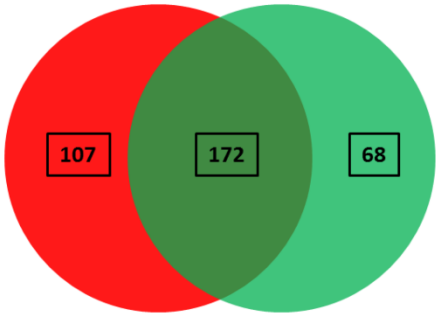
A)



B)

	RhCMV 68-1		RhCMV Δpp65ab	
Source	viral	host	viral	host
% peptides	63.4	36.6	69.9	30.1
mol% protein	64.8	35.2	68.5	31.5

C)

Protein ID	wt	Δpp65
s100 calcium-binding protein a11	1.64	0.00
s100 calcium-binding protein a11	1.14	0.00
annexin a5	1.04	0.00
cd81	0.80	0.00
programmed cell death protein 6	0.65	0.00
annexin a4	0.56	0.00
gtp-binding protein smg p21b	0.55	0.00
lrrn4 c-terminal-like protein	0.50	0.00
40s ribosomal protein s26	0.47	0.00
autosomal recessive hypercholesterolemia protein	0.45	0.00
s100 calcium-binding protein a4	0.41	0.00
cytokeratin-16	0.39	0.00
chronic lymphocytic leukemia deletion region gene 6 protein homolog	0.33	0.00
copine i	0.32	0.00
annexin a11	0.32	0.00
60s ribosomal protein l13	0.31	0.00
elongation factor 1-alpha-like 3	0.31	0.00
60s ribosomal protein l34	0.29	0.00

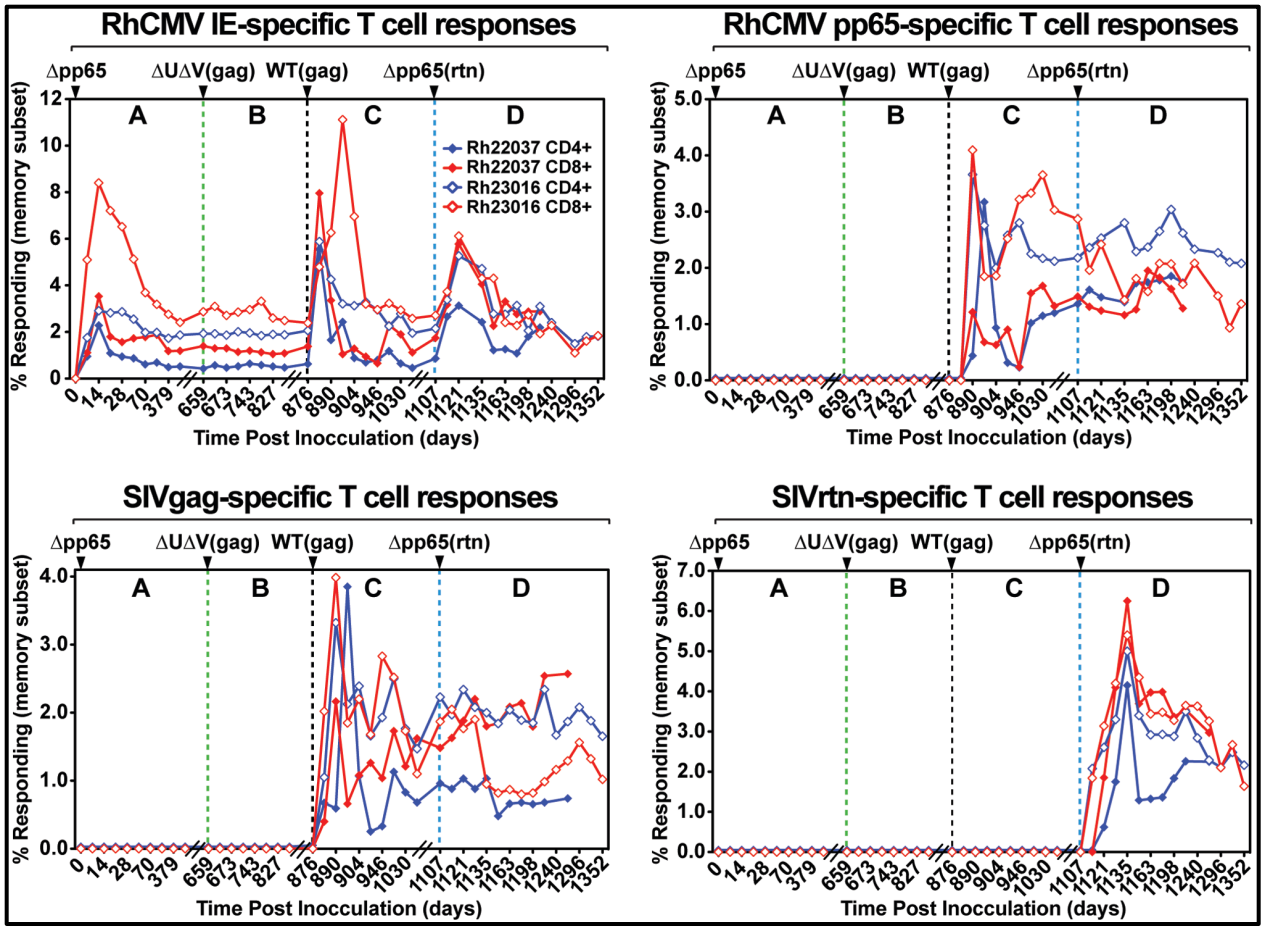
Protein ID	wt	Δpp65	Fold Change
60s ribosomal protein l6	0.33	0.04	8.68
sodium potassium-transporting atpase subunit alpha-1	0.48	0.06	7.59
4f2 cell-surface antigen heavy chain	0.45	0.07	6.54
40s ribosomal protein s27a	0.90	0.18	5.13
hemoglobin alpha chain	1.02	0.21	4.75
cytokeratin-9	1.09	0.26	4.22
glucose transporter type erythrocyte brain	0.41	0.11	3.73
elongation factor 1-alpha 2	0.36	0.10	3.71
40s ribosomal protein s27a	0.33	0.09	3.64
annexin a1	0.46	0.14	3.23
cytokeratin-10	1.88	0.67	2.79
annexin a2	1.41	0.51	2.78
casein kinase ii subunit alpha	0.50	0.19	2.57
cytokeratin-2e	0.53	0.23	2.36
fibronectin	0.36	0.17	2.10
vesicle transport through interaction with t-snares homolog 1a	0.82	0.40	2.04

D)

Protein ID	wt	Δpp65
marvel domain-containing protein 1	0.00	0.85
intestinal membrane a4 protein	0.00	0.61
class i histocompatibility gogo-b*0101 alpha chain	0.00	0.57
trypsinogen c	0.00	0.44
heterogeneous nuclear ribonucleoprotein x	0.00	0.33
transmembrane protein 179b	0.00	0.33
ilm mineralization protein	0.00	0.32
aurora ipl1-related kinase 1	0.00	0.28
integral membrane protein 2b	0.00	0.27
vitronectin	0.00	0.26
glyceraldehyde-3-phosphate dehydrogenase	0.00	0.25
hla-b-associated transcript 3	0.00	0.25

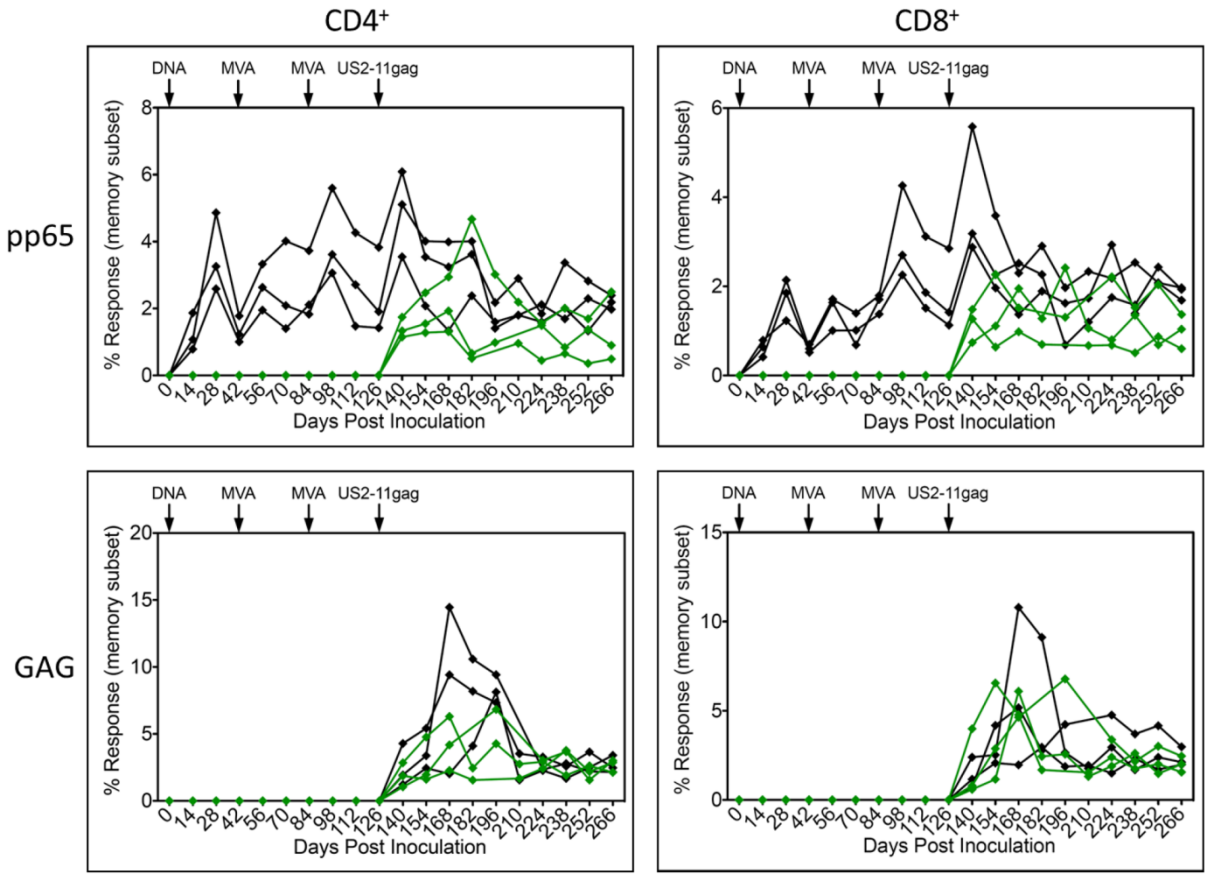
Protein ID	wt	Δpp65	Fold Change
cd155	0.17	0.86	5.06
peptidyl-prolyl cis-trans isomerase a	0.24	1.19	4.89
l-lactate dehydrogenase b chain	0.07	0.31	4.44
l-lactate dehydrogenase c chain	0.06	0.26	4.44
l-lactate dehydrogenase a chain	0.06	0.25	4.44
mapk-activating protein pm28	0.08	0.35	4.23
syntaxin-12	0.08	0.28	3.38
histone h3	0.23	0.76	3.33
histone h3	0.22	0.74	3.33
mhc class i antigen patr-a*0401	0.20	0.62	3.10
eukaryotic initiation factor 4a-ii	0.10	0.27	2.76
mhc class i antigen a*69	0.19	0.47	2.45
mhc class i antigen e	1.93	4.61	2.39
sorting nexin-17	0.47	1.10	2.33
beta-2-microglobulin	0.26	0.60	2.31
histone h2b type 1-j	0.25	0.54	2.18
histone h2b type 1-f j l	0.25	0.54	2.18
histone h2b 291b	0.25	0.54	2.18
mhc class i antigen e	1.11	2.39	2.16
lysosomal-associated membrane protein 3	0.53	1.11	2.11
histone h2a q	0.75	1.57	2.10
serine protease 11	0.22	0.45	2.05

Supplemental Figure 1. Comparison of host proteins contained in WT and Δpp65ab virions. A) The total number of host proteins found in RhCMV WT and Δpp65ab virions and the overlapping proteins found in both samples are shown. B) All peptides and proteins found in WT and Δpp65ab virions as shown here separated into either host or viral proteins dependent on their origin. C) Host proteins with a minimum abundance of 0.25mol% of the total amount of host proteins were ranked by abundance into two groups, proteins that had significant abundance in WT virions and were not found in Δpp65ab virions (upper panel) and host proteins that were found in both virions, but with at least two fold higher abundance in the WT. D) Similar to C), host proteins were ranked by abundance, but only proteins are shown that were either present in Δpp65ab virions and not in the WT (upper panel) or host proteins that were found in both virions, but with at least two fold higher abundance in Δpp65ab.

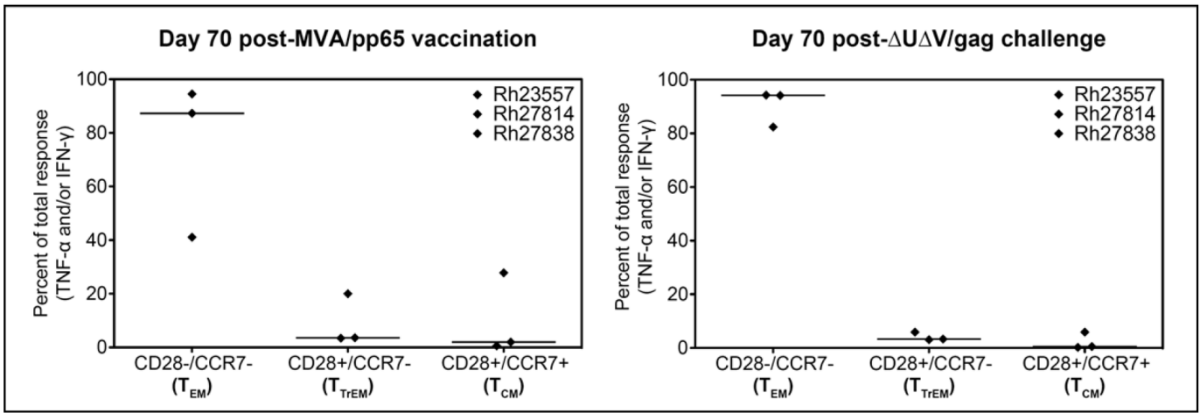


Supplemental Figure 2. Δpp65ab establishes primary and secondary infections and protects against super-infection with ΔUS2-11.. A) Two RhCMV sero-negative male RM (RM1 and RM2) were infected s.c with 10^7 PFU of Δpp65ab at day 1. $CD4^+$ (blue) and $CD8^+$ (red) T-cell responses were monitored in broncho-alveolar lavages (BAL) by intracellular cytokine staining (ICCS) at the indicated days using overlapping peptides of pp65ab and IE1/2) On day 659 the two animals were inoculated s.c. with 10^7 PFU of ΔUS2-11gag (green dotted line) and the T cell response to SIVgag was measured in addition. Note the absence of a T cell response to SIVgag or pp65 and a lack of boosting of responses to IE1. C) On day 876, the two RM were inoculated with 10^7 PFU of WTgag (black dotted line) and the T cell response was monitored by ICCS. Note the appearance of *de novo* responses to SIVgag and pp65 and a boosting of the T cell response to IE1. D) On day 1107 the two RM were inoculated with 10^7 PFU of Δpp65ab-rtn

(blue dotted line). Using overlapping 15mer peptides a *de novo* response to SIVrev/tat/nef was detectable indicating super-infection. Also note a boosting of the IE1 response but not of pp65 or SIVgag-specific responses.



Supplemental Figure 3. T cells induced by heterologous prime/boost vaccination with pp65b do not protect against super-infection with ΔUS2-11. Three CMV-negative RM were vaccinated with 1mg of pND/pp65b and boosted with MVApp65b at 6 and 12 weeks after the initial vaccination (black). As controls three CMV-negative RM were vaccinated with the parental pND plasmid not expressing any antigen and boosted with WT MVA at 6 and 12 weeks after the initial vaccination (green). At 18 weeks after the initial DNA vaccination both groups of animals were challenged with 10^7 PFU of ΔUS2-11gag. The left two panels show the specific T-cell responses to pp65 whereas the right two panes show specific T-cell responses to SIV gag. T-cells were isolated from broncho-alveolar lavages (BAL).



Supplemental Figure 4. Pp65b-specific T cells induced in naïve RM after DNA prime and MVA boost vaccination show mostly effector memory (T_{EM}) phenotype at the time of RhCMV ΔUΔV challenge. T cells were isolated from peripheral blood drawn from the three RM described in Figure 6 (Supplemental Figure 3) at the times indicated above each dot plot. The memory phenotype of the total pp65b response was determined by flow cytometry using the cell surface markers CD28 and CCR7 as previously described (1).

1. Hansen, S.G., Vieville, C., Whizin, N., Coyne-Johnson, L., Siess, D.C., Drummond, D.D., Legasse, A.W., Axthelm, M.K., Oswald, K., Trubey, C.M., et al. 2009. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nature medicine* 15:293-299.