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Supplemental Information

IRF4 Transcription Factor-Dependent

CD11b⁺ Dendritic Cells in Human and Mouse

Control Mucosal IL-17 Cytokine Responses

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Supplemental Inventory

1. Supplemental Figures and Tables

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2. Supplemental Experimental Procedures

3. Supplemental References

Supplementary figure 1 Sorting strategy for mouse lung and small intestinal DC



Supplementary figure 1 Sorting strategy for mouse lung and small intestinal DC Sorting strategy of lung CD103⁺ and CD11b⁺ DC as well as CD11b⁺ Macs from steady state WT mice (A). Sorting strategy of small intestinal CD103⁺, CD103⁺CD11b⁺ and CD11b⁺ DC as well as CD11b⁺ Macs from steady state WT mice (B). Supplementary figure 2 Chimerism of blood granulocytes in mixed BM chimeras



Supplementary figure 2 Chimerism of blood granulocytes in mixed BM chimeras Blood chimerism of granulocytes of FLT3^{+/-}, CSF-1R^{+/-} and IRF4^{+/-} mixed BM chimeras 4 weeks after irradiation (mean \pm SEM, n=4) (A).

Supplementary figure 3 Flow cytometric analysis of mouse colonic and small intestinal lamina propria



Supplementary figure 3 Flow cytometric analysis of mouse colonic and small intestinal

Iamina propria Analysis of the colonic lamina propria DC compartment for the depicted markers by flow cytometry. Dot plots were gated as indicated (n=5) (A). DC and macrophage populations within the SI-LP were labeled with antibodies specific for the indicated markers, or an isotype-matched control antibody and analyzed by flow cytometry (n=3) (B).



Supplementary figure 4 Lung CD24+CD11b⁺ DCs and gut LP CD24+CD103+CD11b⁺ DCs are absent in *Itgax-cre Irf4*^{IVII} but not in any other organ

Supplementary figure 4 Lung CD24⁺CD11b⁺ DCs and gut LP CD24⁺CD103⁺CD11b⁺ DCs are absent in *Itgax-cre Irf4*^[11] but not in any other organ Bar graphs depict percentages of GFP⁺ cells in the MHCII⁺CD11c⁺ fraction of spleen, lung and small intestine of *Itgax-cre Irf4*^[11] mice (mean ± SEM, n=4) (Å). Bar graphs depict the percentage of the indicated cell populations in the indicated organs in *Itgax-cre Irf4*^[11] mice (mean ± SEM, n=4) (Å). Bar graphs depict the percentage of the indicated cell populations in the indicated organs in *Itgax-cre Irf4*^[11] mice (mean ± SEM, n=4) (Å). Bar graphs depict the percentage of the indicated cell populations in the indicated organs in *Itgax-cre Irf4*^[11] mice (mean ± SEM, n=4) (B-D).Cell populations within the lung (E) and SI-LP (H) of WT control littermate (upper panel) and *Itgax-cre Irf4*^[11] (loack pars) mice in the total Dapi CD45⁺SSC^{Iow}MHCII⁺CD11c⁺ CD11b⁺ fractions of the indicated tissues (F-J, n=7, mean ± SEM). Bar graphs depict percentage of DC and macrophage populations in WT (white bars) *Itgax-cre Irf4*^[11] (black bars) mice in the total Dapi⁺CD45⁺SSC^{Iow}MHCII⁺CD11c⁺ OT1b⁺ fractions of the indicated tissues (F-J, n=7, mean ± SEM). Bar graphs depict percentage of DC and macrophage populations in WT (white bars) *Itgax-cre Irf4*^[11] (black bars) mice in the total Dapi⁺CD45⁺SSC^{Iow}MHCII⁺CD11c⁺ OT1b⁺ fractions of the indicated tissues (K-O, n=7, mean ± -SEM).



► IL-17A

Supplementary figure 5 CD24+CD11b+ DCs show no developmental defect but enhanced apoptosis in the lung

Supplementary figure 5 CD24+CD11b+ DCs show no developmental defect but enhanced

Supplementary figure 5 CD24+CD11b+ DCs show no developmental defect but enhanced apoptosis in the lung Bar graphs depict percentages of GFP+ cells in the indicated cell population of *ltgax-cre Irf4^{luii}* mice (mean \pm SEM, n=4) (A). WT (white bars) and *ltgax-cre Irf4^{luii}* (black bars) BM was stained for the depicted precursor populations and their abundance was quantified as % of live cells (B, n=3, mean \pm SEM). Lung CD11b+ DCs were stained with Mitotracker red and analyzed for mitochondrial fragmentation. Images show WT and *ltgax-cre Irf4^{luii}* CD11b+ DCs from the lung stained with Mitotracker red (C) (representative image, scale bar represents 10µm). Bar graph depict percentage of cells with fragmented mitochondria within the population (D) (n \geq 100 cells, mean \pm SEM). Dut blots show 6h PMA/Ionomycin restimulated lung, lung LN, small intestine and mesenteric LN of WT or *ltgax-cre Irf4^{luii}* mice. Depicted tissues were gated for DAPI-CD3+CD4+ and analyzed for IL-17A+ and IFN-g expression (n=2) (E). Langerin-DTR mice were injected, or not, with DT (day -1) and intranasally infected (day 0) with A. *tumigatus*. Mice were sacrificed 7 days after infection. Dot plots show CD3+CD4+T-cells from the indicated tissues after 6h of PMA/ Ionomycin restimulation. Cells were labeled with antibodies specific for IL-17A+ and IFN-g arg graphs show the percentage of IL-17A+ or IFN-g+ CD3+CD4+T-cells in the indicated organs 7 days after infection with A. *tumigatus* (n=2, mean \pm SEM) (F).

Supplementary figure 6 Gating strategy for human DC subsets



PBMC, sorted DC subsets, A. fumigatus hyphae stimulated, autologous CD4+T-cells

PBMC, sorted DC subsets, A. fumigatus hyphae stimulated, autologous naive CD4+T-cells



Supplementary figure 6 Gating strategy for human DC subsets Gating and sorting strategy for PBMC, human lung and human small intestine (A). Sorted DC subsets derived from human PBMC stimulated with the indicated stimuli for 24hs. Depicted cytokines were measure by CBA (n=3, mean \pm -SEM) (B). DC subsets and bulk and naïve CD4+ T-cells were cocultured for 10 days in the presence of A. Fumigatus hyphae, restimulated and analyzed for the expression of IFN-g and IL-17A (n=3, mean \pm SEM) (C, D).

Supplementary table 1 Transcription factors differentially expressed between splenic CD8a^{+/} lung CD103⁺ DCs and splenic CD4⁺ / lung CD11b⁺ DC revealed using the SAM algorithm

Entrez_ID	Gene Symbol	Gene Name
21413	TCF4	transcription factor 4
15900	IRF8	interferon regulatory factor 8
100045165	GM4223	predicted gene 4223; similar to Csf3r protein
12986	LOC100045165	predicted gene 4223; similar to Csf3r protein
100043091	CSF3R	predicted gene 4223; similar to Csf3r protein
15902	ID2	inhibitor of DNA binding 2
381319	BATF3	basic leucine zipper transcription factor, ATF-like 3
14581	GFI1	growth factor independent 1
22433	XBP1	X-box binding protein 1
14255	FLT3	FMS-like tyrosine kinase 3
270110	IRF2BP2	interferon regulatory factor 2 binding protein 2
16600	KLF4	Kruppel-like factor 4 (gut)
16363	IRF2	interferon regulatory factor 2
13653	EGR1	early growth response 1
19698	RELB	avian reticuloendotheliosis viral oncogene related B
12399	RUNX3	runt related transcription factor 3
12982	CSF2RA	colony stimulating factor 2 receptor, alpha
20850	STAT5A	signal transducer and activator of transcription 5A
22778	IKZF1	IKAROS family zinc finger 1
12978	CSF1R	colony stimulating factor 1 receptor
20375	SFP11	SFFV proviral integration 1
12983	CSF2RB	colony stimulating factor 2 receptor, beta
12984	CSF2RB2	colony stimulating factor 2 receptor, beta 2
16364	IRF4	interferon regulatory factor 4
20848	LOC100045296	similar to Stat3B
100045296	STAT3	signal transducer and activator of transcription 3

Supplementary table 2 Differentially expressed genes of mouse CD11b⁺ DC compared to mouse CD11b⁺ Mac. All genes upregulated 1.5fold or more

Entrez_ID	Gene Symbol	Gene Name
11630	AIM1	absent in melanoma 1
11658	ALCAM	activated leukocyte cell adhesion molecule
11676	ALDOC	aldolase C, fructose-bisphosphate
11732	ANK	progressive ankylosis
11799	BIRC5	baculoviral IAP repeat-containing 5
11828	AQP3	aquaporin 3
12040	BCKDHB	branched chain ketoacid dehydrogenase E1
12257	TSPO	translocator protein
12306	ANXA2	similar to Annexin A2 (Annexin II)
12315	CALM3	calmodulin 3
12332	CAPG	capping protein (actin filament), gelsolin-like
12337	CAPN5	calpain 5
12443	CCND1	cyclin D1
12444	CCND2	cyclin D2
12481	CD2	CD2 antigen
12519	CD80	CD80 antigen
12550	CDH1	cadherin 1
12579	CDKN2B	similar to Cyclin-dependent kinase 4 inhibitor B
12615	CENPA	centromere protein A
12700	CISH	cytokine inducible SH2-containing protein
12702	SOCS3	suppressor of cytokine signaling 3
12775	CCR7	chemokine (C-C motif) receptor 7
12827	COL4A2	collagen, type IV, alpha 2
13007	CSRP1	cysteine and glycine-rich protein 1
13169	DBNL	drebrin-like
13449	DOK2	docking protein 2
13482	DPP4	dipeptidylpeptidase 4
13587	EAR2 EMB1	eosinophil-associated, ribonuclease A family
13/30	EMPI	epinenai memorane protein 1
1380/	ENU2 EDUA2	enorase 2, gamma neuronal
13830	BETHAZ RCI 11A	Epi receptor A2
14020	ETRI 2	conclusion factor II (thrombin) recentor like 2
14004	1 2KL2 PTK7	Coagmanon ractor in (unonnonn) receptor-nice 2 PTK2 protein turosine kinase 2
14065	FSCNI	fascin homolog 1
14000	FCGR2R	Ecrecentor IoG low affinity IIb
14150	FGL2	fibringen-like protein 2
14194	FH1	fumarate hydratase 1
14204	11.411	interleukin 4 induced 1
14268	FNI	fibronectin 1
14700	GNG10	guanine nucleotide binding protein
14726	PDPN	podoplanin
14793	CDCA3	cell division cycle associated 3
15000	H2-DMB2	histocompatibility 2, class II, locus Mb2
15001	H2-OA	histocompatibility 2, O region alpha locus
15002	H2-OB	histocompatibility 2, O region beta locus
15108	HSD17B10	hydroxysteroid (17-beta) dehydrogenase 10
15270	H2AFX	H2A histone family, member X
15277	HK2	hexokinase 2
15460	HR	hairless
16164	IL13RA1	interleukin 13 receptor, alpha 1
16174	IL18RAP	interleukin 18 receptor accessory protein

16178	IL1R2	interleukin 1 receptor, type II
16193	IL6	interleukin 6
16364	IRF4	interferon regulatory factor 4
16407	ITGAE	integrin alpha E, epithelial-associated
16421	ITGB7	integrin beta 7
16438	ITPRI	inositol 1,4,5-triphosphate receptor 1
16452	JAK2	Janus kinase 2
16498	KCNAB2	potassium voltage-gated channel
16541	NAPSA	napsin A aspartic peptidase
16643	KLRD1	killer cell lectin-like receptor, subfamily D, member 1
16911	LMO4	LIM domain only 4
16952	ANXA1	annexin A1
16985	LSP1	lymphocyte specific 1
16995	LTB4R1	leukotriene B4 receptor 1
17304	MFGE8	milk fat globule-EGF factor 8 protein
17427	MNS1	meiosis-specific nuclear structural protein 1
17532	MRAS	muscle and microspikes RAS
17750	MT2	metallothionein 2
17768	MTHFD2	methenyltetrahydrofolate cyclohydrolase
17939	NAGA	N-acetyl galactosaminidase, alpha
18074	NID2	nidogen 2
18105	NQO2	NAD(P)H dehydrogenase, quinone 2
18441	P2RVI	purinergic receptor P2Y G-protein coupled 1
18476	PAFAH1R3	platelet-activating factor acetylhydrolase
18479	PAKI	n21 protein (Cdc42/Rac)-activated kinase 1
18569	PDCD4	programmed cell death 4
18636	CFP	complement factor properdin
18817	PLK1	polo-like kinase 1 (Drosophila)
18824	PLP2	proteolipid protein 2: predicted gene 13669
19088	PRKAR2B	protein kinase, cAMP dependent regulatory, type II beta
19354	RAC2	RAS-related C3 botulinum substrate 2
19378	ALDH1A2	aldehyde dehydrogenase family 1, subfamily A2
20190	RYRI	ryanodine receptor 1, skeletal muscle
20194	S100A10	S100 calcium binding protein A10 (calpactin)
20200	S100A6	S100 calcium binding protein A6 (calcyclin)
20249	SCD1	stearoyl-Coenzyme A desaturase 1
20295	CCL17	chemokine (C-C motif) ligand 17
20299	CCL22	chemokine (C-C motif) ligand 22
20312	CX3CL1	chemokine (C-X3-C motif) ligand 1
20344	SELP	selectin, platelet
20351	SEMA4A	semaphorin 4A
20454	ST3GAL5	ST3 beta-galactoside alpha-2,3-sialyltransferase 5
20525	SLC2A1	solute carrier family 2, member 1
20527	SLC2A3	solute carrier family 2, member 3
20698	SPHK1	sphingosine kinase 1
20708	SERPINB6B	serine (or cysteine) peptidase inhibitor
20877	AURKB	aurora kinase B
20893	BHLHE40	basic helix-loop-helix family, member e40
21417	ZEB1	zinc finger E-box binding homeobox 1
21753	TES	testis derived transcript
21754	TESK1	testis specific protein kinase 1
21844	TIAM1	T-cell lymphoma invasion and metastasis 1
21936	TNFRSF18	tumor necrosis factor receptor superfamily, member 18

21948	CD70	CD70 antigen
21950	TNFSF9	tumor necrosis factor (ligand) superfamily, member 9
21953	TNNI2	troponin I, skeletal, fast 2
22032	TRAF4	TNF receptor associated factor 4
22038	PLSCR1	phospholipid scramblase 1
22041	TRF	transferrin
22142	TUBA1A	predicted gene 7172
22248	UNC119	unc-119 homolog (C. elegans)
22330	VCL	vinculin
22337	VDR	vitamin D receptor
22368	TRPV2	transient receptor potential cation channel
22793	ZYX	zyxin
23792	ADAM23	a disintegrin and metallopeptidase domain 23
23912	RHOF	ras homolog gene family, member f
24050	3-SEP	septin 3
26411	MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1
26568	SLC27A3	solute carrier family 27 (fatty acid transporter), member 3
26914	H2AFY	H2A histone family, member Y
27049	ETV3	ets variant gene 3
27357	GYG	glycogenin
27528	D0H4S114	DNA segment, human D4S114
30057	TIMM8B	translocase of inner mitochondrial membrane 8 homolog b
30928	ZFP238	zinc finger protein 238
50784	PPAP2C	phosphatidic acid phosphatase type 2C
53313	ATP2A3	ATPase, Ca++ transporting, ubiquitous
53318	PDLIM3	PDZ and LIM domain 3
53608	MAP3K6	mitogen-activated protein kinase kinase kinase 6
53860	SEP9	septin 9
53877	EAR4	eosinophil-associated, ribonuclease A family, member 4
54563	NUP210	nucleoporin 210
56149	GRASP	General receptor for phosphoinositides 1
56177	OLFM1	olfactomedin 1
56297	ARL6	ADP-ribosylation factor-like 6
56349	NET1	predicted gene 8990; neuroepithelial cell transforming gene 1
56437	RRAD	Ras-related associated with diabetes
56524	MPP6	membrane protein, palmitoylated 6
56526	SEP 6	septin 6
57262	RETNLA	resistin like alpha
58200	PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A
58861	CYSLTR1	cysteinyl leukotriene receptor 1
64008	AQP9	aquaporin 9
64095	GPR35	G protein-coupled receptor 35
64136	SDF2L1	stromal cell-derived factor 2-like 1
66259	CAMK2N1	calcium/calmodulin-dependent protein kinase II inhibitor 1
66422	DCTPP1	dCTP pyrophosphatase 1
66815	CCDC109B	coiled-coil domain containing 109B
66874	1200014J11RIK	similar to RIKEN cDNA 1200014J11 gene
66922	RRAS2	related RAS viral (r-ras) oncogene homolog 2
67044	HIGD2A	HIG1 domain family, member 2A
67102	D16ERTD472E	DNA segment, Chr 16, ERATO Doi 472, expressed
67166	ARL8B	ADP-ribosylation factor-like 8B
67267	2900010M23RIK	hypothetical protein LOC675054
67647	4930523C07RIK	RIKEN cDNA 4930523C07 gene

67739	SLC48A1	solute carrier family 48 (heme transporter), member 1
67784	PLXND1	plexin D1
67800	DGAT2	diacylglycerol O-acyltransferase 2
67846	TMEM39A	transmembrane protein 39a
67941	RPS27L	ribosomal protein S27-like
68026	2810417H13RIK	predicted gene 15428
68870	1190002A17RIK	RIKEN cDNA 1190002A17 gene
69029	1500032L24RIK	RIKEN cDNA 1500032L24 gene
69165	CD209B	CD209b antigen
69189	1810033B17RIK	RIKEN cDNA 1810033B17 gene
69399	1700025G04RIK	RIKEN cDNA 1700025G04 gene
69635	DAPK1	death associated protein kinase 1
69698	2310046K01RIK	RIKEN cDNA 2310046K01 gene
69797	1600029114RIK	thioredoxin domain containing 6
69810	CLEC4B1	C-type lectin domain family 4, member b1
69863	TTC39B	tetratricopeptide repeat domain 39B
69982	SPINK2	serine peptidase inhibitor, Kazal type 2
70025	ACOT7	acyl-CoA thioesterase 7
70031	CMTM8	CKLF-like MARVEL transmembrane domain containing 8
70536	QPCT	glutaminyl-peptide cyclotransferase (glutaminyl cyclase)
71268	LRRFIP2	leucine rich repeat (in FLII) interacting protein 2
71302	ARHGAP26	RIKEN cDNA 9630014M24 gene
71532	9030418K01RIK	RIKEN cDNA 9030418K01 gene
72084	PIGX	phosphatidylinositol glycan anchor biosynthesis, class X
72324	PLXDC1	plexin domain containing 1
72333	PALLD	palladin, cytoskeletal associated protein
72865	CXXIC	CAAX box 1 homolog C (human)
73385	FAM177A	RIKEN cDNA 1700047117 gene 2
73835	IFITM5	interferon induced transmembrane protein 5
74015	FCHO1	FCH domain only 1
74134	CYP2S1	cytochrome P450, family 2, subfamily s, polypeptide 1
74145	F13A1	coagulation factor XIII, A1 subunit
74340	AHCYL2	S-adenosylhomocysteine hydrolase-like 2
75292	PRKD3	protein kinase D3
75766	TM7SF4	transmembrane 7 superfamily member 4
75863	CLEC4G	C-type lectin domain family 4, member g
76062	5830428M24RIK	RIKEN cDNA 5830428M24 gene
76263	GSTK1	glutathione S-transferase kappa 1
76737	CRELD2	cysteine-rich with EGF-like domains 2
77605	H2AFV	H2A histone family, member V
78416	RNASE6	ribonuclease, RNase A family, 6
78826	P2RY10	purinergic receptor P2Y, G-protein coupled 10
80885	NIACRI	niacin receptor 1
80891	FCRLS	Fc receptor-like S, scavenger receptor
80914	UCK2	uridine-cytidine kinase 2
93671	CD163	CD163 antigen
93725	EAR10	eosinophil-associated, ribonuclease A family, member 9
94353	HMGN3	high mobility group nucleosomal binding domain 3
98256	KMO	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)
98752	FCRLA	Fc receptor-like A
103149	UPB1	ureidopropionase, beta
103711	PNPO	pyridoxine 5'-phosphate oxidase
104175	SBK1	SH3-binding kinase 1
		5

105348	GOLM1	golgi membrane protein 1
105841	DENND3	DENN/MADD domain containing 3
106795	TCF19	transcription factor 19
106840	UNC119B	unc-119 homolog B (C. elegans)
107221	GPR120	G protein-coupled receptor 120
107766	HAAO	3-hydroxyanthranilate 3,4-dioxygenase
108673	CCDC86	coiled-coil domain containing 86
108956	APOL7C	predicted gene 8221; apolipoprotein L 7c
109305	ORAII	ORAI calcium release-activated calcium modulator 1
110033	KIF22	kinesin family member 22
170752	BCO2	beta-carotene oxygenase 2
170776	CD209C	similar to SIGNR2; CD209c antigen
170779	CD209D	CD209d antigen
170786	CD209A	CD209a antigen
207777	BZRAP1	benzodiazapine receptor associated protein 1
208638	SLC25A38	hypothetical protein LOC100048873
216233	SOCS2	suppressor of cytokine signaling 2
216864	MGL2	macrophage galactose specific lectin 2
217835	RIN3	Ras and Rab interactor 3
217946	CDCA7L	cell division cycle associated 7 like
224143	KTELC1	KTEL (Lys-Tyr-Glu-Leu) containing 1
225912	CYBASC3	cytochrome b, ascorbate dependent 3
226419	DYRK3	dual-specificity tyrosine phosphorylation regulated kinase 3
227659	SLC2A6	solute carrier family 2, member 6
229323	GPR171	G protein-coupled receptor 171
233038	NCCRP1	non-specific cytotoxic cell receptor protein 1 homolog
233046	RASGRP4	RAS guanyl releasing protein 4
233406	PRC1	protein regulator of cytokinesis 1
234577	CPNE2	copine II
237250	GM221	predicted gene 221
238377	GPR68	G protein-coupled receptor 68
238875	GAPT	Grb2-binding adaptor
240047	MMP25	matrix metallopeptidase 25
243958	SIGLECG	sialic acid binding Ig-like lectin G
246277	CSAD	cysteine sulfinic acid decarboxylase
270066	SLC35E1	solute carrier family 35, member E1
270152	AMICA1	adhesion molecule, interacts with CXADR antigen 1
271844	PLA2G4F	phospholipase A2, group IVF
272636	ESYT3	family with sequence similarity 62
276891	TIMD4	T-cell immunoglobulin and mucin domain containing 4
320782	TMEM154	transmembrane protein 154
381269	MREG	melanoregulin
384009	GLIPR2	GLI pathogenesis-related 2
414084	TNIP3	TNFAIP3 interacting protein 3
433470	AA467197	expressed sequence AA467197
664968	2210411K11RIK	RIKEN cDNA 2210411K11 gene
671466	GM14024	predicted gene 14024
100034251	GM11428	predicted gene 11428
100042480	NHSL2	NHS-like 2;

Supplementary table 3 Differentially expressed genes of mouse CD11b⁺ Mac compared to mouse CD11b⁺ DC. All genes upregulated 1.5fold or more

I	Entrez_ID	Gene Symbol	Gene Name
	11421	ACE	angiotensin I converting enzyme
	11432	ACP2	acid phosphatase 2, lysosomal
	11491	ADAM17	a disintegrin and metallopeptidase domain 17
	11520	PLIN2	adipose differentiation related protein
	11655	ALASI	aminolevulinic acid synthase 1
	11727	ANG	angiogenin, ribonuclease, RNase A family, 5
	11747	ANXA5	annexin A5
	11810	APOBEC1	apolipoprotein B
	11816	APOE	apolipoprotein E
	11821	APRT	adenine phosphoribosyl transferase
	11852	RHOB	ras homolog gene family, member B
	11910	ATF3	activating transcription factor 3
	12259	CIQA	complement component 1, q subcomponent
	12260	CIQB	complement component 1, q subcomponent
	12262	CIQC	complement component 1, q subcomponent
	12266	C3	complement component 3
	12475	CD14	CD14 antigen
	12512	CD63	CD63 antigen
	12514	CD68	CD68 antigen
	12517	CD72	CD72 antigen
	12520	CD81	CD81 antigen
	12571	CDK6	cyclin-dependent kinase 6
	12585	CDR2	cerebellar degeneration-related 2
	12608	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta
	12642	CH25H	cholesterol 25-hydroxylase
	12751	TPP1	tripeptidyl peptidase I
	12767	CXCR4	chemokine (C-X-C motif) receptor 4
	12818	COL14A1	collagen, type XIV, alpha 1
	12819	COL15A1	collagen, type XV, alpha 1
	12978	CSF1R	colony stimulating factor 1 receptor
	12986	CSF3R	predicted gene 4223
	13014	CSTB	cystatin B
	13030	CTSB	cathepsin B
	13032	CTSC	cathepsin C
	13170	DBP	D site albumin promoter binding protein
	13421	DNASE1L3	deoxyribonuclease 1-like 3
	13537	DUSP2	dual specificity phosphatase 2
	13650	RHBDF1	rhomboid family 1 (Drosophila)
	13653	EGR1	early growth response 1
	13733	EMR1	EGF-like module containing
	13808	ENO3	enolase 3, beta muscle
	13822	EPB4.1L2	erythrocyte protein band 4.1-like 2
	13849	EPHX1	epoxide hydrolase 1, microsomal
	14129	FCGR1	Fc receptor, IgG, high affinity I
	14281	FOS	FBJ osteosarcoma oncogene
	14289	FPR2	formyl peptide receptor 2
	14345	FUT4	fucosyltransferase 4
	14388	GAB1	growth factor receptor bound protein 2
	14456	GAS6	growth arrest specific 6
	14562	GDF3	growth differentiation factor 3
	14594	GGTA1	glycoprotein galactosyltransferase alpha 1, 3
	14710	GNGT2	guanine nucleotide binding protein

14744	GPR65	G-protein coupled receptor 65
14825	CXCL1	chemokine (C-X-C motif) ligand 1
15081	H3F3B	predicted gene 14383
15199	HEBP1	heme binding protein 1
15205	HES1	hairy and enhancer of split 1 (Drosophila)
15284	HLX	H2.0-like homeobox
15446	HPGD	hydroxyprostaglandin dehydrogenase 15 (NAD)
15478	HS3ST3A1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1
15483	HSD11B1	hvdroxysteroid 11-beta dehydrogenase 1
15511	HSPA1B	heat shock protein 1B
15531	NDST1	N-deacetylase/N-sulfotransferase 1
15900	IRF8	interferon regulatory factor 8
15903	ID3	inhibitor of DNA binding 3
15985	CD79R	CD79B antigen
16000	IGF1	insulin-like growth factor 1
16007	CYR61	cysteine rich protein 61
16010	ICERPA	inculin like growth factor binding protain 4
16152	101-01-4	interleukin 10
16153	IL 10P A	interleukin 10
10154	IL IORA	
16155	ILIOKB	interieukin 10 receptor, beta
16408	IIGAL	integrin alpha L
16419	IIGB5	integrin beta 5
16449	JAGI	Jagged I
16598	KLF2	Kruppel-like factor 2 (lung)
16653	KRAS	similar to GTPase KRas precursor
16658	MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene family
16783	LAMP1	lysosomal-associated membrane protein 1
16784	LAMP2	lysosomal-associated membrane protein 2
16905	LMNA	lamin A
16988	LST1	leukocyte specific transcript 1
17064	CD93	CD93 antigen
17085	LY9	lymphocyte antigen 9
17087	LY96	lymphocyte antigen 96
17105	LYZ2	lysozyme 2
17127	SMAD3	MAD homolog 3 (Drosophila)
17260	MEF2C	myocyte enhancer factor 2C
17289	MERTK	c-mer proto-oncogene tyrosine kinase
17329	CXCL9	chemokine (C-X-C motif) ligand 9
17381	MMP12	matrix metallopeptidase 12
17386	MMP13	matrix metallopeptidase 13
17387	MMP14	matrix metallopeptidase 14 (membrane-inserted)
17474	CLEC4D	C-type lectin domain family 4, member d
17684	CITED2	Cbp/p300-interacting transactivator
17872	PPP1R15A	protein phosphatase 1
17921	MYO7A	myosin VIIA
18008	NES	nestin
18022	NFE2	nuclear factor, erythroid derived 2
18173	SLC11A1	solute carrier family 11
18175	NRAP	nebulin-related anchoring protein
18412	SQSTM1	sequestosome 1
18590	PDGFA	platelet derived growth factor, alpha
18669	ABCB1B	ATP-binding cassette, sub-family B, member 1B
18689	PHXR4	per-hexamer repeat gene 4

18751	PRKCB	protein kinase C, beta
18805	PLD1	phospholipase D1
18807	PLD3	phospholipase D family, member 3
18858	PMP22	peripheral myelin protein 22
18987	POU2F2	POU domain, class 2, transcription factor 2
19039	LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein
19141	LGMN	legumain
19159	СҮТНЗ	cytohesin 3
19229	PTK2B	PTK2 protein tyrosine kinase 2 beta
19261	SIRPA	signal-regulatory protein alpha
19340	RAB3D	RAB3D, member RAS oncogene family
19703	RENBP	renin binding protein
19731	RGL1	ral guanine nucleotide dissociation stimulator,-like 1
20230	SATB1	special AT-rich sequence binding protein 1
20293	CCL12	chemokine (C-C motif) ligand 12
20302	CCL3	chemokine (C-C motif) ligand 3
20310	CXCL2	chemokine (C-X-C motif) ligand 2
20317	SERPINF1	serine (or cysteine) peptidase inhibitor, clade F, member 1
20363	SEPP1	selenoprotein P, plasma, 1
20393	SGK1	serum/glucocorticoid regulated kinase 1
20715	SERPINA3G	serinepeptidase inhibitor, clade A, member 3G
20719	SERPINB6A	serine peptidase inhibitor, clade B, member 6a
20728	SPIC	Spi-C transcription factor (Spi-1/PU.1 related)
21452	TCN2	transcobalamin 2
21743	INMT	indolethylamine N-methyltransferase
21809	TGFB3	transforming growth factor, beta 3
21810	TGFBI	transforming growth factor, beta induced
21812	TGFBR1	transforming growth factor, beta receptor I
21813	TGFBR2	transforming growth factor, beta receptor II
21817	TGM2	transglutaminase 2, C polypeptide
21824	THBD	thrombomodulin
21858	TIMP2	tissue inhibitor of metalloproteinase 2
21897	TLRI	toll-like receptor 1
21926	TNF	tumor necrosis factor
21928	TNFAIP2	tumor necrosis factor, alpha-induced protein 2
21985	TPD52	similar to Tpd52 protein; tumor protein D52
22022	TPST2	protein-tyrosine sulfotransferase 2
22234	UGCG	UDP-glucose ceramide glucosyltransferase
22249	UNC13B	unc-13 homolog B (C. elegans)
22259	NR1H3	nuclear receptor subfamily 1, group H, member 3
22329	VCAMI	vascular cell adhesion molecule 1
22341	VEGFC	vascular endothelial growth factor C
23849	KLF6	Kruppel-like factor 6
23872	ETS2	E26 avian leukemia oncogene 2. 3' domain
23880	FYB	FYN binding protein
23886	GDF15	growth differentiation factor 15
23890	GPR34	G protein-coupled receptor 34
23962	OASL2	2'-5' oligoadenylate synthetase-like 2
24055	SH3BP2	SH3-domain binding protein 2
24088	TLR2	toll-like receptor 2
24099	TNFSF13B	tumor necrosis factor (ligand) superfamily, member 13b
26433	PLOD3	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3
26930	PPNR	per-pentamer repeat gene
		r r · · · · · · · · · · · · · · · · · ·

26943	SERINC3	serine incorporator 3
27052	AOAH	acyloxyacyl hydrolase
27226	PLA2G7	phospholipase A2, group VII
27261	DOK3	docking protein 3
29819	STAU2	staufen (RNA binding protein) homolog 2 (Drosophila)
30794	PDLIM4	PDZ and LIM domain 4
50778	RGS1	regulator of G-protein signaling 1
50794	KU F13	Kruppel-like factor 13
50934	SLC748	solute carrier family 7
52076	TMEM38R	transmembrane protein 38B
52120	HGSNAT	heparan-alpha-glucosaminide N-acetyltransferase
52120	CAMK1	calcium/calmodulin-dependent protein kinase I
52588	TSPAN14	tetraspanin 14
52855	I AID 1	laukocuta associated Ig like recentor 1
52701	TI P5	toll like recentor 5
53945	SI C4041	solute carrier family 40 member 1
54123	IRE7	interferon regulatory factor 7
54125	HRCDS	prostaclandin D2 synthese 2 homotonoistic
54612	ST2CAL6	ST2 hete gelesteside alpha 2.2 sightfransferese 6
54711	DI ACL2	nlaiomorphia adapome gana lika 2
54725	CADMI	cell adhesion melacula 1
55042	SERTIDI	SEDTA domain containing 1
55095	CYCL12	sherrabias (C.V.C. matif) lisend 12
55985	CACLIS	Chinese encharing metain 1
56210	GKAP1	G kinase anchoring protein 1
50512	NUPRI SDDV2	nuclear protein 1
56504	SKPKS	C targe leasting demonin familie 4 membres a
50019	CLEC4E	c-type fectin domain family 4, member e
56/44	PF4	platelet factor 4
50807	SCAMP 5	secretory carrier memorane protein 5
5/200	CACL14	chemokine (C-X-C motif) ligand 14
58185	KSAD2	radical S-adenosyl methionine domain containing 2
58218	TREM3	triggering receptor expressed on myeloid cells 3
60406	SAP30	sin3 associated polypeptide
63959	SLC29A1	solute carrier family 29, member 1
65256	ASB2	ankyrin repeat and SOCS box-containing 2
66168	GRINA	glutamate receptor
66566	2310079N02RIK	RIKEN cDNA 2310079N02 gene
66610	ABI3	ABI gene family, member 3
66868	MFSD1	major facilitator superfamily domain containing 1
66881	PCYOXI	prenylcysteine oxidase 1
66961	NEATT	RIKEN cDNA 2310043N10 gene
67168	LPAR6	purinergic receptor P2Y, G-protein coupled, 5
67263	ZSWIM6	zinc finger, SWIM domain containing 6
67399	PDLIM7	PDZ and LIM domain 7
67603	DUSP6	dual specificity phosphatase 6
67865	RGS10	regulator of G-protein signalling 10
0/893	I MEM80A	transmembrane protein 86A
08682	SLC44A2	solute carrier family 44, member 2
08728	IKP33INP2	transformation related protein 53 inducible nuclear protein 2
69068	1810011O10RIK	KIKEN CDNA 1810011010 gene
69226	SNX24	sorting nexing 24
70564	5730469M10RIK	RIKEN cDNA 5730469M10 gene
70720	6330407A03RIK	RIKEN cDNA 6330407A03 gene

71602	MYO1E	myosin IE
71712	DRAMI	RIKEN cDNA 1200002N14 gene
73149	CLEC4A3	C-type lectin domain family 4, member a3
74048	4632428N05RIK	RIKEN cDNA 4632428N05 gene
74096	HVCN1	hydrogen voltage-gated channel 1
74136	SEC14L1	SEC14-like 1 (S. cerevisiae)
74191	P2RY13	purinergic receptor P2Y, G-protein coupled 13
74747	DDIT4	DNA-damage-inducible transcript 4
74772	ATP13A2	ATPase type 13A2
74777	SEPN1	selenoprotein N, 1
76408	ABCC3	ATP-binding cassette, sub-family C, member 3
77976	NUAK1	NUAK family, SNF1-like kinase, 1
80281	CTTNBP2NL	CTTNBP2 N-terminal like
83433	TREM2	triggering receptor expressed on myeloid cells 2
83490	PIK3AP1	phosphoinositide-3-kinase adaptor protein 1
93695	GPNMB	glycoprotein (transmembrane) nmb
98878	EHD4	EH-domain containing 4
99543	OLFML3	olfactomedin-like 3
100198	H6PD	hexose-6-phosphate dehydrogenase
100213	RUSC2	RUN and SH3 domain containing 2
101488	SLCO2B1	solute carrier organic anion transporter family, member 2b1
102657	CD276	CD276 antigen
103210	D630004K10RIK	RIKEN cDNA D630004K10 gene
103724	TBC1D10A	TBC1 domain family, member 10a
104099	ITGA9	integrin alpha 9
104252	CDC42EP2	CDC42 effector protein (Rho GTPase binding) 2
106952	ARAP3	ArfGAP with RhoGAP domain
107607	NOD1	nucleotide-binding oligomerization domain containing 1
108089	RNF144A	ring finger protein 144A
109225	MS4A7	membrane-spanning 4-domains, subfamily A, member 7
110454	LY6A	lymphocyte antigen 6 complex, locus A
114606	TLE6	transducin-like enhancer of split 6
140488	IGF2BP3	insulin-like growth factor 2 mRNA binding protein 3
140497	AF251705	cDNA sequence AF251705
140795	P2RY14	purinergic receptor P2Y, G-protein coupled, 14
170460	STARD5	StAR-related lipid transfer (START) domain containing 5
170625	SNX18	sorting nexin 18
170743	TLR7	toll-like receptor 7
170744	TLR8	toll-like receptor 8
192187	STAB1	stabilin 1
192654	PLA2G15	phospholipase A2, group XV
192678	RASSF3	Ras associationdomain family member 3
195522	ZFP691	zinc finger protein 691
207521	DTX4	deltex 4 homolog (Drosophila)
209200	DTX3L	deltex 3-like (Drosophila)
210530	LEPREL1	leprecan-like 1
210808	9030625A04RIK	RIKEN cDNA 9030625A04 gene
213002	IFITM6	interferon induced transmembrane protein 6
213573	EFCAB4A	EF-hand calcium binding domain 4A
215900	FAM26F	family with sequence similarity 26, member F
216805	FLCN	folliculin
216869	ARRB2	arrestin, beta 2
216991	ADAP2	ArfGAP with dual PH domains 2

217333	TRIM47	tripartite motif-containing 47
218454	LHFPL2	lipoma HMGIC fusion partner-like 2
218624	IL31RA	interleukin 31 receptor A
218793	UBE2E2	ubiquitin-conjugating enzyme E2E 2
224454	ZDHHC14	zinc finger, DHHC domain containing 14
224794	ENPP4	ectonucleotide pyrophosphatase 4
224840	TREML4	triggering receptor on myeloid cells-like 4
225884	BC021614	cDNA sequence BC021614
226421	5430435G22RIK	RIKEN cDNA 5430435G22 gene
226519	LAMCI	laminin, gamma 1
226691	A1607873	expressed sequence AI607873
227612	A830007P12RIK	RIKEN cDNA A830007P12 gene
227620	UAP1L1	UDP-N-acteylglucosamine pyrophosphorylase 1-like 1
228545	VPS18	vacuolar protein sorting 18 (yeast)
228608	SMOX	spermine oxidase
229595	ADAMTSL4	ADAMTS-like 4
229599	GM129	predicted gene 129
231507	PLAC8	placenta-specific 8
231633	TMEM119	transmembrane protein 119
231991	CREB5	RIKEN cDNA 9430076C15 gene
232288	FRMD4B	FERM domain containing 4B
232801	LILRA5	predicted gene 4878
232984	B3GNT8	beta-1.3-N-acetylglucosaminyltransferase 8
233016	BLVRB	biliverdin reductase B (flavin reductase (NADPH))
233079	FF4R2	free fatty acid recentor 2
233571	P2RY6	pyrimidinergic receptor P2Y G-protein coupled 6
233979	TPCN2	two nore segment channel 2
234595	SI C3847	nredicted gene 3599; solute carrier family 38, member 7
239027	ARHGAP22	Rho GTPase activating protein 22
239827	PIG7	nhosnhatidylinositol glycan anchor biosynthesis class 7
243725	PPP1P0A	protein phosphatase 1 regulatory (inhibitor) subunit 9A
243723	F4M55D	family with sequence similarity 55 member D
244055	CM4090	predicted cope 4090
243190	CCP4	E manten LC law officity W
246236	CD207	CD207 anti-
246278	CD207	
246696	SLC25A28	solute carrier family 25, member 28
246/07	EMILIN2	elastin microfibril interfacer 2
268973	NLRC4	NLR family, CARD domain containing 4
269181	MGAT4A	mannoside acetylglucosaminyltransferase 4, isoenzyme A
269799	CLEC4A1	C-type lectin domain family 4, member a1
269951	IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial
270118	MAML2	similar to Maml2 protein; mastermind like 2 (Drosophila)
319939	TNS3	tensin 3
320024	NCEH1	arylacetamide deacetylase-like 1
327957	A430084P05RIK	RIKEN cDNA A430084P05 gene
328314	GM5086	predicted gene 5086
330635	3830612M24	hypothetical protein 3830612M24
381484	GM5150	predicted gene 5150; similar to SIRP beta 1 like 1 protein
383435	MS4A14	membrane-spanning 4-domains, subfamily A, member 14
436440	GPR31C	predicted gene 8593
574428	ZMYND15	zinc finger, MYND-type containing 15
100038947	LOC100038947	signal-regulatory protein beta 1
100039795	ILDR2	immunoglobulin-like domain containing receptor 2
100043272	5430417L22RIK	similar to F32B6.11

Supplementary table 4 Real-Time PCR primers used in this article

Target	Primer 5'-3'	Species
Csf-1r forward	CGAGGGAGACTCCA GCTACA	mouse
Csf-1r reverse	GACTGGAGAAGCCA CTGTCC	mouse
Flt3 forward	GAGCGACTCCAGCT ACGTC	mouse
Flt3 reverse	ACCCAGTGAAAATAT CTCCCAGA	mouse
Irf4 forward	ACAGCACCTTATGGC TCTCTG	mouse
Irf4 reverse	ATGGGGTGGCATCAT GTAGT	mouse
Irf8 forward	CCTATGACACACACC ATTCAGC	mouse
Irf8 reverse	AGAGACGGCAGCCT TCAA	mouse
Hprt foward	TCCTCCTCAGACCGC TTTT	mouse
Hprt reverse	CCTGGTTCATCATCG CTAATC	mouse
Id2 forward	GACAGAACCAGGCG TCCA	mouse
Id2 reverse	AGCTCAGAAGGGAA TTCAGATG	mouse
Batf3 forward	AGAAGGCTGACAAG CTCCAC	mouse
Batf3 reverse	CCTTCAGCTTCGAAA TCTCC	mouse
Il-23p19 forward	AACAGCCAGTTCTGC TTGC	mouse
Il-23p19 reverse	AGGGAGGTGTGAAG TTGCTC	mouse
Ccr7 forward	CAGGGAAACCCAGG AAAAAC	mouse
Ccr7 reverse	TCATCTTGGCAGAAG CACAC	mouse
II-23p19 forward	CCCAAGGACTCAGG GACAAC	human
II-23p19 reverse	TCAGACCCTGGTGG ATCCTT	human

Supplemental Experimental Procedures Mice

C57BL/6 (CD45.2⁺) mice were purchased from the Biological Resource Center (BRC), Agency for Science, Technology and Research (A*STAR), Singapore. Congenic C57BL/6 B6.129S1-*Irf4*^{tm1Rdf}/J</sup> (*Irf4*^{fl/fl}), B6.Cg-Tg(Itgax-cre)1-1Reiz/J $(CD45.1^{+}),$ (Itgax-cre), B6.129P-Cx3cr1^{tm1Litt}/J mice were purchased from the Jackson Laboratory (Jackson Laboratory, Bar Harbor, USA). DC-specific Cre recombinase mouse (Itgax-cre) was crossed with the Irf4^{fl/fl} to produce mice which are deficient for IRF4 in the DC lineage (Itgax-cre Irf4^{fl/fl}). Itgax-cre Irf4^{fl/fl} were negative (tested by Q-PCR) for segmented filamentous bacteria (SFB) (Ivanov et al., 2009). OTII-Rag1 mice (Barnden et al., 1998) were obtained from Taconic (Taconic Farms, USA) through the NIAID Exchange Program, NIH line number 4234. Fucci-492 mice (Sakaue-Sawano et al., 2008) were purchased from the Riken BioResource Center (Ibaraki, Japan). Langerin-DTR mice were provided by Dr. Bernard Malissen (Centre d'Immunologie de Marseille-Luminy, France). Lysozyme-GFP mice were kindly provided by Dr. Thomas Graf. All mice were bred and maintained in our animal facility, and analyzed between 8-12 weeks of age. All experiments and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of A*STAR (Biopolis, Singapore) in accordance with the guidelines of the Agri-Food and Veterinary Authority (AVA) and the National Advisory Committee for Laboratory Animal Research (NACLAR) of Singapore.

Restimulation of T-Cells

Murine T-cells were restimulated for 6h using 50ng/ml Phorbol myristate acetate (PMA, Sigma) and 1ng/ml Ionomycin (Iono, Sigma), Brefeldin A was added after 2h of stimulation. Cells were stained intracellularly as described below.

Flow Cytometry and Cell Sorting (Igs and Clones in Supp)

Flow cytometry was performed on an LSR II or FACS Canto (Becton Dickinson, San Jose, USA) and analyzed with FlowJo software (Tree Star, Ashland, USA). Fluorochrome or biotin-conjugated monoclonal antibodies (mAbs) specific to mouse IA/IE (M5/114.15.2), CD103 (2E7), SIRPa (P84), CD11b (M1/70), CD11c (N418), CD45 (30F11), CD45.1 (A20), CD45.2 (104), CD115 (AFS98), Gr-1 (RB6-8C5), F4/80 (A3-1), CD3 (17A2), CD4 (L3T4), CD24 (M1/69), CD64 (X54-5/7.1) CD26 (H194-112), CD172 (P84), EpCAM (G8.8), IL-17A (TC11-18H10.1), IFN- \Box (XMG1.2) and IRF4 (3E4) the corresponding isotype controls and the secondary reagents were purchased either from BD Biosciences (San Jose, USA), Ebiosciences (San Diego, USA) or Serotech (Raleigh, USA). For human tissue analysis the following antibodies were used CD3, CD7, CD19, CD20, CD56, CD2 (TS1/8), IFN- \Box (4S.B3), IL-17A (eBio64DEC17), CD45 (HI30), HLA-DR (Q10052), CD11c (B-ly6), CD14 (M5E2), CD16 (3G8), CD1c (AD5-8E7), CD1a (HI149), CD64 (10.1), CD11b (ICRF44), CD141 (AD5-14H12) CD172 (B4B6), BTLA (MIH26), CX3CR1 (2A9-1) and IRF4 (3E4).

Quantitative Real-Time PCR

Real-Time PCR was performed as described previously (Haniffa et al., 2012). Primer pairs used are described in Supplementary Table 4.

In Vivo Reconstitution Assays

 $FLT3^{-/-}$ C57BL/6 BM was provided by Dr. Ihor Lemischka (Mount Sinai School of Medicine, New York, USA). IRF4^{-/-} C57BL/6 bone marrow was provided by Dr. Axel Kallies (Walter and Eliza Hall Institute, Australia) and Dr Tak Mak (University of Toronto, Canada). *CSF-* $IR^{-/-}$ C57BL/6 fetal liver was provided by Dr. E. Richard Stanley (Albert Einstein College of Medicine, New York, USA). Recipient CD45.1⁺ C57BL/6 mice were lethally irradiated (2x 600 rad, 3 hr apart using a Cesium source) and reconstituted with a mixture of CD45.1⁺ WT BM and CD45.2⁺ BM or fetal liver cells isolated from WT or mutant mice. Engraftment was assessed by measuring the % of donor cells among blood Ly6C/G⁺ granulocytes 4 weeks after transplantation. Mice were analyzed two months post-transplant and the proportion of lung, LLN and splenic DC and MAC subset derived from WT (CD45.1) and KO (CD45.2) cells was determined. To enforce engraftment of the different donor BMs a 90:10 ratio was used for *FLT3^{-/-}* BM.

Nanostring analysis of mouse DC subsets

10000 FACS sorted small intestinal dendritic cells were lysed and analyzed using the nCounter GX mouse inflammation Kit and the nCounter system (Nanostring Technologies, Seattle, USA). Cut-off for non-specific probe binding was the mean of individual lanes negative probes supplied with the kit. Counts were normalized to the geometric means of the supplied positive controls and 5 housekeeping genes, as recommended by the manufacturer. Normalized counts from \log_{10} transformed mean derived from triplicate analysis are shown.

Mitotracker Red Staining

Purified mouse lung CD11b⁺ DCs were sorted as described before and were stained for 30min at 37°C with 12.5nM of Mitrotracker Red and counterstained with DAPI. Images were acquired on an Olympus FV-1000 confocal system.

OTII Proliferation Assay

DCs and macrophages were FACS-sorted from lungs of mice immunized intratrachealy with ova 18h before. OTII T cells were negatively selected from the spleens of OTII- $Rag1^{-/-}$ mice using the CD4⁺ T cell enrichment kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Enriched T cells were washed with PBS, and labeled with 1uM CFSE (Invitrogen). DCs and T-cells were cultured for 72h at a ratio of 1:20 and proliferation was analyzed using flow cytometry.

Cytospins and Scanning Electron Microscopy Imaging

For cytospin, purified cells were spun onto glass slides, dried overnight, stained in Field stain A and B and rinsed in distilled water. Images were analyzed using a Nikon Eclipse E800 microscope (Nikon, Japan) at 10×60 -fold magnification. Scanning electron microscopy was done as described previously (Haniffa et al., 2012).

Microarray Analysis

In order for cross-species comparison to be made, human orthologs of mouse signatures were first obtained via the R HomoVert package (ver. 0.4.1) (http://labs.fhcrc.org/fero/R/HomoVert.html), which uses a Homologene conversion table (Build 64) (http://www.ncbi.nlm.nih.gov/homologene). Enrichment of the human ortholog lung CD11b⁺ DC signature within the human blood CD1c⁺ DC signature was calculated

using a one-tailed hypergeometric test. The same enrichment test was carried out between the human ortholog lung CD11b⁺ MAC signature and the human blood CD14⁺ monocyte signature. Statistical significance was assessed on a p-value cutoff ($\alpha < 0.01$). Statistical Analyses were carried out using Bioconductor's "lumi" package via the R Programming Language (version 2.12.2) and Accelrys' Pipeline Pilot (www.accelrys.com). The microarray Expression Omnibus data are available in the Gene (GEO) database (http://www.ncbi.nlm.nih.gov/gds) under the accession number GSE46680.

Stimulation of Human DCs and Monocytes

FACS sorted PBMC derived CD1c⁺ DCs and CD14⁺ monocytes were cultured in 96-well bottomed plates. Cells were stimulated for 24 with *A. fumigatus* hyphae (ratio 1:10), CD40L (1µg/ml, dimerized, Alexis) + LPS (0.1mg/ml), Poly(I:C) (25µg/ml, InvivoGen) or a TLR7/8 agonist (1µg/ml, CL075 (InvivoGen)). Supernatants were collected 24h after stimulation and analyzed for the presence of IL-1 β , IL-6, IL-8, IL-10, IL-12p70. Cytokines were detected using BD Cytometric Bead arrays and analyzed with BD-FCAP Array software v1.0. IL-23p19 was measured by ELISA (Ebioscience).

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