Supporting Information

Belgacem and Borodinsky 10.1073/pnas.1018217108

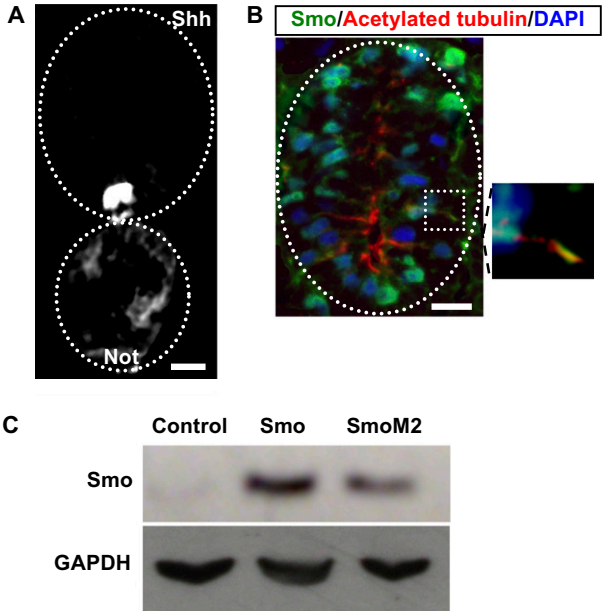

Fig. S1. (*A* and *B*) Expression of Shh and Smo in the developing spinal cord. Transverse sections from stage-24 embryos were processed for immunostaining. Outlined are the notochord (Not) and neural tube. (*B*) (*Right*) Enlarged view (2.5× magnification) of the squared region in the left panel showing expression of Smo at the spinal cell primary cilium. (Scale bars, 20 μm.) (*C*) Effectiveness of Smo and SmoM2 misexpression and specificity of the Smo antibody. Embryos at the two-cell stage were microinjected with 300 pg mRNA encoding human Smo or 150 pg mRNA encoding SmoM2 along with 20 mg/mL dextran-Alexa Fluor 594 conjugate. Protein extracts from 10 neural tubes from stage-24 (26-h-old) embryos injected with Smo, SmoM2, or tracer only (Control) were processed for Western blots, and membranes were probed with anti-Smo and anti-GAPDH.

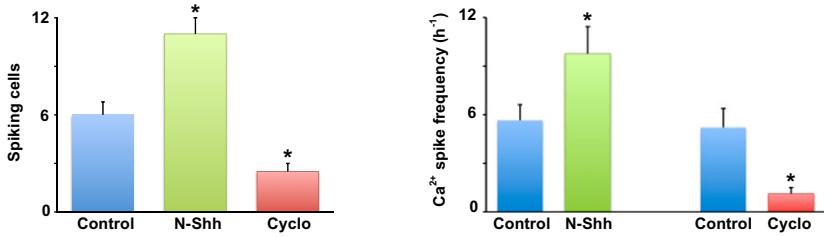

Fig. S2. Shh signaling regulates neuronal Ca^{2+} spike activity in vitro. Neuronal cultures grown for 7 h were loaded with 1 μM fluo4-AM and imaged for 30 min before (Control) and after addition of drugs. Values are mean ± SEM incidence of spiking cells and frequency of spikes per cell during the recording before and after the addition of 100 nM *N*-Shh or 20 μM cyclopamine (Cyclo). $n = 5$ cultures per experimental group; $^*P < 0.05$ compared with control using unpaired (incidence of spiking cells) and paired (frequency) *t* test.

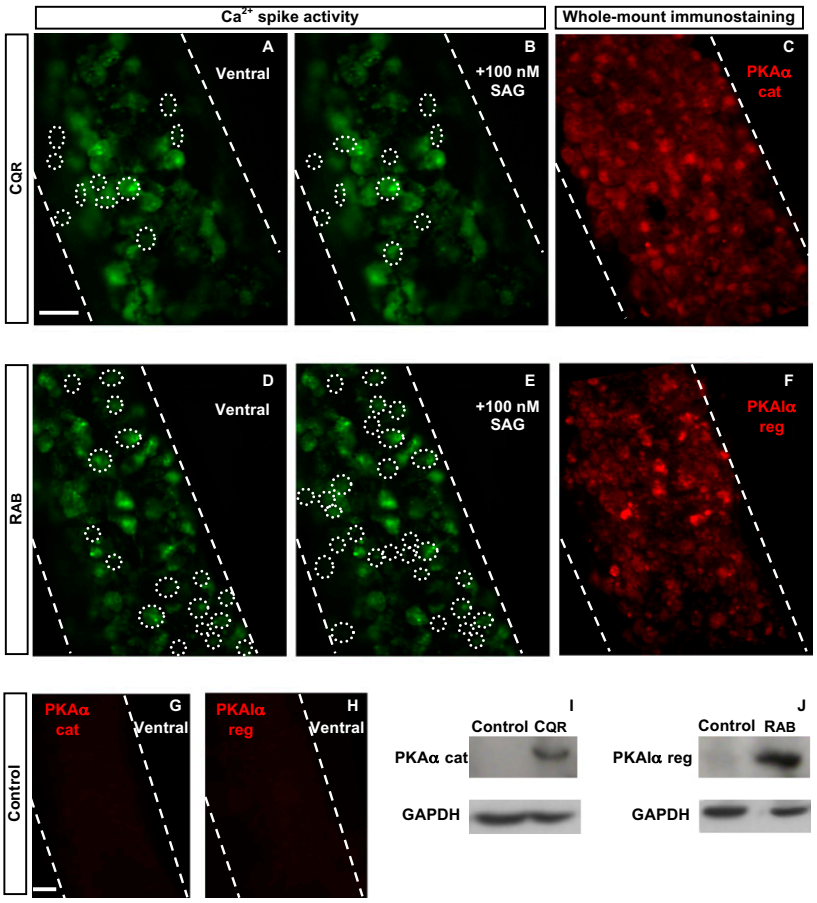

Fig. S3. Overexpression of dominant negative and constitutively active forms of PKA in developing embryos. (*A*–*F*) Four nanoliters of 250 ng/μL mRNA encoding constitutively active (CQR) (*A*–*C*) or dominant negative (RAB) (*D*–*F*) forms of PKA along with 20 mg/mL dextran-Alexa Fluor 594 conjugate were microinjected and electroporated into neural tubes of stage-19 embryos. Ca^{2+} spike activity (*A*, *B*, *D*, and *E*) was assessed 6 h after electroporation followed by whole-mount immunostaining for PKAα catalytic subunit (*C*) and PKAIα regulatory subunit (*F*). Outlined cells exhibited Ca^{2+} spikes during 30-min recordings, before (*A* and *D*) and after (*B* and *E*) the addition of 100 nM SAG. (*G* and *H*) Whole-mount immunostaining of tracer-only electroporated embryos for PKAα catalytic subunit (*G*) and PKAIα regulatory subunit (*H*). (Scale bars, 20 μm.) (*I* and *J*) Protein extracts from 10 neural tubes from CQR-, RAB-, and tracer only-(Control) injected stage-24 embryos were processed for Western blots.

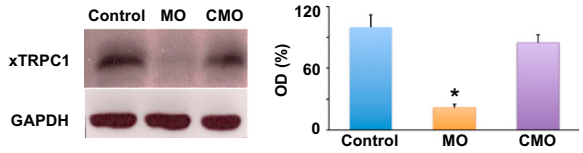

Fig. S4. Molecular knockdown of TRPC1 expression in developing embryos. Embryos at the two-cell stage were microinjected with 100 pg *x*TRPC1 morpholino (MO) or control morpholino (CMO) along with 20 mg/mL dextran-Alexa Fluor 594 conjugate. Protein extracts from 10 neural tubes of stage-24 (26-h-old) embryos injected with MO, CMO, or tracer only (Control) were processed for Western blots, and membranes were probed with anti-*x*TRPC1 and anti-GAPDH. Graph represents mean ± SEM percent of normalized OD from three experiments; $*P \leq 0.0001$.

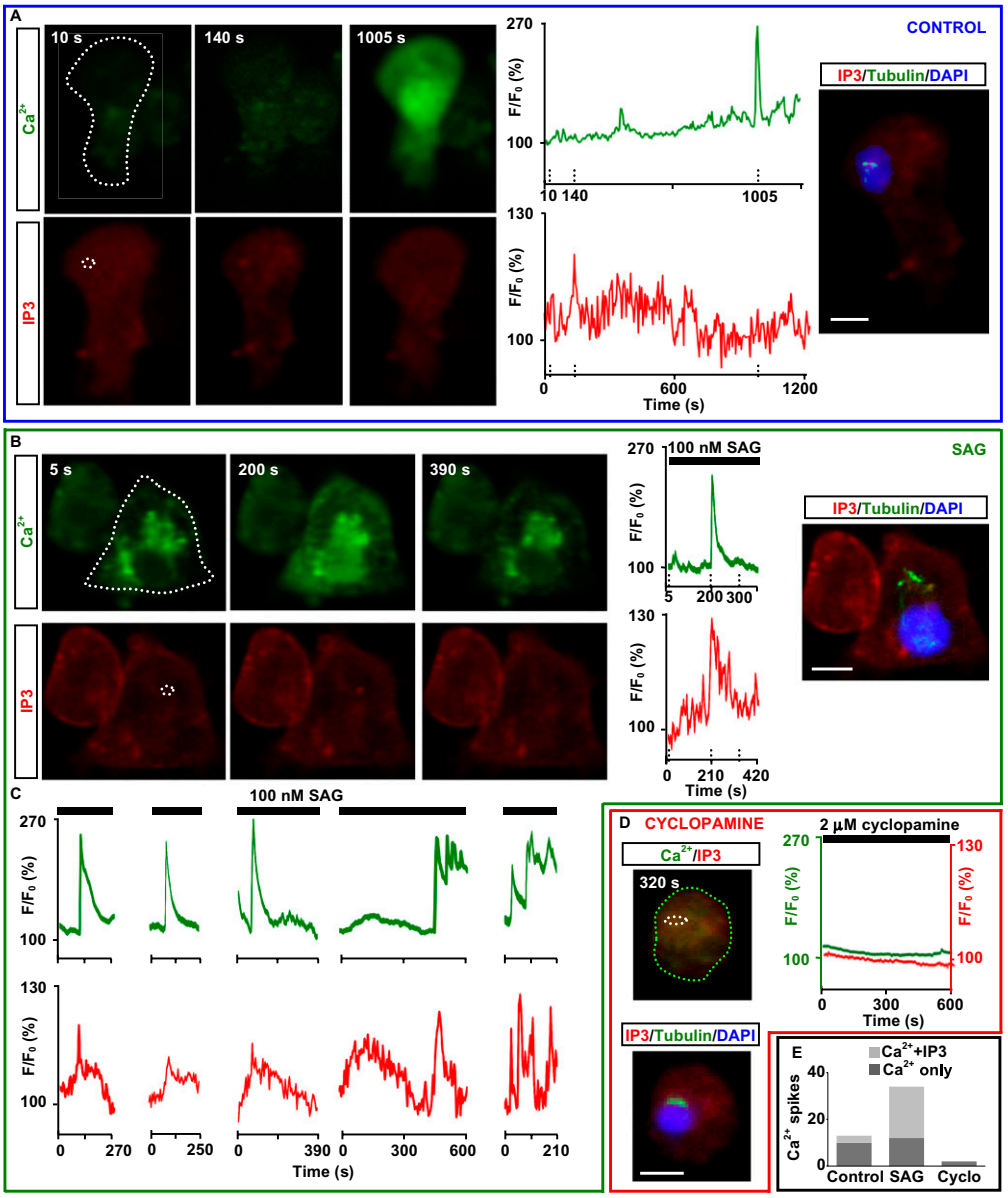

Fig. S5. The incidence of concurrence between IP3 transients at the primary cilium and Ca^{2+} spikes is highest when Shh signaling is enhanced. Neuronal cultures derived from embryos overexpressing Smo and mRFP-PHPLC grown for 7 h were Ca^{2+} and IP3 imaged in the presence of vehicle only (blue box) (*A*), 100 nM SAG (green box) (*B* and *C*), or 2 μM cyclopamine (red box) (*D*). Images in control and SAG correspond to the time points indicated by vertical dashed lines in traces. The traces represent the changes in fluorescence intensity for Ca^{2+} and IP3 probes in regions of interest indicated in *Upper Left A*, *B*, and *D*. (*C*) Traces for additional examples of correlated Ca^{2+} spikes and focal IP3 transients in the presence of SAG. *A* and *B Right* and *D Lower* show the same cell stained with DAPI (blue) and anti-acetylated tubulin (green) and overlapped with IP3 image (red), corresponding to the peak of the transient in *A* and *B* and to the 320 s-image in *D*. (Scale bars, 10 μm.) (*E*) Number of Ca^{2+} spikes concurrent (light gray) or not concurrent (dark gray) with IP3 transients at the primary cilium in the presence of 100 nM SAG, SAG+2 μM cyclopamine (Cyclo), or vehicle only (Control). $n \geq 10$ cells per condition.

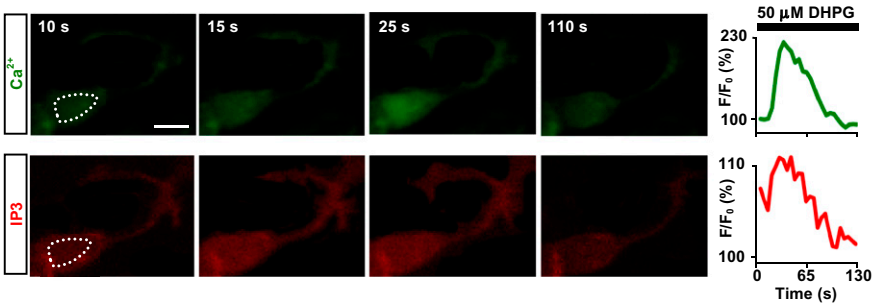

Fig. S6. Group I metabotropic glutamate receptor (mGluR) agonist elicits global IP3 transients correlated with Ca^{2+} transients. Cells were cultured for 9 h followed by simultaneous Ca^{2+} (*Upper*) and IP3 (*Lower*) imaging in the presence of 50 μM dihydroxyphenylglycol (DHPG) applied at 10 s. Results reveal global IP3 elevation throughout the cytosol that precedes the Ca^{2+} transient by several seconds. $n = 5$. (Scale bar, 10 μm.)

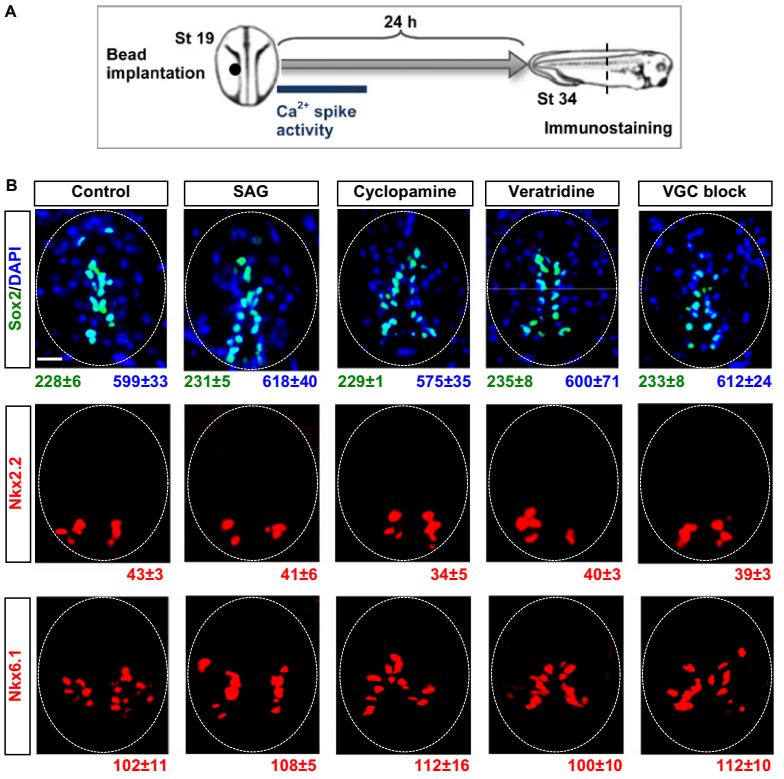

Fig. S7. Total number of cells and the number of progenitors are not affected by Ca^{2+} spike activity or Shh signaling during late stages of spinal cord development. (*A*) Diagram of the experimental design: Beads loaded with the agents indicated in *B* were implanted at stage 19. Embryos were allowed to grow for 1 d and were processed for immunostaining. (*B*) Transverse sections from stage-34 embryos; dorsal is at the top. VGC block, voltage-gated Na^+ and Ca^{2+} channel blockers. Numbers are mean ± SEM immunopositive cells or DAPI-labeled nuclei/100 μm of spinal cord. $n \geq 20$ sections/embryo, five embryos per experimental group. (Scale bar, 20 μm.)

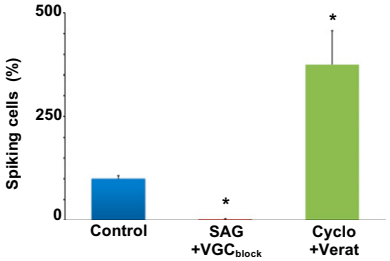

Fig. S8. Na^+ and Ca^{2+} channel modulators override Shh action on Ca^{2+} spike activity. Ventral neural tubes were exposed and loaded with fluo4-AM. Ca^{2+} imaging was done before and after the addition of the combination of 100 nM SAG and Na^+ and Ca^{2+} voltage-gated channel blockers (SAG+VGC_{block}) or with the combination of 20 μM cyclopamine and 1 μM veratridine (Cyclo+Verat). Values are the mean ± SEM percent incidence of spiking cells during 30-min recording before (Control) and after addition of indicated drugs. $n = 5$ stage-24 (26-h postfertilization) embryos per experimental group; $^*P < 0.01$.