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Supplementary Materials for

Protein lysine de-2-hydroxyisobutyrylation by CobB in prokaryotes

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Other Supplementary Material for this manuscript includes the following:

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Table S5 (Microsoft Excel format). The PRM result of comparison of the quantities of K326ac and K343hib in T vector and CobB-overexpressing *P. mirabilis*.

Supplementary Materials

Figure Legends

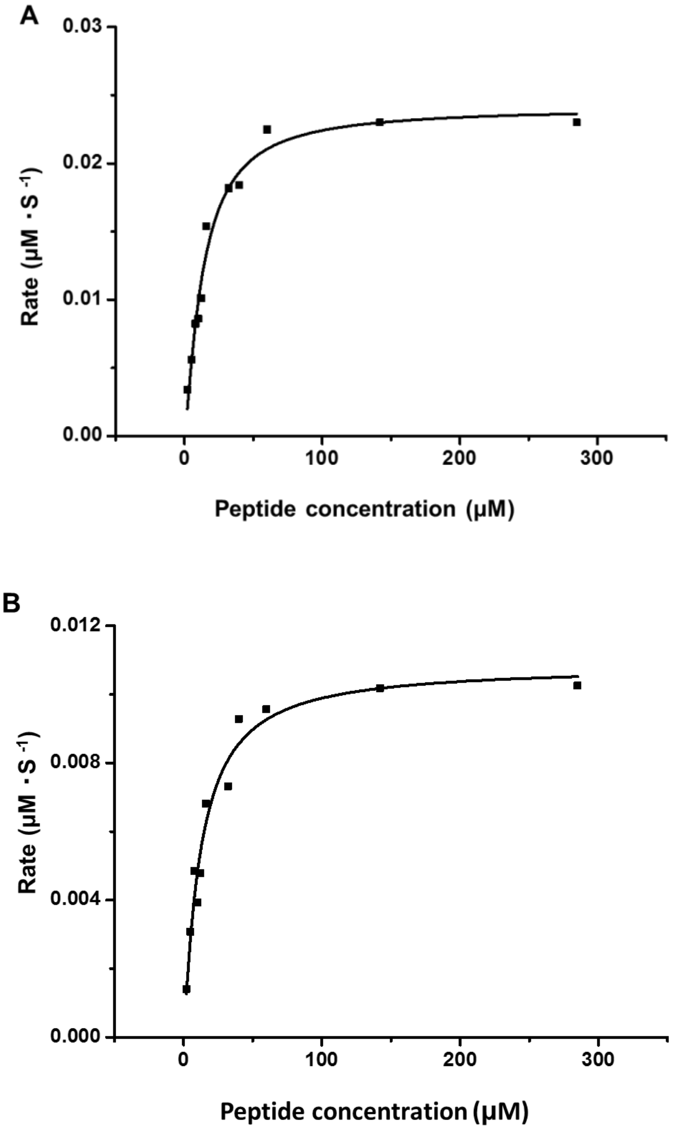


Fig. S1

Fig. S1. Kinetic curves of enzymatic reaction. (**A**) Drawing the kinetic curves of CobB by OriginPro 8. (**B**) Drawing the kinetic curves of mutated CobB (Y55F) by OriginPro 8.

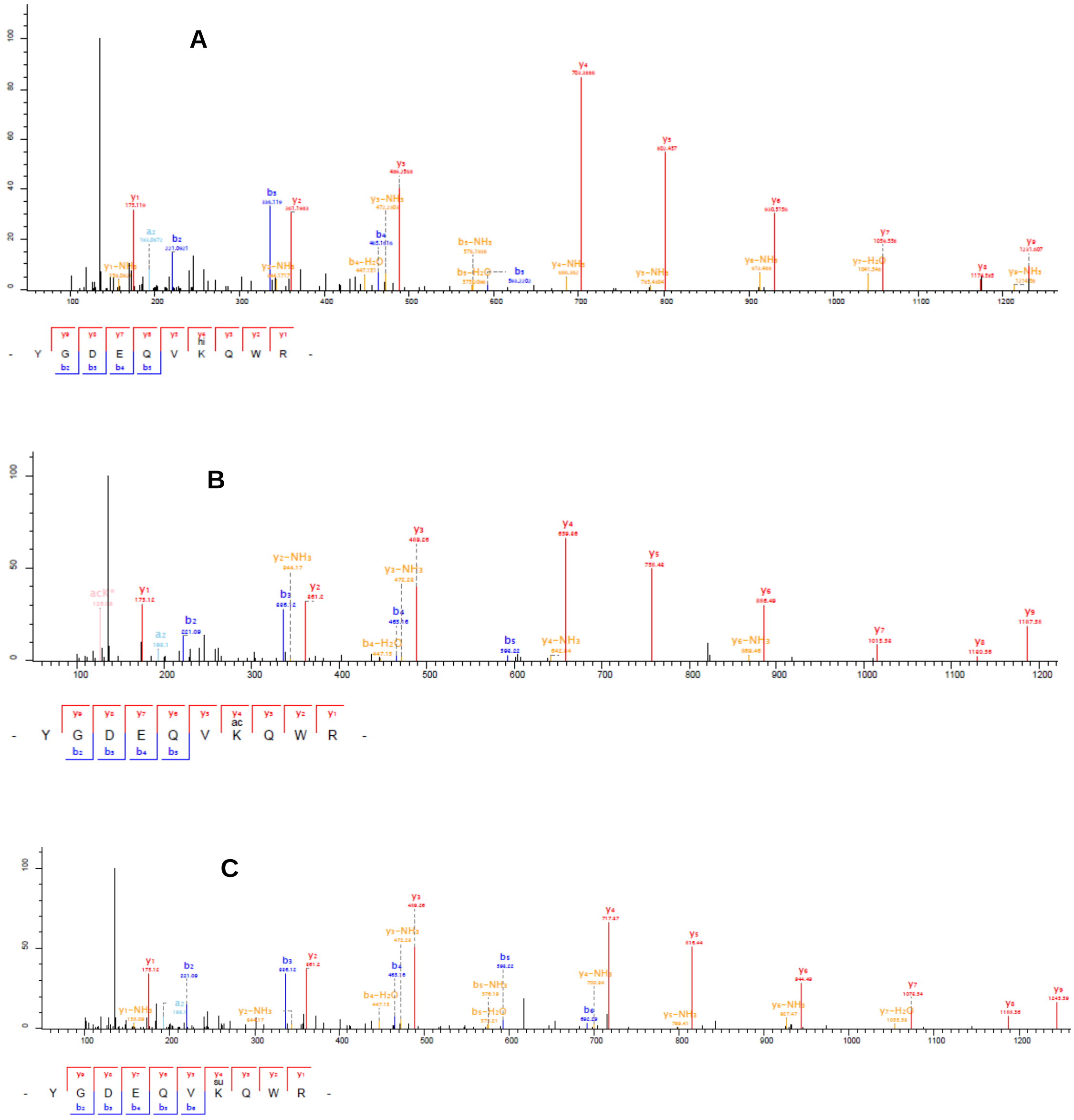
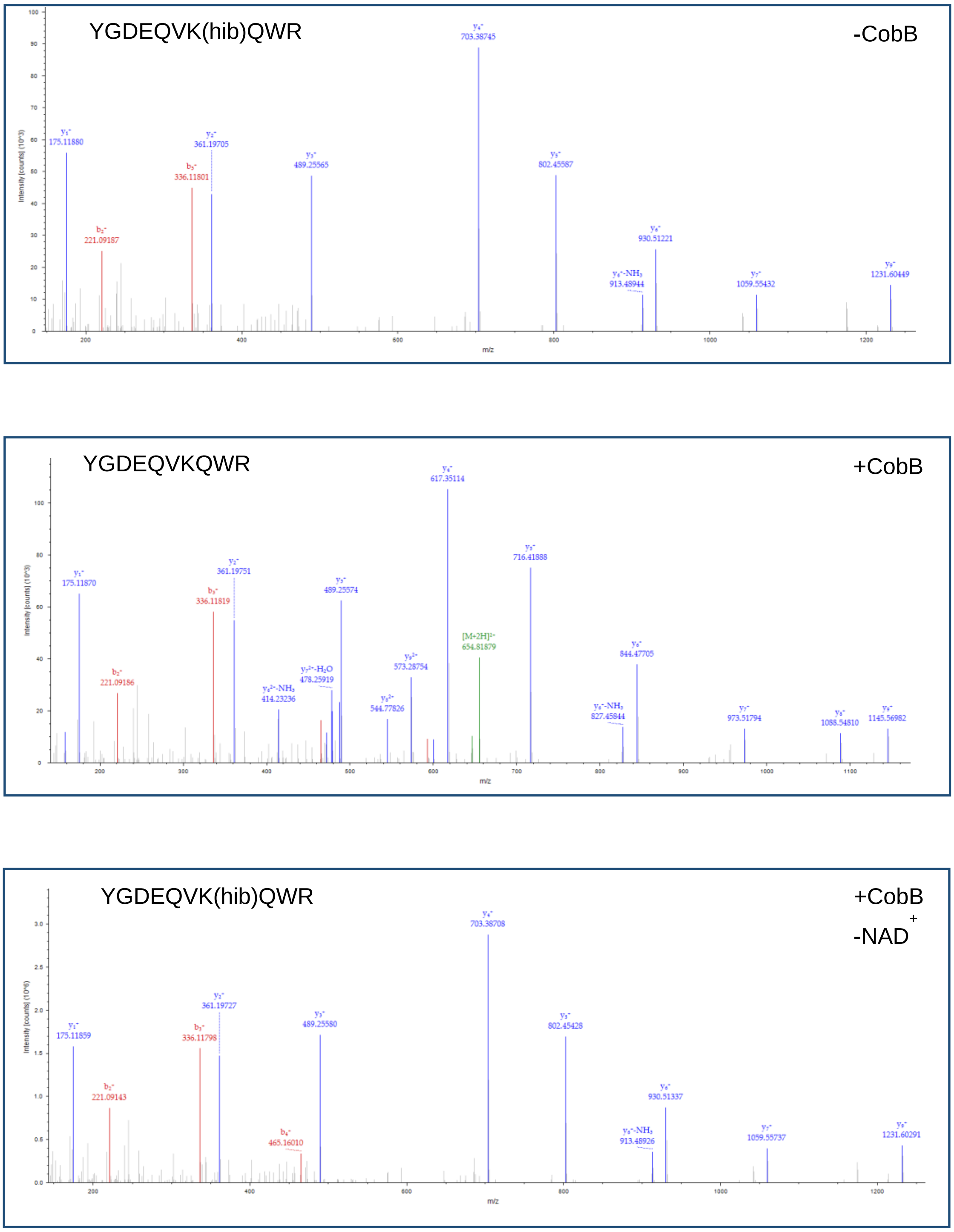
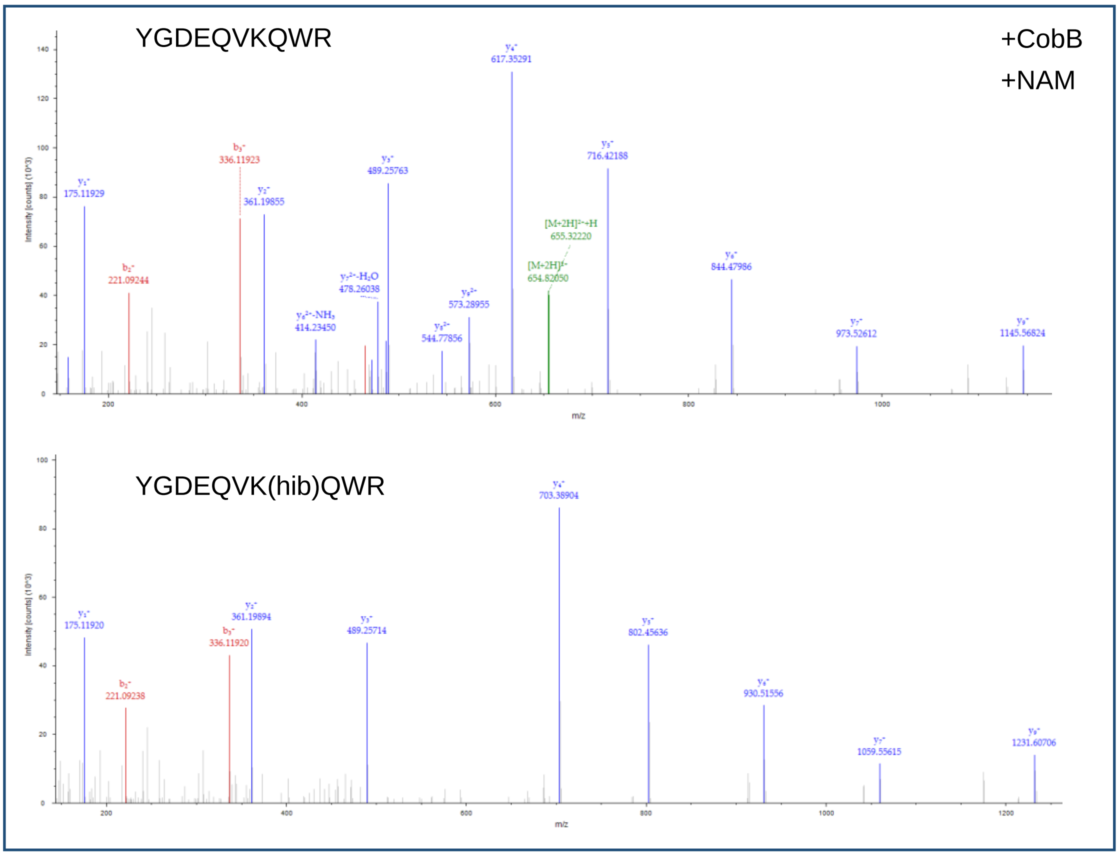


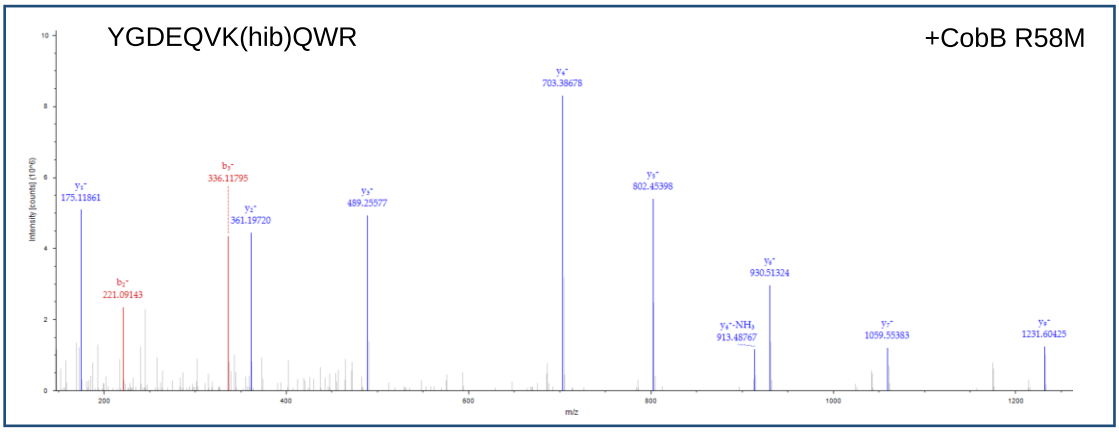
Fig. S2

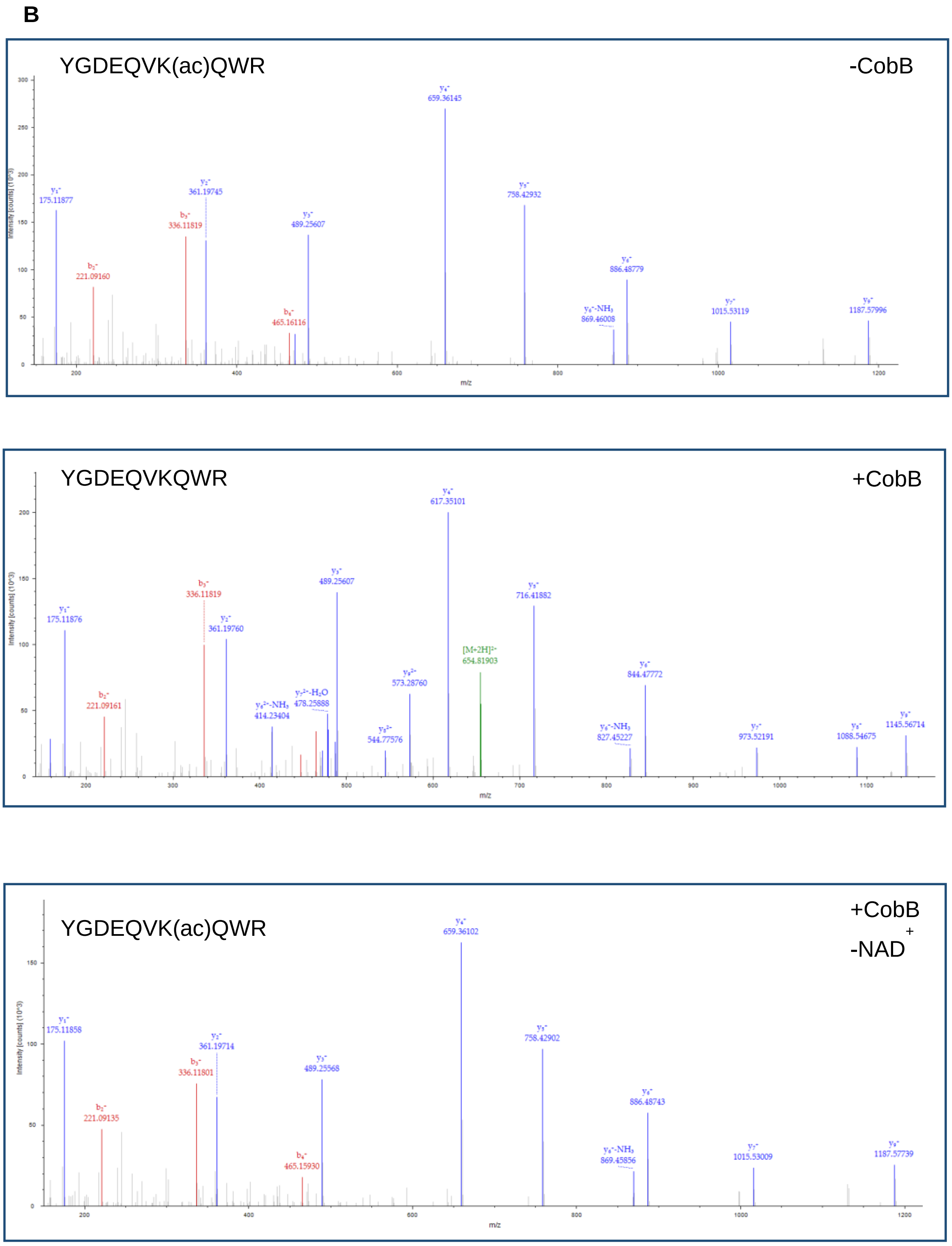
Fig. S2. MS/MS spectrum of lysine-acylated peptides (YGDEQVKQWR) identified in *P. mirabilis.* (**A**) MS/MS spectrum of YGDEQVK(hib)QWR. (**B**) MS/MS spectrum of YGDEQVK(ac)QWR. (C) MS/MS spectrum of YGDEQVK(succ)QWR.

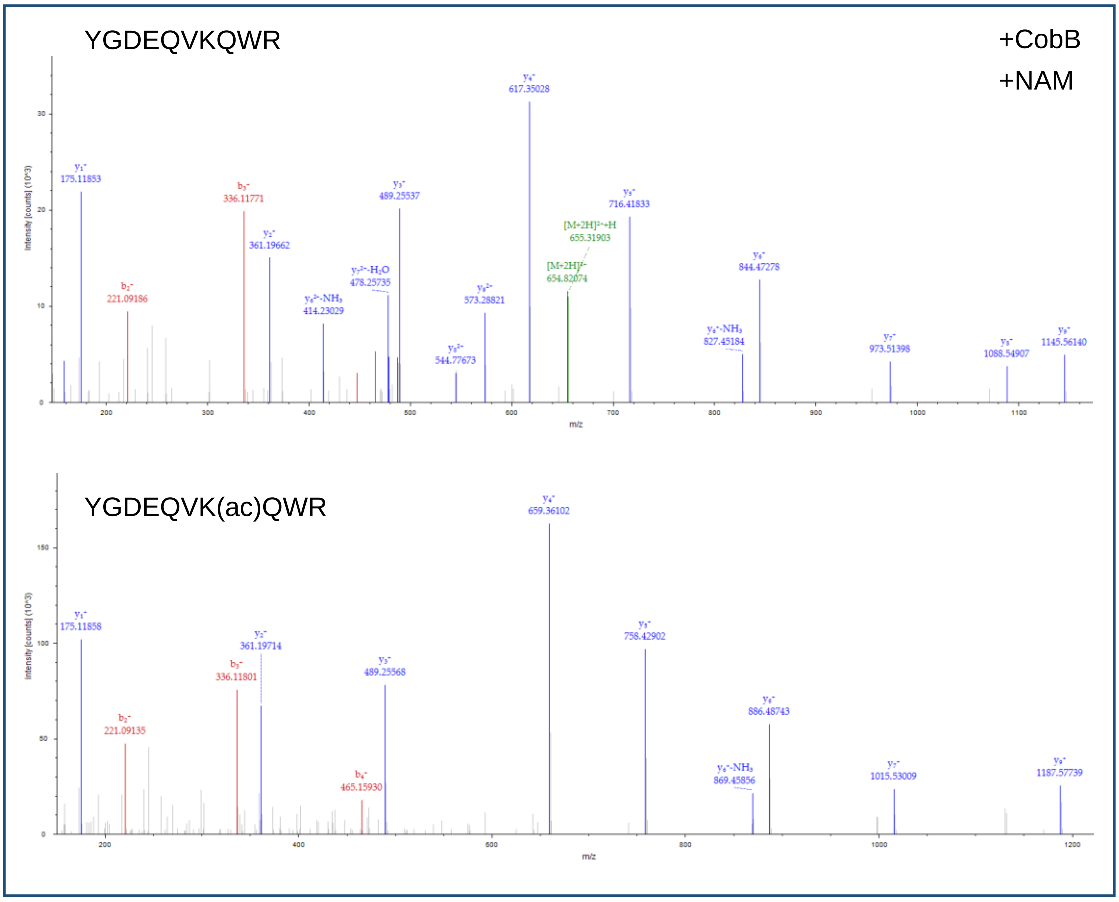
A


YGDEQVK(hib)QWR
-CobB
Intensity [counts] (10^3)
m/z
y1+ 175.11880
b2+ 221.09187
b3+ 336.11801
y2+ 361.19705
y3+ 489.25565
y4+ 703.38745
y5+ 802.45587
y6+-NH3 913.48944
y6+ 930.51221
y7+ 1059.55432
y8+ 1231.60449
YGDEQVKQWR
+CobB
y1+ 175.11870
b2+ 221.09186
b3+ 336.11819
y2+ 361.19751
y6 2+-NH3 414.23236
y7 2+-H2O 478.25919
y3+ 489.25574
y8 2+ 544.77826
y9 2+ 573.28754
y4+ 617.35114
[M+2H]2+ 654.81879
y5+ 716.41888
y6+-NH3 827.45844
y6+ 844.47705
y7+ 973.51794
y8+ 1088.54810
y9+ 1145.56982
YGDEQVK(hib)QWR
+CobB
-NAD+
Intensity [counts] (10^6)
y1+ 175.11859
b2+ 221.09143
b3+ 336.11798
y2+ 361.19727
b4+ 465.16010
y3+ 489.25580
y4+ 703.38708
y5+ 802.45428
y6+-NH3 913.48926
y6+ 930.51337
y7+ 1059.55737
y8+ 1231.60291


YGDEQVKQWR
+CobB
+NAM
Intensity [counts] (10^3)
y1+ 175.11929
b2+ 221.09244
b3+ 336.11923
y2+ 361.19855
y6 2+-NH3 414.23450
y7 2+-H2O 478.26038
y3+ 489.25763
y8 2+ 544.77856
y9 2+ 573.28955
y4+ 617.35291
[M+2H]2+ 654.82050
[M+2H]2+ +H 655.32220
y5+ 716.42188
y6+ 844.47986
y7+ 973.52612
y8+ 1145.56824
m/z
YGDEQVK(hib)QWR
Intensity [counts] (10^3)
y1+ 175.11920
b2+ 221.09238
b3+ 336.11920
y2+ 361.19894
y3+ 489.25714
y4+ 703.38904
y5+ 802.45636
y6+ 930.51556
y7+ 1059.55615
y8+ 1231.60706
m/z


YGDEQVK(hib)QWR
+CobB R58M
Intensity [counts] (10^6)
y1+ 175.11861
b2+ 221.09143
b3+ 336.11795
y2+ 361.19720
y3+ 489.25577
y4+ 703.38678
y5+ 802.45398
y6+-NH3 913.48767
y6+ 930.51324
y7+ 1059.55383
y8+ 1231.60425
m/z


B
YGDEQVK(ac)QWR
-CobB
Intensity [counts] (10^3)
300
250
200
150
100
50
0
y1+ 175.11877
b2+ 221.09160
b3+ 336.11819
y2+ 361.19745
b4+ 465.16116
y3+ 489.25607
y4+ 659.36145
y5+ 758.42932
y6+-NH3 869.46008
y6+ 886.48779
y7+ 1015.53119
y9+ 1187.57996
200
400
600
800
1000
1200
m/z
YGDEQVKQWR
+CobB
Intensity [counts] (10^3)
200
150
100
50
0
y1+ 175.11876
b2+ 221.09161
b3+ 336.11819
y2+ 361.19760
y6 2+-NH3 414.23404
y7 2+-H2O 478.25888
y3+ 489.25607
y8 2+ 544.77576
y9 2+ 573.28760
y4+ 617.35101
[M+2H]2+ 654.81903
y5+ 716.41882
y6+-NH3 827.45227
y6+ 844.47772
y7+ 973.52191
y8+ 1088.54675
y9+ 1145.56714
200
300
400
500
600
700
800
900
1000
1100
m/z
YGDEQVK(ac)QWR
+CobB
-NAD+
Intensity [counts] (10^3)
150
100
50
0
y1+ 175.11858
b2+ 221.09135
b3+ 336.11801
y2+ 361.19714
b4+ 465.15930
y3+ 489.25568
y4+ 659.36102
y5+ 758.42902
y6+-NH3 869.45856
y6+ 886.48743
y7+ 1015.53009
y9+ 1187.57739
200
400
600
800
1000
1200
m/z


YGDEQVKQWR
+CobB
+NAM
Intensity [counts] (10^3)
y1+ 175.11853
b2+ 221.09186
b3+ 336.11771
y2+ 361.19662
y6 2+-NH3 414.23029
y7 2+-H2O 478.25735
y3+ 489.25537
y9 2+ 544.77673
y9 2+ 573.28821
y4+ 617.35028
[M+2H]2+ 654.82074
[M+2H]2++H 655.31903
y5+ 716.41833
y6+-NH3 827.45184
y6+ 844.47278
y7+ 973.51398
y8+ 1088.54907
y9+ 1145.56140
m/z
YGDEQVK(ac)QWR
Intensity [counts] (10^3)
y1+ 175.11858
b2+ 221.09135
b3+ 336.11801
y2+ 361.19714
b4+ 465.15930
y3+ 489.25568
y4+ 659.36102
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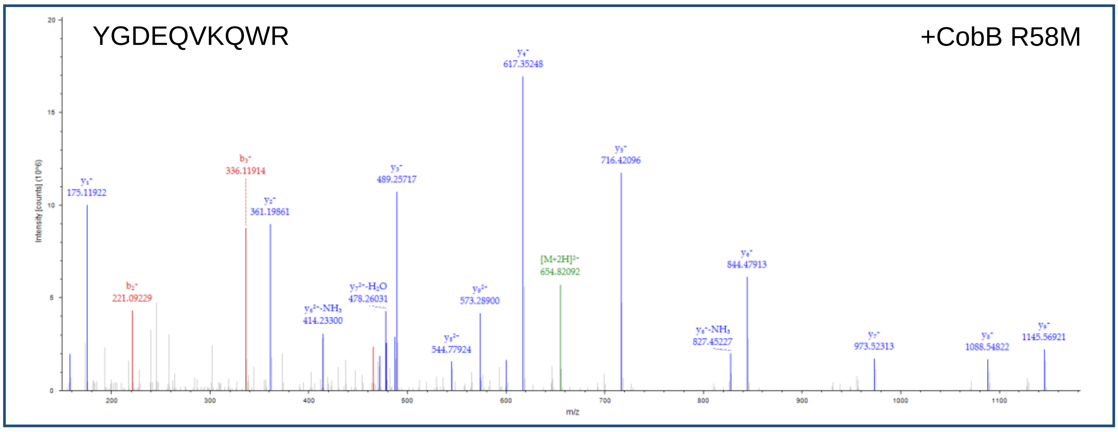
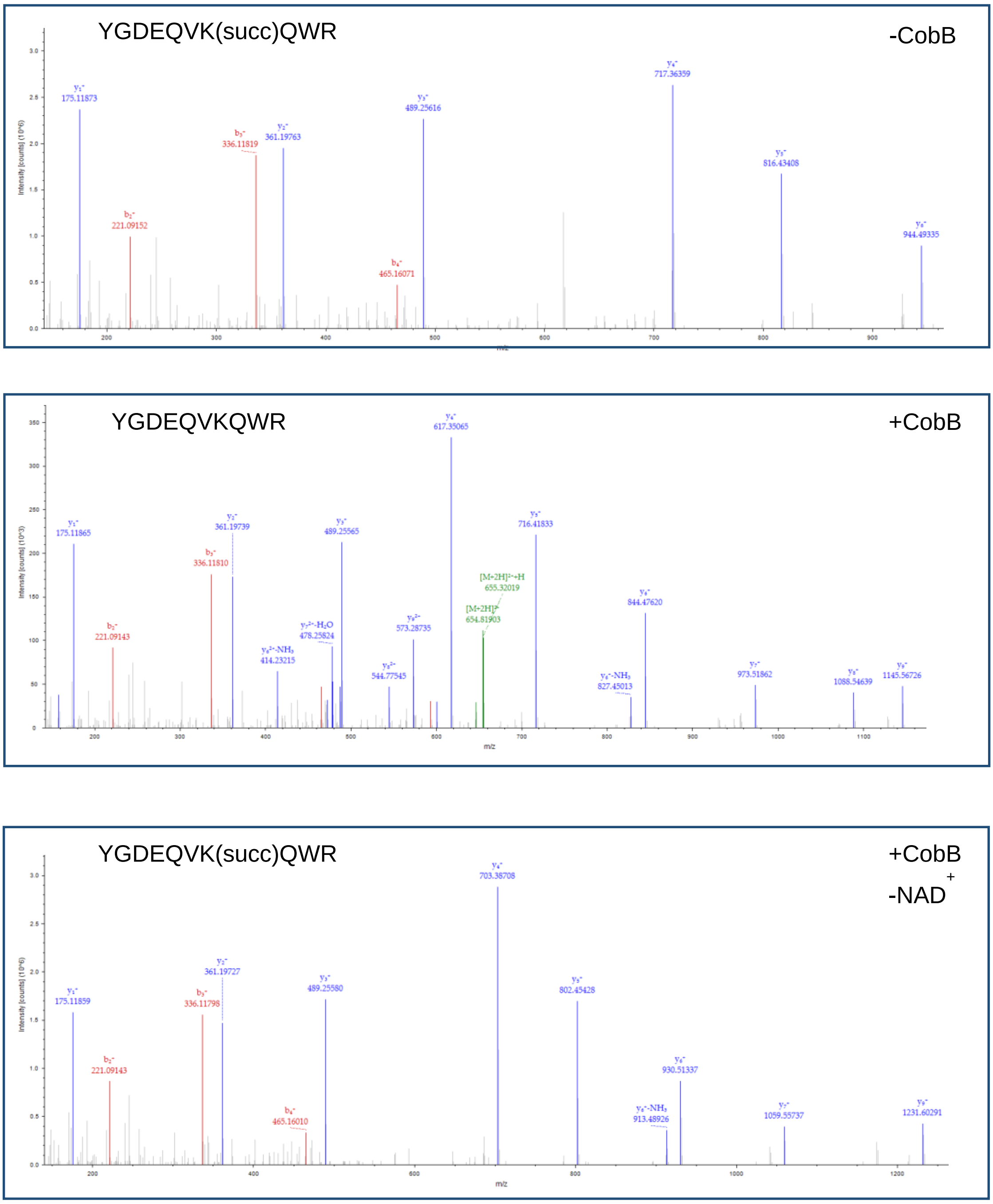
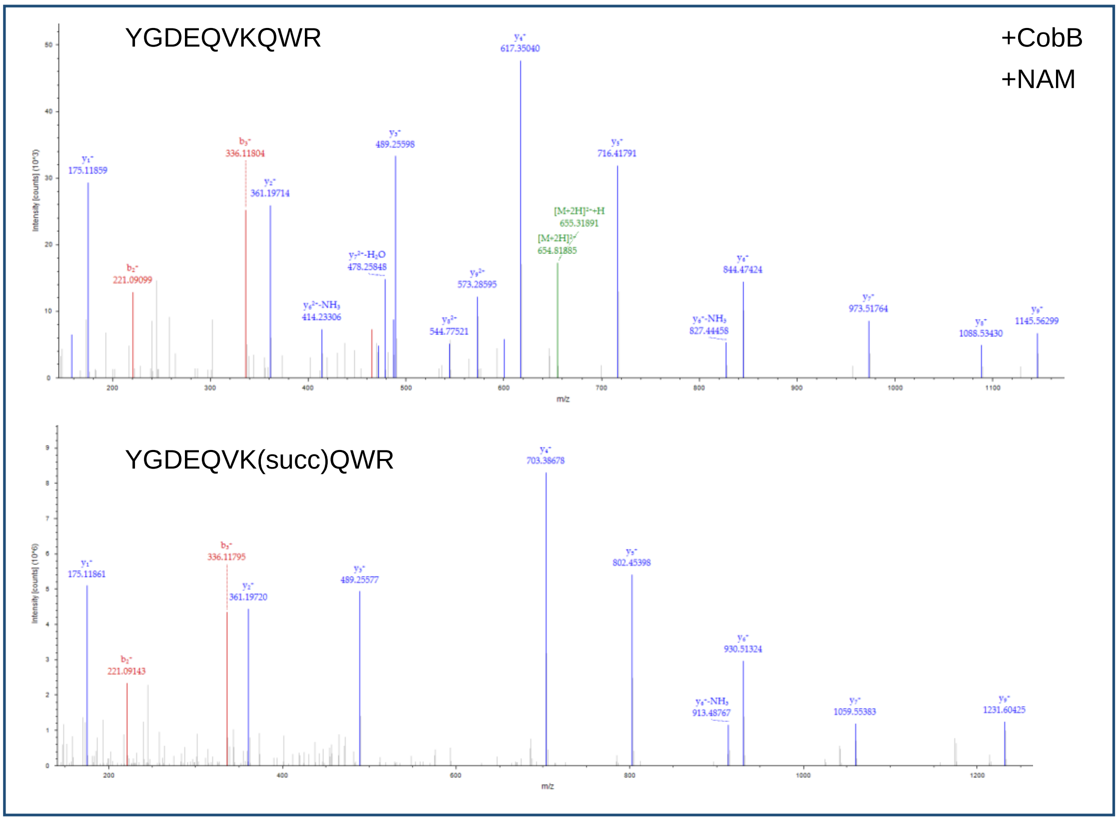
y6+-NH3 869.45856
y6+ 886.48743
y7+ 1015.53009
y9+ 1187.57739
m/z


YGDEQVKQWR
+CobB R58M
Intensity [counts] (10^6)
y1+ 175.11922
b2+ 221.09229
b3+ 336.11914
y2+ 361.19861
y6 2+-NH3 414.23300
y7 2+-H2O 478.26031
y3+ 489.25717
y9 2+ 544.77924
y9 2+ 573.28900
y4+ 617.35248
[M+2H]2+ 654.82092
y5+ 716.42096
y6+-NH3 827.45227
y6+ 844.47913
y7+ 973.52313
y8+ 1088.54822
y9+ 1145.56921
m/z

C


YGDEQVK(succ)QWR
-CobB
Intensity [counts] (10^6)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
200
300
400
500
600
700
800
900
m/z
y_1^+ 175.11873
b_2^+ 221.09152
b_3^+ 336.11819
y_2^+ 361.19763
b_4^+ 465.16071
y_3^+ 489.25616
y_4^+ 717.36359
y_5^+ 816.43408
y_6^+ 944.49335
YGDEQVKQWR
+CobB
Intensity [counts] (10^3)
0
50
100
150
200
250
300
350
200
300
400
500
600
700
800
900
1000
1100
m/z
y_1^+ 175.11865
b_2^+ 221.09143
b_3^+ 336.11810
y_2^+ 361.19739
y_6^{2+}-NH_3 414.23215
y_7^{2+}-H_2O 478.25824
y_3^+ 489.25565
y_8^{2+} 544.77545
y_9^{2+} 573.28735
y_4^+ 617.35065
$[M+2H]^{2+}$ 654.81903
$[M+2H]^{2+}$+H 655.32019
y_5^+ 716.41833
y_6^+-NH_3 827.45013
y_6^+ 844.47620
y_7^+ 973.51862
y_8^+ 1088.54639
y_9^+ 1145.56726
YGDEQVK(succ)QWR
+CobB
-NAD^+
Intensity [counts] (10^6)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
200
400
600
800
1000
1200
m/z
y_1^+ 175.11859
b_2^+ 221.09143
b_3^+ 336.11798
y_2^+ 361.19727
b_4^+ 465.16010
y_3^+ 489.25580
y_4^+ 703.38708
y_5^+ 802.45428
y_6^+-NH_3 913.48926
y_6^+ 930.51337
y_7^+ 1059.55737
y_9^+ 1231.60291



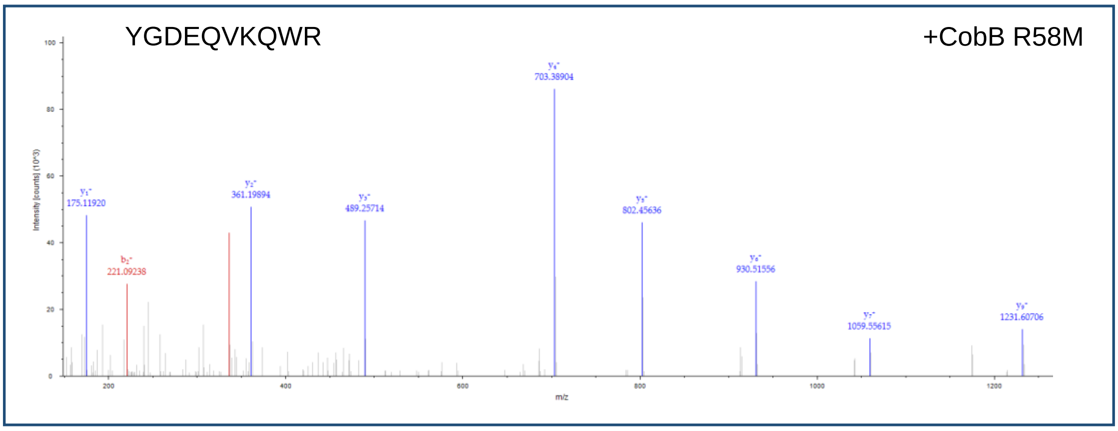


Fig. S3

Fig. S3. MS/MS spectrum of 2-hydroxyisobutyrylated, acetylated, and succinylated peptides under different incubation conditions. The incubation conditions contain: -CobB, +CobB, +CobB and –NAD $^+$, +CobB and +NAM, +CobB R58M, +: presents existence, -: presents absence. (**A**) MS/MS spectrum present PD searching results of CobB and 2-hydroxyisobutyrylated peptides incubation under different conditions. (**B**) MS/MS spectrum present PD searching results of CobB and acetylated peptides incubation under different conditions. (**C**) MS/MS spectrum present PD searching results of CobB and succinylated peptides incubation under different conditions.

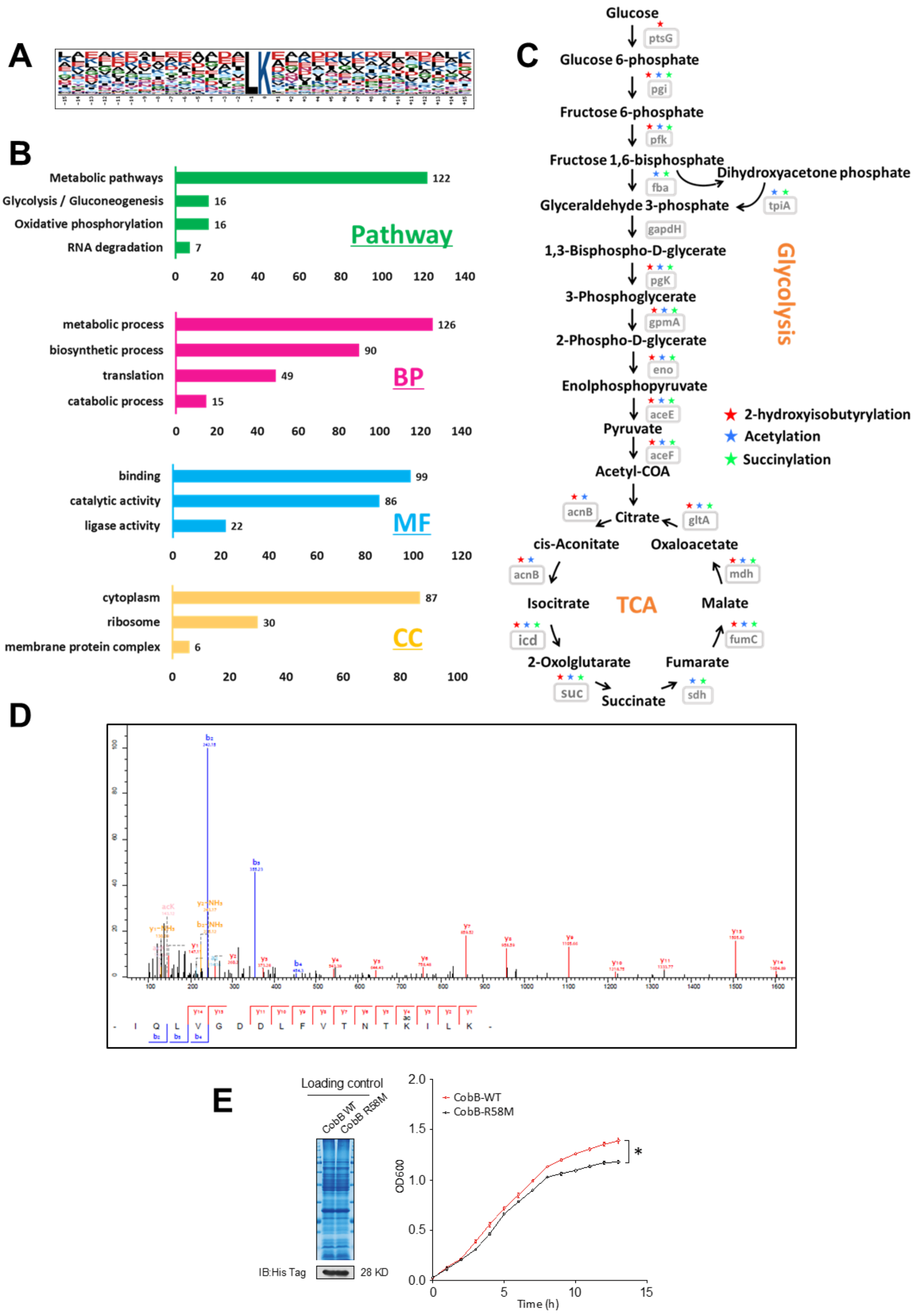


Fig. S4

Fig. S4. Comparative analysis of Khib, Kac, and Ksucc in *P. mirabilis*. (**A**) Sequence Logo representation of significant motifs of peptides detected three acylation identified by Motif-X software. The motifs with significance of p < 0.000001 are shown. The motifs take acyllysine as center and show the distribution of 15 amino acids in both sides. (**B**) Analysis the biological process, pathway, molecular function and cellular component of common acylated proteins in *P. mirabilis* by DAVID. (**C**) Khib, Kac and Ksucc enzymes in central metabolic network in *P. mirabilis*. (**D**) MS/MS Spectrogram of ENO pepetide containing K326ac. (**E**). Measurement growth curve of WT CobB and mutated CobB overexpressing *P. mirabilis* that transformed CobB WT and mutation (R58M) vector.

Table S1. The quantification result of H3K9hib and H3K9 probe enrichment in *P. mirabilis* protein lysate.

Table S2. The identification result of Kac of *P. mirabilis*.

Table S3. The identification result of Ksucc of *P. mirabilis*.

Table S4. The quantification result of 2-hydroisobutyrylated proteomes with CobB KO *E. coli* and WT *E. coli*.

Table S5. The PRM result of comparison of the quantities of K326ac and K343hib in T vector and CobB-overexpressing *P. mirabilis*.

Table S6. The list of reagents, bacterial strains, and plasmids used in this paper.

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REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit pan anti-2-hydroxyisobutyryllysine polyclonal antibody	PTM Biolabs	Cat# PTM-801
Rabbit pan anti-acetyllysine polyclonal antibody	PTM Biolabs	Cat# PTM-105
Rabbit pan anti-succinyllysine polyclonal antibody	PTM Biolabs	Cat# PTM-401
Rabbit pan anti-malonyllysine polyclonal antibody	PTM Biolabs	Cat# PTM-901
Rabbit pan anti-glutaryllysine polyclonal antibody	PTM Biolabs	Cat# PTM-1151
Mouse pan anti-crotonyllysine monoclonal antibody	PTM Biolabs	Cat# PTM-502
Rabbit pan anti-β- hydroxybutyryllysine polyclonal antibody	PTM Biolabs	Cat# PTM-1201
Anti-acetyllysine antibody beaded agarose	PTM Biolabs	Cat# PTM-104
Anti-succinyllysine antibody conjugated agarose beads	PTM Biolabs	Cat# PTM-402
6×His tag antibody (His. H8)	Thermo Fisher	Cat# MA1-21315
Bacterial Strains and Plasmids		
Proteus mirabilis ATCC29906	University of Lodz	N/A
BL21 (DE3) competent cells	TIANGEN	Cat# CB105
DH5α competent cells	TIANGEN	Cat# CB101
Escherichia coli MG1655	Tolo Biotech	N/A
npdA KO *Escherichia coli* MG1655	Tolo Biotech	N/A
pET28a vector	Solarbio	Cat# P3110
pTrc99a vector	addgene	KL-ZL-0193
pGEM-T Easy Vector	Promega	Cat# A1360
Synthetic Peptides		
ARTKQTAR-K(hib)-STGGKAC	SciLight Biotechnology	N/A
YGDEQV-K(hib)-QWR	SciLight	N/A

	Biotechnology	
GIANSILI-K(hib)-FNQIGSLTETLAAIK	SciLight Biotechnology	N/A
YGDEQV-K(ac)-QWR	SciLight Biotechnology	N/A
IQLVGDDLFVTNT-K(ac)-ILK	SciLight Biotechnology	N/A
YGDEQV-K(succ)-QWR	SciLight Biotechnology	N/A
ARTKQTARKSTGGKAC	SciLight Biotechnology	N/A
PCR Primers		
ENO-pET28a-F: cgcggatcctccAAAATCGTTAAAG	Reported	N/A
ENO-pET28a-R: CCGCTCGAGTTATGCTTGGCCTTTAACTTC	Reported	N/A
ENO-K343T-pET28a-F: CTAACTCTATCCTGATCACATTCAACCAA	Reported	N/A
ENO-K343T-pET28a-R: GTGATCAGGATAGAGTTAGCAATACCTTT	Reported	N/A
ENO-K326Q-pET28a-F: TTGTAACTAATACTCAGATCCTAAAAGAAG	This paper	N/A
ENO-K326Q-pET28a-R: GAGTATTAGTTACAAACAGGTCGTC	This paper	N/A
ENO-His-T vector-F: ACGCGTCGACGGATATGTGAGAAGAG	This paper	N/A
ENO-His-T vector-R: CATGCATGCTTATGCGTGGTGGTGGTGGTGGTGTTGGCCTTTAACTTC	This paper	N/A
ENO-K343T-T vector-F: CTAACTCTATCCTGATCACATTCAACCAA	This paper	N/A
ENO-K343T-T vector-R: GTGATCAGGATAGAGTTAGCAATACCTTT	This paper	N/A

ENO-K326Q-T vector-F: TTGTAACTAATACTCAGATCCTAAAAGAAG	This paper	N/A
ENO-K326Q-T vector-R: GAGTATTAGTTACAAACAGGTCGTC	This paper	N/A
npdA-pET28a-F: CGCGGATCCAATTTACCCAAAGTAGTG	This paper	N/A
npdA-pET28a-R: CCGCTCGAGTTATTGCGTCAAATCAGCTTTAG	This paper	N/A
npdA-Y94F-pet28a-F: AGCTGGTTCAGCAGTTTTTCAATGAGCGCC	This paper	N/A
npdA-Y94F-pet28a-R: AAAAACTGCTGAACCAGCTTAGGGTTGCG	This paper	N/A
npdA-R97M-pet28a-F: TTCAGCAGTTTTACAATGAGATGCGTCGTCAATTA	This paper	N/A
npdA-R97M-pet28a-R: CATCTCATTGTAAAACTGCTGAACCAGCTTAGGGT	This paper	N/A
npdA-His-T vector-F: ACGCGTCGACAACTTCGTCATCGTCGGCTTAGGAAGTTTC	This paper	N/A
npdA-His-T vector-R: CATGCATGCTTAGTGGTGGTGGTGGTGGTGTTGCGTCAAATCAG	This paper	N/A
npdA-His-pTrc99a-F: CGCGGATCCATGAAACTTAAACTTCGTCATCGTCGG	This paper	N/A
npdA-His-pTrc99a-R: GCTCTAGATTAGTGGTGGTGGTGGTGGTGTTGCGTCAAATCAG	This paper	N/A
npdA-R58M-pTrc99a-F: TTCAGCAGTTTTACAATGAGATGCGTCGTCAATTA	This paper	N/A

npdA-R58M-pTrc99a-R: CATCTCATTGTAAAACTGCTGAACCAGCTTAGGGT	This paper	N/A
Reagent		
HisPur Ni-NTA Resin	Thermo SCIENTIFIC	Cat# 88221
Fast Mutagenesis System	TRANS	Cat# FM111
PEP Colorimetric/Fluorometric Assay Kit	SIGMA-ALDRICH	Cat# MAK102
SILAC^{TM} L-Lysine HCL	Invitrogen	32461
U-$^{13}C_6$-L-Lysine HCL	Invitrogen	32457