Supplemental Table 1: Non-synonymous polymorphisms detected before and after introduction of ACTs. Mixed genotypes are considered mutant.

Polymorphism	1999-2006 (N=638)	2012-2016 (N = 716)	p-value
L457I	1 (0.15 %)	0 (0.00%)	0.49
I465T	0 (0.00%)	1 (0.14%)	1.00
C469F*	0 (0.00%)	1 (0.14%)	1.00
C469Y**	0 (0.00%)	1 (0.14%)	1.00
W470R	0 (0.00%)	1 (0.14%)	1.00
M472V	0 (0.00%)	1 (0.14%)	1.00
G496S	1 (0.15 %)	0 (0.00%)	0.49
S522C**	0 (0.00%)	1 (0.14%)	1.00
G533A	0 (0.00%)	2 (0.28%)	0.50
I540T	0 (0.00%)	2 (0.28%)	0.50
V555A	4 (0.59%)	1 (0.14%)	0.21
Y558H	0 (0.00%)	1 (0.14%)	1.00
K563E	0 (0.00%)	1 (0.14%)	1.00
W565R‡	1 (0.15 %)	0 (0.00%)	0.49
E567K‡	1 (0.15 %)	0 (0.00%)	0.49
A569T	0 (0.00%)	1 (0.14%)	1.00
A569S	0 (0.00%)	1 (0.14%)	1.00
P574L*	0 (0.00%)	1 (0.14%)	1.00
A578S†	3 (0.44%)	7 (0.98%)	0.34
V581I	1 (0.15 %)	0 (0.00%)	0.49
N594K	0 (0.00%)	1 (0.14%)	1.00
G595S	1 (0.15 %)	0 (0.00%)	0.49
K610E ‡	1 (0.15 %)	0 (0.00%)	0.49
P615S	1 (0.15 %)	0 (0.00%)	0.49
A617T	0 (0.00%)	1 (0.14%)	1.00
L618S ‡	1 (0.15 %)	0 (0.00%)	0.49
L619S	0 (0.00%)	1 (0.14%)	1.00
G638R	0 (0.00%)	1 (0.14%)	1.00
A675V*	0 (0.00%)	1 (0.14%)	1.00

*Polymorphisms identified as candidate artemisinin resistance mutations

**Low frequency variants reported to be associated with delayed clearance but without statistical significance due to the low number of cases.

†Not associated with artemisinin resistance.

‡Newly reported

Supplemental Table 2. Nucleotide and amino acid haplotype diversity of *Plasmodium falciparum* isolates from seven sites in Uganda before and after the introduction of ACTs. Mixed genotypes were treated as mutant genotypes.

Site	ACT usage	N	Nucleotide diversity			No. of amino acid haplotypes	Haplotype Diversity (h_d) (mean ± SD)
			π		θs		
Apac	Pre-ACT	100	0.00009 ± 0.00005	<	0.00085 ± 0.00000	4	0.059 ± 0.033
	Post-ACT	19	-	NA	-	1	-
Arua	Pre-ACT	86	0.00014 ± 0.00007	<	0.00117 ± 0.00064	5	0.091 ± 0.043
	Post-ACT	40	0.00029 ± 0.00017	<	0.00138 ± 0.00077	4	0.146 ± 0.075
Jinja	Pre-ACT	99	0.00006 ± 0.00004	<	0.00057 ± 0.00041	3	0.040 ± 0.027
	Post-ACT	99	0.00018 ± 0.00007	<	0.00142 ±0.00070	6	0.118 ± 0.044
Kampala	Pre-ACT	100	0.00009 ± 0.00005	<	0.00057 ± 0.00000	3	0.059 ± 0.033
	Post-ACT	65	0.00018 ± 0.00008	<	0.00124 ± 0.00068	5	0.120 ± 0.055
Kanungu	Pre-ACT	100	0.00009 ± 0.00005	<	0.00057 ± 0.00000	3	0.059 ± 0.033
	Post-ACT	118	0.00030 ± 0.00012	<	0.00220 ± 0.00091	7	0.131 ± 0.043
Mubende	Pre-ACT	99	0.00018 ± 0.00007	<	0.00142 ± 0.00070	6	0.118 ± 0.044
	Post-ACT	66	0.00013 ± 0.00007	<	0.00093 ± 0.00057	4	0.090 ± 0.048
Tororo	Pre-ACT	99	0.00012 ± 0.00006	<	0.00114 ± 0.00062	5	0.080 ± 0.038
	Post-ACT	309	0.00020 ± 0.00005	<	0.00396 ± 0.00000	18	0.119 ± 0.026
Overall	Pre-ACT	683	0.00011 ± 0.00002	<	0.00372 ± 0.00109	19	0.072 ± 0.014
	Post-ACT	716	0.00020 ± 0.00003	<	0.00678 ± 0.00167	32	0.116 ± 0.017

Supplemental Table 3. Results for testing the hypothesis that all mutations in *K13PD* are selectively neutral.

Site	ACT usage	N	Fu and Li's D^*		Tajima's *D*	
			D	p-value	*D*	p-value
Apac	Pre-ACT	100	-3.86	p < 0.02	-1.61	NS
	Post-ACT	19	NA	NA	NA	NA
Arua	Pre-ACT	86	-3.46	p < 0.02	-1.80	p < 0.05
	Post-ACT	40	-3.38	p < 0.02	-1.88	p < 0.05
Jinja	Pre-ACT	99	-2.85	p < 0.05	-1.38	NS
	Post-ACT	99	-3.28	p < 0.02	-1.87	p < 0.05
Kampala	Pre-ACT	100	-1.08	NS	-1.30	NS
	Post-ACT	65	-3.69	p < 0.02	-1.83	p < 0.05
Kanungu	Pre-ACT	100	-1.09	NS	-1.30	NS
	Post-ACT	118	-2.12	NS	-2.05	p < 0.05
Mubende	Pre-ACT	99	-3.28	p < 0.02	-1.87	p < 0.05
	Post-ACT	66	-3.26	p < 0.02	-1.66	NS
Tororo	Pre-ACT	99	-7.98	p < 0.02	-1.78	NS
	Post-ACT	309	-7.77	p < 0.02	-2.40	p < 0.01
Overall	Pre-ACT	683	-7.98	p < 0.02	-2.28	p < 0.01
	Post-ACT	716	-9.26	p < 0.02	-2.53	p < 0.01

*Outgroup is the *P. praefalciparum* kelch protein K13 gene sequenced from the G01 strain (PPRFG01_1345600)

Supplemental Table 4. Tests for adaptive selection acting on *K13PD* in samples collected before and after the implementation of ACTs.

			McDonald and Kreitman test result					Codon-based Test of Neutrality results	
			Interspecies fixed difference		Intraspecific difference				
Site	**ACT exposure**	N	Syn	N.S	Syn	N.S.	*p-value*	dN-dS	*p-value*
Apac	Pre-ACT	100	4	0	1	2	0.143	-0.428	0.669
	Post-ACT	19	4	0	0	0	NA	NA	NA
Arua	Pre-ACT	86	4	0	0	4	**0.029**	2.152	**0.033**
	Post-ACT	40	4	0	1	3	0.143	-0.139	0.889
Jinja	Pre-ACT	99	4	0	1	1	0.333	-0.717	0.475
	Post-ACT	99	4	0	3	2	0.444	-1.197	0.234
Kampala	Pre-ACT	100	4	0	1	1	0.333	-0.406	0.686
	Post-ACT	65	4	0	2	2	0.429	-1.014	0.312
Kanungu	Pre-ACT	100	4	0	2	0	NA	-1.483	0.141
	Post-ACT	118	4	0	5	3	0.491	-1.603	0.112
Mubende	Pre-ACT	99	4	0	1	4	**0.048**	0.339	0.735
	Post-ACT	66	4	0	1	2	0.143	-0.398	0.692
Tororo	Pre-ACT	99	4	0	2	2	0.429	-1.018	0.311
	Post-ACT	309	4	0	7	11	0.090	-1.327	0.187
Overall	Pre-ACT	683	4	0	7	11	0.090	-1.137	0.258
	Post-ACT	716	4	0	14	18	0.104	-1.104	0.272

Supplemental Table 5: Sequence reference information.

Site	N	Years	Associated Publication	Sequencing	Accession Numbers
Apac	100	2003-2004	Francis et al 2006 JID	New	MN071745.1-MN071844.1
	19	2013	Yeka et al 2016 JID	Published	MN072169.1-MN072187.1
Arua	84	2004	Francis et al 2006 JID	New	MN071845.1-MN071930.1
	43	2016	Asua et al 2018 AAC	Published	MH789087.1-MH789129.1
Jinja	96	2002-2003	Francis et al 2006 JID	New	MN071931.1-MN072029.1
	51	2012-2015	Tumwebaze 2017 JID	Published	MN072188.1-MN072238.1
	48	2016	Asua et al 2018 AAC	Published	MH789130.1-MH789177.1
Kampala	98	1998-1999	Dorsey et al 2001 JID	New	MN072031.1-MN072129.1
	65	2014	Cooper et al 2015 AAC	Published	KR055739.1-KR055753.1; KR055755.1-KR055804.1
Kanungu	100	2003	Francis et al 2006 JID	New	MN071546.1-MN071645.1
	18	2013-2014	Yeka et al 2016 JID	Published	MN072130.1-MN072147.1
	55	2012-2015	Tumwebaze 2017 JID	Published	MN072239.1-MN072291.1
	48	2016	Asua et al 2018 AAC	Published	MH789178.1-MH789225.1
Mubende	98	2002-2003	Francis et al 2006 JID	New	MN071646.1-MN071744.1
	21	2013	Yeka et al 2016 JID	Published	MN072148.1-MN072168.1
	45	2016	Asua et al 2018 AAC	Published	MH789316.1- MH789360.1
Tororo	97	2003-2004	Francis et al 2006 JID	New	MN071447.1-MN071545.1
	161	2008-2012	Conrad et al 2014 PLoS ONE	Published	KM187879.1-KM187898.1; KM187900.1-KM187938.1; KM187940.1-KM187944.1; KM187946.1-KM187955.1; KM187957.1-KM187960.1; KM187962.1-KM187982.1; KM187984.1-KM188041.1; KM188045.1-KM188048.1
	57	2014-2015	Conrad et al 2017 JID	Published	MF285352.1-MF285358.1; MF285360.1-MF285373.1; MF285375.1-MF285379.1; MF285381.1-MF285383.1; MF285385.1-MF285387.1; MF285389.1; MF285391.1-MF285394.1; MF285396.1-MF285398.1; MF285401.1-MF285402.1; MF285404.1-MF285407.1; MF285409.1-MF285413.1
	55	2016	Rasmussen et al 2017 AAC	Published	MF477020.1- MF477022.1; MF477024.1- MF477075.1
	52	2012-2015	Tumwebaze et al 2017 JID	Published	MN072292.1-MN072342.1
	48	2016	Asua et al 2018 AAC	Published	MH789361.1- MH789408.1