Supporting Information

Spragg and Emerman 10.1073/pnas.1316839110

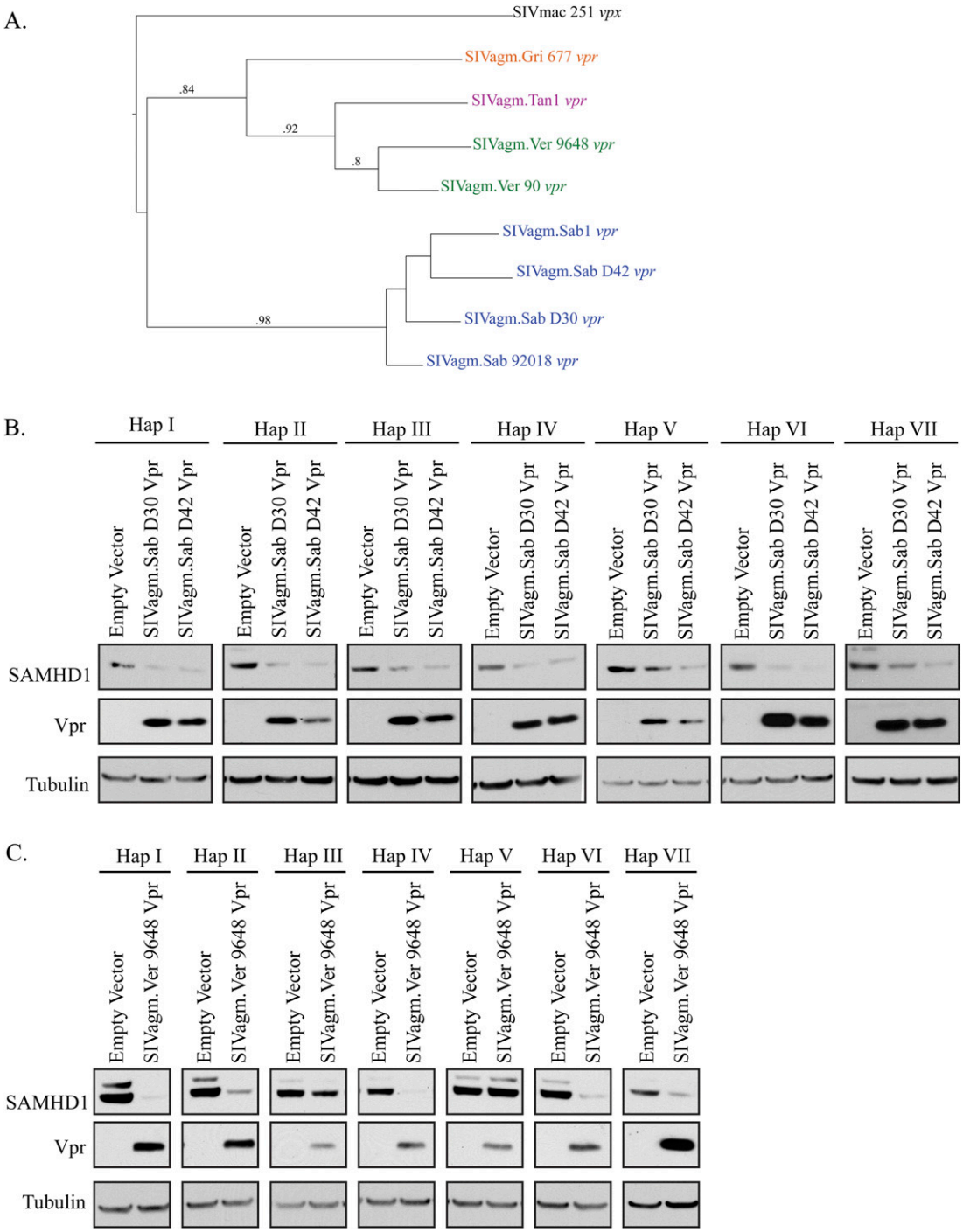

Fig. S1. Multiple African Green Monkey simian immunodeficiency virus (SIVagm) Vpr proteins of independent origins exhibit identical patterns of antagonism. (*A*) SIVagm *vpr* sequences cluster with their lineage, and lineages are distinct from one another. SIVagm *vpr* sequences were aligned using MUltiple Sequence Comparison by Log-Expectation (MUSCLE) (1) and subjected to phylogenetic analysis using PhyML (2) to construct a maximum likelihood tree with 100 bootstrap replicates using the General Time Reversible (GTR) model and four gamma rate categories. (*B*) Western blot analysis of 293T cells cotransfected with HA-tagged AGM SAMHD1 and either FLAG–Vpr or empty vector. The SIVagm Vpr proteins were tested for their ability to degrade seven AGM SAMHD1 variants. Vpr proteins from the SIVagm.Sab D30 and D42 strains have the ability to cause degradation of AGM SAMHD1 variants encoded by all seven

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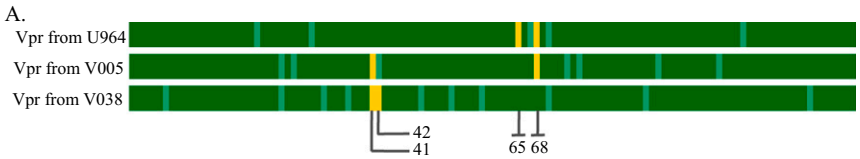
haplotypes. This pattern of specificity is shared by SIVagm.Sab1 and SIVagm.Sab92018 Vpr proteins, which have the capacity to fully or partially (haplotype VII) degrade all AGM SAMHD1 variants. (*C*) Western blot analysis of 293T cells cotransfected with HA-tagged AGM SAMHD1 and either FLAG-Vpr from SIVagm. Ver9648 or empty vector. SIVagm.Ver9648 does not degrade the SAMHD1 variants encoded by haplotypes III and V but degrades all other variants. This phenotype is identical to SIVagm.Ver90 Vpr specificity.

1. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32(5):1792–1797.
2. Guindon S, et al. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59(3):307–321.



Animal	Vpr Mutation	Number of clones
U964	A65T M68T	17 of 19
V005	K41N	4 of 21
V038	K41X	5 of 21
V038	L42V	12 of 21

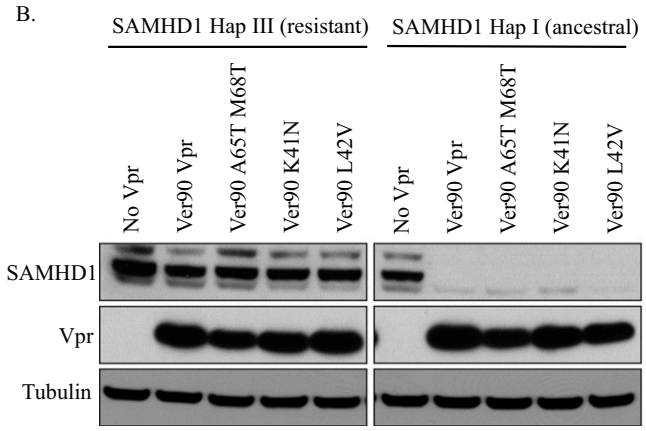


Fig. S2. Mutations acquired in SIVagm.Ver90 *vpr* after 6 mo to 1 y of replication in three sabaeus monkeys expressing a resistant SAMHD1 variant. Sabaeus monkeys U964, V005, and V038 were genotyped, and two were found to be homozygous for *SAMHD1* haplotype III, which is resitant to SIVagm.Ver Vpr. The third (V005) is heterozygous for this resistant *SAMHD1* and for the sensitive haplotype II. In a prior study (1), these three animals were experimentally infected with virus collected from a naturally infected vervet monkey, SIVagm.Ver90. *Vpr* sequences were amplified by RT-PCR from the inoculating sera and from plasma samples collected from the experimentally infected animals at both 6 mo and 1 y postinfection. Approximately 10 *vpr* clones per time point were sequenced, and sequence alignments were prepared using MUSCLE (2). (*A*) A schematic of the *vpr* gene and the mutations acquired in each animal. Light green bars denote changes seen in only one or two clones. Yellow bars denote changes observed in over two clones out of ~20. Table shows specific changes observed in *vpr*. A dominant *vpr* clone in the U964 animal contained two amino acid–altering mutations relative to *vpr* sequences of SIVagm.Ver90. These occur at amino acid positions 65 and 68, and most clones contained no additional amino acid alterations. The *vpr* sequences cloned from the other two animals contained numerous changes at amino acid positions 41 and 42, and again the majority of clones with these changes contained no other amino acid variation. Further, position 41 of *vpr* from virus in the V038 animal exhibited five separate amino acid alterations, but none were observed more than once. (*B*) SIVagm. Ver90 Vpr point mutants representing all commonly observed clones were tested both for a gain of ability to degrade the AGM SAMHD1 haplotype III variant and for maintenance of function in degrading ancestral SAMHD1. The 293T cells were cotransfected with HA-tagged SAMHD1 and FLAG-tagged Vpr, and degradation was measured by Western blotting. Probing for tubulin serves as a loading control.

1. Goldstein S, et al. (2006) Comparison of simian immunodeficiency virus SIVagmVer replication and CD4+ T-cell dynamics in vervet and sabaeus African green monkeys. *J Virol* 80(10): 4868–4877.
2. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32(5):1792–1797.