Supporting Information

Plasmodium falciparum Sir2A preferentially hydrolyzes medium and long chain fatty acyl lysine

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Synthesis of acyl peptides. Solvents were purchased from Fisher Scientific unless otherwise specified and peptide synthesis reagents and Fmoc-protected amino acids and derivatives were purchased from Creosalus Inc.

To facilitate the detection of the peptides by ultra-violet (UV) light absorption, two Trp residues were added to the C-terminal of the peptides. The H3K9 (NH_2-KQTARK*STGGWW-COOH) backbone was prepared using standard solid phase peptide synthesis (SPPS) at room temperature (RT). Wang resins SS (100-200 mesh, 1% DVB, 10 mmole/g) were placed into a peptide synthesis vessel along with 5 mL of anhydrous dichloromethane (DCM) for 5 hours. The first activated amino acid solution was freshly prepared with 0.32 mmoles of Fmoc-W-OH, 0.32 mmoles of O-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), 0.133 mmoles of 4-dimethylaminopyridine (DMAP), 0.64 mmoles of diisopropylethylamine (DIEA, added last) and an appropriate amount of anhydrous *N*,*N*'-dimethylformamide (DMF). The resin was incubated with the solution overnight at RT. The resins were then washed with DMF (5 times) before incubating with a cocktail of acetic anhydride, pyridine and DMF (2:1:3 ratio (v/v)) for 30 minutes to block remaining amino groups on the resin. Kaiser test was used to test the success of the coupling. Once coupling was confirmed, 20% (v/v) piperidine in DMF was used to remove Fmoc. All subsequent activated amino

acid derivatives were freshly prepared with 0.24 mmoles of the amino acid, 0.24 mmole of HBTU, 0.21 mmole of N-hydroxybenzotriazole (HOBT), 0.48 mmole of DIEA in DMF and reaction time was 2 hours at RT.

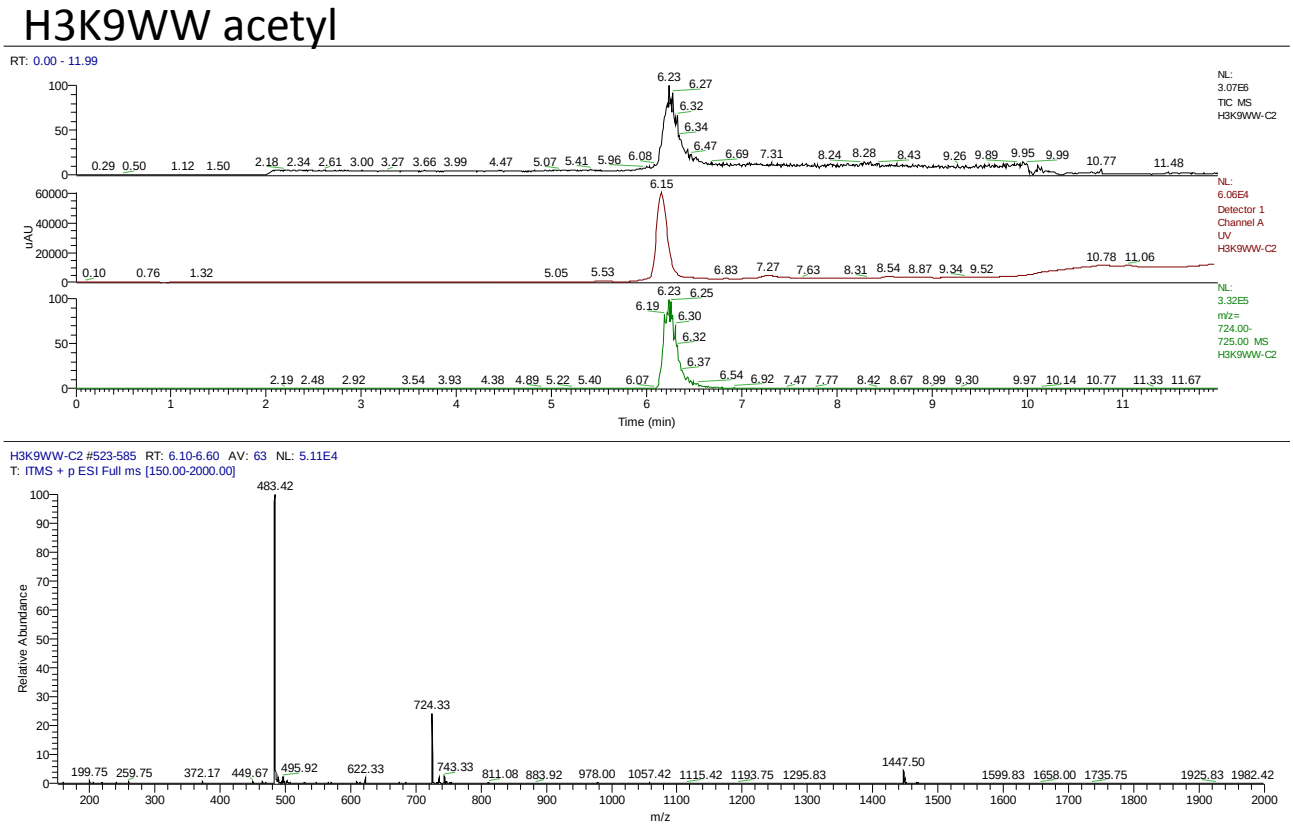
The lysine to be modified by different acyl groups (K*) was protected with allyl carbamate (Alloc) on the side chain while the N-terminal K was protected by Boc. After all the peptide coupling steps, the Alloc group was removed by incubating the resin in a cocktail of DCM, morpholine (Sigma, 2.5%) and glacial acetic acid (5%) with a 1:1 weight ratio of the original resin and tetrakis(triphenylphosphine)palladium(0) for 4 hours under nitrogen. Palladium was removed with 0.5% (v/v) DIEA in DCM and 0.02 M of diethyldicarbamate in DMF. The resin was then incubated with solutions for putting on different acyl groups. The acylation solutions contained 0.24 mmoles of fatty acids (acetic anhydride, butyric acid, octanoic acid, or myristic acid), 0.24 mmole of HBTU, 0.21 mmole of HOBT, 0.48 mmole of DIEA in DMF. The resin was then washed with DMF and the peptides were cleaved with a mixture of trifluoroacetic acid (TFA), 5% (v/v) water, 5% (w/v) phenol, 2.5% (v/v) ethanedithiol and 5% (v/v) thioanisole. TFA was removed from the filtered peptide solution and the peptides were precipitated out by the addition of ether and lyophilized. The crude peptides were dissolved in water and purified by HPLC (Beckman Coulter System Gold 125P solvent module and 168 Detector) using a TARGA C18 column (250 x 20 mm, 10 µM, Higgins Analytical, Inc.) with mobile phase A (water with 0.1% (v/v) TFA) and B (acetonitrile with 0.1% (v/v) TFA) at a gradient of 20% B to 100% B in 50 minutes and a flow rate of 10 mL/min.

Supplementary Table 1. Crystallographic Data Collection and Refinement Statistics

	PfSir2A-myrH3K9	PfSir2A-myrH3K9-NAD
Data collection		
Space group	$P2_12_12$	$P2_12_12$
Cell dimensions		
a, *b*, *c* (Å)	32.51, 103.33, 105.51	32.17, 102.73, 105.18
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	50-2.40	50-2.20
R_{sym} or R_{merge} (%)	14.9 (75.3)	12.9 (74.8)
I / σI	23.83 (1.75)	24.7 (2.21)
Completeness (%)	98.5 (97.2)	99.9 (99.9)
Redundancy	8.2 (4.4)	6.7 (5.0)
Refinement		
Resolution (Å)	50-2.40	50-2.20
No. reflections	18972	23688
R_{work} / R_{free} (%)	22.38 /27.61	20.24/24.65
No. of protein residues	263	263
No. of ligand/ion molecules		
Myristoyl H3K9	1	1
NAD	--	1
Glycerol	1	1
Zn	1	1
No. of water	25	75
R.m.s deviations		
Bond lengths (Å)	0.047	0.024
Bond angles (°)	2.10	2.07

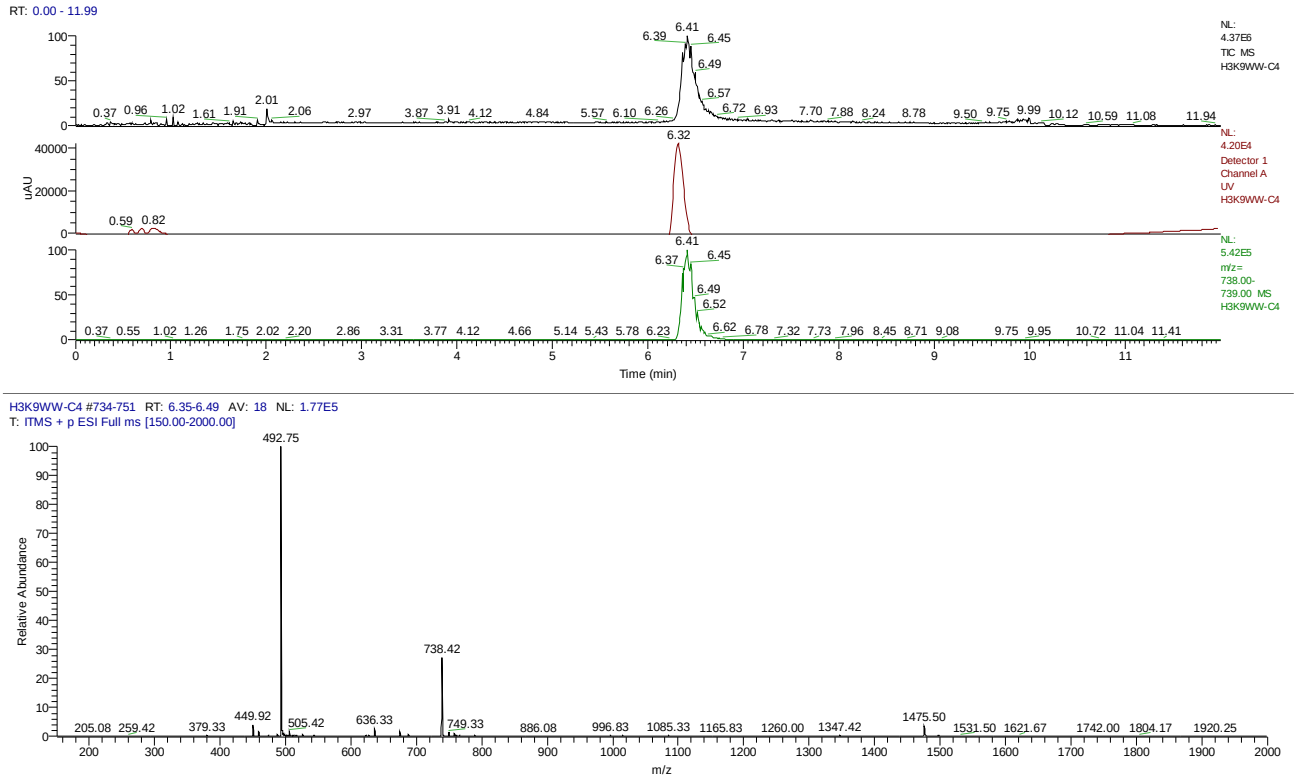
Numbers showed in the parentheses are for the highest resolution shell.

Supplementary Figure 1: Identity and purity of the acyl peptides checked by LC-MS:



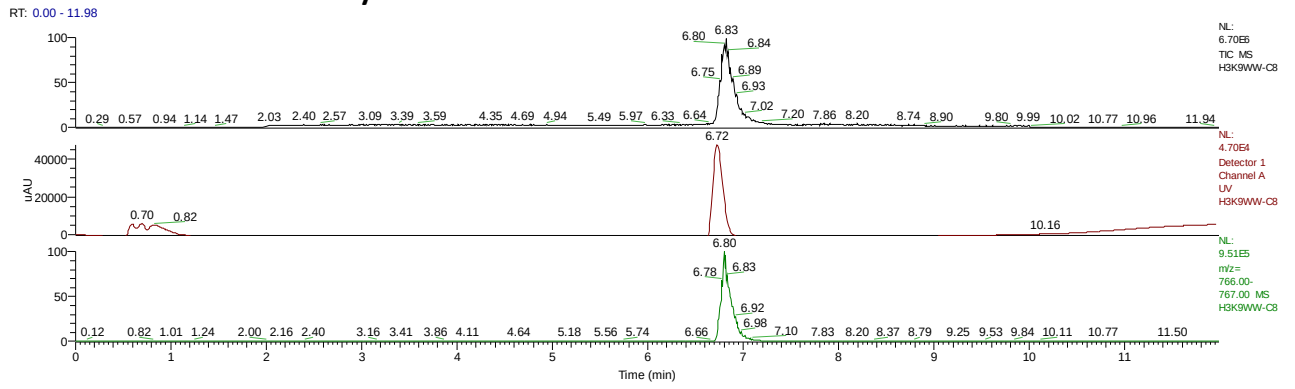
The observed *m/z* for H3K9WW acetyl peptide are 1447.50 ($[M+H]^{+}$, predicted 1447.60); 724.33 ($[M+2H]^{2+}$, predicted 724.8); and 483.42 ($[M+3H]^{3+}$, predicted 484.5). The black trace shows the total ion count trace, the maroon trace shows the UV trace at 280 nm, and the green trace shows the ions with *m/z* from 724 to 725 for $[M+2H]^{2+}$.

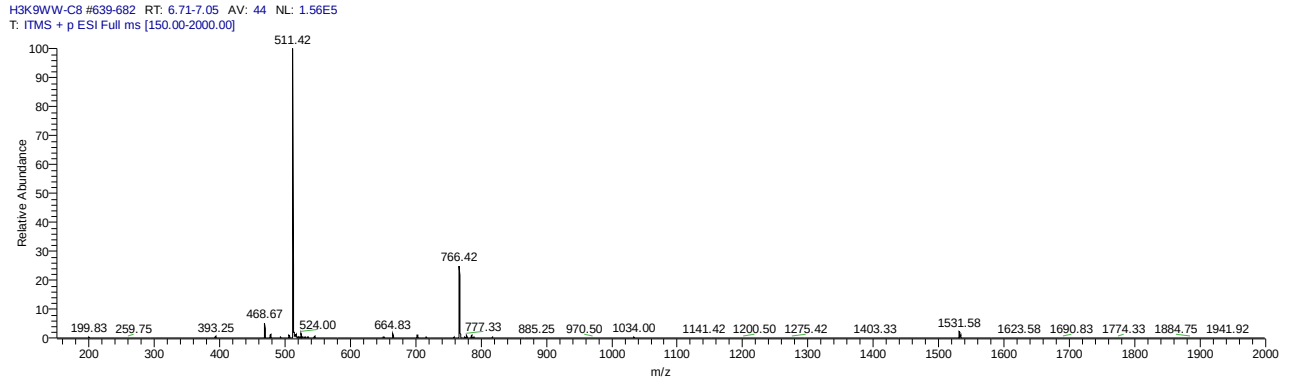
H3K9WW butyryl



The observed *m/z* for the H3K9WW butyryl peptide are 1475.50 ($[M+H]^{+}$, predicted 1475.65); 738.42 ($[M+2H]^{2+}$, predicted 738.8); and 492.75 ($[M+3H]^{3+}$, predicted 493.8). The black trace shows the total ion count trace, the maroon trace shows the UV trace at 280 nm, and the green trace shows the ions with *m/z* from 738 to 739 for $[M+2H]^{2+}$.

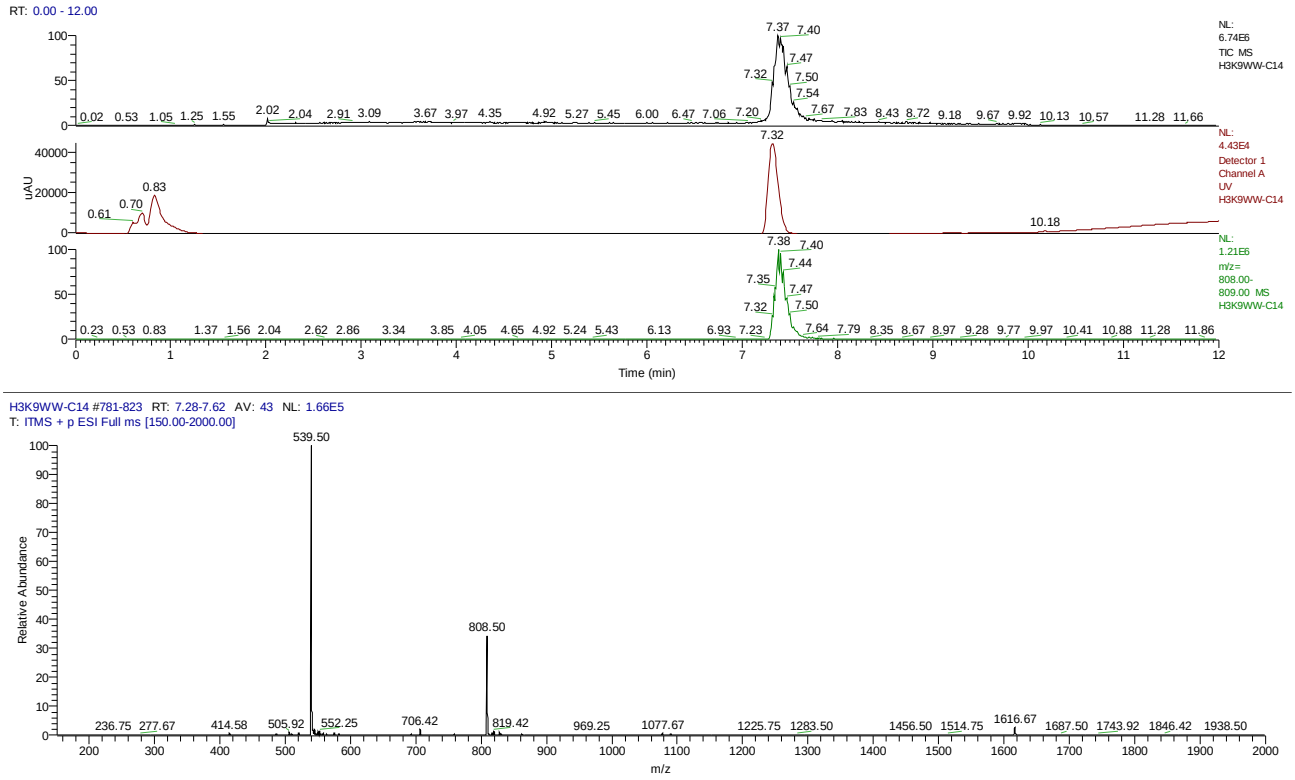
H3K9WW octanoyl





The observed *m/z* for H3K9WW octanoyl peptide are 1531.58 ($[M+H]^{+}$, predicted 1531.76); 766.42 ($[M+2H]^{2+}$, predicted 766.9); and 511.42 ($[M+3H]^{3+}$, predicted 512.6). The black trace shows the total ion count trace, the maroon trace shows the UV trace at 280 nm, and the green trace shows the ions with *m/z* from 766 to 767 for $[M+2H]^{2+}$.

H3K9WW myristoyl



The observed *m/z* for the H3K9WW myristoyl peptide are 1616.67 ($[M+H]^{+}$, predicted 1615.9), 808.5 ($[M+2H]^{2+}$, predicted 808.95), and 539.5 ($[M+3H]^{3+}$, predicted 540.6). The black trace shows the total ion count trace, the maroon trace shows the UV trace at 280 nm, and the green trace shows the ions with *m/z* from 808 to 809 for $[M+2H]^{2+}$.