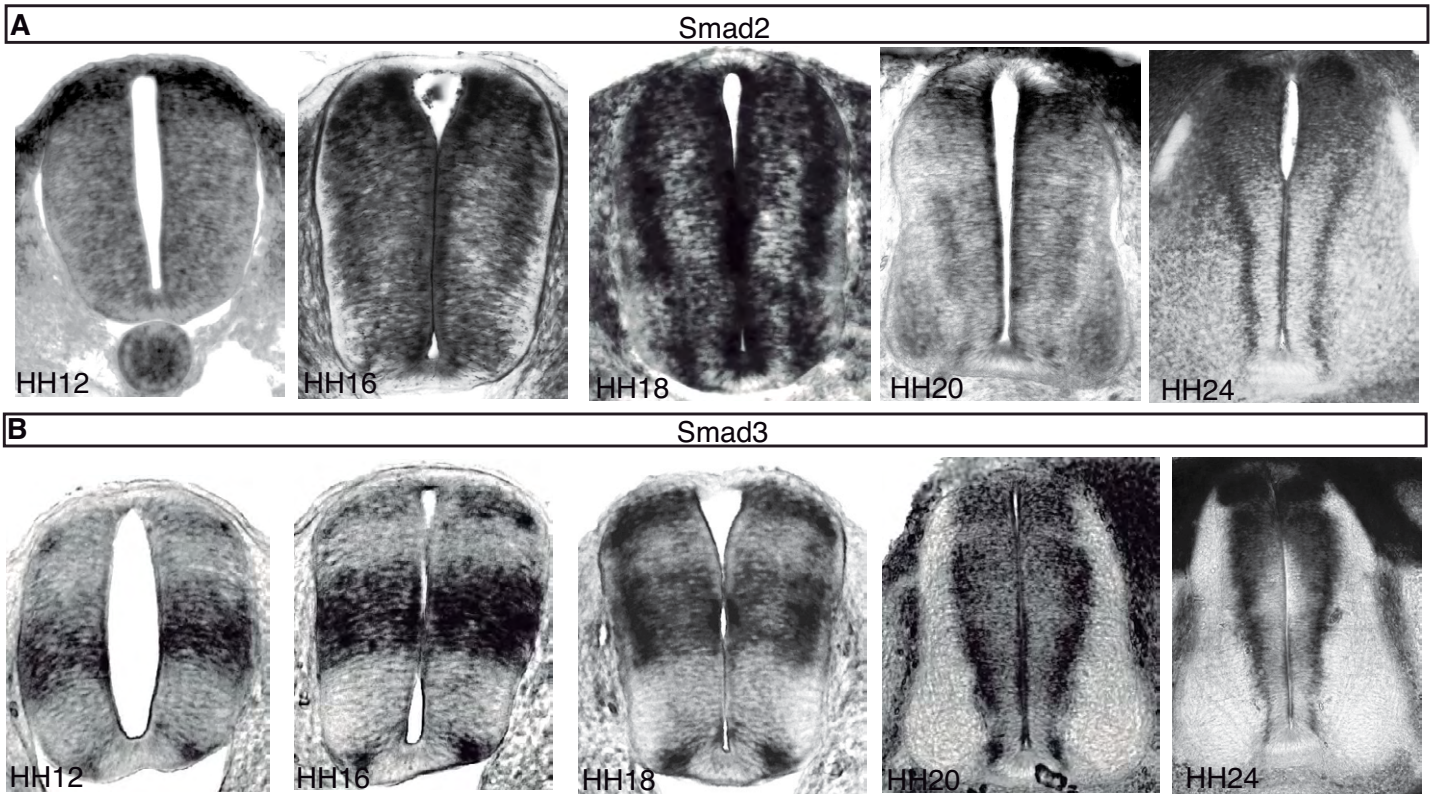

Fig. S1. Smad2 and Smad3 mRNA expression. In-situ hybridization using a Smad2 and Smad3 specific probes at the indicated developmental stages. (A) Smad2 is expressed in the ventricular zone with increased expression at the transition zone at later stages. (B) Smad3 is expressed at the intermediate NT in early stages. Three different regions of high Smad3 expression appear at later stages, as well as increased expression at the transition zone.

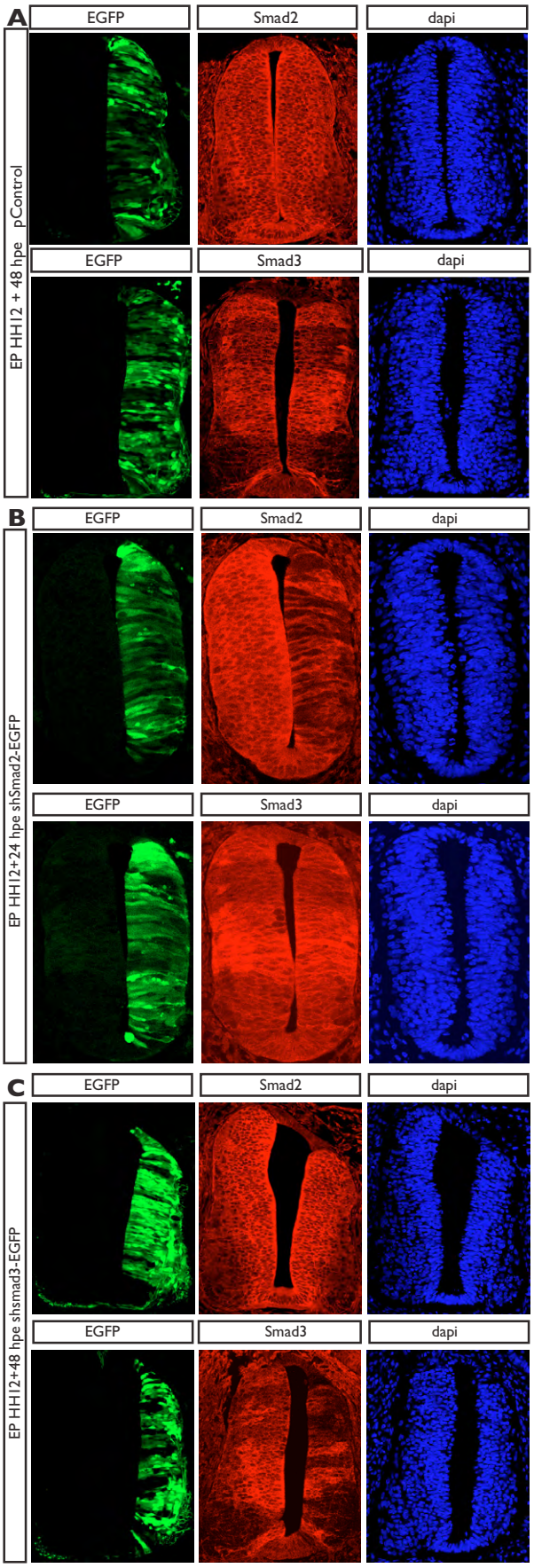

Fig. S2. shSmad2 and shSmad3 strongly reduce expression of their specific targets, without affecting the expression levels of the other R-Smad. (A) Electroporation of control, (B) Electroporation of shSmad2 showing strong reduction in Smad2 electroporated cells, while Smad3 levels remain unchanged. (C) Electroporation of shSmad3 showing strong reduction of Smad3 in electroporated cells, while Smad2 levels remain unchanged. Average pixel intensity measured 48 hpe are shown in Fig. 2I.

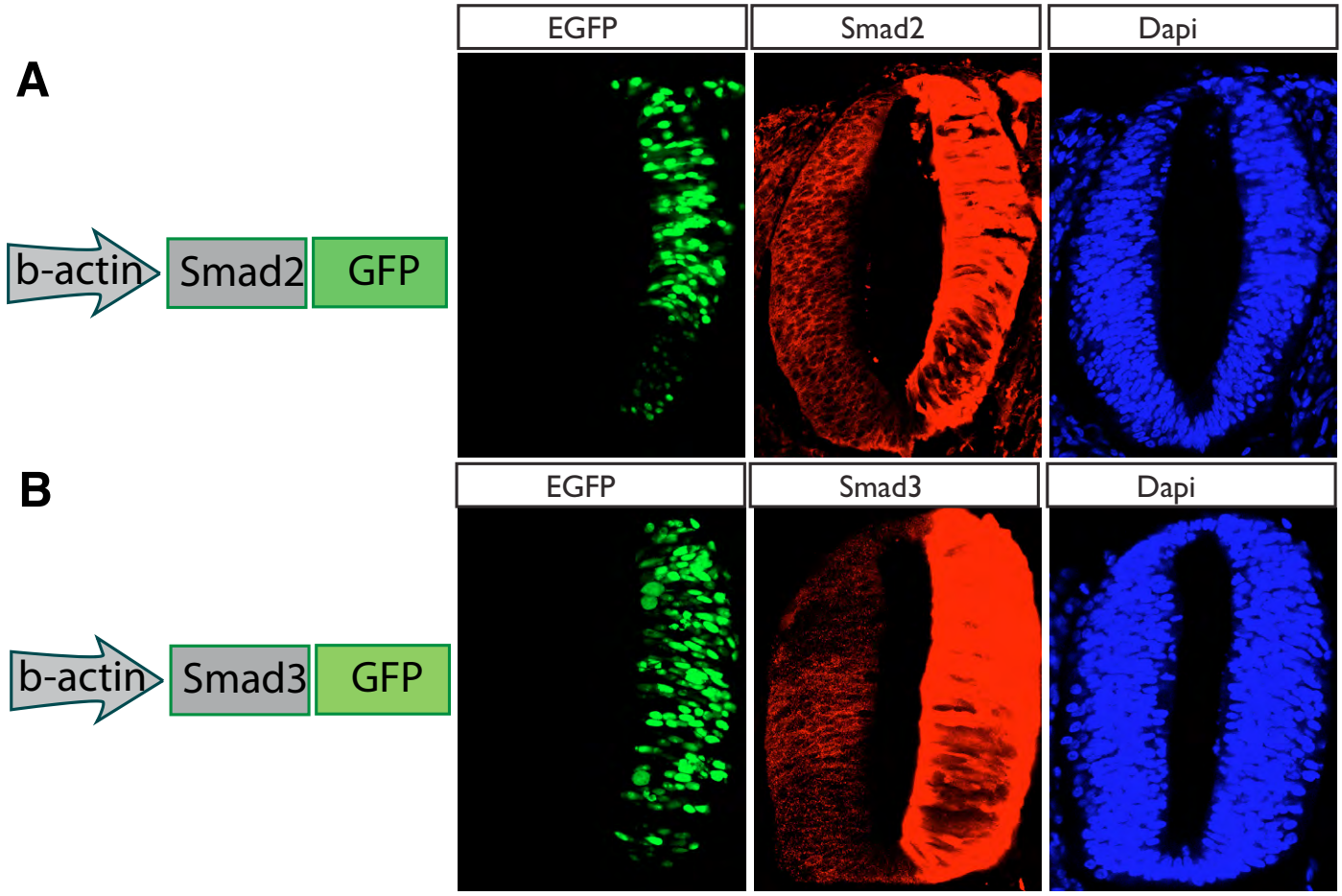

Fig. S3. Changes in Smad2 and Smad3 expression after gain-of-function experiments. (A) Immunofluorescence staining of Smad2 after electroporation of Smad2. (B) Immunofluorescence staining of Smad3 after electroporation of Smad3. Average pixel intensity measured 48 hpe are shown in Fig. 2H.

Table S1. Parameters for the Numerical Model

Parameter	Value	Units
t_{max}	60	min
$[TGF\beta R]$	149	nM
$MM_{TGF\beta R}$	1.03	nM
$[S_2]_{WT}^{t=0}$	100	nM
$[S_3]_{WT}^{t=0}$	100	nM
$[S_4]_{WT}^{t=0}$	100	nM
$[S_2]_{GOF}^{t=0}$	1216	nM
$[S_3]_{GOF}^{t=0}$	403	nM
$[S_2]_{LOF}^{t=0}$	17.4	nM
$[S_3]_{LOF}^{t=0}$	3.3	nM
k_1^+, k_2^+	3.5	s^{-1}
MM_S	1.03	nM
k_1^-, k_2^-	1	s^{-1}
k_3^+, k_4^+	0.1	$\text{nM}^{-1} s^{-1}$
k_3^-, k_4^-	1	s^{-1}
$k_5^+,$	1	$\text{nM}^{-1} s^{-1}$
k_5^-	0.1	s^{-1}
k_7^+	0.38	s^{-1}
k_8^+	19	s^{-1}