SUPPLEMENTARY FIGURES for

Deciphering the molecular basis of mycobacteria and lipoglycan recognition by the C-type lectin Dectin-2

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Figure S1. Structures of the lipoglycans (A) and mannodendrimers (B) used in this study.

A) The correspondence between the structures and the symbols is shown for SaeLM.

B) Only one of the six branches substituting the N3P3 core is represented.

Figure S2. Dectin-2 agonist activity in LM Mtb and 19 kDa MLP fractions relies on ManLAM.

A, C, D) NF-κB activation induced by LM Mtb and 19 kDa MLP fractions in HEK-Dectin-2 or HEK-TLR2 cells. Mannoconjugates (from 1 to 1000 ng in A; from 300 to 10 ng in C; from 20 to 0.2 ng in D) were tested for their capacity to induce NF-κB activation in HEK-Dectin-2 (A) or HEK-TLR2 (C, D) cells. Conditions are the same as in Figure 2. - ManLAM, ManLAM-depleted.

B) Monosaccharide analysis of LM Mtb fraction. Partial capillary electropherograms of APTS derivatives of monosaccharides obtained from total acid hydrolysis of ManLAM Mtb (trace a) and LM Mtb (trace b). Peaks: Ara*, arabinose-APTS; Man*, mannose-APTS; C7*, internal standard, mannoheptose-APTS.

Figure S3. Generation of a *pimE* knock-out mutant of *M. tuberculosis* H37Rv (A) and analysis of phosphatidyl-*myo*-inositol mannosides composition (B).

A) Allelic replacement was confirmed by PCR using the set of primers (Rv1159.1 and Rv1159.2; see Materials and Methods for details). The wild-type 1,295 bp amplification signal (wt) is replaced by a 1,526-bp amplification signal in the mutant clones (Δ) due to the insertion of a 1.2 kb- kanamycin resistance cassette between the two SmaI restriction sites of *pimE*.

B) Negative ion mode MALDI-MS spectra of the total lipids from WT Mtb H37Rv and its *pimE* mutant (H37Rv Δ*pimE*). PIM_2, PIM_4, PIM_6, phosphatidyl-*myo*-inositol di-, tetramannosides; PIM_6: phosphatidyl-*myo*-inositol hexamannosides; Ac_1PIM, acylated PIM (3 acyl chains in total); Ac_2PIM, di-acylated PIM (4 acyl chains in total).

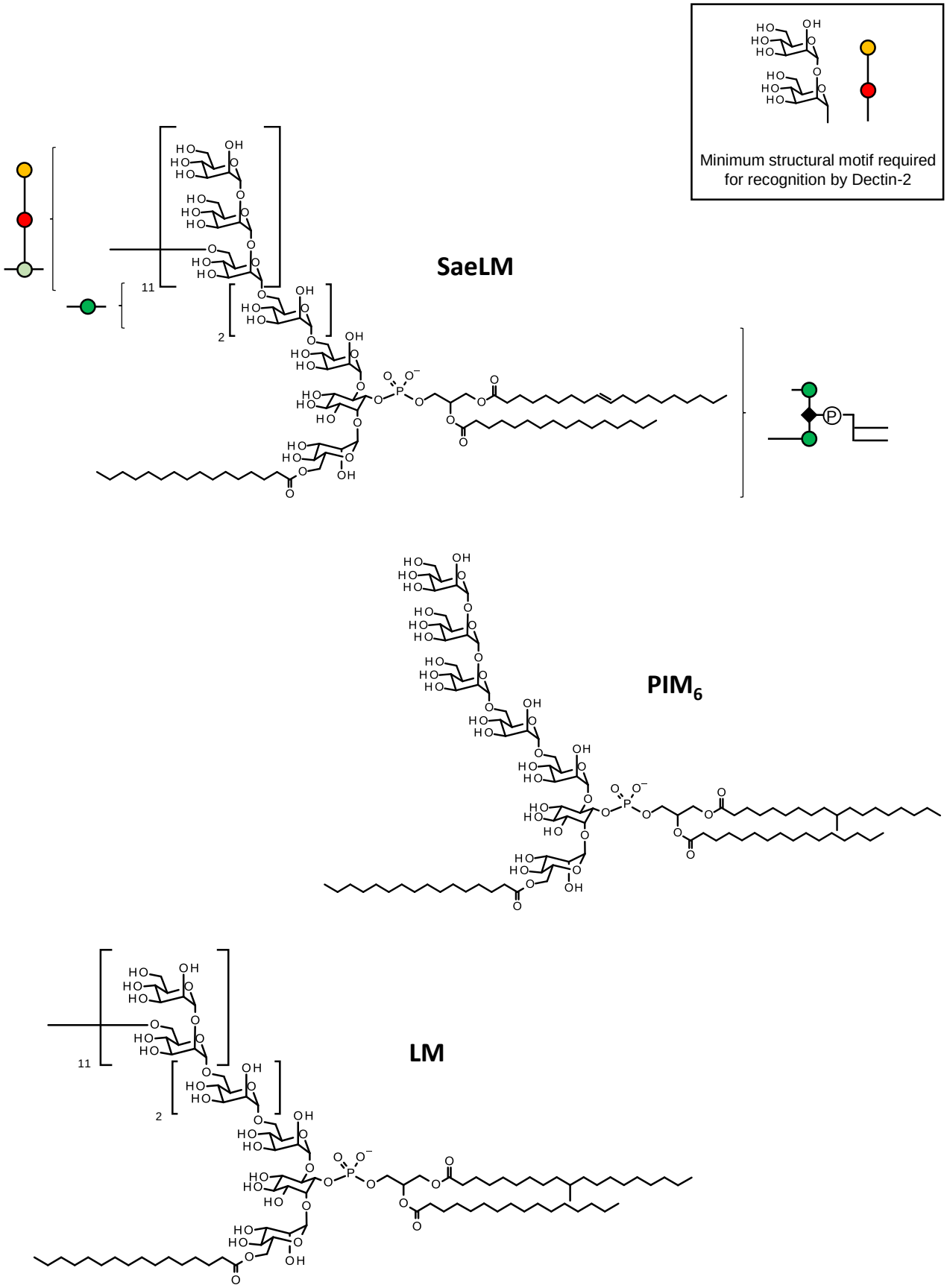

Minimum structural motif required for recognition by Dectin-2
SaeLM
PIM6
LM

Fig. S1A

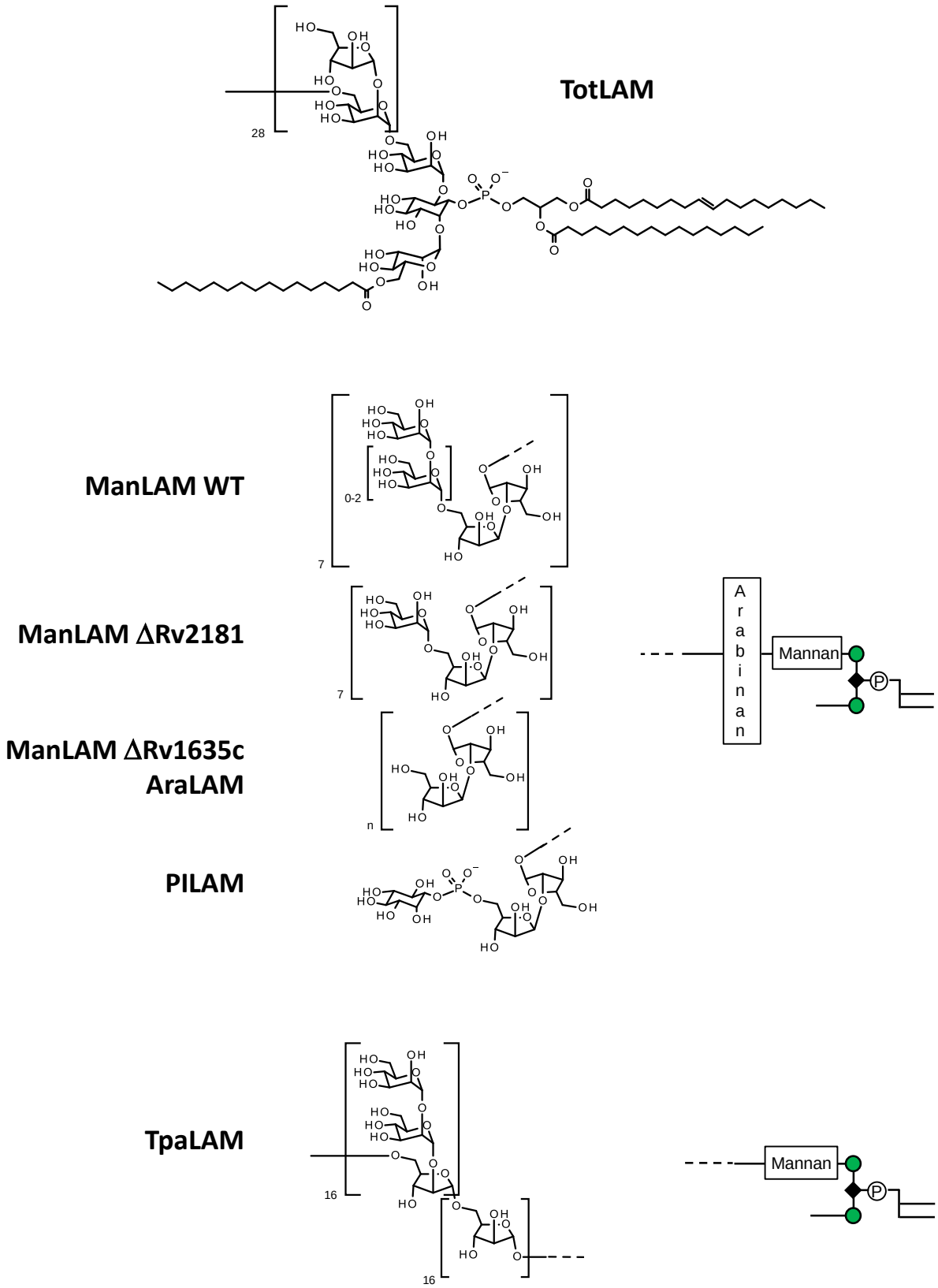


Fig. S1A (continues)

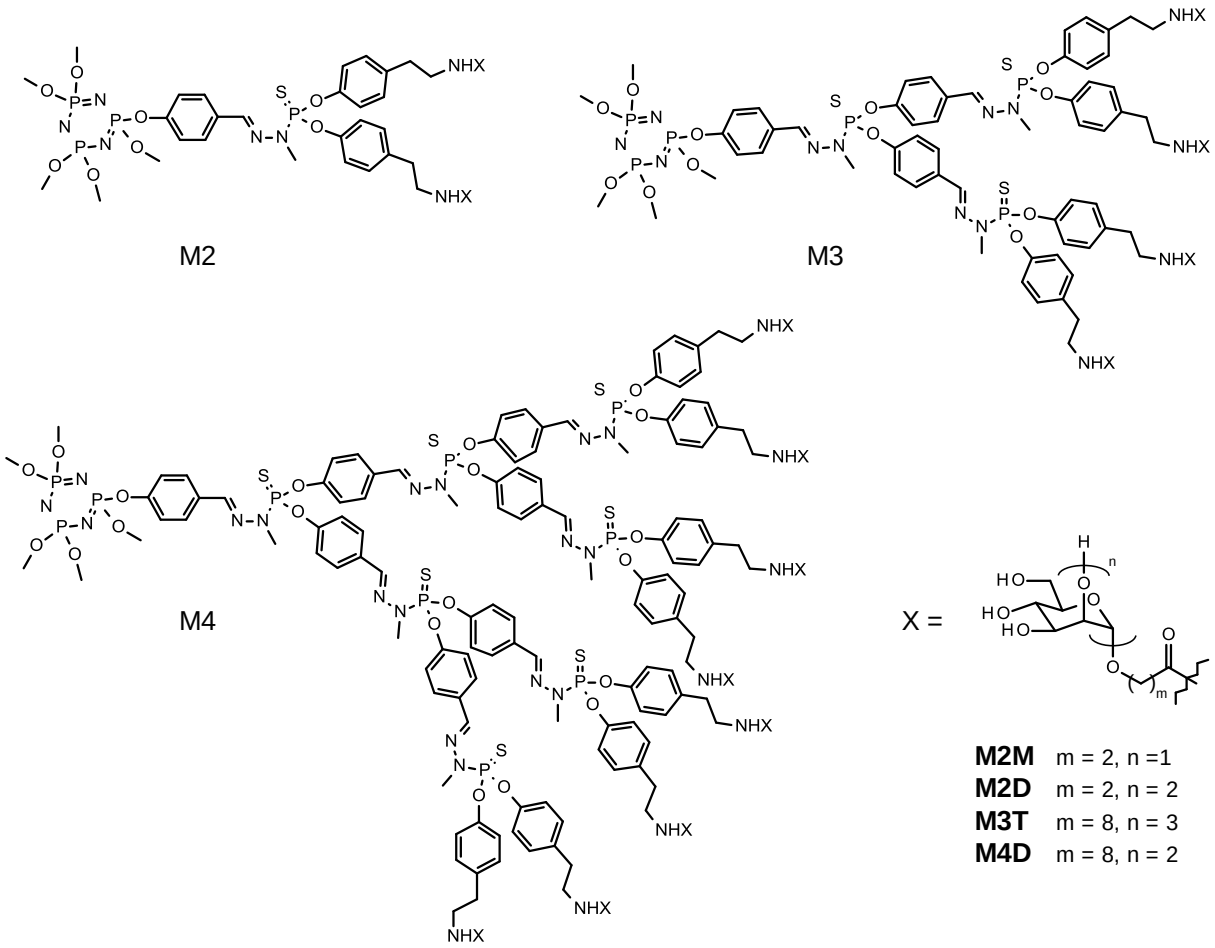


Fig. S1B

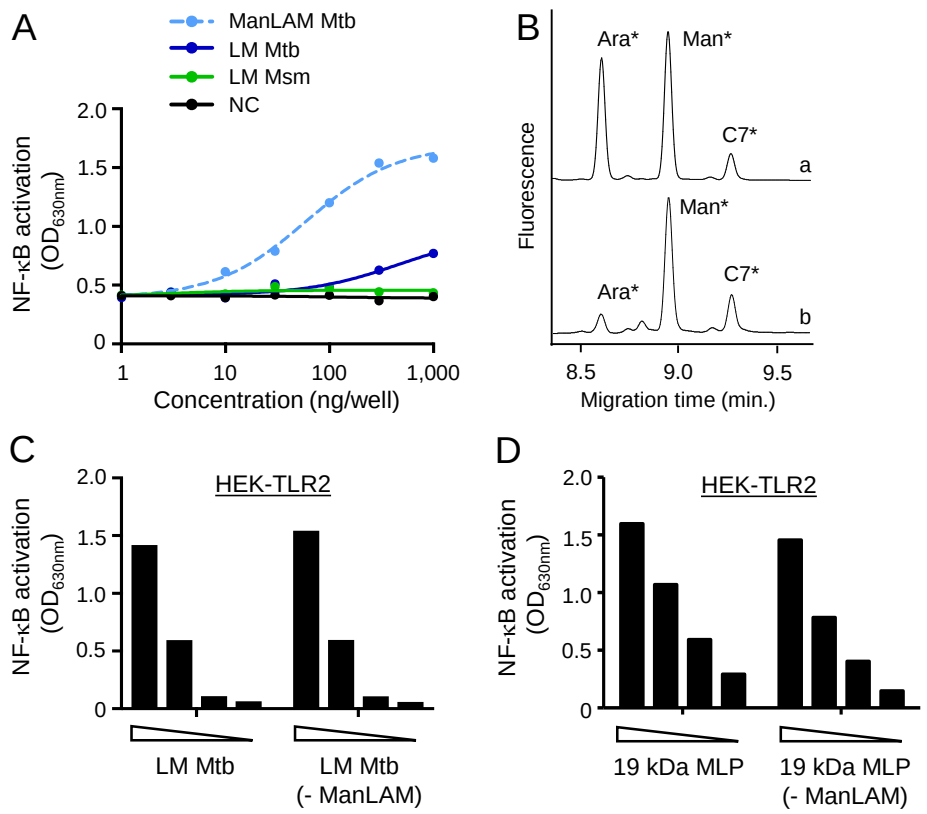
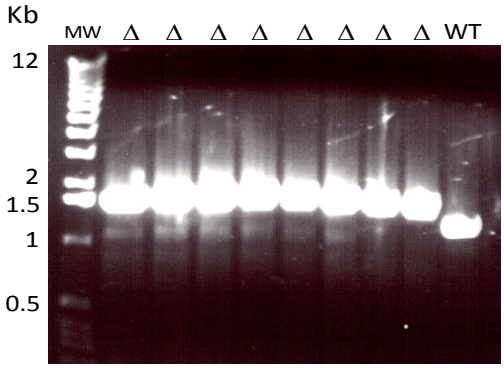
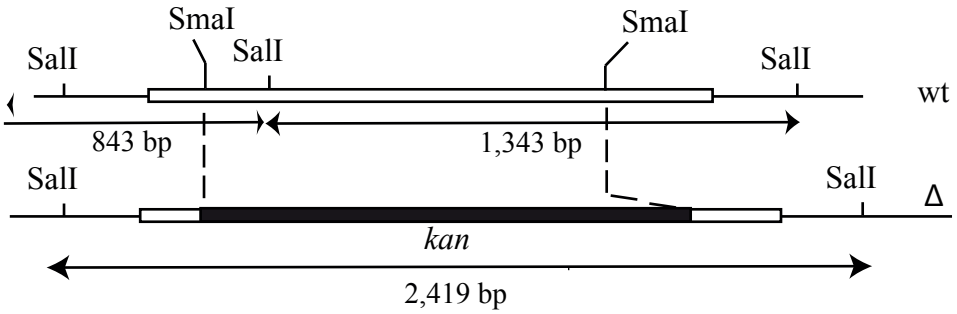

A
ManLAM Mtb
LM Mtb
LM Msm
NC
NF-κB activation (OD630nm)
2.0
1.5
1.0
0.5
0
1
10
100
1,000
Concentration (ng/well)
B
Ara*
Man*
C7*
a
Man*
Ara*
C7*
b
Fluorescence
8.5
9.0
9.5
Migration time (min.)
C
HEK-TLR2
NF-κB activation (OD630nm)
LM Mtb
LM Mtb (- ManLAM)
D
HEK-TLR2
NF-κB activation (OD630nm)
19 kDa MLP
19 kDa MLP (- ManLAM)

Fig S2

A


Kb
MW
Δ
Δ
Δ
Δ
Δ
Δ
Δ
Δ
WT
12
2
1.5
1
0.5


SmaI
SalI
SalI
SmaI
SalI
wt
843 bp
1,343 bp
SalI
SalI
Δ
kan
2,419 bp

B

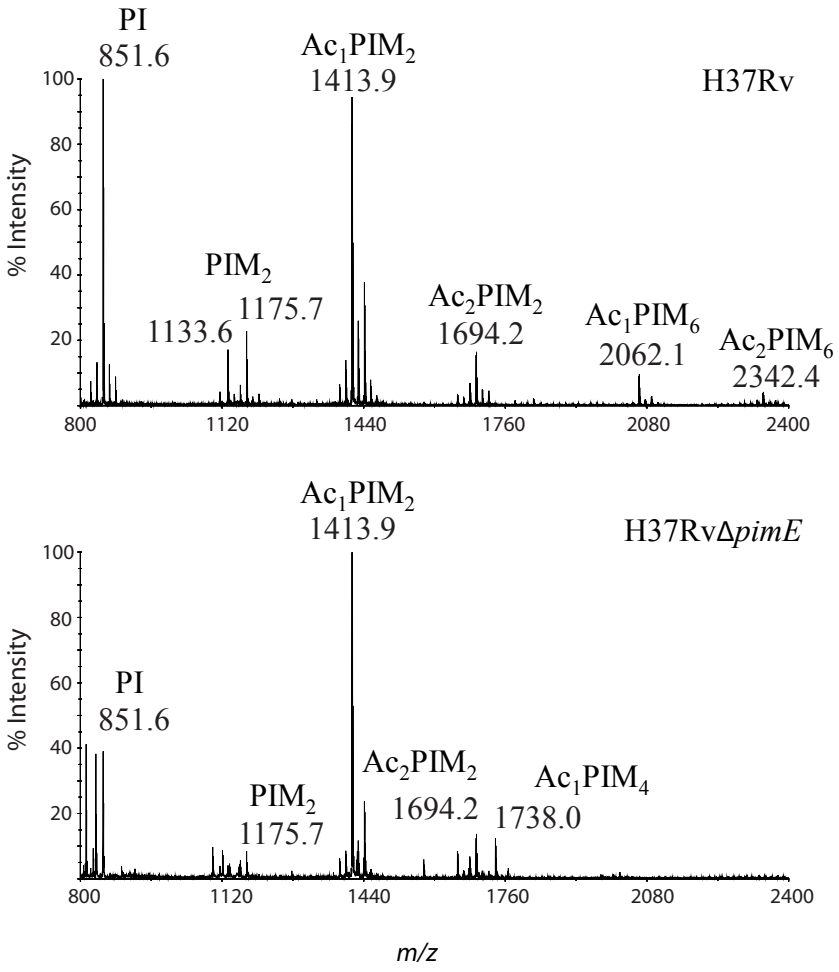

PI
851.6
Ac1PIM2
1413.9
H37Rv
% Intensity
100
80
60
40
20
PIM2
1133.6
1175.7
Ac2PIM2
1694.2
Ac1PIM6
2062.1
Ac2PIM6
2342.4
800
1120
1440
1760
2080
2400
Ac1PIM2
1413.9
H37RvΔpimE
PI
851.6
Ac2PIM2
1694.2
Ac1PIM4
1738.0
PIM2
1175.7
m/z

Fig. S3