Supplementary Materials for

Diversity of *glpK* gene and its effect on drug sensitivity in *Mycobacterium bovis*

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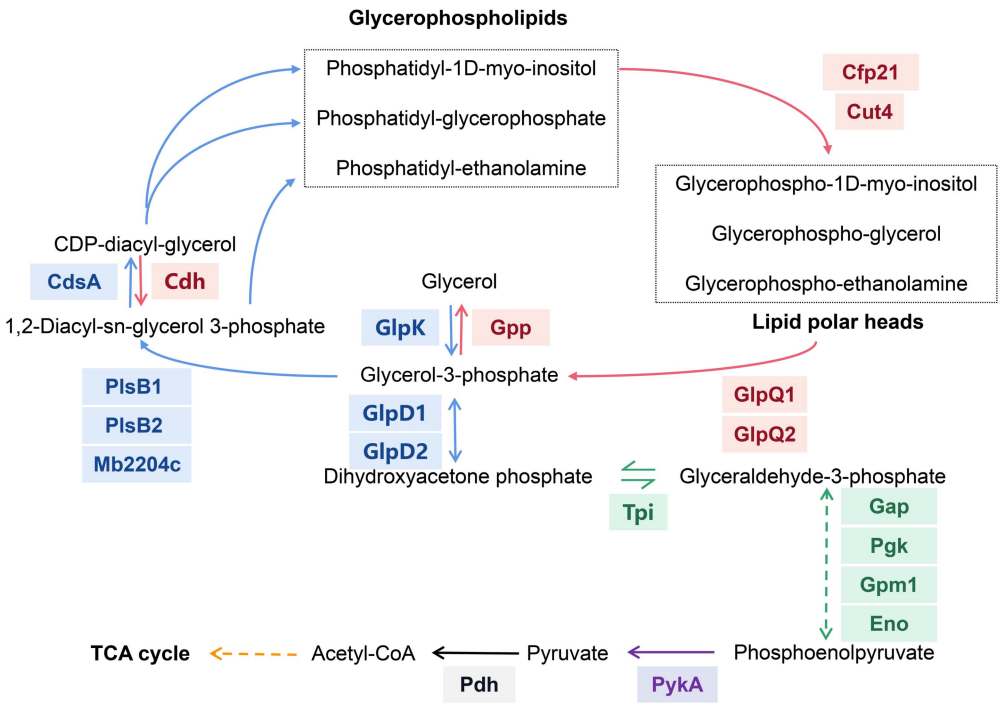
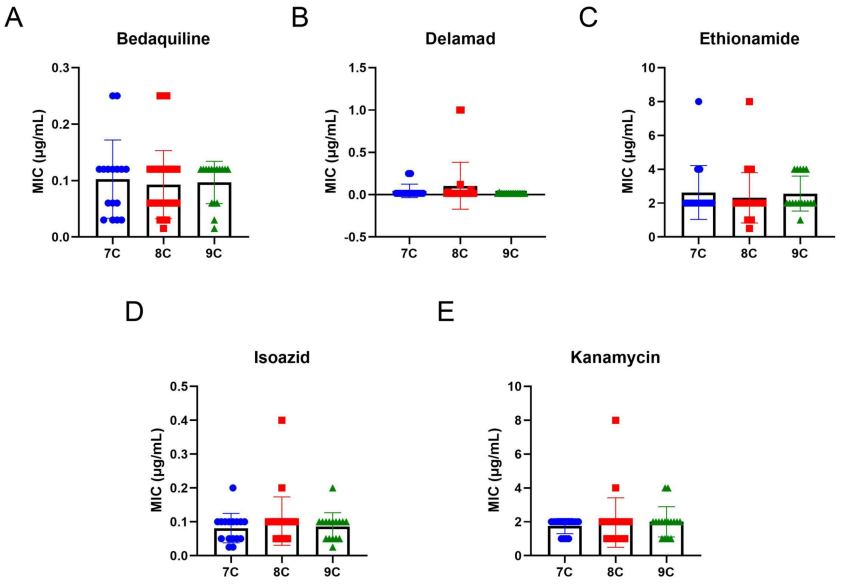


Supplementary Figure 1 Glycerophospholipid recycling and glycerol metabolic pathways of *Mycobacterium bovis*. Enzymes and their encoding genes are color coded to reflect their dedicated pathways: glycerophospholipid catabolism (red), glycerophospholipid anabolism (blue), glycolysis and gluconeogenesis (green), glycolysis (purple). Cdh, CDP-diacylglycerol pyrophosphatase; CdsA, phosphatidate cytidylyltransferase; Cfp21, cutinase precursor; Cut4, cutinase precursor; Eno, enolase; Gap, glyceraldehyde 3-phosphate dehydrogenase; GlpD, glycerol-3-phosphate dehydrogenase; GlpK, glycerol kinase; GlpQ1, glycerophosphodiester phosphodiesterase; GlpQ2, glycerophosphodiester phosphodiesterase; Gpm1, 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase; Gpp, glycerol phosphate phosphatase; Mb2204c, 1-acylglycerol-3-phosphate O-acyltransferase; Pdh, pyruvate dehydrogenase; Pgk, phosphoglycerate kinase; PlsB1, acyltransferase; PlsB2, glycerol-3-phosphate acyltransferase; PykA, pyruvate kinase; TCA cycle, tricarboxylic acid cycle; Tpi, triosephosphate isomerase.



Supplementary Figure 2 The *glpK* mutants had no significant difference in the sensitivity to five antibiotics. (A-E) MIC was conducted for different *glpK* mutants of *M. bovis* isolates against (A) Bedaquiline, (B) Delamanid, (C) Ethionamide, (D) Isoniazid, and (E) Kanamycin. Significant differences of MIC were calculated by using unpaired t test (2 tailed).

Supplementary Table 1 Minimal inhibitory concentration distribution (expressed in μg/mL, with respective cut-off points) of 14 drugs against clinical *Mycobacterium bovis* isolates from China.

Isolate	*GlpK* HT	AMK (1.0)	BDQ (0.25)	CFZ (1.0)	DLM (0.2)	EMB (5.0)	ETH (5.0)	INH (0.2)
B1	10C	<=0.25	0.12	0.12	<=0.015	1	2	0.05
B2	10C	0.5	0.12	0.25	<=0.015	1	2	0.05
B3	11C	<=0.25	0.06	0.12	<=0.015	1	2	0.05
B4	11C	0.5	0.12	0.5	<=0.015	2	4	0.1
B5	7C	<=0.25	0.12	0.12	<=0.015	1	2	0.05
B6	7C	<=0.25	0.25	0.12	<=0.015	1	2	0.1
B7	7C	<=0.25	0.06	0.12	<=0.015	1	2	<=0.025
B8	7C	<=0.25	0.06	0.12	<=0.015	1	2	0.1
B9	7C	<=0.25	0.12	0.25	<=0.015	1	2	0.1
B10	7C	<=0.25	0.03	0.12	<=0.015	1	2	<=0.025
B11	7C	<=0.25	0.12	0.12	<=0.015	1	**8**	0.1
B12	7C	<=0.25	0.06	0.12	<=0.015	1	2	0.05
B13	7C	<=0.25	0.25	0.25	**0.25**	1	2	0.2
B14	7C	<=0.25	0.03	<=0.06	<=0.015	0.5	2	0.05
B15	7C	<=0.25	0.12	0.12	<=0.015	1	2	0.1
B16	7C	<=0.25	0.12	0.12	<=0.015	1	4	0.1
B17	7C	0.5	0.12	0.25	**0.25**	2	4	0.1
B18	7C	<=0.25	0.03	<=0.06	<=0.015	0.5	2	0.05
B19	7C	<=0.25	0.03	<=0.06	<=0.015	0.5	2	0.05
B20	7C	<=0.25	0.12	<=0.06	<=0.015	1	2	0.1
B21	8C	<=0.25	0.03	<=0.06	<=0.015	0.5	1	0.05
B22	8C	<=0.25	0.06	0.12	<=0.015	1	2	0.1
B23	8C	<=0.25	0.12	0.25	<=0.015	1	2	0.1
B24	8C	<=0.25	0.06	0.12	<=0.015	1	2	0.05
B25	8C	<=0.25	0.06	0.12	<=0.015	1	2	0.05
B26	8C	0.5	0.12	0.5	0.12	1	2	0.1
B27	8C	<=0.25	0.12	0.12	<=0.015	1	2	0.1
B28	8C	<=0.25	0.06	0.12	<=0.015	1	2	0.1
B29	8C	<=0.25	0.12	0.12	<=0.015	2	**8**	0.1
B30	8C	0.5	0.12	0.12	<=0.015	1	4	0.1
B31	8C	<=0.25	0.12	0.25	0.06	1	2	0.1
B32	8C	<=0.25	0.06	<=0.06	<=0.015	0.5	2	0.05
B33	8C	<=0.25	0.12	0.25	<=0.015	1	2	0.1
B34	8C	<=0.25	<=0.015	<=0.06	0.03	0.25	0.5	0.05
B35	8C	<=0.25	0.06	<=0.06	<=0.015	1	1	0.05

B36	8C	<=0.25	0.12	<=0.06	<=0.015	1	2	0.1
B37	8C	<=0.25	0.06	0.25	<=0.015	2	2	0.2
B38	8C	0.5	0.25	0.5	**>1**	2	2	0.1
B39	8C	<=0.25	0.03	<=0.06	<=0.015	0.25	1	0.05
B40	8C	<=0.25	0.06	<=0.06	<=0.015	0.5	2	0.1
B41	8C	0.5	0.12	0.5	<=0.015	1	2	0.1
B42	8C	0.5	0.25	0.5	**>1**	1	4	**0.4**
B43	8C	<=0.25	0.06	0.12	<=0.015	1	4	0.1
B44	8C	0.5	0.03	0.25	<=0.015	1	2	0.1
B45	9C	0.5	0.12	0.25	<=0.015	2	2	0.05
B46	9C	<=0.25	0.12	0.12	<=0.015	2	4	0.1
B47	9C	<=0.25	<=0.015	<=0.06	<=0.015	1	2	0.05
B48	9C	0.5	0.12	0.25	0.03	2	4	0.1
B49	9C	<=0.25	0.03	0.12	<=0.015	0.25	1	<=0.025
B50	9C	0.5	0.12	0.25	<=0.015	1	4	0.1
B51	9C	0.5	0.12	0.12	<=0.015	2	4	0.1
B52	9C	0.5	0.12	0.25	<=0.015	1	2	0.1
B53	9C	0.5	0.12	0.25	<=0.015	2	2	0.1
B54	9C	<=0.25	0.12	0.12	<=0.015	1	2	0.05
B55	9C	0.5	0.12	0.25	<=0.015	2	2	0.1
B56	9C	0.5	0.06	0.25	<=0.015	2	2	0.1
B57	9C	0.5	0.12	1	<=0.015	1	4	0.2
B58	9C	<=0.25	0.06	0.25	<=0.015	1	2	0.05
B59	9C	0.5	0.12	0.25	<=0.015	1	2	0.1
B60	9C	<=0.25	0.06	0.12	<=0.015	1	2	0.05
Number of resistant isolates		0	0	0	4	0	2	1
Proportion of resistant isolates		0.00%	0.00%	0.00%	6.67%	0.00%	3.33%	1.67%
Isolate	***GlpK* HT**	**KAN (2.5)**	**LEV (1.0)**	**LZD (1.0)**	**MXF (0.5)**	**PAS (4.0)**	**RFB (0.5)**	**RIF (1.0)**
B1	10C	2	0.5	0.5	0.5	0.25	<=0.06	0.25
B2	10C	2	0.5	0.5	0.25	0.25	<=0.06	0.25
B3	11C	2	0.5	0.5	0.25	0.25	0.12	0.25
B4	11C	2	0.5	0.5	0.5	1	0.12	0.25
B5	7C	2	0.25	0.5	0.25	0.25	<=0.06	0.12
B6	7C	2	0.25	0.5	0.25	<=0.12	<=0.06	0.12
B7	7C	2	0.25	0.25	0.12	<=0.12	<=0.06	<=0.06
B8	7C	2	0.25	0.25	0.12	<=0.12	<=0.06	0.25
B9	7C	2	0.25	0.5	0.25	0.25	<=0.06	0.25
B10	7C	2	0.25	0.25	0.25	<=0.12	<=0.06	<=0.06
B11	7C	<=1	0.25	0.5	0.25	<=0.12	<=0.06	<=0.06
B12	7C	<=1	0.25	0.25	0.25	0.25	0.12	0.25
B13	7C	2	0.5	0.5	0.5	0.5	<=0.06	0.12

B14	7C	<=1	0.25	0.25	0.12	<=0.12	<=0.06	<=0.06
B15	7C	2	0.25	0.5	0.12	<=0.12	<=0.06	<=0.06
B16	7C	2	0.5	0.5	0.25	1	0.12	0.25
B17	7C	2	0.5	0.5	0.5	1	<=0.06	0.12
B18	7C	<=1	0.25	0.25	0.12	<=0.12	<=0.06	<=0.06
B19	7C	2	0.25	0.25	0.12	<=0.12	<=0.06	<=0.06
B20	7C	2	0.25	0.25	0.25	<=0.12	<=0.06	0.25
B21	8C	<=1	<=0.12	0.12	0.12	<=0.12	<=0.06	<=0.06
B22	8C	2	0.5	0.5	0.25	0.5	<=0.06	<=0.06
B23	8C	**4**	0.25	0.5	0.25	0.25	<=0.06	<=0.06
B24	8C	2	0.25	0.25	0.25	<=0.12	<=0.06	<=0.06
B25	8C	<=1	0.25	0.25	0.12	<=0.12	<=0.06	<=0.06
B26	8C	2	0.5	1	0.5	0.5	<=0.06	<=0.06
B27	8C	2	0.5	0.5	0.25	0.5	<=0.06	0.12
B28	8C	<=1	0.5	0.5	0.5	0.25	0.12	0.5
B29	8C	<=1	0.5	0.5	0.25	0.5	<=0.06	0.12
B30	8C	2	0.5	0.5	0.25	2	0.12	0.25
B31	8C	2	0.5	0.5	0.25	0.5	<=0.06	<=0.06
B32	8C	<=1	0.25	0.12	0.12	<=0.12	<=0.06	0.12
B33	8C	2	0.5	0.5	0.25	0.25	0.12	0.5
B34	8C	<=1	0.25	0.06	<=0.06	<=0.12	<=0.06	<=0.06
B35	8C	2	0.25	0.25	0.12	<=0.12	<=0.06	<=0.06
B36	8C	2	0.25	0.5	0.12	<=0.12	<=0.06	<=0.06
B37	8C	2	0.5	0.5	0.25	0.5	<=0.06	0.25
B38	8C	**8**	0.5	1	0.25	0.5	<=0.06	0.12
B39	8C	<=1	0.25	0.12	<=0.06	<=0.12	<=0.06	<=0.06
B40	8C	<=1	0.25	0.25	0.12	<=0.12	<=0.06	0.12
B41	8C	2	0.5	0.5	0.5	1	0.25	0.5
B42	8C	2	0.5	1	0.25	1	<=0.06	0.12
B43	8C	<=1	0.25	0.5	0.12	<=0.12	<=0.06	<=0.06
B44	8C	2	0.5	0.5	0.25	<=0.12	<=0.06	0.12
B45	9C	2	0.5	0.5	0.5	1	0.12	0.5
B46	9C	<=1	0.5	0.5	0.5	0.25	0.12	0.25
B47	9C	<=1	0.25	0.25	0.12	<=0.12	<=0.06	0.12
B48	9C	**4**	0.5	0.5	0.5	1	0.12	0.25
B49	9C	<=1	<=0.12	0.06	<=0.06	<=0.12	<=0.06	<=0.06
B50	9C	2	0.5	0.5	0.25	2	<=0.06	0.12
B51	9C	2	0.5	0.5	0.25	1	0.12	0.25
B52	9C	2	0.5	1	0.5	2	0.5	0.5
B53	9C	2	0.5	1	0.5	2	0.12	0.25
B54	9C	2	0.25	0.5	0.25	0.25	0.12	0.25
B55	9C	**4**	0.5	1	0.5	1	<=0.06	0.12
B56	9C	2	0.5	0.5	0.5	1	0.12	0.5

B57	9C	<=1	0.5	1	0.5	2	0.25	1
B58	9C	2	0.25	0.5	0.12	0.5	<=0.06	0.12
B59	9C	2	0.5	0.5	0.5	1	0.25	0.5
B60	9C	2	0.5	0.25	0.5	0.25	0.12	0.25
Number of resistant isolates		4	0	0	0	0	0	0
Proportion of resistant isolates		6.67%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Bold lines show resistant strains.

Supplementary Table 2 Analysis of drug-resistance gene mutations of *Mycobacterium bovis*.

Isolate	*GlpK* HT	Classification				Drug-resistance gene mutations	
		DLM	KAN	ETH	INH	Gene	Mutation
B11	7C	S	S	**R**	S	ethA	–
B13	7C	**R**	S	S	S	fbiA, fbiB, fbiC, fgd, ddn	–
B17	7C	**R**	S	S	S	fbiA, fbiB, fbiC, fgd, ddn	–
B23	8C	S	**R**	S	S	eis, rrs	–
B29	8C	S	S	**R**	S	ethA	–
B38	8C	**R**	**R**	S	S	fbiA, fbiB, fbiC, fgd, ddn, eis, rrs	–
B42	8C	**R**	S	S	**R**	fbiA, fbiB, fbiC, fgd, ddn, katG	–
B48	9C	S	**R**	S	S	eis, rrs	–
B55	9C	S	**R**	S	S	eis, rrs	–