

SUPPLEMENTAL MATERIAL

Desch et al., <http://www.jem.org/cgi/content/full/jem.20110538/DC1>

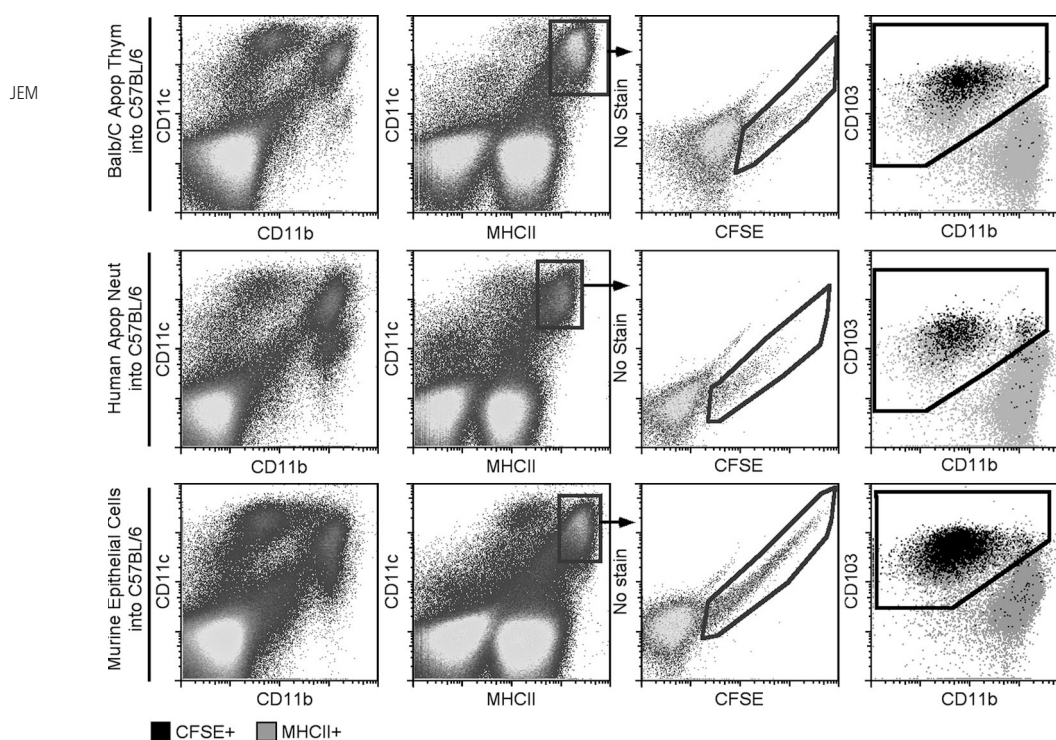


Figure S1. Gating strategy for CFSE⁺ DCs in the LLN 24 h after i.n. delivery of CFSE-labeled apoptotic BALB/c thymocytes, human neutrophils, or murine lung epithelial cells (MLE 12). (first column) Live cells from one LLN plotted as CD11c versus CD11b display heterogeneous DC populations. (second column) Live cells were plotted as CD11c versus MHCII, and the gate identifies all migratory DCs. To assess frequency of migratory DCs acquiring CFSE-labeled apoptotic cells, gated migratory DCs were plotted as an empty channel (no stain) versus CFSE, and then all CFSE⁺ cells were gated. LLN overlays of gated CFSE⁺ migratory DCs (black) over all migratory DCs (gray) were plotted as CD103 versus CD11b.

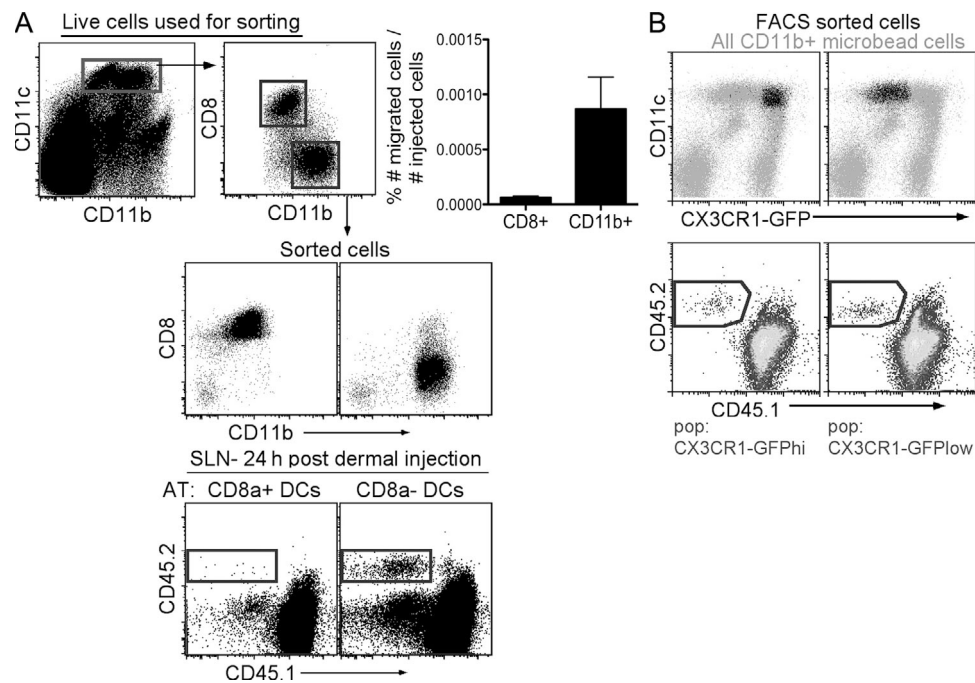


Figure S2. Splenic CD8⁺ DCs do not migrate down afferent lymphatics. (A) Sorted CD45.2 splenic DCs were adoptively transferred into the dermis of CD45.1 mice. 24 h after injection, skin-draining LNs (SLNs) were analyzed for migration. Bar graph demonstrates the percentage of cells that migrated to the LLN over the total number of cell injected. Error bars indicate SEM. (B) Splenic cells were positively selected for the expression of CD11b. CD11c⁺CD11b⁺ cells were then FACS sorted for high and low levels of CX3CR1 expression, using CX3CR1^{gfp/gfp} mice (top). Sorted CD45.2 splenic DCs were adoptively transferred into the back skin of CD45.1 mice. 24 h after injection, skin-draining LNs were analyzed for migration (bottom). Representative data of three (A) or two (B) independent experiments are shown.

Supplement Table I. Gene expression analysis (normalized signal intensity) using Affymetrix gene array of candidate PS receptors, bridging molecules, TLRs and chemokine receptors by pulmonary DCs, pulmonary macrophages, and splenic CD4 T cells.

Gene	Pulmonary CD103 ⁺ DC			Pulmonary CD11b ^{hi} DC			Pulmonary Macrophages			Splenic CD4 ⁺ T cell		
CD36	1790	1775	1645	410	208	832	1515	1786	2130	13	13	13
Clec9a	1568	1249	1193	20	24	30	37	35	68	14	15	12
DEC205	1021	1046	1360	107	130	156	2571	2286	2411	369	600	554
TIM-1	150	119	131	30	29	30	27	27	25	37	25	23
TIM-3	547	524	404	268	190	355	361	416	429	16	18	20
TIM-4	750	785	631	85	57	110	35	36	38	38	37	38
Mertk	65	102	130	68	57	79	2740	2524	2970	30	26	26
Annexin 1	2740	2930	2537	1979	1372	2133	3372	4216	4180	23	26	33
Annexin 5	2043	2198	2241	1872	1251	1497	3838	4232	3969	454	603	605
Tlr3	1932	1803	1643	87	71	106	88	94	93	32	31	27
CCR9	139	140	80	40	35	37	35	22	37	151	136	154
CXCR3	1434	1407	1098	132	111	92	139	95	91	776	713	826
XCR1	1806	1552	1351	44	50	42	43	46	48	51	52	47
Cadm1	567	600	485	117	113	101	70	62	68	73	69	65
Clec4a1	23	23	17	536	333	928	20	32	21	15	14	11
Tlr7	20	33	35	326	184	719	702	765	717	45	45	49
Tlr2	574	594	519	1188	671	919	3229	2824	3006	64	80	81

Supplement Table II. Validation of gene array through the confirmation of mRNA expression for known surface proteins, transcription factors, and growth factors associated with pulmonary DCs, splenic DCs, pulmonary macrophages and splenic CD4 T cells.

Gene	Pulmonary CD103 ⁺ DC			Pulmonary CD11b ^{hi} DC			Splenic CD8 ⁺ DC			Splenic CD8 ⁻ DC			Pulmonary Macrophages			Splenic CD4 ⁺ T cell		
CD11c	2521	2207	2472	2835	1915	2074	2245	2776	2912	4130	3448	4123	4364	4329	4184	36	52	42
Siglec F	185	237	391	125	143	117	91	78	72	75	90	101	2519	2212	2021	92	91	76
CCR7	731	578	1223	614	492	966	1223	1688	1903	1235	2071	452	98	69	98	1597	1377	1612
CD11b	67	66	71	2614	1850	2567	69	66	95	818	660	797	49	41	37	62	50	57
CX3CR1	96	111	125	1127	987	1499	256	174	563	364	265	162	90	77	93	96	114	90
CD103	2770	2626	2114	68	54	50	395	588	280	30	44	33	39	32	32	69	53	66
Langerin	500	488	350	33	40	37	166	131	139	35	40	42	38	37	38	42	37	28
CD8a	60	58	71	63	76	48	3869	5270	3657	61	60	60	49	55	51	70	57	47
CD4	50	55	65	64	98	51	114	155	224	1641	1363	1463	46	45	35	1974	2132	1982
H2-Aa	11157	10242	10708	10369	10153	10283	8571	8201	10407	11528	8149	9376	179	387	397	26	27	18
H2-Ab1	10543	10867	10932	12037	10150	9858	8695	9225	9977	11296	8455	10800	227	330	337	44	41	32
CD74	11503	11287	11931	12995	12008	10754	7461	9413	8894	9874	8042	11096	538	759	623	77	54	71
Batf3	309	390	317	205	226	157	429	349	394	384	357	368	110	118	112	99	94	67
Flt3	2559	2486	2402	1089	1030	895	3864	3605	4644	3008	2235	2439	58	47	51	53	66	38
Irf8	3481	3233	3253	308	336	389	3514	4402	3105	196	196	113	331	347	328	97	135	98
Id2	3753	3556	3124	1677	1207	1255	2305	2305	2646	1737	905	1471	706	849	773	394	454	465
MCSFR	193	215	252	2543	1616	2557	209	189	467	1134	956	425	783	893	806	54	47	39
Irf4	122	100	167	723	644	667	147	180	491	528	464	451	40	46	38	84	94	101