

Figure S1. Overexpression of mPtch1D499AA and ggPtch1D513A mutants.

Cross-sections of chicken neural tubes electroporated with *pMES-mPtch1D499A* (A) and a chicken antiporter Ptch1 mutant, *pMES-ggPtch1D513A* (B). Electroporated cells are labeled in green (GFP) and sections are stained with antibodies to Pax7 (A, C), Hb9 (B) as labeled in red or represented in the corresponding gray scale image ('), and DAPI nuclear in blue. Arrows in A, A', C, and C' indicate a cell autonomous induction of Pax7 expression by the Ptch1 antiporter mutants. Scale bar ~50 μm (C') is 50 μm .

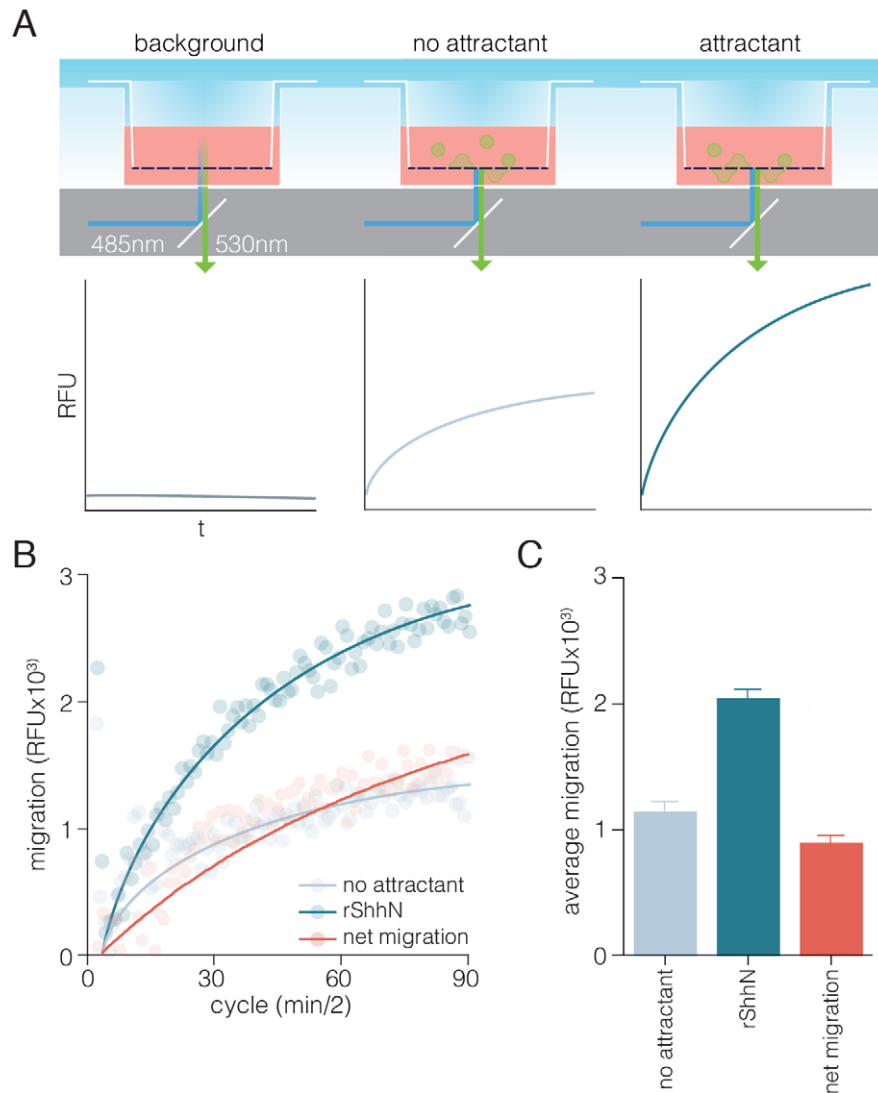


Figure S2. Summary of the modified Boyden chamber migration assay and analysis of results

(A) A Fluoroblok Transwell plate and insert setup is used to measure fluorescence from labeled cells that have migrated through a fluorescence blocking membrane with 8 μm pores. Background fluorescence is measured in time from a well containing medium and these values are subtracted from all other measurements. The 'no attractant' control measures basic cell movement (i.e. movement other than that towards the chemoattractant) for every cell type, transfectant, or other experimental condition. These values are then subtracted from those obtained in the presence of chemoattractant in the bottom compartment of the Transwell setup to yield the specific migration towards a given attractant. Representation as formula; net migration = $(\text{RFU}_{\text{attractant}} - \text{RFU}_{\text{BKG}}) - (\text{RFU}_{\text{no att}} - \text{RFU}_{\text{BKG}})$. (B) An example of a chemotaxis experiment using rShhN is shown. Background values are already subtracted, and starting points of migration are set to $y=0$. To yield specific net migration (indicated in red), values for the no attractant control are subtracted from the chemotaxis towards 5 nM recombinant ShhN. Note that line curves are fitted, rather than plotted means for clarity. (C) The average of migration measured over time as shown in panel B plotted as bar graphs.

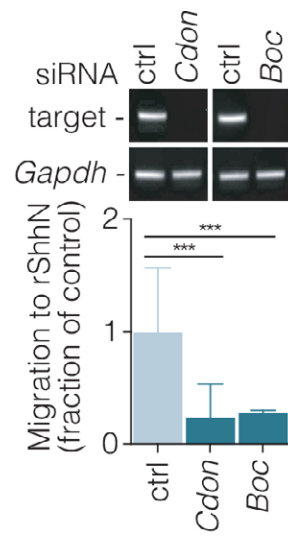


Figure S3. Fibroblast chemotaxis to rShhN requires Cdon and Boc

Ptch1/MEFs were transiently transfected with siRNA against *Cdon*, *Boc*, or scrambled control and knockdown was assessed by RT-PCR. Net migration to ShhN was assessed as for Figure 3. Shown is average net migration from 3 experiments, \pm SEM; *, $p < 0.05$; ***, $p < 0.005$. Statistical significance was assessed by Student's t-test.