

Supplementary information, Figure S3 N-terminal structure is important for GSDMD-N to form oligomer and trigger pyroptosis. (A) Viabilities of $Gsdmd^{-/-}$ RAW-asc cells reconstituted with Flag-GSDMD and $3 \times Flag$ -GSDMD respectively after primed with LPS for 4 hours followed by no treatment or Nig stimulation. Data represent the mean \pm s.d. of two independent experiments. (B) Immunoblot analysis of cell lysates of $3 \times Flag$ -GSDMD reconstituted $Gsdmd^{-/-}$ RAW-asc cells. 4%–12% gradient SDS-PAGE and non-reducing condition were used. (C) Deconvolution microscopy of DMSO-treated CHO cells expressing different GSDMD-N-HBD*-HA deletion mutants. Cells were analyzed as **Figure 5D**. Scale bar, 5 μ m.