**A GFP activation assay for highly sensitive detection of off-target cleavage**

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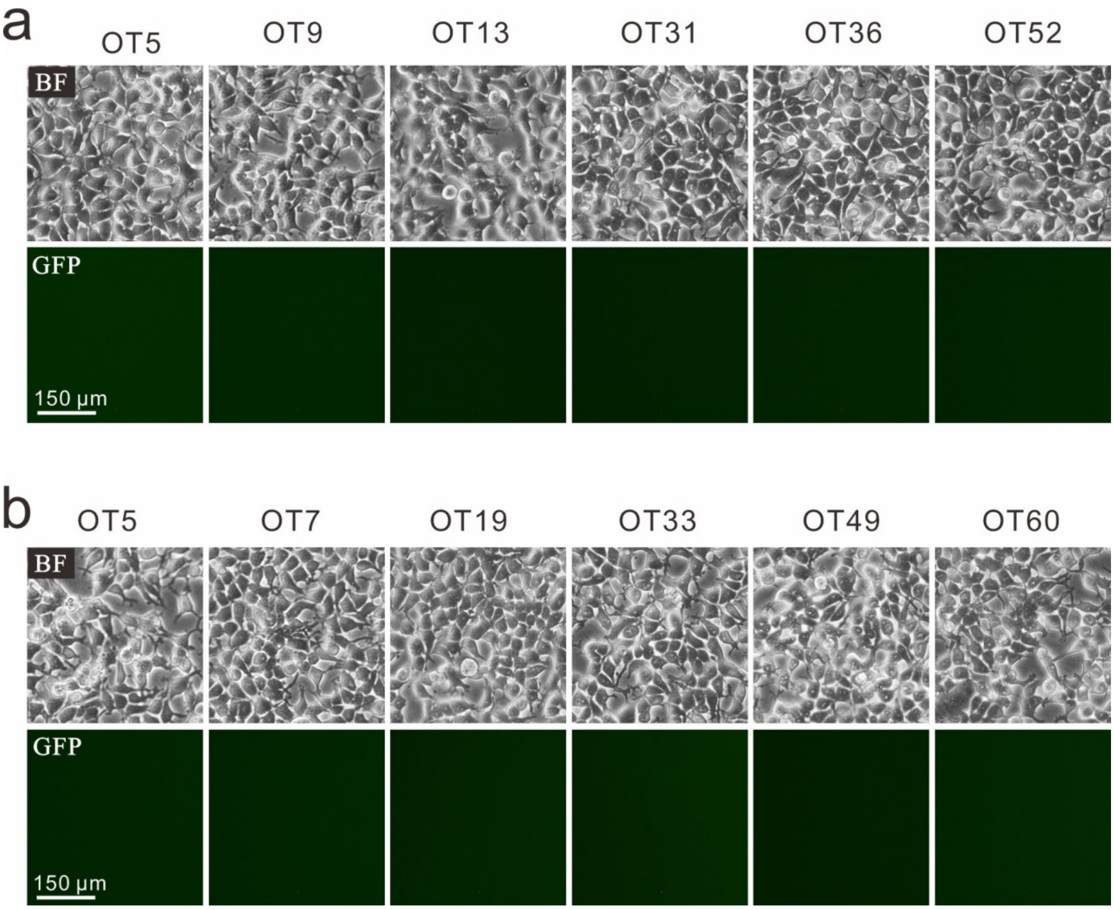



**Supplementary Figure 1. Negative controls for the GFP activation assay. a,** Negative controls for *EMX1* off-targets. Only SpCas9 expressing vector is transfected into the cells. **b,** Negative controls for *VEGFA* site 1 off-targets. Only SpCas9 expressing vector is transfected into the cells.

**EMX1-OT9-Indel**

```
ATGGAGTACAAGCAGATGAAAAACGGGTGAGCAAG
ATGGAGTACAA-----------ACGGGTGAGCAAG
ATGAAGTCCGAG-----GAAA---GGGTGAGCAAG
ATGGAGTACAAGCAGATGAA-----GGTGAGCAAG
ATGGAGTACAAGCAGATGAA--ACGGGTGAGCAAG
ATGGAGTACAAGCAGATGGA--ACGGGTGAGCAAG
ATGGAGTACAAGC-----------GGGTGAGCAAG
ATGGAGTACAAGCAGATGA-----------GCAAG
ATGGAGTACAAGCAG-----------GTGAGCAAG
ATGGAGTACAAGCAGATGAAAAAACGGGTGAGCAAG
ATGGAGTACAAGCAGATGAGCAAGTACAAACGGGTGAGCAAG
```

**EMX1-OT13-Indel**

```
ATGGAGGCCAAGCAGAAAGAAAAAGGGTGAGCAAG
ATGGAGGCCAAGCAGAAAGAAAAAAGGGTGAGCAAG
ATGGA-----------------AAGGGTGAGCAAG
ATGGAGGCCAAGCAGAAAGA--AAGGGTGAGCAAG
ATGGAGGCCAAGCAGAAA--AAAAGGGTGAGCAAG
ATGGAGGCCAAGCAGAA-G-AAAAGGGTGAGCAAG
ATGGACTCCGAGCAG-CAGA-GATGGGTGAGCAAG
ATGGAGGCCAAGCAGAAA-----AGGGTGAGCAAG
ATGGAGGCCAAGCAGAAAGA-----GGTGAGCAAG
ATGGAGGCCATGGAG-----------GTGAGCAAG
ATGGAGGCTTTGCCT-----------GTGAGCAAG
```

**EMX1-OT31-Indel**

```
ATGGACTCCGAGCAGCAGAAGGATGGGTGAGCAAG
ATGG-----------------GATGGGTGAGCAAG
ATGGAC-CCT-------------TGGGTGAGCAAG
ATGGACTCCGAGCAGCAGA-----------GCAAG
ATGGACTCCGAGCAGCAGAA-----------CAAG
ATGGACTCCGAGCAGCAG--------GTGAGCAAG
ATGGACTCCGAGCAGCAGA--------TGAGCAAG
ATGGACTCCGAGCAGCAGAT-----GGTGAGCAAG
ATGGACTCCGAGCAGCAGAA--ATGGGTGAGCAAG
ATGGACTCCGAGCAGCAGA--GATGGGTGAGCAAG
ATGGACTCCGAGCAGCAGAAAGGATGGGTGAGCAAG
```

**EMX1-OT36-Indel**

```
ATGGAGTTAGAGCAGAGGAAGAGAGGGTGAGCAAG
ATGGAGTTAGAGCAGAGGAAAGAGAGGGTGAGCAAG
ATGGAG-----------------AGGGTGAGCAAG
ATGGAGTTAGAGCAGAG--------------CAAG
ATGGAGTTAGAG-----------AGGGTGAGCAAG
ATGGAGTTAGAGCAGAGGA-----------GCAAG
ATGGAGTTAGAGCAGAGG--------GTGAGCAAG
ATGGAGTTAGAGCAGAGGAG--------CAGCAAG
ATGGAGTTAGAGCAGAGGA--------TGAGCAAG
ATGGAGTTAGAGCAGAGGA-----GGGTGAGCAAG
ATGGAGTTAGAGCAGAGGAA-----GGTGAGCAAG
ATGGAGTTAGAGCAGAGGAA--GAGGGTGAGCAAG
```

**EMX1-OT52-Indel**

```
ATGGTGTCAGAGCAGAAAAAGAGTGGGTGAGCAAG
ATGGTGTCAGAGCAGAAAAAAGAGTGGGTGAGCAAG
ATGG-----------------AGTGGGTGAGCAAG
ATGGTGT-----------------GGGTGAGCAAG
ATGGTGTCAGAGCAGA--------------GCAAG
ATGGTGTCAGAG--------------GTGAGCAAG
ATGGTGTCAGAG-----------TGGGTGAGCAAG
ATGGTGTCAGAGCAGAAAA-----------GCAAG
ATGGTGTCAGAGCAGAA-----GTGGGTGAGCAAG
ATGGTGTCAGAGCAG--------TGGGTGAGCAAG
ATGGTGTCAGAGCAGAAA--GAGTGGGTGAGCAAG
```

**Supplementary Figure 2. Indel sequences are detected by deep sequencing for *EMX1* off-targets.**

**VEGFA-OT5-Indel**

```
ATGGGGGGCAGGGAGATTGCTCCTGGGTGAGCAAG
ATGGGGGGCAGGGAGATTGCCTCCTGGGTGAGCAAG
ATGGGGGGCAGGGAGATTGC--CTGGGTGAGCAAG
ATGGGGGG-----------------GGTGAGCAAG
ATGGGGGGCAGGGAGA--------------GCAAG
ATGGGGGGCAGGGAGAT--------------CAAG
ATGGGGGGCAGGG--------------TGAGCAAG
ATGGGGGGC--------------TGGGTGAGCAAG
ATGGGGGGCAGGGAGATTGC-----------CAAG
ATGGGGGGC-----------TCCTGGGTGAGCAAG
ATGGGGGGCAGGGAA--------TGGGTGAGCAAG
ATGGGGGGCAGGGAGA-----CCTGGGTGAGCAAG
```

**VEGFA-OT7-Indel**

```
ATGGTAAGTAAGGGAAGTTTGCTCCTGGGTGAGCAAG
ATGGTAAGTAAGG----------------TGAGCAAG
ATGGTAAGTAAGGGAAG----------------CAAG
ATGGTAAGTAAGGGAAGTTT-------------CAAG
ATGGTAAGTAAGGGAAGT-------------AGCAAG
ATGGTAAGTAAGGGAAGTTTG----------AGCAAG
ATGGTAAGTAAG----------TCCTGGGTGAGCAAG
ATGGTAAGTAAGGGAAGTTTG-------GTGAGCAAG
ATGGTAAGTAAGGGAAGTAAG-------GTGAGCAAG
ATGGTAAGTAAGGGAAGTTTGC----GGGTGAGCAAG
ATGGTAAGTAAGGGAAGTTA----CTGGGTGAGCAAG
ATGGTAAGTAAGGGAAGTTTGC-CCTGGGTGAGCAAG
ATGGTAAGTAAGGGAAGTTTG-TCCTGGGTGAGCAAG
```

**VEGFA-OT19-Indel**

```
ATGGGGAGGAGAGAGTTTGCTCTCTGGTGAGCAAG
ATGGGGAGGAGAGAGTTTGC--TCTGGTGAGCAAG
ATGGGGAGGAGAGAGTTTGCTTCTCTGGTGAGCAAG
```

**VEGFA-OT33-Indel**

```
ATGAGAGGGGTGGAGTTTGTTCCAGGGTGAGCAAG
ATGAGAGGGGTGGAGTTTGTTTCCAGGGTGAGCAAG
```

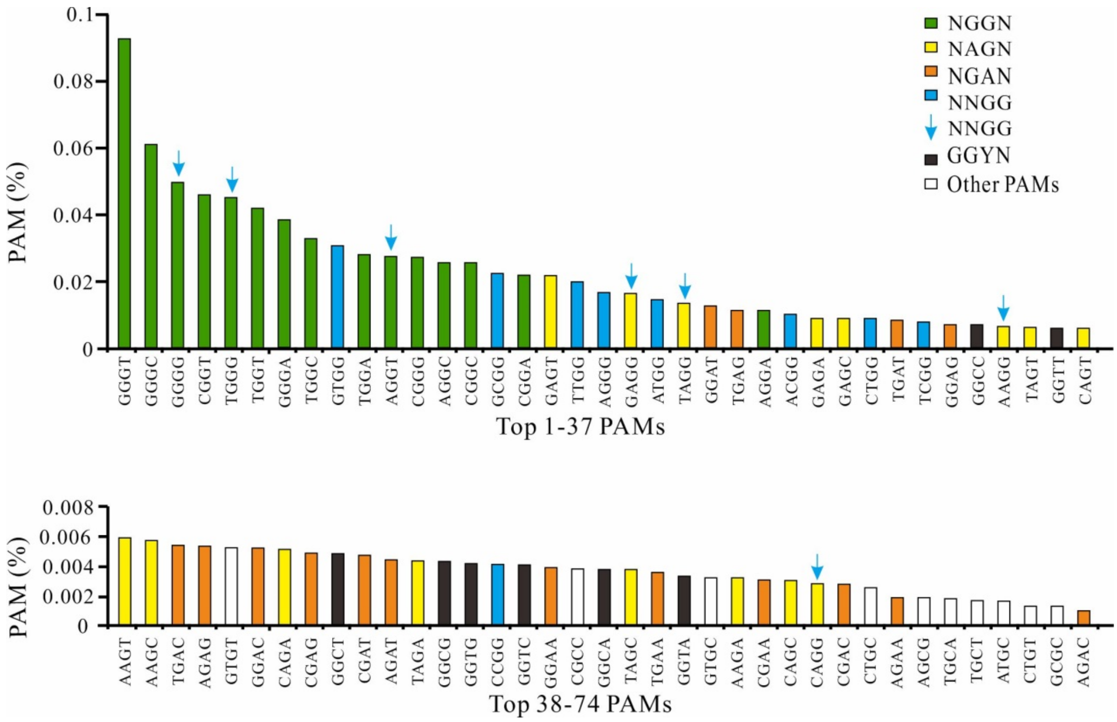
**VEGFA-OT49-Indel**

```
ATGGGGGAGGGGGAGATGGCTCCCGGGTGAGCAAG
ATGGGGGAGG-----------------TGAGCAAG
ATGGGGGAGGGGGAGA--------------GCAAG
ATGGGGGAGGGGG--------------TGAGCAAG
ATGGGGGAGGGGGAGATG-----------AGCAAG
ATGGGGGAGGGGGAGATGGC-----------CAAG
ATGGGGGAGGGGGAGATGGC--------GAGCAAG
ATGGGGGAGGGGGAGATGG--------TGAGCAAG
ATGGGGGAGGGG--------TCCCGGGTGAGCAAG
ATGGGGGAGGGGGAG-----TCCCGGGTGAGCAAG
ATGGGGGAGGGGGAGATG--TCCCGGGTGAGCAAG
ATGGGGGAGGGGGAGATGGC--CCGGGTGAGCAAG
```

**VEGFA-OT60-Indel**

```
ATGGAGAGGTGGGGTGATTTGCTCCAGGGTGAGCAAG
ATGGAGAGGTGGG----------------TGAGCAAG
ATGGAGAGGTGGGGT----------------AGCAAG
ATGGAGAGGT-------------CCAGGGTGAGCAAG
ATGGAGAGGTGGGGTGATTTG----------AGCAAG
ATGGAGAGGTGGGGTGATTTGC----GGGTGAGCAAG
ATGGAGAGGTGGGGTGATT----CCAGGGTGAGCAAG
ATGGAGAGGTGGGGTGATTTGC-CCAGGGTGAGCAAG
ATGGAGAGGTGGGGTGATTTG-TCCAGGGTGAGCAAG
ATGGAGAGGTGGGGTGATTTGCGCTCCAGGGTGAGCAAG
ATGGAGAGGTGGGGTGATTTGCCTTGCTCCAGGGTGAGCAAG
```

**Supplementary Figure 3. Indel sequences are detected by deep sequencing for *VEGFA* site 1 off-targets.**




**Supplementary Figure 4. PAM frequency of the top 74 PAMs.** NNGG PAM overlapped with other PAMs is indicated by arrows.

ACGCGTGTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAACGATG
AGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCGATT
GGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAACAGACGG
GTCTGACATGGATTGGACGAACCACTGAATTGCCGCATTGCAGAGATATTG
TATTTAAGTGCCTAGCTCGATACAATAAACGGGTCTCTCTGGTTAGACCAGA
TCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTC
AATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTG
ACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCT
CTAGCAGTGGCGCCCGAACAGGGACCTGAAAGCGAAAGGGAAACCAGAG
CTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGA
GGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGA
AGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGA
TCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAA
ATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAA
TCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCT
ACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACA
GTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAG
GAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGACCACCG
CACAGCAAGCGGCCACTGATCTTCAGACCTGGAGGAGGAGATATGAGGGA
CAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTA
GGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAA

GAGCAGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGA
AGCACTATGGGCGCAGCCTCAATGACGCTGACGGTACAGGCCAGACAATTA
TTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCG
CAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGC
AAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGAT
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AGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTGTG
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AATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAG
TGAACGGATCTCGACGGTTAACTTTTAAAAGAAAAGGGGGGATTGGGGGG
TACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACT
AAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTATCGATACTAGTA
AGGATCTGCGATCGCGACATTGATTATTGACTAGTTATTAATAGTAATCAATT
ACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTAC
GGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTC
AATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGT
CAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTG
TATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTA
CATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACA
TCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACC
CCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTC
CAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTG
TACGGTGGGAGGTCTATATAAGCAGTCTAGAGATCCGACGCCGCCATCTCTA
GGCCCGCGCCGGCCCCCTCGCACAGACTTGTGGGAGAAGCTCGGCTACTC
CCCTGCCCCGGTTAATTTGCATATAATATTTCCTAGTAACTATAGAGGCTTAA
TGTGCGATAAAAGACAGATAATCTGTTCTTTTTAATACTAGCTACATTTTACA
TGATAGGCTTGGATTTCTATAAGAGATACAAATACTAAATTATTATTTTAAAA
AACAGCACAAAAGGAAACTCACCCTAACTGTAAAGTAATTGTGTGTTTTGA
GACTATAAATATGCATGCGAGAAAAGCCTTGTTTGCCACCATGGAACGGCT
CGGAGATCATCATTGCGNNNNNNNGTGAGCAAGGGCGAGGAGCTGTTCAC
CGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACA
AGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTG
ACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC
CTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGAC
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CATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGC
AGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTG
CTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCAAGCTGAGCAAAGA
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CGGTGCCTGAATCTAGGTCGACAATCAACCTCTGGATTACAAAATTTGTGA
AAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACG
CTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCT
CCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGT
TGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCA
CTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTT
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GGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTC
TGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACC
TTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCT
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GCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCC
TTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTA
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ATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACA
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CTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTC
CAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCG
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GCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATG
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GCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCG
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CTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGG
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AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTG
GTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCT
CTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCA
AGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATC
TTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATT
TTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAA
AATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAG
TTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTT
CATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGG
CTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACC
GGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCA
GAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCG
GGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGC
CATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTC
AGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGC
AAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTG
GCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTG
TCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTC
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ACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGG
AAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATC
CAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACT
TTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAA
AAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTT
TCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATAT
TTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCG
AAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTAT
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GGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGT
GTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTA
CTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGA
AAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAA
GGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGG
ATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACG
ACGTTGTAAAACGACGGCCAGTGCCAAGCTG

**Supplementary Figure 5. Sequence of GFP reporter construct for PAM screening.** CMV promoter was shown in blue; target sequence was shown in red; GFP sequence was shown in green.

**Supplementary information, Table S1. Primers and gRNA used in this study**

| **Name** | **Primer sequence** | **Description** |
|---|---|---|
| PAM library | gcgagaaaagccttgtttgccaccATGGAACGGCTCGGAGATCATCATTGCGNNNNNNNgtgagcaagggcgagga | Oligonucleotide for construction of PAM library |
| AAVS1--1-F | ccg GTAGAGGCGGCCACGACCTG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| AAVS1--1-R | aac CAGGTCGTGGCCGCCTCTAC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| AAVS1--2-F | ccg GTGAGAATGGTGCGTCCTAG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| AAVS1--2-R | aac CTAGGACGCACCATTCTCAC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--1-F | ccg GAAAGTCAGAAGGCTGTTGA | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--1-R | aac TCAACAGCCTTCTGACTTTC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--2-F | ccg GCCTTCGTAGATTTTTGGGT | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--2-R | aac ACCCAAAAATCTACGAAGGC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |

| | | |
|---|---|---|
| COMMD2--3-F | ccg CGTGGGGGCGGTGTTCTGTG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--3-R | aac CACAGAACACCGCCCCCACG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--4-F | ccg CAGAAGGCTGTTGAGTGCCA | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--4-R | aac TGGCACTCAACAGCCTTCTG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--5-F | ccg GGAAAGGGGAAGGCTCCGC A | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--5-R | aac CGGCAGTCCGTCGTACCCAT | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--6-F | ccg GCTCCGCAGGCTTCTAGGGC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| COMMD2--6-R | aac GCCCTAGAAGCCTGCGGAGC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--1-F | ccg GACTGAGGTTGGCTAGCGCG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--1-R | aac CGCGCTAGCCAACCTCAGTC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--2-F | ccg CGTGTGCAGCGCTCATGGCG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--2-R | aac CGCCATGAGCGCTGCACACG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--3-F | ccg GGTAGTGCAGGGGCGCGCGG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--3-R | aac CCGCGCGCCCCTGCACTACC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |

| | | |
|---|---|---|
| HGH1--4-F | ccg CAGGCGCGCTGGCAGCCCGG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--4-R | aac CCGGGCTGCCAGCGCGCCTG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--5-F | ccg GACTCGGGCCTGGAGCGGCT | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--5-R | aac AGCCGCTCCAGGCCCGAGTC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--6-F | ccg CACACGGCGCCGGCTCGCGA | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--6-R | aac TCGCGAGCCGGCGCCGTGTG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--7-F | ccg CGGAGCCGGCAGACTCGGGC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--7-R | aac GCCCGAGTCTGCCGGCTCCG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--8-F | ccg TGGAGCGGCTGGTGCGCGCG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| HGH1--8-R | aac CGCGCGCACCAGCCGCTCCA | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| FGG--1-F | ccg GAAGCACAGTGCCAGGAACC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| FGG--1-R | aac GGTTCCTGGCACTGTGCTTC | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| FGG--2-F | ccg TTCCCAGTGATATCATGGAT | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| FGG--2-R | aac ATCCATGATATCACTGGGAA | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |

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| FGG--3-F | ccg ATCACTGGGAAAGGTAACTG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| FGG--3-R | aac CAGTTACCTTTCCCAGTGAT | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| FGG--4-F | ccg AGTAATGTAAAGGAGAAAGT | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| FGG--4-R | aac ACTTTCTCCTTTACATTACT | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| PARP12--1-F | ccg TGAAGGCAAGAAACTGCGTG | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| PARP12--1-R | aac CACGCAGTTTCTTGCCTTCA | Oligonucleotide pairs for construction of gRNA expression plasmid on epiCRISPR vector |
| EMX1-sg-F | cacc Gagtccgagcagaagaagaa | Oligonucleotide pairs for construction of gRNA expression plasmid on PX459 vector |
| EMX1-sg-R | aaac TTCTTCTTCTGCTCGGACTC | Oligonucleotide pairs for construction of gRNA expression plasmid on PX459 vector |
| VEGFA-sg-F | cacc gggtgggggggagtttgctcc | Oligonucleotide pairs for construction of gRNA expression plasmid on PX459 vector |
| VEGFA-sg-R | aaac GGAGCAAACTCCCCCCACCC | Oligonucleotide pairs for construction of gRNA expression plasmid on PX459 vector |
| AAVS1-deep-F | ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNgctctgggcggaggaatatg | Primers for amplification of the endogenous targets (The frist-step PCR) |
| AAVS1-deep-R | ACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNtccgtgcgtcagttttacct | Primers for amplification of the endogenous targets (The frist-step PCR) |
| COMMD2-deep-F | ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNcttcctgcctcaagtggaca | Primers for amplification of the endogenous targets (The frist-step PCR) |
| COMMD2-deep-R | ACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNaacaagaccctaggactcgc | Primers for amplification of the endogenous targets (The frist-step PCR) |
| HGH1-deep-F | ACACTCTTTCCCTACACGAC | Primers for amplification of the |

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| | GCTCTTCCGATCTNNNNggcct gcacgagacattg | endogenous targets (The frist-step PCR) |
| HGH1-deep-R | ACTGGAGTTCAGACGTGTGC TCTTCCGATCTNNNNttggctgag gttggagagc | Primers for amplification of the endogenous targets (The frist-step PCR) |
| FGG-deep-F | ACACTCTTTCCCTACACGAC GCTCTTCCGATCTNNNNagattg ttaacctgaaagagaaggt | Primers for amplification of the endogenous targets (The frist-step PCR) |
| FGG-deep-R | ACTGGAGTTCAGACGTGTGC TCTTCCGATCTNNNNgacttaatg ggtagccactttct | Primers for amplification of the endogenous targets (The frist-step PCR) |
| PARP12-deep-F | ACACTCTTTCCCTACACGAC GCTCTTCCGATCTNNNNgagca cctctgaaatgtggc | Primers for amplification of the endogenous targets (The frist-step PCR) |
| PARP12-deep-R | ACTGGAGTTCAGACGTGTGC TCTTCCGATCTNNNNcagagcca acacaggcag | Primers for amplification of the endogenous targets (The frist-step PCR) |
| Deep-F1 | ACACTCTTTCCCTACACGAC GCTCTTCCGATCTNNNN gcgagaaaagccttgttt | Primers for amplification of the endogenous targets (The frist-step PCR) |
| Deep-R1 | ACTGGAGTTCAGACGTGTGC TCTTCCGATCTNNNN ctgaacttgtggccgtttac | Primers for amplification of the endogenous targets (The frist-step PCR) |
| P5-adapter0-F | AATGATACGGCGACCACCGA GATCTACAC ACATCG ACACTCTTTCCCTACACGAC | Primers for amplification of the endogenous targets (The second-step PCR) |
| P7-adapter3-R | CAAGCAGAAGACGGCATACG AGAT CACTGT GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter4-R | CAAGCAGAAGACGGCATACG AGAT ATTGGC GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter5-R | CAAGCAGAAGACGGCATACG AGAT GATCTG GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter6-R | CAAGCAGAAGACGGCATACG AGAT TACAAG GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |

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| P7-adapter7-R | CAAGCAGAAGACGGCATACG AGAT CGTGAT GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter8-R | CAAGCAGAAGACGGCATACG AGAT GCCTAA GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter9-R | CAAGCAGAAGACGGCATACG AGAT TCAAGT GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter10-R | CAAGCAGAAGACGGCATACG AGAT CTGATC GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter11-R | CAAGCAGAAGACGGCATACG AGAT AAGCTA GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter12-R | CAAGCAGAAGACGGCATACG AGAT GTAGCC GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter13-R | CAAGCAGAAGACGGCATACG AGAT TTGACT GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter14-R | CAAGCAGAAGACGGCATACG AGAT GGAACT GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter15-R | CAAGCAGAAGACGGCATACG AGAT TGACAT GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter16-R | CAAGCAGAAGACGGCATACG AGAT GGACGG GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter17-R | CAAGCAGAAGACGGCATACG AGAT CTCTAC GTGACTGGAGTTCAGACGTG | Primers for amplification of the endogenous targets (The second -step PCR) |

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| P7-adapter18-R | CAAGCAGAAGACGGCATACG AGAT GCGGAC GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter19-R | CAAGCAGAAGACGGCATACG AGAT TTTCAC GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter20-R | CAAGCAGAAGACGGCATACG AGAT GGCCAC GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter21-R | CAAGCAGAAGACGGCATACG AGAT CGAAAC GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter22-R | CAAGCAGAAGACGGCATACG AGAT CGTACG GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter23-R | CAAGCAGAAGACGGCATACG AGAT CCACTC GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| P7-adapter24-R | CAAGCAGAAGACGGCATACG AGAT GCTACC GTGACTGGAGTTCAGACGTG TG | Primers for amplification of the endogenous targets (The second -step PCR) |
| Deep-F1 | ACACTCTTTCCCTACACGAC GCTCTTCCGATCTNNNNN gcgagaaaagccttgttt | Primers to amplify the random PAM for deep sequencing |
| Deep-R1 | ACTGGAGTTCAGACGTGTGC TCTTCCGATCTNNNNN ctgaacttgtggccgtttac | Primers to amplify the random PAM for deep sequencing |
| P5-adapter0-F | AATGATACGGCGACCACCGA GATCTACAC ACATCG ACACTCTTTCCCTACACGAC | Primers to amplify the random PAM for deep sequencing |
| P7-adapter3-R | CAAGCAGAAGACGGCATACG AGAT CACTGT GTGACTGGAGTTCAGACGTG TG | Primers to amplify the random PAM for deep sequencing |
| EMX1-OT5-F | AagtccgagGagaGgaagaaAGGgtga gcaagggcgaggag | Primers for plasmid construction |

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| EMX1-OT9-F | GagtAcAagcagaTgaaAaaCGGgtgagcaagggcgaggag | Primers for plasmid construction |
| EMX1-OT13-F | GagGccAagcagaaAGaAaaAGGgtgagcaagggcgaggag | Primers for plasmid construction |
| EMX1-OT31-F | GaCtccgagcagCagaagGaTGGgtgagcaagggcgaggag | Primers for plasmid construction |
| EMX1-OT36-F | GagtTAgagcagaGgaagaGAGGgtgagcaagggcgaggag | Primers for plasmid construction |
| EMX1-OT52-F | GTgtcAgagcagaaAaagaGTGGgtgagcaagggcgaggag | Primers for plasmid construction |
| VEGFA-OT5-F | gggGgCAgggagAttgctccTGGgtgagcaagggcgaggag | Primers for plasmid construction |
| VEGFA-OT7-F | GTAAgtAAgggAagtttgctccTGGgtgagcaagggcgaggag | Primers for plasmid construction |
| VEGFA-OT19-F | gggAggAgAgagtttgctcTCTGgtgagcaagggcgaggag | Primers for plasmid construction |
| VEGFA-OT33-F | AgAggggTggagtttgTtccAGGgtgagcaagggcgaggag | Primers for plasmid construction |
| VEGFA-OT49-F | gggGAgggggagAtGgctccCGGgtgagcaagggcgaggag | Primers for plasmid construction |
| VEGFA-OT60-F | GAGAggtggggTgatttgctccAGGgtgagcaagggcgaggag | Primers for plasmid construction |
| cozak-R | CATggtggcaaacaaggctt | Primers for plasmid construction |
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| **Name** | **gRNA sequence** | **Description** |
| AAVS1--sg1 | GTAGAGGCGGCCACGACCTG | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| AAVS1--sg2 | GTGAGAATGGTGCGTCCTAG | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| COMMD2--sg1 | GAAAGTCAGAAGGCTGTTGA | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| COMMD2--sg2 | GCCTTCGTAGATTTTTGGGT | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| COMMD2--sg3 | CGTGGGGGCGGTGTTCTGTG | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| COMMD2--sg4 | CAGAAGGCTGTTGAGTGCCA | gRNA for off-target decetion of SpCas9 in endogenous site of human |

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| COMMD2--sg5 | GGAAAGGGGAAGGCTCCGCA | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| COMMD2--sg6 | GCTCCGCAGGCTTCTAGGGC | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| HGH1--sg1 | GACTGAGGTTGGCTAGCGCG | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| HGH1--sg2 | CGTGTGCAGCGCTCATGGCG | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| HGH1--sg3 | GGTAGTGCAGGGGCGCGCGG | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| HGH1--sg4 | CAGGCGCGCTGGCAGCCCGG | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| HGH1--sg5 | GACTCGGGCCTGGAGCGGCT | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| HGH1--sg6 | CACACGGCGCCGGCTCGCGA | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| HGH1--sg7 | CGGAGCCGGCAGACTCGGGC | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| HGH1--sg8 | TGGAGCGGCTGGTGCGCGCG | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| FGG--sg1 | GAAGCACAGTGCCAGGAACC | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| FGG--sg2 | TTCCCAGTGATATCATGGAT | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| FGG--sg3 | ATCACTGGGAAAGGTAACTG | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| FGG--sg4 | AGTAATGTAAAGGAGAAAGT | gRNA for off-target decetion of SpCas9 in endogenous site of human |
| PARP12--sg1 | TGAAGGCAAGAAACTGCGTG | gRNA for off-target decetion of |

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| | | SpCas9 in endogenous site of human |
| EMX1-sg | gagtccgagcagaagaagaa | gRNA for off-target detection by GFP reporter-based approach |
| VEGFA-sg | gggtgggggggagtttgctcc | gRNA for off-target detection by GFP reporter-based approach |
| GFP-qPCR-F | AAGGACGACGGCAACTACAA | The primer pair for qPCR |
| GFP-qPCR-R | TCTGCTTGTCGGCCATGATA | The primer pair for qPCR |