ADVANCED SCIENCE

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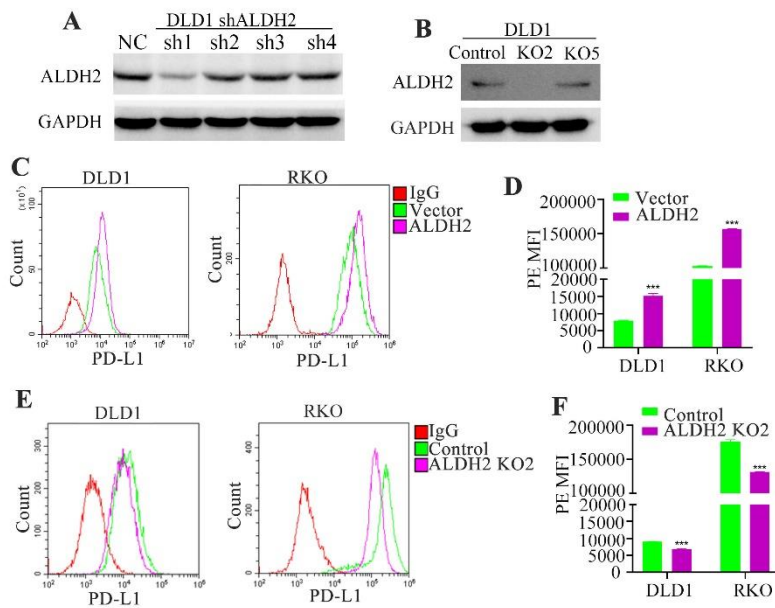## Supporting Information

Aldehyde dehydrogenase 2 mediates alcohol-induced colorectal cancer immune escape through stabilizing PD-L1 expression

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Supplementary figure 1
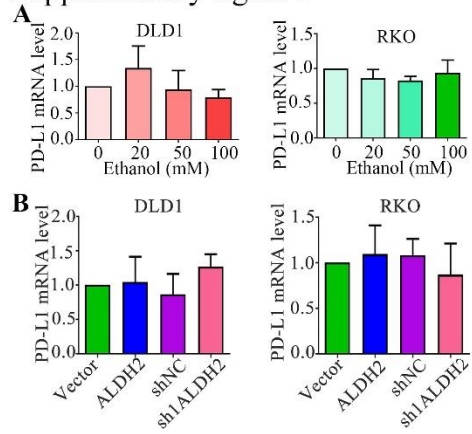
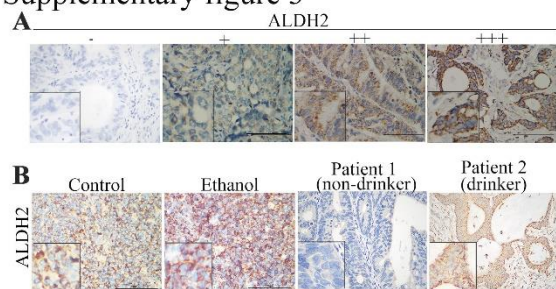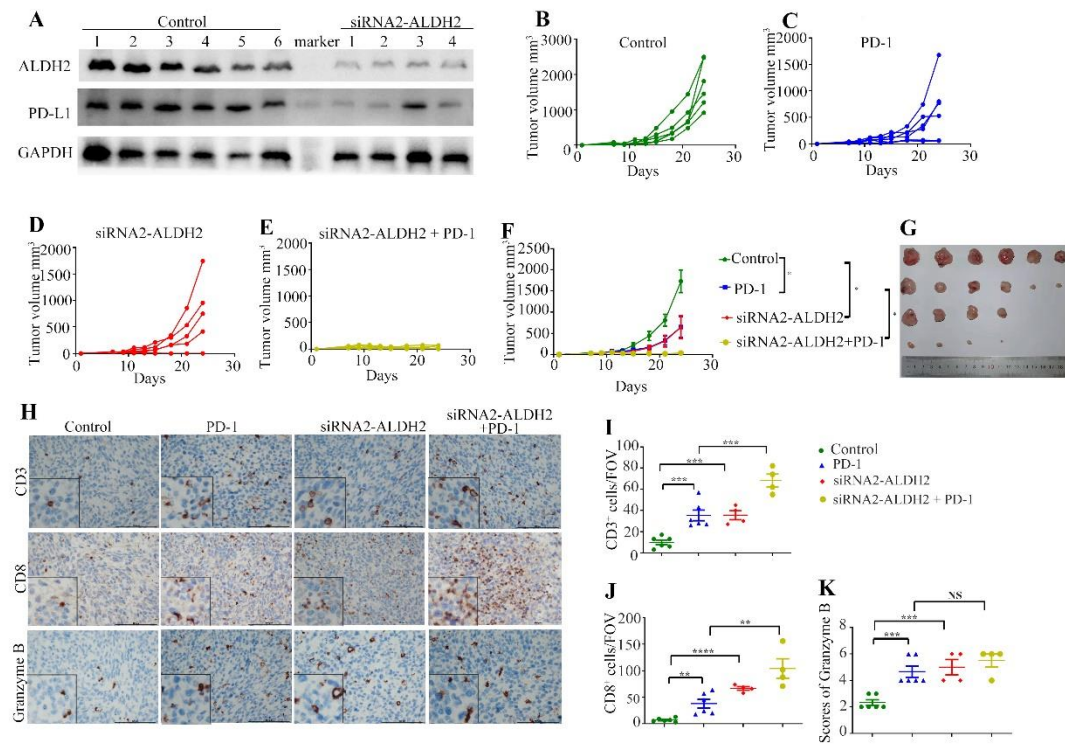# Supplementary figure 2

Supplementary figure 3

Supplementary figure 4

A

	Control						marker	siRNA2-ALDH2			
	1	2	3	4	5	6		1	2	3	4

ALDH2

PD-L1

GAPDH

Supplementary Table 1

ALDH2 shRNA (human)	sequences
sh1 ALDH2 F:	5'-CCGGGGACATGGTCCTCAAATGTCTCTCGAGAGACATTTGAGGACCATGTCCTTTTTG-3'
sh1 ALDH2 R:	5'-AATTCAAAAAGGACATGGTCCTCAAATGTCTCTCGAGAGACATTTGAGGACCATGTCC-3'
sh2 ALDH2 F:	5'-CCGGGCAGATCATTCCGTGGAATTTCTCGAGAAATTCCACGGAATGATCTGCTTTTTG-3'
sh2 ALDH2 R:	5'-AAttCAAAAAGCAGATCATTCCGTGGAATTTCTCGAGAAATTCCACGGAATGATCTGC-3'
sh3 ALDH2 F:	5'-CCGGGAAACGTGGTTGTGATGAAGGCTCGAGCCTTCATCACAACCACGTTTCTTTTTG-3'
sh3 ALDH2 R:	5'- AATTCAAAAAGAAACGTGGTTGTGATGAAGGCTCGAGCCTTCATCACAACCACGTTTC-3'
sh4 ALDH2 F:	5'-CCGGGCAGATCATTCCGTGGAATTTCTCGAGA-3'
sh4 ALDH2 R:	5'-AATTCCACGGAATGATCTGCTTTTTG-3'

Supplementary Table 2

ALDH2 shRNA (mouse)	sequences
sh1 ALDH2 F:	5'-CCGGCCTGAAATGTCTCCGCTATTACTCGAGTAATAGCGGAGACATTTCAGGTTTTTG-3'
sh1 ALDH2 R:	5'-AATTCAAAAACCTGAAATGTCTCCGCTATTACTCGAGTAATAGCGGAGACATTTCAGG-3'
sh2 ALDH2 F:	5'-CCGGCGGGCCAATGATTCTAAGTATCTCGAGATACTTAGAATCATTGGCCCGTTTTTG-3'
sh2 ALDH2 R:	5'-AATTCAAAAACGGGCCAATGATTCTAAGTATCTCGAGATACTTAGAATCATTGGCCCG-3'
sh3 ALDH2 F:	5'-CCGGCCAGTGATGCAAATCCTCAAACTCGAGTTTGAGGATTTGCATCACTGGTTTTTG-3'
sh3 ALDH2 R:	5'-AATTCAAAAACCAGTGATGCAAATCCTCAAACTCGAGTTTGAGGATTTGCATCACTGG-3'

Supplementary Figure 1. PD-L1 mRNA expression. **A,** qPCR validation of PD-L1 expression after treatment with alcohol at different concentrations for 48 h. **B**, Expression of PD-L1 mRNA in vector, ALDH2, shNC, and sh1 ALDH2-transfected DLD1 and RKO cells.

Supplementary Figure 2. ALDH2 upregulated PD-L1 protein expression on cell membrane surface. **A,** Western blot analysis showing the down-expression of ALDH2 by 4 different shRNA in DLD1 cells. **B**, Western blot analysis the effective knockout of ALDH2 by two sgRNA. **C, E**, Flow cytometry assay showing the cell membrane surface expression of PD-L1 after ectopic ALDH2 overexpression or deletion in DLD1 and RKO cells. **D**, **F**, Histograms showing the mean fluorescence intensity value (MFI) $\pm$ SD values from three biological replicates in the flow cytometry assay. (Student's t test, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.)

Supplementary Figure 3. IHC staining images showing the different ALDH2 protein expression in tumor tissues obtained from CRC patients. **A,** Rating of IHC intensity staining comparing relative ALDH2 expression (-, +, ++, +++ representing negative, weak, intermediate and strong staining intensity, respectively). **B**, The expression of ALDH2 in tumor tissues of CRC patients with or without alcohol drinking history.

Supplementary Figure 4. Another siRNA of ALDH2 enhanced efficacy of PD-1 blockade in CT26 tumor model. **A,** Western blot analysis showing ALDH2 and PD-L1 protein expressions in tumor tissues from control and siRNA2-ALDH2 treated mice. **B-E,** Tumor volume of mice treated with control (n = 6), anti-PD-1 mAb (n = 6), siRNA2-ALDH2 (n = 6) and siRNA2-ALDH2 combined anti-PD-1 mAb therapy (n = 6) as indicated were measured every two days and plotted individually. **F**, The tumor volume curves of four different groups. **G**, Photo showing tumors excised from different treatment groups at termination of study. H, Representative IHC staining results of CD3, CD8, and granzyme B from four treatment groups as indicated. **I, J** The number of CD3 and CD8 positive cells per field of vision (FOV) in tumor tissues from IHC analysis (400×). **K**, The immunoreactivity score of granzyme B in tumor tissues of different treatment groups (400×). Error bars represent SD. (Student's t test, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P <$

0.0001.)