

Supplementary Information

A Plant-Produced *in vivo* deglycosylated full-length Pfs48/45 as a Transmission-Blocking Vaccine Candidate against malaria

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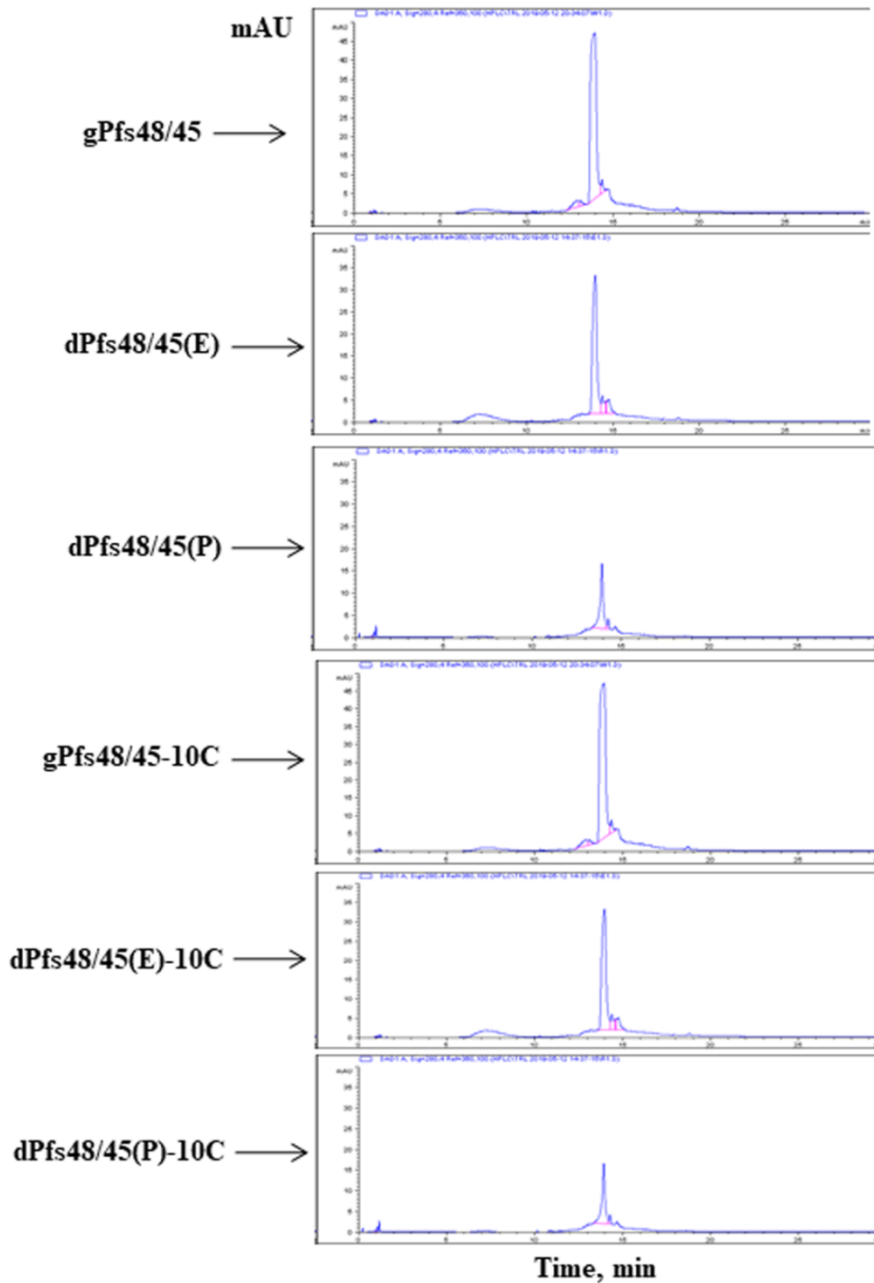
Supplementary Table S1: SMFA results^a

Feed #	Sample name	Mean oocysts	%TRA ^b			
			estimate	95% CI Lo	95% CI Hi	p-value
1	Negative control	18.1				
	dPfs48/45 (E)	0.1	99	98	100	0.001
	dPfs48/45 (P)	1.7	91	79	96	0.001
	dPfs48/45-10C (E)	4.3	76	48	91	0.002
2	Negative control	12.4				
	dPfs48/45-10C (P)	5.8	53	1	78	0.050
	g Pfs48/45	14.4	-16	-151	45	0.721
	g Pfs48/45-10C	20.9	-68	-269	22	0.196
3	Negative control	48.2				
	dPfs48/45 (E)	1.0	98	96	99	0.001
	dPfs48/45 (P)	23.2	52	-4	78	0.066
	dPfs48/45-10C (E)	37.1	23	-68	64	0.460
	dPfs48/45-10C (P)	28.2	41	-21	72	0.155
	g Pfs48/45	50.8	-5	-129	53	0.858
	g Pfs48/45-10C	63.3	-31	-186	39	0.497

^a Purified IgGs were tested at 0.75 mg/ml with complement, and 20 mosquitoes were dissected per group for tests, and 40 mosquitoes for control.

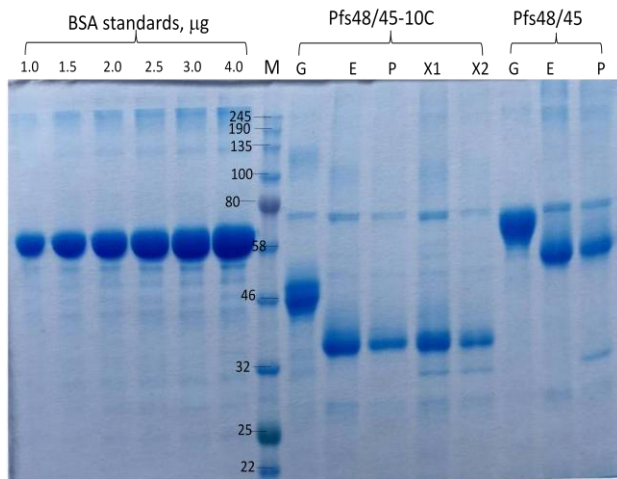
^b The best estimates of % inhibition in oocyst density (%TRA), the 95% confidence intervals (95%CI), and p-values from single or multiple feeds were calculated using a zero-inflated negative binomial random effects model (ZINB model) described previously³⁶.

Supplementary Figure S1. HPLC analysis of plant produced Pfs48/45 (a) and Pfs48/45-10C (b) proteins. All peaks in the chromatogram was detected at 280 nm. The purity was calculated as a percentage of peak area in relation to total area of peaks.



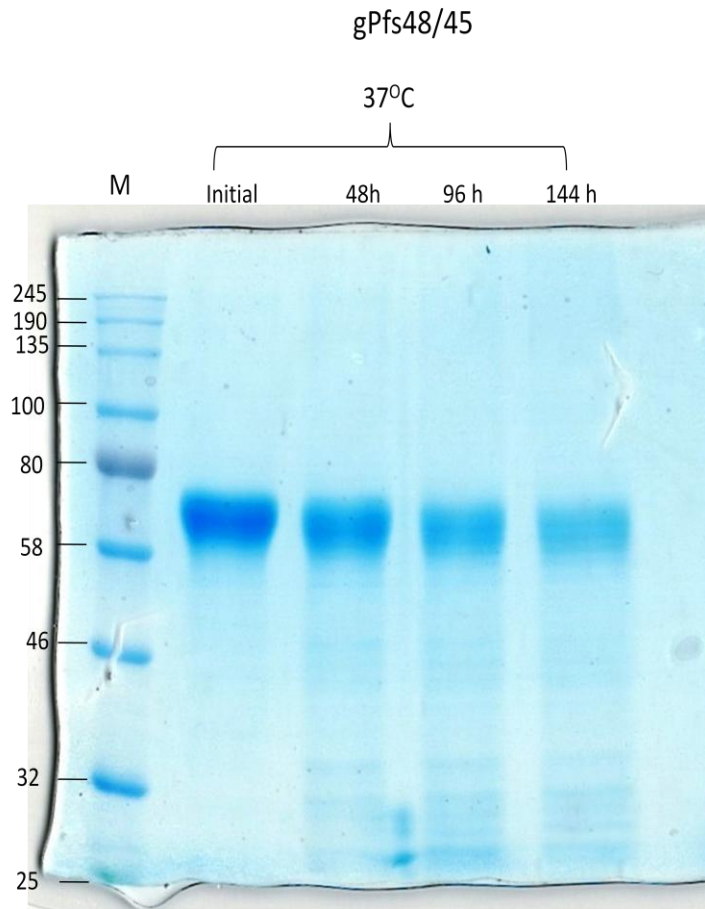
Full-length gels and blots

1. Full-length gels

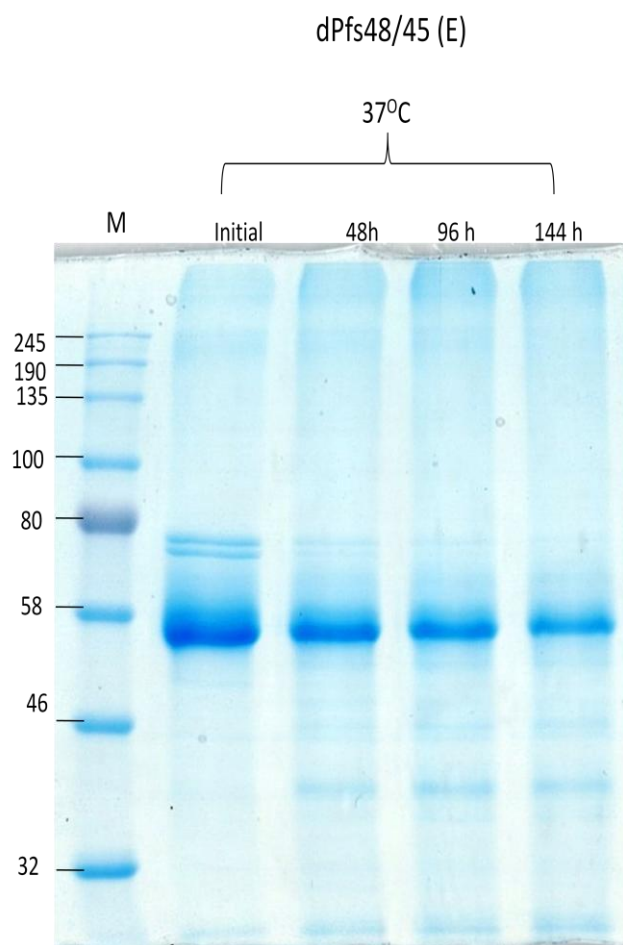


Full-length gel 1. SDS-PAGE analysis of purified, plant produced Pfs48/45 and Pfs48/45-10C variants. Figure 2 (a) indicated as "Pfs48/45" was cropped from this gel (Pfs48/45, Lanes G, E and P along with prestained protein standards, M).

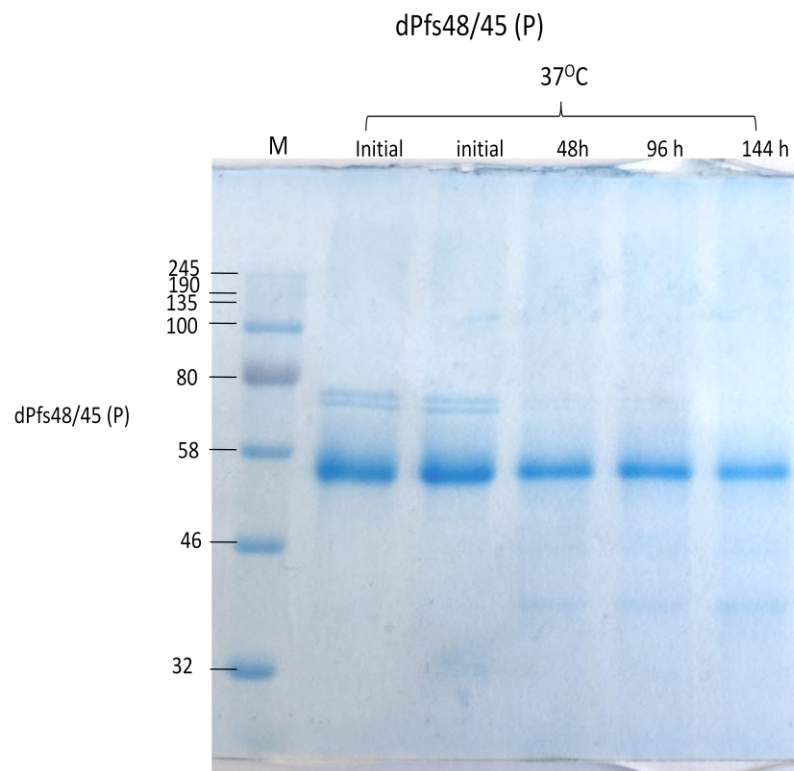
X1, X2: combined last fractions of Pfs48/45-10C-E and Pfs48/45-10C-P, respectively, eluted from anti-FLAG column. M: color prestained protein standard (cat. no. P7712 S New England Biolabs)



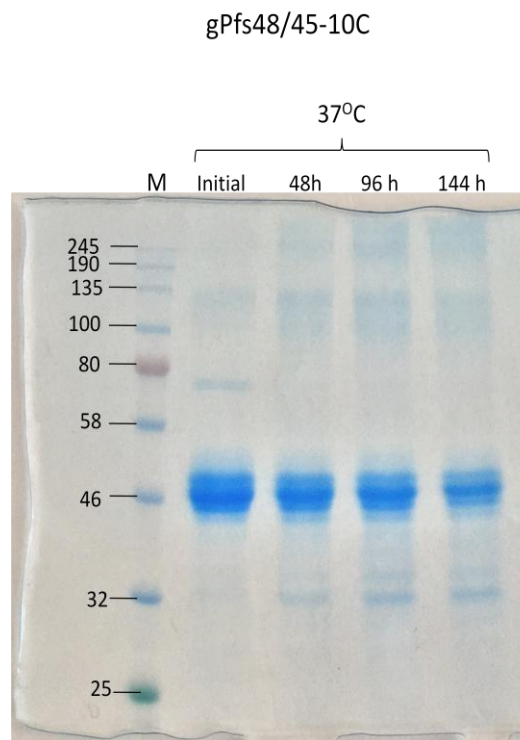
Full-length gel 2. Figure 3 (a), indicated as "gPfs48/45" was cropped from this gel. (in the previous version of the manuscript). Full-length gel 2. was used in this version of the manuscript as suggested by reviewer #3.



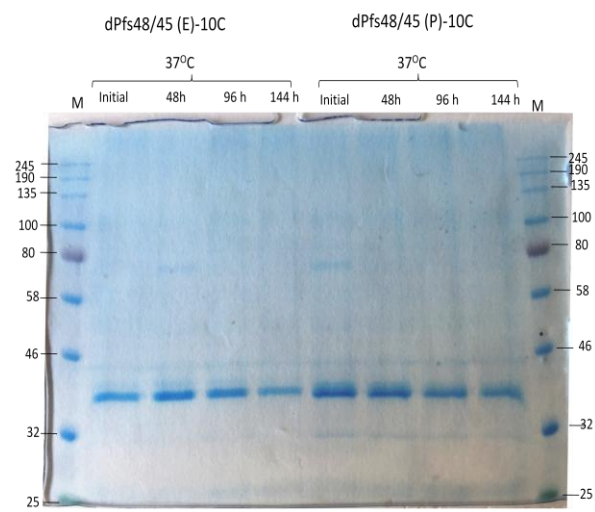
Full-length gel 3. Figure 3 (a), indicated as "dPfs48/45 (E)" was cropped from this gel. (in the previous version of the manuscript). Full-length gel 3. was used in this version of the manuscript as suggested by reviewer #3.



Full-length gel 4. Figure 3 (a), indicated as "dPfs48/45 (P)" was cropped from this gel (in the previous version of the manuscript). Full-length gel 4. was used in this version of the manuscript as suggested by reviewer #3.

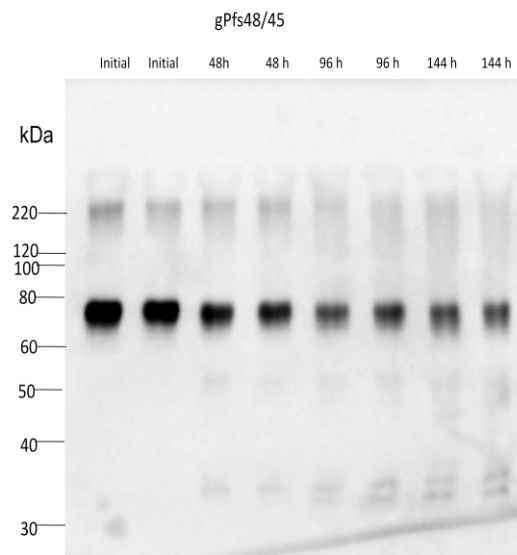


Full-length gel 5. Figure 4 (a), indicated as "gPfs48/45-10C" was cropped from this gel.

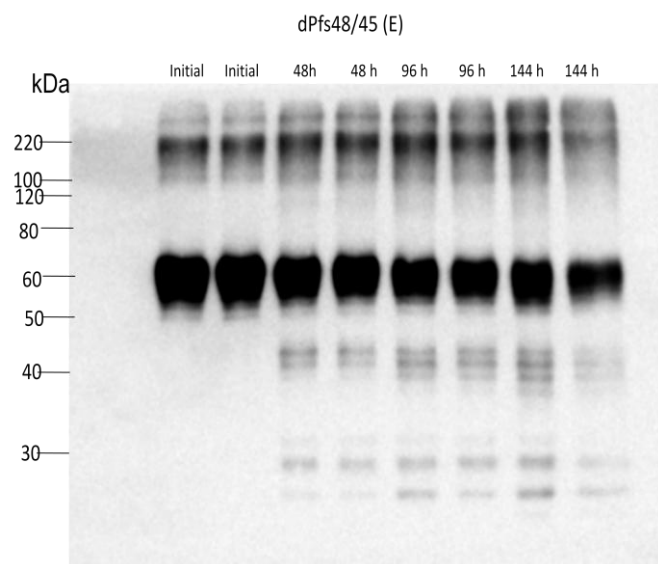


Full-length gel 6. Figure 4 (a), indicated as "dPfs48/45 (P)-10C" and "dPfs48/45 (P)-10C" were cropped from this gel.

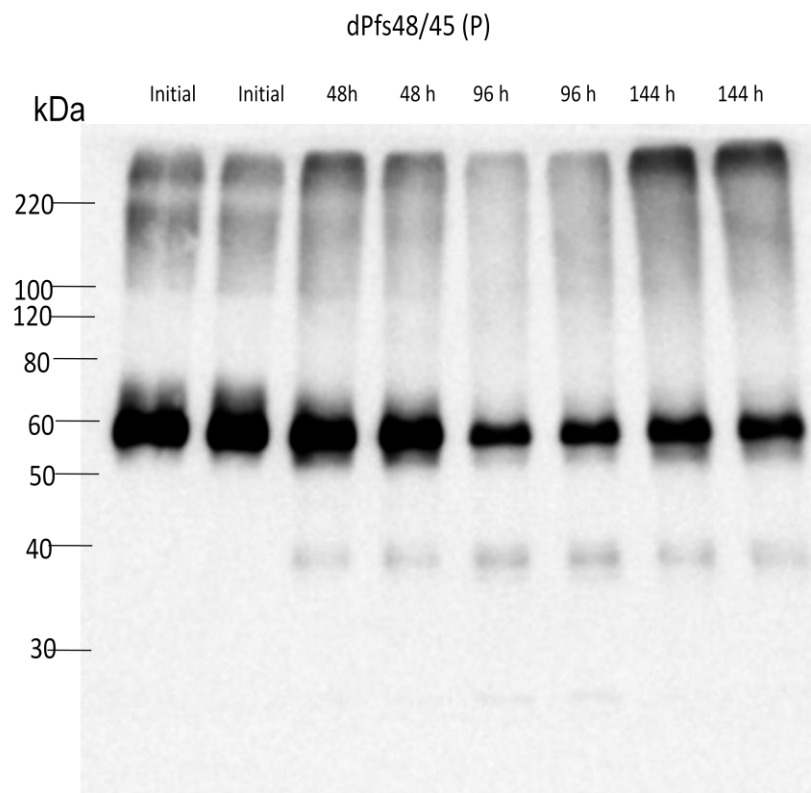
Full-length blots



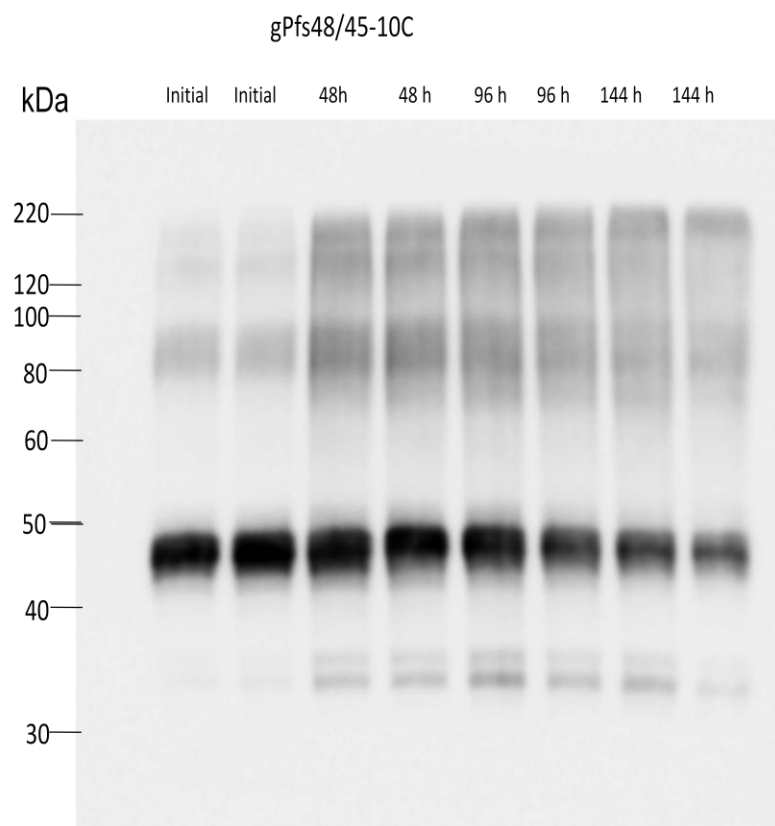
Full-length blot 1. Figure 4 (b), indicated as "gPfs48/45" was cropped from this blot.



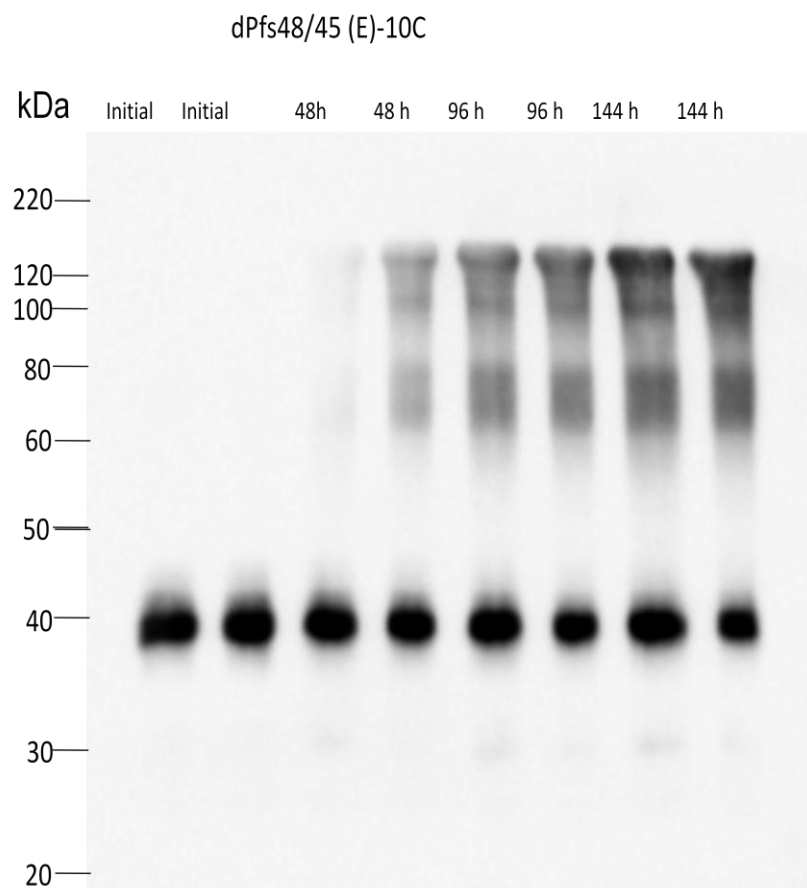
Full-length blot 2. Figure 4 (b), indicated as "dPfs48/45(E)" was cropped from this blot.



Full-length blot 3. Figure 4 (b), indicated as "dPfs48/45(P)" was cropped from this blot.

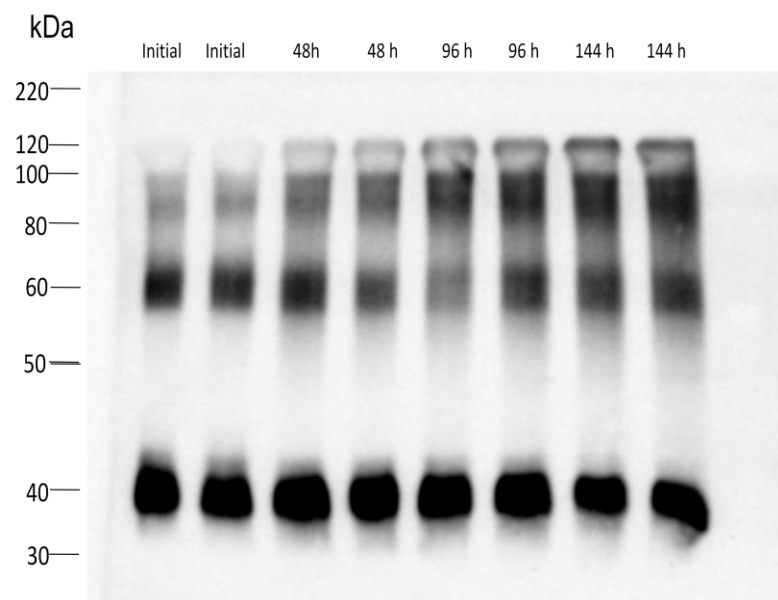


Full-length blot 4. Figure 4 (b), indicated as "gPfs48/45-10C" was cropped from this blot.



Full-length blot 5. Figure 4 (b), indicated as "dPfs48/45(E)-10C" was cropped from this blot.

dPfs48/45 (P)-10C



Full-length blot 6. Figure 4 (b), indicated as "dPfs48/45(P)-10C" was cropped from this blot.