Le Dréau et al., http://www.jcb.org/cgi/content/full/jcb.201307031/DC1

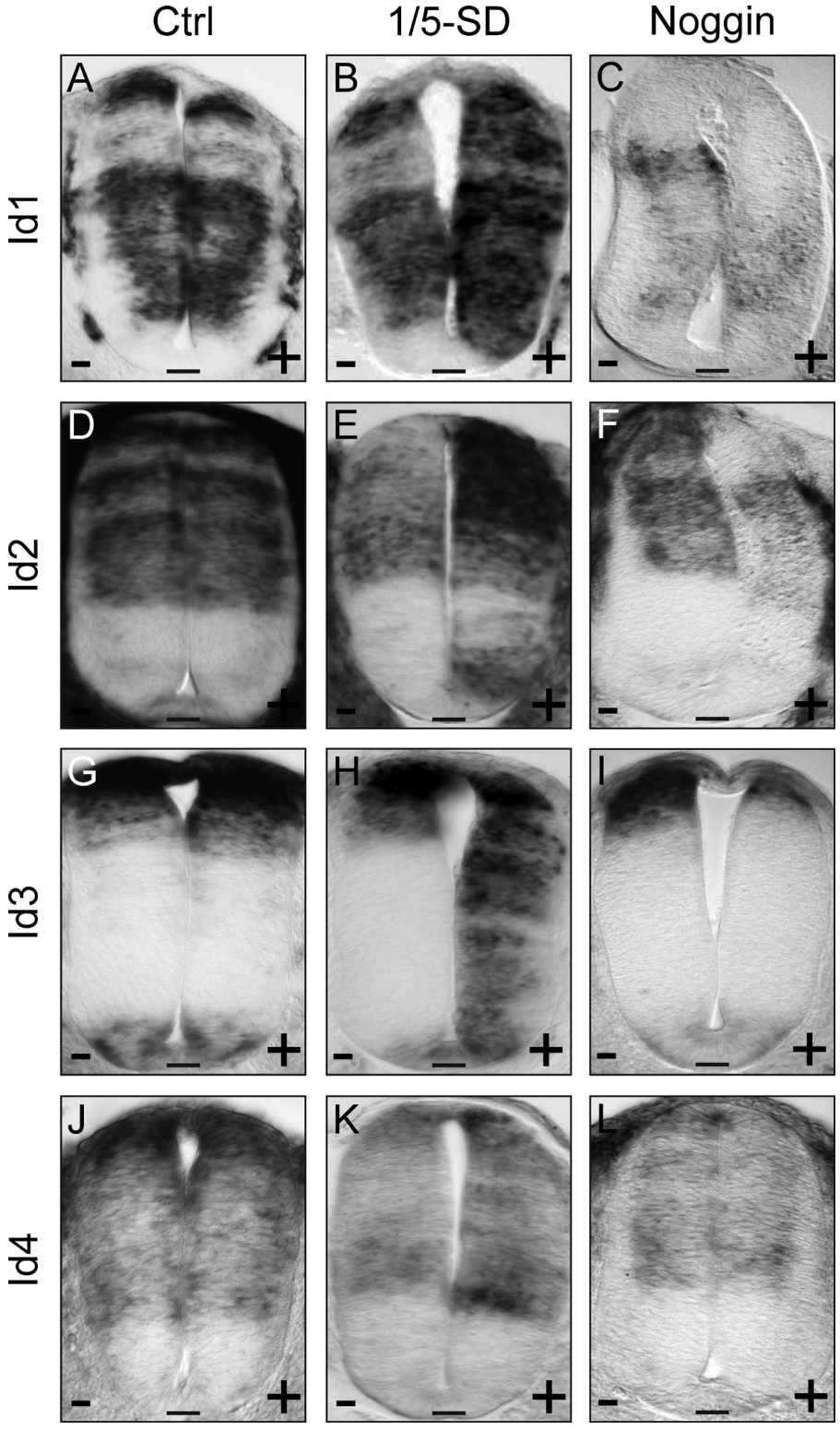


Figure S1. **Analysis of the expression of *Id* genes in response to modulations of the canonical BMP activity.** Relative to Fig. 2. (A–L) The expression of the chick *Id1* (A–C), *Id2* (D–F), *Id3* (G–I), and *Id4* (J–L) genes was assessed by in situ hybridization in sections of neural tubes recovered at 24 hpe of HH14 embryos with control constructs (A, D, G, and J) or constructs overexpressing the constitutively active mutants SMAD1/5-SD (B, E, H, and K) or the BMP antagonist Noggin (C, F, I, and L). Comparison of the signal intensities detected in the nonelectroporated (left, −) and electroporated (right, +) sides of representative neural tube sections revealed that *Id1*, *Id2*, and *Id3* mRNA levels are modulated in response to variations in canonical BMP activity. Ctrl, control. Bars, 25 µM.

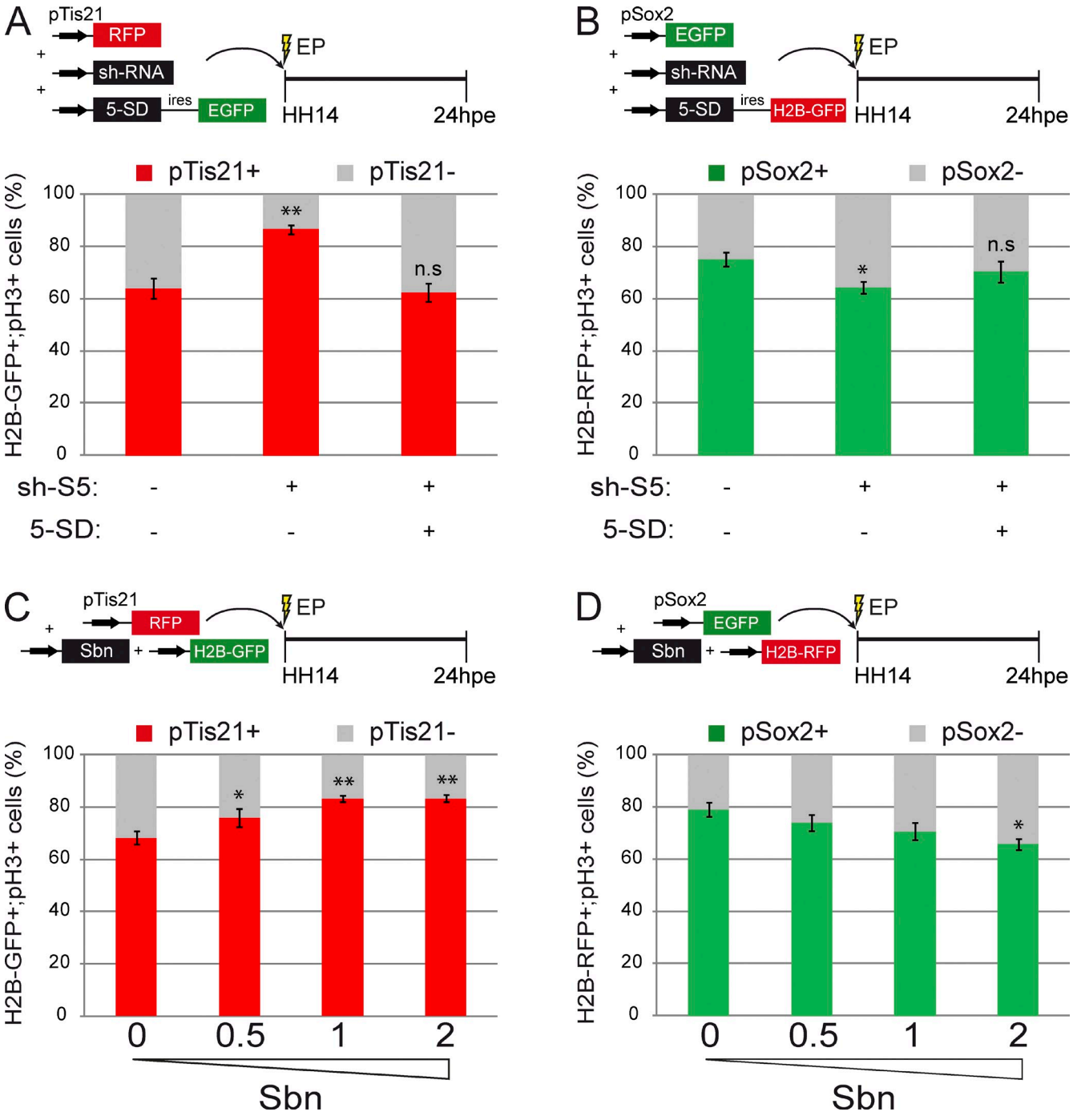


Figure S2. **Proportions of pTis21⁺ and pSox2⁺ divisions obtained in response to experiments of rescue and dose-dependent effects of SMAD1/5 inhibition.** Relative to Fig. 3. (A and B) The proportions of pTis21⁺ (A) and pSox2⁺ (B) divisions were assessed in mitotic electroporated (H2B-GFP⁺;pH3⁺ [A] or H2B-RFP⁺;pH3⁺ [B]) progenitors 24 h after coelectroporation with a control construct (Ctrl) or the Smad5 shRNA construct (sh-S5) alone or combined with the constitutively active SMAD5-SD mutant (5-SD). (C and D) The proportions of pTis21⁺ (C) and pSox2⁺ (D) divisions were assessed in mitotic electroporated (H2B-GFP⁺;pH3⁺ [C] or H2B-RFP⁺;pH3⁺ [D]) progenitors 24 h after coelectroporation with increasing concentrations of the dominant-negative Smad5 mutant Somitabun (Sbn), using combinations of 0:2, 0.5:1.5, 1:1, and 2:0 µg/µl pCS2/Somitabun and empty pCS2 constructs. EP, electroporation. Error bars show means ± SEM. *, $P < 0.05$; **, $P < 0.01$.

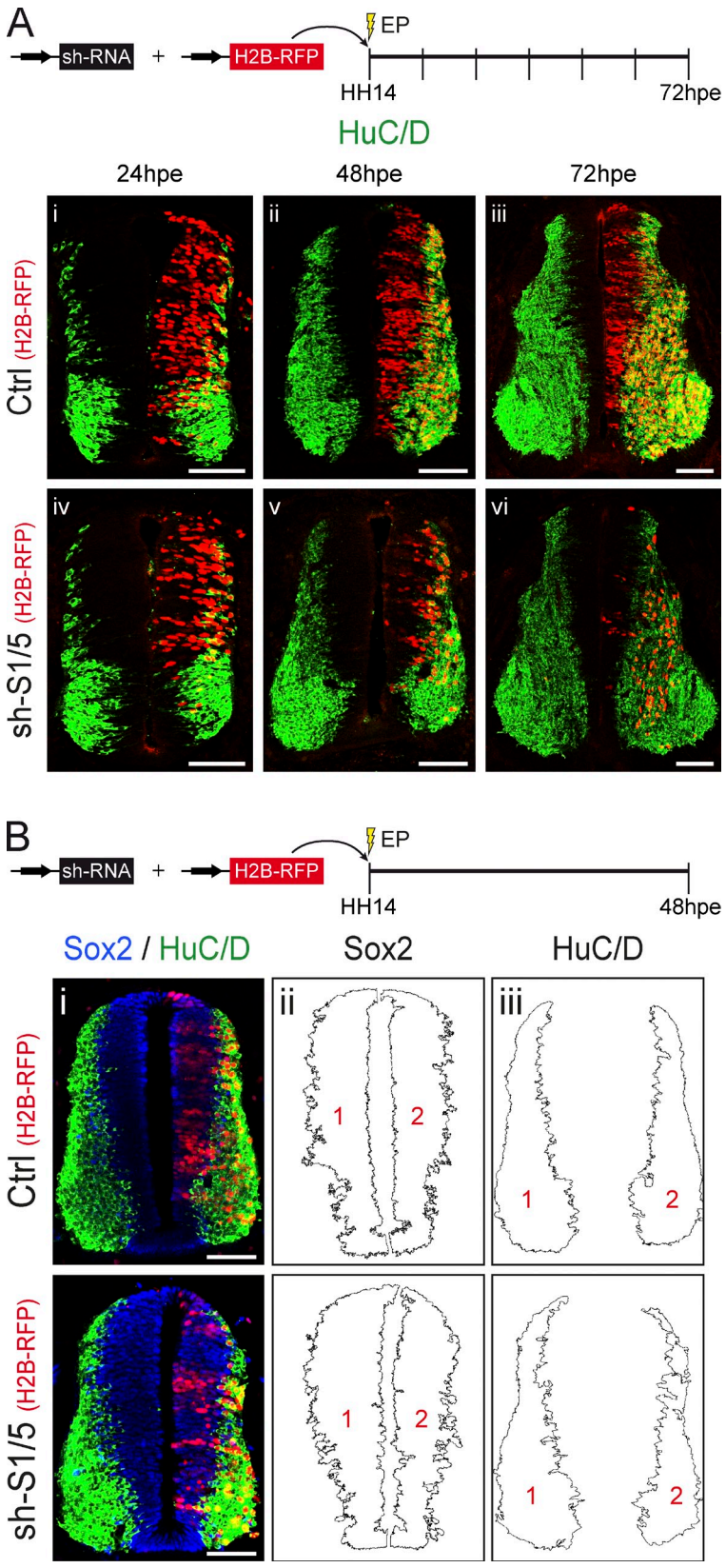
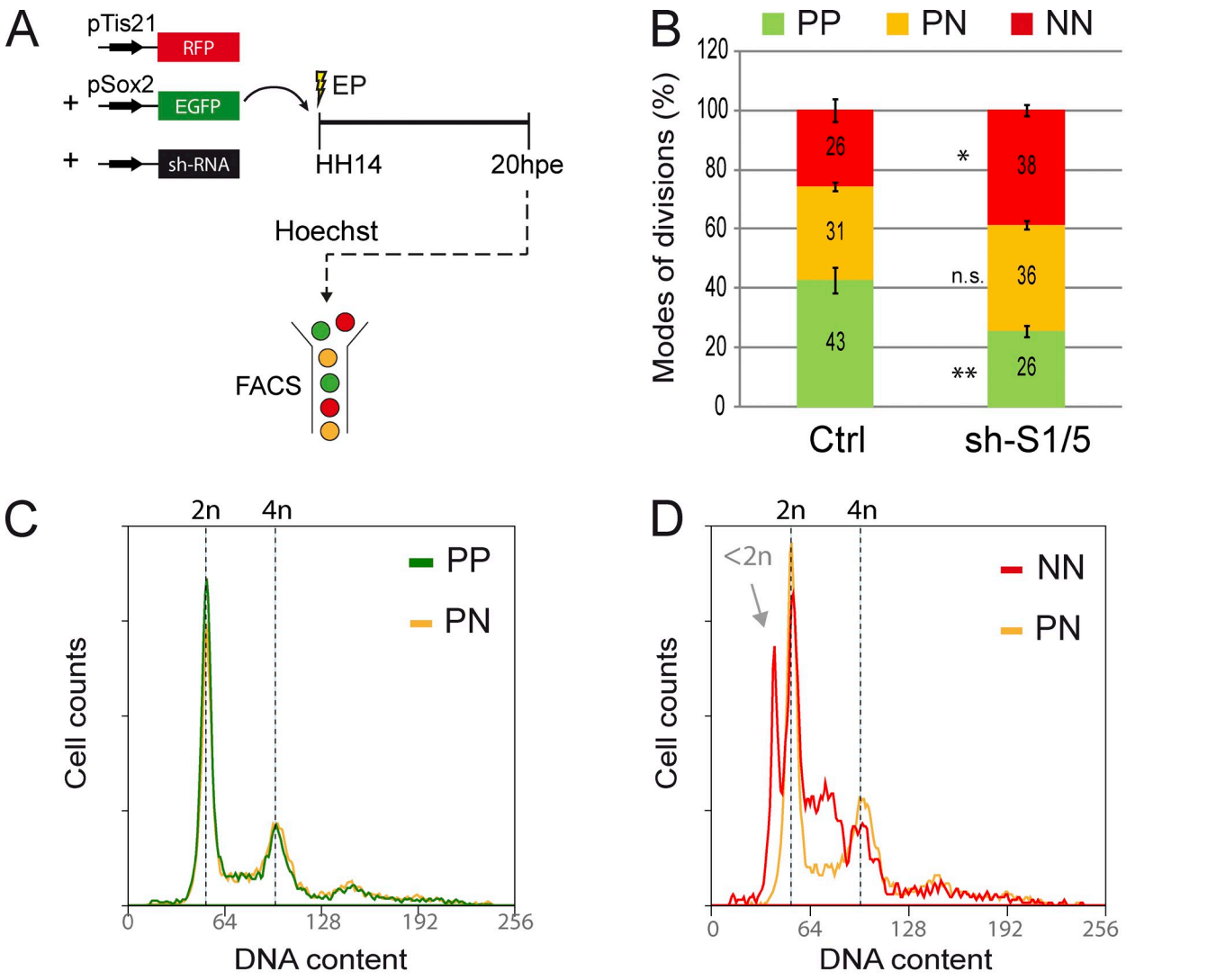


Figure S3. **Cell-autonomous and overall effects of SMAD1/5 inhibition on the neuronal differentiation of spinal progenitors.** Relative to Fig. 5. (A) Representative transverse sections obtained after electroporation of HH14 embryos with control (Ctrl, i–iii) or shRNA constructs against *cSmad1* or *cSmad5* (sh-S1/5, iv–vi) are shown 24 hpe (i and iv), 48 hpe (ii and v), and 72 hpe (iii and vi), demonstrating the premature differentiation of sh-S1/5 electroporated (H2B-RFP$^+$) cells into neurons (HuC/D$^+$). (B, i) Representative transverse sections of a chick neural tube at 48 hpe of HH14 embryos with control or sh-S1/5. Sox2, HuC/D, and H2B-RFP stain, respectively, the neural progenitors, differentiating neurons, and electroporated cells. The Sox2$^+$ (ii) and HuC/D$^+$ (iii) areas corresponding, respectively, to the VZ (containing the progenitors) and the MZ (formed by the differentiating neurons) were defined and measured using ImageJ processing. The areas measured for the electroporated side (2) were standardized to their contralateral controls (1) and are presented as ratios of the size of the VZ (Sox2$^+$) or MZ (HuC/D$^+$) areas (see Fig. 5 E). EP, electroporation. Bars, 50 µM.



E

diploid cycle — aneuploid cycle

	PP		PN		NN			NN	
(%)	Ctrl	sh-S1/5	Ctrl	sh-S1/5	Ctrl	sh-S1/5	(%)	Ctrl	sh-S1/5
G1	56 ± 1	57 ± 1	49 ± 1	51 ± 3	46 ± 3	44 ± 4	G1	36 ± 2	32 ± 3
S	29 ± 1	32 ± 2	33 ± 2	35 ± 3	38 ± 5	40 ± 6	S	60 ± 2	64 ± 2
G2-M	15 ± 2	11 ± 2	18 ± 2	14 ± 2	16 ± 2	16 ± 2	G2-M	4 ± 1	4 ± 2

Figure S4. **Analysis of the cell cycle distribution of the PP, PN, and NN progenitors.** Relative to Fig. 7. (A) Methodology used to analyze by flow cytometry the cell cycle distribution of PP, PN, and NN divisions in dissociated cells processed for Hoechst incorporation at 20 h after coelectroporation of pTis21:RFP, pSox2:EGFP, and control or Smad1/5 shRNA vectors. (B) Proportions of total $GFP^+;RFP^-$ (PP), $GFP^+;RFP^+$ (PN), and $GFP^-;RFP^+$ (NN) cells obtained at 20 hpe with control (Ctrl) or Smad1/5 shRNA (sh-S1/5). (C and D) Overlays of representative DNA content profiles obtained for $GFP^+;RFP^-$ (PP) and $GFP^+;RFP^+$ (PN; C) or $GFP^+;RFP^+$ (PN) and $GFP^-;RFP^+$ (NN; D) cells electroporated with control constructs. (E) Cell cycle distribution obtained for $GFP^+;RFP^-$ (PP), $GFP^+;RFP^+$ (PN), and both diploid and aneuploid $GFP^-;RFP^+$ (NN) cells electroporated with control or Smad1/5 shRNA (sh-S1/5). EP, electroporation. Error bars show means ± SEM. *, $P < 0.05$; **, $P < 0.01$.