

Supplement Fig 1

Optimized 7	ATGGAATTT TACAAA ACT TTCTTCTCGCA CTTT ACGAATACG TTT AACTCGGAA TACATC
Original 7	ATGGAATTTTATAAGAACTTTTTTTTTCACATTTTACAAAACCTTTAATAGTGAATATATT
Optimized 67	TTTGAC CTGAAAGGTAGCACG AAAATCGAT GACAAC GAAATCGCATCA TTTATCAAATCG
Original 67	TTTGACTTAAAGGGTAGTACAAAAATTGATGATAATGAAATTGCGAGTTTTATTAAGTCT
Optimized 127	AACGATCTGTGCGAAACG CAAGAAAATTGTCGAA CTGTACATCG AAAAGAAA ATTAAC
Original 127	AATGATTTGTGTGAAAAATGATAAGAAGATAGTAGAATTATATATTGAGAAGAAAATAAA
Optimized 187	AAAATCATGCTGATC AAA TAC ATGGAA CGT AAAAACAAA ACCTG TTT CGCGGC AAA ATT
Original 187	AAGATTATGTTAATAAAAATATATGGAAAGAAAAACAAAACATTATTTAGAGGAAAGATA
Optimized 247	CAT CTGATGCTGGT TTT ATCAGCCCGCTG TGGATT TTC TATATG CTGTACCTGAG CAAA
Original 247	CATTTAATGTTAGTATTTATATCACCATTATGGATTTTTTATATGTTATATTTATCAAAA
Optimized 307	ACCTGACGGCGGTATC TTT ACG TCT ATTGCCGTCCTG TGATTTTCTTT AACTTTTTC
Original 307	ACATTAACAGCAAGAATATTTACCTCTATAGCTGTACTATGTATTTCTTTAATTTCTTT
Optimized 367	GCC TCTTTCCTGCTGC ATA ACTTC GAATGGAAA CCG AAA TTCTTTTCATCATC GAAAAA
Original 367	GCCTCCTTTTTACTTCACAATTTTGAATGGAAACCCAAATTTTTTTTTATAATTGAAAA
Optimized 427	ATGGAT CAC TT CGGCATC TTT CTG ATGATTAGT GGT TCC CTGCTGCCGGTGCAGG CACTG
Original 427	ATGGATCATTTCCGGAATTTTCTAATGATTAGTGGATCCTTGTTACCAGTTCAAGCTTTA
Optimized 487	CTGTTCAAC AAA ATCAA CT GCTGTTC TTTATT TCACTGCAATTC TTT GCTATCCTGTTC
Original 487	TTGTTAATAAAAATTAAGTTATTATTTTTTATTTCTCTTCAGTTTTTTGCAATATTATTT
Optimized 547	GGCTGCCTGATTG TTTTCTTTAGTTGT TTCAGCTCTGGT AAT CGT TTT ATCCGC TCCATG
Original 547	GGATGCCTTATCGTTTTCTTTAGTTGTTTTTCATCGGAAATAGATTTATAAGGTCCATG
Optimized 607	ATCTTCACC ATT GCGGGCCTGCTGCAC ATT ATC TTTATT CGCGAC TAT GTCTCACTGCTG
Original 607	ATTTTACTATTGCTGGATTACTACATATTATTTTATTAGAGATTATGTATCATTATTA
Optimized 667	TACGGCAA GAATTT ATCCTGCTGATTCTGCTGGCGT GCTGATATT ATCGGTGCCGTG
Original 667	TATGGAAAGGAATTTATCTTTTAAACTTCTGGGTGTTTTATATATTATAGGAGCAGTT
Optimized 727	ATCTACTCGGT TAAA AAACCGAAC ATTTTT CCGGGC ATT CTG GAATTT CATGAAGTG TTC
Original 727	ATATATCTGTGAAAAAGCCAAATATTTTTCCAGGTATTTTGGAAATTCATGAAGTTTTC
Optimized 787	CATATT TGCTGTCTGGGTCTGCTGTC TGC ACCTTCGTTCTG AATTGT AGCGT GATTAAA
Original 787	CATATTTGTTGTCTAGGAAGTGCTGTATGCACATTTGTCCATAAATGTAGTGTATTAAA
Optimized 847	CGTACC
Original 847	AGAACA

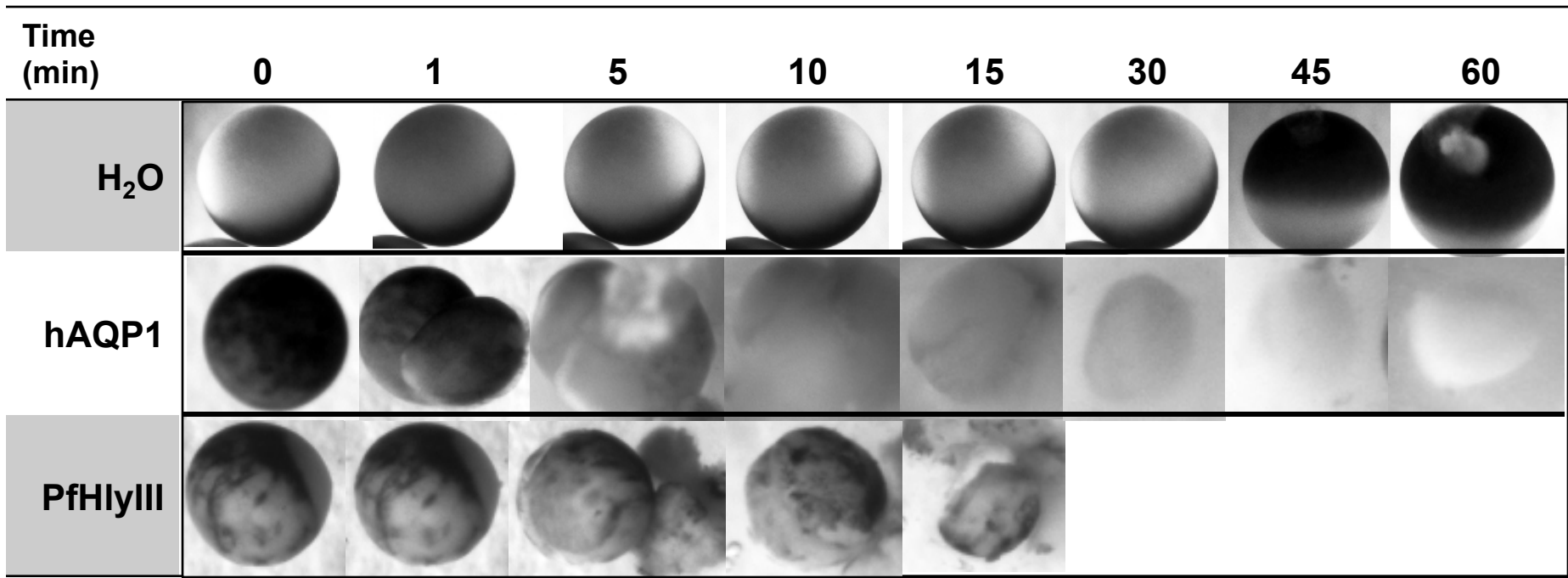
Supplement Fig. 1 Nucleotide sequence of optimized versus native *P. falciparum* hemolysin showing changes in red.

M.tuberculosis..NP_215601.1
M.avium..ZP_05215609.1
E.histolytica..XP_654314.2
E.dispar..XP_001735342.1
S.aureus..NP_372694.1
S.lugdunensis..ADC86995.1
S.pneumoniae..ZP_01825080.1
S.agalactiae..ZP_08650398.1
N.meningitidis..EGC63223.1
N.gonorrhoeae..ZP_06153818.1
E.faecalis..NP_815393.1
E.faecium..ZP_05678486.1
B.cereus..P54176.1
B.anthraxis..YP_028350.1
V.cholerae..ZP_01974604.1
V.vulnificus..AAP50516.2
V.parahaemolyticus..NP_799427.1
Y.pestis..ZP_06204569.1
Y.enterocolitica..YP_001007557.1
E.coliO104:H4..EGR73138.1
E.coliO157:H7..EFX34143.1
S.dysenteriae..EDX36078.1
E.cloacae..YP_003614707.1
S.enterica..EGA37169.1
B.bovis..XP_001609898.1
T.gondii..EEE24257.1
P.berghei..XP_678678.1
P.knowlesi..XP_002260320.1
P.vivax..XP_001615833.1
P.falciparum..XP_001348702.2

TTLAYTAATITMFTVSATYH---RVNW-KSATARNWMKRADHSMIFVFIAGSYTPFALL
STLIYAAATVALFAVSATYH---RVKW-KTEAARTRMKRLDHSMI FVFIAGSYTPFARL
AILCFCLASFTLYFNSTTYHLLNILLPN--SICLRYFFQRLDHITIYIMISGCYLCFI FT
AILCFCLASFTLYFNSTTYHLLNILLPN--SICLRYFFQRLDHITIYIMISGCYLCFI FT
SMSIYVISIFMMFISSTIYH---SMQN--ETPHKYILRIIDHSMIYVAISGTYTPILLT
SVSIFVISIFLMFISSTIYH---TMKN--NSIHKYVLRIIDHSMIYVAISGTYTPVLLH
GVSIFVISLFLMFLSSTIYH---SMAY--GSTHKYVLRIIDHSMIYVAIAGSYTPVVL T
GTSIFVTSLFLMFLSSSIYH---SMTY--NSLQKYVLRMIDHSMIYIAIAGSYTPVAL S
SVSVYGISLLLLYLSSSLYH---GIAA---GKLK SILKKT DHCMIYVLIAGSYTPFAL V
SVSVYGISLLLLYLSSSLYH---GIAA---GKLK SILKKT DHCMIYVLIAGSYTPFAL V
SYAIYGSM LILLFLSSTL FH---SLIF---TRAKKVFQVFDHSSIFLLIAGSYTPFCL I
SYAIYGSM LILLFLTSTL FH---SLIF---TKAKKVFQVFDHSSIFLLIAGSYTPFCL L
AFTVYGVSMFLLYLFSTLLH---SIHH---PKVEKLFTILDHSAIYLLIAGTYTPFLL I
AFTVYGVSMFLLYLFSTLLH---SIHH---PKVEKLFTILDHSAIYLLIAGTYTPFLL I
SMSIYGGSMIALFLASTLYH---AIPY---QRAKRWLKTFDHC AIYLLIAGSYTPFLL V
SMAIYGSSIIVLFLASTLYH---AIPH---PKAKRWLKTFDHS AIYLLIAGSYTPFLL V
SMAIYGSSIIVLFLASTLYH---AIPH---PKAKRWLKTFDHS AIYLLIAGSYTPFLL V
SYSLYGSSIILLFLASTLYH---AIPH---RRAKLWLQKFDHCAIYILIAGTYTPFLL V
SYSLYGSSIILLFLASTLYH---AIPH---RKAKLWLQKFDHCAIYILIAGTYTPFLL V
SYSLYGGS MILLFLASTLYH---AIPH---QRAKMWLKKFDHCAIYLLIAGTYTPFLL V
SYSLYGGS MILLFLASTLYH---AIPH---QRAKMWLKKFDHCAIYLLIAGTYTPFLL V
SYSLYGGS MILLFLASTLYH---AIPH---QRAKMWLKKFDHCAIYLLIAGTYTPFLL V
SYSLYGGS MILLFLASTLYH---AIPH---QRAKIWLKKFDHCAIYLLIAGTYTPFLL V
SYSLYGGS MILLFLASTLYH---AIPH---QRAKIWLKKFDHCAIYLLIAGTYTPFLL V
EYIVAFVCCILNFAASATLH---YSRW--NLNSLDLCLKADYAFIFLMIGGSATPATIA
AAAISCFTFVWNFMASALLH---CFEWTHRPGIYQLLHKLDHAGIFMVISGSTTPIPML
FTSVALMCIFFNFFASFLH---NFEW--TPKY YFLIEKLDHIGIFLMISGSALPAAAL
FTSIAALCMFFNFFASFLH---NFEW--KPELYFLIEKIDHIGIFLMISGSCLPVPAL
FTSIAALCMFFNFFASFLH---NFEW--KPELFFLIEKIDHMGIFLMISGSCLPVPAL
FTSIAVLCIFFNFFASFLH---NFEW--KPKFFF IIEKMDHFGIFLMISGSLLPVQAL

. * * *: *:* *. *
↑ ↑ ↑ ↑
D I I G

Supplement Figure 2 alignment of conserved regions of hemolysin III



Supplementary Figure 3. Time course of hAQP1 and PfHlyIII expressing oocytes rupturing in hypotonic buffer (water) compared to water –injected controls.