Supplementary Information

The biosynthetic pathway for a thousand-year-old natural food colorant and citrinin in *Penicillium marneffei*

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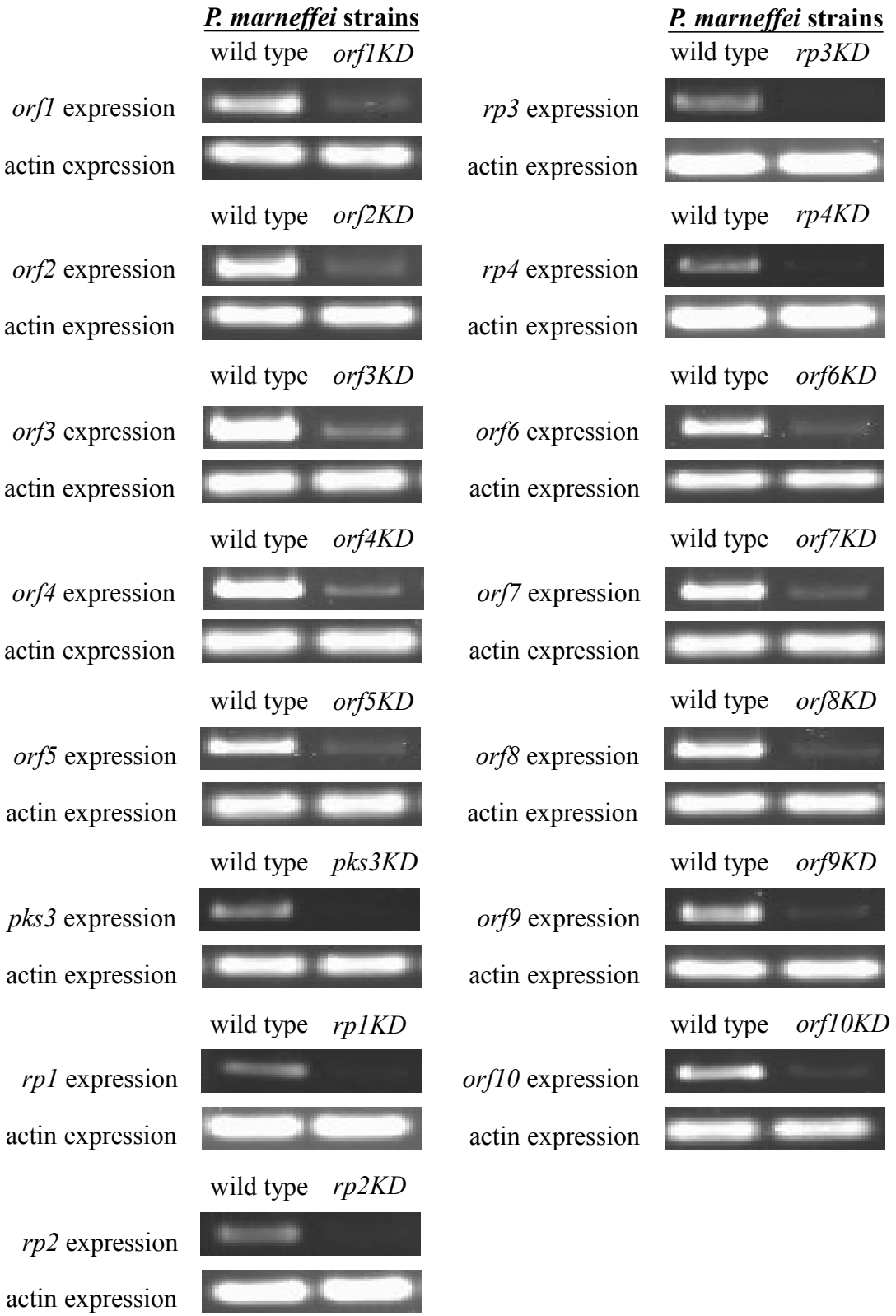
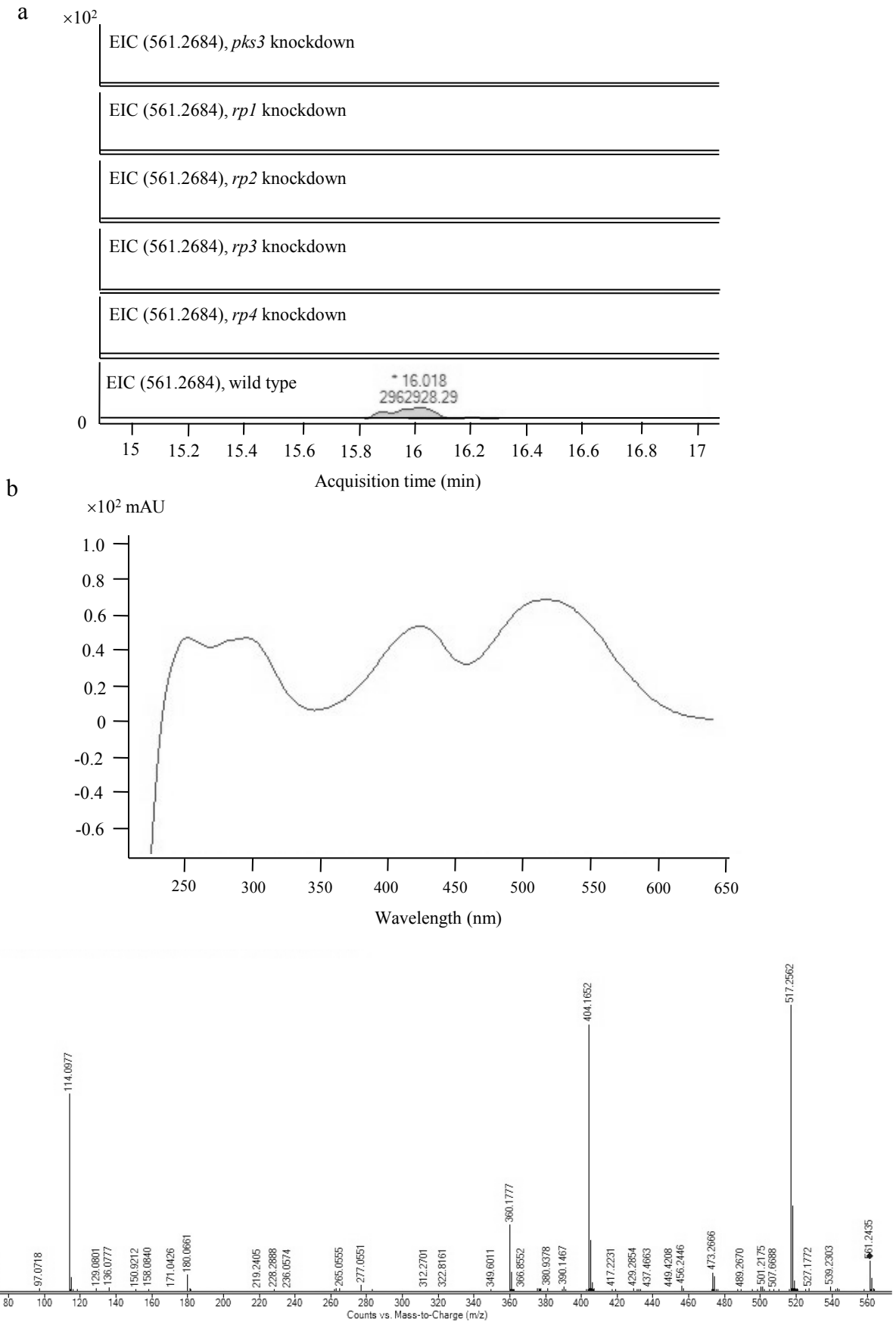
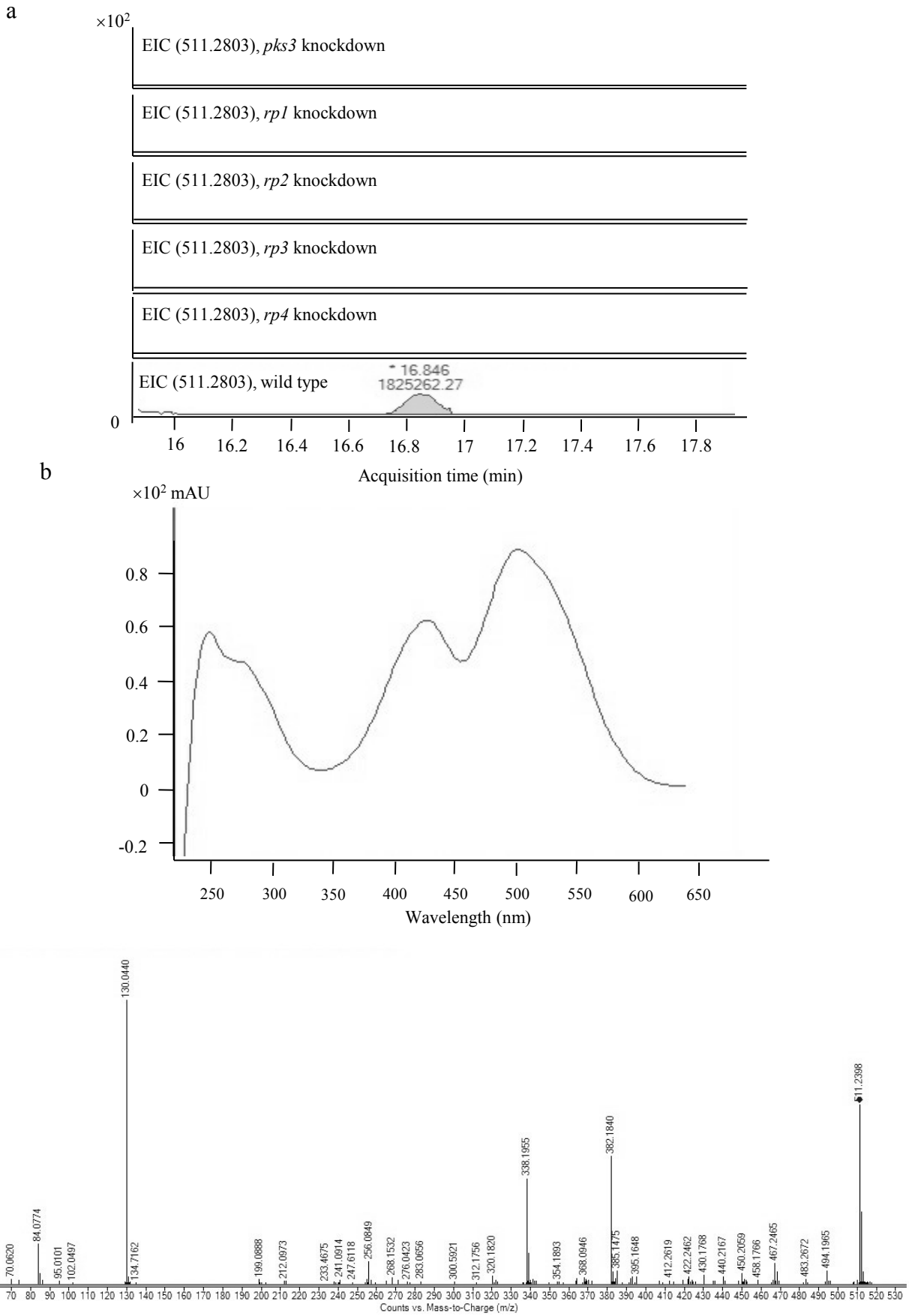


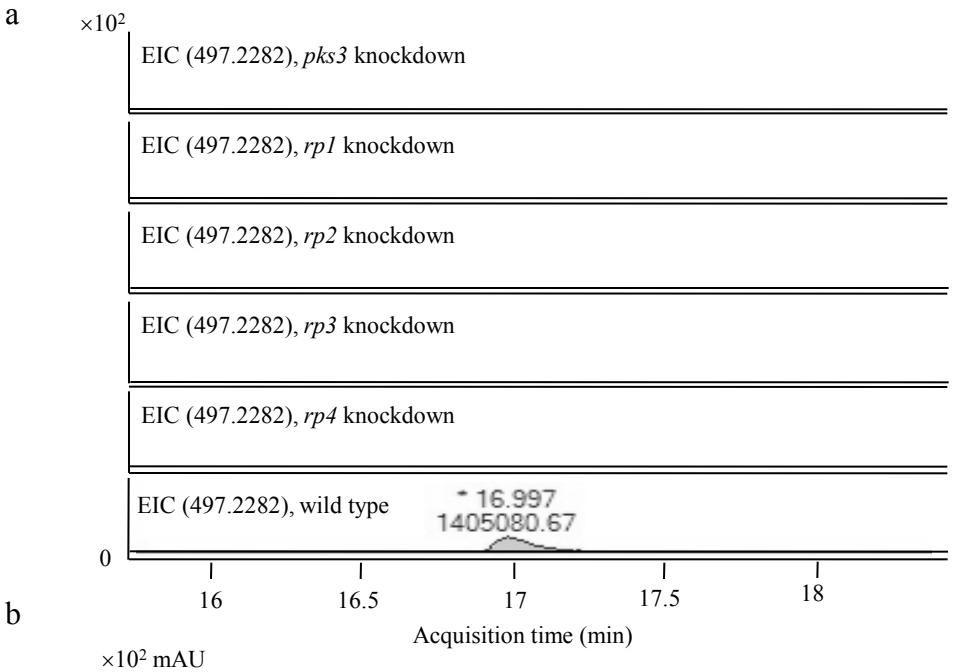
Supplementary Figure S1: Expression of *pks3* and the 14 genes upstream and downstream of *pks3* in the corresponding knockdown mutants of *Penicillium marneffei*.

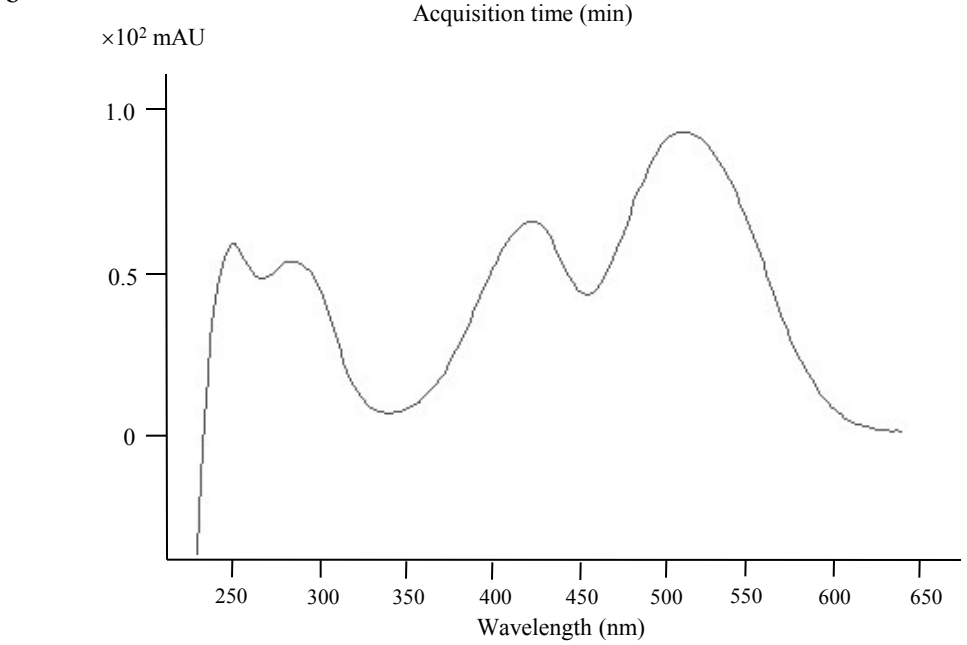


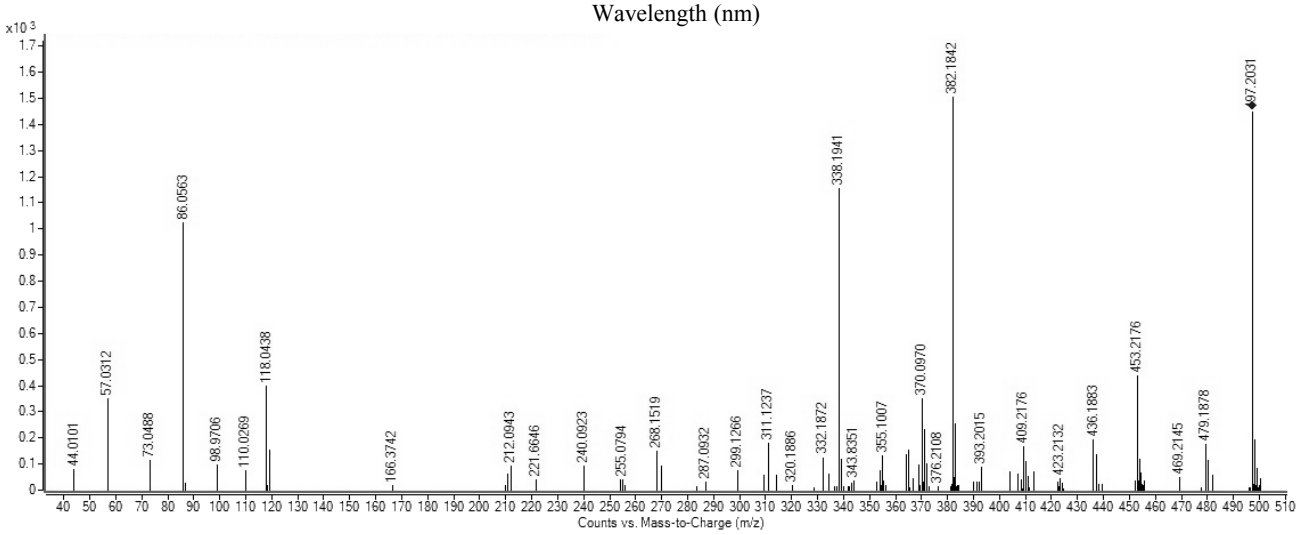
Supplementary Figure S2: Detection of arginine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of arginine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



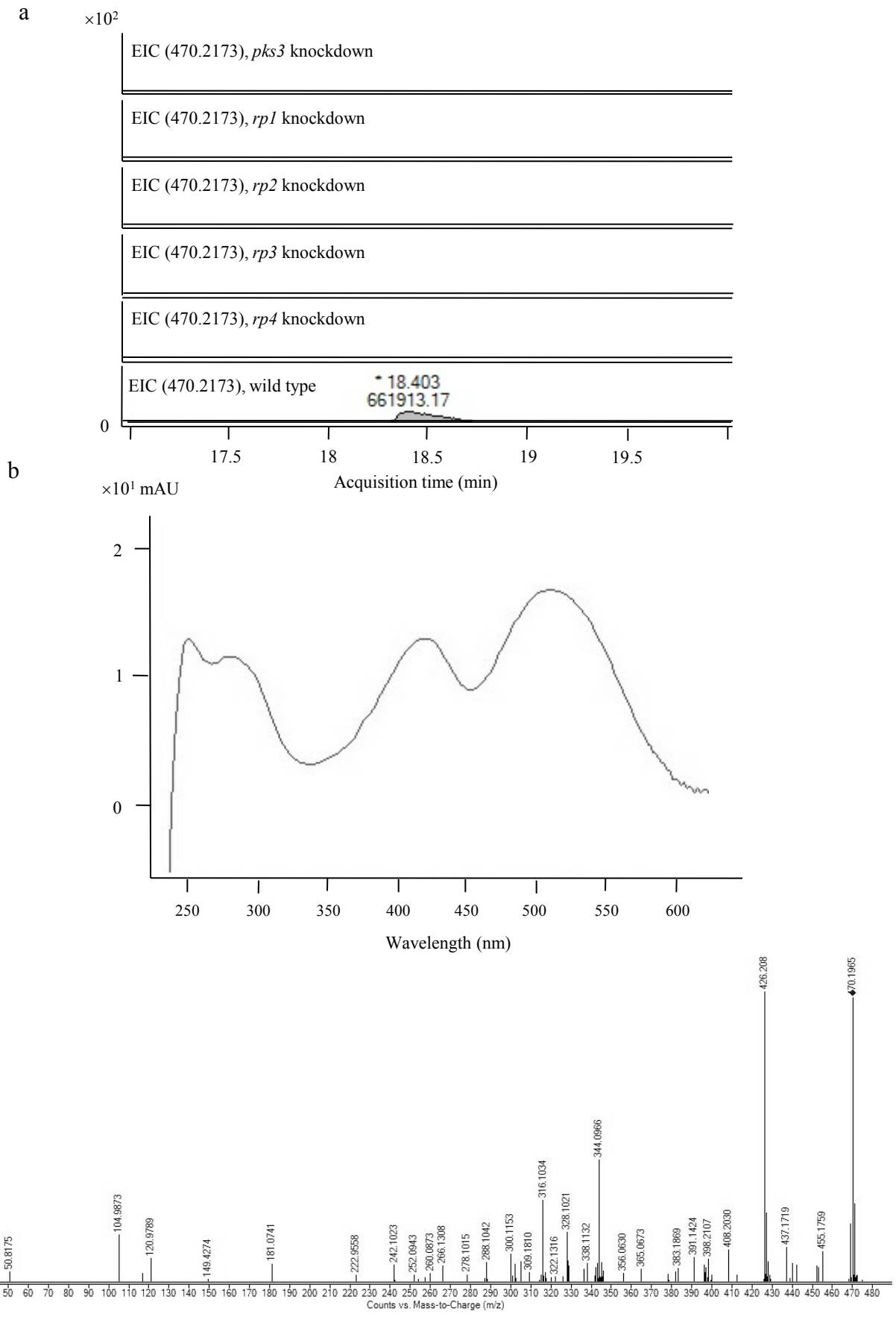
Supplementary Figure S3: Detection of lysine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of lysine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



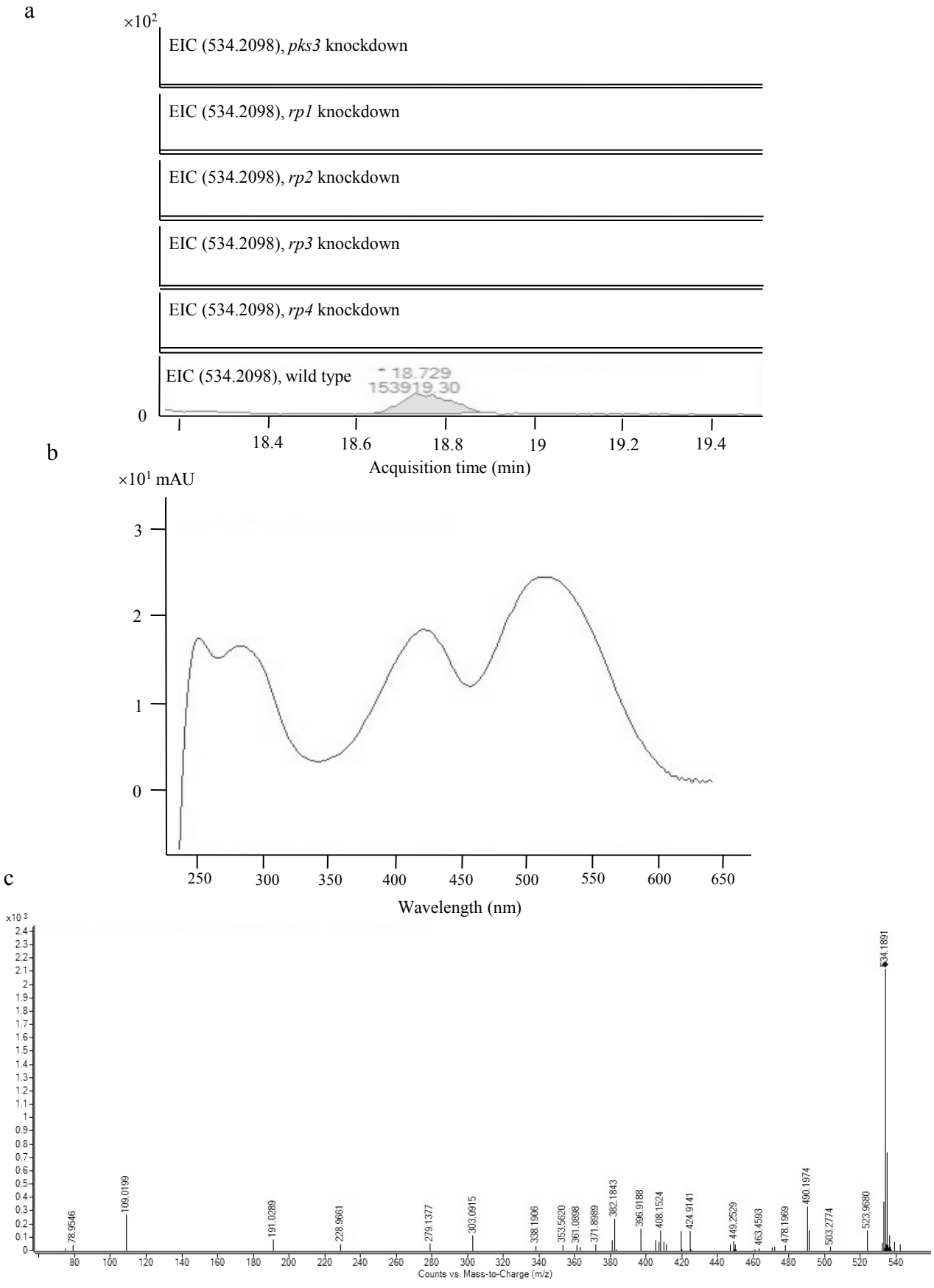




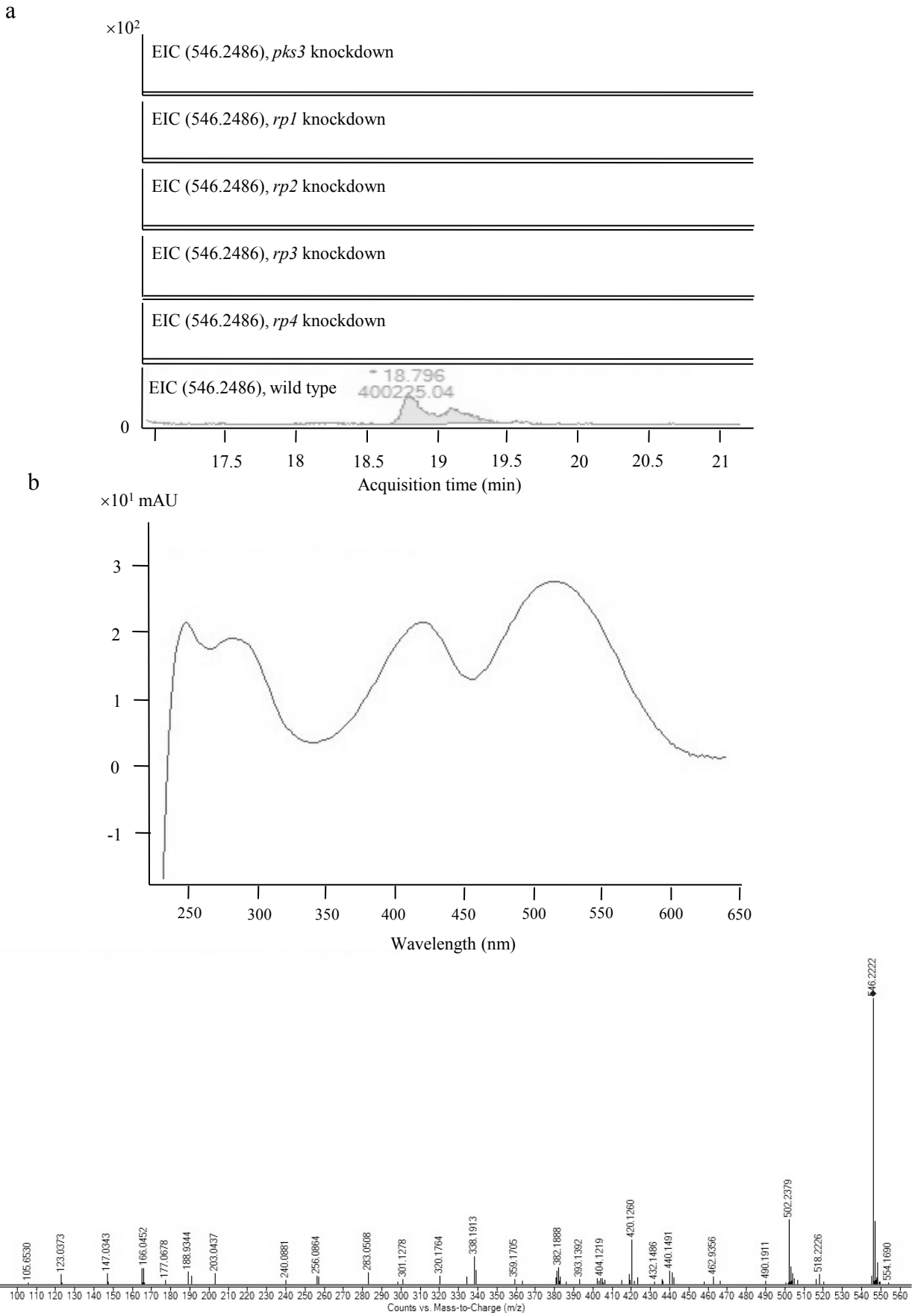
Supplementary Figure S4: Detection of asparagine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of asparagine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



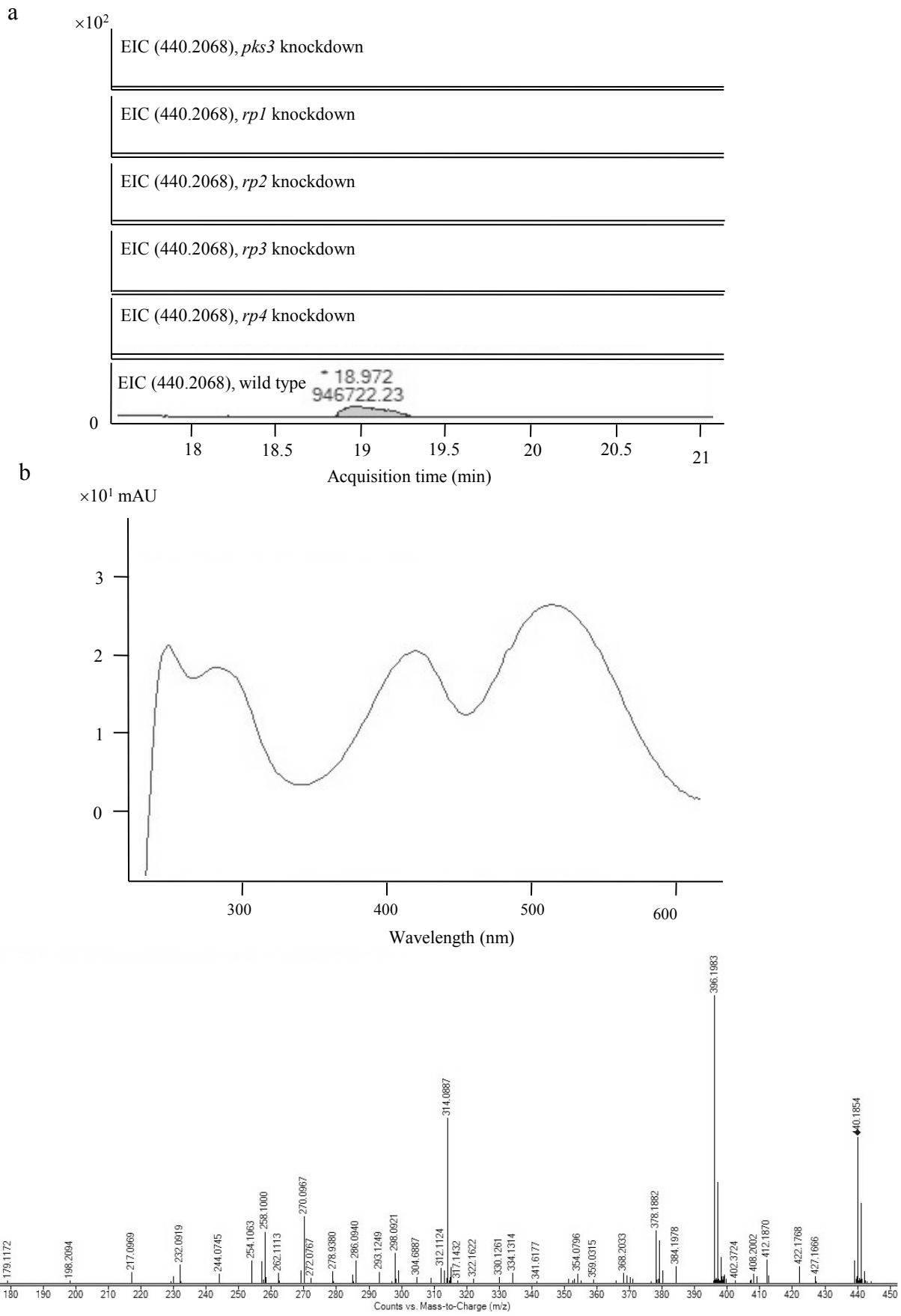
Supplementary Figure S5: Detection of serine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of serine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



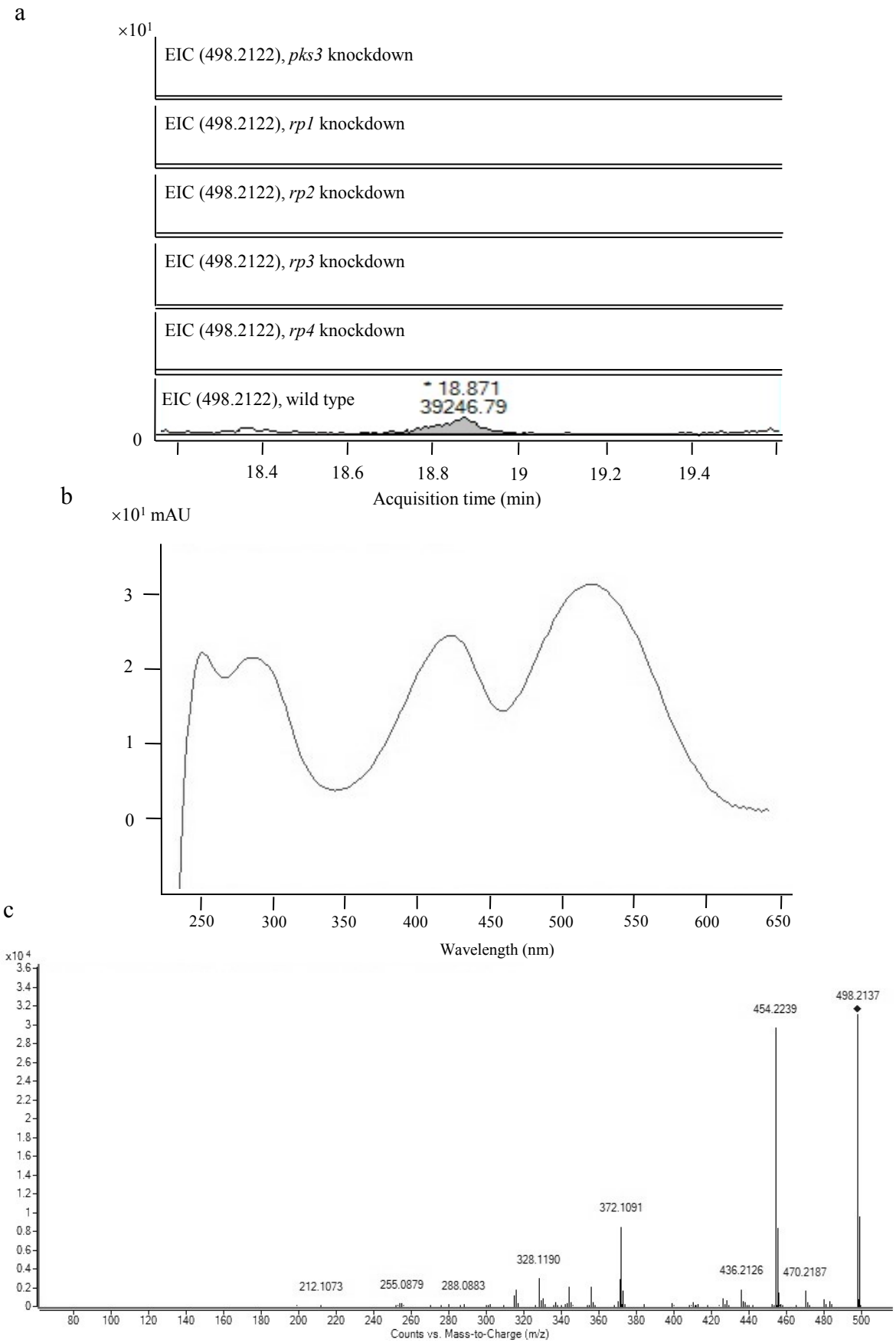
Supplementary Figure S6: Detection of glutamic acid conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of glutamic-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



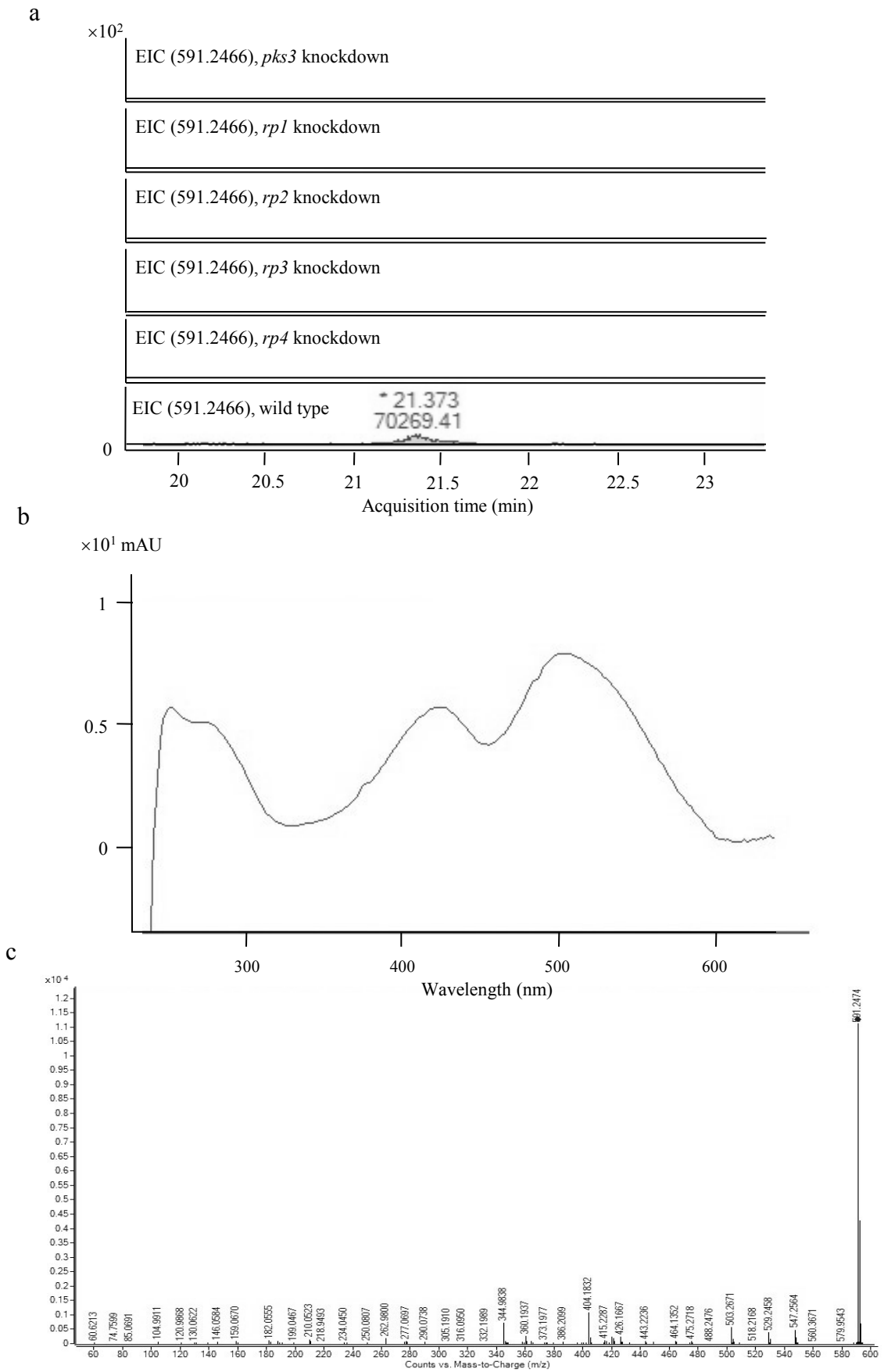
Supplementary Figure S7: Detection of tyrosine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of tyrosine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



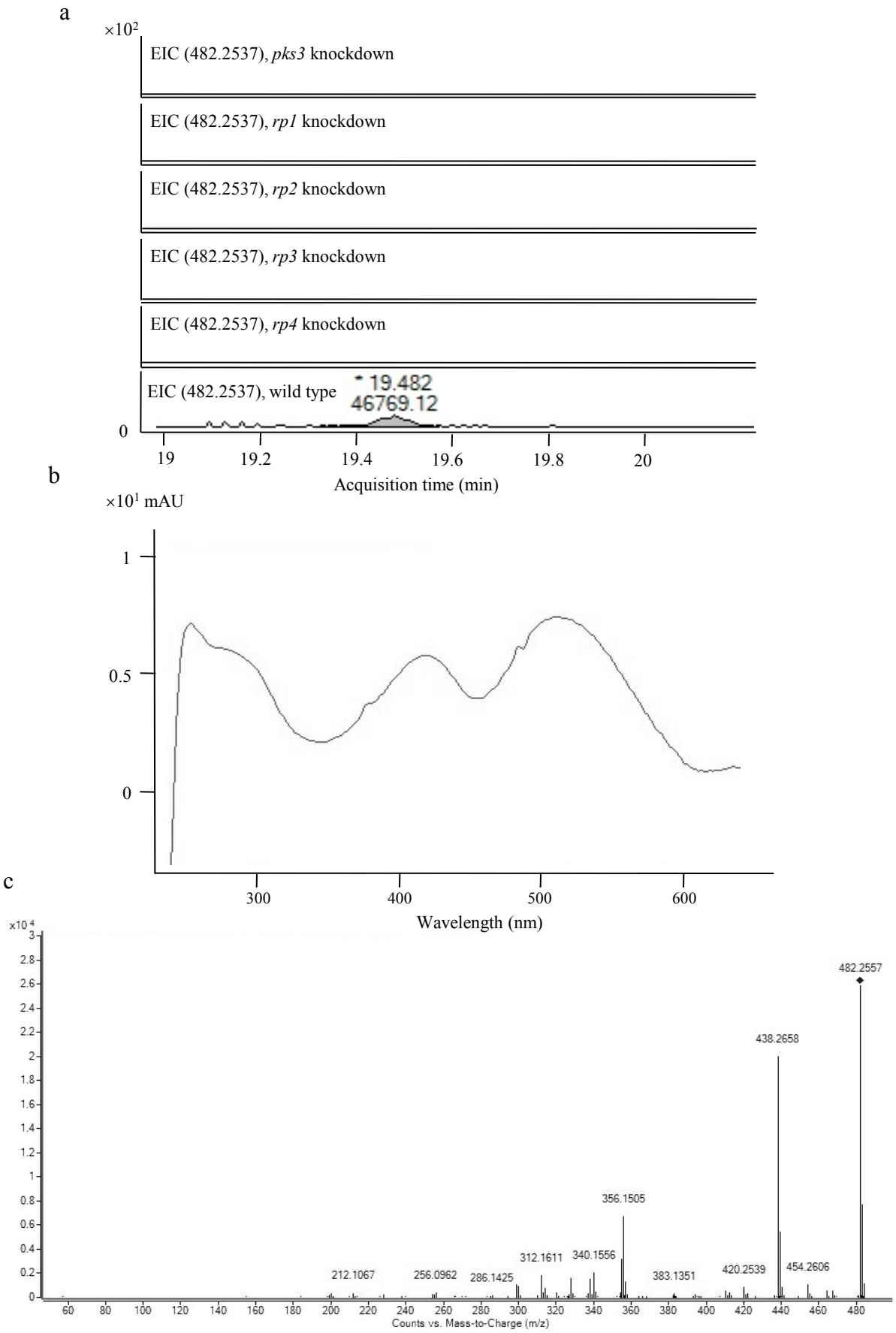
Supplementary Figure S8: Detection of glycine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of glycine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



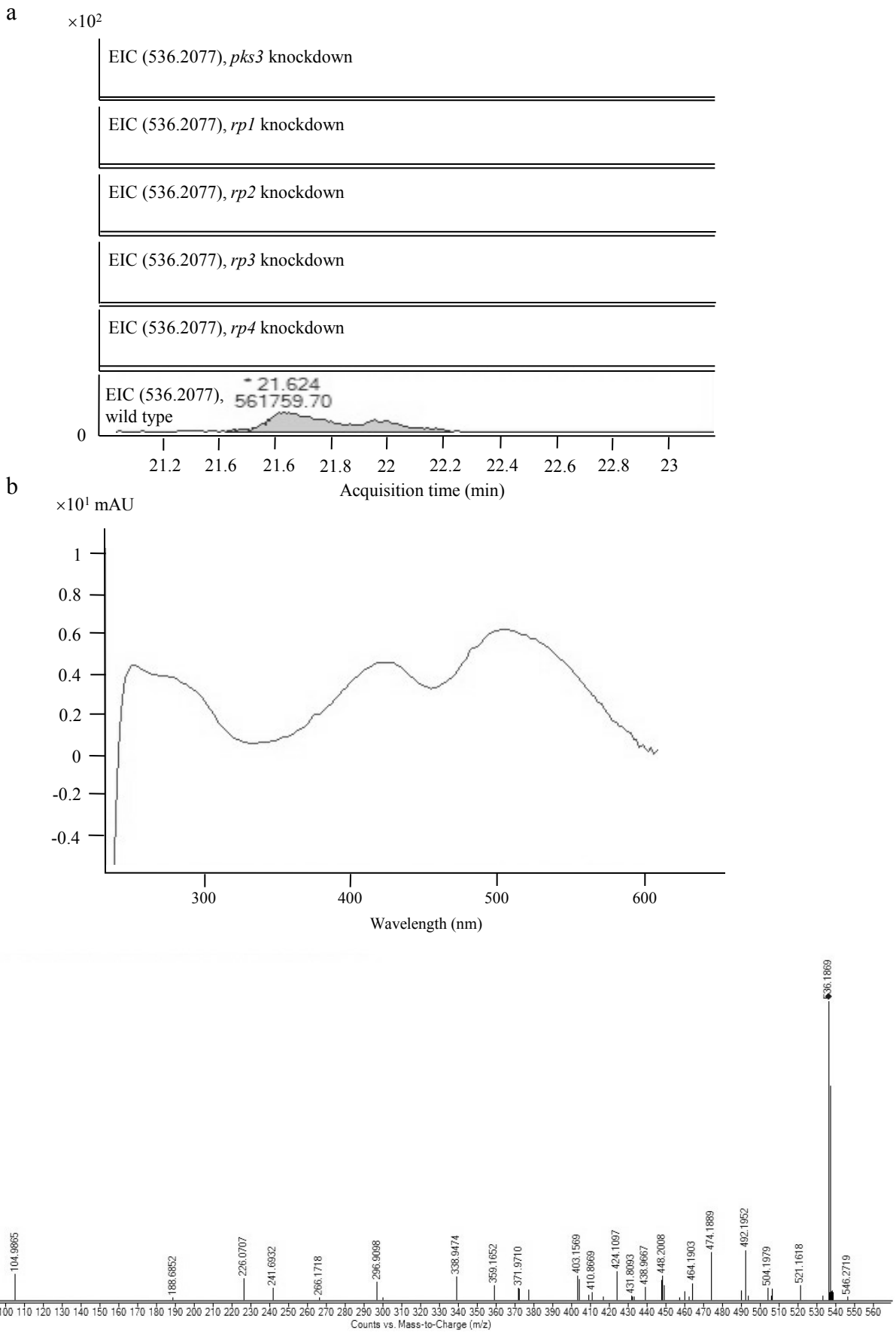
Supplementary Figure S9: Detection of aspartic acid conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of aspartic acid-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



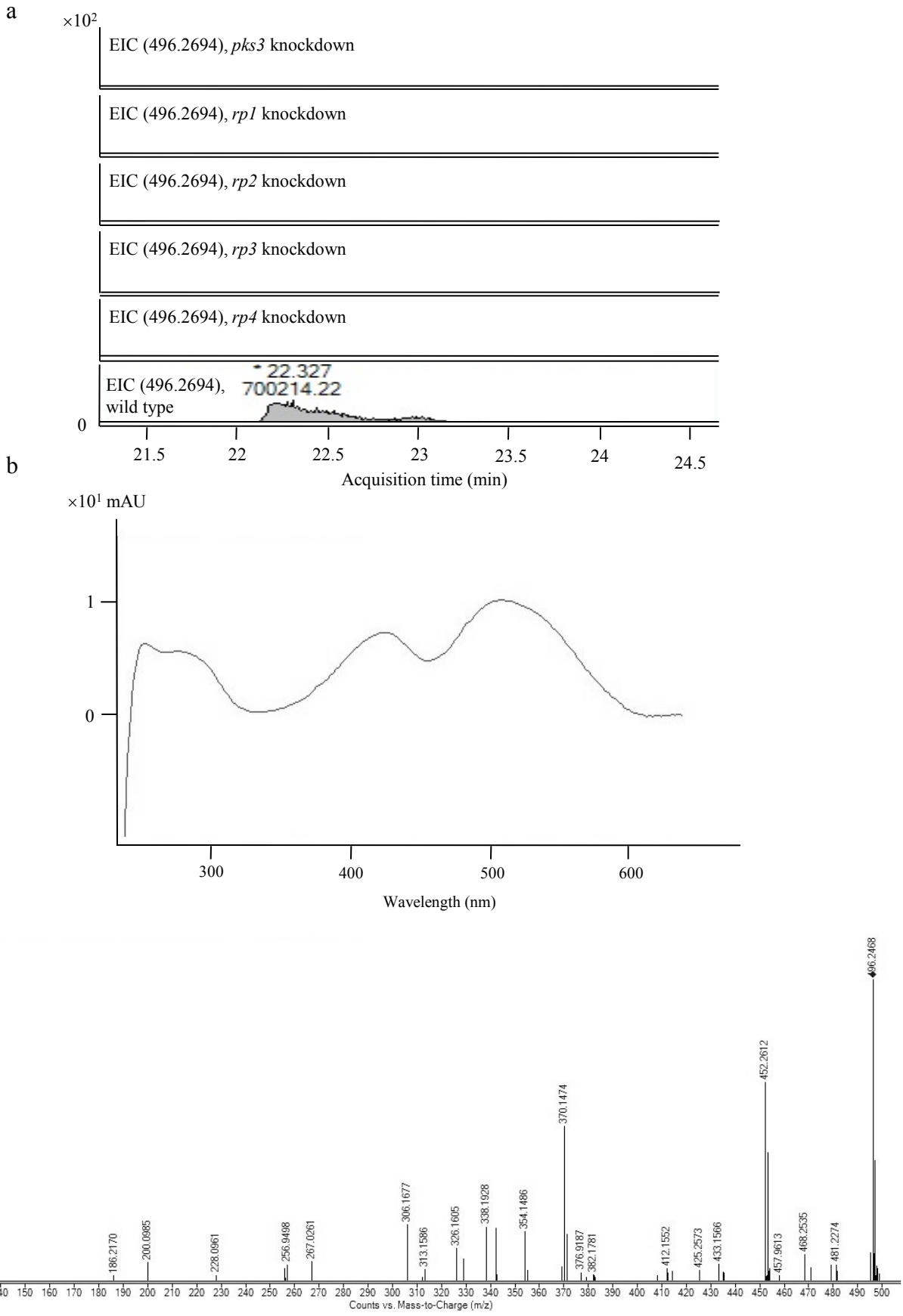
Supplementary Figure S10: Detection of tryptophan conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of tryptophan-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



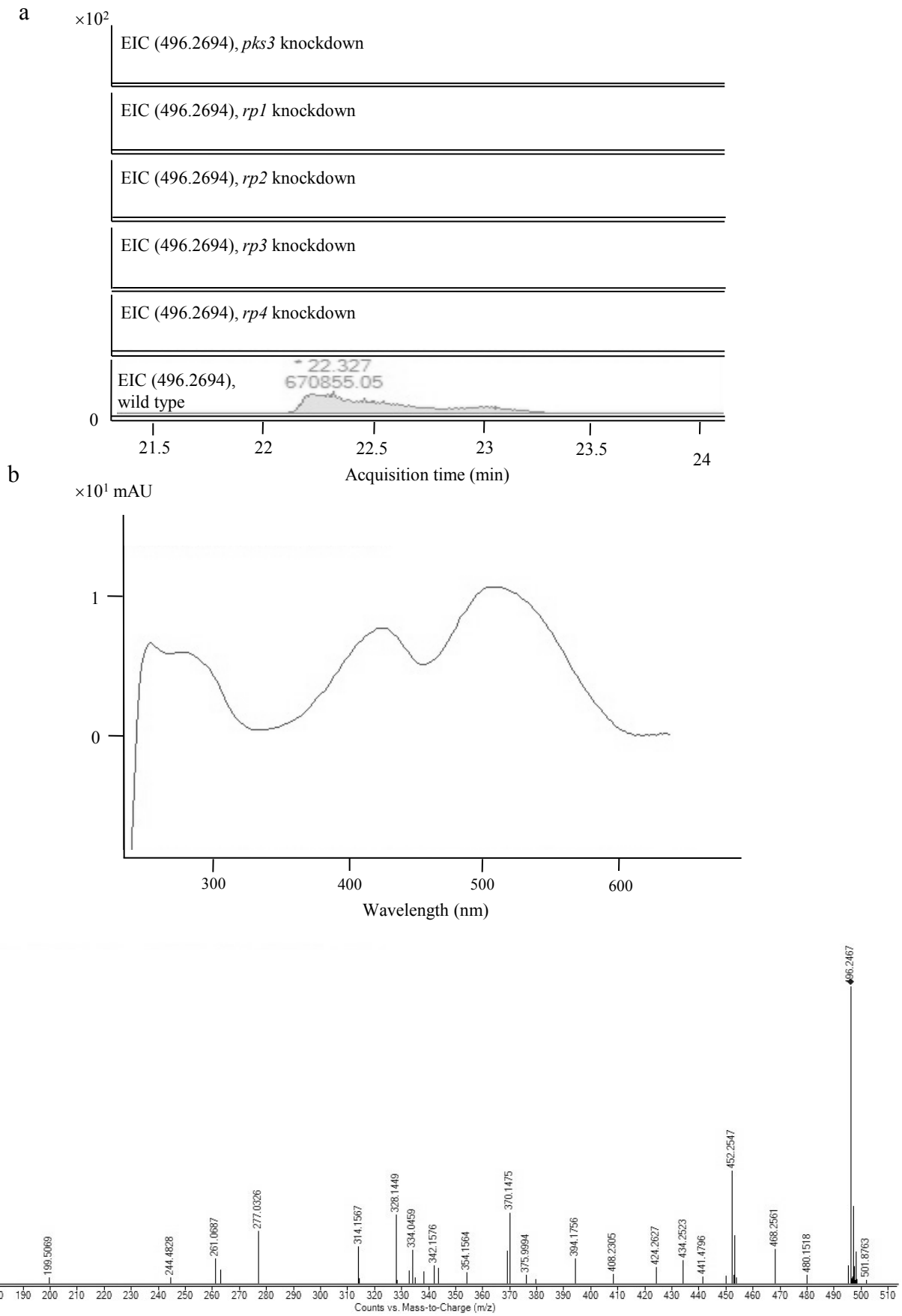
Supplementary Figure S11: Detection of valine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of valine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



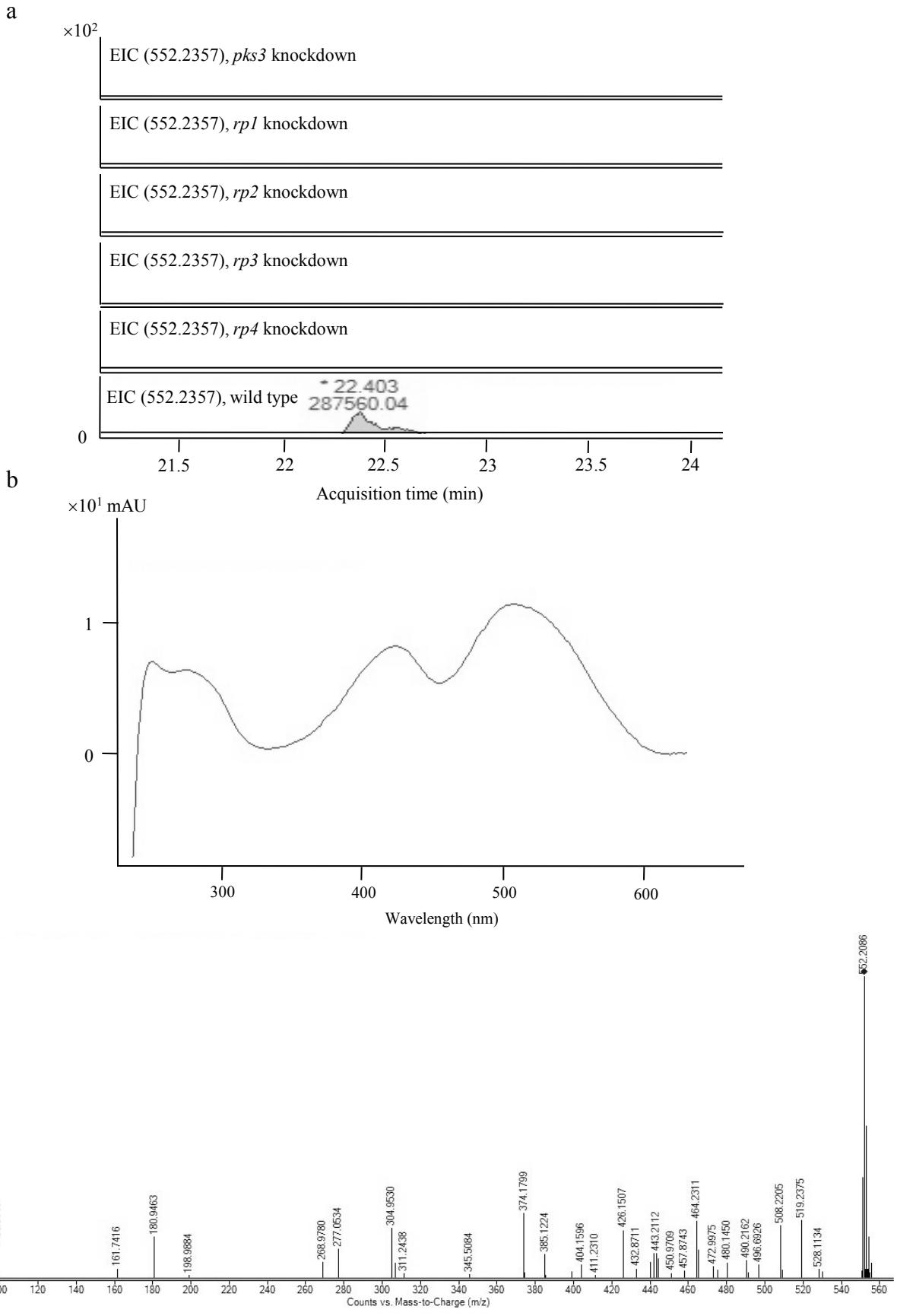
Supplementary Figure S12: Detection of methionine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of methionine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



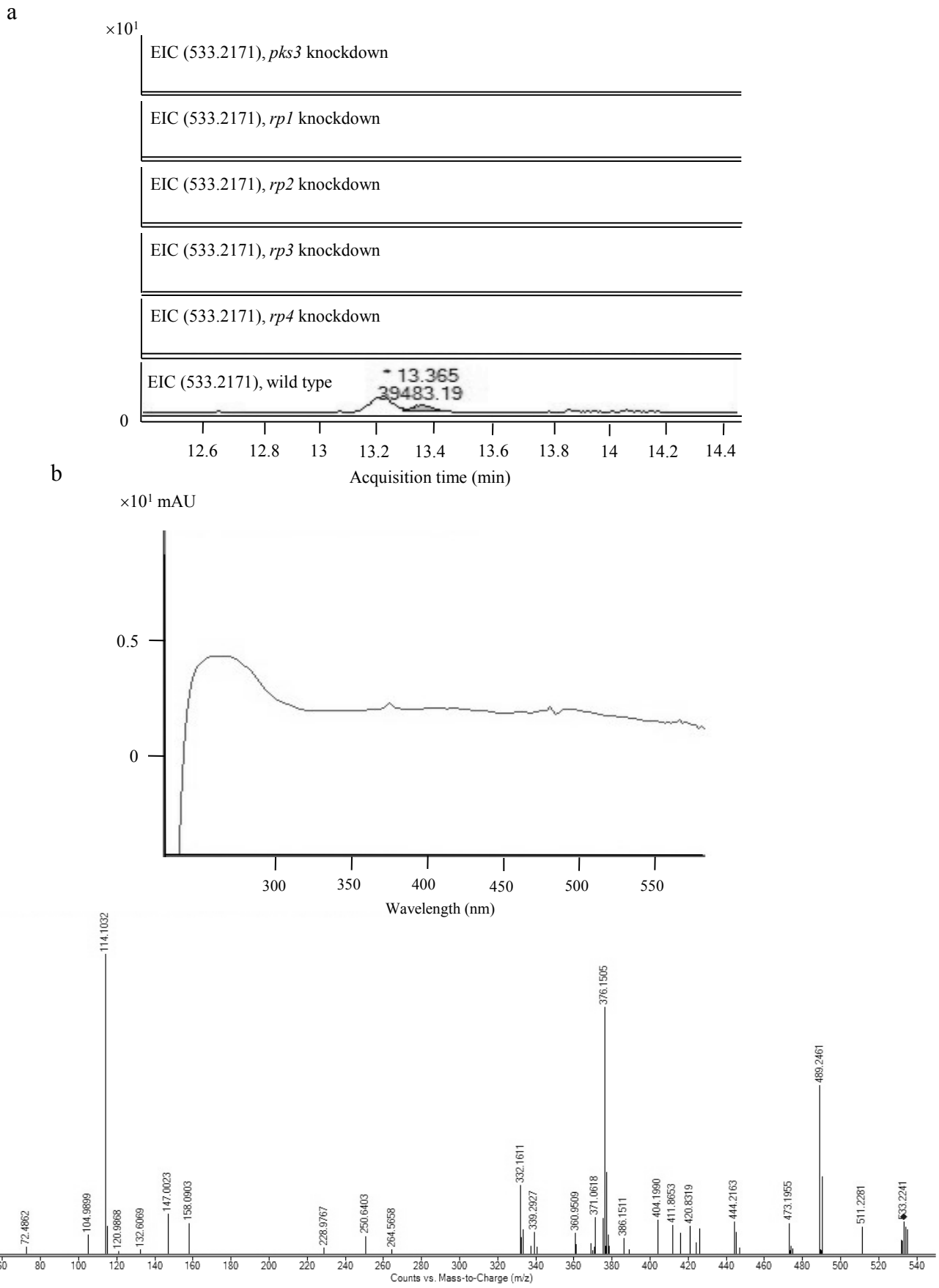
Supplementary Figure S13: Detection of leucine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of leucine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



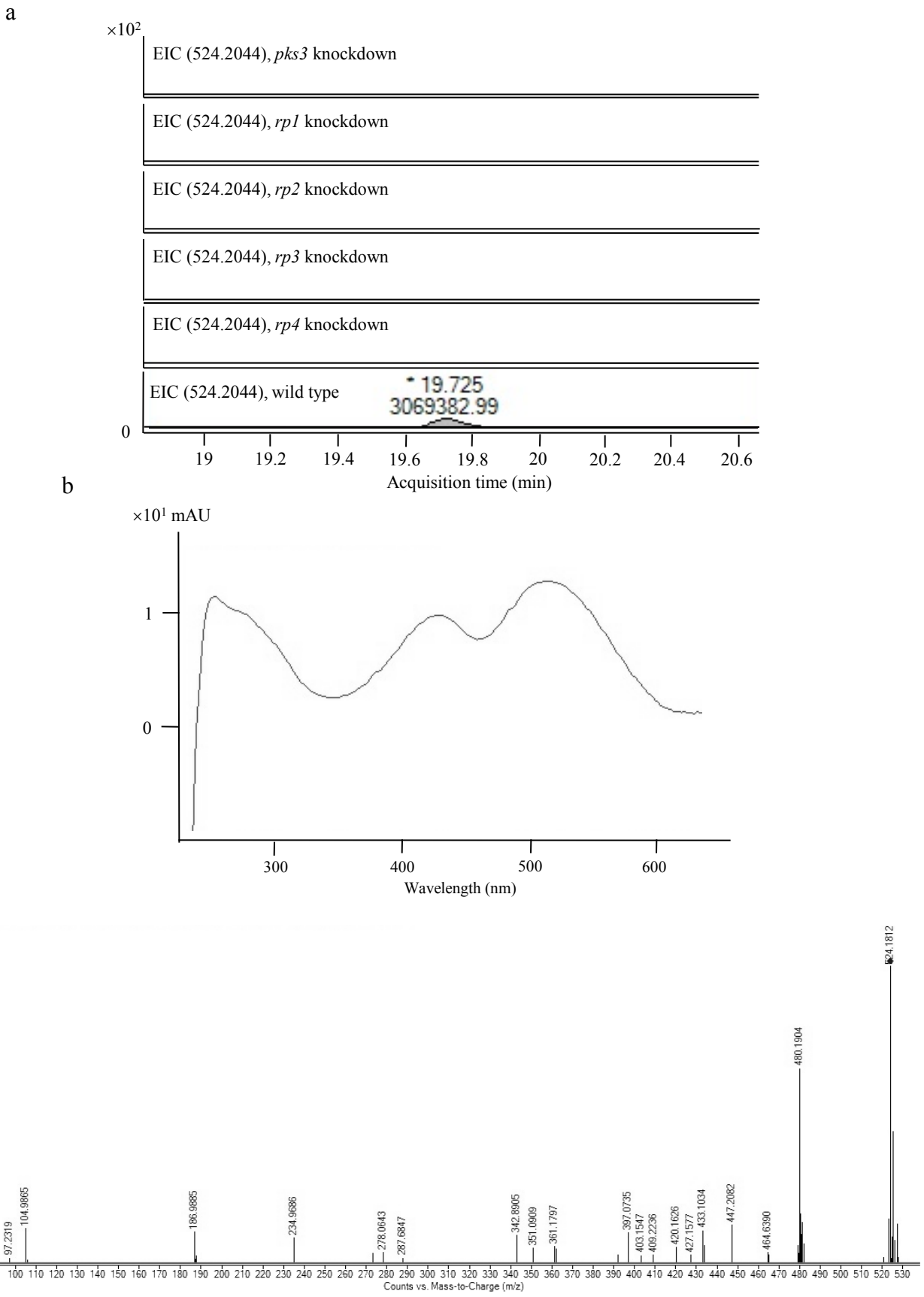
Supplementary Figure S14: Detection of isoleucine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of isoleucine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



Supplementary Figure S15: Detection of phenylalanine conjugated with monascorubrin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of phenylalanine-monascorubrin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



Supplementary Figure S16: Detection of arginine conjugated with rubropunctatin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of arginine-rubropunctatin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.



Supplementary Figure S17: Detection of phenylalanine conjugated with rubropunctatin in *P. marneffei*. (a) Extracted ion chromatogram, (b) UV absorption spectrum and (c) MS/MS fragmentation pattern showing the presence of phenylalanine-rubropunctatin conjugate detected in wild type *P. marneffei* but not in *pks3* or *rp1* to *rp4* knockdown mutants.

Supplementary Table S1: Relative expression of all genes involved in biosynthesis of red pigment in different knockdown mutants of *Penicillium marneffei.*

P. marneffei strains	Relative expression				
	pks3	*rp1*	*rp2*	*rp3*	*rp4*
Wild type	1	1	1	1	1
pks3 KD mutant	<0.01	0.13	0.08	0.05	0.05
rp1 KD mutant	0.05	0.15	0.12	0.01	0.10
rp2 KD mutant	0.54	0.91	0.23	0.90	0.52
rp3 KD mutant	0.98	1.06	0.30	0.33	0.73
rp4 KD mutant	0.44	0.62	0.22	2.10	0.29

Supplementary Table S2: UV-Vis spectroscopic absorption maxima of culture filtrates in *Penicillium marneffei*.

Sample	Absorption maxima			
wild type	373	405	499	553
orf1 knockdown	373	411	501	553
orf2 knockdown	373	416	499	556
orf3 knockdown	373	416	500	558
orf4 knockdown	373	404	498	556
orf5 knockdown	372	414	503	558
pks3 knockdown	---	---	---	560
rp1 knockdown	---	---	---	559
rp2 knockdown	372	404	---	561
rp3 knockdown	372	404	---	561
rp4 knockdown	372	411	---	559
orf6 knockdown	373	417	500	557
orf7 knockdown	372	417	496	553
orf8 knockdown	374	416	499	554
orf9 knockdown	376	417	499	556
orf10 knockdown	373	409	494	557
medium	---	---	---	560

Supplementary Table S3: Molecular formula, mass-to-charge ratio and retention time of different amino acids conjugated with monascorubrin or rubropunctatin in culture filtrate of *Penicillium marneffei* detected by UHPLC-MS analysis.

Name	Molecular Formula	Mass-to-charge ratio	Retention time (min)
Arginine-monascorubrin	$C_{29}H_{38}N_4O_6$	538.2791	16.037
Arginine-rubropunctatin	$C_{27}H_{34}N_4O_6$	510.2478	13.407
Asparagine-monascorubrin	$C_{27}H_{32}N_2O_7$	496.2282	18.901
Aspartic acid-monascorubrin	$C_{27}H_{31}NO_8$	497.2050	19.584
Glutamic acid-monascorubrin	$C_{28}H_{33}NO_8$	511.2206	19.205
Glycine-monascorubrin	$C_{25}H_{29}NO_6$	439.1995	19.533
Isoleucine-monascorubrin	$C_{29}H_{37}NO_6$	495.2621	23.197
Leucine-monascorubrin	$C_{29}H_{37}NO_6$	495.2621	22.941
Lysine-monascorubrin	$C_{29}H_{38}N_2O_6$	510.2730	16.913
Methionine-monascorubrin	$C_{28}H_{35}NSO_6$	513.2185	22.279
Phenylalaine-monascorubrin	$C_{32}H_{35}NO_6$	529.2464	23.209
Phenylalaine rubropunctatin	$C_{30}H_{31}NO_6$	502.2224	19.730
Serine-monascorubrin	$C_{26}H_{31}NO_7$	469.2101	18.901
Tryptophan-monascorubrin	$C_{34}H_{36}N_2O_6$	568.2573	20.696
Tyrosine-monascorubrin	$C_{32}H_{35}NO_7$	545.2414	19.348
Valine-monascorubrin	$C_{28}H_{35}NO_6$	481.2464	22.157

Supplementary Table S4: Primers and plasmids used for gene knockdown experiments in the present study.

Gene	Primer	RE site	F/R	Sequence (5'-3')	Plasmid
orf1	LPW17686	*Xho*I	F	CCGCTCGAGTATGTCCGGGCACAGACAAG	pPW2284
	LPW17687	*Hind*III	R	CCCAAGCTTGGGAGTGGGACGCAGTTGACACC	
	LPW17688	*Kpn*I	F	GGGGTACCTATGTCCGGGCACAGACAAG	
	LPW17689	*Bgl*II	R	GAAGATCTAGTGGGACGCAGTTGACACC	
orf2	LPW17690	*Xho*I	F	CCGCTCGAGGGGAGAACGTGCTTTTGCTC	pPW2285
	LPW17691	*Hind*III	R	CCCAAGCTTGGGGAAGGAGCCGGTGACTTCAA	
	LPW17692	*Kpn*I	F	GGGGTACCGGGAGAACGTGCTTTTGCTC	
	LPW17693	*Bgl*II	R	GAAGATCTGAAGGAGCCGGTGACTTCAA	
orf3	LPW17694	*Xho*I	F	CCGCTCGAGCGACACATACGATCATCCAGCA	pPW2286
	LPW17695	*Hind*III	R	CCCAAGCTTGGGTGTCGCTACCAGTTGGCTCA	
	LPW17696	*Kpn*I	F	GGGGTACCCGACACATACGATCATCCAGCA	
	LPW17697	*Bgl*II	R	GAAGATCTTGTCGCTACCAGTTGGCTCA	
orf4	LPW17698	*Xho*I	F	CCGCTCGAGCGATACTCCGTGTGAAAGACTGC	pPW2287
	LPW17699	*Hind*III	R	CCCAAGCTTGGGGTGGCTGTGGTCAAGCAGTG	
	LPW17700	*Kpn*I	F	GGGGTACCCGATACTCCGTGTGAAAGACTGC	
	LPW17701	*Bgl*II	R	GAAGATCTGTGGCTGTGGTCAAGCAGTG	
orf5	LPW17702	*Xho*I	F	CCGCTCGAGCGTTTTCGTTACCGGAGGTT	pPW2288
	LPW17703	*Hind*III	R	CCCAAGCTTGGGAGCATGGGTAGGAATGACCG	
	LPW17704	*Kpn*I	F	GGGGTACCCGTTTTCGTTACCGGAGGTT	
	LPW17705	*Bgl*II	R	GAAGATCTAGCATGGGTAGGAATGACCG	
pks3	LPW9873	*Xho*I	F	CCGCTCGAGCCTTCTCTTTCGGATCTCTTC	pPW1294
	LPW9874	*Kpn*I	F	GGGGTACCCCTTCTCTTTCGGATCTCTTC	
	LPW9875	*Bgl*II	R	GAAGATCTGCCTAATGTCAAGCTTTTCG	
rp1	LPW 18206	*Xho*I	F	CCGCTCGAGCTGCTGGCGATACCGAGTTC	pPW2422
	LPW 18207	*Hind*III	R	CCCAAGCTTGGGGCAAGGCATCAGCTCAATGA	
	LPW 18208	*Kpn*I	F	GGGGTACCCTGCTGGCGATACCGAGTTC	
	LPW 18209	*Bgl*II	R	GAAGATCTGCAAGGCATCAGCTCAATGA	
rp2	LPW 17706	*Xho*I	F	CCGCTCGAGCATGGGCTACTCGGTTTGGA	pPW2289
	LPW 17707	*Hind*III	R	CCCAAGCTTGGGGTTCGCCTTTGGAGTTCTGC	
	LPW 17708	*Kpn*I	F	GGGGTACCCATGGGCTACTCGGTTTGGA	
	LPW 17709	*Bgl*II	R	GAAGATCTGTTCGCCTTTGGAGTTCTGC	
rp3	LPW 18210	*Xho*I	F	CCGCTCGAGGCAGTAATCGGTTGGGTTCG	pPW2423
	LPW 18211	*Hind*III	R	CCCAAGCTTGGGCGCATGGAACTGAAGGATGA	
	LPW 18212	*Kpn*I	F	GGGGTACCGCAGTAATCGGTTGGGTTCG	
	LPW 18213	*Bgl*II	R	GAAGATCTCGCATGGAACTGAAGGATGA	
rp4	LPW 18214	*Xho*I	F	CCGCTCGAGAAAGTCAATGACCCTGCCGA	pPW2424
	LPW 18215	*Hind*III	R	CCCAAGCTTGGGGTCAAAGACCTGGCTGGCAC	
	LPW 18216	*Kpn*I	F	GGGGTACCAAAGTCAATGACCCTGCCGA	
	LPW 18217	*Bgl*II	R	GAAGATCTGTCAAAGACCTGGCTGGCAC	

Gene	Primer	RE site	F/R	Sequence (5'-3')	Plasmid
orf6	LPW18683	*Xho*I	F	CCGCTCGAGATAGACCTGAAATGGTTCCGATGG	pPW2425
	LPW18684	*Hind*III	R	CCCAAGCTTGGGGTGATGTAAATCCTCATCGAGCAG	
	LPW18685	*Kpn*I	F	GGGGTACCATAGACCTGAAATGGTTCCGATGG	
	LPW18686	*Bgl*II	F	GAAGATCTGTGATGTAAATCCTCATCGAGCAG	
orf7	LPW18426	*Xho*I	R	CCGCTCGAGAGGCTCCTTTCTCCTCCGAT	pPW2426
	LPW18427	*Hind*III	F	CCCAAGCTTGGGTGAACGTGGATTGTGGGAAG	
	LPW18428	*Kpn*I	R	GGGGTACCAGGCTCCTTTCTCCTCCGAT	
	LPW18429	*Bgl*II	F	GAAGATCTTGAACGTGGATTGTGGGAAG	
orf8	LPW18430	*Xho*I	R	CCGCTCGAGCGCTGTGACCGAAATGGAT	pPW2427
	LPW18431	*Hind*III	F	CCCAAGCTTGGGGGAGTCACACCGCAGATAATGA	
	LPW18432	*Kpn*I	R	GGGGTACCCGCTGTGACCGAAATGGAT	
	LPW18433	*Bgl*II	F	GAAGATCTGGAGTCACACCGCAGATAATGA	
orf9	LPW18434	*Xho*I	F	CCGCTCGAGCTGGTTGGAGGTGCCTTGA	pPW2428
	LPW18435	*Hind*III	R	CCCAAGCTTGGGGCCCATACTGTGCGAATCGTA	
	LPW18436	*Kpn*I	F	GGGGTACCCTGGTTGGAGGTGCCTTGA	
	LPW18437	*Bgl*II	R	GAAGATCTGCCCATACTGTGCGAATCGTA	
orf10	LPW18438	*Xho*I	F	CCGCTCGAGGAGGAGGAAATTGACCAGACTGG	pPW2429
	LPW18439	*Hind*III	R	CCCAAGCTTGGGGGTCAGACCTTCCTTGCCGT	
	LPW18440	*Kpn*I	F	GGGGTACCGAGGAGGAAATTGACCAGACTGG	
	LPW18441	*Bgl*II	R	GAAGATCTGGTCAGACCTTCCTTGCCGT	