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Supplemental Information

Targeted Deletion of an Entire Chromosome Using CRISPR/Cas9

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Figure S1 Gonadal phenotypes of E15.5 mouse embryos from CRISPR/Cas9 zygote injection**.** Phenotypically male (blue box) and female (pink box) gonads are shown with their sex chromosome dosage.

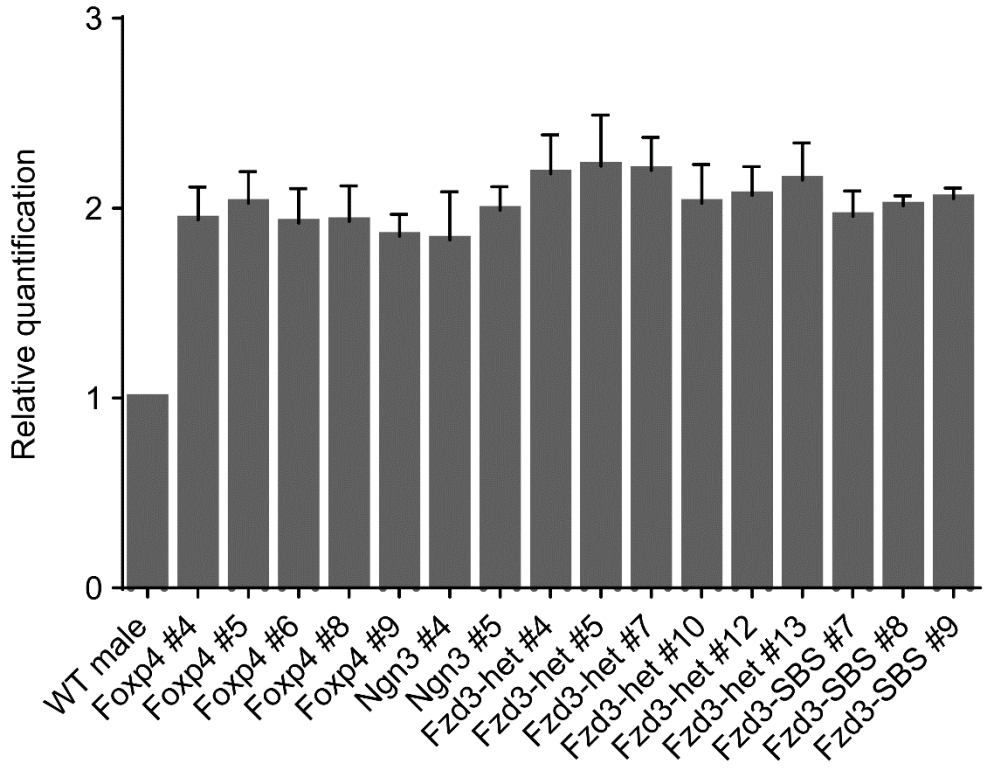

Figure S2 Assessment of X chromosome copy number by *Sox3* qPCR in female mice injected with autosomal gRNAs**.** Sixteen phenotypically female founder mice generated from injection of autosomal gRNAs targeting *Ngn3, Foxp4* and *Fzd3* genes had two copies of the X chromosome. These data indicate that spontaneous loss of the X chromosome does not occur in zygotes injected with CRISPR/Cas9 reagents. Data were presented as mean ± SD from $n \geq 3$ replicates.

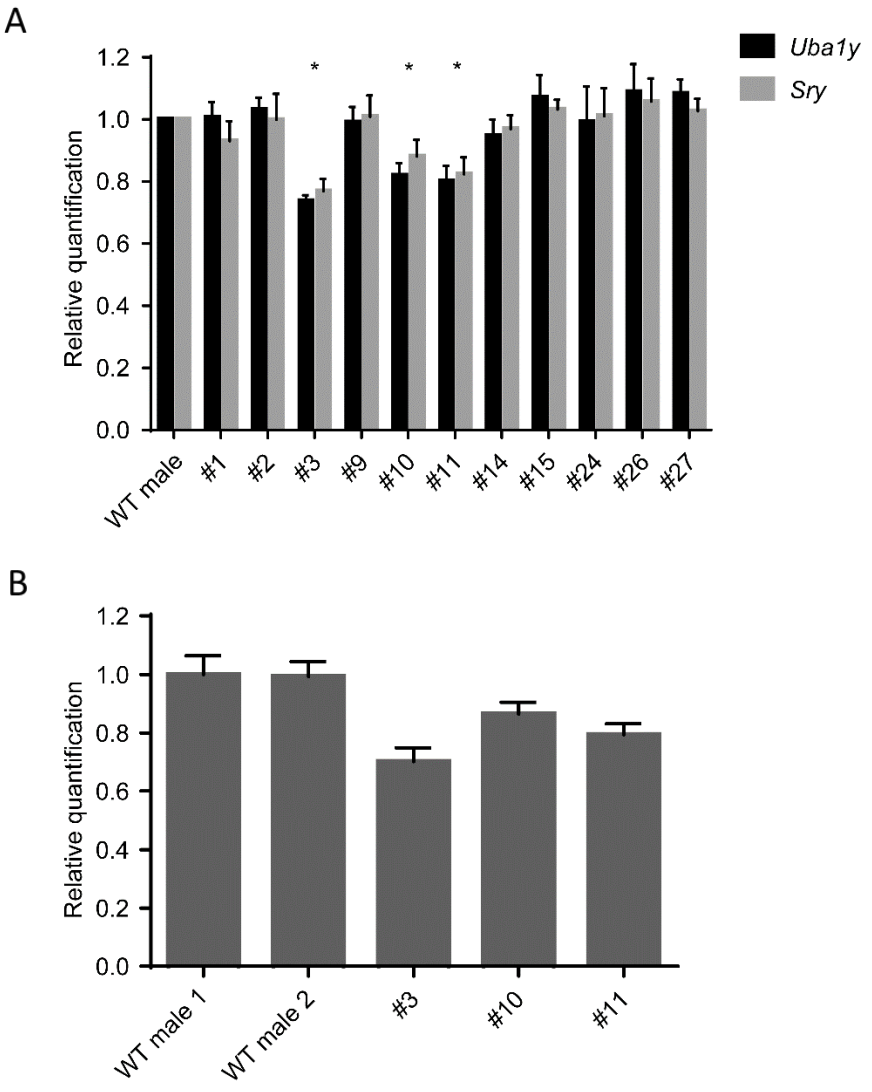

Figure S3 Assessment of Y chromosome dosage in males generated from gRNA centro 41X zygote injection. (**A**) qPCR analysis of *Uba1y* and *Sry* (which are located on Y short arm) revealed males with reduced Y dosage (asterisks) suggesting mosaic XY-XO. (**B**) Confirmation of the mosaicism by qPCR of *Gm28186* (which is located on Y long arm). Data were presented as mean ± SD from n ≥ 3 replicates.

Table S1 | List of gonadal phenotypes and genotypes of all mice generated from gRNA centro 41X injection

Identifier	Gonadal phenotype	Genotype	Additional info
#1	Male	XY	
#2	Male	XY	
#3	Male	XY, XO	Mosaic, with more than 70% XY cells
#4	Female	XO	
#5	Female	XX	
#6	Female	XX	
#7	Female	XX	
#8	Female	XX	
#9	Male	XY	
#10	Male	XY, XO	Mosaic, with more than 70% XY cells
#11	Male	XY, XO	Mosaic, with more than 70% XY cells
#12	Female	XX	
#13	Female	XX	
#14	Male	XY	
#15	Male	XY	
#16	Female	XX	
#17	Female	XX	
#18	Female	XX	
#19	Female	XO, XY_{short_arm}	Mosaic, half XO, half contain truncated Y short arm
#20	Female	XO, XY_{short_arm}	Mosaic, half XO, half contain truncated Y short arm
#21	Female	XO	
#22	Female	XO, XY_{short_arm}	Mosaic, half XO, half contain truncated Y short arm
#23	Female	XX	
#24	Male	XY	
#26	Male	XY	
#27	Male	XY	
#28	Female	XX	

Table S2 | On-targets and potential off-targets all the gRNAs used in this study (provided in excel file)

Table S3 | The two-way ANOVA statistical analysis of *Uba1Y* and *Erdr1* qPCR related to Figure 1B (provided in excel file)

Materials and Methods

gRNA screening and plasmid construction. sgRNAs were identified by manual screening of Y chromosome sequences using the CCTop gRNA design tool http://crispr.cos.uni-heidelberg.de/ provided by Stemmer *et al*. (2015). This tool was also used to predict the off-target potentials containing PAM sequences NGG and NAG (Supplementary information, Table S2). PX459.V2.0 (pSpCas9(BB)-2A-Puro, Addgene #62988) plasmid was used for Cas9 and sgRNA expression. PX459.V2.0 containing sgRNA was prepared as previously described by Ran *et al*. (2013). For dual gRNA centro 2X and long arm 2X, an additional U6-sgRNA cassette was added to the *NotI* site to allow simultaneous expression of two different gRNAs from single plasmid. Plasmid preparations were performed using PureLink® HiPure Plasmid Midiprep Kit (Life Technologies).

Cell culture and transfection. R1 mouse embryonic stem cells were cultured in 15% FCS/DMEM supplemented with 2 mM Glutamax (Gibco), 100 µM non-essential amino acid (Gibco), 100 µM 2-mercaptoethanol (Sigma), 3 µM CHIR99021 (Sigma) 1 µM PD0325901 (Sigma) and LIF (generated in-house). One million of ES cells were nucleofected with 3 µg of plasmid DNA using the Neon™ Transfection System 100 µL Kit (Life technologies) at 1400 V, 10 ms and 3 pulses according to the manufacturer's protocol. 24 hr after transfection, selection was conducted by adding puromycin (2 µg/ml) to the media for the next 48 hr. Surviving cells were cultured for 4-7 days without selection before harvesting.

DNA extraction and qPCR. Genomic DNA was extracted from 1-2 million ES cells or tail tissue using High Pure PCR Template Preparation Kit (Roche) according to the manufacturer's instructions. qPCRs were performed using Fast SYBR Green Master Mix (Applied Biosystems) on an Applied Biosystems 7500 StepOnePlus machine. *Sox1* qPCR was used as internal reference control to normalize qPCR value in all qPCR analyses.

FISH analysis. Cells were cultured in media containing 0.1 µg colcemid (Roche) for 1-2 hours, harvested and incubated in 0.075 M KCl hypotonic solution for 20 minutes. The cells were then fixed using methanol-acetic acid (3:1) solution, dropped onto slides and dried. FISH staining was performed using Mouse IDetect™ Chromosome Y Paint Probe (Empire Genomics) according to the manufacturer's instructions. Y signals were counted from both metaphase and interphase spreads.

Mouse zygote injection. All the experiments involving animal use have been approved by the University of Adelaide Animal Ethics Committee. Cas9 mRNA was produced by *in vitro* transcription of *XhoI*-linearized pCMV/T7-hCas9 (Toolgen) using mMESSAGE mMACHINE® T7 ULTRA Transcription Kit (Ambion). sgRNA centro 41X was generated according to a previously described protocol.[1] In brief, PCR was performed using a T7 containing forward primer 5'-TTAATACGACTCACTATAGAGGAGTTAATATAAAAAACA-3' and a reverse primer 5'- AAAAGCACCGACTCGGTGCC-3' and the PX459.V2 centro 41X plasmid template. The product was purified by QIAquick PCR Purification Kit (Qiagen) and used as a template for in vitro transcription using the HiScribe™ T7 Quick High Yield RNA Synthesis Kit (NEB). RNA purification was conducted using RNeasy Mini Kit (Qiagen). Cas9 mRNA (200 ng/µl) and sgRNA centro 41X (100 ng/µl) were injected to the cytoplasm of C57BL/6N zygotes using a Femtojet microinjector. The survival rate of injected zygotes was 89.4% (93/104). 57 zygotes were transferred into 3 pseudo pregnant females (19/recipient). 27 embryos with normal appearance were harvested at E15.5 for gonadal assessment and tissue collection.

List of gRNA sequences used

Name	gRNA sequences 5'-3'	Position in Y
Centro 2X	Left: GGATAAATGTTACATGCAA Right: GATAATAGTTTACTATTCTAA	4.064.613 4.163.810
Centro 41X	GGAGTTAATATAAAAAACA	4.065.169 to 4.159.436
Centro 140X	GAAGAATTACAATGAAAAATA	4.065.349 to 4.161.710
Long arm 2X	Left: GTCCTCTACGTCTATCAGGA Right: GTTTCCAGCCGGGTTTCTTAC	4.312.132 4.412.892
Long arm 8X	GTTCTATGTCAATTTAGGTGG	4.313.453 to 17.275.105
Long arm 45X	GACTGGGTTCTCCTAATCCTT	4.417.594 to 90.167.758
Long arm 116X	GTGGAATTGTGATCTAGATA	5.726.265 to 88.887.822
Long arm 298X	GGCAAAGCACTTCTGCACC	4.596.490 to 90.662.856
Neo	GGCAGCGCGGCTATCGTGGC	None in mouse

Red highlight indicates additional G was added to the gRNAs

List of qPCR primers

qPCR	F (5'-3')	R (5'-3')	Position
Uba1y (Y short arm)	GGCCACAGACTTGGGCCGAC	TGCCTTGTGGTGCCTGTGGC	chrY: 831.667 - 831.891 & 681.963 - 682.187
Erdr1 (Y long arm)	CTGACTGCGTACAGAAATGTCC	GGAAGACACACACACATCTGCA	chrY: 90.822.353 - 90.822.426 & 90.816.416 - 90.816.489
Sry (Y short arm)	CATTTATGGTGTGGTCCCGTG	ATCTTCAATCTCTGTGCCTCCT	chrY: 2.663.492 - 2.663.607
Gm28186 (Y long arm)	CATGCCTCAGACCCCTCAAG	TCTGACCATTGTGACTCAGAC	chrY: 4.372.957 - 4.373.114
Xist	GCCATCCTCCCTACCTCAGAA	CCTGACATTGTTTTCCCCCTAA	chrX
Dmd	ACAGCAAGCAAGCAAGGCTTC	CTCACCAGTGACCCTATGATTGC	chrX
Sox3	CGTTGCCTTGTACCGAAGAT	CGGGACTTCTCGCTTTTGTA	chrX
Sox1	GACTTGCAGGCTATGTACAACATC	CCTCTCAGACGGTGGAGTTATATT	Chr8

Supplemental Reference:

1. Wang, H. et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell* **153**, 910-918 (2013).