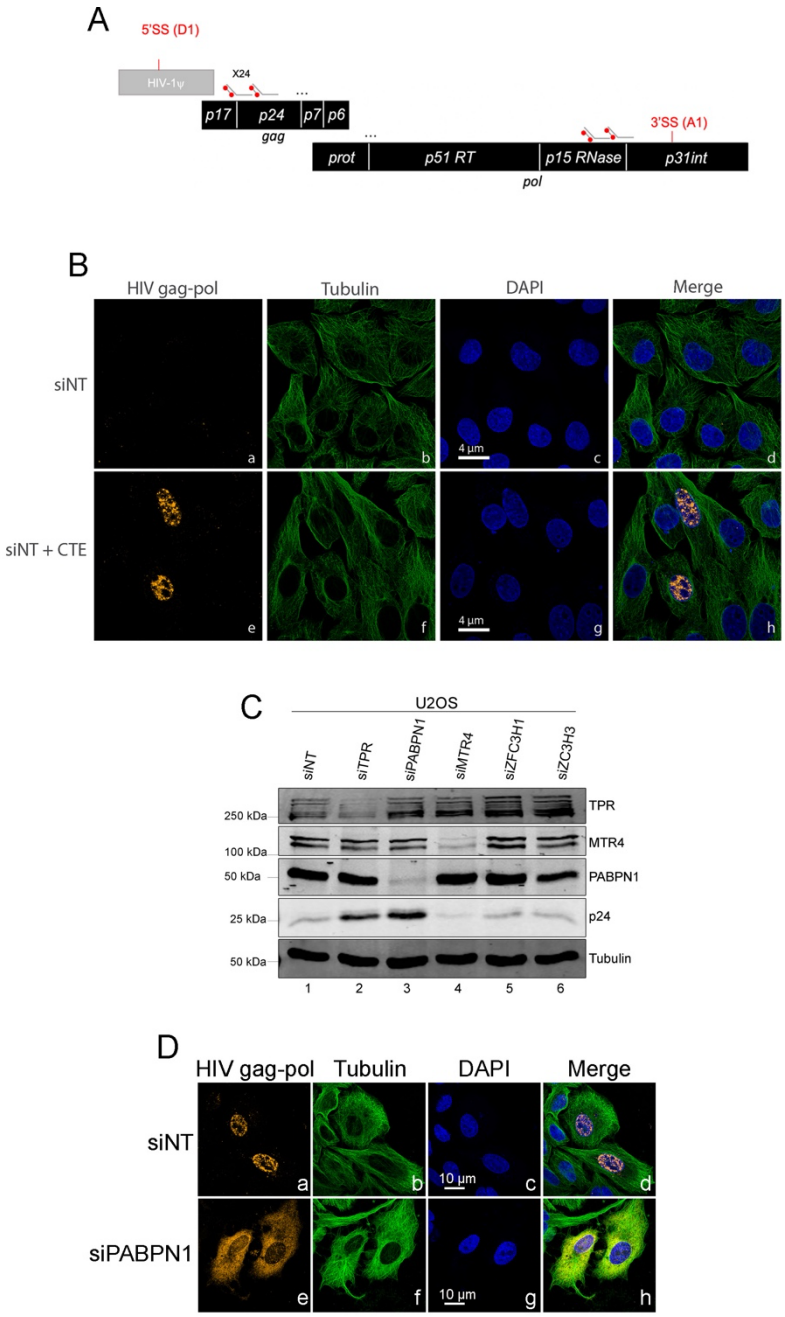

Supplemental Figure 6. Analysis of *gag-pol* unspliced RNA localization by single-molecule fluorescent in situ hybridization (smFISH). (A) A set of 24 primary probes complementary to *gag-pol* sequences located between 5' and 3' splice sites (SS) were pre-hybridized in vitro with the Cy3-labeled (red circles) secondary probe via the FLAP sequence. Resulting duplexes were subsequently hybridized to target RNA on fixed cells. (B) Specific detection of unspliced *gag-pol* transcripts by smFISH in U2OS cells. smFISH analysis of unspliced *gag-pol* transcripts in mock-transfected U2OS cells (a-d) and U2OS cells transfected with the Gag-Pol-CTE construct (e-h). (C) Western blot analysis of the indicated proteins using total extracts prepared from U2OS cells that were previously co-transfected with the Gag-Pol-CTE construct and the indicated siRNAs. (D) Unspliced gag-pol transcripts accumulate in the cytoplasm of PABPN1-deficient HeLa cells. Deconvoluted images of HeLa cells that were previously co-transfected with the wild-type Gag-Pol-CTE construct and either nontarget control (a-d) or PABPN1-specific (e-h) siRNAs were simultaneously analyzed by smFISH using Cy3-labeled probes for *gag-pol* sequences (a and e) and immunostaining for the cytosolic Tubulin (b and f). DNA stained with DAPI shows the nucleus of each cell (c and g). Scale bar size are indicated.