

1 **SUPPLEMENTAL INFORMATION**

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3 **Structures of the gasdermin D C-terminal domains reveal mechanisms of autoinhibition**

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12 The supplemental data include three figures and one table.

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15 **Figure S1. Related to Figure 1. Structures of the hGSDMD, mGSDMD, mGSDMA3, and**
16 **hGSDMB.**

17 (A) Representative electron density maps for mGSDMD (gold) contoured at 1 sigma.

18 (B) Representative electron density maps for hGSDMD (cyan) contoured at 1 sigma.

19 (C) Superposition of structures for hGSDMD (cyan), mGSDMD (gold), mGSDMA3 (gray) and
20 hGSDMB (green). Helices are represented as cylinders. The β sheet and α 11 helix common for
21 GSDMDs and mGSDMA3 but absent in hGSDMB are marked with a red oval. Structural differences
22 near the β 1- β 2 loop are marked with a black square.

23 (D) Highlight of major structural differences near the β 1- β 2 loop between hGSDMB and other
24 gasdermin structures. The $C\alpha$ atom of residue F48 from mGSDMA3 is shown as a gray sphere.

25 (E & F) Highlight of major structural differences near the α 7' helix region between mGSDMA3 (gray in
26 E), GSDMDs (cyan and gold in F) and hGSDMB (green in F).

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28 **Figure S2. Related to Figure 2. The gasdermin N- and C-domain interface.**

29 (A-C) Electrostatic charge surface of the N- and C-domains for hGSDMD (A), mGSDMD (B) and
30 mGSDMA3 (C). The full-length hGSDMD and mGSDMD structural models were created using the full-
31 length mGSDMA3 as a template. The surface representations on top of the three panels are the same
32 as those in Figure 2. The open-book views of the N- and C-domains show charge-charge
33 complementarity with the N-domains largely positively charged (blue), and the C-domains negatively
34 charged (red). The electrostatic charge surface is displayed on a scale of $-5kT/e$ (red) to $5 kT/e$ (blue).
35 Crucial residues at the site I interface are marked.

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37 **Figure S3. Related to Figure 4. Propidium iodide uptake by macrophages expressing WT or**
38 **mutant mGSDMD.**

39 Propidium iodide uptake by macrophages reconstituted with WT mGSDMD or the Y376D mutant, in
40 the presence or absence of *Salmonella*. Significant increase in PI uptake was only observed in the
41 presence of *Salmonella* infection. The average \pm SD is shown for three independent experiments.
42 Asterisk denotes P value <0.05 calculated by one-way ANOVA.

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45 **Table S1. Related to Figure 1. X-ray Diffraction Data Collection and Refinement Statistics**

	mGSDMD-C (SeMet)	mGSDMD-C	hGSDMD-C
Data Collection			
Spacegroup	P1	P1	P2 ₁ 2 ₁ 2
Unit cell (a, b, c) (Å)	45.3, 56.8, 82.0	45.3, 56.8, 81.8	47.8, 108.5, 45.9
(a, b, γ) (°)	94.8, 102.5, 98.2	94.9, 102.8, 99.0	90, 90, 90
Wavelength (Å)	0.9793	0.9800	0.9800
Resolution (Å) (Last shell)	50-2.02 (2.07-2.02)	50-1.76 (1.81-1.76)	50-2.90 (2.98-2.90)
No of reflections (total/unique)	196629/50375	220325/75173	37766/5664
Completeness (%) (Last shell)	97.9 (96.0)	97.1 (95.8)	99.9 (100)
<I/σ(I)> (Last shell)	12.0 (1.8)	10.1 (2.1)	17.5 (1.8)
R _{meas} (%) ^a (Last shell)	8.7 (77.7)	8.8 (84.6)	10.2 (95.9)
CC ½ (%) ^b (Last shell)	99.8 (70.3)	99.5 (55.5)	99.8 (86.5)
SigAno ^c	0.88		
SAD Phasing			
Se sites (found/total)	8/8		
FOM ^d (Initial/DM/model building)	0.23/0.45/0.88		
Refinement			
Number of atoms (protein/solvent)		6021/587	1445/0
Rmsd bonds length (Å)/angles (°)		0.007/1.016	0.008/1.195
R _{work} / R _{free} (%) ^e		16.1/18.6	23.2/29.0
Ramachandran plot favored/disallowed ^f		99.0/0.0	91.5/0.0
PDB code		6AO3	6AO4

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48 ^a R_{meas} = $\sum_h \{N_h / [N_h - 1]\}^{1/2} \sum_i |I_i(h) - \langle I(h) \rangle| / \sum_h \sum_i I_i(h)$, where I_i(h) and <I(h)> are the ith and mean
49 measurement of the intensity of reflection h, and N_h is the multiplicity (Diederichs and Karplus, 1997).

50 ^b CC_{1/2} = $\sum (x - \langle x \rangle) (y - \langle y \rangle) / [\sum (x - \langle x \rangle)^2 \sum (y - \langle y \rangle)^2]^{1/2}$ where x and y are randomly split half datasets.

51 This is the Pearson's correlation coefficient of randomly split half datasets (Karplus and Diederichs,
52 2012).

53 ^c SigAno = $|F(+)-F(-)| / \sigma$ where $F(+)$ and $F(-)$ are structure factor estimates obtained from the merged
54 intensity observations in each parity class, and σ is the standard deviation.

55 ^d FOM = $\int P(\alpha) \exp(i\alpha) d\alpha / \int P(\alpha) d\alpha = \langle \cos(\Delta\alpha) \rangle$ where $P(\alpha)$ is the probability of the phase α being
56 the best phase and the $\Delta\alpha = \alpha_{\text{best}} - \alpha$ is the error in the phase angle at α . The figure of merit is the
57 expected value of the cosine of the phase error.

58 ^e $R_{\text{work}} = \sum_h ||F_{\text{obs}}(h)| - |F_{\text{calc}}(h)|| / \sum_h |F_{\text{obs}}(h)|$, where $F_{\text{obs}}(h)$ and $F_{\text{calc}}(h)$ are the observed and calculated
59 structure factors, respectively; R_{free} is the R value obtained for a test set of reflections consisting of a
60 randomly selected 5% subset of the data set excluded from refinement.

61 ^f Values from the Molprobit server (<http://molprobit.biochem.duke.edu/>).

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64 **Table S2. Related to STAR Methods and the Key Resources Table. Oligonucleotides used in**
 65 **this study.**

Oligonucleotides	SOURCE	IDENTIFIER
hGSDMD-FL Forward: CGGGATCCATGGGGTCCGGCCTTTGAGCGG	This paper	N/A
hGSDMD-FL Reverse: CGCGTGCACCTAGTGGGGCTCCTGGCTCAG	This paper	N/A
hGSDMD-C Forward: CGGGATCCGTCCTGCGGAGGGAGCGTTC	This paper	N/A
hGSDMD-N Forward: CAGAGAACAGATTGGTGGATCCATGGGGTCCGGCCT TTGAGCGGG	This paper	N/A
hGSDMD-N Reverse: GGTGCTCGAGTGCGGCCGCTCATGTGCGGGGTGG CTGGAAGGTC	This paper	N/A
mGSDMD-FL Forward: CGGGATCCATGCCATCGGCCTTTGAGAAAG	This paper	N/A
mGSDMD-FL Reverse: CCGCTCGAGCTAACAAGGTTTCTGGCCTAG	This paper	N/A
mGSDMD-C Forward: CGGGATCCGGGATTGATGAGGAGGAATTAAT	This paper	N/A
mGSDMD-N Forward: CAGAGAACAGATTGGTGGATCCATGCCATCGGCCT TTGAGAAAG	This paper	N/A
mGSDMD-N Reverse: GGTGCTCGAGTGCGGCCGCTCATGAGGAGGGCTC AAAGGCCTC	This paper	N/A
mGSDMD-FL L292D Forward *: CGACTATGCTGAGGTGAAGGCTTGCTC	This paper	N/A
mGSDMD-FL L292D Reverse: CCCTGGAAGTCTGCCGCCTCAATTAAT	This paper	N/A
mGSDMD-FL Y376D Forward *: GACCTGCTGGGAGCACTGGCTGTGCTGAGT	This paper	N/A
mGSDMD-FL Y376D Reverse: GAAGATAGGGGCTGCGAGTTCCGGCAC	This paper	N/A
mGSDMD-FL A380D Forward *: GGGAGATCTGGCTGTGCTGAGTGAAAC	This paper	N/A

mGSDMD-FL A380D Reverse: AGCAGGTAGAAGATAGGGGCTGCGAGT	This paper	N/A
mGSDMD-FL T387D Forward *: GACCAGCAGCAGCTGCTAGCTAAGGCTC	This paper	N/A
mGSDMD-FL T387D Reverse: TTCACCTCAGCACAGCCAGTGCTCCC	This paper	N/A
mGSDMD-FL L391D Forward: CTAGCTAAGGCTCTGGAGACAACG	This paper	N/A
mGSDMD-FL L391D Reverse *: GTCCTGCTGCTGGGTTTTCACTCAGCACAGC	This paper	N/A
mGSDMD-FL W442D Forward *: GATGTCTTGCTAGAAGAATGTGGCCTA	This paper	N/A
mGSDMD-FL W442D Reverse: GGTGGGATTCTTTTCATCCCAGCAG	This paper	N/A
mGSDMD-FL V443D Forward *: TGGGACTTGCTAGAAGAATGTGGCCTAAG	This paper	N/A
mGSDMD-FL V443D Reverse: GGTGGGATTCTTTTCATCCCAGCAG	This paper	N/A

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67 * Red-colored nucleotides mark the mutated codons.

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Figure S1

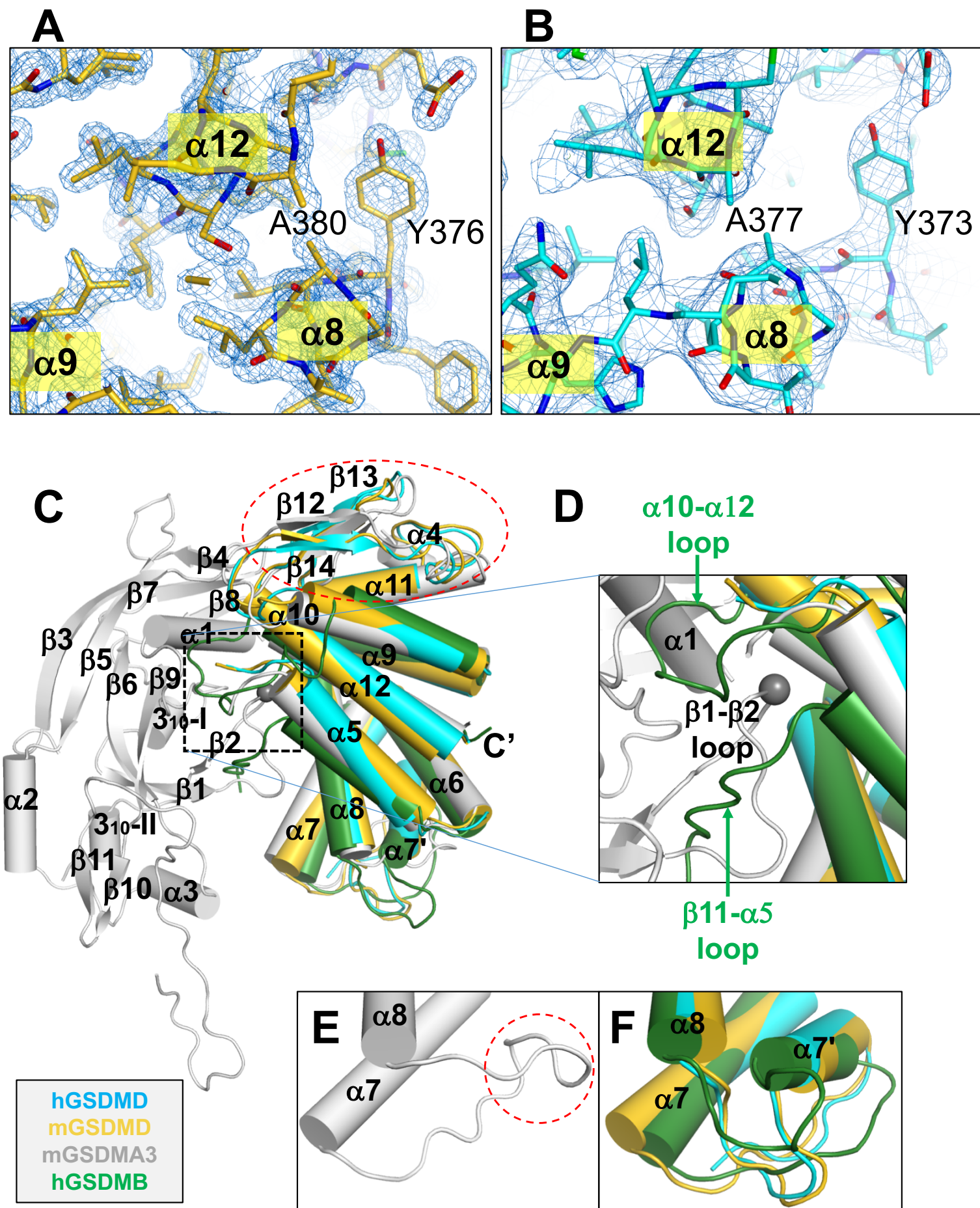
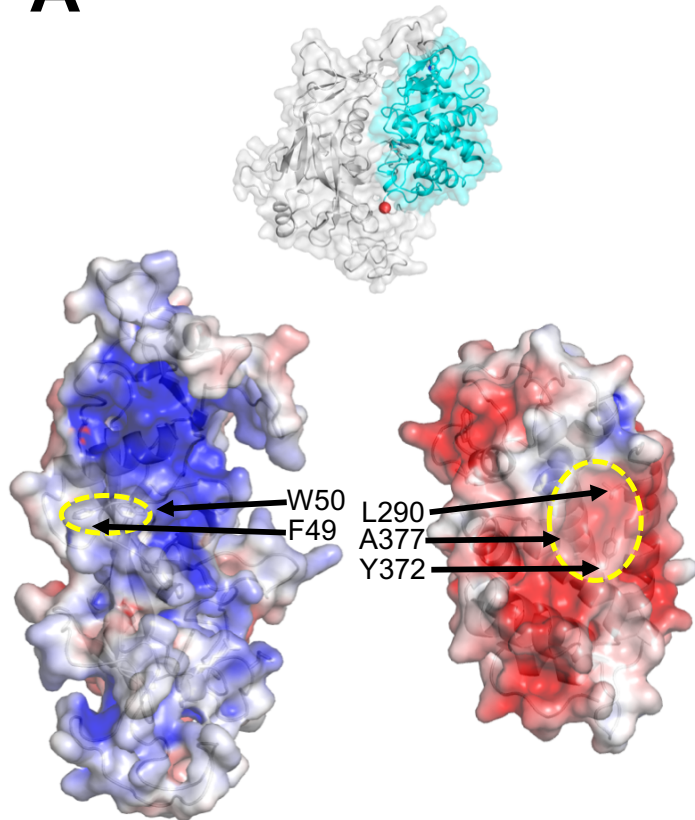
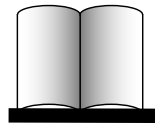
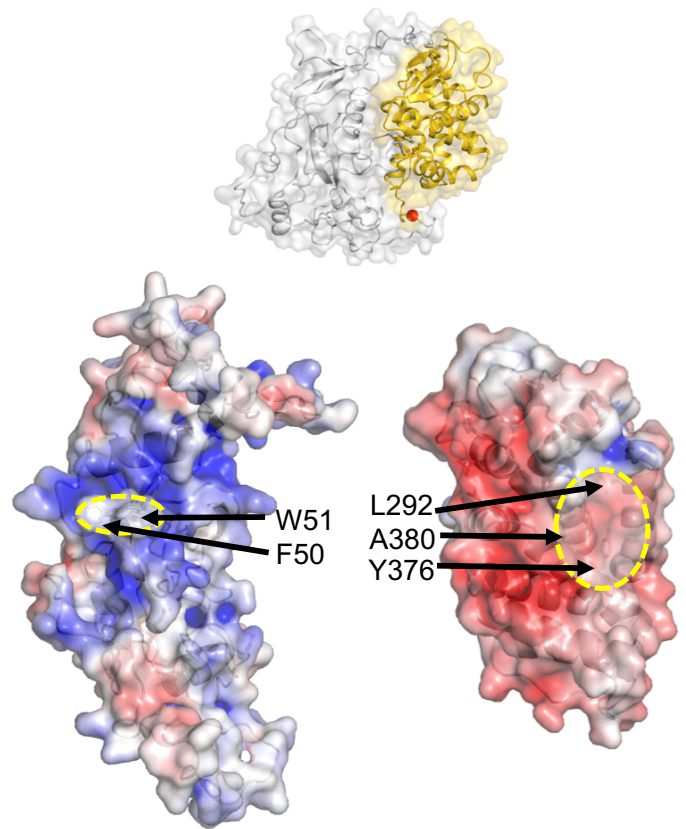


Figure S2

A hGSDMD FL model



B mGSDMD FL model



C mGSDMA3 (5B5R)

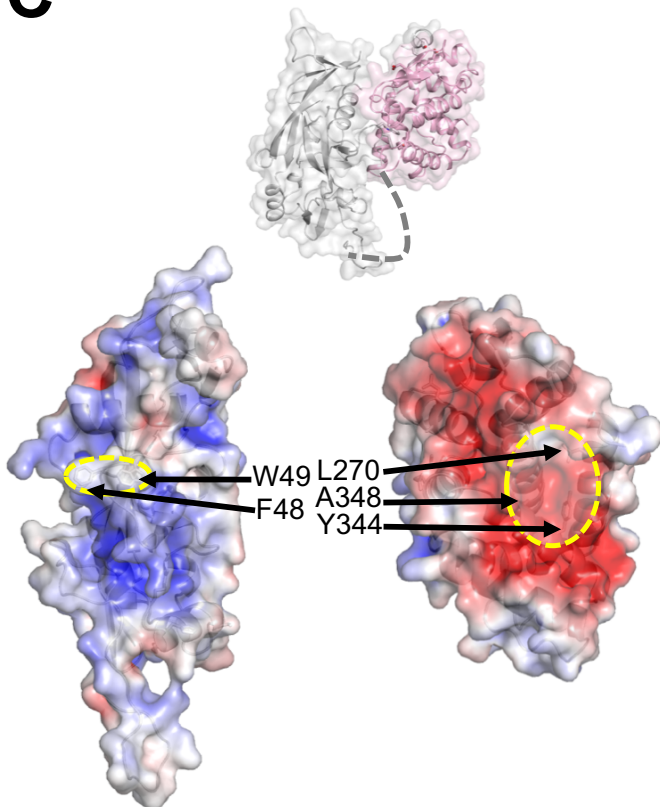


Figure S3

