SUPPLEMENTAL DATA

A significant role for tumor necrosis factor in *Nlrp3* inflammasomopathies

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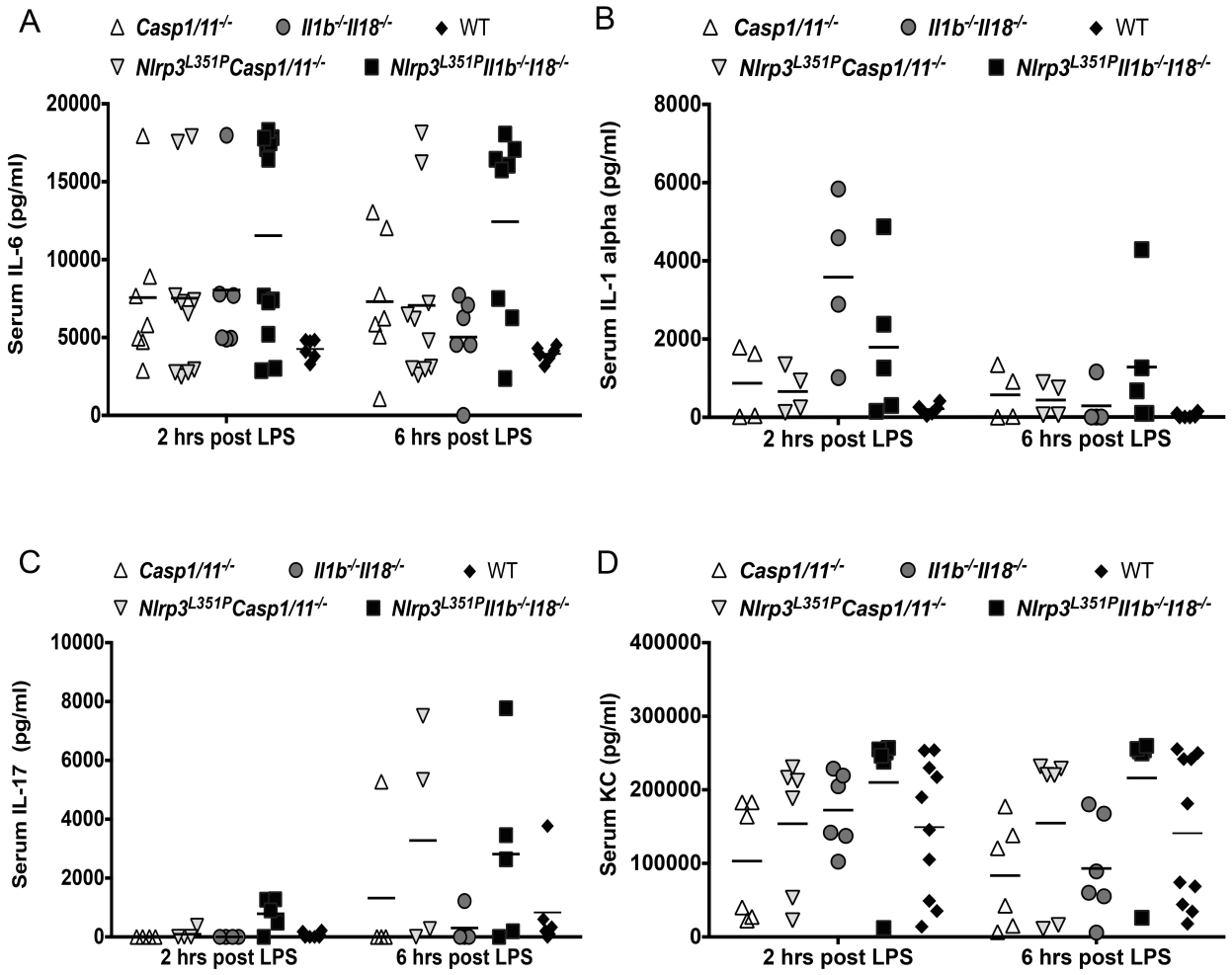
Supplemental Table 1. Sequences of primers used for quantitative PCR

Gene	Species	Sequence
Arg1	mouse	FWD 5'-CTC CAA GCC AAA GTC CTT AGA G-3' REV 5'-AGG AGC TGT CAT TAG GGA CAT C-3'
ASC	mouse	FWD 5'-CTT GTC AGG GGATGA ACT CAA AA-3 REV 5'-GCC ATA CGA CTC CAG ATA GTA GC-3'
pro-Caspase 1	mouse	FWD 5'-ACA AGG CAC GGG ACC TAT G-3' REV 5'-TCC CAG TCA GTC CTG GAA ATG-3'
CXCL1	mouse	FWD 5'-CTG GGA TTC ACC TCA AGA ACA TC-3' REV 5'- CAG GGT CAA GGC AAG CCT C-3'
CXCL2	mouse	FWD 5'-CCA ACC ACC AGG CTA CAG G-3' REV 5'- GCG TCA CAC TCA AGC TCT G-3'
F4/80	mouse	FWD 5'-TGA CTC ACC TTG TGG TCC TAA-3' REV 5'-CTT CCC AGA ATC CAG TCT TTC C-3'
iNOS	mouse	FWD 5'-GTT CTC AGC CCA ACA ATA CAA GA-3' REV 5'-GTG GAC GGG TCG ATG TCA C-3'
Ly6c	mouse	FWD 5'-GCA GTG CTA CGA GTG CTA TGG-3' REV 5'-ACT GAC GGG TCT TTA GTT TCC TT-3'
MPO	mouse	FWD 5'-AGT TGT GCT GAG CTG TAT GGA-3' REV 5'-CGG CTG CTT GAA GTA AAA CAG G-3'
NLRP3	mouse	FWD 5'- ATT ACC CGC CCG AGA AAG G-3' REV 5'- TCG CAG CAA AGA TCC ACA CAG-3'
pro-IL-1β	mouse	FWD 5'-GAA ATG CCA CCT TTT GAC AGT G-3' REV 5'-CTG GAT GCT CTC ATC AGG ACA-3'
pro-IL-18	human	FWD 5'-TCT TCA TTG ACC AAG GAA ATC GG-3' REV 5'-TCC GGG GTG CAT TAT CTC TAC-3'

Supplemental Table 2. NOMID patient characteristics and clinical symptoms

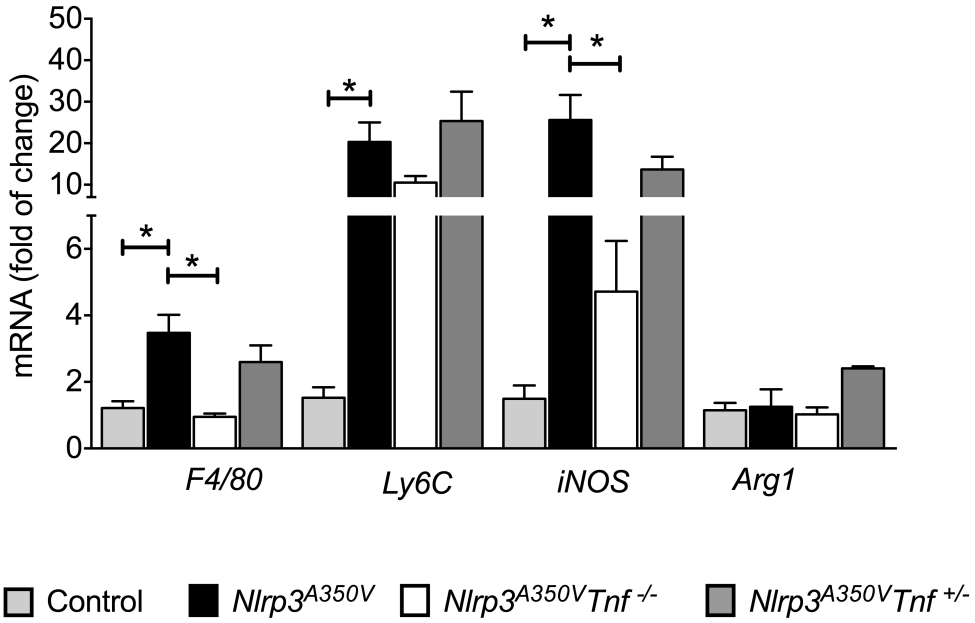
#	Visit	Age (yrs)	M / F	Race	Remission	CRP (mg/dl)	Joint disease	Eye disease	Hearing loss	Developmental delay	Aseptic Meningitis
1	Baseline	3.4	M	Asian	n/a	7.24	No	Conjunctivitis	Mild	No	Yes
	Follow-up	6.4			Yes	0.11					
2	Baseline	1.8	M	Caucasian	n/a	5.62	Patellar overgrowth, mild contractures	Conjunctivitis	No	No	Yes
	Follow-up	5.2			Yes	0.03					
3	Baseline	7.0	F	Caucasian	n/a	3.90	No	Conjunctivitis	Mild-moderate	No	Yes
	Follow-up	8.5			Yes	0.44					
4	Baseline	5.6	M	Asian	n/a	3.19	Bony overgrowth	Conjunctivitis	Severe	No	Yes
	Follow-up	9.7			Yes	1.66					
5	Baseline	15.9	F	Hispanic/ White	n/a	4.81	Patellar overgrowth	Conjunctivitis blindness	Severe	Yes (s/p stroke)	Yes
	Follow-up	16.4			No	2.32					
6	Baseline	8.5	M	Hispanic/ White	n/a	4.90	No	Conjunctivitis h/o transient uveitis	Severe	No	Yes
	Follow-up	16.1			Yes	0.27					

n/a, not applicable

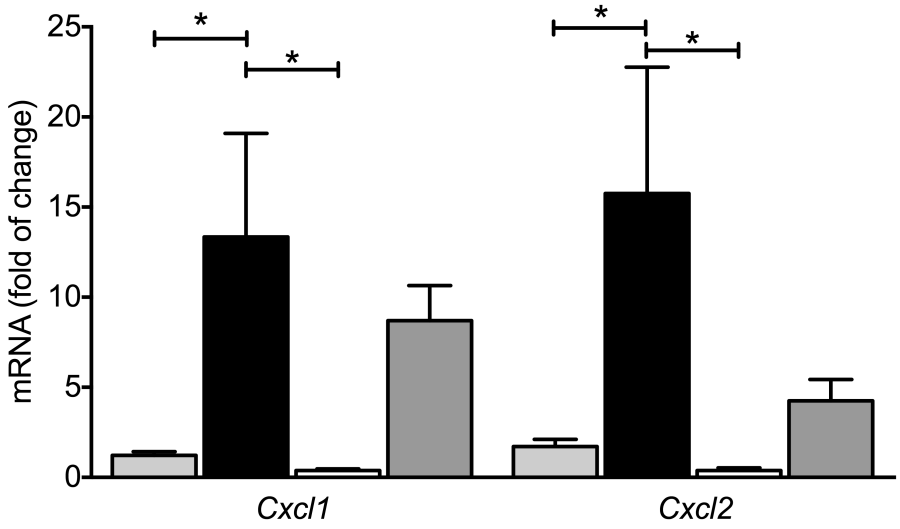


Supplemental Figure 1. Pro-inflammatory cytokines are not significantly elevated in *Nlrp3^{L351P} Il1b$^{-/-}$ Il18$^{-/-}$* mice following stimulation with LPS. Serum IL-6 (A), IL-1 alpha (B), IL-17 (C) and KC (D) showed no significant differences in *Nlrp3^{L351P} Il1b$^{-/-}$ Il18$^{-/-}$* mice at both 2 and 6 hours post LPS (5 µg/g) as compared to *Nlrp3^{L351P} Casp1/11$^{-/-}$* mice. Each point is representative of an individual mouse, mean is represented by horizontal bar. Kruskal-Wallis test and Dunn's Multiple Comparison Test was used to determine statistical significance. Data represent mean ± SEM.

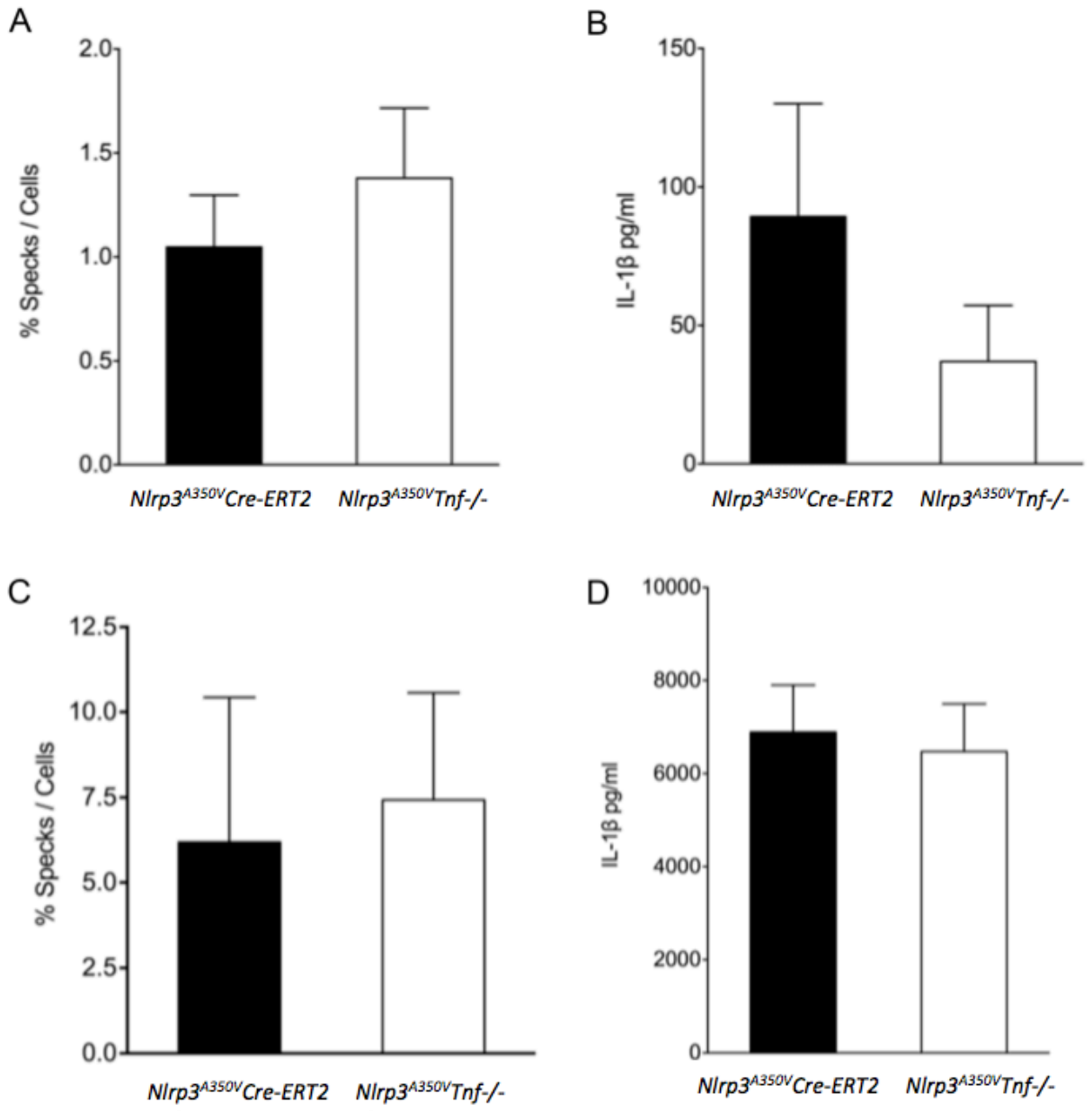
A



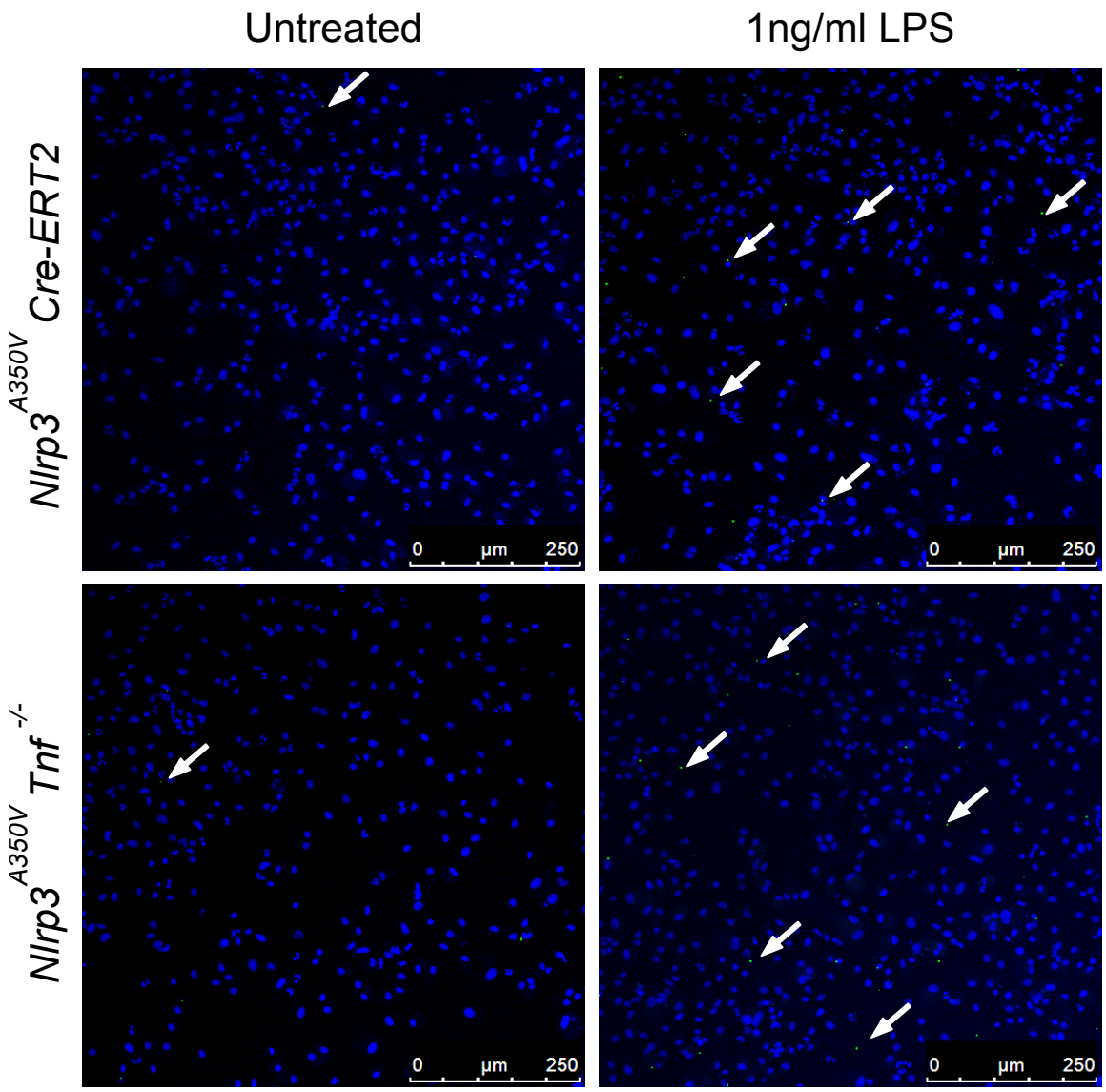
B



Supplemental Figure 2. Knockout of TNF reduces mRNA expression of inflammatory macrophages and neutrophil markers in skin. Expression of *F4/80 and iNos* was significantly raised in *Nlrp3*^{A350V} mice as compared to littermate controls and was attenuated in *Nlrp3*^{A350V} *Tnf* $^{-/-}$ mice, while a similar trend was seen for *Ly6c*. No significant differences were seen in expression of *Arg1 (*A). *Cxcl1* and *Cxcl2* expression is similarly elevated in *Nlrp3*^{A350V} mice and significantly reduced in *Nlrp3*^{A350V} *Tnf* $^{-/-}$ animals (B) (n=12 for control, *Nlrp3*^{A350V} and *Nlrp3*^{A350V} *Tnf* $^{-/-}$ mice and n=8 for *Nlrp3*^{A350V} *Tnf* $^{+/-}$ and *Tnf* $^{-/-}$ animals for each marker). Kruskal-Wallis test and Dunn's Multiple Comparison Test was used to determine statistical significance. Data represent mean ± SEM.



Supplemental Figure 3. No significant differences in ASC specking or mature IL-1β with or without addition of LPS comparing *Nlrp3^{A350V}CreT and Nlrp3^{A350V} Tnf$^{-/-}$* BMDCs. Inflammasome activation, quantified by ASC specking revealed no significant difference in the percentage of ASC specks/nuclei in *Nlrp3^{A350V} Tnf $^{-/-}$* and *Nlrp3^{A350V}CreT* cells (A) (n=3 mice per group with 6 images taken per well at 10X magnification). Mature IL-1β was not significantly reduced in supernatants from *Nlrp3^{A350V} Tnf $^{-/-}$* and *Nlrp3^{A350V}CreT* cells (B) (n=8 mice per group). Likewise, addition of 1ng/ml LPS revealed no significant difference in the percentage of ASC specks/nuclei (C) (n=3 mice per group with 6 images taken per well at 10X magnification). IL-1β had similar values in supernatants from *Nlrp3^{A350V} Tnf$^{-/-}$* and *Nlrp3^{A350V}CreT* cells (D) (n=8 mice per group). Student's unpaired t-test (2 tailed) was utilized for statistical analysis. Data represent mean ± SEM.



Supplemental Figure 4. Visualization of ASC specks in BMDC's from *Nlrp3^{A350V}CreT and Nlrp3^{A350V} Tnf$^{-/-}$* cells. ASC specks were stained and represented as green specks as indicated by the white arrows above. Stimulation with LPS (1ng/ml) similarly increased the number of ASC specks in *Nlrp3^{A350V}CreT and Nlrp3^{A350V} Tnf$^{-/-}$* cells. Images are representative of three mice per group with 6 images taken per well at 10x magnification. Scale bar represents length of 250um.