Supplemental Figures and Tables.

"Aldehyde dehydrogenase-2 regulates nociception in rodent models of acute inflammatory pain"

Zambelli VO, Gross ER, Chen CH, Gutierrez VP, Cury Y, Mochly-Rosen D

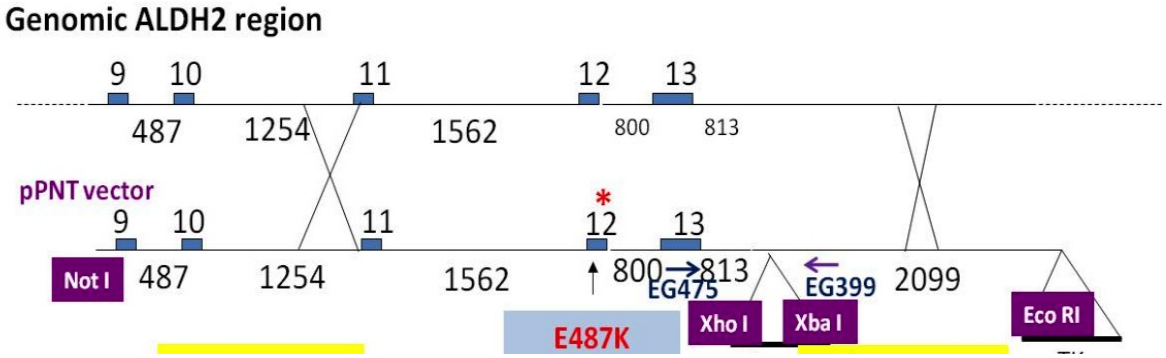

Fig S1. Scheme for generation of ALDH2*2 (E487K) knock-in mice. The details regarding the protocol for the mutant mouse generation is described in the methods section.

A

	Latency to fall (seconds)	
Speed (rpm)	WT	*1/*2
4	60±0	60±0
8	60±0	60±0
15	58±2	57±3
20	59±1	57±2
24	59±1	59±1
33	60±0	60±0
40	48±5	49±4

B

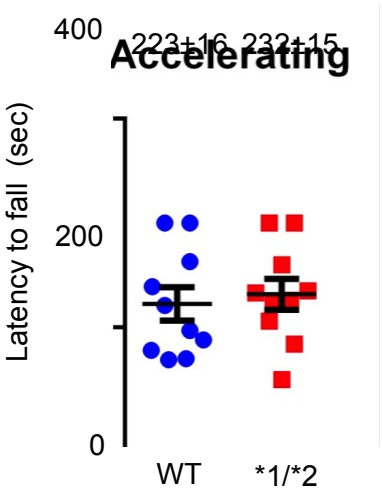

Fig S2. Rotarod data. **A.** Individual data points and speeds used for rotarod data presented in Fig 1D. Shown are data for wild type (WT; ALDH2*1/*1) and heterozygotic mice (ALDH2*1/*2) **B.** Scatogram of results from dynamic rotarod testing, with increased rotation speed from 4 to 40rpm over 5 minutes (n=10 mice/group, tested once per animal, mean±SEM).

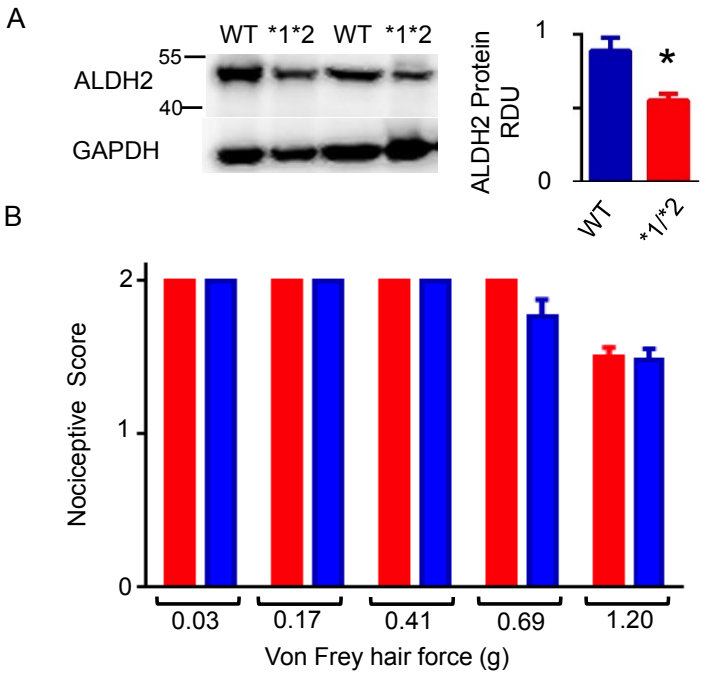

Fig S3. ALDH2 protein level in mouse paw and additional nociceptive data. **A.** Left, a representative Western blot for ALDH2 protein expression in mouse hind paw. Right, a histogram of averaged data from 7 mice/treatment group, biological replicates, expressed as relative densitometry units, RDU, *P<0.01 assessed by t-test. **B.** Baseline nociceptive threshold in mice for both wild type ALDH2 (blue bars) and heterozygous mice (ALDH2*1/*2; red bars) using the scoring technique for von Frey hairs (n=6-8 mice/treatment group, mean ± SEM).

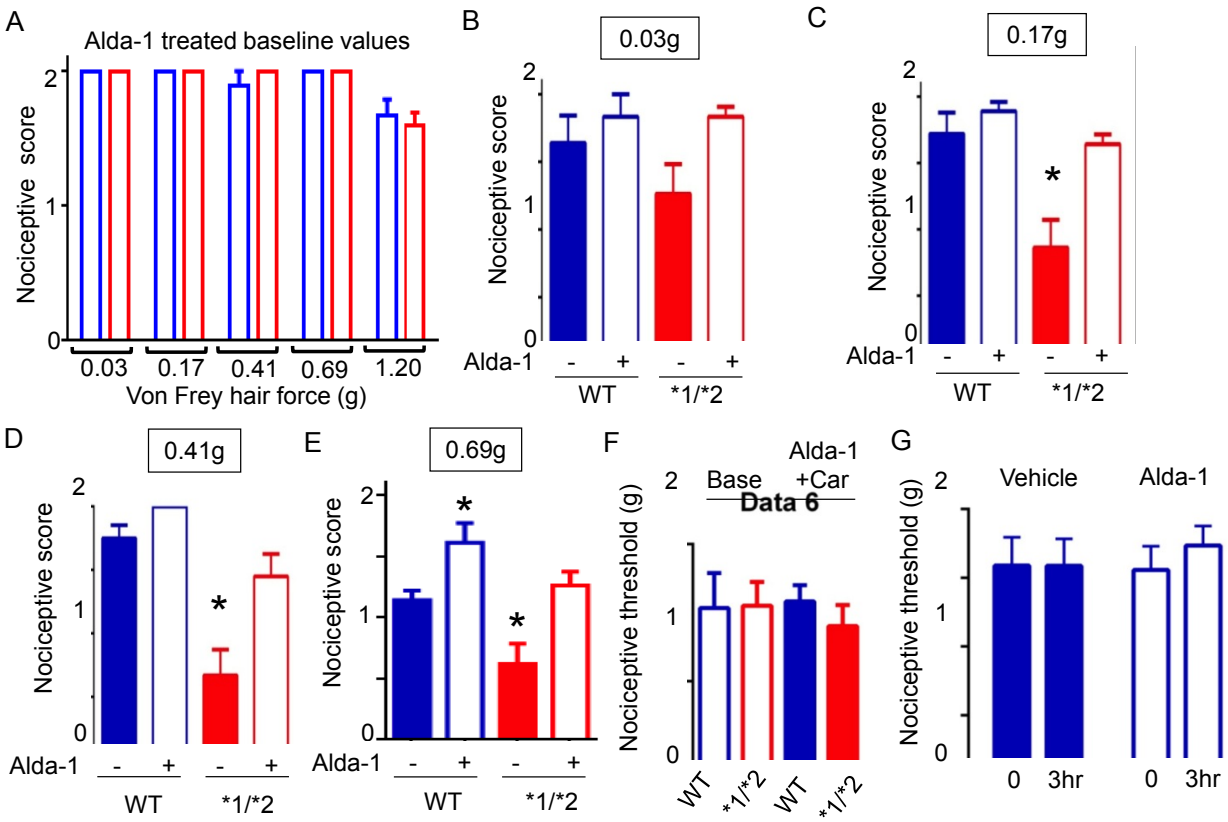

Fig S4. Nociceptive testing. A. Additional baseline nociceptive threshold measurements in mice using von Frey hairs (0.03, 0.17, 0.41, 0.69 and 1.20 grams, for both wild type ALDH2 (WT, blue bars) and heterozygous ALDH2*1/*2 mice (red bars) treated with Alda-1 (1mg/kg) using the scoring technique described in Figure 2D. **B-E.** Effect of Alda-1 on nociceptive tolerance for different von Frey hairs (boxed number) for wild type ALDH2 (blue bars) and heterozygous ALDH2*1/*2 mice (red bars) after carrageenan-induced insult. Alda-1 was administered at 3 time points; see **Fig. 2B**. (n=6-7 mice/treatment group; *P<0.001). **F.** Alda-1 effect on nociceptive threshold during a carrageenan-induced inflammatory insult using the "up-down" method. **G.** Nociceptive threshold without an inflammatory insult using the up-down technique for wild type mice treated with Alda-1 as described in Fig. 2A. (n=8/treatment group). All data are presented as mean ± SEM, *P<0.05 as assessed by one-way ANOVA with Tukey's correction.

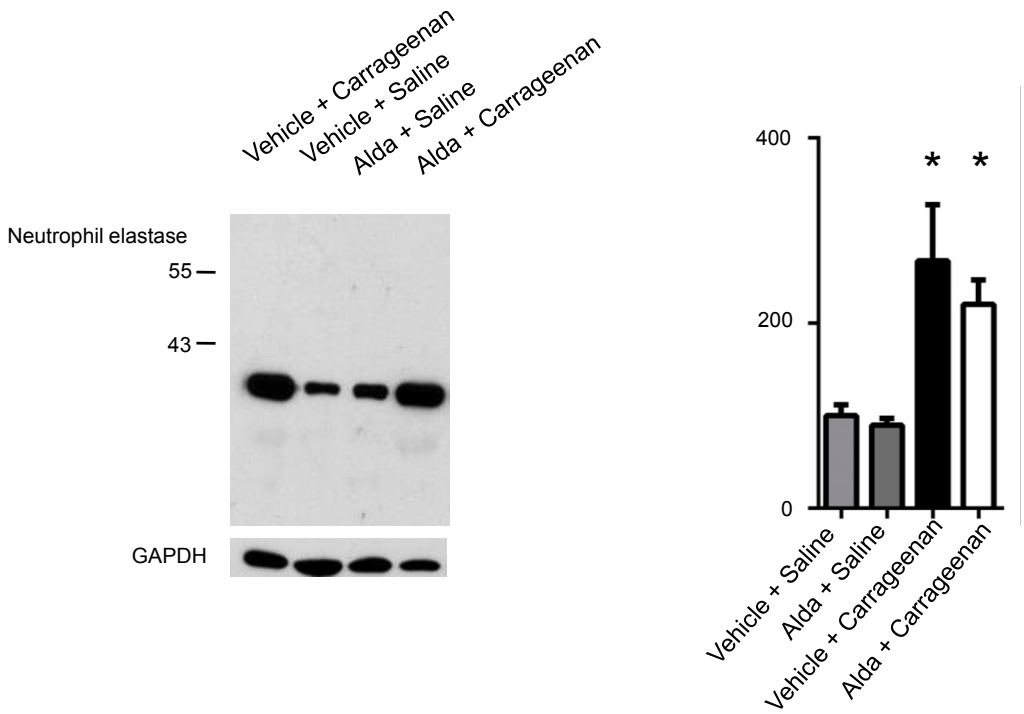

Fig. S5. Neutrophil elastase protein levels. Left, representative Western blot for neutrophil elastase. Right, histogram of neurtophil elastase levels for carageenan treated and carageenan-nontreated groups (n=6 biological replicates/group, mean ± SEM, expressed as a percentage change from vehicle alone *P<0.05 compared to respective carageenan treated groups assessed by t-test).

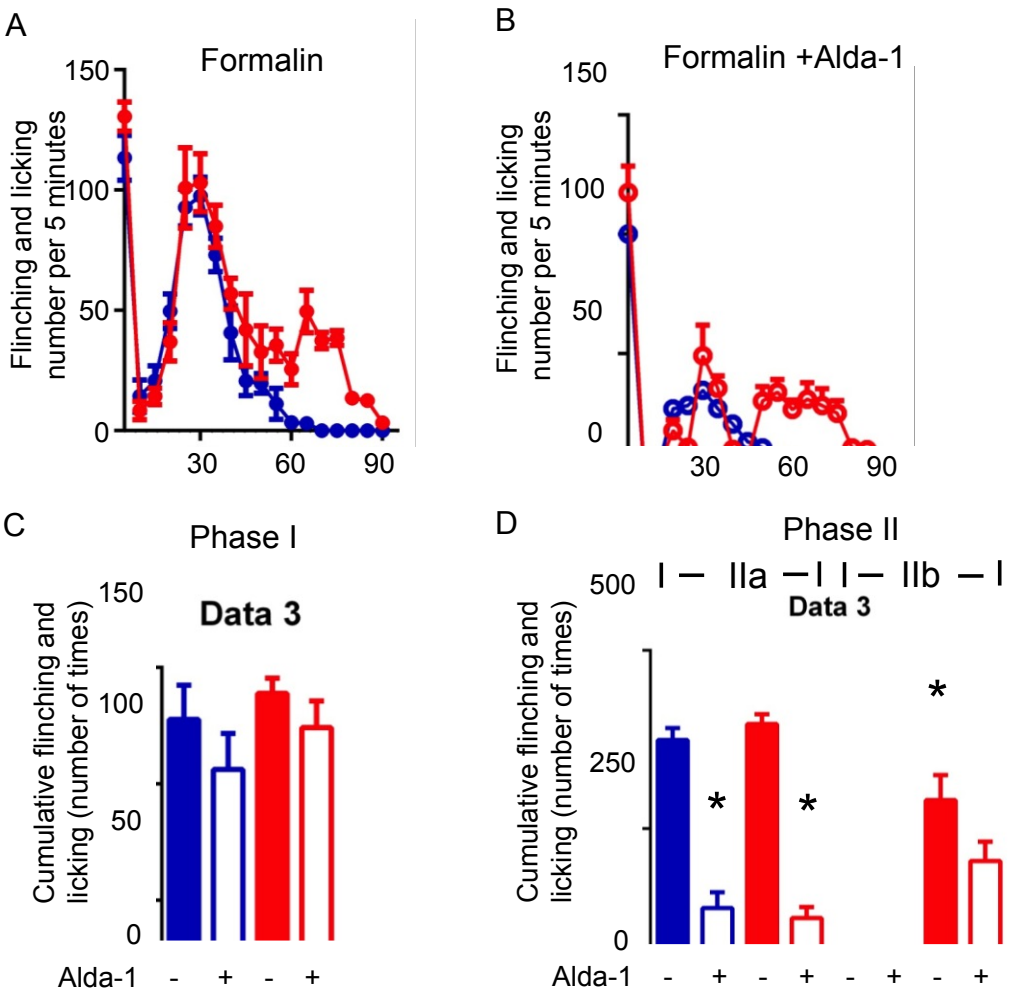

Fig S6. Formalin-induced pain model. A. Flinching and licking for wild type mice (red) and ALDH2*1/*2 mice (blue) when subjected to 2% formalin injected into the paw. **B.** Mice treated as in (**A.**) were also given Alda-1 with flinching and licking responses were measured. **C.** Cumulative phase I flinching and licking responses for each group. **D.** Phase IIa and IIb cumulative flinching and licking responses. n=8 animals/treatment group, blue data points and bars, wild type mice; red data points and bars, ALDH2*1/*2 mice, mean ± SEM, *=P<0.01 as assessed by one-way ANOVA with Tukey's correction.

	% Enzyme Activity	
Isozyme	Vehicle	Alda-1
ALDH1A1	100 ± 4.6	91 ± 5.1
ALDH2 *1	100 ± 2.3	193 ± 4.3**
ALDH2 *2	100 ± 7.4	202 ± 11.0**
ALDH3A1	100 ± 4.7	99 ± 6.9
ALDH4A1	100 ± 1.6	96 ± 2.0
ALDH5A1	100 ± 1.9	97 ± 6.6
ALDH7A1	100 ± 3.1	96 ± 3.7

Table S1. ALDH activation by Alda-1. 10 μg of the indicated human recombinant ALDH isozyme were used in standard ALDH enzymatic assays using 10 mM acetaldehyde as substrate. DMSO was the vehicle of Alda-1. The Alda-1 concentration was 20 μM in each assay. Data were collected and analyzed from 3 technically independent assays using recombinant ALDH enzymes (mean ± SEM, **P<0.01 as assessed by t-test).