SUPPLEMENTAL DATA

Figure S1.

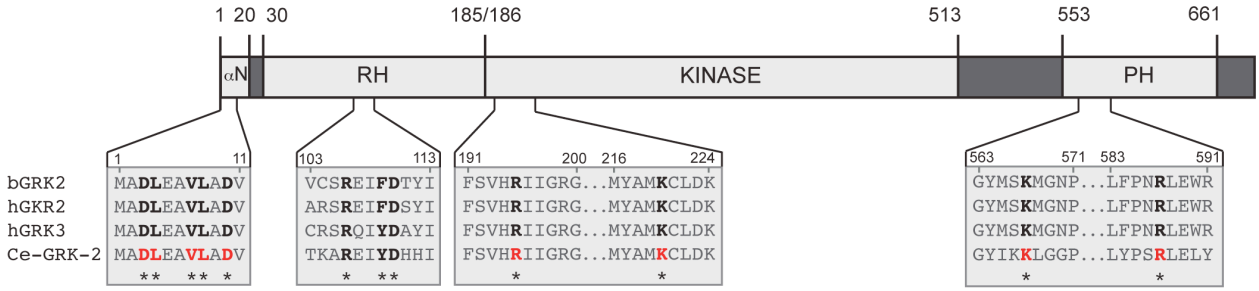

FIGURE S1. **Summary of the sites required for Ce-GRK2 chemosensory function *in vivo*.** Site-directed mutagenesis revealed that Ce-GRK-2 residues Asp3, Leu4, Val7/Leu8, Asp10, Arg195, Lys220, Lys567 and Arg587 (shown in red) contribute to its chemosensory function. Changing Ce-GRK-2 residues Arg106, Tyr109 or Asp110 (shown in black, bold) did not disrupt its ability to restore ASH-mediated chemosensory responses to *Ce-grk-2* mutant animals. Each residue targeted by site directed mutagenesis is indicted by an asterisk.

Figure S2.

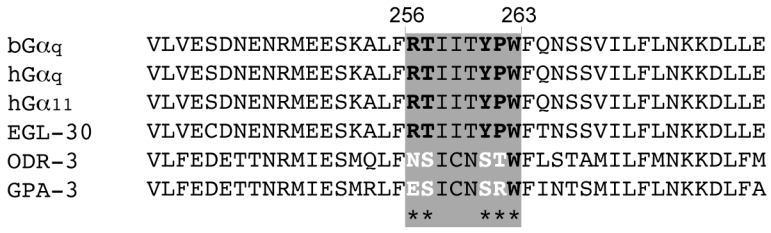

FIGURE S2. **Alignment of the GRK2-binding domain of mammalian Gα$_{q/11}$ subunits with *C. elegans* ASH Gα subunits.** The GRK2-binding domain of bovine Gα$_q$ (bGα$_q$), human Gα$_q$ (hGα$_q$) and human Gα$_{11}$ (hGα$_{11}$) is conserved in the *C. elegans* Gα$_{q/11}$ ortholog EGL-30. The described mammalian GRK2-binding site is highlighted by the grey box. Residues in mammalian Gα$_{q/11}$ that form hydrogen bonds with the GRK2 RH domain, and are required for interaction *in vitro* and in cell culture (1,2), are indicated by an asterisk. Key GRK2-binding residues within this domain are not conserved in the primary ASH sensory Gαs ODR-3 and GPA-3 (shown in white), which are both most similar to the Gα$_{i/o}$ family.

Plasmid Construction

All constructs were sequenced following site-directed mutagenesis.

Site-directed mutagenesis (QuikChange, Stratagene) was used to correct amino acid 20 to serine in *Ce-grk-2* cDNA in pHA#343 (3) to make pFG8. pHA#343 incorrectly contains a glycine at this position.

pFG44: The ~3kb upstream promoter sequence of *Ce-grk-2* (3) was amplified from genomic sequence using primers JFW3 (5'- gcgcg**cctgcagg**cgacagtttccatagtgattgg-3') and DMF3 (5'-aaa**ggatcc**ttttgttctgcaaaatcgaattg-3') to incorporate 5' SbfI and 3' BamHI sites, respectively. The promoter was inserted into these sites of pPD49.26 (Fire Lab *C. elegans* Vector Kit, Addgene).

grk-2p::*grk-2* (pFG45): The ~3kb upstream promoter sequence of *Ce-grk-2* (3) was isolated by SbfI/BamHI digest of pFG44 and placed between the corresponding sites in pFG8.

grk-2p::*grk-2(D3K)* (pFG46), *grk-2p*::*grk-2(L4K)* (pFG47), *grk-2p*::*grk-2(V7A/L8A)* (pFG48), *grk-2p*::*grk-2(D10A)* (pFG49), *grk-2p*::*grk-2(R195A)* (pFG84), and *grk-2p*::*grk-2(K567E)* (pFG89): Site-directed mutagenesis was used to introduce amino acid changes in the wild-type *Ce-grk-2* cDNA in pHA#343 (3). An additional round of site-directed mutagenesis was used to correct Gly20 to serine. Mutant *Ce-grk-2* cDNAs were isolated by Asp718/Sac1 digest and inserted into these sites in pPD49.26 (Fire Lab *C. elegans* Vector Kit, Addgene). The ~3kb *Ce-grk-2* upstream promoter sequence (3) was isolated by SbfI/BamHI digest of pFG44 and placed between the corresponding sites in pPD49.26, upstream of the *Ce-grk-2* cDNAs.

grk-2p::*grk-2(R016A)* (pFG85), *grk-2p*::*grk-2(Y109I)* (pFG86), and *grk-2p*::*grk-2(D110A)* (pFG86): Site-directed mutagenesis was performed in pMST258, pMST259 and pMST260 to correct Gly20 to serine. Mutant *Ce-grk-2* cDNAs were isolated by Asp718/Sac1 digest and inserted into these sites in pPD49.26 (Fire Lab *C. elegans* Vector Kit, Addgene). The ~3kb *Ce-grk-2* upstream promoter sequence (3) was isolated by SbfI/BamHI digest of pFG44 and placed between the corresponding sites in pPD49.26, upstream of the *Ce-grk-2* cDNAs.

grk-2p::*grk-2(K220R)* (pFG88): Site-directed mutagenesis used to correct Gly20 to serine in pHA#348 (*Ce-grk-2(K220R)* cDNA in pPD49.26). The corrected mutant *grk-2(K220R)* cDNA was isolated by Asp718/SacI digest and inserted into these sites in pPD49.26 (Fire Lab *C. elegans* Vector Kit, Addgene). The ~3kb *Ce-grk-2* upstream promoter sequence (3) was isolated by SbfI/BamHI digest of pFG44 and placed between the corresponding sites in pPD49.26, upstream of the *Ce-grk-2(K220R)* cDNA.

grk-2p::*grk-2(R587Q)* (pFG90) and *grk-2p*::*grk-2(K576E/R587Q)* (pFG91): Site-directed mutagenesis was used to introduce the R587Q change into pFG45 and pFG89. The R587Q change introduces a Sac1 restriction site into the *Ce-grk-2* cDNA sequence.

SUPPLEMENTAL REFERENCES

1. Tesmer, V. M., Kawano, T., Shankaranarayanan, A., Kozasa, T., and Tesmer, J. J. (2005) Snapshot of activated G proteins at the membrane: the $G\alpha_q$-GRK2-$G\beta\gamma$ complex. *Science* **310**, 1686-1690
2. Sterne-Marr, R., Tesmer, J. J., Day, P. W., Stracquatanio, R. P., Cilente, J. A., O'Connor, K. E., Pronin, A. N., Benovic, J. L., and Wedegaertner, P. B. (2003) G protein-coupled receptor Kinase 2/$G\alpha_{q/11}$ interaction. A novel surface on a regulator of G protein signaling homology domain for binding $G\alpha$ subunits. *J Biol Chem* **278**, 6050-6058
3. Fukuto, H. S., Ferkey, D. M., Apicella, A. J., Lans, H., Sharmeen, T., Chen, W., Lefkowitz, R. J., Jansen, G., Schafer, W. R., and Hart, A. C. (2004) G protein-coupled receptor kinase function is essential for chemosensation in *C. elegans*. *Neuron* **42**, 581-593