Figure S1. Changes in chromatin accessibility and gene expression over time for select genes in MDDCs during infection with HIV-1-GFP

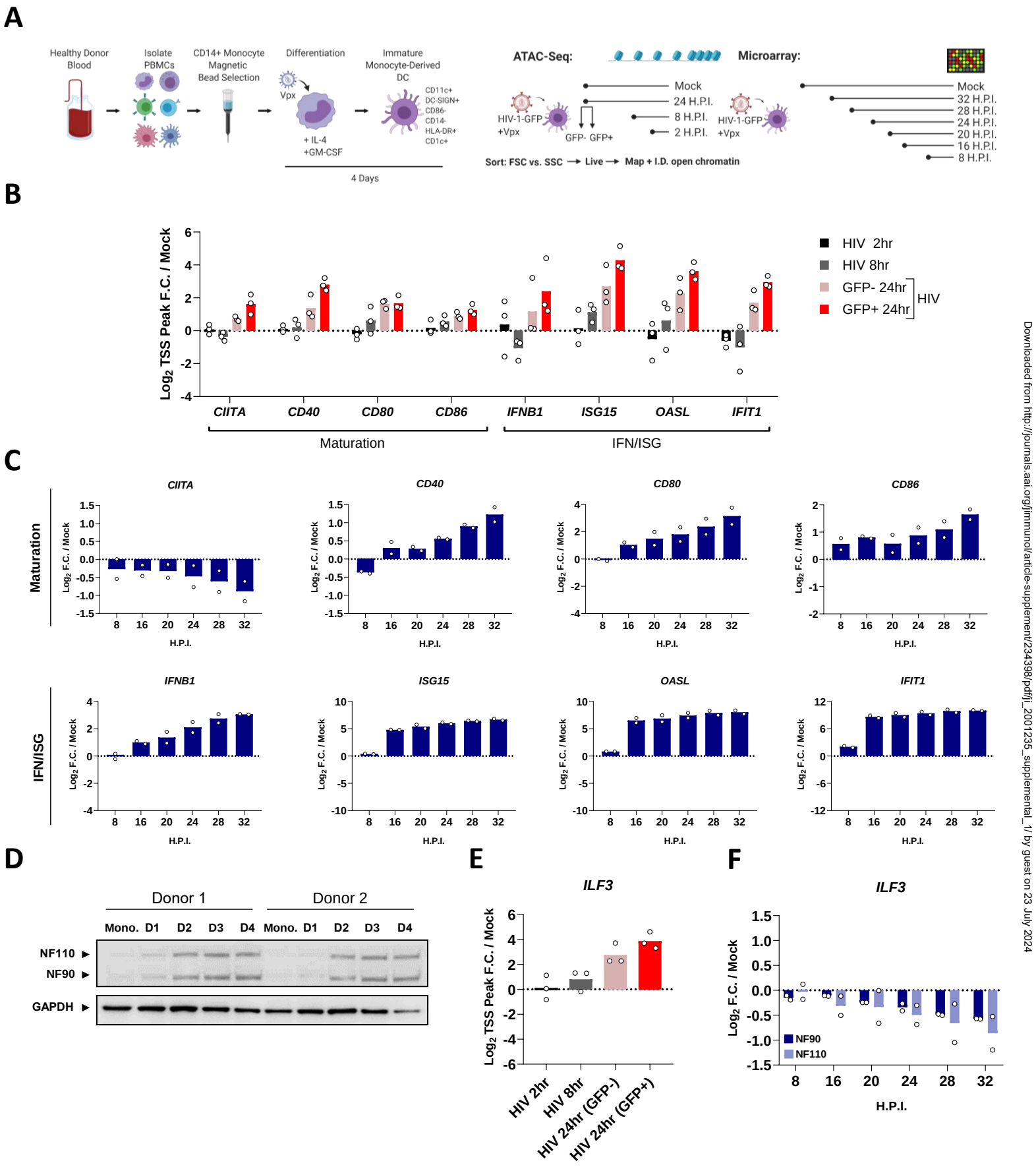

Figure S1. Changes in chromatin accessibility and gene expression over time for select genes in MDDCs during infection with HIV-1-GFP. (A) Schematic illustrating MDDC derivation and subsequent HIV-1-GFP infection at different timepoints for ATAC-seq and transcriptome analysis as described in (18). (B) Log2 fold change compared to mock infection of the normalized signal at the transcription start sites (TSS) of the indicated genes, averaged over 3 independent donors, at 2, 8, or 24 (GFP+/GFP-) hours following of HIV-1-GFP infection of MDDCs (See Materials and Methods). (C) Expression changes relative to mock infection for genes at the indicated time points following HIV-1-GFP infection of MDDCs, averaged over two donors, for the genes shown in (B). (D) Western blot from 2 donors of CD14+ monocytes (Mono. Day 0) differentiated with IL-4 and GM-CSF through Day 4 with samples taken every 24 hours. Stained for ILF3 and GAPDH as a loading control. (E) Log2 fold change of ILF3's TSS peak as described in (B). (F) Log2 of ILF3 gene expression, separated by isoform (NF90 and NF110) as described in (C).

Figure S2. ILF3 regulates expression of genes associated with myeloid cell maturation.

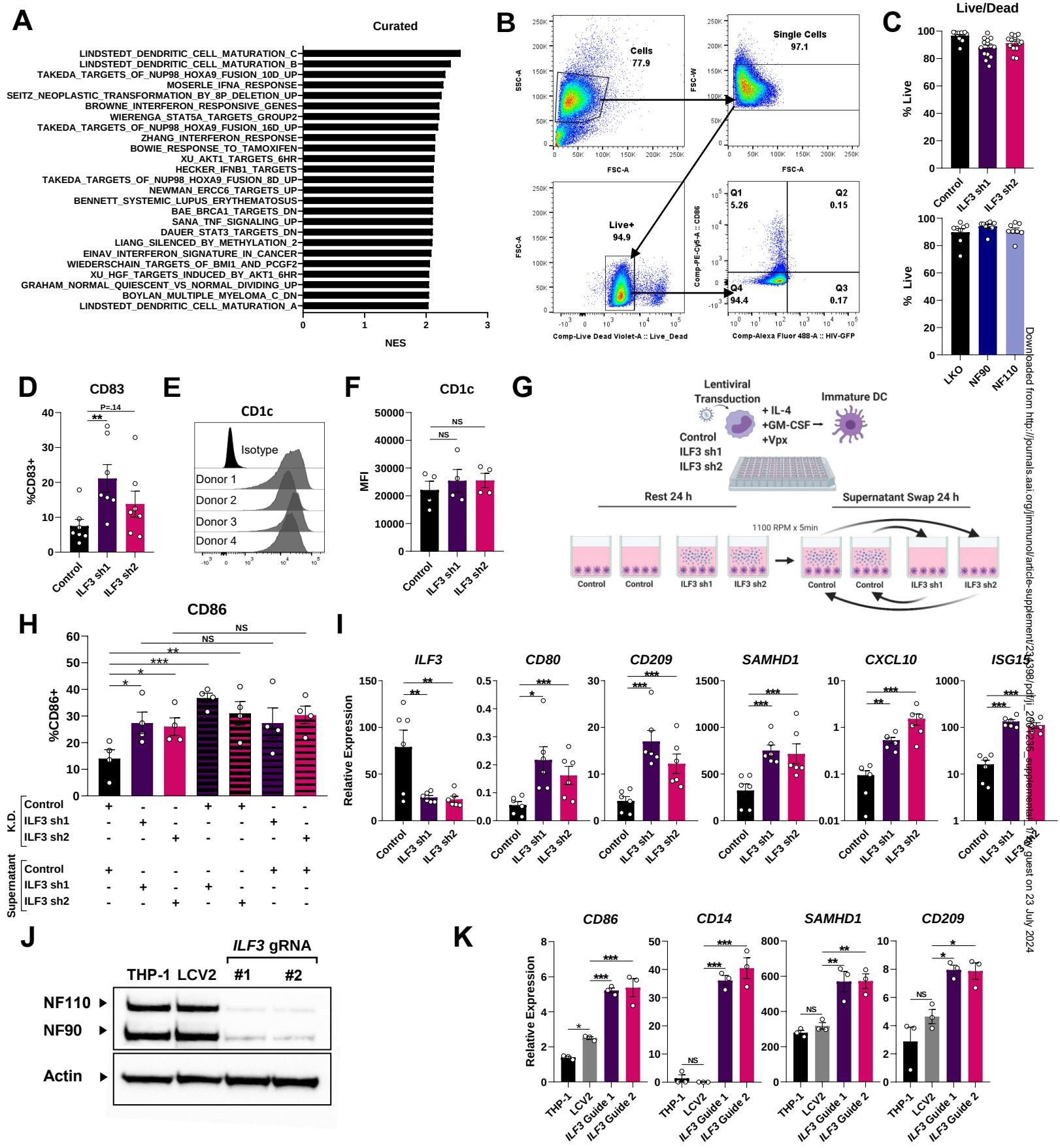

Figure S2. ILF3 regulates expression of genes associated with myeloid cell maturation. (A) Top 25 Curated gene sets (gene set member size 200 > x > 15) with an FDR < 0.05 from GSEA analysis of sh1+sh2 ILF3 vs Control shRNA ranked by t-test. Results represented as Normalized Enrichment Scores. (B) Gating scheme for all flow cytometry experiments listed in this manuscript. Day 6 MDDCs were prepared as described for flow cytometry analysis (see Materials and Methods). Relevant cells were gated on SSC vs. FSC, then singlets were selected, then "live" cells were selected via an amine reactive fixable viability dye, and subsequent surface markers, intracellular markers, and GFP from HIV-1-GFP were analyzed. (C) Live/Dead staining of % Live MDDCs in either ILF3 knockdown (Control, ILF3 sh1, ILF3 sh2) or NF90/NF110 overexpression experiments (LKO, NF90, NF110). (D) Quantification of CD83 expression on live-gated MDDCs, n=7 donors over 2 separate experiments. (E) Histogram of CD1c expression across n=4 donors compared to relevant isotype control (Donor 1 used as a representative isotype example) using control MDDCs. (F) CD1c MFI quantification in control, ILF3 sh1, and ILF3 sh2 conditions across n=4 donors. (G) Illustration of MDDC supernatant swap experiment in control or ILF3 knockdown MDDCs. MDDCs were transduced with control shRNAs or shRNAs targeting ILF3. At day 4 post-transduction, the culture media was replaced with fresh media and the cells were cultured for an additional 24 hours. Media was then exchanged across conditions as indicated and the cells were cultured for another 24 hours before scoring surface expression of CD86 by flow cytometry. (H) Quantification of CD86 expression in the experiment described in G. n = 4 donors. (I) qPCR analysis of the indicated genes following shRNA knockdown of ILF3 in THP-1 cells. n = 6 independent transductions. Statistics were calculated using a one-way ANOVA with Dunnet's test for multiple comparisons. (J) Western blot depicting expression of NF90, NF110, and actin from whole cell lysates of parental THP-1 cells compared to cells transduced with a non-targeting lentiCRISPR control vector (LCV2) or two independent vectors targeting ILF3, at day 9 post-transduction. (K) qPCR analysis of targets from Day 9 CRISPR knockout of ILF3 in THP-1 cells. n = 3 technical replicates, one-way ANOVA using Dunnet's test for multiple comparisons.

Figure S3. ILF3 regulates innate responses to HIV-1-GFP and cGAMP.

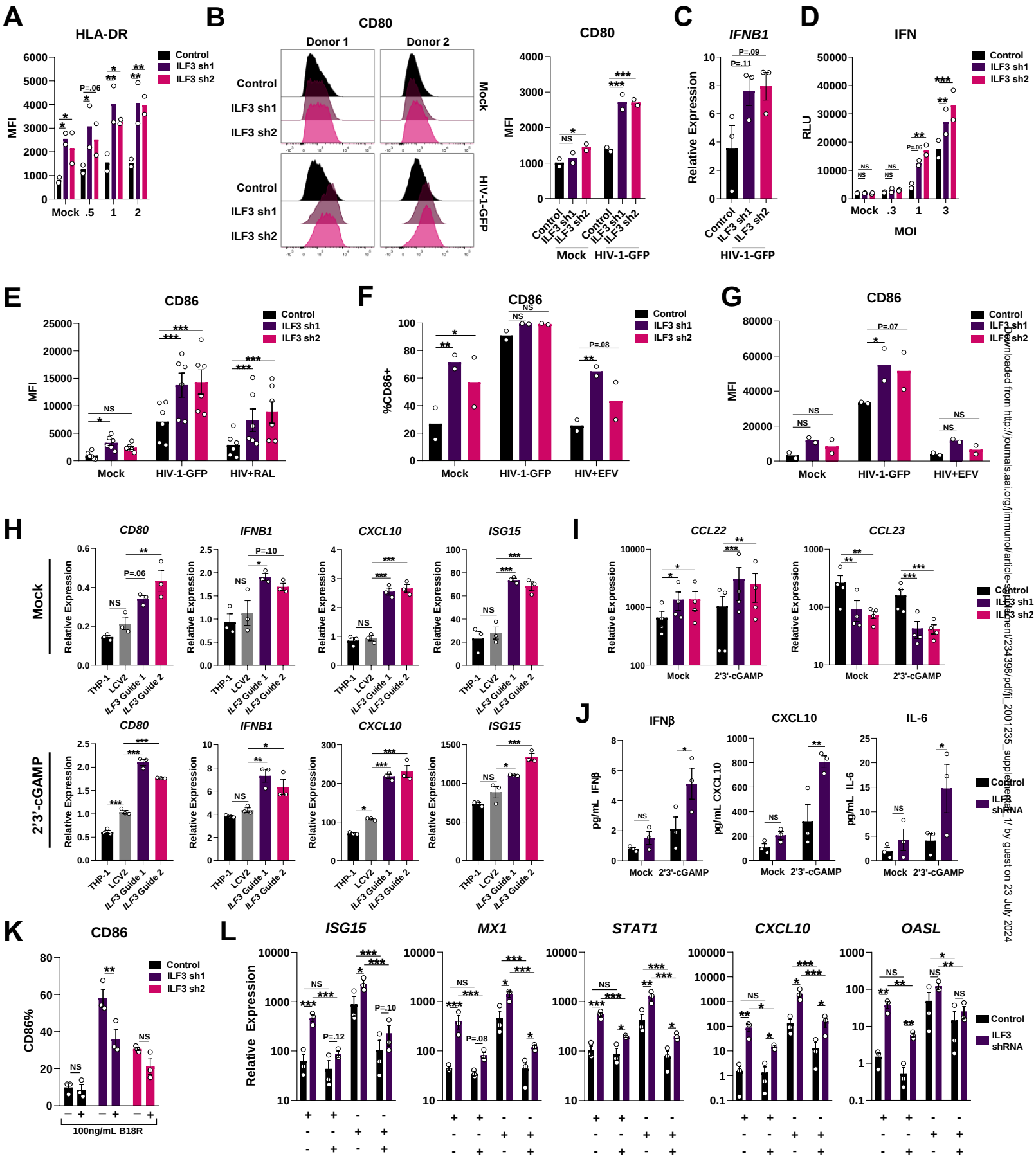

Figure S3. ILF3 regulates innate responses to HIV-1-GFP and cGAMP. (A) Flow cytometry analysis of HLA-DR MFI from n=2 donors in MDDCs at the indicated MOIs of HIV-1-GFP 48 hours post-infection, (B) Histogram and flow cytometry analysis of %CD80+ cells at an HIV-1-GFP MOI of 2, 48 hours post-infection. n = 2 donors. (C) qPCR expression of IFNB1 in HIV-1-GFP-infected MDDCs at an MOI of 1. n=3 donors. (D) Type I IFN bioassay of supernatants from MDDCs infected with HIV-1-GFP at the indicated MOIs for 48 h. n = 2 donors. (E) Flow cytometry analysis of CD86 MFI in MDDCs infected with HIV-1-GFP (MOI = 3) for 48 h, ± RAL. n = 6 donors from 2 experiments. (F) Flow cytometry analysis of CD86%+ in MDDCs infected with HIV-1-GFP (MOI = 3) for 48 h, ± EFV. n = 2 donors. (G) Flow cytometry analysis of CD86 MFI from (E). (H) qPCR analysis of targets from day 9 CRISPR knockout of ILF3 in THP-1 cells untreated or treated with 2'3'-cGAMP (3ug/mL, 7 hours). n = 3 technical replicates. (I) qPCR expression of DC maturation chemokine markers CCL22 and CCL23 in mock or 1μg/mL 2'3'-cGAMP-treated control or ILF3 knockdown MDDCs in n=4 donors. (J) ELISAs for IFNβ, CXCL10, and IL-6 secretion in control or ILF3 knockdown MDDCs in mock or 1μg/mL 2'3'-cGAMP-treated conditions in n=4 donors. (K) Flow cytometry analysis of CD86 expression on live-gated control or ILF3 knockdown MDDCs 48 hours post-media refresh on day 4. n=3 donors were mock or B18R-treated at 100ng/mL at t=0 and t=24. (L) qPCR analysis of ISGs in control or ILF3 knockdown MDDCs. Cell were either mock or 1μg/mL 2'3'-cGAMP-treated for 7 hours. B18R was added 1 hour prior to 2'3'-cGAMP stimulation at 100ng/mL. For (A-G, I-L), statistics were calculated with a paired mixed-effects model using Dunnet's test for multiple comparisons. For (H) a one-way ANOVA was used with Dunnet's test for multiple comparisons.

Figure S4. Overexpression of NF110 impacts expression of non-coding RNAs and protein coding genes associated with cholesterol homeostasis.

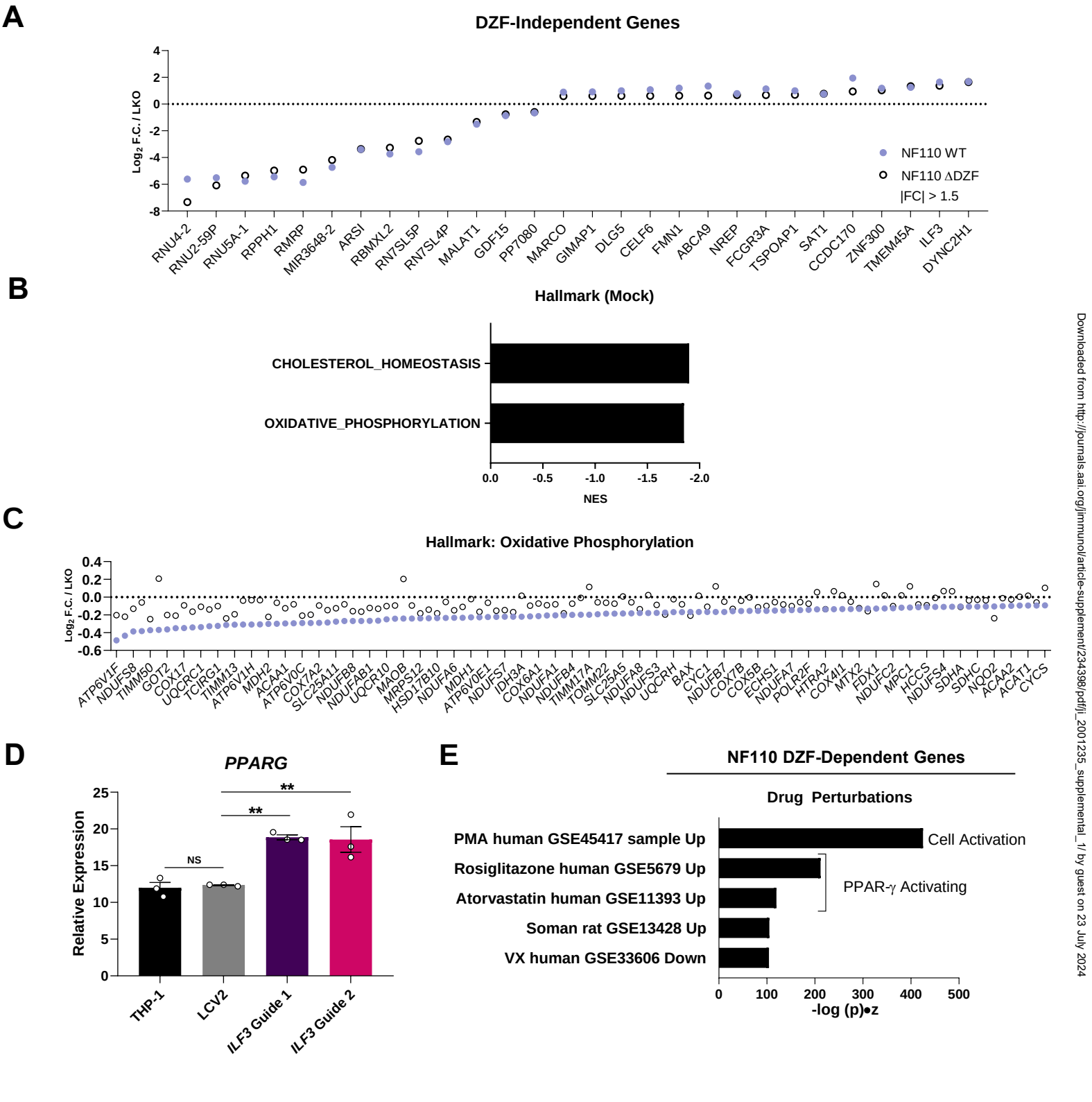

Figure S4. Overexpression of NF110 impacts expression of non-coding RNAs and protein coding genes associated with cholesterol homeostasis. (A) Dot plot of DZF-independent genes as defined as more than 50% of the expression change in a gene induced by overexpression of NF110 being retained when NF110ΔDZF is overexpressed with an FDR < 0.05, Avg Log2 CPM > 1. NF110 wt differential gene expression represented in closed circles and NF110ΔDZF is represented in open circles. Points represent averaged data from n = 4 donors. (B) GSEA Hallmark gene set analysis (gene sets containing 15 < x < 200 members) of untreated MDDCs overexpressing NF110 wt, ranked by t-test, represented as Normalized Enrichment Score, FDR < 0.05. (C) Hallmark Oxidative Phosphorylation core enrichment from (B) represented as NF110 wt or ΔDZF compared to LKO control, with no set FDR. Every other gene is labeled on the x-axis due to space constraints. (D) qPCR analysis of targets from Day 9 CRISPR knockout of ILF3 in untreated THP-1 cells. Representative of 3 technical replicates, using a one-way ANOVA using Dunnet's test for multiple comparisons. (E) EnrichR Drug Perturbation analysis of NF110 DZF-dependent genes (defined as less than 50% of the expression change in a gene induced by overexpression of NF110 being retained when NF110ΔDZF is overexpressed with an FDR < 0.05). Scores represented as Combined Score (-log (p) • z).