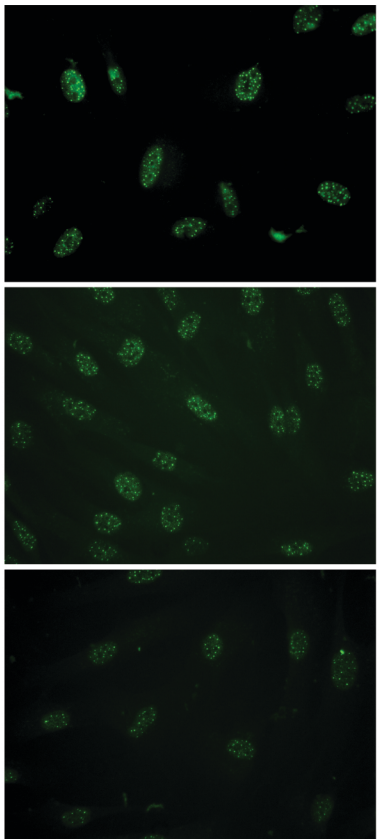
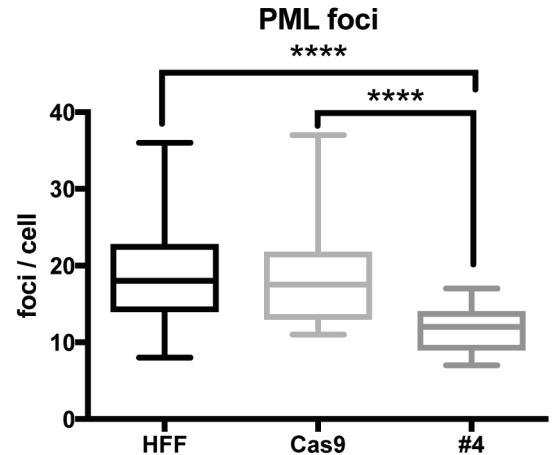
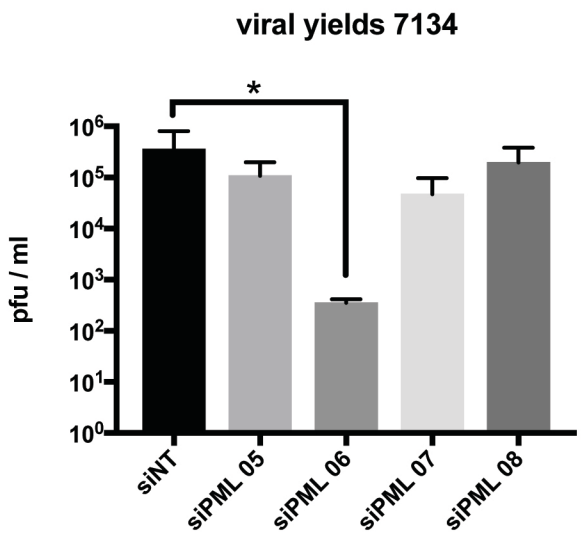
Suppl. Fig. 1

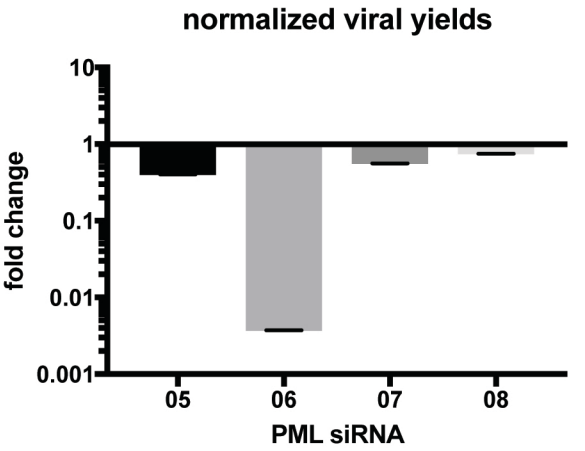




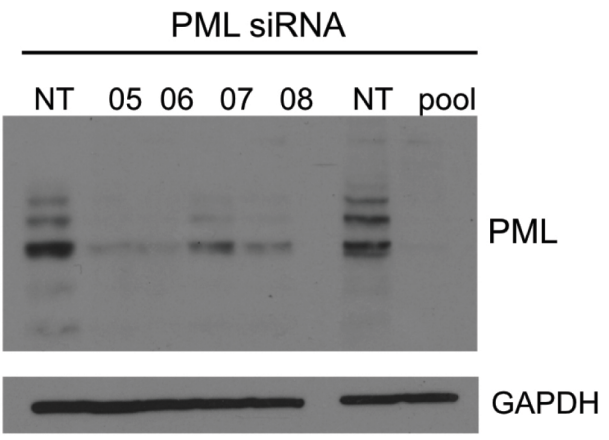
Suppl Fig. 2

A

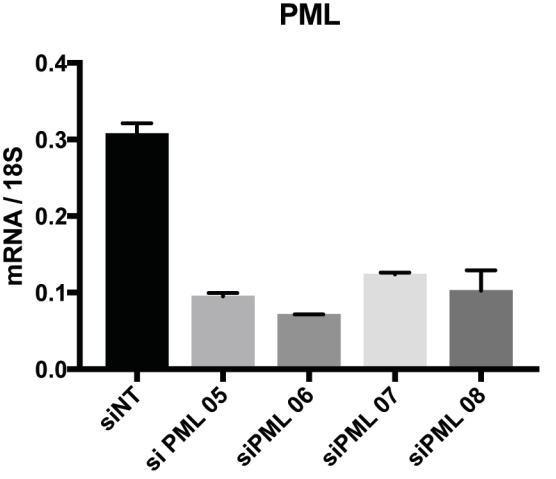




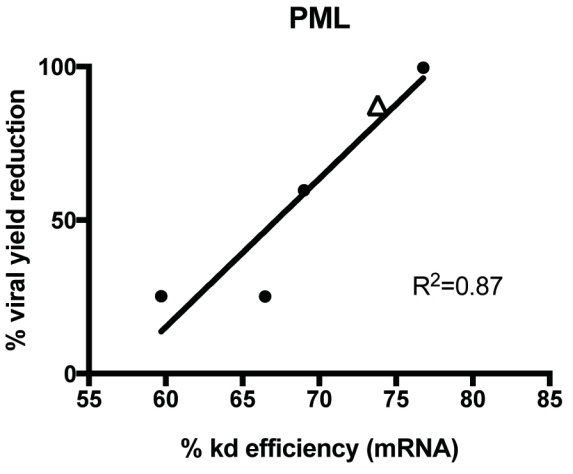
B



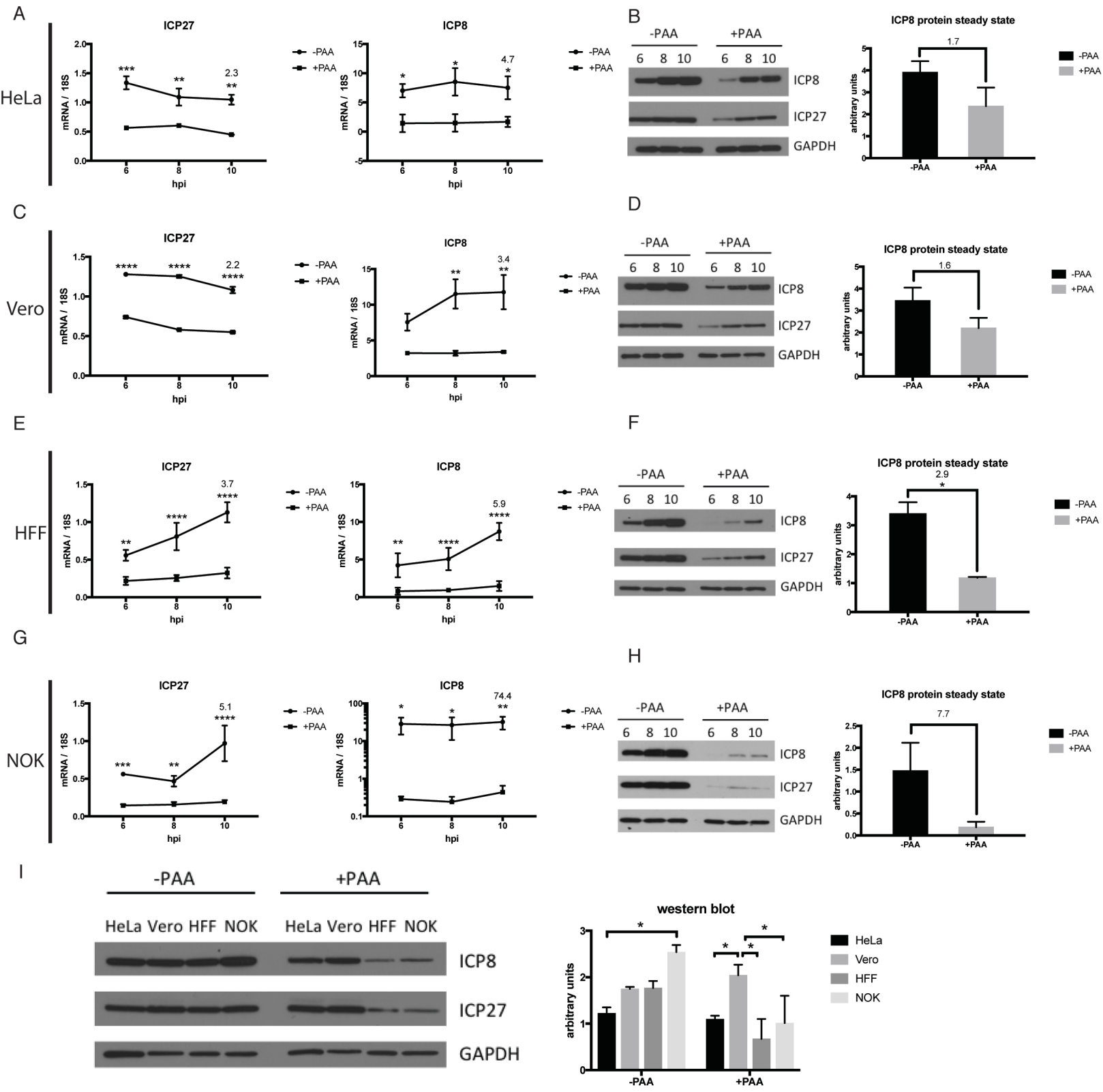
C



D



Suppl. Fig. 3


A
HeLa
ICP27
ICP8
mRNA / 18S
hpi
-PAA
+PAA
B
-PAA
+PAA
6 8 10
ICP8
ICP27
GAPDH
ICP8 protein steady state
arbitrary units
1.7
C
Vero
D
1.6
E
HFF
F
2.9
G
NOK
H
7.7
I
HeLa Vero HFF NOK
western blot
HeLa
Vero
HFF
NOK

Suppl. Fig. 1: The number of PML foci is reduced in absence of IFI16. HFF, Cas9, or IFI16 ko cells were cultivated on coverslips, and immunofluorescence staining was performed as described with an antibody specific for PML. PML foci were counted in an unbiased fashion and plotted. Statistical analysis via t-test was performed as described.

Suppl. Fig. 2: Deconvolution of the PML siRNA pool shows a correlation between PML gene expression and reduction in viral yields. HFF cells were treated with the 4 single siRNAs specific for PML that comprise the PML siRNA pool or non-targeting siRNA. A) Cells were infected with 7134 virus at an MOI of 0.1, viral yields were collected at 48 hpi and titrated on U2OS cells. Yields were plotted as PFU/ml or normalized to non-targeting siRNA. B) Immunoblot of cell lysates prior to infection (NT, 05, 06, 07, 08) compared to cell lysates from an experiment where the PML pool had been used (NT, pool) and probed with antibodies specific for PML and GAPDH. C) Samples for PML transcript analysis were harvested prior to infection and analyzed via qRT-PCR and plotted relative to 18S rRNA. D) Plot of means of the reduction in viral yields in % due to single siRNA treatment versus the % knockdown efficiency. Results obtained in a different experiment for the PML pool is shown as a triangular symbol. The data shown are from at least 3 independent experiments.

Suppl. Fig. 3: Viral gene expression in HFF and NOK cells is largely dependent on progeny viral DNA. HeLa, Vero, HFF, or NOK cells were seeded in respective media at equal densities and infected with 7134 virus at an MOI of 5 in presence or

absence of PAA. Samples for protein and mRNA analysis were collected at 6-10 hpi. A) *ICP27* and *ICP8* mRNA levels in HeLa cells were assessed by qRT-PCR and plotted relative to 18S rRNA. B) Immunoblot of HeLa cell lysates developed with antibodies specific for ICP8, ICP27, and GAPDH. ICP8 signals at 10 hpi were quantified and plotted relative to GAPDH. C) As in A, but with Vero cells. D) As in B, but with Vero cells. E) As in A, but with HFF cells. F) As in B, but with HFF cells. G) As in A, but with NOK cells. H) As in B, but with NOK cells. I) 10 h samples of the four different cell types were analyzed in parallel on one immunoblot probed for ICP8, ICP27, and GAPDH. ICP8 signals were quantified and plotted relative to GAPDH. For statistical analysis, 2-way ANOVA with multiple comparisons was performed in A), C), E), G), and I). T-tests were done for statistical analysis in B), D), F), and H).

Supplementary Table 1: PML siRNA putative off-target genes

target gene	**PML siRNA treatment effect on mRNA levels**
TACC1	unchanged
PRPF31	unchanged
ECT2L	not expressed in HFF