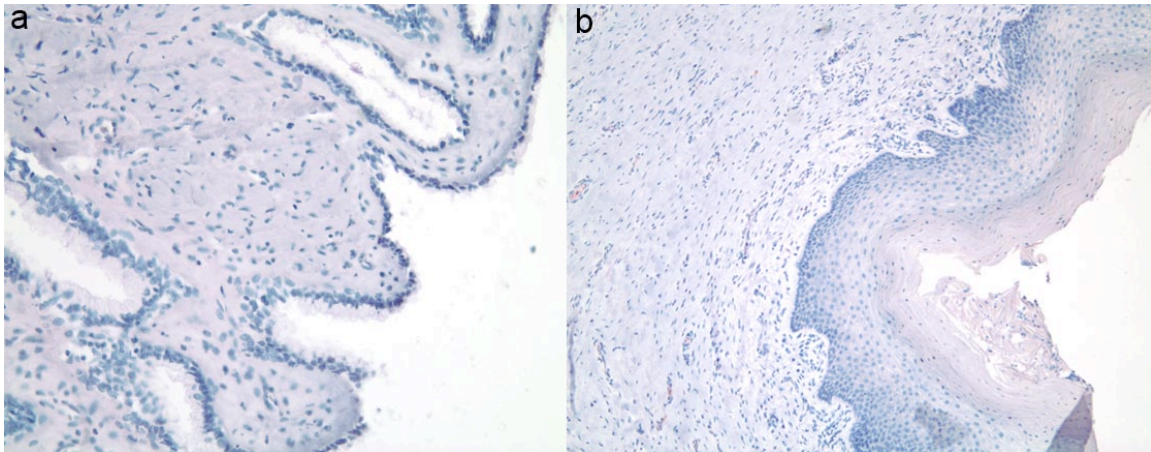


## Supplemental Information

### Supplemental Figure 1.

IgG staining of cervix (a) and vagina from a SIV-negative unvaccinated animal. IgG<sup>+</sup> plasma cells are at low density compared to vaccinated animals (compare with vaccinated animals, Fig. 3a, b and Fig. 6a, b).



## Supplemental Figure 2.

Soluble trimeric gp41 (sgp41t) construct used in RIHC to detect trimeric gp41 antibodies in cells. **(a, b)** Synthesis and schematic structure of gp41 (modified from refs. 18 and 33). The recombinant gp41 ectodomain was synthesized in vitro using a pCMV plasmid that included gp160 residues 554-676 (SIV<sub>mac239</sub> numbering), flanked N-terminally by an IgK signal sequence to target the protein to the ER for glycosylation and secretion from transfected 293F cells; and C-terminally by a strep tag for affinity purification, using StrepTactin resin, and detection by RIHC with a monoclonal antibody to the strep tag. More strongly conserved residues (red fill) and less conserved residues (no fill) estimated from a clustalW alignment using a selection of 21 HIV and SIV Env sequences. Residues belonging to the immunodominant clusters I and II (33) are enclosed by thick red circles. The recombinant purified protein eluted at 66KDa by size exclusion chromatography, consistent with a trimeric quaternary structure, likely the 6-helix bundle as previously solved by X-ray crystallography and NMR (18) shown in the inset. **(c)** Recombinant gp41 protein was bound to ELISA plates and used to capture the monoclonal IgG antibodies whose specificities in WB are shown in Fig. 2b and indicated below the graph in this figure **(c)**. Anti-SIVgp41 monoclonal antibodies (3.8F, 4.9C and 1.7H) bound to gp41t, but no binding was observed with a gp120 antibody or an HIV gp41 cluster I antibody F240 (shown as absorbance 450nm).

## Supplemental Figure 2

a

### sgp41t expression and purification

gp41:



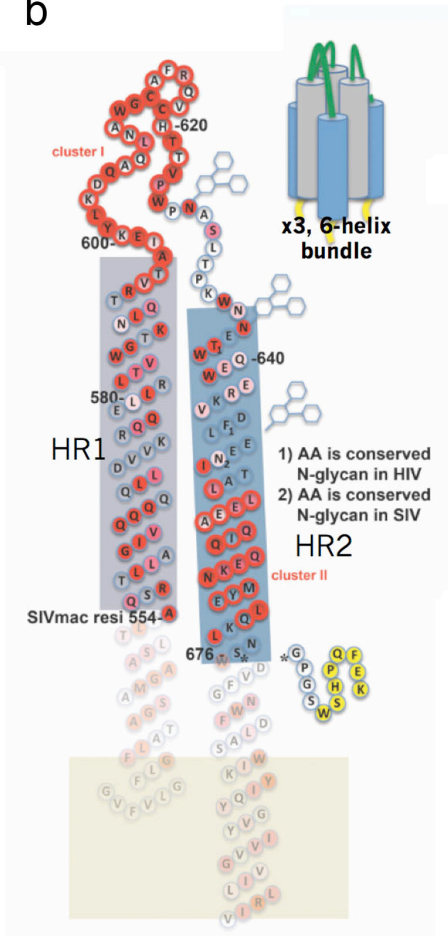
sgp41 construct:



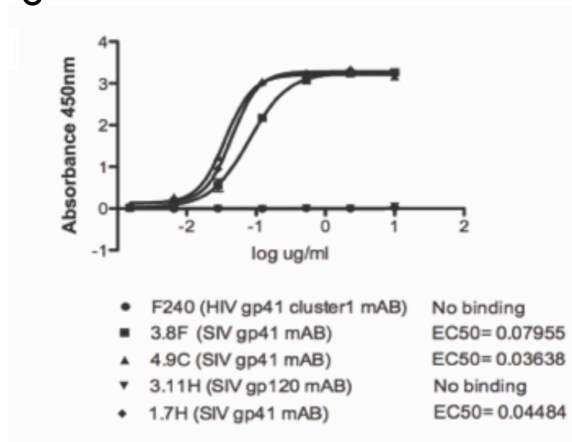
- 293F secretion via Igk leader allows N-linked glycosylation

- Construct contains immunodominant epitopes cluster I and II (in red)
- Affinity purified via strep tag and size exclusion
- Forms a trimer (by size exclusion) consistent with the post-fusion 6 helix bundle conformation solved by X-ray crystallography

b

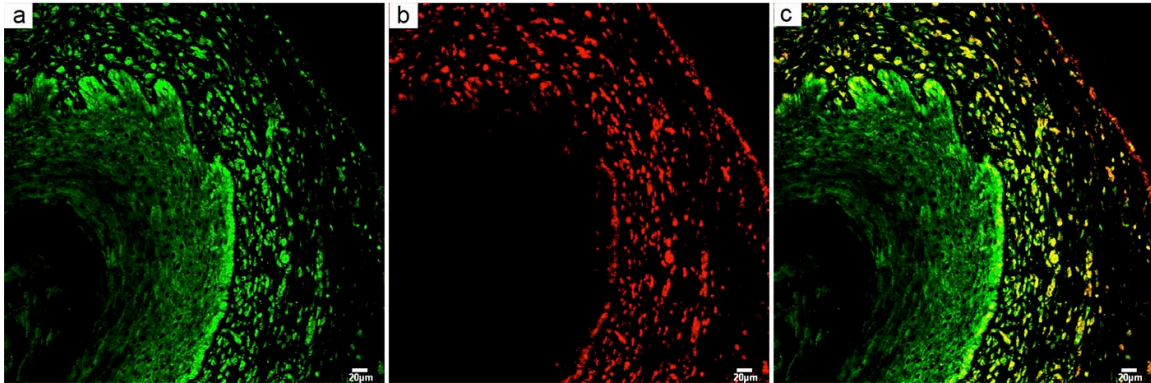


c



### Supplemental Figure 3.

CD138<sup>+</sup>IgG<sup>+</sup> submucosal plasma cells beneath vaginal epithelium at 20 weeks. a. IgG<sup>+</sup> (green) cells in the submucosa and IgG<sup>+</sup> FcRn<sup>+</sup> basal vaginal epithelium (see Fig. 6). b. CD138<sup>+</sup> plasma cells only in submucosa. c. Merged confocal image of IgG<sup>+</sup>CD138<sup>+</sup> plasma cells.



**Supplemental Table 1.** IgG and IgA concentrations in cervical tissue extracts and serum.

Values shown for four individual animals each at 5 and 20 weeks along with averages

and ratios of serum to cervical tissue extracts and IgG to IgA in cervix.

	<b>Cervical Tissue IgG (mgm/ml)</b>	<b>Serum IgG (mgm/ml)</b>	<b>Serum Cervix Ratio IgG</b>	<b>Cervix 20w/5w Ratio</b>	<b>Cervix IgA (mgm/ml)</b>	<b>Cervix IgG/IgA Ratio</b>
<b>Animal</b>						
5w A08-124	0.040	3.31			0.0004	
5w A09-457	0.071	3.18			0.0002	
5w A09-458	0.057	3.90			0.0002	
5w A09-569	0.032	3.77			0.0002	
<b>5 w Average</b>	0.050	3.54	70.1		0.00025	200
20w A08-264	0.026	4.07			0.0004	
20w A10-68	0.052	4.20			0.0002	
20w A10-69	0.082	3.82			0.0004	
20w A10-125	0.052	3.66			0.0004	
<b>20 w Average</b>	0.053	3.94	74.4	1.06	0.00035	150