Supplementary Information for
Transgenic expression of *Nix* converts genetic females into males and allows automated sex sorting in *Aedes albopictus*

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Other supplementary materials for this manuscript include the following:

Supplementary Data 1 (separate file): Model output from all statistical analyses and performance assessment

Supplementary Data 2 (separate file): Wing length measurements (Fig. 3b)

Supplementary Data 3 (separate file): qPCR data (Fig. 4)

Supplementary Data 4 (separate file): COPAS data for SM9 line (Fig. 6a)

Supplementary Data 5 (separate file): COPAS data for 1.2G line (Fig. 6b)

Supplementary Data 6 (separate file): COPAS data for 3.1G line (Fig. 6c)

Supplementary Note 1: Sequence of the *Ae. albopictus* genomic region amplified for cloning *Nix* promoter.

In the sequence below, *Nix* promoter sequence is included in the 1,916 bp sequence highlighted in grey, the 5' UTR sequence of the *Nix* gene is not highlighted, while the ATG start codon is highlighted in blue.

TCGCATTTTATGAGTAAAGGCCCATTACTCATATATGGGTAAAGTGCTTTTTG
TAAAAAATGAGTAAATCGATTTACTCATAAATTATAATTTCACCGTGTTTACT
TTTCGTCTTTTGTTATATTTTTGAACTGTTAACTATTTTGTATTTATCATTTAAT
TCATACAAAATTTTGCGTTTATTTTGTAGTTTATTGCAATCTACGAAAATTATT
AATGAATTTTTACATGTTTCAAATACTCTTAATCATTTTCCTCCTGGAAAACA
GACCTTCCGAGACGTAGCTCTTTGAAGTTTTCATAATGATCCATGTATGAAAT
TTTCCCCTCCGGCTGTTGTTCATCGCGCAGTTGTTCCTGTTCTTCCGTTCCTCG
TTCTTCCGGCGCTTGAAGCCTTGGTGCAGTCGGAACTGTGGAAAAGACTGAT
ATATTTTTATTTCACGGTCGTTAATGAAAATGATGCTGTCACTATGGAACTTA
CCTGCAAATTCCATGCTTTTGTAGCCTTTCTTGTAGCTCCACATGATGATTTGT
TGCTCCTGTATTATCTTCGAGTACCAAACCTTGTGAAACGGAAATAATCCATC
GTAAATGAAAATTGAACGGTATCAATTTATCCAGGAAGTACTTACCGATTAA
AATATCAATGTATTTGGGAACCTCGACTCTAATCCGCGTGGGAATCCGATGG
ACGCGGTATCGGTCATTGAACCCGATGCTGAAGGGCCTCGACTGGCTATAGC
GGTGCCGTGAAAATCAAAATATCTAAAATTTCCCGATAGTGTTATGATCAGG
GGATCCGCATGCGGCAAGGTAGGCCTACTGCTCATCTTTTGCGAGTGTGTTAG
TGTGACTGGCAACGCGACCAGAAGTCTAGTCGAATCCTGCTTGGAAATCGGT
TTGTTTTGTCGATGGGGGTGGCCCGCGAAATCGCATTGTGTTGTTTGGGAAGG
CCCGCTCGATGCATGTTAGGTAGGCGTAGTTTGTTCGAGTTTGACGTGCCTGC
ACCCTCCCTGGTTATGATGGCGCACTAATGCATTCCGAGAAAAGTTGCTAACT
TGAAAATTTTCATCCAAATATGGGTAAGTTCATTTACCCATGTTTGGGTATAG
CGATTTTACCTATAATATGGGCAAATTTGACCGTTTTTCAAAGGTATATTTTA
CCCATATTATGGGTAATGCATATGAAACCCAAATATGGGTAAATCAACTACT
GATCTATGGGTAAACTGATCTTAGCGTGTATGTTTCACACGTGACATTCACTG
GTTTTACAATTGGCTTCTTTAGCAGTGTTGGGATAATTCCATATTATGAATCT
GCATGCCAAACTGGGCCGAAATCCAAATTTTCATCAATTTTGGTGCACGGGA
ACCTATTTAAATATCAATTTGAAGTTTGTATGGGAGCGATTTGTCGAATCACC
CCTCGTTGCATTTTGTACTGGGCGGAGCTGTCAAACAGTTGCCTAGCTGTCAA
AAGGTGATTTCGAATAATCTCTTTGAAATTGATTTTAGGTATCAAAATAAAGT
TCTAAAAATCTGAAAAAAATCATAGTGGCTCAGAAAAAGGTGCTCTTTCGTA
TAAAATCAAAAAATGAACACTTTTTTCAAAATTTAAAAACCCAATTTTCGCAA
ATCTATAGGGCTCTGCACGAAGTTCTCTCCCTCTCTTTCGCTCTCATTGAGATT
TTGTAAACAACAAGGCCAGGAAATGTCAAAATCCCATACAAAATCAAAACA
GTGCAGTGCCCTATATGTAAAACACATCACTCCGACGTGTAAATTTTTTTGAG
TGTTGATTTAATCAAAGTGAATAAAAATATTAGTTTTATGACATACTTGTTTT
CTGAGTGTAGCAAAAATATGAAAACACATTTTTGTACTTTGAATGTTAAGCGT
GTATGCTTTTTGTGTCAATATGTCAATTGTTAAACCCATGTTAATAGTTTTAAT
TTTTTTTTAATCAAATTCTTTTTTTAAGTAATG

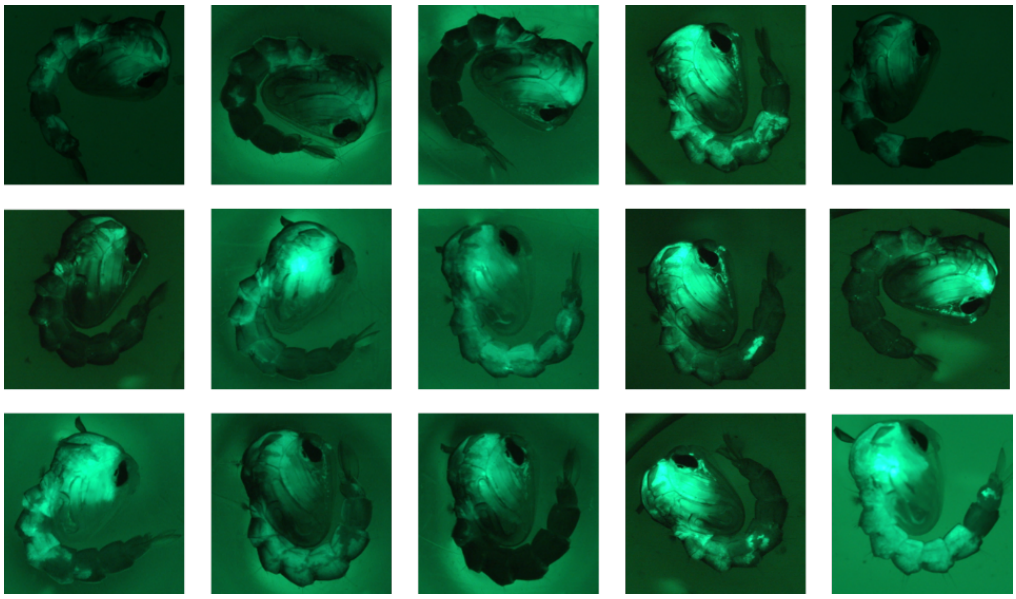
Supplementary Note 2: Male-specific amplification pattern of one of the copies of the *myo-sex* orthologue.

PCR amplification was performed using primers Myosex369-F and Myosex369-R targeting the end of the first intron of *myo-sex*. The expected product with this primer pair was 997 bp as provided below. The discovered male-specific product carries a 664 bp deletion (highlighted in grey) and is present in genetic males only.

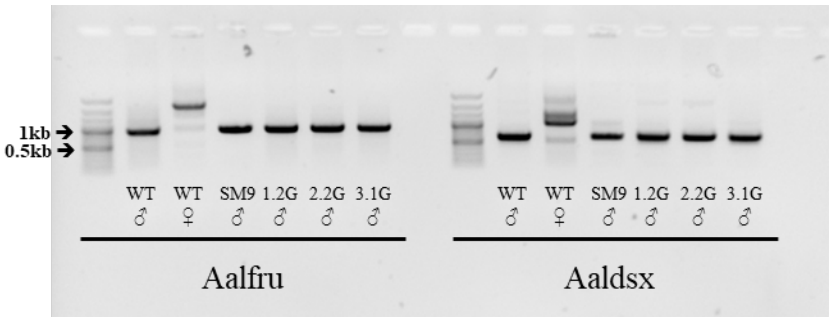
AGGCCATACTAACCTTCCGTAAATCACGCGGTTGGCCACTACCGTCAGCACC
ACGATAATGCTGAGTACAAAATTTAGATTTCTTCAACGTGTTGTACAAAATAC
AAAAGCGCGATCGCTCGAGGGCTAAGAATTAATGAAGAAAATTGCAAATCA
ATTACATATTTATACAAAATCATAACAATAGGTATAGATGTTTGCAAAGATGT
TCGTTGACAGCTTGAATCTCGAAACAGAGATGAATAGATTGAACTATAAAAC
AAACTCGATCAAAAATAACACAGCGTAACAAAAATAACTTTTTGTATGTCTC
TAGAGCAAACTTACGTGTCTCCGAAGGATTTTGGGCCGCTGAATCCGAATCT
GGGCTCAGATTTGCTCTAACACGTCACAATTTTGAGCTATACCTCAATTTATA
GGGCAAAATATGCGATTTTGGGCTTTTTTGACTGCAAGCCATTAAGCAAGGA
AATATTTTTTTAAGCAATCAAAAGGTTAATTGGTCAATTAACATCTAAATTAC
GACTCATGCAAAATATTTCGTTTTACCAAATCGAATTTGATAGTTTTAAGCGA
TTTATGTTAGGTACGATATTTCCCATATAAGTCAGCCTCCAAAAGTTGCATGC
AAGTTTTCATGCTAACATAAAATGCTTAAATCCATCAAATTTGATTAGGTAAA
ACGAAATATTTTGCATGAGTCGTTAATTTAGATGTTAGTTGACCAATTAACCT
TTTGATTGCTTAAAAAAAATATTTCCTTGCTTAATGGCTTGCAGTCAAAAAAG
CCCAAAATCGCATATTTTGCCCTATAAATTGAGGTATAGCTCAAAATTGTGAC
GTGTTAGAGCAAATCTAAGCCCGGATTCGGATTCAGCGGCCCAAAATCCTTC
GGAGACACATAAGTTTGCTCTTGAGACAAAAAAAATGTTGCGCTGTGTAATC
TGTTAAAAACATTGTCCTACATTTTTTGCGCTCCATTGTTACTTCATTGTAT

Supplementary Figure 1: Intersex phenotypes in SM9 line prior to purification. The two pupae on the left side of the picture are a control male and a control female from SM9 line. All four pupae on the right are representative GFP-expressing intersex individuals with deformed genitalia found in the SM9 line prior to elimination of additional non-fully masculinizing transgene insertions. Pictures were taken under a binocular microscope with white light.

Supplementary Figure 2: Detection of lines composed exclusively of genetic females in lines carrying the *Nix*-OpIE2-GFP plasmid. a) PCR amplification of genomic DNA from pooled phenotypic males using primers EM1926-EM1927. This primer pair is located within the second intron of *Nix*, absent from our construct, and thus amplifies only DNA from genetic males. Genomic DNA was extracted from pooled pupae and analysed in the following order: WT females ("F", negative control), WT males ("M", positive control), SM9 partially masculinized female ("9*"), males or pseudo-males from selected lines arising from injection of the first OpIE2-GFP -marked *Nix* expressing plasmid (numbers 2 to 12), negative control without DNA template ("T-"). This PCR allowed identification of line SM9 as composed exclusively of genetic females. **b)** Screening of 10 SM9 individual males (labelled 1 to 10) with primer pair Nix-833F-Nix-833R amplifying any *Nix*-bearing genomic DNA [1] and primer pair EM1926-EM1927 for endogenous *Nix* only. The following controls were used: "M" is WT male positive control, "F" is WT female negative control, "p" is the pooled SM9 male DNA from top panel, and "T-" is a negative control without template DNA. This PCR confirmed that all phenotypic males in the SM9 line were pseudo-males. PCR products were resolved on a 0.8% agarose gel with 0.2µg/mL ethidium bromide.



Supplementary Figure 3: Gallery of male SM9 pupae arising from CRE-injected embryos, showing local demasculinization in the posterior pole. All adults that emerged from these pupae had male heads and female genitalia. Pictures were taken under a binocular fluorescence microscope using a GFP filter.



Supplementary Figure 4: Sex-specific *fruitless* and *doublesex* splicing patterns. mRNAs from WT male, WT female, SM9 pseudo-male, 1.2G pseudo-male, 2.2G pseudo-male, and 3.1G pseudo-male pupae were extracted, reverse-transcribed into cDNA, which was used for PCR. Products were resolved on a 1.5% agarose gel with 0.2µg/mL ethidium bromide. Left side: RT-PCR with *fruitless* sex-specific primers for which the male product is expected at 987 bp and the female product at 2010 bp. Right side: RT-PCR with *doublesex* primers, for which the male product is expected at 620 bp and the female product at 1062 bp.

Supplementary Table 1: Sequences of primers used in this study.

Name	5' – 3' sequence	Use
EM1926	CCCTCAATTTTCCGCCAACTATT	655bp amplicon in intron 2 for detection of endogenous *Nix*
EM1927	AATCTTTGGTGCGCCGTGTC	
Myosex369-F	AGGCCATACTAACCTTCCGT	Genomic amplification of a non-coding part of the *myo-sex* copy from scaffold 369 (end of intron 1).
Myosex396-R	ATACAATGAAGTAACAATGGAGCG	
EM2145	CAAGTTGGTGACGATCCCGA	For RT-qPCR of mRNA from *myo-sex* orthologues
EM2146	GTTGGGTAGAGCAACGGTGA	
EM2147	CGCCGGAAAAACGTATCCACT	For RT-PCR of mRNA from LOC115254984, a candidate *myo-fem* orthologue
EM2148	GCTGGTTCCAGGTTAGTTGG	
EM2149	CCCGTGCTGAAGAGTTGGAG	For RT-PCR of mRNA from LOC115254986, a candidate *myo-fem* orthologue
EM2150	GTGGACAGACGTTGCTTAGT	
EM2151	GTAGGCATCTACGAGCCCAA	For RT-PCR of mRNA from LOC109402113, a candidate *myo-fem* orthologue
EM2152	CCAACCTGTACCACTGGCTT	
EM2153	CATTGGAAACATTCCCGCCG	For RT-PCR or RT-qPCR of mRNA from *Nix*
EM2154	ACTGCCGGTTTCACATCACA	
EM2170	ACGTGCCGAAGAATTGGAAG	For RT-qPCR of mRNA from LOC109402113, putative *myo-fem* orthologue
EM2171	TTCTAAGGCAACACACTTCTGA	
EM2174	CGTGCCACCCTTCTTGGTAA	For RT-qPCR of mRNA from LOC115254984, candidate *myo-fem* orthologue
EM2175	CCTCCAACTCTTCTGCACGG	

Supplementary Table 2: Sex ratios of transgenic versus WT lines

Line	Replicate	Males	Females
WT	1	125	116
WT	2	128	128
WT	3	74	67
SM9	1	936	745
SM9	2	677	590
SM9	3	430	308
1.2G	1	889	1027
1.2G	2	477	423
1.2G	3	1787	1802
3.1G	1	1246	1224
3.1G	2	339	306
3.1G	3	887	641

Supplementary Table 3: Flight ability of SM9 transgenic pseudo-males versus WT males.

Line	Replicate	Number failed	Number successed
WT	1	55	31
WT	2	38	36
WT	3	79	11
SM9	1	35	41
SM9	2	34	65
SM9	3	50	14

Supplementary Table 4: Hatching rate of eggs from the SM9 versus WT lines

Replicate	Line	Larvae	Eggs
1	WT	373	641
2	WT	150	309
3	WT	115	201
1	SM9	138	286
2	SM9	82	147
3	SM9	153	264

Supplementary Table 5: Fertility of the SM9 versus WT lines

Replicate	Line	Number of progeny
1	WT	2224
2	WT	1400
3	WT	1581
1	SM9	2013
2	SM9	1479
3	SM9	858

Supplementary Table 6: Competitiveness of transgenic versus WT males

Comparison	Replicate	Number of transgenic progeny	Number of non-transgenic progeny
SM9 vs WT	1	22	364
SM9 vs WT	2	129	1912
SM9 vs WT	3	211	1540
SM9 vs WT	4	57	421
SM9 vs WT	5	157	1401
1.2G vs WT	1	586	2681
1.2G vs WT	2	530	4296
1.2G vs WT	3	474	3571
1.2G vs WT	4	187	1875
3.1G vs WT	1	16	281
3.1G vs WT	2	99	1042
3.1G vs WT	3	71	895
3.1G vs WT	4	175	1165
3.1G vs WT	5	73	710