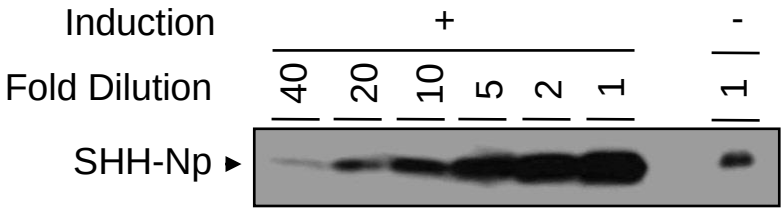
Figure S1

A:



B:

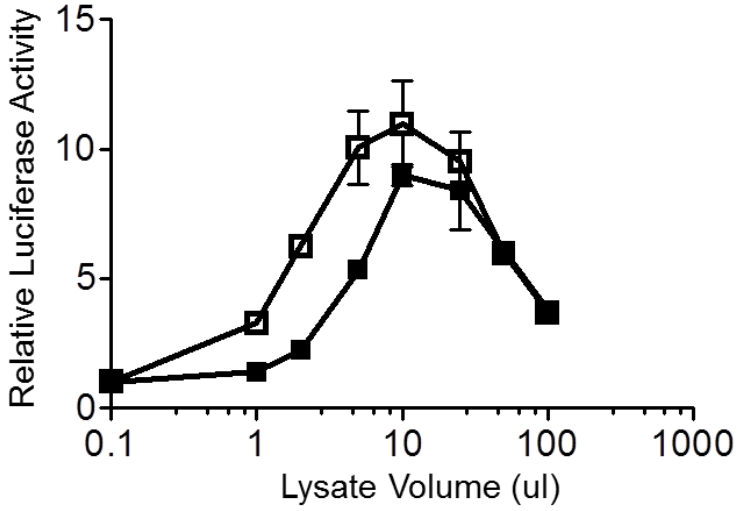


Figure S1: Induction of SHH-I cells produces large amounts of lower activity SHH-Np, that is related to Figure 1. (**A**) An immunoblot of lysates from SHH-I cells induced with (+) or without muristerone (-). (**B**) Aliquots of cellular lysate both from muristerone induced (open square) and uninduced (colored square) SHH-I cells, were incubated with Light-II reporter cells to assay SHH-Np activity. We estimated the linear portion of this assay to be between 2-5 ml of lysate (see Figure 1B). Error bars represent the SD in one representative experiment.

Figure S2

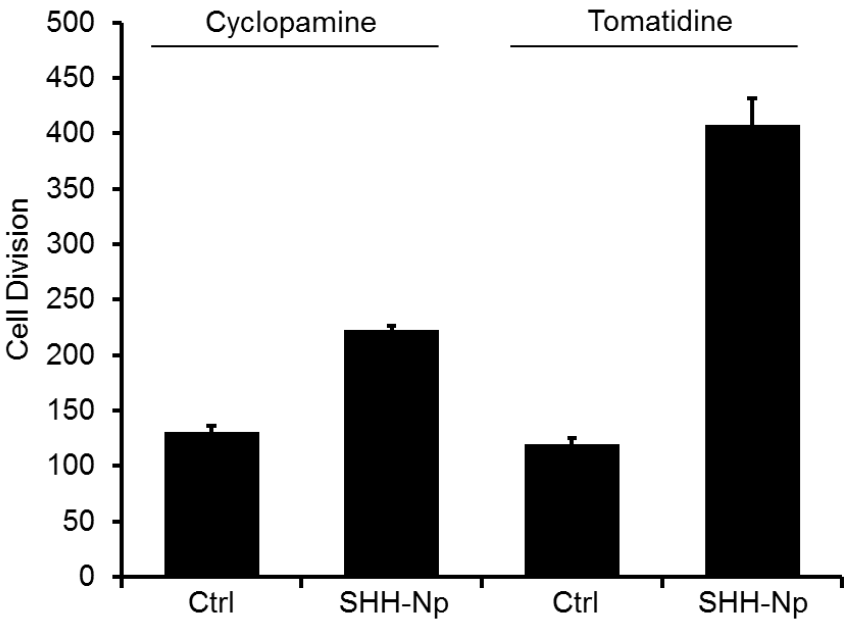


Figure S2: Granule neuron precursor cell proliferation assay, that is related to Figure 2. The ability of purified SHH-Np to induce the proliferation of primary granular neuron precursor cells is measured here, using a BrdU incorporation assay. The use of the SHH pathway antagonist cyclopamine validates this assay as SHH dependent. Tomatidine, an inactive analog of cyclopamine, is used as a negative control. Error bars represent the SD in one representative experiment.

Figure S3

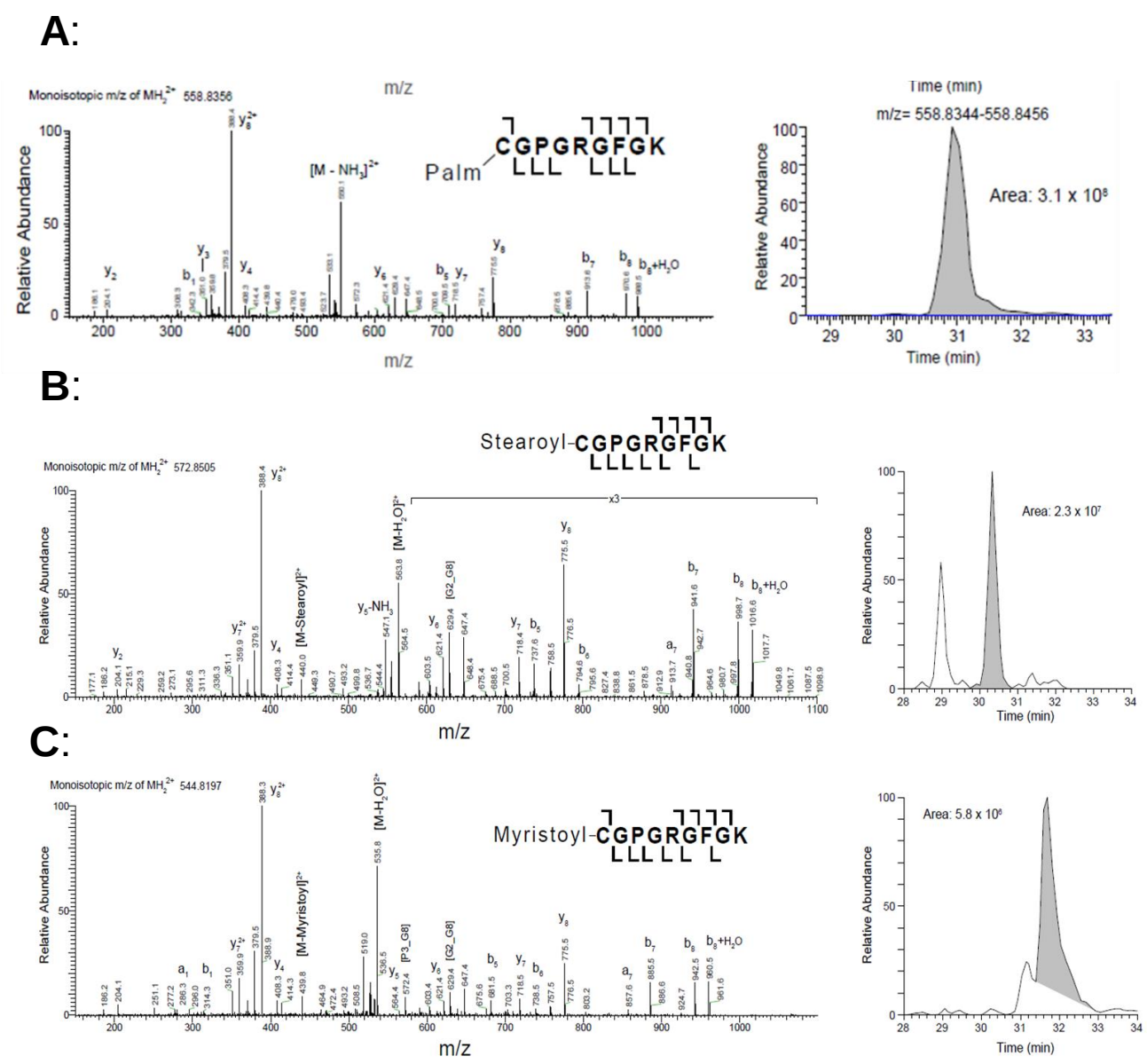


Figure S3: Sequencing and modifications of the amino-terminal SHH-Np peptides, that is related to Figure 3. A representative analysis of the amino-terminal SHH peptide is depicted here (left panels). These peptides were obtained by subjecting purified SHH-Np to endoproteinase Lys C digestion followed by LC-MS/MS analysis. A chromatographic peak corresponding to the sequenced peptide is also shown (right panels). Ions corresponding to backbone fragmentations and parent neutral losses are labeled, resulting in the indicated coverage over the peptide sequence, indicated by brackets for C-terminal y ions (┗) and N-terminal b-ions (┓), corresponding to cleavage at the backbone amide bond. A spectrum of fatty acid modified amino-terminal peptides of SHH-Np were identified in a similar manner. In this example, the amino-terminal peptide is modified by palmitate (Palm) (**A**), stearoyl (**B**) and myristoyl (**C**).

Table S1

Modification	Saturation	Relative abundance (%)			Hydrophobicity (Log P)
		10% FBS Uninduced	10% FBS Induced	Serum deprived Induced	
Palmitoyl	(C16:0)	58.0	37.4	N/D	7.15
Palmitoleyl	(C16:1)	29.9	3.4	72.2	6.64
Stearoleyl	(C18:1)	5.0	55.9	N/D	7.70
Myristoleyl	(C14:1)	3.2	N/D	25.9	5.57
Stearoyl	(C18:0)	3.2	3.3	N/D	8.22
Myristoyl	(C14:0)	0.7	N/D	1.9	6.09

Table S1: Fatty acid modifications identified on purified SHH-Np, that is related to Figure 3. Fatty acid modifications of SHH-Np, along with respective saturation state, relative abundance of speciated SHH-Np and hydrophobicity values (LogP), are shown in the table. The purified SHH-Np was isolated from SHH-I cells grown under three different cellular contexts: 10% FBS without muristerone induction of SHH expression, 10% FBS and muristerone induction of SHH expression, and serum deprivation and muristerone induction of SHH expression. N/D means not detected above 1%.

Supplemental Experimental Procedures

Cerebellar Granular Neuronal Precursors culture and BrdU staining: The isolation and culture of Cerebellar Granular Neuronal Precursors (CGNP) from P5 mice was done following the papain extraction protocol (Lee et al., 2009). Cells were placed in poly-D-lysine coated chamber slides for 2 h in Neurobasal-A-medium plus Glutamax, B27 and KCl 250uM prior to add NP40 (1:300), purified SHH-Np (1:300), tomatidine (10uM) or cyclopamine (10uM). 24 h later BrdU to a concentration 10uM was added and incubated overnight. Cells were fixed in Paraformaldehyde 4 % 1 h. Immunostaining for BrdU was done as previously described (Fernandez et al., 2010) with minor changes. The Cell Signaling BrdU antibody was used 1:1000 in BSA 5 % overnight and secondary Alexa Fluor 594 used 1:500 was incubated the same way. Nuclei were counterstained with Hoechst 1:10000 (Sigma) and samples were mounted with ProLong (Invitrogen). At least 8 independent fields were counted per experimental condition under the 20x objective of an Olympus IX7I microscope.

Purification of SHH-Np:

Buffer A: 10 mM sodium phosphate, 350 mM NaCl buffer, pH 7.4.

Buffer B: 1% Tx-100, 10 mM sodium phosphate, 150 mM NaCl buffer, pH 6.5.

Buffer C: 1% Tx-100, 10 mM sodium phosphate, 150 mM NaCl buffer, pH 5.0.

Buffer D: 0.2% Np-40, 10 mM sodium phosphate, 300 mM NaCl buffer, pH 5.5.

Buffer E: 0.2% Np-40, 10 mM sodium phosphate, 450 mM NaCl buffer, pH 7.8.

Buffer F: 0.1% Np-40, 10 mM sodium phosphate,150 mM NaCl buffer, pH 7.4.

Buffer G: 0.1% Np-40, 10 mM sodium phosphate,150 mM NaCl buffer, pH 2.7*

*To obtain saturating concentrations of SHH-Np, we used a 0.01% Np-40, 10 mM sodium phosphate,150 mM NaCl buffer, pH 2.7, in 50% FBS to elute SHH-Np.

Supplemental References

Fernandez, C., Tatard, V.M., Bertrand, N., and Dahmane, N. (2010). Differential modulation of Sonic-hedgehog-induced cerebellar granule cell precursor proliferation by the IGF signaling network. Developmental neuroscience *32*, 59-70.

Lee, H.Y., Greene, L.A., Mason, C.A., and Manzini, M.C. (2009). Isolation and culture of post-natal mouse cerebellar granule neuron progenitor cells and neurons. Journal of visualized experiments : JoVE.