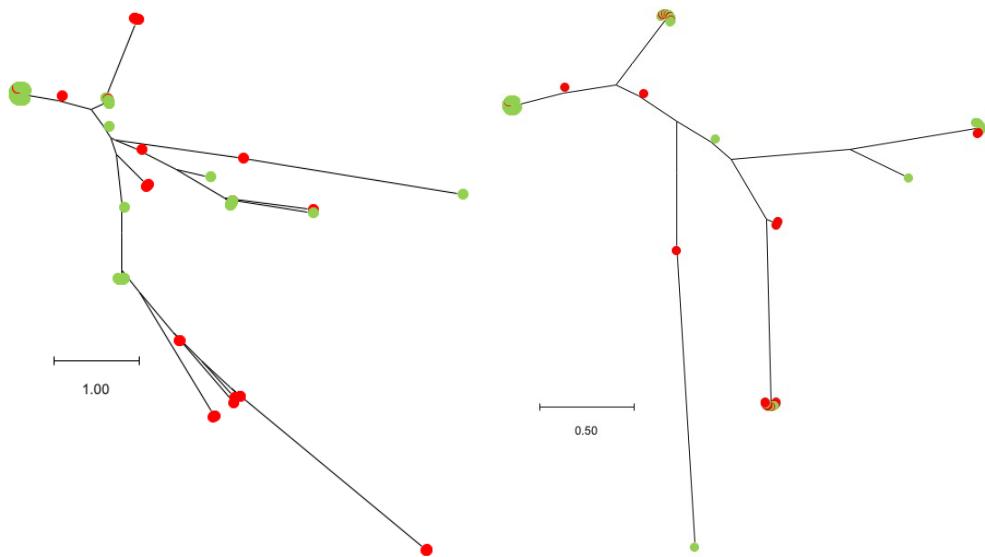
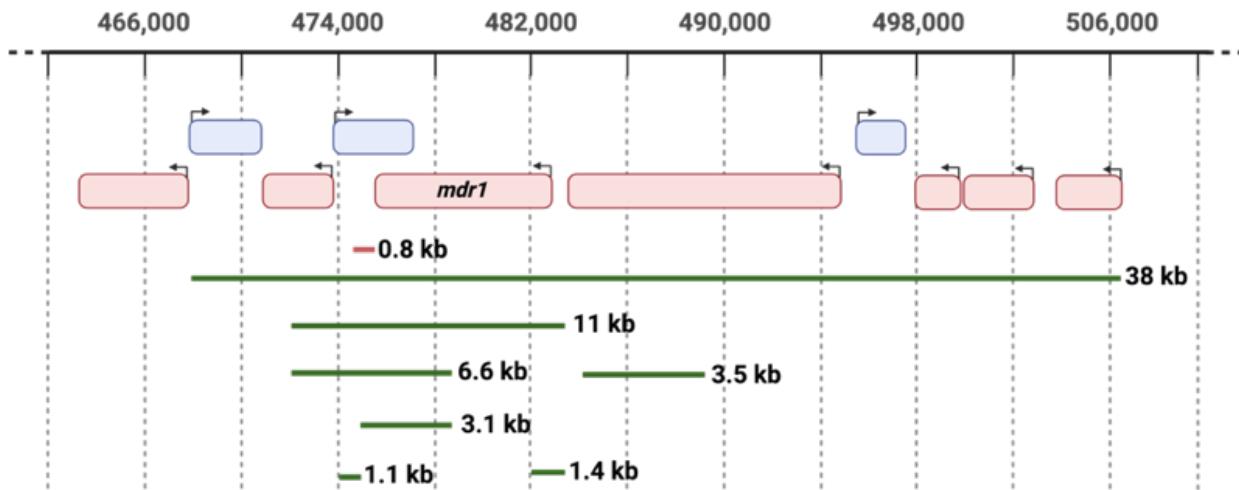


Supplemental Figures

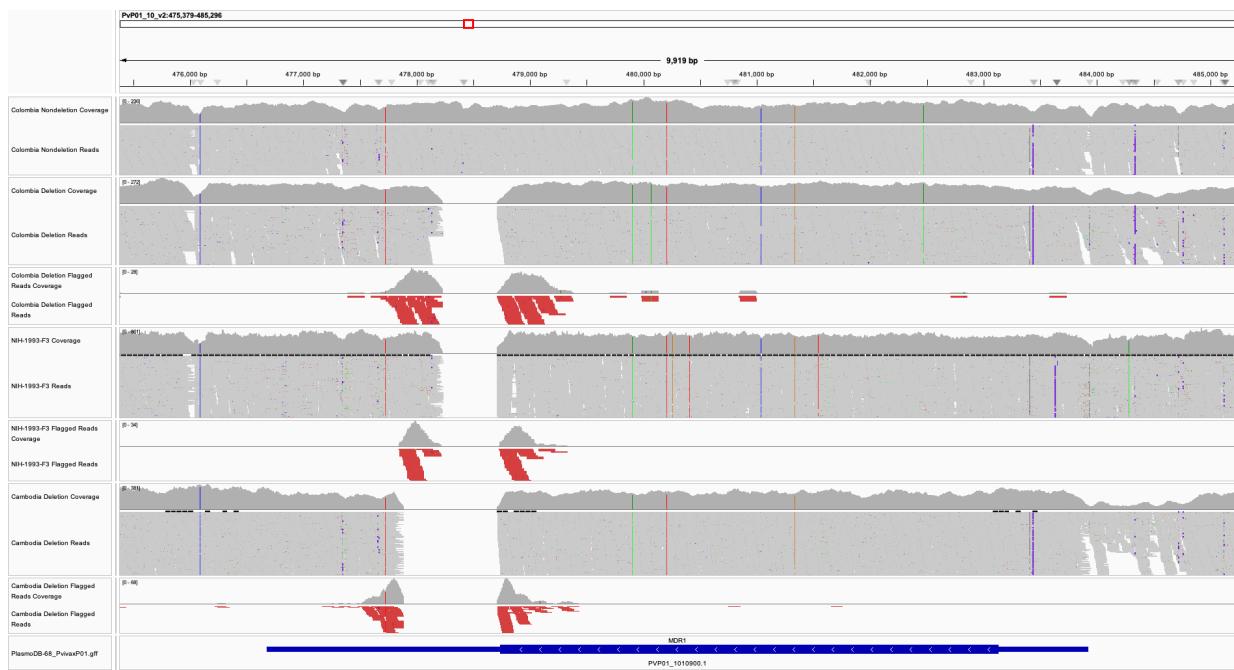
Supplemental Figure 1. Genetic relationships among the *mdr1* protein-coding sequences of isolates with and without the deletion. The figure shows Neighbor-Joining trees reconstructed using the number of differences in the nucleotide (**left**) and amino acid (**right**) between *mdr1* protein-coding sequence from monoclonal samples. The green dots represent samples carrying the *mdr1* deletion, while the red dots indicate isolates without deletions. The scale bar represents one nucleotide (left) or amino acid (right) difference.



Supplemental Figure 2. Diagram illustrating the location of one deletion and seven putative tandem duplications in the *mdr1* region that were detected amongst the MalariaGEN PV4 samples. Blue boxes represent annotated genes on the plus strand while red boxes represent annotated genes on the minus strand. Detected tandem duplications are depicted using green horizontal lines and the detected deletion is depicted using a red horizontal line.



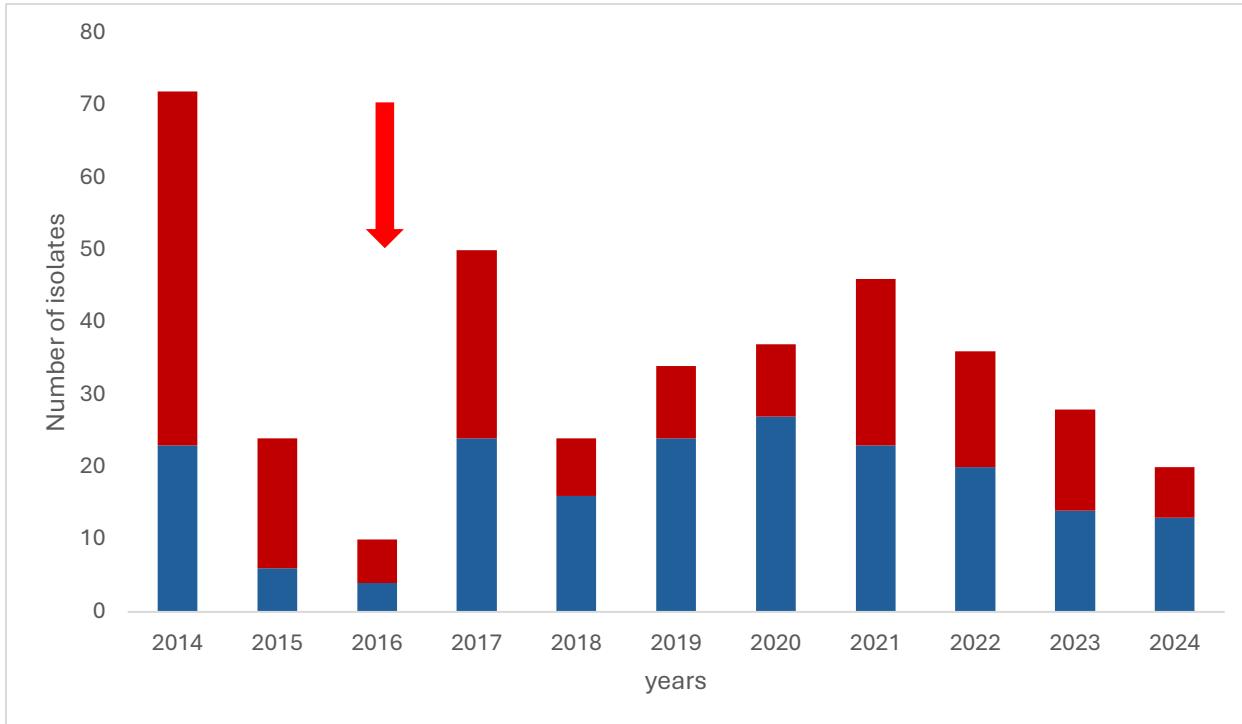
Supplemental Figure 3. Screenshot of whole genome sequencing reads mapped to the *mdr1* locus. Each track represents data from a different *P. vivax* isolate: from top to bottom, a MalariaGen Columbian isolate without a deletion, a MalariaGen Columbian isolate with a deletion, NIH-1993-F3 (a strain derived from Salvador 1), a Cambodian sample with a deletion. Note that while the deletion appears identical in the two South American isolates, the deleted region is significantly larger in the Cambodian isolate.



Supplemental Figure 4. Alignment of the P01 reference sequence downstream of the *mdr1* protein-coding gene with the sequences generated by Sanger sequencing of amplicons spanning the *mdr1* deletion in NIH-1993-F3 and one representative Cambodian isolate with the deletion. The 3' end of the *mdr1* protein-coding region is highlighted in green, while the TGTACA motif is highlighted in blue. Note that *P. vivax* parasites from Southeast Asia and South America carry overlapping but different deletions in the 3' end of the *mdr1* coding sequence.

PvP01 Amplicon	...TTTGTGCGCAGCTT TGTACA CACGACT... (325 bp) ...TGCACGTATG TGTACA CATCCCCAT... (465 bp) ...GCACGATG TGTACA CGCGAAGGGGACCGCGCA GATGAATCGGT ...
Sail1 Amplicon	...TTTGTGCGCAGCTT TGTACA CACGACT... (325 bp) ...TGCACGTATG-----... (465 bp) ... TGTACA CGCGAAGGGGACCGCGCA GATGAATCGGT ...
Cambodia Deletion Amplicon	...TTTGTGCGCAGCTT-----... (780 bp) ...----- TGTACA CGCGAAGGGGACCGCGCA GATGAATCGGT ...

Supplemental Figure 5. Longitudinal survey of the *mdr1* deletion in *P. vivax* isolates collected in Eastern Cambodia between 2014 and 2024. The blue stacks represent the *P. vivax* isolates carrying the deletion downstream of *mdr1* while the red stacks show the number of isolates without the deletion. The red arrow indicates the change in national treatment guidelines from DHA-PPQ to As-MQ.



Supplemental Table**Table S2**

Primer Pairs	Primers	Sequence
<i>mdr1</i> deletion sequencing	Forward Primer (5'-3')	AAAGGCACCGAACTGAAACG
	Reverse Primer (5'-3')	GAACCGGCTCCTTGTACAG
Amplify with or without deletion	Forward Primer (5'-3')	TGCTGAACGATGCGCTTATT
	Reverse Primer (5'-3')	GAACCGGCTCCTTGTACAG
Amplify only if nondeleted	Forward Primer (5'-3')	TTTACGCTGGAATGTGGCAC
	Reverse Primer (5'-3')	GAACCGGCTCCTTGTACAG