

## SUPPLEMENTAL MATERIAL

for

### Uncovering newly identified aldehyde dehydrogenase 2 genetic variants that lead to acetaldehyde accumulation after an alcohol challenge

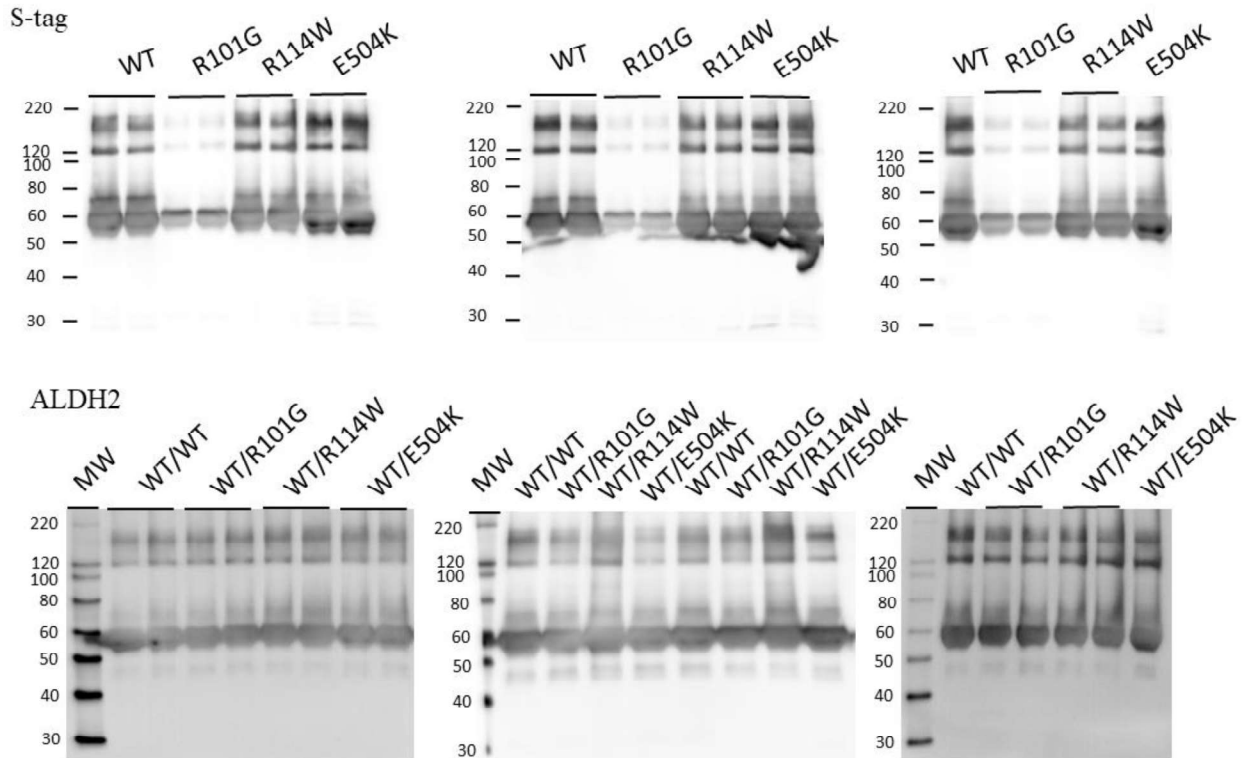
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Supplemental Figure 1.



**Supplemental Figure 1.** Western blots for ALDH2 quantification from two different protein batches per enzyme. **A.** S-tag western blots. The R101G variant did not form ALDH2 dimers and a tetramer, whereas other ALDH2 variants expressed in the S-tag site formed dimers and tetramers. **B.** Western blots probed with an ALDH2 antibody. Both ALDH2 dimers and tetramers were detected. MW = molecular weight markers

**Supplemental Table 1.** ALDH2 primers for exome sequencing

<b>Exon</b>	<b>Forward Primer (5'- to -3')</b>	<b>Reverse Primer (5'- to -3')</b>	<b>Amplicon size/bp</b>	<b>Annealing temperature/ °C</b>
1	TAGCGCCACCCGCTT CGCTTGCATCA	TGCGGGGGACTCGGGC CGGAAAACAAA	351	58
2	GGTAGTCAGTATTAG GTTGACAGCTGG	TTAACACCGTTGTCTGC AGACAAG	311	58
3	TGTGCAGCGATATGC TGATGACC	GCTGCGAGCCTATTCA CCACAT	283	58
4	TTGGAGAGACCATG GCAATAGTCCAG	TTACCTCCTAACAACG TTGCCTCCC	324	58
5	CAGAAAGACTCAGC TGGACCAGTTTG	TTGTCAGAGCCCATCTT CTTACCTGCC	344	58
6	CCAGTGTAGTTCTCT GAGGAAGCT	TAAGAGGGAGCCACTC TGGTTCACAT	332	58
7	GACCACATGTGTCCT TGGCAGA	GGAGCTCACCTTGAGC ATGTCGT	318	58
8	TCTCTCGTGGTCCAG TTGCTCACT	ACAGCAACCAACGCCA TTGGGCA	328	58
9	AGCAGGCATCAACC CTTACAGT	TTCTCATGCTGGCTCTA GACA	347	58

10	CTGCATAATTCTAAG CCTGAAGCCT	CATCAGGATGCCAGGC TGAAGTGT	318	58
11	TTCCCCTGGAAGTGT TAGAGCATGGCT	ATTCCAGGATGGTGAC CACCAGATTC	376	58
12	GTCCTGGGAGTGTA A CCCATAA	AACAGACCCCAATCCC CCAGCA	266	58

**Supplemental Table 2.** Demographics including age, weight, and sex of participants given an alcohol challenge

	Wild type	<i>rs671</i>	<i>rs747096195</i>	<i>rs190764869</i>
Number of participants	8	8	1	1
Age (years)	28±2	29±2	60	25
Weight (pounds)	147±8	146±8	138	143
Gender (Male/Female)	4/4	4/4	0/1	0/1
Smoker	None	None	None	None

**Supplemental Table 3.** Soft ionization parameters for selective ion flow mass spectrometry

<b>Metabolite</b>	<b>Reagent Ion</b>	<b>Reaction Ratio</b>	<b>Mass (m/z)</b>
Acetaldehyde	H <sub>3</sub> O <sup>+</sup>	3.7 x 10 <sup>-9</sup>	45, 63
	NO <sup>+</sup>	6.0 x 10 <sup>-9</sup>	43, 61

**Supplemental Table 4.** Sequences of oligonucleotide primers used to mutate ALDH2

<b>ALDH2</b>	<b>Forward Primer (5'- to -3')</b>	<b>Reverse Primer (5'- to -3')</b>	<b>Amplicon size/bp</b>	<b>Annealing temperature</b>
R101G	GACGCATCACACGGGGG CCGGCTGCTG	CAGCAGCCGGCCCCC GTGTGATGCGTC	27	55°C
R114W	CTGATCGAGTGG GAC CGGACCTACCTGGCG	CGCCAGGTAGGTCCA GTCCCGCTCGATCAG	30	55°C
E504K	GGGCTGCAG GCATAC ACTAAGGTGAAACTGT C	GACAGTTTTCACCTT AGTGTATGCCTGCAG CCC	33	55°C