

Expanded View Figures

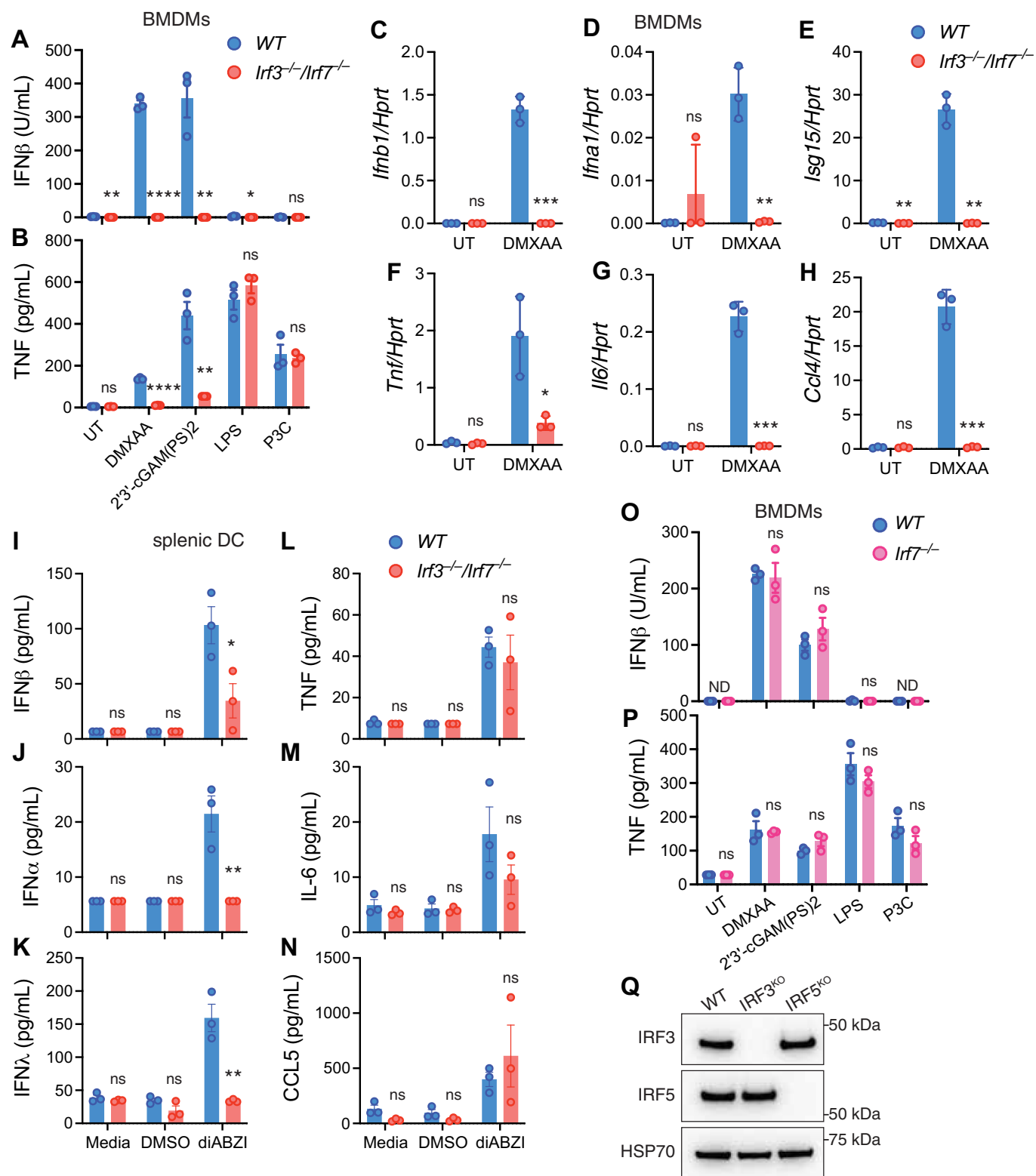
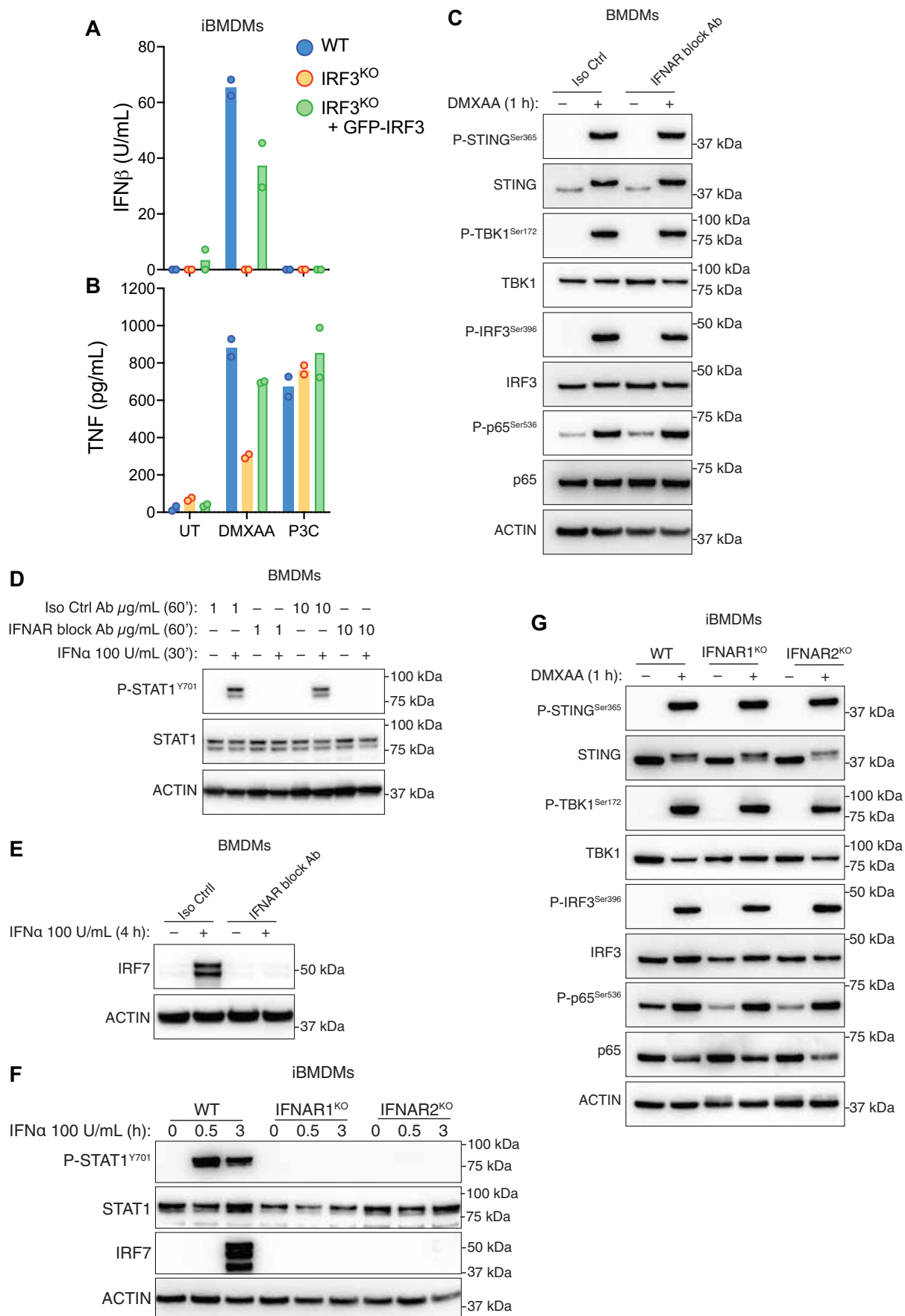


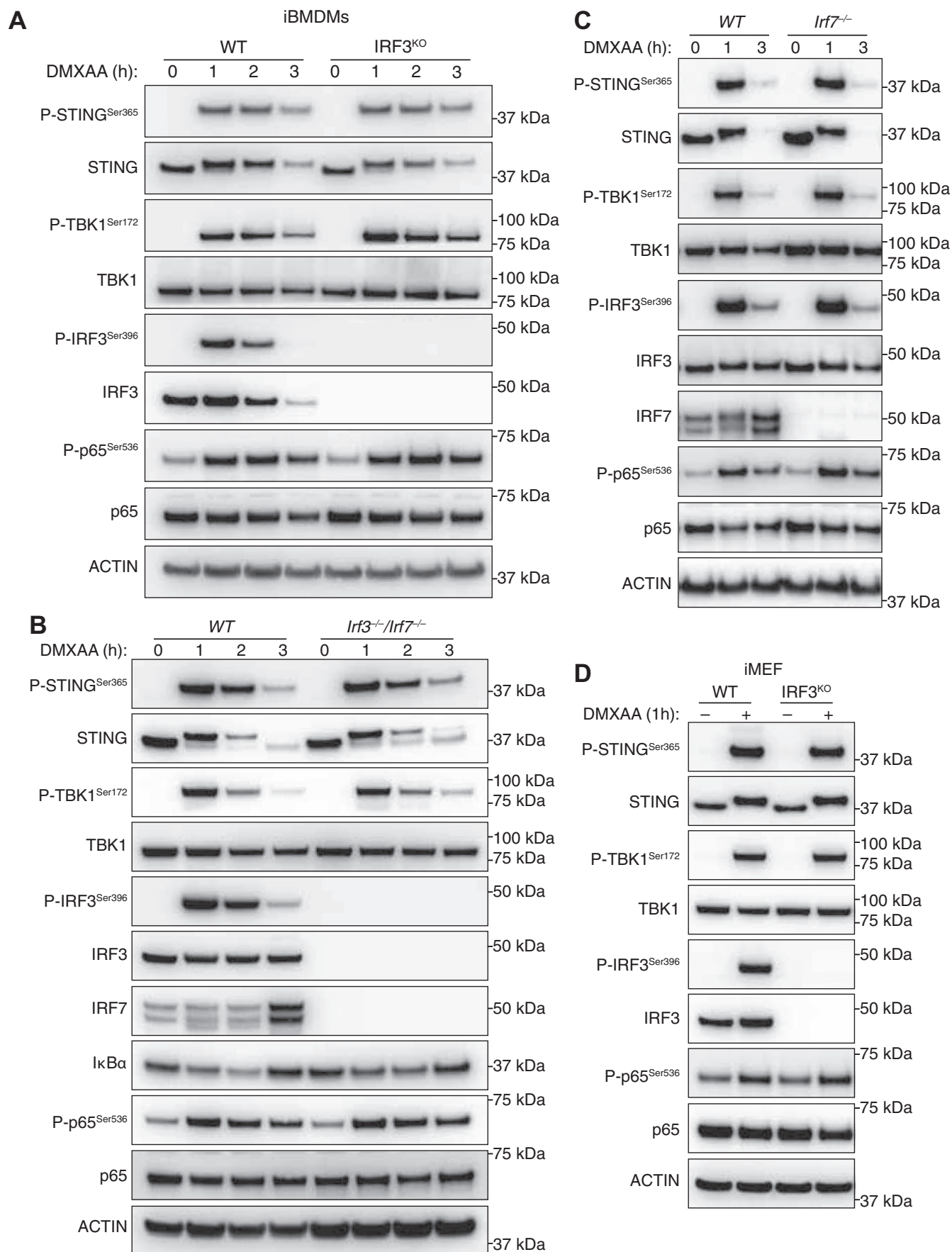
Figure EV1. Lack of IRF3, but not IRF7, impacts the ability of macrophages to produce IFNs and inflammatory cytokines.

(A, B) Wild-type (WT) and *Irf3*^{-/-}/*Irf7*^{-/-} primary BMDMs were left untreated (UT) or stimulated with 50 µg/mL DMXAA, 10 µg/mL 2'3'-cGAM(PS)2, 200 ng/mL LPS or 200 ng/mL Pam3CysK4 (P3C) for 4 h before cell supernatants were collected and levels of secreted IFNβ and TNF were measured by ELISA. Data shown as mean ± SEM from three combined biological experiments. Statistical analysis between genotypes for each condition group was performed using unpaired Student's *t* test. **P* < 0.05 (A *P* = 0.027), ***P* < 0.01 (A *P* = 0.0025, *P* = 0.0034; B *P* = 0.0042), *****P* < 0.0001 (B *P* = 2.4839E-06). ns= non-significant (A *P* = 0.37; B *P* = 0.28, *P* = 0.32, *P* = 0.76). (C-H) Wild-type (WT) and *Irf3*^{-/-}/*Irf7*^{-/-} primary BMDMs were left untreated (UT) or stimulated with 50 µg/mL DMXAA for 4 h before cells for RNA isolation and the expression of IFN and pro-inflammatory genes were analysed by qPCR as indicated. Data shown as mean ± SEM from three combined biological experiments. Statistical analysis between genotypes for each condition group was performed using unpaired Student's *t* test. **P* < 0.05 (F *P* = 0.02), ***P* < 0.01 (D *P* = 0.0011), ****P* < 0.001 (C *P* = 0.0001, E *P* = 0.00005, *P* = 0.00022; G *P* = 0.00011; H *P* = 0.00015). ns= non-significant (C *P* = 0.66; D *P* = 0.37; F *P* = 0.3; G *P* = 0.86; H *P* = 0.92). (I-N) Primary bulk splenic DCs from wild-type (WT) and *Irf3*^{-/-}/*Irf7*^{-/-} mice were left in fresh media or stimulated with DMSO control or 0.2 µM diABZI for 18 h before cell supernatants were collected to measure levels of secreted cytokines by LEGENDplex™ assay. Data shown as mean ± SEM from three combined biological experiments. Statistical analysis between genotypes for each condition group was performed using unpaired Student's *t* test. **P* < 0.05 (I *P* = 0.04), ***P* < 0.01 (J *P* = 0.008; K *P* = 0.004). ns= non-significant (K *P* = 0.31, *P* = 0.11; L *P* = 0.37; M *P* = 0.28, *P* = 0.76, *P* = 0.22; N *P* = 0.12, *P* = 0.5). (O, P) Wild-type (WT) and *Irf7*^{-/-} primary BMDMs were left untreated (UT) or stimulated with 50 µg/mL DMXAA, 10 µg/mL 2'3'-cGAM(PS)2, 200 ng/mL LPS or 200 ng/mL Pam3CysK4 (P3C) for 4 h before cell supernatants were collected and levels of secreted IFNβ and TNF were measured by ELISA. Data shown as mean ± SEM from three combined biological experiments. Statistical analysis between genotypes for each condition group was performed using unpaired Student's *t* test. ns= non-significant (O *P* = 0.83, *P* = 0.27, *P* = 0.37; P *P* = 0.15, *P* = 0.79, *P* = 0.14, *P* = 0.24, *P* = 0.16). ND not determined. (Q) Wild-type (WT), IRF3^{KO} and IRF5^{KO} iBMDMs were lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments.



◀ **Figure EV2. Blocking or deletion of IFNAR abolishes type I IFN responses.**

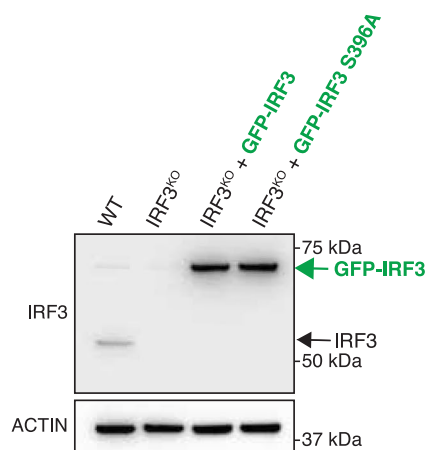
(A-B) Wild-type (WT), IRF3^{KO} and IRF3^{KO} expressing GFP-IRF3 were left untreated (UT) or stimulated with 50 µg/mL DMXAA or 200 ng/mL Pam3CysK4 (P3C) for 4 h before cell supernatants were collected and levels of secreted IFNβ and TNF were measured by ELISA. Data shown as mean from two biological experiments. (C) Wild-type (WT) primary BMDMs were pre-treated for 1 h with 10 µg/mL isotype control antibody or IFNAR blocking antibody before being left untreated (-) or stimulated for 1 h with 50 µg/mL DMXAA (+). Cells were then lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments. (D) Wild-type (WT) primary BMDMs were pre-treated for 1 h with an isotype control antibody or IFNAR blocking antibody as indicated. Cells were then left untreated (-) or stimulated for 30 min with 100 U/mL IFNα (+). Cells were then lysed for immunoblot with the indicated antibodies. Data shown is representative of 3 biological experiments. (E) Wild-type (WT) primary BMDMs were pre-treated for 1 h with 10 µg/mL isotype control antibody or IFNAR blocking antibody. Cells were then left untreated (-) or stimulated for 4 h with 100 U/mL IFNα (+) before they were lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments. (F) Wild-type (WT), IFNAR1^{KO} and IFNAR2^{KO} iBMDMs were left untreated (0) or stimulated for 0.5 or 3 h with 100 U/mL IFNα before cells were lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments. (G) Wild-type (WT), IFNAR1^{KO} and IFNAR2^{KO} iBMDMs were left untreated (-) or stimulated for 1 h with 50 µg/mL DMXAA (+). Cells were then lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments.



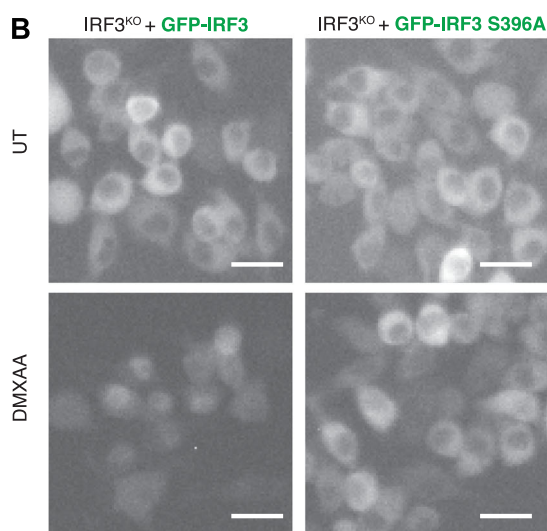
**Figure EV3. STING signalling is unchanged in the absence of IRF3 and/or IRF7.**

(A) Wild-type (WT) and IRF3^{KO} iBMDMs were left untreated (0) or stimulated for 1, 2 or 3 h with 50 µg/mL DMXAA. Cells were then lysed for immunoblot with the indicated antibodies. Data shown is representative of 3 biological experiments. (B) Wild-type (WT) and *Irf3*^{-/-}/*Irf7*^{-/-} primary BMDMs were left untreated (0) or stimulated for 1, 2 or 3 h with 50 µg/mL DMXAA. Cells were then lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments. (C) Wild-type (WT) and *Irf7*^{-/-} primary BMDMs were left untreated (0) or stimulated for 1 and 3 h with 50 µg/mL DMXAA. Cells were then lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments. (D) Wild-type (WT) and IRF3^{KO} iMEFs were left untreated (-) or stimulated (+) with 50 µg/mL DMXAA for 1 h. Cells were then lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments.

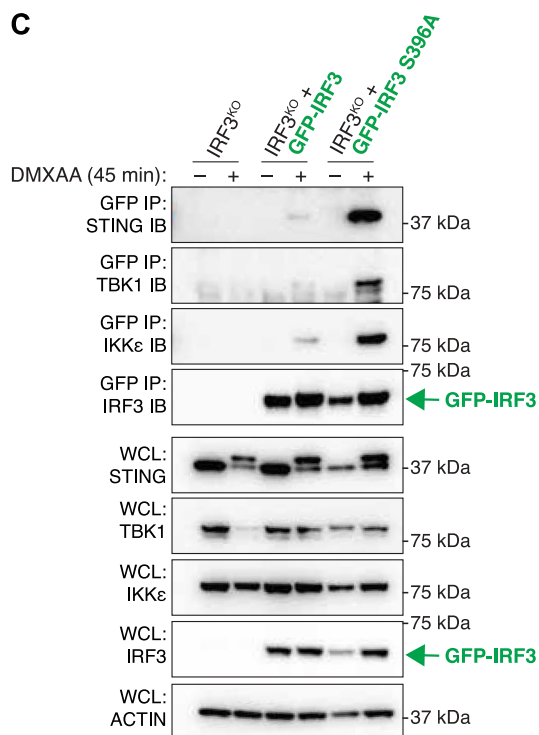
A



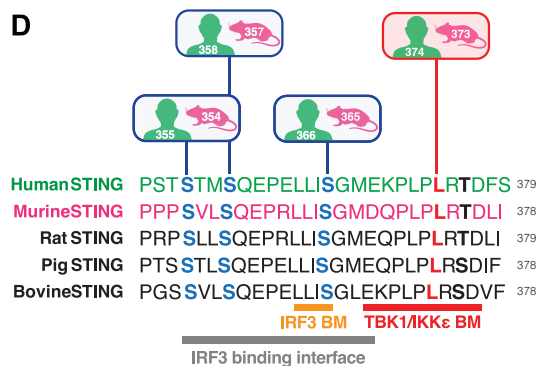
B



C



D



E

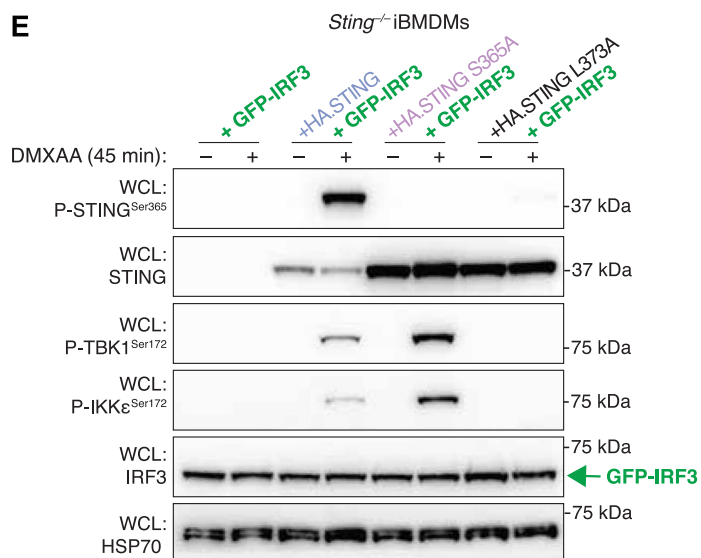


Figure EV4. The role of IRF3 in non-IFN STING responses is independent of its canonical function as a transcription factor for IFNs.

(A) Wild-type (WT) iBMDMs, IRF3^{KO} iBMDMs and those expressing either GFP-IRF3 or GFP-IRF3 S396A were lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments. (B) IRF3^{KO} iBMDMs expressing GFP-IRF3 or GFP-IRF3 S396A were left untreated (UT) or stimulated with 50 µg/mL DMXAA for 4 h before IRF3 localisation was examined by fluorescence microscopy. Scale bar = 40 µm. Data shown is representative of three biological experiments. (C) IRF3^{KO} iBMDMs expressing GFP-IRF3 or GFP-IRF3 S396A were left untreated (-) or stimulated for 45 min with 50 µg/mL DMXAA (+). Cells were lysed and a portion of the whole cell lysate (WCL) underwent immunoblot with the indicated antibodies. The remaining lysate underwent GFP immunoprecipitation (IP) before immunoblotting with the indicated antibodies. Data representative of 2 biological experiments. (D) Multiple sequence alignment of part of the CTT from human (green), murine (pink), rat (black), pig (black) and bovine (black) STING. Conserved phospho-serine (S) residues are highlighted in blue, the conserved lysine (L) residue at position 374 (human STING) is highlighted in red, the threonine (T) residue at position 376 (human STING) previously identified to be phosphorylated is highlighted in black (serine residues in pig and bovine). Red line indicates the TBK1/IKKε binding motif (BM); orange line indicates the IRF3 BM; grey line indicates the IRF3 binding interface. (E) *Sting*^{-/-} iBMDMs expressing GFP-IRF3 as well as either HA.STING, HA.STING S365A or HA.STING L373A were left untreated (-) or stimulated for 45 min with 50 µg/mL DMXAA (+). Cells were lysed and a portion of the whole cell lysate (WCL) underwent immunoblot with the indicated antibodies. Data representative of three biological experiments.

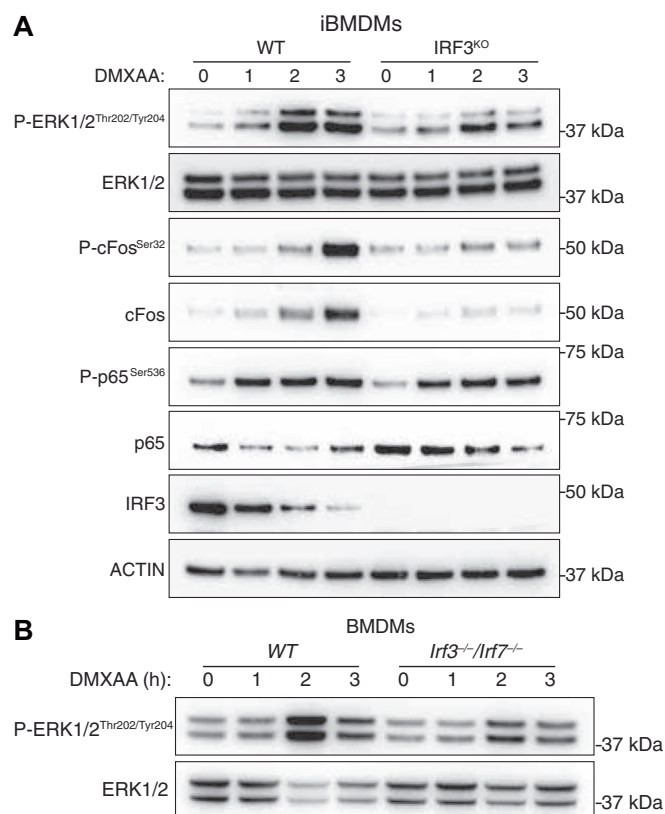


Figure EV5. Loss of IRF3 affects ERK1/2 and cFOS activity.

(A) Wild-type (WT) and IRF3^{KO} iBMDMs were left untreated (0) or stimulated for 1, 2 or 3 h with 50 µg/mL DMXAA before cells were lysed for immunoblot with the indicated antibodies. Data shown is representative of two biological experiments. (B) Wild-type (WT) and *lrf3^{-/-}/lrf7^{-/-}* primary BMDMs were left untreated (0) or stimulated for 1, 2 and 3 h with 50 µg/mL DMXAA. Cells were then lysed for immunoblot with the indicated antibodies. Data shown is representative of three biological experiments.