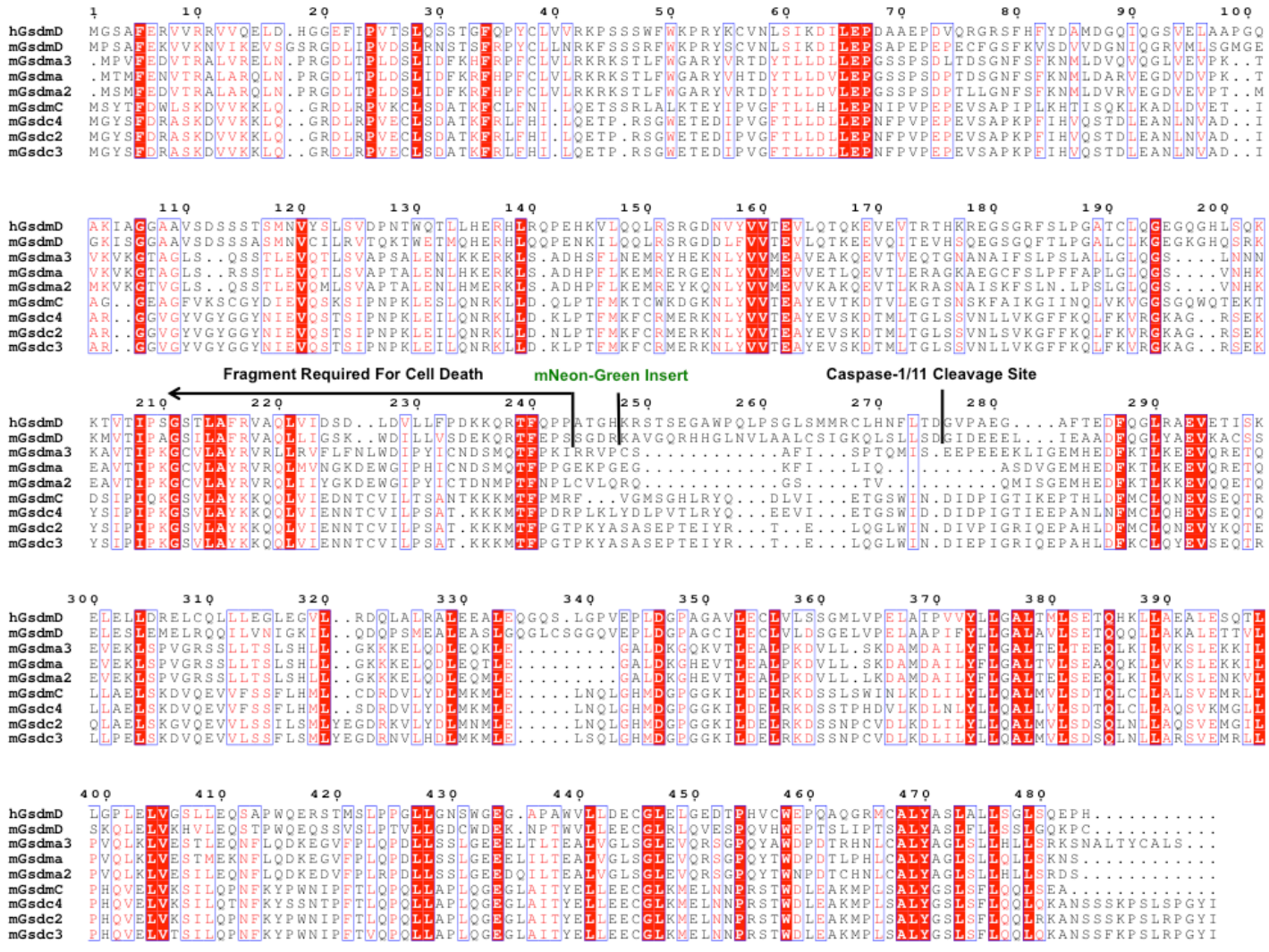
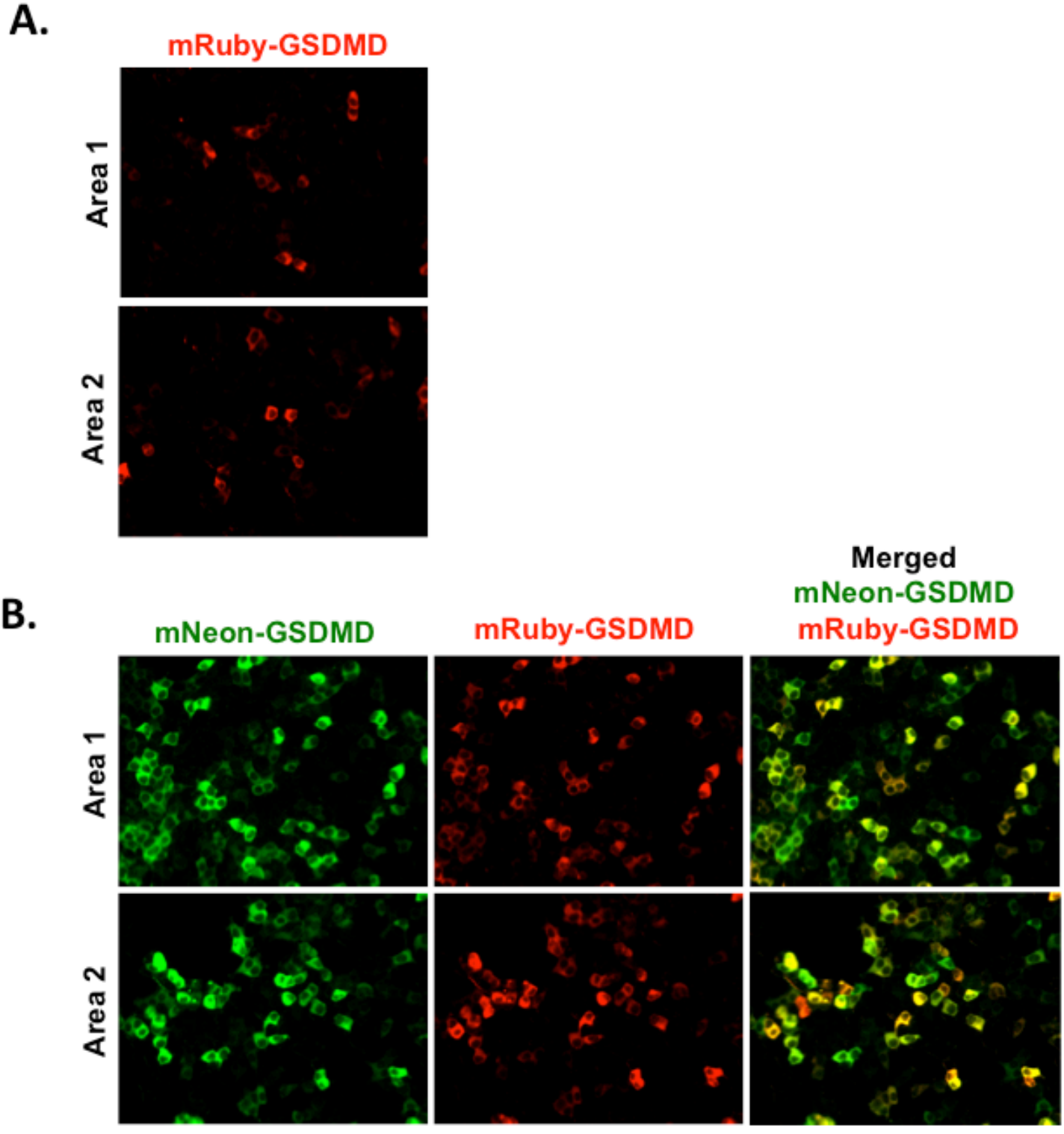
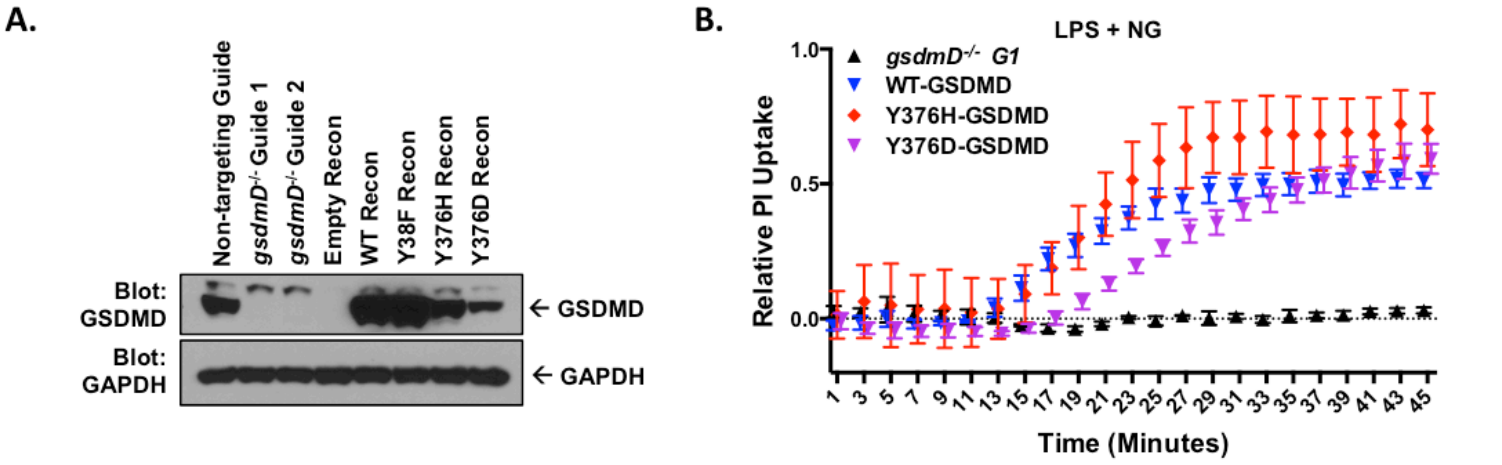
# Rathkey *et al.*, Suppl. Fig 1



Rathkey *et al.*, Suppl. Fig 2



Rathkey *et al.*, Suppl. Fig 3



**SUPPLEMENTAL INFORMATION**

**Supplementary Figure 1: Alignment of gasdermin D with gasdermin family members.** Sequence alignment between GSDMD and family members was conducted using Clustal Omega and the figure generated with ESPript 3.0. The GSDMD fragment demonstrated by Shi *et al.* Nature 2015 to cause death, the mNeon-Green insertion site, and the caspase-1/11 cleavage site are indicated.

**Supplementary Figure 2: mRuby-gasdermin D.** (A) Epifluorescent imaging of mRuby-GSDMD expressed in HEK-293T cells using calcium phosphate transfection. (B) Epifluroescent imaging of HEK-293T cells co-expressing mNeon-GSDMD and mRuby-GSDMD. Imaging is representative of three independent transfections.

**Supplementary Figure 3: Expression and pore formation of Y376H/D GSDMD in macrophages.** *Gsdmd*$^{-/-}$ guide 1 iBMDM cells were reconstituted with untagged WT, Y38F, Y376H, and Y376D GSDMD. (A) Western blot analysis of GSDMD levels in reconstituted cell lines. (B) Pore formation measured as PI uptake in WT-GSDMD and Y376H/D-GSDMD cell lines. Data represents the mean ± SE of three independent experiments and a total of six technical replicates.

**Supplementary Figure 4: Video of live cell imaging of mNeon-GSDMD.** Live cell confocal imaging of mNeon-GSDMD reconstituted iBMDM cells primed with 200ng/mL LPSand stimulated with 10μM nigericin. Video shown at 600x speed. Still images available in Figure 6. Live cell imaging is representative of three independent experiments.