

Engineered virus-like particles for transient delivery of prime editor ribonucleoprotein complexes in vivo

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Supplementary Information

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Supplementary Table 1. Sequences of pegRNAs, epegRNAs, and ngRNAs used in this study.

Supplementary Table 2. Sequences of primers used for genomic DNA amplification and amplicons analyzed with high-throughput sequencing.

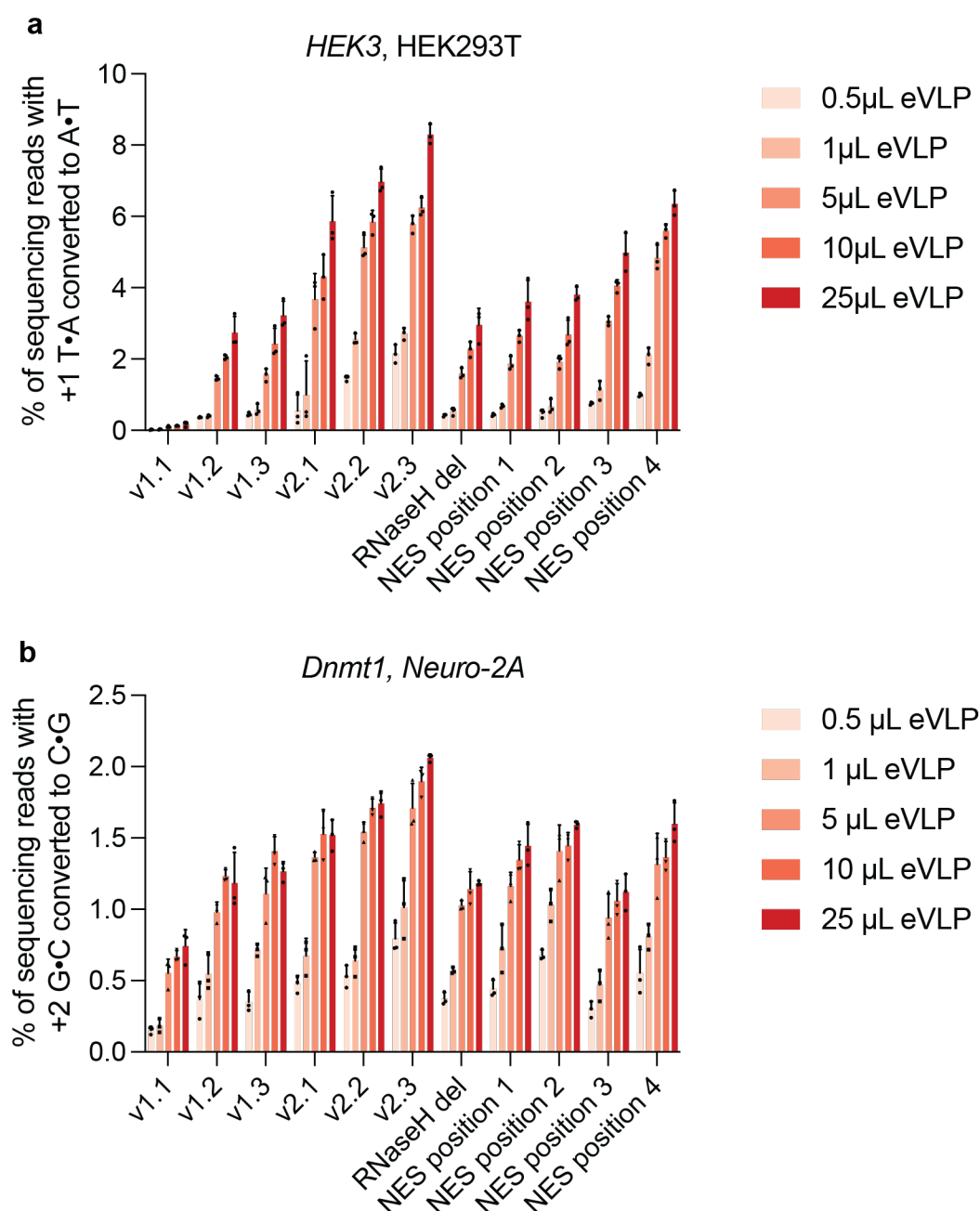
Supplementary Table 3. Sequences of primers used for RT-qPCR.

Supplementary Table 4. epegRNA and ngRNA optimization for *rd6* model correction.

Supplementary Table 5. epegRNA and ngRNA optimization for *rd12* model correction.

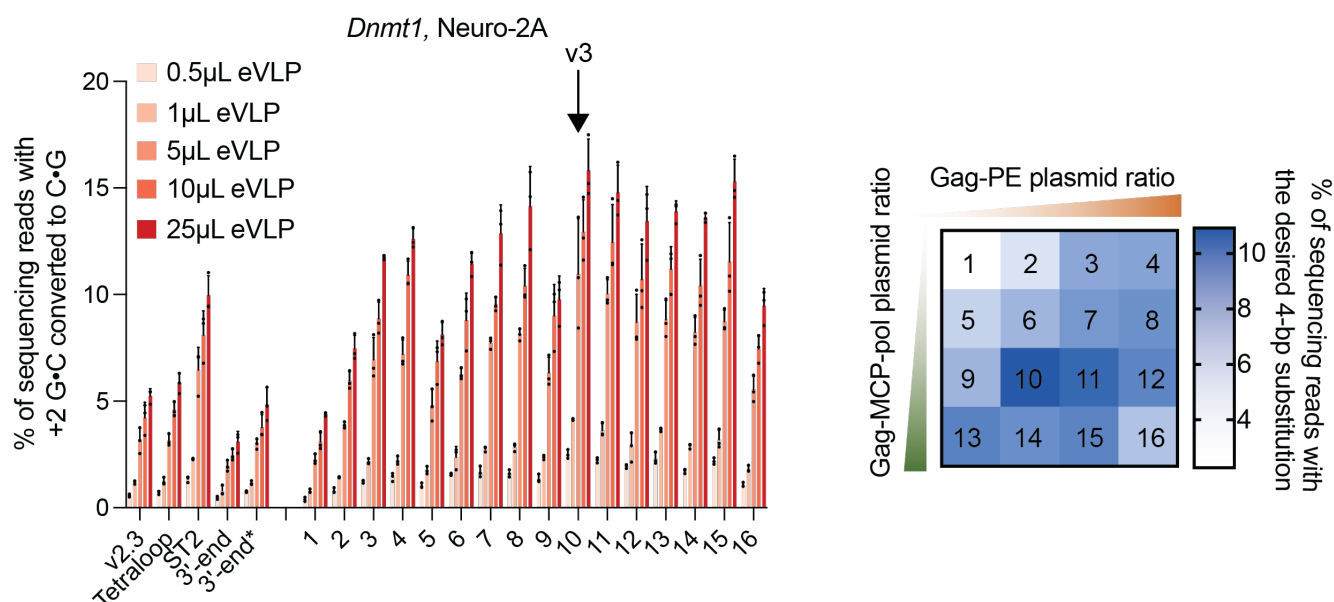
Supplementary Table 6. Sequences of top 10 CIRCLE-seq nominated OT sites for *rd6*.

Supplementary Table 7. Sequences of top 10 CIRCLE-seq nominated OT sites for *rd12*.



Supplementary Figure 1. Data showing all PE-eVLPs produced and tested in parallel for Fig. 1. a, Prime editing efficiencies of “v1.1”, “v1.2”, “v1.3”, “v2.1”, “v2.2”, “v2.3”, “RNaseH del”, “NES position 1”, “NES position 2”, “NES position 3”, “NES position 4” PE-eVLPs at *HEK3* locus in HEK293T cells. Fig 1b, 1d, 1f and 1g show a subset of data from this experiment. Fig. 1b shows results of “v1.1”, “v1.2” and “v1.3” PE-eVLPs fitted to four-parameter logistic curves using nonlinear regression. Fig. 1d shows results of “v1.3”, “RNaseH del”, and “v2.1” PE-eVLPs fitted to four-parameter logistic curves using nonlinear regression. Fig. 1f shows results from “v2.1”, “NES position 1”, “NES position 2”, “NES

position 3", "NES position 4", and "v2.3" PE-eVLPs fitted to four-parameter logistic curves using nonlinear regression. Fig. 1g shows results of "v1.1", "v1.3", "v2.1", "v2.2", and "v2.3" PE-eVLPs fitted to four-parameter logistic curves using nonlinear regression. **b**, Prime editing efficiencies of "v1.1", "v1.2", "v1.3", "v2.1", "v2.2", "v2.3", "RNaseH del", "NES position 1", "NES position 2", "NES position 3", "NES position 4" PE-eVLPs at *Dnmt1* locus in Neuro-2A cells. Fig 1b, 1d and 1g show a subset of data from this experiment. Fig. 1b shows results of "v1.1", "v1.2" and "v1.3" PE-eVLPs fitted to four-parameter logistic curves using nonlinear regression. Fig. 1d shows results of "v1.3", "RNaseH del", and "v2.1" PE-eVLPs fitted to four-parameter logistic curves using nonlinear regression. Fig. 1g shows results of "v1.1", "v1.3", "v2.1", "v2.2", and "v2.3" PE-eVLPs fitted to four-parameter logistic curves using nonlinear regression. Values shown in all graphs represent the average prime editing efficiency of three biological replicates and error bars represent the standard deviation.



Supplementary Figure 2. Data showing all PE-eVLPs produced and tested in parallel for Fig. 2. Prime editing efficiencies of “v2.3”, “Tetraloop”, “ST2”, “3'-end”, “3'-end*” PE-eVLPs, and PE-eVLPs 1-16 tested for stoichiometry optimization in Fig. 2d (number ID is shown on the right to match Fig. 2d and Supplementary Fig 2), at *Dnmt1* locus in Neuro-2A cells. Figs. 2c, 2d, and 2e show a subset of data from this experiment. Fig. 2c show prime editing efficiencies of “v2.3”, “Tetraloop”, “ST2”, “3'-end”, and “3'-end*” PE-eVLPs fitted to four-parameter logistic curves using nonlinear regression. Fig. 2d shows prime editing efficiencies of PE-eVLPs 1-16 at 5 μL dose, shown as heatmap. Fig. 2e shows prime editing efficiencies of “v2.3” PE-eVLP and PE-eVLP 10 (which is v3 PE-eVLPs) fitted to four-parameter logistic curves using nonlinear regression. Values shown in all graphs represent the average prime editing efficiency of three biological replicates and error bars represent the standard deviation.

Supplementary Note 1. epegRNAs have reduced binding affinity to Cas9 compared to sgRNAs.

We previously observed that the apparent affinity of Cas9 for sgRNAs, pegRNAs, and epegRNAs decreases in that order¹. The reduced binding affinity can be attributed to the addition of a 3' extension in pegRNAs and an additional structured 3' end motif in epegRNAs that adds steric hindrance to the Cas9 binding. As a result, while the enhanced stability of epegRNAs improves prime editing efficiencies compared to pegRNAs, epegRNAs might be improved further by enhancing their binding affinity to Cas9.

Supplementary Note 2. MCP insertion does not impair eVLP production.

To first assess whether incorporation of an MCP domain into gag-pol would disrupt the formation of eVLPs, we transfected the producer cells with a 3:1 ratio of wild-type gag-pol plasmid to gag-MCP-pol plasmid, as well as the rest of the components for PE-eVLP production. Prime editing efficiency of a 4-bp substitution at *Dnmt1* locus in N2A cells was maintained upon the addition of gag-MCP-pol, indicating that the MCP fusion did not significantly impair eVLP production (Extended Data Fig. 2c).

Supplementary Note 3. Western blot analysis of protein content in the producer cell lysates and successive generations of PE-eVLPs.

To assess whether the P4-PE construct indeed improves prime editor expression in producer cells, we transfected eVLP producer cells with gag-pol and cargo fusion plasmids (gag-PE or P4-PE), and performed western blots of producer cell lysates 48 h after transfection. We indeed observed that P4-PE protein levels were substantially higher than gag-PE levels in producer cells (Extended Data Fig. 4a), consistent with our hypothesis that the smaller P4-PE fusion protein improved protein production levels. We also performed western blot analysis on lysed v1.3, v2.3, v3 and v3b PE-eVLPs to assess the amount of cargo packaged in each generation of PE-eVLPs. Consistent with the PE-eVLP protein quantification results from ELISA, we observed improvement in the prime editor protein packaging over successive generations of PE-eVLPs from v1.3 to v3 (Extended Data Fig. 4d). Comparing v3 and v3b PE-eVLPs, we did not observe notable differences in the prime editor protein packaging, consistent with the comparable editing efficiencies observed for v3 and v3b PE-eVLPs at multiple target sites tested (Fig. 3e). The advantage in producer cell expression levels offered by the v3b PE-eVLP architecture may be partially counteracted by less efficient cargo recruitment via P3-P4 coiled-coil peptide interaction compared to the direct gag-PE fusion in v3 PE-eVLPs. Nonetheless, these findings confirmed that the v3b PE-eVLP architecture improves cargo expression in the producer cells and mediates cargo packaging into eVLPs with comparable efficiency to the v3 PE-eVLP architecture.

Supplementary Sequence 1. PEmax with a 6-amino acid deletion at the C-terminus of MMLV RT.

Key: NLS-Cas9 nickase (R221K, N393K, H840A)-linker-MMLV RT (6 amino acid del)-NLS-cmycNLS

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Supplementary Sequence 2. Gag-PE in v3 PE3-eVLPs.

Key: Gag(MA+p12 domain)-3xNES-Gag(CA+NC domain)-linker-engineered protease
cleavage site-linker- PEmax with 6 amino acid deletion at C-terminus of MMLV RT (NLS-
Cas9 nickase (R221K, N393K, H840A)-linker-MMLV RT (6 amino acid del)-NLS-cmycNLS)

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GCCCTGCTGCAGACCCTGGGAAACCTGGGATATAGGGCATCCGCCAAGAAGGCCCAGATCTGTCAGAAGC
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Supplementary Sequence 3. Gag-MCP-pol in v3 PE-eVLPs.

Key: Gag-linker-MCP-linker-pol

ATGGGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCAGTGGAAAGATGTCGAGCGGATCGCTCAC
AACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTC
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CAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACCTGGACCGACCGGTGGTACCTCACC
CTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGAACCTAGAACCTCGCTGGAAAGG
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TAG

Supplementary Sequence 4. P4-PE in v3b PE-eVLPs.

Key: P4-linker-3xNES-engineered protease cleavage site-linker- PEmax with 6 amino acid deletion at C-terminus of MMLV RT (NLS-Cas9 nickase (R221K, N393K, H840A)-linker-MMLV RT (6 amino acid del)-NLS-cmycNLS)

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A

Supplementary Sequence 5. Gag-P3-pol in v3b PE-eVLPs.

Key: Gag-linker-P3-linker-pol

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TAG

Supplementary Sequence 6. Gag-COM-pol in v3b PE-eVLPs.

Key: Gag-linker-COM-linker-pol

ATGGGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCAGTGGAAAGATGTCGAGCGGATCGCTCAC
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Supplementary References

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