Supplementary Information

1. Reagent formulation

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Stub1 (WB 1:1000)   | CST    | Cat# 2080  |
| Mdm2 (WB 1:1000)    | CST    | Cat# 27883-1-AP |
| Yy1 (1:1000)        | CST    | Cat# #46395 |
| β-Actin (WB 1:1000) | CST    | Cat# 3700  |
| Reagents            |        |            |
| Puromycin           | Sigma  | Cat# P8833 |
| BsmBI               | NEB    | Cat# R0580 |
| Gibco™ DMEM, high glucose | Gibco | Cat#11584486 |
| GlutaMAX™ Supplement | Gibco | Cat# 35050061 |
| Trypsin-EDTA (0.25%) | Gibco | Cat# 25200056 |
| ESGRO® Recombinant Mouse LIF Protein | Sigma | Cat# ESG1107 |
| KnockOut™ Serum Replacement | Gibco | Cat# 10828028 |
| Embryonic stem-cell FBS | Gibco | Cat# 16141079 |
| D-PBS               | Gibco  | Cat# 14040141 |
| 2-Mercaptoethanol   | Sigma  | Cat# M7522 |
| MEM Non-Essential Amino Acids Solution (100X) | Gibco | Cat# 11140050 |
| Sodium Pyruvate (100 mM) | Gibco | Cat# 11360070 |
| Penicillin-Streptomycin (10,000 U/mL) | Gibco | Cat# 15140122 |
| EmbryoMax™ 0.1% Gelatin Solution | Millipore | Cat# ES-006 |
| T4 DNA Ligase       | Thermo | Cat# EL0011 |
| RIPA buffer         | Millipore | Cat# 20188 |
| SDS-PAGE gel        | Epizyme | Cat# PG112 |
| Ampicillin          | Beyotime Biotechnology | Cat# ST007 |
| **Tris-HCl** | Sinopharm Chemical Reagent | Cat# 73509461 |
| **SDS** | Amresco | Cat# 0227-1KG |
| **Glycerol** | Sinopharm Chemical Reagent | Cat# 10010618 |
| **Bromophenol blue** | Sangon Biotech | Cat# A500922-0025 |
| **Agarose** | Biowest | Cat# BY-R0100 |
| **Yeast extract** | OXOID | Cat# LP0021 |
| **Tryptone** | OXOID | Cat# LP0042 |
| **NaCl** | Sangon Biotech | Cat# 7647-14-5 |
| **Agar powder** | Solarbio | Cat# A8190 |
| **2 × Taq Plus Master Mix** | Vazyme | Cat# P211-01 |
| **Calcium chloride dihydrate** | Sigma | Cat# C7902 |
| **Lipofectamine™ 2000 Transfection Reagent** | Thermo | Cat# 11668019 |
| **Opti-MEM™** | Gibco | Cat# 31-985-062 |

**Experimental Model: Cell line**

R1 embryonic stem cells (Mouse) | ATCC | SCRC-1011™ |

**Bacterial and virus strains**

Trans5α Chemically Competent Cells | TransGen | Cat# 431675 |

**Critical commercial assays**

GeneJet Gel Extraction Kit | Thermo | Cat# K0692 |

TIANamp Genomic DNA Kit | Tiangen | Cat# DP304-03 |

GeneJet Plasmid Miniprep Kit | Thermo | Cat# K0503 |

**Recombinant DNA**

lentiCRISPRv2 | Addgene | Cat# 52961 |

pGL3-U6-sgRNA-PGK-Puromycin | Addgene | Cat# 51133 |

pST1374-NLS-Flag-Linker-Cas9 | Addgene | Cat# 44758 |

**Oligonucleotides** | Table 4-6 |
1.1. Formulation of LB medium and agar plate:

i. Prepare LB buffer and autoclave at 121°C for 15 min (Table 2). The LB buffer can be stored at 4°C.

Note: Add ampicillin to the LB buffer before use (100 μg/mL).

ii. To prepare agar plate, add 15g agar powder to 1LB and autoclave at 121°C (Table 2). Cool adequately, add ampicillin (100 μg/mL) and keep it at room temperature to solidify. The LB buffer can be stored at 4°C for around 2 months.

Table 2. Formulation of LB medium and agar plate.

| Reagent   | Amount  |
|-----------|---------|
| Yeast extract | 5g      |
| Tryptone  | 10g     |
| NaCl      | 10g     |
| ddH2O     | to 1L   |
| **Total** | **1L**  |

Table 3. Formulation of 2X Laemmli sample buffer.

| Reagent                | Amount to add | Final concentration (2X) |
|------------------------|---------------|--------------------------|
| 10% (w/v) SDS          | 4 mL          | 4%                       |
| Glycerol               | 2 mL          | 20%                      |
| 1 M Tris-Cl (pH 6.8)   | 1.2 mL        | 120 mM                   |
| H2O                    | 2.8 mL        |                          |
|                        |               | Add bromophenol blue to a final concentration of 0.02% (w/v). |

Note: Store the 2X Laemmli sample buffer at room temperature.

2. Single Guider RNA (sgRNA) design and synthesis

Timing: 5 minutes

2.1. sgRNA design

Input target gene symbol/gene ID/transcript ID/genomic DNA sequence depending on the different CRISPR/Cas9 gRNA designing tools. For instances, CRISPick (Sanson et al., 2018;
i. Use valid gene symbol (here, we proceed to knock out three genes, namely Mdm2, Stub1 and Yy1, and to simplify our description, we used Mdm2 as an example). Alternatively, use transcript ID or exon sequences of genomic DNA from Ensembl (https://ensembl.org/index.html).

CRITICAL: Selection of sgRNAs with higher specificity is recommended to minimize off-target effects. The specificity of a gRNA can be assessed by the BLAT tool in the UCSC genome browser (http://genome.ucsc.edu) or Blast tool (https://blast.ncbi.nlm.nih.gov).

ii. Check the specificity of a sgRNA using 20nt gRNA sequence plus PAM motif NGG (protospacer adjacent motif) by BLAT tool in the UCSC genome browser or Blast tool in NCBI. For example, Mdm2 sgRNA 1: 5′-TCGGAACAAGACTCTGGT (20nt) + 5′-TGG (PAM).

Note. Typically, design at least 2 sgRNAs that target two different gene coding regions of the desired gene.

2.2. sgRNA synthesis

2.2.1. One vector system (Lentiviral backbone): The following sgRNA oligonucleotide primers were designed for pLentiCRISPR V2 (Sanjana et al., 2014; Shalem et al., 2014) (Figure 1):

i. sgRNA oligonucleotide

sgRNA Forward: 5′-CACC[NT]NNNNNNNNNNNNNNNNNNN-3′
sgRNA Reverse: 5′-AAAC[NT]NNNNNNNNNNNNNNNNNNN-3′

Note: The highlighted region (green) in the sgRNA oligonucleotide represents the 20nt sgRNA sequence of the forward primer and reverse-complementary sequence of this sgRNA in the
reverse primer. The sgRNA oligonucleotide must not contain NGG PAM. The overhangs: CACC and AAAC (black) following BsmBI digestion of pLentiCRISPR V2 are added to the 5′ end of each sgRNA primer respectively. G (red) after the overhang CACC is added to the 5′ region of forward sgRNA, and its complementary C (red) is added to the 3′ end of the reverse sgRNA primer because U6 promoter requires G to express the sgRNA. Copy and paste the sgRNAs of the desired gene (Mdm2, Stub1 and Yy1) separately into the highlighted region (Table 4).

Table 4. Lists of sgRNA oligonucleotides.

| Oligonucleotides | Forward (Sequence 5′-3′) | Reverse (Sequence 5′-3′) |
|------------------|--------------------------|--------------------------|
| Mdm2-sgRNA 1     | CACCG TCAGCAAGAGACTCTGGT | AAAC ACCAGAGTCCTTGTTCCGA C |
| Mdm2-sgRNA 2     | CACCG CAGGCTCGGATCAAAGGACA | AAAC TGTCCTTTGATCCGAGCCTG C |
| Stub1-sgRNA 1    | CACCG GAAGCGCTGGAACAGTATCG | AAAC GATACCTGCTCCAGCGCTTC C |
| Stub1-sgRNA 2    | CACCG GGAGATGGAGAGTTATGATG | AAAC CATCATAACTCTCCATCTCC C |
| Yy1-sgRNA 1      | CACCG AGATATGACCATGAAACAG | AAAC CTGTTTCATGGTCAATATCT C |
| Yy1-sgRNA 2      | CACCG CGACCCGGGAAATAGAAAGT | AAAC ACTCTTATTCCCGGCTCG C |

2.2.2. Two-vector system (non-lentiviral backbone): The sgRNA oligonucleotide primers are designed for pGL3-U6-sgRNA-PGK-puromycin in a two-vector system as follows (Figure 1):

i. sgRNA oligonucleotide (Two vector system)

sgRNA Forward: 5′-CCGGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN-3′
sgRNA Reverse: 5′-AAACNNNNNNNNNNNNNNNNNNNNNNNNNNNC-3′

Note: The highlighted region (green) in the sgRNA oligonucleotide represents the 20nt sgRNA sequence of the forward primer, and the reverse-complementary sequence of this sgRNA in the reverse primer. The sgRNA oligonucleotide should not contain NGG PAM. The overhangs: CCGG and AAAC (black) following Bsa I digestion of pGL3-U6-sgRNA-PGK-puromycin were added to the 5′ end of each sgRNA primer respectively. G (red) after the overhang CACC is added to the 5′ region of forward sgRNA, and its complementary C (red) is added to the 3′ end of the reverse sgRNA primer because U6 promoter requires G to express the sgRNA. Copy and paste the sgRNAs of the desired gene separately into the highlighted region (Table 5).
Table 5. Lists of sgRNA oligonucleotides.

| Oligonucleotides | Forward (Sequence 5’-3’) | Reverse (Sequence 5’-3’) |
|------------------|--------------------------|--------------------------|
| Mdm2-sgRNA 1    | CCGG TGCGAACAAAGAGACTCTG | AAAC ACCAGAGTCCTCTTGTCCTCGA C |
| Mdm2-sgRNA 2    | CCGG CAGGCTCGGATCAAGAGCA AC | AAAC TGTCCTTTGATACCGGAGCTG C |
| Stub1-sgRNA 1   | CCGG GAAGCGCTGGAACAGTATCG | AAAC CGATACTGTTCACGCGCTTC C |
| Stub1-sgRNA 2   | CCGG GGAGATGGAGATGCTATGAG | AAAC CATCATAACTCTCATCTCC C |
| Yy1-sgRNA 1     | CCGG AGATATGGACCAGAAGACG | AAAC CTGTTCATGGTCAATATCTC C |
| Yy1-sgRNA 2     | CCGG GCACCCGGGGAATAAGAAGT | AAAC ACTTCATTCCCGGOGTCG C |

2.3. Order the designed sgRNA oligonucleotides.

Note. In fact, de-salted standard oligos are sufficient for efficient cloning.

2.4. Preparation of single and two-vector constructs

Timing: 2 days

2.4.1. Annealing oligo pair:

a. Forward and reverse oligonucleotides of sgRNA were dissolved in ddH₂O and diluted to a final concentration of 10μM. The oligos were mixed as follow:

| Component                  | Amount |
|----------------------------|--------|
| Forward oligo (10μM)       | 1μl    |
| Reverse oligo (10μM)       | 1μl    |
| T4 ligation buffer (NEB) (10X) | 1μl    |
| ddH₂O                      | 7μl    |
| **Total**                  | 10μl   |

Note: T4 ligation buffer (NEB) or any high salt appropriate buffer can be used.

b. The oligo mix can be annealed by heating at 95°C for 5 minutes and cooling at room temperature (~25°C).
2.4.2. Digestion of plentiCRISPRv2 or pGL3-U6-sgRNA-PGK-puromycin

a. Preparation of lentiCRISPRv2-BsmBI digestion mix:

| Component         | Amount |
|-------------------|--------|
| plentiCRISPRv2    | 1μg    |
| BsmBI             | 0.5μl  |
| NEbuffer3.1 (10X) | 2μl    |
| ddH2O             | 16.5μl |
| **Total**         | **20μl** |

b. Preparation of pGL3-U6-sgRNA-PGK-puromycin-BsaI digestion mix:

| Component                        | Amount |
|----------------------------------|--------|
| pGL3-U6-sgRNA-PGK-puromycin      | 1μg    |
| BsaI                             | 0.5μl  |
| 10X Buffer G                     | 2μl    |
| ddH2O                            | 16.5μl |
| **Total**                        | **20μl** |

c. Keep the plentiCRISPRv2-BsmBI digestion mix at 55°C for 1 hour or pGL3-U6-sgRNA-PGK-puromycin-BsaI digestion at 37°C for 30 minutes.

d. Run the digestion mix on 1% (wt/vol) agarose gel (gel electrophoresis).

Note: Successful digestion will appear in two bands/fragments for plentiCRISPRv2 on the gel: 1. larger band (~13kb) and 2. shorter band (~2kb, filler piece).

e. Cut the gel containing larger fragments for plentiCRISPRv2 or a single fragment of pGL3-U6-sgRNA-PGK-puromycin and purify the digested plasmid using GeneJET gel extraction kit. Dissolve the extracted plasmid into 10μl ddH2O.
2.4.3. Ligation of sgRNA oligonucleotides

a. Prepare the ligation reaction for each sgRNA as follows:

| Component                                                      | Amount (μl) |
|---------------------------------------------------------------|-------------|
| BsmBI-digested plentiCRISPRv2 (from step 2.2)                 | 2           |
| BsmBI-digested pGL3-U6-sgRNA-PGK-puromycin (from step 2.2)   |             |
| sgRNA (from step 1)                                           | 8           |
| 10X T4 DNA ligase buffer                                      | 2           |
| T4 DNA ligase                                                 | 1           |
| ddH2O                                                         | 7           |
| Total                                                         | 20          |

b. Incubate the sgRNA-lentiCRISPRv2/ pGL3-U6-sgRNA-PGK-puromycin ligation reaction mix at 22°C for 1 h.

c. Add the sgRNA- lentiCRISPRv2 ligation reaction mix/ pGL3-U6-sgRNA-PGK-puromycin ligation into an Eppendorf tube containing Trans5α chemically competent cells, incubate the mixture on ice for 30 min, followed by heat shock at 42°C water bath for the 90s, and immediate re-incubation on ice for 5 min.

**Note:** To reduce the chances of potential homologous recombination, the transformation of lentiviral plasmids into recombination-deficient bacteria (e.g., Stbl3) is recommended.

d. Add 50-100μl LB to the ligation-bacteria mix from step 2.3.c and incubate the mixture at 37°C for 45 minutes in a shaker.

e. Spread the mixture from step 2.3.d onto an agar LB dish (ampicillin) and incubate at 37°C for 1 day.

f. Pick up ~3-5 bacterial colonies from step 2.3.e, grow single colony into liquid LB (100μg/mL ampicillin) and incubate bacterial suspension at 37°C for 12-16 hours.

g. Extract plasmid DNA from the bacteria using GeneJET plasmid miniprep kit as per manufacturer’s instruction.
h. Confirm the gRNA sequence of plentiCRISPR-sgRNA plasmid/ pGL3-U6-sgRNA-PGK-puromycin-sgRNA plasmid by Sanger sequencing. Using U6 primer sequence each bacterial colony.

**Note**: The 20bp gRNA sequence should be placed between the U6 promoter and the remainder of the gRNA scaffold in the plentiCRISPR v2 construct.

2.5. Functional validation of knockout cells

2.5.1. Determination of indel frequency of a sgRNA

| Oligonucleotides       | Forward (Sequence 5'-3')                  | Reverse (Sequence 5'-3')                  | Amplicon size |
|------------------------|-------------------------------------------|-------------------------------------------|---------------|
| Amplicon-Mdm2-gRNA1    | ATGTGCAATACCAACATGTCTG                   | GTCTCACTAATGGATCTCTCTCTCTAG              | 550bp         |
| Amplicon-Mdm2-gRNA2    | GAAAGAAACCTTACATTTCTCTG                  | GTTCACGAAAGGGTCCAG                       | 553bp         |
| Amplicon-Stub1-gRNA1   | ATCTCCAGTTCTCTATATCCAG                   | TGAGATATGGAATGAGCTCAC                    | 550bp         |
| Amplicon-Stub1-gRNA2   | TGTACTACACTAACCAGGGGC                   | TGACCCAGAAATCACAGAGC                     | 550bp         |
| Amplicon-Yy1-gRNA1     | GCTGGTCTCTGCTAGAGCTAAAC                 | GCTCCCTTCACACATCAAC                      | 554bp         |
| Amplicon-Yy1-gRNA2     | ATCTCGTGCAGCGCGCGAG                     | GCTTCGGCTCGCACAAACTA                     | 560bp         |
| Amplicon-Cas9          | ACAAGTTCATCAAGGCCCATC                   | GAATCTGGCTGTTCCTCGTC                    | 269bp         |
2.6. Verifying the ‘Off-target Effects’ of a sgRNA

Table 7. Genome-wide *in-silico* analysis of sgRNA plus PAM sequence.

| sgRNA Name          | Overlapping with PAM sequence (Entire mouse genome) | Full coverage (Entire mouse genome) |
|---------------------|-----------------------------------------------------|-------------------------------------|
| Mdm2-sgRNA 1        | No                                                  | Mdm2 (only sgRNA target site)      |
| Mdm2-sgRNA 2        | No                                                  | Mdm2 (only sgRNA target site)      |
| Stub1-sgRNA 1       | No                                                  | Stub1 (only sgRNA target site)     |
| Stub1-sgRNA 2       | No                                                  | Stub1 (only sgRNA target site)     |
| Yy1-sgRNA 1         | No                                                  | Yy1 (only sgRNA target site)       |
| Yy1-sgRNA 2         | No                                                  | Yy1 (only sgRNA target site)       |

I. Mdm2 (Partial coverage of sgRNA sequence +No coverage of PAM sequence) (partial coverage)

Range 2: 93600689 to 93600704

| Score     | Expect | Identities       | Gaps     | Strand       |
|-----------|--------|------------------|----------|--------------|
| 32.2 bits(16) | 3.3    | 16/16(100%)      | 0/16(0%) | Plus/Minus   |

Features: 18974 bp at 5' side: netrin-4 precursor80848 bp at 3' side: ubiquitin carboxyl-terminal hydrolase 44 isoform x1

Query 6       ACAAGAGACTCTGGTT 21
Sbjct 93600704 ACAAGAGACTCTGGTT 93600689

II. Mdm2 (Partial coverage of sgRNA sequence +No coverage of PAM sequence) (partial coverage)

Range 3: 19728780 to 19728794

| Score     | Expect | Identities       | Gaps     | Strand       |
|-----------|--------|------------------|----------|--------------|
| 30.2 bits(15) | 13     | 15/15(100%)      | 0/15(0%) | Plus/Plus    |

Features: 2012 bp at 5' side: solute carrier family 35 member d3 isoform x17878 bp at 3' side: peroxisomal biogenesis factor 7 isoform 2

Query 8       AAGAGACTCTGGTTG 22
Sbjct 19728780 AAGAGACTCTGGTTG 19728794
III. Mdm2 (Partial coverage of sgRNA sequence + No coverage of PAM sequence) (partial coverage)

Range 4: 54329247 to 54329261

| Score   | Expect | Identities     | Gaps    | Strand |
|---------|--------|----------------|---------|--------|
| 30.2 bits(15) | 13     | 15/15(100%)    | 0/15(0%)| Plus/Plus |

Features:
378127 bp at 5' side: mannosyl-oligosaccharide 1,2-alpha-mannosidase ia1564420 bp at 3' side: protein broad-minded

Query 9 AGAGACTCTGGTTGG 23
Sbjct 54329247 AGAGACTCTGGTTGG 54329261

2.6.1. Design primers of Mdm2:

| Table 8. List of PCR primers. |
|-------------------------------|
| Oligonucleotides | Forward (Sequence 5'-3') | Reverse (Sequence 5'-3') | Amplicon size |
|-------------------|---------------------------|---------------------------|---------------|
| I. Mdm2 partial sequence homology gRNA1_ubiquitin carboxyl-terminal hydrolase 44 isoform x1 | GGGCAGCAAAGATATTAGTGG | GGATAGGATTTGGTAAAGTGG | 404bp |
| II. Mdm2 partial sequence homology gRNA1_ubiquitin carboxyl peroxisomal biogenesis factor 7 isoform 2 | AAACCCTCCTTGCCCTCCATC | TCTTCTTGTTCCGCCCCTTC | 510bp |
| III. Mdm2 partial sequence homology gRNA1_protein broad-minded biogenesis factor 7 isoform 2 | CATTACAGCTCGAAGCAGAC | TCCGATGATGACCTTCTTCC | 488bp |
2.6.2. Analysis of sequencing results by Blast

I. Mdm2 partial sequence homology gRNA1_ ubiquitin carboxyl-terminal hydrolase 44 isoform x1

| Score | Expect | Identities | Gaps | Strand |
|-------|--------|------------|------|--------|
| 712 bits(385) | 0.0 | 385/385(100%) | 0/385(0%) | Plus/Minus |
| Query 32 | GGGCACAAAAGAATTAGTGGGCCAGAAGGCTTTCAGCTCAGCACAGGCATTGGCTGAGAG | 91 | 19304 |
| Sbjct 19363 | GGGCACAAAAGAATTAGTGGGCCAGAAGGCTTTCAGCTCAGCACAGGCATTGGCTGAGAG | |
| Query 92 | GACAGTGTGACAGTGGCAGACACAGACGCTGAGTCGCTGCGCAGGGCAACAGGATACGCT | 515 | 19244 |
| Sbjct 19303 | GACAGTGTGACAGTGGCAGACACAGACGCTGAGTCGCTGCGCAGGGCAACAGGATACGCT | |
| Query 152 | GGCTATGCACCTCCAAATAACTCTATATATGGATGGTGAGGGGAAGAGGCGAGGCTA | 211 | 19184 |
| Sbjct 19243 | GGCTATGCACCTCCAAATAACTCTATATATGGATGGTGAGGGGAAGAGGCGAGGCTA | |
| Query 212 | ACCGACATTCCTGGAGATGCGCGTGTGGCCCAAAGATAAAGAGCAGAGTAGAGACCTGAT | 271 | 19124 |
| Sbjct 19183 | ACCGACATTCCTGGAGATGCGCGTGTGGCCCAAAGATAAAGAGCAGAGTAGAGACCTGAT | |
| Query 272 | AAAAGGGGACATATTTCTGGTAGATGAGAGATCGTGGGGAAGACTCCAGCCAC | 331 | 19084 |
| Sbjct 19123 | AAAAGGGGACATATTTCTGGTAGATGAGAGATCGTGGGGAAGACTCCAGCCAC | |
| Query 332 | AGCCTTGGAAAAAGAGACCTGTGGTGCCAGGAGACAAATATTACGGAGGCGAGCT | 391 | 19004 |
| Sbjct 19063 | AGCCTTGGAAAAAGAGACCTGTGGTGCCAGGAGACAAATATTACGGAGGCGAGCT | |
| Query 392 | GAGACGCGAGAATGAGACGGTTCCC | 416 | |
| Sbjct 19003 | GAGACGCGAGAATGAGACGGTTCCC | 18979 |
II. Mdm2 partial sequence homology gRNA1 - ubiquitin carboxyl peroxisomal biogenesis factor 7 isoform 2

| Score | Expect | 0.0 | Identities | Gaps | Strand Plus/Minus |
|-------|--------|-----|------------|------|------------------|
| 856 bits(463) | 0.0     | 463/463(100%) | 0/463(0%) |       |                  |
| Query 1 | CCGCTGCGCTTCTTCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 60 |       |       |                  |
| Sbjct 22670 | CCGCTGCGCTTCTTCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 226161 |       |       |                  |
| Query 61 | CGTTCAGTCCACGAGCTGCGGTGGTGCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 120 |       |       |                  |
| Sbjct 22610 | CGTTCAGTCCACGAGCTGCGGTGGTGCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 22551 |       |       |                  |
| Query 121 | AGACCCACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 180 |       |       |                  |
| Sbjct 22550 | AGACCCACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 223691 |       |       |                  |
| Query 181 | TGTCGCTTGGGCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 240 |       |       |                  |
| Sbjct 22490 | TGTCGCTTGGGCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 22431 |       |       |                  |
| Query 241 | CAACAGAACCCCTCCTCCTCTCTTCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 300 |       |       |                  |
| Sbjct 22430 | CAACAGAACCCCTCCTCCTCTCTTCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 22371 |       |       |                  |
| Query 301 | TGTCGCTTGGGCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 360 |       |       |                  |
| Sbjct 22370 | TGTCGCTTGGGCTGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 22231 |       |       |                  |
| Query 361 | AGACCCACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 420 |       |       |                  |
| Sbjct 22310 | AGACCCACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 22251 |       |       |                  |
| Query 421 | GTGGGCGGAGGACGAGGGACAGGACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 463 |       |       |                  |
| Sbjct 22250 | GTGGGCGGAGGACGAGGGACAGGACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 22288 |       |       |                  |

III. Mdm2 (Partial coverage of sgRNA sequence + No coverage of PAM sequence) (partial coverage)

| Score | Expect | 0.0 | Identities | Gaps | Strand Plus/Minus |
|-------|--------|-----|------------|------|------------------|
| 963 bits(521) | 0.0     | 521/521(100%) | 0/521(0%) |       |                  |
| Query 1 | AAGACGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 60 |       |       |                  |
| Sbjct 157940 | AAGACGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 157881 |       |       |                  |
| Query 61 | AAACGTTCCACGAGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 120 |       |       |                  |
| Sbjct 157800 | AAACGTTCCACGAGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 157821 |       |       |                  |
| Query 121 | ACTTTAGTCGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 180 |       |       |                  |
| Sbjct 157820 | ACTTTAGTCGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 157761 |       |       |                  |
| Query 181 | GTGGCATTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 240 |       |       |                  |
| Sbjct 157760 | GTGGCATTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 157701 |       |       |                  |
| Query 241 | AAGACGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 300 |       |       |                  |
| Sbjct 157790 | AAGACGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 157641 |       |       |                  |
| Query 301 | TTCCATCATATTCTGATGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 360 |       |       |                  |
| Sbjct 157640 | TTCCATCATATTCTGATGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 157581 |       |       |                  |
| Query 361 | CAGAGGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 420 |       |       |                  |
| Sbjct 157580 | CAGAGGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 157521 |       |       |                  |
| Query 421 | CAGAGGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 480 |       |       |                  |
| Sbjct 157520 | CAGAGGCTGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 157461 |       |       |                  |
| Query 481 | TCATCATATTCTGATGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 521 |       |       |                  |
| Sbjct 157460 | TCATCATATTCTGATGACGAGAAGGAGGACACGTCGCAGCAGGACACCCACAGTGGTGCTGTGACCAATGACAGGCAAGACG | 157420 |       |       |                  |
References

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