Ubiquitination and deubiquitination of NP protein regulates influenza A virus RNA replication

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Supplementary information

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Supplementary Materials and Methods

Virus strains and cell culture.

The A/WSN/33 (WSN) strain of influenza virus (H1N1) was grown in MDCK cells maintained in minimal essential medium alpha medium (MEM alpha medium, Invitrogen) with 10% fetal bovine serum and incubated at 37℃ with 5% CO_2. A549 and 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum and incubated at 37℃ with 5% CO_2. For single-cycle virus infections, A549 cells were infected with the WSN virus at a multiplicity of infection (MOI) of 5 PFU/cell. The infected cells were incubated in DMEM containing 2% fetal calf serum.

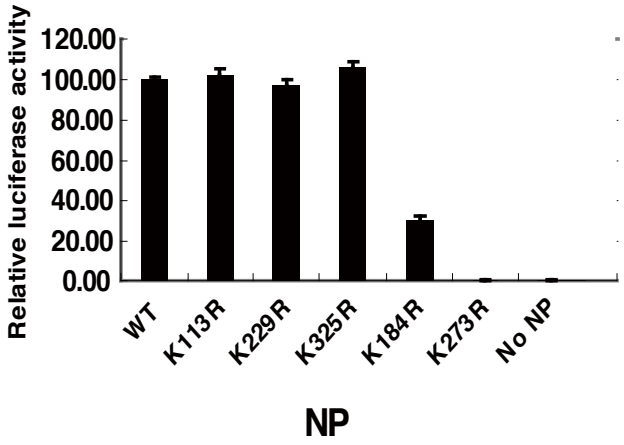
Plasmid construction.

The pCI-USP11 was constructed as follows. The DNA sequences corresponding to NCBI accession number NM_004651 (Lin *et al*, 2008) were amplified by PCR and cloned into the *Nhe*I and *Eco*RI sites of pCI vector (Promega). The pCI-USP11s, which expresses a wobble mutant of USP11, was constructed by using the jumping-PCR method (Higuchi *et al*, 1988). The catalytically deficient mutant of USP11 (pCI-USP11mt) was constructed by substituting two cysteine (C275S and

C283S) residues within the Cys conserved domain of USP11 with serine by the jumping-PCR method. pCAG.2-NP-2xHA, for the expression of the NP protein, was constructed by inserting the *Eco*RI and *Xho*I sites of a modified pCAG.2-HA-MCS-HA vector. All substitution mutations of NP were constructed also by using the jumping-PCR method. The pPolI-Luc produces a virus-like RNA in which the coding region for firefly luciferase is flanked by the packing signals of the NS segment. This plasmid was constructed by inserting the firefly luciferase gene flanked by the 3' and 5' untranslated regions (UTRs) of the NS segment of A/WSN/33 virus into the *Bsm*BI sites of pPolI_BsmBI modified vector, which contains the human RNA polymerase I promoter and mouse RNA polymerase I terminator separated by *Bsm*BI sites.

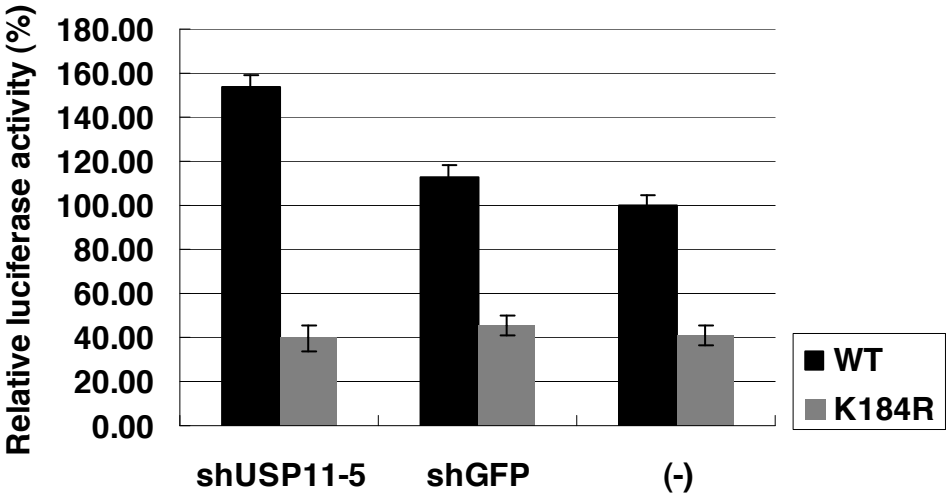
Supplementary Figures

Fig. S1



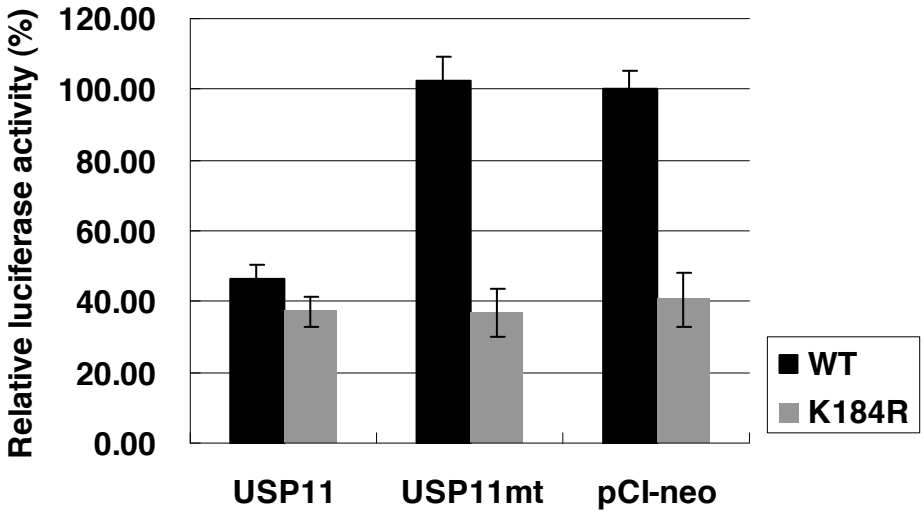
Supplementary Figure S1. K184 of NP is important for influenza A virus genome RNA replication using the mini-replicon reporter system (Li *et al*, 2009b). 293T cells were transfected with pPolI-Luc and plasmids for the expression of the viral PB2, PB1, PA and NP (wild-type or mutant). *Renilla* luciferase was used as an internal control. As a negative control, 293T cells were transfected with the same plasmids, in the absence of the NP expression plasmid. Luciferase assay was performed at 48 hrs post-transfection. The data were normalized relative to the values detected in the cells transfected with wild-type NP. Values are means ± SEM of three separate experiments.

Fig. S2



Supplementary Fig. S2. USP11 inhibits viral RNA replication through deubiquitinating on K184 of NP. USP11 knockdown or control cells were transfected with pPolI-Luc and plasmids for the expression of the viral PB2, PB1, PA and NP (wild-type or K184R). *Renilla* luciferase was used as an internal control. Luciferase assay was performed at 48 hrs post-transfection. The data were normalized relative to the values detected in the 293T cells transfected with wild-type NP. Values are means ± SEM of three separate experiments.

Fig. S3



Supplementary Figure S3. USP11 inhibits viral RNA replication through deubiquitinating on K184 of NP. 293T cells were transfected with pPolI-Luc and plasmids for the expression of the viral PB2, PB1, PA and NP (wild-type or K184R) and USP11 or USP11 mutant. *Renilla* luciferase was used as an internal control. Luciferase assay was performed at 48 hrs post-transfection. The data were normalized relative to the values detected in the cells transfected with wild-type NP. Values are means ± SEM of three separate experiments.

Supplementary Table II. Primer used for the influenza A virus quantitative RT-PCR procedures.

Primer	Sequence (5'-3')[a]
Tagged vRNA-RT	CCCGATAAACACTCACCGACTCACATCGAAATCATGGCGACCAAAGGCACC
Tagv-F	CCCGATAAACACTCACCGACTC
V-R	GCTCCACTTTCCATCTACTCTCC
vRNA-Luc-RT	AGCAAAAGCAGGGTGACAAAGACTAGTA
Luc/F	GTGTTGGGCGCGTTATTTATCGG
Luc/R	GAGGTAGATGAGATGTGACGAACG

[a] Underlined sequences are tag sequences that are not present in influenza A virus genome.