

Supplementary Materials

Unveiling Interleukin-40: A Novel Regulator of Macrophage and B Cell Function in Allergic Asthma

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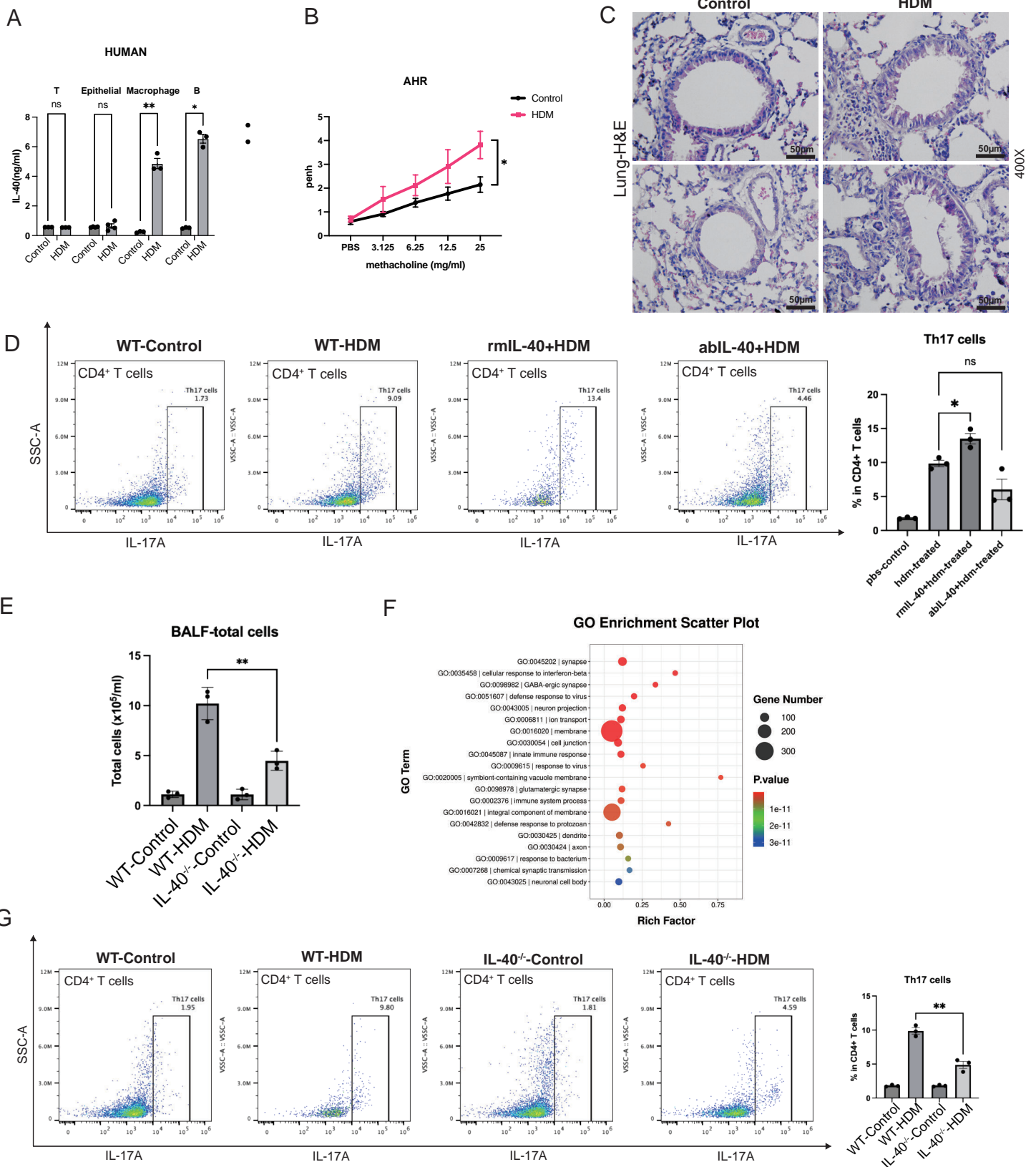
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The Chinese University of Hong Kong

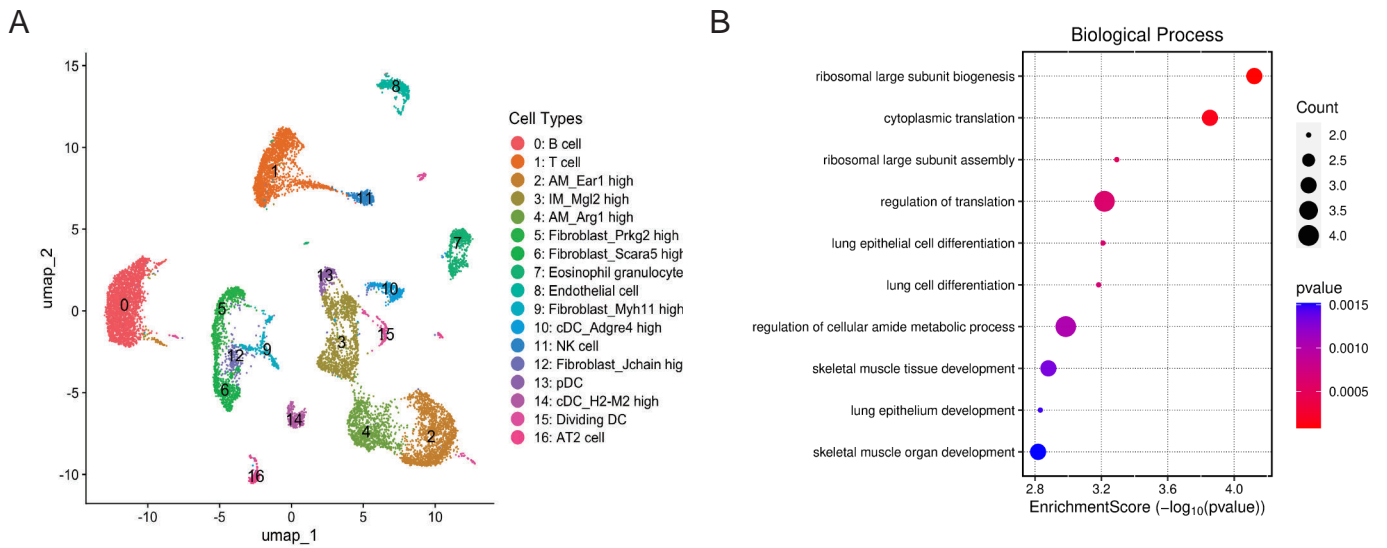
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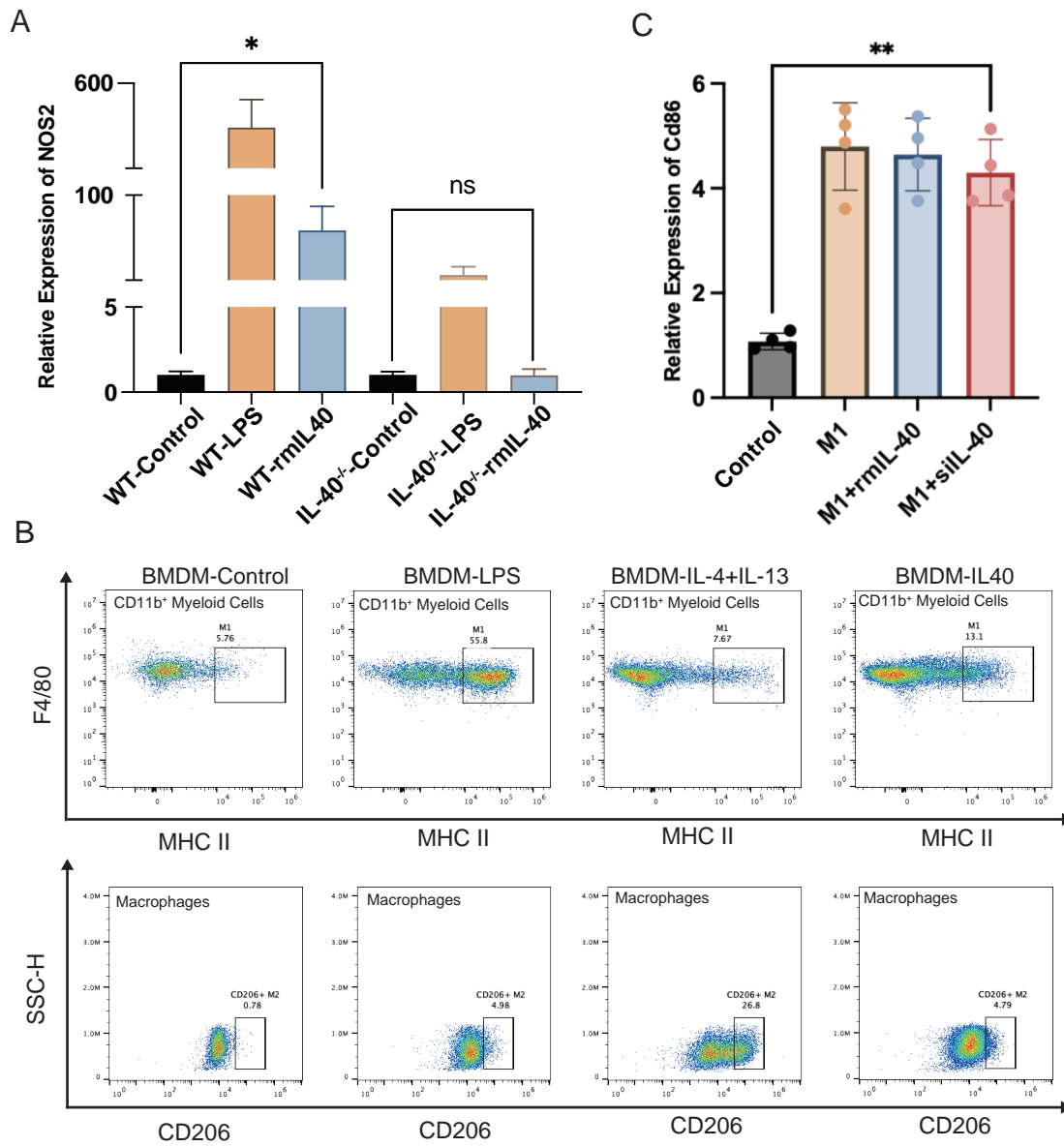
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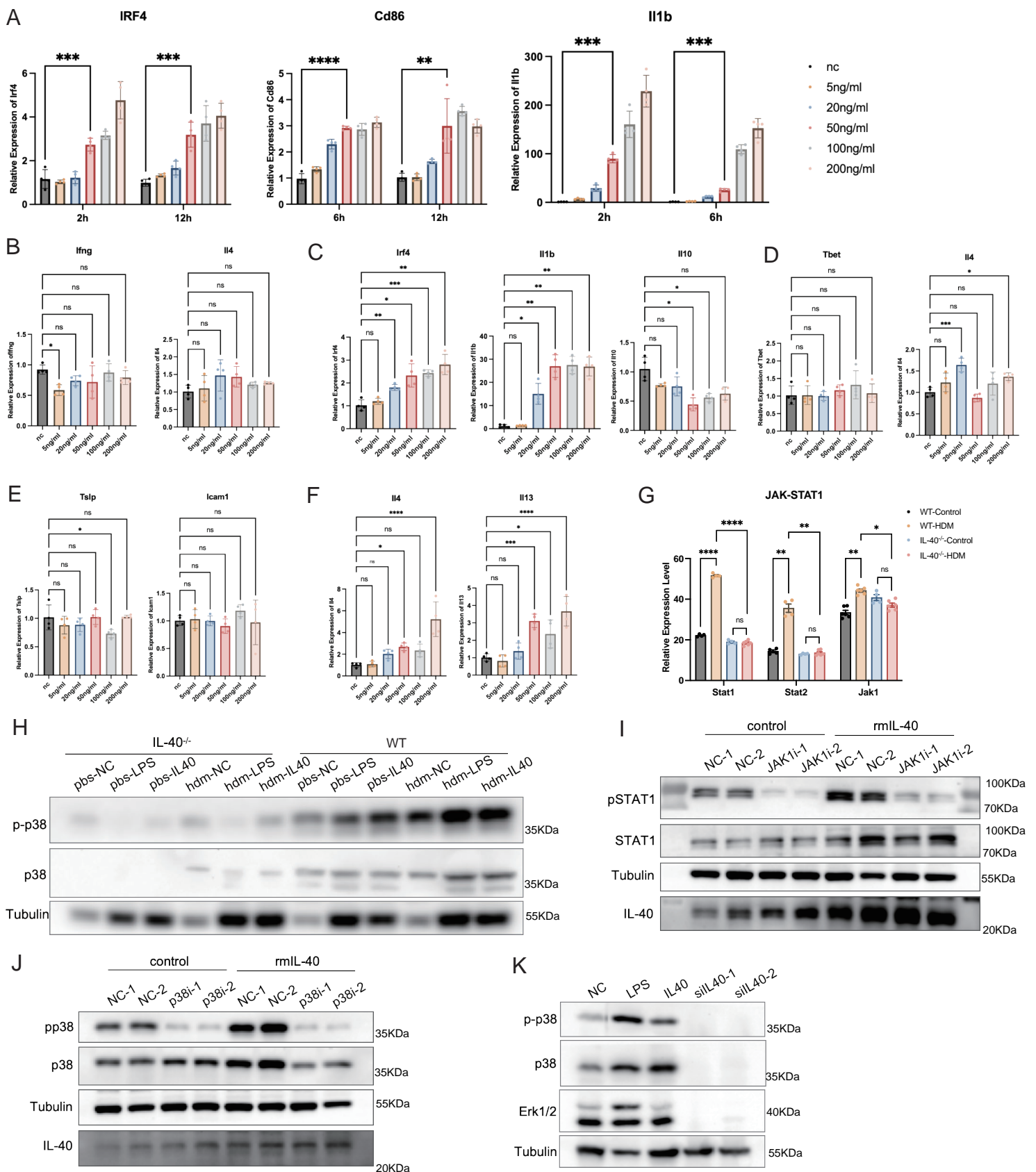
Supplementary Figure 1. IL-40 expression and its effects on airway inflammation and immune cell subsets. **A** Concentrations of IL-40 in sorted human T cells, epithelial cells, macrophages, and B cells measured by ELISA (n=4). **B** AHR to methacholine challenge in control and HDM-treated mice (n=4-5). **C** Representative H&E-stained lung sections from control and HDM-treated groups. **D** Proportion of Th17 cells in lung tissue across groups. **E** Bulk RNA sequencing of lung tissue from WT and IL-40^{-/-} mice, followed by Gene Ontology (GO) analysis of differentially expressed genes (DEGs). **F** Proportion of Th17 cells in lung tissue from WT and IL-40^{-/-} mice. **G** Total cell number in BALF across different groups of mice. Data are representative of at least three independent experiments. Values are expressed as the mean ± SEM. *P < 0.05, **P < 0.01, and ns for not significant by Mann-Whitney test.



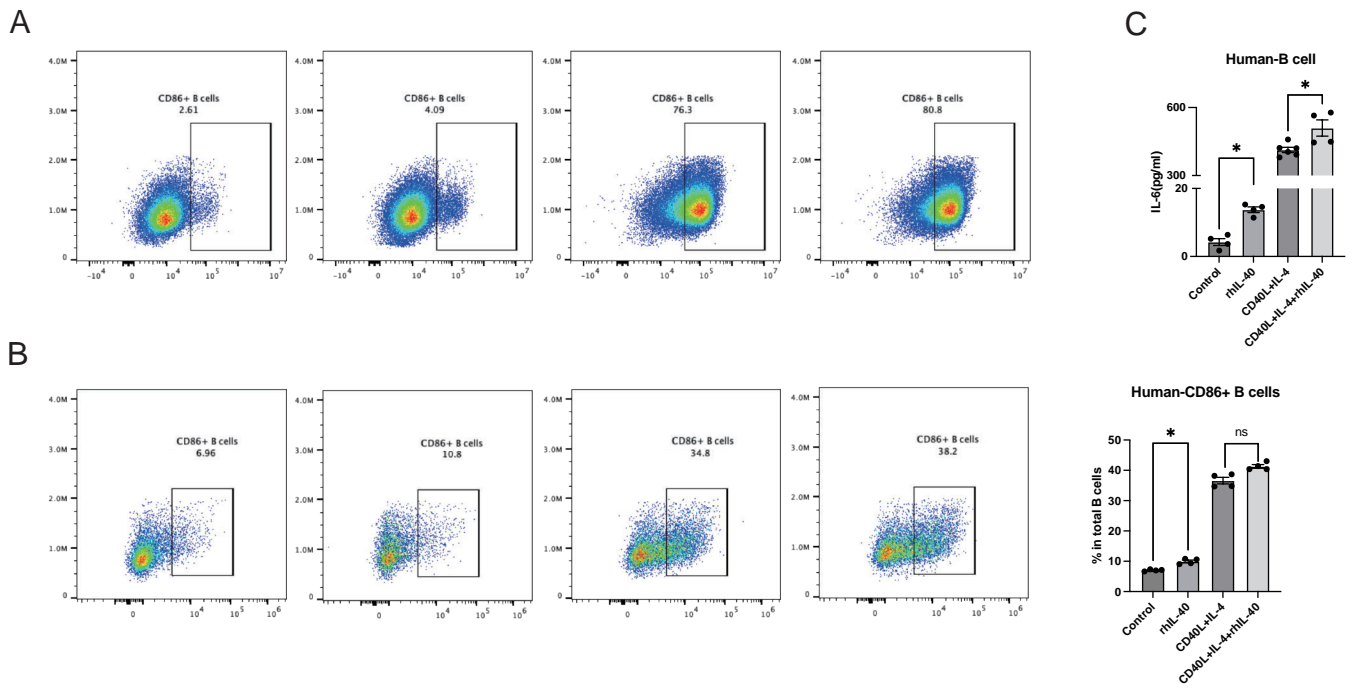
Supplementary Figure 2. IL-40 regulates lung cell clusters and B cell subsets at single-cell resolution. **A** UMAP visualization of annotated pulmonary cell clusters from single-cell RNA sequencing of lung tissue in all mouse groups. **B** Gene ontology (GO) analysis of downregulated differentially expressed genes (DEGs) in cluster 0 (B cell) from IL-40^{-/-} HDM-stimulated mice.



Supplementary Figure 3. IL-40 regulates macrophage polarization. **A, C** Relative expression levels of *NOS2* and *Cd86* in treated BMDMs measured by qRT-PCR (n=4). **B** Flow cytometry analysis of macrophage subtypes following respective stimulations. Values are expressed as mean ± SEM. *P < 0.05; **P < 0.01; ns, not significant by Mann-Whitney test.

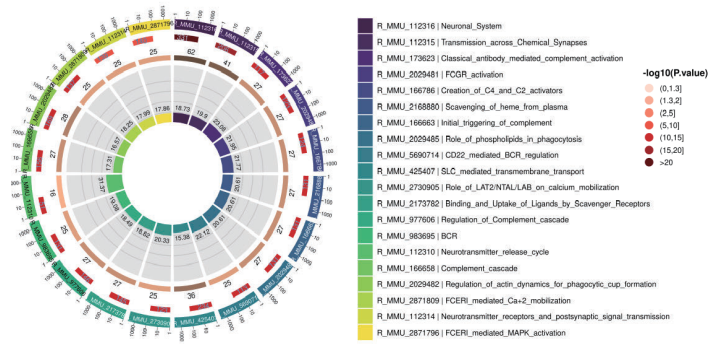


Supplementary Figure 4. IL-40 broadly regulates immune gene and protein expression in multiple cell types. **A** Relative expression levels of *Irf4*, *Cd86* and *Il1b* in treated mouse BMDMs ($n=3-4$) measured by qRT-PCR. **B** Relative expression levels of *Irfng* and *Il4* in 12h-treated primary T cells isolated from mice thymus ($n=3-4$) measured by qRT-PCR. **C** Relative expression levels of *Irf4*, *Il1b* and *Il10* in 12h-treated human PBMC derived macrophages ($n=3-4$) measured by qRT-PCR. **D** Relative expression levels of *Tbet* and *Il4* in 12h-treated human T cells isolated from PBMC ($n=3-4$) measured by qRT-PCR. **E** Relative expression levels of *Tslp* and *Icam1* in 12h-treated HBepiC ($n=4$) measured by qRT-PCR. **F** Relative expression levels of *Il4* and *Il13* in 12h-treated mouse T cells from BMDM & T cell coculture sample. **G** Gene expression analysis by bulk RNA-seq in lung tissue from asthmatic and control mice. **H**, **I**, **J** Western blot analysis of protein expression in treated mouse BMDMs. **K** Western blot analysis in RAW264.7 cells following indicated treatments. Data are representative of at least three independent experiments. Values are expressed as the mean \pm SEM. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ and ns: not significant by the Mann-Whitney test.

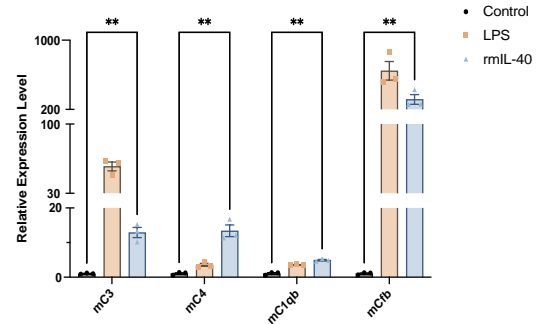


Supplementary Figure 5. IL-40 regulated B cell activation. **A** CD86+ B cells were gated within the CD19⁺B220⁺ population in B cells isolated from mouse spleen and **B** human PBMC, representative dot plots and quantification of CD86+ B cells as a percentage of total B cells are shown (n=4-6). **C** Concentrations of IL-6 in cell lysates from human B cells measured by ELISA (n=3-4). Data are representative of at least three independent experiments. Values are expressed as the mean \pm SEM. *P < 0.05 and ns: not significant by the Mann-Whitney test.

A



B



Supplementary Figure 6. IL-40 regulates complement pathway gene expression in macrophages. **A** Circular visualization of Reactome pathway enrichment analysis for complement-related genes. **B** Relative expression levels of complement pathway genes in macrophages measured by qRT-PCR (n=3-5). Data are representative of at least three independent experiments. Values are expressed as the mean \pm SEM. **P < 0.01 by the Mann-Whitney test.

Supplementary Table 1. Patient Demographics

Sample ID	Gender	Age	Asthma	AR	Eczema
BC 0065	M	18.0	1	1	0
BC 0532	M	10.5	1	1	0
BC 0076	M	15.1	1	1	0
BC 0630	M	12.1	1	1	0
BC 0481	F	5.2	1	1	0
BC 0051	M	11.4	1	1	0
BC 0069	F	17.1	1	1	0
BC 0446	F	11.8	1	1	0
BC 0085	F	11.8	1	1	0
BC 0048	M	9.2	1	1	0
BC 0056	M	6.8	1	1	0
BC 0426	M	13.3	1	1	0
BC 0500	F	13.7	1	1	0
BC 0474	M	9.8	1	1	0
BC 0853	F	15.3	1	1	0
BC 2282	F	20.1	1	1	0
A 542	M	12.2	1	1	0
A 543	M	6.7	1	1	0
A 545	M	6.4	1	1	0
A 548	M	16.3	1	1	0
A 551	F	7.1	1	1	0
A 555	M	10.9	1	1	0
A 556	F	16.6	1	1	0
A 558	M	9.3	1	1	0
A 562	F	9.4	1	0	0
A 564	F	8.3	1	1	0
A 565	M	8.0	1	1	0
A 567	M	7.7	1	0	0
A 569	M	8.8	1	0	0
A 572	M	12.2	1	1	0
A 575	F	9.1	1	1	0
A 576	F	14.7	1	1	0
A 577	M	10.3	1	1	0
A 580	M	15.3	1	0	0
A 583	F	16.9	1	1	0

A 585	F	8.5	1	1	0
A 586	M	17.4	1	1	0
A 587	M	6.5	1	1	0
A 590	M	8.1	1	1	0
A 597	M	11.5	1	1	0
A 600	M	7.1	1	1	0
A 608	F	12.7	1	1	0
A 609	F	17.3	1	1	0
A 611	M	7.4	1	1	0
A 614	F	7.2	1	0	0
A 615	M	9.7	1	1	0
A 617	F	11.5	1	1	0
A 618	M	16.8	1	1	0
A 620	M	11.0	1	0	0
A 623	M	8.6	1	0	0
A 624	F	7.5	1	0	0
A 632	M	17.9	1	1	0
A 634	M	10.6	1	1	0
A 638	M	13.8	1	1	0
A 641	M	13.5	1	0	0
A 642	M	11.2	1	1	0
A 643	M	10.4	1	1	0
A 645	F	6.2	1	1	0
A 648	M	10.0	1	1	0
A 650	M	11.6	1	1	0
A 656	M	9.9	1	1	0
A 671	F	21.9	1	1	0
A 673	F	9.1	1	1	0
A 678	F	11.2	1	1	0
A 684	M	16.4	1	1	0
A 685	M	10.0	1	1	0
A 687	F	13.4	1	1	0
A 750	F	11.3	1	1	0
A 753	F	9.9	1	1	0
A 758	F	10.1	1	1	0
A 761	M	16.1	1	1	0
A 765	M	9.9	1	0	0
A 770	M	10.0	1	1	0

BC 1585	F	11.4	1	0	0
BC 2760	M	17.5	1	1	0
BC 2798	M	16.0	1	1	0

Supplementary Table 2. Primer sequences for qRT-PCR.

Genes	Primer sequences (5'-3') (Forward)	Primer sequences (5'-3') (Reverse)
Mice Gapdh	TGGAGATGGTGAAAGAGG TG	GTCCGTGGAAATGGTGGC
Mice C3	AAGCATCAACACACCCAAC A	CTTGAGCTCCATTCGTGA
Mice C4	AGCGTGTTTCCAGCTCAAA G	GTCCTAAGGCCTCACACC TG
Mice C4b	ACCCCCAGTACTTGCTGGA C	ACCCTGTAGAGCAGAGCC TCTAA
Mice C1qb	TCTGGGAATCCACTGCTGT C	AGACCTCACCCCCTGTG TC
Mice Cfb	CAAGCAGCACAAGGAACA GT	CCTTGGGCCTTTGTAGCAT C
Mice Arg1	CATTGGCTTGCGAGACGTA GAC	GCTGAAGGTCTCTTCCATC ACC
Mice Cd86	ACGTATTGGAAGGAGATTA CAGCT	TCTGTCAGCGTTACTATCC CGC
Mice Nos2	ACTACTGCTGGTGGTGACA A	GAAGGTGTGGTTGAGTTC TCTAAG
Mice Cd206	CTCTGTTCAGCTATTGGAC GC	CGGAATTTCTGGGATTCA GCTTC
Mice 6030468B1 9Rik-1	CATTGCTGACAGGATGCAG AAGG	TGCTGGAAGGTGGACAGT GAGG
Mice 6030468B1 9Rik-2	GCCTGCAGCTGAGACACTG	GCACCTATGGACCCAGCA
Mice 6030468B1 9Rik-3	GAATGGTGGGCGTGTTCTT G	GCTCGGGCCTCTGCTC
Human Gapdh	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATC C
Human C17orf99-1	CAAGGCACGGGAGGAAGA AA	ACAGCAGGTTATGAGCAC CC
Human C17orf99-1	CCTGGGCTGTTCTGCTTGG	CGGCCTTTGGGGAAAAC TC