Supporting Information

Chen et al. 10.1073/pnas.1414657112

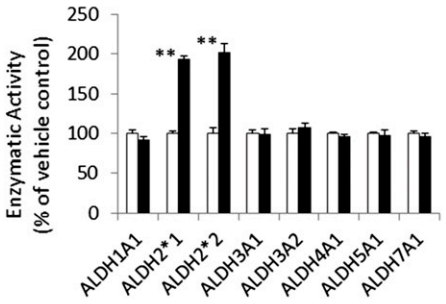
Materials and Methods

Full-length ALDH cDNA clones for ALDH3A1, ALDH3A2, ALDH4A1, and ALDH7A1 isozymes were purchased from ATCC with the following GenBank accession numbers: BC004370, BC002430, BC007581, and BC002515, respectively. Full-length ORF of each isozyme was obtained by PCR and subcloned into pTrcHis expression vector with a His-tag at the N terminus. ALDH1A1, ALDH2*1, ALDH2*2, ALDH3A1, ALDH3A2, ALDH4A1, ALDH5A1, and ALDH7A1 recombinant proteins were expressed in *Escherichia coli* and purified by affinity Ni-columns (HisTrap column, GE Healthcare Sciences), using methods as previously described (1, 2). All purified ALDH recombinants enzymes were stored at −80 °C in Ni-column elution buffer containing 50% (vol/vol) glycerol.

1. Estey T, Piatigorsky J, Lassen N, Vasiliou V (2007) ALDH3A1: A corneal crystallin with diverse functions. *Exp Eye Res* 84(1):3–12.

2. Wymore T, et al. (2004) Molecular recognition of aldehydes by aldehyde dehydrogenase and mechanism of nucleophile activation. *Proteins* 57(4):758–771.

A

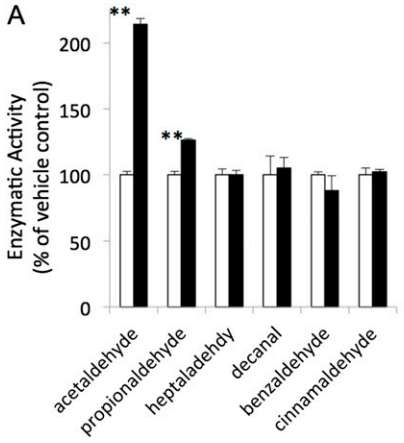


B

	Specific Activity (μmole/min/mg)		
Isozyme	Vehicle	Alda-1	
ALDH1A1	498 ± 23	453 ± 26	
ALDH2*1	2885 ± 67	5580 ± 126**	p<0.01
ALDH2*2	544 ± 40	1099 ± 60**	p<0.01
ALDH3A1	698 ± 33	690 ± 49	
ALDH3A2	69 ± 4	73 ± 4	
ALDH4A1	206 ± 3	199 ± 4	
ALDH5A1	1747 ± 34	1688 ± 116	
ALDH7A1	309 ± 10	296 ± 12	

Fig. S1. Selectivity of Alda-1 for ALDH2. (*A*) Alda-1 (20 μM) selectively increases the activity of wild-type ALDH2*1/*1 and mutant ALDH2*1/*2 recombinant human enzymes by twofold relative to vehicle control, without affecting the activity of six other members of the ALDH superfamily (5–20 mg of each enzyme, using 10 mM acetaldehyde as a substrate); data are presented as percentage of control. (*B*) Same as in *A*, but data are expressed as enzyme-specific activity (μmol NADH/min/mg protein) based on kinetic production of NADH. Data from three independent assays were collected and analyzed ($n = 3$; $^{**}P < 0.01$; bars represent the mean ± SD).

A



B

	Specific Activity (μmole/min/mg)		
Substrate	Vehicle	Alda-89	
Acetaldehyde	656 ± 86	1408 ± 58**	p<0.01
Propionaldehyde	929 ± 45	1164 + 42**	p<0.01
Heptaldehyde	1668 ± 71	1674 ± 55	
Decanal	1216 ± 171	1272 ± 98	
Benzaldehyde	2968 ± 57	2616 ± 330	
Cinnamaldehyde	5881 ± 295	6002 ± 122	

Fig. S2. Alda-89 enhances ALDH3A1 metabolism of short-chain aliphatic aldehyde. (*A*) Accelerated metabolism by Alda-89 was only observed when small aliphatic aldehydes, such as acetaldehyde and propionaldehyde, were used as a substrate. Alda-89 had no effect on the metabolism of either longer chain aliphatic aldehydes, such as heptaldehyde (C7) or decanal (C10), or aromatic aldehydes, such as benzaldehyde or cinnamaldehyde. Data from three independent assays were collected and analyzed ($n = 3$; $^{**}P < 0.01$; bars represent the mean ± SD). (*B*) Same as in *A*, but data are expressed as enzyme-specific activity (μmol NADH/min/mg protein) based on kinetic production of NADH.

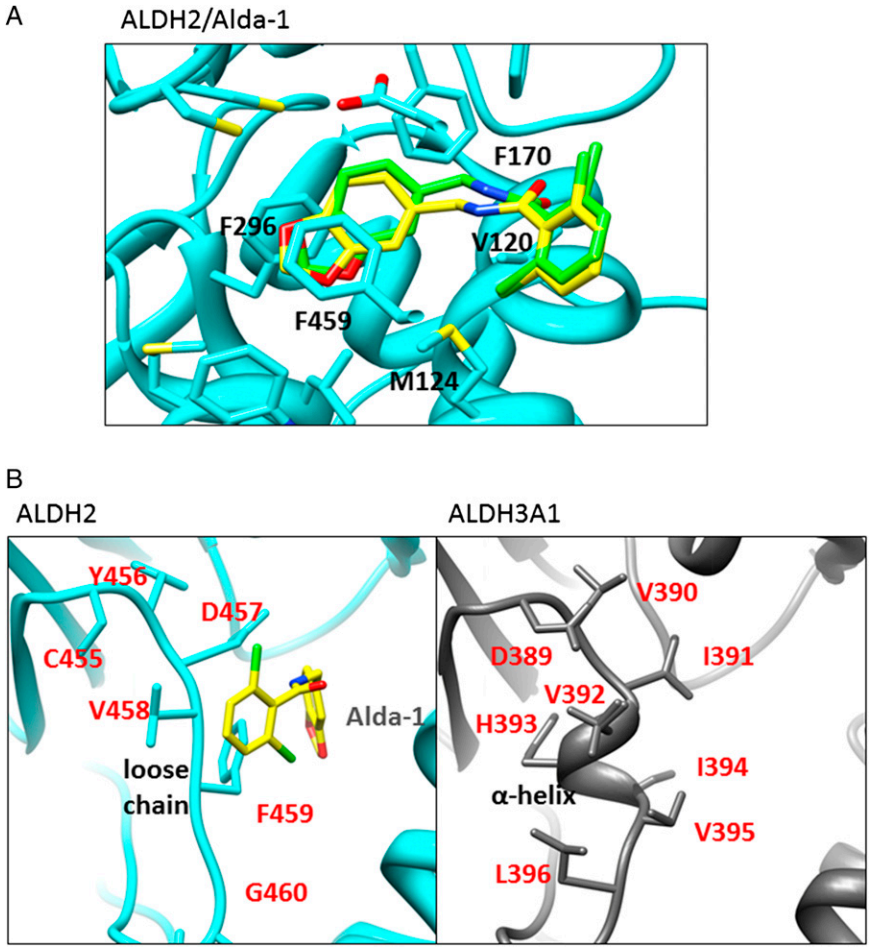


Fig. S3. Docked model of Alda-1 bound to ALDH2. (*A*) Confirmation of a docking similar to the orientation as the previously published cocrystal structure of Alda-1 and ALDH2 (1). Superposition of docked Alda-1 (green sticks) on a cocrystal of ALDH2 with Alda-1 (yellow sticks). ALDH2 is shown in blue ribbon and sticks. (*B*) In ALDH2, close to the Alda-1 binding site, amino acids 455–460 form a loose chain, whereas the corresponding amino acids (389–396) in ALDH3A1 form an α-helix.

1. Chen CH, et al. (2008) Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart. *Science* 321(5895):1493–1495.

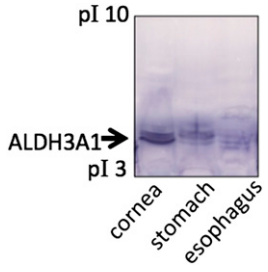


Fig. S4. IEF in-gel ALDH enzyme activity obtained from esophagus and stomach. Detection of ALDH3A1 enzymatic activity was carried using isoelectric gel electrophoresis and in-gel activity staining, as described in the *Materials and Methods*. One hundred micrograms protein from cornea (the richest source of ALDH3A1 (1), stomach, and esophagus tissue homogenates were loaded onto precast PhastGel IEF, 3–10 (GE Healthcare Sciences). After 40 min of focusing, gels were incubated with acetaldehyde, NAD^+-coupled nitroblue tetrazolium/phenazine methosulfate solution, and color reaction developed as a result of formazan formation by ALDH3A1 was monitored as described in the *Materials and Methods*. The arrow indicates the position of ALDH3A1 from cornea; bands in this position are also seen in the stomach and, to a lesser extent, in the esophagus, indicating the presence of ALDH3A1 in these tissues.

1. Estey T, Piatigorsky J, Lassen N, Vasiliou V (2007) ALDH3A1: A corneal crystallin with diverse functions. *Exp Eye Res* 84(1):3–12.

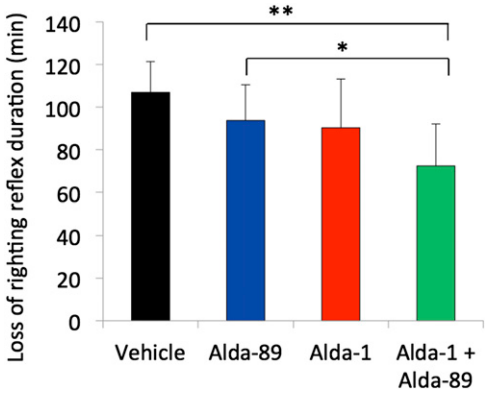


Fig. S5. Effect of Alda-1 and Alda-89 on ethanol-induced loss of self-righting reflex (LORR) in mice. Alda-1, Alda-89 (90 mg/kg), and ethanol (3.3 g/kg) were administered to the ALDH2*1/*2 mice, as described in the *Materials and Methods*. After the mice have lost their righting reflex, they were placed on their back in their home cage and monitored. The duration of LORR was defined as the time from the loss of righting reflex to the time when the mice regained the ability to turn onto all four paws on their own. In this study, treatment of Alda-1 and Alda-89 alone did not have a significant effect on the duration of LORR. However, the combined treatment of Alda-1 with Alda-89 significantly reduced the time for LORR duration from 106 min in the control vehicle treatment group and from 94 min in the Alda-89 alone treatment group to 72 min, respectively ($n = 10$; $^*P < 0.05$,$^{**}P < 0.01$; bars represent the mean ± SD).

Table S1. Summary data of behavioral recovery measured by rearing activity from wild-type male C57BL/6 mice recovering from 3.3 kg/g acute ethanol treatment (total counts of 10 animals), as presented in Fig. 3*D*

	Time, min				
Treatment	0	30	60	90	120
Sham (no ethanol)	12	40	62	79	90
Alda-1 + Alda-89	0	27**	55**	79**	80**
Alda-1	0	8	46	56*	57
Alda-89	0	8	12	19	39
Vehicle (no Alda)	0	1	7	13	25

$n = 10$. $^*P < 0.05$. $^{**}P < 0.01$ vs. vehicle-treated group.

Table S2. Summary data of behavioral recovery measured by rearing activity from mutant ALDH2*1/*2 C57BL/6 mice recovering from 2 kg/g acute ethanol treatment (total counts of 10 animals), as presented in Fig. 4*D*

	Time, min										
Treatment	0	30	60	90	120	150	180	210	240	270	300
Sham (no ethanol)	8	34	60	74	88	95	94	101	100	108	106
Alda-1 + Alda-89	3	3	3	6*	14**	14*	22*	31**	57**	62*	71*
Alda-1	0	2	2	4*	6*	6*	12	18*	28*	32	40
Alda-89	0	3	2	2	8	6	14*	16	24	24	34
Vehicle (no Alda)	0	0	0	0	0	0	5	4	8	18	29

$n = 10$. $^*P < 0.05$. $^{**}P < 0.01$ vs. vehicle-treated group.