ADVANCED MATERIALS

Supporting Information

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SARS-CoV-2 RBD Neutralizing Antibody Induction is Enhanced by Particulate Vaccination

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Supplementary Data

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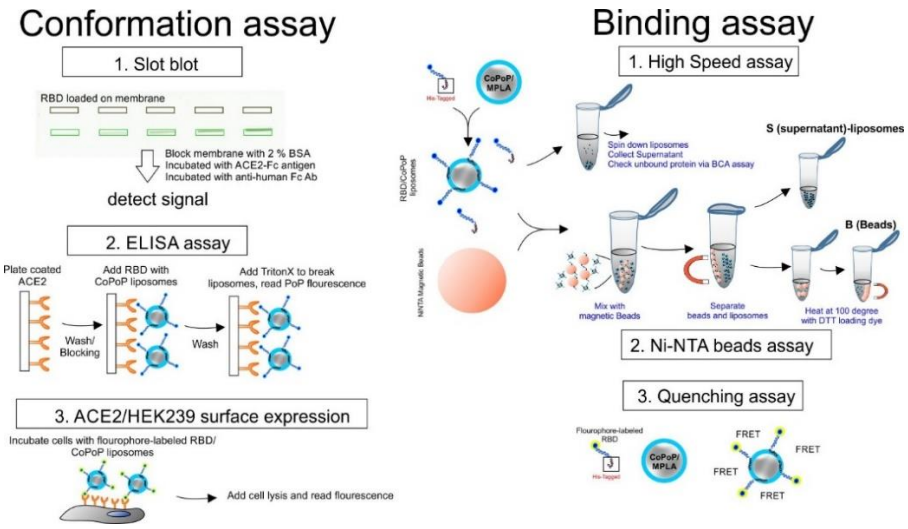
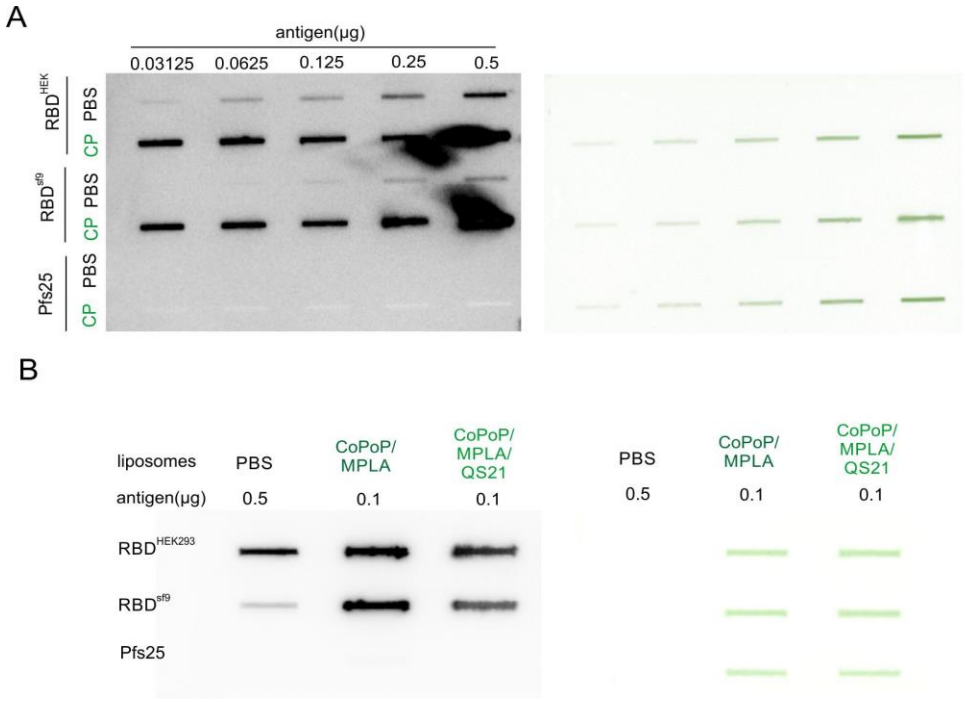
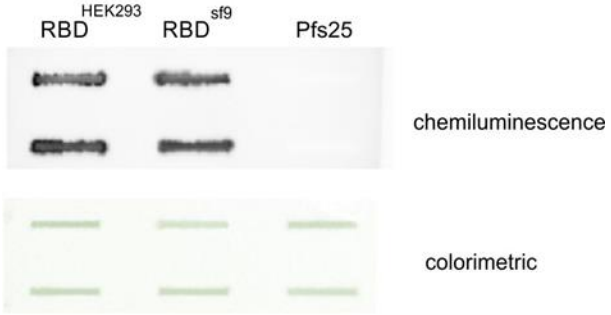


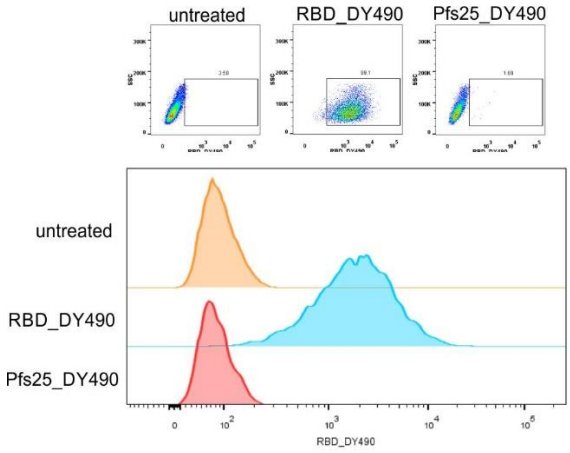
Supplementary Figure S1. Schematic representation of binding assay and conformation assay of His-tagged Receptor binding domain (RBD) with Cobalt-porphyrin-phospholipid (CoPoP) liposomes.



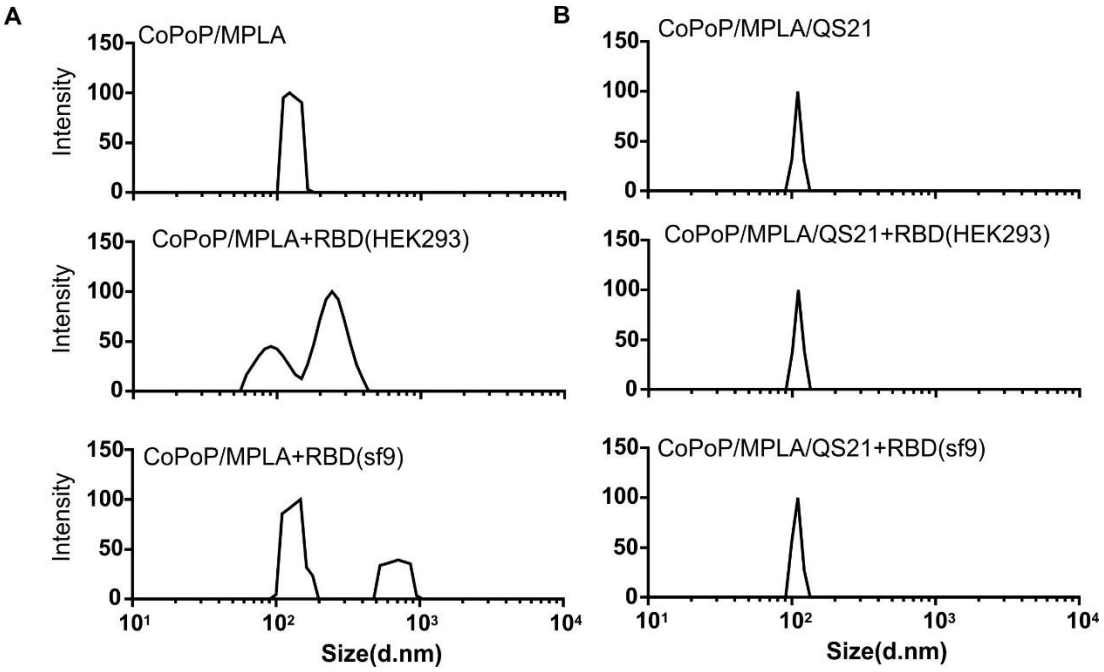
Supplementary Figure S2. RBD on liposome surface recognizes hACE2 using a slot blot. (A) Different doses of free receptor binding domain (RBD) or RBD with Cobalt-porphyrin-phospholipid (CoPoP)/ monophosphoryl Lipid A (MPLA) liposome on membrane. *Plasmodium falciparum* protein Pfs25 was used as a negative control. (B) Comparison between RBD with CoPoP/MPLA liposomes and RBD with CoPoP/MPLA/QS-21 liposomes.



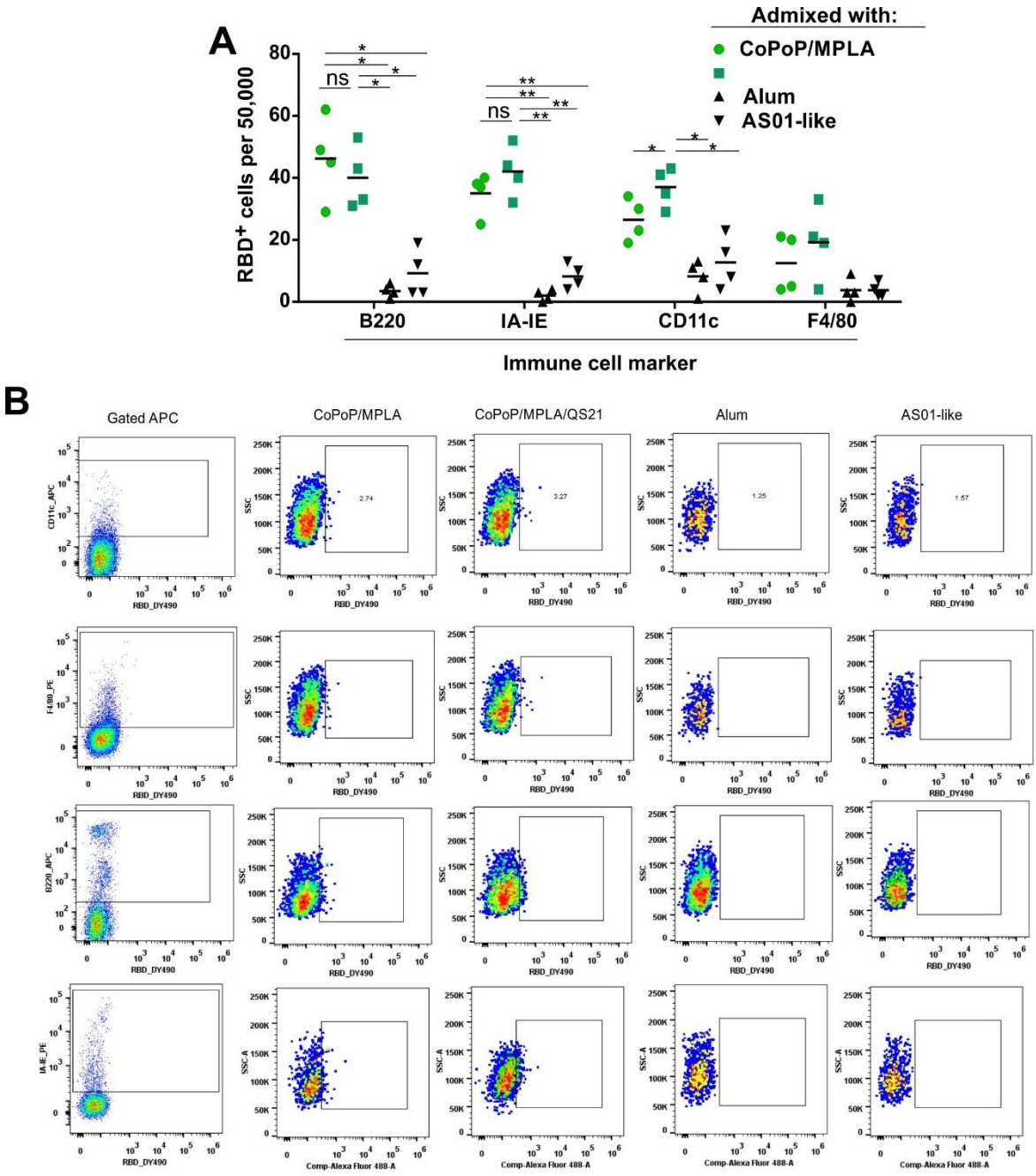
Supplementary Figure S3. RBD on CoPoP liposome surface is recognized by specific antibodies on Slot Blot.



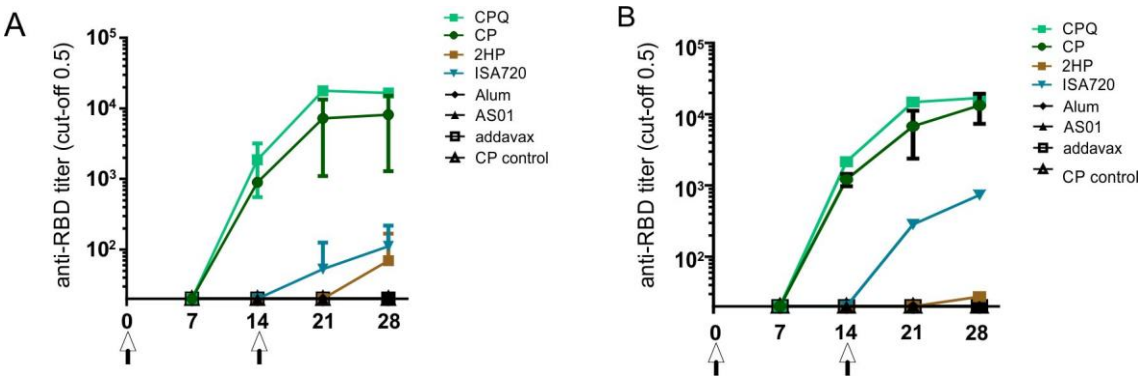
Supplementary Figure S4. RBD_DY490 binds to ACE2/HEK293.



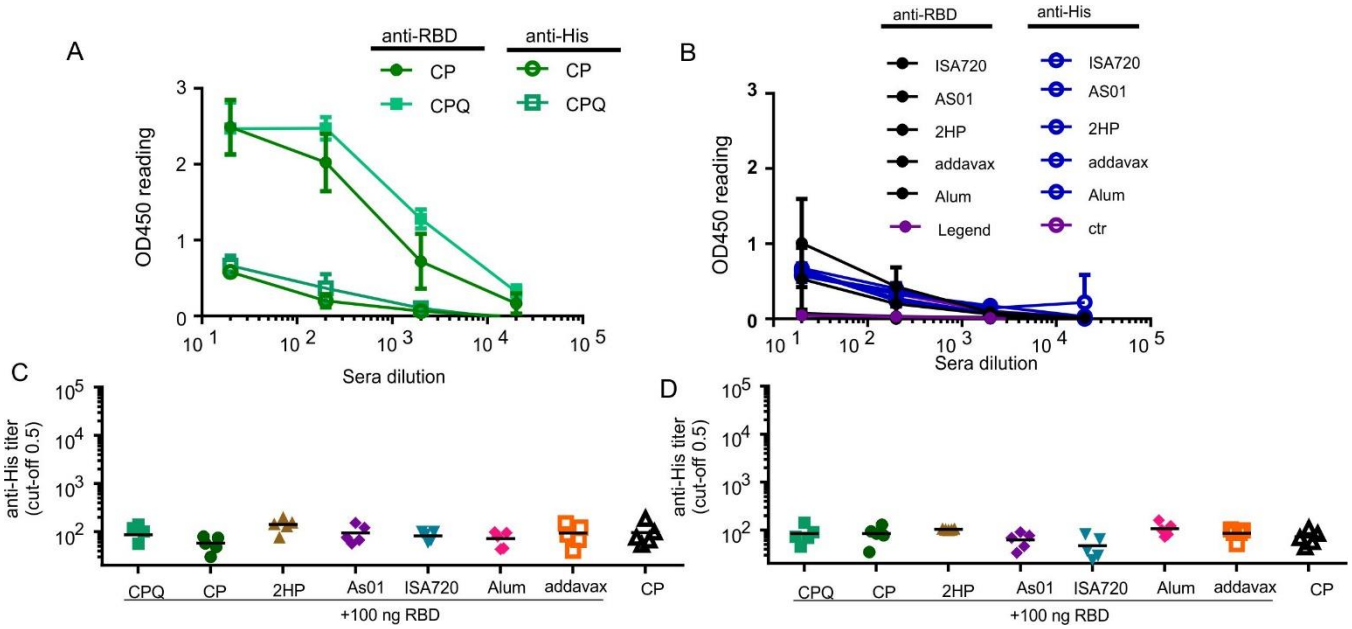
Supplementary Figure S5. Liposomal distribution was measured using DLS. (A) CoPoP/MPLA and (B) CoPoP/MPLA/QS-21 liposomes. Liposomes were incubated with RBD(HEK293) or RBD(sf9) for 3 hours at room temperature.



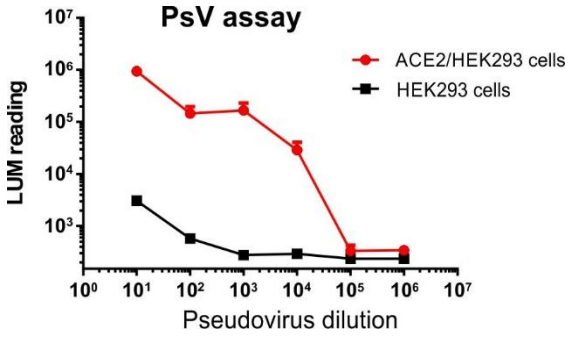
Supplementary Figure S6. Gating strategy for APC uptake of RBD in lymph nodes. (A) RBD uptake in immune cells within draining lymph nodes *in vivo* following intramuscular immunization of mice. Labeled RBD uptake was assessed with flow cytometry and co-staining with the indicated surface markers**. (B)** Cells were gated by SSC-FSC then CD11c-APC, F4/80-PE, B220-APC and IA-IE- positive antigen uptake was assessed by using DY490 labeled RBD. Representative plots are shown from biologically independent experiments with n=5 mice. Bar graphs in A and D show mean +/- std. dev. for n=3 measurements. Data were analyzed by one-way ANOVA followed by Tukey's post hoc analysis adjusting for multiple comparisons, p*<0.05, p**<0.01.



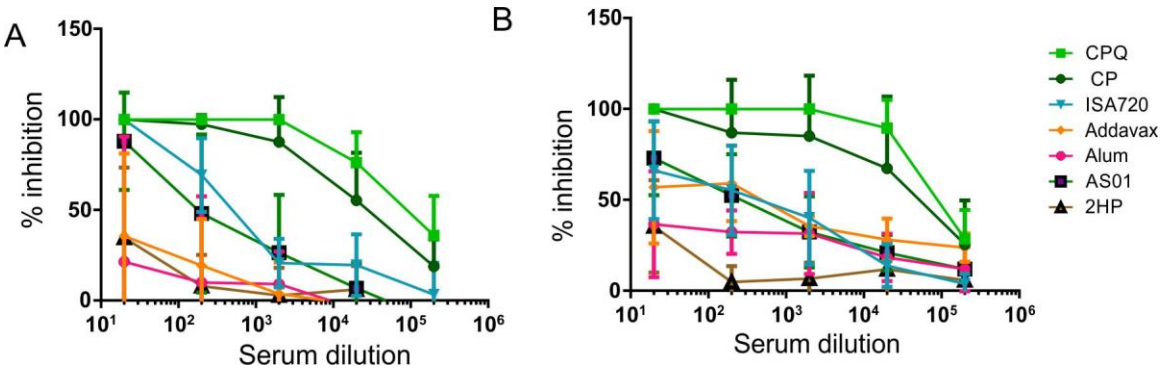
Supplementary Figure S7. Kinetics of antibody response of the RBD in outbred mice. Mice were immunized with (A) 100 ng RBD-HEK293 and (B) 100 ng RBD-sf9 at the time points indicated by arrows (n=5 mice per group).



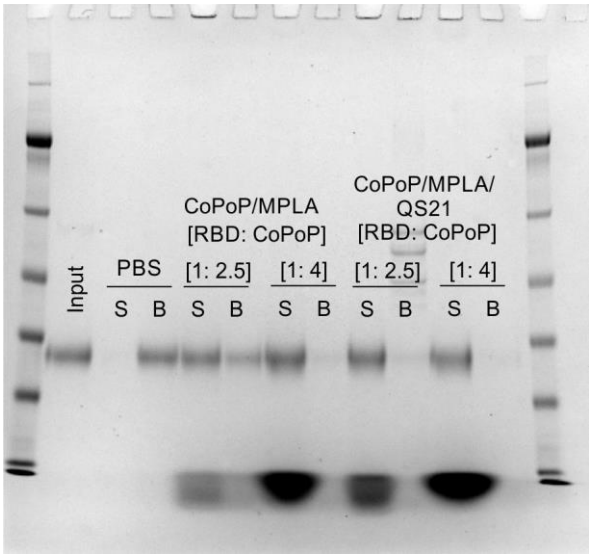
Supplementary Figure S8. Anti-His-tag titer response of the RBD in outbred mice. Mice were immunized with (**A**) 100 ng RBD-HEK293 admixed with CoPoP liposomes, and anti-His-tag were measured by ELISA. (**B**) 100 ng RBD-HEK293 admixed with indicated adjuvants, including ISA720, AS01-like, PoP liposomes, Addavax, Alum. And anti-His-tag titer was measured. Anti-His-tag titer level was measured in mice immunized with (**C**) RBD-HEK293 and (**D**) RBD-sf9. Each group contain n=5 mice.



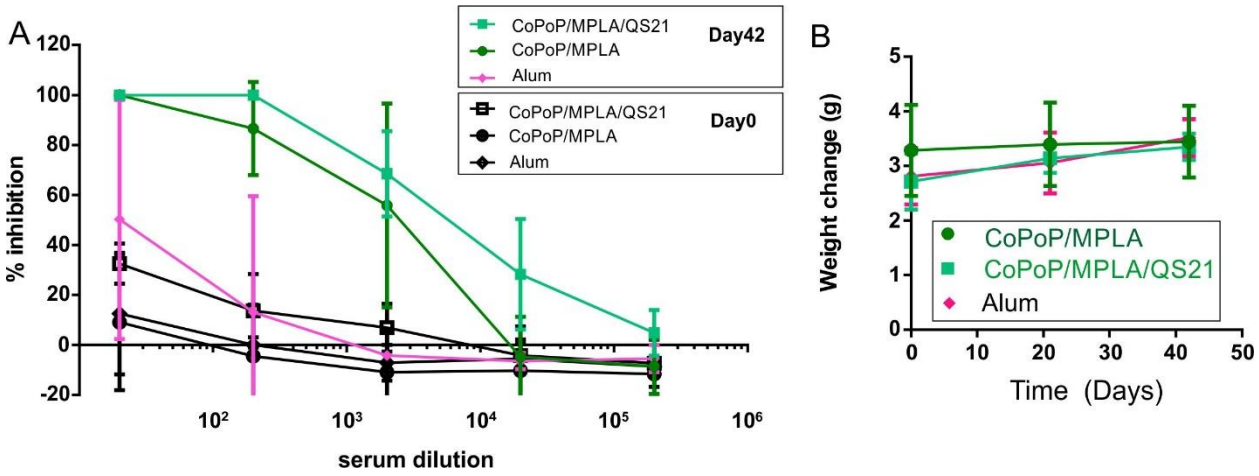
Supplementary Figure S9. Pseudovirus entry in ACE2/HEK293 and HEK293 cells.



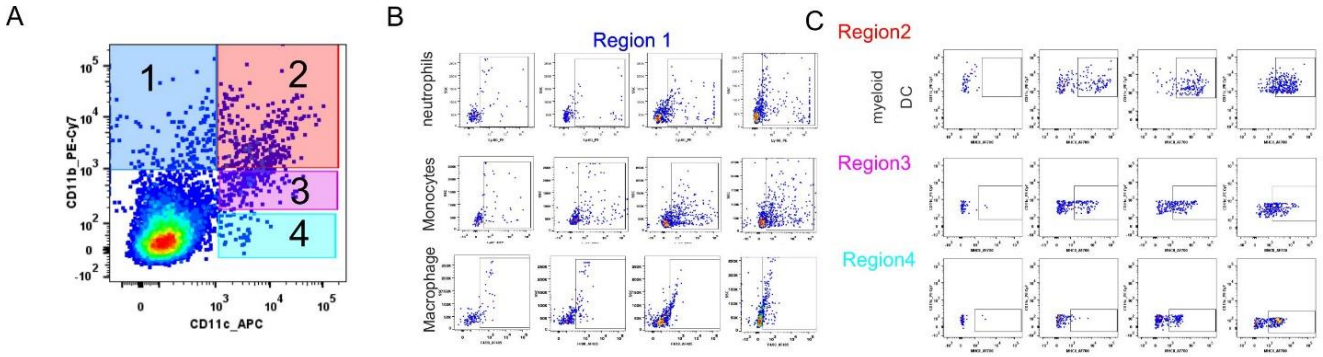
Supplementary Figure S10. Sera dilution and percentage of inhibition of pseudovirus entry into ACE-HEK293 cells. Sera from mice immunized with (A) RBD-HEK293 antigen or (B) RBD-sf9 admixed with indicated adjuvant.



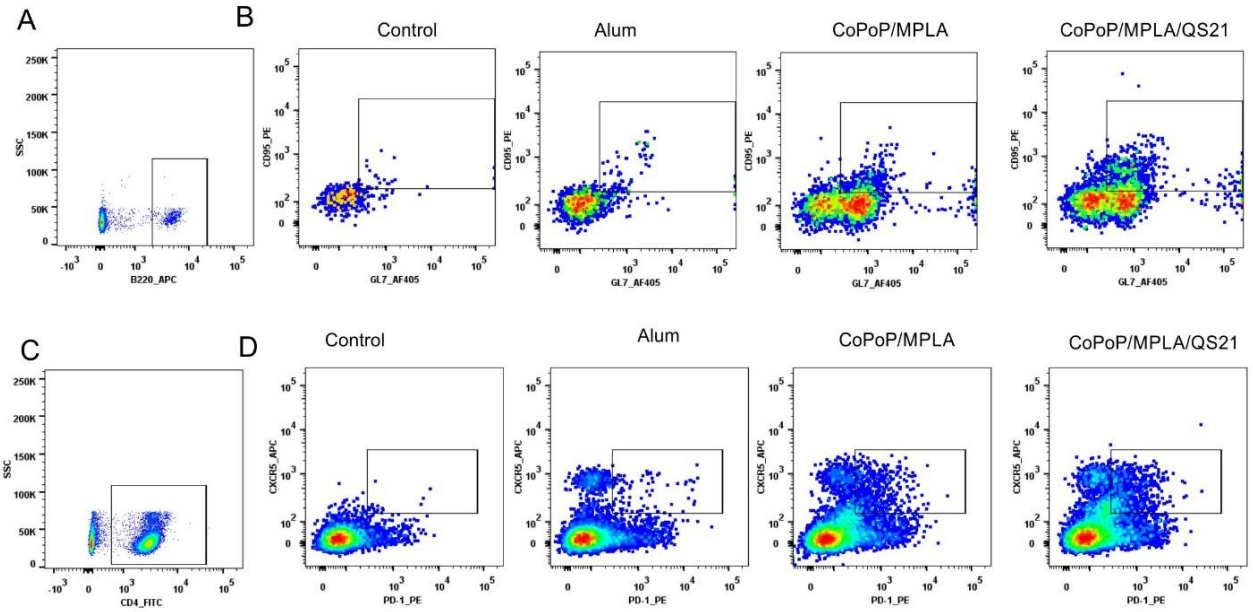
Supplementary Figure S11. Binding ability of His-tagged RBD to CoPoP liposomes used for rabbit immunization.



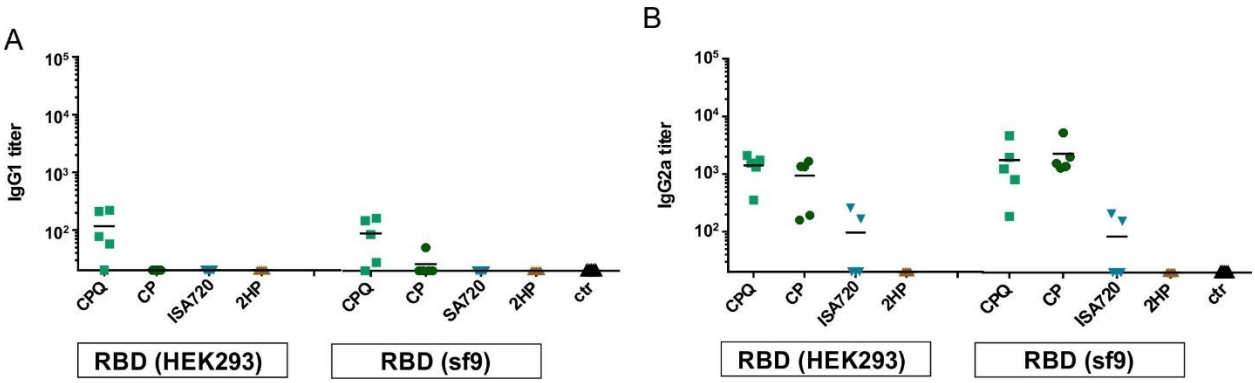
Supplementary Figure S12. Rabbits were immunized with 20µg of RBD with CoPoP liposomes or Alum on Day0 and Day42. (A) Immunized sera were incubated with PsV at different dilution factors. A dose dependent inhibition was observed from the final bleeding sera from rabbit immunized with CoPoP liposomes but not the Alum group. (B) Weight of rabbit post-immunization.



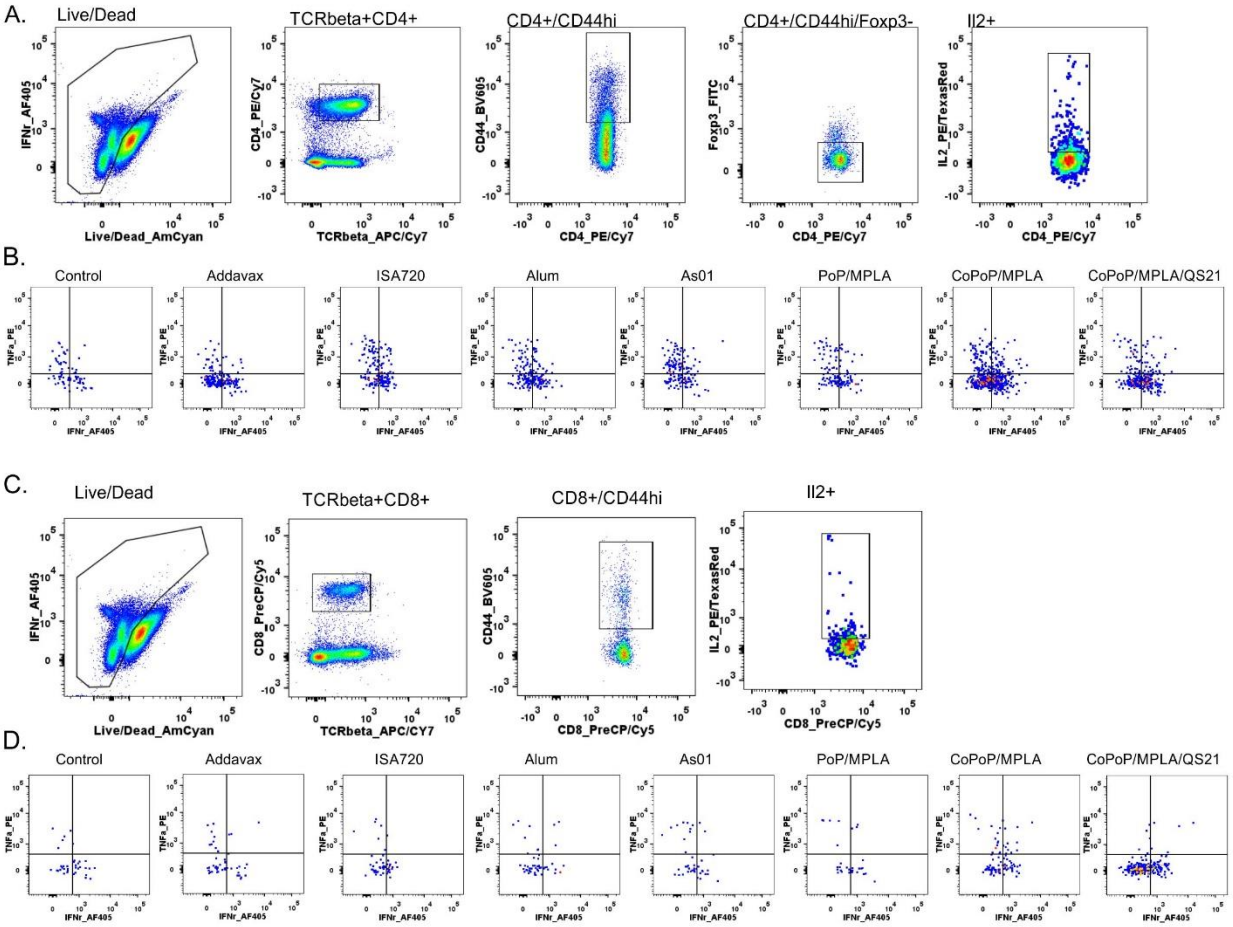
Supplementary Figure S13. Recruitment of immune cells in the draining lymph node. (A) Dot plot of lymph node cells collected 48 hours after CoPoP/MPLA liposome injections. x-y axis refers to CD11c-APC and CD11b-PE cy7. (B) Region 1 includes macrophages, infiltrating monocytes, neutrophils and eosinophils. **(C)** Region 2 represents mDC, Region 3 represents $CD11b^{low}$ DC and Region 4 represents $CD11b^{-}$ DC.



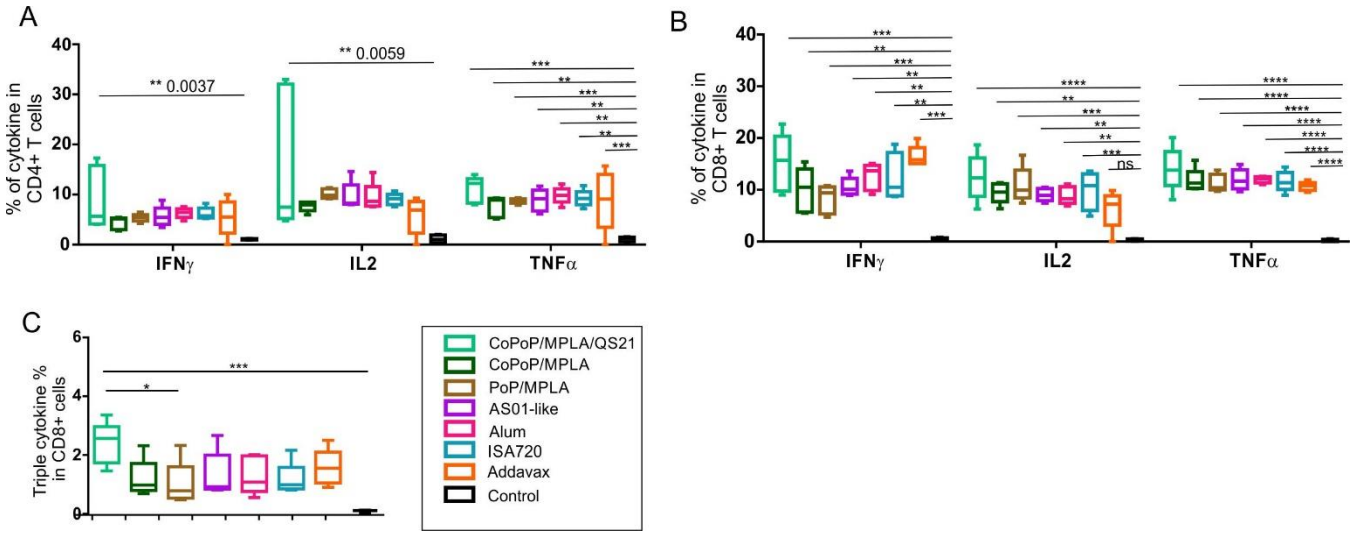
Supplementary Figure S14. Gating strategy for germinal center (GC) activation. GC cells ($GL7^+CD95^+$; within the $B220^+$ cell population) were gated with (A) B220 surface marker to identified B cells, followed by (B) gating $GL7^+CD95^+$ population. Tfh cells ($CXCR5^+PD\text{-}1^+$; within the $CD4^+$ cell population), cells were gated with (C) CD4 surface marker to identify $CD4^+$ T cells, followed by (D) gating $CXCR5^+PD\text{-}1^+$.



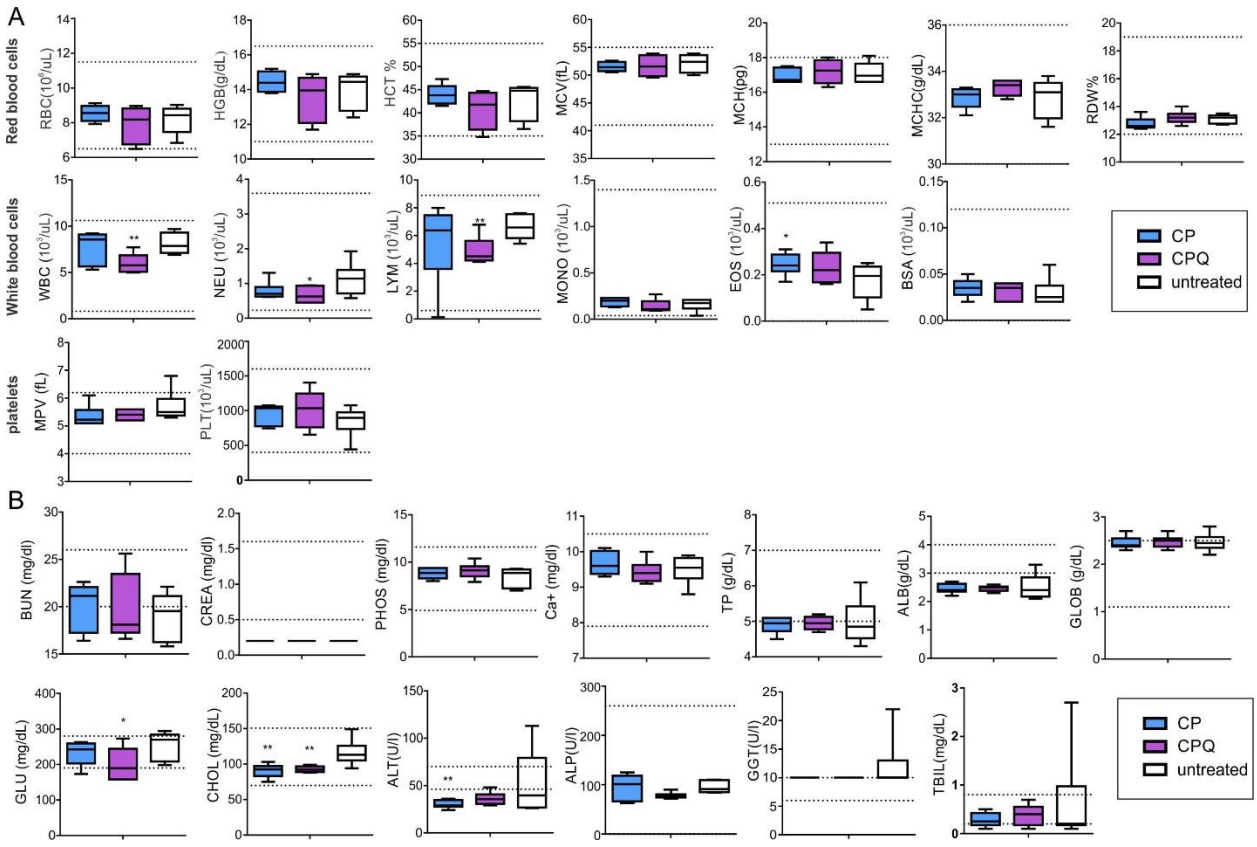
Supplementary Figure S15. Isotype ratios of CoPoP liposomes, ISA720 and PoP liposomes. (A) IgG1 and (B) IgG2a.



Supplementary Figure S16. Gating of CD4$^+$ T cells and CD8$^+$ T cells. (A) Live and Dead cells were gated, then TCRbeta$^+$CD4$^+$ T cells were gated, followed by gating CD44^{high} population. Later on, Foxp- population were gated, and IL2$^+$ cells were gated, followed by gating (B) TNFα$^+$IFNγ$^+$ cell population. (C) Live and Dead cells were gated, then TCRbeta$^+$CD8$^+$ T cells were gated, followed by gating CD44^{high} population. Later on, IL2$^+$ cells were gated, followed by gating (D) TNFα$^+$IFNγ$^+$ cell population.



Supplementary Figure S17. Splenocytes were collected from immunized mice and stimulated with RBD antigen. Intracellular staining of signal cytokine in (A) $CD4^+$ T cells and (B) $CD8^+$ T cells. Triple cytokines in (C) $CD8^+$ T cells.



Supplementary Figure S18. Tolerability of CoPoP liposomes with 1 μg His-tagged RBD in CD-1 mice. Mice were treated with "CoPoP/MPLA+RBD" (1 μg Pfs25, 4 μg CoPoP and 1.6 μg MPLA) or "CoPoP/MPLA/QS-21+RBD" (1 μg Pfs25, 4 μg CoPoP and 1.6 μg MPLA and 1.6 μg QS-21). Values

show mean +/- std. dev for n=6 mice per group). A. Complete blood count parameters are as follows for red blood cells: RBC (red blood cell count), HGB (hemoglobin), HCT (hematocrit); MCV (mean cell volume), MCH (mean cell hemoglobin), MCHC (mean cell hemoglobin concentration) and RDW (red cell distribution width). White blood cell parameters are as follows: WBC (white blood cells), NEU (neutrophils), LYM (lymphocytes), MONO (monocytes); EOS (eosinophils), BAS (basophils). Platelet parameters are as follows: PLT (platelet) and MPV (mean platelet volume). B. Serum markers with their general descriptions: Kidney function markers are as follows: BUN (blood urea nitrogen), CREA (creatinine), PHOS (phosphorus), Ca^{+} (calcium). Pancreas function markers are as follows: Protein TP (total protein), ALB (albumin), GLOB (globulin) other GLU (glucose), CHOL (cholesterol). Liver function markers are as follows: ALT (alanine aminotransferase), ALP (alkaline phosphatase), ALB (albumin), TBIL (total bilirubin). The line in the box represent the median and the whiskers issuing from the box extend to the group minimum and maximum value. The length of the box represents the interquartile range. Unpaired two-sided student's T-test were used for statistical analysis, * represents the comparison between CoPoP liposomes to control with $p<0.1$ and ** represents the comparison between CoPoP liposomes to control with $p<0.05$.

Table S1. Comparison of complete blood count (CBC) parameters between untreated mice and mice treated with CoPoP liposome in CD-1 mice. Mice were treated with "CoPoP/MPLA+RBD" (1 µg RBD, 4 µg CoPoP and 1.6 µg MPLA) or "CoPoP/MPLA/QS-21+RBD" (1 µg Pfs25, 4 µg CoPoP and 1.6 µg MPLA and 1.6 µg QS-21). Values show mean +/- standard deviation for mice (n=6 per group). CBC parameters are as follows for red blood cells: RBC (red blood cell count), HGB (hemoglobin), HCT (hematocrit); MCV (mean cell volume); MCH (mean cell hemoglobin), MCHC (mean cell hemoglobin concentration) and RDW (red cell distribution width). White blood cell parameters are as follows: WBC (white blood cells), NEU (neutrophils), LYM (lymphocytes), MONO (monocytes), EOS (eosinophils), BAS (basophils). Platelets parameters are as follows: PLT (platelet) and MPV (mean platelet volume).

CBC	Control Mean ± SD [range]	CoPoP/MPLA Mean ± SD [range]	CoPoP/MPLA/QS-21 Mean ± SD [range]	Normal range
RBC (10^6/uL)	8.19±0.80 [6.84-9.02]	8.54±0.47 [7.93-9.13]	7.91 ±1.04 [6.49-8.96]	6.5 - 11.5
HGB (g/dL)	13.97±1.05 [12.4-14.9]	14.45±0.58 [13.8-15.2]	13.55 ±1.31 [11.7-14.9]	11 - 16.5
HCT %	42.63±3.94 [36.5-45.6]	43.98±2.18 [41.5-47.3]	40.70 ±4.02 [34.8-44.7]	35 – 55
MCV (fL)	52.13±1.58 [50-53.9]	51.52±0.85 [50.5-52.6]	51.67 ±1.92 [49.6-53.9]	41 – 55
MCH (pg)	17.12±0.58 [16.6-18.1]	16.92±0.42[16.6-17.5]	17.20 ±0.70 [16.3-18]	13.0 – 18
MCHC(g/dL)	32.85±0.83 [31.6-33.8]	32.87±.45 [32.1-33.3]	33.30 ±0.33[32.8-33.6]	30 – 36
RDW%	13.12±0.31 [12.7-13.5]	12.77±.44 [12.4-13.6]	13.21 ±0.46 [12.6-14]	12 – 19
WBC (10^3/uL)	8.11±1.15 [6.90-9.68]	7.74 ±1.77 [5.31-9.07]	5.98 ±1.02 [4.98-6.56] **	0.80 - 10.6

NEU (10^3/uL)	1.13±0.47 [0.58-1.93]	0.79 ±0.26 [0.61-1.31]	0.67 ±0.22 [0.45-0.95] *	0.23 - 3.6
LYM (10^3/uL)	6.62±0.88 [5.42-7.62]	5.49±2.85 [0.14-7.99]	4.89 ±1.01 [4.11-6.78] **	0.6 - 8.9
MONO (10^3/uL)	0.16±0.06 [0.04-0.21]	0.19±0.05 [0.13-0.23]	0.14 ±0.07 [0.09-0.27]	0.04 - 1.4
EOS (10^3/uL)	0.17±0.08 [0.05-0.25]	0.25±0.05 [0.17-0.31] *	0.23 ±0.07 [0.16-0.34]	0.00 - 0.51
BAS (10^3/uL)	0.03±.0.02[0.02-0.05]	0.04±0.01 [0.02-0.04]	0.03 ±0.01 [0.02-0.06]	0.00 - 0.12
MPV (fL)	5.7±0.56 [5.30-6.80]	5.36±0.38 [5.1-6.1]	5.4 ±0.18 [5.2-5.6]	4-6.2
PLT (10^3/uL)	849±215.92 [444-1079]	956.17±149.11 [746-1075]	1020.67 ±276.96 [656-1405]	400 - 1600

* indicates values that differ significantly ($p < 0.1$) between control and CoPoP groups based on a two-sided student's T-test.

** indicates values that differ significantly ($p<0.05$) between control and CoPoP groups based on a two-sided student's T-test.

Table S2. Comparison of Blood Chemistry Panel between untreated or CoPoP liposome-treated CD-1 mice. Mice were treated with "CoPoP/MPLA+RBD" (1 µg RBD, 4 µg CoPoP and 1.6 µg MPLA). Values show mean +/- standard deviation for mice (n=6 per group). Serum markers with their general description are as follows: Kidney function markers are as follows: BUN (blood urea nitrogen), CREA (creatinine), PHOS (phosphorus), Ca^{+} (calcium). Pancreas function markers are as follows: Protein TP (total protein), ALB (albumin), GLOB (globulin) other GLU (glucose), CHOL (cholesterol). Liver function markers are as follows: ALT (alanine aminotransferase), ALP (alkaline phosphatase), ALB (albumin), TBIL (total bilirubin).

	Control Mean ± SD [range]	CoPoP/MPLA Mean ± SD [range]	CoPoP/MPLA/QS-21 Mean ± SD [range]	Normal range
BUN (mg/dl)	19.03±2.49 [15.8-22.1]	20.12±2.54 [16.4-22.6]	19.9±3.63 [16.6-25.6]	20.0 - 26.0
CREA (mg/dL)	0.2±0.0 [0.2-0.2]	0.2±0.0 [0.2-0.2]	0.2±.0 [0.2-0.2]	0.5 - 1.6
PHOS (mg/dL)	8.42±1.01 [7-9.3]	8.82±0.55 [8-9.4]	9.1±0.82 [7.9-10.4]	4.9-11.6
Ca+ (mg/dL)	9.50±0.39 [8.8-9.9]	9.67±0.34 [9.3-10.1]	9.43±.31 [9.1-10]	7.9 - 10.5
TP (g/dL)	4.98±0.62 [4.3-6.1]	4.90±.23 [4.5-5.1]	4.95±0.19 [4.7-5.2]	5.0 - 7.0
ALB (g/dL)	2.52±.44 [2.1-3.3]	2.45±.18 [2.2-2.7]	2.47±0.1 [2.3-2.6]	3.0 - 4.0
GLOB (g/dL)	2.47±0.20 [2.2-2.8]	2.45±.14 [2.3-2.7]	2.48±0.13 [2.3-2.7]	1.1-2.5
GLU (mg/dl)	221.5±111.38 [198-294]	232±34.19 [173-263]	200.5±45.44 [158-273] *	190 - 280
CHOL (mg/dl)	116.00±18.31 [94-149]	90.67±9.48 [75-103] **	92.67±4.23 [88-99] **	69.6-150.81
ALT(GPT) (U/I)	52.17±33.87 [26-113]	30.17±4.26 [24-36]	36.17±6.68 [29-48]	46-70
ALP (U/I)	95.00±12.03 [84-110]	95.50±25.57 [63-125]	77.67±6.28 [72-90] **	0 - 260
GGT (U/I)	12.00±4.9[10-22]	10.00±.0 [10-10]	10.00±0 [10-10]	6.0 - 10.0
TBIL (mg/dL)	0.63±1.02 [0.1-2.7]	0.28±0.15 [0.1-0.5]	0.38±.22 [0.1-0.7]	0.2 - 0.8

* indicates values that differ significantly ($p< 0.1$) between control and CoPoP groups based on a two-sided student's T-test.

** indicates values that differ significantly ($p<0.05$) between control and CoPoP groups based on a two-sided student's T-test.

Table S3 Summary of murine immunization data

Sample	anti-RBD titer	Pseudovirus	ACE2-RBD % inhibition	Live virus titer
CP-RBD-HEK	1920	13649	99.5209	>=1280
CP-RBD-HEK	6990	830	98.2914	>=1280
CP-RBD-HEK	1780	31530	98.0564	>=1280
CP-RBD-HEK	12500	22257	98.2101	>=1280
CP-RBD-HEK	17600	13884	92.985	>=1280
CPQ-RBD-HEK	15900	21718	99.6203	>=1280
CPQ-RBD-HEK	13500	26627	99.8282	>=1280
CPQ-RBD-HEK	17500	72583	96.6552	>=1280
CPQ-RBD-HEK	18100	20301	100.099	>=1280
CPQ-RBD-HEK	17600	12906	99.3401	>=1280
AS01-RBD-HEK	20	20	26.8148	80
AS01-RBD-HEK	20	406.5	23.7778	10
AS01-RBD-HEK	20	20	30.0556	160
AS01-RBD-HEK	20	223.9	28.8056	320
AS01-RBD-HEK	20	284.8	27.9259	80
I720-RBD-HEK	280	363.7	27.537	--
I720-RBD-HEK	20	942.9	24.0278	--
I720-RBD-HEK	20	45.75	34.0926	--
I720-RBD-HEK	125	75.7	32.3981	--
I720-RBD-HEK	110	270.5	21.4907	--
Alum-RBD-HEK	20	1015	32.6296	0
Alum-RBD-HEK	20	20	34.9074	0
Alum-RBD-HEK	20	20	38.4352	10
Alum-RBD-HEK	20	20	22.6204	0
Alum-RBD-HEK	20	20	30.5093	10
Addavax-RBD-HEK	20	20	24.713	--
Addavax-RBD-HEK	244	20	21.7685	--
Addavax-RBD-HEK	20	20	21.3241	--
Addavax-RBD-HEK	20	119.5	29.7407	--
Addavax-RBD-HEK	44.2	212	27.5278	--
2HP-RBD-HEK	20	20	33.6296	--
2HP-RBD-HEK	20	20	29.0648	--
2HP-RBD-HEK	20	20	31	--
2HP-RBD-HEK	20	21.4	31.7315	--
2HP-RBD-HEK	20	201.9	26.0833	--
CP-RBD-sf9	96.0043	21100	108625	>=1280
CP-RBD-sf9	99.5751	14500	74189	>=1280
CP-RBD-sf9	99.4124	5350	32079	>=1280

CP-RBD-sf9	68.2155	9730	180	640
CP-RBD-sf9	97.3423	15900	17916	160
CPQ-RBD-sf9	92.09	19100	21711	>=1280
CPQ-RBD-sf9	97.7943	16600	22481	>=1280
CPQ-RBD-sf9	98.9604	19600	109680	>=1280
CPQ-RBD-sf9	98.5717	15200	167340	>=1280
CPQ-RBD-sf9	99.0689	14100	167683	>=1280
AS01-RBD-sf9	25.0949	20	330.4	--
AS01-RBD-sf9	25.0678	20	27.82	--
AS01-RBD-sf9	31.3596	20	20	--
AS01-RBD-sf9	31.7754	20	212.4	--
AS01-RBD-sf9	30.4466	20	20	--
ISA720-RBD-sf9	27.8883	1760	20	--
ISA720-RBD-sf9	25.3932	116	1051	--
ISA720-RBD-sf9	36.1689	1740	272	--
ISA720-RBD-sf9	33.3484	20	619.5	--
ISA720-RBD-sf9	24.3536	20	25.36	--
Alum-RBD-sf9	17.6365	20	2254	40
Alum-RBD-sf9	27.6365	20	20	80
Alum-RBD-sf9	20.5603	20	20	160
Alum-RBD-sf9	40	20	20	0
Alum-RBD-sf9	13.4339	20	602.2	160
Addavax-RBD-sf9	23.6422	56.7	13269	--
Addavax-RBD-sf9	20.1724	20	49.87	--
Addavax-RBD-sf9	28.2256	20	20	--
Addavax-RBD-sf9	21.7026	20	20	--
Addavax-RBD-sf9	18.8865	20	20	--
2HP-RBD-sf9	30.9619	20	20	--
2HP-RBD-sf9	28.0419	20	100	--
2HP-RBD-sf9	36.1237	20	237.6	--
2HP-RBD-sf9	18.9929	20	20	--
2HP-RBD-sf9	29.7053	20	20	--
CP control	24.1457	20	20	--
CP control	19.6167	20	20	--
CP control	22.5909	20	20	--
CP control	14.5995	20	20	--
CP control	16.3442	20	20	--

Table S4: Summary of rabbit immunization data.

	anti-RBD titer	Pseudovirus	ACE2-RBD % inhibition	Live virus titer
CPQ-RBD-HEK	6170	4113.987	89.5679	>=1280
CPQ-RBD-HEK	11600	2366.538	91.9906	>=1280
CPQ-RBD-HEK	1480	1202.183	97.2067	>=1280
CPQ-RBD-HEK	19800	138010.9	97.9931	>=1280
CP-RBD-HEK	15200	288.3075	40.6708	>=1280
CP-RBD-HEK	18600	3764.018	76.8487	160
CP-RBD-HEK	36000	2423.331	21.9309	320
CP-RBD-HEK	25500	5224.041	98.6169	80
Alum-RBD-sf9	20	20	3.38998	160
Alum-RBD-sf9	171	20	0	320
Alum-RBD-sf9	20	530.0465	0	320
Alum-RBD-sf9	690	20	5.52341	640