SUPPLEMENTAL FIGURE LEGENDS

Figure S1. PQBP1 association with incoming HIV-1 virus particles, Related to Figure 1.

(A) Specificity of PQBP1 antibody (Sigma) and lack of effect on overall cGAS expression was confirmed by IF and/or western blot of THP-1 cells expressing indicated shRNAs (top) or MDDCs treated with indicated siRNAs (bottom). Scale bars: 10 μm (top), 25 μm (bottom). (B) Identification of viral particles from infected THP-1 cells was done with python with skimage, scipy, pims, and trackpy tools. In short, cells were subjected to a binary threshold so that no extracellular signals (viruses or cell debris) would confound the analysis. Viral particles were detected using thresholding and watershed segmentation methods or by using trackpy. Each panel is 221.8 μm squareThe masks of the pixels that are positive for viral particles are then quantified for each independent channel (PQBP1, cGAS, iGFP, IN label, etc.) and (C) for each field of view that was imaged, the fraction of total virions (IN) showing positive PQBP1 signal (compared to controls using secondary antibodies only) were graphed. (D) Quantification of PQBP1 and IN distance distribution analysis. Left, probability distribution of distances, *P(d)*, from each virion (IN) to the nearest PQBP1 (blue). *P(d)* for uniformly distributed jitter *J=1 μm* (yellow) becomes similar to the distribution obtained by random shuffling of puncta (purple). Arrows indicate reasonable arbitrary thresholds (δ) to be applied to *d* values in determining associations of PQBP1 puncta with corresponding IN. Data were obtained from 24 independent images (N=24,471). Right, the percentage of IN spots that have a PQBP1 within a chosen threshold, δ (0.1 μm < δ < 0.75 μm). See PQBP1 and IN distance distribution analysis section under Star Method for detail.

Figure S2. Mapping of the capsid interaction domain in PQBP1, Related to Figure 2.

(A) $PQBP1_{1\text{-}46}$ and $PQBP1_{47\text{-}265}$ with N-terminal eGFP or C-terminal sfGFP produced in a cell-free protein expression mixture before (left) and after (middle) purification by Ni-NTA

chromatography followed by dialysis. Recombinant PQBP1 was labelled at C60 with AF488-maleimide (right). (B) Top panels, representative fluorescence traces of either AF488 labelled PQBP1, CPSF6 peptide or dATP (red traces) and AF568-labelled CA A204C particles (blue traces). Inclusion of hexacarboxybenzene (HCB) or use of either N74D or R18G double mutants with CA A204C is indicated. Bottom panels, analogous to the top, except eGFP-fused PQBP1 truncation constructs were utilized. (C) PQBP1 co-pellets preferentially with CA assemblies containing the R18 ring. Left, assembled CA_{A204C} and $CA_{R18G/A204C}$ were incubated with PQBP1, centrifuged and supernatant (S) and pellet (P) fractions were analyzed using reducing SDS-PAGE with Coomassie staining. Right, bar graph of the intensity of the PQBP1 band relative to the intensity of the CA band in the pellet fraction. Two independent co-pelleting experiments were performed with two repeats per experiment. (D) Fluorescence resonance energy transfer (FRET) assay was used to measure interactions between $PQBP1_{1-46}$-YFP and CypA-dsRed. THP-1 cells, either wild type or stably expressing eYFP, $PQBP1_{1-46}$-eYFP or $PQBP1_{1-104}$-eYFP, were infected with HIV-1 virus packaged with CypA-DsRed for 1.5 hrs, followed by PFA fixation and FRET analysis. Signal intensity distribution of CypA-dsRed positive foci for indicated THP-1 clones infected with the virus (left) and a western blot depicting expression of both eYFP and PQBP1-eYFP proteins are shown.

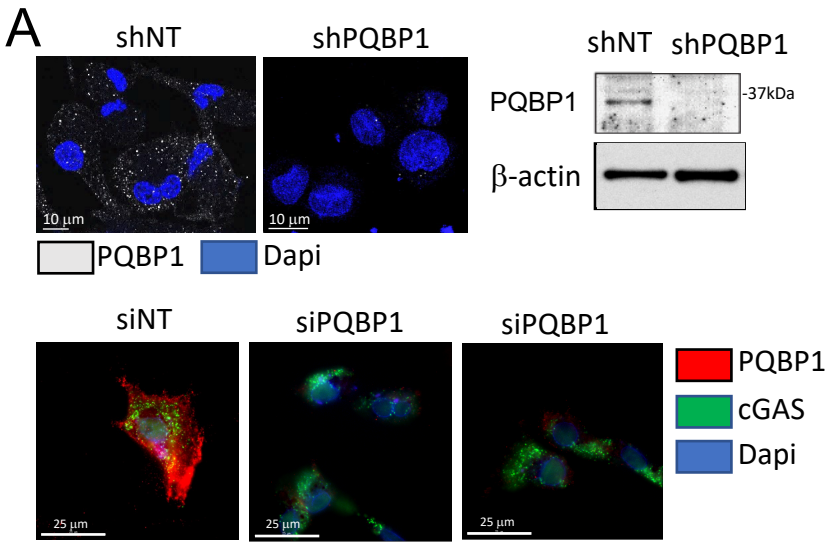
Figure S3. PQBP1 is required for cGAS recruitment to incoming virus particles, Related to Figure 3. (A) The specificity of the anti-cGAS antibody was confirmed by IF and western blot on cGAS knock out in THP-1 cells. Scale bar, 10 μm. (B) Time lapsed imaging to monitor the structural integrity of an incoming viral core. MDDCs were infected with HIV-1 virus with iGFP fluid phase marker (iGFP+IN-mRuby3 at low MOI) where iGFP and IN-mRuby signal intensities of each virion were monitored for one hour. See Video S1. A representative kinetic profile of the signal intensities of a virion where the loss of GFP signal is linked to virion fusion and capsid integrity loss (top). Panels of each virion, identified by both time-lapsed and corresponding IF x-

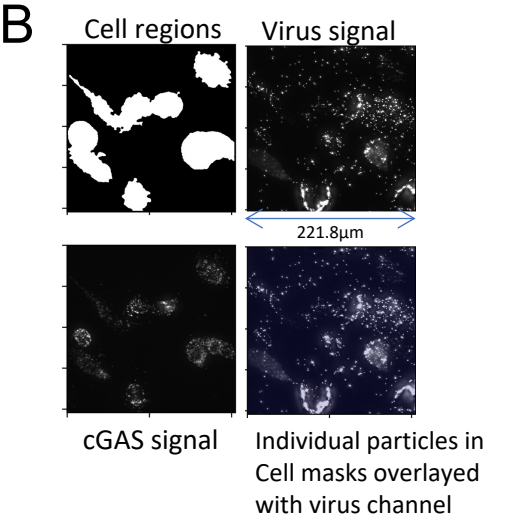
y-z position of the respective foci point label were categorized based on capsid integrity and are color coded for the signals of PQBP1 (green), cGAS (blue), IN-mRuby3 (red). The RGB merged images and iGFP (gray in the individual boxes) are also shown (middle, particle B to G). Scale bars, 5 and 0.2 μm as shown. 3D reconstruction of point E and point F foci are shown where we see the proximity and overlapping of PQBP1/cGAS complexes with the viral particle. 3D Ruler/ScaleBar indicates size in μm in the pictures. (C) The knockdown efficiency of siRNAs against PQBP1 is demonstrated by both RT-qPCR and IF analysis (left and right respectively). MFI of cGAS signal intensity per THP-1 cell treated with either non-targeting (NT) or PQBP1 specific siRNAs and infected with HIV-1 virus, as described in Figure 3C. Scale bar, 60 μm. Mean and error bar (-/+ 1.5*IQR) are shown. Boxes denote the interquartile range. T-test (two-tailed, equal variation), *p<0.05; Dunn's column comparison **** p<0.0001, * p<0.05. (D) Increase in cGAMPs in the cytosol of infected cells. PMA-THP-1 cells, either mock infected or infected with HIV-1 luciferase virus or transfected with either HT-DNA or cGAMP for 3 hours, were stained for cGAMP (green), p24 (red), IRF3 (magenta) and dapi (blue). The white contour line indicates the boundary of the nucleus. Scale bar, 10 μm. (E) PMA-THP-1, transfected with non-targeting siRNAs (NT) or siRNA targeting PQBP1, were challenged with HIV-1, stained for cGAMP (green), cGAS (red), and DAPI (blue). Knockdown efficiencies of both mRNA and protein level of PQBP1 were shown below. Scale bar, 5 μm. (F) The cells treated as in (E) were analyzed IFN β mRNAs induction post 16 hours of the infection. Averages and SEM are shown, One-way ANOVA, ** p<0.01, * p<0.05.

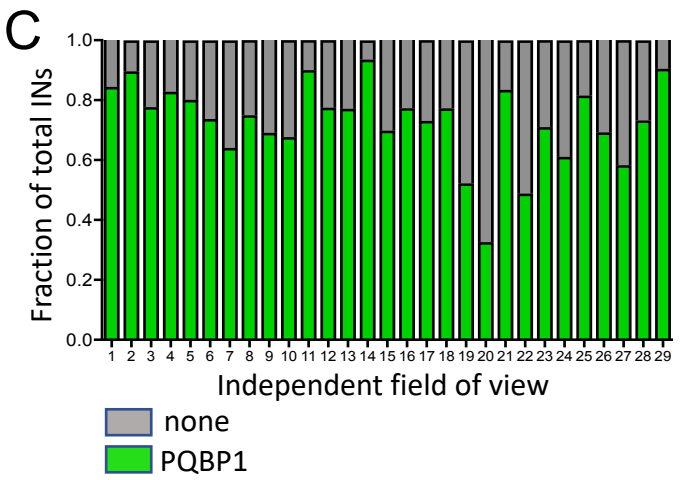
Figure S4. NONO is not required for PQBP1-dependent cGAS sensing during the early steps of infection, Related to Figure 4. (A) Knockdown efficiencies of siRNA-targeted genes in MDDCs were quantified by RT-qPCR. (B) MFIs of cGAS signal per infected MDDC, subjected to indicated siRNA treatments, are shown. (C) PMA-differentiated THP-1 cells, subjected to

siRNA-mediated targeting were infected with were with either mock of HIV-1 in the presence of VLP-Vpx for 2 hrs, followed by post-fixation IF imaging. The levels of NONO and PQBP1 proteins (left) and expression levels of Flag-cGAS (red), p24 (green) and DAPI (blue) in cells used for proximal ligation assays, as in Figure 4 (right), are shown. Scale bars, 20 μm (A table at the bottom of the graph shows # of INs analyzed for each condition. (D) Representative images of the infected MDDCs treated with indicated siRNAs (left) and MFI of indicated protein signals per cells (right), analyzed in Figure 4D and 4F, are shown. Scale bar, 10 μm. Mean and SEMs are shown. One-way ANOVA, ***p<0.001, **p<0.01, *p<0.05. ns denotes no significance.

Figure S5. Distinct domain of PQBP1 interact with the capsid and cGAS, Related to Figure 5. (A) HIV-1 infection (+) enhances co-immunoprecipitation of cGAS or Flag-cGAS with PQBP-IP in MDDCs or PMA-THP-1, respectively. The original uncropped images of the blots in Figure 5B are shown. See Figure 5B for details. Cells were infected with HIV-1 luciferase virus in the presence of VLP-Vpx and subjected to co-IPs at 3 hrs post infection. Endogenous cGAS or Flag-cGAS co-precipitating with PQBP1 was assayed. Normal IgG (NS) or antibody against PQBP1 were used as indicated. Non-specific (ns) bands and the cGAS band (arrow) are indicated. The lines between cGAS and PQBP1 blots indicate corresponding samples. 1x and 3x inputs indicate relative amount of input lysates. Sources of cGAS antibodies are indicated. (B) Either full-length or truncated PQBP1-YFP proteins were co-expressed with MBP-cGAS in 293T cells and subjected to anti-MBP pull down. *ns denotes non-specific protein. A schematic of the PQBP1 protein is shown. WWD and E/D denote the WW domain and the acidic residue-rich domain, respectively.


A
shNT
shPQBP1
10 µm
10 µm
PQBP1
Dapi
shNT shPQBP1
PQBP1
-37kDa
β-actin
siNT
siPQBP1
siPQBP1
25 µm
25 µm
25 µm
PQBP1
cGAS
Dapi


B
Cell regions
Virus signal
221.8µm
cGAS signal
Individual particles in
Cell masks overlayed
with virus channel


C
Fraction of total INs
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Independent field of view
none
PQBP1

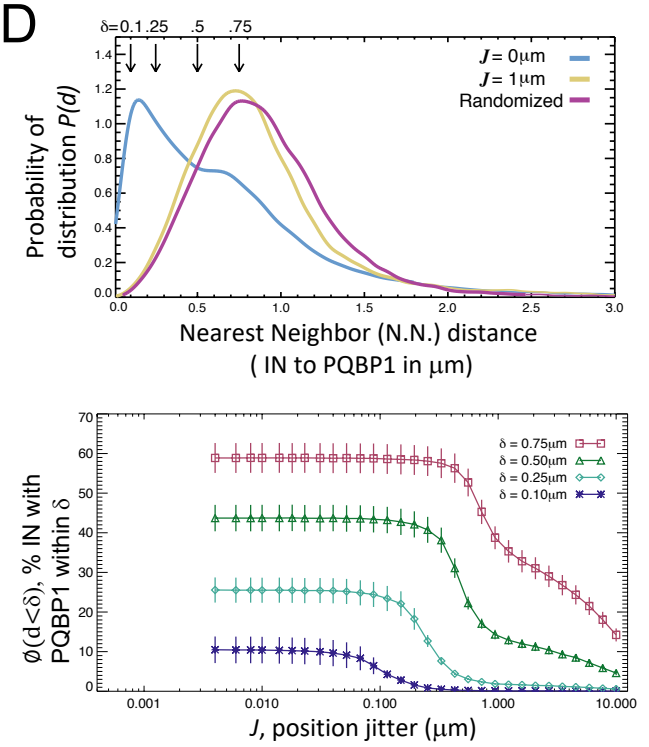

D
δ=0.1 .25 .5 .75
J = 0µm
J = 1µm
Randomized
Probability of
distribution P(d)
0.0 0.5 1.0 1.5 2.0 2.5 3.0
Nearest Neighbor (N.N.) distance
(IN to PQBP1 in µm)
δ = 0.75µm
δ = 0.50µm
δ = 0.25µm
δ = 0.10µm
ø(d<δ), % IN with
PQBP1 within δ
0.001 0.010 0.100 1.000 10.000
J, position jitter (µm)

Figure S1

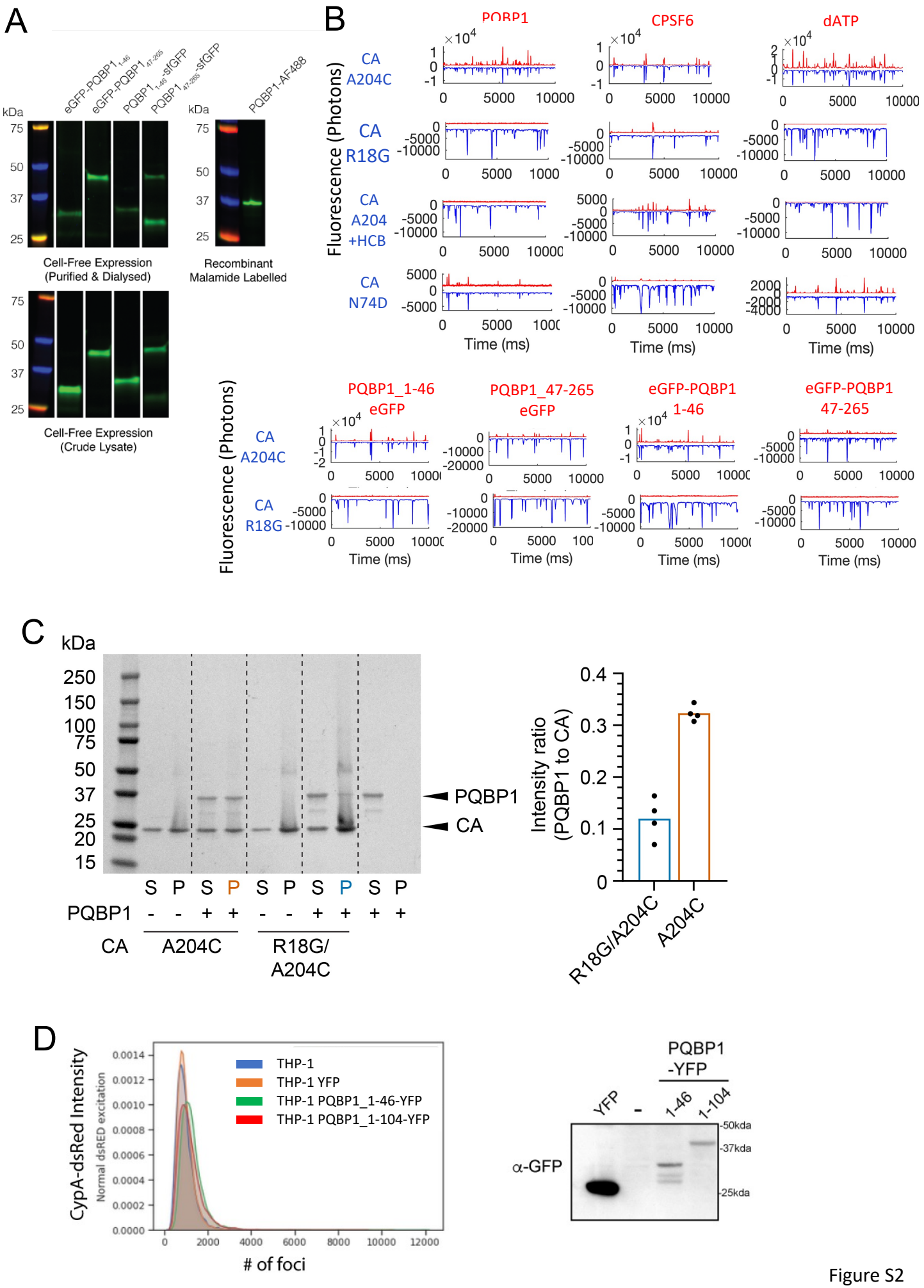

A
kDa
eGFP-PQBP1 1-46
eGFP-PQBP1 47-265
PQBP1 1-46 -sfGFP
PQBP1 47-265 -sfGFP
PQBP1-AF488
75
50
37
25
Cell-Free Expression (Purified & Dialysed)
Recombinant Malamide Labelled
Cell-Free Expression (Crude Lysate)
B
PQBP1
CPSF6
dATP
CA A204C
CA R18G
CA A204 +HCB
CA N74D
Fluorescence (Photons)
Time (ms)
PQBP1_1-46 eGFP
PQBP1_47-265 eGFP
eGFP-PQBP1 1-46
eGFP-PQBP1 47-265
C
kDa
250
150
100
75
50
37
25
20
15
S P S P S P S P S P
PQBP1 - - + + - - + + + +
CA A204C R18G/ A204C
PQBP1
CA
Intensity ratio (PQBP1 to CA)
R18G/A204C
A204C
D
CypA-dsRed Intensity
Normal dsRED excitation
# of foci
THP-1
THP-1 YFP
THP-1 PQBP1_1-46-YFP
THP-1 PQBP1_1-104-YFP
PQBP1 -YFP
YFP
-
1-46
1-104
α-GFP
50kda
37kda
25kda

Figure S2

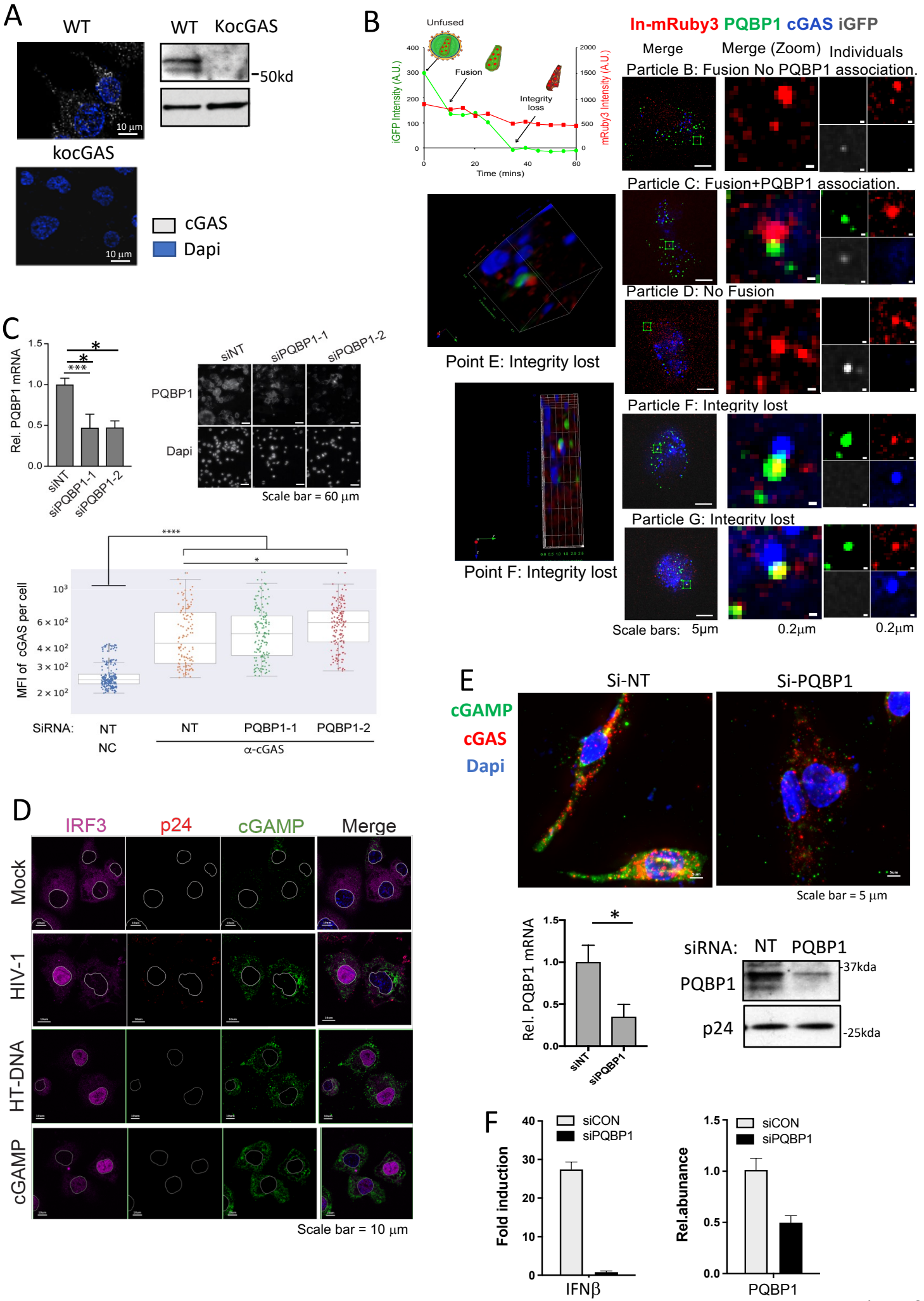

A
WT
WT KocGAS
50kd
10 µm
kocGAS
10 µm
cGAS
Dapi
B
In-mRuby3 PQBP1 cGAS iGFP
Merge
Merge (Zoom)
Individuals
Unfused
Fusion
Integrity loss
iGFP Intensity (A.U.)
mRuby3 Intensity (A.U.)
Time (mins)
Particle B: Fusion No PQBP1 association.
Particle C: Fusion+PQBP1 association.
Particle D: No Fusion
Particle F: Integrity lost
Particle G: Integrity lost
Point E: Integrity lost
Point F: Integrity lost
Scale bars: 5µm 0.2µm 0.2µm
C
Rel. PQBP1 mRNA
siNT
siPQBP1-1
siPQBP1-2
PQBP1
Dapi
Scale bar = 60 µm
MFI of cGAS per cell
SiRNA:
NT
NC
NT
PQBP1-1
PQBP1-2
α-cGAS
D
IRF3
p24
cGAMP
Merge
Mock
HIV-1
HT-DNA
cGAMP
Scale bar = 10 µm
E
Si-NT
Si-PQBP1
cGAMP
cGAS
Dapi
Scale bar = 5 µm
Rel. PQBP1 mRNA
siNT
siPQBP1
siRNA:
NT
PQBP1
PQBP1
p24
37kda
25kda
F
siCON
siPQBP1
Fold induction
IFNβ
Rel.abunance
PQBP1

Figure S3

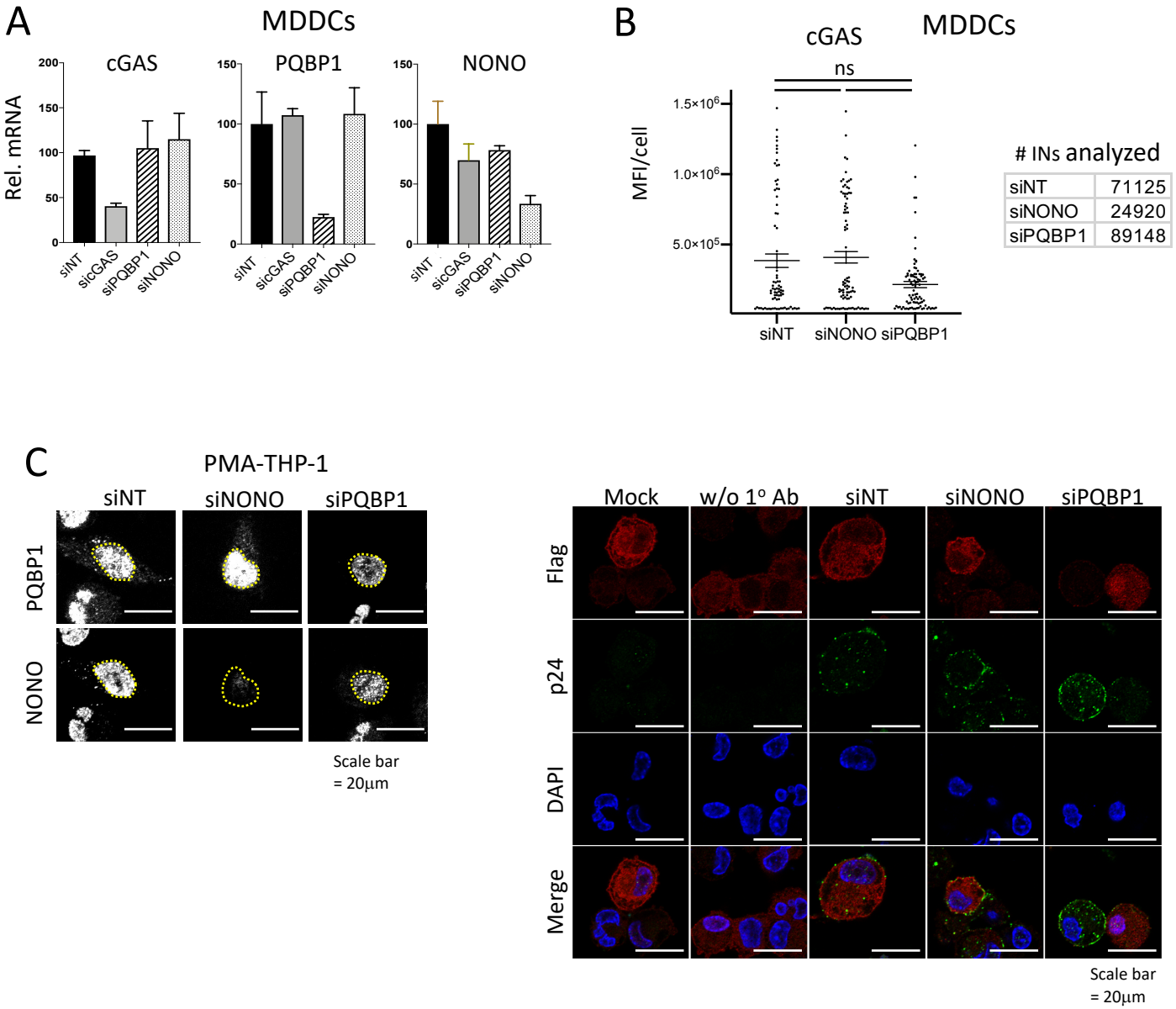
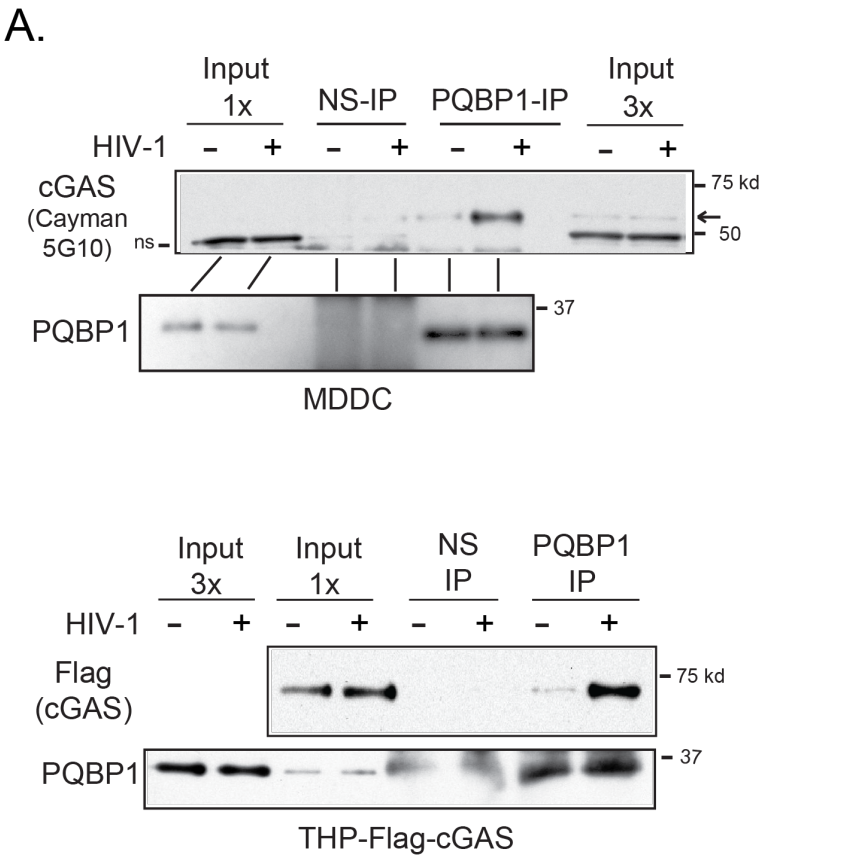

A
MDDCs
cGAS
PQBP1
NONO
Rel. mRNA
siNT
siCGAS
siPQBP1
siNONO
B
cGAS
MDDCs
ns
MFI/cell
siNT
siNONO
siPQBP1
INs analyzed
siNT 71125
siNONO 24920
siPQBP1 89148
C
PMA-THP-1
siNT
siNONO
siPQBP1
PQBP1
NONO
Scale bar = 20µm
Mock
w/o 1° Ab
Flag
p24
DAPI
Merge
Scale bar = 20µm
D
siNT
siNONO
siPQBP1
cGAS
PQBP1
NONO
Scale bar= 10 µm
PQBP1
NONO
MFI*10⁴/cell
**
***
MDDCs

Figure S4


A.
Input 1x
NS-IP
PQBP1-IP
Input 3x
HIV-1
cGAS (Cayman 5G10)
ns
75 kd
50
PQBP1
37
MDDC
Input 3x
Input 1x
NS IP
PQBP1 IP
HIV-1
Flag (cGAS)
75 kd
PQBP1
37
THP-Flag-cGAS

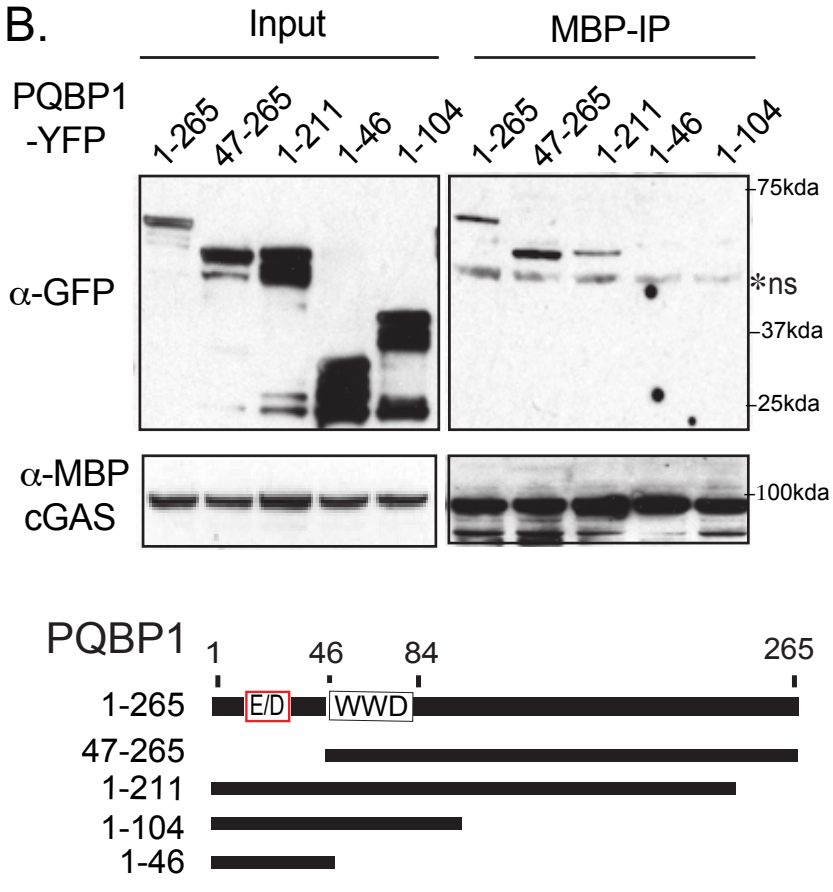

B.
Input
MBP-IP
PQBP1 -YFP
1-265
47-265
1-211
1-46
1-104
α-GFP
75kda
*ns
37kda
25kda
α-MBP cGAS
100kda
PQBP1
1
46
84
265
E/D
WWD
1-265
47-265
1-211
1-104
1-46

Figure S5