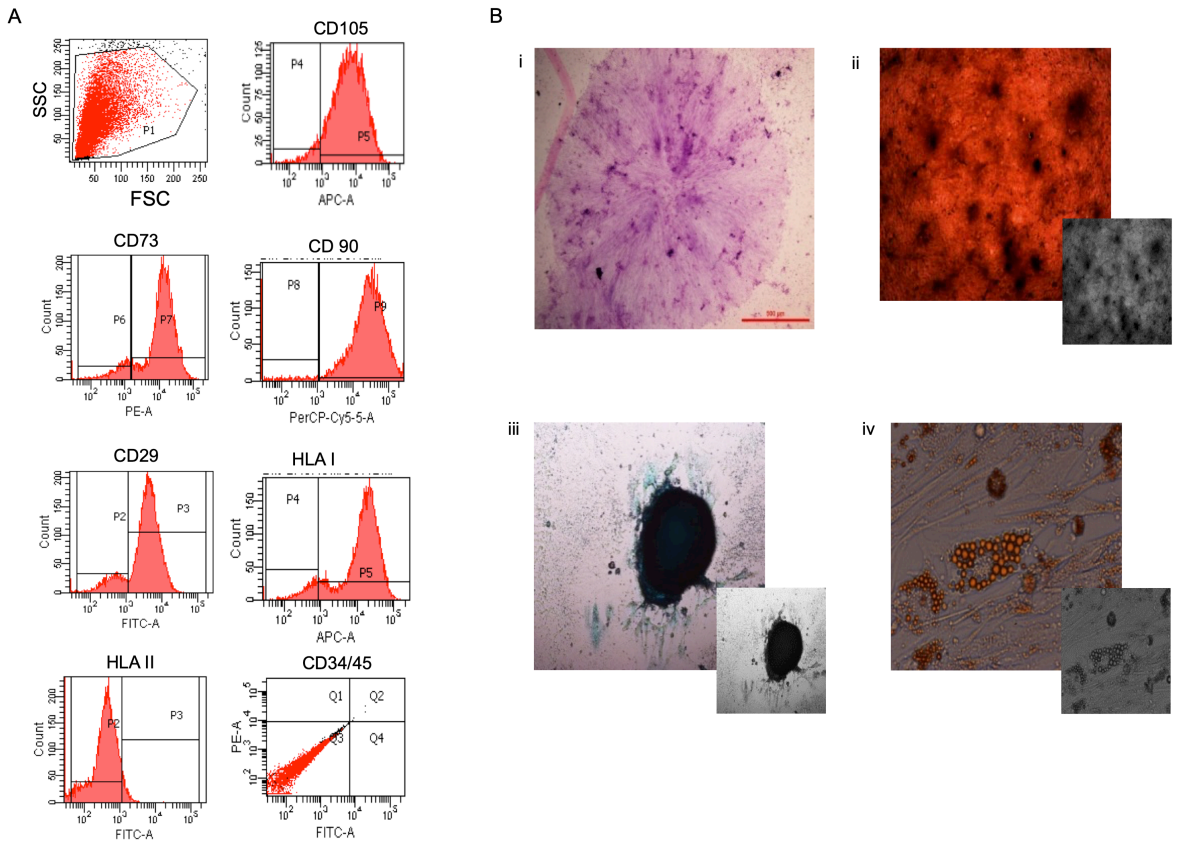
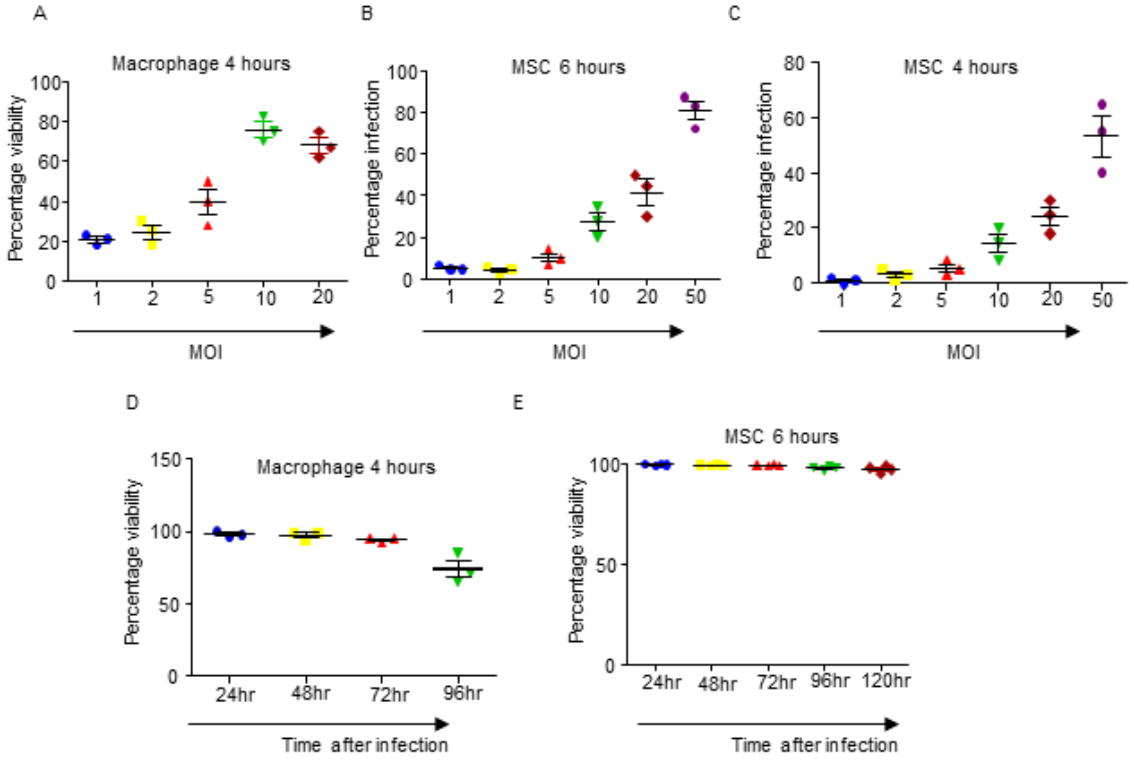
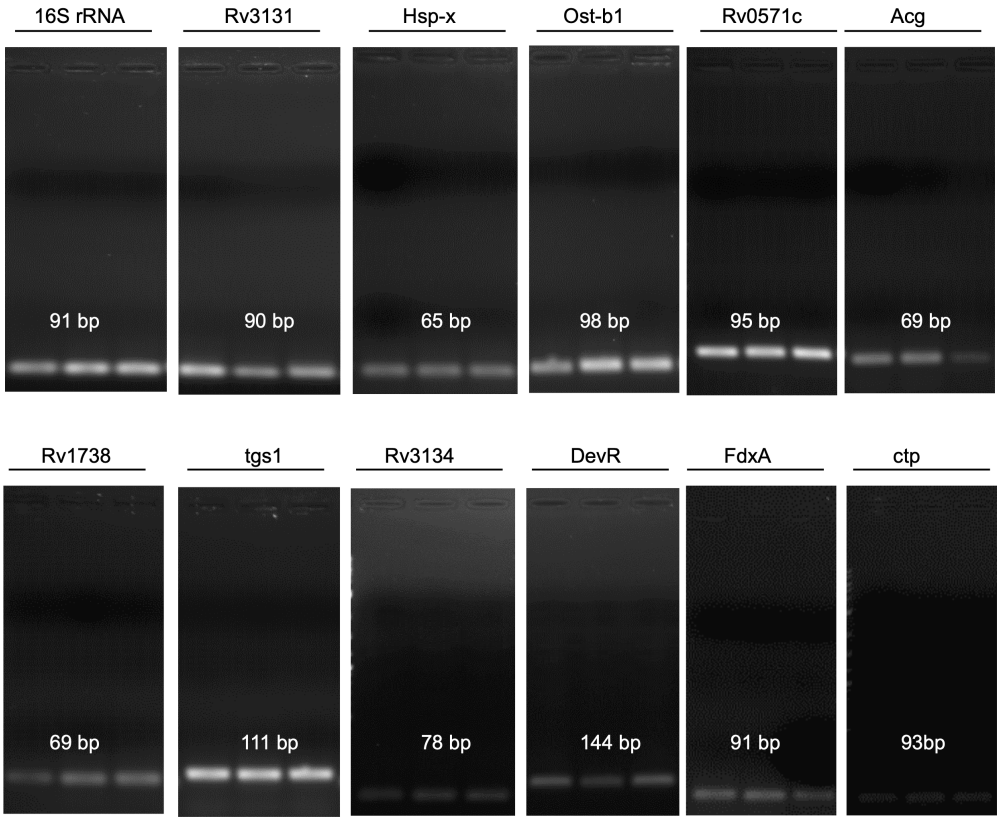
Supplemental Figures and Legends



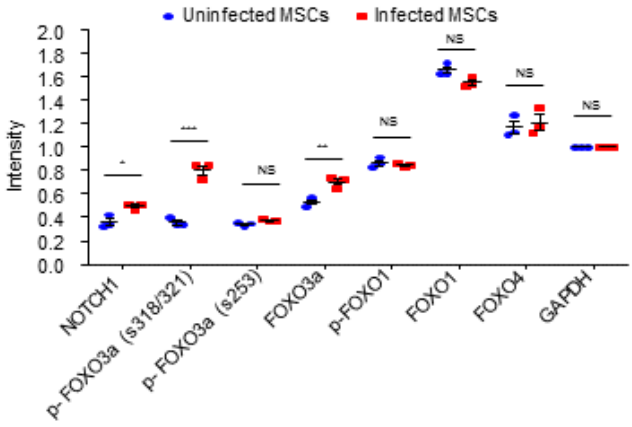
Supplemental Figure 1: Characterization of human BM-MSCs. (A) BM-MSCs were stained with antibodies against stem cell markers CD105, CD73, CD90, CD29, HLAI, HLAII and CD34/45. The MSCs stained positive for CD105, CD73, CD90, CD29, HLAI, and HLAII and negative for hematopoietic stem cell marker CD34/45. **(B)** (i) Colony Forming Unit-Fibroblast (CFU-F) assay for bone marrow derived MSCs. MSCs were subjected to trilineage differentiation, (ii) Alizarin Red S staining post osteogenic differentiation, (iii) Alcian Blue staining post chondrogenic differentiation, and (iv) Oil red O staining post adipogenic differentiation (20X magnification).



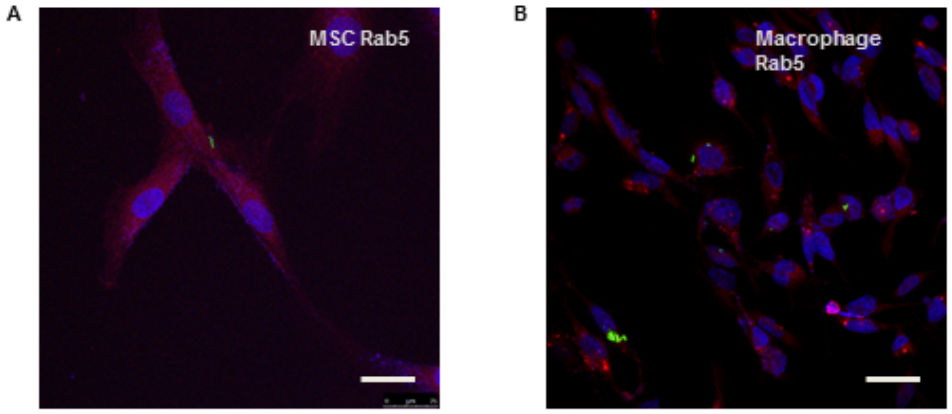
Supplemental Figure 2. Standardization of MOI in MSCs and macrophages. (A) Percentage infection in macrophages after 4 hours of infection by FACs using *M.tb*-GFP strain. **(B)** Percentage infection in MSCs after 6 hours of infection by FACs using *M.tb*-GFP strain. **(C)** Percentage infection in MSCs after 4 hours of infection by FACs using *M.tb*-GFP strain. **(D)** Percentage viability of macrophages at different time points after infection (up to 96 hours) at MOI 1:10 for 4 hours. After 96 hours the macrophages detached from the culture dish. **(E)** Percentage viability of MSCs at different time points after infection (up to 120 hours) at MOI 1:50 for 6 hours. These experiments are representative of three independent experiments.



Supplemental Figure 3: Agarose gel images of PCR products of dormancy-related genes. Agarose gel images showing specific amplification of *M.tb* dormancy genes. bp stands for base pairs (Supporting Figure 1C and D).



Supplemental Figure 4: FOXO3 and NOTCH 1 expression are associated with quiescence in MSCs. Relative intensity profile of proteins from forkhead signaling pathway generated using image J by normalizing the density of each protein to that of GAPDH. Statistical analyses were conducted using two-way ANOVA followed by Bonferroni post-test .Error bars represent S.E.M. *** represents P<0.001, ** P<0.01 and *P<0.05. P>0.05 is taken as non-significant (NS). This experiment was repeated three times.



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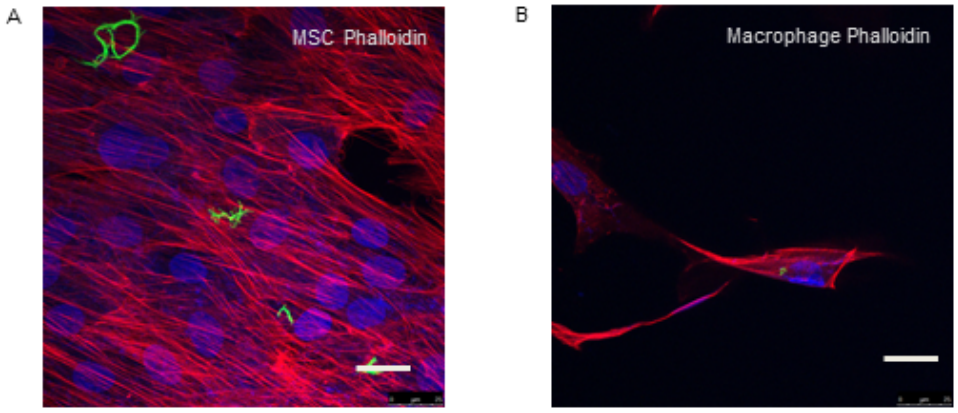
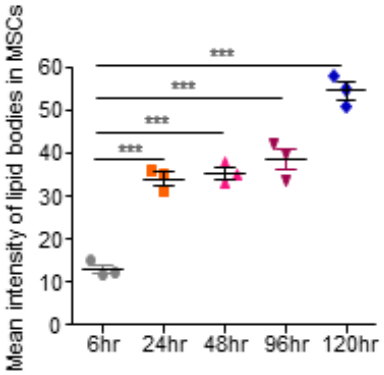
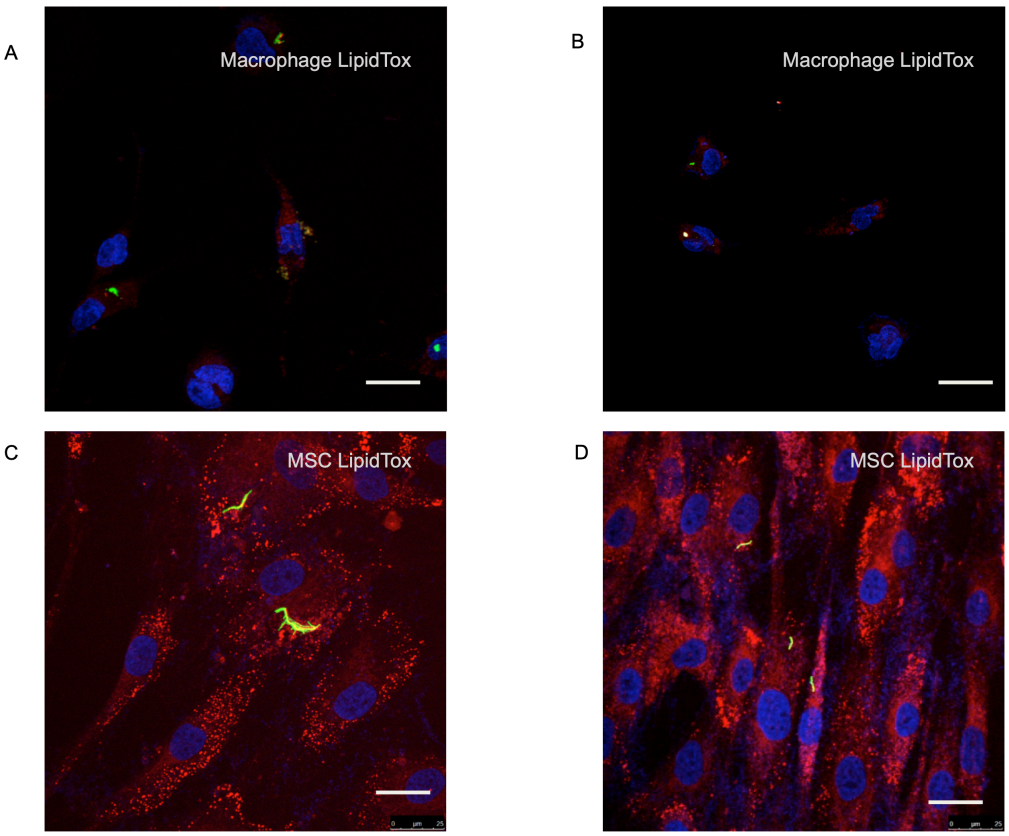
Supplemental Figure 5: Co-localization of *M.tb* with Rab5 in MSCs and macrophages. (A) Confocal microscopy image (bar=25 μM) showing *M.tb* localization in early-endosomes in MSCs. Rab5 is an early-endosomal marker. **(B)** Confocal microscopy image (bar=25 μM) showing *M.tb* localization in early-endosomes in macrophages. Each image is a representation of at least 30 fields. These data are representative of three independent experiments.



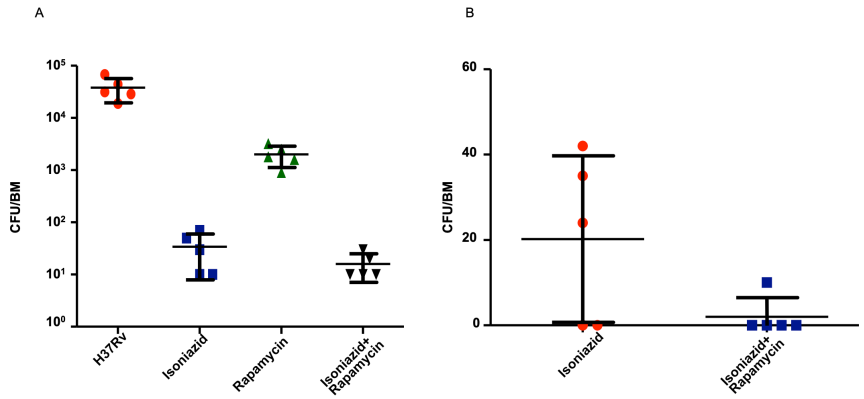
Supplemental Figure 6: Co-localization of *M.tb* with phalloidin in MSCs and macrophages. (A) Confocal microscopy image (bar=25 μM) showing *M.tb* localization in the cytosol of MSCs. Phalloidin is a cytosolic marker which binds F-Actin. Each image is a representation of at least 30 fields. **(B)** Confocal microscopy image (bar=25 μM) showing *M.tb* localization in the cytosol of macrophages. These data are representative of three independent experiments.



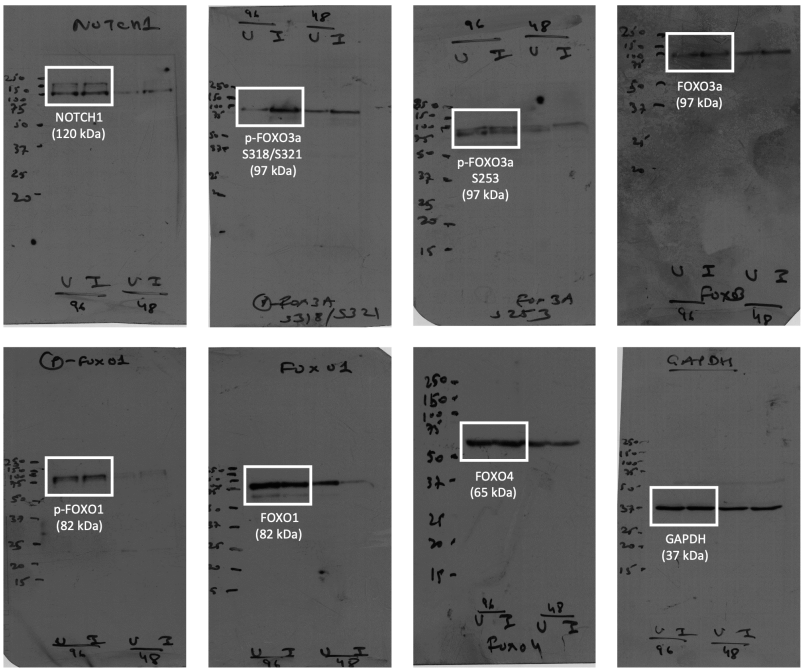
Supplemental Figure 7: Intensity of lipid content in MSCs increases drastically over time. Time dependent intensity profile of lipid-bodies inside MSCs post-infection with *M.tb*. Each bar represents mean intensity of 3 independent experiments with 20 fields each. Image represents the average of 30 fields and was analyzed using Leica Application Suite Software. Statistical analyses were conducted using one-way ANOVA followed by Tukeys post-test. These data are representative of three independent experiments. Error bars represent S.E.M. *** represents $P<0.001$, ** $P<0.01$ and *$P<0.05$. $P>0.05$ is taken as non-significant (NS).



Supplemental Figure 8: Co-localization of *M.tb* with lipid bodies in MSCs and macrophages. (A&B) Confocal microscopy images (bar=25 µM) showing *M.tb* localization in lipid bodies in macrophages. **(C&D)** Confocal microscopy images (bar=25 µM) showing *M.tb* localization in lipid bodies in MSCs. Images were taken in Leica confocal SP5 microscope using 60X objective. These data are representative of three independent experiments.



Supplemental Figure 9. *M.tb* burden in bone marrow after isoniazid, rapamycin and isoniazid+rapamycin treatment and reactivation of the disease upon dexamethasone treatment. (A) *M.tb* burden in bone marrow (BM) isolated from mice treated with or without isoniazid, rapamycin or isoniazid+rapamycin (n=5). **(B)** *M.tb* reactivation in BM isolated from mice treated with isoniazid and isoniazid+rapamycin followed by dexamethasone treatment (n=5). This data is representative of two independent experiment (n=5).



Supplemental Figure 10: Unedited gel picture to support Western-blots (Supporting Figure 1I).

Supplementary Information

Materials and Methods

Ethics Statement

The human studies were ethically approved by the Institutional Committee for Stem Cell Research, All India Institute of Medical Sciences (AIIMS, New Delhi, India). Animal experiments were performed according to the guidelines approved by the Institutional Animal Ethics Committee of All India Institute of Medical Sciences (AIIMS), New Delhi, and International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India adopting the guidelines of the Department of Biotechnology (Government of India).

Reagents

Rapamycin (Cat. No. R8781), isoniazid (Cat. No. I3377), phorbol 12-myristate 13 acetate (PMA) (Cat. No. P1585) and paraformaldehyde (Cat. No. P6148) were obtained from Sigma Aldrich Co (St Louis, USA). The immunosuppressant dexamethasone was also obtained from Sigma, Cat. No. D1756. Triacsin C, used to inhibit lipid synthesis was purchased from A.G Scientific, Inc. (Cat. No. T1242). Primers were purchased from Integrated DNA technologies (IDT), USA. Primary antibodies used were Rab 5 (SantaCruz, Cat. No. sc-46692) and Phalloidin (Invitrogen, Cat. No. A22287). Alexa Fluor-conjugated secondary antibody to Rab5 (Invitrogen, Cat. No. R37121) and lipidTox (Invitrogen, Cat. No. H34477) were purchased from Invitrogen molecular probes, USA. All antibodies used for flow cytometry were obtained from BD Bioscience and eBiosciences. Antibodies for Western blotting were procured from Cell Signaling Technologies (CST, 9946T). Murine Sca1, CD11b and CD45 antibodies were purchased from Biolegend (Cat. No. 108107, 101207 and 103129, respectively)

Animals

C57BL/6 strain of mice (6-8 week old) was used throughout the study. All animals were kept in institutional animal facility following Govt. of India guidelines. All mice used for the study were sacrificed at the particular time point by asphyxiation in carbon dioxide chamber according to institutional guidelines and Department of Biotechnology, Government of India guidelines and regulations.

Isolation and differentiation of human Mesenchymal Stem Cells (MSCs)

MSCs were isolated from the bone marrow (BM) of healthy human donors according to the institutional ethical guidelines. Cryopreserved human BM-MSC (n=5) were revived and cultured in low glucose containing Dulbecco's Modified Eagle's Medium (LG-DMEM, Gibco, Gaitherburg, MD, USA) supplemented with 10 % Fetal Bovine Serum (Gibco, Gaitherburg, MD, USA) and incubated at 37° Celsius and 5% CO_2 in a humified CO_2 incubator. For the experiments, MSCs were used at passage number 3 throughout the study. For all experiments cells were seeded at the required cell density, 24 hours prior to the experiment. Cells were stained with human MSC surface markers CD105 (eBioscience 17-1057), CD29 (eBioscience 14-0299), CD73 (BD 550257), CD90 (BD 555597), HLA-I (BD 555555) and HLA-II (BD 555560) and Hematopoietic Stem Cell Marker CD34/45 (BD 341071) and acquired on BD FACS LSRII and data was analysed with Flow Jo (Tree star, USA). MSCs were subjected to tri-lineage differentiation for characterization of human MSCs.

Peripheral Blood Mononuclear Cell (PBMC) isolation from blood and differentiation to macrophages

Blood was collected from healthy donors in heparin-coated tubes and diluted in DPBS (Gibco, 14190250) at a ratio of 1:2. This blood DPBS diluent was layered onto Ficoll-Paque™ Plus (Cat. No. GE17-1440-02) and centrifuged at 800g for half an

hour. The interphase cells containing the lymphocytes and monocytes were transferred to a new tube and subjected to RBC lysis. The PBMCs were then plated on Poly-D-lysine coated flasks and allowed to stay at 37˚ Celsius and 5 % CO_2 for 3-4 hours in DMEM without FBS. The non-adherent cells were washed with DPBS and the adherent population was treated with 50 ng/ml Macrophage Colony Stimulating Factor (MCSF) from R&D Systems. The media was changed every 2 days for 7-8 days. The attached macrophages were trypsinised and seeded for CFU experiment at 10,000 cells per well. $CD45^{-}Sca1^{+}$ and $CD45^{+}CD11b^{+}$ markers were used for sorting the MSCs and macrophage populations, respectively.

Bacterial Culture

H37Rv strain of *M.tb* used for infection was a kind gift from Colorado State University. *M.tb* was grown up to mid-log phase in 7H9 media (Middlebrooks, Difco™) supplemented with 10% OADC (Ovalbumin, Dextrose and Catalase, Difco™, USA), 0.05% Tween-80 and 0.2% glycerol. Cultures were cryopreserved in 20 % glycerol and preserved at -80˚C until used for infection.

***M.tb* infection and estimation of Colony Forming Units (CFU)**

Human BM-MSCs were infected at MOI of 1:50 for 6 hours and THP-1 (Human monocytic cell line) (ATCC®TIB-202™) and PBMC derived macrophages at MOI of 1:10 for 4 hours to obtain equal internalization of bacteria at 0 hours. After infection, cells were treated with 100 µg/ml of Gentamicin (Gibco, USA) for 2 hours followed by washing and incubation at 37 ˚ C and 5% CO_2 in complete LG-DMEM and complete RPMI-1640 (Invitrogen) medium, respectively. For in vivo experiments mice were infected with low-dose aerosol infection, with approximately 200 CFU per mouse. 15 ml of *M. tuberculosis*, H37Rv ($20x10^6$ bacteria/ml) in single cell suspension was used in a nebulizer chamber to deliver the desired CFU of bacteria to

the lungs of mice kept in the aerosol chamber for a 15 minute cycle. In vitro bacterial colony numbers were counted by lysing 10,000 cells per well by using 250 µl of 0.05% SDS buffer and incubating for 5 minutes for both MSCs and macrophages. Dilutions were made and plated on 7H11 plates supplemented with 10% OADC (Difco, BD). The colonies were counted on day 21 and data was plotted.

Flow Cytometry

For flow cytometry cells were harvested, pelleted and washed twice with FACs buffer. Cells were stained with the antibodies for surface markers and incubated for half an hour. After incubation cells were washed twice with PBS and suspended in PBS. Acquisition was done on BD FACS LSRII and data were analysed with Flow Jo (Tree star, USA). MSCs ($CD45^{-}Sca1^{+}$) were sorted from bone marrow of *M.tb* infected mice and macrophages ($CD45^{+}CD11b^{+}$) were sorted from the lungs of *M.tb* infected mice.

Transcriptomics and data analysis

Total RNA was isolated from MSCs infected with H37Rv using Trizol method (Invitrogen). RNA quality was analysed by Agilent Bioanalyser and by agarose gel electrophoresis. Sequencing libraries were prepared using Illumina Platform to generate ~6 GB data per sample. The fastq files were analyzed using Tuxedo protocol. The files were aligned against (GRCh37/hg19) version of the human genome. ClustVis, online tool was used for generating heat maps (1).

Real Time PCR

RNA was isolated using Trizol method. cDNA was prepared using iScript cDNA synthesis kit (Cat. No. 1708890, Bio-Rad, USA) for MSCs, and for bacterial cDNA synthesis, RevertAid First Strand cDNA synthesis kit (Cat. No. K1622, Thermo Fischer Scientific, USA) was used. Real Time PCR (qPCR) was performed in 10 µl

reaction volume according to the manufacturer's instructions using CFX96 Real Time PCR Detection System (Bio-Rad, USA). All reactions were normalized to GAPDH for human MSCs and 16S rRNA for *M.tb*. Primer sequences have been provided as supplemental table 1.

Supplemental Table 1 showing list of primers

Gene Name	**Primer Sequence (5'→3')**
Rv0001 F	GCGACGTAGACGTGCTGTTG
Rv0001 R	GGCATTGTGCAAGG TGTTGA
dnaA F	CCAGACACCACAACCGACAA
dnaA R	TGGCCAACTGTGCTGGTTATC
ftsZ F	GCAGCGACTTGGGCTT GTTC
ftsZ R	GGGTGAGCGGCGTCTTGTAC
parB F	GCGTAAGCCGATTCAGATGCC
parB R	CC GACGCGAACTCCACCAC
smc F	TGAACTGGATCAAGGCGAGGTC
smc R	CAACGCGGCCACAGTACG
Rv0058 F	GAGAGGCGACGCGTACTGGG
Rv0058 R	ATGACAACGTCGGCATCTTG
Rv0169 F	CCGGAATCGTTGGCGGCGCG
Rv0169 R	TGCATAAGGCGAGCCAACCT
dnaN F	CGGTGAGACGGTGGTTTTG
dnaN R	CGACGCCGACCACTTCAG
parA F	TCACCACCGTGATCCTTACCA
parA R	CTTGATCGGCGAGCT TTGTC
ftsQ F	TGACGTTGGCCGATGGCCGCG
ftsQ R	GCCTGGCTGGGTCAACAGCGC
rodA F	TGACCTTCCTCAAGGACCAC
rodA R	GCCGAAAAGAAGATCAGCAG
pbpA F	GTGAGGCATTCGTCAAATCA
pbpA R	TTTTGGCCGATACTGGTCAT
Rv0571c F	ACG GTG CGG ACA AGG TGG TGC TG
Rv0571c R	CGC CAA ACA CAC CAC CTC ATC GG
narK2 F	CTG GTA CCA GCC GGC GCG
narK2 R	AAC CGC GGG GTG AAG AAC GC
Rv1738 F	CGA CGA ACA CGA AGG ATT GA
Rv1738 R	ACA CCC ACC AAT TCC TTT TCC
ctpF F	CAG CAC CAC GGT CAT CTG
ctpF R	ATC TCA CCG TGG GGT GTC
otsB1 F	GAT CTA CCC TGA GCG CTG TC
otsB1 R	GAT ATC GGA GCG CAG GAC
fdxA F	TGT CCG GTC GAC TGT ATC TAT GA

fdxA R	GGC AGG CCG GTT TGC
hspX F	CGC ACC GAG CAG AAG GA
hspX R	ACC GTG CGA ACG AAG GAA
acg F	GCT TTT GAG ACT TCT GAG GGC ATA
acg R	GGT GAC CCG GTC ACT TTC G
tgs1 F	TGG CTG CCG GGC CTT TCC C
tgs1 R	GCA GGG CCA AAG GTC CTC C
Rv3131 F	CGA TCA GGC CGA TGT CGC CTT
Rv3131 R	TCA CCT CCT GGC ACC GGC C
devR F	CCG ATC TGC GCT GTC TGA TC
devR R	GTC CAG CGC CCA CAT CTT T
Rv3134c F	CTG GCT GGG TCG GCC TTA
Rv3134c R	GCT GAC CTG GGA GGT TGT CG
Human PCNA F	GGCTCTAGCCTGACAAATGC
Human PCNA R	CTAGCTGGTTTCGGCTTCAG
Human CCNA1 F	GCCACCTGCAGTTCTTCTTC
Human CCNA1 R	CAACGTGCAGAAGCCTATGA
Human FoxO3 F	GGCGGACTTTGTGTTTGTTT
Human FoxO3 R	AAGCCACCTGAAATCACACC
Human SKP2 F	GTAGAGACGGGGTTTCACCA
Human SKP2 R	GCAGTTGCTCATGCCTGTAA
Human gapdh F	GGCCTCCAAGGAGTAAGACC
Human gapdh R	AGGGGTCTACATGGCAACTG
Human notch1 F	GGAGGCATCCTACCCTTTTC
Human notch1 R	TGTGTTGCTGGAGCATCTTC

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107 **Confocal Microscopy**

108 BM-MSCs and THP-1 cells were seeded on glass coverslips (Cat. No. 2845-22,
109 Corning) and infected with *M.tb*-GFP at the MOI mentioned above. After 6 hours and
110 4 hours of infection respectively, cells were washed twice with media supplemented
111 with 10 % FBS and treated with 100 μg/ml gentamicin for 1 hour. After 2 hours, cells
112 were washed twice, supplemented with fresh media and kept at 37˚C and 5% CO_2. At
113 the desired time point, the cells were washed twice with PBS and fixed in 2%
114 Paraformaldehyde in PBS, pH 7.4 for 15-20 minutes. After fixation cells were washed
115 three times with PBS at pH 7.4 and kept at 4˚C until used for staining. Staining was
116 done using anti-Rab 5 (Cat. No. sc-46692), Phalloidin (Invitrogen, Cat. No. A22287)
117 and LipidTox (Invitrogen, Cat. No. H34477) according to the manufacturer's

protocol. Images were taken on Leica confocal SP5 microscope using 40X and 60X objectives. Analysis of images was done using the Leica Application Suite software.

Transmission Electron Microscopy (TEM)

MSCs were cultured in 100 mm culture dish in LG-DMEM medium with 10% FBS. Cells at 70% confluency were infected with *M.tb* at MOI of 1:50. Cells were incubated at 37℃ in 5% CO_2. At 96 hours' time point, infected cells were washed three times with PBS. Following this, cells were harvested (using a scraper) and fixed using 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, and 4% PFA for 24 hrs at 4^oC (2, 3) and processed for sectioning at Advanced Instrumentation Research Facility (AIRF), JNU, New Delhi. Ultra-thin sections were cut (50-60 nm) and collected on nickel grid. Electron microscopic images were taken using a transmission electron microscope facility at AIIMS, New Delhi.

Reactivation Experiment

Mice infected with *M.tb* strain H37Rv following the low-dose aerosol infection model (~220 CFU per mice) were treated with 5 mg/kg INH administered *ad libitum* (in the drinking water) every day for 16 weeks starting from 15 days post infection. These mice were then rested for 15 days followed by treatment with dexamethasone (5 mg/kg administered intraperitoneally) three times per week for 30 days. Five mice from each group were then sacrificed and CFUs and RT-PCR for latency and replicative genes were estimated from lung and bone marrow to determine the reactivation rate of latent mycobacteria.

Western Blot

Whole cell lysates were prepared from uninfected and *M.tb* infected MSCs by using RIPA lysis buffer using Protease and Phosphatase inhibitor cocktail (Thermo Scientific, Cat. No. 78441) and Western Blot analysis was done using Forkhead

Signaling Kit (CST, 9946T). 40 µg of protein was loaded into each well and electrophoresed on 12% SDS-PAGE and transferred to Nitrocellulose Membrane. Blots were probed with antibodies against NOTCH-1, p-FOXO3a(s318/321), p-FOXO3a(s253), FOXO1, p-FOXO1, FOXO4 and GAPDH.

Statistical Analysis

Statistical Analysis was performed using GraphPad 5.0 software. Student two-tailed t-test and two-way ANOVA followed by Bonferroni post-test were used to determine statistical significance. Error bars represent S.E.M .*** represents $P<0.001$, ** $P<0.01$ and *$P<0.05$. $P>0.05$ is considered non-significant (NS). A P value less than 0.05 was considered significant.

Study Approval

This study was ethically approved by the Institutional Committee for Stem Cell Research, All India Institute of Medical Sciences (AIIMS), New Delhi, India; (Reference number: IC-SCR/47/16(R)).

References:

1. Metsalu T, Vilo J. ClustVis: A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. *Nucleic Acid Res*. 2015;43(W1): W566-W570.
2. Knight M, et al. Lipid droplet formation in *Mycobacterium tuberculosis* infected macrophage requires IFN-γ/HIF-1α signaling and supports host defense. *PLoS Pathog.* 2018;14(1):e1006874.
3. Fujimoto T, Parton RG. Not Just Fat: The Structure and Function of the Lipid Droplet. Cold Spring Harbor Perspectives in Biology. 2011;3(3):a004838–a004838. pmid:21421923