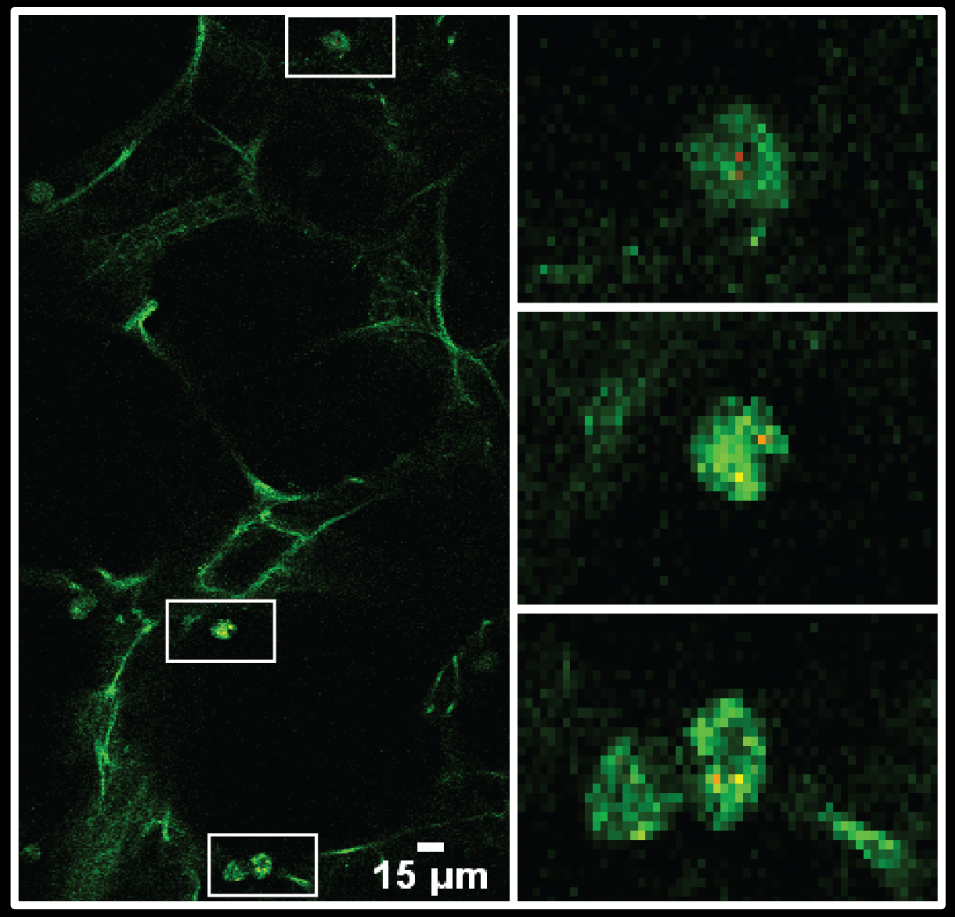
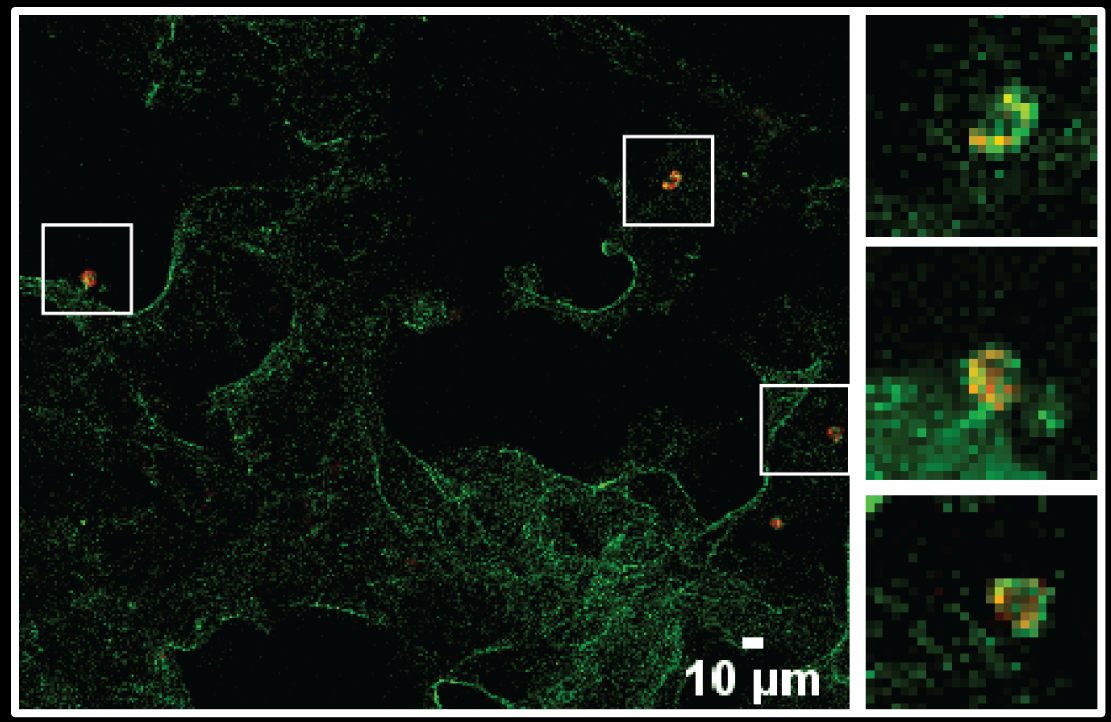
Confocal images of initial asymptomatic course of mycobacterial infection *in situ* in human and monkey lungs

BCG infected human lung slice



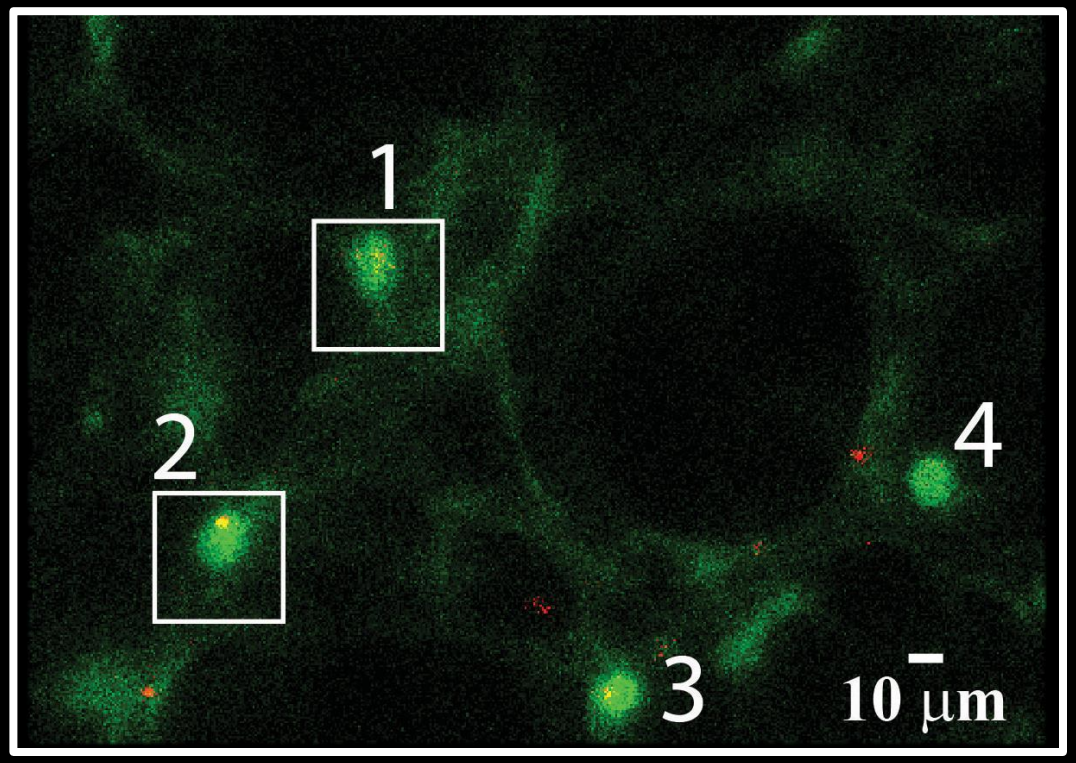
BCG infected marmoset lung slice



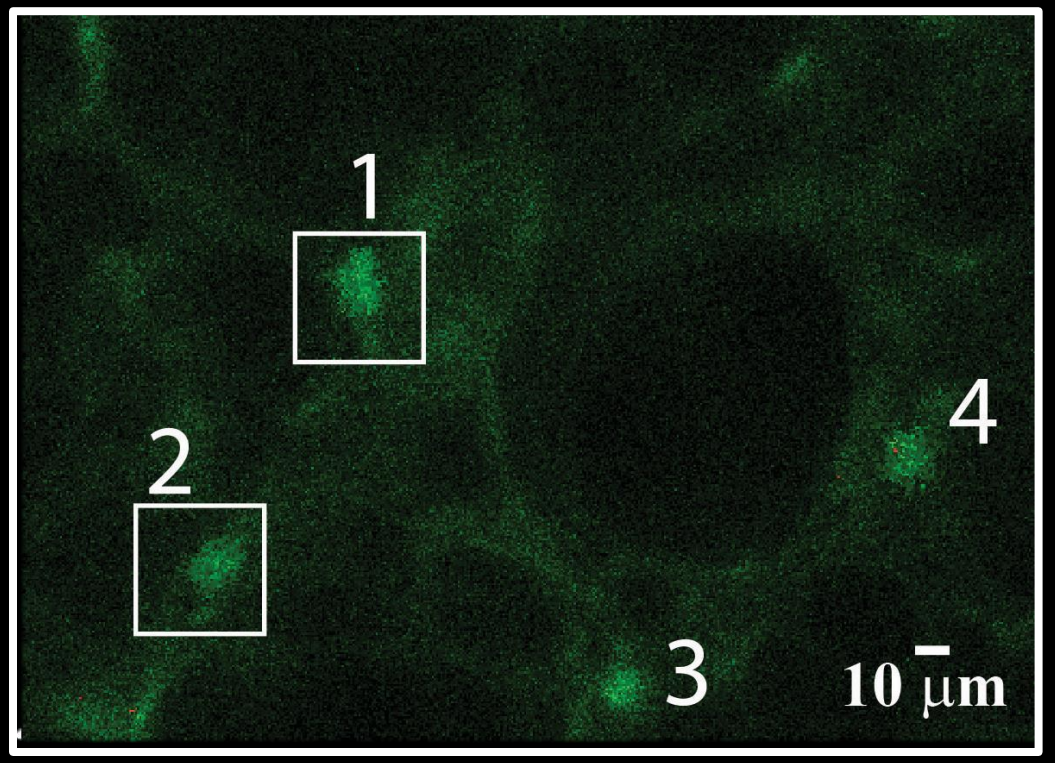
BCG: Bacillus Calmette–Guérin, vaccine strain of tuberculosis
CD11c: alveolar macrophage marker
Lung tissues give autofluorescence under green channel

Slice macrophages clear intracellular BCG as *in vivo*

1 day after infection



5 day after infection



BCG: Bacillus Calmette–Guérin, vaccine strain of tuberculosis

CD11c: alveolar macrophage marker

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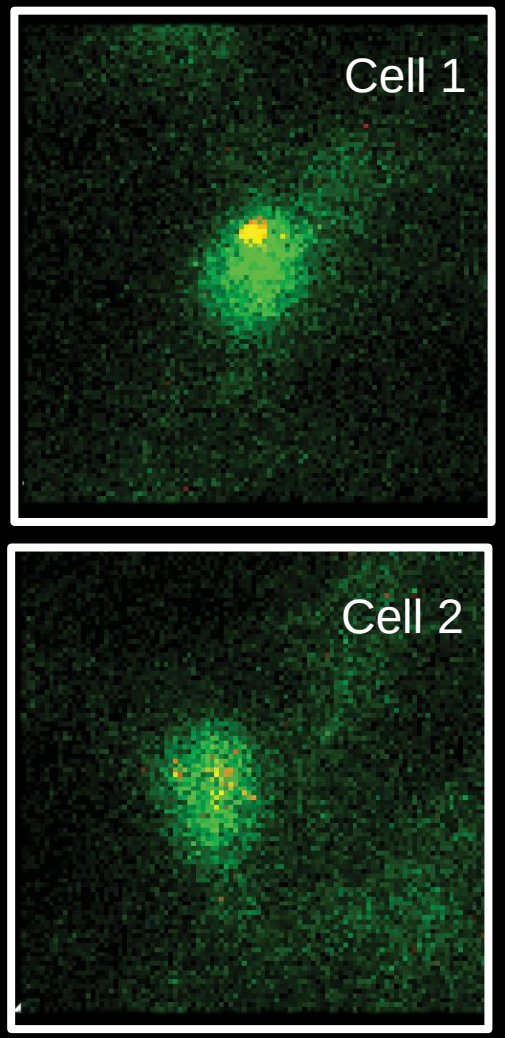
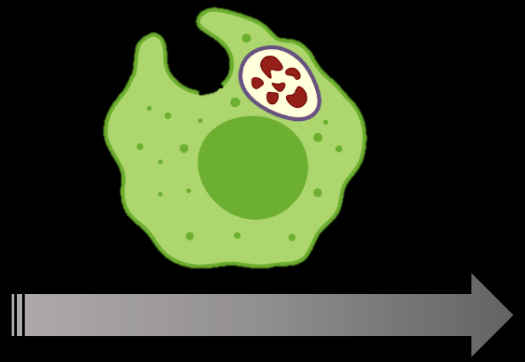
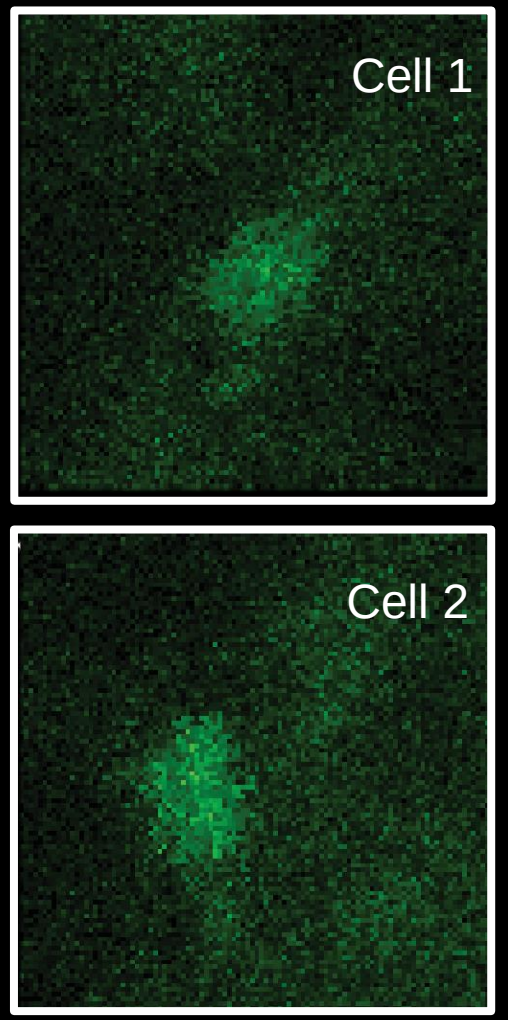




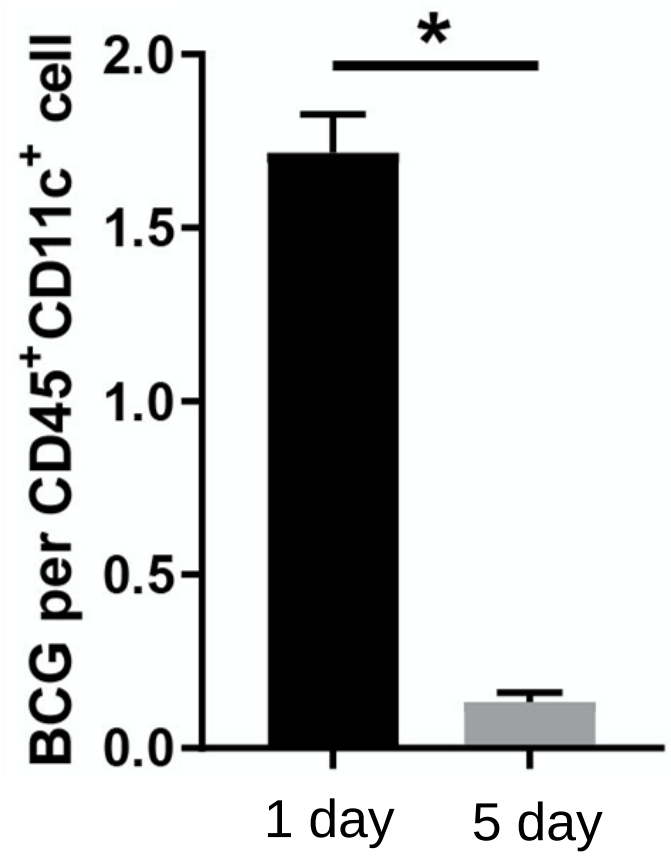
5 day post infection



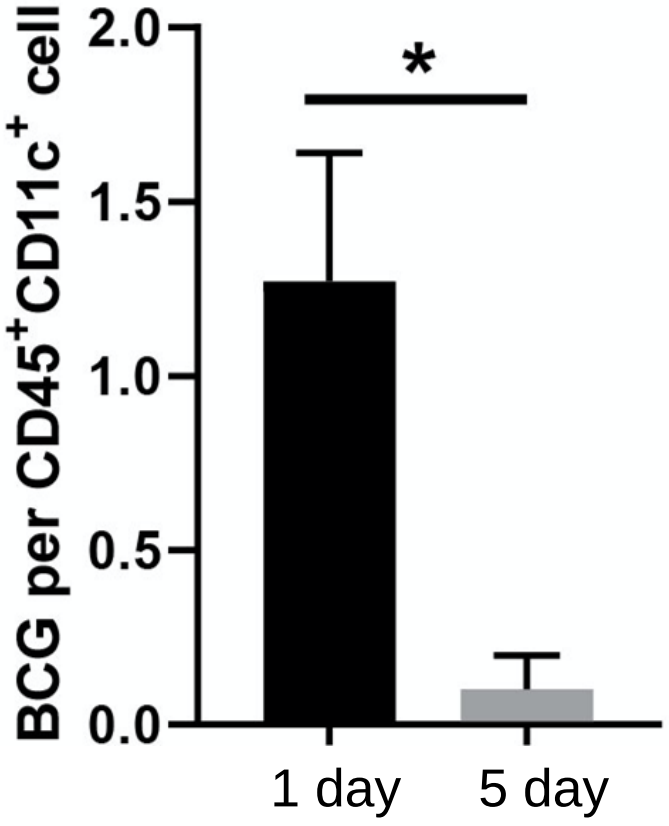
Slice macrophages cleared intracellular BCG as *in vivo*

Intracellular pathogen number

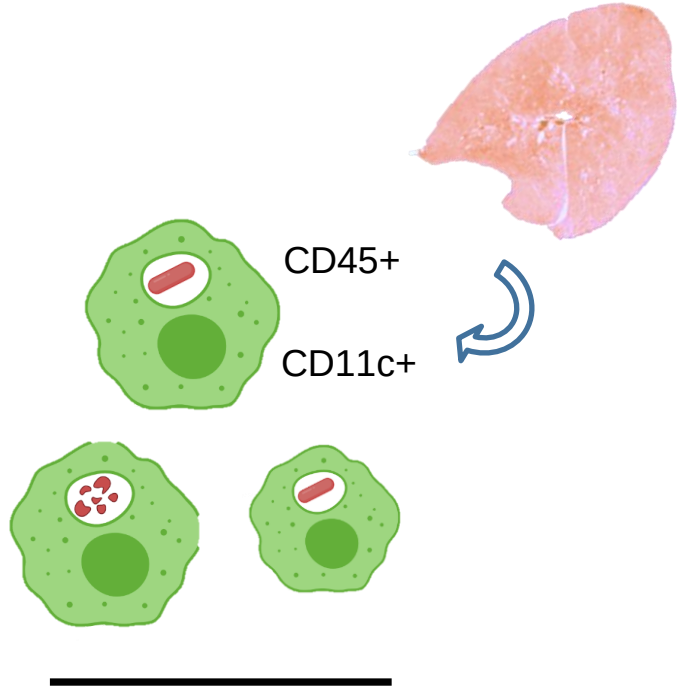
DNA fragments



Colony-forming unit, CFU



Time post infection

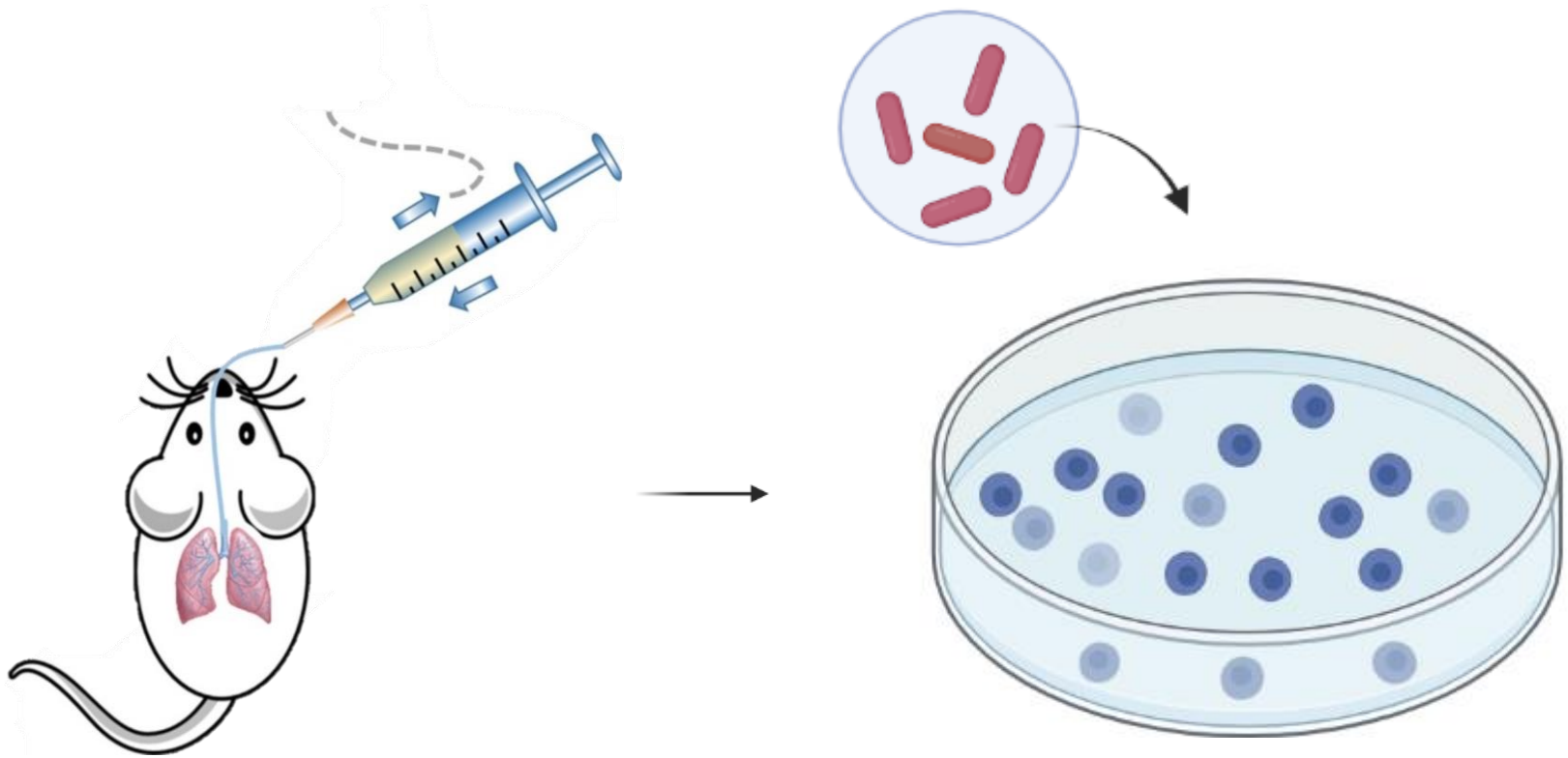


Lung slice macrophages

In vitro cultured primary macrophages permitted intracellular BCG to proliferate

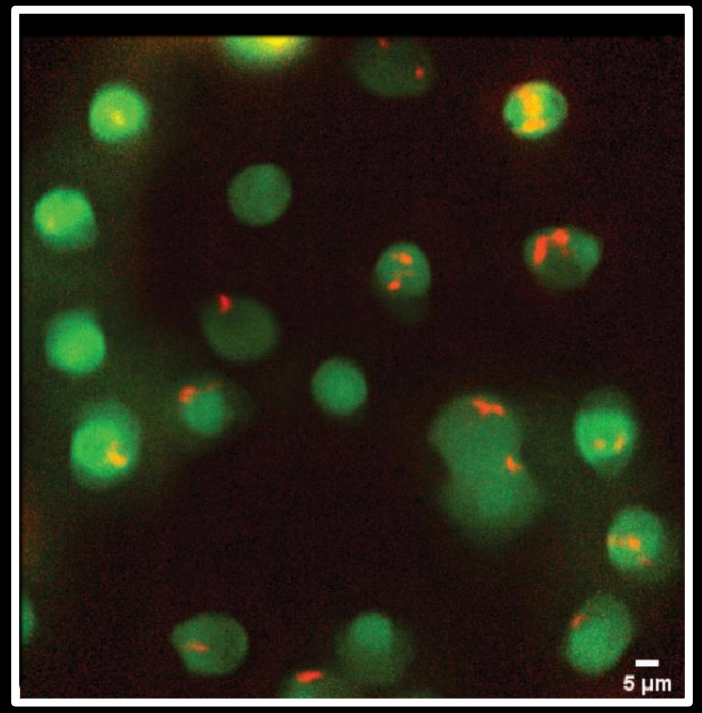
Bronchoalveolar lavage (BAL)

In vitro culture and infection start simultaneously

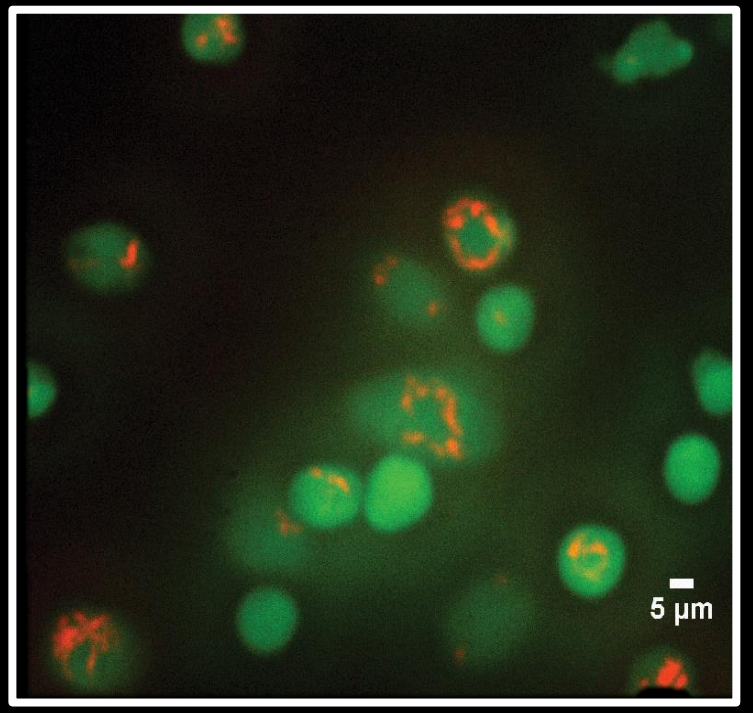


In vitro cultured primary macrophages permitted intracellular BCG to proliferate

1 day post infection



5 day post infection



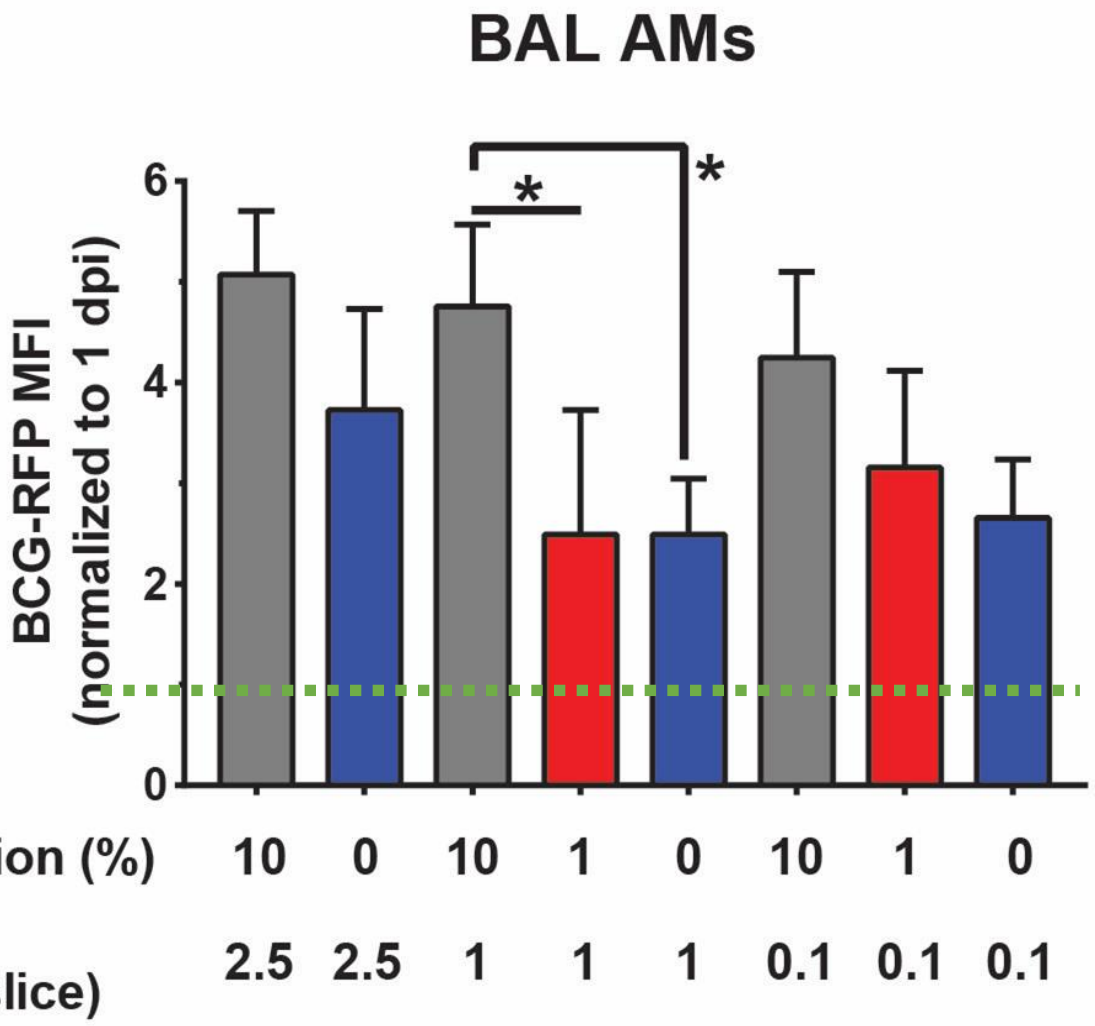
BCG: Bacillus Calmette–Guérin, vaccine strain of tuberculosis

CD11c: alveolar macrophage marker

In vitro cultured primary macrophages (BAL AMs) permitted intracellular BCG to proliferate

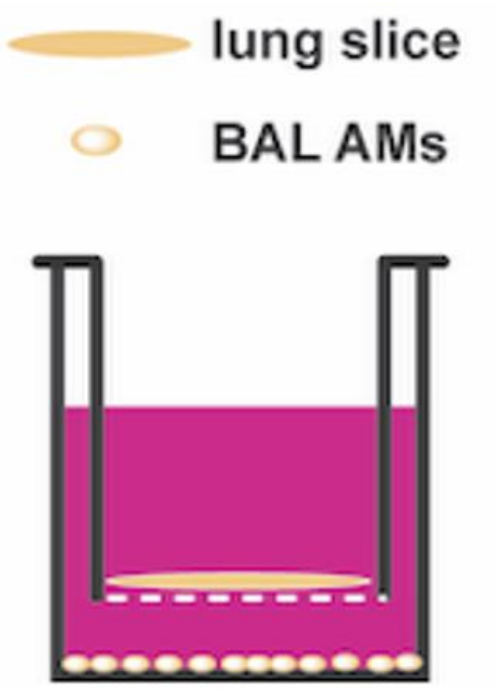
Not due to inter-lab variation

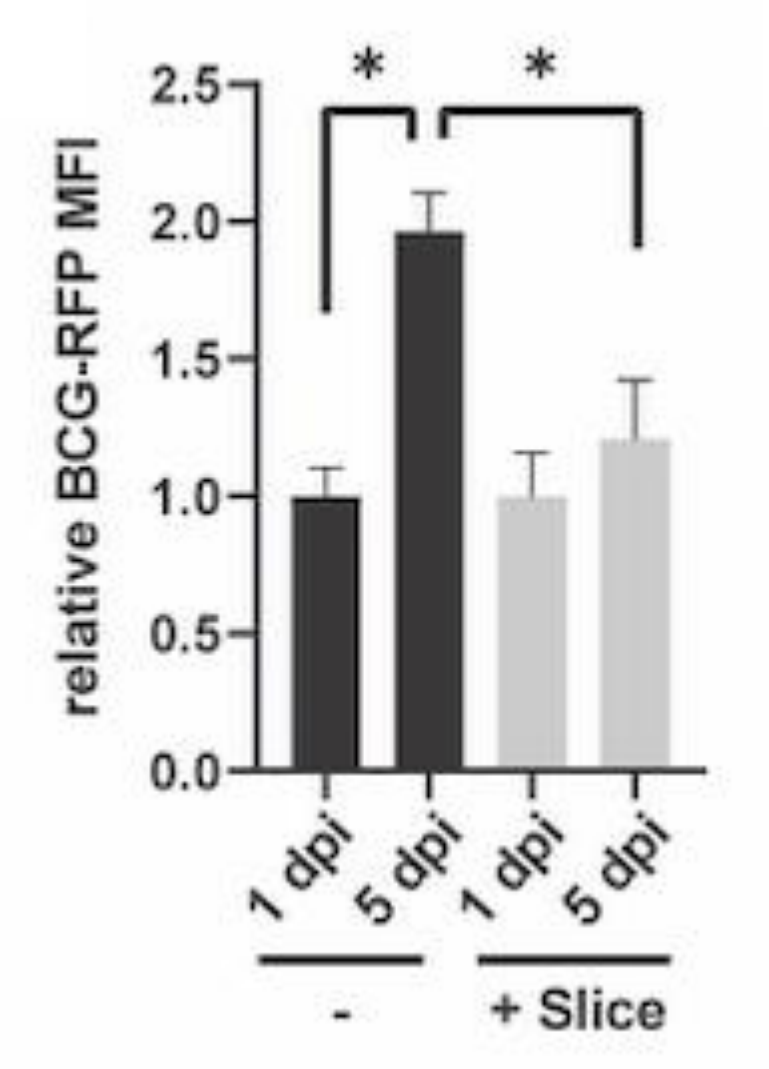
- **BAL AMs: the primary lung alveolar macrophages (AMs) prepared by bronchioalveolar lavage (BAL)**
- **BCG-RFP MFI: mean fluorescence intensity of the RFP carried by BCG, the vaccine strain of tuberculosis**
- **5 dpi (days post infection)**

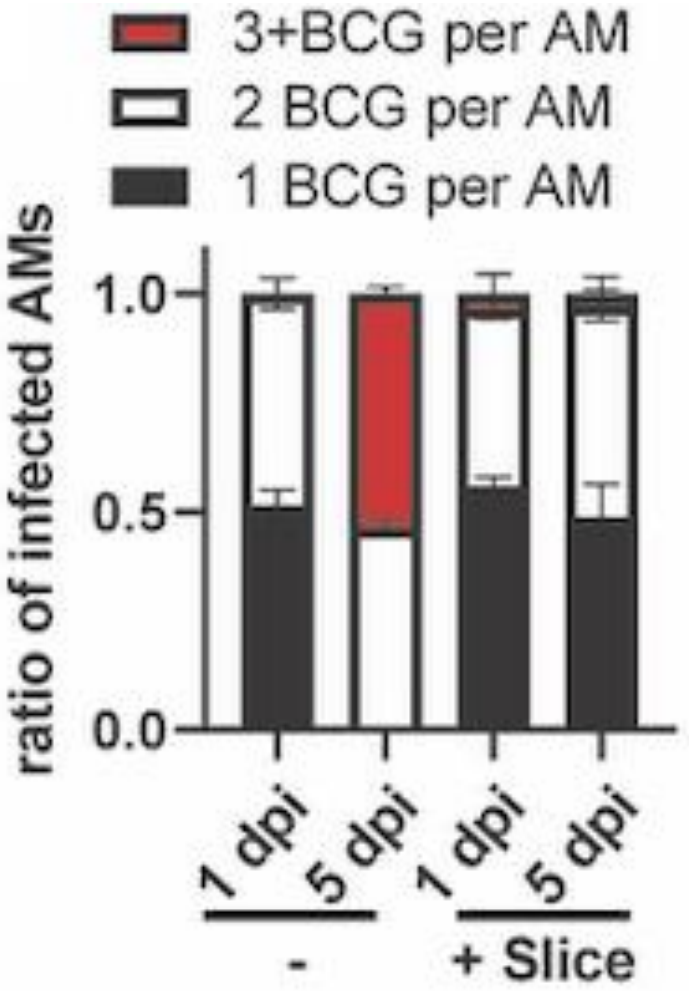


No reported *ex vivo* or *in vitro* models that have lung macrophages able to kill BCG

Coculturing with lung slices prevented intracellular BCG replications in *in vitro* cultured lung macrophages



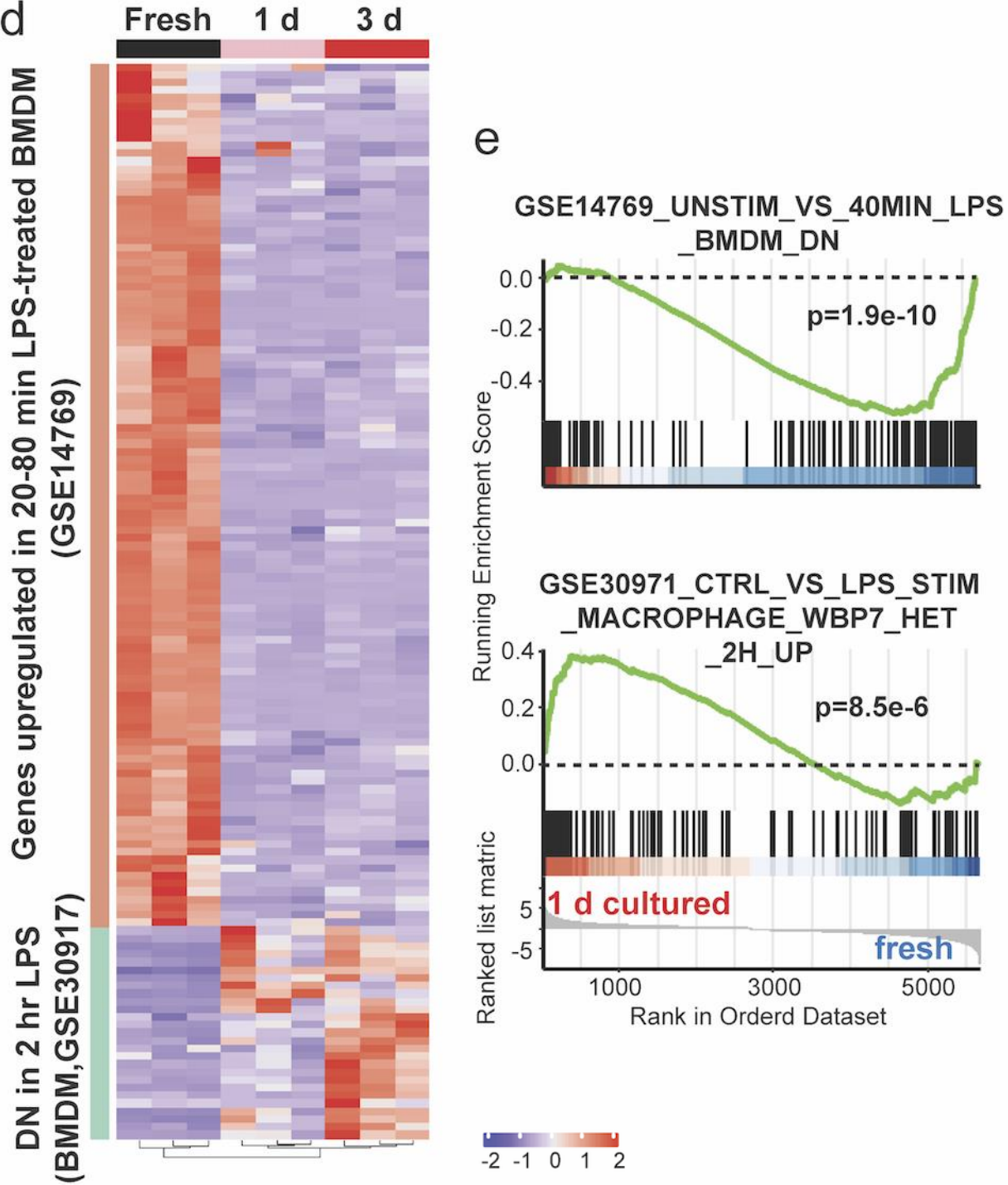




- **BAL AMs: the primary lung alveolar macrophages (AMs) prepared by bronchioalveolar lavage (BAL)**
- **BCG-RFP MFI: mean fluorescence intensity of the RFP carried by BCG, the vaccine strain of tuberculosis**

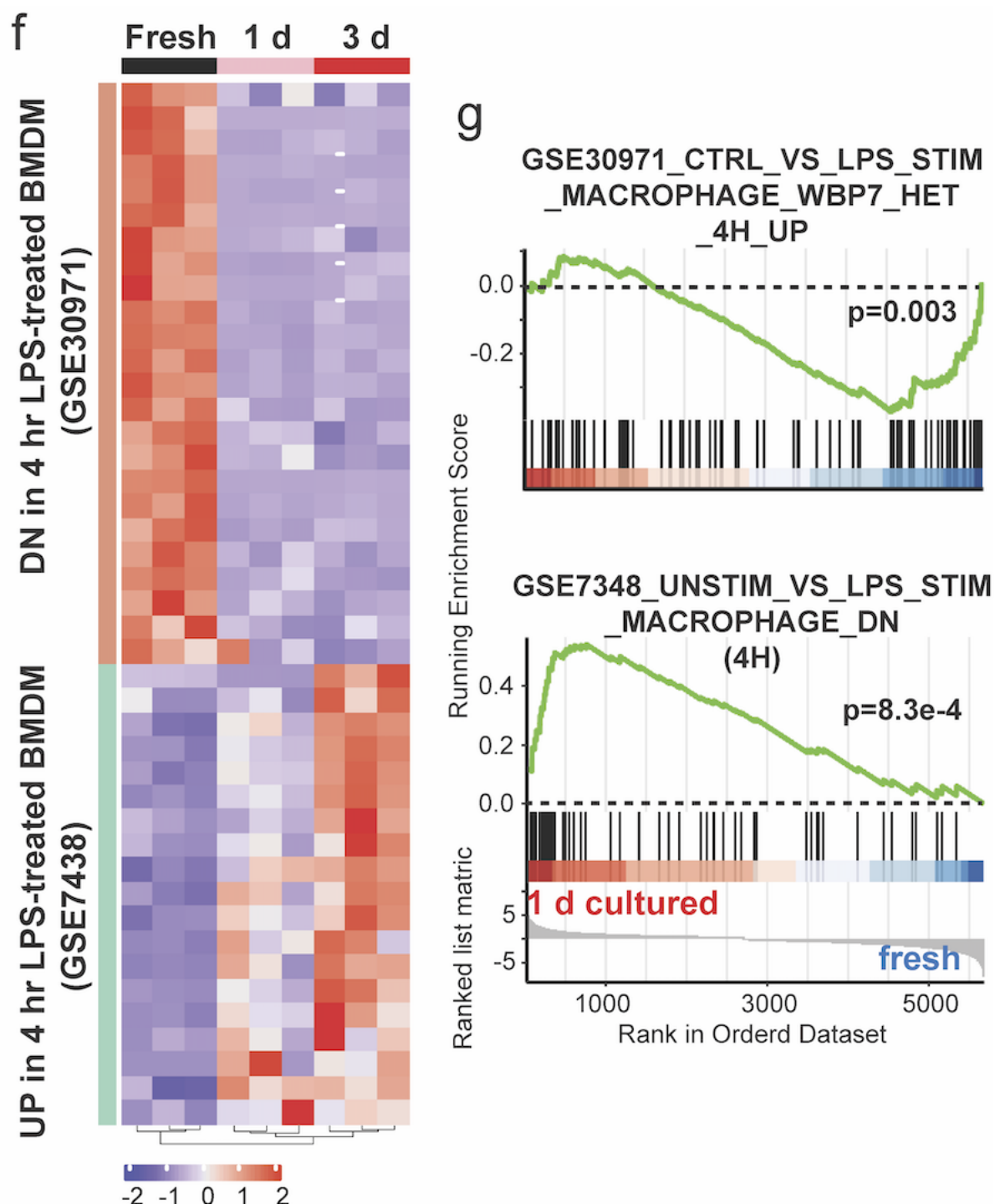
powerful and comprehensive pathway analysis pipeline

Common GSEA practice	**Powerful and comprehensive GSEA**
Call a pathway significant by seeing one geneset related with good p value	Evaluate the significance of potential signaling pathways by scrutinizing all the genesets related to the signaling programmatically and biologically
Evaluate the significance of pathway term only by its individual statistics. Ignore biological relation among genesets	Explore the synergy, antagonism and network among genesets to refine and enhance pathway analysis
Only pay attention to pathways with super small p value	Take into account thousands pathways with p value < 0.05



(d) Expression of the core enrichment genes in the below gene sets in fresh isolated, one-day and three-day *in vitro* cultured macrophages: (upper part) the set of genes upregulated in bone marrow derived macrophages (BMDM) treated with 10 ng/mL LPS for no longer than two hours, and (lower part) the set of genes downregulated in BMDM treated with 10 ng/mL LPS for two hours.

(e) Representative GSEA plots consistent with the heatmap demonstrating that upon 10 ng/mL LPS treatment for no longer than two hours, BMDM upregulated the genes enriched in fresh AMs (upper), and downregulated those enriched in one-day or longer *in vitro* cultured AMs (lower).

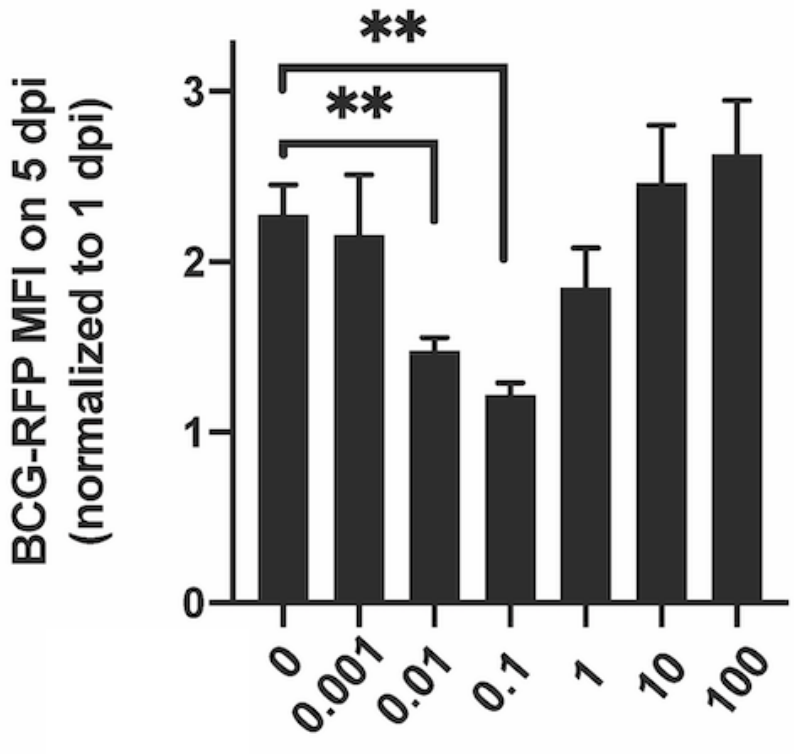


(f) Expression of the core enrichment genes in the below gene sets in fresh isolated, one-day and three-day *in vitro* cultured macrophages: (upper part) the sets of genes downregulated in BMDM treated with 10 ng/mL LPS for four hours, and (lower part) the of genes upregulated in BMDM treated with 10 ng/mL LPS for four hours.

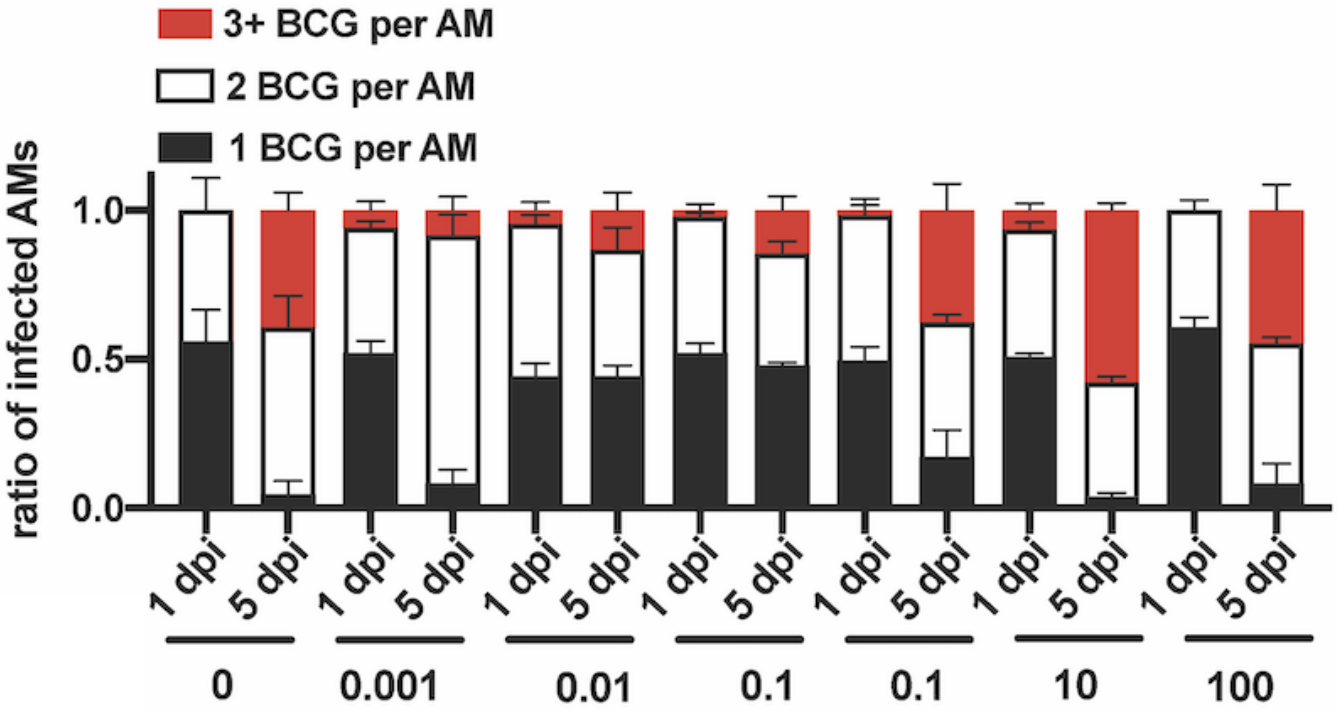
(g) Representative GSEA plots demonstrating that upon 10 ng/mL LPS treatment for four hours, BMDM downregulated the genes enriched in fresh AMs (upper), and upregulated those enriched in one-day *in vitro* cultured AMs (lower).

Experiments validated the role of the singaling revealed by previous pathway analysis

h



i



Distribution plot depicting portion of the macrophages containing one, two or more intracellular BCG

Confidential data upon request