

Supplemental table 1: Strains

Strain list			
Strain #	Nickname	Genotype	Figure panel
CB1527, CB1291, CB1529, CB1531	PstP _{Msmeg} T134A	mc2155 ΔPstP::lox L5::pCT94- p766tetON6- PstPsmegT134A	1B
CB1520, CB1523, CB1360, CB1361, CB1525, CB1439	PstP _{Msmeg} T134E	mc2155 ΔPstP::lox L5::pCT94- p766tetON6- PstPsmegT134E	1B
CB1430, CB1431, CB1514, CB1515, CB1515, CB1516, CB1517, CB1518, CB1432	PstP _{Msmeg} T138A	mc2155 ΔpstP::lox L5::pCT94- p766tetON6- PstPSmeg_T138A	1B
CB1405, CB1363, CB1364, CB1365	PstP _{Msmeg} T138E	mc2155 ΔPstP::lox L5::pCT94- p766tetON6- PstPsmegT138E	1B
CB1437, CB1438, CB1292	PstP _{Msmeg} WT	mc2155 ΔpstP::lox L5::pCT94- p766tetON6- PstPSmegWT	1B, 2A-D, 3A-F, 4A-D
CB1290, CB1300, CB1301	PstP _{Msmeg} T171A	mc2155 ΔpstP::lox L5::pCT94- p766tetON6- PstPSmeg_T171A	1B, 2A-D, 3A-F, 4A-D
CB1366, CB1367, CB1368	PstP _{Msmeg} T171E	mc2155 ΔPstP::lox L5::pCT94- p766tetON6- PstPsmegT171E	1B, 2A-D, 3A-F, 4A-D
CB1709	his-SUMO-CwIM _{Mtb}	BL21 Codon Plus/ pET-his-SUMO-CwIM	5A
CB1710	his-PstPcWT _{Mtb}	BL21 Codon Plus/ pET28-his-PstPc Mtb	5A
CB1711	PstPcT174E _{Mtb}	BL21 Codon Plus/ pET28-his-PstP(TB cyto) T174E	5A
CB1069	His-MBP-PknB _{Mtb}	BI21 Codon Plus/pHMGWA-His- MBP-PknB(KD)	5A

Supplemental table 2- Plasmids

Plasmid list			
In strain #	Plasmid name	Used in strains	Reference for parent vector
CB1284	p1206-p766TetON6-PstP _{Msmeg} T134A	CB1527, CB1291, CB1529, CB1531	this paper
CB1354	pCB1285-p766tetON6-PstP _{Msmeg} T134E	CB1520, CB1523, CB1360, CB1361, CB1525, CB1439	this paper
CB1282	p1207-p766TetON6-PstP _{Msmeg} T138A	CB1430, CB1431, CB1514, CB1515, CB1516, CB1517, CB1518, CB1432	this paper
CB1355	pCB1285-tetON6-PstP _{Msmeg} T138E	CB1405, CB1363, CB1364, CB1365	this paper
CB1285	p1210-p766TetON6-PstP _{Msmeg} WT	CB1437, CB1438, CB1292	this paper
CB1283	p1208-p766TetON6-PstP _{Msmeg} T171A	CB1290, CB1300, CB1301	this paper
CB1356	pCB1285-p766tetON6-PstP _{Msmeg} T171E	CB1366, CB1367, CB1368	this paper
CB174	pL5 PTetO Msm PonA1 truncation A-FLAG clone 1	in all of the strains above	(1)
CT298	pET-his-SUMO-CwIM	CB1709	this paper
CT216	pET28-his-PstPc Mtb	CB1710	this paper
CT216	pET28-PstP(TB cyto) T174E	CB1711	this paper
	pJV53		(2)
CB1175 (KP37-24)	pKM55 ΔpstP::loxP L5::p750TetOFF-pstP _{Mtb} WT-DAS-Zeo		this paper, courtesy of Kenan Murphy

1. **Kieser KJ, Boutte CC, Kester JC, Baer CE, Barczak AK, Meniche X, Chao MC, Rego EH, Sassetti CM, Fortune SM, Rubin EJ.** 2015. Phosphorylation of the Peptidoglycan Synthase PonA1 Governs the Rate of Polar Elongation in Mycobacteria. *PLoS Pathog* **11**:e1005010.
2. **van Kessel JC, Hatfull GF.** 2008. Mycobacterial recombineering. *Methods Mol Biol* **435**:203–215.

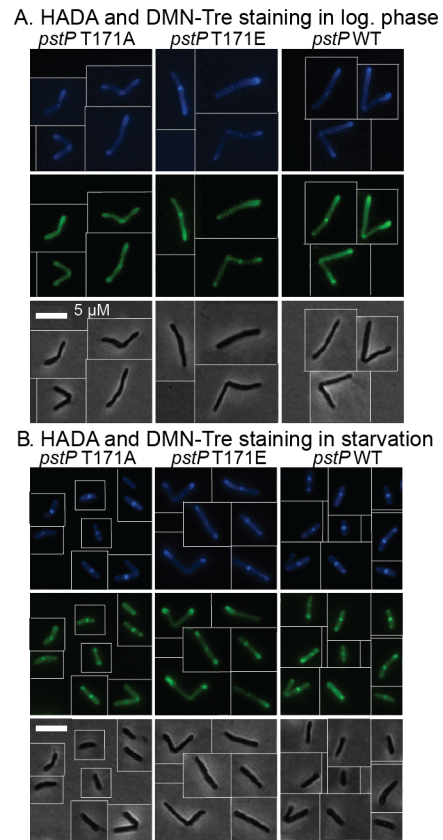
Supplemental table 3- Primers

Primer list			
Strain #	Feature	Primer	Primer #
CB1284	p1206-p766TetON6-PstP _{Msmeg} T134A-strep	TGCTTAATTAAGAAGGAGATATACATatgaccctcgttctccgctacg	FS 11
		GAAGTGGGGGTGGCTCCAGTCGGCGCCGGTGGAGTGtgacaccgcccggcagttcgtccc	FS 12
		TAGGGTCCCCAATTAATTAGCTAAAGCTTtcaCTTCTCGAACTGGGGGTGGCTCCAGTCG	FS 13
		cagatcaccaaggacgacGCgttcgtgcagaccctcgtc	FS 14
		gacgagggtctgcacgaaCGCgtcgtccttggtgatctg	FS 15
		CGTTTAATACTGCATGCACTCTAGAgctaccaggcctagatctggggacc	FS 21
		cgtagcggagaacgaggggtcatATGaggagagccactttgtacaagaaag	FS 22
CB1354	pCB1285-p766tetON6-PstP _{Msmeg} T134E-strep	tttctgtacaaagtggctctcctCATatgaccctcgttctccgctac	FS 40
		acagatcaccaaggacgacGAGttcgtgcagaccctcgt	FS 41
		acgaggggtctgcacgaaCTCgtcgtccttggtgatctg	FS 42
		TAGGGTCCCCAATTAATTAGCTAAAGCTTtcaCTTCTCGAACTGGGGGTGGCTCCAGTCG	FS 12
CB1282	p1207-p766TetON6-PstP _{Msmeg} T138A-strep	TGCTTAATTAAGAAGGAGATATACATatgaccctcgttctccgctacg	FS 10
		GAAGTGGGGGTGGCTCCAGTCGGCGCCGGTGGAGTGtgacaccgcccggcagttcgtccc	FS 11
		TAGGGTCCCCAATTAATTAGCTAAAGCTTtcaCTTCTCGAACTGGGGGTGGCTCCAGTCG	FS 12
		gacgacacctcgtgcagGCCctcgtgcagagggccgc	FS 15
		gcgccctcgtgcagagGGCctgcacgaaggtgctcgtc	FS 16
		CGTTTAATACTGCATGCACTCTAGAgctaccaggcctagatctggggacc	FS 21
		cgtagcggagaacgaggggtcatATGaggagagccactttgtacaagaaag	FS 22
CB1355	pCB1285-tetON6-PstP _{Msmeg} T138E-strep	TAGGGTCCCCAATTAATTAGCTAAAGCTTtcaCTTCTCGAACTGGGGGTGGCTCCAGTCG	FS 40
		tttctgtacaaagtggctctcctCATatgaccctcgttctccgctac	FS 43
		gacgacacgttcgtgcagGAGctcgtgcagagggccgc	FS 44
		gcgccctcgtgcagagCTCctgcacgaacgtgctcgtc	FS 12
CB1285	p1210-p766TetON6-PstP _{Msmeg} WT-strep	TGCTTAATTAAGAAGGAGATATACATatgaccctcgttctccgctacg	FS 10
		GAAGTGGGGGTGGCTCCAGTCGGCGCCGGTGGAGTGtgacaccgcccggcagttcgtccc	FS 11
		TAGGGTCCCCAATTAATTAGCTAAAGCTTtcaCTTCTCGAACTGGGGGTGGCTCCAGTCG	FS 15
		CGTTTAATACTGCATGCACTCTAGAgctaccaggcctagatctggggacc	FS 21
		cgtagcggagaacgaggggtcatATGaggagagccactttgtacaagaaag	FS 22
CB1283	p1208-p766TetON6-	TGCTTAATTAAGAAGGAGATATACATatgaccctc	FS 10

	PstP _{Msmeg} T171A-strep	gttctccgctacg	
		GAACTGGGGGTGGCTCCAGTCGGCGCCGGT GGAGTGtgacaccgcccgagttcgtccc	FS 11
		TAGGGTCCCCAATTAATTAGCTAAAGCTTcaC TTCTCGAACTGGGGGTGGCTCCAGTCG	FS 12
		ggccacgaggtcgagccgGCGctgatcatgcgcgaggcc	FS 17
		ggcctcgcgcatgatcagCGCcggtcgacctcgtggcc	FS 18
		CGTTTAATACTGCATGCACTCTAGAgctaccaggc ctagatctggggacc	FS 21
		cgtagcggagaacgagggatATGaggagagccactttgtac aagaaag	FS 22
CB1356	pCB1285-p766tetON6- PstP _{Msmeg} T171E-strep	TAGGGTCCCCAATTAATTAGCTAAAGCTTcaC TTCTCGAACTGGGGGTGGCTCCAGTCG	FS 12
		tttctgtacaaagtggctctctCATatgaccctcgttccgctac	FS 40
		ggccacgaggtcgagccgGAGctgatcatgcgcgaggcc	FS 45
		ggcctcgcgcatgatcagCTCcggtcgacctcgtggcc	FS 46
CT216	pET28-his-PstPcWT _{Mtb}	cctggtgccgcgcgagccatggtgcgcggtgacctggtcct gcat	FS 33
		gtggtggtggtggtggtgctcgagtcaccgtcgccccaccaccgt ggcc	FS 34
CT216	pET28-PstPc(TB cyto)T174E	cctggtgccgcgcgagccatggtgcgcggtgacctggtcct gcat	FS 33
		gtggtggtggtggtggtgctcgagtcaccgtcgccccaccaccgt ggcc	FS 34
		gttgaccggccatgaggtcgaaccgGAGctgacctgcgagaa gccccgccccggtgat	CB1460
		atcaccggcgcggtctctcgatggtcagCTCcggttcgacctc atggccggtcaac	CB1461
CT298	pET-his-SUMO-CwIM _{Mtb}	Tcacagagaacagattggtgatccatgccaggtccgcgccccg aa	CB1272
		gtgctcgacaagcttattactcgagtaagaaccgccgagctacc	CB1273
KP35-79	ΔpstP-hyg L5::p46- pstP _{Mtb} WT-strep	CGGATCGGCAAGACGGTAATCGAGCTGCGCC CGTGAGCCCGCGCACGCGAGGAGCAGACGC TCTAGAACTAGTGGATCC	S1256- SMEG- PstP- P1b
		GCGGGCGTGACGGTAACCGGCGACTGGGGT TGCGTCGTCATTCTCCTCCTTTCTTACTTCT AGACTCGAGGTACCG	S1257- SMEG- PstP- P2b
		CCAACGGCACTTACCTTGACAGGGCGAAGGT GACAACAGCAGTAAAGGTTCCCATTGGCGCG CCGGTGC GGATCGGCAAGACGGTAATCG	S1258- SMEG- PstP- P3b
		TCGACGATCAACAGGGCCACGGTGGTGATCA GCGCCGCGAACCCAGCAGCAGCAGTTCCG CATTGCGCCGGTTCGGGAGCGGCGGCGTGA CGGTAAC	S1259- SMEG- PstP- P4b
		AACTCGACGGCATGGGCAC	S1260- SMEG- PstP-del Int-For2
		GCGAGATGCCAGGAAGGAG	S1261- SMEG-

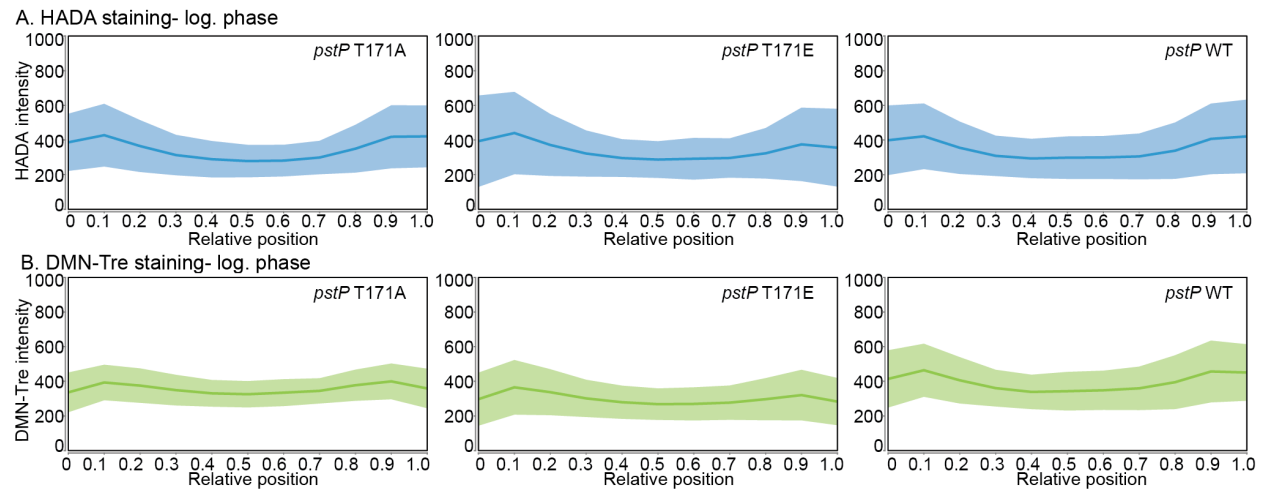
			PstP-del Int-Rev2
		GCGCGGTGATGGTGAGAC	S1121- SMEG- PstP del P6- ExtFor
		GCAGGCTCGCGTAGGAATCATCC	S486- Hyg-N- out
		GAACTGCTCGCCTTCACCTTCC	S538- Hyg-C- out
		TTCAAGAACACCACCACGAGC	S1122- SMEG- PstP del P7 ExtRev

Figure S1



(A) and (B) Representative micrographs of log. phase cells (A) and starved cells in HdB with no glycerol (B) from *pstP* allele strains (WT, T17A and T171E) stained with the fluorescent dyes HADA (blue) and DMN-Tre (green). Corresponding phase images are shown on the bottom panel. The scale bar applies to all images.

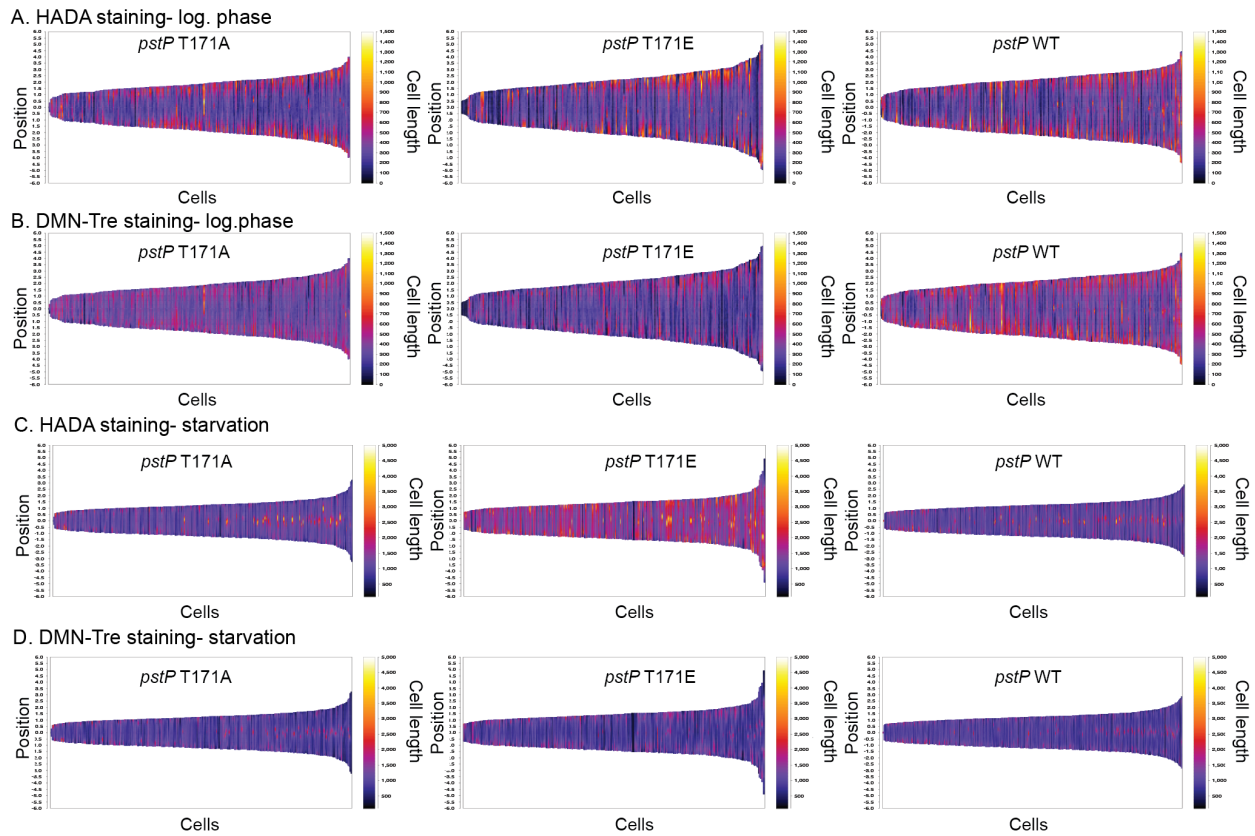
Figure S2



(A) and (B) Intensity profiles of HADA (A) and DMN-Tre (B) signal in cells (pole to pole) from *pstP* allele strains (WT, T17A and T171E) in log. phase. Shaded region represents standard deviation. Solid line represents mean of intensities.

Biological triplicates of each *pstP* allelic variant were analyzed. At least 265 cells from each of the *pstP* allelic variant (at least 62 cells from each biological triplicate strain of each genotype) in log. phase were used to plot the signal intensities.

Figure S3



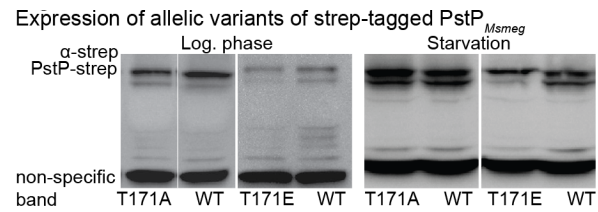
(A) and (B) Demographs showing intensities of fluorescent dyes HADA (A) and DMN-Tre (B) signal in individual cells from *pstP* allele strains (WT, T17A and T171E) in log phase.

(C) and (D) Demographs showing intensities of HADA (C) and DMN-Tre (D) signal in individual cells starved in HdB with no glycerol for 5.5 hours from *pstP* allele strains (WT, T17A and T171E).

Biological triplicates of each *pstP* allelic variant were analyzed. Signal intensities from at least 265 cells from each *pstP* allelic variant (at least 62 cells from each biological triplicate of each genotype) in log phase were plotted in the demograph (A) and (B). Signal intensities from at least 300 cells from each *pstP* allelic variant (at least 100 cells

from each biological triplicate strain of each genotype) in starvation were plotted in the demograph (C) and (D).

Figure S4



α-strep Western blots of allelic variants of strep-tagged PstP^{Msmeg} in log. (left panel) and starvation phase (right panel). A non-specific band at the bottom in all strains was seen in the blots.