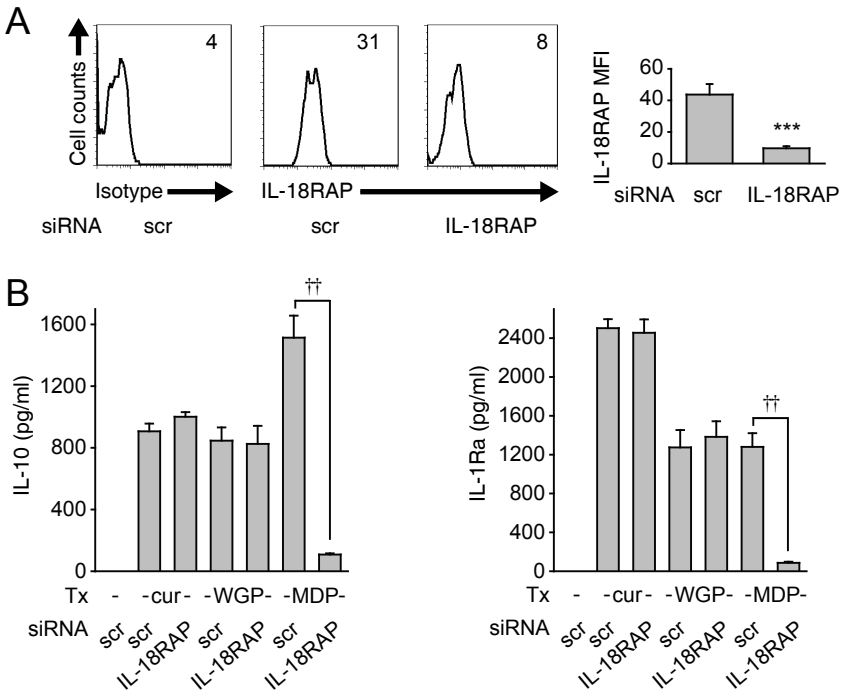
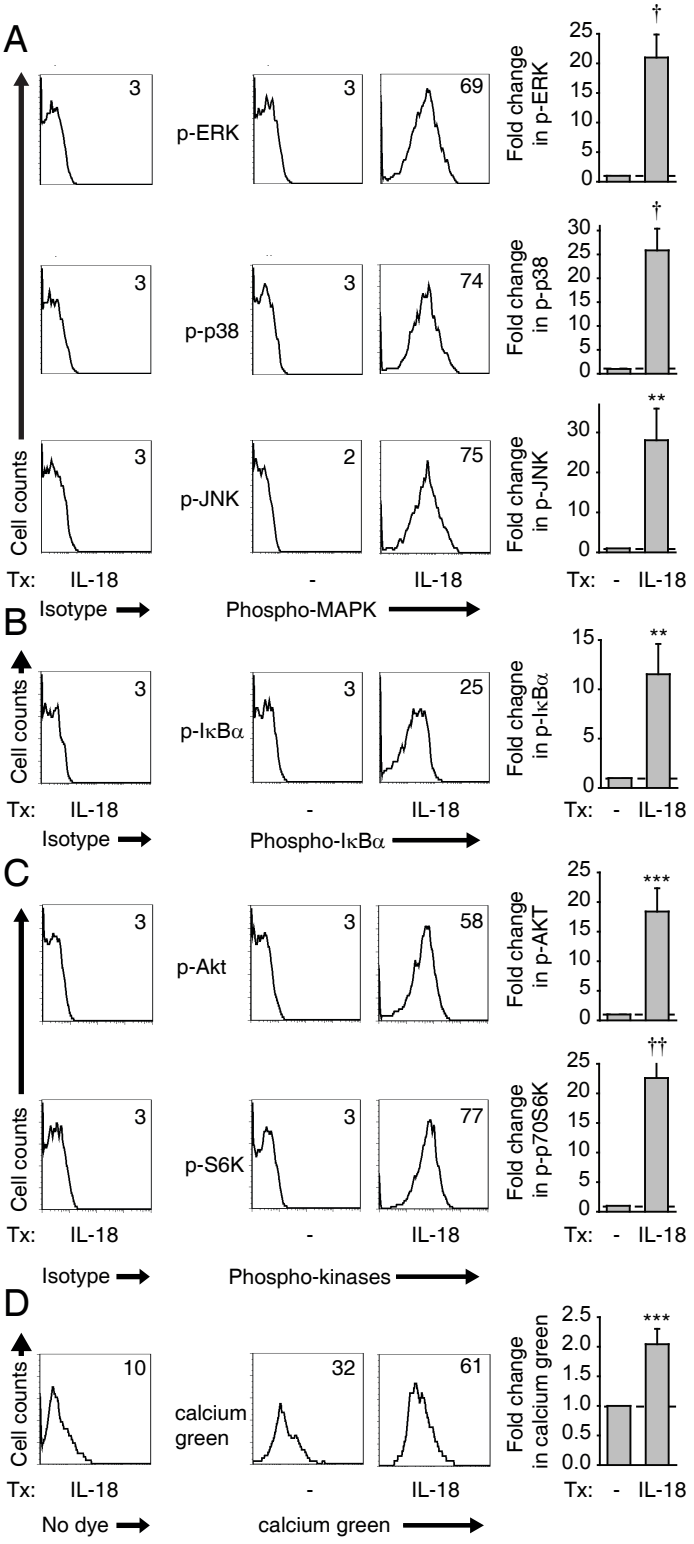
Supplementary Figure 1



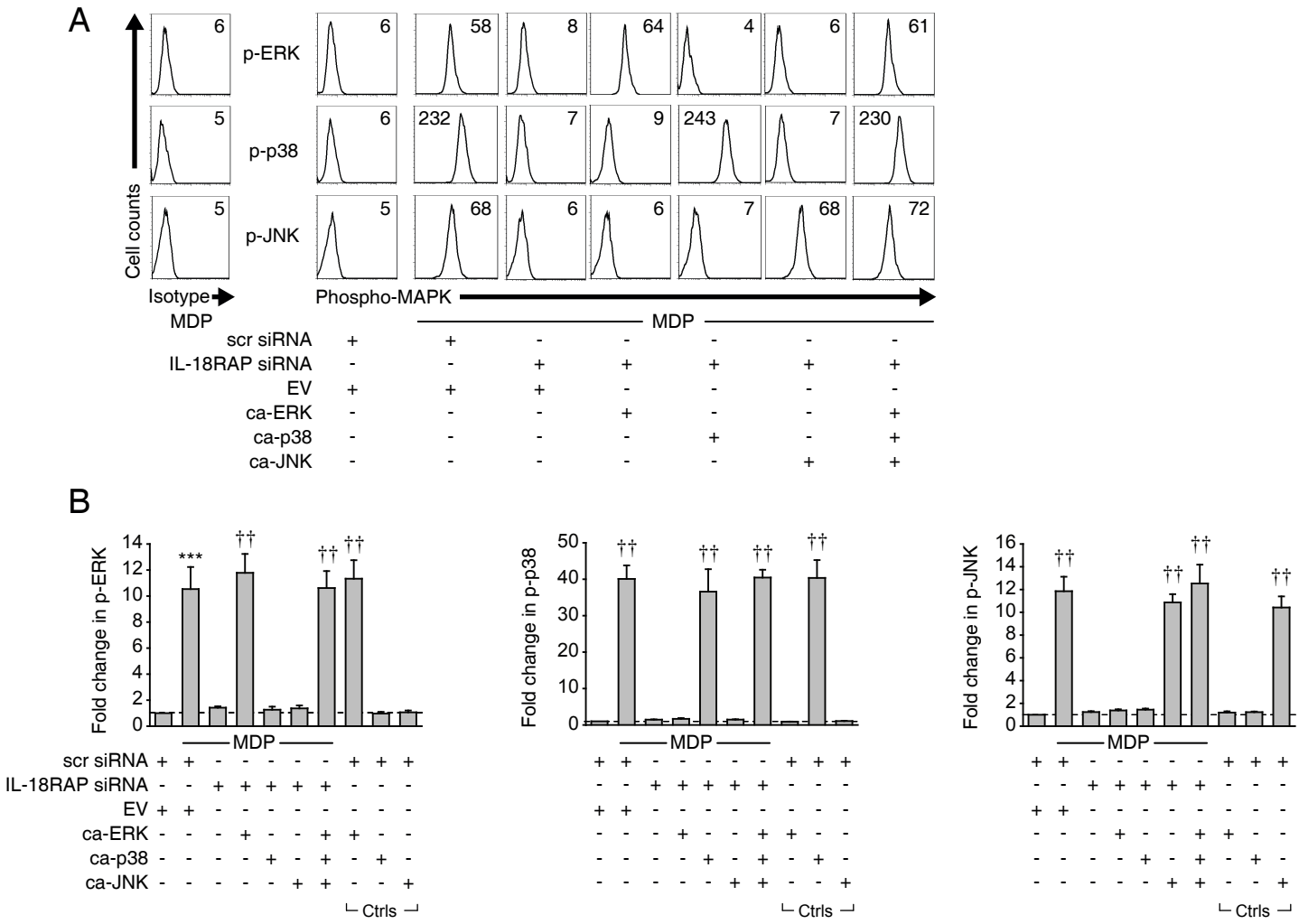
Supplementary Figure 1. MDM knocked-down for IL-18RAP can respond to other stimuli. **(A)** Human MDM (n=4) were transfected with scrambled or IL-18RAP siRNA for 48h. *(Left):* Representative flow cytometry plot of IL-18RAP expression from one of four individuals. (*Right*) Summarized data (n=4) are represented as IL-18RAP surface expression by MFI. **(B)** Human MDM were transfected with scrambled or IL-18RAP siRNA for 48h, then stimulated with 100 μg/ml curdlan (cur) or 100 μg/ml dispersible whole glucan particles (WGP) (Invivogen, San Diego, CA) for 24h. IL-10 and IL-1Ra secretion (n=4). Treatment with 100 μg/ml MDP is shown as a positive control for IL-18RAP knock-down effects. Tx, treatment; scr, scrambled. ***, $p<0.001$; ††, $p<1x10^{-5}$.

Supplementary Figure 2



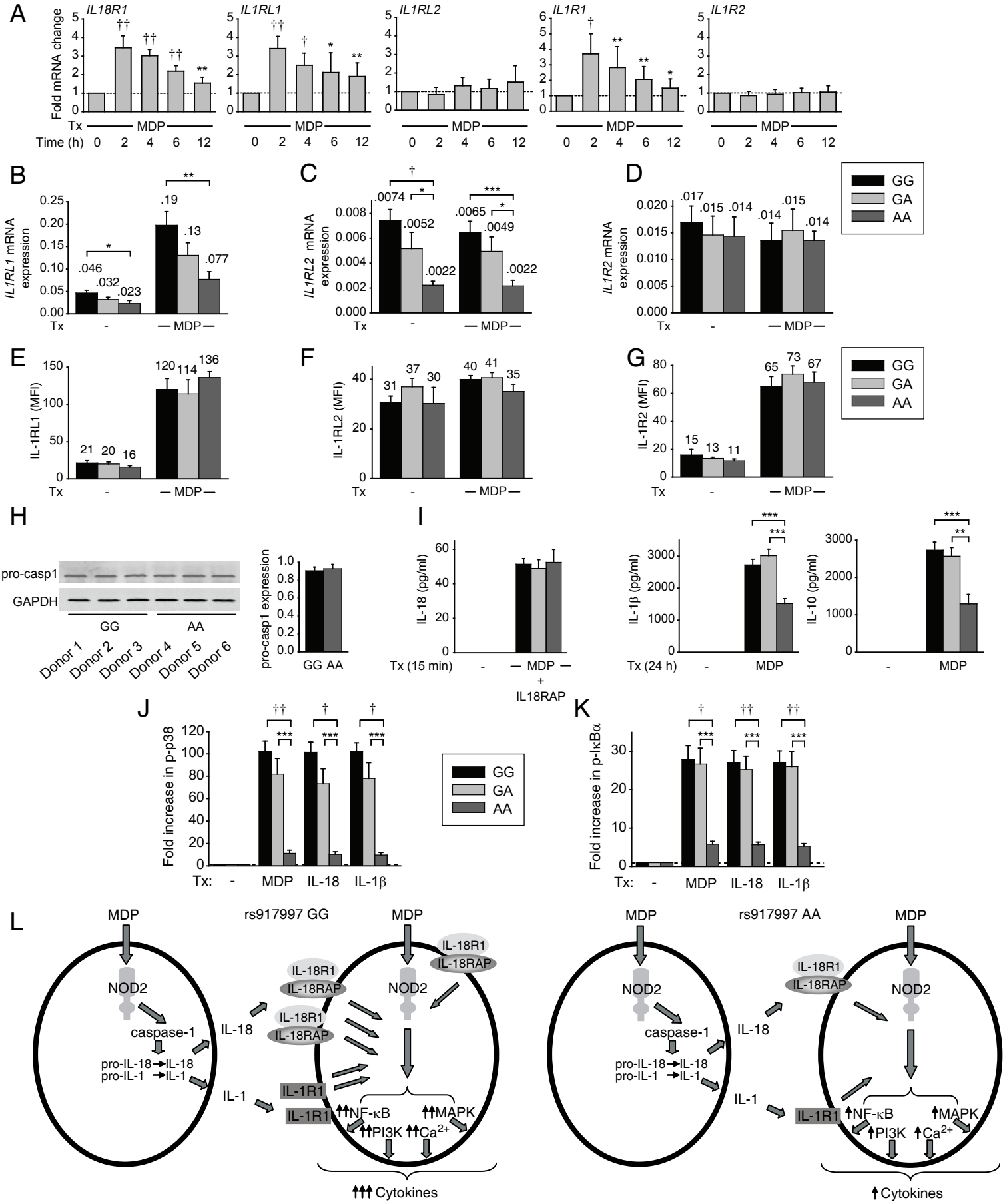
Supplementary Figure 2. IL-18 activates MAPK, NF-κB, PI3K and induces calcium flux in primary human MDM. Human MDM (n=8) were stimulated for 15 min with 10 ng/ml IL-18**.** *Left*: Representative flow cytometry plots with MFI values for **(A)** phospho-ERK, phospho-p38, phospho-JNK, **(B)** phospho-IκBα, **(C)** phospho-Akt and phospho-p70S6K or **(D)** calcium green. *Right:* Summarized data are represented as the fold phospho-protein or calcium flux induction normalized to untreated cells (represented by the dotted line at 1) + SEM. Tx, treatment. **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplementary Figure 3



Supplementary Figure 3. MAPK activation rescues the decreased cytokine secretion following NOD2 stimulation in the absence of IL-18RAP signaling in MDM. (A,B) MDM (n=7) were transfected with scrambled or IL-18RAP siRNA and/or 5 μg pMCL-MKK1 (R4F) (leading to constitutively active ERK kinase, ca-ERK), pCDNA3-Flag MKK6(glu) (leading to constitutively active p38 kinase, ca-p38), pSRα-3HA-JNKK2-JNK1 WT (leading to constitutively active JNK, ca-JNK), the three vectors in combination, or empty vector (EV) and left untreated (controls) or 48h later stimulated with 100 μg/ml MDP for 15 min and analyzed by flow cytometry for phospho-kinase induction. **(A)** Representative histograms with indicated MFI and isotype controls. (**B**) Data are represented as the fold MAPK activation normalized to EV and scrambled siRNA transfected cells (represented by the dotted line at 1) + SEM. Significance is shown relative to untreated, EV, scrambled siRNA transfected cells. ***, $p<0.001$; ††, $p<1x10^{-5}$. Tx, treatment; p-, phospho-; ctrls, controls.

Supplementary Figure 4



Supplementary Figure 4. NOD2 stimulation induces IL-18R1, IL-1RL1, IL-1RL2, IL-1R1 and IL-1R2 expression and MDM from rs917997 **AA risk carriers show decreased NOD2-, IL-18- and IL-1-mediated p38 and NF-κB activation relative to GA and GG carriers. (A)** MDM (n=8) were stimulated for the indicated time points with 100 μg/ml MDP. Summarized data are represented as the fold *IL18R1*, *IL1RL1*, *IL1RL2*, *IL1R1* and *IL1R2* induction normalized to untreated cells (represented by the dotted line at 1) + SEM. **(B-G)** MDM from rs917997 GG, GA or AA carriers were left untreated or stimulated with 100 μg/ml MDP for **(B,C,D)** 2h or **(E,F,G)** 6h. *IL1RL1* **(B),** *IL1RL2* **(C)** or *IL1R2* **(D)** mRNA expression (change in CT values normalized to GAPDH and represented as a linear scale). IL-1RL1 **(E),** IL-1R2 **(F)** or IL-1R2 **(G)** surface protein expression (R&D Systems), with average MFI values above bars. For **(B,C,E,D,G)** rs917997 GG, GA or AA carriers from n=16, n=15 and n=9, respectively. For **(F)** rs917997 GG, GA or AA carriers n=10, n=10 and n=6, respectively. **(H)** Pro-caspase-1 expression by Western blot from 3 representative rs917997 GG and AA donors. Summarized data (rs917997 GG [n=10] and AA [n=6] carrier MDM, respectively) are represented as the relative expression of pro-caspase-1 normalized to GAPDH+SEM. **(I)** MDM from rs917997 GG or AA carriers (n=10 and n=6, respectively) were (*left*) left untreated or incubated with anti-IL-18RAP (300 ng/ml) blocking antibody for 1h to prevent IL-18 consumption, and then stimulated with 100 μg/ml MDP for 15 min to assess early IL-18 secretion, or (*right*) treated with 100 μg/ml MDP for 24h to confirm genotype-dependent regulation of long-term cytokine secretion as a control. Supernatants were assessed for indicated cytokines. **(J,K)** MDM from rs917997 GG, GA or AA carriers (n=10, n=9 and n=8, respectively) were stimulated for 15 min with 100 μg/ml MDP, 10 ng/ml IL-18 or 10 ng/ml IL-1β**.** Summarized data are represented as the fold phospho-kinase induction normalized to untreated cells + SEM. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$. **(L)** A model for the role rs917997 in autocrine IL-18 and IL-1 signaling pathways and cytokine induction upon NOD2 stimulation. MDP stimulation of NOD2 in MDM rapidly induces caspase-1 activation which cleaves pre-existing pro-IL-18 stores to result in early IL-18 secretion (within 15 min). This autocrine IL-18 secretion stimulates IL-18RAP which results in enhanced MAPK, NF-κB, and PI3K signaling and an increased calcium flux. Similar amplification of MDP-mediated signaling by autocrine IL-1 occurs. The increased signaling associated with autocrine IL-18 and IL-1, in turn, results in a dramatic increase in cytokine secretion. Rs917997 AA carriers have decreased IL-18RAP, IL-18R1 and IL-1R1 expression on MDM, decreased IL-18- and IL-1-induced signaling and decreased PRR-induced cytokine secretion.