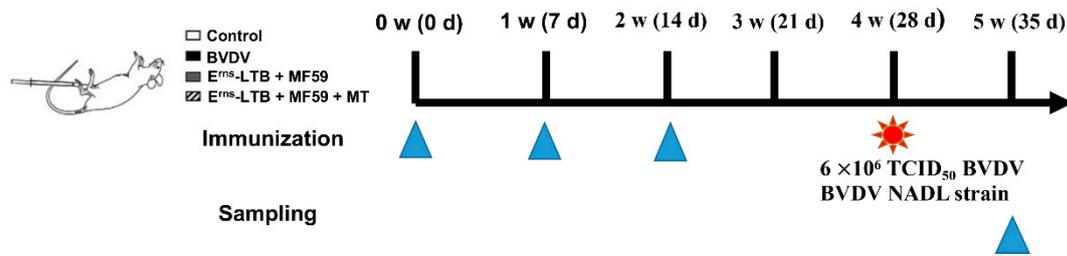


# Appendix for “Melatonin as immune potentiator for enhancement of subunit vaccine efficacy against bovine viral diarrhoea virus”.

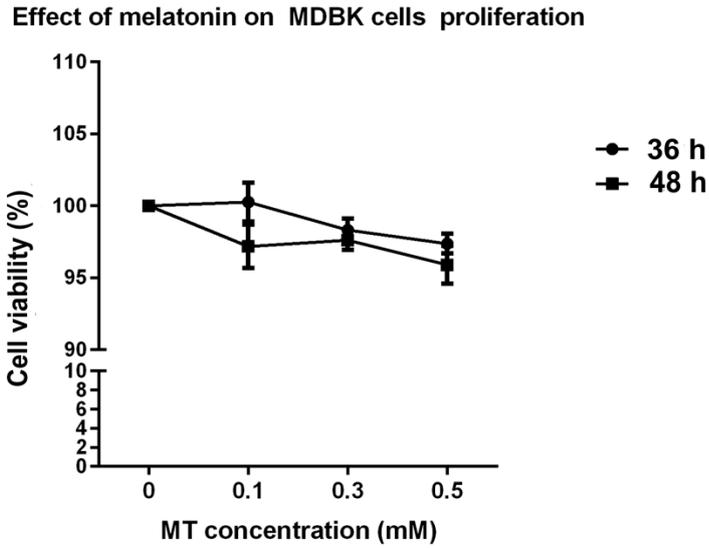
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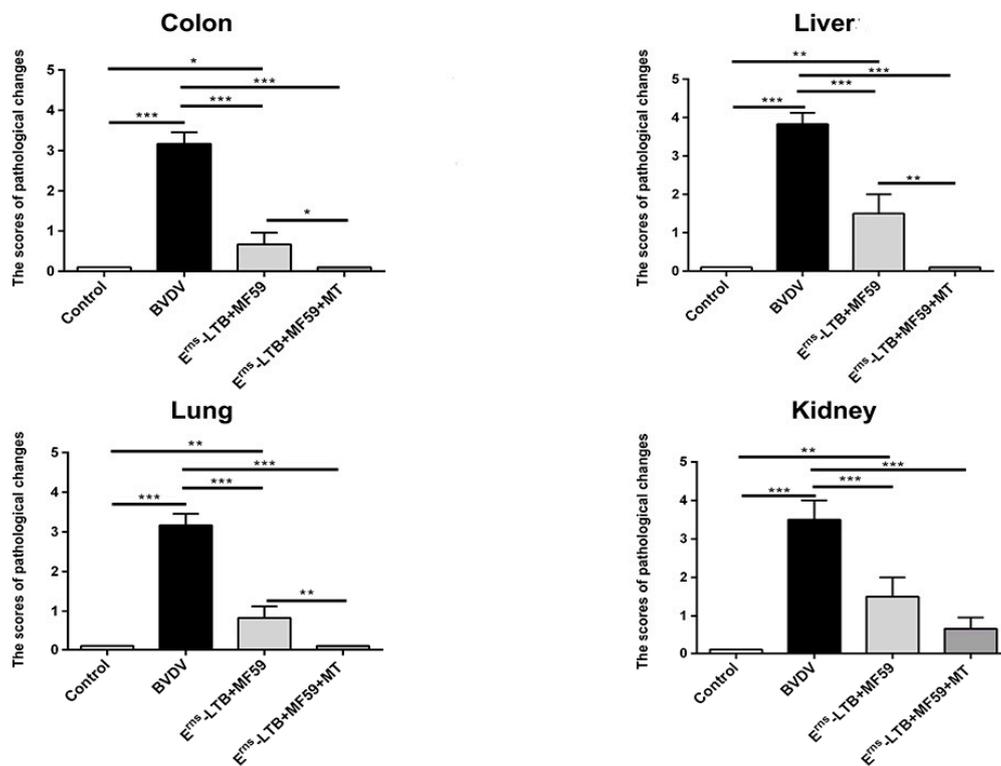
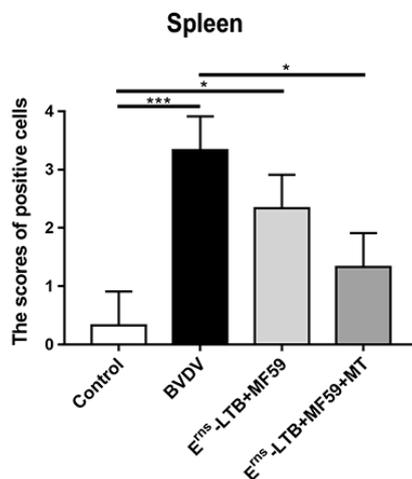
\*Correspondence: [jiufeng\\_wang@hotmail.com](mailto:jiufeng_wang@hotmail.com); +86 010 6273 1094



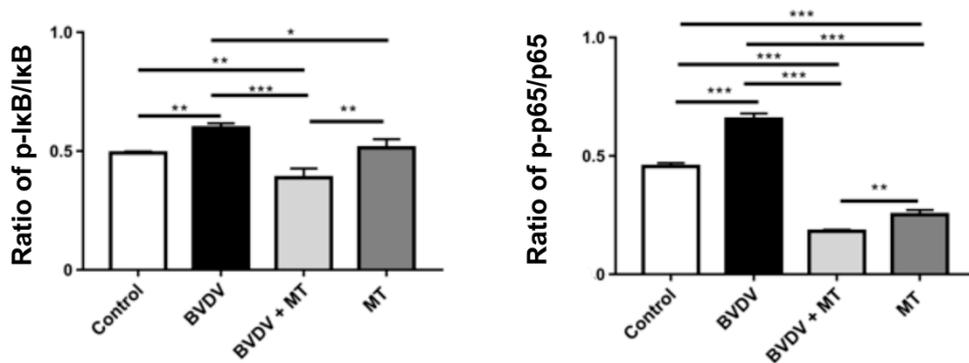
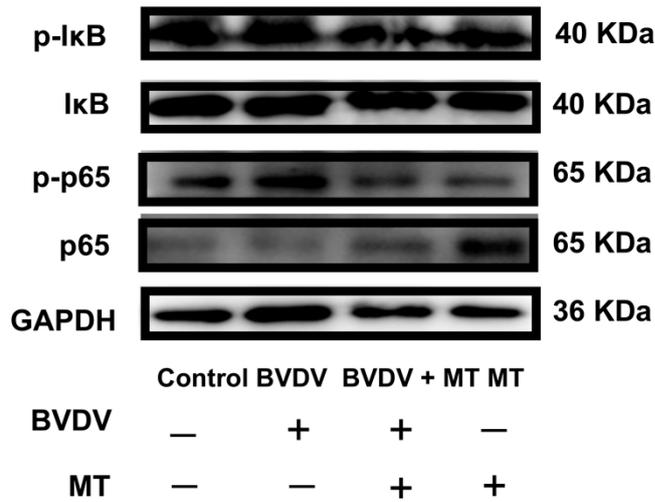
**Figure S1.** Immunization and sampling process. Thirty-two female BALB/c mice were divided into four groups of  $n = 8$  per group. The different groups were immunized three times intraperitoneally with PBS (control and BVDV groups), with MF59 adjuvanted recombinant E<sup>rms</sup>-LTB protein or MF59 + MT adjuvanted recombinant E<sup>rms</sup>-LTB protein. Vaccination took place on days 0, 7 and 14. Four weeks after the primary immunization, the mice were challenged intraperitoneally with  $6 \times 10^6$  TCID<sub>50</sub> BVDV NADL strain. The lung, spleen, liver, kidney, colon of each mouse were collected one week later. The cartoon depicted the method of immunization, and the immunization and sampling schedule were shown in Figure S1.



**Figure S2.** The effects of different concentrations of melatonin on cell viability. MDBK cells were treated with 0.1, 0.3, 0.5 mM melatonin for 36 h and 48 h, respectively. The relative cell viability was evaluated by CCK8 Kit according to the manufacturer's instructions. The values in each column represent the mean  $\pm$  SEM of three individual experiments detected in triplicate.

**A****B**

**Figure S3.** The scores of pathological changes and number of BVDV antigens after the BVDV challenge. (A) On day 7 post-infection, the colon, lung, liver, and kidney of mice in four groups were immediately fixed in 4% formaldehyde. The histopathological changes were evaluated by a veterinary pathologist and scored 0 to 4 in a blinded study. (B) The detection of the BVDV antigen was scored from 0 to 4 according to the number of positive cells per section. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .



**Figure S4.** The comparison of the NF- $\kappa$ B related protein expressions in MDBK cells. Relative protein expression levels were based on Western-Blot band intensity; protein levels of p-p65, p-I $\kappa$ B were normalized respectively with p65, I $\kappa$ B. Representative Western-Blot image of NF- $\kappa$ B p-p65, p-I $\kappa$ B, E2, p65, I $\kappa$ B, GAPDH expression levels in the total protein of cells. The values in each column represent the mean  $\pm$  SEM of three individual experiments detected in triplicate. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .



## CERTIFICATE OF ENGLISH EDITING

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commissioned to us has been carefully edited by a native English-speaking editor of MogoEdit, and the grammar, spelling, and punctuation have been verified and corrected, except the Figure and table captions. Based on this review, we believe that the language in this paper meets academic journal requirements.

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*Gang Zhang*

Dr. Gang Zhang  
Founder & CEO of MogoEdit

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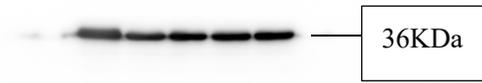


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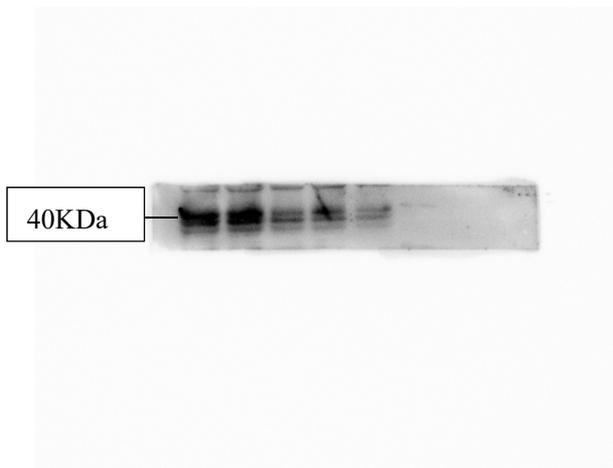
Uncropped blots  
Figure 1  
GAPDH-36 KDa



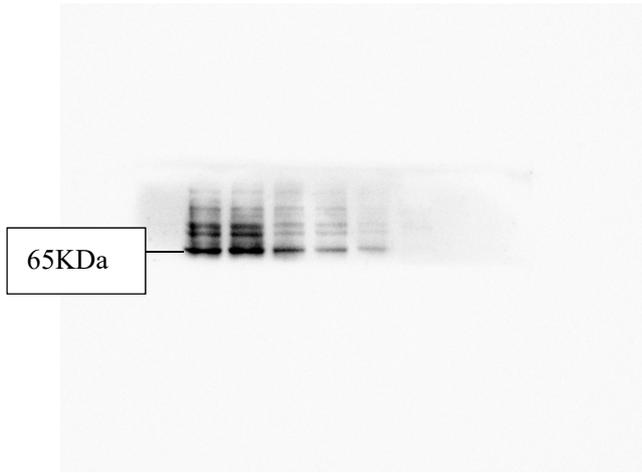
IκB-40 KDa



p-IκB-40 KDa



p-p65-65 KDa



p65-65KDa

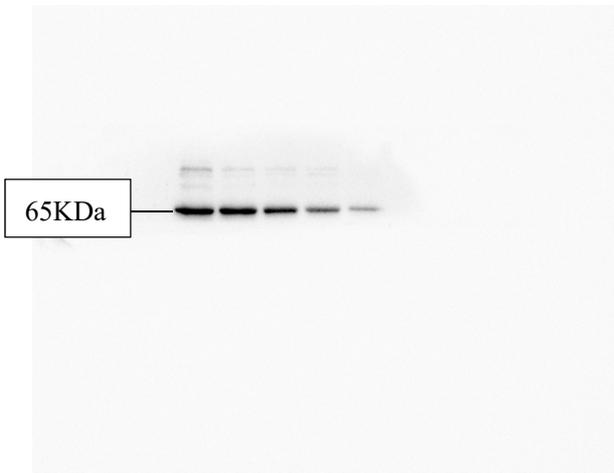
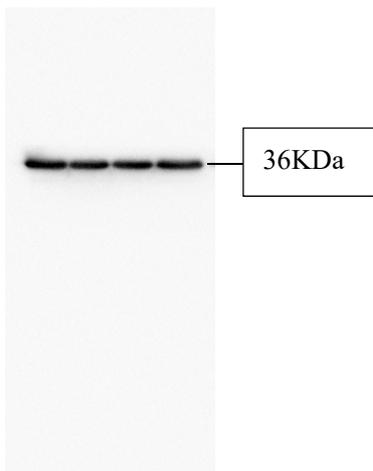
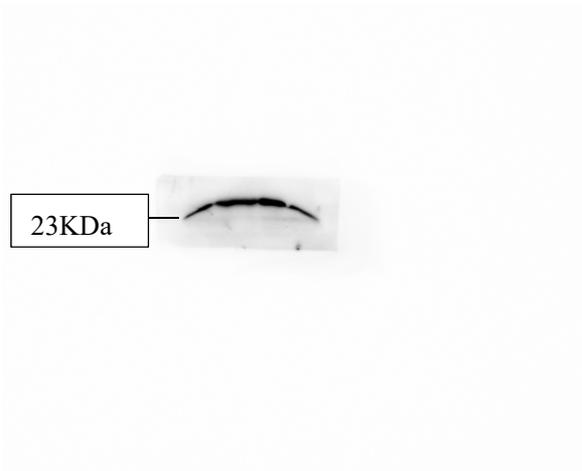


Figure 5  
GAPDH-36 KDa



Caludin1-23KDa



Occludin-59KDa

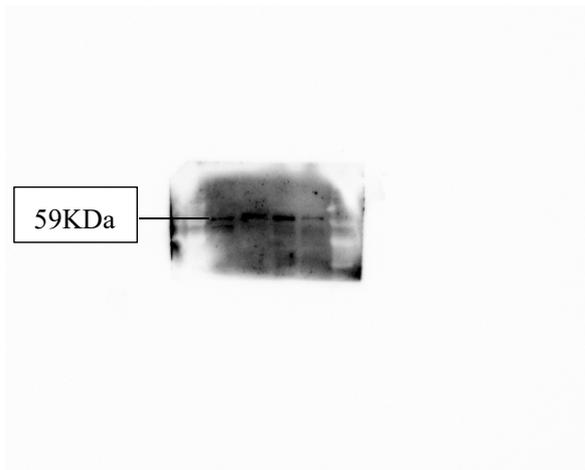
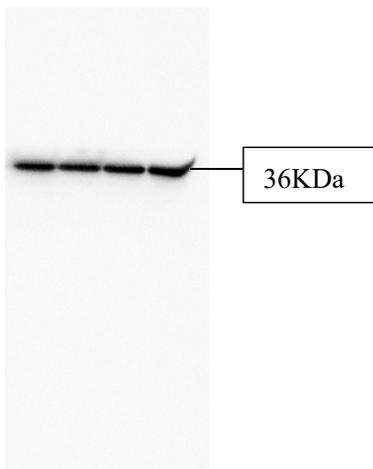
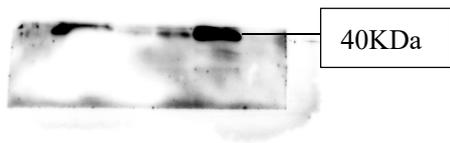


Figure 6

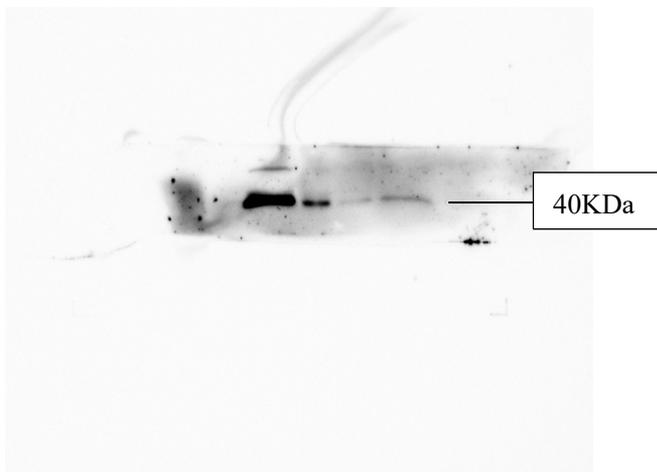
GAPDH-36 KDa



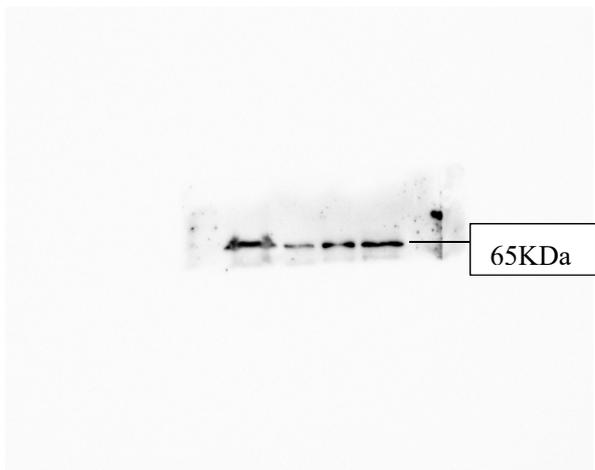
IκB-40KDa



p-IκB-40 KDa



p-p65-65 KDa



p65-65 KDa

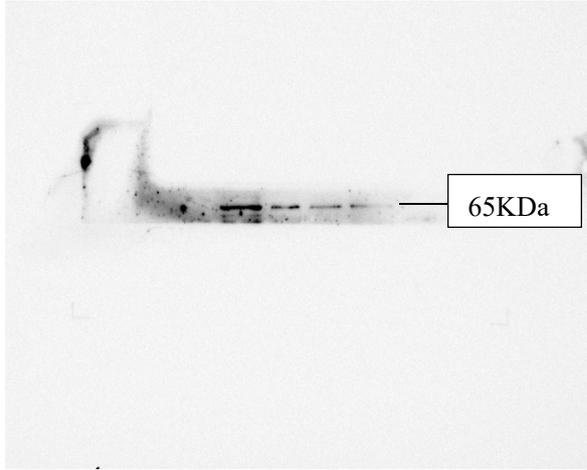
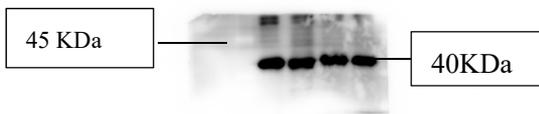


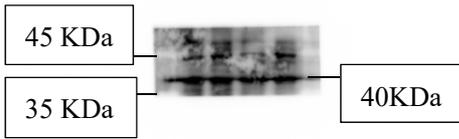
Figure S4  
GAPDH-36 KDa



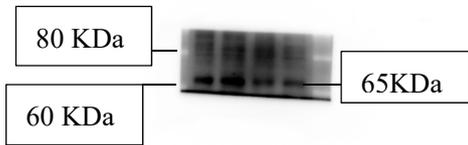
IκB-40 KDa



p-IκB-40 KDa



p-p65-65 KDa



p65-65 KDa

