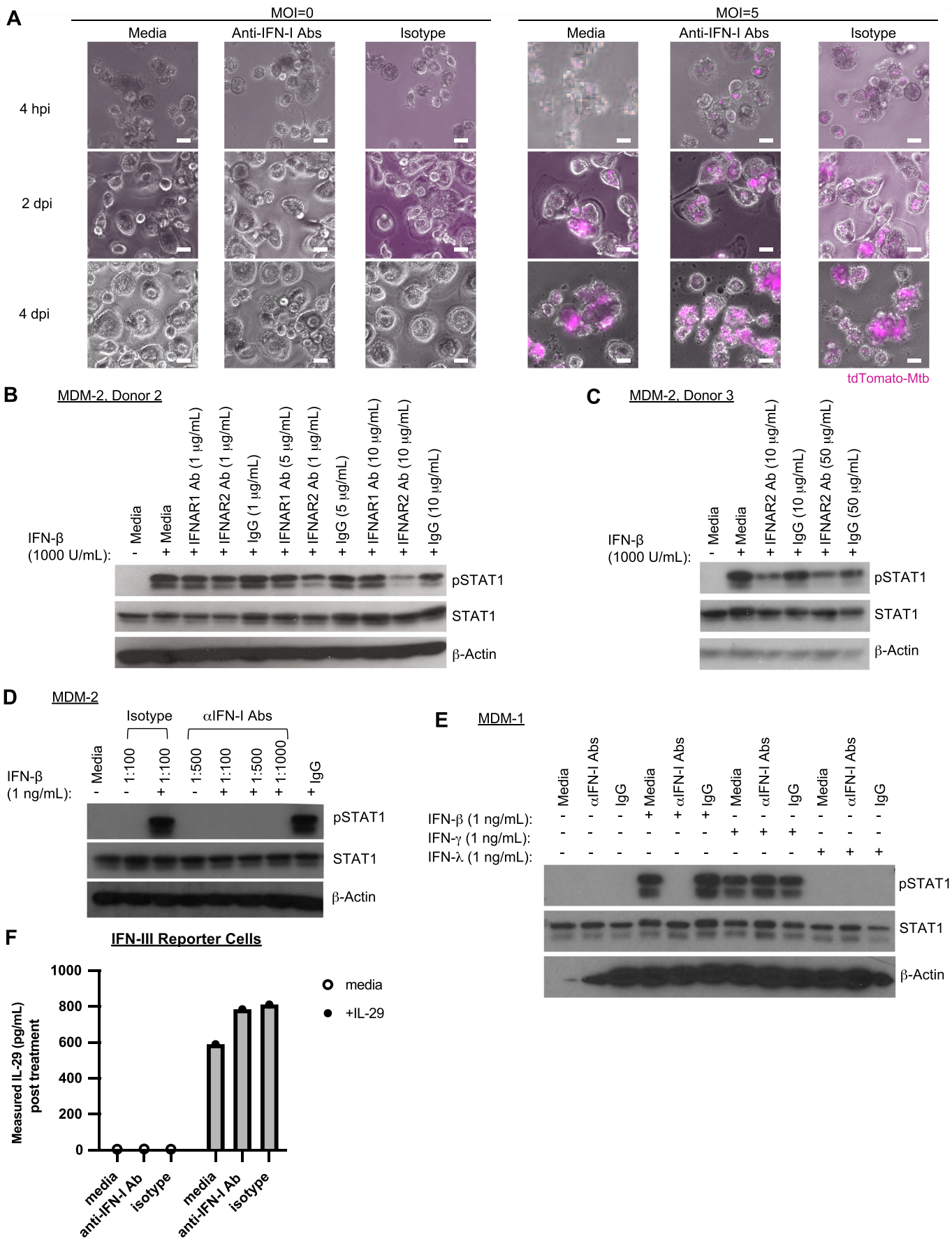
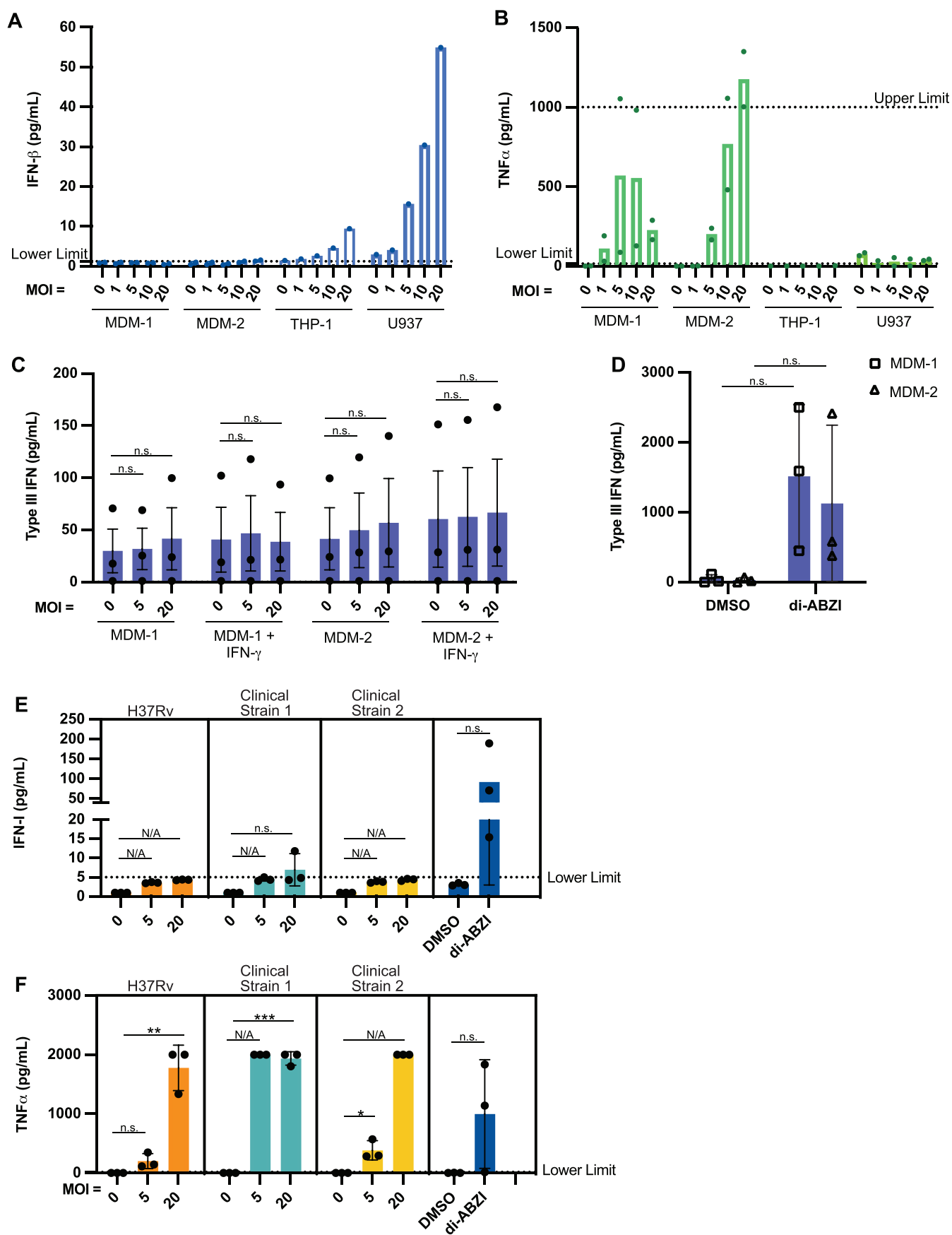


Expanded View Figures

Figure EV1. Neutralizing IFN-I Ab mix specifically ablates IFN-I signaling.

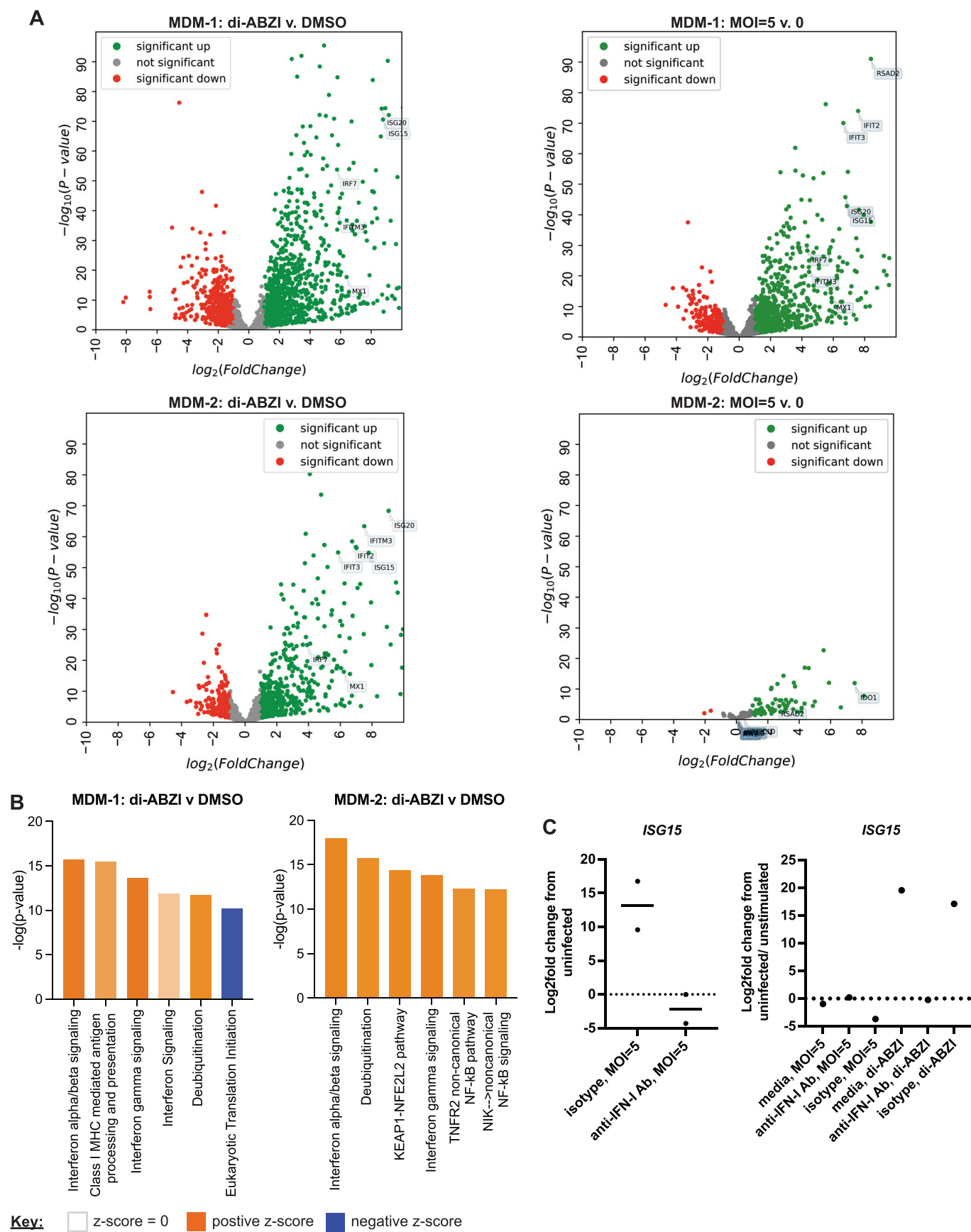
(A) MDM-2 ($n = 1$) were treated with anti-IFNAR1 mAb, anti-IFNAR2 mAb, IgG, or vehicle control (medium) for 2 h, then infected with tdTomato-Mtb. Images were taken at given timepoints post infection at 20X. Scale bars = 20 μm . (B, C) MDM-2 ($n = 1$) were treated with mAbs against IFNAR1, IFNAR2, IgG isotype control, or vehicle control (media) for 2 h, stimulated with IFN- β for 15–20 min, and then lysates were collected for western blot with indicated antibodies. (D) MDM-2 ($n = 1$) were treated with IFN-I neutralizing Ab mixture (see Methods), IgG, or vehicle control (media) for 2 h and stimulated with the indicated concentrations of IFN- β for 15–20 min. Lysates were then collected for western blot with indicated antibodies. (E) MDM-1 ($n = 1$) were treated with IFN-I neutralizing Ab mixture, IgG, or vehicle control (medium) for 2 h and stimulated with IFN- β , IFN- γ , or IFN-I for 15–20 min. Lysates were then collected for western blot with indicated antibodies. (F) IFN-III HEKBlue 293 cells ($n = 1$) were treated with IFN-I neutralizing Ab mixture, IgG, or vehicle control (medium) for 2 h and then stimulated with 1000 pg/mL of IFN-I1 (IL-29) overnight. The level of IFN-III signaling was measured via QUANTI-Blue assay. The concentration of IFN-I was estimated by performing a sigmoidal, 4 parameter interpolation from an IL-29 standard curve, using Prism's software. Data Information: Each • represents the average of 3 technical replicates, while n indicates biological replicates.





◀ **Figure EV2. Mtb-infected MDMs do not secrete more IFN-III than uninfected MDMs at 1 d post infection nor detectable IFN-I upon infection with clinical isolates of Mtb.**

MDM-1 and MDM-2 ($n = 2$) and 10 ng/mL PMA differentiated THP-1 cells and U937 cells were infected with Mtb H37Rv. 1 d post infection, supernatants were collected. (A) IFN- β was measured by a high sensitivity ELISA. (B) TNF α was measured by ELISA. (C, D) MDM-1 and MDM-2 ($n = 3$) were primed with or without 2.5 ng/mL IFN- γ for 1 day and then (C) infected with Mtb strain H37Rv (C) or (D) treated with 3 μ M di-ABZI. Supernatant was collected 1 d post infection. IFN-III expression in the supernatant was measured via the IFN-III HEKBlue reporter assay and quantified by performing a sigmoidal, 4 parameter interpolation from an IFN- λ 1 (IL-29) standard curve. (E, F) MDM-2 ($n = 3$) were differentiated for only 1 week before infection with Mtb H37Rv, clinical strain 1, clinical Mtb strain 2, or treatment with 3 μ M ABZI. (E) IFN-I expression in the supernatant was measured via the IFN-I HEKBlue reporter assay, and (F) TNF α expression was measured via ELISA. Data Information: (A-F) Bar graphs report the mean $\pm$ SEM. Each • represents an individual donor with 2-3 technical replicates per donor. Values too low to be interpolated were assigned a value of 1 pg/mL. Statistical significance was determined using a one-tailed paired, t -test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s. indicates no significance; N/A indicates not applicable). N/A was assigned if all values between two groups being tested were below the limit of detection (E) or if it was not possible to compute t -test because all values per group were the same (F). In each panel, n indicates biological replicates.



◀ **Figure EV3. Mtb-infected MDM-1 upregulate ISGs in an IFN-I dependent manner.**

(A) MDM-1 and MDM-2 ($n = 3$) were infected with Mtb H37Rv or treated with 3 μ M di-ABZI or vehicle controls (equal volume of DMSO as in 3 μ M di-ABZI). Lysates were collected 1 day post-treatment or post-infection for RNA sequencing. Volcano plots show $\log_2$ fold changes of ISGs determined by Interferome between stimulated/infected MDM-1 compared to unstimulated MDM-1 and the corresponding $-\log_{10}$ adjusted p -values. (B) Ingenuity Pathway Analysis (QIAGEN) of the top 6 canonical pathways upregulated in di-ABZI treated MDM-1 (left) and di-ABZI treated MDM-2 (right) determined by $-\log(\text{adjusted } p\text{-value})$ and absolute z -scores >3 . (C) Mtb-infected MDM-1 from 2 donors (left) and 1 donor (right) were treated with anti-IFN-I neutralizing Abs or IgG2a isotype Ab for 2 h pre-infection, infected with Mtb H37Rv and again treated with the respective Ab after Mtb was washed out 4 h post infection. Lysates were collected 1 d post infection. *ISG15* expression was measured by RT-qPCR. Data Information: (A) Each • represents 1 gene. Statistical significance was determined using the DESeq2 package, which uses the Wald test and Benjamini-Hochberg method to correct the FDR (A), and using QIAGEN's IPA analysis, which employs Fisher's exact test and the Benjamini-Hochberg method to control the false discovery rate (Krämer et al, 2013) (B). (C) Each • represents the average value of 4 technical replicates per donor, while n indicates biological replicates.

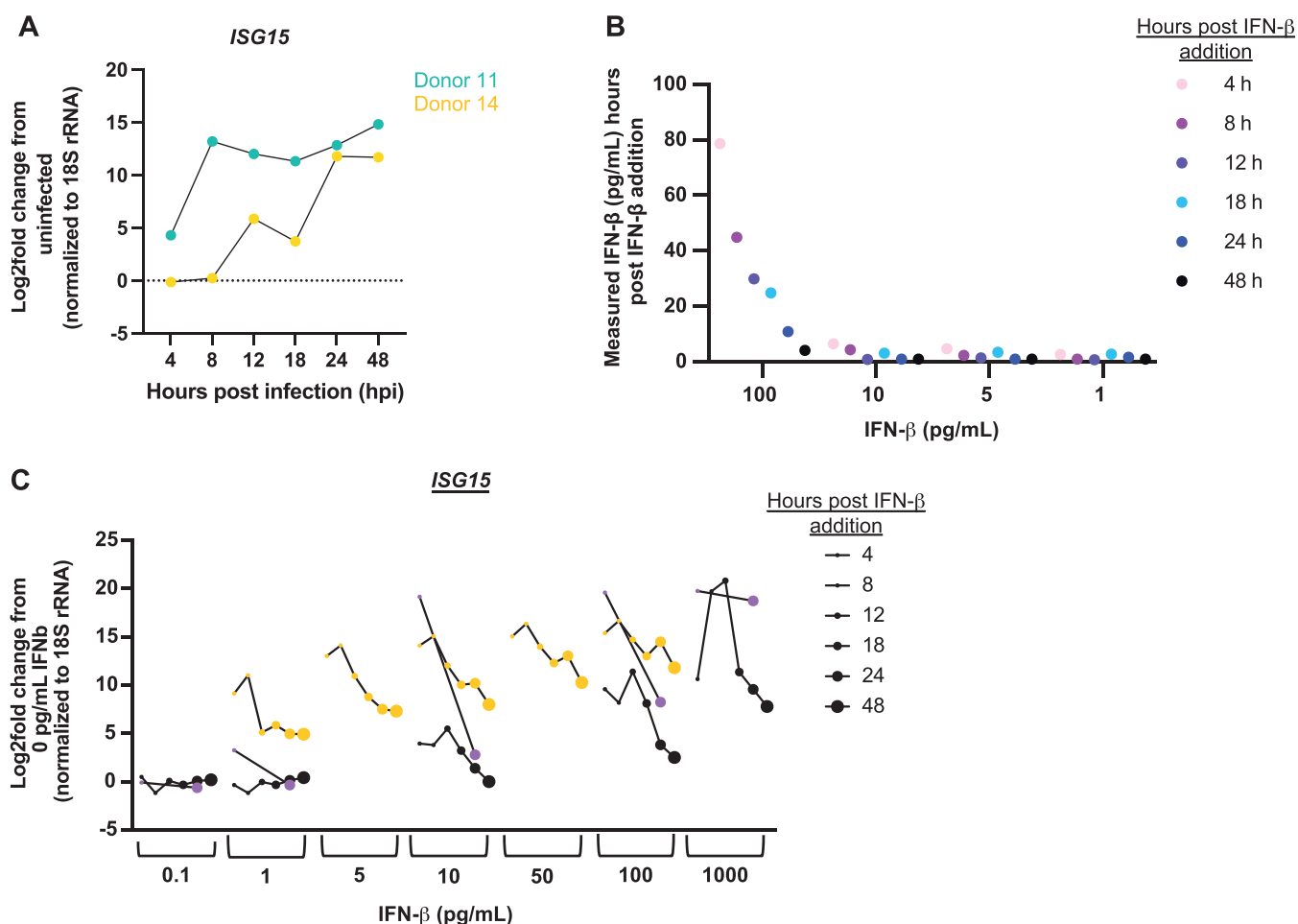
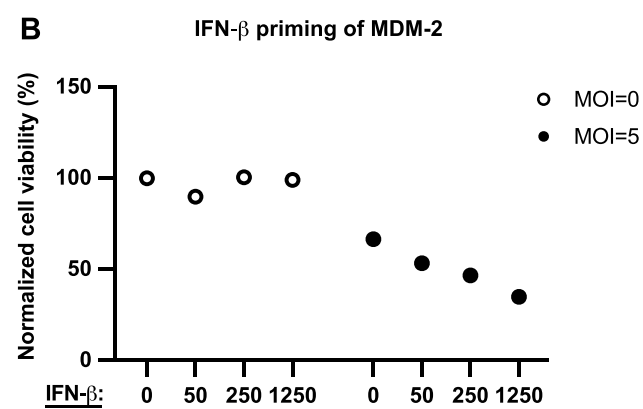
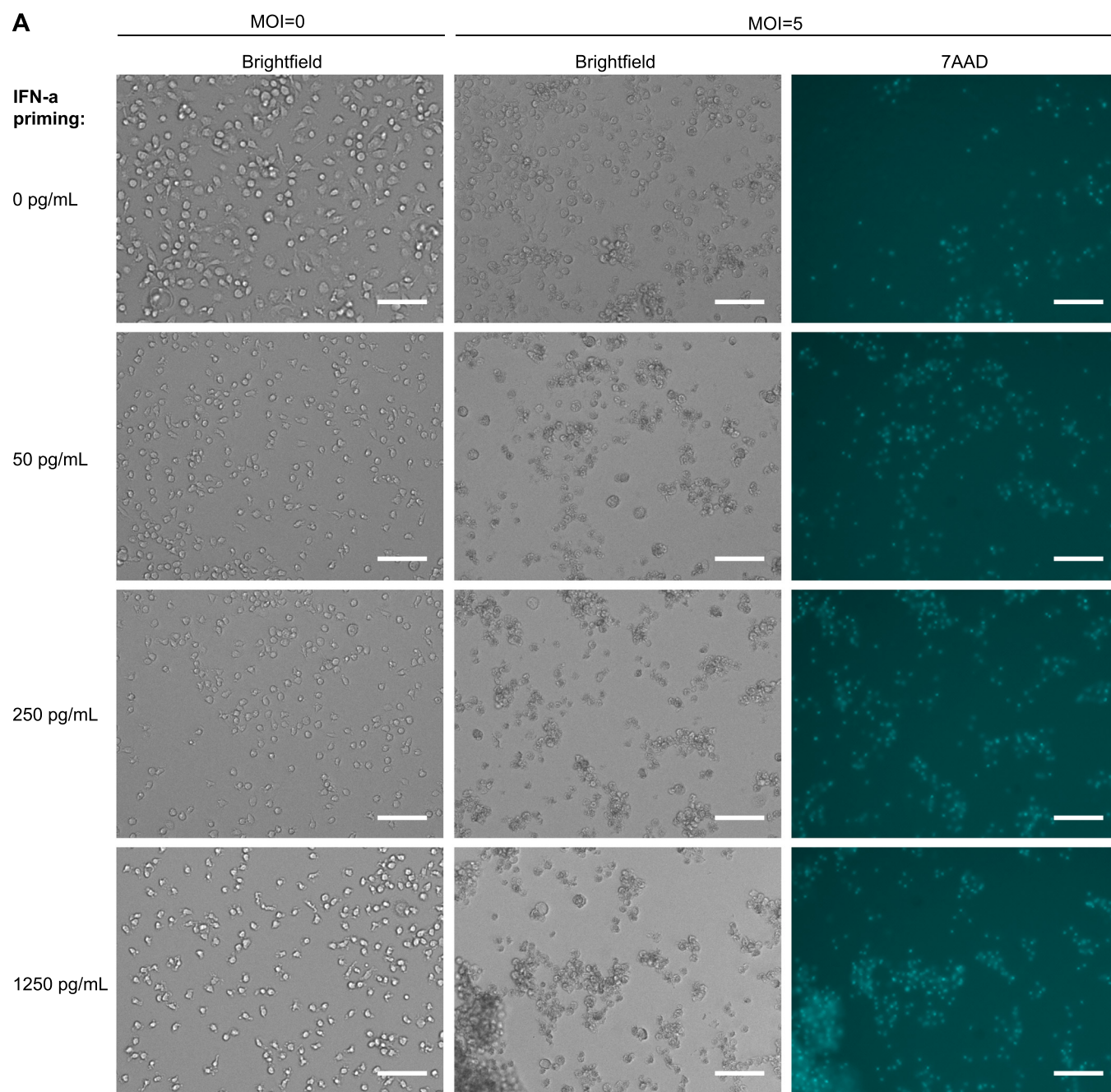


Figure EV4. MDM-1 consume IFN-β.

(A) MDM-1 ($n = 2$) were infected with Mtb H37Rv and lysates were collected at indicated timepoints. *ISG15* expression was measured by RT-qPCR. (B) MDM-1 ($n = 1$) were stimulated with 1–100 pg/mL IFN-β, supernatants were collected at indicated timepoints, and IFN-I expression was measured via the IFN-I HEKBlue reporter assay. IFN-I was quantified by performing a sigmoidal, 4 parameter interpolation from an IFN-β standard curve. (C) MDM-1 ($n = 1$ –3) were treated with IFN-β and lysates were collected at indicated timepoints. *ISG15* expression was measured by RT-qPCR. Each • represents the average value of 3–4 technical replicates per donor, while n indicates biological replicates. Data Information: Each • represents the average value of 3–4 technical replicates per donor (A), the average value of 2 technical replicates per donor (B), and the average value of 3–4 technical replicates per donor (C), n indicates biological replicates.



**Figure EV5. Exogenous IFN-I exacerbates Mtb-induced MDM death.**

(A) Representative images of MDM-1 ($n = 1$) that were primed with IFN- α 1 day pre-infection, infected with Mtb H37Rv, stained with 7-aminoactinomycin D (7-AAD) and imaged at 10 $\times$ 2 d post infection. Scale bars = 100 μ m. (B) MDM-2 ($n = 1$) were primed with IFN- β 1 day pre-infection, infected with Mtb H37Rv, and cell viability was measured via CellTiter Glo assay. Data Information: (B) Dot plot reports mean value of 3 technical replicates per 1 donor, n indicates biological replicates.