

Supplementary data table

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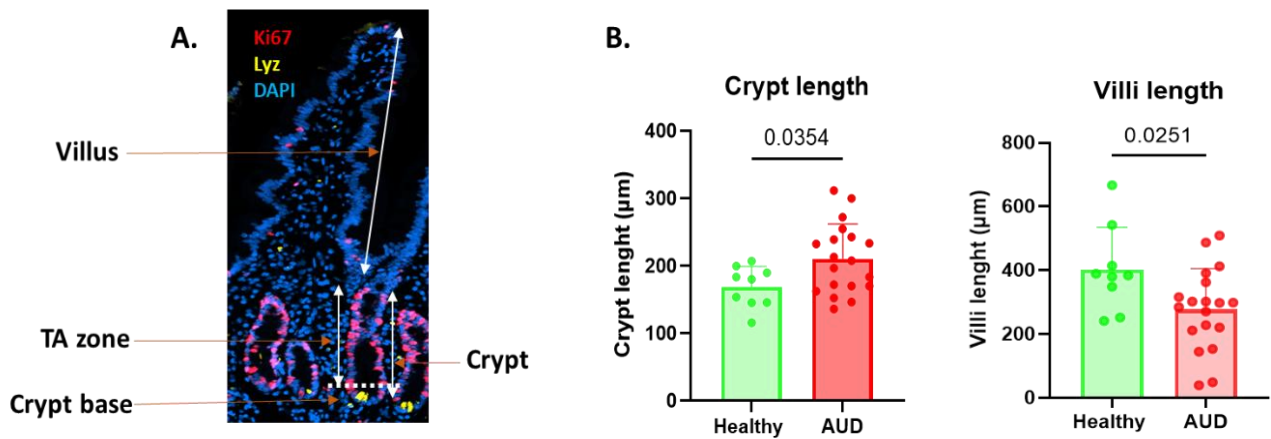
Supplementary table 1: Primers used to perform RT-qPCR

Genes	Orientation	Primers (5'-3')
Cyclin B1	Forward	CCCTGCTGCAACCTCCAA
	Reverse	CTGCAGTTGTTCACTGACTTTGTTAC
CD44	Forward	TGGACAGGACAGGACCTCCT
	Reverse	TTCTTCCAAGCCTTCATGTG
MUC2	Forward	TTCTTCCTATCTTGCTCCTGATG
	Reverse	AATGGAACAGATGTTGAAGTGCT
LGR5	Forward	CCTTCATAAGAAAGATGCTGGAAT
	Reverse	GTTTAATGGGGGAAATGTACAGAG
Axin 2	Forward	AGTGTGAGGTCCACGGAAAC
	Reverse	TGGCTGGTGCAAAGACATAG
KLF4	Forward	CGGACATCAACGACGTGAG
	Reverse	GACGCCTTCAGCACGAACT
SPDEF	Forward	CAGTGCCCGGTCATTGACA
	Reverse	CAGCCGGTATTGGTGCTCT

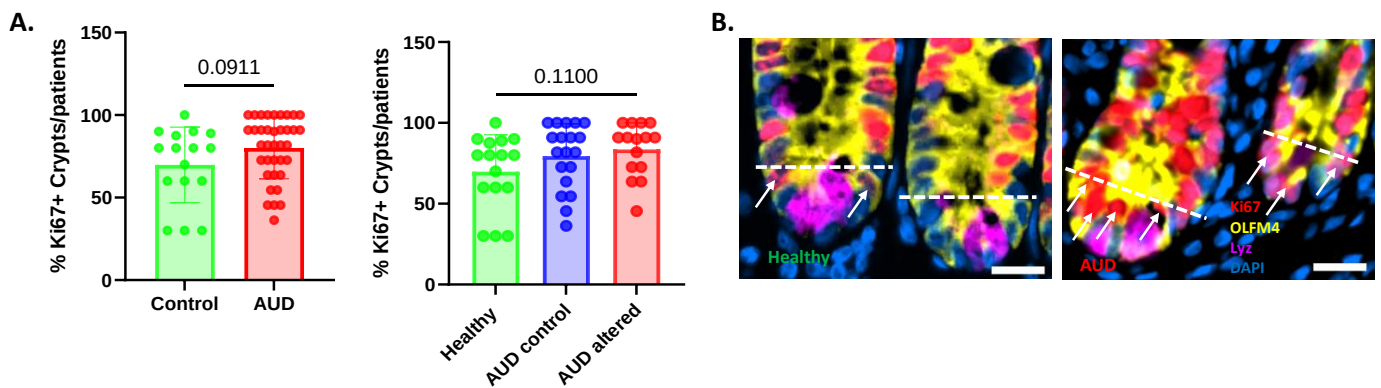
Supplementary table 2: Antibodies used for Immunofluorescence. RT: Room Temperature; ON: Overnight.

Antibodies	Dilution	Species	Supplier	Catalogue n°
Mucin2 (MUC2)	1:200, ON, 4°C	Mouse Monoclonal	Santa Cruz	Sc-515032
Lysozyme (LYZ)	1:400 ON, 4°C	Rabbit Monoclonal	Abcam	Ab223503
Olfactomedin 4 (OLFM4)	1:200 ON, 4°C	Rabbit Monoclonal	Cell Signaling	14369S
E-cadherin (E-cad)	1:100 ON, 4°C	Mouse Monoclonal	Dako	M3612
chromogranin A (CHGA)	1:200 ON, 4°C	Mouse Monoclonal	Cell Signaling	36468S
Axin 2	1 :200 ON, 4°C	Rabbit Recombinant monoclonal	Abcam	ab109307
Nuclear Factor of Activated T Cells 1 (NFATC1)	1:350 1h, RT	Rabbit Recombinant Monoclonal	ThermoFisher scientific	MA532686
CD44	1 :400 1h, RT	Mouse Monoclonal	Cell Signaling	#3570S
Phospho-p44/42 MAPK (pErk1/2) Thr202/Tyr204	1 :400 ON, 4°C	Rabbit Monoclonal	Cell Signaling	#4370S

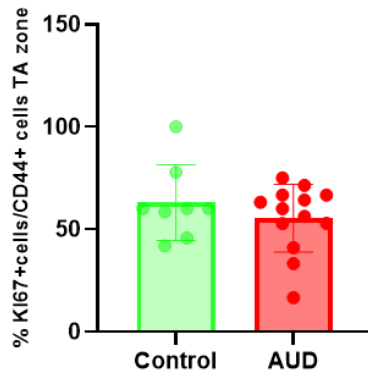
Phospho-EGF Receptor (pEGFR) Tyr1068	1 :400 ON, 4°C	Rabbit Monoclonal	Cell Signaling	#3777S
c-Jun phospho Ser63(pcJun (S63))	1 :400 ON, 4°C	Rabbit Monoclonal	Abcam	ab32385
Ki67	1 :400 ON, 4°C	Mouse Monoclonal	BECTON DICKINSON BENELUX SA (BD biosciences)	Cat. 556003
Dako EnVision+ System- HRP Labelled Polymer (Anti- mouse)	Drops 1h, RT	Mouse Secondary Ab	DAKO	K4001
Dako EnVision+ System- HRP Labelled Polymer (Anti- rabbit)	Drops 1h, RT	Rabbit Secondary Ab	DAKO	K4003
Alexa Fluor™ 488 tyramide	1:200 10 min, RT	-	ThermoFisher scientific	B40953
Alexa Fluor™ 594 tyramide	1:200 10 min, RT	-	ThermoFisher scientific	B40957
CF® 754 tyramide	1:1000 10 min, RT	-	VWR	Cat. 96090
CF®555 tyramide	1:1000 10 min, RT	-	VWR	Cat. 96021
Alexa Fluor™ 488 donkey anti-mouse (Anti- Ms)	1:1500 ON, 4°C	Donkey Secondary Ab	Invitrogen	A21202
Alexa Fluor™ 594 donkey anti-rabbit (Anti- Rb)	1:1500 ON, 4°C	Donkey Secondary Ab	Invitrogen	A21207



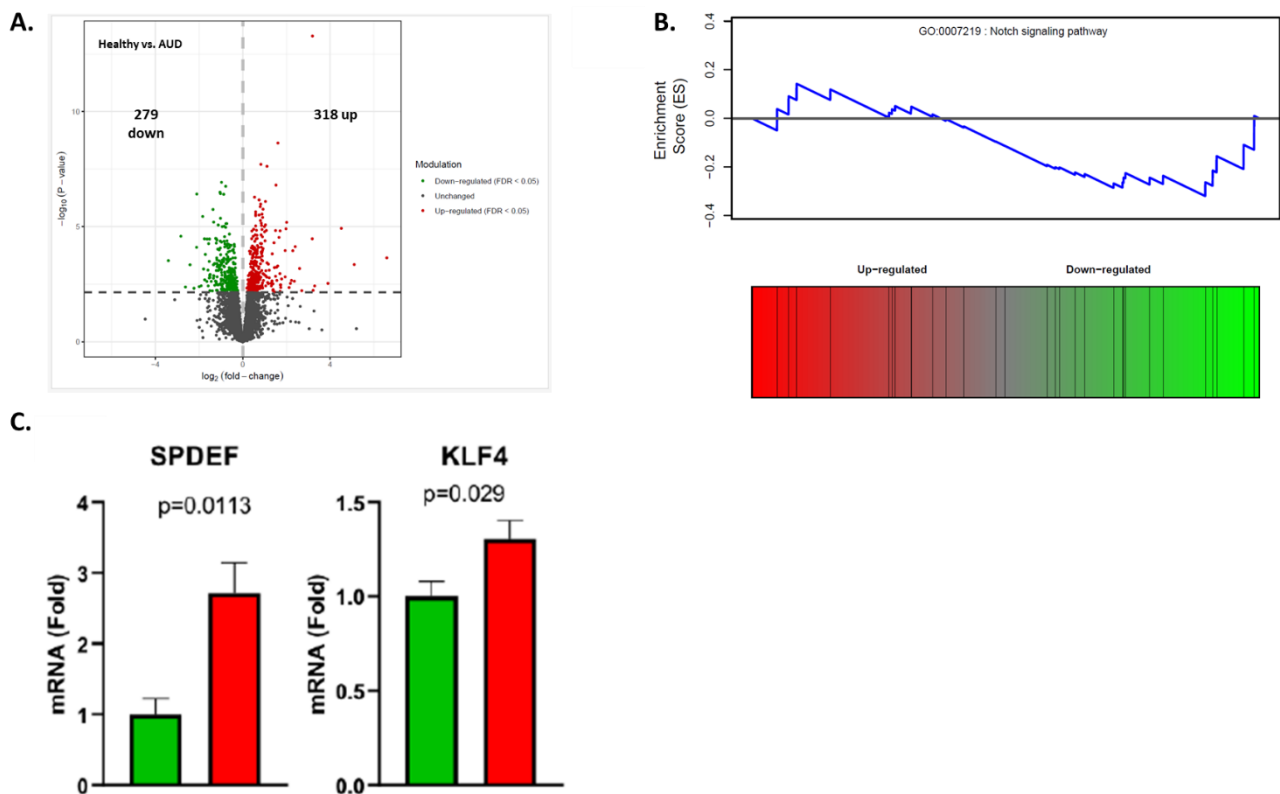
Supplementary Figure 1. Crypt/villus length altered in AUD. **A.** Immunofluorescence imaging showing the boundaries of the crypt, the transit-amplifying (TA) zone, and the villus. Ki67 (Proliferation) in red, Lyz (Paneth cells) in yellow, DAPI (nuclei) in blue. **B.** Crypt length (μm) significantly increased while Villi length significantly decreased in AUD.



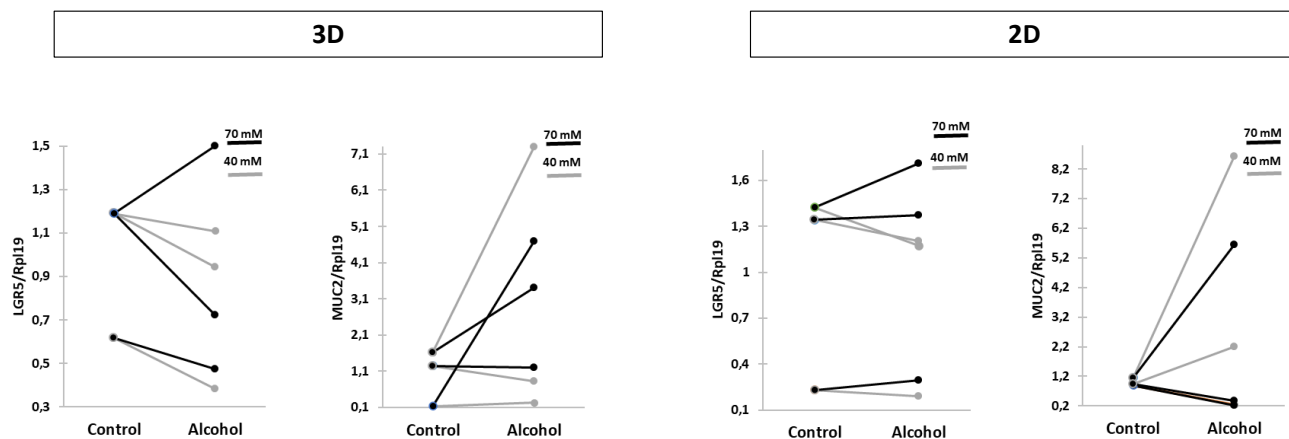
Supplementary Figure 2. Increased cell proliferation in AUD. **A.** Bar graphs indicate the total number of Ki67+ crypts (Crypts containing at least one proliferating cell) per patient in percentage. **B.** Immunofluorescence imaging showing proliferating cells (Ki67, red) double positive for OLFM4 (Yellow) marker that stains stem and stem-like cells (arrows). Lyz (Paneth cells) in yellow to identify the crypt base, DAPI (nuclei) in blue.



Supplementary Figure 3. CD44 protein does not drive TA zone hyperproliferation. Bar graphs indicate the percentage of double positive Ki67+/CD44+ cells per total CD44+ cells in TA zone with no significant difference observed between Healthy controls and AUD.



Supplementary Figure 4. Impaired differentiation and Goblet cells expression in AUD. **A.** Bulk proteomic volcano plot. A high number of proteins (597) was differentially regulated (adjusted $p < 0.05$): 279 proteins were downregulated and 318 proteins upregulated (fig 4C). **B.** Enrichment analysis of proteomic data showing downregulation of the Notch signaling pathway involved in epithelial cell differentiation in AUD vs. healthy subjects. **C.** Gene expression of SPDEF and KLF4 by qPCR, transcription factors implicated in terminal differentiation of Goblet Cells confirms their upregulation in AUD patients (red bars) compared to healthy subjects (green bars) in qPCR.



Supplementary Figure 5. Gene expression of epithelial cell markers in ethanol treated enteroids in the 3D and 2D model. No significant difference was observed in LGR5 (Stem cells) gene expression in both models. MUC2 gene expression was more variable and increased in two individuals at 70 mM in the 3D model.