

Long noncoding RNA *MIR4435-2HG* enhances metabolic function of myeloid dendritic cells from HIV-1 elite controllers

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Supplemental Material

Supplementary Figure Legends

Supplementary Figure 1. The expression of lncRNAs in ECs. **(A)** The gating strategy for the identification of mDCs. **(B)** Venn diagram showing the overlaps of differentially expressed lncRNAs which were upregulated and downregulated in mDCs between EC vs. HIVN and EC vs. HAART. The overlapped lncRNAs were further filtered based on expression intensities (TPM+1 value and BaseMean) and fold changes. **(C)** The expression intensities (TPM+1) of the three other filtered candidate lncRNAs in mDCs were compared among ECs (n=20), HIVNs (n=15) and HAARTs (n=13). Kruskal-Wallis test was used as the statistical test. **(D)** The expression (TPM+1) of *MIR4435-2HG*, ENSG00000260257, ENSG00000222041 and ENSG00000269893 in monocytes, measured in RNAseq, was compared among ECs (n=20), HIVNs (n=15) and HAARTs (n=13). Kruskal-Wallis test was used as the statistical test.

Supplementary Figure 2. Oxidative phosphorylation in KEGG pathway analysis. **(A)** DEGs involved in Oxidative Phosphorylation predicted by IPA in Figure 1D were analyzed for KEGG pathway using DAVID tool. Genes marked with red stars were found in our RNAseq dataset.

Supplementary Figure 3. Correlation of MitoTracker or ROS co-expression with CD86 and *MIR4435-2HG* expression. **(A)** Correlation analysis using Spearman test was done between *MIR4435-2HG* expression and the frequency of MitoTracker⁺ CD86⁺ mDCs in ECs with (n=6) or without (n=6) Poly(I:C) stimulation. **(B)** Same as in (A), but the correlation was done between *MIR4435-2HG* expression and the frequency of ROS⁺ CD86⁺ mDCs.

Supplementary Figure 4. mDC phenotypes in ECs. (A) The frequencies of total mDCs ex vivo were compared between ECs (n=8) and HIVNs (n=13). Mann-Whitney was used as the statistical test. (B) The MFIs of *MIR4435-2HG*, determined by PrimeFlow in ex vivo analyzed pDCs and NK cells from ECs (n=8) and HIVNs (n=13) were compared. Unpaired t test was used as the statistical test. (C) Correlation analyses using Spearman test were done between MFIs of *MIR4435-2HG* and the mDC activation markers. (D) The *MIR4435-2HG* MFI was compared between activation marker-expressing mDCs and non-expressing mDCs in ECs and HIVNs. Wilcoxon matched pairs signed rank test was used to compare MFI between mDCs, whereas Mann Whitney test was used to compare MFI between cohorts. Red dots indicate ECs and blue dots indicate HIVNs.

* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001

Supplementary Figure 5. Seven clusters in mDCs. (A) The frequencies of the seven clusters in each cohort. Mann Whitney test was used to compare the frequencies of each cluster between ECs and HIVNs. (B) Heatmap displaying correlation matrix of expression of markers on mDCs in cluster 1 to 7 using Spearman correlation test. Selected correlation with cutoff of $R^2 \geq 0.3$ and P value < 0.05 were shown.

* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001

Supplementary Figure 6. *MIR4435-2HG* knockdown in mDCs. (A) The expression intensities (TPM+1) of *MIR4435-2HG* in mDCs (n=5) were compared between cells nucleofected with *MIR4435-2HG* siRNA and scramble siRNA (negative control). Wilcoxon matched pairs signed rank

test was used as the statistical test. **(B)** Heatmap displaying DEGs (FDR-adjusted P value < 0.05) involved in the mTOR pathway between mDCs nucleofected with *MIR4435-2HG* siRNA or scramble siRNA after 2µg/ml Poly(I:C) stimulation for 24 hours, measured using RNAseq. **(C)** Comparison of canonical pathways predicted by IPA for *MIR4435-2HG* correlated DEGs (EC vs. HIVN vs. HAART) from Figure 1D and *MIR4435-2HG* knocked-down vs. Scramble from Figure 4C. **(D)** The knockdown efficiency was shown as the fold change of *MIR4435-2HG* expression in mDCs post 24 hours of knockdown using nucleofected *MIR4435-2HG* siRNA was calculated relative to scramble siRNA as negative control. The Ct value was measured by RT-PCR and calculated using Livak ($2^{-\Delta\Delta Ct}$) method. Paired t test was used as the statistical test. **(E)** The pseudocolor plots displaying the viability of mDCs after nucleofection of siRNAs for 24 hours.

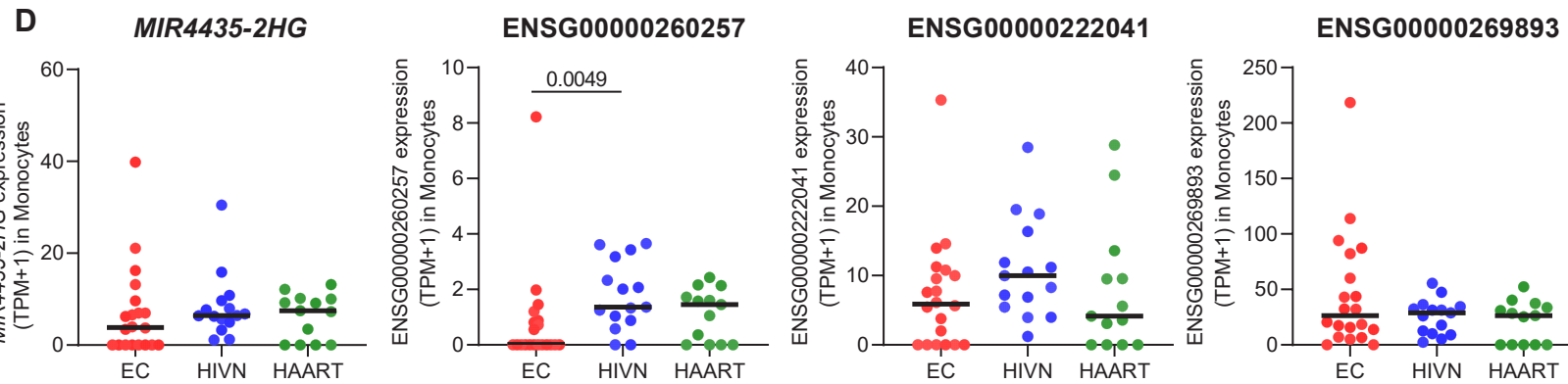
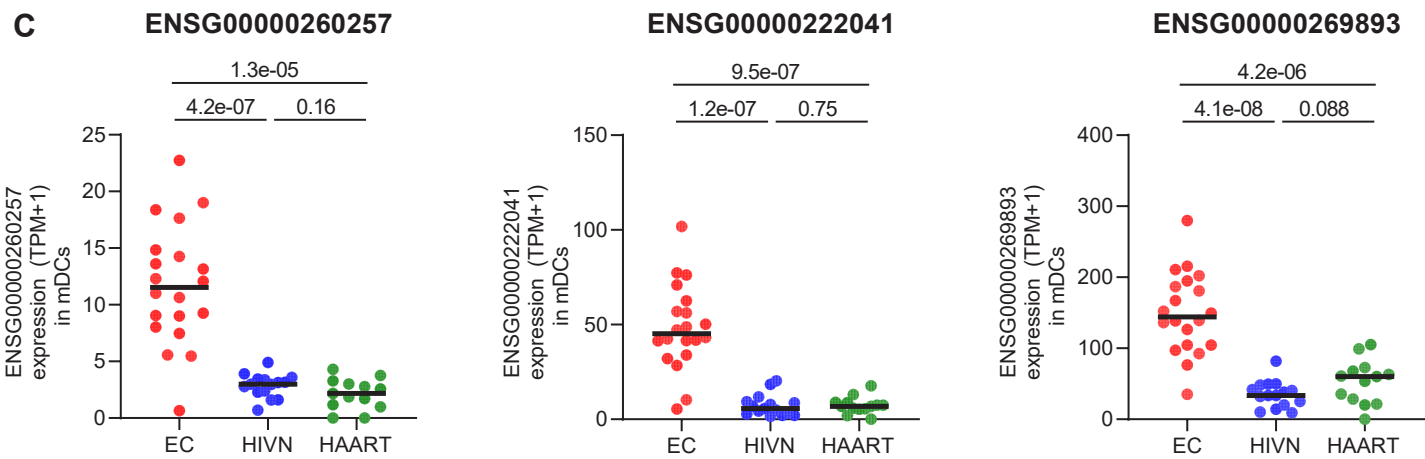
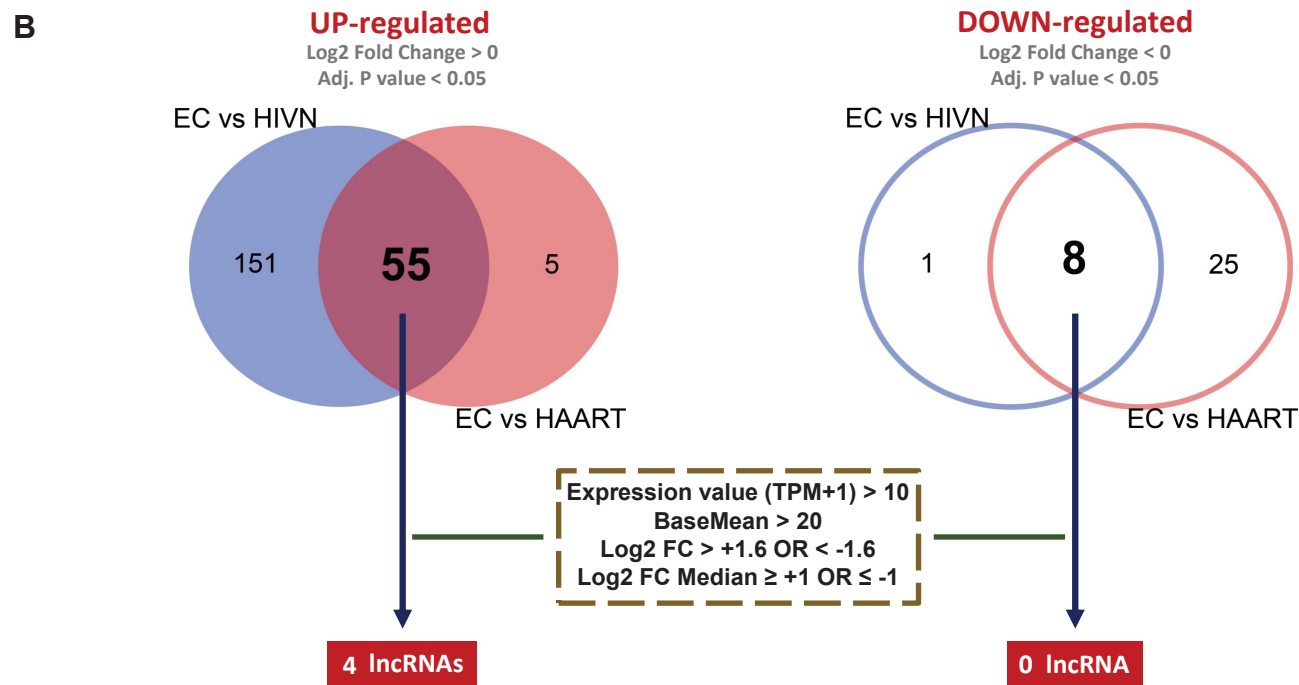
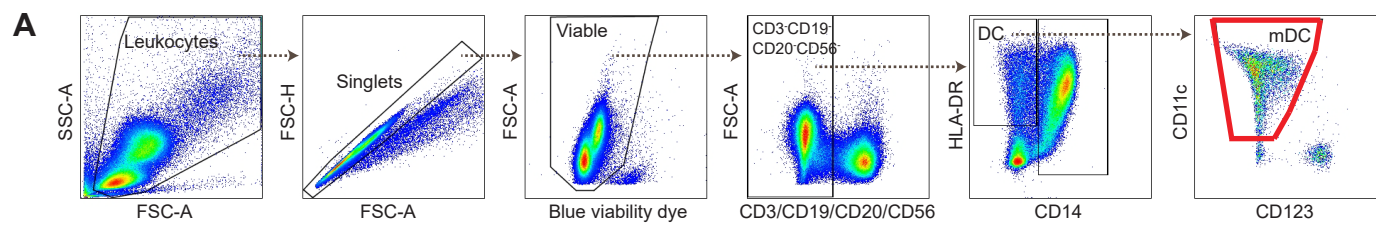
* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001

Supplementary Figure 7. TLR4 and TLR7/8 activation post *MIR4435-2HG* knockdown. **(A)** The fold changes of frequencies of mDCs co-expressing MitoTracker or ROS and activation markers (CD40, CD83 and CD86) were compared following 24 hours of *MIR4435-2HG* knockdown and LPS (TLR4 ligand) stimulation for another 24 hours. Wilcoxon matched pairs signed rank test was used as the statistical test. **(B)** Same as is (A), but the mDCs were stimulated with CL097 (TLR7/8 ligand). ns = not significant.

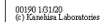
Supplementary Figure 8. Differentially enriched regions for H3K27me3 and H3K4me3. **(A)** Heatmap displaying significant (FDR-adjusted P value < 0.05) H3K27me3 (left) and H3K4me3 (right) enriched genomic loci corresponding to specific genes in ECs (n=4) vs. HIVNs (n=4). **(B)**

Volcano plot showing H3K27me3 (left) and H3K4me3 (right) enriched genomic loci in ECs vs. HIVNs. Red dots represent $-\log(P \text{ value}) > 1.3$ and $\log_2 \text{ fold change} > 1$.

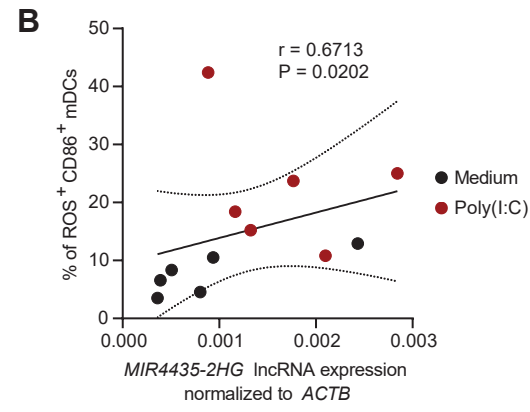
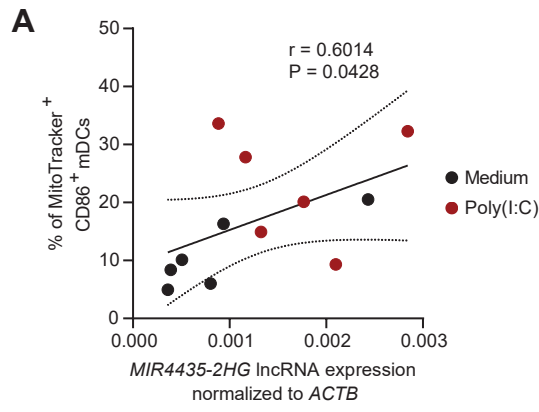
Supplementary Figure 1



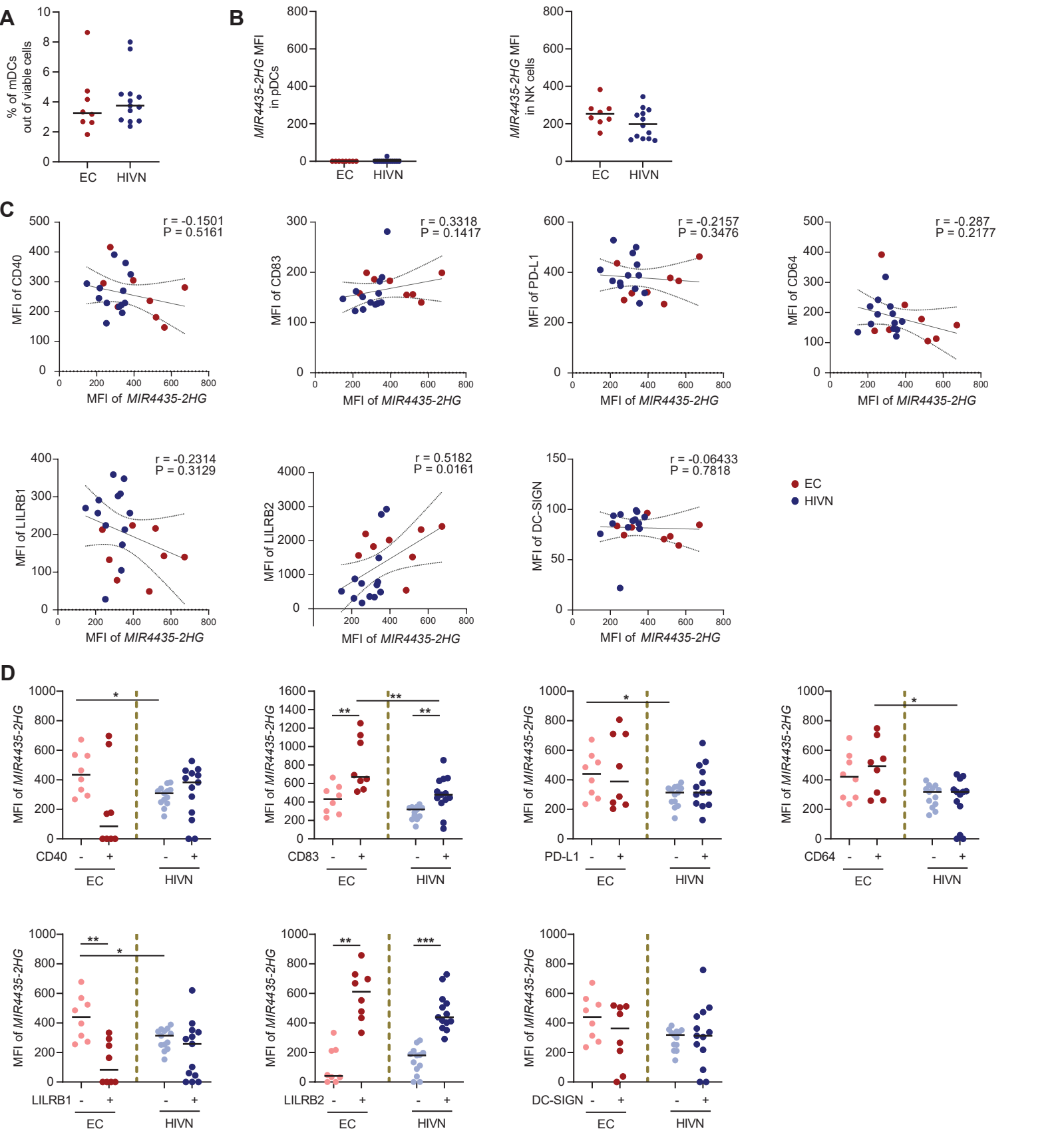
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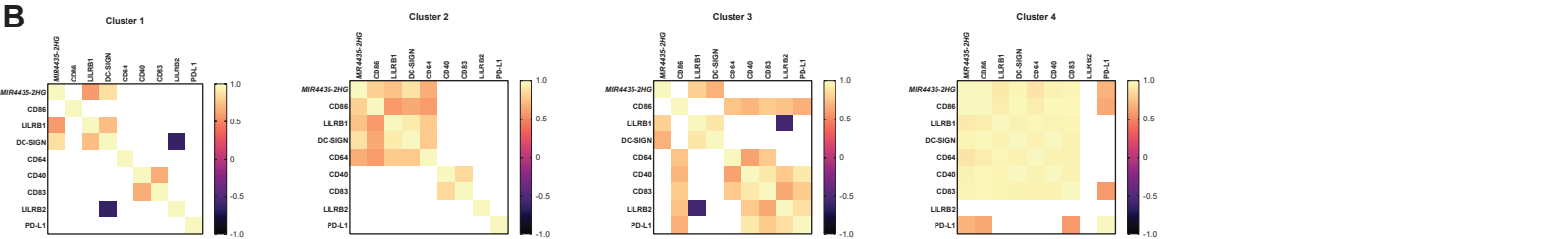
Supplementary Figure 3



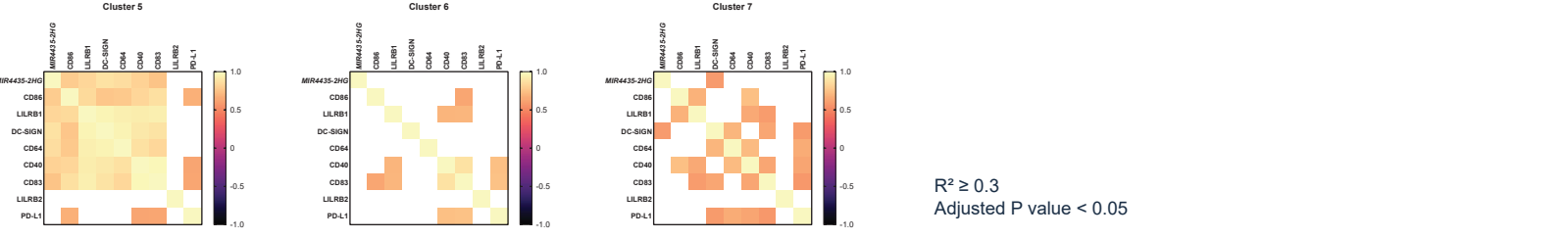
Supplementary Figure 4



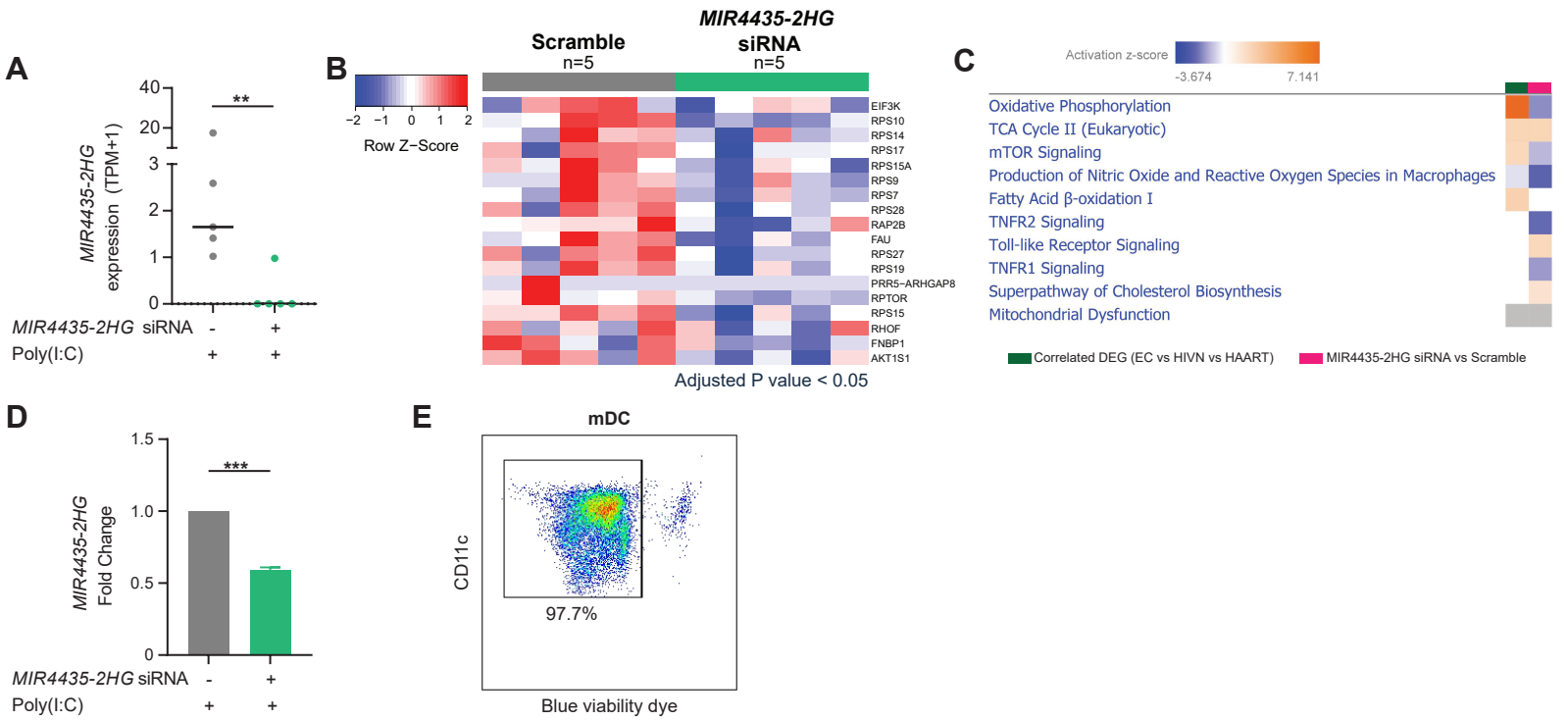
A Cluster 1 Clu



Cluster 5

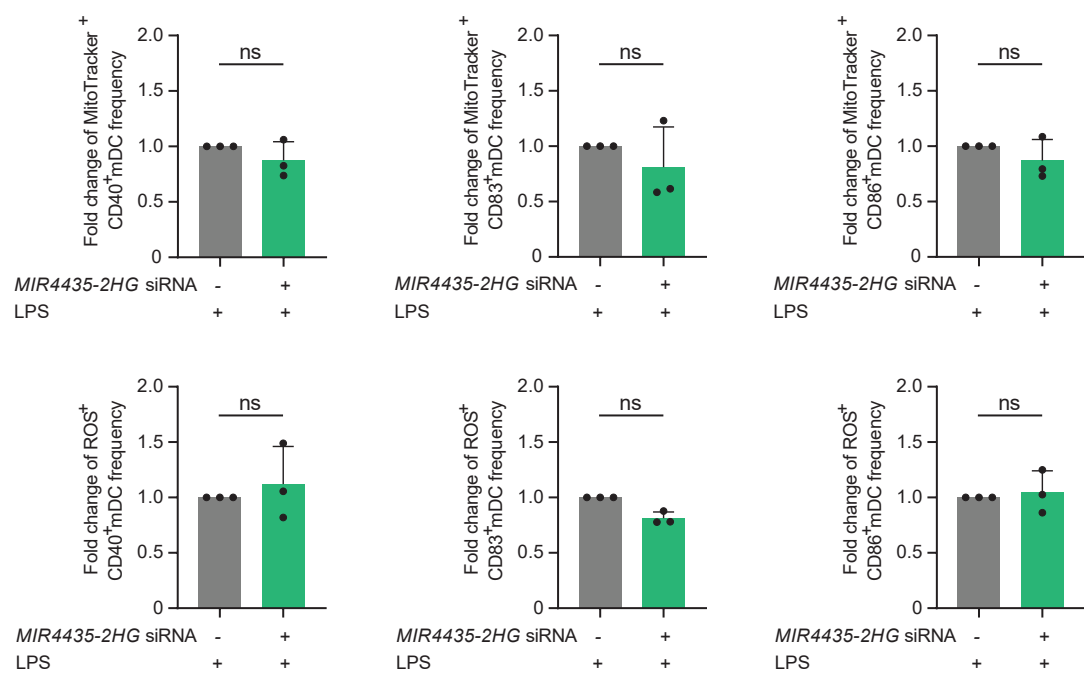


Supplementary Figure 6

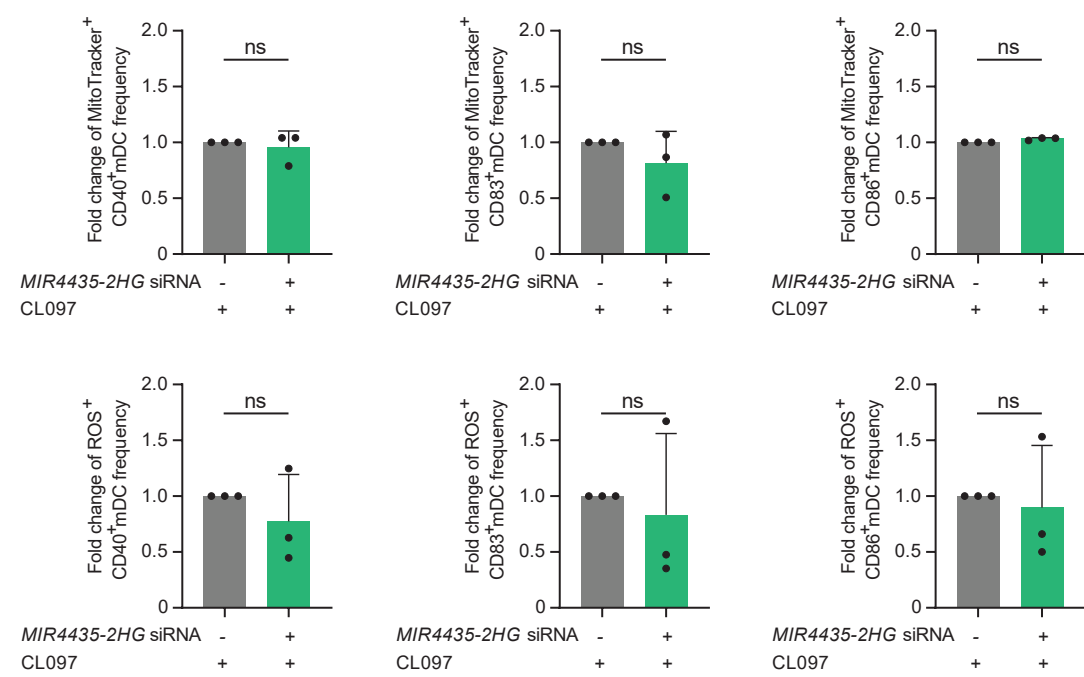


Supplementary Figure 7

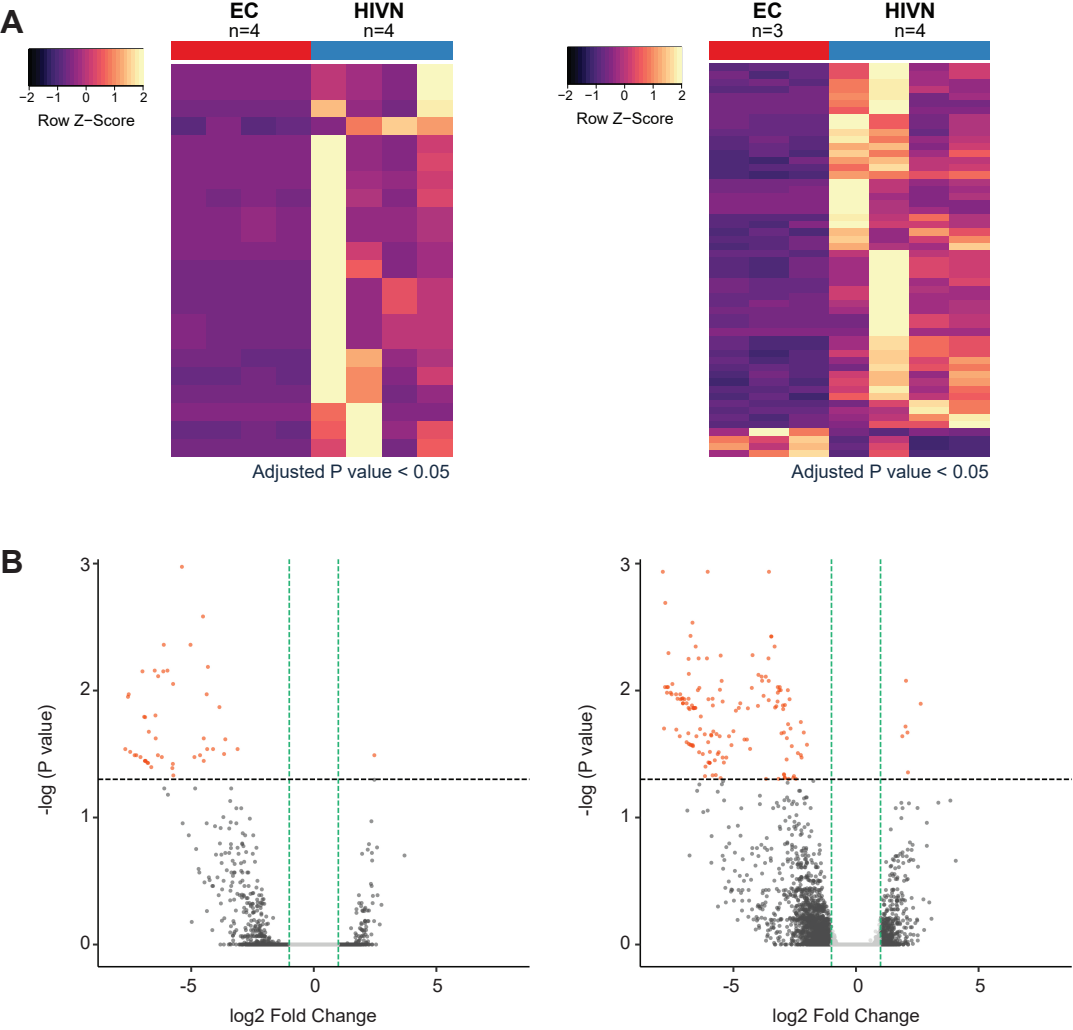
A



B



Supplementary Figure 8



Supplementary Table 1

Clinical and demographic characteristics of study cohorts

	Elite controllers (ECs)	HIV-1 negative donors (HIVNs)	HAART-treated individuals (HAARTs)	Chronic progressors (CPs)
Number of participants	23	15	13	9
Age in years*	50 (47-58)	30 (23-64)	53 (30-70)	53 (37-61)
Female (%) [‡]	13	53.3	7.7	22.2
CD4 counts (cells/mm ³)*	882 (407-2282)	N/A [†]	909 (398-1367)	528 (61-682)
Viral loads (copies/ml)*	under limit of detection	N/A [†]	under limit of detection	53500 (2650-169000)
HLA-B*27/B*57 (%) [#]	28.3	10	3.9	11.1
Time since diagnosis (year)*	18 (3-31)	N/A [†]	11 (1-27)	8 (1-25)

*Median with range.

[‡]P = 0.9701, using Chi-square test.

[#]P = 0.023, using Chi-square test for comparison among all four cohorts. ECs vs HAARTs, nominal P = 0.0119 (Chi-square test). No statistical difference for other group comparisons.

[†]Not Applicable

Supplementary Table 2

H3K27ac, H3K27me3 and H3K4me3 differentially enriched genes in mDCs from ECs

H3K27ac	H3K27me3	H3K4me3
Ensembl ID	Ensembl ID	Ensembl ID
ENSG00000104237	ENSG00000283839	ENSG00000273217
ENSG00000145934	ENSG00000259753	ENSG00000217128
ENSG00000177535	ENSG00000178852	ENSG00000169894
ENSG00000070770	ENSG00000169891	ENSG00000236438
ENSG00000261386	ENSG00000185010	ENSG00000163606
ENSG00000280214	ENSG00000157625	ENSG00000184384
ENSG00000133640	ENSG00000044115	ENSG00000251598
ENSG00000151067	ENSG00000179456	ENSG00000245008
ENSG00000112208	ENSG00000102054	ENSG00000182118
ENSG00000226803	ENSG00000222736	ENSG00000137101
ENSG00000145439	ENSG00000272970	ENSG00000115020
ENSG00000198677	ENSG00000198363	ENSG00000197694
ENSG00000125633*	ENSG00000082438	ENSG00000105929*
ENSG00000235066*	ENSG00000215196	ENSG00000260596
ENSG00000152894	ENSG00000176788	ENSG00000091972
ENSG00000102805	ENSG00000136040	ENSG00000168818
ENSG00000005812	ENSG00000163191	ENSG00000011114
ENSG00000283208	ENSG00000229021	ENSG00000178562
ENSG00000285080	ENSG00000138678	ENSG00000236816
ENSG00000137842	ENSG00000181790	ENSG00000258913
ENSG00000128815	ENSG00000221955	ENSG00000132514*
ENSG00000178209	ENSG00000185420	ENSG00000071242
ENSG00000141564*		ENSG00000148158
ENSG00000214425		ENSG00000117713
ENSG00000168038*		ENSG00000236453
ENSG00000115750		ENSG00000242086
ENSG00000139697		ENSG00000283426
ENSG00000136451*		ENSG00000215421
ENSG00000228804*		
ENSG00000113916*		
ENSG00000128908		
ENSG00000259617		

* Higher enrichment in ECs.

Supplementary Table 3

RT-PCR primer pairs

	5' → 3'
<i>MIR4435-2HG</i> forward	GATCTTCACAGCACAGTTCCT
<i>MIR4435-2HG</i> reverse	GGTTGGAAAAGATGCTGGTGA
<i>RPTOR</i> forward	CAGAGCTGGAGGATGAAGG
<i>RPTOR</i> reverse	CGATCCAGCATTCCAAGC
<i>ACTB</i> forward	CTGGAACGGTGAAGGTGACA
<i>ACTB</i> reverse	CGGCCACATTGTGAACTTTG

Supplementary Table 4***Antibodies for flow cytometry***

No	Markers	Fluorophores	Clone	Catalog number	Company
1	CD3	BUV395	UCHT1	563546	BD
2	CD19	BUV395	HIB19	740287	BD
3	CD20	BUV395	2H7	563782	BD
4	CD56	BUV395	NCAM16.2	563554	BD
5	CD14	BUV805	M5E2	612902	BD
6	CD40	BUV737	5C3	741847	BD
7	CD40	AF700	5C3	334328	Biolegend
8	HLA-DR	BUV661	G46-6	612980	BD
9	PD-L1 (CD274)	BUV563	MIH1	741423	BD
10	CD64	BV785	10.1	305044	Biolegend
11	Siglec6 (CD327)	BV711	767329	747910	BD
12	CD86	BV650	IT2.2	305428	Biolegend
13	CD16	BV480	3G8	566108	BD
14	DC-SIGN (CD209)	BV421	9E9A8	330118	Biolegend
15	AXL	BB700	108724	747866	BD
16	CD123	BB660	7G3	624295	BD
17	CD141	BB630	1A4	624294	BD
18	CD1c	PE-Cy7	L161	331516	Biolegend
19	CD11c	PE-Cy5.5	Bu15	MHCD11C18	Invitrogen
20	CD11c	BV421	Bu15	337226	Biolegend
21	LILRB1	PE	292305	FAB20171P-100	R&D
22	CD83	APC-Cy7	HB15e	305330	Biolegend
23	LILRB2	AF700	287219	FAB2078N-100	R&D