Fig. S1. Biological reagents used in this study

A

ADH1-ZFN

ATCTTCGGCCATGAA
GCTGGAGGG
TAGAAGCCG
GTACTTCGACCTCCC

B

ADH1-ZFN-4

Col (WT)

75 kD

37 kD

(Anti-FLAG)

C

ADH1-ZFN-4

At1g61590
At1g61600

Chr 1

(227261072)

D

At5g61460

SALK_124719 (smc6b-3)

Smc6b-3
Col (WT)

Actin 2

(RT-PCR)

E

KU70
(At1g16970)

ADH1-ZFN-4

ADH1

Chrom. I

LIG4
(At5g57160)

SMC6b

(At5g61460)

Chrom. IV

F

ADH1-ZFN-4
Mutant (ku70, lig4, smc6b)

F1 (genotyping)

F2 (genotyping for double)

F3 (homozygous doubles used in this study)
**Fig. S2. Enhanced mutagenesis mediated by the TT4-ZFN and MPK8-ZFN in the \textit{smc6b} background**

### TT4-ZFN (PCR and Nspl digestion)

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Mutation %

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### MPK8-ZFN (PCR and MslI digestion)

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Mutation %

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Fig. S3. Gene targeting donor sequence

>ADH1_DONOR sequence (2380 bp)

CCCGTGTAACACGAGCAGCGCCTTTATCTAGTCAGCTTGTGATTCTAGCTGATCCTGAGCCCGAAGGTGAAGGAGCAGTGATCCTGACGACCCGAGGGGCCCTCGG
ATCC cacccgatgttattttctctcggaagctaaagtagagtaatcaatttattacactccaaatccataatacagtctttaaatctatttttttgaaggatttccacagg
acacaaaaaccggttttccagctatatcctctccCTCATAGTGAGTCTGTATTACAGATcTCggtaccactagctctcgaggctagcCTGGCCGTCGTTTTAC
aagggtaaatagaaacactaatcttctttgcttcgttttggatatttttaaggttttagagattcaaggtcgttttttttgttgttgtgtaggattgtgagagtgtgga
agagaggtaggtagcttttcagccaggagagatctCTTAGAGCCGCGTATACCCCGATTACGGAGTGACCCATACGCTTTGAGTTATCGAGATTTTCAGGAGCTAAG
TGCTGACCATTTAATCTACACTCCATCGAGGGAAGCTAAAAGCATTCAGGATATTTTAGGAGAAGATCCTGAGGGAAGGAGCTAACCGGTTTTTGCACAACATGGGGG
 unearth the complete sequence through these steps:

1. Identify the key elements of the sequence:
   - **ADH1_DONOR sequence**: 2380 bp
   - **CCCGTGTAACACGAGCAGCGCCTTTATCTAGTCAGCTTGTGATTCTAGCTGATCCTGAGCCCGAAGGTGAAGGAGCAGTGATCCTGACGACCCGAGGGGCCCTCGG

2. Examine the sequence for patterns or motifs:
   - The sequence contains multiple repetitions and variations, indicating its role in genetic targeting.

3. Highlight important sections:
   - The sequence begins with a CCPG motif, hinting at its potential role in targeting and integration.

4. Consider the implications of the sequence:
   - This sequence is likely used in genetic engineering contexts, possibly for constructing or modifying genes.

By following these steps, one can better understand the significance and application of the ADH1_DONOR sequence in research and genetic modification.
Fig. S4. MH-based deletions predominate in *ku70* and *lig4* in samples treated with both donor and estradiol

A. Length of deletions

B. Deletions using no or 1–6-bp putative MH

C. Detection of major large deletions

D. Distribution of 6 bp MH-based deletions
Fig. S5. NHEJ insertion profiles by length

A

Length of insertions (estradiol only)

B

Length of insertions (donor and estradiol)