Supplemental materials and methods

Cell proliferation assays

EMT6 and B16-F10 cells were seeded in 96-well plates and incubated at 37 ℃ overnight. The proliferation rate of both ALDH2 knockout and control tumor cells were measured at 0, 24, 48 and 72 h, using the Cell Counting Kit-8 (CCK-8; Beyotime, Shanghai, China).

Real-time Quantitative PCR

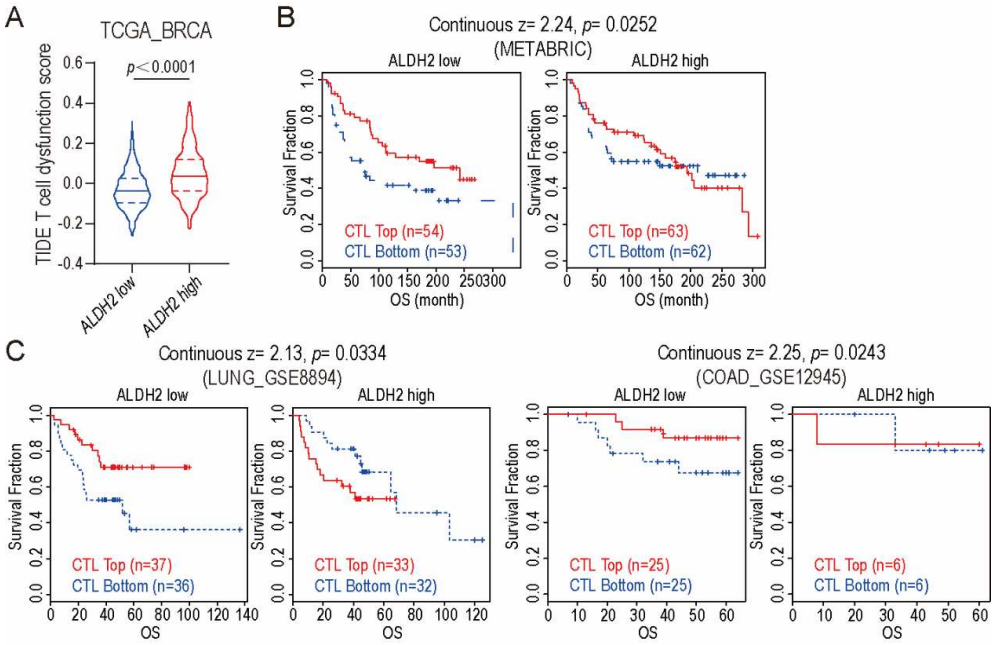
Total cellular RNA was extracted from the samples using Trizol reagent (Invitrogen) and converted into cDNA using GoScript™ Reverse Transcription (Promega) and random primers, following the manufacturer's guidelines. The resulting cDNA was then utilized as a template for amplifying target gene transcripts by real-time PCR, using SYBR Green PCR Master Mix (Invitrogen) on an ABI PRISM 7500HT Sequence Detection System (Applied Biosystems). GAPDH was used as the normalization control, and the primer details are listed in supplemental table 1. The following agents were used: Nodinitib-1 (ML130, HY-18639, MCE; 10 μM) and QNZ (S4902, Selleck; 10 μM).

Supplemental table 1

Primer (human)	Sequences
VISTA Forward	5'- ATCCCTGCTCTTCGCTCTCT -3'
VISTA Reverse	5'- CCTCGGGACAGACATACAGG -3'
VISTA promoter Forward	5'- CTCTCTCTGATGTTTCTGAGACCC -3'
VISTA promoter Reverse	5'- CACTCACTCACTCATTGGTTTGTC -3'
GAPDH Forward	5'- TGTTGCCATCAATGACCCCTT -3'
GAPDH Reverse	5'- CTCCACGACGTACTCAGCG -3'

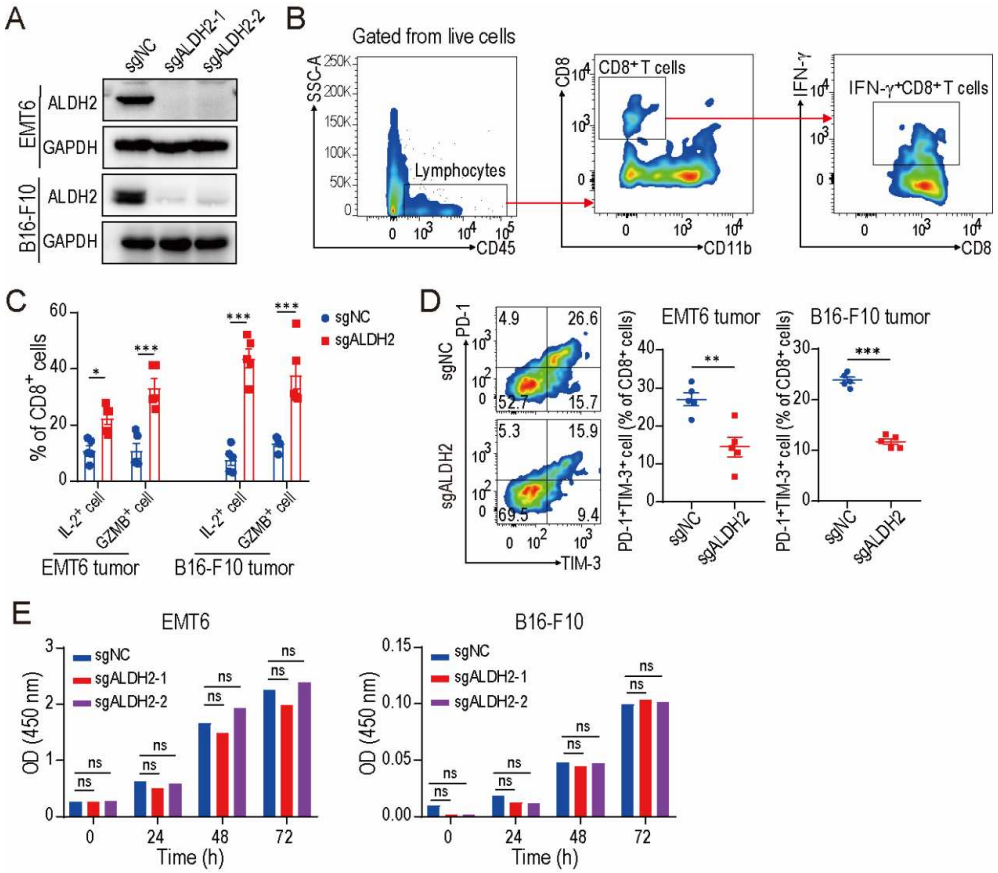
ALDH2 Forward	5'- ATGGCAAGCCCTATGTCATCT -3'
ALDH2 Reverse	5'- CCGTGGTACTTATCAGCCCA -3'
Primer (mouse)	Sequences
Vista Forward	5'- GACAGGTGGCCTCTCACC -3'
Vista Reverse	5'- TTTTCGATTCCTTGGGTGTT -3'
Gapdh Forward	5'- AGGTCGGTGTGAACGGATTTG -3'
Gapdh Reverse	5'- TGTAGACCATGTAGTTGAGGTCA -3'
Aldh2 Forward	5'- GACGCCGTCAGCAGGAAAA -3'
Aldh2 Reverse	5'- CGCCAATCGGTACAACAGC -3'
ALDH2 sgRNA (human)	Sequences
ALDH2 Forward 1	5'- CACCGCCAGTGGACGGATTGACGGT -3'
ALDH2 Reverse 1	5'- AAACACCGTCAATCCGTCCACTGG C -3'
ALDH2 Forward 2	5'- CACCGCTACACACGCCATGAACCTG -3'
ALDH2 Reverse 2	5'- AAACCAGGTTCATGGCGTGTGTAG C -3'
ALDH2 sgRNA (mouse)	Sequences
Aldh2 Forward 1	5'- CACCGACGGGACCGGACCTACCTAG -3'
Aldh2 Reverse 1	5'- AAACCTAGGTAGGTCCGGTCCCGTC -3'
Aldh2 Forward 2	5'- CACCGCCGGCTGTTGTACCGATTGG -3'
Aldh2 Reverse 2	5'- AAACCCAATCGGTACAACAGCCGGC -3'

Supplemental figure legends



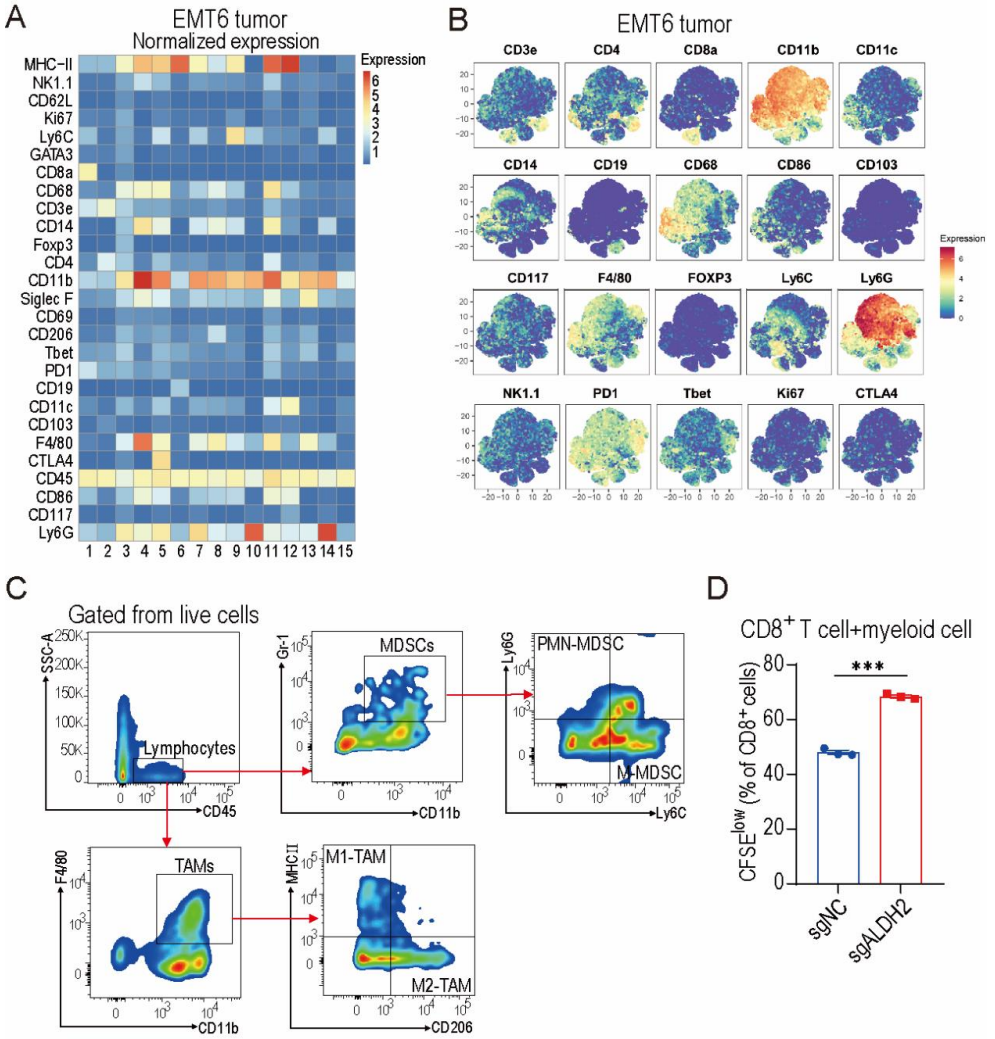
Supplemental figure 1. The correlation between ALDH2 and T cell dysfunction in multiple cancers.

(A) T cell dysfunction scores of ALDH2 low and ALDH2 high infiltrations in breast cancer assessed by TIDE system based on TCGA breast cancer (TCGA_BRCA) database (low: n=407; high: n=526; t-test). (B)The Kaplan–Meier survival analysis of breast cancer patients in ALDH2 low and ALDH2 high groups, categorized based on CTL infiltration level. High CTL infiltration, CTL-Top; Low CTL infiltration, CTL-Bottom. (C)The Kaplan–Meier survival analysis of lung and colon cancer patients in ALDH2 low and ALDH2 high groups, categorized based on CTL infiltration level. Mean±SEM.



Supplemental figure 2. Blocking ALDH2 inhibits CD8$^+$ T cell dysfunction *in vivo*.

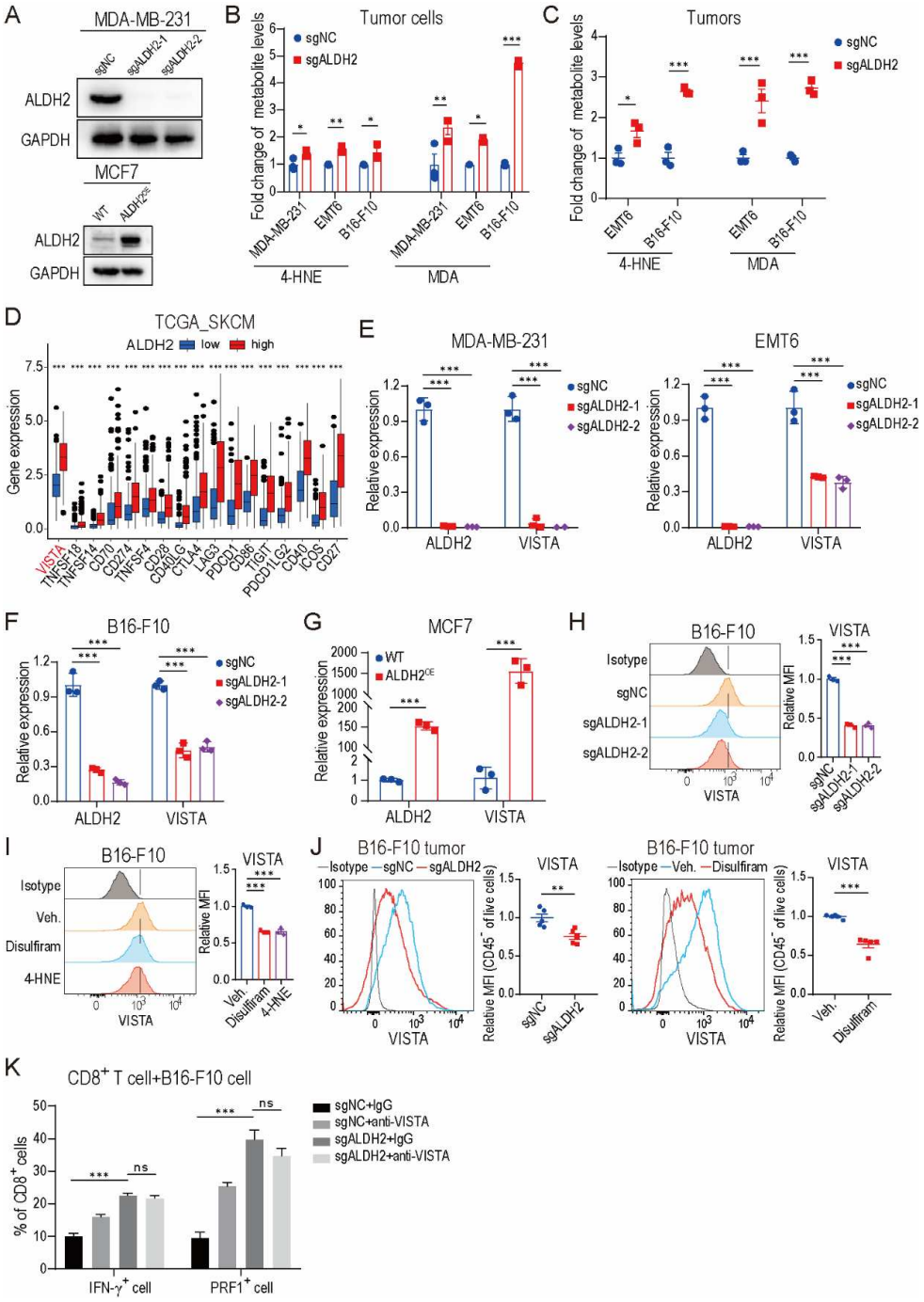
(A) Western blot analysis of ALDH2 expression on ALDH2 knockout and control tumor cells. GAPDH was used as loading controls. (B) Representative gating strategy used to identify cytokines secreted from CD8$^+$ T cells (CD45$^+$CD11b$^-$CD8$^+$), e.g., IFN-γ (CD45$^+$CD11b$^-$CD8$^+$IFN-γ$^+$). (C) Flow cytometry analysis of GZMB$^+$ and IL-2$^+$ CD8$^+$ T cells from EMT6 tumor and B16-F10 tumor (n=5, two-way ANOVA). (D) Representative contour plots and percentage of PD-1$^+$ Tim-3$^+$ CD8$^+$ T cells in EMT6 (left) and B16-F10 (right) tumors (n=5, t test). (E) CCK8 assay showing *in vitro* proliferation of EMT6 and B16-F10 tumor cells with different levels of ALDH2 expression (two-way ANOVA). All *in vitro* experiments were performed at least three times. Mean±SEM; * p<0.05; ** p<0.01; *** p<0.001; ns, not significant.



Supplemental figure 3. Profiling of immune microenvironment in EMT6 tumors by CyTOF.

(A) Heatmap displaying normalized marker expression of each immune cluster. (B) Density t-SNE plots of an equal number of $CD45^+$ tumor-infiltrating leukocytes in both ALDH2 knockout and control EMT6 tumors. (C) Representative gating strategy used to identify the subpopulations of MDSCs ($CD45^+CD11b^+Gr\text{-}1^+$) and TAMs ($CD45^+CD11b^+F4/80^+$), including M1 macrophage ($CD45^+CD11b^+F4/80^+MHCII^+CD206^-$), M2 macrophage ($CD45^+CD11b^+F4/80^+MHCII^-CD206^+$), M-MDSC ($CD45^+CD11b^+Gr\text{-}1^+LY6C^+LY6G^-$), and PMN-MDSC ($CD45^+CD11b^+Gr\text{-}1^+LY6C^-LY6G^+$).

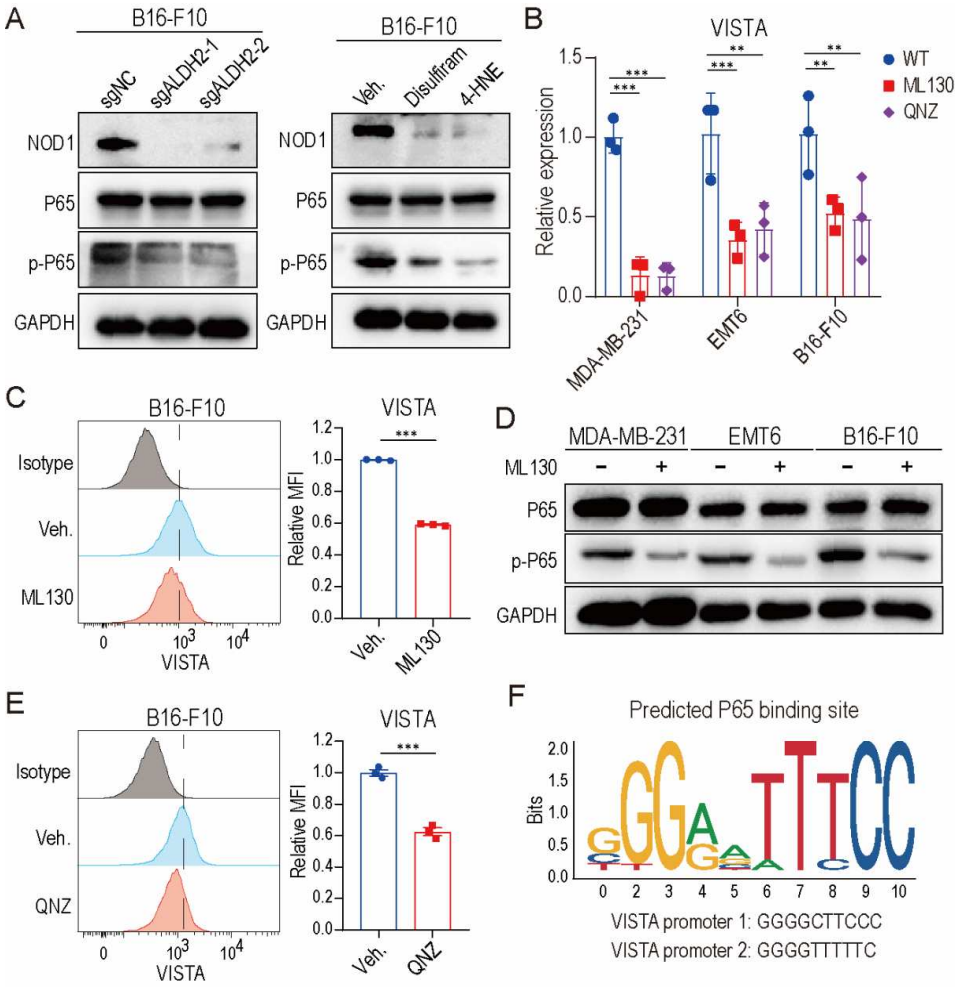
(D) Representative evaluation of $CD8^+$ T cell proliferation based on CFSE dilution *in vitro*. CD3/CD28 activated T cells were co-cultured myeloid cells isolated from sgALDH2 tumors or sgNC tumors before flow cytometry analysis (n=3, t test). Mean±SEM; ***p<0.001.



Supplemental figure 4. Blocking ALDH2 down-regulates transcriptional and membrane levels expression of VISTA.

(A) Western blot analysis of ALDH2 expression on ALDH2 knockout/overexpression tumor cells and

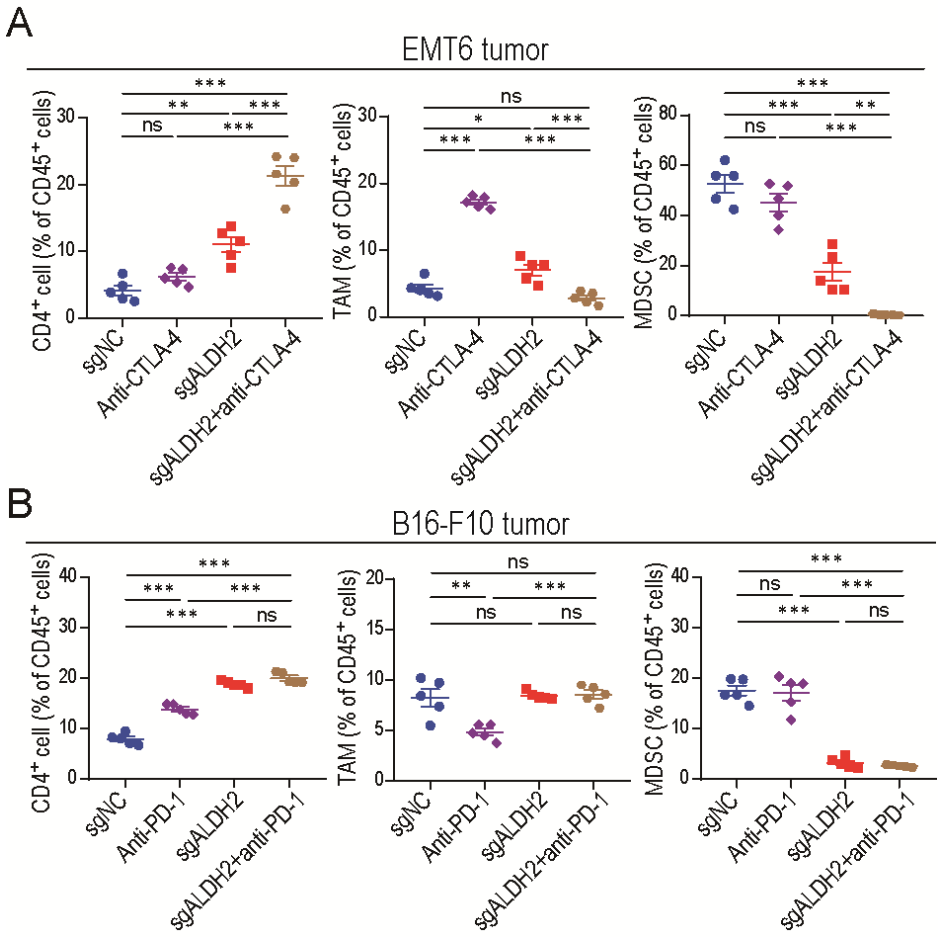
their respective control tumor cells. GAPDH was used as loading controls. (B-C) Metabolites levels of 4-HNE and MDA in ALDH2-knockout vs control tumor cells (B) and ALDH2-knockout vs control tumors (C) (n=3, two-way ANOVA). (D) Expression of immune checkpoints among ALDH2-low and ALDH2-high groups in melanoma patients based on TCGA database. (E-F) Real-time Quantitative PCR analysis of ALDH2 and VISTA expression on ALDH2 knockout and control breast cancer (E) and melanoma (F) tumor cells (two-way ANOVA). GAPDH was used as a control for normalization. (G) Real-time Quantitative PCR analysis of ALDH2 and VISTA expression on ALDH2 overexpression and control breast cancer tumor cells (two-way ANOVA). (H) Flow cytometry analysis of VISTA expression on ALDH2 knockout and control melanoma tumor cells (one-way ANOVA). (I) Flow cytometry analysis of VISTA expression on melanoma cells treated with disulfiram, 4-HNE or vehicle (one-way ANOVA). (J) Flow cytometry analysis of VISTA expression on $CD45^-$ cells in ALDH2 knockout, disulfiram-treated or control B16-F10 tumors (n=5, t test). (K) Percentages of IFN-γ^+ cell and $PRF1^+$ cell of $CD8^+$ T cells co-cultured with B16-F10 pre-treated with IgG or anti-VISTA (10 mg/mL) antibodies (two-way ANOVA). All *in vitro* experiments were performed at least three times. Mean±SEM; * p<0.05; ** p<0.01; *** p<0.001, ns, not significant.



Supplemental figure 5. ALDH2 induces VISTA expression by stimulating NOD/NF-κB activation.

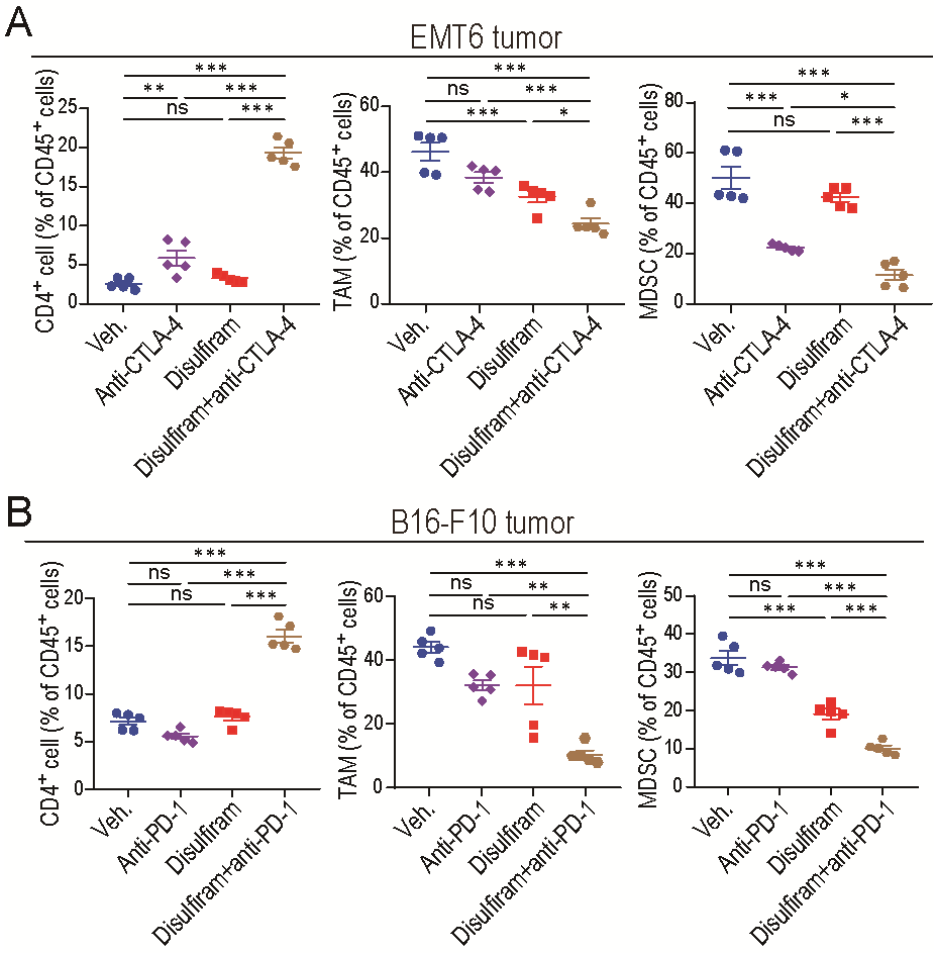
(A) Western blot analysis of NOD1, total P65 and p-P65 expression on ALDH2 knockout (left) or ALDH2 inhibitors (disulfiram and 4-HNE)-treated (right) melanoma cells. GAPDH was used as loading controls. (B) Real-time Quantitative PCR analysis of VISTA expression on breast cancer and melanoma tumor cells treated with ML130, QNZ or vehicle (two-way ANOVA), GAPDH was used as a control for normalization. (C) Flow cytometry analysis of VISTA expression on melanoma cells treated with ML130 or vehicle (t test). (D) Western blot analysis of total P65 and p-P65 expression on ML130 or vehicle-treated tumor cells. GAPDH was used as loading controls. (E) Flow cytometry

analysis of VISTA expression on melanoma cells treated with QNZ or vehicle (t test). (F) Predicted P65 binding sites on VISTA promoters by JASPAR database. All *in vitro* experiments were performed at least three times. Mean±SEM; ***p*<0.01; ****p*<0.001.



Supplemental figure 6. Inhibiting ALDH2 synergizes with ICB therapy.

(A) Percentages of $CD4^+$ T cell, TAM, and MDSC of $CD45^+$ live cells in control, ALDH2 knockout, anti-PD-1-treated or anti-PD-1-treated plus ALDH2 knockout EMT6 tumors (n=5, one-way ANOVA). (B) Percentages of $CD4^+$ T cell, TAM, and MDSC of $CD45^+$ live cells in control, ALDH2 knockout, anti-CTLA-4-treated or anti-CTLA-4-treated plus ALDH2 knockout B16-F10 tumors (n=5, one-way ANOVA). Mean±SEM; * p<0.05; ** p<0.01; *** p<0.001; ns, not significant.



Supplemental figure 7. Disulfiram reshapes the tumor immune landscape.

(A) Percentages of $CD4^+$ T cell, MDSC, and TAM of $CD45^+$ live cells in EMT6 tumors treated with vehicle, disulfiram, anti-CTLA-4, or disulfiram+anti-CTLA-4 (n=5, one-way ANOVA). (B) Percentages of $CD4^+$ T cell, MDSC, and TAM of $CD45^+$ live cells in B16-F10 tumors treated with vehicle, disulfiram, anti-PD-1, or disulfiram+anti-PD-1 (n=5, one-way ANOVA). Mean±SEM; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; ns, not significant.