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Supplementary Materials for

In vivo reprogramming of pathogenic lung TNFR2^{+} cDC2s by IFNβ inhibits HDM-induced asthma

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The PDF file includes:

Figs. S1 to S8
Table S1

Other Supplementary Material for this manuscript includes the following:

Table S2

Supplemental Methods and Materials

Supplemental table 1: Key resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies and Secondary		
Anti-mouse CD4-PE/Cy7 (clone: GK1.5)	BioLegend	Cat#100422
Anti-mouse IL-4-APC (clone: 11B11)	BioLegend	Cat#504106
Anti-mouse IL-17a-PE (clone: TC11-1810.1)	BioLegend	Cat#506903
Anti-mouse IL-4 – APC (clone: 11B11)	BioLegend	Cat#504105
Anti-mouse CD45-PercP/Cy5.5 (clone: 30-F11)	Biolegend	Cat#103131
Anti-mouse CD45.1-APC (clone: A20)	BioLegend	Cat#110713
Anti-mouse MHCII(I-A/I-E)-Brilliant Violet 421 (clone: M5/114.15.2)	BioLegend	Cat#107636
Anti-mouse MHCII(I-A/I-E)- Alexa Fluor (clone: M5/114.15.2)	BioLegend	Cat#107622
Anti-mouse/human CD11b- PE/Cy7 (clone: M1/70)	BioLegend	Cat#101216
Anti-mouse/human CD11b- Brilliant Violet 605 (clone: M1/70)	BioLegend	Cat#101237
Anti-mouse CD64- PerCP/Cy5.5 (clone: X54-5/7.1)	BioLegend	Cat#139307
Anti-mouse pSTAT3 (Y705) mAb (D3A7)	Cell Signaling Technology	Cat#:9145
Rabbit (DA1E) mAb IgG XP® Isotype Control	Cell Signaling Technology	Cat#:3900
CD26 PECy7 (clone: H194-112)	Biolegend	Cat#137810
SiglecF APC (clone: S17007L)	Biolegend	Cat#155507
Anti-mouse/human Arg1-FITC	RD systems	Cat#IC5868F
CD172a APC (clone: P84)	Biolgend	Cat#144013
Ly6G FITC (clone: RB6-8C5)	BioLegend	Cat#108405
Anti-mouse LAP (TGFβ1)-Brilliant Violet 421 (clone:TW7-16B4)	BioLegend	Cat#141407
Anti-mouse LAP (TGFβ1)-FITC (clone:TW7-16B4)	BioLegend	Cat#141413
Anti-mouse OX40L-PE (clone: RM134L)	BioLegend	Cat#108805
Hamster IgG	BioXcell	Car#BE0091
Anti-mouse TNFR2- PE (Clone:TR75-89)	BioLegend	Cat#113405
Anti-mouse TNFR2-APC (Clone: REA228)	Miltenyi Biotec	Cat#130-104-698
Anti-mousPDL1-Brilliant Violet 421 (clone: 10F.9G2)	BioLegend	Cat#124315
Anti-mouse/human pSTAT1 (clone: 58D6)	Cell Signaling Technology	Cat#:9167
Anti-mouse pErk1/2 (Thr202/Tyr204) mAb (D13.14.4E)	Cell Signaling Technology	Cat#:4370
Anti-mouse ICOSL-PE (clone: HK5.3)	BioLegend	Cat#107405
Anti-mouse CD36	Biolegend	Cat# 102616

Anti-mouse IL-10 – APC (clone: JESS-16E3)	BioLegend	Cat#505016
Anti-mouse Foxp3-Pacific Blue (clone: MF-14)	BioLegend	Cat#26410
Anti-mouse T1ST2- APC (clone: D1H4)	BioLegend	Cat#146605
Anti-mouse F4/80- PerCP/Cy5.5 (clone: BM8)	BioLegend	Cat#123127
Anti-mouse IgG-HRP	Southern Biotech	Cat#1033-05
Anti-mouse IgE-HRP	Southern Biotech	Cat#1110–05
Phospho-AMPK (Thr172) rabbit mAb	Cell Signaling Technology	Cat#2535
GLUT1 (clone: SA0377)	Invitrogen	Cat#MA5-31960
pS6 Ribosomal protein (S235/236) (clone: 2F9)	Cell Signaling Technology	Cat#4856S
Anti-CPT-1A rabbit antibody	ProteinTech	Cat#15184–1-AP
Anti-PPARγ rabbit antibody	CST	Cat#2435
Anti-human CD1c- PE/Cy7 (clone: L161)	BioLegend	Cat#331515
Anti-human CD14- PerCP/Cy5.5 (clone: 63D3)	BioLegend	Cat#367109
Anti-human CD206- FITC (clone: 15-2)	BioLegend	Cat#321103
Anti-human TNFR2-APC (clone: 3G7A02)	BioLegend	Cat#358405
Anti-human HLA-DR-APC/Cy7 (clone: L243)	BioLegend	Cat#307617
Anti-human PDL1- Brilliant Violet 421 (clone:29E.2A3)	BioLegend	Cat#329713
Anti-human TGFβ1- PE (clone: Tw4-2F8)	BioLegend	Cat#349603
Anti-human Arginase 1- PE (clone: 14D2C43)	BioLegend	Cat#369703
Anti-human IL-4- PE (clone: G077F6)	BioLegend	Cat#355003
Chemicals, ELISA kits and Recombinant Proteins		
OVA	Invivogen	cat#vac-pova
DNase I	Roche	Cat#10104159001
MitoTracker Green FM	Thermo Fisher Scientific	Cat# M7514
Erk1/2 inhibitor (GDC-0994,)	Selleckchem	Cat # S7910
Etomoxir	Sigma	Cat # E1905
Brefeldin A	Biolegend	Cat#423303
Recombinant mouse IFNα1	R&D	Cat # 10148-IF
Recombinant murine IFNβ	R&D	Cat#8234-MB/CF
Recombinant human IFNβ	R&D	Cat # 8499-IF
C1-BODIPY 500/510 C12	Life Technologies,	Cat# D-382
Anti-TNFR2 monoclonal antibody (clone: TR75-54.7)	BioXcell	Cat#BE0247
Isotype Control (Armenian Hamster IgG)	BioXcell	Cat#BE0091
Anti-CD25 mAb (PC-61.5.3)	BioXcell	Cat#BE0012
Isotype Control (Rat IgG1,κ)	BioXcell	Cat#BE0088
Experimental Models: Organisms/Strains		
Mouse: B6.CD45.1	Jackson Laboratory	Cat#002014

Mouse: ERK2$^{fl/fl}$	Jackson Laboratory	Cat#019112
Mouse: STAT3$^{fl/fl}$	Jackson Laboratory	Cat#016923
Mouse: IFNAR1$^{-/-}$	Jackson Laboratory	Cat#028288
Mouse: CD11c^{Cre}	Jackson Laboratory	Cat#008068
Software and Algorithms		
FlowJo version 10.1r1	FlowJo	http://www.flowjo.com
Prism6	GraphPad	http://www.graphpad.com

Supplemental Figures.

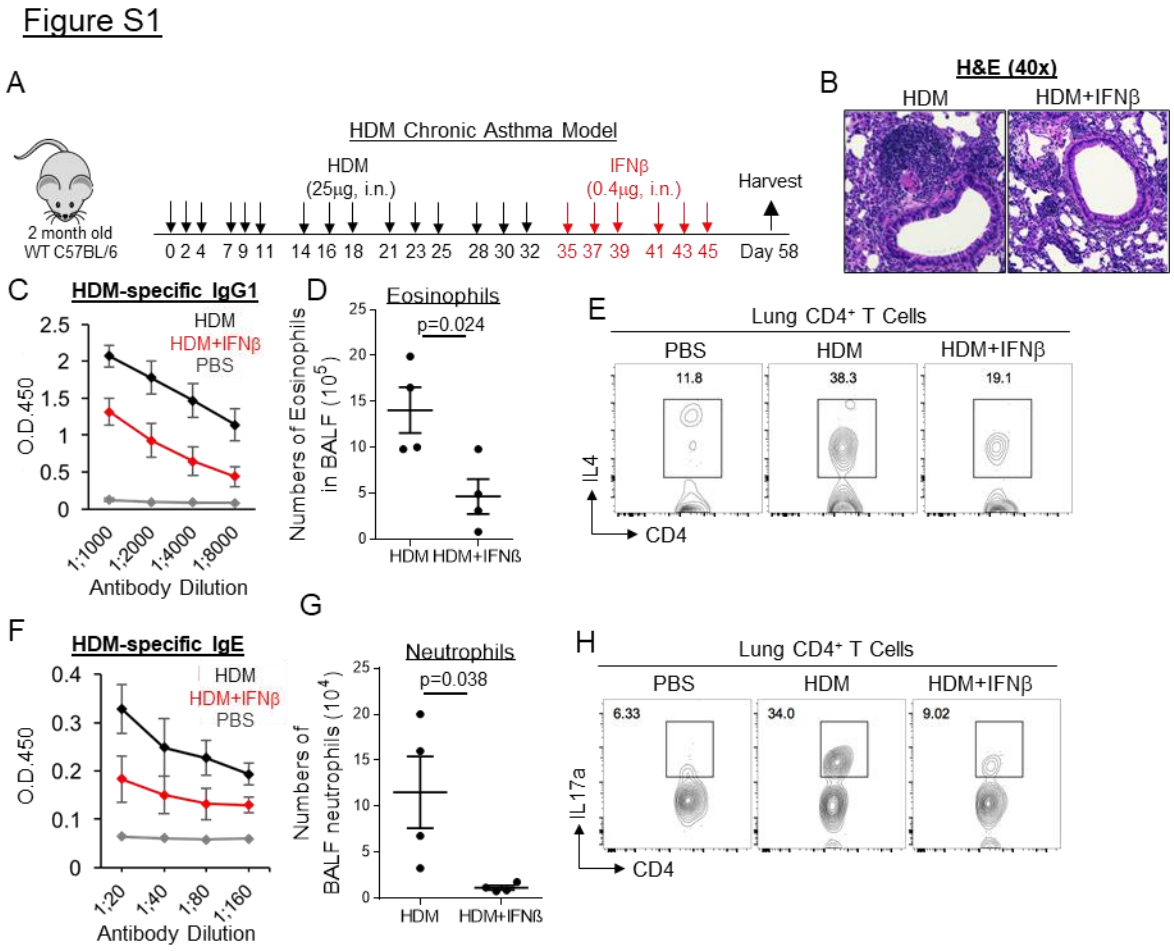

Figure S1. Inhaled IFNβ alleviated HDM-induced chronic asthma. **A.** Experimental protocol for treating HDM-induced chronic asthma. Mice were intranasally (*i.n*) administered PBS or IFNβ (0.4µg). **B.** Representative H&E staining of lung sections from asthmatic mice treated with PBS or IFNβ. (n=3-4 mice per group). Data are representative of 3 independent experiments. **C, F.** Serum HDM-specific IgG1 and BALF HDM-specific IgE from asthmatic mice treated with IFNβ (n=3-4 mice per group). Data are representative of 3 independent experiments. **D.** Absolute numbers of eosinophils in the BALF of asthmatic mice treated with IFNβ (n=3-4 mice per group). Data were representative of 3 independent experiments. **E.** Flow cytometry plots of IL4 producing lung $CD4^+$ T cells from asthmatic mice treated with IFNβ (n=3-4 mice per group). Data are representative of 3 independent experiments. **G.** Absolute numbers of neutrophils in the BALF of asthmatic mice treated with IFNβ (n=3-4 mice per group). Data were representative of 3 independent experiments. **H.** Flow cytometry plots of IL17 producing lung $CD4^+$ T cells from asthmatic mice treated with IFNβ (n=3-4 mice per group). Data are representative of 3 independent experiments. Graphs represent the mean with error bars indication s.e.m. *P* values determined by unpaired student *t*-test.

Figure S2

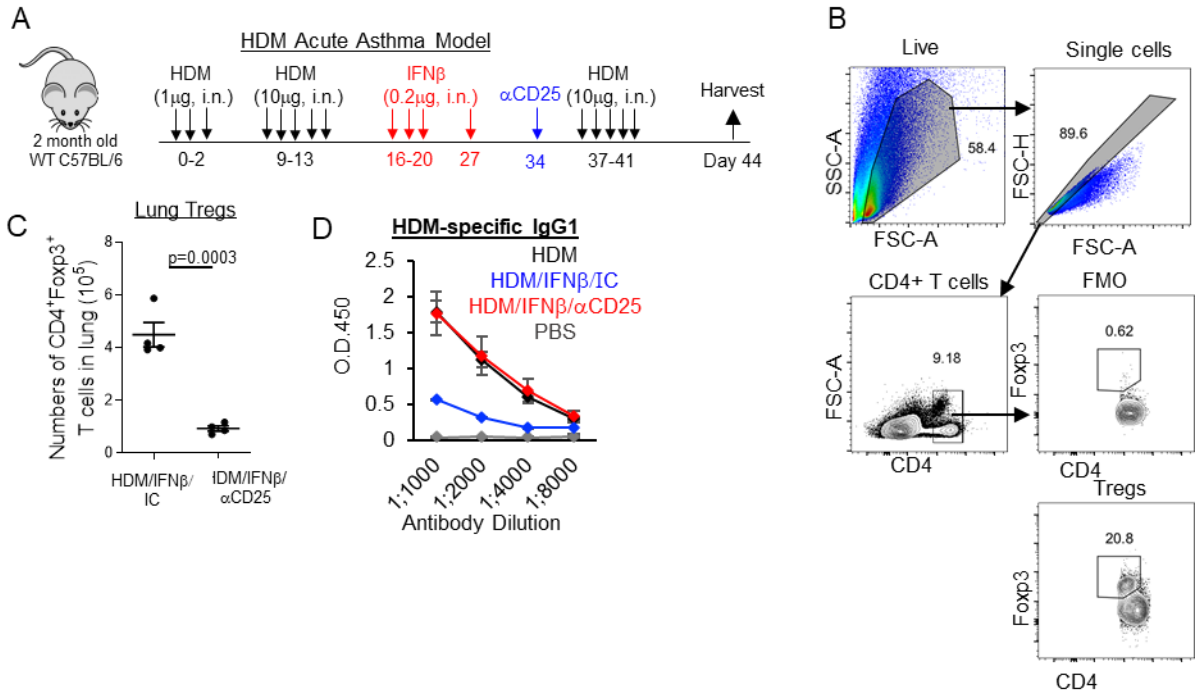

Figure S2. IFNβ efficacy depends on Tregs in HDM mice. A. An experimental protocol for Treg depletion in IFNβ-treated asthmatic mice. Anti-CD25 mAb (PC-61.5.3, 100μg in 40μl PBS) or rat IgG1 isotype control were administered (*i.n.*) on day 34. Asthma exacerbation was induced with daily HDM treatment on day 37-41. The mice were harvest on day 44. (n=4 mice/group). **B.** Lung Tregs was gated on live $CD4^{+}Foxp3^{+}$ cells. **C.** The number of lung Tregs on day 44 were enumerated. Data are representative of 2 independent experiments. **D.** Serum HDM-specific IgG1 in asthmatic mice treated with anti-CD25 mAb or isotype control from mice in **A**. Data are representative of 2 independent experiments. Graphs represent the mean with error bars indication s.e.m. *P* values determined by unpaired student *t*-test.

Figure S3

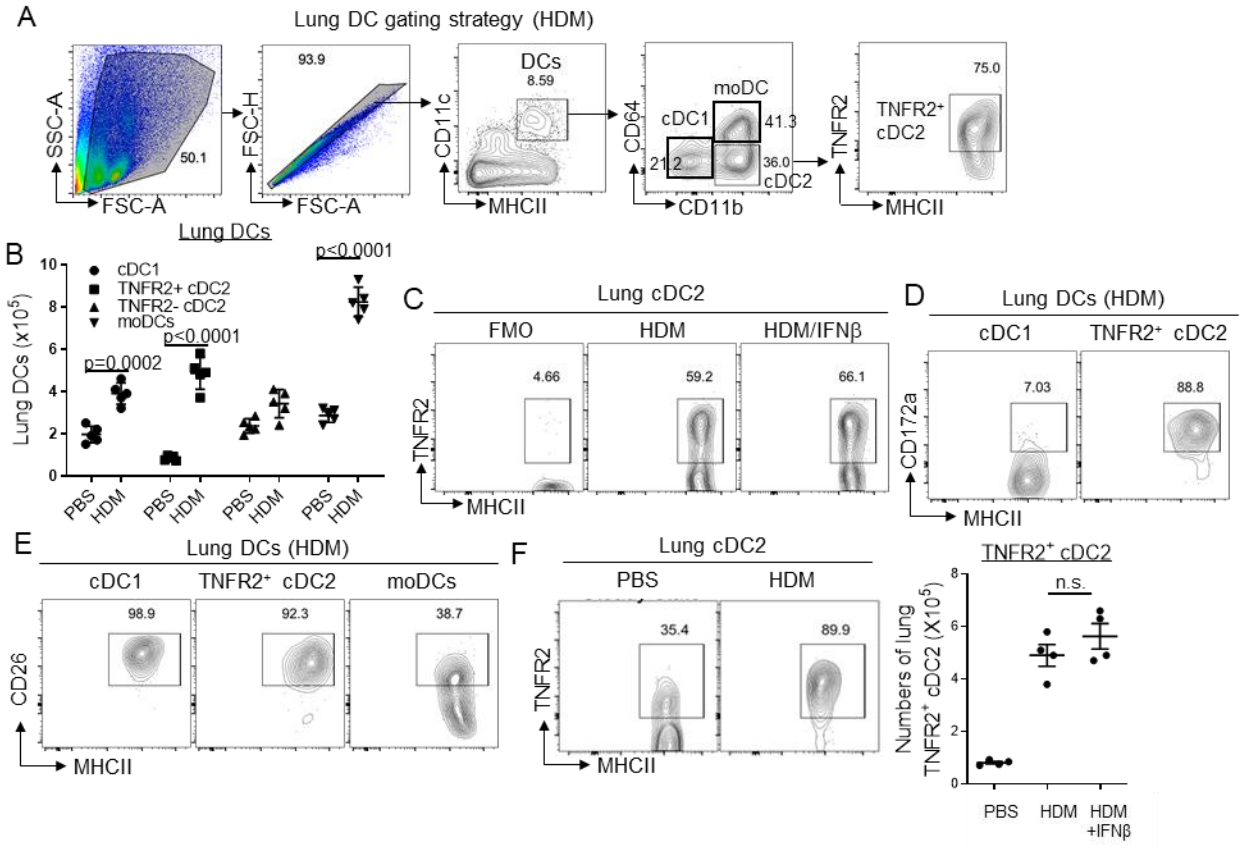

Figure S3. Gating strategy of lung TNFR2$^+$ cDC2 in HDM-induced asthmatic mice. A. Lung TNFR2$^+$ cDC2 was gated on live CD64$^-$CD11B$^+$TNFR2$^+$MHC II$^+$CD11C$^+$ cells. **B, C, F**. Numbers of lung cDC1, moDCs, TNFR2$^-$, TNFR2$^+$ cDC2 in C57BL/6J mice treated with PBS, HDM or HDM/IFNβ. (n=4-5 mice/group). Data are representative of 3 independent experiments. **D-E.** Flow analysis of CD172a (SIRPα) and CD26 expression on indicated DCs subsets. (n=4-5 mice/group). Data are representative of 3 independent experiments. Graphs represent the mean with error bars indication s.e.m. *P* values determined by two-way ANOVA Sidak's multiple comparison (**B**) or one-way ANOVA Tukey's multiple comparison test (**F**).

Figure S4

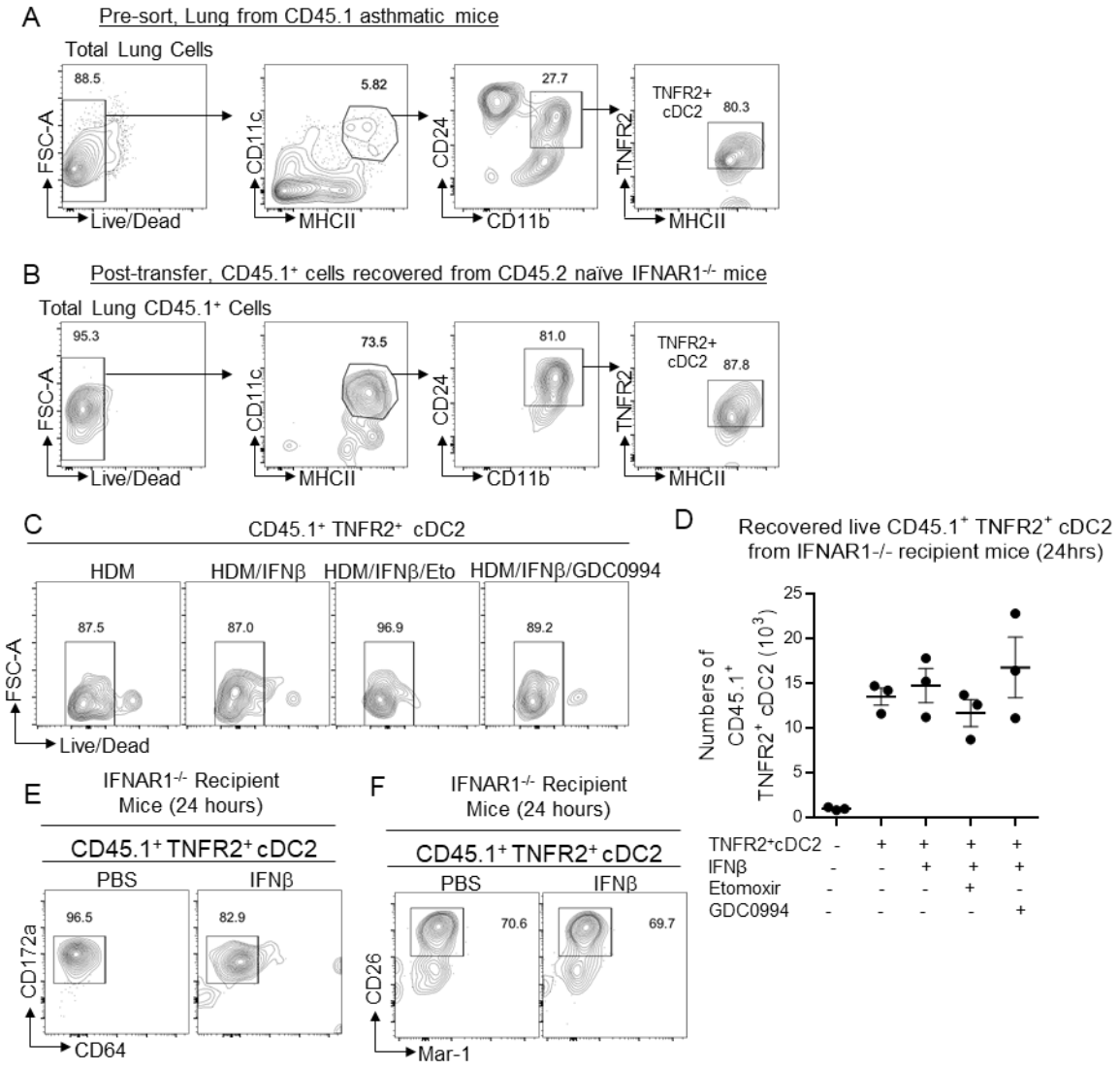

Figure S4. Sorting strategy of lung TNFR2$^+$ cDC2 for the adoptive transfer. A-B, E-F. Flow analysis of pre-sort and post-transfer of lung CD45.1$^+$ TNFR2$^+$ cDC2 population (n=3 mice/group). Data are representative of 2 independent experiments. **C.** Sorted CD45.1 TNFR2$^+$ cDC2 from HDM mice were treated with IFNβ, IFNβ+Etomoxir, or IFNβ+GDC0994 or untreated (HDM) for 30mins in culture. Cell viability were determined by Propidium Iodide stain. Data are representative of 2 independent experiments. **D**. Recipient mice were harvested after 24hrs. Recovered lung CD45.1 cells were enumerated. (n=3 mice/group). Data were representative of 2 independent experiments. Graphs represent the mean with error bars indication s.e.m. *P* values determined by one-way ANOVA Tukey's multiple comparison test.

Figure S5

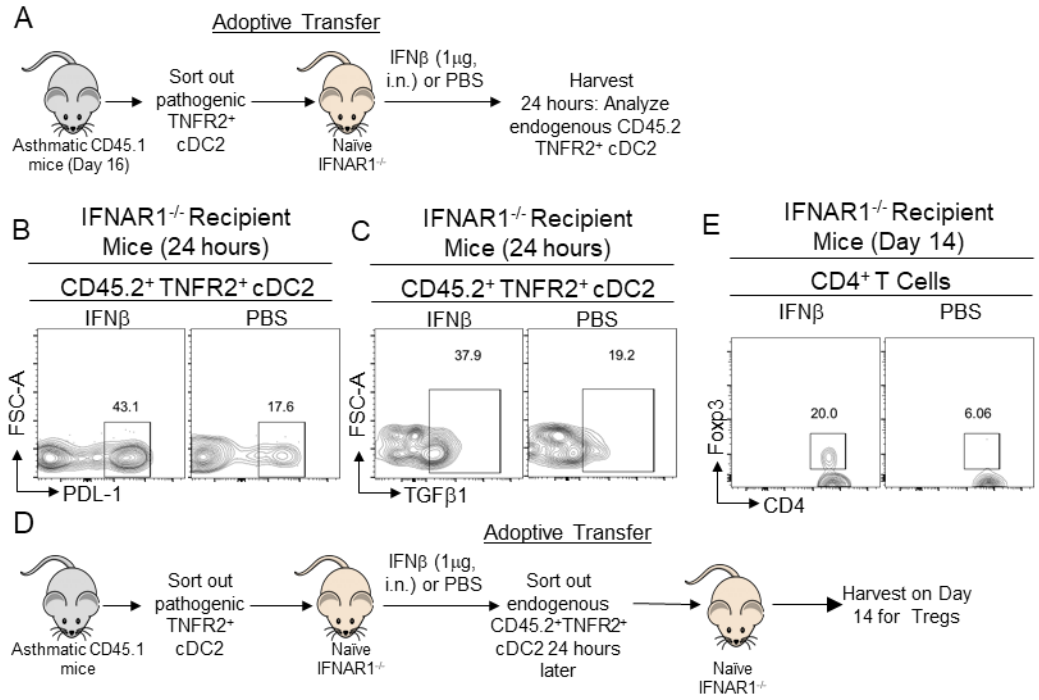

Figure S5. IFNβ-reprogrammed WT TNFR2^{+} cDC2 primes bystander IFNAR1$^{-/-}$ TNFR2^{+} cDC2 to generate lung Tregs. A. A cartoon illustrate the adoptive transfer experiment. **B-C**. Flow analysis of CD45.2^{+} endogenous TNFR2^{+} cDC2 population (n=3 mice/group). Data are representative of 2 independent experiments. **D.** A cartoon illustrate the consecutive adoptive transfer experiments. **E.** Flow analysis of lung Tregs on day 14 in the IFNAR1$^{-/-}$ recipient mice receiving sorted CD45.2^{+} TNFR2^{+} cDC2 population. (n=3 mice/group). Data are representative of 2 independent experiments.

Figure S6

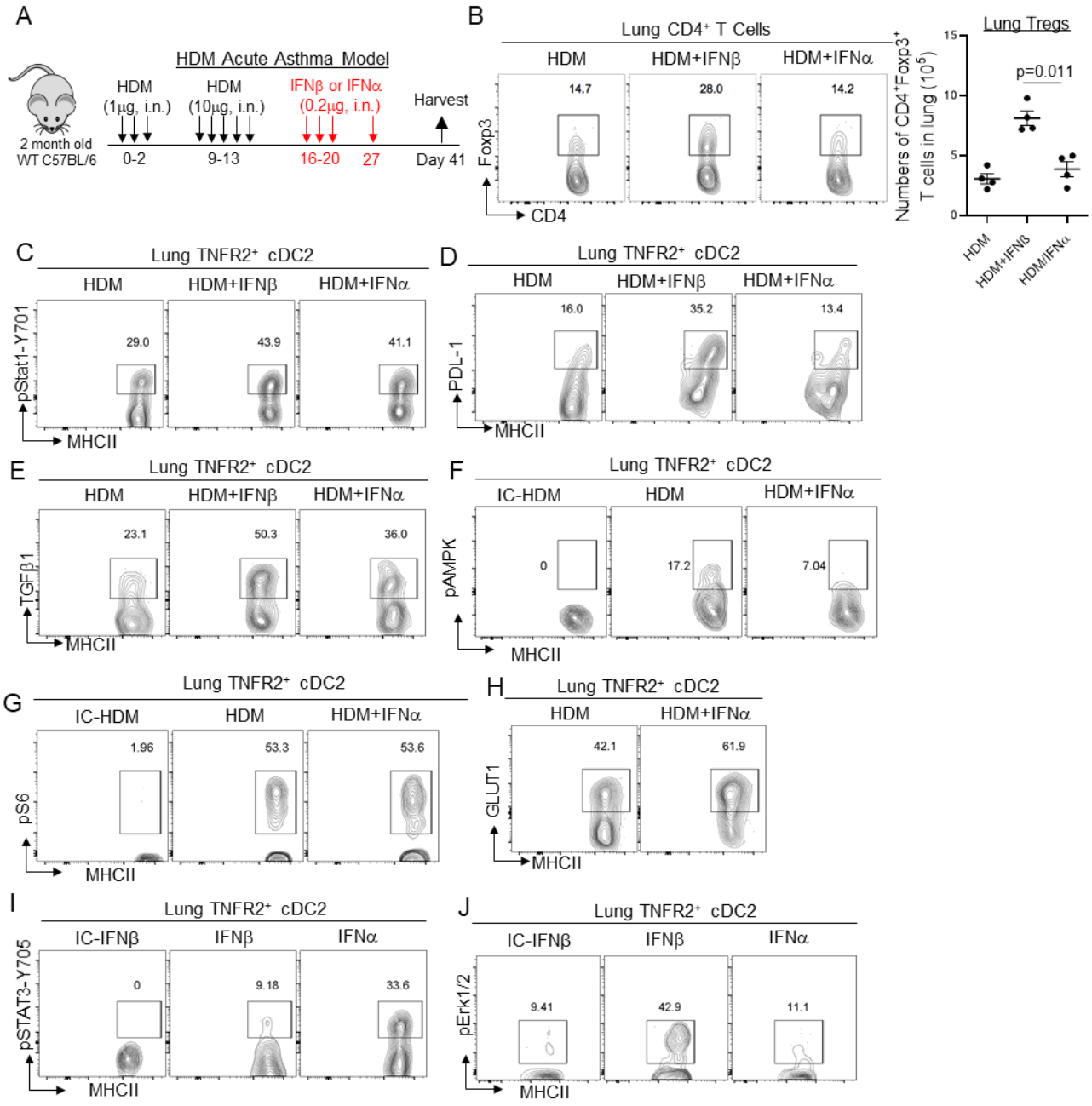

Figure S6. IFNα does not generate lung Tregs in HDM mice and cannot reprogram pathogenic lung TNFR2$^+$ cDC2s *in vivo*. A. Experimental protocol for HDM-induced acute asthma. Mice were intranasally (*i.n*) administered IFNβ (0.2µg) or IFNα1 (0.2µg). **B.** Flow cytometry analysis (left) and absolute number (right) of lung Tregs in asthmatic mice treated with PBS, IFNβ or IFNα1 (0.2µg) (n=4 mice per group). Data are representative of 2 independent experiments. **C-E.** Flow cytometry analysis of pSTAT1, PD-L1 and TGFβ1 expression in lung TNFR2$^+$cDC2 from asthmatic mice treated with IFNβ or IFNα1 as in **A.** Data are representative of 2 independent experiments. **F-H.** Flow cytometry analysis of pS6, pAMPK

and GLUT1 expression in lung TNFR2^{+} cDCs of WT mice 2 days after 100μg HDM and IFNα1 (1μg) administration (*i.n.*). (n=3 mice per group). Data are representative of 2 independent experiments. **I-J.** Flow cytometry analysis of p-ERK1/2 and p-STAT3 in lung TNFR2^{+} cDC2 of C57BL/6J mice treated (*i.n.*) with IFNβ or IFNα1 (1μg) for 24hrs (n=4 mice per group). Data are representative of 2 independent experiments. IC: isotype control. Graphs represent the mean with error bars indication s.e.m. *P* values determined by one-way ANOVA Tukey's multiple comparison test.

Figure S7

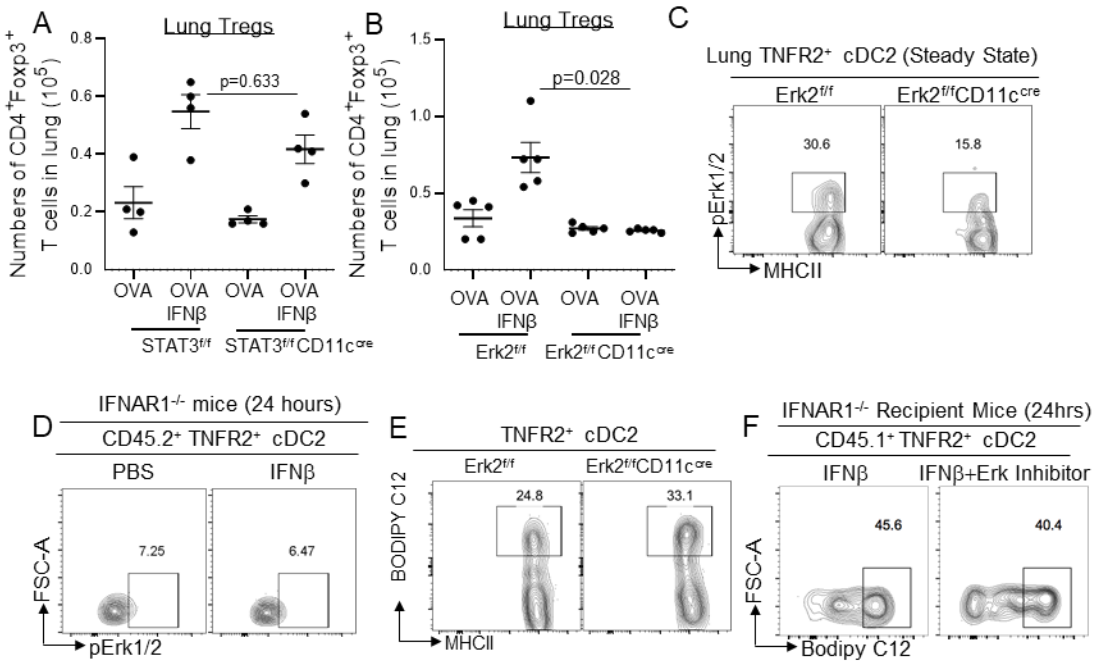

Figure S7. Erk2 expression in CD11c^{+} cells is required for Tregs induction but is dispensable for fatty acid uptakes. A-B. Quantification of CD4^{+}Foxp3^{+} Tregs in STAT3$^{f/f}$, STAT3 $^{f/f}$CD11c^{cre} (**A**), and ERK2$^{f/f}$, ERK2$^{f/f}$CD11c^{cre} (**B**) mice treated (*i.n.*) with one dose of OVA (2μg) or OVA/IFNβ (0.2μg). Lungs were harvested on day 14. (n=4~5 mice per group). Data are representative of 2 independent experiments. **C.** Flow cytometry analysis of pErk1/2 in TNFR2^{+} cDC2s from Erk2$^{f/f}$ and Erk2 $^{f/f}$CD11c^{cre} mice at steady state. (n=3 mice per group). Data are representative of 3 independent experiments. **D**. Lung CD45.1^{+}TNFR2^{+}cDC2s from HDM-induced asthmatic mice were adoptively transferred into IFNAR1$^{-/-}$ CD45.2 recipient mice. Recipient mice were treated with IFNβ (1μg). Flow cytometry analysis of pErk1/2 in CD45.2 TNFR2^{+}cDC2s following IFNβ treatment. (n=3 mice per group). Data are representative of 2 independent experiments. **E.** Flow cytometry analysis of BODIPY C_{12} uptake in TNFR2^{+} cDC2 of Erk2$^{f/f}$ and Erk2$^{f/f}$CD11c^{cre} mice. (n=3 mice per group). Data are representative of 3 independent experiments. **F.** CD45.1^{+}TNFR2^{+}cDC2 were sorted out of asthmatic mice, treated with GDC-0994 (50nM) for 30 mins. The cells were then adoptively transferred (*i.n.*) into naïve IFNAR1$^{-/-}$ mice. Recipient mice were then treated with IFNβ (*i.n*, 1μg). Flow cytometry analysis of BODIPY C_{12} uptake in CD45.1^{+}TNFR2^{+}cDC2. (n=3 mice per group). Data are representative of 2 independent experiments. Graphs represent the mean with error bars indication s.e.m. *P* values determined by one-way ANOVA Tukey's multiple comparison test.

Figure S8

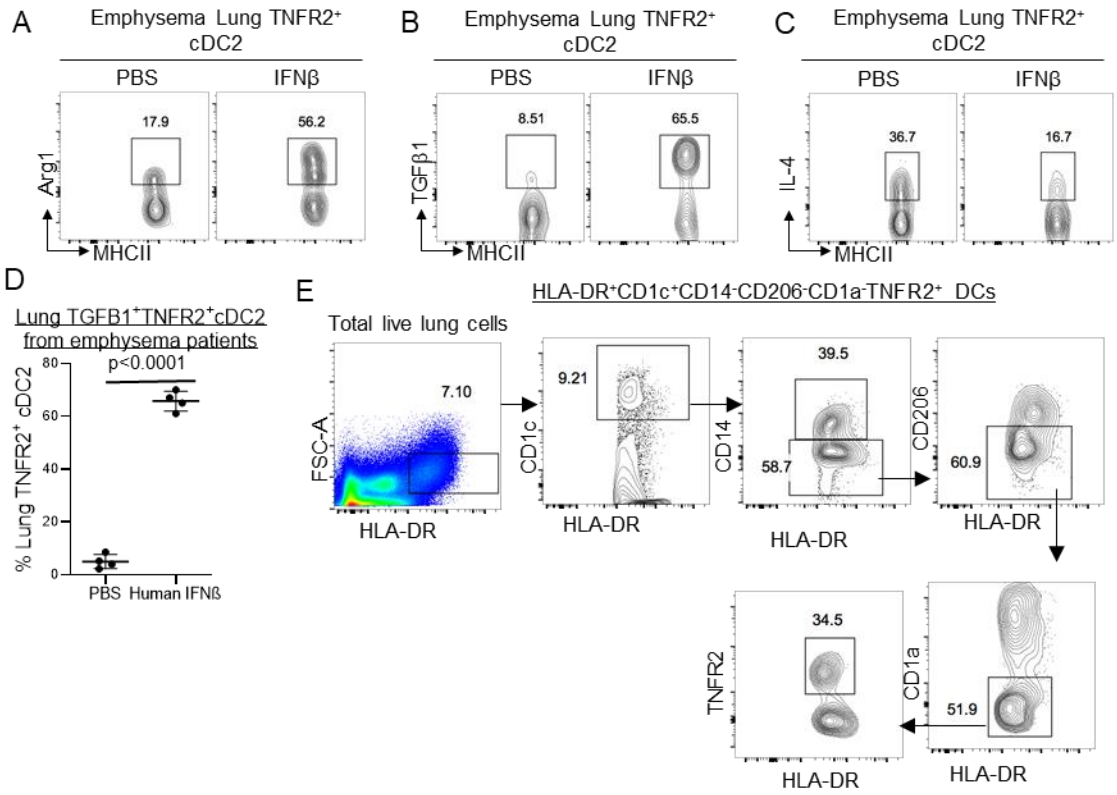

Figure S8. IFNβ enhances tolerogenic markers on pathogenic human lung TNFR2$^+$ cDC2s. A-D. Flow cytometry analysis and frequency of human lung TNFR2$^+$ cDC2s from emphysema patients treated with human recombinant IFNβ (0.4μg) or PBS for 24hrs *ex vivo*. **E.** Human lung cDC2 were gated on live, HLA-DR$^+$CD1c$^+$CD1a$^-$CD14$^-$CD206$^-$ cells as before[3] (n=4 emphysema patients). Graphs represent the mean with error bars indication s.e.m. *P* values determined by unpaired Student's *t*-test.