Supplemental Fig. 1

Copurification of the large domain of polymerase II

Upper panel: To reconstitute viral RNPs for purification by the Strep procedure, HEK 293T cells were transfected with expression plasmids encoding the viral nucleoprotein fused C-terminally to STREP-tag (NP-Strep), untagged NP, the polymerase subunits PA, PB1 and PB2 and a plasmid expressing influenza virus minigenome transcripts under the control of a polymerase-I (PolI) promoter. After 24 hours, cell extract (CE) was prepared and the Strep purification was carried out as described in Materials and Methods. The final eluate and cell extract were analyzed by Western blot for the presence of the large subunit of the polymerase II using a phosphospecific antibody (aH14, BAbC0) recognizing the multiple Ser-5-phosphorylated sites at the C-terminal domain of this protein.

Lower panel: Strep purification of N-terminally tagged non-structural protein (NS1) of influenza virus (A/WSN/33) from HEK 293T cells. The purification and Western blot analysis were carried out as described for the upper panel.