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Supplemental information

Type I interferon decreases macrophage energy metabolism during mycobacterial infection

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Figure S1

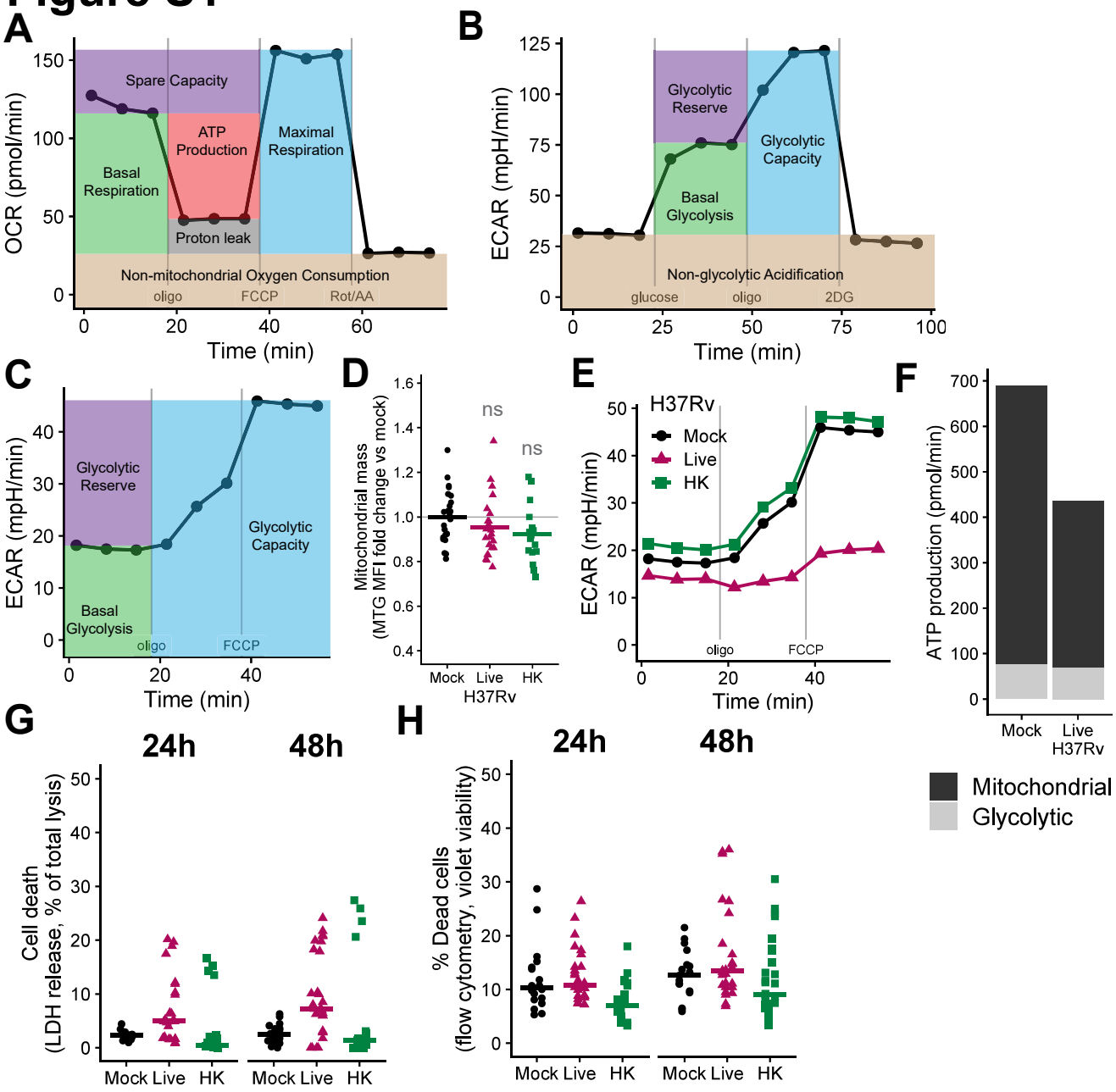

Figure S1. Live H37Rv decreases BMDM metabolism more than HK H37Rv. Related to Figure 1.

(A-C) Derivation of metabolic parameters from Agilent Seahorse extracellular flux analyzer measurements. (A) The mitochondrial stress test measures oxygen consumption rate (OCR) at baseline in the presence of mitochondrial substrates and glucose and then sequentially injects Oligomycin (oligo; 5 μM), FCCP (3 μM), and Rotenone + Antimycin A (Rot/AA; each 1 μM) to determine mitochondrial respiratory parameters, including non-mitochondrial oxygen consumption, basal respiration, ATP generation, proton leak, maximal mitochondrial respiration, and spare respiratory capacity. (B) The glycolytic stress test measures extracellular acidification rate (ECAR) at baseline in glucose-free media and then sequentially injects glucose (10 mM), oligomycin (oligo; 5 μM), and 2 deoxy-glucose (2-DG; 50 mM) to determine glycolytic parameters, including non-glycolytic acidification, basal glycolysis, maximal glycolytic capacity, and glycolytic reserve. (C) Approximations of glycolytic parameters can be derived from ECAR measurements taken during the mitochondrial stress test.

(D) The MitoTracker Green (MTG) mean fluorescence intensity (MFI) for each field of view was measured in CellProfiler (McQuin et al., 2018) and normalized to mock infected wells for the same infection conditions as Figure 1C,D. Bars represents mean of two independent experiments and statistics shown for comparisons to mock infected conditions.

(E) ECAR of WT BMDMs under same conditions as Figure 1G for a single representative plate.

(F) ATP production rates were quantified using the Agilent Seahorse XF real time ATP rate assay on BMDM either mock-infected or infected with MOI 10 live H37Rv for 48 hours.

(G,H) Cell death in WT BMDM either mock infected or infected with live H37Rv or HK H37Rv at an MOI 10 was calculated using an LDH assay (G) or violet viability (H).

ANOVA followed by Tukey's honest significant difference test for multiple comparisons was used to assess significance. Adjusted p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure S2

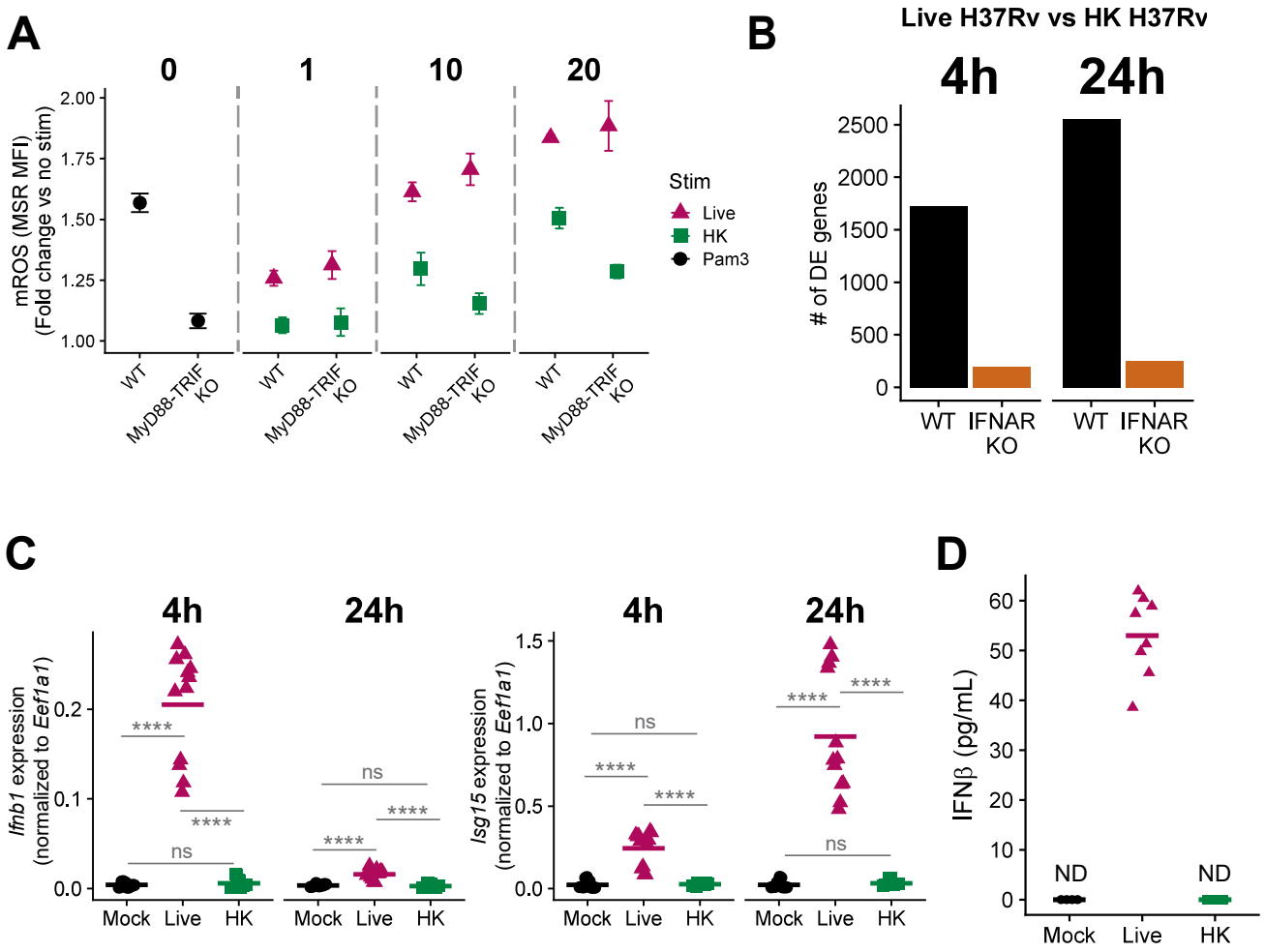

Figure S2. Type I IFN signaling dominates the BMDM transcriptional response to live Mtb and correlates with metabolic changes. Related to Figure 2.

(A) Mitochondrial reactive oxygen species (mROS) quantified by flow cytometric measurement of mitoSOX Red (MSR) fluorescence. WT or MyD88-Trif double KO BMDM were infected with either live H37Rv or heat killed (HK) H37Rv for 24 hours at the MOI indicated at top of graph. Pam3CSK4 (TLR2/TLR1 ligand; 300 ng/mL) was used as a control for MyD88-TRIF ablation. Points represent the mean for four technical replicates and error bars the SEM for one of two independent experiments.

(B) The number of DE genes (FDR <0.001, |log2FC| > 1) identified by edgeR analysis of RNAseq comparing infection conditions (live H37Rv vs HK H37Rv; MOI 10) at 4 or 24 hours post infection in WT or IFNAR KO BMDM.

(C) Expression of *Ifnb1* (left) and *Isg15* (right) was measured with RT-qPCR in WT BMDM either mock infected or infected with live H37Rv or HK H37Rv at an MOI 10 for the indicated number of hours. Expression was normalized to the housekeeping gene *Eef1a1*.

(D) IFNβ ELISA on culture supernatants collected 24 hours after mock infection or infection with MOI 10 live or HK H37Rv in WT BMDM. ND = not detected.

ANOVA followed by Tukey's honest significant difference test for multiple comparisons was used to assess significance. Adjusted p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure S3

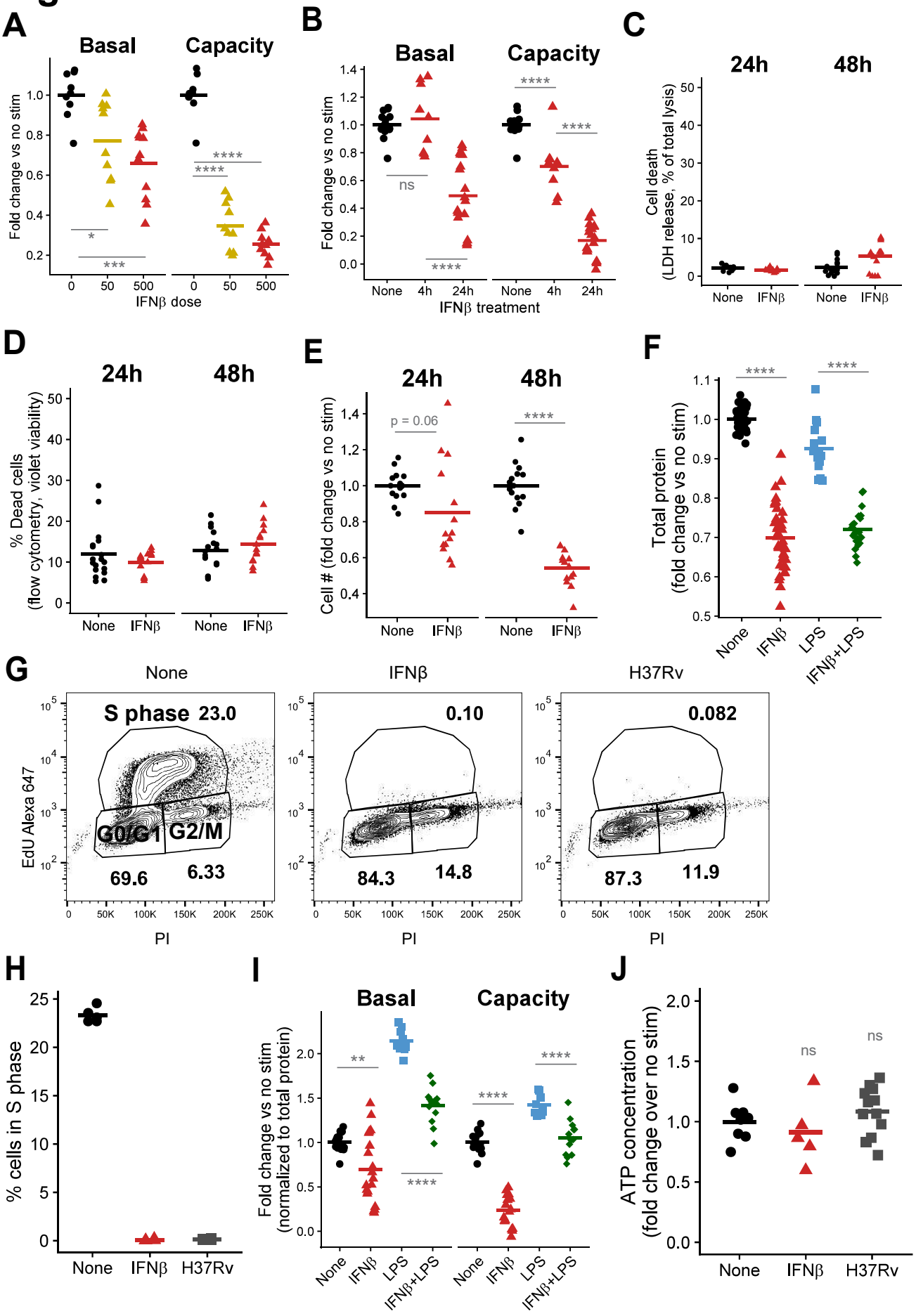
A
Basal
Capacity
Fold change vs no stim
IFNβ dose
B
Basal
Capacity
Fold change vs no stim
ns
IFNβ treatment
C
24h
48h
Cell death
(LDH release, % of total lysis)
None
IFNβ
D
24h
48h
% Dead cells
(flow cytometry, violet viability)
E
24h
48h
Cell # (fold change vs no stim)
p = 0.06
F
Total protein
(fold change vs no stim)
None
IFNβ
LPS
IFNβ+LPS
G
None
IFNβ
H37Rv
S phase 23.0
0.10
0.082
EdU Alexa 647
G0/G1
G2/M
69.6
6.33
84.3
14.8
87.3
11.9
PI
H
% cells in S phase
I
Basal
Capacity
Fold change vs no stim
(normalized to total protein)
J
ATP concentration
(fold change over no stim)
ns

Figure S3. IFNβ restrains BMDM glycolytic machinery and proliferation. Related to Figure 3.
(A) The extracellular acidification rate (ECAR) after addition of glucose (Basal glycolysis) or at maximum glycolysis (Capacity) in WT BMDM treated with either 50 or 500 U/mL IFNβ for 24 hours. 500 U/mL IFN treated data shown for comparison (appears in Figure 3A). Data for 50 U/mL shown for three independent experiments.
(B) Basal glycolysis and glycolytic capacity in WT BMDM treated with 500 U/mL IFNβ for either 4 or 24 hours. 24h IFN treated data shown for comparison (appears in Figure 3A). Data shown for 4h treatment from 3 independent experiments.
(C,D) Cell death in WT BMDM either untreated or treated with 500 U/mL IFNβ was calculated using an LDH assay (C) or violet viability (D).
(E) Flow cytometric counts (# live cells counted in 10 seconds during constant flow rate) for WT BMDM with no treatment or treatment with 500 U/mL IFNβ for the indicated time.
(F) WT BMDMs either untreated or treated with 500 U/mL IFNβ, 10 ng/mL LPS, or both for 24 hours were lysed and total protein was measured by a BCA assay. Fold change was calculated compared to untreated controls.
(G) Flow cytometric analysis of EdU incorporation to assess DNA synthesis and propidium iodide (PI) to assess DNA content in WT BMDM either untreated, treated with 500 U/mL IFNβ, or infected with an MOI10 of H37Rv for 24 hours.
(H) Quantification of cells in S-phase (i.e. EdU-positive) from experiments shown in (G). Bars show the mean of 5 biological replicates across 2 independent experiments.
(I) Normalized glycolytic parameters in WT BMDM treated as in Figure 3B. Basal glycolysis and glycolytic capacity were normalized per well to total protein shown in (F). Each dot represents a single well and the bar is the mean from 3 (LPS alone), 5 (IFNβ), or 4 (both) independent experiments.
(J) ATP concentration measured with bioluminescent ATP kit from WT BMDM either untreated, treated with 500 U/mL IFNβ, or infected with an MOI10 of H37Rv for 24 hours. Bars represent the mean of at least 4 biological replicates across 2 independent experiments. Statistical signicance assessed for comparisons to untreated BMDM.
ANOVA followed by Tukey's honest significant differences test for multiple comparisons was used to assess significance. Adjusted p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure S4

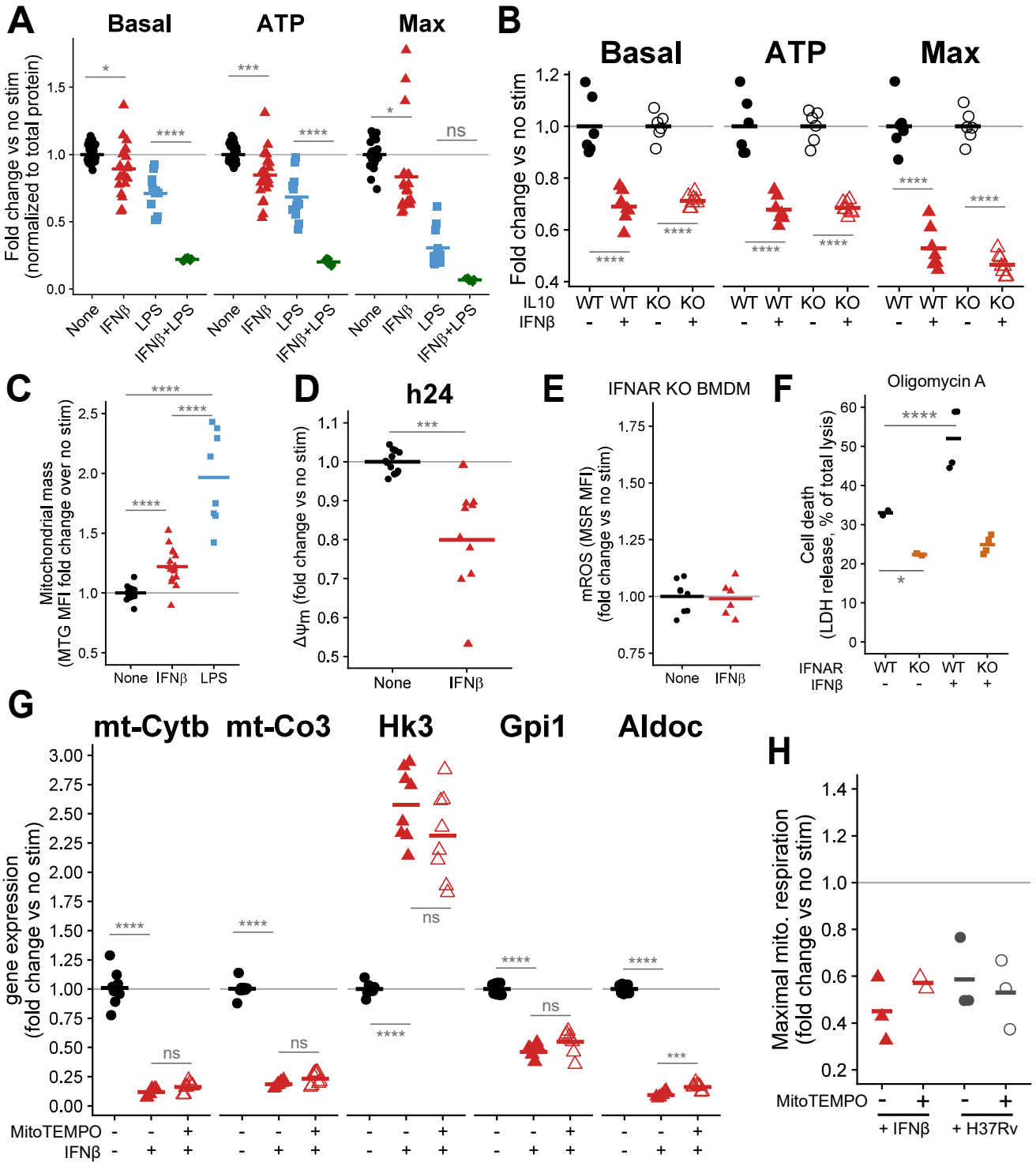
A
Basal
ATP
Max
Fold change vs no stim (normalized to total protein)
None
IFNβ
LPS
IFNβ+LPS
B
Basal
ATP
Max
Fold change vs no stim
IL10
IFNβ
WT WT KO KO
C
Mitochondrial mass (MTG MFI fold change over no stim)
None IFNβ LPS
D
h24
Δψm (fold change vs no stim)
None IFNβ
E
IFNAR KO BMDM
mROS (MSR MFI) (fold change vs no stim)
None IFNβ
F
Oligomycin A
Cell death (LDH release, % of total lysis)
IFNAR
IFNβ
WT KO WT KO
G
mt-Cytb
mt-Co3
Hk3
Gpi1
Aldoc
gene expression (fold change vs no stim)
MitoTEMPO
IFNβ
H
Maximal mito. respiration (fold change vs no stim)
MitoTEMPO
+ IFNβ
+ H37Rv

Figure S4. Interferon β impairs BMDM mitochondrial metabolism. Related to Figure 4.
(A) Mitochondrial parameters were normalized to total protein per well. Each dot represents a single well and the bar represents the mean from 4 (LPS conditions), 6 (IFNβ), or 3 (both) independent experiments.
(B) Mitochondrial parameters in WT or IL10 KO BMDM either untreated or treated with 500 U/mL IFNβ for 24 hours. Each point is a single well and the bar is the mean across two independent experiments.
(C) Mitochondrial mass measured with MitoTracker Green (MTG) MFI (flow cytometry) in WT BMDM either untreated or treated with 500 U/mL IFNβ or 10 ng/mL LPS for 48h. The bar is the mean across 3 experiments.
(D) $\Delta\psi_m$ calculated by normalizing TMRM fluorescence to MTG fluorescence measured by flow cytometry. The bar is the mean across two independent experiments.
(E) Quantification of mitochondrial reactive oxygen species (mROS) in IFNAR KO BMDM untreated or treated with 500 U/mL IFNβ for indicated times. mROS measured with MitoSOX Red (MSR) MFI (flow cytometry) normalized to untreated controls at each timepoint. Each point represents a single well and the bar is the mean from two independent experiments.
(F) Cell death (LDH release into supernatant normalized to total lysis) in WT and IFNAR KO BMDM treated with 1 uM oligomycin A and either untreated or treated with 500 U/mL IFNβ for 48 hours.
(G) Expression of *mt-Cytb*, *mt-Co3*, *Hk3*, *Gpi*, and *Aldoc* was measured with RT-qPCR in WT BMDM either untreated or treated with 500 U/mL IFNβ for 24 hours. Some IFNβ-treated wells also received 200 uM MitoTEMPO. Expression normalized to the housekeeping gene *Eef1a1*. Bars represent the means across two independent experiments .
(H) Maximal mitochondrial respiration was calculated from OCR values from an extracellular flux analyzer during a mitochondrial stress test after 24 hours of treatment with 500 U/mL IFNβ or MOI 10 H37Rv +/- 200 uM MitoTEMPO. ANOVA followed by Tukey's honest significant differences test for multiple comparisons was used to assess significance with >2 groups; Student's t test used for 2 groups. (Adjusted) p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure S5

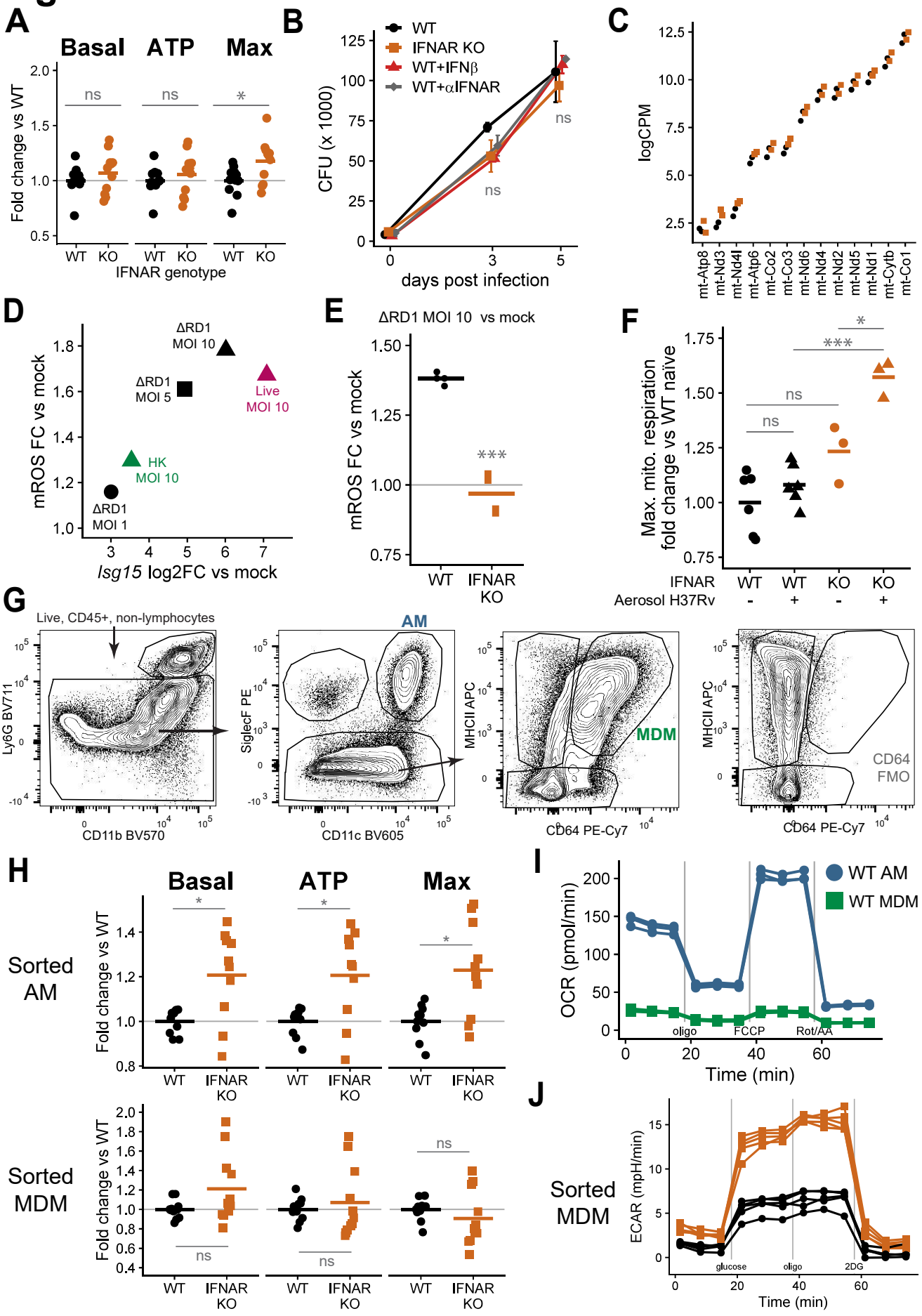
A
Basal
ATP
Max
Fold change vs WT
ns
ns
*
WT
KO
IFNAR genotype
B
WT
IFNAR KO
WT+IFNβ
WT+αIFNAR
CFU (x 1000)
days post infection
ns
ns
C
logCPM
mt-Atp8
mt-Nd3
mt-Nd4l
mt-Atp6
mt-Co2
mt-Co3
mt-Nd6
mt-Nd4
mt-Nd2
mt-Nd5
mt-Nd1
mt-Cytb
mt-Co1
D
ΔRD1 MOI 10
ΔRD1 MOI 5
Live MOI 10
HK MOI 10
ΔRD1 MOI 1
mROS FC vs mock
Isg15 log2FC vs mock
E
ΔRD1 MOI 10 vs mock
mROS FC vs mock
***
WT
IFNAR KO
F
Max. mito. respiration fold change vs WT naïve
*
***
ns
ns
IFNAR
Aerosol H37Rv
WT
WT
KO
KO
-
+
-
+
G
Live, CD45+, non-lymphocytes
Ly6G BV711
CD11b BV570
AM
SiglecF PE
CD11c BV605
MHCII APC
MDM
CD64 PE-Cy7
MHCII APC
CD64 FMO
CD64 PE-Cy7
H
Basal
ATP
Max
Sorted AM
Sorted MDM
Fold change vs WT
*
*
*
ns
ns
ns
WT
IFNAR KO
I
WT AM
WT MDM
OCR (pmol/min)
oligo
FCCP
Rot/AA
Time (min)
J
Sorted MDM
ECAR (mpH/min)
glucose
oligo
2DG
Time (min)

Figure S5. Type I IFN modulates macrophage metabolism in vivo. Related to Figure 5.
(A) Quantified mitochondrial parameters from an XF mitochondrial stress test on untreated WT and IFNAR KO BMDM.
(B) WT or IFNAR KO BMDM were left untreated or treated with 250 U/mL IFNβ or 10 μg/mL anti-IFNAR (clone MAR1-5A3) antibody. All conditions were infected with H37Rv at MOI 5 and colony forming units (CFU) of H37Rv was measured using 7H10 plate dilutions.
(C) Log2 counts per million (logCPM) calculated using edgeR from RNAseq data for protein coding genes of mtDNA for WT (black) and IFNAR KO (orange) BMDM. Each dot is a biological replicate.
(D) WT BMDM were mock infected or infected with heat killed H37Rv, live H37Rv, or live ΔRD1 H37Rv. mROS was measured with MitoSOX Red (MSR) MFI (flow cytometry) at 24 hours post infection and Isg15 expression normalized to Eef1a1 at 4 hours post infection was measured with RT-qPCR.
(E) WT or IFNAR KO BMDM were mock infected or infected with MOI 10 ΔRD1 H37Rv and mROS measured with MSR MFI (fow cytometry).
(F) Maximal mitochondrial respiration was quantified from an XF mitochondrial stress test on WT and IFNAR KO AM isolated by plated BAL from mice either uninfected or infected with a high-dose aerosol challenge of H37Rv as in Figure 5G. One of two independent experiments.
(G) Gating scheme for FACS sorting alveolar macrophages (AM) and monocyte derived macrophages (MDM) from aerosol infected mice. Total events were first gated on time, singlets, size (forward and side scatter), exclusion of a viability dye, and CD45 positivity before being gated as shown. Gate for MDM was based on fluorescence minus one (FMO) samples for both CD64 and MHC; CD64 FMO is shown on the far right.
(H) Mitochondrial parameters quantified for sorted AM (top) or sorted MDM (bottom). Points are technical replicates across 9 plates from 3 independent experiments and bars are the mean.
(I) OCR during a mitochondrial stress test comparing sorted WT AMs and MDMs. Each line is a technical replicate (across three XF plates). Shown for one of three independent experiments.
(J) In two experiments, glycolytic parameters were confirmed with a dedicated glycolysis stress test. Each line shows a technical replicate (across three XF plates) from one representative experiment.
ANOVA followed by Tukey's honest significant differences test for multiple comparisons was used to assess significance with >2 groups; Student's t test used for 2 groups. (Adjusted) p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure S6

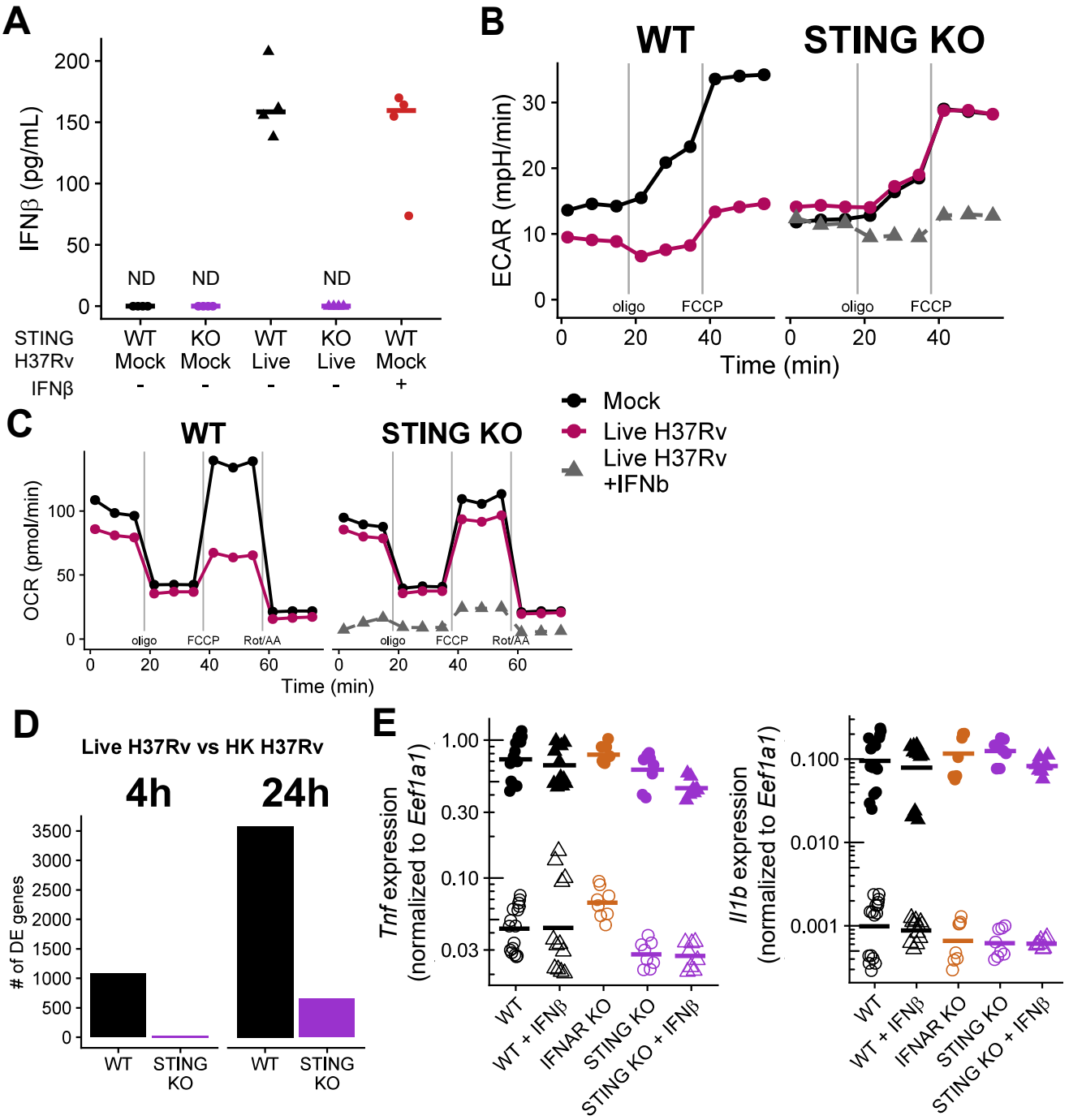

Figure S6. STING signaling is upstream of mitochondrial damage during Mtb infection. Related to Figure 6.
(A) IFNβ ELISA on supernatants of WT or STING KO BMDM infected with MOI 10 H37Rv or treated with 500 U/mL IFNβ for 24 hours. ND = not detected.
(B) Extracellular acidification rate (ECAR) of WT or STING KO BMDMs either mock infected or infected with live H37v at an MOI of 10 for 24 hours. Infected STING KO BMDM were left untreated or treated with 500 U/mL IFNβ. A single representative plate of 2 independent experiments is shown [see Figure 6B].
(C) Oxygen consumption rate (OCR) of WT or STING KO BMDMs in the same conditions as (B). A single representative plate of 2 independent experiments is shown [see Figure 6C].
(D) The number of DE genes (FDR <0.001, |log2FC| > 1) comparing infection conditions (live H37Rv vs HK H37Rv; MOI 10) at 4 or 24 hours post infection in WT or STING KO BMDM.
(E) Expression of *Tnf* (left) or *IL1b* (right) in WT, IFNAR KO, or STING KO BMDM either mock infected (open symbols and dashed line) or infected with MOI 10 live H37Rv (filled symbols and solid line) for 4 hours. WT and STING KO BMDM were either left untreated or treated with 500 U/mL IFNβ. Gene expression was quantified by RT qPCR. Each point represents a technical replicate and the bars are the mean from 2 experiments from each KO genotype.

Table S1

Oligonucleotides for Key Resources Table		
mt-Cytb TaqMan Gene expression assay	Thermo Fisher Scientific	Cat#4331182, ID#Mm04225271_g1
mt-Co3 TaqMan Gene expression assay	Thermo Fisher Scientific	Cat#4331182, ID#Mm04225261_g1
Ifnb1 TaqMan Gene expression assay	Thermo Fisher Scientific	Cat#4331182, ID#Mm00439552_s1
Isg15 TaqMan Gene expression assay	Thermo Fisher Scientific	Cat#4331182, ID#Mm01705338_s1
Il1b TaqMan Gene expression assay	Thermo Fisher Scientific	Cat#4331182, ID#Mm01336189_m1
Tnf TaqMan Gene expression assay	Thermo Fisher Scientific	Cat#4331182, ID#Mm00443258_m1
Hk3 TaqMan Gene expression assay	Thermo Fisher Scientific	Cat#4331182, ID#Mm01341942_m1
Aldoc TaqMan Gene expression assay	Thermo Fisher Scientific	Cat#4331182, ID#Mm01298116_g1
Gpi1 TaqMan Gene expression assay	Thermo Fisher Scientific	Cat#4331182, ID#Mm01962484_u1
Eef1a1 forward primer for custom TaqMan assay: 5' GCAAAAACGACCCACCAATG 3'	Integrated DNA Technologies	N/A
Eef1a1 reverse primer for custom TaqMan assay: 5' GGCCTGGATGGTTCAGGATA 3'	Integrated DNA Technologies	N/A
Eef1a1 probe for custom TaqMan assay: 5' /56-FAM/CACCTGAGCAGTGAAGCCAG/36-TAMSp/3'	Integrated DNA Technologies	N/A