Supporting Information

Tan et al. 10.1073/pnas.1221700110

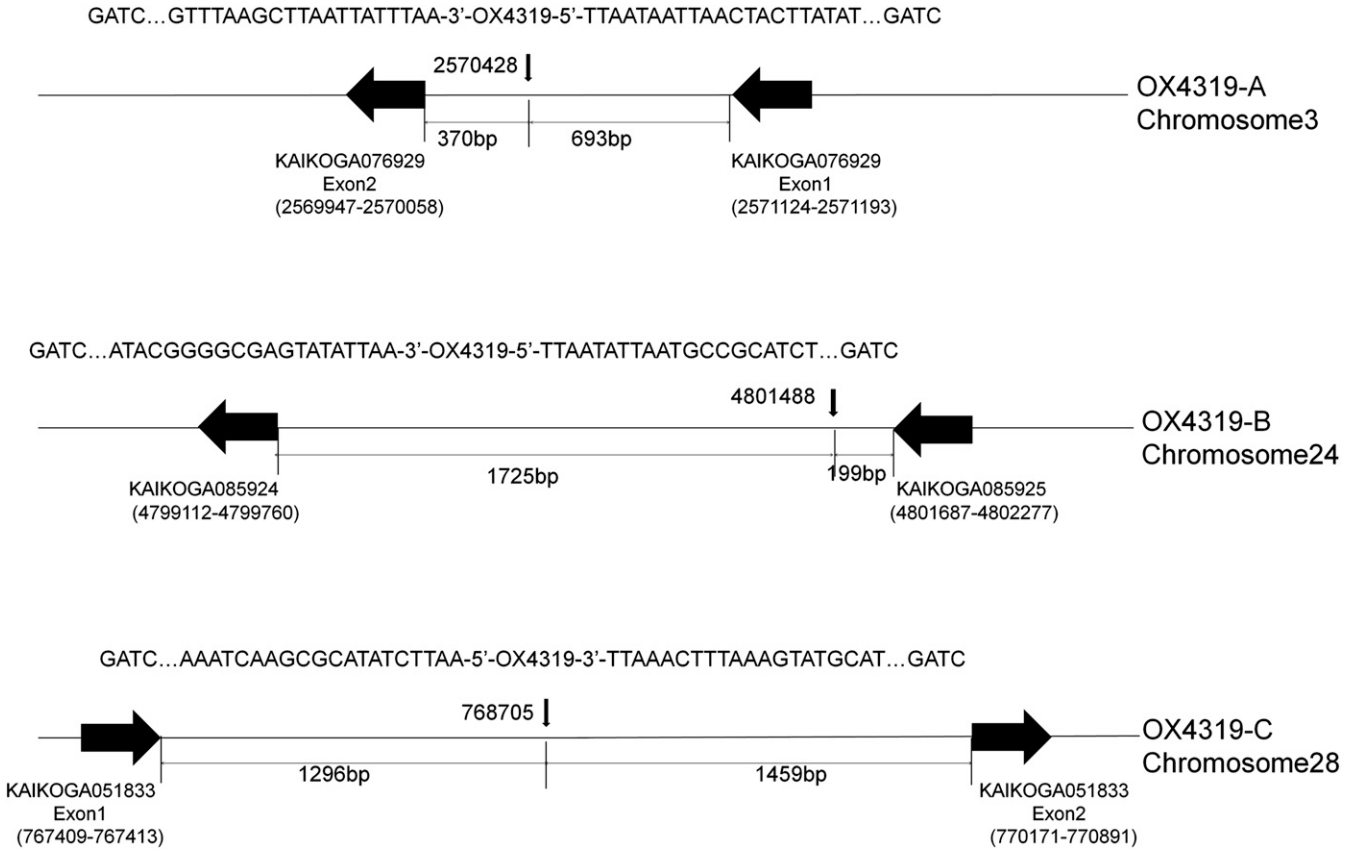

Fig. S1. Genomic insertion of OX4319 construct. Genomic insertion of the OX4319 construct in transgenic silkworm lines, as revealed by inverse PCR and sequencing. Three distinct integration events were identified in three transgenic lines OX4319-A, OX4319-B, and OX4319-C. The transgene integration site in OX4319-A lies in the chromosome 3, in the first intron of KAIKOGA076929, a putative bric-a-brac paralog. The transgene integration site in OX4319-B lies in chromosome 24, between two putative olfactory receptors, KAIKOGA085924 and KAIKOGA085925. The transgene integration site in OX4319-C lies in chromosome 28, in the first intron of KAIKOGA051833, a putative endonuclease-reverse transcriptase. Chromosome localization and partial genomic DNA sequences between the Sau3AI site and the 3′- or the 5′- insert boundaries of the vector are shown. All insertions had the TTAA insertion site found in canonical *piggyBac* insertions at the 3′- and 5′- insert boundary.

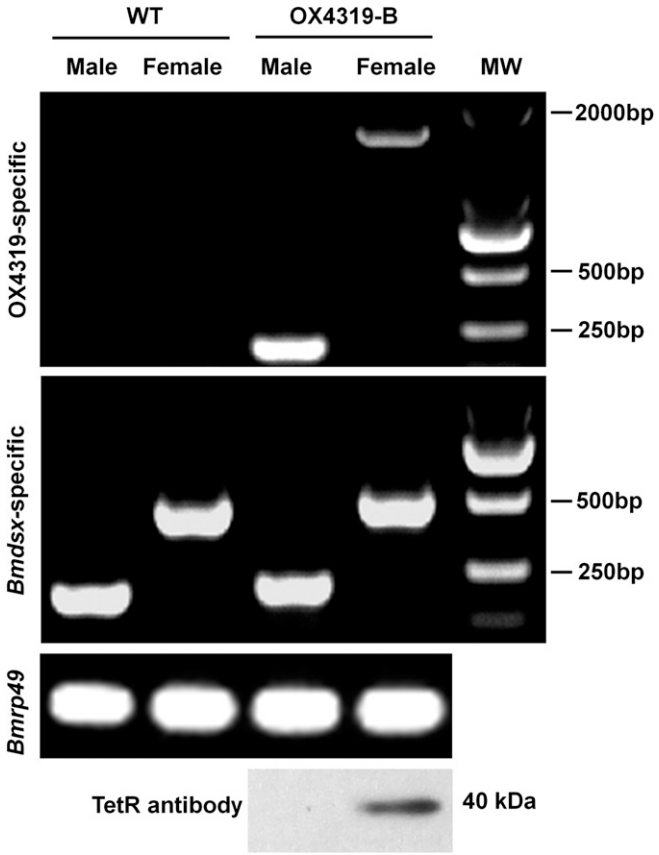

Fig. S2. Sex-specific alternative splicing. Reverse transcription gene amplification (RT-PCR) was performed to investigate sex-specific alternative splicing of the transgene; 220- and 1,551-bp amplicons corresponding to the expected male- and female-specific splicing forms of the *doublesex* tetracycline-repressible transactivator sequence (*dsx*-tTAV) were detected in OX4319-B transgenic males and females, respectively. No such amplicons were detected in WT animals (WT). OX4319-specific primers used here were the sense primer binding to *Pectinophora gossypiella doublesex* (*Pgdsx*) exon 2 and the reverse primer binding to *Pgdsx* exon 5. The identity of these transcripts was confirmed by sequencing, and structure corresponded to that shown diagrammatically in Fig. 1. On the contrary, sex-specific splicing of the endogenous *Bombyx mori doublesex* (*Bmdsx*) was detected in both WT and OX4319-B animals. *Bmdsx*-specific primers used here were the sense primer binding to *Bmdsx* exon 2 and the antisense primer binding to *Bmdsx* exon 5. A 207-bp amplicon was detected in males and a 457-bp amplicon was detected in females. A 136-bp fragment for *Bombyx mori* ribosomal protein 49 (*Bmrp49*) was used as an internal control and was detected as expected all animals. Immunoblot analyses were performed with whole-body homogenates of transgenic animals using a commercial antibody against the TetR domain of the tTAV. tTAV protein was detected only in females (results were similar for all three lines OX4319-A, OX4319-B, and OX4319-C; data shown are from OX4319-B).