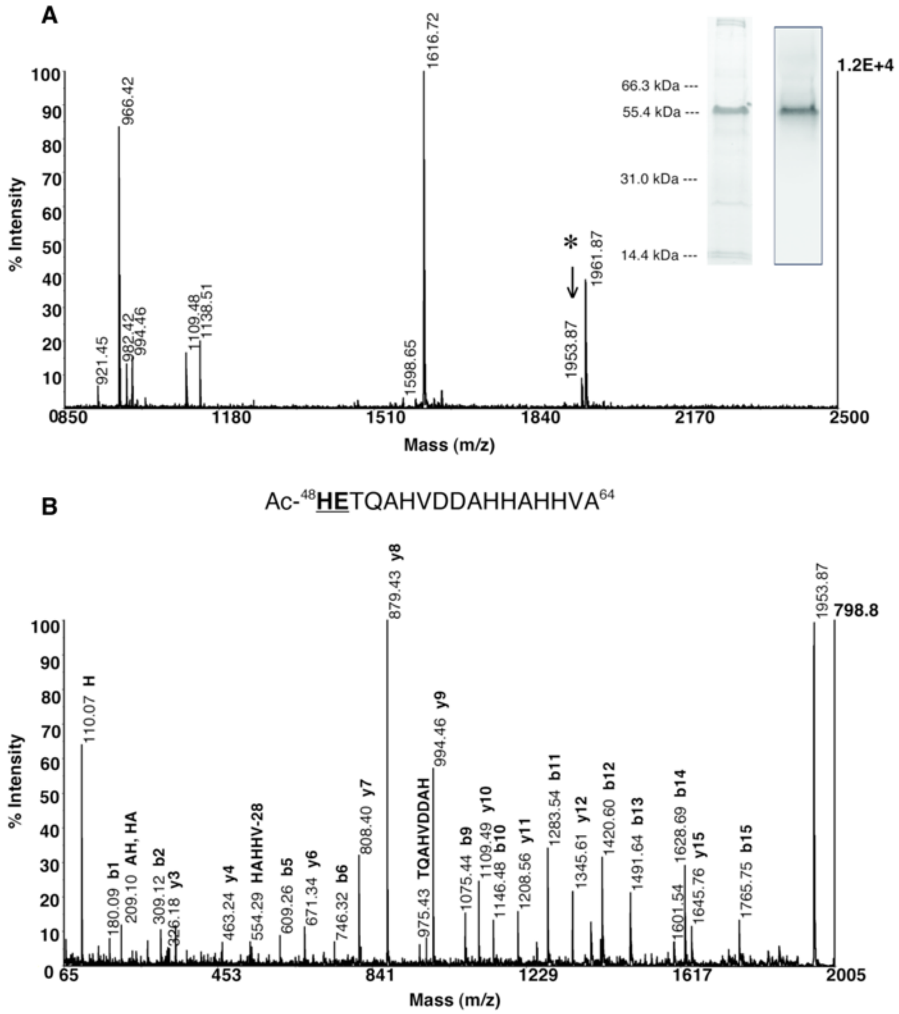
Supplementary Figure Legend

Figure S1. Overexpression of *Pf*HRPIImyc does not affect its PEXEL processing. (A) Overexpressed *Pf*HRPIImyc enriched from the saponin supernatant of trophozoite-infected RBCs was subjected to an Asp-N in-digestion and analyzed by MALDI-TOF MS. The inset Coomassie-stained gel (left) and anti-c-Myc Western blot (right) confirms the enriched protein. The gel band was excised for proteolysis because of contaminating red cell proteins. The peptide fragment at *m/z* 1953.87 (*) was selected for tandem MS analysis. (B) *De novo* sequencing of *Pf*HRPIImyc *m/z* 1953.87. CID analysis reveals that *m/z* 1953.87 is the most N-terminal peptide fragment and demonstrates that the PEXEL in *Pf*HRPIImyc is also cleaved after residue 47 (^{45}RLL↓) and the mature N-terminus is N-acetylated (Ac-HE^{49}). Residues in the sequenced peptide that are bold and underlined are a part of the *Pf*HRPIImyc PEXEL motif.

Figure S1



A
1.2E+4
% Intensity
100
90
80
70
60
50
40
30
20
10
1616.72
966.42
921.45
982.42
994.46
1109.48
1138.51
1598.65
1953.87
1961.87
*
66.3 kDa ---
55.4 kDa ---
31.0 kDa ---
14.4 kDa ---
0850
1180
1510
1840
2170
2500
Mass (m/z)
B
Ac-48HETQAHVDDAHHAHHVA64
798.8
1953.87
879.43 y8
110.07 H
994.46 y9
180.09 b1
209.10 AH, HA
309.12 b2
326.18 y3
463.24 y4
554.29 HAHHV-28
609.26 b5
671.34 y6
746.32 b6
808.40 y7
975.43 TQAHVDDAH
1075.44 b9
1109.49 y10
1146.48 b10
1208.56 y11
1283.54 b11
1345.61 y12
1420.60 b12
1491.64 b13
1601.54
1628.69 b14
1645.76 y15
1765.75 b15
0
65
453
841
1229
1617
2005
Mass (m/z)

Table S1. Peptide fragments were obtained from an Asp-N solution digest of *Pf*HRPII purified from saponin supernatant and analyzed by MALDI-TOF MS. The *m/z* and corresponding amino acid sequence in the full-length sequence is according to GenBank accession no. **AAC47453**.

m/z	Amino acid sequence represented
966.42	65-100, 191-199, 266-274
994.46	56-64
1138.51	275-285
1223.53	218-229, 248-259
1253.54	179-190, 206-265
1598.69	185-199, 260-274
1616.72	134-148, 164-178
1855.79	206-265
1913.84	65-100
1941.87	56-73
1953.87	48-64
2085.92	266-285
2200.95	179-199, 254-274
2458.05	206-259

amino acids in *Pf*HRPII = 309
amino acids after PEXEL processing = 262
amino acids covered = 184
sequence coverage = 184/262 = 70%

Table S2. Peptide fragments were obtained from an Asp-N in-gel digest of *Pf*HRPIImyc enriched from saponin supernatant and analyzed by MALDI-TOF MS. The *m/z* and corresponding amino acid sequence in the full-length sequence is according to GenBank accession no. **AAC47453** and a C-terminal c-Myc tag.

m/z	**Amino acid sequence represented**
966.42	65-100, 191-199, 266-274
982.42	119-127, 149-157
994.46	56-64
1109.48	55-64
1138.51	275-285
1253.54	179-190, 206-265
1598.65	185-199, 260-274
1616.72	134-148, 164-178
1953.87	48-64
1961.87	101-118

\# amino acids in *Pf*HRPIImyc = 319
\# amino acids after PEXEL processing = 272
\# amino acids covered = 220
sequence coverage = 220/272 = 81%

Table S3. Peptide fragments were obtained from an Asp-N in-gel digest of *Pf*HRPIImyc enriched from the ER of BFA-treated ring parasites and analyzed by MALDI-TOF MS. The *m/z* and corresponding amino acid sequence in the full-length sequence is according to GenBank accession no. **AAC47453** and a C-terminal c-Myc tag.

m/z	**Amino acid sequence represented**
863.40	48-54
966.42	65-100, 191-199, 266-274
1109.48	55-64
1138.51	275-285
1223.53	218-229, 248-259
1253.54	179-190, 206-265
1584.68	119-133, 149-163
1598.69	185-199, 260-274
1616.72	134-148, 164-178
1770.78	275-291
1855.79	206-265
1913.84	65-100
1953.87	48-64
1961.87	101-118
2200.95	179-199, 254-274

\# amino acids in *Pf*HRPIImyc = 319
\# amino acids after PEXEL processing = 272
\# amino acids covered = 238
sequence coverage = 238/272 = 88%

Table S4. Peptide fragments were obtained from an Asp-N in-gel digest of KAHRP(1-60)-GFP enriched from saponin supernatant and analyzed by MALDI-TOF MS. The *m/z* and corresponding amino acid sequence is according to Wickham *et al.* [12]. The amino acid numbering starts from the N-terminus after PEXEL processing.

m/z	**Amino acid sequence represented**
1172.53	185-194
1370.80	122-133
1463.71	202-214
1518.68	26-40
1728.81	107-121
1890.03	122-137
*1941.95	221-238
2155.10	195-214
^2182.23	160-177
2203.07	202-220
*2440.20	87-106
2565.21	138-159
2580.43	1-23
^2851.55	160-184

amino acids in processed KAHRP(1-60)-GFP = 243
amino acids covered = 184
sequence coverage = 184/243 = 76%

*The peptides have corresponding peptides with oxidized Met (+16.00) at 1957.95 and 2456.20, respectively.
^NG turns into DG during the ionization process to add 1.01 amu.