

**Supplementary information**

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**Structural basis of NINJ1-mediated plasma membrane rupture in cell death**

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**Supplementary Table 1. Summary of MD simulations**

<b>Simulation system</b>	<b>Resolution</b>	<b>Simulation times</b>
<i>Stability of filaments &amp; rings</i>		
Double filament 2x10 hNINJ1 <sub>39-152</sub>	CG	50 $\mu$ s, 50 $\mu$ s
Double filament 2x10 hNINJ1 <sub>39-152</sub> , with DDM	CG	50 $\mu$ s, 50 $\mu$ s
Single filaments at membrane patch 2x10 hNINJ1 <sub>39-152</sub>	CG	20 $\mu$ s, 20 $\mu$ s
Single filaments at membrane patch 2x5 hNINJ1 <sub>39-152</sub>	AA	1 $\mu$ s, 1 $\mu$ s
Single filament in solution, restrained helices $\alpha$ 3 & $\alpha$ 4, AlphaFold2 shape of $\alpha$ 1+ $\alpha$ 2 hNINJ1 <sub>39-152</sub>	AA	1 $\mu$ s
45-er ring hNINJ1 <sub>39-152</sub>	CG	150 $\mu$ s, 150 $\mu$ s, 150 $\mu$ s, 150 $\mu$ s
45-er ring hNINJ1 <sub>76-152</sub> (helices $\alpha$ 3 & $\alpha$ 4 only)	CG	150 $\mu$ s, 150 $\mu$ s, 150 $\mu$ s
<i>Membrane binding of the N-terminus</i>		
Monomeric hNINJ1 <sub>20-152</sub> , asymmetric membrane	CG	10x40 $\mu$ s
Monomeric hNINJ1 <sub>20-152</sub> , symmetric membrane	CG	10x40 $\mu$ s

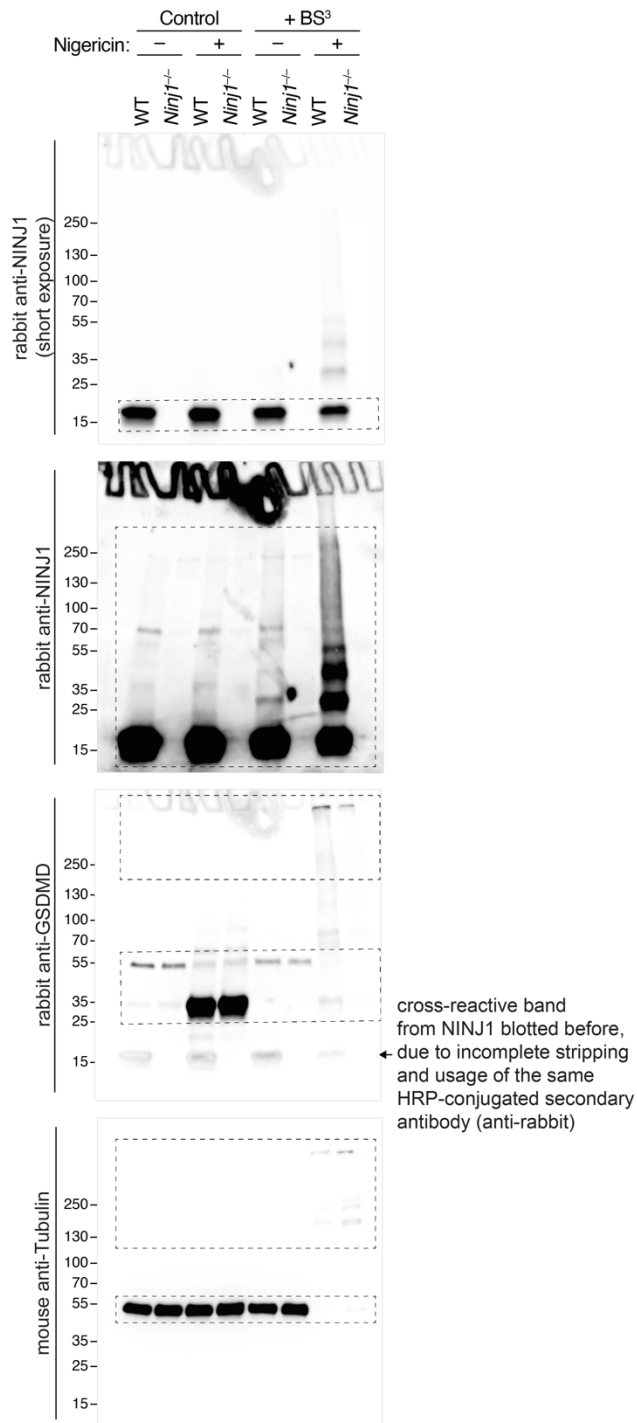
**Supplementary Table 2. Analysis of NINJ1 evolutionary couplings.<sup>a</sup>**

Pair number	Residue 1	Residue 2	Probability	Score	d <sub>C<math>\alpha</math>-C<math>\alpha</math></sub> inter [Å]	d <sub>C<math>\alpha</math>-C<math>\alpha</math></sub> intra [Å]
1	G95	F127	98.7	4.35	8.5	12.4
2	S46	V102	91.6	2.38	11.9	14.9
3	K44	G95	77.4	1.23	8.0	16.5
4	N107	H112	75.0	1.1	9.8	10.8
5	M51	F127	68.2	0.76	6.1	20.2
6	I54	G124	66.7	0.7	11.1	16.8
7	S43	V102	64.9	0.62	7.5	17.5
8	I92	I128	63.8	0.56	8.5	12.9
9	K103	N120	62.5	0.51	8.8	17.6

<sup>a</sup>All evolutionary couplings among the 100 with highest score that have a closer spatial proximity for intermolecular distance than the intramolecular distance. See Methods for details

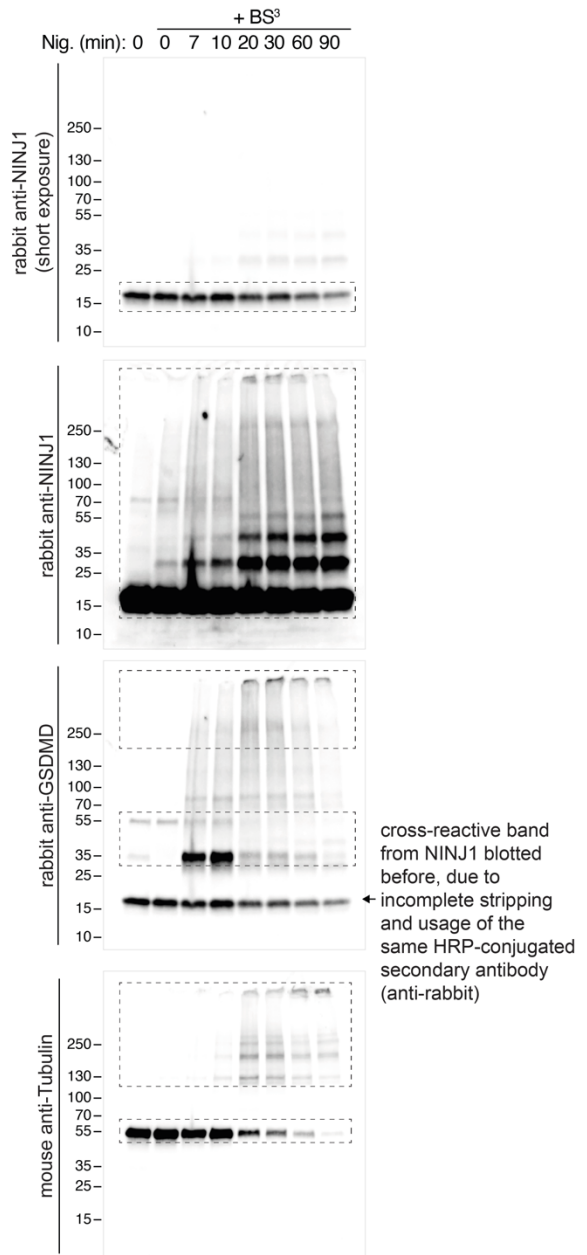
**Fig. 1a**

Order of antibody incubation (all in the same membrane):  
 1) anti-NINJ1  
 2) anti-GSDMD  
 3) anti-Tubulin



**Fig. 1b**

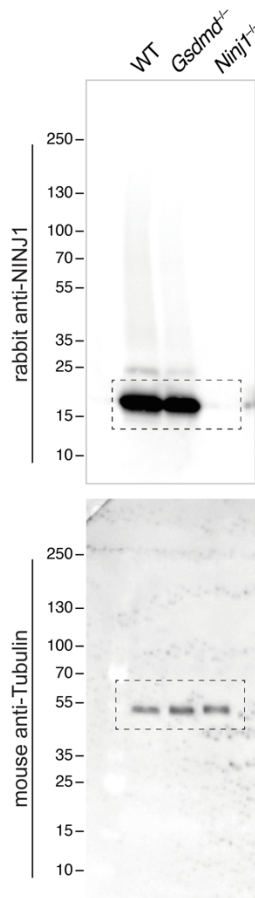
Order of antibody incubation (all in the same membrane):  
 1) anti-NINJ1  
 2) anti-GSDMD  
 3) anti-Tubulin



**Supplementary Figure 1. Uncropped gels of Figure 1.**

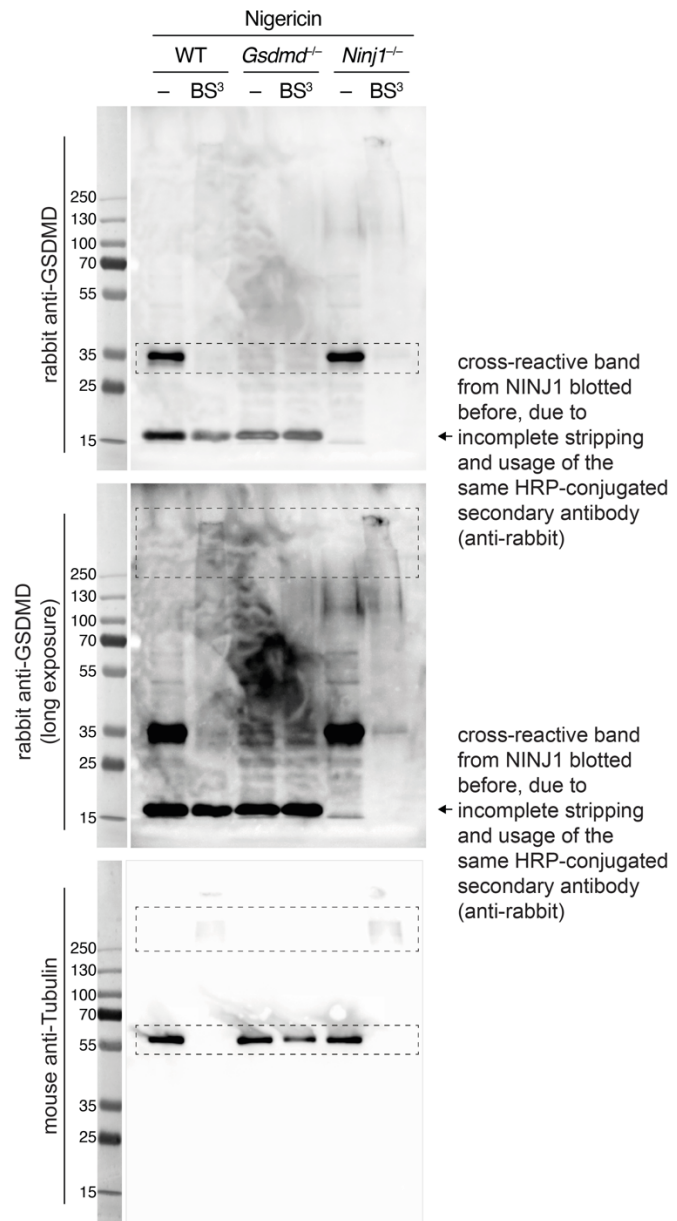
### ED 1c

Order of antibody incubation (all in the same membrane):  
 1) anti-NINJ1  
 2) anti-Tubulin



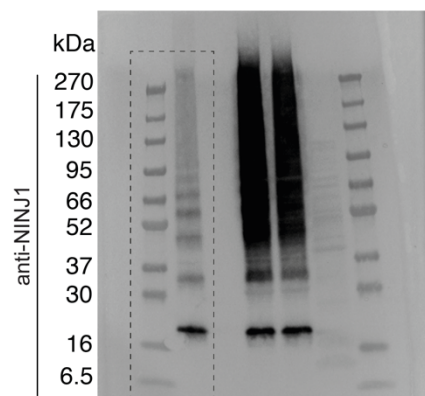
### ED 1e

Order of antibody incubation (all in the same membrane):  
 1) anti-NINJ1 (not shown. Done to validate knock-out cells)  
 2) anti-GSDMD  
 3) anti-Tubulin



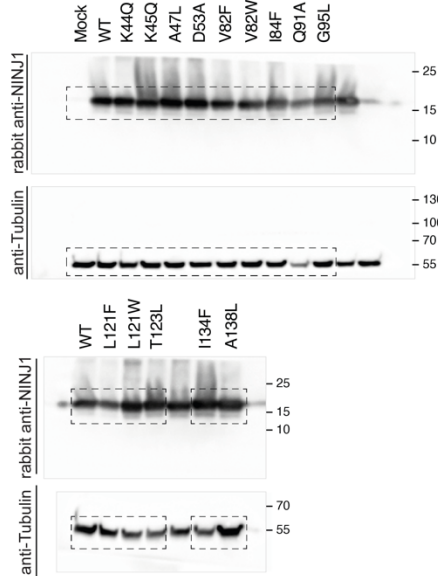
Supplementary Figure 2. Uncropped gels of Extended Data Figure 1.

## ED 5c

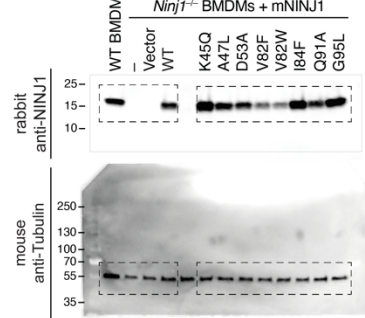


**Supplementary Figure 3.** Uncropped gel of Extended Data Figure 5.

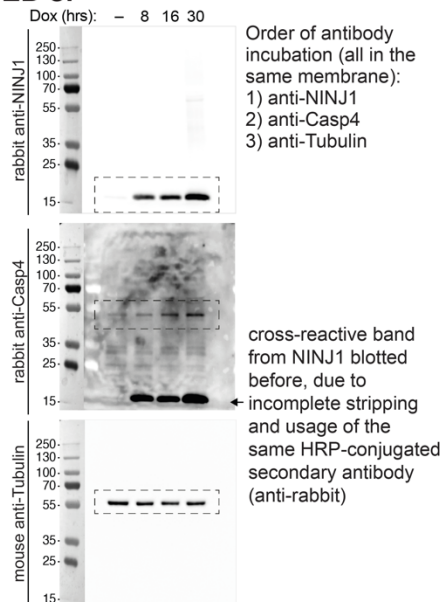
### ED 8b



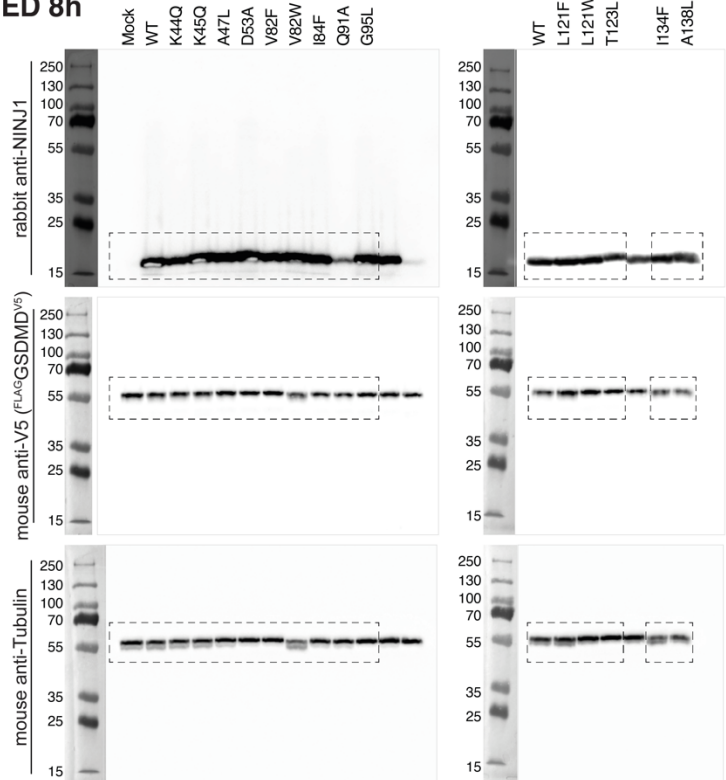
### ED 8e



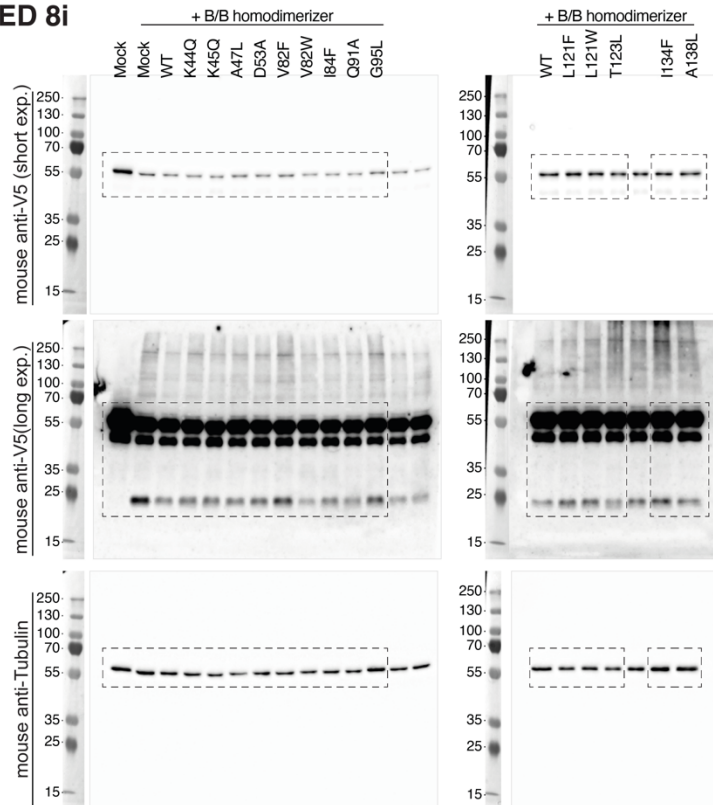
### ED 8f



### ED 8h



### ED 8i



Supplementary Figure 4. Uncropped gels of Extended Data Figure 8.