Knockout mice created by TALEN-mediated gene targeting

To the Editor:
Phenotypic analysis of gene-specific knockout mice has transformed our understanding of in vivo gene functions. Generation of knockout mice, however, remains a time-consuming and expensive process. Transcription activator-like (TAL) effector nucleases (TALENs) are highly effective in inducing mutations at specific genomic loci, and consequently TALEN-mediated mutagenesis in zygotes is a potential alternative to conventional gene targeting in mice. However, to the best of our knowledge, gene knockout mice have yet to be created using TALENs. Here, we report the generation of mice with a genetic knockout of the progesterone immunomodulatory binding factor 1 (Pibf1) or selenoprotein W, muscle 1 (Sepw1) gene using TALENs.

To target these genes in the mouse genome, we designed and synthesized highly active TALENs specific to exon 2 of Pibf1 (Pibf1- TALEN; Fig. 1a, Supplementary Figs. 1 and 2) and exon 1 of Sepw1 (Sepw1- TALEN, Supplementary Figs. 3 and 4). When tested in mouse NIH3T3 cells, Pibf1- TALEN efficiently induced small deletions at the target site (Supplementary Fig. 1b). Each TALEN mRNA pair was injected into the cytoplasm of mouse pronuclear-stage embryos to produce mutant founders (F0) with mutations in Pibf1 (Fig. 1, Table 1 and Supplementary Fig. 5) or Sepw1 (Supplementary Table 1 and Supplementary Fig. 6a,b).

Most TALEN-induced mutations were deletions of variable lengths that induced frameshifts in the Pibf1 and Sepw1 genes (Fig. 1b, Supplementary Figs. 5b and 6b). In-frame mutations, as a result of deletions and substitutions of specific amino-acid residues, were also frequent (Fig. 1c). Such mutations will be beneficial for studying putative domain- or amino acid residue-specific functions of the gene products. Insertional mutations were only observed in two instances (Supplementary Fig. 5b,c).

To investigate the dose-dependent effects of the TALEN mRNAs, two different concentrations of Pibf1- TALEN mRNA (50 ng/µl and 20 ng/µl) were used, yielding 29 mutants (55.8%) from 52 newborns (Fig. 1b, Table 1 and Supplementary Fig. 5). Bi-allelic mutations were observed in seven mutant mice (Fig. 1c and Supplementary Fig. 5b). The mutation rate was approximately proportional to the injection dose of Pibf1- TALEN mRNA. Injection of a high concentration (50 ng/µl) of Pibf1- TALEN mRNA yielded 10/13 (76.9%) mutant F0 mice, whereas injection of a lower dose (20 ng/µl) yielded 19/39 (48.7%) F0 mutant mice (Table 1). Bi-allelic mutations were also found more frequently in the high-dose (6/8 F0) than in the low-dose (1/19 F0; Fig. 1c and Supplementary Fig. 5) group, indicating that Pibf1- TALEN activity was dose-dependent. Furthermore, relatively large deletions were more frequently observed in mutant founders obtained by high-dose injection than in those by low-dose injection (Fig. 1c and Supplementary Fig. 5b).

In contrast to the mutation rate, the number of mutant mice produced by the low-dose injection (19 mutants/176 transplanted embryos, 10.8%) was ~2.5-fold larger than...
the number produced by the high-dose injection (10 mutants/243 transplanted embryos, 4.1%; Table 1). This phenomenon is reminiscent of the toxicity of zinc-finger nucleases (ZFNs) induced by generation of nonspecific double-strand breaks at off-target sites. Indeed, IgM mutant rats obtained by intracytoplasmic injection of IgM-TALEN mRNA had modifications at an off-target site. Although we did not detect off-target effects of Pibf1-TALEN by T7E1 assays (Supplementary Table 2 and Supplementary Fig. 7), we cannot rule out the possibility that nonspecific effects might have caused embryonic toxicity.

To validate TALEN-induced mutations, we measured the level of Sepw1 protein in tail biopsies. No expression of Sepw1 protein was detected in a Sepw1 mutant founder possessing bi-allelic null mutations at their start codon (Supplementary Fig. 6c).

We crossed three F0 Pibf1 mutants to wild-type mice and determined the genotypes of the F1 offspring. All the mutations observed in F0 mice were transmitted through the germline (Supplementary Fig. 8). These results indicate that TALEN-introduced mutant alleles were stably inherited by their F1 progeny.

Mosaicism was rarely detected in both Pibf1 (Supplementary Fig. 5b, c) and Sepw1 mutants (Supplementary Fig. 6b), indicating that TALENs are primarily active at the one-cell stage. To demonstrate that Pibf1-TALEN is active at this stage, we amplified the start codon (Supplementary Fig. 6c), indicating that the activity of Pibf1-TALEN was not sustained in blastomeres at the two-cell stage. This pattern is reminiscent of the maternal-to-zygotic transition (MZT) that actively eliminates maternal-provided gene products. Although more detailed studies should be conducted that are designed to provide more direct evidence for TALEN activity at different developmental stages, our results suggest that TALEN activity is not likely to be maintained after the first cleavage of one-cell embryos.

Our study establishes that TALEN-mediated gene targeting is an efficient method for creating heritable null mutations in a specific locus of the mouse genome. We also provide evidence that TALEN activity in one-cell embryos is sufficient to induce mutations. Taken together, these data suggest that TALEN-mediated *in vivo* mutagenesis might expedite the creation of genetically engineered mouse models and thereby help to accelerate functional genomic research.

**Note:** Supplementary information is available at http://www.nature.com/doifinder/10.1038/nbt.2477.

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**AUTHOR CONTRIBUTIONS**

H.-W.L. and J.-S.K. wrote the manuscript. All the other authors performed the experiments.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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**Table 1** TALEN-mediated Pibf1 gene targeting in C57BL/6J mice.

<table>
<thead>
<tr>
<th>Dose of TALEN-Pibf1 mRNA (ng/µl)</th>
<th>Number of injected zygotes</th>
<th>Number of surviving zygotes</th>
<th>Two-cell embryos</th>
<th>Transferred embryos</th>
<th>Newborns</th>
<th>Founders*</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>276</td>
<td>263 (95.3%)</td>
<td>262 (99.6%)</td>
<td>243</td>
<td>13 (5.3%)†</td>
<td>10 (76.9%)††</td>
</tr>
<tr>
<td>20</td>
<td>183</td>
<td>176 (96.2%)</td>
<td>176 (100%)</td>
<td>176</td>
<td>39 (22.2%)†</td>
<td>19 (48.7%)</td>
</tr>
</tbody>
</table>

Percentages were calculated using the number in each column as the numerator and the number in the column to its left as the denominator. *Determined by T7E1 assays. †Two pups were cannibalized at birth. ††T7E1 assays were conducted using genomic DNA samples from newborns, including the two cannibalized pups.

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**Fig. 1**

A
d
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