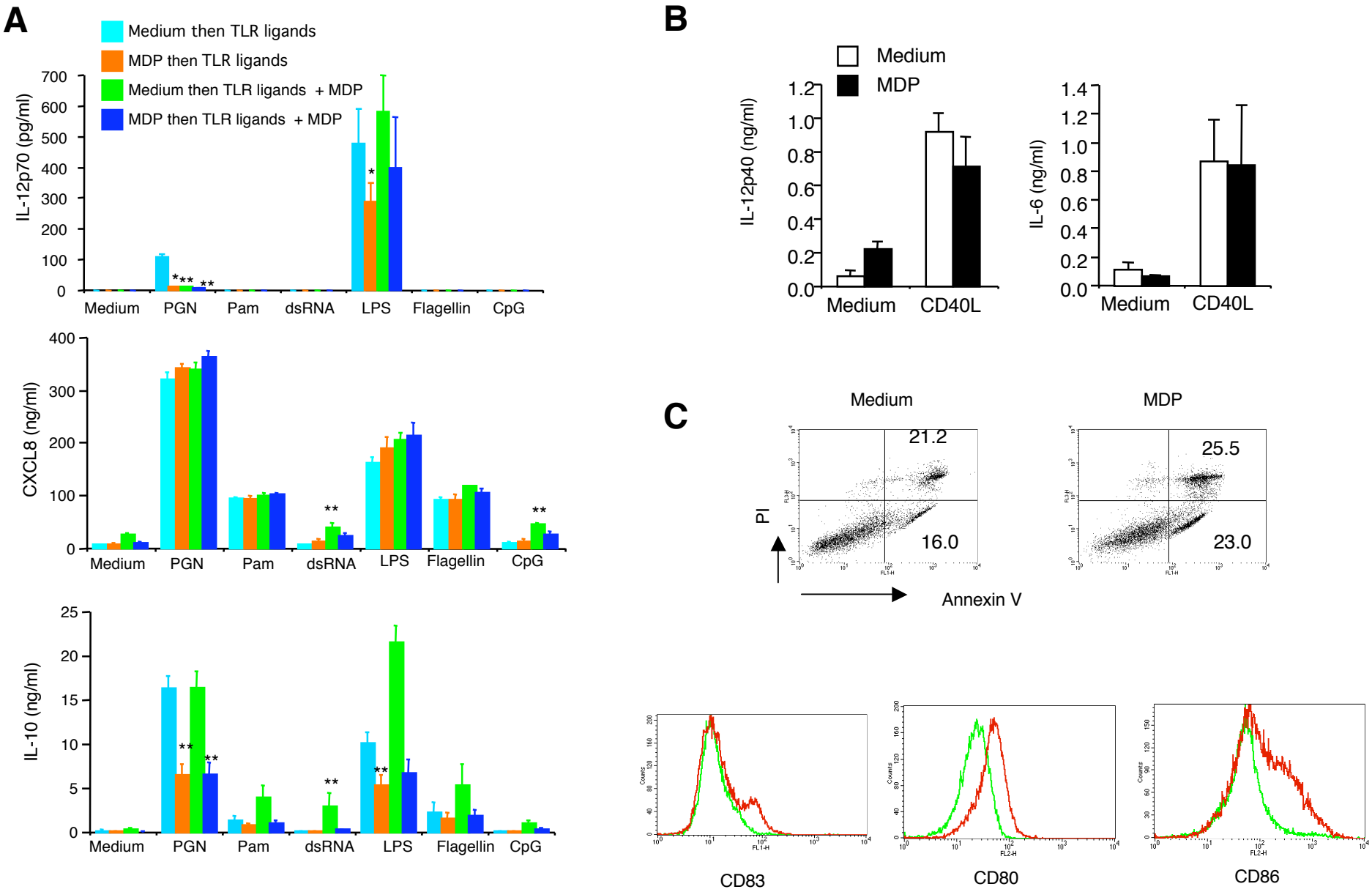
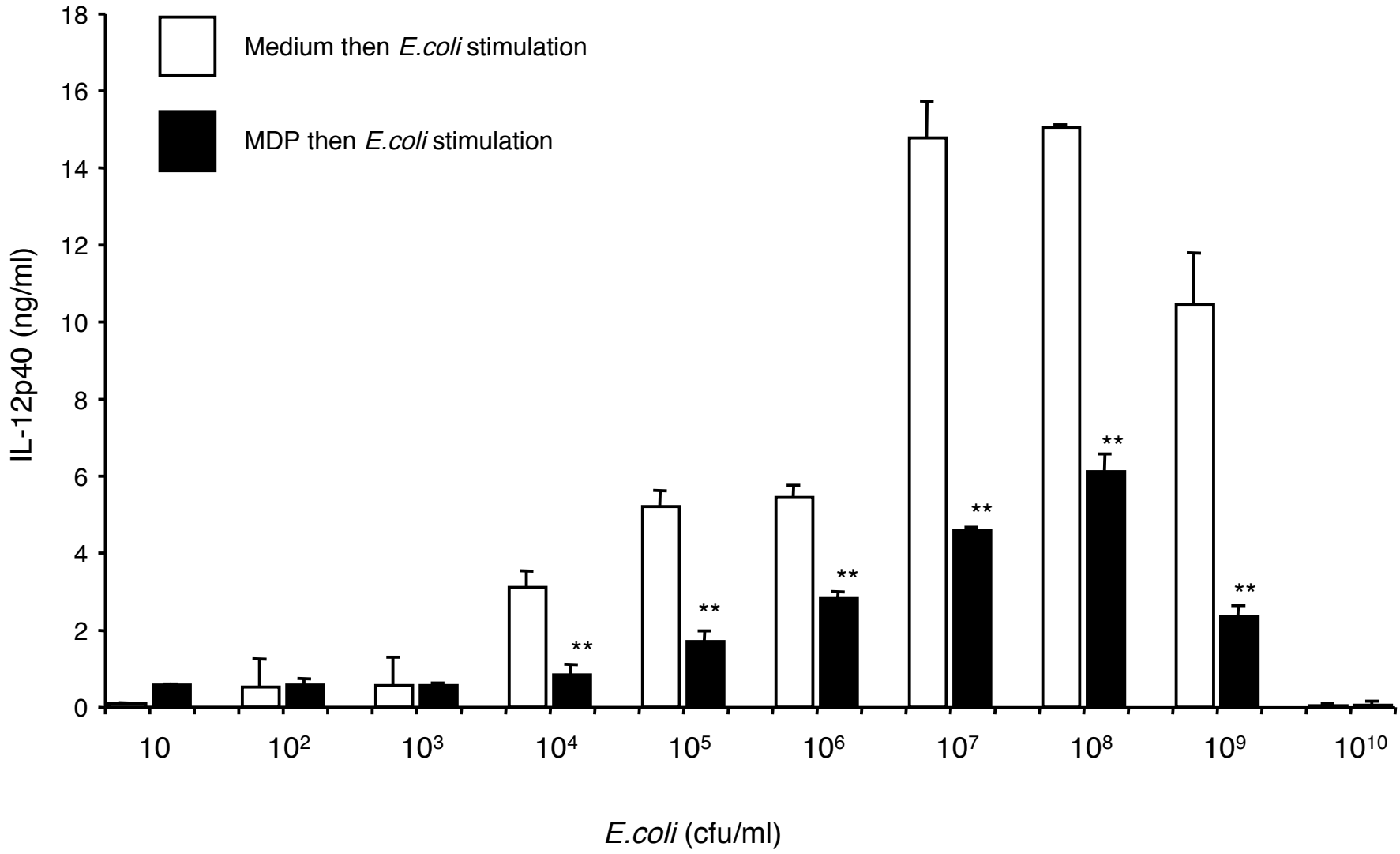
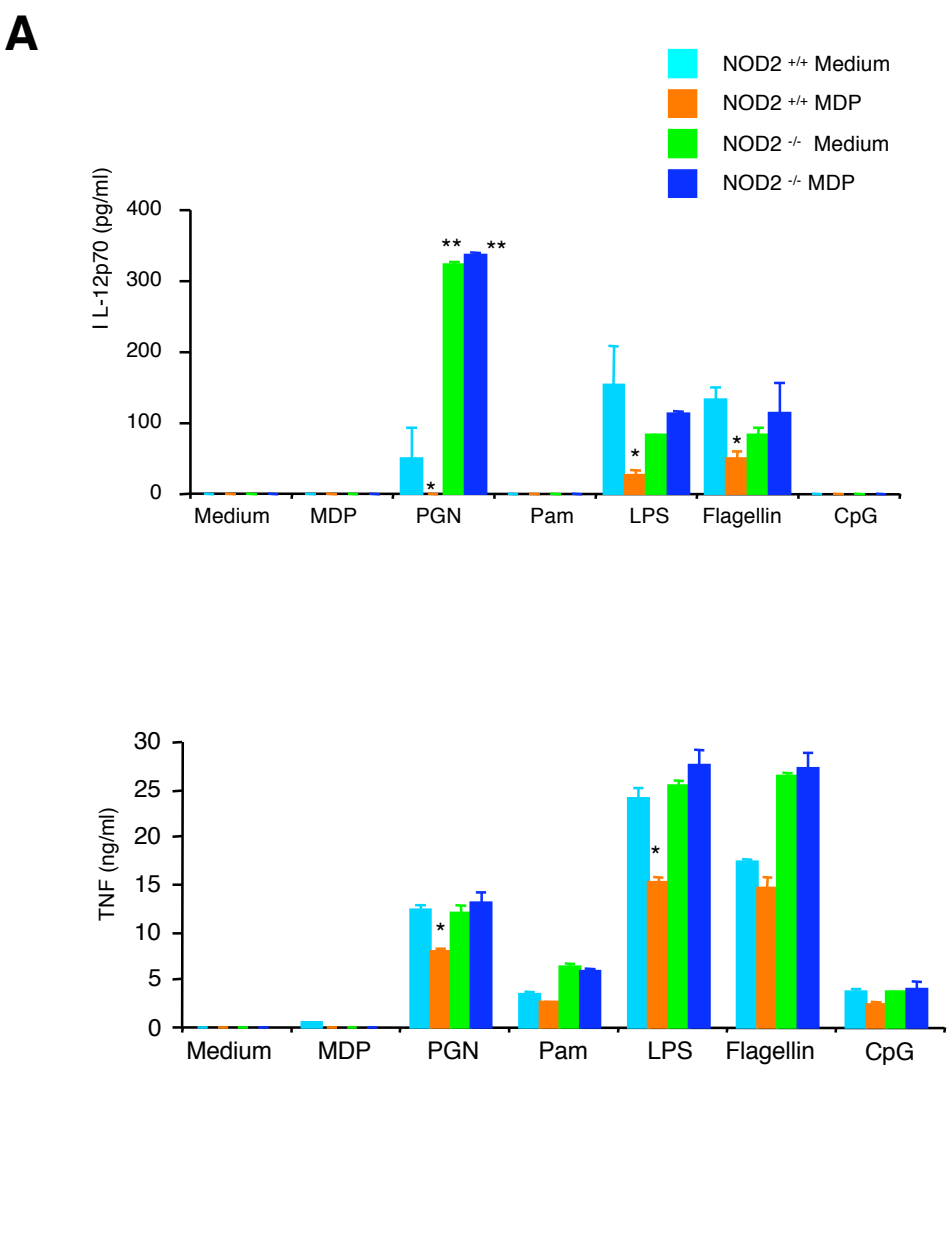
Supplemental Figure 1

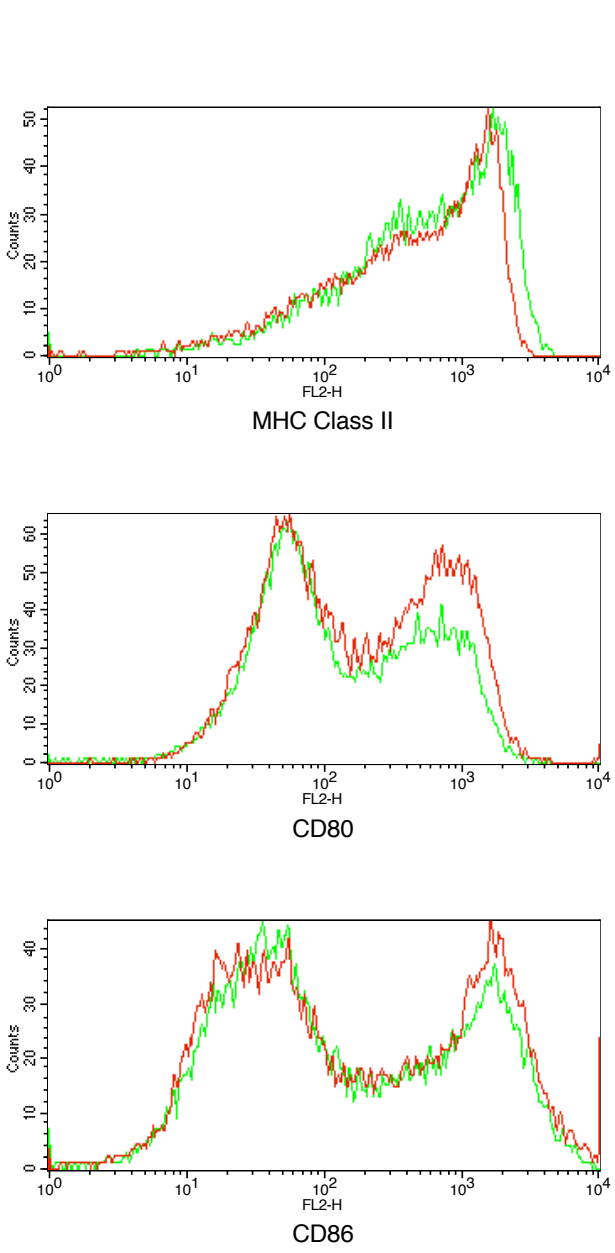


Supplemental Figure 2

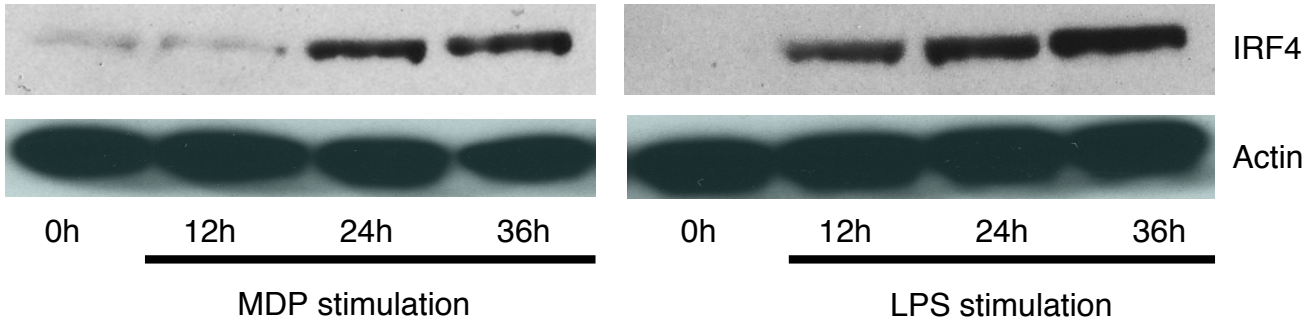


Supplemental Figure 3

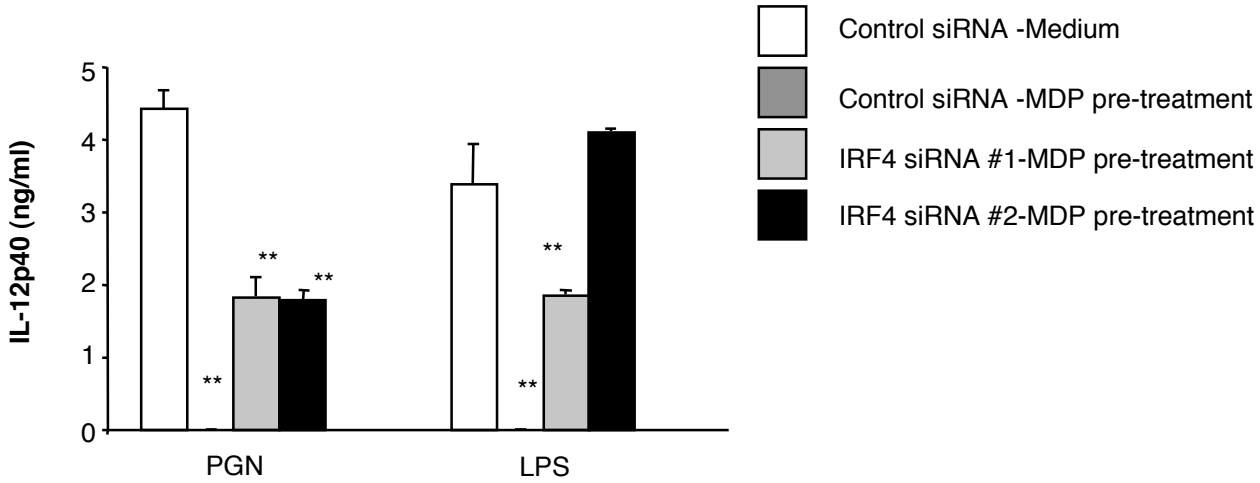




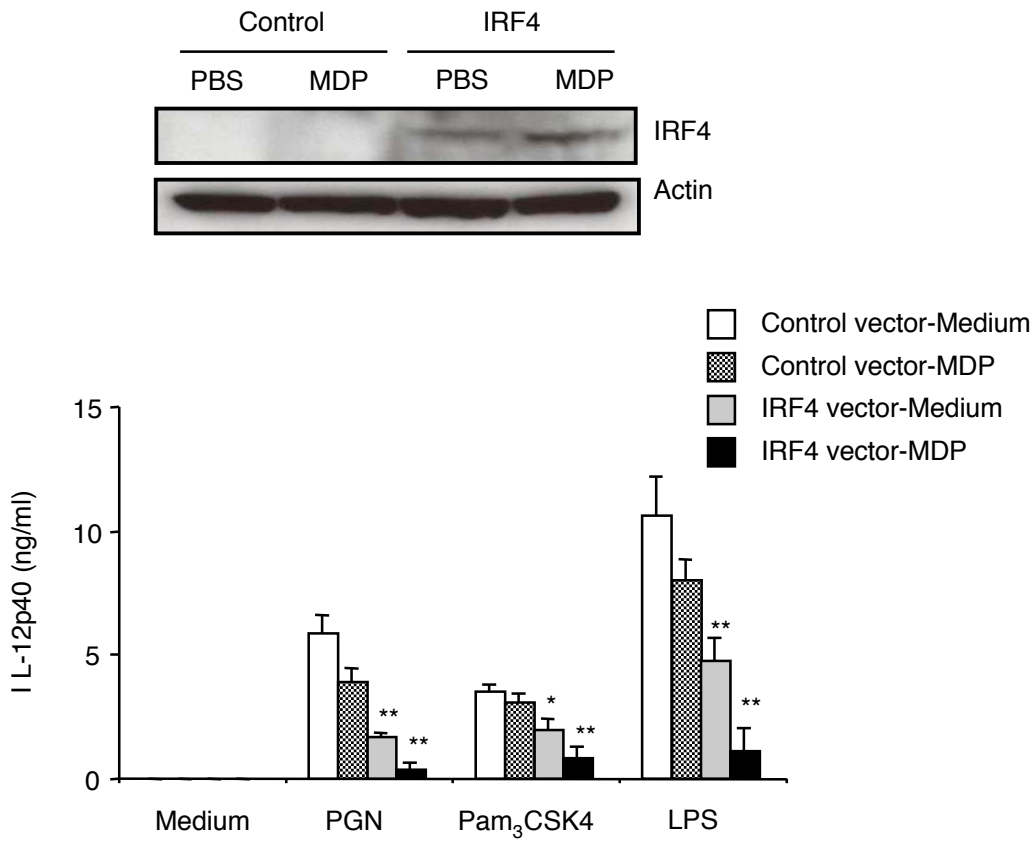
A


IRF4
Actin
0h
12h
24h
36h
0h
12h
24h
36h
MDP stimulation
LPS stimulation

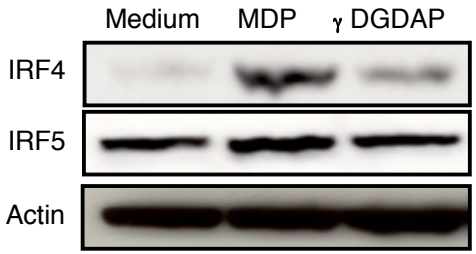
B


IL-12p40 (ng/ml)
0
1
2
3
4
5
**
**
**
**
PGN
LPS
Control siRNA -Medium
Control siRNA -MDP pre-treatment
IRF4 siRNA #1-MDP pre-treatment
IRF4 siRNA #2-MDP pre-treatment

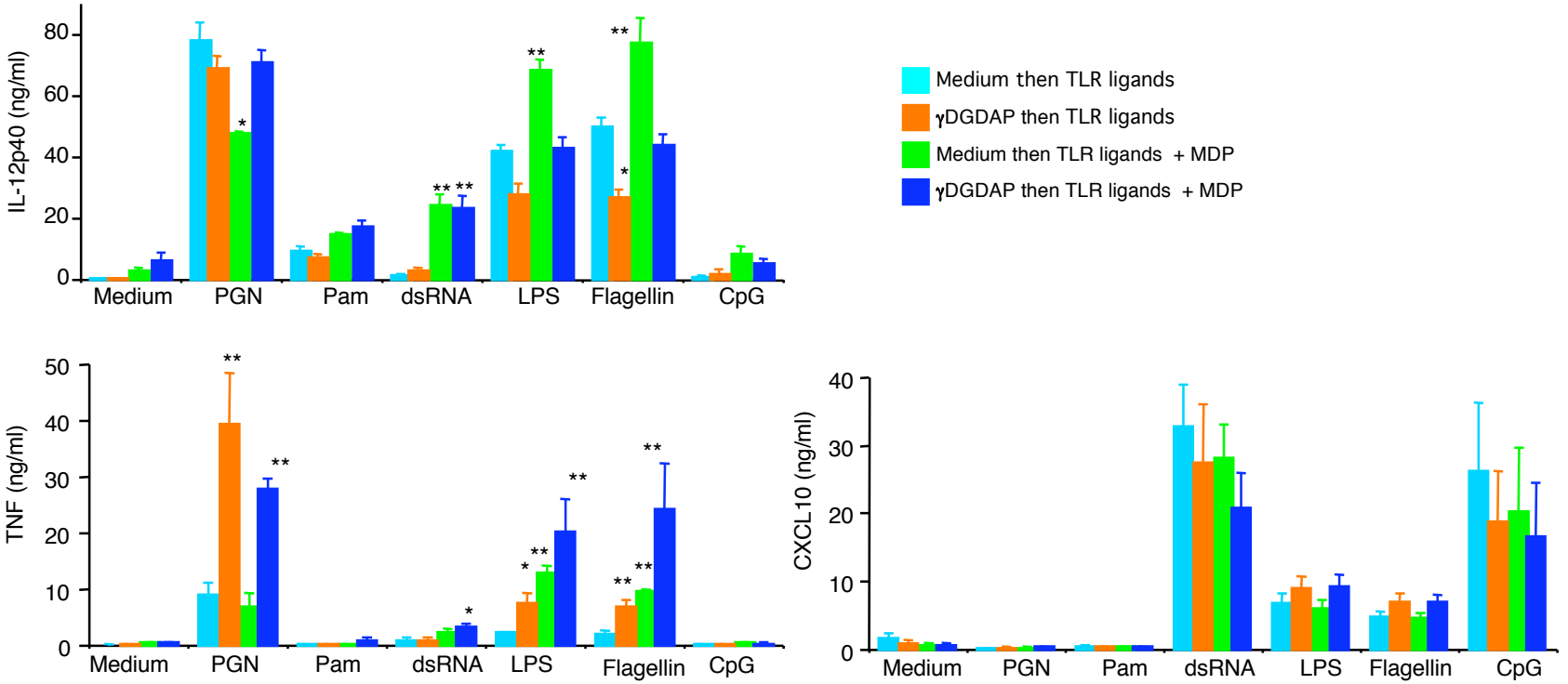
Supplemental Figure 5



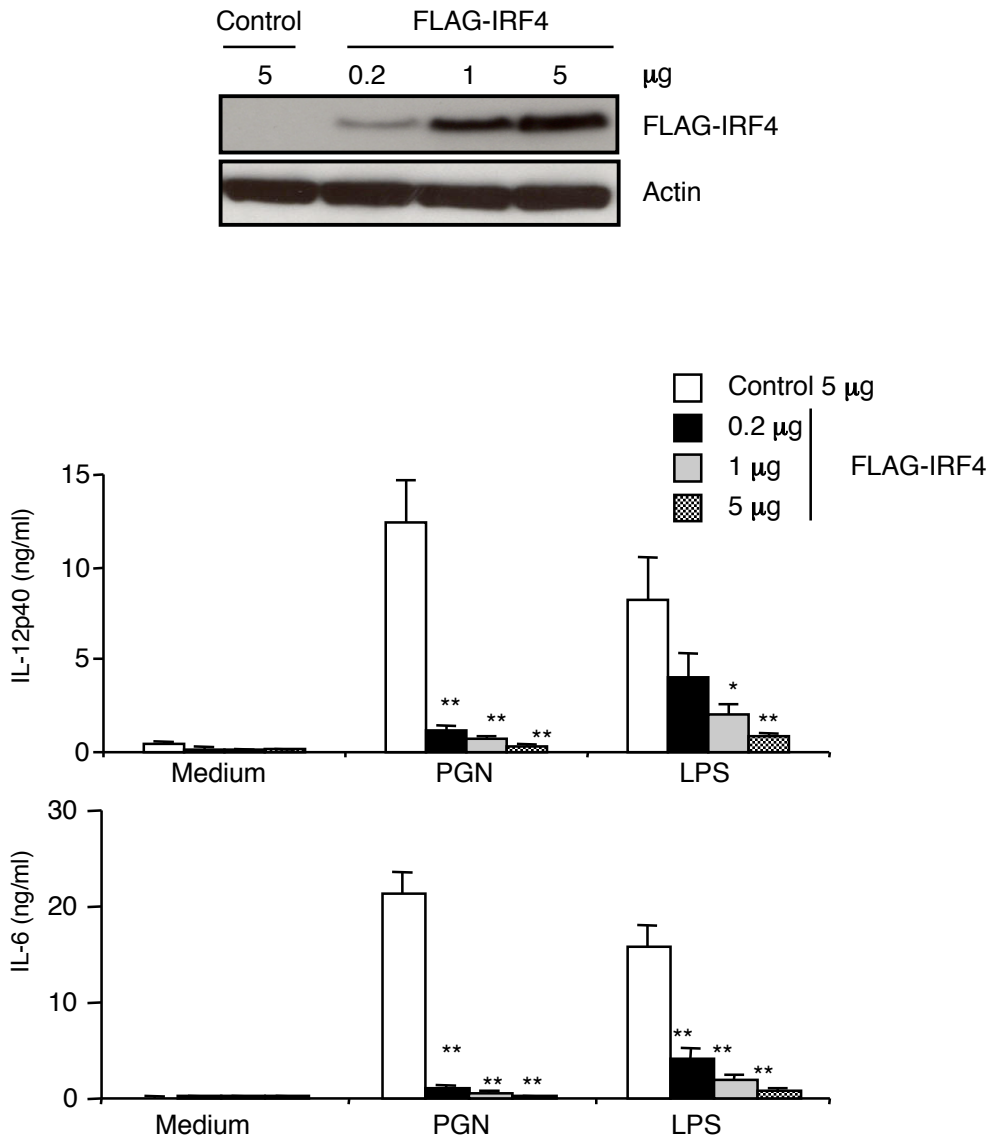
A


Medium
MDP
γ DGDAP
IRF4
IRF5
Actin

B


IL-12p40 (ng/ml)
TNF (ng/ml)
CXCL10 (ng/ml)
Medium
PGN
Pam
dsRNA
LPS
Flagellin
CpG
Medium then TLR ligands
γDGDAP then TLR ligands
Medium then TLR ligands + MDP
γDGDAP then TLR ligands + MDP

Supplemental Figure 7



Supplemental Figure Legends

Supplemental Figure 1. NOD2 pre-stimulation does not induce cell exhaustion or cell death. (A) IL-12p70, CXCL8, and IL-10 production by human DCs pre-incubated with MDP or medium and then stimulated with TLR ligands in combination with MDP as described in Figure 6A. * $P<0.05$, ** $P<0.01$ when compared with the concentrations of cytokines produced by DCs pre-incubated with medium and stimulated with TLR ligands (light blue bar). (B) Production of IL-12p40 and IL-6 in human DCs pre-incubated with MDP or medium and then stimulated with CD40 ligands (10μg/ml). (C) Human DCs stimulated with MDP or medium for 24 hrs were stained with FITC-conjugated Annexin V and propidium iodide (PI). The number in each quadrant shows the percentage of cells (top). PI-negative DCs were stained with FITC-conjugated anti-CD83, CD80, or CD86 Ab. Red and green colors show the expression in cells with MDP and medium, respectively.

Supplemental Figure 2. MDP pre-treatment suppresses the IL-12p40 production of human DCs in response to bacterial stimulation. Human DCs were pre-incubated with MDP or medium for 24hrs and then stimulated with various concentrations of *E.coli*. Culture supernatants were collected at 24hrs and analyzed for IL-12p40 production by ELISA. ** $P<0.01$ when compared with the concentrations of IL-12p40 produced by DCs pre-incubated with medium and stimulated with *E.coli*.

Supplemental Figure 3. MDP pre-stimulation suppresses TLR-induced cytokine secretion in mouse BMDC, but MDP stimulation has no effect on BMDC maturation markers. (A) $CD11c^+$ DCs ($1x10^6$/ml) derived from bone marrow cells from NOD2-intact ($NOD2^{+/+}$) and NOD2-deficient ($NOD2^{-/-}$) mice were pre-incubated with MDP (50μg/ml) or medium alone for 24hrs and stimulated with broad range of TLR ligands. Culture supernatants were collected at 24hrs and analyzed for cytokine production by ELISA. * $P<0.05$, ** $P<0.01$ when sups are compared to NOD2-intact DCs pre-incubated with medium and stimulated with TLR ligands (light blue bar). (B) $CD11c^+$ DCs

derived from NOD2-intact mice were pre-incubated with MDP (50μg/ml) or medium alone for 24hrs and then their co-stimulatory molecules were stained and analyzed by flow cytometry. Red and green colors show the expression in cells with MDP and medium, respectively.

Supplemental Figure 4. Silencing of IRF4 with IRF4-siRNA reverses MDP-mediated suppression. (A) Human DCs were stimulated with either MDP (10μg/ml) or LPS (1μg/ml). Cell lysates were collected at the indicated time points and IRF4 expression was assessed by immunoblot. (B) Human DCs were transfected with either control scrambled siRNA or two different IRF4-specific siRNA (2μg). After transfection, cells were washed and either left alone or pre-stimulated with MDP for 24hrs, and then stimulated with either PGN (10μg/ml) or LPS (1μg/ml) after washing. Culture supernatants were collected at 24hrs and analyzed for IL-12p40 production by ELISA. ** P <0.01 when compared with the concentrations of cytokines by DCs transfected with control siRNA and pre-incubated with medium (white bar).

Supplemental Figure 5. Susceptibility to MDP suppression is restored by IRF4 reconstitution. THP1 cells were transfected with a vector expressing FLAG-tagged human IRF4 or a control vector (1μg) by the Amaxa nucleofection method; the cells were then pre-incubated with medium or MDP for 24hrs and then stimulated with PGN, Pam_3CSK4, and LPS. Expression of IRF4 was analyzed 48hrs after the transfection by immuno-blotting with anti-FLAG Ab (top); culture supernatants were collected at 24hrs and analyzed for cytokine production by ELISA (bottom). * P <0.05, ** P <0.01 when compared with the concentrations of cytokines by cells transfected with a control vector and pre-incubated with medium (white bar).

Supplemental Figure 6. Pre-activation of NOD1 does not lead to reduced cytokine production upon re-stimulation with TLR ligands. Human DCs were pre-incubated with γDGDAP or medium for 24hrs and then stimulated with broad range of TLR ligands in combination with MDP for 24hrs. (A) Expression

of IRF4 and IRF5 in human DCs stimulated with MDP, γDGDAP, or medium for 24hrs. (B) Production of cytokines and chemokines measured by ELISA. * $P<0.05$, ** $P<0.01$ when compared with the concentrations of cytokines by DCs pre-incubated with medium and stimulated with TLR ligands (light blue bar).

Supplemental Figure 7. PGN-signaling is more sensitive to IRF4-mediated suppression than LPS-signaling. Expression of transfected IRF4 in human DCs (top); DCs prepared from three healthy donors were transfected with various doses of FLAG-tagged IRF4 cDNA; whole cell extract were prepared 24hrs after transfection followed by immuno-blotting. IL-12p40 (middle) and IL-6 (bottom) production by IRF4 transfected DCs; after transfection, cells were stimulated with PGN or LPS for 24hrs. Culture supernatants were analyzed for cytokine production by ELISA. ** $P<0.05$, ** $P<0.01$*, compared to control vector-transfected cells.