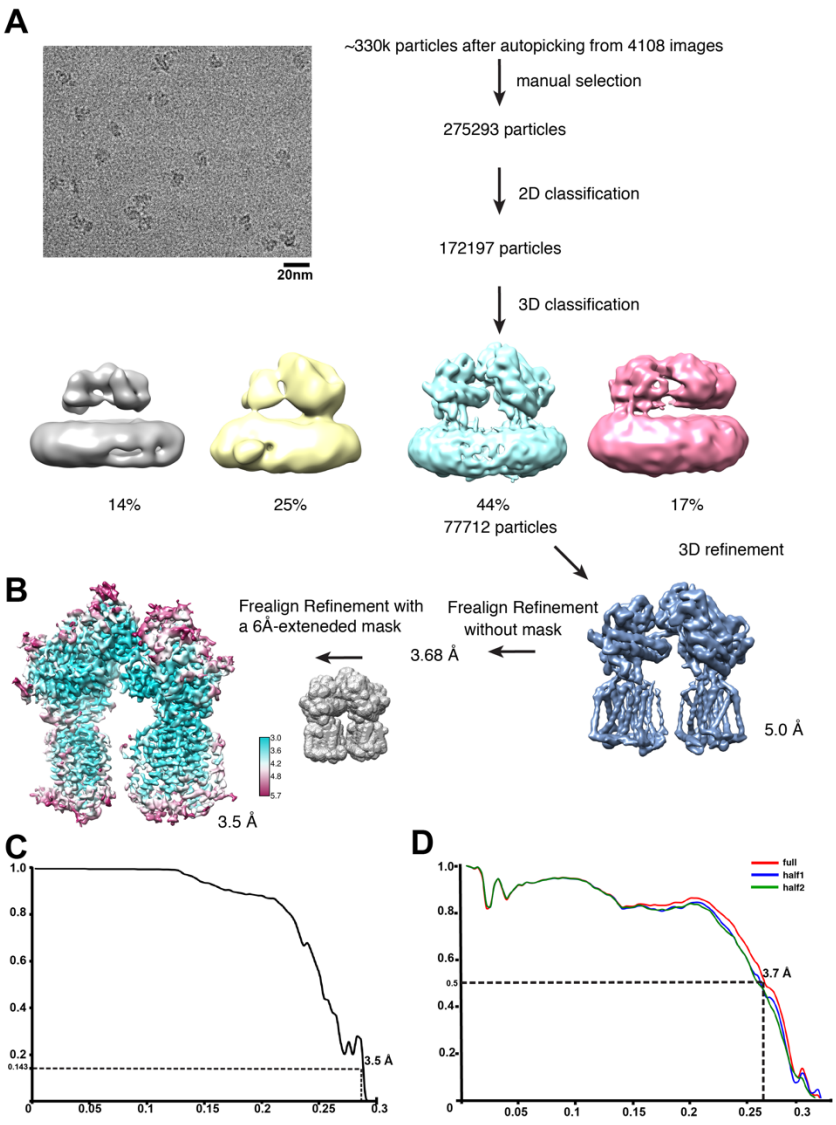

Fig. S1 Data processing and Model quality assessment.

(A) The data processing work-flow for the 2:1 PTCH1*–SHH-N complex with a representative electron micrograph at -2.0 µm defocus (left top). (**B)** Density maps of structure colored by local resolution estimation using blocres. (**C)** Fourier shell correlation (FSC) curve of the structure with FSC as a function of resolution using Frealign output. (**D)** The FSC curves calculated between the refined structure and the half map used for refinement, the other half map and the full map.

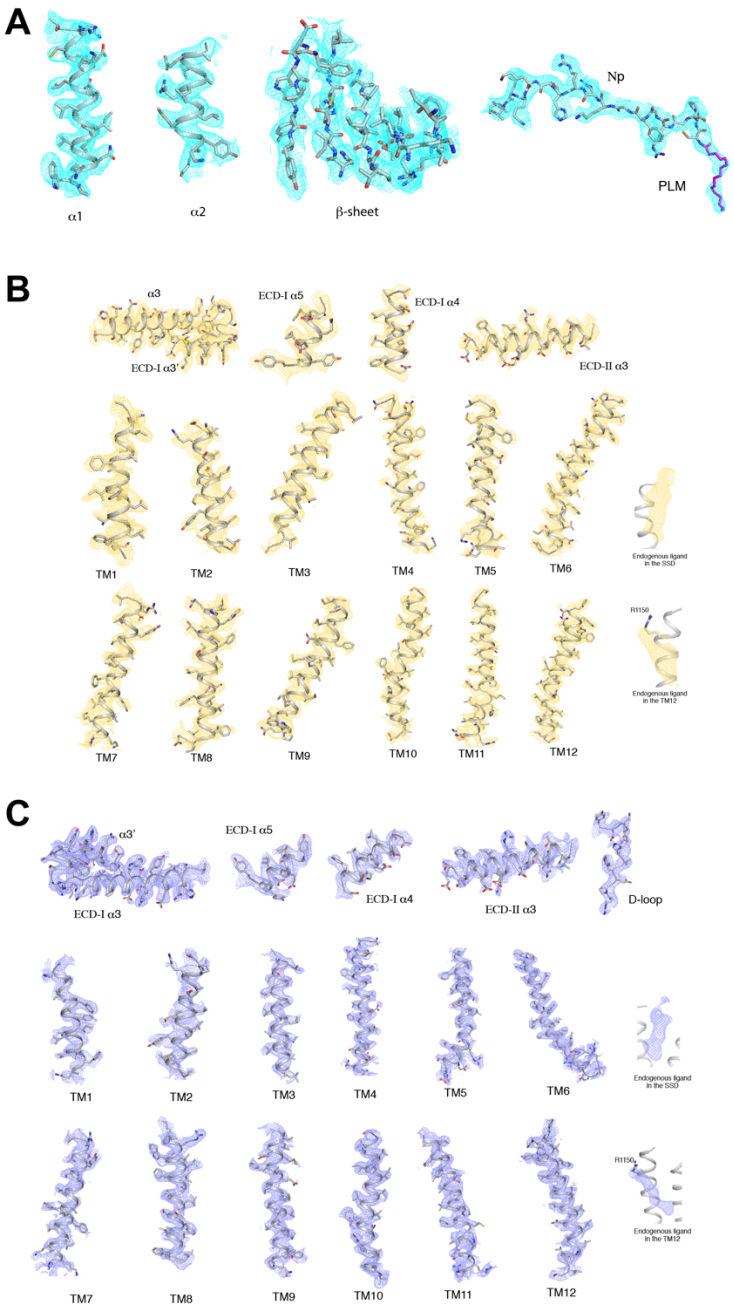

Fig. S2 An atomic resolution model of SHH-N and PTCH1s in the complex.

(A) EM density map and model of SHH-N are shown for major structural elements, the N-terminal peptide (Np) and the palmitate moiety (PLM). (**B**) PTCH1-A; (**C**) PTCH1-B. EM density map and model are shown for all 12 TMs, major structural elements in the ECDs, and two endogenous molecules (only map).

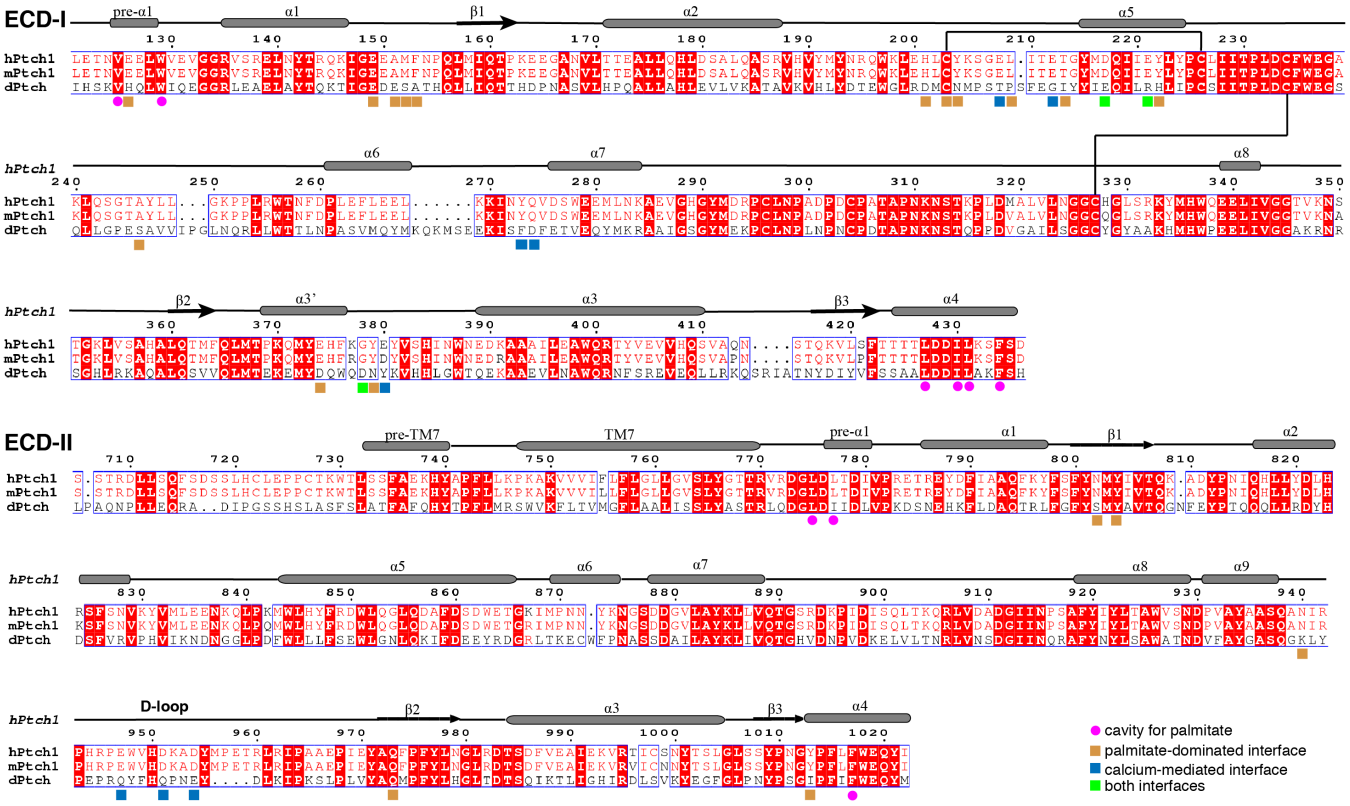

Fig. S3 Sequence alignment of the ECDs of human PTCH1, mouse PTCH1, and *Drosophila* PTCH.

The residue numbers of hPTCH1 are indicated above the protein sequence. The secondary structures of ECDs are labeled and the specific residues for binding the SHH-N have been indicated by the circles or squares.

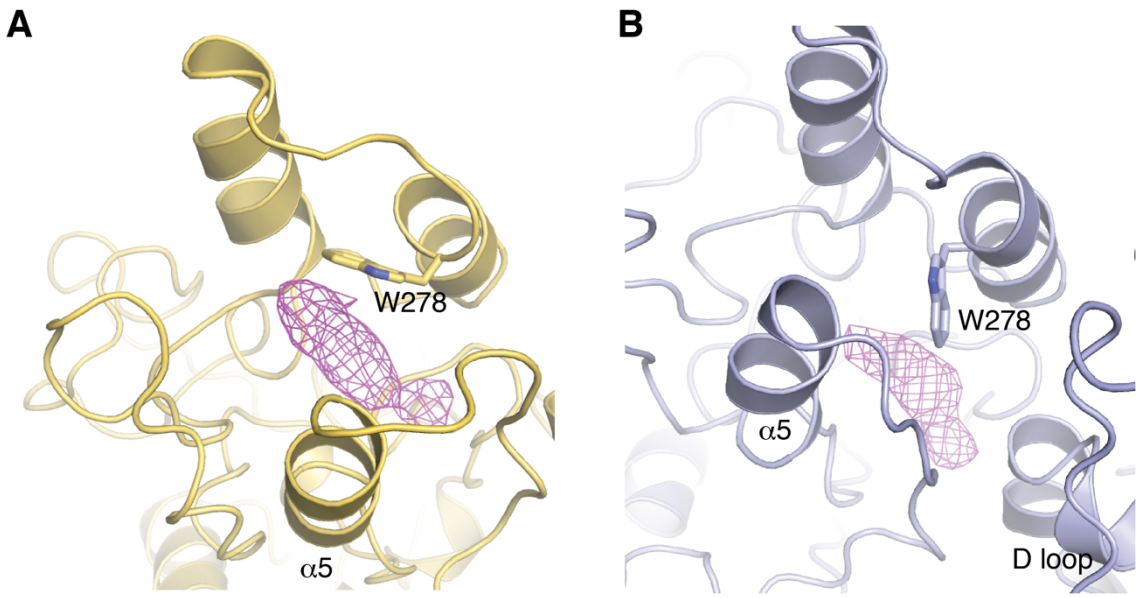

Fig. S4 The rod-like density in ECD-I.

(A) PTCH1-A; **(B)** PTCH1-B. The unidentified molecule is shown as mesh at 5σ level.

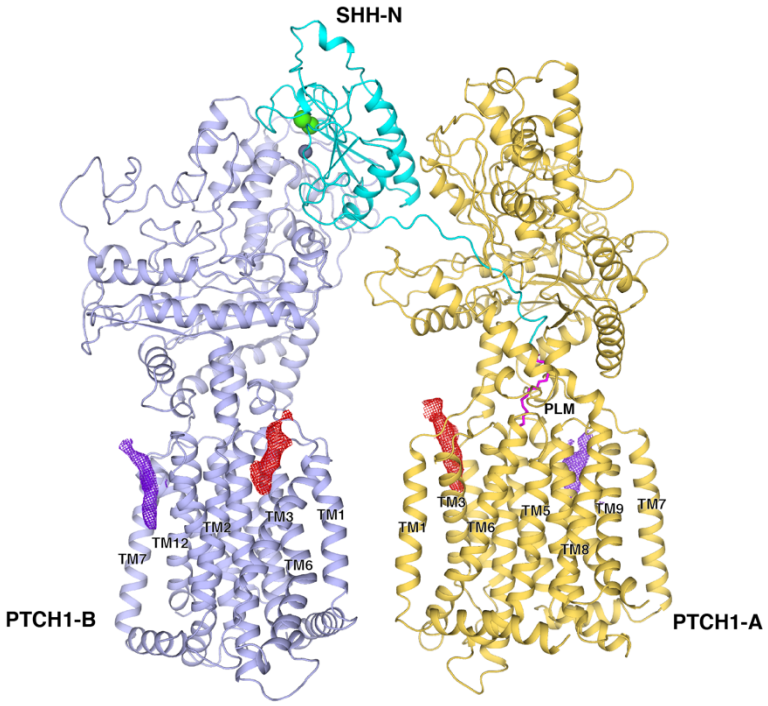

Fig. S5 The sterol-like densities in the transmembrane domains shown as mesh at 2.5σ level.

Sterol-like densities in the SSDs and near TMs12 are colored in red and purple, respectively.

Table S1 Statistics of data collection and refinement.

	PTCH1*–SHH-N
Data Collection	
Microscope	Titan Krios (FEI)
Voltage (kV)	300
Detector	K2 Summit (Gatan)
Pixel size (Å)	1.0
Defocus range (μm)	-1.2 to -2.2
Movies	4108
Frames/movie	50
Dose rate (electrons/pixel/s)	8
Total dose (electrons/Å^2)	80
Number of particle	275,293
Model composition	
Non-hydrogen atoms	16671
Protein residues	2120
Ligand	4
Refinement	
Number of particle for refinement	77,712
Resolution (Å)	3.5
RMS deviations	
Bond lengths (Å)	0.0047
Bond angles (°)	1.1589
Ramachandran plot (%)	
Favored	90.3
Allowed	9.7
Disallowed	0.0
Clashscore, all atoms	5.53
Molprobity score	2.04