

# **Supplementary Material**



## SUPPLEMENTARY FIGURE 1

Supplementary Figure 1. Gating strategy for the identification of T cell subsets in peripheral blood of a representative gag mRNA/LNP vaccinated macaque. (A) Lymphocyte

gate with side scatter/forward scatter. (B) Single cell gate excluding doublets. (C) CD3<sup>+</sup> gate for T cells. (D) CD4<sup>+</sup> and CD8<sup>+</sup> gates. (E) CD4<sup>+</sup> and CD8<sup>+</sup> memory (CD28, CD95) T cell subsets. (F) Dot plots showing medium-only stimulation (upper panel) and Gag-specific (lower panel) IFN- $\gamma^+$  cells within the total CD3<sup>+</sup> population. (G) Dot plots showing medium-only stimulation (upper panel) and Gag-specific (lower panel) IFN- $\gamma^+$  cells within CD4<sup>+</sup> and CD8<sup>+</sup> T cell memory subsets.

#### SUPPLEMENTARY FIGURE 2



Supplementary Figure 2. Identification of antigen-specific lymphocytes in bronchioalveolar (BAL) fluid from mRNA/LNP vaccinated macaques. (A- H) Gating strategy for the identification of antigen-specific lymphocyte subsets in BAL of a representative *gag* mRNA/LNP vaccinated macaque. (A) BAL with side scatter/forward scatter. (B) Singlets. (C) CD3 gate for T lymphocytes. (D) Dot plots showing medium-only stimulation (upper panel) and Gag-specific (lower panel) IFN- $\gamma^+$  cells within the total CD3<sup>+</sup> population. (E) CD4 and CD8 gates for CD3<sup>+</sup> lymphocytes. (F) CD4<sup>+</sup> (left panel) and CD8<sup>+</sup> (right panel) memory (CD28, CD95) T cells. (G) Dot plots showing medium-only stimulation (upper panels) and Gag-specific (lower panels) IFN- $\gamma^+$  and TNF $\alpha^+$  memory T lymphocytes within the CD4 (left panel) and CD8 (right panel) memory subsets. (H) Table showing the antigen-specific responses in BAL in the low and high dose mRNA/LNP vaccinated macaques at 2 weeks afer the 3<sup>rd</sup> and the 2<sup>nd</sup> vaccination, respectively. A sample is considered positive when value is higher than 0.05 and at least 2-fold over the medium-only control

#### SUPPLEMENTARY FIGURE 3



## Supplementary Figure 3. Changes in body temperature upon mRNA/LNP vaccinations.

Body temperatures (in Fahrenheit) were measured in macaques on day 1, 2, 4 after each mRNA/LNP vaccination. The mRNA/LNP vaccines were administered as (A) low (n =15) and (B) high dose (n=5) and the data were plotted overtime. The individual animals (grey lines) and median (red lines) were shown. The tables list the number of animals with at least  $1^{\circ}$  F increase and the median temperature change with [IQR].



**Supplementary Figure 4. HIV CE/CE+gag DNA vaccination of rhesus macaques.** Macaques were vaccinated with CE/CE+Gag DNA following the same schedule used for the mRNA/LNP vaccination (Figures 1, group 3). The DNA vaccine (dose: 2 mg prime, 2+2 mg boost) contained IL-12 DNA as vaccine adjuvant and was administered by IM injection followed by electroporation. Plot shows vaccine-induced Gag Ab measured over time as reciprocal endpoint titers (log). The last time points of blood collection were week 95 and 101, respectively, for 3 animals each and these time points were combined plotted as week 98.

### SUPPLEMENTARY FIGURE 5



Supplementary Figure 5. Differential expression analysis comparing changes after the  $2^{nd}$  and  $3^{rd}$  vaccination. Mean log2 fold changes (Log2FC) of cytokine levels are shown comparing levels at day 2 to day 1 for all the 15 animals receiving the mRNA/LNP vaccine. Volcano plots of data shown in Figure 3D depict differentially expressed analytes upon the vaccination 2 (A) and vaccination 3 (B) at day 2 in comparison to day 1. Red dots indicate significant upregulation; blue dots indicate significant downregulation (adjusted p value <0.05 represented by the broken horizontal line).

Animal	Vaccine	Age	Sex	Dose mRNA/ LNP	Dose gag DNA	Dose IL-12 DNA	Dose mRNA /LNP	Dose gag DNA	Reporte
				(µg)	(mg)	(mg)	(µg)	(mg)	d
DGHG	CE RNA	5	M	25					Fig.1
16C190	CE RNA	4	F	25					Fig.1
DGTE	CE RNA	4	M	25					Fig.1
ZM34	CE RNA	6	F	25					Fig.1
ZN21	CE RNA	5	F	25					Fig.1
16C102	gag RNA -> gag DNA	4	F	25				2	Fig.1->5
16C141	gag RNA -> gag DNA	4	F	25				2	Fig.1->5
DGRK	gag RNA -> gag DNA	4	М	25				2	Fig.1->5
HXN	gag RNA -> gag DNA	5	F	25				2	Fig.1->5
ZM12	gag RNA -> gag DNA	6	F	25				2	Fig.1->5
16C275	CE RNA->CE+gag RNA	4	F	25					Fig.1
16C286	CE RNA->CE+gag RNA	4	F	25					Fig.1
DGRW	CE RNA->CE+gag RNA	5	М	25					Fig.1
ZM07	CE RNA->CE+gag RNA	6	F	25					Fig.1
ZN09	CE RNA->CE+gag RNA	5	F	25					Fig.1
LL25	gag RNA	4	M	100					Fig.2
LM45	gag RNA	4	M	100					Fig.2
LM88	gag RNA	4	M	100					Fig.2
LR02	gag RNA	4	M	100					Fig.2
LR33	gag RNA	4	M	100					Fig.2
I I10		(	м		2		25		E: 5>(
LII9 LD44	gag DNA -> gag RNA	0	M		2		25		Fig. $3 - > 0$
LK44	gag DNA -> gag RNA	4	M		2		25		F1g. 5->6
LTIO	gag DNA -> gag RNA	4	M		2		25		F1g. 5->6
LTH	gag DNA -> gag RNA	4	M		2		25		Fig. 5->6
LT16	gag DNA -> gag RNA	4	M		2		25		Fig. 5->6
5698ª	CE->CE+gag DNA	5	М		2->2+2 <sup>b</sup>	0.2			Fig.1
5699ª	CE->CE+gag DNA	5	М		2->2+2 <sup>b</sup>	0.2			Fig.1
5700 <sup>a</sup>	CE->CE+gag DNA	6	М		2->2+2 <sup>b</sup>	0.2			Fig.1
570 <sup>a</sup>	CE->CE+gag DNA	6	М		2->2+2 <sup>b</sup>	0.2			Fig.1
5702 <sup>a</sup>	CE->CE+gag DNA	6	М		2->2+2 <sup>b</sup>	0.2			Fig.1
5703ª	CE->CE+gag DNA	6	M		2->2+2 <sup>b</sup>	0.2			Fig.1
L982°	gag DNA	13	М		1	0.2			Fig.2
P574°	gag DNA	6	F		1	0.2			Fig.2
R067°	gag DNA	3	М		1	0.2			Fig.2
R288°	gag DNA	3	М		1	0.2			Fig.2
71.601		2			2	0.0			<b>F</b> ' (
ZM01	$gag DNA \rightarrow gag KNA$	3	M		2	0.2			Fig.6
ZIVI04	$gag DNA \rightarrow gag KNA$	2			2	0.2			Fig.6
ZIVIUO	gag DINA -> gag KINA	3	IVI		2	0.2	1		г1g.0

## Supplementary Table 1. Animal description and vaccination details

<sup>a</sup>Hu X, et al. J Immunol: 197:3999-4013 (2016) <sup>b</sup>2 mg CE DNA prime ->2 mg CE DNA+2mg gag DNA boost <sup>c</sup>Kulkarni V, et al. PLoS One:9 e111085 (2014).

		Analytes with minimal	
Analytes with changes (N=35)	Eotaxin	or no change (N=9)	IL-16
	Eotaxin-2		IL-1α
	Eotaxin-3		IL-2
	Fractalkine		MDC
	GRO-a		SDF-1a
	I-TAC		CTACK
	IFN-α2a		ENA-78
	IL-12/IL-23p40		MIP-3ac
	IL-15		FLT3L°
	IL-17A/F	Analytes below detection threshold (N=17)	G-CSF
	IL-17B	, <i>í</i>	GM-CSF
	IL-17C		I-309
	IL-17D		IFN-γ
	IL-17F		IL-10
	IL-18		IL-12p70
	IL-1Ra		IL-13
	IL-22 <sup>a</sup>		IL-17A
	IL-23		IL-1β
	IL-2Ra <sup>b</sup>		IL-4
	IL-6		IL-5
	IL-7		IL-8
	IL-9*		MCP-3
	IP-10		MIP-5
	M-CSF		TARC
	MCP-1		TNF-α
	MCP-2		TNF-β
	MCP-4		
	MIF		
	MIP-1α		
	MIP-10 MIP-30		
	ТРО		
	TRAIL		
	VEGF-A		
	YKL-40		

## Supplementary Table 2. Cytokines and Chemokines (N=61) tested

<sup>a</sup>Analyte below threshold of detection in high-dose vaccine <sup>b</sup>Analyte absent from MSD kit used in high dose analysis <sup>c</sup>Analyte affected in high-dose mRNA/LNP vaccine group only