Table S1 (related to Figure 1). Hindlimb phenotypes in sibling *Shh*-CKO embryos with or without *Bax* function for different Tamoxifen administration times (Hoxb6CreER activation).

Shh-CKO;*Bak*$^{-/-}$;Hoxb6CreER+	1 digit (KO)	2 digits	3 digits	4 digits	5 digits	% rescue
tamoxifen E9.5						
Bax[+]†	12/12	0	0	0	0	0 %
Bax[-]	16/16	0	0	0	0	0 %
tamoxifen E9.5+3h (see also Fig 1)						
Bax[+]	28/28	0	0	0	0	0 %
Bax[-]	13/31	0	2/31	14/31	2/31	58 %
tamoxifen E9.5+6h (E9.75)						
Bax[+]	28/38	0	2/38	8/38	0	26 %
Bax[-]	10/32	1/32	3/32	16/32	2/32	66 %

†Bax[+] denotes *Bax*$^{C/+}$ genotype and Bax[-] denotes *Bax*$^{C/C}$ genotype, which becomes negative following Cre recombination.

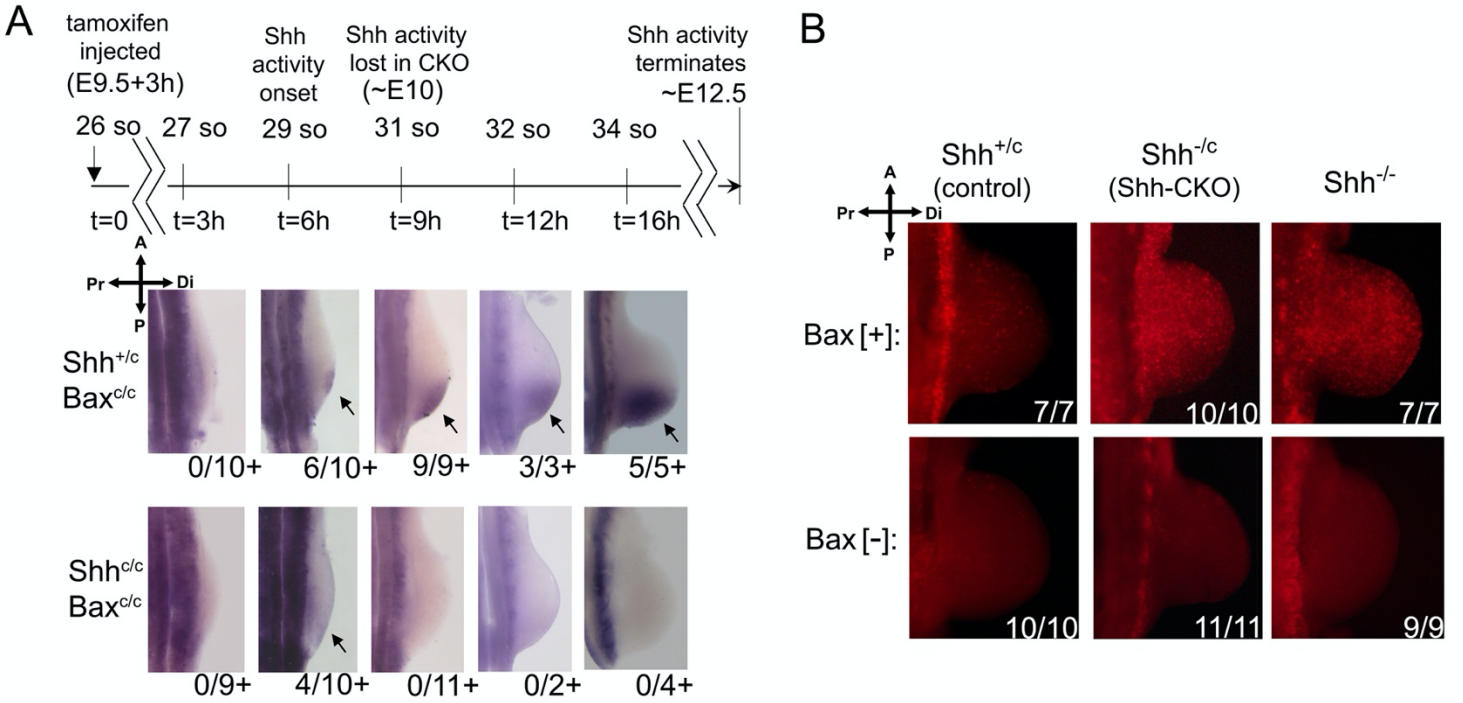

Figure S1 (related to Figure 1). Duration of Shh activity and enforced cell survival in *Shh*-CKO;*Bax*-CKO embryos. (A) Assay of Shh activity (direct response) by *Gli1* RNA expression after tamoxifen treatment (at times post-treatment indicated by timeline) in control (*Shh*$^{+/c}$;*Bax*-CKO, upper panels) and in *Shh*-CKO;*Bax*-CKO hindlimb buds (lower panels). Note that Shh expression initiates at about 6-8 hrs after the time of tamoxifen injection (at 29 so). No activity is detected in either control (n=10) or *Shh*-CKO;*Bax*-CKO (n=9) hindlimbs at 3h after tamoxifen dosage. Shh activity was first detected at 6h after tamoxifen injection (29 so) in a subset of control (6/10, arrow) and *Shh*-CKO;*Bax*-CKO (4/10, arrow) hindlimb buds, and became consistent and strong by 9h (n=9) and later (arrows) in control (31 so), but was absent in all *Shh*-CKO;*Bax*-CKO embryos (n=11) at 9h and later. Limb buds in all panels oriented with anterior at top and distal at right. so, somite. **(B)** Lysotracker staining for cell death at E10.75, following *Bax/Bak* and *Shh* removal with tamoxifen at E9.5+3h (as in panel **A**). In control hindlimbs with *Bax/Bak* function present (Bax [+], *Bax*$^{+/C}$; upper panels) all *Shh*-CKO embryos (10/10) have extensive anterior apoptosis at the same level as *Shh* null mutant (*Shh*$^{-/-}$, n=7). In hindlimbs with *Bax/Bak* function deleted (Bax [-], *Bax*$^{C/C}$; lower panels), no apoptosis is detected in either *Shh*-CKO (11/11) or in *Shh*$^{-/-}$ null (9/9) hindlimb buds. Note that *Hoxb6CreER* is not expressed in somites(Nguyen *et al.*, 2009), where apoptosis remains present. Compass indicates limb bud orientation in all panels.

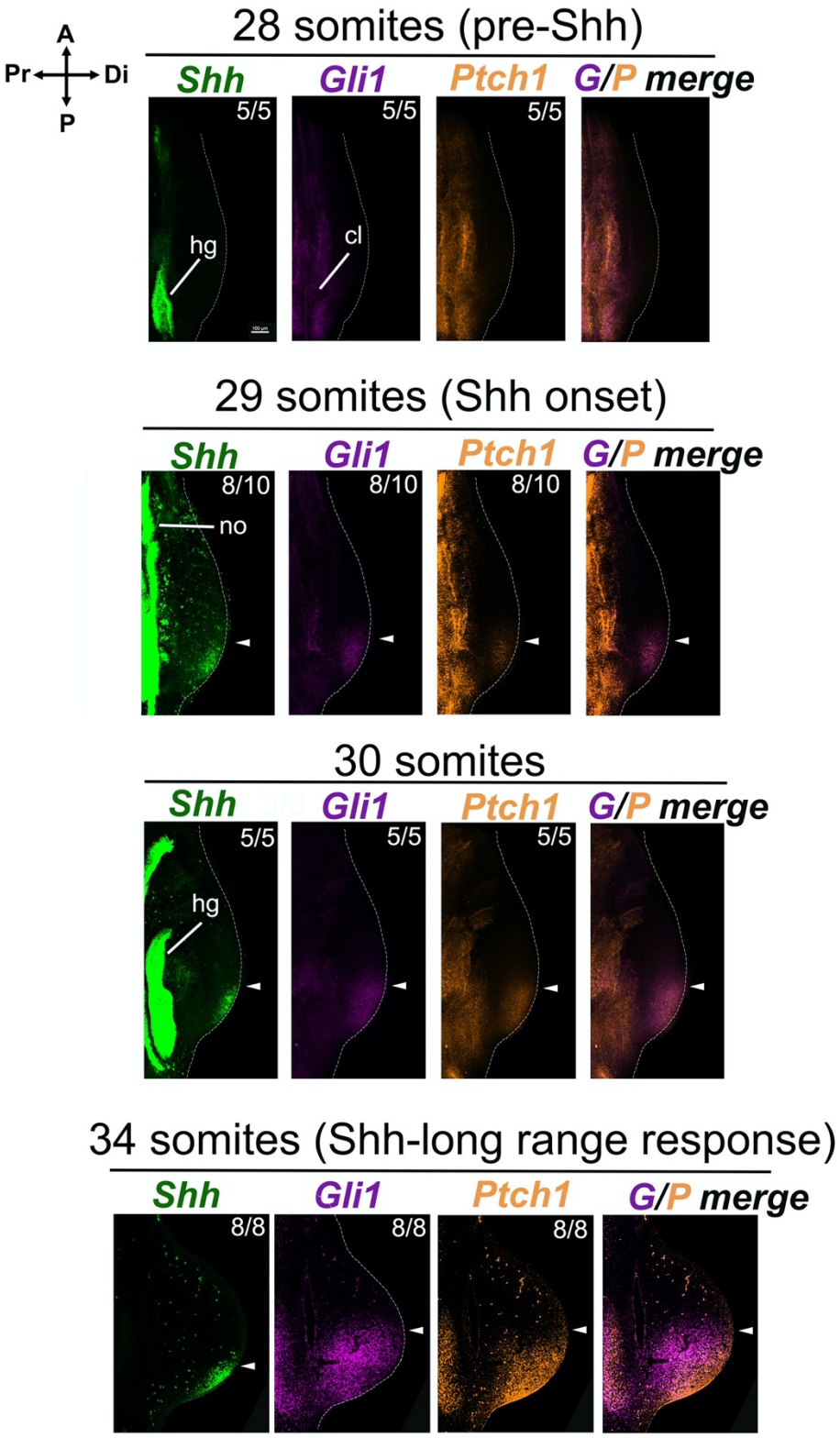

Figure S2 (related to Figure 3). A-P extent of Shh response is coincident using either Gli1, or Ptch1 RNA reporters at early stages spanning and following Shh expression onset.

Shh expression and activity was assayed by *Shh* (green), *Gli1* (purple), and *Ptch1* (orange) RNA in situ HCR(Choi *et al.*, 2018) at somite stages indicated, in normal "control" embryos ($Shh^{+/-}$), but

with *Bax/Bak* alleles deleted (*Bax*-CKO) by tamoxifen injection at E9.5+3h (as in Figures 1B,C; 3). *Shh* RNA and response (*Gli1, Ptch1*) were first detected at the 29 somite stage (*Shh* expression onset), in a substantial subset (8/10); the remainder (2/10) were negative for all probes. By 30 somites (2h later), hindlimb Shh expression and response became robust in all embryos (5/5). The A-P extent of Shh, *Gli1,* and *Ptch1* RNAs were all very similar at these stages (29-30 somites; merged panels, and arrows). Later at the 34 somite stage, when lineage tracing reveals long-range Shh activity (see Figure 2), both *Gli1* and *Ptch1* RNA extend beyond *Shh* RNA (ZPA) to a comparable anterior A-P level (arrows). Numbers analyzed with result shown are indicated at top of each panel, with the remainder negative for expression. no, notochord and hg, hindgut axial sources of Hh ligands and cl, cloacal-urogenital region, also responsive to local Shh and Ihh signaling (Haraguchi *et al.*, 2007; Perriton *et al.*, 2002). Scale bar = 100 μm (top left panel).

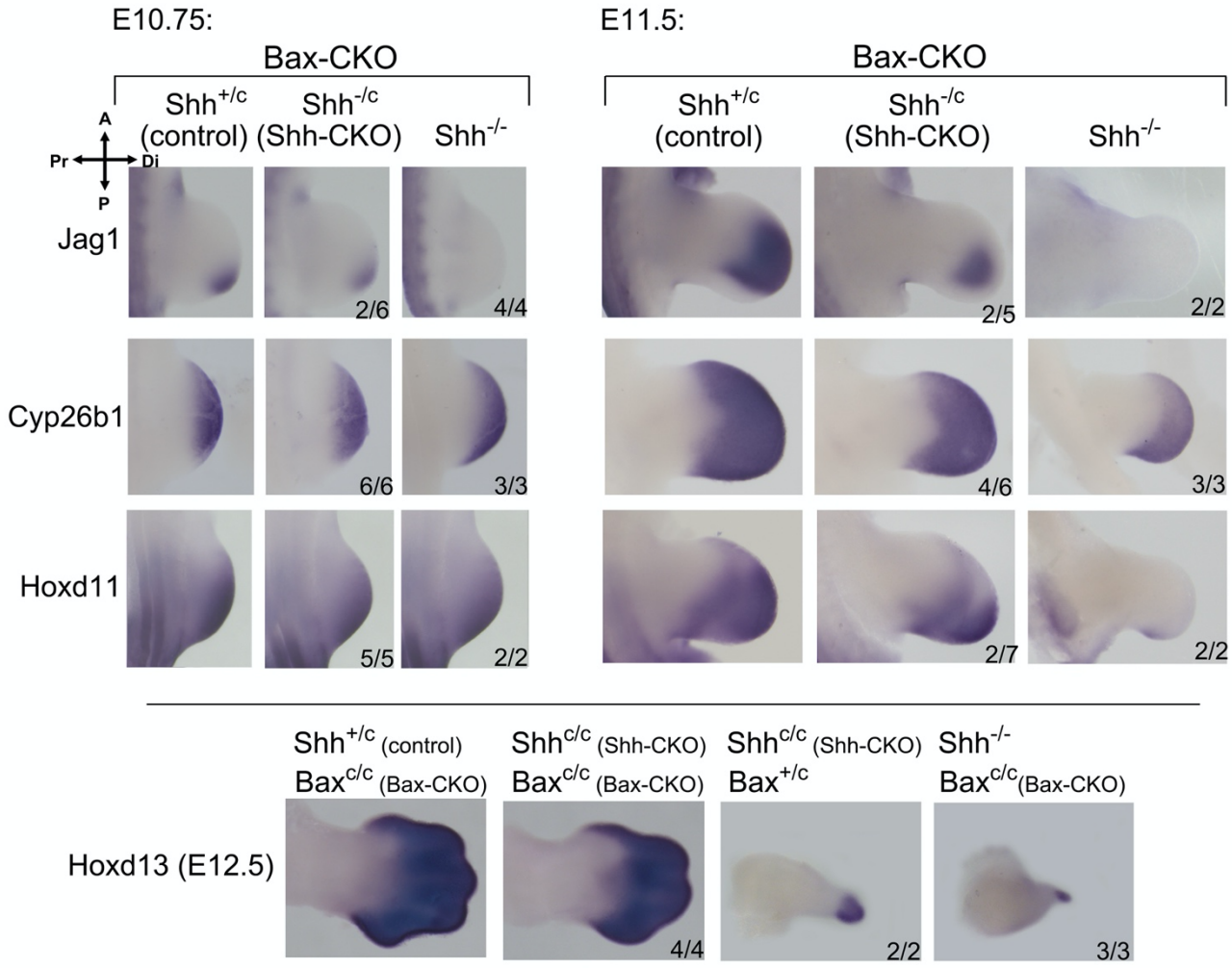

Figure S3 (related to Figure 5). Expression of Shh target genes implicated in limb bud outgrowth and digit patterning is maintained in *Shh*-CKO;*Bax*-CKO. *Jag1*, *Cyp26b1*, and *Hoxd11* expression at E10.75 and E11.5 are sustained in a subset (about 50%) of *Shh*-CKO;*Bax*-CKO hindlimbs. In null $Shh^{-/-}$;*Bax*-CKO limbs, *Cyp26b1* and *Hoxd11* expression were preserved at E10.75, but markedly reduced or lost by E11.5. Mutant numbers analyzed with the result shown are indicated in each panel. In remaining *Shh*-CKO;*Bax*-CKO embryos, expression was unchanged from $Shh^{-/-}$;*Bax*-CKO. The lower set of panels show *Hoxd13* expression at E12.5, which is maintained in *Shh*-CKO;*Bax*-CKO with phenotypic rescue of footplate (4/4), and in the digit rudiment of litter mate *Shh*-CKO;$Bax^{+/-}$ hindlimbs (2/2) similarly to the null $Shh^{-/-}$;*Bax*-CKO. Compass indicates limb bud orientation in all panels.

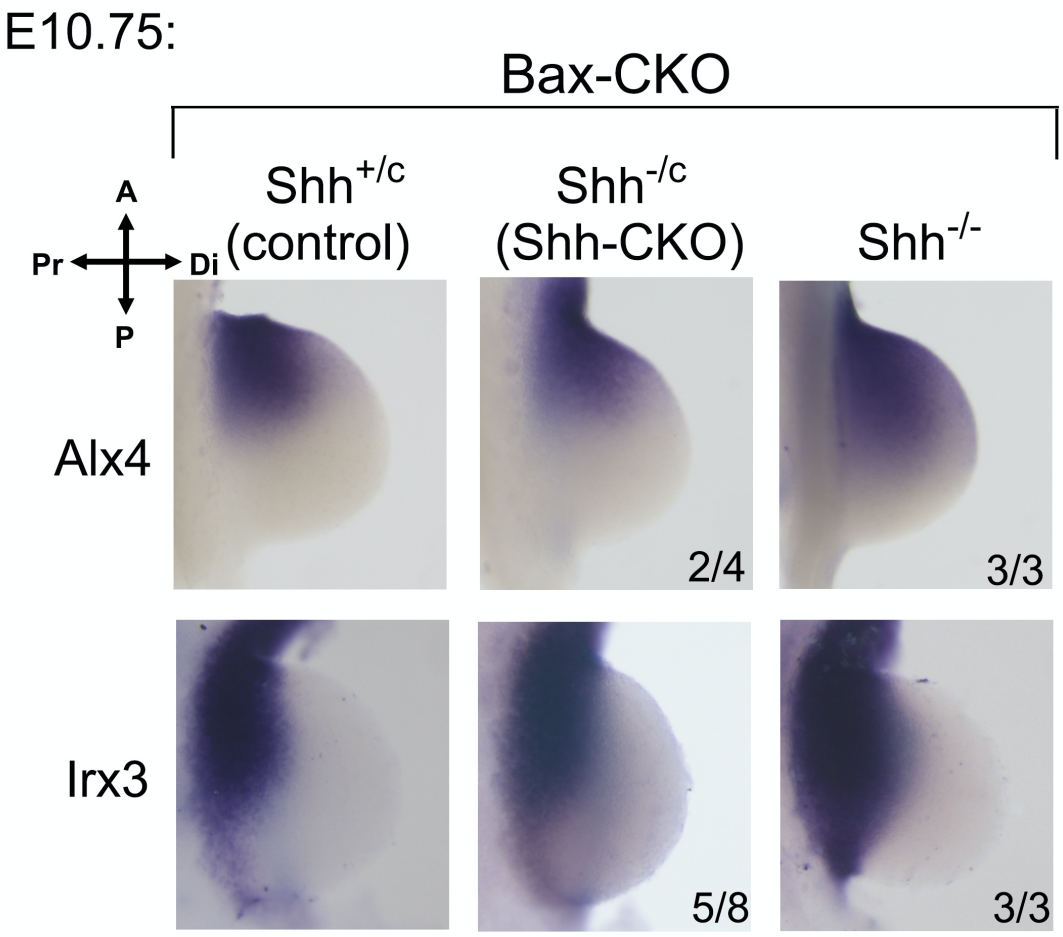

Figure S4 (related to Figure 5). Expression of anterior limb bud patterning regulators Alx4 and Irx3 is maintained in *Shh*-CKO;*Bax*-CKO. *Alx4* and *Irx3* expression at E10.75 remain anteriorly restricted similar to control limb buds in a subset (about 50%) of *Shh*-CKO;*Bax*-CKO hindlimbs. In contrast, in null $Shh^{-/-}$;*Bax*-CKO limbs, *Alx4* and *Irx3* expression had already extended into the posterior limb bud by E10.75. Mutant numbers analyzed with the result shown are indicated in each panel. In remaining *Shh*-CKO;*Bax*-CKO embryos, expression was very similar to the null $Shh^{-/-}$;*Bax*-CKO. Compass indicates limb bud orientation in all panels.

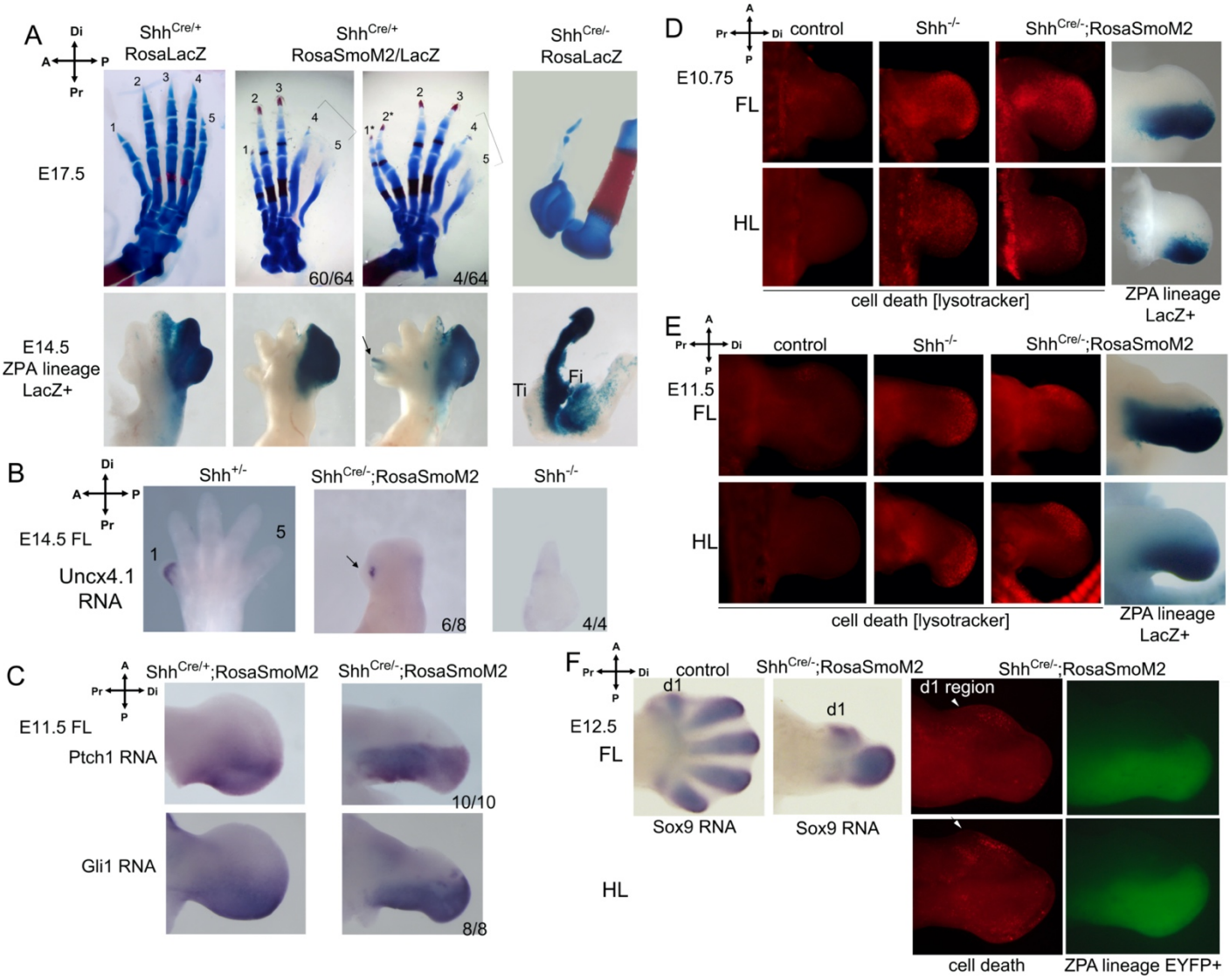

Figure S5 (related to Figure 6). A bona fide digit 1 is restored in *Shh*$^{cre/-}$;Shh-SmoM2+ limbs and is not a consequence of cryptic anterior Hedgehog ligand/pathway activation. For (**A)** - (**B),** all limbs oriented with anterior (digit 1, tibia) at left, distal at top of panel. **(A)** Skeletal staining (E17.5) and ZPA lineage tracing (E14.5) of control *Shh*$^{cre/+}$;Shh-SmoM2 (*Shh*+) embryos. In control *Shh*$^{cre/+}$;Shh-SmoM2+, posterior digits are dysmorphic and uninterpretable because of constitutive Hedgehog pathway activation (brackets in middle 2 panels), and a small percentage of limbs have preaxial polydactyly (*, 4/64), related to ectopic anterior Shh activation revealed by ZPA lineage tracing at E14.5 (LacZ+, arrow). The single digit in *Shh* null arises entirely from ZPA descendants (right panels). Ti: tibia; Fi: fibula. **(B)** *Uncx4.1* RNA expression in E14.5 forelimbs. *Uncx4.1* is expressed exclusively in digit 1 in control forelimbs (*Shh*$^{+/-}$; left panel), and is also expressed in the rescued digit 1 in *Shh*$^{cre/-}$;Shh-SmoM2+ (6/8; middle panel, arrow), but is not

detected in *Shh*$^{-/-}$ limbs (n=4; right panel). For (**C**) - (**F**), all limb buds are oriented with anterior at top, distal at right of panel. **(C)** *Ptch1* and *Gli1* RNA expression in E11.5 forelimbs. *Ptch1* and *Gli1* are expressed in the posterior limb buds in *Shh*$^{cre/-}$;Shh-SmoM2+, consistent with cell-autonomous Shh pathway activation in ZPA domain, but no expression is detected in the anterior limb bud (n=10, *Ptch1*; and n=8, *Gli1*). **(D, E)** Lysotracker staining (for cell death) and LacZ+ detection of ZPA lineage in *Shh*$^{cre/-}$;Shh-SmoM2+ limb buds. Apoptosis persists in anterior non-ZPA descended limb bud in *Shh*$^{cre/-}$;Shh-SmoM2+ limb buds similar to *Shh* null (*Shh*$^{-/-}$) at E10.75 (D) and E11.5 (E). **(F)** Lysotracker staining (for cell death) and EYFP+ detection of ZPA lineage in *Shh*$^{cre/-}$;Shh-SmoM2+ limb buds at E12.5 showing relationship of residual anterior apoptosis compared with early forming digit condensations visualized by Sox9 RNA in wildtype control and *Shh*$^{cre/-}$;Shh-SmoM2+. Note that digit 1 condensation position at anterior-proximal limb bud border correlates with apoptosis negative zone in the mutant limb bud at same stage (white arrowheads). *Rosa*^{LacZ} or *Rosa*^{EYFP} Cre-reporters were used to mark ZPA lineage. Compass indicates limb bud orientation in all panels.