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*Supplementary Materials*

# An Amphipathic Cell-Penetrating Peptide-Aided Delivery of Cas9 RNP for Gene Editing and Correction

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**Table S1.** Single guide RNA sequences used in this work.

| Target | sgRNA targeting sequence |
|---|---|
| Stoplight construct | GGACAGUACUCCGCUCGAGU |
| HDR stoplight construct | GCUUACUUGUACAGCUCGUCC |
| CCR5 | UGACAUCAAUUAUUAUACAU |
| Non-target sgRNA | GUUAAUGUGGCUCUGGUUCU |

**Table S2.** PCR primers used for amplification of the Stoplight and *CCR5* loci for both T7E1 and TIDE experiments.

| Primer name | Sequence 5′- 3′ |
|---|---|
| Stoplight Forward | GAAGGGCGAGATCAAGCAGA |
| Stoplight Reverse | GGTCTTGTAGTTGCCGTCGT |
| *CCR5* Forward | CAACAGAGCCAAGCTCTCCAT |
| *CCR5* Reverse | CCTGGGAGAGACGCAAACAC |

**Table S3.** Single stranded HDR DNA template sequence. DNA mismatches encoding the mutation, are specified with small letter.

| HDR template name | Template sequence 5′-3′ |
|---|---|
| HDR stoplight 81bp | ACGACGCCCGTGAAAAGCTCTTCACCCTTAGACACGGCTTgCTTGT ACAGCTCGTCCAaGCCGCCCGTAGAATCCCTGCCT |
| Alexa 647 labeled HDR template | /Alex647N/ ACGACGCCCGTGAAAAGCTCTTCACCCTTAGACACGGCTTGCTTG TACAGCTCGTCCAAGCCGCCCGTAGAATCCCTGCCT |

**Table S4.** crRNA sequence used in this research.

| Target | crRNA sequence |
|---|---|
| HPRT | CCUGACAAUCGAUAGGUACCGUUUUAGAGCUAUGCU |

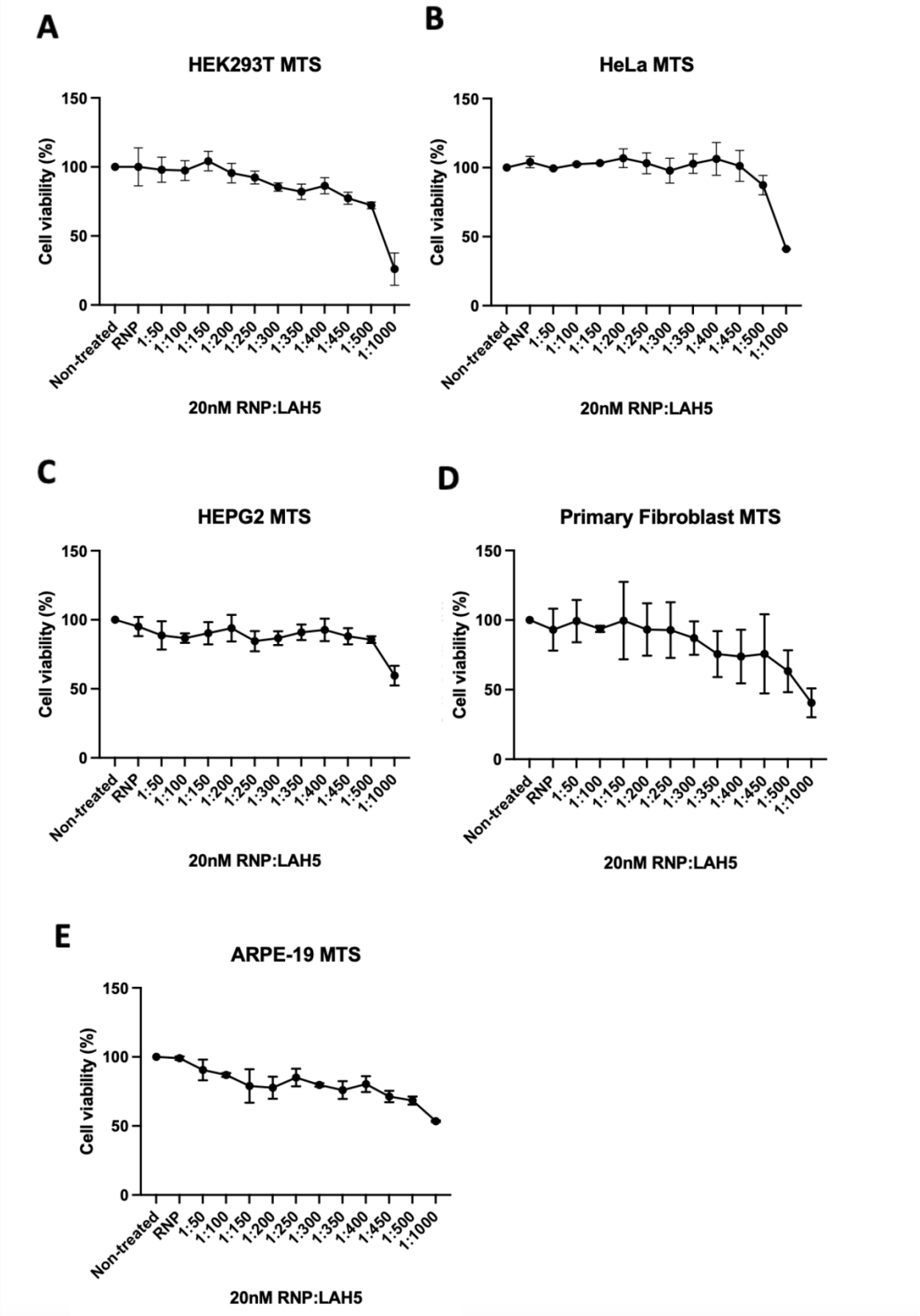
A
HEK293T MTS
Cell viability (%)
150
100
50
0
Non-treated
RNP
1:50
1:100
1:150
1:200
1:250
1:300
1:350
1:400
1:450
1:500
1:1000
20nM RNP:LAH5
B
HeLa MTS
Cell viability (%)
20nM RNP:LAH5
C
HEPG2 MTS
Cell viability (%)
20nM RNP:LAH5
D
Primary Fibroblast MTS
Cell viability (%)
20nM RNP:LAH5
E
ARPE-19 MTS
Cell viability (%)
20nM RNP:LAH5

**Figure S1.** Cytotoxicity study using MTS assay on multiple cell lines: **(A)** HEK293T, **(B)** HeLa, **(C)** HEPG2, **(D)** Primary fibroblast, and **(E)** ARPE-19. RNP/LAH5 nanocomplexes were prepared at 1:50 to 1:1000 M/M ratios 20nM RNP per well in 96-well plates. The medium was then replaced with 100 µl of 20nM RNP complexed with increasing concentrations of LAH5 peptide contained Opti-MEM. 24 h after treatment, an MTS assay was performed. Non-treated cells were used as the negative control and Triton X-100 (1%) treated cells were used as a positive control. The bars are shown as mean ± SD (n = 3).

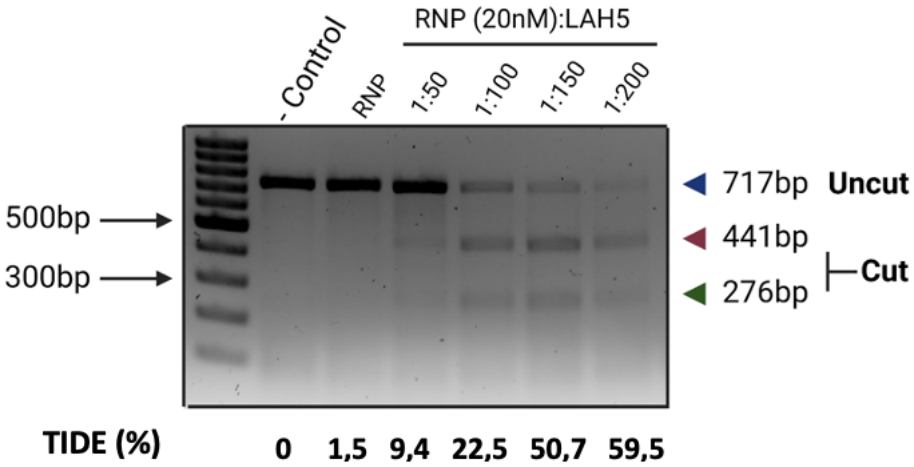


**Figure S2.** The efficiency of gene editing was assessed using ARPE-19 cell line, and the *CCR5* gene was chosen as a target. Percentage indel measurements in *CCR5 locus* DNA isolated from RNP/LAH5 treated ARPE-19 cells and % of gene editing calculated by TIDE and T7E1 assay (uncut, 717 bp; cut, 441 bp 276 bp; Cas9, 20 nM; *CCR5* sgRNA, 20 nM; increasing ratios of LAH5).

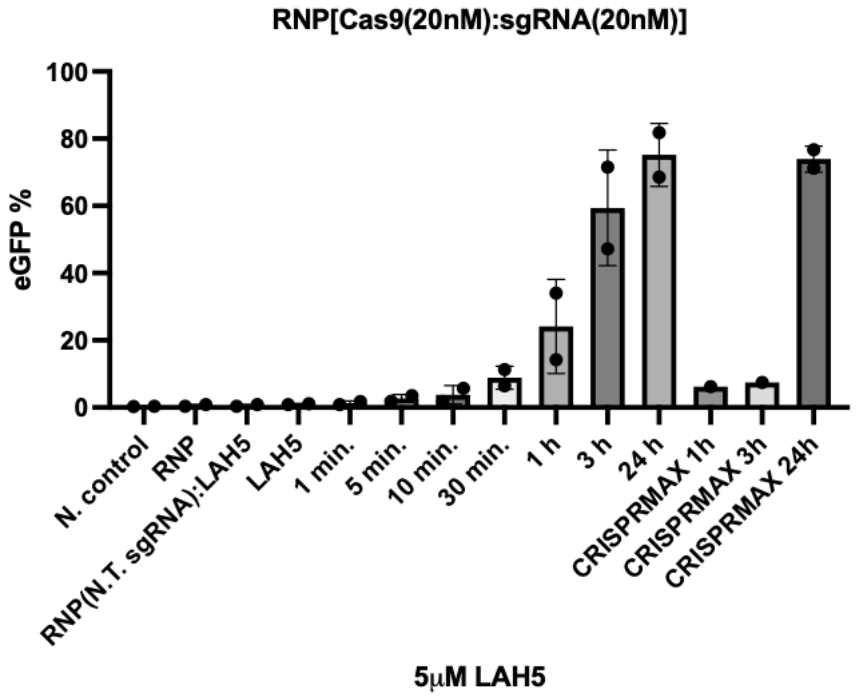


**Figure S3.** HEK293T stoplight cells were treated with RNP/LAH5 nanocomplexes during different incubation times with 20nM RNP and LAH5 (5μM) peptide. 48h following the transfection gene editing efficiency (eGFP+ cells) was calculated by flow cytometry Data are presented as mean ± SD (n = 2).

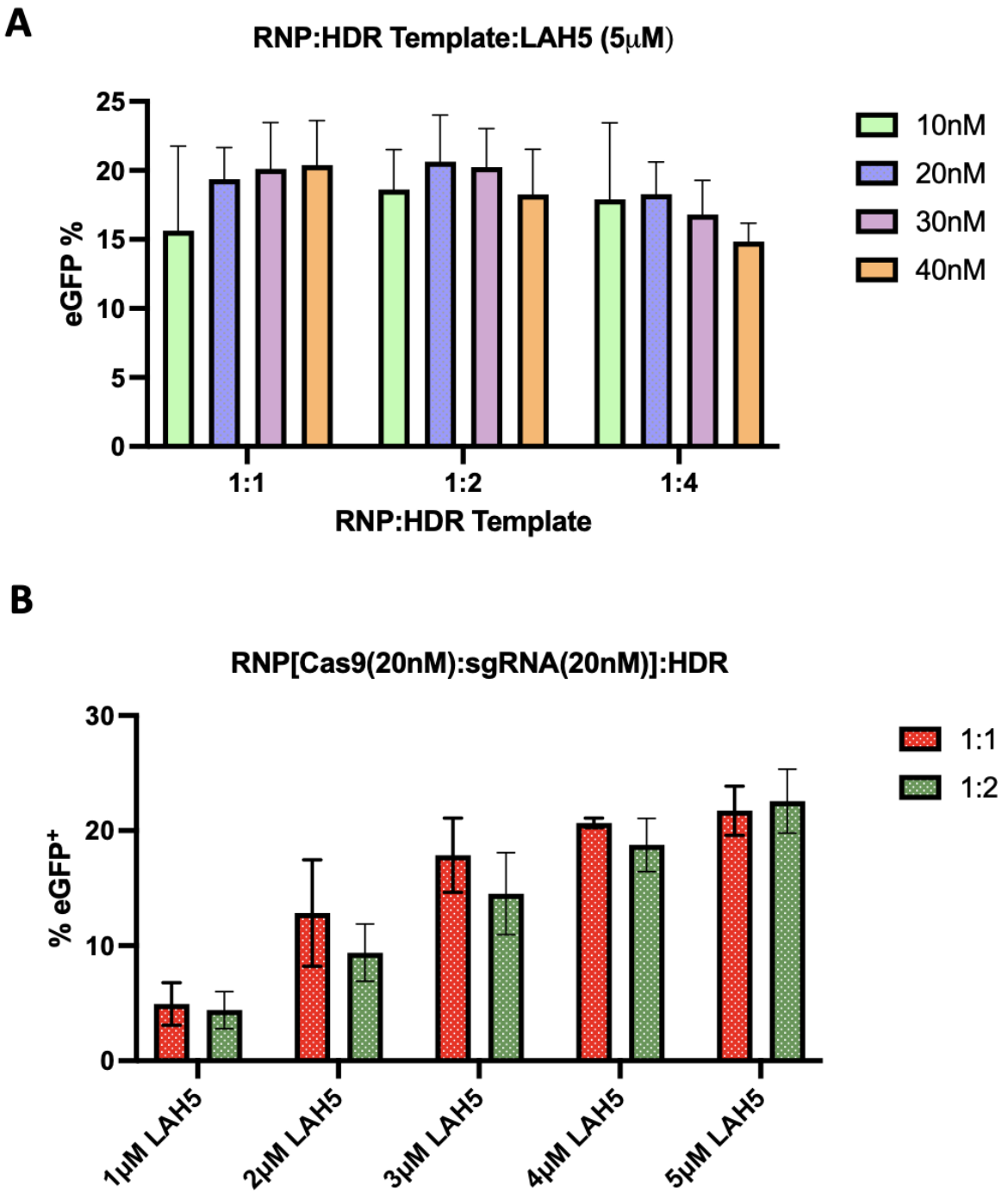


**Figure S4.** LAH5 peptide-mediated gene correction efficiency using the HDR stoplight reporter system (**A)** To evaluate the impact of increasing concentrations of RNP/HDR template HEK293T HDR stoplight cells were treated with a range of 10nM to 40nM RNP/HDR template at 1:1, 1:2 and 1:4 ratios. These various RNP/HDR template concentrations were complexed with 5μM of LAH5 peptide. HDR efficiency was quantified by flow cytometry depending on % of eGFP positive expression (**B)** LAH5 peptide dose-dependent effects on gene correction were tested by treating the cells RNP/HDR template/LAH5 nanocomplexes, which were prepared using RNP (20nM)/HDR template (20nM) and RNP (20nM)/HDR template (40nM), and increasing concentration of LAH5 peptide range at 1uM to 5uM). Data presented as mean ± SD (n = 3).

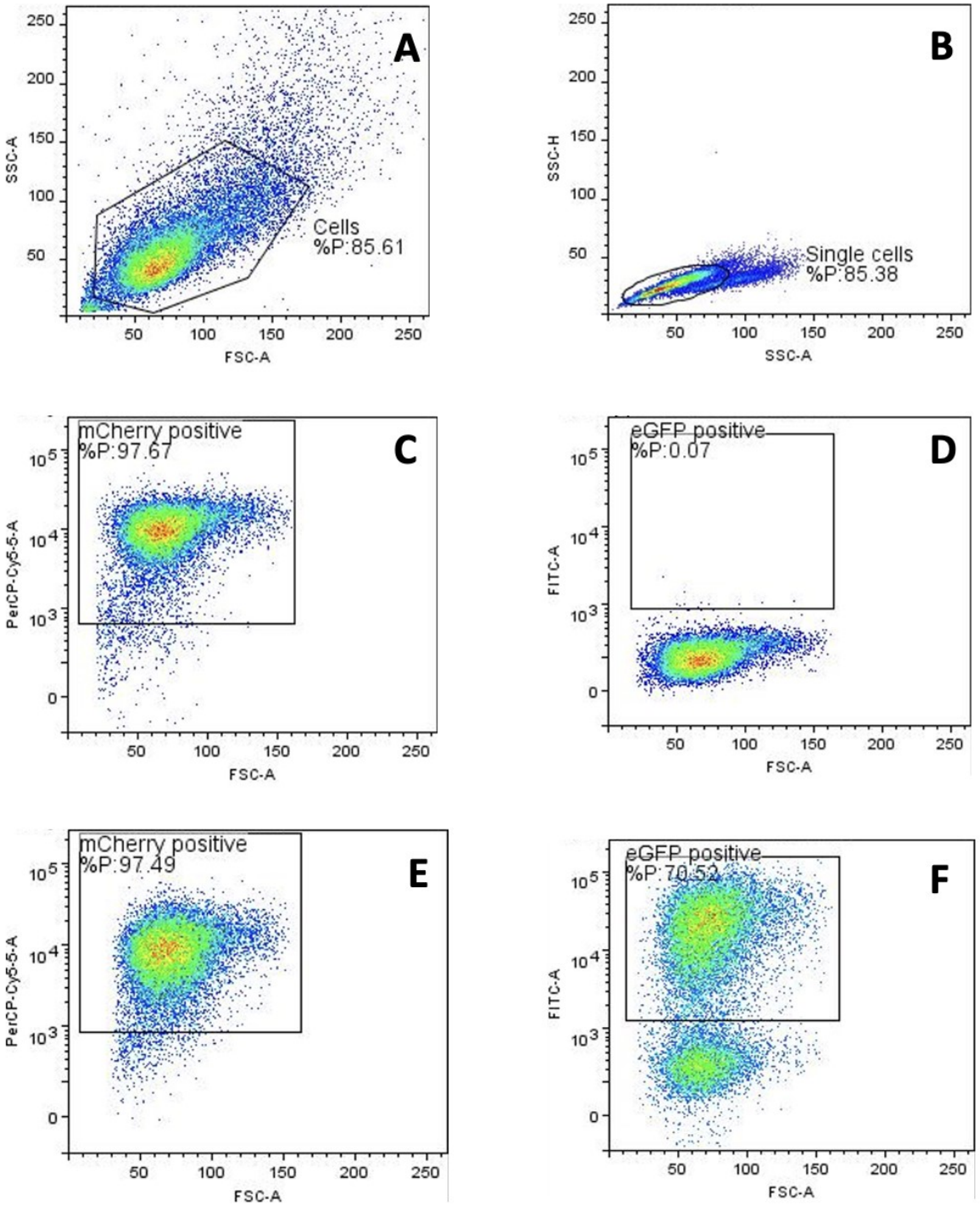


**Figure S5.** (A) and (B) gating strategy to select single cells recruited in all flow cytometry experiments using HEK293T reporter cells (stoplight and HDR stoplight). Then, in negative control samples (C) mCherry positive cells are determined and gated using mCherry signals. (D) eGFP positive cells calculated within mCherry positive cell population FSC-A and eGFP positive signals. The gating strategy of the cell samples transfected 1:200 ratio of RNP/LAH5 nanocomplexes shown at (E) mCherry positive and (F) eGFP positive.

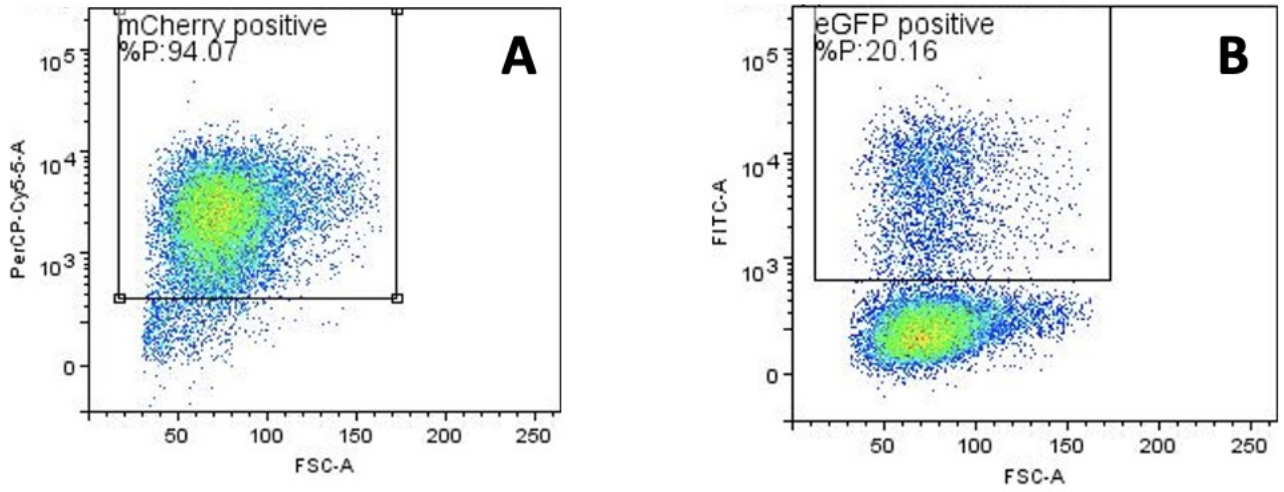


**Figure S6.** The gating strategy represented HE 293T HDR stoplight cells treated with RNP/HDR template/LAH5 nanocomplexes prepared at 1:1:250 ratio **(A)** selection of mCherry positive population and **(B)** eGFP positive gene-corrected cells.