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Supplemental information

Safety and efficiency modifications of SIV-based integrase-defective lentiviral vectors for immunization

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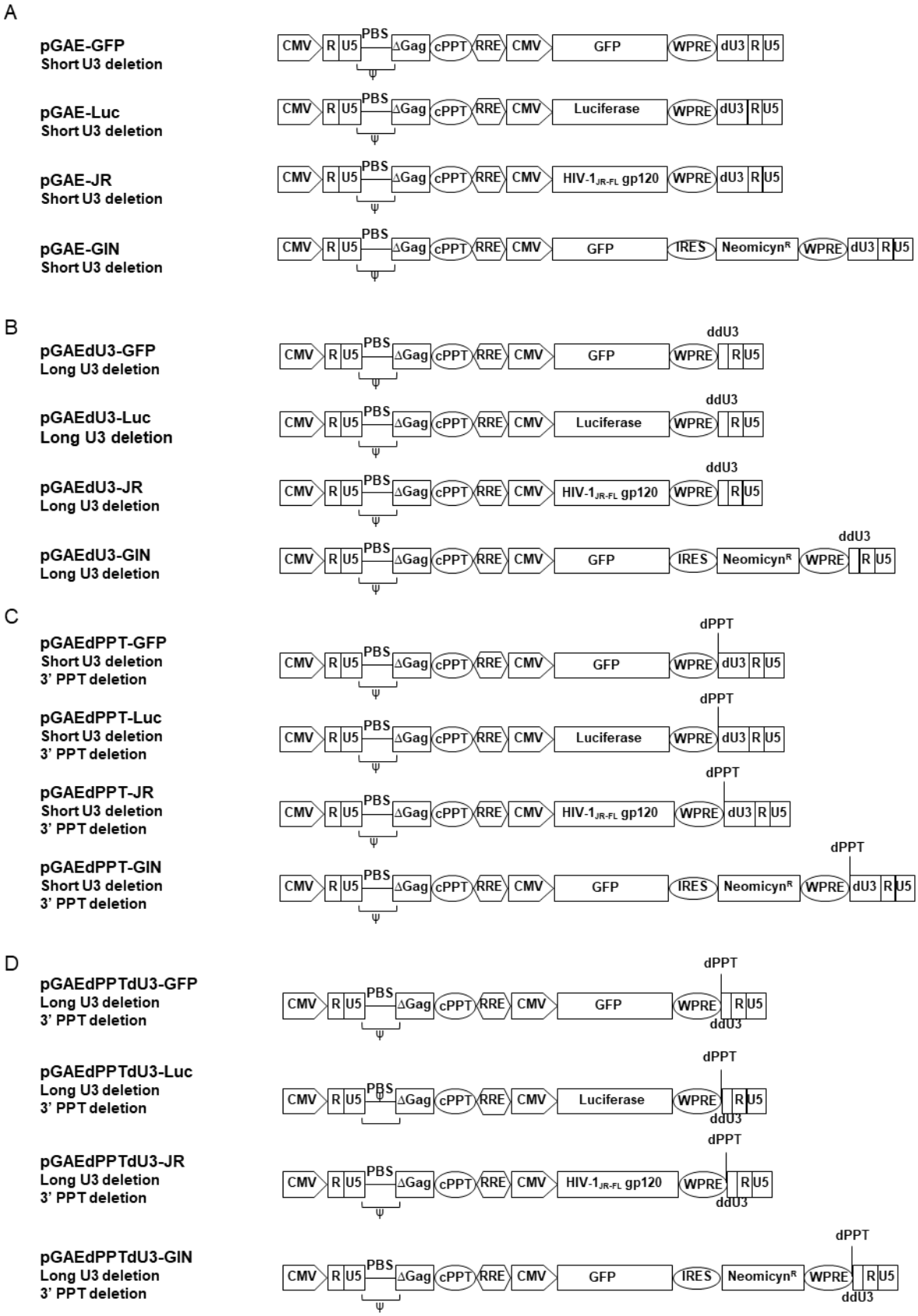
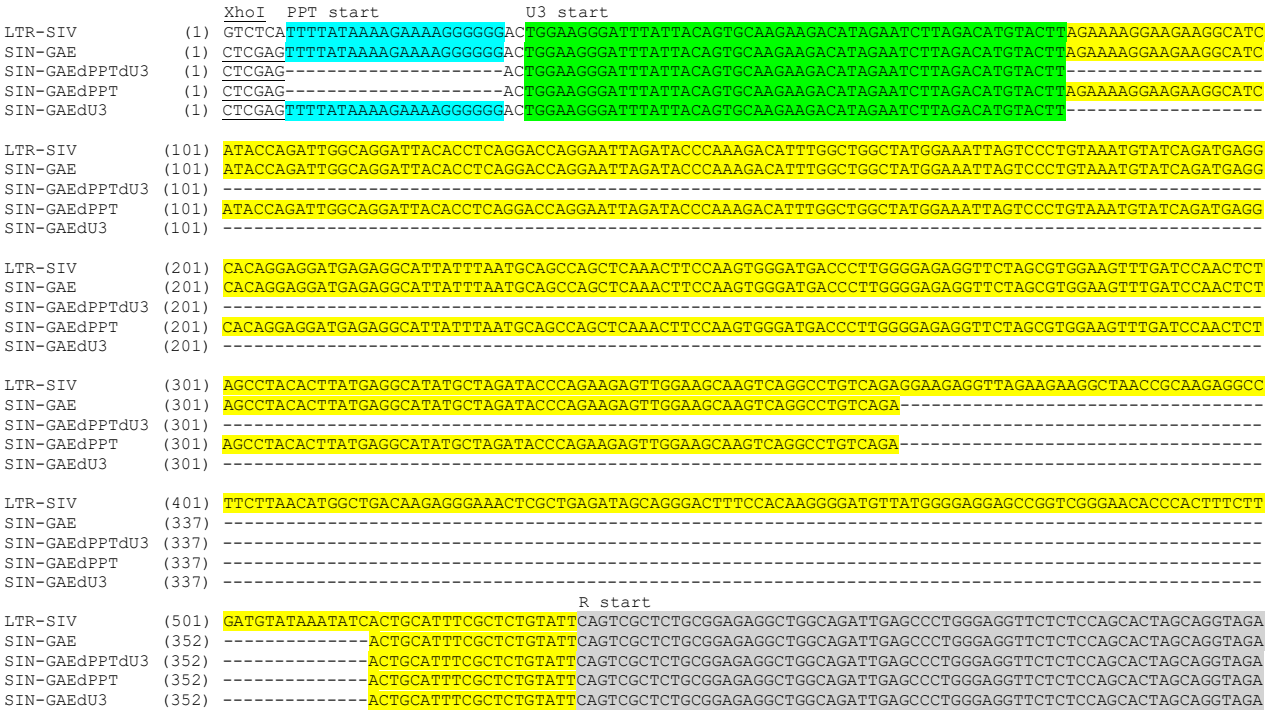


Figure S1. **Schematic representation of SIV-based self-inactivating (SIN) transfer vectors used in this study**. Lentiviral transfer vectors expressing GFP, Luciferase, HIV-1_{JR-FL} gp120 Envelope and the NeoR gene from (A) parental SIN-GAE, (B) SIN-GAEdU3, with indicated extended U3 deletion (ddU3), (C) SIN-GAEdPPT, with indicated deletion of 3' PPT (dPPT), and (D) SIN-GAEdPPTdU3, containing both dPPT and ddU3 deletions. CMV, cytomegalovirus immediate-early promoter; R, repeat element; U5, 5' untranslated region; U3, 3' untranslated region; PBS, primer binding site; SD, splice donor site; Ψ, packaging signal; cPPT, central polypurine tract; RRE, Rev response element; SA, splice acceptor site; IRES, internal rimbosomal entry site; dU3, SIN deletion in U3 region of 3' LTR; ddU3, extended SIN deletion in U3 region of 3' LTR; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element. See Methods for details on construction.



Light Blue: 3' PPT sequence in SH (full-length $SIV_{SIVmac251}$ 3' LTR) and self-inactivated (SIN) GAE and GAEdU3 plasmids.
Green: U3 nucleotides in all transfer vector plasmids
Yellow: U3 nucleotides in SH (full-length $SIV_{SIVmac251}$ 3' LTR) and self-inactivated (SIN) GAE and GAEdPPT plasmids.
Grey: beginning of Repeat region

Figure S2. **Sequence alignment of SIV full-length LTR (SH), and SIN-GAEs deleted or not in U3 and 3' PPT sequences**. SIV-LTR parental sequence is from SIVmac251, GenBank acc. no. M19499

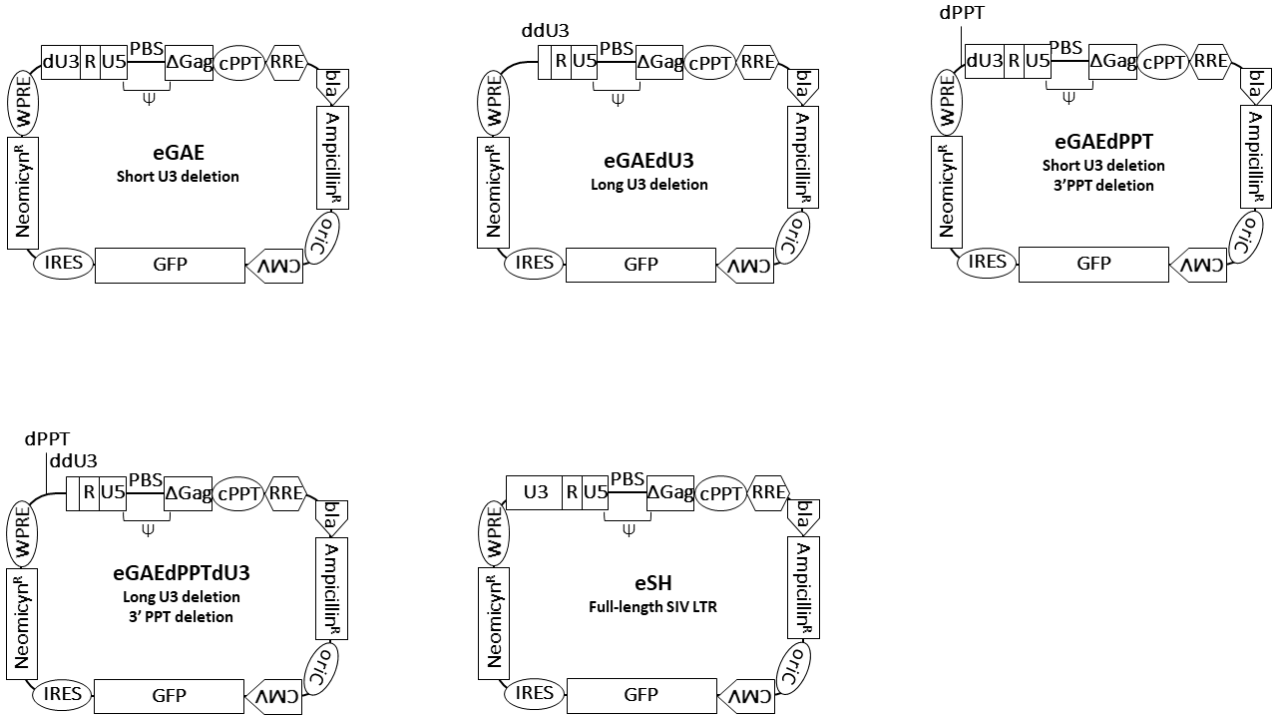


Figure S3. Schematic representation of episomal transfer vectors used in this study. Vectors cassette contain a single LTR, full length (eSH) or in SIN configuration (eGAE-X), the reporter GFP protein and the Neomycin resistance gene under the control of the cytomegalovirus immediate early promoter (CMV). Additional cis-acting elements include the Rev-response element (RRE), the central polypurine tract (cPPT), the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) and the 3' polypurine tract (PPT). The E. Coli Ori bacterial origin of replication (OriC) and the ampicillin resistance gene (Amp) are included for plasmid replication in bacterial cells. See Methods for details on construction.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Construction of transfer vector plasmids

A schematic depiction of the lentiviral transfer vectors is provided in supplemental **Figure S1**. A DNA sequence deleted in 3'PPT and containing the U3-R region of the LTR with extended deletion in the U3 region (deleted from –20 to –453 from the R region) was synthesized (GenScript, Piscataway, NJ, USA) and inserted in pUC57 plasmid to produce the pUC57-dPPTddU3 plasmid. A fragment of DNA from plasmid pUC57-dPPTddU3 was obtained after XhoI/XbaI digestion and inserted into the corresponding restriction sites from pGAE-GFP plasmid to obtain pGAEdPPTdU3-GFP plasmid.

A DNA sequence containing the U3-R region of the LTR with extended deletion in the U3 region (deleted from –20 to –453 from the R region) was synthesized (GenScript, Piscataway, NJ, USA) and inserted in pUC57 plasmid to produce the pUC57-ddU3 plasmid. A fragment of DNA from plasmid pUC57-ddU3 was obtained after XhoI/AvrII digestion and inserted into the corresponding restriction sites from pGAEdPPTdU3-GFP plasmid to obtain pGAEdU3-GFP plasmid.

Plasmid pGAEdPPT-GFP was obtained after amplification of dU3 from pGAE-GFP using a primer pair excluding the 3'PPT and containing suitable restriction sites for cloning into pGAE-GFP plasmid (Forward primer: G3-XhoI-For 5'-gactcgagactggaagggatttattac-3'; Reverse primer: G3-AvrII-Rev 5'-tacctaggtgggtaccatgctagggat-3'). The resulting PCR fragment was cloned into pCR2.1 plasmid (Invitrogen, Carlsbad, CA) to obtain pCR2.1-dU3-dPPT and sequenced to confirm identity. A fragment of DNA from plasmid pCR2.1-dU3-dPPT was obtained after XhoI/AvrII digestion and inserted into the corresponding restriction sites from pGAE-GFP plasmids to obtain pGAEdPPT-GFP plasmid.

Transfer vector plasmids expressing Luciferase were constructed by replacing the CMV-GFP plasmid region of pGAE-GFP, pGAEdU3-GFP, pGAEdPPT-GFP and pGAEdPPTdU3-GFP with a fragment of DNA containing the CMV-Luc region from plasmid pTY2CMV-Luc[1] using ClaI/XhoI restriction enzymes.

Transfer vector plasmids expressing HIV-JR-FL gp120 coding sequence were constructed by replacing the GFP plasmid region of pGAE-GFP, pGAEdU3-GFP, pGAEdPPT-GFP and pGAEdPPTdU3-GFP with a fragment of DNA containing JREnv from plasmid pTY2-JREnv[2] using AgeI/XhoI restriction enzymes.

Transfer vector plasmids expressing GFP-IRES2-Neo were constructed by replacing the GFP plasmid region of pGAE-GFP, pGAEdU3-GFP, pGAEdPPT-GFP and pGAEdPPTdU3-GFP with a fragment of DNA containing GFP-IRES-Neo from plasmid pGAE-GFP-IRES2-Neo[3] using AgeI/EcoRV restriction enzymes.

Construction of episomal transfer vector plasmids

A schematic depiction of the episomal lentiviral transfer vectors is provided in supplemental **Figure S3**. A fragment of plasmid DNA containing the E. coli bacterial origin of replication (OriC) and ampicillin-resistance gene (Amp) was inserted between the two LTRs of pGAE-GIN, pGAEdU3-GIN, pGAEdPPT-GIN and pGAEdPPTdU3-GIN plasmids upstream the CMV promoter by a three-way ligation to eliminate plasmid DNA backbone sequences to produce the pGAE-AmpOri-CMV-GFP-IRES-Neo (pGAE-AO-GIN), pGAEdU3-AmpOri-CMV-GFP-IRES-Neo (pGAEdU3-AO-GIN), pGAEdPPT-AmpOri-CMV-GFP-IRES-Neo, (pGAEdPPT-AO-GIN) and pGAEdPPTdU3-AmpOri-CMV-GFP-IRES-Neo (pGAEdPPTdU3-AO-GIN) plasmids, where all transfer vector plasmid contain the CMV promoter upstream the 5' U5-R region. These transfer vectors were used to produce IN defective lentiviral vectors that were used to transduce 293T Lenti-X cells. After transduction, infected cells were subjected to Hirt extraction[4] of circular vector genomes. Hirt extracted episomal DNA was subject to bacteria transformation, clone isolation and restriction digest analysis to isolate 1-LTR circular episomal transfer vectors eGAE, eGAEdU3, eGAEdPPT and eGAEdPPTdU3 expressing GFP and NeoR (**Figure S3**). Episomal transfer vector plasmid eGAE-AO-GIN maintains the 3' PPT and contains a short 149 base pair (bp) deletion in the U3 region of the LTR (deleted from –20 to –169 from the R region). Episomal transfer vector plasmid eGAEdPPTdU3, is deleted in the 3' PPT and contains a long 433 base pair (bp) deletion in the U3 region of the LTR (deleted from –20 to –453 from the R region). Episomal transfer vector plasmid eGAEdPPT is deleted in the 3' PPT and contains a short 149 base pair (bp) deletion in the U3 region of the LTR (deleted from –20 to –169 from the R region).). Episomal transfer vector plasmid eGAEdU3 maintains the 3' PPT and contains a a long 433 base pair (bp) deletion in the U3 region of the LTR (deleted from –20 to –453 from the R region). Plasmid containing one full length SIVMac251 LTR (eSH) was obtain by three-way ligation substituting SIN-LTR from eGAE with the corresponding fragment from pBK.1 plasmid (a gift from Dr. Suresh Arya) containing a full length SIVMac251 LTR.

SUPPLEMENTAL REFERENCES

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