THE COMPANY OF
Biologists

RESEARCH ARTICLE

WT1 regulates the development of the posterior taste field

Yankun Gao*, Eneda Toska*, Dane Denmon, Stefan G. E. Roberts and Kathryn F. Medler‡

ABSTRACT

Despite the importance of taste in determining nutrient intake, our understanding of the processes that control the development of the peripheral taste system is lacking. Several early regulators of taste development have been identified, including sonic hedgehog, bone morphogenetic protein 4 and multiple members of the Wnt/β-catenin signaling pathway. However, the regulation of these factors, including their induction, remains poorly understood. Here, we identify a crucial role for the Wilms' tumor 1 protein (WT1) in circumvallate (CV) papillae development. WT1 is a transcription factor that is important in the normal development of multiple tissues, including both the olfactory and visual systems. In mice, WT1 expression is detectable by E12.5, when the CV taste placode begins to form. In mice lacking WT1, the CV fails to develop normally and markers of early taste development are dysregulated compared with wild type. We demonstrate that expression of the WT1 target genes *Lef1*, *Ptch1* and *Bmp4* is significantly reduced in developing tongue tissue derived from *Wt1* knockout mice and that, in normal tongue, WT1 is bound to the promoter regions of these genes. Moreover, siRNA knockdown of WT1 in cultured taste cells leads to a reduction in the expression of *Lef1* and *Ptch1*. Our data identify WT1 as a crucial transcription factor in the development of the CV through the regulation of multiple signaling pathways that have established roles in the formation and patterning of taste placodes.

KEY WORDS: Taste, Circumvallate papillae, WT1, Mouse

INTRODUCTION

The sense of taste is mediated by taste receptor cells that detect chemicals in the oral cavity and translate that information into an output signal that is sent to the brain. Taste receptor cells are housed in taste buds, which each comprise 50-150 taste cells. In mammals, most taste buds are located within specialized areas called papillae that are set within discrete areas of the tongue (Mistretta, 1991). There are three types of taste papillae on the tongue: (1) fungiform papillae that are dispersed on the anterior portion of the tongue and house one or two taste buds each; (2) circumvallate (CV) papillae that are located on the back of the tongue; and (3) foliate papillae, which are found on both sides of the tongue. Both the foliate and CV papillae house hundreds of taste buds. Although most taste buds are located within these papillae on the tongue, there also are some on the palate and throat (Kinnamon, 1987). Taste papillae comprise a mesenchymal core that is surrounded by an invaginated epithelium, where the taste buds are located (Kapsimali and Barlow, 2013).

Several signaling proteins, including members of the sonic hedgehog (SHH), bone morphogenetic protein 4 (BMP4) and

Department of Biological Sciences, University at Buffalo, Buffalo, NY 14260, USA.
*These authors contributed equally to this work

‡Author for correspondence (kmedler@buffalo.edu)

Wnt/β-catenin signaling pathways are required for normal peripheral taste development (Beites et al., 2009; Bitgood and McMahon, 1995; Iwatsuki et al., 2007; Jung et al., 1999; Liu et al., 2007), but the factors controlling the expression of these pathways in the taste system have not been identified. In this study, we show that expression of the transcription factor Wilms' tumor 1 (WT1) is coincident with the emergence of CV taste placodes, the first discernible feature of the developing CV papillae. We found that *Wt1* knockout mice exhibit significant defects in the anatomical specializations associated with CV papillae development. We show that WT1 regulates the expression of BMP4 as well as genes from the SHH and Wnt/β-catenin signaling pathways. Our data demonstrate for the first time that WT1 is required for the normal development of the posterior taste field.

RESULTS

WT1 is expressed in the developing CV papillae

Previous PCR analysis of mRNA isolated from mouse tissues detected *Wt1* mRNA in the embryonic and adult tongue (Armstrong et al., 1993). Considering that WT1 affects the development of other sensory tissues (Wagner et al., 2002, 2005), we initiated a more thorough analysis of its expression in the developing mouse taste system. We first determined where WT1 was localized within the developing tongue using immunocytochemical analysis to characterize expression in the developing CV papillae (Fig. 1).

At embryonic day (E) 12.5, the peripheral taste system begins to form as a thickening of epithelial cells, termed placodes (Barlow, 2000; Mistretta, 1972). Analysis of E12.5 tongues where the placode is developing demonstrated that WT1 (Fig. 1A and Fig. 2A) is expressed at the same time as cytokeratin 8 (detected with anti-Troma1; Fig. 1B), an embryonic taste precursor cell marker (Mbiene and Roberts, 2003), and GAP43 (Fig. 1C), which labels the gustatory nerve that innervates the taste buds (Mbiene and Mistretta, 1997). We were unable to detect WT1 expression in E11.5 embryos (data not shown). By E14.5, the invaginations that will become the CV crypts had formed (Fig. 1A and Fig. 2A); WT1 expression became less diffuse and was present in the epithelia of the developing papillae and the surrounding tissue.

By birth [postnatal day (P) 0], WT1 expression was largely confined to the epithelium of the taste bud (Fig. 1A and Fig. 2A). Higher magnification revealed that WT1 expression is both cytoplasmic and nuclear in the cells that are forming the taste papillae (Fig. 2A, bottom panels). This is consistent with previous observations that WT1 can be present in both the nucleus and cytoplasm (Hohenstein and Hastie, 2006; Niksic et al., 2004; Vajjhala et al., 2003). WT1 was also detected in adult CV papillae (supplementary material Fig. S1A) but was primarily restricted to the taste buds. Immunoblotting of extracts from isolated adult taste cells confirmed the expression of WT1 (supplementary material Fig. S1B). Thus, WT1 is expressed from the early placode stage of taste development and continues to be expressed into adulthood. WT1 expression in the developing taste placode was independently confirmed using a different WT1 antibody (supplementary material Fig. S2).

DEVELOPMENT
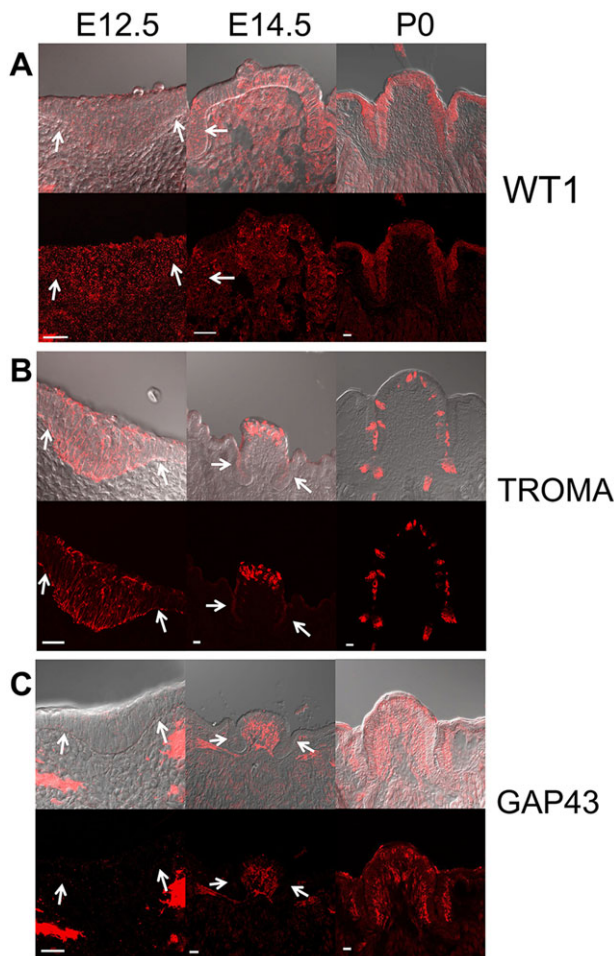
Fig. 1. WT1 is expressed in the developing mouse CV papillae.
Immunocytochemical analysis of the developing taste placode at the indicated time points. Antibodies to detect WT1 (A), Troma1 (B) and GAP43 (C) were used. Immunoreactivity (red) is shown alone in the bottom panel and merged with the DIC image in the top panel. Arrows indicate the edges of the placode (E12.5) or the crypt formation that is part of the CV papillae that will ultimately be lined with taste buds (E14.5). Scale bars: 20 μm.

Fig. 2. Expression pattern of WT1 in the developing CV. (A) WT1 immunocytochemical analysis was performed as in Fig. 1A except that Hoechst stain was included to visualize nuclei. The samples were imaged at low magnification (top, immunoreactivity alone; middle, overlay with DIC image). WT1 is expressed in the mesenchymal cells of the developing tongue, with more intense staining within the placode, and then becoming largely localized to the epithelial layer of the taste buds. Bottom row shows high magnification images of anti-WT1 labeling with Hoechst nuclear staining (blue). Both nuclear and cytoplasmic labeling are evident for WT1. (B) A negative control in which anti-WT1 is omitted but secondary antibody was added. Left, immunolabeling; right, overlay with DIC image. Scale bars: 20 μm in A, top and middle rows; 5 μm in A, bottom row; 50 μm in B.

WT1 localizes to the promoters of several target genes that are important for taste development

Although the mechanisms controlling the development of the peripheral taste system are not well defined, several early regulators have been identified, including SHH, BMP4 and multiple members of the Wnt/β-catenin signaling pathway. *Lef1* (which encodes a major effector of canonical Wnt signaling), *Ptch1* (SHH receptor) and *Bmp4* (a TGFβ ligand) play crucial roles in taste papillae formation (Iwatsuki et al., 2007; Zhou et al., 2006) and have been identified and established as WT1 target genes in the embryonic kidney (Hartwig et al., 2010). We therefore considered the possibility that WT1 regulates the expression of these genes during taste development and examined the embryonic expression of *Lef1*, *Ptch1* and *Bmp4* in the developing CV papillae. We were able to detect expression of all three proteins during mid-gestation, when the initial anatomical specializations that comprise the CV papillae are forming (Fig. 3A).

We then used chromatin immunoprecipitation (ChIP) to determine whether the WT1 binding sites upstream of the *Lef1*, *Ptch1* and *Bmp4* genes are bound by WT1 in the developing peripheral taste system. Tongues were isolated from embryonic mice of a BALB/c background at E15.5 and subjected to ChIP
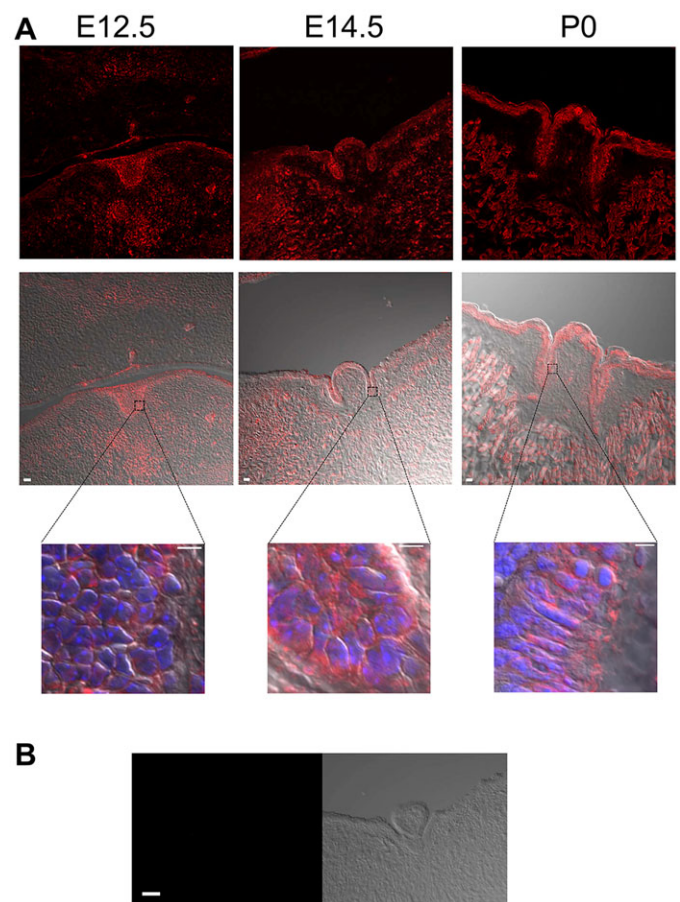
analysis with either control IgG or WT1 antibodies (Fig. 3B). We found that WT1 was bound at the promoter regions of *Lef1*, *Ptch1* and *Bmp4* in the developing tongue and not at a control region 3′ of each gene. Similar results were obtained for ChIP assays performed using E15.5 tongues isolated from the *Wt1* knockout strain that was purchased from Jackson Labs and not backcrossed into the BALB/c background (supplementary material Fig. S3). Follow up experiments confirmed that WT1 expression overlaps with that of all three of these proteins during development (Fig. 3C).

WT1 is required for normal CV development

To determine if WT1 is required for normal taste development, we obtained *Wt1* knockout (KO) mice that contain a mixed genetic background. These WT1-KO embryos do not survive beyond mid-gestation but backcrossing the WT1-KO mutation into a BALB/c background for one generation allows some embryos to survive until birth (Herzer et al., 1999). Herzer et al. (1999) concluded that the WT1-KO phenotype is less severe in the BALB/c background, which suggests some epigenetic control of the penetrance of the *Wt1*
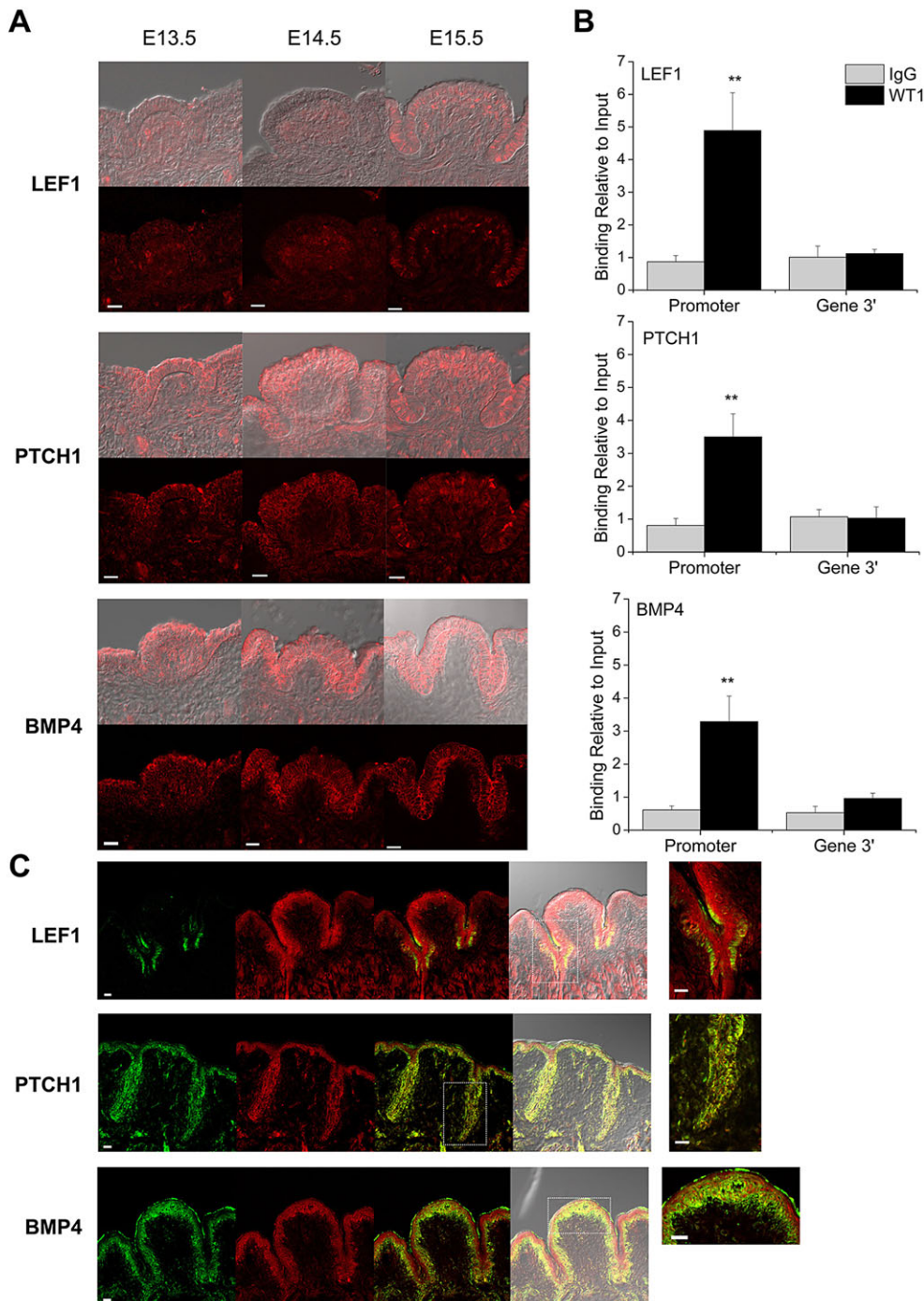
Fig. 3. WT1 regulates the expression of *Lef1*, *Ptch1* and *Bmp4* in the developing mouse taste system. (A) Immunocytochemical analysis of embryonic tongue sections using anti-LEF1, anti-PTCH1 or anti-BMP4 at the indicated time points. Immunoreactivity (red) is shown in the bottom panel and merged with the DIC image in the top panel. (B) Tongues isolated from E15.5 mice were used to perform ChIP with either control IgG or anti-WT1. qPCR was used to amplify the WT1 binding site regions of each gene promoter or control genomic region located 3′ to each gene. **P<0.01 (Student's *t*-test); error bars indicate s.d. (*n*=3). (C) Co-expression with WT1 (red) was evaluated for LEF1, PTCH1 or BMP4 (each green) in E18.5 embryos. An overlay is shown with and without the corresponding DIC image. The boxed region is magnified to the right. Scale bars: 20 μm.

mutation. We found that most WT1-KO embryos in this background still die mid-gestation (only 8% of surviving embryos were WT1-KO, *n*=263 embryos). Analysis of P0 BALB/c WT1-KO mice revealed that the cells forming the CV papillae failed to stain with WT1 antibodies (Fig. 4A). Analysis of E12.5 WT1-KO embryos revealed that the developing placode that will eventually become the CV papillae was significantly impaired (Fig. 4B-E). Using the Troma1 antibody, we labeled cells in the placodes at E12.5 (Fig. 4B). All sections of the KO and wild-type embryos within the vicinity of the CV express Troma1, but the KO embryos lacked the typical epithelial thickening that indicates the onset of CV papillae development. Thus, WT1 is required for normal CV placode formation.

Immunocytochemical analysis to detect other important markers in peripheral taste development further established the vital role of WT1. SOX2 (Fig. 4C), SHH (Fig. 4D) and GAP43 (Fig. 4E) labeling confirmed that, at E12.5, there is a lack of epithelium specialization and loss of organization in the surrounding tissue of WT1-KO embryos. SOX2 labeling was essentially lost, whereas SHH labeling was no longer specific to the developing placode. GAP43 labeling was used to identify the gustatory nerve that normally innervates the papillae during development. In WT1-KO embryos from the BALB/c background, we were unable to detect any GAP43 labeling at E12.5 (Fig. 4E). We therefore analyzed GAP43 expression at E13.5 and E14.5 in WT1-KO embryos compared with wild type (supplementary material Fig. S4).
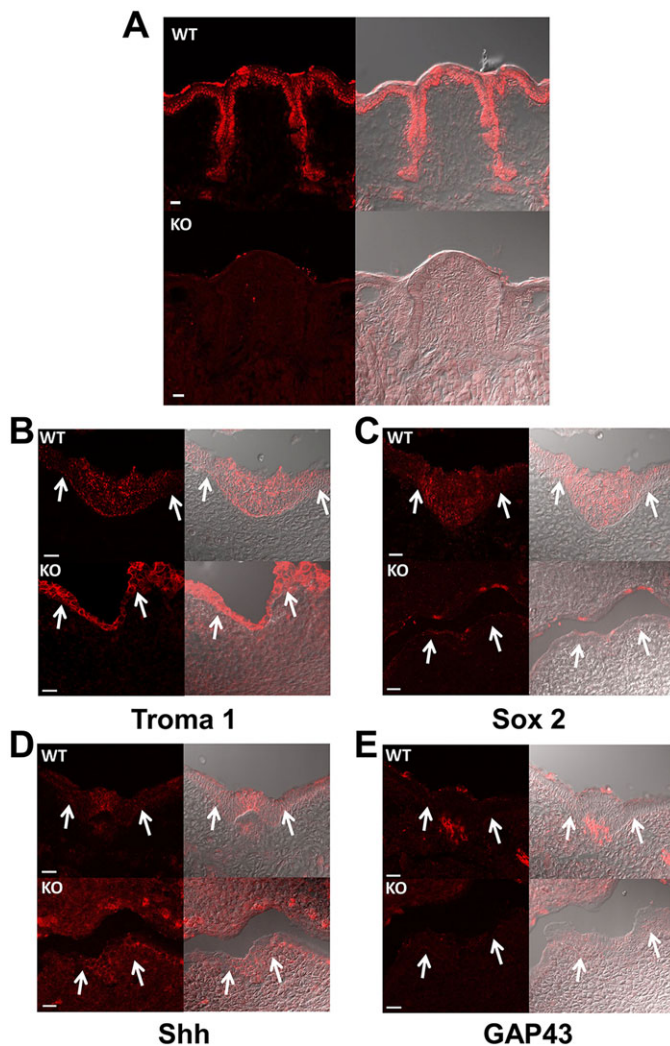
Fig. 4. *Wt1* **knockout mice in the BALB/c background are defective at the early placode stage of CV papillae development.** (A) Immunocytochemical analysis of WT1 in the taste buds of P0 wild-type (WT) and *Wt1* knockout (WT1-KO) mice. Anti-WT1 labeling (red, left) is shown merged with the DIC image (right). (B) Immunocytochemical analysis of Troma1 expression in E12.5 CV from WT1-KO and wild-type embryos. Sections were also analyzed for the known early taste markers SOX2 (C), SHH (D) and GAP43 (E). Arrows indicate the edges of the placode. Scale bars: 20 μm.

Consistent with the impaired CV development, the WT1-KO embryos showed a delay in innervation of the taste bud. Of the WT1-KO embryos from the mixed genetic background, all died mid-gestation (~E15), but we were able to analyze some embryos at E12.5 when the placodes are beginning to form. In this more severe WT1-KO phenotype, the CV placode was completely disrupted (supplementary material Fig. S5).

WT1 regulates the expression of *Lef1*, *Ptch1* and *Bmp4* in the developing taste system

Our data so far suggest that WT1 is required for normal development of the CV papillae. Furthermore, we demonstrated that WT1 is coexpressed with the products of the known WT1 target genes *Lef1*, *Ptch1* and *Bmp4*, and that WT1 localizes to the promoter regions of these genes in the developing tongue. Analysis of LEF1, PTCH1 and BMP4 expression in E14.5 wild-type and KO embryos from the BALB/c background revealed reduced expression of these proteins in the KO embryos (Fig. 5A-C). LEF1 expression at

this time is low in the wild-type embryo, but is further reduced in the KO. PTCH1 expression is relatively widespread at this point in development, but in the KO embryo, is reduced in the epithelia that are forming the papillae. BMP4 expression appears to be largely absent in the KO embryo, in contrast to the wild type. Thus, the loss of WT1 is associated with significant disruption LEF1, PTCH1 and BMP4 expression.

We next prepared mRNA from the tongues of wild-type or WT1-KO E13.5 embryos and analyzed the expression of *Lef1*, *Ptch1* and *Bmp4* by qPCR (Fig. 5, right panels). *Lef1*, *Ptch1* and *Bmp4* mRNA levels were significantly reduced in tongues derived from WT1-KO embryos as compared with wild type. We conclude that WT1 is essential for the normal development of the peripheral taste system and that it is first required during initial placode formation. Although these data demonstrate that WT1 is regulating the expression of *Lef1*, *Ptch1* and *Bmp4*, we cannot conclude that these are the only genes affected by the absence of WT1. Further studies are needed to determine if other factors that are crucial in the development of the CV papillae are also regulated by WT1.

Aberrant CV papillae development in the WT1-KO mouse

We also examined the CV papillae in the BALB/c wild-type and WT1-KO pups that survived to P0. At birth in the wild type, the CV papillae are formed but the taste buds are not yet mature (Kapsimali and Barlow, 2013). Analysis of Troma1-expressing cells revealed defective expression in CV papillae from the KO mice. The Troma1-expressing cells were not confined to the epithelial layer and were aberrantly localized to the papillae crypt (Fig. 6A): whereas 75% of the Troma-expressing cells were found in the CV epithelium of wild-type mice, only 25% of these cells were localized in the epithelium of WT1-KO mice.

We have shown above (supplementary material Fig. S4) that GAP43 expression is significantly delayed in the developing CV of the WT1-KO embryo. When we analyzed GAP43 expression at P0, the gustatory nerve was branched around the taste bud areas but failed to innervate the individual taste buds forming in the WT1-KO embryos as compared with wild type (Fig. 6B). Thus, even though the basic papillae structure ultimately developed in these KO embryos, the CV papillae did not develop normally.

ChIP analysis revealed that WT1 still binds to the promoters of *Lef1*, *Ptch1* and *Bmp4* at P0 in the BALB/c mice (supplementary material Fig. S6A), and analysis of mRNA from wild-type and WT1-KO P0 taste tissue found that *Lef1*, *Ptch1* and *Bmp4* expression was significantly reduced in the WT1-KO specimens compared with wild type (supplementary material Fig. S6B).

WT1 is required for the normal expression of *Lef1*, *Ptch1* and *Bmp4* in primary taste cell cultures

Our data suggest that WT1 affects CV papillae development by regulating the expression of target genes involved in signaling pathways that have known roles in taste development. To better understand the role of WT1 in the peripheral taste system, we used primary taste cells in culture as a model to study WT1 function. WT1 expression is robust in the cultured mouse taste cells, in particular the type II taste cells that express TRPM5 (Fig. 7A). Cultured taste cells were transfected with either control or WT1 siRNA. After 48 h, immunocytochemical analysis found that WT1 was significantly reduced after transfection with WT1 siRNA but not control siRNA (Fig. 7B). Real-time PCR analysis also revealed a significant reduction in *Wt1* mRNA levels after transfection with WT1 siRNA, as well as a significant decrease in *Lef1* and *Ptch1* expression (Fig. 7C). Since WT1 does not bind to
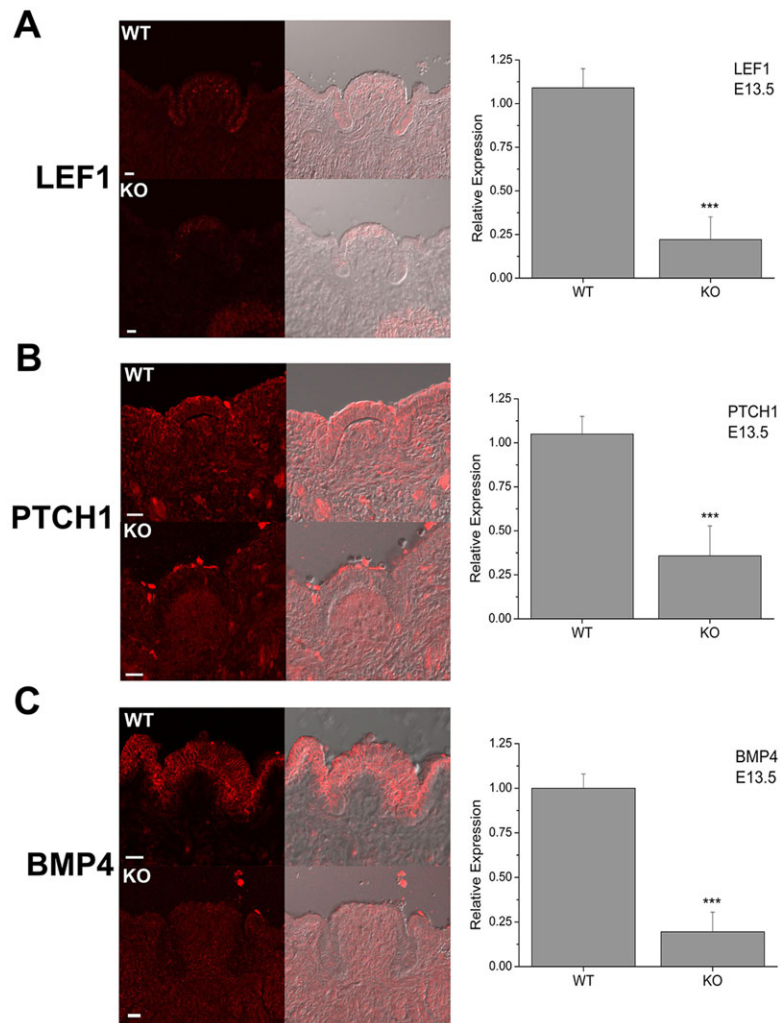
Fig. 5. WT1 is required for the normal expression of *Lef1*, *Ptch1* and *Bmp4* in the developing CV papillae. Immunocytochemical analysis of LEF1 (A), PTCH1 (B) and BMP4 (C) expression in wild-type and WT1-KO embryos at E14.5. Immunoreactivity (red) is shown alone (left) and merged with the DIC image (right). Bar charts show results of qPCR analysis of *Lef1*, *Ptch1* and *Bmp4* in cDNA prepared from E13.5 tongue taste tissue. Data are shown as mRNA relative to *Gapdh*. ***$P<0.001$ (Student's *t*-test); error bars indicate s.d. (*n*=3).

the *Bmp4* promoter in adult mice, we did not analyze BMP4 expression in the cultured taste cells. We conclude that WT1 actively regulates both *Lef1* and *Ptch1* in taste cells.

DISCUSSION

Multiple factors have been shown to play a role in peripheral taste development, the best characterized of which are the Wnt/β-catenin and SHH pathways. The Wnt/β-catenin pathway is required for fungiform taste placode formation, whereas the SHH pathway is an important negative regulator of placode formation (Bitgood and McMahon, 1995; DasGupta and Fuchs, 1999; Iwatsuki et al., 2007; Jung et al., 1999; Liu et al., 2007, 2004). Although both of these signaling pathways are crucial for normal development of the peripheral taste system (Kapsimali and Barlow, 2013), the initial induction factor for these pathways has not been identified. Our results suggest that WT1 is required for the normal induction of *Ptch1* and *Lef1* expression during CV papillae development. These factors are crucial components of the SHH and Wnt pathways that regulate peripheral taste development, although their function is better defined in fungiform than in CV papillae development (Kapsimali and Barlow, 2013). WT1 was also required to regulate BMP4 expression in embryonic taste cells. We note that WT1 expression is sustained in adult taste buds, suggesting its continued requirement for the formation of new taste cells and/or their maintenance.

Previous studies found that SOX2 is required for the formation of the placode in the fungiform papillae (Okubo et al., 2006). Our results suggest that the level and expression pattern of SOX2 is disturbed in the development of the CV of WT1-KO mice. Although *Sox2* has not yet been reported as a WT1 target gene, it is possible that there is some level of connection between these factors in the developing CV papillae. CV papillae formation depends on a balance of expression between Sprouty and FGF genes, which regulate receptor tyrosine kinase signaling to control the number of CV papillae formed (Petersen et al., 2011). Whether there is any relationship between these genes and WT1 expression is currently unknown, but since loss of WT1 expression causes aberrant CV formation, it would be interesting to investigate the potential relationship between these signaling molecules.

In the developing CV, WT1 expression coincides with that of LEF1, PTCH1 and BMP4, which is consistent with a role for WT1 in their expression. We note, however, that LEF1 expression is not evident in all the regions of the placode that express WT1, suggesting that other factors are also important. Indeed, we and others have previously shown that the function of WT1 in transcriptional activation is modulated by its interaction with BASP1 and that this is both cell type- and gene promoter-specific (Essafi et al., 2011; Goodfellow et al., 2011; Green et al., 2009). It will therefore be important to study the potential role of BASP1 in modulating WT1 function during CV formation. Indeed, BASP1 is
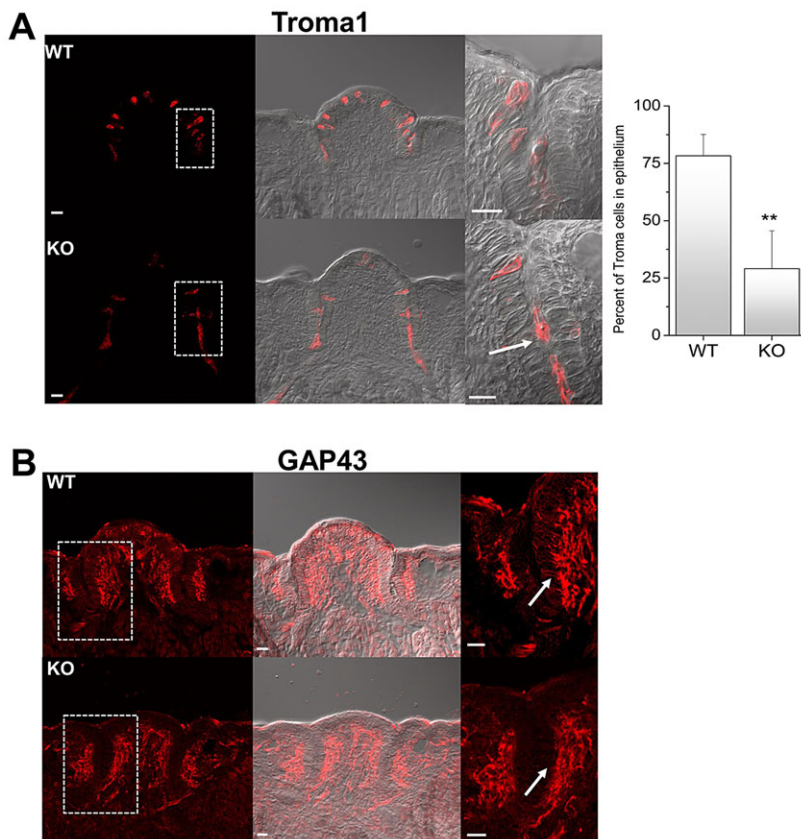
Fig. 6. Defective CV papillae development in BALB/c WT1-KO mice. (A) CV papillae of P0 wild-type and WT1-KO mice were analyzed using Troma1 antibody. Immunoreactivity (red) is shown alone (left) and merged with the DIC image (right). The boxed crypt regions are magnified to the far right. Note the excess localization of Troma1-immunopositive cells in the crypt of WT1-KO mice (arrow) as compared with wild type. The bar chart shows the percentage of Troma1-expressing cells localized within the epithelium for wild-type and WT1-KO mice. **$P<0.01$ (Chi square analysis); error bars indicate s.d. (n=406 for wild type, n=366 for KO). (B) As in A, except that anti-GAP43 was used. Note that the GAP43 immunoreactivity in the wild-type CV section (arrow) is missing in the WT1-KO CV section (arrow). Scale bars: 20 μm.

highly expressed in developing neuronal tissues (Carpenter et al., 2004) and might have a role in the developing taste system. Future studies are needed to address this question.

WT1 plays a crucial role in reciprocal mesenchyme-epithelial transitions, with the cell type direction specified by WT1 co-factors such as BASP1 (Essafi et al., 2011). It will be interesting to determine if WT1 engages its co-factors to regulate the mesenchymal-epithelial interactions that are important in the normal development of the taste buds (Kapsimali and Barlow, 2013). WT1 controls the development of many organ systems and has been shown to be important in the normal development of other peripheral sensory systems including the olfactory epithelium and retinal ganglia (Wagner et al., 2002, 2005). Our data have revealed that WT1 is required for the normal development of the peripheral taste system, in particular the posterior taste field that encompasses CV papillae formation. Taken together with the earlier findings, our data support an extensive role for WT1 in the formation of sensory tissues.

MATERIALS AND METHODS
Taste cell isolation
Two types of WT1-KO mice were used in these experiments. WT1-KO mice from Jackson Labs (stock number 010911) have a mixed genetic background containing C57BL/6, Swiss Webster and B6129SF1/J. These mice have an endogenous *Wt1* coding region replaced with the gene for green fluorescent protein (GFP) and die between E12 and E15. Where indicated, *Wt1* heterozygous mice were crossed with BALB/c mice for one generation and heterozygous offspring were mated to obtain WT1-KO mice. These WT1-KO embryos can survive longer *in utero* and in some litters survive until birth. Of the 263 embryos collected from the BALB/c backcrossed mice, 21 WT1-KO embryos were collected. Some of these embryos were breaking apart and were not usable. A total of seven KO embryos survived to birth.

Animals were cared for in compliance with the University at Buffalo Animal Care and Use Committee. Taste-enriched tissue was collected from tongues at E15.5 for the qPCR and ChIP experiments. In these experiments, the back half of the tongue was collected and homogenized for analysis. For the taste samples collected from P0 mice, the epithelium was peeled and the CV was closely cropped to minimize the amount of non-taste tissue included. Isolated taste receptor cells were harvested from the adult CV papillae as previously described (Hacker et al., 2008) for qPCR, western blot and ChIP experiments.

Immunocytochemistry
Immunocytochemical analysis was performed as previously described (Rebello et al., 2011; Starostik et al., 2010). Antibodies were: anti-WT1 [89 (Toska et al., 2012); 1:50], anti-WT1 (C-19; sc192; 1:50), anti-SHH (sc-9024; 1:50), anti-GAP43 (sc10786; 1:50) from Santa Cruz Biotechnology, anti-Troma1 from Developmental Studies Hybridoma Bank (Iowa City, IA, USA; 1:50), anti-LEF1 (C1285; 1:50; from Signal Transduction), anti-SOX2 (AB97959; 1:50), anti-BMP4 (AB155033; 1:50), anti-WT1 (AB89901; 1:50) and anti-PTCH1 (AB53715; 1:50) from Abcam. Secondary antibodies were purchased from Jackson ImmunoResearch. Some experiments were performed with anti-WT1 that had a fluorophore directly attached using the Mix-n-Stain CF Dye antibody labeling kit (Biotium). In all images, only the brightness and contrast were adjusted. All data are shown as a single 0.1 μm slice from a confocal image except where indicated in the figure legend. If comparisons were made between sections, the laser intensity and brightness/contrast were matched between the conditions being compared.

RNA and ChIP analysis
Total RNA was prepared using the Qiagen RNeasy kit, and cDNA was prepared using the Bio-Rad cDNA synthesis kit. ChIP assays were performed as described previously (Hartkamp et al., 2010). Primers for RNA analysis and ChIP analysis are listed in supplementary material Table S1. All experiments consisted of at least three biological repeats. Data for both the ChIPs and qPCR are reported as average value with standard deviation.
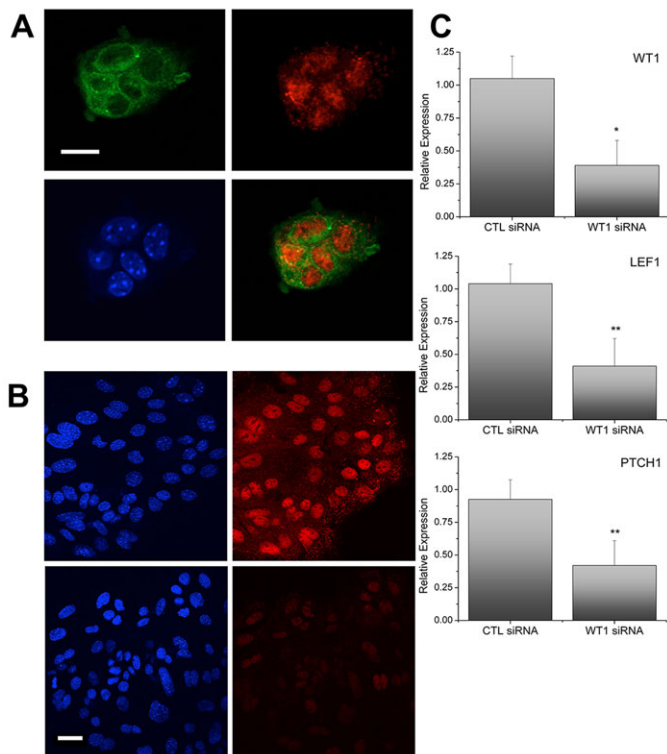
Fig. 7. WT1 controls the expression of *Lef1* and *Ptch1* in adult cultured taste cells. (A) Primary taste cells were isolated from TRPM5-GFP mice and grown in culture for 4-5 days. GFP expression (green) is present in all cells expressing TRPM5 (top left), which is a marker for type II taste cells. Immunocytochemical analysis was performed with anti-WT1 (antibody 89; red, top right). Hoechst was used to stain nuclei (blue, bottom left). Merged images are shown in bottom right. (B) Cultured taste cells were transfected with control (top row) or WT1 siRNA (bottom row) and analyzed with anti-WT1 (antibody 89; red) and Hoechst nuclear staining (blue). (C) qPCR was used to detect *Wt1*, *Lef1* and *Ptch1* in cDNA prepared from cultured taste cells that had been subjected to siRNA transfection to knockdown WT1. Data are mRNA relative to *Gapdh*. *$P<0.05$, **$P<0.01$ (Student's *t*-test); error bars indicate s.d. (*n*=3). Scale bars: 20 μm.

Primary cell culture and transfections

Isolated mouse taste receptor cells were cultured in Iscove's modified Dulbecco's medium on treated coverslips as previously described (Ozdener et al., 2006). After 4 days of culture, cells were transfected with i-Fect (Neuromics) containing control siRNA (Thermo Scientific) or WT1 siRNA (Qiagen). After 48 h, cells were harvested and immunocytochemical or mRNA analysis was performed.

Competing interests
The authors declare no competing financial interests.

Author contributions
K.F.M. and S.G.E.R. conceived the study, designed the experiments, analyzed the data and prepared the manuscript. Y.G., E.T. and D.D. designed and performed experiments, analyzed the data and edited the manuscript.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.105676/-/DC1

References

Armstrong, J. F., Pritchard-Jones, K., Bickmore, W. A., Hastie, N. D. and Bard, J. B. L. (1993). The expression of the Wilms' tumor gene, Wt1, in the developing mammalian embryo. *Mech. Dev.* **40**, 85-97.

Barlow, L. A. (2000). *Gustatory System Development.* New York: Wiley-Liss.

Beites, C. L., Hollenbeck, P. L. W., Kim, J., Lovell-Badge, R., Lander, A. D. and Calof, A. L. (2009). Follistatin modulates a BMP autoregulatory loop to control the size and patterning of sensory domains in the developing tongue. *Development* **136**, 2187-2197.

Bitgood, M. J. and McMahon, A. P. (1995). Hedgehog and Bmp genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. *Dev. Biol.* **172**, 126-138.

Carpenter, B., Hill, K. J., Charalambous, M., Wagner, K. J., Lahiri, D., James, D. I., Andersen, J. S., Schumacher, V., Royer-Pokora, B., Mann, M. et al. (2004). BASP1 is a transcriptional cosuppressor for the Wilms' tumor suppressor protein WT1. *Mol. Cell. Biol.* **24**, 537-549.

DasGupta, R. and Fuchs, E. (1999). Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. *Development* **126**, 4557-4568.

Essafi, A., Webb, A., Berry, R. L., Slight, J., Burn, S. F., Spraggon, L., Velecela, V., Martinez-Estrada, O. M., Wiltshire, J. H., Roberts, S. G. E. et al. (2011). A wt1-controlled chromatin switching mechanism underpins tissue-specific wnt4 activation and repression. *Dev. Cell* **21**, 559-574.

Goodfellow, S. J., Rebello, M. R., Toska, E., Zeef, L. A. H., Rudd, S. G., Medler, K. F. and Roberts, S. G. (2011). WT1 and its transcriptional cofactor BASP1 redirect the differentiation pathway of an established blood cell line. *Biochem. J.* **435**, 113-125.

Green, L. M., Wagner, K. J., Campbell, H. A., Addison, K. and Roberts, S. G. E. (2009). Dynamic interaction between WT1 and BASP1 in transcriptional regulation during differentiation. *Nucleic Acids Res.* **37**, 431-440.

Hacker, K., Laskowski, A., Feng, L., Restrepo, D. and Medler, K. (2008). Evidence for two populations of bitter responsive taste cells in mice. *J. Neurophysiol.* **99**, 1503-1514.

Hartkamp, J., Carpenter, B. and Roberts, S. G. E. (2010). The Wilms' tumor suppressor protein WT1 is processed by the serine protease HtrA2/Omi. *Mol. Cell* **37**, 159-171.

Hartwig, S., Ho, J., Pandey, P., MacIsaac, K., Taglienti, M., Xiang, M., Alterovitz, G., Ramoni, M., Fraenkel, E. and Kreidberg, J. A. (2010). Genomic characterization of Wilms' tumor suppressor 1 targets in nephron progenitor cells during kidney development. *Development* **137**, 1189-1203.

Herzer, U., Crocoll, A., Barton, D., Howells, N. and Englert, C. (1999). The Wilms tumor suppressor gene wt1 is required for development of the spleen. *Curr. Biol.* **9**, 837-840.

Hohenstein, P. and Hastie, N. D. (2006). The many facets of the Wilms' tumour gene, WT1. *Hum. Mol. Genet.* **15** Suppl. 2, R196-R201.

Iwatsuki, K., Liu, H.-X., Gronder, A., Singer, M. A., Lane, T. F., Grosschedl, R., Mistretta, C. M. and Margolskee, R. F. (2007). Wnt signaling interacts with Shh to regulate taste papilla development. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 2253-2258.

Jung, H.-S., Oropeza, V. and Thesleff, I. (1999). Shh, Bmp-2, Bmp-4 and Fgf-8 are associated with initiation and patterning of mouse tongue papillae. *Mech. Dev.* **81**, 179-182.

Kapsimali, M. and Barlow, L. A. (2013). Developing a sense of taste. *Semin. Cell Dev. Biol.* **24**, 200-209.

Kinnamon, J. C. (1987). Organization and innervation of taste buds. In *The Neurobiology of Taste and Smell* (ed. t. E. Finger and W. L. Silver), pp. 277-297. New York: Wiley.

Liu, H.-X., MacCallum, D. K., Edwards, C., Gaffield, W. and Mistretta, C. M. (2004). Sonic hedgehog exerts distinct, stage-specific effects on tongue and taste papilla development. *Dev. Biol.* **276**, 280-300.

Liu, F., Thirumangalathu, S., Gallant, N. M., Yang, S. H., Stoick-Cooper, C. L., Reddy, S. T., Andl, T., Taketo, M. M., Dlugosz, A. A., Moon, R. T. et al. (2007). Wnt-beta-catenin signaling initiates taste papilla development. *Nat. Genet.* **39**, 106-112.

Mbiene, J.-P. and Mistretta, C. M. (1997). Initial innervation of embryonic rat tongue and developing taste papillae: nerves follow distinctive and spatially restricted pathways. *Acta Anat. (Basel)* **160**, 139-158.

Mbiene, J.-P. and Roberts, J. D. (2003). Distribution of keratin 8-containing cell clusters in mouse embryonic tongue: evidence for a prepattern for taste bud development. *J. Comp. Neurol.* **457**, 111-122.

Mistretta, C. M. (1972). Topographical and histological study of the developing rat tongue, palate and taste buds. In *Third Symposium on Oral Sensation and Perception. The Mouth of the Infant* (ed. J. F. Bosma), pp. 163-187. Springfield, IL: Charles C Thomas.

Mistretta, C. M. (1991). Developmental neurobiology of taste. In *Smell and Taste in Health and Disease* (ed. X.-M. Getchell, R. Doty, L. Bartoshuk and J. Snow), pp. 35-64. New York: Raven Press.

Niksic, M., Slight, J., Sanford, J. R., Caceres, J. F. and Hastie, N. D. (2004). The Wilms' tumour protein (WT1) shuttles between nucleus and cytoplasm and is present in functional polysomes. *Hum. Mol. Genet.* **13**, 463-471.

Okubo, T., Pevny, L. H. and Hogan, B. L. M. (2006). Sox2 is required for development of taste bud sensory cells. *Genes Dev.* **20**, 2654-2659.

Ozdener, H., Yee, K. K., Cao, J., Brand, J. G., Teeter, J. H. and Rawson, N. E. (2006). Characterization and long-term maintenance of rat taste cells in culture. *Chem. Senses* **31**, 279-290.

Petersen, C. I., Jheon, A. H., Mostowfi, P., Charles, C., Ching, S., Thirumangalathu, S., Barlow, L. A. and Klein, O. D. (2011). FGF signaling regulates the number of posterior taste papillae by controlling progenitor field size. *PLoS Genet.* **7**, e1002098.

Rebello, M. R., Aktas, A. and Medler, K. F. (2011). Expression of calcium binding proteins in mouse type II taste cells. *J. Histochem. Cytochem.* **59**, 530-539.

Starostik, M. R., Rebello, M. R., Cotter, K. A., Kulik, A. and Medler, K. F. (2010). Expression of GABAergic receptors in mouse taste receptor cells. *PLoS ONE* **5**, e13639.

Toska, E., Campbell, H. A., Shandilya, J., Goodfellow, S. J., Shore, P., Medler, K. F. and Roberts, S. G. E. (2012). Repression of transcription by WT1-BASP1 requires the myristoylation of BASP1 and the PIP2-dependent recruitment of histone deacetylase. *Cell Rep.* **2**, 462-469.

Vajjhala, P. R., Macmillan, E., Gonda, T. and Little, M. (2003). The Wilms' tumour suppressor protein, WT1, undergoes CRM1-independent nucleocytoplasmic shuttling. *FEBS Lett.* **554**, 143-148.

Wagner, K.-D., Wagner, N., Vidal, V. P. I., Schley, G., Wilhelm, D., Schedl, A., Englert, C. and Scholz, H. (2002). The Wilms' tumor gene Wt1 is required for normal development of the retina. *EMBO J.* **21**, 1398-1405.

Wagner, N., Wagner, K.-D., Hammes, A., Kirschner, K. M., Vidal, V. P., Schedl, A. and Scholz, H. (2005). A splice variant of the Wilms' tumour suppressor Wt1 is required for normal development of the olfactory system. *Development* **132**, 1327-1336.

Zhou, Y., Liu, H.-X. and Mistretta, C. M. (2006). Bone morphogenetic proteins and noggin: inhibiting and inducing fungiform taste papilla development. *Dev. Biol.* **297**, 198-213.

DEVELOPMENT