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Fertile offspring from sterile sex chromosome trisomic mice§

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Abstract

Having the correct number of chromosomes is vital for normal development and health. Sex chromosome trisomy (SCT) affects 0.1% of the human population and is associated with infertility. We show that during reprogramming to induced pluripotent stem cells (iPSC), fibroblasts from sterile trisomic XXY and XYY mice lose the extra sex chromosome, by a phenomenon we term trisomy-biased chromosome loss (TCL). Resulting euploid XY iPSCs can be differentiated into the male germ cell lineage and functional sperm that can be used in intracytoplasmic sperm injection to produce chromosomally normal, fertile offspring. Sex chromosome loss is comparatively infrequent during mouse XX and XY iPSC generation. TCL also applies to other chromosomes, generating euploid iPSCs from cells of a Down syndrome mouse model. It can also create euploid iPSCs from human trisomic patient fibroblasts. The findings have relevance to overcoming infertility and other trisomic phenotypes.

The mammalian sex chromosomes have specialized roles in male (XY) and female (XX) germ cell development (1). Sex chromosome abnormalities are the most common genetic cause of human infertility (2). In the SCTs Klinefelter (XXY) and Double Y (XYY) syndrome, spermatogenesis is disrupted by excess X- and Y-genes, respectively (2). XYY men are commonly fertile due to spontaneous loss of the extra sex chromosome (mosaicism). In XXY men, mosaicism is less common. Testicular sperm retrieval has enabled reproduction from some young Klinefelter men, but is less successful in older patients (3, 4). XXY and XYY individuals without XY germ cells are infertile.

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To study SCT-infertility, we generated adult XXY and XYY mice carrying the fluorescent reporter transgenes *Blimp1-mVenus* (BV) and *Stella-ECFP* (SC) (5) to monitor differentiation of pluripotent stem cells into primordial germ cell-like cells (PGCLCs) (6). XXY males were created by mating a wildtype female to a sex chromosome variant male that produces XY-containing sperm (fig. S1). Generation of XYY mice requires inheritance of a Y chromosome from both parents. We therefore used a paternally inherited wild type Y chromosome, and a maternally inherited Y^{d1} chromosome that does not express the testis-determinant *Sry* (fig. S1) (7). As shown previously (8, 9), the spermatogenesis phenotype in both models recapitulated that in SCT men, with arrest at the prospermatogonial stage in XXY mice and at pachynema in XYY mice (fig. S2). Spermatogenesis was normal in euploid XY BVSC transgenic siblings.

Next, we established fibroblasts from SCT and control XY and XX mice (Fig. 1A). DNA-FISH for the X-gene *Slx* and Y-gene *Sly* confirmed that passage 4 (P4) SCT and control fibroblasts had retained their original sex chromosome complements (Fig. 1B, fig. S3A). Fibroblasts were reprogrammed to induced pluripotent stem cells (iPSCs) (10) in a doxycycline (Dox)-inducible manner. DNA-FISH was performed in resulting P2 iPSCs (Fig. 1A).

A high proportion of SCT-derived iPSC lines exhibited sex chromosome loss. From XXY mice we observed XY, XX and XO iPSCs (Fig. 1C, 1E). The incidence of loss was similar for the X and Y chromosome ($p=0.062$, Mann-Whitney test). From XYY mice we observed XY and XO iPSCs (Fig. 1D-E). Y chromosome loss in XYY males occurred at a similar frequency to that observed for the X and Y chromosome combined in XXY males ($p=0.089$, Mann-Whitney test). We then compared the incidence of sex chromosome loss between SCT-derived and euploid XY- and XX-derived iPSCs. Sex chromosome loss was more common in SCT- than euploid-derived iPSCs (Fig. 1E), irrespective of the cutoff used to define sex chromosome loss (fig. S12D).

Sex chromosome loss could occur during reprogramming of SCT cells, or during iPSC propagation to P2, perhaps conferring a proliferative advantage to resulting euploid cells. Indeed, sex chromosome instability has been observed in pluripotent stem cells (11, 12). To test the latter hypothesis, we analyzed sex chromosome stability between P2 to P6 in iPSCs with highly parental (>90%) complements (fig. S4A). We observed sex chromosome loss in XX and XXY iPSC lines ($p<0.01$ and 0.05 respectively, Wilcoxon signed-rank test), but not in XY and XYY iPSC lines ($p=0.21$ and 0.66 respectively, Wilcoxon signed-rank test). However, no iPSC line showed more than a 15% decrease in parental complement (fig. S4B). Furthermore, sex chromosome loss between P2 and P6 was not trisomy-biased (fig. S4B). SCT-derived euploid XY iPSCs also exhibited no proliferative advantage over XXY or XYY iPSCs (fig. S5). Since SCT fibroblasts were also karyotypically stable (Fig. 1B and fig. S3A), chromosome loss is likely induced during iPSC reprogramming and is thus distinct from sex chromosome instability in pluripotent stem cells (11, 12). We refer to the phenomenon as trisomy-biased chromosome loss (TCL).

We next determined whether euploid XY iPSCs derived from SCT fibroblasts would form functional sperm. We selected highly euploid (≥80% of cells XY) P6 iPSCs adapted to Dox-

free medium (fig. S6). For our XYY experiments, only XY iPSC lines that retained the wild type Y rather than the Y^{d1} chromosome were used for PGCLC experiments (fig. S7). Karyotyping confirmed that all SCT-derived XY iPSC lines and a control XY iPSC line were euploid (fig. S8). These iPSC lines were differentiated (6) through epiblast-like state to create PGCLC aggregates positive for BV and SC (Fig. 2A). BV-positive PGCLCs (table S1) were isolated by fluorescence-activated cell sorting (FACS; Fig. 2B) and transplanted into germ-cell deficient W/W^{v} (*Kit* mutant) testes (13).

Spermatogenesis in recipients was evaluated 9-10 weeks post-transplantation. Teratomas, which are observed following iPSC-derived PGCLC transplantation (6), were present in 29% of XXY-derived and 50% of XYY-derived transplanted lines (fig. S9). Reconstitution of spermatogenesis, revealed by the presence of spermatogenic colonies (Fig. 2C) and by histology (Fig. 2D) was observed for all XXY- and XYY-derived iPSC lines used (Table 1). Thus, SCT-derived XY iPSCs can differentiate to germ cells *in vitro* and complete spermatogenesis post-transplantation.

We asked whether sperm created via transplantation could support reproduction. Intracytoplasmic sperm injection (ICSI) using sperm from two XXY- and two XYY-derived XY iPSC lines (Fig. 2E, fig. S10A) generated zygotes that developed into 2-cell embryos *in vitro* (efficiency 76.7-87.3%, Fig. 2F, fig. S10B, table S2) and grossly normal offspring when transplanted into recipients (efficiency 46.9-59.4%, Fig. 2G, fig. S10C, table S2). PCR genotyping confirmed that the offspring were derived from the transplanted PGCLCs (fig. S10D). Pups from XXY- and XYY-derived iPSC lines showed comparable growth to those derived from control XY iPSCs (fig. S10E). Notably, the XXY- and XYY-derived pups had euploid (XY or XX) complements (fig. S11). Three matured males and three females from each XXY- and XYY-derived iPSC line were mated to each other, and all were fertile (Fig. 2H, fig. S10F). Thus, sperm from SCT-derived XY iPSCs give rise to chromosomally normal, healthy, and fertile offspring.

We addressed if TCL is specific to trisomy of sex chromosomes. Since mouse models with trisomy for a complete autosome are not available (14), we repeated our experiments in male Tc1 transchromosomic mice, a Down syndrome model with an accessory human chromosome 21 (hChr.21; 15). Tc1 mice carry a hChr.21-inserted neomycin resistance cassette, selection for which reduces hChr.21 mosaicism (15). We therefore first enriched adult Tc1 fibroblasts for the presence of hChr.21 using the neomycin analog G418 (Fig. 3A). DNA-FISH showed that the vast majority (≥96%) of Tc1 fibroblasts retained hChr.21 (Fig. 3B, fig. S3B). These Tc1 fibroblasts were reprogrammed without G418 selection, and the resulting iPSCs were analyzed at P2. Ten of the 16 iPSC lines generated (62.5%) showed loss of hChr.21 in ≥10% of cells (Fig. 3C-D, fig. S12D). In contrast, following G418 removal, hChr21 was retained in Tc1 fibroblasts cultured for the same period as that used for iPSC reprogramming (18 days), and in P6 iPSC lines that had highly parental (>90% hChr. 21 positive) complements at P2 (fig. S12). We conclude that loss of hChr.21 in Tc1 cells is promoted by reprogramming rather than G418 removal, and therefore that TCL also affects an accessory chromosome.

We next asked whether TCL occurs in human cells. Instances of chromosome loss have been observed during human trisomic cell culture (16, 17), but its prevalence and relationship to reprogramming has not been systematically analyzed. We selected human Klinefelter syndrome, Down syndrome, and euploid XY and XX fibroblast lines exhibiting minimal mosaicism (fig. S13A, S13D), reprogrammed them, and determined the chromosome complements of resulting iPSC lines. We observed XY and XX iPSCs from Klinefelter syndrome fibroblasts, and euploid iPSCs from Down syndrome fibroblasts (fig. S13, B-C and F-G). Chromosome loss was more common in trisomic than in disomic cells, demonstrating that TCL also occurs during human reprogramming. However, the frequency of highly euploid iPSC lines was lower than that observed in trisomy-derived mouse iPSCs (fig. S13, F-H).

We have shown that TCL produces euploid iPSCs from SCT and autosomal trisomic mice and patients (fig. S12E). In mice, the resulting “corrected” iPSCs can form functional sperm, enabling production of chromosomally euploid offspring from infertile SCT individuals. TCL complements existing iPSC-therapies for chromosome abnormalities (17–21). The mechanisms that cause TCL are unknown. Cellular stresses associated with reprogramming may select against trisomic cells, permitting emergence of euploid cells. We observed less frequent TCL in human than in mouse cells (fig. S12D and S13H). Even if rare, TCL could offer treatments for infertile SCT patients for whom alternative approaches are unsuccessful. However, the clinical use of human germ cells made *in vitro* should be carefully considered ethically and legally (22–24). Furthermore, complete *in vitro* spermatogenesis will have to be developed in order to avoid the risk of teratoma formation arising through germ-cell transplantation.

TCL also permits production of female iPSCs from males, offering potential for the genetic dissection of sexual dimorphisms (25). By creating isogenic iPSC lines that differ only with respect to their sex chromosomes, sex differences identified during iPSC disease modelling could be attributed to X or Y chromosomal effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Hughes JF, Page DC. The Biology and Evolution of Mammalian Y Chromosomes. Annu Rev Genet. 2015; 49:507–527. [PubMed: 26442847]
2. Heard E, Turner J. Function of the sex chromosomes in mammalian fertility. Cold Spring Harb Perspect Biol. 2011; 3:a002675. [PubMed: 21730045]

3. Ramasamy R, et al. Successful Fertility Treatment for Klinefelter's Syndrome. J Urol. 2009; 182:1108–1113. [PubMed: 19616796]
4. Greco E, et al. Birth of 16 healthy children after ICSI in cases of nonmosaic Klinefelter syndrome. Hum Reprod. 2013; 28:1155–1160. [PubMed: 23493114]
5. Ohinata Y, Sano M, Shigeta M, Yamanaka K, Saitou M. A comprehensive, non-invasive visualization of primordial germ cell development in mice by the Prdm1-mVenus and Dppa3-ECFP double transgenic reporter. Reproduction. 2008; 136:503–14. [PubMed: 18583473]
6. Hayashi K, Ohta H, Kurimoto K, Aramaki S, Saitou M. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell. 2011; 146:519–32. [PubMed: 21820164]
7. Capel B, et al. Deletion of Y chromosome sequences located outside the testis determining region can cause XY female sex reversal. Nat Genet. 1993; 5:301–307. [PubMed: 8275095]
8. Hunt PA, et al. Germ cell loss in the XXY male mouse: altered X-chromosome dosage affects prenatal development. Mol Reprod Dev. 1998; 49:101–11. [PubMed: 9444653]
9. Mahadevaiah SK, Evans EP, Burgoyne PS. An analysis of meiotic impairment and of sex chromosome associations throughout meiosis in XYY mice. Cytogenet Cell Genet. 2000; 89:29–37. [PubMed: 10894932]
10. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126:663–76. [PubMed: 16904174]
11. Eggan K, et al. Male and female mice derived from the same embryonic stem cell clone by tetraploid embryo complementation. Nat Biotechnol. 2002; 20:455–459. [PubMed: 11981557]
12. Robertson EJ, Evans MJ, Kaufman MH. X-chromosome instability in pluripotential stem cell lines derived from parthenogenetic embryos. J Embryol Exp Morphol. 1983; 74:297–309. [PubMed: 6886600]
13. Mintz B, Russell ES. Gene-induced embryological modifications of primordial germ cells in the mouse. J Exp Zool. 1957; 134:207–237. [PubMed: 13428952]
14. Sheppard O, Wiseman FK, Ruparelia A, Tybulewicz VLJ, Fisher EMC. Mouse models of aneuploidy. Sci World J. 2012; 2012:214078.
15. O'Doherty A, et al. An aneuploid mouse strain carrying human chromosome 21 with Down syndrome phenotypes. Science. 2005; 309:2033–7. [PubMed: 16179473]
16. Briggs JA, et al. Integration-free induced pluripotent stem cells model genetic and neural developmental features of down syndrome etiology. Stem Cells. 2013; 31:467–78. [PubMed: 23225669]
17. Maclean GA, et al. Altered hematopoiesis in trisomy 21 as revealed through in vitro differentiation of isogenic human pluripotent cells. Proc Natl Acad Sci U S A. 2012; 109:17567–72. [PubMed: 23045682]
18. Li LBB, et al. Trisomy Correction in Down Syndrome Induced Pluripotent Stem Cells. Cell Stem Cell. 2012; 11:615–619. [PubMed: 23084023]
19. Jiang J, et al. Translating dosage compensation to trisomy 21. Nature. 2013; 500:296–300. [PubMed: 23863942]
20. Bershteyn M, et al. Cell-autonomous correction of ring chromosomes in human induced pluripotent stem cells. Nature. 2014; 507:99–103. [PubMed: 24413397]
21. Amano T, et al. Correction of Down syndrome and Edwards syndrome aneuploidies in human cell cultures. DNA Res. 2015; 22:331–342. [PubMed: 26324424]
22. Irie N, et al. SOX17 is a critical specifier of human primordial germ cell fate. Cell. 2015; 160:253–268. [PubMed: 25543152]
23. Sasaki K, et al. Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells. Cell Stem Cell. 2015; 17:178–94. [PubMed: 26189426]
24. Ishii T, Pera RAR, Greely HT. Ethical and legal issues arising in research on inducing human germ cells from pluripotent stem cells. Cell Stem Cell. 2013; 13:145–8. [PubMed: 23910081]
25. Arnold AP, Chen X, Itoh Y. What a difference an X or Y makes: Sex chromosomes, gene dose, and epigenetics in sexual differentiation. Handb Exp Pharmacol. 2012; 214:67–88.

26. Burgoyne PS, Mahadevaiah SK, Perry J, Palmer SJ, Ashworth A. The Y* rearrangement in mice: new insights into a perplexing PAR. Cytogenet Cell Genet. 1998; 80:37–40. [PubMed: 9678332]
27. Hunt PA, Eicher EM. Fertile male mice with three sex chromosomes: evidence that infertility in XYY male mice is an effect of two Y chromosomes. Chromosoma. 1991; 100:293–9. [PubMed: 1860375]
28. Carey BW, et al. Reprogramming of murine and human somatic cells using a single polycistronic vector. Proc Natl Acad Sci U S A. 2009; 106:157–62. [PubMed: 19109433]
29. D'Hulst C, Parvanova I, Tomoiaga D, Sapar ML, Feinstein P. Fast quantitative real-time pcr-based screening for common chromosomal aneuploidies in mouse embryonic stem cells. Stem Cell Reports. 2013; 1:350–359. [PubMed: 24319669]
30. Nagy A, Gertsenstein M, Vintersten K, Behringer R. Karyotyping Mouse Cells. Cold Spring Harb Protoc. 2008; doi: 10.1101/pdb.prot4706
31. Nakaki F, et al. Induction of mouse germ-cell fate by transcription factors in vitro. Nature. 2013; 501:222–6. [PubMed: 23913270]
32. Kanatsu-Shinohara M, et al. Allogeneic offspring produced by male germ line stem cell transplantation into infertile mouse testis. Biol Reprod. 2003; 68:167–73. [PubMed: 12493709]
33. Kimura Y, Yanagimachi R. Mouse oocytes injected with testicular spermatozoa or round spermatids. Development. 1995; 121:2397–2405. [PubMed: 7671805]
34. Brambrink T, et al. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. Cell Stem Cell. 2008; 2:151–9. [PubMed: 18371436]

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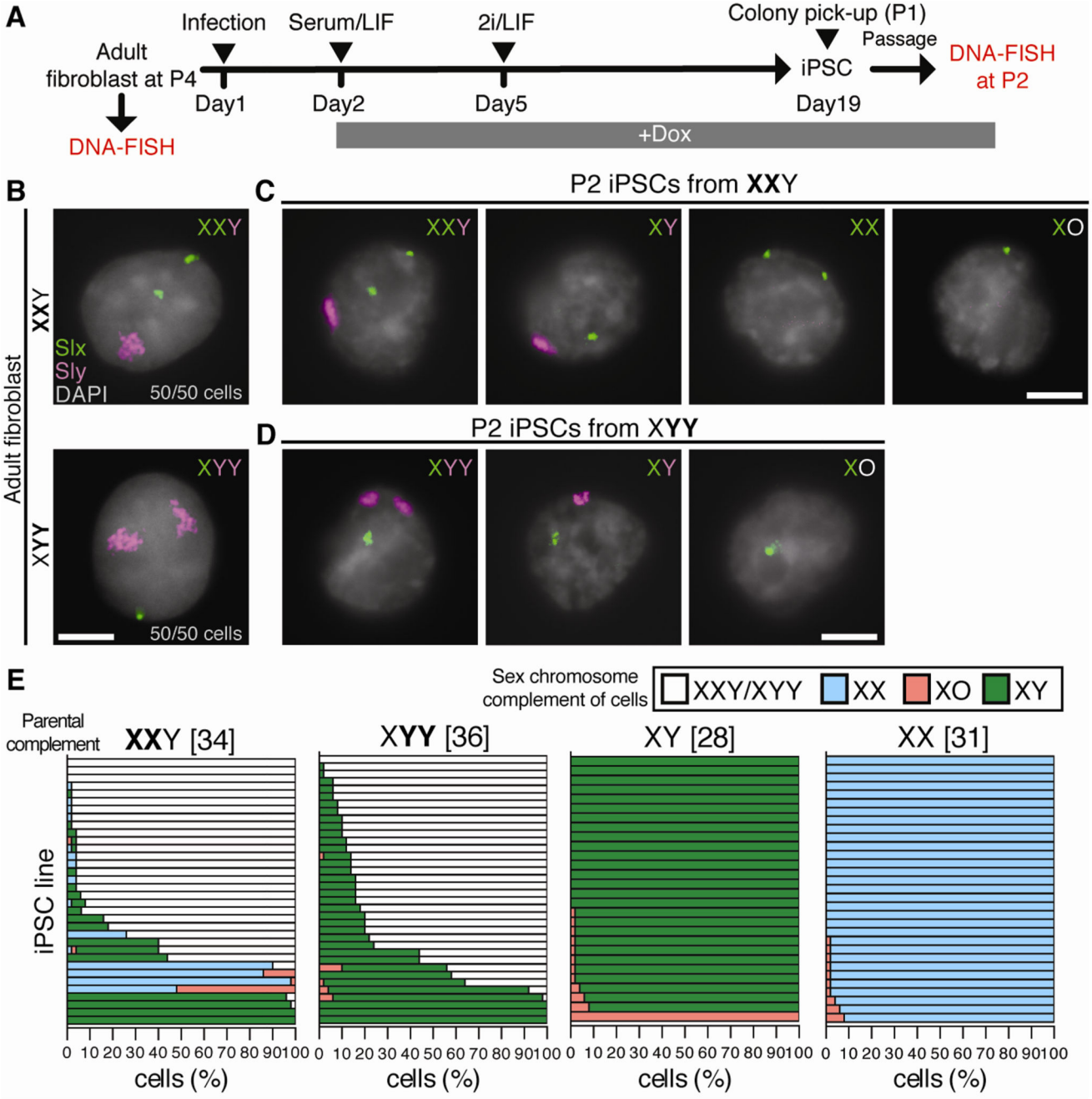

Fig. 1. Chromosome loss through iPSC reprogramming of SCT fibroblasts.

(**A**) Experimental scheme to generate XXY, XYY, XY and XX iPSCs. 2i: inhibitors of GSK3ß and Mek1/2. LIF: leukemia inhibitory factor. (**B-D**) *Slx* (green) and *Sly* (magenta) DNA-FISH of (B) fibroblasts and (C, D) P2 iPSCs from XXY and XYY mice (n = 50 cells). Scale bars: 5 μm. (**E**) P2 iPSC sex chromosome complements. Each bar represents an iPSC line and the percentage of cells exhibiting each complement (n = 50 cells per line). Numbers in brackets show number of iPSC lines examined. Data for two animals combined.

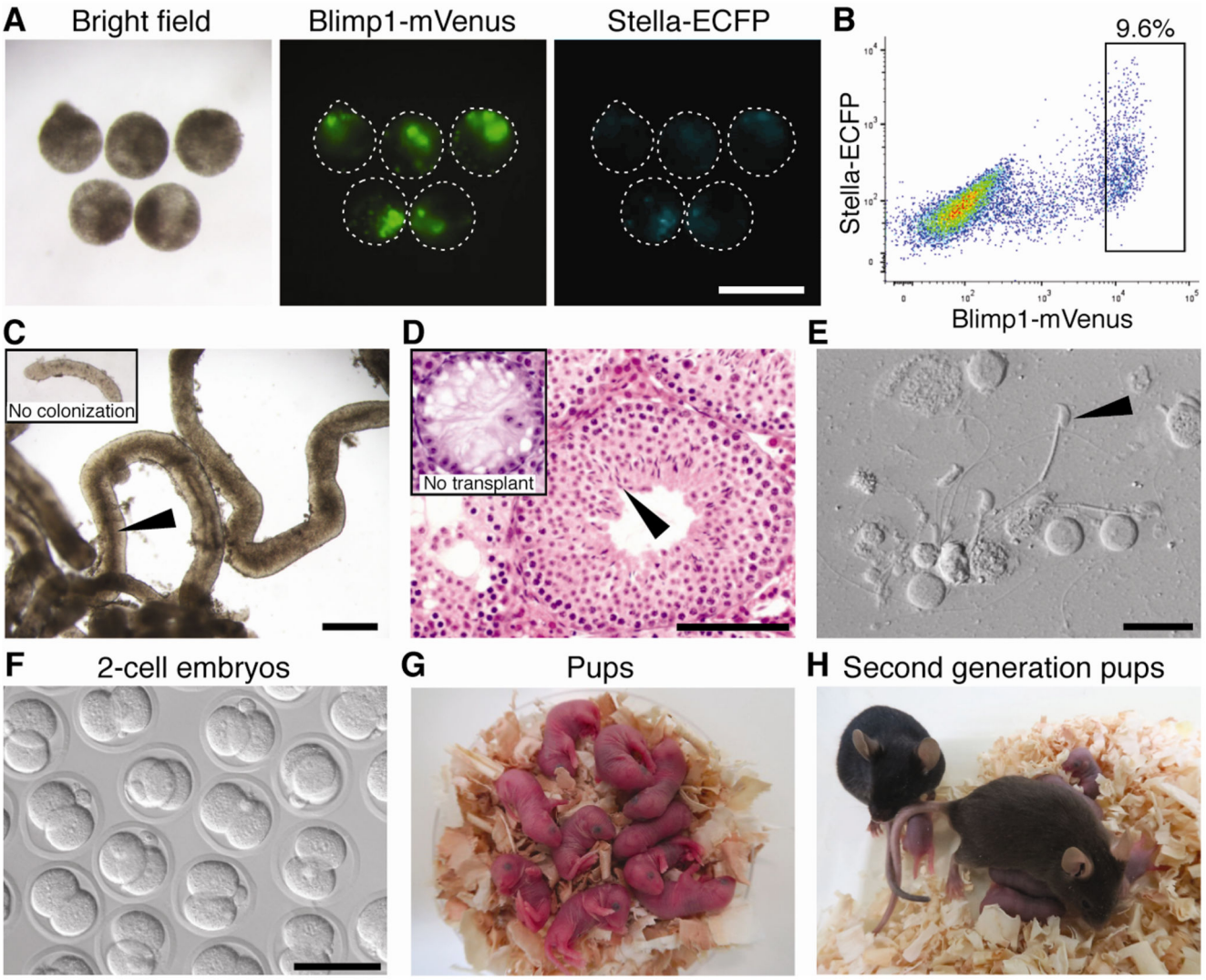

Fig. 2. PGCLC derivation and spermatogenesis from SCT-derived euploid iPSCs.

(**A**) PGCLC aggregates from iPSC line XXY 7-29-1. Green: Blimp1-mVenus. Cyan: Stella-ECFP. (**B**) FACS of resulting aggregates. Rectangle: population used for transplantation. (**C**) Tubule from transplanted testis. Dark region (arrowhead) indicates area with spermatids. Inset: area without spermatogenic colonies. (**D**) Histology of transplanted testis stained with hematoxylin and eosin. Inset: *W/W*^{v} testis without transplantation. Arrowhead: elongated spermatids. (**E**) Sperm (arrowhead) isolated from transplanted testis. (**F-G**) 2-cell embryos (F), and pups (G) produced by ICSI from XXY-derived sperm. (**H**) Pups from iPSC line XXY 7-29-1-derived male and female. Scale bars: 400 (A,C), 100 (D,F), 25 (E) μm.

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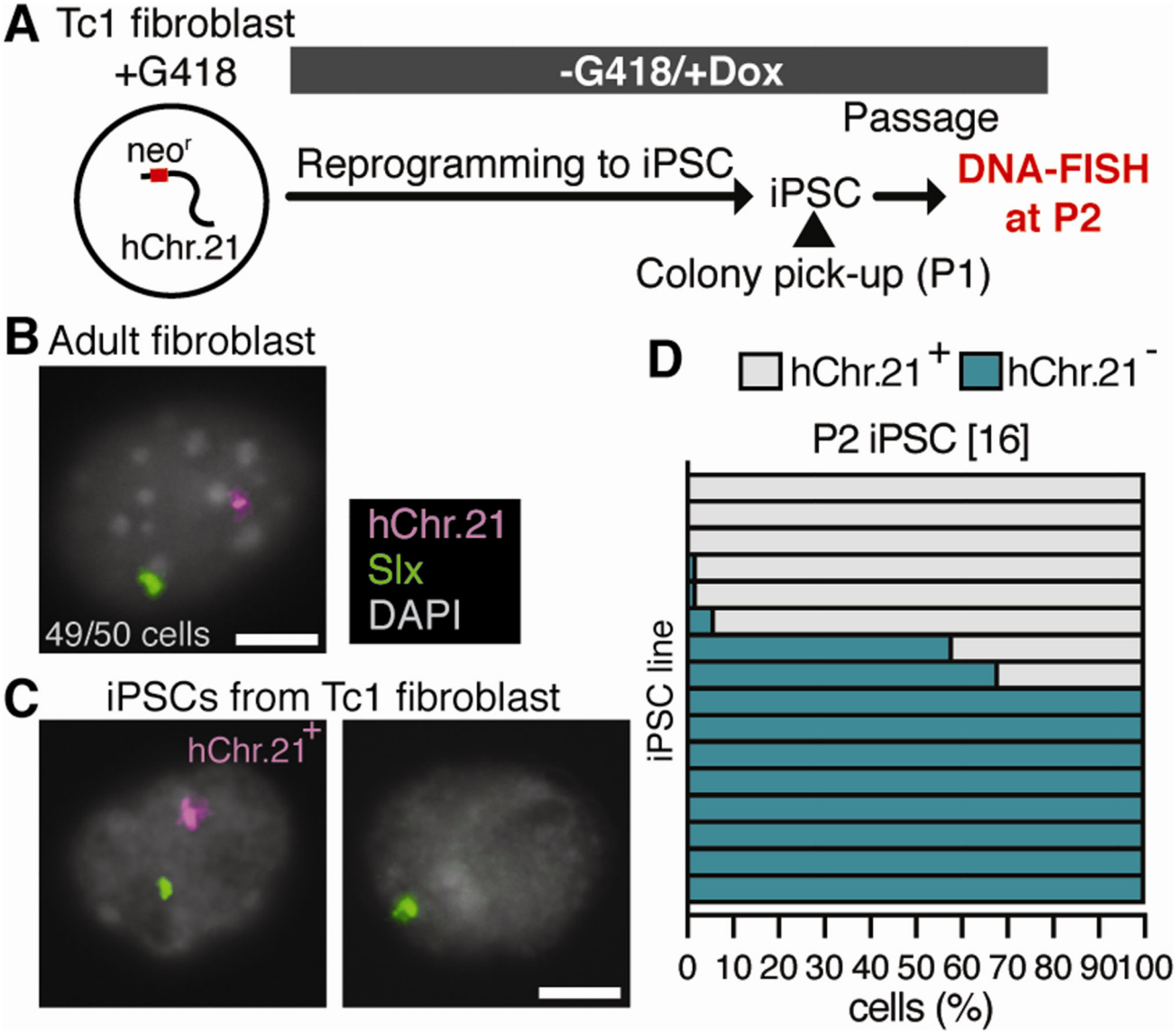

Fig. 3. Accessory chromosome 21 loss during iPSC reprogramming.

(**A**) Experimental scheme to generate Tc1 iPSCs. (**B-C**) *Slx* (green) and hChr.21 (magenta) DNA-FISH of (B) fibroblasts and (C) P2 iPSCs from Tc1 mice (n = 50 cells). Scale bars: 5 μm. (**D**) P2 iPSC chromosomal complements. Each bar represents an iPSC line and the percentage of cells exhibiting each complement (n = 50 cells per line). Numbers in brackets show number of iPSC lines examined. Data for two animals combined.

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Table 1
Spermatogenesis from transplanted PGCLCs.

Sex chromosome complement	iPSC line name	No. of testes transplanted	No. of testes dissected †	No. of testes with spermatogenesis	No. of spermatogenic colonies in each testis
XY from XXY	XXY 7-6	10	8	1	1 *
	XXY 7-7-1	6	6	3	1,1,1
	XXY 7-7-2	2	1	1	13
	XXY 7-8-2	6	5	4	3,3,4,8
	XXY 7-15-2	4	3	2	7,10
	XXY 7-21-2	6	5	3	2,3,15
	XXY 7-29-1	6	4	4	2,6,8,16
XY from XYY §	XYY 5-4	4	3	3	1,9,9
	XYY 5-5	6	6	2	2,10
	XYY 5-8	6	6	3	2,2,3
	XYY 7-3	6	4	3	5,11,14
Control XY	XY 6-6	6	4	3	1,15,20

† Some testes were not dissected and were used for histology analyses.

§ XY iPSC lines with wild type Y chromosome were used for transplantation.

* Sperm were very few.