## Materials and Methods

### Monkeys

All animals were housed at the Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility of Guangdong Landao Biotechnology Co., Ltd. (Landao). Five- to 8-year-old healthy cynomolgus monkeys (*M. fascicularis*) were used for this study. All institutional and national guidelines for the care and use of laboratory animals were followed. All animal protocols were approved in advance by the Institutional Animal Care and Use Committee of Landao.

### *In vitro* transcription

gRNAs cloned into pDR274 vector (Addgene, Watertown, MA, USA). Oligos used for gRNA cloning are listed in **Supplementary information, Table S1**. The gRNA was transcribed using the MEGAshortscript T7 kit (Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. Cas9 mRNA was transcribed using the mMESSAGE mMACHINE T7 ULTRA kit (Life Technologies) following the manufacturer's instructions.

### Preparation, microinjection, and embryos transfer

Embryos of *M. fascicularis* were produced as described in our previous report[20]. Briefly, female adult monkeys at menses were intramuscularly administrated 1.4 μg rhFSH (recombinant human follitropin alfa; GONAL-F, Merck Serono, Geneva, Switzerland) twice per day for eight days followed by 80 μg rhCG (recombinant human chorionic gonadotropin alfa; OVIDREL, Merck Serono) on day 9. Cumulus-oocyte complexes were collected by laparoscopic follicular aspiration at 33–36 h after rhCG administration and oocytes were stripped of cumulus cells by pipetting after brief exposure (<1 min) to hyaluronidase (0.5 mg/mL). Metaphase II (MII, first polar body is present) oocytes were subjected to intracytoplasmic sperm injection for fertilization. At 10–12 h later, a mixture of Cas9 mRNA (100 ng/μL) and gRNA (50 ng/μL each) was injected into the cytoplasm of monkey zygotes. The zygotes were cultured for 7 days to collect blastocysts for genotyping or cryopreserve in liquid nitrogen. For transfer, surrogate mothers with two consecutive normal menstrual cycles (26–32 days) and with a freshly ovulated follicle and uterus with the normal echo in uterus cavity checked by ultrasound immediately before embryo transfer were selected as surrogate mothers. Three to four blastocysts thawed were transferred into one oviduct of each recipient via laparoscopy with a fixed polythene catheter.

### Whole-genome amplification

Whole genome amplification of the embryos was performed using the PEPLI-g Midi Kit (Qiagen, Hilden, Germany). Briefly, blastocysts were transferred into PCR tubes containing reconstituted buffer D2 (7 μL), and then incubated at

65°C for 10 min before adding Stop solution (3.5 μL) and MDA master mix (40 μL) and incubation at 30°C for 8 h. The DNA preparation was diluted with $ddH_2O$ (3:100), and 1 μL of the diluted DNA was used for PCR analysis.

**Genotyping**

Each embryo was transferred into a PCR tube containing 1 μL lysis buffer and then incubated at 65°C for 3 h followed by 95°C for 10 min. The lysis product was amplified by PCR. Genotyping was conducted using a genotyping kit (KK7351, KAPA Biosystems, Wilmington, MA, USA) following the manufacturer's instructions. The primers used are listed in **Supplementary information, Table S2**.

**qPCR**

Total RNA from the blood or ear of monkeys was extracted using Trizol (15596-018, Invitrogen, Carlsbad, CA, USA). First-strand cDNAs were synthesized using the RevertAid First Strand cDNA Synthesis Kit (K1621, Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Real-time PCR was performed using the KAPA SYBR FAST Universal qPCR kit (KAPA Biosystems, KK4601) following the manufacturer's instructions. The primers used are listed in **Supplementary information, Table S3.**

**Capillary electrophoresis**

Capillary electrophoresis was performed using the Minicap System (SEBIA, Evry Cedex, France) according to the manufacturer's instructions.

**Plasma erythropoietin, bilirubin, iron, and serum ferritin analysis**

Blood samples from the monkeys were centrifuged at 1,700 rpm for 5 min. Plasma was evaluated using a UniCel Dxi 800 Immunoassay System (Beckman Coulter, Brea, CA, USA). Bilirubin was detected using the VITROS® 5.1 FS Chemistry System (Johnson & Johnson, New Brunswick, NJ, USA). Serum iron and ferritin were detected using an AU5821 Chemistry Analyzer (Beckman Coulter).

**Statistical analysis**

Statistical calculations were performed with GraphPad Prism software (GraphPad, Inc., La Jolla, CA, USA).

| **Supplementary information, Table S1. Primers used cloning of gRNA** |
| --- |
| HBB-G1-FP taggGTTGCCCATAACAGCATC |
| HBB-G1-RE aaacGATGCTGTTATGGGCAAC |
| HBB-G2-FP taggTGCTGTTATGGGCAACCCTA |
| HBB-G2-RE aaacTAGGGTTGCCCATAACAGCA |
| HBB-G3-FP taggTACCCTTGAGGTTGTCC |
| HBB-G3-RE aaacGGACAACCTCAAGGGTA |

**Supplementary information, Table S2. Genotyping primers**

| | FP | RP |
|---|---|---|
| HBB | TGGGCAGGTTGGTATCAAGG | TCCTGAGAGTTCCACACTGA |
| HBD | CAGGTTACTGGTAGTCCACCC | AGAAAAGTGGAGCATCTCCTG |
| G1-OT1 | TGAATTTTCCATTGTCCCCACCT | ATATGTCCCCCAAAGCTCGTT |
| G1-OT2 | GAAGCAGCCTCTCCTGTTGT | AGGGGGAAGGGGAATGAAGA |
| G1-OT3 | TCTTCCCACTACCGACTCCA | AACTGTTTGATGTTCTCCTTACTTTT |
| G1-OT4 | TTGACTTCTTCTGGAACCCTGC | GAAAGTATCTCGTTTGATGAGGGG |
| G1-OT5 | GCTCTTCACTGTCCCCACTT | GACAGTAGACCCCATGCTCC |
| G1-OT6 | CCTGTAAGAAGGGCAGGCAT | GTGGTCCTCAGTGGGACTCT |
| G1-OT7 | AGAGGAGGACAATGGCATAACTA | AGCCTGAAAAGACCCGGTG |
| G1-OT8 | ATACGAGCATCTGCACAAGAGAA | TGCATTACTGGTAGTTTCTCCCC |
| G1-OT9 | TGTGACCTTCATGGAACAAATTAGA | CCCCCAGACACCCCATCAG |
| G1-OT10 | AGCCACCAAGAGCTGTAGATAAA | CTCCTAGTCTCTTGTGGACGA |
| G2-OT1 | TCTCGTGAGCCCTTGTTTCC | GGCACAGCTCAGAGGTCATT |
| G2-OT2 | GAGCAAACTAAAATCAAAGCAAG | TTAACAAATTCACCAGTGACACC |
| G2-OT3 | TTTTGGCATTTGGTGCAGGG | CAGCTCTTCACTGTCCCCAC |
| G2-OT4 | CTCGTGGTAAGGCATTGGGT | CTGTCCACACGGCACAAAAG |
| G2-OT5 | GTCCCTATCCTTGGGTCCCT | CCTTGGTCAGTTCATCCCCC |
| G2-OT6 | GAAACGCCCTGTGAACTCGT | GCAAGGGATTTCATGCGGTTT |
| G2-OT7 | TGGCCTCAGTGGCATTTGAA | ACTATGGATGCTGTGGTGGC |
| G2-OT8 | TCGTAGTTGTCCGATGGTGAAA | TCTGTGTGGAGCTAGGTGACTA |
| G2-OT9 | CCAATGCATTCCTCACTGCG | CCTTGACCGAGGACCAGGAT |
| G2-OT10 | CCAATGCATTCCTCACTGCG | CCTTGACCGAGGACCAGGAT |
| G3-OT1 | AAAAGCAAAACCTGAATTTCCTG | CTTGAGCCCTGTCACCTCTGTTC |
| G3-OT2 | GAGTAAGTCTGGGCTGCGG | TGGTGCACATTACCAGGATGT |
| G3-OT3 | AGGACCCTCGTCACCTTTCT | GCGGTTGGTAGAGGTTGTCT |
| G3-OT4 | CCATCGCAAGTGCTCTTTGTA | TTCAAAATTGTACCAATCCATCCAA |
| G3-OT5 | AGAGTTTCGCTCTTGTTGCTCAG | GCAACCACAGTTCCCTATCTCAA |
| G3-OT6 | GTAGCGTGCAGACAAGCAAAG | TGTCTGCAAGAAGCTACGTC |
| G3-OT7 | AGTGAAGAGCCACGTGAGTAAA | TCCTCAGGACAGAACGGCT |
| G3-OT8 | GGCCAATCAACAAAACGTGC | CGTGTTGGCCTGAATGGCTA |
| G3-OT9 | AGCAGGGCAGTAGATACCATC | ACTCGAAGTCTACCCACTCCAG |
| G3-OT10 | AGAGGATGACAATGCCCAGGAA | TATGTGCCCAGACACGGTATGAT |
| Deletion-primer 1 | GGTTACTGGTAGTCCACCCT | CCCCATTCTAAACTGTACCCTG |
| Deletion-primer 2 | AGGTTACGAGACATGGAAGCTG | TTCCCCATTCTAAACTGTACCCTG |
| Inversion-primer 1 | AAAGTAAAAAGAGAAAAGTGGAGC | TAATCATAGAAAAGAAGGGGAAAG |
| Inversion-primer 2 | AGGCGGTTGAGAGAGAAAGAG | TAATCATAGAAAAGAAGGGGAAAG |
| Inversion-primer 3 | AGGACCAGCATAAAAGGGAGA | GGCATGTGGAGACAGAGAAGA |
| Inversion-primer 4 | TGAGCATGTGTAGACAGGGAA | CAAGGTGAACGTGGATGAAGT |

| **Supplementary information, Table S3. Primers used for qPCR** | |
|---|---|
| GAPDH-FP | GGAAGGTGAAGGTCGGAGTC |
| GAPDH-RP | ACTTGCCATGGGTGGAATCA |
| HBA-FP | TTAAGGGCCACGGCAAGAAG |
| HBA-RP | CAGAGTCACCAGCAGGCAG |
| HBB-FP | TGCCTTTAGTGATGGCCTGAA |
| HBB-RP | ATCCACATGCAGCTTGTCAC |
| HBG-FP | GGCAAGAAGGTGCTGACTTC |
| HBG-RP | AGTCACCAGCACATTTCCCAG |