**Table S4:**

Recombination between two visible markers.

Recombination is induced between w+ (CIGARmCherry,w+ at ZH attP 102F) and svspa-pol (on chromosome Dp(1;4)1021, y+(left arm of 4th chromosome) svspa-pol).

CTGTTGATAAGCACGCAATC is the sgRNA-3 used for the experiment.

Direct injection of sgRNA-3/Cas9 complexes

**PCR Primers**

For sequencing the primer **AACTACCATCAGGACTTTCAAG** was used

PCR product (**using primers**):

**AACTACCATCAGGACTTTCAAG**TAATTTAAATAGGCTGAACCTTTTCGGTCTGAAACTTGTGAATTGCACACTATATGTATTTTAAAAGGCAAATGAAATGTTAAATCTACATTAAAATTGATGTTATTTTTGAATAAAAATTCAAGATTTGTAAACAATATTTCCGCATCTTTCGCATCCTTTTTTAAGTTCCAAACATTCCTGATCTGCTAAGATAGGTTAAAGTATTTCGGGGAATCTTCAACTTTA**CTGTTGATAAGCACGCAATCGGG**ATATTAGACTTCTGCAGTTGCTTGCAATTTTTTAAGCCTTCACAATTATATTTATATTTTGAACCATTTTTTGATATTATAAATTAAACTTATTCATGTATTAATACTATTTATTAATCGTCTTTAAGTAAAGAATGAGCAATTATGCTCATACATACTTAAAAAAAAAACCATGTGTTACCAAACTTAGGGAAGTGCGTGGAAGACGGATATAAAGCAAAGTGACGTTATACATGATTTTACATTTACATTAAATTCTTATATGACAATTTATGTTTTTA**GAGTCGTAGGCGTTATATAGTT**

**Sequences of the target sites of the recovered recombinants:**

Genotype of the recovered animals (lines):

1-1: yw; CIGARmCherry,102F,**w+,** **svspa-pol** / Dp(1:4)1021, y+, svspa-pol

52-1: yw; Dp(1:4)1021, y+ , **sv+** / Dp(1:4)1021, y+, svspa-pol **(**🡪 **w-)**

Original GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATC**GGG**ATATTAGACTTCTGCAG

 sgRNA-3 PAM

line:

1-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

7-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

18-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

41-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

46-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

52-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

53-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

59-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

61-2 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

70-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

75-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

85-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

85-2\* GGGGAATCTTCAACTTTACTGTTGATAAGCA------CGGGATATTAGACTTCTGCAG

85-3\* GGGGAATCTTCAACTTTACTGTTGATAAGCA------CGGGATATTAGACTTCTGCAG

91-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

93-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

108-2 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

112-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

115-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

115-2 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

117-1 GGGGAATCTTCAACTTTACTGTTGATAAGCACGCAATCGGGATATTAGACTTCTGCAG

Except for the recombinants 115-1 and 115-2, the recombination events monitored are truly independent

\* Sequences shown below.

Sequence trace 85-2:



Sequence trace 85-3:





Sequence alignment of an unrearranged target site and target site with 6bp indel (using blastn: https://blast.ncbi.nlm.nih.gov )