Supplementary Materials for

Targeting the MALT1–MIF–IL-21/IL-17 axis restores glucocorticoid sensitivity in infection-associated steroid-resistant asthma

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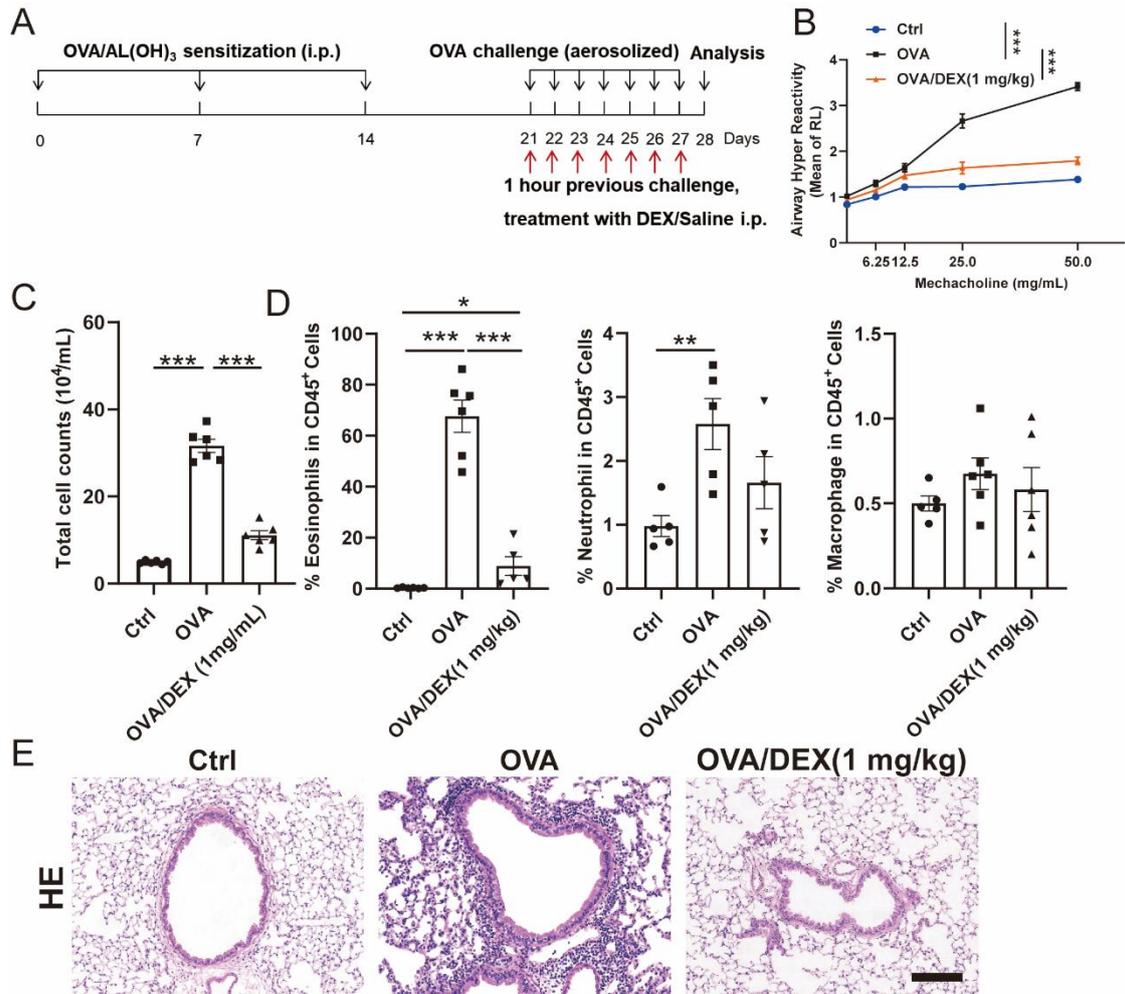
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This PDF file includes:

Supplementary Figures 1 to 12

Figure S1. DEX can alleviate steroid-sensitive asthma induced by OVA. A. Flow chart for a steroid- sensitive mouse model establishment. B. The airway hyperresponsiveness of mice was measured by lung resistance (RL) (n = 5-6).　C. Numbers of total inflammatory cell in bronchoalveolar lavage fluid (BALF) (n = 5-6). D. Frequencies of neutrophils, eosinophils and macrophage in CD45$^+$ cells from pulmonary single-cell suspension through flow cytometry (n = 5-6). E. Representative histological analysis of lung sections with HE, PAS and masson staining, scale bar = 200 μm. Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*p < 0.05; **p < 0.01; ***p < 0.001, as analyzed by one-way ANOVA and with post-hoc test. All experiments were performed in triplicates unless otherwise stated.
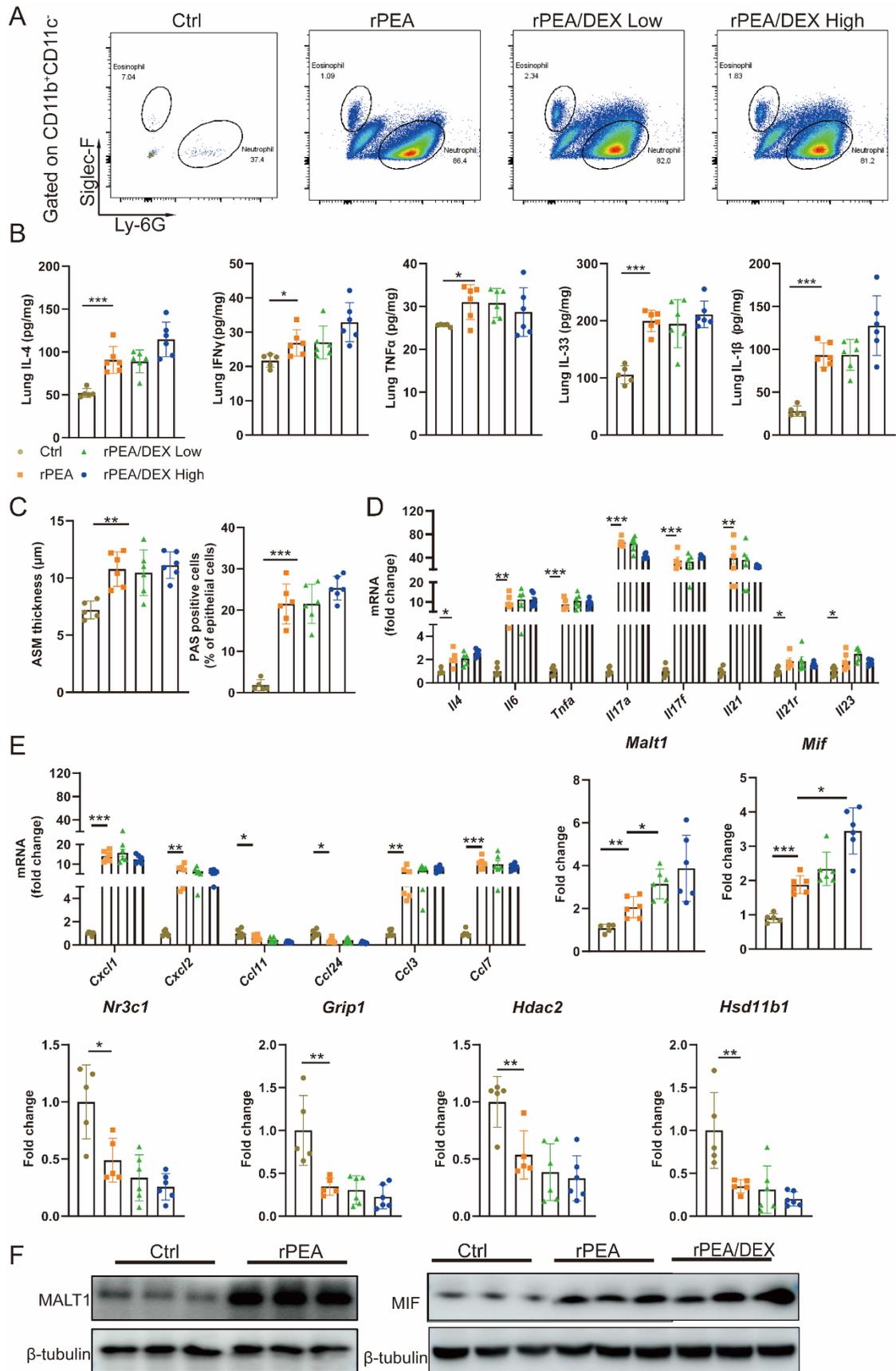
Figure S2. Validation of MALT1 and MIF in an asthma mouse model. A. Frequencies of neutrophils and eosinophils in CD45$^+$ cells from BALF suspension through flow cytometry. B.

ELISA analysis of cytokines in lung lysates (n =5-6). C. ASM thickness and PAS positive cells of HE and PAS stain. D and E. Real-time QPCR analysis mRNA levels in lungs (n = 5-6). F. Immunoblotting analysis of MALT1 and MIF levels in lungs with β-tubulin as internal control. Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*p < 0.05; **p < 0.01; ***p < 0.001, as analyzed by one-way ANOVA and with post-hoc test. All experiments were performed in triplicates unless otherwise stated.
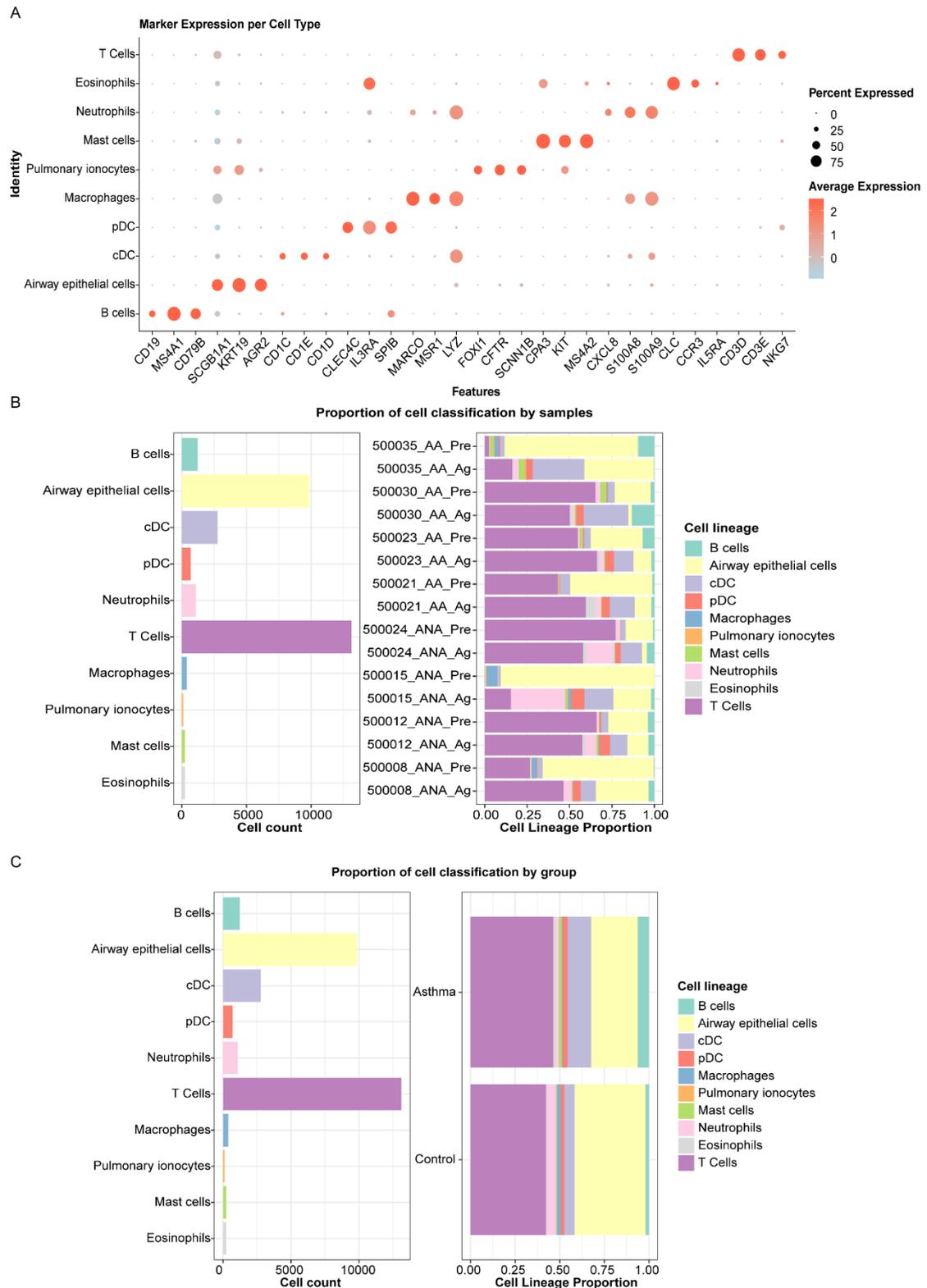
Figure S3. Cell Type Annotation and Proportional Distribution in Single-Cell Transcriptomics (GSE193816). (A) Dot plot showing marker gene expression across annotated cell types. Dot size represents the percentage of cells expressing each marker, while color intensity reflects average expression levels. (B) Bar plots illustrating the total cell counts (left) and relative proportions (right) of each cell lineage across individual samples. (C)

Comparison of cell lineage composition between asthma and control groups. Total cell counts (left) and stacked proportions (right) reveal shifts in immune and epithelial populations associated with disease status.

Figure S4. Combined treatment of MI-2 and DEX can effectively alleviate steroid-resistant asthma. A. Frequencies of GATA3⁺cell, Rorγ t⁺ cell and T-bet⁺ cell from pulmonary single-cell suspension through flow cytometry (n = 5-6). B. Immunofluorescence staining of lung slides with anti-CD74 antibody, scale bar = 50 μm. C and D. Real-time QPCR analysis mRNA levels in lungs (n = 5-6). E. Immunofluorescence staining of lung slides with anti-MPO and anti-MALT1 antibody, scale bar = 50 μm. Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*p < 0.05; **p < 0.01; ***p < 0.001, as analyzed by one-way ANOVA and with post-hoc test. All experiments were performed in triplicates unless otherwise stated.
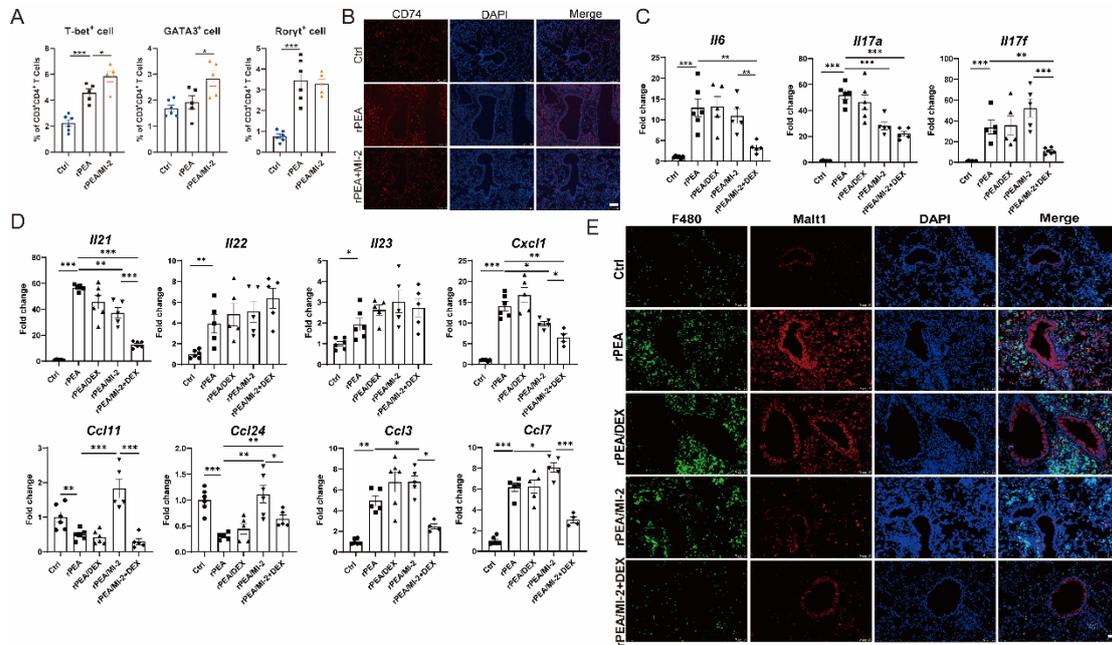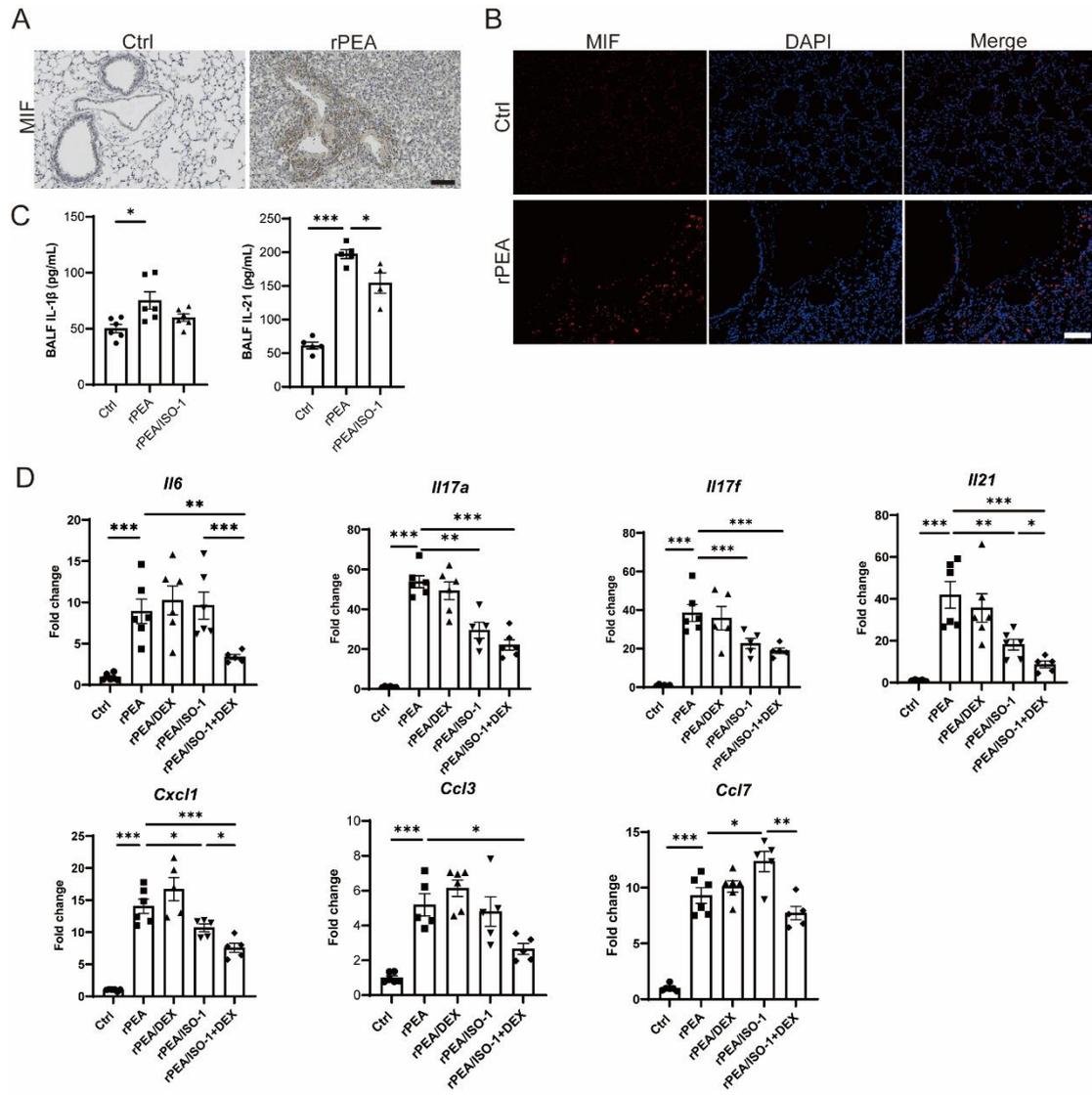
Figure S5. Combined treatment of ISO-1 and DEX can effectively alleviate steroid-resistant asthma. A. The expression of MIF was detected by immunohistochemistry in lung tissue, cale bar = 50 μm. B. Immunofluorescence staining of lung slides with anti-MIF antibody, scale bar = 50 μm. C. ELISA analysis of cytokines in BALF (n =5-6). D. Real-time PCR of mRNA levels normalized to GAPDH (n =5-6). Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*p < 0.05; **p < 0.01; ***p < 0.001, as analyzed by one-way ANOVA and with post-hoc test. All experiments were performed in triplicates unless otherwise stated.

A

Gated on CD11b⁺CD11c⁻

Siglec-F / Ly-6G

WT Ctrl — Eosinophil 8.37, Neutrophil 77.3
WT rPEA — Eosinophil 1.49, Neutrophil 86.6
Il17a⁻/⁻ Ctrl — Eosinophil 8.90, Neutrophil 81.7
Il17a⁻/⁻ rPEA — Eosinophil 2.59, Neutrophil 81.5

B

GATA3⁺ cell — % of CD3⁺CD4⁺ T Cells
T-bet⁺ cell — % of CD3⁺CD4⁺ T Cells
Rorγt⁺ cell — % of CD3⁺CD4⁺ T Cells

- WT Ctrl   *Il17a⁻/⁻* Ctrl
- WT rPEA   *Il17a⁻/⁻* rPEA

C

Lung (pg/mg): IFN-γ, IL-4, IL-17A, IL-21, IL-23, IL-6, TNF-α, IL-1β, IL-33

D

	WT Ctrl			WT rPEA			*Il17a⁻/⁻* Ctrl			*Il17a⁻/⁻* rPEA		
Il4	0.85	0.81	1.35	2.05	1.46	1.05	0.72	0.94	0.63	1.23	1.97	2.69
Il6	1.17	0.74	0.63	7.80	10.96	6.61	1.85	0.48	1.25	5.48	7.08	7.97
Il17a	1.48	1.18	0.28	39.25	27.86	39.31	0.03	0.09	0.08	0.05	0.06	0.09
Il17f	0.99	0.89	0.83	23.04	33.72	39.55	0.87	1.94	1.65	211.70	71.28	207.28
Il21	0.66	0.80	1.41	29.16	26.85	85.39	2.54	0.76	2.47	29.57	26.10	47.05
Il23	1.21	0.63	0.63	1.58	2.31	2.90	0.60	0.82	0.87	1.25	3.18	1.54
Cxcl1	1.04	1.05	1.07	17.78	14.85	12.40	0.79	0.96	0.69	11.73	7.75	9.37
Cxcl2	0.77	1.20	1.33	6.97	8.01	8.24	1.12	1.26	0.82	2.93	5.55	3.44
Ccl11	0.97	0.72	1.02	0.40	0.45	0.46	0.84	0.82	1.10	0.73	0.69	0.60
Ccl24	0.67	1.01	1.04	0.34	0.26	0.23	1.08	1.03	0.71	0.24	0.55	0.37
Ccl3	0.89	1.33	1.36	4.42	6.16	7.08	0.74	0.76	0.52	5.04	4.87	6.08
Ccl7	1.54	0.96	0.81	10.60	11.36	10.17	0.55	0.24	0.40	4.81	3.75	3.47

E

	F4/80	DAPI	Merge
WT Ctrl			
WT rPEA			
Il17a⁻/⁻ Ctrl			
Il17a⁻/⁻ rPEA			

Figure S6. Absence of IL-17A enhances the hormone sensitivity of severe asthma. A. Frequencies of neutrophils and eosinophils in CD45⁺ cells from BALF through flow cytometry. B. Frequencies of GATA3⁺cell, Rorγt⁺ cell and T-bet⁺ cell from pulmonary single-cell suspension through flow cytometry (n = 5-6). C. ELISA analysis of cytokines in lung (n =5-6). D. Real-time PCR of mRNA levels normalized to GAPDH (n =5-6). E.

Immunofluorescence staining of lung slides with anti-F4/80, scale bar = 50 μm. Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*p < 0.05; **p < 0.01; ***p < 0.001, as analyzed by one-way ANOVA and with post-hoc test. All experiments were performed in triplicates unless otherwise stated.

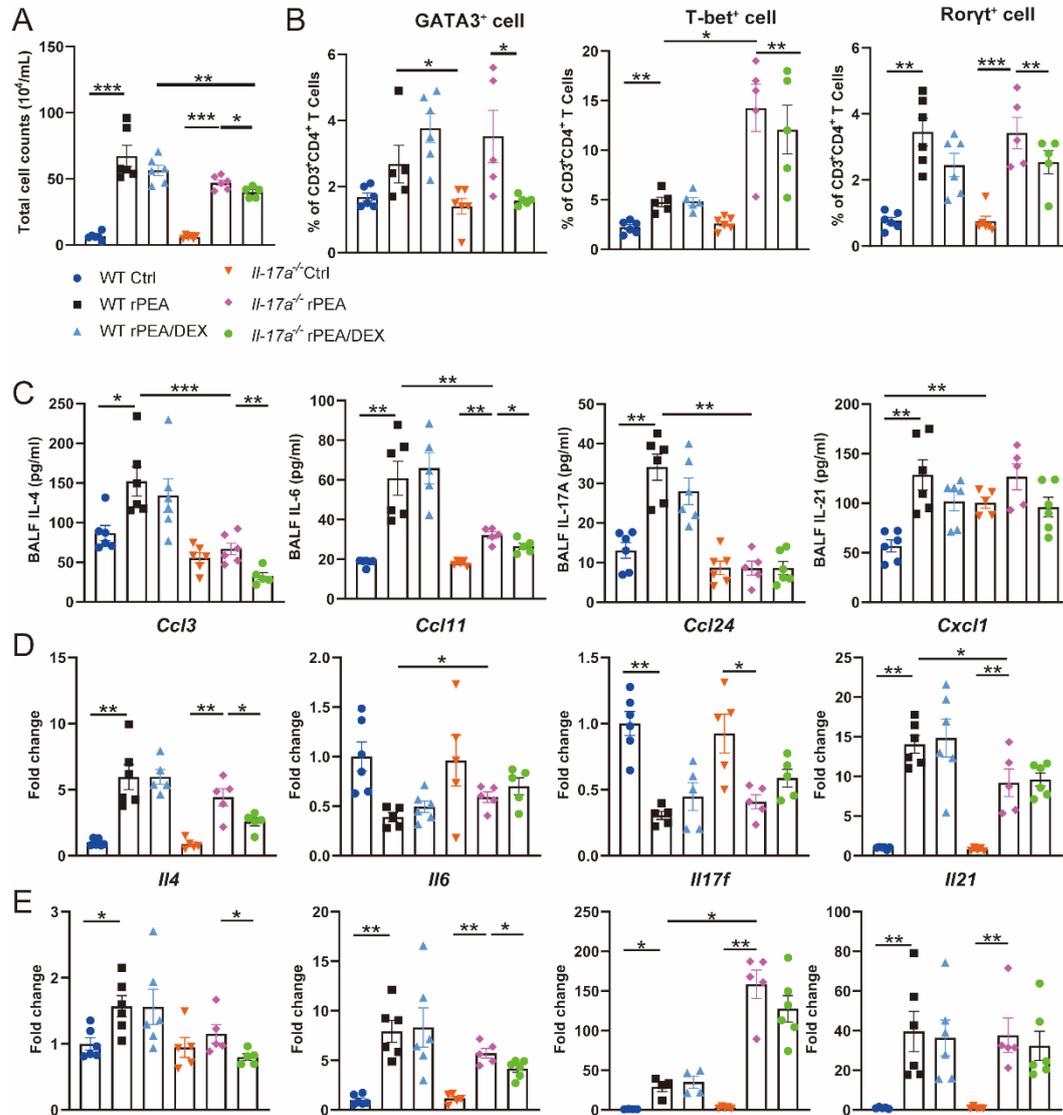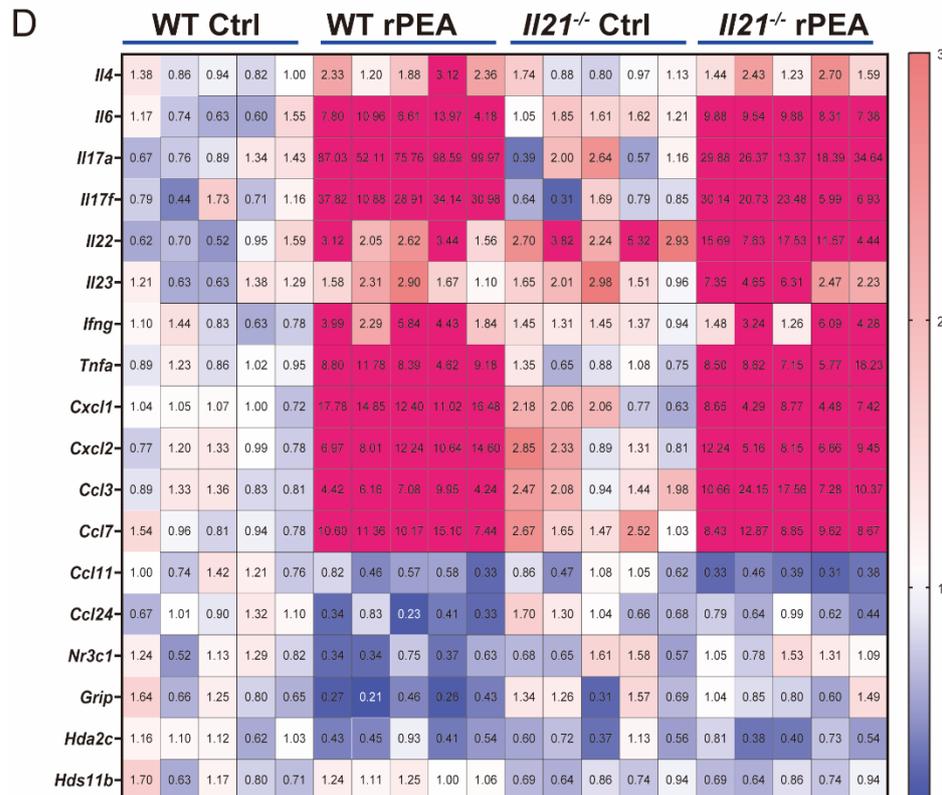**Figure S7. DEX can effectively alleviate steroid-resistant asthma in *Il17a*^{-/-} mouse.** A. Numbers of total inflammatory cell in bronchoalveolar lavage fluid (BALF) (n = 5-6). B. Frequencies of GATA3^{+}cell, Rorγt[+] cell and T-bet[+] cell from pulmonary single-cell suspension through flow cytometry (n = 5-6). C. ELISA analysis of cytokines in BALF (n =5-6). D. Real-time PCR of mRNA levels normalized to GAPDH (n =5-6). Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*p < 0.05; **p < 0.01; ***p < 0.001, as analyzed by one-way ANOVA and with post-hoc test. All experiments were performed in triplicates unless otherwise stated.
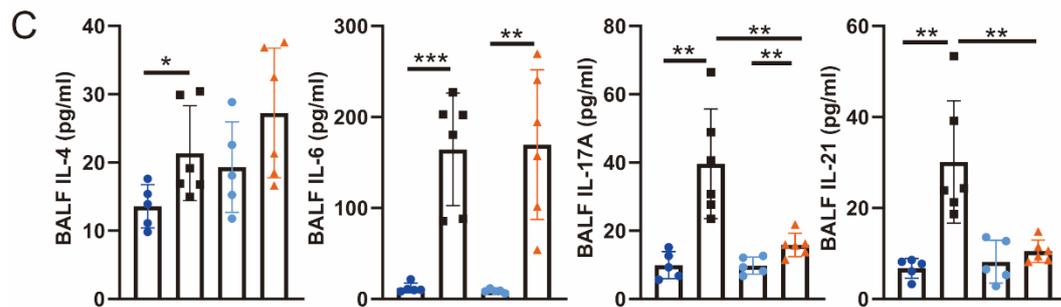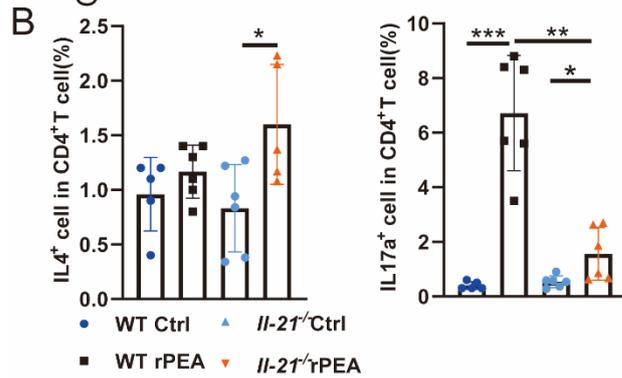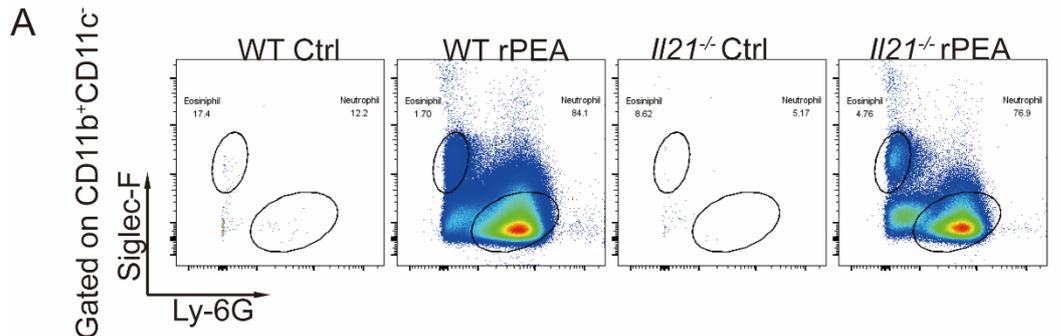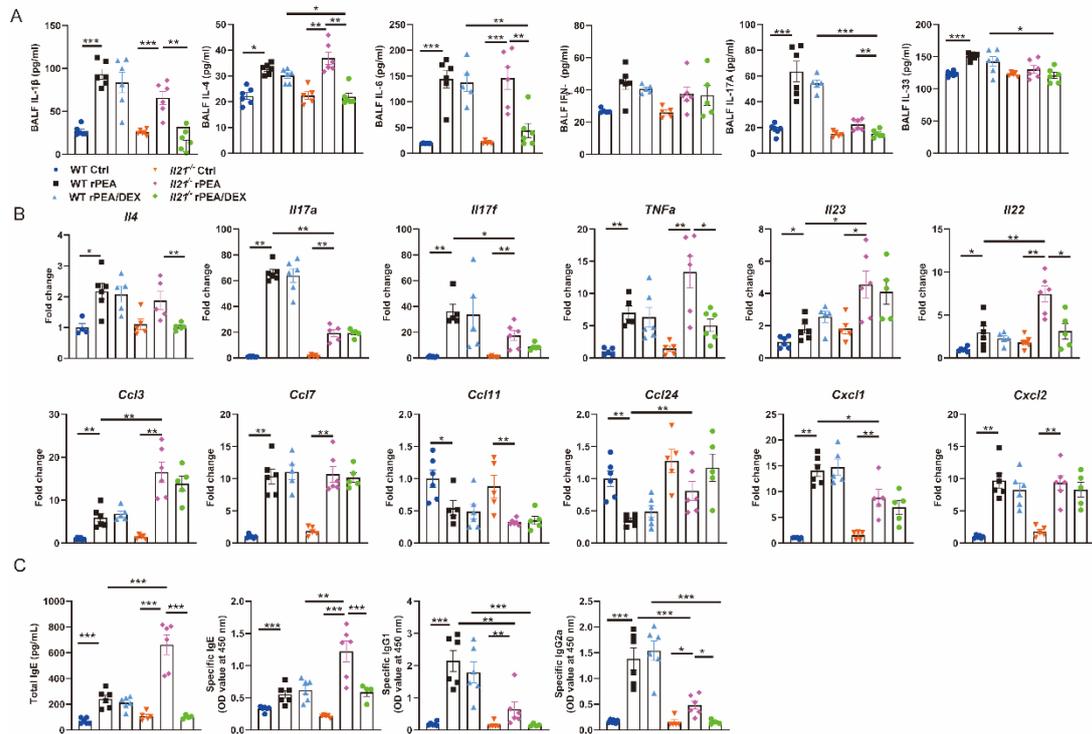
A

Gated on CD11b⁺CD11c⁻ — Siglec-F vs Ly-6G

WT Ctrl	WT rPEA	$Il21^{-/-}$ Ctrl	$Il21^{-/-}$ rPEA
Eosiniphil 17.4 / Neutrophil 12.2	Eosiniphil 1.70 / Neutrophil 84.1	Eosiniphil 8.62 / Neutrophil 5.17	Eosiniphil 4.76 / Neutrophil 76.9

B

IL4⁺ cell in CD4⁺ T cell (%) *

IL17a⁺ cell in CD4⁺ T cell (%) *** ** *

● WT Ctrl ▲ $Il-21^{-/-}$ Ctrl
■ WT rPEA ▼ $Il-21^{-/-}$ rPEA

C

BALF IL-4 (pg/ml) *

BALF IL-6 (pg/ml) *** **

BALF IL-17A (pg/ml) ** ** **

BALF IL-21 (pg/ml) ** **

D

|	WT Ctrl					WT rPEA					$Il21^{-/-}$ Ctrl					$Il21^{-/-}$ rPEA				
Il4	1.38	0.86	0.94	0.82	1.00	2.33	1.20	1.88	3.12	2.36	1.74	0.88	0.80	0.97	1.13	1.44	2.43	1.23	2.70	1.59
Il6	1.17	0.74	0.63	0.60	1.55	7.80	10.96	8.61	13.97	4.18	1.05	1.85	1.61	1.62	1.21	9.88	9.54	9.88	8.31	7.38
Il17a	0.67	0.76	0.89	1.34	1.43	87.03	52.11	75.76	98.59	99.97	0.39	2.00	2.64	0.57	1.16	29.88	26.37	13.37	18.39	34.64
Il17f	0.79	0.44	1.73	0.71	1.16	37.82	10.88	28.91	34.14	30.98	0.64	0.31	1.69	0.79	0.85	30.14	20.73	23.48	5.99	6.93
Il22	0.62	0.70	0.52	0.95	1.59	3.12	2.05	2.62	3.44	1.56	2.70	3.82	2.24	5.32	2.93	15.69	7.63	17.53	11.57	4.44
Il23	1.21	0.63	0.63	1.38	1.29	1.58	2.31	2.90	1.67	1.10	1.65	2.01	2.98	1.51	0.96	7.35	4.65	6.31	2.47	2.23
Ifng	1.10	1.44	0.83	0.63	0.78	3.99	2.29	5.84	4.43	1.84	1.45	1.31	1.45	1.37	0.94	1.48	3.24	1.26	6.09	4.28
Tnfa	0.89	1.23	0.86	1.02	0.95	8.80	11.78	8.39	4.82	9.18	1.35	0.65	0.88	1.08	0.75	8.50	8.62	7.15	5.77	18.23
Cxcl1	1.04	1.05	1.07	1.00	0.72	17.78	14.85	12.40	11.02	16.48	2.18	2.06	2.06	0.77	0.63	8.65	4.29	8.77	4.48	7.42
Cxcl2	0.77	1.20	1.33	0.99	0.78	6.97	8.01	12.24	10.64	14.60	2.85	2.33	0.89	1.31	0.81	12.24	5.16	8.15	6.66	9.45
Ccl3	0.89	1.33	1.36	0.83	0.81	4.42	6.16	7.08	9.95	4.24	2.47	2.08	0.94	1.44	1.98	10.66	24.15	17.56	7.28	10.37
Ccl7	1.54	0.96	0.81	0.94	0.78	10.60	11.36	10.17	15.10	7.44	2.67	1.65	1.47	2.52	1.03	8.43	12.87	8.85	9.62	8.67
Ccl11	1.00	0.74	1.42	1.21	0.76	0.82	0.46	0.57	0.58	0.33	0.86	0.47	1.08	1.05	0.62	0.33	0.46	0.39	0.31	0.38
Ccl24	0.67	1.01	0.90	1.32	1.10	0.34	0.83	0.23	0.41	0.33	1.70	1.30	1.04	0.66	0.68	0.79	0.64	0.99	0.62	0.44
Nr3c1	1.24	0.52	1.13	1.29	0.82	0.34	0.34	0.75	0.37	0.63	0.68	0.65	1.61	1.58	0.57	1.05	0.78	1.53	1.31	1.09
Grip	1.64	0.66	1.25	0.80	0.65	0.27	0.21	0.46	0.28	0.43	1.34	1.26	0.31	1.57	0.69	1.04	0.85	0.80	0.60	1.49
Hda2c	1.16	1.10	1.12	0.62	1.03	0.43	0.45	0.93	0.41	0.54	0.60	0.72	0.37	1.13	0.56	0.81	0.38	0.40	0.73	0.54
Hds11b	1.70	0.63	1.17	0.80	0.71	1.24	1.11	1.25	1.00	1.06	0.69	0.64	0.86	0.74	0.94	0.69	0.64	0.86	0.74	0.94

Figure S8. Absence of IL-21enhances the hormone sensitivity of severe asthma. A.
Frequencies of neutrophils, eosinophils and macrophage in CD45^{+} cells from BALF through
flow cytometry. B. Frequencies of IL-4^{+}cell and IL-17a^{+} cell from pulmonary single-cell
suspension through flow cytometry (n = 5-6). C. ELISA analysis of cytokines in BALF (n =5-
6). D. Real-time PCR of mRNA levels normalized to GAPDH (n =5-6). Data are presented as
mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*p < 0.05; **p < 0.01; ***p < 0.001, as
analyzed by one-way ANOVA and with post-hoc test. All experiments were performed in
triplicates unless otherwise stated.

Figure S9. DEX can effectively alleviate steroid-resistant asthma in *Il21^{-/-}* mouse. A.
ELISA analysis of cytokines in BALF (n =5-6). B. Real-time PCR of mRNA levels
normalized to GAPDH (n =5-6). Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$,
*** $p < 0.001$*p < 0.05; **p < 0.01; ***p < 0.001, as analyzed by one-way ANOVA and with
post-hoc test. All experiments were performed in triplicates unless otherwise stated.
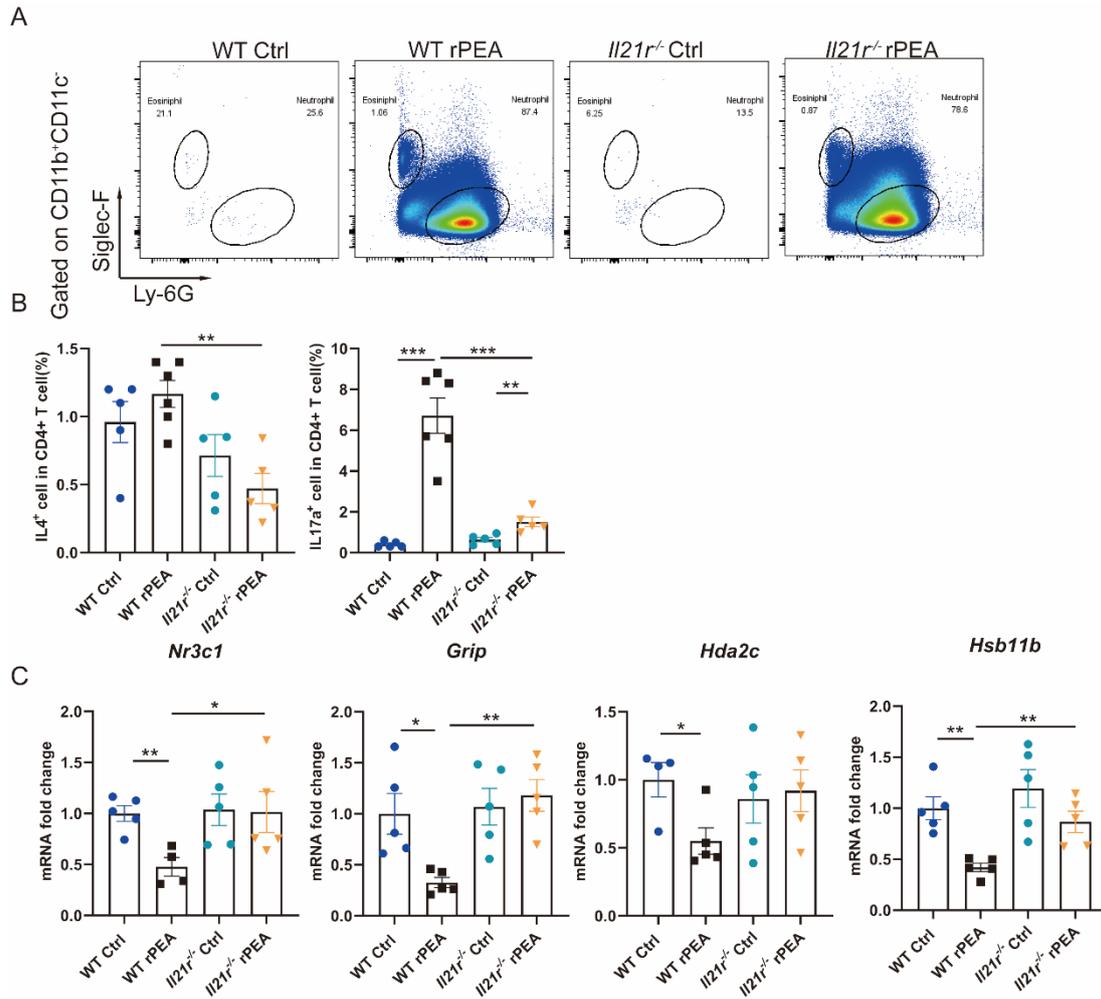
Figure S10. Absence of IL-21R enhances the hormone sensitivity of severe asthma. A. Frequencies of neutrophils and eosinophils in CD45^{+} cells from BALF through flow cytometry. B. Frequencies of IL-4^{+}cell and IL-17a^{+} cell from pulmonary single-cell sus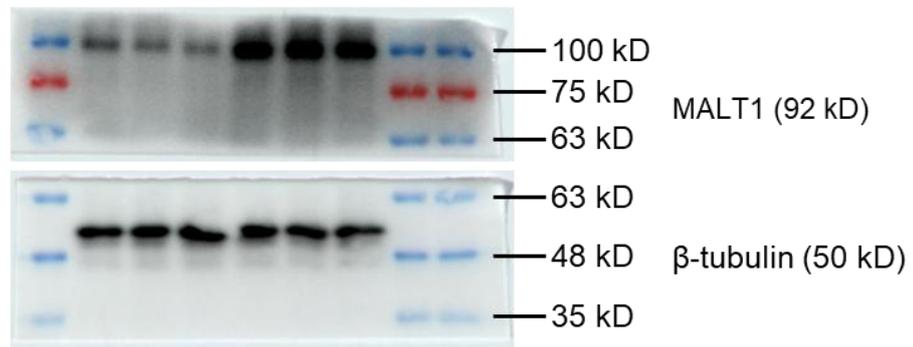pension through flow cytometry (n = 5-6). C. Real-time PCR of mRNA levels normalized to GAPDH (n =5-6). Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*p < 0.05; **p < 0.01; ***p < 0.001, as analyzed by one-way ANOVA and with post-hoc test. All experiments were performed in triplicates unless otherwise stated.
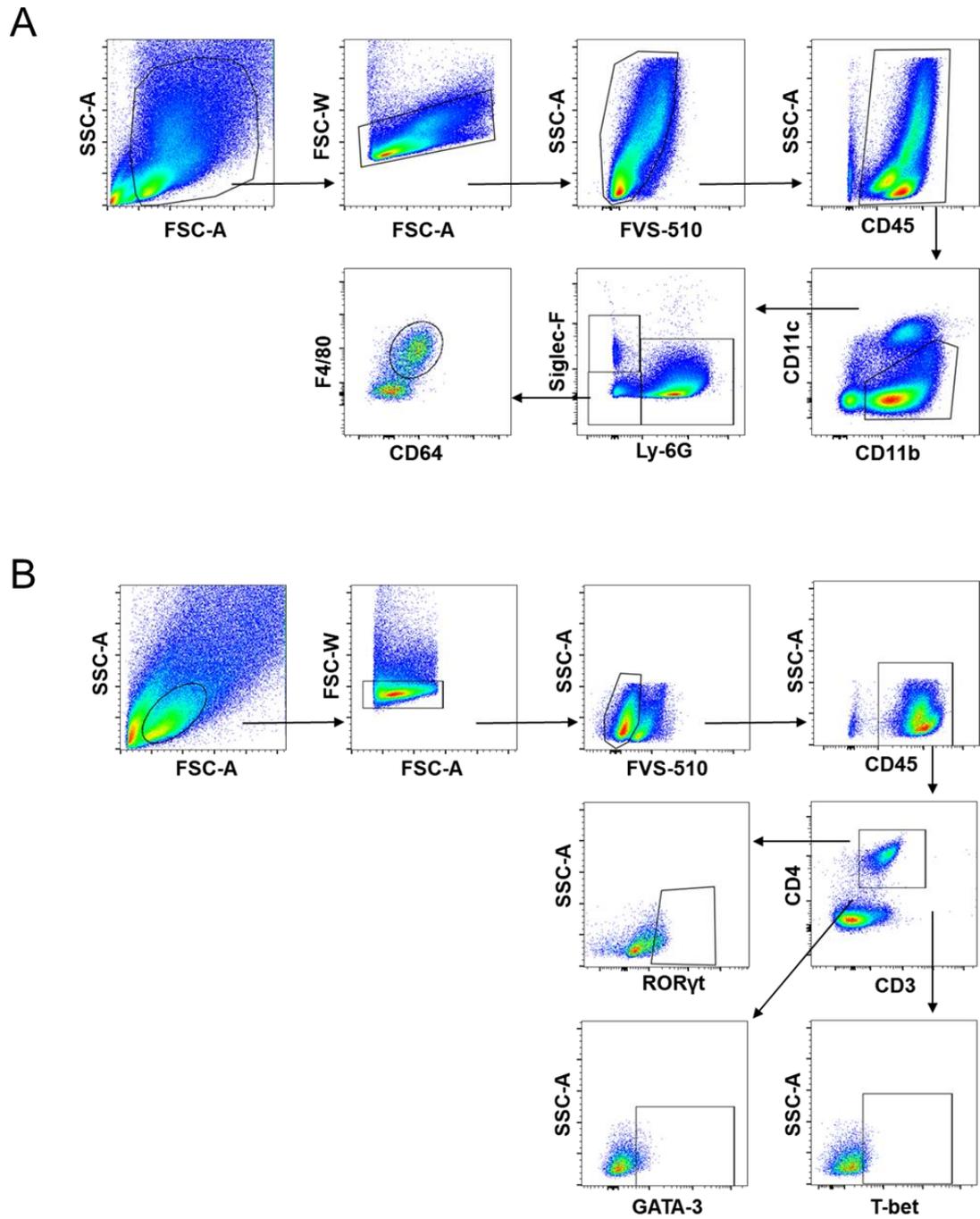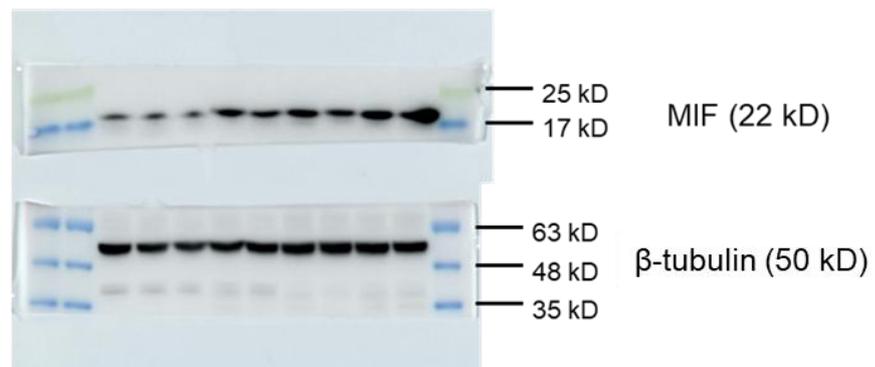
Figure S11. Gating strategies in flow cytometry analysis. Gating strategies to define neutrophils and eosinophils (A), TH1 (T-bet), TH2 (GATA3) and TH17 (Rorγt) cells (B) from pulmonary single - cell suspension through flow cytometry.
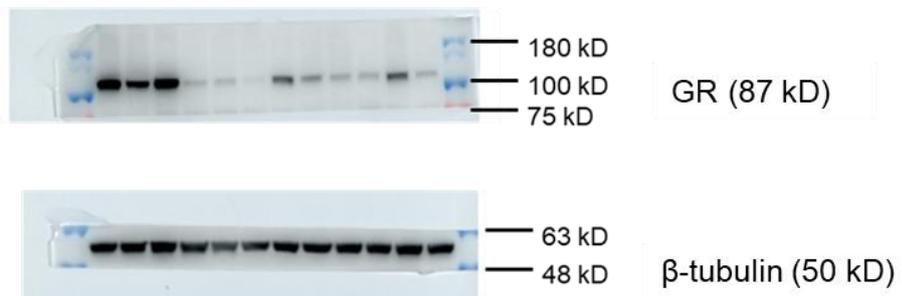
Figure S2F

100 kD — 75 kD — 63 kD — MALT1 (92 kD)

63 kD — 48 kD — 35 kD — β-tubulin (50 kD)

Figure S2F

25 kD — 17 kD — MIF (22 kD)

63 kD — 48 kD — 35 kD — β-tubulin (50 kD)

Figure 4M

180 kD — 100 kD — 75 kD — GR (87 kD)

63 kD — 48 kD — β-tubulin (50 kD)

Figure S12. Uncropped images of Western blots. Uncropped images related to different immunoblotting results as indicated.