



**Figure S2. Analysis of Synthetic DK<sup>L</sup>A and Digestion Products.** (A) HPLC chromatogram of synthetic lipoyl tripeptide DK<sup>L</sup>A eluting from a C18 column on a HP1090 instrument (Konishi et al., 1996). The only peak eluted at 10.89 min and contained 100% of the area, indicating there were no byproducts from synthesis that were not pure DK<sup>L</sup>A. (B) LC-MS mass spectrum of Anaspec synthesized DK<sup>L</sup>A injected onto a C18 column. DK<sup>L</sup>A exhibited a peak at 521.7 Da (C) LC-MS mass spectrum of aminopeptidase M digested DK<sup>L</sup>A injected onto a C18 column and analyzed in positive ion mode on a nanoAcuity/Qtof premier instrument. DK<sup>L</sup>A exhibited a peak at 521.2381 Da, DK<sup>L</sup> exhibited a peak at 450.1994 Da, and K<sup>L</sup> exhibited a peak at 335.1571 Da.

**Figure S3. Heterologous Expression of *L. monocytogenes* Lipoate Ligases in *E. coli*.** *E. coli* TM131 transformed with an empty IPTG inducible vector or vector containing a C-terminal histidine-tagged *E. coli* LplA, *L. monocytogenes* LplA1 or LplA2. Strains were grown in LB with 1mM IPTG. Bacterial pellets were lysed with SDS-PAGE buffer, boiled and analyzed by SDS-PAGE, followed by immunoblot with **A**) an anti-his antibody and **B**) an anti-lipoic acid antibody. The bands appearing at 37kDa on the anti-histidine blot correspond to the predicted size of *E. coli* LplA and *L. monocytogenes* LplA1, and the 75kDa band in the anti-lipoic acid blot corresponds with lipoylated E2-PDH from *E. coli*.

# Figure S1

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Lm LplA1 -----MYFIDNNEKDPRLNLAVEEFILTELNLDEPVLLFYINKPSIIIGRNQN 49
Lm LplA2 -----MIYLDNEDVLDQAYNFAMEEYALRSLDENETYFMFYRMKPTIIVGKNQN 49
Sp LplA  XGSDKIHSHHHXKYIINHSNDTAFNIALEEYAFKHLLEDQIFLLWINKPSIIVGRHQ 60
Ec LplA  -----MSTLRLISDSYDPWFNLAVEECIFRQMPATQRVLFWRNADTVVIGRAQN 51
          . . * * : : : : : : : : : : : : : : : : : : : : : : : : : : : :
          . . * * : : : : : : : : : : : : : : : : : : : : : : : : : : : :

Lm LplA1 TVEEIDTEYVEKNDVIVVRRRLS GGGAVYHDEGNLNF SFITEDDGESFHNFAKFTQPIVEA 109
Lm LplA2 TLEEINHPFVKDHHIDVLRRLS GGGAVYHDEGNISFSMITKDDGNSFQNF AKFTPEPVIRA 109
Sp LplA  TIEEINRDYVRENGIEVVRRIS GGGAVYHDLNNLNYTIISKEDENKAFDFKSFSTPVINT 120
Ec LplA  PWKECNTRMEEDNVRLARRSS GGGAVFHDLGNTCFTFMAGKPE---YDKTISTSIVLNA 108
          . : * : : . . . : : * * * * * : : * . * : : : : . : : : : : : : : : :

Lm LplA1 LKRLGVNAELKGRNDLLIDGF---KVSGNAQFATKGKMFSGHGLM YDLNLDNVAASLKP 165
Lm LplA2 LRKLGVAELSGRNDIEVNGK---KISGNAQFATKGRLYSHGLLFDVDLSMLEKALQV 165
Sp LplA  LAQLGVKAEFTGRNDLEIDGK---KFCGNAQAYINGRIXHHGCLLFDVDLSVLANALKV 176
Ec LplA  LNALGVSAEASGRNDLVVKTVEGDRKVSGSA YRETKDRGFHGHGLLLNADLSRLANYLNP 168
          * * * * . * * : : . * * * * : : * * * : : : * : : * :

Lm LplA1 RKDKIESKGIKSVRSRVANISDFMDQEMTTEEFRDLLLLYIFGVEKVEDVKEYKLTAADW 225
Lm LplA2 DPEKYLKSGVKSVRSRVTTIREHLAEDIDILTFKQILLESIF---ETKDIPRYTFTEADK 222
Sp LplA  SKDKFESKGVKSVRARVTNIINELPKKITVEKFRDLLLEYXK--KEYPEXTEYVFSEEL 234
Ec LplA  DKKKLAAGKITSVRSRVTNLTELTPGITHEQVCEAITEAFFAHYGERVEAEIISPKNKTPD 228
          . * : * * : * * : * * : : : : : : : : : : : : : : .

Lm LplA1 EKIHEISAKRYGNWDWNYGKSPKFDLTRTKRFPVGAVDVRLNVQKGVITDIKIFGDFFGV 285
Lm LplA2 QGIEKLRTERYRNWDWTYGKSPKATIKRKKRFPAGTIEFQVSLEKGQVKEATIYGDFFGT 282
Sp LplA  AEINRIKDTKFGTWDWNYGKSPEFNVRRGIKFTSGKVEVFANVTESKIQDIKIYGDFFGI 294
Ec LplA  LPNFAETFARQSSWEWNFGQAPAFSHLLDERFTWGGVELHFDVEKGHITRAQVFTDSLNP 288
          : . * * : * * : * * : * * : * * : * * : * * : * * : * * : * * :

Lm LplA1 KNVADIEEKLVTNTTYKREVLAEALVDIDVKEYFGNITKDEFLLDLY--- 331
Lm LplA2 EDVAELAEKIIGCRFERKSIQNAWQEINAKDYFGGIEKEAILDMLFE--- 329
Sp LplA  EDVAAVEDVLRGVKYEREDVLKALKTIDITRYFAGISREEIAEAVVG--- 341
Ec LplA  APLEALAGRLQGCLYRADMLQEQEALLVDFPEQEKELELSAWMAGAVR 338
          : : : . : . : : : : : : : : : : : : : : : : : : : : : : : : :


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## Lm LplA1 vs Lm LplA2

Identity: 46.8%  
Similarity: 70.9%

## Lm LplA1 vs Ec LplA

Identity: 27.7%  
Similarity: 49.5%

## Lm LplA2 vs Ec LplA

Identity: 22.5%  
Similarity: 40.7%

## Sp LplA vs Ec LplA

Identity: 22.4%  
Similarity: 40.5%

## Lm LplA1 vs Sp LplA

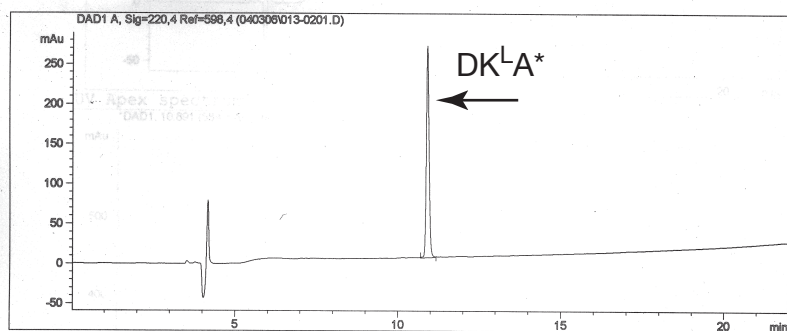
Identity: 45.6%  
Similarity: 65.8%

## Lm LplA2 vs Sp LplA

Identity: 43.8%  
Similarity: 66.6%

# Figure S2

## A

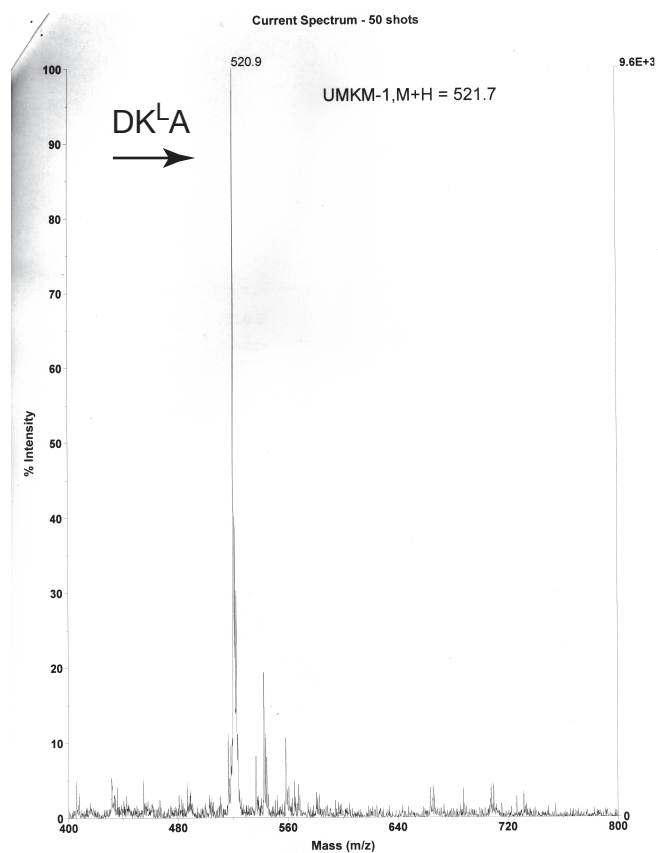


Area Percent Report

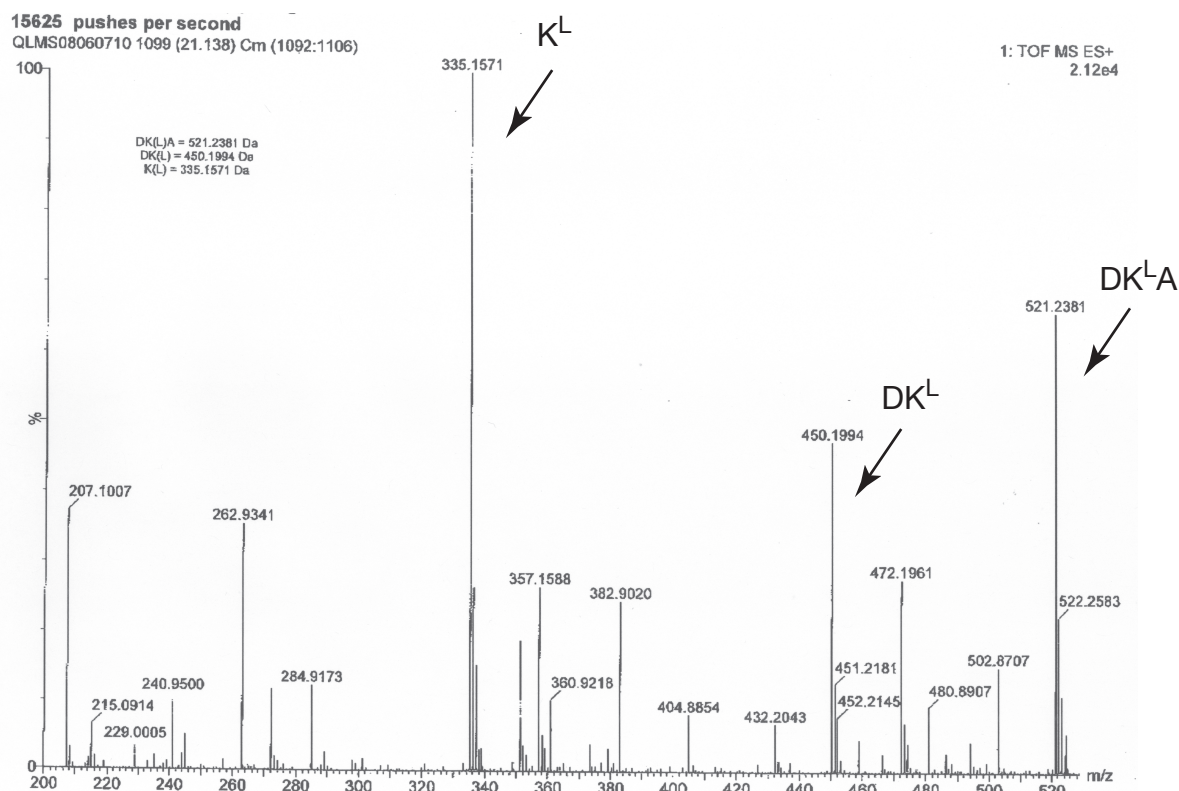
| Meas. RT<br>min | Area<br>mAU*sec | Height<br>mAU | Area<br>% |
|-----------------|-----------------|---------------|-----------|
| 10.89           | 1535.6          | 265.5         | 100.0     |

DK<sup>L</sup>A\* = peak corresponding to lipoyl-peptide represents 100% of all HPLC elutants from injection

## B

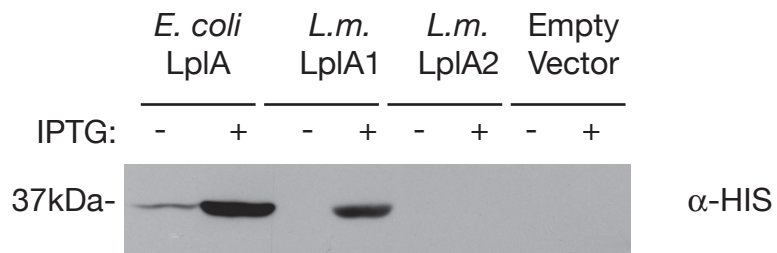


## C



# Figure S3

## A



## B

