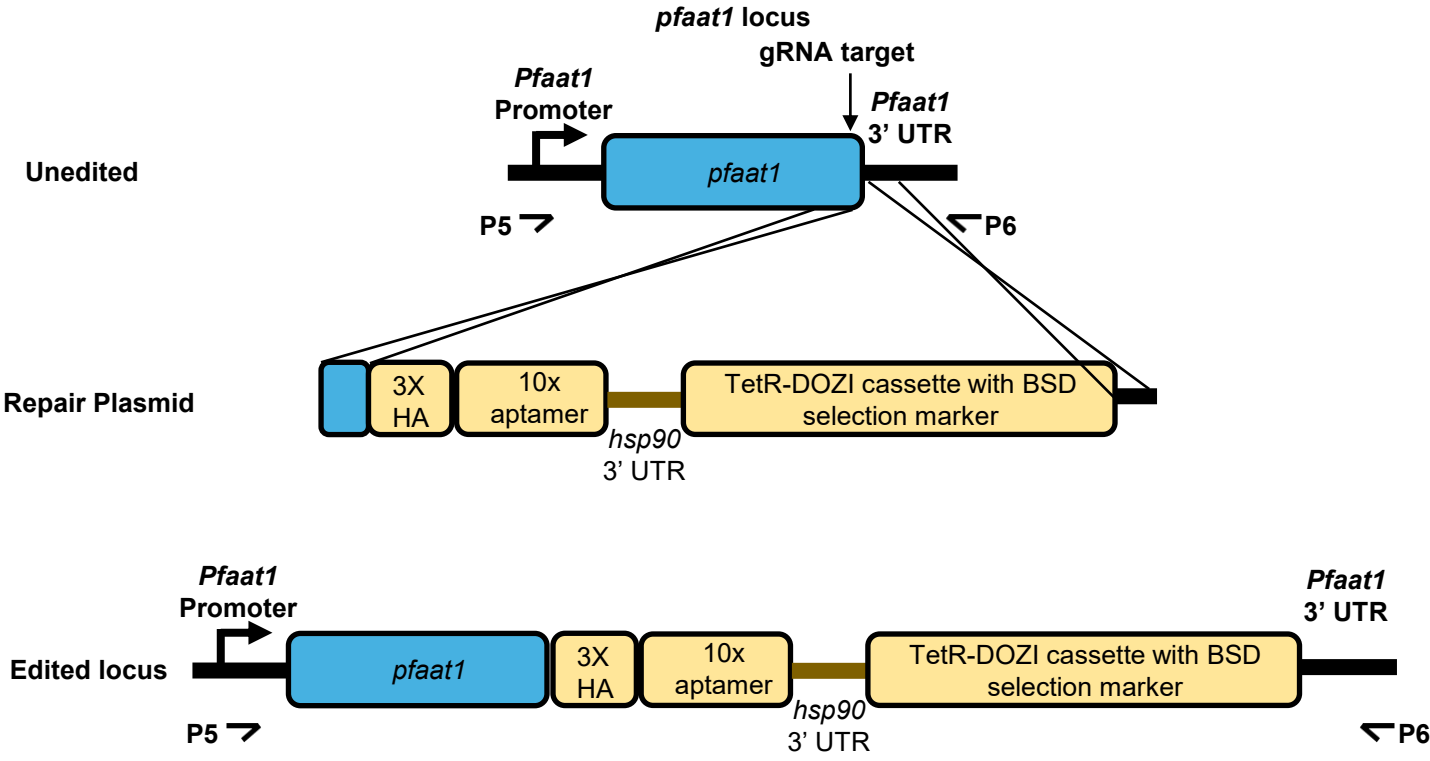
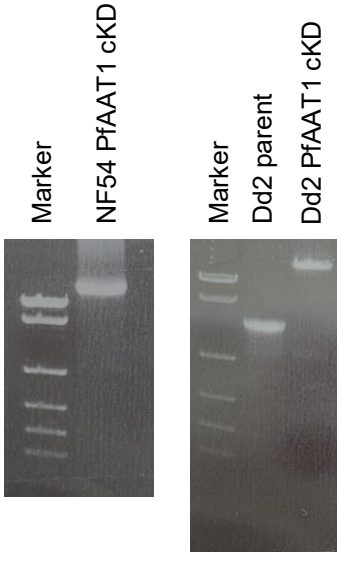
Supplementary Fig. 1

a.



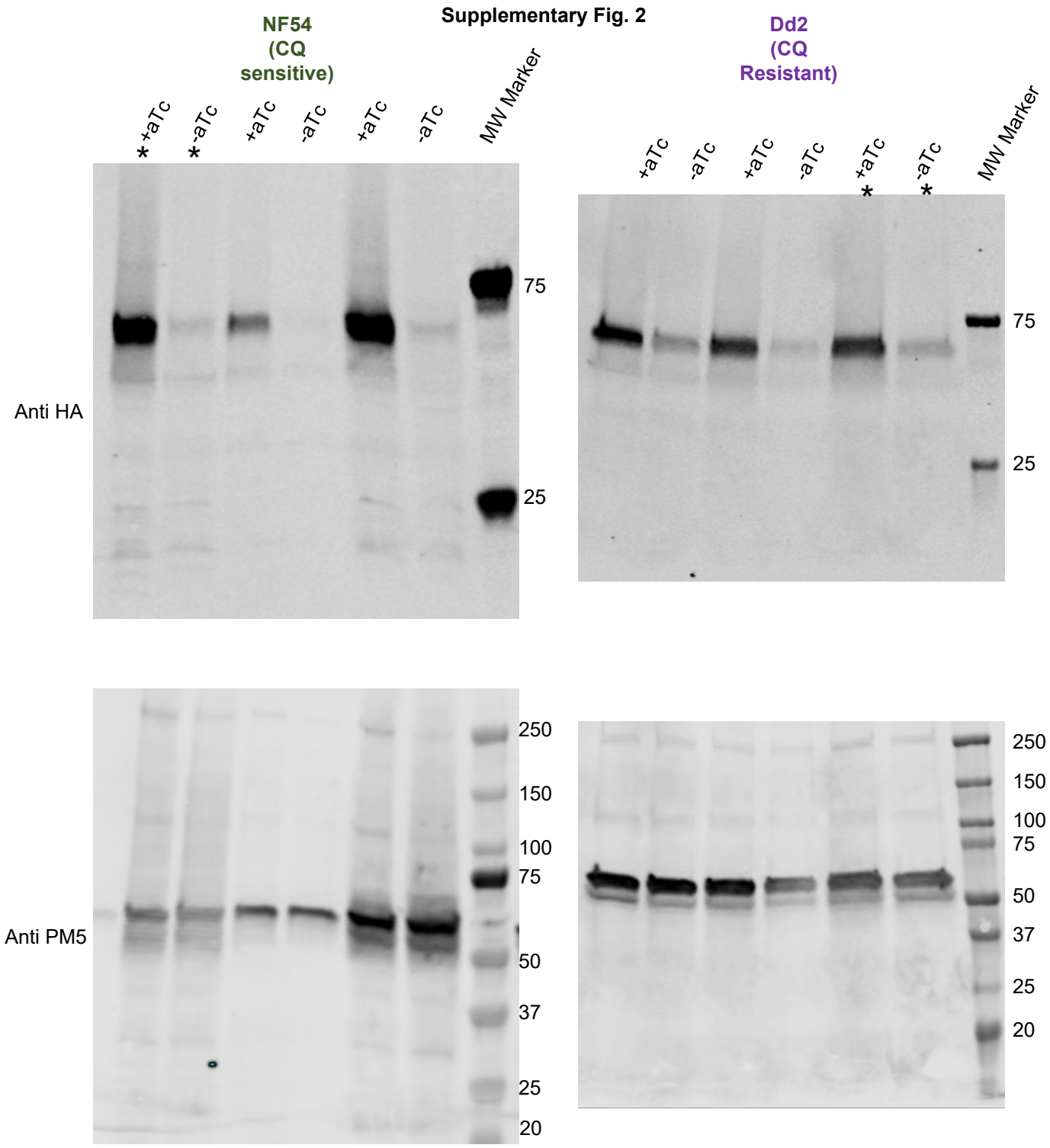
b.



Supplementary Fig. 1. Editing of the *pfcrt* locus.

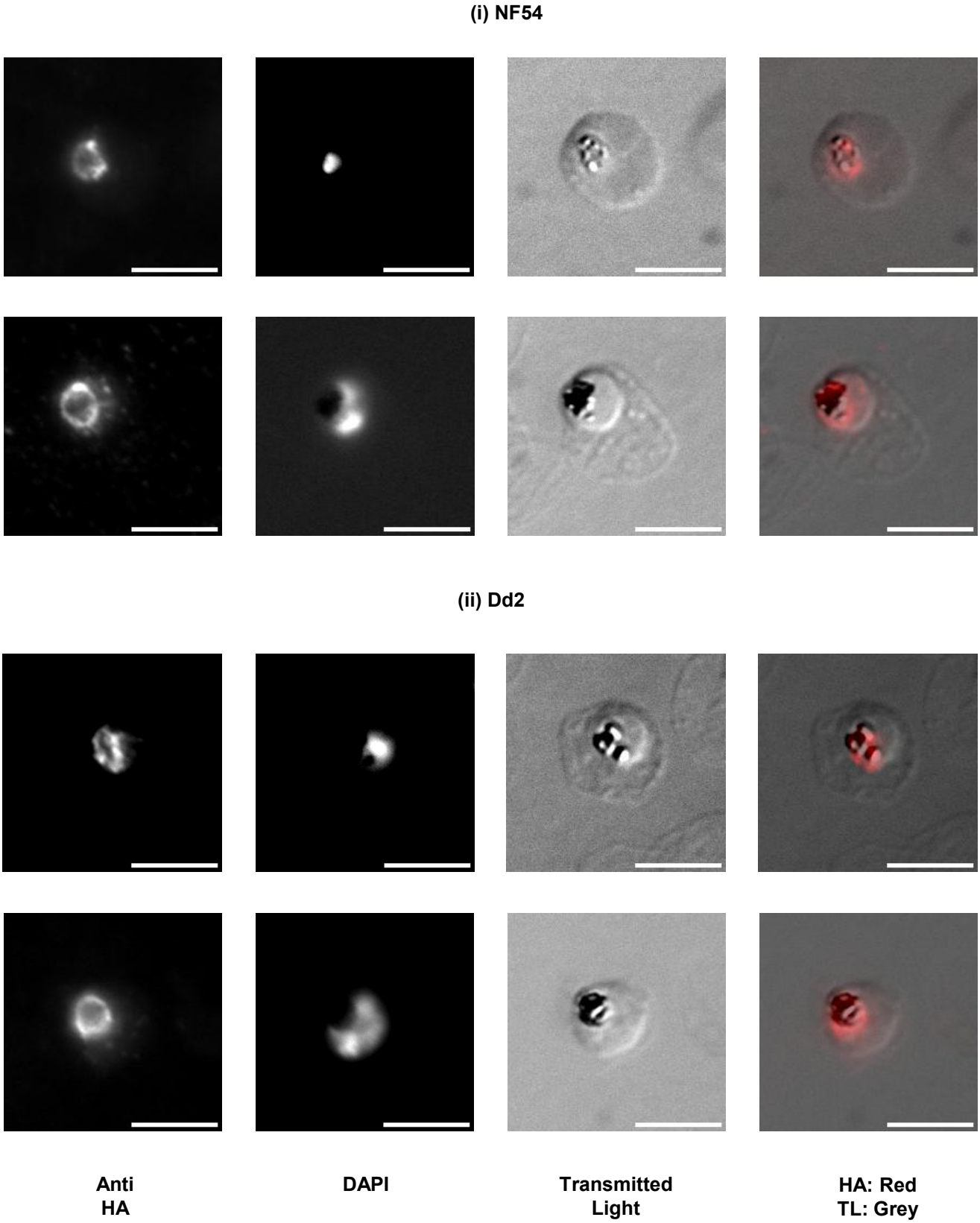
a, Schematic representation of the CRISPR–Cas9–mediated genome editing strategy for epitope tagging and addition of a tunable aptamer cassette at the endogenous *pfcrt* locus. 5′ and 3′ homology regions between the genomic sequence and the repair cassette are indicated by connecting lines. Location for diagnostic PCR primer set (P5 and P6) are indicated. From the unedited *pfaat1* locus, it amplifies a product of 3226 bp and from edited locus a product of 10986 bp.

b, Diagnostic PCR of genomic DNA confirming successful editing.



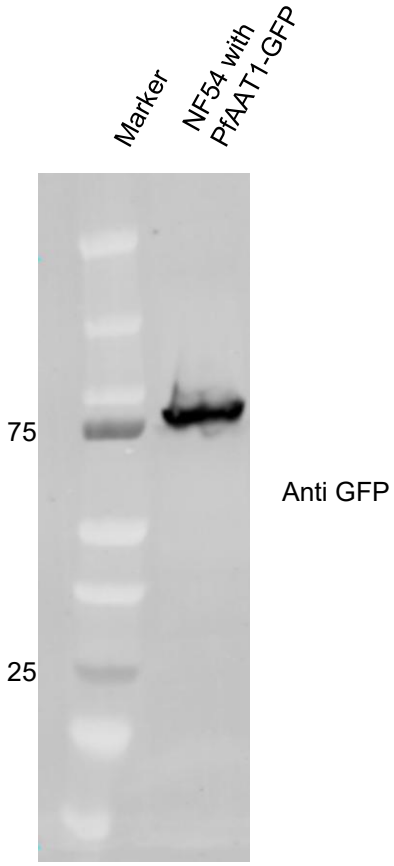
Supplementary Fig. 2. Full western blot images of PfAAT1 conditional knockdown in parental NF54 and Dd2 strains.
Three biological replicates of the knockdowns are shown. Lanes marked with asterisks (*) correspond to those shown in Fig. 1b. Molecular weights of the marker bands are indicated.

Supplementary Fig. 3



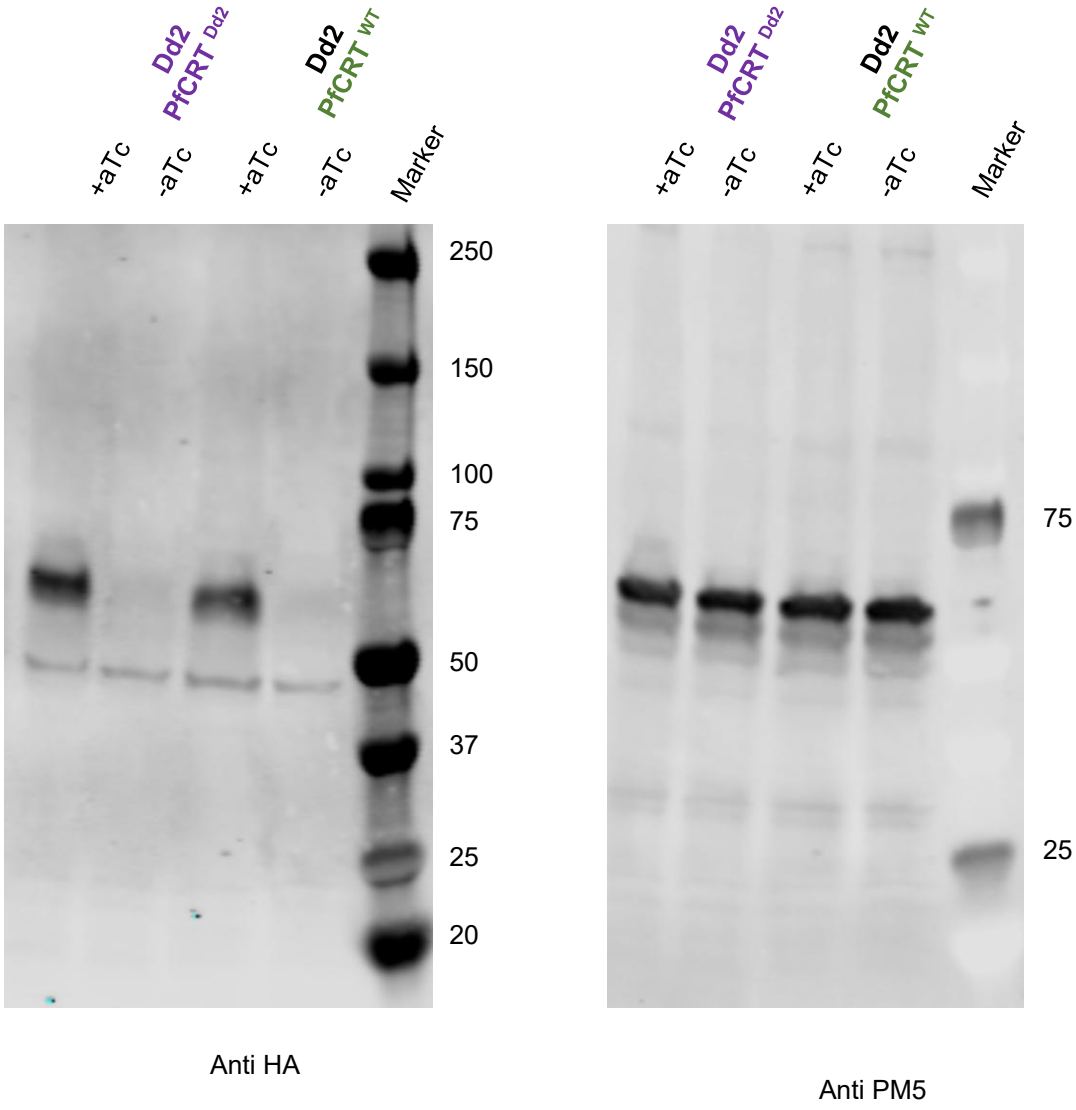
Supplementary Fig. 3. Localization of 3×HA-tagged PfAAT1 by immunofluorescence assay. Representative images of (i) NF54 and (ii) Dd2 parasites expressing 3×HA-tagged PfAAT1 under +aTc conditions. Scale bars, 5 μm.

Supplementary Fig. 4



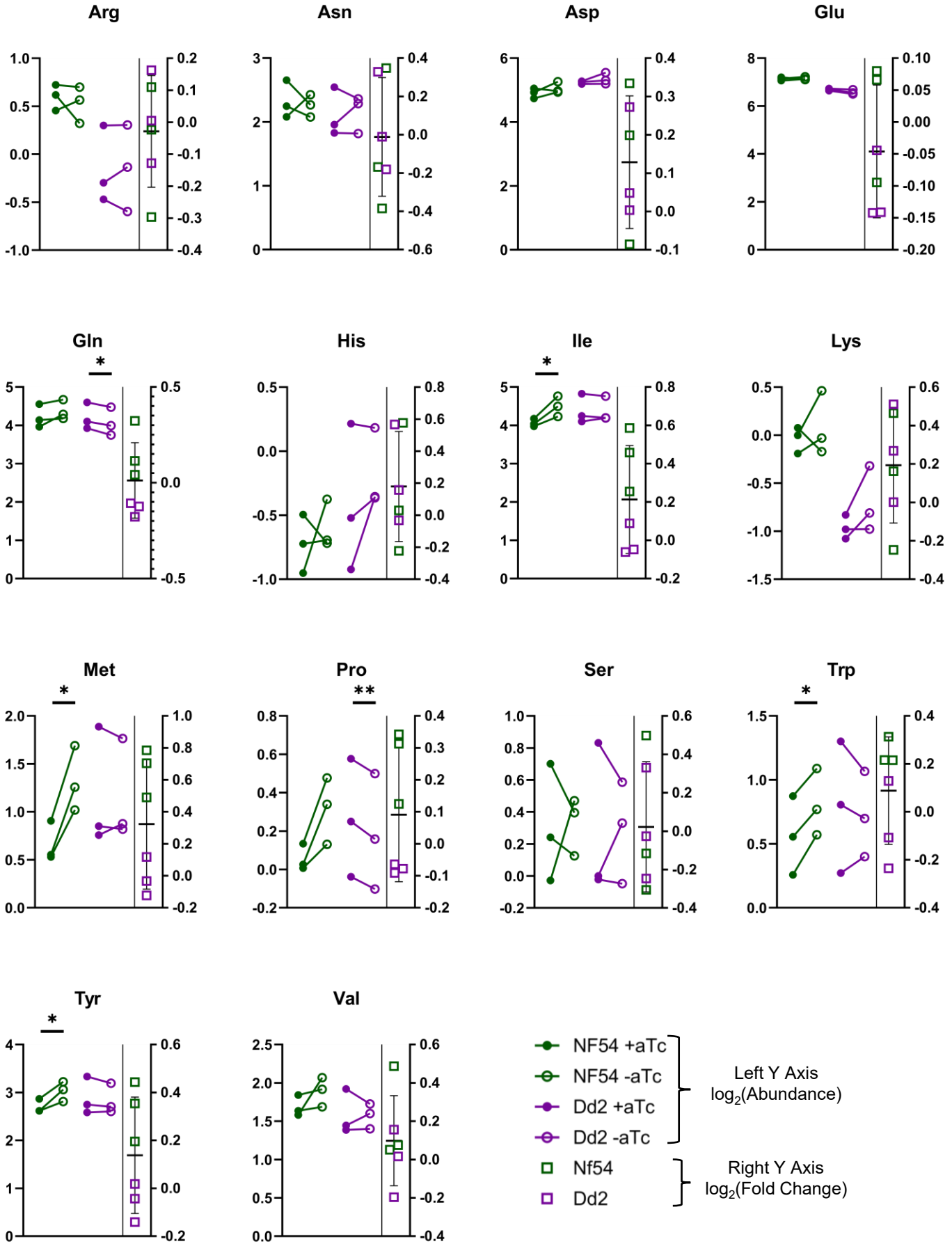
Supplementary Fig. 4. Western blot showing expression of GFP-tagged PfAAT1.
Note the higher molecular weight of GFP-tagged PfAAT1 compared to the 3×HA-tagged protein. Molecular weight markers are indicated.

Supplementary Fig. 5



Supplementary Fig. 5. Full western blot images of PfAAT1 conditional knockdown in an isogenic Dd2 parasite pair.
Molecular weights of the bands in the marker lane are indicated on the sides. Data are representative of two independent experiments.

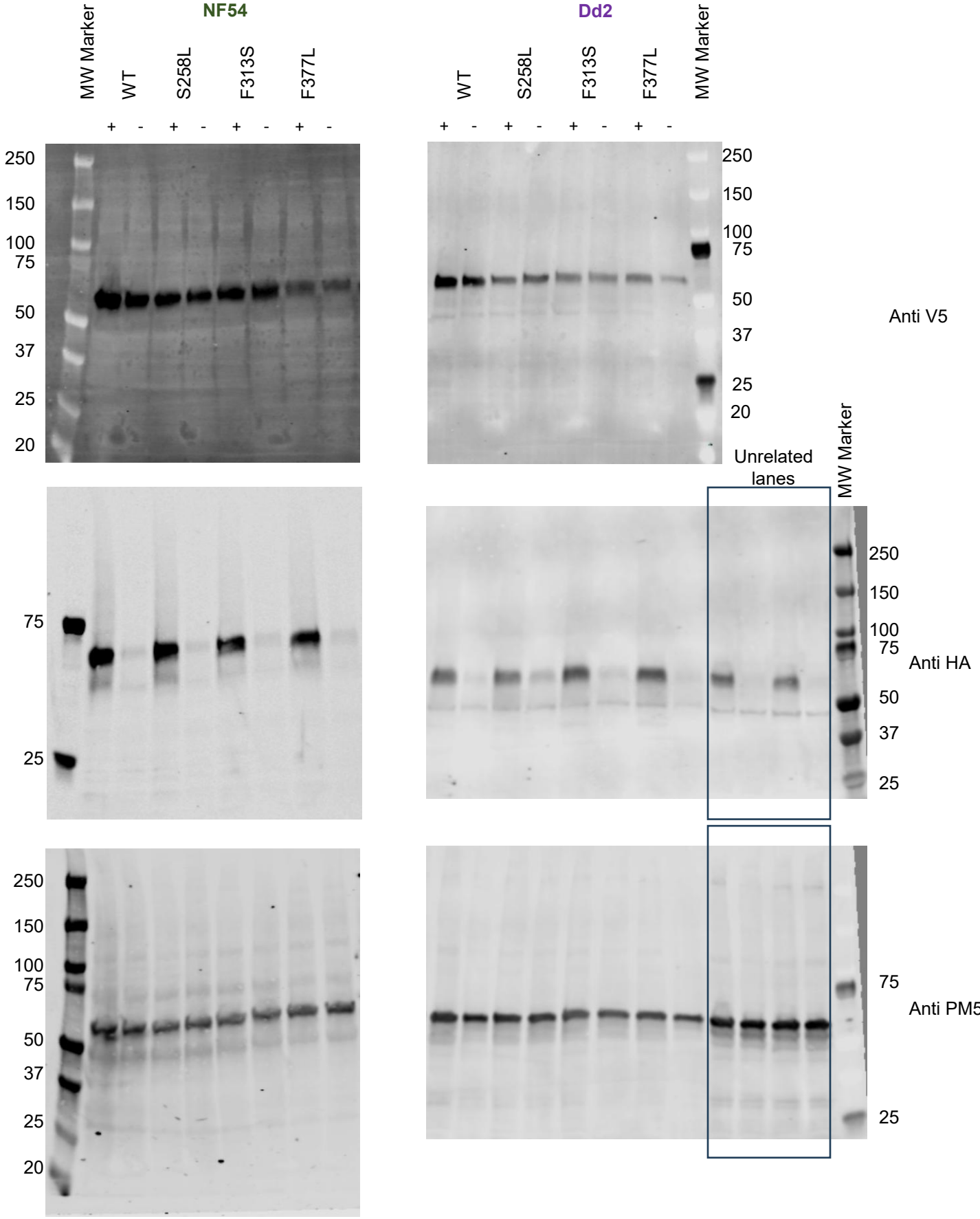
Supplementary Fig. 6



Supplementary Fig. 6. Comparison of amino acid abundance between WT and PfAAT1 knockdown conditions.

Abundance of individual amino acids in NF54 (green) and Dd2 (purple) parasites under +aTc and −aTc conditions. Left y axis shows $\log_2$-transformed, median-normalized abundance. Paired samples (n = 3 per strain) are connected by lines. Statistical significance within each strain was assessed using paired t-tests and is indicated above the paired comparisons. Right y axis shows $\log_2$ fold change (−aTc versus +aTc) for each paired sample (n = 6 total). Individual fold-change values are plotted alongside the mean ± 95% confidence interval. Statistical significance of the combined dataset was assessed using a paired t-test and is indicated above the fold-change values (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). Only statistically significant differences are annotated.

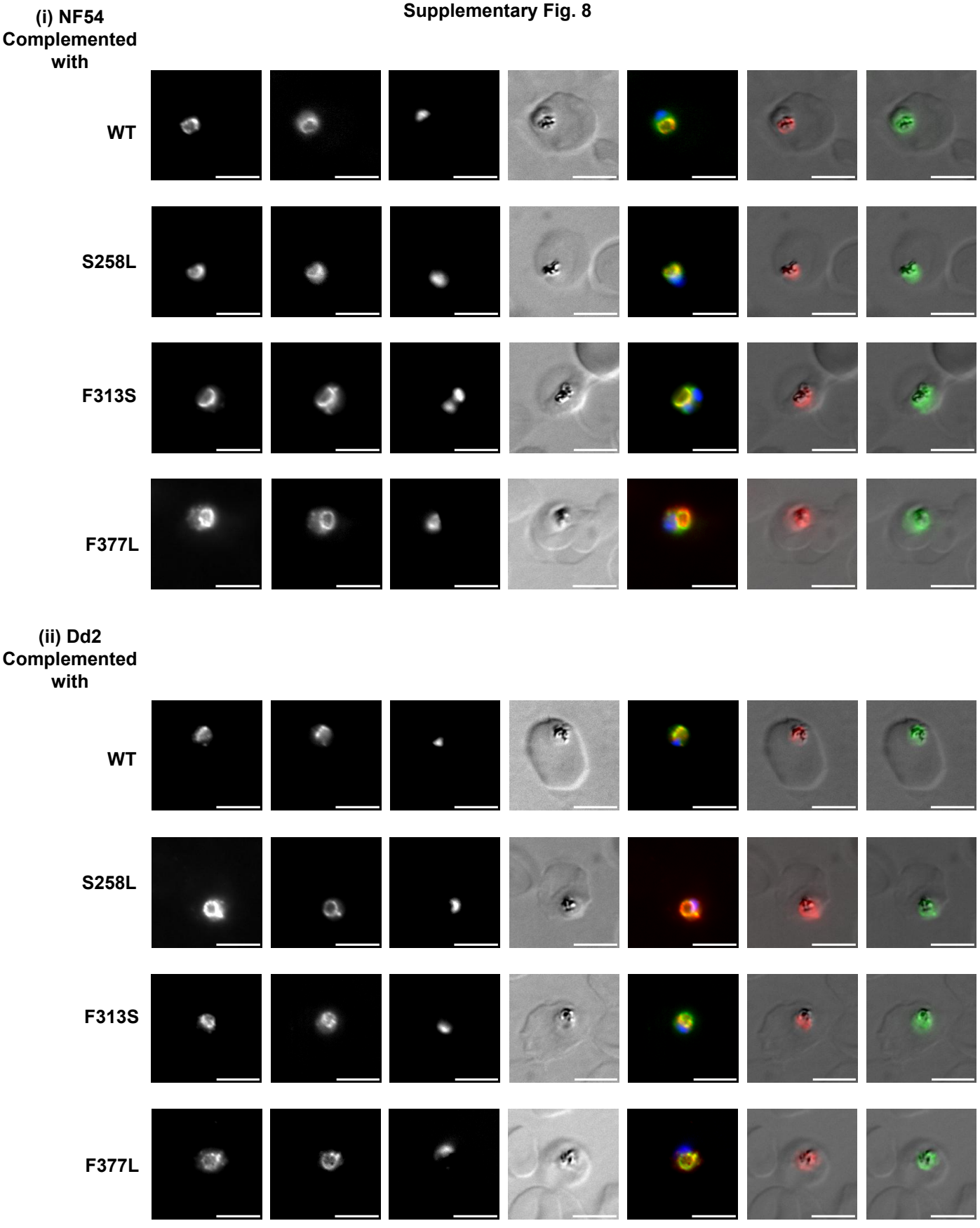
Supplementary Fig. 7



Supplementary Fig. 7. Full western blot images of PfAAT1 conditional knockdown in NF54 and Dd2 strains expressing an N-terminally V5-tagged second copy of PfAAT1 from the cg6 locus.

Molecular weights of the bands in the marker lane are indicated on the sides. Data are representative of two independent experiments.

Supplementary Fig. 8



Supplementary Fig. 8. Localization of endogenous and second-copy PfAAT1 by immunofluorescence assay.

Representative images showing the localization of different PfAAT1 variants (V5-tagged) in NF54 (i) and Dd2 (ii) PfAAT1 (3×HA-tagged) conditional knockdown lines. Composite fluorescence images (column 5) are shown to illustrate the extent of colocalization between the endogenous and second-copy proteins. Anti-V5 and anti-HA channels are individually overlaid on the transmitted light channel (columns 6 and 7) to highlight their localization around the hemozoin crystal (visible as dark regions in the transmitted light channel). Scale bars, 5 μm.

Supplementary Fig. 9

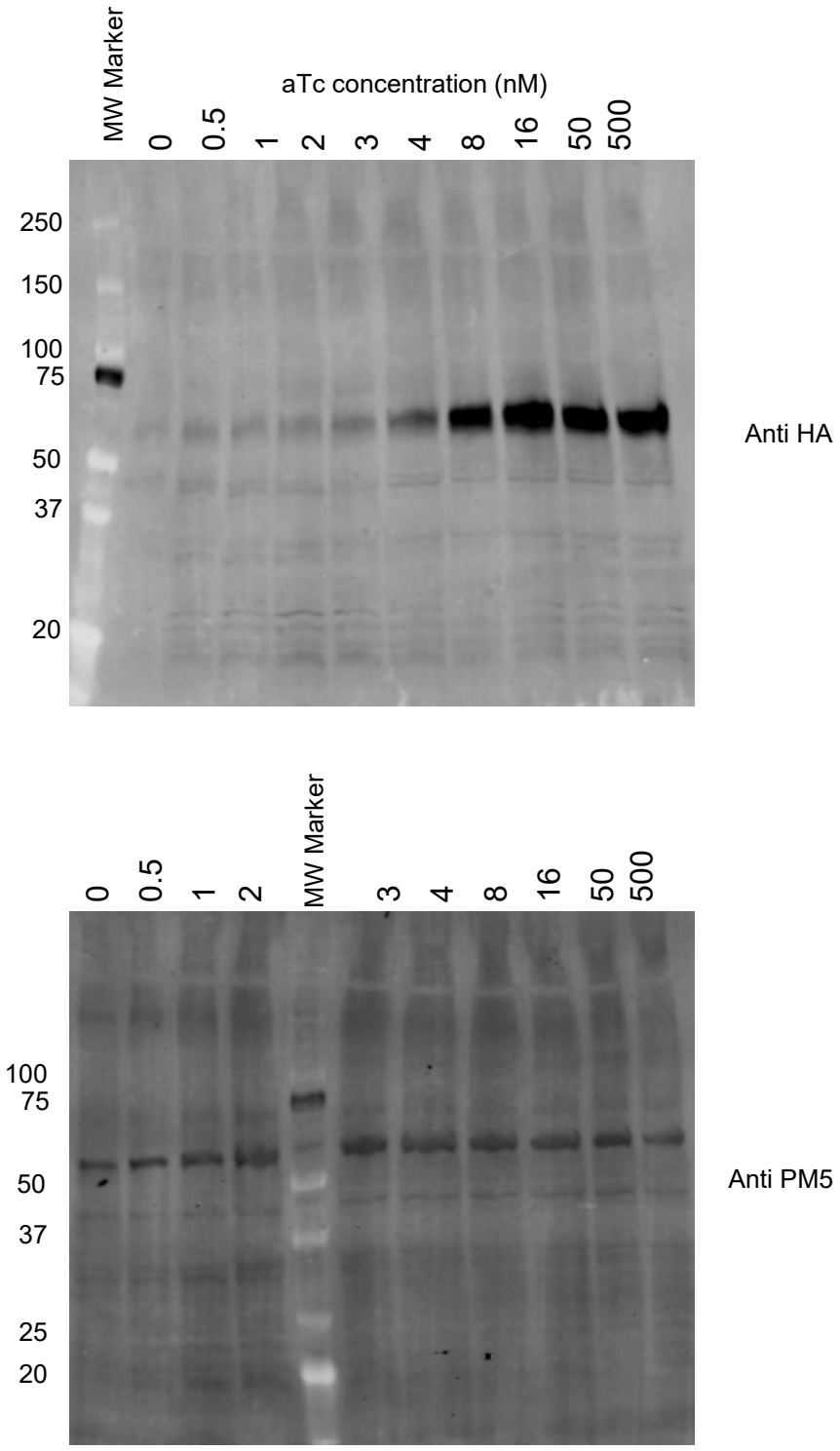
		pfaat1 allele		
		S258L & OR F313S	Other SNPs	WT
pfcrt allele	WT	4871	142	1468
	K76T	8721	62	43

P value and statistical significance

Test	Chi-square
Chi-square, df	2157, 2
P value	<0.0001

Supplementary Fig. 9. Contingency table and chi-square statistics for assessing linkage between specified *pfcrt* and *pfaat1* alleles.

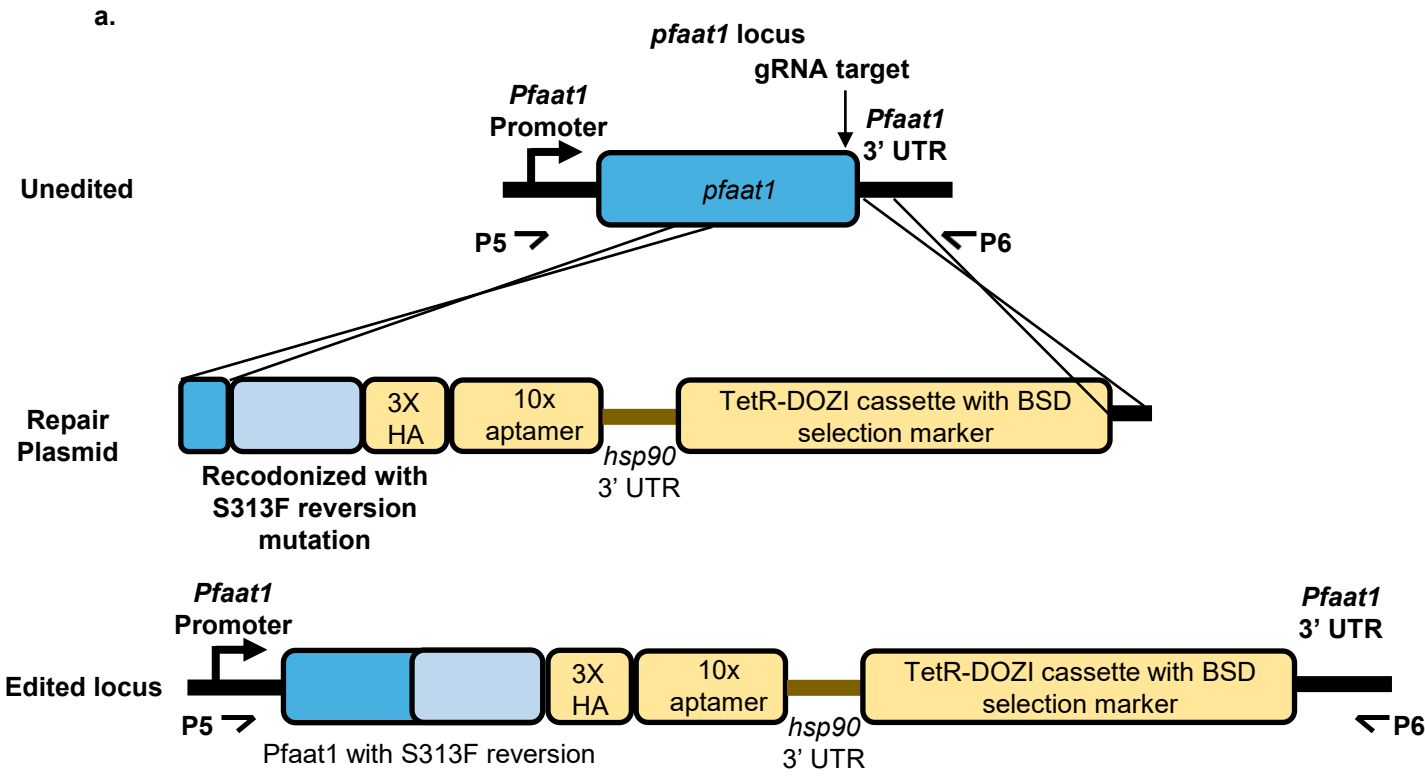
Supplementary Fig. 10

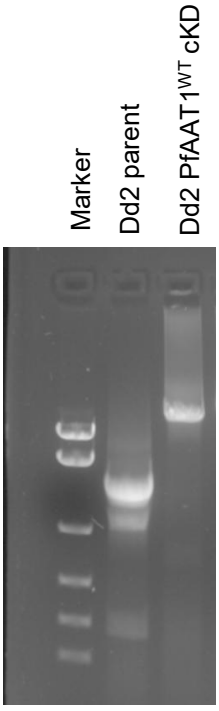


Supplementary Fig. 10. Full western blot images showing tuning of PfAAT1 expression in Dd2 PfAAT1 conditional knockdown parasites.

Samples were collected 48 h post–aTc washout and subsequent readdition at specified concentrations.

Supplementary Fig. 11

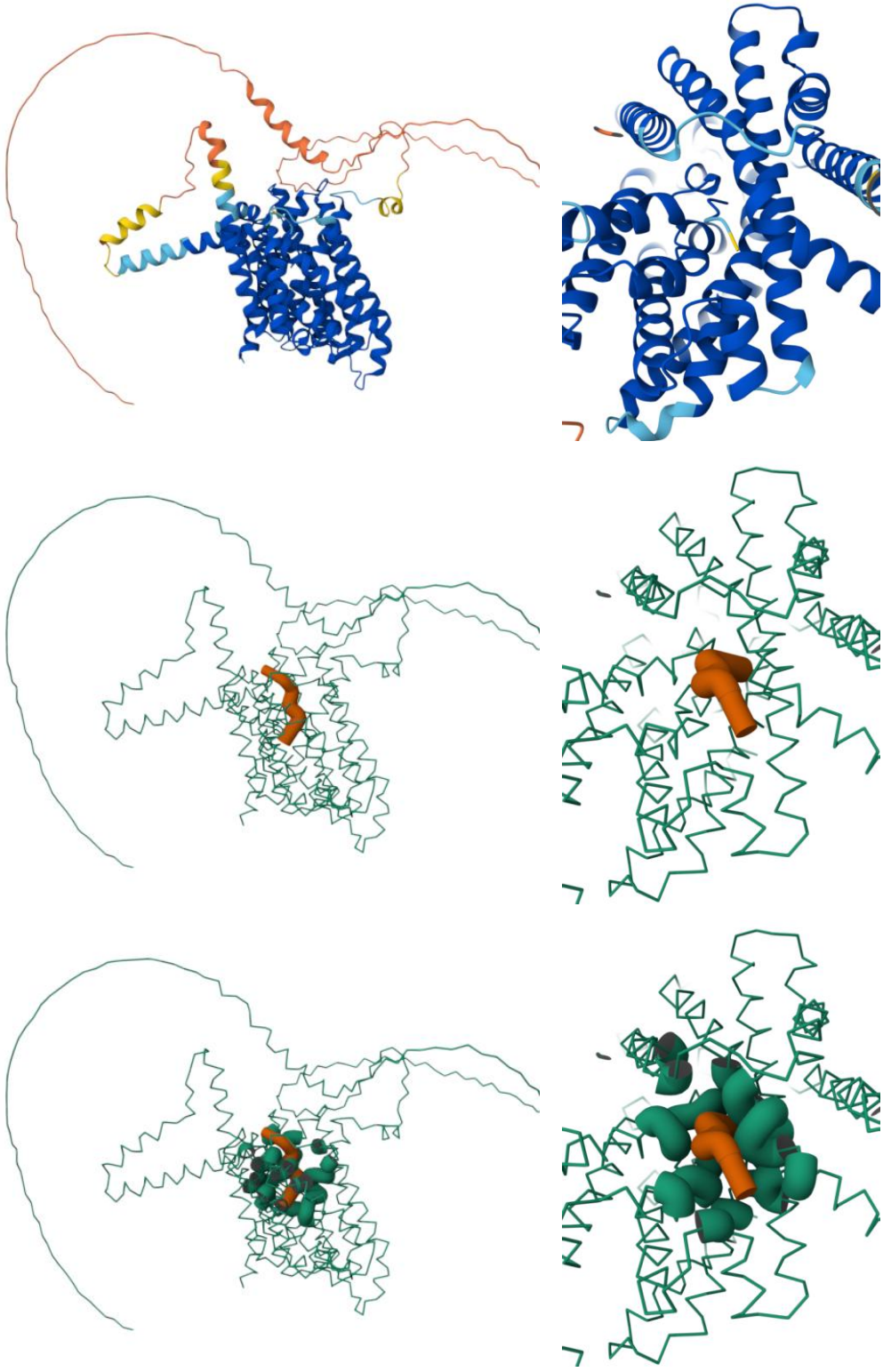




Supplementary Fig. 11. Schematic representation of endogenous PfAAT1 allele-swapping strategy. a, This strategy is similar to that shown in Supplementary Fig. 1, except that the 5′ homology region between the repair cassette and the endogenous locus is positioned further upstream within the gene. Following the 5' homology region, the remaining coding sequence is recodonized in the repair cassette, which includes sequences containing phenylalanine at position 313. Location for diagnostic PCR primer set (P5 and P6) are indicated. From the unedited pfaat1 locus, it amplifies a product of 3226 bp and from edited locus a product of 10986 bp.

b, Diagnostic PCR from genomic DNA of the recombinant parasite with primer pair P5-P6.

Supplementary Fig. 12



Supplementary Fig. 12: AlphaFold prediction of PfAAT1 in complex with the Hb-derived hexapeptide VDPVNF from orthogonal views.

Columns 1 and 2 show vertical and horizontal orientations, respectively.

Row 1, Cartoon representation. Colors indicate pLDDT confidence scores (>90, blue; 70–90, light blue; 50–70, yellow; <50, red).

Row 2, PfAAT1 backbone (green) with putty (brown) representation of the hexapeptide.

Row 3, PfAAT1 residues within 5 Å of the hexapeptide are highlighted in putty representation. Residue positions along the primary sequence are: 180–186, 231, 251–252, 255–256, 259, 362, 368–369, 372, 447–454, 553, and 557.

Supplementary Table 1.

Oligo	Sequence	Purpose
G1	ATTTAAGGTTGTCACTACAACGG	gRNA
P1	CATTGACTGTGCCGGCCGGCCTCTGAATATAC AAATGATCCATACC	Forward primer for LHR amplification
P2	CGTCATAAGGGTAGGCGATCGCTAAAATTAAA TTTAAGGTTGTTACGAC	Reverse primer for LHR amplification
P3	CAAACCCGGAATTCGAGCTCGGGAACATAGTT TTTCTTTTTCTTTTTTGAAATACC	Forward primer for RHR amplification
P4	GACCTAGGGATAACAGGGTAATAGGGGTCTG TAAGTATTTAATCATGAC	Reverse primer for RHR amplification
P5	CATCTTCCCTTACATTATATATATTGTAAACAT ATTAGATAACAGTGTTTTGGG	Forward Primer for diagnostic PCR
P6	CTTTTGCCCTCTACATATTATTCAATCATATAA TTAGTATATAATAAAAATATTTCAG	Reverse Primer for diagnostic PCR
P7	CGAATAAACACGATTTTTTCTCGAGATGAATAA AAAGTATGGTACGTCCTCGAATAAC	Forward primer for GFP tagged PfAAT1 amplification
P8	CATAATGTGCTGCACCTGGCCTAGGTAAAATT AAATTTAAGGTTGTCACTACAACG	Reverse primer for GFP tagged PfAAT1 amplification
P9	TCGAATAAACACGATTTTTTCTCGAGATGGGA AAACCGATTCCTAACCCATTATTGGGCCTGGA TAGCACTAATAAAAAGTATGGTACGTCCTCGA AT	Forward primer for v5 tagged PfAAT1 amplification
P10	ATATTATATAACTCGACGCGGCCGCTTATAAA ATTAAATTTAAGGTTGTC	Reverse primer for v5 tagged PfAAT1 amplification
P11	CATTGACTGTGCCGGCCGGCCGATATATCTG ATGGGGATTATACTAATGATG	Forward primer for LHR amplification for PfAAT1 allelic swap
P12	CAAGGAGAAGAGGCTGAATATAAGAAAGTGAT TTAATGATCCGACTTTATTTCTAAATG	Reverse primer for LHR amplification for PfAAT1 allelic swap
P13	CATTTAGAAATAAAGTCGGATCATTAAATCACT TTCTTATATTCAGCCTCTTCTCCTTG	Forward primer for human codon optimized Part of PfAAT1 amplification
P14	CGTCATAAGGGTAGGCGATCGCCAAGATAAG ATTCAAAGTTGTAACGAC	Reverse primer for human codon optimized Part of PfAAT1 amplification