Additional file 2. Supplementary figures S1-S9.



Figure S1. *Drosophila suzukii* **NULLO protein alignment**. DsNULLO is aligned with orthologs from *Drosophila biarmipes* [DbNULLO] (GenBank: XP_016948821.1), *Drosophila hydei* [DhNULLO] (GenBank: XP_023161247.1), *Drosophila kikkawai* [DkNULLO] (GenBank: XP_017022675.1), *Drosophila melanogaster* [DmNULLO] (GenBank: NP_511067.3), *Drosophila miranda* [DmirNULLO] (GenBank: XP_017156218.1), *Drosophila navojoa* [DnNULLO] (GenBank: XP_017964581.1), *Drosophila navojoa* [DnNULLO] (GenBank: XP_016982709.1), *Drosophila serrata* [DoNULLO] (GenBank: XP_022210380.1), *Drosophila rhopaloa* [DrNULLO] (GenBank: XP_016982709.1), *Drosophila serrata* [DserNULLO] (GenBank: XP_020805612.1) and *Drosophila willistoni* [DwNULLO] (GenBank: XP_002071148.1). Identical amino acids are shaded black and conservative changes are indicated in gray. All proteins contain a consensus site for N-terminal myristoylation (M) followed by a positively charged cluster (P). The remainder of the protein contains five conserved regions of amino acids (A–E) separated by short non-conserved regions.

Consensus DsSRY-a DmSRY-a DvSRY-a DhSRY-a DøSRY-a		XX LV LA KE KS		10 TCX TCS TCS LCT ACL SCL	D L E L D I R M L	X E A E A E E K D C	2 G Y 9 G Y 9 G Y 9 G A 1 S R 1 G -	0 S - S - C H / N -	TGN TGN TGN TAK GD - SN	I X W M G W I G W I A W I N W	30 LNE LNE LNE	F C F C F C F C	A T F A T F A T F A A F A K F A F	L D L D L D H T L I	40 F A S F A S F A S F A S F A N F A N		KAX KAR KAR KSY QIY VKS	P P P I S N	50 E V E V E L N 0	APS APS APS APK IEP LEY	X - G - G - D -	 	 G N 	60 X X A N I R	X X L E L D I H - S - A		F F Y C	70 C L C L C L C F C L C F C L
Consensus DsSRY-a	TQV	/ T C I	80 T H	LER		K T E	90 A S -	- X I	G - X	X X A - Q M	100 R Q		L D R	1 D	10 W C L	R R L		S L	120 T Q		N - N -		130 V T V A	P V P V	K X I		14 H S F	40 E V E
DmSRY-a DvSRY-a DhSRY-a DgSRY-a		/ T C I T C I I C V 1 C V	T H T Q R Q R H 150	LER LES LEY		S M E	A P - G T L E S - T A - 160	A A - S - S	G A Q G L R G S R	- H M L L A I P A I M A 170	TR TR SHL SHH	A H F H F H F H F H F	LDR LDR VDR	D D K R 180	WCL WCL VCL VCL	R R L R R L N R L Q R L		S L S V C L A L 190	T Q Y Q L S T P	E G A E T V K S A A	N - E A G - A -	I A 	V T S T A P G T 200	PV PV QD QN	K N K K - K N A T N A	E D E D F E G F E G	H S F L S F V S F I S Y 210	- V E - V E - L K - L K
Consensus		. A L	X	DDY	XEH	(L A	Q X	NX	SX -	- 1 L	E E X	(І Т	EXS	КО	L X S	XVN	NHL	XX	HX	LAF	A N	V A	LQ	S D	кк /	ALS	ALC	T
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Consensus	VLR	C X X	E X	EEX	GLF	230			- N S	XXR	K L E	A L	SLE	R A	LYX	LES	S Y L	200 N E	A L	LXL	XF	T S	, 	DX	E X)	s v	XKL	RN
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Consensus	X L Q >	(ХК	S X A	XAL	DEL	ISE	FD	XNX	D R I	QQI	GI	XA	A F	XQD	K	IK T	I V	R S	CLA	S L	S S	LD	A C	IV	P A L	Q L >	х х х
DsSRY-a	ALQ		- K	NAN	GAO	R E L		F D	T N M		QQ	G V	AI	A F	S Q D				R S		S L	E S	LD	A C			Q L P	ES
DvSRY-a DhSRY-a DgSRY-a		S N T S N T T R T 360	CE E- K	S S G A S V A		G L C C C C C C C C C C C C C C C C C C		F D F D F D		D R I D R A D R A		G G G	F A I Y A I F A I	A F A F A F	A P N A P N	V K L K L K	K T K T K T 400		R S R S R S		S L S F S F 410	E S E Y E Y		A C T S T S	i V L I I L I I	2 A F 2 S L 2 S L 420	QLC QS- HG-	2 T T
Consensus	NXS	×	EJ	LEZ	HEN	V Q E	L X	FR	N V	HEI	I D S	S R A		NY	LDM	LX	E S	ХХ	ХЕ	K X X	XK	S H	LX	ΥJ	XQN	ALG F	V	V E H
DsSRY-a DmSRY-α DvSRY-α DhSRY-α DgSRY-α	V S S T S S S L A S N A S N A S	H H V F H H A F Y H A D L H S D L H S	E I E V D I E L E L				LLL LMN MSK MST	F R F R F R F K	N V N V Q A L T A L			C S C S R A S R A S R A	L I N L I N F V A F V G	N Y N Y C Y C Y	L D M L D M L D I L Q I L H M	L G E L A E L T N	ES ER AL TG	Q H D N H	Q E Q D A E I E A E	KLY KQF	- K - K D K	S H S H G Y A K Q		V I Q V D L H	VQF VQF AQN LQN	R G S R G G M G N M A F M G F	V L V V V V S I L Y I	/ E H / D H / Q H L E H / E H
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DvSRY-a DhSRY-a DgSRY-a	FQLF	R A N N V N R G V N Q	Q E K T K V	LIA VLP LL-	Q	G K G H Q H	RLH EQL ENL	Q D Q N Q N	FVA FVK FVR	ILH IVR ILR			M C M C	S V A S A S	H V D Q V E Q V Q	P E P	R R R L	K R K R K R	F K		N I T I	L A L R L R	K L K L K F	R D H V H N	V C · H I V S L /	V D E A A A	E N K E S K	(P T (P A
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DmSRY-a DvSRY-a				R D N	LEF	P D S Y E R	AMS	S E	AQV	P S S N	NEK	RTF KSM	V R S T T S	S R T K	s													
DhSRY-a DgSRY-a	G F R A N E F 570	- E K A P H P H	E Q R E	P L E E T D 580	V E V	E E	T T C S Y T	P 1 590	Q G L Q S L	V A S M A S	P S F P S F	R S I R S I 600	L Y N L Y E	Т Н Т Q	K Q R 	S K \ 610	V N S	S K 	L E 	N F N 620	T D 	Q M 624	Q S	E Y 	L K I	_ K V	Q N C	: L R
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Figure S2. *Drosophila suzukii* **SERENDIPITY-** α (**SRY-** α) **protein alignment**. DsSRY- α is aligned with orthologs from *Drosophila grimshawi* [DgSRY- α] (GenBank: XP_001995347.1), *Drosophila hydei* [DhSRY- α] (GenBank: XP_023168708.1), *Drosophila nelanogaster* [DmSRY- α] (GenBank: NP_524580.1) and *Drosophila virilis* [DvSRY- α] (GenBank: XP_002056142.1). The putative transmembrane domain is underlined and the region of similarity with proteins of the ERM family is boxed.

	1 10	20	30	40	50	60	70 80	
Consensus DoBNK DmirBNK DsBNK DmBNK DkBNK DwBNK DvBNK	M S I S T F N F Q F Y N M S I S T F N F Q F Y N M S I S T F N F Q F Y N M S I S T F N F Q F Y N M S I S T F N F Q F Y N M S I S T F N F Q F Y N M S I S T F N F Q F Y N M S I S T F N F Q F Y N M S I S T F N F Q F Y N	Y K P P X P Q F G S - - - A M G S - - A M G S S P - - A A G I S S P D - - A A G I S S P D - - A A G I S I O T G I S P D F G S P D F G S P G S P G F G S P S P G S P G S P S P G S P S P S P S P S P S P S P S P S P S	L G T G S G P G S	T S R S S S S T S R S S S T S R S S S A S R S S S A S R S S S G S T S R T S I N S - T I G S G S S S	S F S E L E M D - S F I S E L E M D I - S F I S E L M D I - S F I S E L M D I - S F I S E L M D I - S F I S E L M D I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I I	Image: Sector 1 Image: Sector 1	S X P S - - - T S P S S P A - - - V T S P T T R P P T T S P S A Q P - T T S P S A P S - - V T S P S A Q S - - V T S P S A P S - - V T S P S A P S - - V T S P S A P S - - V T S P S A P S - - V T S P S A P S - - V T S P S Q Q M - P T T S P	K
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Figure S3. *Drosophila suzukii* **BOTTLENECK (BNK) protein alignment.** DsBNK is aligned with orthologs from *Drosophila grimshawi* [DgBNK] (GenBank: EDV90810.1), *Drosophila hydei* [DhBNK] (GenBank: XP_023168586), *Drosophila kikkawai* [DkBNK] (GenBank: XP_017034088.1), *Drosophila melanogaster* [DmBNK] (GenBank: NP_524604.2), *Drosophila miranda* [DmirBNK] (GenBank: XP_017140437.1), *Drosophila obscura* [DoBNK] (GenBank: XP_022213695.1), *Drosophila virilis* [DvirBNK] (GenBank: XP_002055918.1) and *Drosophila willistoni* [DwBNK] (GenBank: XP_002072000.1). Highly conserved regions are marked with red lines.

Consensus DsSLAM DmSLAM DnSLAM	1 10 MPESHSYKLKR MPESHSYKLKR	20 TSTTROOTNTKM TSTTROOTNTKM	30 VLSNSTPN VLSNSTPNN	40 - XKKHNELIVVD - QSKHNRMVVD - QSKHNRMVVD - QKPHHELIMD	50 TAAMNSXDLSE TAAMKNEDLSK TAAMNSDDLSE TAAMNSDDLSE	60 LLQLNALEIEER LVQLNTEIEQR LLQLNALEIEER LLQLNALEIEER	70 - MAR SIMIRH G D A M - MAR SIMIRH G D V M - MAR SIMIRH G D A M - MAR SIMIRH - SIM	80 DACGLLRATMI NAFGLLQATMI TACGLLRATMI DACGLLRATMI	100 References X ISISS References T F SSI References S S S	110 DDDRFLTALEHQN DDDRFLTAIKHQN DDDRFLTALEYQN DDDRFLSALEHON	120 SIM X HERSI RIVOVIDI SI L UHER R RIVOVIDI SIMA KER R RIVOVIDI SIMA KERSIK S RIVOVIDI
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Figure S4. Drosophila suzukii **SLOW-AS-MOLASSES (SLAM)** protein alignment. DsSLAM is aligned with orthologs from Drosophila grimshawi [DgSLAM] (GenBank: XP 001988112.1), Drosophila pseudoobscura [DpSLAM] (GenBank: XP 001355861.2), Drosophila melanogaster [DmSLAM] (GenBank: NP 001285668.1), and Drosophila busckii [DbuSLAM] (GenBank: ALC38995.1). Highly conserved regions are marked with red lines.

DsSLAM DpSLAM DpSLAM DgSLAM DbuSLAM

DTLLGRRAVEN DTLMDRRAVEN



Figure S5. Reverse-transcriptase (RT)-PCR to evaluate the reference (a) and cellularization (b) genes through the development

of *Drosophila suzukii*. Primer sequences for reference genes *TATA binding protein (TBP), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), arginine kinase (AK), a-Tubulin (\alpha-Tub) and Histone H3 (His3) (Zhai et al., 2014; Li and Handler, 2017), as well as for four cellularization genes can be found in "Additional file 1". Embryos collected at different time points after egg laying (in hours), larvae (first, second and third instar), pupae (2 days after prepupae), adult female and male (1 and 5 days old). Additional 0-0.5 h samples were used for cellularization genes. The PCR product sizes are 140 bp for <i>AK*, 130 bp for *GAPDH*, 189 bp for α -Tub, 129 bp for His3, and 182 bp for *TBP*, 161 bp for *sry-a*, 155 bp for *nullo*, 159 bp for *bnk*, and 149 bp for *slam*. M is the molecular weight ladder.



Figure S6. Alignment of the *Ds_sry-a* and *Dm_sry-a* 5' flanking sequences. The upstream flanking sequence from *Ds_sry-a* (165 bp, before it reaches the upstream gene DS10_00012896, the ortholog of *D. melanogaster janus A*) and *Dm_sry-a* (167 bp) together with the 5'-UTR (annotated in green) are compared. The 5' flanking sequences of *Ds_sry-a* and *Dm_sry-a* contain three and four conserved motifs that confer blastoderm-specific expression, respectively. Both 5' flanking sequences contain two TAGteam motifs (annotated in yellow), and a TATA box (TATATAAA) 23 bp upstream of the putative transcription start site. The *Ds_sry-a* 5' flanking sequence was fused to DsRed-NLS in V205 to act as a gene promoter for the *in vitro* test.

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Ds_nullo 5' flanking sequence Dm_nullo 5' flanking sequence	G A T G C - T C T G G T G A A T C T T	TTAGGAA GTGGGGA	T T T A T C A / G C C C C A C A A C	АТТАТ АА - - Т БССБС АА АА Т	Т А С А Б А Т Т А Т Т С А Б Т С С А /	ТАПТАТАААТ АСП БИСССССИ	. ТАТА СПАТТТ . ТА САТТТССА	астаталта G Т Т С Маспаса С А	C <u>T T G A</u> <u>A T G A A</u> G T T G A C A <mark>A T G A A</mark>	GCTTAAAGCA ATGCTCTCTGCG	С G С ПС С А Т Т С G G С С	A A A A G A A C A C A G G G
	120	130	140	150	160	170	180	190	200	210 22	3 230	
Ds_nullo 5' flanking sequence Dm_nullo 5' flanking sequence	A T A C A A A A A T T G A G T	GTTAGCT ATCAGCT	CACTANTA TGCATATGC	СТТАСАА ГСТССТСАС	ATTACTT/ TTCACAATC	GTAAACATAA GTAAACATAA	TTAACTAAAAAA	TTTTAAGTTCC TTAGTTTC	ТТТА СААТТСТТ ССАААССТСАА	Т Т С С Т G А А Т Т Т Т Т Т Т G А А С G С Т	GTAATACTTA TTGAAAATATA	A C A T C / T T - A A C
	240	250	260	270	280	290	300	310	320	330 TAGteam motif	340 356	D
Ds_nullo 5' flanking sequence Dm_nullo 5' flanking sequence	TTTAGTATTC TCTAGTAAAA	A A B A T T T	T C T II T G T G G A A A A II G A A G T	A T T A T G T A G G A G T A T A C	A G G A A A II G G C C C A A C T II C G		С G C C C C A A A A С Т C A A A A A A A A A A A A A A A A A A	AACCCATTT AAATACGATTC	A TITIAC TAC TITITAC A CTAC	T G C C T G G A A T T T T A C C G G G A A T A T	TCAAATGAATT TCAAATGAATT	ТТАССТ ТТССССТ
	360	370	380	390	400	410	420	430	440	450	490	470
Ds_nullo 5' flanking sequence Dm_nullo 5' flanking sequence	TTTCGTTTGC TTTCGTTTGC	ATCCCTC		G G A <mark>A A A A G C G</mark> C G A A A A G C G	G A A A A T G G A O G A A A A T A G A O	CACTACCGAAC CACTGCTGG	Т G G A <mark>С С А С А А</mark> (С С А С А А (G C C G C A C C C C - G C C G C A C T C T C	TTCAACCTAC CATTCCCCCAAC	G T <u>T G A C C T T C G T</u> A A T G A C C T T C G T	CCTTTGGCCTC CCTTTGGCCTC	G A A T T T G T A T T T
	480	490	500	510	520	530	540	550	560	570	580	5 <u>9</u> 0
Ds_nullo 5' flanking sequence Dm_nullo 5' flanking sequence	– <u>C C A G A G G A A</u> I C C A G A G G A A	<u>A A C A G C G</u> A A A C A G C G.	<u>A A C A A G A G A A</u> A A C A A G A G A G A J	<u>A G G T A T T T T G</u> A G G T A T T T T G	T G T T T C G A T T G T T T C A A T	T T T A A T G C A A 1 T T T A A T A C A A 1	<u>T A G A G G C T G C /</u> T A G A G G T T G C /	<u>А Т С G A A A G C T G</u> А Т С G A T - <u>G C T G</u>	<u>C A A A</u> – <u>T A A G T G G</u> C A A A A T A A T T G G	<u>A A G A T G A G C G G A</u> A A G C T G A G T G G A	<u>A A T T C A G G G T C</u> A A T T C A G G G C C	АТТ А С А С А
	600	610	620	630	640	650	660	670	680	eșo	700	710
Ds_nullo 5' flanking sequence Dm_nullo 5' flanking sequence	<u>G G G G T T T C C</u> / G G G G T T T C C		<u>С С СААТТАА</u> С С ТААТТАА	<u> </u>	G A G T A C T C G G G T G T A G A C G G	<u>G G G C T A C G A A A</u> G G G T T A C - A A A	T G G G A T T T T A / G G C G A T T T T A	A C C A G A G C G A G T G C A C A G C A A G	A A A A T G T C G G T G A A A A T	ΤΤΤΤΤΑ G Α ΤΑ G G	TAAAAGTGAAG	ΤΑΤΑΑΤ
	720	730	740	750	7	40 7j	ro 780	790	890	810	820	830
Ds_nullo 5' flanking sequence Dm_nullo 5' flanking sequence	АТАТ <mark>АТТА</mark> АА АТТА СС	ATAATTTT STGATATC	Т С Т Т А Т А Т Т Т С	F A A A T T T A C G	С А Т А Т Т Т Т А Т Т б	Т Т С А С А С Т С А Т Т Т С А	AGCATTTCA GCATTTA	ATTTCATCTA ATTTCTATCTA		G C T A T T T C T C T C T C T A T T - C T C A C	A G T G T A T T T G T A G T G T A G C T G T	<u>G C A C T</u> T G C A T T A
	840	850	8	60	870	880	890 9	óo aic	920	930	940	950
s_nullo 5' flanking sequence m_nullo 5' flanking sequence	<u> </u>	C <u>ATTATT</u> A ATTATT	TTCTCCGGC TTCTTCAAC	T G C T T - T T A T G G C T T A C T G	C G C A G T T C C C C A C A G C C C	C A C T <u>G T A C A T A</u> C G C G G T A C A T A	TGTCCACTTA	<u>T A A T A A T G G C A</u> T A A T G A A G G C A	<u>A A G C A G C T T A T G</u> A A G C C G C T T A T G	<u>TCCTCGCCCAC</u> TCCTCGCCCAC	<u>CAATTACTTAT</u> CAATTACTTAT	АСАСТС АС
	960	9	970	980	990	1,000	1,010	1,020 1	.030 1.040	1,050	1,060	1,070
s_nullo 5' flanking sequence m_nullo 5' flanking sequence	C A G G C A C G C A 		T C A C T T A C A C T C A T C T A C A	E A C A C T A A C A C C A G C A	<u>CACAC</u> TCGC/ CACACACACAC	A C A C A A A A G A C A A C A C A C A C G C A	A A G G C A G A G G G A G A G A C A G A G G G A G A C A G A G G	A G A A T T G A T G A G A A T T G A T G	A A G G C T - C T A C T A A G G C A G C T G C T	<u>GACATGCAAAG</u> GACATGCAAAG	<u>C T G A T T G A C A C</u> C T G A T T G A C A C	ACAAAG ACAAAG
	1,080		1,090	1,100	1,110	1,120	1,130	1,140	1,150 1,1	0 1,170	1,180	1
0s_nullo 5' flanking sequence 0m_nullo 5' flanking sequence	<u>G A G C A T T C T</u> G A G C A T T C T	5 C C A <u>G G A T</u> A C T G <mark>G G A T</mark>	<u>G C T T A A C C A ⁻</u> G C T T A A C C A ⁻	<u> </u>	A A C T C G C A G A C C T C G A C C I		S A A C C C G G A C C (С Т С С G С С А Т С С А С С С А А Т С Т С С	ААТТ ССП GАТТТ Т G C C ССП СТ G C C		CATCTACACAG CAAGTGCAGAG	A G A G A A C A A A A A
	1,2	200	1,210	1,220	1,230	1,240	1,250	1,260	1,270	1,280 1,290	1,300	
s_nullo 5' flanking sequence m_nullo 5' flanking sequence	С С А Т G С Т G А И Т С А Т А G G G Т И	AGTTC - T AGTTC - T	Т АА G С А Т G Т A С АА Т G Т Т G Т /	A G C A G A C T G A C C A C A T T T	G T A C A A A A C G T T G A A G C T	A G G T T G G T T C 1 A		A A T A C G G C T T G	GTTTGGTAGAAAA GTTTAGCAG	G G T T A A A A T T A A T T T G A T T T A A	А А G T T C T T A A A T A G T A C T T A A G	GATT H T ACCA H C
	1,310	1,320	1,330	1,340	1,350	1,360	1,370	1,380	1,390	1,400 1,4	0 1,420	
s_nullo 5' flanking sequence n_nullo 5' flanking sequence			G G C A T T T A T / G T C C G T T A T ·	A T G T A THURG G T THURG A	ТТТССААТ СТСТССАСТ		TATGAATAAT/ TATGG	атататын аасса син тбс	T G C A A G T T T T T C T G C A A T T C A T T A		11 G T T T C G T 11 T A 11 G C G A H G A	CAGCCA.
Dr. Julio 5' flanking sequence Dm. Julio 5' flanking sequence	1,430	1,440	1,450	1,460	1,470	1,480	1,490	1,500	1,510	1,520	1,530 1,54	10
s_nullo 5' flanking sequence m_nullo 5' flanking sequence	G C T G C A A T T C G A C T T A A G T C	G C G C A C A C A C A C A C A C A C A C	A T T I C C C C C C C A C I G C C A G C	FIGICIGIA TIGAICA CICICITIGAIA GI	G C C A A G T C T ⁻ G T G G A G G C - ·	T A A G T G G C T G T 	A CCACCCCCCC TCCATTGCTCC	C C T T C T G G G A A T G G C C A T C G	Т С Т Т G С С С G – С С П Т С G С С С С А Т С	<u>A G A A G T G C A G</u> C C A G A A C T G C A G	ACCCACTACAG - CTCCT TACAG	CGAGCA TGGGCA
	1,550	1,560	1,570	1,580	1,590	1,600	1,610	1,620	1,630	1,640	1,650	1,660
s_nullo 5' flanking sequence m_nullo 5' flanking sequence	TTATGTCTCC	<u>A G G A T G T</u> A G G A T G T	Б G АТА Т G A G A C АТА Т	<u>С С Т G G С С А G</u> С С Т G G С С А G	C G A G C A G C A C G A G C A G C G	5	A T G A C A G G T C A A T G A C A G G C C A	<u>A T T A A A T G C C C</u> A T T A A A T G C C C	G T HGCCT G CT CC	G G C C A A C C T C G T G A C C A G C C T C A T	C C C T A T T T C G T T A T G G C G A	CCCTCA
	1,670	1,680	1,690	1,700	1,710	1,720	1,730	1,740	1,750	1,760	1,770	1,780
_nullo 5' flanking sequence n_nullo 5' flanking sequence	GTCGACCO GAGTTAACCO	I C G A A T C G C A A		C T T G C A C G T C T T G C A C G T	<u>GCAAGGATT</u> GCAAGGATT	<u>G C A G G G G C A A 1</u> G C A G G G G G C A A 1		GCGACTGTTG GCGACTCTTG	<u>C A A A C C C C G A A</u> C A A A A C C C C G A A	<u>A T C G C G G C C A A A</u> A T C G T G G C C A A A	T G T T G C C T C A T T G T T G C C T C A T	AAAAA
	1,290	1,400	1,810	1,820	1,630	1,840	1,850	1,860	1,870	1,880	1,890	1,900
s_nullo 5' flanking sequence n_nullo 5' flanking sequence	<u> </u>	<u>A G G G G A T T .</u> A G G G G A T T .	<u>A T G G T C A G G G</u> A T G G T C A G G G	5 T <u>T A G G G G T</u> C G C T A G G G G T T	A G A A T T T T A A G G A T T T T A	<u>T G A A G G T C G C G</u> T G A A G G T C G C G	5 C G G G T A G C G A 7 5 C G G G T A G C G A 7	<u>A T A A G A C C T G A</u> A T A A G A C C T G A	<u>1 C C C T A T T A G G</u> T C C C T A T T A G G G	Т С С Т G С С С А С С С	<u>C A G G T A C C T G A</u> C G G G T A C C T G A	TCCTTT
	1,910	1,920 TATA box	1,930	1,94) IJ	950 1,9	60 1,970	1,980	1,990 S'UTR	2,000	2,010	2,020
s_nullo 5' flanking sequence m_nullo 5' flanking sequence	<u>A G A T T T T A G C</u> A G A T T T T T C G C	GTATATA G-TATATA TATAbox		C C A G T G G C A T C G A T G G C A	ATCAGCATC	A G T T G T C C A A C A G T T G T C C A A C	A A C A G T T A C A A A C A G T T A C A	G G A T A A C A T C C G C T A A C A A C	GILGTITTGAAAGA GTI-TICTCCAAAAA S'UTR	A G A AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	GAGTATTACAA . ТАТТА АТАТ	A G A G C G A T T A C G
	2,030	2,040) 2,6	250 2	.çeo	2,070 5'L	2,080 2,1	290 2,10	0 2,110	2,120	2,130 2,13	15
s_nullo 5' flanking sequence m_nullo 5' flanking sequence		A T A A C A G A T A C C A T	С А А А Т С А <mark>А А</mark> (Т С Т С А А G <mark>А А</mark>)	GATA <mark>CAAA</mark> GT ATCT <mark>CAAA</mark>	GTGCCCATT	A C A C C A A A C G A	TTCGCAAAGA	GTGACCATCAC	ATTTGAATTTTA	ΤΑΑΑΑΑΟΟΤΑΑΘ	ΤΤΤΤΓΑΑΑΑΑΑ	(

Figure S7. Alignment of Ds nullo the and Dm nullo 5' flanking sequences. The upstream flanking sequence from Ds nullo (1876 bp) and *Dm nullo* (1753 bp) together with the 5'-UTR (annotated in green) are compared. The 5' flanking sequences of Ds nullo sequence contains one TAGteam motif (annotated in yellow), whereas that of Dm nullo contains none. The TATA box (TATATAT) is 24 bp upstream of the putative transcription start site. The Ds nullo 5' flanking sequence was fused to DsRed-NLS in V206 to act as a gene promoter for the *in vitro* test.

	1	10	40	30	1	0	50	60 0	10		0 ¹	30	190	10		40	120	140
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence	G G A G G T G G A A A C A G C G A G	G C A A T G A A G G A G A A	<u>, </u>	A G C T T A G A A G C T T A G A	GAGGCGAGA GAGGTGAG	A G C T G T G G C T C T G G	<u>С С Т А Т С А Т</u> С С Т А Т С А Т	<u>ТАТААТТ</u> ТАТААТТ	<u>Т С А С А С С Т</u> Т С А С А С С Т	A A T C C C G G A A T C C G G C	<u>G A G A T T A C</u> G A G A T T A C	<u>T T T T T C A A</u>	<u> </u>	<u>T A T T A T C C C</u> T A T T A T C C C	<u>Т Т G C A C T T</u> Т G G C A C T T	<u>G C T G C G T T G</u> G C T G C G T T G	C T C G G C A T A C A A G G C A T A	<u>A A A G T</u> A A A G T
	19	0	160	170	180		190	200	210	-	220	230	240	250	260		270	290
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence	G A A A A G A A A G A A A - G A A A			H H H H H G G	<u> </u>	$\frac{1}{3}$	стасс 	сассса 		T C C T C G C A T C C T T G C A	8818888	<u>TCCGGGTG</u>	CTTGGCCA	TTAAAGGTC	A C A G T T A A A C A G T T A A	 } 2 	CTTGCTG CTGCGCTG	<u>C A A A C</u> C A A A C
	290		300	310	320		390	340	350	360		370	380	390	400	410		420
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence	TTGGCTGTC	TTSS HT8	ТСТ СТСТС	ĊŦĂĊŦĊŦŦ		H T E E T A A	TTTCCTT		88887777	<u>******</u>	 	ATTAAGT G	E A A A G E T G	A E G T T E E E A	A T G G A C A C	CG-AAACAG TGCAAACGT	G A G C C C C T G T G C C C A T	G G A A G G A A A G
	430		440	450	410	470		490	490	spo		sio	520	530	540	spo	5	590
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence	G <u>A C C C</u> G <u>G G A</u> A A C C C A G G A	TEEGETT	A A G G A T A A A G G A T A	TTGCTCC1	CATGCTGG GACGCTGG		T T A A A T T G	<u>C T G G C G A</u> C T G G C G A	<u>A G T T T T C G</u> A G T T T T C G	T G G C A A A G T G G T G A A G	T C C T G G C C T C C C G G G A	<u>A A G A T T T C</u> A A G A T T T C		T C C G G C A T C C C T G A C A T C	CCCGAGAGA CCTGGAGA	GTTGAGTTG GTTGTGGTT	A G G T T G C A C C A A A .	- G T G A A G C G G
	570	58	10	590	600	670		620	630	640	650		660	670	680	690	700	
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence	A G <mark>G A C A T T C</mark> T T <mark>G A C A T T C</mark>	ATTGCA ATTGCAT	TTTGCAG	<u>G A A T A T T</u> G A A T A C T T	TATGGGTAC TAATAT	TTTTAAA G	G T T T G A C T C T T T G T C T	АТААТС G 	T G T A C T T C		<u>A A T T A G</u> G A A A T T A G	G Т <u>С Т А А С Т</u> <mark>С Т А А С Т</mark>	A A C T G A T G A A T A A A T A	CATCATTT CTTTAATTG	G T G G C G A G	TTTCGAGA TTTAC-AGA		C T T ∏ T T A G ∏ G
	710	720		730	740	750	760		770	780	790		800	810	820	830	840	
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence		A G T T C C Z G G T Z		СТТССАСТ СТАСТ	C T T G T G G C A G C A		G C G T T T C A A C T T T T C A	A A C A G A A T	G C A G T A A G	CAATAAGT		T G M A T A M A M T C G M G	ТАПТТАСС ССПАТССС	A T T T A T A T G A G C T G A A T T	T T C T G A C T C C C T G A A T	TCATATTGA TCATATTC	ACTATTGG ACAAAT	C A A T A
	850	860		870	890	890	500		ajo	920	aĝo	940		950	ało	970	990	
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence	T T A C T A H A A H C A	ACTTTGA ATTTAA		ТААСТТСТ	C A C T T C T T A		A F F A C F A F	TTCAA AG	A A A A C T C T G A A C C C G C			222555	∓≑≑ ⋩∓⋧⋧∓	Т & & T ? & 3 T	TAAT TAAT G	* 	AABATEE	<u>G A C A C</u>
	990	1,000	1,01	10	1,020	1,030	1,040	,	.050	1,960	1,070	1,010	1,	.090	1,100	1,110	1,120	
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence		<u>C C T T T</u> C T C C T T T - T	<u>GCATAG</u> C GCACAGA	A A T T T C G G A T T T T C G G			A A T T G C G T G A T T G C G T	ATT-CGC ATTTCTC	CTTCAGTA TTTGAATA	A A T G A A A A T A T G G A A A	T T G C A G T	G T A T T T T A G T A T G C T T		T T T T T C A H H A C A C C G T H H				TTTG TTTG
	1,190	1,140	1,150		1,160	1,170	1,180	1,190		1,200	1,210	1,220	1,230		240	1,250	1,260	
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence	C C A G C T G T C T C G G C T G T G	<u>A A T T G C</u> A A T T G C A	<u>G A G T G T C</u> G A G T G T C	G A C T G A A T G G G T G A A T	ТАТТ G G C Z	ATTAATTS	CATIGATI	<u>T T G A T T G</u> T T G A T T T	T A A T G A A C T A A C G A G C		<u>G A A A C G T C</u> G A A A C G T C	A A C A G C T A A A C A G T G T	A G A G T G G G A A G T G G -	<u>C C A T A A A A C</u> C C A T A A A C C	C C G G A G G G C C A G C G G -	ATTATCTTC ATTG-CTTG	<u>T A G A A A C C</u> T A G A A A C C	T <u>T G A A</u> C T G A A
	1,270	1,280	1,290	1.3	00	1,310	1,320	1,330	1.	340	1,350	1,360	1,370	1,380		1,390	1,400	1,410
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence	EATETETE	CGAATTA CGT-TTA		 }}\$\$\$\$	ttatsa fta	A G T A C C C G A T A C C C	T & & T & & B & F & A & F & A & F & A & F & A & A & A	TTTT33	GAAAGGAC GT-GGTC	ETTATAAA	AGTGCGEG	gattage ê	A A A A G A T T G T C A G T C T	TGTGTAA TTTGCCAAA	A A T T G G T G		<u> </u>	ATTCG ACTCG
		1,420	1,430	1,440	1.4	150	1,460	1,470	1,490		1,490	1,500	1,510	1.520	1.	530	1,540	1,550
Ds bok 5' flanking sequence	CGTGCTCGG	STTGCAA	ТТАССББ	G A A A T						eam			GAATGGAG		G 6	1974 G G G G G G G G G		
Dm_bnk_5' flanking sequence	ččtčtčč č	ĞŦŦĞĊÂĂ	t t á č č č č	GAAATCGC	тсстттдас	БААА́ П СТТ	č č t t t t t t	à Ĉ T T T Ĉ Â	TĂ <mark>ĞĊTTĂĞ</mark> TAGU	GTAGTGAT	C T C A G G T A TAGteam	ст ттссс	GAATTAAG	ттасстсс	GTATCGC	A T C GGGAT		TGAAC
	1,50	50	1,570	1,580	1,590		1,400	1,610	1,620	1	,¢30	1,640	1,650	1,660 1	ies			
Ds_bnk 5' flanking sequence Dm_bnk_5' flanking sequence	T C C C C G G A C T T T T G G C A C	C A G C T G T C A G C T G	C C G G T G T C G G G G G T	Т G A A A C T A G A A A A C T G			<u> G A A</u> T C C <u>C A</u> G A A G T G C A			C G T T C C T G C G T T C C T G	G C A A C A G C G T C T C A A C	AGCACATT	C G T T T A T C C G T A C T T C	A G C C T T C A A A A C C G T C A A	C			

Figure S8. Alignment of the *Ds_bnk* and *Dm_bnk* 5' flanking sequences. The upstream flanking sequence from *Ds_bnk* (1581 bp) and *Dm_bnk* (1523 bp) together with the 5' UTR (annotated in green) are compared. The 5' flanking sequences of *Ds_bnk* and *Dm_bnk* contain three and four TAGteam motifs (annotated in yellow), respectively, and the TATA box (TATATAAA) is 25 bp upstream of the putative transcription start site. The *Ds_bnk* 5' flanking sequence was fused to DsRed-NLS in V208 to act as a gene promoter for the *in vitro* test.



Figure S9. Alignment of the *Ds_slam* and *Dm_slam* **5' flanking sequences**. The upstream flanking sequence from *Ds_slam* (822 bp) and *Dm_slam* (887 bp) together with the 5' UTRs (annotated in green) are compared. Neither the *Ds_slam* nor *Dm_slam* 5' flanking sequences contain TAGteam motifs or a TATA box. The *Ds_slam* 5' flanking sequence was fused to DsRed-NLS in V207 to act as a gene promoter for the *in vitro* test.