Response to comments from referee 1:

Referee comment: The study supports a very novel idea about pathogenesis of tuberculosis. The problem is case selection. Diagnosis of tuberculosis is not according to pathological finding or molecular detection. These criteria are not mentioned in material and method. I don’t decide to describe these here, but the study doesn’t support it and so the result and conclusion harm from this issue. This issue that the lymph nodes doesn’t have Mtb bacillus but molecular assay have been positive, it doesn’t support that these patients suffer from tuberculosis. If criteria was complete to confirm tuberculosis disease (not infection), and the study report these findings, I think about it, but now, I don’t accept the results and conclusion. I get an example for you; sarcoidosis in clinical and radiological is very similar to TB. The pathological finding could be granulomatous or non-granulomatous. In recent studies, MTB DNA has detected up to 30%, while culture has been negative for Mtb and the patients were clinically approved as sarcoidosis. So, the criteria for tuberculosis disease must be confirmed by culture (gold standard), positive smear or molecular assay beside of clinical finding and appropriate response to anti-TB. (Sarcoidosis and tuberculosis: the same disease with different manifestations or similar manifestations of different disorders. Dheeraj Gupta)

Author: Thank you for your valuable comments and we totally agree. These cases were diagnosed and treated as TB cases with good response to treatment. The diagnostic criteria were a combination of clinical, microbiological and histological criteria. Many cases were negative for AFB staining and culture results could not be confirmed for all cases. Therefore PCR was done afterwards just to have a further confirmation, especially for cases where culture was negative or results were not available. The diagnostic criteria are added in the text.

Referee comment: Also, the control group is very important. Did they check molecular assay to roll out existence of Mtb DNA? Did they perform PPD or interferon gamma assay to roll out LTBI (latent tuberculosis infection)?

Author: Yes. The negative controls were negative with nested-PCR for M.tuberculosis complex. PPD or interferon gamma assay was not performed on controls.

Referee comment: Both abstract and introduction is very long and boring, so finally the reader loss his/her focuses on issue.

Author: This is revised and shortened and made more focused.

Referee comment: In introduction, when the author tries to clarify the importance of in vivo finding, doesn’t describe that animal model is very different from human model, murine model is very different in dissemination and granuloma formation.(The best model is primates, the best capable and similar to human is rabbit/guinea pig,etc) (The tuberculous granuloma: an unsuccessful host defence mechanism providing a safety shelter for the bacteria? Silva Miranda M)

Author: This is revised in the text.
Referee comment: In result section is mentioned that the samples was evaluated if they contain granuloma, it should be mention in material method that which sample entered to study and what is the selection criteria.

Author: Selection criteria were confirmed TB cases based on the combined clinical, microbiological, and histological criteria. This information is added in the text. Controls were known non-TB controls. Absence of mycobacterial DNA was further confirmed by negative nested-PCR for M.tuberculosis IS6110.

Response to comments from referee 2:

Referee comment: How were the antigens selected for immunohistochemical analysis?

Author: The proteins are selected because these are the major secreted and the major somatic antigens.

Referee comment: No information is provided about the controls. Since many control samples yielded positive signal, it is important to ascertain if the controls had latent TB infection (positive tuberculin skin test or IGRA). If there is no evidence of Mtb exposure, this would suggest that the signal is non-specific.

Author: The negative controls included foreign-body granulomas of the skin, Colon cancer, normal tonsilar tissue, lung tissues from autopsy of ischemic heart disease as listed in table 1. All these controls were obtained from the archives of Department of Pathology, Haukeland University Hospital, Norway and no information is available on the exposure to TB or latent infection. Based on the quality of staining we suggest that the staining in negative controls is non-specific. Based on the localization and distribution in the tissue section it was possible to distinguish between true positive staining as in TB cases and the non-specific staining in non-TB controls (mentioned in results section) when evaluated by experienced persons, and this information can be used for academic purpose. However we chose to present the non-specific staining as positive in the controls in order to highlight that these antigens are not good candidates for diagnostic purposes.

Referee comment: How many sections and high-power fields were examined per case for immunostaining? The “+” system does not seem very quantitative and the cutoffs for number of cells infected are arbitrary.

Author: One section per case was used for counting of positive cells and three areas per section were counted. There was large intra-observer variability in counting due to the nature of staining. Due to this error in counting data is presented as semi-quantitative rather than in absolute numbers and percentages. This information is added in the text.

Referee comment: Antigen expression is not normalized based on mycobacterial number. Given that acid-fast bacilli could not be detected in the lymph nodes, while large numbers of bacilli were present in lung lesions, it is likely that differences in levels of antigen expression reflect different bacterial loads rather than different metabolic states of the organisms, as the authors suggest.
Author: Agree, and the statement about metabolic states is modified.

Referee comment: Simply because antigen are detected in the tissues does not mean these are protective and could be useful for informing vaccine studies. On the contrary, the high levels of expression in the lung tissue with large numbers of bacilli would suggest otherwise. Also, it is not clear how these findings could be “important for the development of new anti-mycobacterial drugs” (lines 107-108). Rather, their utility seems more for diagnostic purposes. The Introduction and Discussion should be modified accordingly.

Author: We agree with the comments and the discussion and introduction is modified.

Referee comment: Extrapulmonary TB accounts for approximately one-fifth of TB cases among immune-competent individuals and up to one-half in HIV-infected individuals.

Author: Thank you and changes are made.