Supporting Information

Gudleski et al. 10.1073/pnas.1000304107

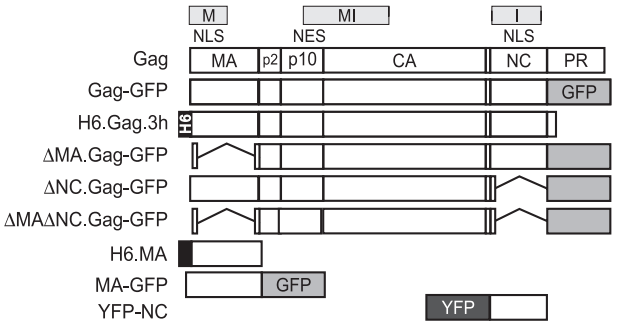

Fig. S1. Schematic of Gag expression constructs. The WT RSV Gag polyprotein contains MA, p2, p10, CA, NC, and PR domains. The nuclear localization domains (NLS), membrane-binding domain (M, residues 1–86), NES (residues 219–229), multimerization interface (MI), and Gag-vRNA interaction (I) domain are denoted. In Gag-GFP derivatives, GFP replaces seven amino acids of NC and the entire PR domain. H6.Gag.3h and H6.MA each contain an N-terminal 6-histidine tag (H6). H6.Gag.3h includes the WT Gag sequence through the first seven amino acids of PR. The ΔMA construct has residues 5–148 deleted. In ΔNC, all but the N-terminal seven amino acids were deleted. MA-GFP and YFP-NC fusion proteins are depicted.

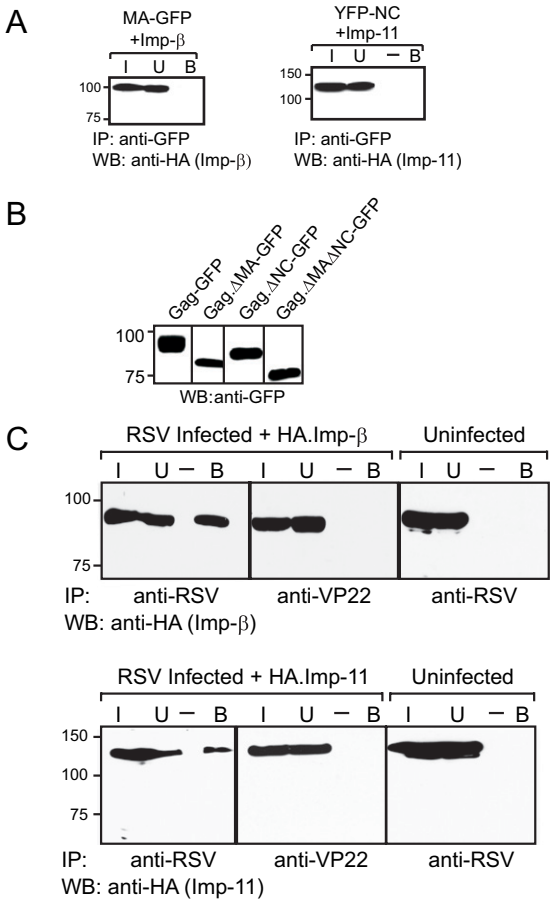

Fig. S2. Coimmunoprecipitation of WT and mutant RSV Gag with imp-β and imp-11. (*A*) Coimmunoprecipitation of imp-11 with YFP-NC and imp-β with MA-GFP. QT6 cells coexpressing MA-GFP and HA.imp-β or YFP-NC and HA.imp-11 were lysed and incubated with anti-HA antibody, immune complexes were precipitated, and proteins eluted were detected by Western blotting using anti-GFP antiserum. The lanes marked by (–) do not contain any protein. Molecular weight standards (kDa) are shown at the left. I, input (3% of total cell lysate); U, unbound (3% of total unbound fraction); B, bound (entire sample eluted from beads); IP, immunoprecipitation antibody; WB, antibody used for Western blotting. (*B*) Expression levels of Gag-GFP, Gag.ΔMA-GFP, Gag.ΔNC-GFP, and Gag.ΔMAΔNC-GFP used in coimmunoprecipitation assays shown in Fig. 1 and *C*. (*C*) Coimmunoprecipitation of imp-β or imp-11 with RSV Gag from RSV-infected cells. RSV-infected QT6 cells were transiently transfected with either HA.imp-β (*Upper Left*) or HA.imp-11 (*Lower Left*). Uninfected cells were included as a negative control (*Upper and Lower Right*). An antibody against HSV-1 VP22 protein was used as an irrelevant antibody control. The HA-tagged importins were detected as described in Fig. 1. RSV Gag was detected with anti-RSV polyclonal antiserum followed by mouse-anti-rabbit FITC-conjugated secondary antibody. Each experiment was performed three times, and representative blots are shown.

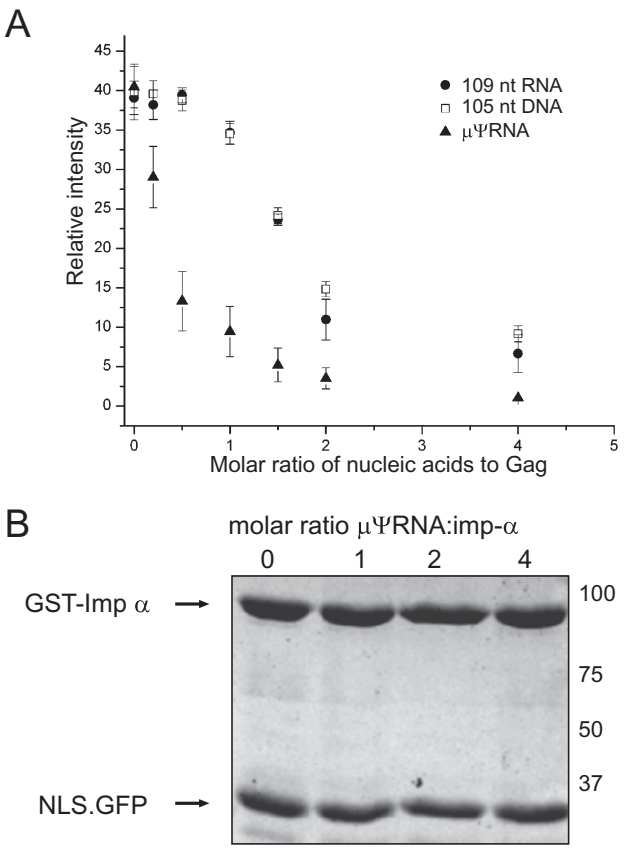

Fig. S3. Quantification of Gag pull-down at various nucleic acid concentrations and in RNA competition control experiment. (*A*) Averages (±SEM) for the H6.Gag.3h band intensities (representative experiment shown in Fig. 2*C*) from three independent experiments were quantified using ImageJ software (1) and plotted against the nucleic acid/protein molar ratio. (*B*) Competition between μΨ-RNA and imp-α for binding to the SV40 T antigen NLS (H6.NLS.GFP). Recombinant GST-tagged imp-α was mixed with increasing amounts of in vitro transcribed μψ-RNA at 4 °C as described in *Materials and Methods* and then incubated with recombinant H6.NLS.GFP protein at an equimolar concentration. After incubation with GST beads, bound proteins were visualized with Coomassie blue. Molecular weight markers are indicated to the right. μΨ-RNA did not inhibit binding of imp-α to the SV40 NLS.

1. Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with ImageJ. *Biophotonics Int* 11:36–42.

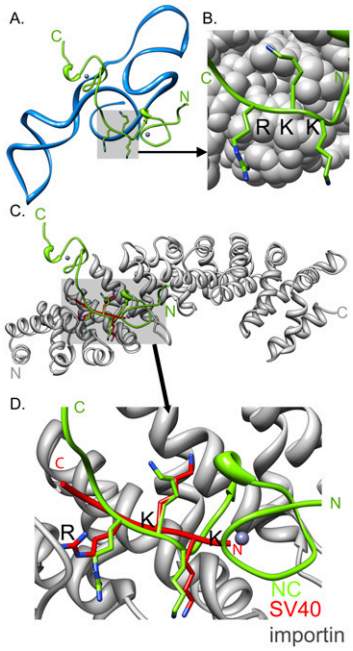

Fig. S4. Comparison of vRNA and imp-α binding sites in NC. (*A*) Ribbon diagram of the RSV NC protein in a binary complex with the μψ-RNA packaging signal (PDB ID code 2IHX, model 13) (1). The RNA is shown in blue, and the NC protein is shown in green. The zinc ions are shown as gray spheres. (*B*) Close-up of the basic residues RKK in NC and their contacts with the μψ-RNA, which are depicted as gray spheres in the space-filling model. (*C*) Ribbon diagram of imp-α (gray ribbon) in a complex with the NLS peptide of the SV40 T antigen (red; PDB ID code 1BK6) (2). The NC protein, in the same orientation as in *A*, has been superimposed onto the equivalent residues of the NLS peptide of SV40 T antigen. In this orientation, the imp-α binding site and the RNA binding site in NC colocalize, suggesting that these binding events are mutually exclusive. (*D*) Close-up of the NLS binding site in imp-α. Side chain residues are shown in stick representation with nitrogen atoms colored blue. The side chain atoms of the basic residues in the SV40 T-antigen NLS and homologous basic residues in NC superimpose well, suggesting that the binding site of RSV NC is preformed in the RNA-bound conformation.

1. Zhou J, McAllen JK, Tailor Y, Summers MF (2005) High affinity nucleocapsid protein binding to the muPsi RNA packaging signal of Rous sarcoma virus. *J Mol Biol* 349:976–988.
2. Conti E, Uy M, Leighton L, Blobel G, Kuriyan J (1998) Crystallographic analysis of the recognition of a nuclear localization signal by the nuclear import factor karyopherin alpha. *Cell* 94: 193–204.

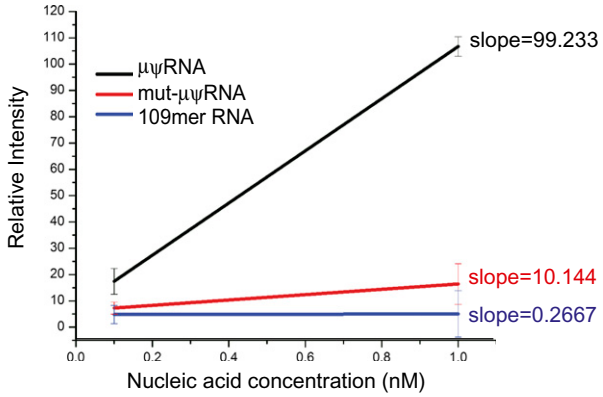

Fig. S5. Analysis of Gag:CRM1:RanGTP complexes stabilized by RNA. Quantitative analysis of data shown in Fig. 4*B*. The average intensities (±SEM) of H6. Gag.3h signals detected by Western blotting from three independent experiments were quantified using ImageJ software (1) and plotted against nucleic acid/protein molar ratios. Analysis of the slope of the curves indicated that μψ-RNA stimulated Gag:CRM1 binding 9.78-fold over mut-μψ-RNA ($P = 0.001$) and 372-fold compared with nonviral 109-nt RNA ($P = 0.0002$).

1. Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with ImageJ. *Biophotonics Int* 11:36–42.