Expanded View Figures

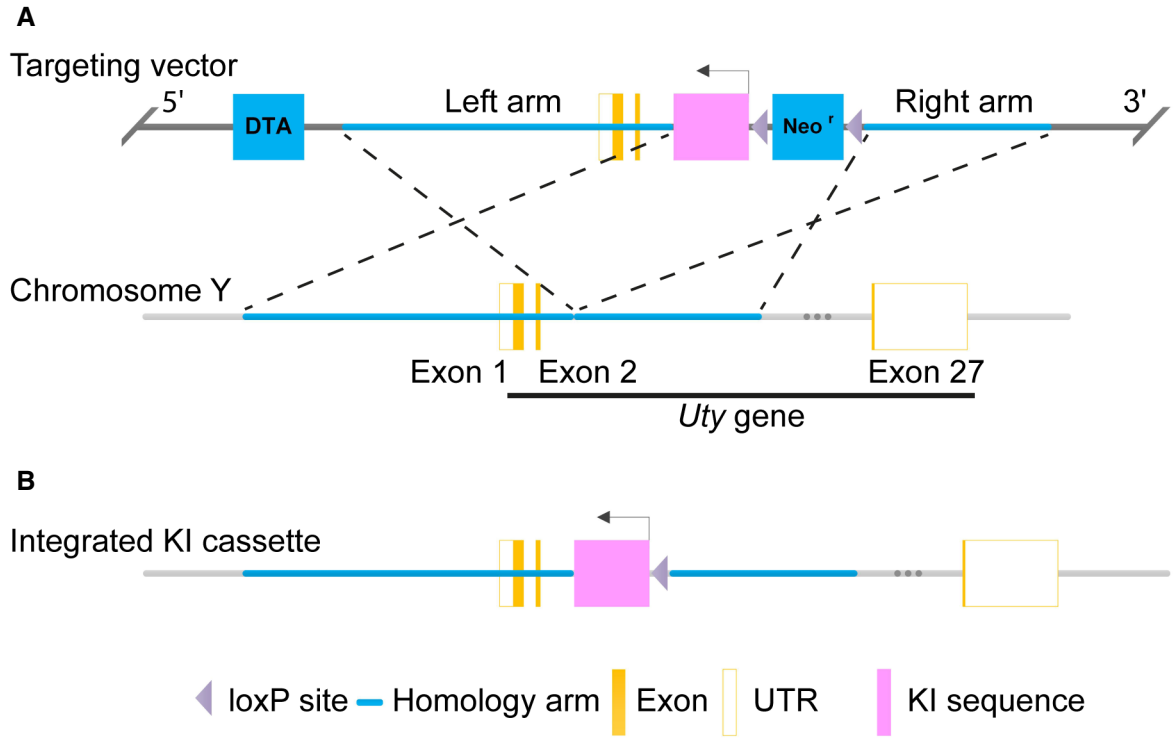
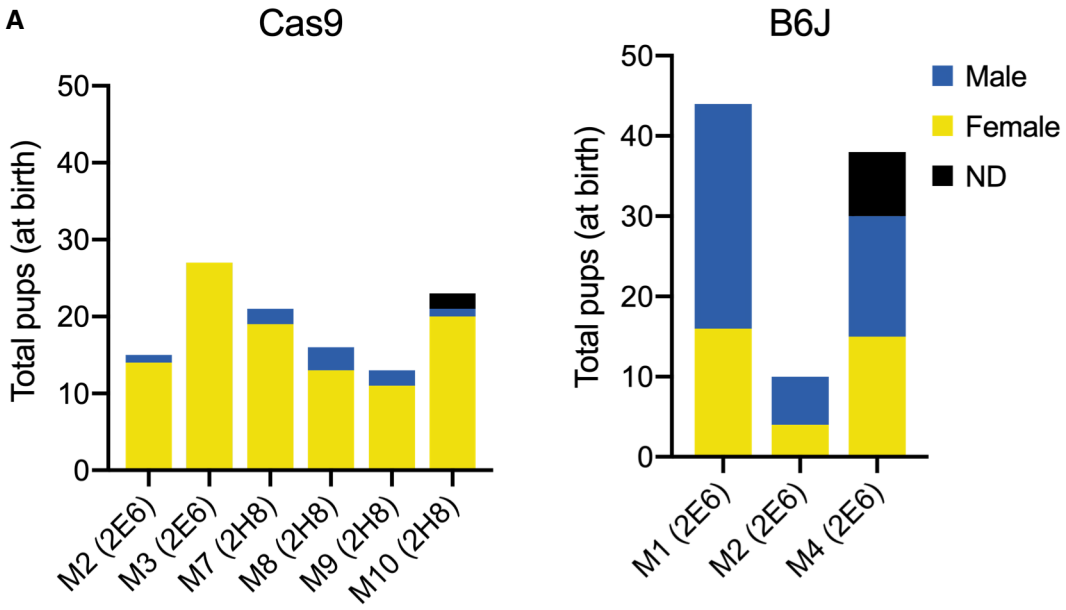


Figure EV1. Schematic summary of Y-line generation.

A Mouse genomic fragments containing homology arms to the *Uty* gene located on mouse chromosome Y were amplified from a BAC clone and sequentially assembled into a targeting vector along with negative and positive selection markers (DTA and Neo, respectively). There are only few mice reported with a transgene on the Y chromosome, one of which is on the *Uty* gene [28], and thus, we chose to target the KI cassette into this gene. The KI cassette, encoding the gRNAs targeting genes *Atp5b*, *Cdc20*, and *Casp8*, each expressed from a U6 promoter, was inserted into the targeting vector, which was designed to integrate in reverse orientation of the 2nd exon of the *Uty* gene.

B The KI cassette integrated into the *Uty* gene and the Neo cassette was self-deleted due to the loxP sites. Details on the linearization of the targeting vector, its transfection into C57BL/6N ES cells, as well as PCR and Southern blot analyses and ES implantation procedures, are provided in the Appendix.

A



B

Cross			Female/Male/ND								
Female	Male		Litter 1	Litter 2	Litter 3	Litter 4	Litter 5	Litter 6	Litter 7	Total	Total
	Number	Clone									
Cas9	2	2E6	4/1/0	2/0/0	2/0/0	6/0/0				14/1/0	41/1/0
	3		4/0/0	4/0/0	9/0/0	5/0/0	3/0/0	2/0/0		27/0/0	
	7	2H8	5/0/0	2/0/0	3/2/0	1/0/0	3/0/0	2/0/0	3/0/0	19/2/0	63/8/2
	8		3/2/0	6/1/0	4/0/0					13/3/0	
	9		3/2/0	1/0/0	3/0/0	3/0/0	1/0/0			11/2/0	
	10		2/0/2	1/1/0	1/0/0	3/0/0	8/0/0	5/0/0		20/1/2	
B6J	1	2E6	1/7/0	3/4/0	5/2/0	1/6/0	3/4/0	3/5/0		16/28/0	35/49/8
	2		1/6/0	3/0/0						4/6/0	
	4		0/0/4	4/3/0	5/3/0	4/3/0	2/1/4	0/5/0		15/15/8	

Figure EV2. Sex of pups from individual Y-line males.

A, B Total pups and their sex resulting from crosses between the indicated males from indicated clones (2E6 and 2H8—detailed in the Appendix) and the indicated females in graphical (A) and numerical (B) representations. ND, not determined due to parental infanticide.

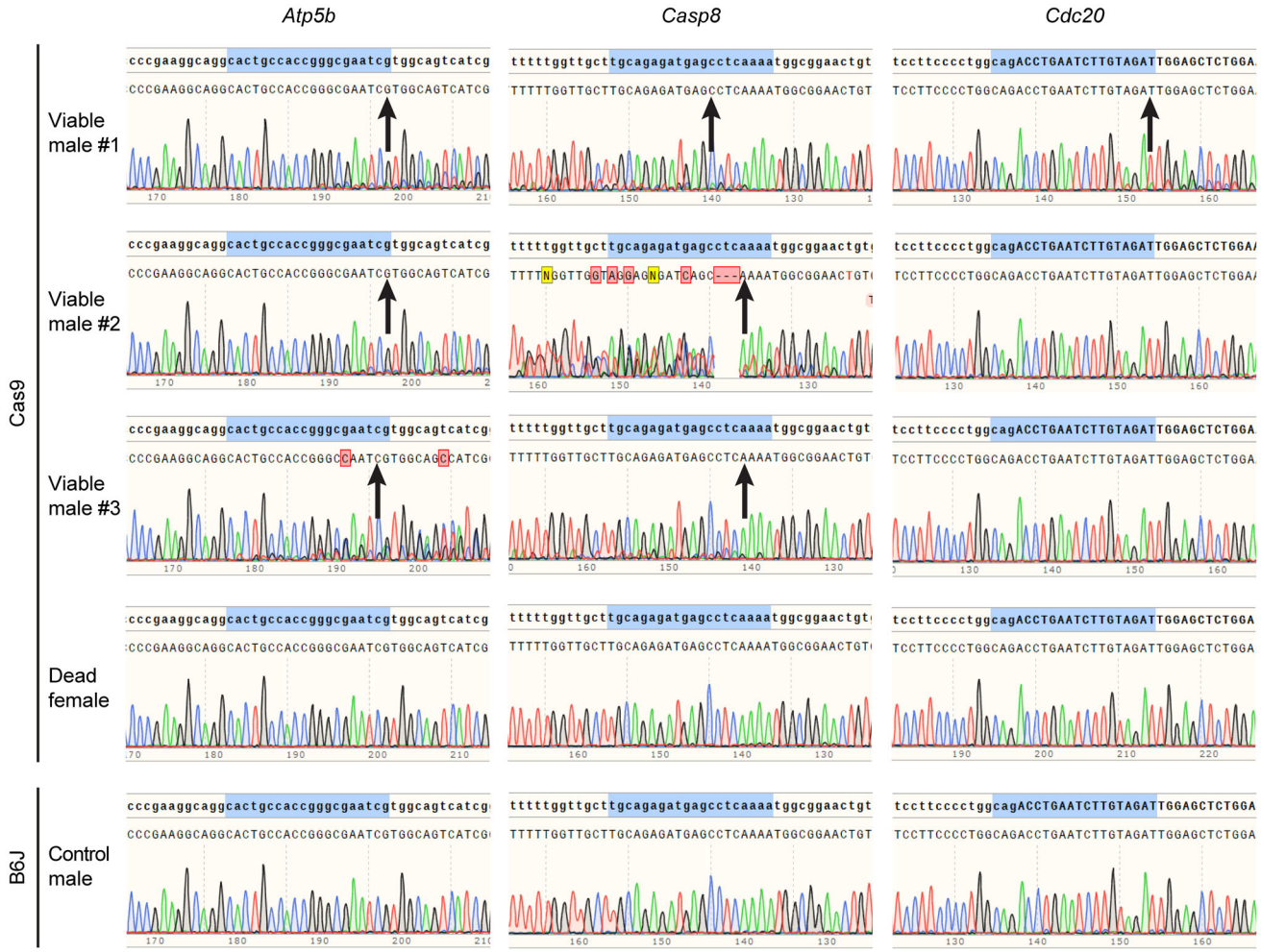


Figure EV3. Partial disruption of target genes in viable male pups.
Chromatograms of Sanger DNA sequencing of the indicated PCR amplified genes from samples of mice taken at P0 from the cross of the Y-line males and the indicated females. Targeted regions homologous to the gRNAs are highlighted in blue. Red boxes represent deviations between the expected and observed sequences. Arrows point to indel locations ($P < 0.001$, two-tailed *t*-test of the variance–covariance matrix of the standard errors), as determined by Tracking of Indels by Decomposition [27].

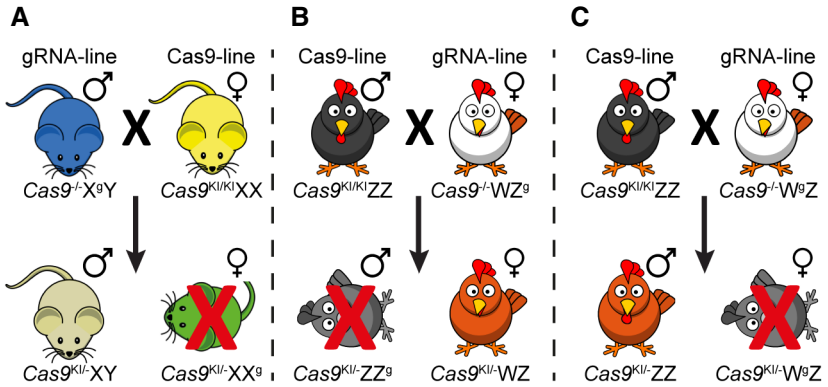


Figure EV4. Alternative biasing of sex in mice and birds.

A–C Schematic illustrations of hypothetical crosses between (A) mice producing only males, (B) birds producing only females, and (C) birds producing only males.