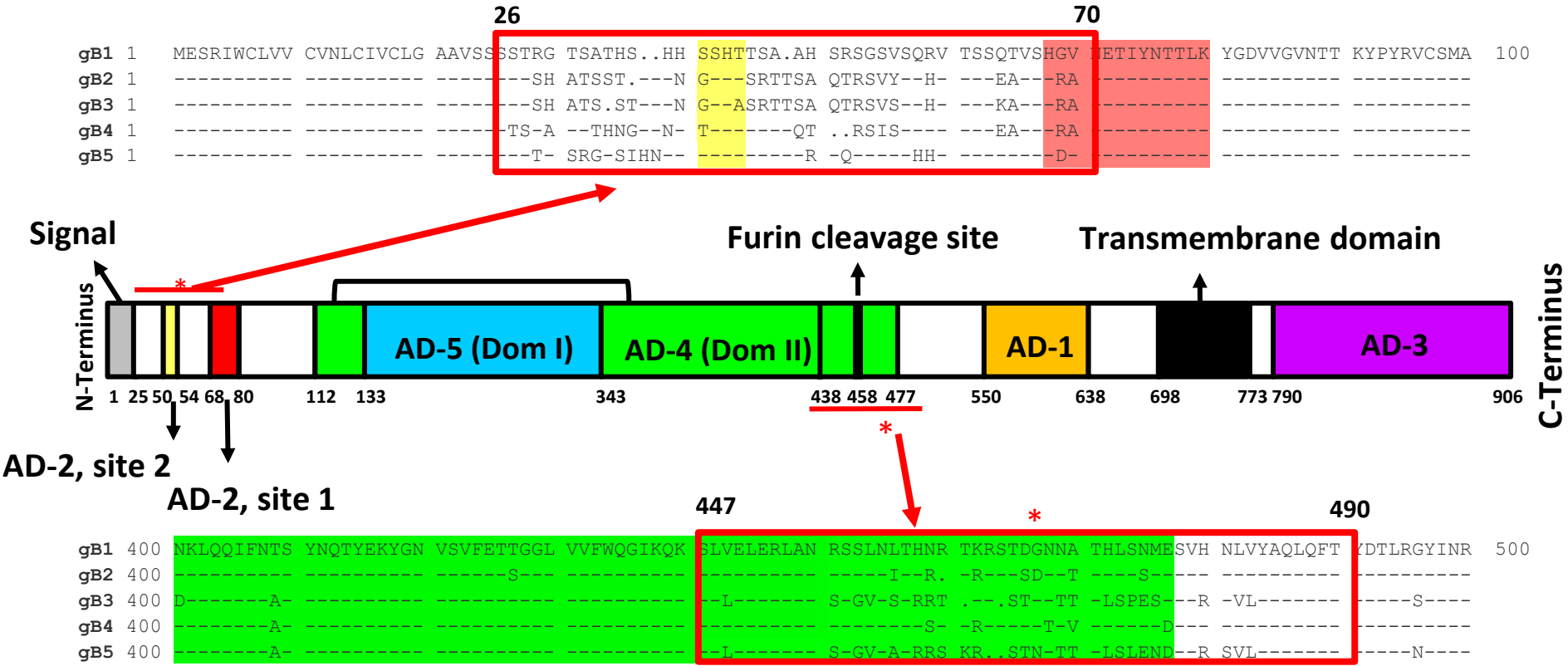
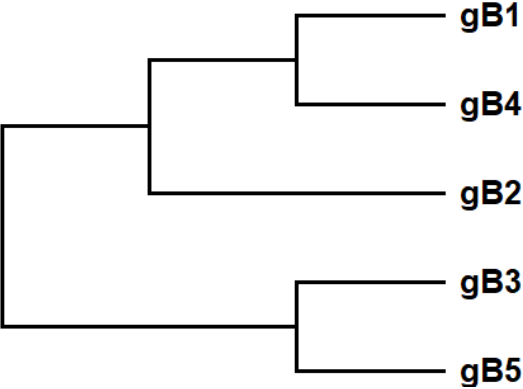


Sup Fig. 1

A



B



gB Genotypes Sequence Alignment (Percent Identify Matrix)					
	gB5	gB3	gB2	gB1	gB4
gB5	100	93.36	92.14	93.04	91.14
gB3	93.36	100	95.35	93.03	93.46
gB2	92.14	95.35	100	95.47	95.24
gB1	93.04	93.03	95.47	100	96.13
gB4	91.14	93.46	95.24	96.13	100

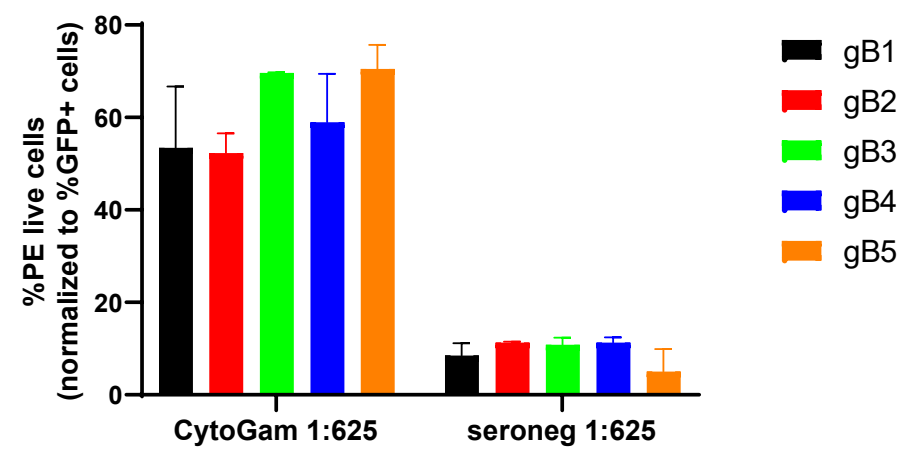
gB1	MESRIWCLVV	CVNLCIVCLG	AAVSSSSSTRG	TSATHS..HH	SSHTTSA.AH	SRSGSVSQRV	TSSQTVSHGV	NETIYNTTLK	YGDVVGVTNT	KYPYRVC SMA		100
gB2	- - - - -	- - - - -	- - - - - SH	ATSST.- --N	G--SRTTSA	QTRSVY--H-	--EA--RA	- - - - -	- - - - -	- - - - -		
gB3	- - - - -	- - - - -	- - - - - SH	ATS.ST--N	G--ASRTTSA	QTRS VS--H-	--KA--RA	- - - - -	- - - - -	- - - - -		
gB4	- - - - -	- - - - -	- - - - - TS-A	--THNG--N-	T-----QT	. . RSIS----	--EA--RA	- - - - -	- - - - -	- - - - -		
gB5	- - - - -	- - - - -	- - - - - T-	SRG-SIHN--	- - - - - R	- Q----HH-	- - - - - D-	- - - - -	- - - - -	- - - - -		
gB1	QGTDLIRFER	NIVCTSMKPI	NEDLD EGIMV	VYKRNI VA HT	FKVR VYQ KVL	TFRRSYAYIH	TTYLLGS NTE	YVAPP MWEIH	HINSHSQ CYS	SYS RV IAGTV		200
gB2	- - - - -	--I- - - -	- - - - -	- - - - -	- - - - -	- - - - - Y	- - - - -	- - - - -	--KFA----	- - - - - G---		
gB3	- - - - -	- - - P - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
gB4	- - - - - D-	- - - P - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
gB5	- - - - -	- - - PV - - -	- - - - -	- - - - -	- - - - -	- - - - - Y	S - - - - -	- - - - -	--KFA----	- - - - - L		
gB1	FVAYHRDSYE	NKT MQLPDD	YSN TH STRYV	TVKDQ WHSRG	STWL YRETCN	LNC MV TI TT A	RSKY PYHF FA	TSTGD VDVIS	P FYNG TN RNA	SYFG ENADKF		300
gB2	- - - - -	- - - I - - -	- - - - -	- - - - -	- - - - -	- - - L - - -	- - - - -	- - - Y - - -	- - - - -	- - - - -		
gB3	- - - - -	- - - L - - -	- - - I - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - T - - -	- - - - -		
gB4	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
gB5	- - - - -	- - - S - - -	- - - - -	- - - - -	- - - H - - -	- - - M - A -	- - - - -	- - - - -	- - - T - - -	- - - - -		
gB1	FIFPNYTIVS	D FGR PN SALE	THR LVAFLER	ADS VISWDIQ	DEKN VT CQLT	FWE ASER TIR	SEA EDSYHFS	SAKM TATFLS	KKQE VNMSDS	ALDC VRDEAI		400
gB2	- - - - -	- - - A - P -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
gB3	- - - - -	- - - A - - P -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - P - - -	V - - - - NQ - L		
gB4	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - E - - -	- - - - -	- - - - -	- - - - -	- - - - - L		
gB5	- - - - -	- - - A - Q -	- - - - -	- - - - -	- - - - -	- - - K - - -	- - - - -	- - - - -	- - - P - - -	V - - - - Q - L		
gB1	NKLQQIFNTS	YNQTYE KYGN	VSVFETT GGL	VVF WQGIKQK	SLVE LERLAN	RSSLNLTHNR	TKR STD GNNA	THLS NMESVH	NLVYA QLQFT	YDTLRGY INR		500
gB2	- - - - -	- - - - - S - - -	- - - - -	- - - - -	- - - - -	- - - I - - R.	- R - - SD - T	- - - S - - -	- - - - -	- - - - -		
gB3	D - - - - A -	- - - - -	- - - - -	- - - - -	- - L - - - -	S - GV - S - RRT	. - . ST - TT	- LS PES - - R	- VL - - - - -	- - - S - - -		
gB4	- - - - - A -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - - S -	- R - - - T - V	- - - - - D - - -	- - - - -	- - - - -		
gB5	- - - - - A -	- - - - -	- - - - -	- - - - -	- - L - - - -	S - GV - A - RRS	KR. . STN - TT	- LS LE ND - - R	SVL - - - - -	- - - N - - -		
gB1	ALAQIAEAWC	VDQR RTLEV F	KEL SKINPSA	ILSAIYNKPI	AARFM GDVLG	LASC VTIN QT	SVKV LRDMNV	KESP GR CY SR	PVVI FN FANS	SYVQ YGQLGE		600
gB2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
gB3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - V - - -	- - - - -		
gB4	- - T - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
gB5	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - TI -	- D - T - - -	- - - T - - -	- H - - - - -		
gB1	DNEILLGNHR	TEECQLPSLK	IFI AGNSAYE	YVDYL FKRM I	DL SSISTV DS	MIALDIDPLE	NTDFRVLELY	SQKE LRSINV	FDLEE IMREF	NSYK QRVKYV		700
gB2	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - S - - -	- - - - -	- - - - -	</	

Sup Figure 1. Schematic representation of HCMV gB sequence with antigenic domains and the most variable regions among 5 gB genotypes.

- (A) The full opening reading frame of HCMV gB is shown from the N-terminus (left) to the C-terminus (right). The gB sequence consists of ectodomain, transmembrane domain (black), and the cytosolic domain. The AD-1 (orange), AD-2 site 1 (red), AD-2 site 2 (yellow), AD-4 (also known as Dom II, green), and AD-5 (also known as Dom I, blue) are in ectodomain, while AD-3 (purple) belongs to cytosolic domain. This figure was adapted from Burke et al. [1], Chandramouli et al. [2], and Nelson et al. [3]. The most variable regions defining the five gB genotypes are codons 26-70 and codons 441-490. These regions were aligned using Clustal W and Clustal X version 2.0 [4] and presented by SeqPublish. The five gB sequences included in the vaccine design are: gB-1 (C327A strain, M60929.1), gB-2 (C336A strain, M60931.1), gB-3 (BE/37/2011 strain, KP745723.1), gB-4 (C194A strain, M60926.2), and gB-5 (17_saliva_7-24-2003 isolate, MK157431.1).
- (B) Phylogenetic tree of full gB open reading frame encoding 5 gB genotypes. The phylogenetic analysis was performed following the bootstrap method with 100 replications by Molecular Evolutionary Genetics Analysis version 11 (Tamura 2011). Clades representing 5 individual gB genotypes are color-coded : gB-1, black circle; gB-2, red square; gB-3, green upward triangle; gB-4, blue diamond; gB-5, orange downward triangle. The percent identity matrix comparing five gB genotypes was generated using Clustal W and Clustal X version 2.0 (94).
- (C) The complete sequence alignment of the five HCMV gB genotypes using Clustal W and Clustal X version 2.0 (94) and presented by SeqPublish. The five gB sequences included in the vaccine design are: gB-1 (C327A strain, M60929.1), gB-2 (C336A strain, M60931.1), gB-3 (BE/37/2011 strain, KP745723.1), gB-4 (C194A strain, M60926.2), and gB-5 (17_saliva_7-24-2003 isolate, MK157431.1).

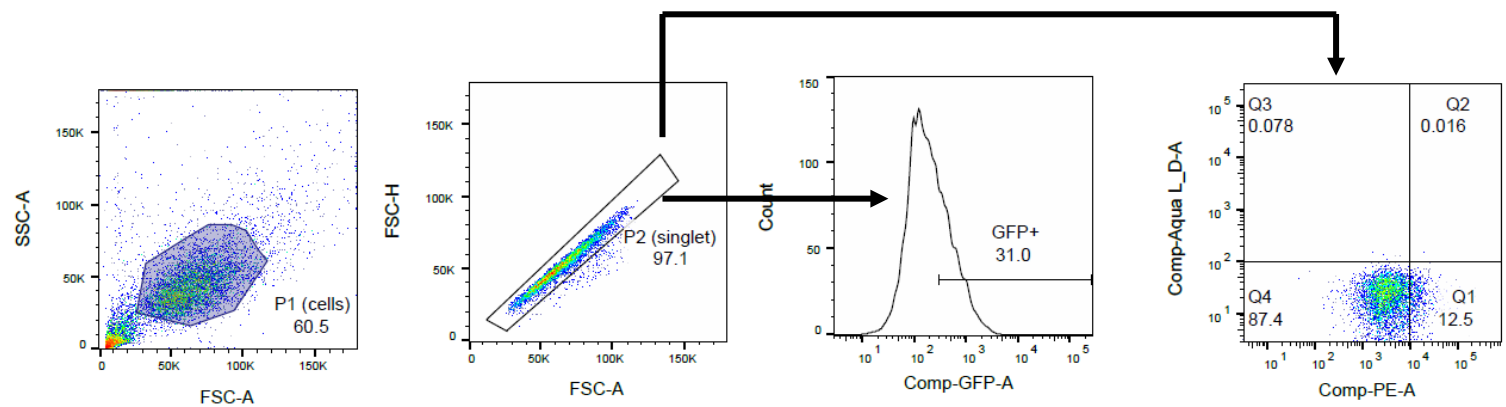
Sup Fig. 2

A Cell-Associated gB Binding



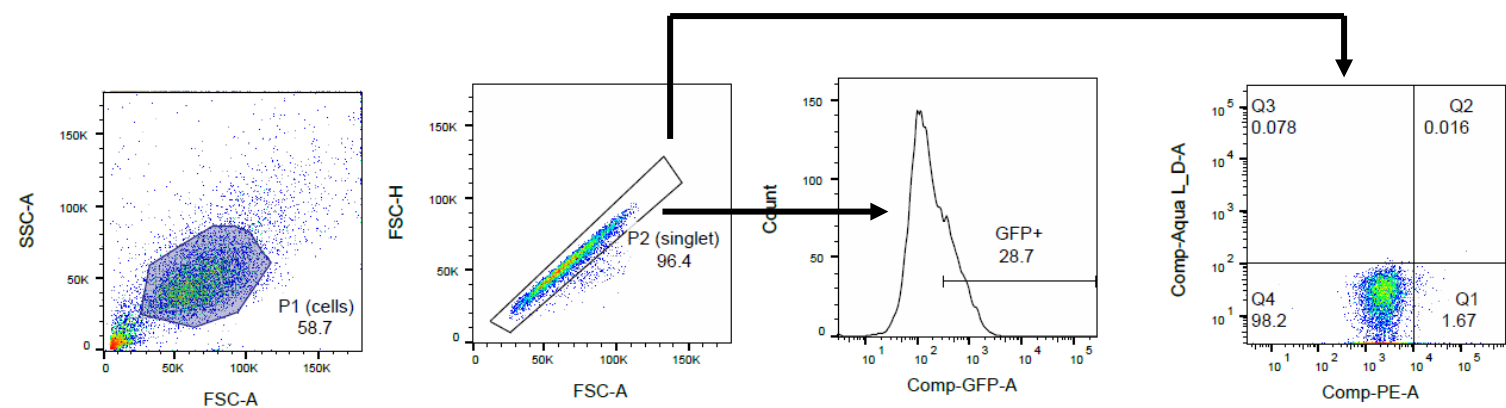
B

CytoGam



C

CMV-negative Serum



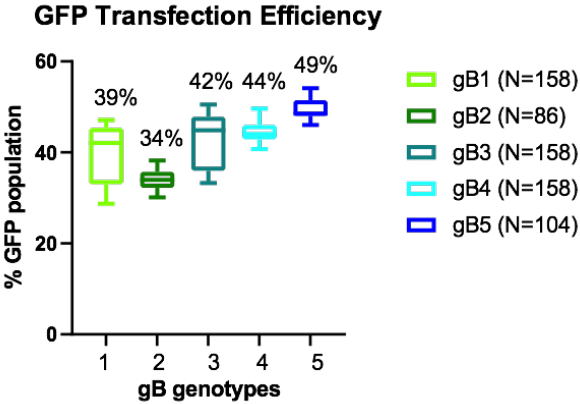
Sup Fig. 2 mRNA-LNPs encoding gB genotype antigens encoding each genotype similar antigenicity.

(A) Transfection efficiency of gB mRNA-LNPs encoding each genotype *in vitro*. mRNA-LNPs encoding each gB genotype were co-transfected with GFP DNA plasmid in HEK 293 T cells *in vitro* to compare the antigenicity of each gB antigen. The transfected cells were incubated with HCMV immune globulin (CytoGam) or seronegative plasma to measure the polyclonal IgG binding to cell-associated gBs. Each data bar represents the average % PE+ live cell population normalized to % GFP+ population in duplicate, with error bar indicating the standard error of mean. Polyclonal IgG binding to gB genotypes are color-coded: gB-1 (black), gB-2 (red), gB-3 (green), gB-4 (blue), and gB-5 (orange). No statistical analysis was performed due to the small sample size.

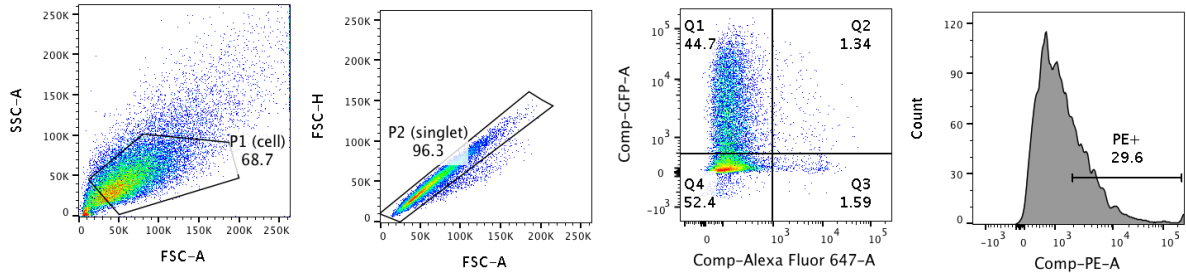
(B-C) Gating strategy of gB antigenicity for CytoGam (B) and HCMV-seronegative plasma (C). The 293T cells were determined by SSC-A and FSC-A, designated as P1 population. The P2 singlet population was later obtained by gating the P1 population through FSC-A and FSC-H. Next, the singlet P2 population was gated by GFP+ population based on the mock transfection control and live PE+ population (Q1) based on the HCMV-seronegative plasma, respectively. The % PE+ live population shown in Q1 was normalized to GFP+ population to eliminate the transfection efficiency effect.

Sup Fig. 3

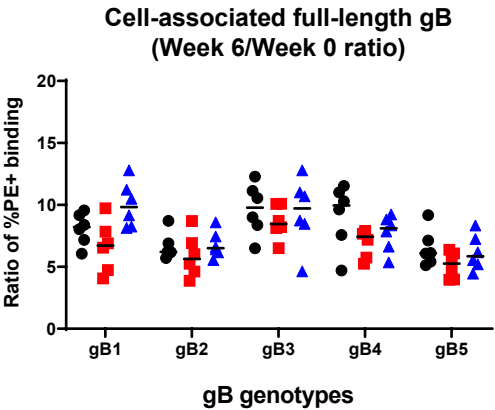
A



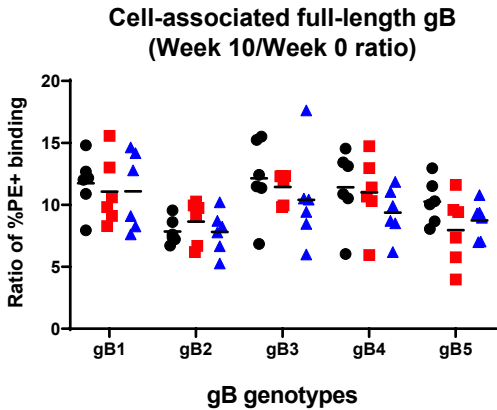
B



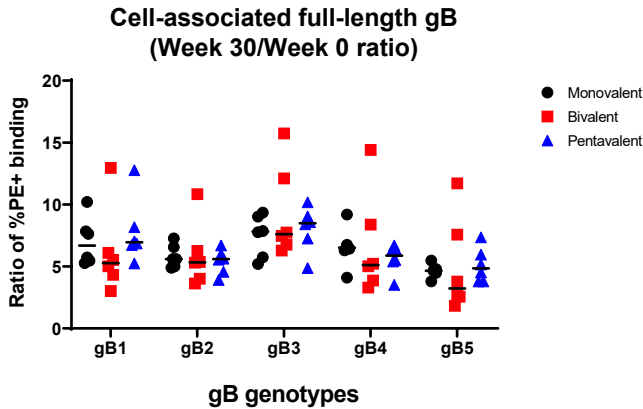
C



D



E



Sup Fig. 3 Monovalent, bivalent, and pentavalent gB mRNA-LNP vaccinated rabbit plasma IgG elicited a similar binding breadth to cell-associated gB of 5 genotypes.

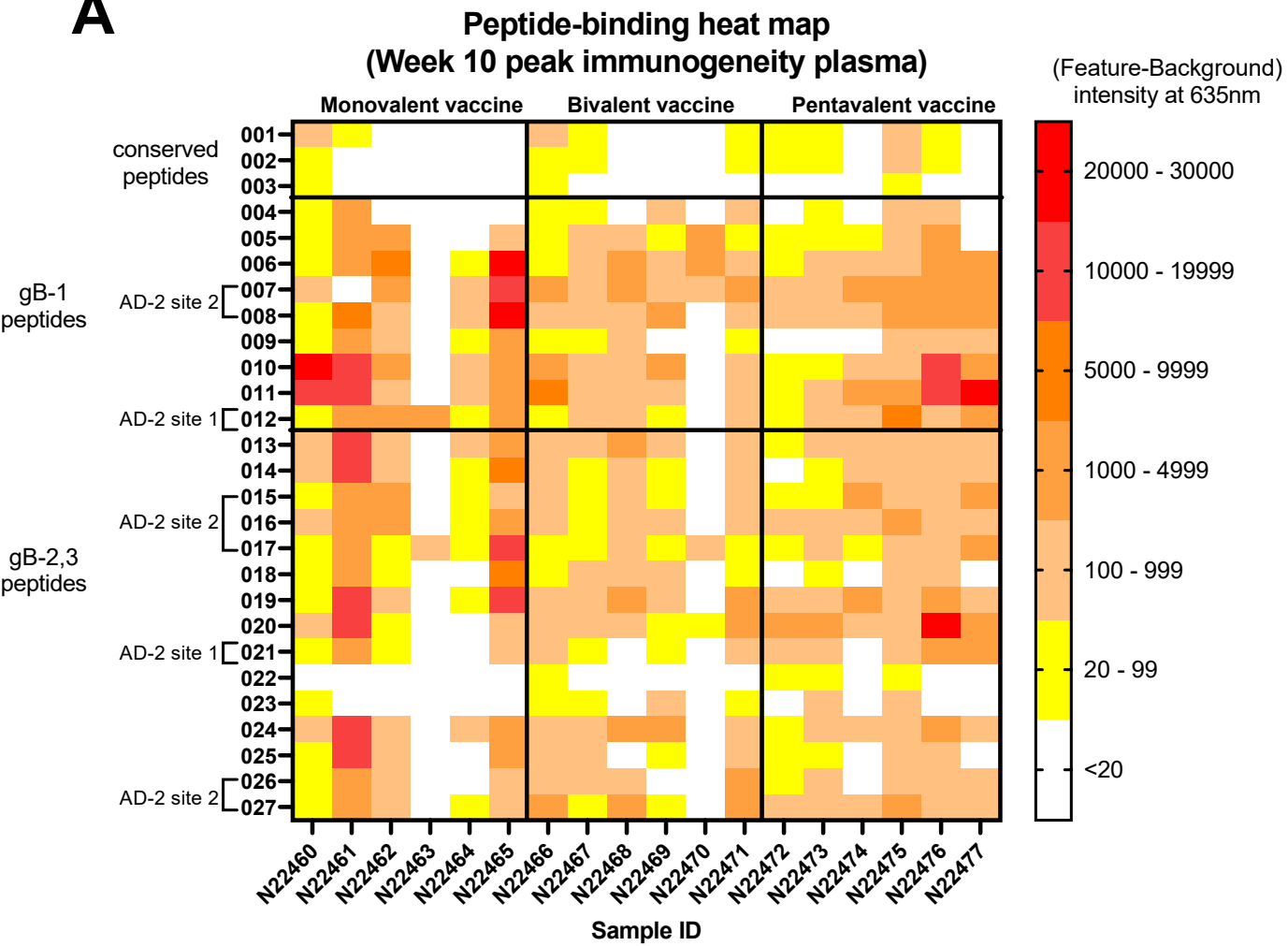
(A) The transfection efficiency of gB-T2A-GFP plasmids encoding 5 genotypes, respectively, was determined by %GFP expression. The %GFP expression was shown by box and whisker plot, with the mean value labeled on top. These values were color-coded based on the gB genotypes: gB-1, lawn green; gB-2, Forest green; gB-3, Paris green; gB-4, sky blue; gB-5, blue.

(B) Gating strategy of gB transfected cell binding assay. The 293T cells were determined by SSC-A and FSC-A, designated as P1 population. The P2 singlet population was later obtained by gating the P1 population through FSC-A and FSC-H. Next, the live GFP+ P2 population was shown in Q1, and the %PE+ population was compared from the Q1 population.

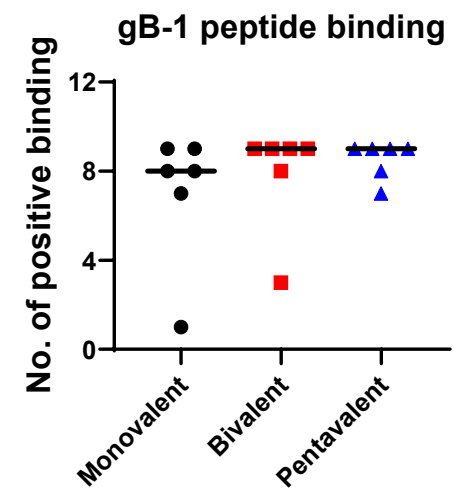
(C-E) Rabbit plasma IgG binding breadth to full-length gB encoding 5 genotypes at week 6 (C), week 10 (D), and week 30 (E) by gB-transfected cell binding assay. Data points are shown as the IgG binding response normalized to that of the week 0 samples to eliminate the discrepancies among the transfection efficiency. Each data point represents the normalized IgG binding response of one individual animal, with the median response labeled by a black line. Black circles: rabbits immunized with monovalent vaccine; red squares: those immunized with bivalent vaccine; blue triangles: those immunized with pentavalent vaccine.

Sup Fig. 4

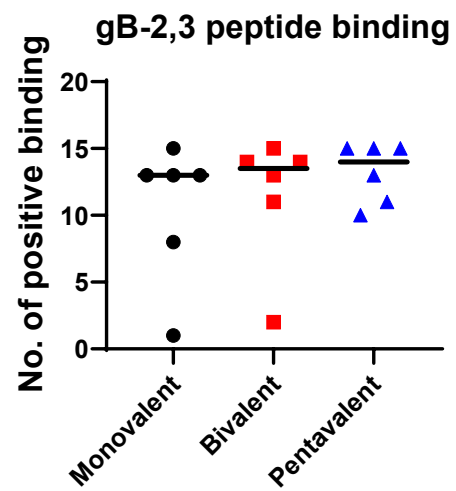
A



B



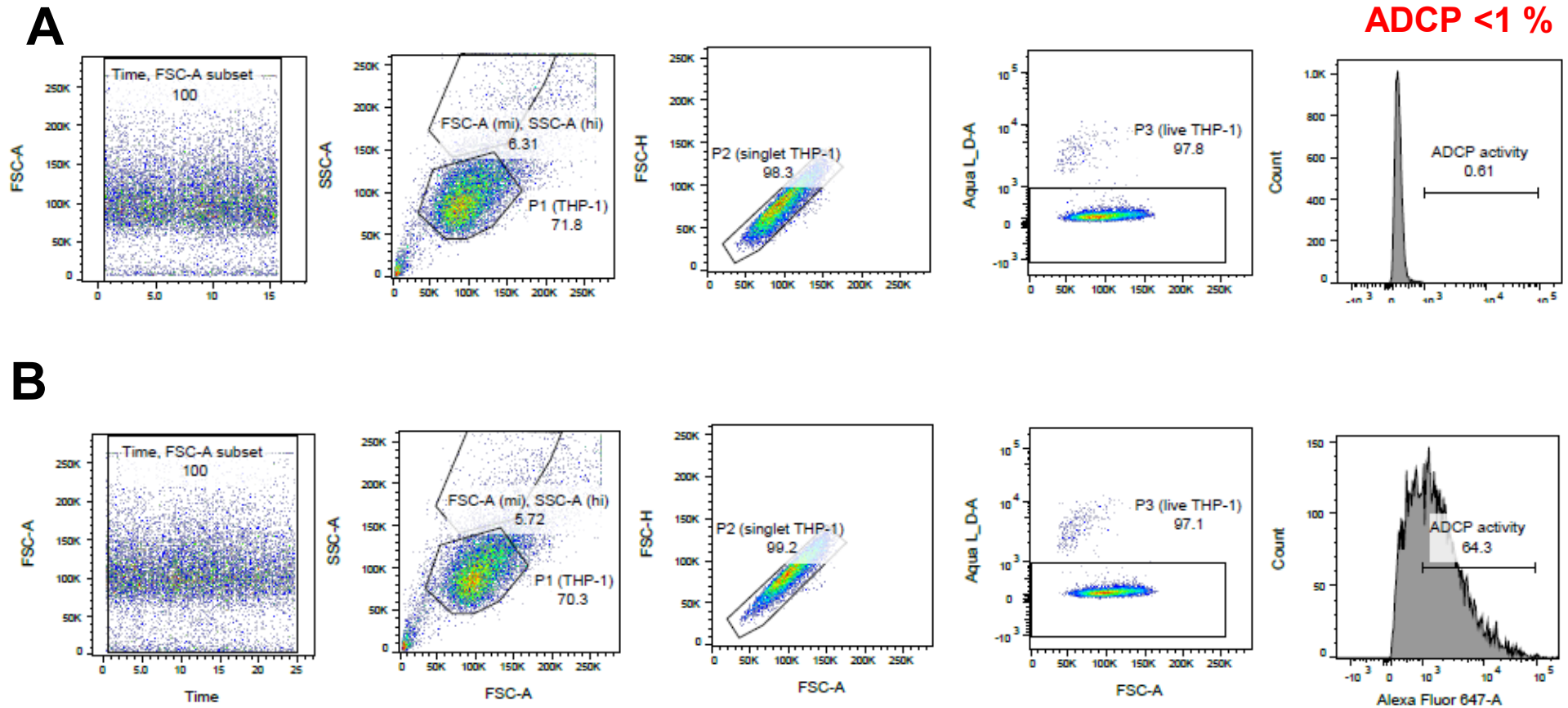
C



Sup Fig. 4 Monovalent, bivalent, and pentavalent gB mRNA-LNP vaccinated rabbit plasma IgG at week 10 did not show a statistical different binding pattern to linear gB peptides of gB-1 or gB-2/3 genotypes.

(A) The breadth of rabbit plasma IgG binding to 15-mer linear gB peptides covering the amino acid codons 1-77 of gB-1 or gB2/3 genotypes (27 unique peptides) at peak immunogenicity (week 10), measured by peptide microarray. Each row indicates a linear gB peptide: 001-003, conserved peptides; 004-012, gB-1 peptides; 013-027, gB2/3 peptides. Each column is one animal from each vaccine group. The plasma IgG binding to linear 15-mer gB peptides was calculated by (feature-background) intensity at 635nm, and the average peptide median binding (feature-background) intensity at 635nm of each of the triplicates was reported. (B-C) The positive binding MFI cutoff was defined by the average binding of the secondary non-specific antibody binding plus two standard deviations. Each data point represents the number of peptides bound by plasma IgG from one individual animal, with the median response labeled by a black line. Black circles: rabbits immunized with monovalent vaccine; red squares: those immunized with bivalent vaccine; blue triangles: those immunized with pentavalent vaccine.

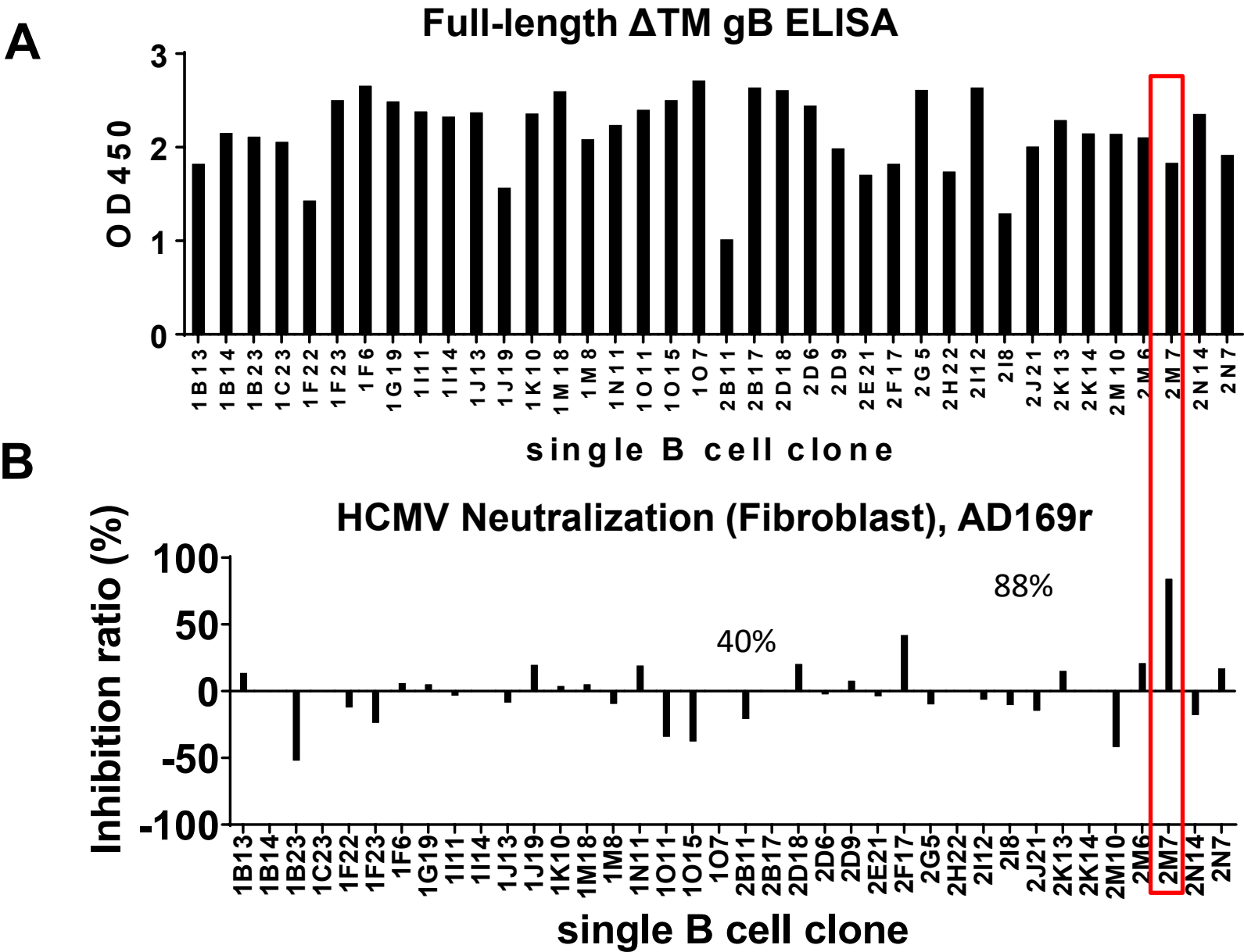
Sup Fig. 5



Sup Fig. 5 Gating strategy of ADCP for negative control (PBS) and positive control (CytoGam)

(A-B) The THP-1 cells were first gated for acquisition time and the cells were determined by SSC-A and FSC-A, designated as P1 population. The P2 singlet THP-1 population was later obtained by gating the P1 population through FSC-A and FSC-H. Next, the live THP-1 cell population was by aqua L/D stain as P3, and the % ADCP activity was determined by the AF647+ cells from the live cell population using the PBS negative control as the threshold. ADCP activity from PBS negative control (A) and CytoGam positive control (B).

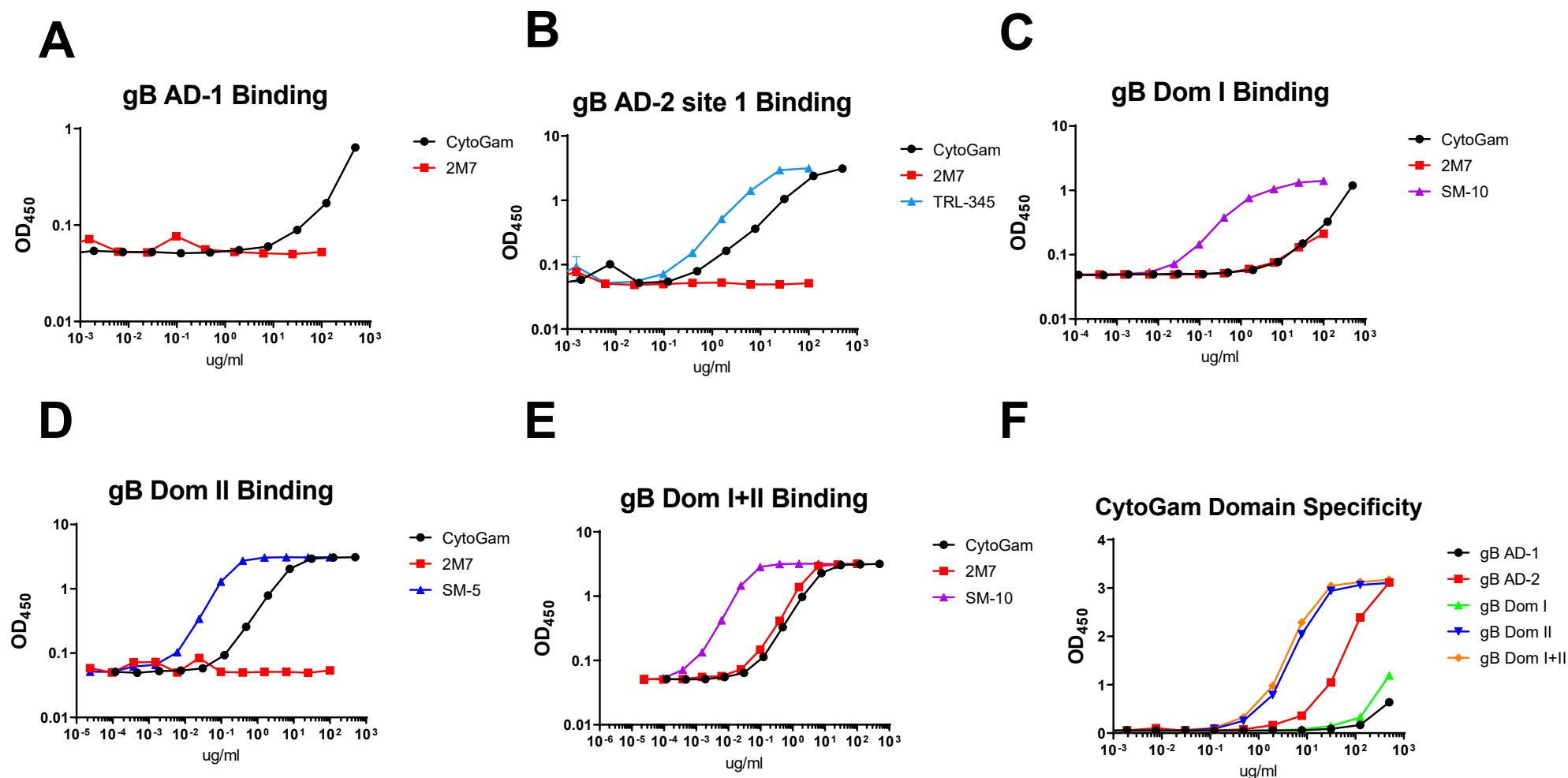
Sup Fig. 6



Sup Fig. 6 Monoclonal antibody screening from memory B cell clones.

(A) Among 600 single memory B cell clones, the supernatant (1:20 dilution) of 38 clones demonstrated a binding response to post-fusion full-length gB by ELISA. (B) The fibroblast neutralization potency of the supernatants from the 38 gB-binding clones (1:20 dilution) was examined by neutralization assay against HCMV AD169r strain. Among 38 mAbs, only 2M7 showed the strongest neutralization potency.

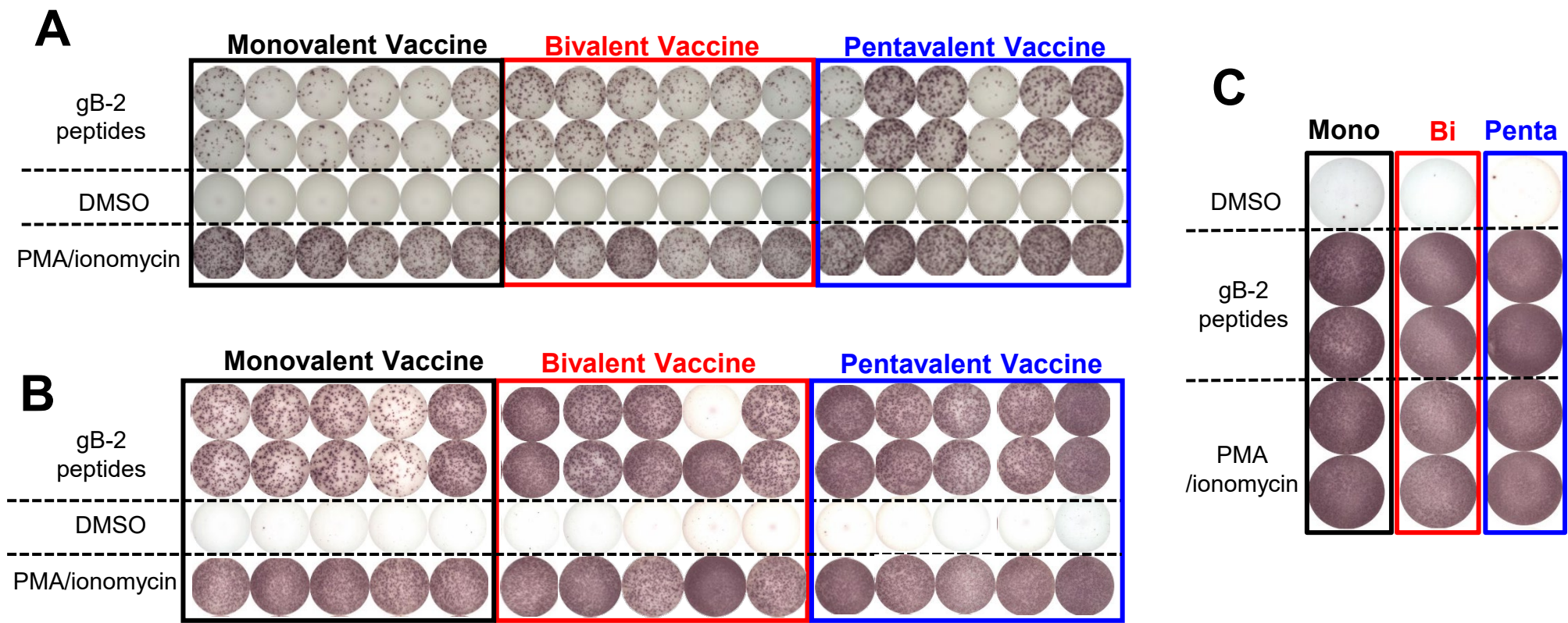
Sup Fig. 7



Sup Fig. 7 Positive controls of gB domain specificity confirmed 2M7 only showed binding to gB Dom I+II.

(A-E) The gB domain specificity of 2M7 was determined by comparing binding to gB AD-1 (A), AD-2 (B), Domain I (C), Domain II (D), and domain I+II (E). The positive controls for each antigen are CytoGam (A-F, black circle), TRL-345 (B, light blue triangle), SM-10 (C, E, purple triangle), and SM-5 (D, blue triangle). (F) The gB domain specificity of CytoGam, measured by ELISA. CytoGam binding to gB domains is color-coded with symbols: black circle, AD-1; red square, AD-2 site 1; green upward triangle, Domain I; blue downward triangle, Domain II; orange diamond, Domain I+II.

Sup Fig. 8



Sup Fig. 8 Representative wells of IFN-r+ cells stimulated with gB-2 peptides, DMSO, or PMA/ionomycin from rabbit PBMCs at week 6, and splenocytes at week 30 or 43.

gB-2-specific IFN-r+ cells from rabbit PBMCs or splenocytes were measured by ELISPOT. gB-2 peptide pool stimulation were performed in duplicate. Black rectangle: rabbits immunized with monovalent vaccine; red rectangle: those immunized with bivalent vaccine; blue rectangle: those immunized with pentavalent vaccine. Representative wells of rabbit PBMCs at week 6 (A), splenocytes at week 30 (B) and week 43 (C). (B) One well from the bivalent vaccine group incubated with gB-2 peptides did not show any binding and thus was removed for analysis. (C) 1 rabbit from each vaccine group received another booster at week 41 and underwent necropsy at week 43.

Sup Fig. 9

**gB3-specific IFN- γ + cells
(Necropsy, Week 30)**



· Neg ctrl (DMSO):19

Bivalent Vaccine

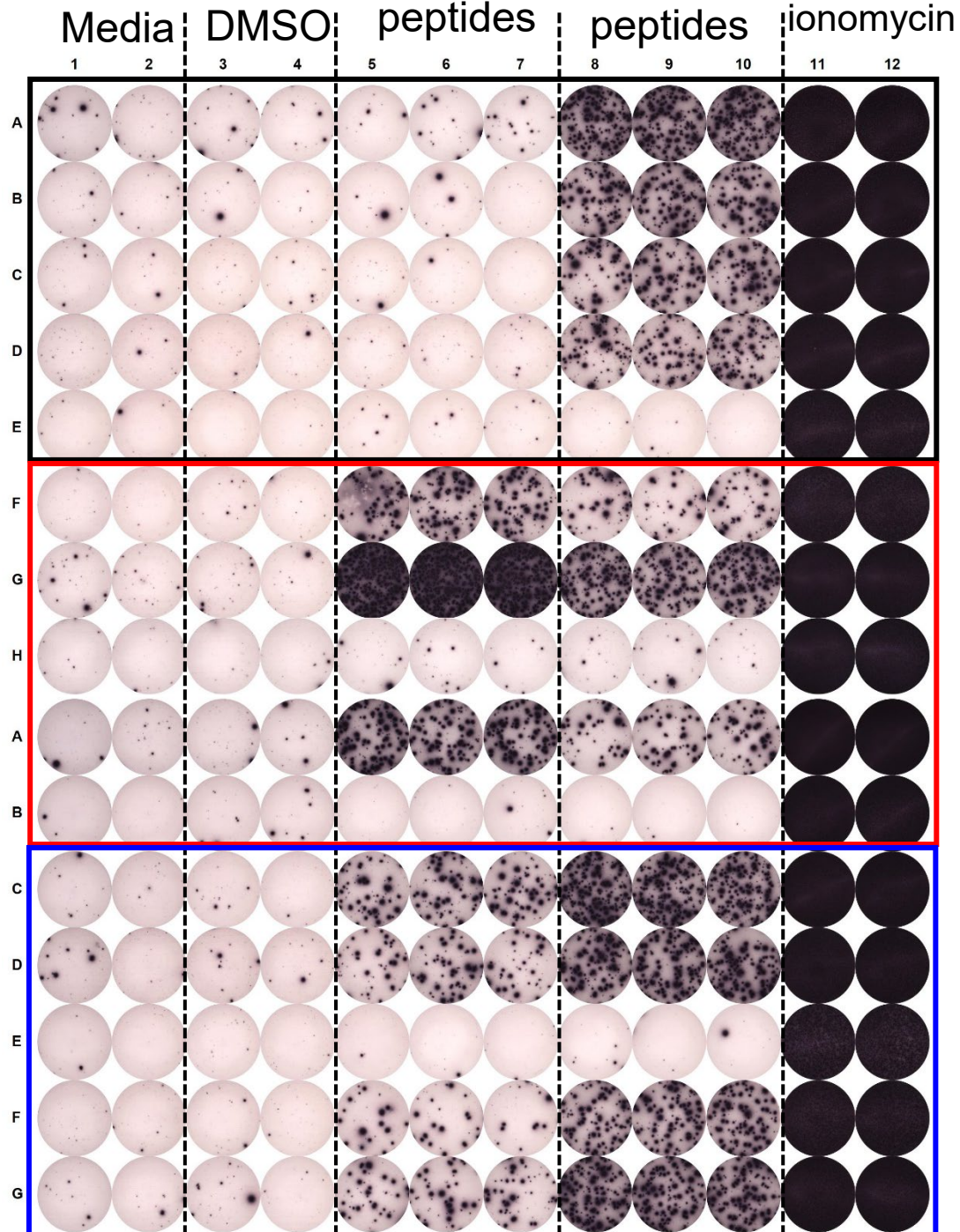
Pentavalent Vaccine

- Monovalent
- Bivalent
- ▲ Pentavalent

gB-3
peptides

gB-5
peptide

PMA/
ionomycin



Sup Fig. 9 Multivalent gB mRNA-LNP vaccine elicited a stronger gB-3 (Toledo strain) but not gB-5-specific IFN-r+ cell response in splenocytes at necropsy.

gB-3 and gB-5-specific IFN-r+ cells from rabbit splenocytes were measured by ELISPOT. Overlapping 15-mer peptide libraries targeting gB-3 and gB-5 variable regions, respectively, were incubated with rabbit splenocytes in triplicate.

(A) Quantification of IFN-r+ spots from rabbit splenocytes stimulated with gB-3 variable regions peptides at necropsy (week 30) from monovalent, bivalent, and pentavalent vaccine groups.

(B) Quantification of IFN-r+ spots from rabbit splenocytes stimulated with gB-5 variable regions peptides at necropsy (week 30) from monovalent, bivalent, and pentavalent vaccine groups. 15 animals underwent necropsy at week 30, while 3 animals (1 animal from each group) underwent necropsy at week 43 due to an extra boost prior to B cell isolation at week 41. Each data point represents one peptide-stimulated well from individual animals, with the lines designating the median. Black circles: rabbits immunized with monovalent vaccine; red squares: those immunized with bivalent vaccine; blue triangles: those immunized with pentavalent vaccine. Kruskal-Wallis test with Bonferroni correction was performed for any overall difference. The assay limitation was determined by the average spot count of the cells incubated with DMSO only (negative control).

(C) Wells of IFN-r+ cells stimulated with gB-3 or gB-5 variable region peptides (in triplicate), DMSO (in duplicate), media (in duplicate) or PMA/ionomycin (in duplicate) from rabbit splenocytes at week 30. Black rectangle: rabbits immunized with monovalent vaccine; red rectangle: those immunized with bivalent vaccine; blue rectangle: those immunized with pentavalent vaccine.

Sup Table 1. Linear overlapping 15-mer gB peptide sequence.

Peptide No.	gB peptide sequence	gB genotype	Peptide No.	gB peptide sequence	gB genotype
001	MESRIWCLVVCVNLC	1,2,3,4,5	015	SSTSHATSSTHNGSH	2,3
002	WCLVVCVNLCIVCLG	1,2,3,4,5	016	ATSSTHNGSHTSRTT	2,3
003	CVNLCIVCLGAAVSS	1,2,3,4,5	017	HNGSHTSRTTSAQTR	2,3
004	IVCLGAAVSSSSTRG	1	018	TSRTTSAQTRSVSSQ	2,3
005	AAVSSSSTRGTSATH	1	019	SAQTRSVSSQHVTSS	2,3
006	SSTRGTSATHSHHSS	1	020	SVSSQHVTSSSEAVSH	2,3
007	TSATHSHHSSHTTSA	1	021	HVTSSSEAVSHRANET	2,3
008	SHHSSHTTSAAHRSR	1	022	WCLVVCVNLCYVCLG	2,3
009	HTTSAAHSRSGSVSQ	1	023	CVNLCYVCLGAVVSS	2,3
010	AHSRSGSVSQRVTSS	1	024	YVCLGAVVSSSSTSH	2,3
011	GSVSQRTVSSQTVSH	1	025	AVVSSSSTSHATSSA	2,3
012	RVTSSQTVSHGVNET	1	026	SSTSHATSSAHNGSH	2,3
013	IVCLGAAVSSSSTSH	2,3	027	ATSSAHNGSHTSRTT	2,3
014	AAVSSSSTSHATSST	2,3			

Sup Table 2. Overlapping 15-mer peptide libraries targeting gB-3 and gB-5 variable regions.

Peptide library targeting gB-3 variable regions		
Peptide No.	gB-3 peptide sequence	Codon
001	SSTSHATSSAHNGSH	26-40
002	HATSSAHNGSHTSRT	30-44
003	SAHNGSHTSRTTSAQ	34-48
004	GSHTSRTTSAQTRSV	38-52
005	SRTTSAQTRSVSSQH	42-56
006	SAQTRSVSSQHVTS	46-60
007	RSVSSQHVTSSSEAVS	50-64
008	SQHVTSSSEAVSHRAN	54-68
009	LANSSGVNSTRRTKR	445-459
010	SGVNSTRRTKRSTGN	449-463
011	STRRTKRSTGNTTTL	453-467
012	TKRSTGNTTTLSELES	457-461
013	TGNTTTLSELESVR	461-465
014	TTLSELESVRNVLY	464-468
015	LESESVRNVLYAQLQ	468-472
016	SVRNVLYAQLQFTYD	472-476

Peptide library targeting gB-5 variable regions		
Peptide No.	gB-5 peptide sequence	Codon
001	SSTTGSRGTSIHNNHH	26-40
002	GSRGTSIHNNHHSSHT	30-44
003	TSIHNNHHSSHTTSA	34-48
004	NHHSSHTTSAARSQS	38-52
005	SHTTSAARSQSGSVS	42-56
006	SAARSQSGSVSHHVT	46-60
007	SQSGSVSHHVTSSQT	50-64
008	SVSHHVTSSQTVSHD	54-68
009	HVTSSQTVSHDVNET	58-72
010	LANSSGVNATRRSKR	447-461
011	SGVNATRRSKRSTNN	451-465
012	ATRRSKRSTNNTTTL	455-469
013	SKRSTNNTTTLSELEN	459-473
014	TNNTTTLSELENSVR	463-477
015	TTLSELENSVRNVLY	467-481
016	LENSVRNVLYAQLQ	471-485
017	SVRSVLYAQLQFTYD	475-489

References for supplementary info:

1. Burke, H. G. & Heldwein, E. E. Crystal Structure of the Human Cytomegalovirus Glycoprotein B. PLoS Pathog 1–21 (2015) doi:10.1371/journal.ppat.1005227.
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