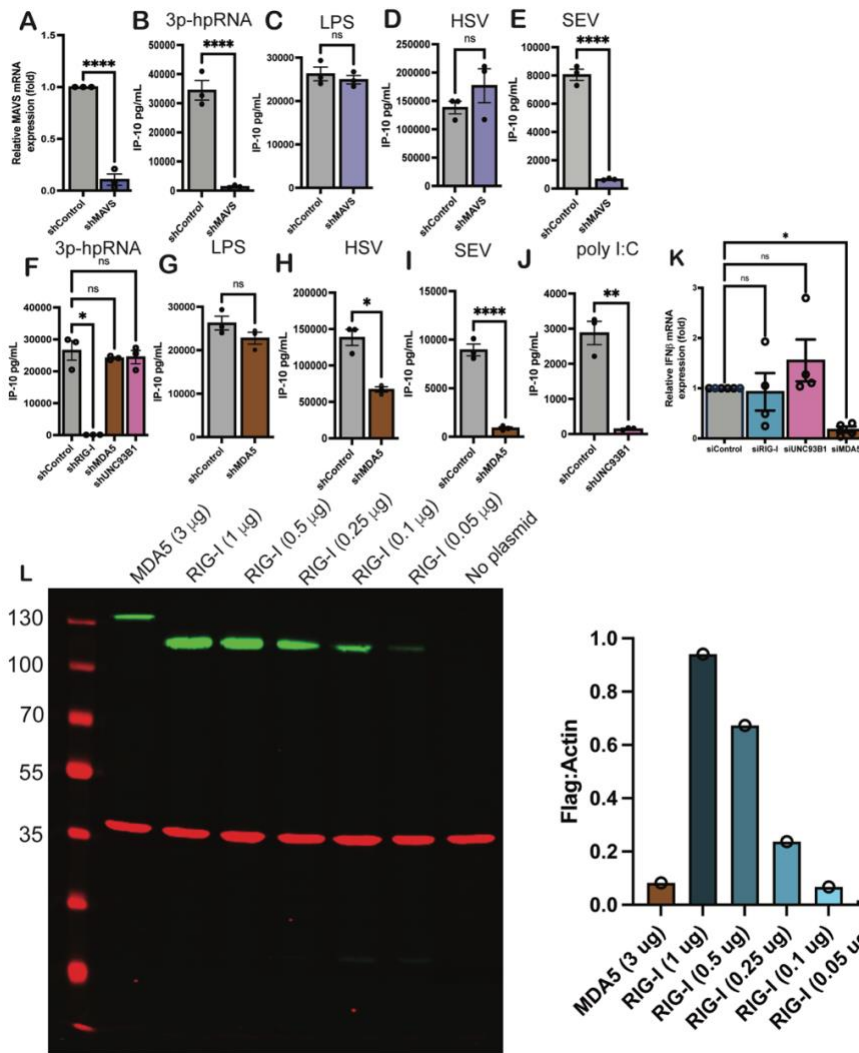
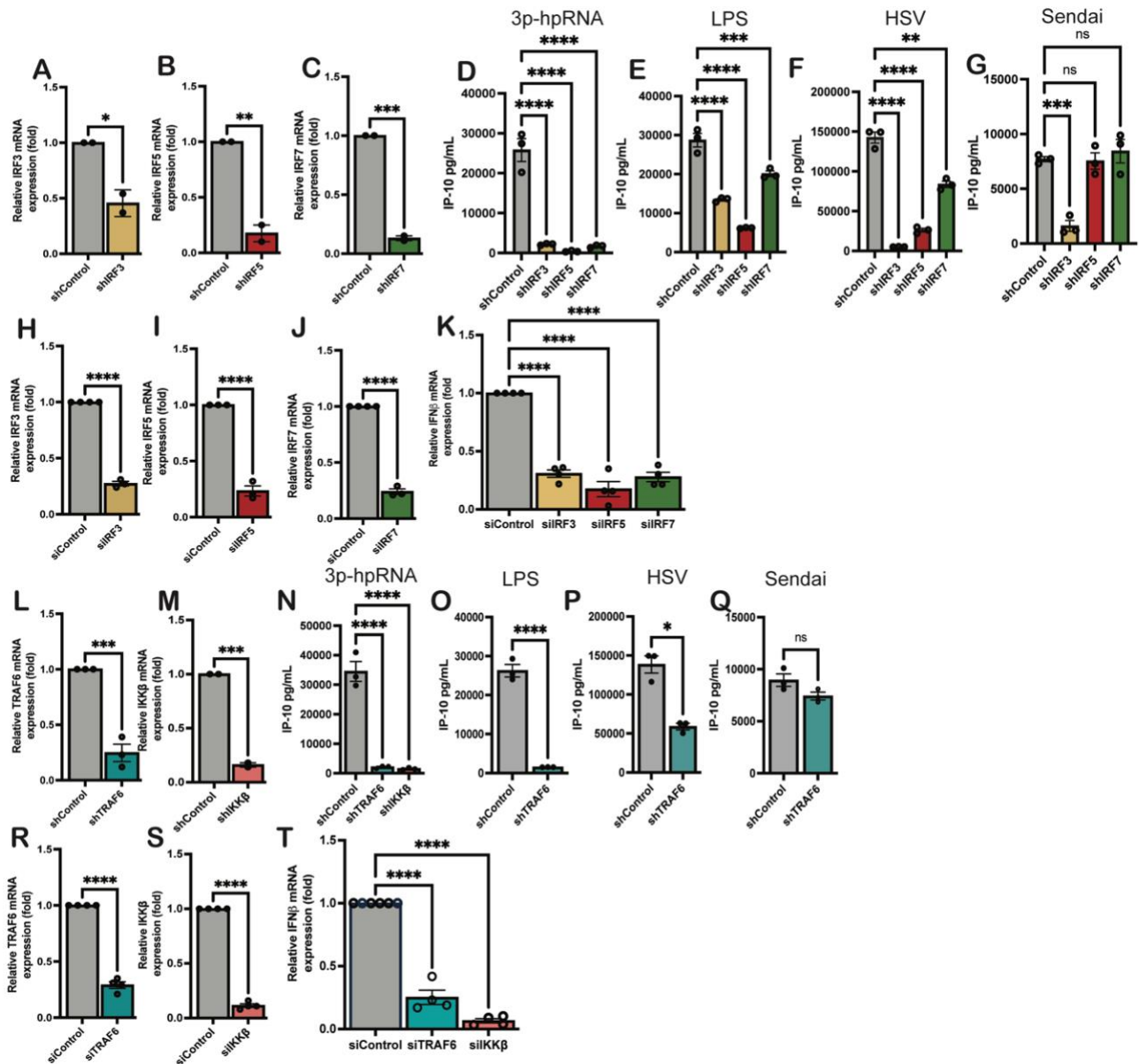


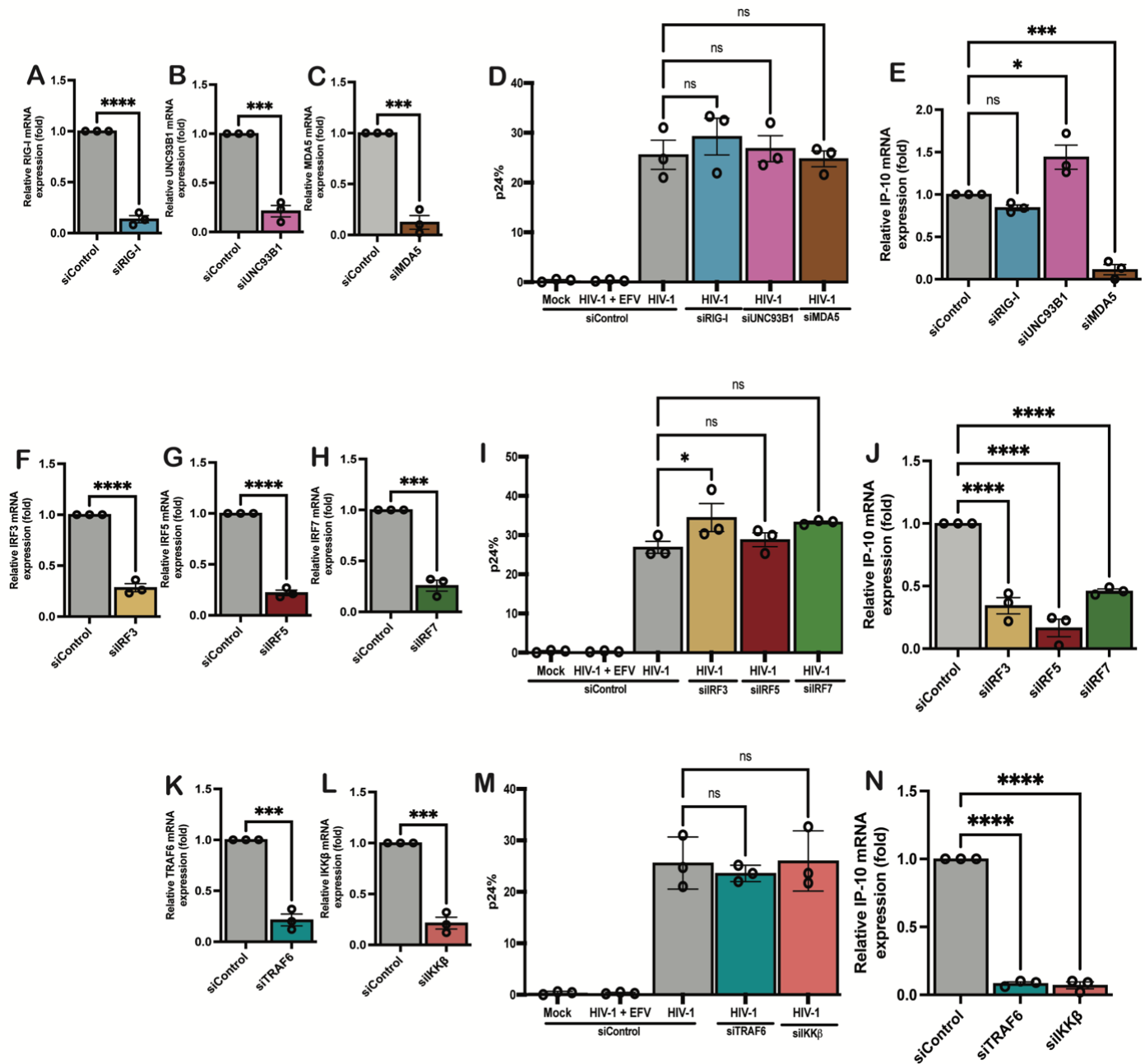
Supplemental Material



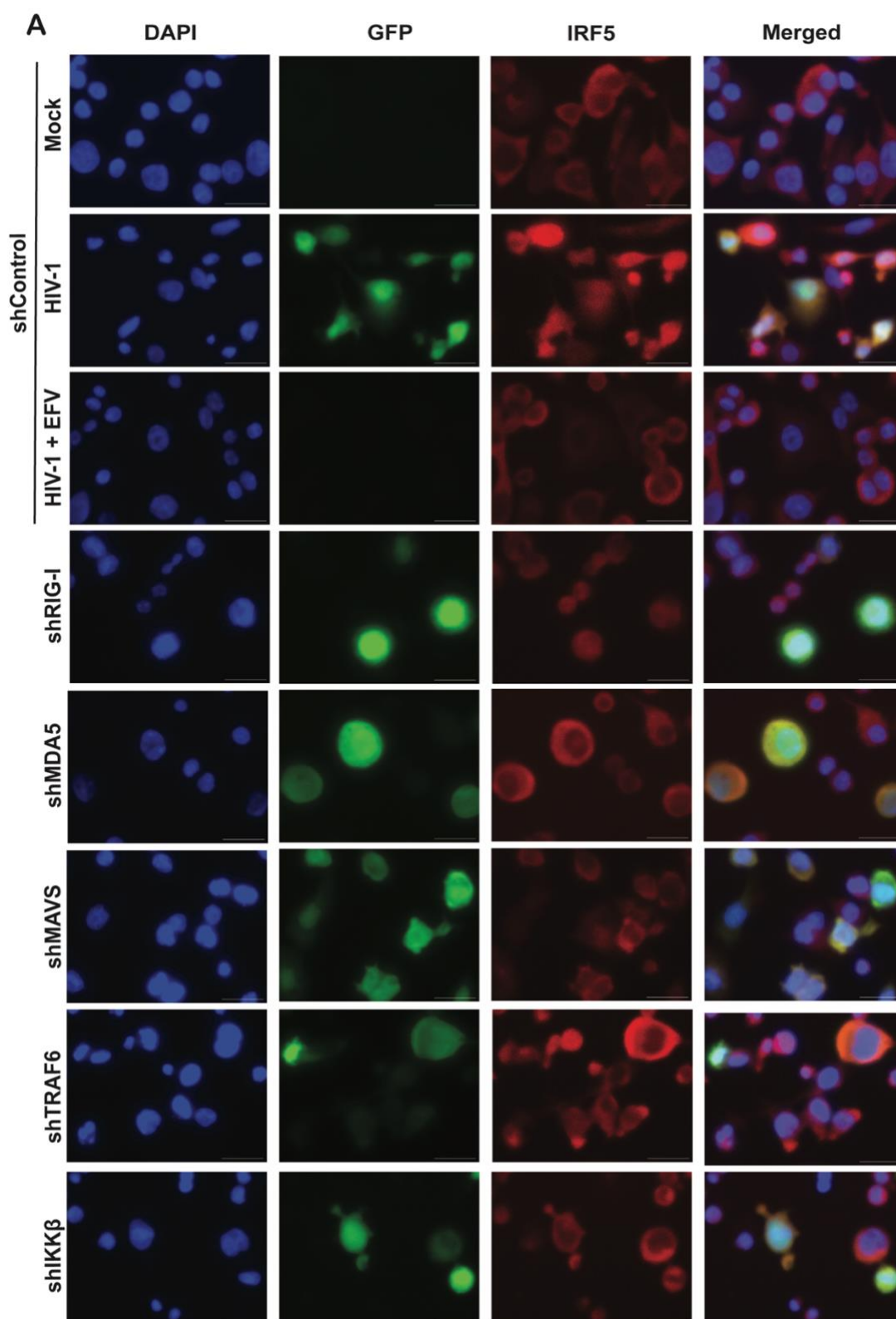
Supplementary Figure 1 (A) Knockdown of MAVS expression in THP-1/PMA macrophages was validated using RT-qPCR. (B-E) MAVS kd THP-1/PMA cells were treated with 3p-hpRNA(2.5 ng/mL), LPS(100 ng/ml), infected with HSV (MOI 0.1) or Sendai virus (SEV)(MOI 1), and supernatants were harvested 24 h post-stimulation or 48 hpi for analysis of IP-10 production via ELISA. (F-J) THP-1/PMA cells with knock-down of RIG-I/MDA5/UNC93B1 expression were treated with (F) 3p-hpRNA(2.5 ng/mL), (G) LPS(100 ng/mL), or infected with (I) HSV (MOI 0.1) or (I) Sendai virus (MOI 1), or treated with (J) HMW polyI:C(10 mg/ml). Supernatants were harvested 24 hours post-stimulation or 48 hpi for analysis of IP-10 production via ELISA. (K) siRNA-transfected MDMs were infected with LaiΔenv GFP/G (MOI 1) in the presence of dNs and harvested for analysis of IFNβ mRNA expression via RT-qPCR at 2 dpi. (L) HEK293T cells were transfected with varying concentrations of RIG-I-Flag, or a single amount of MDA5-Flag expression plasmids. Expression of MDA5/RIG-I-Flag was assessed via Western blot. Flag intensity was normalized to that of actin and plotted as a graph. Data is displayed as mean ± SEM with each dot representing an experiment (A-J) or individual donor (K). Statistical significance assessed via unpaired t-test (A-E,F-J) or 1-way ANOVA (K) with Dunnett's multiple comparisons analysis. *: p < 0.05; **: p < 0.01, ****: p < 0.0001, ns = not significant.



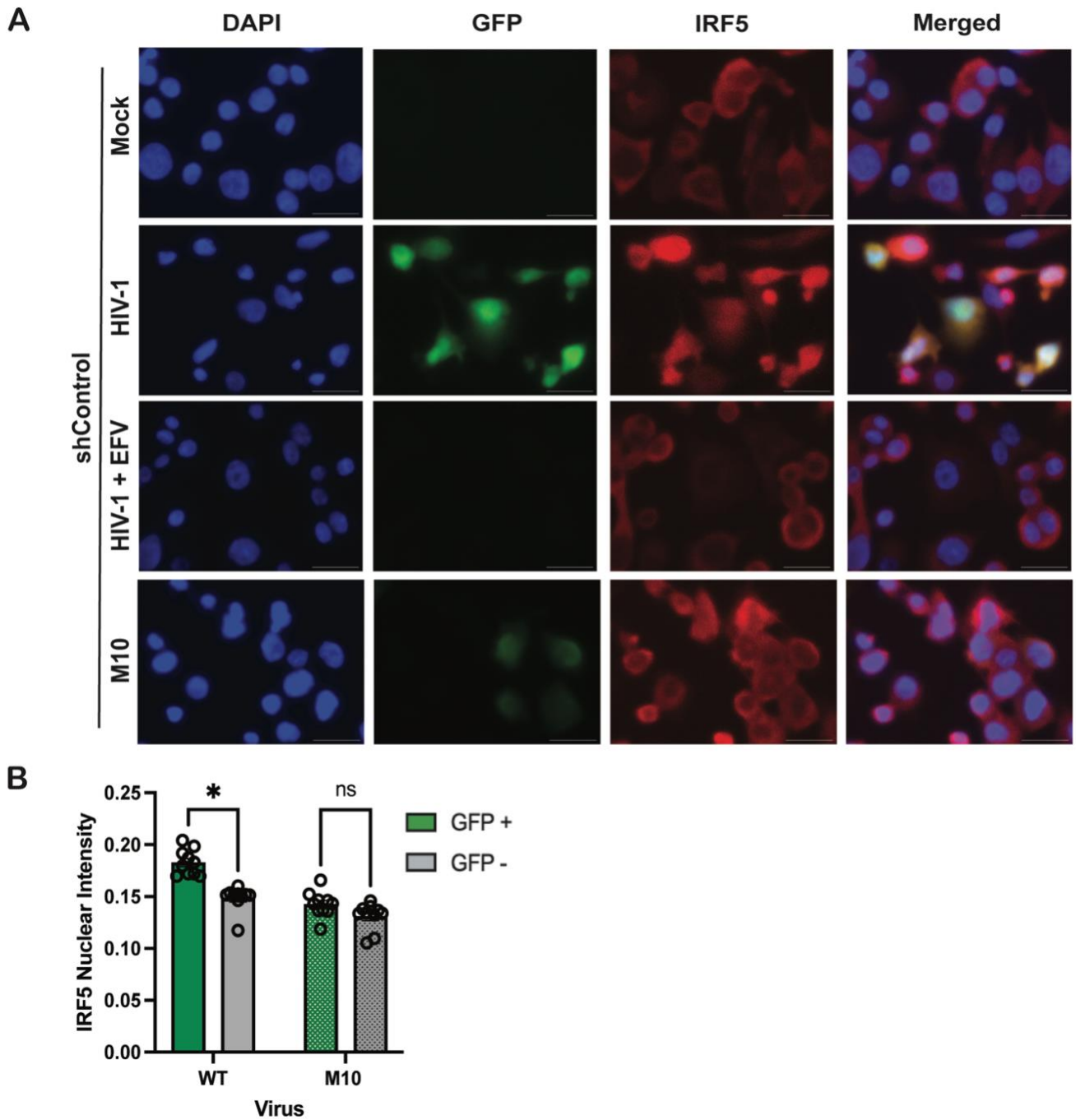
Supplementary Figure 2 Knockdown of (A)IRF3, (B)IRF5, or (C)IRF7 expression in THP-1/PMA macrophages was validated by RT-qPCR for (n=3). Functional knockdown of IRF expression in THP1/PMA macrophages was validated using (D)3p-hpRNA(2.5 ng/ml), (E)LPS(100ng/ml), (F)HSV (MOI 0.1) infection or (G)Sendai virus (MOI 1) infection. MDMs were transfected with siRNA targeting (H)IRF3, (I)IRF5, or (J)IRF7 for 48 hours and knockdown validated using RT-qPCR(n=4). siRNA transfected MDMs were infected with LaiΔenvGFP/G (MOI 1) in the presence of dNs and harvested for analysis of (K)IFNβ mRNA expression via RT-qPCR at 2 dpi. Knockdown of (L)TRAF6 and (M)IKKβ expression in THP-1/PMA macrophages was validated using RT-qPCR, and functionally validated by treatment with (N)3p-hpRNA(2.5 ng/mL), (O)LPS(100 ng/mL), infection with (P)HSV (MOI 0.1) or (Q)Sendai virus (MOI 1). Cell supernatants were harvested 24 h post-stimulation or 2 dpi for analysis of IP-10 production via ELISA. (R-S)MDMs were transfected with siRNA against (R)TRAF6 or (S)IKKβ and mRNA expression was measured by RT-qPCR (n=4).(T)MDMs transfected with siRNA targeting TRAF6 or IKKβ and infected with LaiΔenvGFP/G (MOI 1) in the presence of dNs and harvested for analysis of IFNβ mRNA expression at 2 dpi. Data is represented as mean ± SEM with each dot representing an individual experiment(A-G,L-Q) or donor(K,T). Statistical significance was assessed using unpaired t-test (A-C,H-J,L-M,O-S) or 1-way ANOVA with Dunnett's multiple comparisons analysis (D-H,K,N,T). *: p < 0.05; **: p<0.01, ***: p < 0.001 ****: p < 0.0001, ns = not significant.



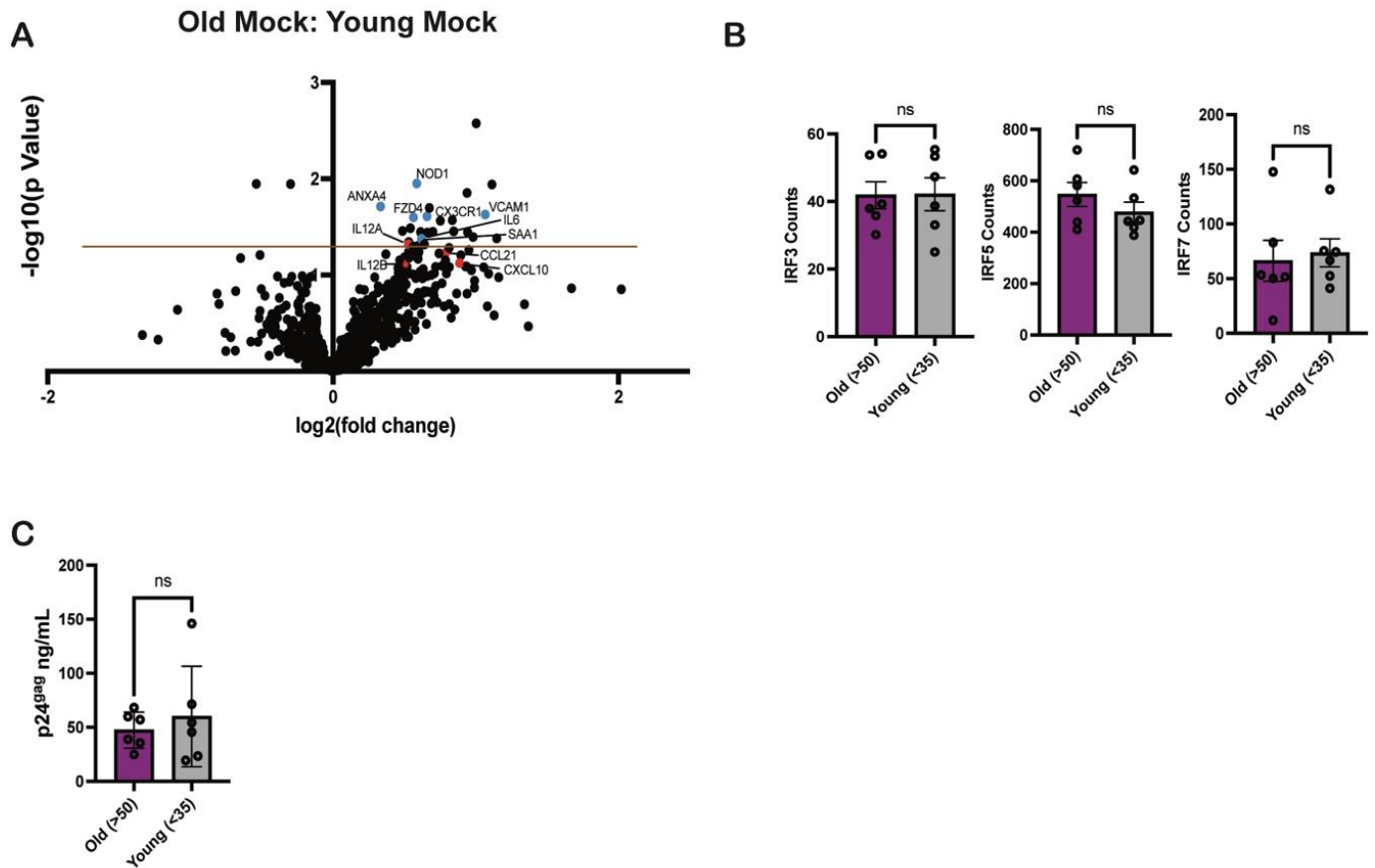
Supplementary Figure 3 MDMs were transfected with siRNA targeting **(A)**RIG-I, **(B)**UNC93B1, or **(C)**MDA5 for 48 hours and knockdown validated using RT-qPCR (n=3). siRNA transfected MDMs were infected with replication competent Lai-YU2env (MOI 1) in the presence of dNs and harvested at 2 dpi. Infection was measured by intracellular p24 staining via **(D)**flow cytometry and **(E)**IP-10 expression was measured by RT-qPCR. MDMs were transfected with siRNA targeting **(F)**IRF3, **(G)**IRF5, or **(H)**IRF7 for 48 hours and knockdown validated using RT-qPCR (n=3). siRNA transfected MDMs were infected with replication competent Lai-YU2 (MOI 1) in the presence of dNs and harvested at 2 dpi for assessment of **(I)**infection and **(J)**IP-10 expression. MDMs were transfected with siRNA targeting **(K)**TRAF6 or **(L)**IKKβ for 48 hours and knockdown validated using RT-qPCR(n=3). siRNA transfected MDMs were infected with replication competent Lai-YU2 (MOI 1) in the presence of dNs and harvested at 2 dp for assessment of **(M)**infection and **(N)** IP-10 expression. Data is represented as mean ± SEM with each dot representing an individual donor (A-N). Statistical significance was assessed using unpaired t-test **(A-C,F-J,K-L)** or 1-way ANOVA with Dunnett's multiple comparisons analysis **(D-E,I-J,M-N)**. *: p < 0.05, ***: p < 0.001 ****: p < 0.0001, ns = not significant.



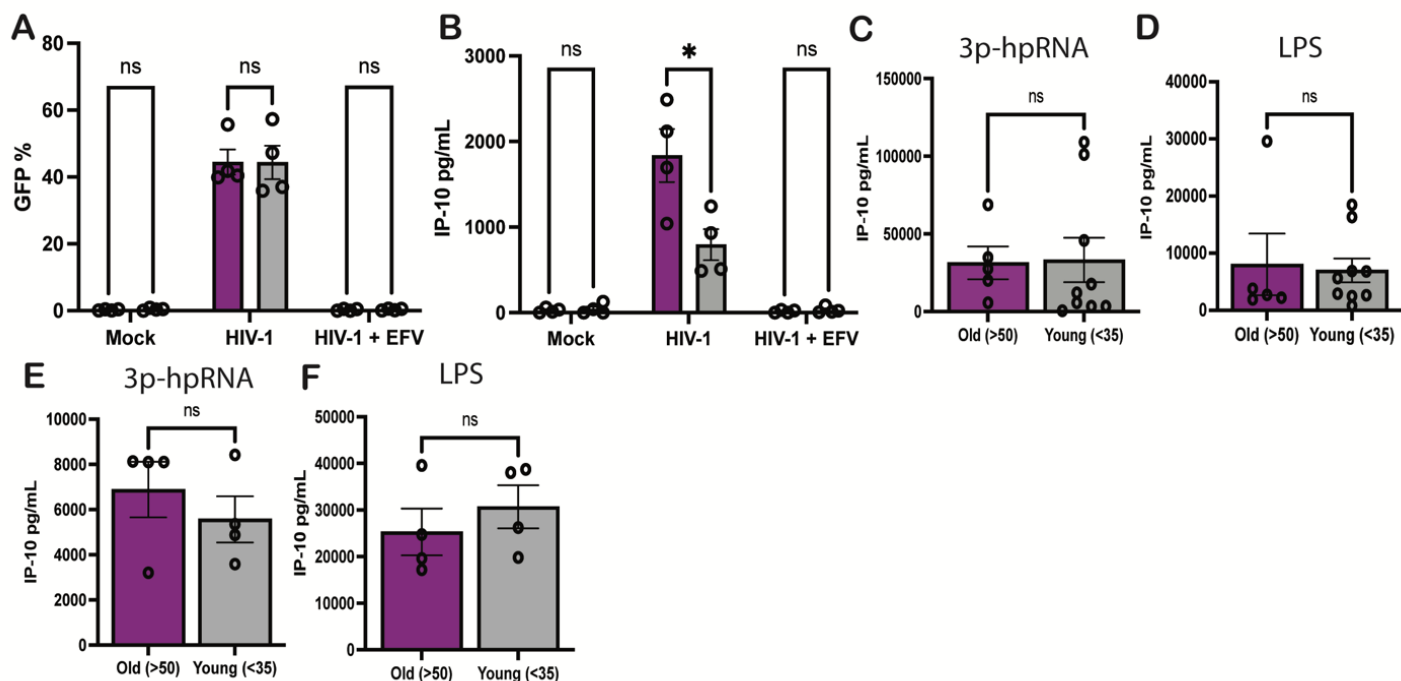
Supplementary Figure 4 THP1/PMA macrophages transduced with lentivectors expressing shRNAs were infected with LaiΔenvGFP/G (MOI 2) ± EFV (1μM) on coverslips. Cells were fixed at 3 dpi and stained to visualize intracellular IRF5 localization and DAPI via immunofluorescence imaging. Scale bar = 100 μm.



Supplementary Figure 5 (A) THP-1/PMA macrophages were infected with Lai Δ envGFP/G (WT or M10 mutant) viruses (MOI 2). At 3 dpi, cells were fixed and stained for IRF5 localization and DAPI to visualize nuclei. Scale bar = 100 μ m. **(B)** Cell Profiler was utilized to assess IRF5 nuclear intensity in infected and uninfected cells. Images from three independent infection experiments were quantified, with each dot representing a field containing approximately 50-150 cells. Representative images for shControl cells infected with WT virus are the same as those in Figure S4. Statistical significance assessed via unpaired t-test **(B)**. *: $p < 0.05$, ns = not significant.



Supplementary Figure 6 (A) RNA isolated from Lai Δ envGFP/G-infected MDMs (MOI 2) was analyzed via Nanostring nCounter using the Myeloid Innate Immunity V2 panel. Baseline expression of the gene panel was calculated using nSolver and plotted as a ratio of Old (Mock) vs. Young (Mock) with significantly upregulated IRFs (red) highlighted. The dashed line represents p-value of 0.05. (B) Raw count values for IRF3, IRF5, and IRF7 were plotted to assess differences in basal mRNA expression. (C) p24gag levels for selected donors in order to ensure equivalent levels of infection measured by ELISA. Significance was assessed via unpaired two-tailed t-test (A-C). ns = not significant.



Supplementary Figure 7 (A-B)MDMs (NY Biologics) were infected with Lai Δ envGFP/G (MOI 1) in the presence of dNs and cells and supernatants were harvested at 3 dpi to assess **(A)**levels of infection and **(B)**IP-10 secretion. MDMs from **(C-D)**HIV/Aging cohortor **(E-F)**NY Biologics were treated with either 3p-hpRNA(2.5 ng/mL) or LPS(100 ng/mL) and supernatant was harvested at 18h post-stimulation to assess levels of IP-10 secretion by ELISA. Statistical significance was assessed via unpaired t-test**(A-F)** *: $p < 0.05$, ns = not significant.

Supplementary Table 1

Group	Old (>50)	Young (<35)
Age (Mean $\pm$ SD)	64 $\pm$ 7.9	29.5 $\pm$ 4.2
Sex (% Male)	50	50
BMI (Mean $\pm$ SD)	28.5 $\pm$ 2.9	30.5 $\pm$ 7.9
Gastrointestinal Disorder (%)	66.7	16.7

Supplementary Table 2

Group	Old (>50)	Young (<35)
Age (Mean $\pm$ SD)	71.75 $\pm$ 7.8	28.0 $\pm$ 4.8
Sex (% Male)	75	75

Table 1 summarizes characteristics of the HIV/Aging Cohort (BMC). Table 2 summarizes characteristics of leukopaks obtained from NY Biologics.