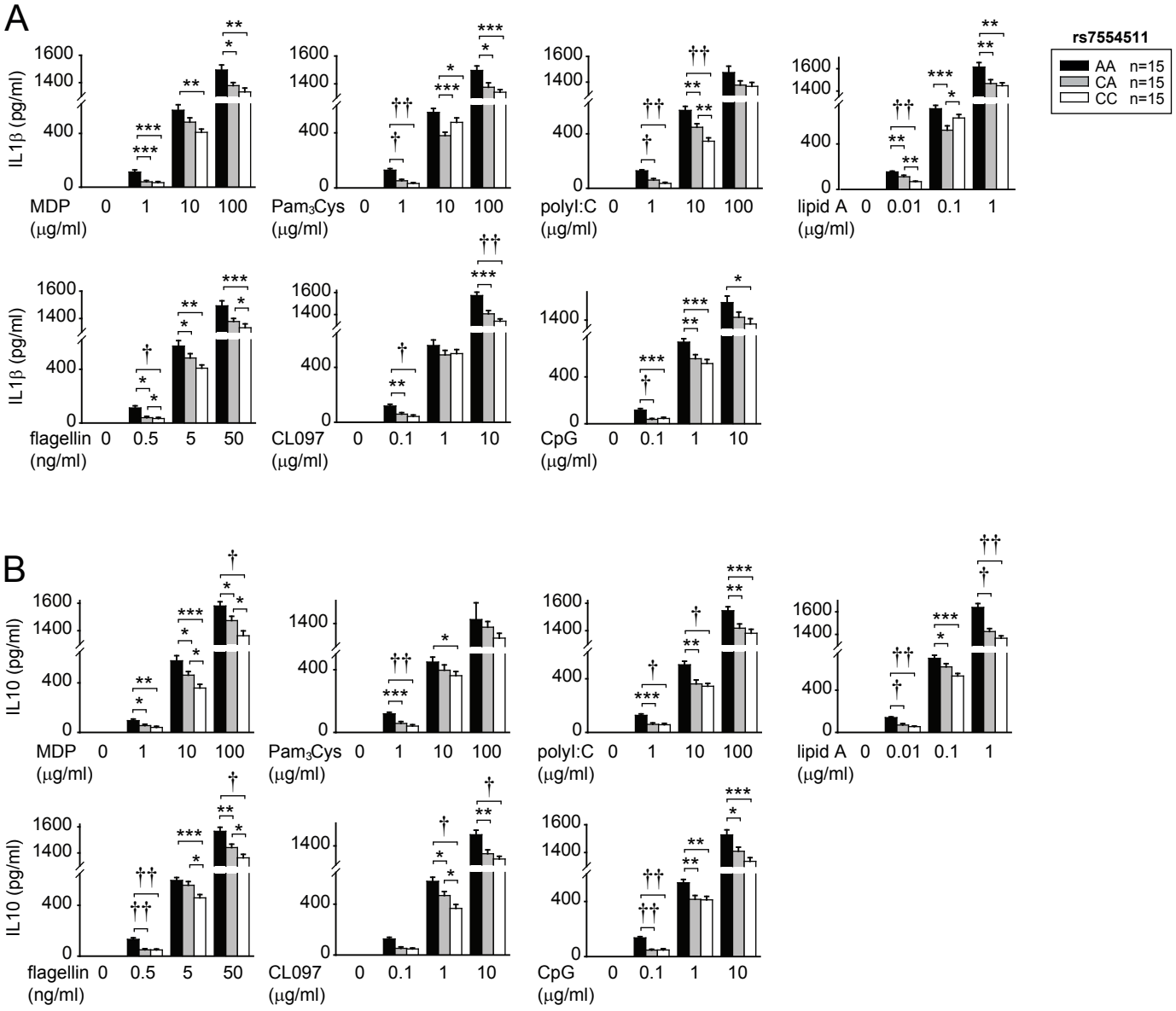
Supplemental Figure 1



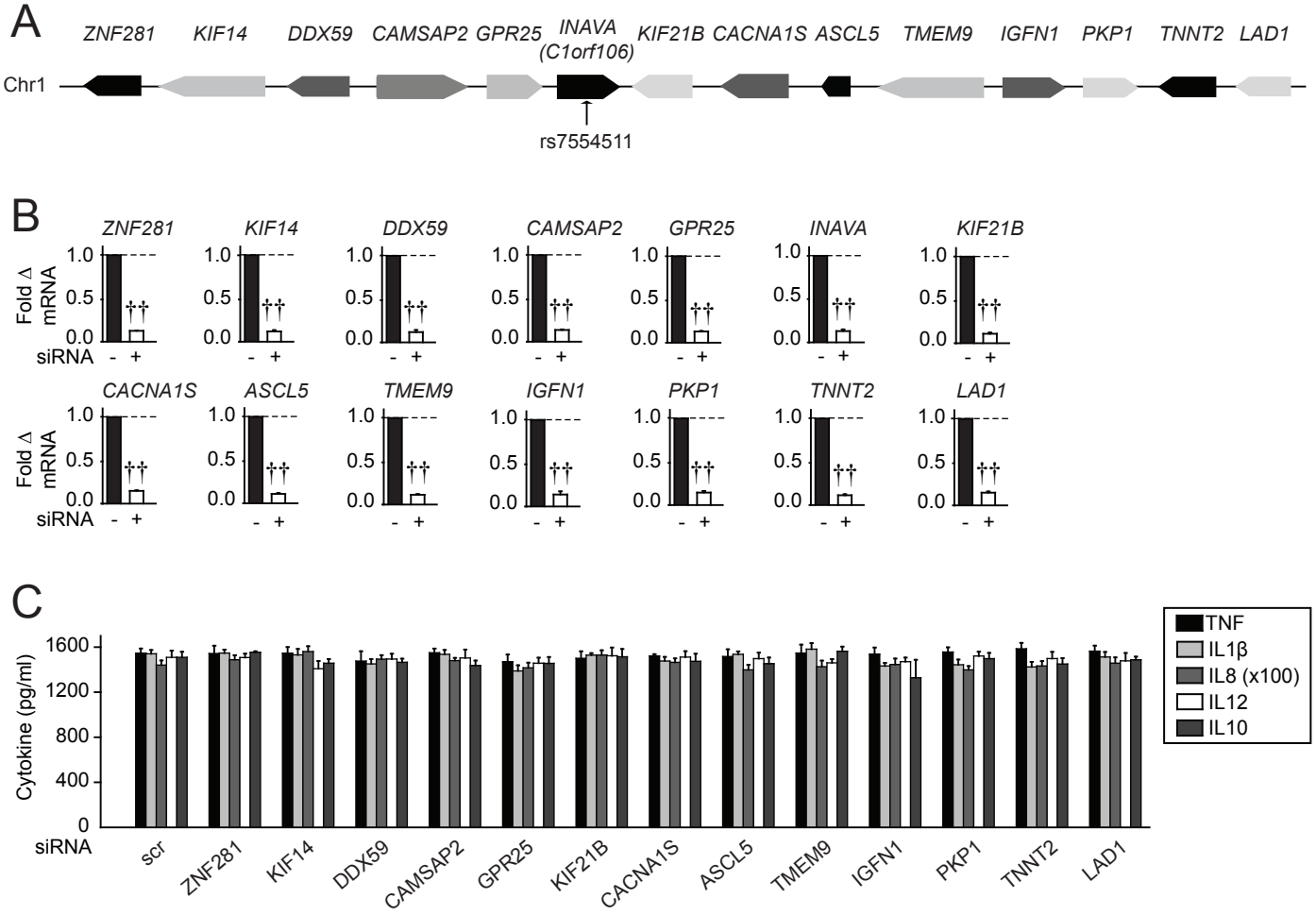
Supplemental Figure 1. Myeloid cells from rs7554511 C risk carriers secrete less IL1β and IL10 upon PRR stimulation. MDMs from rs7554511 AA, CA and CC carriers (n=15/genotype) were treated for 24h with the indicated doses of MDP (NOD2), Pam3Cys (TLR2), polyI:C (TLR3), lipid A (TLR4), flagellin (TLR5), CL097 (TLR7), or CpG DNA (TLR9). Shown is **(A)** IL1β and **(B)** IL10 secretion. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplemental Figure 2

Marker	Significance
rs7554511*	1.64E-04
rs35730213*	1.64E-04
rs55838263*	1.64E-04
rs12132298*	1.64E-04
rs12132349*	1.64E-04
rs59655222*	1.64E-04
rs41299637*	1.64E-04
rs12131796*	1.64E-04
rs10800746	1.80E-04
rs7522462	2.12E-04
rs12126806	2.18E-03
rs10920091	2.33E-03
rs296539	4.01E-03
rs296547	4.01E-03
rs11589573	4.72E-03
rs378672	5.09E-03
rs3208703	5.50E-03
rs12118735	5.50E-03
rs12140420	5.50E-03
rs11584383	5.81E-03
rs6427868	6.05E-03
rs10494828	6.46E-03
rs12142704	6.46E-03
rs2297909	6.93E-03
rs11579874	7.60E-03
rs169850	9.31E-03
rs6692427	1.52E-02
rs41269929	1.52E-02
rs1572789	NS
rs59682551	NS

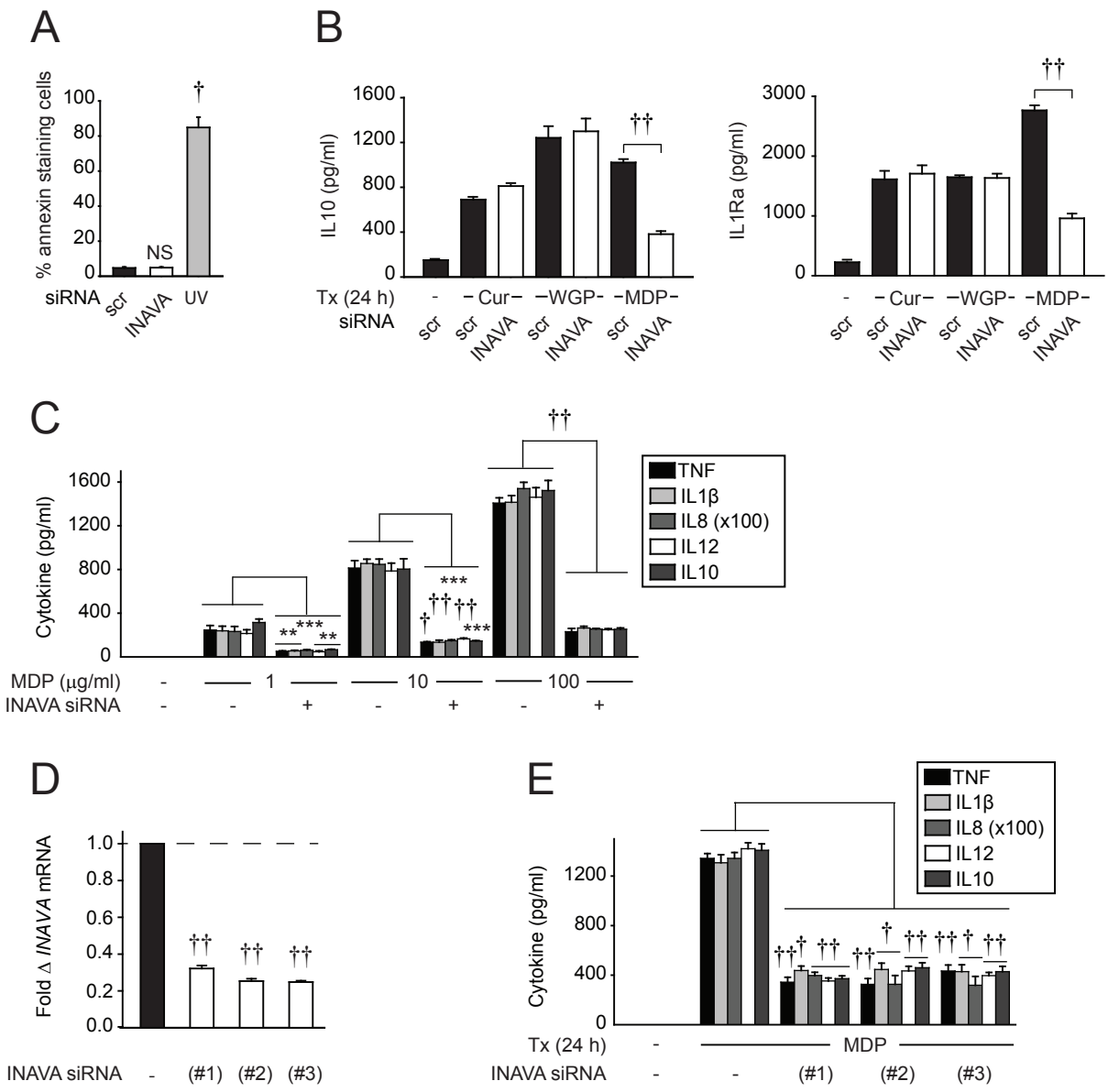
Supplemental Figure 2. Effects of *INAVA* region polymorphisms on NOD2-induced IL1β secretion in primary human MDMs. MDMs from major allele and minor allele homozygote carriers (a cohort of n=100) were assessed for IL1β secretion upon 1 μg/ml MDP stimulation and stratified on polymorphisms in the *INAVA* region using the Mann-Whitney U test. Bonferroni-Holm correction was utilized to account for multiple comparisons. Shown is significance for the 30 most significant markers. *Note that the first 8 polymorphisms are in linkage disequilibrium. NS, not significant.

Supplemental Figure 3



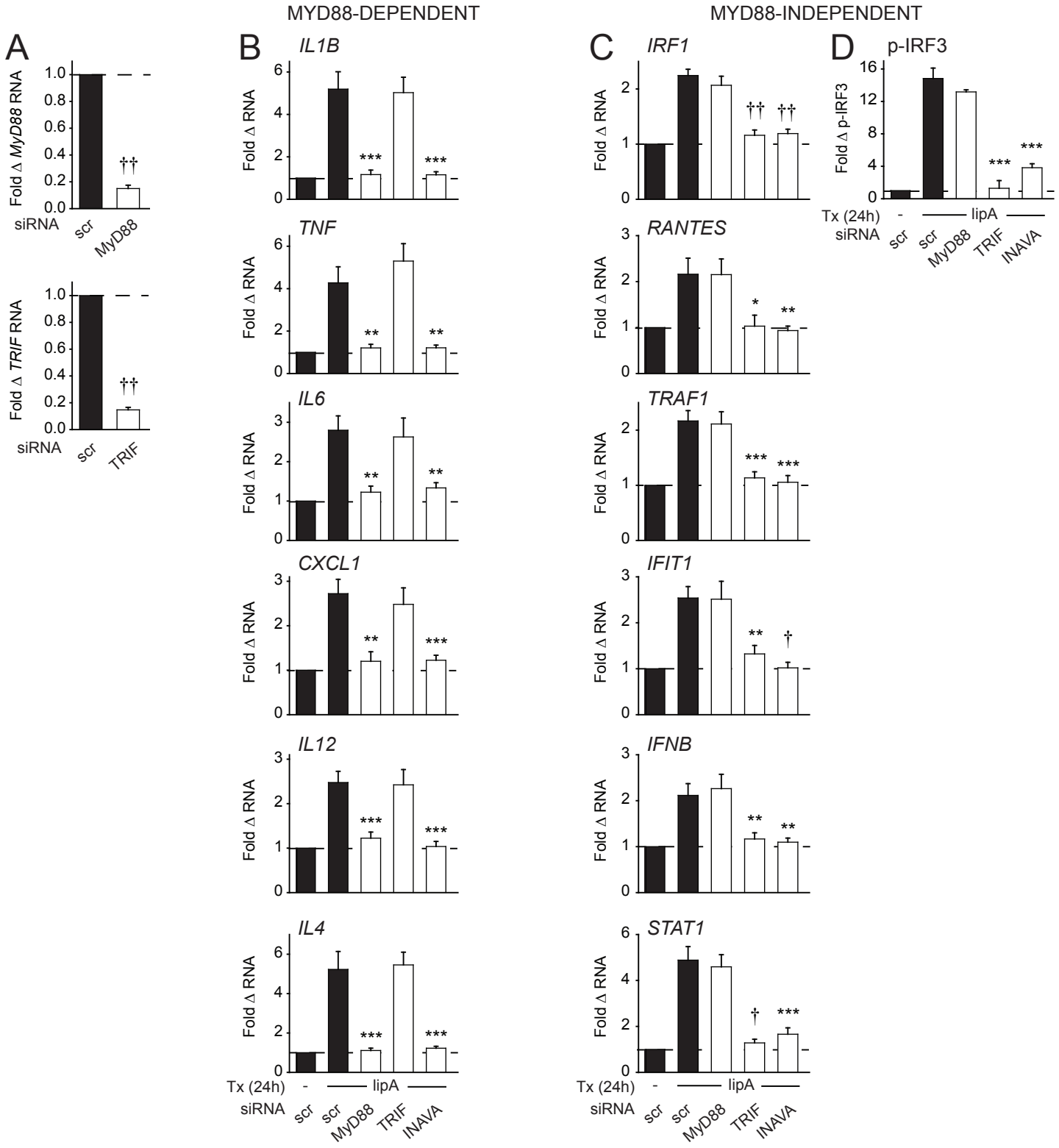
Supplemental Figure 3. Multiple genes in the rs7554511 region do not regulate NOD2-induced cytokines. **(A)** Representation of genes within ~500kb from rs7554511. To enable viewing of the genes in the region, gene positions are not drawn to scale. **(B-C)** MDMs were transfected with scrambled or the indicated siRNAs. **(B)** mRNA expression for each respective gene was normalized to scrambled siRNA-transfected cells + SEM (n=4). **(C)** The transfected MDMs were treated with 100μg/ml MDP for 24h and cytokine secretion + SEM (n=4) was examined. Tx, treatment; scr, scrambled. ††, $p<1x10^{-5}$.

Supplemental Figure 4



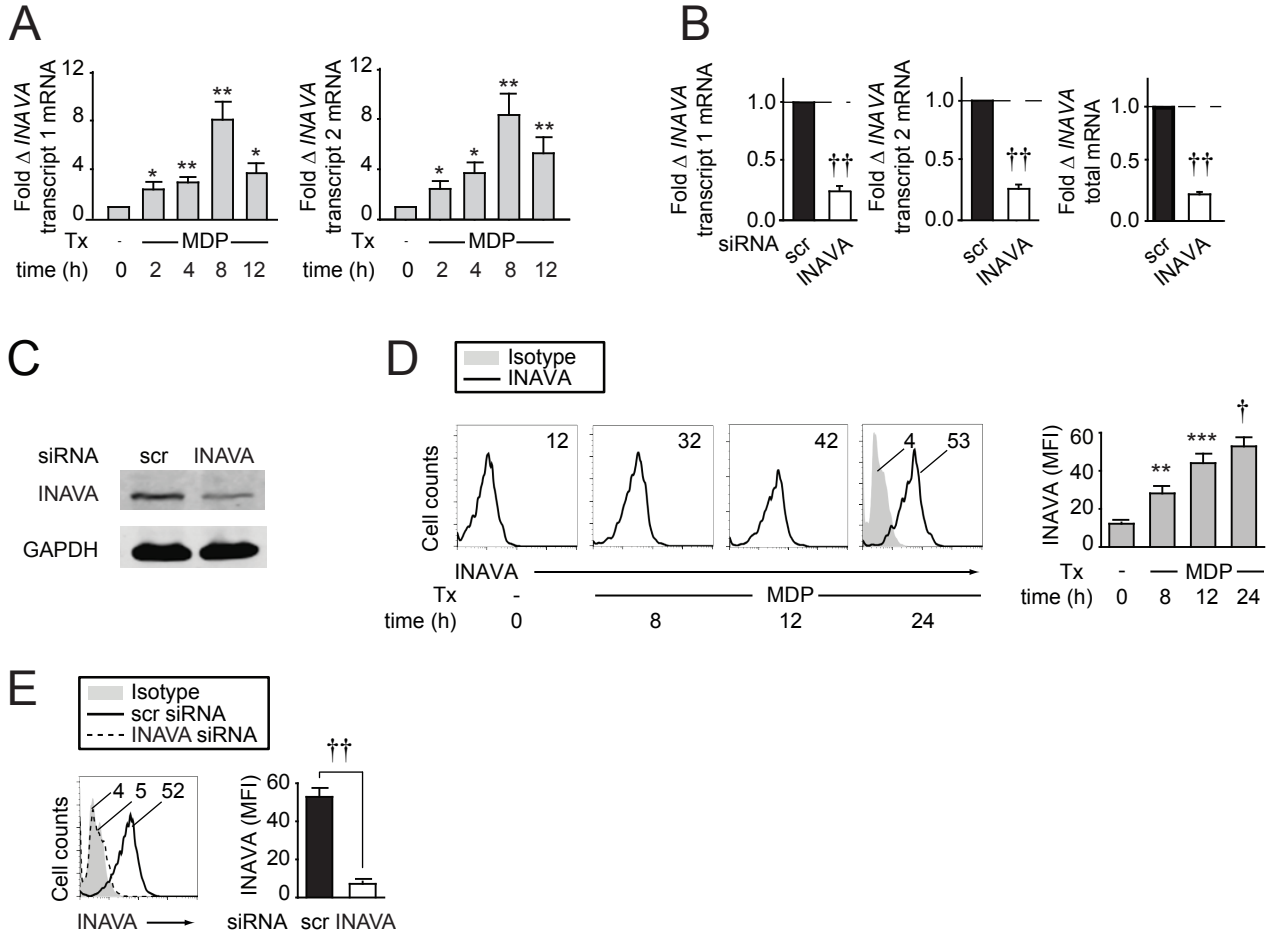
Supplemental Figure 4. Cell viability and responses to dectin ligands are intact upon knockdown of INAVA in MDMs. MDMs (n=4) were transfected with scrambled or INAVA siRNA. **(A)** Transfected cells (n=4) were stained with annexin V (BD Biosciences). Shown is percent dead cells + SEM. UV stimulation at 50-100 J/m^2 was used as a positive control. Significance is shown compared to scrambled siRNA-transfected cells. **(B)** Transfected cells (n=4) were treated with 100μg/ml curdlan (cur) (Invivogen), 100μg/ml dispersible whole glucan particles (WGP) (Invivogen) or 100μg/ml MDP for 24h. IL10 or IL1Ra secretion + SEM. **(C)** MDMs (n=4) were transfected with scrambled or INAVA siRNA, treated with 1, 10 or 100μg/ml MDP for 24h and examined for cytokine secretion + SEM. **(D-E)** MDMs were transfected with scrambled or INAVA siRNA [three different single siRNAs: J-020280-05 (#1), J-020280-06 (#2), J-020280-07 (#3), Dharmacon]. **(D)** *INAVA* mRNA (n=4) was normalized to scrambled siRNA-transfected cells + SEM. **(E)** Transfected cells (n=4; similar results were seen in an additional n=12) were treated with 100μg/ml MDP for 24h. Shown is cytokine secretion + SEM. Tx, treatment; NS, not significant; scr, scrambled. **, p<0.01; ***, p<0.001, †, p<1x10^{-4}; ††, p<1x10^{-5}.

Supplemental Figure 5



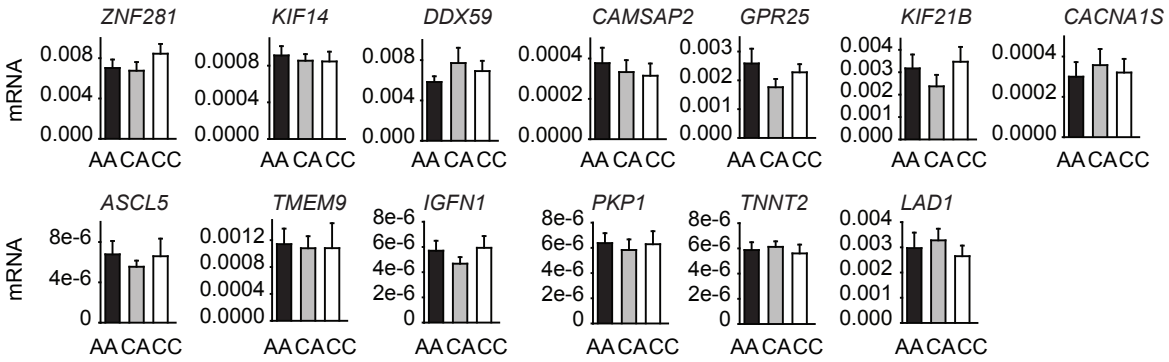
Supplemental Figure 5. INAVA regulates both MyD88-dependent and MyD88-independent signaling. (A) MDMs (n=6) were transfected with scrambled or the indicated siRNA. mRNA expression for each respective gene was normalized to scrambled siRNA-transfected cells+SEM. **(B-D)** MDMs (n=4) were transfected with scrambled or the indicated siRNA. **(B-C)** The transfected MDMs were treated with 0.1 μg/ml lipid A for 4h. Shown is mRNA expression for MyD88-dependent and -independent genes normalized to scrambled siRNA-transfected cells + SEM. **(D)** The transfected MDMs were treated with 0.1 μg/ml lipid A for 15 min. Summarized data are represented as the fold phospho-IRF3 (Cell Signaling Technology, clone D6O1M) induction as assessed by flow cytometry normalized to scrambled siRNA-transfected cells (represented by the dotted line at 1) + SEM. Tx, treatment; scr, scrambled. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplemental Figure 6



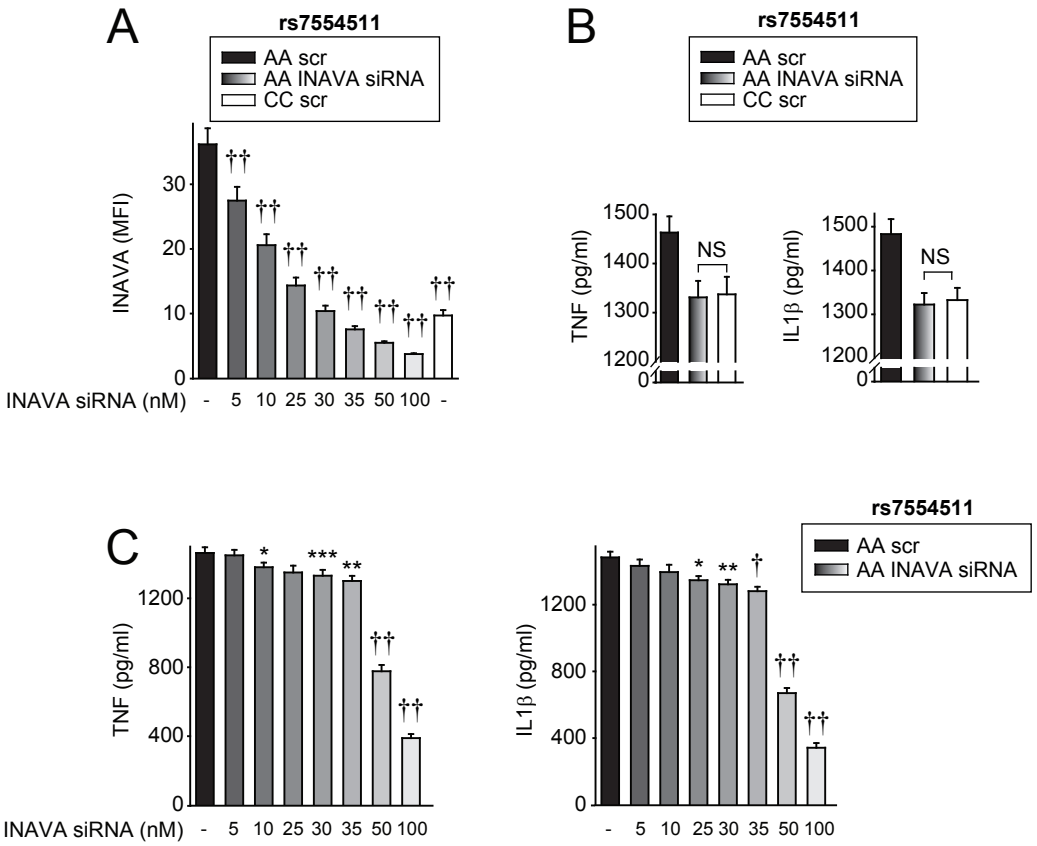
Supplemental Figure 6. NOD2 stimulation increases INAVA mRNA and protein expression. **(A)** MDMs were treated with 100μg/ml MDP for the indicated times and mRNA expression for the two *INAVA (C1orf106)* transcripts (transcript 1: NM_018265; transcript 2: NM_001142569) was normalized to GAPDH. Mean + SEM (n=10). **(B)** MDMs were transfected with scrambled or INAVA siRNA, and mRNA expression for the two *INAVA* (*C1orf106*) transcripts, as well as the total *INAVA* mRNA, was normalized to scrambled siRNA-transfected cells. Mean + SEM (n=4). **(C)** MDMs were transfected with scrambled or INAVA siRNA. Shown is a representative Western blot of INAVA (C1orf106) expression (Abcam). **(D)** MDMs (n=8) were treated with 100μg/ml MDP for the indicated times and INAVA (C1orf106) protein expression (Proteintech, cat #21506-1-AP) was assessed by flow cytometry. **(E)** Shown as a control for antibody specificity in **'D'** is scrambled compared to INAVA siRNA-transfected MDMs. (*Left*) Representative flow cytometry for INAVA protein expression with mean fluorescent intensity (MFI) indicated. (*Right*) Summarized data for MFI + SEM (n=8). Tx, treatment; scr, scrambled. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplemental Figure 7



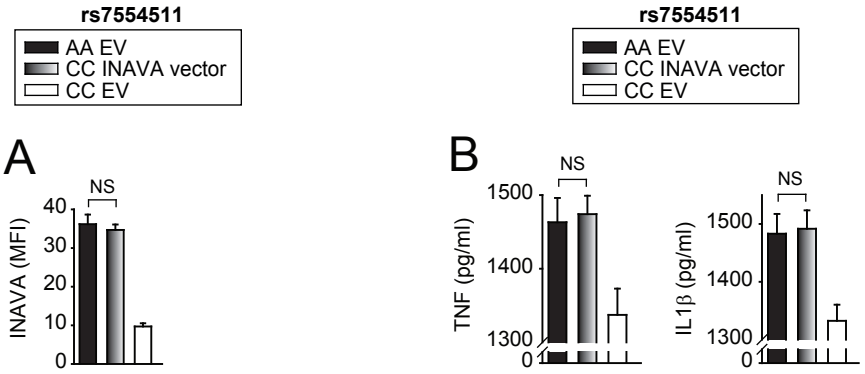
Supplemental Figure 7. The rs7554511 polymorphism does not regulate mRNA expression of additional genes in the region. MDMs from rs7554511 AA, CA, and CC carriers (n=8/genotype) were left untreated or treated with 100μg/ml MDP for 8h. mRNA expression of the indicated genes was assessed (expressed as change in CT values normalized to GAPDH and represented as a linear scale) + SEM.

Supplemental Figure 8



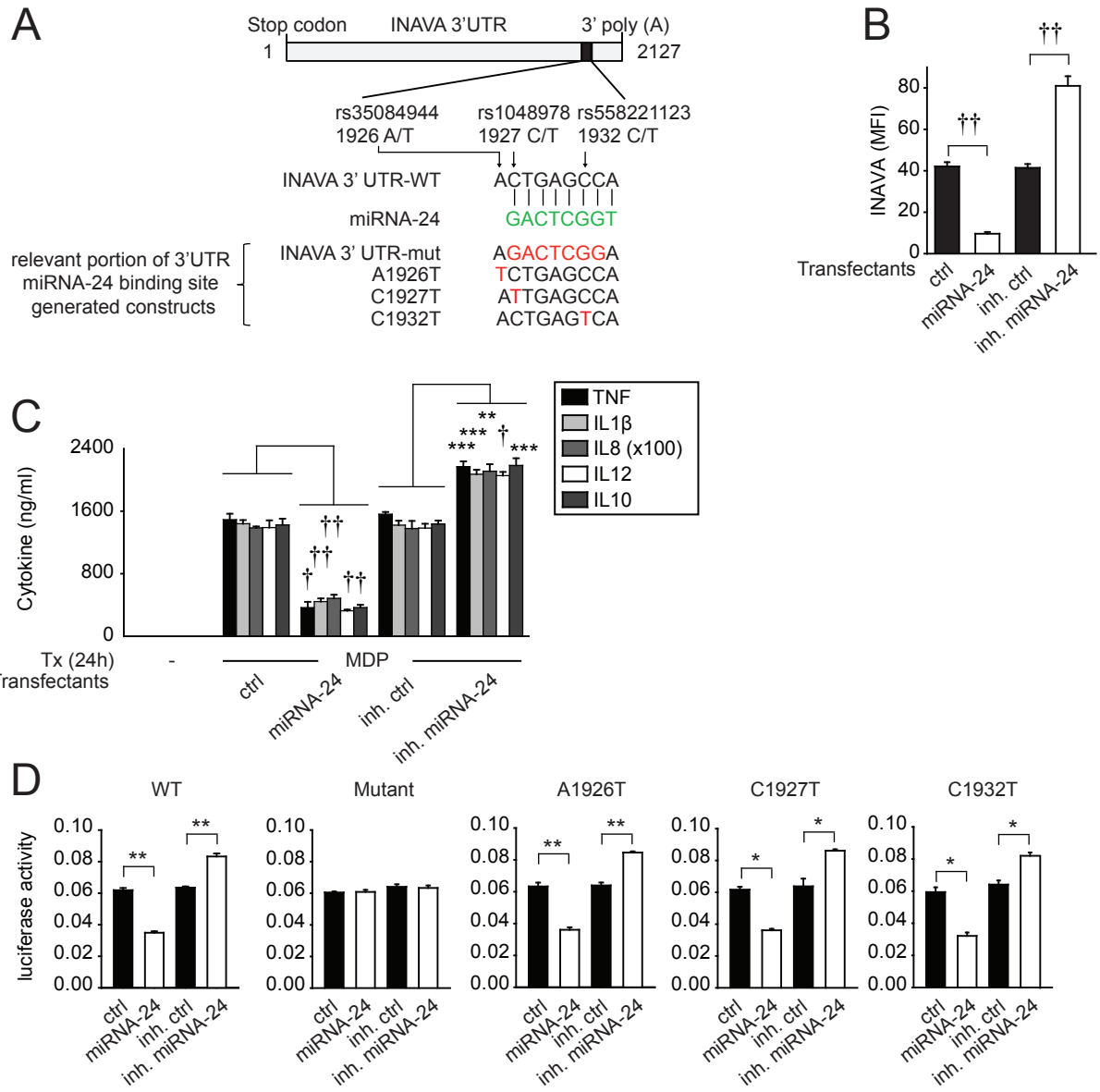
Supplemental Figure 8. Modulation of INAVA expression in rs7554511 AA genotype carriers regulates cytokine secretion levels. (A) MDMs from rs7554511 AA carriers (n=15) were transfected with scrambled or INAVA siRNA at the indicated concentrations, then treated with 100μg/ml MDP for 24h. Rs7554511 CC carriers (n=15/genotype) transfected with scrambled siRNA are shown for comparison. Shown is summarized MFI+SEM for INAVA expression assessed by flow cytometry. **(B)** MDMs from rs7554511 AA or CC carriers (n=15/genotype) were transfected with 30 nM scrambled or INAVA siRNA, treated with 100μg/ml MDP for 24h, and assessed for cytokine secretion+SEM. **(C)** MDMs from rs7554511 AA (n=15) were transfected with scrambled or INAVA siRNA at the indicated concentrations, then treated with 100μg/ml MDP for 24h and assessed for cytokine secretion+SEM. NS, not significant; scr, scrambled. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplemental Figure 9



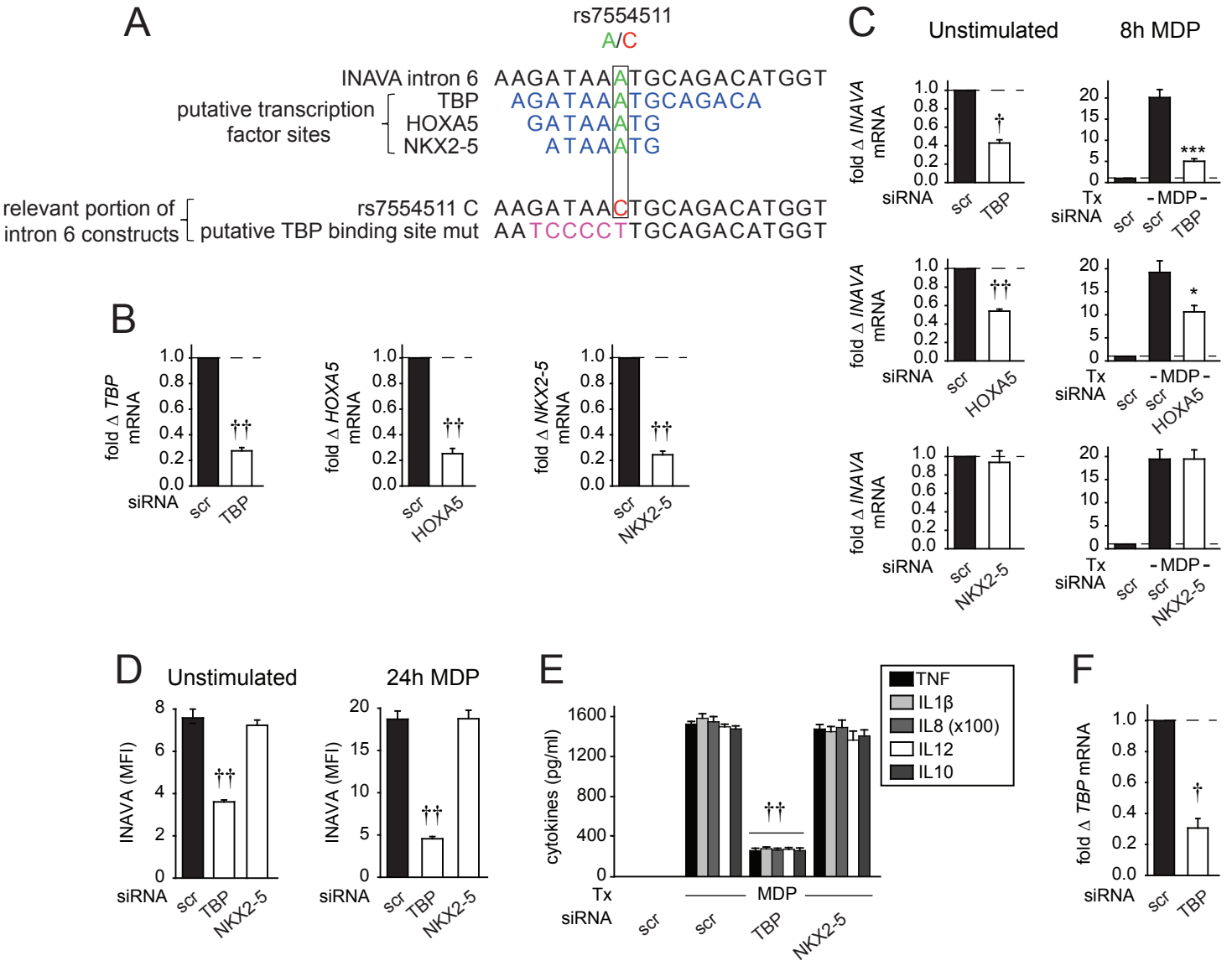
Supplemental Figure 9. Modulation of INAVA expression in rs7554511 CC carriers regulates cytokine secretion levels. MDMs from AA or CC rs7554511 carriers (n=15/genotype) were transfected with 4.5μg empty vector (EV) or INAVA vector, then treated with 100μg/ml MDP for 24h. **(A)** Shown is summarized MFI+SEM for INAVA expression assessed by flow cytometry and **(B)** cytokine secretion + SEM. NS, not significant.

Supplemental Figure 10



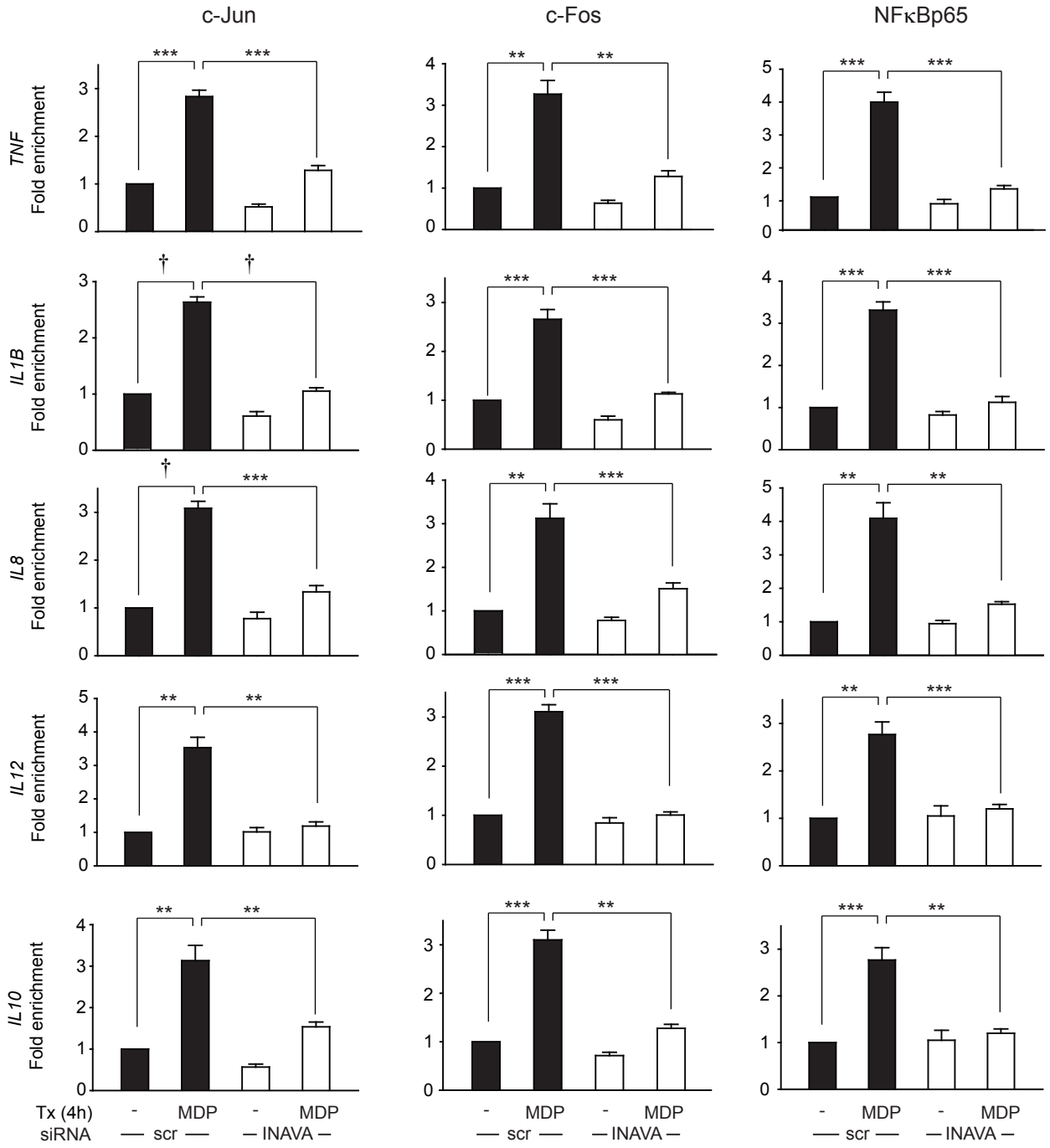
Supplemental Figure 10. miRNA-24 regulates INAVA expression. (A) Shown is the 3' UTR region of the *INAVA* gene, along with the region predicted to bind miRNA-24 per TargetScan. Nucleotides mutated in the designed constructs are shown in red. **(B-C)** MDMs were transfected with miRNA-24 mimic, miRNA-24 hairpin inhibitor (Dharmacon) or appropriate controls. The transfected MDMs were treated with 100μg/ml MDP for 24h. **(B)** Flow cytometry for INAVA expression. Summarized data are represented as INAVA mean fluorescent intensity (MFI)+SEM (n=4). **(C)** Cytokine secretion+SEM (n=4). **(D)** A portion of the INAVA 3' UTR (from nucleotides 1639 to 2106) was subcloned into pmirGLO vector (Promega, Madison, WI). Site-directed mutagenesis was used to mutate the predicted miRNA-24 binding site (CTGAGCC to GACTCGG, mut), and to generate 3 constructs corresponding to dbSNP-reported variants in the region (A1926T, C1927T and C1932T) as shown in '**A**'. The indicated INAVA variants and miRNA-24 mimic or miRNA-24 hairpin inhibitor were transfected into HEK293 cells along with Renilla. Luciferase activity was assessed. Of note is that these 3 variants were not in LD with rs7554511 as per dbSNP data and/or our sequencing of DNA from 50 individuals distributed across the 3 rs7554511 genotypes. Inh., inhibitor; ctrl, control. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplemental Figure 11



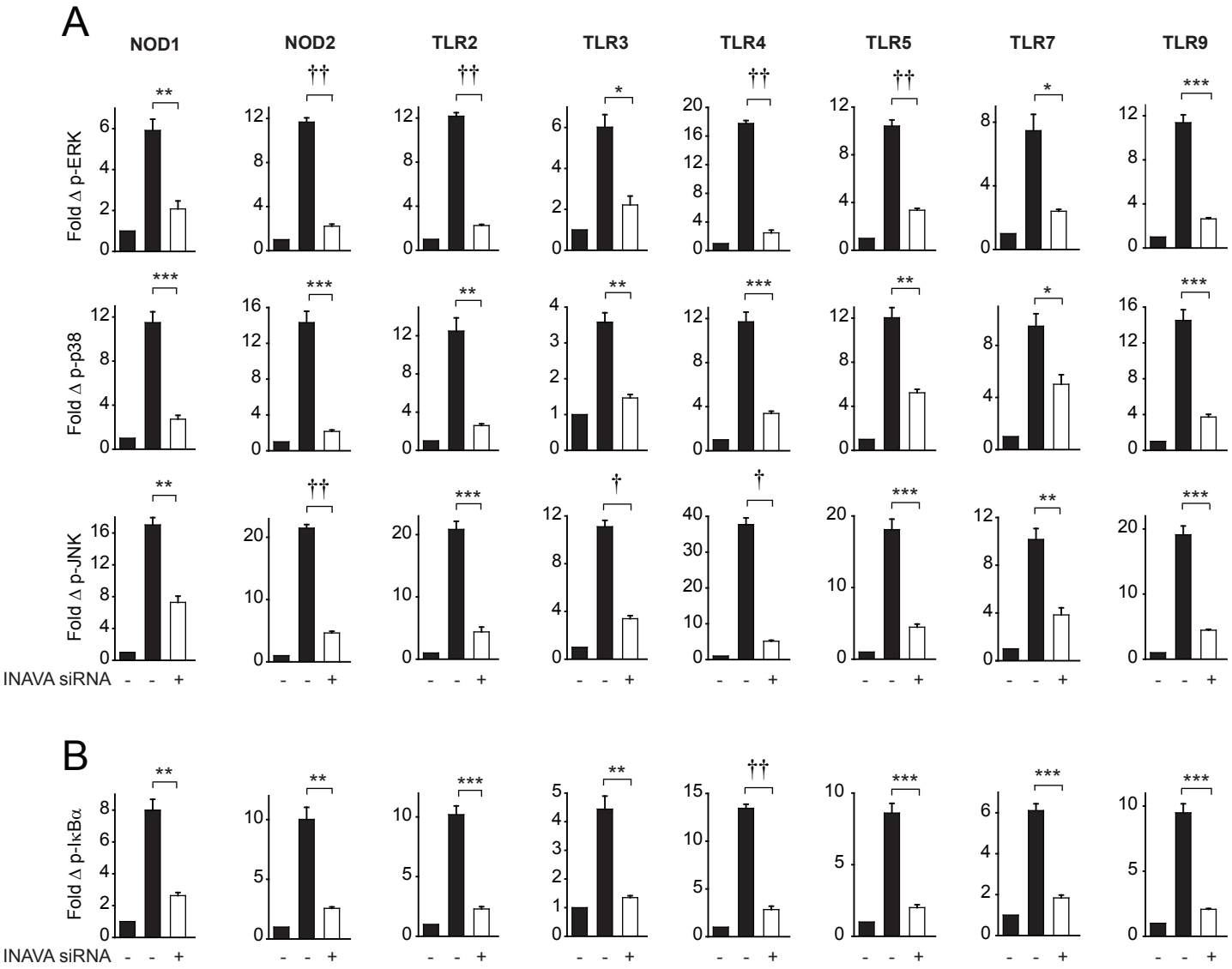
Supplemental Figure 11. The transcription factor TBP regulates INAVA expression in MDMs. (A) Shown is a portion of the intron 6 region within the *INAVA* gene containing the rs7554511 SNP and predicted to bind the indicated transcription factors (per JASPAR database), along with the relevant sequence portions of the designed constructs. The rs7554511 A variant is shown in green and the C risk variant in red; predicted transcription factor binding sites are shown in blue. **(B)** MDMs (n=8) were transfected with scrambled or the indicated siRNA. Shown is mRNA expression for the indicated genes normalized to scrambled siRNA-transfected cells + SEM. **(C)** MDMs (n=8, similar results seen in an additional n=4) were transfected with scrambled or the indicated siRNA and left untreated or treated with 100 μg/ml MDP for 8h. Shown is *INAVA* mRNA expression for each respective gene normalized to scrambled siRNA-transfected cells + SEM. Note the unstimulated and 8h treated scales are different due to the increase in *INAVA* expression with MDP treatment. **(D-E)** MDMs were transfected with scrambled or the indicated siRNA and left untreated or treated with 100 μg/ml MDP for 24h. Shown is **(D)** INAVA mean fluorescent intensity (MFI) as assessed by flow cytometry + SEM (n=10, similar results for MDP-treated cells seen in an additional n=10) and **(E)** cytokine secretion (n=4) + SEM. **(F)** HEK293 cells (replicate of 6) were transfected with scrambled or TBP siRNA. Shown is *TBP* mRNA expression. Tx, treatment; scr, scrambled. *, $p<0.05$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplemental Figure 12



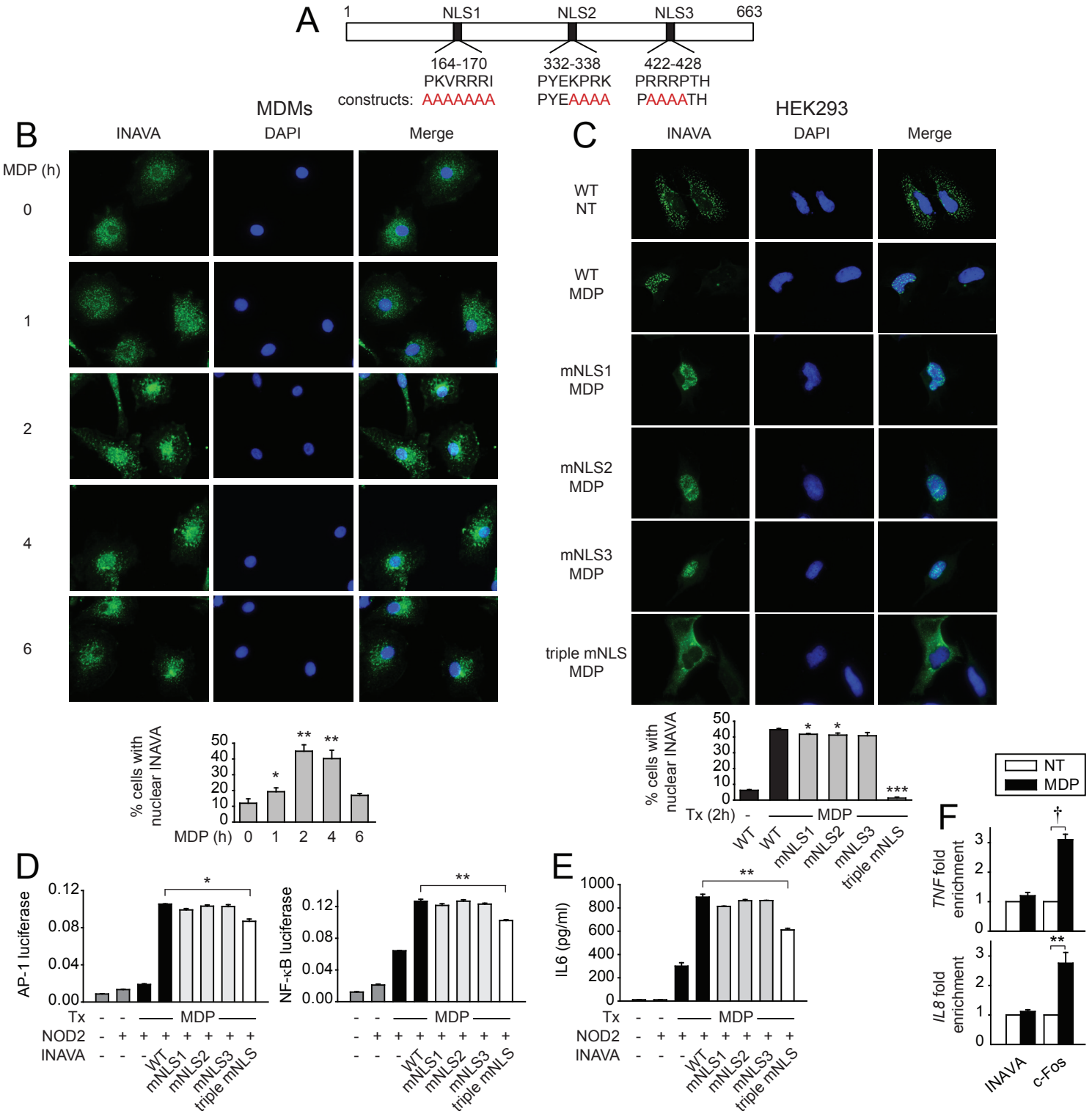
Supplemental Figure 12. INAVA is required for optimal NOD2-induced recruitment of c-Jun, c-Fos or NFκBp65 to cytokine promoters. Human MDMs (n=6) were transfected with scrambled or INAVA siRNA and then treated with 100μg/ml MDP for 4h. Binding of c-Jun, c-Fos, and NFκBp65 to the *TNF*, *IL1B*, *IL8*, *IL12* and *IL10* promoters was assessed by ChIP. Antibodies used include c-Jun (clone D), c-Fos (clone K-25), NFκBp65 (clone A) (Santa Cruz Biotechnology), or rabbit IgG (EMD Millipore). Data are represented as the fold enrichment normalized to untreated, scrambled siRNA-transfected cells. Similar results were observed in an additional n=4. Tx, treatment; scr, scrambled. **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$.

Supplemental Figure 13



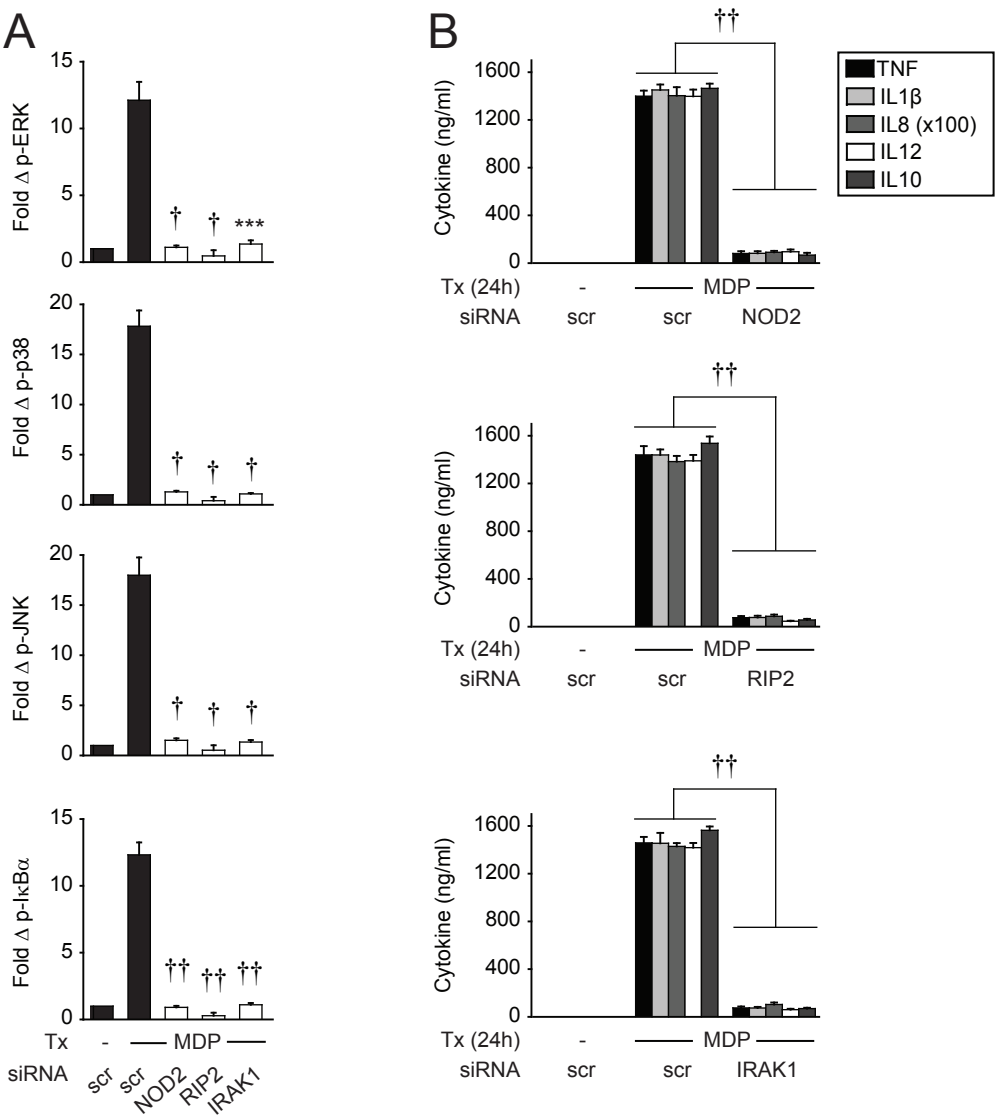
Supplemental Figure 13. INAVA contributes to signaling through multiple PRRs. MDMs (n=4) were transfected with scrambled or INAVA siRNA. The transfected MDMs were treated with 100µg/ml TriDAP (NOD1), 100µg/ml MDP (NOD2), 10µg/ml Pam3Cys (TLR2), 100µg/ml polyI:C (TLR3), 0.1µg/ml lipid A (TLR4), 5ng/ml flagellin (TLR5), 1µg/ml CL097 (TLR7), or 10µg/ml CpG DNA (TLR9) for 15 min and assessed for phospho-protein induction by flow cytometry. Summarized data are represented as the fold **(A)** phospho-MAPK or **(B)** phospho-IκBα protein induction normalized to scrambled siRNA-transfected cells + SEM. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplemental Figure 14



Supplemental Figure 14. INAVA translocates to the nucleus upon NOD2 stimulation. **(A)** Shown is the region of the *INAVA* gene with predicted nuclear localization signals (NLSs). The amino acids mutated in the designed constructs are shown in red. **(B)** MDMs were treated with 100μg/ml MDP for the indicated times. **(C)** HEK293 cells were transfected with NOD2 + the indicated INAVA mutant NLS (mNLS) constructs and treated with 100μg/ml MDP for 2h. **(B-C)** Cells were immunostained for INAVA (green) and nucleus (DAPI, Thermofisher; blue). Shown are representative micrographs and summary graphs indicating percent cells with INAVA nuclear translocation + SEM. Data represent three independent experiments with 100-120 cells quantified per each experiment. **'C'** Significance is shown compared to WT INAVA-transfected, MDP-treated cells. **(D)** HEK293 cells were transfected with NOD2, a Renilla reporter, AP-1 or NFκB luciferase reporters, and empty vector or the indicated INAVA mutant NLS constructs. Transfected cells were treated with 100μg/ml MDP for 6h and activation of AP-1 and NFκB luciferase reporters was assessed and normalized to Renilla. Mean + SEM for triplicates. Representative of 3 independent experiments. **(E)** HEK293 cells were transfected with NOD2 and empty vector or the indicated mutant INAVA constructs, and then treated with 100μg/ml MDP for 24h. Secreted IL6 was assessed + SEM for triplicates. Similar results were observed in an additional 2 independent experiments. **(F)** Human MDMs (n=6) were treated with 100μg/ml MDP for 4h. Binding of INAVA or c-Fos to the *TNF* and *IL8* promoters was assessed by ChIP. Data are represented as the fold enrichment normalized to untreated cells. Tx, treatment. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$.

Supplemental Figure 15



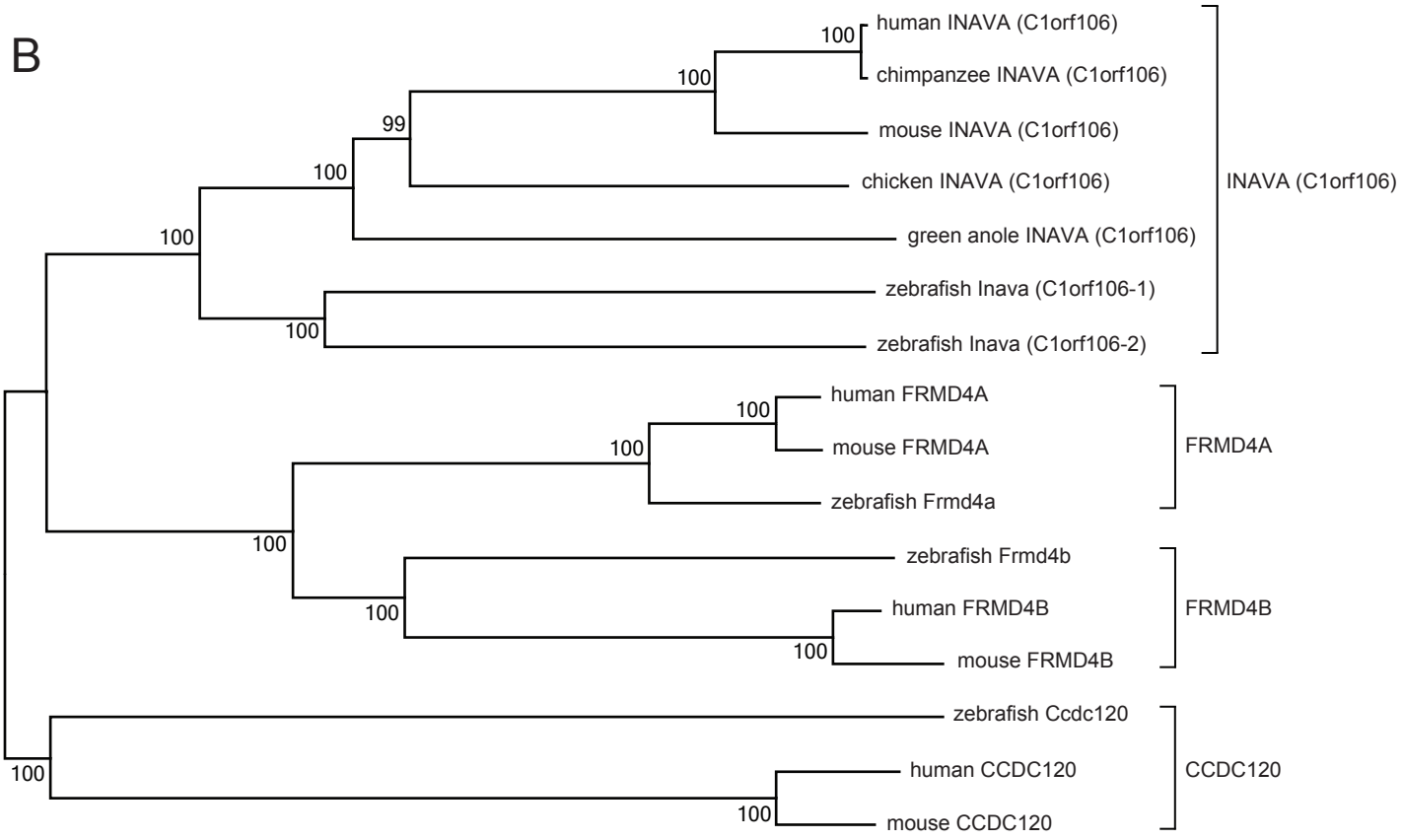
Supplemental Figure 15. NOD2, RIP2 and IRAK1 are required for optimal MDP-induced signaling and cytokine secretion. (A-B) MDMs (n=4) were transfected with scrambled or the indicated siRNA and left untreated or treated with 100 μg/ml MDP for **(A)** 15 min or **(B)** 24h. Shown is **(A)** phospho-protein induction normalized to scrambled siRNA-transfected cells + SEM or **(B)** cytokine secretion + SEM. Tx, treatment; scr, scrambled. ***, $p<0.01$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplemental Figure 16

A

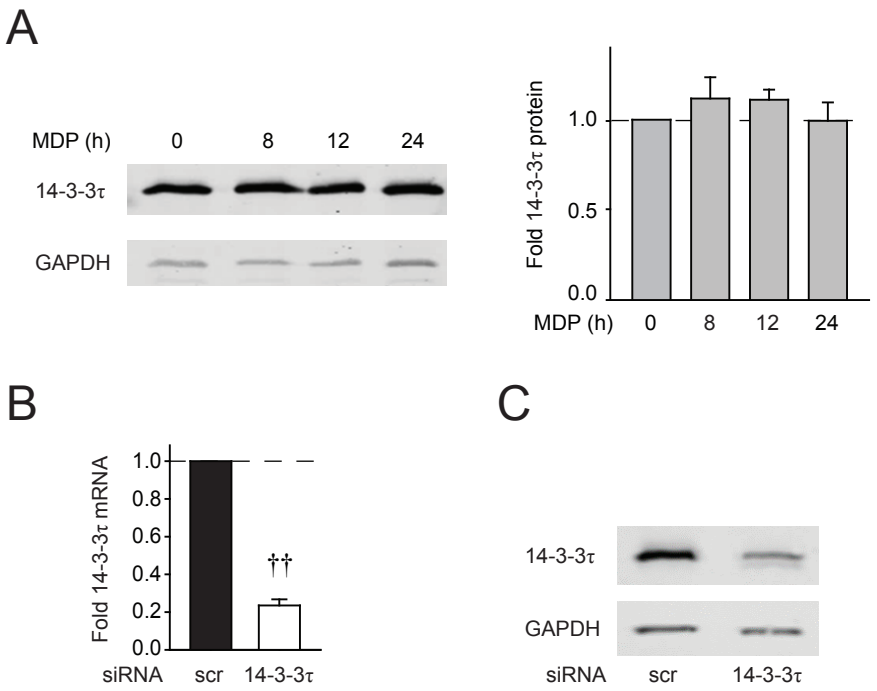
Kingdom	Species	Copy	Accession numbers	Identity to human INAVA (C1orf106) (BC106877.2)
Mammalia	*Pan troglodytes*	1	XP_016790848	99.7%
	Mus musculus	1	NP_083148	85.5%
Aves	*Gallus gallus*	1	XP_015154275	51.0%
Reptilia	*Anolis carolinensis*	1	XP_008108005	52.9%
Teleosts	*Danio rerio*	2	NP_001073474	37.4%
			XP_009302242	35.9%

B



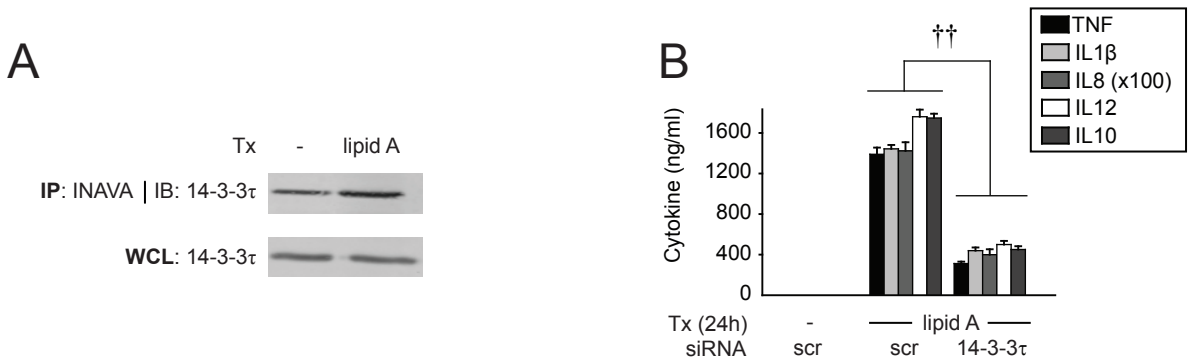
Supplemental Figure 16. Identity and phylogeny of INAVA. (A) INAVA (C1orf106) protein sequence identity across different organisms. **(B)** Neighbor-joining phylogenetic tree of INAVA protein. Numbers on the interior branches indicate bootstrap confidence values derived from 1000 replicates. INAVA homologs are shown as species followed by gene names. Genbank accession numbers for human, chimpanzee, mouse, chicken, green anole and zebrafish for INAVA (C1orf106) are the same as in **'A'** and other sequences are as follows: human FRMD4A (NP_060497); human FRMD4B (NP_055938); human CCDC120 (NP_001156793); mouse FRMD4A (NP_766063); mouse FRMD4B (AAH58262); mouse CCDC120 (NP_997085); zebrafish Frmd4a (NP_001122007); zebrafish Frmd4b (XP_009294836); zebrafish Ccdc120 (NP_001070919).

Supplemental Figure 17



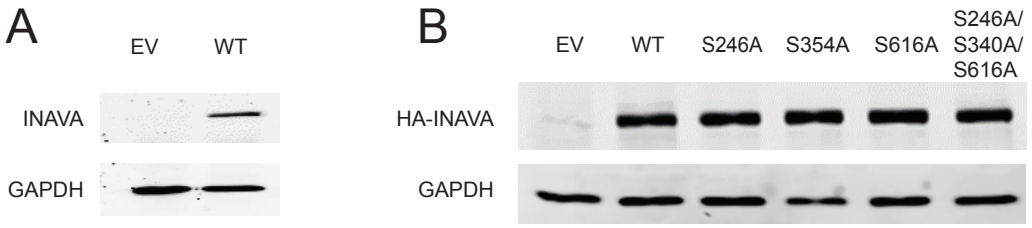
Supplemental Figure 17. 14-3-3τ expression is not regulated upon NOD2 stimulation. (A) MDMs (n=4) were treated for the indicated times with 100μg/ml MDP. (*Left*) Representative 14-3-3τ protein expression by Western blot. (*Right*) Summary of densitometric quantification of 14-3-3τ protein normalized to GAPDH and untreated cells (n=3). **(B-C)** MDMs were transfected with scrambled or 14-3-3τ siRNA. **(B)** 14-3-3τ mRNA was normalized to scrambled siRNA-transfected cells. Mean + SEM (n=4). **(C)** 14-3-3τ protein was assessed by Western blot. GAPDH was used as a loading control. Scr, scrambled. ††, $p<1x10^{-5}$.

Supplemental Figure 18



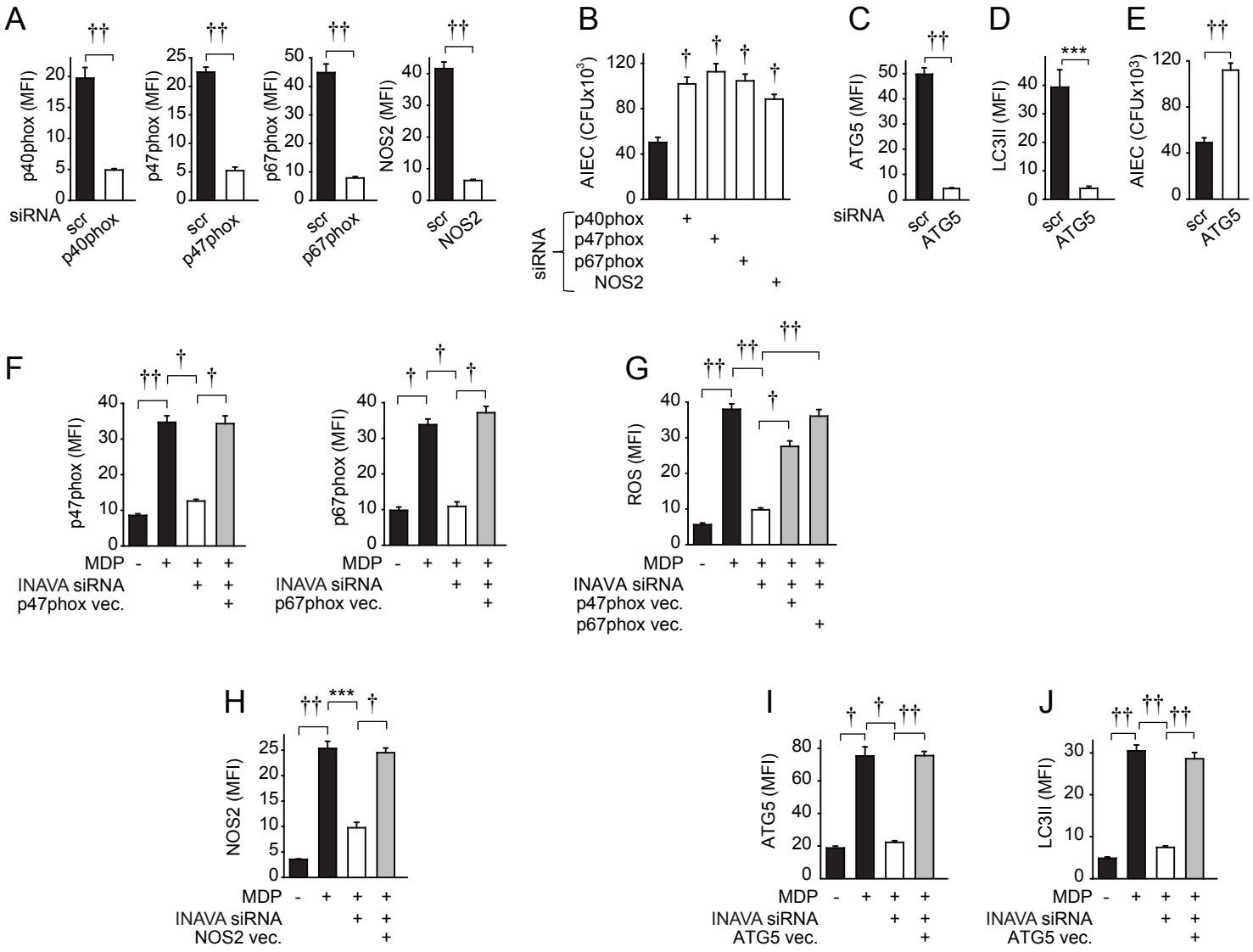
Supplemental Figure 18. 14-3-3τ is recruited to INAVA following TLR4 stimulation. (A) MDMs were treated 0.1μg/ml lipid A for 15 min. INAVA was immunoprecipitated and recruitment of 14-3-3τ was assessed by Western blot. Equivalent 14-3-3τ expression is shown in whole cell lysates. **(B)** MDMs (n=4) were transfected with scrambled or 14-3-3τ siRNA and left untreated or treated with 0.1 μg/ml lipid A for 24h. Shown is cytokine secretion. Tx, treatment; scr, scrambled. ††, $p<1x10^{-5}$.

Supplemental Figure 19



Supplemental Figure 19. INAVA mutant constructs express at equivalent levels. **(A)** HEK293 cells were transfected with empty vector (EV) or WT INAVA. INAVA expression was assessed by Western blot using an anti-INAVA (C1orf106) antibody (Abcam). GAPDH was used as a loading control. Representative of 3 independent experiments. **(B)** HEK293 cells were transfected with empty vector (EV) or the indicated INAVA constructs and levels of expression were assessed by Western blot with anti-HA antibody (Abcam, clone HA.C5). GAPDH was used as a loading control. Representative of 3 independent experiments.

Supplemental Figure 20



Supplemental Figure 20. PRR-induced ROS, RNS and autophagy pathways cooperate to clear bacteria in human macrophages. (A,C,D) MDMs (n=4) were transfected with scrambled or the indicated siRNA and treated with 100μg/ml MDP for 48h. Protein expression was assessed by flow cytometry with summary plots of mean fluorescent intensity (MFI)+SEM. **(B,E)** MDMs (n=6) were transfected with scrambled or the indicated siRNA, treated with 100 μg/ml MDP for 48h, and co-cultured with AIEC at 10:1 MOI. Shown are bacterial CFU + SEM. **(F-J)** MDMs (n=4 per each measure) were transfected with scrambled or INAVA siRNA ± p47phox-, p67phox-, NOS2- or ATG5-expressing vectors or empty vector, then left untreated or treated with 100μg/ml MDP for 48h. **(F, H-J)** Protein or **(G)** ROS expression was assessed by flow cytometry with summary plots of MFI+SEM shown. Scr, scrambled; tx, treatment; vec, vector. ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

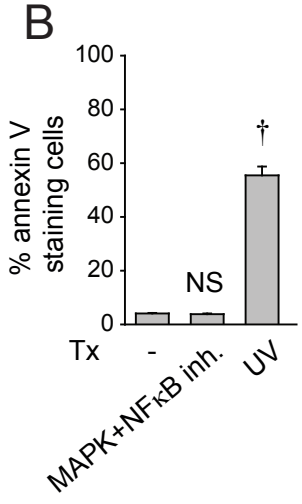
Supplemental Figure 21

A

MDP upregulated transcripts

	MDP					Curdlan		
	scr siRNA	INAVA siRNA		MAPK NFκB inhibitors		scr siRNA	INAVA siRNA	
Gene transcript	fold Δ	fold Δ	p value	fold Δ	p value	fold Δ	fold Δ	p value
ICAM1	10.7±1.11	2.72±0.41	5.29E-04	1.17±0.14	1.47E-04	0.94±0.04	0.94±0.09	NS
TLR2	10.1±0.63	4.98±0.42	5.47E-04	2.09±0.36	3.44E-05	1.06±0.05	0.95±0.13	NS
IL6	9.64±0.98	2.72±0.59	9.33E-04	2.29±0.22	3.36E-04	1.07±0.08	1.10±0.15	NS
CDC42EP2	9.50±1.12	1.05±0.16	3.03E-04	0.95±0.04	2.70E-04	1.16±0.13	0.89±0.01	NS
A20	9.46±0.51	2.68±0.46	6.15E-05	1.01±0.23	5.22E-06	0.83±0.06	0.80±0.11	NS
GPR132	9.33±0.61	1.12±0.24	1.57E-05	0.76±0.09	8.59E-06	0.78±0.22	0.92±0.01	NS
TRAF1	9.18±1.12	2.27±0.50	1.34E-03	0.95±0.20	3.56E-04	0.92±0.18	0.87±0.12	NS
RASL11A	9.01±1.41	1.16±0.22	1.49E-03	0.85±0.12	1.17E-03	0.84±0.14	1.18±0.08	NS
CSF2	8.93±0.59	2.48±0.48	1.46E-04	1.82±0.24	2.93E-05	0.92±0.09	0.98±0.12	NS
SLC7A2	8.76±0.63	4.22±0.69	2.87E-03	2.01±0.35	8.76E-05	0.89±0.08	0.90±0.09	NS
NKX3-1	8.75±0.74	3.82±0.47	1.35E-03	0.98±0.12	4.59E-05	0.98±0.09	0.88±0.10	NS
MARCSL1	8.63±0.66	1.80±0.23	6.55E-05	1.81±0.12	5.25E-05	0.82±0.09	0.90±0.11	NS
IL1A	8.55±0.31	2.11±0.50	3.30E-05	0.95±0.10	3.74E-07	0.87±0.11	1.03±0.12	NS
GPR84	5.72±0.70	1.31±0.29	1.08E-03	1.02±0.22	6.49E-04	1.03±0.11	1.20±0.14	NS
IFIT1	5.50±0.61	1.46±0.38	1.33E-03	1.09±0.09	3.79E-04	1.05±0.22	1.10±0.24	NS
OLR1	5.39±0.82	1.45±0.39	4.75E-03	1.02±0.17	1.93E-03	0.97±0.20	0.99±0.18	NS
IL1B	5.24±0.34	1.15±0.19	4.28E-05	1.05±0.14	2.80E-05	0.99±0.09	1.26±0.09	NS
S100A3	5.18±0.40	1.26±0.12	8.51E-05	0.92±0.06	4.47E-05	0.88±0.03	1.16±0.08	NS
MMP11	4.97±0.53	1.05±0.46	1.40E-03	1.05±0.29	6.13E-04	1.04±0.15	1.18±0.18	NS
ICOSL	4.92±0.22	2.17±0.41	1.01E-03	1.06±0.08	3.34E-06	0.76±0.09	1.06±0.09	NS
CD83	4.91±0.64	1.24±0.25	1.79E-03	0.99±0.20	1.13E-03	0.99±0.11	1.07±0.17	NS
MMP19	4.66±0.68	1.23±0.21	2.93E-03	1.02±0.14	1.89E-03	0.92±0.06	1.18±0.25	NS
MMP14	4.64±0.44	2.40±0.38	8.75E-03	0.99±0.08	1.90E-04	0.91±0.10	1.03±0.19	NS
SLC1A2	4.61±0.75	1.28±0.18	4.96E-03	1.04±0.16	3.51E-03	0.97±0.21	1.05±0.07	NS
SOCS3	4.58±0.50	1.28±0.25	1.00E-03	1.13±0.13	5.29E-04	0.90±0.05	1.03±0.07	NS
SLC27A4	4.57±0.15	1.30±0.29	5.87E-05	1.04±0.15	3.19E-06	1.14±0.15	1.15±0.06	NS
CD40	4.56±0.46	1.16±0.20	4.94E-04	1.02±0.14	3.06E-04	0.96±0.05	0.92±0.07	NS
CKB	4.44±0.30	2.49±0.43	1.03E-02	1.98±0.19	4.52E-04	1.07±0.20	1.01±0.13	NS
CD69	4.27±0.49	2.39±0.53	3.99E-02	2.01±0.35	9.51E-03	0.72±0.13	1.03±0.14	NS
SLC2A6	4.20±0.49	2.04±0.31	1.01E-02	1.71±0.19	3.29E-03	0.80±0.05	0.87±0.07	NS
TNF	3.87±0.65	1.30±0.31	1.15E-02	1.00±0.08	4.52E-03	0.92±0.22	1.20±0.23	NS

B



Supplemental Figure 21

C

MDP and curdlan upregulated transcripts

	MDP					Curdlan		
	scr siRNA	C1orf106 siRNA		MAPK NFκB inhibitors		scr siRNA	C1orf106 siRNA	
Gene transcript	fold Δ	fold Δ	p value	fold Δ	p value	fold Δ	fold Δ	p value
MMP12	9.84±0.62	1.18±0.18	1.07E-05	0.96±0.09	7.68E-06	8.61±0.67	8.77±0.51	NS
CXCL1	9.44±0.73	2.47±0.39	1.54E-04	2.11±0.15	6.57E-05	8.17±0.77	9.32±0.84	NS
RELB	8.87±0.62	4.99±1.16	2.60E-02	3.58±0.10	1.54E-04	3.57±0.68	4.11±0.59	NS
MMP10	8.78±0.70	1.97±0.15	7.79E-05	0.82±0.09	2.90E-05	3.33±0.29	3.69±0.18	NS
CCL5	6.95±0.38	2.22±0.33	7.97E-05	1.86±0.16	1.72E-05	3.53±0.52	4.67±0.76	NS
NFKB2	5.26±0.70	1.38±0.25	2.02E-03	1.02±0.12	1.01E-03	2.20±0.22	2.65±0.43	NS
CXCL2	4.91±0.42	2.61±0.31	4.49E-03	1.30±0.28	3.72E-04	8.16±0.61	8.08±0.94	NS
NFKBIZ	4.74±0.57	1.29±0.24	1.35E-03	1.11±0.19	8.94E-04	8.99±1.09	8.27±0.51	NS
CXCL11	4.37±0.33	1.02±0.12	8.12E-05	0.91±0.13	6.98E-05	3.67±0.30	3.59±0.11	NS
TNFAIP3	4.13±0.39	2.28±0.37	1.33E-02	1.09±0.23	5.19E-04	3.75±0.24	3.20±0.23	NS
REL	3.83±0.14	1.09±0.15	1.17E-05	0.94±0.10	3.05E-06	3.16±0.39	3.73±0.19	NS
CSF1	2.37±0.21	1.24±0.33	2.86E-02	1.10±0.12	2.05E-03	4.09±0.54	4.56±0.43	NS

D

curdlan upregulated transcripts

	MDP					Curdlan		
	scr siRNA	INAVA siRNA		MAPK NFκB inhibitors		scr siRNA	INAVA siRNA	
Gene transcript	fold Δ	fold Δ	p value	fold Δ	p value	fold Δ	fold Δ	p value
MCLON3	1.76±0.49	2.03±0.43	NS	2.11±1.07	NS	13.9±4.96	15.7±3.78	NS
PPAP2B	0.93±0.06	1.27±0.17	NS	0.78±0.06	NS	8.60±0.85	8.79±1.10	NS
MPO	1.08±0.12	1.19±0.19	NS	1.01±0.13	NS	8.30±1.09	8.73±1.81	NS
EGR1	0.97±0.17	0.93±0.08	NS	0.90±0.24	NS	7.54±1.07	8.04±0.60	NS
PRDM1	1.22±0.14	1.01±0.26	NS	1.12±0.20	NS	4.60±0.39	4.17±0.38	NS
F5	0.91±0.12	0.85±0.11	NS	0.85±0.10	NS	3.97±0.27	3.70±0.24	NS
FABP4	0.90±0.09	1.10±0.25	NS	0.74±0.05	NS	3.80±0.39	4.40±0.67	NS
HSPA1A	0.98±0.13	0.88±0.07	NS	1.00±0.21	NS	3.76±0.40	4.04±0.37	NS
THBS1	0.96±0.19	1.01±0.27	NS	0.90±0.17	NS	3.67±0.63	4.42±0.46	NS
ADAMTS1	0.95±0.07	1.11±0.13	NS	1.13±0.16	NS	3.66±0.40	5.15±0.67	NS
EGR3	0.90±0.12	0.84±0.13	NS	0.88±0.09	NS	3.56±0.25	3.93±0.31	NS
HSPA1B	1.06±0.11	1.10±0.22	NS	0.98±0.12	NS	1.90±0.11	1.94±0.17	NS

Supplementary Figure 21. INAVA is required for optimal expression of a broad range of NOD2-induced transcripts. MDMs (n=4) were transfected with scrambled or INAVA siRNA, or pretreated for 1 h with 0.1 μM JNK inhibitor II, PD98059 (ERK inhibitor), SB202190 (p38 inhibitor) and BAY 11-7082 (NFκB inhibitor) (EMD Biosciences), then treated with 100μg/ml MDP (NOD2 ligand) or 100μg/ml curdlan (dectin ligand) for 4h. mRNA at 4h is normalized to scrambled siRNA-transfected, untreated cells. Fold mRNA change of indicated transcripts classified according to: **(A)** MDP-upregulated transcripts, **(C)** MDP- and curdlan-upregulated transcripts, and **(D)** curdlan-upregulated transcripts. Significance is compared to scr siRNA-transfected, MDP- or curdlan-treated cells. **(B)** MDMs (n=4) were treated for 4h with 0.1 μM JNK inhibitor II, PD98059, SB202190 and BAY11-7082. Cell death was assessed by flow cytometry staining with annexin V+SEM. 50-100 J/m^2 UV-treated cells are shown as a positive control. Tx, treatment; inh., inhibitors; NS, not significant. †, $p<1x10^{-4}$.

Supplemental Table 1

Primers for Main Figures

Gene	Primer sequence
INAVA common	FWD: 5' CCTTGGGAGAGAGGGCTTCG 3' REV: 5' GCAGAGTTACACCTGGCTGATGAT 3'
INAVA isoform 1	FWD: 5' ATGCTGCAAATGCCGAAGTTA 3' REV: 5' AGCTTCAGGACCTGTTTGTCC 3'
INAVA isoform 2	FWD: 5' ATTAGTTCAGGGAAGGACTTGCTC 3' REV: 5' CTGCCTACCTTGCCGTCCCT 3'
GAPDH	FWD: 5' GGCATGGACTGTGGTCATGAG 3' REV: 5' TGCACCACCAACTGCTTAGC 3'
p40phox	FWD: 5' GAGAGGTGAACTCAGCCTGG 3' REV: 5' TTCAAAGTCACTCTCGGCCC 3'
p47phox	FWD: 5' AGTACCGCGACAGACATCAC 3' REV: 5' CGCTCTCGCTCTTCTCTACG 3'
p67phox	FWD: 5' TGCCAAAAGGTGGGGACATT 3' REV: 5' CAAGAGAGCTGCCAGGAGAC 3'
p22phox	FWD: 5' CGAGCGGCATCTACCTACTG 3' REV: 5' GCTTGATGGTGCCTCCGAT 3'
gp91phox	FWD: 5' AAGTGCCCAAAGGTGTCCAA 3' REV: 5' CCCAACGATGCGGATATGGA 3'
NOS2	FWD: 5' CGCAGAGAACTCAGCCTCAT 3' REV: 5' TGCCTTGAGAACTTCGGGAC 3'
ATG5	FWD: 5' ACTGAAAGGGAAGCAGAACCAT 3' REV: 5' ATGCCATTTCAGTGGTGTGC 3'
ATG10	FWD: 5' CCGCAGTCCCATCATTCAGT 3' REV: 5' ATGATAACCCTCTCCGCTCG 3'
IRGM	FWD: 5' AGGCCTCACCTCCTACTGAG 3' REV: 5' CAGGTCCCACAACACCACAT 3'

Primers for Supplemental Figures

Gene	Primer sequence
ZNF281	FWD: 5' ATGACCACCATGGCACTGAG 3' REV: 5' TCTGGCTTTGGCCTTTTTGC 3'
KIF14	FWD: 5' TTTCCCTACAGGCGACCAC 3' REV: 5' GACCTGGAATGCTGAGGAACT 3'
DDX59	FWD: 5' CTCCCAAAACCCGGGGAAGT 3' REV: 5' TGACCCGCTCGTCAGGA 3'
CAMSAP2	FWD: 5' GGAGGTCAAAAGGCTCGTTATC3' REV: 5' GCCATCTGTCCCATCCTTCA 3'
GPR25	FWD: 5' CCAACCCGCTCATCTACCTC 3' REV: 5' AACGGAACACGGAACTGTCG 3'
KIF21B	FWD: 5' GCAATGACCGCAATGTCTTCTCT 3' REV: 5' CCCTCAGGACCTCCGACAAAG 3'
CACNA1S	FWD: 5' GGCTACTTTGGAGACCCCTG 3' REV: 5' GAGGCCAGGAAAGTGTCGAT 3'
ASCL5	FWD: 5' ATAACTTCTGCCGGGCTCTG 3' REV: 5' CGCATAGGCGTCGTAGTAGG 3'
TMEM9	FWD: 5' TCCCAGAAGGACTGTTGTAGC 3' REV: 5' CCACCACGGACAGGTAGATG 3'
IGFN1	FWD: 5' CAGCTGTACACCCAGGGTAA 3' REV: 5' AGCATTTTTCCCCTCTGGCA 3'
PKP1	FWD: 5' AGGAGGAACTCATTGCCGAC 3' REV: 5' AGCTCAGGTTCCTCAAGCAG 3'
TNNT2	FWD: 5' ATCCGGAATGAGCGGGAGAA 3' REV: 5' CCACTTTTCCGCTCTGTCTTC 3'
LAD1	FWD: 5' TGGAGGCCTTGCTTTCTAGC 3' REV: 5' TGGCAAGCCACCACCTTAAT 3'
MyD88	FWD: 5' CTATTGCCCCAGCGACATCC 3' REV: 5' GGCCTTCTAGCCAACCTCTTTT 3'
TRIF	FWD: 5' CCCTTGCCTATTCTGGAGCC 3' REV: 5' ACAGAAAGTTGGAGTGGCGT 3'
IL1B	FWD: 5' ACAGATGAAGTGCTCCTTCCA 3' REV: 5' GTCGGAGATTCGTAGCTGGAT 3'
TNF	FWD: 5' CCCCAGGGACCTCTCTCTAATC 3' REV: 5' GGTTTGCTACAACATGGGCTACA 3'
IL6	FWD: 5' CACCATCCTGAGGGAAGAGG 3' REV: 5' CGTCGGCACCCAAGAATT 3'
CXCL1	FWD: 5' TTGAGTCCCAACAGTCCACC 3' REV: 5' CTGGCTTAGAACAAAGGGGCT 3'
IL12p40	FWD: 5' CCTCACCCCCACCTCTCTAA 3' REV: 5' GGGCTGTTAAGAAGCCACCT 3'

IL4	FWD: 5’ CTTTGCTGCCTCCAAGAACAC 3’ REV: 5’ GCGAGTGTCCTTCTCATGGT 3’
IRF1	FWD: 5’ AAGGGGTGTGGCCTTTTTAGA 3’ REV: 5’ TGTCCCTGTTCACCCCCAAAG 3’
RANTES	FWD: 5’ TCATTGCTACTGCCCTCTGC 3’ REV: 5’ TCGGGTGACAAAGACGACTG 3’
TRAF1	FWD: 5’ TAAAGTGGTGGCACCTGACC 3’ REV: 5’ ACCCTATCTTGCCTGGGACT 3’
IFIT1	FWD: 5’ CTGGCTAAGCAAAACCCTGC 3’ REV: 5’ AGCCTATGGAGGAAGGCTGT 3’
IFNB	FWD: 5’ TGCTCTGGCACAACAGGTAG 3’ REV: 5’ AGCCTCCCATTCAATTGCCA 3’
STAT1	FWD: 5’ TGCGCGCAGAAAAGTTTCATTT 3’ REV: 5’ CTACGCACAGCACGTTAGGT 3’
TBP	FWD: 5’ GTGACCCAGCATCACTGTTTC 3’ REV: 5’ GAGCATCTCCAGCACACTCT 3’
HOXA5	FWD: 5’ AGTCATGACAACATAGGCGGC 3’ REV: 5’ CGGGTCAGGTAACGGTTGAA 3’
NKX2-5	FWD: 5’ AAGTGTGCGTCTGCCTTTCC 3’ REV: 5’ TTTCAGGCTTTCTTTTCGGCT 3’
TNF promoter CHIP	FWD: 5’ GGACTCAACACAGCTTTTCCC 3’ REV: 5’ TGGGAGGGGCTTCAGAAA 3’
IL1B promoter CHIP	FWD: 5’ CGTGGGAAAATCCAGTATTTTAATG 3’ REV: 5’ CAAATGTATCACCATGCAAATATGC 3’
IL8 promoter CHIP	FWD: 5’ GACAGCAGAGCACACAAGCT 3’ REV: 5’ TCAGGAAGGCTGCCAAGA 3’
IL12 promoter CHIP	FWD: 5’ ATCATCTCCCCAGGTCTGTG 3’ REV: 5’ TGCACCTCTCCCCACATT 3’
IL10 promoter CHIP	FWD: 5’ CAGAAGTTCATGTTCAAC 3’ REV: 5’ TCCTTCTCTAACCTCTCT 3’
YWHAQ	FWD: 5’ CCACGGTGCTGGAATTGTTG 3’ REV: 5’ TTCGATCATCACCACACGCA 3’
ICAM1	FWD: 5’ TCTTCCTCGGCCTTCCCATA 3’ REV: 5’ AGGTACCATGGCCCCAAATG 3’
TLR2	FWD: 5’ GGCCAGCAATTACCTGTGTG 3’ REV: 5’ AGGCGGACATCCTGAAACCT 3’
CDC42EP2	FWD: 5’ CCTTGGGCCAACTTTGTTCG 3’ REV: 5’ CAGCCAGAGGAAACACTCGT 3’
A20	FWD: 5’ CAGAAGAAACTCAACTGGTGTCG 3’ REV: 5’ GCCGTCACCGTTCGTTTT 3’
GPR132	FWD: 5’ GCCGCTGCCTTTTCCTACTA 3’ REV: 5’ CGTGGCCAGCACGTAGATAA 3’
RASL11A	FWD: 5’ AGCATGTCCGGGCACTTTC 3’ REV: 5’ GCACGATCATTGCGCTCTTG 3’
CSF2	FWD: 5’ GGGAGCATGTGAATGCCATC 3’ REV: 5’ GGCTCCTGGAGGTCAAACAT 3’

SLC7A2	FWD: 5’ AATATGTCGTCGCAGCTGGT 3’ REV: 5’ CTGCCTCTTACTCACTCTGGC 3’
NKX3-1	FWD: 5’ CCGAGCCAGAAAGGCACTTG 3’ REV: 5’ CTTAGGGGTTTGGGGAAGCC 3’
MARCSL1	FWD: 5’ GGCTACAGAGCCATCCACTC 3’ REV: 5’ TGACCTCACAAGGACAGCAC 3’
IL1A	FWD: 5’ CTTCTGGGAAACTCACGGCA 3’ REV: 5’ AGCACACCCAGTAGTCTTGC 3’
GPR84	FWD: 5’ AGGACTGCTCTTTGGGTGAG 3’ REV: 5’ GTTCCACATGATAGAGGCTGAGT 3’
OLR1	FWD: 5’ GCGACTCTAGGGGTCCTTTG 3’ REV: 5’ CCTGGGATAATTGCATGCCC 3’
S100A3	FWD: 5’ CTTCAAGGACTGCCCCTCAG 3’ REV: 5’ CTGATCGCAGAAGACCTGGG 3’
MMP11	FWD: 5’ GATCGACTTCGCCAGGTACT 3’ REV: 5’ CCCCGATAGTCCAGGTCTCA 3’
ICOSL	FWD: 5’ CGTGTACTGGATCAATAAGACGG 3’ REV: 5’ TGAGCTCCGGTCAAACGTGGCC 3’
CD83	FWD: 5’ GAACGCGCGGGCATAAAAG 3’ REV: 5’ AGGGCAAGTCCACATCTTCG 3’
MMP19	FWD: 5’ TCGCCTCGAACACAATGGAT 3’ REV: 5’ CTTCTTGGGGAAGCCAGGAG 3’
MMP14	FWD: 5’ CGCTGCCATGCAGAAGTTTT 3’ REV: 5’ TCATGGCCTTCATGGTGTCT 3’
SLC1A2	FWD: 5’ GGTGATGTCAGCTCTCGACG 3’ REV: 5’ CAGGGAGGGATTGCAAGGTT 3’
SOCS3	FWD: 5’ ACCTGAACTCGCACCTCCTAC 3’ REV: 5’ CAACCCCTGGTTTGTGCA 3’
SLC27A4	FWD: 5’ ATTGGTGAACTGTGCCGCTA 3’ REV: 5’ GAAGTTGCCCAGGCTACAGT 3’
CD40	FWD: 5’ GCTGCTGAATGATGGGTATGG 3’ REV: 5’ GTCACCACTCTTCGAGCTGT 3’
CKB	FWD: 5’ TCATCGAGATGGAACAGCGG 3’ REV: 5’ CCAAGGGTGACGGAAGTCTC 3’
CD69	FWD: 5’ GAGCTGGACTTCAGCCCAAA 3’ REV: 5’ CCACTTCCATGGGTGACCAG 3’
SLC2A6	FWD: 5’ CCTGCAGTCCATCTTCGACA 3’ REV: 5’ AAACATGATGGCCGCTGAGA 3’
MMP12	FWD: 5’ AACCAACGCTTGCCAAATCC 3’ REV: 5’ TTTCCCACGGTAGTGACAGC 3’
RELB	FWD: 5’ GCTCTACTTGCTCTGCGACA 3’ REV: 5’ GGCCTGGGAGAAGTCAGC 3’
MMP10	FWD: 5’ CCTGGGTTTTCCTCCAACCA 3’ REV: 5’ TCTAGGGAAGCCTTGCTCCA 3’
CCL5	FWD: 5’ GACACCACACCCTGTGCT 3’ REV: 5’ TACTCCTTGATGTGGGCACG 3’

NFKB2	FWD: 5’ ACACCGTTGTACAAAGATACGC 3’ REV: 5’ GCCCGGCTCTGTCTAGTG 3’
CXCL2	FWD: 5’ CGTTCTCGGAGAGCCACAG 3’ REV: 5’ AGGGGCGCTCCTGCT 3’
NFKBIZ	FWD: 5’ CCTGGGAGCATGATTGTGGA 3’ REV: 5’ AAGTAGCTCAGGTTGAGCGG 3’
CXCL11	FWD: 5’ GTTCAAGGCTTCCCCATGTTC 3’ REV: 5’ ATAAGCCTTGCTTGCTTCGAT 3’
TNFAIP3	FWD: 5’ GCATACAACTGAAACGGGGC 3’ REV: 5’ GGGGTGTGATCTCTCTTGGC 3’
REL	FWD: 5’ CGAACCCAATTTATGACAACCG 3’ REV: 5’ TTTTGTTTCTTTGCTTTATTGCCG 3’
CSF1	FWD: 5’ GGCGTTGGGCACTATCCAAG 3’ REV: 5’ ATTCCGACTATGCCCGGCTC 3’
MCLON3	FWD: 5’ GCTCTCCTCCCGTCTGACTCT 3’ REV: 5’ CTGCAGCTACTCACAACTACCT 3’
PPAP2B	FWD: 5’ AGGATTTGCTCAAGGAGCCC 3’ REV: 5’ AGGGAGAGCGTCGTCTTAGT 3’
MPO	FWD: 5’ TGGGGGTTCCCTTCTTCTCT 3’ REV: 5’ AGGACAGCTGGAGCAGCA 3’
EGR1	FWD: 5’ CTGACCGCAGAGTCTTTTCCT 3’ REV: 5’ GAGTGGTTTGGCTGGGGTAA 3’
PRDM1	FWD: 5’ AGCCAGACGGTTAACACAGA 3’ REV: 5’ CTCTTGCCTCTCCGCAACA 3’
F5	FWD: 5’ CTCCGGGCTGTCCCAG 3’ REV: 5’ TAGAACTGCCTTAGCTGTGCC 3’
FABP4	FWD: 5’ TGGGCCAGGAATTTGACGAA 3’ REV: 5’ CACATGTACCAGGACACCCC 3’
HSPA1A	FWD: 5’ GAGCAGGTGTGTAACCCCAT 3’ REV: 5’ TGAAGCTCCAAAACAAAAACAGC 3’
THBS1	FWD: 5’ CCATGCTTATTTGTTCTCTACTGGC 3’ REV: 5’ TAAGCCTAGGCCTGAGCAAC 3’
ADAMTS1	FWD: 5’ CTCCAACTTTGCGCTGGAAG 3’ REV: 5’ AGCTCCCGGAGTCACTAAGA 3’
EGR3	FWD: 5’ CCGGTGACCATGAGCAGTTT 3’ REV: 5’ TCGTTGGTCAGACCGATGTC 3’
HSPA1B	FWD: 5’ GAGCAGGTGTGTAACCCCAT 3’ REV: 5’ ACAGCAGCAAAGTCCTTGAGT 3’