Supporting Information

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Fig. S1. BICD2 required for productive HIV-1 infection. (*A* and *B*) HeLa TZM-bl cells depleted of two dynein adaptor proteins, Hook3 and ZW10, by siRNA transfection (*A*) and subsequent infection with HIV-1 pseudotyped with JRFL and HXB2 envelope proteins (*B*). (*C* and *D*) Infectivity measurements performed and plotted as above in HeLa TZM-bl cells following infection with R7 Δ EnvGFP pseudotyped with envelope proteins from VSV or A-MLV (*C*) or B-MLV pseudotyped with VSV or A-MLV envelope proteins (*D*). (*E*) Infectivity measurements in THP1 cells following infection with R7 Δ EnvGFP pseudotyped with envelope proteins from VSV or A-MLV. Data from single independent experiments (left graph in every panel) and normalized and average data from three or more independent experiments (right graph in every panel) are shown (\pm SD). ****P* < 0.001**P* < 0.05; ns, not significant.



Fig. S2. Viral fusion unaffected in BICD2 knockout cells. Control and BICD2 knockout HeLa TZM-bl cells infected with JRFL pseudotyped R7 Δ EnvGFP containing Blam-Vpr. Cells loaded with Blam substrate CCF2 2 h following synchronized infection. (A) FACS analysis showing the percent of cleaved CCF2. Data are representative of three independent experiments. (B) Means (\pm SEM) of results from three independent experiments. ns, not significant.

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Fig. S3. BICD2 required for targeting HIV-1 virions to nuclear import sites. Control and BICD2 knockout HeLa TZM-bl cells were synchronously infected with JRFLg pseudo typed HIV-1 reporter virus (MOI of 0.3). Cells fixed 0, 1, or 3 h postinfection and immunostained with antibodies to Nup358 (green) and HIV-1 capsid protein p24 (red). (*A*) A representative image at 3 h postinfection. Nup358 and CA colocalization in control cells is shown in the middle. (*B*) The fraction of Nup358 signal in the cytoplasm at the indicated time postinfection. (*C*) Quantification of CA and Nup358 localization in the control and knockout cells. (*D*) PLA of Nup358 and CA performed on the BICD2 knockout cells 3 h following synchronous infection. (*E*) Quantification of average fold increase in PLA puncta; 20 or more cells were analyzed in each experiment. Error bar represent the SEM. Data are representative of three independent experiments. ****P* < 0.001, **P* < 0.05; ns, not significant.