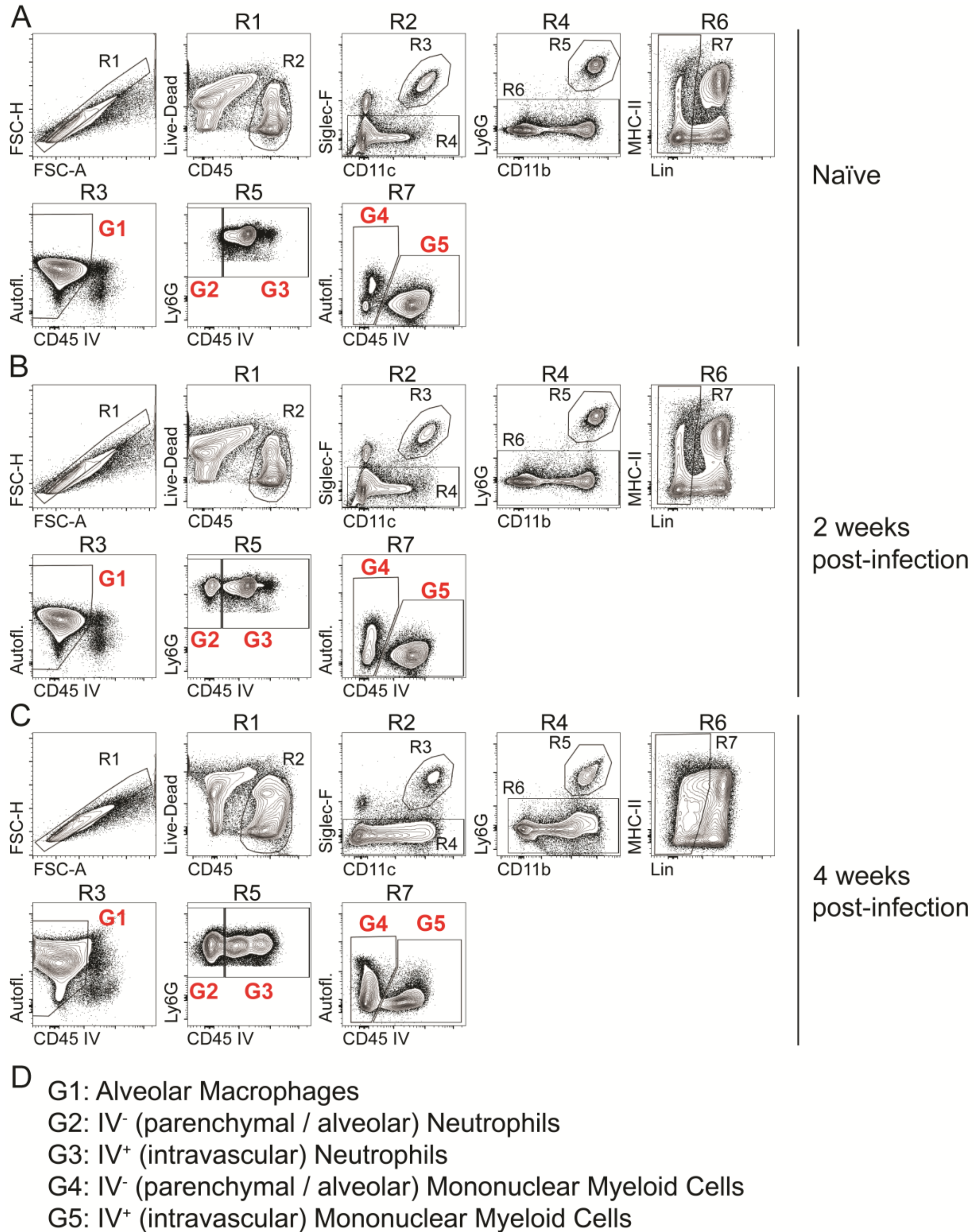

Figure S1: Gating strategy used for analysis of murine pulmonary myeloid cells. Cells were phenotyped according to their expression of CD45, viability dye, CD11c, Siglec-F, CD11b, Ly6G, I-A/I-E (MHC-II), Lin (lymphocytic lineage markers – TCRβ, TCRγδ, CD19 and NK1.1)

and intravascular CD45.1/2 staining. Representative dot plots of cells isolated from lungs of naïve (A), or Mtb-infected mice at 2 (B) and 4 (C) weeks post-infection (wpi); (D) nomenclature used throughout the text to identify specific cell populations as defined in Fig. S1A, S1B and S1C.

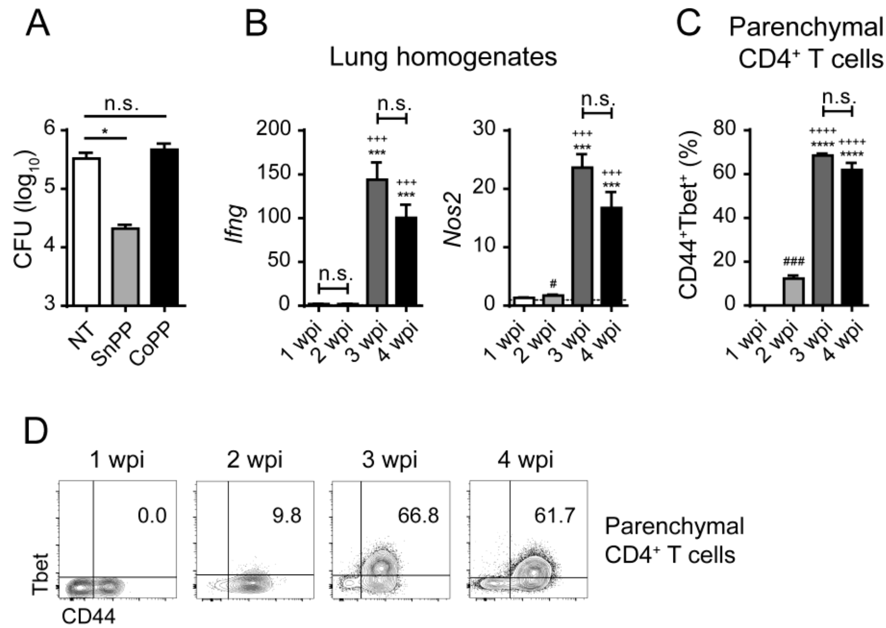

Figure S2: Outcomes of in vivo treatment of Mtb-infected mice with HO-1 inhibitor or inducer and time course of pulmonary IFNγ and NOS2 expression as well as CD44⁺Tbet⁺CD4⁺ T cell recruitment in Mtb-infected mice. (A) CFU loads in lung homogenates of Mtb-infected C57BL/6 mice intraperitoneally treated or not with tin protoporphyrin (SnPP - 5 mg/kg/day – HO-1 inhibitor) or cobalt protoporphyrin (CoPP - 5 mg/kg/day – HO-1 inducer), daily for 21 days starting at 28 days post-infection (n = 4 mice/group) (B) Quantification of mRNA for IFNγ (left) and NOS2 (right) by real time PCR in lung homogenates obtained from Mtb-infected C57BL/6 mice at 1, 2, 3 and 4 weeks post-infection (wpi) (n = 4 mice/group) - Actb F: 5’ AGC TGC GTT TTA CAC CCT TT 3’; Actb R: 5’ AAG CCA TGC CAA TGT TGT CT 3’; Ifng F: 5’ CGA TCT TGG CTT TGC AGC T 3’; Ifng R: 5’ CCT TTT TCG CCT TGC TGT TG 3’; Nos2 F: 5’ CGA AAC GCT TCA CTT CCA A 3’; Nos2 R: 5’ TGA GCC TAT ATT GCT GTG GCT 3’; (C) Frequencies of $CD44^{+}Tbet^{+}$ cells among $CD4^{+}$ T lymphocytes measured by flow cytometry in cells obtained from lungs of Mtb-infected C57BL/6 mice at 1, 2, 3 and 4 wpi (n = 4 mice/group); (D) Representative dot plots

from data shown in Fig. 2B (flow cytometry data concatenated from 4 samples). The data are presented as the means ± standard error (A, B and C), and dot plots from concatenated data (D). Data are representative of 2 (A, C and D) or 3 (B) independent experiments. Statistical analysis: unpaired Student’s t test. (A) * = $p< 0.05$, n.s = non-significant. (B and C) # = $p< 0.05$ and ### = $p<0.001$ compared to 1 wpi, *** = $p<0.001$ and **** = $p<0.0001$ as compared to 1 wpi; +++ = $p<0.001$ and ++++ = $p<0.0001$ as compared to 2 wpi, n.s = non-significant.

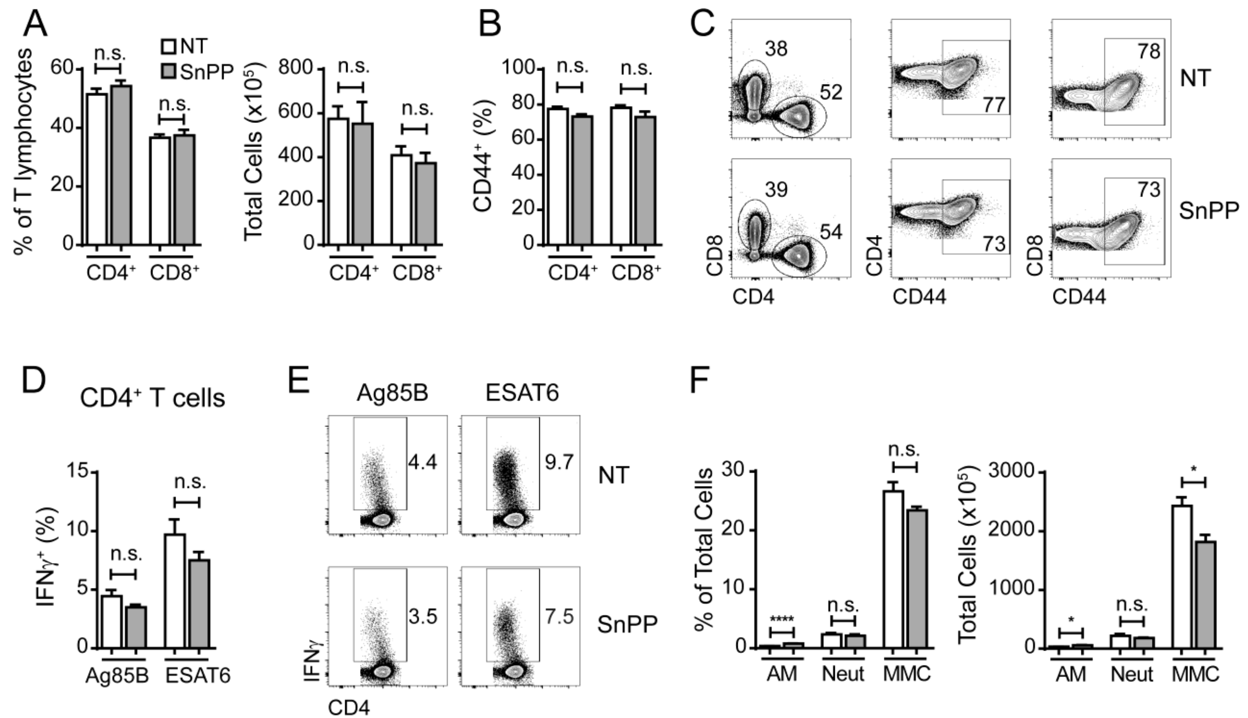

Figure S3: HO-1 inhibition has no significant effect on IFNγ production by CD4⁺ T cells.

(A) Frequencies of $CD4^+$ and $CD8^+$ cells among T lymphocytes (left) and total number of $CD4^+$ and $CD8^+$ T lymphocytes (right) and (B) frequencies of $CD44^+$ cells among $CD4^+$ and $CD8^+$ T lymphocytes obtained from lungs of Mtb-infected C57BL/6 treated or not for 21 days with SnPP, as measured by flow cytometry (n = 4 mice/group); (C) Representative dot plots of data shown in Fig. S2A and B (flow cytometry data concatenated from 4 samples); (D) Frequencies of $IFN\gamma^+$ cells in $CD4^+$ T lymphocytes obtained from lungs of Mtb-infected C57BL/6 mice treated or not for 21 days with SnPP (cells were stimulated for 5 hours with synthetic peptides representing the immunodominant Mtb epitopes Ag85B ($Ag85B_{280-294}$) or ESAT-6 ($ESAT\text{-}6_{4-17}$) in the presence of a brefeldin and monensin prior to staining) (n = 4 mice/group); (E) Representative dot plots from data shown in Fig. S2D (flow cytometry data concatenated from 4 samples). (F) Frequency of total (left) and total number (right) of alveolar macrophages (AM) and IV- parenchymal / alveolar neutrophils (Neut) and mononuclear myeloid cells (MMC) in

lungs of Mtb-infected C57BL/6 mice treated or not for 21 days with SnPP, gated as detailed in Fig. S1 (n = 4 mice/group). Data expressed as mean ± standard error (A, B, D and F) and dot plots from concatenated data (C and E). Data are representative of 2 independent experiments. Statistical analysis: Student's t test. * = $p < 0.05$, **** = $p < 0.0001$, n.s. = non-significant.

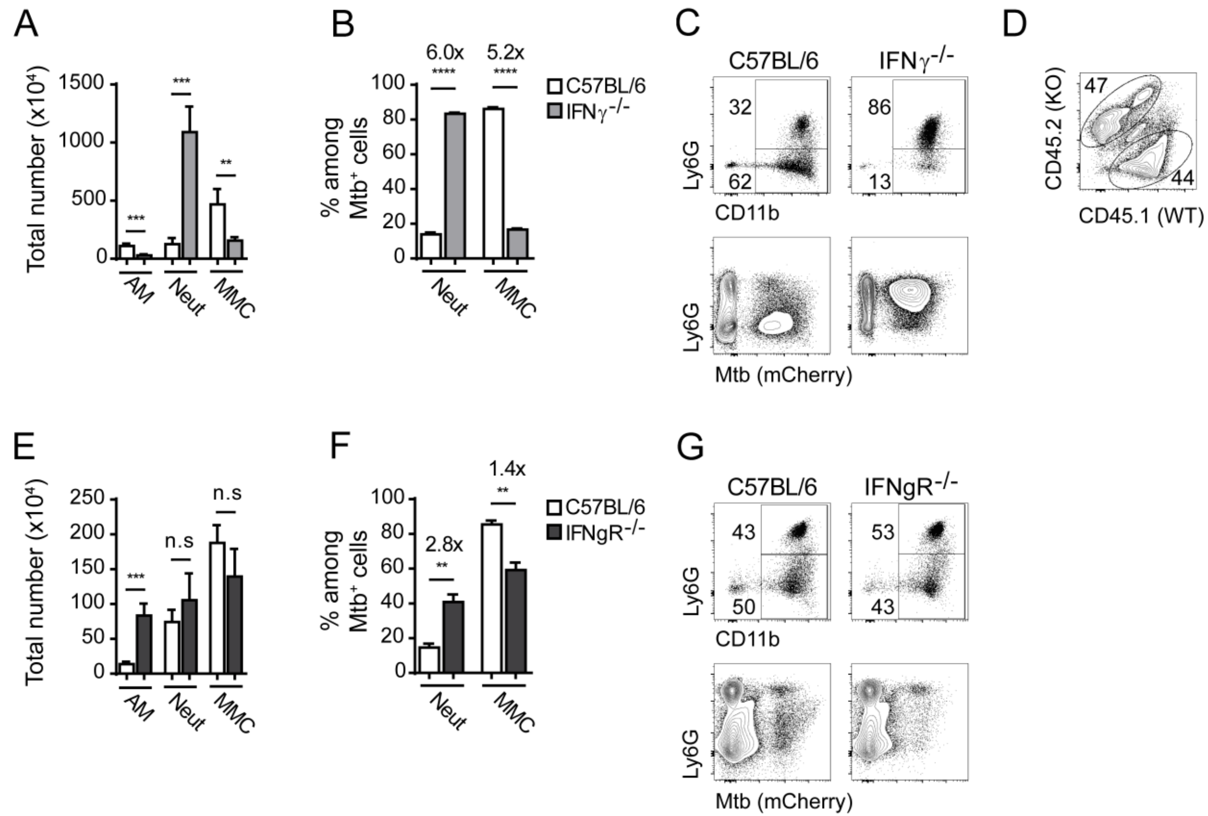

Figure S4: Validation of mixed bone marrow chimera approach for assessing the role of IFNγ signaling on HO-1 expression in vivo. (A) Total number of alveolar macrophages (AM), parenchymal / alveolar neutrophils (Neut) and parenchymal / alveolar mononuclear myeloid cells (MMC), detected by flow cytometry and gated as detailed in Fig. S1, in lungs from C57BL/6 or IFNγ$^{-/-}$ Mtb-infected mice at 4 weeks post-infection (wpi) (n = 4 mice/group); (B) Frequencies represented by parenchymal / alveolar neutrophils (Neut) and parenchymal / alveolar mononuclear myeloid cells (MMC) among mCherry Mtb^{+} cells in lungs of Mtb-infected C57BL/6 or IFNγ$^{-/-}$ mice at 4 wpi (n = 4 mice/group); (C) Representative dot plots showing frequencies of neutrophils and CD11b^{+} mononuclear myeloid cells (top) and mCherry Mtb staining in Ly6G^{+} and Ly6G^{-} cells (bottom), selected from the CD45 IV^{-} subset in gate R4 as detailed in Fig. S1, in lungs from C57BL/6 or IFNγ$^{-/-}$ Mtb-infected mice at 4 wpi (flow cytometry data concatenated from 4 samples); (D) Representative dot plots showing the

frequencies of WT and IFNgR$^{-/-}$ donor cells recovered from lungs of Mtb-infected chimeric recipient mice at 4 wpi (bone marrow chimeras described in Fig. 2F) (flow cytometry data concatenated from 4 samples); (E) Total number of alveolar macrophages (AM), parenchymal / alveolar neutrophils (Neut) and parenchymal / alveolar mononuclear myeloid cells (MMC) detected by flow cytometry among donor WT or IFNgR$^{-/-}$ cells in lungs of Mtb-infected chimeric recipient mice at 4 wpi (n = 4 mice/group); (F) Frequencies represented by parenchymal / alveolar neutrophils (Neut) and parenchymal / alveolar mononuclear myeloid cells (MMC) among mCherry Mtb^{+} WT and IFNgR$^{-/-}$ donor cells in lungs from Mtb-infected chimeric recipient mice at 4 wpi (n = 4 mice/group); (G) Representative dot plots showing frequencies of neutrophils and CD11b^{+} mononuclear myeloid cells (top) and mCherry Mtb staining in Ly6G^{+} and Ly6G^{-} cells (bottom), gated from the CD45 IV^{-} subset in gate R4 as detailed in Fig. S1, in donor WT or IFNgR$^{-/-}$ cells obtained lungs of Mtb-infected chimeric recipient mice at 4 wpi (flow cytometry data concatenated from 4 samples). Data expressed as mean ± standard error of mean (A, B, E and F) and dot plots from concatenated data (C, D and G). Data are representative of 2 (D, E and F) or 3 (A, B and C) independent experiments. Statistical analysis: Student’s t test. ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$, n.s. = non-significant.

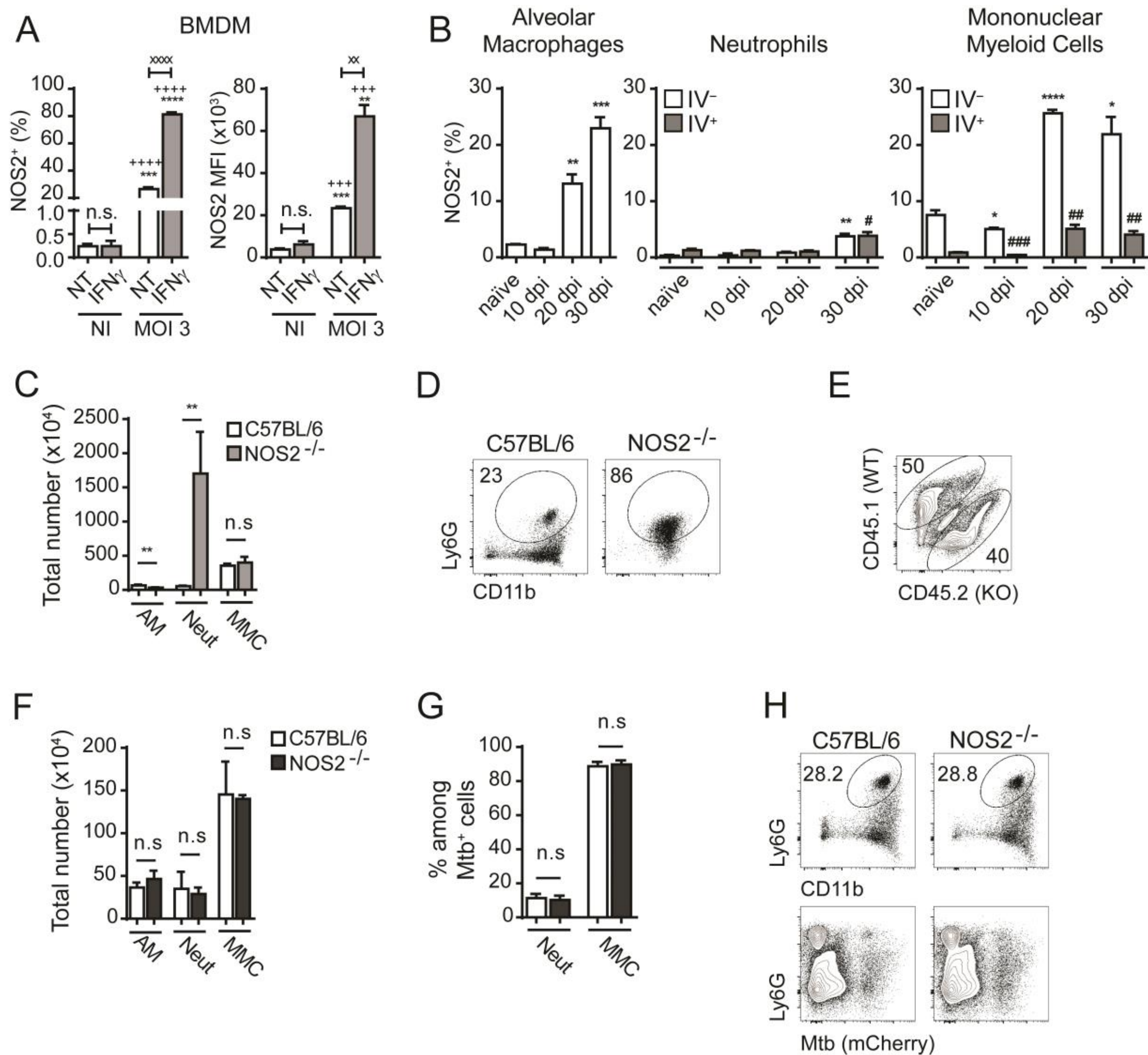

Figure S5: Induction of NOS2 expression in vitro and in vivo by Mtb infection and validation of mixed bone marrow chimera approach for assessing the role of NOS2 signaling on HO-1 expression in vivo. (A) Frequency of NOS2$^+$ (left) and median fluorescence intensity of NOS2 staining (right) in C57BL/6 bone marrow derived macrophages (BMDM) 24 hours after infection or not (NI – non-infected) with Mtb at a multiplicity of infection of 3 (MOI:3) and treatment or not with 250 U/ml of IFNγ (triplicates); (B) Frequencies of NOS2$^+$ alveolar macrophages (left), IV$^-$ and IV$^+$ neutrophils (middle) and IV$^-$ and IV$^+$ mononuclear myeloid cells (right), in lungs from naïve or Mtb-infected mice at 10, 20 and 30 days post-

infection (dpi), detected by flow cytometry and gated as detailed in Fig. S1 (n = 4 mice/group); (C) Total number of alveolar macrophages (AM), parenchymal /alveolar neutrophils (Neut) and parenchymal /alveolar mononuclear myeloid cells (MMC), detected by flow cytometry and gated as detailed in Fig. S1 in lungs from Mtb-infected C57BL/6 or NOS2$^{-/-}$ mice at 4 weeks post-infection (wpi) (n = 4 and 3 mice/group); (D) Representative dot plots showing frequencies of neutrophils selected from the CD45 IV^{-} subset in gate R4 as detailed in Fig. S1, in lungs from Mtb-infected C57BL/6 or NOS2$^{-/-}$ mice at 4 wpi (flow cytometry data concatenated from 4 and 3 samples); (E) Representative dot plots showing the frequencies of WT and NOS2$^{-/-}$ donor cells recovered from lungs of Mtb-infected chimeric recipient mice at 4 wpi (bone marrow chimeras described in Fig. 3C) (flow cytometry data concatenated from 3 samples); (F) Total number of alveolar macrophages (AM), parenchymal / alveolar neutrophils (Neut) and parenchymal / alveolar mononuclear myeloid cells (MMC), detected among donor WT or NOS2$^{-/-}$ cells in lungs of Mtb-infected chimeric recipient mice at 4 wpi (n = 3 mice/group); (G) Frequencies represented by parenchymal / alveolar neutrophils (Neut) and parenchymal / alveolar mononuclear myeloid cells (MMC) among mCherry Mtb^{+} WT and NOS2$^{-/-}$ donor cells in lungs from Mtb-infected chimeric recipient mice at 4 wpi (n = 3 mice/group); (H) Representative dot plots showing frequencies of neutrophils (top) and mCherry Mtb staining in Ly6G^{+} and Ly6G^{-} cells (bottom), gated from the CD45 IV^{-} subset in gate R4 as detailed in Fig. S1, in donor WT or NOS2$^{-/-}$ cells obtained chimeric recipient mouse lungs at 4 wpi (flow cytometry data concatenated from 3 samples). Data expressed as mean ± standard error of mean (A, B, C F and G) and dot plots from concatenated data (D, E and H). Data are representative of 2 independent experiments. Statistical analysis: Student's t test. (A) ** = $p<0.01$, *** = $p<0.001$ and **** = $p<0.0001$ as compared to NI – NT; +++ = $p<0.001$ and ++++ = $p<0.0001$ as compared to NI –

IFNγ; xx = $p<0.01$ and xxxx = $p<0.0001$, n.s. = non-significant; (B) * = $p<0.05$, ** = $p<0.01$ and **** = $p<0.0001$ as compared to naïve IV^-; # = $p<0.05$, ## = $p<0.01$ and ### = $p<0.001$ as compared to naïve IV^+; (C, F and G) ** = $p<0.01$, n.s. = non-significant.

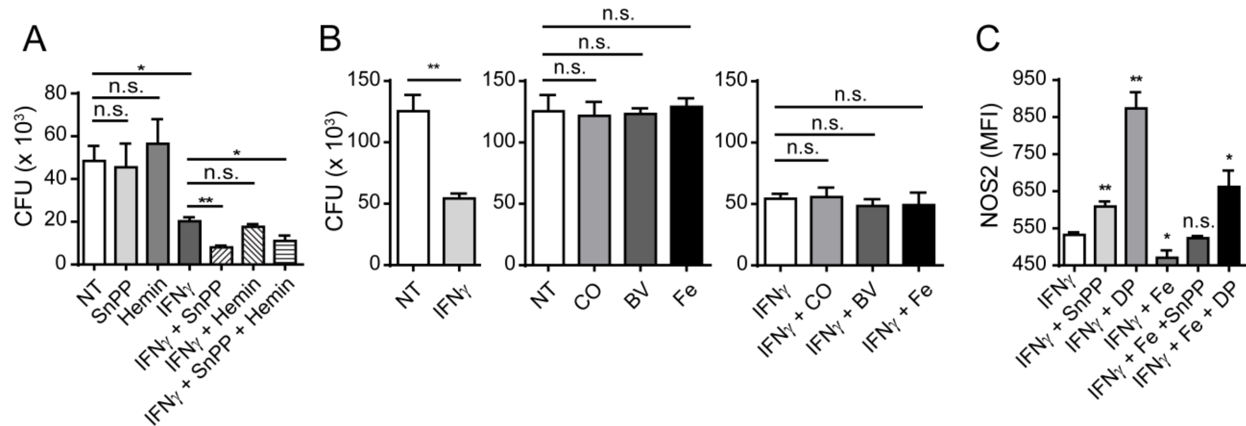

Figure S6: Effects of heme and HO-1 enzymatic reaction products on macrophage bacterial levels *in vitro* and of iron chelation on NOS2 expression. (A) CFU loads obtained from C57BL/6 BMDM 96 hours post-infection and stimulation or not with hemin (2.5 µM), IFNγ (250 U/mL), IFNγ (250 U/mL) plus hemin (2.5µM) or IFNγ (250 U/mL) plus SnPP (1µM) plus hemin (2.5µM) (triplicates); (B) CFU loads obtained from C57BL/6 BMDM 96 hours post infection and stimulation or not with (left): IFNγ (250 U/mL), (middle): CORM2 (5µM) - CO, biliverdin (5 µM) -BV or $FeSO_4$ (5 µM) – Fe, (right): IFNγ (250 U/mL), IFNγ (250 U/mL) plus CORM2 (5µM) – IFNγ + CO, IFNγ (250 U/mL) plus biliverdin (5 µM) – IFNγ + BV or IFNγ (250 U/mL) plus $FeSO_4$ (5 µM) – IFNγ + Fe (triplicates); (C) NOS2 expression measured by flow cytometry (MFI – Median of Fluorescence Intensity) in C57BL/6 BMDM 24 hours post-infection and stimulation with IFNγ (10 U/ml) in the presence or absence of SnPP (1 µM), 2,2' bipyridine (2,2' dipyridyl - DP – 50 µM), $FeSO_4$ (Fe – 5 µM), FeSO4 (Fe – 5 µM) + SnPP (1 µM) or $FeSO_4$ (Fe – 5 µM) + 2,2' bipyridine (2,2' dipyridyl - DP – 50 µM) (triplicates). Data expressed as mean ± standard error of mean. Data are representative of 2 independent experiments. Statistical analysis: Student's t test. (A and B) * = $p<0.05$, ** = $p<0.01$, n.s. = non-significant; (C) * = $p< 0.05$, ** = $p<0.01$, n.s. = non-significant, all compared to IFNγ alone.

Table S1: Antibody clones used for flow cytometry, sorting and western blot

Molecule	Clone	Manufacturer
CD4	GK1.5	eBioscience/ThermoFisher Scientific
CD8α	53-6.7	eBioscience/ThermoFisher Scientific
CD11b	M1/70	eBioscience/ThermoFisher Scientific
CD11c	N418	eBioscience/ThermoFisher Scientific
CD19	1D3	BD Biosciences
CD44	IM7	eBioscience/ThermoFisher Scientific
CD45	30-F11	eBioscience/ThermoFisher Scientific
CD45.1	A20	eBioscience/ThermoFisher Scientific
CD45.2	104	eBioscience/ThermoFisher Scientific
CD64	X54-5/7.1	eBioscience/ThermoFisher Scientific and Biolegend
I-A/I-E	M5/114.15.2	eBioscience/ThermoFisher Scientific
Ly6C	HK1.4	Biolegend
Ly6G	1A8	Biolegend
NK1.1	PK136	BD Biosciences
TCRβ	H57-597	BD Biosciences
TCRγδ	GL3	BD Biosciences
Siglec-F	E50-2440	BD Biosciences
HO-1	ADI-SPA-895	Enzo Life Sciences
IFNγ	XMG1.2	eBioscience/ThermoFisher Scientific

NOS2	CXNFT	eBioscience/ThermoFisher Scientific
Tbet	4B10	eBioscience/ThermoFisher Scientific
B-actin (1E5)	Polyclonal	Cell Signaling Technologies
Anti-rabbit IgG-HRP	Polyclonal	ThermoFisher Scientific
F(ab')2 donkey anti-rabbit IgG	Polyclonal	ThermoFisher Scientific

eBioscience/ThermoFisher Scientific (Waltham, MA), ThermoFisher Scientific (Waltham, MA), Biolegend (San Diego, CA), BD Biosciences (San Jose, CA), Enzo Life Sciences (Farmingdale, NY), Cell Signaling Technologies (Danvers, MA).