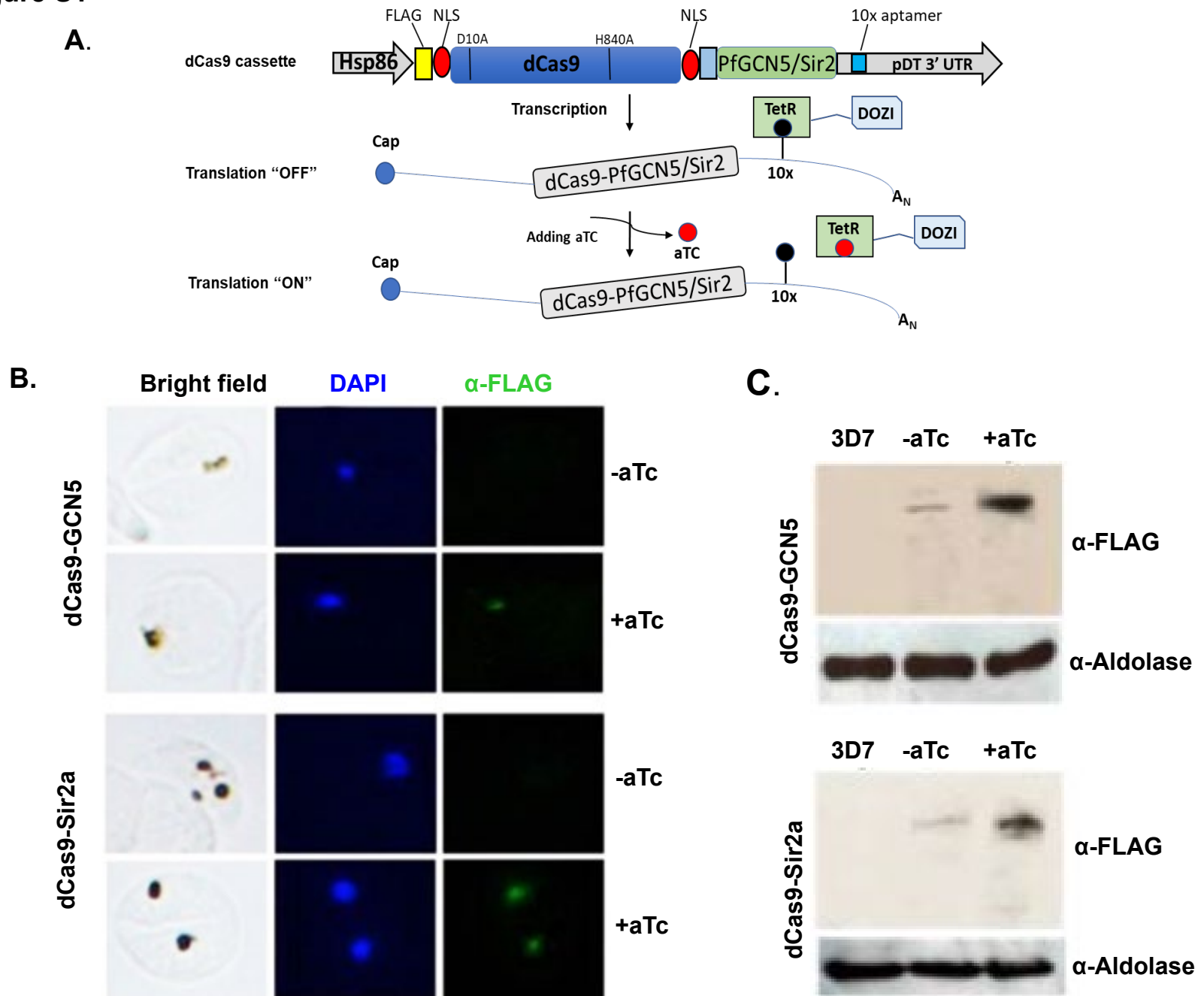
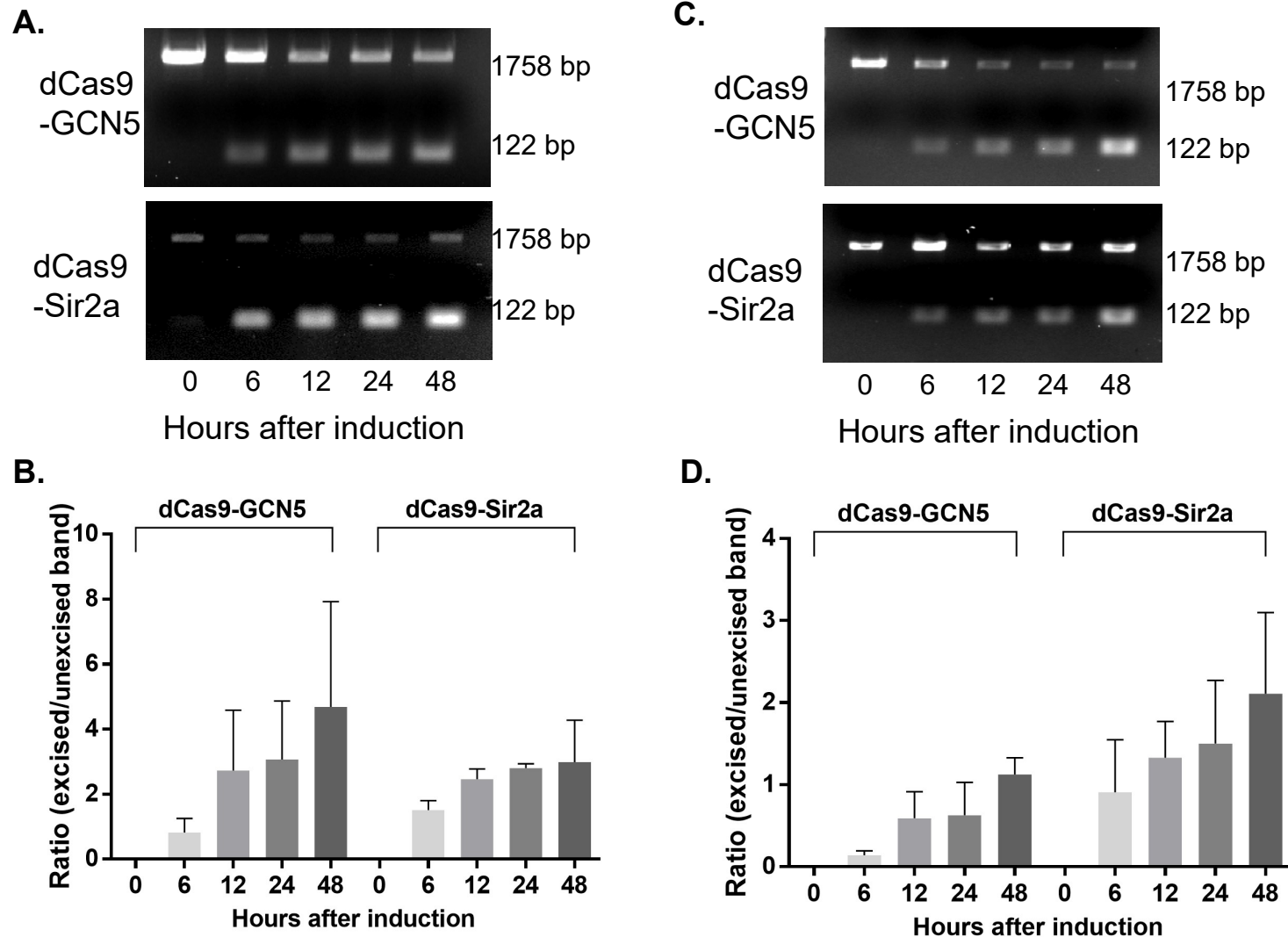


**Figure S1****Figure S1. TetR-DOZI mediated the inducible expression of the dCas9-GCN5/Sir2a.**

**A.** A diagram illustrates the TetR-DOZI inducible CRISPR/dCas9 system, in which 10x aptamer motifs are inserted into 3' UTR of the expression cassette. After transcription, TetR-DOZI binds aptamer motifs to block the translational process (translation "OFF"), whereas adding the tetracycline analog aTc causes TetR-DOZI to be released from the aptamer, inducing protein translation (translation "ON").  $A_N$  indicates the polyA tail of the mRNA. **B** and **C.** immunofluorescence images (**B**) and Western blot (**C**) show efficient induction of dCas9-GCN5 (upper panel) and dCas9-Sir2a (lower panel) protein expression by the addition of aTc.

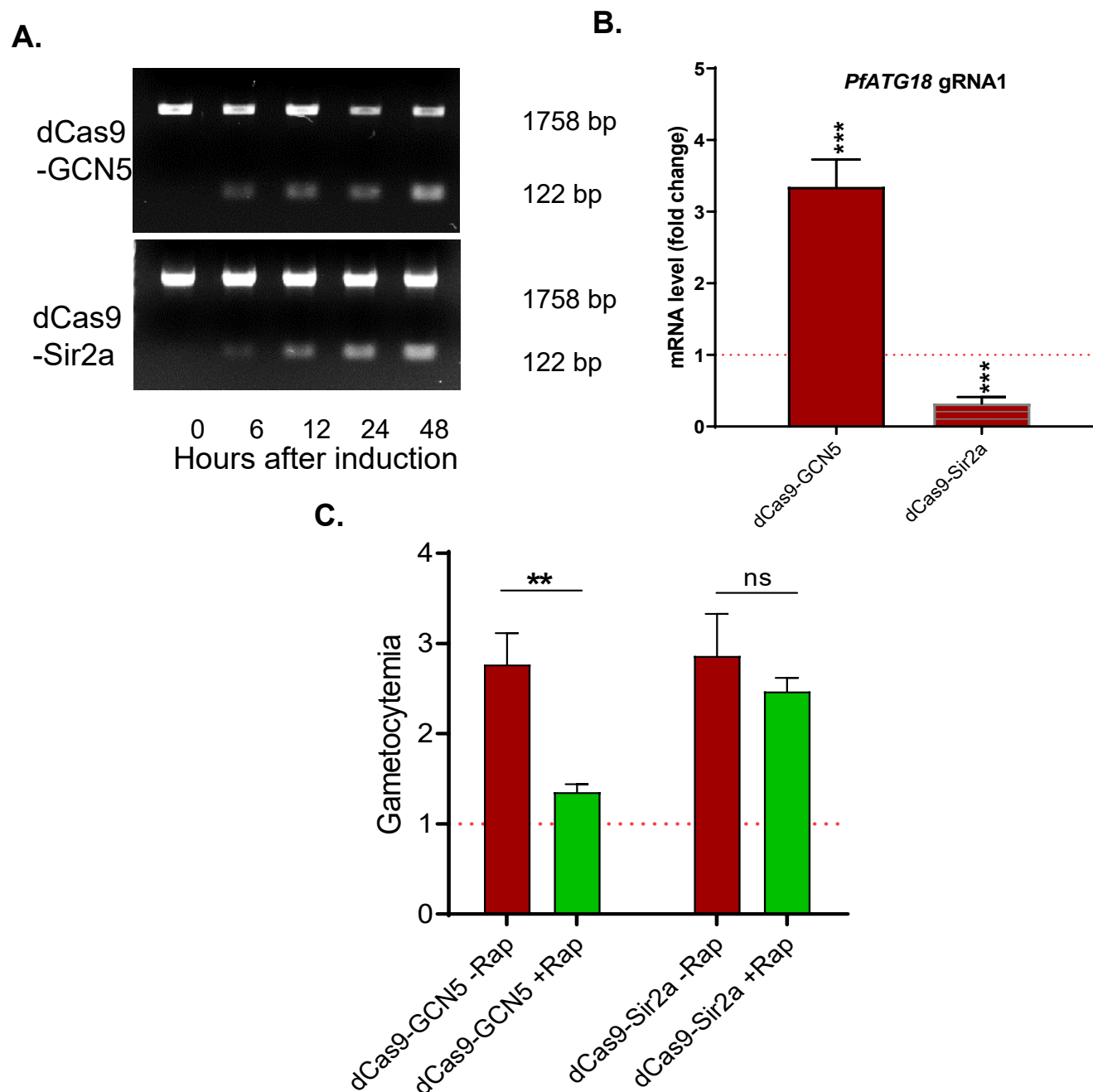
**Figure S2**



**Figure S2. The dynamics of DiCre excision at trophozoite and schizont stages.**

After Rap treatment at trophozoite (**A, B**) and schizont (**C, D**) stage, the efficiency of DiCre excision of *loxPed* GFP cassette was measured by PCR with specific primers shown in Figure 1A. The gradual decrease and increase of unexcised- and excised-bands at the size of 1758bp and 122bp were shown in agarose gels (**A, C**), respectively. Three biological replicates were performed for the experiments in **A** and **C** to depict a bar graph showing the ratio of excised- to unexcised-bands over time elapsed after Rap treatment (**B, D**). The pixel intensities of DNA bands were measured using ImageJ.

**Figure S3**



**Figure S3. Regulation of *PfATG18* by the inducible dCas9 system at the sexual stage**

**A.** The efficiency of DiCre excision of *loxPed* GFP cassette in Day 5 (stage III) gametocytes was measured by PCR with specific primers shown in Figure 1A. The gradual decrease and increase of unexcised- and excised-bands at the size of 1758bp and 122bp were shown in agarose gels, respectively. **B.** Compared to gametocytes without Rap induction, the changes of *PfATG18* transcriptions were analyzed by RT-qPCR in dCas9-Sir2a and dCas9-GCN5 parasite lines with *PfATG18* gRNA1 48h after Rap induction. The paired T-tests were conducted (\*\*\*:  $P < 0.001$ ) from three replicates. **C.** Three replicates of gametocyte growth rates were measured after up-or down-regulation of *PfATG18* at the gametocyte stage by Rap induction (+Rap) compared to the DMSO treated (-Rap) gametocytes. (ns: not significant; \*\*:  $P < 0.01$ , one-way ANOVA).

**Table S1** Transcriptional expression levels of eight selected genes during IDC based on the transcriptomic profile by RNAseq

The data were extracted from 3D7 iRBC RNA-seq showing the scaled TPM ( transcripts per million) level (Bártfai R et al. H2A.Z demarcates intergenic regions of the plasmodium falciparum epigenome that are dynamically marked by H3K9ac and H3K4me3. PLoS Pathog. 2010 Dec 16;6(12):e1001223. doi: 10.1371/journal.ppat.1001223. PMID: 21187892)

| Gene ID       | Product Description                   | Gene Name | Hour 5 | Hour 10 | Hour 15 | Hour 20 | Hour 25 | Hour 30 | Hour 35 | Hour 40 |
|---------------|---------------------------------------|-----------|--------|---------|---------|---------|---------|---------|---------|---------|
| PF3D7_0709000 | chloroquine resistance transporter    | CRT       | 11.2   | 14.26   | 17.82   | 38.05   | 135.28  | 138.67  | 83.89   | 17.84   |
| PF3D7_0727300 | DNA (cytosine-5)-methyltransferase    | DNMT      | 1.01   | 3.49    | 5.75    | 16.06   | 19.44   | 33.83   | 22.25   | 13.81   |
| PF3D7_0823300 | histone acetyltransferase GCN5        | GCN5      | 8.74   | 12.2    | 16.35   | 46.15   | 220.1   | 228.1   | 276.25  | 108.77  |
| PF3D7_1012900 | autophagy-related protein 18          | ATG18     | 15.27  | 9.86    | 11.29   | 29.47   | 110.81  | 134.58  | 108.69  | 42.64   |
| PF3D7_1118600 | histone acetyltransferase MYST        | MYST      | 18.18  | 7.31    | 6.1     | 15.67   | 53      | 65      | 60      | 24.21   |
| PF3D7_1219100 | clathrin heavy chain, putative        | CHC       | 2.18   | 3.58    | 7.45    | 19.08   | 72.64   | 93.72   | 119.21  | 52.05   |
| PF3D7_1222600 | AP2 domain transcription factor AP2-G | AP2-G     | 5.06   | 3.48    | 2.97    | 5.46    | 2.06    | 1.47    | 6.74    | 5.06    |
| PF3D7_1343700 | kelch protein K13                     | Kelch13   | 23.88  | 18.34   | 18.3    | 34.56   | 87.2    | 79.33   | 66.91   | 51.34   |

**Table S2.** TSS and gRNA locations of eight selected genes

| Gene Name | Gene ID       | Essential gene (Y/N) | major TSS site (-ATG bp) | sgRNA | gRNA position (-ATG bp) |
|-----------|---------------|----------------------|--------------------------|-------|-------------------------|
| MYST      | PF3D7_1118600 | Y                    | 203                      | gRNA1 | 630                     |
|           |               |                      |                          | gRNA2 | 1884                    |
| DNMT      | PF3D7_0727300 | N                    | 402                      | gRNA1 | 581                     |
|           |               |                      |                          | gRNA2 | 1696                    |
| CHC       | PF3D7_1219100 | Y                    | 431                      | gRNA1 | 1420                    |
|           |               |                      |                          | gRNA2 | 936                     |
| GCN5      | PF3D7_0823300 | Y                    | 342                      | gRNA1 | 794                     |
|           |               |                      |                          | gRNA2 | 1459                    |
| k13       | PF3D7_1343700 | Y                    | 880                      | gRNA1 | 1294                    |
|           |               |                      |                          | gRNA2 | 1600                    |
| ATG18     | PF3D7_1012900 | Y                    | 1599                     | gRNA1 | 88                      |
|           |               |                      |                          | gRNA2 | 757                     |
|           |               |                      |                          | gRNA3 | 1409                    |
| AP2-G     | PF3D7_1222600 | N                    | 489                      | gRNA1 | 176                     |
|           |               |                      |                          | gRNA2 | 1815                    |
|           |               |                      |                          | gRNA3 | 2779                    |
| CRT       | PF3D7_0709000 | Y                    | 837                      | gRNA1 | 93                      |
|           |               |                      |                          | gRNA2 | 509                     |
|           |               |                      |                          | gRNA3 | 1204                    |

**Table S3.** Primer information

| Primer name                             | Sequence  |
|---|---|
| <b>primers for plasmid construction</b> |   |
| Loxp-GFP-Loxp-F                         | TTCGAATAAACTCGAGATAACTTCGTATAGCATAACATTATACGAAGTTATGTCGACATGAGTAAAGGAGAAGAACTTTTC |
| Loxp-GFP-Loxp-R                         | TATAGTCCATCTCGAGATAACTTCGTATAATGTATGCTATACGAAGTTATCCCGGGTATTTGTATAGTTCATCCATGC    |
| Aptamer F                               | GCTGCCATATCCTTAATTAACGAGTTGTAACTAGAGGCCTGG  |
| Aptamer R                               | TACATTTTATACTTAATTAAGGTTAACTGGCCAAGATCTCCC  |
| Hsp86-F                                 | ACGAAGTTATCCCGGGTATATAATATATTTATGTACTCACAATGG                                     |
| Hsp86-R                                 | ATACAAATAACCCGGGGGCATGCTATTTGATGAATTAAC   |
| <b>primers for excision event</b>       |   |
| Ex-F                                    | CTTCCCACATTTTGAATAAAActcg   |
| Ex-R                                    | GACCTTCCGCTTCTTCTTTGG   |
| <b>sgRNA primers</b>                    |   |
| AP2G sgRNA1-F                           | TAAGTATATAATATTGTAACATCTATATATAAGTGTTTTAGAGCTAGAA                                 |
| AP2G sgRNA1-R                           | TTCTAGCTCTAAAACACTTATATATAGATAGTTACAATATTATATACTTA                                |
| AP2G sgRNA2-F                           | TAAGTATATAATATTACATTAATAAATTCATAATAGTTTTAGAGCTAGAA                                |
| AP2G sgRNA2-R                           | TTCTAGCTCTAAAACACTTATGAATTTATTAATGTAAATATTATATACTTA                               |
| AP2G sgRNA3-F                           | TAAGTATATAATATTGTGTACAACATAAATGCACGTTTTAGAGCTAGAA                                 |
| AP2G sgRNA3-R                           | TTCTAGCTCTAAAACGTCATTATTAGTTGTACACAATATTATATACTTA                                 |
| CRT sgRNA1-F                            | TAAGTATATAATATTAGTCTAAAAATATAATATATGTTTTAGAGCTAGAA                                |
| CRT sgRNA1-R                            | TTCTAGCTCTAAAACATATATTATATTTTTAGACTAATATTATATACTTA                                |
| CRT sgRNA2-F                            | TAAGTATATAATATTAACCTGGGAAAGTAAAAAAGTTTTAGAGCTAGAA                                 |
| CRT sgRNA2-R                            | TTCTAGCTCTAAAACCTTTTTACTTTCCCAAGTTAATATTATATACTTA                                 |
| CRT sgRNA3-F                            | TAAGTATATAATATTAATAAATAATCGGAAAAATGTTTTAGAGCTAGAA                                 |
| CRT sgRNA3-R                            | TTCTAGCTCTAAAACATTTTTCCGATTATTTAATATTATATACTTA                                    |
| ATG18 sgRNA1-F                          | TAAGTATATAATATTTGGTAAAAATATAAAAAGTTTTAGAGCTAGAA                                   |
| ATG18 sgRNA1-R                          | TTCTAGCTCTAAAACCTTTTTATATATTTTACCACAAATATTATATACTTA                               |
| ATG18 sgRNA2-F                          | TAAGTATATAATATTTAATAACATATAAATCAACAGTTTTAGAGCTAGAA                                |
| ATG18 sgRNA2-R                          | TTCTAGCTCTAAAACGTTGATTTATATGTTATTAATATTATATACTTA                                  |
| ATG18 sgRNA3-F                          | TAAGTATATAATATTTAATAAGGTTTTGAAGGGTAGTTTTAGAGCTAGAA                                |
| ATG18 sgRNA3-R                          | TTCTAGCTCTAAAACCTACCCTTCAAACCTTATTAATATTATATACTTA                                 |
| MYST sgRNA1-F                           | TAAGTATATAATATTGAAAAACAATTATATGTTTAGTTTTAGAGCTAGAA                                |
| MYST sgRNA1-R                           | TTCTAGCTCTAAAACATAAATGTTTTCAATATTATATACTTA  |
| MYST sgRNA2-F                           | TAAGTATATAATATTGACGCTAATGTTGAATGAACGTTTTAGAGCTAGAA                                |
| MYST sgRNA2-R                           | TTCTAGCTCTAAAACGTTCAATCAACATTAGCGTCAATATTATATACTTA                                |
| DNMT sgRNA1-F                           | TAAGTATATAATATTTATATGTGTTCAAAGGCATAGTTTTAGAGCTAGAA                                |
| DNMT sgRNA1-R                           | TTCTAGCTCTAAAACATATGCTTTGAACACATATAAATATTATATACTTA                                |
| DNMT sgRNA2-F                           | TAAGTATATAATATTGGTTCAGTATACCATAAACAGTTTTAGAGCTAGAA                                |
| DNMT sgRNA2-R                           | TTCTAGCTCTAAAACGTTTATGGTACTGAACCAATATTATATACTTA                                   |
| GCN5 sgRNA1-F                           | TAAGTATATAATATTACTTAAATATTATCAAATGGTTTTAGAGCTAGAA                                 |
| GCN5 sgRNA1-R                           | TTCTAGCTCTAAAACCTTTGATAATATTAAGTATAATATTATATACTTA                                 |
| GCN5 sgRNA2-F                           | TAAGTATATAATATTACTATCATAATAAGTAATGTTTTAGAGCTAGAA                                  |
| GCN5 sgRNA2-R                           | TTCTAGCTCTAAAACACTTATTATGATAGTGAATATTATATACTTA                                    |
| CHC sgRNA1-F                            | TAAGTATATAATATTATAAATAAGCAATCATTTCTGTTTTAGAGCTAGAA                                |
| CHC sgRNA1-R                            | TTCTAGCTCTAAAACAGAAATGATTGCTTATTATAATATTATATACTTA                                 |
| CHC sgRNA2-F                            | TAAGTATATAATATTAATAAATTACCATGAAAAATAGTTTTAGAGCTAGAA                               |
| CHC sgRNA2-R                            | TTCTAGCTCTAAAACATTTTTCATGGTAATTTTTAATATTATATACTTA                                 |
| K13 sgRNA1-F                            | TAAGTATATAATATTGTTATCATTCAAAAATATATGTTTTAGAGCTAGAA                                |
| K13 sgRNA1-R                            | TTCTAGCTCTAAAACATATATTTTTGAATGATAACAATATTATATACTTA                                |
| K13 sgRNA2-F                            | TAAGTATATAATATTTACCATGTTGAATTGGTGACGTTTTAGAGCTAGAA                                |
| K13 sgRNA2-R                            | TTCTAGCTCTAAAACGTCACCAATTCAACATGGTAAATATTATATACTTA                                |
| <b>primers for qPCR</b>                 |   |
| AP2G-F                                  | TTGTTTCATGGAGATGTAGATGGTG   |
| AP2G-R                                  | TTTGTCTTTGGGATTACTTTTTTTT   |
| Clathrin-F                              | ATACATCCGGACAATTATCTCCAC  |
| Clathrin-R                              | TTGTAAAACAACCGGTTTTACCAA  |
| DNMT-F                                  | CAACCACTTGTTGTCATGTGGCAA  |
| DNMT-R                                  | TTTTGCGTTCATATGCTTGGTTGG  |
| GCN5-F                                  | TGTTATTACCTCTTCCTTATTGTC  |
| GCN5-R                                  | TTTCTTCTCTTCATCATATTTAT   |
| K13-F                                   | TATTAATGTTGGTGGAGCTATTTT  |
| K13-R                                   | CCTTGTTTATCTCTGGTTACATGA  |
| MYST-F                                  | AAGAGCAAAGAAAAAACAGGTGAC  |
| MYST-R                                  | TAAAGCGTTTGGAAAAATTAAGC   |

|                         |                             |
|-------------------------|-----------------------------|
| CRT-F                   | GTTGTATACAAGGTCCAGCAATAGC   |
| CRT-R                   | CCAAATAGGTAGCCAAACAAAGTTACG |
| atg18-F                 | CCAGGATTATGGTTGTTTGTG       |
| atg18-R                 | GTTAGTTTCGCTATTTCCCG        |
| Gexp5-F                 | CCAATGGAATCATGTACACAT       |
| Gexp5-R                 | GTCTATAAAATCTGTACGCGTC      |
| Pfs16-F                 | CGAAAGTTCATACCATCTTTAGC     |
| Pfs16-R                 | GTTGAAGGGGATCCTTTTCC        |
| seryl-tRNA synthetase F | AAGTAGCAGGTCATCGTGGTT       |
| seryl-tRNA synthetase R | TTCGGCACATTCTTCATAA         |