Expanded View Figures

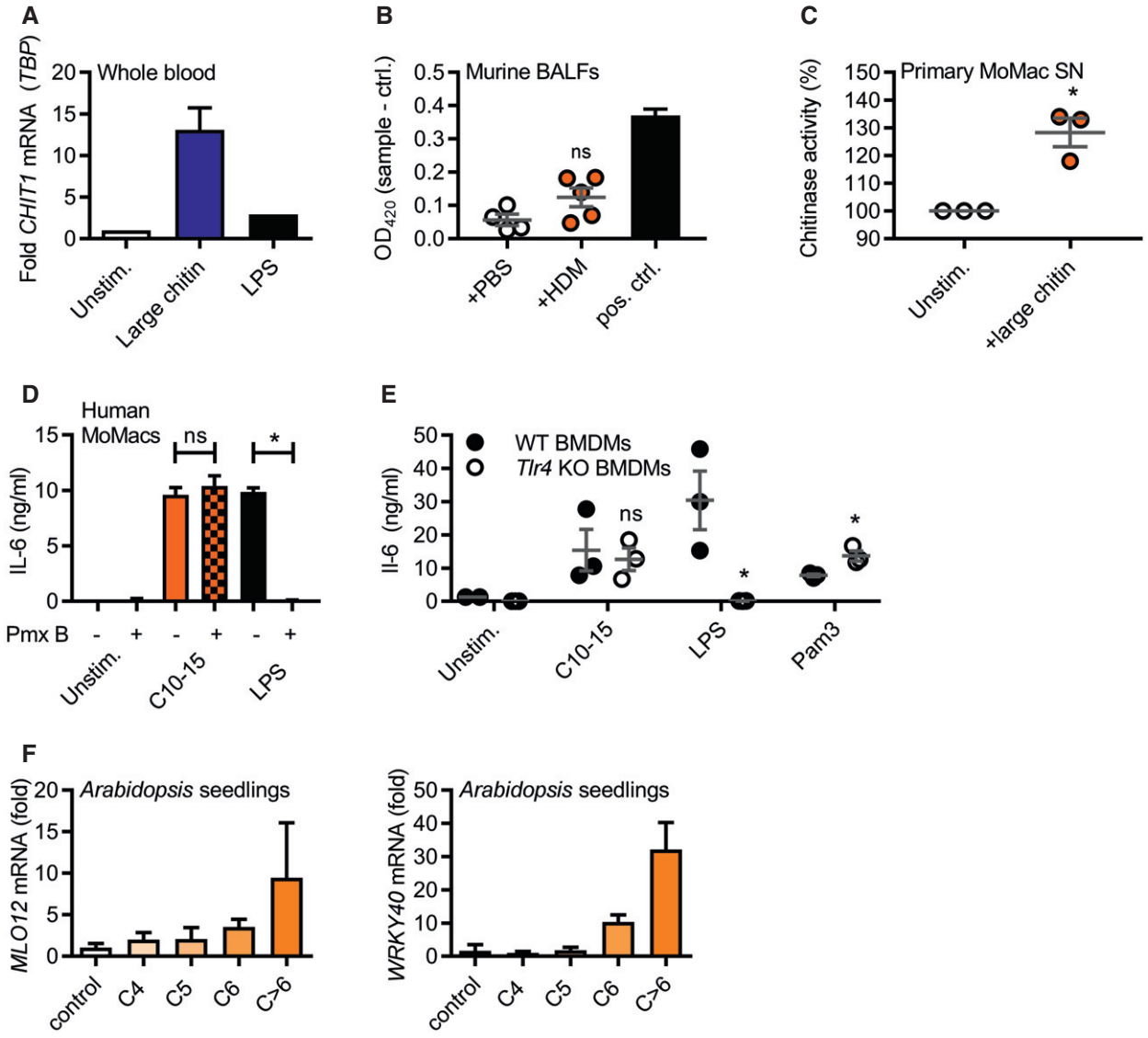

Figure EV1. Chitin treatment results in chitinase and target gene induction in mammalian and plant cells, respectively.

A mRNA induction of *CHIT1* relative to *TBP* and unstimulated conditions in human whole blood treated with large chitin (n = 2).

B Chitin azure degradation indicative of chitinase activity in murine BALF from mice previously treated with PBS or house-dust mite allergen (HDM) *in vivo* (n = 5). *S. griseus* recombinant chitinase was used as a positive control.

C Primary human MoMacs were stimulated with C10-15 or large chitin for 24 h and supernatants harvested and incubated with chitin azure (n = 3).

D, E Cytokine release induced by chitin is independent of endotoxin. (D) IL-6 release from stimulated human MoMacs stimulated in the absence or presence of polymyxin B (Pmx B, n = 3), and (E) IL-6 release from WT or *Tlr4* KO BMDMs (n = 3) are shown for one representative batch of C10-15.

F Target gene induction in *A. thaliana* seedlings. *MLO12* and *WRKY40* mRNA induction (n = 3).

Data information: (B, C, and E) represent data (mean ± SEM) combined from n biological replicates (mice or donors, each donor represented by one dot). In (A, D, and F), one representative of "n" (given in brackets for each panel) independent experiments is shown (mean ± SD). *$P < 0.05$ according to one-sample t-test (B), Kruskal–Wallis test with Dunn's correction (C), and Student's t-test (D, E).

Figure EV2. Chitin oligomers initiate cellular activation in a TLR2-dependent manner.

A *IL6* or *TNF* mRNA induction relative to *TBP* in human MoMacs (n = 3).
B Relative CD62L shedding (n = 8, histograms shown for one representative donor) and IL-8 release (n = 7–13) from human PMNs upon 1- or 4-h stimulation, respectively.
C *TLR2* mRNA knock-down efficiency upon Ctrl, *MYD88* or *TLR2* siRNA transfection in human MoMacs (see Fig. 2c), relative to Ctrl siRNA.
D TLR2 immunoblot and flow cytometry in *TLR2* CRISPR-edited THP-1 cells (n = 1 each). Arrowheads denote endogenous TLR2 (glycosylated).
E NF-κB activity in empty vector (EV) or NOD2, Dectin-1, or TLR9-transfected HEK293T cells (n = 2–3).
F TNF release (relative to unstimulated) from WT or *Clec7a* KO (Dectin-1 deficient) immortalized macrophages (iMacs, n = 2).

Data information: (B and C) represent data (mean ± SEM) combined from 4 to 13 donors (each dot represents one donor). In (A, C, E, and F), one representative of "n" (given in brackets for each panel) independent experiments is shown (mean ± SD). *$P < 0.05$ according to Wilcoxon signed rank sum (B), one-way ANOVA with/without Dunnett's correction (A, C), or Student's t-test (E, F).

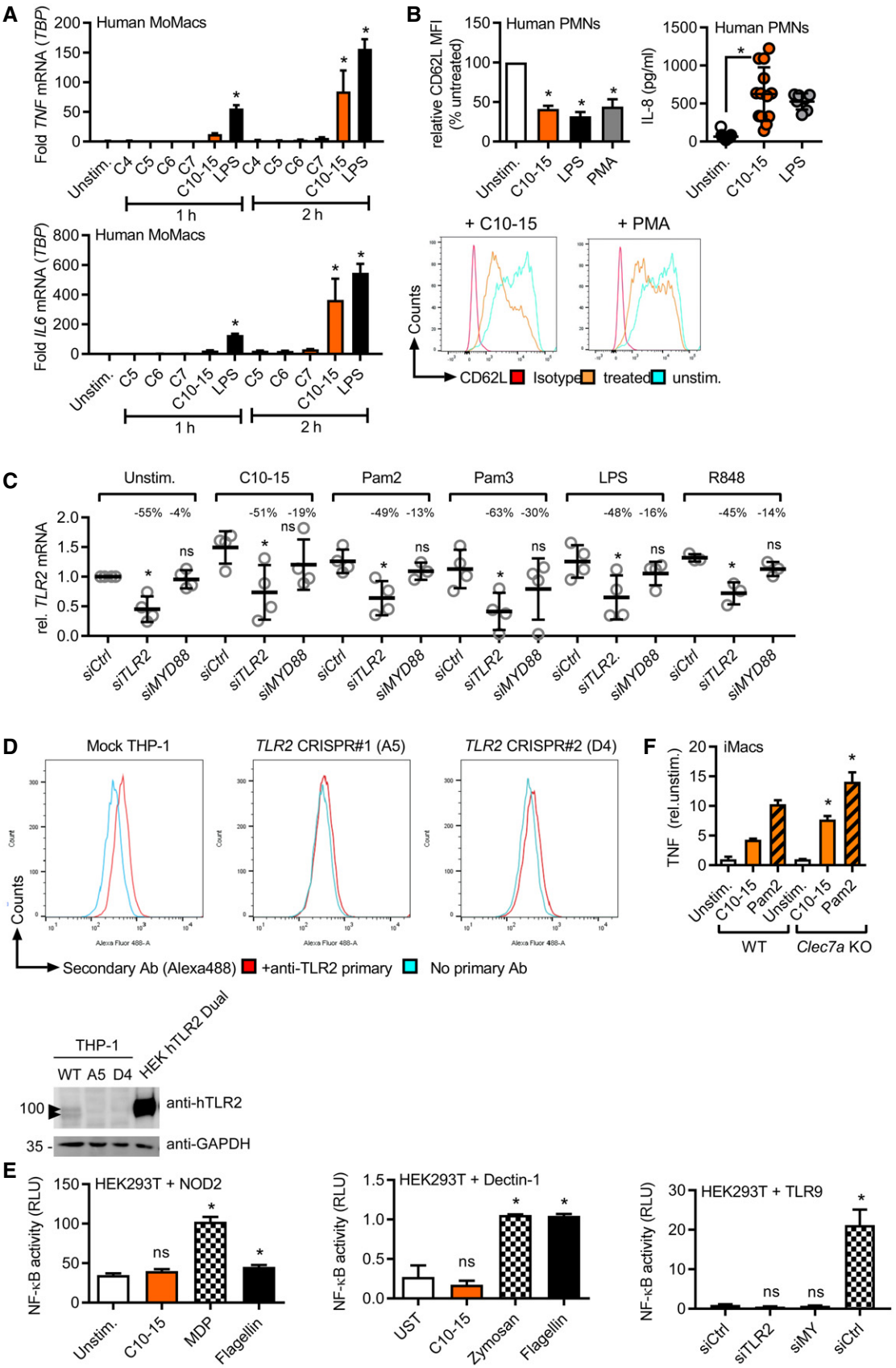
A
Human MoMacs
Fold TNF mRNA (TBP)
Unstim. C4 C5 C6 C7 C10-15 LPS C4 C5 C6 C7 C10-15 LPS
1 h
2 h
Human MoMacs
Fold IL6 mRNA (TBP)
B
Human PMNs
relative CD62L MFI (% untreated)
Unstim. C10-15 LPS PMA
Human PMNs
IL-8 (pg/ml)
Unstim. C10-15 LPS
+ C10-15
+ PMA
Counts
CD62L Isotype treated unstim.
C
Unstim. C10-15 Pam2 Pam3 LPS R848
-55% -4% -51% -19% -49% -13% -63% -30% -48% -16% -45% -14%
rel. TLR2 mRNA
siCtrl siTLR2 siMYD88
D
Mock THP-1
TLR2 CRISPR#1 (A5)
TLR2 CRISPR#2 (D4)
Counts
Alexa Fluor 488-A
Secondary Ab (Alexa488) +anti-TLR2 primary No primary Ab
THP-1
WT A5 D4
HEK hTLR2 Dual
100
anti-hTLR2
35
anti-GAPDH
F
iMacs
TNF (rel.unstim.)
Unstim. C10-15 Pam2 Unstim. C10-15 Pam2
WT Clec7a KO
E
HEK293T + NOD2
NF-κB activity (RLU)
Unstim. C10-15 MDP Flagellin
HEK293T + Dectin-1
UST C10-15 Zymosan Flagellin
HEK293T + TLR9
siCtrl siTLR2 siMY siCtrl

Figure EV2.

Figure EV3. Zymosan contains chitin as a TLR2-stimulatory component and chitin oligomers induce distinct gene expression profiles.

A NF-κB activity in TLR2-HEK293T cells using zymosan with or without o/n incubation with *S. griseus* chitinase or chitinase alone. Note: *S. griseus* chitinase contained TLR2 activity in the absence of an additional stimulant. Incubation of zymosan with this chitinase led to a reduction in this residual level of TLR2 activity.

B MALDI-TOF-MS analysis of chitinase-treated zymosan supernatants ($n = 2$).

C Whole-blood microarray intensities (excerpt) of the 10 genes contributing most strongly to PC1 and PC2, respectively (see Expanded View methods and Code EV1).

D Relative cytokine mRNAs in whole-blood stimulation ($n = 5$–13).

Data information: In (A and B), one representative of two independent experiments is shown (mean ± SD). (C and D) represent data (mean ± SEM) combined from 3 to 13 donors (each box/dot represents one donor). *$P < 0.05$ according to one-way ANOVA with Sidak's correction (A).

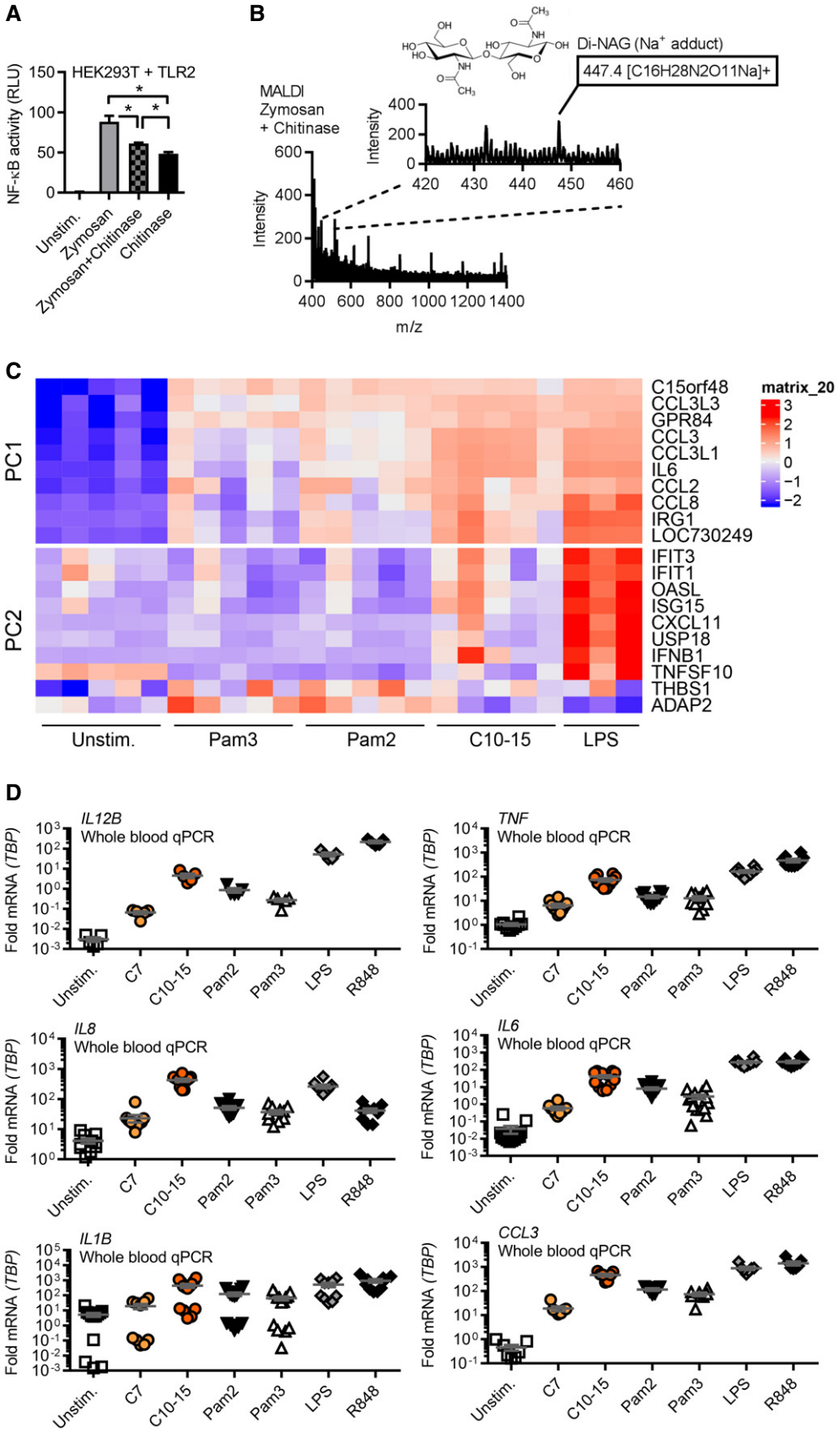
A
HEK293T + TLR2
NF-κB activity (RLU)
Unstim.
Zymosan
Zymosan+Chitinase
Chitinase
B
Di-NAG (Na+ adduct)
447.4 [C16H28N2O11Na]+
MALDI
Zymosan
+ Chitinase
Intensity
m/z
C
PC1
PC2
C15orf48
CCL3L3
GPR84
CCL3
CCL3L1
IL6
CCL2
CCL8
IRG1
LOC730249
IFIT3
IFIT1
OASL
ISG15
CXCL11
USP18
IFNB1
TNFSF10
THBS1
ADAP2
matrix_20
Unstim.
Pam3
Pam2
C10-15
LPS
D
IL12B
TNF
IL8
IL6
IL1B
CCL3
Whole blood qPCR
Fold mRNA (TBP)
Unstim.
C7
C10-15
Pam2
Pam3
LPS
R848

Figure EV3.

Figure EV4. TLR2 recognition depends on residues in the hydrophobic channel of the TLR2 ectodomain.

A Microscale thermophoresis analysis of Alexa647-labeled C10-15 and hTLR2 protein (n = 3).

B–D Modeling (B), functional NF-κB activity (C), and expression levels (D) in empty vector (EV) or TLR2-transfected HEK293T cells (n = 2) for TLR2 WT or point mutants designed to narrow the hydrophobic pocket. In (B), a blue dotted line delineates the hydrophobic pocket, here shown in cross-section. Black mesh signifies original surface profile in pdb 2z7x, and red mesh the predicted surface of the respective point mutant. In (C), dotted lines signify the levels of NF-κB activity observed for WT TLR2 for the respective ligand. (D) Expression of TLR2 mutants verified by anti-Flag immunoblot (n = 1).

E Docking grid used for placing of a chitin 5-mer.

F Close-up on the gatekeeper F349 which restricts access to the cavity in "closed position" (red) but allows access in "open" rotamer position (orange).

G Docking poses of a 5-mer (C5) in the grid specified in e and with F349 in "open" rotamer position.

H Potential interactions of chitin 6-mer (C6) hydrophobic methyl groups with aromatic side chains in the TLR2 hydrophobic cavity.

Data information: In (A, C, and D), one representative of "n" (given in brackets for each panel) independent experiments is shown (mean ± SD). *$P < 0.05$ according to two-way ANOVA with Dunnett's correction compared to WT (top sub-panel, indicated by dotted lines in each sub-panel) in (C).

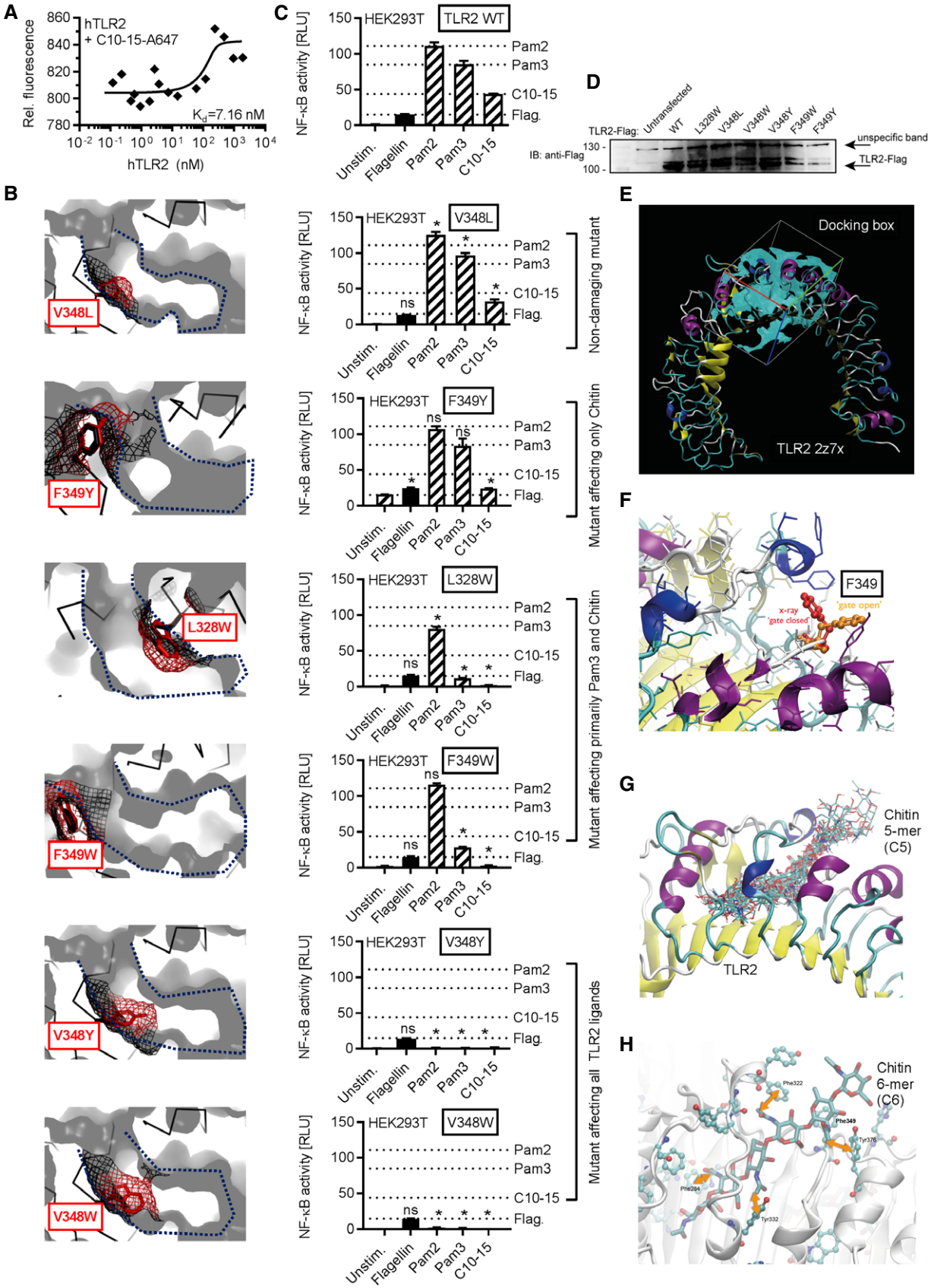
A
Rel. fluorescence
hTLR2
+ C10-15-A647
Kd=7.16 nM
hTLR2 (nM)
B
V348L
F349Y
L328W
F349W
V348Y
V348W
C
NF-κB activity [RLU]
HEK293T
TLR2 WT
V348L
F349Y
L328W
F349W
V348Y
V348W
Pam2
Pam3
C10-15
Flag.
Unstim.
Flagellin
ns
Non-damaging mutant
Mutant affecting only Chitin
Mutant affecting primarily Pam3 and Chitin
Mutant affecting all TLR2 ligands
D
TLR2-Flag: Untransfected
WT
L328W
V348L
V348W
V348Y
F349W
F349Y
IB: anti-Flag
unspecific band
TLR2-Flag
E
Docking box
TLR2 2z7x
F
F349
'gate open'
x-ray
'gate closed'
G
Chitin
5-mer
(C5)
TLR2
H
Chitin
6-mer
(C6)
Phe322
Phe349
Tyr376
Phe284
Tyr332

Figure EV4.

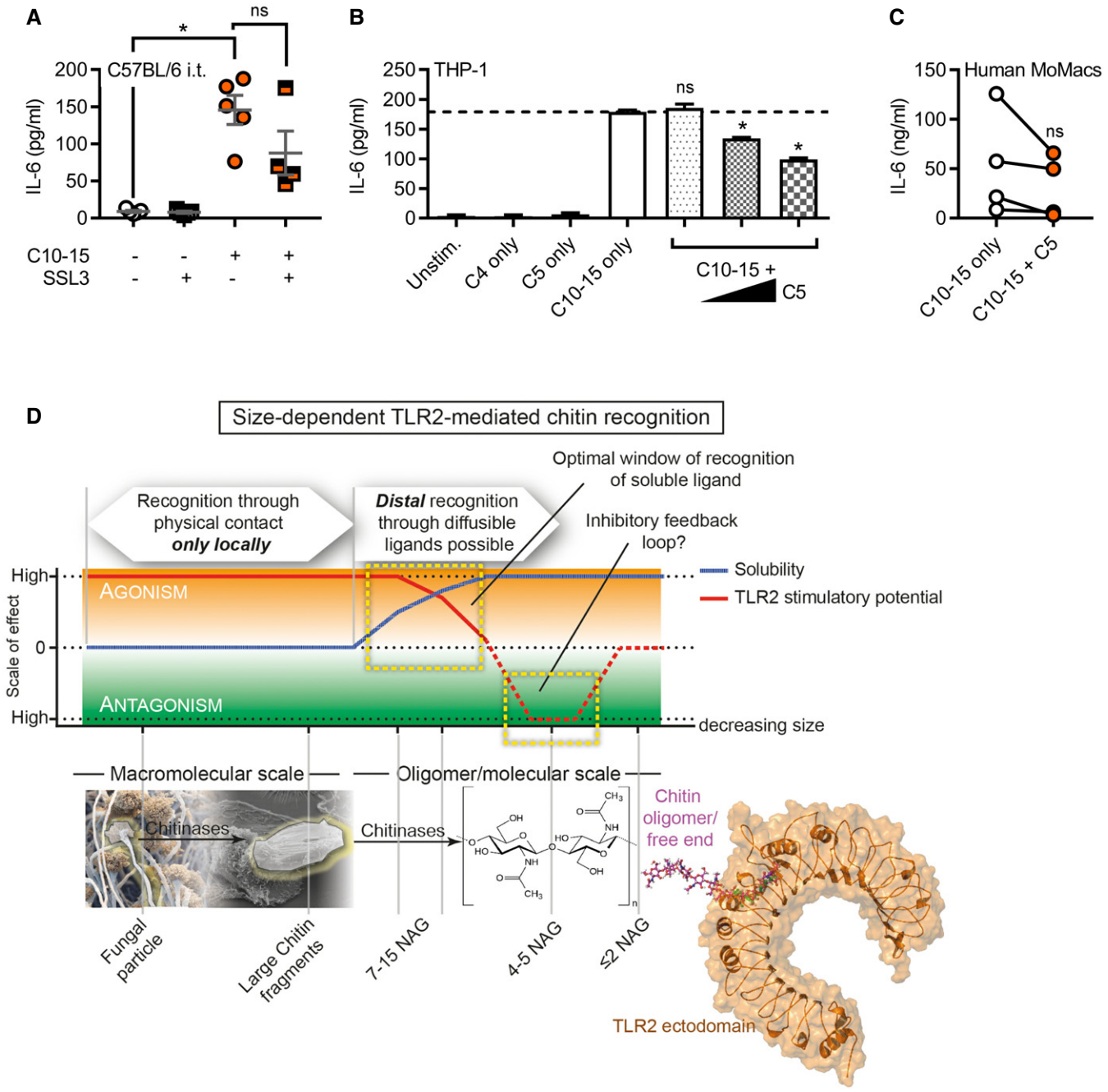

Figure EV5. Size-dependent immuno-modulatory effects of oligomeric chitin.

A BALF IL-6 in C57BL/6 mice (n = 4–5/group) upon C10-15 administration without or with SSL3.

B, C IL-6 release from THP-1 cells (B) or primary MoMacs (C) without or with C5 (n = 3 or 4).

D Schematic representation of size-dependent recognition of chitin oligomers by TLR2. Through the activity of chitinase or physical fragmentation, a range of different sized fragments and oligomers is generated. TLR2 recognition is possible for both particulate chitin as well as diffusible oligomers. As recognition can occur in a window where both activatory potential and solubility are high, distal recognition of fungal pathogens is possible, while the minimum length required for agonism (6 NAG) ensures that mono- or disaccharides are prevented from receptor activation. As activating oligomers are cleaved, soluble fragments with antagonistic properties may arise, representing an inherent negative regulatory loop.

Data information: (A and C) represent data (mean ± SEM) combined from "n" biological replicates (human donors or mice, respectively; each dot represents one biological replicate). In (B), one representative of three independent experiments is shown (mean ± SD). *$P < 0.05$ according to one-way ANOVA with Dunnett's multiple comparison (A), Student's t-test (B), or Wilcoxon signed rank sum (C) test.