

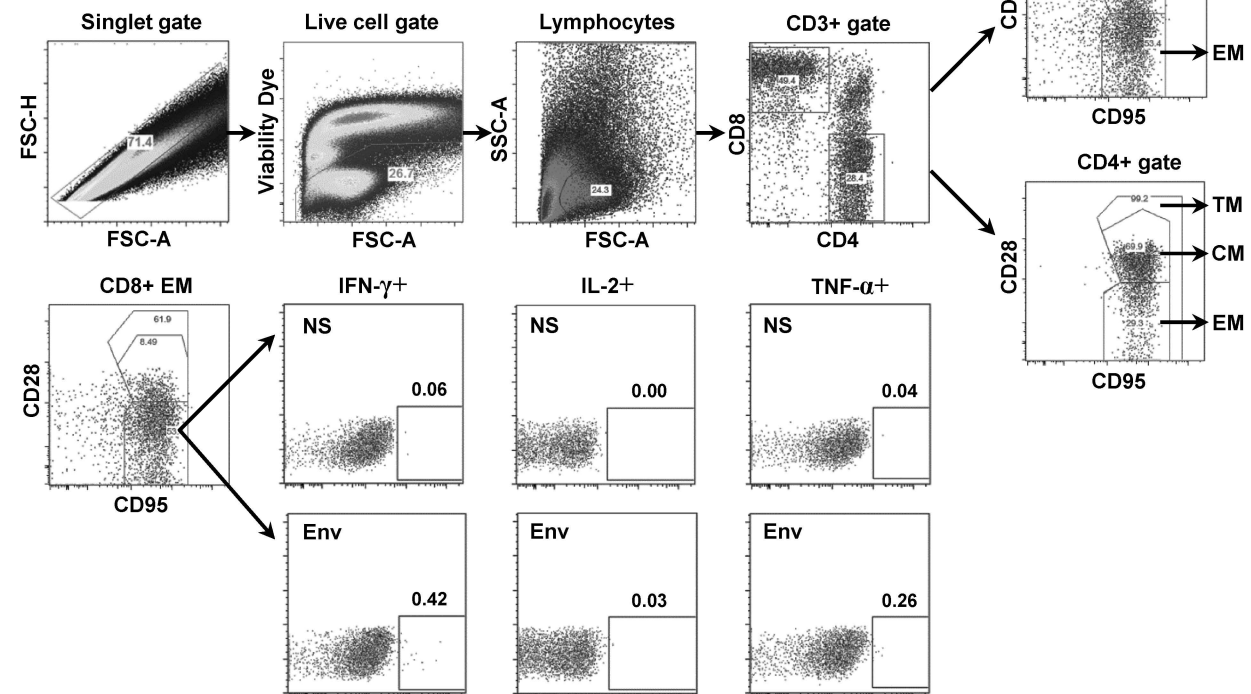
Supplemental Figure Legends.

Figure S1. Representative ICS staining of BAL and rectal tissue. Gating and staining for individual cytokines with no stimulation (NS) or with Env peptide stimulation (Env) are shown.

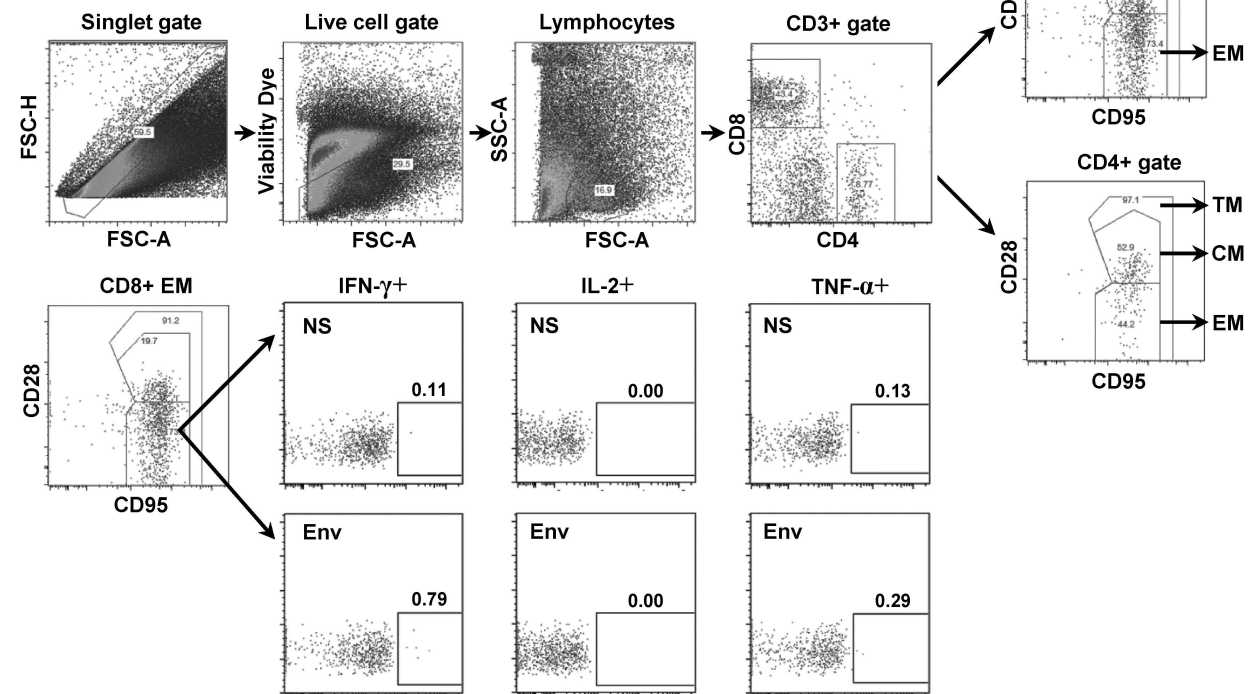
Figure S2. Evidence of infection with one or two variants following low-dose rectal challenge. Phylogenetic tree of viral inoculum (black) and sequences from each control animal (colored) representing full-length *env* sequences obtained by SGA at day 7 (d7) or day 14 (d14) post challenge. For 8 out of 9 animals only a single viral lineage was identified. For animal G402 (green), there are two distinct viral lineages as well as evidence of viral recombination.

Figure S3. Kaplan-Meier curves illustrating percent uninfected macaques versus number of SIV_{mac251} challenges for individual immunization groups and controls.

(A) Representative ICS data from BAL at wk28 (animal G408, IN/IT)



(B) Representative ICS data from Rectal sample at wk28 (animal G401, IR)



1:500 i.r.

0.002

G419 d14 1/1

G423 d7 1/1

G402 d14 1/2

Recombinants

G421 d7 1/1

G420 d14 1/1

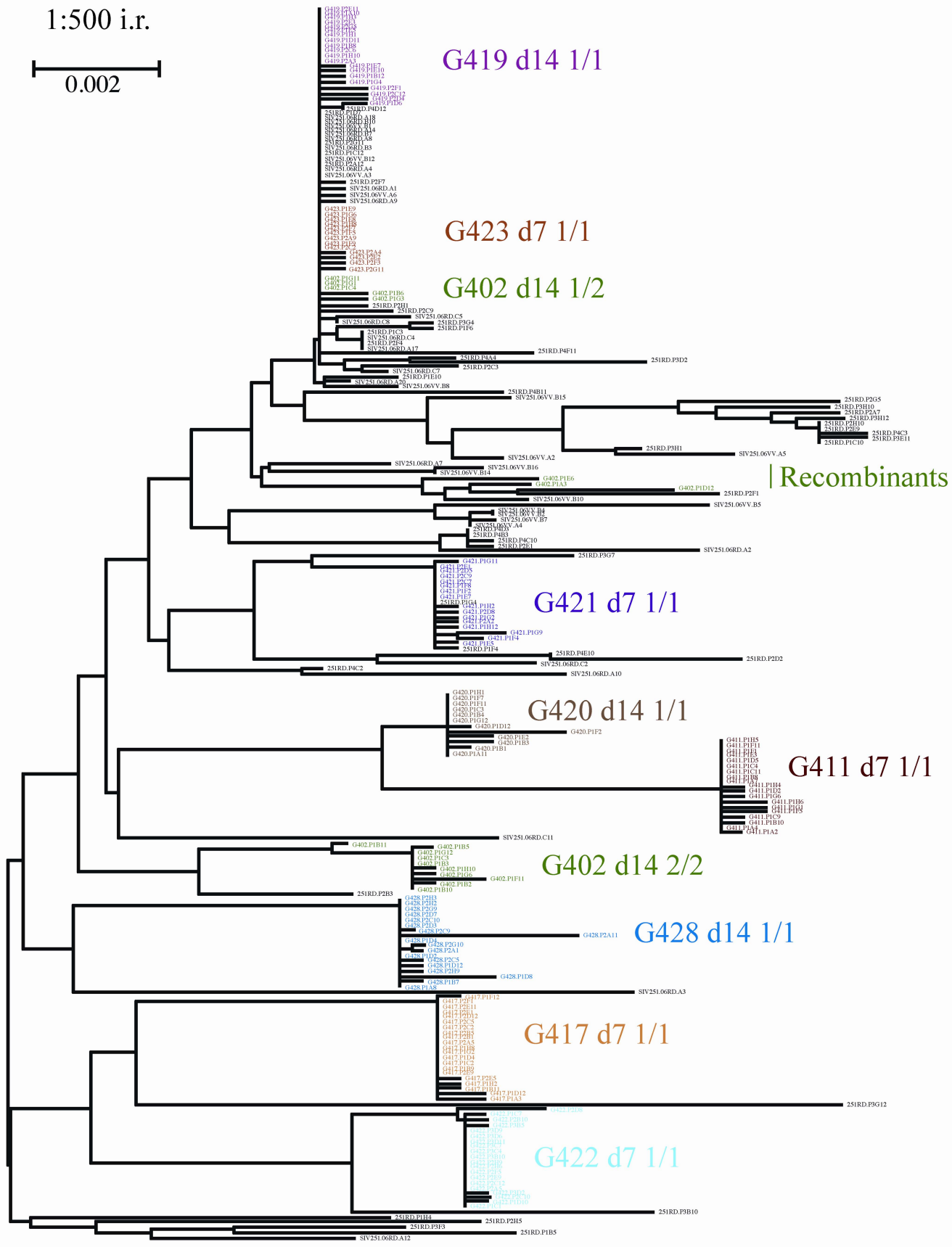
G411 d7 1/1

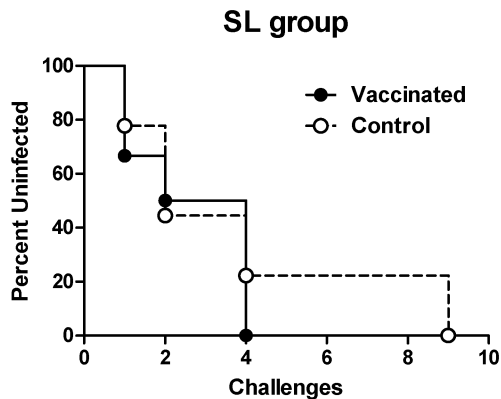
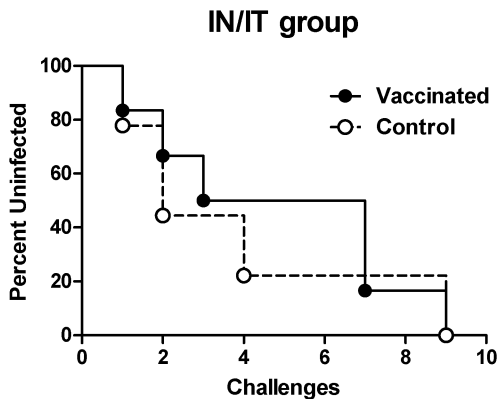
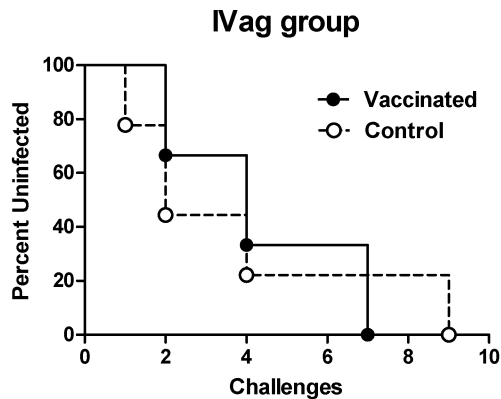
G402 d14 2/2

G428 d14 1/1

G417 d7 1/1

G422 d7 1/1



A.**B.****C.****D.**