

NLRP3 inflammasome has a protective effect against oxazolone-induced colitis: a possible role
in ulcerative colitis

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Supplementary Figure 1. Effects of caspase-1 deficiency on oxazolone-induced colitis. A:

Changes in body weight during the experimental period. Round represents WT, wild-type. B, C:

Comparison of histological score between wild-type and caspase-1^{-/-} mice on days 1 and 3.

Histological scores were calculated according to the criteria (Supplementary Table 3). D, E:

Histological findings in wild-type (D) and caspase-1^{-/-} mice (E) with colitis. Compared to

wild-type mice, caspase-1^{-/-} mice exhibited severe colitis.

Supplementary Figure 2. The influence of cohousing on OXA-induced colitis in NLRP3^{-/-}

mice and caspase-1^{-/-} mice. The negative effects of NLRP3 deficiency on body weights (A) and

on the histological score of mice (B) were not attenuated by the cohousing of wild-type (WT)

mice with NLRP3^{-/-} mice. Histological findings in wild-type (C) and NLRP3^{-/-} mice (D) with

colitis. Compared to wild-type mice, NLRP3^{-/-} mice exhibited severe colitis. The negative

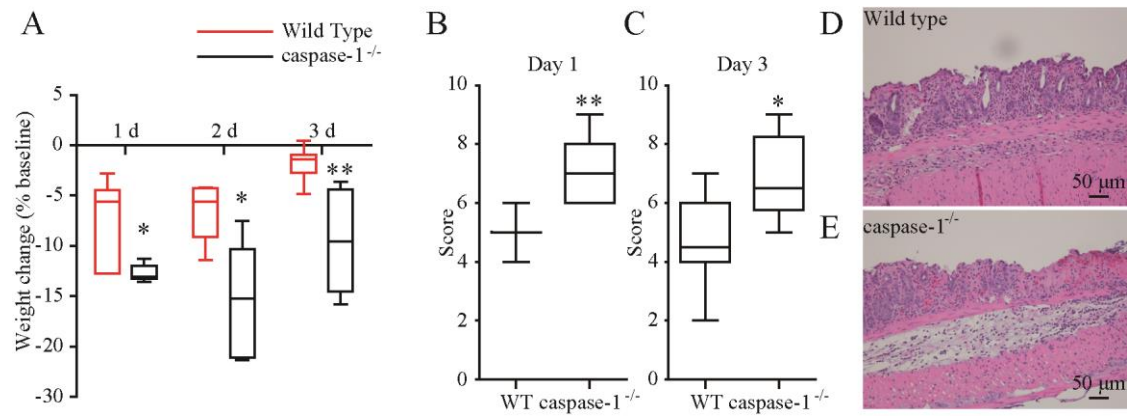
effects of caspase-1 deficiency on body weights (E) and on the histological score of mice (F)

were not attenuated by the cohousing of wild-type mice with caspase-1^{-/-} mice. Histological

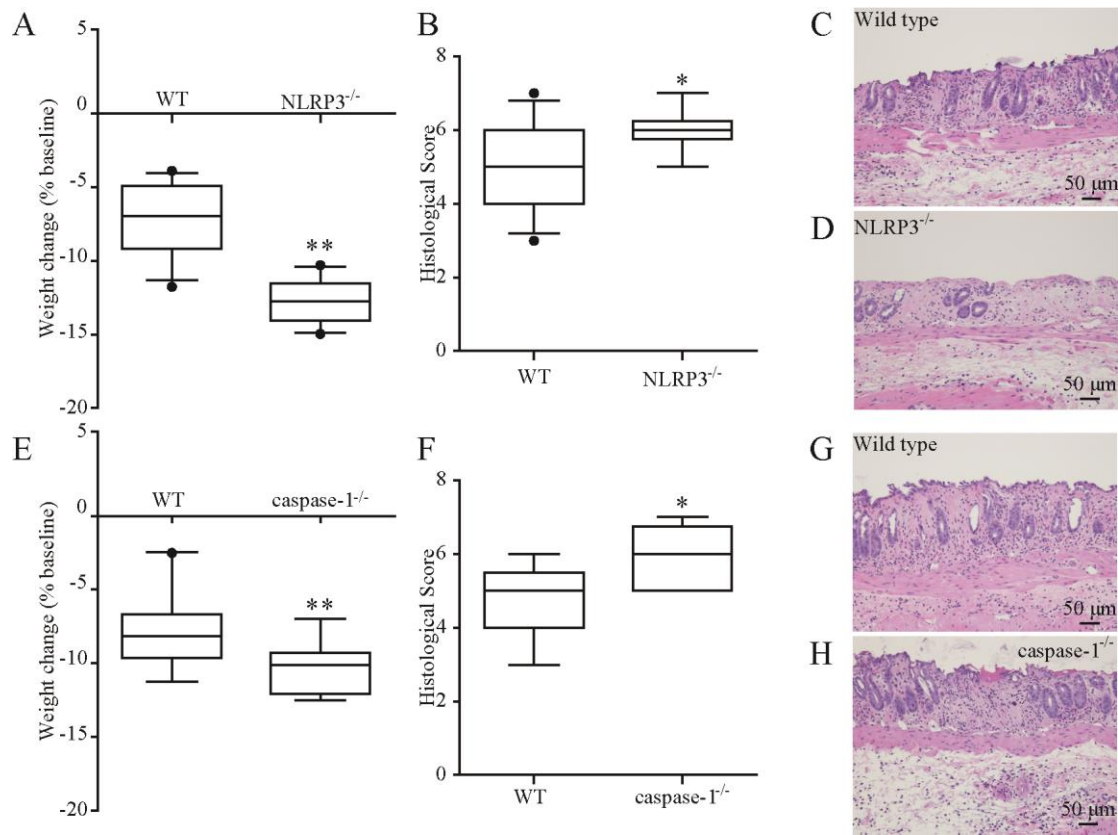
findings in wild-type (G) and caspase-1^{-/-} mice (H) with colitis. Compared to wild-type mice,

caspase-1^{-/-} mice exhibited severe colitis.

Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Table 1. Matts' histopathological grading system

Grade 1	normal appearance
Grade 2	some infiltration of the mucosa or lamina propria with either round cells or polymorphs
Grade 3	much cellular infiltration of the mucosa, lamina propria, and submucosa
Grade 4	presence of crypt abscesses, with much infiltration of all layers of the mucosa
Grade 5	ulceration, erosion, or necrosis of the mucosa, with cellular infiltration of some or all of its layers

Supplementary Table 2. Antibodies Used

	Source information	Dosage	Use
NLRP3	mouse monoclonal (AdipoGen)	1:200 dilution	IHC (human)
cleaved caspase-1	rabbit polyclonal (EnoGene)	1:200 dilution	IHC (human)
CD68	rabbit polyclonal (LifeSpan BioSciences)	1:200 dilution	IHC (human)
CD68	mouse monoclonal (Abcam)	1:100 dilution	IHC (human)
MUC2	rabbit polyclonal (GeneTex)	1:800 dilution	IHC (mouse)
NLRP3	mouse monoclonal (AdipoGen)	1:1000 dilution	WB (mouse)
caspase-1	rabbit polyclonal (Santa Cruz)	1:200 dilution	WB (mouse)
IL-1 β	goat polyclonal (R&D Systems)	1:500 dilution	WB (mouse)
IL-18	rabbit polyclonal (Abnova)	1:500 dilution	WB (mouse)
CDX2	mouse monoclonal (Santa Cruz)	1:100 dilution	WB (mouse)
β -actin	mouse monoclonal (Sigma-Aldrich)	1:5000 dilution	WB (mouse)

Supplementary Table 3. Summary of checked specific pathogens.

		Charles River Laboratories International, Inc.	Laboratory Animal Center, Graduate School of Medicine, Osaka City University
Bacteria			
	Pneumonia Virus of Mice	○	○
	Reovirus	○	
	Mouse Hepatitis Virus	○	○
	Murine Norovirus	○	
	Theiler's Murine Encephalomyelitis Virus	○	
	Minute Virus of Mice	○	
	Epizootic Diarrhea of Infant Mice Virus	○	
	Mouse Parvovirus (1 and 2)	○	
	Lymphocytic Choriomeningitis Virus	○	○
	Hantaan Virus	○	
	Mouse Adenovirus (1 and 2)	○	○
	Polyoma Virus	○	
	Mouse Pneumonitis Virus	○	
	Mouse Cytomegalovirus	○	
	Ectromelia virus	○	○
	Mouse Thymic Virus	○	
	Sendai Virus		○
Virus			
	Coryne bacterium kutscheri	○	○
	Pseudomonas aeruginosa	○	
	Salmonella spp.	○	○
	Streptococcus pneumoniae	○	
	Pasteurella pneumotropica	○	○
	Citrobacter rodentium	○	
	Helicobacter hepaticus	○	
	Helicobacter bilis	○	

	Helicobacter spp.	<input type="radio"/>	
	Streptobacillus moniliformis	<input type="radio"/>	
	Mycoplasma pulmonis	<input type="radio"/>	<input type="radio"/>
	Clostridium piliforme	<input type="radio"/>	<input type="radio"/>
	CAR Bacillus	<input type="radio"/>	
Parasite, Fungus			
	Ectoparasites	<input type="radio"/>	
	Gastrointestinal protozoa		
	-Spirochete	<input type="radio"/>	<input type="radio"/>
	-Giardia	<input type="radio"/>	<input type="radio"/>
	-Non-Pathogenic Protozoa	<input type="radio"/>	
	Pinworms(Aspicularis, Syphacia)	<input type="radio"/>	<input type="radio"/>
	Encephalitozoon cuniculi	<input type="radio"/>	

Supplementary Table 4. Histological injury score

Histological appearance	score
I Enterocyte loss	
Normal	0
Loss of single cell	1
Loss of groups of cells	2
Frank ulceration	3
II Crypt inflammation	
Normal	0
Single inflammatory cell	1
Cryptitis	2
Crypt abscess	3
III Lamina propria mononuclear cells	
Normal	0
Slight increase	1
Moderate increase	2
Marked increase	3
IV Neutrophils	
Normal	0
Slight increase	1
Moderate increase	2
Marked increase	3
V Epithelial hyperplasia	
Normal	0
Mild	1
Moderate	2
Pseudopolyp	3

Supplementary Table 5. Primers and Probes

Gene		Primer and Probe
MUC2	Probe	5'-FAM-TCTGCATTGACTGGCGGAACCACACC-TAMRA-3'
	Primer (forward)	5'-ACCCTCTGTGCCAAGGAAGG-3'
	Primer (reverse)	5'-TGGGCACTTCACAGAGCAGA-3'
CDX2	Probe	5'-FAM-TTCCGCATCCACTCGCAGGTTTCG- TAMRA-3'
	Primer (forward)	5'-CCGCCGAACAGCTGTCCC-3'
	Primer (reverse)	5'-TCCTGGTTTTCACTTGGCTTCC-3'
TNF- α	Probe	5'-FAM-AATGGGCTTTCCGAATTCACTGGAGC-TAMRA-3'
	Primer (forward)	5'-TCATGCACCACCATCAAGGA-3'
	Primer (reverse)	5'-GAGGCAACCTGACCACTCTCC-3'
IL-1 β	Probe	5'-FAM-ATGATCCCAATGAGTCGGCTGGAGA-TAMRA-3'
	Primer (forward)	5'-GCTGCTACTCATTCACTGGCAA-3'
	Primer (reverse)	5'-TGCTGCTGGTGATTCTCTTGTA-3'
IL-18	Probe	5'-FAM-TTCACAGAGAGGGTCACAGCCAGTCCT-TAMRA-3'
	Primer (forward)	5'-CCTGTGTTTCGATATGACTGA-3'
	Primer (reverse)	5'-ACAGGAGAGGGTAGAGACATTTTACTA-3'
NLRP3	Probe	5'-FAM-AGGCCGGAATTCACCAACCCAGCT-TAMRA-3'
	Primer (forward)	5'-TGCCTTGGGAGACTCAGGAG-3'
	Primer (reverse)	5'-CAGAGGTCAGAGCTGAACAACA-3'
caspase-1	Probe	5'-FAM-TGGCACATTTCCAGGACTGACTGGGACC-TAMRA-3'
	Primer (forward)	5'-GGACATCCTTCATTCATCCTCAGAAACA-3'
	Primer (reverse)	5'-TTTCTTTCCATAACTTCTGGGCTTT-3'
IL-4		Mm00445259_m1 (Thermo Fisher Scientific Inc.)
IL-13		Mm00434204_m1 (Thermo Fisher Scientific Inc.)
IFN- γ		Mm01168134_m1 (Thermo Fisher Scientific Inc.)