Supplemental materials

Discordant activity of kaempferol towards dengue virus and Japanese encephalitis virus

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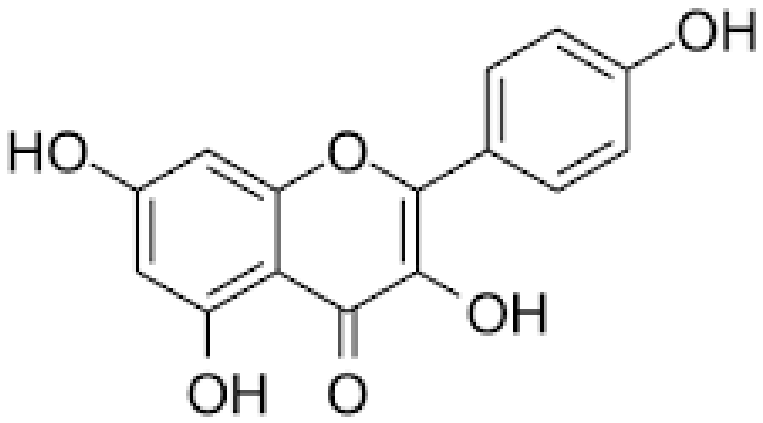
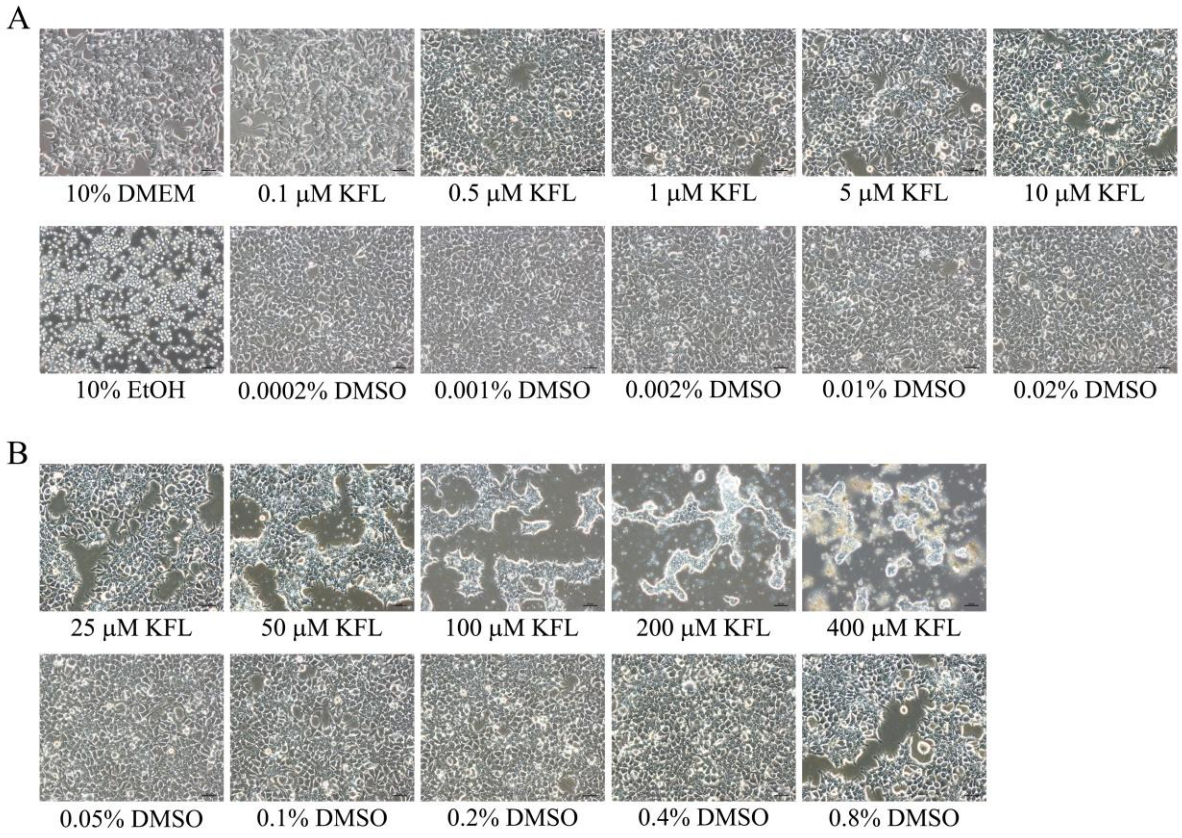
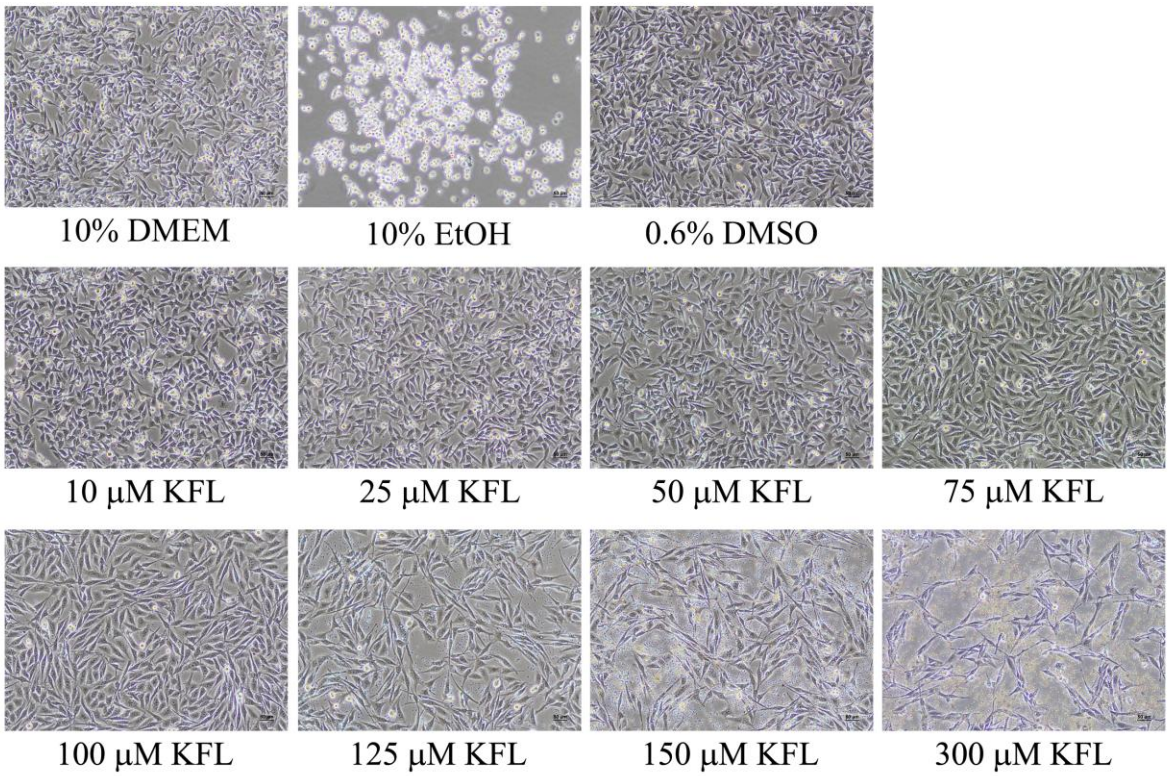


Supplemental Figure S1. Structure of kaempferol.



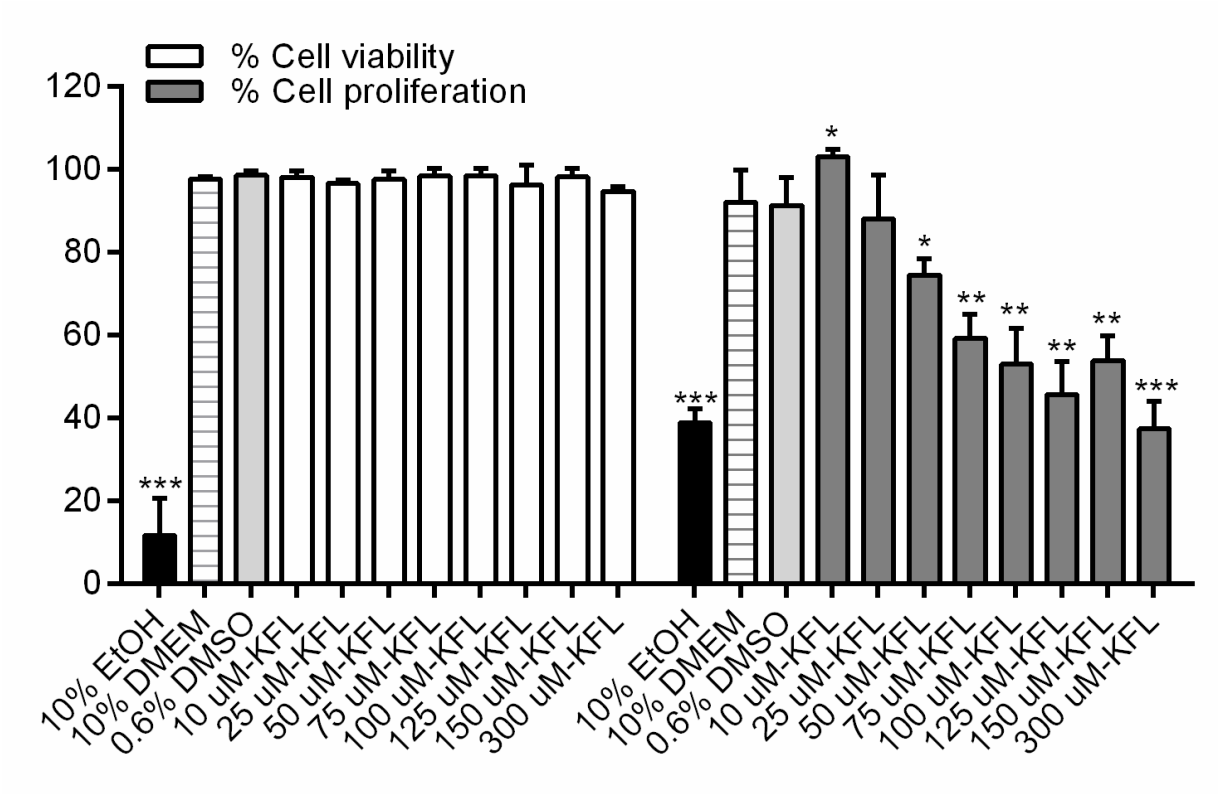
Supplemental Figure S2. Morphological changes of HEK293T/17 cells after treatment with kaempferol

HEK293T/17 cells were treated with various concentrations of DMSO or kaempferol for 24 hrs after which the cell morphology was observed under an inverted microscope. Cells that were treated with 10% EtOH were used as a positive control. All experiments were undertaken independently in duplicate. Magnification: 200 X, Scale bar 20 μm. Enlargements of representative panels can be found in Supplementary Figures S5-26.



Supplemental Figure S3. Morphological changes of BHK-21 cells after treatment with kaempferol

BHK-21 cells were treated with various concentrations of DMSO or kaempferol for 24 hrs. The cytotoxicity of kaempferol on cell morphology was observed under the inverted microscope. Cells that were treated with 10% EtOH were used as a positive control. All experiments were undertaken independently in duplicate. Magnification: 200 X, Scale bar 20 μm. Enlargements of representative panels can be found in Supplementary Figures S27-37.

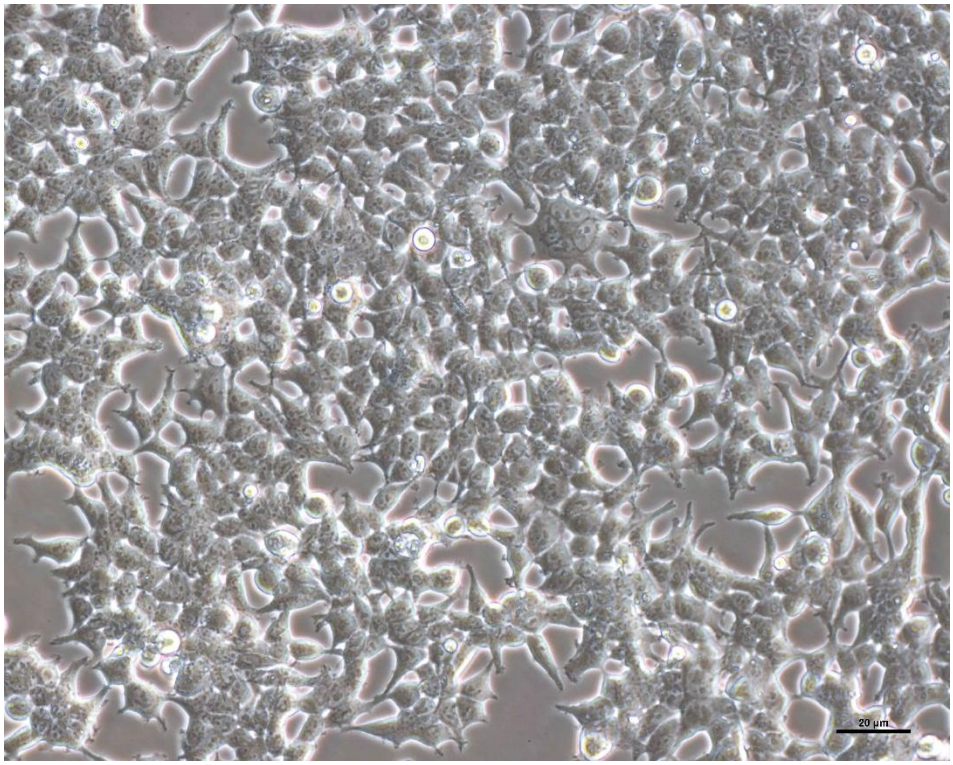


Supplemental Figure S4. Trypan blue staining and proliferation assay in BHK-21 cells.

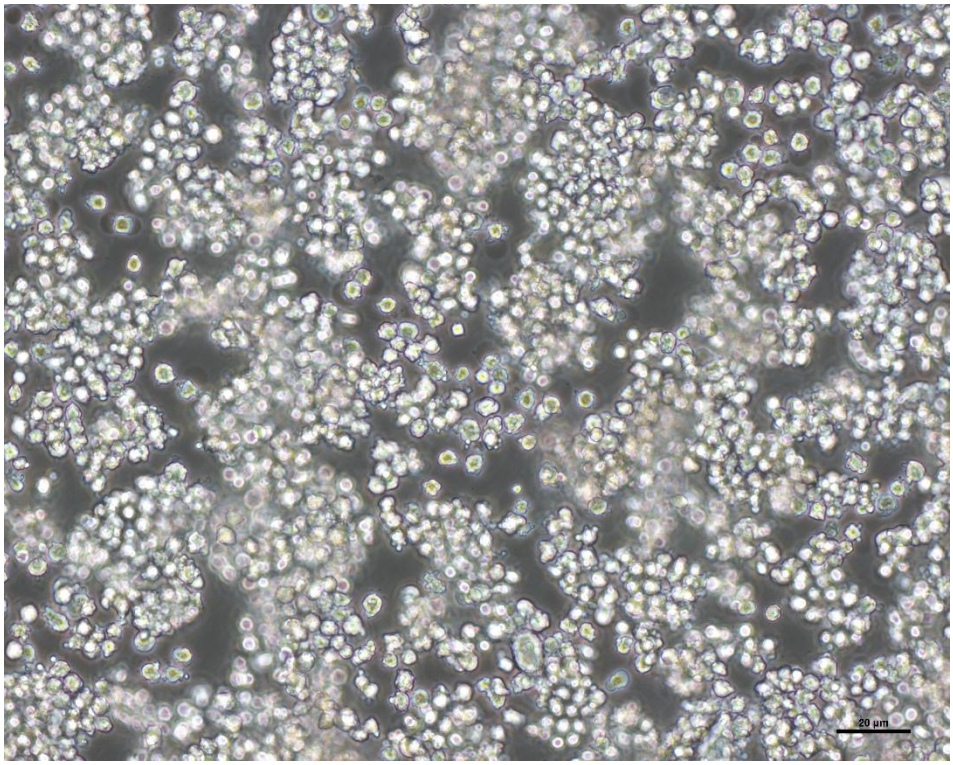
BHK-21 cells were treated with various concentrations of DMSO or kaempferol for 24 hrs. The cytotoxicity of kaempferol was assessed by a trypan blue exclusion assay and by a cell proliferation assay (total cell count). Results are presented as percentage of cell viability from 4 replicates at 24 h post treatment. Negative (10% FBS in DMEM) and positive (10% EtOH) controls were included. Error bars represent mean ± SD *; p value < 0.05, **; p value <0.01 and ***; p value <0.001. All statistics were determined by comparison with the DMSO control.

Figure S5-26. Effect of Kaempferol on HEK293/17 cell morphology

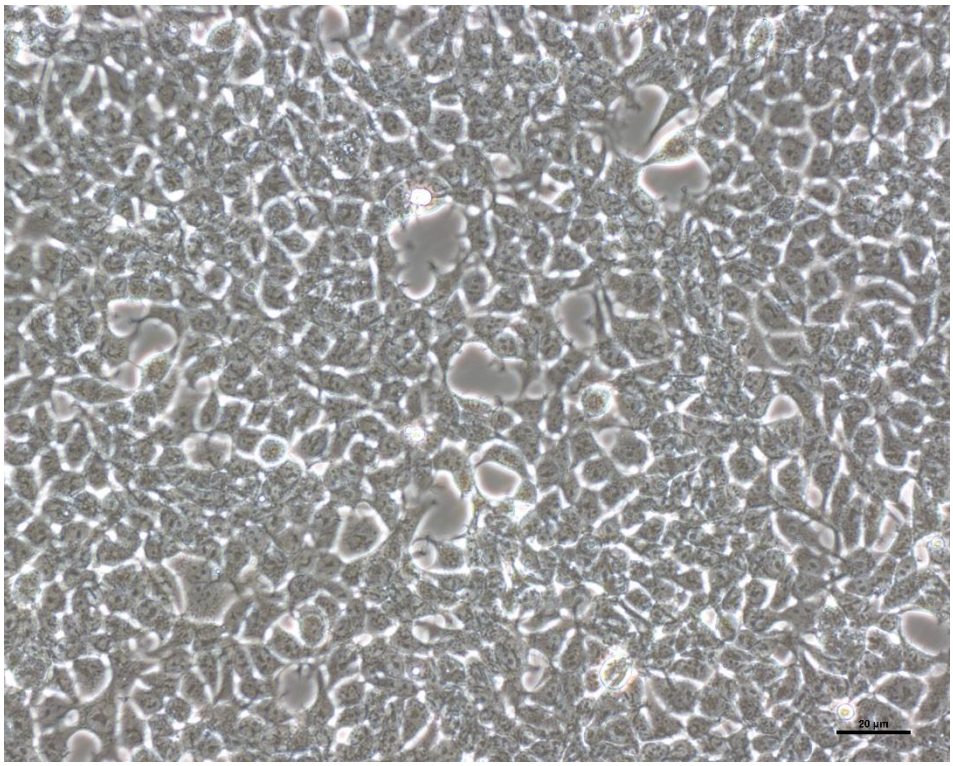
S5. 10% DMEM



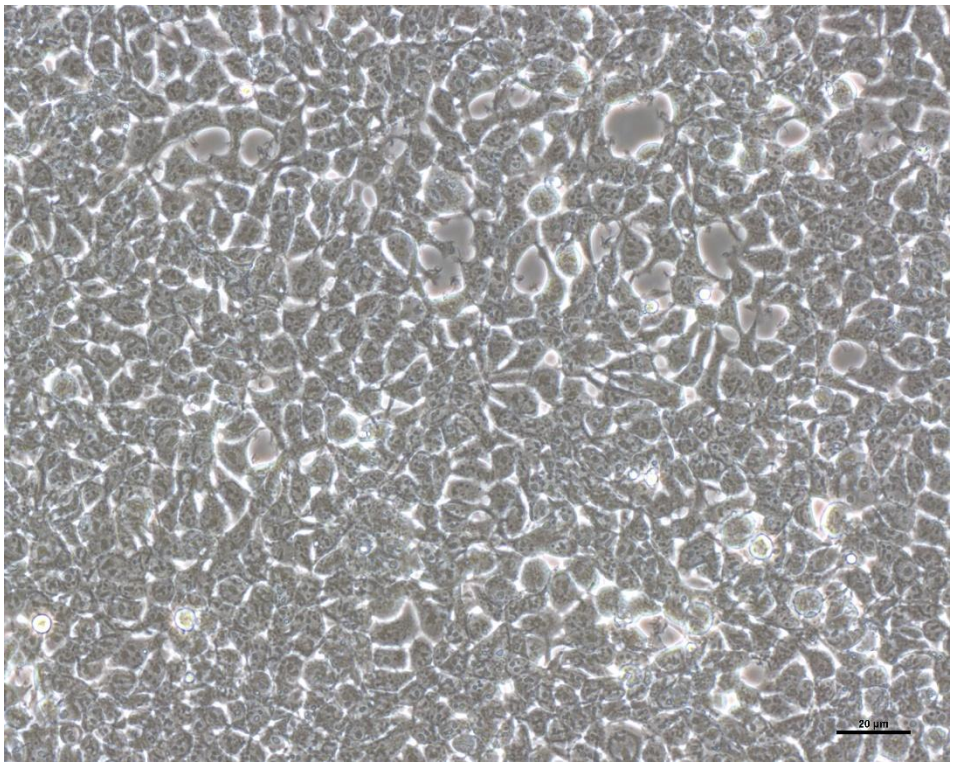
S6. 10% EtOH



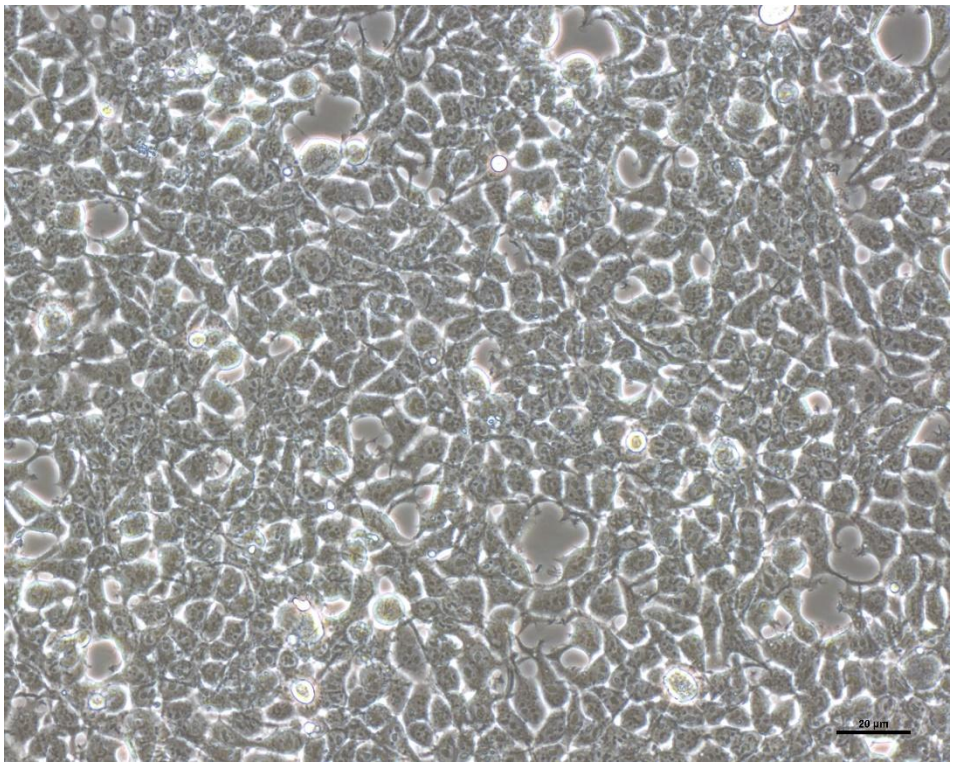
S7. 0.0002% DMSO



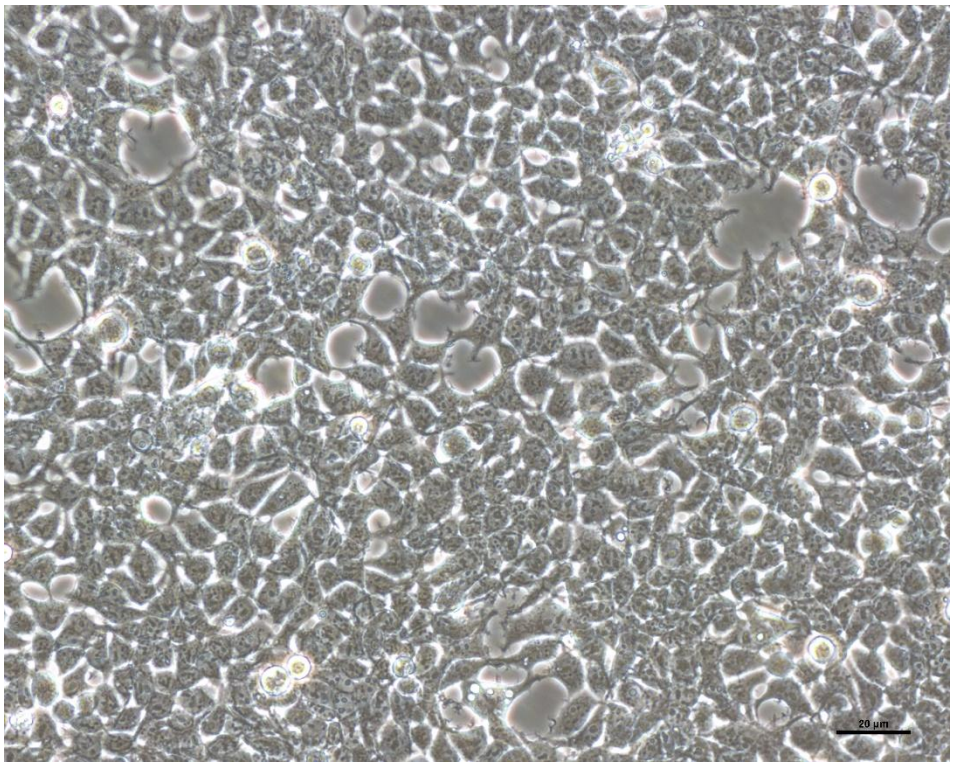
S8. 0.001% DMSO



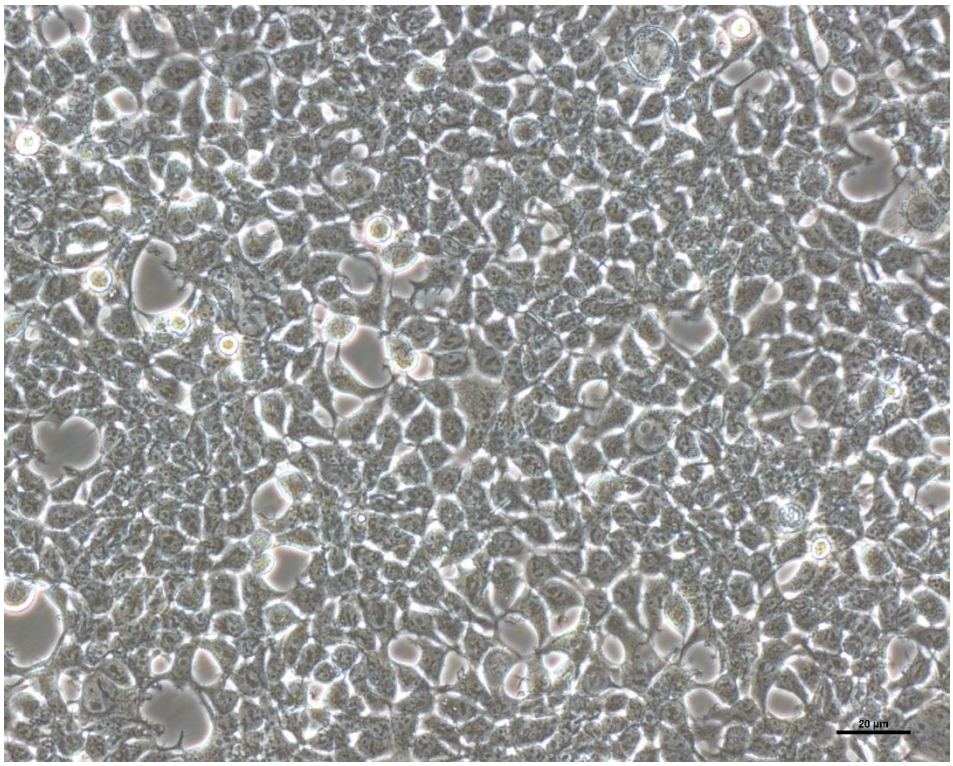
S9. 0.002% DMSO



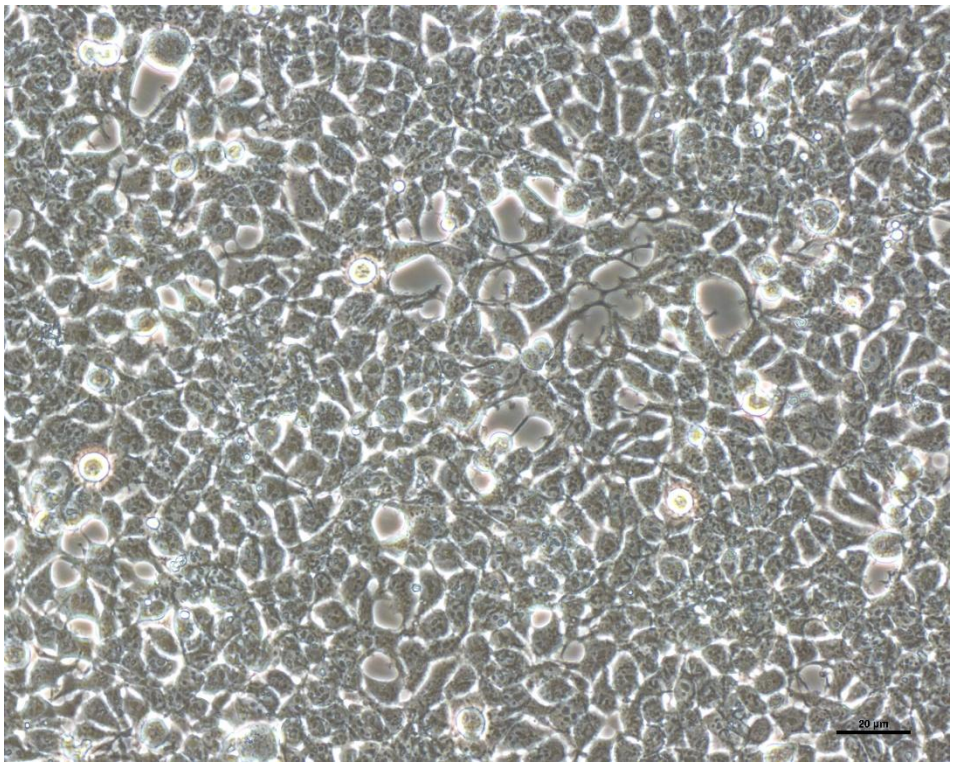
S10. 0.01% DMSO



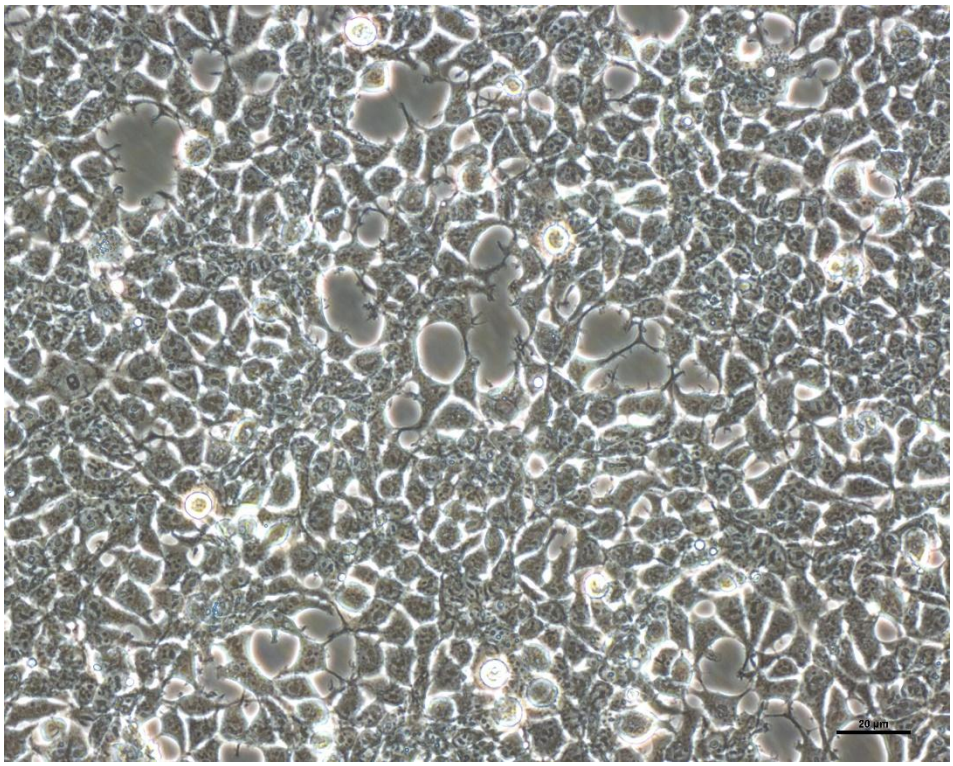
S11. 0.02% DMSO



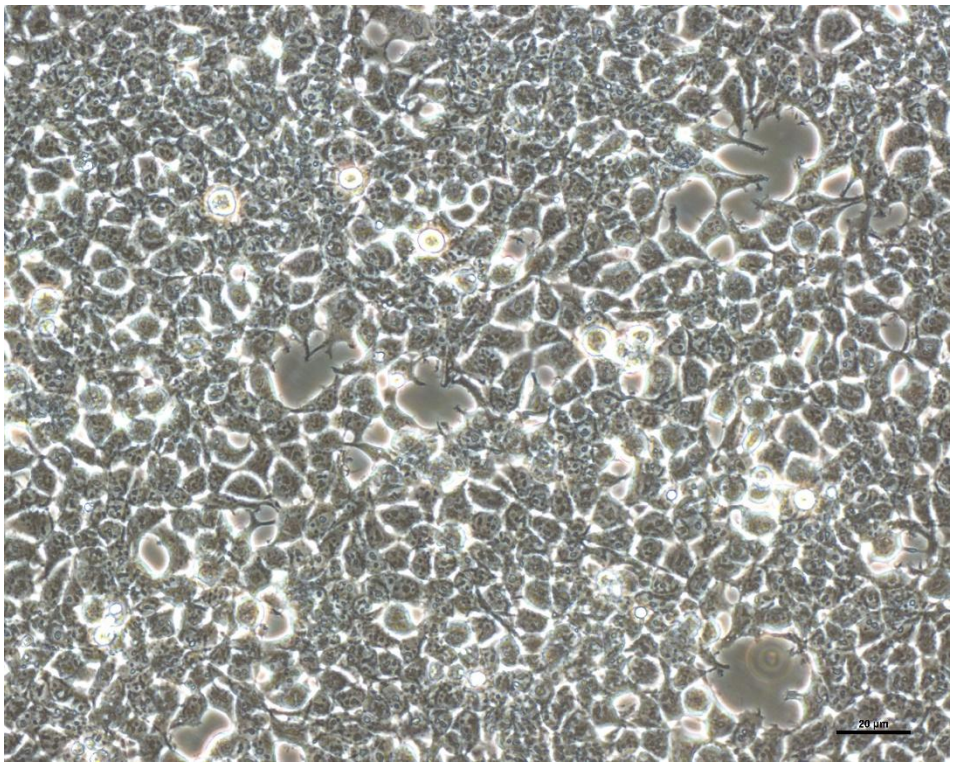
S12. 0.05% DMSO



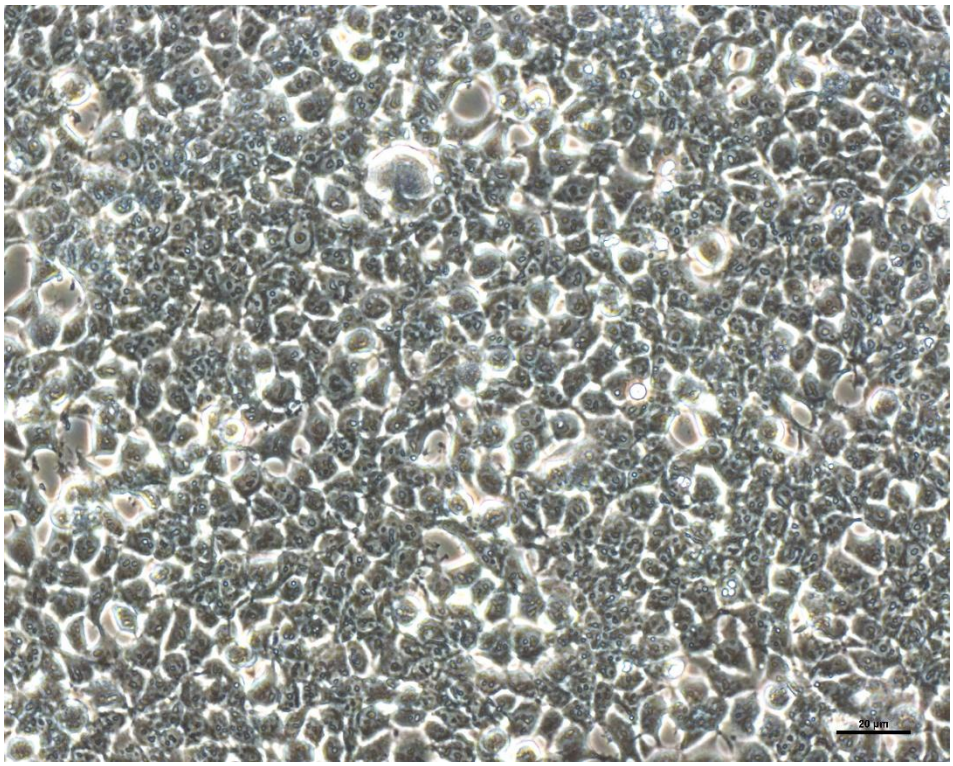
S13. 0.1% DMSO



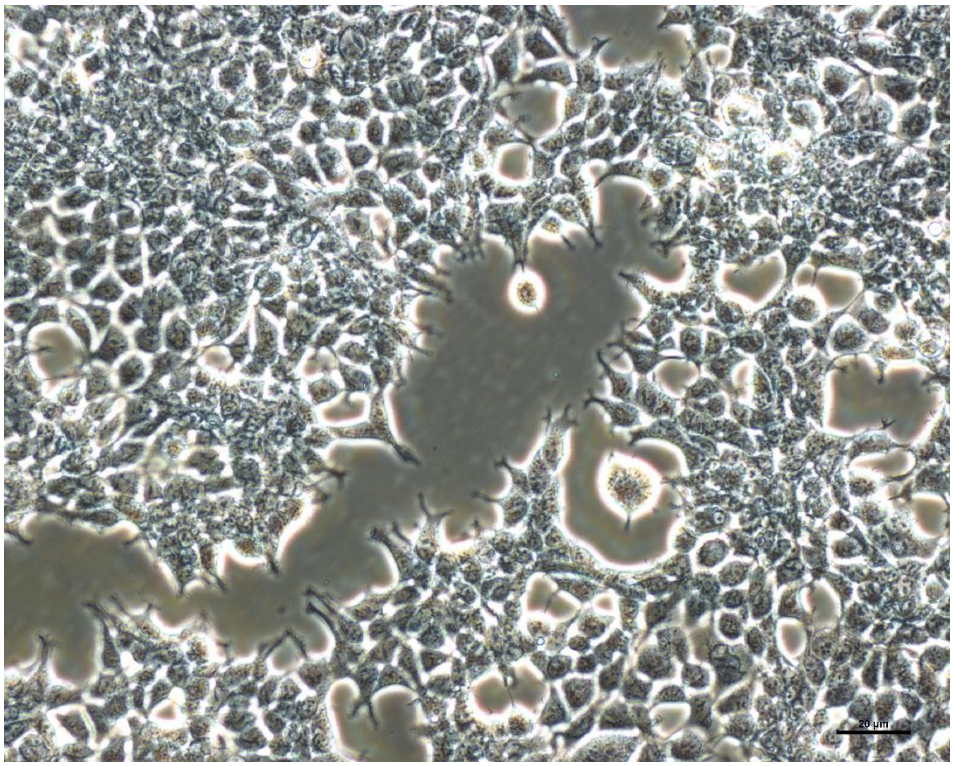
S14. 0.2% DMSO



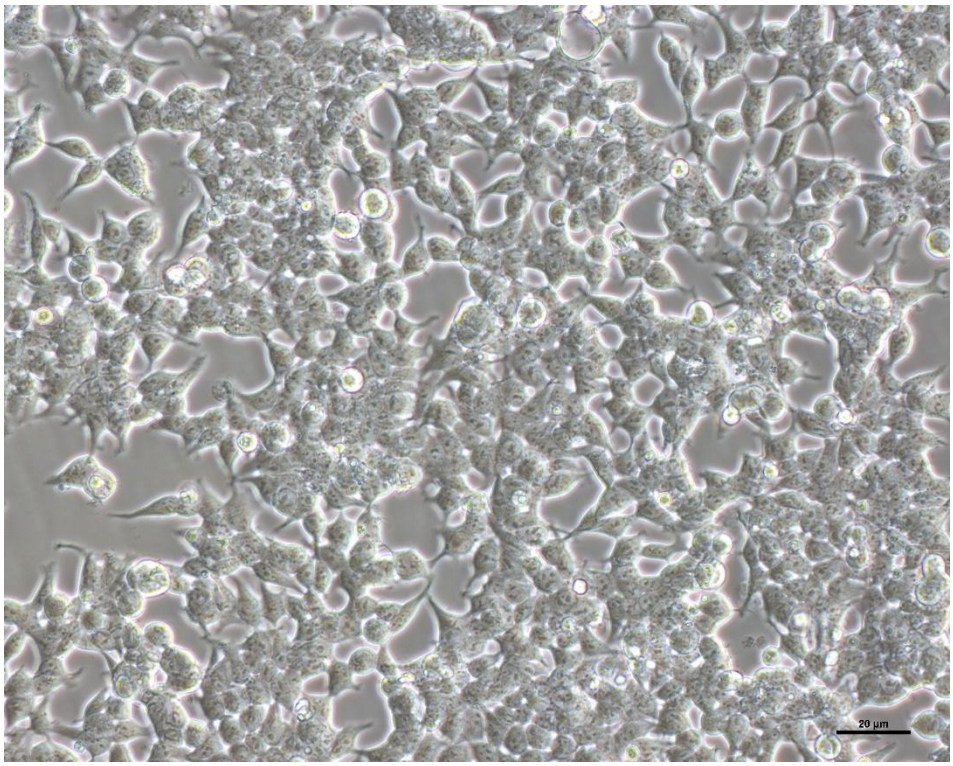
S15. 0.4% DMSO



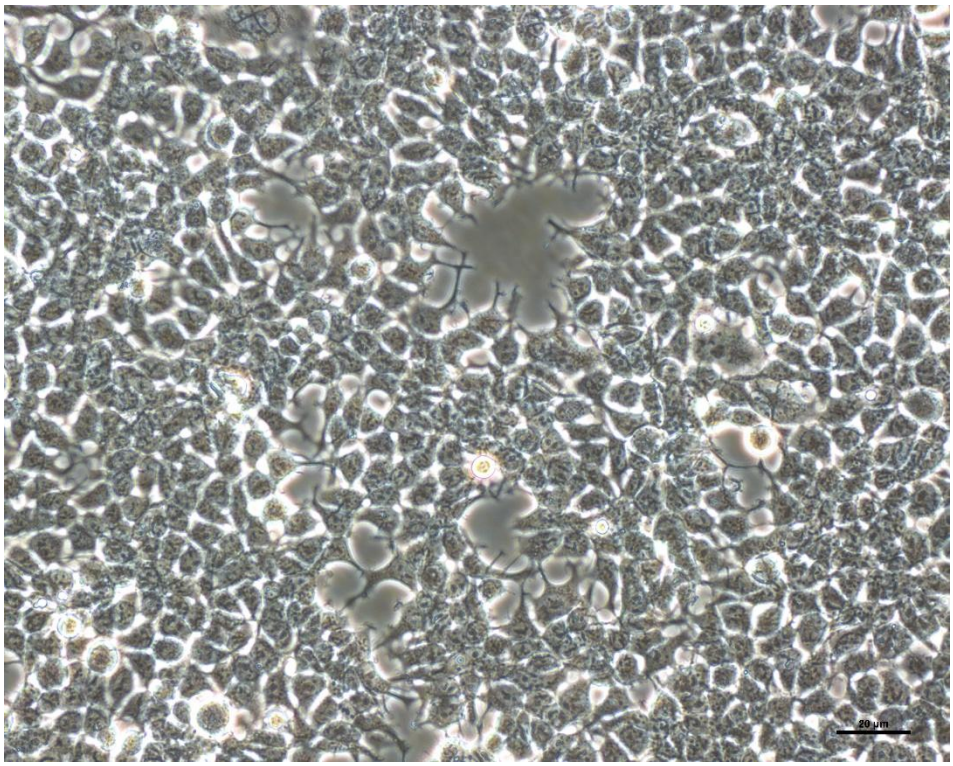
S16. 0.8% DMSO



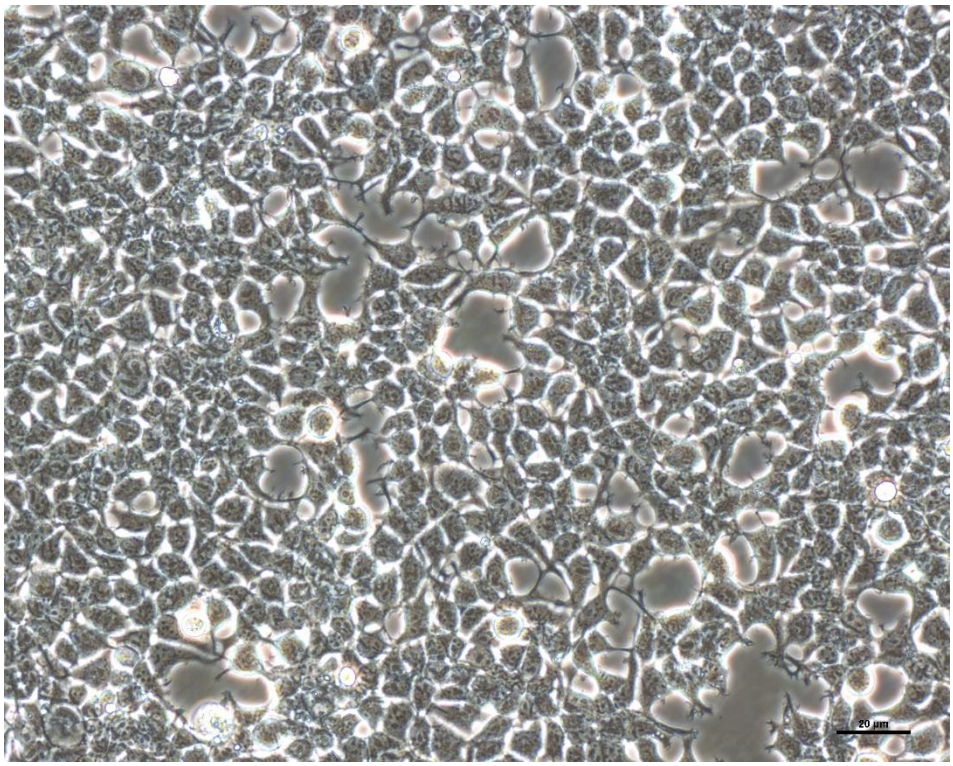
S17. 0.1 µM Kaempferol



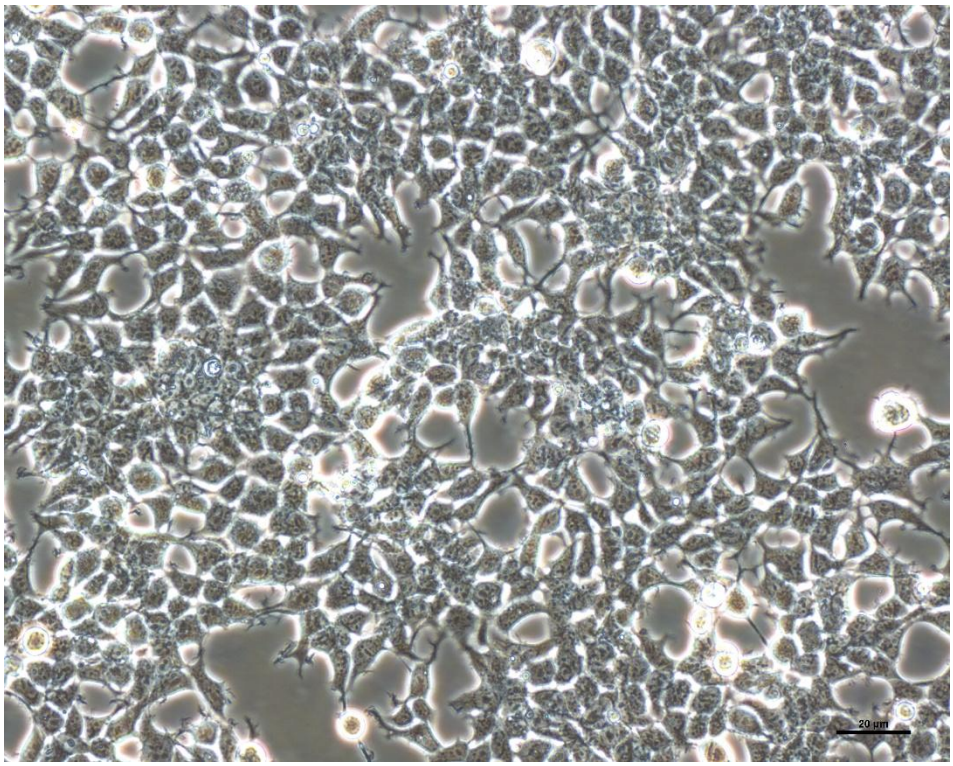
S18. 0.5 µM Kaempferol



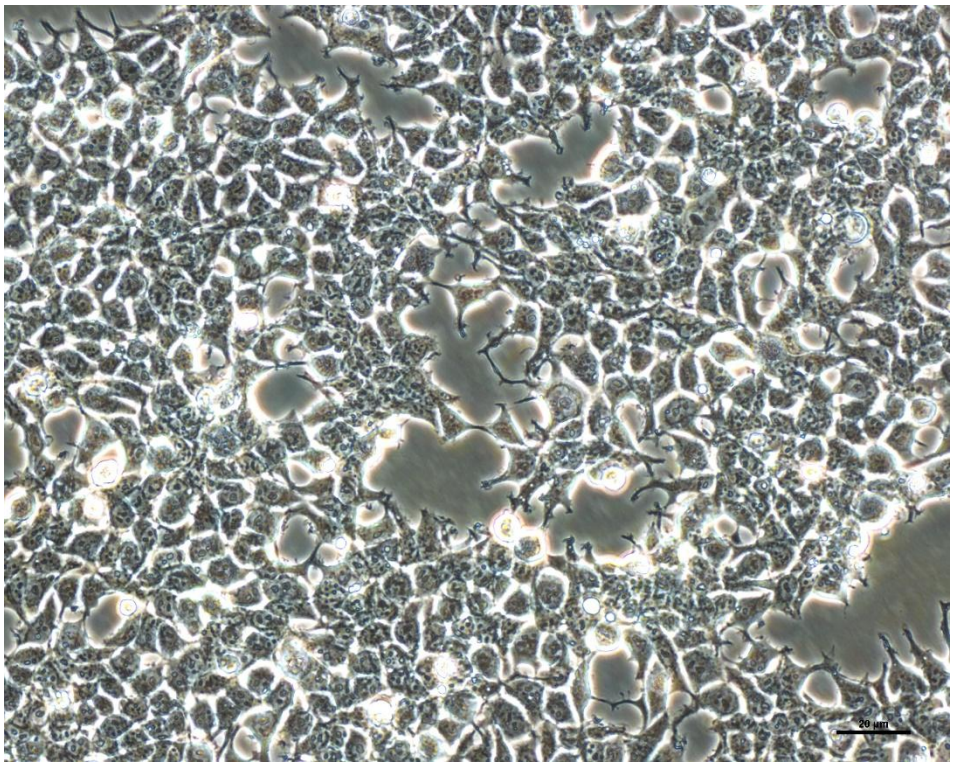
S19. 1 µM Kaempferol



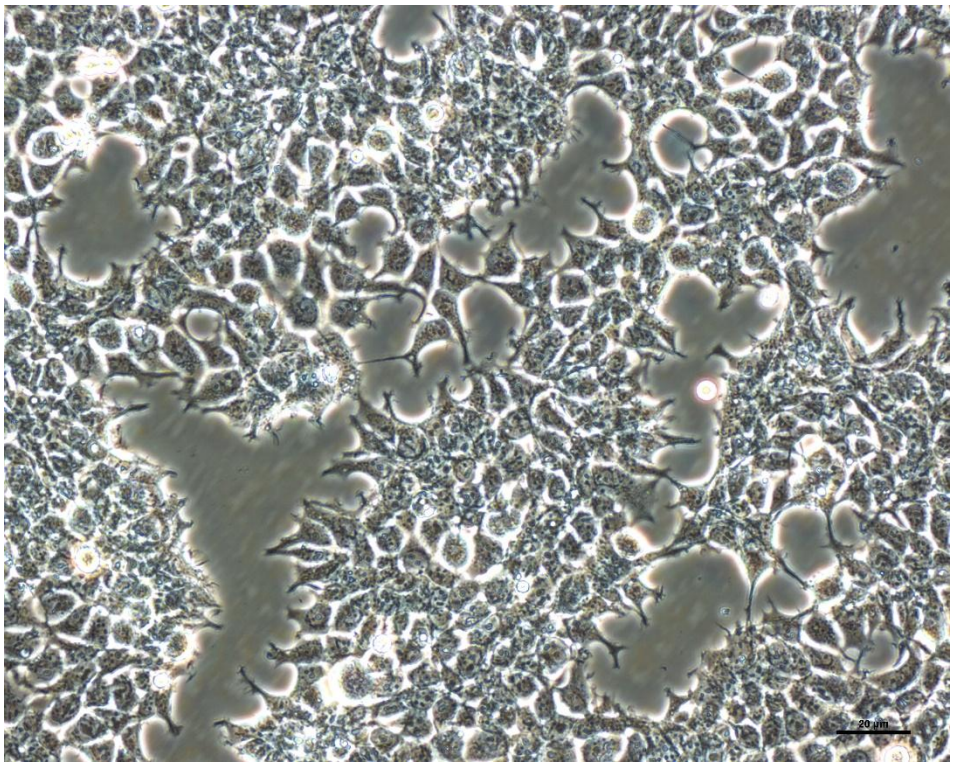
S20. 5 µM Kaempferol



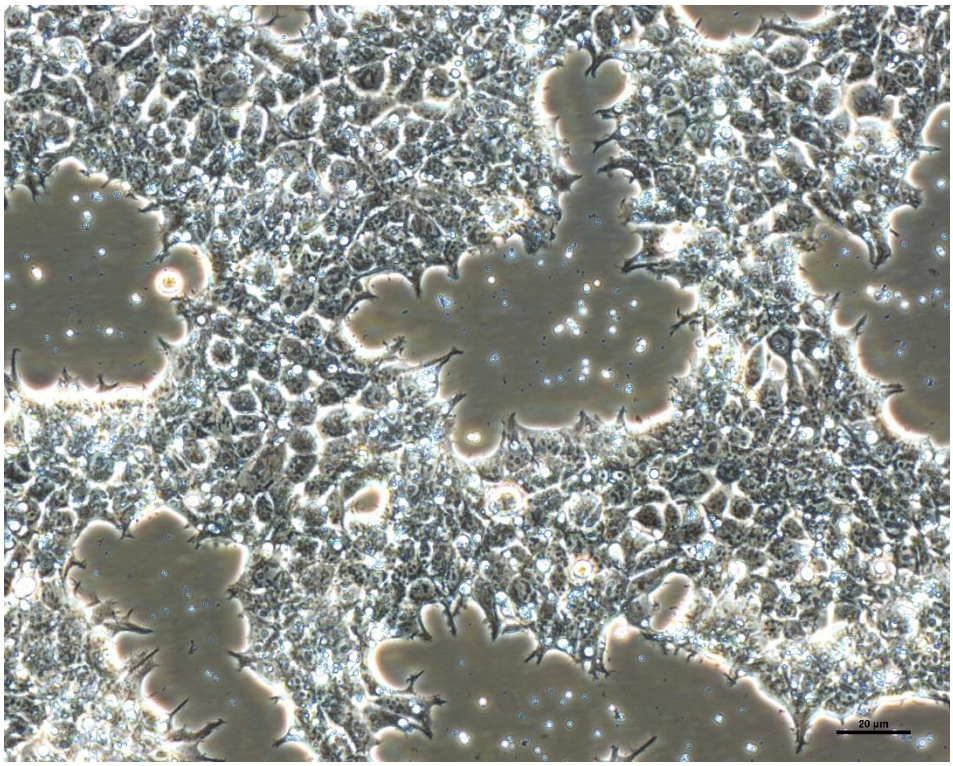
S21. 10 µM Kaempferol



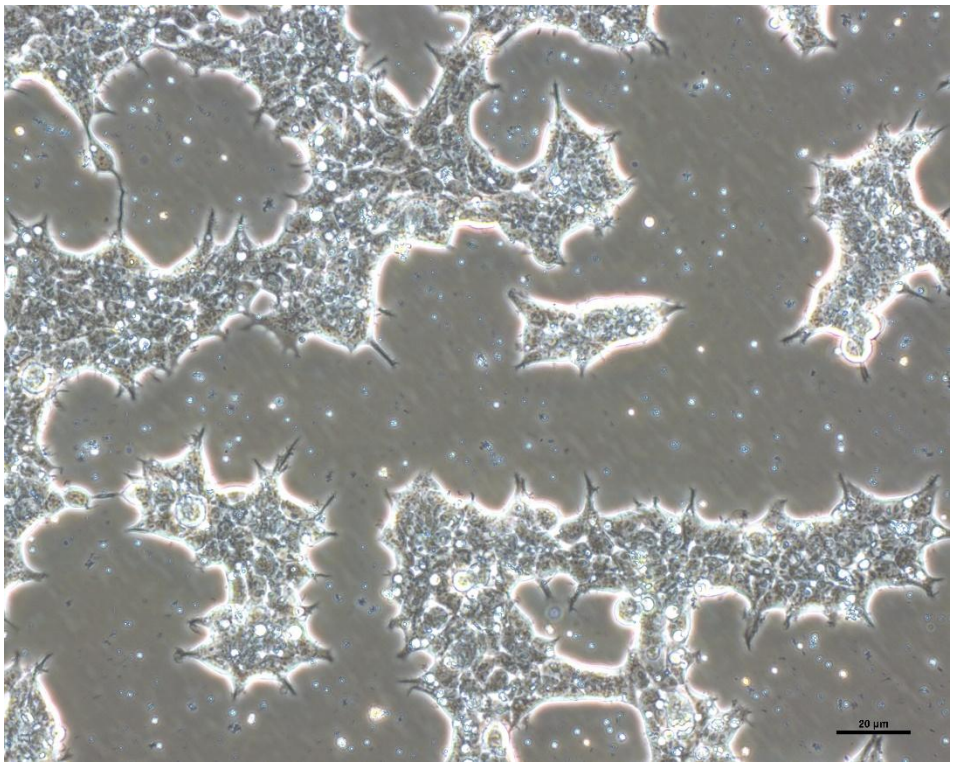
S22. 25 µM Kaempferol



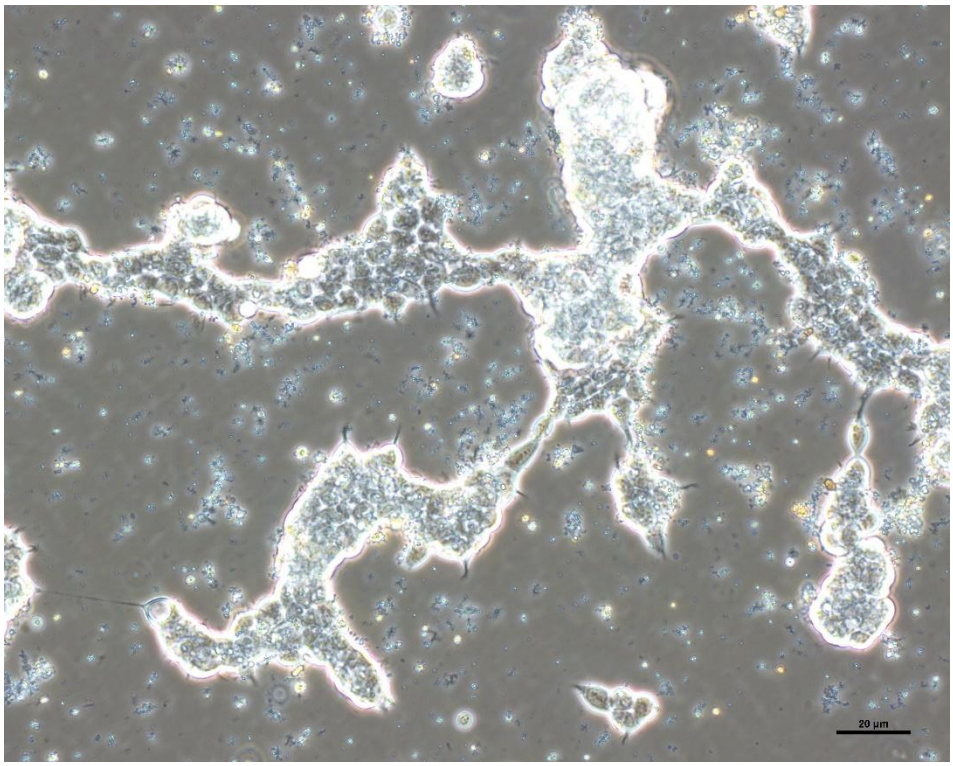
S23. 50 µM Kaempferol



S24. 100 µM Kaempferol



S25. 200 µM Kaempferol



S26. 400 µM Kaempferol

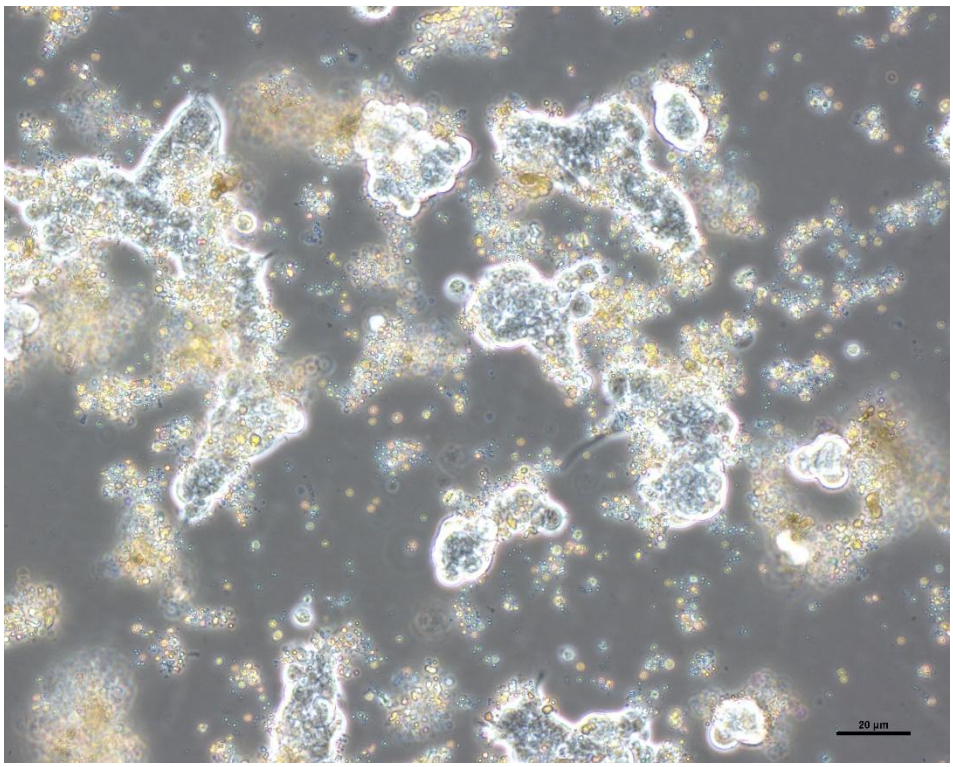
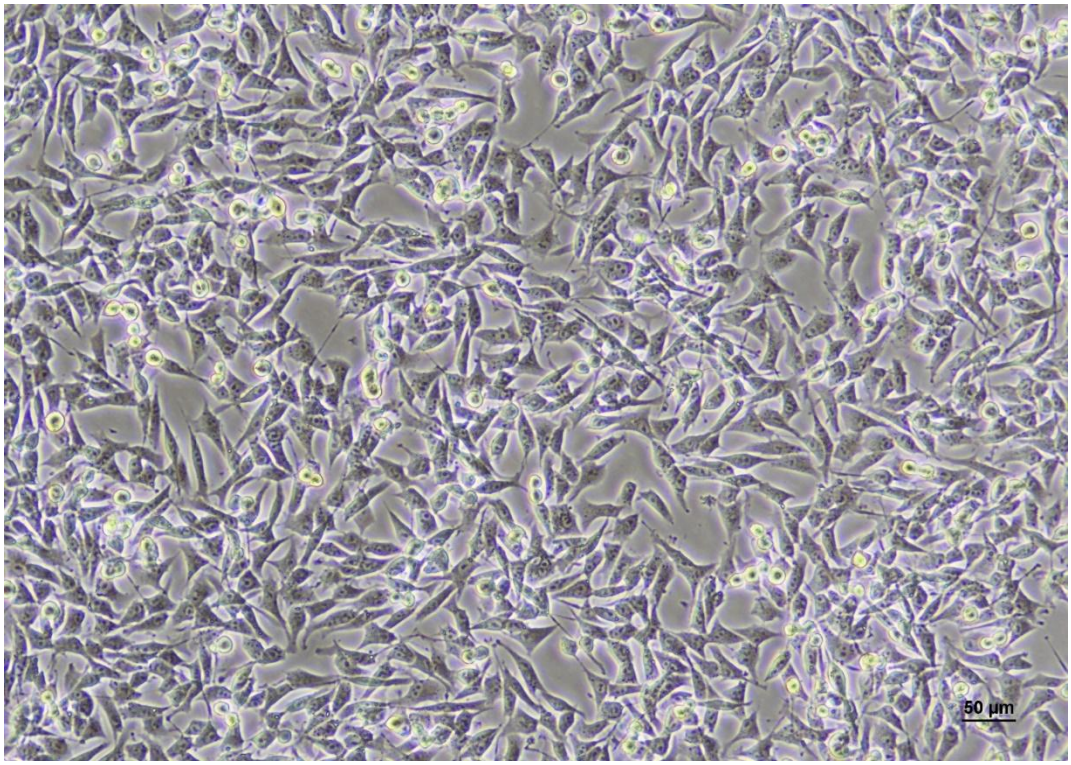
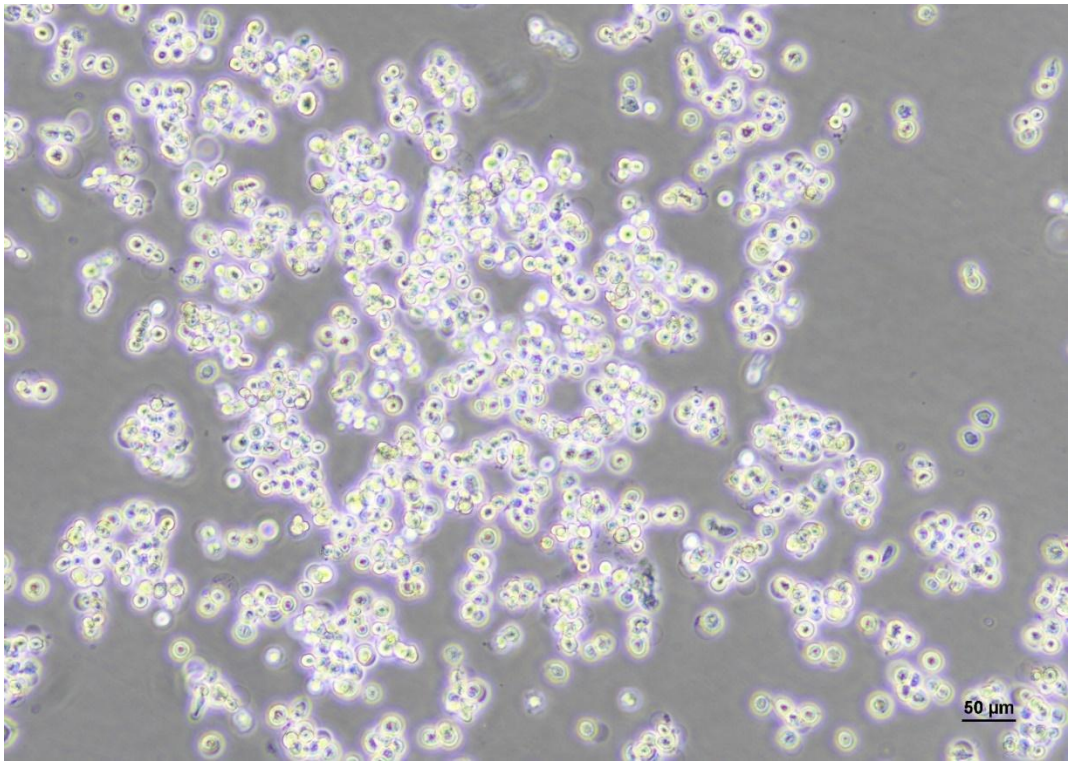


Figure S27-37. Effect of Kaempferol on BHK-21 cell morphology

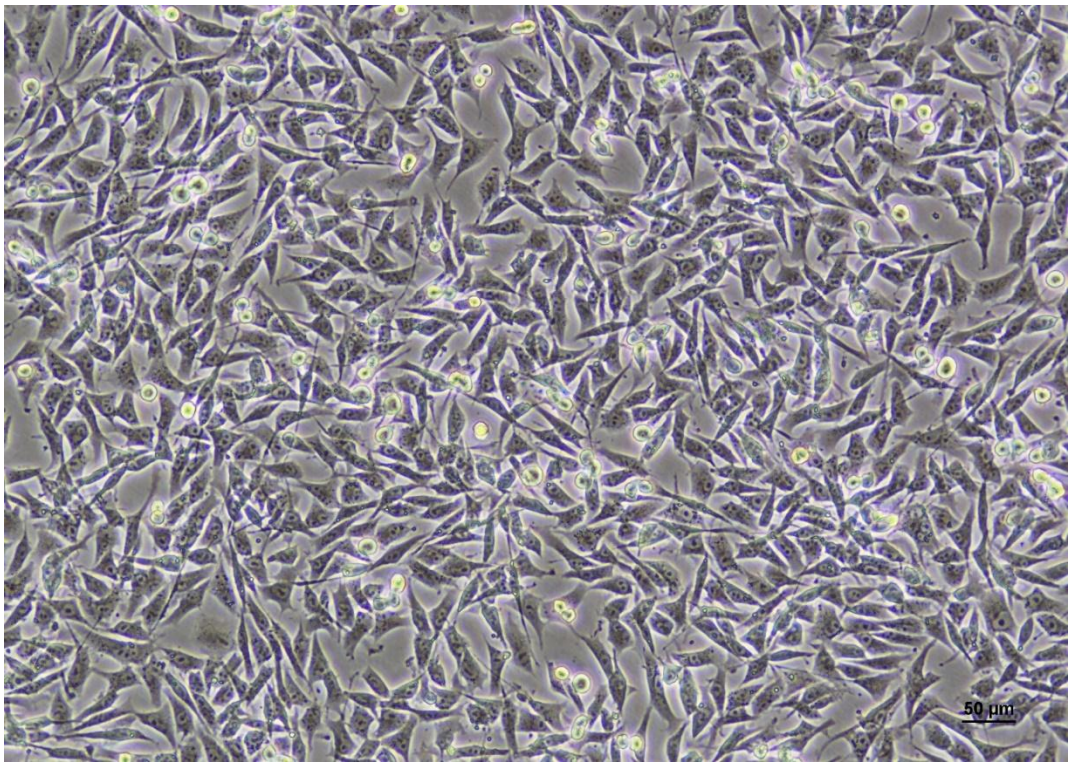
S27. 10% DMEM



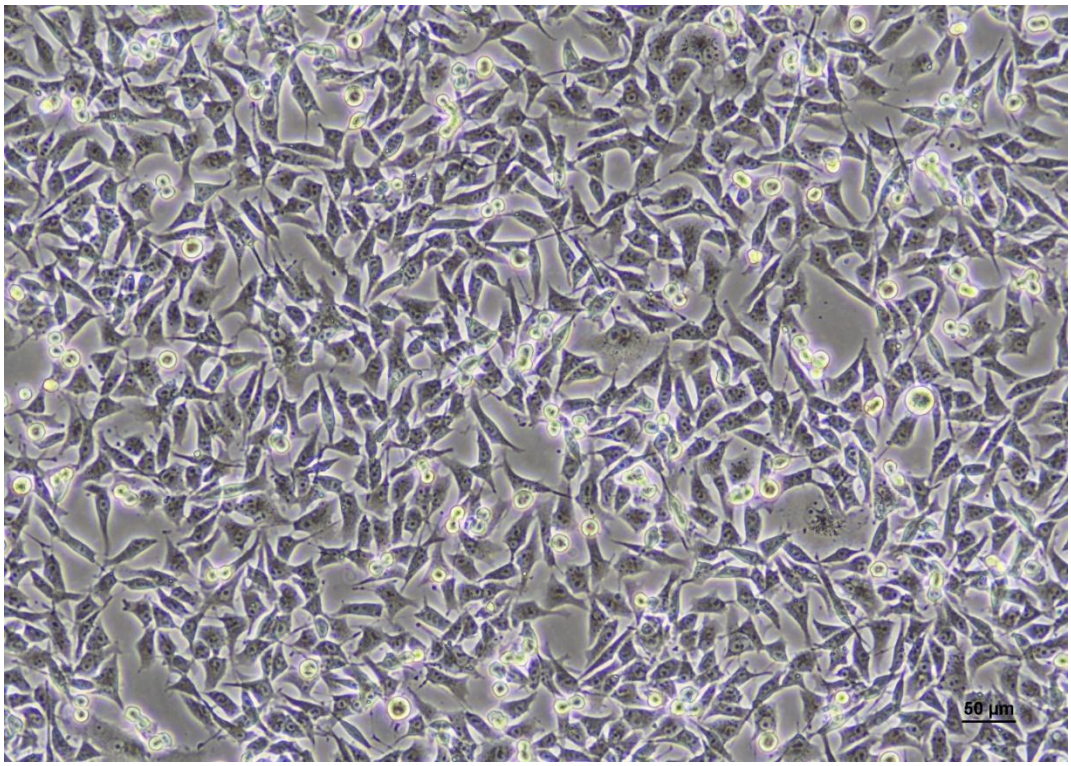
S28. 10% EtOH



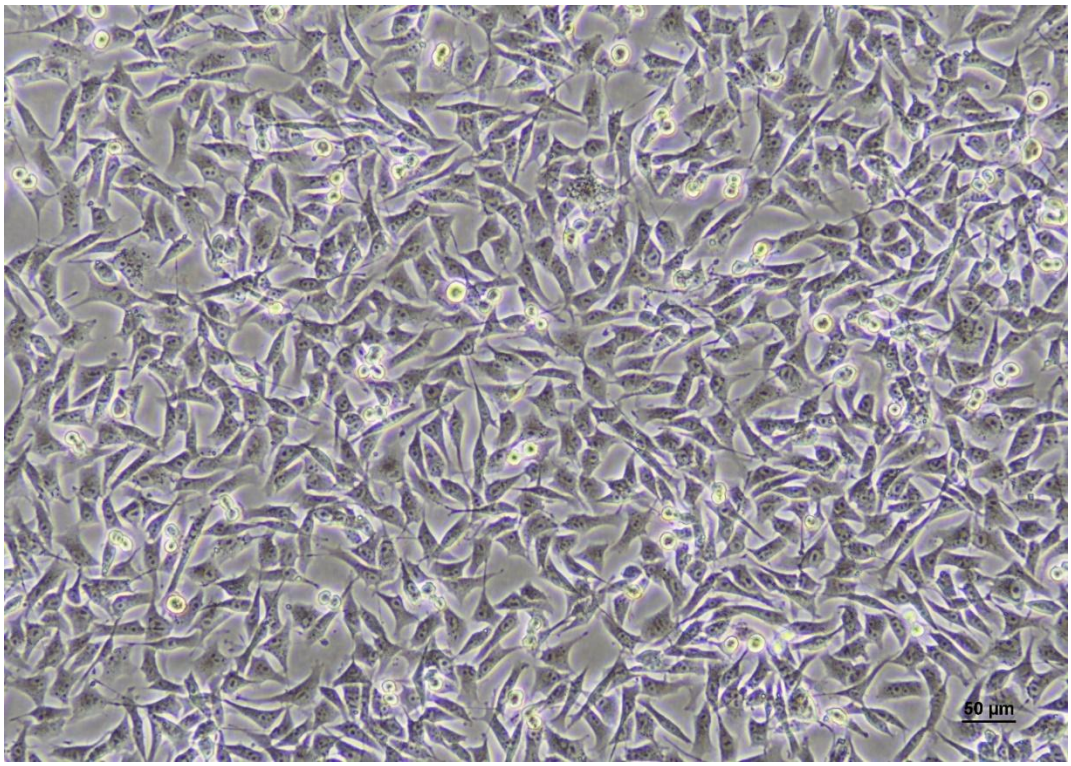
S29. 0.6% DMSO



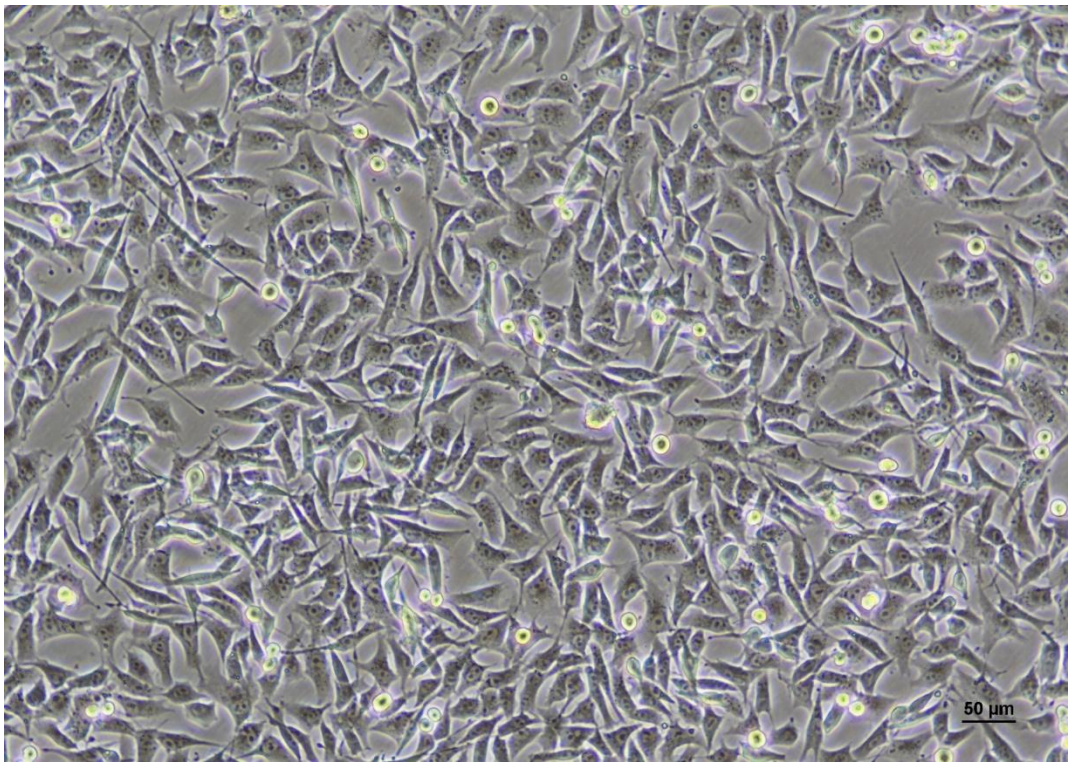
S30. 10 µM Kaempferol



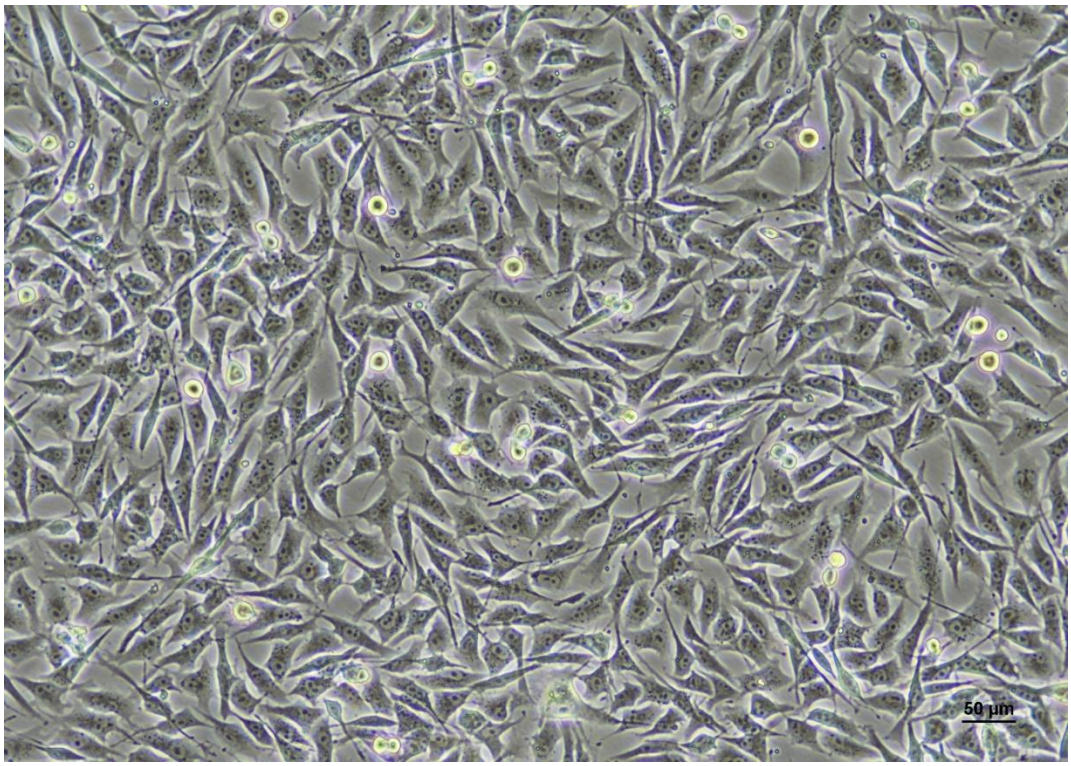
S31. 25 µM Kaempferol



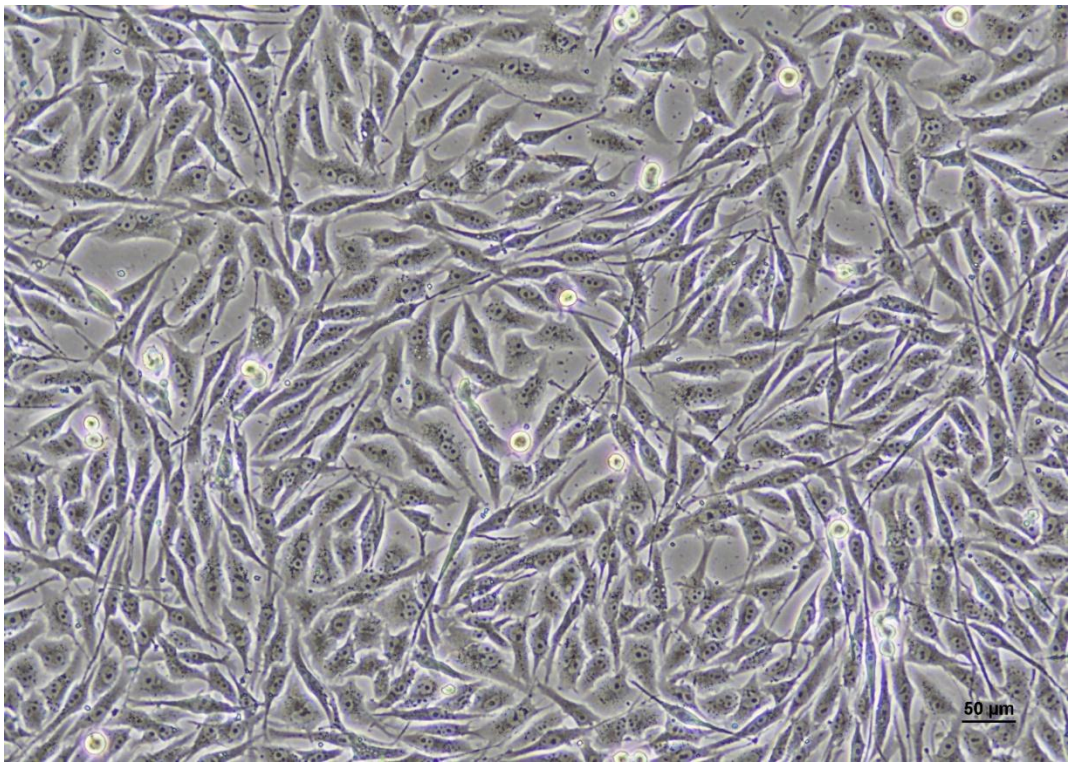
S32. 50 µM Kaempferol



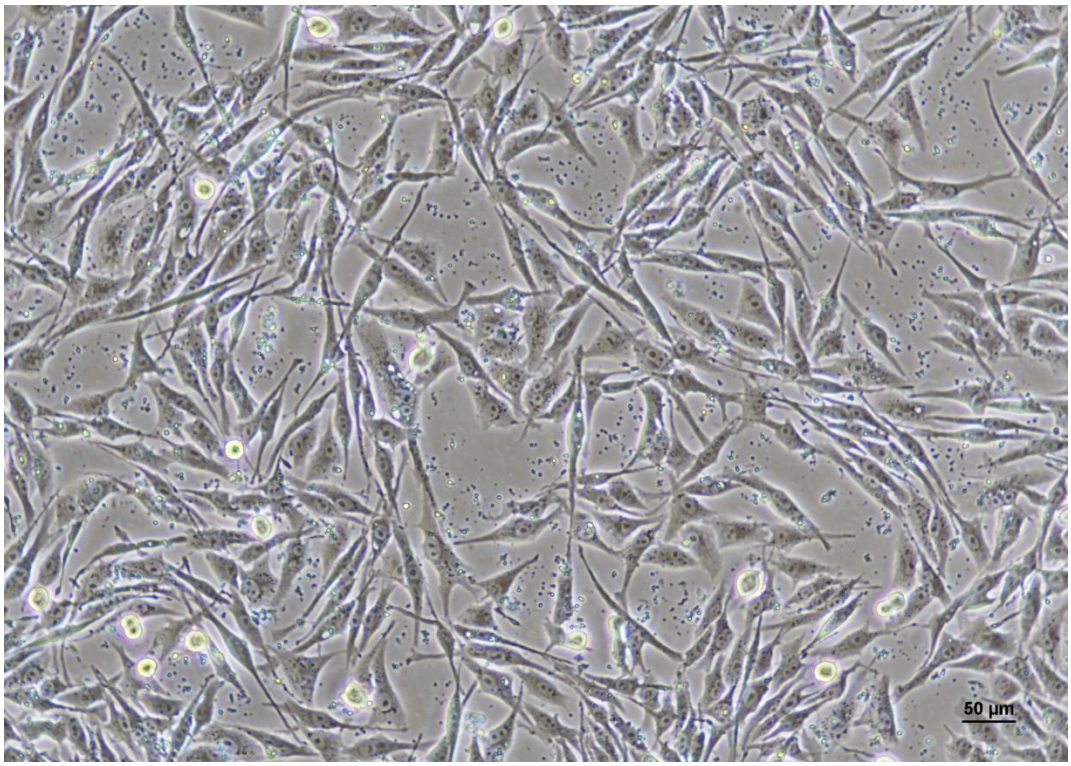
S33. 75 µM Kaempferol



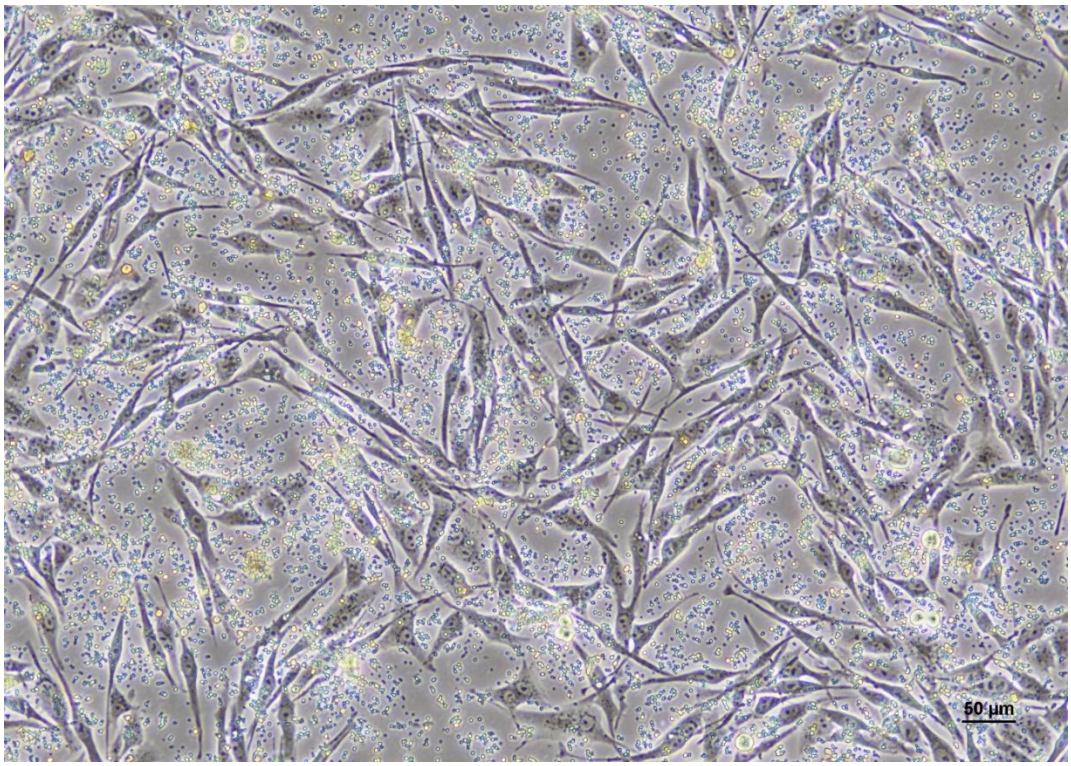
S34. 100 µM Kaempferol



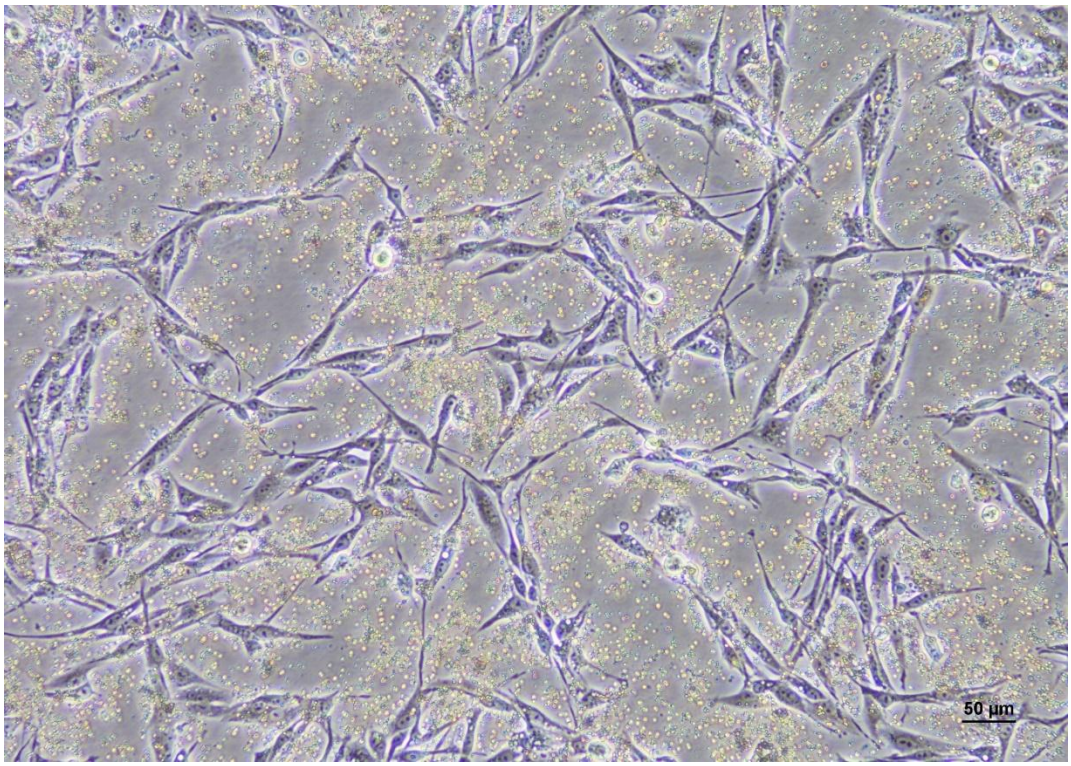
S35. 125 µM Kaempferol



S36. 150 µM Kaempferol

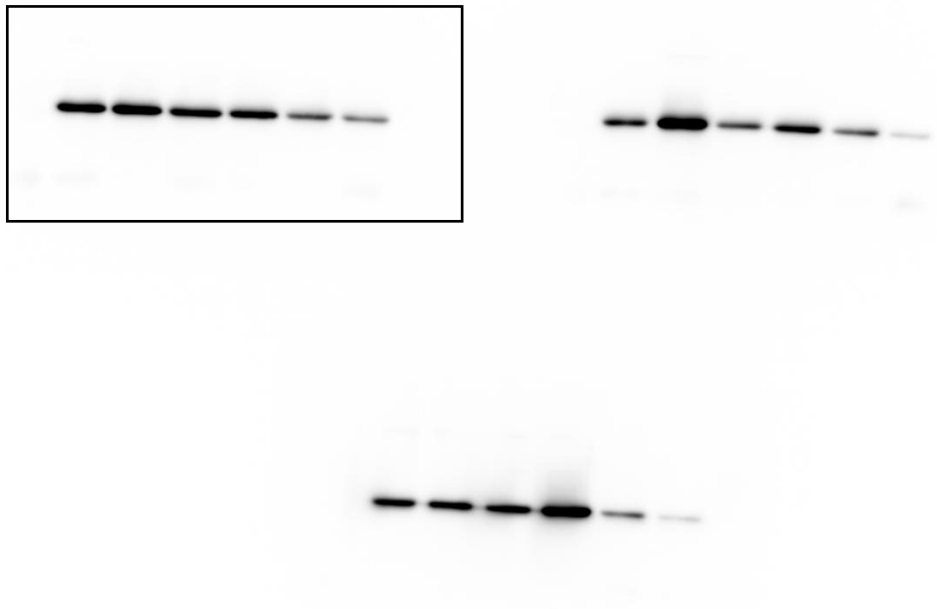


S37. 300 µM Kaempferol

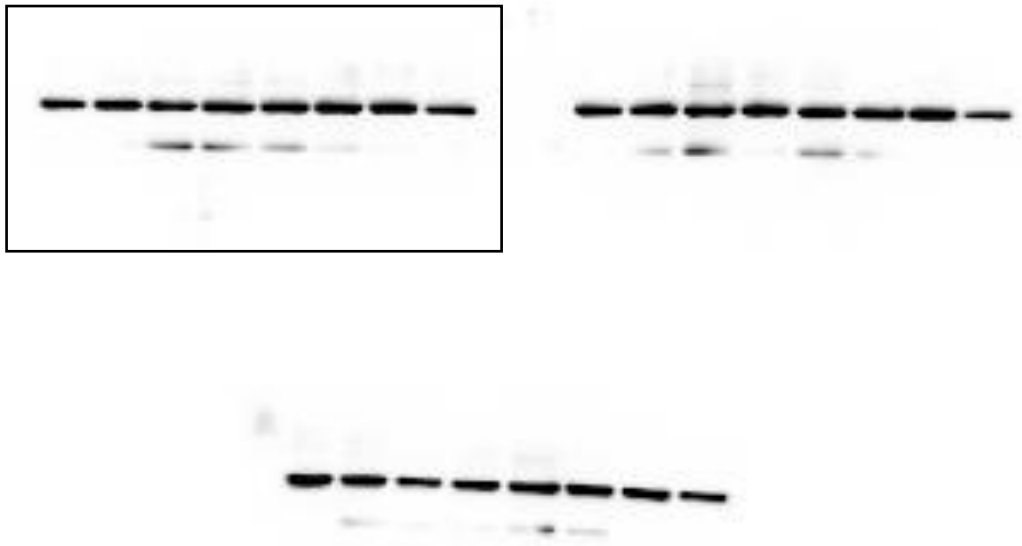


Supplementary Table 1. Primer sequences.

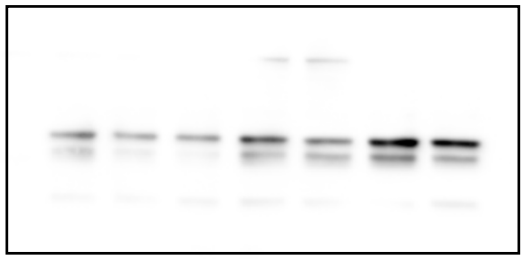
Primer	Sequence (5'-3')
GRP78-F	AGACTTTGACCAGCGTGTCATGGA
GRP78-R	TTCAAATTTGGCCCGAGTCAGGGT
GAPDH-F	GAACATCATCCCTGCCTCTAC
GAPDH-R	CCTGCTTCACCACCTTCTT
β actin-F	GAAGATGACCCAGATCATGT
β actin-R	ATCTCTTGCTCGAAGTCCAG



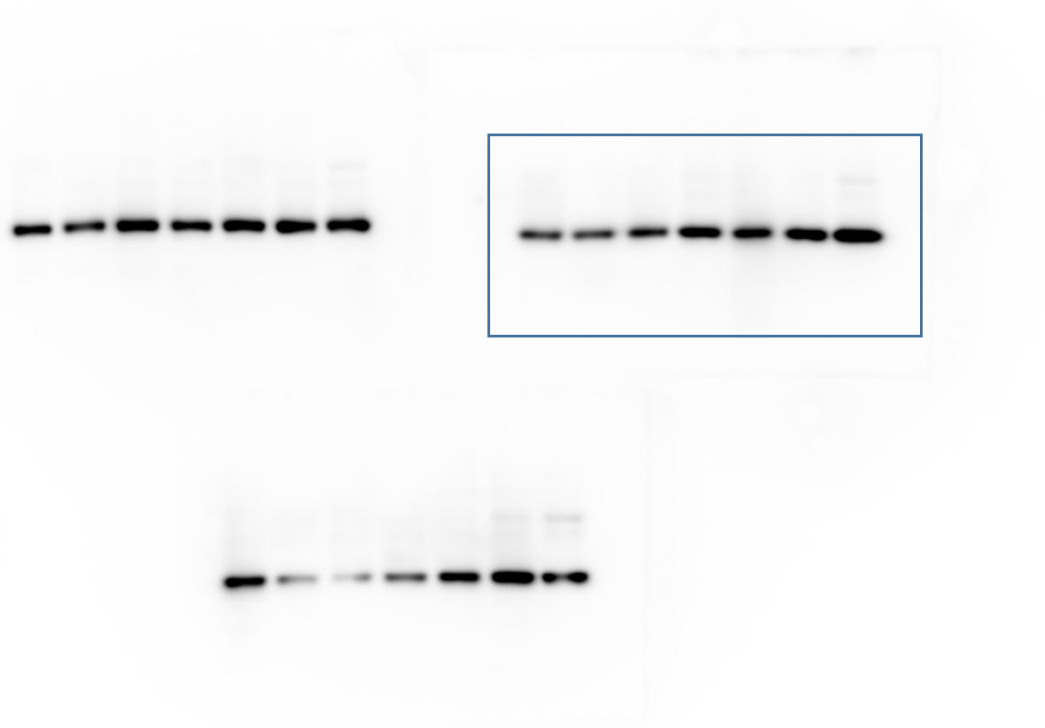
E protein of JEV with treatment of kamepferol



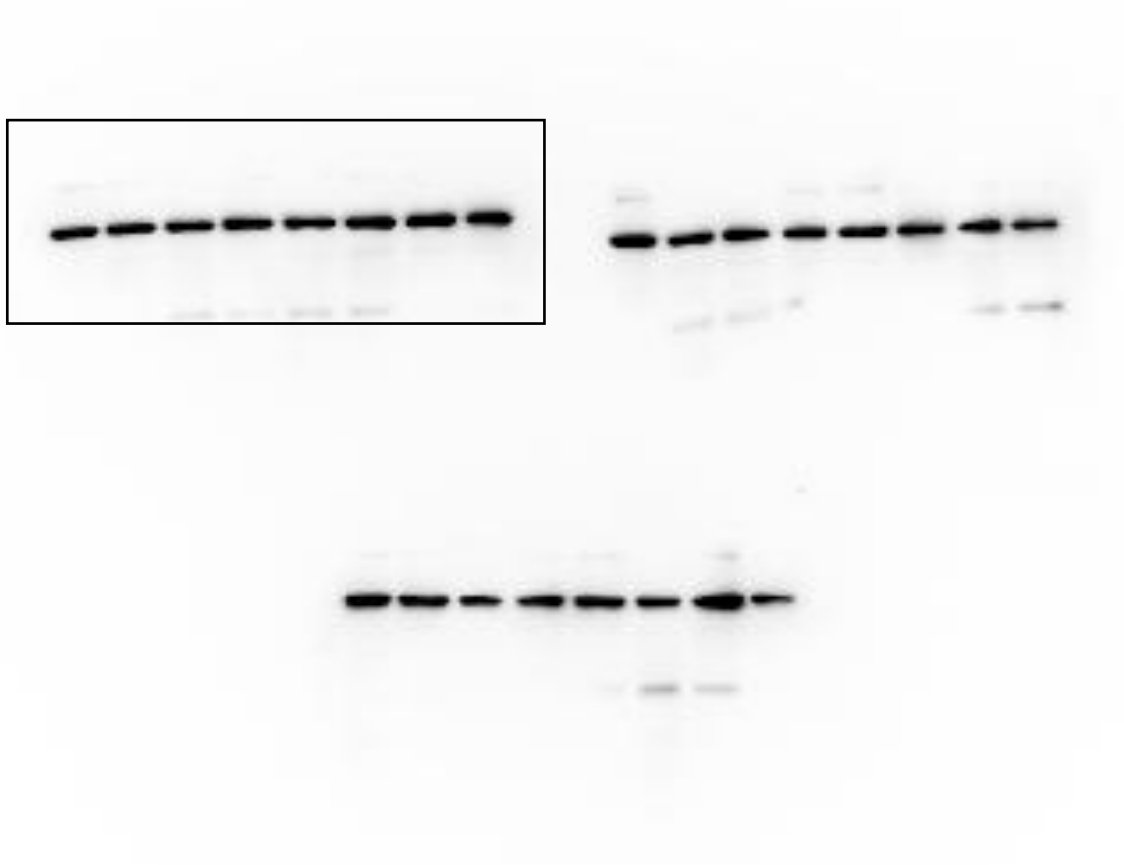
HSP70 of BHK-21 cells infected by JEV followed by treatment with kaempferol



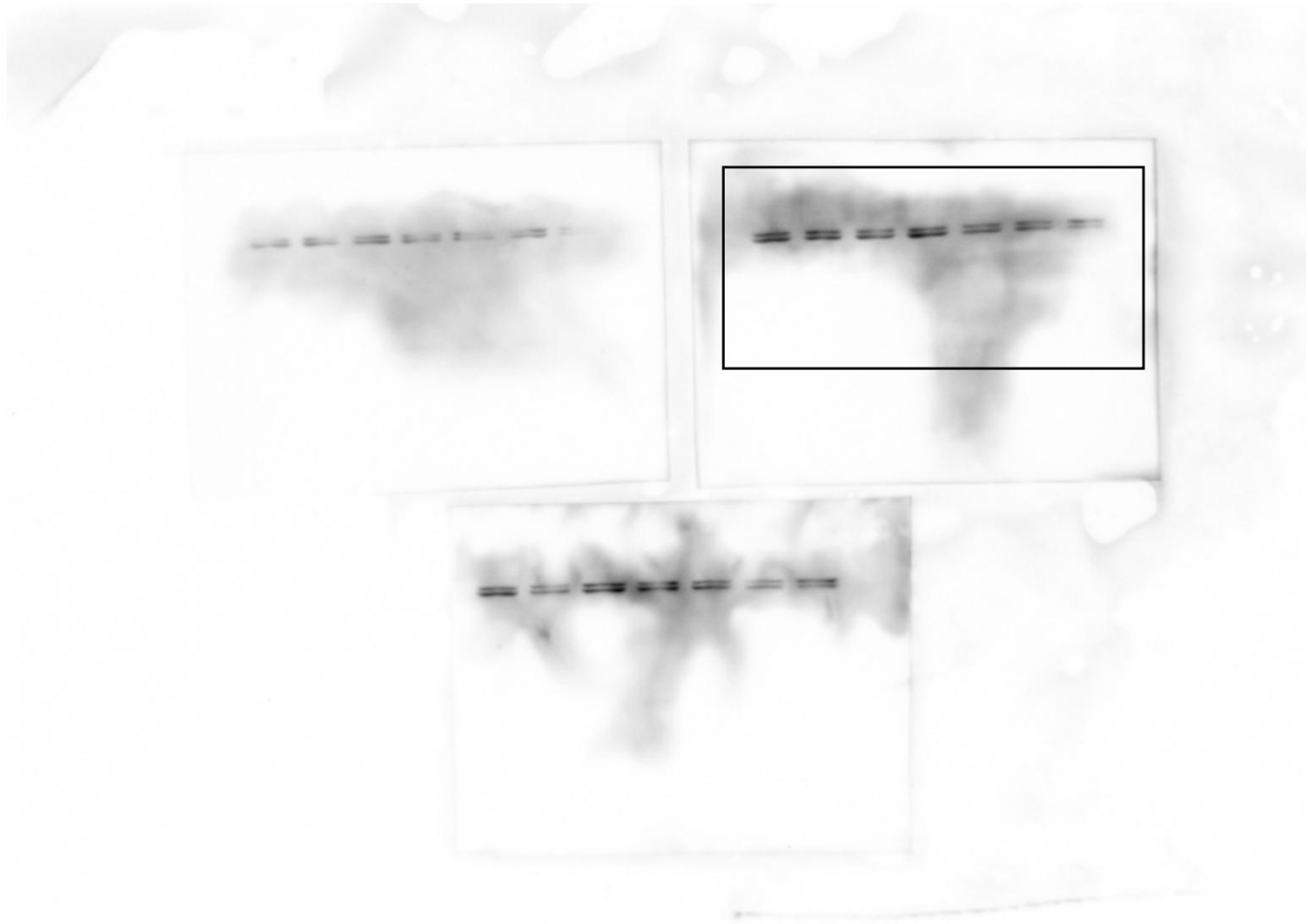
E protein of DENV with treatment of kamepferol



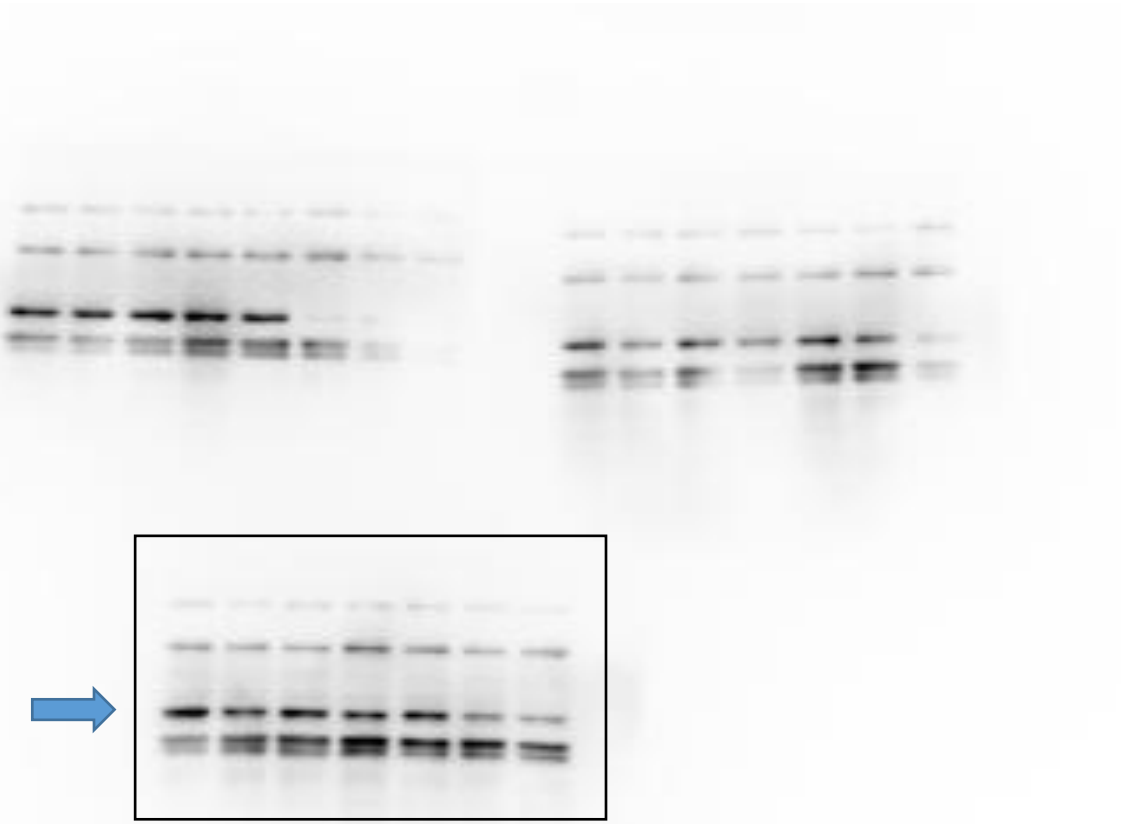
NS1 protein of DENV 2 treated with kaempferol



HSP70 of BHK-21 cells infected by DENV followed by treatment with kaempferol

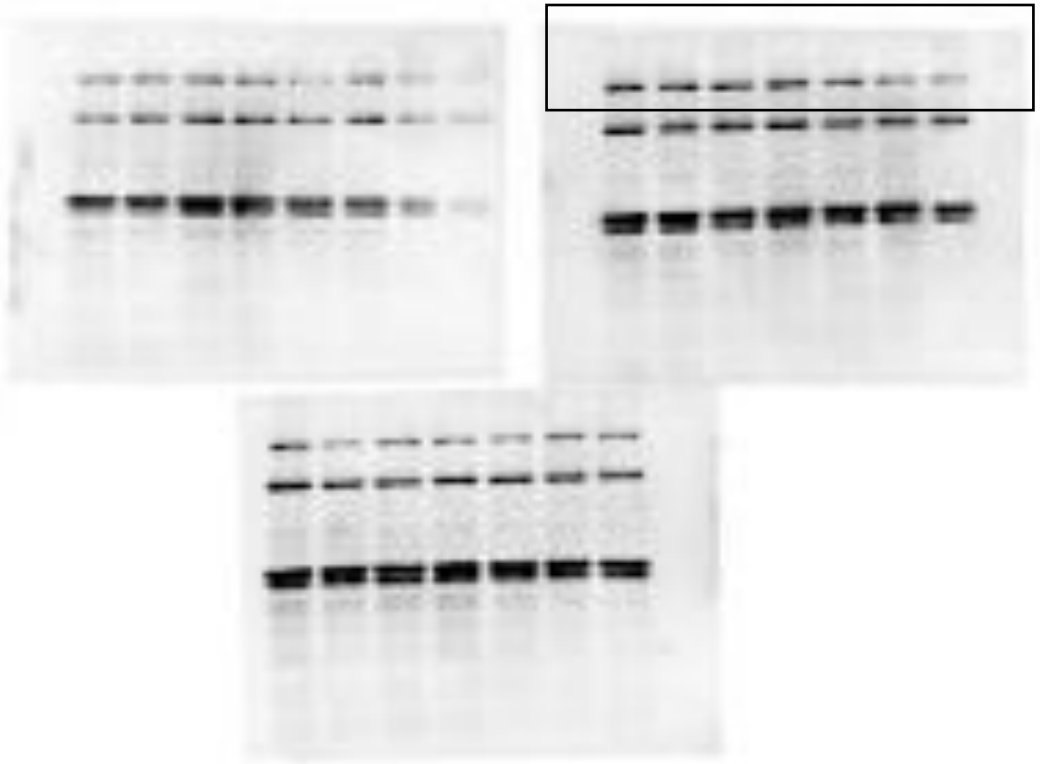


GRP 78 for control



Actin for control

GAPDH for control



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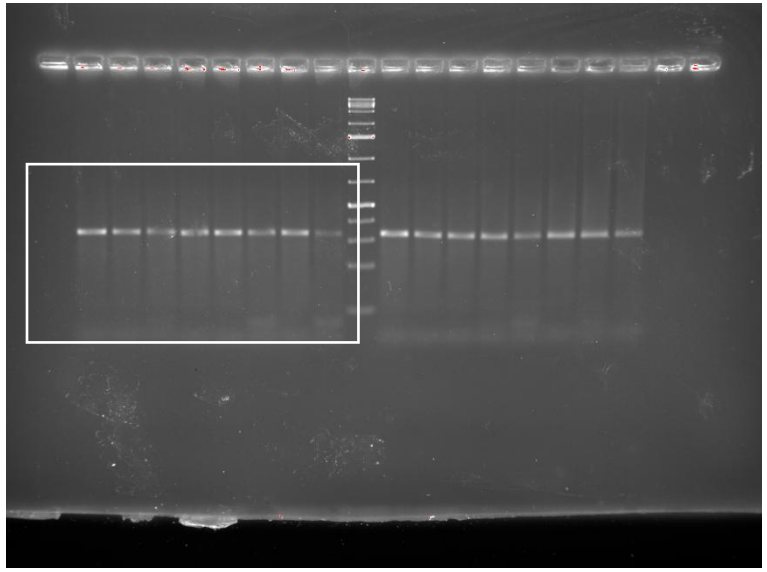
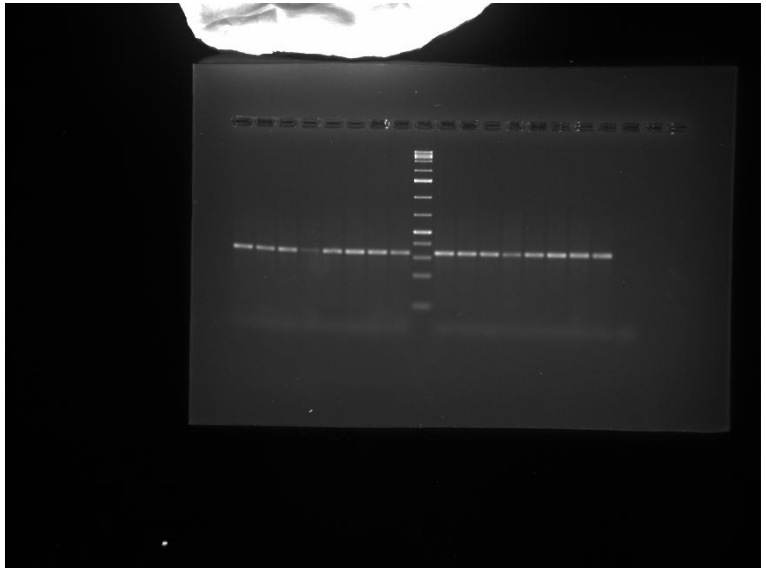
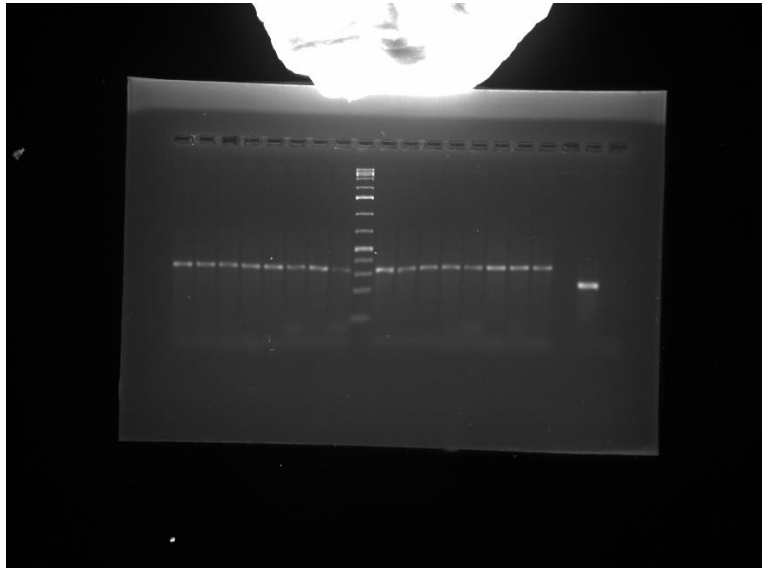
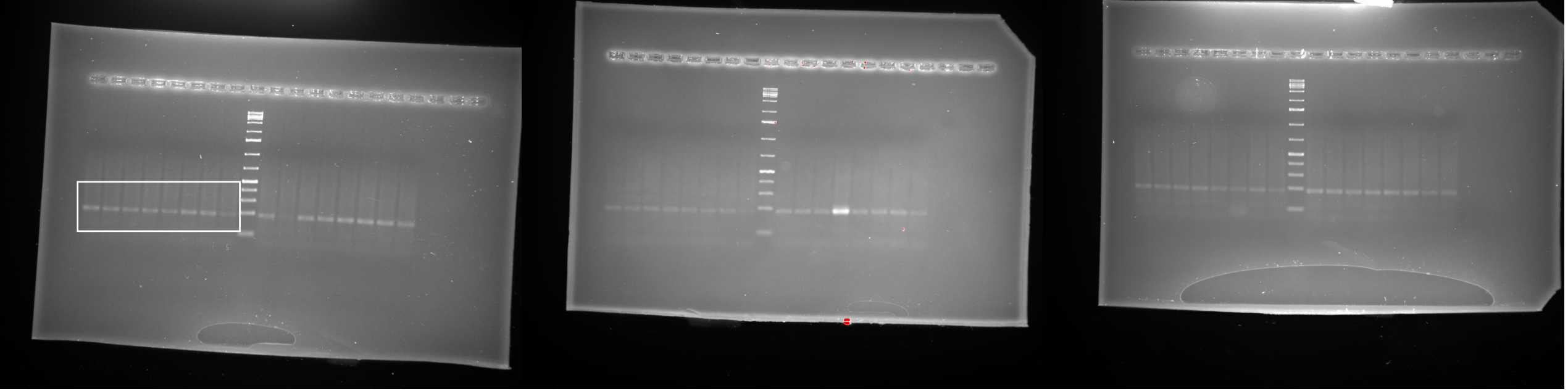
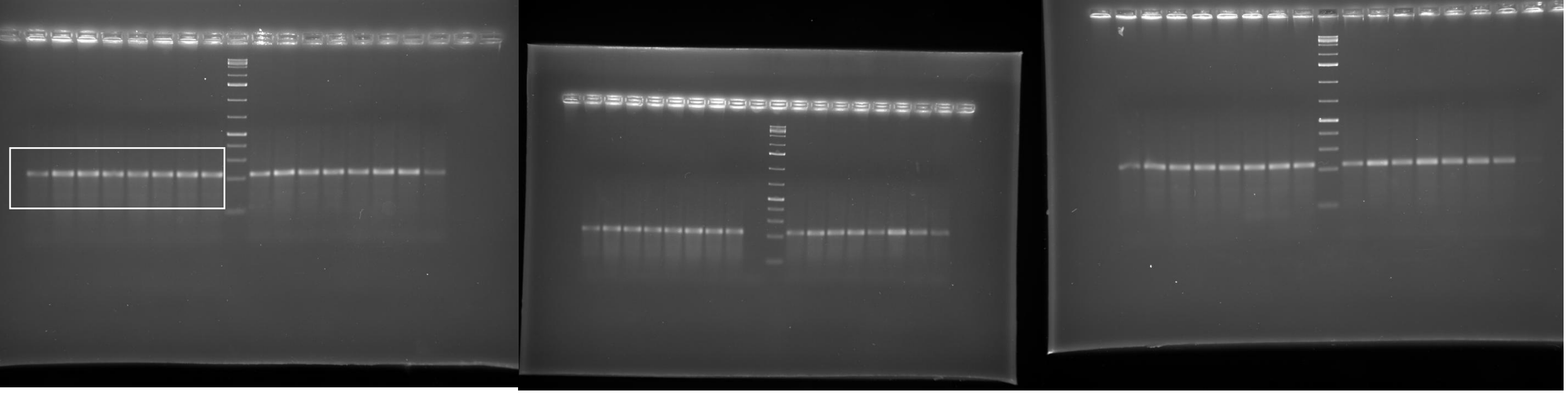




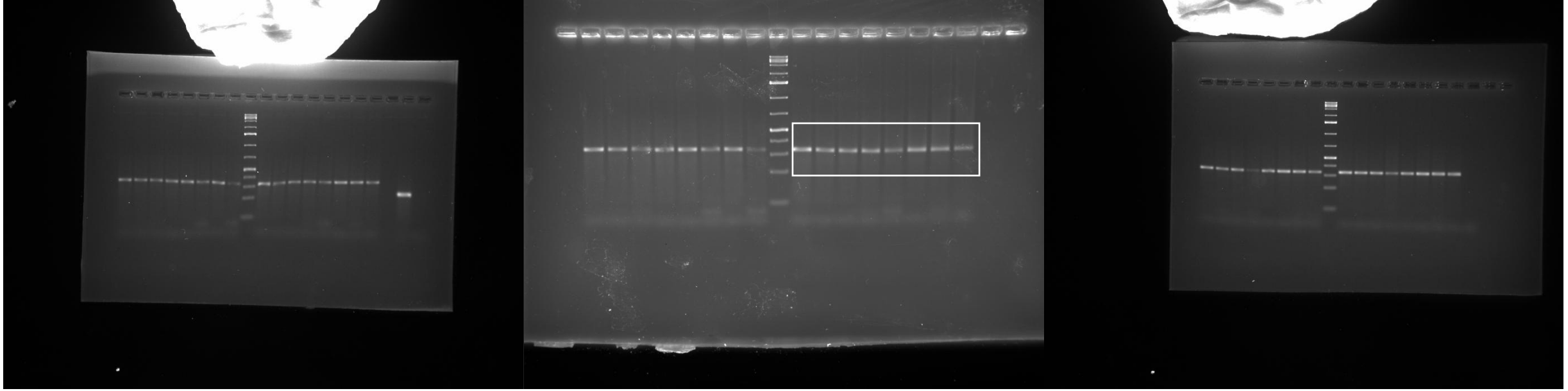
Actin of DENV



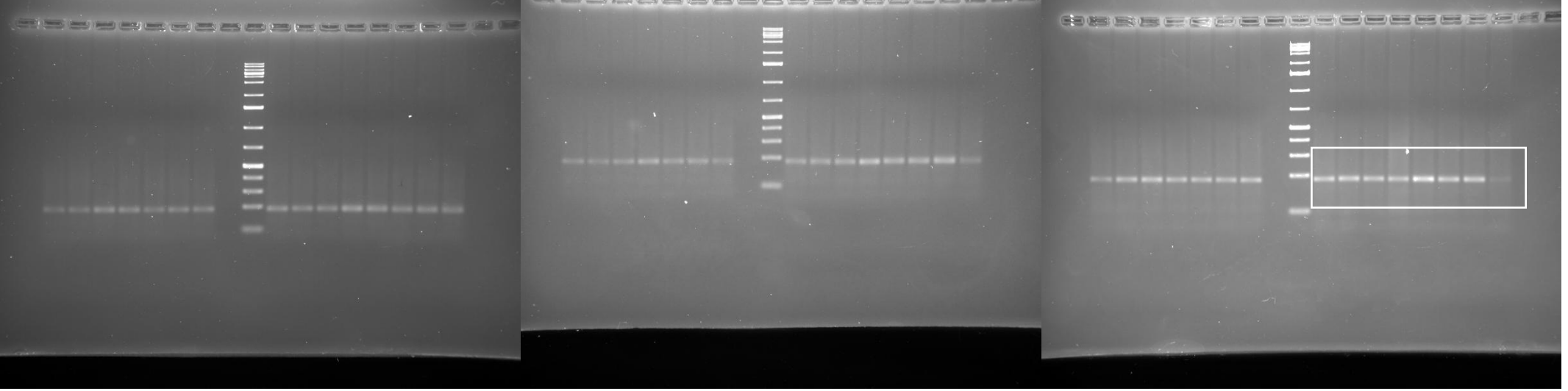
GAPDH of DENV



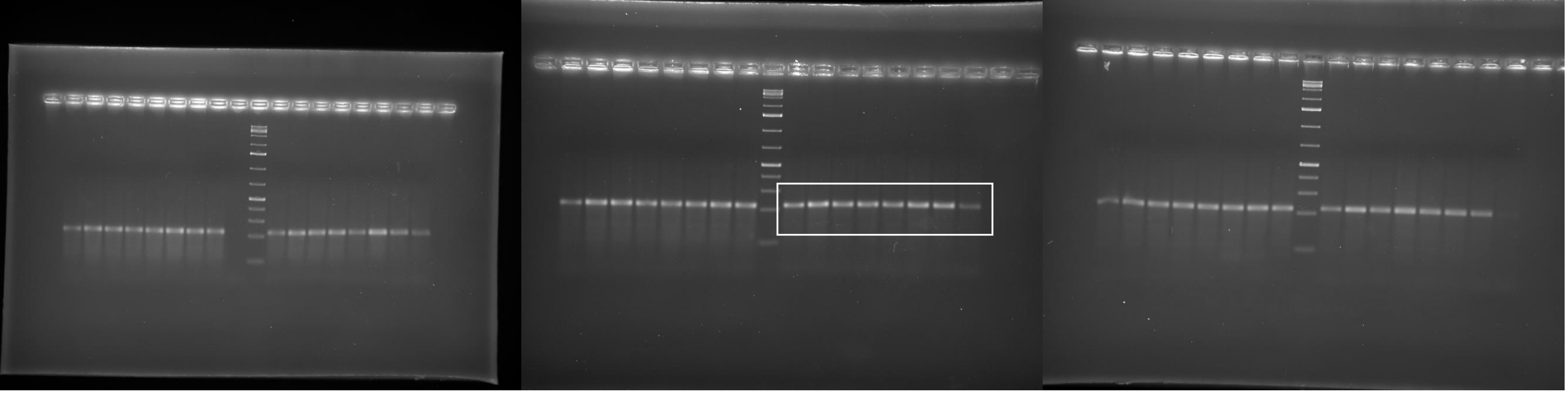
GRP78 of DENV



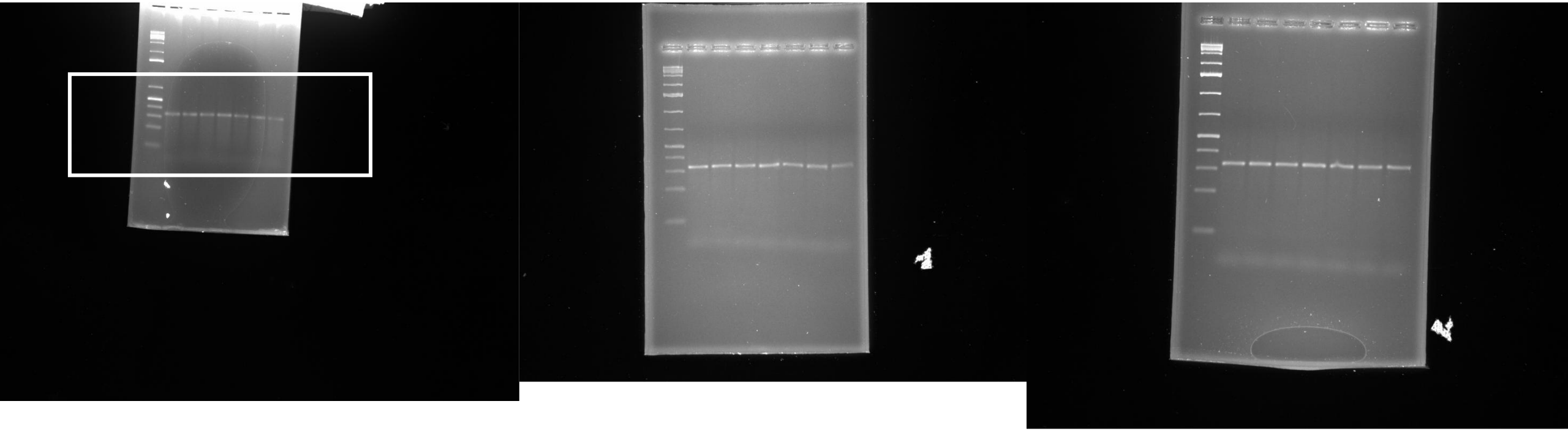
Actin of JEV



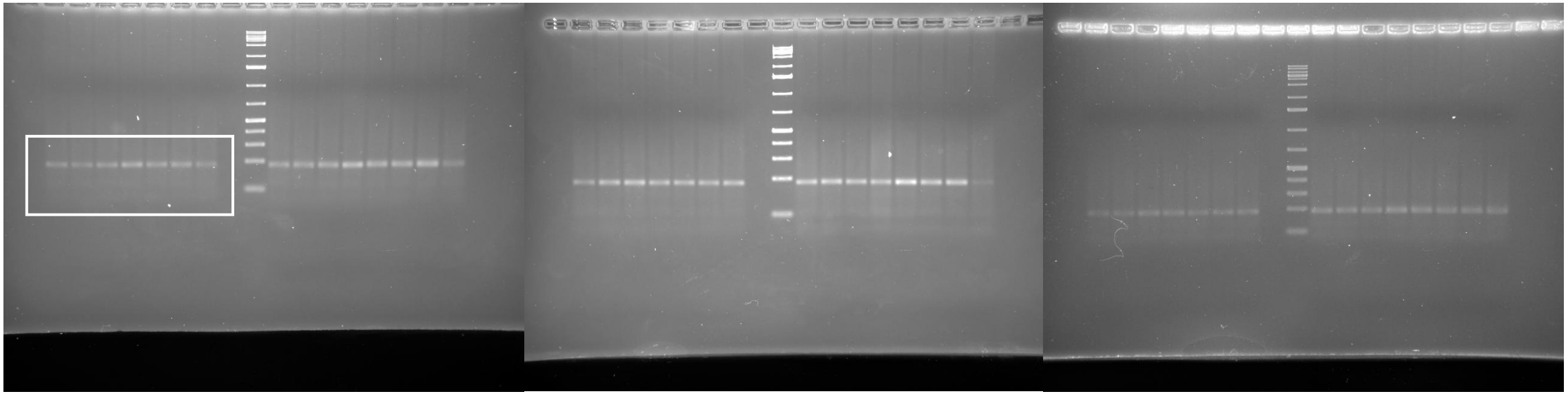
GAPDH of JEV



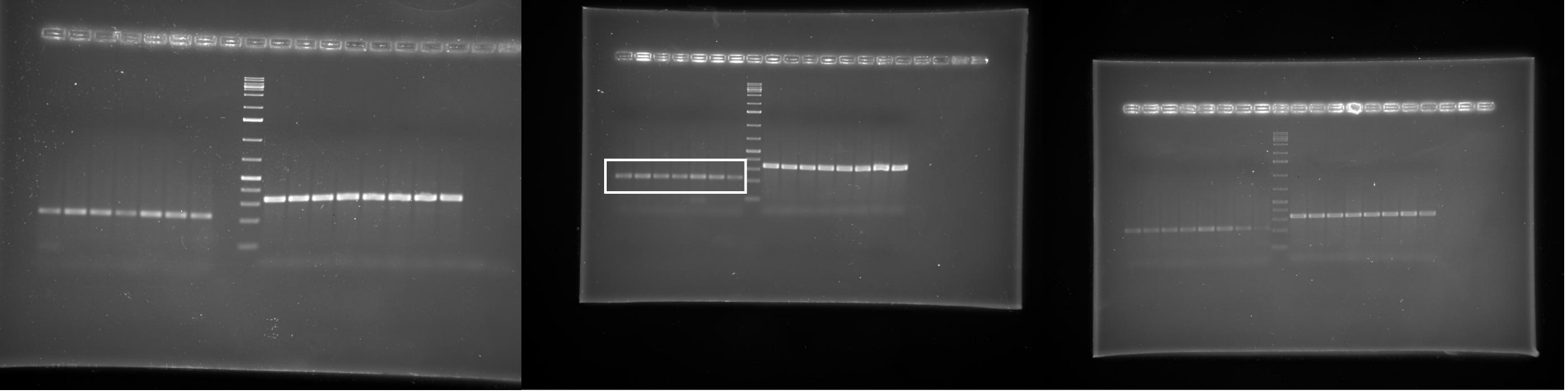
GRP78 of JEV



Actin control

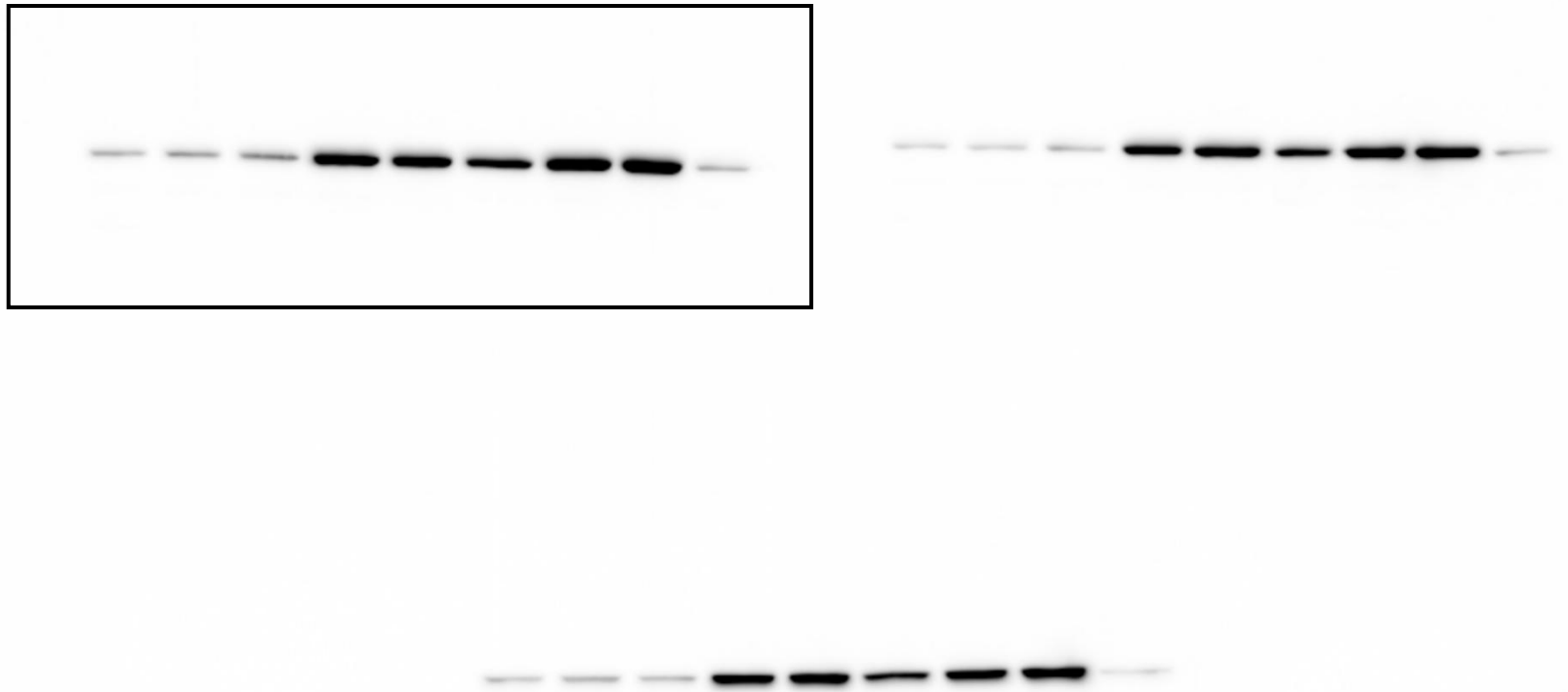


GAPDH control



GRP78 control

GRP78 protein



HSP70 protein