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Supplemental information

Aryl hydrocarbon receptor activity downstream of IL-10 signaling is required to promote regulatory functions in human dendritic cells

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Figure S1

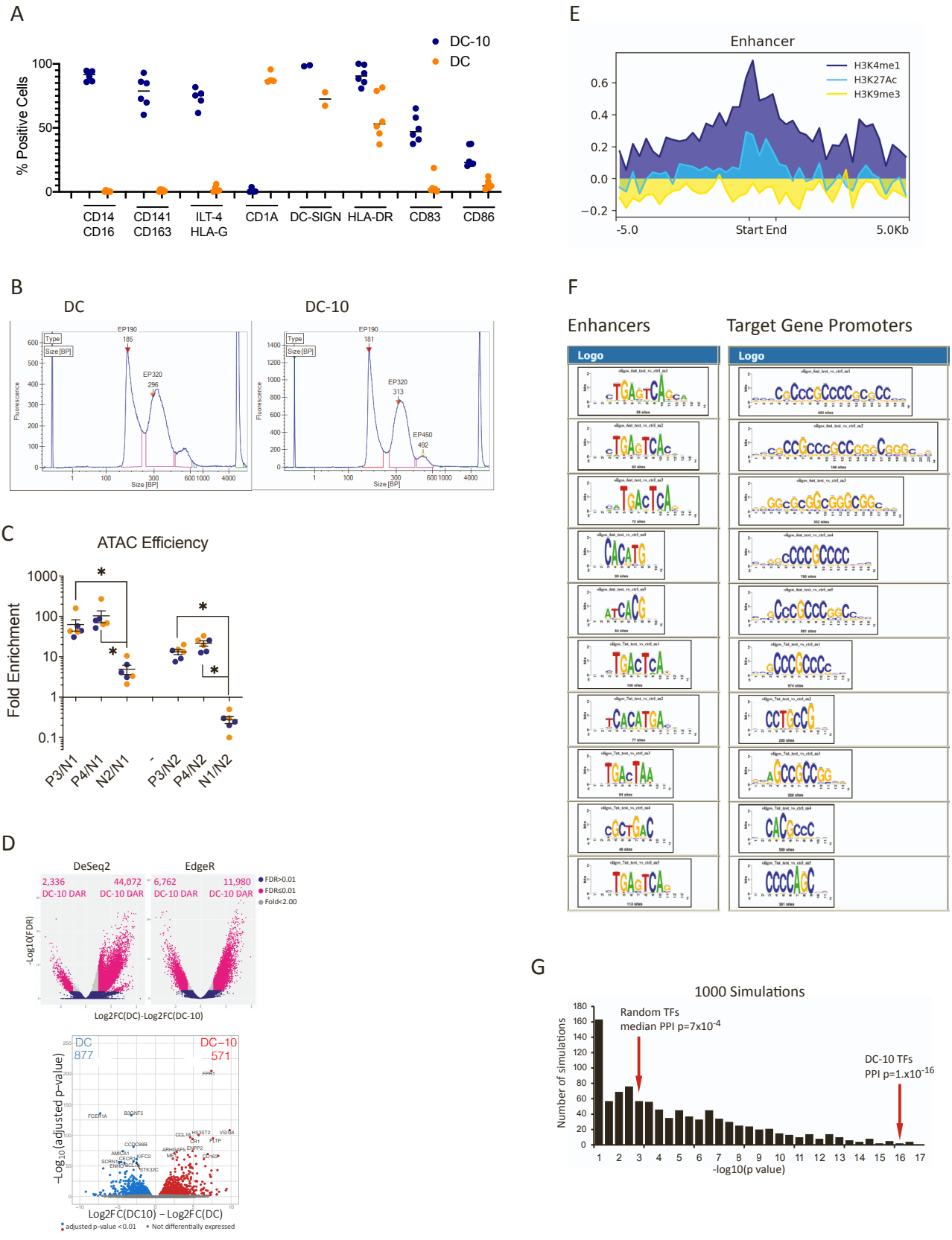

A
DC-10
DC
% Positive Cells
100
50
0
CD14
CD16
CD141
CD163
ILT-4
HLA-G
CD1A
DC-SIGN
HLA-DR
CD83
CD86
B
DC
DC-10
Type
Size [BP]
EP190
185
EP320
296
EP450
492
181
313
Fluorescence
Size [BP]
C
ATAC Efficiency
Fold Enrichment
1000
100
10
1
0.1
P3/N1
P4/N1
N2/N1
P3/N2
P4/N2
N1/N2
D
DeSeq2
EdgeR
2,336
DC-10 DAR
44,072
DC-10 DAR
6,762
DC-10 DAR
11,980
DC-10 DAR
FDR>0.01
FDR≤0.01
Fold<2.00
-Log10(FDR)
Log2FC(DC)-Log2FC(DC-10)
DC
877
DC-10
571
-Log10(adjusted p-value)
Log2FC(DC10) – Log2FC(DC)
adjusted p-value <0.01
Not differentially expressed
E
Enhancer
H3K4me1
H3K27Ac
H3K9me3
0.6
0.4
0.2
0.0
−0.2
-5.0
Start End
5.0Kb
F
Enhancers
Target Gene Promoters
Logo
Logo
G
1000 Simulations
Random TFs
median PPI p=7x10-4
DC-10 TFs
PPI p=1.x10-16
Number of simulations
-log10(p value)

Figure S1. Relative to Figure 1 *IL-10 Impacts Chromatin and Transcription in DCs*

A) Flow Cytometry profile of DC-10 (blue) and DC (orange) samples used for ATAC- and RNA-seq (n=6). Percentage of cells positive for indicated markers is shown.

B-C) ATAC Efficiency of tagmentation (B) and enrichment of accessible genomic sites (C) in DC-10 (blue) and DC (orange) samples. B) Electropherograms of purified ATAC libraries showing DNA sizes corresponding to 1-, 2- and 3-nucleosome stretches for DC and DC-10 samples. C) Droplet Digital PCR analysis with primers amplifying accessible (P3, P4) or non-accessible (N1, N2) regions in hematopoietic cells (Grbesa et al. 2017, *Journal of Visualized Experiments*, https://doi.org/10.3791/56313). Fold enrichment is expressed as the ratio between positive and negative signals (number of positive molecules *per* mL in accessible regions/number of positive molecules *per* mL in non-accessible regions) normalized to the average of the ratios between negative and negative signals (number of positive molecules *per* mL in non-accessible regions/number of positive molecules *per* mL in non-accessible regions; set as 1). *p*-values by Wilcoxon matched pairs test.

D) ATAC-seq (top) and RNA-seq (bottom) volcano plots of DC-10 vs. DC samples. Top plots display differentially accessible genomic regions (DAR) (pink) by DESeq2 and edgeR algorithms. Bottom, differentially expressed genes (DEG) (red) by DESeq2 with top 10 DEG name per group indicated. The number of DAR and DEG per group are indicated.

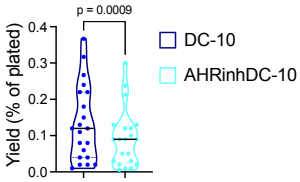
E) Feature alignment of ChIP-seq results obtained for the 3 indicated histone marks in DC-10 on the 107 DC-10 enhancers (start-end of the sequences are indicated).

F) Motif logos of the 10 most enriched motifs in enhancer and target gene promoter regions found using the RSAT tool.

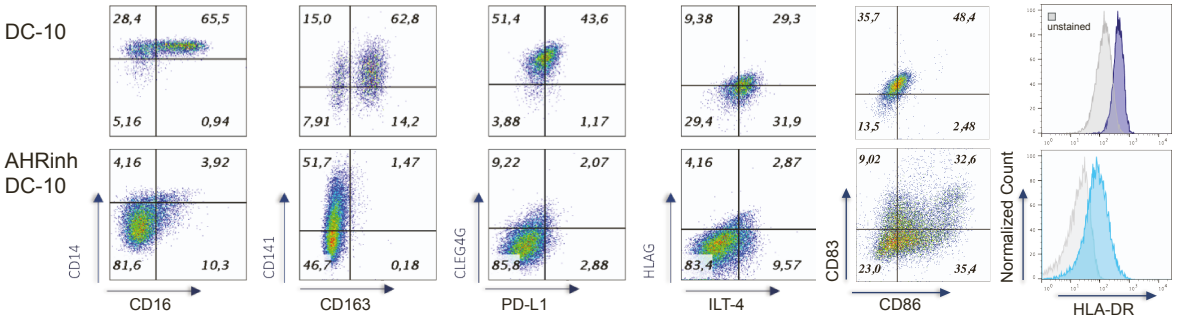
G) PPI *p*-values obtained by the STRING tool in 1000 simulations performed with TFs randomly chosen from the ENCODE human TF list.

Figure S2

A



B



C

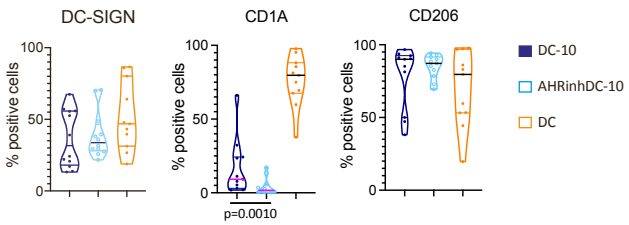


Figure S2. Relative to Figure 2 *AHR activity characterizes IL-10 induced tolDC*

$CD14^+$ monocytes isolated from peripheral blood (PB) were differentiated *in vitro* for 7 days in the presence of GM-CSF, IL-4 and IL-10 with (AHRinhDC-10) or without (DC-10) the AHR inhibitor (AHRinh) CH223191 (20μM).

A) Cell yield upon 7 days of DC-10 (blue) and AHRinhDC-10 (light blue) differentiation. Yield is expressed as number of recovered cells/number of plated cells (n=21).

B) Representative flow cytometric plots showing the expression of the indicated markers in DC-10 (top) and AHRinhDC-10 (bottom).

C) Percentage of DC-10 (blue), AHRinhDC-10 (light blue) and DC (orange) expressing the DC differentiation markers DC-SIGN (CD209), CD1a and CD206 analyzed by flow cytometry (n=11-12).

p values by Wilcoxon matched pairs test.

Figure S3

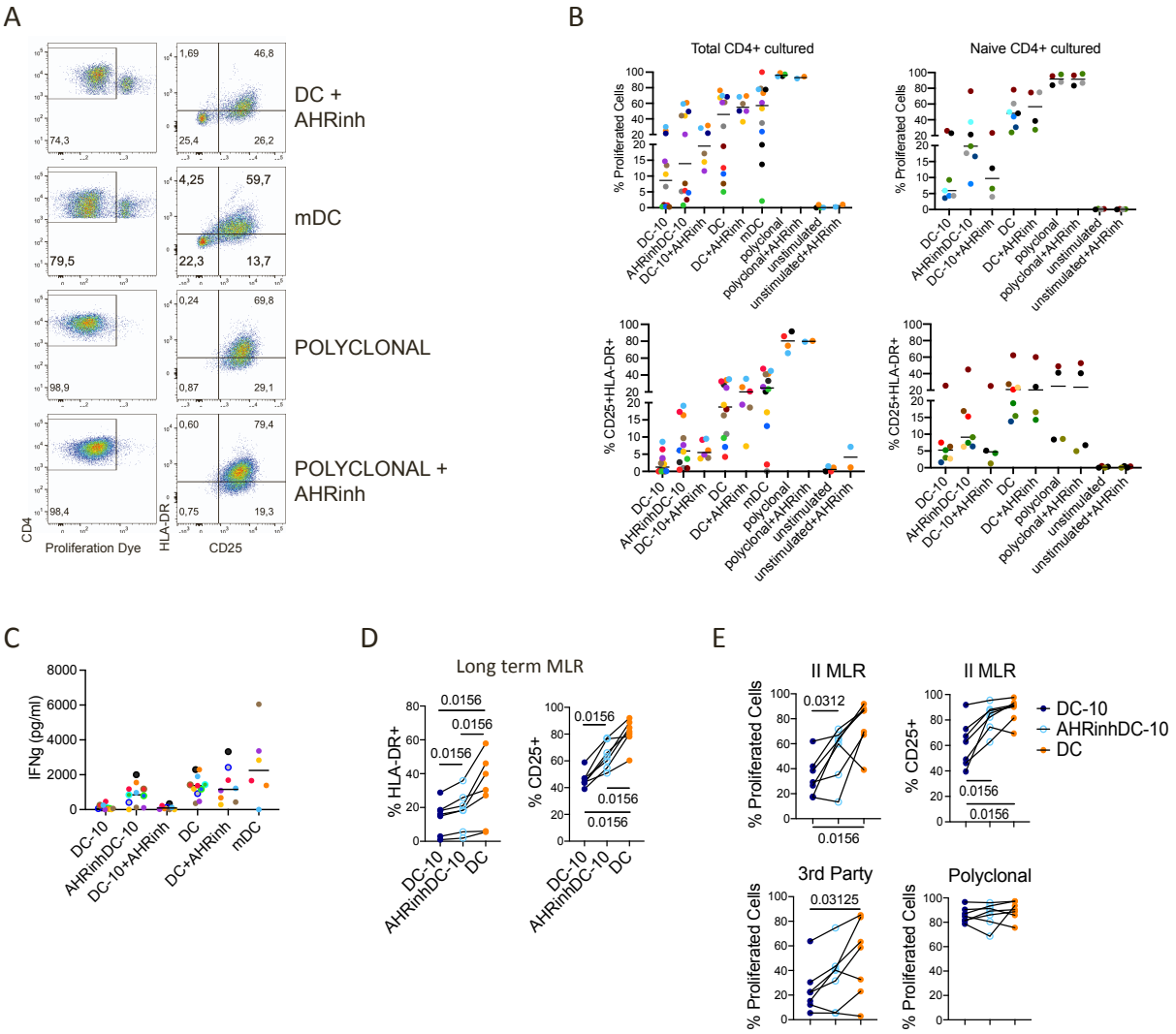

A
DC + AHRinh
mDC
POLYCLONAL
POLYCLONAL + AHRinh
CD4
Proliferation Dye
HLA-DR
CD25
B
Total CD4+ cultured
Naive CD4+ cultured
% Proliferated Cells
% CD25+HLA-DR+
DC-10
AHRinhDC-10
DC-10+AHRinh
DC
DC+AHRinh
mDC
polyclonal
polyclonal+AHRinh
unstimulated
unstimulated+AHRinh
C
IFNg (pg/ml)
D
Long term MLR
% HLA-DR+
% CD25+
E
II MLR
3rd Party
Polyclonal
% Proliferated Cells
% CD25+
DC-10
AHRinhDC-10
DC

Figure S3. Relative to Figure 3 ***AHR activation is necessary for IL-10 induced tolerogenic functions in tolDCs generated in vitro***

A-C) Short-term MLR. Allogeneic $CD4^+$ T cells were stimulated *in vitro* with the indicated DC. After 5 days, T cell proliferation and activation were tested by flow cytometry and IFNg production in culture supernatants was tested by ELISA.

A) Representative flow cytometric plots showing the expression of the indicated markers in $CD4^+$ T cells stimulated with DC in the presence of the AHR inhibitor CH223191 (20μM), mDC or with anti-CD3/anti-CD28 coated beads (Polyclonal) in the absence or presence of AHR inhibitor CH223191 (20μM).

B) Raw percentages of proliferating (top) and $CD25^+$HLA-DR^+ (bottom) $CD4^+$ cells in MLR of total (left) and naïve (right) $CD4^+$ cells stimulated by the indicated DC. Each color represents an independent donor, line at median.

C) IFNg ELISA quantification in culture supernatants of T cells stimulated by the indicated DC. Each color represents an independent donor, line at median.

D-E) Long-term MLR and II MLR. Long-term MLR: allogeneic $CD4^+$ T cells were stimulated by the indicated DC. The percentage of proliferating and Tr1 cells was assessed after 10 days by flow cytometry. II MLR: $CD4^+$ T cells primed in I MLR by the indicated DC were re-stimulated with mature DC derived from the same donor used in I MLR. After 3 days of re-stimulation, proliferation by flow cytometry and cytokine production by ELISA on culture supernatants were assessed.

D) Raw percentages of $CD25^+$ and HLA-DR^+ cells in I long-term MLR of T cells stimulated with the indicated DC.

E) Top, raw percentages of proliferating (left) and $CD25^+$ (right) $CD4^+$ cells after II MLR. Bottom, percentage of proliferating $CD4^+$ upon stimulation with third party donor mature DC (left) and polyclonal stimulation by anti-CD3/anti-CD28 coated beads (right). Blue dots = T cells stimulated in I MLR by DC-10; light blue dots = T cells stimulated in I MLR by AHRinhDC-10; orange dots = T cells stimulated in I MLR by DC.

p-values by Wilcoxon matched pairs test.

Figure S4

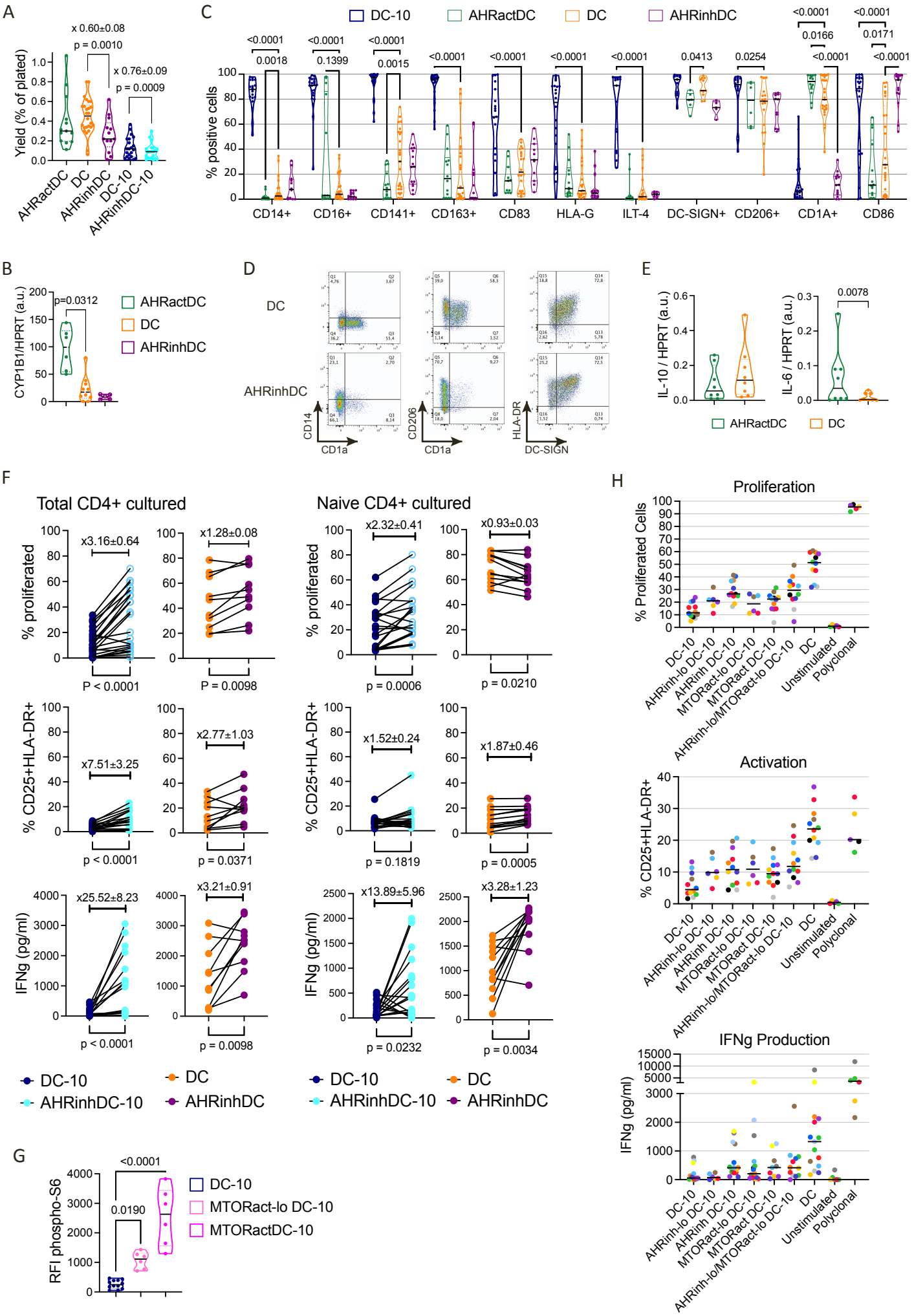

A
x 0.60±0.08
p = 0.0010
x 0.76±0.09
p = 0.0009
Yield (% of plated)
AHRactDC
DC
AHRinhDC
DC-10
AHRinhDC-10
C
DC-10
AHRactDC
DC
AHRinhDC
% positive cells
CD14+
CD16+
CD141+
CD163+
CD83
HLA-G
ILT-4
DC-SIGN+
CD206+
CD1A+
CD86
B
p=0.0312
CYP1B1/HPRT (a.u.)
D
DC
AHRinhDC
CD14
CD1a
CD206
HLA-DR
DC-SIGN
E
IL-10 / HPRT (a.u.)
IL-6 / HPRT (a.u.)
0.0078
F
Total CD4+ cultured
Naive CD4+ cultured
% proliferated
% CD25+HLA-DR+
IFNg (pg/ml)
x3.16±0.64
x1.28±0.08
x2.32±0.41
x0.93±0.03
P < 0.0001
P = 0.0098
p = 0.0006
p = 0.0210
x7.51±3.25
x2.77±1.03
x1.52±0.24
x1.87±0.46
p < 0.0001
p = 0.0371
p = 0.1819
p = 0.0005
x25.52±8.23
x3.21±0.91
x13.89±5.96
x3.28±1.23
p < 0.0001
p = 0.0098
p = 0.0232
p = 0.0034
DC-10
AHRinhDC-10
DC
AHRinhDC
H
Proliferation
% Proliferated Cells
Activation
IFNg Production
DC-10
AHRinh-lo DC-10
AHRinh DC-10
MTORact-lo DC-10
MTORact DC-10
AHRinh-lo/MTORact-lo DC-10
DC
Unstimulated
Polyclonal
G
RFI phospho-S6
<0.0001
0.0190
DC-10
MTORact-lo DC-10
MTORactDC-10

Figure S4. Relative to Figure 4 *AHR induced tolerogenic features are dependent on IL-10 and MTOR pathway inhibition*

$CD14^+$ monocytes isolated from peripheral blood (PB) were differentiated *in vitro* for 7 days in the presence of GM-CSF, IL-4 and IL-10 (DC-10), GM-CSF and IL-4 without (DC) or with the AHR agonist ITE (30μM) (AHRactDC) or the AHR inhibitor CH223191 (20μM) (AHRinhDC).

A) Cell yield upon 7 days of AHRactDC (green), DC (orange) and AHRinhDC-10 (plum) differentiation. Yield is expressed as number of recovered cells/number of plated cells. To allow direct comparison, yield of DC-10 (blue) and AHRinhDC-10 (light blue), already shown in Fig. S2A, are reported. *p*-values by Wilcoxon matched pairs test and mean fold change±SEM are indicated.

B) Expression of the CYP1B1 gene in AHRactDC (green), DC (orange) and AHRinhDC (plum) by ddPCR. The ratio of molecules per μL amplified by CYP1B1 primers / molecules per μL amplified by HPRT primers is shown in arbitrary units (n=6-10). *p*-values by Wilcoxon matched pairs test.

C) Violin plots displaying the median +/- interquartile range of the percentage of positive cells expressing the indicated marker by flow cytometry in AHRactDC (green), DC (orange) and AHRactDC (plum) at 7 days of differentiation (n=11-25). p values by Mixed effect model with Geisser-Greenhouse correction followed by Sidak's multiple comparison test (comparing each group against DC).

D) Representative flow cytometry plots showing the expression of the indicated markers in DC (top panels) and AHRinhDC (bottom panels).

E) Expression of the IL-10 (top) and IL-6 (bottom) gene in AHRactDC (green) and DC (orange) by ddPCR. The ratio of molecules per μL amplified by IL-10 or IL-6 primers / molecules per μL amplified by HPRT primers is shown in arbitrary units (n=8). *P*-values by Wilcoxon matched pairs test.

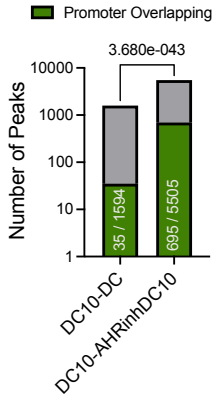
F) Side-by-side comparison of T cell proliferation, activation and IFNg production obtained in short term MLR assays by stimulating T cells with DC-10 (blue) vs AHRinhDC-10 (light blue) (n=16-22) and DC (orange) vs AHRinhDC (plum) (n=10-12). Results after co-culture of total (left panels) and naive (right panels). Percentages of proliferating (top) and $CD25^+HLA\text{-}DR^+$ activated (middle) CD4+ cells, assessed by flow cytometry; IFNg production (bottom), assessed in the culture supernatant by ELISA. *p*-values by Wilcoxon matched pairs test and mean fold change±SEM are indicated.

G) Phosphorylated-S6 (phospho-S6) fluorescence intensity (RFI, relative to unstained) DC-10 (blue), MTORact-lo DC-10 (pink) and MTORactDC-10 (magenta), assessed by flow cytometry at day 2 of differentiation. P values by Kruskal-Wallis test followed by Dunn's multiple comparison test (n=6-12).

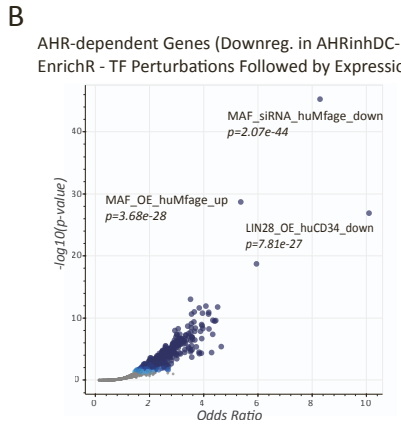
H) Raw percentages of proliferating (top) and $CD25^+HLA\text{-}DR^+$ (middle) $CD4^+$ cells, and IFNg quantification in culture supernatants (bottom) in MLR of $CD4^+$ cells stimulated by the indicated stimulus. Each color represents an independent donor, line at median.

Figure S5

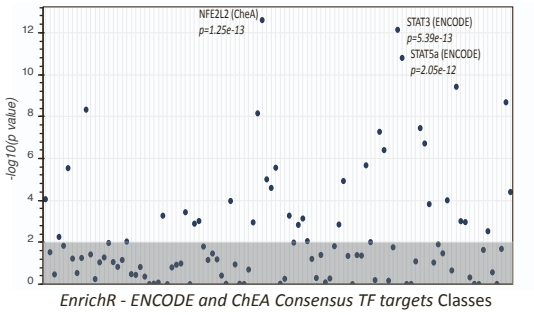
A



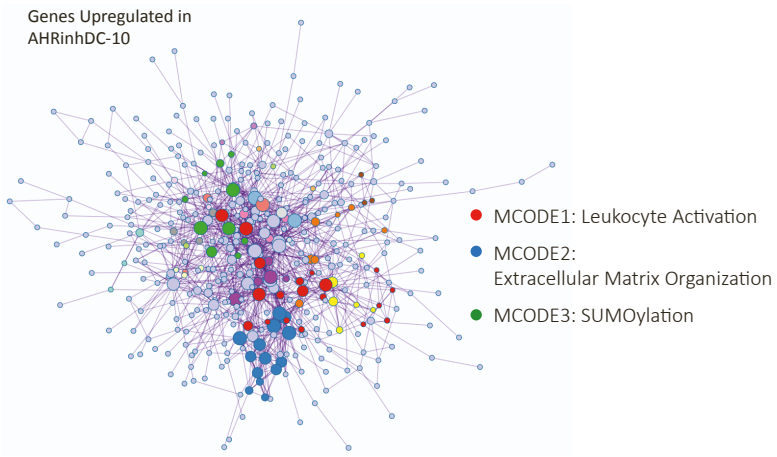
B



C



D



E

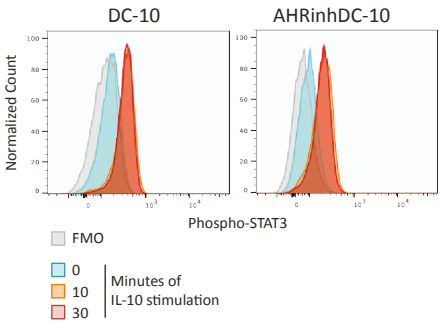


Figure S5. Relative to Figure 5 *AHR activity controls IL-10-induced gene expression patterns*
A) Number of DC-10-specific peaks obtained from the comparison with DC (*in vitro* DC-10 vs. DC; DC10-DC) and AHRinhDC-10 (*in vitro* DC-10 vs. AHRinhDC-10; DC10-AHRinhDC10) (grey) and fraction of these peaks overlapping with mapped coding gene promoters (green bars). *p*-value by Fisher's exact test.
B, C) Enrichr gene enrichment analysis of genes downregulated in AHRinhDC-10 compared to DC-10. Top 3 classes with relative *p*-values are shown. B) Volcano plot of transcription factor perturbation followed by gene expression categories and C) Manhattan plot of ENCODE and ChEA transcription factor consensus target genes categories.
D) Gene enrichment analysis of genes upregulated in AHRinhDC-10 compared to DC-10: protein-protein interaction network components by MCODE Metascape algorithm. Top 3 MCODE component nodes are colored. MCODE1 (red) = GO:0002274 (myeloid leukocyte activation), GO:0002366 (leukocyte activation involved in immune response), GO:0002263 (cell activation involved in immune response); MCODE2 (blue) = R-HSA-1474244 (Extracellular matrix organization), R-HSA-1474290 (Collagen formation), R-HSA-1650814 (Collagen biosynthesis and modifying enzymes); MCODE3 (green) = R-HSA-3108232 (SUMO E3 ligases SUMOylate target proteins), R-HSA-2990846 (SUMOylation), R-HSA-3899300 (SUMOylation of transcription cofactors). GO = Gene Ontology; R-HSA = Reactome Gene Sets. GO categories associated to all MCODEs are described in Table S5.
E) Representative flow cytometry histograms of phospho-STAT3 analysis in DC-10 (left) and AHRinhDC-10 (right) incubated with IL-10 for the indicated times. Fluorescence minus one (FMO) signal is also shown.

Figure S6

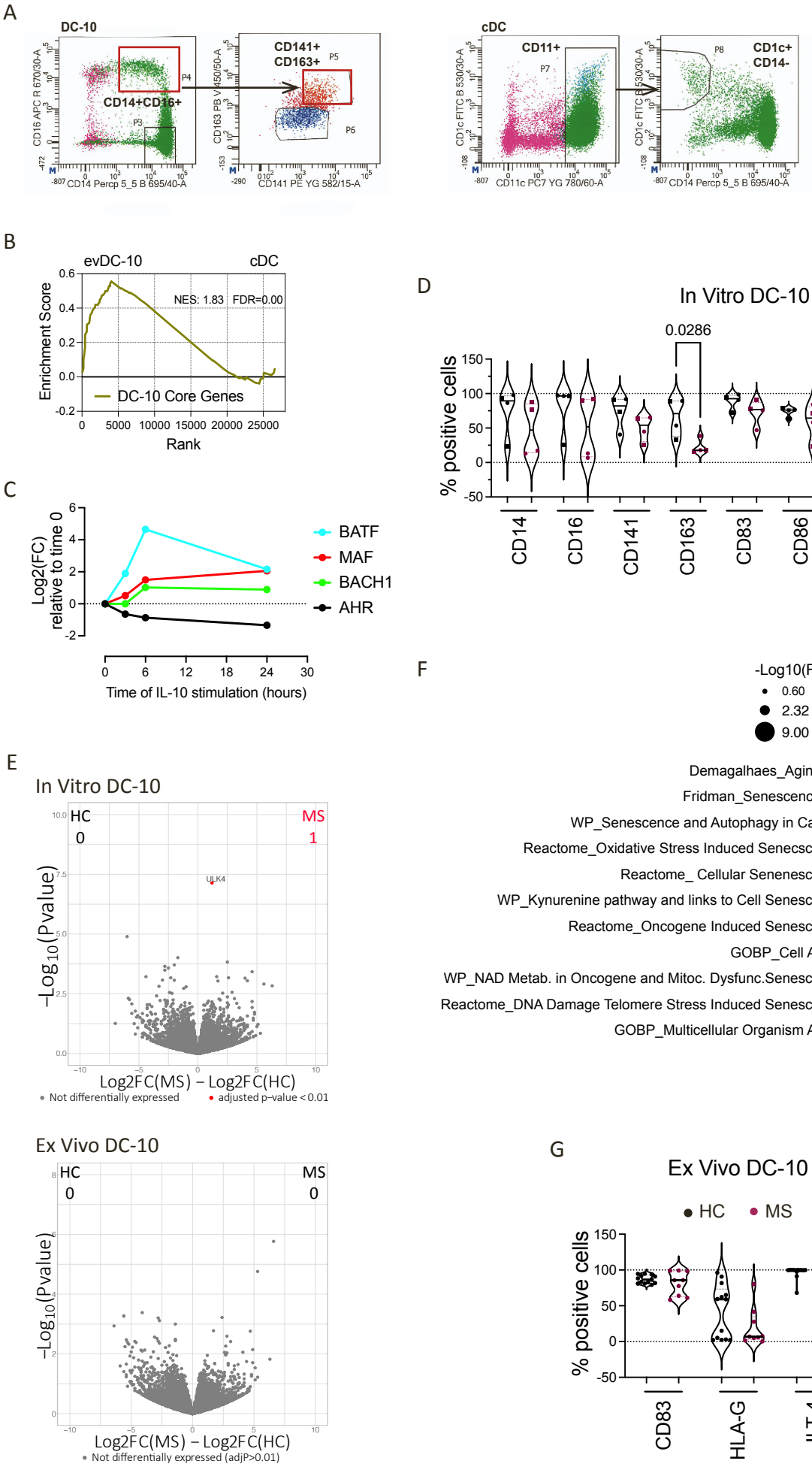

A
DC-10
CD14+CD16+
CD141+
CD163+
cDC
CD11+
CD1c+
CD14-
B
evDC-10
cDC
NES: 1.83 FDR=0.00
Enrichment Score
DC-10 Core Genes
Rank
C
Log2(FC) relative to time 0
BATF
MAF
BACH1
AHR
Time of IL-10 stimulation (hours)
D
In Vitro DC-10
0.0286
HC
MS
% positive cells
CD14
CD16
CD141
CD163
CD83
CD86
HLA-DR
HLA-G
ILT-4
E
In Vitro DC-10
HC
0
MS
1
ULK4
−Log10(Pvalue)
Log2FC(MS) − Log2FC(HC)
Not differentially expressed
adjusted p-value < 0.01
Ex Vivo DC-10
HC
0
MS
0
−Log10(Pvalue)
Log2FC(MS) − Log2FC(HC)
Not differentially expressed (adjP>0.01)
F
-Log10(FDR)
0.60
2.32
9.00
Monocytes
NES
Demagalhaes_Aging Up
Fridman_Senescence Up
WP_Senescence and Autophagy in Cancer
Reactome_Oxidative Stress Induced Senecscence
Reactome_ Cellular Senenescence
WP_Kynurenine pathway and links to Cell Senescence
Reactome_Oncogene Induced Senescence
GOBP_Cell Aging
WP_NAD Metab. in Oncogene and Mitoc. Dysfunc.Senescence
Reactome_DNA Damage Telomere Stress Induced Senescence
GOBP_Multicellular Organism Aging
MS
HC
G
Ex Vivo DC-10
HC
MS
% positive cells
CD83
HLA-G
ILT-4

Figure S6. Relative to Figure 6 *IL-10/AHR signature is active in vivo and is altered in autoimmune conditions*

A) Representative flow cytometry plots showing the sorting strategy for evDC-10 (left panels, red gates) and cDC (right panels) isolation from peripheral blood. Arrows represent sub-gating. Antibody and fluorochrome are shown on the axes.

B) GSEA of 91 DC-10 Core Genes (Table S1) in evDC-10 vs. cDC transcriptomes. Normalized enrichment score (NES) and FDR q value are reported. Leading-edge subset genes: 44.

C) Kinetic of expression upon IL-10 stimulation of the indicated transcription factors expressed as Log2 (Fold Change) versus time 0 monocytes (dotted line), assessed in Microarray data GSE82316.

D) Percentage of cells positive for the indicated markers in DC-10 differentiated from monocytes isolated from HC and MS donors (n=4). *p*-value by Mann-Whitney test.

E) RNA-seq volcano plots displaying differentially expressed genes (DEG) (red, adjusted p<0.01) by DESeq2, with top 10 DEG per group indicated, in MS vs. HC *in vitro* (top) and *ex vivo* (bottom) DC-10. The number of DEG in each group is indicated.

F) Normalized enrichment score (NES, color scale) and -Log10(FDR) (dot size) by GSEA of the indicated senescence-associated molecular signatures (see table S3) in the transcriptomes of MS and HC monocytes. FDR > 0.25 are not shown.

G) Percentage of circulating DC-10 expressing the indicated markers in healthy controls (HC) and multiple sclerosis (MS) donors by flow cytometry (n=9-13).