Supplementary information, Figure S7

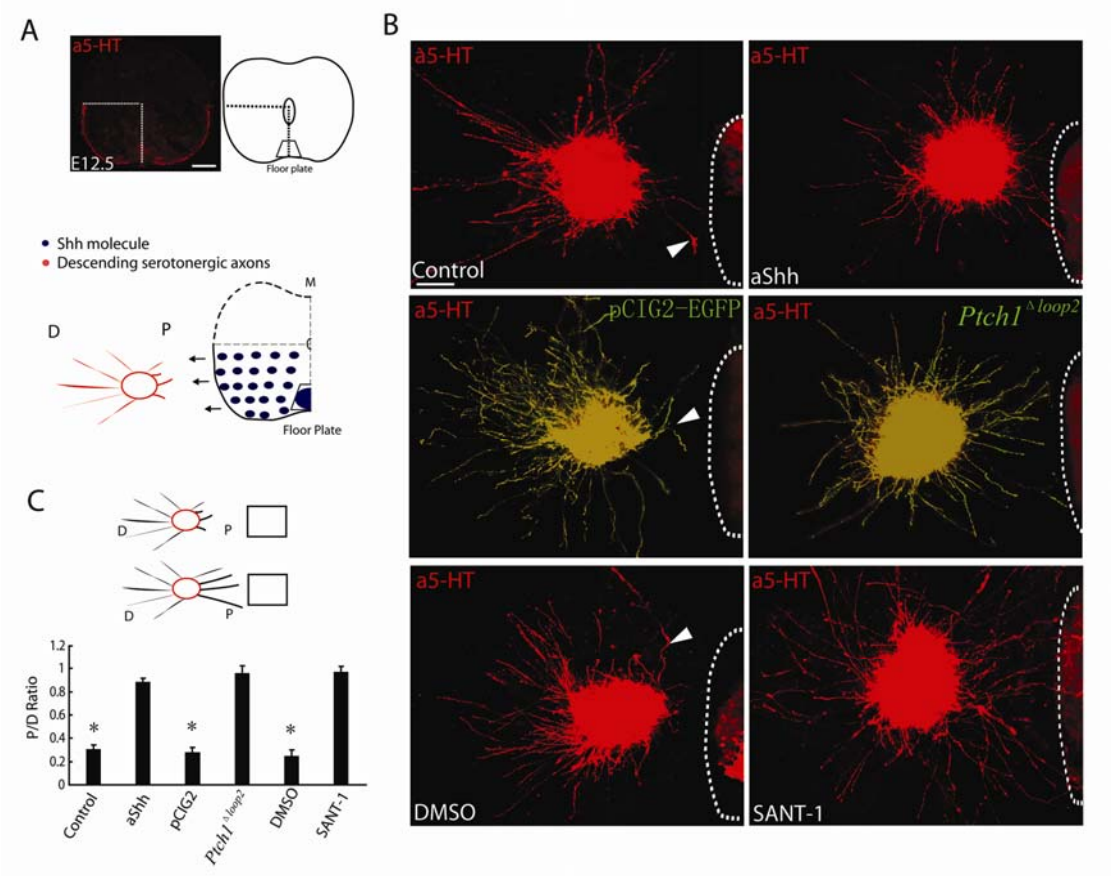

Figure S7 M-L repulsion is mediated by Shh signaling. **(A)** The M-L co-culture assay procedure. The region of the ventral spinal cord with descending serotonergic RST axons was revealed by 5-HT immunostaining of coronal sections of the E12.5 upper cervical spinal cord (outlined by the dashed line). Scale bar: 200 μm. E12.5 CRN explants and slices of the ventral spinal cord were dissected and co-cultured in the M-L functional assay. In the *ex utero* electroporation procedure, the *Ptch1*$^{\Delta loop2}$ construct was electroporated into the CRN of E12.5 embryos before dissection. After culturing, 5-HT immunostaining was conducted to confirm that serotonergic RST axons were growing from the CRN explants. **(B)** E12.5 ventral spinal cords repelled the serotonergic RST axons from the CRN explants. This repulsion was effectively eliminated when the Shh antibody (5E1) or SANT-1 were added to the co-culture system or when *Ptch1*$^{\Delta loop2}$ was expressed in serotonergic RST axons by co-culturing *Ptch1*$^{\Delta loop2}$-electroporated CRN with ventral spinal cords. White arrowheads indicate deflected axons. White dashed lines outline the boundary of ventral spinal cords.

Scale bar: 200 μm. **(C)** Quantification of axon outgrowth. Repulsion was measured using the P/D ratio. Blocking the Shh signaling components effectively abolished ventral spinal cord repulsion of serotonergic RST axons. The experiment was replicated at least five times. Statistical analysis was performed using the Student's *t*-test. Results are presented as the mean ± S.E.M. Control group: 0.298 ± 0 .044; Shh Antibody group: 0.878 ± 0.039; pCIG2-EGFP group: 0.275 ± 0.049; $Ptch1^{\Delta loop2}$ group: 0.952 ± 0.073; DMSO group: 0.241 ± 0.060; SANT-1 group: 0.962 ± 0.058. Asterisks indicate values that differed significantly from the values of the control groups ($P < 0.05$).