## Title

AIM2 Inflammasome-Mediated Pyroptosis in Enterovirus A71-Infected Neuronal Cells Restricts Viral Replication

## Authors

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Viral antigen positive- Alexa Fluor 488

**Supplementary Figure S1 SK-N-SH cells infected at MOI of 10.** Flow cytometry analysis of SK-N-SH cells infected with EV-A71/13903 at 48 and 72 hours post-infection (hpi). Each graph is a representative set of similar triplicate results.



Supplementary Figure S2 Transfection controls showed credible results. (a) Gene expression of GAPDH in siRNA GAPDH and siRNA Negative transfected SK-N-SH cells at 48 hpt. GAPDH expression were significantly decreased (\*p<0.05) in about 99% of SK-N-SH cells. siRNA Negative showed basal level expression of GAPDH. (b) Flow cytometry analysis of transfection efficiency and cell viability were determined using siGLO and 7-AAD, respectively. siGLO showed 86% cells to be successfully transfected at the 48 hpt, and 7-AAD staining showed only 1 - 8% of cell death at 24 to 144 hpt. All data represents the mean±standard deviation of a set of triplicates.



**Supplementary Figure S3 AIM2 and 7-AAD were not expressed in mock-infected SK-N-SH cells.** Flow cytometry of AIM2 expression, 7-AAD staining and viral antigens in SK-N-SH cells staining. Mock-infected SK-N-SH cells were negative for all stains. Positive controls for all staining was acceptable.



Supplementary Figure S4 Disease and function pathways analysis indicates the overlap between cell death and cell movement pathways. Most genes were involved in both cell death (green arrows) and cellular movement (blue arrows) mechanisms, including caspase-1 and inflammatory mediators, IL-6, CXCL10 and TNF- $\alpha$ . AIM2 and CARD16 is only involve in cell death mechanisms. All illustrated genes were up-regulated in SK-N-SH cells.