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Supplemental Information

Pan-retroviral Nucleocapsid-Mediated Phase Separation Regulates Genomic RNA Positioning and Trafficking

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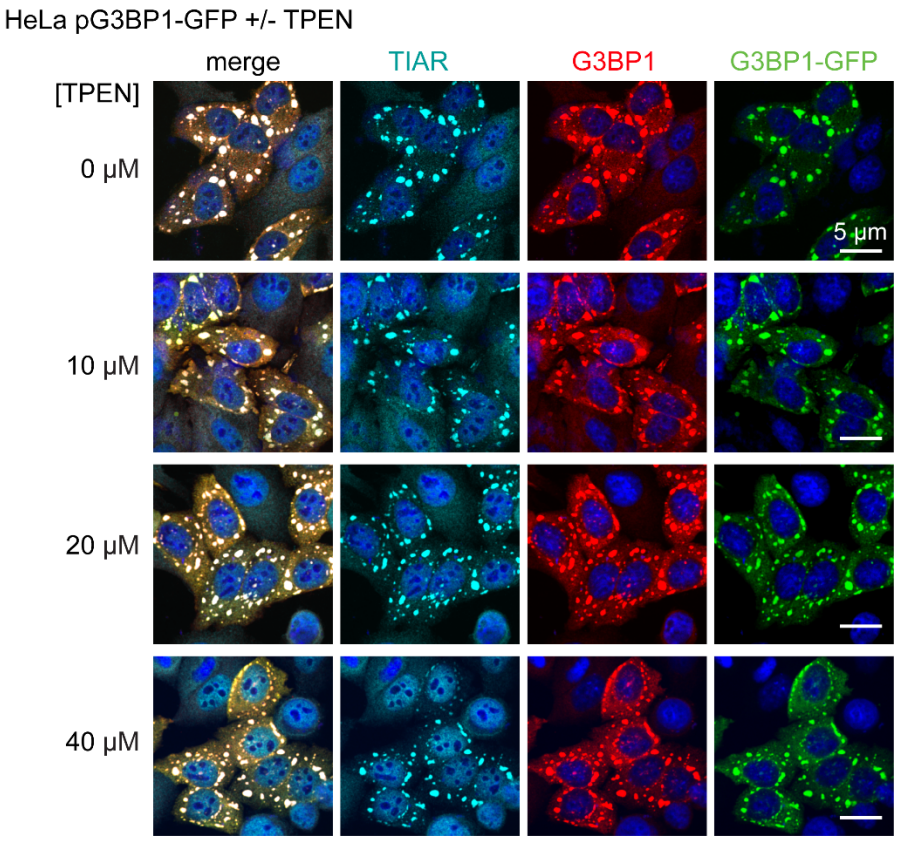
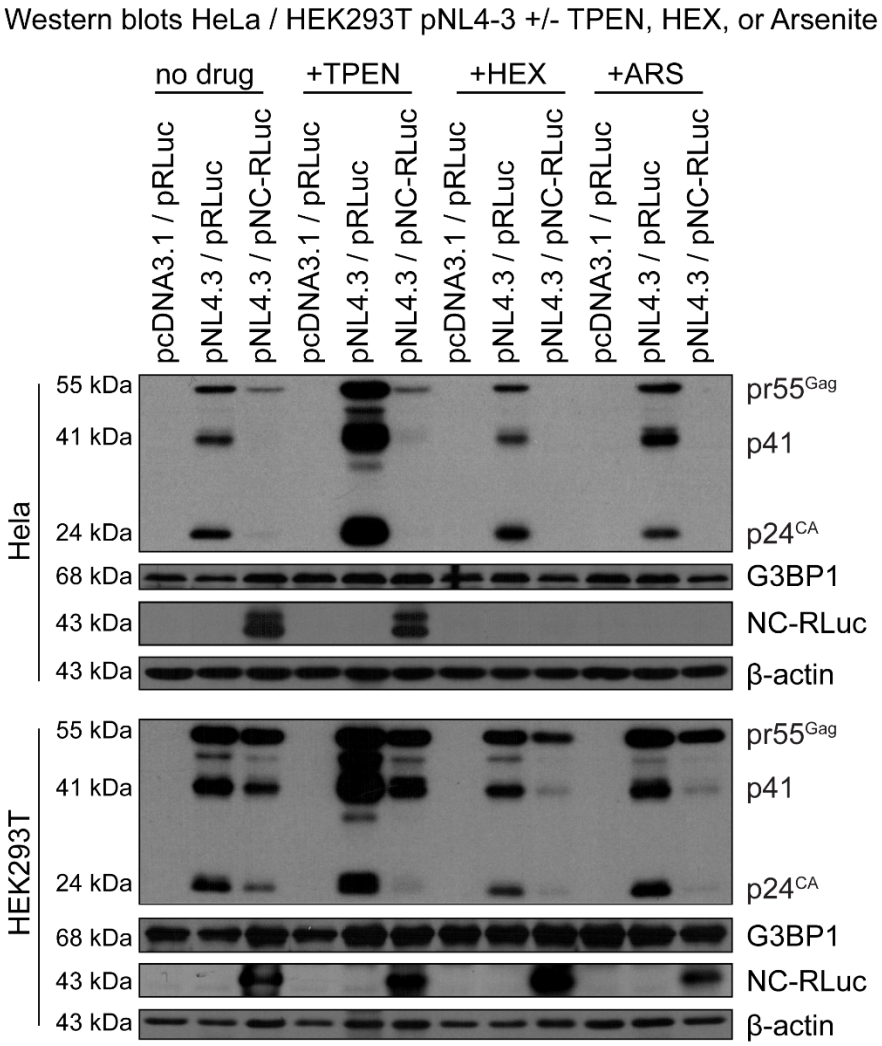
Supplemental Figures and Supplemental Figure Legends

Supplemental Figure S1



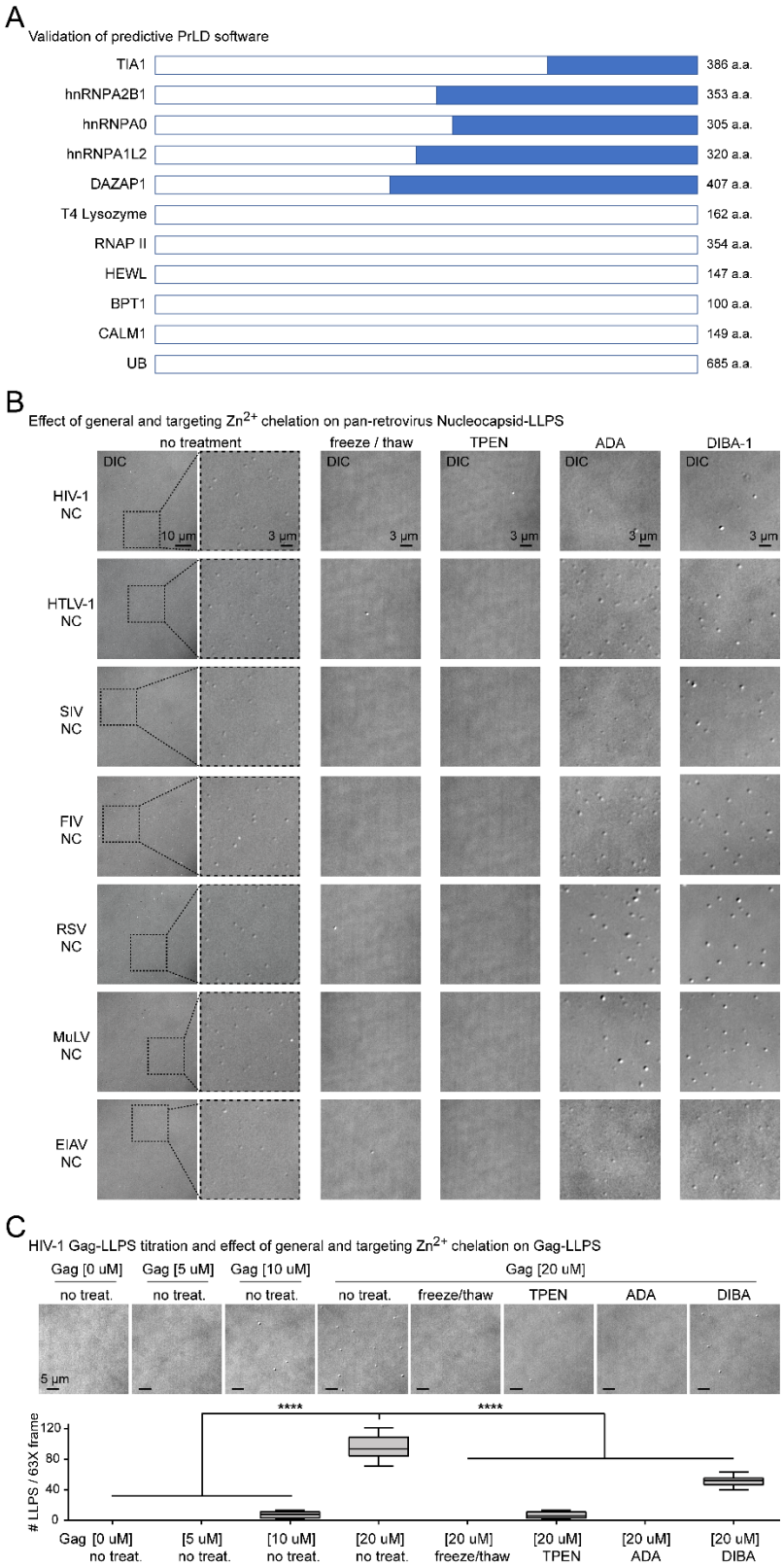
Supplemental Figure S1, Related to Figure 2: G3PB1 overexpression induced SGs are not disrupted by Zn^{2+} chelation. HeLa cells transfected with pG3BP1-GFP (*green*) and treated with TPEN [0 to 40 μM], to observe that G3BP1 induced SGs were not affected by the treatment. Endogenous G3PB1 (red) and TIAR (cyan) were also labeled, albeit endogenous G3PB1 levels are much lower than those of those of G3BP1-GFP (*n* = 4). TPEN, N,N,N′,N′-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine; μm, micron.

Supplemental Figure S2



Supplemental Figure S2, Related to Figure 4: Western blots for Zn^{2+} chelation leads to cellular retention of HIV-1 Gag and decreased virus production. Representative Western blots of cell lysates from HeLa or HEK293T cells co-transfected with proviral construct pNL4-3 (or pcDNA3.1 control) and pNC-RLuc (p2-p1/Rluc) (or pRLuc control (pRluc-N1)) for 24 h, and treated using TPEN [20 µM], ARS [500µM], or HEX [3.5 %], demonstrating significant increase in cellular Gag expression levels from treatment with TPEN (*n* = 3). TPEN, N,N,N′,N′-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine; ARS, arsenite; HEX, 1,6-hexanediol.

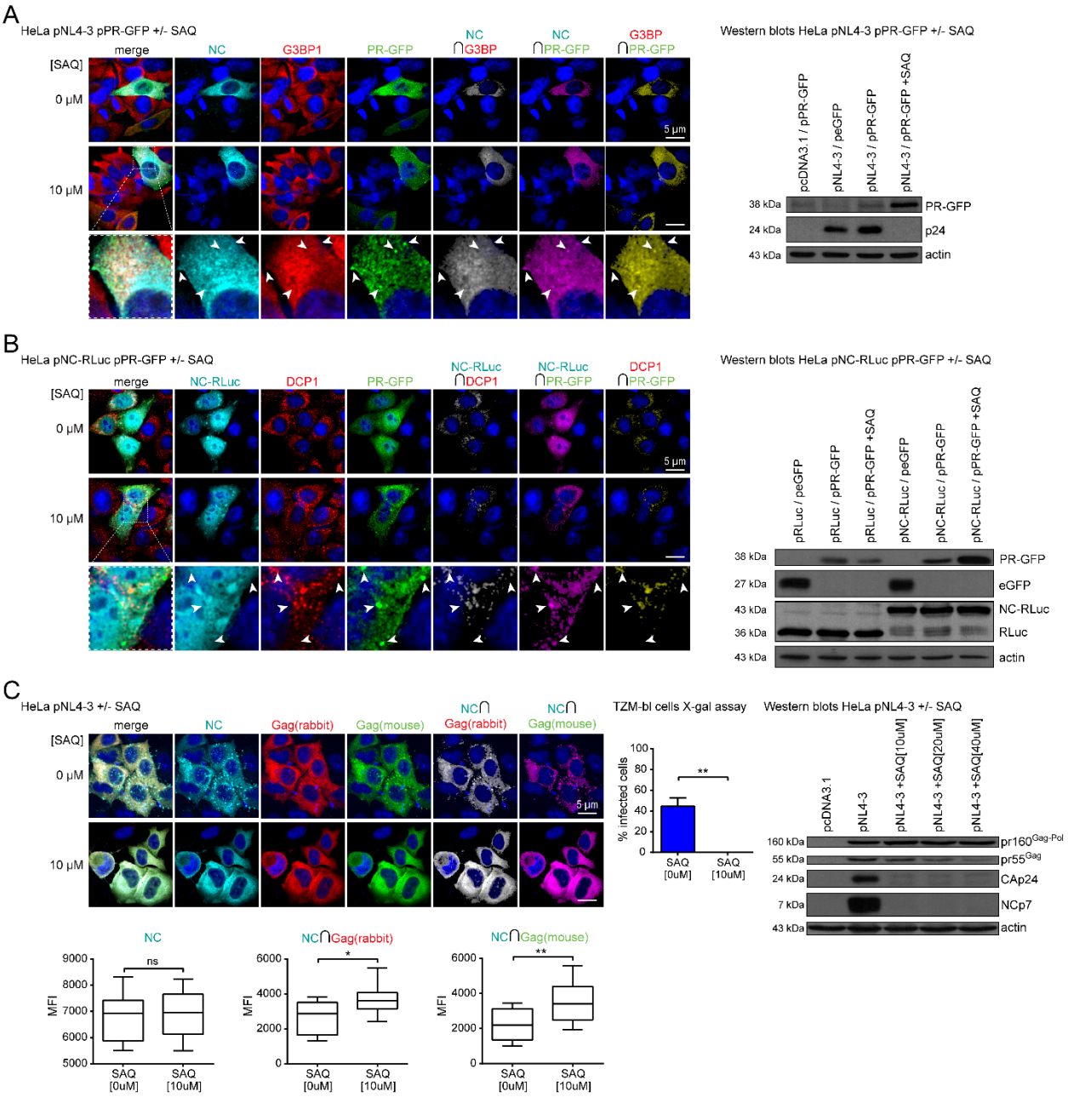
Supplemental Figure S3



Supplemental Figure S3, Related to Figure 6: PrLD informatics and pan-retrovirus NC and HIV-1 Gag proteins condensing into Zn^{2+}-dependent LLPSs. (**A**) Illustration of predicted positions of PrLDs (*blue*) from various protein amino acid sequences used to validate the ability of PLAAC, PONDR and MobiDB to properly predict PrLD locations.TIA1, T-cell intracellular antigen 1; hnRNP A2B1, Heterogeneous nuclear ribonucleoproteins A2/B1 isoform; hnRNP A0, Heterogeneous nuclear ribonucleoprotein A0; hnRNP A1L2, Heterogeneous nuclear ribonucleoprotein A1-like 2; DAZAP1, DAZ-associated protein 1; Lysozyme, T4 lysozyme mutant Arg 96 -> His; RNA pol II, RNA polymerase II second largest subunit; HEWL, Hen egg white lysozyme; BPT1, Bos Taurus pancreatic trypsin inhibitor precursor; CALM1, Calmodulin; UB, Ubiquitin. (**B**) Expanded representative images from Figure 6E. Ability of purified Zn^{2+}-loaded NC proteins, from several retroviruses tested, to phase separate into spherical 0.5-2 µm NC-LLPS at the 10 µM concentration, as visualized by laser microscopy and DIC, demonstrating pan-retrovirus NC-LLPS sensitivities to general and specific Zn^{2+} ejecting treatments; where all NC proteins are sensitive to several cycles of freeze/thaw or to TPEN treatment, but only a few NC-LLPSs are sensitive to selective treatment by both ADA and DIBA-1 (HIV-1, SIV, MuLV), and two are sensitive to DIBA-1 only (HTLV-1, RSV) (n = 3). (**C**) Ability of purified HIV-1 Gag protein to phase separate into spherical 0.5-2 µm Gag-LLPS at the 10 and 20 µM concentrations, as visualized by laser microscopy and DIC (10 vs. 20 µM Gag protein, $p < 0.0001$, 95% CI -93.3 to -80.6) (n = 3), demonstrating Gag-LLPS sensitivity to freeze/thaw cycles, general Zn^{2+} chelation (TPEN) and specific Zn^{2+} ejecting treatments (ADA and DIBA-1) (no treat. vs. freeze/thaw, $p < 0.0001$, 95% CI 87.3 to 101.1; no treat. vs. TPEN, $p < 0.0001$, 95% CI 81.4 to 94.0; no treat. vs. ADA, $p < 0.0001$, 95% CI 87.9 to 100.6; no treat. vs. DIBA-1, $p < 0.0001$, 95% CI 36.2 to 49.0) (n = 4). HIV-1, human

immunodeficiency virus 1; SIV, simian immunodeficiency virus; FIV, feline immunodeficiency virus; EIAV, equine infectious anemia virus; HTLV-1, human T-cell leukemia virus 1; RSV, Rous sarcoma virus; MuLV, murine leukemia virus; DIC, differential interference contrast; µm, micron; size bars, 3 µm; TPEN, N,N,N′,N′-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine; ARS, arsenite; ADA, azodicarbonamide; DIBA (DIBA-1), 2,2′-dithiobisbenzamide-1.

Supplemental Figure S4



Supplemental Figure S4, Related to Figure 7: Cellular colocalization of HIV-1 NC and pNC-RLuc with HIV-1 PR is unaltered by protease inhibitor causing loss of cellular NC. (**A**) HeLa cells transfected with pNL4-3 and pPR-GFP were treated with SAQ [0-10μM] for effects on colocalization between NC *(cyan)* and PR-GFP *(green)* colocalization in cells (-drug vs. 10 μM SAQ, NC-PR, p = 0.3251, two-tailed t test, t=0.99 df=32, 95% CI ; NC-RLuc-PR, p = 0.2024, two-tailed t test, t=1.30 df=38, 95% CI). G3BP1 (*red*) was used to explore SAQ induced PR-GFP cytoplasmic bodies *(white arrows)* (*n* = 3). Western blot *(right)* is included to demonstrate increase in $p24^{Gag}$ from PR-GFP expression, and loss of $p24^{Gag}$ from SAQ treatment. (**B**) HeLa cells transfected with pNC-RLuc and pPR-GFP were treated with SAQ [0-10μM] for effects on colocalization between NC-RLuc *(cyan)* and PR-GFP *(green)* colocalization in cells. Dcp1 (*red*) was used to explore SAQ induced PR-GFP cytoplasmic bodies *(white arrows)* (*n* = 3). Western blot *(right)* is included to demonstrate expression of NC-RLuc (non-specific bands from previous blotting) and PR-GFP. (**C**) HeLa cells transfected with pNL4-3 with SAQ [0-40μM] for effects on colocalization between NC *(cyan)* able to recognize NC, Gag-Pol, and $pr55^{Gag}$, and two different Gag antibodies, both demonstrating increased colocalization between NC and Gag from SAQ treatment *(bottom graphs)*, indicating that NC is cleaved by active protease in cells in non-treatment conditions (-drug vs. 10 μM SAQ, NC-Gag(rabbit), p = 0.0106, two-tailed t test, t=2.82 df=20, 95% CI; NC-Gag(mouse), p = 0.0064, two-tailed t test, t=3.04 df=20, 95% CI) (*n* = 3). TZM-bl cells X-gal assay graph *(top middle panel)*, demonstrates SAQ treatment on virus titer (infectivity) (p = 0.0001, two-tailed t test, t=38.01, df=10, 95% CI). Western blot *(right)* is included to demonstrate NC cleavage by PR in cell lysates from cells stripped of viruses by Trypsin/EDTA treatment, in addition elimination of NC by SAQ treatment, and decreased $pr55^{Gag}$ from increasing

concentrations of SAQ treatment. Box plot horizontal lines indicate median, and whiskers are minimum to maximum. SAQ, saquinavir; µm, micron; RLuc, Renilla Luciferase; ∩, colocalization (intersection); MFI, mean fluorescence intensity; *, $p < 0.05$; **, $p < 0.01$; two tailed t test.