Table S1. The TaqMan gene expression probes used in this study.

Table S2. The shRNA constructs used in this study.

Figure S1. HH signaling components are expressed in NSCLC cell lines. (A) Q-PCR was used to determine the expression of the HH signaling pathway effectors *SMO* and *GLI2* and the HH target genes *GLI1* and *PTCH1*, relative to *GAPDH* expression in the indicated NSCLC cell lines. **(B)** Q-PCR was used to determine the expression of the HH family members *SHH*, *DHH* and *IHH* relative to *GAPDH* in the indicated NSCLC cell lines. For comparison, the highest level of expression for each indicated gene across all cell lines was set to 1. All error bars in **(A)** and **(B)** show ± standard error of mean (SEM) of three separate experiments.

Figure S2. Validation of the shRNAs used in this study: effectors. A549 cells were transduced with the indicated shRNAs, then RNA was extracted from these cells three days later. The expression of the indicated genes was determined by Q-PCR using inventoried TaqMan probes. The data were normalized to expression of *GAPDH* and are shown after setting the control levels to 1. The result shown is from a representative Q-PCR experiment performed in triplicate.

Figure S3. Validation of the shRNAs used in this study: HH isoforms. A549 cells were transduced with the indicated shRNAs, then RNA was extracted from these cells three days later. The expression of the indicated genes was determined by Q-PCR using inventoried TaqMan probes. The data were normalized to expression of *GAPDH* and are shown after setting the control levels to 1. The result shown is from a representative Q-PCR experiment performed in triplicate.

Figure S4. HH signaling is required for NSCLC proliferation and survival. (A). A549 and HOP62 cells were transduced with the indicated GLI1 and SMO shRNAs. Five days later cell proliferation was determined using a MTT assay (1). The result shown is from a representative experiment performed in triplicate. The data is normalized to cells infected with pLKO.1 control virus (Ctrl) and are shown +/- S.D. The result shown is from a representative experiment performed in triplicate. **(B)** HH activity regulates cell viability in NSCLC cells. HOP62 and A549 cells were transduced with the indicated shRNA and the release of lactate dehydrogenase (LDH), a general measure of cell death, determined 5 day post-infection using an *in vitro* LDH based Toxicology Assay Kit Tox-7 (Sigma-Aldrich, St. Louis, MO, USA). The data were normalized to total LDH levels and are shown +/-S.D. after setting its level to 1 for the control shRNA. The result shown is from a representative experiment performed in triplicate. **(C)** HH pathway knockdown induces apoptosis in NSCLC cells. HOP62 and A549 cells were transduced with indicated shRNA. Four days post-infection, the cells were analyzed for apoptosis using an Annexin V-FITC Apoptosis Detection Kit (Sigma- Aldrich). The cells were stained with Annexin V-FITC and propidium iodide (PI) and analyzed by FACS. The viable cells were negative for both Annexin V and PI (Q3). Cells undergoing apoptosis were positive for Annexin-V, but negative for PI (Q4); cells already in the late stages of apoptosis were positive for both Annexin-V and PI (Q2). Damaged or necrotic cells were negative for Annexin-V and positive for PI (Q1).

Figure S5. NSCLC cell lines elaborate a functional HH signaling pathway. A549 cells were transfected with indicated amount of SHH expression plasmid or a control vector pcDNA3.1 (Ctrl). Five days later RNA was extracted and the expression of HH target genes *GLI1* and *PTCH1* determined by Q-PCR. These results were then normalized to the expression of *GAPDH*. The expression of the

respective genes is shown relative to the cells transfected with a control vector pcDNA3.1 (A549-Ctrl). The result shown is from a representative Q-PCR experiment performed in triplicate.

Figure S6. HH signaling in lung tumors xenografts. The tumors formed by A549 cells infected with control shRNA (Ctrl shRNA) and *GLI1* shRNA #1 or #5 in nude mice experiment shown in Figure 5 were evaluated for HH target gene expression by Q-PCR using human or mouse specific probes (human *GLI1*: *hGLI1*; human *PTCH1*: *hPTCH1*; mouse *GLI1*: *mGLI1*; mouse *PTCH1*: *mPTCH1*) as previously described (2). The expression of the respective genes is shown relative to the expression of human or mouse specific *GUSB* - a house-keeping gene. All error bars indicate ± SEM (n=3).

References:

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2. Yauch RL, Gould SE, Scales SJ et al. A paracrine requirement for hedgehog signalling in cancer. Nature 2008;455:406-10.