**Supplemental Information**

## Additional Material and Methods

*Cell line, Expression constructs, and other reagents*

HEK293, A549, and Hop-62 cells (ATCC) were maintained as described (1, 2). Expression constructs used were as follows: GFP (pEGFP, Clonetech), N-terminal Flag tagged human GLI1 (CβF-GLI1, gift from Dr. M Cole, Dartmouth College), N-terminal Xpress tagged mouse GLI2 (pcDNA-GLI2, gift from Dr. H Sasaki, Osaka University, Japan), N-terminal Flag tagged human GLI3 (a Flag coding epitope sequence inserted into 5'of GLI3 ORF in pRK-GLI3 (3)). HEK293 cells were transient transfected with these constructs as described (1). Protein expression was assayed 48 hr post-transfection. Antibodies used were as follows: rabbit polyclonal Flag (Sigma), mouse monoclonal Xpress (Invitrogen), rabbit polyclonal GLI1 (Abcam), and the rabbit polyclonal GLI1 antibody mentioned in the main body of the text.

*Lentiviral RNA interference*

Production of lentivirus and subsequent viral infection were as described previously (4). A549 cells were infected with lentivirus expressing control non-target shRNA (Sigma) or two shRNAs targeting different regions of human *GLI1* (Open Biosystem). Cells were split on day 3 post viral infection, mounted onto glass cover slides, and incubated overnight. Immunohistochemistry was performed as described previously (5). An immunoblot for GLI1 was performed on the same set of cells to confirm the GLI1 knockdown.

**Legends for Supplemental Figures**

**Figure S1.** Characterization of the GLI1 antibody used in this study. (A) Immunoblot in HEK293 cells transiently transfected with constructs expressing GFP (lane 1), Flag-GLI1 (lane 2), Xpress-GLI2 (lane 3), or Flag-GLI3 (lane 4). Each cell lysate was split into three aliquots and immunoblotted for GLI1, Flag, or Xpress separately. (B) Immunoblot for GLI1 in a known low-GLI1 (Hop-62) and high-GLI1 (A549) non-small cell lung cancer cell line (1). (C) A549 cells were infected with lentivirus expressing control shRNA or two distinct *GLI1* shRNAs for 4 days. Immunohistochemistry for GLI1 showed decreased nuclear staining in cells receiving *GLI1* shRNAs. An immunoblot performed on the same set of cells confirmed the GLI1 knockdown. (D) Immunohistochemistry of GLI1 in a histologically confirmed normal skin sample (left) and a basal cell carcinoma specimen (right). Basal cell carcinomas are known to have elevated GLI1 levels (6). (E) Comparative immunohistochemistry were performed on the bladder cancer TMA used in this study, using a second GLI1 antibody (Abcam). The specificity of this antibody was characterized as described (1). Similar results were obtained with both GLI1 antibodies (data not shown). A representative comparison between the ability of the two GLI1 antibodies to detect nuclear Gli1 in a bladder cancer sample is shown.

**Figure S2.** Bladder cancers showed different levels of GLI1 staining. Representative immunohistochemical stained sample for each GLI1 score (top panel), and related patient information (bottom panel), are shown. A subset of nuclear GLI1 positive cancer cells are indicated by the arrows. The images shown for the GLI1 scores "Less than 1%" and "25 – 50 %" are the same ones shown in Figure 4A.

**Figure S3.** Cyclopamine abolished *GLI1* induction by SHH conditioned media (SHH CM). NIH3T3 cells were treated with SHH CM or control media for 4 hr. Cyclopamine (5 μM) or the inactive analog

tomatidine (5 μM), were added to the SHH CM during the treatment. Real-time PCR was performed to determine the expression of the HH target gene, *GLI1*, relative to expression of *18S* rRNA.

**Figure S4.** Arsenic affects the expression of *GLI2* and *GLI3*. RNA was extracted from NIH3T3 cells treated with or without 0.5 μM, 1 μM, or 5 μM sodium arsenite for 8 weeks. The expression levels of *GLI2* and *GLI3* were examined by real-time PCR relative to the expression of *18S* rRNA. Gene expression in untreated control cells was set to 1. Error bars indicate s.e.m from five independent experiments. The asterisk indicates significant changes compared to control cells.

## References


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