

Supporting Information

Revealing the antibacterial power of hydrogen-releasing PdH nanohydride against drug resistant *Staphylococcus aureus*: An in-depth mechanism study

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Fig. S1 High-resolution TEM image of PdH nanohydride presenting a cubic shape with a mean diameter of about 10 nm.

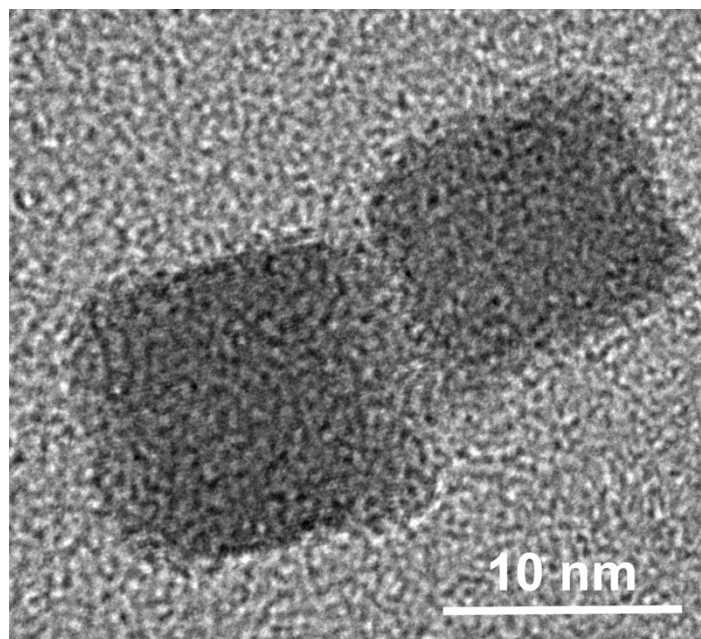


Fig. S2 Changes in UV-Vis spectra of MB under different conditions. (A) Pd at 37 °C. (B) PdH at 4 °C. (C) PdH at 37 °C.

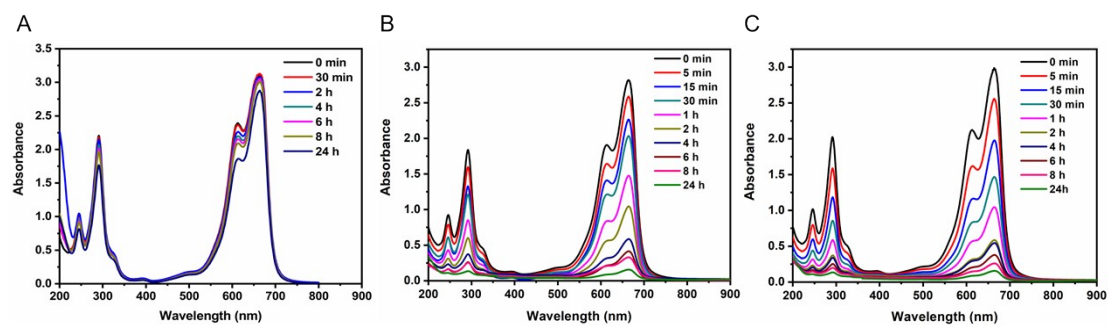


Fig. S3 The standard curve of MB showing the relationship of concentration with the UV-Vis absorbance value at 664 nm.

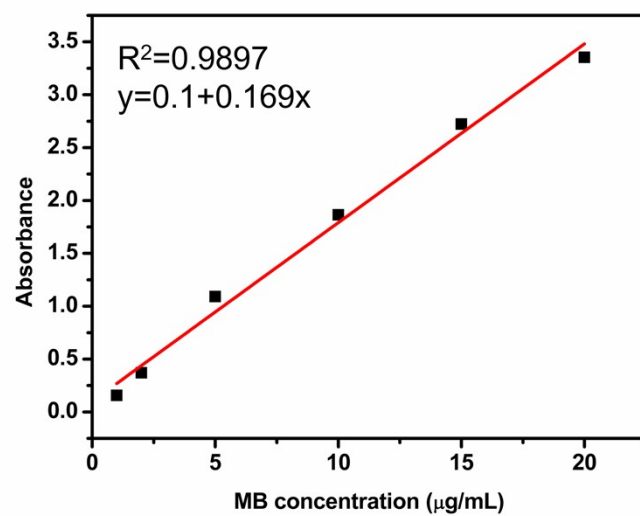


Fig. S4 (A) Crystal violet assay showing the anti-biofilm activity of different treatments. (B) Histograms showing the changes in OD₅₅₀ underwent different treatments.

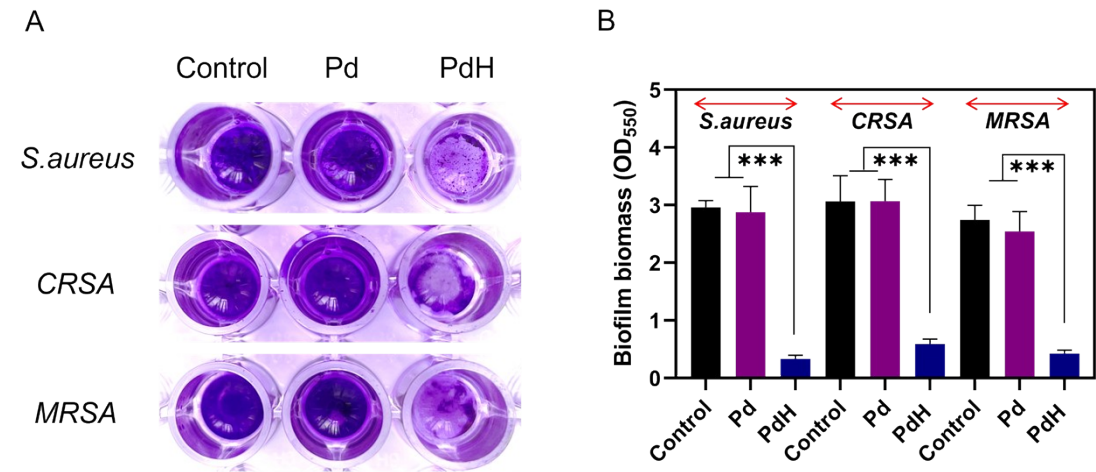


Fig. S5 OD₂₆₀ value of *S. aureus* and *CRSA* underwent different treatments.

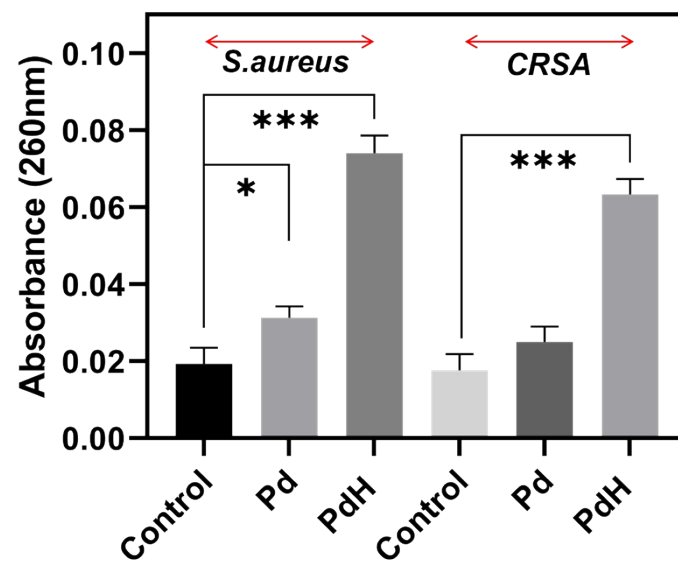


Fig. S6 Genome analysis on the antibacterial mechanism of H₂ against *CRSA*. (A) KEGG analysis showing the Top 20 functional pathway enrichment of *CRSA*, underwent PdH treatment as expressed by KEGG-analysis. (B) Heat map analysis of differentially expressed genes in *CRSA*. Red color represents gene up-regulation and green color represents gene down-regulation.

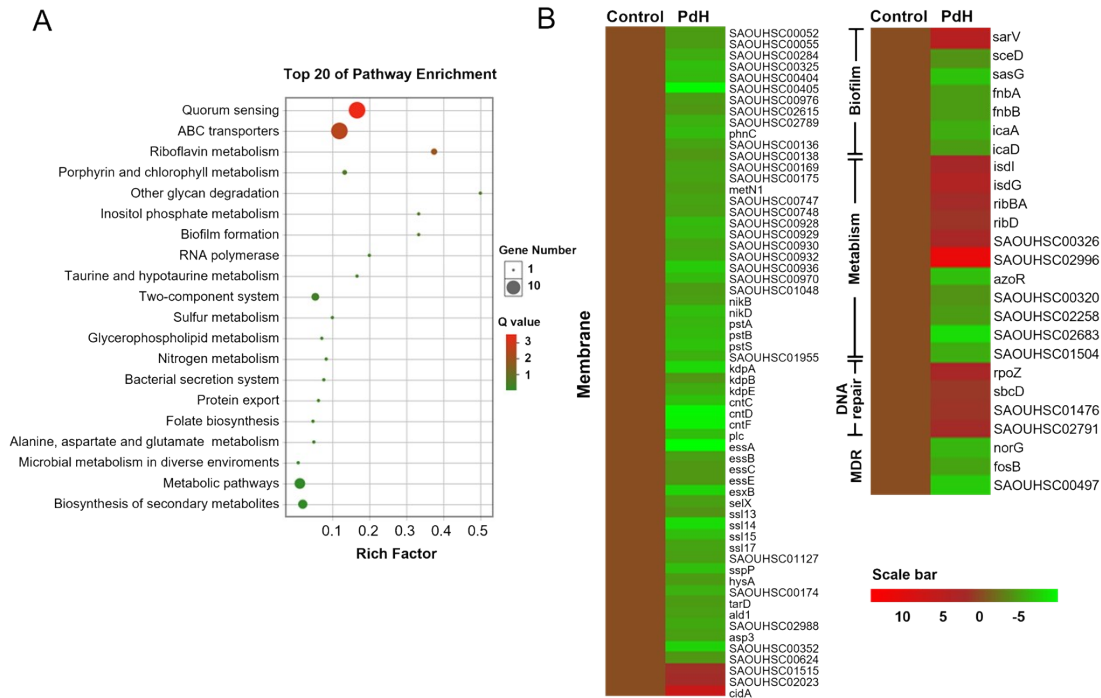


Fig. S7 Evaluation on the cell viability of NIH-3T3 cells underwent Pd and PdH treatment with different concentrations.

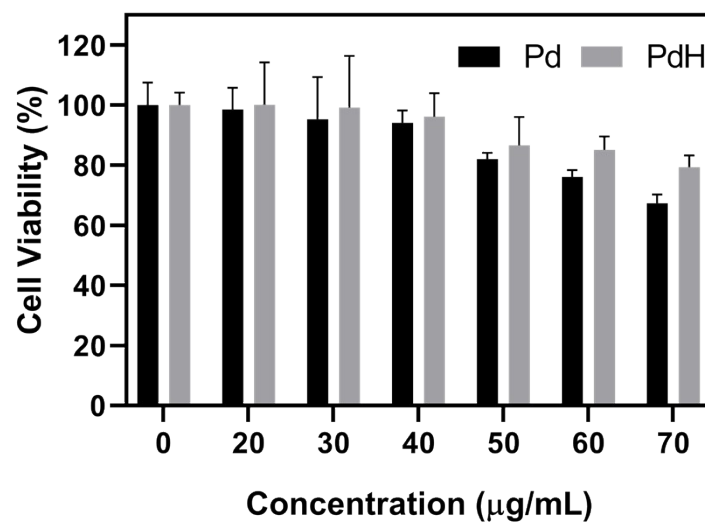


Table S1 The differentially expressed functional genes in *CRSA* and *MRSA* after PdH treatment.

Bacteria	Gene ID	Gene names	Protein names	Control	PdH	Fold
<i>CRSA</i>	SAOUHSC_00052	<i>SAOUHSC00052</i>	lipoprotein	30.898	13.458	-2.296
<i>CRSA</i>	SAOUHSC_00055	<i>SAOUHSC00055</i>	lipoprotein	15.259	6.644	-2.297
<i>CRSA</i>	SAOUHSC_00284	<i>SAOUHSC00284</i>	5'-nucleotidase, lipoprotein e(P4) family	16.815	5.344	-3.146
<i>CRSA</i>	SAOUHSC_00325	<i>SAOUHSC00325</i>	Efem/EfeO family lipoprotein	23.590	5.968	-3.953
<i>CRSA</i>	SAOUHSC_00404	<i>SAOUHSC00404</i>	lipoprotein	26.886	7.141	-3.765
<i>CRSA</i>	SAOUHSC_00405	<i>SAOUHSC00405</i>	lipoprotein	9.112	1.383	-6.589
<i>CRSA</i>	SAOUHSC_00976	<i>SAOUHSC00976</i>	Fe/B12 periplasmic-binding domain-containing protein	27.568	12.007	-2.296
<i>CRSA</i>	SAOUHSC_02615	<i>SAOUHSC02615</i>	UPF0060 membrane protein	84.751	40.124	-2.112
<i>CRSA</i>	SAOUHSC_02789	<i>SAOUHSC02789</i>	lipoprotein	23.812	7.270	-3.275
<i>CRSA</i>	SAOUHSC_00104	<i>phnC</i>	Phosphonates import ATP-binding protein PhnC	23.580	6.373	-3.700
<i>CRSA</i>	SAOUHSC_00136	<i>SAOUHSC00136</i>	ABC transporter domain-containing protein	22.557	8.494	-2.656
<i>CRSA</i>	SAOUHSC_00138	<i>SAOUHSC00138</i>	ABC transmembrane type-1 domain-containing protein	20.170	9.152	-2.204
<i>CRSA</i>	SAOUHSC_00169	<i>SAOUHSC00169</i>	Peptide ABC transporter, permease protein	18.972	7.011	-2.706
<i>CRSA</i>	SAOUHSC_00175	<i>SAOUHSC00175</i>	Multiple sugar-binding transport ATP-binding protein	31.832	11.614	-2.741
<i>CRSA</i>	SAOUHSC_00423	<i>metN1</i>	Methionine import ATP-binding protein MetN 1	54.284	23.700	-2.291
<i>CRSA</i>	SAOUHSC_00747	<i>SAOUHSC00747</i>	Iron-regulated surface determinant protein F	34.922	12.794	-2.730
<i>CRSA</i>	SAOUHSC_00748	<i>SAOUHSC00748</i>	ABC transporter domain-containing protein	23.700	9.599	-2.469
<i>CRSA</i>	SAOUHSC_00928	<i>SAOUHSC00928</i>	Oligopeptide ABC transporter, substrate-binding protein	23.606	6.537	-3.611
<i>CRSA</i>	SAOUHSC_00929	<i>SAOUHSC00929</i>	Oligopeptide ABC transporter, substrate-binding protein	37.556	10.510	-3.574
<i>CRSA</i>	SAOUHSC_00930	<i>SAOUHSC00930</i>	Oligopeptide ABC transporter, substrate-binding protein	29.955	10.747	-2.787
<i>CRSA</i>	SAOUHSC_00932	<i>SAOUHSC00932</i>	Oligopeptide ABC transporter, substrate-binding protein	56.188	19.282	-2.914
<i>CRSA</i>	SAOUHSC_00936	<i>SAOUHSC00936</i>	ABC transporter, ATP-binding protein	24.324	5.513	-4.412
<i>CRSA</i>	SAOUHSC_00970	<i>SAOUHSC00970</i>	ABC transporter, ATP-binding protein	22.749	6.095	-3.732

<i>CRSA</i>	SAOUHSC_01048	<i>SAOUHSC01048</i>	Spermidine/putrescine ABC transporter, permease protein	34.152	14.069	-2.428
<i>CRSA</i>	SAOUHSC_01380	<i>nikB</i>	Nickel import system permease protein NikB	22.962	8.959	-2.563
<i>CRSA</i>	SAOUHSC_01378	<i>nikD</i>	Nickel import system ATP-binding protein NikD	23.332	5.934	-3.932
<i>CRSA</i>	SAOUHSC_01386	<i>pstA</i>	Phosphate transport system permease protein PstA	22.807	6.669	-3.420
<i>CRSA</i>	SAOUHSC_01385	<i>pstB</i>	Phosphate import ATP-binding protein PstB	21.644	5.989	-3.614
<i>CRSA</i>	SAOUHSC_01389	<i>pstS</i>	Phosphate-binding protein PstS	37.476	9.159	-4.092
<i>CRSA</i>	SAOUHSC_01955	<i>SAOUHSC01955</i>	Amino acid ABC transporter, ATP-binding protein	22.473	6.757	-3.326
<i>CRSA</i>	SAOUHSC_02312	<i>kdpA</i>	Potassium-transporting ATPase potassium-binding subunit	26.216	5.271	-4.974
<i>CRSA</i>	SAOUHSC_02311	<i>kdpB</i>	Potassium-transporting ATPase ATP-binding subunit	42.886	20.320	-2.111
<i>CRSA</i>	SAOUHSC_02315	<i>kdpE</i>	Transcriptional regulatory protein KdpE	12.975	4.155	-3.123
<i>CRSA</i>	SAOUHSC_02765	<i>cntC</i>	Metal-staphylopine import system permease protein CntC	20.975	5.278	-3.974
<i>CRSA</i>	SAOUHSC_02764	<i>cntD</i>	Metal-staphylopine import system ATP-binding protein CntD	14.361	2.293	-6.263
<i>CRSA</i>	SAOUHSC_02763	<i>cntF</i>	cntF	18.188	2.948	-6.169
<i>CRSA</i>	SAOUHSC_00051	<i>plc</i>	1-phosphatidylinositol phosphodiesterase	24.713	6.547	-3.775
<i>CRSA</i>	SAOUHSC_00260	<i>essA</i>	Type VII secretion system accessory factor EsaB	8.194	1.209	-6.777
<i>CRSA</i>	SAOUHSC_00261	<i>essB</i>	Type VII secretion system protein EssB	29.197	11.461	-2.548
<i>CRSA</i>	SAOUHSC_00262	<i>essC</i>	Type VII secretion system protein EssC	38.252	17.604	-2.173
<i>CRSA</i>	SAOUHSC_00266	<i>essE</i>	Type VII secretion system protein EsaE	59.211	27.472	-2.155
<i>CRSA</i>	SAOUHSC_00265	<i>esxB</i>	Type VII secretion system extracellular protein B	44.606	9.197	-4.850
<i>CRSA</i>	SAOUHSC_00354	<i>selX</i>	Enterotoxin-like toxin X	21.039	8.341	-2.522
<i>CRSA</i>	SAOUHSC_00386	<i>ssl3</i>	Staphylococcal superantigen-like 3	45.725	22.703	-2.014
<i>CRSA</i>	SAOUHSC_00389	<i>ssl4</i>	Staphylococcal superantigen-like 4	21.963	4.219	-5.205
<i>CRSA</i>	SAOUHSC_00390	<i>ssl5</i>	Staphylococcal superantigen-like 5	22.893	6.032	-3.795
<i>CRSA</i>	SAOUHSC_00392	<i>ssl7</i>	Staphylococcal superantigen-like 7	16.288	6.111	-2.666
<i>CRSA</i>	SAOUHSC_01127	<i>SAOUHSC01127</i>	Stap_Strp_tox_C domain-containing protein	40.755	15.699	-2.596
<i>CRSA</i>	SAOUHSC_02127	<i>sspP</i>	Staphopain A	16.784	4.371	-3.840

CRSA	SAOUHSC_02463	<i>hysA</i>	Hyaluronate lyase	9.819	4.207	-2.334
CRSA	SAOUHSC_00174	<i>SAOUHSC00174</i>	Lysostaphin	45.477	13.519	-3.364
CRSA	SAOUHSC_00645	<i>tarD</i>	Glycerol-3-phosphate cytidyltransferase	56.885	24.756	-2.298
CRSA	SAOUHSC_01452	<i>ald1</i>	Alanine dehydrogenase 1	44.620	17.322	-2.576
CRSA	SAOUHSC_02988	<i>SAOUHSC02988</i>	Accessory Sec system protein Asp1	22.115	7.329	-3.018
CRSA	SAOUHSC_02989	<i>asp3</i>	Accessory Sec system protein Asp3	18.220	7.014	-2.598
CRSA	SAOUHSC_00352	<i>SAOUHSC00352</i>	Integrase-like protein	12.956	2.731	-4.744
CRSA	SAOUHSC_00624	<i>SAOUHSC00624</i>	Integrase/recombinase	19.187	9.100	-2.108
CRSA	SAOUHSC_01515	<i>SAOUHSC01515</i>	Probable autolysin PH	1.584	2.259	2.259
CRSA	SAOUHSC_02023	<i>SAOUHSC02023</i>	Bifunctional autolysin	18.348	45.086	2.457
CRSA	SAOUHSC_02851	<i>cidA</i>	Holin-like protein CidA	3.010	18.944	6.293
CRSA	SAOUHSC_02532	<i>sarV</i>	HTH-type transcriptional regulator SarV	40.770	178.711	4.383
CRSA	SAOUHSC_02333	<i>sceD</i>	Probable transglycosylase SceD	32.300	15.888	-2.033
CRSA	SAOUHSC_02798	<i>sasG</i>	Surface protein G	44.126	10.993	-4.014
CRSA	SAOUHSC_02803	<i>fnbA</i>	Fibronectin-binding protein A	24.468	10.003	-2.446
CRSA	SAOUHSC_02802	<i>fnbB</i>	Fibronectin binding protein B	9.687	4.011	-2.415
CRSA	SAOUHSC_03002	<i>icaA</i>	Poly-beta-1,6-N-acetyl-D-glucosamine synthase	14.413	4.528	-3.183
CRSA	SAOUHSC_03003	<i>icaD</i>	Poly-beta-1,6-N-acetyl-D-glucosamine synthesis protein IcaD	27.679	11.696	-2.366
CRSA	SAOUHSC_00130	<i>isdI</i>	Heme oxygenase (staphylobilin-producing) 2	35.955	98.288	2.734
CRSA	SAOUHSC_01089	<i>isdG</i>	Heme oxygenase (staphylobilin-producing) 1	4.733	18.415	3.890
CRSA	SAOUHSC_01887	<i>ribBA</i>	Riboflavin biosynthesis protein RibBA	13.809	35.742	2.588
CRSA	SAOUHSC_01889	<i>ribD</i>	Riboflavin biosynthesis protein RibD	17.258	36.883	2.137
CRSA	SAOUHSC_00326	<i>SAOUHSC00326</i>	Deferrochelataase/peroxidase	10.781	32.316	2.997
CRSA	SAOUHSC_02996	<i>SAOUHSC02996</i>	Accessory Sec system protein Asp3	7.014	18.220	9.636
CRSA	SAOUHSC_00173	<i>azoR</i>	FMN-dependent NADH:quinone oxidoreductase	119.534	30.936	-3.864
CRSA	SAOUHSC_00320	<i>SAOUHSC00320</i>	FMN-dependent NADPH-azoreductase	284.412	140.762	-2.021
CRSA	SAOUHSC_02258	<i>SAOUHSC02258</i>	Nitroreductase domain-containing protein	417.756	173.132	-2.413
CRSA	SAOUHSC_02683	<i>SAOUHSC02683</i>	Assimilatory nitrite reductase [NAD(P)H],	8.555	1.623	-5.271

			small subunit			
<i>CRSA</i>	SAOUHSC _01504	<i>SAOUHSC01</i> 504	Ferredoxin	57.389	18.959	-3.027
<i>CRSA</i>	SAOUHSC _01177	<i>rpoZ</i>	DNA-directed RNA polymerase subunit omega	63.898	198.90 9	3.113
<i>CRSA</i>	SAOUHSC _01341	<i>sbcD</i>	Nuclease SbcCD subunit D	19.245	38.510	2.001
<i>CRSA</i>	SAOUHSC _01476	<i>SAOUHSC01</i> 476	MazG domain-containing protein	17.148	36.316	2.118
<i>CRSA</i>	SAOUHSC _02791	<i>SAOUHSC02</i> 791	Pyrophosphohydrolase	34.668	90.060	2.598
<i>CRSA</i>	SAOUHSC _00064	<i>norG</i>	HTH-type transcriptional regulator NorG	33.237	9.728	-3.417
<i>CRSA</i>	SAOUHSC _02609	<i>fosB</i>	Metallothiol transferase FosB	17.401	6.487	-2.682
<i>CRSA</i>	SAOUHSC _00497	<i>SAOUHSC00</i> 497	HTH-type transcriptional regulator NorG	25.684	5.900	-4.353
<i>MRSA</i>	SAOUHSC _00054	<i>SAOUHSC00</i> 054	Uncharacterized lipoprotein	1.865	0.849	-2.198
<i>MRSA</i>	SAOUHSC _02615	<i>SAOUHSC02</i> 615	UPF0060 membrane protein	30.843	10.021	-3.078
<i>MRSA</i>	SAOUHSC _00667	<i>SAOUHSC00</i> 667	ABC transporter ATP- binding protein	5.663	1.374	-4.122
<i>MRSA</i>	SAOUHSC _00746	<i>SAOUHSC00</i> 746	Iron-regulated surface determinant protein F	6.052	2.692	-2.248
<i>MRSA</i>	SAOUHSC _01311	<i>SAOUHSC01</i> 311	ABC transporter, ATP- binding protein	2.445	1.151	-2.123
<i>MRSA</i>	SAOUHSC _02640	<i>hrtA</i>	Putative hemin import ATP-binding protein HrtA	310.449	117.90 1	-2.633
<i>MRSA</i>	SAOUHSC _02763	<i>cntF</i>	Metal-staphylopine import system ATP- binding protein CntF	11.681	5.408	-2.160
<i>MRSA</i>	SAOUHSC _00261	<i>essB</i>	Type VII secretion system protein EssB	15.662	7.151	-2.190
<i>MRSA</i>	SAOUHSC _00269	<i>essG</i>	Type VII secretion system protein EsaG	5.585	2.661	-2.099
<i>MRSA</i>	SAOUHSC _02167	<i>scn</i>	Staphylococcal complement inhibitor	308.171	118.72 7	-2.596
<i>MRSA</i>	SAOUHSC _00174	<i>SAOUHSC00</i> 174	Holin, phage phi LC3 family	59.635	26.752	-2.882
<i>MRSA</i>	SAOUHSC _01462	<i>gpsB</i>	Cell cycle protein GpsB	3447.48 8	1288.8 87	-2.675
<i>MRSA</i>	SAOUHSC _02170	<i>SAOUHSC02</i> 170	Peptidoglycan hydrolase	45.439	15.451	-2.941
<i>MRSA</i>	SAOUHSC _02464	<i>SAOUHSC02</i> 464	Lysostaphin	183.486	90.890	-2.019
<i>MRSA</i>	SAOUHSC _02834	<i>srtA</i>	Sortase A	575.820	226.99 4	-2.537
<i>MRSA</i>	SAOUHSC _00352	<i>SAOUHSC00</i> 352	Integrase-like protein	35.269	12.185	-2.894
<i>MRSA</i>	SAOUHSC _02239	<i>SAOUHSC02</i> 239	Integrase	148.588	68.424	-2.172
<i>MRSA</i>	SAOUHSC _01515	<i>SAOUHSC01</i> 515	Probable autolysin PH	0.090	1.078	11.991
<i>MRSA</i>	SAOUHSC _02710	<i>hlgB</i>	Gamma-hemolysin component B	5.618	13.098	2.332
<i>MRSA</i>	SAOUHSC _02532	<i>sarV</i>	HTH-type transcriptional regulator SarV	11.201	27.235	2.432

<i>MRSA</i>	SAOUHSC _02566	<i>sarR</i>	HTH-type transcriptional regulator SarR	334.029	1013.7 89	3.035
<i>MRSA</i>	SAOUHSC _02669	<i>sarZ</i>	HTH-type transcriptional regulator SarZ	237.909	478.40 6	2.011
<i>MRSA</i>	SAOUHSC _00666	<i>sarA</i>	Transcriptional regulator SarA	1.632	0.503	-3.248
<i>MRSA</i>	SAOUHSC _02576	<i>ssaA</i>	Secretory antigen SsaA	27.944	12.283	-2.275
<i>MRSA</i>	SAOUHSC _02970	<i>argR</i>	Arginine repressor	8.434	3.492	-2.415
<i>MRSA</i>	SAOUHSC _00130	<i>isdI</i>	Heme oxygenase (staphylobilin-producing) 2	75.358	283.99 3	3.769
<i>MRSA</i>	SAOUHSC _00365	<i>ahpC</i>	Alkyl hydroperoxide reductase C	851.104	2391.3 06	2.810
<i>MRSA</i>	SAOUHSC _00487	<i>hslO</i>	33 kDa chaperonin	92.258	193.57 4	2.098
<i>MRSA</i>	SAOUHSC _01139	<i>bshC</i>	Putative cysteine ligase BshC	13.240	29.338	2.216
<i>MRSA</i>	SAOUHSC _01282	<i>SAOUHSC01</i> 282	Glutathione peroxidase	181.736	400.79 6	2.205
<i>MRSA</i>	SAOUHSC _00833	<i>SAOUHSC00</i> 833	Nitroreductase domain- containing protein	270.852	126.06 8	-2.148
<i>MRSA</i>	SAOUHSC _00873	<i>SAOUHSC00</i> 873	NifU domain-containing protein	112.235	49.678	-2.259
<i>MRSA</i>	SAOUHSC _01504	<i>SAOUHSC01</i> 504	Probable autolysin PH	1.078	0.090	-2.532
<i>MRSA</i>	SAOUHSC _01655	<i>fur</i>	Ferric uptake regulation protein	68.475	30.597	-2.238
<i>MRSA</i>	SAOUHSC _02369	<i>rpoE</i>	Probable DNA-directed RNA polymerase subunit delta	65.090	131.07 4	2.014
<i>MRSA</i>	SAOUHSC _01036	<i>rpoY</i>	DNA-directed RNA polymerase subunit epsilon	38.363	165.32 3	4.309
<i>MRSA</i>	SAOUHSC _01177	<i>rpoZ</i>	DNA-directed RNA polymerase subunit omega	34.167	107.81 9	3.156
<i>MRSA</i>	SAOUHSC _01469	<i>nth</i>	Endonuclease III	367.236	816.77 5	2.224
<i>MRSA</i>	SAOUHSC _02334	<i>SAOUHSC02</i> 334	Single-stranded DNA- binding protein	64.510	171.57 3	2.660
<i>MRSA</i>	SAOUHSC _00444	<i>SAOUHSC00</i> 444	Nucleoid-associated protein	89.040	238.50 6	2.679
<i>MRSA</i>	SAOUHSC _02861	<i>SAOUHSC02</i> 861	Methylated-DNA-- protein-cysteine methyltransferase	71.482	170.93 3	2.391
<i>MRSA</i>	SAOUHSC _00998	<i>fntA</i>	Teichoic acid D-alanine hydrolase	7.005	2.629	-2.665
<i>MRSA</i>	SAOUHSC _00992	<i>SAOUHSC00</i> 992	Transcriptional regulator, MarR family, putative	56.720	21.207	-2.675