Supplementary Information

12-hour phenotypic drug susceptibility testing for *Mycobacterium tuberculosis* variant *bovis* BCG

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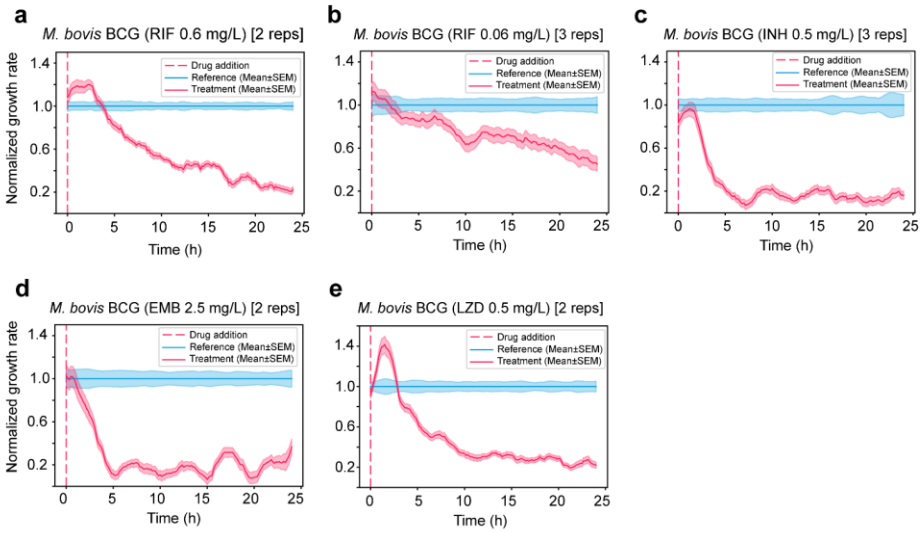
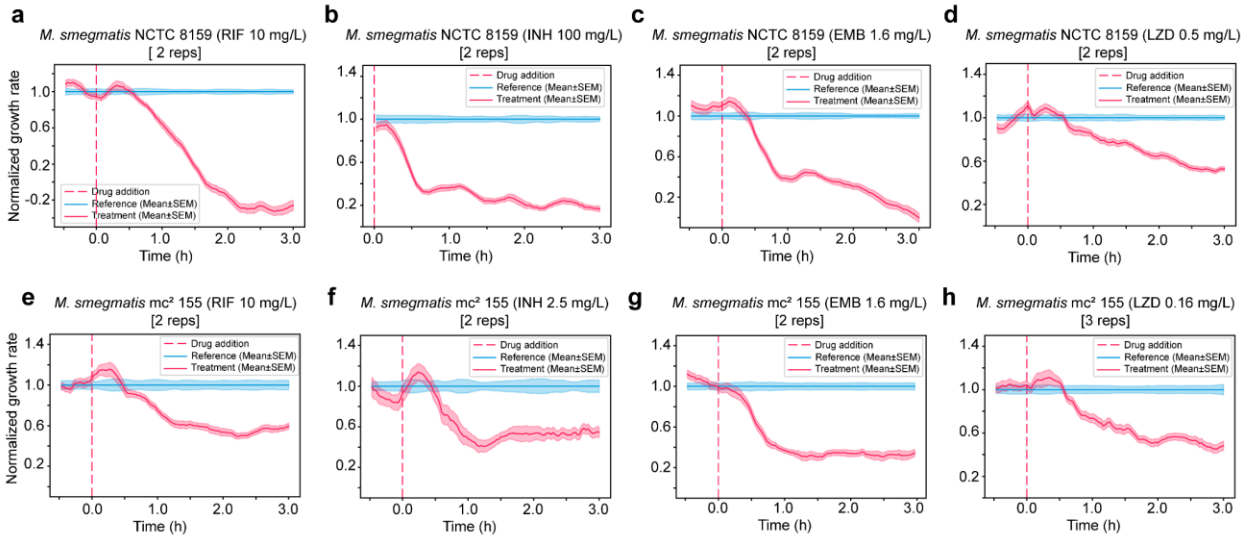
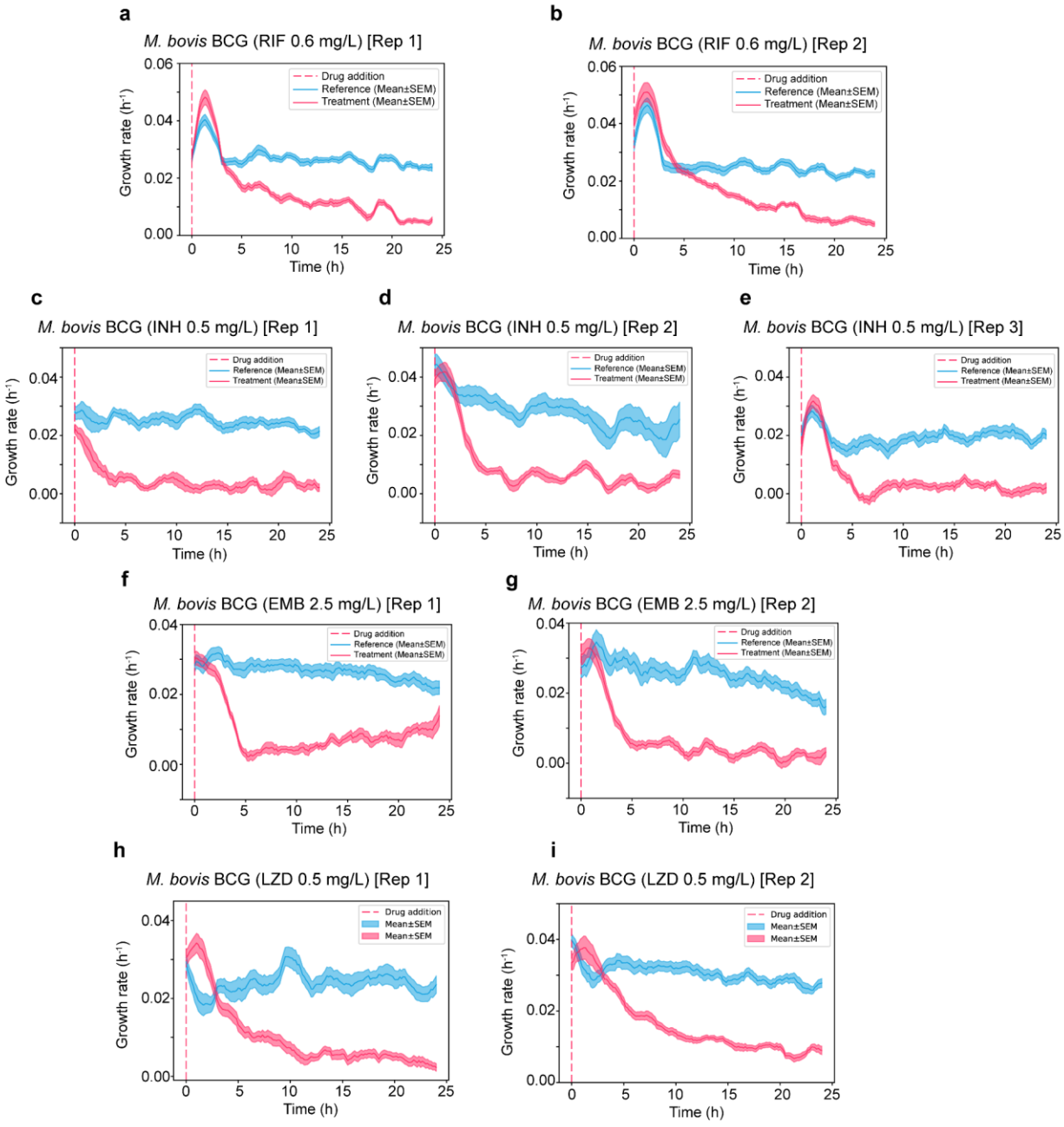


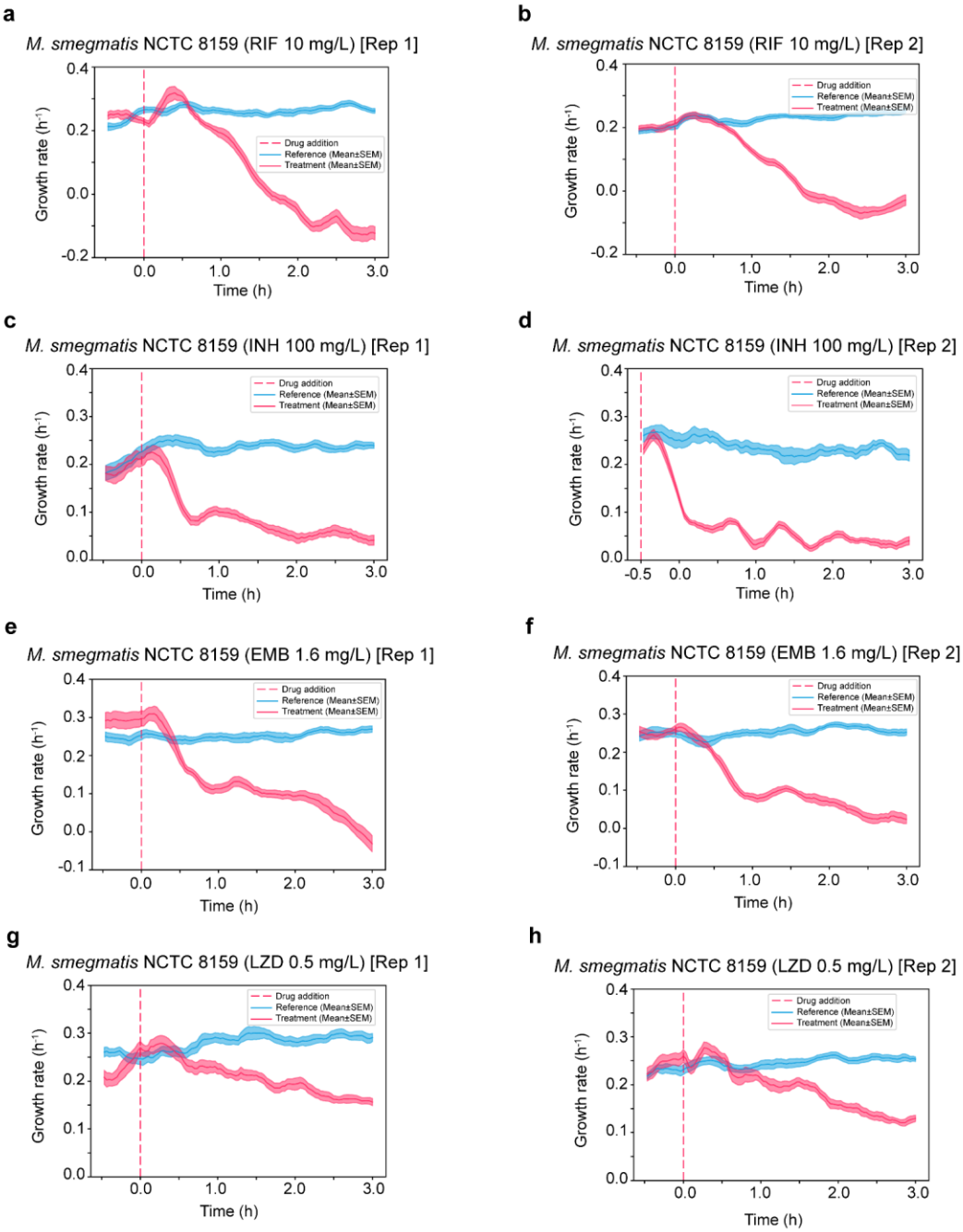
Supplementary Figure 1. pDST assays detecting fast response of *M. bovis* BCG to (**a**) rifampicin (RIF) 0.6 mg/L, (**b**) rifampicin (RIF) 0.06 mg/L, (**c**) isoniazid (INH) 0.5 mg/L, (**d**) ethambutol (EMB) 2.5 mg/L, and (**e**) linezolid (LZD) 0.5 mg/L. Data were pooled from the number of biological replications indicated in each graph.



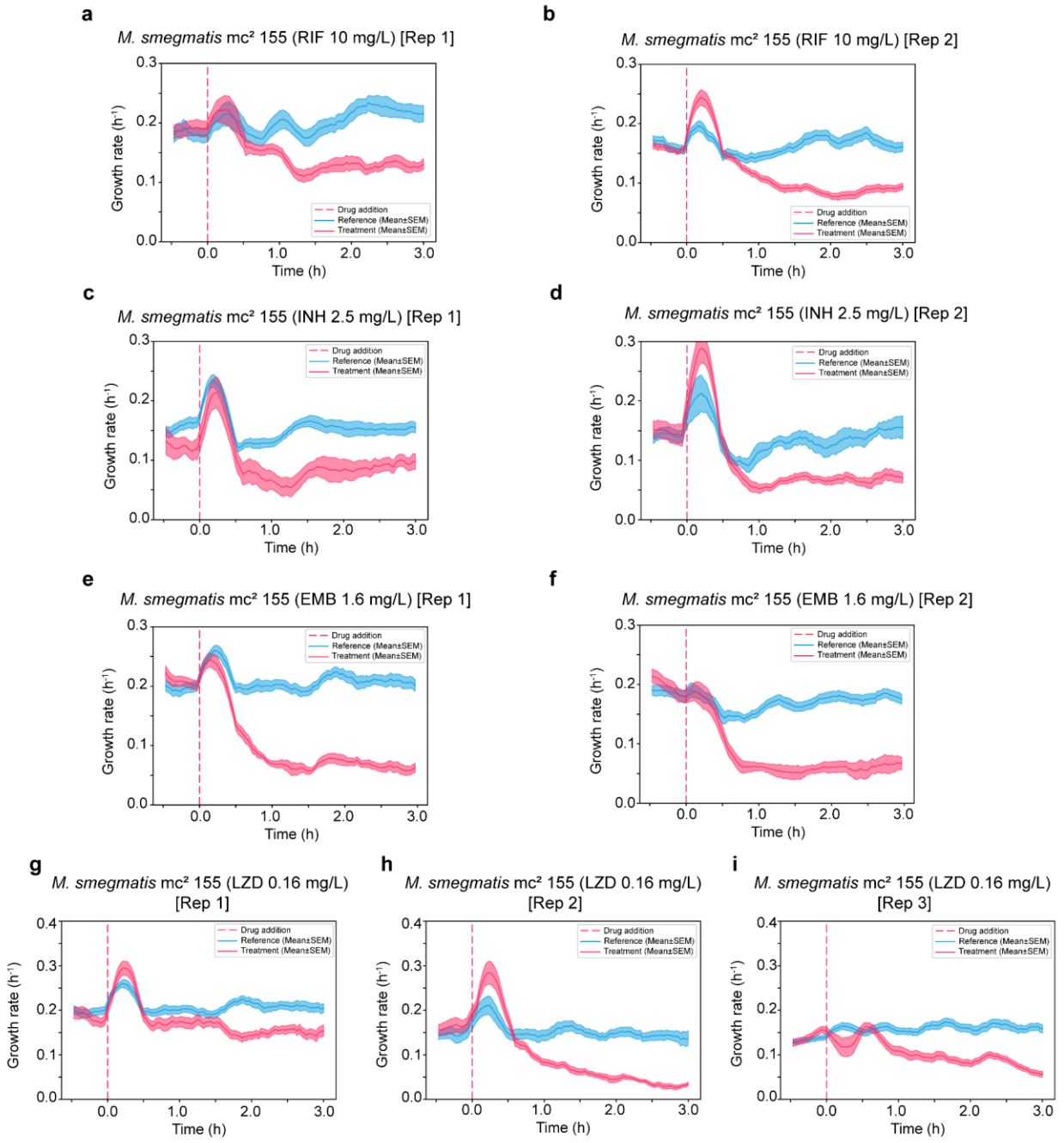
Supplementary Figure 2. pDST assays detecting fast response of *M. smegmatis* NCTC 8159 to (**a**) rifampicin (RIF) 10 mg/L, (**b**) isoniazid (INH) 100 mg/L, (**c**) ethambutol (EMB) 1.6 mg/L, and (**d**) linezolid (LZD) 0.5 mg/L; and *M. smegmatis* mc^2 155 to (**e**) rifampicin (RIF) 10 mg/L, (**f**) isoniazid (INH) 2.5 mg/L, (**g**) ethambutol (EMB) 1.6 mg/L, and (**h**) linezolid (LZD) 0.16 mg/L. Data were pooled from the number of biological replications indicated in each graph.



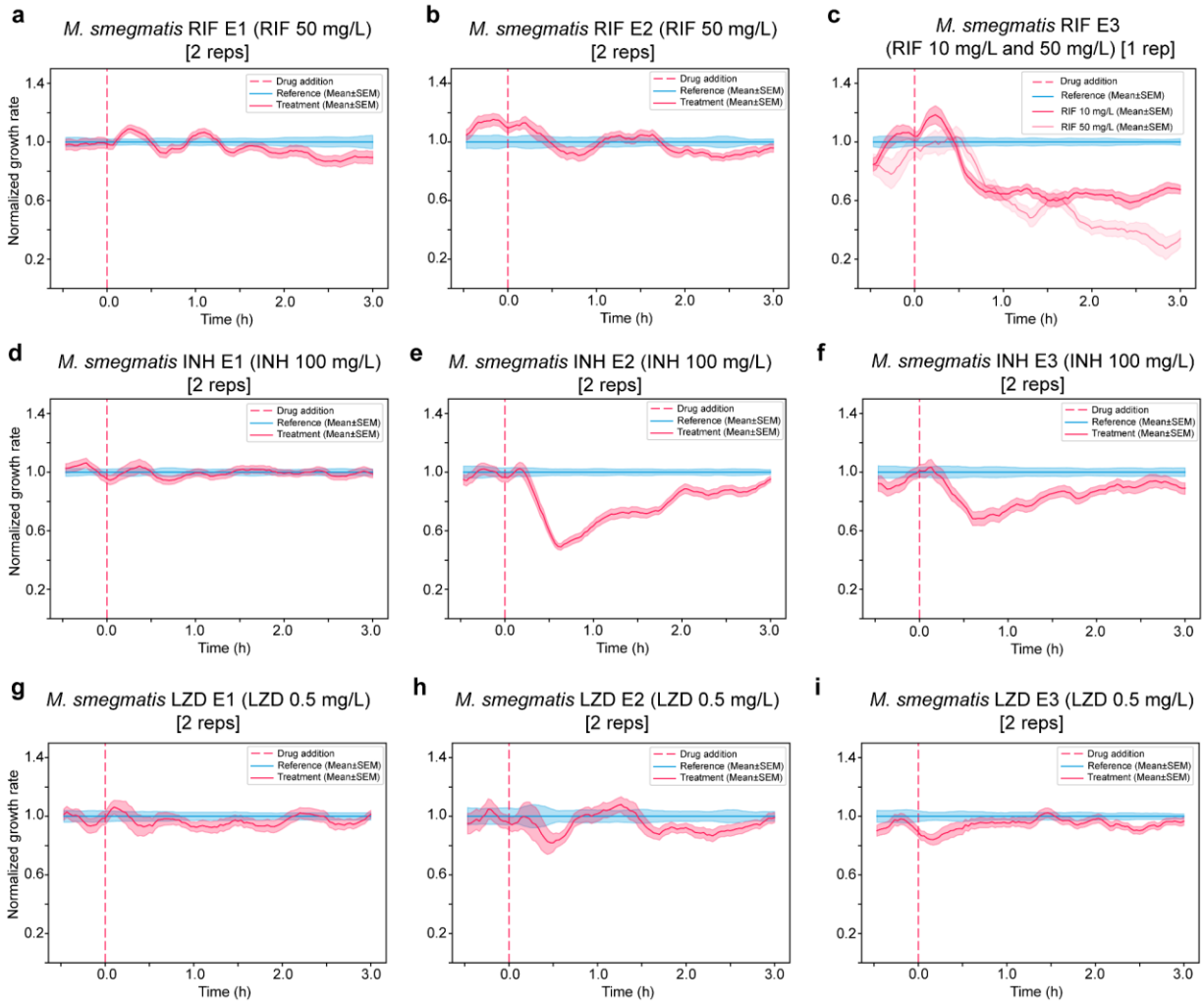
Supplementary Figure 3. The growth rates of *M. bovis* BCG in response to drug treatments were measured across two or three biological replicates from different fast pDST experiments. **a - b**. *M. bovis* BCG in rifampicin (RIF) 0.6 mg/L. **c - e**. *M. bovis* BCG in isoniazid (INH) 0.5 mg/L. **f - g**. *M. bovis* BCG in ethambutol (EMB) 2.5 mg/L. **h - i**. *M. bovis* BCG in linezolid (LZD) 0.5 mg/L.



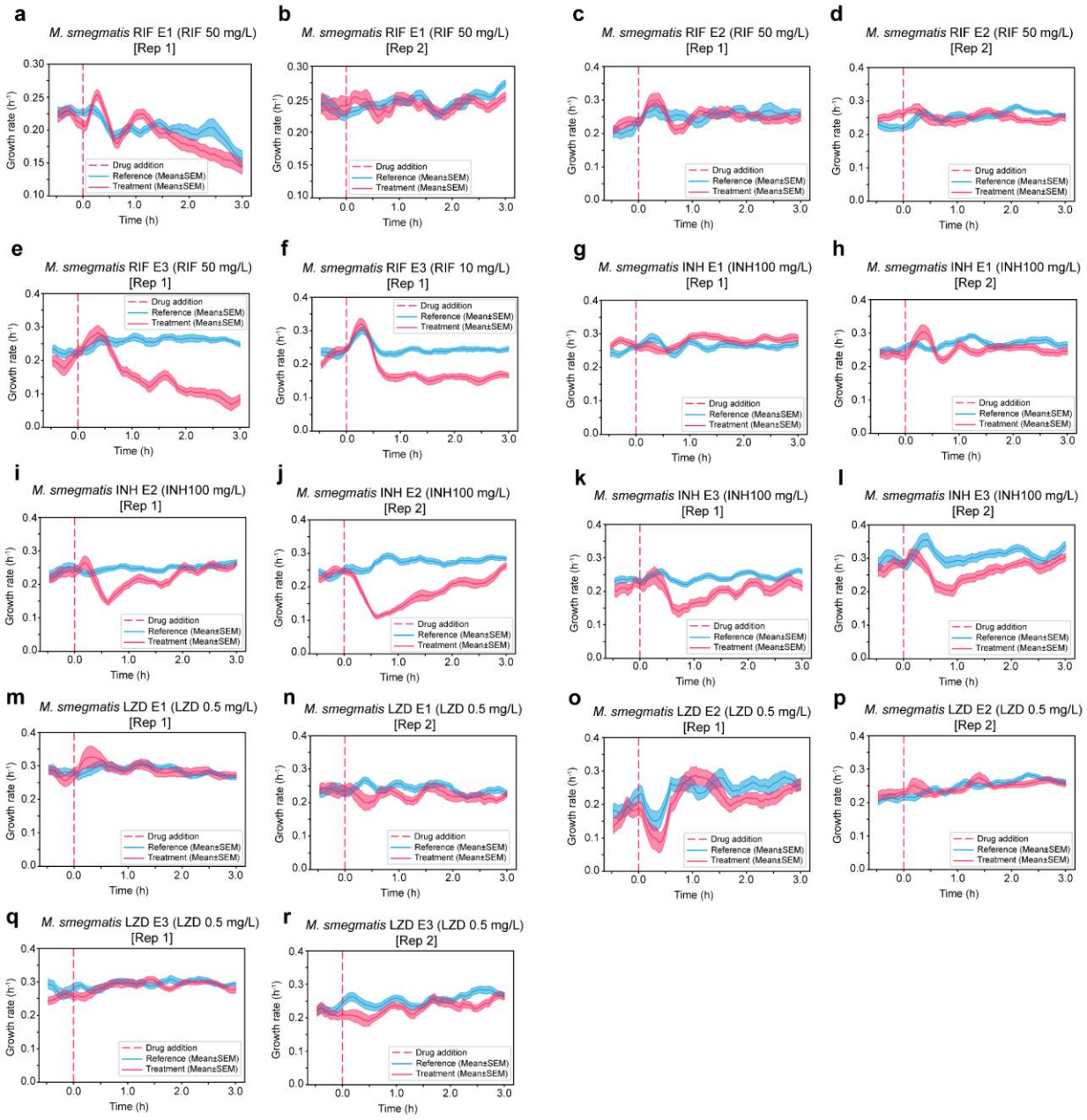
Supplementary Figure 4. The growth rates of *M. smegmatis* NCTC 8159 in response to drug treatments were measured across two biological replicates from different fast pDST experiments. **a - b**. *M. smegmatis* NCTC 8159 in rifampicin (RIF) 10 mg/L. **c - d**. *M. smegmatis* NCTC 8159 in isoniazid (INH) 100 mg/L; In replication 2, the drug was added after 30 min instead of 1 hour like other measurements. **e - f**. *M. smegmatis* NCTC 8159 in ethambutol (EMB) 1.6 mg/L. **g - h**. *M. smegmatis* NCTC 8159 in linezolid (LZD) 0.5 mg/L.



Supplementary Figure 5. The growth rates of *M. smegmatis* mc^2 155 in response to drug treatments were measured across two or three biological replicates from different fast pDST experiments. **a - b**. *M. smegmatis* mc^2 155 in rifampicin (RIF) 10 mg/L. **c - d**. *M. smegmatis* mc^2 155 in isoniazid (INH) 2.5 mg/L. **e - f**. *M. smegmatis* mc^2 155 in ethambutol (EMB) 1.6 mg/L. **g - h**. *M. smegmatis* mc^2 155 in linezolid (LZD) 0.16 mg/L.



Supplementary Figure 6. Fast detection of resistant strains. pDST profiles of lab-evolved resistant strains derived from *M. smegmatis* NCTC 8159 including (**a**) *M. smegmatis* RIF E1, (**b**) *M. smegmatis* RIF E2, (**c**) *M. smegmatis* RIF E3 in rifampicin treatment, (**d**) *M. smegmatis* INH E1, (**e**) *M. smegmatis* INH E2, (**f**) *M. smegmatis* INH E3 in isoniazid treatment, and (**g**) *M. smegmatis* LZD E1, (**h**) *M. smegmatis* LZD E2, (**i**) *M. smegmatis* LZD E3 in linezolid treatment. Data were pooled from the number of biological replications indicated in each graph.



Supplementary Figure 7. The growth rates of resistant strains of *M. smegmatis* NCTC 8159 in response to drug treatments were measured across two biological replicates from different fast pDST experiments. **a - b**. *M. smegmatis* NCTC 8159 RIF E1 in rifampicin (RIF) 50 mg/L. **c - d**. *M. smegmatis* NCTC 8159 RIF E2 in rifampicin (RIF) 50 mg/L. **e - f**. *M. smegmatis* NCTC 8159 RIF E3 in rifampicin (RIF) 50 mg/L and 10 mg/L, respectively. **g - h**. *M. smegmatis* NCTC 8159 INH E1 in isoniazid (INH) 100 mg/L. **i - j**. *M. smegmatis* NCTC 8159 INH E2 in isoniazid (INH) 100 mg/L. **k - l**. *M. smegmatis* NCTC 8159 INH E3 in isoniazid (INH) 100 mg/L. **m - n**. *M. smegmatis* NCTC 8159 LZD E1 in linezolid (LZD) 0.5 mg/L. **o - p**. *M. smegmatis* NCTC 8159 LZD E2 in linezolid (LZD) 0.5 mg/L. **q - r**. *M. smegmatis* NCTC 8159 LZD E3 in linezolid (LZD) 0.5 mg/L.

Table 1. Training/fine-tuning parameters of models for mycobacteria using Omnipose network [1]

No.	Model	Training parameters
1.	Omnipose	default Omnipose model for bacteria phase contrast (bact_omnitorch_0)
2.	Mycobact_1	trained from scratch, 4000 epochs, nclasses=2, learning_rate=0.1, 240 images
3.	Mycobact_2	trained from scratch, 4000 epochs, nclasses=3, learning_rate=0.05, 240 images
4.	Mycobact_3	trained from scratch, 4000 epochs, nclasses=3, learning_rate=0.05, 738 images
5.	Mycobact_4	trained from scratch, 8000 epochs, nclasses=3, learning_rate=0.01, 738 images
6.	Mycobact_5	fine-tuned from default Omnipose model for bacteria phase contrast, 1300 epochs, nclasses=3, learning_rate=0.1, 240 images
7.	Mycobact_6	fine-tuned default Omnipose model for bacteria phase contrast, 4000 epochs, nclasses=3, learning_rate=0.1, 240 images

Table 2. MIC values of the tested strains were determined using the resazurin microtiter assay (REMA)[2] and EUCAST broth microdilution assay (BMDA)[3] (unit: mg/L)

No.	Strain	MIC				Remarks
		RIF	INH	EMB	LZD	
1.	*M. bovis* BCG WT	0.06	0.5	2	0.5	ATCC - 35734
2.	*M. smegmatis* mc^2 155 WT	10	2.5	1.6	0.16	From Leif Kirsebom
3.	*M. smegmatis* NCTC 8159 WT	10	50	1.6	0.5	[4]
4.	*M. smegmatis* NCTC 8159 RIF E1	>100 (50*)				
5.	*M. smegmatis* NCTC 8159 RIF E2	>100 (50)				
6.	*M. smegmatis* NCTC 8159 RIF E3	50 (50)				
7.	*M. smegmatis* NCTC 8159 INH E1		>100 (100)			
8.	*M. smegmatis* NCTC 8159 INH E2		>100 (100)			
9.	*M. smegmatis* NCTC 8159 INH E3		>100 (100)			
10.	*M. smegmatis* NCTC 8159 LZD E1				50 (0.5)	
11.	*M. smegmatis* NCTC 8159 LZD E2				12.5 (0.5)	
12.	*M. smegmatis* NCTC 8159 LZD E3				50 (0.5)	

(*) Values in the parentheses are the lab-evolved selective concentrations [4]

Movies of the segmentation performance are in the SI PowerPoint file (SI - Segmentation performance of Omnipose on mycobacteria data).

References

1. Cutler, K. J. *et al.* Omnipose: a high-precision morphology-independent solution for bacterial cell segmentation. *Nat. Methods* **19**, 1438–1448 (2022).
2. Palomino, J.-C. *et al.* Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* **46**, 2720–2722 (2002).
3. Schön, T. *et al.* Antimicrobial susceptibility testing of Mycobacterium tuberculosis complex isolates - the EUCAST broth microdilution reference method for MIC determination. *Clin. Microbiol. Infect.* **26**, 1488–1492 (2020).
4. Maeda, T., Kawada, M., Sakata, N., Kotani, H. & Furusawa, C. Laboratory evolution of Mycobacterium on agar plates for analysis of resistance acquisition and drug sensitivity profiles. *Sci. Rep.* **11**, 15136 (2021).