



**FIG. S1** Expression of recombinant proteins in *E. coli* JM 109 cells. (i) Whole cell proteins of induced clones stained with Coomassie blue, (ii) Western blots of whole cell proteins of induced clones probed with *M. bovis*-specific calf sera. Lanes: 1, PageRuler prestained protein ladder (Thermo Scientific); 2, pGEX-4T-1-*milA*-AB (125.5 kDa); 3, pGEX-4T-1-*milA*-CD (127 kDa); 4, pGEX-4T-1-*milA*-A (72.8 kDa); 5, pGEX-4T-1-*milA*-B (69 kDa); 6, pGEX-4T-1-*milA*-C (71.9 kDa); 7, pGEX-4T-1-*milA*-D (73.3 kDa); 8, pGEX-4T-1-*milA*-cd (94.3 kDa); 9, pGEX-4T-1-*milA*-ab (92.9 kDa); 10, purified GST (26 kDa).

Consensus	E	-	-	-	-	-	S	<b>E</b>	<b>K</b>	<b>V</b>	<b>K</b>	<b>Y</b>	<b>V</b>	<b>A</b>	<b>L</b>	<b>G</b>	<b>D</b>	<b>S</b>	<b>I</b>	<b>S</b>	<b>A</b>	<b>G</b>	<b>F</b>	<b>D</b>	<b>S</b>	<b>Q</b>	<b>L</b>	<b>D</b>	<b>K</b>	<b>D</b>	<b>Y</b>	<b>P</b>	<b>G</b>	<b>N</b>	<b>Y</b>	<b>D</b>											
MBOVPG45_0710	E	-	-	-	-	-	S	<b>E</b>	<b>K</b>	<b>V</b>	<b>K</b>	<b>Y</b>	<b>V</b>	<b>A</b>	<b>L</b>	<b>G</b>	<b>D</b>	<b>S</b>	<b>I</b>	<b>S</b>	<b>A</b>	<b>G</b>	<b>F</b>	<b>D</b>	<b>S</b>	<b>Q</b>	<b>L</b>	<b>D</b>	<b>Q</b>	<b>D</b>	<b>Y</b>	<b>P</b>	<b>G</b>	<b>N</b>	<b>Y</b>	<b>D</b>											
MMB_0654	E	-	-	-	-	-	S	<b>E</b>	<b>K</b>	<b>V</b>	<b>K</b>	<b>Y</b>	<b>V</b>	<b>A</b>	<b>L</b>	<b>G</b>	<b>D</b>	<b>S</b>	<b>I</b>	<b>S</b>	<b>A</b>	<b>G</b>	<b>F</b>	<b>D</b>	<b>S</b>	<b>Q</b>	<b>L</b>	<b>D</b>	<b>K</b>	<b>D</b>	<b>Y</b>	<b>P</b>	<b>G</b>	<b>N</b>	<b>Y</b>	<b>D</b>											
MAGa6830	E	-	-	-	-	-	N	<b>E</b>	<b>K</b>	<b>V</b>	<b>K</b>	<b>Y</b>	<b>V</b>	<b>A</b>	<b>L</b>	<b>G</b>	<b>D</b>	<b>S</b>	<b>I</b>	<b>S</b>	<b>A</b>	<b>G</b>	<b>F</b>	<b>D</b>	<b>S</b>	<b>H</b>	<b>L</b>	<b>D</b>	<b>K</b>	<b>D</b>	<b>Y</b>	<b>P</b>	<b>G</b>	<b>N</b>	<b>Y</b>	<b>D</b>											
MCSF7_01871	K	-	-	-	-	-	D	<b>D</b>	<b>K</b>	<b>V</b>	<b>N</b>	<b>Y</b>	<b>L</b>	<b>A</b>	<b>L</b>	<b>G</b>	<b>D</b>	<b>S</b>	<b>I</b>	<b>T</b>	<b>A</b>	<b>G</b>	<b>W</b>	<b>G</b>	<b>Q</b>	<b>L</b>	<b>P</b>	<b>Q</b>	<b>D</b>	<b>Y</b>	<b>Q</b>	<b>G</b>	-	-	K												
MFE_02570	K	-	-	-	-	-	E	<b>N</b>	<b>S</b>	<b>I</b>	<b>K</b>	<b>Y</b>	<b>L</b>	<b>A</b>	<b>L</b>	<b>G</b>	<b>D</b>	<b>S</b>	<b>I</b>	<b>S</b>	<b>A</b>	<b>G</b>	<b>F</b>	<b>N</b>	<b>A</b>	<b>Q</b>	<b>L</b>	<b>D</b>	<b>K</b>	<b>D</b>	<b>Y</b>	<b>P</b>	<b>G</b>	-	-	Q											
MYPU130	S	G	F	I	D	N	N	S	T	L	S	N	D	K	R	L	I	K	<b>D</b>	<b>K</b>	<b>I</b>	<b>R</b>	<b>F</b>	<b>V</b>	<b>T</b>	<b>F</b>	<b>G</b>	<b>D</b>	<b>S</b>	<b>I</b>	<b>A</b>	<b>A</b>	<b>G</b>	<b>F</b>	<b>N</b>	<b>A</b>	<b>K</b>	<b>M</b>	<b>L</b>	<b>D</b>	<b>Y</b>	<b>V</b>	<b>P</b>	<b>G</b>	<b>E</b>	<b>Y</b>	<b>D</b>
mhp677																		<b>E</b>	<b>V</b>	<b>N</b>	<b>Y</b>	<b>L</b>	<b>A</b>	<b>L</b>	<b>G</b>	<b>D</b>	<b>S</b>	<b>L</b>	<b>T</b>	<b>A</b>	<b>G</b>	<b>F</b>	<b>N</b>	<b>E</b>	<b>E</b>	<b>T</b>	<b>Y</b>	<b>R</b>	<b>D</b>	<b>F</b>	<b>Q</b>	<b>G</b>	<b>T</b>	<b>L</b>	<b>D</b>		

Consensus	K	E	K	N	E	X	T	G	J	S	F	P	T	Y	L	A	S	F	I	Q	X	S	N	-	X	N	N	K	L	E	S	Y	T	N	Y	A	S	S	G	T	T	L	E	D	W	X	F		
MBOVPG4_0710	K	E	K	N	E	V	T	G	I	S	F	P	T	Y	L	A	S	F	I	K	N	S	N	A	D	N	S	K	L	E	S	Y	T	N	Y	A	S	S	G	T	T	L	E	D	W	K	F	164	
MMB_0654	K	E	K	N	E	V	T	G	I	S	F	P	T	Y	L	A	S	F	I	E	N	S	N	A	D	N	S	K	L	E	S	Y	T	N	Y	A	S	S	G	T	T	L	E	D	W	K	F	164	
MAGA6830	K	E	K	N	E	I	T	G	I	S	F	P	T	Y	L	A	S	F	I	Q	R	S	N	R	E	N	N	K	L	E	S	Y	T	N	Y	A	S	S	G	T	T	L	E	D	W	K	F	162	
MCSF7_01871	L	I	D	N	Q	V	T	G	I	S	F	P	T	Y	L	A	S	F	I	Q	Q	S	-	-	E	E	D	K	L	N	S	F	T	N	L	A	V	S	G	T	F	N	D	W	N	S	162		
ME_F_02570	L	V	N	S	K	I	E	G	L	S	F	P	T	Y	L	A	S	F	I	Q	Q	L	-	-	A	D	K	K	L	E	S	F	D	N	K	A	V	S	G	T	T	F	K	Q	W	N	L	159	
MPYU3130	P	N	T	K	Q	I	T	G	L	S	Y	S	S	Y	I	A	D	Y	I	I	N	D	L	D	-	-	P	N	K	L	E	S	F	K	N	F	A	F	S	G	T	T	L	K	D	W	N	-	191
mhp677	K	D	G	N	-	L	S	G	Q	S	Y	P	A	Y	F	A	Y	L	Q	K	L	N	-	-	K	N	S	L	V	S	Y	D	N	L	A	I	S	G	T	T	T	E	N	W	L	Y	110		

Consensus	<b>L</b>	<b>L</b>	T	V	D	<b>D</b>	X	-	X	-	X	-	X	X	B	X	X	<b>K</b>	<b>L</b>	A	Y	Y	K	S	<b>K</b>	<b>F</b>	<b>G</b>	X	D	L	I	X	X	A	K	X	X	E								
MBOVPG45_0710	<b>L</b>	<b>L</b>	T	V	D	S	K	L	K	Q	L	-	-	-	A	E	N	D	K	N	<b>K</b>	<b>L</b>	A	Y	Y	K	S	<b>K</b>	<b>F</b>	<b>G</b>	K	D	I	I	K	K	A	E	V	R	E					
MMB_0654	<b>L</b>	<b>L</b>	T	V	D	E	S	K	L	K	E	L	-	-	-	A	E	K	D	K	N	<b>K</b>	<b>L</b>	A	Y	Y	K	T	<b>K</b>	<b>F</b>	<b>G</b>	E	D	I	I	E	R	A	K	Q	V	K	E			
MAGa6830	<b>L</b>	<b>L</b>	T	V	D	D	K	S	L	K	E	L	-	-	-	Q	T	K	N	P	T	<b>K</b>	<b>L</b>	A	Y	Y	K	V	<b>K</b>	<b>F</b>	<b>G</b>	D	N	L	I	E	K	A	K	V	K	D				
MCSF7_01871	<b>I</b>	<b>L</b>	T	Q	E	Y	-	-	-	-	-	-	-	-	-	K	T	L	N	A	T	<b>K</b>	<b>L</b>	V	K	L	T	K	<b>Y</b>	<b>F</b>	<b>G</b>	S	N	L	D	D	F	Y	T	K	L	V	S			
MFE_02570	<b>L</b>	<b>L</b>	N	Y	D	P	N	-	-	-	-	-	-	-	-	K	P	L	D	P	K	<b>D</b>	<b>L</b>	A	E	L	N	K	<b>Y</b>	<b>F</b>	<b>G</b>	T	D	L	V	N	I	K	N	D	L	I	A			
MYPU3130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
mbo677	<b>I</b>	<b>I</b>	N	P	T	K	Y	P	N	G	K	M	S	D	N	P	L	V	T	N	Y	S	G	N	<b>K</b>	<b>Y</b>	<b>N</b>	<b>E</b>	<b>I</b>	<b>G</b>	<b>S</b>	<b>V</b>	<b>F</b>	<b>G</b>	<b>D</b>	<b>F</b>	<b>N</b>	<b>K</b>	<b>D</b>	<b>S</b>	<b>V</b>	<b>P</b>	<b>G</b>	<b>L</b>	<b>V</b>	<b>E</b>

Consensus	E	L	X	K	A	N	L	X	T	I	T	L	G	A	N	D	F	M	E	L	V	X	X	K	L	K	D	-	-	-	P	K	F	I	X	Q	I	Q	K	X	K	A	N	P	X	N	
MBOVPG45_0710	E	L	Q	K	A	N	L	V	T	I	T	L	A	A	N	D	F	M	E	L	V	A	K	K	L	K	N	-	-	-	P	E	F	I	K	Q	I	Q	N	I	K	A	N	P	T	N	
MMB_0654	E	L	Q	K	A	N	L	V	T	I	T	L	A	A	N	D	F	M	E	L	V	A	K	K	L	K	D	-	-	-	P	K	F	I	E	Q	I	Q	N	I	K	A	N	P	T	N	
MAGa6830	E	L	Q	K	A	N	L	V	T	I	T	L	A	A	N	D	F	M	E	L	V	S	E	K	L	K	D	-	-	-	P	K	F	L	E	N	I	Q	K	I	K	A	N	P	T	N	
MCSF7_01871	Q	I	K	E	A	N	L	L	T	V	T	L	G	A	N	D	A	L	H	-	L	L	T	K	E	L	T	K	I	P	F	A	Q	L	V	D	Q	I	I	K	N	K	Y	N	Y	A	Q
MFE_02570	K	I	K	N	A	N	L	I	T	V	T	L	G	A	N	D	F	L	K	D	L	L	F	K	M	V	S	N	M	P	I	L	D	L	I	K	Q	I	K	N	N	L	D	Y	N	M	
MPYU3130	R	L	T	N	S	N	L	L	L	N	L	G	A	N	D	F	M	S	L	A	F	E	K	F	K	E	-	-	-	K	D	V	S	L	L	M	K	K	D	A	S	T	N	D			
mhp677	K	V	K	K	A	N	L	L	T	M	S	V	G	A	N	D	P	F	L	A	I	F	N	E	F	K	K	W	A	S	I	I	K	P	K	S	E	A	K	K	L	D	P	N	E		

Consensus	A I A G V S S A - - - - -	<b>F Q D T F X E M K K R I D X F I X E L K K X N K T E N V N F I G</b>
MBOVPG45_0710	A I A G V S S A - - - - -	<b>F Q D T F K E M K K R I D D F S A E L K K H V K T E N V N F I G</b>
MMB_0654	A I A G V S S A - - - - -	<b>F Q D T F K E M K K R I D D F S A E L K K H V K T E N V N F I G</b>
MAGa6830	A I A N V S S A - - - - -	<b>F Q D T F N S M K K R I D E F S V E L K K Y V K T E N V N F I G</b>
MCSF7_01871	I V Q L I N N T - - - - -	<b>F Q T V F D A I A Q R Q N L F I Q K L K E I N P N L N V N F I G</b>
MFE_02570	L V S H F D Q V - - - - -	<b>F R T L F E Q L E N R Q K Q F I Q T I K S L N D K V N I N F I A</b>
MYPU3130	L I G V F L P I - - - - -	<b>L S S I R H E M G I R Y K E L I S H I R K Y N K N V T I N L I G</b>
mbo672	P A N F I A F V G M L I K A E V N K V I E F I N T N L D N I L I F I Z A I N P K I S T N I L G	<b>P A N F I A F V G M L I K A E V N K V I E F I N T N L D N I L I F I Z A I N P K I S T N I L G</b>

Consensus	<b>Y P L P L P H L F V L L D X Y L F K - N N G S S I V S Q M A X N L I N K N I Q S Q V T K N K</b>
MBOVPG4_0710	<b>Y P L P L P H L F V L L D G Y L F K - N N G S S I V S Q M A V D A L N K N I Q S Q V T K N K</b> 333
MMB_0654	<b>Y P L P L P H L F V L L D G Y L F K - N N G S S I V S Q M A V E S L N K N I Q S Q V T K N K</b> 333
MAGa6830	<b>Y P L P L P H L F V L L D G Y L F K - N N N S S I V S Q M A I N L L N T N I Q S Q V T K N N</b> 331
MCSF7_01871	<b>Y P L P M S F I G K L L D K Y I A N - N F G V S I Q L S Q I L I N L L N R K I A F G A R V N N</b> 329
MFE_02570	<b>Y P L P L T F T I M D L I D K A I N K G D T N N A L S I G Q T I L D L L N K K L K Y T A I S Q K</b> 329
MPYU3130	<b>Y P M P A P A L K L F N V M V N K L L G E S V K I G N D T T L L G F L L N F I N S S I K D Q V D L Y E</b> 408
mhp677	<b>Y K L P N S G F I K I L K Y L L Y T - Y A K I E T D F I N E I P E K I N K I I R E S A I K N K</b> 303

Block IV		*	*	
Consensus	- X N F I N P Y D F S F W T D N - - I N K L S P T L F D I H P X S F G Y K K M A Q D V F I K L			
MBOVPG45_0710	- F N F I N P Y D F S F W T D N S N I N K L S P T L F D I H P G S F G Y K K M A Q D V F I K L			389
MMB_0654	- F N F I N P Y D F S F W T D N S N I N K L S P T L F D I H P G S F G Y K K M A Q D V F I K L			387
MGa6830	- F N F I N S Y D F D F W T K D S N I N R L S P T L F D I H P G S F G Y K K M A Q D V F I K L			383
MCSF7_01871	- V N F I N A Y D A E Y W I N N - - V E K L A P L L F D I H P S A Y G Y K K M A M D I F T K L			383
MFE_02570	- T Y Y I N P Y Q S S F W K Q N - - L K L L T P N I F D I H P S S F G Y K K M A M E V F V K L			462
MPYU3130	N V N F V N A Y D P L W L K H - - K N D F T D V A F D I H P N E I G Y K K M A Q E I F L K L			357
mhp677	- V N Y I D V Y D K C S I W N D S - - D K N L M A K N F D I H P S I Q G Y K K I A H Q L L L K L			

**FIG. S2** Multiple sequence alignment of region 1 of mycoplasma immunogenic lipase (MilA) with homologues in other mycoplasmas and the mycoplasma GDSL carboxyesterases.

Numbers on the right indicate the position of the adjacent amino acid. Identical amino acids are shaded black. Grey shading indicates at least one substitution with a very similar amino acid. Light grey shading indicates at least one substitution with a similar amino acid. Dashed lines indicate gaps in the amino acid sequence alignment. GDSL-like lipase conserved sequence blocks are underlined. Asterisks (\*) indicate the active site residues and the arrowheads indicate the conserved amino acids of the SGNH\_hydrolase family. The sequences included in the alignment are: MBOVPG45\_0710 (MilA), *M. bovis* PG45; MMB\_0654, *M. bovis* strain Hubei-1; MAGa6830, *M. agalactiae* PG2; MCSF7\_01871, *M. columbinum* SF7; MFE\_02570, *M. fermentans* JER; MYPU3130, *M. pulmonis*; and mhp677, *M. hyopneumoniae*.