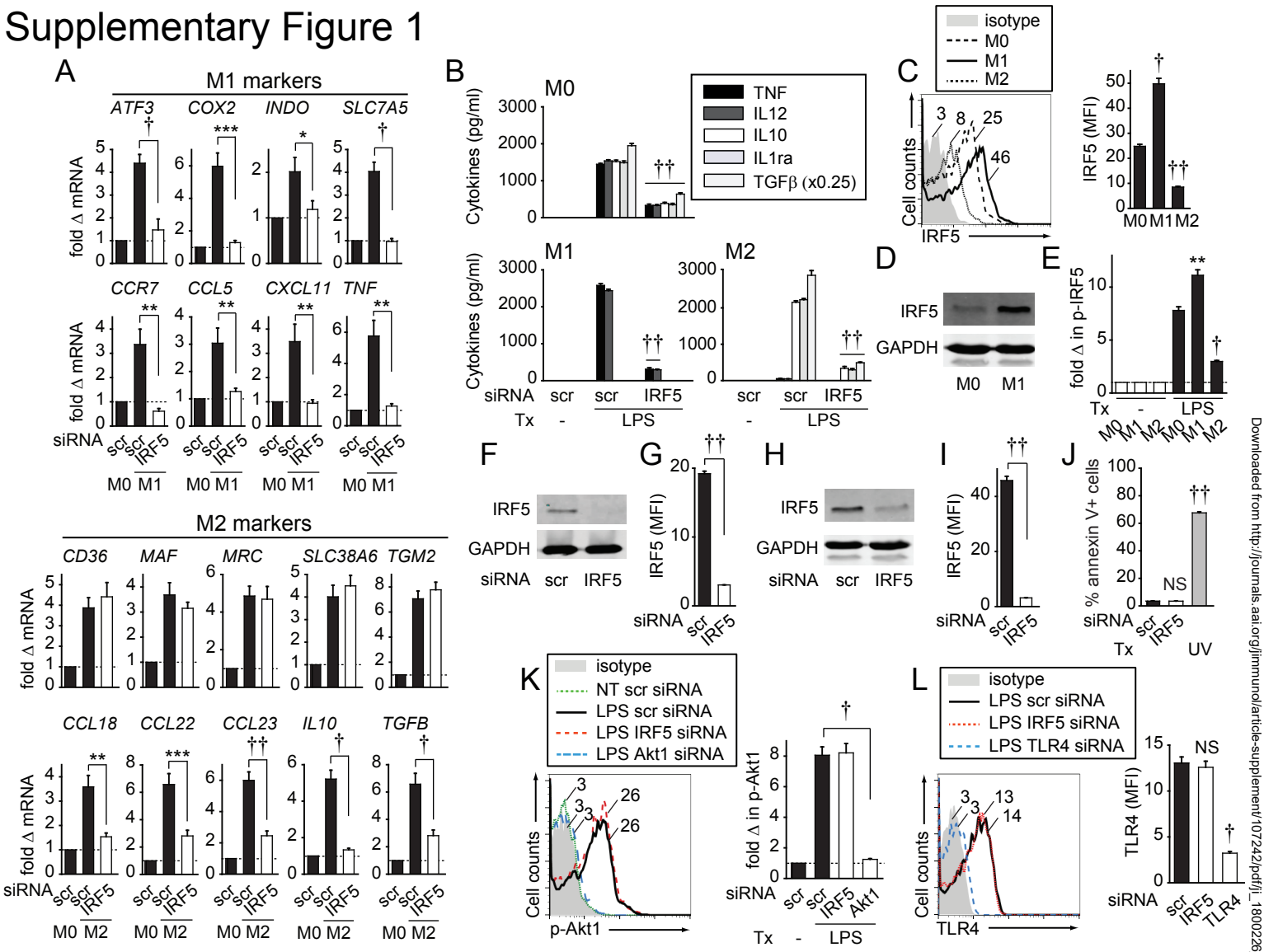
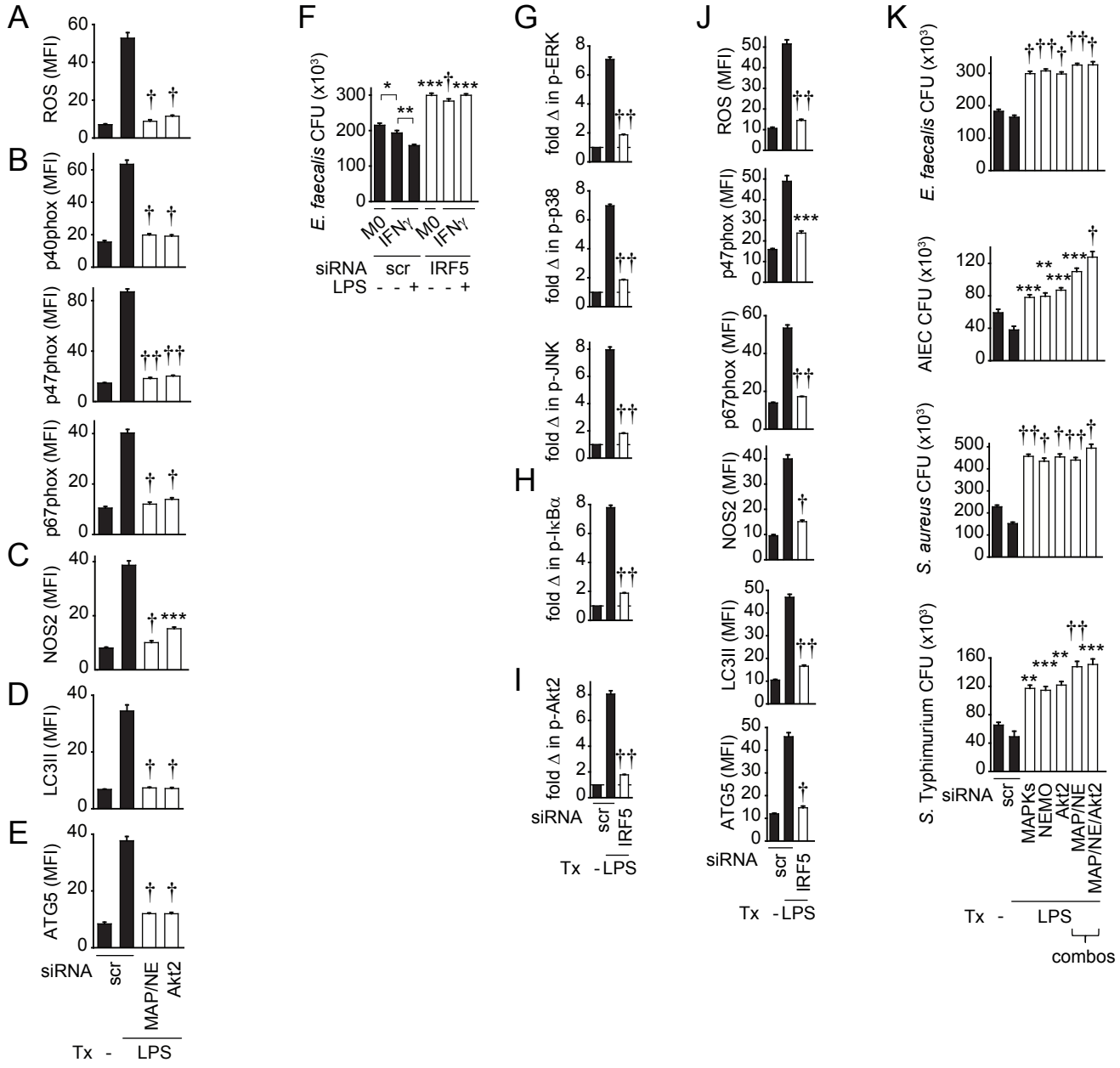
Supplementary Figure 1



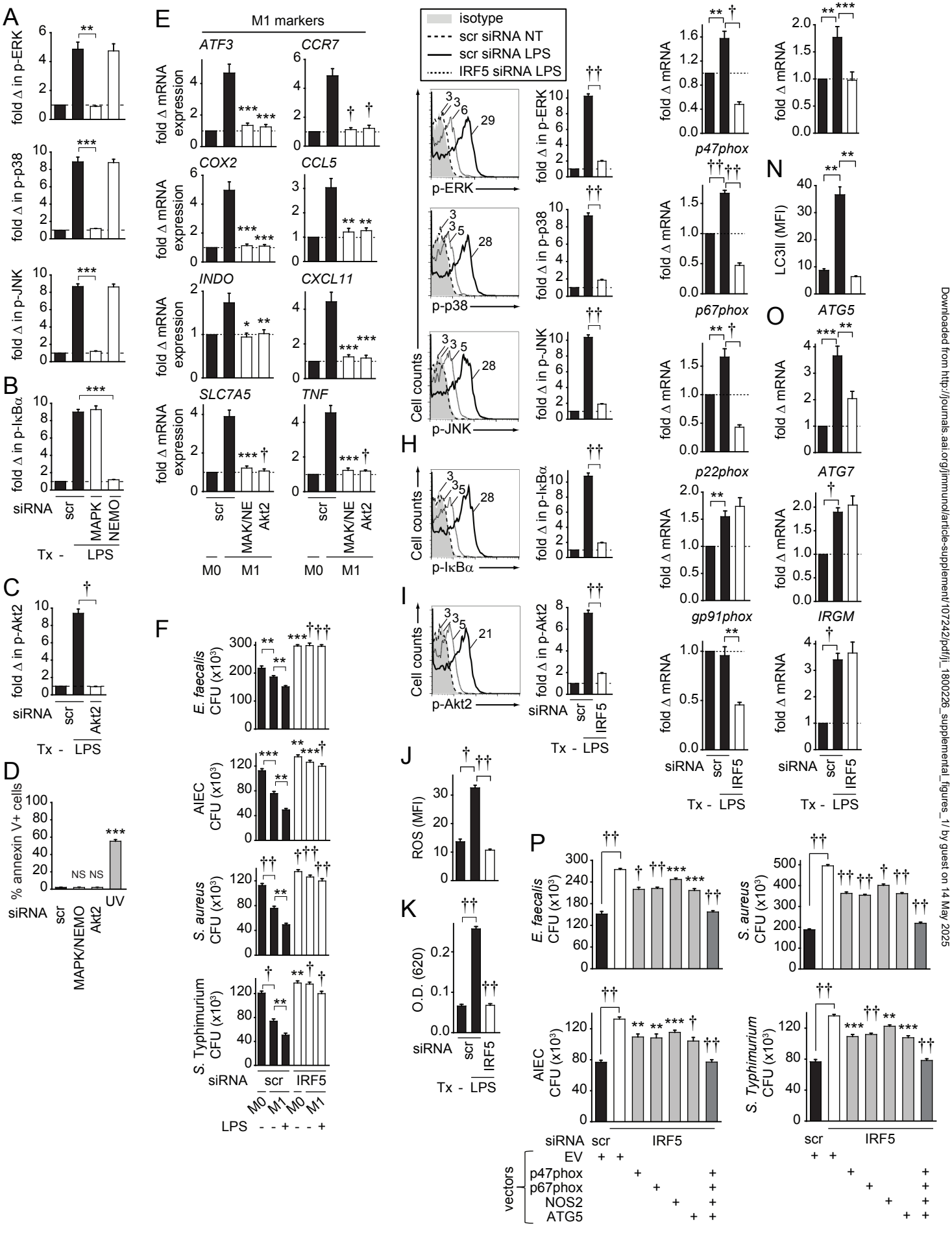
Supplementary Figure 1. IRF5 is required for M1 macrophage differentiation and for a subset of M2 macrophage markers. **(A-B)** MDMs were transfected with scrambled or IRF5 siRNA, and then left untreated (M0) or polarized into M1 (as per Materials and Methods) or M2 (with 20 ng/ml IL4 (R&D Systems)) macrophages for 24h. **(A)** Fold mRNA change (n=8 donors) of M1 or M2 markers normalized to non-polarized, scrambled siRNA-transfected macrophages (represented by the dotted line at 1) + SEM. **(B)** Cells (n=6) were treated with LPS for 24h. Cytokine secretion + SEM. ELISA was performed using antibodies against TNF, IL10 (BD Biosciences), IL12p40, IL1Ra (eBioscience) or TGFβ (Biolegend). Significance is compared to scrambled siRNA-transfected cells for each respective condition or as indicated. To keep cytokines on the same axis, a multiplier was applied for the lower levels of TGFβ. **(C-D)** MDMs were left untreated (M0) or polarized into M1 or M2 macrophages and assessed for IRF5 expression by: **(C)** flow cytometry (n=6, similar results seen in an additional n=16 for M0 and M1 macrophages) with mean fluorescense intensity (MFI) values and a summary graph with MFI + SEM. Isotype control is shown for M1 macrophages, or **(D)** Western blot. GAPDH served as loading control. **(E)** MDMs (n=6) were left untreated (M0) or polarized into M1 or M2 macrophages, treated with 0.1 μg/ml LPS for 15min and assessed for phospho-IRF5 expression (Thermofisher) by flow cytometry. MFI + SEM. **(F-I)** MDMs were transfected with scrambled or IRF5 siRNA. **(F-G)** IRF5 knockdown efficacy was assessed by: **(F)** Western blot. GAPDH expression served as loading control, or **(G)** flow cytometry (n=8, similar results were seen in an independent n=12) with MFI + SEM. **(H-I)** Cells were polarized into M1 macrophages and IRF5 protein expression was assessed by: **(H)** Western blot or **(I)** flow cytometry (n=6, similar results seen in an independent n=6) with MFI + SEM. **(J)** MDMs (n=6) treated as in **(F-G)** were assessed for cell death by annexin V by flow cytometry with 50-100 J/m^2 UV-treated cells shown as a positive control. **(K)** MDMs (n=6) were transfected with scrambled or IRF5 siRNA and then treated with 0.1 μg/ml LPS for 15min. Akt1 phosphorylation was measured by flow cytometry with phospho-Akt1 (Abcam). Summarized data are represented as the fold phospho-protein change normalized to untreated cells (represented by the dotted line at 1) + SEM. Akt1 siRNA is included as a positive control. **(L)** MDMs (n=6) were transfected with scrambled or IRF5 siRNA, polarized into M1 macrophages for 24h and treated with LPS for 48h. TLR4 surface expression by flow cytometry with PE-labeled anti-TLR4 (eBioscience). Representative flow cytometry, and summarized MFI + SEM. TLR4 siRNA is included as a positive control. Isotype control is shown for scrambled siRNA-transfected, LPS-treated cells in '**K-L**'. Tx, treatment; scr, scrambled. NS, not significant. *, p<0.05; **, p<0.01; ***, p<0.001; †, p<1x10^{-4}; ††, p<1x10^{-5}.

Supplementary Figure 2



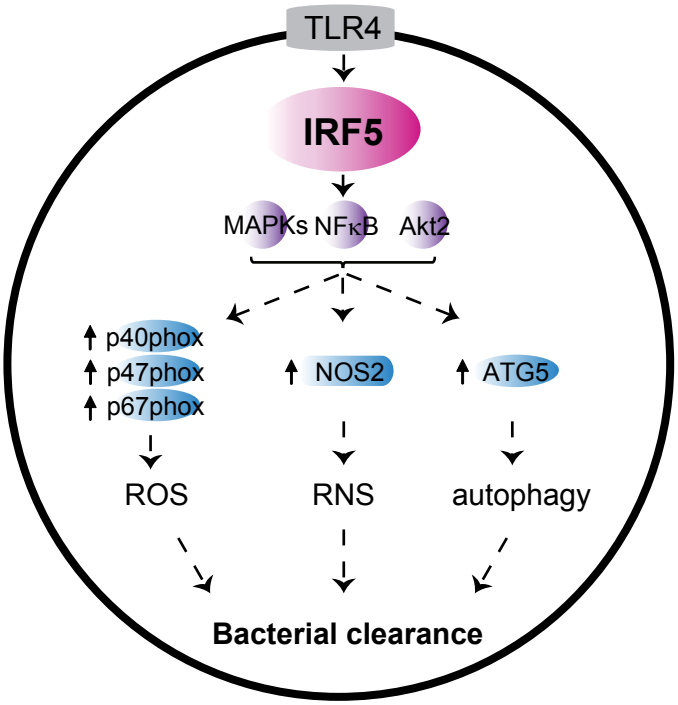
Supplementary Figure 2. IRF5 is required for optimal bacterial clearance pathways in IFNγ-conditioned human MDMs. (A-E) MDMs (n=6, similar results in additional n=6 for MAPK/NEMO) were polarized into M1 macrophages, transfected with scrambled siRNA, a combination of ERK, p38, JNK and NEMO (MAP/NE) siRNA, or Akt2 siRNA, then left untreated or treated with 0.1 μg/ml LPS for 48h. **(A)** ROS, **(B)** p40phox, p47phox and p67phox, **(C)** NOS2, **(D)** LC3II and **(E)** ATG5 protein expression was analyzed by flow cytometry. MFI + SEM. **(F-J)** MDMs were polarized with 20 ng/ml IFNγ and then transfected with scrambled or IRF5 siRNA. **(F)** Cells were then left untreated or treated with 0.1μg/ml LPS for 48h and co-cultured with *E. faecalis*. Colony forming units (CFU)+SEM. Significance is compared to scrambled siRNA-transfected cells for each respective condition (i.e. M0, IFNγ and IFNγ+LPS) or as indicated. **(G-I)** Cells (n=6) were treated with 0.1μg/ml LPS for 15min. Summarized data of fold phospho-protein normalized to untreated cells (represented by the dotted line at 1)+SEM. **(J)** Cells (n=6) were then left untreated or treated with 0.1μg/ml LPS for 48h. Summary graph for ROS or MFI change of the indicated proteins as detected by flow cytometry with MFI+SEM. **(K)** MDMs (n=6) were polarized with 20 ng/ml IFNγ, transfected with scrambled or combined ERK, p38, JNK (MAPKs), NEMO (NE) or Akt2 siRNA alone or in various combinations as indicated, then left untreated or treated with 0.1μg/ml LPS for 48h and co-cultured with *E. faecalis,* AIEC, *S. aureus* or *S.* Typhimurium. CFU+SEM. Significance is compared to scrambled siRNA-transfected, LPS-treated cells or as indicated. Tx, treatment; scr, scrambled. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplementary Figure 3



Supplementary Figure 3. IRF5 deficiency prior to M1 polarization results in impaired M1 differentiation and reduced bacterial clearance pathways. (A-C) MDMs (n=4 donors) were polarized into M1 macrophages, transfected with scrambled siRNA, a combination of ERK, p38, and JNK (MAPK) siRNA, NEMO siRNA, or Akt2 siRNA and then left untreated or treated with 0.1 μg/ml LPS for 15 min. Flow cytometry data are represented as the fold phospho-kinase change normalized to untreated cells (represented by the dotted line at 1) + SEM. **(D)** Cell death was assessed by annexin V by flow cytometry with 50-100 J/m^2 UV-treated cells shown as a positive control. **(E)** MDMs (n=8, similar results in an independent n=8 for MAPK/NEMO) were transfected with scrambled siRNA, a combination of ERK, p38, JNK and NEMO (MAP/NE) siRNA or Akt2 siRNA, and left untreated (M0) or polarized to M1 macrophages. Fold mRNA change of M1 markers normalized to non-polarized macrophages (represented by the dotted line at 1) + SEM. Significance is compared to scrambled siRNA-transfected, M1-polarized macrophages or as indicated. **(F)** MDMs (n=6, similar results in n=12-16) were transfected with scrambled or IRF5 siRNA and then left untreated (M0) or polarized into M1 macrophages. Cells were then left untreated or treated with 0.1μg/ml LPS for 48h and co-cultured with *E. faecalis*, AIEC, *S. aureus* or *S.* Typhimurium. Colony forming units (CFU)+SEM. Significance is compared to scrambled siRNA-transfected cells for each respective condition (i.e. M0, M1 and M1+LPS) or as indicated. **(G-P)** MDMs were transfected with scrambled or IRF5 siRNA and polarized into M1 macrophages. **(G)** Cells (n=6, similar results seen in an independent n=6) were treated with 0.1μg/ml LPS for 15 min. (*Left*): Representative flow cytometry with mean fluorescent intensity (MFI) values for: **(G)** phospho-ERK, phospho-p38, phospho-JNK, **(H)** phospho-IκBα or **(I)** phospho-Akt2. (*Right*): Summarized data of fold phospho-protein change normalized to untreated cells (represented by the dotted line at 1)+SEM. Isotype control for scrambled siRNA-transfected, LPS-treated cells. **(J-O)** Cells were left untreated or treated with 0.1μg/ml LPS for 48h. **(J,N)** Summary graph for ROS and LC3II change as detected by flow cytometry with MFI+SEM (n=6, similar results were seen in an independent n=4 for ROS). **(K)** Nitroblue tetrazolium+SEM (n=6). **(L,M,O)** Fold mRNA change compared to scrambled siRNA-transfected, untreated cells+SEM (n=6, similar results were seen in an additional n=14). **(P)** MDMs (n=4) were transfected with scrambled or IRF5 siRNA + p47phox-, p67phox-, NOS2-, or ATG5-expressing vectors alone or in combination, or empty vector (EV), polarized into M1 macrophages, then treated with 0.1μg/ml LPS for 48h. Cells were cultured with *E. faecalis*, AIEC, *S. aureus* and *S.* Typhimurium. CFU+SEM. Significance is compared to IRF5 siRNA, EV-transfected cells or as indicated. Tx, treatment; scr, scrambled; NS, not significant. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$.

Supplementary Figure 4



Supplementary Figure 4. A model showing IRF5 roles in bacterial clearance. PRR stimulation in M1 macrophages results in IRF5-dependent MAPK, NFκB and Akt2 activation, which in turn, leads to upregulation of the p40phox, p47phox and p67phox NADPH oxidase subunits, NOS2 and ATG5. These pathways, in turn, result in upregulation of ROS, RNS, and autophagy, respectively, which cooperate for optimal intracellular bacterial clearance in macrophages. LPS-treated, M1 macrophages from high IRF5-expressing rs2004640/rs2280714 TT/TT immune-mediated disease risk carriers demonstrate increased upregulation of bacterial clearance pathways and increased bacterial clearance compared to GG/CC carriers, which is due to the increased IRF5 expression in these carriers.