



Fig. S1. Construction and characterization of PD1-based DNA and influenza-based vaccines. (a) DNA vaccine expressing SARS-CoV-2 RBD antigen fused to a human soluble PD1 domain (PD1-RBD-DNA) was constructed using the pVAX plasmid as the backbone. The protein expression was under the control of a CMV promoter and contained a human tissue plasminogen activator (tPA) secretory signal sequence to promote antigen secretion. A $(G_4S)_3$ linker sequence was placed in between the soluble PD1 domain and the antigen in PD1-RBD. The whole gene was codon optimised. (b) HEK 293T cells were transfected with the PD1-RBD-DNA vaccine or mock using PEI. Expression of the recombinant antigen was determined in supernatants 2 days post-transfection by Western blot analysis. The membrane was probed with rabbit anti-SARS-CoV-2 Spike antibody and mouse anti-human PD-1 antibody, respectively. The antigen-antibody complexes were detected with anti-rabbit IRDye 800CW (green) and anti-mouse IRDye 680RD (red). Lanes are identified by the legend, and the numbers in kDa indicate marker sizes (c) Supernatants from HEK 293T cells transfected with PD1-RBD-DNA (blue) or RBD alone (gray) were co-cultured with HEK 293T cells transiently transfected with the human PD-L1 expression vector. Supernatant from mock transfected HEK 293T cells (dashed) were used as the negative control. The binding of the recombinant antigens to PD-L1 was detected using rabbit anti-SARS-CoV-2 Spike antibodies, followed by AF647-labelled anti-rabbit secondary antibodies. Half offset histograms depict the binding of RBD. Relative geometric mean fluorescence intensity (gMFI) was shown. (d) SARS-CoV-2 RBD antigen was fused to an NS1 gene-deleted NS segment. The RBD domain was conjugated with a human tPA to promote antigen secretion and a V5 tag for detection (e) Eight pHW2000 plasmids containing the DeINS1-RBD segment and the other 7 Influenza virus genomic segments, together with an NS1 expression plasmid, were transfected into a 293T/MDCK cell mixture. Virus supernatant was collected 72 h later and designated passage 0 (P0) virus and subsequently passaged in MDCK cells 5 times (P1-P5). Cell lysates were subjected to detection of SARS-CoV-2 RBD and influenza NP.