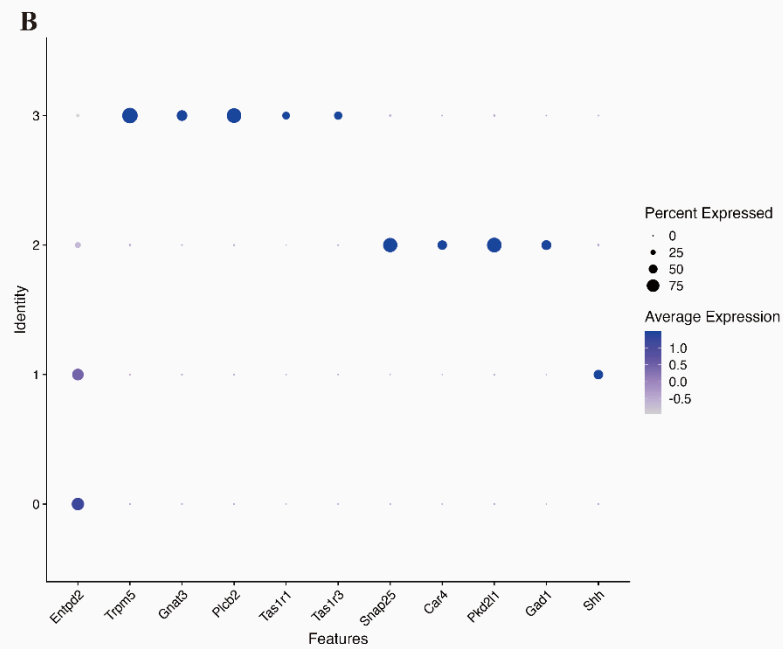
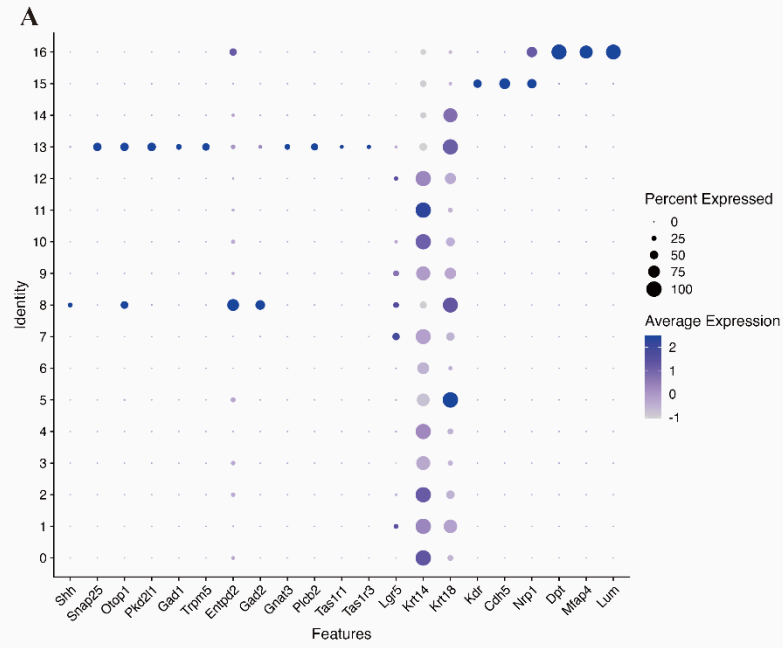


Exploring the Role of Ccn3 in Type III Cell of Mice Taste Buds

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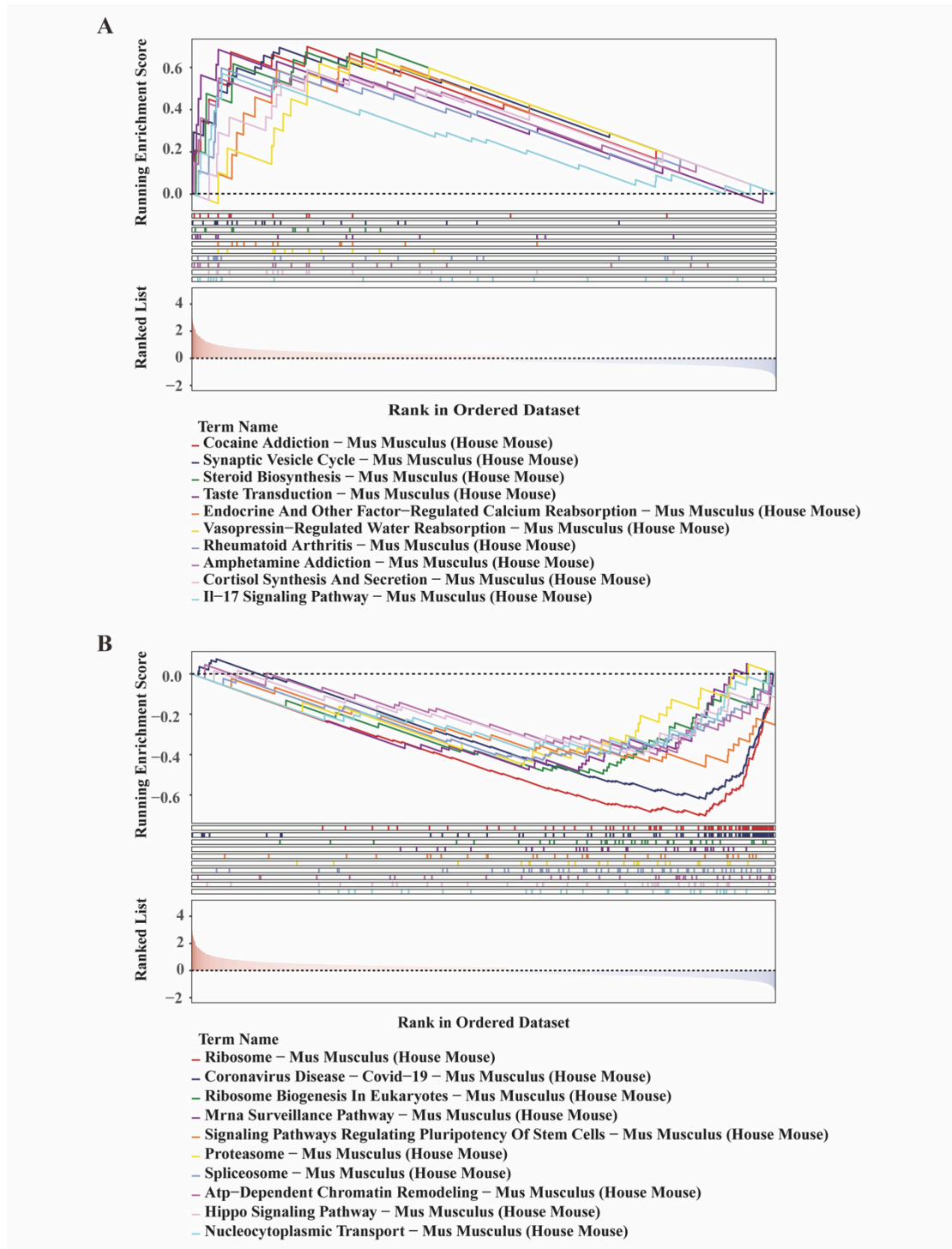
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Supplementary Figure 1. Dot Plot of marker genes.

(A) Dot Plot illustrating the expression of marker genes across various cell subsets in epithelial cells from the CvP and the FoP of the mouse tongue. Cluster 8 and 13 are taste cell clusters. The markers corresponding to the cell annotation are presented in Supplementary Table 9. (B) Dot Plot illustrating the expression of marker genes across various cell subsets in taste cells from the CvP and the FoP of the mouse tongue. Cluster 0 is Type I taste cell, cluster 1 is Type IV taste cell, cluster

2 is Type III taste cell, and cluster 4 is Type II taste cell. The markers corresponding to the cell annotation are presented in Supplementary Table 10. Row data from (Vercauteren Drubbel & Beck, 2023).



Supplementary Figure 2. Predicting the potential functions of *Ccn3*+Type III and *Ccn3*-Type III cell subgroups.

(A) GSEA enrichment analysis showing the top ten genes sets enriched in *Ccn3*+Type III cell subgroups. (B) GSEA enrichment analysis showing the top ten genes sets enriched in *Ccn3*-Type III cell subgroups. Row data from (Vercauteren Drubbel & Beck, 2023).

Supplementary Table 1. Primers for RT-PCR

	Forward	Reverse	product
<i>Ccn3</i>	CAGACCCCAACAACCAGACT	TCAACTCCTACGGTGGCTTC	325bp
<i>Gnat3</i>	TGCTTTGAAGGAGTGACGTG	GTAGCGCAGGTCATGTGAGA	341bp
<i>Actb</i>	GGTTCCGATGCCCTGAGGCTC	ACTTGCGGTGCACGATGGAGG	370bp

Supplementary Table 2. Primers for DNA templates for ISH probes

	Forward	Reverse	product
<i>Ccn3</i>	AGCCTGTAACAGCCCTAGCA	ATTTCTGGTGCGGAGACAC	827bp
AS probe	AGCCTGTAACAGCCCTAGCA	CTAATACGACTCACTATAGGGAGA ATTTCTGGTGCGGAGACAC	
S probe	CTAATACGACTCACTATAGGGAGA AGCCTGTAACAGCCCTAGCA	ATTTCTGGTGCGGAGACAC	

Supplementary Table 3. Primers for qRT-PCR

	Forward	Reverse	product
<i>Gapdh</i>	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG	150bp
<i>Entpd2</i>	ATGGCTGGAAAGTTGGTGTC	TCTTGGGTAGGGACGCACA	92bp
<i>Gnat3</i>	AGGGCATCTGAATACCAGCTCAA	CTGATCTCTGGCCACCTACATCAA	196bp
<i>Ca4</i>	TTGTCCTGCTAGGACAAAGGTGAA	TCCGATAATGCACGCACCTC	140bp
<i>Otop1</i>	TCACGGCCTTCTGTTTCTT	GCCAAAGACAATTCCTCCA	101bp
<i>Ccn3</i>	AGCCTGTAACAGCCCTAGCA	CGCAGAGATGCAGAGACTTG	111bp

Supplementary Table 4. Two-sample Student's t-test results of taste cell numbers in FuP.

Cell type	Degrees of Freedom	T-value	P-value
Type II cells	176	-1.05	0.296
Type III cells	150	0.306	0.760

*:P<0.05, **:P<0.01, ***: P<0.001

Supplementary Table 5. Two-sample Student's t-test results of qRT-PCR.

Gene	Group	Degrees of Freedom	T-value	P-value
<i>Entpd2</i>	FuP	9	-1.3822	0.2002
<i>Gnat3</i>	FuP	9	-1.1991	0.2611
<i>Ca4</i>	FuP	9	-0.9271	0.9282
<i>Otop1</i>	FuP	9	-1.7645	0.1145
<i>Ccn3</i>	FuP	9	7.9719	<0.001***
<i>Entpd2</i>	CvP	10	-1.3874	0.1954
<i>Gnat3</i>	CvP	10	-0.0596	0.9537
<i>Ca4</i>	CvP	10	0.4331	0.6741
<i>Otop1</i>	CvP	10	-0.561	0.5872
<i>Ccn3</i>	CvP	10	11.178	<0.001***

*:P<0.05, **:P<0.01, ***: P<0.001

Supplementary Table 6. Two-way ANOVA results of CT nerve recordings.

Tastant	Effect	Degree of Freedom	F Value	p Value
HCl	genotype	1.00	3.76	0.057
	concentration	4.00	158.18	<0.001***
	interaction	4.00	1.05	0.391
Sucrose	genotype	1.00	0.71	0.403
	concentration	4.00	98.464	<0.001***
	interaction	4.00	0.779	0.543
MPG	genotype	1.00	0.0199	0.888
	concentration	3.00	45.6768	<0.001***
	interaction	3.00	0.2961	0.828
Quinine	genotype	1.00	1.756	0.19
	concentration	4.00	140.694	<0.001***
	interaction	4.00	0.295	0.88
NaCl	genotype	1.00	1.124	0.293
	concentration	4.00	144.323	<0.001***
	interaction	4.00	0.338	0.852

*:P<0.05, **:P<0.01, ***: P<0.001

Supplementary Table 7. Repeated ANOVA results of behavioral lick responses.

Tastant	Effect	Degree of Freedom	F Value	p Value
HCl	genotype	2.00	0.953	0.408
	concentration	5.00	2040.773	<0.001***
	interaction	10.00	0.719	0.704
Citric acid	genotype	2.00	0.0128	0.987
	concentration	5.00	3926.921	<0.001***
	interaction	10.00	0.549	0.849
NaCl	genotype	2.00	0.131	0.878
	concentration	5.00	1872.2	<0.001***
	interaction	10.00	1.05	0.413
Quinine	genotype	2.00	0.292	0.751
	concentration	4.00	1963.566	<0.001***
	interaction	8.00	0.622	0.756
Sucrose	genotype	2.00	0.104	0.902
	concentration	4.00	1368.71	<0.001***
	interaction	8.00	1.8	0.096
MSG	genotype	2.00	1.34	0.292
	concentration	4.00	1500.18	<0.001***
	interaction	8.00	0.323	0.954

*:P<0.05, **:P<0.01, ***: P<0.001

Supplementary Table 8. Results of one-way ANOVA on 10 μ M capsaicin behavioral lick responses.

	Degree of Freedom1	Degree of Freedom2	F Value	p Value
Value	2.00	8.63	182	<.001**

Tukey Post-Hoc Test – Value

		WT	<i>Ccn3</i> -KO	<i>Ccn3/Trpv1</i> -KO
WT	Mean difference	—	-0.181	-30.2
	p-value	—	0.989	<.001**
<i>Ccn3</i> -KO	Mean difference	—	—	-30.1
	p-value	—	—	<.001**
<i>Ccn3/Trpv1</i> -KO	Mean difference	—	—	—
	p-value	—	—	—

*:P<0.05, **:P<0.01, ***: P<0.001

Supplementary Table 9. The marker corresponding to the cell annotated manually for the first time.

Taste cell	<i>Shh/Snap25/Otop1/Pkd2l1/Gad1/Trpm5/Entpd2/Gad2/Gnat3/Plcb2/Tas1r1/Tas1r3/Krt18</i>
Epithelial cell	<i>Krt14</i>
Epithelial progenitor cell	<i>Krt14/Lgr5</i>
Endothelial cell	<i>Kdr/Cdh5/Nrp1</i>
Fibroblasts cell	<i>Dpt/Mfap4/Lum</i>

Supplementary Table 10. The marker corresponding to the cell annotated manually for the second time.

Type I taste cell	<i>Entpd2</i>
Type II taste cell	<i>Trpm5/ Gnat3/Plcb2/Tas1r1/Tas1r3</i>
Type III taste cell	<i>Snap25/Otop1/Pkd211/Gad1</i>
Type IV taste cell	<i>Shh</i>