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Supplemental information

HIV-1 exploits the Fanconi anemia pathway for viral DNA integration

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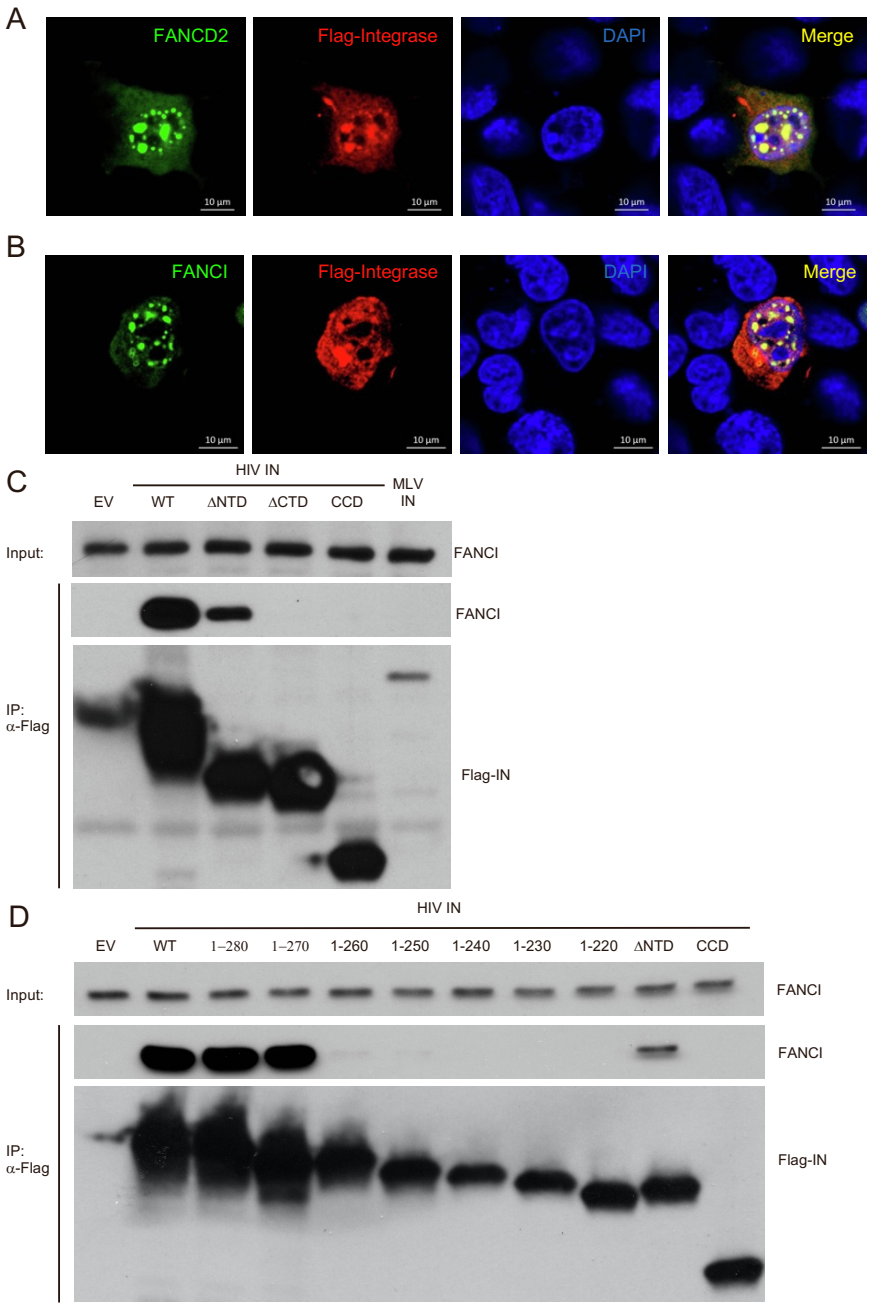

Figure S1. HIV-1 IN interacts with FANCI, related to Figure 1.
(A and B) Intracellular colocalization of HIV-1 IN with FANCD2 (A) and FANCI (B). 293A cells stably expressing flag-tagged HIV-1 IN were fixed, permeabilized and stained with anti-flag, anti-FANCD2, and anti-FANCI antibodies. Images are representative of at least three similar experiments. Scale bar size: 10 μm.
(C and D) 293A cells were transfected with empty expression vector (EV), or plasmids expressing Flag-tagged WT HIV-1 IN, or HIV-1 IN with indicated deletions, or MLV IN. Cell lysates were treated with Benzonase. Flag-tagged IN were immunoprecipitated by the anti-Flag antibody. The co-immunoprecipitated FANCI was probed by Western blot. ΔNTD: HIV-1 IN with NTD deletion; ΔCTD: HIV-1 IN with CTD deletion; CCD: HIV-1 IN catalytic core domain.

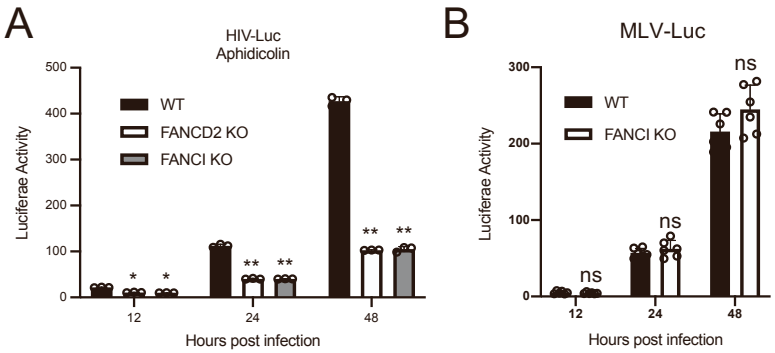

Figure S2. FANCD2 and FANCI are required for HIV-1 DNA integration, related to Figure 2.
(A) FANCD2 and FANCI are required for HIV-1 DNA integration in non-dividing cells. Wild-type (WT) 293A cells or 293A cells with knockout of indicated genes were treated with Aphidicolin for 24 hours and then infected with VSV-G pseudotyped HIV-Luc virus. Luciferase activities were measured at indicated time points post infection.
(B) FANCI is not required for the infection of MLV. Indicated cells were infected with VSV-G pseudotyped MLV-Luc virus. Luciferase activities were measured at indicated time post infection.
Data are represented as mean ± SD from three independent experiments (n = 3). ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$.

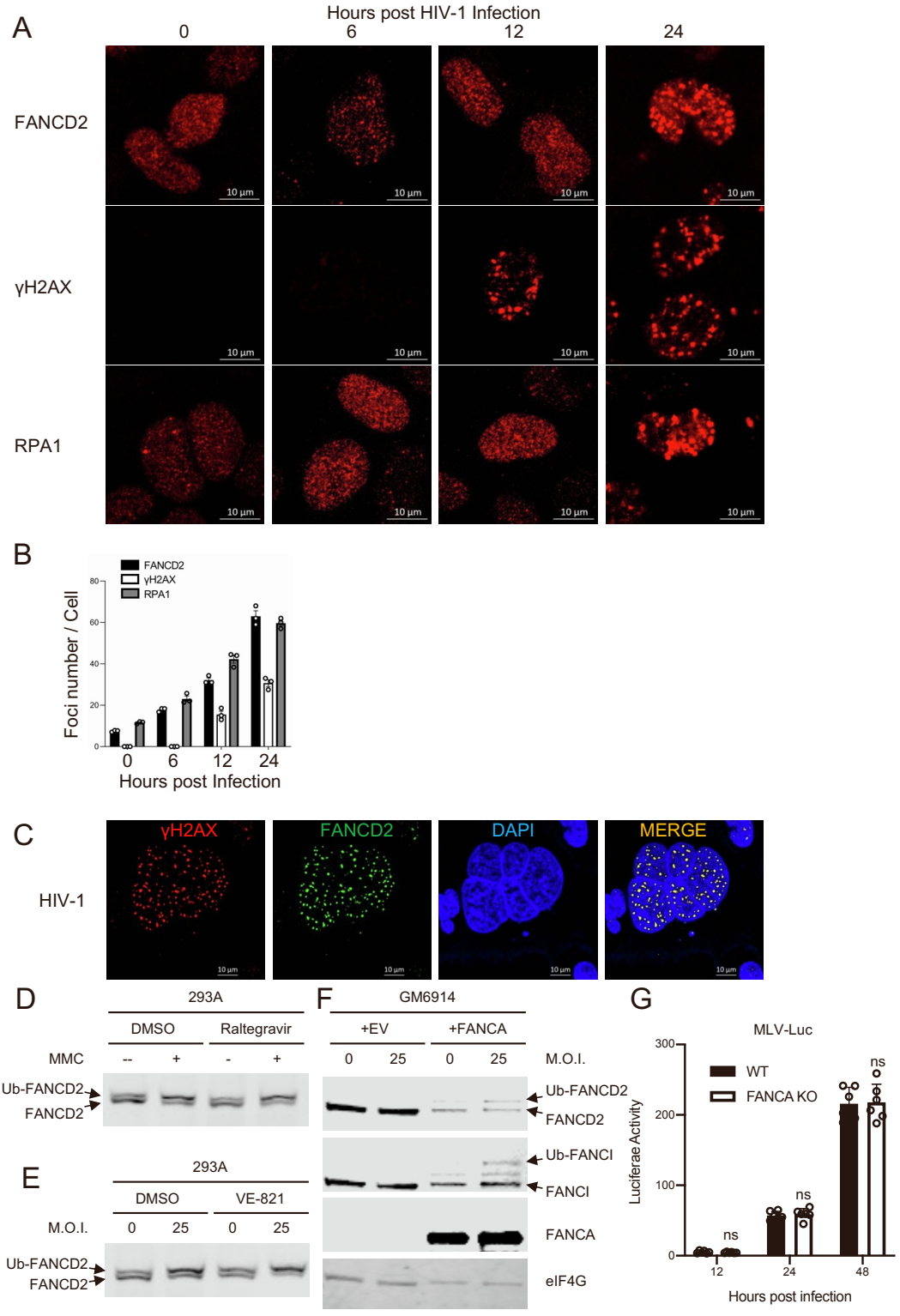

Figure S3. HIV-1 infection activates the FA pathway, related to Figure 3.
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(A) 293A cells were infected with VSV-G pseudotyped HIV-mCherry reporter virus (M.O.I 25). Cells were then processed for the immunostaining of FANCD2, γH2AX, and RPA1 at indicated time post infection. Images are representative of at least three similar experiments. Scale bar size: 10 μm.
(B) Quantitation of average FANCD2, γH2AX, and RPA1 foci numbers per cell. Foci with fluorescence intensity >= 200, size >= 75 pixel^2 were counted. Data are mean ± SD from three representative images (n = 3).
(C) 293A cells were infected with VSV-G pseudotyped HIV-mCherry reporter virus (M.O.I 25) for 24 hours. Cells were then processed for the immunostaining of FANCD2 and γH2AX. Images are representative of at least three similar experiments. Scale bar size: 10 μm.
(D) HIV-1 Integrase inhibitor Raltegravir had no effect on the FA pathway activation by MMC. 293A cells were treated with MMC and Raltegravir. The mono-ubiquitination of FANCD2 was determined by Western blot.
(E) Cells were infected with VSV-G pseudotyped HIV-mCherry reporter virus at indicated M.O.I and treated with DMSO or ATR inhibitor VE-821. The mono-ubiquitination of FANCD2 was determined by Western blot.
(F) Patient-derived FANCA-deficient cells (GM6914) stably transduced with empty expression vector (+EV) or wild-type FANCA (+FANCA) were infected with VSV-G pseudotyped HIV-mCherry virus at indicated M.O.I, and the expression of indicated proteins were determined by Western blot.
(G) FANCA is not required for the infection of MLV. Parental 293A cells (WT) and FANCA KO cell lines were infected with VSV-G pseudotyped MLV-Luc virus. Luciferase activities were measured at indicated time post infection. Data are represented as mean ± SD from three independent experiments (n = 3). ns: $p > 0.05$.

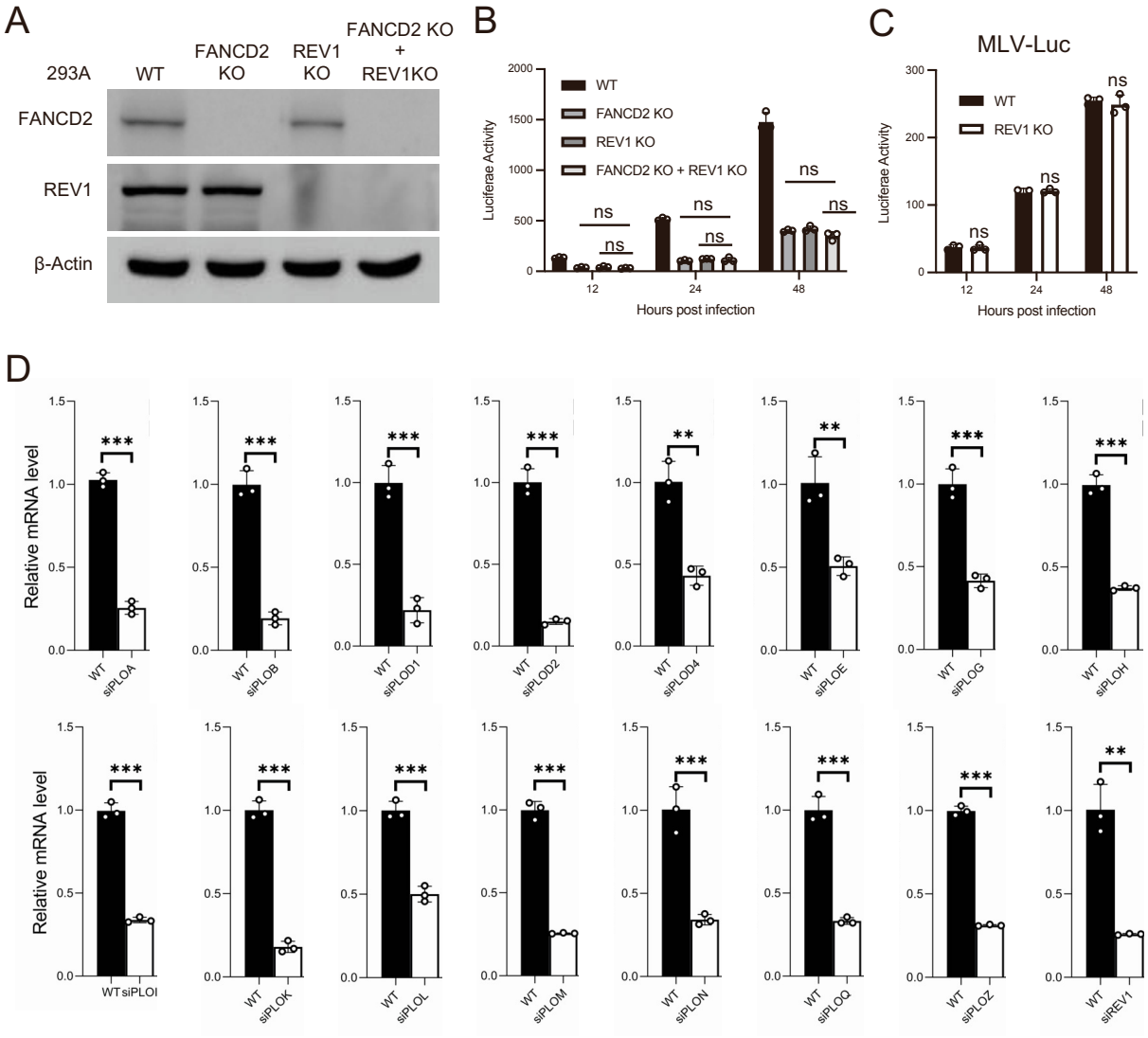

Figure S4. REV1 is required for HIV-1 DNA integration, related to Figure 4.
(A) The expression of indicated proteins in knockout cell lines were determined by Western blot.
(B) Parental 293A cells (WT), and 293A cells with knockouts of indicated genes were infected with VSV-G pseudotyped HIV-Luc virus. Luciferase activities were measured at indicated time post infection.
(C) REV1 is not required for the infection of MLV. Parental 293A cells (WT) and REV1 KO cell lines were infected with VSV-G pseudotyped MLV-Luc virus. Luciferase activities were measured at indicated time post infection.
(D) RT-qPCR to determine the relative mRNA levels of target gene in cells transfected with indicated siRNA. WT: cells transfected with non-targeting control siRNA.
Data are represented as mean ± SD from three independent experiments (n = 3). ns: $p > 0.05$.

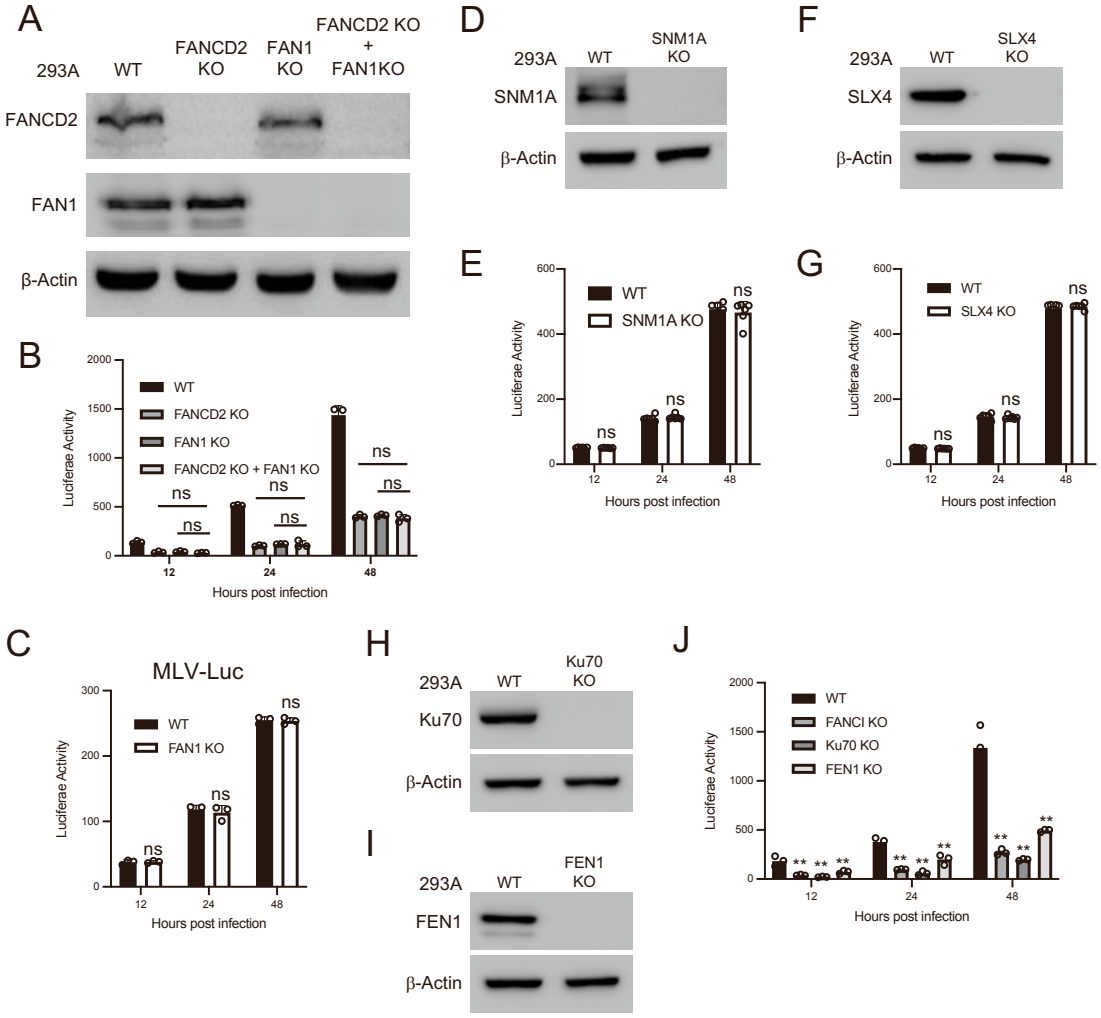

Figure S5. Flap nuclease FAN1 is required for HIV-1 DNA integration, related to Figure 5.
(A, D, F, H, and I) The expression of indicated proteins in knockout cell lines were determined by Western blot.
(B) Parental 293A cells (WT), and 293A cells with knockouts of indicated genes were infected with VSV-G pseudotyped HIV-Luc virus. Luciferase activities were measured at indicated time post infection.
(C) FAN1 is not required for the infection of MLV. Parental 293A cells (WT) and FAN1 KO cell lines were infected with VSV-G pseudotyped MLV-Luc virus. Luciferase activities were measured at indicated time post infection.
(E and G) SNM1A (B) or SLX4 (D) is not required for HIV-1 infection. Indicated cells were infected with VSV-G pseudotyped HIV-Luc virus. Luciferase activities were measured at indicated time post infection.
(J) Knockout of Ku70 or FEN1 inhibited HIV-1 infection. Parental 293A cells (WT), and 293A cells with knockouts of indicated genes were infected with VSV-G pseudotyped HIV-Luc virus. Luciferase activities were measured at indicated time post infection.
Data are represented as mean ± SD from three independent experiments (n = 3). ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$.