SUPPLEMENTARY METHODS

Protease analysis:

Serum MCPT1 was determined using the eBioscience Ready Set Go Kit (eBioscience, CA, USA).

Histological assessment

Paraffin embedded formalin fixed lung lobes were sectioned and subjected to Periodic Acid-Schiff (PAS) and picro sirus red staining. Sections were scored and assessed as previously described[1].

Total Collagen

Total collagen was assessed in lung homogenate using the Sircol™ Soluble Collagen assay (Biocolor, Carrickfergus, UK) according to manufacturer's instructions.

Airway smooth muscle assessment

Paraffin embedded formalin fixed lung lobes were sectioned and immunohistochemically stained with rabbit anti-mouse smooth muscle actin (Abcam, Cambridge, UK) as previously described[2]. Lung sections were imaged at x20 magnification and the size of the airway smooth muscle layer was measured. Briefly, the thickness of 10 airways were measured per mice and 10 measurements were taken per airway. The data is presented as an average. Data was blinded for analysis.

SUPPLEMENTARY FIGURE LEGENDS

Table S1: Flow cytometry antibodies used in the study

Lineage negative (Lin^-) cells were defined as: $CD11b^-CD19^-TCR\text{-}\beta^-TER119^-GR1^-CD3^-CD4^-CD8^-$

Figure S1: T_{FH} accumulate over time in the mLN following HDM exposure.

Adult female BALB/c mice were exposed to either 25 µg house dust mite (HDM), 10 µg *alternaria alternata (*ALT) or 25 µl phosphate buffered saline (PBS), 3 times a week for up to 5 weeks. Flow cytometry was used to determine the frequency of T_{FH} within cellular compartments following allergen exposure. T_{FH} were defined as $CXCR5^+PD1^+Foxp3^-CD4^+$. Representative flow plots of T_{FH} in PBS, ALT or HDM treated animals, pre-gated on $CD4^+CD3^+Foxp3^-CD44^{hi}CD62L^-$ lymphocytes. **A)** Mediastinal lymph node (mLN), **B)** Spleen, **C)** Lung tissue. Data is representative of n=5 per time-point and 2 independent experiments.

Figure S2: T_{FH} are not found in the circulation and do not accumulate over time.

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Figure S3: T_{FH} accumulate over time following HDM exposure.

Adult female BALB/c mice were exposed to either 25 µg house dust mite (HDM), 10 µg *alternaria alternata (*ALT) or 25 µl phosphate buffered saline (PBS), 3 times a week for up to 5 weeks. Flow cytometry was used to determine the frequency of germinal centre (GC) B cells within cellular compartments following allergen exposure. GC B cell were defined as $CD38^{-}GL7^{+}FAS^{+}CD19^{+}B220^{+}$ cells Representative flow plots of GC B cells in PBS, ALT or HDM treated animals, pre-gated on $CD19^{+}B220^{+}$ lymphocytes. **A)** Mediastinal lymph node (mLN), **B)** Lung tissue, **C)** Spleen. Data is representative of n=5 per time-point and 2 independent experiments.

Figure S4: Schematic of experimental design of ICOS-L blockade during chronic allergic airway disease.

Adult female BALB/c mice were exposed (i.n) to 25 µg house dust mite (HDM), 10 µg *alternaria alternata (*ALT) or 25 µl phosphate buffered saline (PBS), 3 times a week for 5 weeks. From the start of week 4 mice were also administered 150 µg anti-ICOS-L (α-ICOS-L) or isotype control (2A3) antibody i.p, 3 times a week. Mice were culled at the end of week 5.

Figure S5: Mast cell degranulation is reduced by ICOS-L blockade during house dust mite driven chronic allergic airway disease.

Adult female BALB/c mice were exposed (i.n) to 25 µg house dust mite (HDM), 10 µg *alternaria alternata (*ALT) or 25 µl phosphate buffered saline (PBS), 3 times a week for 5 weeks. From the start of week 4 mice were also administered 150 µg anti-ICOS-L (α-ICOS-L) or isotype control (2A3) antibody i.p, 3 times a week. Mice were culled at the end of week 5. Serum MCPT1 was determined by ELISA **A)** HDM study, **B)** ALT study. * *P<0.05*, ***p<0.01*, ****p<0.001.* Representative data. n=4-6.

Figure S6: Lung eosinophil proportions were unchanged by ICOS-L blockade during chronic allergic airway disease.

Adult female BALB/c mice were exposed (i.n) to 25 µg house dust mite (HDM), 10 µg *alternaria alternata (*ALT) or 25 µl phosphate buffered saline (PBS), 3 times a week for 5 weeks. From the start of week 4 mice were also administered 150 µg anti-ICOS-L (α-ICOS-L) or isotype control (2A3) antibody i.p, 3 times a week. Mice were culled at the end of week 5. Flow cytometry was used to determine the frequency of eosinophils in the lungs following allergen exposure. Eosinophils were defined as $CD68^{-}CD11c^{-}CD11b^{+}Siglecf^{hi}$. **A)** HDM study, **B)** ALT study. Statistical significance was determined using a Mann Whitney U test. * *P<0.05*, ***p<0.01*, ****p<0.001.* Data shown is pooled from two independent experiments, n=4-12. ALT data is based on one study, n=4-6.

Figure S7: Therapeutic ICOS-L blockade does not alter mucus hyper-secretion, collagen deposition or airway smooth muscle thickening.

Adult female BALB/c mice were exposed (i.n) to 25 µg house dust mite (HDM), 10 µg *alternaria alternata (*ALT) or 25 µl phosphate buffered saline (PBS), 3 times a week for 5 weeks. From the start of week 4 mice were also administered 150 µg anti-ICOS-L (α-ICOS-L) or isotype control (2A3) antibody (i.p) 3 times a week. Mice were culled at the end of week 5. **A-B)** Representative images of Periodic Acid-Schiff (PAS) staining on parafilm embedded formalin fixed (PEFF) lung sections used to score mucus hyper-secretion. **A)** HDM+2A3**, B)** HDM+α-ICOS-L**, C)** Quantification of PAS scoring. **D-E)** Representative staining of Pico-Sirus Red on PEFF lung used to determine collagen I and III deposition. **D)** HDM+2A3**, E)** HDM+α-ICOS-L, **F)** Total collagen in the lungs determined by Sircol soluble collagen assay. **G-H)** Representative staining of airway smooth muscle cells and myofibroblasts identified by immunohistochemical staining of α-smooth muscle actin (α-SMA). **G)** HDM+2A3**, H)** HDM+α-

ICOS-L, **I)** Airway smooth muscle layer thickness was measured from α-SMA stained sections. Images were taken at x20 magnification. Statistical significance was determined using a Mann Whitney U test. n=8-12.

Figure S8: IL-17A$^+$ CD4$^+$ T cells are not directly targeted by ICOS-L blockade.

Adult female BALB/c mice were exposed to either 25 µg house dust mite (HDM), 10 µg *alternaria alternata (*ALT) or 25 µl phosphate buffered saline (PBS), 3 times a week for up to 5 weeks. Flow cytometry was used to determine the frequency of lung cellular populations. **A)** Representative gating of IL-17A$^+$ CD4$^+$ T cells following allergen and 2A3 or α-ICOS-L treatment. These populations were quantified **B)** Proportions of lung IL-17A$^+$ CD4$^+$ - HDM study, **C)** Number of lung IL-17A$^+$ CD4$^+$ - HDM study**, D)** Proportions of lung IL-17A$^+$ CD4$^+$ - ALT study, **E)** Number of lung IL-17A$^+$ CD4$^+$ - ALT study. Statistical significance was determined using a Mann Whitney U test. * *P<0.05*, ***p<0.01*, ****p<0.001.* HDM data shown is pooled from two independent experiments, n=8-12. ALT data is based on one study, n=4-6.

Figure S9: IL-13$^+$ and IL-17A$^+$ CD4$^+$ ILCs are not directly targeted by ICOS-L blockade.

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IL-13$^+$ ILCs – HDM study, **B)** IL-13$^+$ ILCs – ALT study**, C)** Representative gating of IL-17A$^+$ ILCs following allergen and 2A3 or α-ICOS-L treatment. **D)** Frequency of IL-17A$^+$ ILCs – HDM study, **E)** Number of IL-17A$^+$ ILCs – HDM study, **F)** Frequency of IL-17A$^+$ ILCs – ALT study, **G)** Number of IL-17A$^+$ ILCs – ALT study. Statistical significance was determined using

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Figure S10: Graphical summary of the effects of blocking ICOS/ICOS-L interactions on established chronic allergic airway disease

1. Saglani S, Mathie SA, Gregory LG, Bell MJ, Bush A, Lloyd CM. Pathophysiological Features of Asthma Develop in Parallel in House Dust Mite–Exposed Neonatal Mice. *American Journal of Respiratory Cell and Molecular Biology* 2009; **41**(3)**:** 281-289.

2. Gregory LG, Mathie SA, Walker SA, Pegorier S, Jones CP, Lloyd CM. Overexpression of Smad2 drives house dust mite–mediated airway remodeling and airway hyperresponsiveness via activin and IL-25. *American journal of respiratory and critical care medicine* 2010; **182**(2)**:** 143-154.

Target	Clone	Supplier	Concentration	Fluorochrome
B220	RA3-6B2	Biolegend	1:200	BV510
CD3e	145-2C11	eBioscience	1:200	PE-CF594
CD4	RM4-5	Biolegend	1:100	APC/CY7
CD8	53-6.7	Biolegend	1:200	BV711
CD11b	M1/70	Biolegend	1:200	BV711
CD11b	M1/70	eBioscience	1:100	PERCP-Cy5.5
CD19	6D5	Biolegend	1:200	BV605
CD19	6D5	eBioscience	1:100	PERCP-Cy5.5
CD38	90	eBioscience	1:200	Alexa700
CD44	IM7	Biolegend	1:200	PERCP-Cy5.5
CD45	30-F11	Biolegend	1:200	FITC
CD62L	MEL-14	Biolegend	1:200	BV605
CD90-2	30-H12	Biolegend	1:100	Alexa700
CXCR5	L138D7	Biolegend	1:50	BV421
FAS	15A7	eBioscience	1:100	PERCP-Cy5.5
Foxp3	11-5773-82	eBioscience	1:100	FITC
GL7	GL7	Biolegend	1:100	FITC
GR1	RB6-8C5	eBioscience	1:100	PERCP-Cy5.5
Ia/Ie	M5/114.15.2	Biolegend	1:300	BV421
IFN-γ	XMG1.2	Biolegend	1:50	BV510
IgD	11-26c.2a	Biolegend	1:100	Pacific Blue
IgM	RMM-1	Biolegend	1:50	APC/CY7
IL-13	eBio13A	eBioscience	1:100	PE/CY7
IL-17A	TC11-18H10.1	Biolegend	1:100	BV605
Nkp46	29A1.4	Biolegend	1:50	BV421
PD1	RMP1-30	Biolegend	1:200	PE/CY7
Siglecf	E50-2440	BD Pharmingen	1:100	PE
TCR-β	H57-597	Biolegend	1:100	PERCP-Cy5.5
TER119	TER119	eBioscience	1:100	PERCP-Cy5.5

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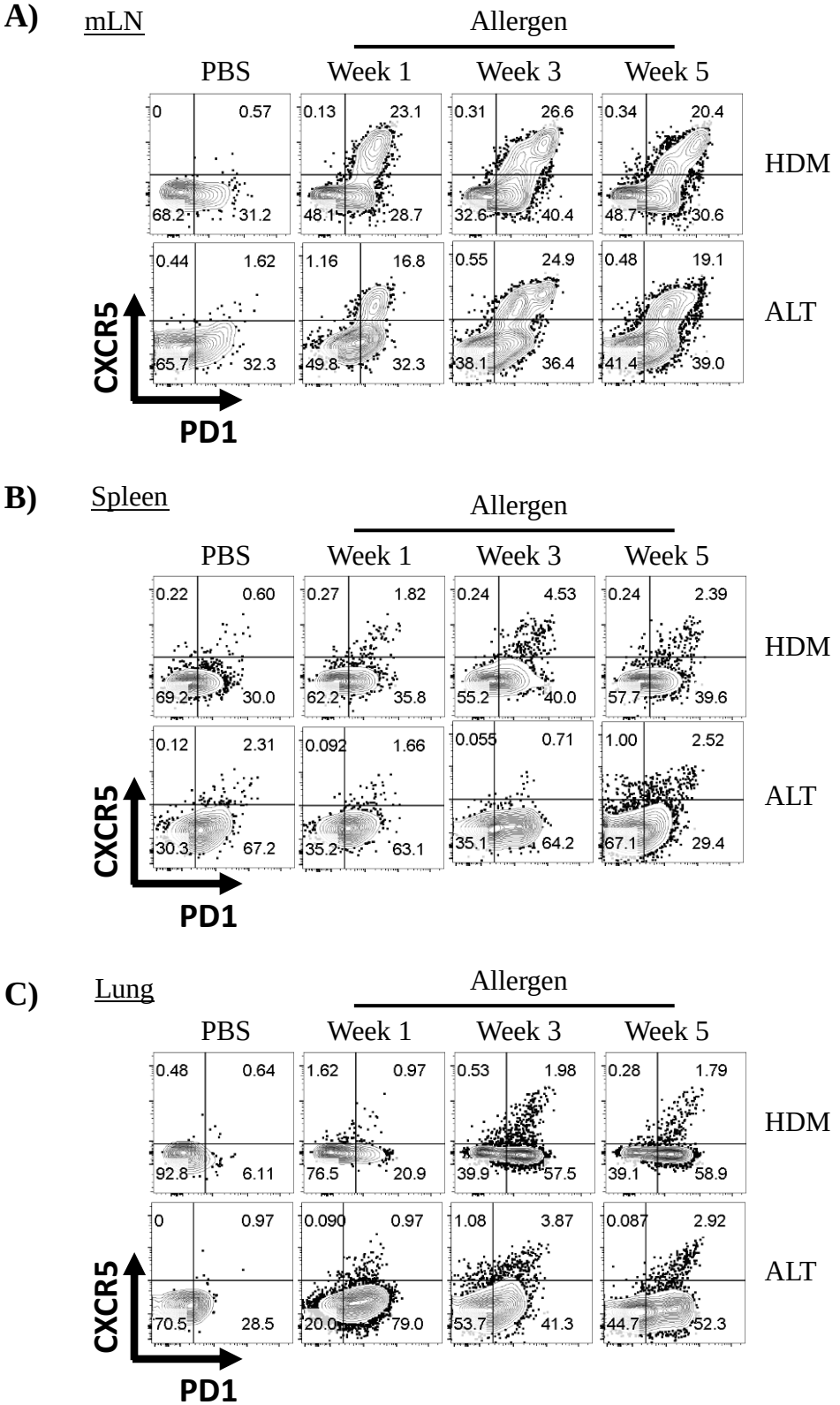

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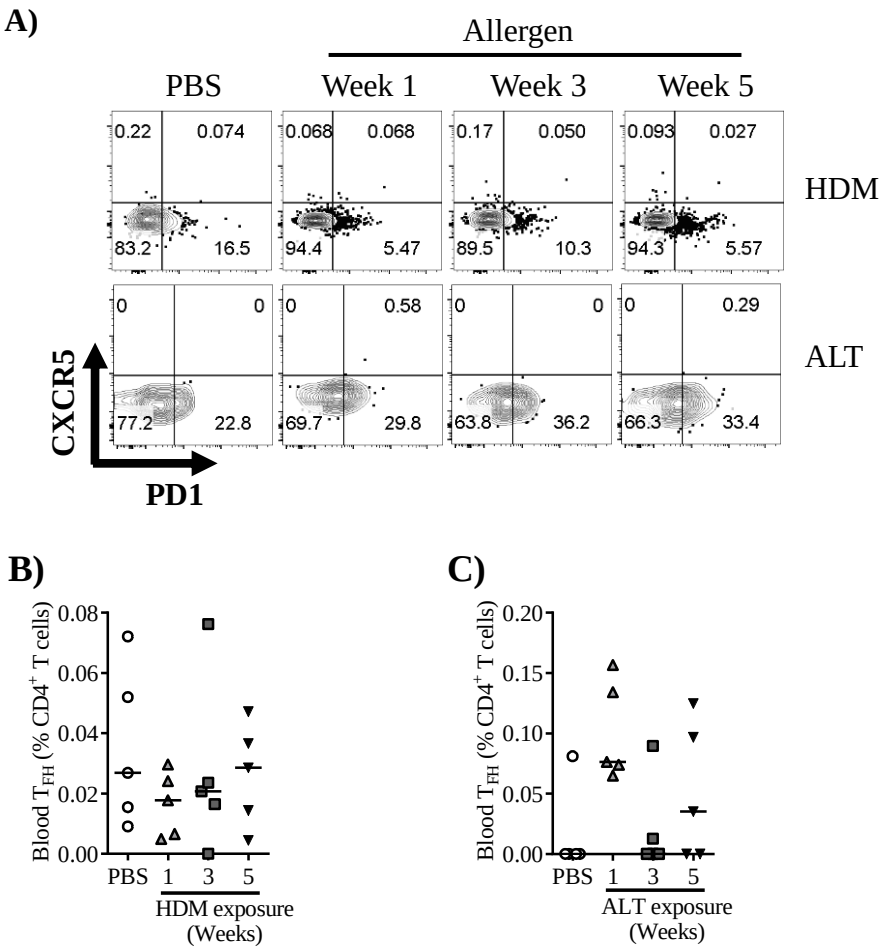

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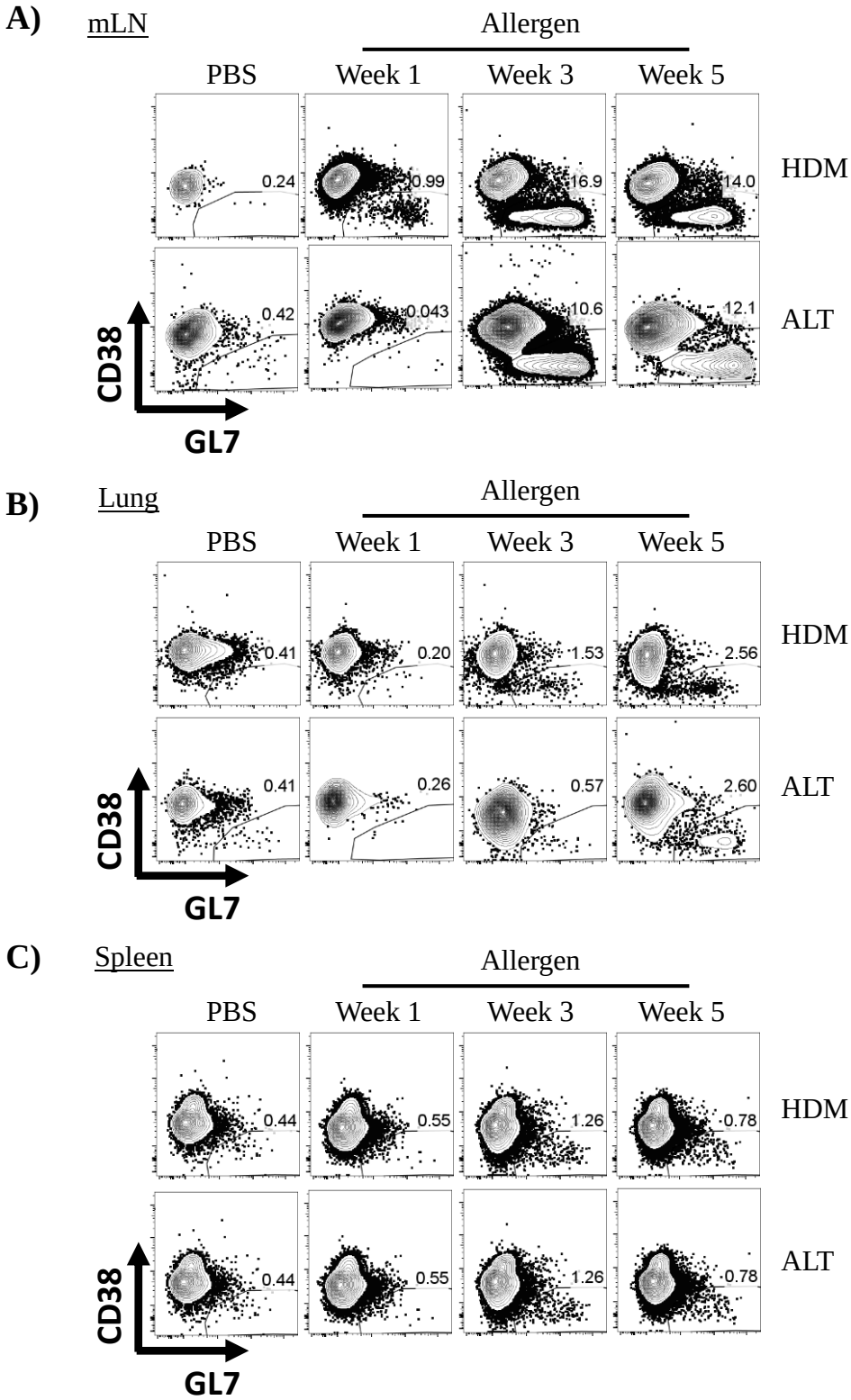

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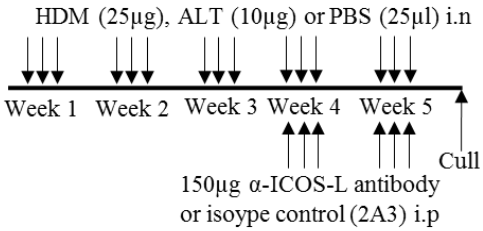

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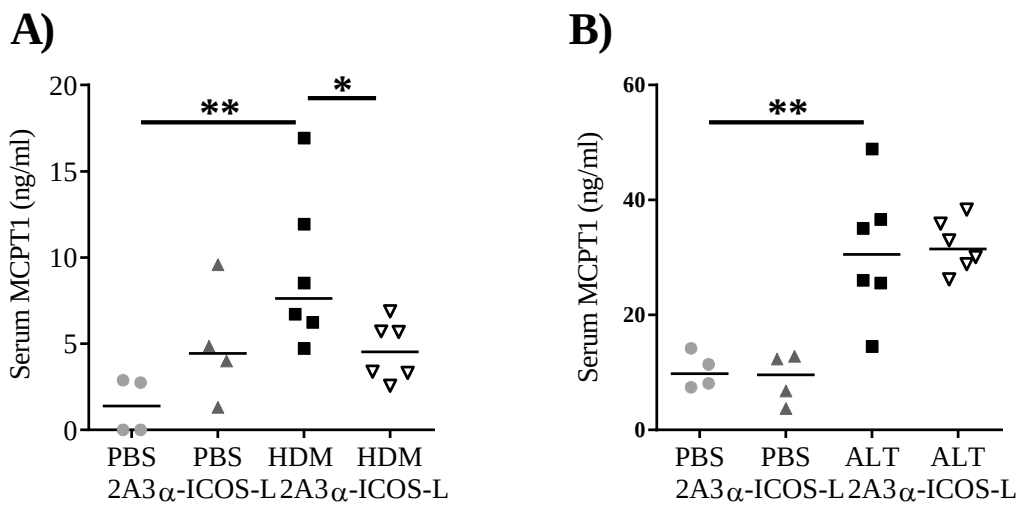

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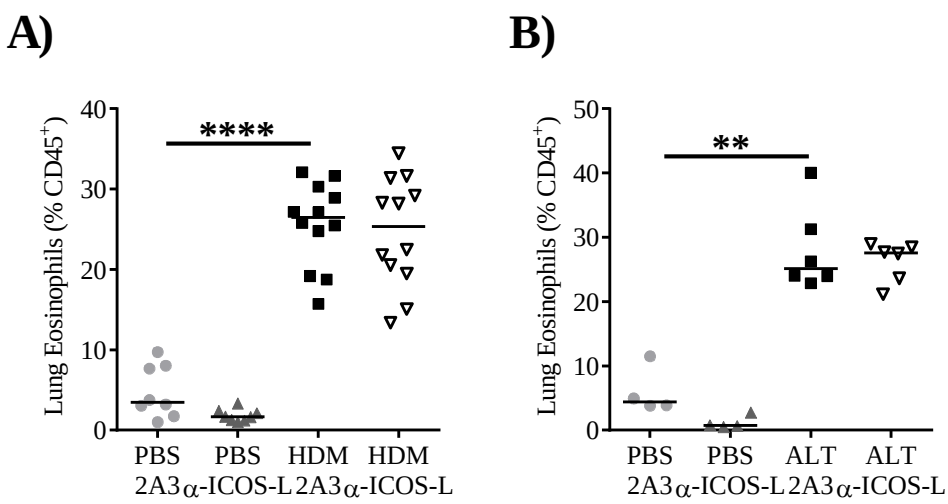

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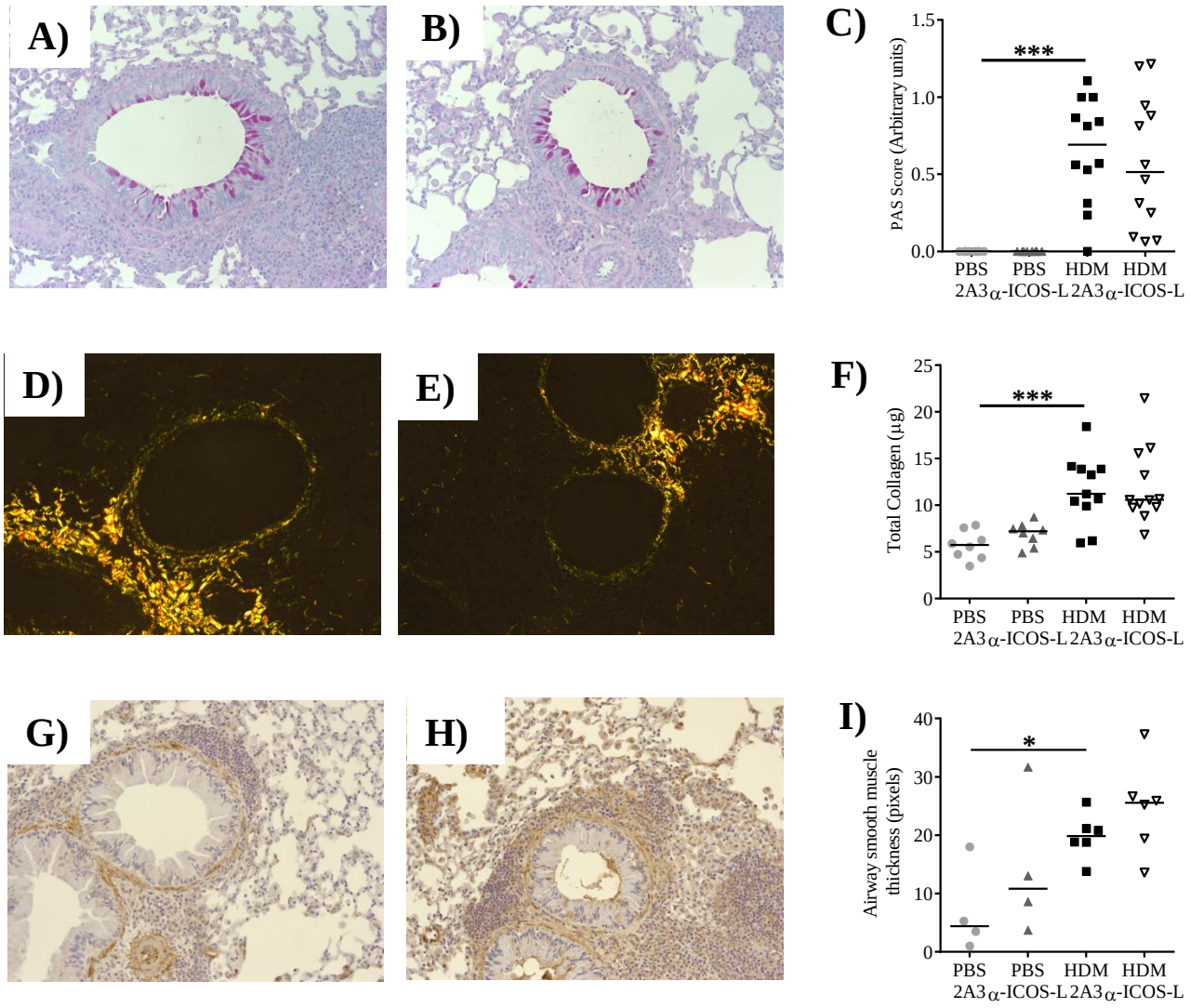

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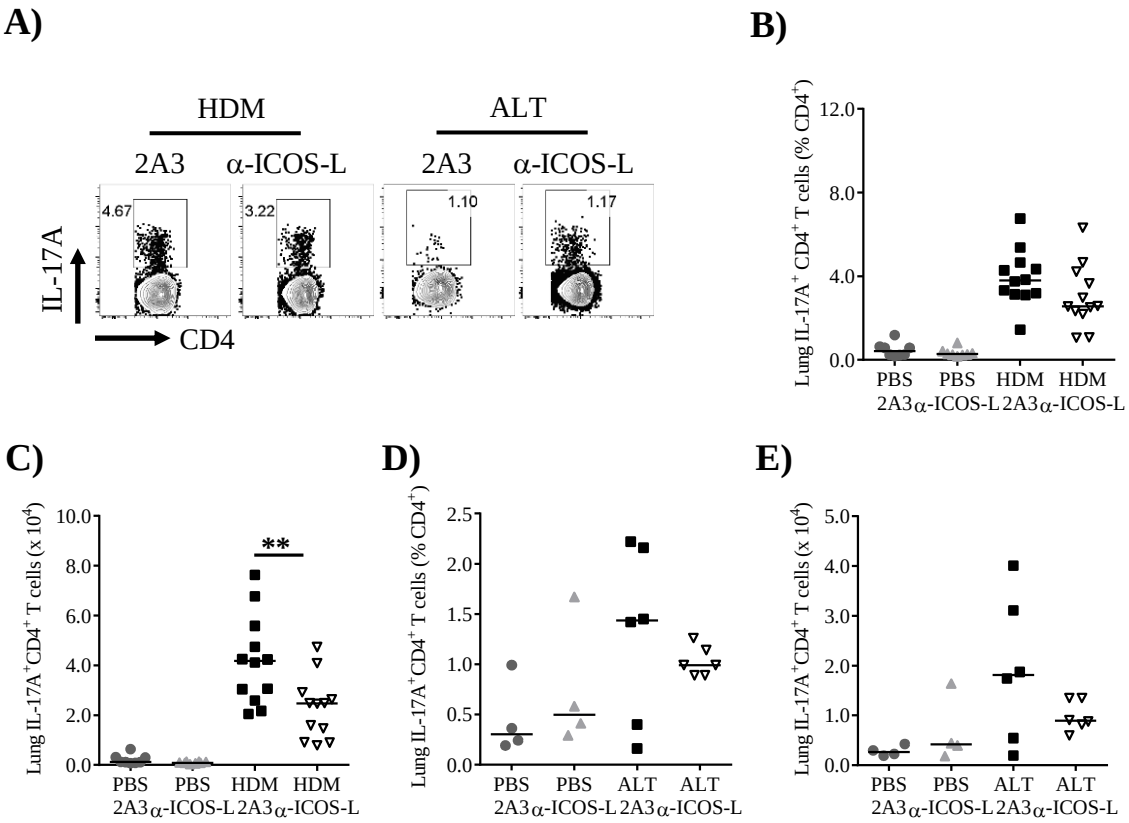

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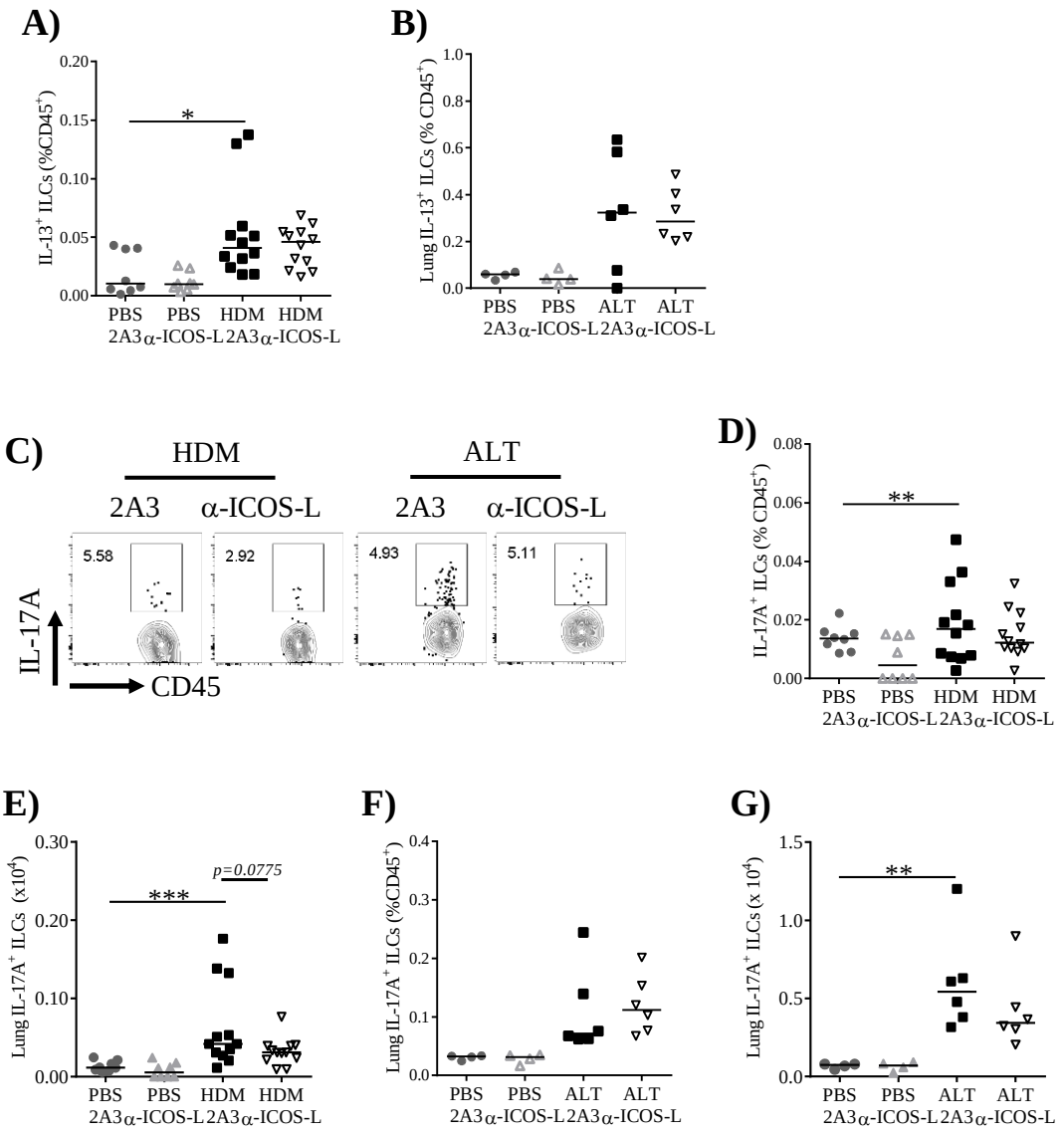

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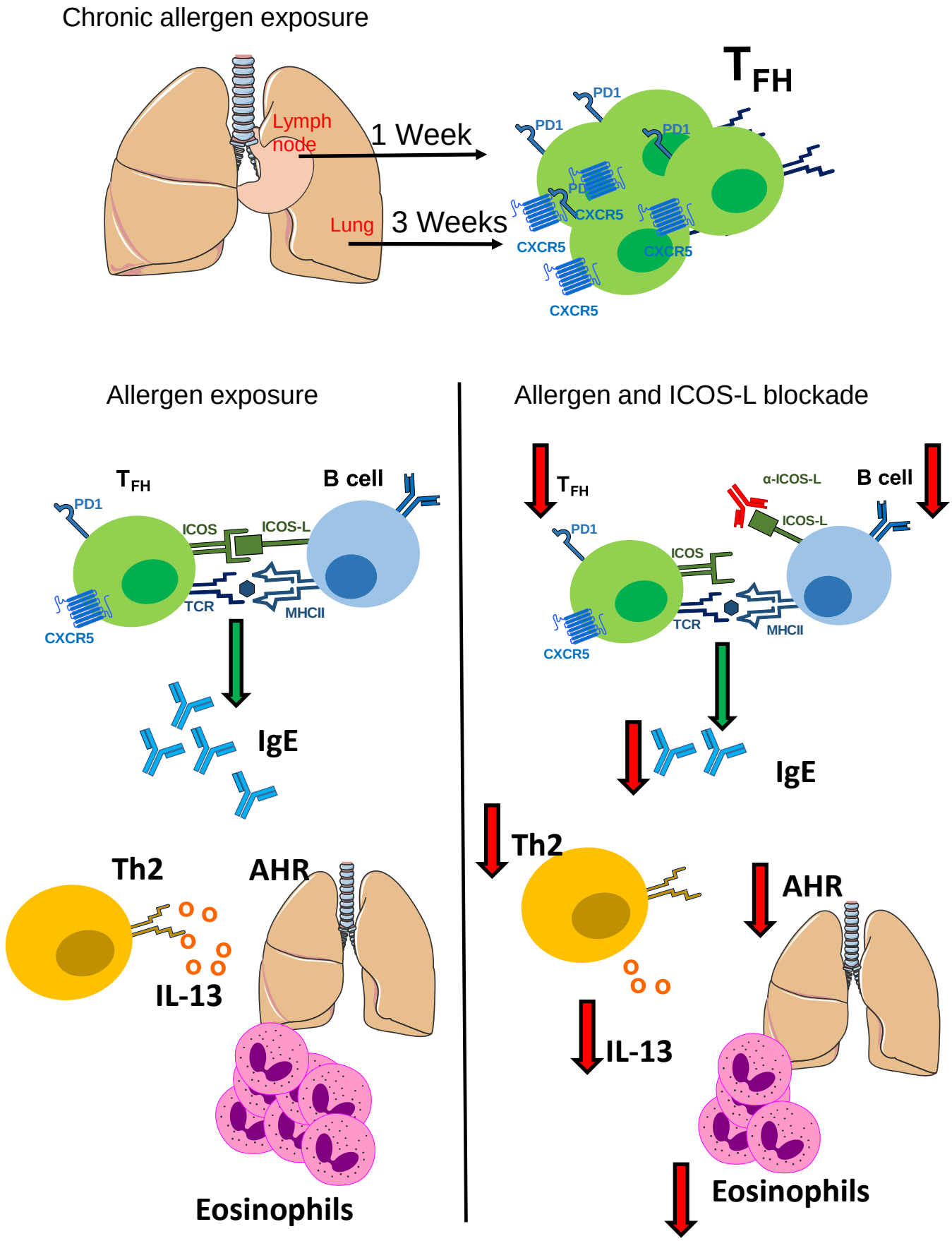

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