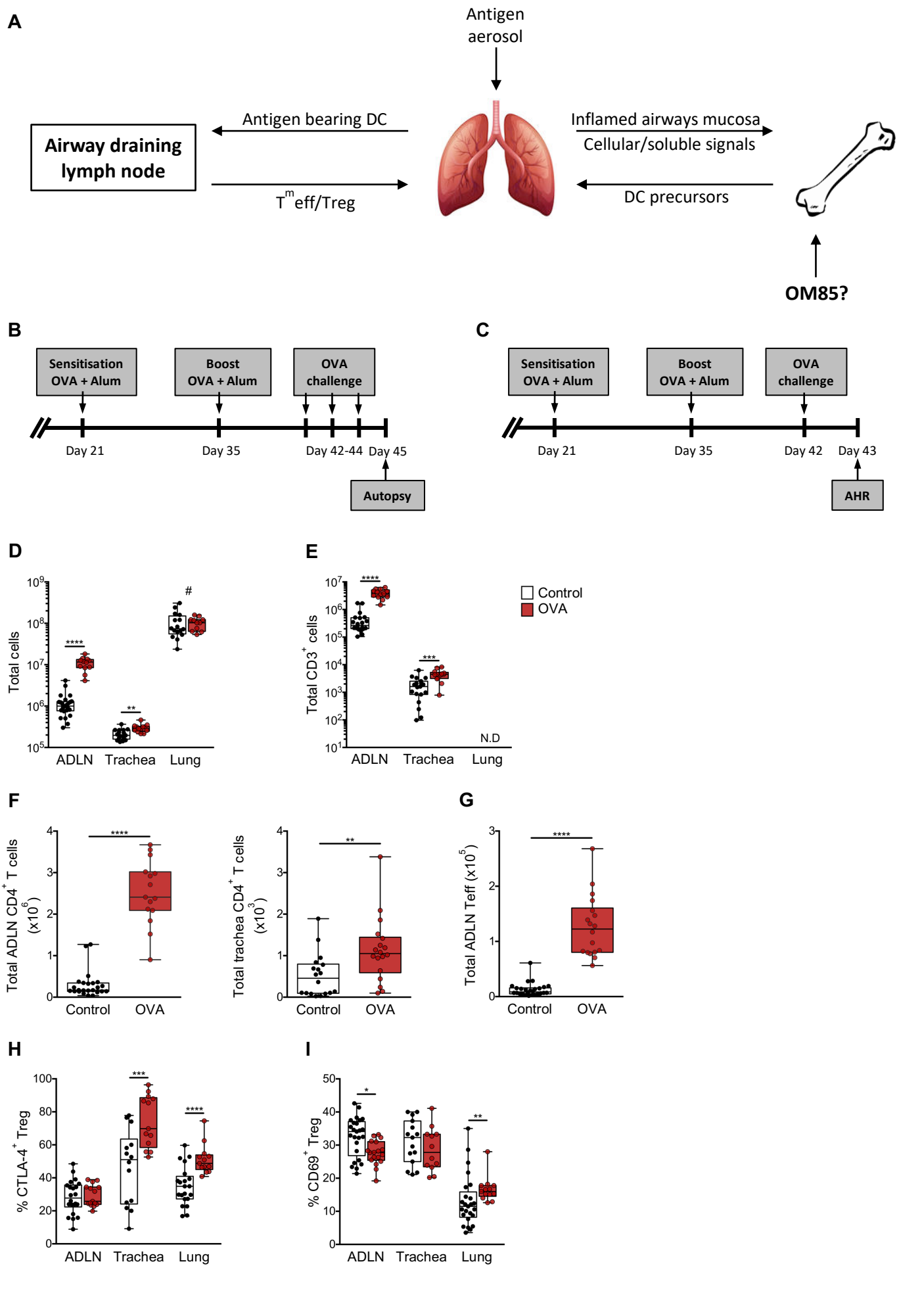
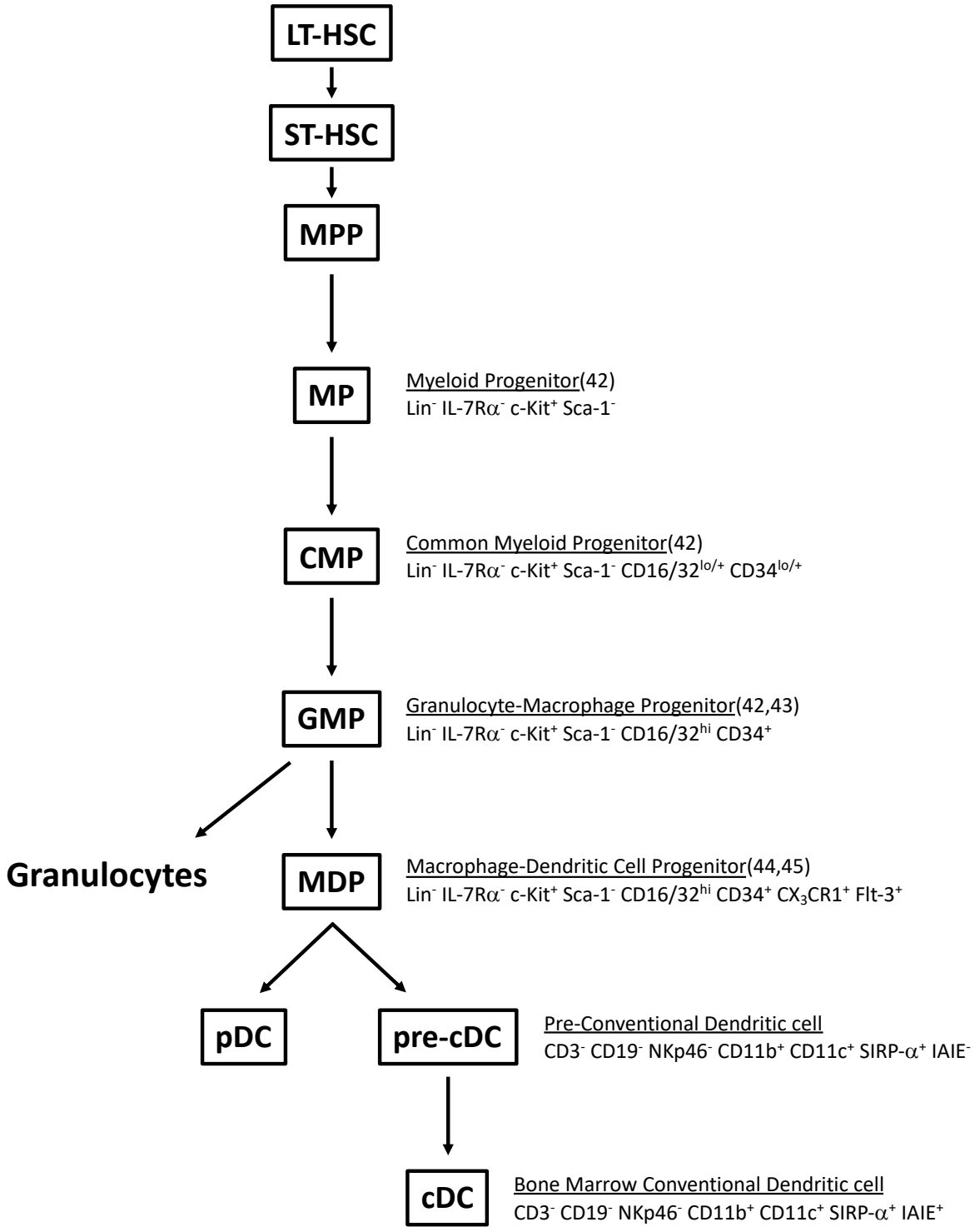
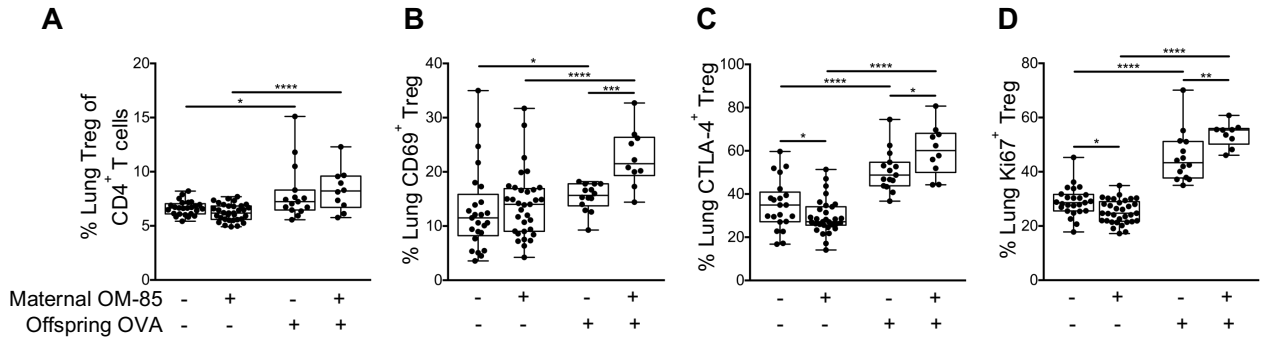

A
Antigen aerosol
Airway draining lymph node
Antigen bearing DC
T^m eff/Treg
Inflamed airways mucosa
Cellular/soluble signals
DC precursors
OM85?
B
Sensitisation OVA + Alum
Boost OVA + Alum
OVA challenge
Day 21
Day 35
Day 42-44
Day 45
Autopsy
C
Sensitisation OVA + Alum
Boost OVA + Alum
OVA challenge
Day 21
Day 35
Day 42
Day 43
AHR
D
Total cells
ADLN
Trachea
Lung
E
Total CD3+ cells
N.D
Control
OVA
F
Total ADLN CD4+ T cells (x10^6)
Total trachea CD4+ T cells (x10^3)
Control
OVA
G
Total ADLN Teff (x10^5)
H
% CTLA-4+ Treg
I
% CD69+ Treg

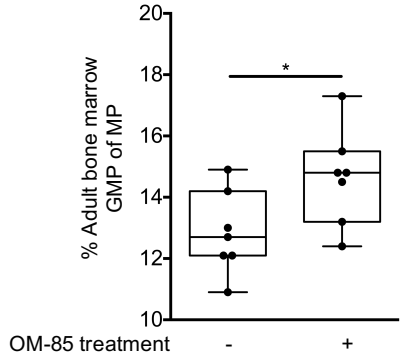
Supplementary Figure 1. Offspring early life sensitisation and aeroallergen challenge T-cell response. (**A**) Sequence of systemic cellular and signalling events occurring as a direct result of aeroallergen exposure. (**B-C**) Kinetics of early life sensitisation disease model employing a prime-boost OVA-Alum regime followed by (**B**) 3 consecutive or (**C**) a single 1% OVA aerosol challenge. (**D**) Total cellularity of ADLN, trachea and peripheral lung samples. (**E**) Absolute numbers of $CD3^+$ cells in ADLN and trachea samples. (**F**) Absolute numbers of $CD3^+CD4^+$ T cells in ADLN and trachea samples. (**G**) Absolute numbers of Teff within ADLN samples. (**H-I**) Analysis of Tregs in ADLN, trachea and peripheral lung showing (**H**) proportion of Treg CTLA-4^+ and (**I**) proportion of Treg $CD69^+$. Data are presented from individual animals comparing naïve controls (white) versus OVA sensitised and aerosol challenged offspring (with sample collection 24 hours post challenge; red) and displayed as box and whisker plot showing median, Q_1 and Q_3 and min to max values of $n \geq 6$ independent experiments. Total peripheral lung cells displayed as cells per milligram of tissue (#; D). Statistical significance was determined using Student's t-test or Mann Whitney test and presented as *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.



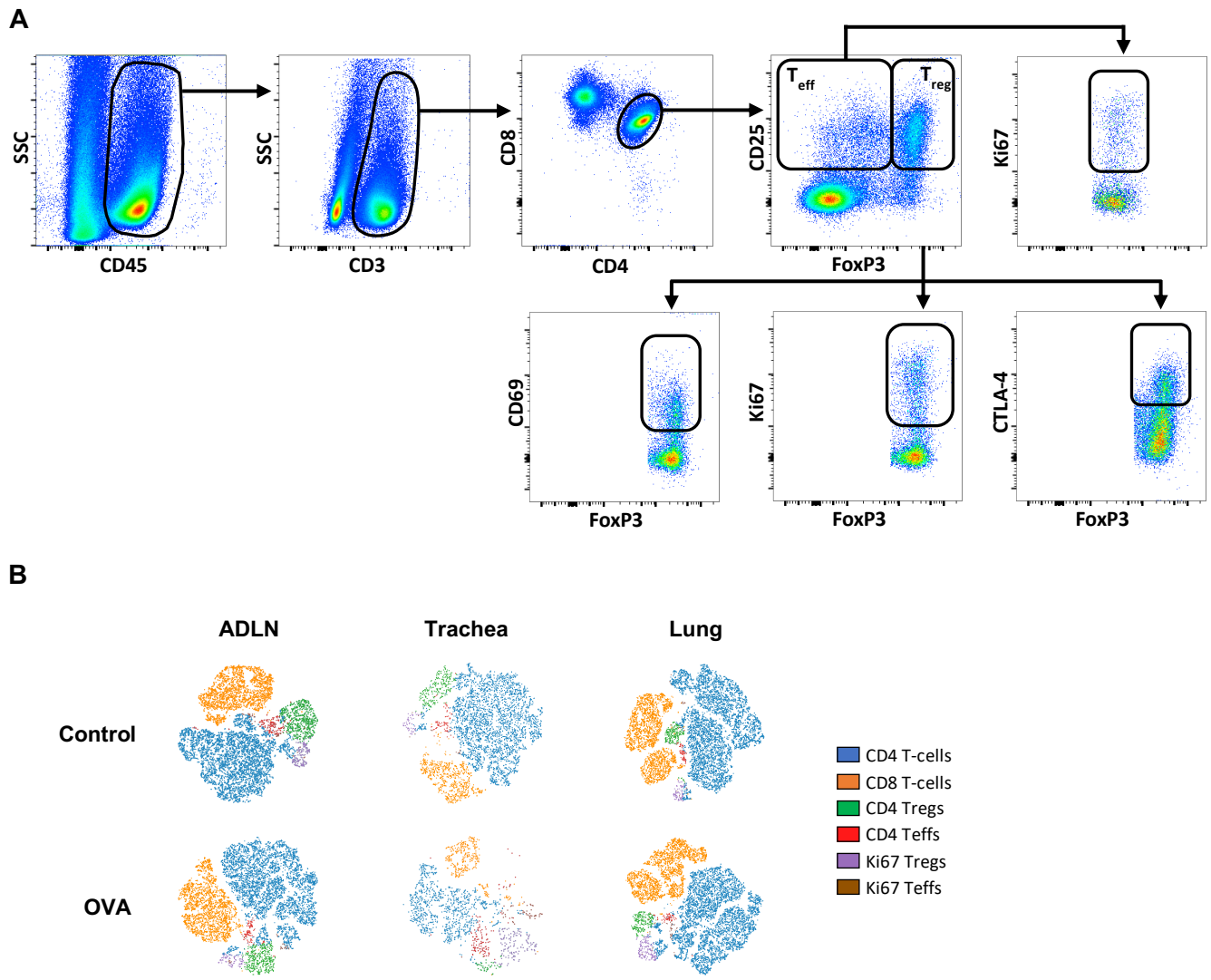
Supplementary Figure 2. Hierarchical commitment of HSPCs to granulocytes, pDCs and cDCs. Developmental pathway within the bone marrow resulting in the generation of terminally committed granulocytes, pDCs and cDCs and the associated cellular markers used to identify individual cell types. LT-HSC = long-term hematopoietic stem cell, ST-HSC = short-term hematopoietic stem cell, MPP = multipotent progenitor.



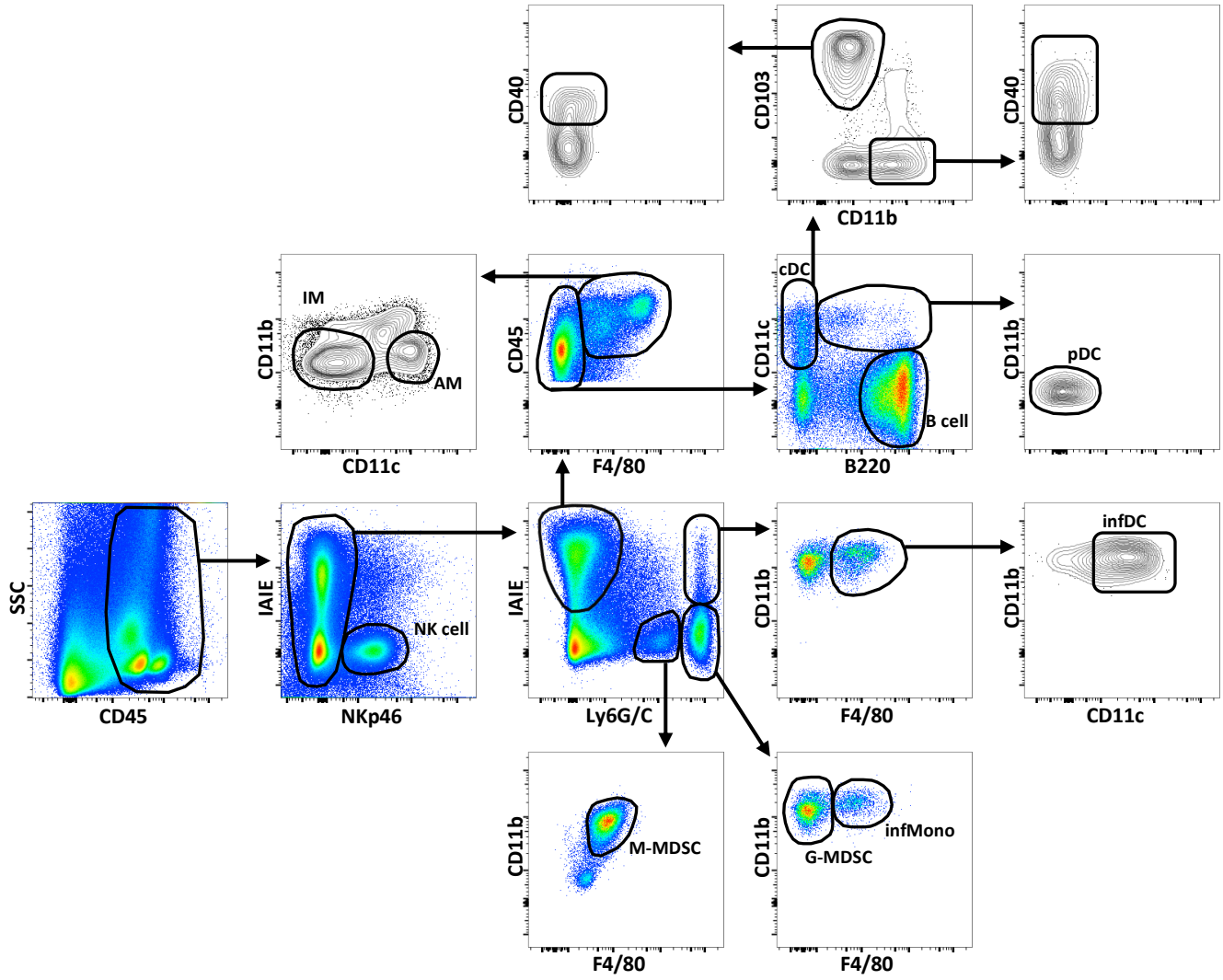
Supplementary Figure 3. Offspring peripheral lung Treg response following maternal OM-85 treatment. (**A-D**) Analysis of Tregs in peripheral lung showing (**A**) Tregs as a proportion of total $CD4^+$ T-cells, (**B**) proportion of Treg $CD69^+$, (**C**) proportion of Treg CTLA-4^+ and (**D**) proportion of Treg $Ki67^+$ in naïve controls versus OVA sensitised and aerosol challenged offspring from OM-85 treated/untreated mothers. Data displayed as box and whisker plot showing median, Q_1 and Q_3 and min to max values of $n \geq 9$ independent experiments. Statistical significance was determined using Student's t-test or Mann-Whitney test and presented as *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.



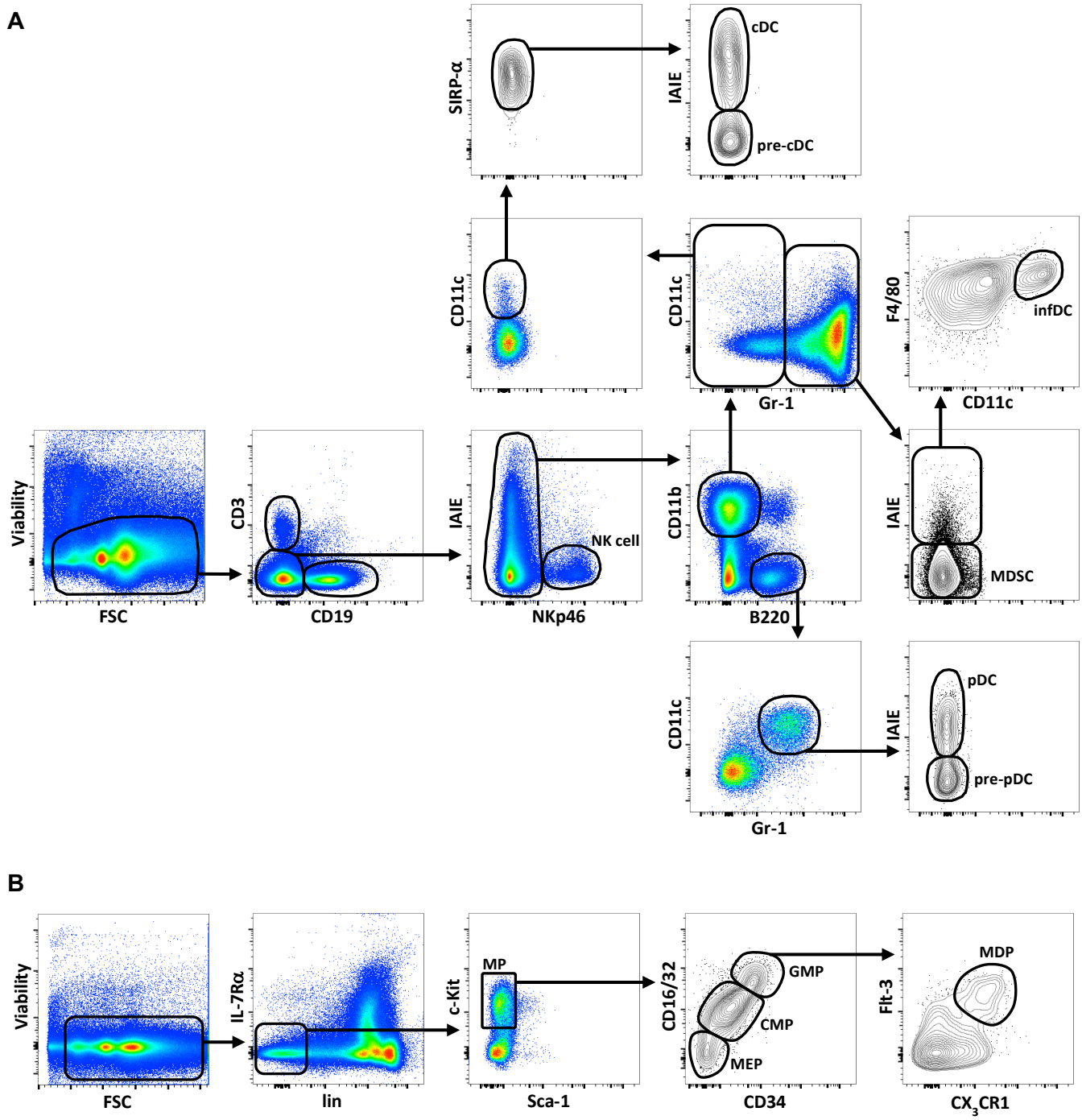
Supplementary Figure 4. Adult bone marrow response to direct OM-85 treatment. Analysis of GMPs in adult bone marrow as a proportion of MPs in naïve control versus OM-85 treated mice. Data displayed as box and whisker plot showing median, Q_1 and Q_3 and min to max values of n=4 independent experiments. Statistical significance was determined using Student's t-test and presented as *$p<0.05$.



Supplementary Figure 5. Immunophenotyping gating strategy to characterize airways T-cell subsets. (**A**) Representative FlowJo® gating strategy used to identify T-cell subsets within ADLN, trachea and peripheral lung digests via multi-colour flow cytometry (**B**) Representative FlowJo® contour plot displaying FoxP3-phycoerythrin fluorescence minus one (FMO) antibody staining control. (**C**) Visual representation (n=2) of viSNE clustering shown for $CD45^{+}CD3^{+}$ cells within ADLN, trachea and peripheral lung single cell suspensions and with analysis of 7 parameters. CD4 T cells ($CD3^{+}CD4^{+}CD8\alpha^{-}$), CD8 T cells ($CD3^{+}CD4^{-}CD8\alpha^{+}$), CD4 Tregs ($CD3^{+}CD4^{+}CD8\alpha^{-}CD25^{+}FoxP3^{+}$), CD4 Teff ($CD3^{+}CD4^{+}CD8\alpha^{-}CD25^{+}FoxP3^{-}$), Ki67 Treg ($CD3^{+}CD4^{+}CD8\alpha^{-}CD25^{+}FoxP3^{+}Ki67^{+}$) and Ki67 Teff ($CD3^{+}CD4^{+}CD8\alpha^{-}CD25^{+}FoxP3^{-}Ki67^{+}$) were delineated based on surface and intracellular marker co-expression.



Supplementary Figure 6. Immunophenotyping gating strategy to characterize myeloid populations. Representative FlowJo® gating strategy used to identify dendritic cell subsets within ADLN, trachea, peripheral lung and bone marrow cultures via multi-colour flow cytometry.



Supplementary Figure 7. Immunophenotyping gating strategy to characterize bone marrow cellular subsets. Representative FlowJo® gating strategies used to identify multiple (**A**) committed myeloid and (**B**) hematopoietic stem and progenitor cell populations within bone marrow samples via multi-colour flow cytometry.

Supplementary Table 1. Airways T cell monoclonal fluorescent-labelled antibody panel

Antibody	Fluorochrome	Clone	Supplier
Rat Anti-Mouse CD3	FITC	17A2	BD Biosciences
Rat Anti-Mouse CD4	V500	RM4-5	BD Biosciences
Rat Anti-Mouse CD8a	BV650	53-6.7	BD Biosciences
Rat Anti-Mouse CD25	APC-Cy7	PC61	BD Biosciences
Rat Anti-Mouse CD45	PerCP	30-F11	BD Biosciences
Hamster Anti-Mouse CD69	PE-Cy7	H1.2F3	BD Biosciences
Hamster Anti-Mouse CTLA-4 (CD152)	BV421	UC10-4B9	BioLegend
Anti-Mouse/Rat FoxP3	PE	FJK-16S	BD Biosciences
Mouse Anti-Ki67	AF700	B56	BD Biosciences

Supplementary Table 2. Myeloid cell monoclonal fluorescent-labelled antibody panel

Antibody	Fluorochrome	Clone	Suppler
Rat Anti-Mouse CD8a	BV650	53-6.7	BD Biosciences
Rat Anti-Mouse CD11b	V500	M1/70	BD Biosciences
Hamster Anti-Mouse CD11c	AF700	HL3	BD Biosciences
Rat Anti-Mouse CD40	BV421	3/32	BD Biosciences
Rat Anti-Mouse CD45	PerCP	30-F11	BD Biosciences
Rat Anti-Mouse CD86	Biotin	PO3	BD Biosciences
Rat Anti-Mouse CD103	PE	M290	BD Biosciences
Rat Anti-Mouse IA-IE	AF647	M5/114.15.2	BD Biosciences
Rat Anti-Mouse B220 (CD45R)	PE-CF594	RA3-6B2	BD Biosciences
Rat Anti-Mouse Ly6G/C	APC-Cy7	RB6-8C5	BD Biosciences
Rat Anti-Mouse NKp46 (CD335)	PE-Cy7	29A1.4	BioLegend
Rat Anti-Mouse F4/80	FITC	BM8	BioLegend
Streptavidin	PE-Cy5	-	BD Biosciences

Supplementary Table 3. Bone marrow hematopoietic stem and progenitor cell monoclonal fluorescent-labelled antibody panel

Antibody	Fluorochrome	Clone	Suppler
Rat Anti-Mouse CD2	Biotin	RM2-5	BD Biosciences
Hamster Anti-Mouse CD3	Biotin	145-2C11	BD Biosciences
Rat Anti-Mouse CD4	Biotin	GKl.5	BD Biosciences
Rat Anti-Mouse CD5	Biotin	53-7.3	BD Biosciences
Rat Anti-Mouse CD8a	Biotin	53-6.7	BD Biosciences
Rat Anti-Mouse CD19	Biotin	1D3	BD Biosciences
Rat Anti-Mouse B220 (CD45R)	Biotin	RA3-6B2	BD Biosciences
Rat Anti-Mouse Gr-1	Biotin	RB6-8CS	BD Biosciences
Rat Anti-MouseTer119	Biotin	TER-119	BD Biosciences
Rat Anti-Mouse CD16/32	PerCP-Cy5.5	2.4G2	BD Biosciences
Rat Anti-Mouse CD34	FITC	RAM34	BD Biosciences
Rat Anti-Mouse IL-7Ra (CD135)	PE-Cy7	SB/199	BD Biosciences
Rat Anti-Mouse Flt-3 (CD135)	PE	A2F10.1	BD Biosciences
Rat Anti-Mouse c-Kit (CD117)	APC-Cy7	2B8	BD Biosciences
Rat Anti-Mouse Sca-1	BV510	D7	BD Biosciences
Rat Anti-Mouse CX3CR1	APC	SA011F11	BioLegend
Rat Anti-Mouse NKG28	BV711	CX5	BD Biosciences
Viability	AF700	-	BD Biosciences
Streptavidin	BV605	-	BD Biosciences

Supplementary Table 4. Bone marrow committed myeloid cell monoclonal fluorescent-labelled antibody panel

Antibody	Fluorochrome	Clone	Suppler
Rat Anti-Mouse CD3	FITC	17A2	BD Biosciences
Rat Anti-Mouse CD11b	BV510	M1/70	BD Biosciences
Hamster Anti-Mouse CD11c	BV711	HL3	BD Biosciences
Rat Anti-Mouse CD19	APC-H7	1D3	BD Biosciences
Rat Anti-Mouse CD24	PE	M1/69	BD Biosciences
Rat Anti-Mouse Gr-1	Biotin	RB6-6B2	BD Biosciences
Rat Anti-Mouse B220 (CD45R)	PerCP-Cy5.5	RA3-6B2	BD Biosciences
Rat Anti-Mouse NKp46 (CD335)	PE-Cy7	29A1.4	BioLegend
Rat Anti-Mouse SIRP-a (CD172a)	APC	P84	BioLegend
Rat Anti-Mouse IA-IE	BV421	M5/114.15.2	BioLegend
Rat Anti-Mouse F4/80	BV785	BM8	BioLegend
Viability	AF700	-	BD Biosciences
Streptavidin	BV605	-	BD Biosciences