

## Supplemental Information

### Biosafety Level 3 setup for multiphoton microscopy *in vivo*

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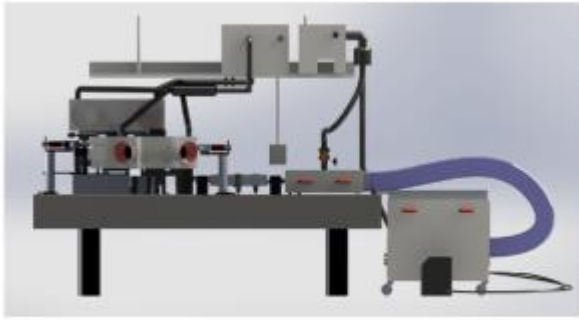
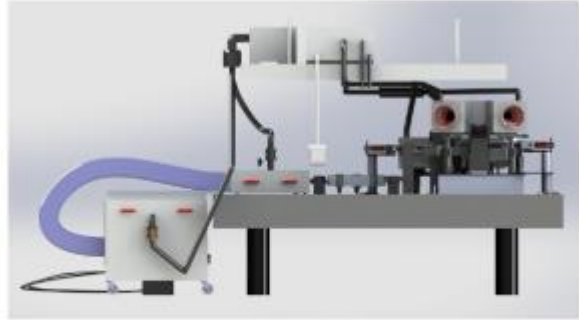
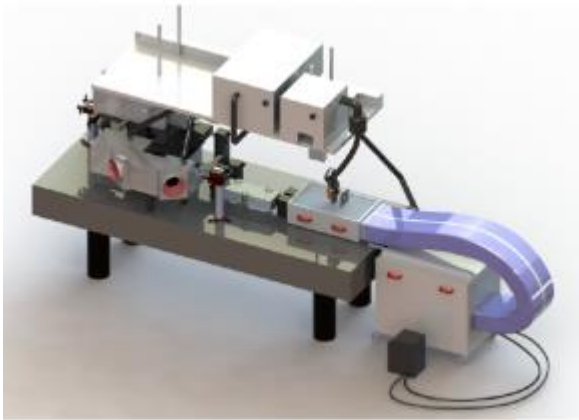
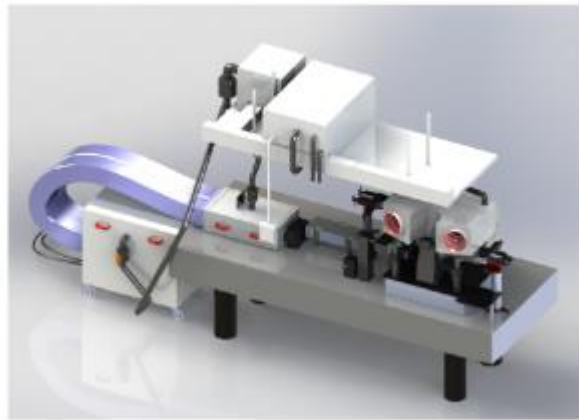
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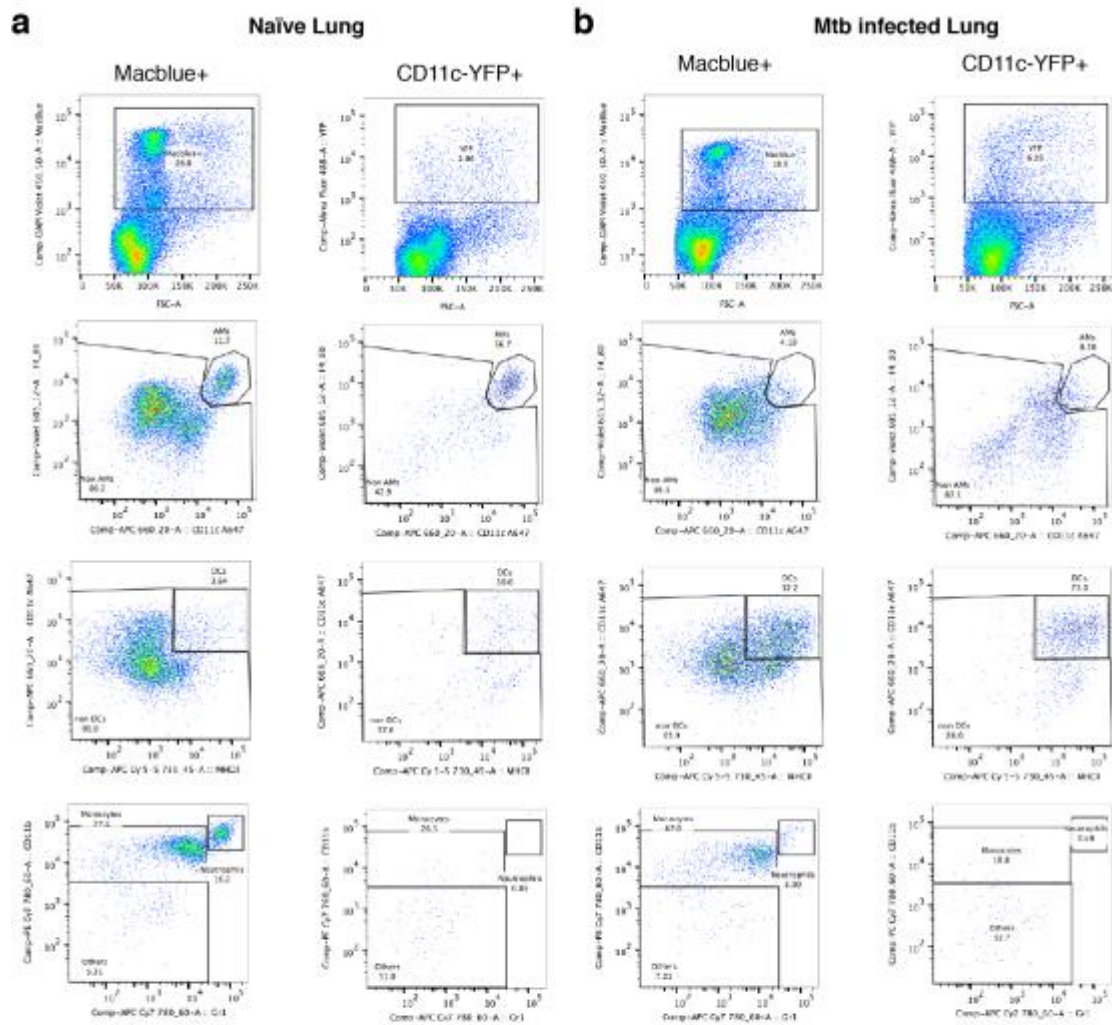
**Supplementary Figure S1. 3D drawings of BSL3 setup for MPM imaging.** The BSL3 setup was rendered digitally using Solidworks software for a detailed description of the assembly of all its parts. Left (a) and right (b) side view. Left (c) and right (d) orthogonal view.

Enable 3D View

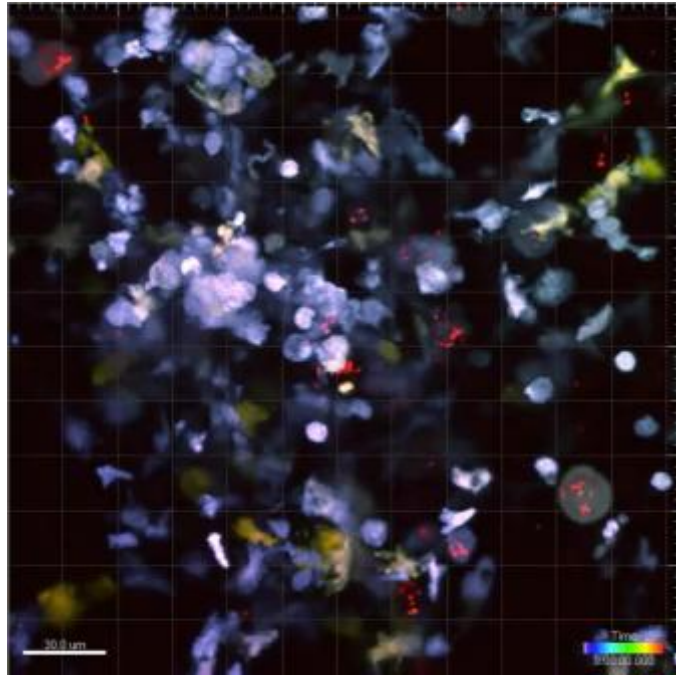
Supplementary Figure S2. Interactive 3D PDF of BSL3 setup for MPM imaging. SolidWorks drawings of the BSL3 setup were converted to 3D PDF file for browsing and detailed description of the assembly of all its parts. See 3D PDF file. In order to obtain the dimensions of biosafety cabinets and other parts please use 3D measurement tool available in Adobe Reader version 11.0.1 or higher while using a scale ratio of 1 model unit = 1000mm. For more detailed instructions please read: [https://helpx.adobe.com/acrobat/using/measuring-3d-objects-pdfs.html#measuring\\_3d\\_objects\\_in\\_pdfs](https://helpx.adobe.com/acrobat/using/measuring-3d-objects-pdfs.html#measuring_3d_objects_in_pdfs)

Supplementary Figure S3. Interactive 3D PDF of objective holder for BSL3 setup for MPM imaging. SolidWorks drawings of the BSL3 setup were converted to 3D PDF file for browsing and detailed description of the assembly of all its parts. See 3D PDF file. In order to obtain the dimensions of biosafety cabinets and other parts please use 3D measurement tool available in Adobe Reader version 11.0.1 or higher while using a scale ratio of 1 model unit = 1000mm. For more detailed instructions please read: [https://helpx.adobe.com/acrobat/using/measuring-3d-objects-pdfs.html#measuring\\_3d\\_objects\\_in\\_pdfs](https://helpx.adobe.com/acrobat/using/measuring-3d-objects-pdfs.html#measuring_3d_objects_in_pdfs)

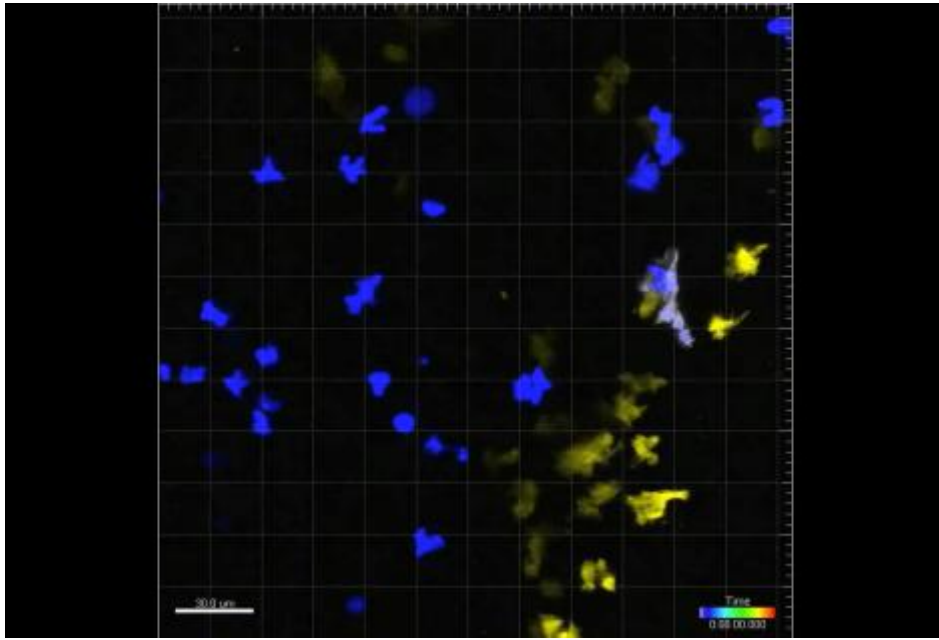




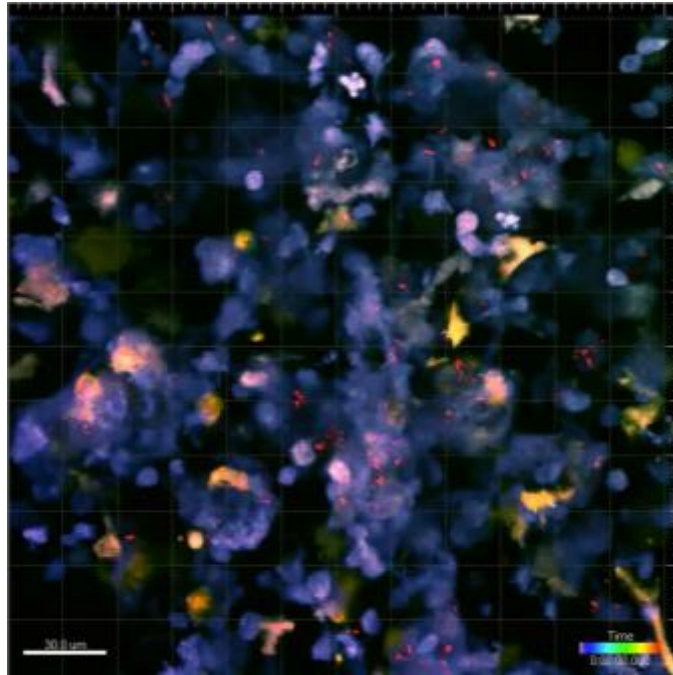
**Supplementary Figure S4. Flow Cytometry gating strategy to define different immune cell types in the mouse lung.** (a) naïve and (b) *Mycobacterium tuberculosis* (Mtb) infected lung. Alveolar macrophages (AM) are F4/80<sup>high</sup> CD11c<sup>high</sup>, Dendritic cells (DCs) are CD11c<sup>high</sup> MHCII<sup>high</sup>, Monocytes are CD11b<sup>high</sup> Gr1<sup>low/int</sup> and Neutrophils are CD11b<sup>high</sup> Gr1<sup>high</sup>.



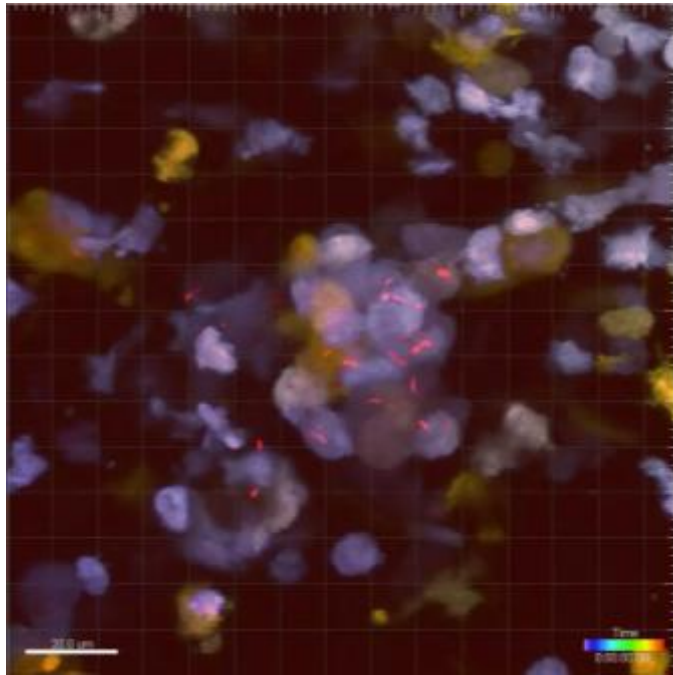
**Supplementary Movie 1. Macblue and CD11c-YFP cell migration in lung after *M. tuberculosis* infection at high negative pressure levels (90Pa).** Macblue x Cd11c-YFP mice were infected with H37Rv-Dsred and 36 later lung slices were obtained using a vibratome and used for MPM. 107 μm 3D stack. See Sup Movie 1 movie file.



**Supplementary Movie 2. Macblue and CD11c-YFP cell migration in the lung before infection.** Macblue x CD11c-YFP mice were sacrificed and lung slices obtained using a vibratome for MPM. 44µm 3D stack. See See Sup Movie 2 movie file.



**Supplementary Movie 3. Macblue and CD11c-YFP cell migration in lung after *M. tuberculosis* infection at low negative pressure levels (20Pa).** Macblue x Cd11c-YFP mice were infected with H37Rv-Dsred and 36 later lung slices were obtained using a vibratome and used for MPM. 90μm 3D stack. See Sup Movie 3 file.



**Supplementary Movie 4. Macblue and CD11c-YFP cell migration in lung after *M. tuberculosis* infection at low negative pressure levels (20Pa) and high zoom.** Macblue x Cd11c-YFP mice were infected with H37Rv-Dsred and 36 later lung slices were obtained using a vibratome and used for MPM. 67μm 3D stack. See Sup Movie 4 file.