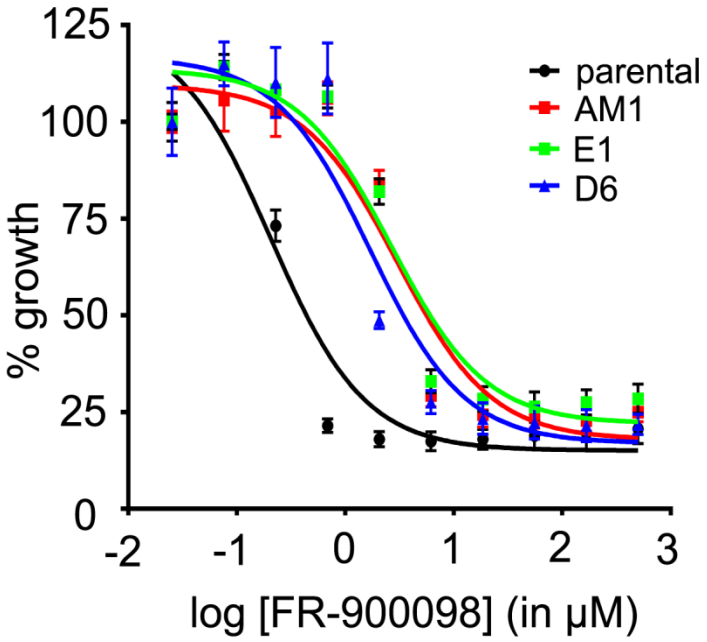
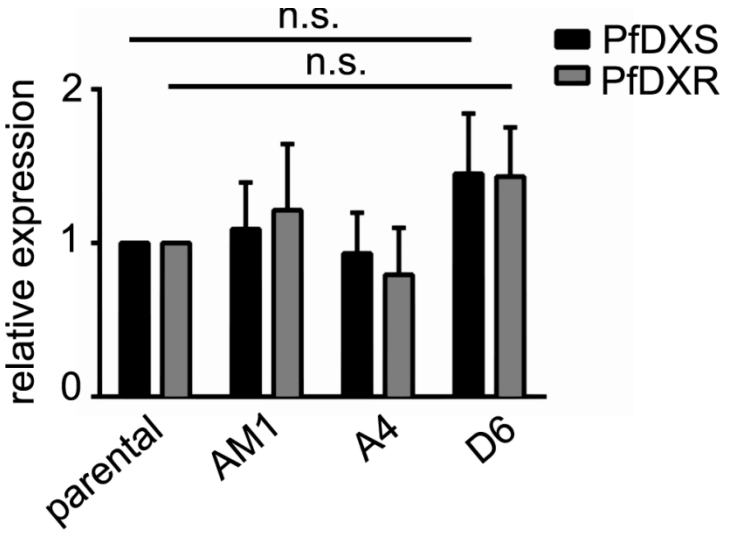
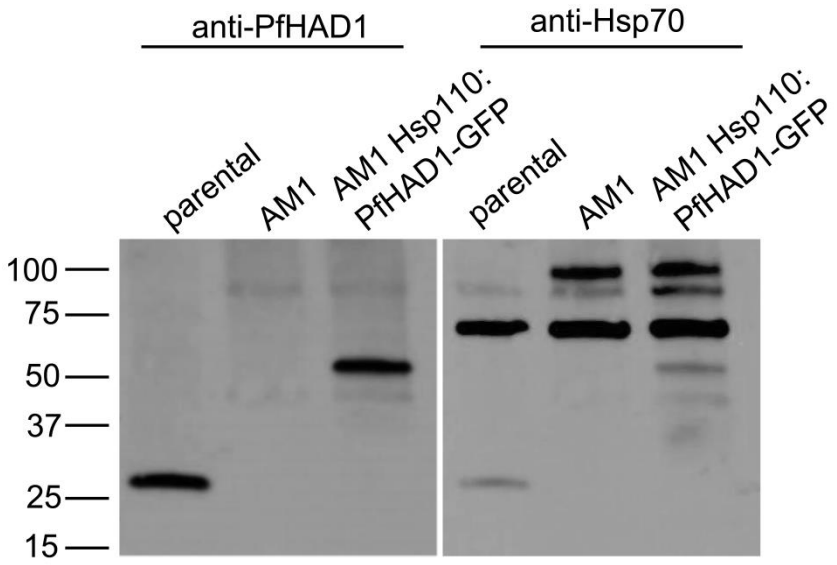


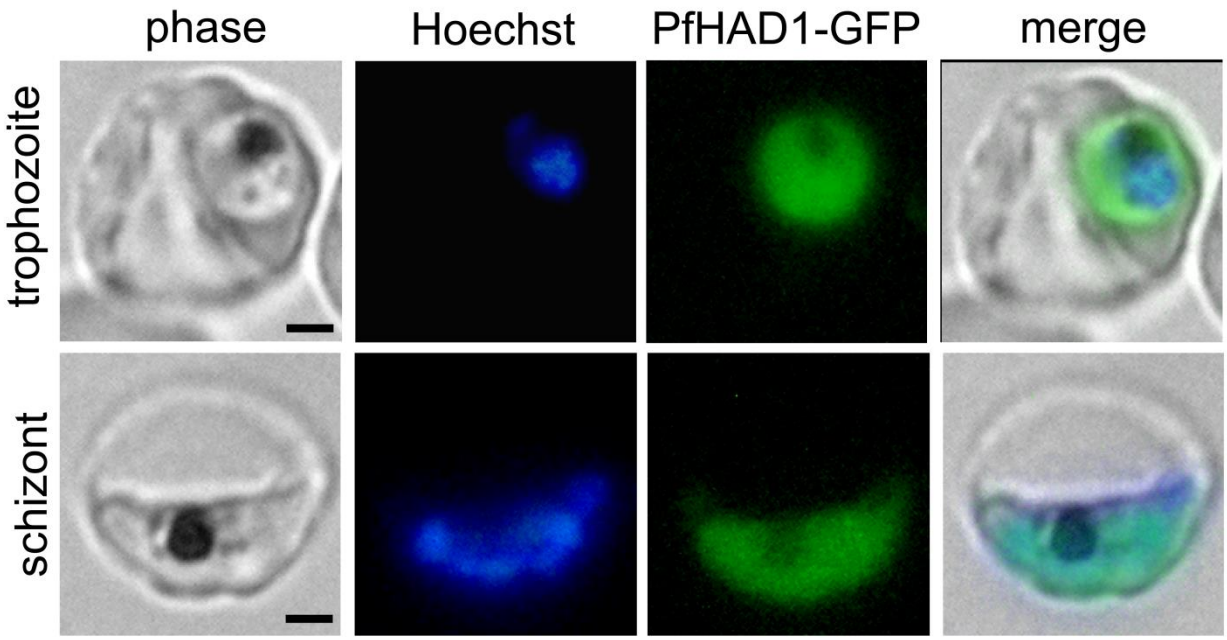
Supplementary Figure 1. FSM^R strains are also resistant to the FSM-related compound, FR-900098. Displayed are the means ± standard error of the mean (SEM) of at least three independent experiments. The parental strain has an IC_{50} of 0.23 ± 0.03 µM against FR-900098. FSM^R strains AM1, E1, and D6 have FR-900098 IC_{50}s of 3.2 ± 0.24 µM, 1.7 ± 0.001 µM, and 2.8 ± 0.17 µM, respectively.



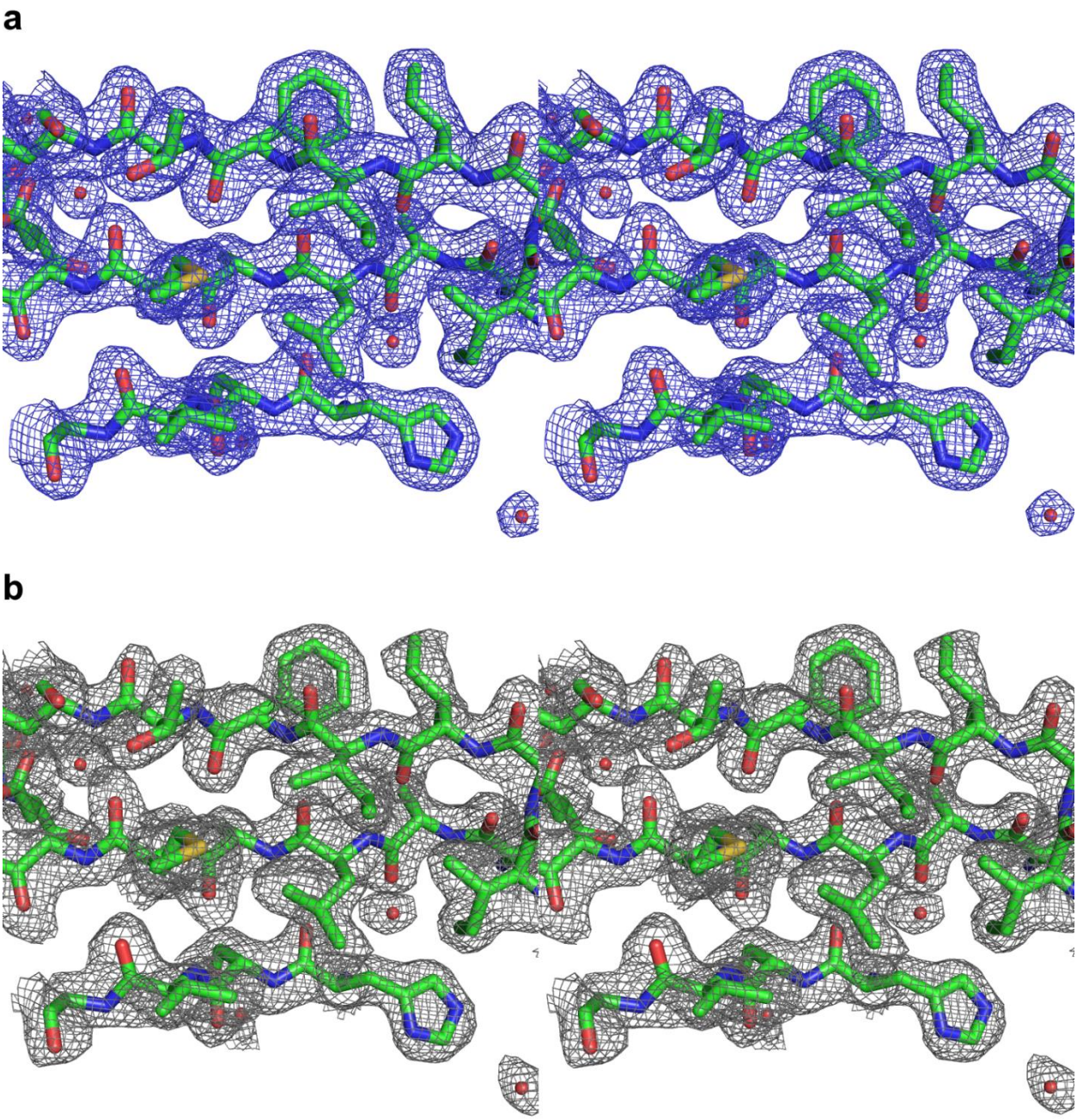
Supplementary Figure 2. **Relative mRNA expression levels of PfDXS and PfDXR are unchanged in FSM^{R} strains.** Data represent means and standard error of the mean (SEM) of at least two independent experiments. “N.s” = not significant. P-values are >0.3 (unpaired Student’s *t*-test) for comparisons shown.



Supplementary Figure 3. Full immunoblots demonstrating expression of PfHAD1-GFP in FSM^{R} strain AM1. Samples shown are the parental strain, FSM^{R} strain AM1, and AM1 Hsp110:PfHAD1-GFP. Marker units are kilodaltons. The blot was probed with anti-PfHAD1 antisera (left), stripped, and re-probed with anti-heat shock protein 70 antisera (right). Expected approximate protein masses: native PfHAD1, 33 kDa; PfHAD1-GFP, 60 kDa; hsp70, 74 kDa. Data are representative of at least three independent experiments.



Supplementary Figure 4. PfHAD1-GFP localization is similar to that of PfHAD1 (Fig. 7). Shown is live microscopy of AM1 Hsp110:PfHAD1-GFP trophozoite and schizont, stained with Hoechst 33258 nuclear stain. Scale bars represent 2 µm.



Supplementary Figure 5. Stereo image of the electron density maps for a representative region of PfHAD1. (a) The 2Fo-Fc electron density map contoured at 1.0 σ is colored blue. **(b)** The composite simulated annealing omit map contoured at 1.0 σ is colored grey.

Supplementary Table 1. FSM IC_{50}s, PfHAD1 alleles, and NCBI database accessions for FSM^R strains.

Strain	FSM IC_{50} (μM)	PfHAD1 change	PfHAD1 protein variant	Polyphen-2 score*	SRA accession #$	Trace Archive TI #s$
parental	1.1	none	wild-type		SRS561734	2338198571-75
E1	8.1	A28T	K10X	n/a*	SRS561733	2338198557-60
B4	2.8	A28T	K10X	n/a		2338198501-4
A1	6.1	C77G	T26R	0.999	SRS561731	2338198460-63
D6	8.7	G89A	G30E	1.000	SRS561730	2338198553-56
A3	4.6	ΔA112	Frameshift, results in L47X	n/a		2338198468-71
D2	1.5	C179A	A60E	1.000		2338198540-43
A2	4.6	T388C	W130R	0.941		2338198464-67
A4	5.8	A403 insertion (+A)	Frameshift, results in E140X	n/a		2338198472-75
MK1	3.7	A443G	Y148C	0.747	SRS561761	2338198569-70
AM1	5.5	T623A	L208X	n/a	SRS561775	2338198484-87
AM2	3.3	T623A	L208X	n/a	SRS561776	2338198488-91
B1	5.9	Δ681-685	Frameshift, results in E240X	n/a	SRS561732	2338198492-95
F1	3.7	Δ681-685	Frameshift, results in E240X	n/a		2338198565-68
D3	4.3	Δ681-685	Frameshift, results in E240X	n/a		2338198544-47
A5	5.4	ΔC698	Frameshift, results in L234X	n/a		2338198476-79
D4	2.0	none	wild-type			2338198548-52
A6	4.1	none	wild-type		SRS561778	2338198480-83
E2	4.6	none	wild-type			2338198561-64
B2	5.0	none	wild-type			2338198496-500
D1	4.7	none	wild-type		SRS561777	2338198536-39

* = Polyphen-2 scores cannot be calculated for truncation mutations. Polyphen-2 is an algorithm for predicting the probability of deleterious effects of missense mutations.

$ = Whole genome sequencing data is deposited in the NCBI BioProject and Sequence Read Archive databases. Representative Sanger sequencing data of the PfHAD1 locus is deposited in the NCBI Trace Archive.

Supplementary Table 2. Kinetic parameters for PfHAD1 with the top three tested substrates and gly3P. Shown are means ± standard error of the mean (SEM) of at least three independent experiments.

Substrate	K_m (mM)	k_{cat} (sec^{-1})	k_{cat}/K_m (M^{-1} sec^{-1})
fru1P	2.5 ± 0.6	5.2 ± 0.9	2.1×10^3
glc2P	6.5 ± 0.8	8.5 ± 0.2	1.3×10^3
man6P	3.6 ± 0.8	3.1 ± 0.3	0.9×10^3
gly3P	4.6 ± 0.7	11 ± 4.3	2.4×10^3

Supplementary Table 3. FSM^R strains possessed increased levels of MEP pathway metabolites. Shown are concentrations of MEP pathway intermediates in *P. falciparum* FSM^R PfHAD1 strains. Data shown are means ± standard error of the mean (SEM) of at least three independent experiments.

Compound (attograms/cell)	DOXP		MEP		CDP-ME		MEcPP	
Strain	**- FSM**	**+ FSM**	**- FSM**	**+ FSM**	**- FSM**	**+ FSM**	**- FSM**	**+ FSM**
parental	0.20 ± 0.01	0.25 ± 0.03	1.03 ± 0.36	1.03 ± 0.38	0.020 ± 0.007	0.015 ± 0.007	16.02 ± 0.81	1.34 ± 0.29
B4	6.58 ± 3.14	18.73 ± 1.02	0.87 ± 0.32	0.27 ± 0.02	0.056 ± 0.004	0.049 ± 0.007	15.62 ± 0.44	3.39 ± 0.21
E1	10.06 ± 1.41	17.26 ± 2.18	3.97 ± 0.29	2.94 ± 0.28	0.057 ± 0.005	0.029 ± 0.005	24.35 ± 3.56	3.82 ± 0.45
A1	17.12 ± 2.52	33.39 ± 2.99	2.77 ± 0.30	0.75 ± 0.10	0.077 ± 0.006	0.056 ± 0.006	55.14 ± 5.01	7.18 ± 0.85
D6	8.50 ± 1.35	13.98 ± 2.79	4.19 ± 0.53	3.05 ± 0.42	0.035 ± 0.004	0.018 ± 0.001	26.65 ± 4.13	4.28 ± 0.82
A3	10.03 ± 0.86	14.86 ± 2.64	1.51 ± 0.10	0.33 ± 0.10	0.053 ± 0.003	0.031 ± 0.003	25.21 ± 2.07	3.07 ± 0.41
A4	11.03 ± 1.54	14.44 ± 5.18	2.02 ± 0.27	0.42 ± 0.18	0.079 ± 0.005	0.038 ± 0.010	33.95 ± 2.85	2.69 ± 0.71
AM1	14.71 ± 1.28	28.2 ± 3.61	1.65 ± 0.14	0.38 ± 0.02	0.064 ± 0.007	0.038 ± 0.002	20.06 ± 1.45	3.11 ± 0.48
A5	10.31 ± 1.24	16.63 ± 3.28	1.10 ± 0.12	0.14 ± 0.02	0.104 ± 0.011	0.044 ± 0.005	12.64 ± 1.53	1.47 ± 0.23