**Supplemental Figure legends**

**Supplemental Figure 1**

**Dynamics of gene expression during *in vivo* DC development in FACS sorted progenitors and DC subsets.**

Heat map representation of gene expression in MPP, MDP, CDP, cDC and pDC obtained by FACS sorting from mice [[1](#_ENREF_1)] using the same 3194 differentially expressed genes as in Fig. 1A. MPP, MDP and CDP, 2, 3 and 3 replicates, respectively; cDC, a panel of CD8+, CD8-CD4+, CD8-CD4-CD11b+ subsets in 5-6 replicates; pDC in 6 replicates (GSE15907) [[1](#_ENREF_1)]. Red, high expression; blue, low expression.

**Supplemental Figure 2**

**Correlation of histone modifications and gene expression.**

The levels of H3K4me1, H3K4me3, H3K27me3 and H3K9me3 modifications at gene promoters (TSS±1kb) or 3’ ends (TES±1kb) were determined for all NCBI Refseq annotated genes in MPP, CDP, cDC and pDC. For each cell type genes were grouped into bins of 100 genes based on their expression levels. The histone modification levels were then averaged for the same bins of 100 genes and depicted in heat map format. Dark colors indicate high levels of modification; light or white colors indicate low or no modification. The corresponding correlation coefficient values between histone modification levels and gene expression levels are shown. Upper panel, all genes; lower panel, differentially expressed (DE) genes from Fig. 1A.

**Supplemental Figure 3**

**Histone modifications and PU.1 binding dynamics of DC key regulatory genes during DC development.**

Occupancy for H3K4me1, H3K4me3, H3K27me3 and PU.1 and mRNA expression profiles (log2 expression) of DC progenitor gene (Gfi1 and Cebpa), cDC genes (Id2, Batf3) and pDC genes (Tcf4, Spib, Irf7r and Irf1) in MPP, CDP, cDC and pDC. Irf8, a central DC gene, is also shown as in Fig. 1D. Arrow indicates the direction of transcription.

**Supplemental Figure 4**

**Changes of epigenetic modifications between MPP and CDP.**

(A-C) Venn diagrams of H3K4me3, H3K27me3 and bivalent marked promoters are shown, including gain or loss of the respective modifications upon MPP to CDP transition. Loss of bivalency refers to resolution of H3K4me3 or H3K27me3 or both marks. Boxplots of mRNA expression from microarray data of MPP, CDP, pDC and cDC are shown below Venn diagrams. *P* values obtained by t-test are indicated. We note that gain or loss of H3K4me3 mark relates well to an increase and decrease of gene expression (A), while H3K27me3 and bivalent marks did not or only marginally affect gene expression (B, C).

**Supplemental Figure 5**

**Epigenetic regulation during DC subset specification.**

(A) Boxplot analysis of mRNA expression and H3K4me3, H3K27me3, H3K4me1 and PU.1 occupancy of differentially expressed genes between cDC and pDC (1111 genes, Fig. 1A; 573 and 538 genes are more abundantly expressed in cDC and pDC, respectively). Changes across DC development (MPP, CDP, cDC and pDC) are shown. H3K4me3 and H3K27me3, TSS±1kb; H3K4me1 and PU.1, TSS±50kb. (B) The percentage of genes with H3K4me3, H3K27me3 or both (bivalent domain) or no modification is shown. Genes with higher expression in cDC versus pDC, upper panel; genes with higher expression in pDC versus cDC, lower panel. The activation or silencing of cDC/pDC specific genes is in line with the H3K4me3 and the bivalent modification, respectively.

**Supplemental Figure 6**

**PU.1 binding peaks in promoters and enhancers.**

(A) The genomic distribution of PU.1 peaks in DC progenitors and subsets. Promoter, TSS±1kb; 3’ Ends, TES±1kb.(B) PU.1 binding peaks were classified by active enhancer (regions modified by both H3K27ac and H3K4me1), poised enhancer (regions only modified by H3K4me1) and active promoter (regions modified by H3K27ac, H3K4me1, H3K4me3 and close to TSS±1kb) in MPP, CDP and cDC. The information on enhancer and promoter annotation was obtained from ChIP-seq analysis of GM-DC (GSE36104).

**Supplemental Figure 7**

**Workflow of regulatory network inference method.**

(A) First we compiled all potential transcription factors and genes from the list of differentially expressed (DE) genes between two cell types, i.e. cDC vs pDC. (B) Next, we collected motifs from differentially expressed (DE) transcription factors (TFs) from public databases. (C) We identify differential peaks (DP; i.e. PU.1 peaks) close to differentially expressed (DE) genes of (A) in the same cell type, i.e. cDC differential peaks close to cDC differential genes. (D) Motif search from (B) within identified peak regions of (C). (E) Enrichment analysis of transcription factor motifs within differential peaks (DP) of (C) indicates transcription factors co-binding with PU.1 on a cell specific manner. (F) Construction of cell type specific transcription factor regulatory networks by connecting enriched transcription factors to its putative targets. For example, Ap1 has an edge to Bcl6 because: Bcl6 is up-regulated in cDC, Bcl6 has an Ap1 binding site on a cDC differential peak close to its promoter and Ap1 binding is enriched within cDC differential peaks.

**Supplemental Figure 8**

**The gene expression pattern of PU.1 co-binding transcription factors.**

Gene expression of potential PU.1 co-binding transcription factors of Fig. 5A in MPP, CDP, cDC and pDC is depicted in heat map format. Red, high expression; blue, low expression. Transcription factors whose enrichment pattern (Fig 5A) fits the expression profiles are marked in red.

1. Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, Pandey G, Leboeuf M, Elpek KG, Helft J, et al: **Deciphering the transcriptional network of the dendritic cell lineage.** *Nat Immunol* 2012.