Reed, et al., http://www.jcb.org/cgi/content/full/jcb.201111012/DC1

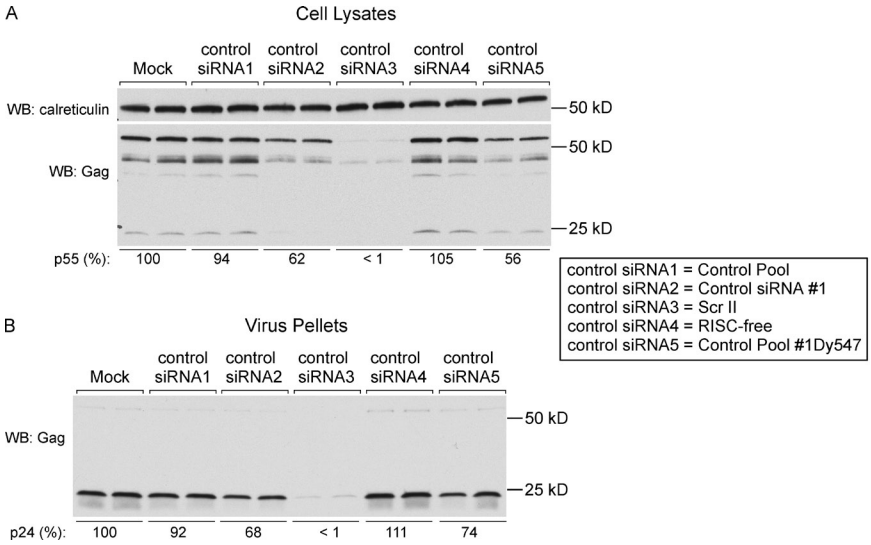

Figure S1. **Some siRNA control oligos reduce steady-state Gag levels.** (A and B) COS-1 cells were transfected twice with the indicated siRNA controls or mock transfected. Subsequently, cells were transfected with a provirus encoding HIV-GFP, and cells and media were harvested for analysis. Numbers below the Gag blots indicate the amount of p55 or p24 Gag as a percentage of the mock-transfected control. Blots are representative of two independent experiments. (A) Equivalent amounts of total protein from cell lysates were analyzed by WB for Gag and then reprobed for calreticulin as a loading control. The total protein concentration of each sample was approximately equivalent suggesting that control siRNAs did not have effects on cell viability (not depicted). RISC, RNA-induced silencing complex. (B) Virus pellets were harvested from the media of transfected cells, and equivalent aliquots of virus pellets were analyzed by WB for Gag.

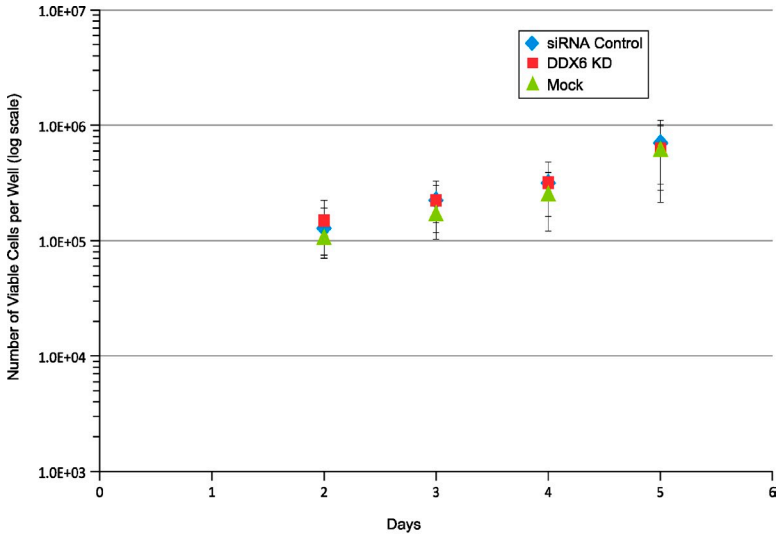

Figure S2. **DDX6 KD has no effect on cell viability.** Extra wells were plated in parallel in a DDX6 siRNA KD experiment and were treated with control or DDX6-specific siRNA or mock treated, as for experimental samples, with harvest on day 5. Cells in one well were harvested on days 2–5 of the experiment, and viable cells were counted using Trypan blue staining. Data are representative of two experiments, with error bars representing SEM.

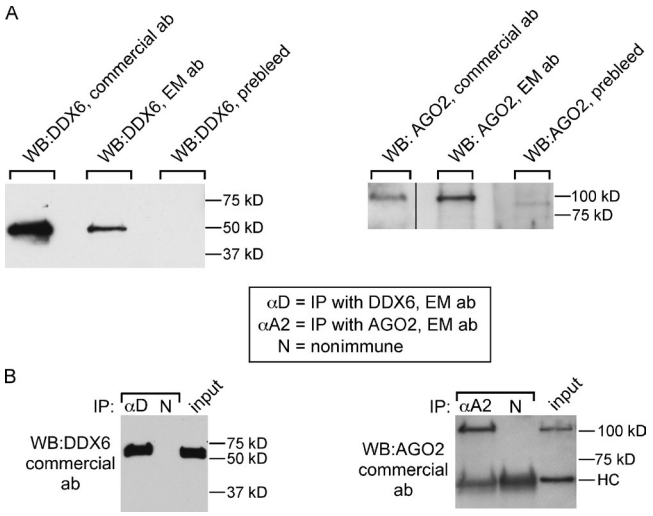

Figure S3. **Validation of antibodies to DDX6 and AGO2 used for IEM.** (A and B) Peptide-specific polyclonal antisera were generated to peptides from DDX6 and AGO2, as described in Materials and methods. Antisera were affinity purified against the relevant peptide, desalted, and concentrated for use in IEM. (A) Aliquots of COS-1 lysate were analyzed in parallel by WB using a commercial antibody (ab), the affinity-purified antibody we generated for IEM (DDX6, EM antibody, or AGO2, EM antibody), or the prebleed from the same rabbit. The black line indicates that an intervening lane has been spliced out. (B) To determine whether the antibodies we generated for IEM detect the expected antigens by IP, lysates of H9-HIV cells were subjected to IP under native conditions using the affinity-purified antibodies indicated (αA2 and αD) or a matched nonimmune antibody followed by WB using the corresponding commercial antibody. Equivalent aliquots of IP inputs are also shown. HC, IgG heavy chain detected by secondary antibody. Blots are representative of three independent experiments.