Immunity, Volume 53

Supplemental Information

Differential IRF8 Transcription Factor

Requirement Defines Two Pathways

of Dendritic Cell Development in Humans

Urszula Cytlak, Anastasia Resteu, Sarah Pagan, Kile Green, Paul Milne, Sheetal Maisuria, David McDonald, Gillian Hulme, Andrew Filby, Benjamin Carpenter, Rachel Queen, Sophie Hambleton, Rosie Hague, Hana Lango Allen, James E.D. Thaventhiran, Gina Doody, Matthew Collin, and Venetia Bigley

Table S1. DC2 and DC3 marker genes identified by Villani et al., (related to Figure S1)

Transcripts identified as DC2 and DC3 marker genes by Single Cell RNA-Seq in Villani et al., and present in our BM Single Cell RNA-Seq dataset, used to cluster BM DCs in Figure S1J,K

DC2 Genes	DC3 Genes			
SLC2A3	MGST1	IL1 RN		
FCGR2B	MTMR11	ASGR1		
PTGS1	VCAN	NLRP12		
CD33	SLC2A3	ADAM15		
AREG	RAB27A	S100A8		
CLEC4A	FCN1	MPP7		
CCR6	LAT2	HNMT		
CD2	LYZ	NR4A2		
MBOAT7	RETN	PID1		
CLEC10A	IL27RA	CD1D		
ENTPD1	RAB3D	LMNA		
ADAM8	BST1	ITGA5		
NR4A2	HBEGF	NLRP3		
PID1	CSF3R	S100A9		
CLIC2	PLBD1	S100A12		
CACNA2D3	CSTA	MNDA		
ETS2	NFE2	NOD2		
CD1D	F13A1	RNASE2		
CD1C	TREM1	PTAFR		
CD1E	EREG	CD14		
ITGA5	IL1B	FPR1		
PEA15	IL13RA1	CD163		
NOD2	CLEC10A	FCER1A		
PTAFR	MICAL2	TMEM173		
PER1	ANXA1	CES1		
FCER1A	CD36	NAIP		
CLEC17A	AOAH			

Table S2. CyTOF Panel (Related to Figures 1 and 5)

Antigens included in the CyTOF panel and the antibody metal conjugate. For antibody details see **Key Resources Table**

Antigen	Metal	Surface/intracellular
CD14	113In	S
CD370/CLECL9A	141Pr	S
AXL	142Md	S
CD123	143Nd	S
CD11b	144Nd	S
CD116	145Nd	S
SIGLEC6	146Nd	S
CD303	147Sm	S
SIRPA	148Nd	S
IRF8	149Sm	IC
FCER1	150Nd	S
CD2	141Eu	S
IRF4	152Sm	IC
CD45RA	153Eu	S
CD38	154Sm	S
CD36	155Gd	S
CD10	156Gd	S
Lineage-FITC (CD3, 19, 20, 56)	157Gd (secondary)	S
CD33	158Gd	S
CD11c	159Tb	S
ID2	160Gd	S
CD90	161Dy	S
SLAN-PE	162Dy (secondary)	S
BTLA	163Dy	S
CD15	164Dy	S
CD141	165Ho	S
CD34	166Er	S
CD115	167Er	S
CD100	168Er	S
CD304	169Tm	S
CD135	170Er	S
CD88	171Yb	S
CD117	172Yb	S
HLA=DR	173Yb	S
CD1c	174Yb	S
CD5	175Lu	S
CX3CR1-APC	176Yb	S
CD16	209Bi	S
CD45 (PBMC)	115In	S
CD45 (BM)	89Y	S
Cisplatin	195Pt	
DNA	191Ir	

Dataset		BM CD34+	BM CD34med	PB pre-DC	
Dataset		Progenitors	pre-DC and DC		
Figures		Figures 3E-J;	1H, S1J-L, S4E-K,	S4K-0	
inguico		S3D-H	S4H-J	34K Q	
	Total cells sequenced	399	260	184	
	ERCC % threshold (adjusted				
Raw	for concentration of ERCC	30%	70%	25%	
Data	spike added)				
Analysis	Mitochondrial % threshold	25%	15%	30%	
and QC	Total features threshold	>2500	>1700	>3000, <5000	
CELLS	Total counts threshold	>50000	>50000	>25000	
	Number of cells retained	262	244	116	
	after cell filtering	202	244	110	
	Number of genes expressed	10701	17050	14459	
Raw	at > 2 counts in > 2 cells	18/91	17850	14458	
Data	Number of genes retained				
Analysis	after removing cell cycle	18181	17237	13920	
and QC	genes				
GENES	Number of protein-coding	12406	12127	10246	
	genes retained	12400	12137	10540	
	Hierarchical Clustering	Figure 3F, S3H	Figure F, S4J	Figure S4N	
	Cells in analysis	262	244	116	
	Genes retained for analysis	6 8/6	6 838	7 265	
	by SC3	0,840	0,838	7,205	
	Clustering solutions	2.10	2.15	2.15	
	explored	2.10	2.13	2.15	
	Clustering solution	10	15	8	
	Average Silhouette width	0.45	0.58	0.27	
	Highest cluster stability	0.63	0.7	0.4	
Whole	index		-	0.05	
dataset	Marker gene p value	0.01	0.01		
(all cells,	threshold				
all	AUROC threshold	0.75	0.85	0.7	
genes)	tSNE	Figure 3E-G	Figure 4E-G, S4I	Figure S4O-P	
	Cells in analysis	262	244	116	
	Genes in analysis	6,846	6,838	7,265	
	PC for tSNE	10	20	5	
	Total variance explained by	25 35		26	
	PCs selected for tSNE				
	Perplexity setting for tSNE	13	15	20	
	Diffusion Map	Figure 3H-I	Figure 4H-I		
	Number of PC selected for	20	10		
	diffusion map		5 1 411		
		Figure 3D (GMP	Figure 1H		
	Hierarchical Clustering	only)	(mature DC only,		
Devetiel	Colle in analyzia	EO	an genes)		
Partial	Cens in analysis	58	ŏŏ		
		6,846	6,838		
subcote)	Clustering solutions				
5003013/	explored	2:15	2:15		
	Clustering solution	Δ	8		
1					
	Average Silhouette width	0.5	0.67		

Table S3. Single Cell analysis parameters (related to Figures 1, 3, 4, S3, S4)

	Highest cluster stability index	0.3	0.8	
	Marker gene p value threshold	0.1	0.01	
	AUROC threshold	0.75	0.85	
	tSNE	Figure S3E-G		
	Cells in analysis	58		
	Genes in analysis	6,846		
	PC for tSNE	5		
	Total variance explained by PCs selected for tSNE	30		
	Perplexity setting for tSNE	13		
	Hierarchical Clustering		Figure S1K (mature DC only, DC2/3 genes)	
	Cells in analysis		88	
Partial	Genes retained for analysis by SC3		61 out of 71 genes found in Villani et al.	
	Clustering solutions explored		2:15	
	Clustering solution		7	
	Average Silhouette width		0.53	
gene subsets)	Highest cluster stability index		0.75	
	Marker gene p value threshold		0.05	
	AUROC threshold		0.75	
	tSNE		Figure S1L (71 DC2/3 genes)	
	Cells in analysis		88	
	Genes in analysis		71	
	PC for tSNE		35	
	Perplexity setting for tSNE		15	

Table S4. Cell input/output and donor details for *in vitro* DC Culture experiments (related to figures 3, 4, S3, S4).

Figure	Input subset	Donor	Input Cell No	Output Cell No				
				Mono	CD14+	CD14-	cDC1	pDC
					DC3	DC2		•
CD34⁺		1	1000	243	393	7515	1690	408
Progenitor		1	3258	529	1614	5617	217	12
analysis		1	3258	554	1081	2037	275	9
3C, S3B		2	496	10	343	1332	44	24
		3	500	868	3520	1250	355	50
		3	3090	53	262	742	180	120
		3	3090	27	265	844	145	33
		3	3090	400	374	288	145	65
		4	2600	208	1588	8185	347	179
		4	1642	0	22	1256	125	138
		5	792	6	295	4664	712	106
	BUIK CD34+	6	800	94	328	1583	91	23
		7	6200	355	2250	2556	195	57
		8	3135	45	838	2783	624	50
		8	3135	53	367	1014	279	8
		8	3248	56	61	424	67	14
		9	3056	46	1477	4577	315	51
		10	1712	6	625	706	11	7
		10	2911	29	1574	2650	17	66
		10	2911	802	4996	1925	43	37
		11	848	0	10	120	10	6
		12	1343	5250	5734	1043	0	0
		13	446	22	1854	343	0	0
		1	3362	122	301	95	0	0
CMP GMP33+		1	1426	31	272	33	0	0
	CMP	2	500	12	0	0	0	0
	CIVIP	4	1558	0	0	1489	0	2
		5	2409	0	210	120	0	0
		14	3040	0	1	0	0	0
		14	1646	51	99	11	1	0
		1	2000	643	4369	11989	1511	51
		2	507	324	1570	397	2	0
	GMP33+	3	500	1538	4467	4346	633	226
		4	1168	142	1572	12966	24	20
		5	1670	144	1169	2211	271	52
		6	669	858	99	39	0	0
		6	70	140	20	0	0	0
		1	1500	126	605	3546	3589	827
		1	2957	150	223	1525	1277	757
	IMPP	2	502	238	618	6479	714	413
		3	428	99	298	7017	4556	3800
		4	215	0	6	9628	358	778
		5	1729	2	21	7262	793	993
		14	1708	7	105	2439	169	86

CD34** 3 558 37 511 3541 269 480 A 364 0 246 1148 495 59 10 502 0 27 30 36 33 11 1709 4 64 163 205 34 6MP123low 4 419 0 10 192 23 52 6MP123low 4 419 0 35 271 709 234 70 0 35 271 709 234 35 36 37 466 159 3 445 0 54 37 466 159 37 134 344 36 37 466 159 37 134 35 36 37 466 159 37 134 36 36 36 36 37 465 132 134 36 36 37 46 37 465			1	3042	268	750	456	451	178
GMP33- 4 364 0 246 1148 495 59 10 502 0 27 30 36 23 GMP123low 3 550 9 278 1502 102 23 52 5 491 1 199 363 145 40 7 700 0 35 271 709 234 1 700 0 35 271 709 234 1 700 0 35 271 709 234 1 2462 0 54 134 130 37 10 953 0 6 10 20 2 14 497 0 0 3 4 4 131 1313 256 75 131 158 303/dow 1 2132 0 12 202 75 191 700			3	558	37	511	3541	2649	480
CMP3- 5 848 22 194 2998 437 77 10 502 0 27 30 36 23 14 1709 4 64 163 205 34 GMP123low 3 550 9 278 1502 119 222 5 491 1 19 363 145 40 1 700 0 35 271 709 234 1 2462 0 54 37 466 159 3 445 0 52 65 394 39 4 252 0 9 191 45 134 5 152 0 34 138 190 37 10 953 0 6 102 202 75 191 123high 303/4low 1 2182 0 12 202 32 2 </th <th>4</th> <th>364</th> <th>0</th> <th>246</th> <th>1148</th> <th>495</th> <th>59</th>			4	364	0	246	1148	495	59
CD34med 10 502 0 27 30 36 23 GMP123low 3 550 9 278 1502 119 222 32 GMP123low 4 419 0 10 192 23 52 5 491 1 19 363 145 40 700 0 35 271 709 234 40 1 2462 0 54 37 466 159 3 445 0 52 65 394 39 4 252 0 34 138 190 37 10 953 0 6 10 20 2 12 1913 26 75 312 158 123high 33/4low 11212 0 0 7 40 30 8 1622 0 1 23 23 2 5		GMP33-	5	848	22	194	2998	437	77
CD34 ^{med} 14 1709 4 64 163 205 34 GMP123low 4 550 9 278 1502 119 222 5 491 1 19 363 145 40 6MP123med 1 2462 0 54 37 466 159 3 445 0 52 34 138 130 37 10 953 0 6 102 22 14 497 0 0 3 4 4 11 2182 0 12 202 75 191 12 218 12 12 12 12 12 12 15 112 12 12 12 12 12 12 12 14 14 14 12 12 12 12 12 12 12 12 12 12 12 12 12 12 12			10	502	0	27	30	36	23
GMP123low 3 550 9 278 1502 119 222 GMP123low 4 419 0 10 192 23 52 5 491 1 19 363 135 425 709 234 1 700 0 35 271 709 234 53 GMP123med 1 2462 0 54 37 466 159 3 445 0 52 65 344 33 130 4 252 0 9 191 45 134 55 152 0 34 138 190 37 10 953 0 6 10 202 75 191 Precursor 123high 1 2182 0 12 202 75 191 93/40m/ 15 1210 0 2 10 181 103			14	1709	4	64	163	205	34
GMP123low 4 419 0 10 192 23 52 5 491 1 19 363 145 40 5 491 1 19 363 145 40 6MP123med 1 2462 0 54 37 466 159 3 445 0 52 65 394 39 131 4 252 0 9 191 45 131 134 135 353 353 351 351 151 151 152 150 17 151 152 0 0 14 145 141 151 <th></th> <th></th> <th>3</th> <th>550</th> <th>9</th> <th>278</th> <th>1502</th> <th>119</th> <th>222</th>			3	550	9	278	1502	119	222
CD34med 5 491 1 19 363 145 40 GMP123med 1 700 0 35 271 709 234 6MP123med 1 2462 0 54 37 466 159 3 445 0 52 65 394 39 6MP123med 4 252 0 9 191 45 134 5 152 0 34 138 190 37 10 953 0 6 10 200 20 20 14 497 0 0 3 4 4 15 1913 26 75 312 158 700 9 11 2182 0 1 23 23 2 16 16 100 1 23 23 2 1 16 101 10 15 15 10 13 16		GMP123low	4	419	0	10	192	23	52
CD34med 1 700 0 35 271 709 234 GMP123med 1 2462 0 54 37 466 159 3 445 0 52 65 394 394 5 152 0 34 138 190 37 10 953 0 6 10 20 2 14 497 0 0 3 4 4 15 1913 26 75 312 158 709 234 11 2182 0 12 202 75 191 Precursor 11 1282 0 1 23 23 2 15 123high 1 1282 0 1 23 23 2 15 1229 0 0 2 10 181 16 1005 0 1 2 4 84 <th></th> <th></th> <th>5</th> <th>491</th> <th>1</th> <th>19</th> <th>363</th> <th>145</th> <th>40</th>			5	491	1	19	363	145	40
CD34med 1 2462 0 54 37 466 159 3 445 0 52 65 394 39 4 252 0 9 191 45 134 5 152 0 34 138 190 37 10 953 0 6 100 20 2 14 497 0 0 3 4 4 11 2182 0 12 202 75 191 11 2182 0 10 73 35 303/4low 8 1622 0 1 22 5 50 17 1126 0 2 11 1 57 50 17 1126 0 1 2 4 84 10 17 536 0 0 1 10 130 17 536 0			1	700	0	35	271	709	234
GMP123med 3 445 0 52 65 394 39 4 252 0 9 191 45 134 5 152 0 34 138 190 37 10 953 0 6 100 20 2 14 497 0 0 3 4 4 15 1913 26 75 312 158 1 2182 0 12 202 75 191 1 2182 0 1 23 23 2 16 1521 0 0 7 40 30 303/4low 8 1622 0 1 1 5 50 17 136 0 0 1 1 1 5 16 1005 0 1 2 4 84 17 536 0 0			1	2462	0	54	37	466	159
GMP123med 4 252 0 9 191 45 134 5 152 0 34 138 190 37 10 953 0 6 10 20 2 14 497 0 0 3 4 4 15 1913 26 75 312 158 7 123high 1 2182 0 0 7 40 30 8 1622 0 0 7 40 30 35 8 1622 0 1 23 23 2 11 1 57 16 1521 0 0 2 10 181 10 30 17 135 129 0 0 2 10 181 16 1005 0 1 6 1 103 56 15 144 0 0			3	445	0	52	65	394	39
CD34med Precursor analysis 4B, S4C 5 152 0 34 138 190 37 10 953 0 6 10 20 2 14 497 0 0 3 4 4 15 1913 26 75 312 158 2034med Precursor analysis 123high 303/4low 1622 0 1 23 23 2 16 1521 0 0 7 40 30 8 1622 0 1 23 23 2 16 1521 0 0 2 5 50 17 1126 0 2 10 181 103 CD2+ pre-pC2 16 1005 0 1 2 4 84 CD123high CD5+ early pre-DC2 17 536 0 0 10 0 0 0 CD123high CD34med SIRPA- 138 144 0		GMP123med	4	252	0	9	191	45	134
CD34med Precursor analysis 4B, S4C 10 953 0 6 100 200 2 14 497 0 0 3 4 4 15 1913 266 75 312 158 Precursor analysis 4B, S4C 1 2182 0 12 202 75 191 123high 303/4low 1 2182 0 0 7 400 30 8 1622 0 0 7 400 30 8 1622 0 0 2 5 50 16 1521 0 0 2 10 181 16 1005 0 1 0 5 50 17 536 0 0 1 0 0 44 14 434 0 0 10 0 0 19 5444 0 14 65 4 1 <			5	152	0	34	138	190	37
CD34med Precursor analysis 4B, 54C 123high 303/4low 121212 1 121212 1 122 1 122 1 122 1 123high 3 123high 303/4low 123high 303/4low 123high 303/4low 121212 1 0 12 202 75 191 1 123high 303/4low 121212 1 0 0 7 40 30 11 12182 0 0 0 7 40 30 11 12122 0 0 0 7 40 30 11 12126 0 0 2 11 1 57 11 1229 0 0 0 2 10 181 11 125 12 0 0 1 0 55 17 136 0 0 1 0 55 17 536 0 0 1 0 0 11 434 0 11 65 4 1 1 11 68 <			10	953	0	6	10	20	2
CD34med Precursor analysis 48,54C 123high 1 2182 0 12 202 75 191 1 2182 0 5 150 73 35 analysis analysis 48,54C 12 202 75 191 1 2182 0 5 150 73 35 8 1622 0 0 7 40 30 8 1622 0 1 23 23 2 16 1521 0 0 2 5 50 17 1126 0 2 10 181 16 1005 0 1 6 1 103 17 536 0 0 1 0 55 17 536 0 0 10 0 1 18 444 0 14 65 4 1 17 536 0 0 10 0 <th></th> <th></th> <th>14</th> <th>497</th> <th>0</th> <th>0</th> <th>3</th> <th>4</th> <th>4</th>			14	497	0	0	3	4	4
CD34med Precursor analysis 4B, S4C 123high 123high 303/4low 12122 12 120 75 191 1 2182 0 5 150 73 35 4B, S4C 12 202 75 191 1 2182 0 5 150 73 35 8 1622 0 0 7 40 30 10 1126 0 2 5 50 0 11 1126 0 2 10 181 11 51229 0 0 2 10 181 15 1229 0 0 2 10 181 16 1005 0 1 6 1 103 17 536 0 0 0 0 0 17 536 0 0 10 0 0 17 536 0 0 10 0 0			15	1913	26	75		312	158
Precursor analysis 4B, S4C 123high 303/4low 12122 0 0 7 40 30 123high 303/4low 1621 0 0 7 40 30 8 1622 0 1 23 23 2 2 16 1521 0 0 2 5 50 17 1126 0 2 11 1 57 CD2+ pre-pDC 16 1005 0 1 2 4 84 17 536 0 0 1 0 55 17 536 0 0 10 0 0 CD123high CD5+ early pre-DC2 5 444 0 14 65 4 1 CD123med CD5+ pre-DC2 548 1 113 108 0 3 CD123med CD5+ pre-DC2 548 0 16 64 159 0 CD123med SIRPA+ 19 170 0	CD34 ^{med}	1	1	2182	0	12	202	75	191
analysis 48, 54C 123high 303/4low 1622 0 0 7 40 30 16 1521 0 0 2 5 50 17 1126 0 2 11 1 57 16 1521 0 0 2 5 50 17 1126 0 2 11 1 57 16 1005 0 1 2 4 84 16 1005 0 1 6 1 103 17 536 0 0 1 0 55 17 536 0 0 10 0 0 CD123high 1 434 0 0 10 0 0 CD123high 1 444 0 14 65 4 1 pre-DC2 8 469 0 5 44 0 0 CD123med	Precursor		1	2182	0	5	150	73	35
48, 54C 303/4low 302 102 0 1 100 100 16 1521 0 0 2 5 50 17 1126 0 2 11 1 57 CD2+ 15 1229 0 0 2 10 181 CD2+ 16 1005 0 1 2 4 84 16 1005 0 1 2 4 84 pre-pDC 16 1005 0 1 0 0 17 536 0 0 1 0 0 CD123high 1 434 0 0 10 0 CD123med 5 444 0 14 65 4 1 pre-DC2 8 469 0 5 41 0 0 CD123med 548 1 113 108 0 0 0	analysis	123high	8	1622	0	0	7	40	30
Image: constraint of the second sec	4B, S4C	303/4low	8	1622	0	1	23	23	2
ID ID <thid< th=""> ID ID ID<!--</th--><th></th><th></th><th>16</th><th>1521</th><th>0</th><th>0</th><th>25</th><th>5</th><th>50</th></thid<>			16	1521	0	0	25	5	50
CD2+ pre-pDC 15 1229 0 0 2 10 181 CD2+ pre-pDC 16 1005 0 1 2 4 84 pre-pDC 16 1005 0 1 6 1 103 17 536 0 0 1 0 55 17 536 0 0 10 0 49 CD123high 1 434 0 0 10 0 0 CD5+ early 5 444 0 14 65 4 1 pre-DC2 8 469 0 5 41 0 0 CD123med 3 548 1 113 108 0 3 CD24med 3 548 1 113 108 0 3 CD34med 1596 0 36 54 156 8 SIRPA- 19 1701 0			10	1126	0	2	11	1	57
CD2+ pre-pDC 16 1005 0 1 2 4 84 pre-pDC 16 1005 0 1 6 1 103 17 536 0 0 1 0 55 17 536 0 0 10 0 49 CD123high CD5+ early pre-DC2 1 434 0 0 10 0 0 8 1194 0 4 97 1 3 CD123med CD5+ early pre-DC2 3 548 1 113 108 0 3 CD123med CD5+ 4 717 0 0 11 2 1 pre-DC2 8 679 0 2 34 0 0 CD34med CD123med SIRPA- 1 683 0 16 64 159 0 CD34med SIRPA+ 3 3818 16 109 39 18 0 0 0 0 <th></th> <th></th> <th>15</th> <th>1229</th> <th>0</th> <th>0</th> <th>2</th> <th>10</th> <th>181</th>			15	1229	0	0	2	10	181
CD2+ 10 1005 0 1 6 1 103 17 536 0 0 1 6 1 103 17 536 0 0 1 0 55 17 536 0 0 10 0 49 CD123high 1 434 0 0 10 0 0 CD5+ early 5 444 0 14 65 4 1 pre-DC2 8 469 0 5 41 0 0 CD123med 3 548 1 113 108 0 3 CD5+ 4 717 0 0 11 2 1 pre-DC2 8 679 0 2 34 0 0 CD34med 1 683 0 16 64 159 0 CD34med SIRPA- 19 1701		CD3+	16	1005	0	1	2	4	84
Inc pice 10 100 100 1 10 100 17 536 0 0 1 0 55 17 536 0 0 0 0 49 CD123high CD5+ early pre-DC2 1 434 0 0 10 0 0 8 1194 0 4 97 1 3 3 CD123med CD5+ pre-DC2 3 548 1 113 108 0 3 CD123med CD5+ pre-DC2 3 548 1 113 108 0 3 CD34med CD123med SIRPA- 1 683 0 16 64 159 0 18 630 0 0 16 29 3 144 194 10 2703 59 295 29 0 0 0 CD34med SIRPA+ 3 3818 16 109 39 18 0 CD34m		nre-nDC	16	1005	0	1	6	1	103
II JJO JO II IO JJO 17 536 0 0 0 0 49 CD123high CD5+ early pre-DC2 1 434 0 0 10 0 0 8 469 0 5 41 0 0 0 0 8 1194 0 4 97 1 3 CD123med CD5+ pre-DC2 3 548 1 113 108 0 3 CD123med CD5+ pre-DC2 3 548 1 113 108 0 3 CD34med CD123med SIRPA- 1 683 0 16 64 159 0 18 630 0 0 16 29 3 3818 16 109 39 18 0 CD34med SIRPA+ 3 3818 16 109 39 18 0 0 0 0 0 0 0 0 <t< th=""><th></th><th>pre-poc</th><th>17</th><th>536</th><th>0</th><th>0</th><th>1</th><th>0</th><th>55</th></t<>		pre-poc	17	536	0	0	1	0	55
CD123high CD5+ early pre-DC2 1 434 0 0 10 0 0 CD123high CD5+ early pre-DC2 5 444 0 14 65 4 1 CD123med CD5+ pre-DC2 8 469 0 5 41 0 0 CD123med CD5+ pre-DC2 3 548 1 113 108 0 3 CD123med CD34med CD123med SIRPA- 3 548 1 113 108 0 0 CD34med CD123med SIRPA- 1 683 0 16 64 159 0 CD34med SIRPA- 18 630 0 0 16 29 3 19 1701 0 13 54 144 194 3 3818 16 109 39 18 0 CD34med SIRPA+ 3 38040 15 27 0 0 0 20 869 18 2557 118 8 <t< th=""><th></th><th></th><th>17</th><th>536</th><th>0</th><th>0</th><th>0</th><th>0</th><th>49</th></t<>			17	536	0	0	0	0	49
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		CD122bigb	1	434	0	0	10	0	
CD31 Carly 3 444 0 14 03 4 1 pre-DC2 8 469 0 5 41 0 0 8 1194 0 4 97 1 3 CD123med CD5+ 3 548 1 113 108 0 3 CD4 4 717 0 0 11 2 1 pre-DC2 8 679 0 2 34 0 0 8 483 0 0 4 0 0 8 483 0 0 4 0 0 CD34med SIRPA- 1 683 0 0 16 64 159 0 CD34med SIRPA+ 19 1701 0 13 54 144 194 M 2703 59 295 29 0 0 SIRPA+CD2+ 4 329 0 2		CD125High	5		0	14	65	4	1
Image: biologic		pre-DC2	8	469	0	5	41	0	0
CD123med CD5+ pre-DC2 3 548 1 113 108 0 3 CD34med CD123med CD123med CD123med SIRPA- 4 717 0 0 11 2 1 CD34med CD123med SIRPA- 683 0 0 4 0 0 1 683 0 16 644 159 0 CD34med CD123med SIRPA- 1 683 0 16 644 159 0 3 3818 16 109 39 18 0 <th></th> <th>pre Dez</th> <th>8</th> <th>110/</th> <th>0</th> <th>1</th> <th>97</th> <th>1</th> <th>3</th>		pre Dez	8	110/	0	1	97	1	3
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		CD122mod	3	5/18	1	113	108	0	3
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$				717	0	0	100	2	1
$\frac{ \mathbf{r} ^2 \mathbf{r} ^2}{ \mathbf{r} ^2 \mathbf{r} ^2} = \frac{ \mathbf{r} ^2}{ \mathbf{r} ^2} = \frac{ \mathbf{r} ^2}{ \mathbf{r} ^2 \mathbf{r} ^2} = \frac{ \mathbf{r} ^2}{ \mathbf$		DC2	4 Q	670	0	2	24	2	0
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		pre-bez	0 Q	/83	0	0	 /	0	0
CD34med CD123med SIRPA- 1 083 0 10 04 139 0 18 630 0 36 54 156 8 19 1701 0 13 54 144 194 CD34med SIRPA+ 3 3818 16 109 39 18 0 CD34med SIRPA+ 8 3040 15 27 0 0 0 10 2703 59 295 29 0 0 15 1802 0 24 30 0 0 20 869 18 2557 118 8 0 3 4045 2 6 1 0 0 3 4045 2 6 1 0 0 10 2724 6 6 1 0 0 10 2724 6 6 1 0 0 SIRPA+CD2- pre-mono </th <th></th> <th></th> <th>0</th> <th>602</th> <th>0</th> <th>16</th> <th>4 64</th> <th>150</th> <th>0</th>			0	602	0	16	4 64	150	0
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		CD34med	<u>۲</u>	1506	0	36	5/1	156	Q Q
SIRPA- 18 0.50 0 0 10 123 3 19 1701 0 13 54 144 194 A 3818 16 109 39 18 0 CD34med 8 3040 15 27 0 0 0 SIRPA+ 10 2703 59 295 29 0 0 15 1802 0 24 30 0 0 20 869 18 2557 118 8 0 SIRPA+CD2+ 4 329 0 21 17 0 0 5 2449 18 37 13 0 0 10 2724 6 6 1 0 0 SIRPA+CD2- 7 3 4942 14 5 0 0 SIRPA+CD2- 10 3176 4 3 1 0 0 <th></th> <th>CD123med</th> <th>18</th> <th>630</th> <th>0</th> <th>0</th> <th>16</th> <th>20</th> <th>2</th>		CD123med	18	630	0	0	16	20	2
CD34med SIRPA+ 3 3818 16 109 39 18 0 10 2703 59 295 29 0 0 10 2703 59 295 29 0 0 15 1802 0 24 30 0 0 20 869 18 2557 118 8 0 SIRPA+CD2+ pre-DC3 3 4045 2 6 1 0 0 SIRPA+CD2+ pre-DC3 3 4045 2 6 1 0 0 SIRPA+CD2+ pre-DC3 3 4942 14 5 0 0 0 SIRPA+CD2- pre-mono 3 4942 14 5 0 0 0 SIRPA+CD2- pre-mono 3 4942 14 5 0 0 0 10 3176 4 3 1 0 0 0 20 1419 6		SIRPA-	10	1701	0	12	5/	23 1//	10/
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$			3	3818	16	109	30	19	194
SIRPA+ 10 2703 59 295 29 0 0 15 1802 0 24 30 0 0 20 869 18 2557 118 8 0 20 869 18 2557 118 8 0 SIRPA+CD2+ pre-DC3 3 4045 2 6 1 0 0 5 2449 18 37 13 0 0 10 2724 6 6 1 0 0 SIRPA+CD2- pre-mono 3 4942 14 5 0 0 0 10 3176 4 3 1 0 0 0 15 4966 1 0 0 0 0 0		(D24mod	2 8	3010	15	27	0	10	0
SIRPA+CD2+ pre-DC3 10 10 10 210 230 230 230 0 0 SIRPA+CD2+ pre-DC3 3 4045 2 6 1 0 0 SIRPA+CD2+ pre-DC3 3 4045 2 6 1 0 0 SIRPA+CD2+ pre-DC3 5 2449 18 37 13 0 0 SIRPA+CD2- pre-mono 3 4942 14 5 0 0 0 SIRPA+CD2- pre-mono 3 4942 14 5 0 0 0 3 4942 14 5 0 0 0 0 SIRPA+CD2- pre-mono 15 4966 1 0 0 0 0		SIRPA+	10	2702	59	27	29	0	0
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$		JINF AT	10	1200	0	295	20	0	0
$\frac{10}{10} = \frac{10}{10} = 10$			20	1002	1Q	24	110	Q	0
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$			20	1015 1015	20	2337 6	110	0	0
pre-DC3 5 2449 18 37 13 0 0 10 2724 6 6 1 0 0 SIRPA+CD2- pre-mono 3 4942 14 5 0 0 0 10 3176 4 3 1 0 0 15 4966 1 0 0 0 0		SIRPA+CD2+	4	329		21	17	0	0
5 2449 18 37 13 0 0 10 2724 6 6 1 0 0 3 4942 14 5 0 0 0 SIRPA+CD2- pre-mono 10 3176 4 3 1 0 0 15 4966 1 0 0 0 0 0		pre-DC3	-	325		~~			
IO 2724 6 6 1 0 0 SIRPA+CD2- pre-mono 3 4942 14 5 0 0 0 10 3176 4 3 1 0 0 15 4966 1 0 0 0 0 20 1410 6 210 53 1 0 0			5	2449	18	37	13	0	0
SIRPA+CD2- pre-mono 3 4942 14 5 0 0 0 10 3176 4 3 1 0 0 15 4966 1 0 0 0 0 20 1410 6 210 53 1 0 0			10	2724	b 14	<u>ь</u>		0	0
pre-mono 15 4966 1 0 0 0 0 0 0 0		SIRPA+CD2-	3 10	4942	14	2	1	0	0
		pre-mono	10	7066	4			0	0
			20	1419	6	810	53	1	0

Figure S1, related to Figure 1



Figure S1: CD1c+DC heterogeneity is evident in human bone marrow

A. Upstream gating steps for the flow cytometric analysis of HC PB monocyte and DC subsets identified PBMC by light scatter properties, excluded doublets, dead and CD45- cells before lin(CD3, CD16, CD19, CD20, CD56)-HLA-DR+ cells were selected for further analysis in **Figure 1A**. Representative example of n=22

B. Histogram of CD14 expression on CD5+BTLA+CD1c+DCs (red), CD14+CD1c+DCs (orange) and CD14+CD88+ monocytes (black), by flow cytometric analysis

C. Correlation of expression fold change of differentially expressed genes by NanoString analysis with the fold change of differentially expressed genes in single cell RNA-Seq analysis described by Villani et al., comparing BTLA+CD1c+DCs (red) (mean of n=3) and BTLA-CD1c+DCs (orange) (mean of n=3) with DC2 and DC3 described by Villani et al. 2017.

D. Correlation of expression fold change of differentially expressed genes by NanoString analysis with the fold change of differentially expressed genes in single cell RNA-Seq analysis described by Villani et al., comparing BTLA-CD1c+DCs(orange) (mean of n=3) and monocytes (black) (mean of n=3) with DC3 and monocytes described by Villani et al. 2017.

E. Intracellular flow cytometric analysis of *in vitro* cytokine elaboration by PB monocytes (black), CD14+CD1c+DC (orange), CD14-CD5-CD1c+DC (gray) and CD5+CD1c+DC (red) from n=9 healthy donors in response to 14hrs stimulation with TLR agonists (CpG, poly(I:C), CL075, LPS). Integrated median fluorescence intensity (iMFI) was calculated by multiplying the frequency of positive cells by the MFI of a given marker. P values were derived from paired two-tailed t-tests (* p<0.05; **p<0.01; ***p<0.005). Bars show mean±SEM and circles represent individual donors.

F. Relative proportions of CD5+CD1c+DCs (red), CD14+CD1c+DCs (orange) and CD14-CD5-CD1c+DCs (gray) in HC PB (n=22) and BM (n=13) expressed as a percentage of the total CD1c+DC (gated as shown in **Figure 1A**). * p=0.046 (Mann Whitney U, two-tailed). Bars show mean±SD and circles represent individual donors.

G. Upstream gating steps for the flow cytometric analysis of HC spleen, skin (dermis) and BM monocyte and DC subsets identified mononuclear cells by light scatter properties, excluded doublets, dead and CD45- cells before lin(CD3, CD16, CD19, CD20, CD56)-HLA-DR+ cells were selected for further analysis in **Figure 1E** (representative example of n=3 for each tissue) and **F** (representative example of n=13)

H. Histograms of BTLA expression on cDC1, CD5+CD1c+DC and pDC from PB, spleen, BM and dermis (pDC absent), by flow cytometric analysis.

I. tSNE visualization of the expression of TFs IRF4 and IRF8 and surface markers across HC PB and BM lin(CD3,19,20,56,161)-HLA-DR+ cells by simultaneous CyTOF analysis as in **Figure 1G**. Heat map shows SIGLEC6 expression.

J. Flow cytometric identification of index sorted human BM DC and monocytes for single cell transcriptomic analysis in **Figure 1H** and **S1K,L**.

K. Hierarchical clustering of single cell transcriptomes of mature DC from BM using 71 DC2 and DC3 marker genes identified in Villani et al. to define 7 clusters used to annotate **Figure S1L** (in SC3 software p<0.05, AUROC>0.75). Heatmap shows log2 expression. Genes enriched in DC2 or DC3 are shown in red and orange type, respectively. The top rows display fluorescence intensity of surface antigens ('Antigens') from index sorted cells.

L. tSNE plots of the first 35 principal components of the DC2 and DC3 single cell transcriptome dataset (as described in J,K) of index sorted pDC, cDC1, CD1c+DC and monocytes. The large panel depicts cells annotated by 7 clusters generated in K. Smaller panels show the expression of key subset-specific antigens taken from indexed flow cytometry.

M. Schematic of the phenotypic definition of DC2 and DC3 in PB and tissues; CD163-CD5+/-(BTLA+ in PB) DC2 and CD163+CD14+/-(BTLA- in PB) DC3.

Figure S2, related to Figure 2

ç

IL-1β

IL-10

IL-12

0

IL-8

TNF





Figure S2: CD14 expression distinguishes between CD1c+DC subsets generated *in vitro*

A. Upstream gating steps for the flow cytometric analysis of *in vitro* derived DC and monocyte subsets. Gates identified live, singlet, cells and excluded CD15+ neutrophils and CD34+progenitors before DC and monocyte identification as shown in **Figure 2A**.

B. Upstream gating steps for the analysis of FACS-purified CD1c+DC subsets after 7 days of culture (**Figure 2E,F**). A representative example of the upstream gating from the culture output of CD14-BTLA-DC3 (n=7).

C. Full gating strategy for FACS-purification of *in vitro* generated DC and monocyte subsets derived from BM CD34+ progenitors after 21days in culture, for NanoString gene expression analysis (**Figure 2G,H**) and TLR elaboration (**Figure 2I**).

D, E. Correlation of expression fold change of differentially expressed genes by NanoString analysis with the fold change of differentially expressed genes in single cell RNA-Seq described by Villani et al. (in a similar analysis to **Figure S1**), comparing D) DC2 (red) and DC3 (orange) or E) DC3 and monocytes (black). PB DC (BTLA+CD1c+DC2 and BTLA-CD1c+DC3) and their culture-derived counterparts (CD14-CD1c+DC2 and CD14+CD1c+DC3, outlined dots) are shown. Fold change derived from mean expression of n=3 for PB and n=3 for culture-derived cells.

F. Intracellular flow cytometric analysis of *in vitro* cytokine elaboration by CD14+1cmonocytes (black), CD14+CD1c+DC3 (orange) and CD14-CD1c+DC2 (red) generated from n=4 BM donors after 21 days in culture, in response to TLR agonists (CpG, poly(I:C), CL075, LPS). Integrated median fluorescence intensity (iMFI) was calculated by multiplying the frequency of positive cells by the MFI of a given marker. P values were derived from paired two-tailed t-tests (* p<0.05). Bars show mean±SEM and circles represent individual donors.



Figure S3. High IRF8 expression defines LMPP-associated DC progenitors

A. Median Fluorescence intensity of Intracellular IRF8 by flow analysis across gates identifying CD34+progenitor populations of HC BM (n=4). HSC, hematopoietic stem cell; MPP, multipotent progenitor; MEP, megakaryocyte erythroid progenitor; MLP, multilymphoid progenitor; LMPP, lymphoid primed multipotent progenitor; CMP, common myeloid progenitor; GMP, granulocyte macrophage progenitor. Bars represent mean±SD.

B. Flow analysis of CD34+CD38+CD10- progenitor populations (as shown in **Figure 3A**) showing the relative levels of CD123 expression across CD33+CD123_{neg-lo} (predicted CMP) and CD33-CD123- (predicted MEP) CD45RA- populations and CD45RA+GMP fractions.

C. Absolute proliferative capacity and differentiation potential of progenitor fractions subjected to 14 days in DC differentiation culture, as depicted in **Figure 3C**, expressed as the number of cells generated per input CD34+ cell. CD15+ neutrophils are included. Bars show mean±SEM, circles represent individual donors.

D. The relative output (ratio) of DC2 and DC3 from the specified CD34+progenitor fractions. Orange bars DC2<DC3; red bars DC2>DC3.

E. Kinetics of DC, monocyte and neutrophil output from progenitor fractions over 14-21 days in DC differentiation culture. CD34+ n=3 (n=2-3/timepoint), CMP n=1, GMP33+ n=3 (n=1-3/timepoint), LMPP n=3 (n=2-3/timepoint), GMP33- n=3 (n=1-2/timepoint), GMP123_{lo} n=2 (n=1-2/timepoint), GMP123_{int} n=3 (n=1-3/timepoint). Dots and bars represent mean–SEM.

F. Flow identification of index sorted human BM progenitors for single cell transcriptomic analysis in **Figures 3D-J and S3G-J.** A tight lin(CD3, 7, 14, 16, 19, 20)-gate was used to select CD34+ progenitors for single cell sorting. This excluded lineage₁₀ CD10+B/NK progenitors, as defined in **Figure 3A**, shown backgated (red dots) onto the pre-sort BM live, singlet, mononuclear cells (gray). Top middle panel shows the same Lin-34+ gate on sorted single cells. Top right panel demonstrates the absence of CD10+B/NK cells in the sorted CD38+CD45RA+ population.

G-I. tSNE visualization of the first 5 principal components (30% total variance) of the transcriptomes of 58 single GMP, analyzed independently of surface phenotype. tSNE plots are annotated by **G**, gate of origin from index-linked flow (**Figure S3F**) or **H**, 4 hierarchical clusters (**Figure 3D**) showing clustering of cells from the CD33-, CD123_{lo}, and CD123_{int} gates (cluster 4) away from clusters 1-3 containing GMP33+ cells. Heatmaps (I) show log2 expression of key DC TF genes on tSNE plot in **G** and **H**.

J. Unsupervised hierarchical clustering of single cell transcriptomes of all progenitor cells, using all protein-coding, non-cell cycle genes (independent of surface antigen expression). Marker genes for 10 clusters generated within SC3 (p<0.01, AUROC>0.75) are displayed, examples of which identify cluster 1, monocyte (*LYZ*, *CSF1R*); cluster 2, granulocyte (*CALR*, *ELANE*), clusters 3-5, HSC and MPP (*AVP*, *PCDH9*), cluster 6, MEP (*GATA1*, *KLF1*), cluster 8, DC (*IRF8*, *TCF4*, *RUNX*), clusters 9-10, LMPP (*SMIM24*). Heatmap shows log2 gene expression. The top rows display fluorescence intensity of surface antigens ('Surface Antigens') from index sorted cells, 'Flow annotation (Flow annot)' denotes the classification of index sorted cells by their surface phenotype (**Figure S3F**).



Figure S4, related to Figure 4

Figure S4. Two trajectories of DC development connect the progenitor compartment with mature DC

A. Heatmaps showing IRF8 expression by intracellular flow cytometric analysis across sequential bivariate plots, using the gating strategy to identify DC and pre-DC shown in **Figure 4A**. Heatmaps generated in FlowJo 10.6.1.

B. Absolute proliferative capacity and differentiation potential of CD34_{int} precursor fractions subjected to 14 days in DC differentiation culture, as depicted in **Figure 4C**, expressed as the number of cells generated per input precursor cell. CD15₊ neutrophils are included. Bars show mean±SEM, circles represent individual donors.

C. The relative output (ratio) of DC2 versus DC3 (left graph) or Monocyte versus DC3 (right graph) from the specified CD34_{int} precursor fractions. Red bars DC2>DC3; orange bars DC3>DC2, black bars with orange outline DC3>monocyte.

D. Mean fluorescence intensity (MFI) of CD34 by flow cytometric analysis of CD2+ and CD2- pDC from BMMC (n=3) and PBMC (n=3). Plots show mean±SEM.

E. CFSE dilution in CD2+ and CD2- pDC from BM and PB after 3 days in DC culture, to assess proliferation. Histograms show CD34+ progenitors and monocytes as controls. Plots shown are representative of n=3 experiments in PB and BM.

F. Kinetics of the output generated from 14 days culture of CD2+ pDC, expressed as the number of cells generated per input cell. n=2 donors with n=2 analyses at each time point. Dots and bars show mean+SEM.

G. Flow cytometric analysis of Lin-HLA-DR+CD1c-CD141-CD123hi PBMC and BMMC showing the location of AXL+CD5+CD123+CD11c- early pre-DC2 (pink) on the bivariate plot of CD2 versus CD303/4, overlying the CD34int CD123hiCD303/4lo population (turquoise).

H. Summary of the proliferative capacity of BM DC precursors. BMMCs were CFSE stained then precursors FACS-purified and cultured in DC-culture conditions for 3 days before flow cytometric analysis of CFSE. Bulk CD34+ progenitors and CD14+ monocytes were included as positive and negative controls, respectively. Bars represent mean±SD of n=3-4 donors, circles represent individual donors.

I. Location, in flow cytometry parameter space, of index-sorted precursor cells purified for scRNA-Seq depicted in **Figure 4E-J**, **S4J-K**).

J. DC subset signature scores generated from Villani et al, mapped across the tSNE plot of precursor and mature DC single cell transcriptomes (**Figure 4E-J**). To generate signature scores, the dataset was mined for expression of cell subset 'signature' genes defined by single cell RNA-Seq in Villani et al., including DC1 (cDC1), DC2 (cDC2a), DC3 (cDC2b), DC5 (AXL+), DC6 (pDC) and monocytes. Normalized counts were then rescaled from 0 to 1 and the average scaled score displayed on t-SNE plots generated as described in the STAR methods. The left panel shows the level of expression of DC subset signature scores. The right panel shows the mapping across tSNE space of similar scores generated from differentially expressed genes after pairwise comparisons of subsets, as indicated.

K. Unsupervised hierarchical clustering of single cell transcriptomes of BM precursor and mature DC subsets and CD14+ monocytes, using all protein-coding, non-cell cycle genes (independent of surface antigen expression). Marker genes for 15 clusters generated within SC3 (p<0.01, AUROC>0.85) are shown. Examples used to identify clusters are marked in red and derived from subset signature genes in Villani et al.. Clusters were used to annotate **Figure 4F, H**. The top rows display fluorescence intensity of surface antigens ('Surface Antigens') from index sorted cells. 'Flow Annotation' denotes the classification of index sorted cells by their surface phenotype (in **Figure S4I**), Cluster color code related to **Figure 4F,H**.

L. The output of *in vitro* culture of DC precursors from PB, FACS-purified using the gating strategy shown in **Figure 4C** showing lineage-specific enrichment from precursors, analogous to the output from phenotypically similar BM precursors. Output is expressed as the proportion of each DC and monocyte population as a percentage of the total cells captured by all DC and monocyte gates. n=3-5 donors for each population. Bars represent mean+SEM, circles represent individual experiments.

M. Proliferative potential of PB DC precursors at day 3 of culture, estimated by CFSE dilution. Bulk CD34+ progenitors and CD14+monocytes were included as positive and negative controls, respectively. The CFSE dilution histograms for each precursor are grouped and ordered according to their proposed position in the developmental trajectory for pDC or DC2 lineages. Results are representative of n=3 experiments.

N. Sorting strategy applied to Lin-HLA-DR+ CD1c-CD141- cells, and location, in flow cytometry parameter space, of subsequently index-sorted PB precursor cells (CD2-pDC excluded) purified for scRNA-Seq analysis depicted in **Figure S4O-R**.

O. Hierarchical clustering of single cell transcriptomes of PB pre-DC populations showing signature genes that identify 8 clusters (p<0.05 and AUROC>0.7), used to annotate **Figure S4P**. These defined CD11c+ pre-DC2 (cluster 1 and 2; marked by *CD1c* and *SIRPA* at RNA level), AXL+CD5+/-CD11c₁₀ early pre-DC2 (clusters 4,5,6,8), CD34_{int}CD123_{int}AXL- cells, marked by *NFIL3* (critical for cDC1 development, Bagadia et al., 2019) and shown to have enriched cDC1 potential in culture (**Figure S4L**) and a small number of AXL-CD5-CD303/4_{hi} pre-pDC (contained within cluster 7, marked by *SERPINF1*, *MZB1*).

P. tSNE of the first 5 principal components (26% total variance) of 116 single cell transcriptomes sampled from PB pre-DC populations, annotated by the gate of origin from index-linked flow cytometry (Index Sort) or by 7 clusters generated from hierarchical clustering **Figure S40**. Pre-DC2 and pre-pDC were polarized in tSNE space, linked by early pre-DC2 which showed variable expression of both pDC and CD1c+DC genes.

Q. Heatmaps show expression of key TF on the tSNE plot from Figure S4P.

R. Mapping of single cell transcriptomes to cell populations identified by Villani et al. Cells identified as early pre-DC2 and pre-DC2(CD123+CD5+CD303/4_{lo}) showed enrichment of gene expression with 'AS' DC (DC5) and DC2; pre-pDC (CD123+CD2+CD303/4_{hi}) mapped to pDC (DC6) and the CD34_{int}CD123_{int}SIRPApopulation (early pre-cDC1) showed enrichment of genes expressed by a CD34_{int}CD100+ population also found in Villani et al. to contain cDC1 potential *in vitro*.

Figure S5, related to Figure 5



Figure S5. Differential IRF8 expression defines the two trajectories of DC development

A. Flow cytometric gating strategy for the FACS-purification of PBMC and BMMC to enrich for CD45+ cells and exclude lin(CD3,19,20,56,161)+ lymphocytes prior to CyTOF analysis (**Figure 5A-E**).

B. Gating of bivariate plots from CyTOF analysis to identify CD34+ progenitor subsets, comparable with flow cytometric analysis in **Figure 3A**.

C. Gating of bivariate plots from CyTOF analysis to identify DC and monocyte subsets and their precursors, comparable with flow cytometric analysis in **Figure 4A**.

D. Heatmap expression of IRF4 and additional key antigens (CD303, CD1c, SIGLEC6) across the tSNE visualization plot shown in **Figure 5A-E**.

E. Heatmaps of the expression (log2) of additional key antigens superimposed across the diffusion map trajectories generated with 14,000 GMP, precursor and mature DC and monocyte cells and 29 markers to infer pseudo-temporal ordering of cells and reconstruct lineage branching, as shown in **Figure 5F**.

F. Back-gating of CD34_{neg-int} DC precursors and DC subsets (defined in **Figure 4A**) on bivariate plots, to relate the DC developmental pathways to standard flow analysis. The relative expression of CD34 and CD123 is visualized across populations comprising lineage-specific developmental pathways as defined by the previous data. Schematic arrows summarize the proposed sequence of maturation of gated populations across the 2D space, from CD34+ progenitor compartment to mature DC populations. The utility of BM as a source material and relative paucity of DC precursors in PB is also illustrated.

G. Lineage-specific CD34_{neg-int} DC precursors and DC subsets from BM, as defined in **Figure 4A**, backgated onto bivariate plots of CD123 versus CD11c and CD123 versus SIRPA/B to visualize the relative expression of these antigens on populations comprising lineage-specific developmental pathways.

Figure S6, related to Figure 6



Figure S6. IRF8hi and IRF8ho pathways are differentially compromised in IRF8 deficiency

A. Flow cytometric gating strategy for whole PB Trucount[™] analysis of a HC (Cont), subject carrying heterozygous *IRF8* mutation (*R83C*) and subject carrying dominant negative mutation (*V426fs*), summarized in **Figure 6B**. Numbers represent the percent of cells from the parent gate.

B-C. Intracellular flow cytometric analysis of *in vitro* cytokine elaboration by monocytes (black bars), CD14+DC3 (orange), CD14-CD5-CD1c+DC (gray) and CD5+DC2 (red) (B) and CD2+pre-pDC and pDC (C) from HC (n=8) and subjects carrying heterozygous *IRF8* mutations (*R83C*, *R291Q*, mean of technical duplicates; and *V426fs*) in response to 14hrs stimulation with TLR agonists (CpG, poly(I:C), CL075, LPS). Integrated median fluorescence intensity (iMFI) was calculated by multiplying the frequency of positive cells by the MFI of a given marker. Bars show mean±SEM. P values from Mann Whitney U analysis (* p<0.05, **p<0.01, ***p<0.001).

Figure S7, related to Figure 7



Figure S7. IRF8 deficiency causes dose-dependent blockade of the IRF8hi pathway

A. Summary of DC and monocyte differentiation pathways from BM (where available) and PB of subjects carrying heterozygous *R83C*, dominant negative *V426fs* and biallelic *R83C/R291Q IRF8* mutations. DC and precursor populations were gated as shown in **Figure 7B**. These were backgated on bivariate plots to visualize the relative expression of CD34 and CD123 on populations comprising lineage-specific developmental pathways. Arrows indicate the proposed sequence of maturation of gated populations from CD34+ progenitor compartment to mature DC populations.