Expanded View Figures

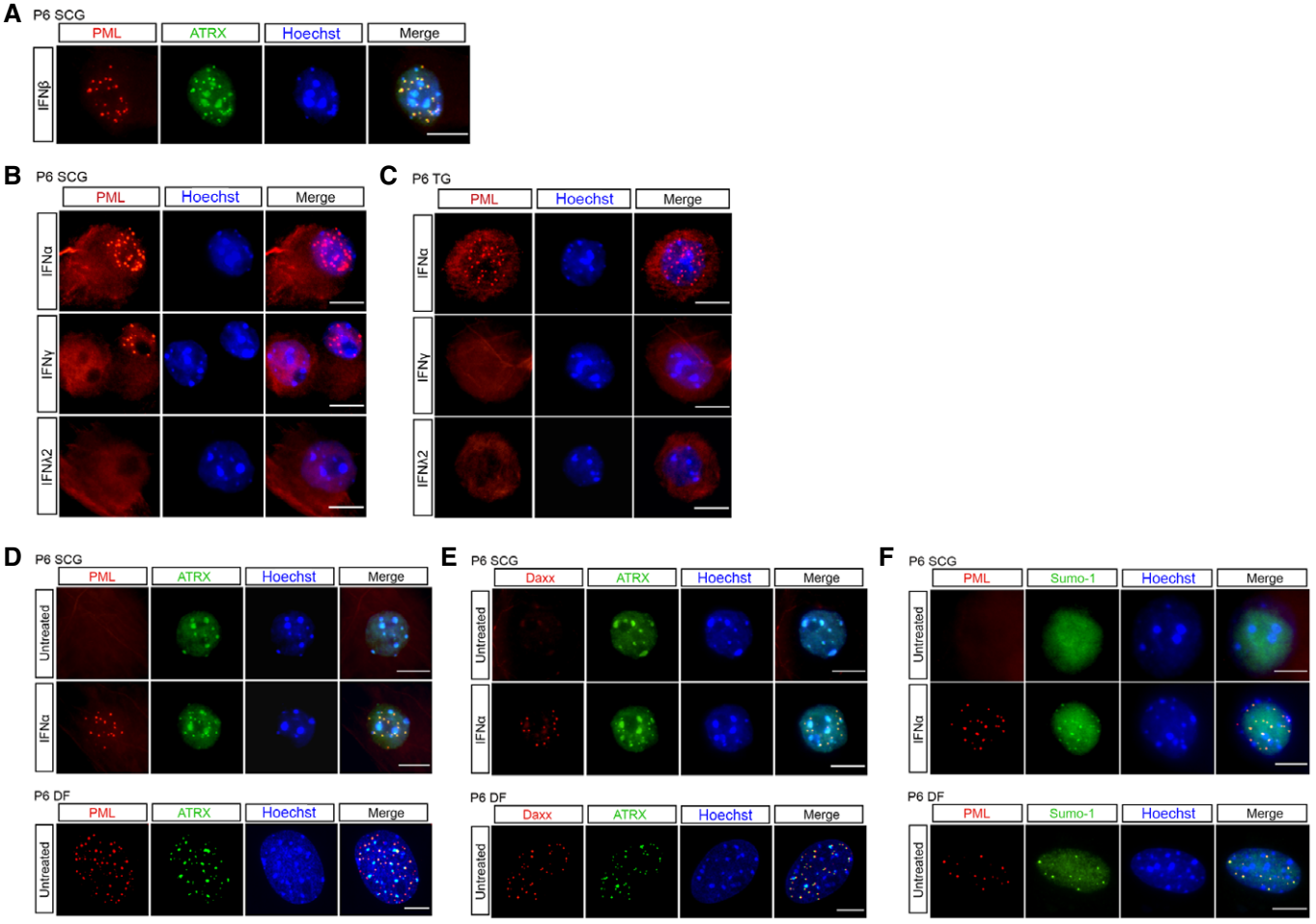

Figure EV1. Type I IFN alters the sub-cellular localization of ATRX, Daxx, and SUMO-1 in primary peripheral neurons.

A Representative images of P6 SCG neurons treated with IFNβ (150 IU/ml) and stained for PML and ATRX.

B Representative images of P6 SCG treated with IFNα (600 IU/ml), IFNγ (500 IU/ml) and IFNλ2 (500 ng/ml) and stained for PML.

C Representative images of P6 TG treated with IFNα (600 IU/ml), IFNγ (500 IU/ml), and IFNλ2 (500 ng/ml) and stained for PML.

D–F Representative images of untreated or IFNα (600 IU/ml)-treated P6 SCG neurons stained for PML and ATRX (D), Daxx and ATRX (E), and PML and SUMO-1 (F). P6 dermal fibroblasts (DF) isolated from the same mice were used as a non-neuronal control (D–F).

Data information: Scale bars, 20 μm.

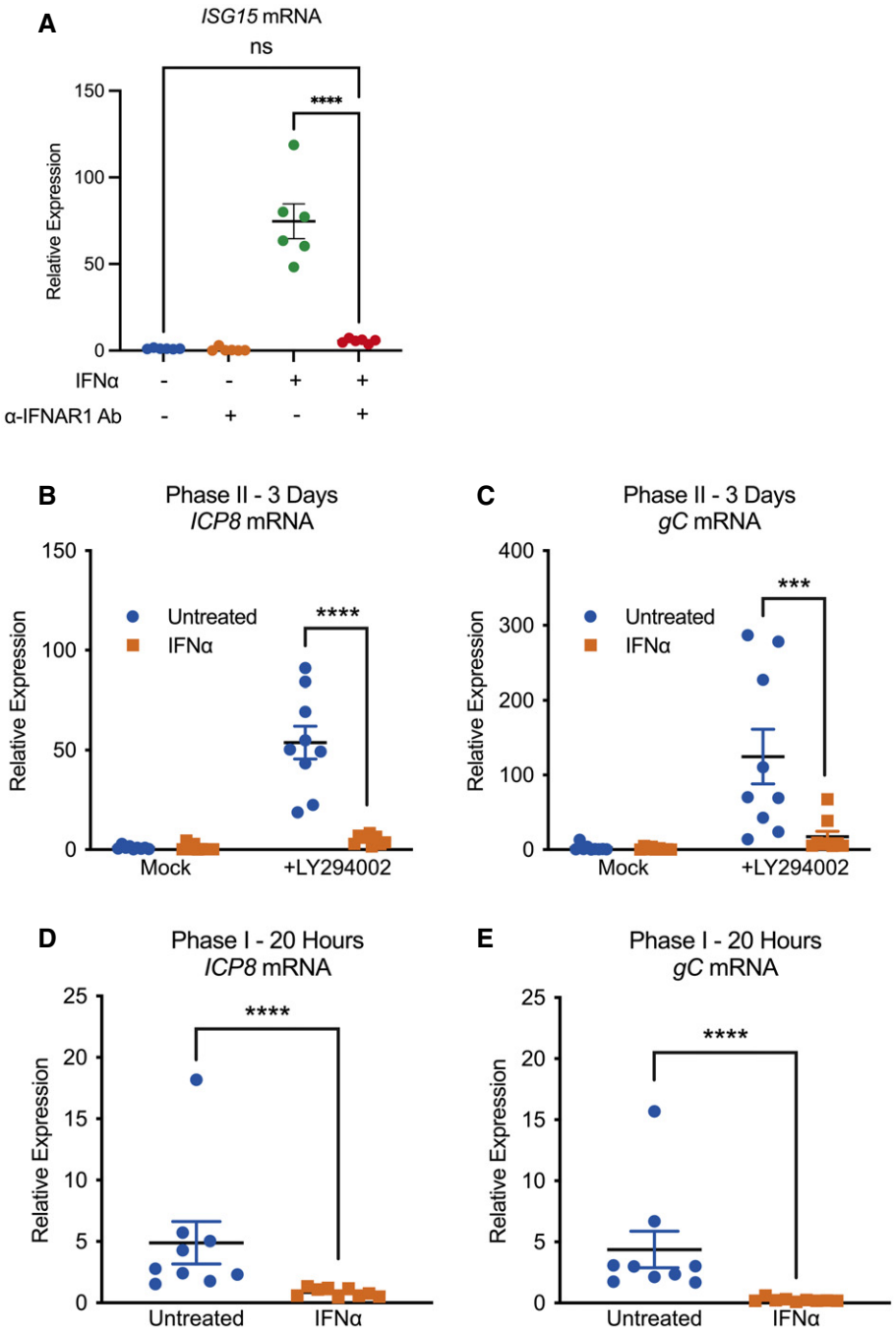

Figure EV2. Type I IFN treatment solely at time of infection inhibits LY294002-mediated reactivation of HSV-1 in primary sympathetic SCG neurons.

A RT–qPCR for *ISG15* mRNA expression in SCG neurons treated with IFNα (600 IU/ml) in the presence or absence of anti-mouse IFNAR1 antibody (1:1,000). $n = 6$ biological replicates.

B, C RT–qPCR for viral mRNA transcripts at 3 days post-LY294002-induced reactivation of SCGs infected with HSV-1 in the presence or absence of IFNα (600 IU/ml). $n = 9$ biological replicates.

D, E RT–qPCR for viral mRNA transcripts at 20 h post-LY294002-induced reactivation in SCGs infected with HSV-1 in the presence or absence of IFNα (600 IU/ml). $n = 9$ biological replicates.

Data information: Data represent the mean ± SEM. Statistical comparisons were made using a one-way ANOVA with Tukey's multiple comparison (A) or a Mann–Whitney test (ns not significant, ***$P < 0.001$, ****$P < 0.0001$).

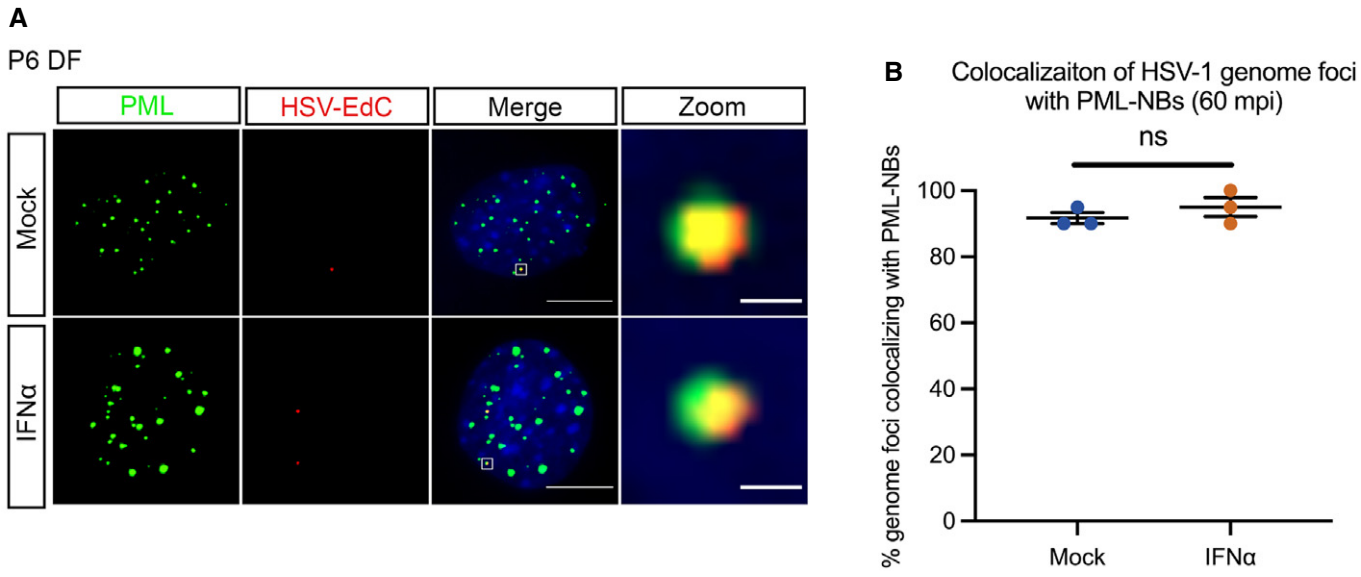

Figure EV3. PML-NBs entrap vDNA in the absence of type I IFN during lytic HSV-1 infection of murine dermal fibroblasts.

A Representative images of vDNA foci detected by click chemistry to PML at 60 min post-infection in P6 dermal fibroblasts lytically infected with HSV-1^{EdC} in the presence or absence of IFNα (600 IU/ml). Scale bar, 20 μm. Zoom scale bar, 1 μm.

B Percent colocalization of vDNA foci detected by click chemistry to PML at 60 mpi in P6 dermal fibroblasts infected with HSV-1^{EdC} in the presence or absence of IFNα (600 IU/ml). Each point represents the percentage of 20 vDNA foci that colocalized to PML from 3 biological replicates.

Data information: Data represent the mean ± SEM. Statistical comparisons were made using a Mann–Whitney test (ns not significant).

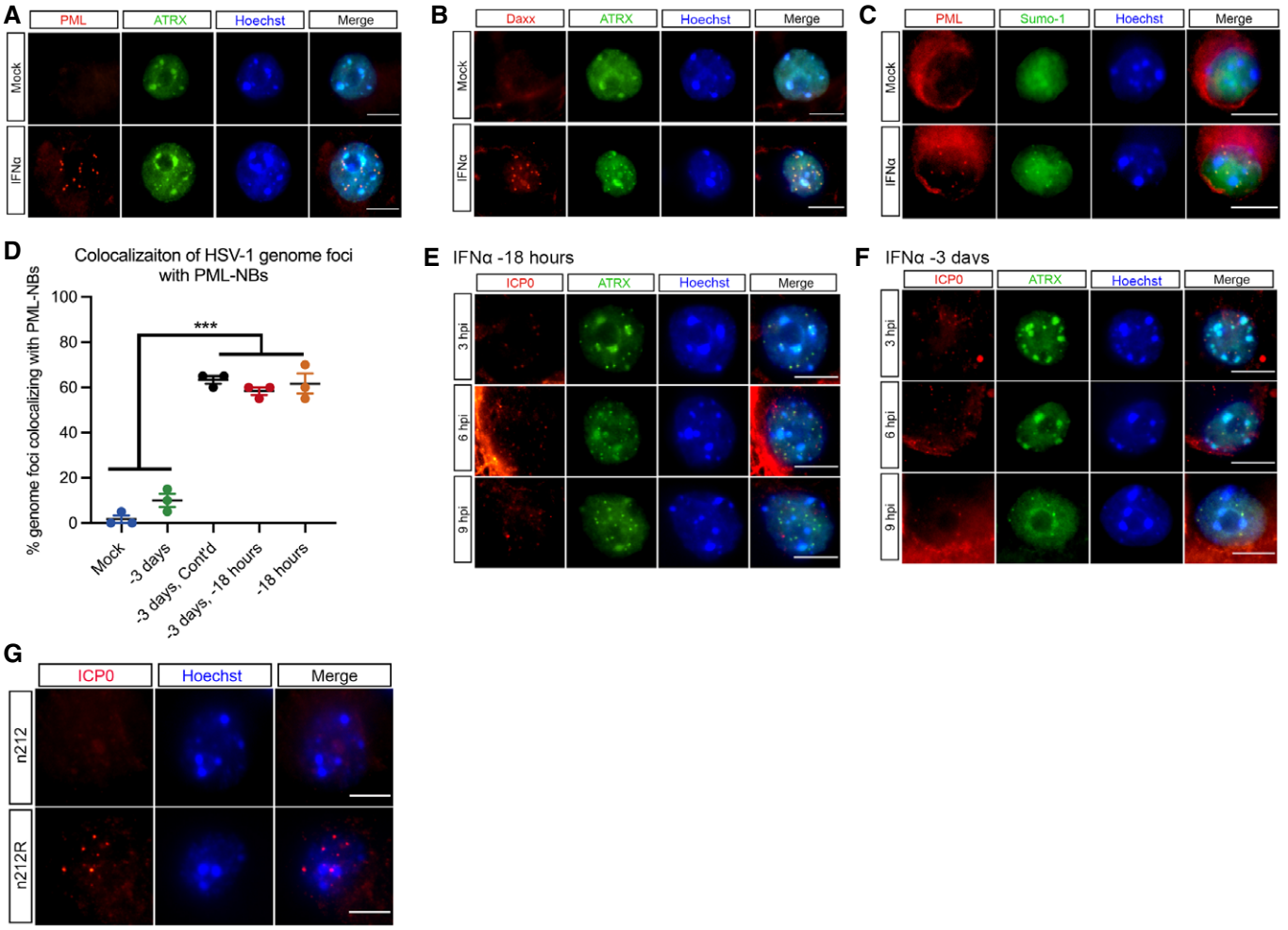

Figure EV4. HSV-1 genomes only associate with PML-NBs when type I IFN is present during initial infection.

A Representative images of untreated or IFNα-treated (600 IU/ml) P6 SCG neurons stained for PML and ATRX at 3 days post-treatment.

B Representative images of untreated or IFNα-treated (600 IU/ml) P6 SCG neurons stained for Daxx and ATRX at 3 days post-treatment.

C Representative images of untreated or IFNα-treated (600 IU/ml) P6 SCG neurons stained for PML and SUMO-1 at 3 days post-treatment.

D Percent colocalization of vDNA foci detected by click chemistry to PML at 3 dpi in SCG neurons infected with HSV-1^{EdC} with or without IFNα (600 IU/ml) present at the time of infection. Each point represents the percentage of 20 vDNA foci that colocalized to PML from 3 biological replicates.

E, F Representative images of HSV-1-infected P6 SCG neurons treated with IFNα (600 IU/ml) for 18 h prior to infection or for 18 h at 3 days prior to infection and stained for ICP0 and ATRX at 3, 6, and 9 h post-infection.

G Representative images of P6 SCG neurons infected with n212 or n212R for 8 h and stained for HSV-1 ICP0.

Data information: Scale bars, 20 μm. Data represent the mean ± SEM. Statistical comparisons were made using a Mann–Whitney test (***$P < 0.001$).

Figure EV5. PML is not required for ISG induction in primary postnatal sympathetic neurons.

A P6 SCG neurons were transduced with either control non-targeting shRNA or shRNA targeting PML for 3 days, then treated with IFNα (600 IU/ml) for 18 h. Total genes > 1.5-fold higher in IFNα (600 IU/ml) treated cells than untreated cells were subdivided into 3 groups: shCtrl-treated neurons only. shPML-treated neurons only. Both shCtrl and shPML neurons (shared).

B Gene expression heat map of top 50 most upregulated genes in P6 neurons transduced with control non-targeting shRNA or shRNA targeting PML for 3 days, then treated with IFNα (600 IU/ml) for 18 h.

C Heat map of the top 25 shared upregulated GO terms in P6 neurons transduced with control non-targeting shRNA or shRNA targeting PML for 3 days, then treated with IFNα for 18 h.

D, E RT–qPCR for *Pml* mRNA expression in SCG neurons transduced with either control non-targeting shRNA or shRNA targeting PML for 3 days, then treated with IFNα (600 IU/ml) for 18 h. $n = 9$ biological replicates.

F Quantification of detectable, IFNα (600 IU/ml)-induced PML puncta in P6 SCG neurons treated with ATO (1 μM) for 2, 6, 18, and 24 h. $n = 20$ cells from 2 biological replicates.

G Number of Us11-GFP expressing neurons at 3 days post-treatment with LY294002 (20 μM), ATO (1 μM) or LY294002 (20 μM) + ATO (1 μM) in P6 SCG neuronal cultures infected with HSV-1 in the presence or absence of IFNα (600 IU/ml). $n = 12$ biological replicates.

Data information: Data represent the mean ± SEM. Statistical comparisons were made using a one-way ANOVA with Tukey's multiple comparison (D–F) or a 2-way ANOVA (G) (ns not significant, ***$P < 0.001$, ****$P < 0.0001$).

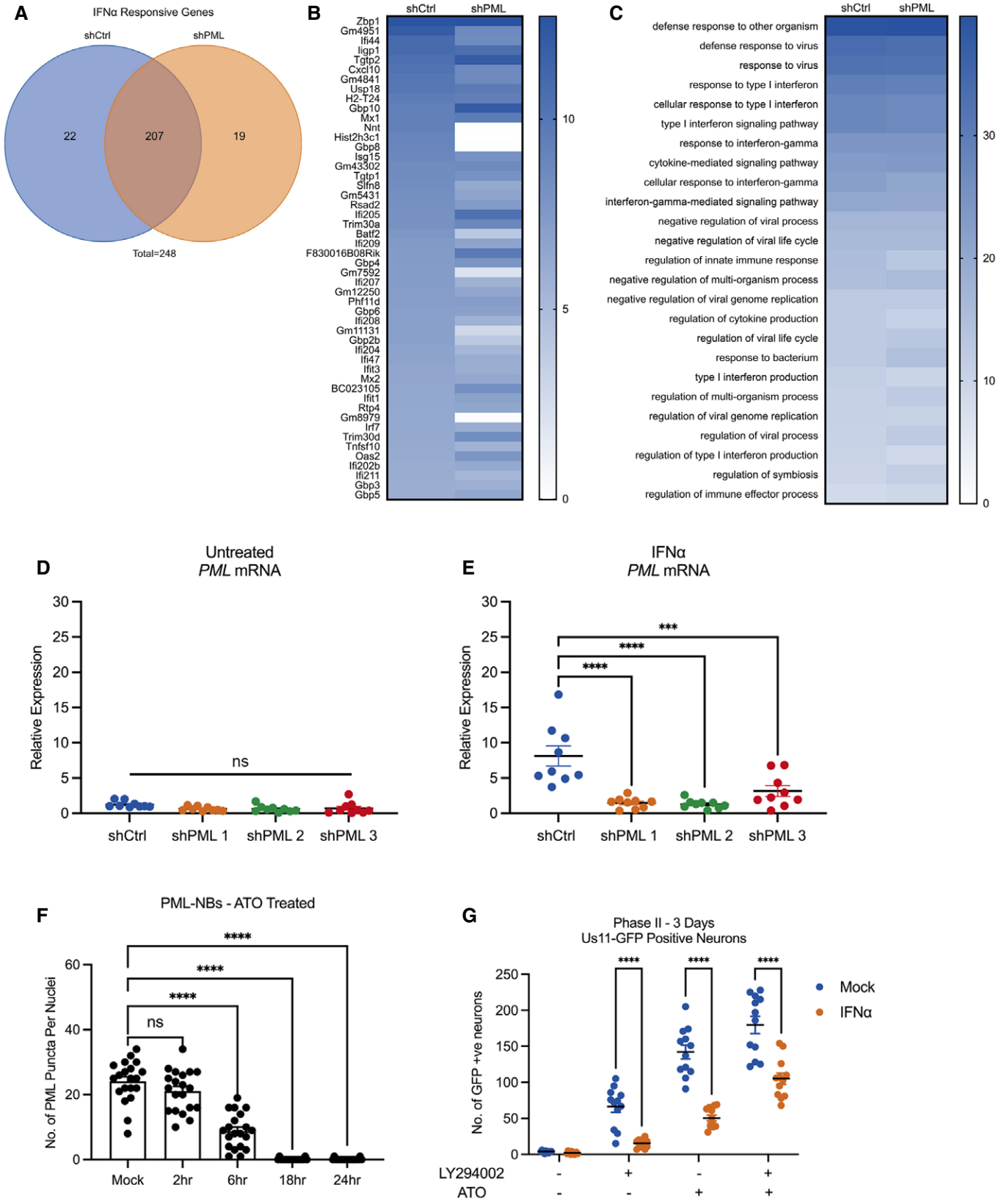
A
IFNα Responsive Genes
shCtrl
shPML
22
207
19
Total=248
B
shCtrl
shPML
Zbp1
Gm4951
Ifi44
Iigp1
Tgtp2
Cxcl10
Gm4841
Usp18
H2-T24
Gbp10
Mx1
Nnt
Hist2h3c1
Gbp8
Isg15
Gm43302
Tgtp1
Slfn8
Gm5431
Rsad2
Ifi205
Trim30a
Batf2
Ifi209
F830016B08Rik
Gbp4
Gm7592
Ifi207
Gm12250
Phf11d
Gbp6
Ifi208
Gm11131
Gbp2b
Ifi204
Ifi47
Ifit3
Mx2
BC023105
Ifit1
Rtp4
Gm8979
Irf7
Trim30d
Tnfsf10
Oas2
Ifi202b
Ifi211
Gbp3
Gbp5
10
5
0
C
shCtrl
shPML
defense response to other organism
defense response to virus
response to virus
response to type I interferon
cellular response to type I interferon
type I interferon signaling pathway
response to interferon-gamma
cytokine-mediated signaling pathway
cellular response to interferon-gamma
interferon-gamma-mediated signaling pathway
negative regulation of viral process
negative regulation of viral life cycle
regulation of innate immune response
negative regulation of multi-organism process
negative regulation of viral genome replication
regulation of cytokine production
regulation of viral life cycle
response to bacterium
type I interferon production
regulation of multi-organism process
regulation of viral genome replication
regulation of viral process
regulation of type I interferon production
regulation of symbiosis
regulation of immune effector process
30
20
10
0
D
Untreated
PML mRNA
Relative Expression
ns
shCtrl
shPML 1
shPML 2
shPML 3
E
IFNα
PML mRNA
Relative Expression
***
****
****
shCtrl
shPML 1
shPML 2
shPML 3
F
PML-NBs - ATO Treated
No. of PML Puncta Per Nuclei
****
****
****
ns
Mock
2hr
6hr
18hr
24hr
G
Phase II - 3 Days
Us11-GFP Positive Neurons
No. of GFP +ve neurons
****
****
****
Mock
IFNα
LY294002
ATO

Figure EV5.