

Supplemental Methods

Generation of transgenes

ptub-FRT-Gal80-FRT (*ptub>Gal80>*) was generated by amplifying the *Gal80* open reading frame by PCR using genomic DNA of *yw*, *FRT19A*, *tub-Gal80* as a template (Lee and Luo, 1999). Primers were designed to add the FRT site GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC to each end of the fragment and introduce a flanking NotI site. The amplified fragment was cloned into the NotI site of *ptub* (Lee and Luo, 1999). Full primer sequences:

Forward:

ACAGCGGCCGCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCAACAT
GGACTACAACAAGAGATC

Reverse:

ACAGCGGCCGCGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGCCTTCTA
GTGGATCCAGACAT

UAS-CD4::spGFP1-10 and *LexAop-CD4::spGFP11* were generated by PCR amplification of *CD4::spGFP1-10* and *CD4::spGFP11* (Feinberg et al., 2008) with XhoI (5') and XbaI (3') sites added to primers. The PCR fragments were then cloned into *pUAST* and *pLOT* respectively. Primer sequences:

Forward: CCCTCGAGATGCCACCTTCAACATCATTG

Reverse: GCTCTAGACTAGCGCCTTCGGTGCCG

Gr5a-LexA:VP16 was generated by PCR amplification of the *Gr5a* promoter from genomic DNA, using primers with flanking Sall (5') and XhoI (3') sites. The PCR product was cut with Sall and XhoI and cloned into *pCasper4* cut with XhoI.

LexA::VP16 was then excised from *Or83b-LexA::VP16* (Lai and Lee, 2006) using XhoI and cloned into *pCasper4-Gr5a* cut with XhoI. Primer sequences:

Forward: GATCTCGTCGACGATTCCTTCTGCGCTCAAAA

Reverse: GAACGTCTCGAGAAATCCTGACTAAACGGCAAA

Inducible activation with VR1

For heat activation of VR1, flies expressing VR1 were starved for 16h and mounted on myristic acid as described above. Extension assays were performed by bringing a probe heated to approximately 150°C within 5mm of fly for 1s.

Table S1: Expression summary for Gal4 behavioral mutants.

Line	Mutant class	Taste sensory neuron expression	non-sensory SOG neuron expression	non-SOG neuron expression
E402	bitter	+	+	+
E441	bitter	+	+	+
E657	bitter	+	+	+
E628	bitter	+	-	+
E806	bitter	+	-	+
E809	bitter	+	-	+
E133	bitter	+	-	-
E419	bitter	+	-	-
E23	bitter	-	+	+
E34	bitter	-	+	+
E175	bitter	-	+	+
E320	bitter	-	+	+
E442	bitter	-	+	+
E496	bitter	-	+	+
E515	bitter	-	+	+
E708	bitter	-	+	+
E734	bitter	-	+	+
E86	bitter	-	-	+
E150	bitter	-	-	+
E157	bitter	-	-	+

E184	bitter	-	-	+
E234	bitter	-	-	+
E239	bitter	-	-	+
E401	bitter	-	-	+
E593	bitter	-	-	+
E596	bitter	-	-	+
E718	bitter	-	-	+
E350	sugar	+	+	+
E67	sugar	-	+	+
E131	sugar	-	+	+
E762	sugar	-	+	+
E548	sugar	-	-	+
E745	sugar	-	-	+
E143	unresponsive	+	+	+
E243	unresponsive	+	+	+
E404	unresponsive	+	+	+
E440	unresponsive	+	+	+
E463	unresponsive	+	+	+
E535	unresponsive	+	+	+
E651	unresponsive	+	+	+
E760	unresponsive	+	+	+
E49	motor	+	+	+
E51	motor	-	+	+
E85	motor	-	+	+
E470	motor	-	+	+
E639	motor	-	+	+
E662	motor	-	+	+

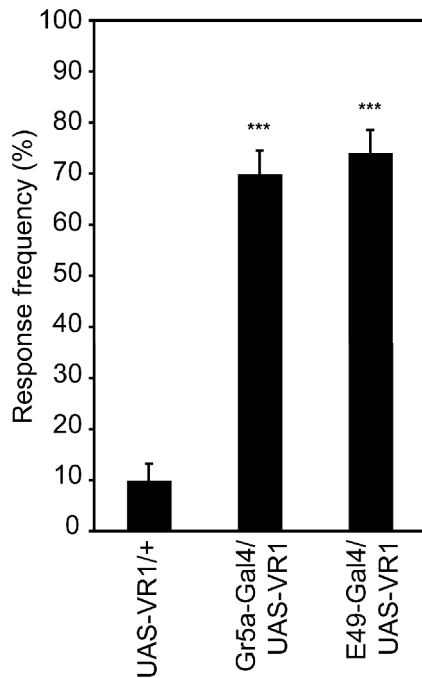


Figure S1: Inducible activation with VR1 evokes rostrum extension.

Flies expressing VR1 in Gr5a-expressing cells or E49 neurons show robust extension in response to heat delivered by a heated probe brought within a few millimeters of the fly. n= 56-76 flies. For quantitation, any extension of the rostrum or rostrum and haustellum is counted as a response; however, in general Gr5a-Gal4/UAS-VR1 flies displayed full extension, while E49-Gal4/UAS-VR1 flies displayed only lifting of the rostrum. Student t-test, *** = $p < 10^{-5}$.

Supplemental Video 1: This movie shows the Proboscis Extension Response (PER) of a wild-type fly following stimulation on the leg with 100mM sucrose (Quicktime; 4.8 MB).

Supplemental Video 2: This movie shows the response of *E49-Gal4, UAS-TNT* flies following stimulation on the leg with 100mM sucrose (Quicktime; 4.9 MB).

Supplemental Video 3: This movie shows the response of *Gr5a-Gal4, UAS-ChR2* flies to alternating pulses of red light (3s pulses; 690nm) and blue light (1s pulses; 480nm) (Quicktime; 8.9 MB).

Supplemental Video 4: This movie shows the response of *E49-Gal4, UAS-ChR2* flies to alternating pulses of red light (3s pulses; 690nm) and blue light (1s pulses; 480nm) (Quicktime; 9.9 MB).