**Supplementary FIGURE 1 – Comparison of expression strategies excluding the plant-based system (presented in box 1)**. Each bar is subdivided proportionally to the number of expressed proteins for flu-VLPs production. *Details for proteins expressed in vector constructs are available in* ***Supplementary Table 2****.* *\* 4 or more*: VLPs produced with multiple HA subtypes using rBV carrying up to 6 genes.

**Supplementary TABLE 1 – Strain origin of influenza proteins used for flu-VLP production.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Host** | **Strain Specs.** | **Strain Subtype** | **Influenza Strain** | **Protein(s)** | **References** |
| **HUMAN INFLUENZA VIRUS** | **Laboratory & Pandemic strain** | **H1N1** | A/Brevig Mission/1/1918 | NA | [123] |
| A/California/04/2009 | HA, NA, M1 | [8,23,31,32,128,130,131,142] |
| A/Puerto Rico/8/1934 | HA, NA, M1 | [21,23,38,40,43,44,58,63,65,66,74,97,100,115,132,137,138,151] |
| A/South Carolina/1/1918 | HA | [123,133] |
| A/WSN/1933 | M1, M2 | [41,124,125] |
| **H3N2** | A/Aichi/2/1968 | HA | [35] |
| A/Aichi/2/1968-x31 | HA | [65] |
| A/Hong Kong/1/1968 | HA, NA, M | [35,137] |
| A/Udorn/72 | HA, NA, M1, M2 | [15,23,24,122,123] |
| **HPAI** | **H5N1** | A/Cambodia/JP52a/2005 | HA, NA | [31] |
| A/Hanoi/30408/2005 | HA, NA | [39] |
| A/Thailand/1(KAN-1)/2004 | HA, NA, M2 | [34,124,125] |
| A/Viet Nam/1203/2004 | HA, NA, M1 | [32,40,58,63,97,124,125,129,136–138,140] |
| A/Indonesia/05/2005 | HA, NA, M1 | [53,63,96,119,136,137,141,146] |
| A/Anhui/1/2005 | HA | [125] |
| **Other Human** | **H1N1** | A/Changchun/01/2009 | HA, NA, M1 | [134] |
| A/Gansu Chengguan/1129/2007 | NA | [31] |
| A/Korea/01-2-9/2009 | HA, NA, M1, M2 | [29] |
| A/New Caledonia/20/1999 | HA | [136] |
| A/New York/312/2001 | M, M1 | [32,133] |
| **H2N2** | A/Singapore/1/1957 | NA | [35] |
| **H3N2** | A/Fujian/411/2002 | HA, NA, M1 | [71] |
| A/Hiroshima/52/2005 | HA | [23] |
| A/New York/55/2004 | HA | [138] |
| A/Taiwan/083/2006 | HA, NA, M1, M2 | [37,39] |
| **H6N1** | A/Taiwan/2/2013 | HA, NA | [37] |
| **H7N2** | A/NewYork/107/2003 | HA | [136,138] |
| **H7N9** | A/Anhui/1/2013 | HA, NA | [24,119,141] |
| A/Shanghai/1/2013 | HA, NA | [24,119] |
| A/Shanghai/2/2013 | HA | [63] |
| A/Taiwan/S02076/2013 | HA, NA, M1 | [118] |
| A/Wuxi/1/2013 | HA, NA, M1 | [33] |
| **H9N2** | A/Hong Kong/1073/99 | HA, NA, M1 | [20] |
| A/Hong Kong/33982/2009 | HA | [63,138] |
| **H10N8** | A/Jiangxi/IPB13a/2013 | HA | [63] |
| **B** | B/Shanghai/361/2002 | HA | [136] |
| B/Yamagata/16/1988 | NA | [21] |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **OTHER INFLUENZA VIRUSES** | **Avian** | **H3N8** | A/pintail/Ohio/339/1987 | HA | [133] |
| **H5N1** | A/mallard/Maryland/802/2007 | HA | [133] |
| A/duck/Hokkaido/Vac-1/2004 | HA, NA, M1 | [135] |
| **HPAI H5N1** | A/goose/Guangdong/1/96 | M1 | [31] |
| A/chicken/WestJava(Sbg)/29/2007 | HA | [64,139] |
| A/chicken/Egypt/121/2012 | HA, NA | [64,139] |
| A/chicken/Korea/Gimje/2008 | HA, M1 | [59] |
| A/chicken/Germany/2014 | HA | [64] |
| A/chicken/Korea/ES/2003 | HA, NA, M1 | [101] |
| A/chicken/Hubei/489/2004 | HA, NA, M1 | [126] |
| **H5N3** | A/duck/France/02166/2002 | HA, NA | [127] |
| **H5N8** | A/mallard/Korea/W452/2014 | HA, NA | [100] |
| **H6N1** | A/partridge/Taiwan/LU1/99 | M1 | [152] |
| **H7N1** | A/chicken/FPV/Rostock/1934 | HA, NA, M2 | [34] |
| A/chicken/Italy/1067/99 | M | [127] |
| A/duck/Taiwan(H7N1) | HA | [152] |
| **H7N3** | A/chicken/Jalisco/CPA1/2012 | HA, NA | [119] |
| A/turkey/Oregon/1971 | HA | [139] |
| **H9N2** | A/chicken/Korea/01310/2001 | HA, M1 | [67] |
| A/turkey/Wisconsin/1/1966 | HA | [139] |
| **Swine** | **H2N3** | A/swine/Missouri/4296424/2006 | HA | [136] |
| **Canine** | **H3N2** | A/canine/Korea/LBM412/2008 | HA, M1 | [95] |
| **Meerkat** | **HPAI H5N1** | A/meerkat/Shanghai/SH-1/2012 | HA, NA, M1 | [117] |
| **Other** | **H7N3** | A/environment/Maryland/261/2006 | HA | [133] |

**Supplementary TABLE 2:**  **Vector construction used for influenza VLP production**. Pseudotyping of influenza-VLPwith the insertion of other viruses structural proteins : human immunodeficiency virus 1 (HIV-1) gag, friend–murine leukemia virus (MLV) Gag or GagPol, bovine immunodeficiency virus (BIV) gag (Bgag), G protein of vesicular stomatitis virus (VSVG).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | PROTEIN(S)  NUMBER | EXPRESSED INFLUENZA GENES | REFERENCES | | |
| Recombinant BV / Insect cells | Bacmam or MVA\* / Mammalian cells | Plasmid / Mammalian cells |
| SINGLE EXPRESSION | **1** | **HA** | [15,59,152] | [40] | [35] |
| **NA** | [15] | [40] | [31] |
| **M1** | [15,24,135] | [40] | [41] |
| CO-EXPRESSION | **2** | **HA - M1** | [59,72,125,133,135,140,152] | [40] |  |
| **HA - NA** |  | [40] |  |
| **NA - M1** | [135] |  |  |
| **NA - M** |  |  | [32] |
| **Gag - HA** |  |  | [34] |
| **3** | **HA - NA - M1** | [8,20,44,71,97,101,117,119,128,135,141,142] | [38] | [38] |
| **HA - NA - M** | [127] |  |  |
| **HA - NA - Bgag** | [63,137] |  |  |
| **4** | **HA - NA - M1 -M2** | [15,58,123] |  |  |
| **NA-M1-M2-VSVG (or VSVG/HA)** | [15] |  |  |
| **HA - NA - M1 -VSVG** |  | [38,44] |  |
| **5** | **triple HA - NA - M1** | [136,138] |  |  |
| **triple HA - NA - Bgag** | [64,139] |  |  |
| **6** | **quadruple HA - NA - Bgag** | [63] |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
| CO-INFECTION /  CO-TRANSFECTION | **2** | **HA + M1** | [21,23,24,65–67,95,96,115,122,130,131,133] |  |  |
| **NA + M1** | [151] |  |  |
| **HA + NA** |  |  | [35] |
| **M1 + M2** |  |  | [41] |
| **HA + Gag / GagPol** |  |  | [34] |
| **3** | **HA + NA + M** | [20] |  | [35] |
| **HA + NA + M1** | [100,118,129,134] |  | [21,31,33] |
| **HA + NA + Gag / GagPol** |  |  | [21,34] |
| **9** | **HA+NA+M1+M2+PB1+PB2+PA+NP+NS2** |  |  | [36] |
| COMBINATION | **3** | **NA - M2 + M1** | [124] |  |  |
| **HA - M1 + NA** | [125] |  |  |
| **HA - NA + M1** | [132] | [40] |  |
| **Gag - HA + NA** |  |  | [34] |
| **4** | **HA - M1 + NA - M2** | [124,125] |  |  |
| **6** | **Double HA – M1 + Triple HA – M1** | [30] |  |  |
| STABLE TRANSFECTION | **2** | **HA – NA** |  |  | [43] |
| **3** | **HA – NA – Gag** |  |  | [74] |
| **HA - NA (STABLE) + M1/Gag (TRANSIENT)** |  |  | [43] |
| **4** | **HA - NA (STABLE) + M1 + M2 (TRANSIENT)** | [29] |  |  |
| **HA – NA – M1 – M2** |  |  | [37,39] |
| **6** | **Double HA (STABLE) + Triple HA – M1** | [30] |  |  |