Supplementary Figures

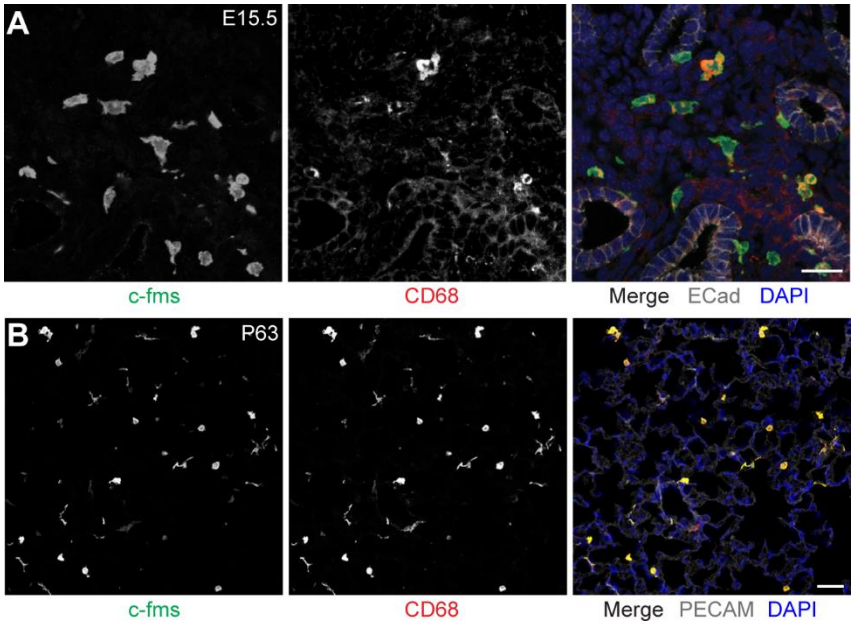

Figure S1. Expression of CD68 in mid-embryonic and adult lung macrophages. **A,B,** Sections of embryonic (E15.5, A) and adult (P63, B) c-fms-EGFP mouse lungs immunostained for GFP (green) to show cytoplasm of c-fms-expressing macrophages, and for macrophage marker microsialin CD68 (red), epithelial marker E-cadherin (grey, **A**) or endothelial marker PECAM (grey, **B**), and counterstained with DAPI (blue, nuclei). CD68 is expressed in all c-fms-EGFP positive macrophages. CD68 expression at E15.5 is speckled and dim (**A**), whereas in the adult it is more prominent (**B**). Bar, 20 um (A), 40 um (B)

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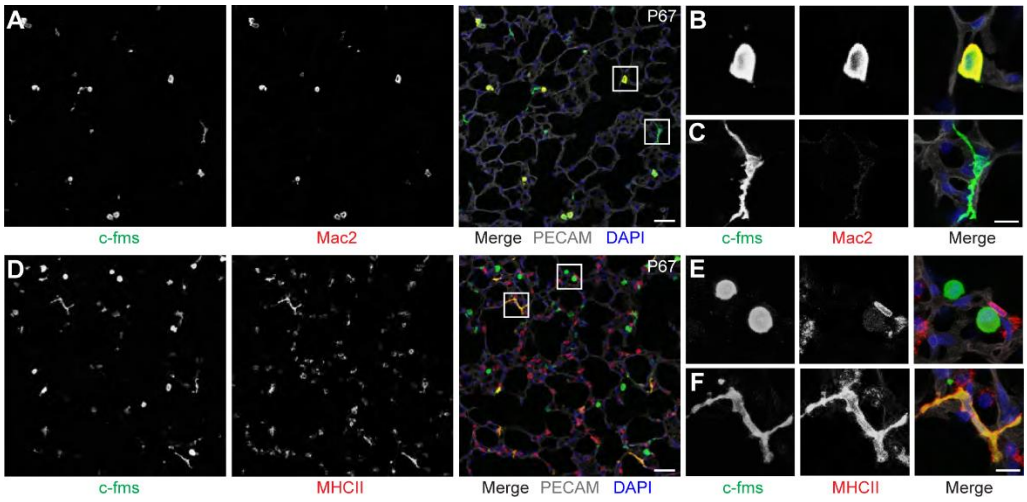

Figure S2. Mac2 and MHCII expression in complementary populations of adult lung macrophages. Sections of adult (P67) c-fms-EGFP mouse lungs immunostained for EGFP (c-fms, green) to show cytoplasm of c-fms-expressing macrophages, and for macrophage markers Mac2 (red, **A-C**) or MHCII (red, **D-F**), endothelial marker PECAM (grey), and counterstained with DAPI (blue). **B** and **C** are close-ups of boxed regions in **A**, and **E** and **F** are close ups of boxed regions in **D**. Note Mac2 is expressed by all of the alveolar macrophages (**A, B**) but none of the interstitial macrophages (**A, C**), and conversely, MHCII is expressed by all of the interstitial macrophages (**D, F**) but none of the alveolar macrophages (**E, F**). Bar, 40 um (A), 10 um (C), 40 um (D), 10 um (F).

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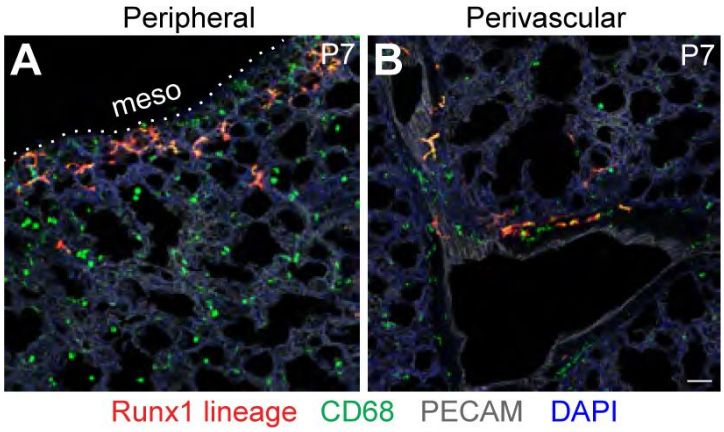

Figure S3. Lineage trace of yolk sac precursors in the juvenile lung. Lineage trace of yolk sac precursors as in Figure 4 using Runx1-CreER>TdTomato (yolk sac) lineage trace (Runx1 lineage, red) induced at E6.75, but lung harvested at juvenile stage (postnatal day P7) and then immunostained for CD68 (green) to visualize all macrophages, for PECAM (blood vessels, grey), and counterstained with DAPI (blue). Peripheral (**A**) and more internal (**B**) lung sections are shown. Note that as in the adult lung (Figure 4), yolk sac derived (Runx1 lineage-labeled) macrophages (yellow) localize to the interstitium peripherally near the mesothelium (meso, **A**) and more internally surrounding a large blood vessel (**B**), indicating that the change from broad interstitial to restricted distribution of yolk sac derived macrophages occurs during the first week of postnatal life. Bar, 40 um

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Table S1. Summary of marker expression in lung macrophages

MΦ marker (synonyms)	Protein/function	Marker expression level[a, b]					
		F4/80 MΦ		Int MΦ	Mac2 MΦ		Alv MΦ
		E13.5	E16.5	Adult	E13.5	E16.5	Adult
csf1r-EGFP (c-fms, MacGreen)	Macrophage colony stimulating factor 1 receptor; tyrosine kinase	+++	+++	+++	+++	+++	+++
Cd68	Microsialin; endosomal/lysosomal membrane protein	+ [c]	++ [c]	+++ [c]	+ [c]	++ [c]	+++ [c]
Lyz2 [d] (Lyz)	Lysozyme C type M; secreted bacteriolytic protein	+	+/++	+/++	+++	+++	+++
Emr1 (Adre1, F4/80)	EGF-like, mucin-like hormone receptor-like 1; cell surface glycoprotein	**+++** [c]	++ [c]	- [c]	(few) + [e]	+/++ [e]	+/++ [e]
H2 [d] (MHCII)	H-2 class II histocompatibility agent	-	-	+++ [c]	-	-	-
Lyve1	Lymphatic vessel endothelial hyaluronic acid; membrane protein	(many) +++ [c,f]	(few) +++ [c,f]	-	-	-	-
Lgals3 (Mac2)	Galactose-specific lectin that binds IgE; acute inflammatory responses	- [c]	- [c]	- [c]	+++ [c]	+++ [c]	+++ [c]
Itgax (CD11c)	Integrin alpha-X, receptor for fibrinogen; cell interactions in inflammatory responses	+	+	+	++	+++	+++
Marco	Macrophage receptor; pattern recognition, bacterial-binding	-	-	-	-	(some) ++	(some) +++
Mrc1 (MMR)	Macrophage mannose receptor; endocytosis of glycoproteins	-	-	-	-	+++	+++

[a] +++, high macrophage (MΦ) marker expression; ++, moderate; +, low; -, none

[b] Fraction of macrophages expressing marker: all, no parenthetical note; (many), >60%; (some), 30-60%; (few), <30%

[c] Based on quantitation of >200 *csf1r-EGFP*+ macrophages

[d] Also expressed in alveolar type II cells

[e] Expression on Mac2+ MΦs variable but increases over time

[f] Transient expression in F4/80 macrophages (peaks at E14.5 and largely absent by E16.5)

Abbreviations: MΦ, macrophage; Int, interstitial; Alv, alveolar

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