

AMPK activation by Aldometanib alleviates inflammation and autoimmune disease via suppressing inflammatory T cell generation

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Supplemental information include:

Table S1-S2

Fig. S1-S12

Table S1. Demographic, serological, and clinical characteristics of SLE patients

Patient ID	Age (years)	Sex	ANA (pattern and intensity)	IgG (mg/dL)	SLEDAI score	Disease activity	Treatment
1150422 (#1)	43	F	Coarse speckled, strongly positive (+++)	310	8	Mild	Methylprednisolone, Hydroxychloroquine, Methotrexate, Tofacitinib
954226 (#2)	50	F	Homogeneous, strongly positive (+++)	314	6	Mild	Methylprednisolone, Hydroxychloroquine, Cyclophosphamide
1149071 (#3)	19	F	Fine speckled, moderately positive (++)	178	6	Mild	Methylprednisolone, Hydroxychloroquine, Mycophenolate mofetil
1152133 (#4)	22	F	Homogeneous and coarse speckled, moderately positive (++)	223	12	Moderate	Hydroxychloroquine, Mycophenolate mofetil, Methylprednisolone, Tacrolimus
1151525 (#5)	48	F	Fine speckled (++), cytoplasmic dense fine speckled (+)	312	6	Mild	Methylprednisolone, Hydroxychloroquine, Mycophenolate mofetil, Rituximab
733248 (#6)	50	M	Coarse speckled, strongly positive (+++)	348	6	Mild	Methylprednisolone, Cyclosporine, Hydroxychloroquine, Rituximab, IVIG
1153617 (#7)	35	F	Fine speckled, strongly positive (+++)	215	7	Mild	Methylprednisolone, Hydroxychloroquine, Mycophenolate mofetil, Dalteparin, Benazepril
1020922 (#8)	49	F	Fine speckled, moderately positive (++)	276	6	Mild	Prednisone, Methotrexate, Hydroxychloroquine, Aspirin
1154414 (#9)	39	F	Fine speckled, strongly positive (+++)	305	5	Mild	Methylprednisolone, Mycophenolate mofetil
704292 (#10)	65	F	Negative	355	6	Mild	Prednisone, Hydroxychloroquine, Mycophenolate mofetil, Zoledronic acid

Table S2. sgRNA sequences targeting the *Prkaa1* gene

Name	Sequence (5'→3')
sgNTC	AACTCGTTAGGCCGTGAAG
sgPrkaa1-1	TTATTGTCACAGGCATATGG
sgPrkaa1-2	ACTTCTGGTGCGGCATAATT
sgPrkaa1-3	GAAGATCGGCCACTACATCC

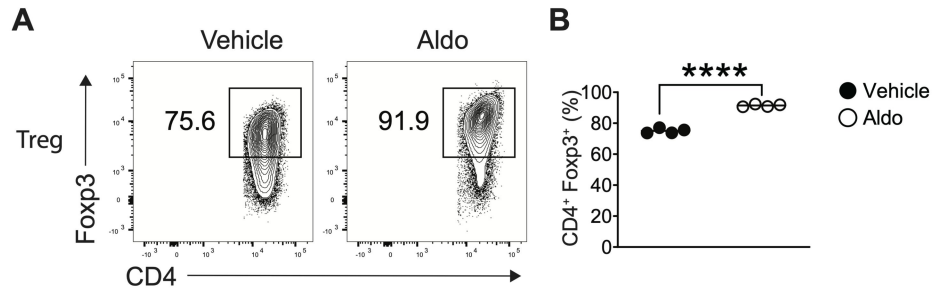


Figure S1. Aldometanib promotes iTreg cell differentiation.

(A and B) Enhanced Treg cell differentiation under 2nM aldometanib treatment (n=4 per group). All statistical analyses were conducted with an unpaired Student's t test. All bar graphs display the mean $\pm$ SD. Statistical significance is indicated as follows: **** $P < 0.0001$.

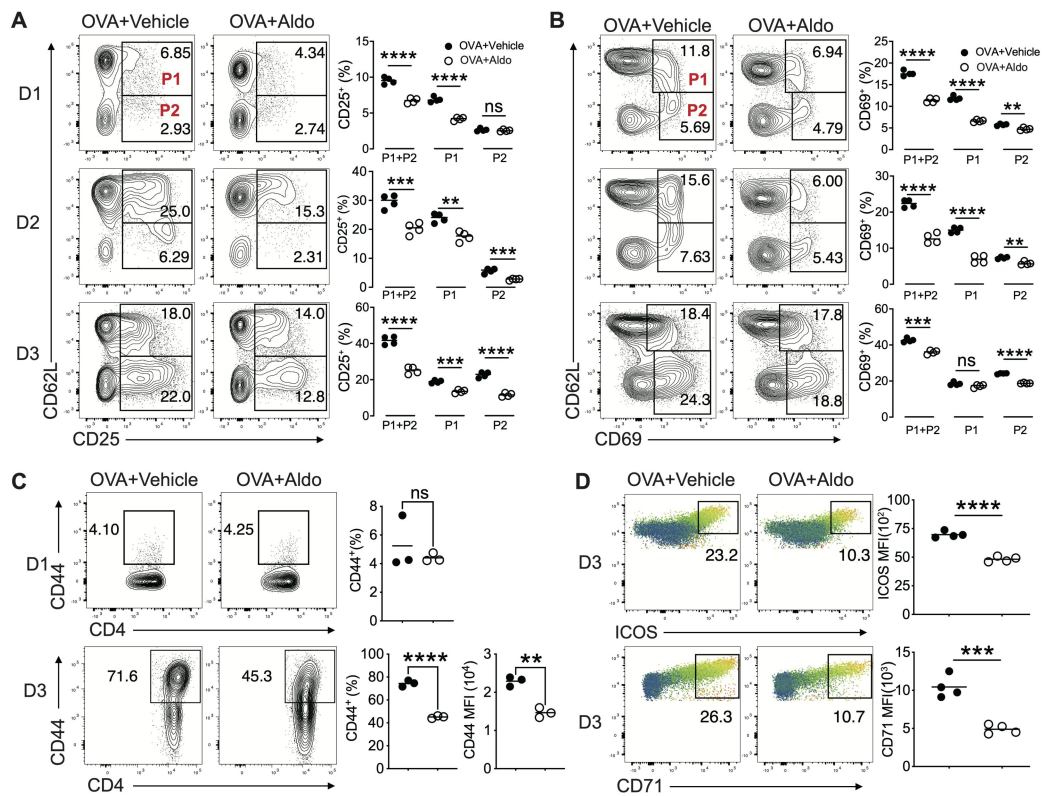


Figure S2. Aldometanib inhibits CD4⁺ T cell activation.

(A, B) Decreased early activation status in CD4⁺ T cells after 2nM aldometanib treatment (n=4). (C, D) Reduced late activation markers (CD44, CD71, ICOS) expressed on CD4⁺ T cells with 2nM aldometanib intervention (Panel C, n=3; Panel D, n=4). All statistical analyses were conducted with an unpaired Student's t test. All bar graphs display the mean $\pm$ SD. Statistical significance is indicated as follows: ns = not significant, ** $P < 0.01$, *** $P < 0.001$,

**** $P < 0.0001$.

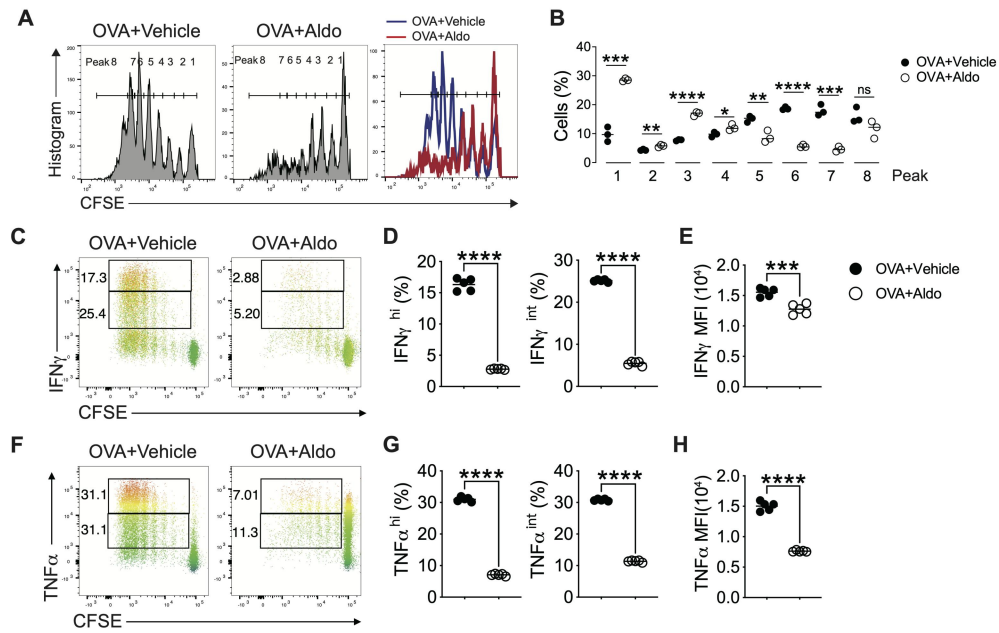


Figure S3. Aldometanib inhibits CD4 $^{+}$ T cell proliferation.

(A and B) CFSE dilution assay to analyze the effect of 2nM aldometanib on CD4 $^{+}$ T cell proliferation (n=3 per group). (C-H) Flow cytometry examines IFN γ (C-E) and TNF α (F-H) expression in CD4 $^{+}$ T cells with 2nM aldometanib (n=5 per group). All statistical analyses were conducted with an unpaired Student's t test. All bar graphs display the mean $\pm$ SD. Statistical significance is indicated as follows: ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

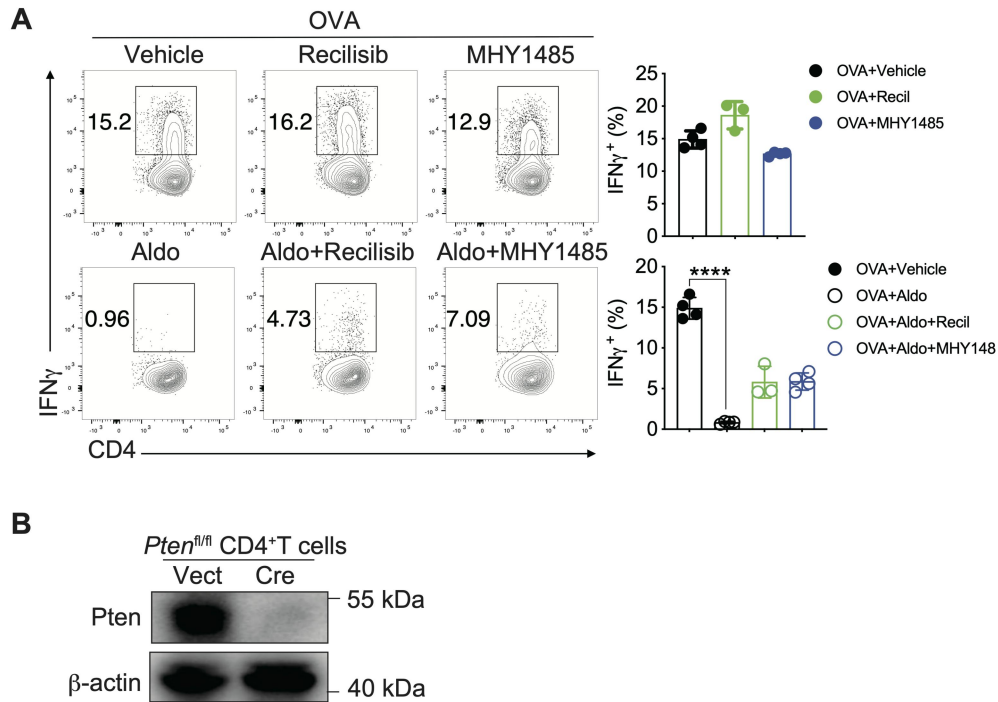


Figure S5. Aldometanib regulates T cell function via AKT/mTOR activity and PTEN-associated programs.

(A) Flow cytometry analysis of the impact of 10 μ M recilisib (Akt activator) and 2 μ M MHY-1485 (mTOR activator) alongside 2nM aldometanib treatment on the IFN γ production ($n = 3\sim 4$). (B) Efficacy of PTEN deletion in *Pten* $^{fl/fl}$ CD4 $^{+}$ T cells with Cre-expressing retrovirus transduction. All statistical analyses were conducted with an unpaired Student's t test. All bar graphs display the mean $\pm$ SD. Statistical significance is indicated as follows: **** $P < 0.0001$.

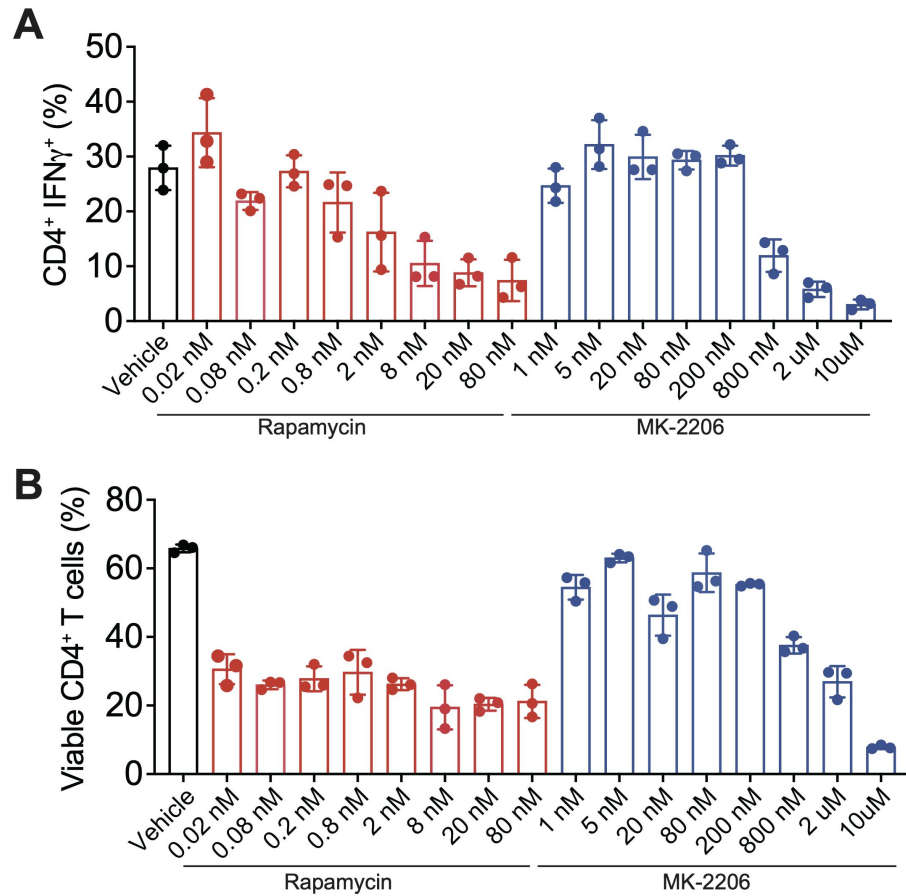


Figure S6. Rapamycin and MK-2206 potently suppressed IFN γ production along with obvious reductions in cell viability.

(A, B) Flow cytometry examining IFN γ secretion (A) and cell viability (B) of CD4⁺ T cells following rapamycin or MK-2206 treatment (n=3 per group).

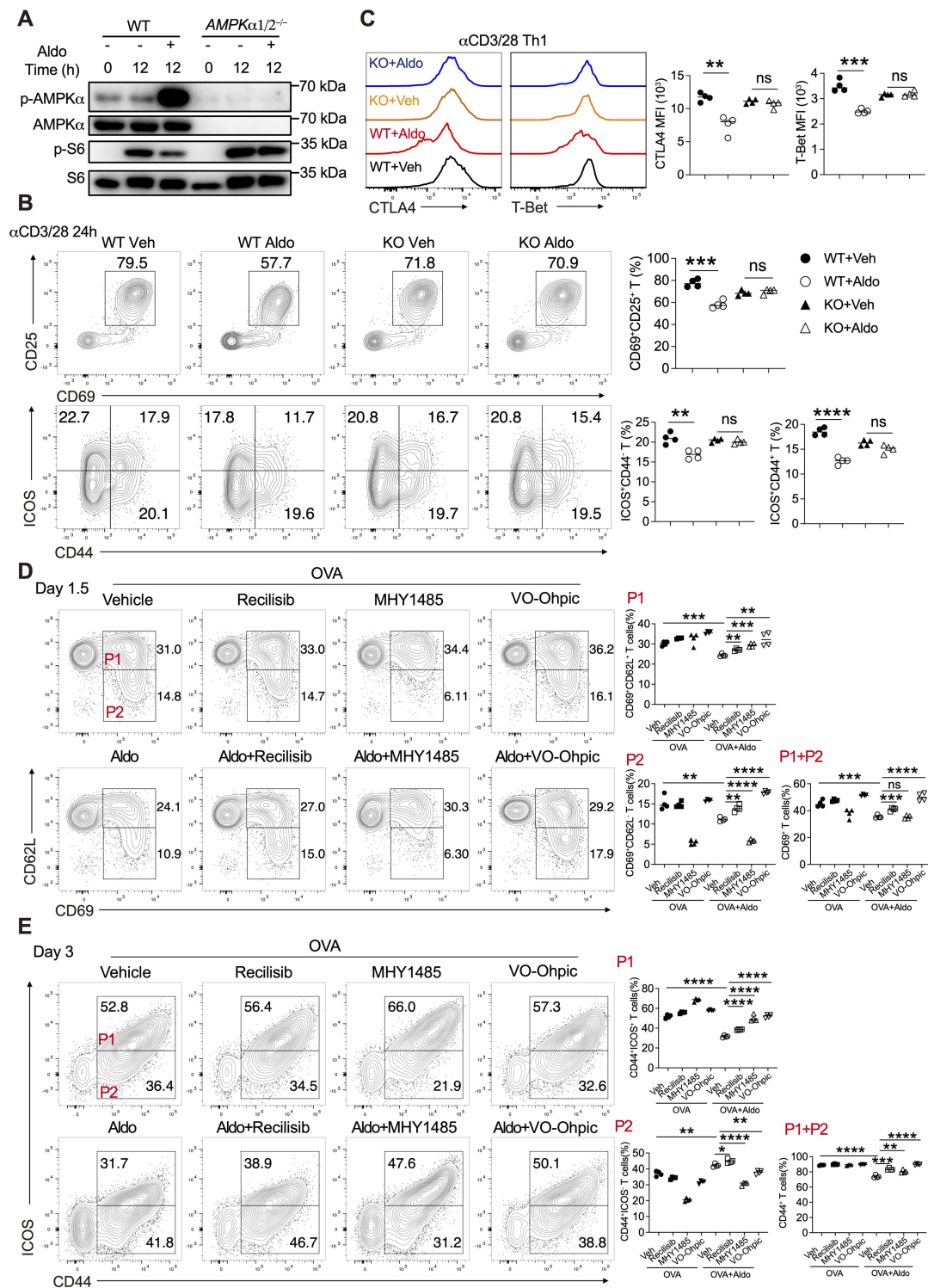


Fig. S7. Aldometanib suppresses T-cell activation via AMPK activation and PTEN-associated programs

(A) Immunoblot analysis of phosphorylation of AMPK α and S6 ribosomal protein in CD4 $^{+}$ T cells isolated from WT and AMPK α 1/2 $^{-/-}$ mice stimulated with anti-CD3/CD28 stimulation in the presence or absence of 2nM aldometanib for 12h. (B) Flow cytometric analysis of early

(upper row, CD25⁺CD69⁺) and late (lower row, ICOS⁺CD44⁻, ICOS⁺CD44⁺) activation of 2nM aldometanib-treated CD4⁺ T cells from WT and AMPK α 1/2^{-/-} mice following anti-CD3/CD28 stimulation for 24h (n=4). (C) Flow cytometric analysis of CTLA-4 and T-Bet expression in 2nM aldometanib-treated CD4⁺ T cells from WT and AMPK α 1/2^{-/-} mice under Th1-polarizing conditions (n=4). (D-E) Flow-cytometric analysis of early-stage (CD25⁺CD69⁺, D) and late-stage (ICOS⁺CD44⁺, E) CD4⁺ T-cell activation in the OVA-OT-II system. Cells were treated with 2 nM aldometanib alone or in combination with 10 μ M recilisib (Akt activator), 2 μ M MHY-1485 (mTOR activator), or 10 μ M VO-Ohpic (PTEN inhibitor) (n=4). All statistical analyses were conducted with an unpaired Student's t test. All bar graphs display the mean $\pm$ SD. Statistical significance is indicated as follows: ns = not significant, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

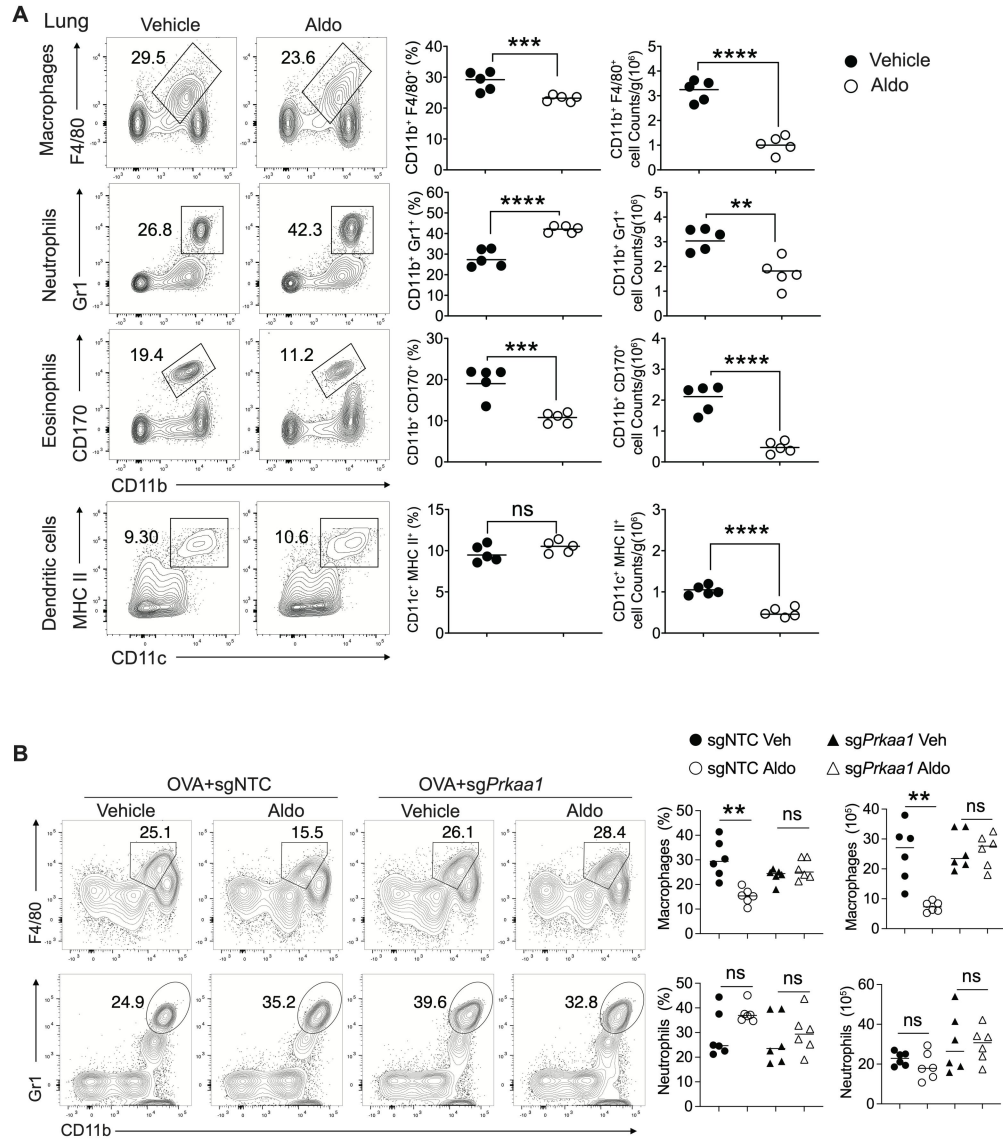


Figure S8. Aldometanib affects innate cell populations in T cell induced lung injury.

(A) Aldometanib regulates innate immune cell proportions and absolute counts in lung (n=5 per group). (B) Aldometanib regulates the proportions and absolute counts of macrophages and neutrophils in lung (n=6 per group). All statistical analyses were conducted with an unpaired Student's t test. All bar graphs display the mean $\pm$ SD. Statistical significance is indicated as follows: ns = not significant, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

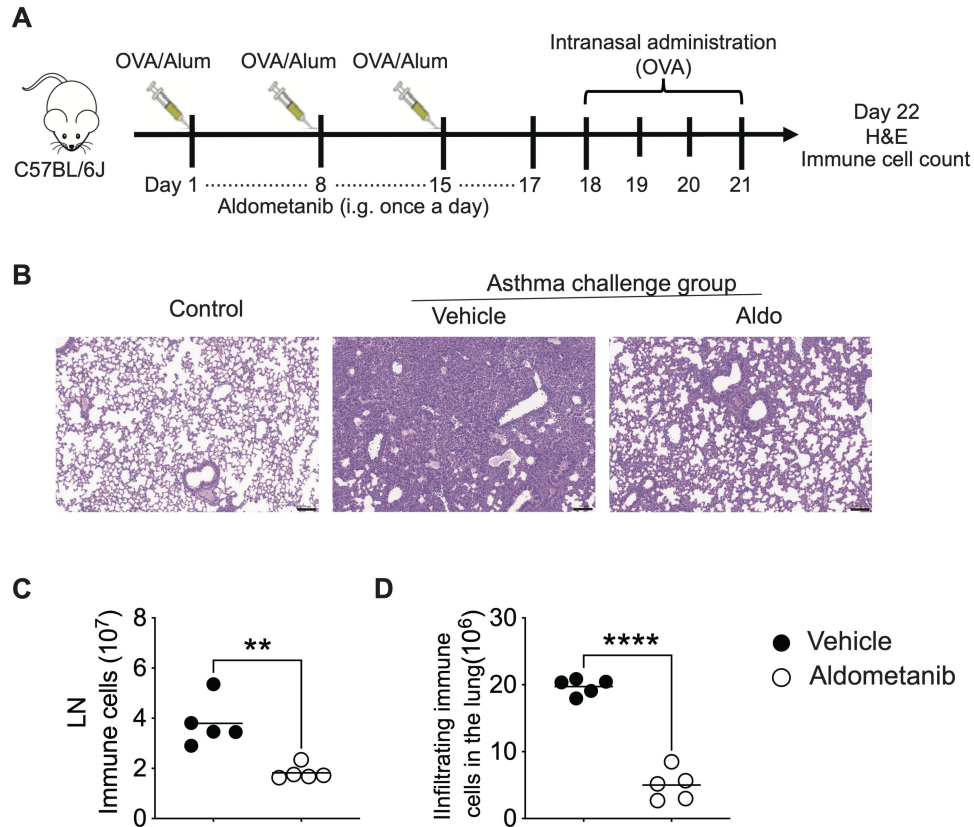


Figure S9. Aldometanib significantly alleviates allergic/ovalbumin (OVA)-induced murine asthma.

(A) Establishment of an allergic/ovalbumin (OVA)-induced asthma model. (B) Effects of aldometanib on the lung tissue H&E staining in the model. Scale bar, 200 μm . (C and D) Immune cell infiltration in lymph nodes (C) and lungs (D) upon aldometanib treatment ($n=5$ per group). All statistical analyses were conducted with an unpaired Student's t test. All bar graphs display the mean $\pm$ SD. Statistical significance is indicated as follows: ** $P < 0.01$, **** $P < 0.0001$.

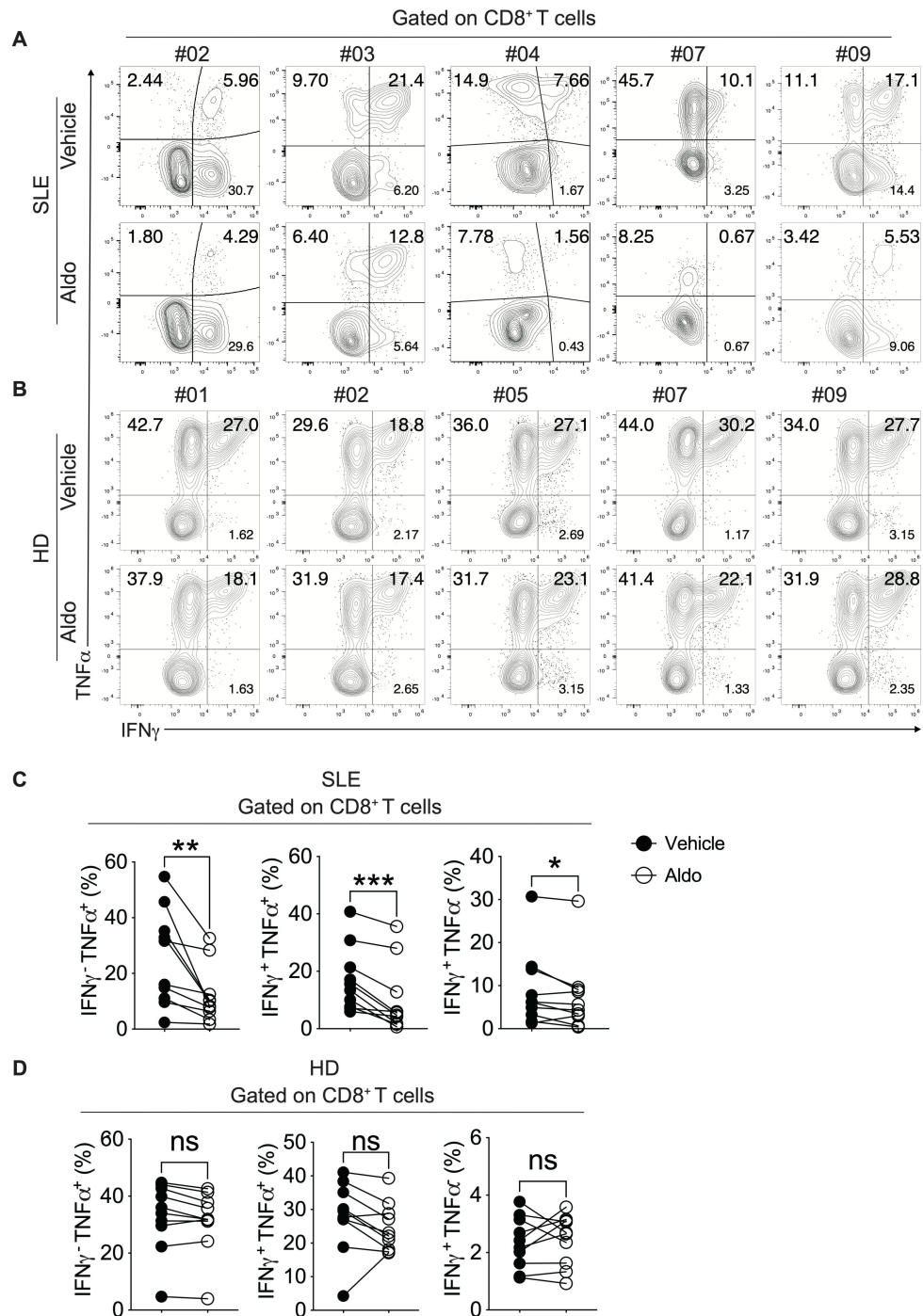


Figure S10. Aldometanib suppresses cytokine production in CD8⁺ T cells derived from SLE patient PBMCs.

(A-D) IFN- γ ⁺ TNF- α ⁺ CD8⁺ T cell frequencies in PBMCs from SLE patients (A, C) and healthy donors (HD) (B, D) following aldometanib treatment (n = 10 per group). All statistical analyses were conducted with a paired Student's t test. All bar graphs display the mean $\pm$ SD. Statistical significance is indicated as follows: ns = not significant, *P < 0.05, **P < 0.01, ***P < 0.001.

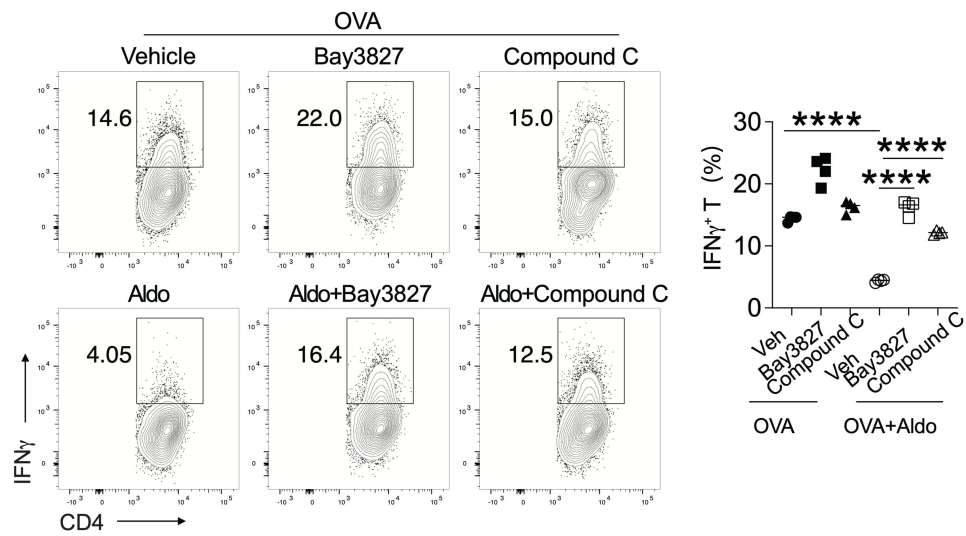


Figure S11. BAY-3827 or compound C reversed the aldometanib-mediated reduction in cytokine production by mouse T cells.

Impact of compound C (2 μ M) and Bay3827 (1 μ M) on cytokine production by CD4⁺ T cells in the OVA-OT-II system (n =4). All statistical analyses were conducted with an unpaired Student's t test. All bar graphs display the mean $\pm$ SD. Statistical significance is indicated as follows: **** P < 0.0001.

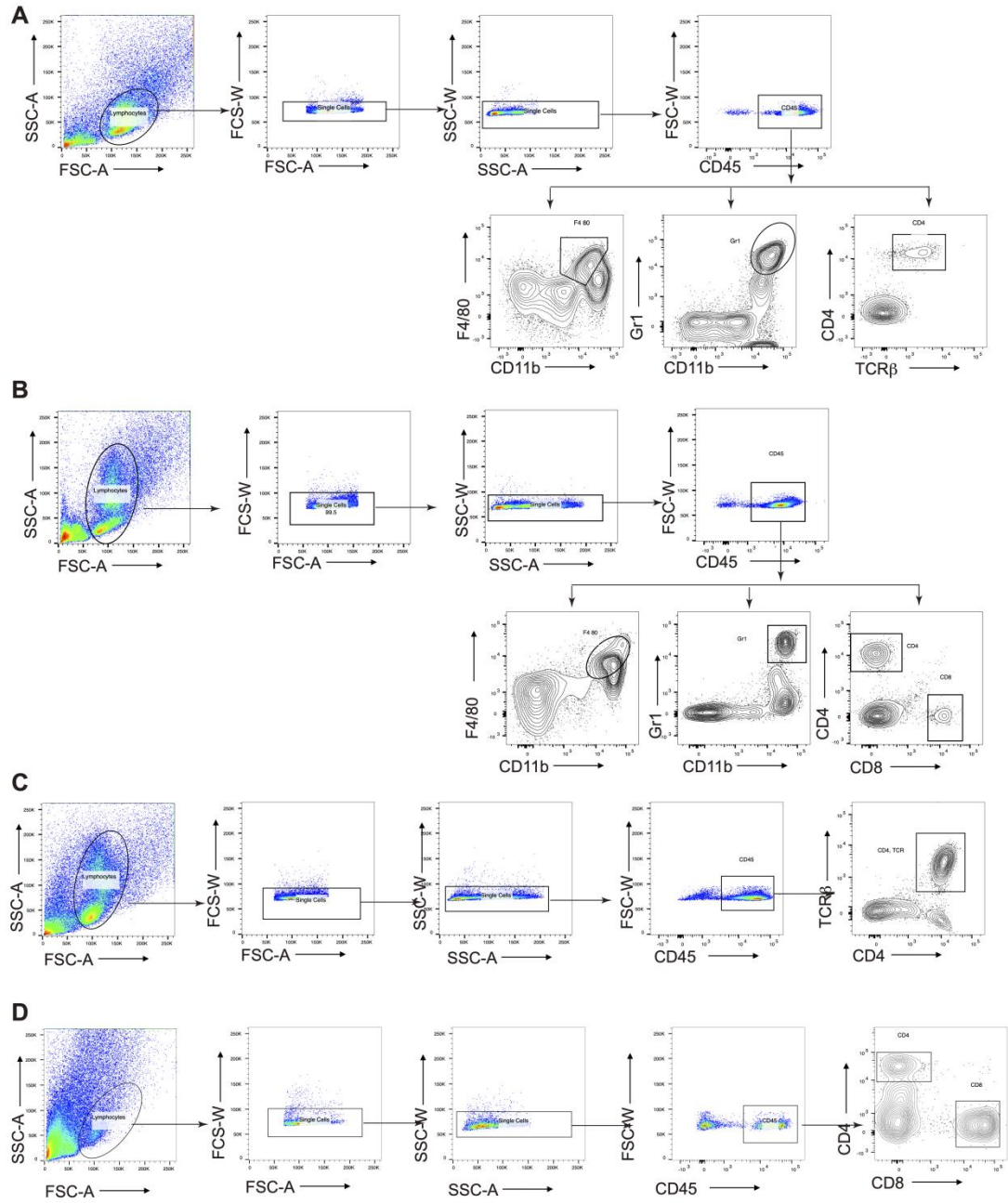


Fig. S12: Gating Strategy.

(A-D) Gating Strategies for identifying tissue-infiltrating T lymphocytes, macrophages and neutrophils isolated from OTII CD4⁺T cell-induced lung injury (A) and allergic lung injury (B), colon T cells (C) and kidney T cells (D).