

SUPPLEMENTARY MATERIALS

Table S1. Immunogenicity study design

| vaccines | mice | Days of the study | | | |
|----------|------|-------------------|----------------|----------------|--------|
| | | Immunization | | Blood sampling | |
| | | I (day 1) | II (day 15) | day 15 | day 29 |
| SGF | 15 | 1 dose | 1 dose | 5 | 10 |
| U3 | 15 | 1 dose | 1 dose | 5 | 10 |
| U4 | 15 | 1 dose | 1 dose | 5 | 10 |
| VG | 15 | 1 dose | 1 dose | 5 | 10 |
| SGU | 15 | 1 dose | 1 dose | 5 | 10 |
| PBS | 15 | 0.5 ml | 0.5 ml | 5 | 10 |

Table S2. Protection study design

| | | Days of the study | | | | | | | |
|------|----|-------------------|----------------|-------------------------------------|---|--|-----------------------------|----------------------|---------------------|
| | | Immunization | | Challenge, day 29 | | | | | |
| mice | | | | Lethal challenge | | Sublethal challenge (sampling on day 32 after challenge) | | | |
| | | I (day 1) | II (day 15) | A/California H1N1pdm09 10LD50 | Survey day 29 to day 43 | A/Brisbane (H1N1) 10MID | A/Kansas (H3N2) 10MID | B/Maryland 100MID | B/ Phuket 100MID |
| SGF | 30 | 1 dose | 1 dose | 10 | | 5 | 5 | 5 | 5 |
| U3 | 30 | 1 dose | 1 dose | 10 | | 5 | 5 | 5 | 5 |
| U4 | 30 | 1 dose | 1 dose | 10 | weight loss and survival rates | 5 | 5 | 5 | 5 |
| VG | 30 | 1 dose | 1 dose | 10 | | 5 | 5 | 5 | 5 |
| SGU | 30 | 1 dose | 1 dose | 10 | | 5 | 5 | 5 | 5 |
| PBS | 30 | 0.5 ml | 0.5 ml | 10 | | 5 | 5 | 5 | 5 |

Table S3. Identification of neuraminidase in influenza virus monovaccines via mass spectrometry. In B/Vic (B/Colorado Victoria), B/Ym (B/Phuket Yamagata), and A/N2 (A/Kansas H3N2) samples, NA was reliably identified, the score exceeded the threshold value. The score for A/N1 (A/Brisbane H1N1) was below the threshold, so it can be assumed that NA is located within this interval. Apparently, this is probably due to the shortage of ions in the spectrum because NA was highly glycosylated.

| | MW (PAGE) | Similar strains with the respective NA subtype | Score / threshold |
|-------|--------------|--|----------------------|
| B/Vic | ~65 kDa | B/Singapore/49H/2010; B/India/P1015910/2010; B/Nairobi/351/2005 | 78 / 74 |
| B/Ym | ~65 kDa | B/Japan/315/2008 | 117 / 74 |
| A/N2 | ~70 kDa | A/Beijing/106/2012; A/Louisiana/06/2011; A/Wisconsin/10/2011 | 78 / 74 |
| A/N1 | ~70 kDa | A/Wisconsin/09/2013; A/California/10/2014; A/Helsinki/771M/2014 | 59 / 74 |

Figure S1. Chromatograms B/Vic, B/Ym, A/N2, and A/N1. Purple color stands for absorption at 280 nm wavelength, while red shows the fractions collected. In all the products, NA was present in fractions No. 3 (see the arrows).

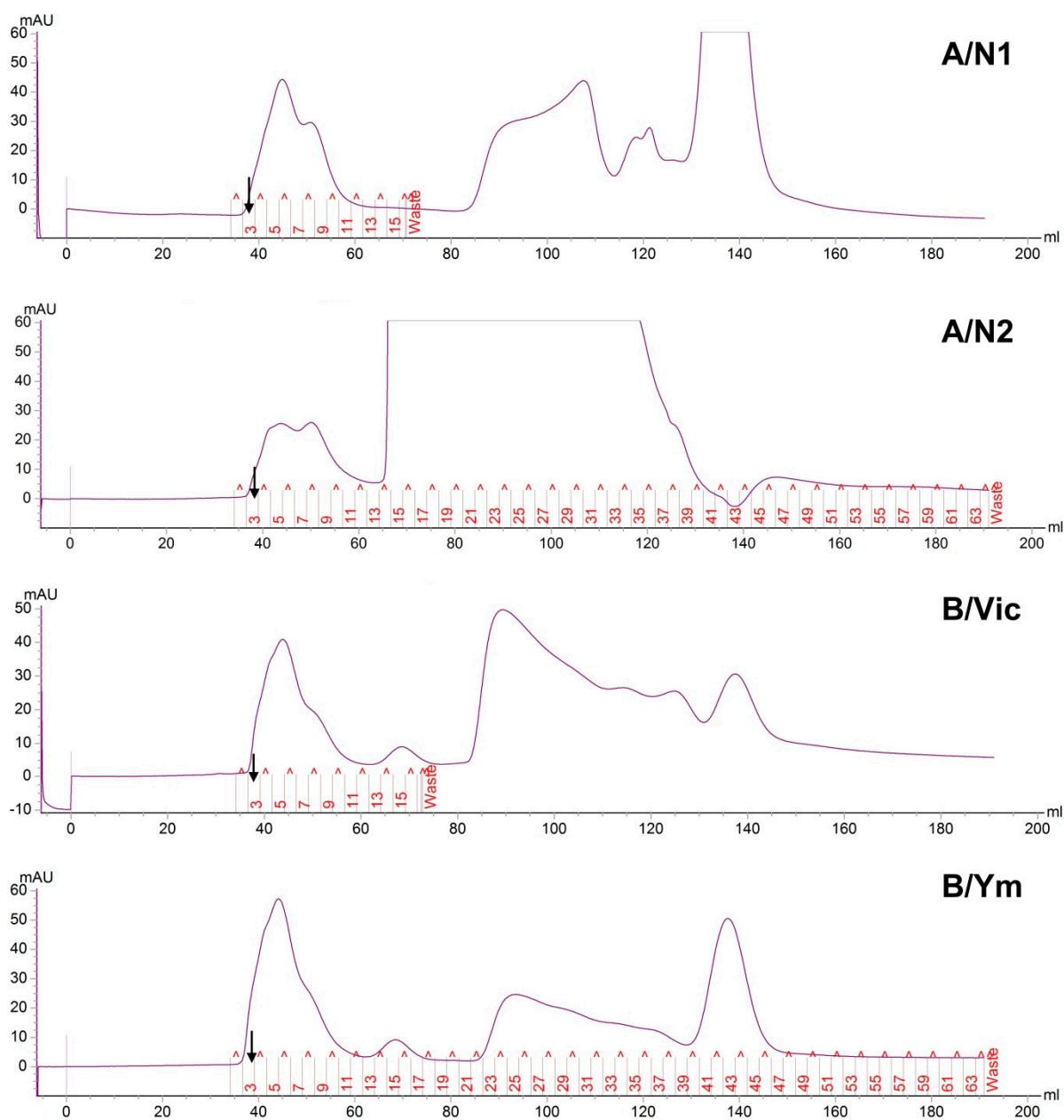


Figure S2. SDS-PAGE of B/Vic, B/Ym, A/N2, and A/N1. M is the molecular-weight size marker. The dots indicate the spots cut out for mass spectrometry analysis. Arrows show the areas where the IV NA was reliably detected. An exclamation mark (!) indicates the spot with the low score / threshold ratio found for A/N1.

