Supporting Information

Lee et al. 10.1073/pnas.0811744106

Fig. S1. Deduced amino acid sequence of Gr93a. The signal peptide at the N terminus (*box*) and the 7 predicted transmembrane segments (*Upper lines*) are indicated.

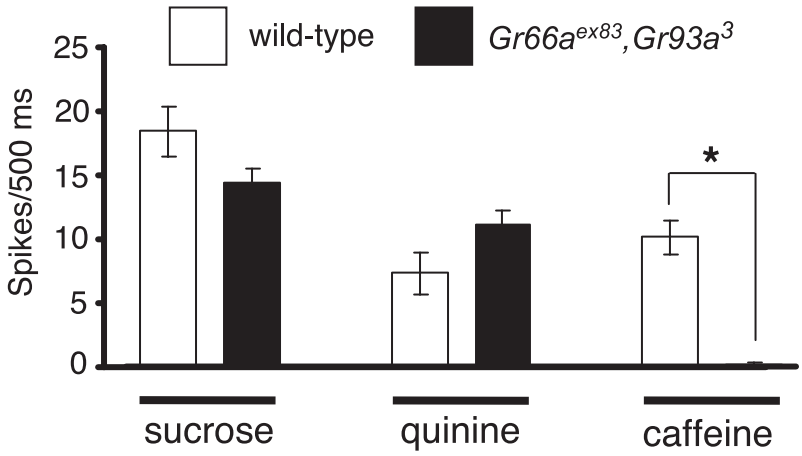

Fig. S2. The *Gr66a* ^{ex83}, *Gr93a*^{3} double-mutant animals show responses to sucrose and quinine, but not caffeine, similar to the *Gr66a* ^{ex83} or *Gr93a*^{3} single-mutant animals. Shown are the average action potentials (spikes/500 ms) produced in S6 sensilla after application of 50-mM sucrose, 1-mM quinine, or 10-mM caffeine. The error bars represent SEMs. The asterisk indicates a statistically significant difference from wild type ($P < 0.0003$).

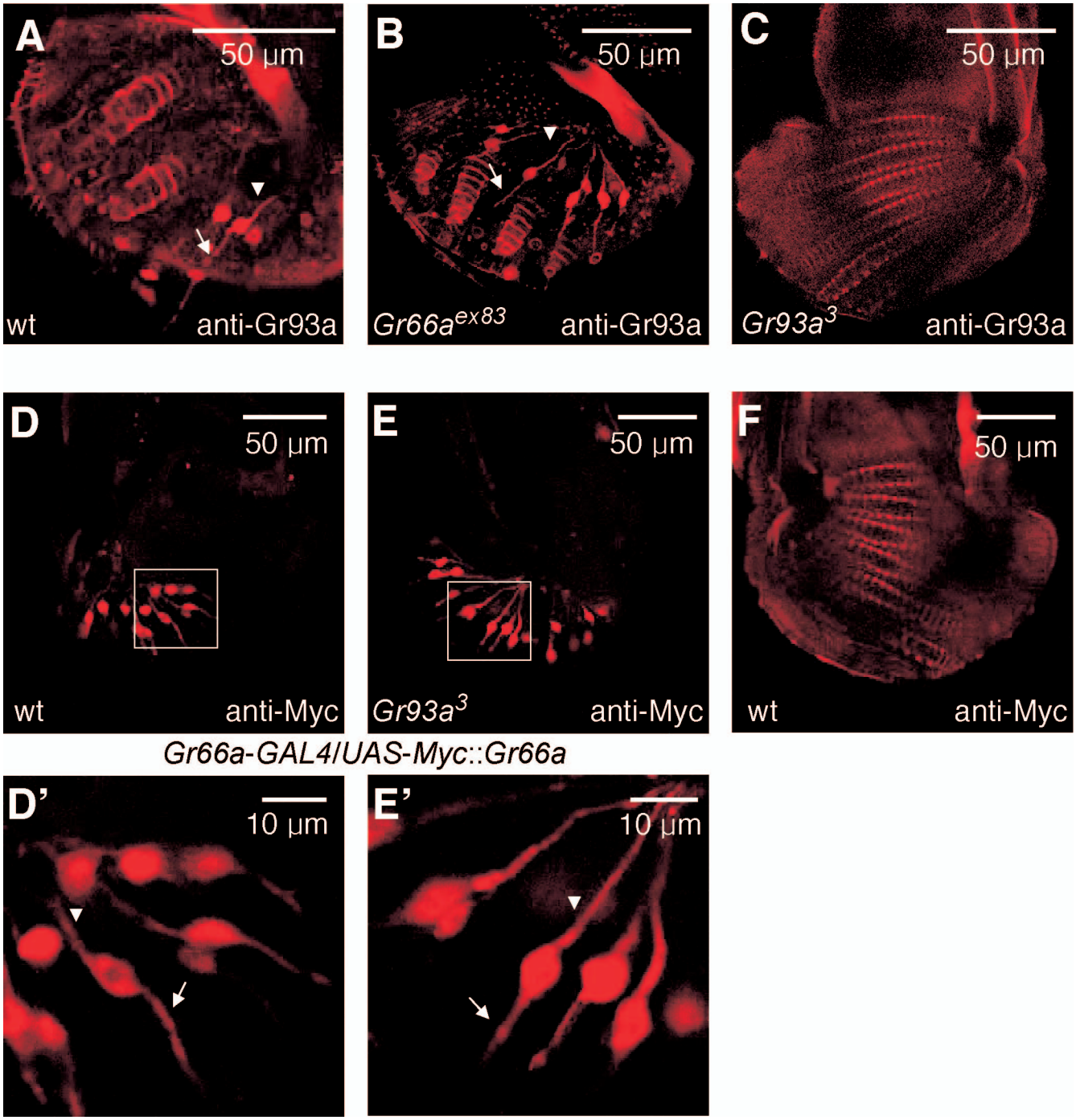

Fig. S3. Normal subcellular localization of the Gr93a and Gr66a proteins in the *Gr66a* and *Gr93a* mutant backgrounds. Shown are fluorescent images of labella viewed by confocal microscopy. (*A*–*C*) Anti-Gr93a staining in (*A*) wild-type, (*B*) $Gr66a^{ex83}$, and (*C*) $Gr93a^{3}$. (*D*–*F*) Anti-Myc staining in (*D*) wild-type flies containing the *Gr66a-GAL4/UAS-Myc*::*Gr66a* transgenes, (*E*) $Gr93a^{3}$ flies contaning the *Gr66a-GAL4/UAS-Myc*::*Gr66a* transgenes, and (*F*) wild-type. (*D'*) and (*E'*) are magnifications of the regions indicated by the boxes in (*D*) and (*E*) respectively. The proteins were expressed in dendrites (*arrows*), axons (*arrowheads*), and cell bodies.

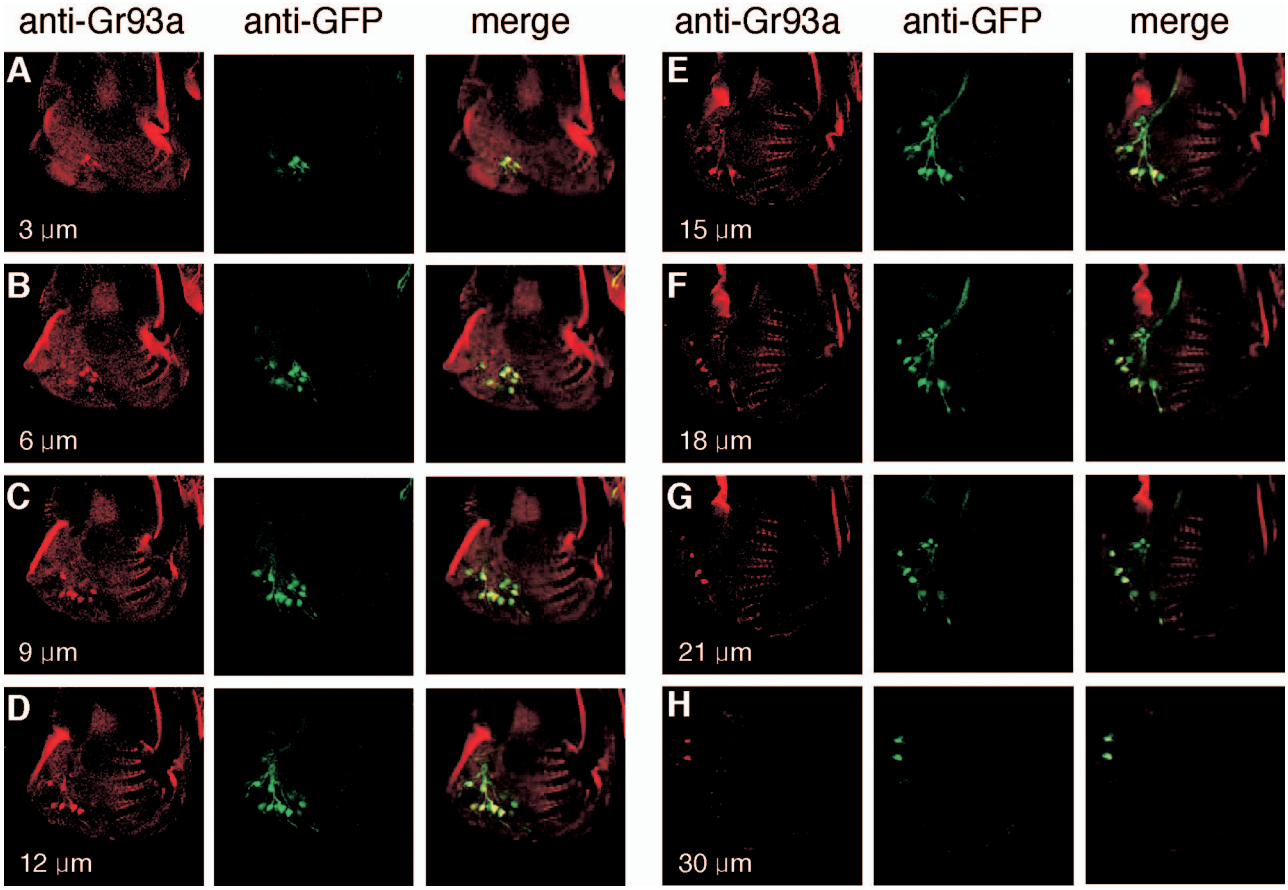

Fig. S4. The Gr93a protein and the Gr66a reporter were coexpressed in 22 GRNs in wild-type labella. (*A–H*) The GFP was expressed using the *UAS-mCD8::GFP* and the *Gr66a-GAL4* transgenes. The anti-Gr93a and anti-GFP were fluorescently stained red and green, respectively. Shown are confocal stacks at the indicated intervals.

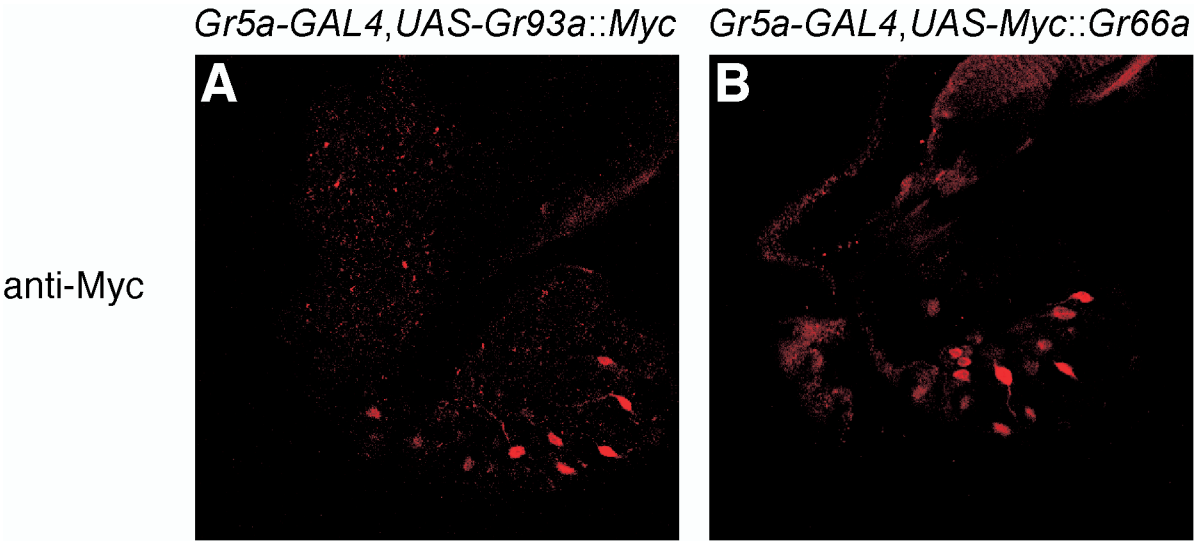

Fig. S5. The Gr93a::Myc and Myc::Gr66a proteins were misexpressed in *Gr5a* GRNs. The immunofluorescent stainings of labella were viewed by confocal microscopy. The *Gr5a* GRNs expressed the following transgenes under the control of the *Gr5a-GAL4*: (*A*) *UAS-Gr93a*::*Myc*, and (*B*) *UAS-Myc*::*Gr66a*.

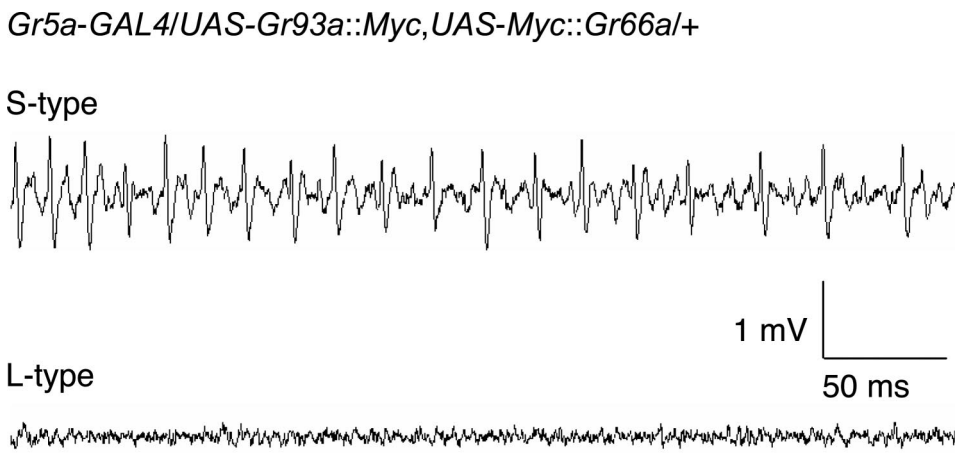

Fig. S6. Caffeine did not induce action potentials in GRNs in L-type sensilla, which misexpress Gr93a and Gr66a. In wild-type animals, presentation of caffeine produces action potentials in S-type but not L-type sensilla. Shown are tip recordings obtained from transgenic animals misexpressing *UAS-Gr93a*::*Myc* and *UAS-Myc*::*Gr66a* in L-type sensilla under control of the *Gr5a-GAL4*. The 10-mM caffeine did not elicit a response in the transgenic L-type L2, L4, and L6 sensilla, but showed normal action potentials in S-type (S6) sensilla.

Table S1. Statistics for the data in Fig. 2*A*

	Wild-type	*Gr93*^{3}	
Sugars	PI	PI	*P* values
Maltose	[1]1.00 ± 0.002	[2]1.00 ± 0.003	0.53
Trehalose	0.95 ± 0.01	0.95 ± 0.01	0.93
Fructose	0.99 ± 0.01	0.99 ± 0.01	0.80
Glucose	0.93 ± 0.02	0.94 ± 0.01	0.49
Sucrose	0.99 ± 0.01	0.98 ± 0.01	0.80
Sucrose (dye switch)	0.99 ± 0.01	0.99 ± 0.01	0.32

The mean PIs are listed. $n \geq 4$ (40–70 flies per experiment) for the behavioral assays. The *P* values are relative to wild type and are based on the unpaired Student *t*-test. [1]0.998. [2]0.995.

Table S2. Statistics for the data shown in Fig. 2*B*

	Wild-type	*Gr93*^{3}	
Tastants	PI	PI	*P* values
Sucrose only	0.09 ± 0.02	0.08 ± 0.03	0.82
Quinine	0.91 ± 0.06	0.98 ± 0.02	0.13
Strychnine	0.96 ± 0.01	0.96 ± 0.02	0.97
Berberine	0.93 ± 0.03	0.92 ± 0.02	0.80
Denatonium	0.98 ± 0.002	0.99 ± 0.01	0.78
Lobeline	0.98 ± 0.01	0.96 ± 0.02	0.34
Papaverine	0.95 ± 0.02	0.92 ± 0.05	0.51
Caffeine	0.97 ± 0.02	0.41 ± 0.05	1.4×10^{-7}

The mean PIs are listed. $n \geq 4$ (40–70 flies per experiment) for the behavioral assays. The *P* values are relative to wild type and are based on the unpaired Student *t*-test.

Table S3. Statistics for the data shown in Fig. 2*C*

	wild-type	*Gr93*^{3}	
Caffeine-dye	PI	PI	*P* values
Caffeine-red	0.92 ± 0.04	0.37 ± 0.04	7.5×10^{-5}
Caffeine-blue	0.92 ± 0.02	0.31 ± 0.05	2.0×10^{-5}

The mean PIs are listed. $n \geq 4$ (40–70 flies per experiment) for the behavioral assays. The *P* values are relative to wild type and are based on the unpaired Student *t*-test.

Table S4. Additional statistics for the data shown in Fig. 2*C*

Values	Wild-type	*Gr93*^{1}	*Gr93*^{1}/*Gr93*^{2}	*Gr93*^{2}/+
PI	0.97 ± 0.02	0.43 ± 0.04	0.56 ± 0.04	0.99 ± 0.004
P values		5.9×10^{-9}	3.9×10^{-7}	0.23

Table S5. Statistics for the data in Fig. 3

	Wild-type	*Gr93*^{3}	
Compounds	Spikes/500 ms	Spikes/500 ms	*P* values
Sucrose	13.6 ± 2.13	15.0 ± 2.10	0.69
Quinine	10.1 ± 0.98	11.1 ± 0.97	0.49
Strychnine	14.5 ± 1.09	11.9 ± 0.69	[1]0.05
Berberine	19.0 ± 3.04	17.6 ± 3.09	0.75
Denatonium	13.5 ± 1.81	15.3 ± 1.49	0.47
Lobeline	15.4 ± 1.90	13.8 ± 1.45	0.51
Papaverine	20.2 ± 4.42	20.2 ± 4.30	1.00
Caffeine	13.8 ± 1.60	1.2 ± 0.64	1.4×10^{-6}

The mean spike frequencies (±SEMs) are listed. $n \geq 6$ for the tip recordings. The *P* values are relative to wild type and are based on the unpaired Student *t*-test. [1]0.054.

Table S6. Statistics for the data shown in Fig. 5*A*

Genotype	PI	*P* values
wild-type	0.97 ± 0.02	
UAS-Gr93a::Myc;Gr93^3	0.37 ± 0.04	
Gr66a-GAL4;Gr93^3	0.46 ± 0.06	
Gr66a-GAL4/UAS-Gr93a::Myc;Gr93^3	0.83 ± 0.05	≤0.0022

The mean PIs (±SEMs) are listed. $n \geq 4$ (40–70 flies per experiment) for the behavioral assays. The *P* values are relative to *UAS-Gr93a::Myc;Gr93^3* and *Gr66a-GAL4;Gr93^3* and are based on the unpaired Student *t*-test.

Table S7. Statistics for the data shown in Fig. 5*B*

Genotype	Spikes/500 ms	*P* values
Wild-type	13.8 ± 1.60	
UAS-Gr93a::Myc;Gr93^3	0.33 ± 0.33	
Gr66a-GAL4;Gr93^3	0.00 ± 0.00	
Gr66a-GAL4/UAS-Gr93a::Myc;Gr93^3	13.0 ± 1.46	$\leq 1.1 \times 10^{-4}$

The mean PIs and spike frequencies (±SEMs) are listed in each table. $n \geq 6$ for the tip recordings. The *P* values are relative to *UAS-Gr93a::Myc;Gr93^3* and *Gr66a-GAL4;Gr93^3* and are based on the unpaired Student *t*-test.

Table S8. Statistics for the data shown in Fig. 6*A*

Methylxanthine		Wild-type	*Gr93*^{3}	*Gr66a*^{ex83}
Caffeine	PI	0.82 ± 0.06	0.35 ± 0.03	0.52 ± 0.01
	P values		6.5×10^{-5}	0.0024
Theophylline	PI	0.81 ± 0.03	0.48 ± 0.06	0.67 ± 0.02
	P values		0.0027	0.0043
Theobromine	PI	0.89 ± 0.03	0.96 ± 0.02	0.94 ± 0.03
	P values		0.12	0.27
Paraxanthine	PI	0.95 ± 0.04	[1]1.00 ± 0.00	0.97 ± 0.01
	P values		0.23	0.56

The mean PIs (±SEMs) are listed. $n \geq 4$ (40–70 flies per experiment) for the behavioral assays. The *P* values are relative to wild-type unless specified otherwise and are based on the unpaired Student *t*-test. [1]0.996 ± 0.004

Table S9. Statistics for the data shown in Fig. 6*B*

Methylxanthine		Wild-type	*Gr93*^{3}	*Gr66a*^{ex83}
Caffeine	Spikes/500 ms	13.8 ± 1.60	1.2 ± 0.64	0.6 ± 0.42
	P values		1.4×10^{-6}	9.8×10^{-7}
Theophylline	Spikes/500 ms	17.4 ± 3.74	0.6 ± 0.38	0.6 ± 0.32
	P values		6.2×10^{-4}	6.1×10^{-4}
Theobromine	Spikes/500 ms	17.4 ± 4.42	15.3 ± 4.43	19.2 ± 2.63
	P values		0.75	0.76
Paraxanthine	Spikes/500 ms	10.9 ± 1.18	14.5 ± 1.83	15.4 ± 2.13
	P values		0.13	0.070

The mean spike frequencies (±SEMs). $n \geq 6$ for the tip recordings. The *P* values are relative to wild-type unless specified otherwise and are based on the unpaired Student *t*-test.