991 **SUPPORTING INFORMATION**

992 **Table 1: List of oligonucleotides used in this study.** All oligonucleotides were purchased from
993 Integrated DNA Technologies. Restriction sites are underlined, InFusion overhangs are
994 italicized, gRNA sequence is in lowercase**.**

Primer number	Oligonucleotide sequence
1	*CGATTTTTTCTCGAG*ATGTCTTTGAGTATTTATGG
2	*ATGTGCTGCACCTGGCCTAGG*TGGTTCTTCTTCCATCTTACGC
3	*CGATTTTTTCTCGAG*ATGAATTCTGTTATGCCTG
4	*ATGTGCTGCACCTGGCCTAGG*CATTCCTGATCTTTTTCTTGG
5	*CGATTTTTTCTCGAG*ATGTTAAAAAATCCAGGTAC
6	*ATGTGCTGCACCTGGCCTAGG*AAATTCATTTTTGATTG
7	*CGATTTTTTCTCGAG*ATGGCAGAAGTAGCAA
8	*ATGTGCTGCACCTGGCCTAGG*TCTACAAATAACTGTGTCATC
9	*CGATTTTTTCTCGAG*ATGAATATTGCTATTGATGAG
10	*ATGTGCTGCACCTGGCCTAGG*ATTGAAGTCGTTTGGAGC
11	*CGATTTTTTCTCGAG*ATGTCCATACACCTGTTGAAC
12	*ATGTGCTGCACCTGGCCTAGG*TTTCTCCTCTCCCATTGATTTCCC
13	*CGATTTTTTCTCGAG*ATGAGTCACTTAATG
14	*ATGTGCTGCACCTGGCCTAGG*TGAGTAAGGACTTCTC
15	*CGATTTTTTCTCGAG*ATGGTAAGCTAAGTTTGG
16	*ATGTGCTGCACCTGGCCTAGG*GAAGGCTTCGTCATAGTCAG
17	AACGGTAAAAATAATAACACGAATTATCAC
18	TATTTCATCTATTTATGGACAATGG
19	CCTATATGCACATATTTCATATTAAGTATATAATATTCCTAGGAACTCATCG CTCGCGATGCTGCCCGACAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAA GGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTAT TATTTCCTATAAAATAATATATATATA
20	*ACGATTTTTT*CTCGAGATGGACAAGAAGTACAGCATCGGC
21	*CCTTGTAGTC*CCTAGGCACTTTCCGCTTTTTCTTAGGATCTTCCACCTTGCGT TTTTTCTTGGGGTCGCCTCCCAGCTGAGACAGGTC
22	CTTTGTTGACTTGTGGTGATGTTGAAGAAAATCCAGGTCCAGACAAGAAGT ACAGCATCG
23	*TGCCATATCC*CTCGAGTTACTTGTCGTCGTCGTCCTTGTAGTCCACTTTCCGC TTTTTCT
24	*CGATATGAAT*TCTAGACATTTTGTAAAAAAAATTAAAATATATTTATATAAT ATTATTTT

25	CAACATCACCACAAGTCAACAAAGAACCTCTACCTTCACCAATGCTGTTCA ACTTCCCAC
26	TAAGTATATAATATTagaaggcttcgtcatagtcaGTTTTAGAGCTAGAA
27	TTCTAGCTCTAAAACtgactatgacgaagccttctAATATTATATACTTA
28	TAAGTATATAATATTatataatttttacatcgaatGTTTTAGAGCTAGAA
29	TTCTAGCTCTAAAACattcgatgtaaaaattatatAATATTATATACTTA
30	GTATACCTTTGAAGACACATCATCAGC*CTTAAG*GTATGGTCATAGCGAGAG AAGC
31	*TTTCTGTTCGGTAACC*GCGAAGGCTTCGTCATAGTCAGGATTTTTGTCACGAG GTTTTGG
32	*CCCTTTCCGGGCGCGCC*GATATATCAATATATCATTATATCAATATATCAAG ATATC
33	GCTTCTCTCGCTATGACCATAC*CTTAAG*GCTGATGATGTGTCTTCAAAGGTA TAC
34	CTTATGACGTACCTGATTATGCAC
35	GTAGACCCCATTGTGAGTACATAAATATATTATATAAACTAGACTAGG

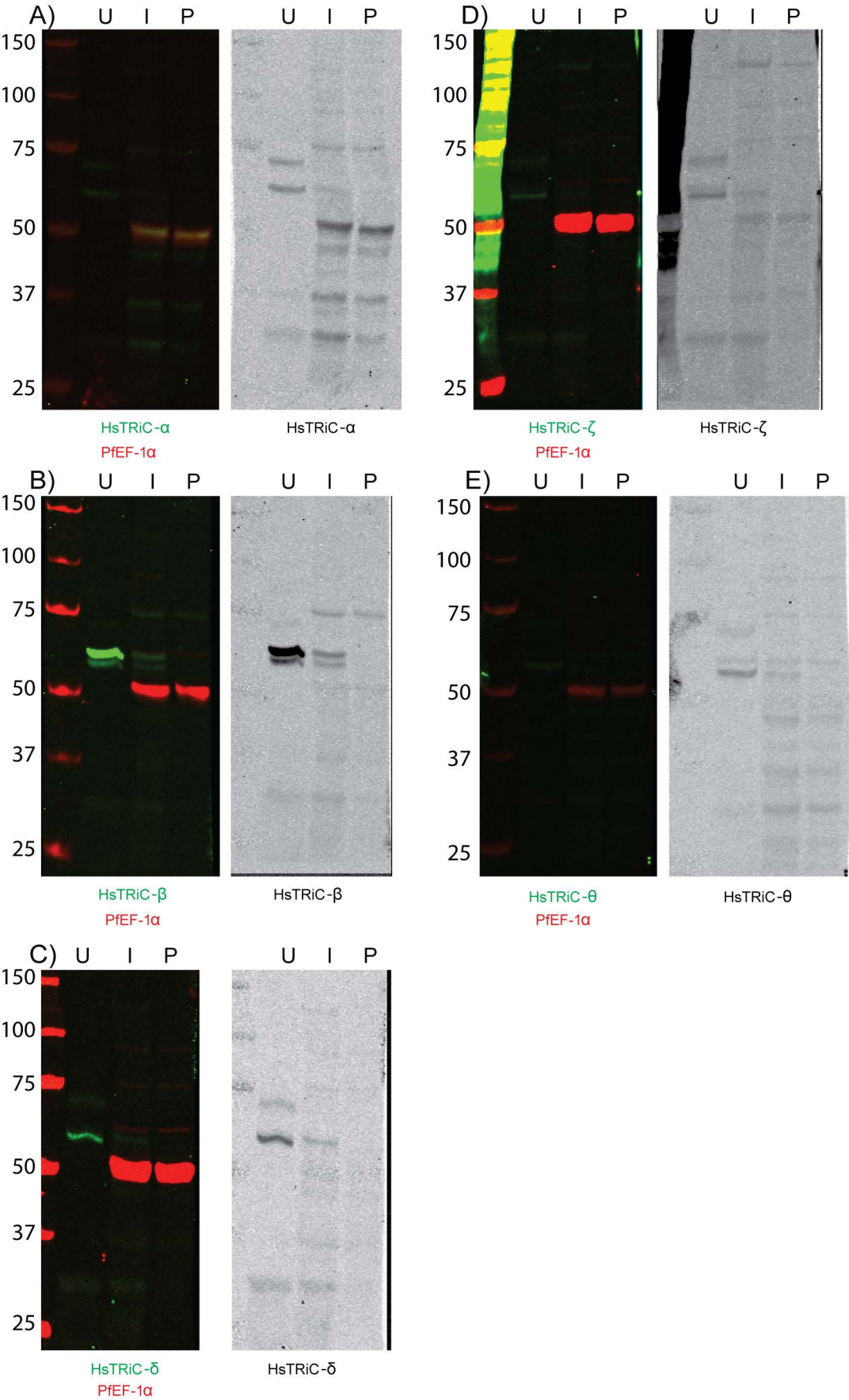
995 **SUPPLEMENTAL FIGURE LEGENDS**

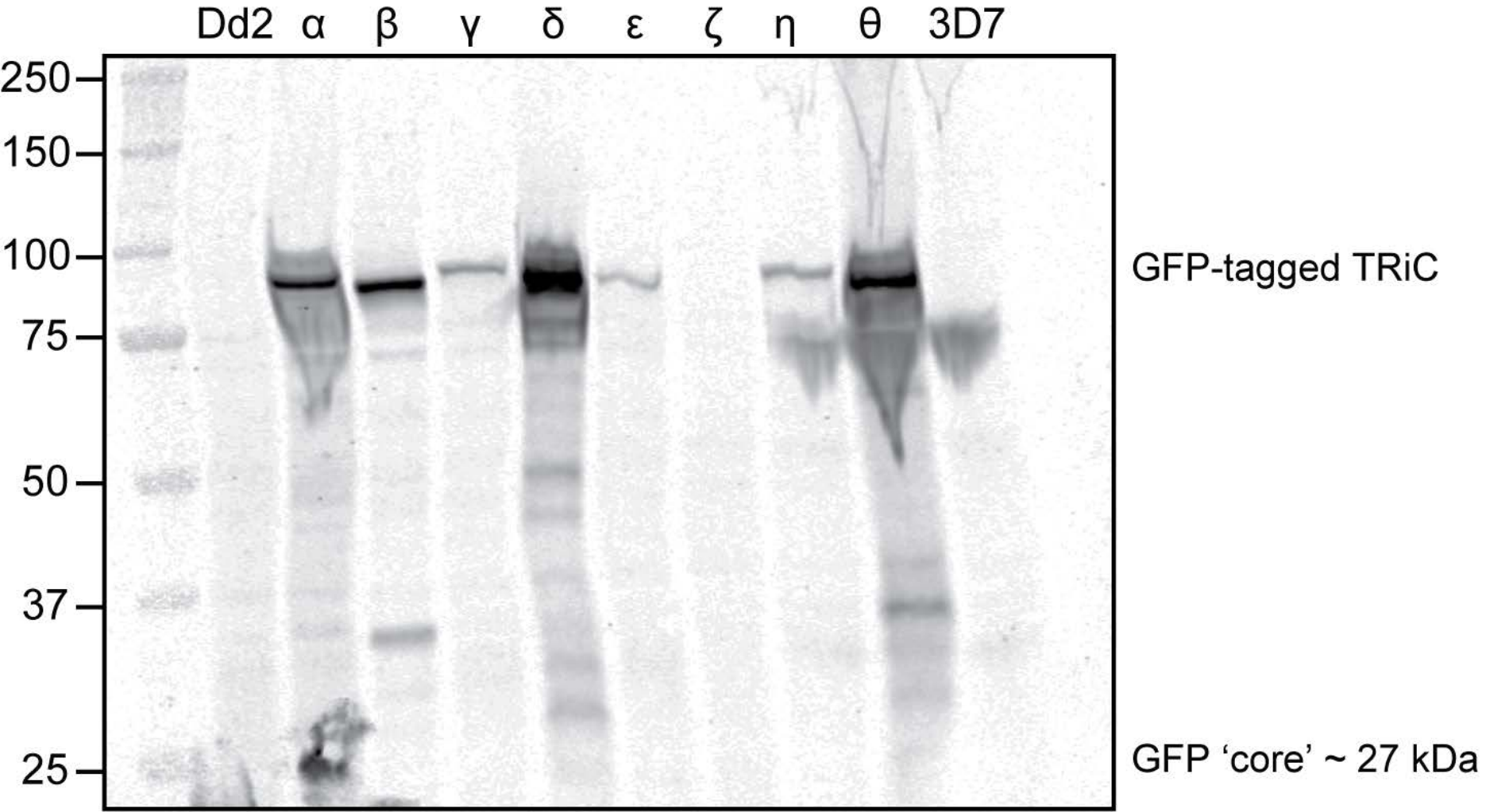
996 **Supplemental Figure 1: Full length blots for Figure 1A- RBC contain TRiC subunits in the**
997 **cytosolic fraction.** As described in Figure 1, lysates from uninfected RBC (U), trophozoite-stage
998 infected RBC (I) or saponin isolated parasites (P) were extracted with RIPA buffer and probed
999 with antibodies against human TRiC subunits A) α, B) β, C) δ, D) ζ and E) θ. PfEF-1α is a
1000 cytosolic parasite protein. Equivalent fractions were loaded in all blots. Immunoblots are
1001 representative of two-four independent experiments. Marker bands are in kDa.

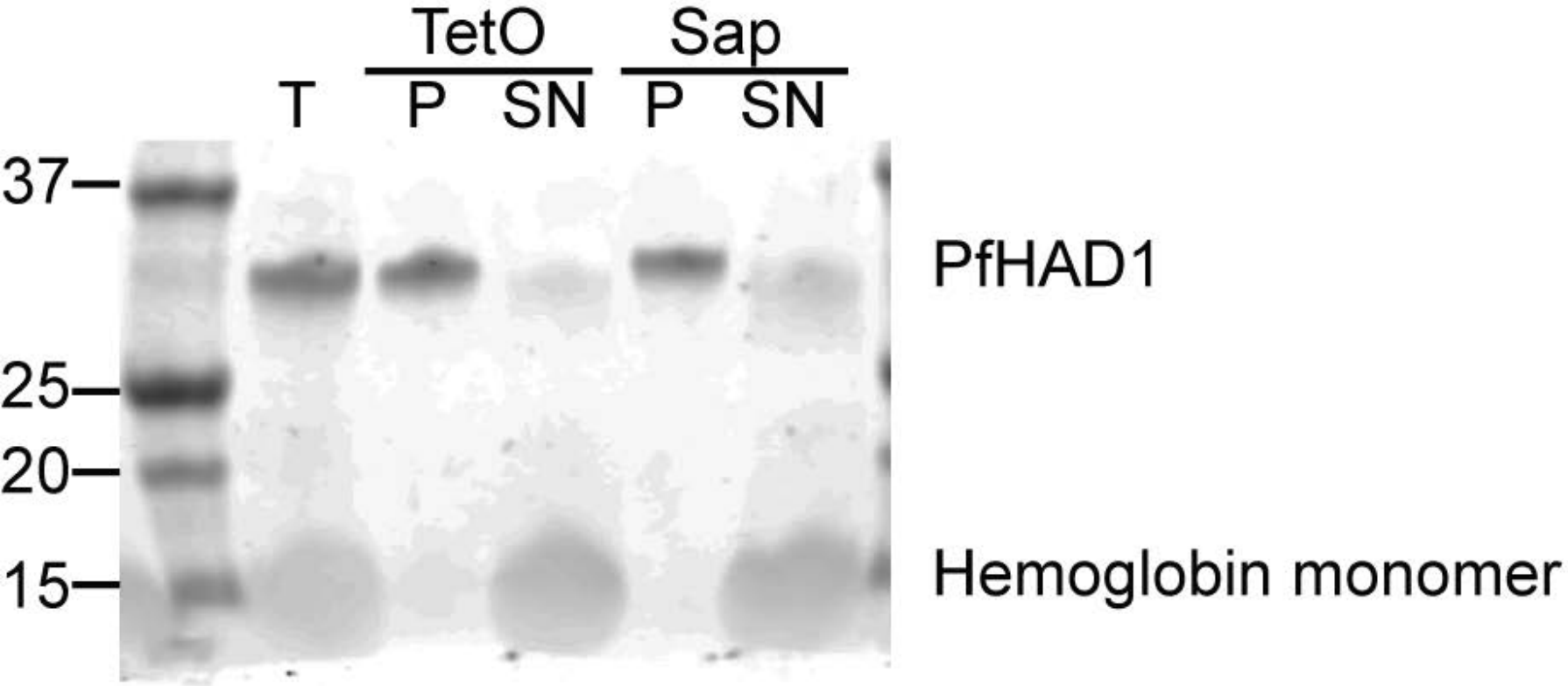
1002

1003 **Supplemental Figure 2: Full length blot for GFP-tagged PfTRiC subunits.** As described in
1004 Figure 3, ectopic expression of GFP-tagged PfTRiC subunits was under the control of the strong,
1005 constitutive HSP86 promoter. Dd2 and 3D7 are parent parasite lines. No ~27 kDa GFP 'core' is
1006 observed. Equivalent fractions were loaded in all blots. Marker bands are in kDa.

Supplemental Figure 3: Release of hemoglobin into the supernatant fraction upon TetO treatment. The activity of TetO was confirmed by visually observing release of hemoglobin into the supernatant fraction. This is also clear in immunoblots as the abundant hemoglobin monomer cross-reacts (independent of the antibodies used) during the Western blotting procedure. Infected RBC samples (T = total sample) were fractionated with 1 HU of TetO (20 min, 37 °C) or 0.05 % saponin (Sap; 20 s, room temperature). PfHAD1 is a soluble parasite protein, remaining in the saponin- and TetO-pellet (P) fractions, whereas hemoglobin is detected in the saponin- and TetO-supernatant (SN) fractions. Equivalent fractions were loaded in all blots. Marker bands are in kDa. This representative blot relates to data presented in Figure 3B panel i).


A)
U I P
U I P
150
100
75
50
37
25
HsTRiC-α
PfEF-1α
HsTRiC-α
D)
U I P
U I P
150
100
75
50
37
25
HsTRiC-ζ
PfEF-1α
HsTRiC-ζ
B)
U I P
U I P
150
100
75
50
37
25
HsTRiC-β
PfEF-1α
HsTRiC-β
E)
U I P
U I P
150
100
75
50
37
25
HsTRiC-θ
PfEF-1α
HsTRiC-θ
C)
U I P
U I P
150
100
75
50
37
25
HsTRiC-δ
PfEF-1α
HsTRiC-δ


Dd2
α
β
γ
δ
ε
ζ
η
θ
3D7
250
150
100
75
50
37
25
GFP-tagged TRiC
GFP 'core' ~ 27 kDa


TetO
Sap
T
P
SN
P
SN
37
25
20
15
PfHAD1
Hemoglobin monomer