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Supplementary Information for

Architecture of the mycobacterial succinate dehydrogenase with a membrane-embedded Rieske FeS cluster

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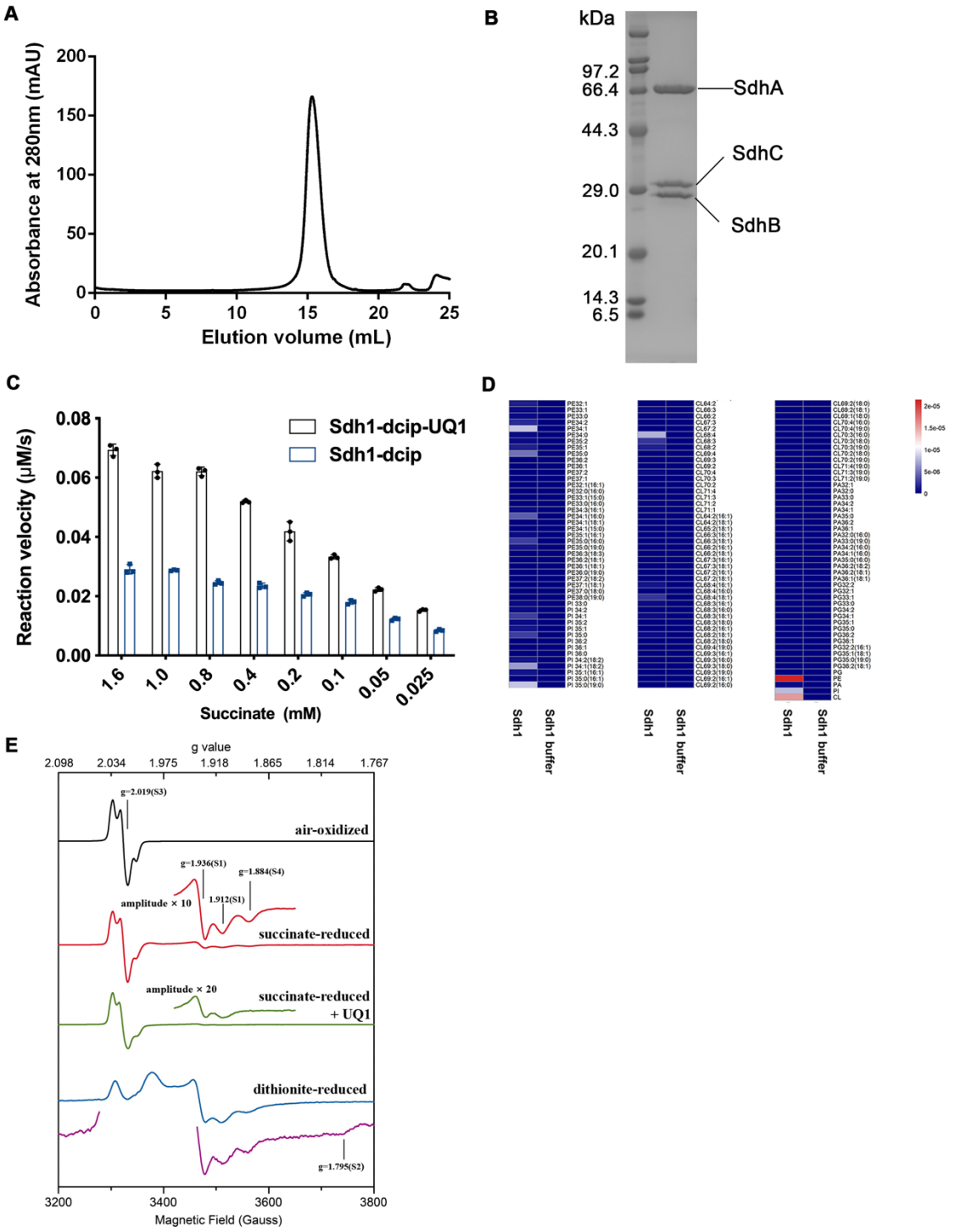


Fig. S1. Purification and component identification of the *Msm* Sdh1. (*A*) Superose 6 increase size-exclusion chromatography elution profile of the Sdh1 complex. (*B*) SDS-PAGE of Sdh1 after size-exclusion. The three components were further confirmed by mass spectrometry. (*C*) Succinate dehydrogenase 1 activity

in the wild-type. Mean values are from technical triplicates and error bars using standard deviation are shown. (*D*) Identification of lipids in the purified protein sample and buffer used in protein purification by mass spectrometry. (*E*) X-band EPR spectroscopic characterization of Sdh1. Isolated Sdh1 protein (air-oxidized) showed characteristic peaks corresponding to oxidized S3 ([3Fe-4S]) cluster at crossover g-value of 2.019 (black trace); Protein sample following addition of 200 μM sodium succinate, frozen after 5 minutes incubation, showed signals arising from reduced S1 ([2Fe-2S], g-values of 1.936 and 1.912) cluster and reduced S4 ([2Fe-2S], g-value of 1.884) cluster (red trace); The succinate-reduced Sdh1 was further incubated with 500 μM UQ1 and the EPR signal corresponding to reduced S4 cluster (g-value of 1.884) was vanished with the addition of UQ1 (green trace); Addition of 1mM dithionite resulted in complete loss of the signal attributable to S3 ([3Fe-4S]) (blue trace) and signal associated with reduced S2 ([4Fe-4S], g-value of 1.795) was observed with higher microwave power (10mW) (purple trace). Spectra were scaled correspondingly to highlight different clusters (red trace and green trace) and part of signals have been omitted to highlight the signal of reduced S2 in dithionite-reduced sample (purple trace).

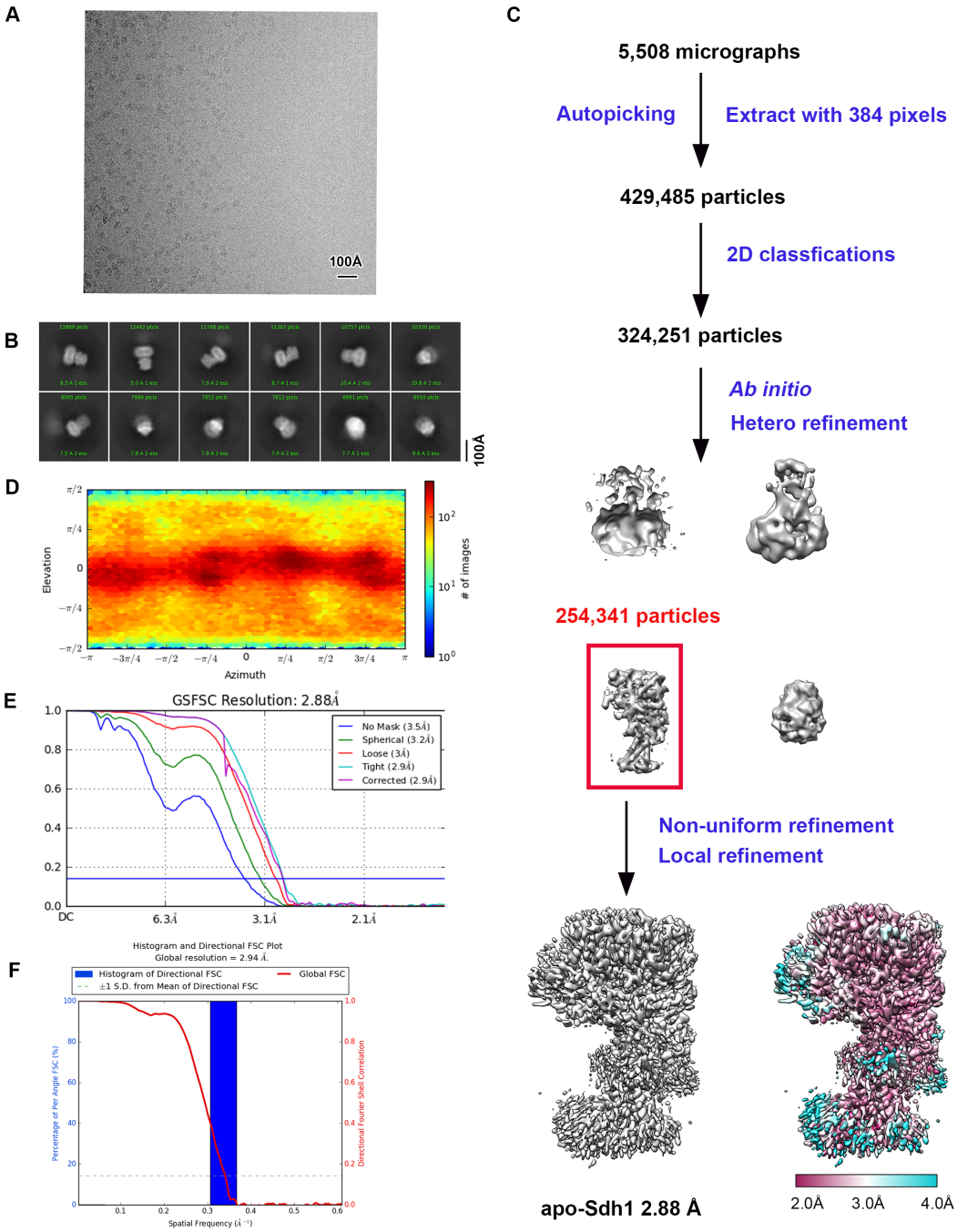


Fig. S2. Cryo-EM data processing of apo-*Msm* Sdh1. (*A*) Representative cryo-EM image of apo-Sdh1. (*B*) Representative 2D classification averages in different orientations. (*C*) Workflow of cryo-EM data processing. The map is colored according to the local resolution. (*D*) Angular distribution heatmap of particles used for the refinement. (*E*) Fourier shell correlation (FSC) of the final 3D reconstruction. (*F*) 3DFSC histogram of the final map.

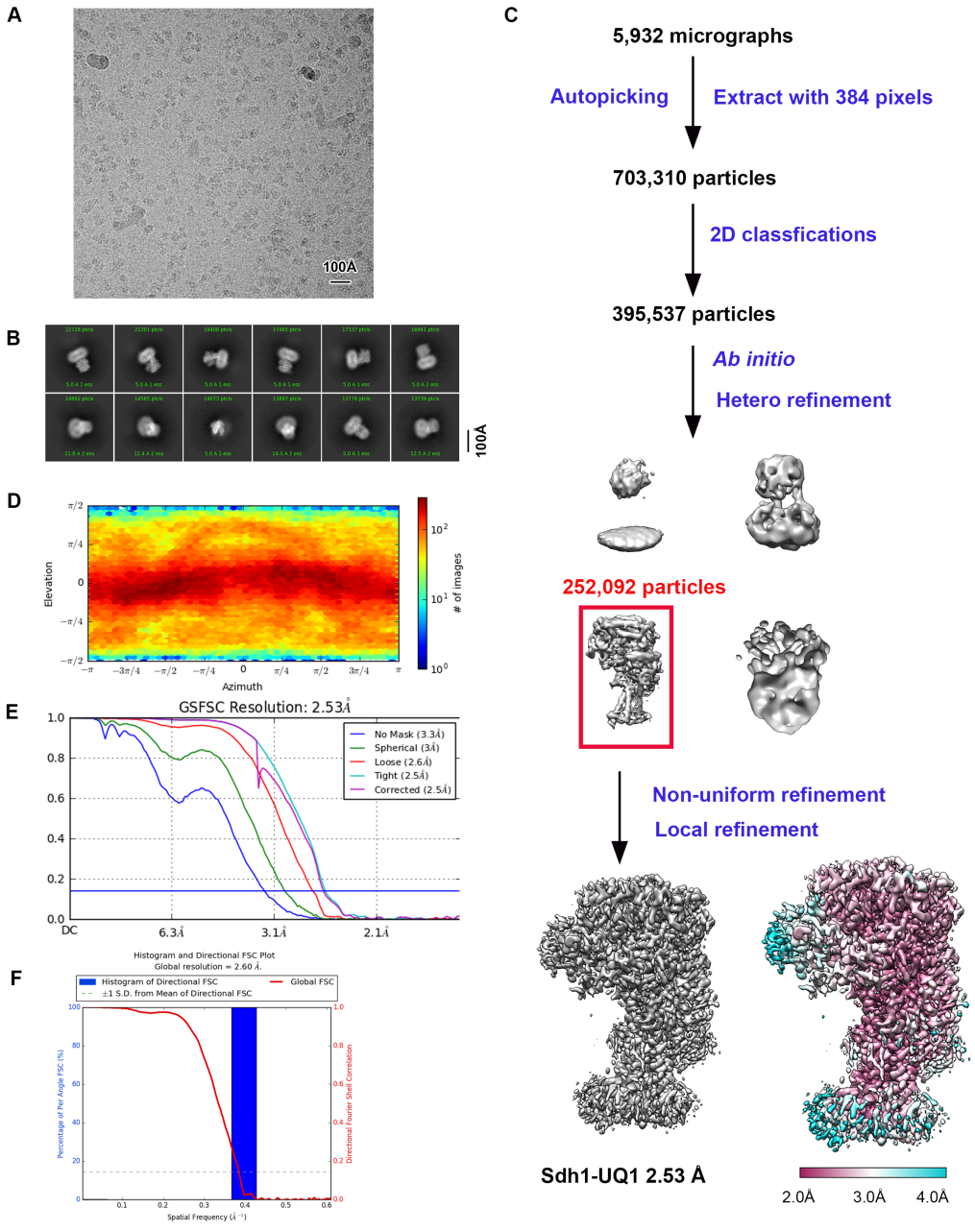


Fig. S3. Cryo-EM data processing of *Msm* Sdh1 bound with UQ1. (*A*) Representative cryo-EM image of the Sdh1-UQ1 complex. (*B*) Representative 2D classification averages showing the complex in different orientations. (*C*) Workflow of the cryo-EM data processing for the Sdh1-UQ1 complex. The map is colored according to local resolution. (*D*) Angular distribution heatmap of particles used for the refinement. (*E*) Fourier shell correlation (FSC) of the final 3D reconstruction. (*F*) 3DFSC histogram of the final map.

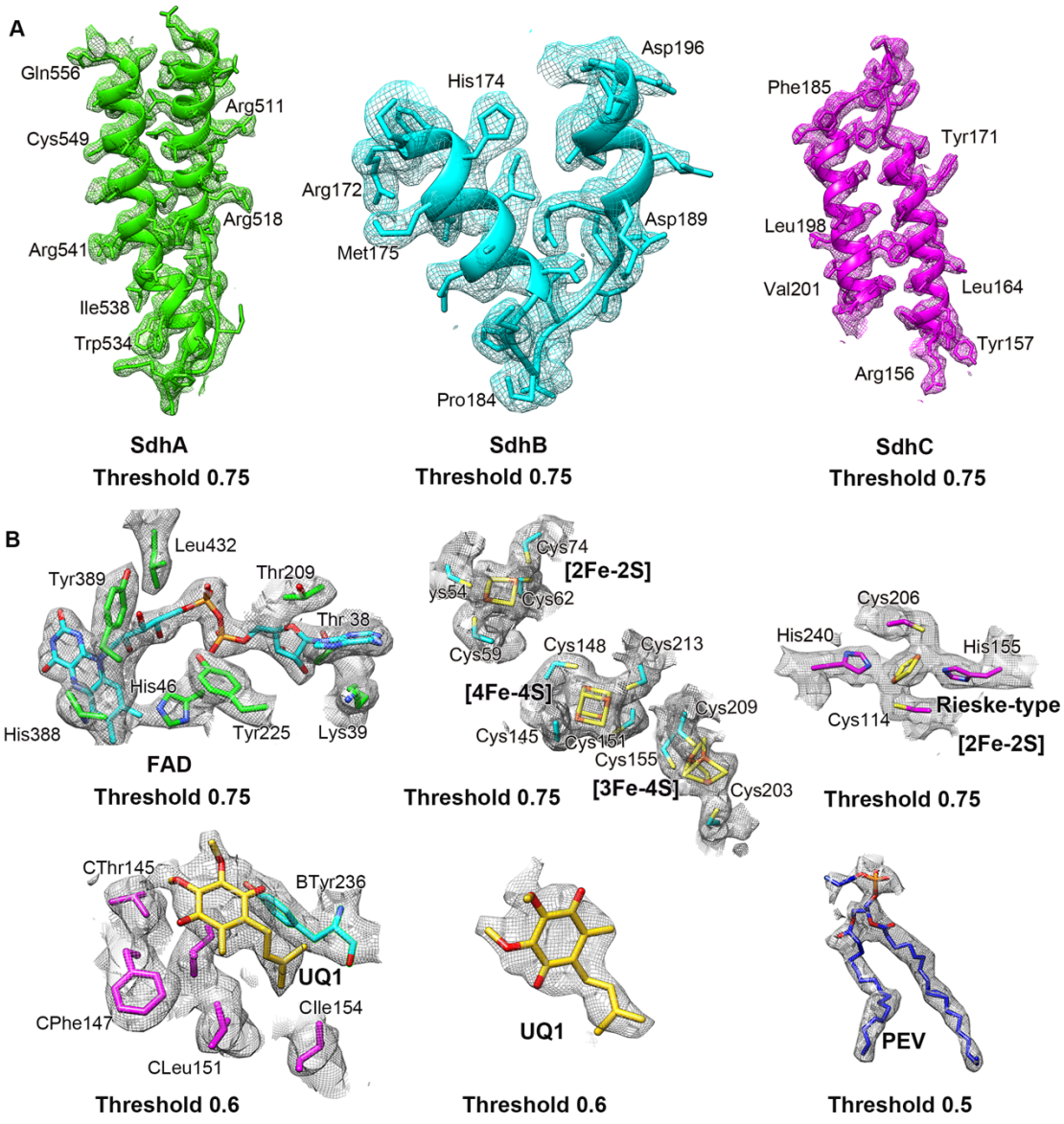
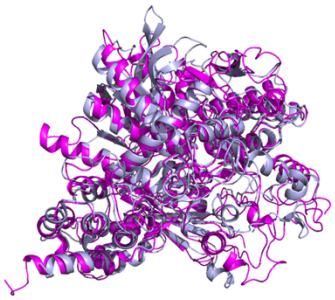


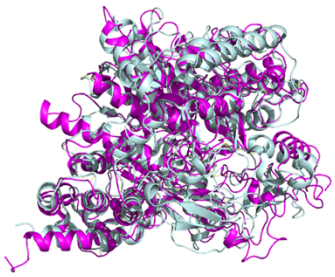
Fig. S4. Cryo-EM maps for the *Msm* Sdh1 structures. (*A*) Cryo-EM densities of representative helixes from the different subunits of apo-Sdh1 at 2.88-Å resolution. (*B*) Cryo-EM densities of the prosthetic groups (FAD, Fe-S clusters), UQ1 with nearby amino acids, UQ1 alone, and phospholipid at 2.53-Å resolution.

A



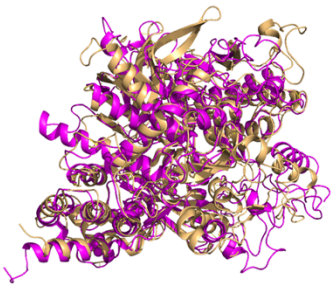
M. smegmatis Sdh1 vs Sdh2

RMSD of 1.006 Å for 499 Cα atoms



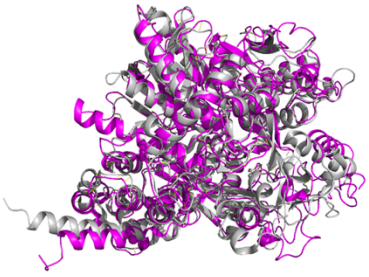
M. smegmatis Sdh1 vs *W. succinogenes* QFR

RMSD of 1.187 Å for 545 Cα atoms



M. smegmatis Sdh1 vs *Sus scrofa* SQR

RMSD of 1.307 Å for 542 Cα atoms



M. smegmatis Sdh1 vs *E. coli* QFR

RMSD of 1.263 Å for 540 Cα atoms

B

Strain	Query Cover	E value	Per. Ident
M. smegmatis Sdh2A	89%	9e-106	38.75%
W. succinogenes FrdA	90%	2e-77	32.18%
S. scrofa SdhA	85%	7e-86	33.16%
E. coli FrdA	84%	6e-88	34.80%

Strain	Query Cover	E value	Per. Ident
M. smegmatis Sdh2B	75%	2e-18	27.36%
W. succinogenes FrdB	90%	3e-26	28.39%
S. scrofa SdhB	90%	2e-25	27.83%
E. coli FrdB	81%	4e-24	27.05%

Fig. S5. Subunits SdhA and B of *Msm* Sdh1 and comparison with type A-D complex IIs. (*A*) Structural superimposition of *Msm* Sdh1 (magenta) with *Msm* Sdh2 (type A, PDB code 6LUM, lightblue), *Wolinella succinogenes* QFR (type B, PDB code 1E7P, palecyan), *Sus scrofa* SQR, (type C, PDB code 1ZOY, orange), and *Escherichia coli* QFR (type D, PDB code 1L0V, grey). (*B*) Sequences alignment of *Msm* Sdh1 with *Msm* Sdh2, *W. succinogenes* QFR, *Sus scrofa* SQR and *E. coli* QFR, respectively.

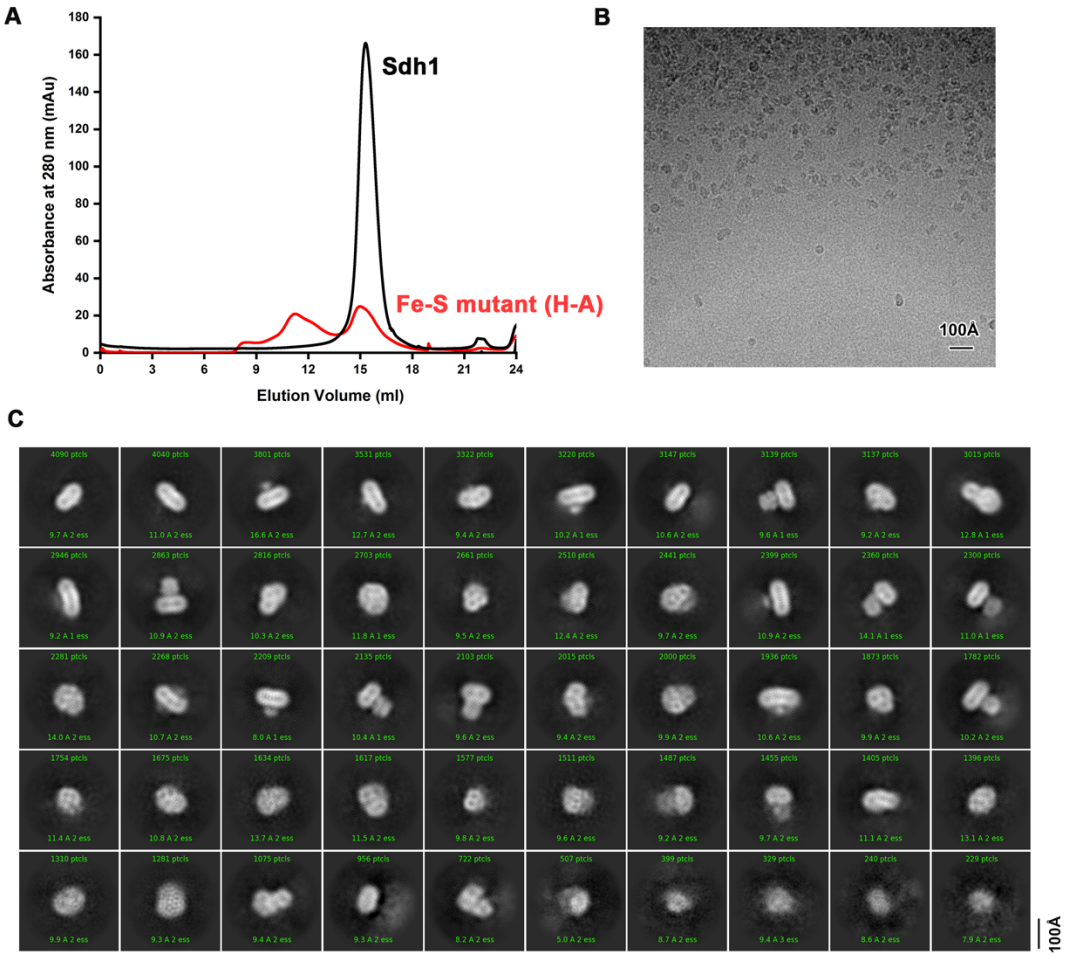


Fig. S6. Purification and cryo-EM data processing of Fe-S mutant (H-A). (*A*) Superose 6 increase size-exclusion chromatography elution profile of the wild-type Sdh1 (black) and Fe-S mutant (H-A) (red). (*B*) Representative cryo-EM image of Fe-S mutant (H-A). (*C*) Representative 2D classification averages in different orientations. For the dataset of Fe-S mutant (H-A), a total of 7,048 image stacks were recorded. 546,799 particles were initially autopicked using the 3D map projections of wild-type Sdh1 as the templates, but only 10,136 complete particles were remained.

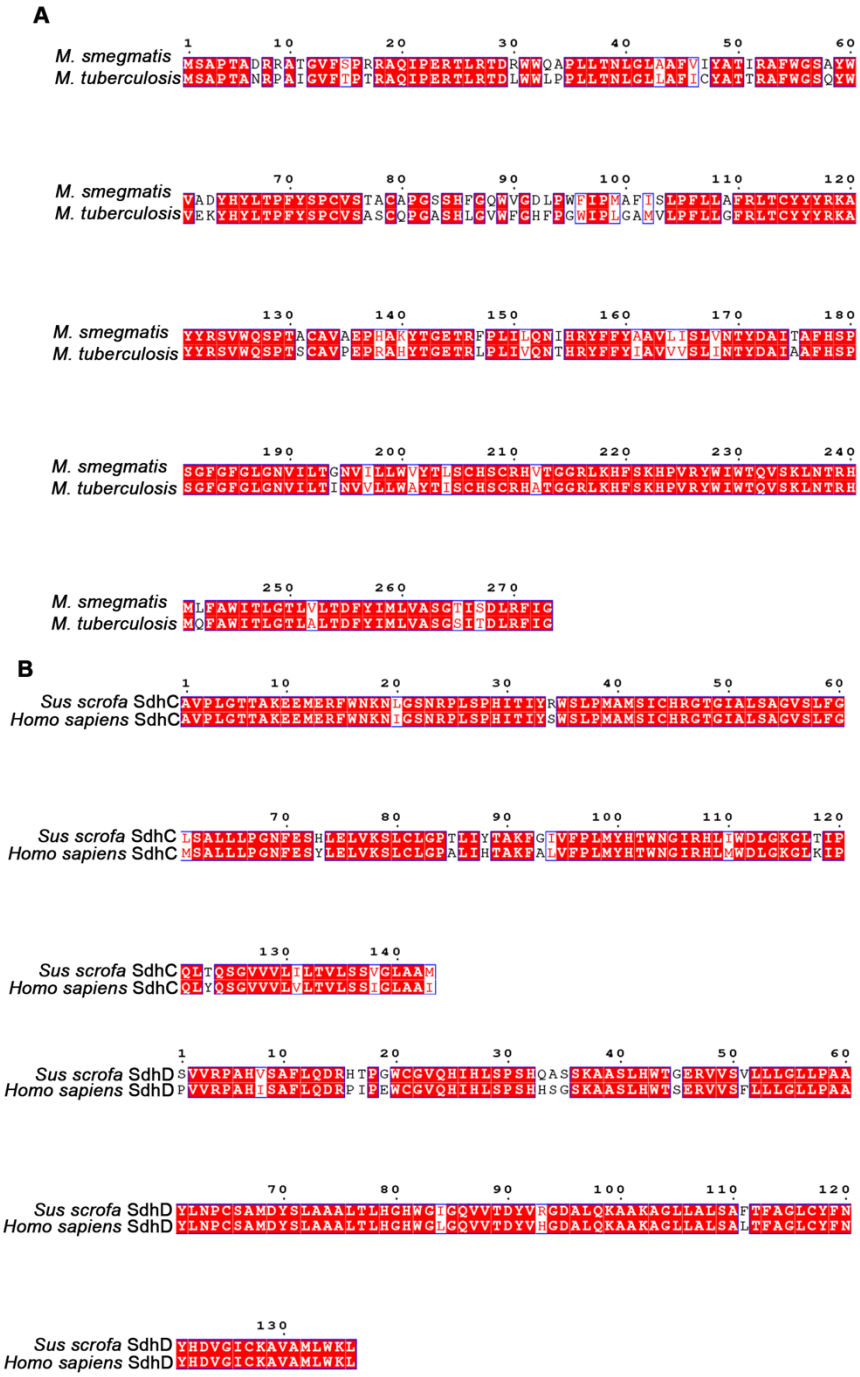


Fig. S7. Sequence alignments of subunits involved in the quinone-binding site. (*A*) Sequence alignment of SdhC from *M. smegmatis* and *M. tuberculosis*. (*B*) Sequence alignments of SdhC and SdhD from *Sus scrofa* and *Homo sapiens*, respectively.

Table S1. Statistics for data collection, image processing and model building

	Apo-Sdh1	Sdh1-UQ1
PDB entry	7D6X	7D6V
EMDB entry	EMD-30595	EMD-30594
Data collection and processing		
Magnification	165,000	165,000
Voltage (keV)	300	300
Electron exposure (e^{-}/Å^2)	60.00	60.00
Defocus range (μm)	-1.2 to -2.2	-1.2 to -2.2
Pixel size (Å)	0.82	0.82
Symmetry imposed	C1	C1
Initial particle images (no.)	429,485	703,310
Final particle images (no.)	254,341	252,092
Map global resolution (Å)	2.88	2.53
Global resolution FSC threshold	0.143	0.143
Map local resolution range (Å)	2.0-6.5	2.0-6.0
Local resolution FSC threshold	0.5	0.5
Refinement		
Model resolution (Å)	2.8	2.4
FSC threshold	0.143	0.143
Model resolution range (Å)	∞ to 2.8	∞ to 2.4
Map sharpening *B* factor (Å^2)	-78.0	-74.7
Model composition		
Non-hydrogen atoms	8723	8793
Protein residues	1097	1104
Ligands	6	7
B factors (Å^2)		
Protein	47.85	21.28
Ligand	32.08	17.23
R.m.s. deviations		
Bond lengths (Å)	0.036	0.007
Bond angles (°)	1.735	1.047
Validation		
MolProbity score	2.14	1.99
Clashscore	10.36	8.5
Poor rotamers (%)	0.22	0.00
Ramachandran plot		
Outliers	0.37	0.09
Allowed	11.94	9.31
Favored	87.70	90.60
EMRinger score		
Model vs. Data		
CC(mask)	0.88	0.89
CC(volume)	0.85	0.84
Mean CC for ligands	0.87	0.87

Table S2. Summary of the model for apo-Sdh1

Subunit Name	Chain	Total residues/range built	% atomic model	Cofactors	Resolution (Å)
SdhA	A	4-281, 316-631	94.0	FAD	2.0~5.0
SdhB	B	2-240	96.0	2Fe-2S 3Fe-4S 4Fe-4S	2.0~4.0
SdhC	C	10-273	96.7	2Fe-2S	2.0~6.0

Table S3. Summary of the model for the Sdh1-UQ1 complex

Subunit Name	Chain	Total residues/range built	% atomic model	Cofactors	Resolution (Å)
SdhA	A	4-285, 313-631	95.1	FAD	2.0-5.0
SdhB	B	2-240	96.0	2Fe-2S 3Fe-4S 4Fe-4S	2.0~3.5
SdhC	C	10-273	96.7	2Fe-2S	2.0~6.0

Table S4. Comparison of succinate dehydrogenase activity for *M. smegmatis* Sdh1

Strain	V_{max} (μM/s)	K_m(μM)	k_{cat} (s^{-1})
Sdh1-dcip	0.029 ± 0.00057	67.66 ± 6.05	0.37 ± 0.01
Sdh1-dcip-UQ1	0.068 ± 0.0013	107.90 ± 7.21	0.87 ± 0.02
Fe-S mutant (H-A)-dcip-UQ1	0.019 ± 0.00040	57.44 ± 5.75	0.24 ± 0.01

Table S5. Primers used in molecular cloning

p261-F	5’-catttgggtgggataa-3’
p261-R	5’-agcattggattggaagta-3’
Msdh1-p-F	5’-tacttccaatccaatgctttgtccaccacgacagagttcgctg-3’
0419-R	5’-tcagtggtgatggtgatggtgatgatggtgatggtggccaatgaatctcaagtcgg-3’
0418-F	5’-catcaccatcaccactgacaggccgactgccaggtgcgc-3’
Msdh1-p-R	5’-ttatcccacccaaatgtcagggccgtgcggcgagtt-3’
155H-A-F	5’-gaacattgcccgatacttcttttacgc-3’
155H-A-R	5’-gtatcgggcaatgttctgcaggatc-3’
240H-A-F	5’-gcgggccatgttgttcgcctggatcac-3’
240H-A-R	5’-aacatggcccgcgtgttgagtttgct-3’