

## Supplemental materials

# Berberine inhibits dengue virus through dual mechanisms

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**Supplemental Figure S1. Chemical structure of berberine.**

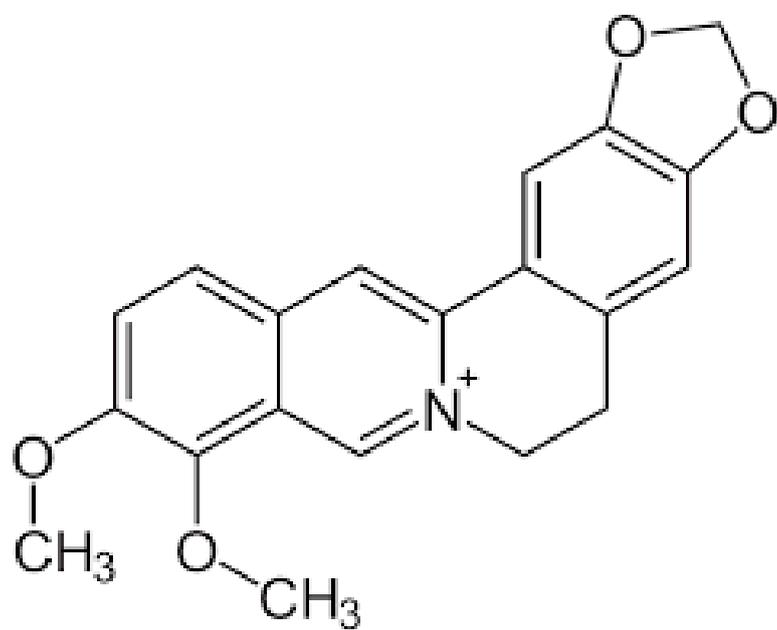
**Supplement Figure S2. Cytotoxicity of BBR towards several cell lines.**

**Supplement Figure S3. BHK-21 morphology after BBR treatment.**

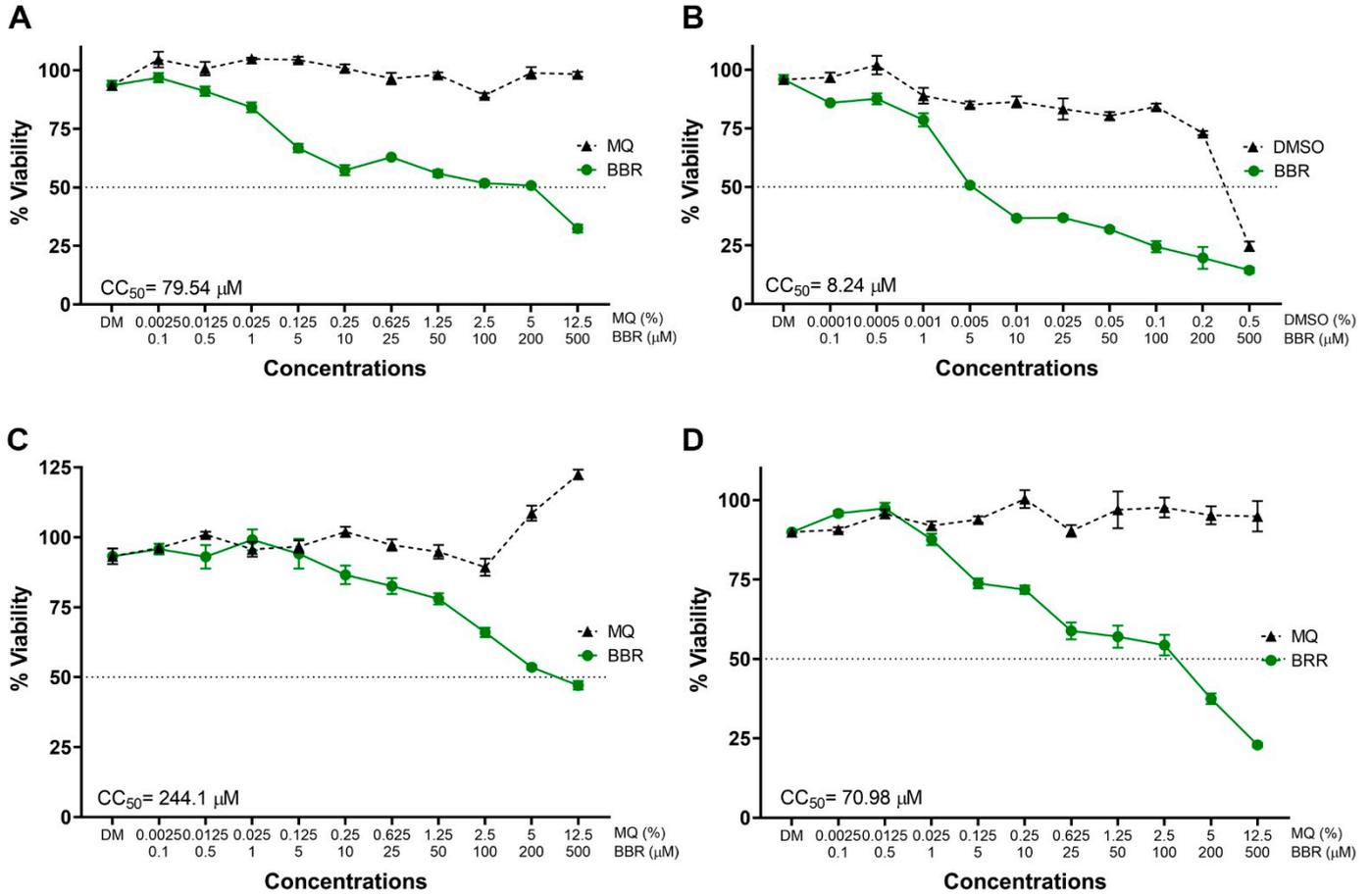
**Supplemental Figure S4. Optimization of DENV infection.**

**Supplemental Figure S5. EC<sub>50</sub> calculation of BBR in DENV infection.**

**Uncropped western blots**

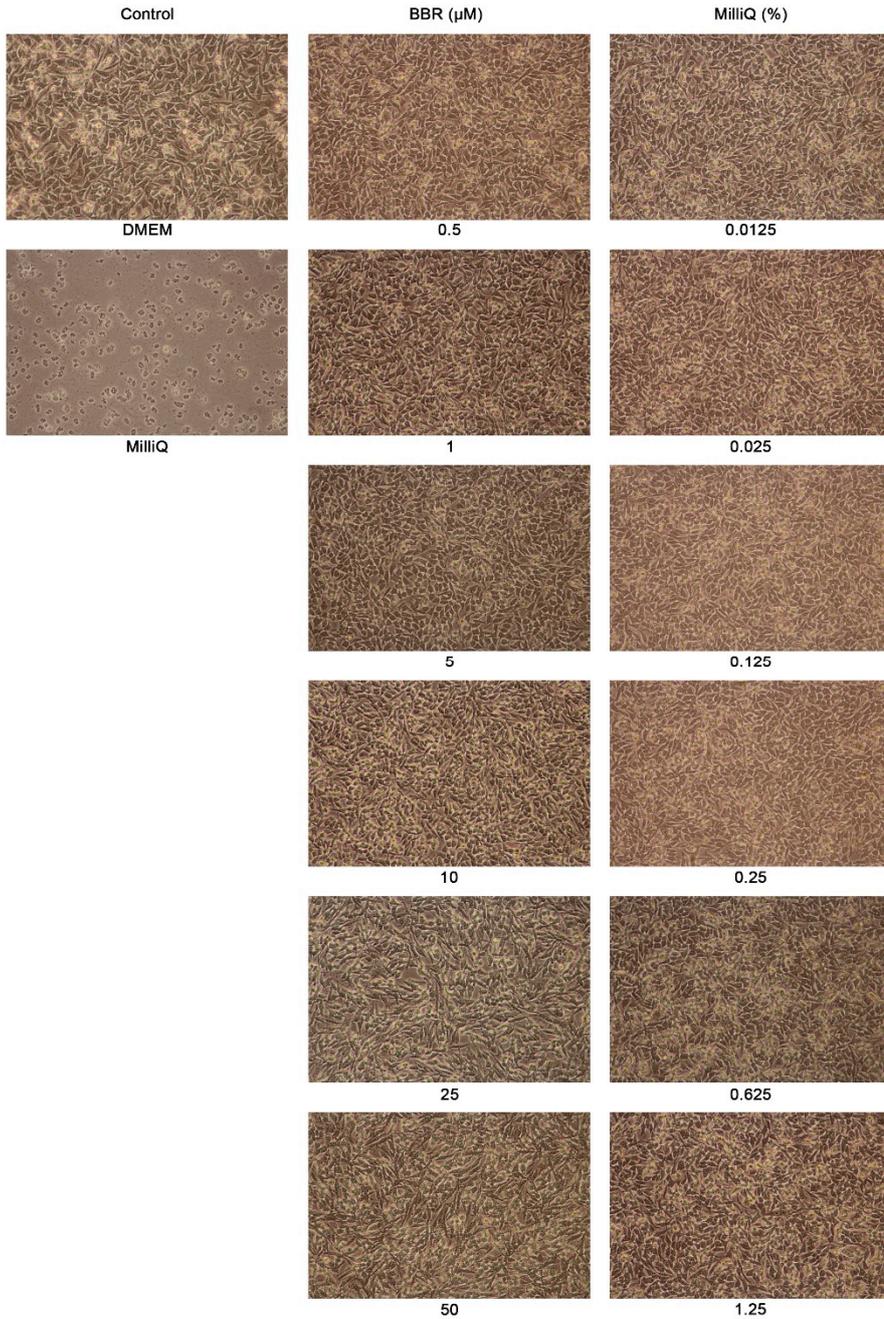


Supplemental Figure S1. Chemical structure of berberine.



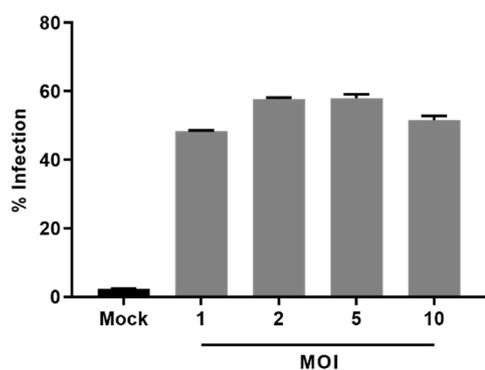
**Supplemental Figure S2. Cytotoxicity of BBR towards several cell lines.**

(A) A549, (B) HEK293T/17, (C) HepG2 and (D) Huh-7 cells were cultured in 96 well plates before being treated with different concentration of BBR or equivalent volume of MQ water in DMEM for 24 h before cell viability was determined by the MTT assay. Experiment was undertaken as 4 independent replicates. Errors bars show  $\pm$  S.E.M. \* $p < 0.0001$ .

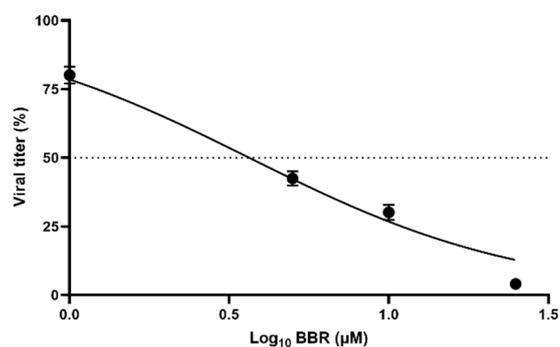


**Supplemental Figure S3. BHK-21 morphology after BBR treatment.**

BHK-21 cells were treated with BBR at concentrations ranging from 0.1  $\mu\text{M}$  – 50  $\mu\text{M}$  or an equivalent volume vehicle for 24 h before observation of cell morphology under an inverting microscope.



**Supplemental Figure S4. Optimization of DENV infection.** BHK-21 cells were infected with DENV 2 at different MOI, and after 24 h, level of infection was determined by flow cytometry.

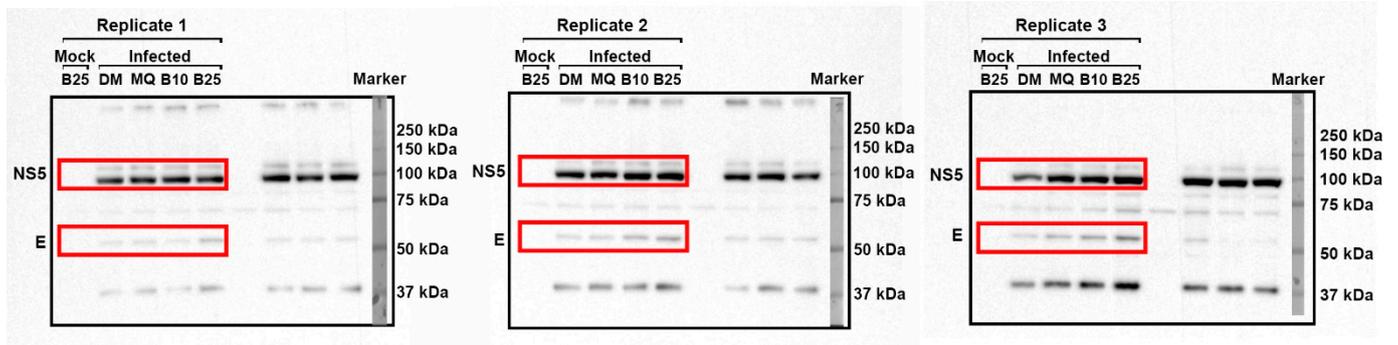


**Supplemental Figure S5. EC<sub>50</sub> calculation of BBR in DENV infection.**

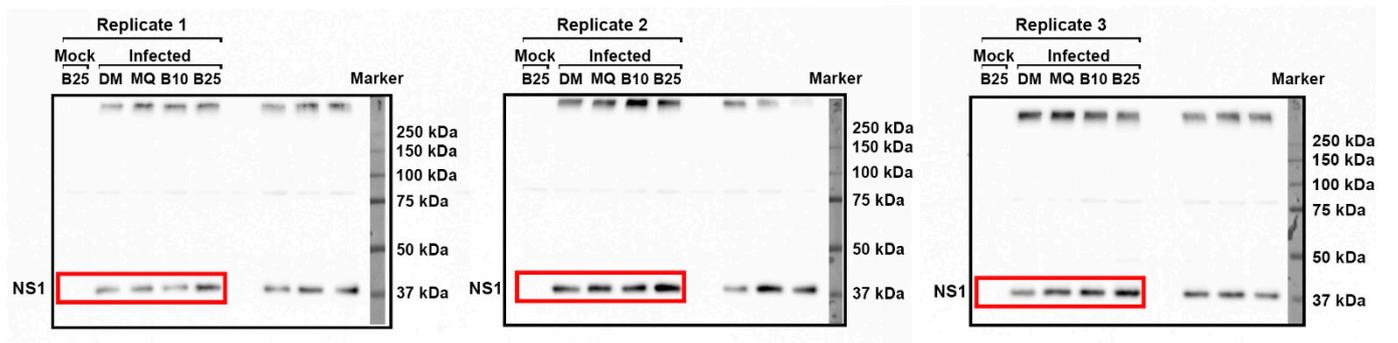
Virus titer data from Figure 2E was used to calculate the EC<sub>50</sub> of BBR towards DENV under conditions of post treatment.

## Uncropped western blots

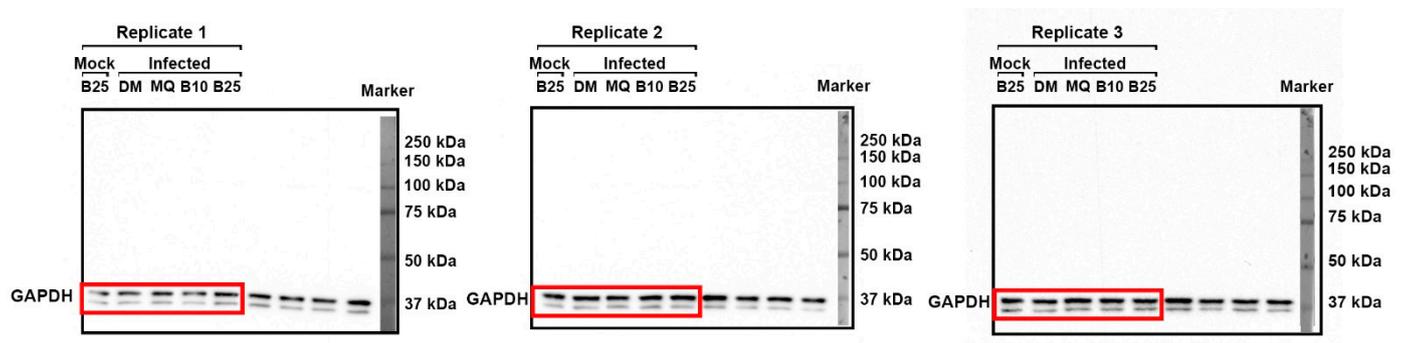
### Western blot of viral protein expression at 24 hours post- treatment



Full western blot membranes of NS5 and E proteins captured by Azure400 (Replicate 1-3 left-right), red boxes indicate bands presented in Fig 3D; NS5 and E proteins.

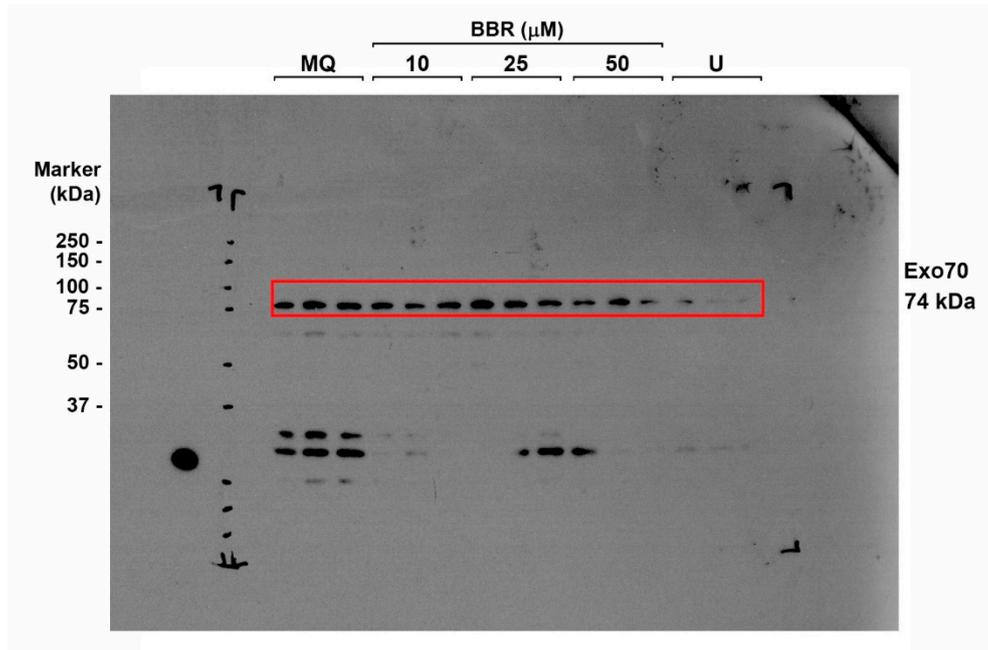


Full western blot membranes of NS1 protein captured by Azure400 (Rep 1-3 left-right), red boxes indicate bands presented in Fig 3D; NS1 protein.

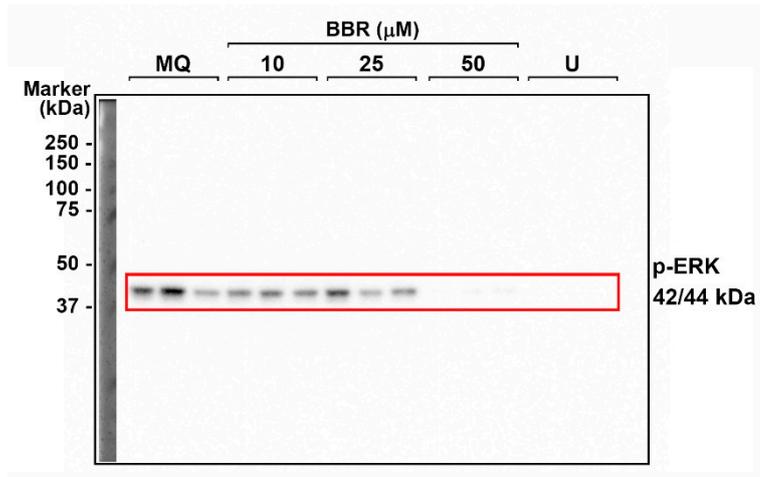


Full western blot membranes of GAPDH captured by Azure400 (Rep 1-3 left to right), red boxes indicate bands presented in Fig 3D; GAPDH.

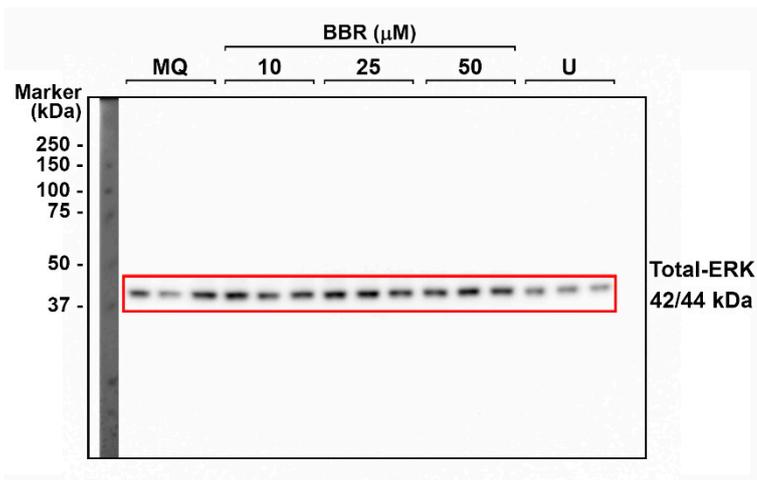
Western blot of BBR effect on Erk1/2 and Exo70



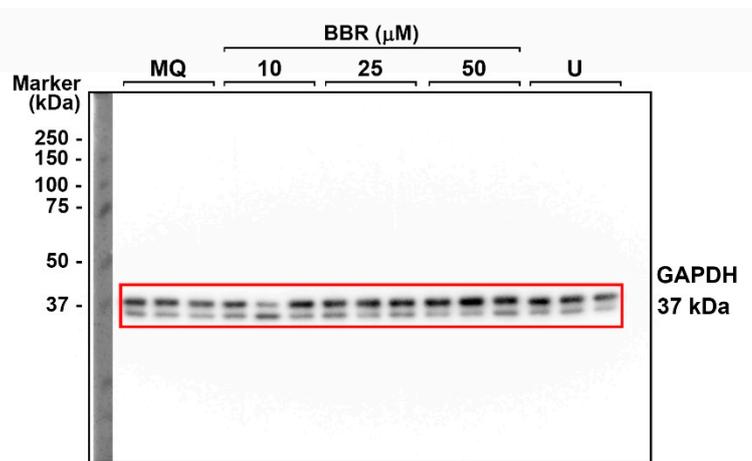
Full western blot membrane of Exo70 captured on X- ray film (Rep 1-3 were in consecutive lane), red box indicates bands presented on Fig 4A; Exo70.



Full western blot membrane of p-ERK captured by Azure400 (Rep 1-3 were in consecutive lane), red boxes indicate bands presented in Fig 4A; p-ERK.



Full western blot membrane of total ERK captured by Azure400 (Rep 1-3 were in consecutive lane), red box indicates bands presented in Fig 4A; Total ERK.

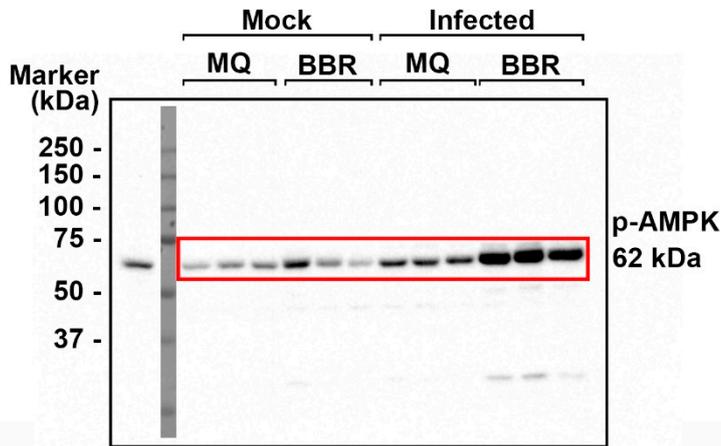


Full western blot membrane of GAPDH captured by Azure400 (Rep 1-3 were in consecutive lane), red box indicates bands presented in Fig 4A; GAPDH

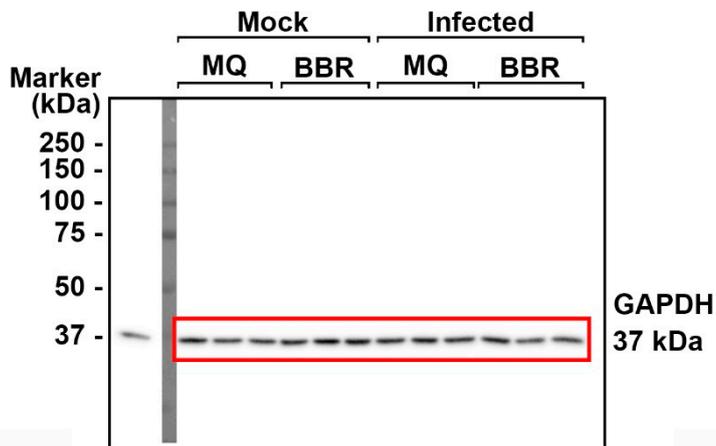
## Western blot of BBR effect on lipid droplets and AMPK

Note: Lane 15 left empty

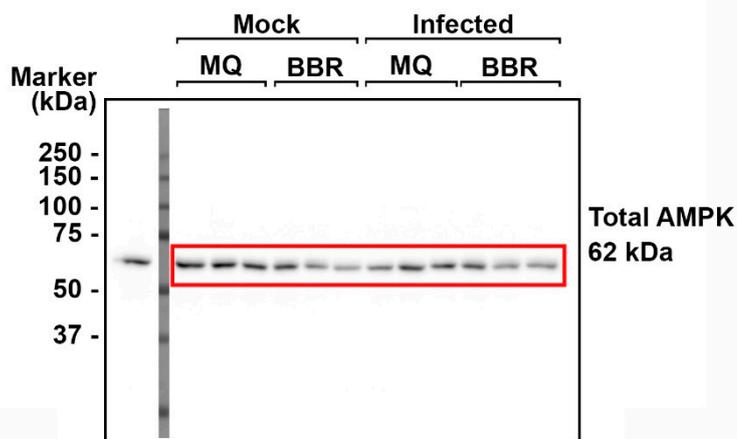
p-AMPK and AMPK were performed on different membranes



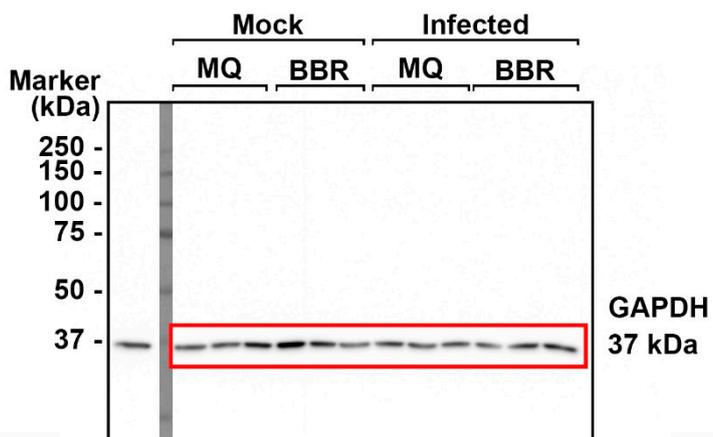
Full western blot membrane of p-AMPK captured by Azure400 (Rep 1-3 were in consecutive lane), red box indicates bands presented in Fig 5C; p- AMPK.



Full western blot membrane of GAPDH (from phospho- membrane) captured by Azure400 (Rep 1-3 were in consecutive lane), red box indicates bands presented in Fig 5C; GAPDH for p-AMPK.



Full western blot membrane of total AMPK captured by Azure400 (Rep 1-3 were in consecutive lane), red box indicates bands presented in Fig 5C; total AMPK..



Full western blot membrane of GAPDH (total- membrane) captured by Azure400 (Rep 1-3 were in consecutive lane), red box indicates bands presented in Fig 5C; GAPDH for total AMPK.