Supplemental Table 1. Clinical validation checklist.

Study goals: To test if lysine acetylation could affect the immunogenicity of peptide derived from HspX.

Study design: Two peptides (6291 and A-6291) were synthesized (GL Biochem Ltd, shanghai) based on the sequence of HspX of *M. tuberculosis* H37Ra. The two peptides share the same sequence, which spanning from residue 62-91 of HspX (PDKDVDIMVRDGQLTIKAERTEQKDFDGRS), except that all three lysines in A-6291 were acetylated according to our mass spectrometry data. ELISPOT test was performed according to the instructions of the manufacture (T-SPOT TB 96 assay, catalogue no. TB.200; Oxford Immunotec). 5 mL of whole blood was drawn from each subject by venipuncture and stored at room temperature. After isolation by ficoll-hypaque (MD Pacific, Tianjin, China) density-gradient centrifugation, the cloudy PBMCs harvested were washed twice using pre-warmed RPMI-1640 (MD Pacific, Tianjin, China) or AIM-V (Invitrogen, Karlsruhe, Germany). Adequate AIM-V was used to resuspend PBMCs at last and blood cell counting plate was applied to take count of the cells. Four wells of the 96-wells microtiter plate pre-coated with monoclonal antibody to interferon-gamma (IFN-γ, provided in the kit), were seeded with PBMCs suspension prepared before. The positive control was stimulated with phytohaemagglutinin (PHA, provided in the kit). A Nil control well contained PBS and medium as stimulants was set in one experiment to detect contamination. The other two were added with identical concentration (10 μg mL^{-1}) of acetylated peptide A-6291 and the normal peptide 6291 resolved in PBS. The plate

was then incubated at 37 ℃ and 5% CO_2 for 16-20 h. After that, the plates were washed thoroughly by the PBS several times after sufficient incubation time, and then incubated with 50 μL of alkaline-phosphatase conjugated anti-IFN-γ monoclonal antibody at 4 ℃ for 1 h. Then the plates were washed thoroughly again with PBS after incubation and 50 μL substrate solution was added to each well. After incubation for 7 minutes at room temperature, the reaction was stopped with tap water and the plates were dried to visualize the spots. Total number and analysis of the spots forming cell (SFC) of each well was performed by using Bioreader 5000 (Dakewei Biotech Company, Shenzhen).

Characterization of patients for immunogenicity tests of peptides derived from HspX

Patient number*	Age	Gender	Disease type	Disease description	Co-morbidities	Potential confounders
1	43	male	tuberculosis	Smear positive, pulmonary tuberculosis	Chronic bronchitis	N/A
2	45	male	tuberculosis	Secondary pulmonary tuberculosis with cavities in the left upper lobe	Lumbar vertebra tuberculosis	N/A
3	62	male	tuberculosis	Secondary pulmonary tuberculosis	Chronic laryngopharyngitis	N/A
4	44	female	tuberculosis	Secondary pulmonary tuberculosis of the right lung	N/A	N/A
5	26	female	tuberculosis	Secondary pulmonary tuberculosis of the left upper lobe	N/A	N/A

6	45	male	tuberculosis	Secondary pulmonary tuberculosis with cavities in the right lung	Type 2 diabetes	N/A
7	68	male	tuberculosis	Secondary pulmonary tuberculosis with cavities in the right upper lobe	N/A	N/A
8	45	male	tuberculosis	Secondary pulmonary tuberculosis	N/A	N/A
9	23	female	tuberculosis	Secondary pulmonary tuberculosis	N/A	N/A
10	24	female	tuberculosis	Hematogenous disseminated pulmonary tuberculosis	Tubercular meningitis	N/A
11	54	female	tuberculosis	Tuberculous pleurisy	N/A	N/A
12	60	female	tuberculosis	Secondary pulmonary tuberculosis	Bronchiectasia	N/A
13	46	male	tuberculosis	Secondary pulmonary tuberculosis of the right upper lobe	N/A	N/A
14	53	male	tuberculosis	Secondary pulmonary tuberculosis	Tuberculous pleurisy	N/A
15	68	male	tuberculosis	Secondary pulmonary tuberculosis	N/A	N/A
16	46	male	tuberculosis	Secondary pulmonary tuberculosis	N/A	N/A
17	25	male	tuberculosis	Secondary pulmonary tuberculosis, multi-drug	N/A	N/A

				resistant		
18	49	male	tuberculosis	Secondary pulmonary tuberculosis, multi-drug resistant	N/A	N/A
19	47	male	tuberculosis	Secondary pulmonary tuberculosis	Enterophthisis	N/A
20	60	male	tuberculosis	Secondary pulmonary tuberculosis	Laryngophthisis	N/A
21	25	female	tuberculosis	Secondary pulmonary tuberculosis of the left lung with cavities	N/A	N/A
22	58	female	tuberculosis	Secondary pulmonary tuberculosis	N/A	N/A
23	56	male	tuberculosis	Secondary pulmonary tuberculosis	N/A	N/A
24	76	male	tuberculosis	Secondary pulmonary tuberculosis	N/A	N/A
25	27	mae	tuberculosis	Secondary pulmonary tuberculosis	N/A	N/A
26	66	male	tuberculosis	Infiltrative pulmonary tuberculosis with cavities	N/A	N/A

*26 tuberculosis patients were recruited from Foshan Fourth People's Hospital for this study, which were diagnosed and classified according to the 1990 edition of Diagnostic Standards and Classification of Tuberculosis published by the American Lung Association. The information of patients get enrolled in this study is listed as above table. This study was approved by and performed under the guidelines of the Ethical Committee of Wuhan Institute of Virology (CAS) (approve notice scanning copy showed below). Written informed consent was directly obtained from each participant.

生物医学研究伦理审查批准证明

批准号：WIVH16201401

项目名称：乙酰化对来源于HspX的人工合成多肽免疫原性的影响

人体材料：血样

申请时间：2014 年 01 月 09 日

伦理委员会审查认为：该申请中人体样本使用设计合理，使用量限于最低需要，研究人员具备相应资质，具备保障样本提供者个人隐私及知情权的措施；各项材料符合《中国科学院武汉病毒研究所涉及人体的生物医学研究伦理审查指南》要求，同意按照申请中的实验方案开展研究。

中国科学院武汉病毒研究所生命科学研究伦理审查委员会

2014 年 01 月 28 日

Approval notice from Institutional Review Board

Approval Number: WIVH16201401

The project Effect of lysine acetylation on immunogenicity of peptide derived from HspX requires human blood as material. An ethical application was submitted to the Institutional Review Board (IRB) dated on Jan 9, 2014.

Based on "Guideline for Biomedical Research Involving Human Subjects, Wuhan Institute of Virology, Chinese Academy of Sciences (CAS)", the IRB confirmed that the scheme of this project was properly designed, the amount of human materials required was limited to the lowest level, the investigators were qualified for carrying out the proposed project, sufficient information would be provided to the material providers, and the providers' privacy and data confidentiality would be adequately protected. In conclusion, the project design was ethically acceptable, and should be performed as described.

IRB, Wuhan Institute of Virology, CAS

01.28.2014