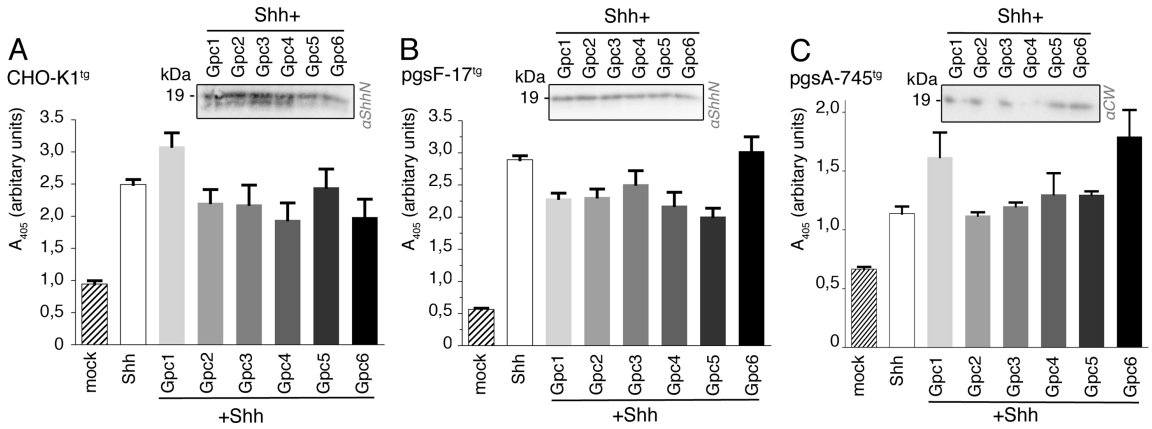

Fig. S1: A-C) Shh release and biofunction in Hhat-transfected CHO-K1^{tg}, pgsF-17^{tg} and pgsA-745^{tg} cells. Gpcs 1-6 and Shh were co-expressed in CHO cells from bicistronic mRNAs. released into serum free media for 24h and the soluble protein amounts were quantified. Supernatants of Gpc/Shh expressing CHO cells, or control media conditioned with (Shh) or without Shh (mock) were added to C3H10T1/2 osteoblast precursor cells, and Hh-dependent cell differentiation was determined. Gpc core proteins do not significantly affect Shh bioactivity in CHO-K1^{tg} cells (with the exception of Gpc1)(A), CHO pgsF-17^{tg} (with the exception of Gpc6)(B) and CHO pgsA-745^{tg} cells (with the exception of Gpc1 and Gpc6)(C). CHO K1^{tg} cells: Shh alone (control): 2.5±0.08 arbitary units (au) (n=9), Gpc1/Shh 3.1±0.2 au, p=0.01, n=8, Gpc2/Shh 2.2±0.2 au p=0.21, n=9, Gpc3/Shh 2.18±0.3 au, p=0.33, n=9, Gpc4/Shh 1.9±0.26 au, p-value 0.06, n=9, Gpc5/Shh 2.4±0.28 au, p=0.89, n=9, Gpc6/Shh 1.987±0.2779 au, p-value 0.0989, n=9. For pgsF-17^{tg} cells: Shh 2.893±0.06 au, n=9, Gpc1/Shh 2.29±0.08 au, p=0.0001, n=9, Gpc2/Shh 2.3±0.12 au, p=0.0007, n=9, Gpc3/Shh 2.5±0.21 au, p=0.1, n=9, Gpc4/Shh 2.18±0.21 au, p=0.0044, n=9, Gpc5/Shh 2±0.13 au, p=0.0001, n=9, Gpc6/Shh 3±0.22 au, p=0.57, n=9 and for pgsA-745^{tg} cells: Shh 1.14±0.06 au, n=9, Gpc1/Shh 1.62±0.2 au, p=0.0416, n=9, Gpc2/Shh 1.1±0.03 au, p=0.8, n=9, Gpc3/Shh 1.2±0.03 au, p=0.37, n=9, Gpc4/Shh 1.3±0.18 au, p=0.4, n=9, Gpc5/Shh 1.3±0.09 au, p=0.03, n=9, Gpc6/Shh 1.8±0.22 au, p=0.01, n=9.

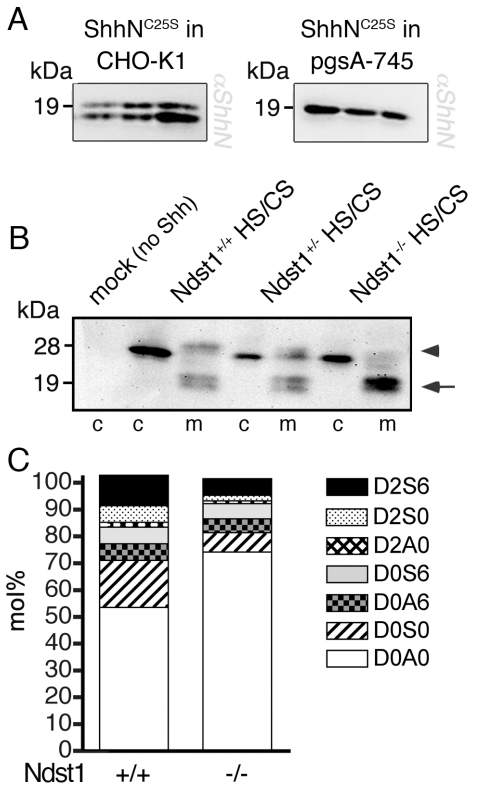

Fig. S2: A) Unlipidated $ShhN^{C25S}$ is secreted from CHO-$K1^{tg}$ and pgs-$A745^{tg}$ cells into the supernatant, but only the CHO-$K1^{tg}$ secreted material is N-terminally processed. This demonstrates that HS-expression is a critical determinant of Shh processing. Processed proteins show increased electrophoretic mobility. **B)** HS was isolated from E18 C57/Bl6 mice, Ndst1 heterozygous or homozygous mutant embryos, and analyzed as described before. Although the processing cellular material was potentiated by all HS isolates, only HS isolated from Ndst1 mutant embryos stabilized the product, resembling results obtained by pooled EHS obtained from E12.5-E18 mouse embryos (Fig. 6). In contrast, HS derived from wildtype littermate controls resembled CHS-facilitated Shh processing. This demonstrates that HS, not the CS fraction also present in the isolates, potentiates Hh processing an agreement with glypicans being HS-proteoglycans, not CS proteoglycans. **C)** HS was isolated and samples digested with heparin lyases. The resulting disaccharides were analyzed by quantitative LC/MS. Values denote the mean % of total disaccharide. The abbreviations used denote the following disaccharides: D0A0 (ΔUA1,4GlcNAc), D0S0 (ΔUA1,4GlcNS), D0A6 (ΔUA1,4GlcNAc-6S), D0S6 (ΔUA1,4GlcNS-6S), D2A0 (ΔUA2S1,4GlcNAc), D2S0 (ΔUA2S1,4GlcNS) and D2S6 (ΔUA2S1,4GlcNS-6S). Overall HS sulfation of the $Ndst1^{-/-}$ material was 1/3 reduced if compared to HS isolated from wild-type or heterozygous littermate controls.

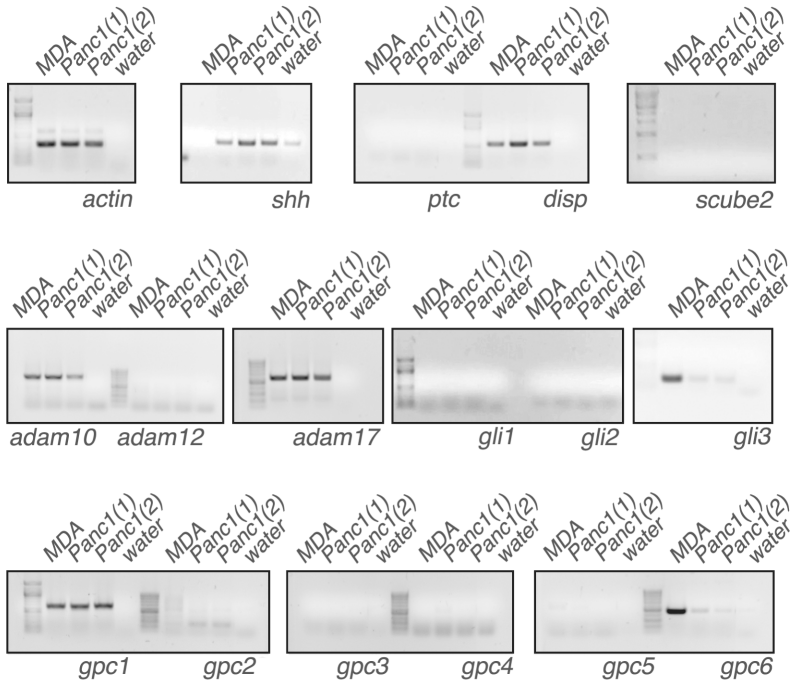

Fig. S3: PANC cells express genes required for Shh production and release, but not for Shh reception, as determined by semiquantitative RT-PCR. Assays were performed in duplicate, and MDA cells served as a control. We detected lack of scube2 and adam12 mRNA expression consistent with previous reports (Creanga et al., 2012; Damhofer et al., 2015; Tukachinsky et al., 2012).

References:

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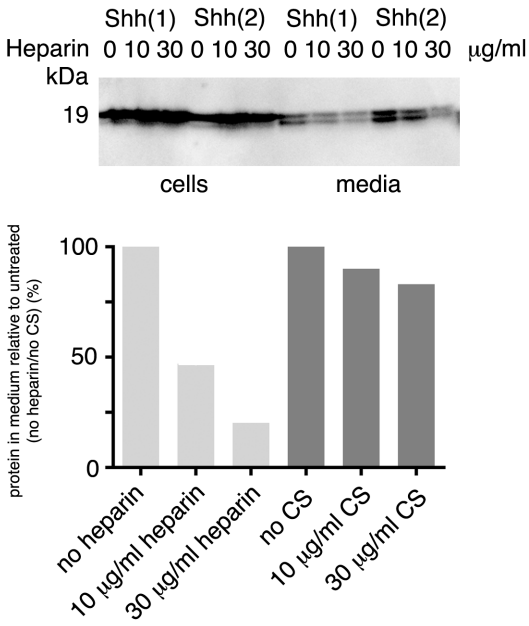

Fig. S4: Shh expression in the presence or absence of 0-30μg/ml heparin and chondroitin sulfate (CS). Heparin blocked Shh processing and release in concentration-dependent manner.