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## Supplemental Information

# A New Tool for CRISPR-Cas13a-Based Cancer Gene Therapy

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**Supplementary Data**

Table S1. Guide sequences of Cas13a for interference experiments.

| Name | Guide sequence | PFS |
|---|---|---|
| No transcript guide | TAGATTGCTGTTCTACCAAGTAATCCAT | N/A |
| EGFP guide | CTGTTGTAGTTGTACTCCAGCTTGTGCC | C |
| mCherry guide | CCTCGAAGTTCATCACGCGCTCCCACTT | C |
| hTERT guide | CCCTCTTTTCTCTGCGGAACGTTCTGGC | C |
| hEZH2 guide | CGGCTTTCTTTATCATCTCGGTGATCCT | C |
| hRelA guide | CCTCTTTCTGCACCTTGTCACACAGTAG | A |
| mTERT guide | ACACGGTCCTTCCCCGGGCTGCTGCGGA | C |
| mEZH2 guide | CCGTCCTTTTTCAGTTGTATCTTTCTGC | C |
| mRelA guide | GGAAAGATGAGGGGAAACAGATCGTCCA | C |
| hTER guide | CCCTCTTTTCTCTGCGGAACGTTCTGGCGAAACACCGATTTAGACTACCCC AAAAACGAAGGGGACTAAAACCGGCTTTCTTTATCATCTCGGTGATCCTG AAACACCGATTTAGACTACCCCAAAAACGAAGGGGACTAAAACCCTCTTT CTGCACCTTGTCACACAGTAG | |
| mTER guide | ACACGGTCCTTCCCCGGGCTGCTGCGGAGAAACACCGATTTAGACTACCC CAAAAACGAAGGGGACTAAAACCCGTCCTTTTTCAGTTGTATCTTTCTGCG AAACACCGATTTAGACTACCCCAAAAACGAAGGGGACTAAAACGGAAAG ATGAGGGGAAACAGATCGTCCA | |

Note: h,human; m, mouse.

Table S2. Oligonucleotides used to construct pDCUg vectors.

| Name | Sequence (5′→3′) |
|---|---|
| NT-F | AGGAAGACTAAAACTAGATTGCTGTTCTACCAAGTAATCCATTTTTGCGTCTTCGA |
| NT-R | TCGAAGACGCAAAAATGGATTACTTGGTAGAACAGCAATCTAGTTTTAGTCTTCCT |
| EGFP-F | AGGAAGACTAAAACCTGTTGTAGTTGTACTCCAGCTTGTGCCTTTTGCGTCTTCGA |
| EGFP-R | TCGAAGACGCAAAAGGCACAAGCTGGAGTACAACTACAACAGGTTTTAGTCTTCCT |
| mCherry-F | AGGAAGACTAAAACCCTCGAAGTTCATCACGCGCTCCCACTTTTTTGCGTCTTCGA |
| mCherry-R | TCGAAGACGCAAAAAAGTGGGAGCGCGTGATGAACTTCGAGGGTTTTAGTCTTCCT |
| hTERT-F | AGGAAGACTAAAACCCCTCTTTTCTCTGCGGAACGTTCTGGCTTTTGCGTCTTCGA |
| hTERT-R | TCGAAGACGCAAAAGCCAGAACGTTCCGCAGAGAAAAGAGGGGTTTTAGTCTTCCT |
| hEZH2-F | AGGAAGACTAAAACCGGCTTTCTTTATCATCTCGGTGATCCTTTTTGCGTCTTCGA |
| hEZH2-R | TCGAAGACGCAAAAAGGATCACCGAGATGATAAAGAAAGCCGGTTTTAGTCTTCCT |
| hRelA-F | AGGAAGACTAAAACCCTCTTTCTGCACCTTGTCACACAGTAGTTTTGCGTCTTCGA |
| hRelA-R | TCGAAGACGCAAAACTACTGTGTGACAAGGTGCAGAAAGAGGGTTTTAGTCTTCCT |
| mTERT-F | AGGAAGACTAAAACACACGGTCCTTCCCCGGGCTGCTGCGGA TTTTGCGTCTTCGA |
| mTERT-R | TCGAAGACGCAAAATCCGCAGCAGCCCGGGGAAGGACCGTGTGTTTTAGTCTTCCT |
| mEZH2-F | AGGAAGACTAAAACCCGTCCTTTTTCAGTTGTATCTTTCTGC TTTTGCGTCTTCGA |
| mEZH2-R | TCGAAGACGCAAAAGCAGAAAGATACAACTGAAAAAGGACGGGTTTTAGTCTTCCT |
| mRelA-F | AGGAAGACTAAAACGGAAAGATGAGGGGAAACAGATCGTCCA TTTTGCGTCTTCGA |
| mRelA-R | TCGAAGACGCAAAATGGACGATCTGTTTCCCCTCATCTTTCCGTTTTAGTCTTCCT |
| hTER | AGGAAGACTAAAACCCCTCTTTTCTCTGCGGAACGTTCTGGCGAAACACCGATTTAGACTAC<br>CCCAAAAACGAAGGGGACTAAAACCGGCTTTCTTTATCATCTCGGTGATCCTGAAACACCGA<br>TTTAGACTACCCCAAAAACGAAGGGGACTAAAACCCTCTTTCTGCACCTTGTCACACAGTAG<br>TTTTGCGTCTTCGA |
| mTER | AGGAAGACTAAAACACACGGTCCTTCCCCGGGCTGCTGCGGAGAAACACCGATTTAGACTA<br>CCCCAAAAACGAAGGGGACTAAAACCCGTCCTTTTTCAGTTGTATCTTTCTGCGAAACACCG<br>ATTTAGACTACCCCAAAAACGAAGGGGACTAAAACGGAAAGATGAGGGGAAACAGATCGTC<br>CATTTTGCGTCTTCGA |

Note: double-stranded hTER and mTER were prepared by PCR amplification using the listed single-stranded oligonucleotides as templates. Note: h, human; m, mouse.

Table S3. Primers for qPCR detection.

| Name | Primer sequence (5′-3′) | Name | Primer sequence (5′-3′) |
|---|---|---|---|
| AAV-F | TGCATGACCAGGCTCAGCTA | AAV-R | GACAGGGAAGGGAGCAGTG |
| Cas13a-F | GGAAAAGTACCAGTCCGCCA | Cas13a-R | GAAGTCCAGGAACTTGCCGA |
| EGFP-F | GACGGCAACTACAAGACCCG | EGFP-R | CTGCCGTCCTCGATGTTGT |
| mCherry-F | CGCGTGATGAACTTCGAGGA | mCherry-R | TCTGCTTGATCTCGCCCTTC |
| hGAPDH-F | ATTTGGTCGTATTGGGCG | hGAPDH-R | CTCGCTCCTGGAAGATGG |
| hTERT-F | GGCACGGCTTTTGTTCAGATG | hTERT-R | GCTGTTCACCTGCAAATCCA |
| hEZH2-F | ACATGCGACTGAGACAGCTC | hEZH2-R | GTCACTGGTCACCGAACACT |
| hRelA-F | CCTGGAGCAGGCTATCAGTC | hRelA-R | ATGGGATGAGAAAGGACAGG |
| mGAPDH-F | TCACCACCATGGAGAAGGC | mGAPDH-R | GCTAAGCAGTTGGTGGTGCA |
| mTERT-F | TCTACCGCACTTTGGTTGCC | mTERT-R | CAGCACGTTTCTCTCGTTGC |
| mEZH2-F | CGAATAACAGTAGCAGACCCAG | mEZH2-R | TGTTTGACACCGAGAATTTGCTT |
| mRelA-F | TGCGATTCCGCTATAAATGCG | mRelA-R | ACAAGTTCATGTGGATGAGGC |
| mCD31-F | ACCGGGTGCTGTTCTATAAGG | mCD31-R | TCACCTCGTACTCAATCGTGG |
| mMKi67-F | CAAGGCGAGCCTCAAGAGATA | mMKi67-R | TGTGCTGTTCTACATGCCCTG |
| mCaspase3-F | CTGACTGGAAAGCCGAAACTC | mCaspase3-R | CGACCCGTCCTTTGAATTTCT |

Note: h, human; m, mouse.

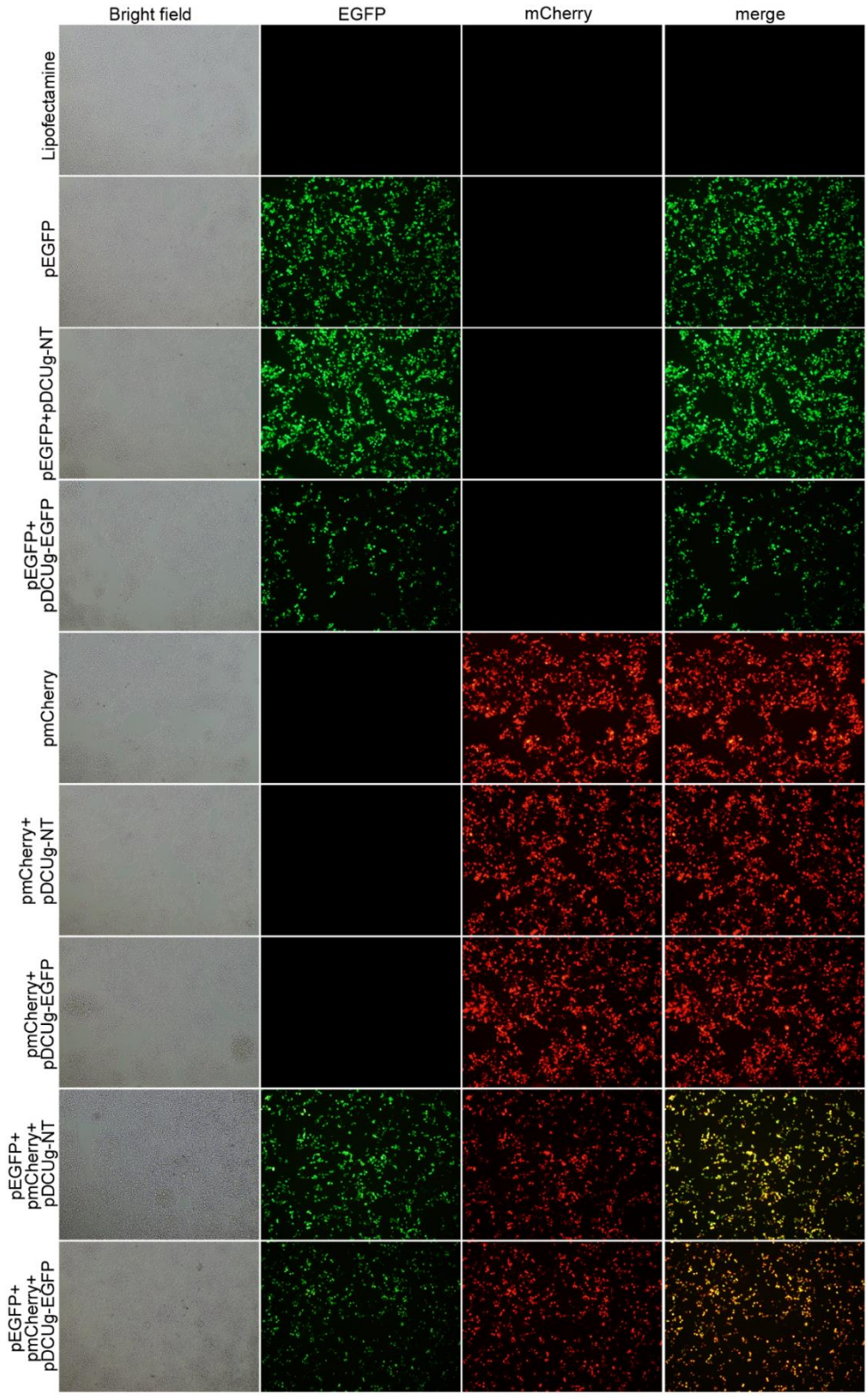



Fig.S1. Interference of ectopic EGFP/mCherry expression with DMP-Cas13a/U6-EGFP in HEK293T cells. Cells were transfected by various plasmid vectors and imaged with fluorescence microscope at 24 h post transfection. pEGFP/mCherry, plasmid expressing EGFP/mCherry under the contol of CMV promoter; pDCUg-NT/EGFP, plasmid expressing DMP-controlled Cas13a and U6-controlled gRNA targeting no transcript (NT) or EGFP transcript. This figure shows the representative images of cells of three independent transfection.

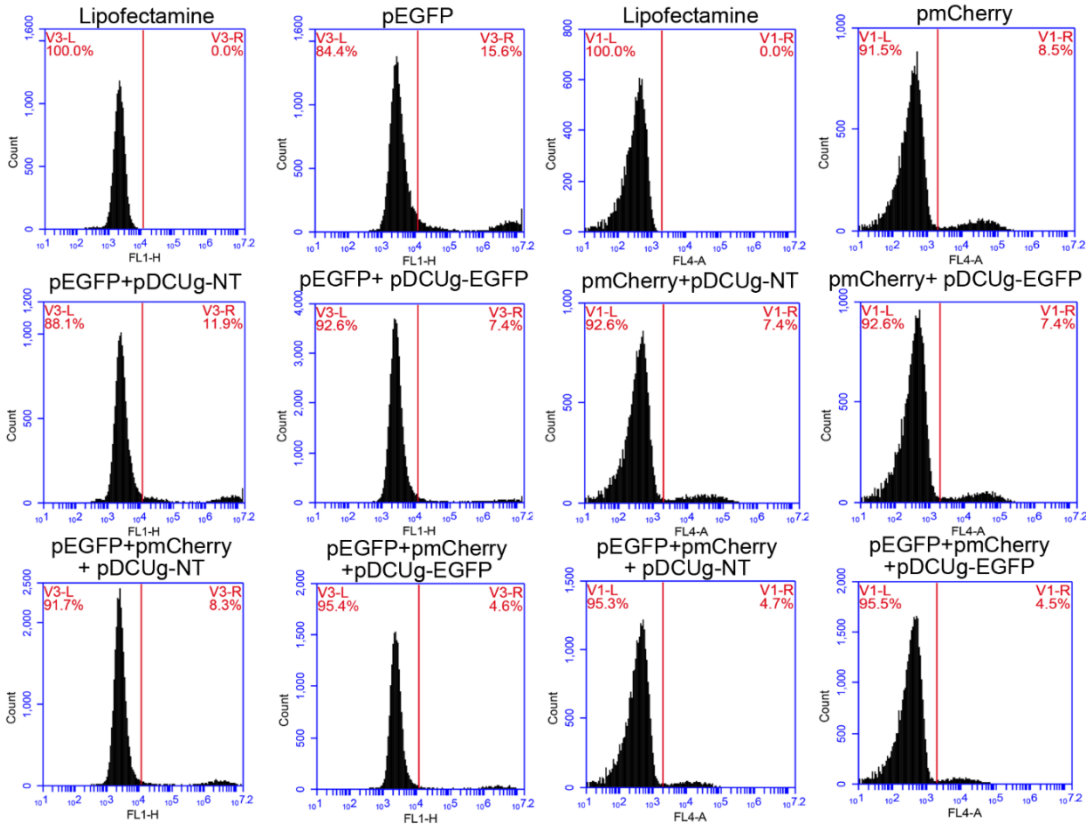



Fig.S2. Interference of ectopic EGFP/mCherry expression with DMP-Cas13a/U6-EGFP in HEK293T cells. Cells were transfected by various plasmid vectors and the fluorescence intensity of cells were quantified with flow cytometry (FC) at 24 h post transfection. The channel FL1 shows the green fluorescence and FL4 the red fluorescence. pEGFP/mCherry, plasmid expressing EGFP/mCherry under the control of CMV promoter; pDCUg-NT/EGFP, plasmid expressing DMP-controlled Cas13a and U6-controlled gRNA targeting no transcript (NT) or EGFP transcript. This figure shows the representative FC results of three independent transfection.

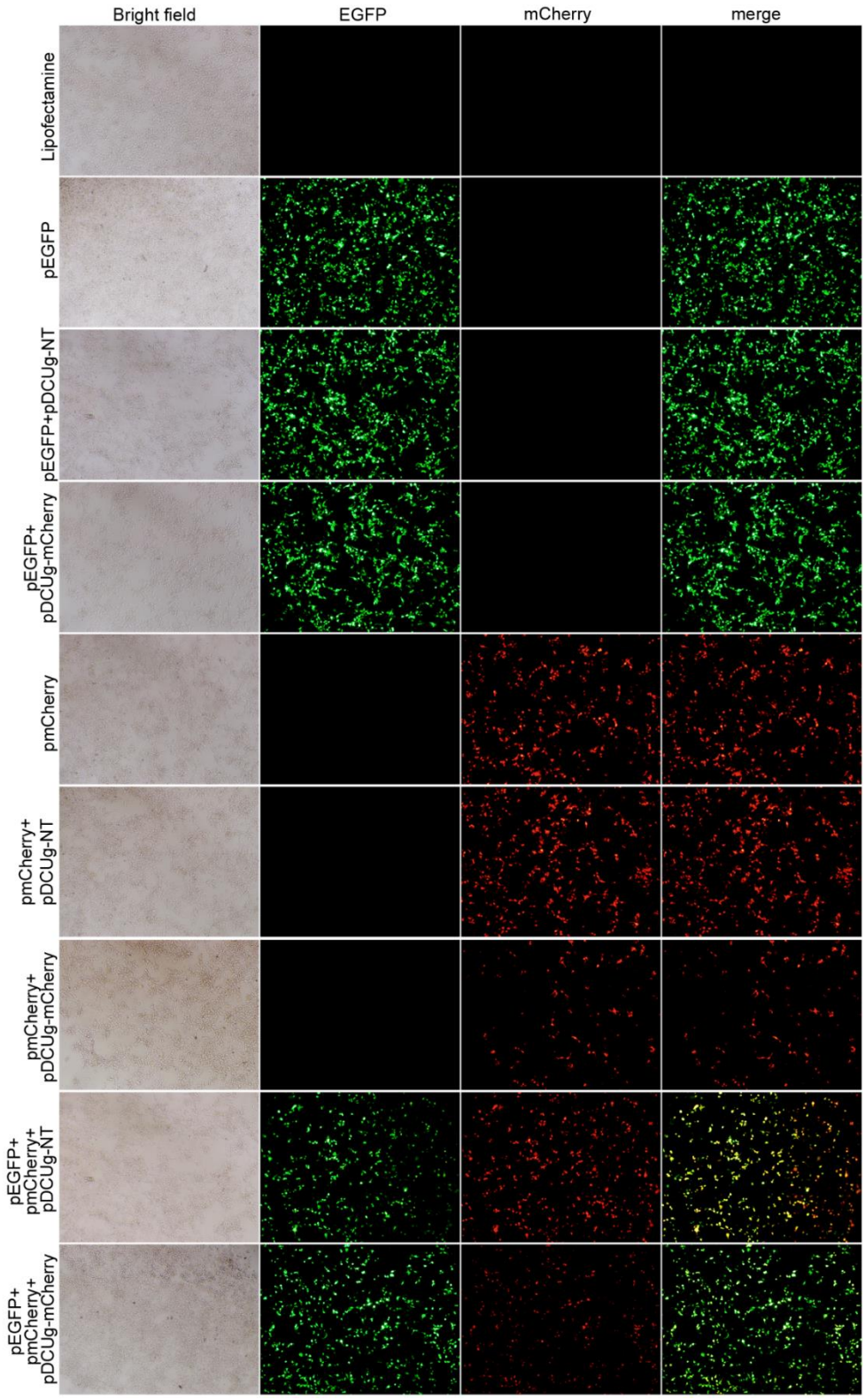



Fig.S3. Interference of ectopic EGFP/mCherry expression with DMP-Cas13a/U6-mCherry in HEK293T cells. Cells were transfected by various plasmid vectors and imaged with fluorescence microscope at 24 h post transfection. pEGFP/mCherry, plasmid expressing EGFP/mCherry; pDCUg-NT/mCherry, plasmid expressing DMP-controlled Cas13a and U6-controlled gRNA targeting no transcript (NT) or mCherry transcript. This figure shows the representative images of cells of three independent transfection.

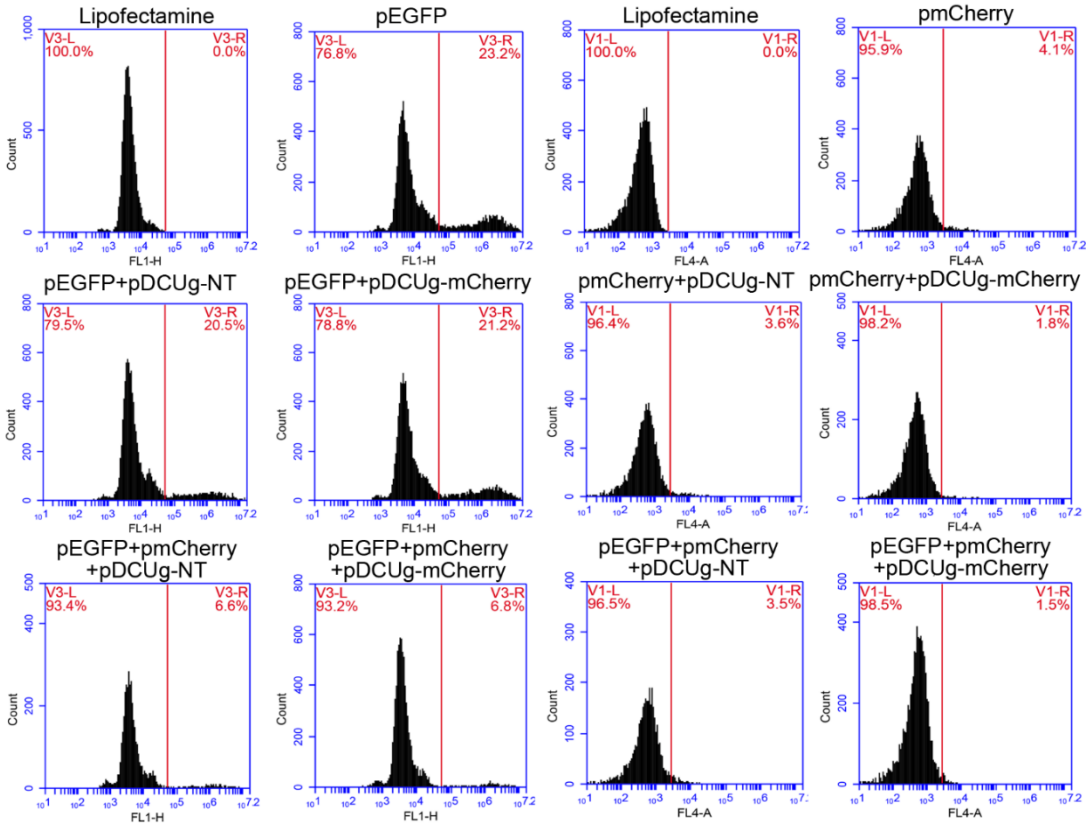



Fig.S4. Interference of ectopic EGFP/mCherry expression with DMP-Cas13a/U6-mCherry in HEK293T cells. Cells were transfected by various plasmid vectors and the fluorescence intensity of cells were quantified with flow cytometry (FC) at 24 h post transfection. The channel FL1 shows the green fluorescence and FL4 the red fluorescence. pEGFP/mCherry, plasmid expressing EGFP/mCherry under the control of CMV promoter; pDCUg-NT/mCherry, plasmid expressing DMP-controlled Cas13a and U6-controlled gRNA targeting no transcript (NT) or mCherry transcript. This figure shows the representative FC results of three independent transfection.

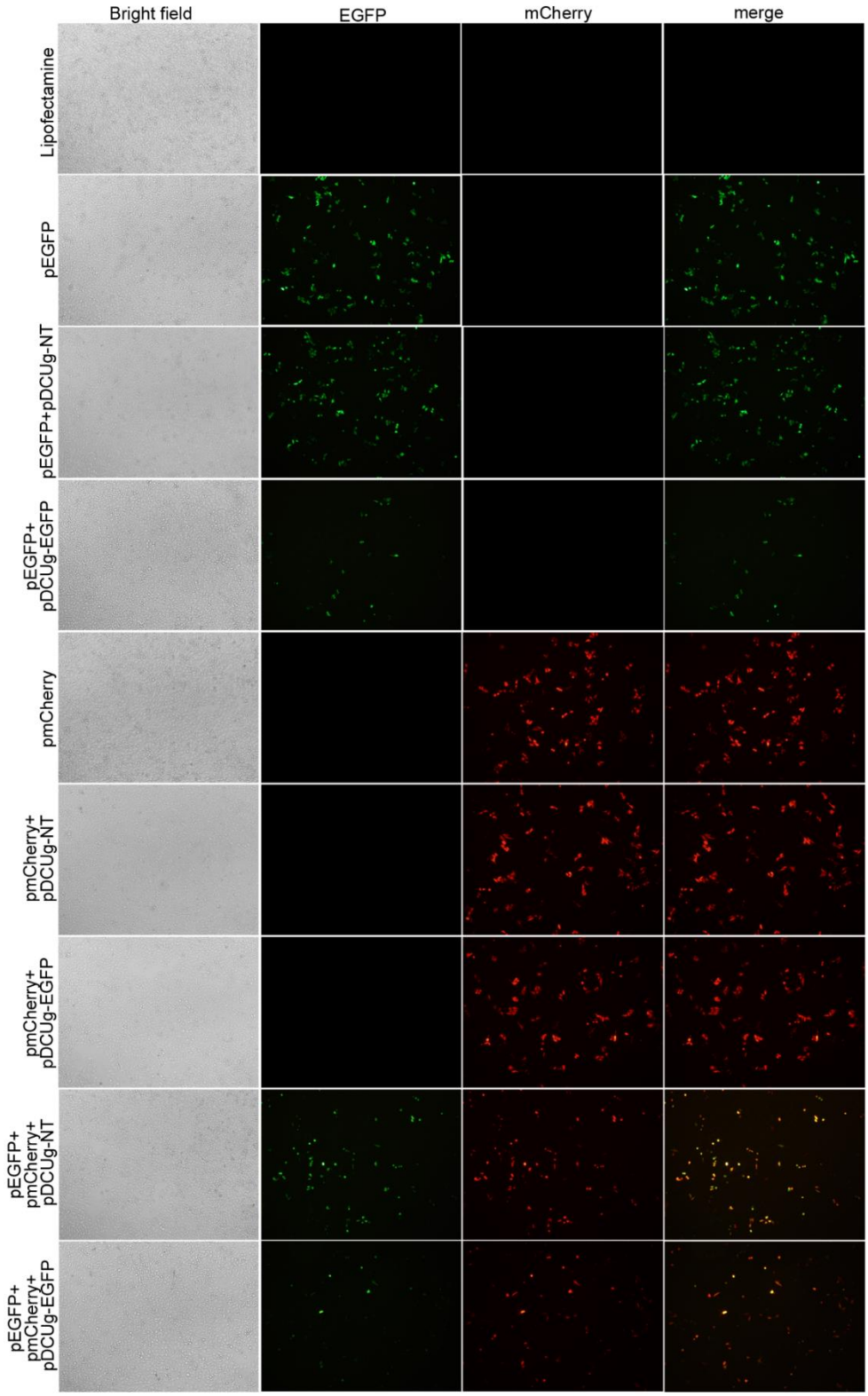



Fig.S5. Interference of ectopic EGFP/mCherry expression with DMP-Cas13a/U6-EGFP in HepG2 cells. Cells were transfected by various plasmid vectors and imaged with fluorescence microscope at 24 h post transfection. pEGFP/mCherry, plasmid expressing EGFP/mCherry under the control of CMV promoter; pDCUg-NT/EGFP, plasmid expressing DMP-controlled Cas13a and U6-controlled gRNA targeting no transcript (NT) or EGFP transcript. This figure shows the representative images of cells of three independent transfection.

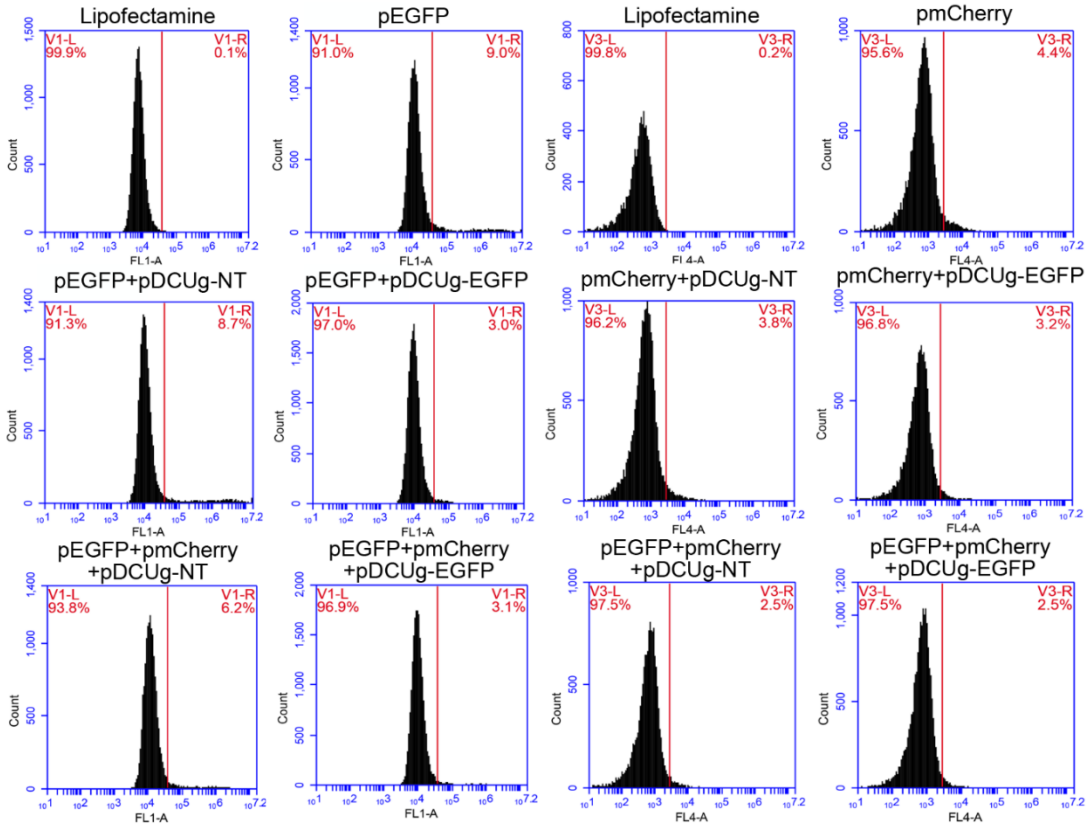



Fig.S6. Interference of ectopic EGFP/mCherry expression with DMP-Cas13a/U6-EGFP in HepG2 cells. Cells were transfected by various plasmid vectors and the fluorescence intensity of cells were quantified with flow cytometry (FC) at 24 h post transfection. The channel FL1 shows the green fluorescence and FL4 the red fluorescence. pEGFP/mCherry, plasmid expressing EGFP/mCherry under the control of CMV promoter; pDCUg-NT/EGFP, plasmid expressing DMP-controlled Cas13a and U6-controlled gRNA targeting no transcript (NT) or EGFP transcript. This figure shows the representative FC results of three independent transfection.

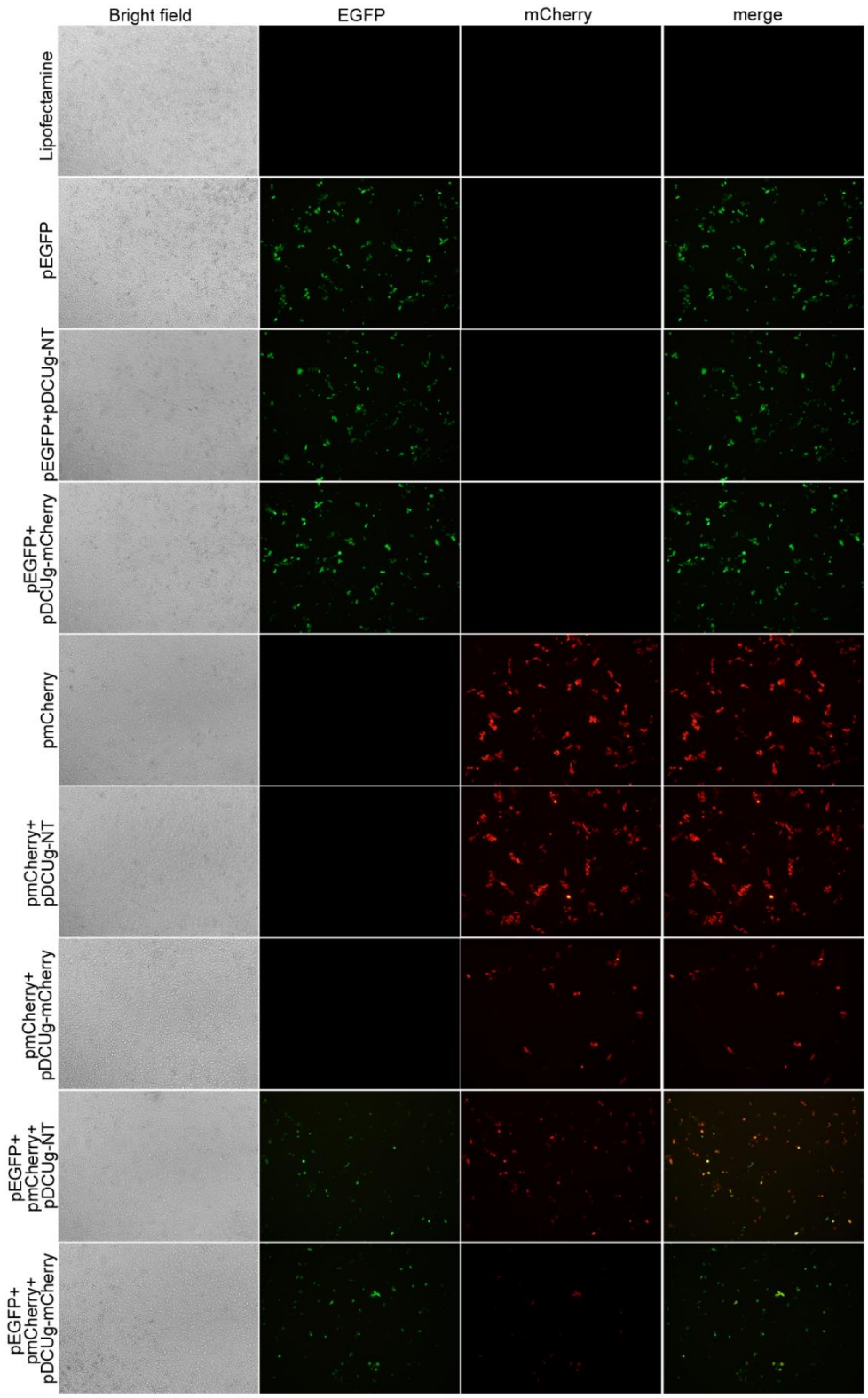



Fig.S7. Interference of ectopic EGFP/mCherry expression with DMP-Cas13a/U6-mCherry in HepG2 cells. Cells were transfected by various plasmid vectors and imaged with fluorescence microscope at 24 h post transfection. pEGFP/mCherry, plasmid expressing EGFP/mCherry under the control of CMV promoter; pDCUg-NT/mCherry, plasmid expressing DMP-controlled Cas13a and U6-controlled gRNA targeting no transcript (NT) or mCherry transcript. This figure shows the representative images of cells of three independent transfection.

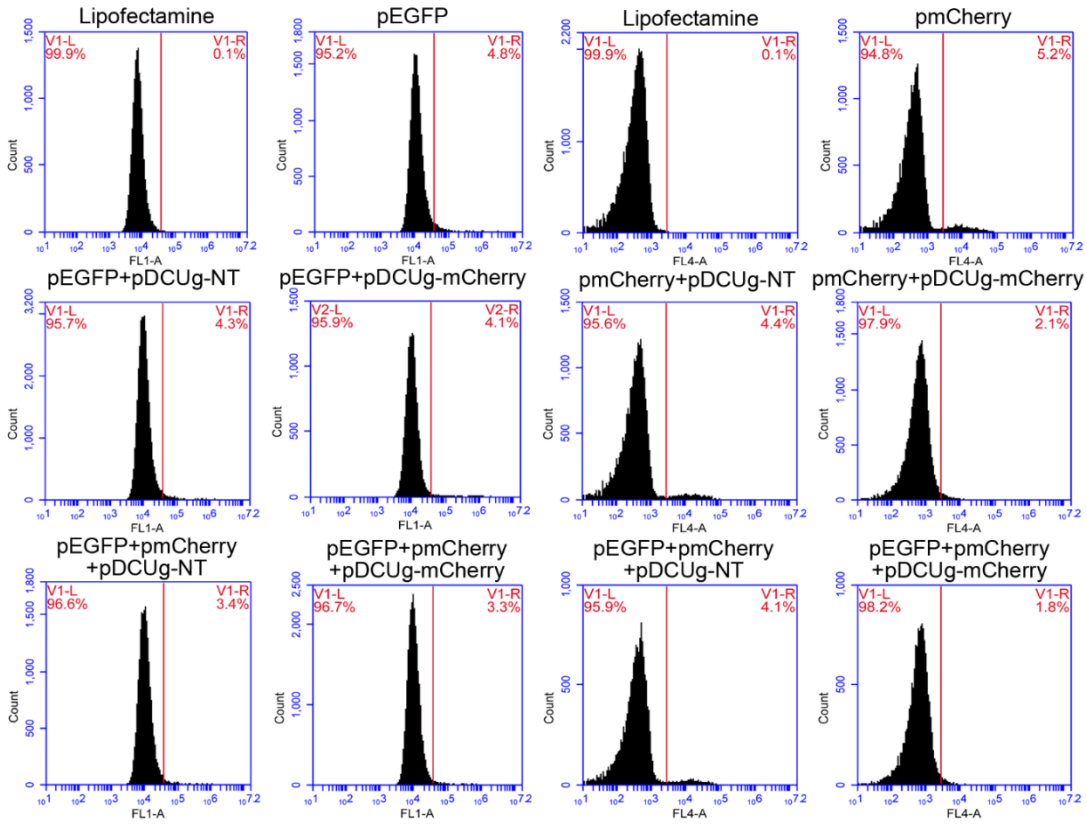



Fig.S8. Interference of ectopic EGFP/mCherry expression with DMP-Cas13a/U6-mCherry in HepG2 cells. Cells were transfected by various plasmid vectors and the fluorescence intensity of cells were quantified with flow cytometry (FC) at 24 h post transfection. The channel FL1 shows the green fluorescence and FL4 the red fluorescence. pEGFP/mCherry, plasmid expressing EGFP/mCherry under the control of CMV promoter; pDCUg-NT/mCherry, plasmid expressing DMP-controlled Cas13a and U6-controlled gRNA targeting no transcript (NT) or mCherry transcript. This figure shows the representative FC results of three independent transfection.

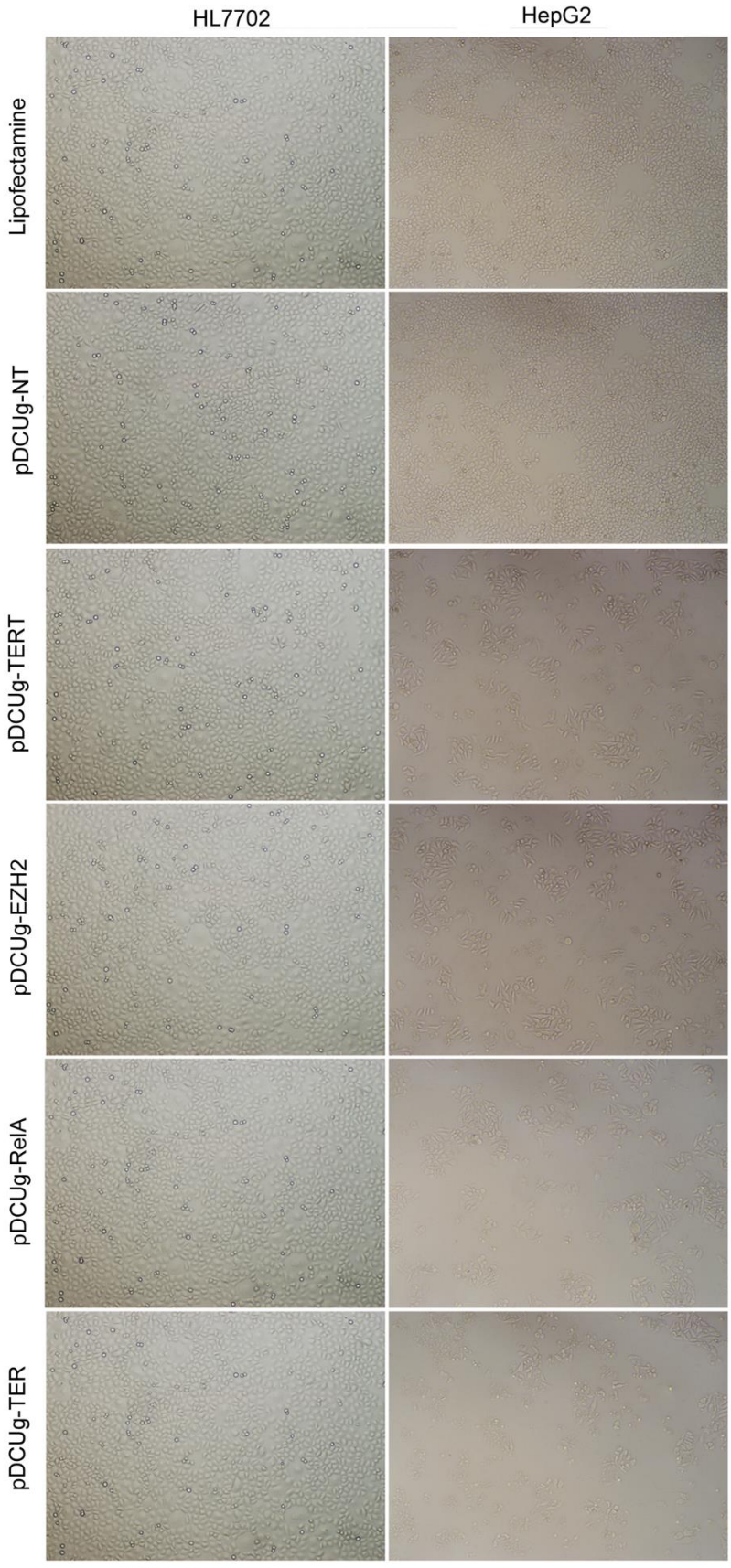



Fig.S9. Interference of endogenous gene expression with DMP-Cas13a/U6-gRNA. Cells were transfected by various vectors and detected at 24 h post transfection. Representative images of cells.

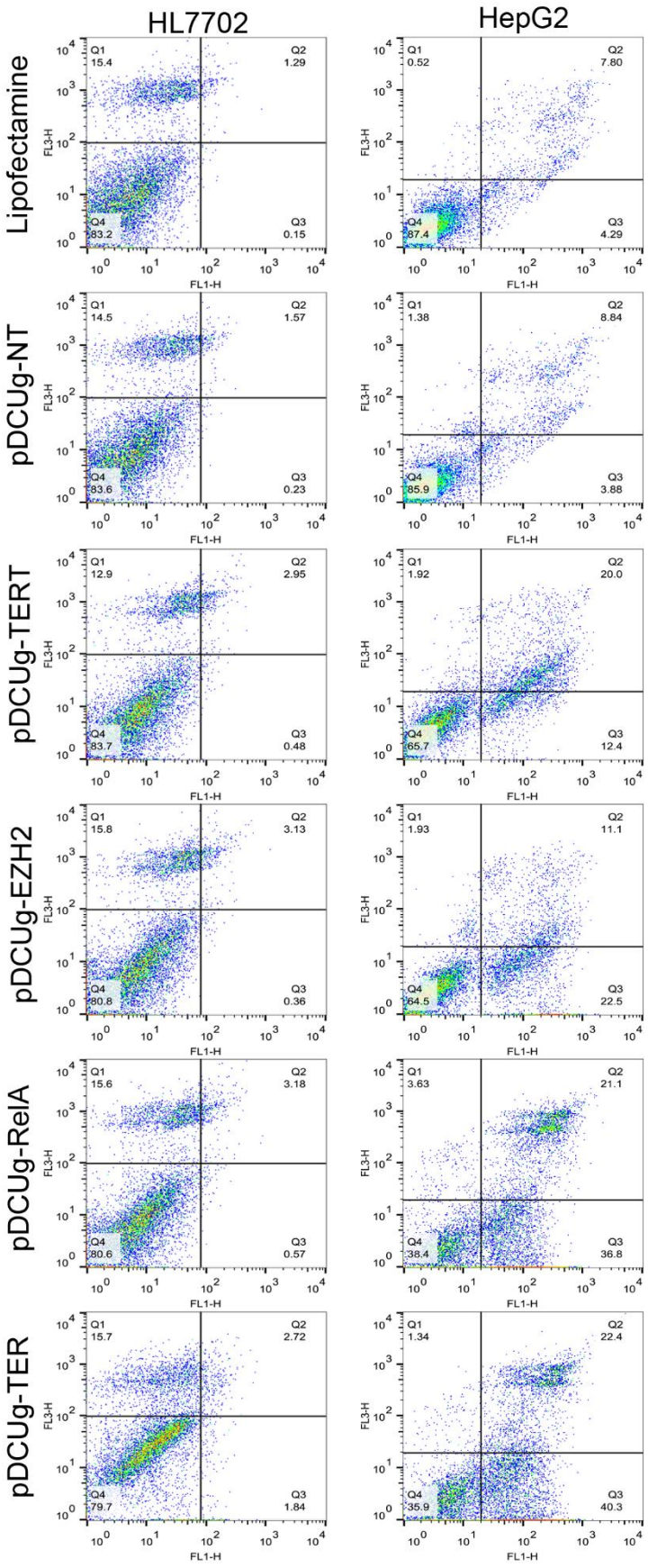



Fig.S10. Interference of endogenous gene expression with DMP-Cas13a/U6-gRNA. Cells were transfected by various vectors and detected at 24 h post transfection. Flow cytometry analysis of cell apoptosis.

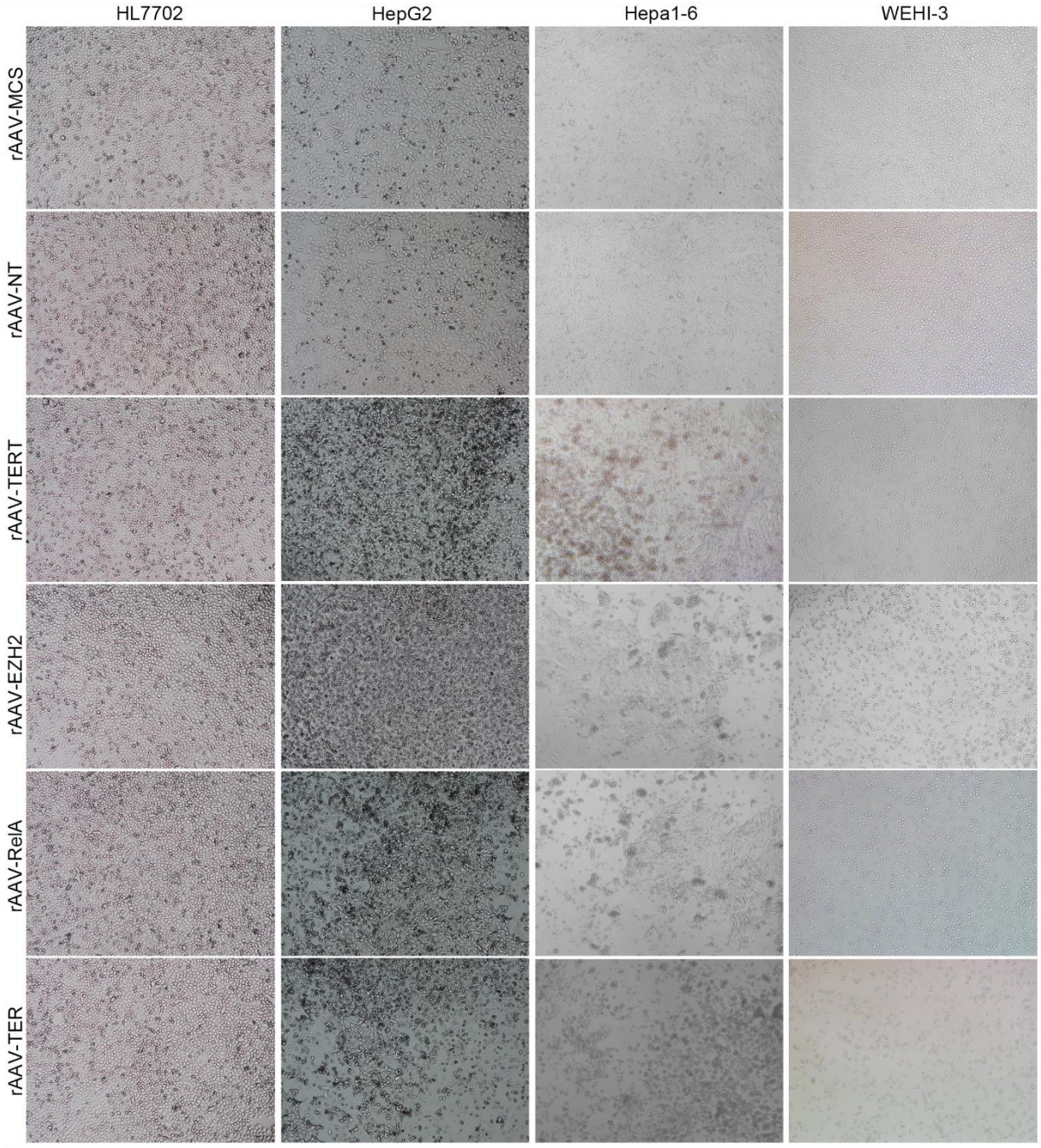



Fig.S11. Antitumor effects of DMP-Cas13a/U6-gRNA virus in vitro. Cells were inoculated in 24-well plate ($1\times10^5$ cells/well) and cultured for 12 h. Cells were then treated with viruses ($5\times10^{10}$ vg/well) including rAAV-MCS, rAAV-NT, rAAV-TERT, rAAV-EZH2, rAAV-RelA, and rAAV-TER, respectively. Cells were cultured for 72 h and imaged at 24 h post transfection.

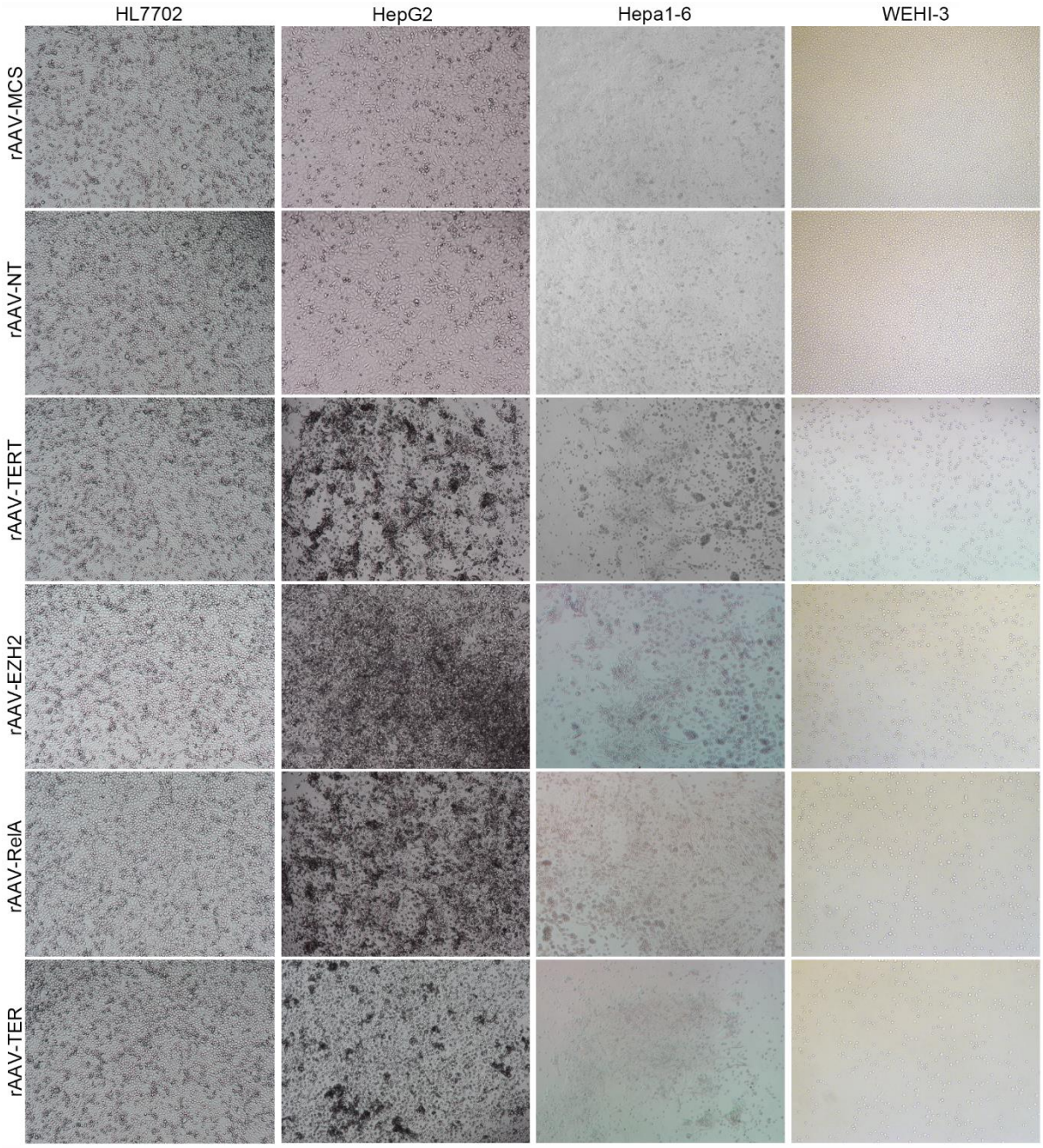
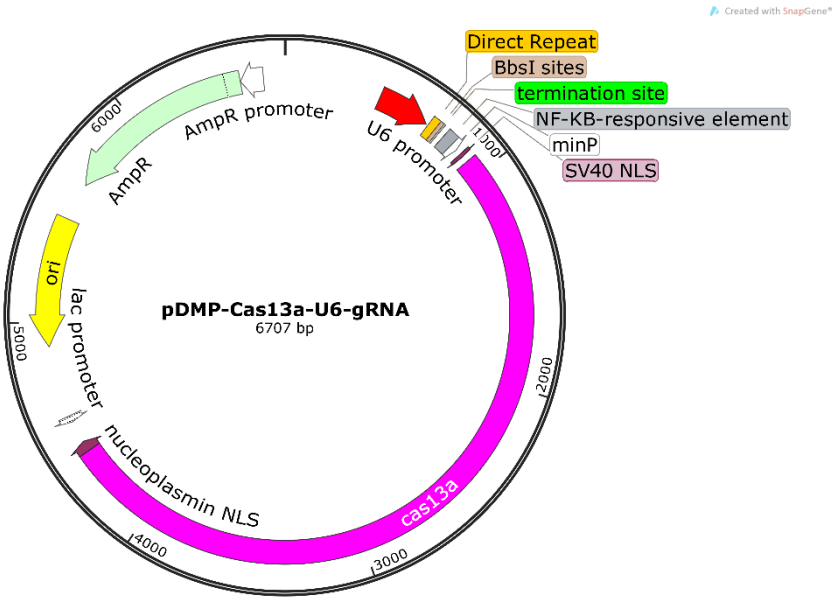



Fig.S12. Antitumor effects of DMP-Cas13a/U6-gRNA virus in vitro. Cells were inoculated in 24-well plate ($1\times10^5$ cells/well) and cultured for 12 h. Cells were then treated with viruses ($5\times10^{10}$ vg/well) including rAAV-MCS, rAAV-NT, rAAV-TERT, rAAV-EZH2, rAAV-RelA, and rAAV-TER, respectively. Cells were cultured for 72 h and imaged at 48 h post transfection.

**Plasmids and the functional sequences**




pDMP-Cas13a-U6-gRNA

U6+Direct repeat+Spacer+DMP+Cas13a+SV40 poly(A) signal

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATT
GGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAAT
TTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTT
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ATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTT
TTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTA

The spacer sequence in above skeleton vector can be replaced by the following sequences for constructing pDMP-Cas13a-U6-gRNAs targeting particular genes:

No transcript (NT): TAGATTGCTGTTCTACCAAGTAATCCAT
hTERT: CCCTCTTTTCTCTGCGGAACGTTCTGGC
hEZH2: CGGCTTTCTTTATCATCTCGGTGATCCT
hRelA: CCTCTTTCTGCACCTTGTCACACAGTAG
hTER:CCCTCTTTTCTCTGCGGAACGTTCTGGCGAAACACCGATTTAGACTACCCCAAAAACGA
AGGGGACTAAAACCGGCTTTCTTTATCATCTCGGTGATCCTGAAACACCGATTTAGACTACCCC
AAAAACGAAGGGGACTAAAACCCTCTTTCTGCACCTTGTCACACAGTAG

mTERT: ACACGGTCCTTCCCCGGGCTGCTGCGGA
mEZH2: CCGTCCTTTTTCAGTTGTATCTTTCTGC
mRelA: GGAAAGATGAGGGGAAACAGATCGTCCA
mTER:AGGAAGACTAAAACACACGGTCCTTCCCCGGGCTGCTGCGGAGAAACACCGATTTAGA
CTACCCCAAAAACGAAGGGGACTAAAACCCGTCCTTTTTCAGTTGTATCTTTCTGCGAAACAC
CGATTTAGACTACCCCAAAAACGAAGGGGACTAAAACGGAAAGATGAGGGGAAACAGATCGT
CCATTTTGCGTCTTCGA