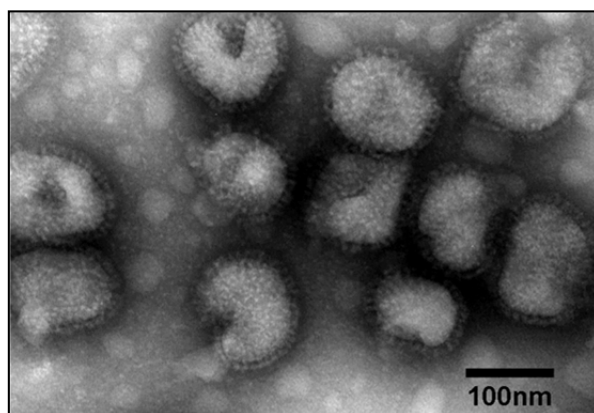
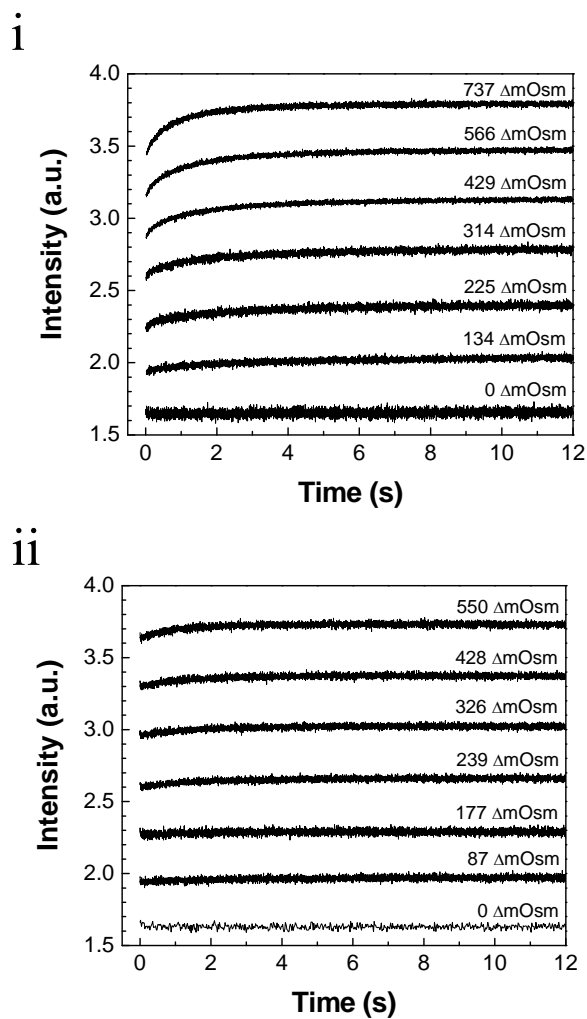


Supporting Information 1



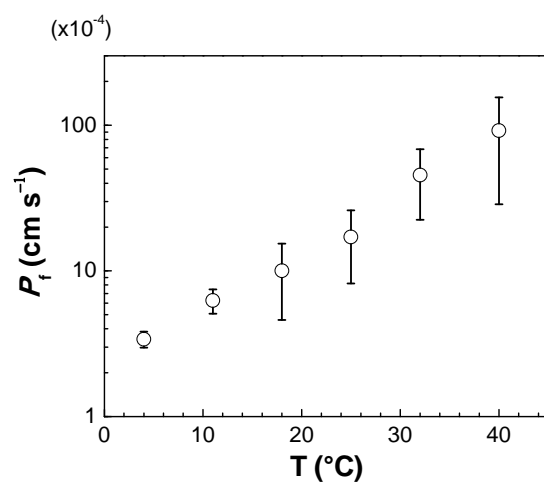
S1 Fig. A Transmission electron microscopy image of inactivated influenza virus. As shown in the micrograph, viruses are spherical in shape and spike-shaped glycoproteins are observed on the surface of the viral envelope. A mean diameter of the virus was measured to be 105 ± 16 nm from the analysis of such micrographs.

Supporting Information 1



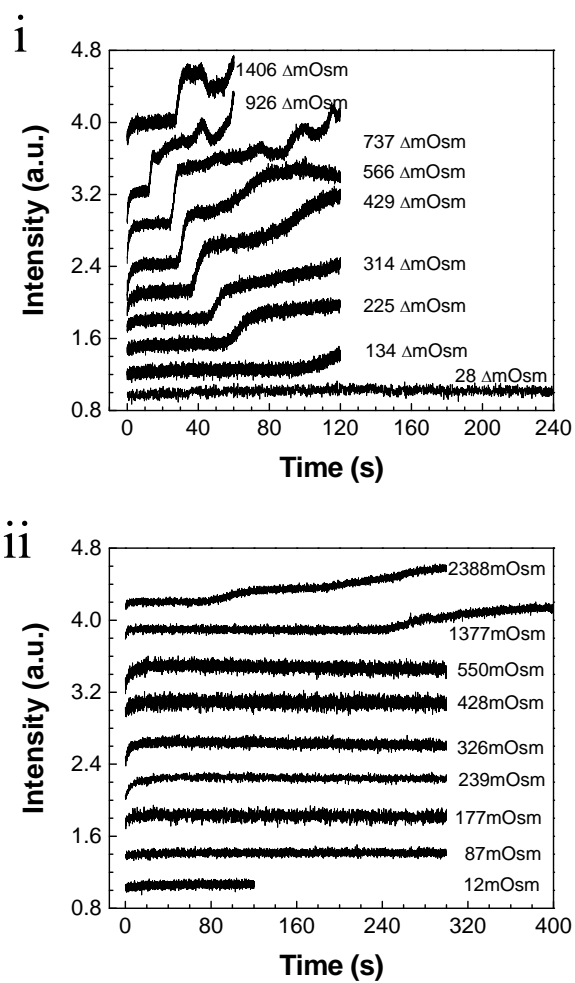
S1 Fig. B Time course of SFLS analysis of inactivated influenza virus exposed to hypertonic solutions. Virus suspensions ($0.2 \mu g \mu L^{-1}$) were abruptly exposed to hyperosmotic solutions of (i) sucrose and (ii) NaCl in a stopped-flow apparatus and the resulting changes in scattered light intensity were monitored at 546 nm as a function of time for 12 s. All measurements were made at 4 °C. Data are presented as the mean \pm standard deviation (SD) for $n > 30$. Hypertonic osmotic differences (ΔC_{os}) are indicated within the plots of (i) and (ii).

Supporting Information 1



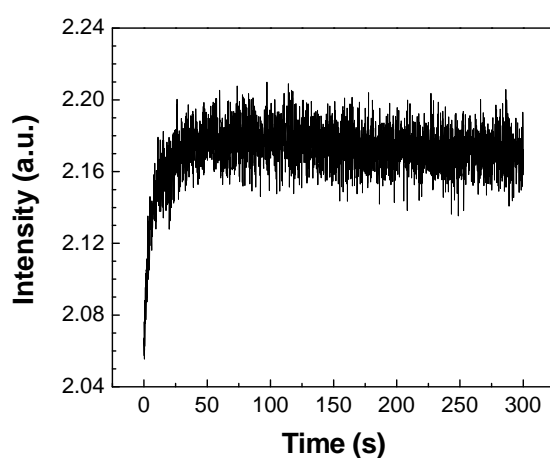
S1 Fig. C Temperature dependence of the osmotic water permeability coefficients of inactivated influenza virus derived from data in Fig. 2. (Mean \pm SD, $n = 15 - 30$.)

Supporting Information 1



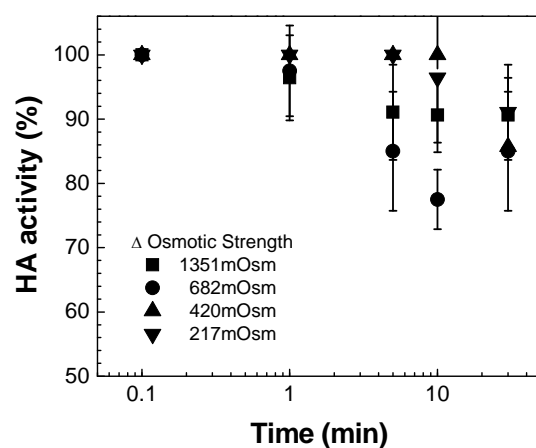
S1 Fig. D Long-term course of SFLS behavior of inactivated influenza virus in response to hyperosmotic gradients by (i) sucrose and (ii) NaCl solution. Hypertonic osmotic differences (ΔC_{os}) are indicated at the right side of each curve.

Supporting Information 1



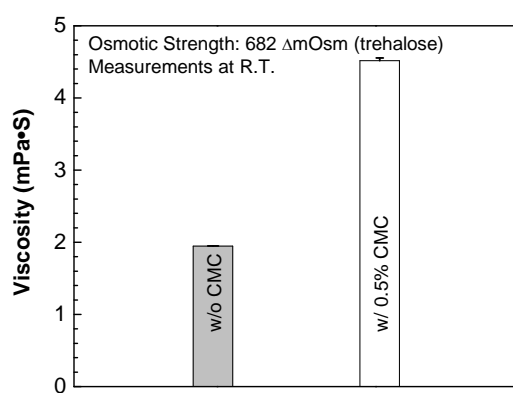
S1 Fig. E Long-term course of SFLS analysis of inactivated influenza virus in response to a hyperosmotic difference of $\Delta C_{os} = 239$ mOsm by NaCl solution. A biphasic intensity increase was not observed from the virus with NaCl solution at this condition. This can be explained by the leakage of the viral envelope to Na^+ Cl^- ions, as indicated by a gradual intensity decrease following the first phase of rapid intensity increase.

Supporting Information 1



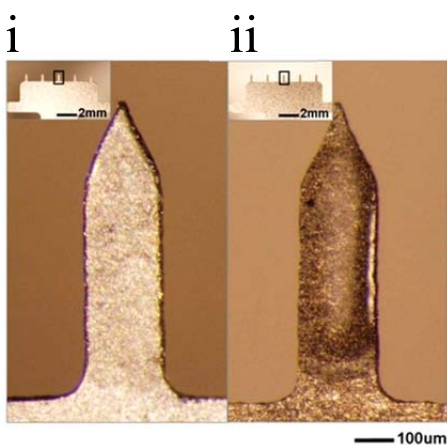
S1 Fig. F HA activity change as a function of incubation time in hypertonic solutions. The effect of osmotic pressure on the activity of live influenza virus was investigated by measuring HA activity change at four osmotic strength differences ($\Delta C_{os} = 217, 420, 682, 1351$ mOsm) using trehalose with the increase of incubation time (10 s, 1 min, 5 min, 10 min, and 30 min). HA activity change was calculated with respect to HA titer of the virus in iso-osmotic solution. All measurements were performed at 4 °C. (Mean \pm SD, $n = 8 - 16$.)

Supporting Information 1



S1 Fig. G Viscosity of the trehalose ($\Delta C_{os} = 682$ mOsm) solution and the trehalose ($\Delta C_{os} = 682$ mOsm) plus CMC (0.5% w/v) solution. (Mean \pm SD, $n = 3$.)

Supporting Information 1



S1 Fig. H Dried vaccine-coated MNs with inactivated influenza virus in formulations of (i) trehalose ($\Delta C_{os} = 682$ mOsm) only and (ii) trehalose (682 Δ mOsm) plus viscosity enhancer CMC (0.5% w/v). Influenza vaccine-coated MNs were air-dried for one day at ambient conditions and reconstituted in DPBS for vaccination of mice.