SUPPLEMENTAL INFORMATION FOR:

Activation of Smoothened in the Hedgehog pathway unexpectedly increases Gαs-dependent cAMP levels in Drosophila

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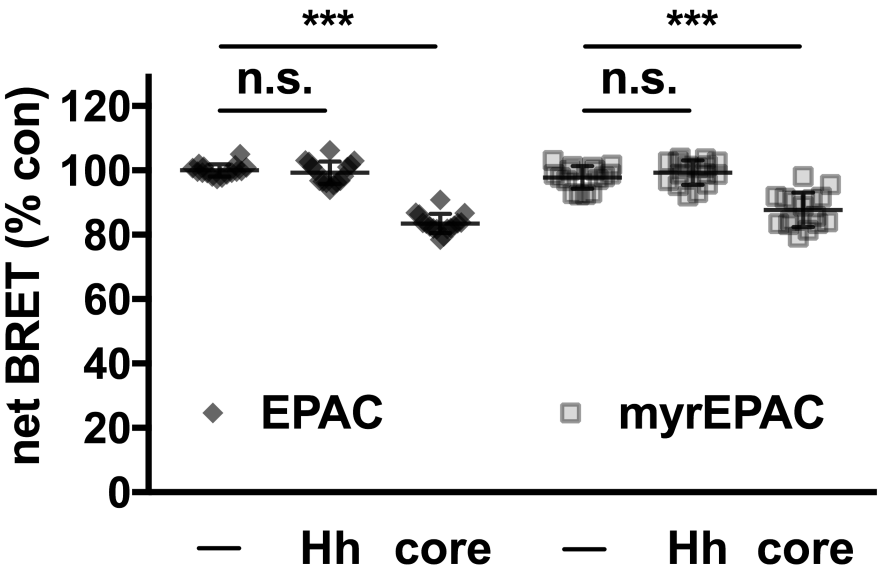

Figure S1. Comparison of Hh/Smo^{core}-dependent changes in total versus membrane cAMP pools. *Left*: using the standard cytoplasmic EPAC cAMP biosensor in a BRET assay, Hh^{N} expressing cells showed no significant change whereas cAMP levels are higher (lower net BRET signal) upon expression of Smo^{core}. *Right*: myrEPAC, an EPAC cAMP biosensor targeted to the plasma membrane by fusion to the N-terminal myristoylation sequence of Drosophila Src64B (1), responds in the same manner to Hh and Smo^{core} expression.

Supplemental Methods: To generate a membrane-localized form of the EPAC-BRET cAMP biosensor, the 14 amino acid myristoylation sequence from Drosophila Src64B (MGNKCCSKRQDQEL**)** was added to the N-terminus of the EPAC-BRET biosensor in the *pMT.puro/GFP10-EPAC-RLucII_T781A,F782A* plasmid (described in (2)) by PCR. This tag has been shown to mediate plasma membrane localization of AKAR3, a similar genetically encoded intramolecular FRET-based biosensor of PKA activity (1). S2 cells were transfected and processed for BRET analysis essentially as described (3), using 100 ng of the plasmids encoding either cytoplasmic or membrane-associated EPAC-BRET proteins.

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References

1. Li, S., Ma, G., Wang, B., and Jiang, J. (2014) Hedgehog induces formation of PKA-Smoothened complexes to promote Smoothened phosphorylation and pathway activation. *Sci Signal* **7**, ra62
2. Cheng, S., Maier, D., and Hipfner, D. R. (2012) Drosophila G-protein-coupled receptor kinase 2 regulates cAMP-dependent Hedgehog signaling. *Development* **139**, 85-94
3. Maier, D., Cheng, S., Faubert, D., and Hipfner, D. R. (2014) A broadly conserved g-protein-coupled receptor kinase phosphorylation mechanism controls Drosophila smoothened activity. *PLoS Genet* **10**, e1004399