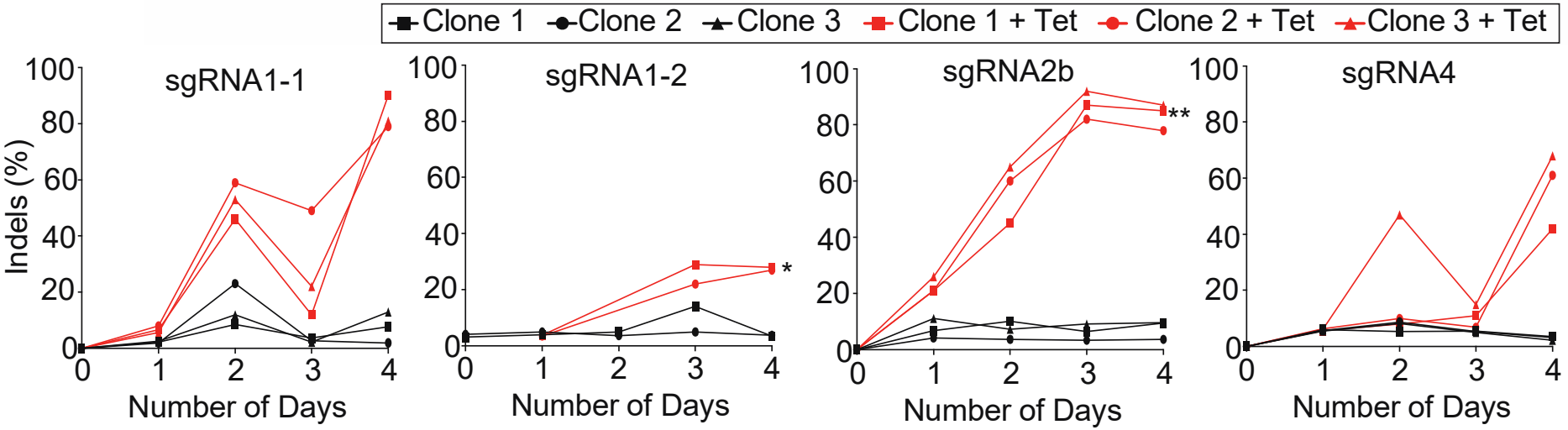
Identifying the most efficient method to inactivate OCT4 using inducible CRISPR/Cas9 System in human ESCs



- Constitutive expression of Cas9 and inducible sgRNA
- Generate isogenic clonal lines for comparative sgRNA analysis

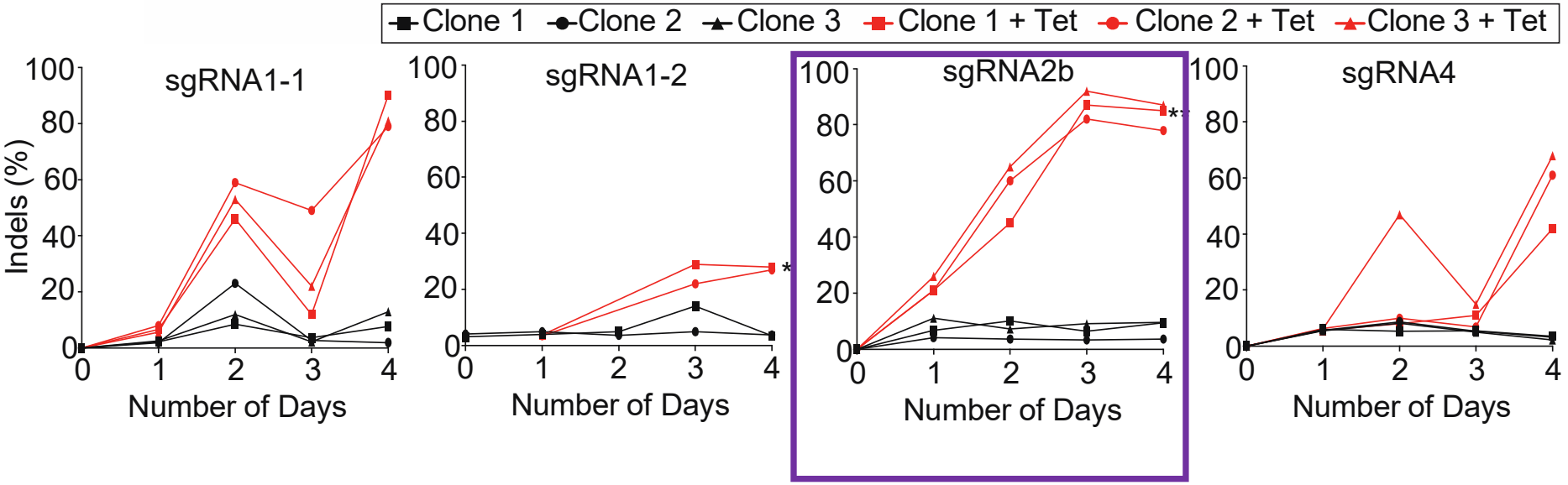
Bertero et al. 2017

Identifying the most efficient method to inactivate OCT4 using inducible CRISPR/Cas9 System in human ESCs



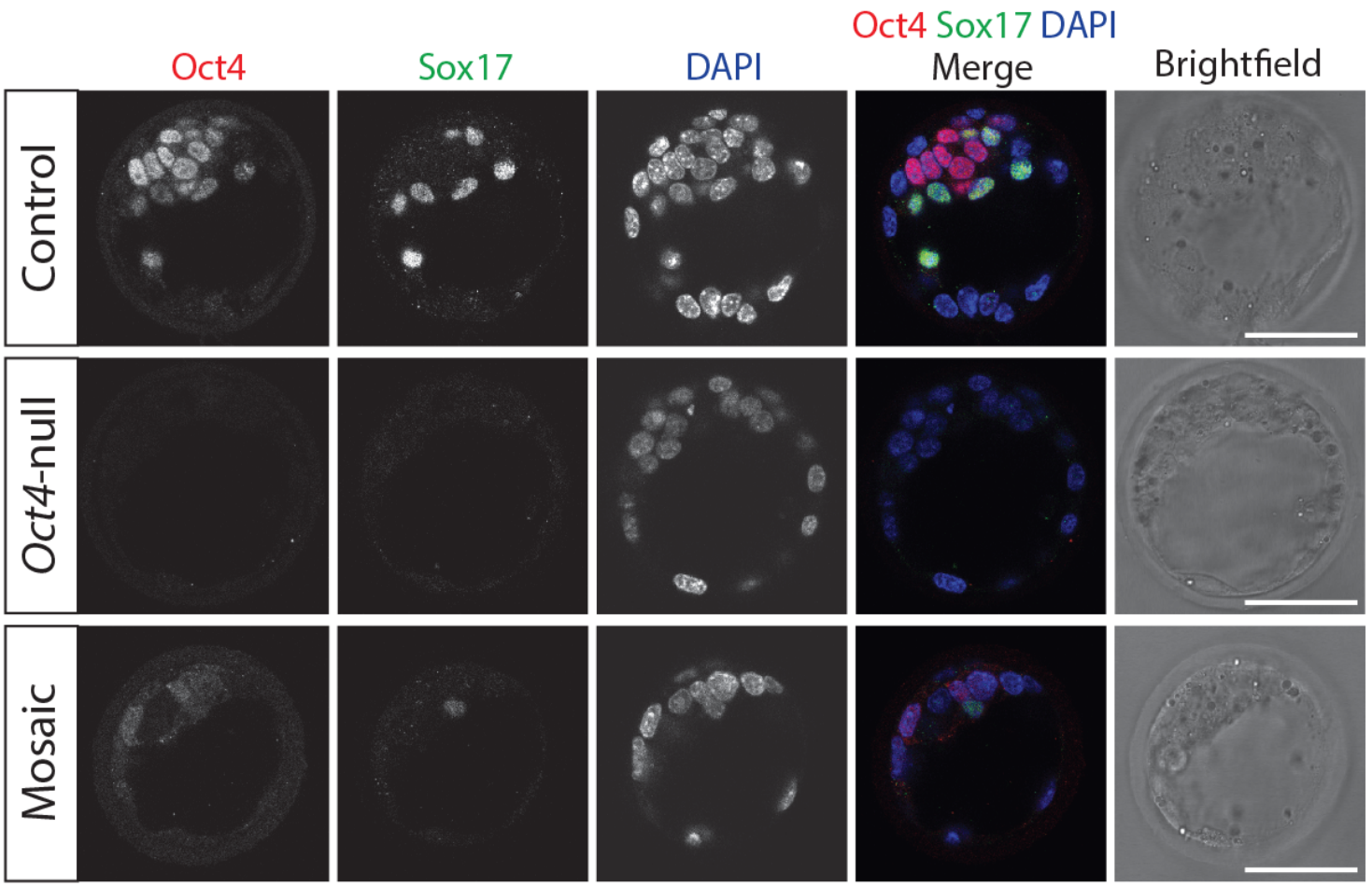

```
Reference:          AGGGGAACCCACCAAATAGAACCCCCAGGGTGAGC
2 bp deletion:      AGGGGAACCCACCAAATAGAACC--CAGGGTGAGC
1 bp deletion:      AGGGGAACCCACCAAATAGAACC-CCAGGGTGAGC
3 bp deletion:      AGGGGAACCCACCAAATAGAACC---AGGGTGAGC
1 bp insertion:     AGGGGAACCCACCAAATAGAACCCCCCAGGGTGAGC
                                          ↓
                                          C
```

Identifying the most efficient method to inactivate OCT4 using inducible CRISPR/Cas9 System in human ESCs

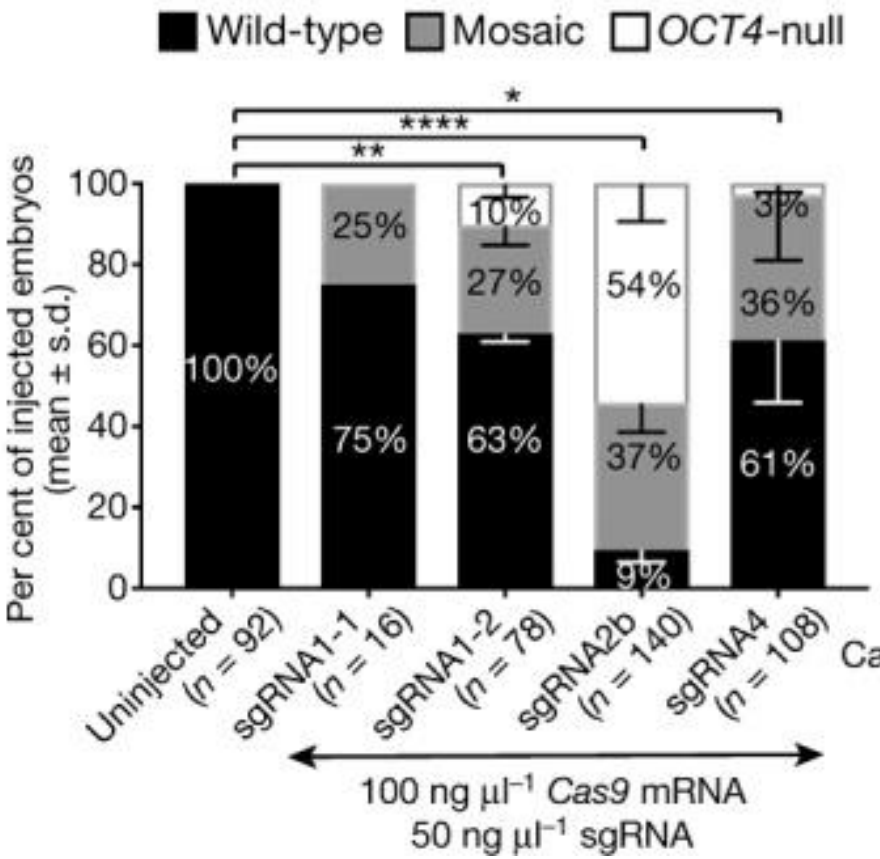


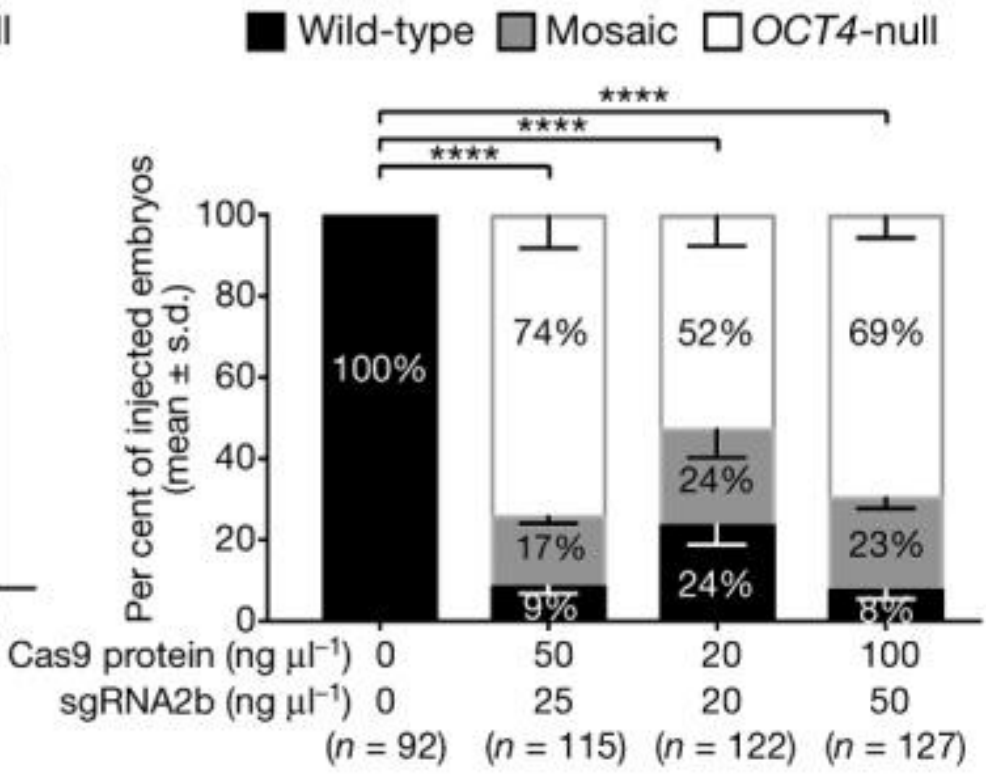

```
Reference:          AGGGGAACCCACCAAATAGAACCCCCAGGGTGAGC
2 bp deletion:      AGGGGAACCCACCAAATAGAACC--CAGGGTGAGC
1 bp deletion:      AGGGGAACCCACCAAATAGAACC-CCAGGGTGAGC
3 bp deletion:      AGGGGAACCCACCAAATAGAACC---AGGGTGAGC
1 bp insertion:     AGGGGAACCCACCAAATAGAACCCCCCAGGGTGAGC
                                          ↓C
```

OCT4 CRISPR/Cas9 targeted mouse embryos recapitulated the null mutation phenotype

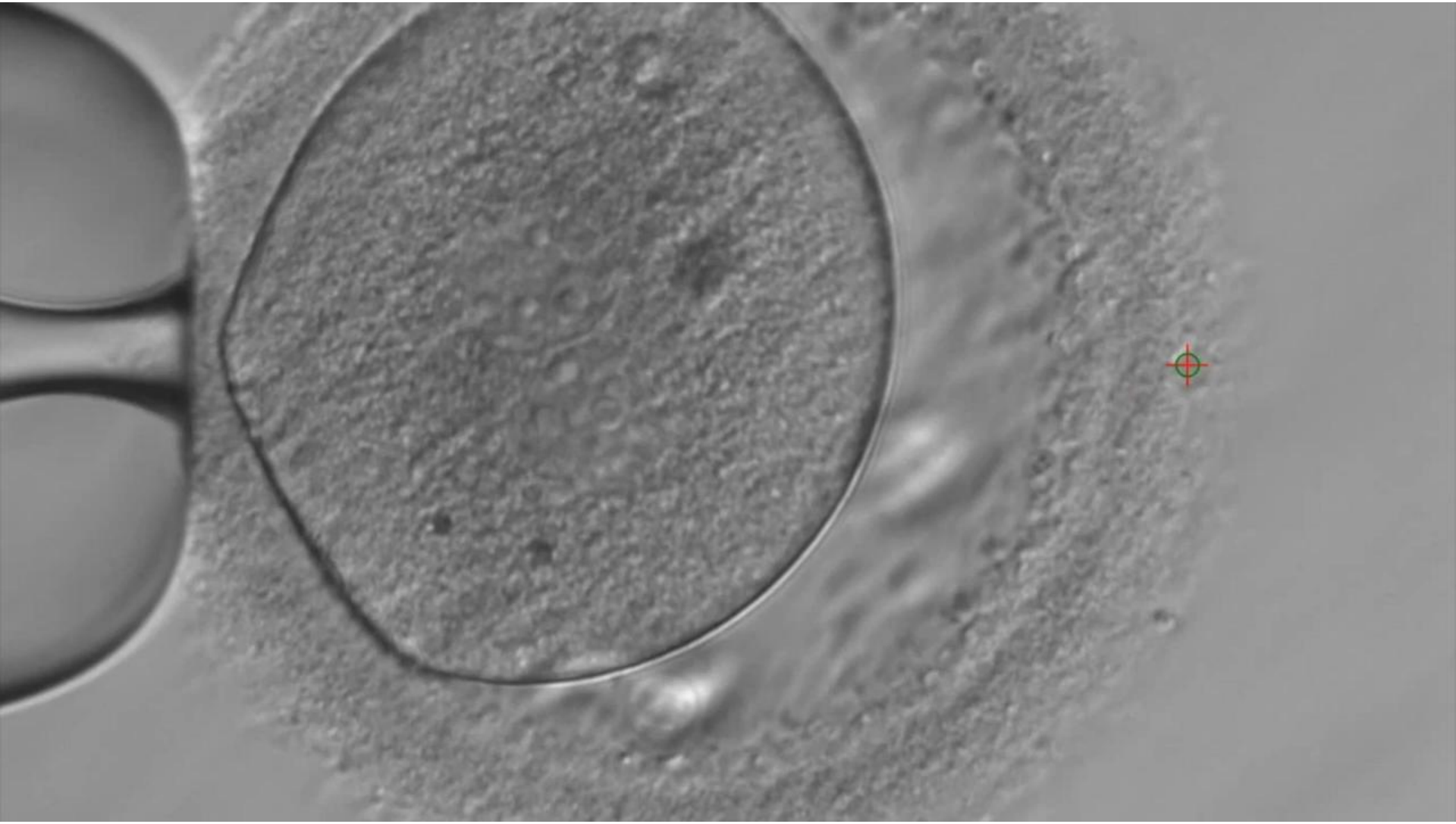


Assessing sgRNA activity and optimizing microinjection methodologies using mouse embryos as a model system



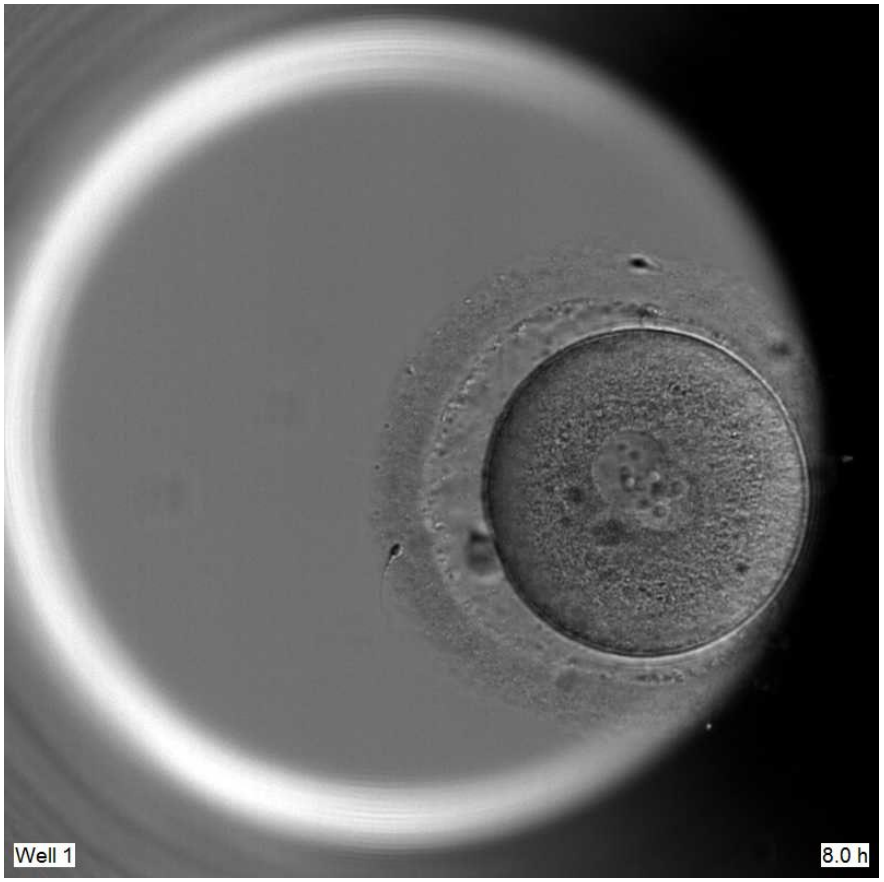


Targeting OCT4 in human embryos using pronuclear microinjection

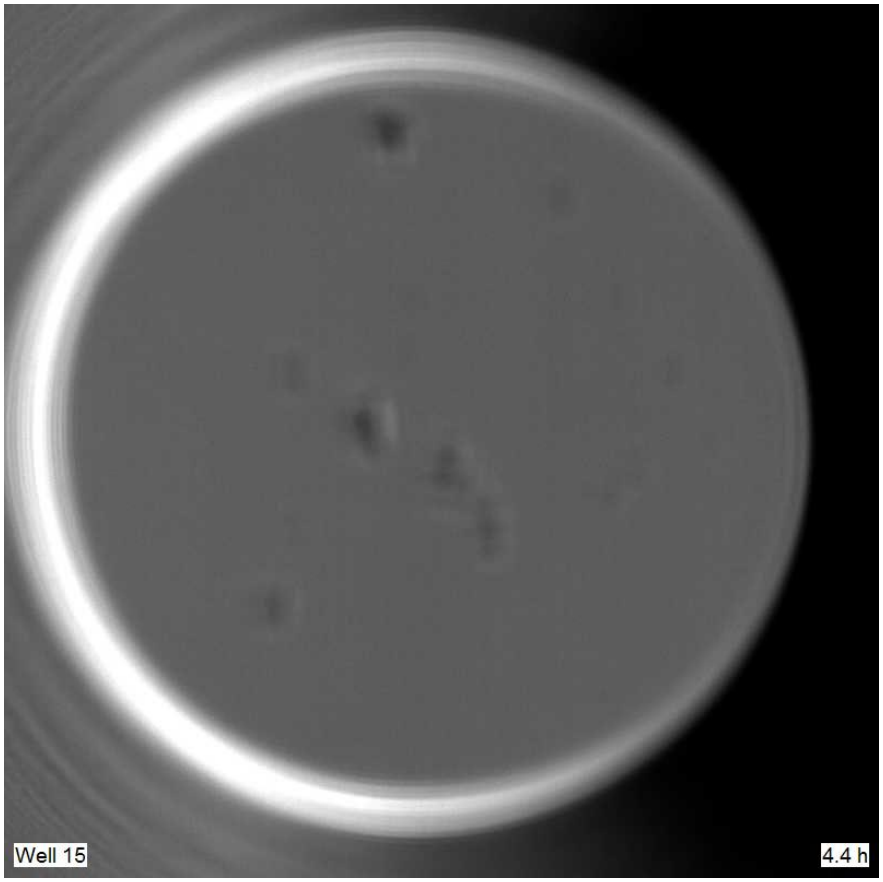


The developmental potential of human embryos is compromised following CRISPR/Cas9-targeting of OCT4

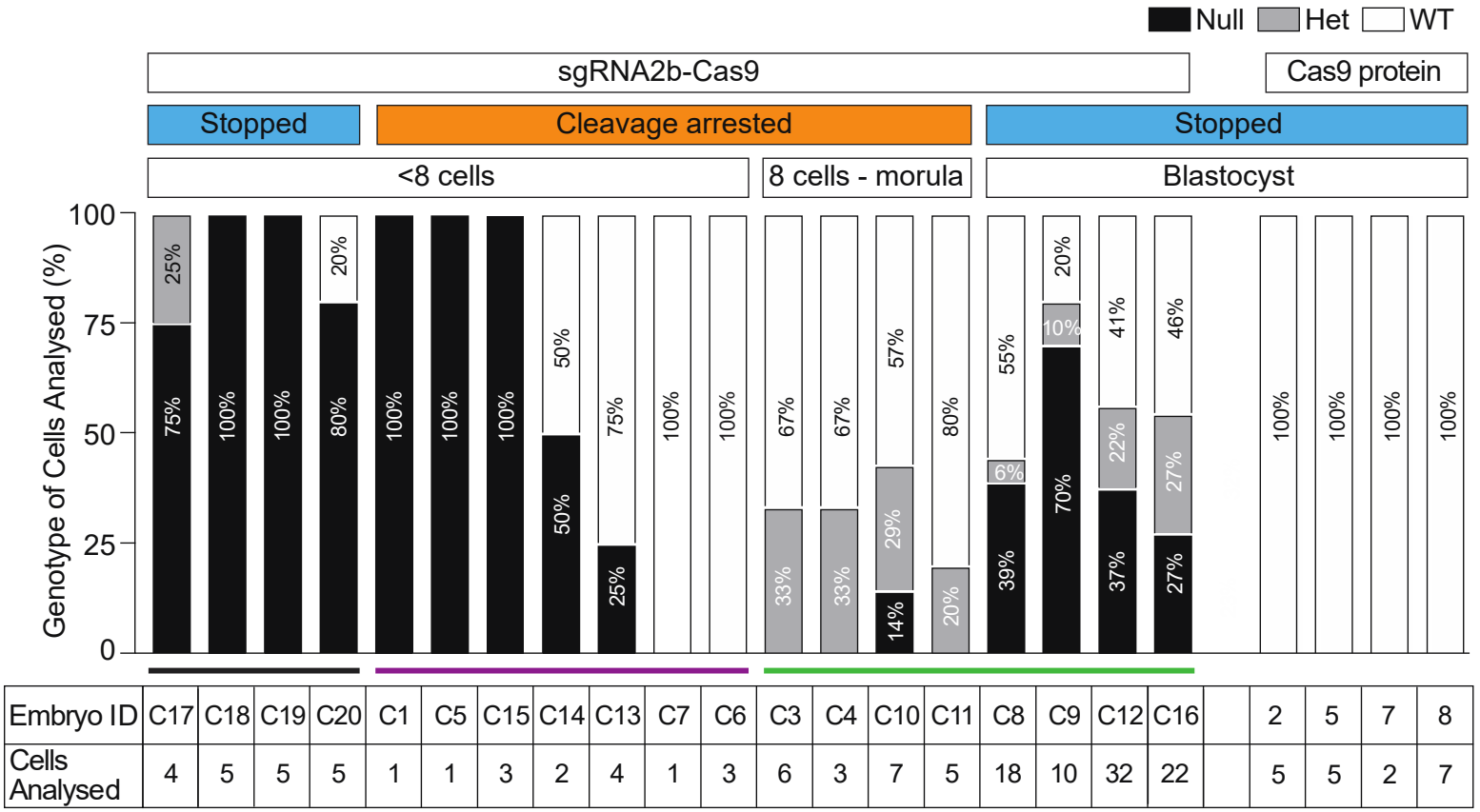
Cas9 only injected controls



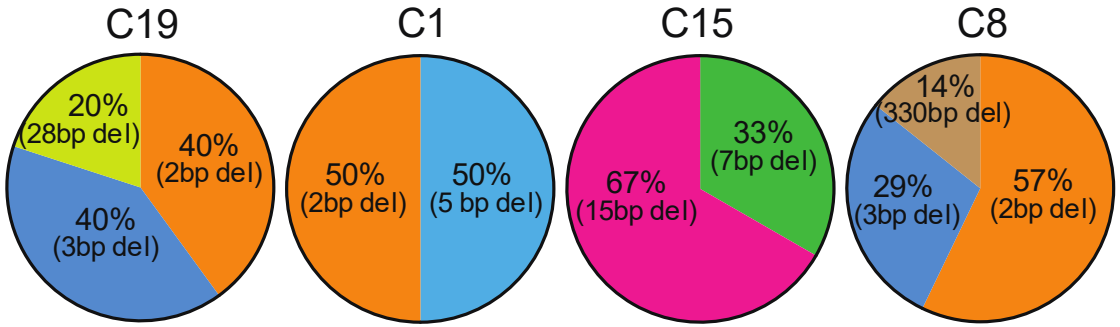
Cas9+guide RNA injected



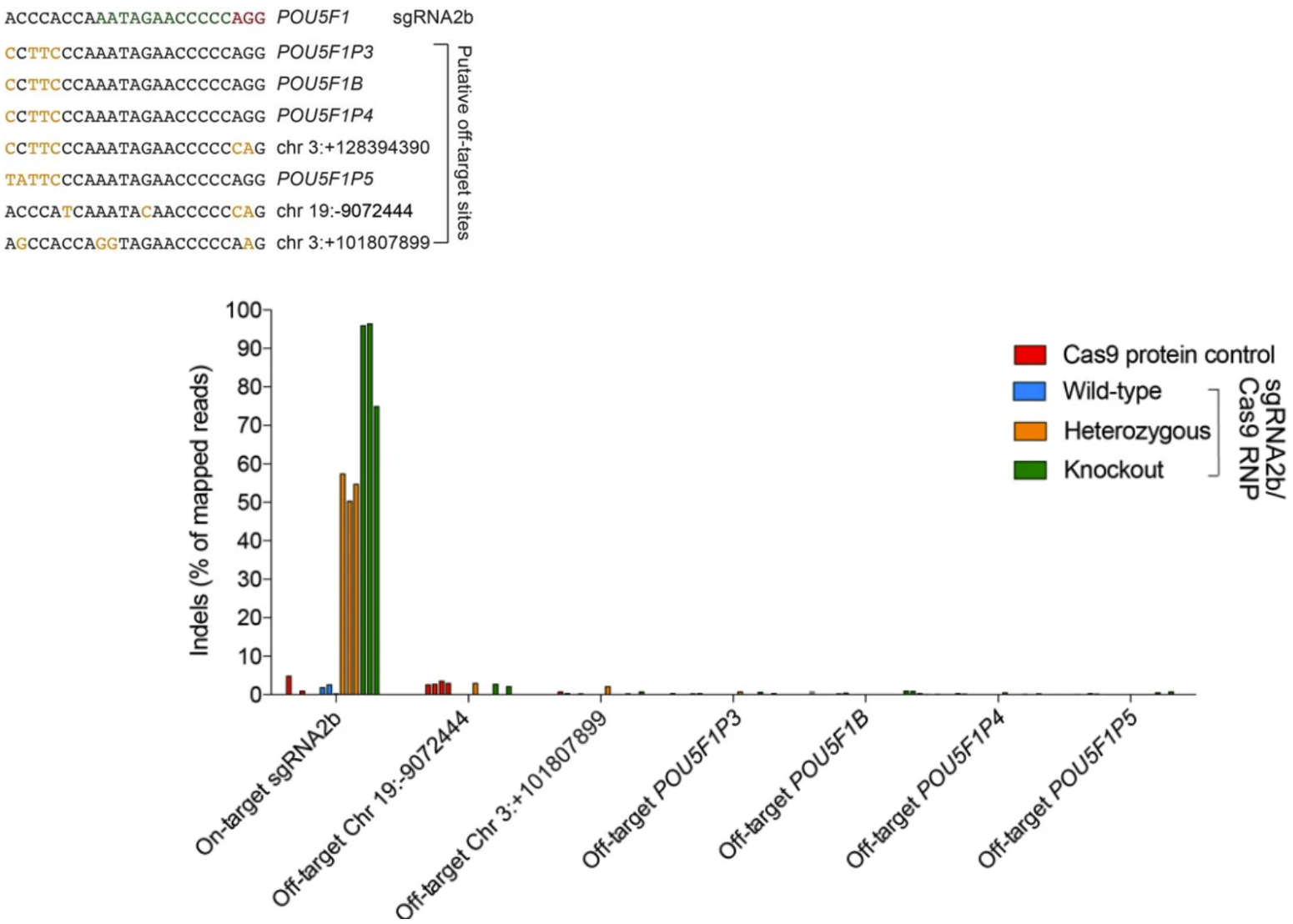
Only mosaic human embryos can make it to advanced stages of development



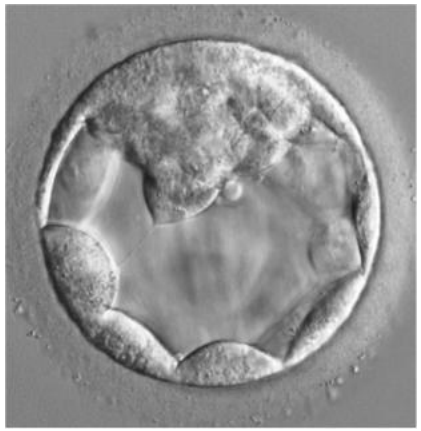
Embryo ID	C17	C18	C19	C20	C1	C5	C15	C14	C13	C7	C6	C3	C4	C10	C11	C8	C9	C12	C16		2	5	7	8
Cells Analysed	4	5	5	5	1	1	3	2	4	1	3	6	3	7	5	18	10	32	22		5	5	2	7

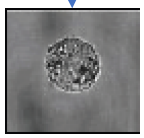


Off-target mutations were undetectable above background PCR error rates confirming the specificity of the sgRNA

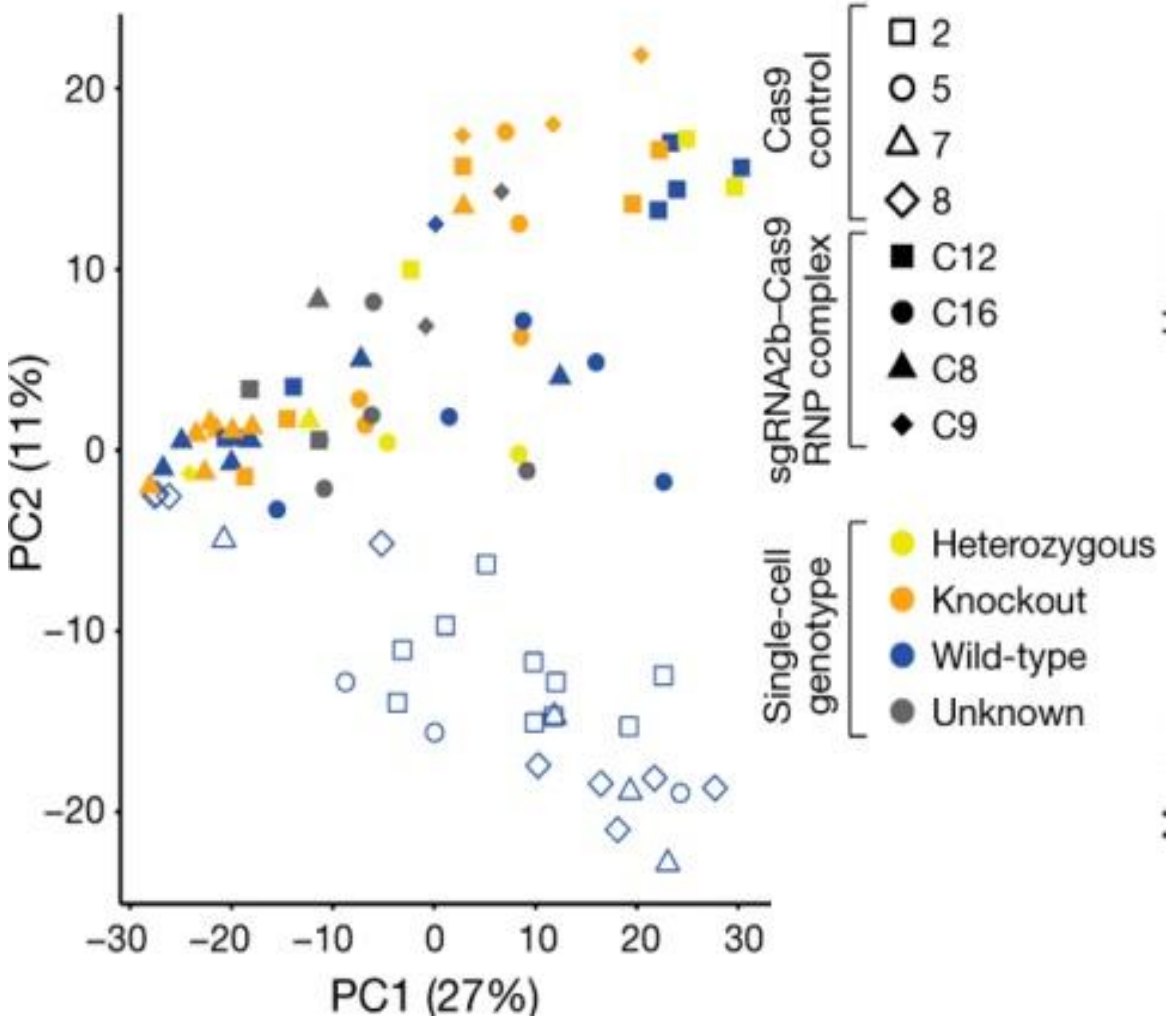


G&T-seq: loss of OCT4 is associated with gene mis-expression in all three lineages

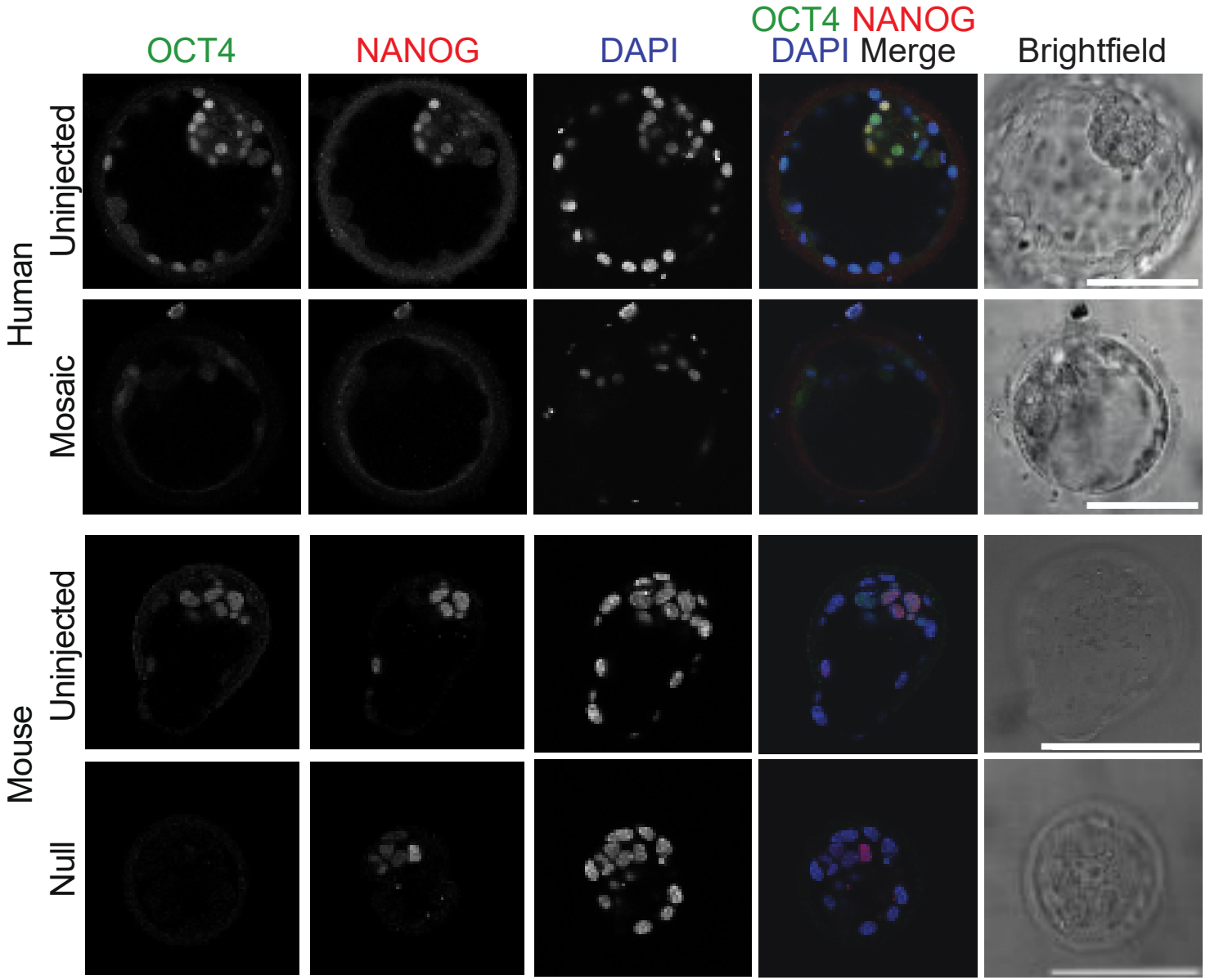




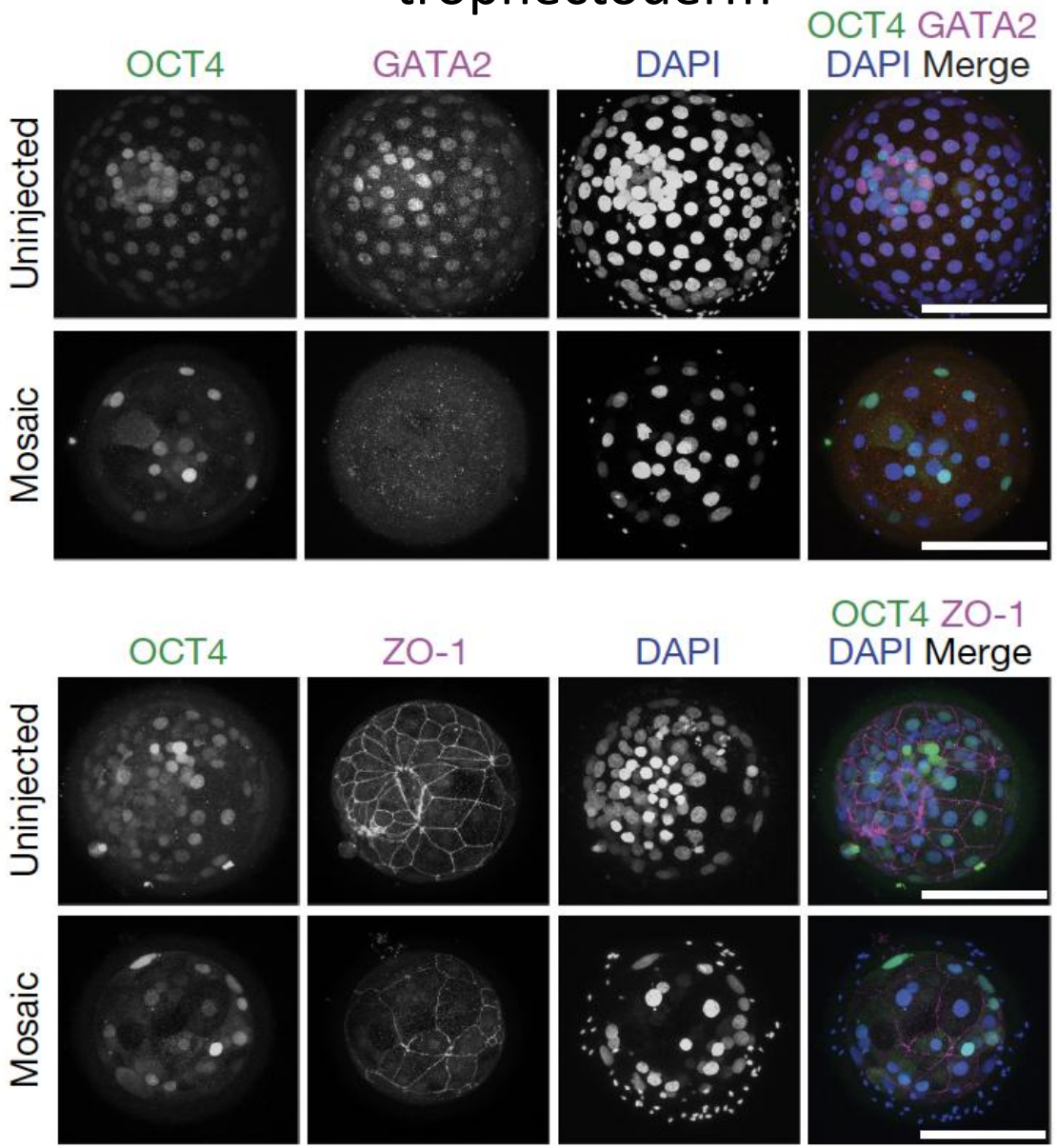
RNA DNA



NANOG expression is lost in OCT4-targeted human embryos but is not affected in mouse embryos



Disruption of OCT4 in the human embryo negatively affects the trophectoderm

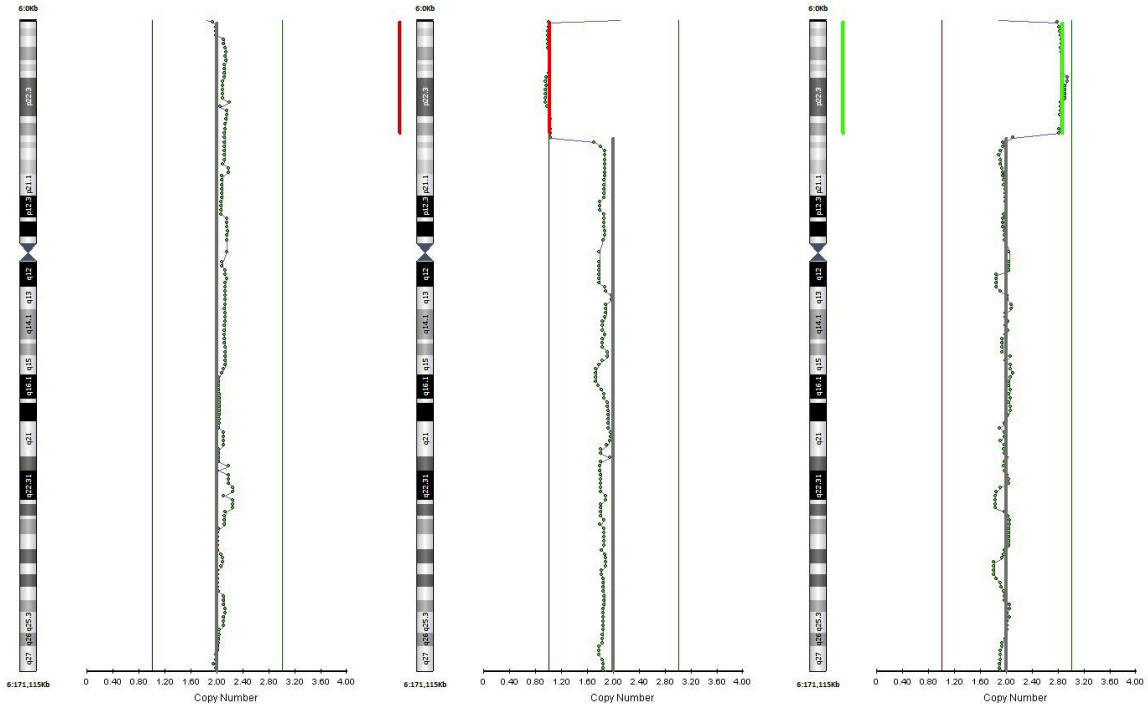


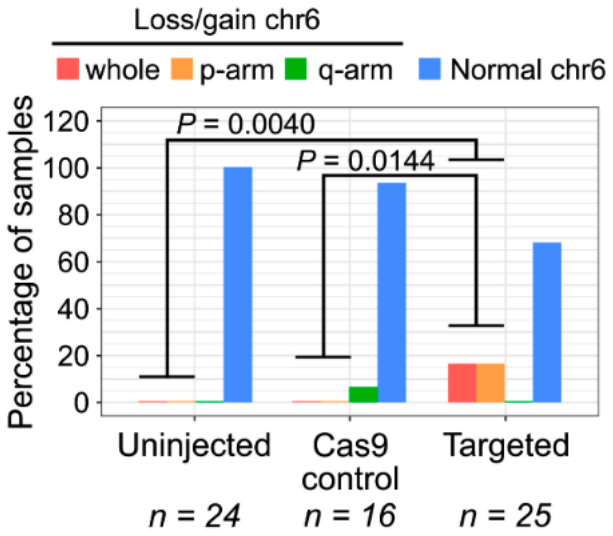
Single cell WGS reveals segmental loss or gains of Chr6 in response to CRISPR/Cas9 targeting

Normal chr6

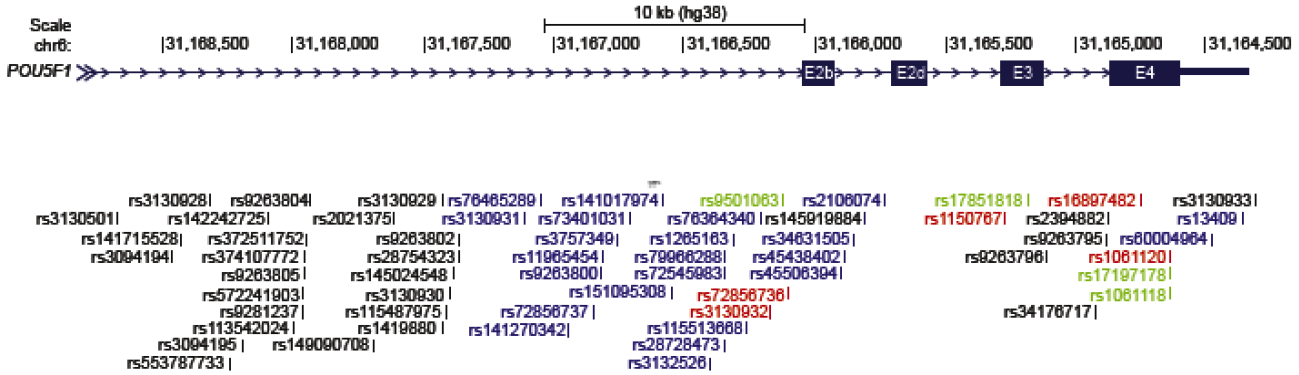
Segmental loss chr6 -6p22.3 (29.1Mb)

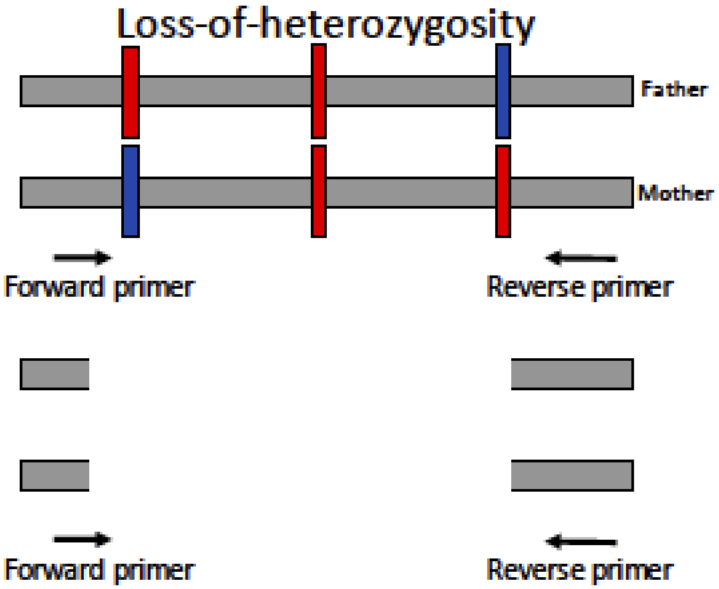
Segmental loss chr6 +6p22.3 (29.1Mb)

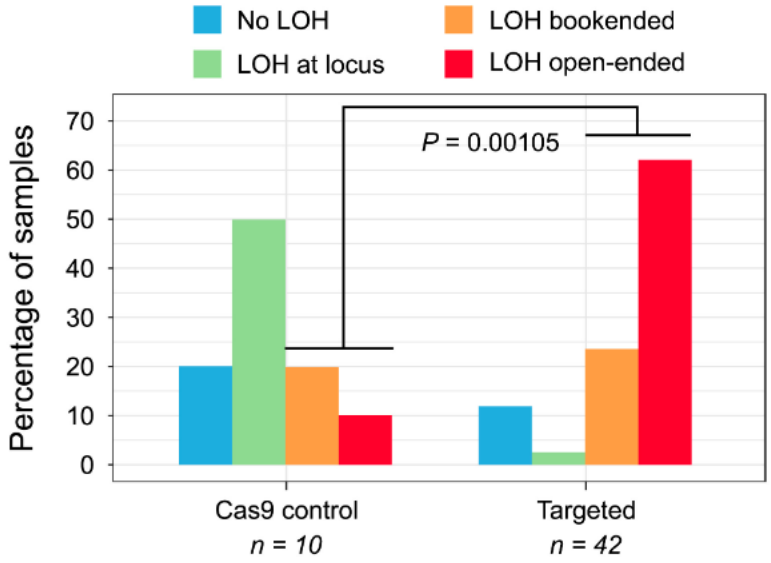




Frequent loss of heterozygosity is observed in CRISPR/Cas9-edited early human embryos



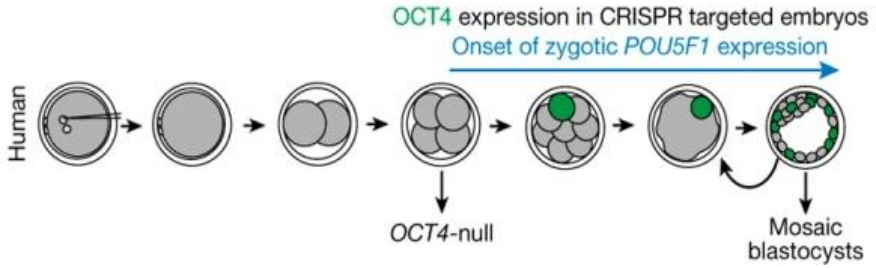




Summary

- Human ES cells can be used to screen sgRNAs for efficiency and mutation spectrum prior to use in human embryos

- OCT4 is required earlier in human development than in the mouse



- OCT4 has a cell non-autonomous role in embryogenesis and disruption causes misexpression of genes in all three lineages in human embryos

- Large deletions highlight need for more basic research to assess safety of genome editing in human embryos

- CRISPR/Cas9 can be applied as a tool to study gene function in human preimplantation development

Acknowledgements

The Francis Crick Institute
Human Embryo and Stem Cell Lab
Kathy Niakan
Sissy Wamaitha
Afshan McCarthy
Rebecca Lea
Paul Blakeley

Sex Chromosome Biology Lab
James Turner
Ben Powell
Valdone Maciulyte

Bioinformatics Facility
Jens Kleinjung

Advanced Sequencing Facility

University of Cambridge
Ludovic Vallier
Kirsten Snijders
Alessandro Bertero

Bourn Hall
Kay Elder

University of Oxford
Dagan Wells
Nada Kubikova

Seoul National University
Jin-Soo Kim
Deasik Kim