

Figure S1. Dual sgRNA-directed mutation of *SRY* in zygotes. (A) Mutation detection in blastocyst by PCR. M, marker; 1#–13# represent different male blastocysts used in this study. M DNA marker. (B) Mutation detection in blastocyst by T-cloning and Sanger sequencing. The WT sequence is shown at the top of the targeting sequence. sgRNA sequences are marked in green and the protospacer adjacent moti (PAM) sequences are in red with underline. WT: wild type; deletions “-”; insertion “+”.

| | SgRNA1 | SgRNA2 | |
|-----------|--|--------|---------|
| SRY GENE | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| HEART | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| LIVER | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (+1bp) |
| | ATACAGGAGGAACACGTAAGT--- <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (-3bp) |
| | ATACAGGAGGAACACGTAAGTG-----ACGGGTCAAGCGACCCATGAAC | | (-11bp) |
| SPLEEN | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (+1bp) |
| | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (+2bp) |
| | ATACAGGAGGAACACGTAAG--- <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (-4bp) |
| | ATACAGGAGGAACACGTAAGT----- <u>AGGAACGGGTCAAGCGACCCATGAAC</u> | | (-8bp) |
| | ATACAGGAGGAACACGTAAGTG-----ACGGGTCAAGCGACCCATGAAC | | (-11bp) |
| LUNG | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| | ATACAGGAGGAACACGTAAGT----- <u>AGGAACGGGTCAAGCGACCCATGAAC</u> | | (-8bp) |
| SKIN | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (+2bp) |
| | ATACAGGAGGAACACGTAAG--- <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (-4bp) |
| | ATACAGGAGGAACACGTAAGT----- <u>AGGAACGGGTCAAGCGACCCATGAAC</u> | | (-8bp) |
| KIDNEY | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| | ATACAGGAGGAACACGTAAGTGA- <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (-1bp) |
| | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (+1bp) |
| | ATACAGGAGGAACACGTAAGTG-----ACGGGTCAAGCGACCCATGAAC | | (-11bp) |
| MUSCLE | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| INTESTINE | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| STOMACH | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| GENITAL | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (WT) |
| | ATACAGGAGGAACACGTAAGTGAG <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (+2bp) |
| | ATACAGGAGGAACACGTAAG--- <u>CGGCCA</u> GGAACGGGTCAAGCGACCCATGAAC | | (-4bp) |
| | ATACAGGAGGAACACGTAAGT----- <u>AGGAACGGGTCAAGCGACCCATGAAC</u> | | (-8bp) |
| | ATACAGGAGGAACACGTAAGTG-----ACGGGTCAAGCGACCCATGAAC | | (-11bp) |

Figure S2. T-cloning and Sanger sequencing for mutation detection of multiple organs in the *SRY*-mutant chimeric rabbits. The PAM sites are underlined and highlighted in red; the target sequences are shown in green; the insert sequences are shown in yellow. Deletions (–) and insertions (+) are shown. WT, wild-type control.

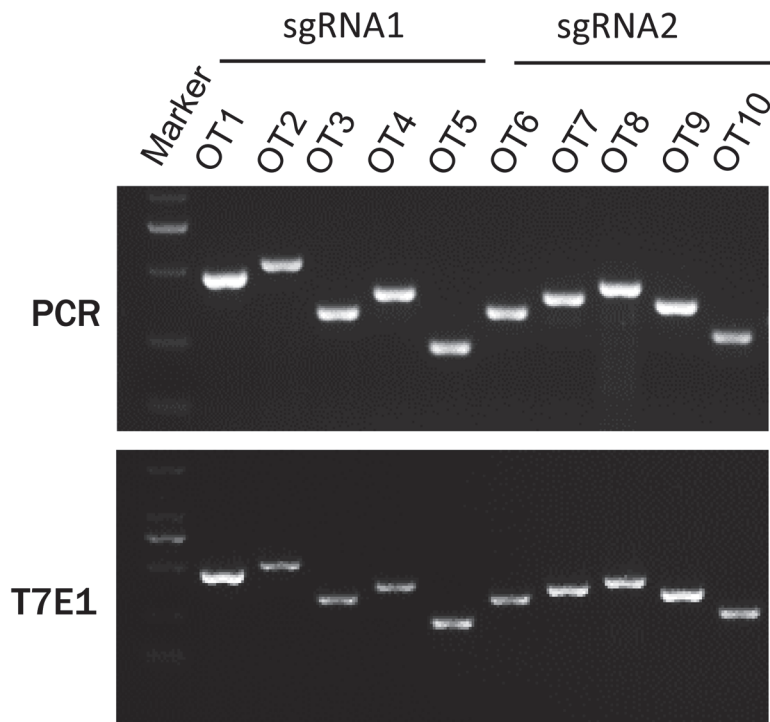


Figure S3. Off-target analysis of the 10 potential off-target sites (POTS) for sgRNA1 and sgRNA2 in the *SRY* KO rabbits.

Table S1: Primers used for construction of sgRNA expression plasmids and PCR mutation detection.

| NO. | Name | Primers | Sequence(5'-3') |
|------------|-------------|----------------|-------------------------|
| 1 | sgRNA1 | gRNA -1-F | TAGGAGGAACACGTAAAGTGAG |
| | | gRNA -1-R | CACCCTCACTTTACGTGTTTCCT |
| 2 | sgRNA2 | gRNA -2-F | TAGGAGGAACACGTAAAGTGAG |
| | | gRNA -2-R | CACCCTCACTTTACGTGTTTCCT |
| 3 | SRY | SRY-F | AGGCATGGAATCCACAATGA |
| | | SRY-R | AATGGGTAGTTTACAGGGAGATG |

1 and 2 were used for the construction of sgRNA expression plasmids; 3 was used for the PCR mutation detection.

Table S2.10 potential off-target sites examined by PCR and primers used for list.

| | sgRNA Sequence | Potential Off Target Sites | Number of mismatch | Position |
|----|--------------------------|----------------------------|--------------------|---------------------|
| s1 | GGAGGAACACGTAAA GTGAG | GGAGGAGGACGTAAAG TGAG | 2 | chr16:-21600211 |
| | | GGAGGAACAAGTAAAG TGGG | 2 | chrUN0:+295810 5 |
| | | GAAGGAGCAGGTAAAG TGAG | 3 | chrUN0:-2242557 |
| | | ACAGGAATAAGTAAAG TGAG | 4 | chr12:+15386370 |
| | | TGTGGAAATGTAAAG TGAG | 4 | chr16:+67886992 |
| s2 | TGGGTCGCTTGACCCG TTCC | TGGGCCGCTTGACCCG GTCC | 2 | chrUN0:+23582 |
| | | TGGGGCTCTTGCCCCG TTCC | 4 | chr15:-4547229 |
| | | TGGGTGGCTGGAGCCG TTCC | 3 | chrUN0:-157366 |
| | | TGTGTGCCTGGACCCG TTCC | 4 | chr5:-1695177 |
| | | TGGGCTGCTAGTCCCGT TCC | 4 | chr1:-194836712 |