Supplemental Table 1

Genotypes

***D. melanogaster* males:**	***D. melanogaster* male genotype**	**Figure**
WT	*+ ; +/+ ; +/+*	*Fig. 1, Extended Data Fig. 1*
fru^{Gal4}>UAS-GFP	*w- ; UAS-GFP/+ ; fru^{Gal4}/+*	*Fig. 2a, Extended Data Fig. 2b-g*
WT fru^{Gal4}>UAS-GCaMP	*w- ; UAS-GCaMP/cyo ; fru^{Gal4}/tm6b*	Fig. 3a-b, 4e, 4g (function), 5c, 5e-f, *Extended Data Fig. 3e, 4i, 6d, 6h, 7c*
ppk23$^{-/-}$ fru^{Gal4}>UAS-GCaMP	*w+, Δppk23 ; UAS-GCaMP/+ ; fru^{Gal4}/+*	Fig. 3b, *Extended Data Fig. 3e, 4i, 6c-d*
ppk23-Gal4>UAS-GCaMP	*w- ; UAS-GCaMP/cyo ; ppk23-Gal4/tm6b*	Fig. 3d, *Extended Data Fig. 3e, 3f*
ppk23-Gal4	*w- ; +/+ ; ppk23-Gal4/+*	Fig. 3e
UAS-CsChrimson	*w-, 20xUAS-IVS-CsChrimson.mVenus ; +/+ ; +/+*	Fig. 3e
ppk23-Gal4>UAS-CsChrimson	*w-, 20xUAS-IVS-CsChrimson.mVenus; +/+ ; ppk23-Gal4/+*	Fig. 3e
fru^{LexA} > LexA-Op-GFP, SplitP1-Gal4>UAS-GCaMP	*w- ; UAS-GCaMP6s, LexAOp-Tomato/SplitP1-Gal4 AD ; Fru-LexA/ SplitP1-Gal4 DBD*	Fig. 4a, 4g (anatomy)
fru^{LexA} > LexAOp-sPA-t2a-sPA-GFP	*w- ; LexAop-SPA-T2A-SPA / LexAop-SPA-T2A-SPA; FruLexA/tm2*	*Fig. 5b, Extended Data Fig. 5a-c*
mAL-Gal4>UAS-GCaMP	*w- ; UAS-GCaMP/+ ; R25E04-Gal4/+*	*Fig. 5d, Extended Data Fig. 6e, 6g*
ppk23-Gal4>UAS-GFP	*w- ; UAS-GFP/cyo ; ppk23-Gal4/tm6b*	*Extended Data Fig. 3a-c*
P1-Gal4>UAS-GCaMP	*w-, UAS-CsChrimson ; +/+ ; R71G01-Gal4/+*	*Extended Data Fig. 4a-b*
fru^{LexA} > LexA-Op-GCaMP, P1-Gal4>UAS-Tomato	*w- ; UAS-tdTomato/LexAOp-GCaMP6s ; R71G01-Gal4/fru-LexA*	*Extended Data Fig. 4g*
WT P1-Gal4 > UAS-GCaMP	*w- ; UAS-GCaMP/cyo ; 71G01-Gal4/tm6b*	*Extended Data Fig. 4h*
ppk23$^{-/-}$ P1-Gal4 >UAS-GCaMP	*w+, Δppk23 ; UAS-GCaMP/+ ; 71G01-Gal4/+*	*Extended Data Fig. 4h*
fru^{LexA} > LexA-Op-GCaMP, vAB3-Gal4>UAS-Tomato	*w- ; UAS-tdTomato/LexAOp-GCaMP6s ; AbdominalB[LDN]-Gal4/fru-LexA*	*Extended Data Fig. 6a*
WT vAB3-Gal4 > UAS-GCaMP	*w-; UAS-GCaMP6s/cyo; AbdB-Gal4/tm2*	*Extended Data Fig. 6a-b*
ppk23$^{-/-}$ vAB3-Gal4 > UAS-GCaMP	*w+, Δppk23; UAS-GCaMP6s/+; AbdB-Gal4/+*	*Extended Data Fig. 6b*

***D. simulans* males:**	***D. simulans* male genotype**	**Figure**
WT	*w+; +/+ ; +/+*	*Fig. 1, 2b, Extended Data Fig. 1*
Gr32a$^{-/-}$	w+; ΔGr32a/ΔGr32a ; +/+	*Fig. 1, Extended Data Fig. 1*
ppk23$^{-/-}$	w+, Δppk23 ; +/+ ; +/+	*Fig. 1, Extended Data Fig. 1*
fru^{GFP}	w+; +/+ ; *fru^{GFP}*/+	*Fig. 2a, 4a, 4g (anatomy), 5b, Extended Data Fig. 2a-g, 5a-d*
fru$^{-/-}$	*w-; +/+ ;* Δfru/Δfru	*Fig. 2b, Extended Data Fig. 2h*
fru^{Gal4}	w+; +/+ ; *fru^{Gal4}*/+	*Fig. 2c, Extended Data Fig. 2a*
fru^{Gal4} > UAS-CsChrimson	w+ ; +/+ ; 20xUAS-IVS-CsChrimson.tdTomato (2039)/ *fru^{Gal4}*	*Fig. 2c*
WT fru^{Gal4} > UAS-GCaMP	w- ; +/+ ; *fru^{Gal4}*/ UAS-GCaMP6s (2186)	Fig. 3a, 3c, 4f, 4h (function), 5c, 5e-f, *Extended Data Fig. 3e, 4j, 6c-d, 6h, 7a-g*
ppk23$^{-/-}$ fru^{Gal4}>UAS-GCaMP	w+, Δppk23 ; +/+ ; *fru^{Gal4}*/ UAS-GCaMP6s (2186)	Fig. 3c, *Extended Data Fig. 3e, 4j*

ppk23-Gal4>UAS-GCaMP	w- ; ppk23-Gal4 (2176)/+; UAS-GCaMP (2186)/+	Fig. 3d, *Extended Data Fig. 3a-f*
ppk23-Gal4	w+ ; ppk23-Gal4 (2176)/+; +/+	Fig. 3e
UAS-CsChrimson	w+; +/+ ; 20xUAS-IVS-CsChrimson.tdTomato (2039)	Fig. 3e
ppk23-Gal4>UAS-CsChrimson	w+ ; ppk23-Gal4 (2176)/+ ; 20xUAS-IVS-CsChrimson.tdTomato (2039)/+	Fig. 3e
P1-Gal4> UAS-CsChrimson	*w+ ; R71G01-Gal4/+;* 20xUAS-IVS-CsChrimson.mVenus/+	*Fig. 4b, 4c, Extended Data Fig. 4a, 4c-f*
UAS-CsChrimson	*w+ ; +/+; UAS-CsChrimson.mVenus/+*	*Extended Data Fig. 4d-f, 6f*
mAL-Gal4>UAS-GCaMP	w- ; R25E04-Gal4 (2176)/+ ; UAS-GCaMP6s (2186)/+	*Fig. 5d, Extended Data Fig. 6e, 6g*
mAL-Gal4>UAS-CsChrimson	w+ ; R25E04-Gal4 (2176)/+ ; *UAS-CsChrimson.mVenus*/+	*Extended Data Fig. 6f*
mAL-Gal4	w+ ; R25E04-Gal4 (2176)/+ ; +/+	*Extended Data Fig. 6f*

Landing Sites: attP 2178 (3R), attP 2176 (2L), attP 2186 (3R) and attP 2039 (3L)

***D. melanogaster, D. simulans, D. sechellia, D. erecta* and *D. ananassae* females:** always wild type (+/+ ; +/+ ; +/+)

Sample Size and Statistics

Figures 1b, d and e
Analysis (1b, d): Courtship Preference = (time spent courting *D. simulans* (sim) female - time spent courting *D. melanogaster* (mel) female)/(total time spent courting)
Analysis (1e): Courtship Preference = (time spent courting *D. simulans* female perfumed with ethanol (+EtOH) - time spent courting *D. simulans* female perfumed with 7,11-HD (7,11-HD))/(total time spent courting)
Null hypothesis: The male spends equal time courting both females thus exhibiting a preference index of zero
All are biological replicates assessed with a two-tailed test

Figure	Species	Condition	Targets	Sample Size (# males tested)	Statistical Test	P-value
1b	mel WT	+ tarsi	mel v sim	13	One-sample t-test	<0.0001
	mel WT	- tarsi	mel v sim	17	One-sample t-test	0.9303
	sim WT	+ tarsi	mel v sim	17	One-sample t-test	0.0009
	sim WT	- tarsi	mel v sim	17	One-sample t-test	0.4521
1d	sim	wild type	mel v sim	19	One-sample t-test	<0.0001
	sim	*Gr32a* $^{-/-}$	mel v sim	20	One-sample t-test	<0.0001
	sim	*ppk23* $^{-/-}$	mel v sim	20	One-sample t-test	0.9931
1e	sim	wild type	EtOH v 7,11-HD	19	One-sample t-test	<0.0001
	sim	*ppk23* $^{-/-}$	EtOH v 7,11-HD	19	One-sample t-test	0.534

Figures 1c
Analysis: Courtship Index = total time male courts a female / total time of the assay
Null hypothesis: The courtship index is equivalent for all groups
All are biological replicates assessed with a two-tailed test
Different letters represent significant differences in courtship behavior

Figure	Genotype	Target female	#	Sample Size (# males tested)	Statistical Test	Multiple comparisons test	Adjusted P-value	Summary	Group
1d	sim wild type	sim	1	25	Kruskal-Wallis test P<0.0001	1v2	>0.9999	ns	1 = A
	sim *Gr32a* $^{-/-}$	sim	2	24		1v3	>0.9999	ns	2 = A
	sim *ppk23* $^{-/-}$	sim	3	24		1v4	<0.0001	****	3 = A
	sim wild type	mel	4	24		1v5	<0.0001	****	4 = B
	sim *Gr32a* $^{-/-}$	mel	5	24		1v6	>0.9999	ns	5 = B
	sim *ppk23* $^{-/-}$	mel	6	24		2v3	>0.9999	ns	6 = A
						2v4	<0.0001	****	
						2v5	<0.0001	****	
						2v6	0.5992	ns	
						3v4	0.0002	***	
						3v5	<0.0001	****	
						3v6	>0.9999	ns	
						4v5	>0.9999	ns	
						4v6	0.0012	**	
						5v6	0.0004	***	

Figure 2b
Analysis: Courtship Preference = (time spent courting *D. simulans* (sim) female-time spent courting *D. melanogaster* (mel) female)/(total time spent courting)
Null hypothesis: The male spends equal time courting both females thus exhibiting a preference index of zero
All are biological replicates assessed with a two-tailed test

Figure	Species	Condition	Target Female	Sample Size (# males tested)	Statistical Test	One-tailed or Two-tailed	P-value
Fig. 2b	sim	wild type	sim v mel	16	One-sample t-test	Two-tailed	<0.0001
	sim	*fru* $^{-/-}$	sim v mel	18	One-sample t-test	Two-tailed	0.5644

Figure 2c
Analysis: Courtship Behavior Index = fraction of time male is displaying courtship behavior
Null hypothesis Wilcoxon: Fraction of time spent displaying courtship behaviors in light and dark is equal
All are biological replicates assessed with a two-tailed test

Figure	Genotype	Target	Sample Size (# males tested)	Statistical Test	Comparison	P-value
Fig. 2c	*fru*^{Gal4}	n/a	5	Wilcoxon matched-pairs signed rank test	with light v without light	0.999
	fru^{Gal4}*>UAS-CsChrimson*	n/a	5	Wilcoxon matched-pairs signed rank test	with light v without light	0.034

Figures 3b-d, 4e, 4f
Analysis: Average peak ΔF/F response to a female stimulus measured in the VNC (3b-d) or LPC (4e, f) per male
Null hypothesis: Responses to *D. simulans* (sim) female and *D. melanogaster* (mel) female are equal
All are biological replicates assessed with a two-tailed test

Figure	Species	Genotype	Stimulus	Sample Size (# males imaged)	Statistical comparison	Statistical Test	P-value

3b	mel	*WT fru^{Gal4}> UAS-GCaMP*	mel and sim females	14	mel v sim females	Wilcoxon matched-pairs signed rank test	0.0002
	mel	*ppk23$^{-/-}$ fru^{Gal4}> UAS-GCaMP*	mel and sim females	6	mel v sim females	Wilcoxon matched-pairs signed rank test	>0.9999
3c	sim	*WT fru^{Gal4}> UAS-GCaMP*	mel and sim females	12	mel v sim females	Wilcoxon matched-pairs signed rank test	0.005
	sim	*ppk23$^{-/-}$ fru^{Gal4}> UAS-GCaMP*	mel and sim females	6	mel v sim females	Wilcoxon matched-pairs signed rank test	0.6875
3d	mel	*ppk23-Gal4> UAS-GCaMP*	mel and sim females	6	mel v sim females	Wilcoxon matched-pairs signed rank test	0.0078
	sim	*ppk23-Gal4> UAS-GCaMP*	mel and sim females	6	mel v sim females	Wilcoxon matched-pairs signed rank test	0.0313
4e	mel	*fru^{Gal4}> UAS-GCaMP*	mel and sim females	9	mel v sim females	Paired t-test	<0.0001
4f	sim	*fru^{Gal4}> UAS-GCaMP*	mel and sim females	12	mel v sim females	Paired t-test	<0.0001

Figures 3e
Analysis: Courtship Index = total time male courts a female / total time of the assay
Null hypothesis: The courtship index is equivalent for all groups
All are biological replicates assessed with a two-tailed test
Different letters represent significant differences in courtship behavior

Figure	Species	Genotype	Target female	#	Sample Size (# males tested)	Statistical Test	Multiple comparisons test	Adjusted P-value	Summary	Group
3e	mel	*ppk23-Gal4*	mel	1	16	Kruskal-Wallis test P<0.0001	1v2	0.4957	ns	1 = A
	mel	*UAS-CsChrimson*	mel	2	17		1v3	<0.0001	****	2 = A
	mel	*ppk23-Gal4> UAS-CsChrimson*	mel	3	17		2v3	0.0037	**	3 = B
	sim	*ppk23-Gal4*	sim	1	24	Kruskal-Wallis test P<0.0001	1v2	>0.9999	ns	1 = A
	sim	*UAS-CsChrimson*	sim	2	21		1v3	<0.0001	****	2 = A
	sim	*ppk23-Gal4> UAS-CsChrimson*	sim	3	23		2v3	<0.0001	****	3 = B

Figures 4b
Analysis: Courtship Index = total time male courts a female / total time of the assay
Null hypothesis: The courtship index is equivalent for all groups
All are biological replicates assessed with a two-tailed test
Different letters represent significant differences in courtship behavior

Figure	Species	Genotype	Target	#	Sample Size (# males tested)	Statistical Test	Multiple comparisons test	Adjusted P-value	Summary	Group
4b	sim	*71G01-Gal4> UAS-CsChrimson*	magnet: pre-stimulation	1	18		1v2	<0.0001	****	1 = A
	sim	*71G01-Gal4> UAS-CsChrimson*	magnet: during stimulation	2	18	Kruskal-Wallis test P<0.0001	1v3	0.0039	**	2 = B
	sim	*71G01-Gal4> UAS-CsChrimson*	magnet: post-stimulation	3	18		2v3	0.0014	**	3 = C
	sim	*71G01-Gal4> UAS-CsChrimson*	sim female: pre-stimulation	1	18		1v2	<0.0001	****	1 = A
	sim	*71G01-Gal4> UAS-CsChrimson*	sim female: during stimulation	2	18	Kruskal-Wallis test P<0.0001	1v3	0.0001	***	2 = B
	sim	*71G01-Gal4> UAS-CsChrimson*	sim female: post-stimulation	3	18		2v3	0.0101	*	3 = C
	sim	*71G01-Gal4> UAS-CsChrimson*	mel female: pre-stimulation	1	20		1v2	<0.0001	****	1 = A
	sim	*71G01-Gal4> UAS-CsChrimson*	mel female: during stimulation	2	20	Kruskal-Wallis test P<0.0001	1v3	0.0005	***	2 = B
	sim	*71G01-Gal4> UAS-CsChrimson*	mel female: post-stimulation	3	20		2v3	0.0011	**	3 = C

Figures 4c
Analysis: Courtship Index = total time male courts a female / total time of the assay
Null hypothesis: The distribution of courtship indices towards a *D. simulans* (sim) female and *D. melanogaster* (mel) female will be identical
All are biological replicates assessed with a two-tailed test

Figure	Species	Genotype	Light Stimulation	Target Female	Sample Size (# males tested)	Statistical Test	P value
4c	sim	*71G01-Gal4> UAS-CsChrimson*	Dim	sim	11	Mann Whitney	>0.9999
	sim	*71G01-Gal4> UAS-CsChrimson*	Dim	mel	11		
	sim	*71G01-Gal4> UAS-CsChrimson*	0.75 uW/mm2	sim	11	Mann Whitney	0.4854
	sim	*71G01-Gal4> UAS-CsChrimson*	0.75 uW/mm2	mel	11		
	sim	*71G01-Gal4> UAS-CsChrimson*	1 uW/mm2	sim	11	Mann Whitney	0.0005
	sim	*71G01-Gal4> UAS-CsChrimson*	1 uW/mm2	mel	11		
	sim	*71G01-Gal4> UAS-CsChrimson*	1.25 uW/mm2	sim	11	Mann Whitney	<0.0001
	sim	*71G01-Gal4> UAS-CsChrimson*	1.25 uW/mm2	mel	11		
	sim	*71G01-Gal4> UAS-CsChrimson*	1.5 uW/mm2	sim	11	Mann Whitney	<0.0001
	sim	*71G01-Gal4> UAS-CsChrimson*	1.5 uW/mm2	mel	11		
	sim	sim *71G01-Gal4>UAS-CsChrimson*	2 uW/mm2	sim	11	Mann Whitney	<0.0001

	sim	sim *71G01-Gal4>UAS-CsChrimson*	2 uW/mm2	mel	11		
	sim	sim *71G01-Gal4>UAS-CsChrimson*	Bright	sim	11	Mann Whitney	<0.0001
	sim	sim *71G01-Gal4>UAS-CsChrimson*	Bright	mel	11		

Figures 4g, h
Analysis: Peak ΔF/F response to a *D. melanogaster* female stimulus per male per tap
Sample size is 2-3 technical replicates per 6 biological replicates

Figure	Species	Genotype	Stimulus	Sample Size (# taps)	Statistical Test	Slope ± SE	95% Confidence Intervals of Slope	Goodness of Fit
4g	mel	*fru^{Gal4}>UAS-GCaMP*	mel females	17	Linear Regression	0.4114 ± 0.1424	0.1079 to 0.7149	0.3575
4f	sim	*fru^{Gal4}>UAS-GCaMP*	mel females	21	Linear Regression	-0.002324 ± 0.06474	-0.1374 to 0.1327	0.0914

Figures 5c, d
Analysis: Average peak ΔF/F response to a female stimulus per male
Null hypothesis: Responses to *D. simulans* (sim) female and *D. melanogaster* (mel) female are equal
All are biological replicates assessed with a two-tailed test

Figure	Species	Genotype	Stimulus	Sample Size (# males imaged)	Statistical comparison	Statistical Test	P-value
5c	mel	*fru^{Gal4}> UAS-GCaMP*	mel and sim females	7	mel v sim female	Paired t-test	0.0031
	sim	*fru^{Gal4}> UAS-GCaMP*	mel and sim females	7	mel v sim female	Paired t-test	0.0093
5d	mel	*25E04-Gal4> UAS-GCaMP*	mel and sim females	6	mel v sim female	Paired t-test	0.005
	sim	*25E04-Gal4> UAS-GCaMP*	mel and sim females	6	mel v sim female	Paired t-test	0.0059

Figures 5f
Analysis: Average peak ΔF/F response to vAB3 stimulation per male
Null hypothesis: Responses in P1 neurons are equal in *D. simulans* (sim) and *D. melanogaster* (mel) males
All are biological replicates assessed with a two-tailed test

Figure	Species	Genotype	Condition	Sample Size (# of males imaged)	Statistical comparison	Statistical Test	P-value
5f	mel	*fru^{Gal4}>UAS-GCaMP*	mAL intact (+)	7	P1 mel male v P1 sim male	Unpaired t-test with Welch's correction	0.0003
	sim	*fru^{Gal4}>UAS-GCaMP*	mAL intact (+)	7			
	mel	*fru^{Gal4}>UAS-GCaMP*	mAL severed (-)	8	P1 mel male v P1 sim male	Unpaired t-test with Welch's correction	0.0318
	sim	*fru^{Gal4}>UAS-GCaMP*	mAL severed (-)	8			

Extended Data Figure 1e
Analysis: Courtship Index = total time male courts a female / total time of the assay
Null hypothesis: The courtship index is equivalent for all groups
All are biological replicates assessed with a two-tailed test

Target females: *D. simulans* (sim), *D. sechellia* (sech), *D. melanogaster* (mel), *D. erecta* (erc) and *D. ananassae* (ana)
Different letters represent significant differences in courtship behavior

Figure	Genotype	Target female	#	Sample Size (# males tested)	Statistical Test	Multiple comparisons test	Adjusted P-value	Summary	Group
Ex. Data Fig. 1e	sim wild type	sim	1	25	Kruskal-Wallis test P=0.0515	1v2	>0.9999	ns	1 = A
	sim *Gr32a*$^{-/-}$	sim	2	24		1v3	0.4081	ns	2 = A
	sim *ppk23*$^{-/-}$	sim	3	24		2v3	0.0572	ns	3 = A
	sim wild type	sech	1	10	Kruskal-Wallis test P=0.0022	1v2	>0.9999	ns	1 = A
	sim *Gr32a*$^{-/-}$	sech	2	13		1v3	0.0438	*	2 = A
	sim *ppk23*$^{-/-}$	sech	3	13		2v3	0.0026	**	3 = B
	sim wild type	mel	1	25	Kruskal-Wallis test P<0.0001	1v2	>0.9999	ns	1 = A
	sim *Gr32a*$^{-/-}$	mel	2	24		1v3	0.0001	***	2 = A
	sim *ppk23*$^{-/-}$	mel	3	24		2v3	<0.0001	****	3 = B
	sim wild type	erc	1	25	Kruskal-Wallis test P=0.0003	1v2	>0.9999	ns	1 = A
	sim *Gr32a*$^{-/-}$	erc	2	24		1v3	0.0037	**	2 = A
	sim *ppk23*$^{-/-}$	erc	3	24		2v3	0.0008	***	3 = B
	sim wild type	ana	1	17	Kruskal-Wallis test P=0.0022	1v2	>0.9999	ns	1 = A
	sim *Gr32a*$^{-/-}$	ana	2	9		1v3	0.0033	**	2 = A
	sim *ppk23*$^{-/-}$	ana	3	16		2v3	0.034	*	3 = B

Extended Data Figure 2h
Analysis 2h: Chaining Index = fraction of time when more than three males were simultaneously courting
Null hypothesis unpaired t-test: The male spends equal time chaining
All are biological replicates assessed with a two-tailed test

Figure	Genotype	Target	Sample Size (# males tested)	Statistical Test	Comparison	P-value
Ex Data Fig. 2h	sim wild type	8 wild type males	8	Unpaired t-test with equal SD, two-tailed	WT v mutant males	0.0004
	sim *fru*$^{-/-}$	8 *fru*$^{-/-}$ males	8			

Extended Data Figure 3a
Analysis: counting number of ppk23+ soma are present in the distal three tarsal segments of the male's foreleg tarsi
Null hypothesis unpaired t-test: Males have equal number of soma
All are biological replicates assessed with a two-tailed test

Figure	Species	Genotype	Sample Size (# males examined)	Statistical Test	Comparison	P-value
Ex Data Fig. 3a	mel	ppk23-Gal4> UAS-GFP	6	Unpaired t-test with equal SD, two-tailed	# ppk23+ soma in mel v sim males	0.7014
	sim	ppk23-Gal4> UAS-GCaMP	6			

Extended Data Figure 3e
Analysis: peak ΔF/F response to individual taps by a male
Null hypothesis: Responses are equivalent for all groups
2-3 technical replicates per several biological replicates assessed with a two-tailed test
Different letters represent significant differences in courtship behavior

Figure	Species	Genotype	Female Stimulus	#	Sample Size (# taps)	Statistical Test	Multiple comparisons test	Adjusted P-value	Summary	Group
Ex Data Fig 3e	mel	*WT fru^{Gal4}> UAS-GCaMP*	mel	1	19	One-way ANOVA P<0.0001	1v2	0.0002	***	1 = A
	mel	*WT fru^{Gal4}> UAS-GCaMP*	sim	2	17		1v3	<0.0001	****	2 = B
	mel	*ppk23$^{-/-}$ fru^{Gal4}> UAS-GCaMP*	mel	3	17		1v4	<0.0001	****	3 = C
	mel	*ppk23$^{-/-}$ fru^{Gal4}> UAS-GCaMP*	sim	4	18		2v3	0.0004	***	4 = C
							2v4	0.0001	***	
							3v4	0.9932	ns	
	sim	*WT fru^{Gal4}> UAS-GCaMP*	mel	1		One-way ANOVA P<0.0001	1v2	<0.0001	****	1 = A
	sim	*WT fru^{Gal4}> UAS-GCaMP*	sim	2			1v3	<0.0001	****	2 = B
	sim	*ppk23$^{-/-}$ fru^{Gal4}> UAS-GCaMP*	mel	3			1v4	<0.0001	****	3 = C
	sim	*ppk23$^{-/-}$ fru^{Gal4}> UAS-GCaMP*	sim	4			2v3	0.00124	**	4 = C
							2v4	0.00232	**	
							3v4	0.997	ns	
	mel	*ppk23-Gal4> UAS-GCaMP*	mel	1	21	One-way ANOVA P<0.0001	1v2	0.0001	***	1 = A
	mel	*ppk23-Gal4> UAS-GCaMP*	sim	2	21		1v3	0.2301	ns	2 = B
	sim	*ppk23-Gal4> UAS-GCaMP*	mel	3	18		1v4	0.0247	*	3 = A
	sim	*ppk23-Gal4> UAS-GCaMP*	sim	4	17		2v3	<0.0001	****	4 = B
							2v4	0.5184	ns	
							3v4	<0.0001	****	

Extended Data Figure 3f
Analysis: Average peak ΔF/F response to bristle stimulation per male
Null hypothesis: Responses are equivalent for all groups
All are biological replicates assessed with a two-tailed test

Figure	Species	Genotype	Chemical Stimulus	Sample Size (# males imaged)	Statistical comparison	Statistical Test	P-value
Ex Data Fig 3f	mel	*ppk23-Gal4> UAS-GCaMP*	7,11-HD	5	Soma A v B	Paired t-test	0.0303
	mel	*ppk23-Gal4> UAS-GCaMP*	EtOH	5	Soma A v B	Paired t-test	0.099
	sim	*ppk23-Gal4> UAS-GCaMP*	7,11-HD	5	Soma A v B	Paired t-test	0.0218
	sim	*ppk23-Gal4> UAS-GCaMP*	EtOH	5	Soma A v B	Paired t-test	0.2075

mel	*ppk23-Gal4> UAS-GCaMP*	7,11-HD	5	mel v sim	Unpaired t-test	0.2834	
sim	*ppk23-Gal4> UAS-GCaMP*	7,11-HD	5				

Extended Data Figure 4b-f, 6f
Analysis: Courtship Index = total time male courts a female / total time of the assay
Null hypothesis: The courtship index is equivalent for all groups
All are biological replicates assessed with a two-tailed test

Figure	Species	Genotype	Target female	#	Sample Size (# males tested)	Statistical Test	Multiple comparisons test	Adjusted P-value	Summary	Group
Ex Data Fig. 4b	mel	*71G01-Gal4> UAS-CsChrimson*	magnet: pre-stimulation	1	6		1v2	<0.0001	****	1 = A
	mel	*71G01-Gal4> UAS-CsChrimson*	magnet: during stimulation	2	6	Kruskal-Wallis test P<0.0001	1v3	0.0428	*	2 = B
	mel	*71G01-Gal4> UAS-CsChrimson*	magnet: post-stimulation	3	6		2v3	0.0428	*	3 = C
Ex Data Fig. 4c	sim	*71G01-Gal4> UAS-CsChrimson*	magnet: 0mm/s	1	11		1v2	0.5369	ns	1 = A
	sim	*71G01-Gal4> UAS-CsChrimson*	magnet: 3 mm/s	2	7		1v3	<0.0001	****	2 = A
	sim	*71G01-Gal4> UAS-CsChrimson*	magnet: 6 mm/s	3	11	One-way ANOVA P<0.0001	1v4	<0.0001	****	3 = B
	sim	*71G01-Gal4> UAS-CsChrimson*	magnet: 10 mm/s	4	11		1v5	<0.0001	****	4 = B
	sim	*71G01-Gal4> UAS-CsChrimson*	magnet: 20 mm/s	5	11		2v3	0.0003	***	5 = B
							2v4	<0.0001	****	
							2v5	<0.0001	****	
							3v4	0.6909	ns	
							3v5	0.2097	ns	
							4v5	0.9076	ns	
Ex Data Fig. 4d	sim	*71G01-Gal4*	magnet: during stimulation	1	5		1v2	>0.9999	ns	1 = A
	sim	*UAS-CsChrimson*	magnet: during stimulation	2	5	Kruskal-Wallis test P<0.0001	1v3	0.0029	**	2 = B
	sim	*71G01-Gal4> UAS-CsChrimson*	magnet: during stimulation	3	18		2v3	0.0017	**	3 = C
	sim	*71G01-Gal4*	magnet: post-stimulation	1	5		1v2	>0.9999	ns	1 = A
	sim	*UAS-CsChrimson*	magnet: post-stimulation	2	5	Kruskal-Wallis test P=0.0002	1v3	0.0173	*	2 = A

	sim	*71G01-Gal4>* *UAS-CsChrimson*	magnet: post-stimulation	3	18		2v3	0.0011	**	3 = B
Ex Data Fig. 4e	sim	*71G01-Gal4*	sim female: during stimulation	1	12		1v2	>0.9999	ns	1 = A
	sim	*UAS-CsChrimson*	sim female: during stimulation	2	12	Kruskal-Wallis test P<0.0001	1v3	<0.0001	****	2 = A
	sim	*71G01-Gal4>* *UAS-CsChrimson*	sim female: during stimulation	3	20		2v3	<0.0001	****	3 = B
	sim	*71G01-Gal4*	sim female: post-stimulation	1	12		1v2	>0.9999	ns	1 = A
	sim	*UAS-CsChrimson*	sim female: post-stimulation	2	12	Kruskal-Wallis test P<0.0001	1v3	<0.0001	****	2 = A
	sim	*71G01-Gal4>* *UAS-CsChrimson*	sim female: post-stimulation	3	20		2v3	<0.0001	****	3 = B
Ex Data Fig. 4f	sim	*71G01-Gal4*	mel female: during stimulation	1	11		1v2	>0.9999	ns	1 = A
	sim	*UAS-CsChrimson*	mel female: during stimulation	2	12	Kruskal-Wallis test P<0.0001	1v3	<0.0001	****	2 = A
	sim	*71G01-Gal4>* *UAS-CsChrimson*	mel female: during stimulation	3	18		2v3	<0.0001	****	3 = B
	sim	*71G01-Gal4*	mel female: post-stimulation	1	11		1v2	>0.9999	ns	1 = A
	sim	*UAS-CsChrimson*	mel female: post-stimulation	2	12	Kruskal-Wallis test P<0.0001	1v3	<0.0001	****	2 = A
	sim	*71G01-Gal4>* *UAS-CsChrimson*	mel female: post-stimulation	3	18		2v3	<0.0001	****	3 = B
Ex Data Fig. 6f	sim	*25E04-Gal4*	sim female	1	19		1v2	>0.9999	ns	1 = A
	sim	*UAS-CsChrimson*	sim female	2	17	Kruskal-Wallis test P<0.0001	1v3	0.0081	**	2 = A
	sim	*25E04-Gal4>* *UAS-CsChrimson*	sim female	3	19		2v3	0.0006	***	3 = B

Extended Data Figure 4h-j, 6b, 6d, 6g
Analysis: peak ΔF/F response to individual taps by a male
Null hypothesis: Responses are equivalent for all groups
2-3 technical replicates per biological replicate assessed with a two-tailed test

Figure	Species	Genotype	Female Stimulus		Sample Size (# of taps)	Statistical Test	Dunn's multiple comparisons test	Adjusted P-value	Summary	Group
Ex Data Fig. 4h	mel	WT *71G01-Gal4> UAS-GCaMP*	mel	1	9	One-way ANOVA P<0.0001	1v2	0.0012	**	1 = A
	mel	WT *71G01-Gal4> UAS-GCaMP*	sim	2	9		1v3	0.0003	***	2 = B
	mel	*ppk23$^{-/-}$ 71G01-Gal4> UAS-GCaMP*	mel	3	9		1v4	0.0006	***	3 = B
	mel	*ppk23$^{-/-}$ 71G01-Gal4> UAS-GCaMP*	sim	4	9		2v3	0.9538	ns	4 = B
							2v4	0.9937	ns	
							3v4	0.9938	ns	
Ex Data Fig. 4i	mel	*WT fru-Gal4> UAS-GCaMP*	mel	1	12	One-way ANOVA P<0.0001	1v2	0.0012	**	1 = A
	mel	*WT fru-Gal4> UAS-GCaMP*	sim	2	12		1v3	<0.0001	****	2 = B
	mel	*ppk23$^{-/-}$ fru-Gal4> UAS-GCaMP*	mel	3	12		1v4	0.0003	***	3 = B
	mel	*ppk23$^{-/-}$ fru-Gal4> UAS-GCaMP*	sim	4	12		2v3	0.8855	ns	4 = B
							2v4	0.9907	ns	
							3v4	0.9705	ns	
Ex Data Fig. 4j	sim	*WT fru-Gal4> UAS-GCaMP*	mel	1	21	One-way ANOVA P<0.0001	1v2	<0.0001	****	1 = A
	sim	*WT fru-Gal4> UAS-GCaMP*	sim	2	21		1v3	<0.0001	****	2 = B
	sim	*ppk23$^{-/-}$ fru-Gal4> UAS-GCaMP*	mel	3	24		1v4	<0.0001	****	3 = C
	sim	*ppk23$^{-/-}$ fru-Gal4> UAS-GCaMP*	sim	4	24		2v3	0.0002	***	4 = C
							2v4	0.0002	***	
							3v4	0.9271	ns	
Ex Data Fig.6b	mel	*WT AbdB-Gal4> UAS-GCaMP*	mel	1	9	Kruskal-Wallis Test P=0.0002	1v2	0.0041	**	1 = A
	mel	*WT AbdB-Gal4> UAS-GCaMP*	sim	2	9		1v3	0.0004	***	2 = B
	mel	*ppk23$^{-/-}$ AbdB-Gal4> UAS-GCaMP*	mel	3	9		1v4	0.0056	**	3 = B
	mel	*ppk23$^{-/-}$ AbdB-Gal4> UAS-GCaMP*	sim	4	9		2v3	>0.9999	ns	4 = B
							2v4	>0.9999	ns	
							3v4	>0.9999	ns	
Ex Data Fig.6d	mel	*WT fru-Gal4> UAS-GCaMP*	mel	1	18	One-way ANOVA P<0.0001	1v2	<0.0001	****	1 = A
	mel	*WT fru-Gal4> UAS-GCaMP*	sim	2	16		1v3	<0.0001	****	2 = B

	mel	*ppk23$^{-/-}$ fru-Gal4> UAS-GCaMP*	mel	3	18		1v4	<0.0001	****	3 = B
	mel	*ppk23$^{-/-}$ fru-Gal4> UAS-GCaMP*	sim	4	18		2v3	0.4977	ns	4 = B
							2v4	0.3786	ns	
							3v4	0.9967	ns	
	sim	*WT fru-Gal4> UAS-GCaMP*	mel	1	22		1v2	<0.0001	****	1 = A
	sim	*WT fru-Gal4> UAS-GCaMP*	sim	2	21	One-way ANOVA P<0.0001	1v3	<0.0001	****	2 = B
	sim	*ppk23$^{-/-}$ fru-Gal4> UAS-GCaMP*	mel	3	18		1v4	<0.0001	****	3 = B
	sim	*ppk23$^{-/-}$ fru-Gal4> UAS-GCaMP*	sim	4	18		2v3	0.858	ns	4 = B
							2v4	0.8457	ns	
							3v4	>0.9999	ns	
Ex Data Fig. 6g	mel	*25E04-Gal4> UAS-GCaMP*	mel	1	19		1v2	0.0001	***	1 = A
	mel	*25E04-Gal4> UAS-GCaMP*	sim	2	15	One-way ANOVA P<0.0001	1v3	0.6114	ns	2 = B
	sim	*25E04-Gal4> UAS-GCaMP*	mel	3	15		1v4	<0.0001	****	3 = A
	sim	*25E04-Gal4> UAS-GCaMP*	sim	4	15		2v3	0.0104	*	4 = B
							2v4	0.2239	ns	
							3v4	<0.0001	****	

Extended Data Figures 6c, h
Analysis: Average peak ΔF/F response to a female stimulus per male
Null hypothesis: Responses to *D. simulans* (sim) female and *D. melanogaster* (mel) female are equal
All are biological replicates assessed with a two-tailed test

Figure	Species	Genotype	Female Stimulus	Sample Size (# of males imaged)	Statistical comparison	Statistical Test	P-value
Ex Data Fig.6c	mel	*ppk23$^{-/-}$ fru-Gal4> UAS-GCaMP*	mel	6	mel female v sim female	Paired t-test	0.7189
	mel	*ppk23$^{-/-}$ fru^{Gal4}> UAS-GCaMP*	sim	6			
	sim	*ppk23$^{-/-}$ fru^{Gal4}> UAS-GCaMP*	mel	5	mel female v sim female	Paired t-test	0.9361
	sim	*ppk23$^{-/-}$ fru^{Gal4}> UAS-GCaMP*	sim	5			
Ex Data Fig. 6h	mel	*fru^{Gal4}>UAS-GCaMP pre-PTX injection*	mel	6	mel female pre v post PTX	Paired t-test	0.0089
	mel	*fru^{Gal4}>UAS-GCaMP post-PTX injection*	mel	6			
	mel	*fru^{Gal4}>UAS-GCaMP pre-PTX injection*	sim	6	sim female pre v post PTX	Paired t-test	0.7516
	mel	*fru^{Gal4}>UAS-GCaMP post-PTX injection*	sim	6			

	sim	*fru^{Gal4}>UAS-GCaMP pre-PTX injection*	mel	6	mel female pre v post PTX	Paired t-test	0.0001
	sim	*fru^{Gal4}>UAS-GCaMP post-PTX injection*	mel	6			
	sim	*fru^{Gal4}>UAS-GCaMP pre-PTX injection*	sim	6	sim female pre v post PTX	Paired t-test	0.3620
	sim	*fru^{Gal4}>UAS-GCaMP post-PTX injection*	sim	6			

Extended Data Figures 7c, e,
Analysis: Average peak ΔF/F response to vAB3 stimulation in a given neural population per male
Null hypothesis: Responses across different conditions are equal
All are biological replicates assessed with a two-tailed test

Figure	Species	Genotype	Neural Population Imaged	#	Sample Size (# males imaged)	Statistical Test	Multiple comparisons test	Adjusted P-value	Summary	Group
Ex Data Fig. 7c	mel	*fru^{Gal4}>UAS-GCaMP pre-severing*	vAB3	1	6	Kruskal-Wallis test P=0.0004	1v2	>0.9999	ns	1 = A
	mel	*fru^{Gal4}>UAS-GCaMP mock severing*	vAB3	2	6		1v3	0.0043	**	2 = A
	mel	*fru^{Gal4}>UAS-GCaMP vAB3 severing*	vAB3	3	6		2v3	0.0242	*	3 = B
	mel	*fru^{Gal4}>UAS-GCaMP pre-severing*	mAL	1	6	Kruskal-Wallis test P=0.0003	1v2	>0.9999	ns	1 = A
	mel	*fru^{Gal4}>UAS-GCaMP mock severing*	mAL	2	6		1v3	0.0062	**	2 = A
	mel	*fru^{Gal4}>UAS-GCaMP vAB3 severing*	mAL	3	6		2v3	0.0175	*	3 = B
	sim	*fru^{Gal4}>UAS-GCaMP pre-severing*	vAB3	1	7	Kruskal-Wallis test P=0.0005	>0.9999	>0.9999	ns	1 = A
	sim	*fru^{Gal4}>UAS-GCaMP mock severing*	vAB3	2	7		0.0074	0.0043	**	2 = A
	sim	*fru^{Gal4}>UAS-GCaMP vAB3 severing*	vAB3	3	7		0.0148	0.0242	*	3 = B
	sim	*fru^{Gal4}>UAS-GCaMP pre-severing*	mAL	1	7	Kruskal-Wallis test P=0.0005	1v2	>0.9999	ns	1 = A
	sim	*fru^{Gal4}>UAS-GCaMP mock severing*	mAL	2	7		1v3	0.0074	**	2 = A
	sim	*fru^{Gal4}>UAS-GCaMP vAB3 severing*	mAL	3	7		2v3	0.0148	*	3 = B
Ex Data Fig. 7e	mel	*fru^{Gal4}>UAS-GCaMP pre-severing*	vAB3		8	mel vAB3 v sim vAB3	Unpaired t-test	0.4508		
	sim	*fru^{Gal4}>UAS-GCaMP pre-severing*	vAB3		8					
	mel	*fru^{Gal4}>UAS-GCaMP pre-severing*	mAL		12	mel mAL v sim mAL	Unpaired t-test	0.1636		
	sim	*fru^{Gal4}>UAS-GCaMP pre-severing*	mAL		8					

Extended Data Figures 7f, g
Analysis: Peak ΔF/F response to an individual stimulation of vAB3
Null hypothesis: Responses across conditions and species are equal

All are technical replicates assessed with a two-tailed test

Figure	Species	Genotype	Neural Population Imaged	#	Sample Size (# stimulations)	Statistical Test	Multiple comparisons test	Adjusted P-value	Summary	Group
Ex Data Fig. 7f	mel	*fru^{Gal4}>UAS-GCaMP pre-severing mAL*	vAB3	1	15	One-way ANOVA P=0.1363	1v2	0.8467	ns	1 = A
	sim	*fru^{Gal4}>UAS-GCaMP pre-severing mAL*	vAB3	2	15		1v3	0.9779	ns	2 = A
	mel	*fru^{Gal4}>UAS-GCaMP post-severing mAL*	vAB3	3	14		1v4	0.3557	ns	3 = A
	sim	*fru^{Gal4}>UAS-GCaMP post-severing mAL*	vAB3	4	16		2v3	0.6811	ns	4 = A
							2v4	0.0956	ns	
							3v4	0.6701	ns	
Ex Data Fig. 7g	mel	*fru^{Gal4}>UAS-GCaMP pre-severing mAL*	P1	1	15	One-way ANOVA P<0.0001	1v2	0.0001	***	1 = A,B
	sim	*fru^{Gal4}>UAS-GCaMP pre-severing mAL*	P1	2	15		1v3	>0.9999	ns	2 = C
	mel	*fru^{Gal4}>UAS-GCaMP post-severing mAL*	P1	3	14		1v4	>0.9999	ns	3 = B
	sim	*fru^{Gal4}>UAS-GCaMP post-severing mAL*	P1	4	16		2v3	<0.0001	****	4 = B
							2v4	0.00147	*	
							3v4	0.4821	ns	

Supplemental Table 2

CRISPR and genotyping primers:

Extended Data Fig. 1d - Sim Gr32a CRISPR

CrSim_Gr32a-F: gaaattaatacgactcactataGGCGAGATTCTTCGCGGATAgttttagagctagaaatagc

Extended Data Fig. 1d - Genotype sim Gr32a mutant

SimSeq_Gr32a-F: CCCGAACACTTGGGTAATTG

SimSeq_Gr32a-R: CGATCCACTGGTTCACATTG

Extended Data Fig. 1d - Sim ppk23 CRISPR

CrSim_ppk23-F: gaaattaatacgactcactataGGTCTGGAACTTCTCCCAGgttttagagctagaaatagc

Extended Data Fig. 1d - Genotype sim ppk23 mutant

SimSeq_ppk23-F:

SimSeq_ppk23-R: CCTGGGCCTCGTCGTAGTTA

Extended Data Fig. 2a - Fru Intron CRISPR

CrSim_FruIntron-F: gaaattaatacgactcactataGGTCCGCGGAAAAGGGCGTAgttttagagctagaaatagc

Extended Data Fig. 2a - Fru Intron attP oligo

GCTTTGGGCGTTTGATTCTCGACGCTTAGCGCTCGGAATTCAGTGCTCAGTTCAGTAGGTGACACCATTGCGCTACG

CCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACTACGCGGCCGCCGTAGGTGTTTTGGTCGGCCCAC

GACGTCTGGCCTATATTGCCACATATGGCAGTATATGCAACTCCTCCCG

Extended Data Fig. 2a - Genotype sim Fru-attP Intron

Sim_FruIntron-ExF: GCTTTGGGCGTTTGATTCT

Sim_FruIntron-ExR: GCACAACCCACATAAATCTCAA

Extended Data Fig. 2a - Genotype sim Fru-GFP

Sim_FruGFP-InR: TTGGGACAACTCCAGTGAAA

Extended Data Fig. 2a - Genotype Fru-GAL4

Sim_FruGAL4-InR: TCGGTTTTTCTTTGGAGCAC

Extended Data Fig. 2h - Fru Exon CRISPR

CrSim_FruExon-F: gaaattaatacgactcactataGGTCCGCGGAAAAGGGCGTAgttttagagctagaaatagc

Extended Data Fig. 2h - Fru Exon attP oligo

GCTTTCAGCCAGAGCCAAATTGTTGGCGACGTCACAGGATTATTTTGGCAATCCATACGCCCTTTTCCGCGGCTACG

CCCCCAACTGAGAGAACTCAAAGGTTACCCCAGTTGGGGCACTACgaattcACCGCCCACAACACTGCGGCCACGCGA

GTCGCCGCTGGGCGTGGGCCACCCTCACGGCCATGGGCACCTGCA

Extended Data Fig. 2h - Genotype sim Fru-attP Exon

Sim_FruExon-F: GAGGCAATCGGTGGCTATAA

Sim_FruExon-R: GGAGGCTTACCTAGGGGATG

attB-SAS-GFP Plasmid:

Forward primer for eGFP with SAS+Kozak sequences and reverse primer for eGFP with SV40 termination sequence

SAS-Kozak-GFP-F:

cggccgcggacatatgcaCACCTGCgatcgtagtgccccaactggggtaacctttgaAAAAGCAGGCTTCAGTCGATCCAACATGGCGACTT

GTCCCATCCCCGGCATGTTTAAATATACTAATTATTCTTGAACTAATTTTAATCAACCGATTTATCTCTCTTCCGCAGCA

AAATGAGTAAAGGAGAAGAACTTTTCAC

GFP-SV40-R:

tacgcccccaacGGGGACCACTTTGTACAAGAAAGCTGGGTGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAA

CCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGC

TGCAATAAACAAGTTCAGTTCCATAGGTTGGAATCTAAA

Nested PCR primers to add on gibson overhangs to GFP PCR product

Gib-GFP-pHDattP-F: acacctgcgatcgtagtgccccaactggggtaacctttgaAAAAGCAGGCTTCAGTCGAT

Gib-GFP-pHDattP-R: tatagcatacattatacgaagttatctacgcccccaacGGGGACCACTTTGTACAAGAAA

Linearize pHD-DsRed-attP plasmid

Gib-Linear-pHDattP-F: gttgggggcgtagataacttc

Gib-Linear-pHDattP-R: tcaaaggttaccccagttgg

oligo for inserting attB

EcoRI-attB: AATTcGGAGTACGCGCCCGGGGAGCCCAAGGGCACGCCCTGGCACCCGCACCGCGGgc

attB-NotI:

ggccgcCCGCGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCg

Sequencing primers for attB-SAS-GFP insertion

DsRed-GFP-Seq-LeftF: CATGCCGAACTCAGAAGTGA

DsRed-GFP-Seq-LeftR: TTGGGACAACTCCAGTGAAA

pHD-GFP-Seq-RightF: TCCAACCTATGGAACTGAACTTG

pHD-GFP-Seq-RightR: CGACGTGTTCACTTTGCTTG

attB-SAS-Gal4 Plasmid:

Forward and reverse primer for Gal4 with Gibson assembly overhang

Gibson-Gal4-F

ATTTATCTCTCTTCCGCAGCAAAGAAAGATGAAGCTACTGTCTTCTATCG

Gibson-Gal4-R

GATCCACTAGTTCTAGAGCGGCGCATAGGCCACTAGTtaaagatc

Forward primer for attB-SAS with Gibson assembly overhang

Gibson-attBSAS-F:

TGGGGTGTCGCCCTTCGCTGAAGCAGGTGgAGCAGGTGgAATTcGGAGTA

Gibson-attBSAS-R:

GCATGCTTGTTCGATAGAAGACAGTAGCTTCATCTTTCTTTGCTGCGGAAGAGAGAT