**Supporting Information**

**A single point mutation in the *Plasmodium falciparum* 3´-5´exonuclease does not alter piperaquine susceptibility**

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Short running title: The E145G mutation in the exonuclease gene does not confer piperaquine resistance in *Plasmodium falciparum*

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**Table S1** Oligonucleotide primer sequences used in this study

|  |  |
| --- | --- |
| **Primer name** | **Sequence (5′ - 3′)** |
| Q5SDM\_G415E\_F | AAGAGGTGAACAACCACTGGGAG |
| Q5SDM\_G415E\_R | CCTTGATGGTGATCACCATATTGTTAAAATC |
| Bsd\_NcoI\_F | GGATCCATGGCCAAGCCTTTGTCTCAAG |
| Bsd\_SacII\_R | AATTCCGCGGCCGCGGTTAGCCCTCCCACACATAAC |
| sgE415G-1F | ATTGTATGGTTATAACGATAAAAG |
| sgE415G-1R | AAACCTTTTATCGTTATAACCATA |
| ExonI\_K283\_F **→** | AAGCAGTTGAAAAATACACTCTTTTTATTATTAAACGATATTGAGTG |
| Screen\_WT\_Rev\_V616 **←** | AATCTATTATATATATTTTATTATTTGTTGAAATAGATAGAACACTA |
| Recodon\_F **→** | ATGGTGATCACCATCAAGGGCG |
| Recodon\_R **←** | TTCCAGCAGCCACTTGAAGAATGT |
| Screen\_3’UTR\_R ← | CGAGGGGATAAGGTTTATTTTGTAGGGAAAAG |
| Pfk13\_outer\_F | CGGAGTGACCAAATCTGGGA |
| Pfk13\_outer\_R | GGGAATCTGGTGGTAACAGC |
| Pfk13\_nested\_F | GCCAAGCTGCCATTCATTTG |
| Pfk13\_nested\_R | GCCTTGTTGAAAGAAGCAGA |
| Pfexo\_E415G\_F | GCACCTCCTATCATCAGATGATACC |
| Pfexo\_E415G\_R | CAAGAAAAGAGGAAGGAACACCTTC |
| Pfcrt\_93\_145\_F | TGCTAAAAGAACTTTAAACAAAATTGG |
| Pfcrt\_93\_145\_R | CAAGAACTACTGGAAATATCCAATC |
| Pfcrt\_218\_F | TCTCGGAGCAGTTATTATTGTTG |
| Pfcrt\_218\_R | ATTTCCCTTGTCATGTTTGAA |
| Pfcrt\_343\_353\_F | CGCATTGTTTTCCTTCTTTAAC |
| Pfcrt\_343\_353\_R | CGGCTAAGAATTTAAAGTAATAAGCAA |
| Pfpm1\_fw | CAATGGTTTCGAACCAGCTT |
| Pfpm1\_rv | GGTAAAAACGGCTTGTTCGAT |
| Pfpm2\_fw | TGGTGATGCAGAAGTTGGAG |
| Pfpm2\_rv | TGGGACCCATAAATTAGCAGA |
| Pfpm3\_fw | CACCTTCATGAAAAATGAAGAATC |
| Pfpm3\_rv | AAGAAAAACCTCCTGCCAAAA |
| PfB-tub\_fw | TGATGTGCGCAAGTGATCC |
| PfB-tub\_rv | TCCTTTGTGGACATTCTTCCTC |
| AF\_for | CCACGATTTATATTGGCAAGTTGATTTAG |
| AR\_rev | CATTTCTACTAAAATTAGCTTTAGCATCATTCACG |
| BF\_for | CGTAGAATCTGCAAGTGTTTTCAAAG |
| BR\_rev | AATGTTATAAATGCAATATAATCAAACGACATCAC |

(Coloured arrows in the left-hand column correspond to primer annotation in Fig 1)

PF3D7\_Exo\_WT ATGACATCACATATTAGTTACAATAAAATAAGAGAAAAAAGGAACATAAGAAGGGTACTG 60

PFB5\_Exo\_WT ATGACATCACATATTAGTTACAATAAAATAAGAGAAAAAAGGAACATAAGAAGGGTACTG 60

PFB8\_Exo\_E415G ATGACATCACATATTAGTTACAATAAAATAAGAGAAAAAAGGAACATAAGAAGGGTACTG 60

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PF3D7\_Exo\_WT AGCGTTTATAATTTTTGTTCATTAAATAGATTCTCATGGTACAGACAAGATGTGAGATTA 120

PFB5\_Exo\_WT AGCGTTTATAATTTTTGTTCATTAAATAGATTCTCATGGTACAGACAAGATGTGAGATTA 120

PFB8\_Exo\_E415G AGCGTTTATAATTTTTGTTCATTAAATAGATTCTCATGGTACAGACAAGATGTGAGATTA 120

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PF3D7\_Exo\_WT GATAATGTGATATATAGAAAGAATTATGAATATATTTATTATAATTGTATTATAAGAAAA 180

PFB5\_Exo\_WT GATAATGTGATATATAGAAAGAATTATGAATATATTTATTATAATTGTATTATAAGAAAA 180

PFB8\_Exo\_E415G GATAATGTGATATATAGAAAGAATTATGAATATATTTATTATAATTGTATTATAAGAAAA 180

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PF3D7\_Exo\_WT TGTAGTAGAAATTATATATCTACACGTAATAATATATATATTAAAAACAAAATATATGAT 240

PFB5\_Exo\_WT TGTAGTAGAAATTATATATCTACACGTAATAATATATATATTAAAAACAAAATATATGAT 240

PFB8\_Exo\_E415G TGTAGTAGAAATTATATATCTACACGTAATAATATATATATTAAAAACAAAATATATGAT 240

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PF3D7\_Exo\_WT ATAATACCATATTTATGTAAAGGTAAAGATGTAAAAAATATAACAAGTAATATAATATTT 300

PFB5\_Exo\_WT ATAATACCATATTTATGTAAAGGTAAAGATGTAAAAAATATAACAAGTAATATAATATTT 300

PFB8\_Exo\_E415G ATAATACCATATTTATGTAAAGGTAAAGATGTAAAAAATATAACAAGTAATATAATATTT 300

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PF3D7\_Exo\_WT TATATTTTACAACATTTAAGTACAAAAAATGTTGTTAATAGTAATGAATATAAAGATAAT 360

PFB5\_Exo\_WT TATATTTTACAACATTTAAGTACAAAAAATGTTGTTAATAGTAATGAATATAAAGATAAT 360

PFB8\_Exo\_E415G TATATTTTACAACATTTAAGTACAAAAAATGTTGTTAATAGTAATGAATATAAAGATAAT 360

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PF3D7\_Exo\_WT ATAAAAAAAATATATTTTAATTTATTGAAATGTTATCATAAAATTTTTGAATATAATAAT 420

PFB5\_Exo\_WT ATAAAAAAAATATATTTTAATTTATTGAAATGTTATCATAAAATTTTTGAATATAATAAT 420

PFB8\_Exo\_E415G ATAAAAAAAATATATTTTAATTTATTGAAATGTTATCATAAAATTTTTGAATATAATAAT 420

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PF3D7\_Exo\_WT GAATATGGAGAATATATATTTAGCCTGTTTAATGATGATATAATTATATCTGTTAGTCCG 480

PFB5\_Exo\_WT GAATATGGAGAATATATATTTAGCCTGTTTAATGATGATATAATTATATCTGTTAGTCCG 480

PFB8\_Exo\_E415G GAATATGGAGAATATATATTTAGCCTGTTTAATGATGATATAATTATATCTGTTAGTCCG 480

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PF3D7\_Exo\_WT TCTTTAAGAAAGAAACAAAAAAATATTATACAAGATATATTATTGAATAGCTTACATTTT 540

PFB5\_Exo\_WT TCTTTAAGAAAGAAACAAAAAAATATTATACAAGATATATTATTGAATAGCTTACATTTT 540

PFB8\_Exo\_E415G TCTTTAAGAAAGAAACAAAAAAATATTATACAAGATATATTATTGAATAGCTTACATTTT 540

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PF3D7\_Exo\_WT TTTTTCCAGAATAATATAACATATAAAAATAAATTAAATATAAATTTGATGTGTGAAATT 600

PFB5\_Exo\_WT TTTTTCCAGAATAATATAACATATAAAAATAAATTAAATATAAATTTGATGTGTGAAATT 600

PFB8\_Exo\_E415G TTTTTCCAGAATAATATAACATATAAAAATAAATTAAATATAAATTTGATGTGTGAAATT 600

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PF3D7\_Exo\_WT ATTAATTATCCAAAGTTTTTGTATGTATTACATTCTATTAATTATGATATGAATATATTA 660

PFB5\_Exo\_WT ATTAATTATCCAAAGTTTTTGTATGTATTACATTCTATTAATTATGATATGAATATATTA 660

PFB8\_Exo\_E415G ATTAATTATCCAAAGTTTTTGTATGTATTACATTCTATTAATTATGATATGAATATATTA 660

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PF3D7\_Exo\_WT AAACAAAATATAAGTGTACAAAATGTAGATTATTTATTTTCTCAGTATATTAATAAAACA 720

PFB5\_Exo\_WT AAACAAAATATAAGTGTACAAAATGTAGATTATTTATTTTCTCAGTATATTAATAAAACA 720

PFB8\_Exo\_E415G AAACAAAATATAAGTGTACAAAATGTAGATTATTTATTTTCTCAGTATATTAATAAAACA 720

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PF3D7\_Exo\_WT AATATATATATTACTACAGCAATTCAATTCGCTTCATTTTTTAAAAATGTCAACATGAAT 780

PFB5\_Exo\_WT AATATATATATTACTACAGCAATTCAATTCGCTTCATTTTTTAAAAATGTCAACATGAAT 780

PFB8\_Exo\_E415G AATATATATATTACTACAGCAATTCAATTCGCTTCATTTTTTAAAAATGTCAACATGAAT 780

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PF3D7\_Exo\_WT ATTTTCACACCTTTTAAGAAACACGGTACATTTAATTATTTCTTATTATTAAAACGTATA 840

PFB5\_Exo\_WT ATTTTCACACCTTTTAAGAAACACGGTACATTTAATTATTTCTTATTATTAAAACGTATA 840

PFB8\_Exo\_E415G ATTTTCACACCTTTTAAGAAACACGGTACATTTAATTATTTCTTATTATTAAAACGTATA 840

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PF3D7\_Exo\_WT GTAAATAAGCAGTTGAAAAATACACTCTTTTTATTATTAAACGATATTGAGTGTCATCGG 900

PFB5\_Exo\_WT GTAAATAAGCAGTTGAAAAATACACTCTTTTTATTATTAAACGATATTGAGTGTCATCGG 900

PFB8\_Exo\_E415G GTAAATAAGCAGTTGAAAAATACACTCTTTTTATTATTAAACGATATTGAGTGTCATCGG 900

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PF3D7\_Exo\_WT TTAAGGCAGGAAATGCTTTTGCACCTCCTATCATCAGATGATACCACAGgtataaaaaaa 960

PFB5\_Exo\_WT TTAAGGCAGGAAATGCTTTTGCACCTCCTATCATCAGATGATACCACAGgtataaaaaaa 960

PFB8\_Exo\_E415G TTAAGGCAGGAAATGCTTTTGCACCTCCTATCATCAGATGATACCACAGgtataaaaaaa 960

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PF3D7\_Exo\_WT aaaaaaatatatatatatatatatatatatttatatatgtatatatgtatatatgtatat 1020

PFB5\_Exo\_WT aaaaaaatatatatatatatatatatatatttatatatgtatatatgtatatatgtatat 1020

PFB8\_Exo\_E415G aaaaaaatatatatatatatatatatatatttatatatgtatatatgtatatatgtatat 1020

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PF3D7\_Exo\_WT atttattttttatttattatttttttttttttttttttttttccttctgacccctttata 1080

PFB5\_Exo\_WT atttattttttatttattatttttttttttttttttttttttccttctgacccctttata 1080

PFB8\_Exo\_E415G atttattttttatttattatttttttttttttttttttttttccttctgacccctttata 1080

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PF3D7\_Exo\_WT gGAATGTGCTTTAACGAATGGAGTCATTTAGCAGCAAAGAAATATTTACTTATGAACAAG 1140

PFB5\_Exo\_WT gGAATGTGCTTTAACGAATGGAGTCATTTAGCAGCAAAGAAATATTTACTTATGAACAAG 1140

PFB8\_Exo\_E415G gGAATGTGCTTTAACGAATGGAGTCATTTAGCAGCAAAGAAATATTTACTTATGAACAAG 1140

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PF3D7\_Exo\_WT TATGAAAATGATCTTAAGGAAAATATAAGAGATCAAGAAATAAATGTTAACATTTTGAAA 1200

PFB5\_Exo\_WT TATGAAAATGATCTTAAGGAAAATATAAGAGATCAAGAAATAAATGTTAACATTTTGAAA 1200

PFB8\_Exo\_E415G TATGAAAATGATCTTAAGGAAAATATAAGAGATCAAGAAATAAATGTTAACATTTTGAAA 1200

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PF3D7\_Exo\_WT AGATCAATTGATTATGTTAAGGATTACGATGACGATAACGATAAAGTTAACAATAACGAT 1260

PFB5\_Exo\_WT AGATCAATTGATTATGTTAAGGATTACGATGACGATAACGATAAAGTTAACAATAACGAT 1260

PFB8\_Exo\_E415G AGATCAATTGATTATGTTAAGGATTACGATGACGATAACGATAAAGTTAACAATAACGAT 1260

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PF3D7\_Exo\_WT AACGATAATGATAACGATTTATATATGGAATATTTTAATTTACCTGAAGACGTTAAAAAT 1320

PFB5\_Exo\_WT AACGATAATGATAACGATTTATATATGGAATATTTTAATTTACCTGAAGACGTTAAAAAT 1320

PFB8\_Exo\_E415G AACGATAATGATAACGATTTATATATGGAATATTTTAATTTACCTGAAGACGTTAAAAAT 1320

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PF3D7\_Exo\_WT GTAAAGTACATAAAATGTGTTGATGATTTTAACAATATGGTTATAACGATAAAA**GAG**GAA 1380

PFB5\_Exo\_WT GTAAAGTACATAAAATGTGTTGATGATTTTAACAATATGGTTATAACGATAAAA**GAG**GAA 1380

PFB8\_Exo\_E415G GTAAAGTACATAAAATGTGTTGATGATTTTAACAATATGGTGATCACCATCAAG**GGC**GAG 1380

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PF3D7\_Exo\_WT GTAAACAATCATTGGGAAAATAATATATACAATAAAAAGGATATGGTTAATTATACAAAT 1440

PFB5\_Exo\_WT GTAAACAATCATTGGGAAAATAATATATACAATAAAAAGGATATGGTTAATTATACAAAT 1440

PFB8\_Exo\_E415G GTGAACAACCACTGGGAGAACAACATCTACAACAAGAAGGACATGGTCAACTACACCAAT 1440

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PF3D7\_Exo\_WT GAAAATTATAATGATAATATATTAACATATGAATATATTAATGAAACACTTAGAAAAGAA 1500

PFB5\_Exo\_WT GAAAATTATAATGATAATATATTAACATATGAATATATTAATGAAACACTTAGAAAAGAA 1500

PFB8\_Exo\_E415G GAGAACTACAACGACAACATCCTGACCTACGAGTACATCAACGAGACACTGAGAAAGGAA 1500

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PF3D7\_Exo\_WT AAAAAAAGATATTATATAGGTATAGATATCGAATGGGATAGTTATAAAAAAAAAAAAAAT 1560

PFB5\_Exo\_WT AAAAAAAGATATTATATAGGTATAGATATCGAATGGGATAGTTATAAAAAAAAAAAAAAT 1560

PFB8\_Exo\_E415G AAGAAGCGGTACTACATCGGCATCGACATCGAGTGGGACAGCTACAAGAAAAAGAAAAAC 1560

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PF3D7\_Exo\_WT ACTGTTAGTGTTCTATCTATTTCAACAAATAATAAAATATATATAATAGATTTATATTAT 1620

PFB5\_Exo\_WT ACTGTTAGTGTTCTATCTATTTCAACAAATAATAAAATATATATAATAGATTTATATTAT 1620

PFB8\_Exo\_E415G ACCGTGTCCGTGCTGAGCATCAGCACCAACAACAAGATCTACATCATCGATCTGTACTAC 1620

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PF3D7\_Exo\_WT ATTGATTATAATTATAAATTTATGATATATACGTTTTTTAAATGGTTATTAGAAAATCCG 1680

PFB5\_Exo\_WT ATTGATTATAATTATAAATTTATGATATATACGTTTTTTAAATGGTTATTAGAAAATCCG 1680

PFB8\_Exo\_E415G ATCGACTACAATTACAAGTTCATGATCTATACATTCTTCAAGTGGCTGCTGGAAAATCCG 1680

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PF3D7\_Exo\_WT TTTATATATAAATTGTTTTTTAATTTCCCTTCGGATATTAAAATAATGTCTTCATATTTT 1740

PFB5\_Exo\_WT TTTATATATAAATTGTTTTTTAATTTCCCTTCGGATATTAAAATAATGTCTTCATATTTT 1740

PFB8\_Exo\_E415G TTTATATATAAATTGTTTTTTAATTTCCCTTCGGATATTAAAATAATGTCTTCATATTTT 1740

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PF3D7\_Exo\_WT CAAAACATATCACATATAAATATATATAACAATATTATAGATTTAAATAATAATATATAT 1800

PFB5\_Exo\_WT CAAAACATATCACATATAAATATATATAACAATATTATAGATTTAAATAATAATATATAT 1800

PFB8\_Exo\_E415G CAAAACATATCACATATAAATATATATAACAATATTATAGATTTAAATAATAATATATAT 1800

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PF3D7\_Exo\_WT ATATATACAAGAAAAGAGGAAGGAACACCTTCTTATAAGAATTATAATATTTTATATTTT 1860

PFB5\_Exo\_WT ATATATACAAGAAAAGAGGAAGGAACACCTTCTTATAAGAATTATAATATTTTATATTTT 1860

PFB8\_Exo\_E415G ATATATACAAGAAAAGAGGAAGGAACACCTTCTTATAAGAATTATAATATTTTATATTTT 1860

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PF3D7\_Exo\_WT GAAACATTGAATAGAGATATGATTCAATCAAATGATGTACATTTATTCAAAGAATTAGTA 1920

PFB5\_Exo\_WT GAAACATTGAATAGAGATATGATTCAATCAAATGATGTACATTTATTCAAAGAATTAGTA 1920

PFB8\_Exo\_E415G GAAACATTGAATAGAGATATGATTCAATCAAATGATGTACATTTATTCAAAGAATTAGTA 1920

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PF3D7\_Exo\_WT CATTCGACACCTTATAATTTTAATAAGAATTTAATGAATAAAATAAAAAAA**AAA**AATAAT 1980

PFB5\_Exo\_WT CATTCGACACCTTATAATTTTAATAAGAATTTAATGAATAAAATAAAAAAA**AAT**AATAAT 1980

PFB8\_Exo\_E415G CATTCGACACCTTATAATTTTAATAAGAATTTAATGAATAAAATAAAAAAA**AAT**AATAAT 1980

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PF3D7\_Exo\_WT AATATTAATATACCAAACAAACAAATGTTCAAATTATATGTTAAAAGTTTAAATGACTTA 2040

PFB5\_Exo\_WT AATATTAATATACCAAACAAACAAATGTTCAAATTATATGTTAAAAGTTTAAATGACTTA 2040

PFB8\_Exo\_E415G AATATTAATATACCAAACAAACAAATGTTCAAATTATATGTTAAAAGTTTAAATGACTTA 2040

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PF3D7\_Exo\_WT TGTATAAAGATATTAAATAAAAAGTTAAATAAAAAATTTCAATTAGCTAACTGGAATATA 2100

PFB5\_Exo\_WT TGTATAAAGATATTAAATAAAAAGTTAAATAAAAAATTTCAATTAGCTAACTGGAATATA 2100

PFB8\_Exo\_E415G TGTATAAAGATATTAAATAAAAAGTTAAATAAAAAATTTCAATTAGCTAACTGGAATATA 2100

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PF3D7\_Exo\_WT AGACCACTTAACCAAGAACAAATAATATATGCATGTATTGATTCATATGTTTTAATAAAG 2160

PFB5\_Exo\_WT AGACCACTTAACCAAGAACAAATAATATATGCATGTATTGATTCATATGTTTTAATAAAG 2160

PFB8\_Exo\_E415G AGACCACTTAACCAAGAACAAATAATATATGCATGTATTGATTCATATGTTTTAATAAAG 2160

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PF3D7\_Exo\_WT ATAGAAGAAATGTTGATAGAAAAAGGTTATATGTCTACATGTGATTCTAACAACAATCAA 2220

PFB5\_Exo\_WT ATAGAAGAAATGTTGATAGAAAAAGGTTATATGTCTACATGTGATTCTAACAACAATCAA 2220

PFB8\_Exo\_E415G ATAGAAGAAATGTTGATAGAAAAAGGTTATATGTCTACATGTGATTCTAACAACAATCAA 2220

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PF3D7\_Exo\_WT ATGATGAATTTATTTCTTCAAAAATATAAGTTCAAAGATAGTACATGGGAATGA 2274

PFB5\_Exo\_WT ATGATGAATTTATTTCTTCAAAAATATAAGTTCAAAGATAGTACATGGGAATGA 2274

PFB8\_Exo\_E415G ATGATGAATTTATTTCTTCAAAAATATAAGTTCAAAGATAGTACATGGGAATGA 2274

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**Fig.S1.** **DNA sequence alignment of *exo* gene from *P. falciparum* 3D7, B5 and B5-r*exo*-E415G-B8 parasites.** The *exo* gene contains 2 exons and one intron. Exon 1 starts from nucleotides 1 to 949, while exon 2 starts from nucleotides 1018 to 2274. The intron is shown in lowercase letters. Grey highlight indicates the recodonized region in B8 line with the amino acid residue 415 change from Glu (codon GAG) to Gly (codon GGC). One single nucleotide mutation at position 1974 from A to T leads to non-synonymous mutation, changing from amino acid Lys to Asn. Guide RNA sequence is shown in yellow highlight while the Protospacer Adjacent Motif (PAM) site is shown in magenta highlight.

Timeline

Description automatically generated

**Fig. S2.** **Gene and amino acid composition of PfEXO**. Schematic diagram of *pfexo* gene and domain structure of PfEXO from 3D7 and B5 lines. *pfexo* gene contains 2 exons (light blue boxes) and 1 intron (red box). PfEXO contains N-terminal signal peptide (green box) and 3′ to 5′ exonuclease domain (navy blue box). Single amino acid mutation from Lys to Asn at residue 614 found in PfEXO from the B5 line is indicated in red circles. The amino acid Glu at residue 415, focused on in this study, is indicated in orange circles.

A screenshot of a computer

Description automatically generated with medium confidence

**Fig S3**. Schematic of *P. falciparum plasmepsin* 2/3 *(pfpm2*/3) gene duplication. (A) Gene model showing the *pfpm* 2/3 breakpoint (dashed lines). Primer positions are labelled in the single copy (top) and multiple copy (bottom) isolates. (B) Amplification primer pairs 1 and 2 are to confirm that regions surrounding the *pfpm* 2/3 amplification are present in all samples, while primer pair 3 is to detect the *pfpm* 2/3 amplification and no PCR products are observed in samples with a single copy of *pfpm* 2/3 amplification. It is noted that ASAP-05 was name isolate 1 in the previous publication1.

**Reference**

1 Boonyalai, N. *et al.* Piperaquine resistant Cambodian Plasmodium falciparum clinical isolates: in vitro genotypic and phenotypic characterization. *Malar J* **19**, 269, doi:10.1186/s12936-020-03339-w (2020).