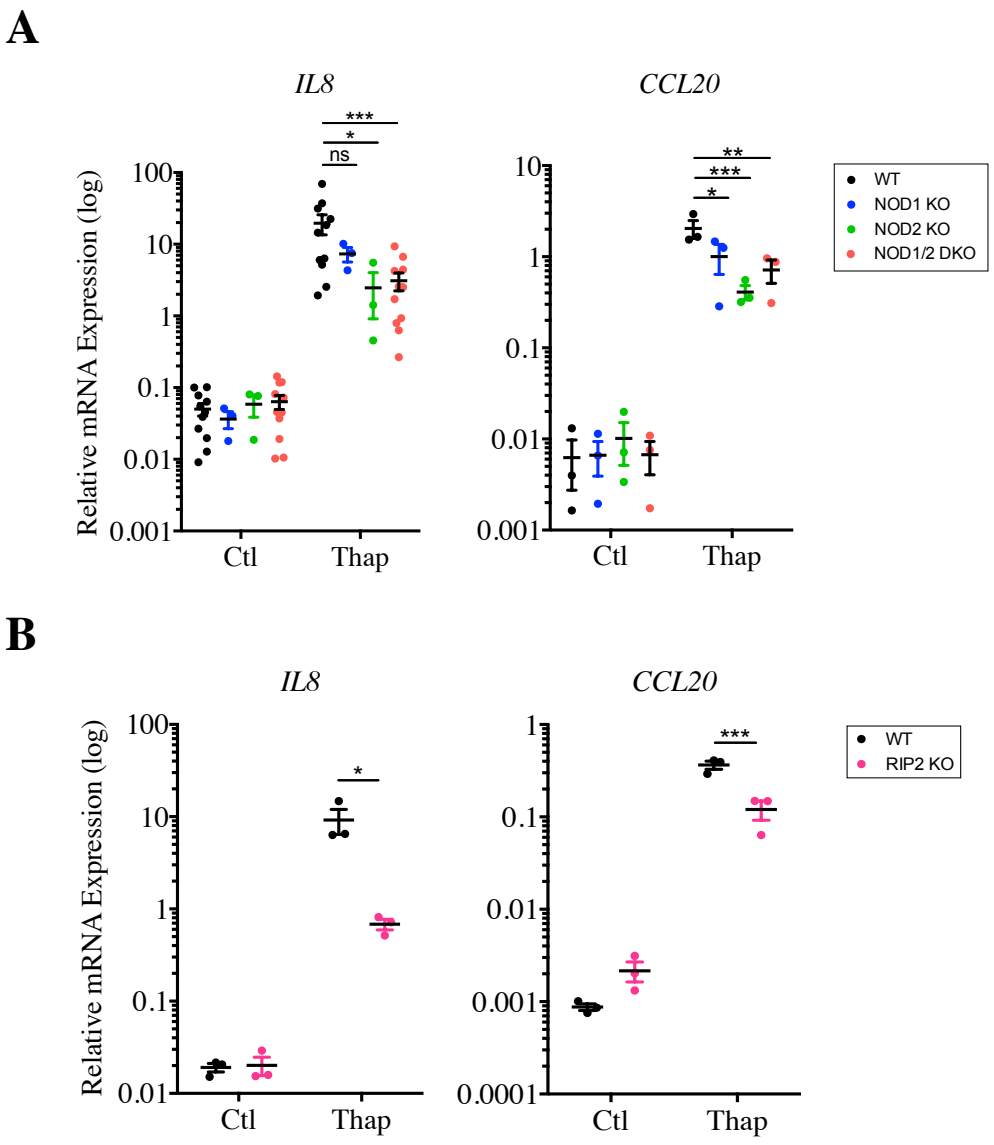

Figure S1. Thapsigargin induces pro-inflammatory cytokine expression in a NOD-dependent manner (**A-B**) Expression of *IL8* and *CCL20* in wild type (WT), *NOD1* knockout (KO), *NOD2* KO and *NOD1/2* double KO (DKO) HCT116 cells (**A**) or WT and *RIPK2* KO HCT116 cells (**B**) following stimulation with 0.1mg/ml thapsigargin for 4 hours. Gene expression analysis was carried out by qRT-PCR. In all data sets, each point is from an independent experiment and is the average of three technical replicates. *, ** and *** represents $p< 0.05$, $p< 0.01$ and $p< 0.001$, respectively. ns - not significant.

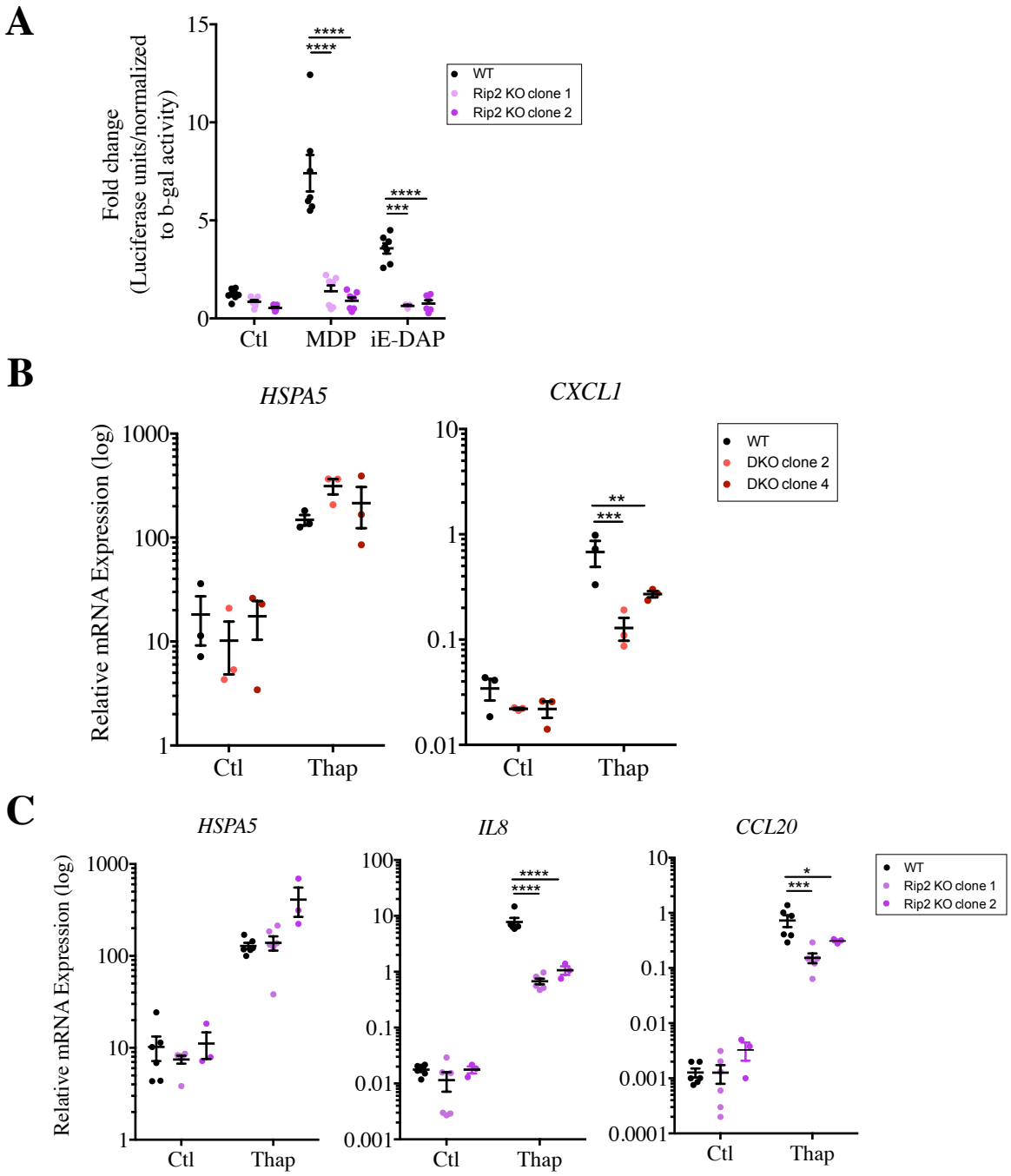

Figure S2. Analysis of distinct clones of CRISPR KO HCT116 cells

(**A**) Wild type (WT) or two distinct clones of *RIPK2* KO HCT116 cells were transfected overnight with Igk-Luci (NF-kB reporter construct) and b-galactosidase expressing construct in the presence or absence of 10mg/ml NOD ligands muramyl dipeptide (MDP) or iE-DAP. (**B**) Expression of *HSPA5* and *CXCL1* in wild type (WT) and two clones of *NOD1/2* double KO (DKO) HCT116 cells following stimulation with 0.1mg/ml thapsigargin for 4 hours measured by qRT-PCR. (**C**) Expression of *HSPA5*, *IL8* and *CCL20* in wild type (WT) and two clones of *RIPK2* KO HCT116 cells following stimulation with 0.1mg/ml thapsigargin for 4 hours. Gene expressions were measured by qRT-PCR. In all data sets, each point is from an independent experiment and is the average of three technical replicates. *, *** and **** represents $p< 0.05$, $p< 0.001$ and $p< 0.0001$, respectively. ns - not significant.

Figure S2

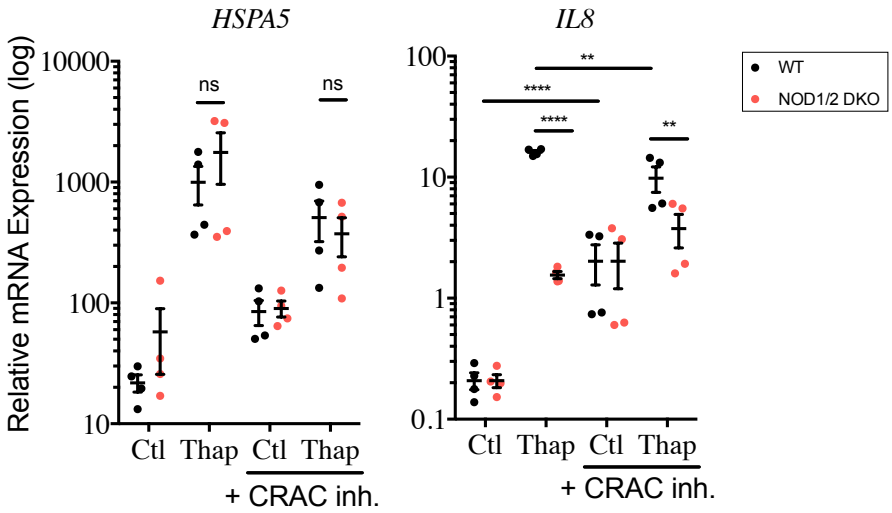

Figure S3. Internalization of extracellular Ca^{2+} by CRAC channels contributes to NOD-dependent pro-inflammatory cytokine expression.

Expression of *HSPA5* and *IL8* in wild type (WT) and *NOD1/2* double KO (DKO) HCT116 cells upon treatment with Thapsigargin (0.1mg/ml) in presence or absence of CRAC inhibitor, GSK7975A (10mM). Gene expression analysis was carried out by qRT-PCR. In all data sets, each point is from an independent experiment and is the average of two technical replicates. ** and **** represents $p< 0.01$ and $p< 0.0001$, respectively. ns - not significant.

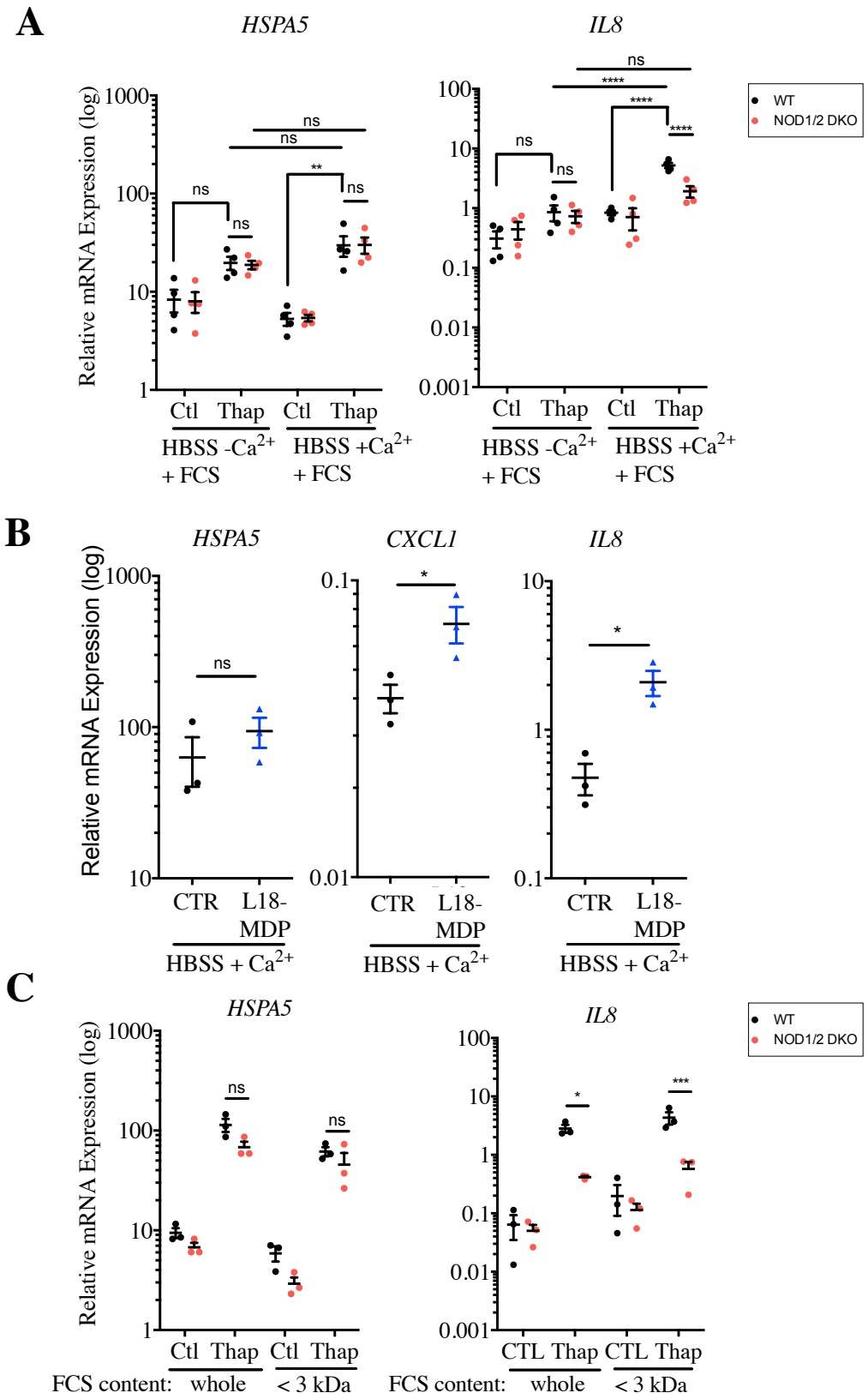
A
HSPA5
IL8
Relative mRNA Expression (log)
WT
NOD1/2 DKO
ns
**
****
Ctl
Thap
HBSS -Ca2+ + FCS
HBSS +Ca2+ + FCS
B
HSPA5
CXCL1
IL8
Relative mRNA Expression (log)
CTR
L18-MDP
HBSS + Ca2+
C
HSPA5
IL8
Relative mRNA Expression (log)
WT
NOD1/2 DKO
CTL
FCS content:
whole
< 3 kDa

Figure S4

Figure S4. Heat-resistant small molecules from cell culture serum trigger NOD-dependent activation of pro-inflammatory signalling induced by thapsigargin

(**A**) Expression of *HSPA5* and *IL8* in wild type (WT) and *NOD1/2* double KO (DKO) HCT116 cells incubated overnight in Hanks' Balanced Salt Solution (HBSS) medium without Ca^{2+} in the presence or absence of 10% fecal calf serum (FCS) following stimulation with 0.1mg/ml thapsigargin for 4 hours. (**B**) Expression of *HSPA5* and *IL8* in wild type (WT) HCT116 cells cultured overnight in Hanks' Balanced Salt Solution (HBSS) medium supplemented with 140mg/ml $CaCl_2$ in absence of 10% fecal calf serum (FCS), following stimulation with L-18 MDP (100ng/ml) for 4 hours (**C**) Expression of *HSPA5* and *IL8* in wild type (WT) and NOD1/2 double KO (DKO) HCT116 cells incubated in DMEM supplemented with either normal 10% FCS (whole) or serum boiled for 10 minutes and filtered with a 3kDa filter, following stimulation with 0.1mg/ml thapsigargin for 4 hours. Gene expressions were measured by qRT-PCR. In all data sets, each point is from an independent experiment and is the average of three technical replicates. *, **, *** and ****, $p< 0.05$, $p< 0.01$, $p< 0.001$ and $p< 0.0001$, respectively. ns - not significant.

Figure S4 Continued

COMPOUND	NEUTRAL FORMULA	M+H	RT	SAMPLE #A	SAMPLE #B
L-ALA-Γ-D-GLN-MDAP	C15H27N5O7	390.1983	9.57	1.00E+06	1.76E+05
L-ALA-Γ-D-GLU-MDAP	C15H26N4O8	391.1823	14.56		4.27E+04
			10.68	1.84E+06	1.39E+05
L-ALA-D-GLN-LYS-D-ALA	C17H32N6O6	417.2456	8.53	7.51E+06	
L-ALA-D-GLU-LYS-D-ALA	C17H31N5O7	418.2296			
L-ALA-D-GLU-MESODAP-D-ALA	C18H31N5O9	462.2194	10.81		2.05E+05
ANHYDROMURNAC-L-ALA-D-GLN	C19H30N4O10	475.2034	10.16	1.10E+05	
ANHYDROMURNAC-L-ALA-D-GLU	C19H29N3O11	476.1874	10.9	4.92E+05	2.05E+05
L-ALA-D-GLN-LYS-D-ALA-D-ALA	C20H37N7O7	488.2827			
L-ALA-D-GLU-LYS-D-ALA-D-ALA	C20H36N6O8	489.2667	9.26		1.01E+06
MURNAC-L-ALA-D-GLN	C19H32N4O11	493.2140			
MURNAC-L-ALA-D-GLU	C19H31N3O12	494.1980			
L-ALA-D-GLN-MESODAP-D-ALA-D-ALA	C21H37N7O9	532.2725	2.33	1.46E+07	5.20E+07
L-ALA-D-GLU-MESODAP-D-ALA-D-ALA	C21H36N6O10	533.2565			
			9.02	5.10E+05	
ANHYDROMURNAC-L-ALA-D-GLU-LYS	C25H41N5O12	604.2824			6.95E+04
MURNAC-L-ALA-D-GLU-LYS	C25H43N5O13	622.2930			1.62E+05
MURNAC-L-ALA-D-GLN-MDAP	C26H44N6O14	665.2988			
GLCNAC-ANHYDROMURNAC-L-ALA-D-GLU	C27H42N4O16	679.2669			
GLCNAC-MURNAC-L-ALA-D-GLN	C27H45N5O16	696.2934	8.03	1.02E+06	
GLCNAC-MURNAC-L-ALA-D-GLU	C27H44N4O17	697.2774			
GLCNAC-ANHYDROMURNAC-L-ALA-D-GLN-MDAP	C34H55N7O18	850.3676			
GLCNAC-MURNAC-L-ALA-D-GLN-MDAP	C34H57N7O19	868.3782	2.32	5.52E+06	4.87E+07
GLCNAC-ANHYDROMURNAC-L-ALA-D-GLU-LYS-L-ALA	C36H59N7O18	878.3989	1.41	7.25E+05	
GLCNAC-MURNAC-L-ALA-D-GLN-LYS-L-ALA	C36H62N8O18	895.4255			
GLCNAC-ANHYDROMURNAC-L-ALA-D-GLN-MDAP-L-ALA	C37H60N8O19	921.4047			
GLCNAC-ANHYDROMURNAC-L-ALA-D-GLU-MDAP-L-ALA	C37H59N7O20	922.3888			6.63E+05
GLCNAC-MURNAC-L-ALA-D-GLN-LYS-L-ALA-L-ALA	C39H67N9O19	966.4626			
GLCNAC-MURNAC-L-ALA-D-GLU-LYS-L-ALA-L-ALA	C39H66N8O20	967.4466			2.42E+06
GlcNac-anhydroMurNac-L-Ala-D-Gln-mDAP-L-Ala-L-Ala	C40H65N9O20	992.4419			
GlcNac-anhydroMurNac-L-Ala-D-Glu-mDAP-L-Ala-L-Ala	C40H64N8O21	993.4259			
GlcNac-MurNac-L-Ala-D-Gln-mDAP-L-Ala-L-Ala	C40H67N9O21	1010.4524			
GlcNac-MurNac-L-Ala-D-Glu-mDAP-L-Ala-L-Ala	C40H66N8O22	1011.4364			

Figure S5. Analysis of peptidoglycan fragments in two fetal calf serum samples by LC-MS
List of the peptidoglycan fragments analyzed by LC-MS from two independent fetal calf serum samples. RT, retention time.

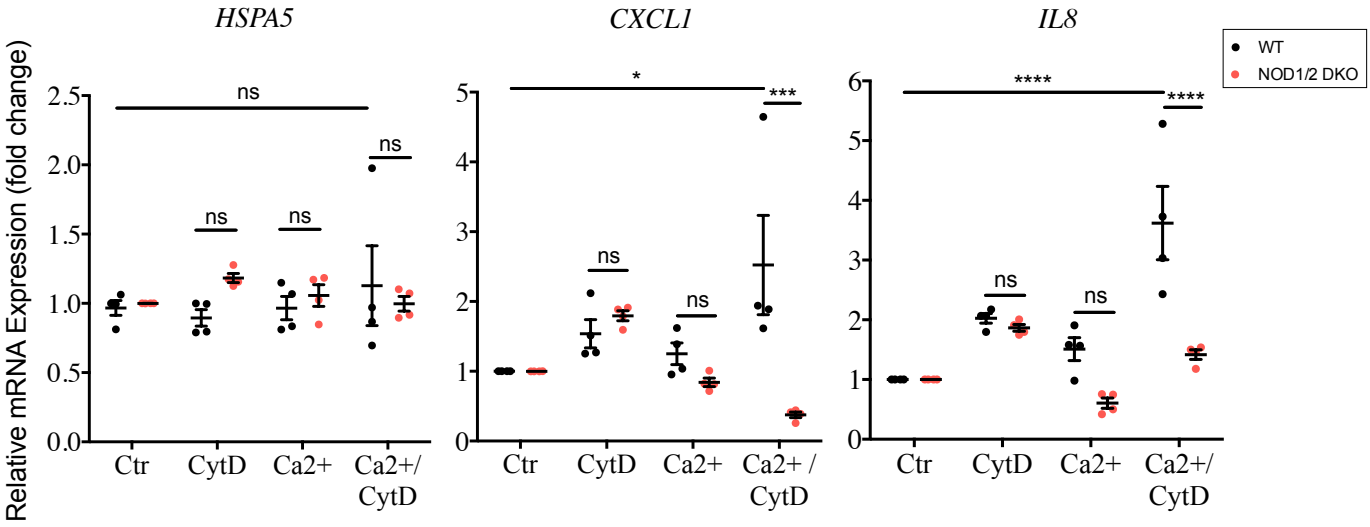

Figure S6. Cytochalasin D triggers NOD-dependent expression of pro-inflammatory genes
Expression of *HSPA5, CXCL1* and *IL8* in wild type (WT) and *NOD1/2* double KO (DKO) HCT116 cells incubated overnight in Hanks' Balanced Salt Solution (HBSS) medium supplemented with 10% fecal calf serum (FCS), in the absence or presence Ca^{2+}, following stimulation with or without 5mM cytochalasin D (CytD) for 4 hours. Gene expressions were measured by qRT-PCR. In all data sets, each point is from an independent experiment and is the average of two technical replicates. * and ****, $p< 0.05$ and $p< 0.0001$, respectively. ns - not significant.

Figure S6