

Supplemental Information

Immunization with HIV-1 gp41 Subunit Virosomes

Induces Mucosal Antibodies Protecting Nonhuman

Primates against Vaginal SHIV Challenges

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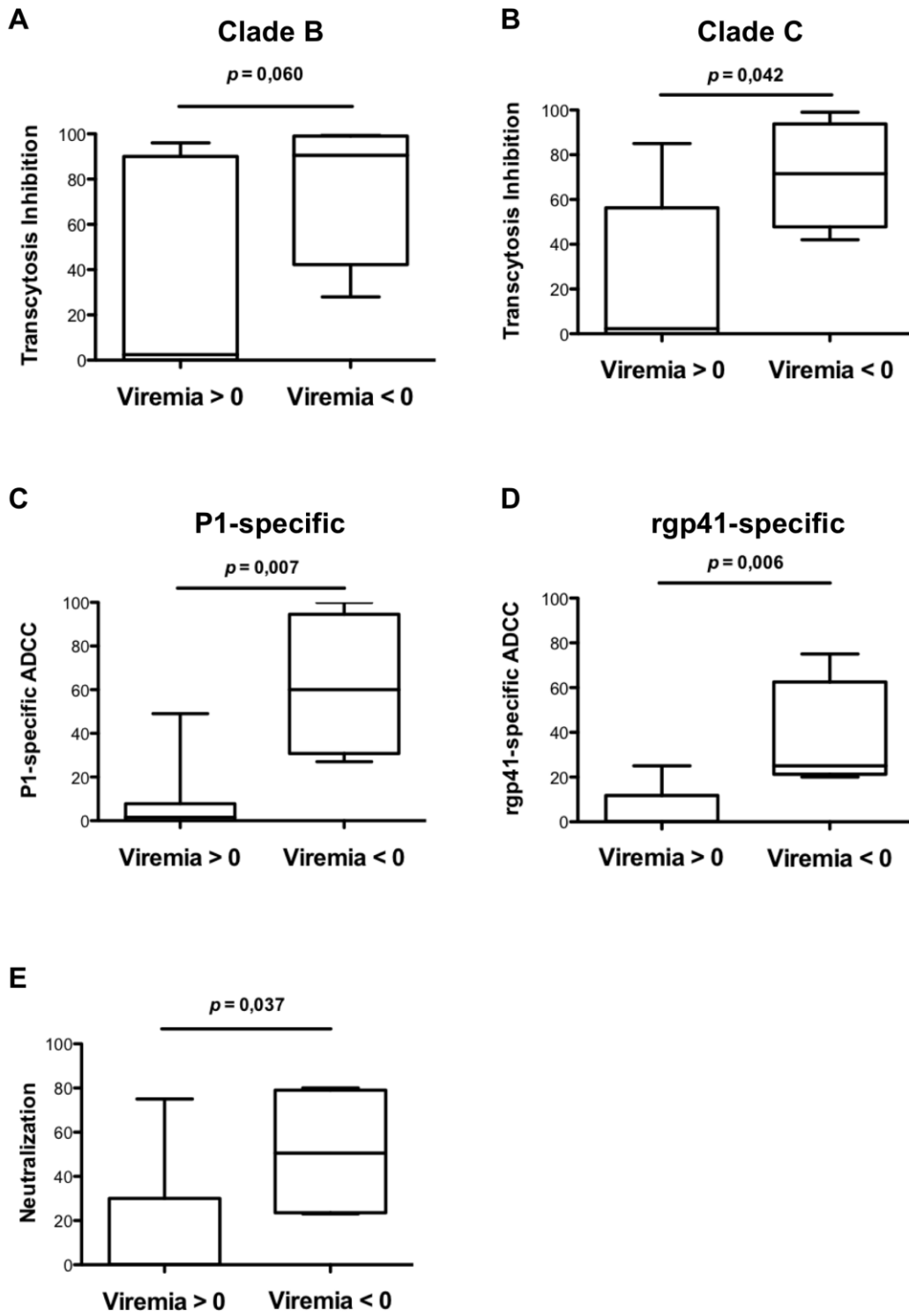


Figure S1

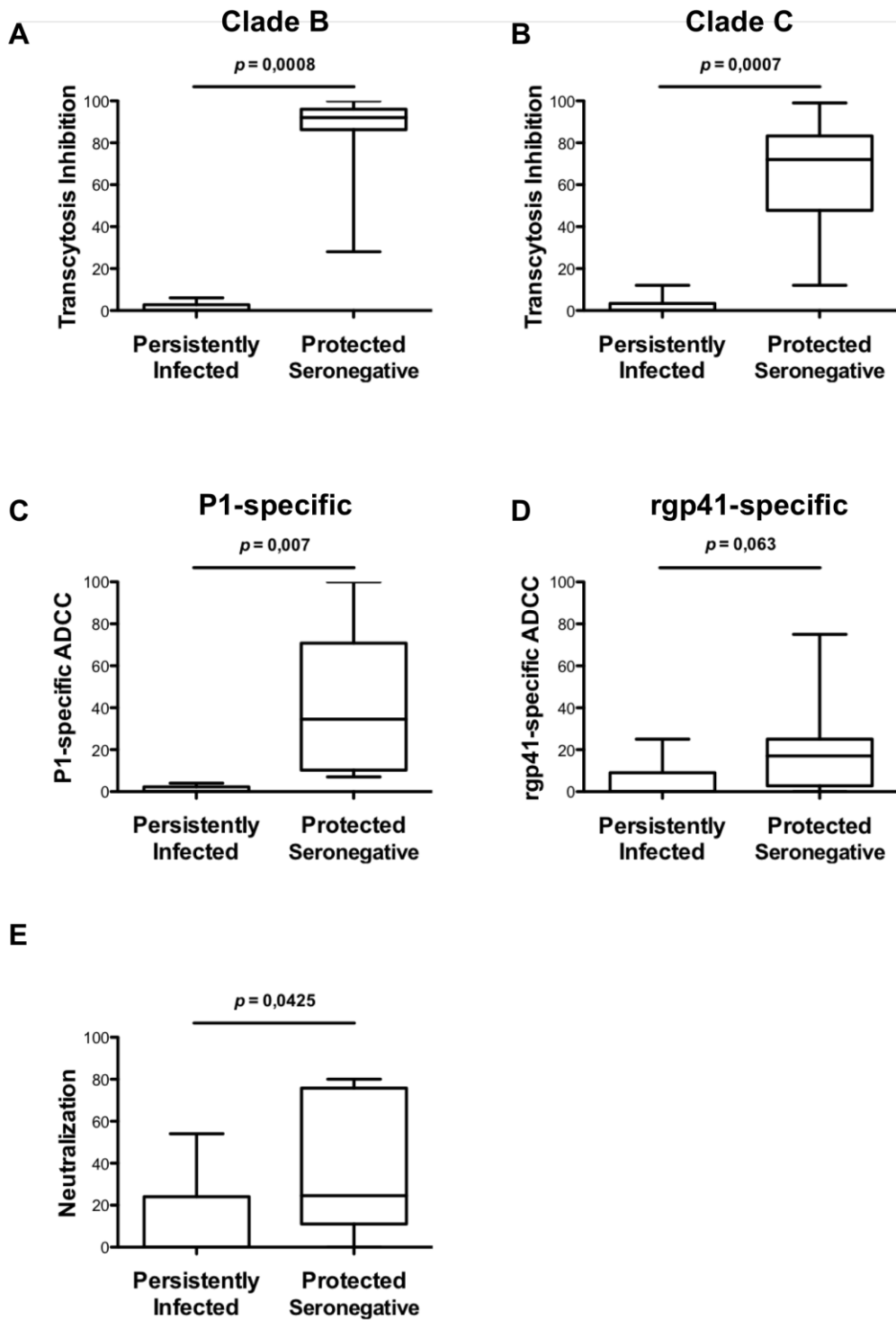
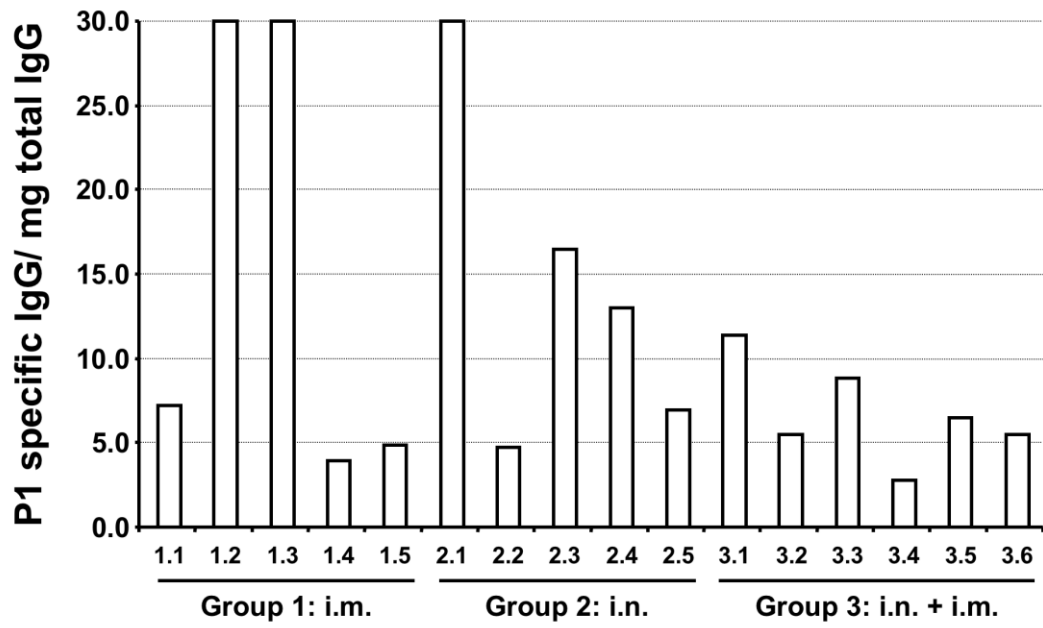


Figure S2

A



B

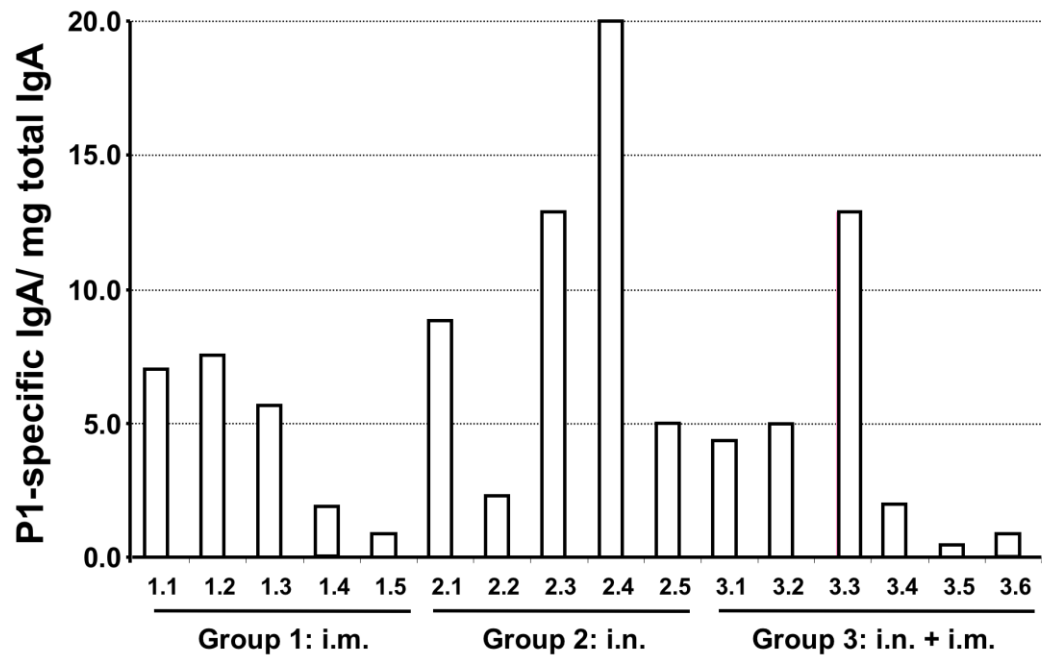
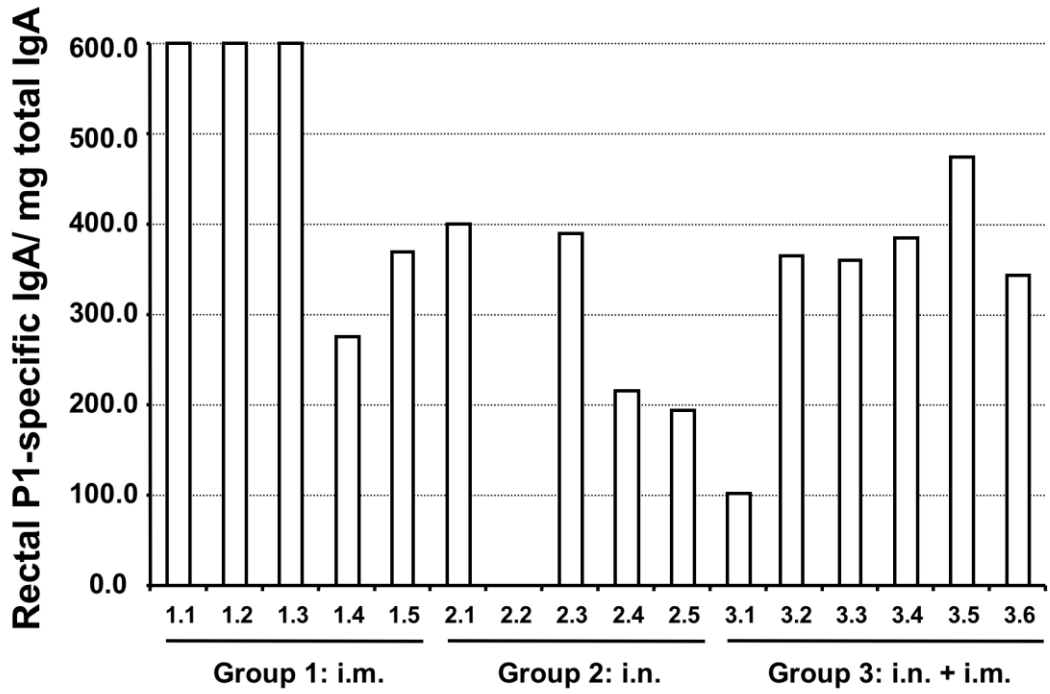


Figure S3

C



D

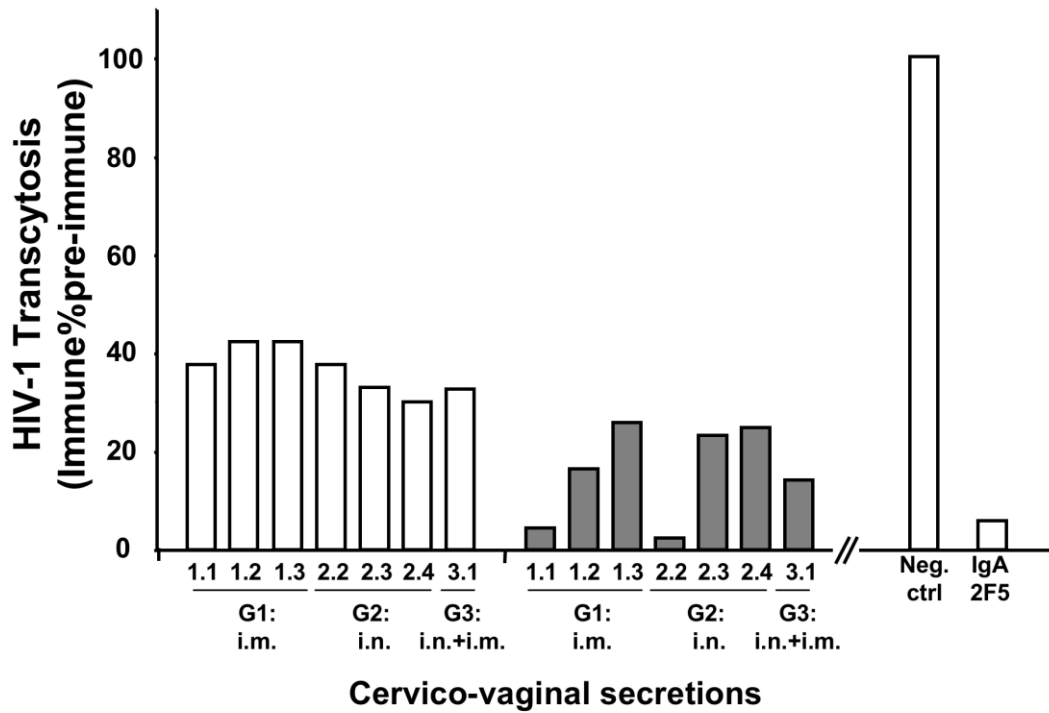


Figure S3

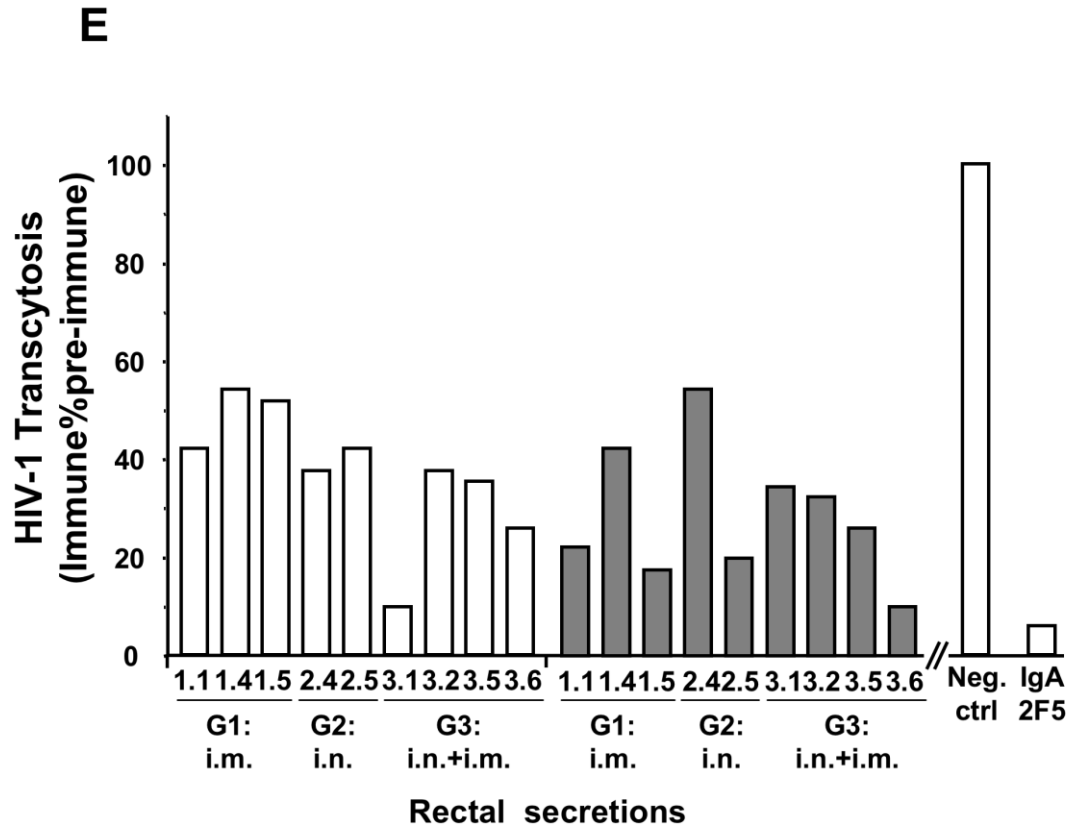


Figure S3

Figure legends

Figure S1: HIV-1 transcytosis-blocking, ADCC and neutralization activities in cervico-vaginal secretions after immunization with P1-and rgp41-virosomes: viremia positive versus viremia negative animals comparison.

For transcytosis-blockade, pre-immune (week 0) and immune (after the fourth immunization) (week 24) CVS at 1:6 dilutions from viremia positive (including blips with viremia <300 RNA copies /ml) and viremia negative animals were pre-incubated with PBMCs infected with primary HIV-1 clade B 93BR029 (Panel A) or clade C 92BR025 (Panel B) prior to addition to the luminal side of polarized epithelial cell monolayers. Transcytosis inhibition is defined by the ratio of neutralization observed in presence of immune CVS related to that observed in presence of pre-immune CVS (week 24% week 0). Values are the mean of at least 2 independent experiments.

For ADCC, pre-immune and immune (after the fourth immunization: week 24) CVS at serial dilutions from 1:3 to 1:27 were tested comparatively for P1- (Panel C) and rgp41- (Panel D) specific ADCC activity as described in Experimental Procedures. Results are expressed as ADCC-specific lysis. Values are the mean of at least 2 independent experiments.

For neutralization, pre-immune and immune (after the fourth immunization: week 24) CVS (1:6 dilution) from viremia positive (including blips with viremia <300 RNA copies /ml) and viremia negative animals were compared for their neutralizing activity against infection of human CD4+T cells by JR-CSF R5 tropic HIV-1 as described in Experimental Procedures (Panel E). Results are expressed as specific neutralization defined by as the ratio of neutralization observed in presence

of immune CVS related to that observed in presence of pre-immune CVS (week 24% week 0). Values are the mean of at least 2 independent experiments.

For all functional tests, statistical analyses were performed first by the Kruskal-Wallis test; pairwise comparisons were performed by the Mann-Whitney U-test, the *p*-values are presented in each panel.

Figure S2: HIV-1 transcytosis-blocking, ADCC and neutralization activities in cervico-vaginal secretions after immunization with P1-and rgp41-virosomes: persistently infected versus protected seronegative animals comparison.

Tests have been performed on week 24 and week 0 samples as indicated in figure SI legend except that analyses compared persistently infected (seropositive) versus seronegative protected animals.

Figure S3 : P1-specific immunogenicity in cervico-vaginal and rectal secretions after virosome-P1 immunization in Rhesus monkey and HIV-1 transcytosis-blocking activity.

Panel A, B and C: P1-specific immunogenicity

Cervico-vaginal secretion IgG (**panel A**), IgA (**panel B**), and rectal secretion IgA (**panel C**) specific for P1 were evaluated by P1-specific ELISA after the last immunization. Results are expressed as P1 specific antibody / mg of total antibody.

Panel D, E : Mucosal secretions of P1-vaccinated macaques block transcytosis of HIV-1 clade B and C.

Pre-immune and immune (after the fourth immunization) cervico-vaginal secretions (**panel D**, dilution 1:6) and rectal secretions (**panel E**, dilution 1:6) from Group 1 (immunized 4X intra-muscular), Group 2 (immunized 4X intra-nasal) and Group 3 (immunized 2X intra-muscular/2X intra-nasal) animals were pre-incubated with PBMCs infected by primary HIV-1 clade B 93BR029 (open bars) or clade C 92BR025 (closed bars) prior to addition to the luminal side of polarized epithelial cell monolayers. After 2 hour contact with the mucosal barrier at 37°C, transcytosis was evaluated in the basal compartment by measuring the amount of p24 translocated and expressed as transcytosis in presence of cervico-vaginal secretion % transcytosis in presence of pre-immune cervico-vaginal secretion. 2F5 IgA (1 µg/mL) was used as positive control. The experiment shown is representative of at least two independent experiments.

Table legends

Table S1. Evaluation of antibodies toward the P1 and rgp41 antigens in each animal after the second immunization, at week 8

Systemic and mucosal responses to P1 and rgp41 in female *Macaca mulatta* monkeys were analyzed after the second vaccination with virosome-rgp41 and virosome-P1 (W8 compared to W0) by antigen-specific ELISA. For mucosal samples, results were expressed as specific IgA (or IgG) percent of total IgA or IgG in each sample so as to account for the variation of total Ig between each mucosal sample. Samples showing a specific signal at least twice the background

were considered positive. The number of animals with specific IgAs or IgGs is shown over the total number of animals in each group. n.a.: not applicable.

Table S2. Evaluation of antibodies toward the P1 and rgp41 antigens in each animal after the third immunization, at week 16

Systemic and mucosal responses to P1 and rgp41 in female *Macaca mulatta* monkeys were analyzed after the third vaccination with virosome-rgp41 and virosome-P1 (W16 compared to W0), as described in Table SI figure legend.

Table S1

Sample		Serum			CVS			RS		
		1: CT	2: i.m.	3: i.m.+i.n.	1: CT	2: i.m.	3:i.m.+i.n.	1: CT	2: i.m.	3:i.m.+i.n.
Monkey goup										
P1	IgA	0/6	2/6	3/5	0/6	0/6	0/5	n.a.	n.a.	n.a.
	IgG	0/6	1/6	1/5	0/6	0/6	0/5	n.a.	n.a.	n.a.
rgp41	IgA	0/6	0/6	0/5	0/6	2/6	3/5	n.a.	n.a.	n.a.
	IgG	0/6	3/6	3/5	0/6	3/6	1/5	n.a.	n.a.	n.a.

Table S2

Sample		Serum			CVS			RS		
		1: CT	2: i.m.	3: i.m.+i.n.	1: CT	2: i.m.	3:i.m.+i.n.	1: CT	2: i.m.	3:i.m.+i.n.
Monkey goup										
P1	IgA	0/6	3/6	3/5	0/6	0/6	0/5	0/6	5/6	3/5
	IgG	0/6	1/6	1/5	0/6	1/6	1/5	0/6	0/6	0/5
rgp41	IgA	0/6	0/6	0/5	0/6	2/6	3/5	0/6	2/6	0/5
	IgG	0/6	5/6	5/5	0/6	2/6	3/5	0/6	0/6	0/5

Supplemental Experimental Procedures

The immunogenicity of virosome-P1 was investigated in female Chinese rhesus monkeys (*Macaca mulatta*). The aim of this study was to determine the best administration route for the induction of systemic and mucosal antibodies against P1 peptide in the vaginal and rectal mucosa. Therefore, four groups of animal were vaccinated as follows: Groups 1, 2, and 3 received the virosome-P1, with 40 µg of P1 per virosome dose, whereas Group 4 received the placebo virosome. Group 1 and 4 received 4 intra-muscular immunizations, Group 2 four intra-nasal immunizations and Group 3 two intra-nasal followed by two intra-muscular immunizations. Animals were vaccinated at weeks 0, 4, 12 and 24. Blood and cervico-vaginal and rectal secretions were collected one week following the immunization date.