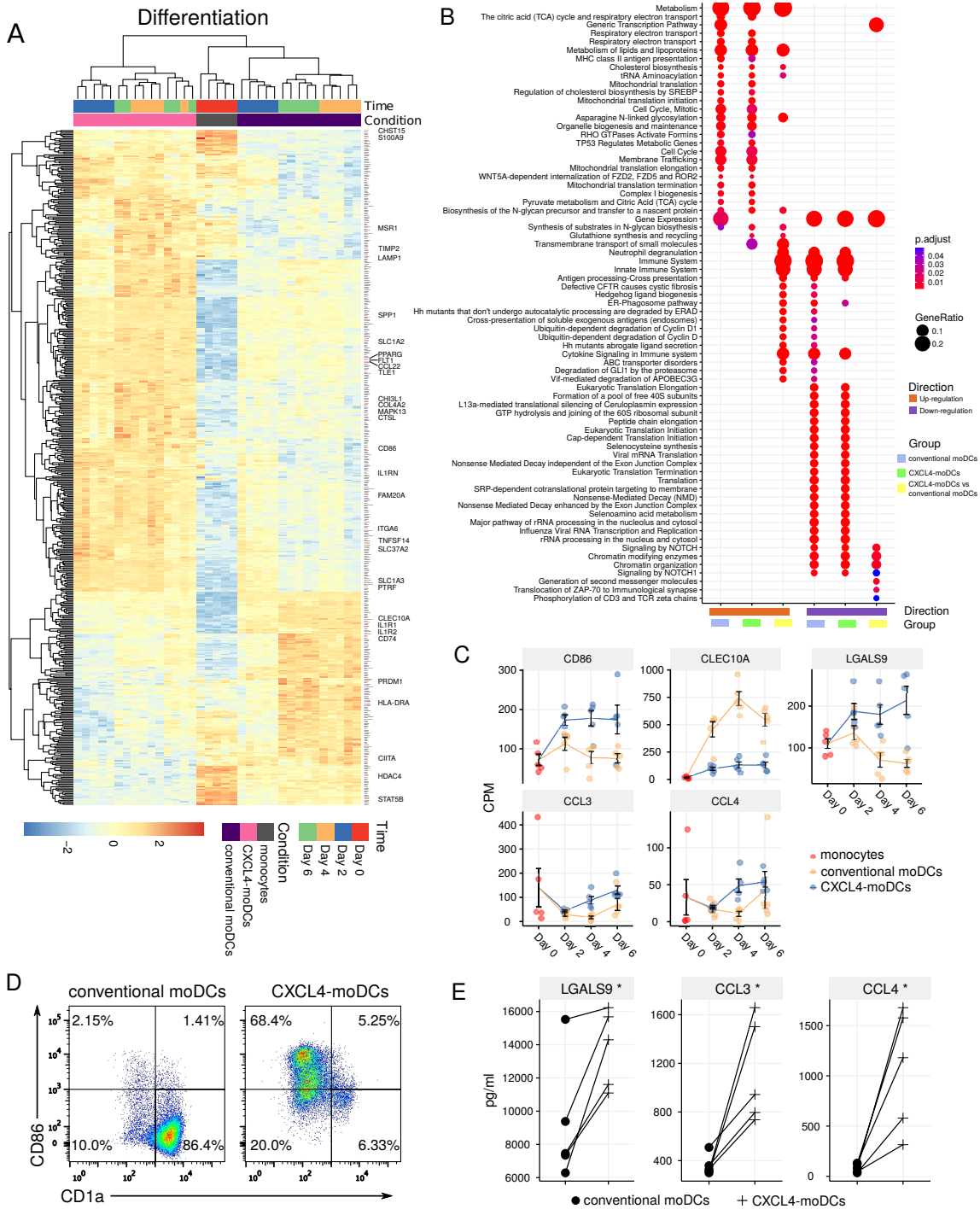


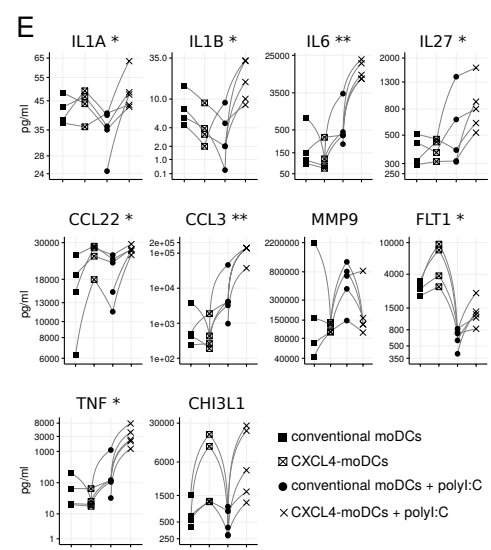
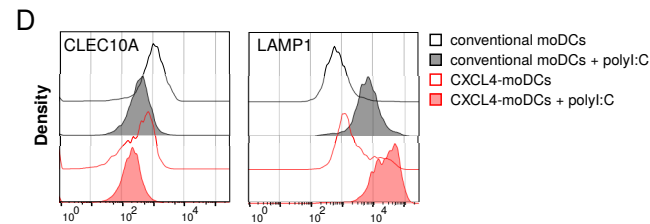
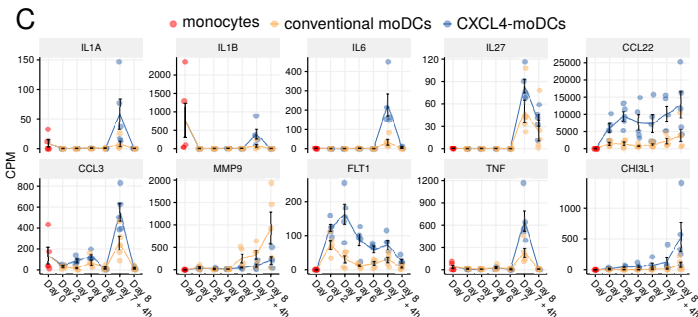
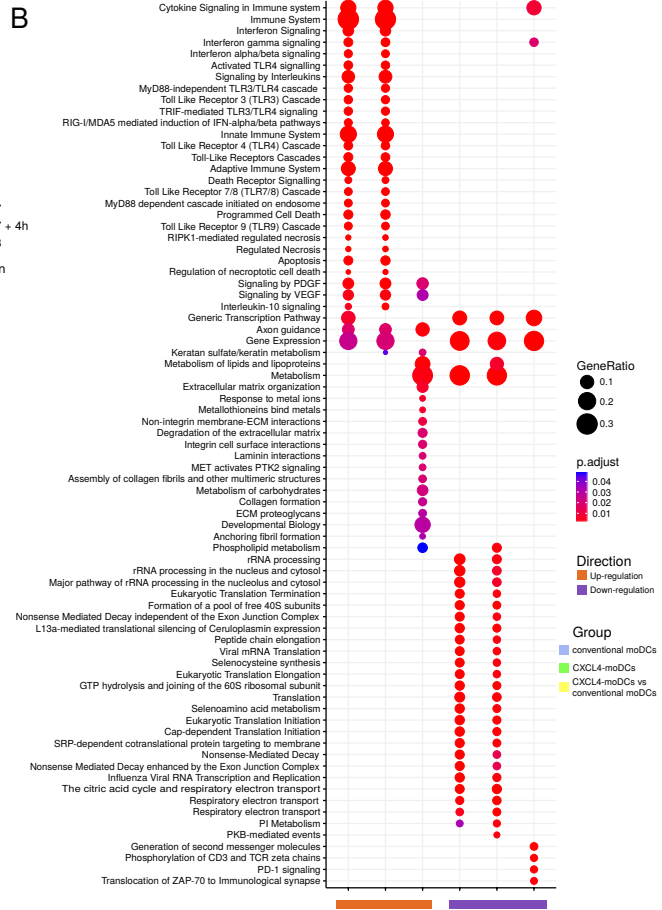
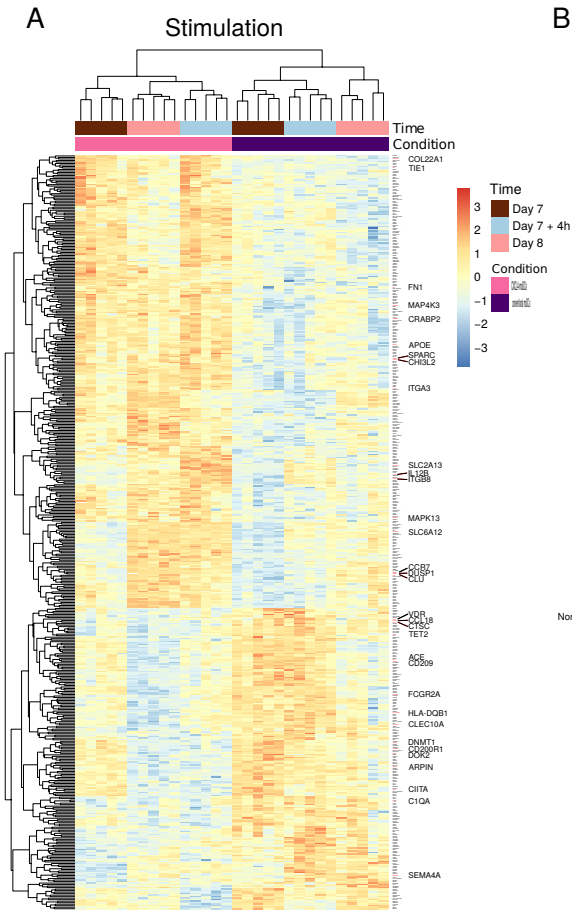
# Supplementary Material

## 1 Supplementary Figures and Tables

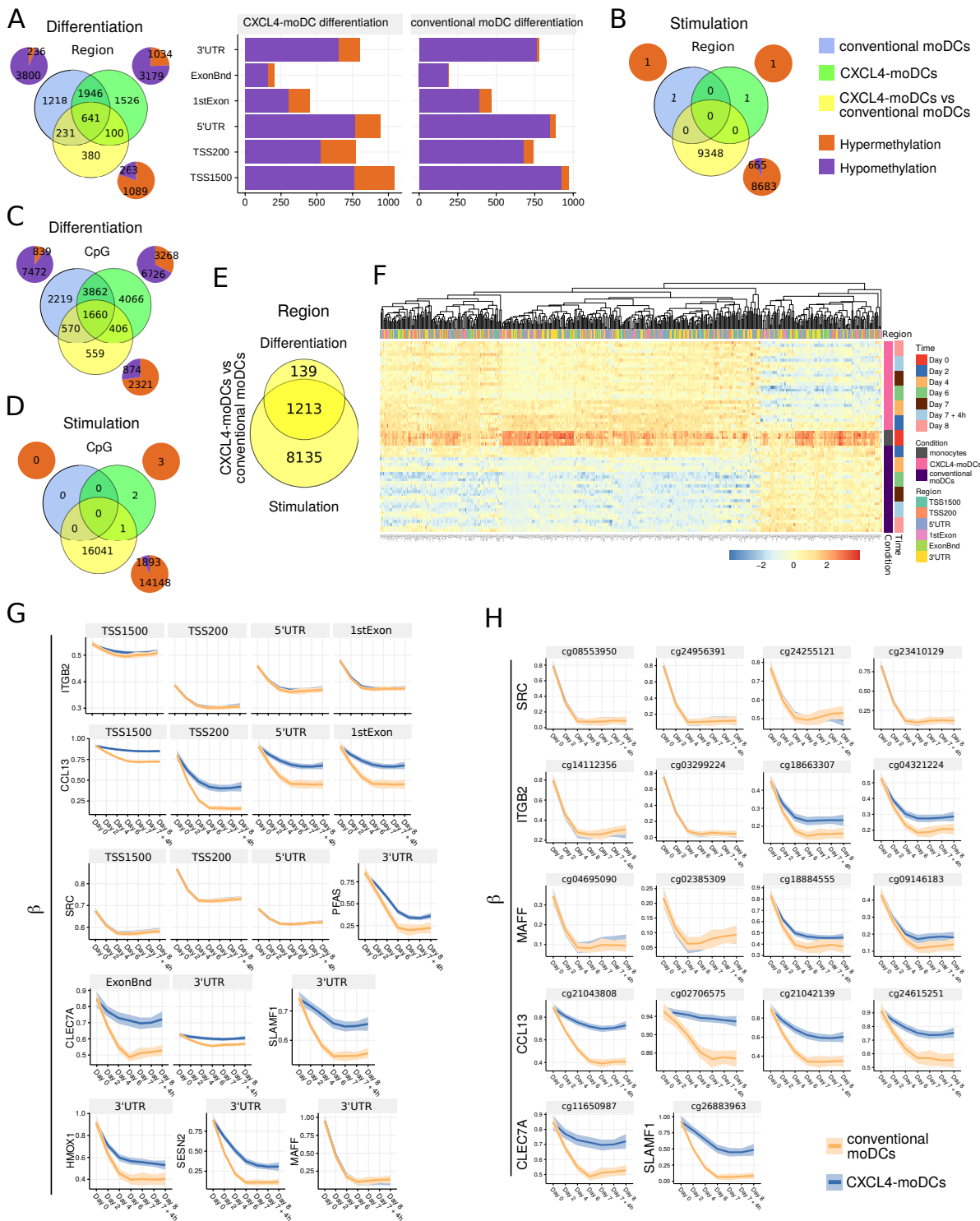
### 1.1 Supplementary Figures



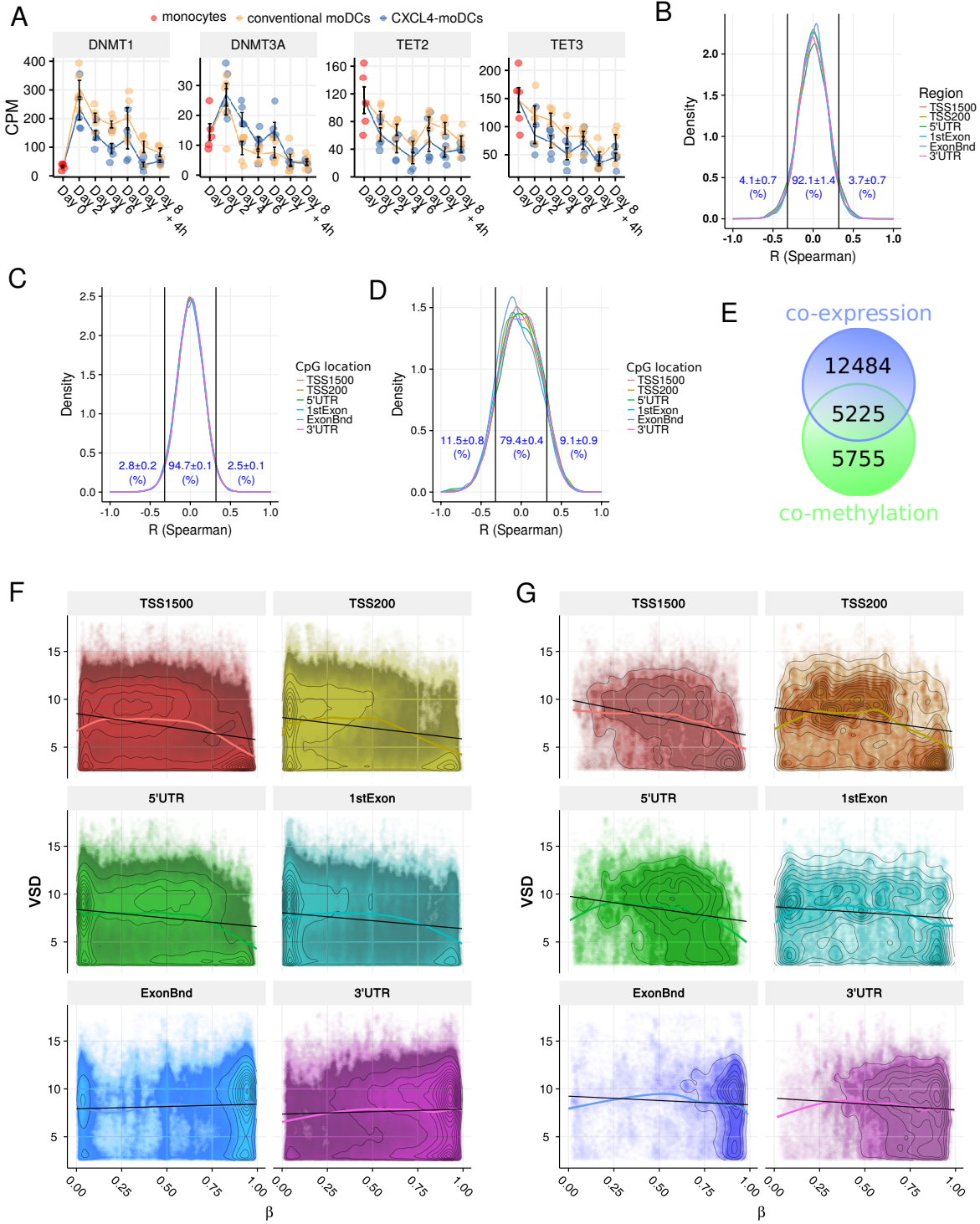
**Figure S1. Differentially expressed genes during differentiation.** (A) Heatmap showing normalized expression (VSD) of top 500 DEGs between CXCL4 moDCs and moDCs during their differentiation (day 0 to day 6). Heatmap color schemes are based on z-scores. Hierarchical clustering dendrograms were calculated using Euclidean distance. (B) Pathway enrichment analysis for the genes which were significantly up- and down-regulated during differentiation, as shown in **Figure 1B**. Up to 30 most significant pathways (FDR adjusted  $p < 0.05$ ) were shown for each set of genes. The size of the circle depicts the gene ratio of DEGs in the pathway to the total number of DEGs in each set of genes. Circle color represents FDR adjusted p values. The full lists of pathways are available in **Table S5**. (C) Gene expression profiles for example genes from top 500 DEGs that were further validated at the protein level. Data are shown as mean of count per million (CPM)  $\pm$  SEM. (D) Flow cytometry dot plot showing expression of CD86 and CD1a. Representative data from 5 HDs are shown. (E) Cytokine production of selected example proteins measured by Luminex using cell-free supernatants collected on day 6. Each symbol represents an individual donor; lines connect the same donor. Paired two-sided Student's *t*-test or two-sided Wilcoxon signed rank sum test (see statistical analyses in materials and methods).; \*  $P < 0.05$ .



**Figure S2. Differentially expressed genes upon polyI:C stimulation.** (A) Heatmap same as **Figure S1** for top 500 DEGs between CXCL4 moDCs and moDCs upon polyI:C stimulation (day 7 to day 8). (B) Pathway enrichment analysis for the genes that were significantly up- and down-regulated upon polyI:C stimulation, shown in **Figure 1C**. Up to 30 most significant pathways (FDR adjusted  $p < 0.05$ ) were shown for each set of genes. The size of the circle depicts the gene ratio of DEGs in the pathway to the total number of DEGs in each set of genes. Circle color represents FDR adjusted  $p$  values. The full lists of pathways are available in **Table S6**. (C) Gene expression profiles for example genes from top 500 DEGs that were further validated on protein level. Data are shown as mean of count per million (CPM)  $\pm$  SEM. (D) Flow cytometry analysis showing the relative expression of CLEC10A and LAMP1 between CXCL4 moDCs and moDCs, unstimulated and stimulated with polyI:C for 24 hours (on day 8). Representative data from 5 HDs are shown. (E) Cytokine production in cell-free supernatants was measured by Luminex for cells with/without polyI:C stimulation for 24 hours on day 8. Each symbol represents an individual donor; lines connect the same donor. Paired two-sided Student's  $t$ -test or two-sided Wilcoxon signed rank sum test (see statistical analyses in materials and methods) was performed for "conventional moDCs + polyI:C" group and "CXCL4-moDCs + polyI:C" group; \*  $P < 0.05$ , \*\*  $P < 0.01$ .

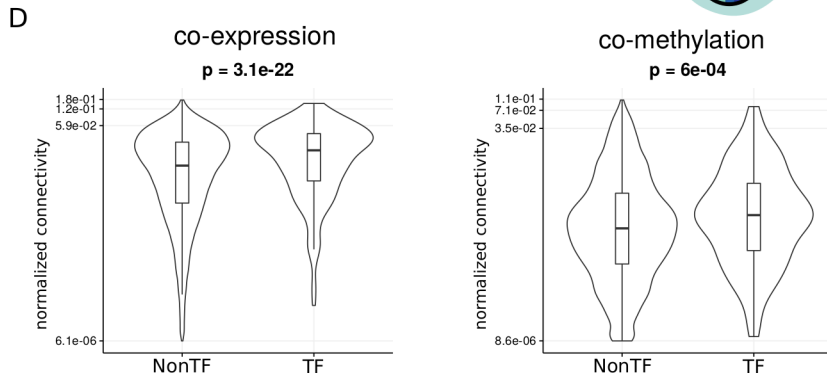
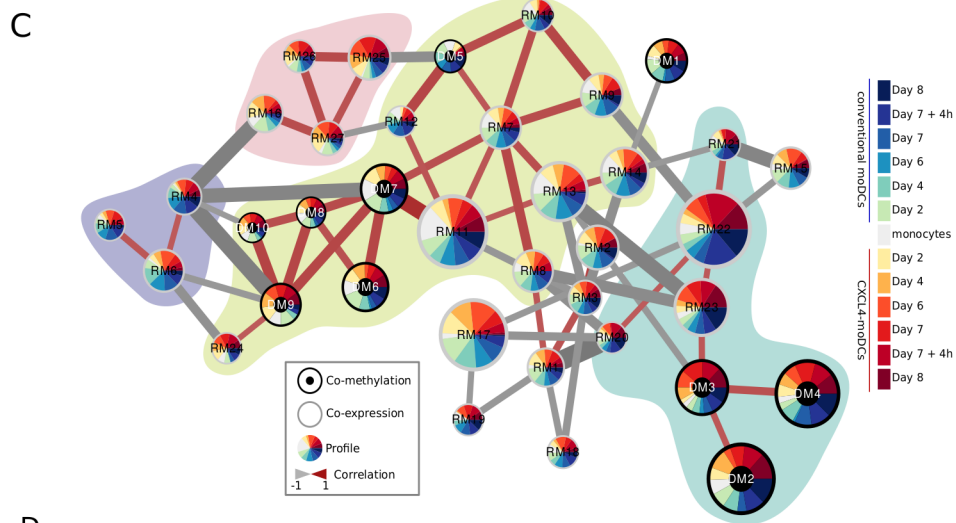
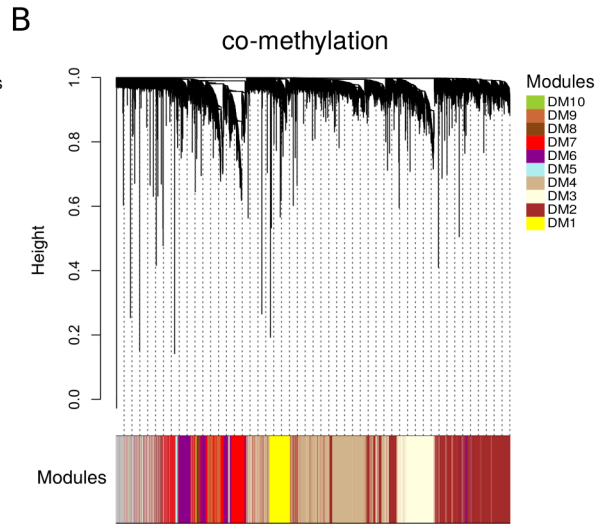
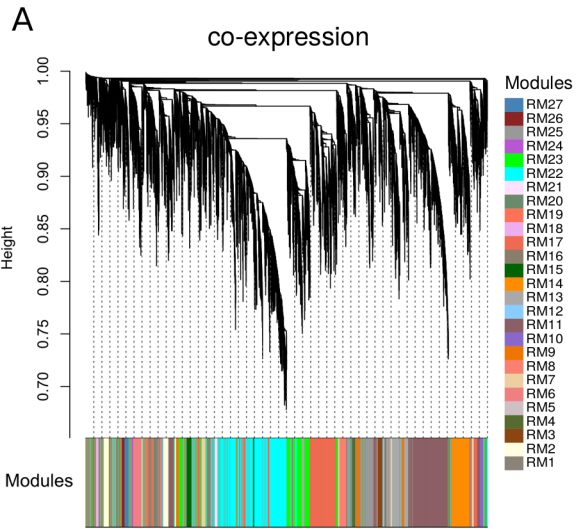


**Figure S3. Dynamics of DNA methylation on region and CpG levels.** Venn diagram shows the overlaps of **(A)** differentially methylated regions (DMRs) and **(C)** CpG sites during differentiation of: monocytes into moDCs (blue); monocytes into CXCL4 moDCs (green), and DMRs between moDCs and CXCL4 moDCs (yellow). In the middle and right panels of **(A)**, bar charts show the distribution of DMRs across 6 regions during CXCL4-moDC differentiation (middle), and during conventional moDC differentiation (right), as a supplement of **Figure 2G**. **(B)** Venn diagram showing DMRs and CpG sites **(D)** in moDC (blue), CXCL4 moDCs (green) and DMR/CpG between moDCs and CXCL4 moDCs (yellow) after polyI:C stimulation. In **(A-D)** pie charts represent the number of hyper-methylated regions (orange) and hypo-methylated regions (magenta). **(E)** Venn diagram showing the overlap of DMRs during differentiation (yellow circle in panel **A**) and upon polyI:C stimulation (yellow circle in panel **B**). **(F)** Heatmap reporting top 500 regions in overlapping DMRs in panel **D**. **(G)** Temporal methylation patterns of selected regions and CpG sites **(H)** found to be differential in different comparisons. Lines represent mean of  $\beta$  values and shades represent 95% confidence interval.

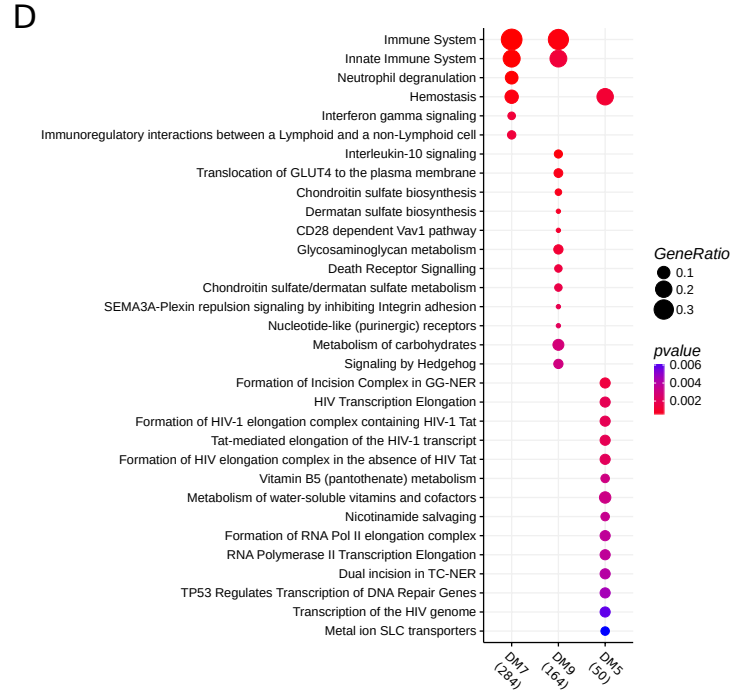
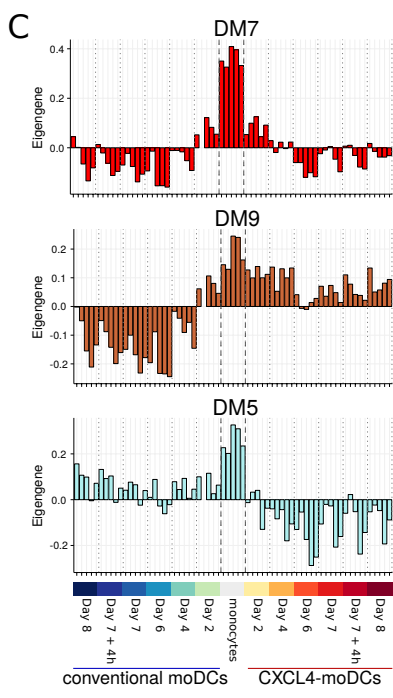
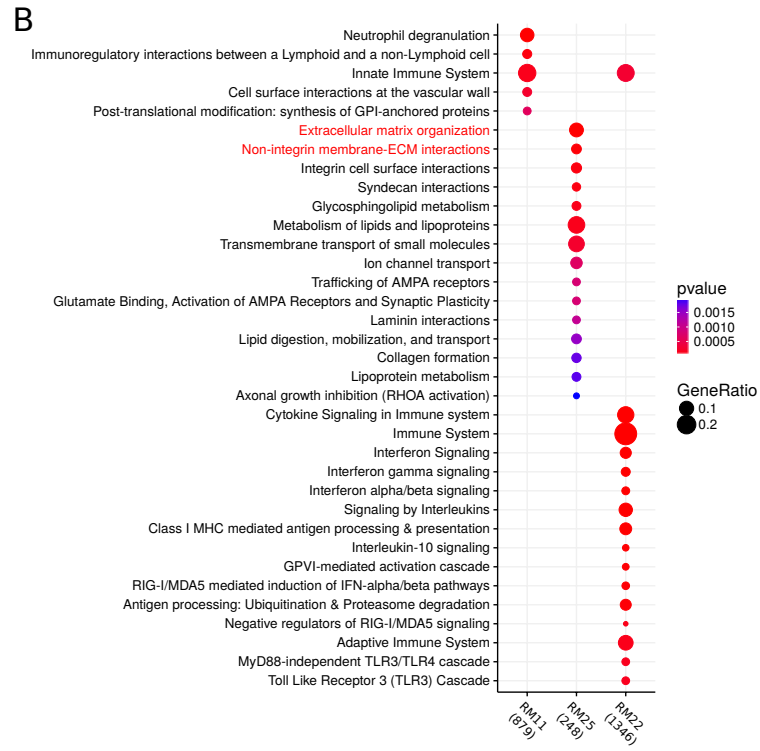
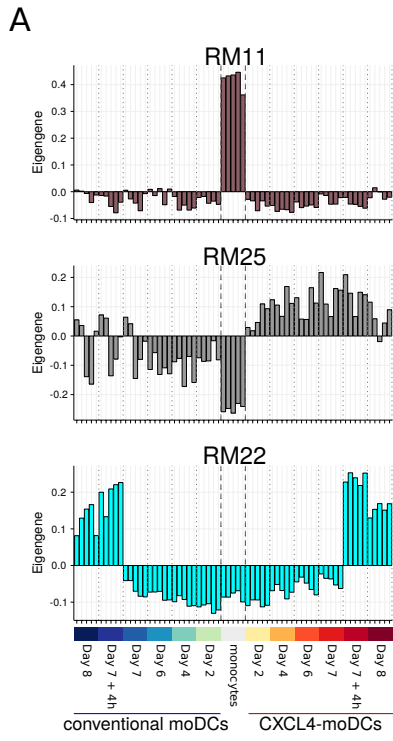


**Figure S4. Comparison of transcriptome and DNA methylome.** (A) Gene expression profile alterations of DNA methyltransferases and DNA demethylases. Data are shown as mean of count per million (CPM)  $\pm$  SEM. (B) The distribution of Spearman correlation coefficients between the methylation levels of all regions (including the regions that are not differentially methylated) and the corresponding gene expression. (C) The distribution of Spearman correlation coefficients between the methylation levels of all CpG sites (including the CpGs that are not differentially methylated) and the corresponding gene expression. (D) The distribution of Spearman correlation coefficients between the methylation levels of differentially methylated CpGs and the corresponding gene expression. In (B to D) the cut-offs (two vertical lines at  $R = \pm 0.32$ ) indicate significant correlation coefficients ( $P < 0.01$ ). Overlap between differentially expressed genes (DEGs) and differentially methylated genes (DMGs) during differentiation and upon polyI:C stimulation. A gene is considered differentially methylated if there is at least one region within this gene that is differentially methylated. (E) Venn diagram shows the overlapping genes between DEGs and DMGs in all comparisons which were used for further analysis. (F) Contour plots show global comparison of  $\beta$  values (x-axis) and VSD values (y-axis) for all the genes. (G) Contour plots show global comparison of  $\beta$  values (x-axis) and VSD values (y-axis) for genes that are differentially expressed and methylated. The black straight lines were obtained by fitting a linear regression model and the smoothing curves were obtained by fitting a non-linear model (see Methods).

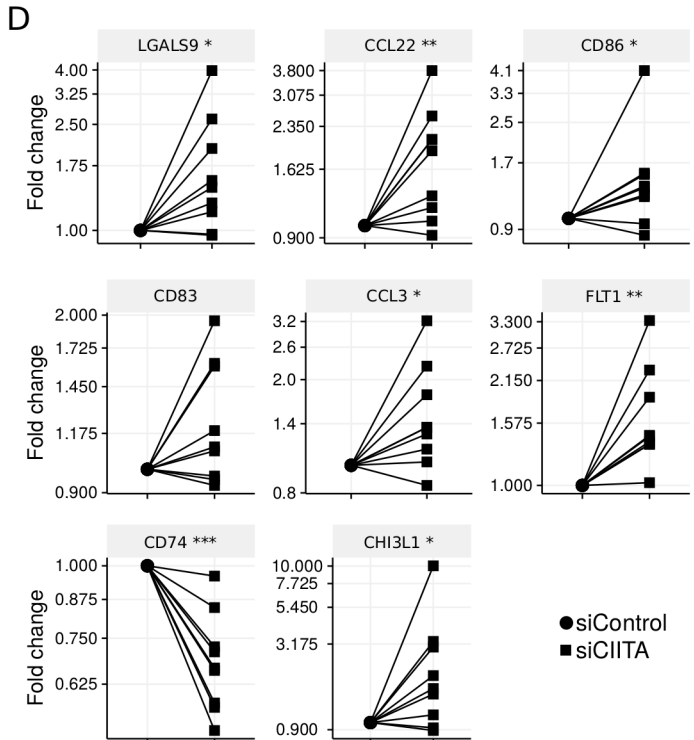
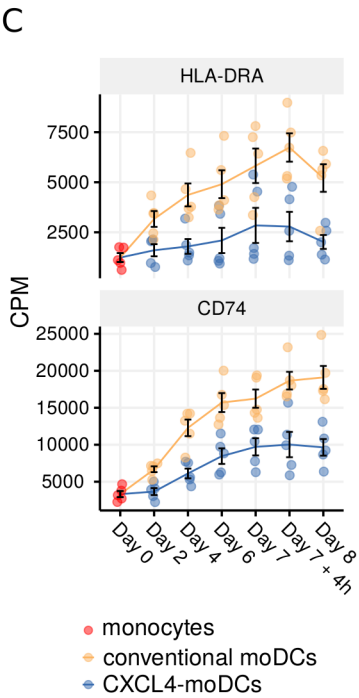
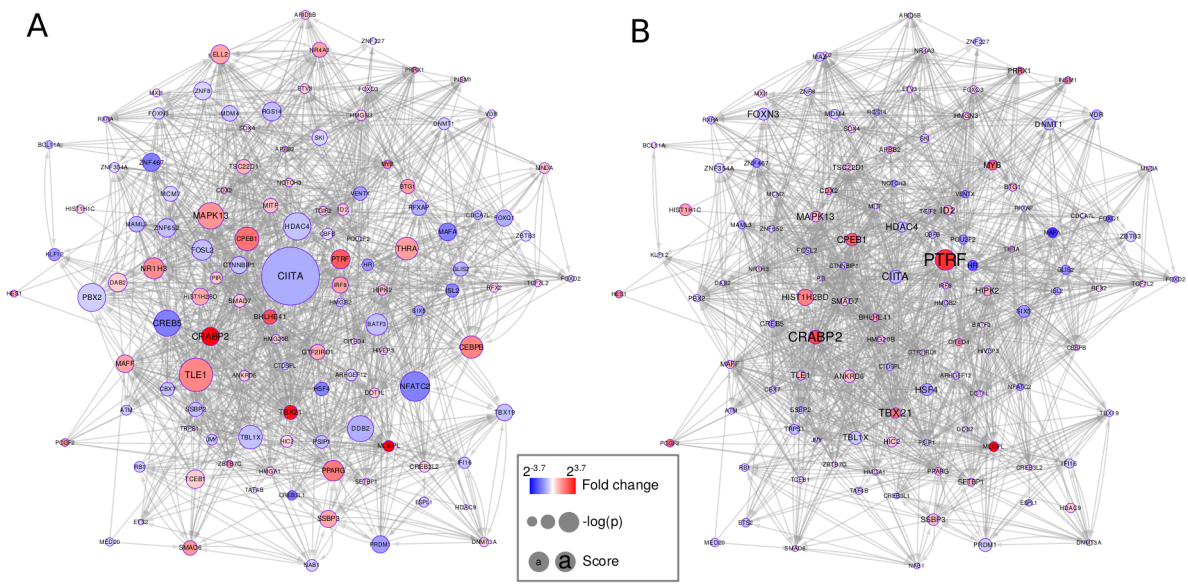




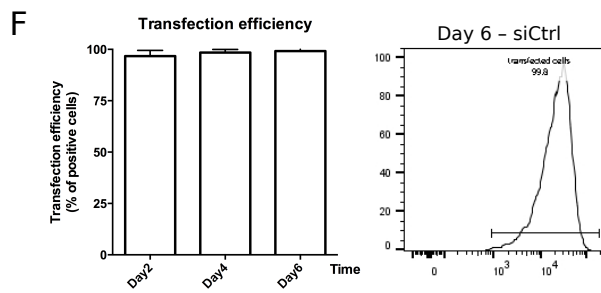
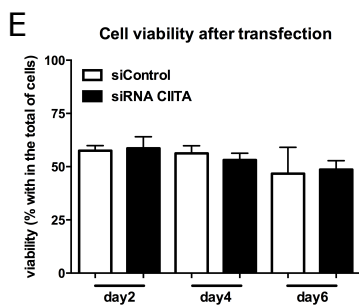
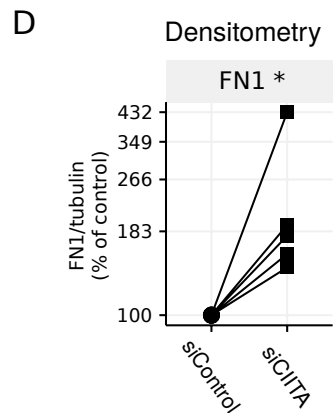
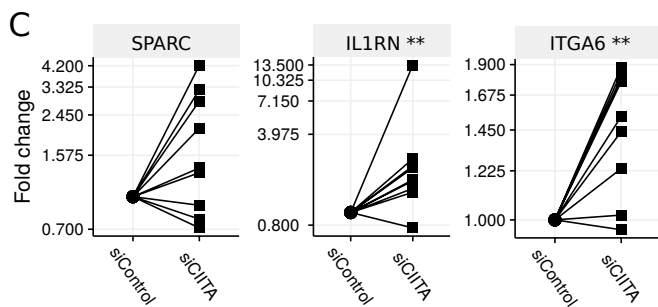
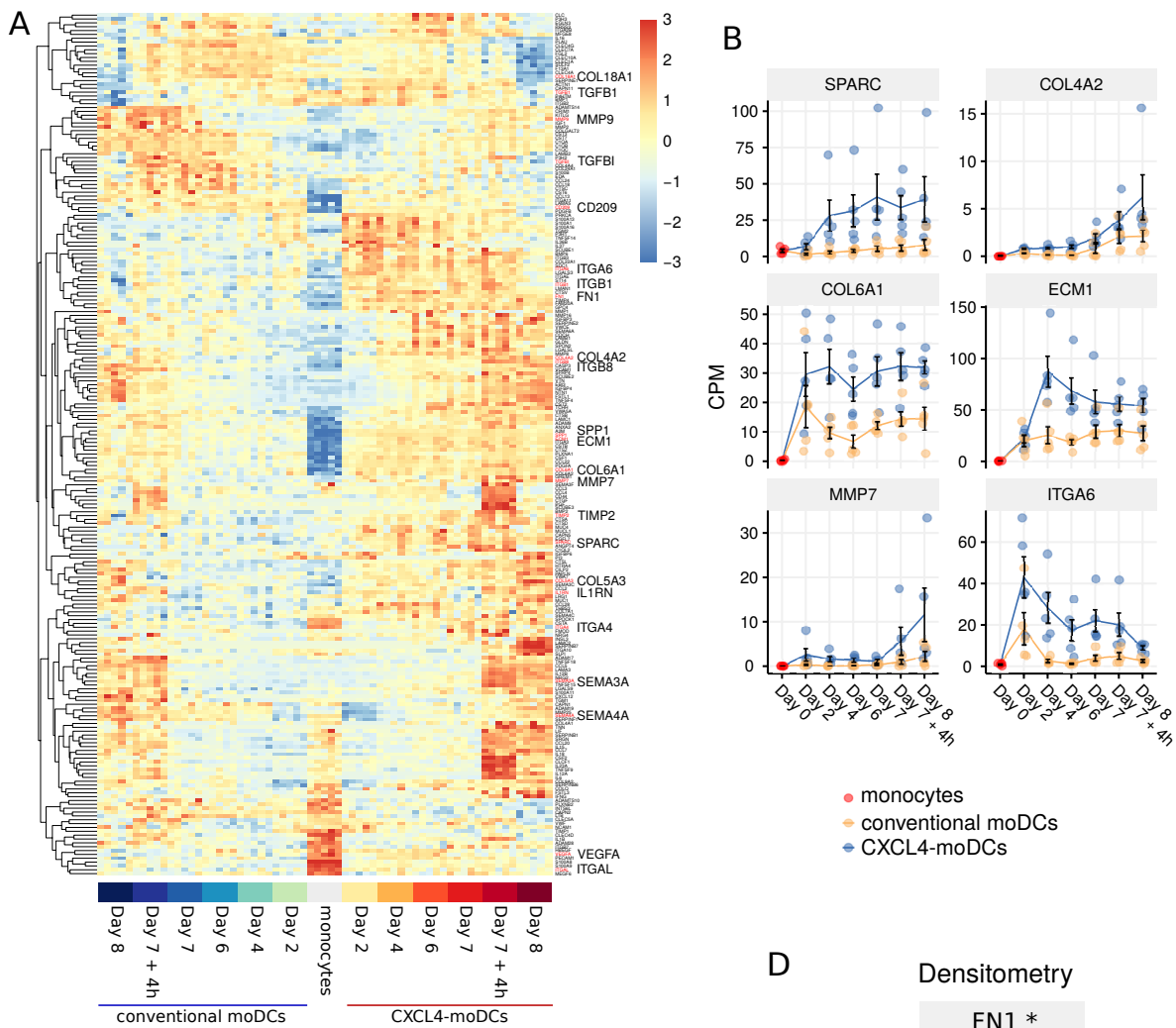
**Figure S5. Modules of co-expression and co-methylation networks.** Hierarchical clustering dendrogram of genes generated using topological overlap matrix (TOM) obtained from **(A)** gene expression or **(B)** methylation data. The co-expression and co-methylation modules were obtained using WGCNA package and are shown with different colors independently. **(C)** Network of co-expression and co-methylation modules based on the correlation of module eigengenes. Each circle (node) represents either co-expression or co-methylation module. Co-methylation modules are denoted as a node with a black circle in the middle, while the other nodes denote co-expression modules. The size of each node depicts the number of genes within that module. Different time and conditions are represented by the colors shown in the legend. The colored pie chart within each node represents the eigengene profile of that module. The edges (lines between two nodes) represent spearman correlation coefficient ( $\rho$ ) of eigengenes between two modules. The thickness of edge depicts the absolute value of  $\rho$  and edges with absolute value of  $\rho < 0.65$  are not shown. Colored shades in the background depict strongly positively correlated modules. **(D)** Violin/box plots comparing the connectivity, normalized by the total connectivity in the corresponding module, for transcription (co-)factors (TF) and other genes (NonTF) in the co-expression (left) and co-methylation (right) networks.



**Figure S6. Characteristics of co-expression and co-methylation modules.** (A) Bar charts show the eigengene of representative co-expression modules. (B) Pathway enrichment analyses for the modules shown in (A). (C) Bar charts of the eigengene of representative co-methylation modules. (D) Enriched pathways analysis for the modules shown in (C). In (B) and (C) the size of the circle depicts the gene ratio of DEGs in the pathway to the total number of DEGs in each set of genes; the colors of the circle represent FDR adjusted p values. In brackets, below the graph, we show the number of genes in each module. The full lists of pathways are available in **Table S8**.

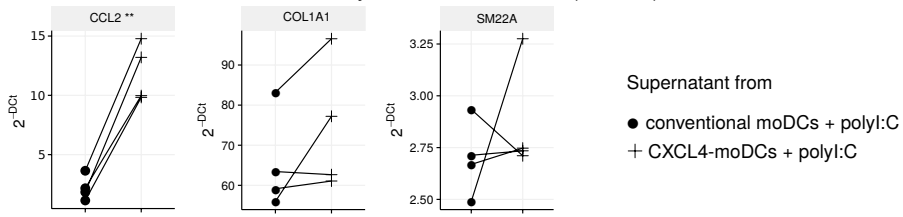
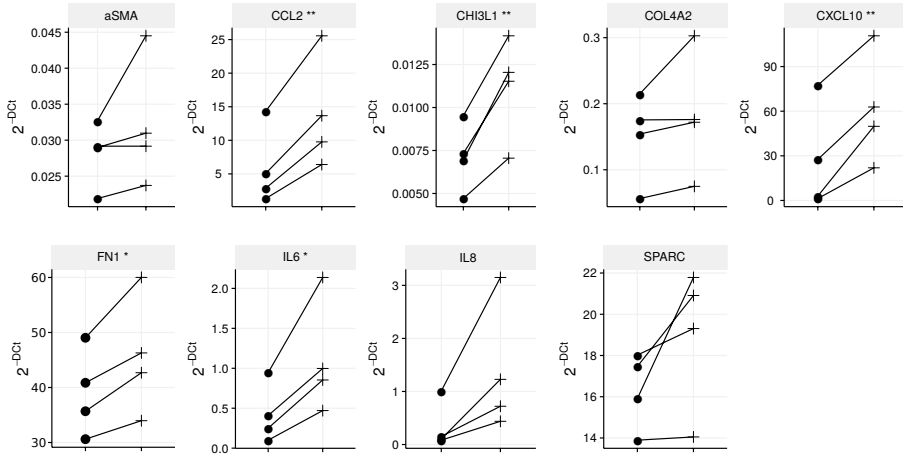
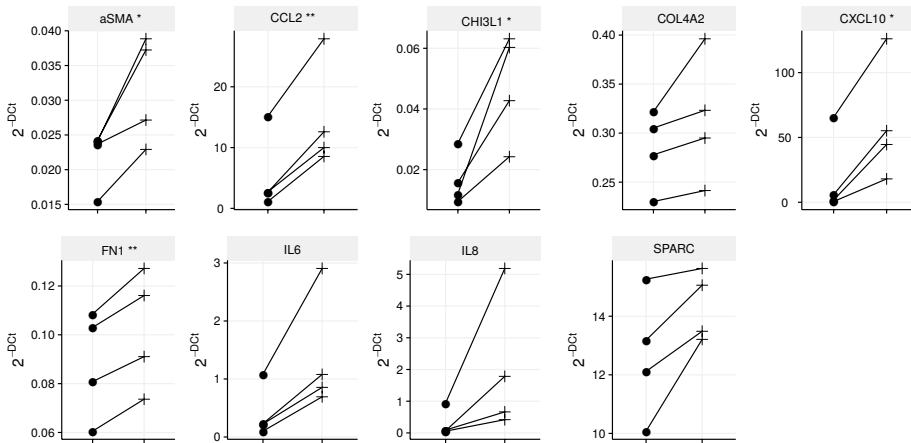


**Figure S7. Enriched transcription regulators obtained using random forest-based gene regulatory network.** Enriched transcription regulator networks **(A)** during differentiation and **(B)** upon stimulation. Red indicates up- regulation and blue indicates down-regulation of TFs in CXCL4 moDCs compared to moDCs. Circle size shows  $(-\log_{10}(p))$  obtained using differential expression analysis. Text size represents the overall score of TF enrichment obtained from RegEnrich (see Methods). Edges (lines) were obtained from top 5% edges in gene regulatory network inferred by random forest. To make the networks comparable same scales and parameters were used. **(C)** Gene expression profile of DEGs downstream to CIITA regulation. **(D)** After transfection of monocytes with Silencer negative control siRNA (siControl) and Silencer CIITA siRNA (siCIITA), cells were differentiated into moDCs for 6 days. On day 6, inflammatory and co-stimulatory genes were analyzed by qPCR. (Data are normalized by the mean expression of *RPL32* and *RPL13A*; fold change relative to siControl). Each symbol represents an individual moDC donor; lines connect the same donor. Paired two-sided Student's *t*-test or two-sided Wilcoxon signed rank sum test (see statistical analyses in materials and methods). \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



**Figure S8. Identification of genes implicated in ECM remodeling.** (A) Heatmap showing differentially expressed genes that play a role in ECM remodeling, identified from pathway enrichment analysis (**Figure S1B** and **Figure S2B**), and CXCL4 responsive co-expression modules (**Figure S5B**). Each column represents a sample and the colors on the bottom denote different time and conditions. The color schemes in the heatmap are shown as z-scores. (B) Expression profiles of example genes implicated in ECM remodeling. Data are shown as mean of count per million (CPM)  $\pm$  SEM. (C) After transfection of monocytes with Silencer negative control siRNA (siControl) and Silencer CIITA siRNA (siCIITA), cells were differentiated into moDCs for 6 days. Genes involved in ECM remodeling/fibrosis analyzed by qPCR on day 6. Data are normalized by the mean expression of RPL32 and RPL13A; fold change relative to siControl. (D) Fibronectin (FN1) and tubulin expression measured by western blot on day 6. Signal intensity of 5 independent experiments was quantified by densitometry analysis. To determine the relative expression of FN1 between siControl and siCIITA, the ratio between the expression of FN1 and tubulin was first calculated. (E-F) Monocytes were transfected with either control non-targeting siRNA (siControl) or with specific siRNA targeting CIITA (siCIITA). Cells were differentiated into moDCs for 6 days. Viability (E) and transfection efficiency (F) was accessed by flow cytometry on days 2, 4 and 6. N=2. In panels (C) and (D) each symbol represents an individual moDC donor; lines connect the same donor. Paired two-sided Student's *t*-test or two-sided Wilcoxon signed rank sum test (see statistical analyses in materials and methods).. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .



**A****Myfibroblast transition (donor 1)****B****Myfibroblast transition (donor 2)****C****Myfibroblast transition (donor 3)**

**Figure S9. Gene expression analysis of healthy myofibroblasts after exposure to cell-free supernatants from CXCL4 moDCs and moDCs.** CXCL4 moDCs and moDCs on day 7 were stimulated with polyI:C for 24 hours. Cell-free supernatants were added to healthy myofibroblasts for 24 hours. Inflammatory and fibrotic gene expression was analyzed by qPCR. Each symbol represents an individual moDC donor (n=4). Lines connect the same moDC donors. **(A)** shows the gene expression for the first fibroblast donor. The remaining measured genes are shown in **Figure 5H**. The gene expression for the **(B)** second and **(C)** third independent fibroblast donors. Paired two-sided Student's *t*-test or two-sided Wilcoxon signed rank sum test (see statistical analyses in materials and methods).. \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001.

## **1.2 Supplementary Tables**

### **Table S1.**

The number of differently expressed genes (DEGs) and differently methylated genes (DMGs) during differentiation and upon polyI:C stimulation.

### **Table S2.**

Primer list for RT-qPCR.

### **Table S3.**

DEGs and p-values by differential expression analysis.

### **Table S4.**

DMRs and p-values by differential methylation analysis.

### **Table S5.**

Full list of enriched pathways for the DEGs during differentiation.

### **Table S6.**

Full list of enriched pathways for the DEGs upon stimulation.

### **Table S7.**

Full list of enriched pathways for the DEGs overlapping during differentiation and upon stimulation.

### **Table S8.**

Full list of enriched pathways for co-expression and co-methylation modules.

### **Table S9.**

Transcription regulators used as TFs for the analysis.