

1 **Title:** Immunization with an adenovirus-vectored TB vaccine containing  
2 Ag85A-Mtb32 effectively alleviates allergic asthma

3 **Journal Name:** Journal of Molecular Medicine

4 **Authors:** Yiling Zhang<sup>1,2,3¶</sup>, Ying Feng<sup>1,2¶</sup>, Liang Li<sup>1,2¶</sup>, Xianmiao Ye<sup>2</sup>,  
5 Jinlin Wang<sup>2</sup>, Qian Wang<sup>2</sup>, Pingchao Li<sup>1,2</sup>, Na Li<sup>1</sup>, Xuehua Zheng<sup>2</sup>, Xiang,  
6 Gao<sup>2</sup>, Chufang Li<sup>1</sup>, Feng Li<sup>4</sup>, Baoqing Sun<sup>1</sup>, Kefang Lai<sup>1</sup>, Zhong Su<sup>2</sup>,  
7 Nanshan Zhong<sup>1</sup>, Ling Chen<sup>1,2\*</sup>, and Liqiang Feng<sup>2\*</sup>

8 **Affiliations:**

9 <sup>1</sup>State Key Laboratory of Respiratory Disease, The First Affiliated  
10 Hospital of Guangzhou Medical University, Guangzhou, China,

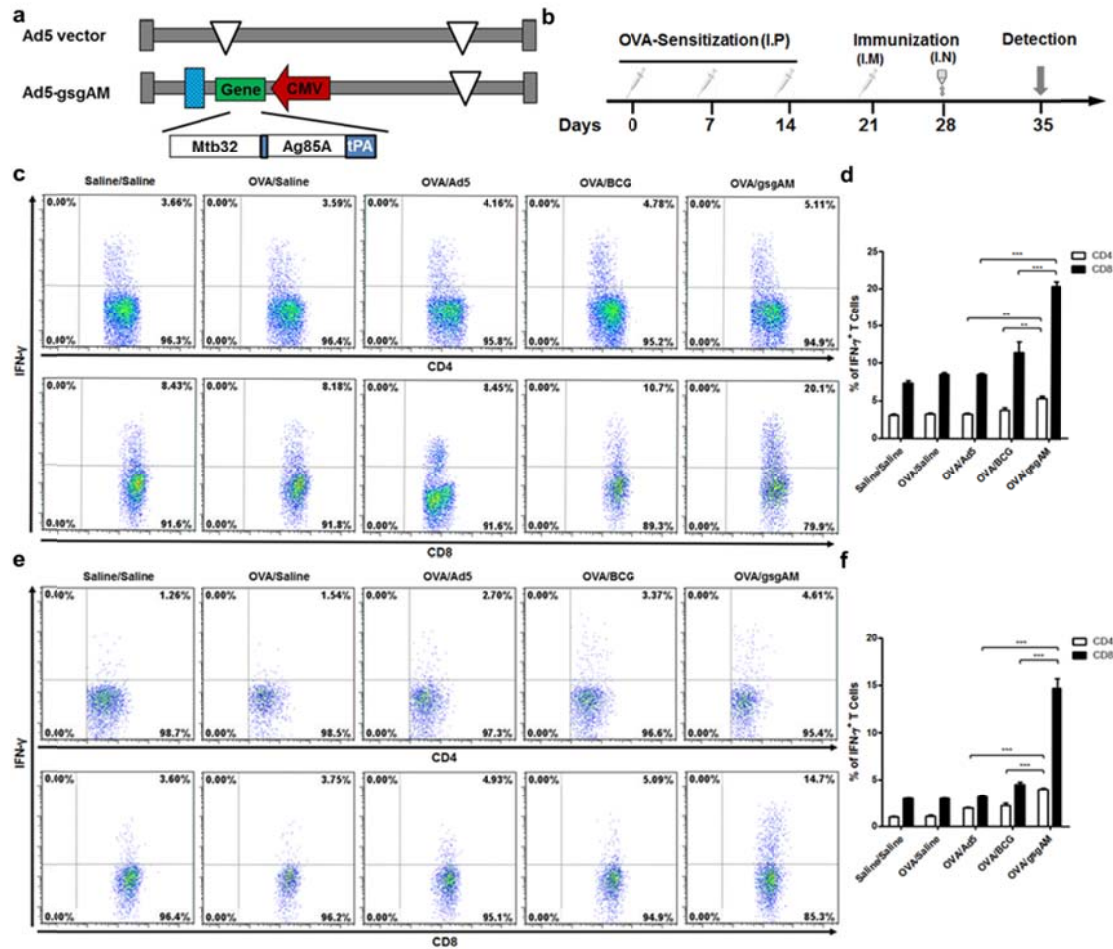
11 <sup>2</sup>Guangzhou Institutes of Biomedicine and Health, Chinese Academy of  
12 Sciences, Guangzhou, China, <sup>3</sup>Department of Respiratory Medicine,  
13 Guizhou Provincial People's Hospital, Guiyang, China. <sup>4</sup>Institute of  
14 Infectious Diseases, Guangzhou Eighth people's Hospital, Guangzhou  
15 Medical University, China.

16 ¶These authors contributed equally to this work.

17 \*Correspondence should be addressed to:

18 L.F. (E-mail: [feng\\_liqiang@gibh.ac.cn](mailto:feng_liqiang@gibh.ac.cn); Tel: +86-20-32015289; Fax:  
19 [+86-20-32015299](tel:+86-20-32015299)), or L.C. (E-mail: [chen\\_ling@gibh.ac.cn](mailto:chen_ling@gibh.ac.cn); Tel:  
20 [+86-20-32015289](tel:+86-20-32015289); Fax: [+86-20-32015299](tel:+86-20-32015299)), Guangzhou Institutes of  
21 Biomedicine and Health, Chinese Academy of Sciences, 190 Kai Yuan  
22 Avenue, Science Park, Guangzhou, China.

24 **Supplementary Figure S1**



25

34 **Figure S1. The immunogenicity of Ad5-gsgAM in OVA-sensitized**  
 35 **mice.** 6-week-old mice were sensitized with OVA and immunized with  
 36 Ad5, BCG, or Ad5-gsgAM through a prime-boost strategy. One week  
 37 after the final immunization, the spleen and lung tissues were dissected  
 38 out for analysis. **a** Diagram of the genomes of Ad5 and Ad5-gsgAM. **b**  
 39 Schedules of OVA sensitization, immunization and detection. **c**  
 40 Representative dot plots of IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> (upper panel) and IFN- $\gamma$ <sup>+</sup>CD8<sup>+</sup>  
 41 (bottom panel) T cells in the splenic lymphocytes stimulated with  
 42 PMA+ionomycin. **d** The percentages of IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> and IFN- $\gamma$ <sup>+</sup>CD8<sup>+</sup> T

34 cells in total CD4<sup>+</sup> or CD8<sup>+</sup> T cells in the spleen. **e** Representative dot  
35 plots of IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> (upper panel) and IFN- $\gamma$ <sup>+</sup>CD8<sup>+</sup> (bottom panel) T  
36 cells in the MLN lymphocytes stimulated with PMA+ionomycin. **f** The  
37 percentages of IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> and IFN- $\gamma$ <sup>+</sup>CD8<sup>+</sup> T cells in total CD4<sup>+</sup> or  
38 CD8<sup>+</sup> T cells in the MLN. Lymphocytes were isolated from the spleens  
39 and lungs of different groups of mice and were stimulated with  
40 PMA+ionomycin for 6h. Then the cells were stained with CD3-PerCP,  
41 CD4-FITC, CD8-APC, and IFN- $\gamma$ -PE, and subjected to FACS analysis.  
42 Representative results from one of three independent experiments are  
43 shown. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .