

Li et al., <http://www.jcb.org/cgi/content/full/jcb.201008087/DC1>

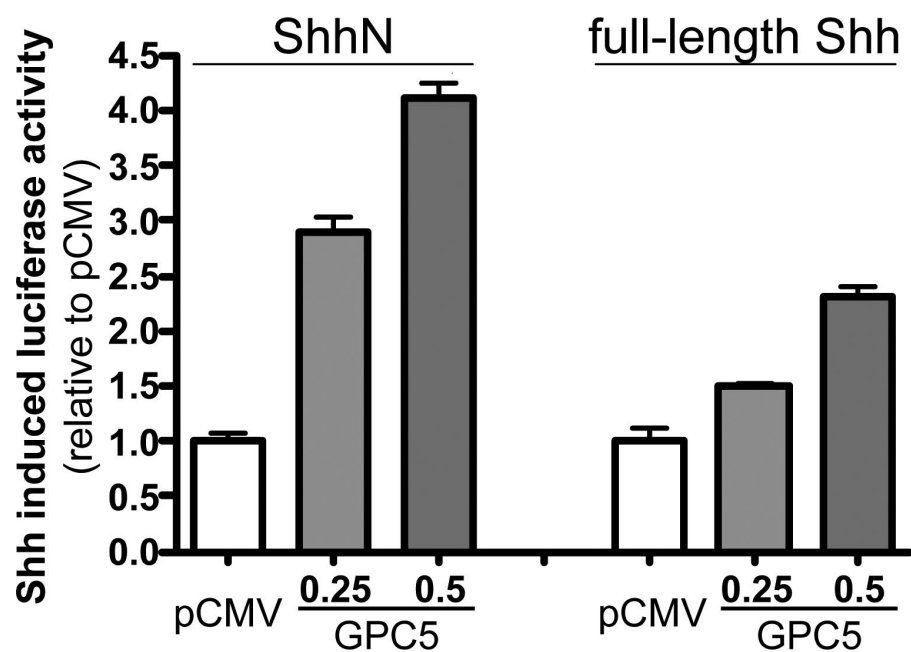


Figure S1. **GPC5 stimulates both ShhN- and full-length Shh-induced signaling.** NIH 3T3 cells were transfected with GPC5 or empty vectors (pCMV) and were incubated with ShhN-, full-length Shh-, or control-conditioned medium for 48 h. A transient luciferase reporter assay was performed as described in Fig. 3 A. Results represent the means  $\pm$  SD of triplicates. The experiment was repeated twice with similar results.

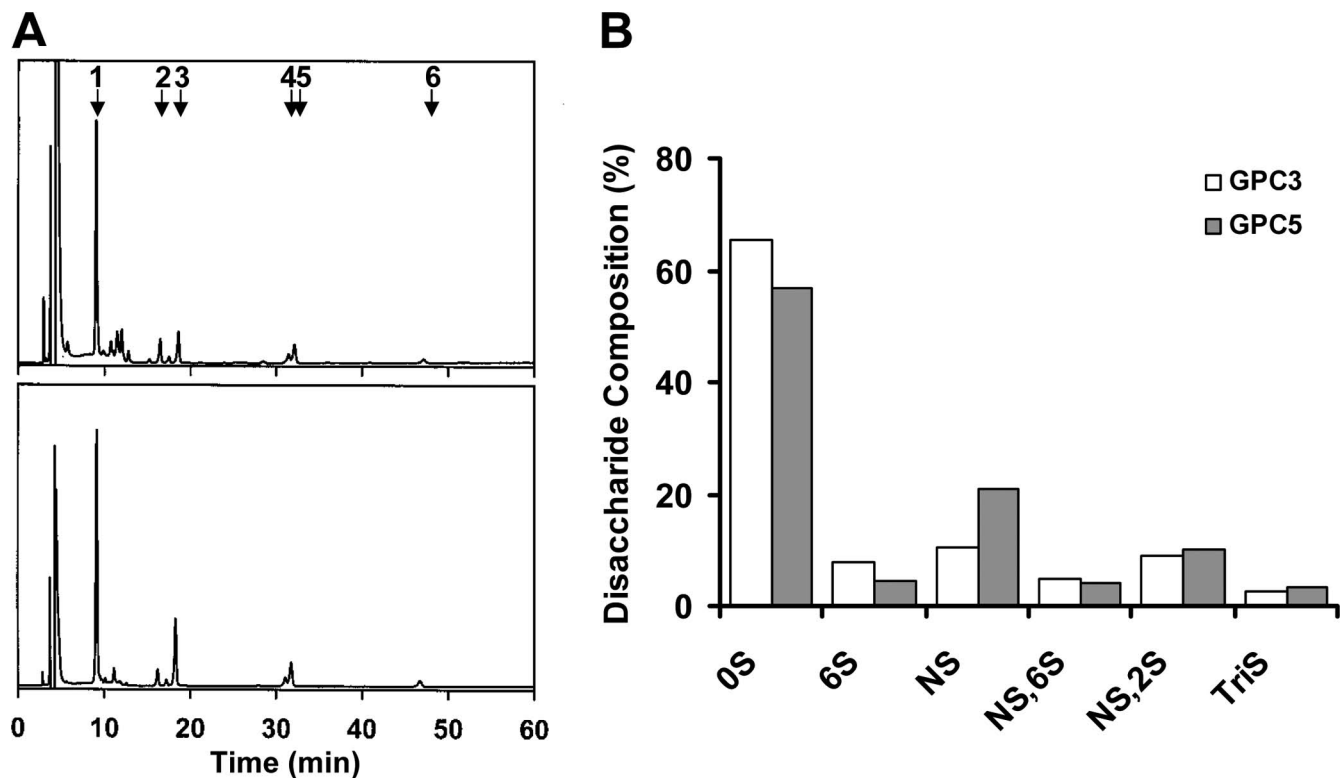


Figure S2. **Disaccharide analysis of the HS chains of GPC3-AP and GPC5-AP purified from transiently transfected RH30 cells.** (A) Disaccharide analysis of the HS chains of purified GPC3-AP (top) and GPC5-AP (bottom) was performed by digestion with HSases followed by HPLC analysis as described in Fig. 9 E. The elution positions of authentic 2AB disaccharide standards derived from HS are indicated by numbered arrows (top): (1)  $\Delta$ HexUA-GlcNAc (OS), (2)  $\Delta$ HexUA-GlcNAc(6-O-sulfate) (6S), (3)  $\Delta$ HexUA-GlcN(2-N-sulfate) (NS), (4)  $\Delta$ HexUA-GlcN(2-N,6-O-disulfate) (NS,6S), (5)  $\Delta$ HexUA(2-O-sulfate)-GlcN(2-N-sulfate) (NS,2S), and (6)  $\Delta$ HexUA(2-O-sulfate)-GlcN(2-N,6-O-disulfate) (TriS). (B) Bar graph displaying the results of the disaccharide analysis.  $n = 1$ .

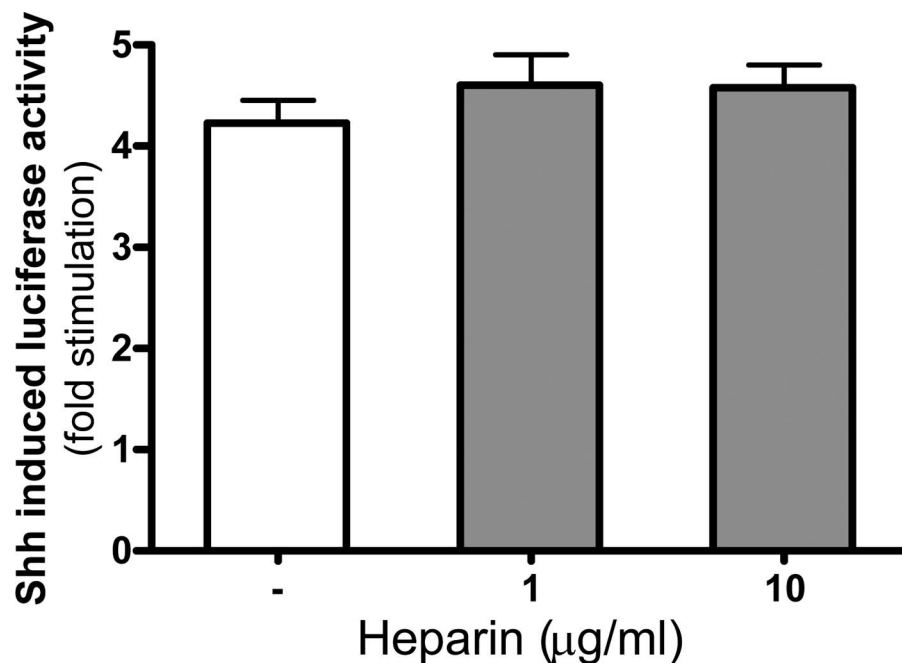


Figure S3. **Heparan does not stimulate Shh-induced signaling.** NIH 3T3 cells were transfected with GPC5 and incubated with ShhN- or control-conditioned medium in the presence or absence of the indicated amounts of heparan. A luciferase assay was then performed as described in Fig. 3 A. The experiment was repeated twice with similar results. Error bars represent the means  $\pm$  SD.

Table S1. Kinetic parameters for the interaction of Shh with immobilized GPC5-AP

Ligand	$k_a$	$k_d$	$K_d$
	$M^{-1}s^{-1}$	$s^{-1}$	$nM$
GPC5-AP	$(1.2 \pm 0.2) \times 10^4$	$(3.6 \pm 0.6) \times 10^{-3}$	$310 \pm 60$

The  $k_a$ ,  $k_d$ , and  $K_d$  values were determined using a 1:1 Langmuir binding model as described in Materials and methods. The results represent the means  $\pm$  SEM of five different concentrations.  $k_a$ , association constant;  $k_d$ , dissociation constant;  $K_d$ , affinity constant.