
Supplementary information

Brain ethanol metabolism by astrocytic ALDH2 drives the behavioural effects of ethanol intoxication

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1 **Supplementary Table 1** Quantitative data for colocalization of GFAP with ALDH2
 2 in mouse cerebellum ($n=3$).

Group	cells		Colocalization GFAP vs. ALDH2	Colocalization /GFAP (%)	Average (mean±s.e.m)
	GFAP	ALDH2			
Mouse					
	71	67	67	94.36	
	84	78	76	90.47	94.67±2.52
	124	124	123	99.19	

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4 **Supplementary Table 2** Quantitative data for colocalization of ALHD1L1 with
 5 ALDH2 in mouse and human cerebellum ($n=3$).

Group	cells		Colocalization ALDH1L1 vs. ALDH2	Colocalization /ALDH1L1 (%)	Average (mean±s.e.m)
	ALDH1L1	ALDH2			
Mouse					
	156	175	108	69.23	
	204	241	116	56.86	65.88±4.56
	116	128	83	71.55	
Human					
	148	156	114	77.03	
	176	191	136	77.27	81.68±4.53
	378	443	343	90.74	

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7 **Supplementary Table 3** Key resources table

Reagent or Resource	Source	Identifier
<u>RNAScope Probes</u>		
Mm ALDH2 Probe	Advanced Cell Diagnostics, Inc.	NM_009656.4
Mm Cre Probe	Advanced Cell Diagnostics, Inc.	KC845567.1
Mm MAP2 Probe	Advanced Cell Diagnostics, Inc.	NM_001039934.1
Mm GFAP Probe	Advanced Cell Diagnostics, Inc.	NM_001131020.1
Mm EGFP Probe	Advanced Cell Diagnostics, Inc.	U55763.1
Mm Pcp2 Probe	Advanced Cell Diagnostics, Inc.	NM_001129804.1
Hs Pcp2 Probe	Advanced Cell Diagnostics, Inc.	NM_174895.2
Hs ALDH2 Probe	Advanced Cell Diagnostics, Inc.	NM_000690.3

Hs ALDH1L1 Probe	Advanced Cell Diagnostics, Inc.	NM_001270364.1
Cyanine 1	Perkin Elmer	NEL756001KT
Cyanine 3	Perkin Elmer	FP1170
Cyanine 5	Perkin Elmer	FP1171
Antifade Reagent	Thermo Fisher Scientific	P36930
<u>Antibodies</u>		
Goat anti-ALDH2	Santa Cruz Biotechnology	sc-48837
Mouse anti-actin	Cell signaling technology	3700S
Rabbit anti-GABA	Abcam	Ab8891
Goat anti-GFAP	Santa Cruz Biotechnology	sc-6171
Alexa 488 donkey anti-rabbit	Thermo Fisher	A21206
Alexa 568 donkey anti goat	Thermo Fisher	A11057
Donkey anti-goat	Abcam	Ab6885
<u>Virus Strains</u>		
AVV5-GFAPCre	Addgene	105550-AAV5
AVV9-Synt1Cre	Addgene	105553-AVV9
<u>Chemicals</u>		
D-AP5	TOCRIS	0106
DNQX	TOCRIS	2312
TTX	BIOTIUM	00061
QX-314	TOCRIS	552233
Bicuculline	EMD Millipore Corp.	14343
Sodium acetate	Sigma-Aldrich	S2889
Ethyl alcohol	Sigma-Aldrich	E-7148
QIAzol® lysis reagents	QIAGEN sciences	79306
TaqMan fast advance Master mix	Thermo Fisher Scientific	4444556
Phosphatase inhibitor Cocktails	Cell signaling technology	5871S
PMSF	Cell signaling technology	8553S
RIPA buffer	Cell signaling technology	9806S

Paraformaldehyde	Alfa Aesar Chemical	J61899
Fetal bovine serum	Gibco	A31604-01
RNA later™ Solu	Thermo Fisher Scientific	Am7021
¹³ C-1-Acetate	Sigma-Aldrich	279293
¹³ C-1-GABA	Sigma-Aldrich	678236
Cyanamide	Sigma-Aldrich	187364

Commercial Assays

Pierce® BCA protein assay kit	Thermo Fisher Scientific	23228
Reverse Transcription kit	QIAGEN sciences	205311
Mitochondrial ALDH2 enzyme activity kit	Abcam	Ab115348
RNAscope® H2O ₂ and protein reagents	Advanced Cell Diagnostics, Inc.	322381
RNAscope® wash buffer solution	Advanced Cell Diagnostics, Inc.	310091
RNAscope® multiplex fluorescent detection reagents v2	Advanced Cell Diagnostics, Inc.	323110
Jess/Wes separation	Jess & Wes	SM-W004
RNeasy® lipid tissue Mini kit	Jess & Wes	DM-001
Anti-mouse detection module	Jess & Wes	DM-002
