Figure S1. Tertiary structure prediction of the vaccine target through EasyModeller 4.0 (A) and Ramachandran Plot analysis (B).
**Figure S2.** Quality factor analysis of modelled 3D structure of LEGPH protein TolB by ERRAT.
Figure S3. Prediction of B cell linear epitope and intrinsic properties for LEGPH protein Tol B using different scales (A: Bepipred, B: Surface accessibility, C: Emini surface, D: Flexibility, E: Antigenicity, F: Hydrophilicity). (For each graph: the x-axis and y-axis represent the position and score; residues that fall above the threshold value are shown in yellow colour; the highest peak in yellow colour identifies the most favoured position).
**Figure S4.** Schematic representation of the *L. pneumophila* vaccine constructs.

The adjuvants are attached at the N-terminal of the construct. The four linkers (EAAAK, GGGS, GPGPG and KK) were used to connect the adjuvant, PADRE and epitopes together.
Figure S5. Secondary structure prediction of designed vaccine V1 using PESIPRED server
Figure S6. 3D modelled structure of vaccine protein V2 (A) and V3 (B) generated via RaptorX server.
Figure S7. Restriction digestion (A) and in silico cloning (B) of the gene sequence of final vaccine construct V1 into pET28a(+) expression vector. Target sequence was inserted between HindIII (173) and BamHI (198).
**Supplementary Table 1:** Unique pathways of *Legionella pneumophila* retrieved from KEGG server.

| Serial. no. | Pathway id | Unique Pathways                          |
|-------------|------------|-------------------------------------------|
| 1           | lpn00121   | Secondary bile acid biosynthesis          |
| 2           | lpn00261   | Monobactam biosynthesis                   |
| 3           | lpn00281   | Geraniol degradation                      |
| 4           | lpn00300   | Lysine biosynthesis                       |
| 5           | lpn00332   | Carbapenem biosynthesis                   |
| 6           | lpn00361   | Chlorocyclohexane and chlorobenzene degradation |
| 7           | lpn00362   | Benzoate degradation                      |
| 8           | lpn00364   | Fluorobenzoate degradation                |
| 9           | lpn00401   | Novobiocin biosynthesis                   |
| 10          | lpn00460   | Cyanoamino acid metabolism                |
| 11          | lpn00473   | D-Alanine metabolism                      |
| 12          | lpn00521   | Streptomycin biosynthesis                 |
| 13          | lpn00523   | Polyketide sugar unit biosynthesis        |
| 14          | lpn00525   | Acarbose and validamycin biosynthesis     |
| 15          | lpn00540   | Lipopolysaccharide biosynthesis           |
| 16          | lpn00550   | Peptidoglycan biosynthesis                |
| 17          | lpn00623   | Toluene degradation                       |
| 18          | lpn00625   | Chloroalkane and chloroalkene degradation |
| 19          | lpn00626   | Naphthalene degradation                   |
| 20          | lpn00627   | Aminobenzoate degradation                 |
| 21          | lpn00643   | Styrene degradation                       |
|   | Code       | Description                                           |
|---|------------|-------------------------------------------------------|
| 22| lpn00680   | Methane metabolism                                    |
| 23| lpn00903   | Limonene and pinene degradation                       |
| 24| lpn00930   | Caprolactam degradation                               |
| 25| lpn01110   | Biosynthesis of secondary metabolites                 |
| 26| lpn01120   | Microbial metabolism in diverse environments          |
| 27| lpn01130   | Biosynthesis of antibiotics                           |
| 28| lpn01220   | Degradation of aromatic compounds                     |
| 29| lpn01501   | beta-Lactam resistance                               |
| 30| lpn01502   | Vancomycin resistance                                |
| 31| lpn01503   | Cationic antimicrobial peptide (CAMP) resistance      |
| 32| lpn02020   | Two-component system                                 |
| 33| lpn02024   | Quorum sensing                                       |
| 34| lpn02020   | Bacterial chemotaxis                                 |
| 35| lpn02040   | Flagellar assembly                                   |
| 36| lpn03070   | Bacterial secretion system                           |
| 37| lpn00550   | Peptidoglycan biosynthesis                           |
**Supplementary Table 2:** KO assigned (KEGG orthlogy) proteins not involved in any metabolic pathway

| Serial no. | KO assignment | Accession no. | Protein name |
|------------|---------------|---------------|--------------|
| 1          | K03797        | tr|Q5ZY73      | Carboxy-terminal protease |
| 2          | K03294        | tr|Q5ZZI4      | Amino acid permease |
| 3          | K09822        | sp|Q5ZYZ0      | Hypothetical protein |
| 4          | K07568        | sp|Q5ZU04      | S-adenosylmethionine:tRNAribosyltransferase-isomerase |
| 5          | K06941        | sp|Q5ZV93      | Dual-specificity RNA methyltransferaseRlmN |
| 6          | K03980        | tr|Q5ZS84      | Probable lipid II flippaseMurJ |
| 7          | K03466        | tr|Q5ZUM7      | Cell division protein FtsK |
| 8          | K03089        | tr|Q5ZS52      | RNA polymerase sigma factor RpoH |
| 9          | K07007        | tr|Q5ZS92      | Hypothetical protein |
| 10         | K03656        | tr|Q5ZRR4      | ATP-dependent DNA helicase Rep |
| 11         | K03837        | tr|Q5ZT44      | Serine transporter |
| 12         | K03086        | tr|Q5ZTO4      | LEGPH RNA polymerase sigma factor RpoD |
| 13         | K03609        | tr|Q5ZUR8      | Site-determining protein |
| 14         | K02600        | tr|Q5ZRV3      | Transcription termination/antitermination protein NusA |
| 15         | K03722        | tr|Q5ZU85      | ATP dependent DNA helicase |
| 16         | K03592        | tr|Q5ZUM2      | Peptide maturation protein PmbA |
| 17         | K19810        | tr|Q5ZYS3      | L-lysine 2,3-aminomutase, radical SAM domain protein |
| 18         | K05837        | tr|Q5ZVR6      | Peptidoglycan glycosyltransferaseMrdB |
| 19         | K03641        | sp|Q5ZV69      | Protein TolB |
| 20         | K03545        | sp|Q5ZUD8      | Trigger factor |
| 21         | K03549        | sp|Q5ZW98      | Probable potassium transport system protein kup 1 |
| 22         | K03549        | sp|Q5ZSY2      | Probable potassium transport system protein kup |
| Serial. no. | Accession no. | KO assignment | Family name | Probability (%) | GO category |
|-------------|---------------|---------------|-------------|----------------|-------------|
| 1           | sp|Q5ZYZ0       | K09822       | Iron-binding | 98.4          | GO:0005506  |
| 2           | tr|Q5ZS92       | K07007       | Iron-binding | 98.9          | GO:0005506  |
| 3           | tr|Q5ZYU2       | K06915       | All DNA-binding | 85.4        | GO:0003677  |
**Supplementary Table 4**: Subcellular localization of nonhomologous essential proteins involved in unique pathways of *Legionella pneumophila*

| Serial. no. | Accession no. | PSORTbv3.0.2 server | CELLO v2.5 server | ngLOC server | PSLpred server | Final result |
|-------------|---------------|---------------------|------------------|--------------|----------------|--------------|
| 1           | tr|Q5ZY73        | Cytoplasmic Membrane | Outer membrane   | Cytoplasmic  | Cytoplasmic  | Cytoplasmic  |
| 2           | tr|Q5ZZI4        | Cytoplasmic Membrane | Inner Membrane   | Inner Membrane | Inner Membrane |
| 3           | sp|Q5ZYZ0        | Cytoplasmic         | Cytoplasmic      | Cytoplasmic  | Cytoplasmic  | Cytoplasmic  |
| 4           | sp|Q5ZU04        | Cytoplasmic         | Cytoplasmic      | Cytoplasmic  | Cytoplasmic  | Cytoplasmic  |
| 5           | sp|Q5ZV93        | Cytoplasmic         | Cytoplasmic      | Cytoplasmic  | Cytoplasmic  | Cytoplasmic  |
| 6           | tr|Q5ZS84        | Cytoplasmic Membrane | Inner Membrane   | Inner Membrane | Inner Membrane |
| 7           | tr|Q5ZUM7        | Cytoplasmic Membrane | Inner Membrane   | Inner Membrane | Inner Membrane |
| 8           | tr|Q5ZS52        | Cytoplasmic         | Cytoplasmic      | Cytoplasmic  | Cytoplasmic  | Cytoplasmic  |
| 9           | tr|Q5ZS92        | Cytoplasmic         | Cytoplasmic      | Cytoplasmic  | Cytoplasmic  | Cytoplasmic  |
| 10          | tr|Q5ZR4         | Cytoplasmic         | Cytoplasmic      | Cytoplasmic  | Cytoplasmic  | Cytoplasmic  |
| 11          | tr|Q5ZT44        | Cytoplasmic Membrane | Inner Membrane   | Inner Membrane | Inner Membrane |
| 12          | tr|Q5ZT04        | Cytoplasmic         | Cytoplasmic      | Cytoplasmic  | Cytoplasmic  | Cytoplasmic  |
|   | Accession    | Location         | Cytoplasmic | Inner Membrane | Inner Membrane | Inner Membrane |
|---|--------------|------------------|-------------|----------------|----------------|----------------|
| 13| tr|Q5ZUR8      | Multiple location |  |  |  |
| 14| tr|Q5ZRV3      | Cytoplasmic      | Cytoplasmic |  |  |
| 15| tr|Q5ZU85      | Cytoplasmic      | Cytoplasmic |  |  |
| 16| tr|Q5ZUM2      | Cytoplasmic      | Outer membrane |  |  |
| 17| tr|Q5ZYS3      | Cytoplasmic      | Cytoplasmic |  |  |
| 18| tr|Q5ZVR6      | Cytoplasmic      | Inner Membrane |  |  |
| 19| sp|Q5ZV69      | Outer membrane   | Inner Membrane | Outer membrane |  |
| 20| sp|Q5ZUD8      | Cytoplasmic      | Cytoplasmic |  |  |
| 21| sp|Q5ZW98      | Cytoplasmic      | Inner Membrane |  |  |
| 22| sp|Q5ZSY2      | Cytoplasmic      | Inner Membrane |  |  |
| 23| sp|Q5ZTN5      | Cytoplasmic      | Inner Membrane |  |  |
| 24| sp|Q5ZRL2      | Cytoplasmic      | Inner Membrane |  |  |
| 25| tr|Q5ZYU2      | Unknown          | Inner Membrane | Cytoplasmic | Periplasmic | Multiple location |
|   | Accession | Membrane Type 1 | Membrane Type 2 | Membrane Type 3 | Membrane Type 4 |
|---|-----------|-----------------|-----------------|-----------------|-----------------|
| 26| tr|Q5ZY66     | Outer membrane  | Outer membrane  | Inner Membrane  | Outer membrane  |
| 27| tr|Q5ZXX9     | Cytoplasmic     | Cytoplasmic     | Cytoplasmic     | Cytoplasmic     |
| 28| tr|Q5ZX71     | Cytoplasmic     | Inner Membrane  | Inner Membrane  | Inner Membrane  |
| 29| tr|Q5ZUP0     | Cytoplasmic     | Cytoplasmic     | Cytoplasmic     | Cytoplasmic     |
| 30| tr|Q5ZXL1     | Cytoplasmic     | Inner Membrane  | Inner Membrane  | Inner Membrane  |
| 31| tr|Q5ZX16     | Cytoplasmic     | Cytoplasmic     | Cytoplasmic     | Cytoplasmic     |
| 32| tr|Q5ZX17     | Cytoplasmic     | Inner Membrane  | Cytoplasmic     | Inner Membrane  |
| 33| tr|Q5ZVR5     | Cytoplasmic     | Outer membrane  | Inner Membrane  | Inner Membrane  |
**Supplementary Table 5:** Details of Protein-Protein Interactions through STRING v11.5

| Target Protein | Predicted Functional Partner | Gene Name | Functions |
|----------------|-------------------------------|-----------|-----------|
| UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase (murE) | UDP-N-acetylmuramate--L-alanine ligase | murC | involved in the pathway peptidoglycan biosynthesis, which is part of Cell wall biogenesis |
| | Peptidoglycan D,D-transpeptidase FtsI | pbpB | Catalyzes cross-linking of the peptidoglycan cell wall at the division septum |
| | Phospho-N-acetylmuramoyl-pentapeptide-transferase | mraY | involved in the first step of the lipid cycle reactions in the biosynthesis of the cell wall peptidoglycan |
| | Probable peptidoglycan glycosyltransferase FtsW | ftsW | Essential cell division protein.; transports lipid-linked peptidoglycan precursors from the inner to the outer leaflet of the cytoplasmic membrane |
| | D-alanine--D-alanine ligase | ddl | involved in cell wall formation; catalyzes the formation of D-alanyl-D-alanine |
| | UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase | murG | Catalyzes the transfer of a GlcNAc subunit on undecaprenyl-pyrophosphoryl-MurNAc-pentapeptide (lipid intermediate I) to form undecaprenyl-pyrophosphoryl-MurNAc- (pentapeptide)GlcNAc (lipid intermediate II); involved in cell wall formation |
| | UDP-N-acetylenolpyruvylglucosamine reductase | murB | involved in the pathway peptidoglycan biosynthesis |
| | UDP-N-acetylg glucosamine 1-carboxyvinyltransferase | murA | involved in the pathway peptidoglycan biosynthesis; adds enolpyruvyl to UDP-N-acetylg glucosamine |
| | UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase | murF | Involved in cell wall formation; catalyzes the final step in the synthesis of UDP-N-acetylmuramoyl-pentapeptide, the precursor of murein |
| | UDP-N-acetylmuramoylalanine--D-glutamate ligase | murD | Catalyzes the addition of glutamate to the nucleotide precursor UDP-N-acetylmuramoyl-L-alanine |
| Protein                                      | Gene  | Function                                                                 |
|----------------------------------------------|-------|---------------------------------------------------------------------------|
| 50S ribosomal protein L15                    | rplO  | Binds to the 23S rRNA                                                    |
| Trigger factor (tig)                         |       | ATP-dependent Clp protease proteolytic subunit                            |
| clpP                                          |       | Cleaves peptides in various proteins in a process that requires ATP hydrolysis; role in the degradation of misfolded proteins |
| 60 kDa chaperonin                            | grol  | Prevents misfolding and promotes the refolding and proper assembly of unfolded polypeptides generated under stress conditions |
| Peptidoglycan D,D-transpeptidase MrdA        | pbrA  | Catalyzes cross-linking of the peptidoglycan cell wall                   |
| 50S ribosomal protein L2                     | rplB  | Required for association of the 30S and 50S subunits to form the 70S ribosome, for tRNA binding and peptide bond formation |
| Two component response regulator             | lpg0715 | Makes several contacts with the 16S rRNA in the 70S ribosome             |
| 50S ribosomal protein L29                    | rpmC  | -                                                                         |
| 50S ribosomal protein L5                     | rplE  | It contacts protein S13 of the 30S subunit (bridge B1b), connecting the 2 subunits in the 70S ribosome; Contacts the P site tRNA |
| 50S ribosomal protein L6                     | rplF  | Binds to the 23S rRNA                                                    |
| 50S ribosomal protein L23                    | rplW  | Binds 23S rRNA; Forms the main docking site for trigger factor binding to the ribosome |
| 50S ribosomal protein L15                    | rplO  | Binds to the 23S rRNA                                                    |
| Probable lipid II flippase MurJ (mviN)       |       | Catalyzes the conversion of dethiobiotin (DTB) to biotin by the insertion of a sulfur atom into dethiobiotin via a radical- based mechanism |
| Biotin synthase                              | bioB  | -                                                                         |
| Leucine aminopeptidase                       | lpg2634 | -                                                                         |
| Uncharacterized protein                      | lpg2633 | -                                                                         |
| Protein TolB (tolB)                          |       | Mediates coordination of peptidoglycan synthesis and outer membrane constriction during cell division |
| TolA colicin import membrane protein        | tola  | -                                                                         |
| Cell division coordinator CpoB               | cpoB  | -                                                                         |
| Protein Description                                      | Gene Symbol | Function Description                                                                                                                                 |
|----------------------------------------------------------|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| Peptidoglycan-associated protein                         | pal         | plays a role in outer membrane invagination during cell division; important for maintaining outer membrane integrity                               |
| Outer-membrane lipoprotein carrier protein               | lolA        | Participates in the translocation of lipoproteins from the inner membrane to the outer membrane                                                     |
| Tol-Pal system protein TolR                               | tolR        | Participates in the translocation of lipoproteins from the inner membrane to the outer membrane                                                     |
| Holliday junction ATP-dependent DNA helicase RuvB         | ruvB        | mediates the Holliday junction migration by localized denaturation and reannealing                                                                     |
| Tol-Pal system protein TolQ                               | tolQ        | Participates in the translocation of lipoproteins from the inner membrane to the outer membrane                                                     |
| Esterase                                                 | lpg1575     | -                                                                                                                                                  |
| Outer membrane protein, OmpA family protein               | lpg0657     | -                                                                                                                                                  |
| Sodium-type flagellar protein                            | lpg2962     | -                                                                                                                                                  |
| Probable potassium transport system protein kup 1 (kup1)  | lpg1189     | -                                                                                                                                                  |
**Supplementary Table 6:** Templates used for homology modelling by EasyModeller 4.0

| Protein accession no. | PDB ID | Identity (%) | Query cover |
|-----------------------|--------|--------------|-------------|
| sp|Q5ZV69 | 4R40 | 41 | 95 |
| | 3IAX | 40 | 99 |
| | 1C5K | 40 | 99 |

**Supplementary Table 7:** ProtParam analysis of LEGPH protein TolB

| Viral Proteins | Accession ID | Molecular Weight | Instability Index | Aliphatic Index | Theoretical pI | No. of Amino acids | Extinction Co-Efficient | Gravy |
|----------------|--------------|------------------|------------------|----------------|----------------|-------------------|------------------------|-------|
| TolB_LEGPH | Q5ZV69 | 45387.0 6 | 38.09 | 89.81 | 8.67 | 419 | 34380 | -0.204 |

**Supplementary Table 8:** Identified epitope clusters among top CTL, HTL and BCL epitopes

| CTL epitope clusters | HTL epitope clusters | BCL epitope clusters |
|----------------------|----------------------|----------------------|
| RIISLFLLFT | TSGRGGSPQVY | SGPQGANSQSSV |
| TQLVADLE | QVIALDLELTQ | AREGDVQEPAWSPY |
| QVIALDLELTQ | TSGRGGSPQVYRLSLA | SWSPNGK |
| IGVQNTGGGP | LTFGNSIDTEPRYSPDG | SGRGGSP |
| LPAREGDVQ | FTSGRGGSPQVYRLSLA | SFEKKKA |
| DVDLSSGSMK | YSLEVADADGHNPQS | IDTEPRYSPDG |
| TFGNSIDTE | RVTFEGNYNARASYT | ADGQISR |
| | GGRSRYSLEVADADG | IISLFLLFTQVIALDLE |
| | ISGPGQGANSQSVST | DLE |
| | VIALDLELTQGINS | GQHLAVVLSK |
| | | YSPDGRS |
Supplementary Table 9: Protein-protein interaction after 100 ns molecular docking complex.

| Receptor | Residues | Position | Bond type | Ligand | Residues | Position | Distance |
|----------|----------|----------|-----------|--------|----------|----------|----------|
| Human TLR2 (Chain B) | ASN | 294 | Hydrogen | Vaccine (Chain D) | ILE | 396 | 2.66 |
| | ILE | 319 | Hydrogen | | GLY | 279 | 2.95 |
| | PHE | 325 | Hydrogen | | GLN | 331 | 3.19 |
| | ASP | 327 | Hydrogen | LYS | 352 | 2.72 |
| | LYS | 347 | Hydrogen | GLY | 281 | 3.19 |
| | GLU | 375 | Hydrogen | ARG | 284 | 2.92 |
| | GLU | 375 | Hydrogen | ARG | 284 | 3.04 |
| | ASP | 327 | Salt Bridge | LYS | 352 | 2.72 |
| | GLU | 375 | Salt Bridge | ARG | 284 | 2.92 |