Identification of a Functional Interaction between the Transmembrane Protein Smoothened and the Kinesin-Related Protein Costal2

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Supplemental Experimental Procedures

Preparation of Lysates

Drosophila melanogaster Oregon^R strain were grown according to established procedures (see [S1]). Four- to six-hr-old embryos were dechorionated with bleach and lysed in hypotonic lysis buffer ([HLB]; 50 mM β-glycerophosphate, 10 mM NaF, 1 mM EGTA, 1 mM DTT [pH 7.6]) by homogenization in a glass dounce with a teflon pestle. S2 cells were lysed in the same buffer by dounce homogenization. The lysates were cleared of nuclei by centrifugation at 5000 or 2000 × g for 20 min and either used immediately or frozen in liquid nitrogen and stored at −80°C.

Reporter Assays

Cl8 cell transfections were carried out using Cellfectin transfection reagent per manufacturers instructions (Invitrogen). 48 hr post-transfection, cells were harvested and processed using the Dual Luciferase Assay Kit per manufacturer's instructions (Promega). ptcΔ136-Luc [S2] was used as a reporter construct for Hh target gene transcription. Luciferase activity was detected with a Zylux luminometer. Renilla-luciferase activity (pRL-TK, Promega) was used to control for transfection efficiency/normalization.

Yeast Two-Hybrid Analysis

Cos2 and the carboxyl-terminal domain of Smo (amino acids 554–1036) cDNAs were each subcloned into both pGAD-C1 (GAL4 activation domain) and pGBDU-C1 (GAL4 DNA binding domain) vectors and transformed into the yeast strain PJ69-4A. cDNAs were also subcloned in antisense orientation to serve as negative control. Matings were preformed as previously described [S3, S4]. Yeast able to grow on both histidine and adenine screening plates were interpreted as true and positive clones.

Supplemental References

S1. Robbins, D.J., Nybakken, K.E., Kobayashi, R., Sisson, J.C., Bishop, J.M., and Therond, P.P. (1997). Hedgehog elicits signal transduction by means of a large complex containing the kinesin-related protein costal2. Cell *90*, 225–234.

S2. Chen, C.H., von Kessler, D.P., Park, W., Wang, B., Ma, Y., and Beachy, P.A. (1999). Nuclear trafficking of Cubitus interruptus in the transcriptional regulation of Hedgehog target gene expression. Cell *98*, 305–316.

S3. James, P., Halladay, J., and Craig, E.A. (1996). Genomic libraries

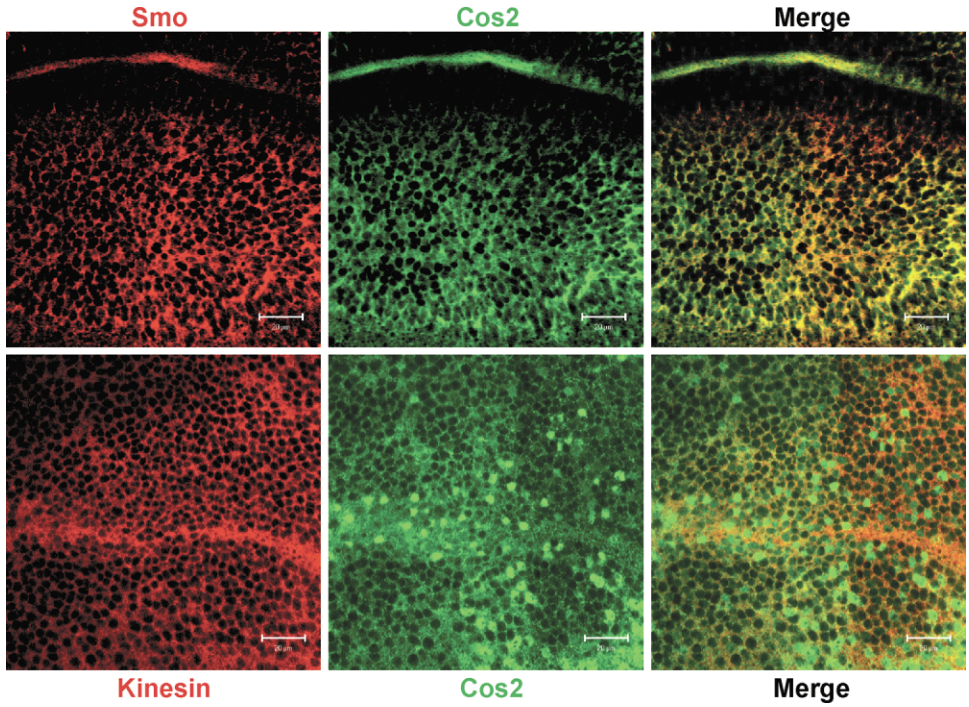

Figure S1. Smo-Cos2 Colocalization Is Similar across the Wing Imaginal Disc

Wild-type wing imaginal discs were immunostained with antibodies directed against Smo (red) and Cos2 (green) (top), or Kinesin (red) and Cos2 (green) (bottom). These images are a lower magnification of the wing pouch in Figure 2C. For each panel, anterior is to the left, and dorsal is toward the top. The staining pattern of Smo is similar to that previously reported [S5]; i.e., the posterior compartment and anterior/posterior boundary stain more intensely. Cos2 levels appear somewhat elevated in the anterior compartment, similar to the staining pattern previously reported using a different Cos2 antibody [S6].

and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics *144*, 1425–1436.

S4. Ascano, M., Jr., Nybakken, K.E., Sosinski, J., Stegman, M.A., and Robbins, D.J. (2002). The carboxyl-terminal domain of the protein kinase fused can function as a dominant inhibitor of hedgehog signaling. Mol. Cell. Biol. *22*, 1555–1566.

S5. Denef, N., Neubuser, D., Perez, L., and Cohen, S.M. (2000). Hedgehog induces opposite changes in turnover and subcellular localization of patched and smoothened. Cell *102*, 521–531.

S6. Sisson, J.C., Ho, K.S., Suyama, K., and Scott, M.P. (1997). Costal2, a novel kinesin-related protein in the Hedgehog signaling pathway. Cell *90*, 235–245.