SUPPLEMENTARY INFORMATION

Supplementary Table and Figures for:

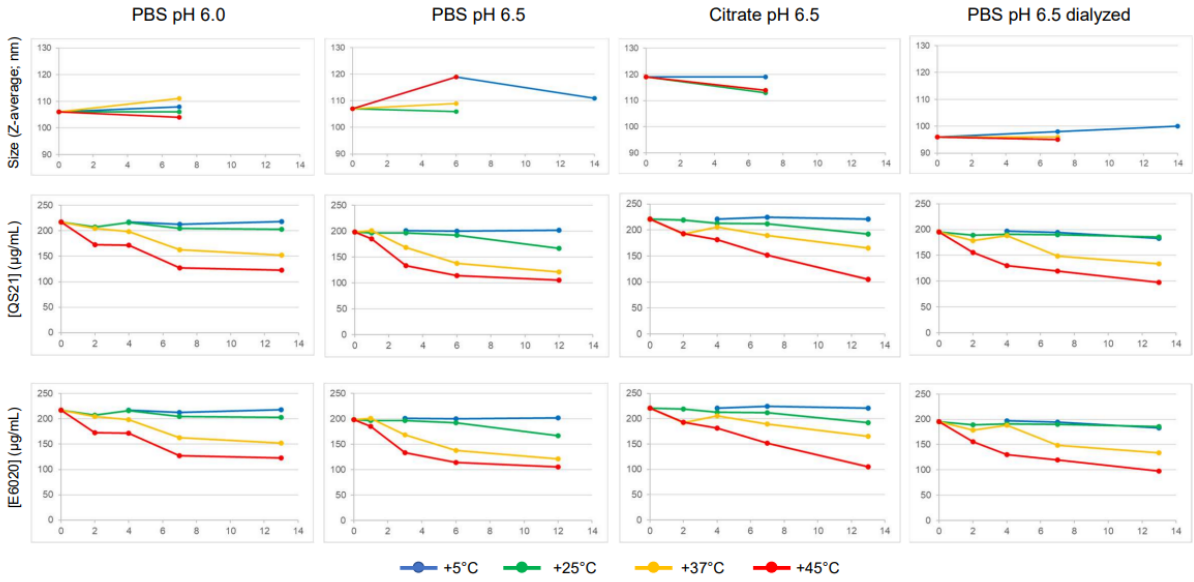
SPA14 liposomes combining saponin with fully synthetic TLR4 agonist provide adjuvanticity to hCMV vaccine candidate.

Supplementary Table 1. Response to SPA14 and AS01B adjuvants in the MIMIC® PTE module.

Assay parameter	Dose	SPA14-20	SPA14-8	AS01B	Mock	L+R	E6020	MPLA	Liposome
% Live cells	**1:20**	62.3	60.1	58.0	70.6	28.4	x	75.0	x
	1:40	67.0	61.0	61.4	x	x	39.3	x	57.2
	1:80	69.1	64.6	68.1	x	x	x	x	x
	1:160	63.4	62.4	65.5	x	x	x	x	x
% CD86+ cells (parent HLA-DR+CD11c+)	**1:20**	16.8	15.3	11.3	5.1	20.1	x	18.3	x
	1:40	17.4	15.3	9.9	x	x	26.5	x	9.3
	1:80	15.3	15.4	9.8	x	x	x	x	x
	1:160	13.7	14.2	7.7	x	x	x	x	x
IL-6 (pg/ml)	**1:20**	2870.0	140.6	53.2	18.6	16651.4	x	3165.7	x
	1:40	1115.9	52.5	33.9	x	x	34390.6	x	15.8
	1:80	252.9	26.5	24.0	x	x	x	x	x
	1:160	54.3	22.8	21.2	x	x	x	x	x
PGE2 (pg/ml)	**1:20**	883.80	53.36	47.0	24.6	3345.3	x	404.9	x
	1:40	271.35	55.54	45.5	x	x	4144.2	x	14.2
	1:80	118.12	45.25	33.0	x	x	x	x	x
	1:160	57.20	46.30	34.5	x	x	x	x	x

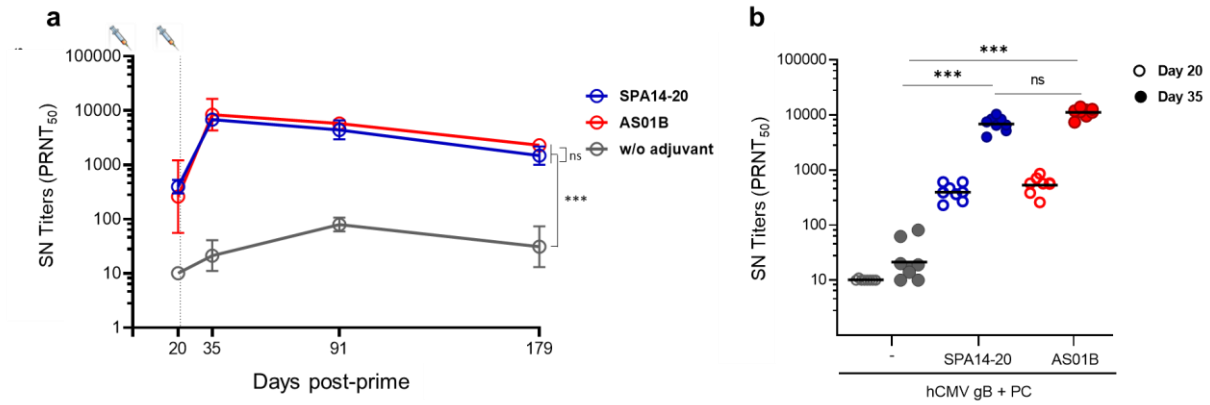
The MIMIC® PTE was treated with indicated human dose dilutions of adjuvants for 48 hrs. The cells were then harvested and evaluated for cell viability and expression of CD86 on CD11c+ HLA DR+ cells. IL-6 secretion was determined as a representative cytokine in the culture supernatants by Luminex based multiplex assay. PGE-2 secretion was determined by ELISA in the culture supernatants. E6020 (200 ng/mL), MPL (5000 ng/mL) and QS21-Liposomes (SPA14 without E6020 at a 1:40 dilution) were used in the assay as benchmarks along with an LPS+R848 positive control. Mean group values are shown; N= 8-15 donors/group.

Supplementary Figure 1



Supplementary Fig. 1. Accelerated heat stability study of SPA14 (preformulation study). *DOPC/Chol/E6020 liposomes (8:2:0.016 mg/mL) were manufactured in water as described in the Material and Methods section and diluted 1:1 volume/volume with a solution of QS21 at 0.4 mg/mL in PBS pH 6.0, PBS pH 6.5 or in 50 mM Citrate buffer pH 6.3 to generate SPA14-8 in different buffers containing 2.5% residual ethanol from the formulation process. Aliquots of the different preparations were placed under nitrogen in capped borosilicate glass vials and stored at 5°C, 25°C, 37°C or 45°C in temperature-controlled incubators. The stability of the different preparations in terms of pH and osmolality (not shown because of no variation), particle size (Z-average by DLS) and QS21 and E6020 chemical integrity by HPLC was followed over 14 weeks and compared to the stability of SPA14-8 dialyzed against PBS pH 6.5 as to remove the traces of residual ethanol from the manufacturing process.*

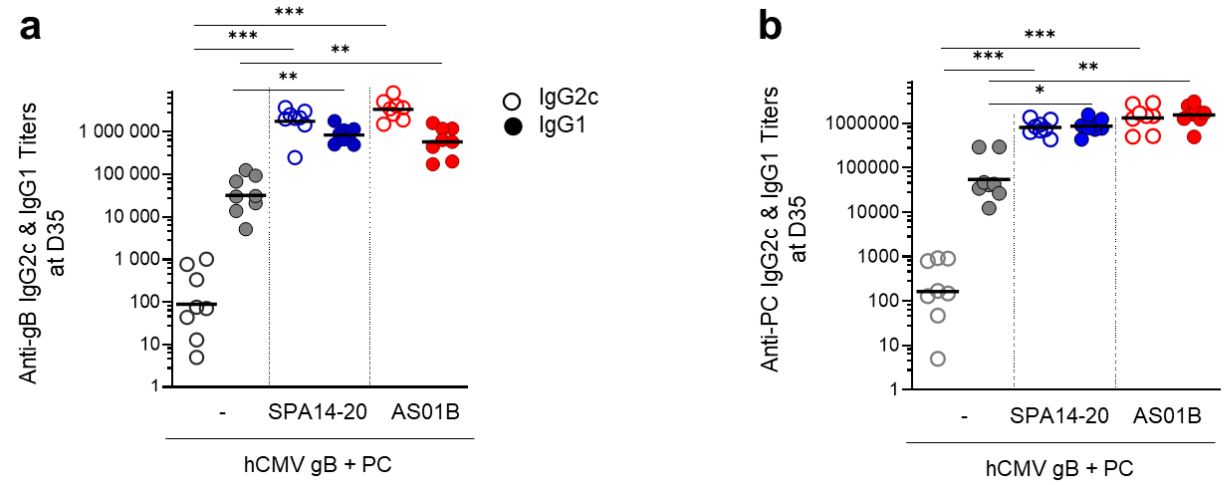
Supplementary Figure 2



Supplementary Fig. 2. Serum neutralizing antibody response to hCMV virus over time in the immunized mice as determined on MRC-5 fibroblasts.

a, *BADrUL131-Y4 CMV virus strain neutralizing titers ($PRNT_{50}$) were determined using MRC-5 fibroblasts and represented over time. For each group, geometric mean of neutralizing titers (GMT) and 95% confidence intervals are represented.* ***b,*** *Individual neutralizing titers from each mouse at D20 (3 weeks after a single immunization; open circles) and D35 (2 weeks after the second immunization; close circles) are shown as scattered plots with group GMTs as horizontal bars. Groups of mice immunized in the absence of adjuvant (○,●), in the presence of SPA14-20 (○,●) or AS01B (○,●) are shown. Group comparisons were done by Tukey or Dunnett adjustment. We applied one- or two-way or repeated ANOVA for group comparisons (* p-value<0.05, ** p-value<0.01, *** p-value<0.001).*

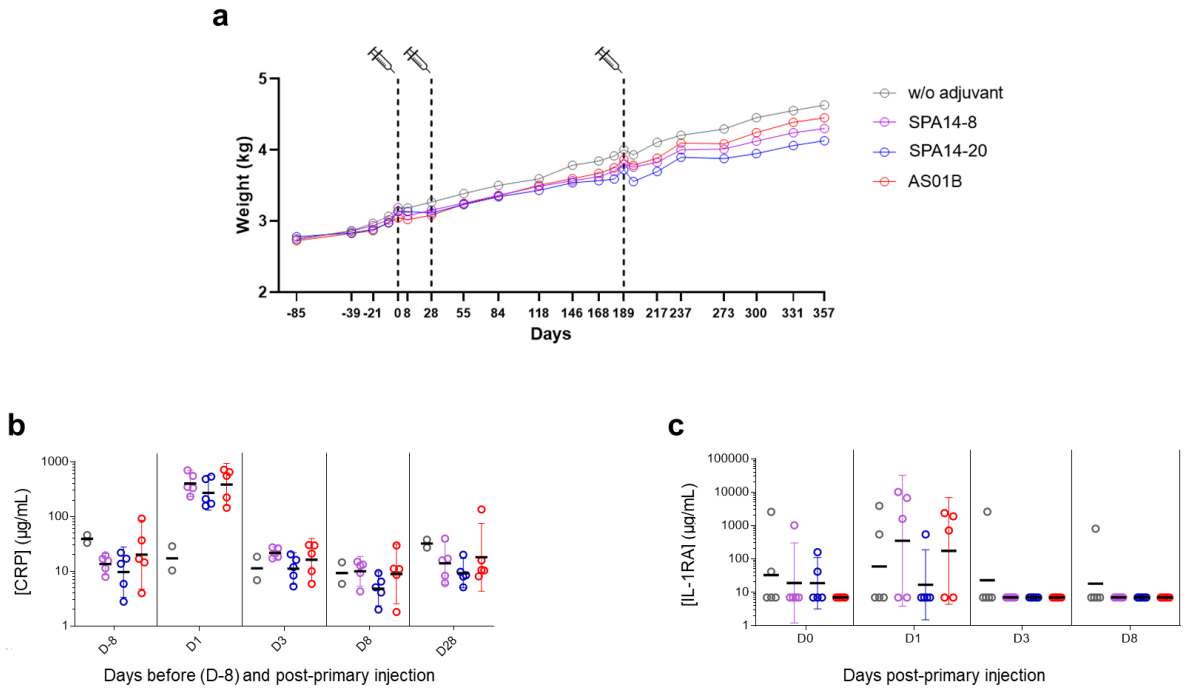
Supplementary Figure 3



Supplementary Fig. 3. Individual D35 serum antibody titers to hCMV antigens in the immunized mice.

a, *IgG1 (filled circles) and IgG2c (open circles) titers specific to CMV-gB and* ***b,*** *to PC as determined two weeks following the second immunization (D35) were reported on a (Log10) scale. For each group, individual titers are shown as scattered plots with group GMTs as horizontal bars. Groups of mice immunized in the absence of adjuvant (○,●), in the presence of SPA14-20 (○,●) or AS01B (○,●) are shown. Group comparisons were done by Tukey adjustment and one-way ANOVA (* p-value<0.05, ** p-value<0.01, *** p-value<0.001).*

Supplementary Figure 4



Supplementary Fig. 4. Monitoring of clinical parameters in the immunized macaques.
a, *Mean body weights* ***b,*** *serum CRP concentrations and* ***c,*** *serum IL-1Ra concentrations were monitored over time following immunization with CMV-gB + PC in the absence of adjuvant (O), in the presence of SPA14-20 (O), SPA14-8 (O) or AS01B (O). Individual CRP and IL-1Ra levels are shown for each group as scattered plots and group geometric means as horizontal bars.*