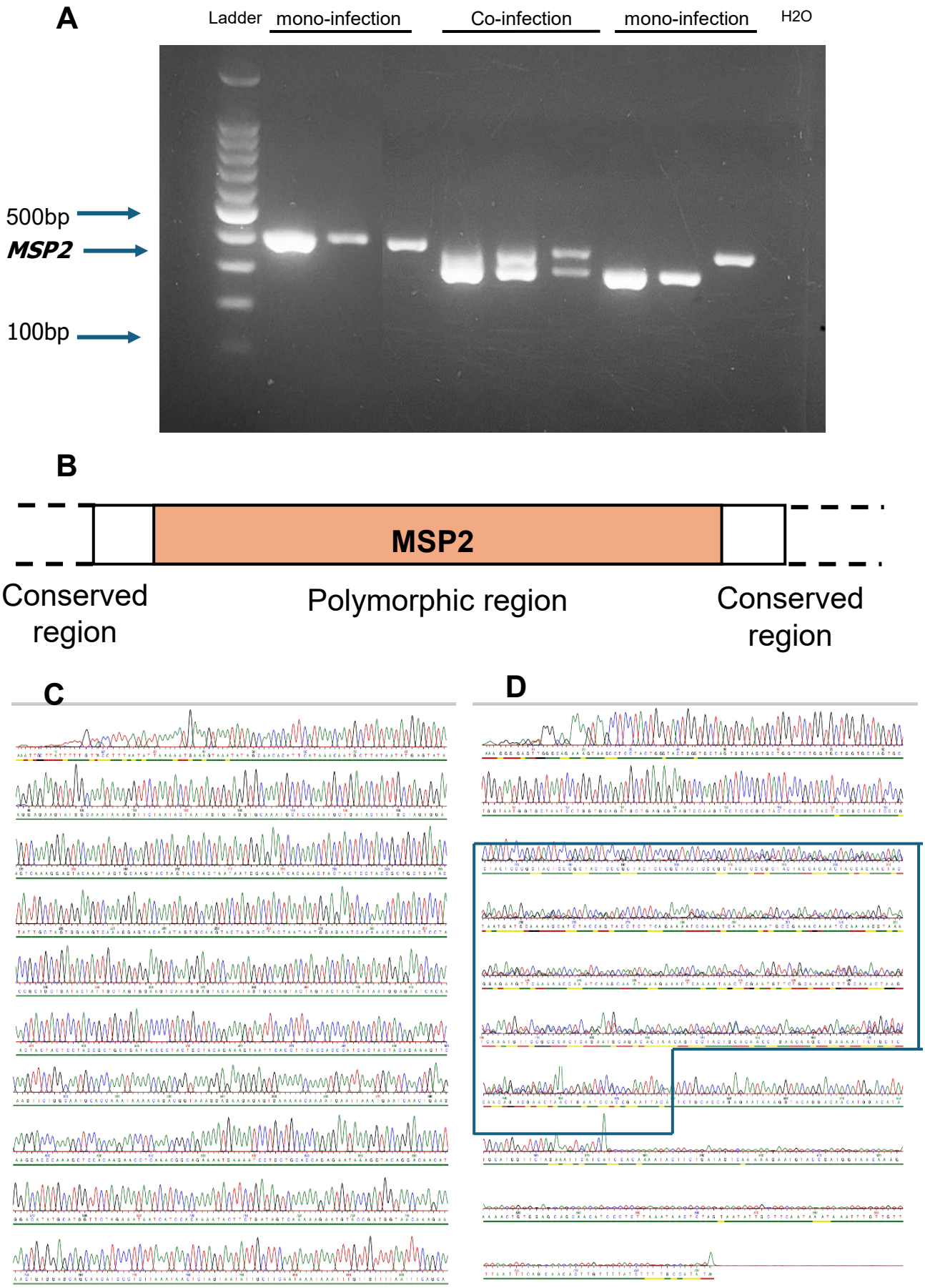

Figure S1: Sequencing of MSP2 is a good is an efficient approach to discriminate coinfection and mono-infection of *Plasmodium falciparum* in clinical isolates

A: MSP2 gel electrophoresis revealed presence of double bands as sign of co-infection.
B: Simplified illustration of MSP2 sequence showing the conserved and polymorphic regions.
Sequencing peaks from Eurofins genomics platform showed high specificity and amplitude of MSP2 gene from mono-infected sample **(C)** as compared to coinfected samples **(D)** which shows a specific signal at conserved N and C terminals and an unspecific sequencing signal at the variable region.

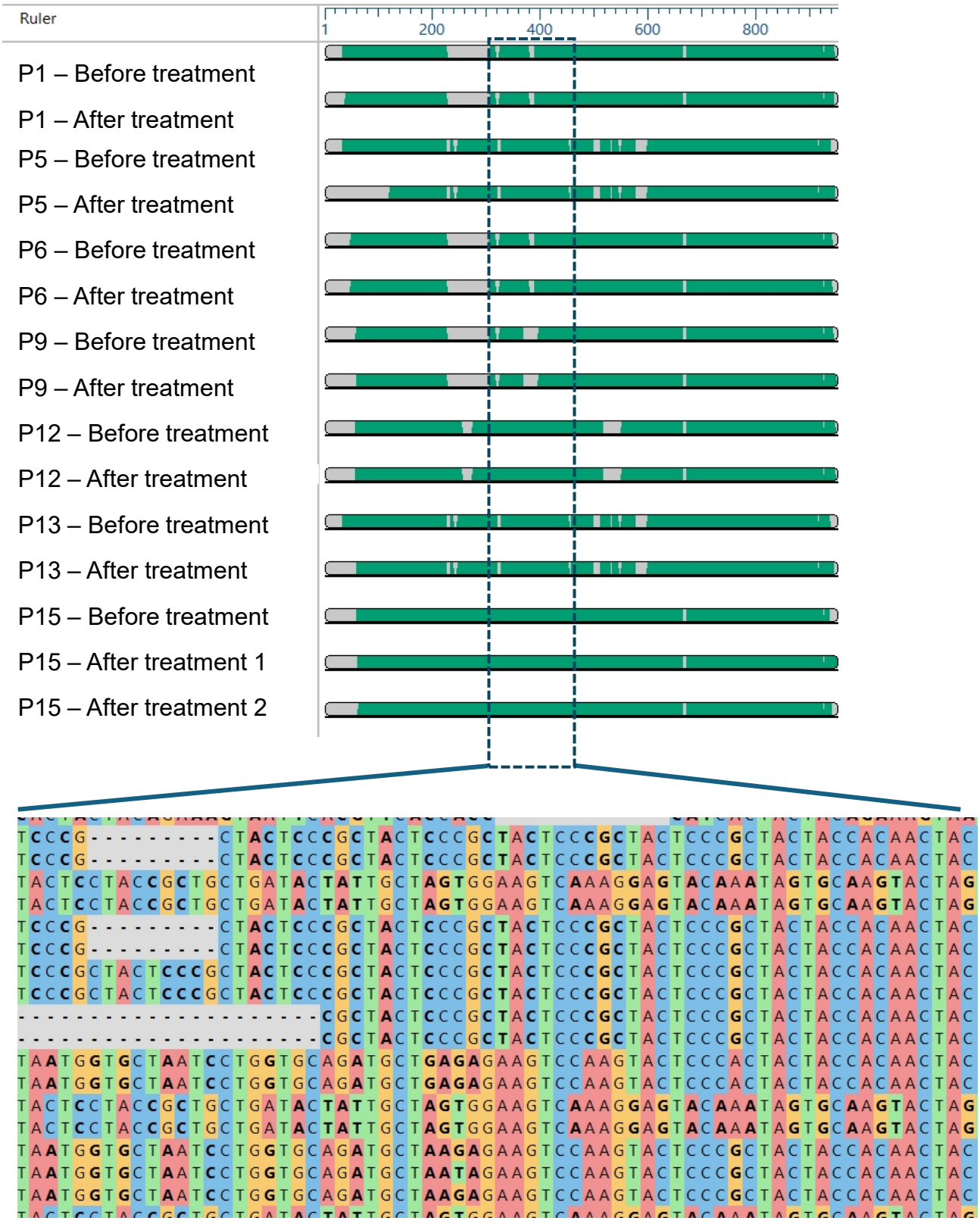

Figure S2: Conserved *MSP2* sequence identity in isolates before and after ACT treatments

Sequence alignment of 15 (all data not shown) mono-infected samples with treatment failure revealed the presence of highly conserved regions at N and C terminals of the sequence and presence of deletions in the highly variable regions (in grey) that enables to differentiate between strains. All sequences before and after treatment showed same or similar pattern with differences less that 50% and could be identified as parasite recrudescence.

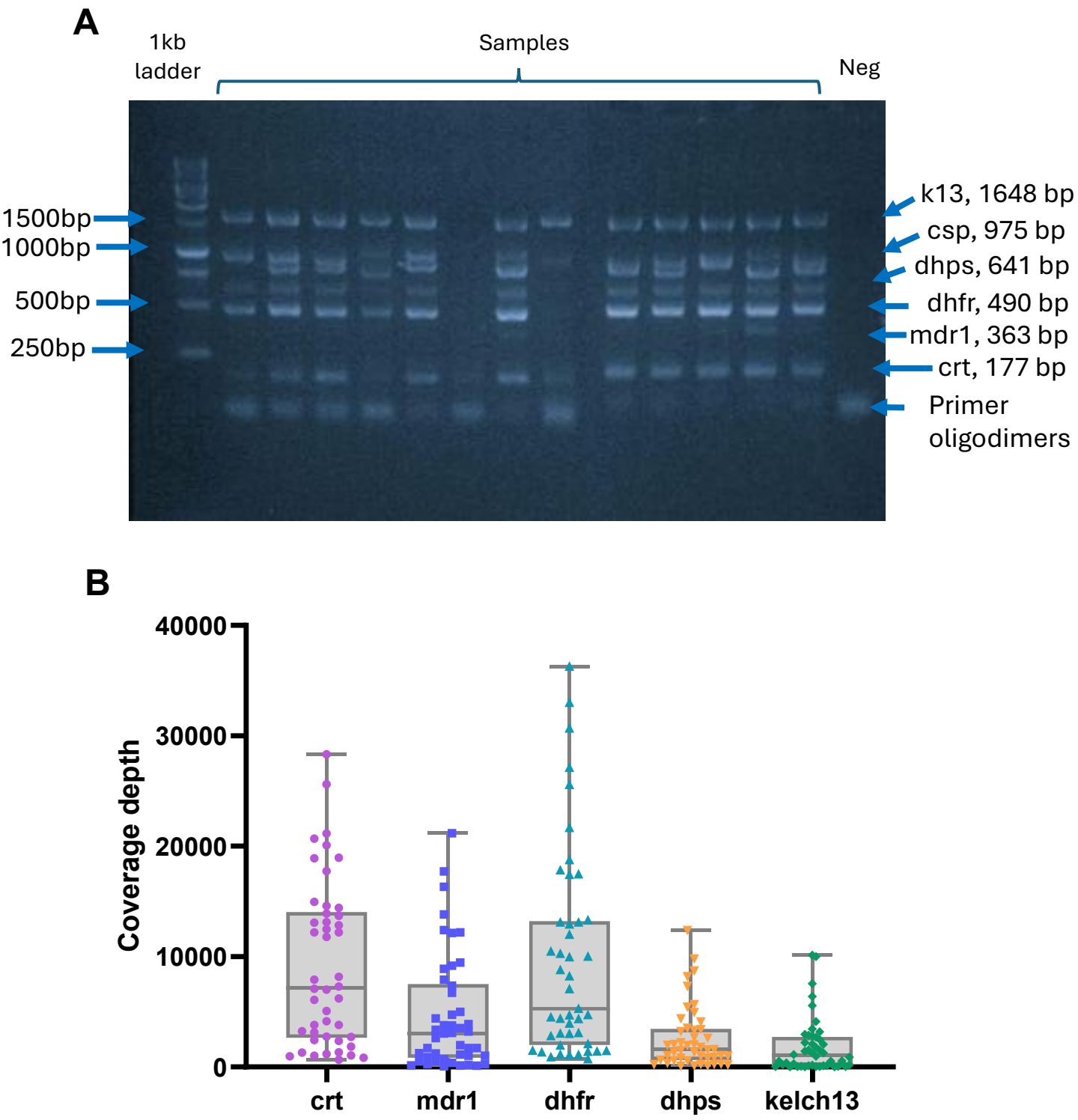

Figure S3: Agarose gel of targeted sequencing and target coverage depth.

Multiplex PCR of *crt, mdr1, dhfr, dhps* and *csp* genes and single *kelch13* PCR from all samples before and after treatment (N=46 – 23 samples before treatment; 19 samples after first treatment and 4 samples after second treatment) were performed, PCR product pooled and revealed on agarose gel (**A**). Purified PCR products were used for library preparation using ONT Native Barcoding Kit 24 V14 (SQK-NBD114.24) and final library loaded on Flongle flow cell R.10.4. and run for more than 20 hours. Raw fastq files generated were processed to obtained VCF files. Coverage depth (**B**) of each variant was assessed with cut off point set at 20X.

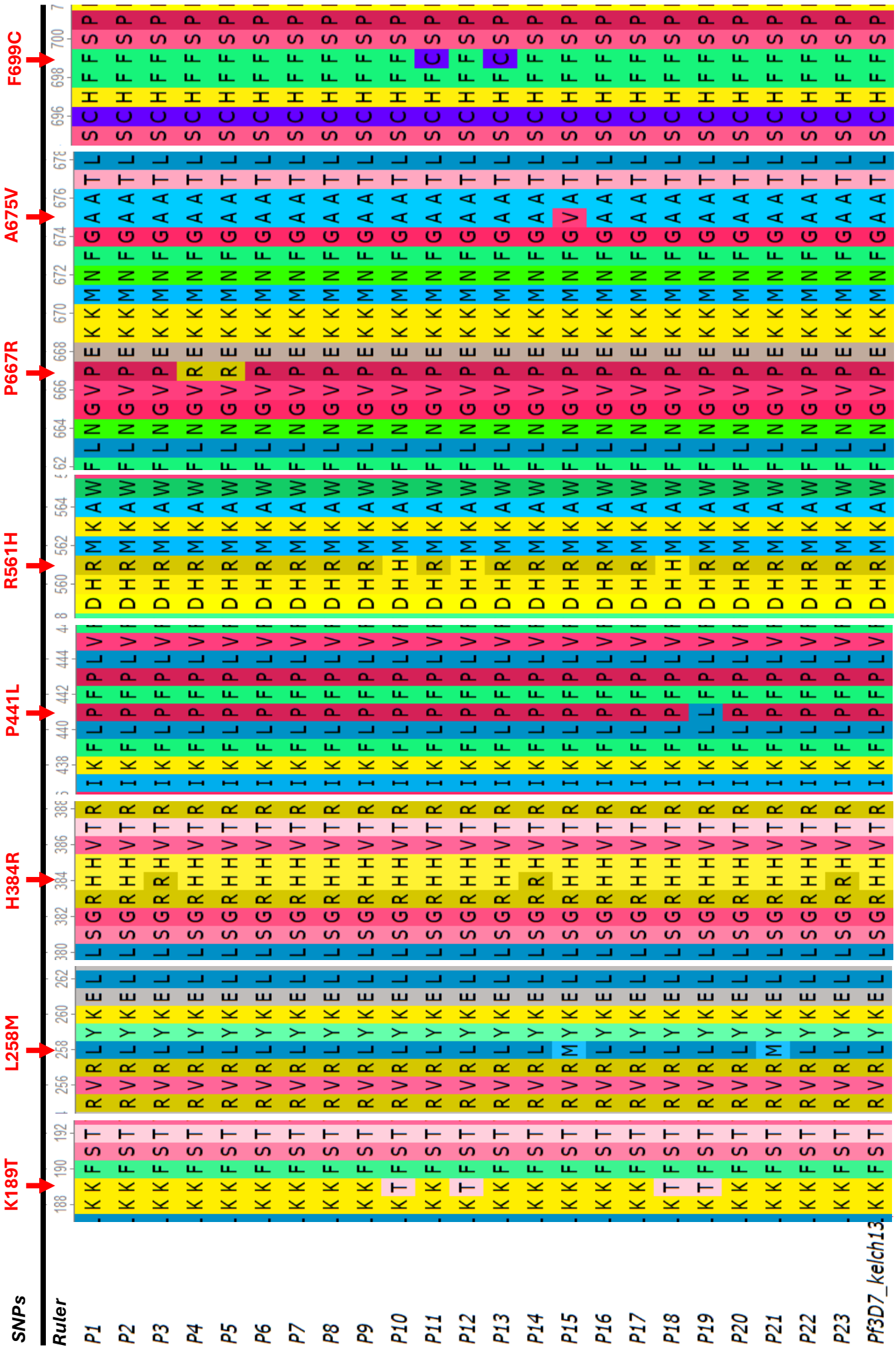

Figure S4: Multiple sequence alignment of Kelch13 sequences.

All 3 domains propeller Kelch 13 were assessed for SNP by Sanger sequencing using the forward primer and Eurofin genomics platform. Red arrow indicates the nucleotide position mutated in isolates with SNP in the K13PD. The alignment of the obtained sequences using PF3D7_1343700 as reference confirmed the presence of 8 different SNPs and the corresponding amino acid substitution were as follows: K189T, L258M, H384R, P441L, R561H, P667R, A675V and F699C.

Table S1: Docking parameters between KELCH13 H384R CULLIN-E3 ligase

Parameters	H384 + CULLIN E3 (wild type interacion)	384R + CULLIN E3 (mutant type interacion)
HADDOCK score	-85.0 +/- 11.9	-71.9 +/- 7.0
Cluster size	16	15
RMSD from the overall lowest-energy structure	1.1 +/- 0.7	23.1 +/- 0.2
Van der Waals energy	-70.7 +/- 2.4	-64.8 +/- 6.0
Electrostatic energy	-405.9 +/- 12.7	-447.1 +/- 21.6
Desolvation energy	-0.5 +/- 4.3	5.2 +/- 2.3
Restraints violation energy	673.0 +/- 106.7	771.9 +/- 33.4
Buried Surface Area	2484.6 +/- 74.2	2380.8 +/- 66.3
Z-Score	-1.9	-1.9

Table S2: Primer pairs used in the study

Gene name	Forward primer	Reverse primer
18Srna	AAA GTT AAG GGA GTG AAG A	AAG ACT TTG ATT TCT CAT AAG G
MSP2	ATG AAG GTA ATT AAA ACA TTG TCT ATT ATA	ATA TGG CAA AAG ATA AAA CAA GTG TTG CTG
PfS25	GAC TGT AAA TAA ACC ATG TGG AGA TT	CCG TTA CCA CAA GTT ACA TTC TTA C
S-tRNA syn	AAG TAG CAG GTC ATC GTG GTT	TTC GGC ACA TTC TTC CAT AA
crt	TGT CTT GGT AAA TGT GCT CA	AGT TGT GAG TTT CGG ATG TT
mdr1	TGT GTT TGG TGT AAT ATT AAA GAA CA	ACA TAA AGT CAA ACG TGC ATT T
dhfr	GTT TTC GAT ATT TAT GCC ATA TGT G	TGA TAA ACA CGG AAC CTC C
dhps	TTT GTT GAA CCT AAA CGT GC	AAC ATT TTG ATC ATT CAT GCA AT
kelch13	GAT GCA GCA AAT CTT ATA AAT GAT GAT TCT	GCC AAG CTG CCA TTC ATT TG