SUPPLEMENTAL FIGURES

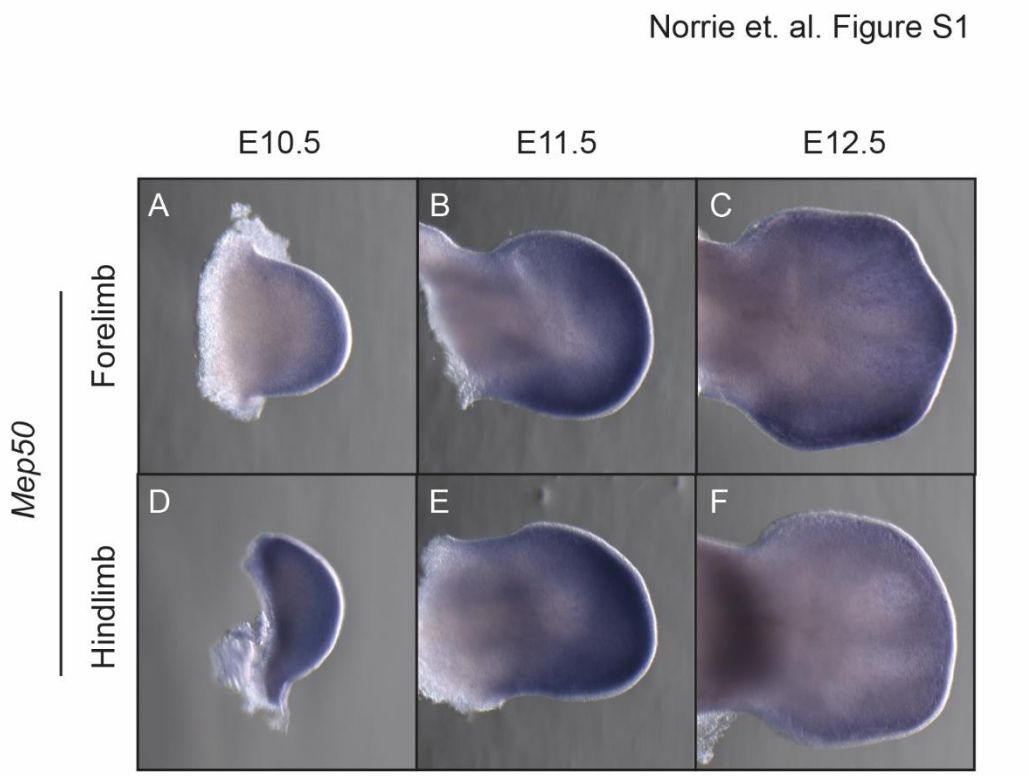

Figure S1. *Mep50* expression in forelimbs and hindlimbs. *In-situ* hybridization of *Mep50* in forelimbs (A-C) and hindlimbs (D-F) at the indicated stages.

Norrie et. al. Figure S2

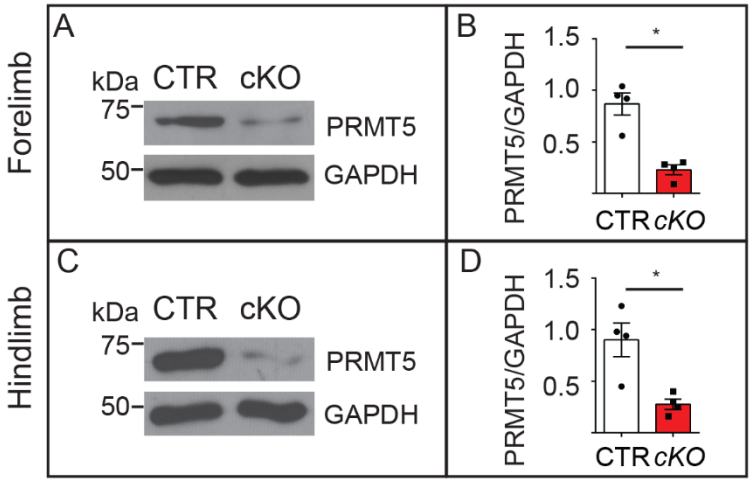

Figure S2. PRMT5 levels are reduced in *Prmt5cKO* limb buds. Western blots for PRMT5 and GAPDH on E11.5 limb lysate from control and *Prmt5cKO* forelimbs (A) and hindlimbs (C). (B, D) Quantification of band intensities (PRMT5 normalized to GAPDH). The asterisk indicates statistically significant changes ($P<0.05$) in *Prmt5cKO* compared with heterozygous or control siblings using a Student's t-test. Error bars indicate standard error of the mean.

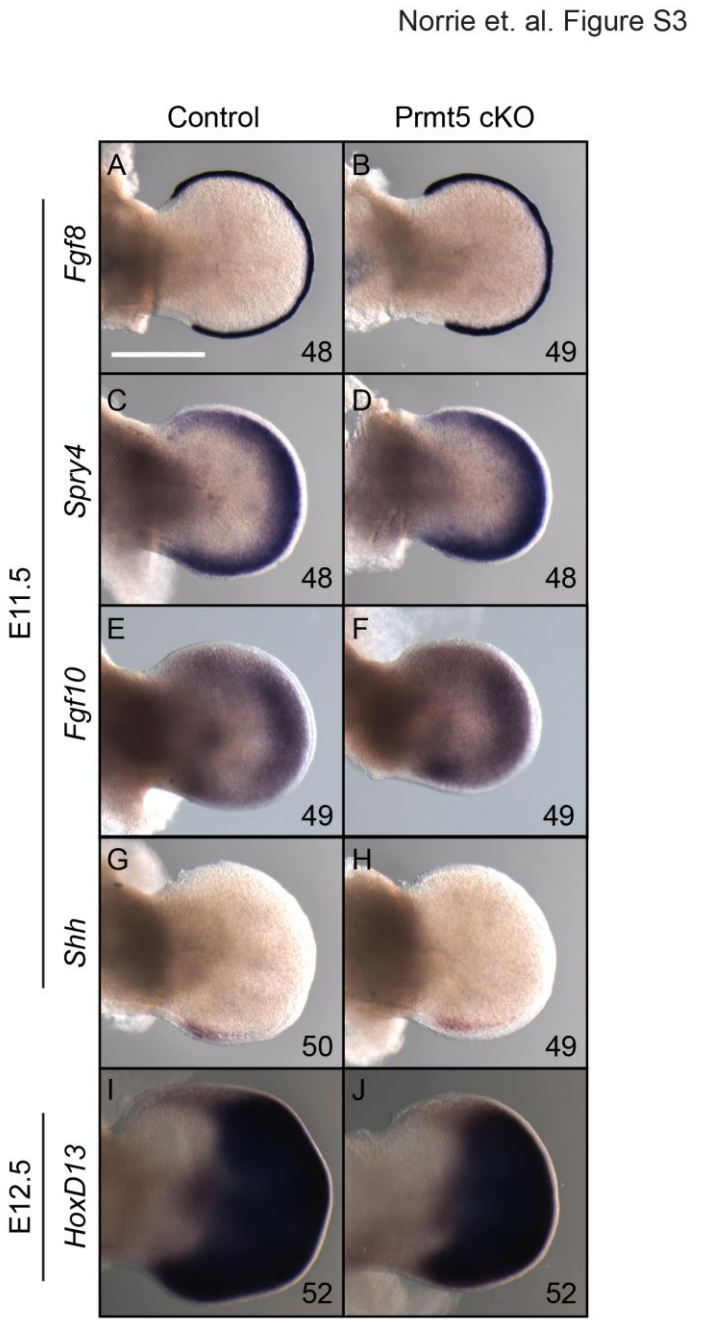

Figure S3. The SHH-FGF feedback loop is spatially unchanged. *In-situ* hybridization on E11.5 control and *Prmt5cKO* forelimbs for the indicated genes. The numbers within the panel indicate the somite stage of the embryo. Scale bar indicates 500µm.

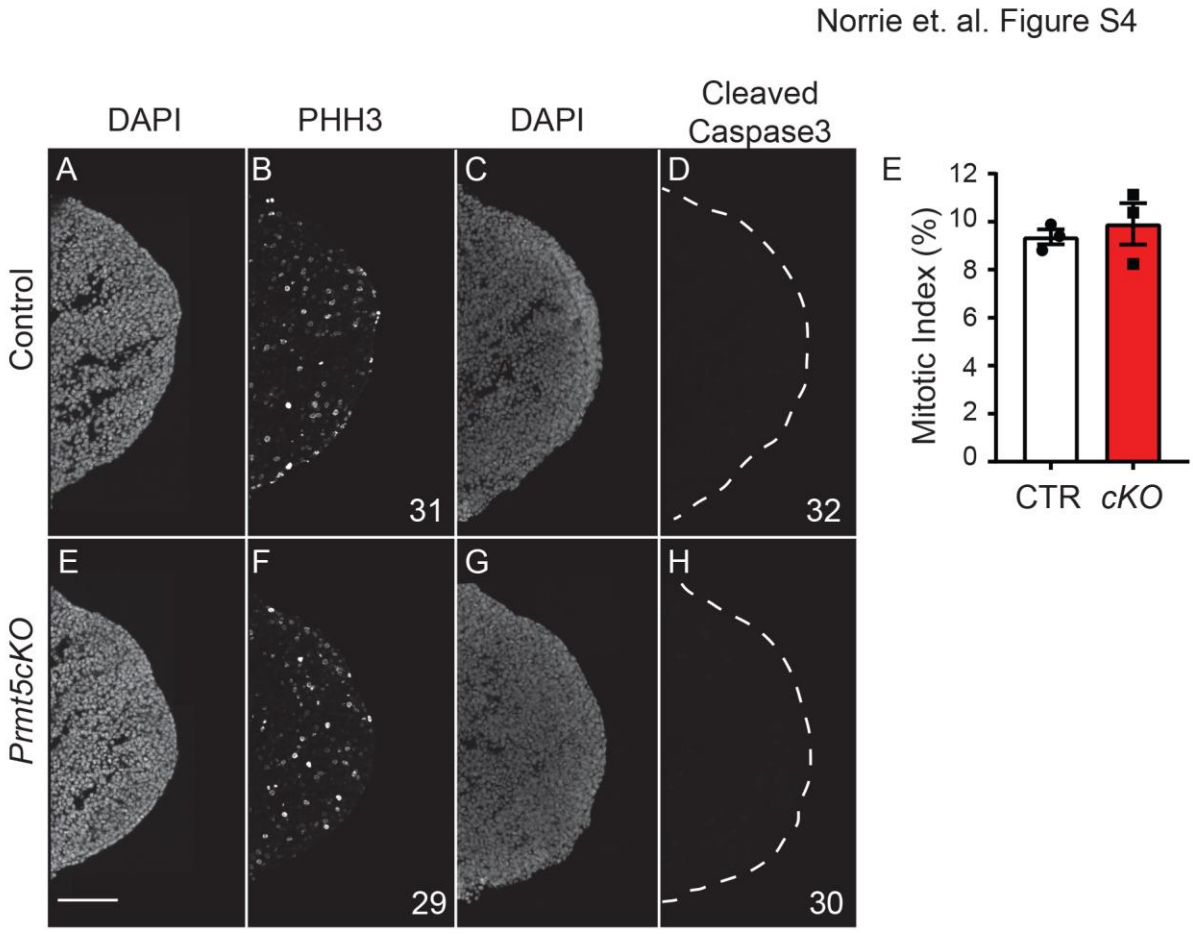

Figure S4. Proliferation and apoptosis in the *Prmt5cKO* at E10.5. Immunostaining sections from E10.5 control and *Prmt5cKO* forelimbs for PHH3 (A, B, E, F) and Cleaved Caspase 3 (C, D, G, H). Mitotic Index (E) was calculated for control (n=3) and Prmt5cKO (n=3) forelimbs. Scale bar indicates 100μm.

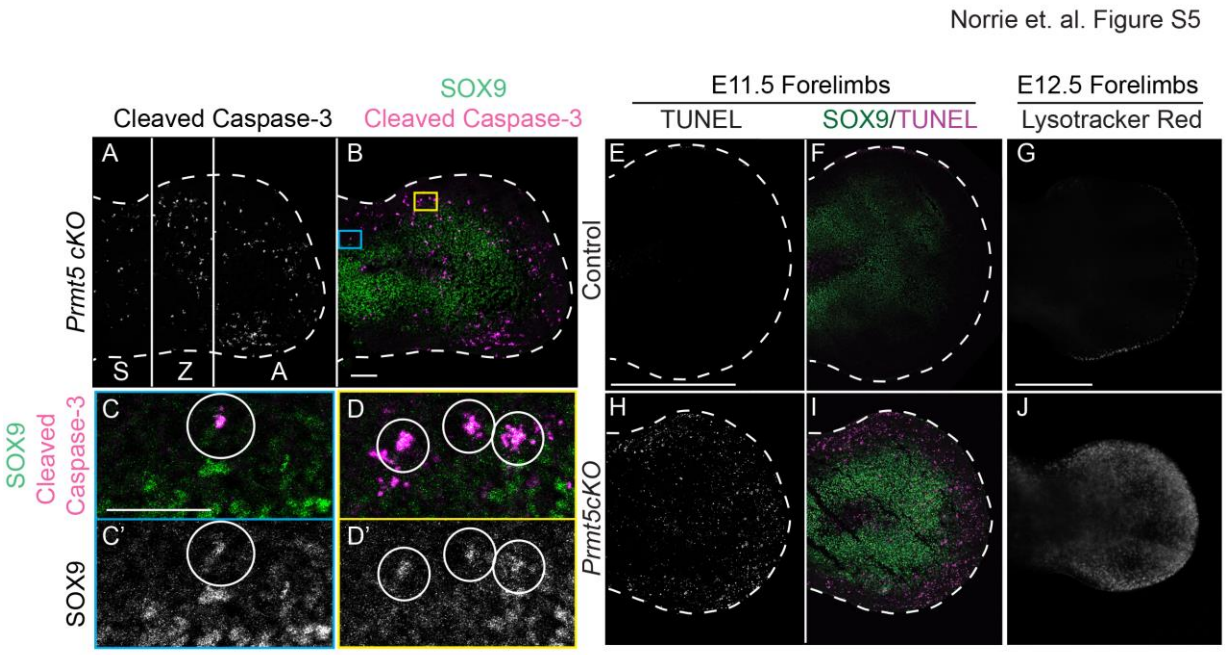

Figure S5. Apoptotic cells co-express SOX9 throughout the proximal distal axis in *Prmt5cKO* forelimbs. Cleaved Caspase-3 immunostaining in a *Prmt5cKO* showing co-expression of SOX9 in the stylopod (S), zeugopod (Z) and autopod (A) elements (A). SOX9 and Cleaved Caspase-3 are colocalized (B) with blue and yellow boxes indicating regions selected for magnified images in C, C' and D, D', respectively. Immunostaining in sections from E11.5 control and *Prmt5cKO* forelimbs for TUNEL (E, H) and TUNEL (pink) and SOX9 (green) double staining (F, I). Wholemount Lysotracker red staining of control and *Prmt5cKO* forelimbs at E12.5 (G, J). Images in panels A-D' are taken from the same section depicted in Figs. 5D, E. Scale bar indicates 100µm in B, 50 µm in C and 500 µm in E and G.

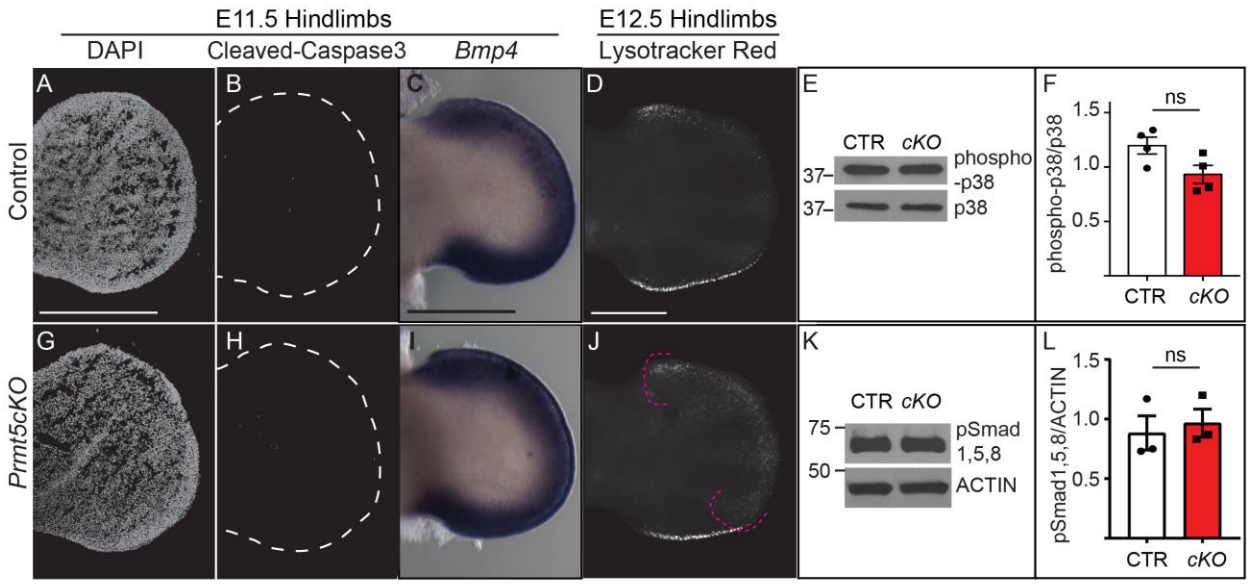

Figure S6. Apoptosis and BMP signaling in the hindlimb. Immunostaining for Cleaved Caspase-3 in control and *Prmt5cKO* hindlimbs at E11.5 (A, B, F, G). Wholemount hindlimb buds stained for Lysotracker red in control and *Prmt5cKO*at E12.5 (C, H). Western blot analysis of phospho-p38 (D) and pSmad1,5,8 (I) levels in E12.5 hindlimbs. Quantification of band intensities for phospho-p38, normalized to pan-p38 (E, n=4) and pSMAD1,5,8, normalized to ACTIN (J, n=3). Scale bar indicates 500µm.

Norrie et. al. Figure S7

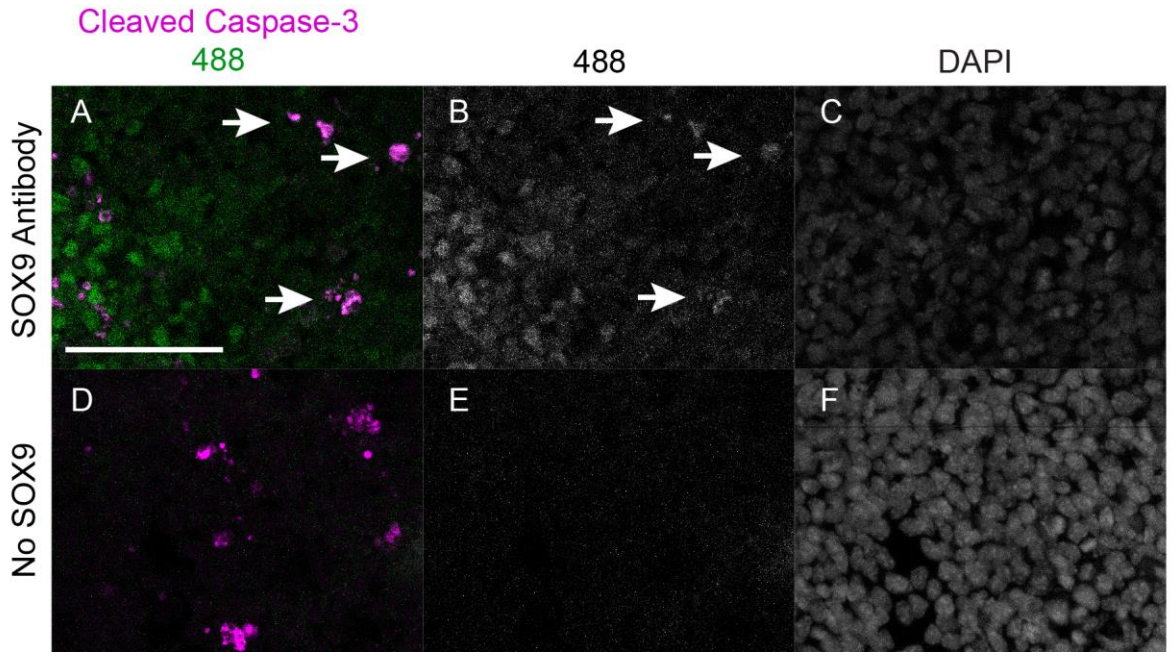

Figure S7. Control for apoptotic cells co-expressing Cleaved Caspase-3 and SOX9. Sections were stained for SOX9 and Cleaved Caspase-3 (A-C) or Cleaved Caspase-3 alone (D-F) and imaged with the 568nm laser (Cleaved Caspase-3 primary and AlexaFluor 568-conjugated anti-rabbit) and the 488nm laser (SOX9 directly conjugated to AlexaFluor 488) to ensure that SOX9 expressing cells did not have bleed-through fluorescence caused by Cleaved Caspase-3 staining. Scale bar indicates 50µm.

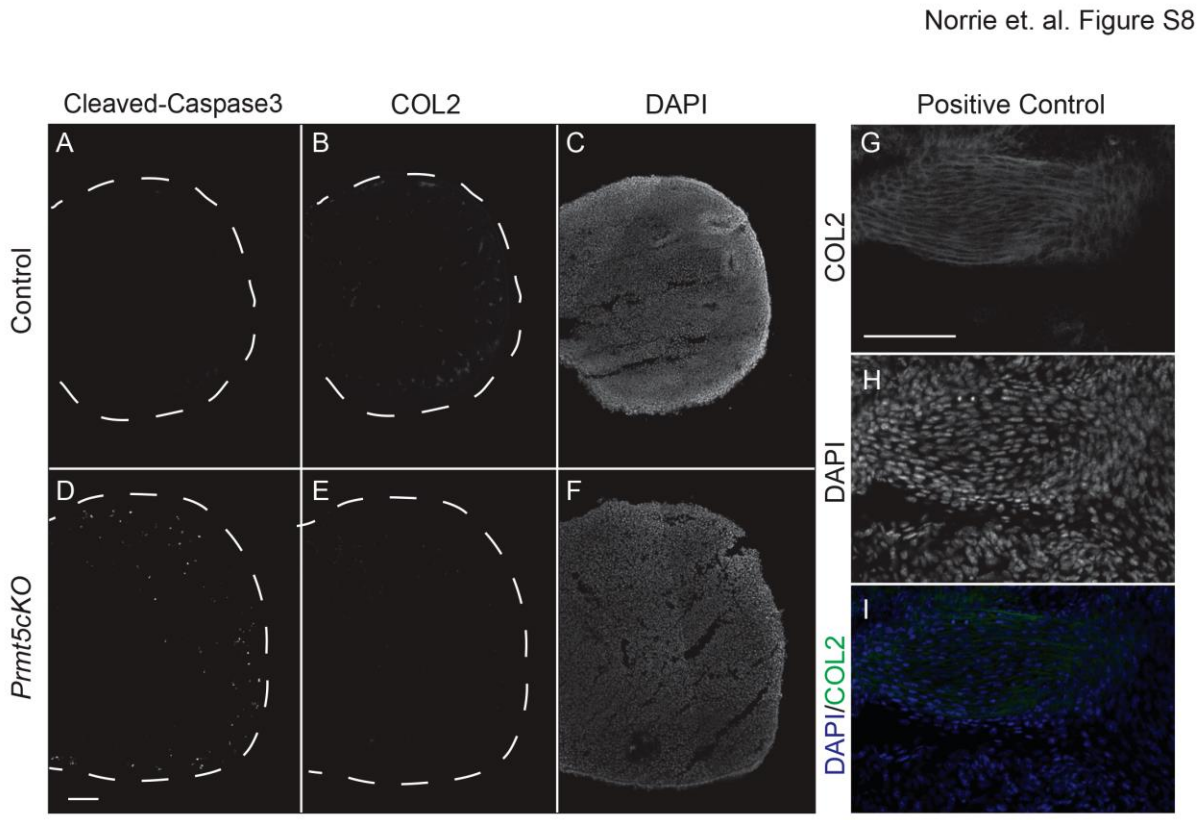

Figure S8. COL2 is not present in apoptotic cells in *Prmt5cKO* limb buds. Immunostaining of control and *Prmt5cKO* forelimbs for Cleaved Caspase-3 at E11.5 (A,D) and COL2 (B,E). While a few cells express very low levels of what is likely background staining in both wild-type and Prmt5cKOs, there is no overlap between this staining and Cleaved Caspase-3 positive cells. As a positive control for COL2 immunostaining, an E18.5 wildtype limb was sectioned and stained alongside the E11.5 limbs. (G-I) Portion of an E18.5 digit positive for COL2.

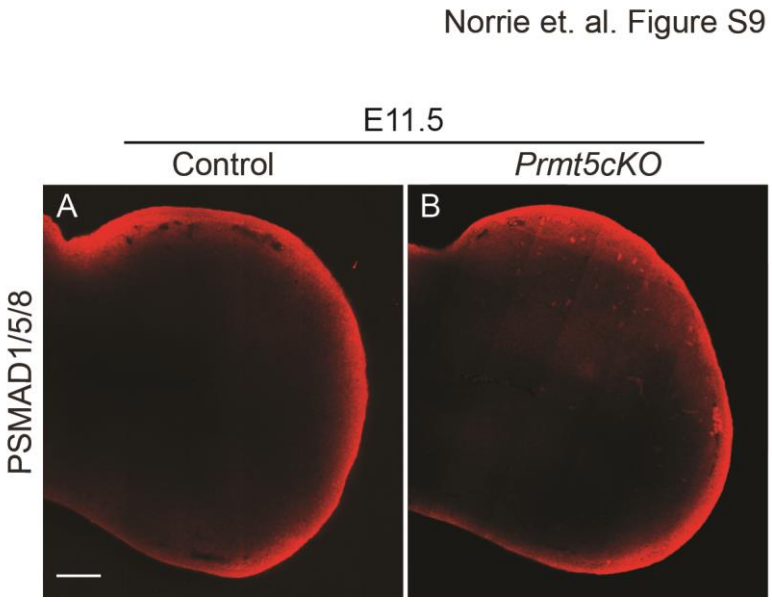

Figure S9. pSmad1,5,8 expression is spatially unchanged. (A, B) Immunostaining for phosphorylated Smad 1,5,8 in control and *Prmt5cKO* forelimbs at E11.5.

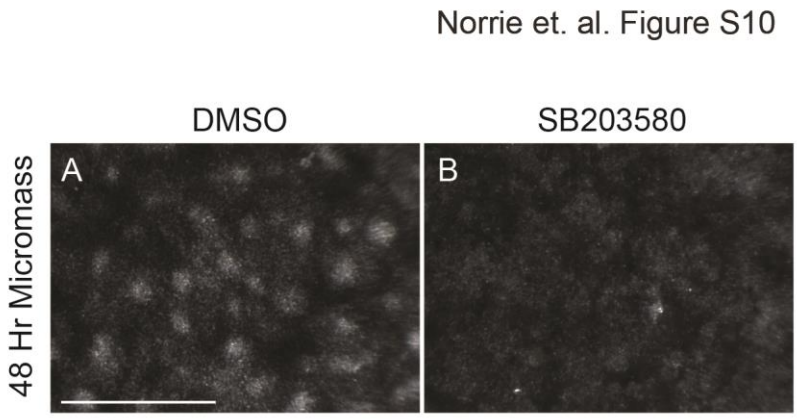

Figure S10. Inhibiting p38 signaling inhibits nodule formation. Images of micromass cultures from paired E10.5 forelimbs (n=4 pairs) after 48 hours of culture and treatment with DMSO (A) or 20μM SB203580, a p38 inhibitor (B). Scale bar indicates 500μm.

SUPPLEMENTAL TABLES

Table S1. Differentially expressed genes in *Prmt5cKOs*. Results from RNA-seq analysis from control and *Prmt5cKO* E11.5 forelimb buds. Genes listed have an average FPKM > 1, a log2 fold change > 1, and a q-value < 0.01. Abbreviation: FPKM, Fragments Per Kilobase of Exon Per Million Fragments Mapped.

Click here to Download Table S1

Table S2. GO terms of differentially expressed genes. GO analysis of the 208 differentially expressed genes. GO terms are listed with the gene names for each category and the associated p-value.

GO Term	Gene Names	p-value
Signal transduction by p53 class mediator genes	*Bbc3, Tnfrsf10b, Bax, Pmaip1, Phlda3, Aen*	1.00E-04
Negative regulation of cellular processes	*Mid1, Id4, Tfap2c, Prmt5, Aplp1, Osr2, Nbl1, Gsc, Phlda3, Bmp4, 4632434I11Rik, Bax, Uty, Bbc3, Ccng1, Fgf8, Vill, Eomes, Rarb, Cd36, Zmat3, Pde1c, Ddit4l, Sesn2, Foxc1, Klk14, Gas2, Glis3, Ndrg2, Sulf1, Pak7, Erdr1, Grem1, Cdkn1a, Cgref1, Myo1f, Gdf5, Pmaip1, Rhbdf2, Ptprv, Nkx3-2*	2.00E-04
Embryonic hindlimb morphogenesis	*Fgf8, Lmbr1, Rarb, Osr2, Bmp4*	2.00E-04
Chondrocyte differentiation	*Gdf5, Sulf1, Rarb, Nkx3-2, Osr2, Bmp4*	4.00E-04
Sensory organ development	*Fgf8, Gsc, C1qb, Chrdl1, Rarb, Miat, Bmp4, Bax, Nkx3-2, Osr2, Foxc1, Kera, Col8a2*	7.00E-04
Telencephalon development	*Fgf8, Id4, Tfap2c, Bax, Rarb, Eomes, Bmp4*	1.40E-03
Extracellular matrix organization	*Csgalnact1, Aplp1, Postn, Egfl6, Sulf1, Grem1, Foxc1*	1.40E-03
Intrinsic apoptotic signaling pathway	*Cdkn1a, Bbc3, Pmaip1, Ptprv, Phlda3, Grem1, Aen*	1.40E-03
Bone morphogenesis	*Csgalnact1, Rarb, Osr2, Col9a1, Bmp4*	1.40E-03
Kidney development	*Fgf8, Bax, Sulf1, Grem1, Osr2, Foxc1, Bmp4*	2.40E-03
BMP signaling pathway	*Fgf8, Zcchc12, Sulf1, Grem1, Nbl1, Bmp4*	2.40E-03
Embryonic skeletal system development	*Gsc, Fuz, Sulf1, Nkx3-2, Osr2, Bmp4*	2.40E-03

Table S3. qRT-PCR primer sequences. A list of forward (F) and reverse (R) primer sequences that were used for quantitative RT-PCR.

Target		Sequence
Acctb	F	GCTGTATTCCCCTCCATCGTG
	R	CACGGTTGGCCTTAGGGTTCAG
Axin2	F	GAGGAGATCGAGGCAGAAGC
	R	CACCTCTGCTGCCACAAAAC
Bmp2	F	CAAAGCAGGACCAGTGGGAA
	R	AGCCCCCTGGAAGGGATTAT
Bmp4	F	ACGTACTCCCAAGCATCACC
	R	GCACAATGGCATGGTTGGTT
Bmp7	F	CGCACTCTCCCTCACAGTAG
	R	AAGACGCCAAAGAACCAAGA
Cdk6	F	TCCCAGGAGAGGAAGACTGG
	R	GCCGTAGGCGGATATCCTTT
Col2a1	F	GATGGCTGGAGGGTATGACG
	R	CAGGTTCACCAGGATTGCCT
Dusp6	F	CGGAAATGGCGATCTGCAAG
	R	GACGACTCGTACAGCTCCTG
Fgf8	F	CCGGACCTACCAGCTCTACA
	R	GGCAATTAGCTTCCCCTTCT
Gli1	F	CCCAGCTCGCTCCGCAAACA
	R	CTGCTGCGGCATGGCACTCT
HoxD13	F	GCACGAGGCATACATCTCCA
	R	GCTGCAGTTTGGTGTAAGGC
Msx2	F	CAAGTGAAGGGGGAGGTGTA
	R	CAGGGACCTGACATGGAGTT
Prdm1	F	ATCCAGCTTCCCTACCGAGT
	R	GGGGGACTACTCTCGTCCTT
Ptch1	F	GACCGGCCTTGCCTCAACCC
	R	CAGGGCGTGAGCGCTGACAA
Shh	F	TCTCGAGACCCAACTCCGAT
	R	GACTTGTCTCCGATCCCCAC
Smad7	F	CCTTCCCTTTGGATCAGCGT
	R	CACTATGAGCCTCTCAGCCG
Sox9	F	TAAGTTCCCCGTGTGCATCC
	R	TTGCCCAGAGTCTTGCTGAG
Spry4	F	GACCCACTCGGGTTCGGGGA
	R	GGGGCGCTCTGCTGTCAAGG

Supplemental Methods

COL2 immunostaining: cryosections were blocked for 30 minutes in block (5% normal goat serum and 2.5% bovine serum albumin) then stained for anti-Cleaved Caspase-3 (Cell signaling #9664 1:250) overnight at 4°C followed by Alexa Fluor 568 goat anti-rabbit secondary (1:250 A11036 Life technologies) for one hour at room temperature, and DAPI (300nM Life Technologies). Sections were loosely mounted with Prolong Gold and imaged on a Zeiss Axiovert Fluorescent light microscope. After imaging, the sections were washed, and equilibrated in 0.02N HCl for 10 minutes at 37°C, permeabilized in 5mg/mL Pepsin in 0.02N HCl for 1 minute at 37°C, then blocked for 30 minutes. Sections were then incubated with a Collagen II antibody (Abcam #3092 1:100) (Meech et al., 2005) overnight at 4°C followed by Alexa Fluor 488 goat anti-mouse secondary (1:250 Life technologies #A11029) for one hour at room temperature, and DAPI (1:5000) for 5 minutes. The slides were imaged on a Zeiss 710 confocal microscope collected as tile scans with a 20X objective.

Micromass cultures were performed as previously described (Lewandowski et al., 2014) the following variations. Pairs of forelimbs were dissected from single embryos and incubated in trypsin EDTA (TE) for 10 minutes. Limb buds resuspended in 100 µl of media and split into 2 wells of a half area 96 well plate. One well was treated with 20µM the p38 inhibitor SB203580 resuspended in DMSO (Barancik et al., 2001; Chen et al., 2016; Engel et al., 2005; Hirose et al., 2003; Kim et al., 2016; Tong et al., 1997) while the other was treated with an equivalent amount of DMSO. Cells were culture for 48 hours and imaged on a Leica M165 stereo microscope.

Supplemental References

Barancik, M., Bohacova, V., Kvackajova, J., Hudecova, S., Krizanova, O., and Breier, A. (2001). SB203580, a specific inhibitor of p38-MAPK pathway, is a new reversal agent of P-glycoprotein-mediated multidrug resistance. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences *14*, 29-36.

Chen, J.C., Hsieh, M.J., Chen, C.J., Lin, J.T., Lo, Y.S., Chuang, Y.C., Chien, S.Y., and Chen, M.K. (2016). Polyphyllin G induce apoptosis and autophagy in human nasopharyngeal cancer cells by modulation of AKT and mitogen-activated protein kinase pathways in vitro and in vivo. Oncotarget.

Engel, F.B., Schebesta, M., Duong, M.T., Lu, G., Ren, S., Madwed, J.B., Jiang, H., Wang, Y., and Keating, M.T. (2005). p38 MAP kinase inhibition enables proliferation of adult mammalian cardiomyocytes. Genes Dev *19*, 1175-1187.

Hirose, Y., Katayama, M., Stokoe, D., Haas-Kogan, D.A., Berger, M.S., and Pieper, R.O. (2003). The p38 mitogen-activated protein kinase pathway links the DNA mismatch repair system to the G2 checkpoint and to resistance to chemotherapeutic DNA-methylating agents. Mol Cell Biol *23*, 8306-8315.

Kim, G.Y., Park, J.H., Kim, H., Lim, H.J., and Park, H.Y. (2016). Coronin 1B serine 2 phosphorylation by p38alpha is critical for vascular endothelial growth factor-induced migration of human umbilical vein endothelial cells. Cellular signalling *28*, 1817-1825.

Lewandowski, J.P., Pursell, T.A., Rabinowitz, A.H., and Vokes, S.A. (2014). Manipulating gene expression and signaling activity in cultured mouse limb bud cells. Developmental Dynamics *243*, 928-936.

Meech, R., Edelman, D.B., Jones, F.S., and Makarenkova, H.P. (2005). The homeobox transcription factor Barx2 regulates chondrogenesis during limb development. Development *132*, 2135-2146.

Tong, L., Pav, S., White, D.M., Rogers, S., Crane, K.M., Cywin, C.L., Brown, M.L., and Pargellis, C.A. (1997). A highly specific inhibitor of human p38 MAP kinase binds in the ATP pocket. Nature structural biology *4*, 311-316.