SONIC HEDGEHOG INDUCED PROLIFERATION REQUIRES SPECIFIC G-ALPHA-INHIBITORY PROTEINS

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LEGENDS FOR SUPPLEMENTARY FIGURES

Supplementary Fig 1. (A) Shh-induced proliferation of CGNPs is strongly enhanced by the active forms of members of the Gαi/o class. CGNPs cells were plated in a saturating concentration of Shh (3 μg/ml) and transfected with different active mutant forms of Gα cloned into pCIG, a nuclear GFP expressing bicistronic vector. After 24 hours, cells were washed, treated for 48 additional hours with the indicated amounts of Shh, and proliferation was analyzed through H3-Thymidin incorporation. (B) Effect of different wild-type Gα proteins expression on Shh induced proliferation. CGNPs cultures treated as above, were transfected with different wild type Gα proteins and 48 h later proliferation measured by BrdU incorporation. (C) CGNPs cultures were transfected with either empty vector (pGHIN), or pGHIN expressing shRNAs directed against Gαi2 and Gαi3 (Gi2UTR2 and Gi3UTR2). The proliferation induced by different amounts of Shh was measured 48 later by H3-Thymidin incorporation.

Supplementary Fig 2. Representative images of cells transfected with Gi1UTR3, Gi2UTR2 or Gi3UTR3. Representative photos of cells transfected with the most efficient shRNA constructs for Gαi1, Gαi2 or Gαi3. The shRNA expressing constructs were electroporated into freshly isolated CGNPs, grown in a saturating concentration of Shh (3 μg/ml) for 48h and the proliferation measured by BrDu incorporation.

Supplementary Fig 3. Representative photos of rescue of proliferation in cells transfected with Gi2UTR2/Gi3UTR3. Freshly isolated CGNPs were electroporated with vectors containing Gαi2UTR2/Gαi3UTR2 and constructs expressing the active forms of the different Gαi class members. Cells were cultured in a saturating concentration of Shh (3 μg/ml) for 48h and proliferation measured by BrdU incorporation..

Supplementary Fig 4. (A) Association between Smo and Gαis was studied through co-immunoprecipitation assay as previously described (38). Fresh isolated CGNPs were electroporated with pCIG vectors expressing either SmoM2-HA and/or different Gαis-FLAG. Cells were cultured with Shh for 48h, lysated in a non denaturing buffer and immunoprecipitated with anti-HA or anti-FLAG antibodies. Western blots developed with these same antibodies failed to show an association between Smo and Gαi. (B) Ciliar location of Gαi3 was not affected by Shh deprivation. CGNPs were transfected with Gαi3QL-FLAG, grown for 48 and Shh starved or not 4h prior fixation. Cilium position and Gαi3QL-FLAG were revealed with anti-ACIII (adenylate cyclase III) and anti-FLAG respectively (white arrows). (C) Ciliar location of Gαi3QL-FLAG was not affected by SmoM2 co-expression. Freshly isolated CGNPs were electroporated with Gαi2QL-FLAG or Gαi3QL-FLAG and a 5x excess of either empty vector or SmoM2. Percentage of transfected cells showing either ciliar or non ciliar Gαi2QL-FLAG or Gαi3QL-FLAG were counted in each case. (D) Smo location at the cilium is not affected by Gαi2/Gαi3 depletion. CGNPs were electroporated with SmoM2-HA and a 5X excess of either empty vector (pGHIN), or pGHIN expressing shRNAs directed against Gαi2 and Gαi3 (Gi2UTR2 and Gi3UTR2). SmoM2 and cilium were developed by anti-HA and anti-Acetylated Tubulin staining respectively.

Supplementary Fig 5. Gαi1 accumulates in the trans-golgi network. CGNPs were transfected with FLAG-Gαi1 and then double-stained with anti-FLAG and anti-ACIII (adenylate cyclase III) or anti-C-PKA (PKA catalytic sub-unit) or anti-TGN38 (a trans-golgi marker). We had previously shown that C-PKA in CGNPs locates at the cilium base surrounding the centrioles. We now observe that Gαi1 distributes around the area positive for C-PKA, coincident with the vesicles of the trans-golgi network.