Supplementary information

Supplementary Table 2. Wild-type and transgenic lines used in this study.

Stock name	Donor plasmid	Parental strain	Species	Method/Reference	Figure
Dsec.07		*Drosophila* Species Stock Center [DSSC] 14021-0248.07	*D. sechellia*		Fig. 1, 2, 4, 5; Extended Data Fig. 1, 2, 5, 7
Dsec.30		DSSC 14021-0248.30	*D. sechellia*		Fig. 1; Extended Data Fig. 2, 7
Dsec.28		DSSC 14021-0248.28	*D. sechellia*		Fig. 1; Extended Data Fig. 1, 2
Dmel CS		*D. melanogaster Canton S*	*D. melanogaster*		Fig. 1, 2, 4, 5; Extended Data Fig. 1, 2, 3, 5, 7
Dmel OR		*D. melanogaster Oregon R*	*D. melanogaster*		Fig. 1; Extended Data Fig. 1, 2
Dmel w^{1118}		*D. melanogaster w1118*	*D. melanogaster*		Fig. 1; Extended Data Fig. 2, 3, 7
Dsim.04		DSSC 14021-0251.004	*D. simulans*		Fig. 1, 2, 4, 5; Extended Data Fig. 1, 2, 5, 7
Dsim.196		DSSC 14021-0251.196	*D. simulans*		Fig. 1; Extended Data Fig. 1, 2
Dsim.195		DSSC 14021-0251.195	*D. simulans*		Fig. 1; Extended Data Fig. 2
DsecIr8a$^{-/-}$/*Orco*$^{1-/-}$			*D. sechellia*	[1]	Fig. 1; Extended Data Fig. 2
DsecOrco^{1}			*D. sechellia*	[1]	Fig. 1; Extended Data Fig. 2
DsimOrco^{1}	*pHD-DsimOrco-3xP3-DsRed*	DSSC 14021-0251.195	*D. simulans*	CRISPR knock-in, this study	Fig. 1; Extended Data Fig. 2
DmelOrco$^{-/-}$		BDSC_23130	*D. melanogaster*		Fig. 1; Extended Data Fig. 2
Dmel Ir8a$^{-/-}$/*Orco*$^{-/-}$/*Ir25a*$^{-/-}$/*Gr63a*$^{-/-}$			*D. melanogaster*	[2]	Extended Data Fig. 2
poxn^{70}		BDSC_60688	*D. melanogaster*		Fig.1, Extended Data Fig. 3
poxn$^{70\text{-}23}$			*D. melanogaster*	[3], gift from Anne von Philipsborn	Fig.1, Extended Data Fig. 3

Dmel Gr64f-Gal4		Bloomington Drosophila Stock Center [BDSC] 57669	*D. melanogaster*		Fig. 1, 2; Extended Data Fig. 3, 5
Dmel Gr66a-Gal4			*D. melanogaster*	[4]	Fig. 1, 3; Extended Data Fig. 3, 6
Dmel Gr66a-Gal4, Gr64f-Gal4		BDSC_57668	*D. melanogaster*	[4], this study	Fig. 1, Extended Data Fig. 3
Dmel Ir25a-Gal4		BDSC_41728	*D. melanogaster*		Fig. 2; Extended Data Fig. 4, 5
Dmel ppk28-Gal4		BDSC_93020	*D. melanogaster*		Fig. 1; Extended Data Fig. 3
Dmel ppk23-Gal4		BDSC_93026	*D. melanogaster*		Fig. 1; Extended Data Fig. 3
Dmel Ir94e-Gal4		BDSC_81246	*D. melanogaster*		Fig. 1; Extended Data Fig. 3
Dmel UAS-Kir2.1		BDSC_6595	*D. melanogaster*		Fig. 1; Extended Data Fig. 3
DsecGr66a^{Gal4}	*pHD-DsecGr66a-T2A-Gal4-3xP3-DsRed*	*Dsec*nanos-Cas9[1]	*D. sechellia*	CRISPR knock-in, this study	Fig. 3, 4; Extended Data Fig. 6, 7
DsecGr66a$^{Gal4(DsRed-)}$		*DsecGr66a^{Gal4}*	*D. sechellia*	CRISPR mediated DsRed disruption, this study	Extended Data Fig. 7
DsecGr64f^{Gal4}	*pHD-DsecGr64f-T2A-Gal4-3xP3-DsRed*	*Dsec*nanos-Cas9[1]	*D. sechellia*	CRISPR knock-in, this study	Fig. 2; Extended Data Fig. 5, 7
DsecGr64f$^{Gal4(DsRed-)}$		*DsecGr64f^{Gal4}*	*D. sechellia*	CRISPR mediated DsRed disruption, this study	Extended Data Fig. 7
DsecIr25a^{Gal4}	*pHD-DsecIr25a-T2A-Gal4-3xP3-DsRed*	*Dsec*nanos-Cas9[1]	*D. sechellia*	CRISPR knock-in, this study	Fig. 2; Extended Data Fig. 4, 5, 6
Dsec unc84::GFP	*pMUH_unc84_2xGFP (Addgene #46023)*	*Dsec-pBac-attPA26*[5]	*D. sechellia*	attB/P integration, this study	Fig. 2, 3; Extended Data Fig. 4, 5, 6
DmelGr66a^{Gal4}	*pHD-DmelGr66a-T2A-Gal4-3xP3-DsRed*		*D. melanogaster*	CRISPR knock-in, this study	Fig. 3; Extended Data Fig. 6
DmelGr64f^{Gal4}	*pHD-DmelGr64f-T2A-Gal4-3xP3-DsRed*		*D. melanogaster*	CRISPR knock-in, this study	Fig. 2; Extended Data Fig. 4
Dmel unc84::GFP			*D. melanogaster*	[6]	Fig. 2, 3; Extended Data Fig. 4, 6,
DsimGr66a^{Gal4}	*pHD-DsimGr66a-T2A-Gal4-3xP3-DsRed*		*D. simulans*	CRISPR knock-in, this study	Fig. 3; Extended Data Fig. 6, 7
DsimGr64f^{Gal4}	*pHD-DsimGr64f-T2A-Gal4-3xP3-DsRed*		*D. simulans*	CRISPR knock-in, this study	Fig. 2; Extended Data Fig. 4
Dsim unc84 ::GFP	*pMUH_unc84_2xGFP (Addgene #46023)*	*Dsim#2176*[7]	*D. simulans*	attB/P integration, this study	Fig. 2, 3; Extended Data Fig.

					4, 6
DsecUAS-GCaMP6s			*D. sechellia*	[5]	Fig. 2, 3, 4, 5; Extended Data Fig. 4, 5, 6, 7, 9
DmelGr39a$^{-/-}$			*D. melanogaster*	[8]	Fig. 4
Dsec-UAS-DmelGr39a.a	*attB-DmelGr39a.a*	*Dsec-pBac-attPA26*[5]	*D. sechellia*	attB/P integration, this study	Fig. 4
Dsec-UAS-DmelGr39a.b	*attB-DmelGr39a.b*	*Dsec-pBac-attPA26*[5]	*D. sechellia*	attB/P integration, this study	Fig. 4; Extended Data Fig. 5
DmelGr39a.a-Gal4		BDSC_57631	*D. melanogaster*	[9]	Fig. 4
Dmel-UAS-DmelGr39a.a	*attB-DmelGr39a.a*	BDSC_24749	*D. melanogaster*	attB/P integration, this study	Fig. 4
Dmel-UAS-DsecGr39a.a	*attB-DsecGr39a.a*	BDSC_24749	*D. melanogaster*	attB/P integration, this study	Fig. 4
Dmel-UAS-DmelGr39a.a $^{\Delta 3}$	*attB-DmelGr39a.a $^{\Delta 3}$*	BDSC_24749	*D. melanogaster*	attB/P integration, this study	Fig. 4
Dsec.UAS-eGFP-Kir2.1	*pBac(10xUAS-eGFP-Kir2.1 - 3P3-DsRed)*[10]		*D. sechellia*	piggyBAC-integration, this study	Extended Data Fig. 7
DsecNsyb-Gal4			*D. sechellia*	[1]	Fig. 5; Extended Data Fig. 9
DsimNsyb-Gal4			*D. simulans*	[11]	Extended Data Fig. 9
DmelNsyb-Gal4			*D. melanogaster*	;nSyb-Gal4 3-1 (2);; Gift from Julie Simpson	Fig. 5; Extended Data Fig. 8, 9
Dsim-UAS-GCaMP6s			*D. simulans*	[7]	Extended Data Fig. 6, 9
Dmel-UAS-GCaMP6s		BDSC_42746	*D. melanogaster*		Fig. 2, 3, 4, 5; Extended Data Fig. 4, 5, 6, 7, 9

Supplementary Table 3. Oligonucleotides used to generate sgRNA expression vectors.

Target	Name	Sequence (5'-3')	Resulting sgRNAs
DsimOrco	*PCR1fwd*	GCGGCCCGGGTTCGATTCCCGGCCGATGCAGCCGAG CAAGTACACGGGCCGTTTTAGAGCTAGAAATAGCAAG	sgRNA1: GCCGAGCAAGTACACGGGCC
	PCR1rev	CTCGACCCAGTACTTGATGGTGCACCAGCCGGGAATC GAACCC	
	PCR2fwd	CCATCAAGTACTGGGTCGAGGTTTTAGAGCTAGAAATA GCAAG	sgRNA2: CCATCAAGTACTGGGTCGAG
	PCR2rev	CCCCCGTTACCGCCGAAGGATGCACCAGCCGGGAAT CGAACCC	
	PCR3fwd	TCCTTCGGCGGTAACGGGGGGTTTTAGAGCTAGAAAT AGCAAG	sgRNA3: TCCTTCGGCGGTAACGGGGG
	PCR3rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACGCCACCGAC ATGGACATGTCTGCACCAGCCGGGAATCGAACCC	sgRNA4: GACATGTCCATGTCGGTGGC
DmelGr66a	*PCR1fwd*	GCGGCCCGGGTTCGATTCCCGGCCGATGCAGATAGCT GGAATTCTGCCACGTTTTAGAGCTAGAAATAGCAAG	sgRNA1: GATAGCTGGAATTCTGCCAC
	PCR1rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACTTACGGGATT TCTCCAGCAGTGCACCAGCCGGGAATCGAACCC	sgRNA2: CTGCTGGAGAAATCCCGTAA
DsimGr66a	*PCR1fwd*	GCGGCCCGGGTTCGATTCCCGGCCGATGCACTGCTG GAGAAATCCCGTAAGTTTTAGAGCTAGAAATAGCAAG	sgRNA1: CTGCTGGAGAAATCCCGTAA

	PCR1rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACTTACGGGATCTCTCCAGCAGTGCACCAGCCGGGAATCGAACCC	sgRNA2: CTGCTGGAGAGATCCCGTAA
DsecGr66a	*PCR1fwd*	GCGGCCCGGGTTCGATTCCCGGCCGATGCAGATAGCTGGAATTCTGCCGCGTTTTAGAGCTAGAAATAGCAAG	sgRNA1: GATAGCTGGAATTCTGCCGC
	PCR1rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACTTACGGGATCTCTCCAGCAGTGCACCAGCCGGGAATCGAACCC	sgRNA2: CTGCTGGAGAGATCCCGTAA
DmelGr64f	*PCR1fwd*	GCGGCCCGGGTTCGATTCCCGGCCGATGCAAAGATTCTTCCGAAGCTGGAGTTTTAGAGCTAGAAATAGCAAG	sgRNA1: AAGATTCTTCCGAAGCTGGA
	PCR1rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACGTGATGTCCGGGTTACCCTTTGCACCAGCCGGGAATCGAACCC	sgRNA2: AAGGGTAACCCGGACATCAC
DsimGr64f	*PCR1fwd*	GCGGCCCGGGTTCGATTCCCGGCCGATGCAAAGATCCTTCCCAAGCTGGAGTTTTAGAGCTAGAAATAGCAAG	sgRNA1: AAGATCCTTCCCAAGCTGGA
	PCR1rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACGTGATGTCCGGGTTACCCTTTGCACCAGCCGGGAATCGAACCC	sgRNA2: AAGGGTAACCCGGACATCAC
DsecGr64f	*PCR1fwd*	GCGGCCCGGGTTCGATTCCCGGCCGATGCACGCAACTTGCCCTCCAGCTTGTTTTAGAGCTAGAAATAGCAAG	sgRNA1: CGCAACTTGCCCTCCAGCTT
	PCR1rev	GAGATGTCCGAGTTACCCTTTGCACCAGCCGGGAATCGAACCC	sgRNA2: AAGGGTAACTCGGACATCTC
	PCR2fwd	AAGGGTAACTCGGACATCTCGTTTTAGAGCTAGAAATAGCAAG	sgRNA3: AAGATCCTTCCAAAGCTGGA
	PCR2rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACTCCAGCTTTGGAAGGATCTTTGCACCAGCCGGGAATCGAACCC	
DsecIr25a	*PCR1fwd*	GCGGCCCGGGTTCGATTCCCGGCCGATGCAACTATACTTCGAATGGCCTCGTTTTAGAGCTAGAAATAGCAAG	sgRNA1: ACTATACTTCGAATGGCCTC
	PCR1rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACGGGCCTGGGTGAAGCTCTTCTGCACCAGCCGGGAATCGAACCC	sgRNA2: GAAGAGCTTCACCCAGGCCC

Supplementary Table 4. Oligonucleotides used for plasmid cloning.

Amplicon	Forward primer (5'-3')	Reverse primer (5'-3')
DmelGr39a.a	GCCGCCCGACGCTTCGGCAAGCTCACTAAGGCGCCTGAGAAGAGCGGCGAACGGG	CCCGTTCGCCGCTCTTCTCAGGCGCCTTAGTGAGCTTGCCGAAGCGTCGGGCGGC
DmelGr39a.b	GATCGGATCCTCGACCATGGGCACAAGAAATCGAAAGCTTCTG	GATCCTCGAGTCAAAATTTATTAATAGACTTTGTGGA
DmelGr39a.a$_{\Delta 3}$	CAACCAACAGCTTTGTCAGGCCTCTGGTCTTCGAGGCAGGCAGCAGATCC	CTGCCTCGAAGACCAGAGGCCTGACAAAGCTGTTGGTTGTAGTGAATCAATCCCGCGG
DsecGr39a.a	GATCGAATTCATGTCAAAAGTCTGCCGGG	GATCCTCGAGTCAAAATTTATTAATAGACTTTGTGG

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