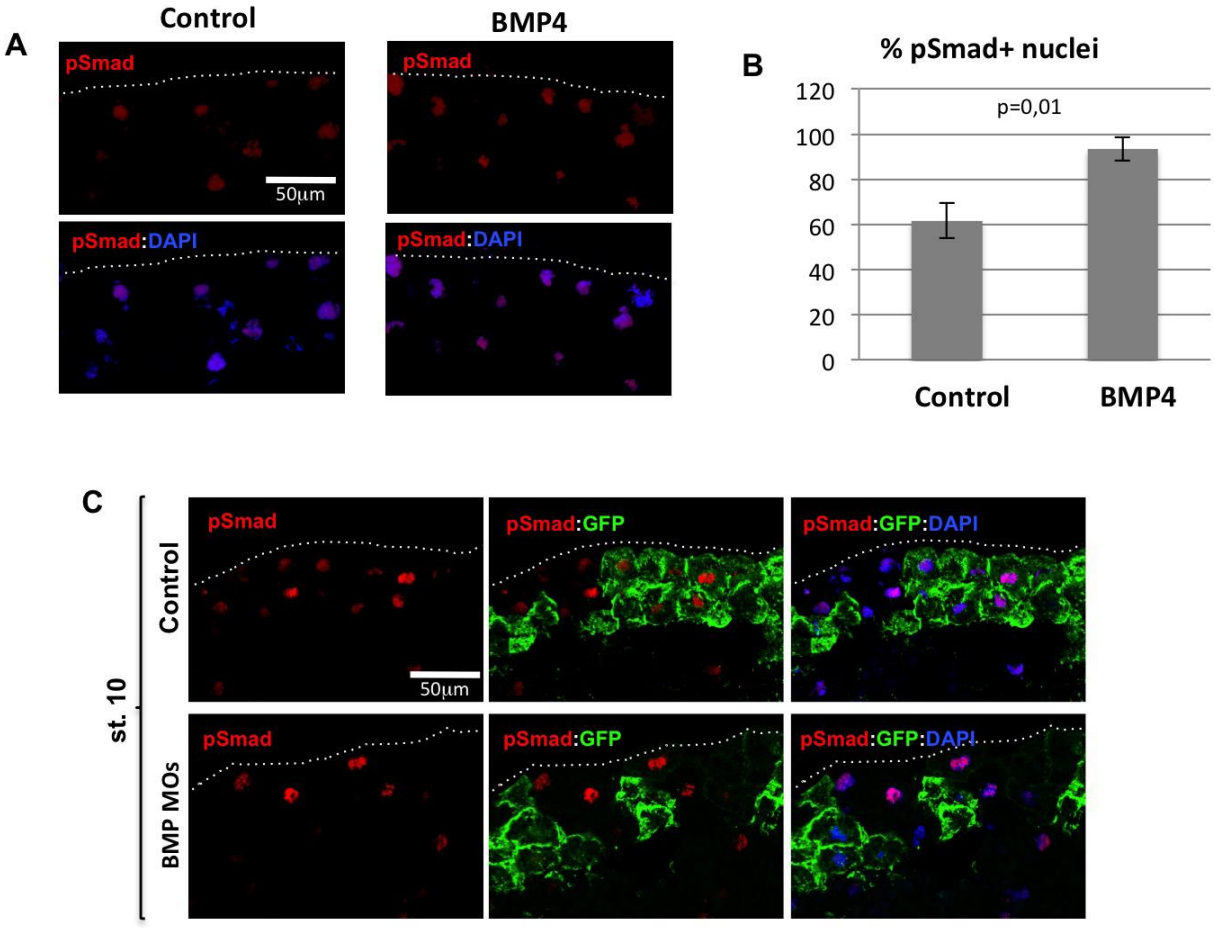

Figure S1: Validation of pSmad1/5/8 staining in response to BMP manipulation.

(A): Injection of stage 9 *Xenopus* embryos with recombinant BMP4 led to increased Smad1/5/8 phosphorylation and nuclear localization. **(B)**: The percentage of nuclei positive for the pSmad signal is significantly higher in BMP-treated than in control embryos (Student's t-test). **(C)**: Knocking down BMP2, BMP4 and BMP7 with specific morpholino oligonucleotides inhibited Smad1/5/8 phosphorylation. Embryos were injected with either GFP mRNA alone (upper row) or GFP mRNA and the BMP2/4/7 MOs (lower row). The pSmad1/5/8 signal was lost in cells that received the BMP2/4/7 MOs, but not in those that received only the GFP mRNA.

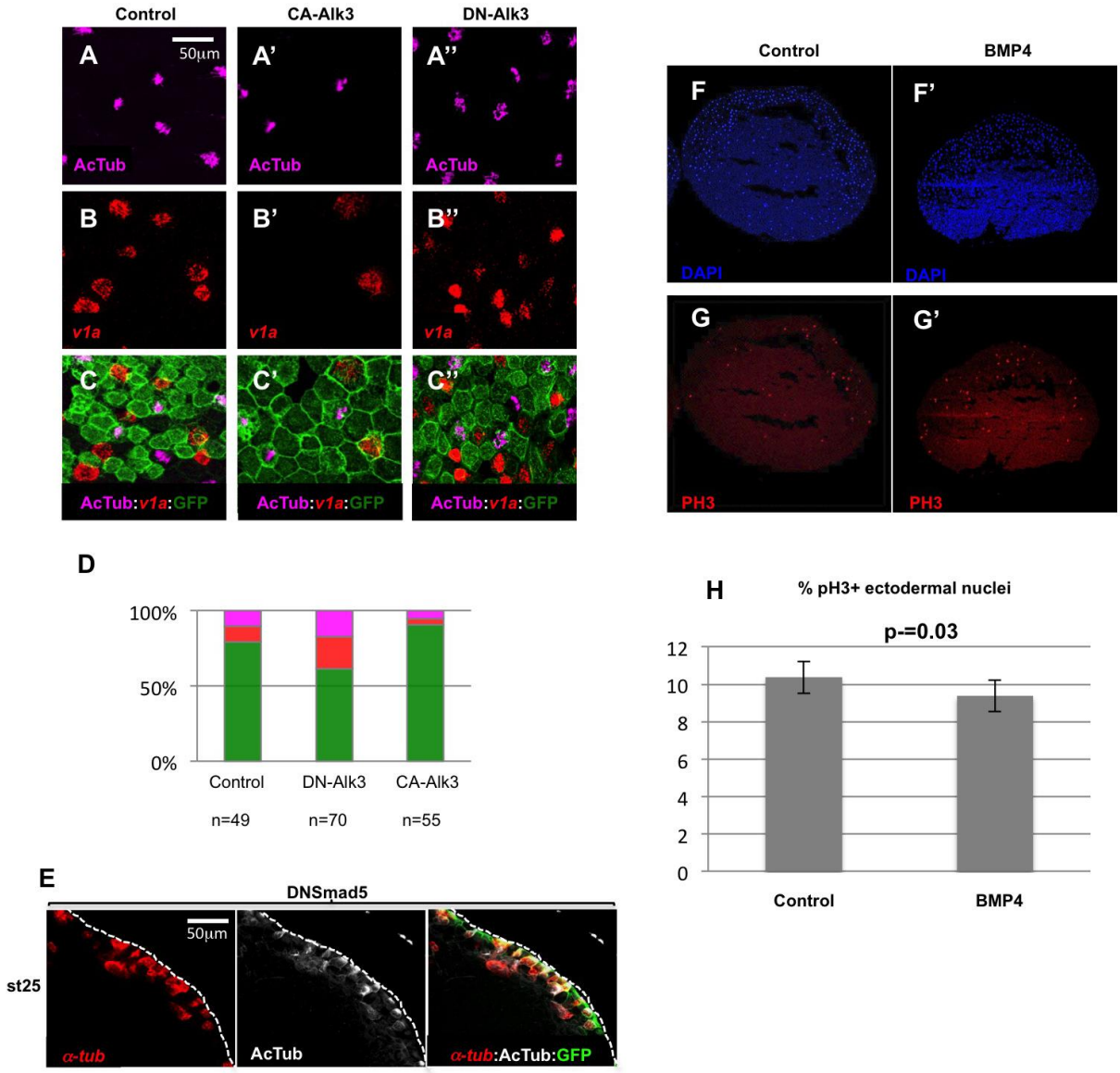

Figure S2: Interfering with the BMP pathway alters the numbers of MCCs and ionocytes in the developing *Xenopus* epidermis.

(A-D): 8-cell stage embryos were injected into the animal ventral blastomeres with synthetic mRNAs coding for GFP, alone (control; A, B, C) or together with mRNAs coding for a constitutively active (CA-Alk3; A', B', C') or a dominant negative (DN-Alk3; A'', B'', C'') version of the BMP receptor ALK3, then hybridized at stage 25 with a probe against the ionocyte marker *v1a* and antibodies against acetylated tubulin and against GFP. Activation of the BMP pathway by CA-Alk3 injection resulted in a decrease in the numbers of MCCs and

ionocytes. Inhibition of the BMP pathway by DN-Alk3 injection results in an increase in the numbers of MCCs and ionocytes. (D): quantification of the different cellular populations in injected epidermal clones. The bars represent the total number of GFP positive cells scored. Magenta: acetylated tubulin positive MCCs; red: *v1a* positive ionocytes; green: GFP positive cells negative for both acetylated tubulin and *v1a*. **(E):** Embryos injected with a synthetic mRNA coding for a dominant negative form of the zebrafish BMP pathway nuclear effector Smad5 (dnSmad5), showed supernumerary *α-tubulin* positive MCC precursors, which only partially managed to intercalate. **(F-H):** Embryos were injected in the blastocoele with BSA (control) or recombinant BMP4 protein (BMP4), fixed at stage 10, cryosectioned and immunostained with an antibody against phosphorylated histone H3 (red), a hallmark of cells in mitosis. DAPI (blue) was used to stain the nuclei. The graph in (H) shows the ratio of phospho-H3 positive nuclei to the total number of ectodermal DAPI stained nuclei. BMP4 injection did not significantly modify the number of mitotic nuclei compared to controls (Student's t-test). In F-G', the animal pole (ectoderm) is up.

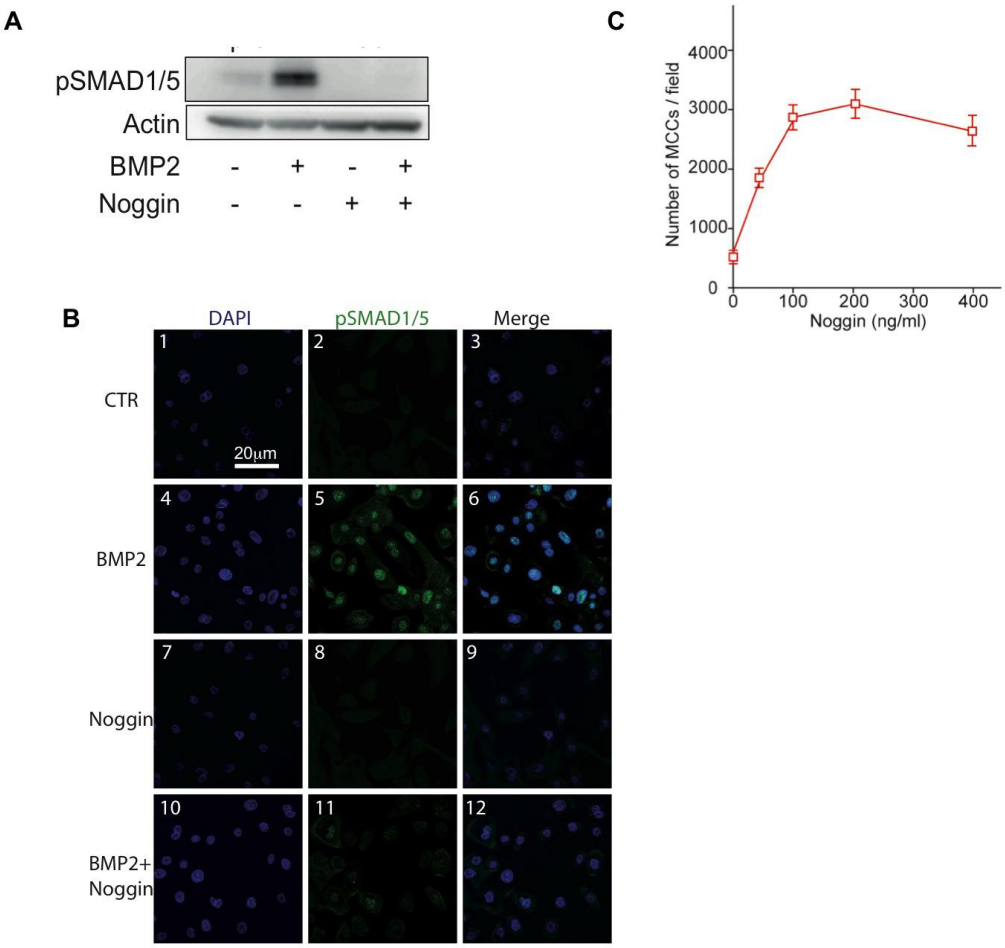

Figure S3: Validation of BMP pathway manipulation in HAECs.

(A): Expression levels of pSMAD1/5 in response to BMP2 (100ng/ml, 2h), Noggin (100ng/ml, 2h), or BMP2+Noggin (100ng/ml each, 2h) in proliferating HAECs. Actin was used as a loading control. **(B)** Proliferating HAECs were stained to identify the specific sub-cellular localization of pSMAD1/5 (in green) in untreated control cells (panels 1-3), BMP2-treated cells (100ng/ml, 2h) (panels 4-6), Noggin-treated cells (100ng/ml, 2h) (panels 7-9) and cells treated with both BMP2 and Noggin (100ng/ml each, 2h) (panels 10-12). Nuclei were stained with DAPI (in blue; panels 1,4,7,10). Data are representative of 3 independent experiments. **(C):** Dose-response curve of Noggin treatment. Regenerating HAECs were chronically treated with different doses of recombinant Noggin. Acetylated-tubulin positive MCCs were counted at LC. 100ng/ml Noggin was the minimal dose to give the maximal effect and used in the rest of the study.

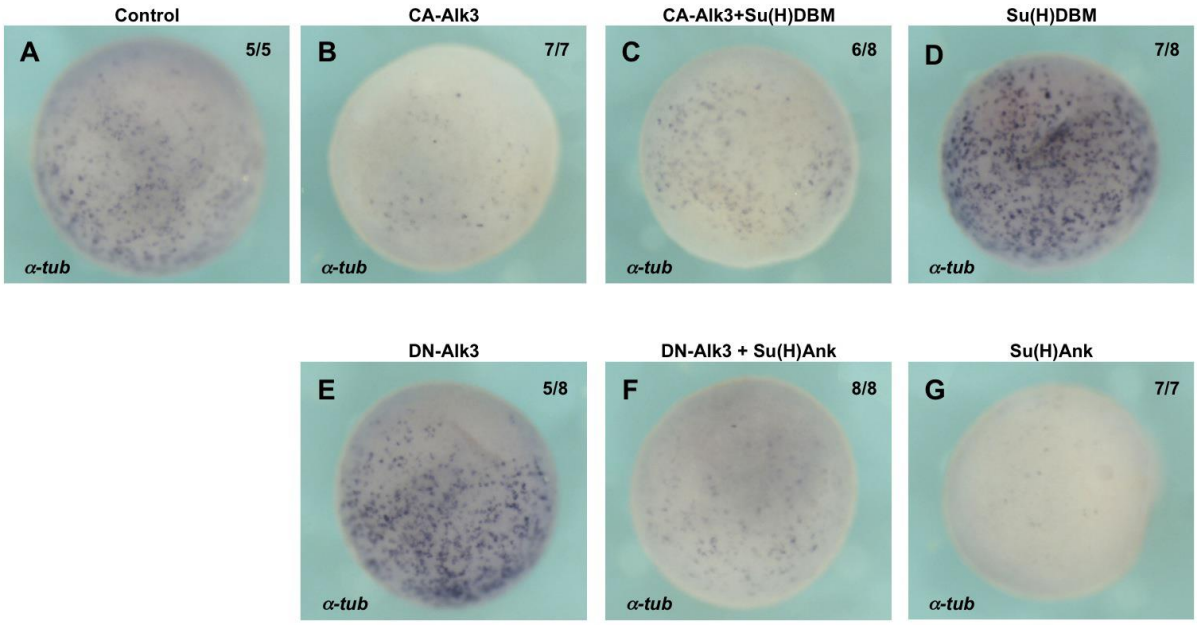

Figure S4: The BMP and Notch pathways interact.

Co-injection of mRNAs coding for a constitutively active form of the BMP receptor Alk3 (CA-Alk3) and a dominant negative form of the transcriptional Notch effector Suppressor-of-hairless (Su(H)DBM) (**C**) mitigated both the loss of α-tubulin positive cells resulting from expression of CA-Alk3 alone **(B)** and their increase following injection of Su(H)DBM alone **(D)**. Co-injection of mRNAs coding for a dominant negative form of the BMP receptor Alk3 (DN-Alk3) and a constitutively active form of the transcriptional Notch effector Suppressor-of-hairless (Su(H)Ank) **(F)** mitigated both the increase in α-tubulin positive cells resulting from expression of DN-Alk3 alone **(E)** and their loss following injection of Su(H)Ank alone **(G)**. The number of embryos showing the phenotype displayed over the total number of embryos examined is indicated.

Table S1: Plasmids, morpholinos, antibodies, drugs and recombinant proteins used.

Plasmid name	**Vector**	**linearized with**	**transcribed with**
dnSmad5	pCS2+	NotI	SP6
CA-Alk3	pCS2+	NotI	SP6
DN-Alk3	pCS2+	NotI	SP6
GFP-CAAX	pCS105	Ase1	SP6

Morpholino Name	**Sequence**
MO-BMP2	5'-GATCCCAGCGACCATTGTCAACCTG-3'
MO-BMP4	5'-CAGCATTCGGTTACCAGGAATCATG-3'
MO-BMP7	5'-TTACTGTCAAAGCATTCATTTTGTC-3'

Antibodies used on *Xenopus*	**Source**	**Reference**	**Dilution**
Monoclonal mouse anti-acetylated tubulin	Sigma	Clone 6-11B-1	1:200
Monoclonal mouse 5G7	Gift from Saburo Nagata (Japan Women's University)		1:500
Monoclonal mouse anti-P63	Abcam	Ab111449	1:100
Polyclonal rabbit anti-pSmad1/5/8	Cell signalling	9511	1:100
Polyclonal chicken anti-GFP	2BScientific	GFP-1020	1:1000
Polyclonal rabbit anti-Serotonin	Millipore	Ab938	1/250
Polyclonal rabbit anti-phosphoH3	Millipore	06-570	1 :250
anti-mouse-Cy5 anti-mouse 647 anti-mouse-561 anti-chick-488 anti-rabbit-561	Molecular Probes		1:500

Antibodies used on HAECs	**N° clone**	**Source**	**Reference**	**Dilution**	
				IF	Blot
Anti-acetylated tubulin	6-11B-1	Sigma-Aldrich	T7451	1 :1000	
Anti-HSP60	K-19	Santa Cruz Biotechnology	sc-1722		1 :5000
Anti-Muc5AC	45M1	Abcam	ab3649	1 :500	
Secondary antibodies		Molecular Probe		1:500	
Anti-pSmad1/5/8	41D10	Cell signaling	9516	1 :100	1 :1000
Anti-Smad1	D59D7	Cell signaling	6944		1 :1000
Anti-Actin	I-19	Santa Cruz Biotechnology	sc-1616		1 :5000

Drug and Recombinant proteins	Source	Reference	Concentration
Dorsomorphin	Sigma-Aldrich	P5499	2mg/ml
BMP2	R&D	355-BM	200ng/ml
Noggin	R&D	6057-NG	200ng/ml or see figures
BMP4	R&D	1128-BM-010	2 to 7ng/embryo

Probe	Vector	linearized	transcribed	Reference
Dll1	pBS	XhoI	T7	Deblandre et al. 1999
FoxA1	pBS	EcoRI	T3	Sinner et al. 2004
FoxJ1	pCS2+	BamHI	T7	Stubbs et al. 2008
Foxi1e	pCMV Sport 6	SalI	T7	Dubaissi et al. 2011
Intelectin-1	pBS	EcoRI	T7	Hayes et al. 2007
Multicilin	pBS	HindIII	T3	Stubbs et al. 2012
Otogelin	pCMV Sport 6	SalI	T7	Hayes et al. 2007
Tph1	pCMV Sport 6	EcoRV	T7	Walentek et al. 2014
α-Tubulin	pBS	NotI	T7	Deblandre et al. 1999
Trim29	pCS2+	EcoRI	T7	Hayes et al. 2007
V1a	pCS107	ClaI	T7	Dubaissi et al. 2011
α-Dystroglycan	pCRII	XhoI	Sp6	Bello et al. 2008

References:

Bello, V. Sirour, C. Moreau, N., Denker, E. and Darribère, T. (2008). A function for dystroglycan in the pronephros development in Xenopus laevis. *Dev. Biol*. **317**,106-120.

Dubaissi, E. and Papalopulu, N. (2011). Embryonic frog epidermis: a model for the study of cell-cell interactions in the development of mucociliary disease. *Dis. Mod. Mech.* **4**, 179-192.

Sinner, D., Rankin, S., Lee, M. and Zorn, A.M. (2004). Sox17 and beta-catenin cooperate to regulate the transcription of endodermal genes. *Development* **131,** 3069-3080.

Stubbs, J.L., Oishi, I., Izpisùa Belmonte, J.C., Kintner, C. (2008). The forkhead protein Foxj1 specifies node-like cilia in Xenopus and zebrafish embryos. *Nat. Genet.* **40**, 1454-1460.