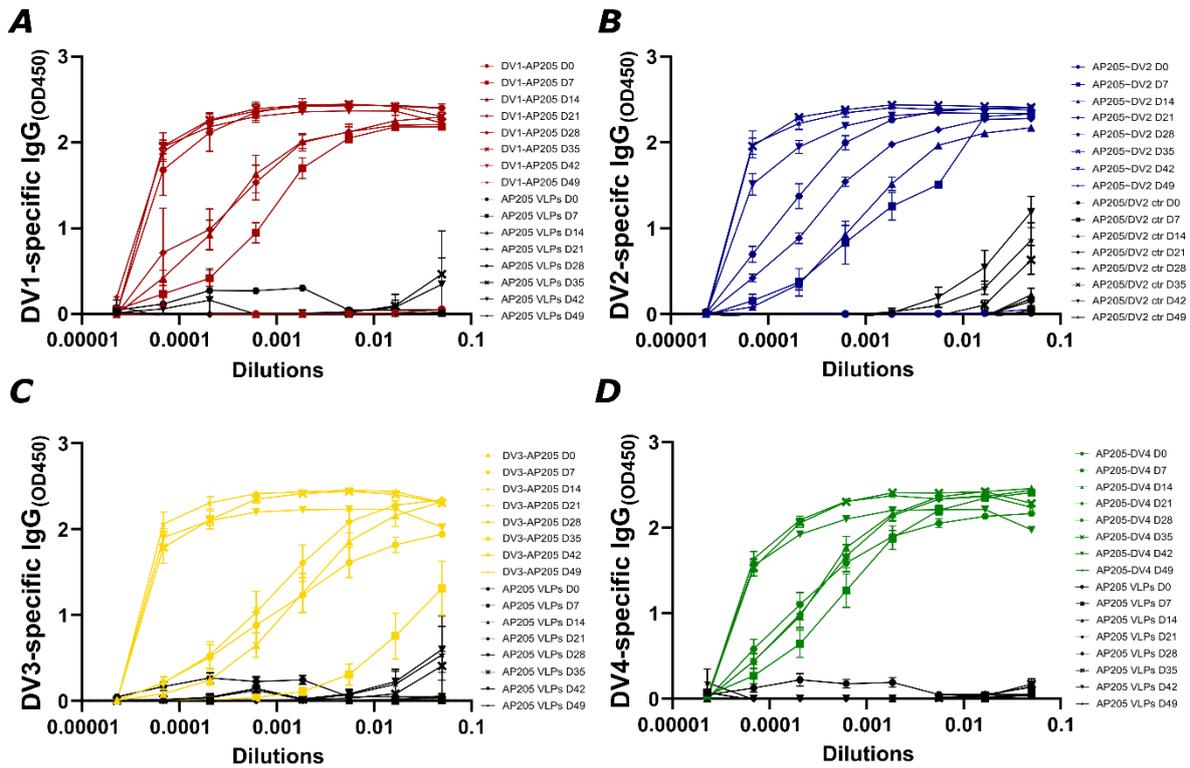


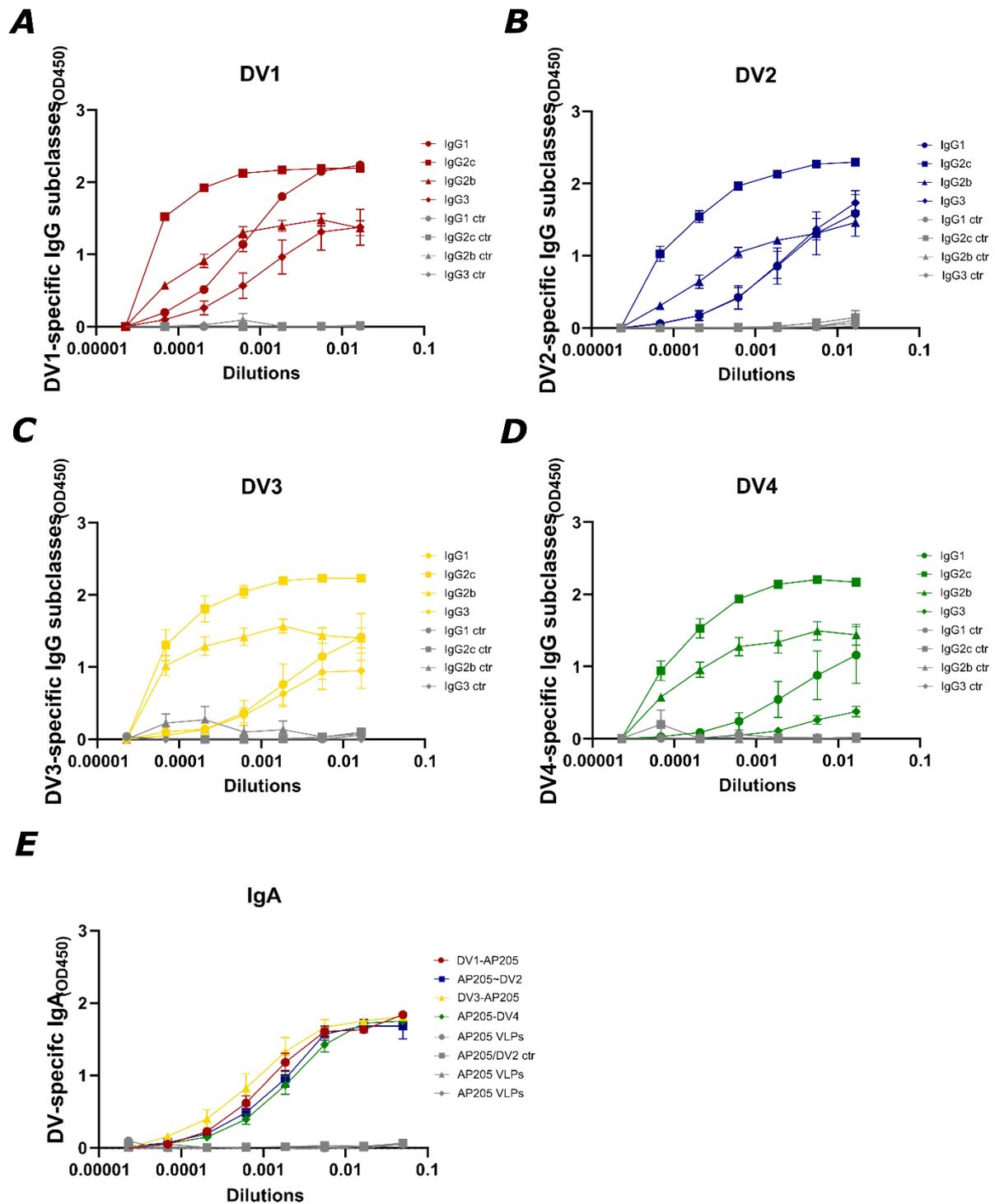
1 **Supplementary Figures**



2

3 **Supplementary Figure S1. Vaccination with the newly developed vaccine candidates induce a**
 4 **strong humoral immune response. A)** DV1-specific IgG titer on days 0, 7, 14, 21, 28, 35, 42 and 49
 5 from mice vaccinated with DV1-AP205 or AP205 VLPs measured by ELISA, OD450 shown. **B)** DV2-
 6 specific IgG titer on days 0, 7, 14, 21, 28, 35, 42 and 49 from mice vaccinated with AP205~DV2 or
 7 AP205 VLPs/DV2 mixture measured by ELISA, OD450 shown. **C)** DV3-specific IgG titer on days 0, 7,
 8 14, 21, 28, 35, 42 and 49 from mice vaccinated with DV3-AP205 or AP205 VLPs measured by ELISA,
 9 OD450 shown. **D)** DV4-specific IgG titer on days 0, 7, 14, 21, 28, 35, 42 and 49 from mice vaccinated
 10 with AP205-DV4 or AP205 VLPs measured by ELISA, OD450 shown. Vaccine groups n = 5, control
 11 group n = 5. One representative of 3 similar experiments is shown.

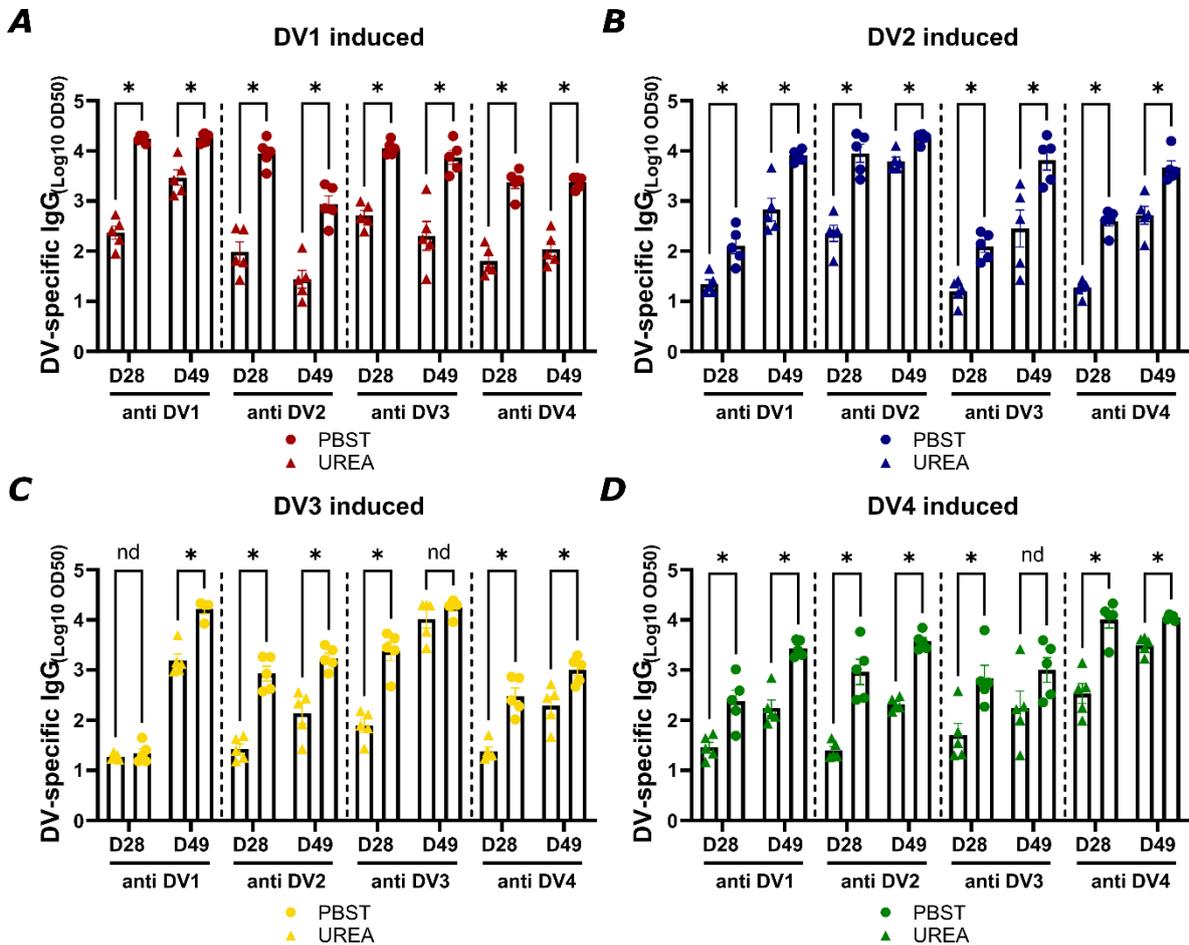
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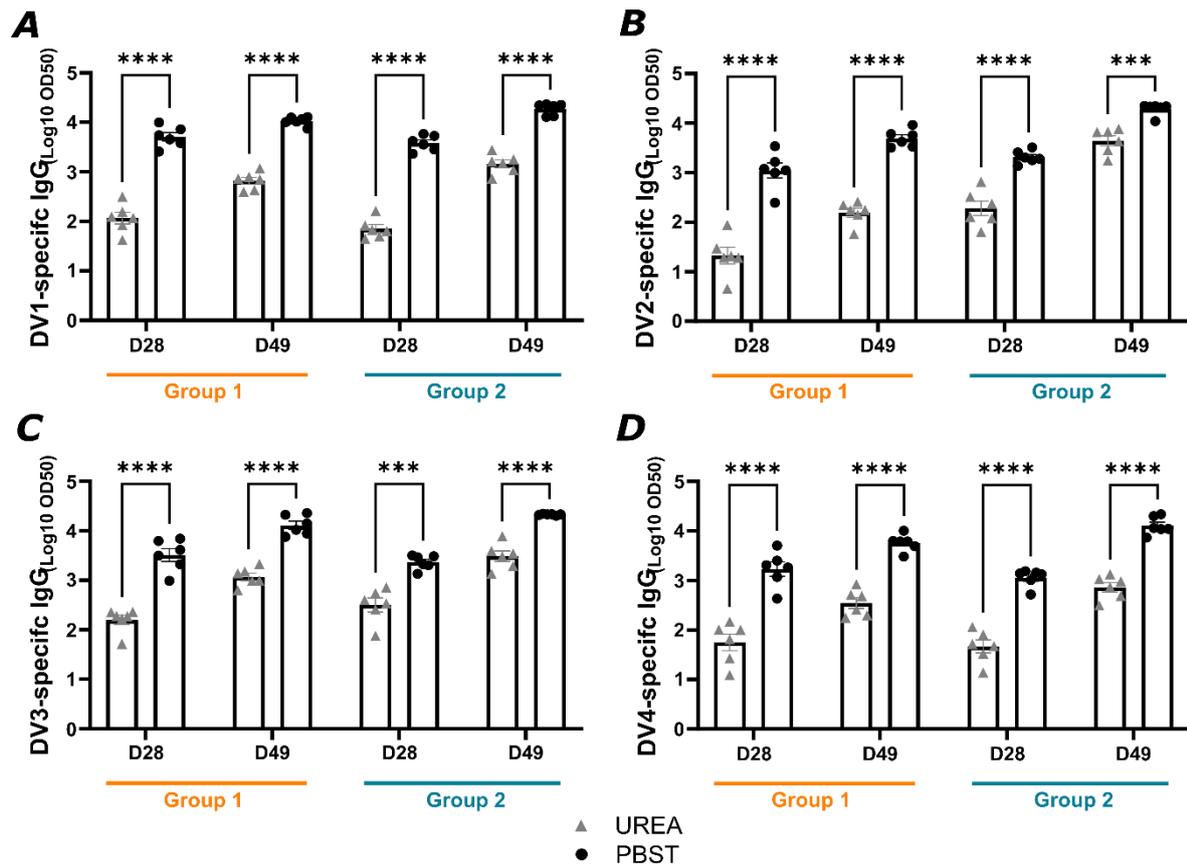
14 **Supplementary Figure S2. Immunization with all four vaccine candidates induces IgG2c and**
 15 **IgG2b dominant Dengue virus Envelope protein domain III specific IgG subclass response and**
 16 **promotes isotype switching to IgA. A)** DV1-specific IgG subclasses titer of day 42 from mice
 17 vaccinated with AP205 VLPs as control and DV1-AP205 measured by ELISA, OD₄₅₀ shown. **B)**
 18 DV2-specific IgG subclasses titer of day 42 from mice vaccinated with AP205 VLPs/DV2
 19 mixture as control and AP205~DV2 measured by ELISA, OD₄₅₀ shown. **C)** DV3-specific IgG
 20 subclasses titer of day 42 from mice vaccinated with AP205 VLPs as control and DV3-AP205
 21 measured by ELISA, OD₄₅₀ shown. **D)** DV4-specific IgG subclasses titer of day 42 from mice
 vaccinated with AP205 VLPs as control and

22 AP205-DV4 measured by ELISA, OD450 shown. **E)** DV-specific IgA titer at day 42 from mice immunized
 23 with AP205 VLPs or AP205/DV2 mixture (for DV2-specific) and DV1-AP205, AP205~DV2, DV3-AP05 and
 24 AP205-DV4 vaccines measured by ELISA, OD450 shown. Control group n = 5, vaccine groups n = 5. One
 25 representative of 2 similar experiments is shown.



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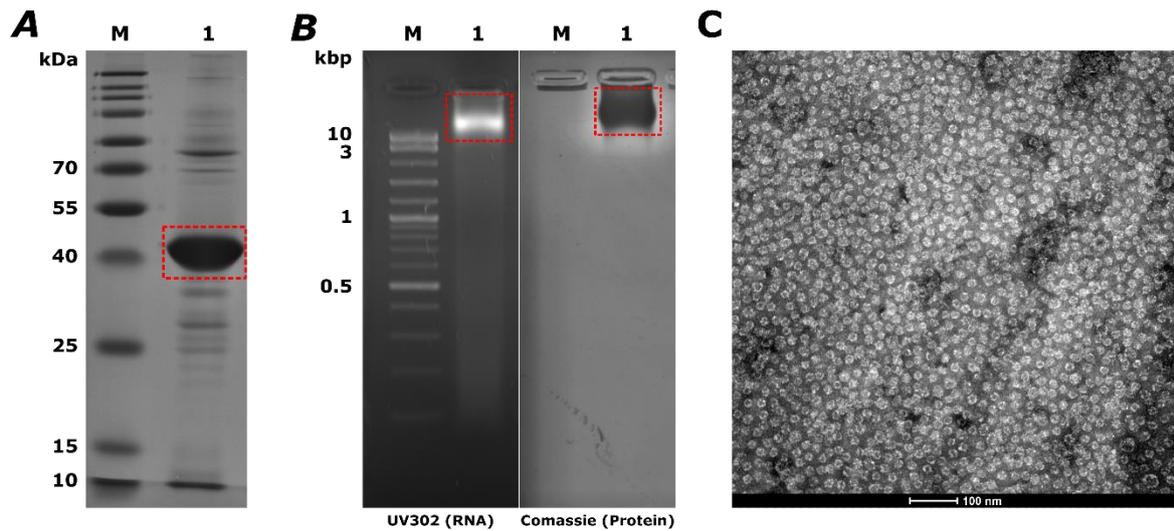
27 **Supplementary Figure S3. Vaccination with DV1-AP205, AP205~DV2, DV3-AP205 and AP205-**
 28 **DV4 elicits high avidity antibodies which are able to recognize the other Dengue serotype EDIII**
 29 **domains due to cross-reactive properties. A)** DV-specific IgG titer of day 28 and 49 from mice
 30 vaccinated with DV1-AP205 measured by ELISA, LOG₁₀ OD₅₀ shown. **B)** DV-specific IgG titer of day 28
 31 and 49 from mice vaccinated with AP205~DV2 measured by ELISA, LOG₁₀ OD₅₀ shown. **C)** DV-specific
 32 IgG titer of day 28 and 49 from mice vaccinated with DV3-AP205 measured by ELISA, LOG₁₀ OD₅₀
 33 shown. **D)** DV-specific IgG titer of day 28 and 49 from mice vaccinated with AP205-DV4
 34 measured by ELISA, LOG₁₀ OD₅₀ shown. After serum incubation one plate was treated with PBS
 35 +0.05% Tween 20 and the other plate with 7M Urea in PBS+0.05% Tween 20. Statistical analysis
 36 (mean ± SEM) using Student's t-test. Vaccine groups n = 5. One representative of 2 similar
 37 experiments is shown. The value of p < 0.05 was considered statistically significant (*p < 0.05,
 38 **p < 0.01, ***p < 0.001, ****p < 0.0001).



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40 **Supplementary Figure S4. Vaccination with all four vaccines compared to vaccination with only**
 41 **two (DV1-AP205/AP205-DV4) tends to increase the humoral immune response with higher**
 42 **avidity. A)** DV1-specific IgG titer of day 49 from group 1 and group 2 measured by ELISA, LOG₁₀ OD₅₀
 43 shown. **B)** DV2-specific IgG titer of day 49 from group 1 and group 2 measured measured by ELISA,
 44 LOG₁₀ OD₅₀ shown. **C)** DV-specific IgG titer of day 49 from group 1 and group 2 measured AP205
 45 measured by ELISA, LOG₁₀ OD₅₀ shown. **D)** DV-specific IgG titer of day 49 from group 1 and
 46 group 2 measured measured by ELISA, LOG₁₀ OD₅₀ shown. After serum incubation one plate was
 47 treated with PBS+0.05% Tween 20 and the other plate with 7M Urea in PBS+0.05% Tween 20.
 48 Statistical analysis (mean ± SEM) using Student's t-test. Group 1 n = 6, group 2 n = 6. One
 49 representative of 2 similar experiments is shown. The value of p < 0.05 was considered
 50 statistically significant (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

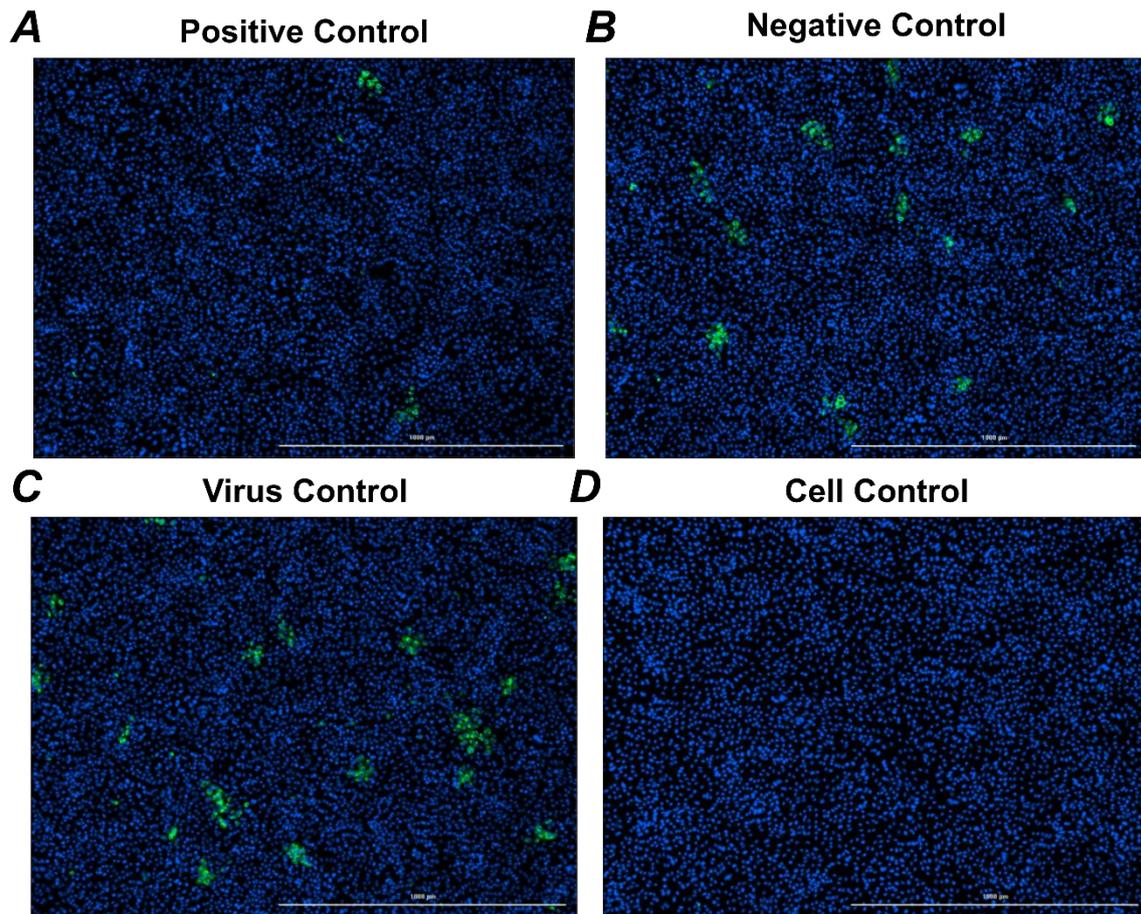
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 53 **Supplementary Figure S5. Stability of DV1-AP205 after 6 months storage at 4°C.** **A)** 12% SDS-PAGE for
 54 DV1-AP205 after 6 months storage at 4°C. M. Protein marker, 1. DV1-AP205. DV1-AP205 indicated in
 55 the red box. Bands were visualized with InstantBlue™ Comassie stain. **B)** Agarose gel analysis to
 56 visualize the packed RNA in the VLPs and the correlating protein staining with Comassie. M. DNA
 57 Ladder, 1. DV1-AP205. DV1-AP205 indicated in the red boxes. **C)** Electron microscopy (EM) of DV1-
 58 AP205. Scale bar 200nm.

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62 **Supplementary Figure S6. Fluorescence Microscopy images of controls on Cells for the**
63 **Neutralization Assays. A) Positive Control. B) Negative Control. C) Virus Control. D) Cell Control.**
64 **DAPI nucleic acid stain is depicted in blue, infected cells are depicted in green. Scale bar 1000 µm.**

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