SUPPLEMENTAL INFORMATION FOR:

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

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Figure S1. Comparison of Hh/Smo<sup>core</sup>-dependent changes in total versus membrane cAMP pools. Left: using the standard cytoplasmic EPAC cAMP biosensor in a BRET assay, Hh<sup>N</sup> expressing cells showed no significant change whereas cAMP levels are higher (lower net BRET signal) upon expression of Smo<sup>core</sup>. 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<sup>core</sup> 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.

References