**Supplementary File 1**

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| Supplementary File 1a. Screen of D10 clones for *Pfftsh1* sequence and actinonin resistance. | | |
| ActR clone | **Nucleotide sequence of PF3D7\_1239700 (*Pf*FtsH1)** | **Actinonin IC50**  **(µM)** |
| *Pf* D10 WT | 1451 CAGAAACTAGTAGT**G**GTGCTTCAAGTGATA 1480 | 2.0 ± 0.2 (n=4) |
| *Pf* D10 ActR Clone 1 | 1451 CAGAAACTAGTAGT**T**GTGCTTCAAGTGATA 1480 | 73.3 ± 2.7 (n=3) |
| *Pf* D10 ACTR Clone2 | 1451 CAGAAACTAGTAGT**T**GTGCTTCAAGTGATA 1480 | 61.6 ± 14.4 (n=2) |
| *Pf* D10 ACTR Clone3 | 1451 CAGAAACTAGTAGT**T**GTGCTTCAAGTGATA 1480 | 51.4 (n=1) |
| *Pf* D10 ACTR Clone4 | 1451 CAGAAACTAGTAGT**T**GTGCTTCAAGTGATA 1480 | 36.2 (n=1) |

**Supplementary File 1b: Descriptive statistics for growth inhibition trials in Figure 1.**

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|  | D10 ActS | D10 r*ftsH1***G**489**G** | D10 r*ftsH1***G**489**C**a | D10 r*ftsH1***G**489**C**b | D10 ActR |
| n | 4 | 6 | 3 | 5 | 3 |
| Mean | 2.024 | 2.894 | 43.88 | 59.19 | 73.29 |
| Median | 2.117 | 2.887 | 43.60 | 58.71 | 71.69 |
|  |  |  |  |  |  |
| SD | 0.32 | 0.68 | 3.61 | 3.78 | 4.64 |
| SEM | 0.16 | 0.28 | 2.08 | 1.70 | 2.68 |
| 95% CI | 1.52-2.53 | 2.18-3.61 | 34.92-52.84 | 54.49-63.88 | 61.76-84.82 |

**Supplementary File 1c:** **Oligos for PCR amplification of potential actinonin targets.**

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| GeneID | Forward primer | Reverse primer |
| PF3D7\_0907900  *Pfpdf* | TCACTCAGGAAAACCACAACT | CCCCCAGAAACAAAAGAACA |
| PF3D7\_1313200  *Pfftsh1* | TCCAATCTAAGAAATATTCGACCCCT | AATGGAGAGAATTCTATGCCTCTT |
| PF3D7\_0804400  *Pfmap* | GCTTCCTGTTGGGGTGTTT | AAAATTGTCATTTTGTTTAACACTT |
| PF3D7\_1239700  *Pffmt* | GAGCAATTGAAAGGATGGAA | TTTTCCAAAACAACAAATAAAACA |
| PF3D7\_1405700  *PfRING* | AAAATCCTCTTCGCACATTTTT | TTGATTATCACAAATGCTCATTCA |

**Supplementary File 1d: Oligos for generation and sequencing of allelic replacement constructs.**

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| *Pfftsh1* primer | Forward primer | Reverse primer |
| sgRNA | TAAGTATATAATATTTTCTGTTTTATTGCATGATGGTTTTAGAGCTAGAA | TTCTAGCTCTAAAACCATCATGCAATAAAACAGAAAATATTATATACTTA |
| Genome segment for allelic replacement | CCTAGGAAATGGGTGCTAGAATGCC | GCATGCAGCGACTAGTAAGAATATTTAT |
| sgRNA sequencing | AAAAAATTCTTGCTTGTTCAGA | CGGCCGCTATTTCATCTATTT |
| Shield mutations | CTTGAAGCACCACTACTTGTTTCAGATTTACCAAAAACAATTTCTTCAGC | GCTGAAGAAATTGTTTTTGGTAAATCTGAAACAAGTAGTGGTGCTTCAAG |
| G489C mutation | ATATATCACTTGAAGCACAACTACTTGTTTCAGATTTACCAAAAACAATTTCTTCAG | CTGAAGAAATTGTTTTTGGTAAATCTGAAACAAGTAGTTGTGCTTCAAGTGATATAT |
| Mutation sequencing | TGAAGCTGGTCATGCTATCG | TGATCTCTTCTCCGGATAAGG |