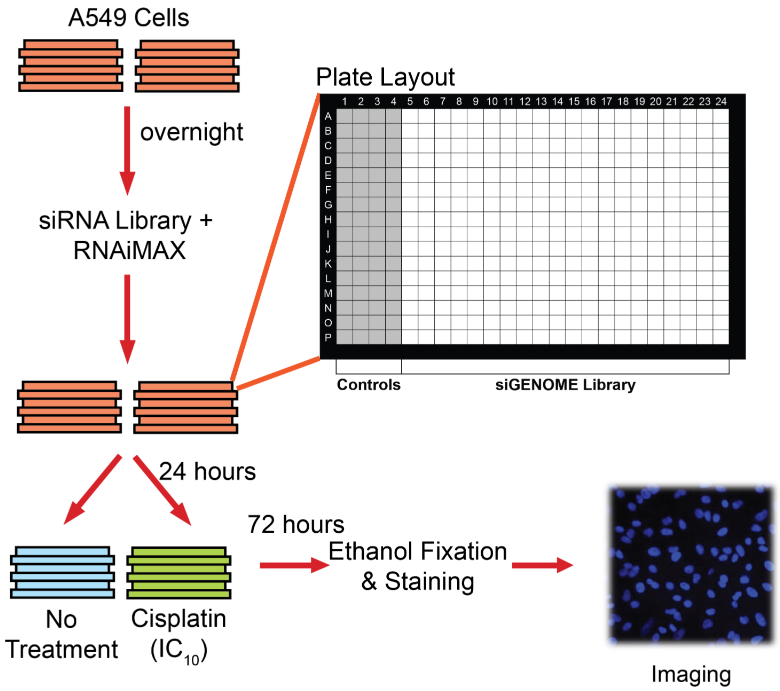
Chromosome-wide genetic manipulations

Jiang-An Yin

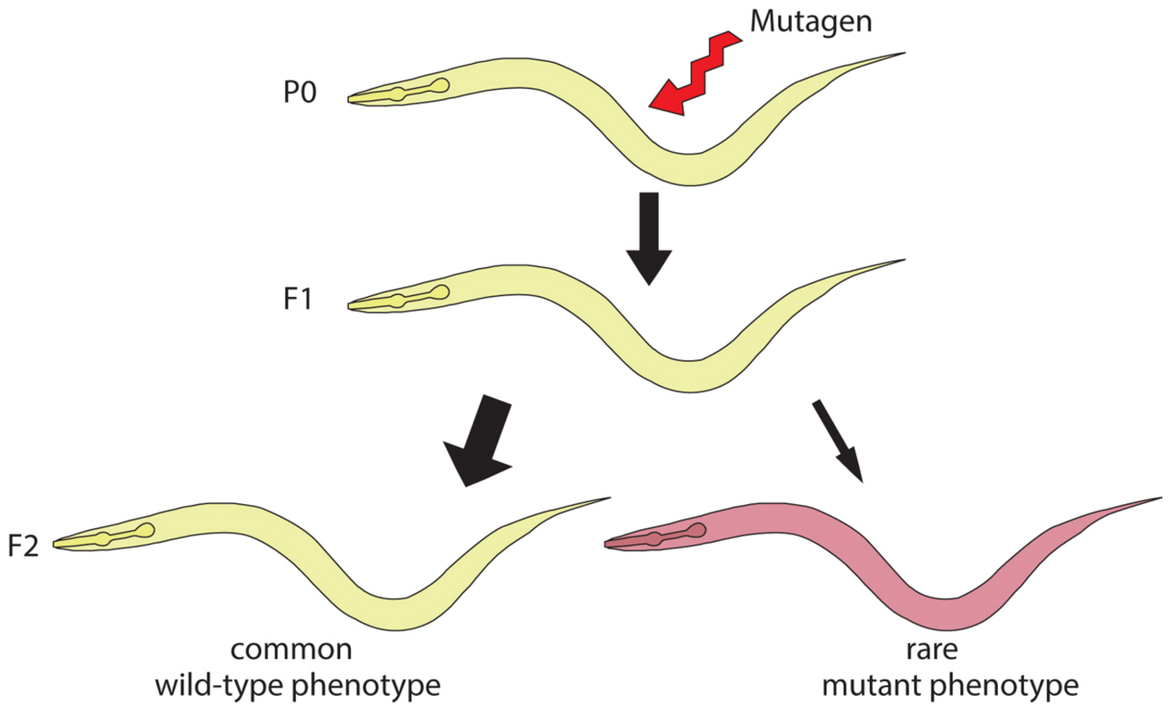
2021-05-04

Traditional genetic perturbations at the gene level

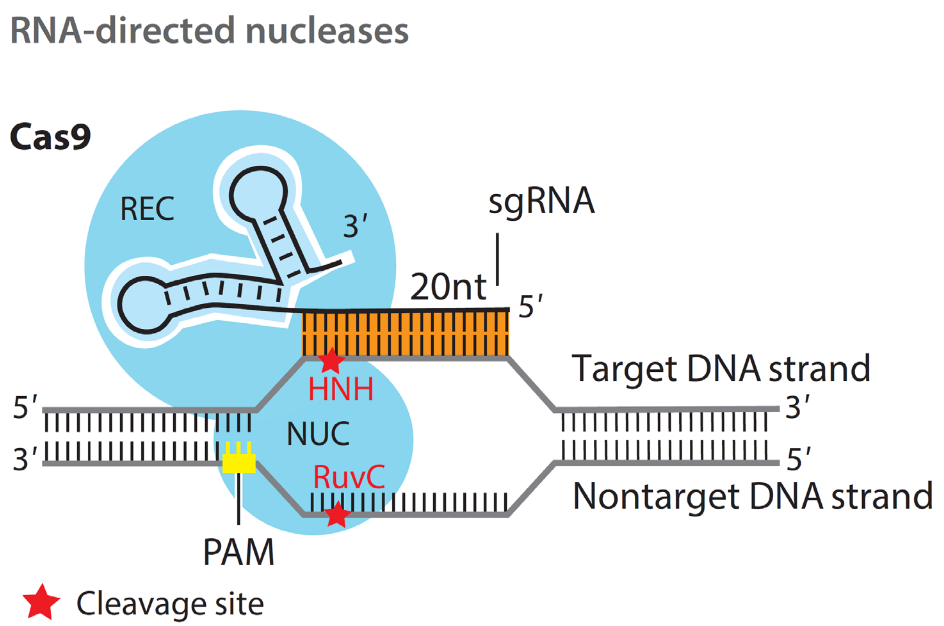
siRNA Screen or ORF

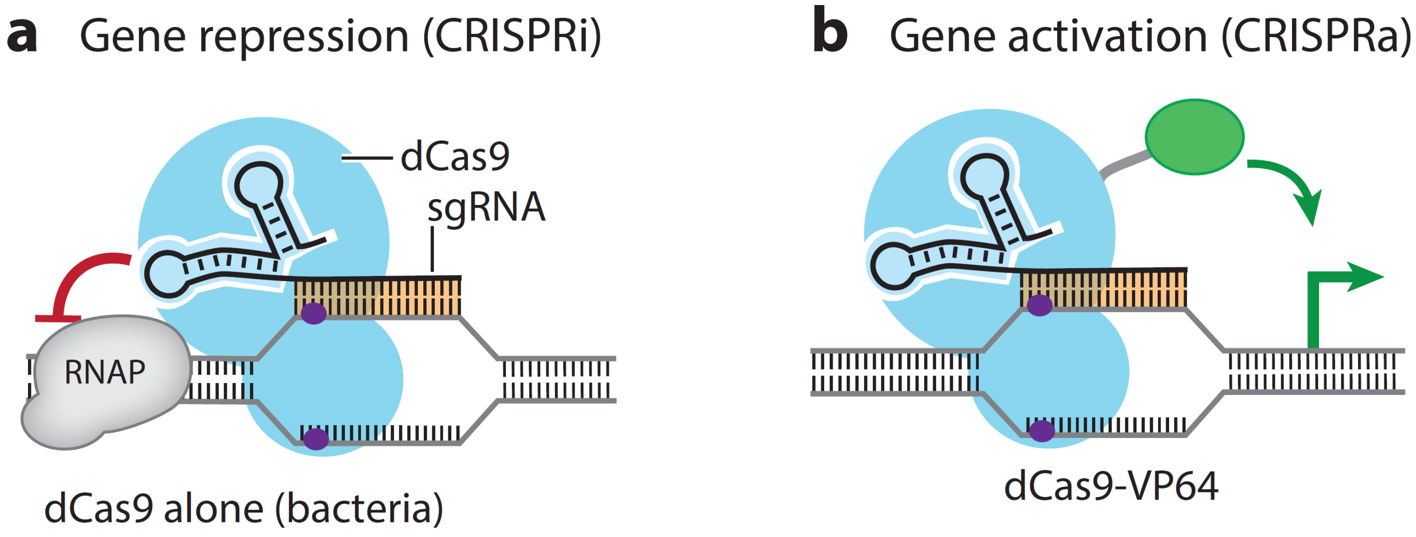


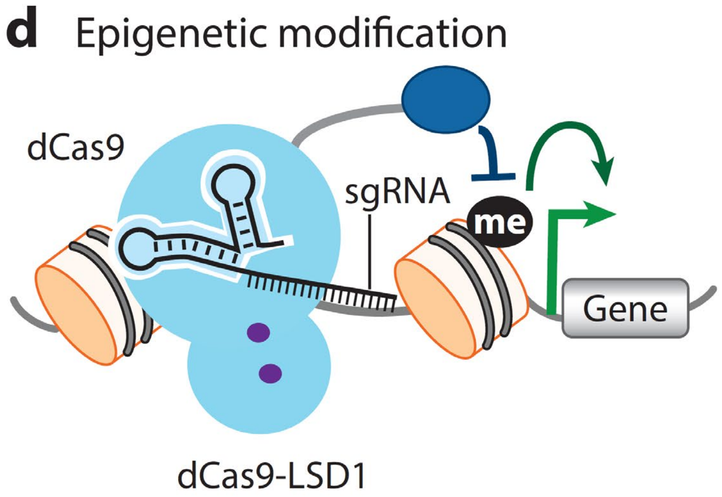
Mutagenesis

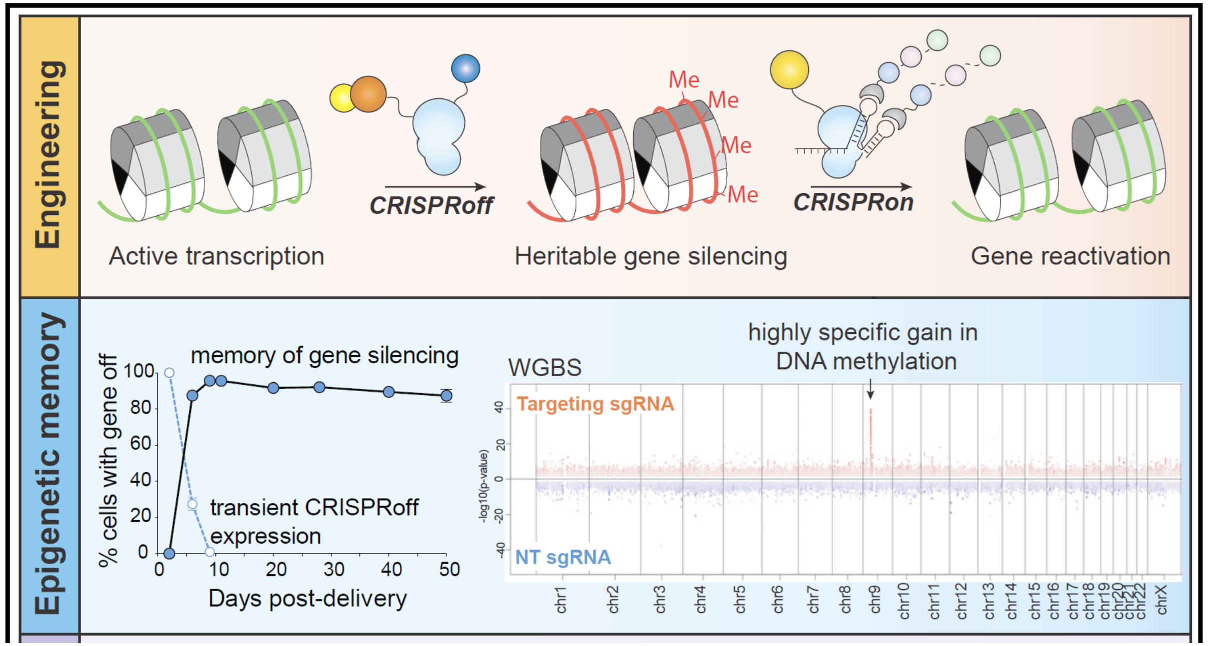


CRISPR-nuclease-based gene perturbation



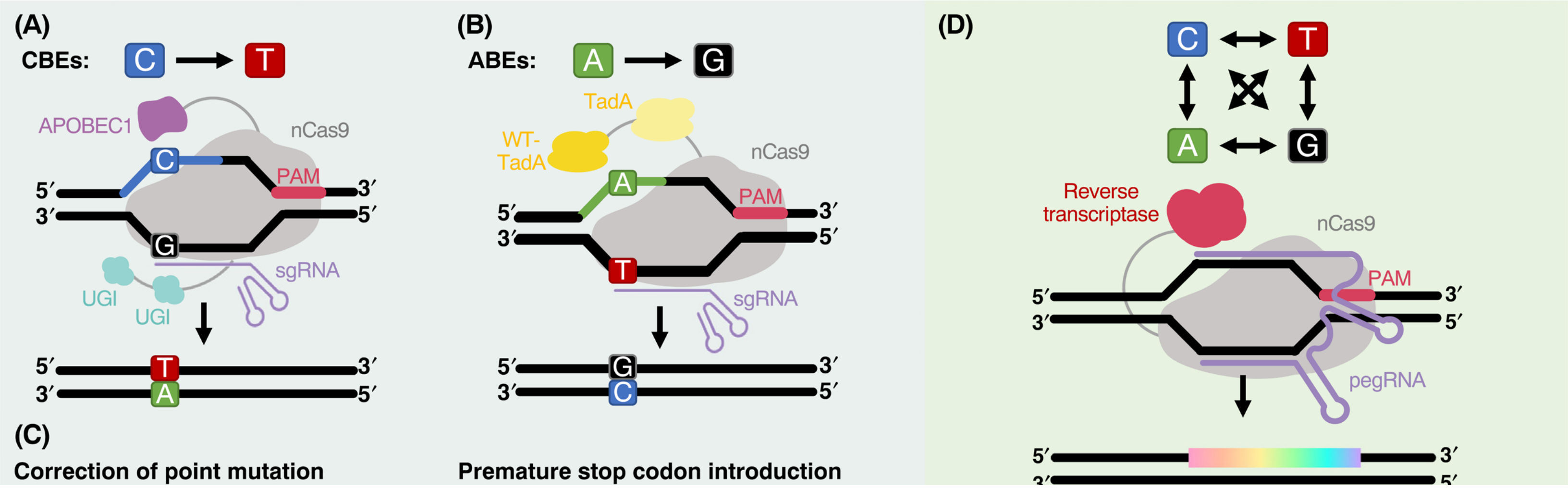


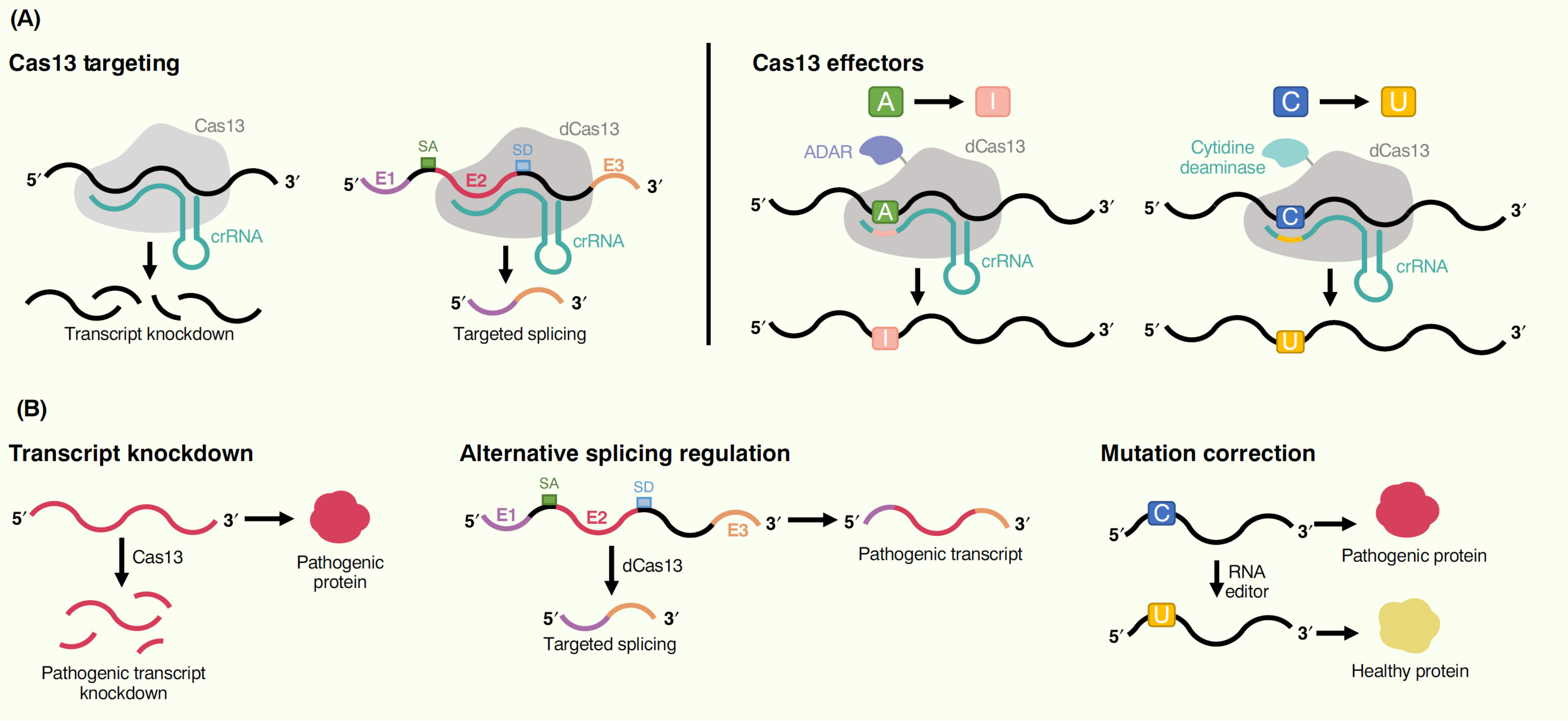




Wang et.al, *Annu. Rev. Biochem.* 2016. 85:227–64.

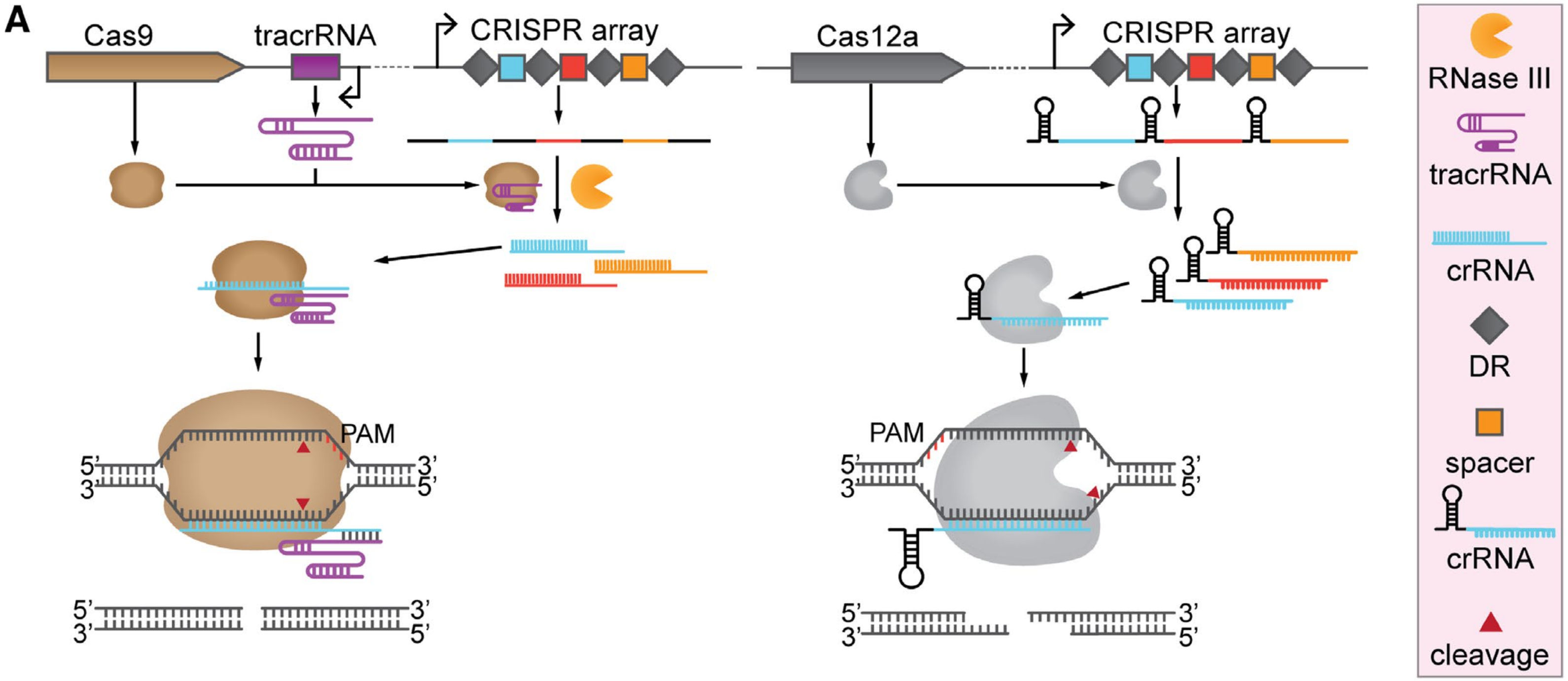
CRISPR base and RNA editors





Trends in Biotechnology

Multiplexed and megabase genetic “perturbations”



ARTICLE

https://doi.org/10.1038/s41467-019-09006-2 OPEN

CRISPR-Cas9 genome editing induces megabase-scale chromosomal truncations

Grégoire Cullot[1,2], Julian Boutin[1,2,3], Jérôme Toutain[4], Florence Prat[1,2], Perrine Pennamen[4], Caroline Rooryck[4],

Aneuploidy, an incorrect number of chromosomes, is the leading cause of miscarriages and mental retardation in humans and is a hallmark of cancer. ... **Immortalization**, the acquisition of the ability to proliferate indefinitely, was also **affected** by the presence of an additional copy of certain chromosomes.

Human trisomy [edit]

Trisomies can occur with any chromosome, but often result in miscarriage, rather tl
pregnancies, occurring in more than 1% of pregnancies; only those pregnancies in
survive.[3] This condition, however, usually results in spontaneous miscarriage in tl

The most common types of autosomal trisomy that survive to birth in humans are:

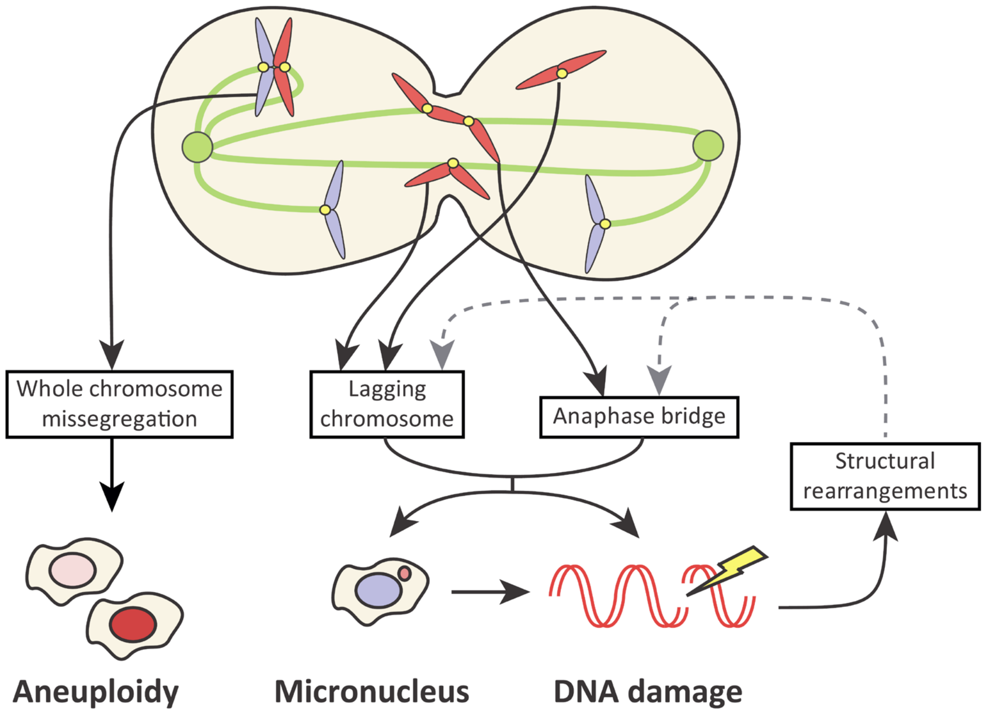
- Trisomy 21 (Down syndrome)
- Trisomy 18 (Edwards syndrome)
- Trisomy 13 (Patau syndrome)
- Trisomy 9
- Trisomy 8 (Warkany syndrome 2)

Of these, Trisomy 21 and Trisomy 18 are the most common. In rare cases, a fetus associated with birth defects, intellectual disability and shortened life expectancy.

Trisomy of sex chromosomes can also occur and include:[4]

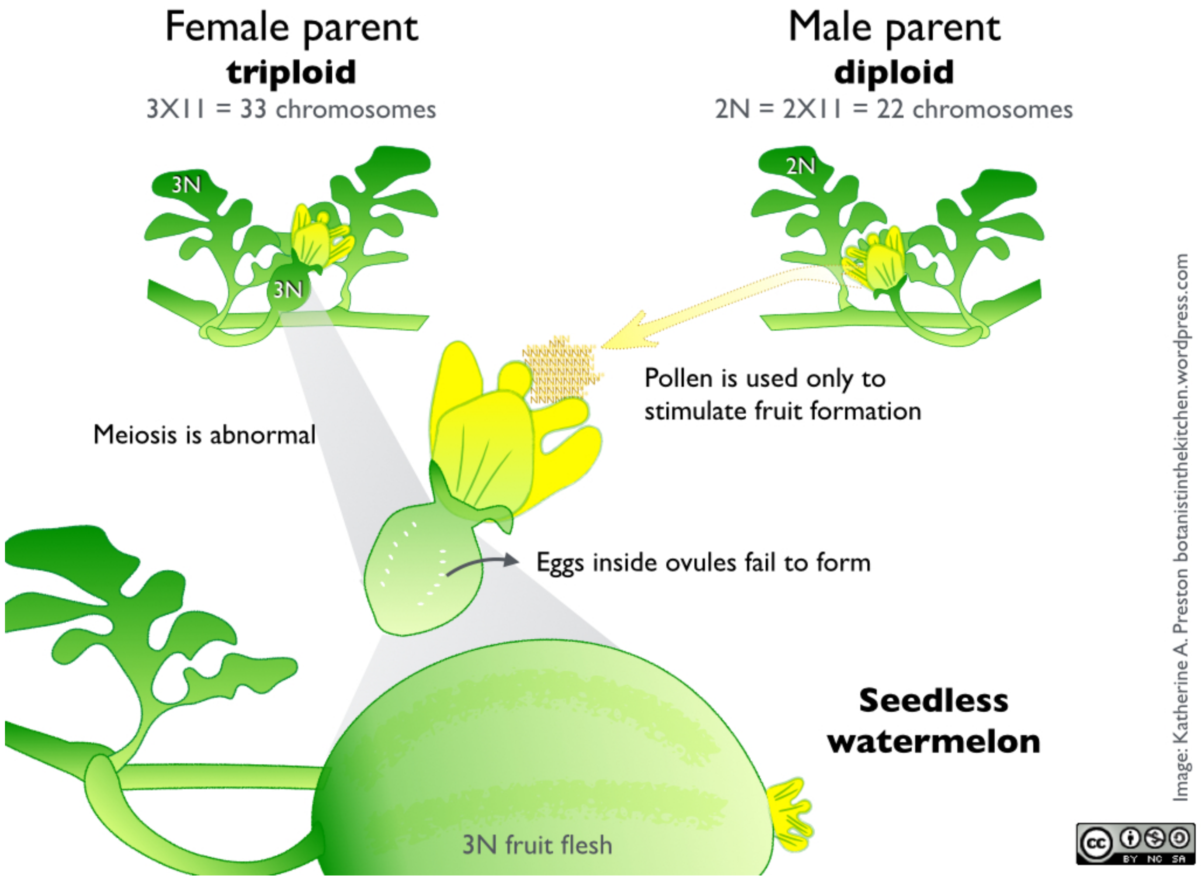
- XXX (Triple X syndrome)
- XXY (Klinefelter syndrome)
- XYY

Down syndrome causes a mental handicap. It may be mild or severe. The average IQ of a young adult with Down syndrome is 50, equivalent to the mental age of an 8- or 9-year-old child, but it truly depends on the person.



How about the chromosomal level manipulations?

Chromosomal set manipulations



Box 6.1 Glossary of terms

- **Androgenesis:** A form of asexual reproduction in which embryos develop without any genetic contribution of egg nucleus and, consequently, progeny have paternally derived genotypes
- **Diploid:** Cells or individuals with two sets of homologous chromosomes, one set from mother (egg) and another set from father (sperm), and thus is shown by the symbol $2n$. A state of diploid condition of cells or individuals is referred to as *diploidy*.
- **Doubled haploid**: Cells or individuals with completely homozygous diploid genotypes, comprising two identical sets of haploid chromosomes.
- **Gynogenesis:** A form of asexual reproduction in which development occurs without any genetic contribution of the father, but it requires sperm to trigger development.
- **Haploid:** Cells or individuals with a single set of chromosomes, and thus is shown by the symbol n. A state of haploid condition of cells or individuals is referred to as *haploidy*.
- **Hybrid:** Progeny with chromosome sets from maternal and paternal species (strains) after fertilization between two different species (strains).
- **Polyploid:** Cells or individuals with extra set(s) of chromosomes are collectively referred to as polyploid. A state of polyploid condition of cells or individuals is referred to as *polyploidy*. Polyploid cells or individuals are described, according to the number of chromosome sets that they have, as: *triploid* (3 sets, $3n$); *tetraploid* (4 sets, $4n$); *pentaploid* (5 sets, 5n); *hexaploid* (6 sets, 6n); *heptaploid* (7 sets, 7n); *octaploid* (8 sets, $8n$); *nanoploid* (9 sets, $9n$); *decaploid* (10 sets, $10n$) and so on. *Autopolyploid* (ex. *autotriploid*) includes homospecific homologous chromosome sets, while *allopolyploid* (ex. *allotriploid*) includes at least one set of heterospecific non-homologous chromosomes.

Two ways of chromosome-wide manipulations in mammals

Silencing of trisomic chromosome

ARTICLE

doi:10.1038/nature12394

Translating dosage compensation to trisomy 21

Jun Jiang[1], Yuanchun Jing[1], Gregory J. Cost[2], Jen-Chieh Chiang[1], Heather J. Kolpa[1], Allison M. Cotton[3], Dawn M. Carone[1], Benjamin R. Carone[1], David A. Shivak[2], Dmitry Y. Guschin[2], Jocelynn R. Pearl[2], Edward J. Rebar[2], Meg Byron[1], Philip D. Gregory[2], Carolyn J. Brown[3], Fyodor D. Urnov[2], Lisa L. Hall[1] & Jeanne B. Lawrence[1]

Removal of chromosomes

CRISPR/Cas9-mediated targeted chromosome elimination

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Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos

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Removal of chromosomes

Trisomy Correction in Down Syndrome Induced Pluripotent Stem Cells

Crc ssell[1,2,*]

CRISPR/Cas9-mediated targeted chromosome elimination

Erwei Zuo[1†], Xiaona Huo[1†], Xuan Yao[1†], Xinde Hu[1†], Yidi Sun[3†], Jianhang Yin[2†], Bingbing He[1,4], Xing Wang[1,4], Linyu Shi[1], Jie Ping[5], Yu Wei[1,6], Wenqin Ying[1], Wei Wei[1,7], Wenjia Liu[1], Cheng Tang[1], Yixue Li[3], Jiazhi Hu[2*] and Hui Yang[1*]

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Translating dosage compensation to trisomy 21

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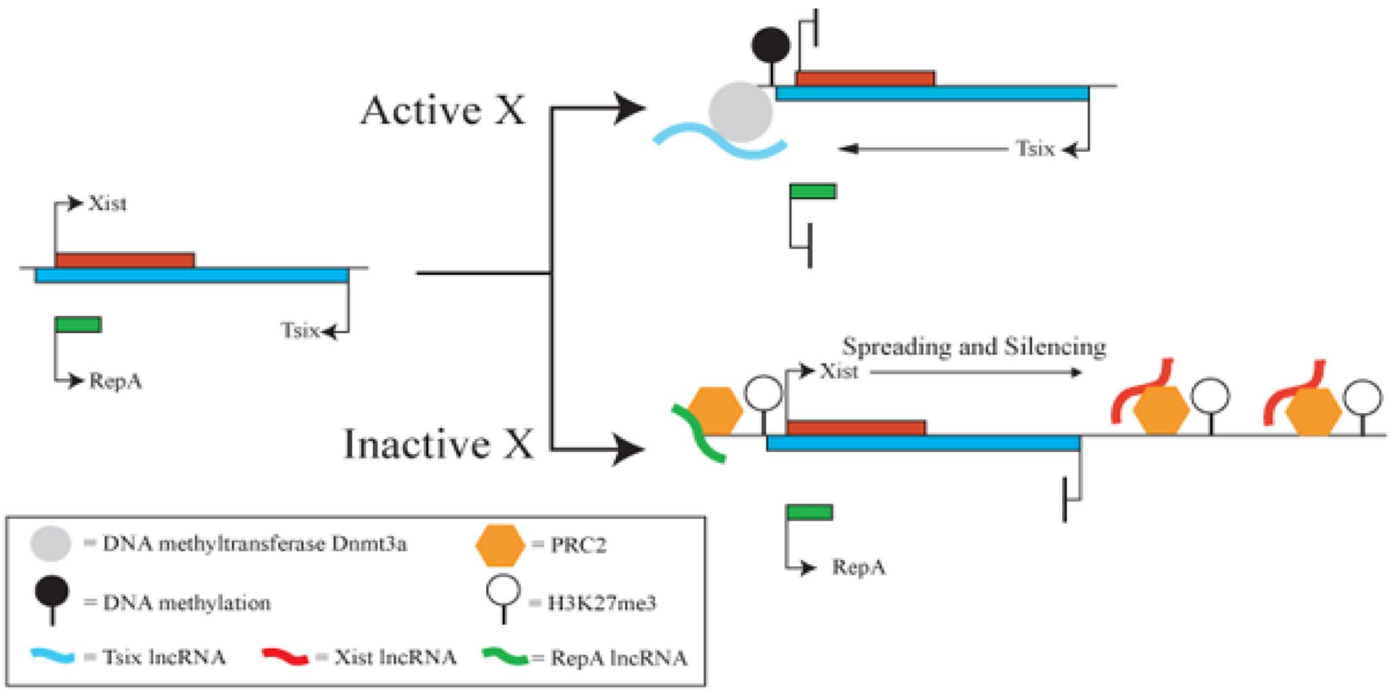
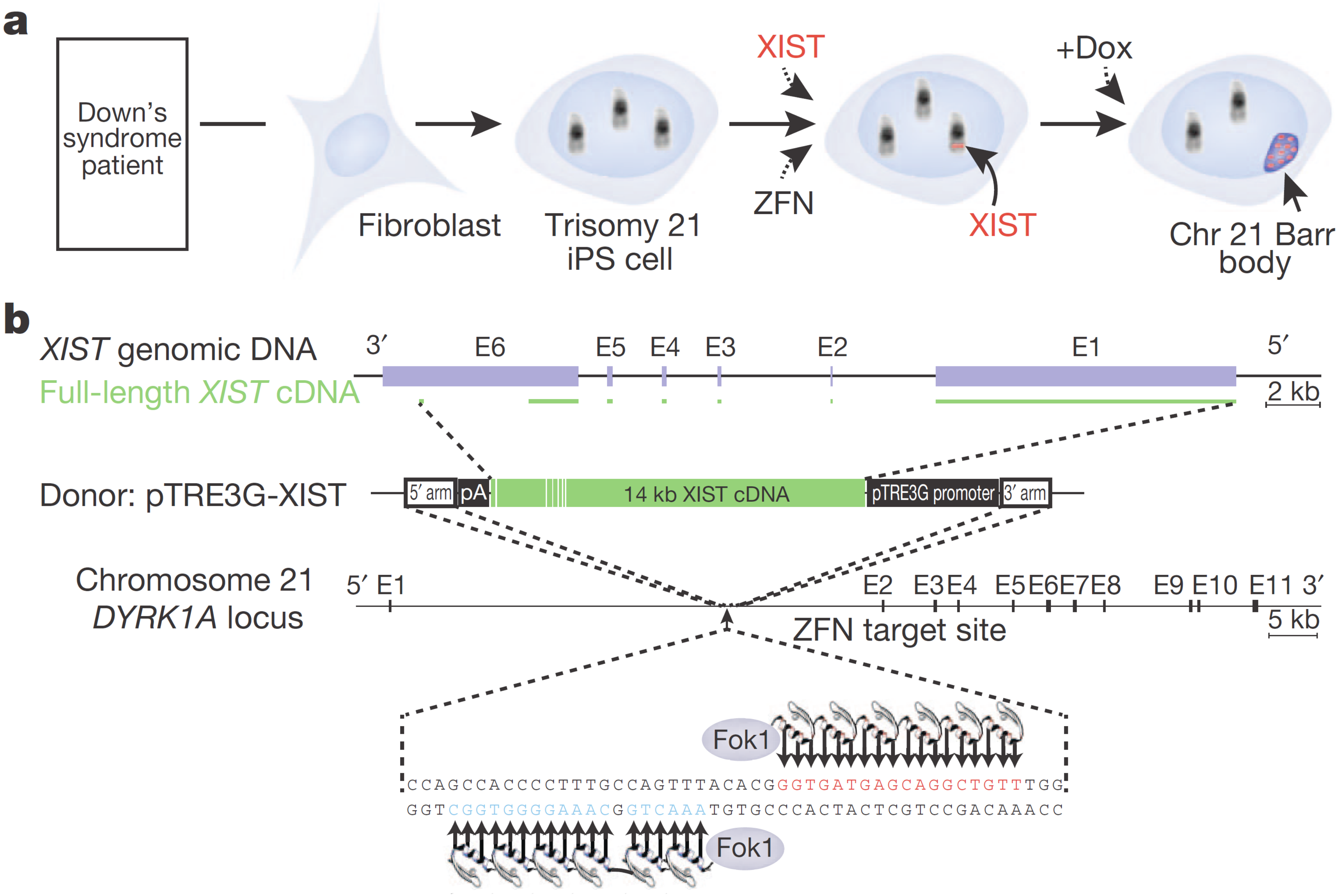


Genome editing integrates XIST into chromosome 21 in trisomic iPS cells



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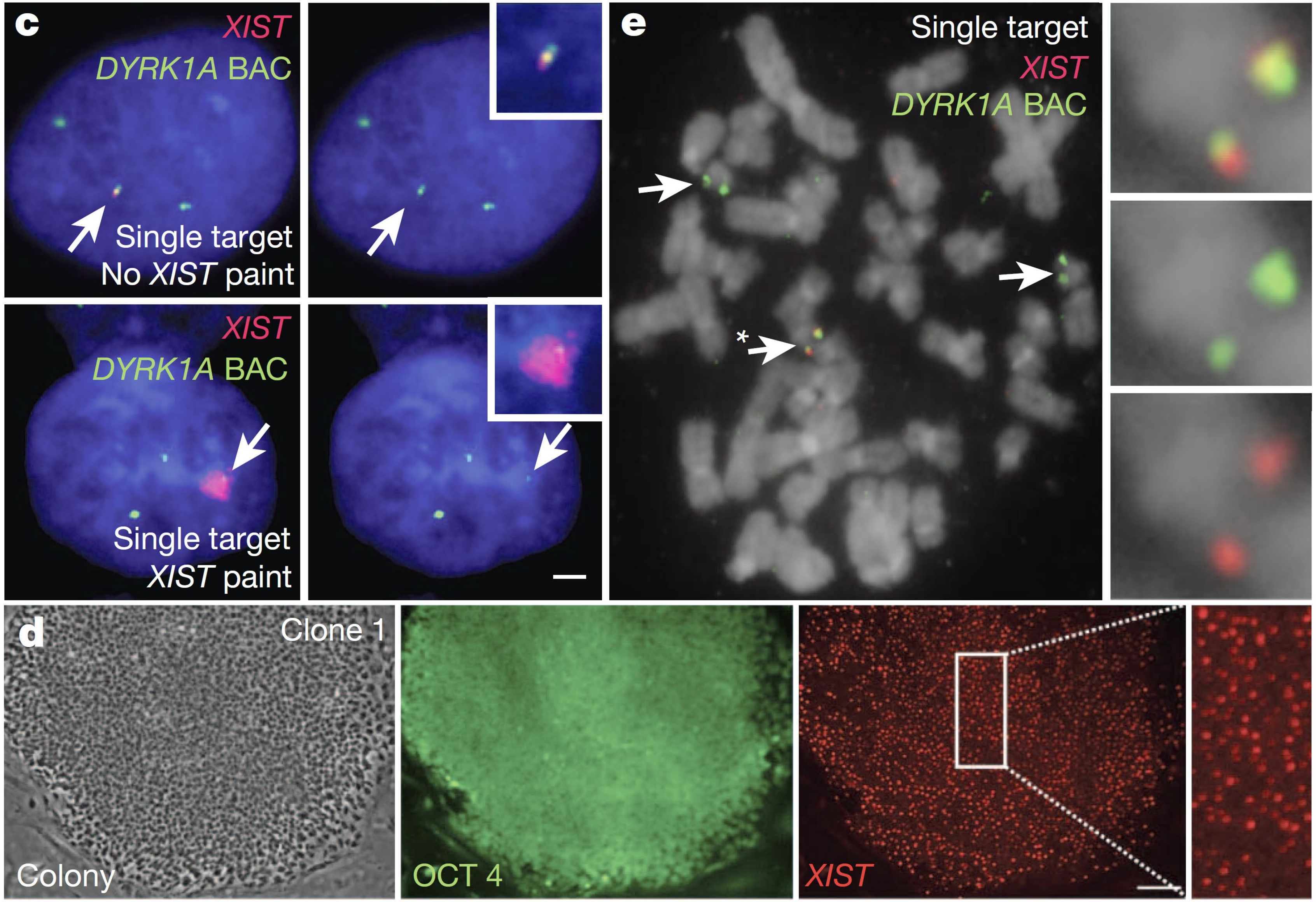
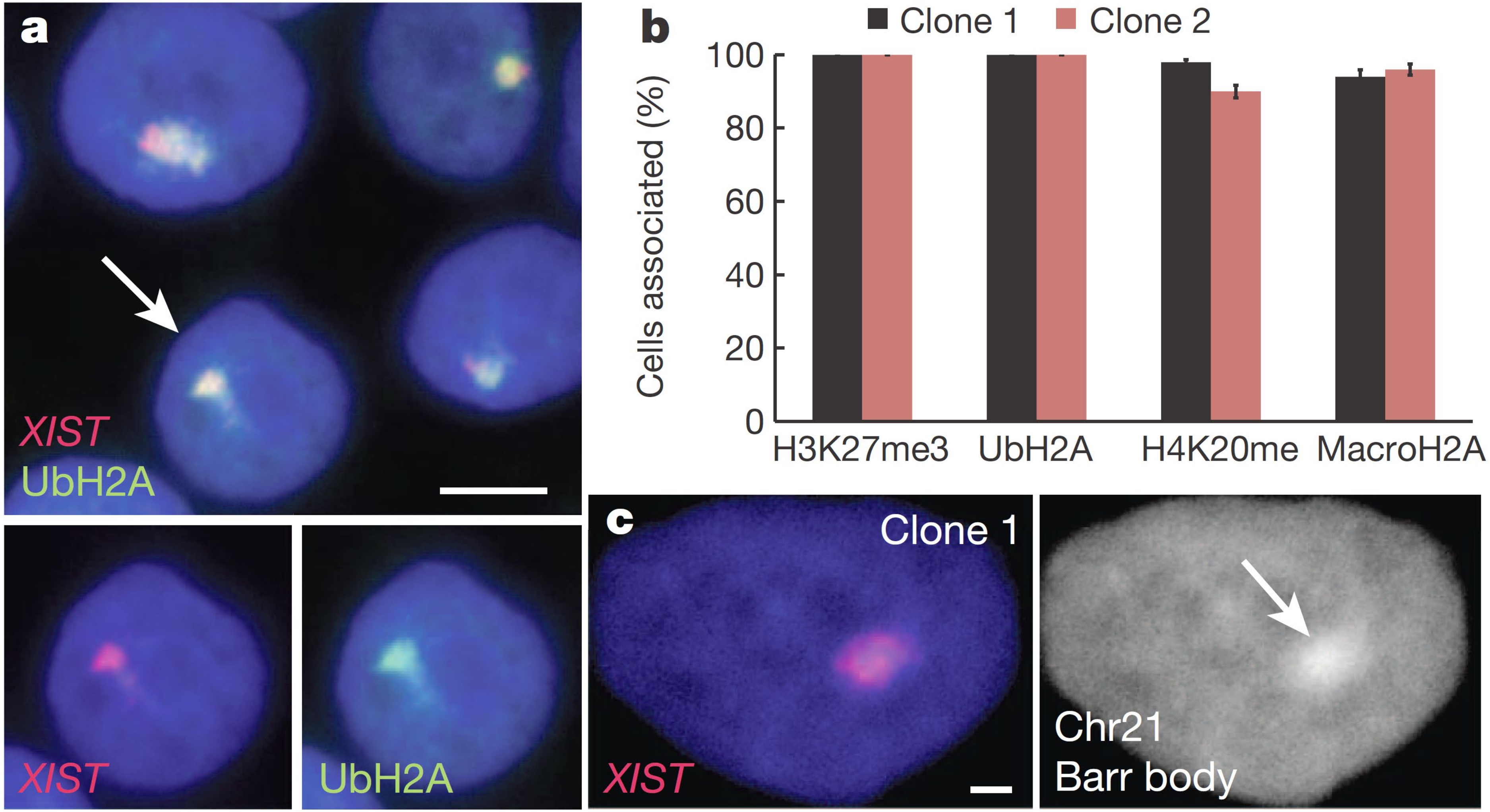
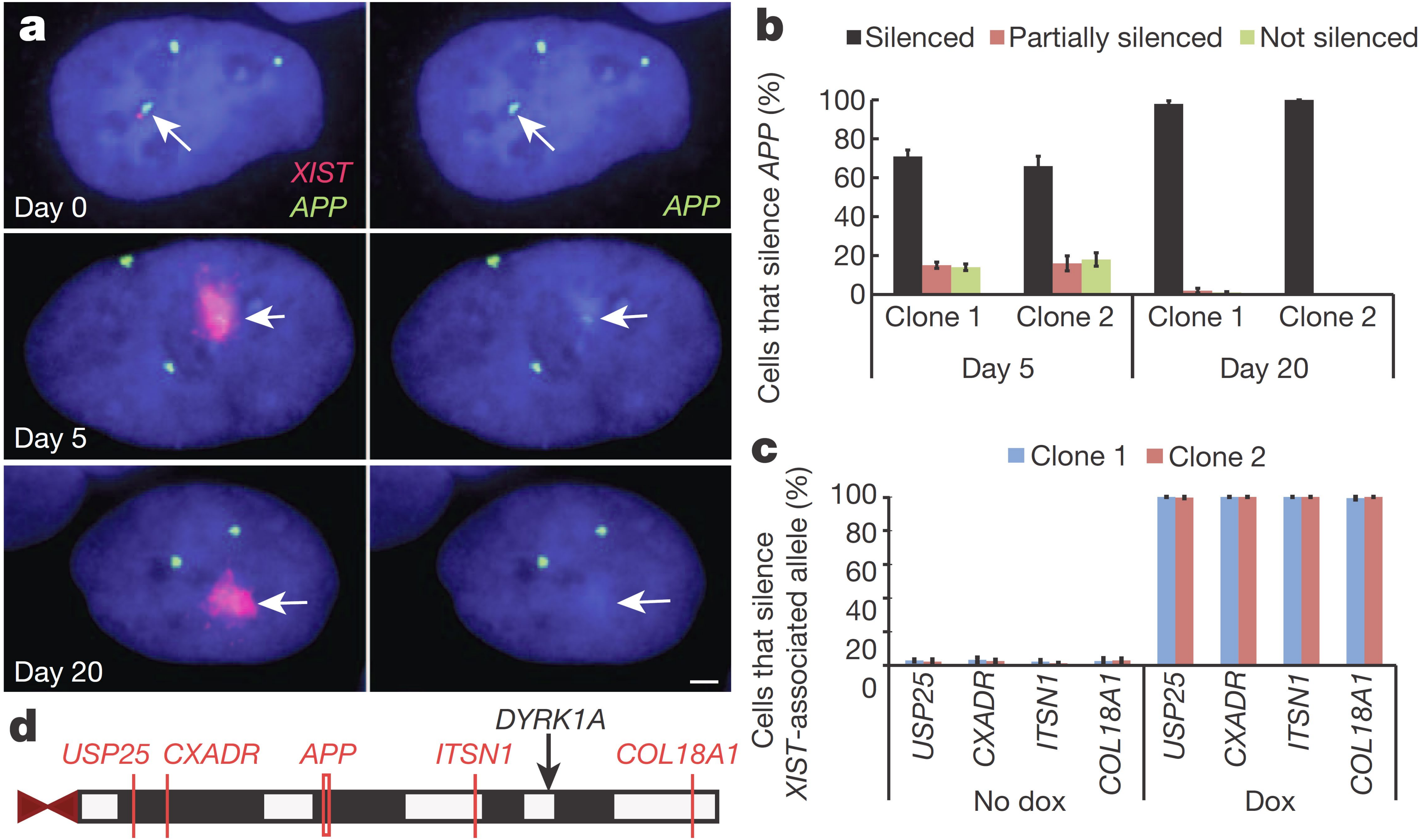


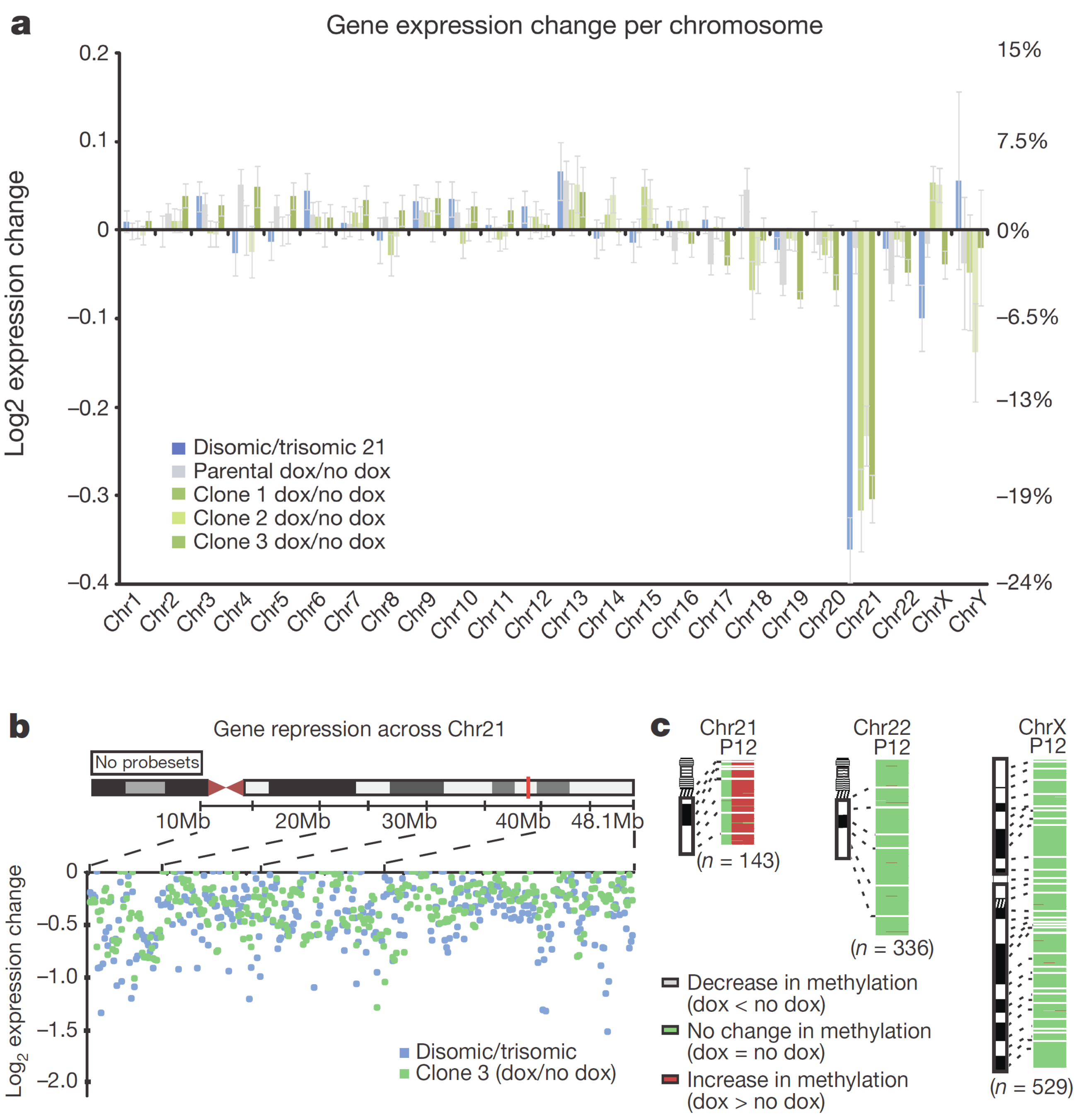
XIST induces heterochromatin modifications and condensed chromosome 21 Barr body



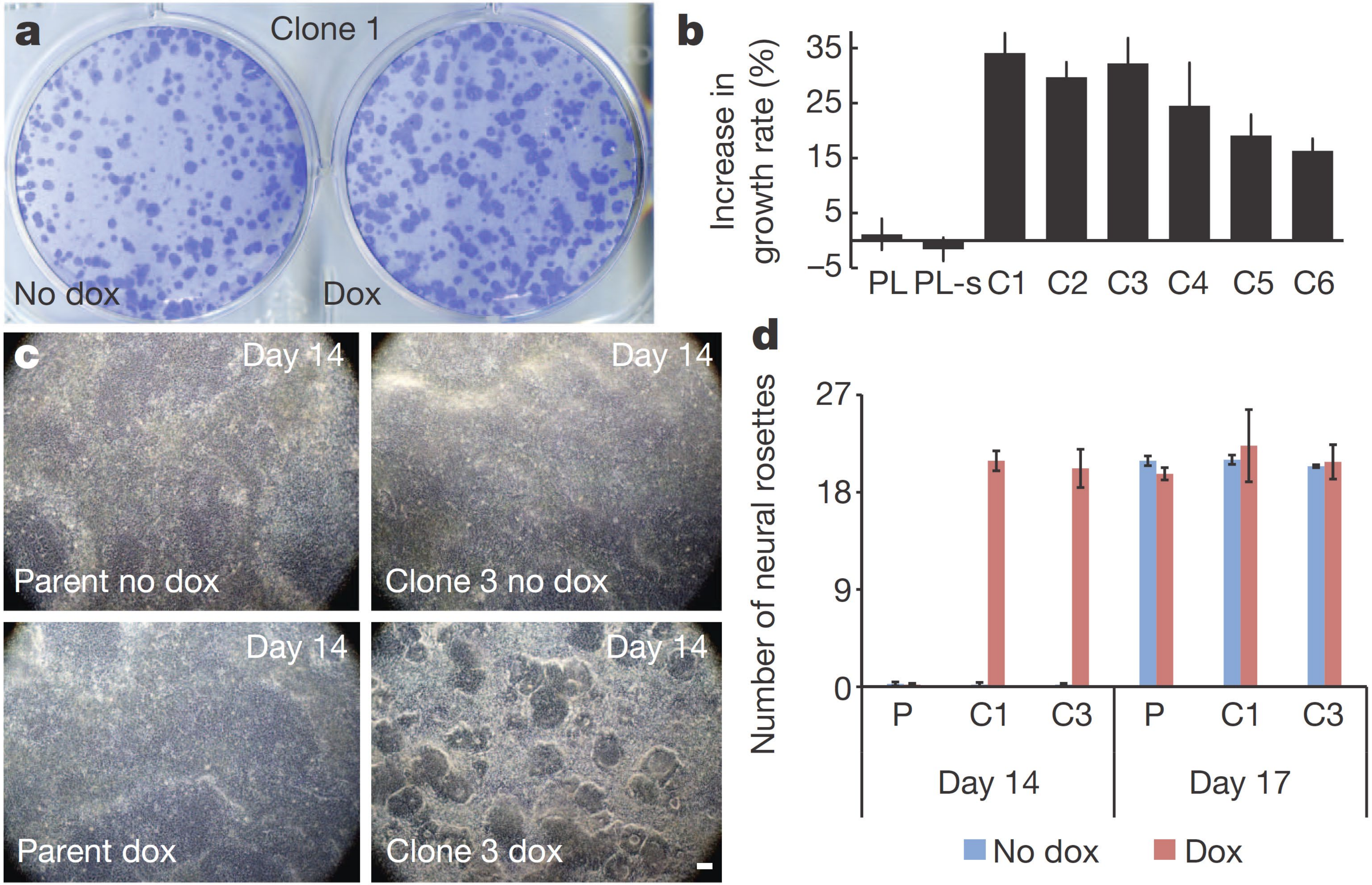
XIST induces long-range silencing in targeted iPS cells



Genomic expression and methylation reveal widespread silencing of chromosome 21



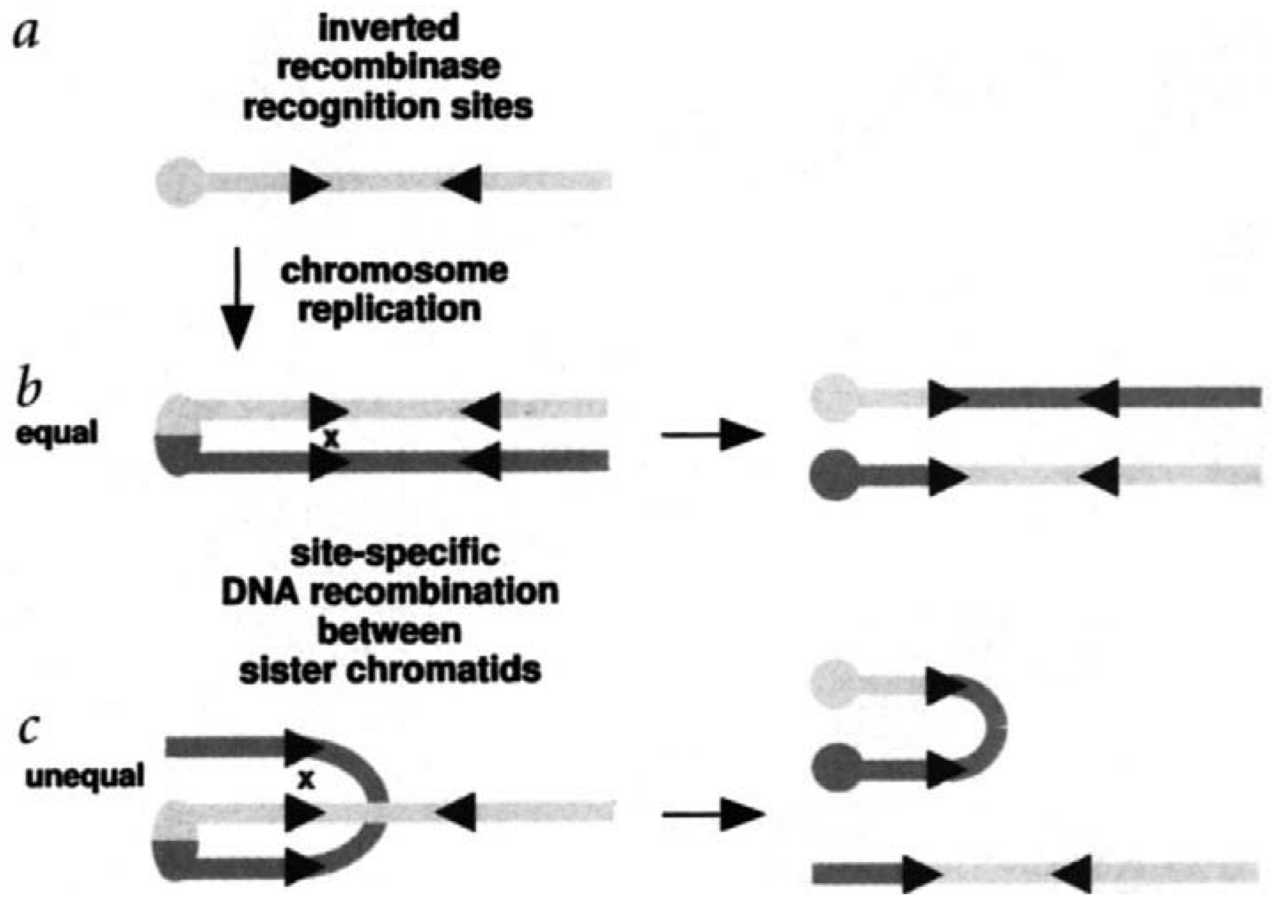
‘Trisomy correction’ affects cell proliferation and neurogenesis



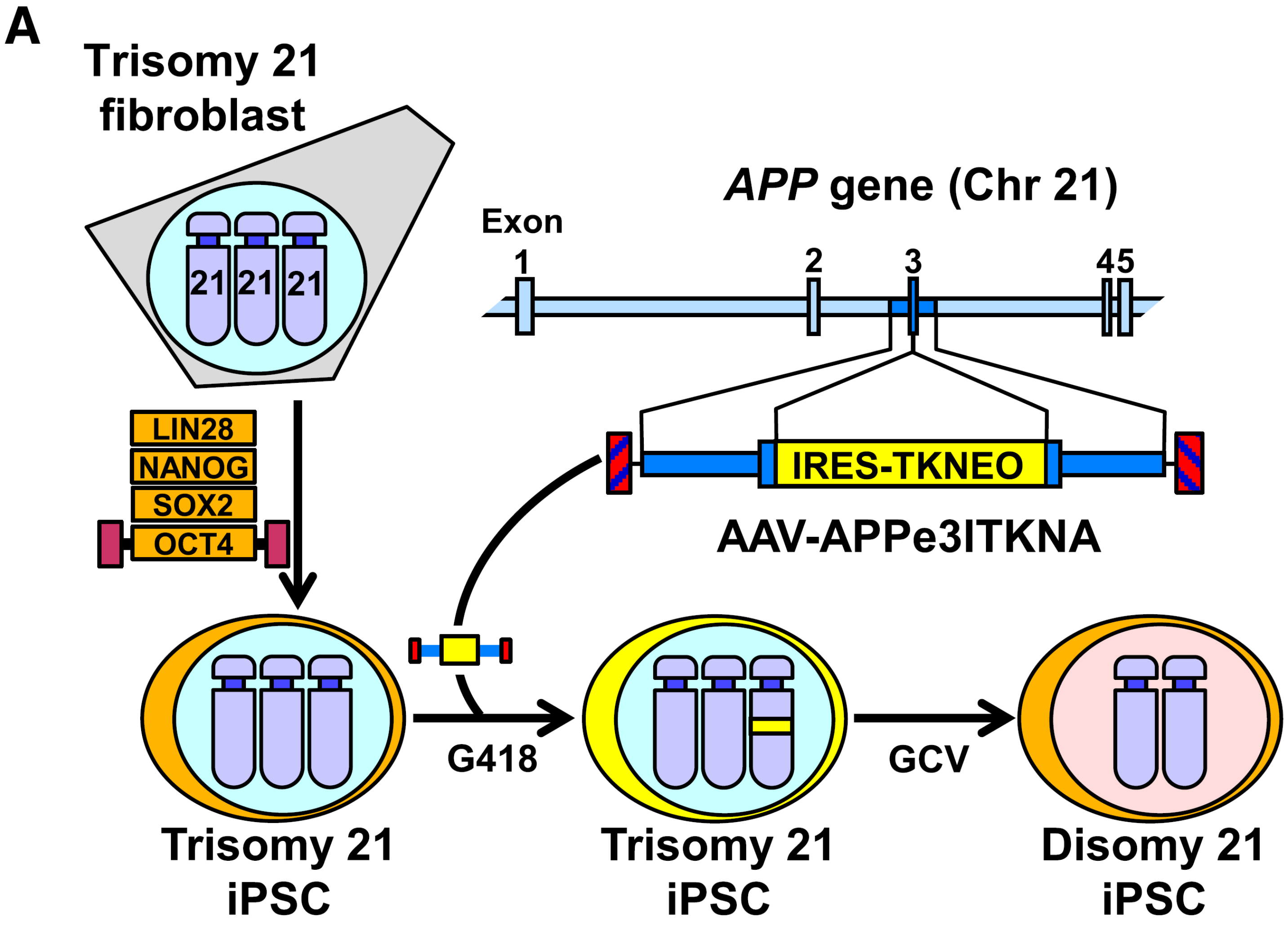
Trisomy Correction in Down Syndrome Induced Pluripotent Stem Cells

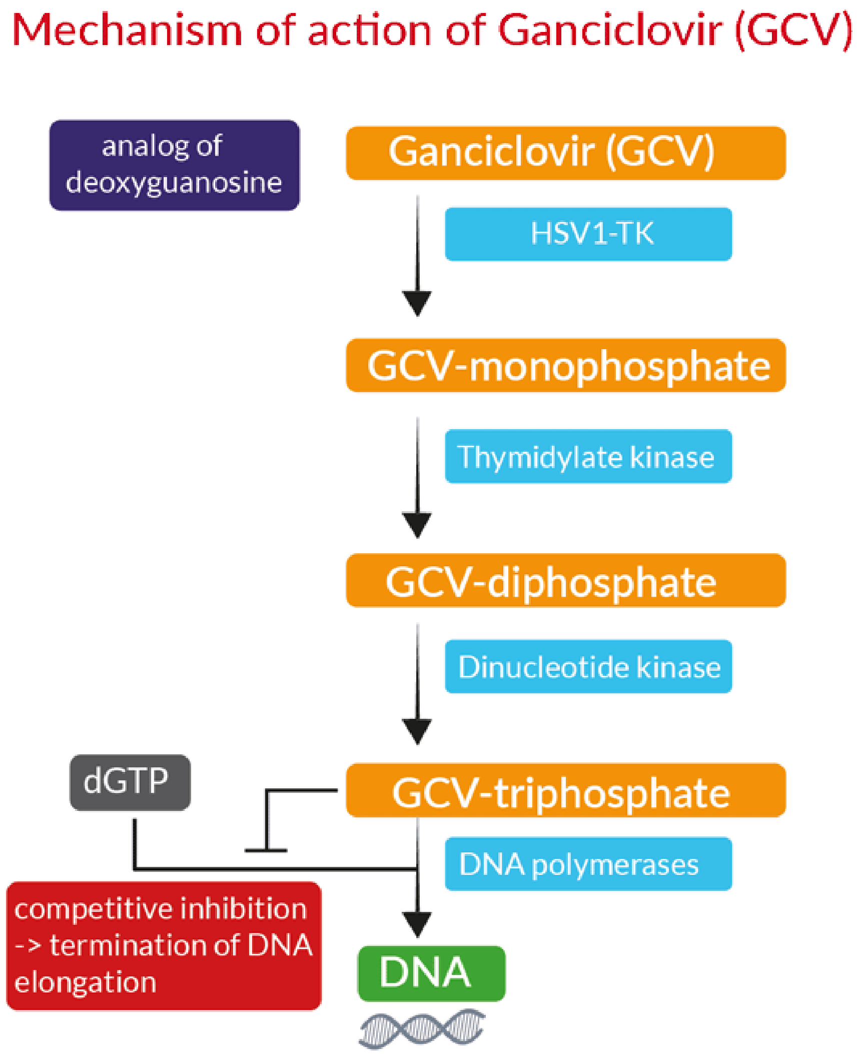
Li B. Li,[1] Kai-Hsin Chang,[1] Pei-Rong Wang,[1] Roli K. Hirata,[1] Thalia Papayannopoulou,[1] and David W. Russell[1,2,*]

Cre-mediated chromosome loss in mice

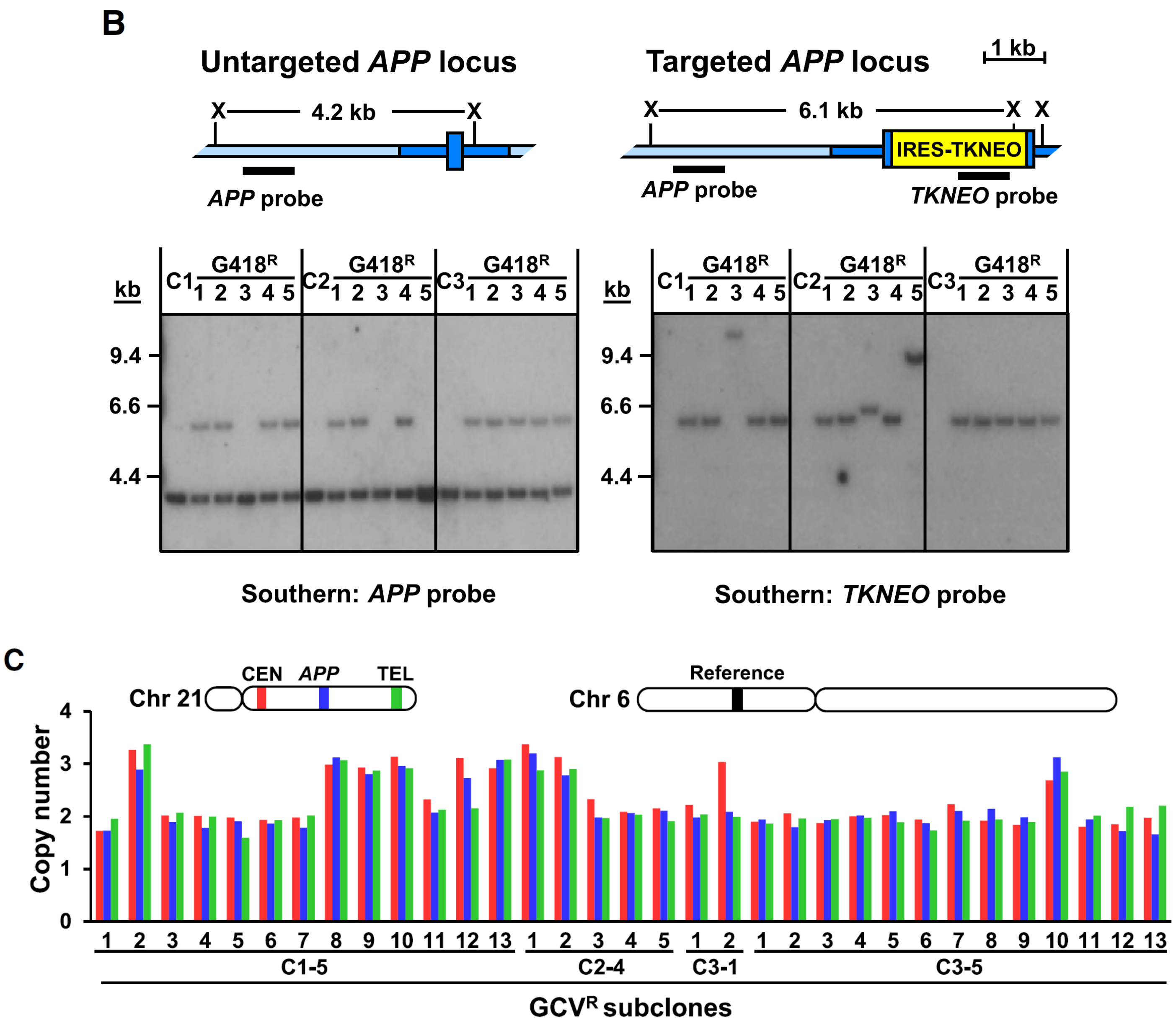


Positive and negative selection of trisomic corrected cells

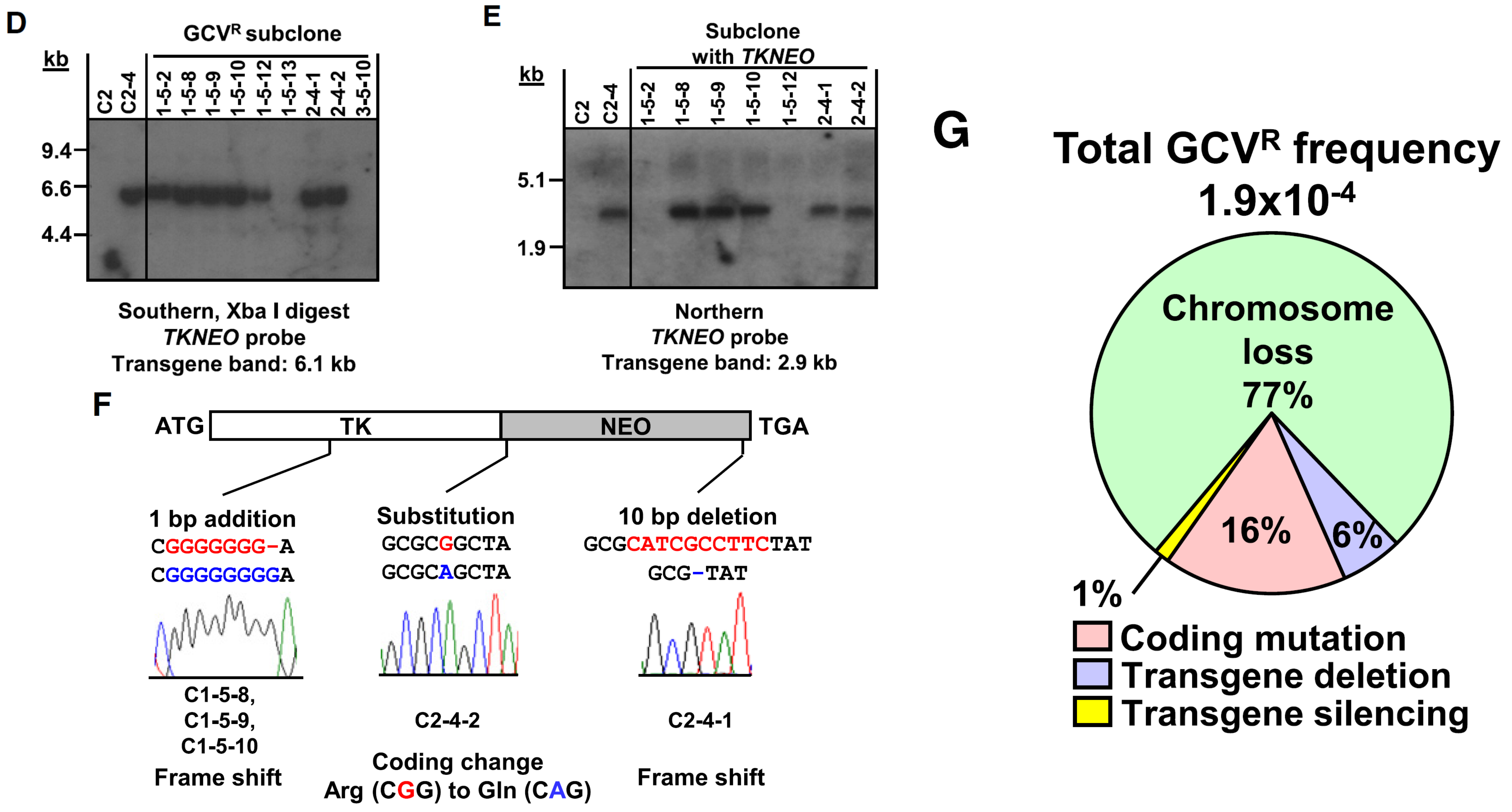




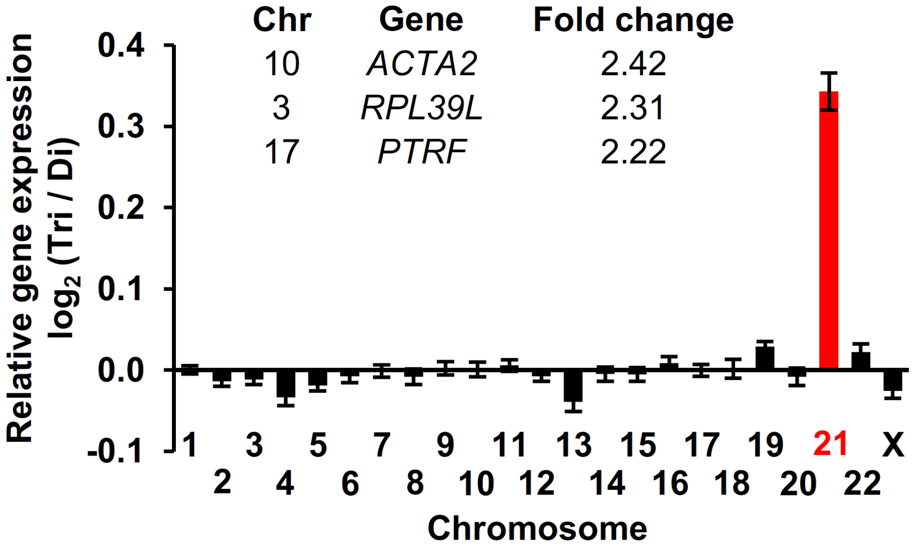
Validation of yield clones

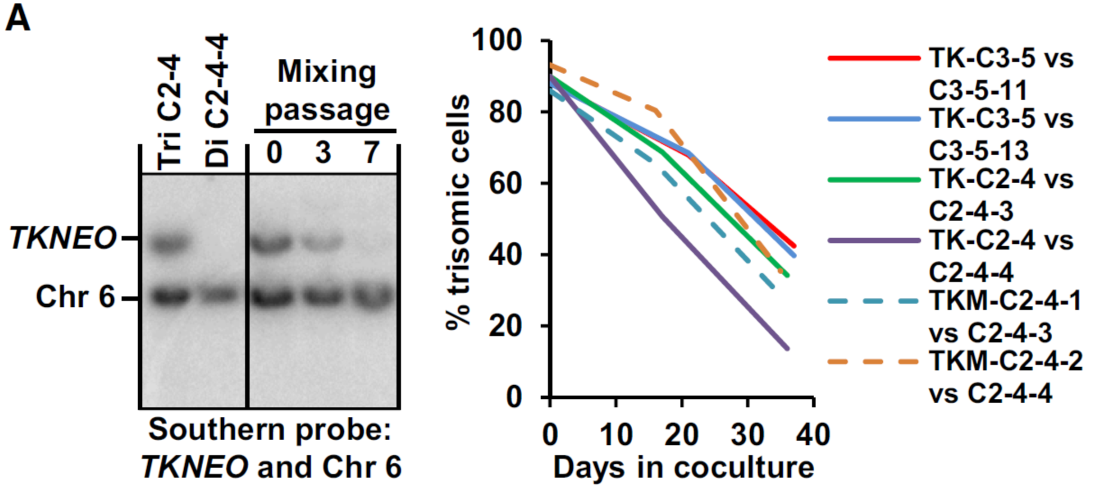


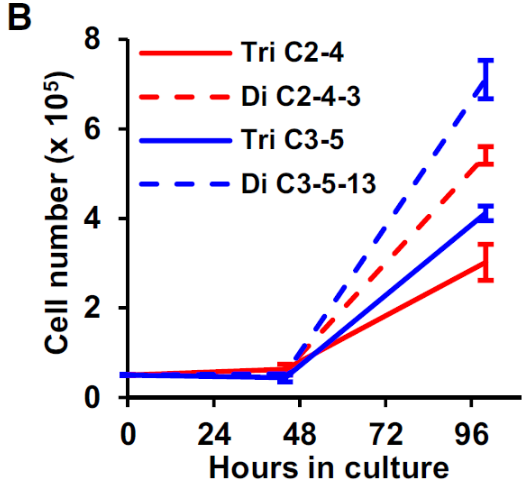
The analysis of unsuccessful clones and total GVR frequency



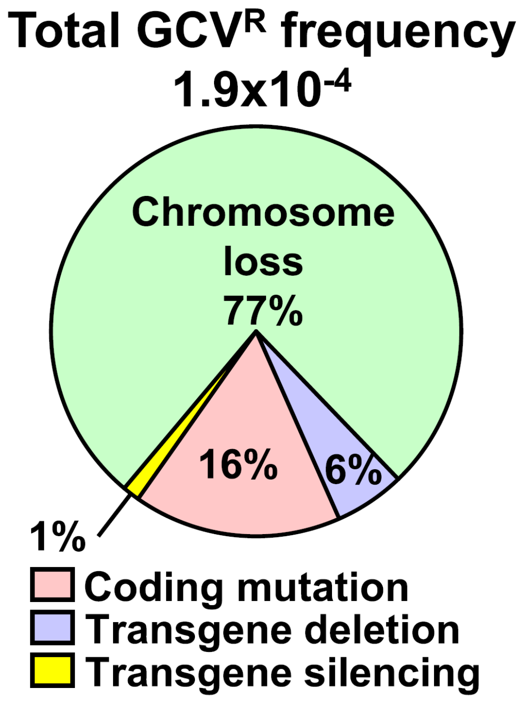
Proliferation and Gene Expression of Trisomic and Disomic iPSCs







CRISPR mediated chromosome elimination
unexpected off-targets two papers



CRISPR/Cas9-mediated targeted chromosome elimination

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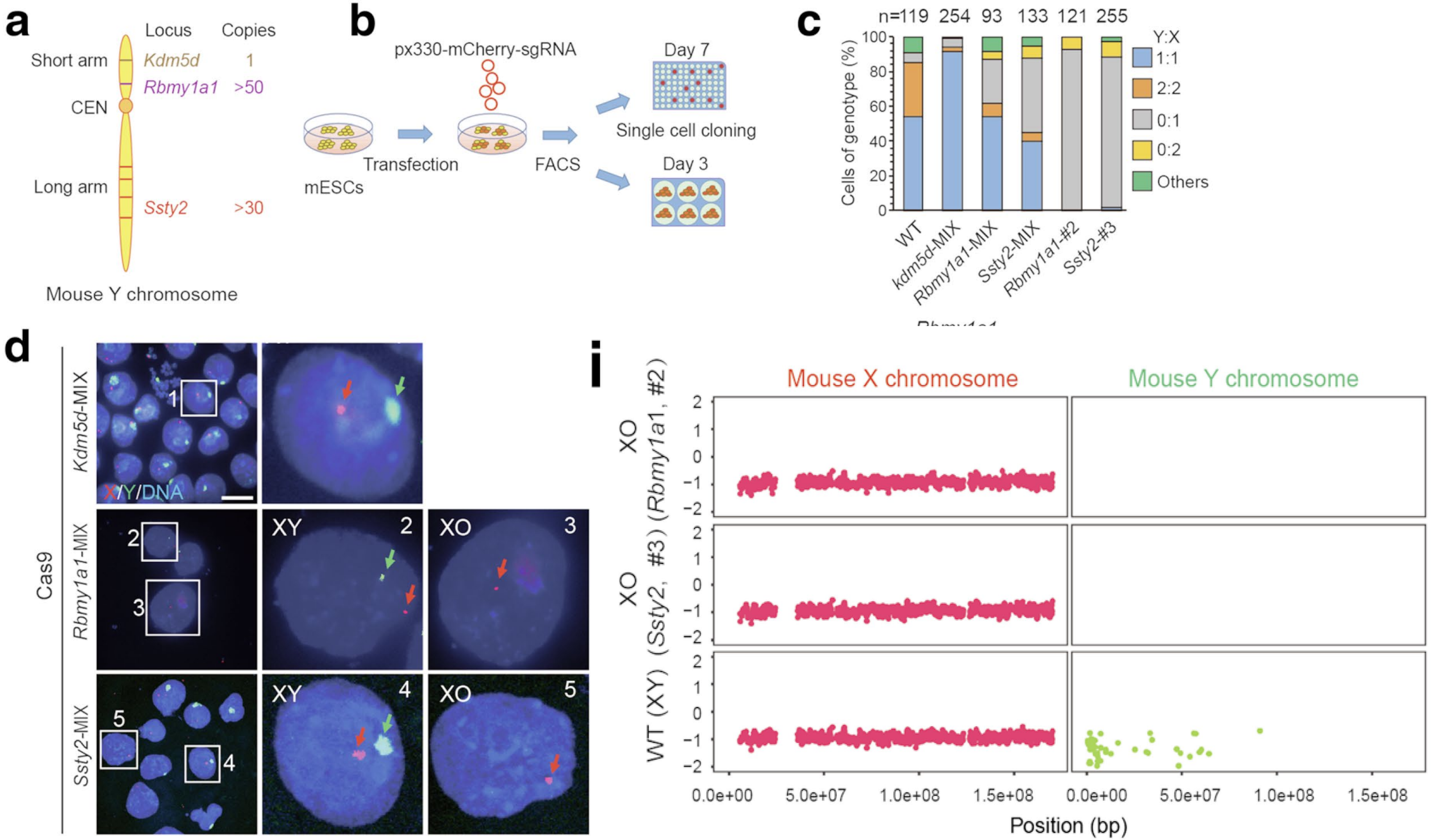
Cell

Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos

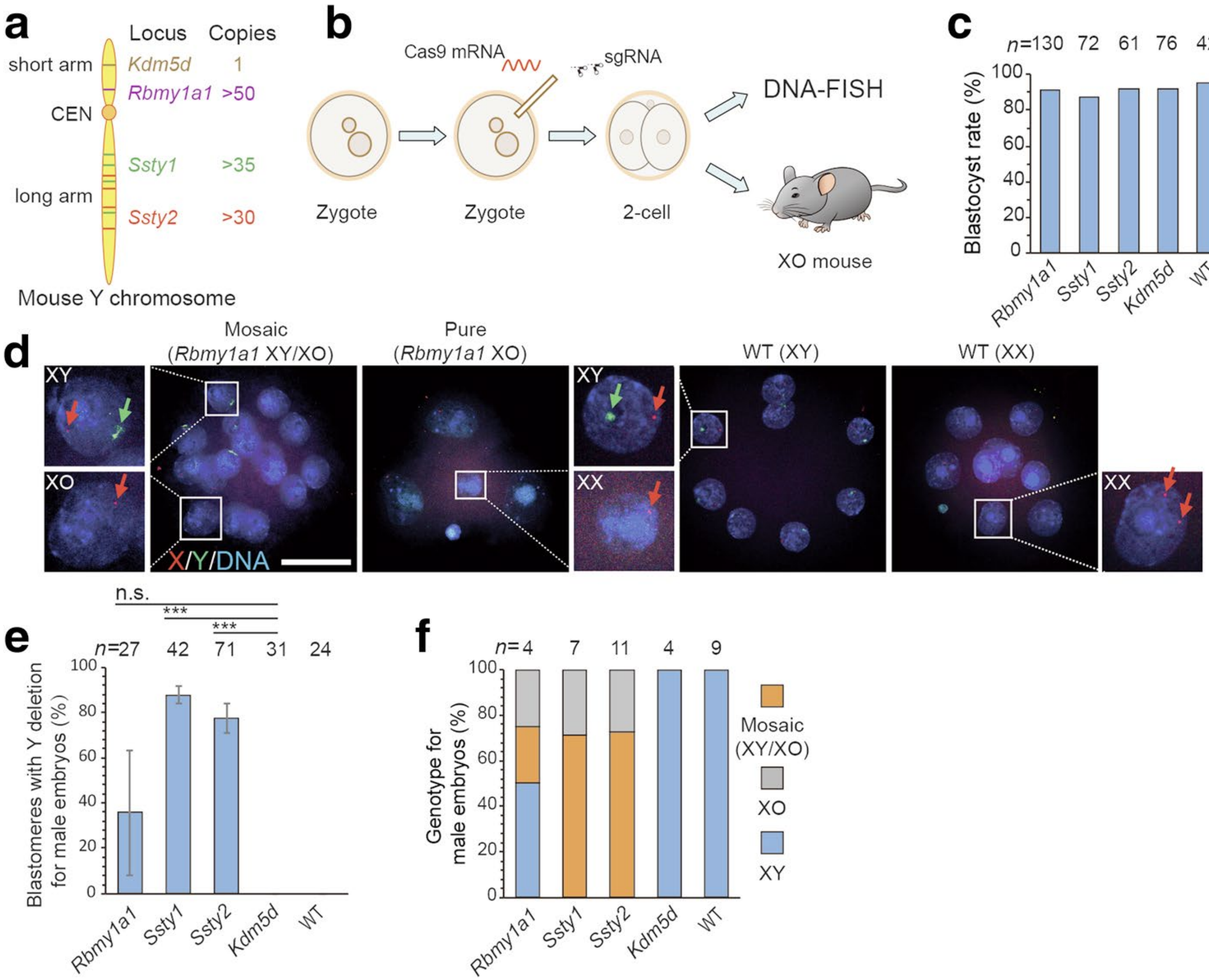
Authors

Michael V. Zuccaro, Jia Xu,
Carl Mitchell, ..., Rogerio Lobo,
Nathan Treff, Dieter Egli

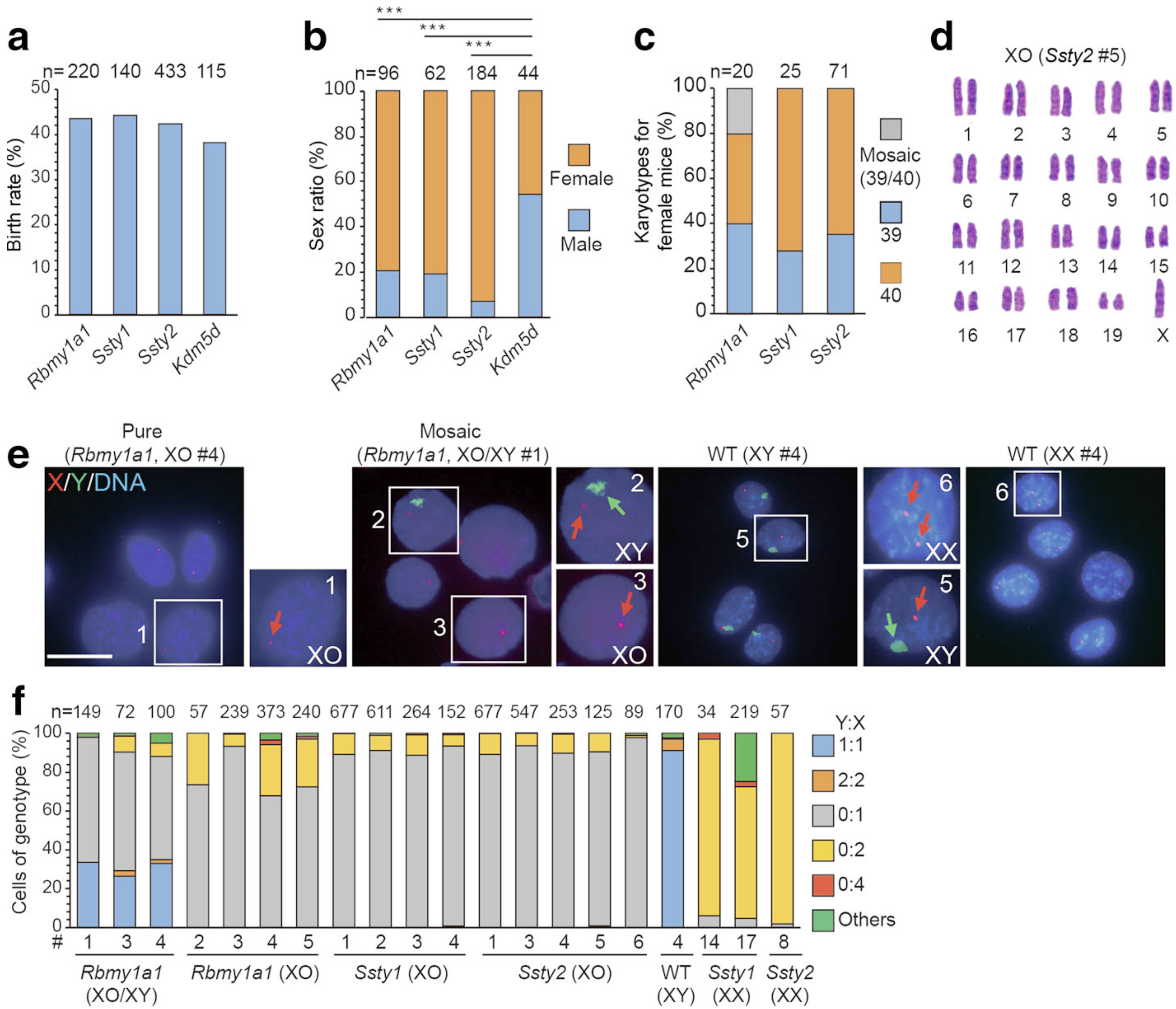
CRISPR/Cas9-mediated Y chromosome elimination in mESCs



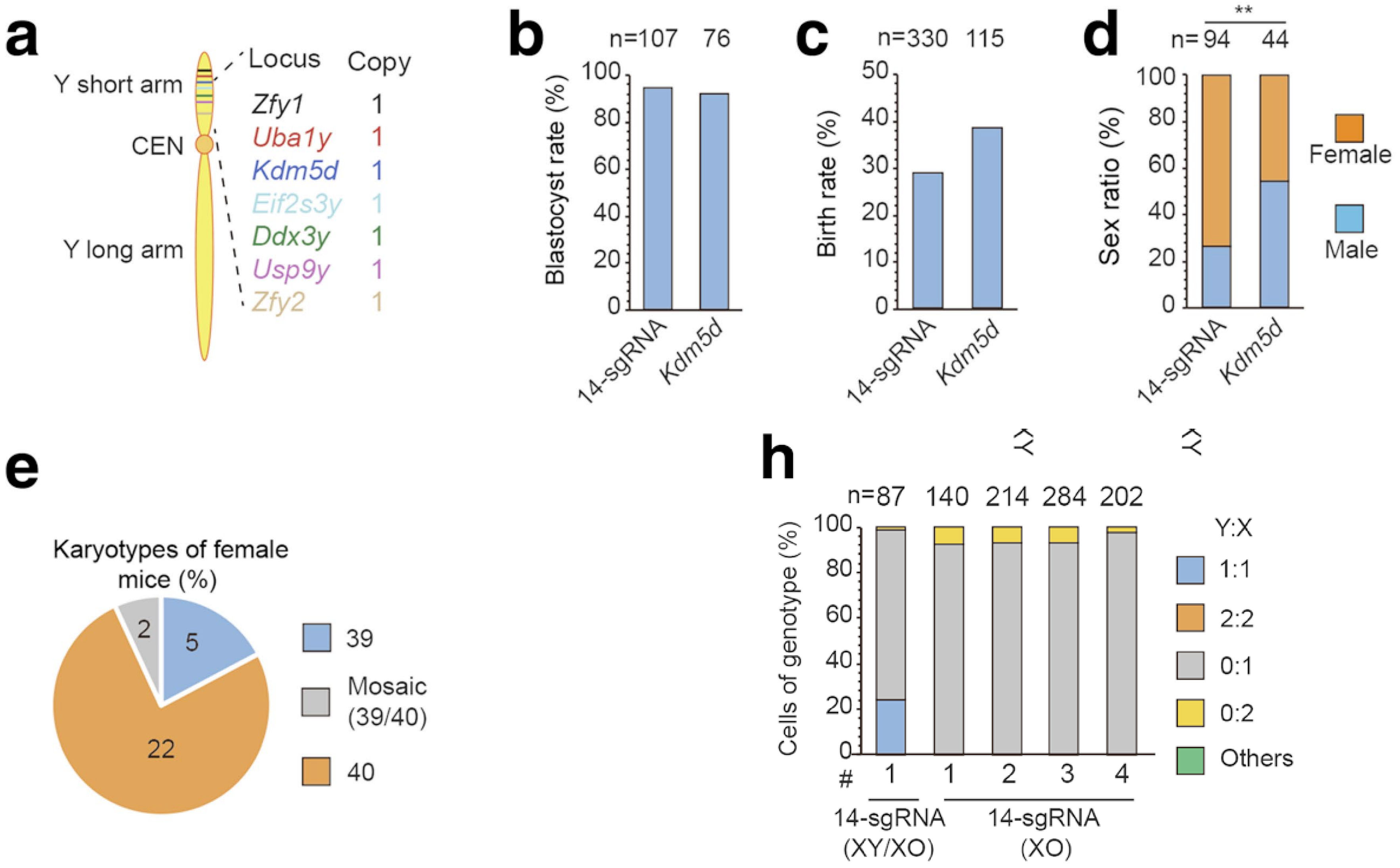
Elimination of the Y chromosome in zygotes by CRISPR/Cas9-mediated gene editing



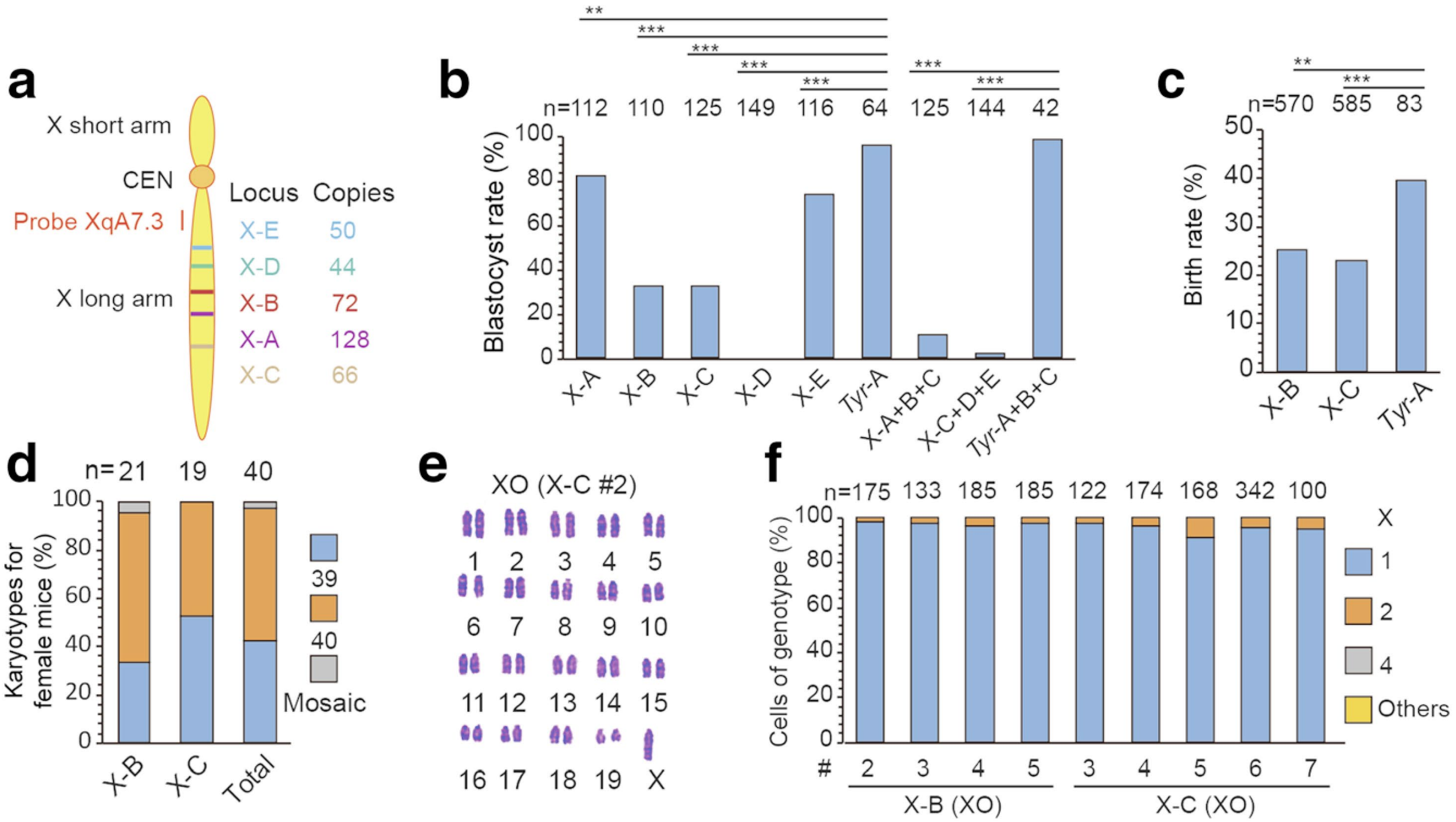
Generation of mouse model with Turner syndrome by Y chromosome elimination



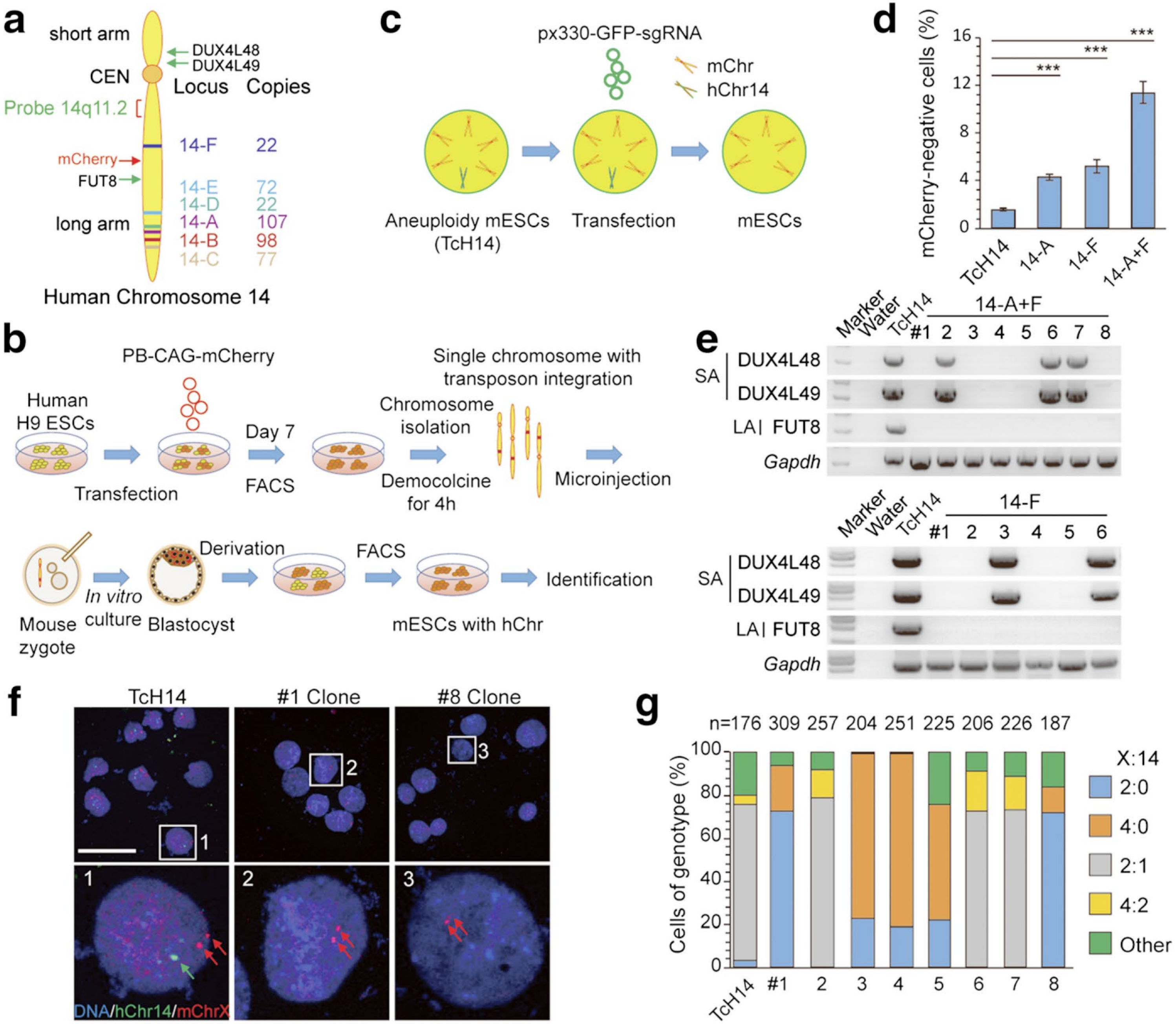
Elimination of mouse Y chromosome in zygotes with an sgRNA cocktail



Generation of mouse model with Turner syndrome by X chromosome elimination

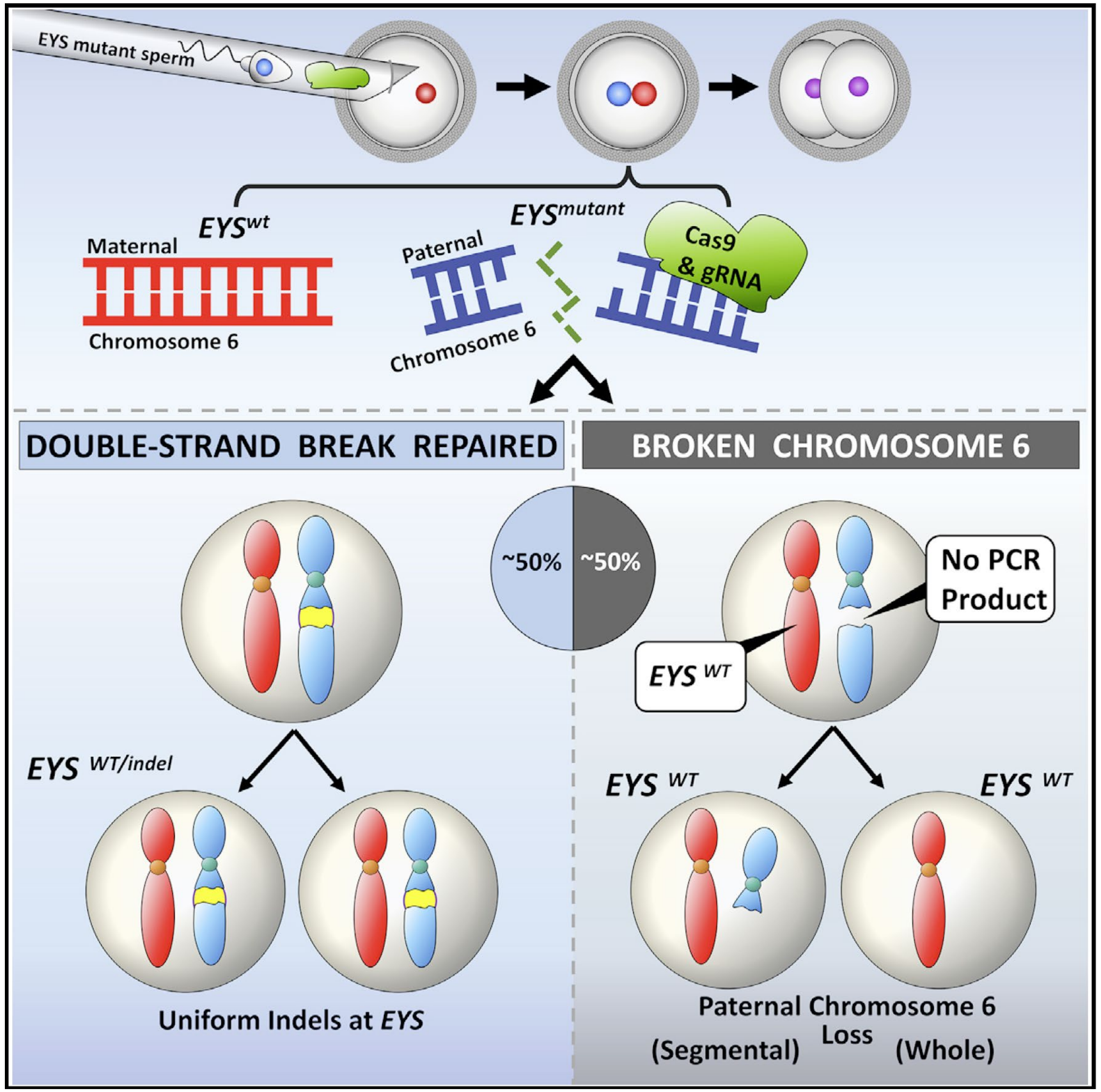


Elimination of human chromosome 14 in cells by CRISPR/Cas9-mediated gene editing



Cell

Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos



Chromosome Transplantation: A Possible Approach to Treat Human X-linked Disorders

Marianna Paulis,[1,2] Lucia Susani,[1,2] Alessandra Castelli,[1,2] Teruhiko Suzuki,[3] Takahiko Hara,[3] Letizia Straniero,[4] Stefano Duga,[2,4] Dario Strina,[1,2] Stefano Mantero,[1,2] Elena Caldana,[1,2] Lucia Sergi Sergi,[5] Anna Villa,[1,5] and Paolo Vezzoni[1,2]

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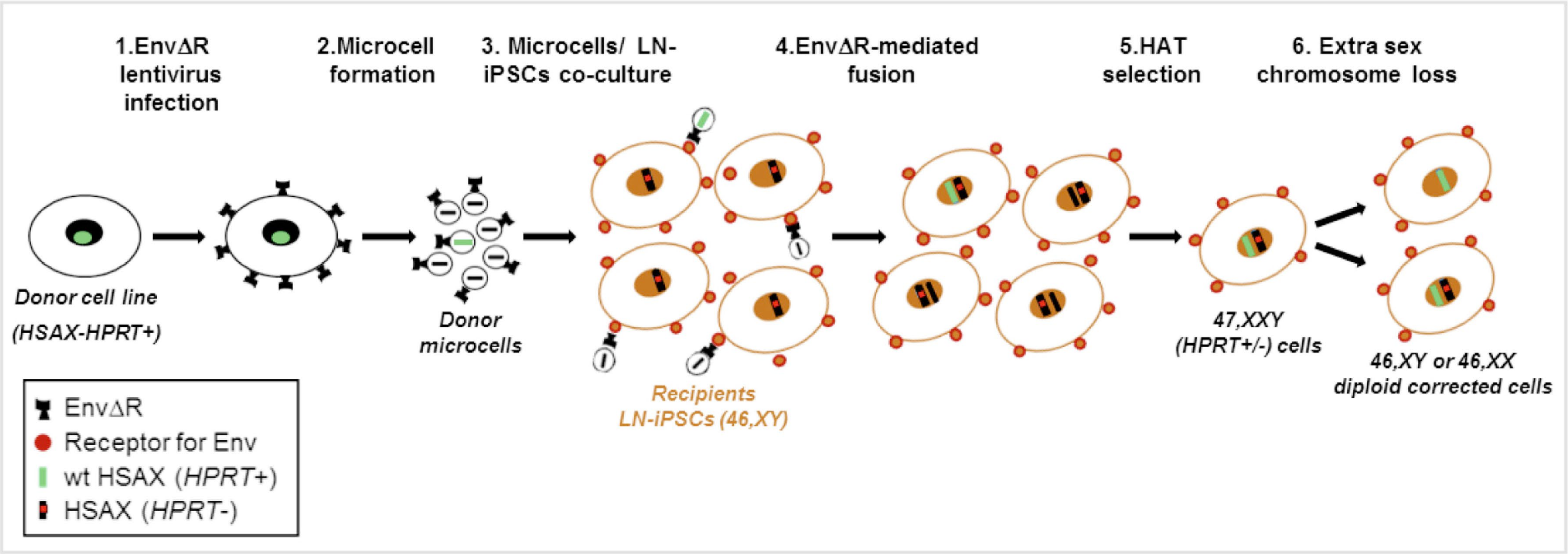

Thanks for your attention!