

**Table S1. Sanger sequencing of palindromic SCF product junctions**

|                                                 |
|-------------------------------------------------|
| <b>TF1+TF2</b>                                  |
| <b>Type #1 product (mESC, n=11; U2OS, n=23)</b> |
| <p> </p>                                        |
| <b>Type #2 product (mESC, n=6)</b>              |
| <p> </p>                                        |
| <b>TF1+TF3</b>                                  |
| <b>Type #1 product (mESC, n=28; U2OS, n=2)</b>  |
| <p> </p>                                        |

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|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>GATGCCCTTCAGCTCGATGCGGTTACACAGGGTGTGCGCCCTCGAACTTCACCTCGGCGCGGGTC<br/> TTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGGCTCCTGGACGTAGCCTTCGGGCATGGCGG<br/> ACTTGAAGAAGTCGTGCTGCTTCGATGTGGTCGGGGTAGCGGCTGAAGCACTGCAC<b>GCCGTAGG</b><br/> <u><b>TCAGGGTGGTCACGAGGGT</b></u></p> <p style="text-align: right;">TF1 ←</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <p><b>Type #2 product (mESC, n=1)</b></p> <p> <div style="display: flex; align-items: center;"> <div style="flex: 1;"> <p><b>TACCGGTCGCCACCATGGT</b></p> <p>TGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGA<br/> TGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGG<br/> <b>CCCACCTCGTGACCACCCTGACCTACGGC</b>GTGCAGTGCTTCAGCCGCTACCCCGACCACATGA<br/> AGCAGCACGACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTT<br/> CAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAAC<br/> CGCATCGAGCTGAAGGGCATCGTAGGGATAACAGGGTAATCA<sup>^</sup>TGATTACCCTGTTATCCCTAC<br/> GATGCCCTTCAGCTCGATGCGGTT<b>CACCAGGGTGTGCGCCCTCGAACTTCACCTCGGCGCGGGTC</b><br/> TTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGGCTCCTGGACGTAGCCTTCGGGCATGGCGG<br/> ACTTGAAGAAGTCGTGCTGCTTCGATGTGGTCGGGGTAGCGGCTGAAGCACTGCAC<b>GCCGTAGG</b><br/> <u><b>TCAGGGTGGTCACGAGGGT</b></u></p> <p style="text-align: right;">TF1 ←</p> </div> <div style="flex: 0.1; text-align: center;"> <p>TF3 →</p> <p>→ TF1</p> </div> <div style="flex: 1;"> <p>TGTTACCGGGGTGGTGCCCATCC</p> </div> </div> </p> |

**Notes:** TF1+TF2 (**top**) and TF1+TF3 (**bottom**) indicate the primer pair used for PCR. The numbers in parentheses denotes the sample numbers for product types in mESC and U2OS cells. Red symbol “<sup>^</sup>” indicates the breakpoint generated by SpCas9-gHRc4. The inverted *GFP* sequences are shown in green on two sides of the breakpoint. The position and direction of the primers are indicated on the sequences. TF1, TF2 and TF3 pointing downwards are above the sequences, and TF1 pointing upwards below the sequences. The arrows indicate the direction of the primers. The shaded area represents the deleted sequences in the palindromic sister chromatid fusion (SCF) products. Red bold letter denotes an inserted base. Microhomology at the junctions is underlined.

**Table S2. The list of gRNAs used in this study**

| sgRNA                   | Sequence (5'-3')                 | PAM | TS |
|-------------------------|----------------------------------|-----|----|
| <b>SpCas9 sgRNA</b>     |                                  |     |    |
| gEJ <sub>C</sub> 5      | TGGATTACCCTGTTATCCCT             | AGG | C  |
| gEJ <sub>W</sub> 3      | AGGGATAACAGGGTAATCCA             | TGG | W  |
| gEJ <sub>W</sub> 4      | GGATGGATCCTAGGGATAAC             | AGG | W  |
| gEJ <sub>W</sub> 6      | TCATGTGAAGGATGGATCCT             | AGG | W  |
| gEJ <sub>W</sub> 5      | CATGTGAAGGATGGATCCTA             | GGG | W  |
| gEJ <sub>W</sub> 7      | CTTGCTGATCATGTGAAGGA             | TGG | W  |
| gEJ <sub>C</sub> 5-C2A  | TGGATTACCCTGTTATCCAT             | AGG | C  |
| gEJ <sub>C</sub> 5-T15A | TGGATAACCCTGTTATCCCT             | AGG | C  |
| gEJ <sub>C</sub> 5-16nt | TTACCCTGTTATCCCT                 | AGG | C  |
| gEJ <sub>W</sub> 7-A1T  | CTTGCTGATCATGTGAAGGT             | TGG | W  |
| gEJ <sub>W</sub> 7-A4C  | CTTGCTGATCATGTGACGGA             | TGG | W  |
| gEJ <sub>W</sub> 7-T15A | CTTGCAGATCATGTGAAGGA             | TGG | W  |
| gEJ <sub>W</sub> 10     | ATAACAGGGTAATCCATGG              | TGG | W  |
| gI-SceI site            | TGGCGGC <sub>gg</sub> TAGGGATAAC | AGG | W  |
| gHR <sub>C</sub> 3      | TAGGGATAACAGGGTAATCA             | AGG | C  |
| gHR <sub>C</sub> 4      | GATAACAGGGTAATCAAGG              | AGG | C  |
| gHR <sub>C</sub> 1      | TCGAGCTGAAGGGCATCGTA             | GGG | C  |
| gHR <sub>C</sub> 2      | AGGGCATCGTAGGGATAACA             | GGG | C  |
| gHR <sub>C</sub> 5      | AACAGGGTAATCAAGGAGGA             | CGG | C  |
| gHR <sub>C</sub> 4-G1C  | GATAACAGGGTAATCAAGC              | AGG | C  |
| gHR <sub>C</sub> 4-G2C  | GATAACAGGGTAATCAACG              | AGG | C  |
| gHR <sub>C</sub> 4-17nt | TAACAGGGTAATCAAGG                | AGG | C  |
| gHR <sub>C</sub> 2-A1T  | AGGGCATCGTAGGGATAACT             | GGG | C  |
| gHR <sub>C</sub> 2-C2A  | AGGGCATCGTAGGGATAAAA             | GGG | C  |
| gHR <sub>C</sub> 2-17nt | GCATCGTAGGGATAACA                | GGG | C  |
| gG <sub>C</sub> 1       | GGCCACAAGTTCAGCGTGTC             | CGG | C  |
| gG <sub>W</sub> 1       | GCGCTCCTGGACGTAGCCTT             | CGG | W  |
| gG <sub>C</sub> 4       | CATGCCCCGAAGGCTACGTCC            | AGG | C  |
| gG <sub>C</sub> 5       | ACCATCTTCTTCAAGGACGA             | CGG | C  |
| gG <sub>C</sub> 7       | GAAGTTCGAGGGCGACACCC             | TGG | C  |
| gG <sub>W</sub> 5       | CAGCTCGATGCGGTTACCC              | AGG | W  |
| gG <sub>C</sub> 9       | GCTGAAGGGCATCGACTTCA             | AGG | C  |

|                     |                       |        |   |
|---------------------|-----------------------|--------|---|
| gG <sub>C</sub> 10  | GAAGGGCATCGACTTCAAGG  | AGG    | C |
| gG <sub>W</sub> 2   | TGAAGAAGATGGTGCCTCC   | TGG    | W |
| gG <sub>W</sub> 6   | CGATGCCCTTCAGCTCGATG  | CGG    | W |
| gG <sub>C</sub> 12  | AGGAGGACGGCAACATCCTG  | GGG    | C |
| gG <sub>W</sub> 7   | TGTACTCCAGCTTGTGCCCC  | AGG    | W |
| gG <sub>C</sub> 14  | ATGGCCGACAAGCAGAAGAA  | CGG    | C |
| gG <sub>C</sub> 15  | AAGCAGAAGAACGGCATCA   | AGG    | C |
| gG <sub>W</sub> 10  | TGCCGTCCTCGATGTTGTGG  | CGG    | W |
| gG <sub>C</sub> 17  | ATCCGCCACAACATCGAGGA  | CGG    | C |
| gR1                 | CCAACCCCTAGACAGAGCAT  | TGG    | C |
| gR2                 | CAGACTTCTAAGATCAGGAA  | AGG    | W |
| gR3                 | GTGTATGTAACATAATCTGTC | TGG    | W |
| gR4                 | ACTGAGCTATTAACCATTAA  | TGG    | C |
| gR4-A1C             | ACTGAGCTATTAACCATTAC  | TGG    | C |
| gR4-A16T            | ACTGTGCTATTAACCATTAA  | TGG    | C |
| gC1                 | TTCCCAGGCATCACCAGTCT  | TGG    | C |
| gC4                 | GCCAGGAAGAGTCCCAAGAC  | TGG    | W |
| gC2                 | AGCAGTGGCCAGGTACAGCT  | CGG    | W |
| gC3                 | CAGACAAGCGAGCAGTGGCC  | AGG    | W |
| gC4-C1T             | GCCAGGAAGAGTCCCAAGAT  | TGG    | W |
| gC4-G16C            | GCCACGAAGAGTCCCAAGAC  | TGG    | W |
| gPnpla3             | GGGATTAGGCCAGAGAAGAG  | GGG    | W |
| gMertk              | CGCCAGGAACCTCACACTTCA | TGG    | W |
| gDNA-PKcs-1         | TGTTGGCTACTGCAGCTGC   | AGG    | C |
| gDNA-PKcs-1a        | CAGACCGCTGCAGTGCTGC   | CGG    | C |
| gKu80-1             | TGTGGATGTGGGGGTTGCCA  | TGG    | C |
| gKu80-2             | TCAATTGGAGATTCTTCACC  | AGG    | W |
| <b>SaCas9 sgRNA</b> |                       |        |   |
| gSa-Gc1             | TCAGATCTCGAGCTCAAGCT  | TCGAAT | C |
| gSa-Gw1             | TCGAAGCTTGAGCTCGAGAT  | CTGAGT | W |
| gSa-Gw2             | AGGATGGATCCTAGGGATAA  | CAGGGT | W |
| gSa-Gw3             | TCATGTGAAGGATGGATCCT  | AGGGAT | W |
| gSa-Gw4             | CCTTGCTGATCATGTGAAGG  | ATGGAT | W |
| gSa-Gw5             | TCGCCCTTGCTGATCATGTG  | AAGGAT | W |

TS: Target strand, complementary to sgRNA.

**Table S3. The list of primers used in this study**

| <b>PCR primers</b>             | <b>Sequence (5'-3')</b>      |
|--------------------------------|------------------------------|
| mCola1-F                       | CCACTGATTGACACGTTGATTG       |
| mCola1-R                       | AGTCACACCCTTTGTCTGGTAT       |
| mRosa26-gR4-F                  | ACAACAATAGATGTATTGAG         |
| mRosa26-gR4-R                  | ACCTTTTACAGATGTGTACA         |
| mKu80-F                        | GGCCTTTGTTTCTAGGCAG          |
| mKu80-R                        | TGGACAAACATAGTCATCAC         |
| mDNA-PKcs-F                    | TAGAGTGCTGAGACGCGCAT         |
| mDNA-PKcs-R                    | GCACTCCTGCCCCAGACTA          |
| TF1                            | ACCCTCGTGACCACCCTGACCTACGGC  |
| TF2                            | GGCCACAAGTTCAGCGTGTCCGGCGAG  |
| TF3                            | TACCGGTGCGCCACCATGGTG        |
| <b>OFF-Target site primers</b> |                              |
| <b>SpCas9-gHRc1</b>            |                              |
| On-Target-P1-F                 | CTAGCGCTACCGGTCGCCAC         |
| On-Target-P1-R                 | TGATCTAGAGTCGCGGCCGC         |
| On-Target-P2-F                 | CGAAGGCTACGTCCAGGAGCGCACCAT  |
| On-Target-P2-R                 | TGTGGCGGATCTTGAAGTTCACCTTGA  |
| OT1-F                          | GCAAAACCTGGAGTAATGTAAGTG     |
| OT1-R                          | CCCTCTTCTATGTGACTGATAGT      |
| OT2-F                          | CCGCTCTGGATTTTCTGTAGC        |
| OT2-R                          | GGCCAATGGGGTTTGGTGCT         |
| OT3-F                          | GACCTACCCAAACTCTACATAG       |
| OT3-R                          | TCCTGTTACTGTTCTCGTTCTC       |
| OT4-F                          | ATGGACCACATGTATGCAGTCC       |
| OT4-R                          | CCACCTGCCAATGGTTTTAGAC       |
| OT5-F                          | GTCTTATGCCTACAGCAACCAT       |
| OT5-R                          | GTAAAGAGCCTATTGGCTGAATGA     |
| OT6-F                          | GCACTCATTCCCTAGTATAAAATCTG   |
| OT6-R                          | CAAGCACTTGGGAGGTAGAAG        |
| <b>SpCas9-gHRc2</b>            |                              |
| On-Target-P1-F                 | CTAGCGCTACCGGTCGCCAC         |
| On-Target-P1-R                 | TGATCTAGAGTCGCGGCCGC         |
| On-Target-P2-F                 | CGAAGGCTACGTCCAGGAGCGCACCAT  |
| On-Target-P2-R                 | TGTGGCGGATCTTGAAGTTCACCTTGA  |
| OT1-F                          | GATCTCCATAGGAGGAAAGAAGT      |
| OT1-R                          | GCTTCATTTGTTAACCAGAGATGG     |
| OT2-F                          | GTCCACTGAGGCATTTTCCCA        |
| OT2-R                          | TCCTCACAGCTACCTGATATTCAA     |
| OT3-F                          | CCCTCTGATCAATTAGATGATGGA     |
| OT3-R                          | CATTGACAACACATGTTACATTTTGTGC |
| OT4-F                          | CCTGATGCTGATGGCACTGA         |
| OT4-R                          | GGGTAGGGTGATTCAATCAATAATG    |

|                       |                                |
|-----------------------|--------------------------------|
| OT5-F                 | CTGTGCCACCTGCTTTCATTA ACT      |
| OT5-R                 | CATTTATCTTGAACCATTTGGCCAC      |
| OT6-F                 | CATAGAGCTTTGAACAGTATCTGTG      |
| OT6-R                 | CCAAGTCAAAGGTTTAGGATTTCTG      |
| <b>SpCas9-gPnpla3</b> |                                |
| On-Target-F           | GCATCTCACTTACACTGCTCAC         |
| On-Target-R           | CAAGACTCAGATCCCAGCTC           |
| OT1-F                 | TAGAAGGGTCCCTAGTCATTCC         |
| OT1-R                 | GTCACATTGTTCTCCTCAGATAC        |
| OT2-F                 | ATTTTAATGTGTTTGTGGGGCAAGAAAC   |
| OT2-R                 | GGAGATTTATGTTTGTGTTTGTGCATCTGA |
| OT3-F                 | CTGGCTATACTCTCCACATAT          |
| OT3-R                 | TTCAGCCAAGTCAGCAGAGC           |
| OT4-F                 | TCTGTGACATCATCCCTTACCC         |
| OT4-R                 | AGGAGATATGAGAAGGCTATAGC        |
| OT5-F                 | CTTGCTATGATCCATCCAGACTC        |
| OT5-R                 | CTCCTTCCATCAGAAGATAGGA         |
| OT6-F                 | CATACTTCCCTAAGAATCTTGATGC      |
| OT6-R                 | AGCTACTTGTAGACTCTGTAGC         |
| OT7-F                 | GTCTGCCTCTGTTCCCTCTA           |
| OT7-R                 | GCATGTAGAAAGATCACCTGTTC        |
| <b>SpCas9-gMertk</b>  |                                |
| On-Target-F           | AAAGCATCTGCGCAGCACTGC          |
| On-Target-R           | CTTCCACATTGATCGTCACACC         |
| OT1-F                 | CAAGTCTCTAGAATTGGAAGCTAC       |
| OT1-R                 | GTGACATCCAGAAACACTCACC         |
| OT2-F                 | TCAGATTAAAGACACCACTCTCCT       |
| OT2-R                 | CACTGAACTCTAACCTTTCTATGG       |
| OT3-F                 | TGACCCACGTGTGGAAGCTG           |
| OT3-R                 | TTCAAAGCAAACAATGTGGAGAAAGTA    |
| OT4-F                 | CTAATGTACTGTAGAATGTGTGCC       |
| OT4-R                 | CTCAGCCTTCTCTAGGACTG           |
| OT5-F                 | GACTATGTGGGTGTGAACAGG          |
| OT5-R                 | AAACA ACTTCACTTGTGTAAAATTTCC   |
| OT6-F                 | ACATATAACACACAGACAGCATAACAC    |
| OT6-R                 | GAAAGCACAGCAAGGAAGAGAC         |