# Rabies virus Large Protein-derived T-cell immunogen facilitates rapid viral clearance and enhances protection against lethal challenge in mice

Shimeng Bai[1,3†], Xinghao Pan[2†], Tianhan Yang[2†], Nan Gao[2], Cuisong Zhu[2], Ai Xia[1], Meiqi Feng[1], Miaomiao Zhang[2], Xiaoyan Zhang[1,2*] and Jianqing Xu[1,2*]

[1] Clinical Center of Biotherapy, Zhongshan Hospital & Institutes of Biomedical Sciences, Fudan University; Shanghai, P. R. China

[2] Shanghai Public Health Clinical Center & Institutes of Biomedical Sciences, Fudan University, Shanghai, P. R. China

[3]Bio-therapeutic Center, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital; The Second Hospital Affiliated with the School of Medicine, Southern University of Science and Technology, Shenzhen, Guangdong, China

*Corresponding authors: Jianqing Xu, xujianqing@fudan.edu.cn; Xiaoyan Zhang, zhangxiaoyan@fudan.edu.cn;

**Description of Supplementary Information Files**

Figure S1. Analysis of RABV-LT antigen expression by Western blot

Figure S2. Analysis of RABV-G-LT antigen expression by Western blot corresponds to the images presented in Figure 2B.

Figure S3. Gating strategy and representative flow cytometry profiles of cytokines secreted splenocytes cells in mice, as presented in Fig. 1 or Fig. 2.

Table S1. HLA Class I and Class II supertypes and their representative HLA Class I or Class II alleles.

Table S2. RABV-LT peptides used in the study

Table S3. RABV-G peptides used in the study

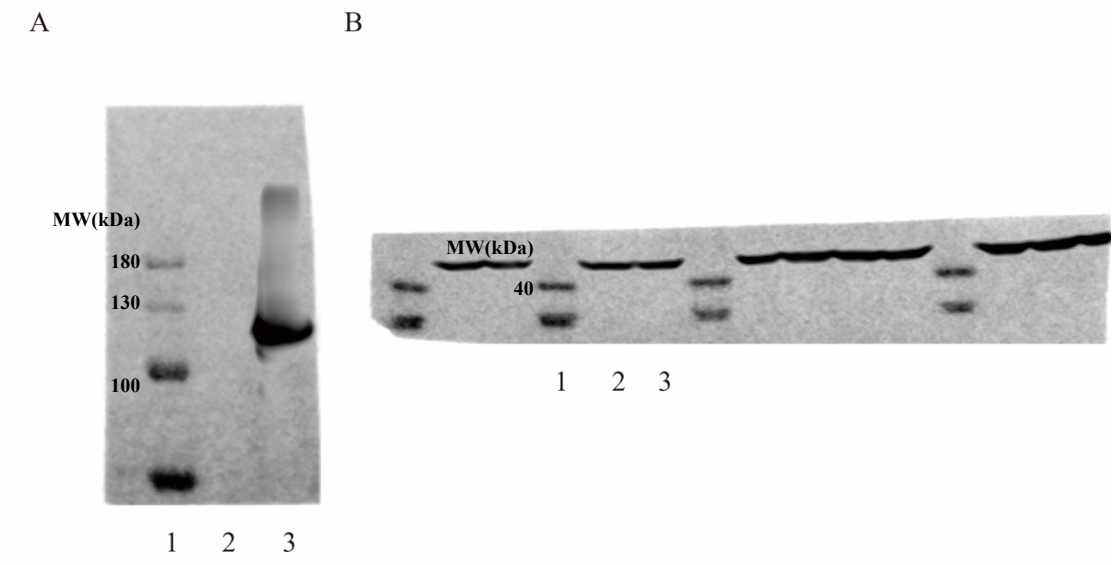


**Figure S1. Analysis of RABV-LT antigen expression by Western blot corresponds to the images presented in Figure 1C. (A)** Original Western blot image showing RABV-LT expression in HEK293T cells transfected with mRNA-LT, detected using Flag tag antibody. **(B)** Original Western blot image of β-actin corresponds to the samples of the lanes in Figure S1A. Lane 1: Thermo Scientific Page Ruler$^{TM}$ Prestained Protein Ladder (No. 26616); Lane 2: Blank lysates; Lane 3: mRNA-RABV-LT lysates.

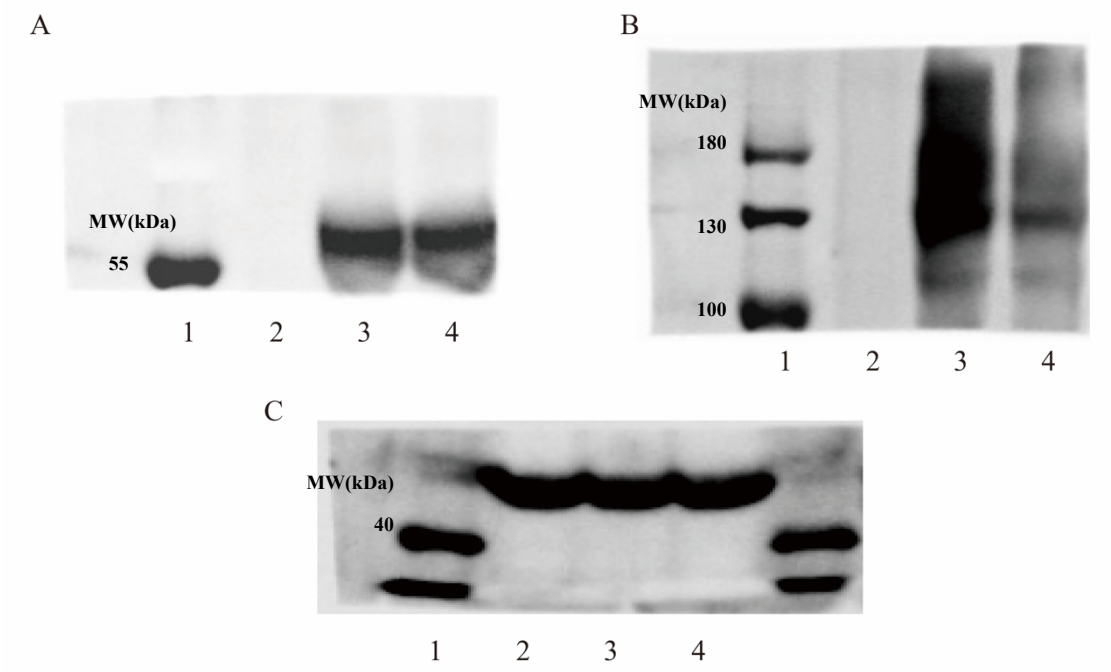


**Figure S2. Analysis of RABV-G-LT antigen expression by Western blot corresponds to the images presented in Figure 2B.** (A) Original Western blot image showing the RABV-G expression in HEK293T cells transfected with mRNA-RABV-G-LT (detected using anti-RABV-G antibody). (B) Original Western blot image showing RABV-LT expression in HEK293T cells transfected with mRNA-RABV-G-LT (detected using Flag tag antibody). (C) Western blot image of β-actin corresponds to the samples of the lanes in Figure S2A, S2B. Lane 1: Thermo Scientific Page Ruler$^{TM}$ Prestained Protein Ladder (No. 26616); Lane 2: Blank lysates; Lane 3: mRNA-RABV-G-LT + 50 μM TMP lysates; Lane 4: mRNA-RABV-G-LT lysates.

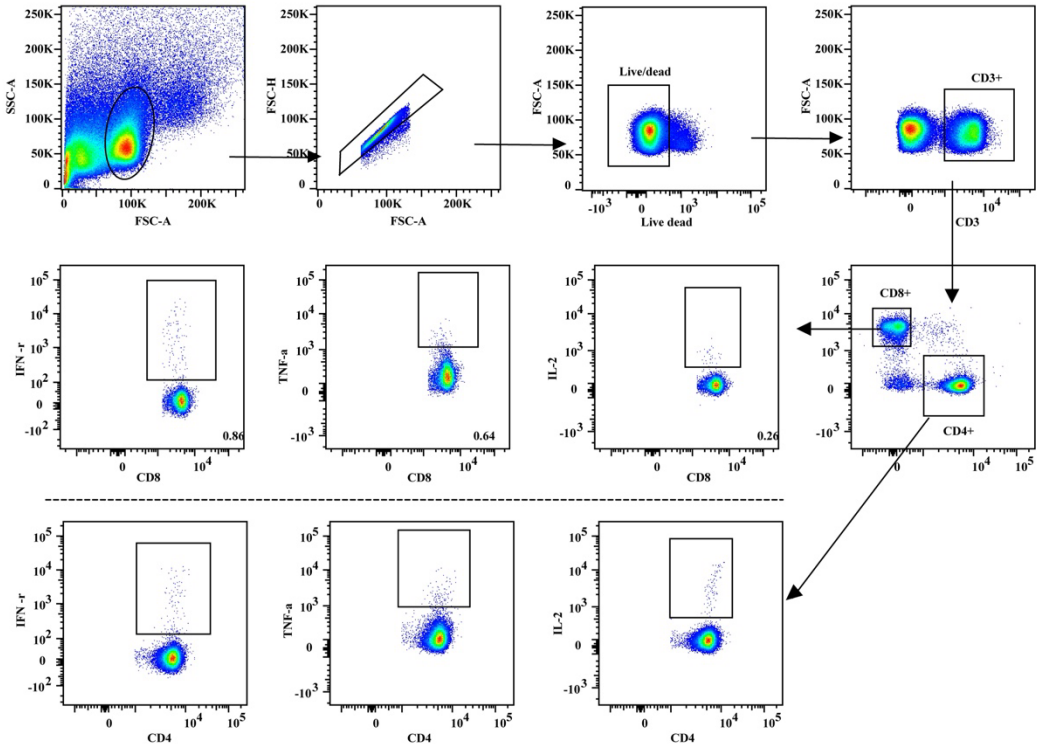


**Figure S3. Gating strategy and representative flow cytometry profiles of cytokines secreted splenocytes cells in mice, as presented in Fig. 1 or Fig. 2.** Cytokine + CD4+ T cells and Cytokine + CD8+ T cells ex vivo were stimulated with RABV-G peptide pools (1 μg/mL) or RABV-LT peptide pools (1 μg/mL) for 6 hours.

**Table S1. HLA Class I and Class II supertypes and their representative HLA Class I or Class II alleles.**

| HLA-I supertype representative alleles | HLA-II supertype representative alleles | |
|---|---|---|
| HLA-A*01:01 (A1) | DRB1*0101 | DRB1*1601 |
| HLA-A*02:01 (A2) | DRB1*0102 | DRB3*0101 |
| HLA-A*03:01 (A3) | DRB1*0103 | DRB3*0202 |
| HLA-A*24:02 (A24) | DRB1*0301 | DRB4*0101 |
| HLA-A*26:01 (A26) | DRB1*0305 | DRB4*0103 |
| HLA-B*07:02 (B7) | DRB1*0401 | DRB5*0101 |
| HLA-B*08:01 (B8) | DRB1*0402 | DRB5*0202 |
| HLA-B*27:05 (B27) | DRB1*0403 | DPA1*0103-DPB1*0201 |
| HLA-B*39:01 (B39) | DRB1*0404 | DPA1*0103-DPB1*0301 |
| HLA-B*40:01 (B44) | DRB1*0405 | DPA1*0103-DPB1*0401 |
| HLA-B*58:01 (B58) | DRB1*0408 | DPA1*0103-DPB1*0402 |
| HLA-B*15:01 (B62) | DRB1*0701 | DPA1*0103-DPB1*0601 |
| | DRB1*0801 | DPA1*0103-DPB1*1101 |
| | DRB1*0803 | DPA1*0103-DPB1*1701 |
| | DRB1*0901 | DPA1*0103-DPB1*2001 |
| | DRB1*1001 | DPA1*0103-DPB1*2301 |
| | DRB1*1101 | DQA1*0102-DQB1*0501 |
| | DRB1*1104 | DQA1*0102-DQB1*0602 |
| | DRB1*1201 | DQA1*0102-DQB1*0604 |
| | DRB1*1301 | DQA1*0103-DQB1*0501 |
| | DRB1*1302 | DQA1*0103-DQB1*0603 |
| | DRB1*1303 | DQA1*0201-DQB1*0201 |
| | DRB1*1401 | DQA1*0201-DQB1*0202 |
| | DRB1*1402 | DQA1*0401-DQB1*0301 |
| | DRB1*1454 | DQA1*0501-DQB1*0201 |
| | DRB1*1501 | DQA1*0501-DQB1*0301 |
| | DRB1*1503 | DQA1*0505-DQB1*0301 |

**Table S2 RABV-LT peptides used in the study.**

| Number | Sequence | Number | Sequence | Number | Sequence |
|---|---|---|---|---|---|
| 1 | LMLYAQTWTSELVQR | 29 | VLRYEREAFSDFRSV | 57 | QKQVNMSYDLIICDE |
| 2 | AQTWTSELVQRDTRH | 30 | EREAFSDFRSVKMTY | 58 | NMSYDLIICDEVTDI |
| 3 | TSELVQRDTRHIGSA | 31 | FSDFRSVKMTYLTLM | 59 | DLIICDEVTDIASIN |
| 4 | VQRDTRHIGSAQGLL | 32 | RSVKMTYLTLMTYQS | 60 | CDEVTDIASINRITL |
| 5 | TRHIGSAQGLLYSIL | 33 | MTYLTLMTYQSHLLL | 61 | TDIASINRITLLMSD |
| 6 | GSAQGLLYSILVAIH | 34 | TLMTYQSHLLLQRVE | 62 | SINRITLLMSDFALS |
| 7 | GLLYSILVAIHDSGY | 35 | YQSHLLLQRVERMRA | 63 | ITLLMSDFALSIDGP |
| 8 | SILVAIHDSGYGTIF | 36 | LLLQRVERMRATLRQ | 64 | MSDFALSIDGPLYLV |
| 9 | AIHDSGYGTIFPVNI | 37 | RVERMRATLRQMSSL | 65 | ALSIDGPLYLVFKTY |
| 10 | SGYGTIFPVNIYILI | 38 | MRATLRQMSSLMRQV | 66 | DGPLYLVFKTYGTML |
| 11 | TIFPVNIYILIGSSI | 39 | LRQMSSLMRQVLGGH | 67 | YLVFKTYGTMLVNPD |
| 12 | VNIYILIGSSICFRP | 40 | SSLMRQVLGGHALSK | 68 | KTYGTMLVNPDYKAI |
| 13 | ILIGSSICFRPLELI | 41 | RQVLGGHALSKRFQN | 69 | TMLVNPDYKAIRAFP |
| 14 | SSICFRPLELISGVI | 42 | GGHALSKRFQNPLIS | 70 | NPDYKAIRAFPSVTG |
| 15 | FRPLELISGVISYIL | 43 | LSKRFQNPLISGLRV | 71 | KAIRAFPSVTGFVTQ |
| 16 | ELISGVISYILLRLD | 44 | FQNPLISGLRVRAVL | 72 | AFPSVTGFVTQVTSS |
| 17 | GVISYILLRLDNHPS | 45 | LISGLRVRAVLNMFP | 73 | VTGFVTQVTSSFSSE |
| 18 | YILLRLDNHPSLYIM | 46 | LRVRAVLNMFPDSKL | 74 | VTQVTSSFSSELYLR |
| 19 | RLDNHPSLYIMLREP | 47 | AVLNMFPDSKLVFNS | 75 | TSSFSSELYLRFSKR |
| 20 | HPSLYIMLREPSLRG | 48 | MFPDSKLVFNSLLVN | 76 | SSELYLRFSKRGKFF |
| 21 | YIMLREPSLRGEIFS | 49 | SKLVFNSLLVNDLMA | 77 | YLRFSKRGKFFRDAE |
| 22 | REPSLRGEIFSIPQK | 50 | FNSLLVNDLMASGTH | 78 | SKRGKFFRDAEYLTS |
| 23 | LRGEIFSIPQKREGN | 51 | LVNDLMASGTHPLPP | 79 | KFFRDAEYLTSSTLR |
| 24 | IFSIPQKREGNRSIL | 52 | LMASGTHPLPPSAIR | 80 | DAEYLTSSTLREMSL |
| 25 | PQKREGNRSILCYLQ | 53 | GTHPLPPSAIRYFQS | 81 | LTSSTLREMSLVLFN |
| 26 | EGNRSILCYLQHVLR | 54 | LPPSAIRYFQSVQKQ | 82 | TLREMSLVLFNCSSK |
| 27 | SILCYLQHVLRYERE | 55 | AIRYFQSVQKQVNMS | 83 | MSLVLFNCSSKSEMQ |
| 28 | YLQHVLRYEREAFS | 56 | FQSVQKQVNMSYDLI | 84 | LFNCSSKSEMQRARS |
| | | | | 85 | SSKSEMQRARSLNYQ |

**Table S3 RABV-G peptides used in the study.**

| Number | Sequence | Number | Sequence |
|---|---|---|---|
| 1 | SPIDIHHLSCP | 15 | GFGKAYTIFNKTLME |
| 2 | TYTNFVGYV | 16 | AYTIFNKTL |
| 3 | PRYEESLHNPYPDYH | 17 | HYKSVRTWNEI |
| 4 | ESLHNPYPDYHWLRT | 18 | LKVGGRCHPHVNGVF |
| 5 | NPYPDYHWL | 19 | RCHPHVNGVFF |
| 6 | NPYPDYHWLRTVRTT | 20 | HVNGVFFNGIILGPD |
| 7 | PDYHWLRTVRTTKES | 21 | HVLIPEMQSSLLQQH |
| 8 | LRTVRTTKESLIIIS | 22 | SSVIPLMHPLADPST |
| 9 | RTTKESLIIISPSVT | 23 | LPNWGKYV L |
| 10 | AMQTSDETKWCPPDQ | 24 | KYVLMTAGAMI |
| 11 | CPPDQLVNL | 25 | GAMIGLVLIFSLMTW |
| 12 | PDQLVNLHDFRSDEI | 26 | RRANRPESKQRSFGG |
| 13 | DALESIMTTKSVSFR | 27 | FGGTGRNVSVTSQSG |
| 14 | KSVSFRRLSHLRKLV | 28 | IPSWESYKSGGQTRL |